SUPPLEMENTAL METHODS

In vitro cell ablation assay

To assess the efficacy of the transgene, cell ablation was monitored by MTT assay and LIVE/DEAD analyses (Invitrogen).

Cells were transfected with *Mos-iCsp3* (2 ng), with or without co-transfection with *pxCAN-Cre* (4 ng) using FuGENE6 (Roche) according to the manufacturer's protocol overnight. Twenty-four hr after transfection, 10 nM AP20187 or mock was applied for 48 hr, followed by measurement of cell-viability by MTT assay (CellTiter 96 Aqueous One solution proliferation assay, Promega). Cells with transfection reagent alone and cells ablated with 70% methanol for 20 min were negative and positive controls. Absorbance from the experimental groups (with or without transfection, with or without AP20187 treatment, six replicates per group) was subtracted from the absorbance of the control ablation group.

The LIVE/DEAD analysis system (Invitrogen) in which cell-viability is measured by calcein fluorescence while nuclei of dying cells are stained by ethidium-D1 (EthD1) was used to assess cell-viability and dying cells simultaneously. Cells were co-transfected with *pxCAN-Cre* (0.5 μg) and *Mos-iCsp3* (0.5 μg) or transfected with the same amount of the parental cell-ablation vector, pSHMFv2Csp3E or empty vector (positive and negative controls) or without vector but with transfection reagent. Twenty-four hr after transfection, AP20187 was added to the medium. At 48 hr the cells were stained with calcein and EthD1 by the manufacturer's protocol. Both the distribution of calcein fluorescence intensity and the number of nuclei positively stained with EthD1 were automatically measured by Metamorph software (Fig. 1).

Screening and expression profile of transgene among individual lines

Transgene expression in individual lines of mice was analyzed by LacZ staining and β-galactosidase assay.

For LacZ staining, six positive male founders were crossed with C57BL/6Jcl females and whole body embryos were harvested between E13.5 and E16.5 followed by LacZ staining. Three lines had robust and ubiquitous LacZ expression. Two positive female founders were crossed with wild type males. Male offspring were mated with wild type females and embryos were used for staining. One strong LacZ positive line was found. Consequently, four lines with strong transgene expression were obtained. These lines were maintained by mating with C57BL/6Jcl mice.

Individual organs were dissected out, weighed, collected in a 1.5 ml tube, homogenized and incubated in protein digestion buffer on ice for one hr for measurement of β-galactosidase activity. Samples were centrifuged after vortexing and β-galactosidase activity of the supernatant was measured according to the manufacturer's (Promega) protocol in a 96 well plate. Absorbance was measured at 750 nm (microplate reader, Model 680, BioRad). The data are shown in Table S1.

Activation of conditional caspase-3 genes by administration of dimerizer, AP20187

To activate inducible caspase-3 and initiate cell ablation a specially designed chemical dimerizer, AP20187, was administrated. For *Ins2-Cre* and *Krt14-CreER* ; *Mos-iCsp3* double transgenics, AP20187 was intraperitoneally injected (10 mg/kg body weight). For *Pou4f3-Cre;Mos-iCsp3* double transgenics, AP20187 was intracerebrospinally injected. Note that the expression of inducible suicide gene that might be expressed in the hypothalamus of *Ins2-Cre;Mos-iCsp3* double transgenics and in the kidney in *Pou4f3-Cre;Mos-iCsp3* double transgenics are not

expected to be activated because the dimerizer cannot penetrate the blood-brain barrier (data from Ariad Pharmaceuticals).

