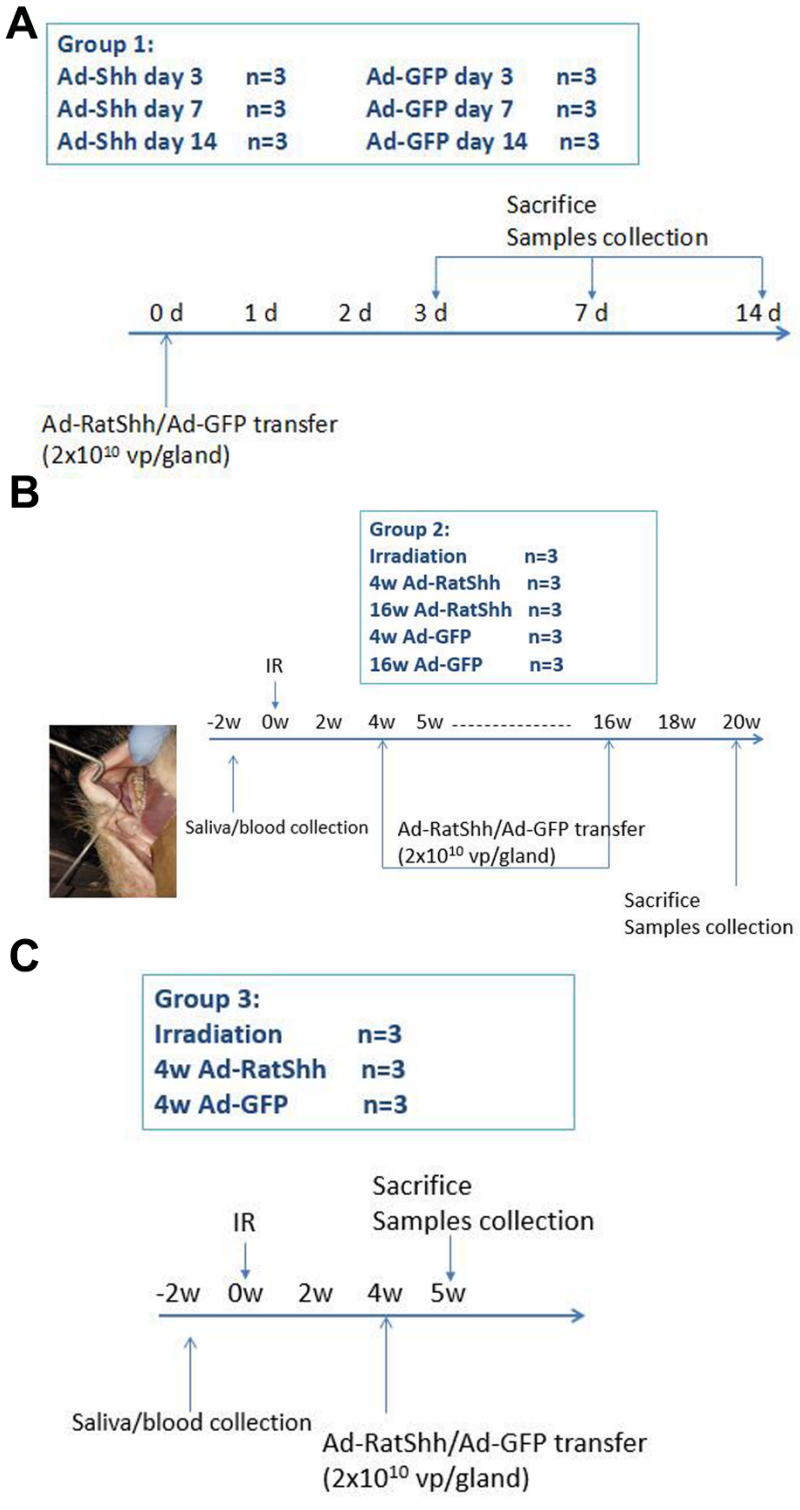
Supplementary table 1: The clinical laboratory analyses of serum.
There were no significant effects of IR or gene transfer on serum chemistry values among different groups except K^+. N=3. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

