Supporting information

Photoswitchable gRNAs for spatiotemporally controlled CRISPR-Cas-based genomic regulation.

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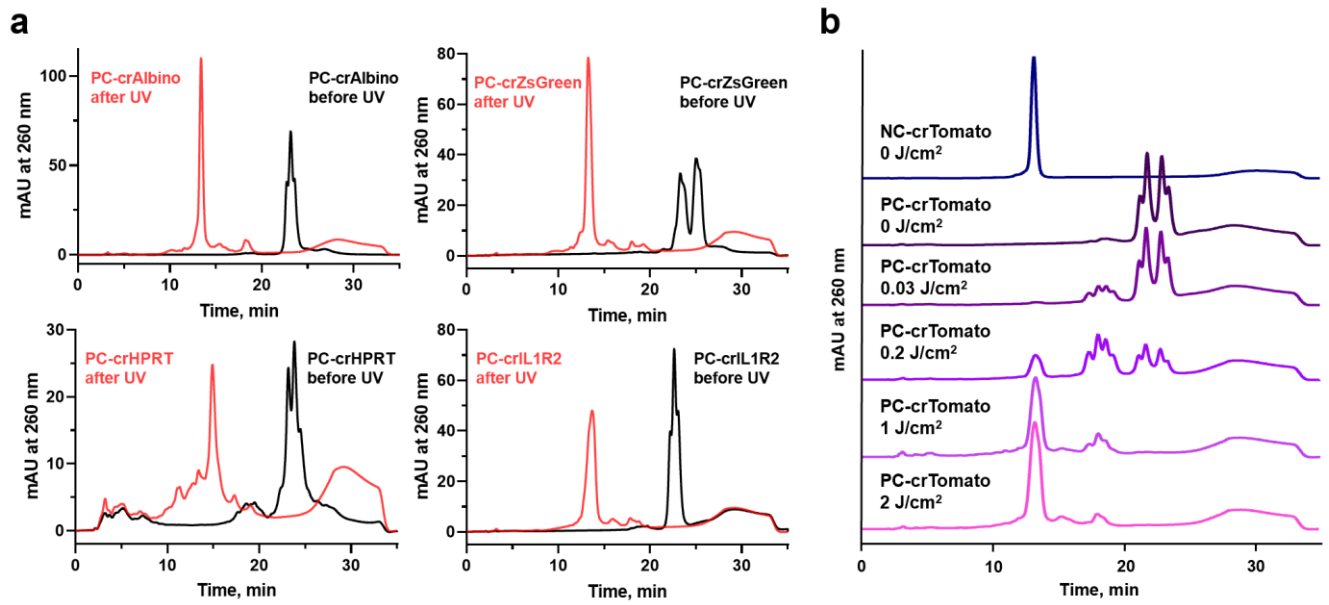
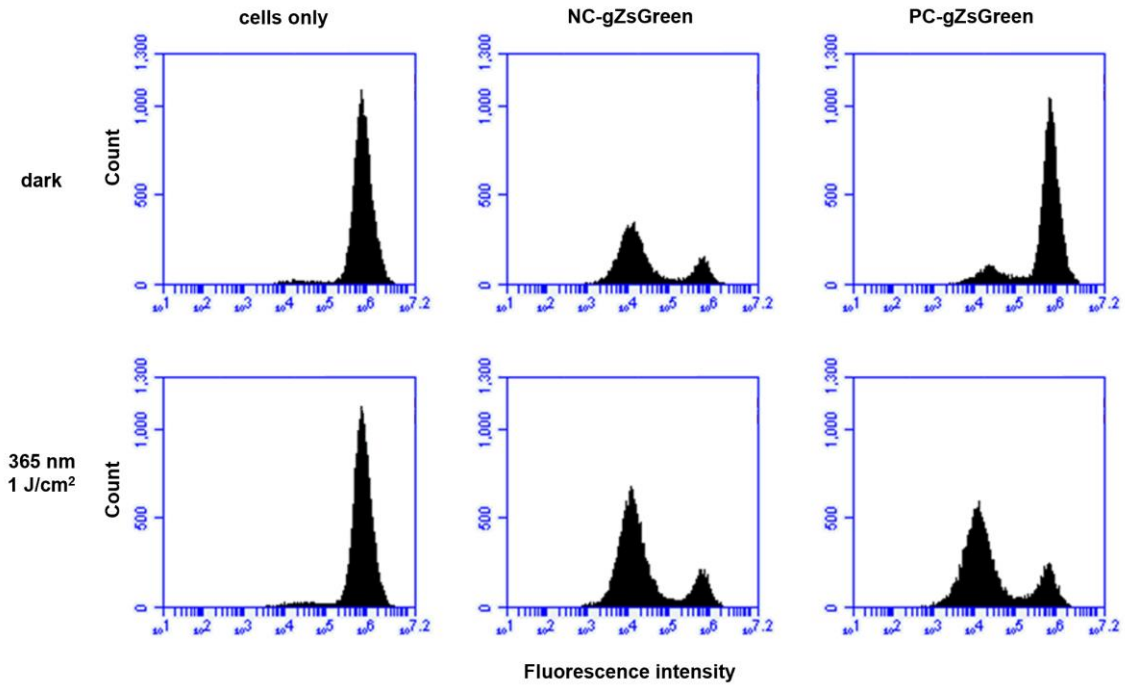
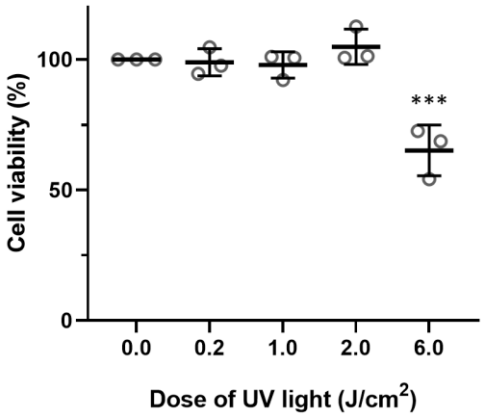
Materials and methods



