

APPLIED SCIENCES AND ENGINEERING

Local control of Cre-regulated genetic modeling in vivo using wireless implantable optoelectronics

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Effective modeling of chronic human diseases in vivo requires precise spatiotemporal regulation of molecular processes at defined tissue locations. Optogenetic and optoelectronic tools can provide such control but targeting internal organs such as the lungs remain challenging. To address this, we developed E-LightR-Cre recombinase, enabling precise, spatially restricted gene activation with no dark-state activity and robust blue light responsiveness. To achieve local activation of E-LightR-Cre in specific organ sites, we engineered wireless, fully implantable optoelectronic devices enabling focal illumination of murine lungs with no discernible organ damage. The size of the activated area is controlled by modulating light intensity and duration. Using implanted optoelectronic devices, we activated E-LightR-Cre in a selected area of mouse lungs and achieved local regulation of targeted genes. Light-regulated expression of the oncogenic KRas-G12D mutant in mouse lungs induced local formation of oncogenic lesions. These results show that our protein engineering and adaptable optoelectronic approaches enable facile application of optogenetic tools for locally controlled disease modeling in vivo.

INTRODUCTION

Strategies for genetic manipulation in vivo largely rely on site-specific DNA recombinases, with the Cre recombinase being the most widely used among them. To achieve conditional regulation of gene expression, Cre is often fused with an inducer-responsive domain, facilitating recombination between loxP-flanked target DNA fragments (1, 2). In the Cre-lox mouse models, some spatiotemporal control can be achieved by selecting a cell type-specific promoter for Cre expression along with adjusting the chemical inducer type, dose, and timing (3–10). However, this strategy triggers genetic modification in all cells where the promoter is active, causing systemic phenotypes that often mask local effects of the gene regulation (8, 11, 12). Chemical induction of engineered Cre in vivo also introduces additional caveats of nonspecific side effects and toxicity (13–15). Direct

delivery of tissue-specific, transiently expressed Cre recombinase improves spatial and temporal regulation by restricting the induction to a specific organ but still lacks the capability to control Cre activity in a locally restricted area within the target organ (16–19). These limitations hamper our ability to model localized physiological and pathological processes in vivo.

Many chronic conditions in humans, including various forms of cancer, originate from molecular alterations localized to specific regions within an organ. Human tumors typically originate at a single site and gradually progress to stages of invasion and dissemination. However, animal models using Cre-mediated conditional expression of an oncogene (19–21) or exposure to carcinogens (22) often generate multiple tumors growing simultaneously at different locations within an organ, making it difficult to study the shifts in tumor microenvironment

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typically associated with clinical nascent tumors (23). Attempting to limit the number of tumors by reducing the Cre recombinase or carcinogen dose decreases the probability of tumor formation and extends the experimental timeline, making early tumor detection time-consuming and cost prohibitive. Alternative approaches, such as direct implantation of tumor cells or tumor tissue, can generate local onset of tumors but bypass the critical early stages of tumor initiation (24). Thus, there is a need for preclinical models of early tumorigenesis that emulate the emergence of spatially localized lesions seen typically in early human tumorigenesis.

With recent advances in optogenetic technologies, photoactivatable Cre recombinases have emerged as a promising solution for local regulation of genetic modifications (25, 26). An ideal optogenetic Cre for spatiotemporal control in vivo must exhibit a strong light-induced recombination activity while demonstrating no background recombination in the dark. Tight control of Cre activity is critical for in vivo optogenetics as leakiness, if allowed to accumulate over time, would negate the ability of the tool in modeling localized chronic diseases. Most reported optogenetic Cre recombinases rely on light-mediated dimerization of split-Cre systems (27–33). They often suffer from unintended spontaneous assembly and imbalanced expression levels of the two components, resulting in suboptimal performance and/or high background activity. A single-component, light-inducible Cre (LiCre) has shown very strong photoactivity in mammalian cell culture (34). However, this system also exhibits targeted DNA binding in the dark, indicating potential background recombination activity (35). All these limitations ultimately compromise the application of existing photoactivatable Cre recombinases for spatiotemporally defined modeling of chronic diseases in vivo.

An essential element required for in vivo optogenetic regulation is a system for local illumination that can offer tunable control over illumination intensity, duration, area, and timing. Tethered fiber optics (36, 37) can physically damage the targeted tissue, whereas the application of external illumination devices is limited due to the poor penetration of light through animal tissue, making it difficult to reach internal organs. In vivo illumination strategies using implantable devices overcome these limitations. The advancement of wireless microscale inorganic light-emitting diodes (μ -ILEDs) controlled via near field communication (NFC)-enabled reliable operation in freely moving animals (38–40). Previous implantable optoelectronic devices have predominantly been developed for applications targeting the central nervous system (38, 39, 41, 42). However, their adaptability for use in other organs and peripheral nervous systems has been limited (40, 43, 44). Wireless device integration with soft, moving organs is further challenging due to the mechanical compliance and biocompatibility of the device materials. Existing devices, largely designed for relatively fixed anatomical environments, are less suited for internal, dynamic organs such as the lungs, potentially compromising device performance and increasing the risk of tissue damage over time.

Here, we report a powerful strategy to model long-term disease processes with spatiotemporal precision. We developed a photoactivatable Cre system E-LightR-Cre, enabling the desired spatiotemporal control of genetic modifications in vivo. E-LightR-Cre ensures robust light-mediated regulation of gene expression with no detectable leaky activity in the dark. To regulate E-LightR-Cre in deep internal organs in vivo, we developed miniaturized wireless, battery-free, fully implantable optoelectronic devices that integrate a soft, flexible, serpentine interconnect with a μ -ILED, mounted on a lightweight,

thin, flexible, printed circuit board. The mechanically decoupled design is intended to accommodate constraints imposed by surrounding anatomy, surgical access, and organ motion, thereby addressing multiaxial deformation challenges associated with prior designs. The use of commercially available fabrication workflows provides a pathway toward scalable, reproducible, and cost-effective device production. A custom-designed software interface (GUI) provides real-time light control, allowing precise spatiotemporal modulation of light by controlling parameters such as duty cycle, operating duration, and power to initiate and regulate genetic modification and oncogenesis locally. Together, this wireless system ensures spatiotemporal optogenetic stimulation in animals with minimal mechanical interference, enabling reliable, continuous experiments for adjustable surgical depth and illumination area.

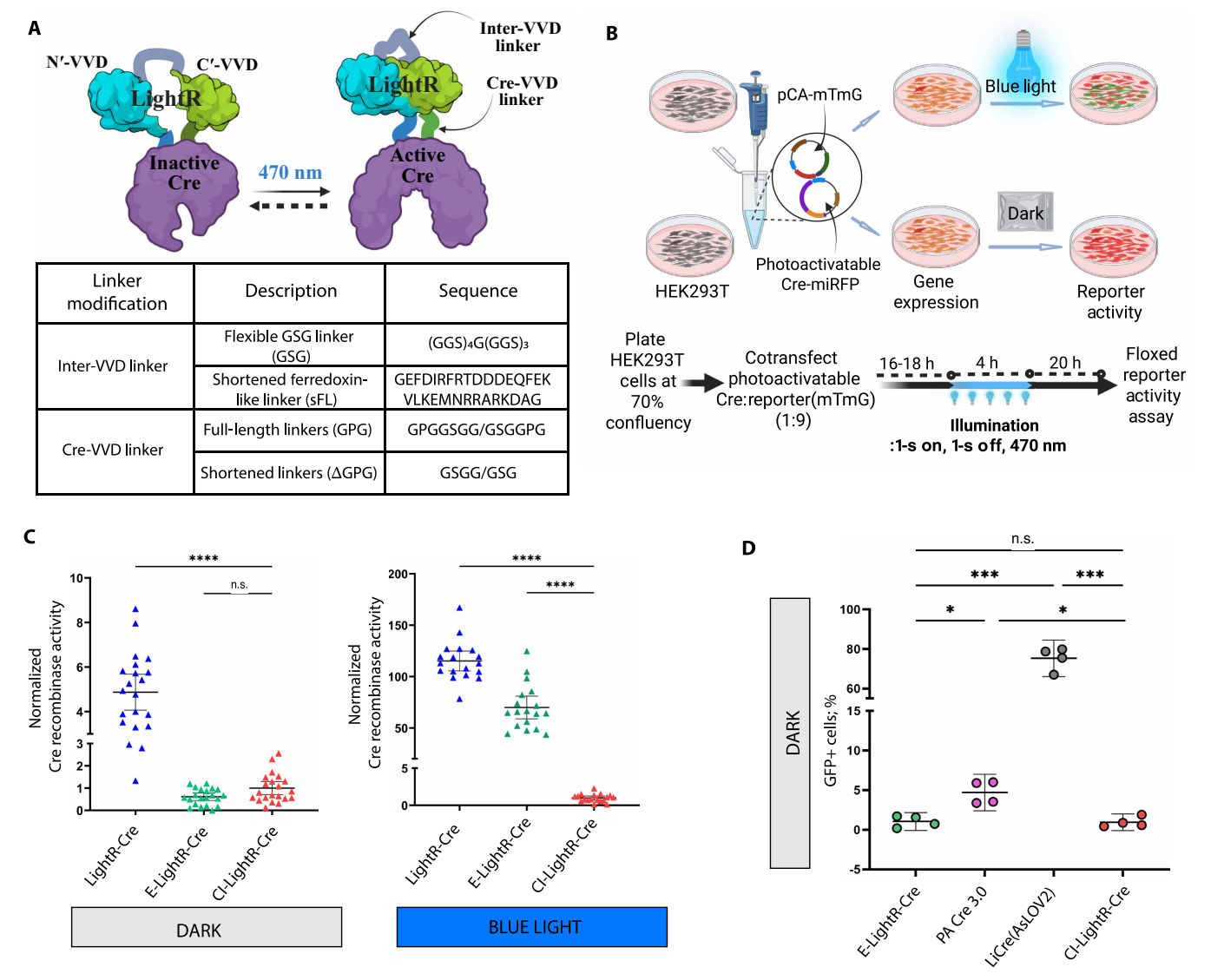
We demonstrate the tunability of localized regulation with these devices in vitro, showcasing their ability to induce local gene expression and trigger oncogenic transformation in a spatially restricted population of cells. Intrathoracic placement of the μ -ILED over the lung enabled localized E-LightR-Cre activation, inducing local expression of a reporter gene. These implants did not affect mouse health, mobility, or lung architecture while providing stable wireless power delivery. Application of these technological advancements allowed us to initiate focal onset of lung adenocarcinoma in LSL-KRas-G12D mice. Together, our study describes a substantial advancement of optogenetic technology to enable better preclinical modeling of human diseases.

RESULTS

Development of light-regulated Cre recombinase demonstrating no leaky activity in the dark

Spatiotemporal control of genetic modeling in vivo requires a light-regulated Cre construct that demonstrates no background activity in the dark. We previously reported the development of light-regulated Cre recombinase LightR-Cre (45) (Fig. 1A). To assess whether LightR-Cre has properties needed for application in vivo, we carefully evaluated regulation of LightR-Cre by blue light. We used mTmG reporter (46) that basally expresses a membrane-targeted tdTomato and switches to expression of membrane-targeted green fluorescent protein (GFP) following Cre-mediated recombination (Fig. 1B). Our analysis confirmed robust activation of LightR-Cre by blue light in human embryonic kidney (HEK) 293T cells (Fig. 1C). However, we detected a low level of background recombination in the dark when compared to the inactive mutant of LightR-Cre [Y324F (29), CI-LightR-Cre] (Fig. 1C and fig. S1A). Assessment of basal leakiness from CI-LightR-Cre indicates that the low-level GFP expression from the mTmG reporter is intrinsic to the reporter construct and independent of Cre activity as the reporter signal shows no significant difference between cells transfected with CI-LightR-Cre or with a non-Cre control vector (fig. S1B).

Even low level of leakiness can interfere with application of light-regulated Cre in vivo to study chronic processes. Therefore, to eliminate the leakiness of LightR-Cre in the dark, we introduced additional modifications to the LightR domain. The LightR domain is composed of two tandemly connected VVD photoreceptor domains that homodimerize in the blue light and dissociate in the dark, creating a light-sensitive clamp that is open in the dark and closes upon illumination (Fig. 1A). Insertion of the LightR domain into Cre at position D153 made it regulatable by light, where the open LightR clamp inactivates Cre in the dark and light-induced closing of LightR rescues



Cre activity (45). We hypothesized that the leakiness of LightR-Cre in the dark state occurs due to spontaneous dimerization of the VVD domains, resulting from high flexibility of the connecting linkers. Thus, to develop a tightly regulated variant of LightR-Cre with no leakiness, we aimed to switch the highly flexible 22 amino acid inter-VVD linker (GSG linker) with a more rigid 31 amino acid sFL linker “GEFDIRFRTDDDEQFEKVLKEMNRRARKDAG” (Fig. 1A). The sFL linker is a shortened version of the previously reported ferredoxin-like linker peptide, which demonstrates reversible unfolding and refolding upon induction and withdrawal of mechanical load, without any hysteresis (47). We hypothesized that the increased rigidity

of the sFL linker would prevent spontaneous homodimerization of the VVD domains in the dark and thus reduce unwanted leakiness of LightR-Cre. In a parallel study, we successfully used the sFL linker to eliminate background leakiness in a light-regulated kinase (48). Also, our previous results show that variation of the linkers connecting an allosteric switch domain to the target enzyme affects the efficiency of regulation (49–51). We proposed that shortening the Cre-VVD junction linkers limits conformational freedom between domains, thereby enhancing allosteric coupling and tightening regulation, reducing basal activity and leakiness while preserving inducible control. Thus, we also replaced the flexible linkers GPGGSGG and GSGGPG

at the N and C termini of the LightR (Cre-VVD linkers; Fig. 1A) to shorter GSGG and GSG, respectively, which can further reduce the leaky activity in the dark. To evaluate the individual contribution of each linker modification, we compared constructs containing combinations of the inter-VVD linkers (GSG or sFL) with Cre-VVD linkers (GPG or Δ GPG) under light and dark conditions. All variants exhibited robust light-induced recombination (fig. S1C). In the dark, shortening the Cre-VVD linker alone did not significantly reduce leakiness. In contrast, incorporation of the ferredoxin-like (sFL) inter-VVD linker markedly suppressed background activity compared to GSG-GPG and GSG- Δ GPG constructs (fig. S1C). Comparison of sFL-GPG and sFL- Δ GPG revealed a modest but significant further reduction in dark-state activity, indicating an additive effect of the two modifications, with the sFL linker serving as the primary contributor to leakiness suppression (fig. S1C). We named the sFL- Δ GPG linker-modified Cre as E-LightR-Cre. Fluorescence microscopy analysis of Cre reporter activity revealed that E-LightR-Cre exhibits nearly eightfold reduced recombination frequency in the dark, compared to the original LightR-Cre (Fig. 1C). Analysis of a constitutively inactive version of LightR-Cre revealed no significant difference between E-LightR-Cre and CI-LightR-Cre in the dark, indicating no detectable leakiness with E-LightR-Cre. Under illuminated conditions, E-LightR-Cre displayed strong DNA recombination activity (Fig. 1C and fig. S1A).