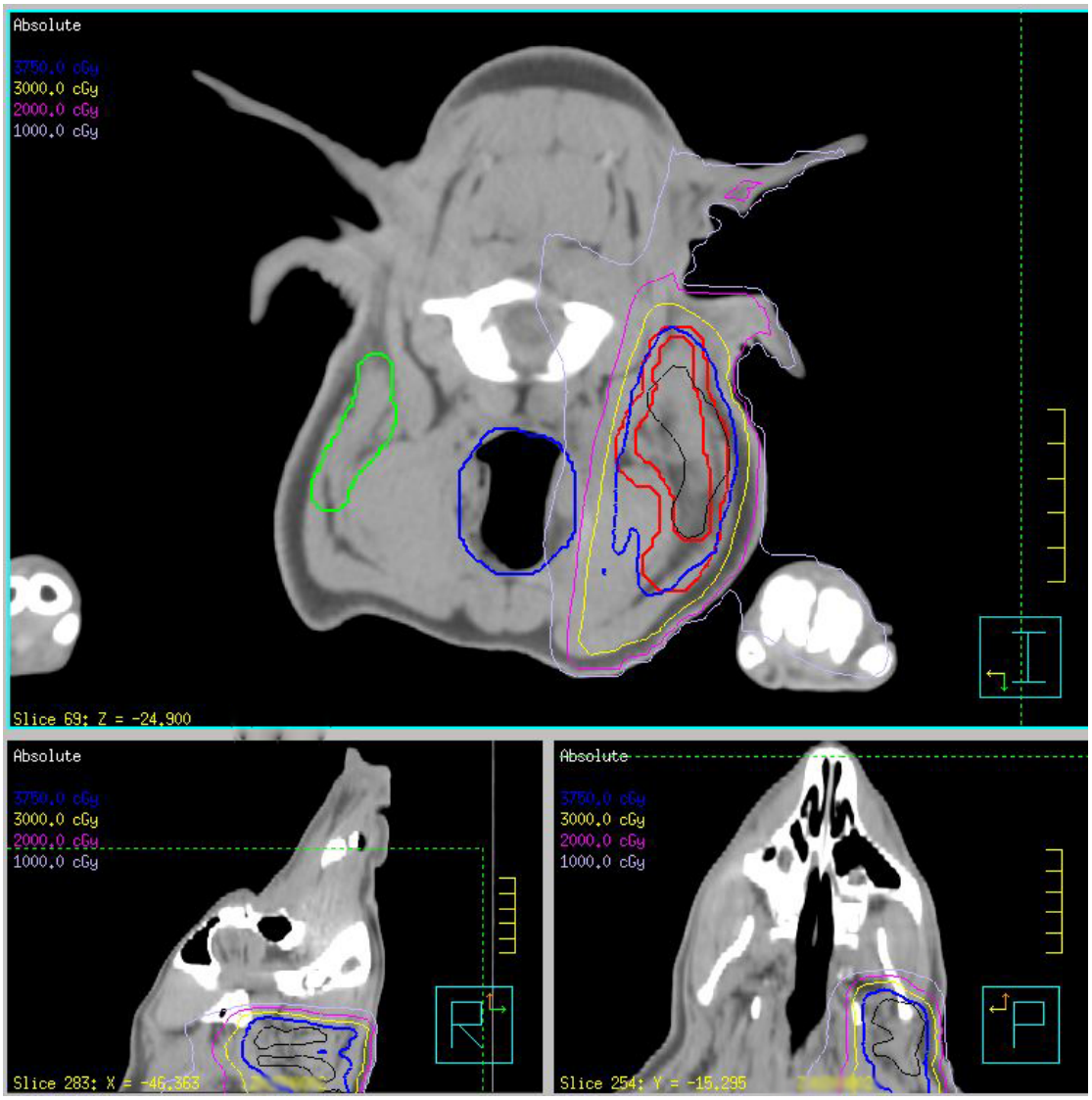
	Pre-IR	Post-IR 1w	Post-IR 5w			Post-IR 20w				
			4w Shh	4w GFP	IR	4w Shh	4w GFP	16w Shh	16w GFP	IR
ALT	37.6	35.78	32.67	31.67	29.33	31	32.33	29.33	30	27.33
(U/L)	±6.11	±4.55	±1.53	±6.51	±3.21	±4.58	±5.86	±2.52	±2.65	±9.02
AST	33.8	28.89	30.67	32.33	29	28.33	33.33	30.67	35.67	31.67
(U/L)	±9.28	±3.22	±8.96	±3.79	±6.24	±6.66	±3.79	±4.04	±6.81	±11.59
ALP	126.4	124.89	131.33	102	102.67	99.67	103	104	104.33	105
(U/L)	±22.46	±19.74	±18.34	±14.11	±16.95	±14.64	±12.29	±5.57	±15.95	±12.49
LDH	355.02	373.82	361.33	336.67	343.47	346.87	346.17	358.2	344.73	382.4
(U/L)	±82.7	±49.69	±31.36	±43.16	±59.47	±54.11	±57.51	±79.02	±51.72	±75.39
Ca^{2+}	2.5	2.49	2.46	2.38	2.29	2.48	2.3	2.30	2.44	2.29
(mmol/L)	±0.08	±0.07	±0.02	±0.31	±0.19	±0.14	±0.05	±0.21	±0.11	±0.06
K^+	3.8	4	4.04	3.87	3.58	4.05	2.89*	3.01*	2.83*	3.14*
(mmol/L)	±1.61	±0.36	±0.33	±0.92	±0.64	±0.44	±0.15	±0.21	±0.19	±0.24
Na^+	138.18	138.79	135.47	146.17	130.1	138.17	137.33	135.53	142.3	134.97
(mmol/L)	±1.61	±1.76	±5.95	±16.5	±9.27	±2.21	±1.85	±4.7	±3.44	±7.46
Cl^-	99.34	100.72	98.47	103.87	92.73	100.77	99.67	99.93	103.43	99.33
(mmol/L)	±2.08	±1.61	±6.20	±9.5	±7.31	±2.84	±1.31	±5.47	±2.64	±7.2

