Supporting Information

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Materials and Methods

Generation of Gal4→Fluc Transgenic (Tg[Gal4(+)]+)) Mice. The plasmid contained a polyadenylation site upstream of 5 consensus repeats of the upstream activation sequence of Gal4 and an adenovirus E1b minimal TATA box driving the firefly luciferase (P. pyralis) gene (derived from pGL3; Promega). Downstream of the expression cassette, the construct contained a SV40 intron and another polyadenylation site to enhance the stability and expression efficiency of the transgene. The purified linear plasmid was injected into pronuclei of fertilized FVB oocytes. Embryos were transferred into pseudopregnant FVB mice. Injections and embryo transfers were performed in the Washington University Mouse Genetics Core. Pups were weaned at 3 weeks of age, and tails were sampled for DNA analysis.

DNA Extraction and PCR. Mouse tail DNA was sampled by incubating the tail snips with SNET buffer [20 mM Tris-HCl, 5 mM EDTA, 400 mM NaCl, 1% (wt/vol) SDS; pH 8.0] that contained 400 µg of RNase A at 37°C for 60 min. DNA was then harvested using a standard phenol/chloroform/isoamyl alcohol extraction protocol. DNA was dissolved in water. To test for the presence of the transgene, a PCR was performed with primers spanning the entire ORF of the luciferase coding sequence. The primers were 5’-ATGGAAGCGCCTAAACTAATAAA GAAAGGCC-3’ at the 5’ end and 5’-CAGGATCTCGTC TCCGCTTCTCTG-3’ at the 3’ end which amplified a 1.6 kb fragment. PCR reactions were carried out in a total volume of 20 µl containing 250 nM of each primer, 2.5 mM MgCl2, either 0.3 µg of mouse genomic DNA or 10 ng of plasmid DNA pGL3-control (Promega) using the commercially available Platinum Pfx Polymerase system with 1x Enhancer (Invitrogen).

Following hydrodynamic injections, to document the presence of the plasmids encoding the interacting protein pair, pGal4BD-p53 and pVP16-TAG, DNA was extracted from mouse livers using a tissue kit (Qiagen). The primers to amplify a 235 bp fragment of Gal4BD were 5’-CGCTACTCTCCCAAAACCAA-3’ and 5’-CAGTCCATCCTGAAACC-ATAC-3’; primers to amplify a 186 bp fragment of VP16 were 5’-GGACGATGCTTATTA GACG-3’ and 5’-AGGGCAATGATACATCTG-3’. PCR products were then fractionated on 1% or 2% agarose gels and visualized with ethidium bromide.

Generation of Recombinant Viruses. An Ad5Gal4BD-VP16 adenovirus (Ad-Gal4) that incorporated a fusion construct (Gal4BD fused to VP16) was generated by the Gene Transfer Vector Core at the University of Iowa. Ad5CMVCre (Ad-Cre) was purchased at the University of Iowa. Ad5CMVCre (Ad-Cre) was purchased from the Gene Transfer Vector Core.

Cell Culture. HeLa cells were grown in DMEM with 10% heat-inactivated FBS and 1% glutamine. Using Fugene 6 (Roche Applied Science, Indianapolis, IN), cells were transfected with pDNA6/5-HisA engineered to contain a concatenated Gal4 promoter driving expression of firefly luciferase in place of a mutant thymidine kinase reporter (1). Stable clones were selected in the presence of 2.5 µg/ml blasticidin and clone #43 was used for experiments (HeLa Fluc #43).

Mouse embryonic fibroblasts (MEF) were isolated on Day 13.5 as described elsewhere (2). Cells were grown in DMEM with 10% FBS, 1% L-glutamine, 1% non-essential amino acid mixture, 1% penicillin/streptomycin, and 0.14 mM 2-mercaptoethanol.

Primary mouse fibroblasts were isolated as described (2). In brief, after shaving, adult abdominal mouse skins (TgGal4(−/−) and control WT mice) were washed and incubated with 0.5% trypsin/ PBS for 60 min in a 37°C water bath to remove the epidermis. The dermal samples were then cut into small pieces, placed into a 6-well plate and covered with a sterile coverslip. Complete DMEM (DMEM with 10% heat-inactivated FBS, 1% L Hepes buffer, 1% L glutamine, 1% non-essential amino acid mixture, 1% penicillin/streptomycin, and 1% sodium pyruvate) was added to each well. After fibroblast cells had grown out of the skin specimens and the well was confluent, the dermal pieces were removed. Fibroblast cells could then be cultured, passaged and frozen.

Bioluminescence Imaging of Cell Lines. HeLa Fluc #43 cells were transiently transfected with pM3-VP16, a positive control vector that expresses a fusion of Gal4 DNA-BD to the VP16, from the Mammalian Matchmaker Two-Hybrid Assay Kit (Clontech). The next day, cells were imaged in colorless solution (2.7 mM KCl, 139 mM NaCl, 8.1 mM NaHPO4, 7H2O, 1.5 mM KH2PO4, 1.8 mM CaCl2, 1 mM MgCl2, and 5.5 mM D-glucose) containing 150 µg/ml D-luciferin as described (3) using a cooled CCD camera (IVIS 100; Caliper Life Sciences). MEFs (P1) were incubated with DMEM containing 4 µg/ml of polybrene and 4 x 107 pfu of Ad-Gal4 for 90 min. After that time, complete medium was added. Cells were imaged after 24 h using 150 µg/ml D-luciferin in colorless solution (acquisition time, 1 min; binning, 8; field of view, 15 cm; f/stop, 1; open filters). Primary mouse skin fibroblast cells were cotransfected using a nucleo- factor device (Amaxa) with pM3-VP16 along with a coad humanized vector pBluc-N3 (BioSignal Packard) encoding Renilla luciferase as a transfection control. Cells first were imaged for Renilla luciferase activity in colorless solution containing a final concentration of 400 nM coelenterazine. To image firefly luciferase, the media was changed to colorless solution containing 150 µg/ml D-luciferin. Images were acquired with a >590 nm bandpass filter. Data analysis was performed as described (3, 4).