Next, we further compared the regulation of E-LightR-Cre to two other blue light-inducible Cre recombinase systems, PA Cre 3.0 (30) and LiCre(AsLOV2) (34), which have shown the most efficient light-mediated regulation of DNA recombination in previous reports. Flow cytometry analysis of the reporter expression in HEK293T cells revealed that the PA Cre 3.0 and the LiCre(AsLOV2) both show significant leaky activity in the dark, when compared to inactive CI-LightR-Cre (Fig. 1D and fig. S1, D and E). E-LightR-Cre exhibited no detectable leaky activity in the dark. The light-induced activity of E-LightR-Cre was comparable to PA Cre 3.0 with no significant difference observed (fig. S1D). Although LiCre(ASLOV2) was found to show the highest level of light-induced activity, the pronounced leakiness in the dark renders its application in vivo very challenging (Fig. 1D and fig. S1, D and E). Overall, our data demonstrate that modified E-LightR-Cre exhibits much improved regulation required for optogenetic experiments in vivo.

Design and operational features of wireless optoelectronic devices for in vivo activation of E-LightR-Cre

We designed a wireless implantable optoelectronic device for localized optogenetic control of lung tissue in freely moving mice by optimizing its mechanical flexibility, biocompatibility, and illumination characteristics. The overall design includes a radio frequency (rf) power harvesting system, composed of an inductive planar coil antenna and a rectifier, coupled to a μ -ILED by a flexible trace and encapsulated in transparent, flexible, and biocompatible materials (Fig. 2). The planar optoelectronic design integrates top and bottom copper (Cu) layers laminated on a 50- μ m-thick polyimide (PI) substrate. A soft, flexible serpentine interconnect [24 mm (*L*) by 1.8 mm (*W*)] provides both vertical and horizontal freedom relative to the connected oval rf receiver coil (10 mm in diameter; Cu traces: six turns, 60 μ m in width, 18 μ m in thickness, 73- μ m spacing) and the integrated μ -ILED [270 μ m (*L*) by 220 μ m (*W*) by 50 μ m (*H*)] for localized optogenetic stimulation. This approach facilitates large-scale, commercial flexible printed circuit board (fPCB) manufacturing processes to increase

throughput and facilitate broader dissemination of wireless implantable optoelectronic tools across the scientific community (Fig. 2A and fig. S2). The serpentine interconnects can be fabricated in customizable lengths, supporting additional applications targeting peripheral nerves or visceral organs (fig. S2B). The device components include a capacitor that provides parallel resonant impedance matching at 13.56 MHz and a Schottky diode half-bridge rectifier to yield a current source for the μ -ILED (Fig. 2B and fig. S3). A second μ -ILED emitting red light (650 nm) resides within the receiver coil to provide a visible indicator of system activation and confirmation of device operation (52). Last, the device is encapsulated in parylene followed by a soft and flexible layer of silicone [Ecoflex; poly(dimethylsiloxane) (PDMS)] for implant comfort. The devices wirelessly harvest power via magnetic inductive coupling to a resonant transmission antenna around an animal enclosure, powered by an rf source at 13.56 MHz (NeuroLux Inc.). The miniaturized device dimensions and ultralightweight, biocompatible design ($\sim$ 60 mg; Fig. 2B) were implemented to ensure successful implantation in adult mice without affecting their health or motion.

Electrical and mechanical properties of the optoelectronic device

Reliable operation of the device under mechanical stress exerted by freely moving animals is critical for successful applications in mouse models. Thus, we evaluated the key electrical and mechanical properties of the devices.

Electrical characterization of the device coil reveals that experimental measurements of scattering parameters (S11), resonant frequency (f_r), and quality (Q) factor at different radii of curvature match the simulated properties of the antenna. These results demonstrate that the device maintains reliable functionality and consistent optical stimulation even under significant mechanical deformation during both in vitro benchtop experiments and simulations (up to a radius of curvature of 4 mm; Fig. 2C) and within the in vivo wireless rf chamber ($L \times W \times H$: 30.5 cm by 25.4 cm by 15.2 cm) for performance during the natural locomotion of small animals (Fig. 2C and fig. S4A). To address the angular misalignment and wireless device powering within the cage under realistic load conditions, we used electromagnetic modeling of the wired antenna cage and acquired quantitative power measurements from the magnetic field, as well as the device's harvested power versus tilt angle as a surrogate for postural changes by the animal (fig. S4, B to D). These results demonstrate that more than sufficient electrical power is captured by the device even at the lowest density location (center of cage) and at up to 70° of tilt (fig. S4D).

To evaluate the mechanical performance of the device coil, we quantified strain distribution in the metal traces of the coil antenna when bent to a 6-mm radius. Simulation results show that the metal traces experience strain levels exceeding the yield point (0.3%) but remain well below the fracture limit for Cu (<1%) (Fig. 2D). We also assessed the mechanical performance of the serpentine interconnects encapsulated by a soft silicone layer under various deformation conditions. Finite element analysis (FEA) was used to simulate serpentine stretching (up to 18%), bending (to a radius of curvature $R = 2.5$ mm), and twisting (up to 720°). This analysis demonstrates that the serpentine coil could accommodate extensive mechanical deformations without yielding as the strain in the metal remains below 0.3% (Fig. 2E), aligning with the types of deformation experienced by soft biological tissues (40, 53). Additional mechanical tests, including tensile and

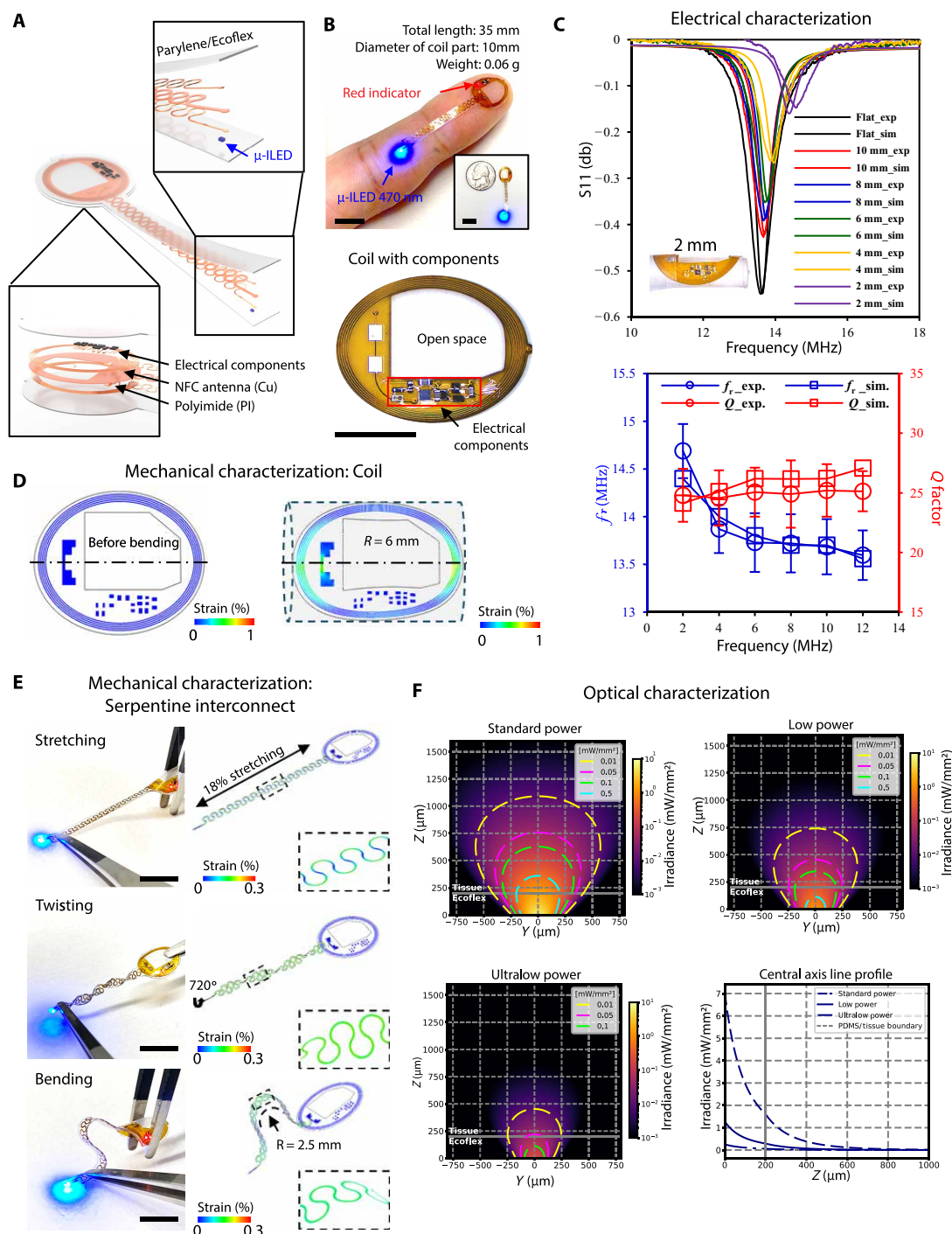


Fig. 2. Development of wireless, battery-free, miniaturized, fully implantable optoelectronic device and characterization of its electrical, mechanical, and optical properties. (A) Schematic illustration of the overall construction highlights a flexible serpentine interconnect with a μ -ILED at the tip end, connected to a receiver coil with matching capacitors, a rectifier, and a separate μ -ILED indicator. (B) Top: Optical image of the device showing the overall design and size, highlighting the soft, flexible, stretchable interconnect with a blue μ -ILED at the tip and a separate red indicator LED. Scale bar, 1 cm. Bottom: Optical image showing the top view of the coil within the electronic circuitry, including all integrated components. Scale bar, 5 mm. (C) Electrical performance analysis: Experimental (exp) and simulated (sim) antenna performance of the coil as a function of bending curvature. Top: Simulated reflection scattering parameter (S_{11}) versus antenna frequency at different curvature radii. Bottom: Comparison of the experimental and simulated values of the resonant frequency (f_r) and Q factor of the antenna at different radii of curvature. (D and E) Mechanical performance analysis: (D) Device coil: distribution of effective strain in the Cu coil of the device body, before (left) and after (right) bending, with a radius of curvature of $R = 6$ mm. (E) Serpentine interconnect: images of the activated devices and corresponding FEA results in stretching, twisting, and bending mode. Scale bars, 1 cm. The color map represents effective strain distribution. (F) Irradiance profiles of blue μ -ILEDs with varying current-limiting resistors for spatiotemporally confined illumination. Each condition is accompanied by a central axis irradiance line plot.

cyclic testing, demonstrate that the encapsulating layers provide effective barrier properties, ensuring sufficient operational stability and stretchability for implantation in vivo. The flexible serpentine interconnects accommodate ~210% strain without causing mechanical or electrical failure of the embedded conductive Cu traces (fig. S5, A and B), far more than the devices would experience in vivo. They also maintain structural integrity and functionality under a wide range of mechanical deformations caused by exaggerated animal locomotion, enduring from -40% bending to +50% stretching over 10,000 repetitive cycles without degradation in performance (fig. S5C). Such deformation applied during in vitro benchtop testing substantially exceeds physiological respiratory strains and represents an accelerated fatigue test under a deliberately conservative mechanical loading envelope (54–56).

Together, these results demonstrate that the device offers reliable functionality and confirmational flexibility without compromising its ability to harvest wireless energy, allowing for long-term, chronic animal experiments.

Optical and thermal properties of the optoelectronic device

The optoelectronic devices were modified to operate the blue μ -ILEDs at varying light intensities to spatiotemporally confine the illumination volume with the goal of achieving E-LightR-Cre-driven local genetic modification in mouse lungs. To achieve this, we engineered devices with varying resistors (R_s), thus providing a range of μ -ILED drive currents used three resistors (R_s) with different current-limiting capacities [$R_s = 200\ \Omega$: 580 μ A, 2 k Ω : 135 μ A, and 10 k Ω : 37 μ A] and named the devices as Standard_power ($I_o = 163\ \mu$ W; previously used in optoelectronic devices and standard for blue μ -ILEDs), Low_power ($I_o = 31.1\ \mu$ W), and Ultralow_power ($I_o = 6\ \mu$ W), respectively.

Simulation of irradiance profiles in mouse lungs shows that the predicted penetrance depth of the tissue and illuminated volume of Low_power and Ultralow_power devices is markedly reduced, thus enabling more localized optogenetic regulation (Fig. 2F and table S1). Irradiance magnitude and light energy delivery dose at the lung surface across the in vivo and in vitro experimental illumination paradigms show the spatial bounds of the irradiation profiles across duty cycles (figs. S6 and S7). Quantitative measurements of illuminated volume within the tissue region, bounded by dose contour thresholds, further support precise and localized light delivery consistent with controlled optogenetic regulation (fig. S7 and tables S1 and S2).

Thermal FEA modeled the μ -ILEDs using a worst-case 100% duty cycle operation and determined the heat rise to be $<0.5^\circ\text{C}$ (fig. S8), representing a conservative upper bound for thermal loading under continuous operation and implying further reduced time-averaged heating under experimental conditions below this limit. In addition, the μ -ILED is physically separated from the surrounding lung tissue by an ~200- μ m PDMS encapsulation layer (not included in the model), which further lowers the peak interface temperature by increasing the distance between the heat source and the tissue boundary. A recent study with detailed analytical and experimental evaluation of thermal behavior in miniaturized optoelectronic implants using the same class of 460- to 470-nm μ -ILEDs showed that thermal loading is dominated primarily by Joule heating within the μ -ILED and interconnects, rather than by optical absorption in tissue (57). Together, these considerations suggest that the device design and operating regime fall within safe limits and are unlikely to cause any potential risk of thermal or photodamage to the adjacent lung tissue.

Local regulation of E-LightR-Cre using the optoelectronic device in vitro

One of the advantages of optogenetic tools is the capability to vary the zone of activation depending on the specific goals of the experiment. Thus, we evaluated whether we could control the area of E-LightR-Cre activation by modulating the illumination intensity and duration of μ -ILED devices. We used the Low_power ($I_o = 31.1\ \mu$ W) and Ultralow_power ($I_o = 6\ \mu$ W) μ -ILED devices to evaluate the area of E-LightR-Cre activation in HEK293T cells cotransfected with the Floxed-STOP-mCherry reporter construct (29). Our data demonstrate that, by adjusting illumination intensity and duration, we can control the area of E-LightR-Cre activation with a minimum average activation radius of less than 2 mm from the center of illumination (Fig. 3A).