Supplementary figure 1: The experiment scheme



(A) To detect whether Ad-Shh could effectively activate Hh pathway, randomly grouped animals received transfer of Ad-Shh or Ad-GFP into one parotid, and then euthanized at 3 different time points (n=3). (B) To examine long-term effects of Ad-Shh, animals were irradiated at one parotid region, and then randomly divided into five groups (n=3) with no

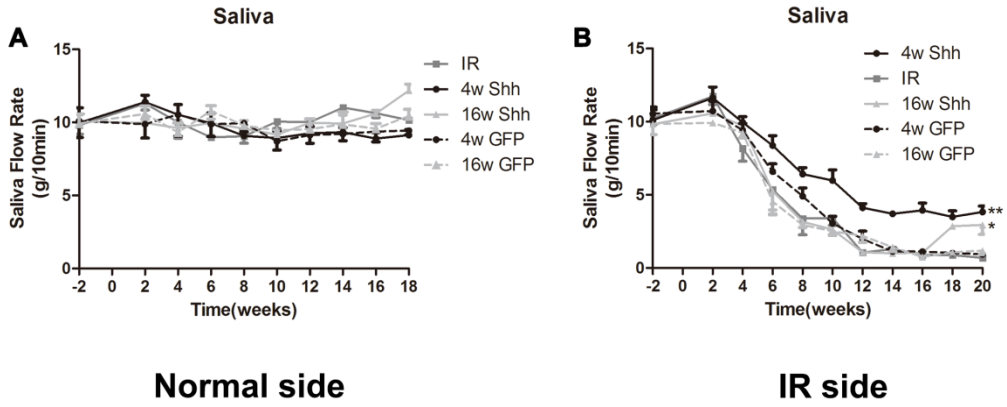
further treatment or transfer of Ad-Shh or Ad-GFP into irradiated parotids 4 or 16 weeks after the irradiation. Saliva and blood samples were collected every two weeks after IR. All animals were sacrificed at week 20. (C) To study the mechanisms, animals were irradiated at one parotid region, randomly divided into 3 groups (n=3) with no further treatment or transfer of Ad-Shh or Ad-GFP into irradiated parotids at Week 4, and were sacrificed at Week 5.



Supplementary figure 2: The irradiation of miniature pigs.

Three-dimensional treatment planning system (Pinnacle3, version 7.6; ADAC Inc., Concord, CA, USA) was used to determine the target area before irradiation. More than 95% of the irradiation dose covered the whole target volume of the parotid gland. The animals were anesthetized and placed at the prone position. With the image guide radiation therapy technology, the animals were irradiated with 6 mV of photon energy at 3.2Gy/min by Elekta Synergy accelerator (Elekta AB, Stockholm, Sweden) and received a single dose of 20 Gy.