SUPPLEMENTAL FIGURES

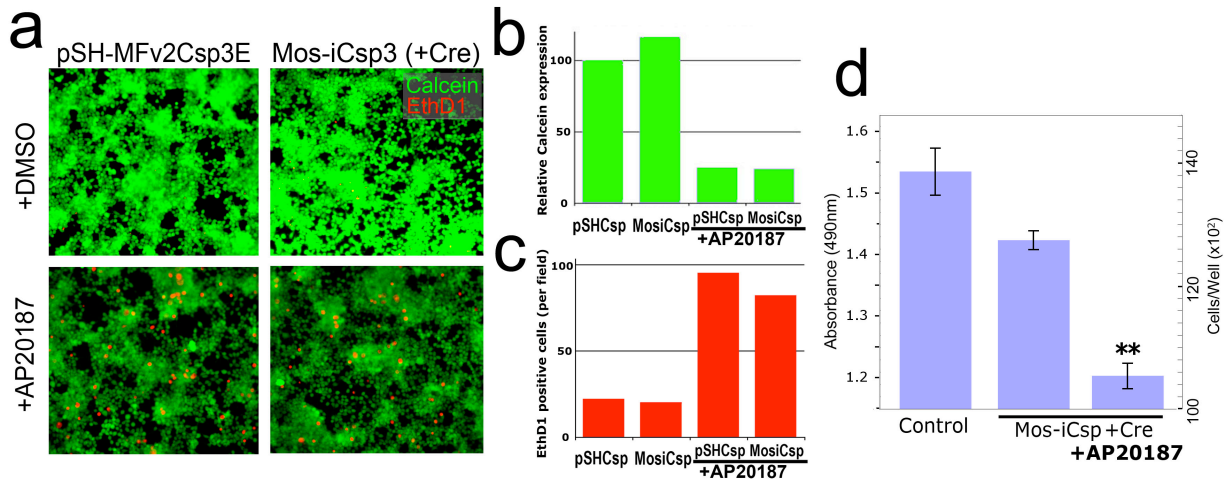

Figure S1: Inducible cell death after expression of *Mos-iCsp3* and *Cre* in vitro. (**a - c**) In vitro transient transfection assays were carried out to evaluate the function of the transgene. Calcein assay showed decreased cell viability when cells were incubated with the dimerizer, AP20187, after co-transfection with *Mos-iCsp3* and *pxCAN-Cre* and the cell death occurred to the same extent as in cells transfected with the parental vector, *pSHMFv2Csp3E*. In parallel, EthD1 positive dying cells were increased (N = 2 experiments). Nontransfected cells treated with AP20187 or DMSO showed no change in EthD1. **(d)** MTT assay after transfection with Mos-iCsp3 and pxCAN-Cre or Mos-iCsp3 alone (control). The decrease in cell number could be seen to be induced by AP20187 treatment.

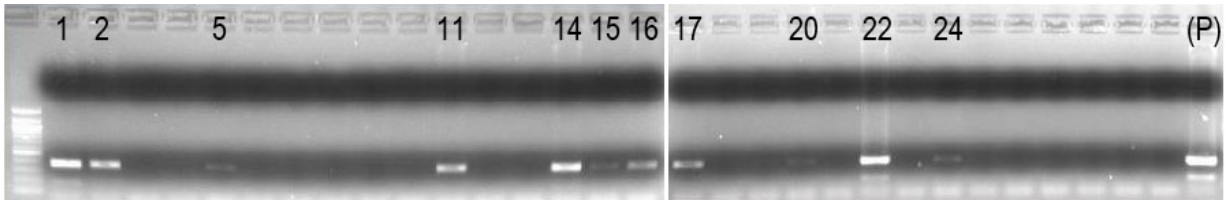

Figure S2: Identification of founder mice. Twenty-four pups were tested for transgene expression by PCR with genomic DNA isolated from tails as the target. Samples for which the transgene were detected are numbered 1, 2, 5, 11, 14, 15, 16, 17, 20, 22, 24; lines corresponding to samples 1, 2, 11, 14, 17, 22 were selected for breeding.