Next, we assessed the ability of E-LightR-Cre to induce specific signaling and oncogenic transformation in a locally illuminated population of cells. We generated reporter constructs that express membrane-targeted tdTomato flanked by loxP sites and followed by GFP-tagged wild-type (WT) KRas or G12D mutant (Fig. 3B). GFP-tagged WT KRas or its oncogenic mutant (G12D) will be expressed only in cells where E-LightR-Cre is activated. Using the optoelectronic device μ -ILEDs, we induced expression of GFP-KRas-G12D or GFP-KRas-WT in a local population of HEK293T cells lacking intrinsic KRas mutations. Cells transfected with the unmodified mTmG reporter construct served as a negative control (Fig. 3B). After 6 hours of local illumination followed by 18 hours of incubation in the dark, GFP-positive cells were observed in the illuminated regions, whereas GFP-negative/tdTomato-positive cells remained at the nonilluminated periphery. To evaluate the effect of oncogenic KRas, GFP-positive and GFP-negative cells from each group were sorted using fluorescence-activated cell sorting (FACS), and total RNA was extracted from the sorted cells for bulk RNA sequencing (RNA-seq) analysis (Fig. 3B). Principal components analysis demonstrated clustering of all GFP-negative cells and visualized individual clusters of GFP-only, GFP-KRas-WT, and GFP-KRas-G12D-expressing cells (fig. S9A). We identified 3798 and 2106 early responding genes differentially expressed in the GFP-KRas-G12D cells compared to GFP-only and GFP-KRas-WT cells, respectively (adj. P value < 0.05 ; table S3). This included an up-regulation of 1219 genes and down-regulation of 2579 genes relative to the GFP-only cells and the up-regulation of 941 genes and down-regulation of 1165 genes relative to the GFP-KRas-WT cells (table S3).

Among the top differentially expressed genes in the GFP-KRas-G12D-positive cells were *egr1*, *arc*, and *vgf* (Fig. 3C and fig. S9B), which have been previously implicated as downstream targets of oncogenic Ras signaling (58–60). Gene ontology (GO) enrichment analysis of the GFP-positive population in the GFP-KRas-G12D cells compared to GFP-only cells revealed up-regulation of transforming growth factor- β (TGF- β) and fibroblast growth factor (FGF) pathways along with down-regulation of aerobic respiration and oxidative phosphorylation (OXPHOS) pathways (Fig. 3D), consistent with previous findings in KRas transformed cells (61–63). The differentially expressed genes within the identified biological pathways are shown in representative heatmap diagrams (Fig. 3E). Analysis of TGF- β pathway genes revealed up-regulation of *jun* and *fos*, consistent with activation of early oncogenic signaling induced by KRas-G12D (64). We also observed up-regulation of *zeb1*, *lats1*, and *lats2* genes, in line with previous reports identifying them as downstream regulators in

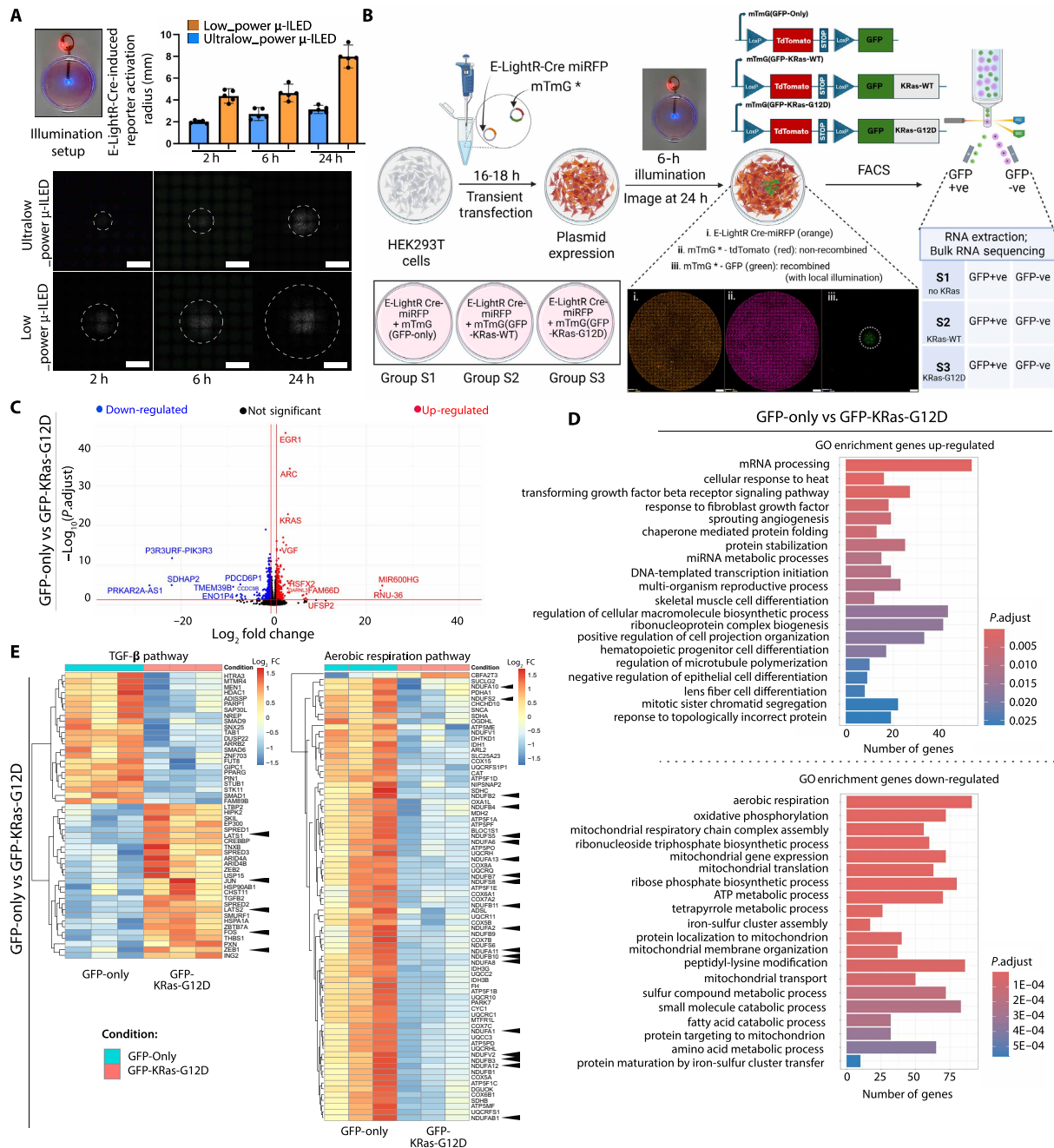


Fig. 3. Characterization of E-LightR-Cre-mediated spatiotemporal control of genetic modeling using a wireless μ -ILED in vitro. (A) Effect of the illumination intensity and duration on spatial extent of activation following localized illumination. Top left: In vitro setup for localized illumination, using wireless μ -ILEDs. The devices were positioned beneath 35-mm culture dishes with the illuminating tip at the center. Bottom: Representative wide-field epifluorescence images of HEK293T cells cotransfected with the E-LightR-Cre-miRFP670 and a Floxed-STOP-mCherry reporter, illuminated with specified μ -ILEDs for indicated durations and subsequently incubated in the dark to complete 24 hours postillumination. Localized mCherry expression zones are outlined by dotted circles. Scale bars, 5 mm. Top right: Quantification of the average radius of E-LightR-Cre-induced mCherry expression zones. Data presented as mean $\pm$ 95% CI, $n = 5$. (B) Experimental design for evaluating the spatiotemporal regulation of KRas-G12D expression in vitro. HEK293T cells cotransfected with E-LightR-Cre-miRFP670 and one of the three indicated reporter constructs were locally illuminated using the Low_power μ -ILED device (470 nm; 10 W, 10 Hz, 20% duty cycle) for 6 hours. Following illumination, cells were incubated in the dark for 18 hours. GFP-positive and GFP-negative cells from each group were sorted by flow cytometry and used for total RNA extraction and bulk RNA-seq analysis ($n = 3$ in each group). Illustration created in BioRender. Bansal, T. (2026) <https://BioRender.com/1qfhsou>. (C) Volcano plot of the top differentially expressed genes comparing GFP-only with GFP-KRas-G12D-expressing cells. Top 17 differentially expressed genes are annotated. (D) GO enrichment analysis of differentially up-regulated (top) or down-regulated (bottom) pathways comparing GFP-only and GFP-KRas-G12D-expressing cells. Significance criteria: adj. P value < 0.05 . (E) Heatmaps of differentially expressed genes under the TGF- β pathway (left) and aerobic respiration pathway (right). Significance criteria: adj. P value < 0.05 . Color-coded scale corresponds to the indicated fold change in gene expression ($\log_2 FC$).

Ras-driven cancers (65–68). Down-regulation of the aerobic respiration pathway, marked by significant reductions in the mitochondrial complex I–IV genes including a major decrease in major complex I subunit genes (*ndufa*, *ndufb*, *ndufs*, *ndufv*, and *ndufab1*), suggests a metabolic switch within 24 hours of oncogenic KRas-G12D expression (Fig. 3E). This aligns with previous reports of suppressed mitochondrial complex I activity and mitochondrial dysfunction induced by mutant KRas (63, 69). When compared to the GFP-KRas-WT, the GFP-KRas-G12D-expressing cells display a similar trend in up-regulation of TGF- β signaling along with down-regulation of aerobic respiration and mitochondrial complex genes, thereby promoting early oncogenic transformation and metabolic reprogramming (fig. S9, C and D). Together, these findings demonstrate that E-LightR-Cre induces KRas-G12D-driven tumorigenic signaling within 24 hours of locally illuminating cells.

We also analyzed the effect of the KRas-G12D-transformed cells on their nontransformed GFP-negative neighbors in the same samples. We observed significant differential expression of several genes in the nonilluminated cells that did not undergo recombination. In the GFP-negative population of the mTmG(GFP-KRas-G12D) group, 13 genes were differentially expressed when compared to the mTmG(GFP-only) cells (adj. *P* value < 0.05; table S3) and 5 genes were differentially expressed when compared to the mTmG(GFP-KRas-WT) cells (adj. *P* value < 0.05; table S4). These data suggest that KRas-G12D expression rapidly (within 24 hours) reprograms the expressing cells and their nontransformed neighbors.

Device implantation and biocompatibility

We used a posterolateral thoracotomy to place the device and achieve optogenetic regulation of E-LightR-Cre in the lungs. The circular rf receiver coil for wireless NFC signal was inserted subcutaneously along the back of the mouse, whereas the stretchable serpentine metallic trace, coated with implantation-compatible Ecoflex/PDMS, was inserted into the thoracic cavity between the sixth and the seventh ribs, positioning the μ -ILED over the left lobe of the lung (see Materials and Methods; Fig. 4A and fig. S10A). Successful implantation with optimal positioning of the device was confirmed by respiratory-gated live and postmortem high-resolution, whole-body micro-computed tomography (micro-CT) imaging, 4 weeks postsurgery (Fig. 4B). The device was retained at its desired position in all mice (fig. S10B) at their respective experimental end points (up to 16 weeks postsurgery).

We assessed five Low_Power optoelectronic devices, explanted after an experimental duration of ~10 weeks and evaluated >2 years poststudy. The devices showed no evidence of delamination of encapsulation or Cu corrosion, indicating a lack of biofluid infiltration (fig. S11). They also exhibited stable forward emitted optical power before implantation (mean $\pm$ SD; 31.1 ± 3.0 μ W) and >2 years post-implantation (33.1 ± 2.7 μ W), with no observable degradation in μ -ILED intensity, indicating long-term stability and biocompatibility of the devices.

Photographic assessment of device biocompatibility with the lung indicated no visible signs of tissue damage at the device contact site (Fig. 4C). The body weight of the animals showed no statistically significant differences in either males (*n* = 3) or females (*n* = 3) when comparing preoperative day 1 to postoperative time points at 3 days, 1 week, and 2 weeks (Fig. 4D). No major postoperative complications were observed in the implanted animals. Locomotor behavior of the mice showed no significant differences in average traveled

distance or average velocity at 1 or 4 weeks postsurgery compared to the day before the surgical procedure (Fig. 4E and fig. S12A). For histopathological assessment of biocompatibility, we analyzed tissue sections from lungs, harvested at 4 weeks postimplantation. Hematoxylin and eosin (H&E) staining demonstrated minimal to no immune or stromal cell infiltration at the device contact site, relative to distant regions within the same lobe and the contralateral right lobes, as assessed by a board-certified pathologist using a blinded semiquantitative scoring system (fig. S12, B and C). CD45 immunostaining showed no significant elevation in pan-leukocyte accumulation in the device contact site, compared to distant sites, supporting the absence of a local inflammatory response (Fig. 4F and fig. S12D). Lung tissue sections from C57BL/6 mouse 6 hours after administration of lipopolysaccharide (LPS; 12 mg/kg body weight) were used as a positive control. We also evaluated any presence of fibrosis by Masson's trichrome staining revealing no significant collagen deposition relative to distant regions within the same lobe or the contralateral right lobes (Fig. 4G and fig. S12E). Lung tissue sections from C57BL/6 mouse 19 days after administration of bleomycin (0.5 U/kg body weight) were used as a positive control. Collectively, these results suggest that the implanted devices do not interfere with natural movement or normal activity levels of mice and do not cause detectable damage to the mouse lungs.

Local control of gene expression in mouse lungs using E-LightR-Cre and wireless μ -ILED

To assess the spatial control of DNA recombination in vivo, we tested local regulation of E-LightR-Cre in the lungs of ROSA26-loxP-STOP-loxP(LoxP)-tdTomato reporter mice (70). Following implantation of the Low_Power μ -ILED device, adenovirus (Ad) expressing either E-LightR-Cre or unmodified WT-Cre was delivered endotracheally for whole-lung distribution. After 24 hours, mice were placed within a wireless rf chamber, and the left lung was locally illuminated by the implanted μ -ILED (see Materials and Methods; Fig. 5A, left, and fig. S13). Following 10 days postillumination, lungs were harvested and cleared using the “Fast-3D clear” protocol (71) adapted for lung tissue (Fig. 5A, left). Light sheet microscopy and subsequent spatial and volumetric analysis of tdTomato expression (72) revealed that E-LightR-Cre activation was tightly restricted to the illumination site, with tdTomato signal confined to a mean volume of 13.78 mm³ encompassing 95% of the detected signal and occupying only 9.69% of the lung volume (Fig. 5B and fig. S14, A to D). In contrast, unmodified WT-Cre drove recombination across the entire lung, yielding a substantially larger mean volume of 132.3 mm³ (92.05% of the lung volume) using the same analytical approach (Fig. 5B and fig. S14, A to D). Additional analysis demonstrated that endotracheal delivery of Cre restricted its activity only to the lungs and did not induce any detectable tdTomato expression in the heart and liver (fig. S15).

Together, these results quantitatively demonstrate the markedly reduced spatial spread achieved with light-gated E-LightR-Cre compared to constitutive WT-Cre, supporting precise spatial control of recombination in vivo.