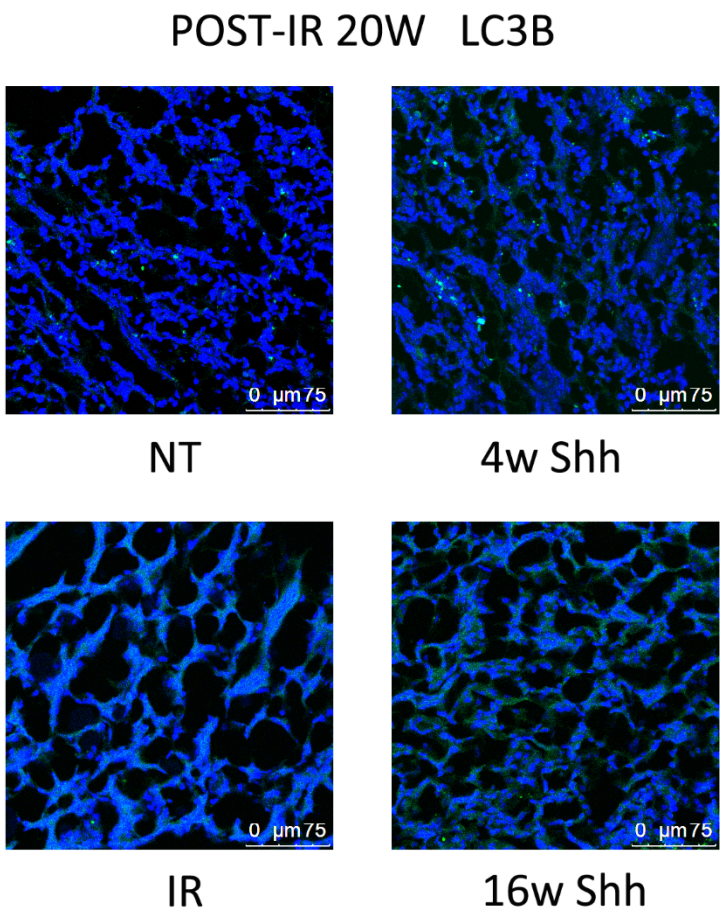
Supplementary figure 3: The saliva flow rate of non-treated and irradiated parotids.



Normal side **IR side**

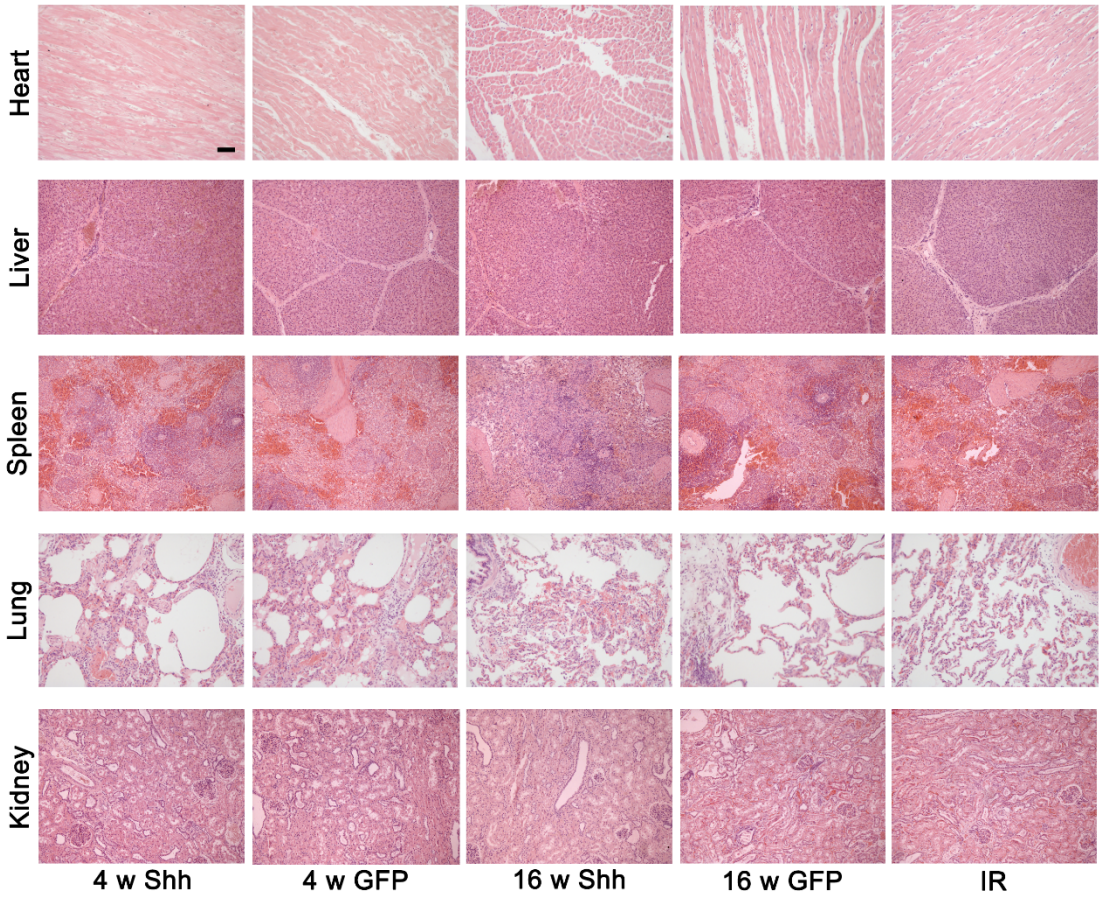
The left parotid of all animals was non-treated (NT) as the internal control, and the saliva flow rates of NT parotids did not change significantly at all time points examined after all treatments at the right side except Ad-Shh delivery at Week 16. In the experimental parotids at the right side, the saliva rate tended to rise after receiving irradiation firstly, then began to drop at Week 2. Although the saliva flow rate still decreasing, the descending range was much smaller after transferring rat-Shh at Week 4. The saliva flow was increased after transferring rat-Shh at Week 16 at both normal side and IR side.

