Supplementary figures

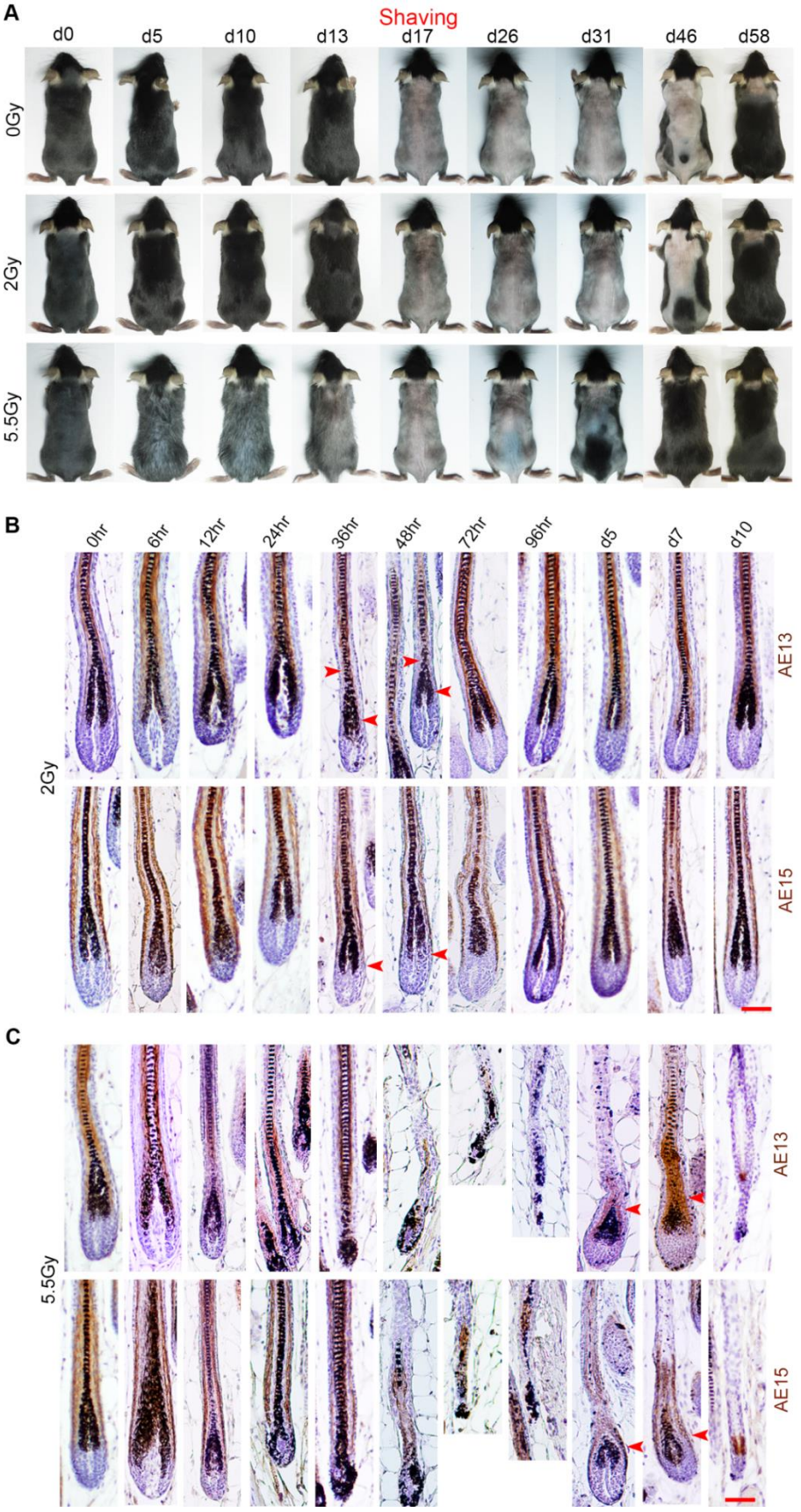


Figure S1. Hair cycles and disruption and restoration of differentiation in anagen HFs after IR.
IRS and hair cortex of anagen HFs were recognized by specific antibodies, AE15 and AE13,

respectively. AE15 stains for trichohyalin and AE13 stains for hair cortex cytokeratin. (A) Long-term observation of hair loss and hair cycle changes after IR. Severe hair loss was induced by 5.5Gy of IR at post-IR d5. After 2Gy and 5.5Gy of IR, HFs in both groups could resume a new anagen in the subsequent hair cycle. Compared with 2Gy, entry into the immediate subsequent anagen was faster at 5.5Gy. Hair was shaved on d17 following IR for visualization of entry into the next anagen. (B) 2Gy-induced changes. Hair cortex was partially disrupted between 36 and 48hrs (red arrowheads, upper panel). IRS was partially disrupted between 36 and 48hrs (red arrowheads, lower panel). (C) 5.5Gy-induced changes. Both hair cortex and IRS were more severely disrupted. At 72hrs, staining for AE13 and AE15 became almost absent. Differentiation toward hair cortex and IRS appeared again between days 5 and 7 following IR (red arrowheads). Scale bar=75μm.