E-LightR-Cre-mediated local stimulation of tumor initiation in mouse lungs

Local regulation of tumor initiation in mice remains a significant challenge, especially in lung cancer models. Our optogenetic approach offers a promising solution to overcome these limitations. To

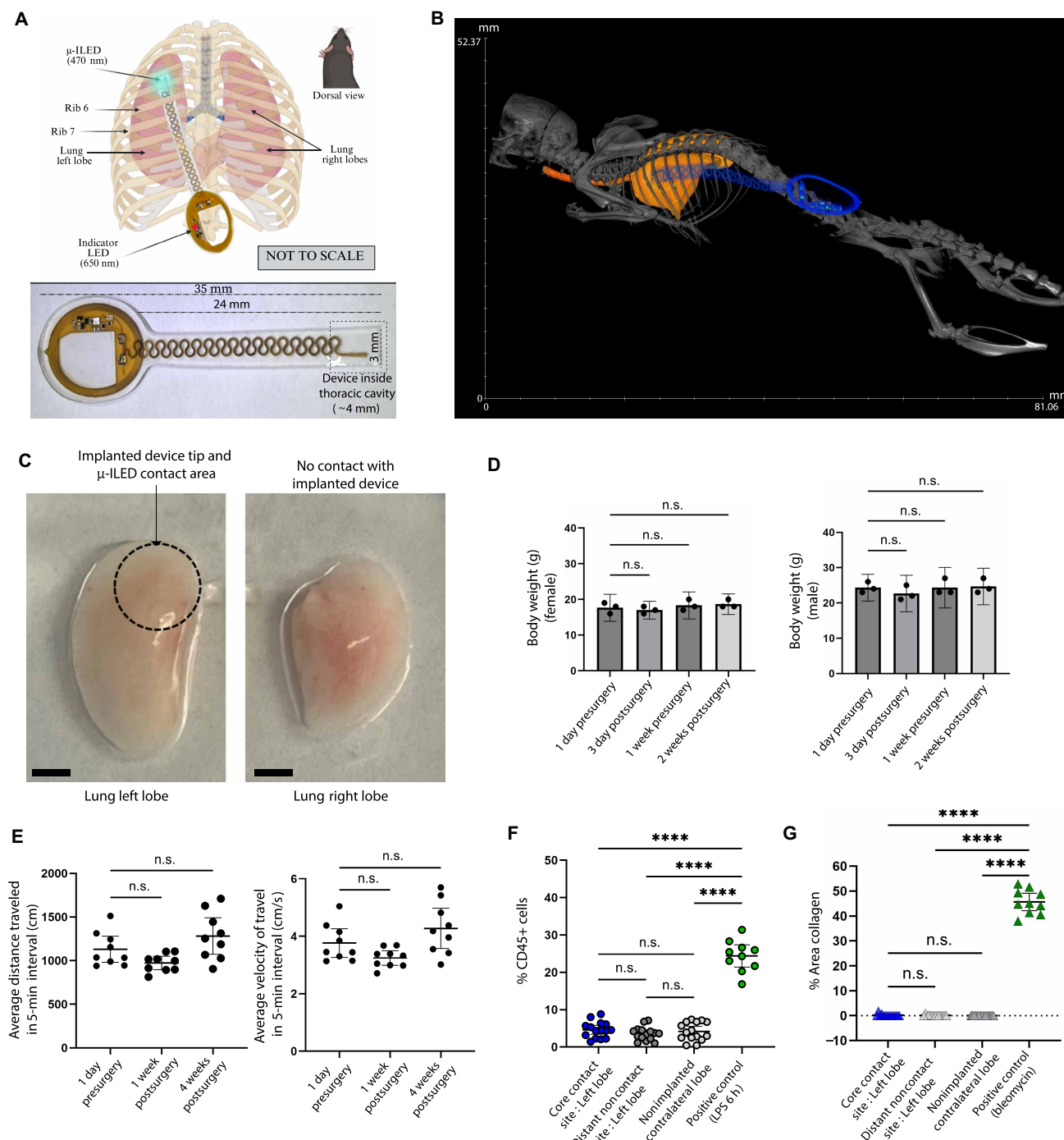


Fig. 4. Surgical implantation of the optoelectronic μ -ILED device. (A) Illustration of the posterolateral, backmounted, intrathoracic implantation of the peripheral wireless μ -ILED device in a mouse. Illustration created in BioRender. Bansal, T. (2026) <https://BioRender.com/uml77ww>. (B) Respiratory-gated in vivo micro-CT imaging shows a backmounted intrathoracic implantation of the wireless μ -ILED device. The stretchable serpentine trace is inserted into the thoracic cavity between sixth and seventh ribs positioning the illuminating tip over the left lung. (C) Representative photograph of the mouse lung left lobe and a contralateral right lobe. The dotted circle indicates the μ -ILED contact area with the intact lung surface. Scale bars, 2 mm. (D) Mouse body weights at indicated times before and after implantation. Statistical analysis conducted using a paired, repeated-measures one-way ANOVA followed by Tukey's multiple comparisons test. Data presented as mean $\pm$ 95% CI ($n = 3$ males and 3 females); n.s., not significant, $P > 0.49$. (E) Average distance traveled (left) and average velocity (right) measured over a 5-min period in a rectangular open-field test at indicated times before and after implantation. Data presented as mean $\pm$ 95% CI ($n = 3$, 5-min time intervals for each time point per mouse). (F and G) Assessment of the device biocompatibility in lung sections, 4 weeks postimplantation. (F) Inflammation quantified as % CD45⁺ cells in indicated lung samples. Data shown as mean $\pm$ 95% CI (five fields of view; $n = 3$ mice per study group). LPS-treated lungs (6 hours) served as a positive control. (G) Fibrosis quantified as % collagen area (Masson's trichrome staining) in indicated lung samples. Data shown as mean $\pm$ 95% CI (five fields of view; $n = 3$ mice per study group). Bleomycin-treated lungs (19 days) served as a positive control. All statistical significance determined using a Brown-Forsythe and Welch one-way ANOVA with Dunnett's multiple comparisons test (n.s., not significant, $P > 0.05$; **** $P < 0.0001$).

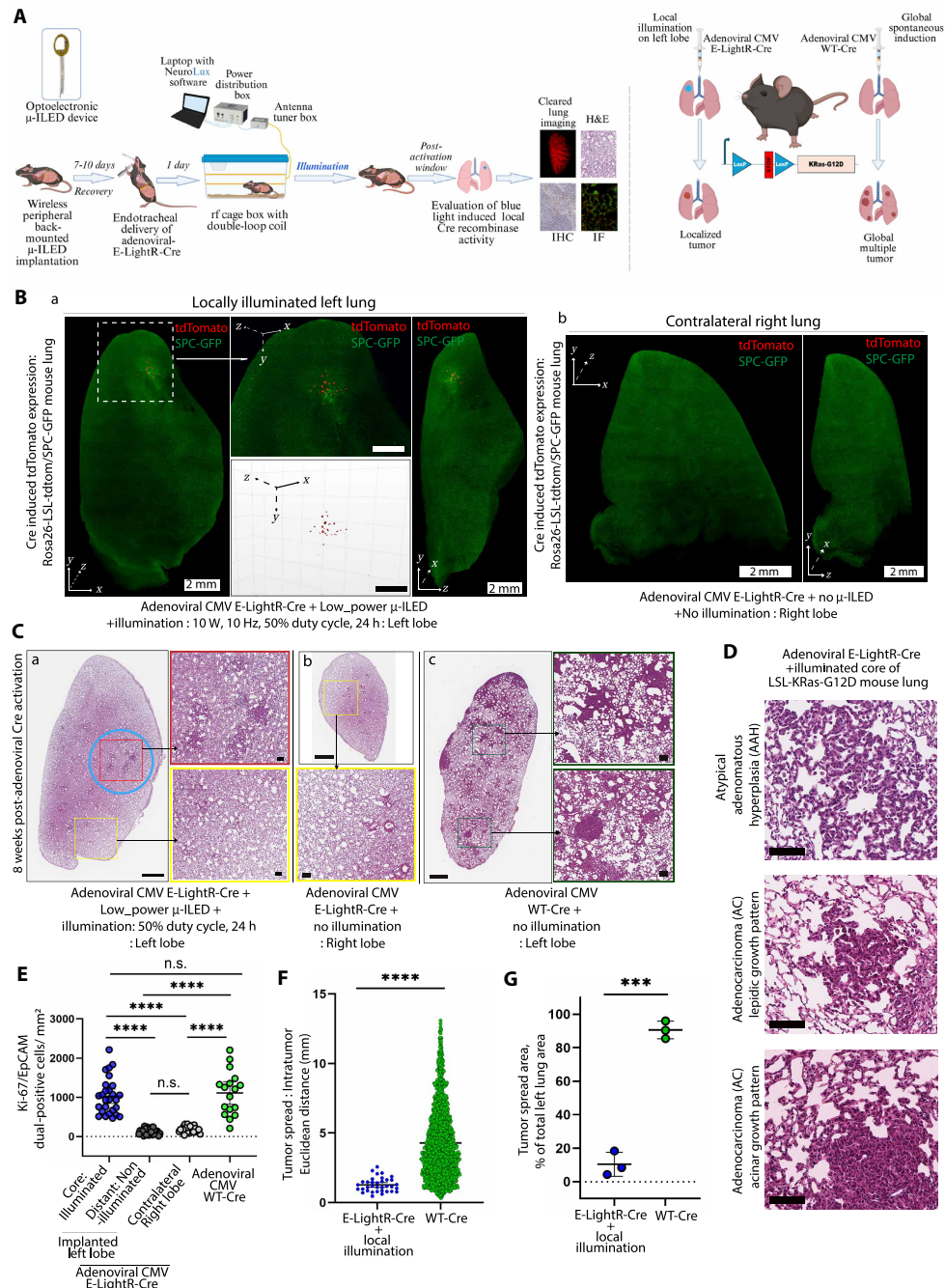


Fig. 5. Light-mediated local genetic modification and spatially restricted tumor initiation in mouse lungs. (A) Schematic workflow for evaluating the spatiotemporal gene regulation using E-LightR-Cre and wireless μ -ILEDs in vivo (left). Study design comparing the global versus local light-controlled tumor stimulation (right). IHC, immunohistochemistry; IF, immunofluorescence. Illustrations created in BioRender. Bansal, T. (2026) <https://BioRender.com/tg26eeq>; <https://BioRender.com/zr61ldp>. (B) Representative light sheet fluorescence microscopy 3D micrographs of cleared, locally illuminated left (a) and contralateral right (b) lungs from Rosa26-LSL tdTomato/SPC-GFP mouse, 10 days after local illumination (Low_power μ -ILED), following E-LightR-Cre delivery. TdTomato expression (red) marks activation, over constitutive SPC-GFP (green). Local activation radius (95% signal area) ≤ 1 mm. Scale bars, 2 mm (whole lobes) and 1 mm (insets). (C) Representative H&E-stained lung sections from LSL-KRas-G12D mice, 8 weeks after Cre induction. (a and b) Locally illuminated left lung (a) and nonilluminated right lung (b) receiving E-LightR-Cre. The illuminated area is outlined (blue). Insets show illuminated (red) and nonilluminated distant (yellow) regions. (c) Left lung receiving WT-Cre. Scale bars, 1 mm (whole lobes) and 100 μ m (insets). (D) Representative H&E images demonstrating lesion types 8 weeks after E-LightR-Cre activation. (E) Quantification of Ki-67/EpCAM dual-positive cells per square millimeter area, from the indicated regions. Data shown as mean $\pm$ 95% CI (matched ROIs; $n = 3$ mice). WT-Cre-treated lungs served as a positive control. Statistical significance determined using a Brown-Forsythe and Welch one-way ANOVA with Dunnett's test (**** $P < 0.0001$; n.s., not significant, $P > 0.05$). (F and G) Tumor spatial distribution analysis of H&E sections from left lungs comparing E-LightR-Cre + local illumination versus WT-Cre-treated mice. (F) Pairwise Euclidean distances between tumor centroids (mean $\pm$ 95% CI; $n = 3$). Statistical significance determined using a Brown-Forsythe and Welch one-way ANOVA with Dunnett's test (**** $P < 0.0001$). (G) Convex hull-based tumor spread measurement (mean $\pm$ SD; $n = 3$). Statistical significance determined using an unpaired two-tailed t test with Welch's correction (*** $P < 0.001$).

evaluate this, we used the widely used LSL-KRas-G12D mouse model, a common tool for studying lung cancer development (19). We tested whether optogenetic control over the expression of the oncogenic KRas-G12D mutant could initiate the development of tumors in a local area of the lungs (Fig. 5A, right).

Following implantation of the Low_power μ -ILED device and endotracheal delivery of adenoviral E-LightR-Cre, the left lobe of the lung was illuminated locally, and the formation of tumors was assessed by histological analysis. Eight weeks after induction, we observed localized neoplasia in the illuminated area of the left lobe, histopathologically characterized as lesions ranging from atypical adenomatous hyperplasia (AAH) to premalignant, in situ adenocarcinoma (AC) with lepidic or acinar growth pattern (Fig. 5, C and D; fig. S16, A to C; and table S5). We found no signs of neoplasia in the distant areas on the left lobe or on the right lung lobes (Fig. 5C and fig. S16, A and B).

Subsequent immunohistochemical analysis revealed a significantly higher number of Ki-67-positive proliferating cells within the light-activated neoplastic mass, compared to the surrounding normal lung tissue, indicating increased proliferation within locally induced tumors (fig. S17, A and B). Immunofluorescence staining further demonstrated elevated EpCAM expression in histopathologically identified neoplastic regions relative to adjacent normal tissue, confirming the epithelial origin of the tumors (73). In addition, the increased presence of dual Ki-67/EpCAM-positive cells in the light-activated core provides further support for the epithelial origin and proliferative nature of the light-induced neoplastic lesions (Fig. 5E and fig. S17C). In contrast, endotracheal delivery of Ad expressing unmodified WT-Cre led to the formation of multiple adenomatous tumors at different stages of growth throughout the lungs within 8 weeks (Fig. 5, C and E, and fig. S17C).

Analysis of tumor spatial distribution, based on pairwise Euclidean distances between tumor centroids and the derived Clark-Evans index of dispersion, demonstrated significant spatial confinement of tumors under light-regulated conditions (Fig. 5F and fig. S17D). Consistent with this, convex hull-based delineation of tumor boundaries showed that neoplastic lesions in the E-LightR-Cre group were restricted to a mean of 10.36% of the left lung area (Fig. 5G and fig. 17E). In contrast, unmodified WT-Cre resulted in a broader spatial distribution, encompassing a mean of 90.60% of the left lung area, as quantified from two-dimensional (2D) projections of H&E-stained left lung sections (Fig. 5G and fig. 17E). Endotracheal delivery of adenoviral E-LightR-Cre in the absence of illumination yielded no detectable tumor formation confirming the absence of leaky recombinase activity (fig. S18).

To assess the molecular resemblance of the optogenetically induced neoplastic lesions with authentic adenocarcinomas, we performed spatial transcriptomics analysis comparing the pathologist-demarcated lesions within the illuminated area of the left lung to randomly selected tissue regions outside of the illuminated area (fig. S19). Principal components analysis demonstrated that clustering of all the lesion regions of interest (ROIs) was distinct from distant nonilluminated ROIs (fig. S20A). We identified 5692 genes differentially expressed in the defined lesion ROIs compared to distant nonilluminated regions (adj. *P* value < 0.05). This included an up-regulation of 2119 genes and down-regulation of 3573 genes in lesions. GO enrichment analysis revealed up-regulation of several metabolic pathways, including OXPHOS and amino acid metabolism, alongside down-regulation of immune activation pathways, indicating early metabolic remodeling coupled with

an immune-suppressed microenvironment (74) (fig. S20B). Consistently, single-sample gene set enrichment analysis (ssGSEA) demonstrated enrichment of cell cycle-associated programs, supporting a proliferative phenotype (75) (fig. S20C). Spatially variable gene signatures in the lesions identified enrichment of canonical primary lung adenocarcinoma markers, including NKX2-1 and NAPS1 along with retention of an alveolar type II (AT2)-like identity (SFTPC) indicating early-stage, premalignant lesions (76, 77) (fig. S21). These spatially resolved molecular features not only corroborate our histological assessment but also closely mirror signatures of early preinvasive lung adenocarcinoma that precedes the emergence of increased cellular and spatial heterogeneity (77).