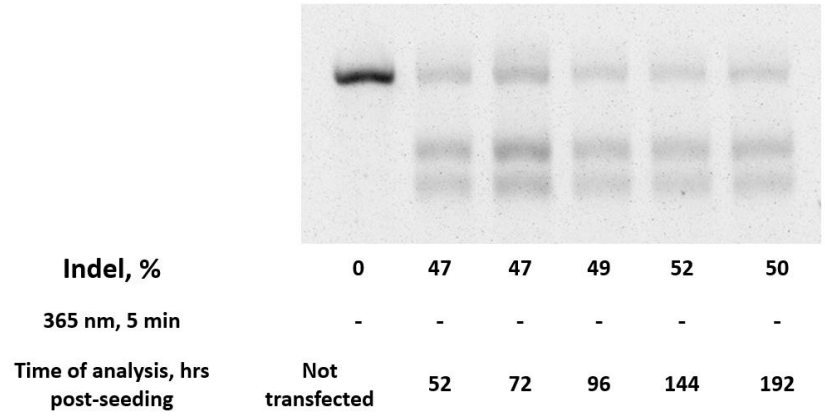
Supporting information Figure 1. HPLC analysis of uncaging of PC-crRNA. (a) HPLC traces of PC-crRNA oligos before and after UV light (365 nm) exposure (2.0 J/cm^2, 10 min exposure). (b) HPLC traces of NC- and PC-crTomato before and after exposure to increasing doses of UV light.



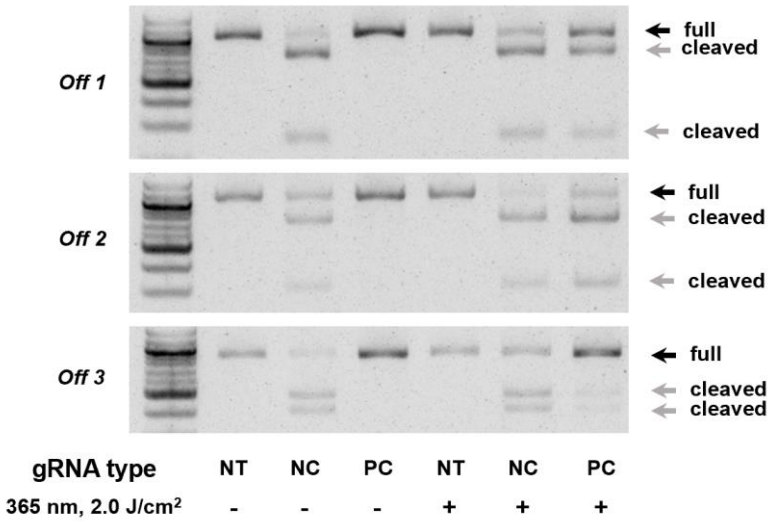
Supporting information Figure 2. ZsGreen protein knockout in HEK-Cas9-ZsGreen cells. Flow cytometry profiles of HEK-Cas9-ZsGreen cells transfected with noncaged or photocaged gZsGreen in the absence or presence of 1.0 J/cm^2 of UV light illumination, cells analyzed 5 days postirradiation.



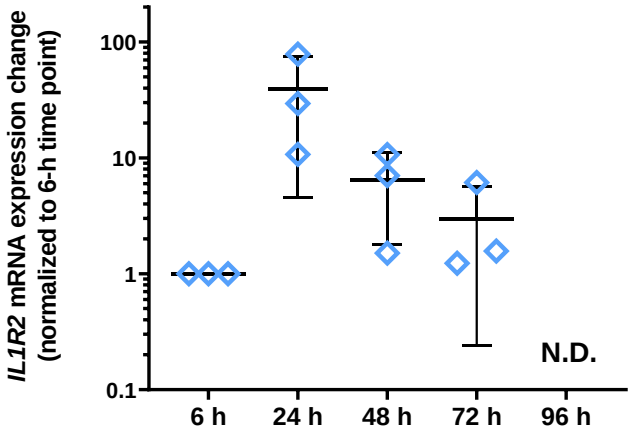
Supporting information Figure 3. UV light (365 nm) cytotoxicity assay on HEK-Cas9 cells. Results are expressed as individual data points overlaid with the mean ± SD (N = 3); ***$p < 0.001$ versus cells kept in the dark according to one-way ANOVA analysis combined with Tukey's (Holm-Sidak) posthoc test.