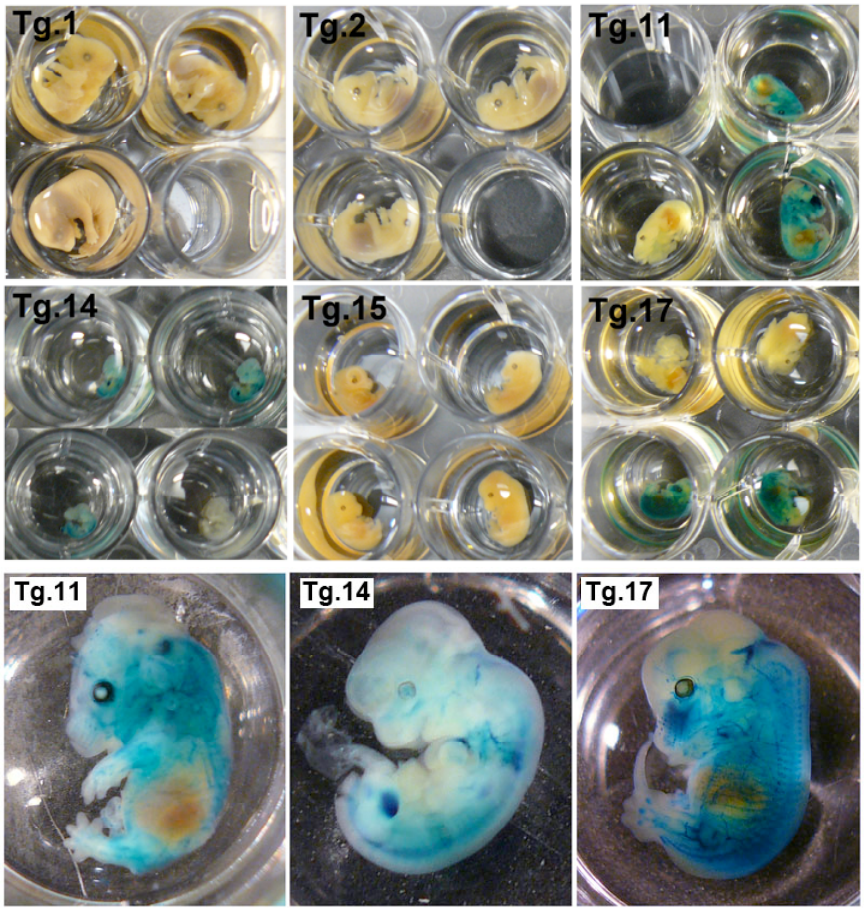

Figure S3: Testing of F1 generation of transgenic lines for LacZ expression. Whole body staining was performed on embryos at E13 to E16. Higher power view is shown below.

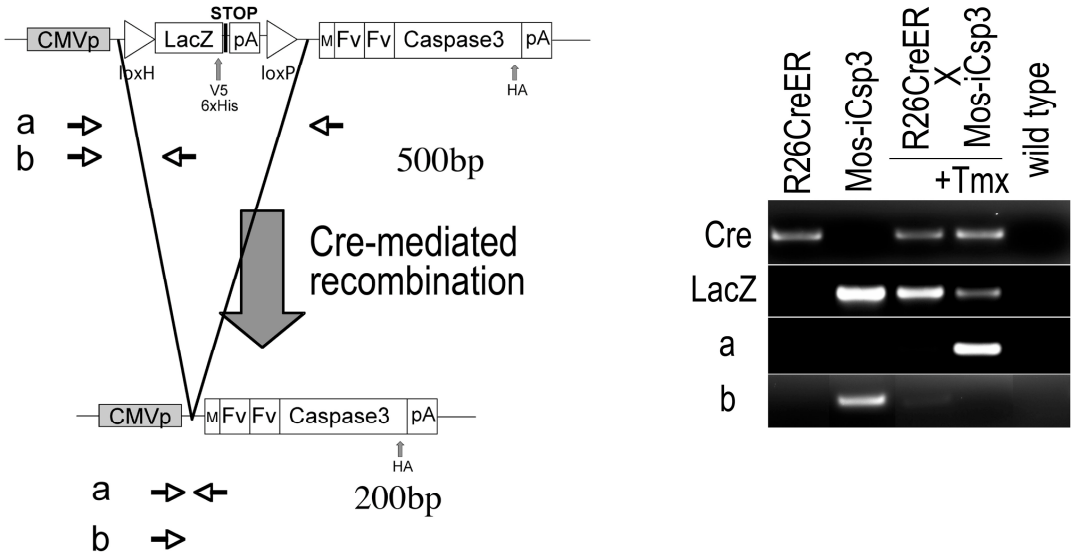

Figure S4: Cre-mediated in vivo recombination. Offspring of *Mos-iCsp3* crossed with *ROSA26-CreER* were intraperitoneally injected with tamoxifen and their tails were genotyped using primers external and internal to LacZ (a and b, respectively). The result showed partial recombination of the transgene upon tamoxifen administration, indicating stochastic recombination between the mismatched lox sites.