These data suggest that locally induced lung tumors accurately recapitulate the spatially constrained progression of lung tumorigenesis from a single initiation site, thereby providing an improved platform to investigate early events in lung cancer development.

DISCUSSION

Despite the promises of optogenetics in spatiotemporal control of genetic modifications, its application in animal models has been hampered by current methodological limitations. Our study overcomes these by developing an optimized E-LightR-Cre recombinase and integrating it with an implantable wireless optoelectronics system for precise spatiotemporal gene regulation in vivo. We demonstrated that E-LightR-Cre has the characteristics critical for in vivo applications: (i) no detectable leaky activity in the dark and (ii) robust activation upon illumination with blue light, thereby preventing unwanted random recombination events that could complicate the analysis. Development of advanced optoelectronic devices and a precise implantation procedure allowed localized E-LightR-Cre activation in vivo, without causing tissue damage. With this, we demonstrated local recombination through reporter gene expression and localized initiation of tumor formation, within the mouse lungs. Our technology thus enables light-guided in vivo regulation of preclinical genetic modeling, in both time and space, opening previously unexplored possibilities for targeted genetic interventions.

Comparison to other optogenetic Cre systems shows that E-LightR-Cre has significantly lower background activity (Fig. 1D and fig. S1, B and C). Two-component systems like Magnet-based PA Cre 3.0 often suffer from imbalanced expression or incomplete cleavage, leading to nonspecific background activity (28, 30). Although LiCre(AsLOV2) overcomes the limitations of split systems and demonstrates strong photoactivity, it exhibits targeted DNA binding in the dark, as previously reported (35), and shows a high dark-state leakiness in our results (Fig. 1D). This makes it less suitable for extended duration of in vivo experiments, where unintended recombination can accumulate over time. Thus, E-LightR-Cre represents a more reliable alternative for light-regulated Cre recombination in vivo.

One of the challenges of blue light-guided in vivo optogenetics has been the limited depth of light penetration using externally applied illumination sources, which is especially problematic when targeting internal organs, particularly within the intrathoracic chamber. The lungs remain among the most challenging organs due to continuous respiratory motion, low structural rigidity, and delicate tissue architecture, all of which complicate stable and localized optical control. To overcome this limitation, we introduced wireless fully implantable optoelectronic devices with reengineered electronic circuitry, and mechanical, optical, and operational properties, that are capable

of localized illumination of mouse lungs in the intrathoracic compartment, without compromising animal mobility, weight, general health, survival, or tissue integrity at the point of contact (Figs. 2 and 4). The platform is fabricated using scalable fPCB manufacturing, providing a reproducible and cost-effective route to device production compared to conventional clean room–based micro- and nanofabrication approaches. From a mechanical standpoint, the implanted device operates under continuous respiratory deformation. Adult mice exhibit respiratory rates of ~120 to 160 breaths per minute (54), corresponding to ~230,000 breathing cycles per day at conservative maximum estimates and ~3.5 million cycles over ~2 weeks of experimental study (Fig. 5). Although the implanted probe with a μ -ILED maintains direct contact with the lung surface during respiratory inflation and deflation, the local strains induced by normal tidal breathing are expected to be limited to ~0 to 5% linear strain, consistent with experimental and morphometric estimates of physiological alveolar deformation during tidal respiration (55, 56). Therefore, the absence of failure in both exaggerated benchtop cyclic testing (fig. S5C) and during millions of *in vivo* breathing cycles over 10 weeks of implantation (fig. S11) indicates a substantial mechanical durability margin for respiratory deformation during *in vivo* tests. Wireless, implantable μ -ILED devices have been explored for preclinical and clinical studies over the past decade (38–44, 53, 78, 79). However, this study reports wireless, spatiotemporally confined optogenetic manipulation of the lung in awake, freely moving small animals. Successful regulation in such a delicate organ suggests that this approach can be broadly adaptable for a wide range of *in vivo* applications, including the spleen and liver, and those with less challenging anatomical and surgical requirements such as muscle and subcutaneous tissue.

Complementing this mechanical robustness, thermal and optical analyses further support safe operating conditions. Thermal simulations performed at 100% duty cycles, far exceeding our experimental conditions, together with a prior report of thermal loading in similar classes of μ -ILEDs (57), indicate a very low likelihood for inducing heating-related tissue damage, inflammation, or other physiological perturbations that could confound biological outcomes such as tumorigenesis. Future studies in tissue phantoms or *ex vivo* lung preparations could provide direct thermal validation. In parallel, irradiance modeling from the reduced power μ -ILEDs, which incorporate duty cycles and illumination durations indicate minimal risk of photodamage and further demonstrate spatially confined light delivery (Fig. 2F and figs. S6 to S8).

With the regulated illumination fields generated by reduced power μ -ILED, we enabled local E-LightR-Cre activation to achieve a fine precision in spatiotemporally restricted Cre reporter activity both *in vitro* (Fig. 3A) and *in vivo* (Fig. 5B and fig. S14). The ability to generate such spatial heterogeneity through localized gene manipulation offers a powerful platform for studying complex cellular environments. Harnessing the specificity of E-LightR-Cre, we selectively restricted the KRas-G12D driver mutation to a defined local group of cells within an otherwise normal cell population. Light-guided, locally restricted expression of oncogenic KRas-G12D in a subpopulation of HEK293T cells *in vitro* revealed significant rewiring of gene expression pathways within 24 hours, indicating broad and rapid reprogramming of the transcriptome. We identified up-regulation of TGF- β pathway and down-regulation of aerobic respiration and OXPHOS pathways in the local GFP-KRas-G12D-expressing cells compared to GFP-KRas-WT or GFP-only cells (Fig. 3, B to E, and fig. S9), consistent with the established KRas-driven transformation events (61, 63).

Within the TGF- β pathway, we observed increased expression of both oncogenic and tumor suppressor gene transcripts. Although the oncogenes signify tumor-initiating signaling, the increased expression of tumor suppressors within 24 hours of KRas-G12D induction aligns well with the premalignant role of TGF- β signaling in early tumor development before transitioning to support epithelial-mesenchymal transition and metastasis (59, 80–85). Similarly, OXPHOS dependency also varies widely across cancer types, stages, and microenvironments (86–90). Our results showing down-regulation of OXPHOS pathway upon KRas-G12D induction may indicate an early metabolic shift synergizing with TGF- β tumor suppressor effects. Alongside spatially restricted signaling in the illuminated area, the small-scale differential gene expression in the nonrecombined periphery of the KRas-G12D transformed cells likely represents the early responses of the tumor environment to the transformed cells (tables S3 and S4). Overall, optogenetic regulation of locally restricted KRas-G12D signaling offers a valuable tool to investigate how localized somatic mutations prime the environment through cell fate–determining signaling and metabolic shifts ultimately driving tumorigenesis (91).

Consistent with previous findings, the unmodified WT-Cre recombinase induced widespread development of advanced lung tumors (11, 19) within 8 weeks (Fig. 5C). Such rapidly progressing multitumor models may not fully recapitulate the early stages of disease development. In contrast, our novel approach involved the application of the μ -ILED devices to activate E-LightR-Cre. This induced localized oncogenic KRas-G12D expression in mouse lungs and led to the development of fewer and slower-progressing focal lesions with fine spatial confinement (Fig. 5 F and G, and fig. S17, D and E). These lesions predominantly exhibit preinvasive signs of atypical adenomatous hyperplasia, as well as *in situ* adenocarcinoma with lepidic and acinar growth patterns (Fig. 5, C and D, and fig. S16, A and B). Spatial transcriptomics analysis further supports a model in which early lung tumorigenesis is characterized by a proliferative dysplastic, premalignant state marked by metabolic reprogramming and lineage fidelity (75). The up-regulation of OXPHOS and purine metabolism suggests an early feature of progression from preinvasive to invasive disease. Concurrent low expression of vascular signatures and immune activation genes may further indicate signs of tumor microenvironment remodeling and establishing immune evasion to promote tumor growth. The retention of AT2-like identity, together with enrichment of NKX2-1–driven transcriptional programs, highlights a lineage-constrained lung-originated tumorigenic state that precedes the emergence of phenotypic heterogeneity and invasiveness (77).

The spatial confinement of the tumor promoting signature also suggests that, when KRas-G12D was induced in a small subset of cells, tumor initiation was notably impaired, likely due to a stronger anti-tumorigenic response from the surrounding tissue. This is consistent with a recent observation that limiting KRas-G12D expression in the mouse pancreas allows surrounding healthy cells to outcompete and eliminate transformed cells from the tissue (92). Therefore, local mutations must overcome greater competition from the surrounding environment to reach the tipping point and generate tumors. By enabling precise spatial restriction of KRas-G12D expression, our model provides a useful preclinical platform to study the early cellular and microenvironmental processes driving lung tumor formation while offering advantages for evaluating early-stage therapeutic targeting potential in relatively less heterogeneous cellular contexts, without the confounding effects of complex cross-talk present in conventional models.

In conclusion, the integration of the optogenetic E-LightR-Cre with a wireless, fully implantable, and biocompatible optoelectronic device offers a major advancement in achieving spatiotemporal control over gene expression in vivo for long-term disease modeling without impairing tissue integrity or animal behavior. In addition, the ability to modulate light parameters, including intensity, duration, and frequency provides tunable control over the spatial extent and timing of gene recombination, offering a powerful tool to dissect complex physiological and pathological processes at high resolution. E-LightR-Cre demonstrates no detectable background activity in the dark and strong activation upon blue light exposure, overcoming a critical limitation of previous optogenetic Cre systems. This high level of control enables precise, localized gene activation within specific subpopulations of cells, allowing researchers to model focal disease processes including, but not limited to early tumor initiation. The ability to target at any loxP-flanked alleles allows for spatiotemporal activation/inactivation of genes regulating apoptosis, differentiation, fibrosis, inflammation, development, and many other biological processes. Together, these features enable spatially controlled interrogation of physiological and pathological processes beyond KRas-mediated oncogenesis.

Although the implementation of optogenetic and optoelectronic strategies has enabled precise spatiotemporal gene activation and disease modeling in vivo, several limitations remain that may require adaptations for broader applicability. Organ-targeted adenoviral delivery of the optogenetic protein achieves relatively homogeneous expression but cannot ensure uniform distribution across all target cells, potentially causing variability in timing and activation strength across experiments. Generating a cell type-specific, knock-in mouse model could resolve this limitation. Moreover, organs exposed to external light may benefit from complementary chemical control in conjunction with optogenetic allosteric to maintain the desired local precision. To accommodate targeting different organs or animals of varying sizes, including younger mice, the optoelectronic devices should need adjustments in scale and dimensions to ensure optimal illumination and precise activation. Addressing these factors will improve reproducibility, precision, and versatility. The modular design of both the engineered allosteric proteins and the wireless optoelectronic platform provide a flexible framework to overcome these limitations and enables seamless adaptation to light-responsive synthetic tools and target tissues, thereby broadening its applicability across diverse biological contexts and disease models with precise spatial control.

MATERIALS AND METHODS

Molecular cloning of DNA constructs

Original mammalian codon-optimized gene, encoding the LightR-Cre (45), was amplified from our previously published construct (Addgene, plasmid #162158; <http://n2t.net/addgene:162158>; RRID: Addgene_162158). To obtain E-LightR-Cre, the inter-VVD linker and the linkers between Cre and LightR domain (Cre-VVD linker) were replaced using modified site-directed mutagenesis, as previously described (50). The E-LightR-Cre-miRFP670 construct was generated in the pN1 vector backbone, same as the original LightR-Cre (45). Amino acid sequence alterations involved in linker modifications are described in Fig. 1A. To generate a constitutively inactive CI-LightR-Cre construct, a single point mutation [Y324F (29); in WT Cre] was introduced into the original LightR-Cre via site-directed mutagenesis.

PA Cre 3.0 (30) in the lentiviral backbone (Lv-SD-PA-Cre-nMag-Opti) was obtained as a gift from M. Yazawa and LiCre(AslOv2) (34) (alternative names: AsLOV2-CreE340AD341A; pGY577) was a gift from G. Yvert (Addgene, plasmid #166663; <http://n2t.net/addgene:166663>; RRID: Addgene_166663). Both constructs were subcloned into the pN1 vector, in-frame with a C-terminal miRFP670 tag, to match the backbone and the promoter strength to the E-LightR-Cre-miRFP670 construct. The cloning procedure involved generation of a megaprimer followed by modified site-directed mutagenesis as previously described (50). The reporter plasmid pCA-mTmG was a gift from L. Luo (Addgene, plasmid # 26123; <http://n2t.net/addgene:26123>; RRID: Addgene_26123), and the reporter plasmid pcDNA3.1_Floxed-STOP-mCherry was a gift from M. Sato (Addgene, plasmid #122963; <http://n2t.net/addgene:122963>; RRID: Addgene_122963). The pCA-mTmG(GFP-KRas-WT) and pCA-mTmG(GFP-KRas-G12D) reporter plasmids were cloned following the protocol for generation of megaprimers and subsequent modified site-directed mutagenesis (50). Briefly, the KRas-WT and KRas-G12D genes were amplified as megaprimers from pBabe-KRas-WT (Addgene, plasmid #75282; <http://n2t.net/addgene:75282>; RRID: Addgene_75282) and pBabe-Kras-G12D (Addgene, plasmid #58902; <http://n2t.net/addgene:58902>; RRID: Addgene_58902), obtained as gifts from C. Der. Using a modified site-directed mutagenesis strategy, the megaprimers were subsequently inserted into the pCA-mTmG vector, C-terminal to GFP, to generate GFP-KRas-WT or GFP-KRas-G12D fusion genes (50).

E-LightR-Cre-miRFP670 was further cloned into the pShuttle plasmid (93). For cloning, the E-LightR-Cre-miRFP pN1 plasmid was used as a template to polymerase chain reaction (PCR) amplify the E-LightR-Cre-miRFP gene, with primers that introduced restriction sites Not I and Hind III on the 5' and 3' end, respectively. This PCR product and the pShuttle plasmid were digested with Not I and Hind III and then ligated to generate the E-LightR-Cre-miRFP-pShuttle construct. Ad production and amplification from the E-LightR-Cre-miRFP-pShuttle construct were done at the CCVR-RRC Viral Vector Core at the University of Illinois Chicago (UIC) to obtain Ad expressing E-LightR-Cre-miRFP. Briefly, pAdEasy-1-containing BJ5183-AD-1 *Escherichia coli* cells were transformed with Pme I-linearized E-LightR-Cre-miRFP-pShuttle for homologous recombination. The resulting recombinant Ad plasmid was verified with Pac I digestion and sequencing of target genes. The recombinant Ad plasmid was linearized with Pac I digestion, and the linearized plasmid was transfected into HEK293 cells. High-titer Ads were purified by cesium chloride (CsCl) gradient centrifugation after multiple rounds of amplification, followed by dialysis against storage buffer [10 mM Tris (pH 8.0), 2 mM MgCl₂, 2 mM CaCl₂, 5% glycerol, and 3% sucrose] at 4°C. Viral titer was determined by a modified plaque-forming assay to generate the plaque-forming unit (PFU)/ml value. The entire process was performed from the RRC Viral Vector Core at the UIC. Adenoviral cytomegalovirus (CMV) WT-Cre-eGFP was a gift from K. Schachtschneider. All prepared constructs were validated by Sanger sequencing performed at the DNA Services (DNAS) facility within the Research Resources Center (RRC) at the UIC. The relevant DNA and amino acid sequence information and plasmid resources are provided in Supplementary Text S3 and tables S6 and S7.