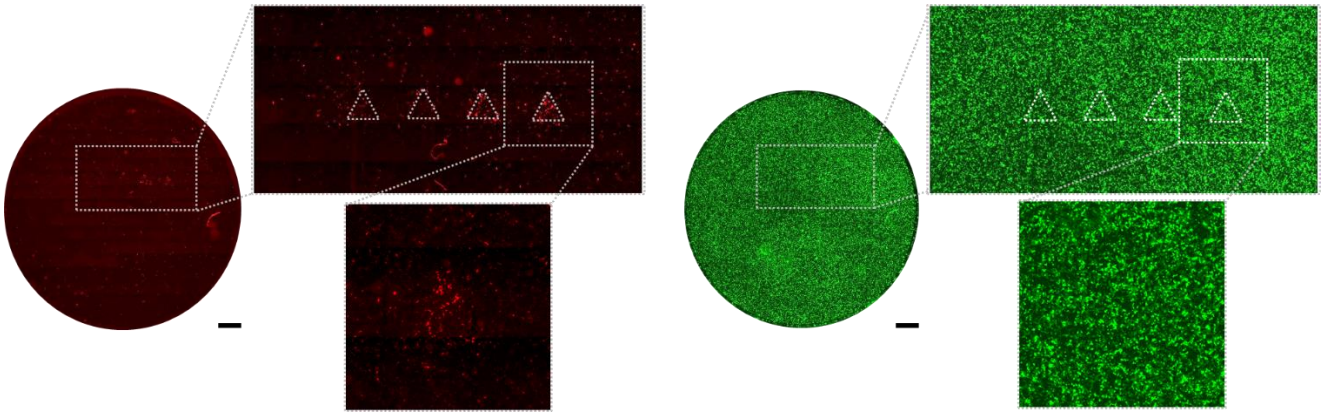
Supporting information Figure 4. Time-dependency of HPRT gene editing in HEK-Cas9 cells transfected with NC-gHPRT. Cells were transfected for 4 h and analyzed for mutations at the indicated time points using the mismatch-based assay.



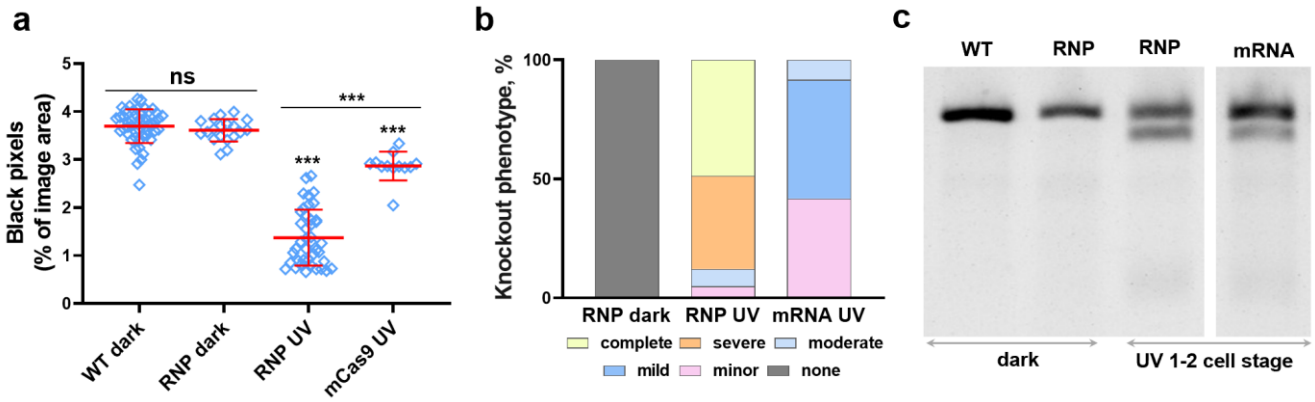
Supporting information Figure 5. Representative gel images of off-target DNA cleavage by NC- or PC-gIL1R2 with 365-nm light illumination (0-2 J/cm^2) *in vitro*. NT – nontreated amplicon (Cas9 only). Off 1, 2, 3 – three off-target sites of gIL1R2 (predicted using CCTop online tool).