Supplementary figure 4: The LC3B expression at Week 20 post irradiation.



The LC3B expression was higher in 4w Shh group than IR and 16w Shh group. The results were similar to those at Week 5 post irradiation.

Supplementary figure 5: The histological structures of vital organs



We checked the histological structures of multiple vital organs (heart, liver, spleen, lungs, kidneys) at week 20 after irradiation. No differences were found among five groups.

Supplementary materials and methods:

Semi-quantitative analysis of IHC and IF:

To calculate the expression of Shh^+ area, acinar cell area, $Masson^+$ area, $ACHE^+$, $Lc3b^+$ and $Chrm1^+$ area, we used Image-Pro Plus version 6.0 software to analyze the sum Integral optical density (IOD) in three glands from three animals in each group and 10 fields in each gland.

CD31 was used as the marker for microvascular endothelial cells. We calculated the number of CD31 positive cells per field at ×200 magnification using Image-Pro Plus version 6.0 software to determine the MVD. For MVD analysis, three glands from three miniature pigs in each treatment group were studied, and 10 fields were counted per gland.

Quantitative RT-PCR

Primers for *Gapdh, hhip, ptched 1, CD31, Gli1, Aqp5, BDNF, NRTN, C-KIT, P21, AMPK, ULK1, Chrm1, Chrm3* and *VEGFA* were listed as below:

GAPDH	F	CCCCTTCATTGACCTCCACT	R	TGGAAGATGGTGATGGCCTT
AQP5	F	CGCTCAACAACAACACGACT	R	GTGACAGACAGGCCAATGGA
CD31	F	GTGTACAGTGAAATCCGGAAAGC	R	TTCTCAGAATGCGGTGTCTCC
Gli1	F	GGAGAAGCCTGAACCTGACT	R	CTCCATGGATGTGTTCGCTG
ptch1	F	ATCAGCTGGAACGAGGACAA	R	GAGCACCTTCAGAGTGGAGT
hhip	F	TGGCGGTGTCTGTGTTAGAC	R	TGGCGGTGTCTGTGTTAGAC
CHRM1	F	GTGCCTAGAGGAAGGGGCT	R	GGTGATCCCAATGAAGGCCA
CHRM3	F	CTTCCAGGTCACAGCACCAT	R	CTTCTGGGCTTGCAGTTTGC
VEGFA	F	TCACCAAGGCCAGCACATAG	R	CAAATGCTTTCTCCGCTCCG
P21	F	AACTTTGAGGTCCCCTTGCC	R	CAGGGCCCTACTTTCACTGG
C-KIT	F	TGCGGATCCCCTCAAAAGAC	R	TCCGCACAGAATGATCCACC
BDNF	F	CGAAGTCTTCCCCAGAGCAG	R	CAGCCTTCATGCAACCGAAG
NRTN	F	GCGTGTGACCCTACCTCACT	R	TTATCACGAGAGTCCTGAGGC
ULK1	F	GGACCCCATCTCCTTCCCC	R	TTCTGCTCAATGCGCTGGTA
AMPK	F	GGCAAAGTGAAGGTTGGCA	R	TGCCTGAAAAGCTTGAGGTTC