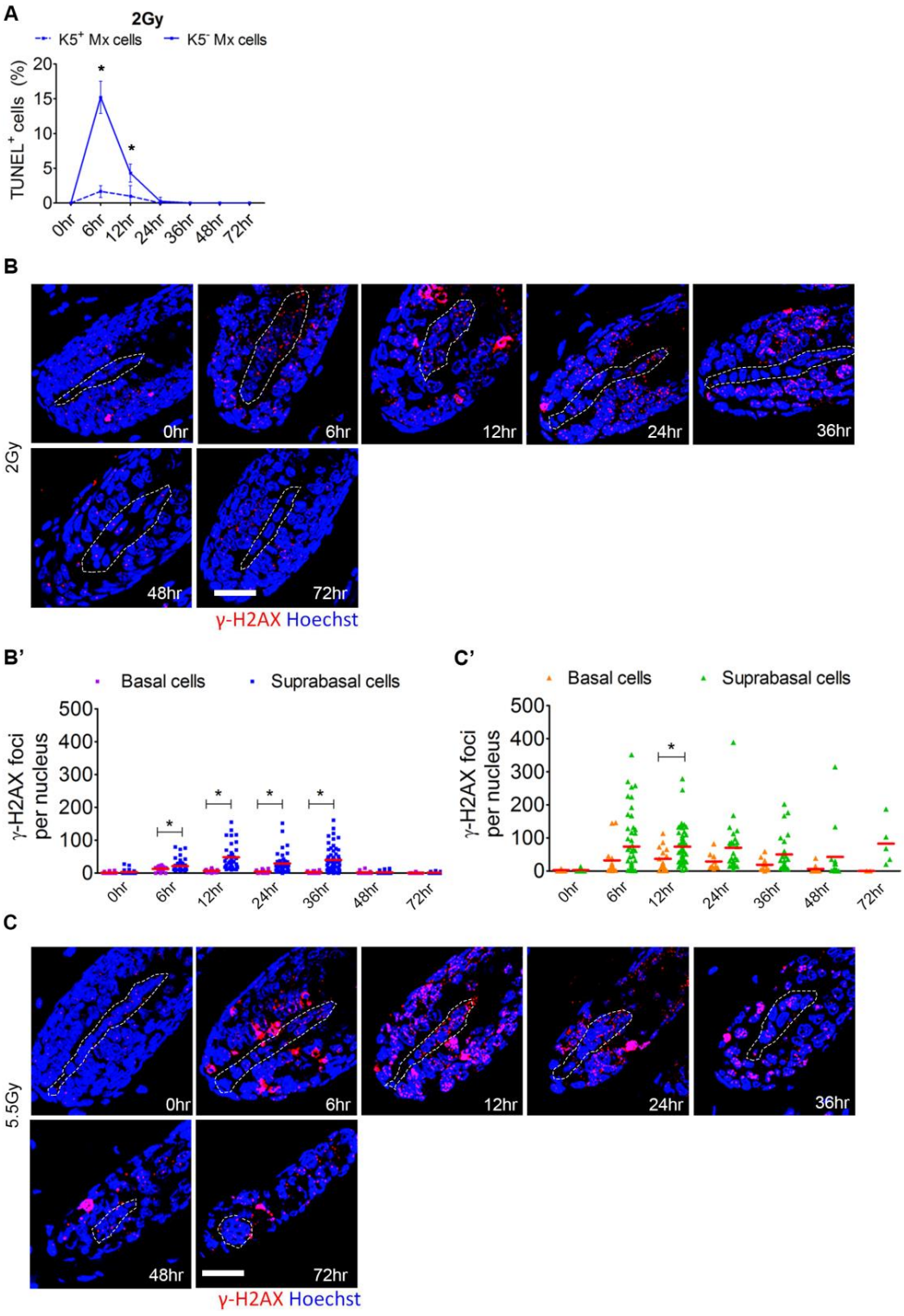
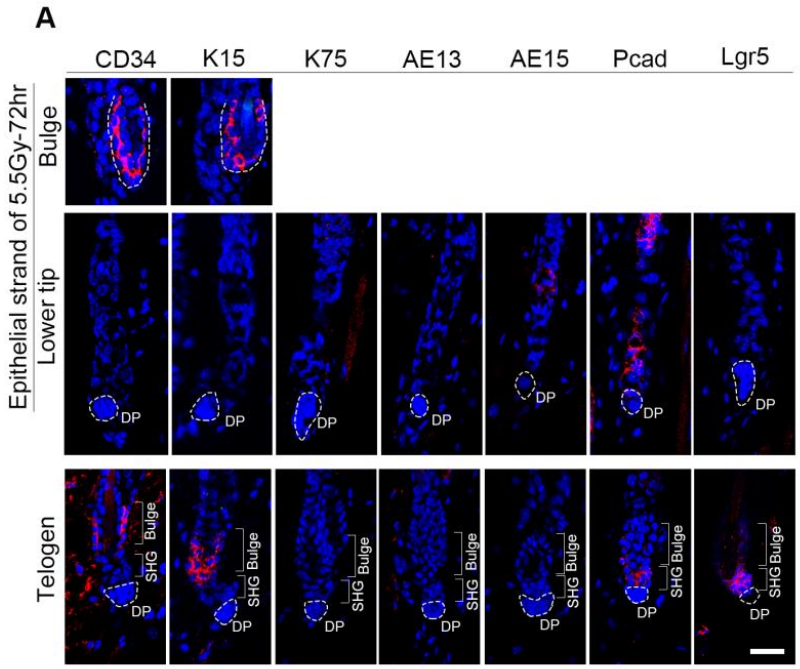