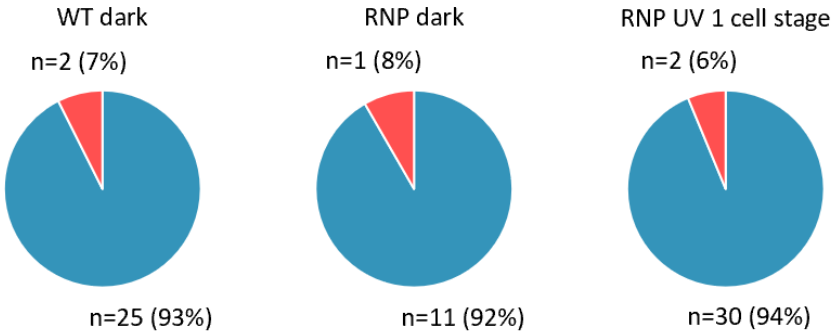
Supporting information Figure 6. Time-dependent expression of the *IL1R2* gene in HEK293FT cells. HEK-dCas9-VPR cells were transfected with gIL1R2 or negative control gTomato, and the *IL1R2* mRNA levels were analyzed by qPCR at 6-96 h post-transfection. The *GAPDH* mRNA expression level served as a reference. (N = 3, mean ± SD, normalized to 6-h expression level). Only gIL1R2 was able to induce detectable *IL1R2* gene expression. N.D. – not detected.



Supporting information Figure 7. Spatial control over gene expression using PC-gRNA. HEK-dCas9-VPR cells cotransfected with PC-gTomato, the tdTomato reporter plasmid, and a GFP plasmid as a transfection control. A four-triangle pattern was drawn on top of the transfected HEK-dCas9-VPR cells using a 375-nm programmable laser (in a 35-mm dish). Red: tdTomato, green: GFP, scale bar 2 mm. The base of the triangles was 1 mm.



Supporting information Figure 8. Comparison of gene editing efficacy in zebrafish embryos using RNP and mRNA format for Cas9 delivery. (a) Automated image analysis of impaired pigment formation in *slc45a2* knockout embryos using Fiji ImageJ software. Results are expressed as individual data points overlaid with the mean ± SD (N = 12 – 49); ***$p < 0.001$, ns – not significant ($p > 0.05$) versus wild-type embryos according to one-way ANOVA analysis combined with Tukey's (Holm-Sidak) posthoc test. Dark – no UV irradiation, only ambient light. (b) Phenotype scoring of zebrafish embryos microinjected with PC-gAlbino and Cas9 protein or mRNA, and globally exposed to the 365-nm UV light (2.0 J/cm^2) immediately after microinjection. The total number of embryos exhibiting each knockout phenotype category was counted for each treatment group (N = 12 – 41). The representative images of each knockout phenotype are shown in the Fig. 4a of the main text. Dark – no UV irradiation, only ambient light. (c) Mismatch-based mutation detection assay of *slc45a2* gene in embryos microinjected with PC-gAlbino and Cas9 protein or mRNA, and globally irradiated with 365-nm UV light (2.0 J/cm^2) immediately after microinjection. (N = 5 embryos per sample). Dark – no UV irradiation, only ambient light.



Supporting information Figure 9. UV light toxicity on zebrafish embryos. Wild-type (WT) or RNP-microinjected zebrafish embryos were either kept in the dark or exposed to 2.0 J/cm^2 of 365-nm UV light immediately after microinjection and their viability was assessed at 48 hpf. Viable embryos are in blue, dead in red. Dark – no UV irradiation, only ambient light.

Materials and methods

Materials

HEK293FT cells, Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose and GlutaMAX™, Opti-MEM® medium, Dulbecco's phosphate buffered saline (DPBS), fetal bovine serum (FBS), penicillin-streptomycin solution, 0.05 % Trypsin-EDTA, nuclease-free water, Lipofectamine® RNAiMAX, Lipofectamine® 2000, Proteinase K, alamarBlue™ cell viability reagent, PCR primers, high-capacity cDNA reverse transcription kit, PowerUp™ SYBR® Green master mix, E-gel® 2 % and 4 % precast agarose gels were purchased from Thermo Fisher Scientific (Walthman, MA). Noncaged CRISPR RNAs (NC-crRNAs) and trans-activating CRISPR RNA (Alt-R® tracrRNA) were purchased from Integrated DNA Technologies (IDT, Coralville, IA). NC-crRNAs were composed of AUGC and synthesized using proprietary Alt-R® modifications. All photocaged CRISPR RNAs (PC-crRNAs) were custom synthesized from commercially available building blocks and are readily accessible from Microsynth (Balgach, Switzerland). Edit-R® Cas9 protein, Edit-R® lentiviral Blast-Cas9-CAG nuclease plasmid and Edit-R® lentiviral hEF1a-Blast-dCas9-VPR particles were obtained from Horizon Discovery Group (Cambridge, UK). Cas9-encoding mRNA was purchased from Oz Biosciences (Marseille, France). PMD2.G and psPAX2 lentiviral packaging plasmids were a gift from Didier Trono (Addgene plasmids # 12259 and # 12260, respectively). ZsGreen lentiviral particles and Guide-it™ mutation detection kit were obtained from Takara Bio (Kusatsu, Japan). Reporter-gT1 plasmid for tdTomato was a gift from George Church (Addgene plasmid # 47320). Pmax-GFP plasmid used as a transfection control was from Lonza (Basel, Switzerland). Acetonitrile, HEPES, 0.5 M EDTA pH 8.0, NaCl and $MgCl_2$ were obtained from Sigma Aldrich (St. Louis, MO). Gel Pilot 1 kb Plus Ladder, QIAquick® PCR purification kit, and RNeasy Mini kit were obtained from QIAGEN (Hilden, Germany). Triethylammmonium acetate 1 M pH 7.0 (TEAA) was acquired from ITW Reagents Division (Glenview, IL). Blasticidin was purchased from Invivogen (San Diego, CA). PVDF filters with 0.45-µm pore size and 100 kDa molecular cut-off Amicon® ultracentrifugal filter concentrators were obtained from Millipore (Burlington, MA). Imaging dishes (35 mm) with a glass bottom and an imprinted 50-µm grid for laser-guided photoactivation experiments were obtained from Ibidi (Martinsried, Germany).