Cell culture and transfection

HEK293T cells were purchased from the American Type Culture Collection (ATCC; CRL-3216). The cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich; St. Louis, MO,

USA) supplemented with 5% fetal bovine serum (FBS) and 1% glutamine to 70% confluency on a plastic dish coated with 1:200 poly-L-lysine and were transfected with jetOPTIMUS (Polyplus-transfection S.A., Illkirch, France) transfection reagent following the manufacturer's recommendations. The ratio of any photoactivatable Cre recombinase to the reporter plasmid in all cotransfection experiments were maintained at 1:9, and the cells were kept in the dark under aluminum foil wrap inside the incubator with 5% CO₂ at 37°C. This condition was maintained for the entire course of the experiment in the dark controls and the light-activated groups except the illumination window.

To enhance detection, the Cre-to-reporter ratio was increased to 1:1 in the dark sample experiments shown in fig. S1C.

Global photostimulation with the LED panel in vitro

Twenty-four hours posttransfection, the HEK293T cells were taken out of the foil covers and stimulated with blue light, using the HQR LED Plant Grow Panel Lamp System (3 mW/cm², 470-nm wavelength). To avoid photothermal toxicity, the cell plates were placed over a perforated plexiglass rack 10 cm above the LED panel and illuminated for 4 hours with 1-s ON/1-s OFF cycle, controlled by an Arduino IDE microcontroller (Arduino Uno) and power relay (IoT Relay, Digital Data Loggers Inc.) system (51). After illumination, the cells were again foil covered and incubated in the dark overnight (~20 hours) in the incubator with 5% CO₂ at 37°C, before analysis (Fig. 1C and fig. S1A).

Fluorescence microscopy analysis of Cre activity following global illumination

In experiments involving LED panel-based global illumination, 20 hours postillumination, the light and dark condition HEK293T cell samples were washed in 1X phosphate-buffered saline (PBS) and stained with Hoechst dye (Hoechst 33342; 20 mM, 1 µg/ml) for 10 min and subsequently fixed with 2% PFA and rinsed with PBS. The procedures were performed under safe red lights until fixation. Fixed HEK293T cells in PBS were imaged using an EVOS Auto 2 Invitrogen fluorescence microscope using a 20X air objective. The mRFP670, tdTomato, GFP, and Hoechst signals were captured via epifluorescence wide-field imaging. Cre activity was measured by calculating the ratio of the number of GFP-expressing cells to the number of tdTomato-expressing cells, reflecting the conversion of the tdTomato reporter to GFP. Image processing and analysis were performed using the NIH ImageJ/Fiji open-source software (94) (fig. S1A).

Flow cytometry analysis of Cre activity following global illumination

In experiments involving LED panel-based global illumination, 20 hours after illumination, the light and dark condition HEK293T samples were washed with 1X PBS, trypsinized with 0.25% Trypsin-EDTA, resuspended in FACS buffer [1% bovine serum albumin (BSA), 2 mM EDTA, and 0.1% sodium azide] and filtered through 70-µm disposable cell strainers to make single-cell suspensions. For flow cytometry, the cell suspensions were run on a CytoFLEX S Flow Cytometer (Beckman) and data analysis was done using the Kaluza Analysis 2.1 (Beckman) software. The data analysis was performed using a gating strategy applied as the percentage of mRFP670⁺, tdTomato⁺, and GFP⁺ cells. It is worth mentioning that, in a 24-hour experiment, we observe no mRFP670⁺/GFP⁺ cells, which are tdTomato⁺.

Optoelectronic device design and fabrication

Figure S2 demonstrates the fabrication procedure of the optoelectronic device. Fabrication of the fPCB began with laser ablation of a sheet of PI/Adhesive/Cu/PI/Cu/Adhesive/PI (12.5/15/12/25/12/15/12.5 µm; PCBway) to pattern both sides with circuit traces, bonding pads, and coil antennas. A separate fPCB with an identical stack-up formed the serpentine interconnect. The fabrication and assembly of the devices began with the soldering of electronic components using a soldering iron and heat air gun at ~176.7°C. A low-temperature soldering paste (CHIPQUIK, TS391AX10) bonded the various electronic components to the fPCB. An ultraviolet (UV)-curing epoxy (UV10TKMed, MasterBond Inc.) coated the contact pads and µ-ILED (length: 270 µm, width: 220 µm, and height: 50 µm; TR2227, Cree Inc.) at the tip to reinforce the solder connections. Chemical vapor deposition formed a uniform coating of parylene-C (thickness: 10 µm; Specialty Coating Systems Inc.) on the assembled device. Polymethyl methacrylate (PMMA A5; MicroChem Inc.; 2000 rpm) was spin coated for 60 s on a silicon wafer and cured at 180°C for 1 min on a hot plate to form a substrate. Next, a silicone layer of Ecoflex 00-30 A and B (Smooth-On Inc., 11:11 weight ratio) mixed with SYLGARD 184 (4:0.4 elastomer-to-curing agent weight ratio) in a 4:1 weight ratio was spin coated onto the substrate to a thickness of 300 µm. The prepared devices were immersed into the uncured silicon to completely encapsulate them, followed by curing at 100°C for 15 min. Last, a customized die was used to uniformly cut out the devices.

Wireless operation of the optoelectronic device

A commercial rf system (NeuroLux Inc., Northfield, IL) wirelessly delivered power to the implanted optoelectronic devices for localized optogenetic stimulation of lung tissue. The system consisted of the following components: (i) a laptop equipped with the customized NeuroLux software to manage device operation, (ii) a Power Distribution Control box for providing wireless power and communication to the devices, (iii) an antenna tuning box designed to optimize power transfer and match the impedance between the source and the antenna, and (iv) a cage box with adjustable double-loop antenna designs to facilitate device operation in various environments (e.g., in vitro and in vivo conditions) (fig. S13).

Electrical validations in benchtop testing and modeling

A transmitting circular coil made of Cu wire (diameter: 15 mm) connected to a vector network analyzer (E5063A, Keysight Technologies Inc.) measured the S11 signal of the device under bending of the receiver coil up to a radius of 2 mm. The *Q* factor was calculated as the ratio between the resonant frequency (*f_r*) and the full width at half maximum (FWHM: Δf).

The numerical model for electromagnetic validations of the device relied on FEA (Ansys HFSS, ANSYS Inc.) to simulate the electromagnetic response as the coil is bent up to a radius of 2 mm. A matching capacitor tunes the coil to a resonant frequency and then geometrical, resistive, and inductive changes caused by bending alter the resonant frequency, the quality factor of the coil, and the magnitude of the scattering parameter. A frequency sweep was performed between 10 and 18 MHz to capture the electromagnetic response and frequency shift as a function of bending radius from 10 to 2 mm. In the mechanical simulation, the elastomeric encapsulation was meshed with solid elements (C3D8R), and the Cu layer was meshed with shell elements (S4R). The total number of elements in the model is ~350,000 to ensure accuracy. In the electromagnetic

simulation, an adaptive meshing strategy was adopted to ensure mesh convergence over a spherical radiation boundary with a radius of 500 mm. The elastic modulus and Poisson's ratio values used in the simulation were 119 GPa and 0.34 for Cu as well as 60 kPa and 0.49 for Ecoflex, respectively. The relative permittivity, relative permeability, and conductivity of Cu used in the simulation were 1, 0.999, and $5.8 \times 10^7 \text{ S m}^{-1}$, respectively.

Electromagnetic simulation

The commercial software ANSYS HFSS (2021 R2) was used to study the magnetic field of double-loop coil transmitter. A current excitation with a frequency of 13.56 MHz and an amplitude of 1.71 A was applied, based on the measured rf current under a 10-W input power. An adaptive mesh (tetrahedron elements) together with a spherical surface (with radius of at least five times compared to the device sizes) as the radiation boundary were adopted to ensure computational accuracy. Cross-sectional magnetic field plots and field strength at the corner and center of the mouse cage were outputted. The dielectric constant, electrical conductivity, and dielectric loss of Cu were 1, $5.8 \times 10^7 \text{ S/m}$, and 0.

Thermal FEA model

The transient heat transfer, incorporating both the thermal load from μ -ILEDs and the optical power-induced body heat flux, follows the governing equation

$$\begin{cases} \rho C_p \frac{\partial T}{\partial t} + \nabla \cdot (-k \nabla T) = Q_{\text{the}} + \phi \mu_a & \text{in } \Omega \\ T = T_{\infty} & \text{on } \partial \Omega \end{cases}$$

where T , ρ , C_p , and k denote the temperature, mass density, heat capacity, and thermal conductivity. Q_{the} is the heat source from the μ -ILEDs, and ϕ is the optical fluence rate from Monte Carlo simulations. The isothermal boundary conditions $T = T_{\infty}$ are applied on $\partial \Omega$. $T_{\infty} = 37^\circ \text{C}$ is the body temperature. The equation was solved via FEA in Abaqus using linear tetrahedral elements (minimum edge length: 20 μm). Material properties (k , C_p , and ρ) were derived from a previous study focused on analyzing and managing thermal loads generated in vivo by miniaturized implantable optoelectronic devices (57).

Mechanical validations in benchtop testing and modeling

Tensile and cyclic tests were used to determine the transition threshold from elastic to plastic deformation. A tensile tester (Tensile Tester, Mark-10 Inc.) measured the stress-strain curves of the serpentine in tension mode, with the sample clamped between the top and bottom of the cantilever and both ends of the serpentine connected to a multimeter to measure electrical continuity. The tensile modulus was calculated by fitting the tangent of the linear elastic region in the stress-strain curve. The cyclic test, using a custom-built stretching stage based on a screw-driven linear stage (ATS100, Aerotech Inc.), applied dynamic mechanical stress to the devices, simulating a full range of natural animal movements: from -40% bending to $+50\%$ stretching for a total of 10,000 consecutive cycles at 1 Hz (40). Sample preparation for cyclic testing involved attaching external Cu wires (diameter: 0.1 mm) to the contact pads of the devices using a soldering iron with low-temperature solder paste (Chip Quik Inc.) and a flux pen (SRA Soldering Products) to measure the resistance across the device. A block of PDMS (10:1 elastomer-to-curing agent ratio; SYLGARD 184, Dow Corning Inc.) was placed on the top and bottom

of the device to protect it from the metal clamps during sample fixation and cyclic testing. To simulate physiological conditions in animal models, the devices were immersed in a PBS solution maintained at body temperature (around 37°C) and a neutral pH of 7.4. Measurements of the electrical resistance across the device before and after these mechanical validations identified any shift from elastic to plastic deformation. Here, no change in electrical resistance indicated elastic deformation, whereas a significant increase in resistance indicated plastic deformation. The numerical modeling of the mechanical durability of the devices relied on the 3D finite element simulation (ABAQUS, Analysis User's Manual 2023) to determine the elastic characteristics of the serpentine and coil, when subjected to stretching and bending deformations. The strain level at the serpentine Cu layers was used to identify the elastic limits during deformation.

Modeled optical properties of murine lung tissue

The absorption (α_0) and scattering (α_s') coefficients of murine lung tissue at 470 nm were extrapolated using an exponential fit for absorption (k_{abs}) and a power-law fit for scattering (a, b) based on 80% inflated lung measurements at 535 and 730 nm (95). The explicit equations are given as $\alpha_0(\lambda) = \alpha_{0,\text{ref}} e^{k_{\text{abs}}(\lambda_{\text{ref}} - \lambda)}$ and $\alpha_s'(\lambda) = a\lambda^b$, which resulted in estimates of $\alpha_0 = 2.2 \text{ cm}^{-1}$ and $\alpha_s' = 135 \text{ cm}^{-1}$, respectively. Under the assumption of predominant forward scattering and a volumetrically weighted anisotropy factor for alveoli and intrinsic tissue taken as $g_a = 0.6$ and $g_t = 0.85^2$, the effective anisotropy factor at 80% inflation was estimated as $g = 0.65$. The radiative transfer equation was used to estimate an effective attenuation coefficient of $\alpha_{\text{eff}} = 30.3 \text{ cm}^{-1}$ through the equation $\alpha_{\text{eff}} = \sqrt{3\alpha_0[\alpha_0 + \alpha_s(1-g)]}$ (96), where the total scattering factor is defined as $\alpha_s = \frac{\alpha_s'}{1-g}$ (97). The index of refraction was assumed to be 1.373 based on deflated measurements in rat lung tissue (98). It is important to note that the $\sim 55\text{-}\mu\text{m}$ -diameter alveoli sacks in mice (99) are modeled as air-filled microspheres and accounted for in the estimated scattering factor at 80% inflation (95, 100).

μ -ILED emission profile modeling

Power was transferred wirelessly to the device through a near field magnetic link between a NeuroLux transmitter coil and an integrated parallel resonant receiver coil tuned to the drive frequency of 13.56 MHz (38). The harvested voltage was rectified and then regulated to 2.8 V using a low dropout regulator to drive the μ -ILED. Three potential current-limiting resistors [$R_s = 200 \Omega$ (580 μA), 2 k Ω (135 μA), 10 k Ω (37 μA)] were selected to establish an appropriate illumination intensity for generating adequate physiological responses while minimizing adverse tissue effects and were labeled as the Standard (163 μW), Low (31.1 μW), and Ultra_low (6 μW) power devices, respectively. The near-field irradiance profiles were subsequently generated using a ray tracing approach and modeled as a Gaussian beam with a width waist of $2W_{0x} = 220 \mu\text{m}$ and $2W_{0y} = 270 \mu\text{m}$. The model assumed transmission from a μ -LED encapsulated in a biocompatible epoxy (Master Bond, UV10TKMed) and coated with a thin 12- μm layer of water-proofing parylene-C into a superstrate composed of a soft 200- μm interfacial layer (Ecoflex 00-30) and murine lung tissue (101). A superstrate of 8 mm³ within the interfacial-tissue layer was discretized into voxels with 5- μm side lengths, and the irradiance $I_{\text{em}}(x, y, z)$ at each voxel was calculated by scaling the normalized Gaussian equation (101)

$$I_{\text{em}}(x, y, z) = I_0 \frac{W_{0x} W_{0y}}{W_x(z) W_y(z)} e^{-2 \left[\frac{x^2}{W_x^2(z)} + \frac{y^2}{W_y^2(z)} \right]} e^{-\alpha_{\text{eff}} z}$$

with $I_0 = \frac{2P_0}{\pi W_{0x} W_{0y}}$, where P_0 is the total forward emitted optical power of the μ -ILED encapsulated with epoxy and parylene. The beam divergence in the x and y directions was encoded in the variables $W_{x,y}(z)$ through the equation $W_{x,y}(z) = W_{0x,y} + z \tan(\theta_{\text{div}/2})$, respectively (102). In addition, the divergence half-angle $\theta_{\text{div}/2}$ was calculated as 32.5° using Snell's law where the indices of refraction of the epoxy and Ecoflex were 1.55 and 1.405, respectively, resulting in a numerical aperture (NA) of 0.833. The effective attenuation in Ecoflex was measured (103) to be $\alpha_{\text{eff}} = 2.4 \text{ cm}^{-1}$; thus, this region was dominated by dispersion. The total dose delivered per voxel was computed by scaling the irradiance by the duty cycle and experiment duration.