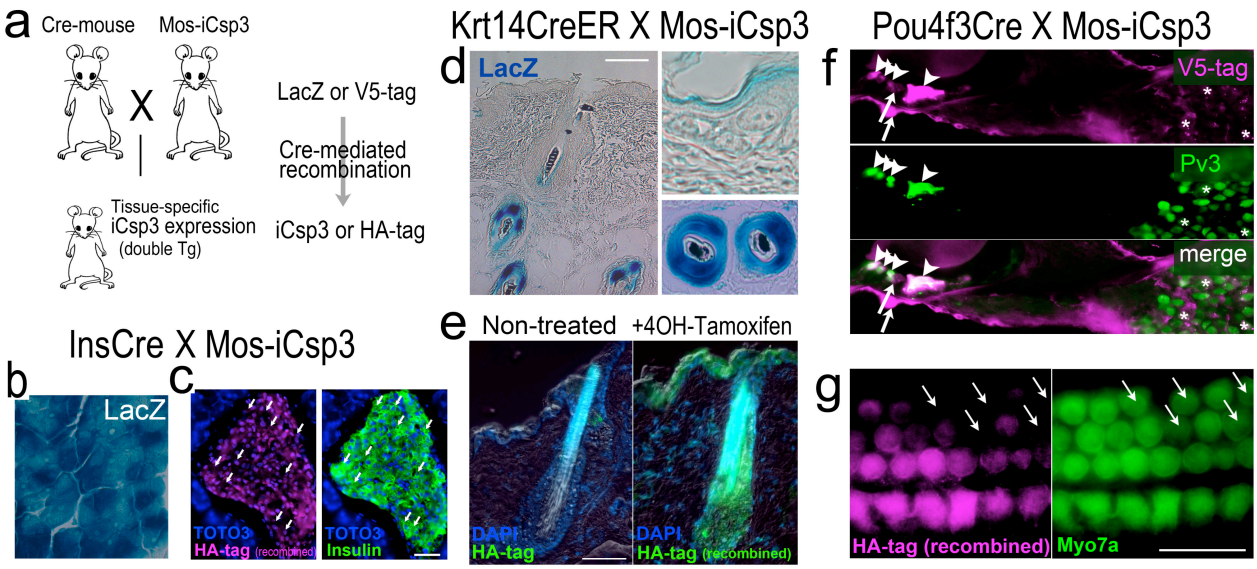

Figure S5: Cre-mediated in vivo recombination in double transgenics. **(a)** Strategy to create tissue-specific mosaic patterned suicide gene expression. (**b – g**) The recombined iCsp3 can be visualized by anti-HA tag immunostaining (**c, e, g**), while the transgene prior to recombination can be visualized either by β-Gal staining or anti-V5 tag immunostaining in the *Mos-iCsp3* transgenic mouse (**b, d, f**). The *Mos-iCsp3* mouse (line 14 in **b**) had strong LacZ expression in pancreas including islets of Langerhans. In the double transgenics of *InsCre;Mos-iCsp3* **(c)**, anti-HA tag staining showed that 72.3 ± 1.5% of insulin-positive β-cells expressed iCsp3 (selected insulin-positive β-cells that did not express HA-tag are marked by the arrows). The Mos-iCsp3 transgenic mouse (line 14 in **d**) showed strong expression in the hair follicles and fairly strong expression in the stratum spinosum and stratum basale of the epidermis. In the double transgenics of *Krt14-CreER;Mos-iCsp3* (**e**), the conditional cell death gene was expressed specifically in the epidermis where 4-OH tamoxifen was topically applied. Anti-HA tag staining showed that 45.3 ± 9.5% of the epidermal cells expressed iCsp3. The *Mos-iCsp3* transgenic mouse (line 17 in **f**) had staining in inner and outer hair cells (arrowheads) but did not express LacZ in the supporting cells and neurons (asterisks). In the double transgenics of *Pou4f3-Cre;Mos-iCsp3* (**g**), anti-HA tag staining showed that 63.5 ± 6.6% of the myosin VIIa-positive hair cells expressed iCsp3, but the supporting cells did not. Scale bars: **(c, g)** 20 μm, **(d, e)** 50 μm.