Animal Studies. Animal care and euthanasia were approved by the Washington University Medical School Animal Studies Committee. Bioluminescence imaging was performed on the IVIS at the indicated time points as described (acquisition time, 1–60 seconds unless otherwise indicated; binning, 4 or 8; field of view, 15 cm; f/stop, 1) (3, 4). Anesthesia was maintained during imaging by nose cone delivery of 2.5% isoflurane. Following imaging, animals were killed by cervical dislocation.

I.V. Injections. Mice were anesthetized with 2.5% isoflurane before tail vein injections of Ad-Gal4 or Ad-Cre [1.4 x 107 pfu/mouse in 100 µl PBS (pH 7.4)]. Imaging of mice was performed by injection of D-luciferin (150 µg/g body weight, i.p.), starting 24 h after virus delivery. On Day 2, after imaging in vivo, some mice were killed by cervical dislocation, organs quickly harvested, and immediately imaged ex vivo.

Inhalational Delivery. For nasal delivery, mice were anesthetized with ketamine/xylazine mixture (87 mg/kg and 13 mg/kg, respec- tively) to prevent contamination of the nose cones. To straighten the airway, mice were hung by their front teeth on twisted surgical tape. Ad-Gal4 or Ad-Cre [2 x 106 pfu/mouse in 10 µl PBS (pH 7.4)] was then added drop-wise onto the nose to ensure that the entire amount was breathed into the respiratory system.
before continuing. Imaging was performed before virus administration (Day 0) and 24 h after virus delivery (Day 1) and followed for 9 days. On Day 3, a few mice were killed by cervical dislocation, the chest cavity quickly opened, and mice imaged to detect signal originating from the lungs.

**Intramuscular Injections.** For intramuscular delivery, mice were anesthetized with 2.5% isoflurane and injected with Ad-Gal4 (2.3 × 10^5 pfu/mouse in 50 μl of PBS) into the right calf muscle. The left calf muscle was injected with vehicle alone or Ad-Cre (2.3 × 10^5 pfu/mouse in 50 μl PBS). Imaging was started 24 h after viral inoculation and followed for 4 days. On Day 3, a few mice were killed by cervical dislocation, calf muscle quickly harvested, and immediately imaged *ex vivo*.

**Intracranial Injections.** Mice were anesthetized with ketamine/xylazine mixture (87 mg/kg and 13 mg/kg, respectively) and fixed in a stereotactic frame (Stoelting). Ad-Gal4 [2 × 10^6 pfu/mouse in 10 μl PBS (pH 7.4)] was injected through a 27-gauge needle over 2 min at 2 mm lateral and posterior to the bregma and 3 mm below the dura. The incision was closed with Vetbond (3M). Imaging was performed prior (Day 0) and 24 h after virus injection (Day 1) and followed for 9 days.

**Hydrodynamic Injections.** *In vivo* transfection of mouse hepatocytes was performed using the hydrodynamic somatic gene transfer method as described (3, 5). Briefly, for the protein-protein interaction experiments, different plasmid combinations of 15 μg of pM-53 together with 15 μg of pVP16-T or pVP16-CP (all from the Mammalian Matchmaker Kit Two-Hybrid Assay Kit; Clontech) and 2 μg of pRLuc-N3 (BioSignal Packard) as injection control were combined as indicated. For the shRNAi experiments, different plasmid combinations of 5 μg of pM-53 together with 5 μg of pVP16-T combined with 50 μg pSuper (shRNAi-control) or 50 μg pRetrosuper-p53 (shRNAi-p53) (3, 6) and 1 μg of pRLuc-N3 as injection control were combined as indicated. Plasmids were diluted in PBS (pH 7.4) in a volume of 1 ml per 10 g body weight and rapidly injected into tail veins of mice (3). Imaging of mice for Fluc and Luc activity, respectively, was performed before and 24 h after somatic gene transfer by injection of coelenterazine (1 μg/g BW, i.v.), followed 4 h later (generally no residual signal) by injection of D-luciferin (150 μg/g BW, i.p.).

**Analysis and Statistics.** Corresponding grayscale photographs and color luciferase images were superimposed and analyzed with LivingImage 2.50 (Xenogen) and Igor (Wavemetrics) image analysis software. Signal intensities for luciferase were obtained from regions of interest defined either with a grid template for cells grown in tissue culture plates or manually for the viral induction sites as well as the hepatocyte transfections. Data were expressed as total photon flux (photons/sec) or as normalized total flux (Fluc photons/sec over Rluc photons/sec). Fold-induction was expressed as normalized posttreatment/pretreatment images. Values (n = indicated animals within each group) were reported as mean ± SEM or mean ± range.