In vitro photostimulation with μ -ILED and fluorescence microscopy analysis

To analyze local regulation of E-LightR-Cre by μ -ILEDs, 70% confluent HEK293T cells were cotransfected with E-LightR-Cre-miRFP670 and Floxed-STOP-mCherry reporter DNA constructs at a 1:9 ratio in 35-mm tissue culture plates and incubated in the dark for 16 to 18 hours. Following the incubation, the 35-mm dishes were illuminated with wireless Low_power μ -ILED and Ultralow_power devices attached beneath each plate with the illuminating tip at the center (Fig. 3A), using a 20% duty cycle, under a 10-W transmitting antenna power, and a 10-Hz frequency of illumination and varying illumination duration (2, 6, or 24 hours). Illumination occurred in the rf chamber to mimic the conditions for subsequent in vivo experiments. A sterile wireless rf chamber ($L \times W \times H$: 30.5 cm by 25.4 cm by 15.2 cm) was placed within the cell culture incubator to enable wireless connection. The cell culture dishes with the μ -ILED devices attached beneath were positioned inside the chamber to activate the μ -ILEDs. The entire setup was handled under safe red light to prevent unintended nonspecific activation. Following illumination, the plates were wrapped in aluminum foil and incubated at 37°C and 5% CO_2 for additional time periods (22, 18, or 0 hours, respectively) to complete the total of 24 hours of incubation from the start of illumination (Fig. 3A).

At the end point of each experiment involving μ -ILED-induced local illumination (Fig. 3A), the 35-mm dishes of HEK293T cells in growth media (DMEM with 5% FBS and 1% glutamine) were transferred to the microscope imaging chamber under safe red lights and imaged using horizontal 2D area scanning using an Olympus IX83 microscope equipped with motorized Olympus SSU ultrasonic stage and the cellVivo Incubation System (Evident Scientific) that maintains the cells at 37°C and 5% CO_2 during imaging. Imaging was performed with an EMCCD camera (Andor iXon Ultra 888; Oxford Instruments) paired with a 4X air objective (UPlanXApo 4X/0.16 NA). The miRFP670 and mCherry signals were captured via confocal imaging using excitation laser lines of 647- and 561-nm wavelengths, respectively. Image acquisition was performed using the cellSens software (version 4.1), enabling automated alignment of multiple images with a 10% overlap. Each cell plate imaging took ~ 10 min. The extent of localized Cre activity was determined by measuring the radius of mCherry reporter expression on each plate. For image processing, the stitching artifact was removed using Fast Fourier

transform (FFT) in ImageJ (94). After that, the diameter of the activated zone was determined using the image processing functionalities in MATLAB (version R2022b). Each image was thresholded for removal of background signal, and the approximate center of the illuminated region was marked. Subsequently, two rectangular regions (400x60 and 60x400 pixels, respectively) were cropped from the binarized mCherry signal distributions. The corresponding pixel values, represented as 2D matrices, were averaged along their shorter dimension to produce two 1D vectors, each 400 elements long, representing the average intensity distributions along the horizontal (x) and vertical (y) directions in the image. The average intensity distributions $I(x)$ and $I(y)$ were then fitted to Gaussian curves as

$$I(x) = a_1 e^{-\frac{(x-b_1)^2}{2c_1^2}}, \quad I(y) = a_2 e^{-\frac{(y-b_2)^2}{2c_2^2}}$$

where a is the height of the curve's peak, b is the position of the center of the peak, c is the SD, and subscripts 1 and 2 represent fit parameters corresponding to x and y directions, respectively. After that, the average radius of the activated zone was calculated as $r = (2c_1 + 2c_2) / 2$, where the half-width (radius) of the activated zone in each direction was assumed to be twice the respective SD, which corresponds to $\sim 95.5\%$ of the total area under the Gaussian curve (104).

Local expression of oncogenic KRas and analysis by FACS and bulk RNA-seq

To stimulate localized expression of GFP-KRas(WT/G12D) constructs, 70% confluent HEK293T cells were cotransfected with E-LightR-Cre-miRFP670 and one of the three constructs: pCA-mTmG(GFP-only), pCA-mTmG(GFP-KRas-WT), or pCA-mTmG(GFP-KRas-G12D) in a 1:9 ratio and incubated in dark for 16 to 18 hours. Following incubation, local illumination was performed using Low_power μ -ILED devices operating at a 10-W power, 10-Hz frequency, and 20% duty cycle for 6 hours. Following illumination, the plates were wrapped in aluminum foil and incubated at 37°C and 5% CO_2 for 18 hours in the dark. At the end points, the cells were harvested under safe red light for FACS of GFP-positive and GFP-negative population (Fig. 3B).

The cells were harvested as single-cell suspensions, and the GFP-positive and GFP-negative cells from these locally illuminated plates were sorted using the MoFlo Astrios (Beckman Coulter). The sorted cells were used for total RNA extraction by TRIzol reagent (Invitrogen, 15596026), the RNA was treated with DNase, and its quality was evaluated with gel quality control (QC). All handling of the live cells was performed under safe red light to avoid unwanted nonspecific activity. Bulk RNA-seq was performed with oligo-dT mRNA directional using Illumina NovaSeq X plus for coding genes at the University of Illinois Urbana-Champaign Genomic Facility.

Bulk RNA-seq analysis

A reference transcriptome index was created using Gencode hg38 annotations v45 to map against. To distinguish transgenic KRas-G12D transcripts from endogenous KRas if any, the Kras-G12D sequence was added to the reference as, even in the absence of DNA recombination, the reporter construct design still leads to transcription of a single mRNA encoding tdTomato, GFP, and KRas-G12D genes. However, KRas-G12D protein is not produced due to the stop codon at the end of tdTomato gene. Sequenced reads were mapped to the reference genome (GRCh38), and their abundances were quantified using Salmon v1.3.0 in quasimapping mode (105). Salmon produced transcript-level quantification files, which were subsequently aggregated into

gene-level counts for downstream analysis. Differential expression analysis was performed using DESeq2 v1.40.2, which models count data based on the negative binomial distribution, suitable for identifying differentially expressed genes across conditions (106). Normalization and dispersion estimation were automatically performed by DESeq2 to account for library size differences and biological variability. Genes with an adjusted *P* value of <0.05 were considered statistically significant. Differentially expressed genes were further analyzed for enrichment in GO Biological Processes using the clusterProfiler package v4.8.2 (107).

Animal model

All animal experiments were performed in accordance with institutional and national regulations and approved by the office of Animal Care and Institutional Biosafety (OACIB) at the UIC (ACC protocol no. 23-199). Mice were maintained in a pathogen-free environment at the University of Illinois Animal Care Facility with a 12-hour dark/12-hour light cycle, a temperature at ~23°C, and a humidity at 40 to 60%. Mice had free access to water and food. For local optogenetics experiments (Fig. 5B and fig. S7A); 8- to 10-week-old male or female Rosa-LSL-tdTomato mice [B6.Cg-Gt(ROSA)26Sor^{tm9(CAG-TdTomato)Hze}/J]; the Jackson Laboratory, strain #007909] (70) or Rosa-LSL-tdTomato/SPC-GFP (obtained from Y.L.; UIC) mice were used (108). For tumor initiation studies (Fig. 5, C to G, and figs. S16 to S21), 8- to 12-week-old male and female LSL-Kras-G12D strain mice (LSL-K-ras G12D/B6.129S4-Kras^{tm4Tyj}/J strain; the Jackson Laboratory, strain #008179) were used (19).

Wireless μ -ILED surgical implantation

The numbers in the text represent the steps shown in fig. S10A. The extended surgical procedure can be found in text S1. Animals were anesthetized with ketamine/xylazine (100/5 mg/kg body weight) administered intraperitoneally, followed by subcutaneous Buprenorphine ER (0.3 mg/kg). (fig. S10A1) The trachea was visualized using a surgical microscope (Amscope MU500-HS) to ensure proper placement of the catheter for ventilation. (fig. S10A2) The animal was intubated with a 20-gauge catheter and placed under isoflurane anesthesia on an isothermally heated surgical stage (37°C) and ventilated with a VentElite Small Animal Ventilator (Harvard Apparatus, Holliston, MA), maintained at 120 bpm, 14 PIP, and a flow volume of 500 to 700 μ l. (fig. S10A3) The posterolateral skin area over the ribs was shaved and cleaned with an alcohol swab, and a vertical 1-cm incision was made on the skin over the fifth rib. (fig. S10A4) The incision was extended through the muscles to expose the ribs and the ventilated lung beneath. (fig. S10A5) A small vertical incision was made in the intercostal muscle between the sixth and the seventh ribs to insert the device tip with the μ -ILED (~3 mm wide) facing the lung. (fig. S10A6) The entire surgical stage was rotated 90° for superior surgical access. A reverse cutting P-3 needle (13 mm, 3/8) with a 6-0 silk suture (#S-N610R13-B; AD Surgical) was passed through the fifth and sixth rib, staying closer to the fifth rib, while avoiding the lung underneath and exiting the thoracic cavity through the incision space between the sixth and seventh ribs. (fig. S10A7) The needle was then passed through the Ecoflex-parylene coating of the optogenetic device, next to the μ -ILED tip, to create a loop, ensuring that the device is oriented with the receiver coil side facing upward, μ -ILED side facing downward, and entering from the top and exiting at the bottom. (fig. S10A8) The needle was reintroduced into the chest cavity, through the incision space between the sixth

and seventh ribs, and made to exit through the intercostal muscle between the fourth and the fifth ribs, staying close to the fifth rib. (fig. S10A9) While holding both ends of the suture, the device was slowly inserted into the chest cavity. (fig. S10A10) Both ends of the suture were carefully pulled further so that the device was positioned underneath the ribs. (fig. S10A11) The two ends of the suture were tied around the fifth rib, securely but not too firmly, to avoid any breakage of the ribs, ensuring to secure the device in position. (fig. S10A12) A second needle with 6-0 silk suture was passed inside the thoracic cavity between the seventh and eighth rib and then exited between the fifth and the sixth rib to suture ribs 6 and 7 together and seal the incision. (fig. S10A13) Before tying off the suture, the initial knot was made without pulling it tight to prepare for sealing the chest cavity. (fig. S10A14) The suture was then tied to completely seal the chest cavity, following an inspiration hold and a gentle squeezing of the chest to remove the excess air from the chest cavity. (fig. S10A15) After resealing the thoracic cavity, the serpentine interconnect was extended posterolaterally through the muscle layers underneath the skin with the help of blunt end forceps creating a cavity right under the skin layer for the device to slide into. (fig. S10A16) This ensured subcutaneous positioning of the NFC-enabled receiver coil and the attached red indicator LED on the back of the mouse for optimal power harvesting and operational monitoring. (fig. S10A17) The surgical incisions in the muscle and skin layers were closed with 5-6 interrupted sutures (6-0 nylon sutures with reverse cutting P-3 needle: 13 mm, 3/8). Halfway through the skin sutures, the isoflurane flow was shut off to gradually allow the animal to come back to consciousness. (fig. S10A18) The animals were then disconnected from the ventilator, and the intubation tube was removed. Once normal spontaneous breathing resumed, the mice were allowed to recover on an isothermal pad with access to food and water. Appropriate postoperative care was provided for a minimum of 3 days following surgery. The animals were observed every week following implantation, until the end of the experiment.

Micro-CT

All procedures were approved by the animal institutional care and use committees at the UIC and Northwestern University (NU). For these procedures, the device-implanted mice were transferred from the UIC to the NU by an approved, specialized courier. Mice with implanted μ -ILED devices were anesthetized with 1-2% isoflurane in oxygen (O₂) during imaging. A Bruker preclinical x-ray micro-CT imaging system (Skyscan 1276, Bruker Belgium, Kontich, Belgium) was used to acquire the CT images. First, respiratory-gated scans were acquired of the lungs with the following parameters: 4 $\times$ 4 binning, 65 kV/200 μ A, 0.25-mm Al filter, 160-ms exposure, 37- μ m voxel size, 3600 projections over 360°, and two frame averages. Images were retrospectively sorted into 4 bins for respiratory gating and reconstructed with filtered backprojection using NRecon v2.2.0.6 (Bruker Belgium, Kontich, Belgium). Then, the mice were overdosed with isoflurane until the respiratory monitor no longer registered any breathing. The whole-body, postmortem scans were acquired with the following parameters: 2 $\times$ 2 binning, 85 kV/200 μ A, 0.5-mm Al filter, 150-ms exposure, 18.5- μ m voxel size, 3600 projections over 360°, and two frame averages. Filtered backprojection reconstruction was used without gating. Amira 2024.2 (FEI Co., Hillsboro, OR) was used to merge the gated lung CT images with whole-body postmortem scans. Then, the device, lungs, and skeleton were segmented, followed by 3D surface rendering.

Behavioral experiments

The detailed protocol is available in text S2. The animals were placed in an arena (30 cm by 18 cm). Each mouse was recorded individually for ~25 min using a smartphone camera (iPhone 13) mounted on a tripod, capturing video at 1080p resolution (1080 × 1920) and 25 frames per second (FPS). Mouse movement was tracked using the Track-Analyzer module in the Animal Tracker API plug-in in ImageJ (109). Briefly, the image sequence was obtained from an open-source MP4-to-JPEG converter and imported into ImageJ. A polygonal area was selected on the image to define the analysis boundary, detected by the Radial Maze selection under the Animal Tracker plug-in, generating a tracking window. Background subtraction was performed using Gaussian Blur, and the mouse was thresholded in the processing window. Analysis started at frame 7501 (5 min) and ended at frame 30,000 (20 min), including a 5-min acclimatization period at the beginning. The thresholded mouse contour was selected to initiate tracking from the Tracker menu. After tracking, analysis zones were selected from the Show Analyzer menu, and the correct frame interval and pixel/unit values were entered to generate raw data for distance and velocity vectors. The data were entered into Microsoft Excel and analyzed for consecutive 5 min time points to measure the average distance traveled and average velocity from three 5-min intervals per video.