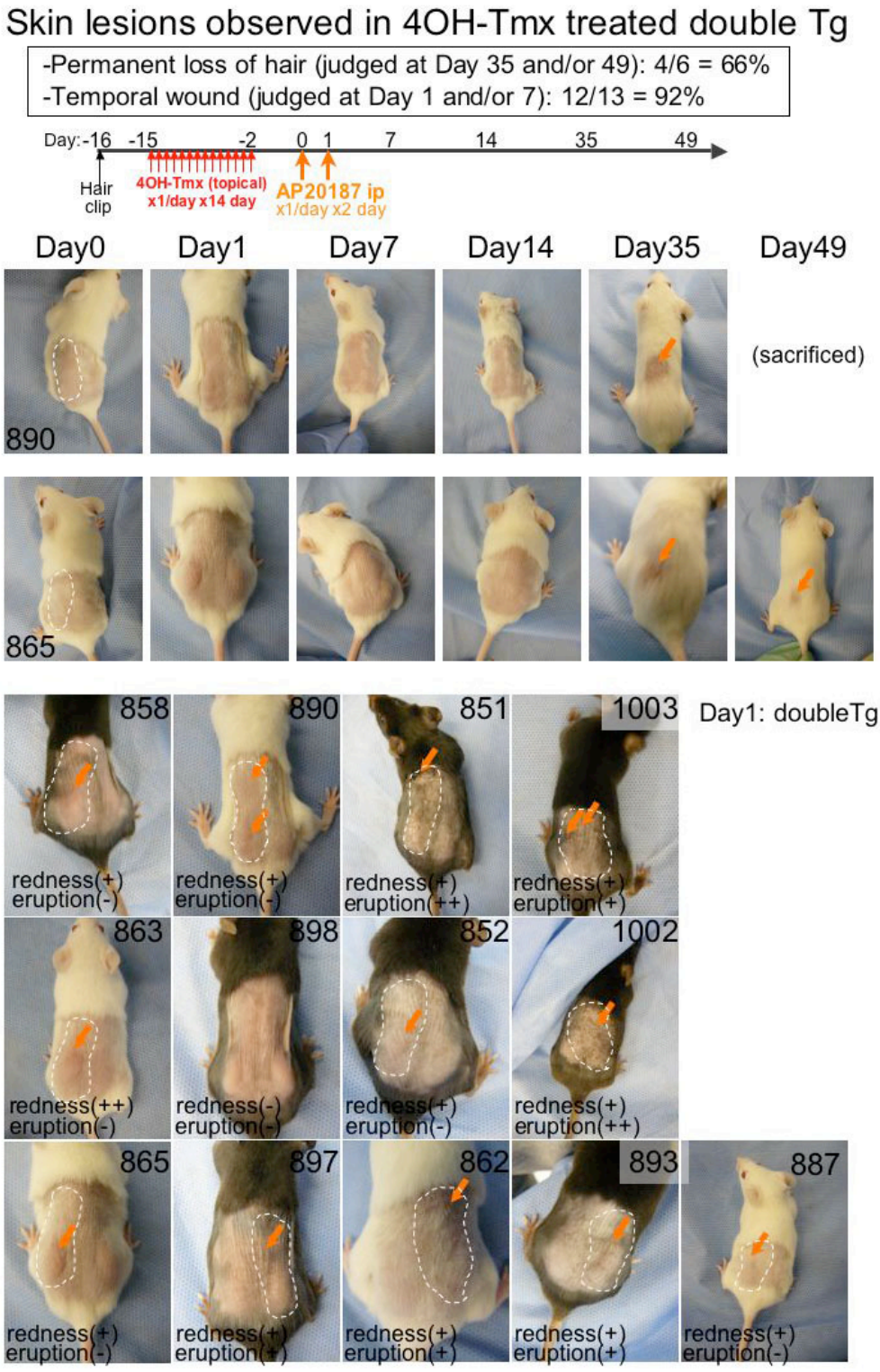

Figure S6: Skin lesions observed in 4OH-tamoxifen treated areas in *Krt14-CreER;Mos-iCsp3* double Tg.