Monitoring of UV-light mediated uncaging of PC-crRNAs using liquid chromatography (LC)

Twenty five microliters of PC-crRNA at 20 μM concentration were placed in a 0.2 mL PCR tube and were either kept in the dark or irradiated by UV light of 365 nm wavelength for 0-2 J/cm^2 (corresponding to 0-10 min of irradiation, respectively), and then diluted with 25 μL of 0.1 M TEAA in nuclease-free water to a final concentration of 10 μM. The UV lamp (UVLMS-38 8-watt, Analytik Jena AG, Jena, Germany) exhibited a power density of (3.3±0.5) mW/cm^2 as measured by UVA/B Light Meter 850009 (Sper Scientific, Scottsdale, AZ). The HPLC analysis was performed on an Agilent 1260 Infinity instrument (Agilent Technologies, Santa Clara, CA) equipped with a variable wavelength detector (VWD) using a Phenomenex Gemini®-NX 5 μm C18 110 Å 150 × 4.6 mm column. The detection wavelength λ = 260 nm, column temperature = 323 K, injection volume = 25 μL and flow rate =1 mL/min using the following gradient of 0.1 M TEAA in water (buffer A) and 0.1 M TEAA in 90 % acetonitrile (buffer B): 0-5 min 11 % B, 5-25 min 11-22 % B, 25-30 min 22 % B, 30-30.5 min 22-11 % B, 30.5-35 min 11 % B.

***In vitro* cleavage assay**

Guide RNAs with or without photocaging groups were produced by annealing equimolar amounts of photocaged or noncaged crRNAs with tracrRNA to obtain a final concentration of 25 μM in nuclease-free water. The mixture was heated to 95 °C for 5 min in a heat block, and slowly cooled to room temperature. Noncaged crRNAs and tracrRNA were Alt-R® modified from IDT.

Three-step PCR (98 °C 2 min, 98 °C 10 s, 60 °C 15 s, 68 °C 1 min, 35 cycles) was performed to amplify *HPRT*, *ZsGreen*, *slc45a2*, *IL1R2* genes and three off-target sites of gIL1R2 (predicted using CCTop online tool) using Guide-it™ mutation detection kit according to manufacturer's instructions on a thermocycler (Alpha Laboratories, UK). Primer sequences were as follows (5′ to 3′): *HPRT* forward TACACGTGTGAACCAACCCG, *HPRT* reverse GTAAGGCCCTCCTCTTTTATTT, *ZsGreen* forward CTCGAGAAGCTTGATCGCGT, *ZsGreen* reverse GACAAGATGTCCTCGGCGAA, *IL1R2* forward GACTTGATGCTGGATTCCCACT, *IL1R2* reverse GATTTCCTAACCGGGTGGTGT, *slc45a2* forward CTGGGAAGTCCAACGCTCAG, *slc45a2* reverse CCTATTGTCCACTCCCAGCA, IL1R2 off-target 1 forward GTCACGCCTTCGGTGAATTG, IL1R2 off-target 1 reverse TGGAGCTGAGGAAAACGGTC, IL1R2 off-target 2 forward GGAAGAGGAGGAGCCTACCA, IL1R2 off-target 2 reverse AGGGGCCACATTTACCAGTG, IL1R2 off-target 3 forward CACCCTAGTCCTATGCTGCG, IL1R2 off-target 3 reverse TGTTCCTTGGCCAGCTTCAT.

PCR products were purified using QIAquick® PCR purification kit and their concentrations were determined using NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Walthman, MA).

In vitro cleavage assay was performed according to manufacturer's protocol (Alt-R® CRISPR-Cas9 system, IDT). Briefly, equimolar amounts of crRNA:tracrRNA duplex and Cas9 enzyme were incubated for 5 min at room temperature to form the ribonucleoprotein complex (RNP). Purified target DNA amplicon (20 nM final concentration) was mixed with RNP complex together with 1 µL of Cas9 buffer (200 mM HEPES, 1 M NaCl, 50 mM $MgCl_2$, and 1 mM EDTA, pH 6.5) to achieve RNP:DNA molar ratio of 10:1. The total volume of the mixture was adjusted to 10 µL with nuclease-free water, which was followed by the incubation at 37 °C for 2 hours. After the incubation, 2 µL of Proteinase K solution (20 mg/mL) was added to the reaction mixture and incubated at 56 °C for 10 min to digest Cas9 protein. Samples were resolved on pre-stained 4 % agarose E-gels® and imaged using Invitrogen™ E-Gel® Imager.