Adenoviral Cre delivery

The mice received a global lung targeted endotracheal delivery of 5×10^8 PFU (in ~30 μ l) of adenoviral CMV-E-LightR-Cre-miRFP670 or unmodified adenoviral CMV-WT-Cre-GFP. For oropharyngeal endotracheal intubation and adenoviral Cre delivery to the lung, the mice were anesthetized with ketamine/xylazine (100/5 mg/kg body weight) administered intraperitoneally. Then, they were laid on an intubation stand (ETI-MSE-01; Kent Scientific) suspended at a 45° angle and held in position with surgical thread secured around their incisors, and the tail immobilized with adhesive tape. A stereo microscope (SM-3TZ-54S-5M; Amscope) with LED intensity adjustable ring light (LED-144 W-ZK; Amscope) was used to allow illumination and magnification of the larynx inside oral cavity. In the next step, the tongue was gently pulled out and held with the recessive hand to visualize the larynx under the microscope. Once the epiglottis and the arytenoid cartilages were visualized, the dominant hand was used to carefully insert a 20-gauge, 1.25-inch catheter over the epiglottis and between the arytenoid cartilages and gently pushed ~5 mm deeper through the trachea. Once the catheter was placed, the intubated mice with the surgical stand were transferred into the biological safety cabinet for endotracheal delivery of the adenoviral load. Administration of the payload inside the catheter automatically delivers it through the catheter to the lungs. The catheter was removed immediately after delivery and the mice were restored from the intubation rack to be put back in their cages with access to food and water. After adenoviral delivery, the mice were maintained following the Institutional Biosafety Committee's guidelines at the UIC.

In vivo illumination using implanted μ -ILED devices

To induce local optogenetic E-LightR-Cre activity in mouse lungs in vivo, we used a 10-W transmitting antenna power and 10-Hz frequency with duty cycles ranging from 20 to 50% in the rf cage. The mice were housed in a standard animal cage that was positioned within a wireless rf chamber ($L \times W \times H$: 30.5 cm by 25.4 cm by 15.2 cm) to power the devices. The red indicator LED allowed monitoring of wireless connection and desired pulsing (figs. S11 and S13).

Lung tissue preparation

Mice were euthanized at the designated time points for each experiment following approved Animal Care Committee (ACC) guidelines at the UIC. The chest cavity was opened using scissors to expose the heart. The right atrium was then cut, and the heart was slowly perfused through the left ventricle, with 10 ml of PBS through a 21-gauge needle. Thereafter, the animals were intratracheally perfused with 10 ml of PBS through a 21-gauge needle. The perfusion was repeated with 5 ml each of 4% PFA in PBS (pH 7.4) transcardially and intratracheally. While the lungs were still being perfused with 4% PFA intratracheally, the trachea was tied with a suture to maintain the lungs under an inflated condition. The lungs were carefully removed from the thoracic cavity, with particular care taken to avoid damaging the lung, which had an implanted μ -ILED device positioned adjacent to the organ and still attached within the thoracic cavity. The extracted lungs were fixed overnight in 4% PFA at 4°C, and each lung lobe was separated from each other the next day, before the subsequent analysis. Heart and liver lobes were also collected from the selected experimental groups (fig. S15) following transcardial perfusion, fixed overnight in 4% PFA, and processed for subsequent analysis.

Lung tissue clearing

To evaluate local gene recombination following local activation of E-LightR-Cre, mouse lungs were cleared for subsequent light sheet microscopy analysis. Lung samples from Rosa-LSL-tdTomato and Rosa-LSL-tdTomato/SPC-GFP mice were collected 10 days postillumination. After 24 hours of fixation in 4% PFA at 4°C, the lung lobes were washed four to five times for 10 min each in PBS and then incubated in RBC lysis buffer (Invitrogen, catalog no. 50-112-9751) overnight at 4°C and washed again four to five times in ultrapure water (Invitrogen, catalog no. 10977015) for 10 min each. To retain the endogenous fluorescence signals, an aqueous-based tissue clearing was performed following the “Fast 3D clear” protocol with some alterations in incubation windows adapted for the mouse lungs (71). For dehydration and delipidation, the tissue was transferred to glass vials, and incubated in a tetrahydrofuran (THF) gradient: 50% THF, pH 9.0 for 1.5 hours, 70% THF, pH 9.0 for 1.5 hours, and 90% THF, pH 9.0 for 18 hours. The samples were then rehydrated in reverse order followed by washing four to five times with ultrapure water for 10 min each. Last, the samples were incubated in Fast 3D aqueous clearing solution with refractive index (RI) 1.512 to 1.515 for 24 to 48 hours at 37°C and then stored in the clearing solution at 4°C before imaging. During all these steps, the incubation chambers were aluminum foil covered to protect from any light-induced signal damage.

Light sheet fluorescence microscopy (LSFM) of cleared lungs

The cleared lung samples were imaged using a Zeiss Z7 light sheet fluorescence microscope equipped with dual sCMOS cameras (PCO. edge 4.2) following a detailed imaging protocol by Yu *et al.* (72). The ZEN 3.1 LS (black edition) imaging software was used. The imaging system was equipped with a 5X, 0.16 NA, variable RI detection objective and two 5X, 0.1 NA, variable RI illumination objectives, all set to RI of 1.51. The cleared lungs were mounted on the sample holder vertically with superglue (Loctite Ultragel control) and imaged in Cargille immersion oil type A with RI = 1.515 (Cargille 16842) in the sample reservoir. The specimen navigator was used to adjust the position of the camera relative to the sample. The light sheet illumination was configured for aligned dual illumination in the desired laser channel. The software-suggested value for Z-plane

distances and 10% for the tile overlap were applied throughout the experiments. Reporter tdTomato was imaged using 561-nm excitation laser. SPC-GFP was imaged using 488-nm excitation laser. Autofluorescence was imaged using 638-nm excitation laser to define the lung edges and airways. For samples containing no SPC-GFP, 488-nm laser was used for background autofluorescence imaging.

Light sheet image processing and image analysis

The original Zeiss imaging file (.czi) was converted using the Imaris File Converter (Oxford Instrument, versions 9.5.0, 10.2.1, and 11.0.0). Views were then stitched together using the Imaris Stitcher (Oxford Instrument, versions 9.5.0, 10.2.1, and 11.0.0) with the “Automatic Align” function. Images and videos were captured and analyzed by Imaris (Oxford Instrument, versions 9.5.0, 10.2.1, and 11.0.0). Representative 3D micrographs were generated on the basis of thresholded fluorescence intensities, using the volume and surface rendering tools of the Imaris software (Oxford Instruments, versions 9.5.0, 10.2.1, and 11.0.0). Local activation was quantified as both radius (encompassing 95% of the total signal area) and 3D volume of tdTomato signal spread from the estimated LED contact point, relative to the total left lung volume measured from the SPC-GFP or autofluorescence channel.

Histological analysis

For histological analysis of mouse lung samples, the left and right lung lobes from different experimental groups were transferred to 70% ethanol after 24 hours of fixation in 4% PFA. The samples were then processed and embedded in paraffin at the Research Histology Core, UIC. Five-micrometer-thick consecutive sections were collected from the paraffin blocks of lung samples, using a microtome (Reichert-Jung 2030 Biocut Manual Microtome). Special attention was paid during embedding and sectioning to obtain sections close to the μ -ILED device attachment surface on the left lung. Additional samples from the heart and liver collected from the specified experimental group were processed and sectioned using the same protocol for subsequent analysis.

Selected sections were stained with H&E following a previously reported protocol (110). The sections were examined blind for histopathological annotation. For light microscopy analysis, histological images were obtained using an Aperio bright-field whole slide scanner and imager using 20X (0.5 μ m/pixel) and 40X (0.25 μ m/pixel) objectives at the Research Histology and Tissue Imaging Core at UIC. The images were visualized and extracted using QuPath (Edinburgh, UK), an open-source quantitative Pathology & Bioimage Analysis software (111).

Histological analysis and spatial quantification of tumors

From the H&E-stained lung section images, we selected pathologist-annotated tumor regions in ImageJ and extracted tumor centroids for spatial analysis.

Spatial distribution analysis

We computed pairwise Euclidean distances (d_{ij}) between tumor centroids with coordinates (x_i, y_i) using

$$d_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$$

We calculated the Clark-Evans dispersion index (R) as $R = \bar{r}_{\text{obs}} / \bar{r}_{\text{exp}}$, where $\bar{r}_{\text{obs}}$ is the mean nearest-neighbor distance and $\bar{r}_{\text{exp}} = \frac{1}{2\sqrt{\lambda}}$ is the expected mean distance under complete spatial randomness, with λ denoting the number of lesions on the lung section. Values of

$R < 1$ indicate clustering, $R \approx 1$ indicates random distribution, and $R > 1$ indicates uniform dispersion.

Tumor area analysis

We generated a convex hull encompassing all tumors on a section using the ImageJ to estimate the minimum bounding area. The ratio of total tumor bounding area to lung section surface area was used to quantify tumor spread. We also quantified tumor burden as the percentage of annotated tumor area relative to total lung area.

Immunohistochemistry (IHC)

IHC staining for Ki-67 (Rabbit polyclonal; Abcam ab15580) was performed on selected sections following standard protocols at HistoWiz Inc. (Brooklyn, NY, USA). Digitized images obtained from HistoWiz were analyzed using the NIH ImageJ/Fiji open-source software to assess the number of Ki-67-positive nuclei in selected ROIs.

Immunofluorescence (IF)

For IF, the sections were deparaffinized in xylene and rehydrated using graded ethanol series and distilled water. The antigen retrieval was performed by boiling the slides in 10 mM sodium citrate buffer (pH 6.0) in a 2100 Antigen Retriever instrument (Electron Microscopy Sciences, Hatfield, PA). After antigen retrieval, the sections were washed in deionized water and PBS for 10 min each and blocked at room temperature (RT) for 2 hours in PBS containing 0.3% Triton X-100 and 1% BSA. The slides were then incubated overnight at 4°C with desired primary antibody combinations diluted in blocking buffer. Following the primary antibody, the sections were washed three times for 5 min with 0.3% Triton X-100 in PBS at RT. The sections were then incubated at RT for 1 hour with compatible fluorescently tagged secondary antibody combinations, dissolved together in blocking buffer. After secondary antibody incubation, the sections were stained with Hoechst dye (Hoechst 33342; 20 mM, 1 μ g/ml) in PBS for 5 min followed by a 5-min PBS wash. The slides were then mounted in Fluoromount-G (SouthernBiotech; catalog no. 0100-01). Images were captured using an Olympus IX83 microscope using an EMCCD camera (Andor iXon Ultra 888, Oxford Instruments) and a 40X silicone immersion oil (UPLANSApo 40X silicone/1.25 NA) objective. Imaging was performed using the 488-nm laser, 561-nm laser, and wide-field 4',6-diamidino-2-phenylindole (DAPI) filter set. All antibodies and dilutions are listed in table S8.

The proportion of proliferating epithelial cells was determined by counting dual-positive EpCAM-positive and Ki-67-positive cells per square millimeter area (Fig. 5E and fig. S17C). The level of inflammatory response was quantified as the percentage of CD45-positive nuclei, determined by counting CD45/DAPI dual-positive cells relative to total number of DAPI signals per field (Fig. 4F and fig. S12D). Image analysis was performed using the NIH ImageJ/Fiji open-source software and QuPath, an open-source quantitative Pathology & Bioimage Analysis software (94, 111).

Spatial transcriptomics (10X Genomics, Visium HD)

The mouse lung tissue sections from 8 weeks after E-LightR-Cre delivery and Low-power μ -ILED driven local activation in LSL-KRASG12D mice were H&E stained and evaluated by pathologist for neoplastic lesions. Tissue sections were processed according to the Visium HD Gene Expression User Guide (CG000685) to generate sequencing libraries. The mouse v2 probe set was used for hybridization. Minimum sequencing depth was estimated on the basis of the percentage of tissue-covered capture area using the recommended

minimum of 275 million read pairs. Libraries were quantified, pooled, and sequenced on Illumina NovaSeq X, generating over 380 million cDNA reads per sample. Base calling and demultiplexing of Visium HD libraries were performed using bcl-convert v4.3.6, producing fastq.gz files with Sanger format quality scores (ASCII offset 33). Data preprocessing was performed using Space Ranger v3.1.2. The 8- μ m binning resolution was selected for downstream analysis to balance spatial granularity with sufficient transcript capture per bin. QC filtering was performed using Scanpy (v1.10.3). Spots were retained if they contained at least 10 total counts. In addition, spots with high mitochondrial transcript content were removed by excluding those with >15% mitochondrial counts. Filtered data were used for all downstream analyses. ROIs were defined using the high-resolution tissue image associated with each sample. Spatial coordinates for each spot were obtained from the spatial embedding and mapped to image pixel space. ROIs were manually selected by overlaying spot coordinates onto the high-resolution histological image and defining rectangular regions based on pixel coordinates corresponding to pathologist identified lesions within illuminated area and normal tissue compartments outside illuminated area. Eight ROIs (four lesions and four normals) were selected per sample for each condition. We ensured that ROIs were of comparable size and contained a similar number of spatial spots across conditions to minimize biases due to sampling depth. To enable robust downstream comparisons, pseudobulk profiles were generated for each annotated region. For each region, gene expression counts were aggregated by summing the raw counts across all spots within that region. Each pseudobulk sample was annotated with its corresponding biological condition (lesion or normal) based on the histology. The resulting counts matrix of samples by genes was analyzed with DESeq2 v1.44.0, using a negative binomial generalized linear model, incorporating regions from different samples as a batch covariate to account for technical variation. Size factor and dispersion estimates were computed with default parameters; Wald tests for contrasts were used. Multiple testing was Benjamini-Hochberg adjusted and genes with false discovery rate (FDR) < 0.05 were considered differentially expressed. Differentially expressed gene sets were stratified by directionality, with up-regulated and down-regulated genes analyzed separately. Overrepresentation analysis of Gene Ontology Biological Process (GO BP) terms was performed using the clusterProfiler R package (v 4.12.6). Statistical significance of enrichment was tested with a hypergeometric test, and P values were Benjamini-Hochberg adjusted. Background (universe) gene set consisted of all genes in the dataset. Enrichment outputs were filtered at FDR < 0.05 . For reducing redundancy, GO terms with similar semantics were collapsed with the simplify function in clusterProfiler (similarity cutoff = 0.7, ranking terms by adjusted P value). For ssGSEA, count data were normalized using the median-of-ratios method implemented in DESeq2 to ensure comparability across samples. Gene sets from the C5 GO BP collection were retrieved from the Molecular Signatures Database (MSigDB) using the msigdb package (v25.1.1), from which cell cycle-related gene sets were selected for downstream analysis. ssGSEA was then performed on each pseudobulk sample/region using the GSVA package (v1.52.3), generating a per-sample enrichment score that reflects the relative activity of each gene set across all samples.

Statistics

Data analyses were performed using GraphPad Prism (version 10.2.2 for windows, GraphPad Software, Boston, Massachusetts, USA). Data

are presented as mean $\pm$ 95% confidence interval (CI) or mean $\pm$ SD. Statistical details for specific experiments including numbers of repeats (n), P values, and statistical tests are specified in the figures and the figure legends. Data were analyzed by a Brown-Forsythe and Welch's one-way analysis of variance (ANOVA) followed by post hoc Dunnett's T3 multiple comparisons test for unpaired data from a Gaussian distribution but unequal variance. A repeated-measures one-way ANOVA with post hoc Tukey's multiple comparisons test was used to analyze three or more paired/matched groups. When comparing between two conditions, an unpaired two-tailed t test (data normal, equal variance) with Welch's correction was used for statistical significance testing. Significance levels are denoted as follows: n.s., not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Supplementary Materials

This PDF file includes:

Supplementary Text S1 to S3
Figs. S1 to S21
Tables S1 to S8

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Local control of Cre-regulated genetic modeling in vivo using wireless implantable optoelectronics

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