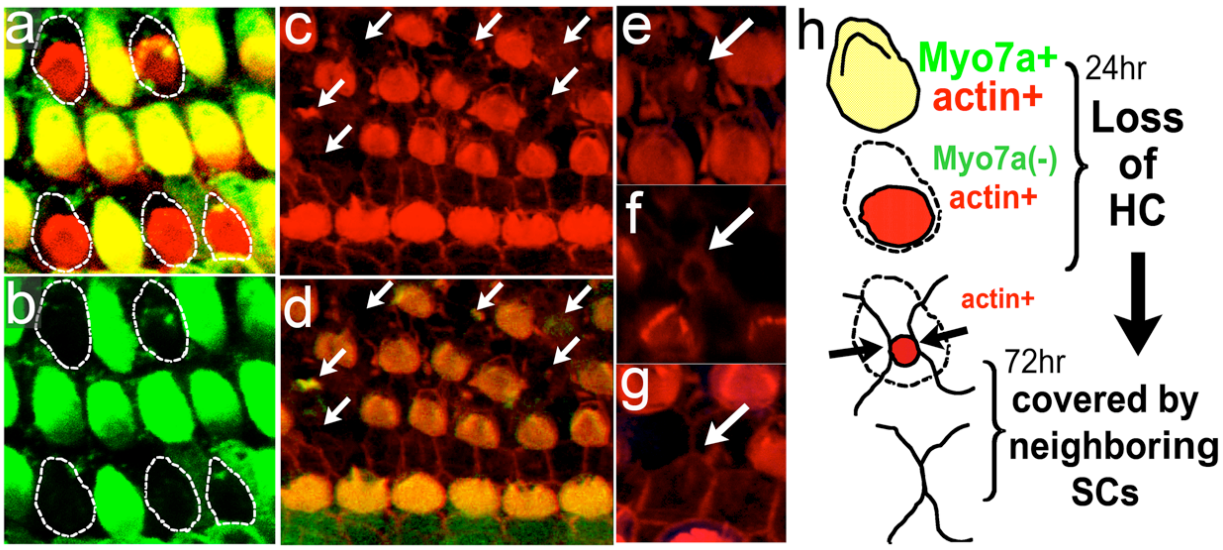

Figure S7: Cre-mediated in vivo recombination in double transgenics. **(a - g)** Tissue responses to hair cell ablation in organ of Corti. Morphological changes during and after ablation were examined by myosin VIIa **(a, b, d)** and F-actin (**a – g**) staining shown in green to label hair cells and red to visualize structures, respectively. Loss of myosin VIIa staining and reduction of the actin-positive area at 24 hr revealed the dying hair cells **(a and b)**. Following cell ablation, the area was covered by surrounding supporting cells revealed by F-actin staining **(c, e - h)**.

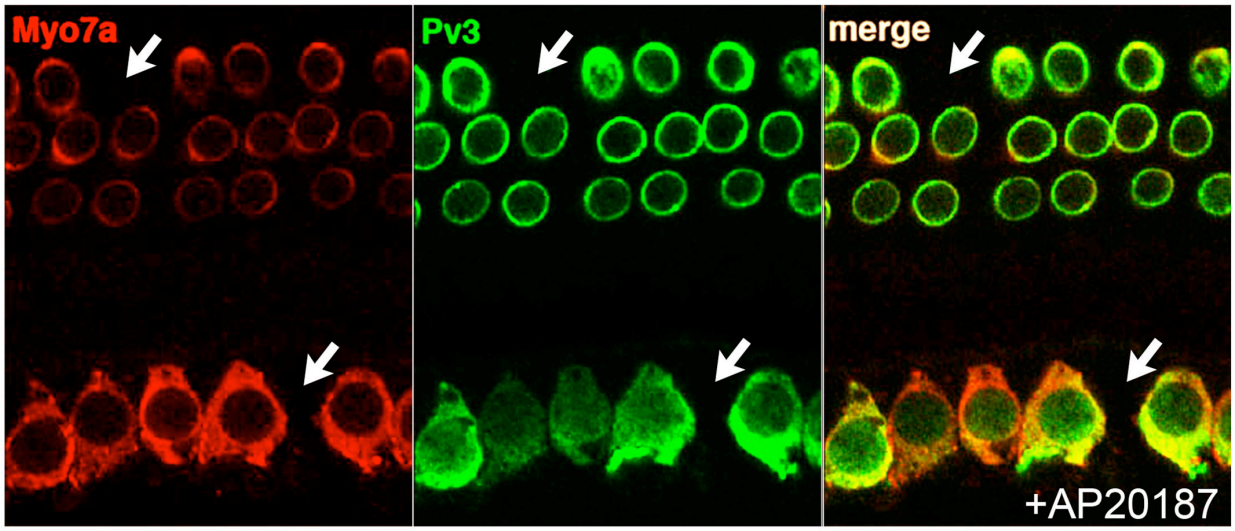

Figure S8: Histological changes in the cochlea after AP20187 treatment in vivo. Mosaic patterned partial hair cell loss was observed in double transgenics (N = 6) treated with AP20187 confirmed by Pv3 and myosin VIIa.