Intensities of the bands were quantified using Fiji ImageJ open-source software [44], and target DNA cleavage efficacy was calculated using the following formula, where $I_{fragment\ 1}$ and $I_{fragment\ 2}$ are intensities of cleaved fragments and $I_{full\ length\ amplicon}$ is the intensity of uncut DNA amplicon:

$$DNA\ cleavage\ (\%) = \frac{I_{fragment\ 1} + I_{fragment\ 2}}{I_{fragment\ 1} + I_{fragment\ 2} + I_{full\ length\ amplicon}} \times 100\ \%\ .$$

Cell culture

HEK293FT cells (human embryonic kidney cell line) were maintained in DMEM supplemented with 4.5 g/L glucose, GlutaMAX™, 10 % FBS and 1 % penicillin/streptomycin (complete medium) in 5 % CO_2 at 37 °C, with regular passaging twice a week using 1:5 split ratio. Each freshly thawed cell line was grown for 2–3 passages before transfection. Cells were regularly tested for mycoplasma contamination.

Cell viability assay after UV-light irradiation

The cytotoxicity of 365-nm light to HEK293FT cells was assessed using alamarBlue™ cell viability reagent according to manufacturer's instructions. The HEK293FT cells were plated in 96-well plate at a seeding density of 4×10^4 cells per 100 µL of complete medium per well. After 4 h incubation, cells were exposed to increasing doses of UV light (UVLMS-38 lamp, 0-30 min, 0-6 J/cm^2), followed by 48 h of further incubation in the dark. The cells were then incubated with 10 % v/v alamarBlue (100 µL/well) in complete medium for 4 h. The supernatants were transferred into new 96-well plate and fluorescence was read by microplate reader SpectraMax

M5 (Molecular Devices, San Jose, CA) at Ex/Em = 570/585 nm (auto cut-off) from the bottom of the plate. The alamarBlue™ solution in complete medium was used as a negative control. The cell viability was calculated according to the following equation, where F_{sample} refers to the fluorescence of cells treated with different doses of UV light, $F_{cell\ only}$ refers to fluorescence of untreated cells, and $F_{Alamar\ Blue}$ refers to the fluorescence of negative control:

$$\text{Cell viability (\%)} = \frac{F_{sample} - F_{Alamar\ Blue}}{F_{cell\ only} - F_{Alamar\ Blue}} \times 100\ \%\ .$$

Lentivirus and cell lines generation

For Cas9 lentiviral particles production, HEK293FT cells were seeded at ~40 % confluency in a T175 flask the day before transfection. Cells were transfected for 4 h with a mixture of plasmids encoding for Cas9 (62.9 μg), pMD2.G (16.9 μg) and psPAX2 (31.3 μg) using Lipofectamine® 2000 according to the manufacturer's instructions. After medium exchange, cells were incubated for another 48 h. The supernatant containing Cas9 lentiviral particles was filtered through a 0.45-μm PVDF filter, 100-fold concentrated using 100 kDa molecular cut-off Amicon® ultracentrifugal filter concentrator, and aliquots were stored at −80 °C.

For generation of HEK-Cas9 or HEK-dCas9-VPR cell lines, HEK293FT cells were seeded at a 1×10^5 cell per well of a 24 well plate in complete medium the day before transduction. Cells were transduced with 10-fold serial dilutions of the viral concentrates in complete medium for 24 h, followed by medium exchange. Successfully transduced cells were selected using 10 μg/mL of blasticidin for 7 days, and the samples with the cell viability of less than 30 % were used for further studies to encourage single gene copy insertions. Monoclonal cell lines were obtained by plating cells in a 96-well plate at 0.5 cell/well seeding density and subsequent colony propagation.

For generation of HEK-Cas9-ZsGreen cell line, HEK-Cas9 cells were seeded at a 1×10^5 cell per well of a 24 well plate the day before transduction. Cells were transduced with 10-fold serial dilutions of the viral concentrate in complete medium for 24 h, followed by medium exchange. The samples with less than 30 % of ZsGreen-positive cells were FACS sorted for ZsGreen expression at the Flow Cytometry Facility of Imperial College London. Monoclonal cell lines were obtained by plating cells in a 96-well plate at 0.5 cell/well seeding density and subsequent colony propagation.

Light-activated gene editing in cells

For the *HPRT* and *IL1R2* gene editing experiments, HEK-Cas9 cells were transfected for 4 h in tissue culture 96-well plates with 12.5 pmol of NC-gRNA or PC-gRNA using Lipofectamine® RNAiMAX as per manufacturer's instructions. For *ZsGreen* gene editing experiments, HEK-Cas9-ZsGreen cells were transfected for 4 h in tissue culture 96-well plates with 12.5 pmol of NC-gRNA or PC-gRNA using Lipofectamine® RNAiMAX. After the transfection medium exchange, the cells were either kept in the dark or exposed to 365-nm UV light for 5 min (1.0 J/cm^2) at the indicated time points, followed by 2 days of further incubation for gene mutation assay or for 5 days for ZsGreen protein expression evaluation by fluorescence microscopy and flow cytometry.

Mutation detection assay

Mutation detection assay was performed using Guide-it™ mutation detection kit according to manufacturer's instructions (Takara Bio, Kusatsu, Japan). Target genes were purified from the genomic DNA using the same primers as for the *in vitro* cleavage assay, followed by amplicon rehybridization and resolvase-mediated mismatched strands cleavage. The products were resolved on 2 or 4 % agarose E-Gels® and imaged by Invitrogen™ E-Gel® Imager. Fiji ImageJ open-source software was used to analyze the intensities of the bands and to calculate the rate of insertions and deletions in the target genes (indel) using the following equations, where $f_{mismatch} = \frac{N_{mismatched\ amplicons}}{N_{all\ amplicons}}$, $f_{mutation} = \frac{N_{mutated\ amplicons}}{N_{all\ amplicons}}$, *N* refers to number of molecules, *I* refers to band intensity:

$$f_{mismatch} = \frac{I_{fragment\ 1} + I_{fragment\ 2}}{I_{fragment\ 1} + I_{fragment\ 2} + I_{full\ length\ amplicon}},$$

$$\text{Indel (\%)} = f_{mutation} \times 100\ \% = \left(1 - \sqrt{1 - f_{mismatch}}\right) \times 100\ \%.$$

Spatially controlled transcription activation

For spatially resolved transcriptional activation of a reporter gene using a photomask, HEK-dCas9-VPR cells (2.5 × 10^5 cells per well of a 24-well plate) were transfected for 4 h with 0.75 μg of reporter tdTomato plasmid [48], 0.375 μg of GFP plasmid, and 9.38 pmol of NC-gRNA or PC-gRNA targeting a sequence upstream of *tdTomato* ORF cassette using Lipofectamine® RNAiMAX. A small portion of the well was illuminated through a tin foil photomask (1.0 J/cm^2) using collinear 365-nm light emitting device OmniCure S1500 (Excelitas

Technologies, Waltham, MA) equipped with a light guide. Fluorescence images were taken 1 day after the irradiation.

For laser-guided activation of gene expression, 6×10^5 HEK-dCas9-VPR cells were seeded into imaging dishes (35 mm) with a glass bottom and an imprinted 50-µm cell location grid 24 h before the experiment. The next day, cells were transfected for 4 h with 0.5 µg of reporter tdTomato plasmid, 0.25 µg of GFP plasmid, and 6.25 pmol of PC-gRNA targeting a sequence upstream of *tdTomato* ORF cassette using Lipofectamine® RNAiMAX. Four triangular shapes with the base of 1 mm were scanned on top of the transfected cells using the laser lithography system Dilase 250 (Kloe, Montpellier, France). The 375-nm laser spot size was 5 µm, the power modulation was 2.6 %, and the scanning speed was 1-8 mm/s. After the exposure, the cells were further incubated for 1 day to allow for the tdTomato expression, which was assessed by fluorescence microscopy.

Temporally controlled transcription activation

To evaluate the time-dependency and the sequence-specificity of the *IL1R2* gene activation, HEK-dCas9-VPR cells (2.5×10^5 cells per well of a 24-well plate) were transfected for 4 h with 1 µg of NC-gRNA targeting a sequence upstream of *IL1R2* gene (crIL1R2) using Lipofectamine® RNAiMAX. The gRNA used for activation of tdTomato cassette served as a negative control. Relative expression of *IL1R2* mRNA was assessed by qRT-PCR 6-96 h post-transfection. Due to undetectable *IL1R2* mRNA expression in the nontransfected HEK-dCas9-VPR cells, cells transfected with the NC-gIL1R2 at the 6 h post-transfection time point were used for normalization.

For temporally resolved endogenous gene activation, HEK-dCas9-VPR cells (2.5×10^5 cells per well of a 24-well plate) were transfected for 4 h with 1.5 µg of NC-gRNA or PC-gRNA targeting a sequence upstream of IL1R2 gene (crIL1R2) using Lipofectamine® RNAiMAX. The gRNA used for activation of *tdTomato* cassette served as a negative control. After transfection medium exchange, cells were either kept in the dark or exposed to 365-nm UV light for 5 min (UVLMS-38 lamp, 1.0 J/cm^2 dose) at the specified time points, followed by 1 day of further incubation. Relative expression levels of the *IL1R2* mRNA were assessed by qRT-PCR, and the cells treated with the NC-gIL1R2 served as a normalization control.

Quantitative real-time PCR analysis (qRT-PCR)

For the *IL1R2* mRNA expression analysis, total RNA was isolated using RNeasy Mini kit (A_{260}/A_{230} >1.8). The expression levels of *IL1R2* mRNA relative to the internal control *GAPDH* mRNA were quantified by two-

step quantitative real-time PCR. One microgram of total mRNA was reverse transcribed in 20 µL of final reaction volume using high-capacity cDNA reverse transcription kit. Quantitative RT-PCR (50 °C 2 min, 95 °C 10 s, 40 cycles × (95 °C 15 s, 60 °C 1 min)) was performed using PowerUp™ SYBR® Green PCR master mix and specific primers for human *IL1R2* (forward CAGGTGAGCAGCAACAAGG, reverse TGCTCCTGACAACTTCCAGA), and *GAPDH* (forward TGGTATCGTGGAAGGACTCATGA, reverse ATGCCAGTGAGCTTCCCGTTCAG) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). Delta delta Ct ($2^{-\Delta\Delta Ct}$) method was used to calculate relative mRNA expression levels. Results are expressed as the *IL1R2* mRNA expression level change between cells treated for 6 h and the following time points for time-dependent gene activation, and between NC-gRNA treated and PC-gRNA treated cells for light-activation experiments.