SUPPLEMENTAL TABLES

Table S1

Transgene expression of each organ measured by ß-galactosidase assay

	Tg.11	Tg.14	Tg.17	wt
forebrain	0.335	0.355	0.282	0.151
brainstem	0.598	0.356	0.427	0.195
cerebrum	0.176	0.356	0.921	0.124
spinal cord	0.288	0.553	0.287	0.211
olfactory bulb	0.805	0.128	0.558	0.133
eye	0.19	0.308	0.64	0.208
temporal bone	0.227	0.703	0.331	0.119
salivary grand	0.194	0.191	0.286	0.182
tongue	0.512	0.853	0.843	0.165
lung	0.232	0.399	0.255	0.368
heart (atrium)	0.193	0.165	0.243	0.334
heart (ventricle)	0.324	0.277	0.652	0.193
diaphragm	0.256	0.791	0.344	0.253
esophagus	0.144	0.234	0.755	0.511
stomach	0.355	0.14	0.547	0.113
ileum	0.182	0.204	0.302	0.122
caecum	0.154	0.166	0.156	0.18
colon	0.24	0.228	0.28	0.135
liver	0.196	0.351	0.419	0.671
spleen	0.844	0.948	0.937	1.014
pancreas	0.753	0.631	0.686	0.165
adrenal gland	0.513	0.374	0.686	0.834
kidney	0.353	0.24	0.96	0.314
ovary	0.212	0.185	0.384	0.176
bladder	0.153	0.175	0.189	0.491
muscle	0.259	0.809	0.826	0.303
skin	0.817	0.773	1.19	0.369
tail	0.935	0.58	0.867	0.167
thymus	0.235	0.179	0.941	
gallbladder	0.131	0.11		

Transgene expression of each organ in *Mos-iCsp3* transgenic mouse measured by β-galactosidase assay. A_{750} is reported (N = 2 per line).

Table S2A

Quantification of transgenes in target cells of double transgenic mouse

Cre-mouse for cross	*Ins-Cre*	*Pou4f3-Cre*	*Krt14-CreER*			
Cell type	β-cell	hair cell	total	epidermis	follicle	bulge
V5 or LacZ-positive/target cell (%)	100.0 ± 0.0	83.6 ± 1.7	92.9 ± 0.4	91.3 ± 1.3	93.7 ± 0.9	100 ± 0.0
HA-tag positive/target cell (%)	72.3 ± 1.4	63.5 ± 6.6	62.9 ± 5.0	74.4 ± 5.2	37.2 ± 4.0	80.0 ± 5.8
Dead cell/target cell (%)	57.4 ± 3.9	45.1 ± 1.6	41.5 ± 0.1	50.1 ± 0.1	28.2 ± 0.1	46.1 ± 0.2

Percentages are given of cells positive for unrecombined transgene (*Mos-iCsp3* single transgenic), cells positive for recombined transgene (double transgenic mouse crossed with respective Cre-mouse), and dead cells after AP20187 treatment. Percentage of cell death in *Ins-Cre;Mos-iCsp3*, *Pou4f3-Cre;Mos-iCsp3* and *Krt14-CreER;Mos-iCsp3* was measured by the decrease of insulin-immunopositive area, number of lost cells in the organ of Corti and TUNEL positive cells in the section (N = 3, 3, 5, respectively).

Table S2B

Quantification of recombination and cell ablation efficiency in target cells of double transgenic mouse

Cre-mouse for cross	*Ins-Cre*	*Pou4f3-Cre*	*Krt14-CreER*			
Cell type	β-cell	hair cell	total	epidermis	follicle	bulge
HA/V5-positive cells (recombination efficiency, %)	72.3 ± 1.4	76.0 ± 7.9	67.7 ± 5.4	81.5 ± 5.7	39.7 ± 4.3	80.0 ± 5.8
Dead/V5-positive cells (cell death ratio, %)	57.4 ± 3.9	53.9 ± 1.9	44.7 ± 0.1	54.9 ± 0.1	30.1 ± 0.1	46.1 ± 0.2
Dead/HA-positive cells (ablation ratio, %)	79.4 ± 5.4	71.0 ± 2.5	66.0 ± 0.2	67.3 ± 0.1	75.8 ± 0.3	57.6 ± 0.3

Based on the results in Table S2A, recombination efficiency (HA /V5-positive), cell death ratio (dead cell/V5-positive) and the ablation ratio (dead cell/HA-positive) by dimerizer treatment were calculated.