Fluorescence microscopy

ZsGreen, GFP, and tdTomato protein expression was assessed using fluorescence microscopy on Z1 AxioObserver (Zeiss, Oberkochen, Germany). Cells were fixed in 2 % paraformaldehyde (PFA) in PBS for 10 min, washed with PBS, and the cell nuclei were stained with Hoechst. Images were taken in bright field, Hoechst 33258 (Ex/Em 352/455), Alexa Fluor 488 (Ex/Em 493/517), and tdTomato (Ex/Em 554/581) channels using 20× objective (NA 0.4). Whole well images were processed and stitched using Zen 2 (blue edition) imaging software.

Flow cytometry analysis

Flow cytometry analysis of ZsGreen knockout in HEK-Cas9-ZsGreen cells was performed using a BD Accuri™ C6 Flow Cytometer (BD Biosciences, San Jose, CA). HEK-Cas9-ZsGreen cells transfected as described in gene editing section were trypsinized and resuspended in 0.5 mL of complete medium, centrifuged and washed with PBS. Cells were fixed with 2 % PFA in PBS for 10 min, centrifuged and resuspended in 0.5 mL of PBS containing 5 % FBS as a blocking reagent. Any debris and damaged cells were excluded in SSC-A versus FSC-A plot, and clumps or doublets were gated out in FSC-H versus FSC-A plot. Flow cytometry data was analyzed *via* BD Accuri™ C6 Software.

Temporally controlled gene editing in zebrafish embryos

Experiments involving zebrafish were conducted in accordance with UK Home Office requirements (ASPA 1986, project licence P5D71E9B0, institution licence 70/2722 X32FDCFC1). The fish were kept in the CBS facility of Imperial College London. Wild-type zebrafish used in this study were reared and maintained according

to standard practices at 28.5°C on a 14-hour light/10-hour dark cycle. Zebrafish embryos were obtained *via* natural mating and were maintained in E2-water supplemented with 0.00003 % methylene blue at 28.5°C.

RNPs were prepared to contain 4 μM of Cas9 protein and 20 μM of NC- or PC-gAlbino in nuclease-free water. For comparison, Cas9 protein in the mixture was replaced with 0.2 g/L of Cas9-encoding mRNA. Single- to double-cell stage embryos of wild-type zebrafish were microinjected with 2.5 nL of these solutions using a pressure microinjector Narishige IM300 (Tokyo, Japan) with pulled borosilicate capillary needles with outer diameter 1.0 mm, inner diameter 0.78 mm, length 100 mm (Harvard Apparatus, Holliston, MA), followed by irradiation with 365-nm light for 10 min (UVLMS-38 lamp, 2.0 J/cm^2) or incubation in the dark. For time-dependent gene editing activation study, the injected embryos were exposed to the UV light at different developmental stages. Alternatively, for spatially resolved gene editing, the microinjected embryos were left at the ambient light (no UV irradiation) until they reached a 12-somite stage. Then they were dechorionated, embedded into 0.5 % low melting agarose in a glass bottom 35-mm dish, and oriented laterally. After that, the eye of the embryo was irradiated for 1 min at 20× objective (HC PL APO NA 0.7) and 4× zoom using a 405-nm laser at 50 % power in a confocal microscope setup (Leica SP5 inverted, Wetzlar, Germany). After 2 days of growth, the embryos were scored for albino-like phenotype in the retina and skin and photographed using a stereomicroscope (Leica M205 FCA, Wetzlar, Germany).

For the mutation detection assay, total genomic DNA of 3-5 embryos per sample was extracted and analyzed using Guide-it™ mutation detection kit. For spatially resolved gene editing, the mutations in the *slc45a2* gene were analyzed in the head and tail parts of the same embryos separately. Three to five embryos were combined for the analysis. Only embryos that developed normally were assayed for mutations.

Automated image analysis of impaired pigment formation in *slc45a2* knockout embryos was performed using Fiji ImageJ software. First, the gaussian blur with a radius of 10 was run on a duplicate image, then the original image was divided by the blurred image to decrease the background variability, and the threshold was set between -3.4e38 and 0.71 in order to create a binary image. Then the dust was cropped out and the relative area occupied by the black pixels was quantified. These processing steps were performed on each image. Wild-type embryos served as a normalization control.

Statistical Analysis

The statistical analysis was performed using OriginPro 2017 software. All experimental groups were compared pairwise using the one-way analysis of variance (ANOVA) followed by Tukey's (Holm-Sidak) posthoc test assuming normal data distribution. The differences between treatment groups were considered statistically significant at *p*-values lower than 0.05.