Figure S2. Comparison of apoptosis and DNA damage response of K5^{+} and K5^{negative} cells in the hair matrix. (A) Quantification of apoptosis detected by TUNEL staining after 2Gy of IR from Figure 2A. More apoptosis was observed in K5^{negative} matrix cells. (B) (B') Comparison of γ-H2AX foci in basal and suprabasal matrix cells after 2Gy of IR. (C) (C') Comparison of γ-H2AX foci in basal and suprabasal matrix cells after 5.5Gy of IR. Dashed line: DP. * p<0.05. Scale bar=25μm.



Summary of immunostaining results.

	SHG	lower tip
CD34	-	-
K15	-	-
K75	-	-
AE13	-	-
AE15	-	-
Pcad	+	+
Sox9	+	+
Lgr5	+	-

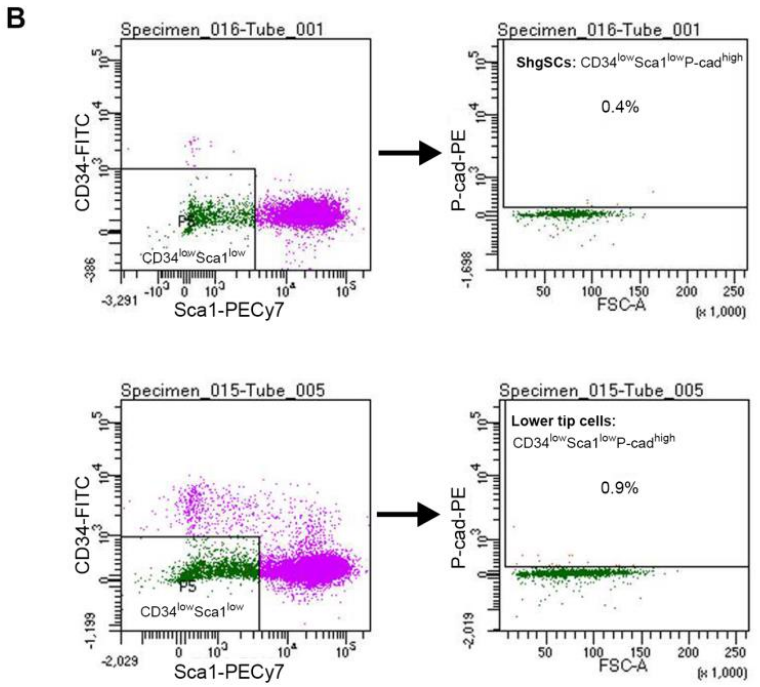


Figure S3. Immunofluorescent comparison of lower tip cells of the epithelial strand at 72hrs after 5.5Gy of IR with BgSCs and ShgSCs and cell sorting. (A) Immunofluorescent images and summary of the results. Telogen HFs from seven-week-old female mice were used for comparison. (B) Strategy for FACS sorting of the lower tip cells and ShgSCs. Cells were sorted with the surface markers CD34, Sca1 and p-cadherin (P-cad). ShgSCs and lower tip cells were sorted as $CD34^{low}$ $Sca1^{low}$ $P\text{-}cad^{high}$ cells. Scale bar=25μm.

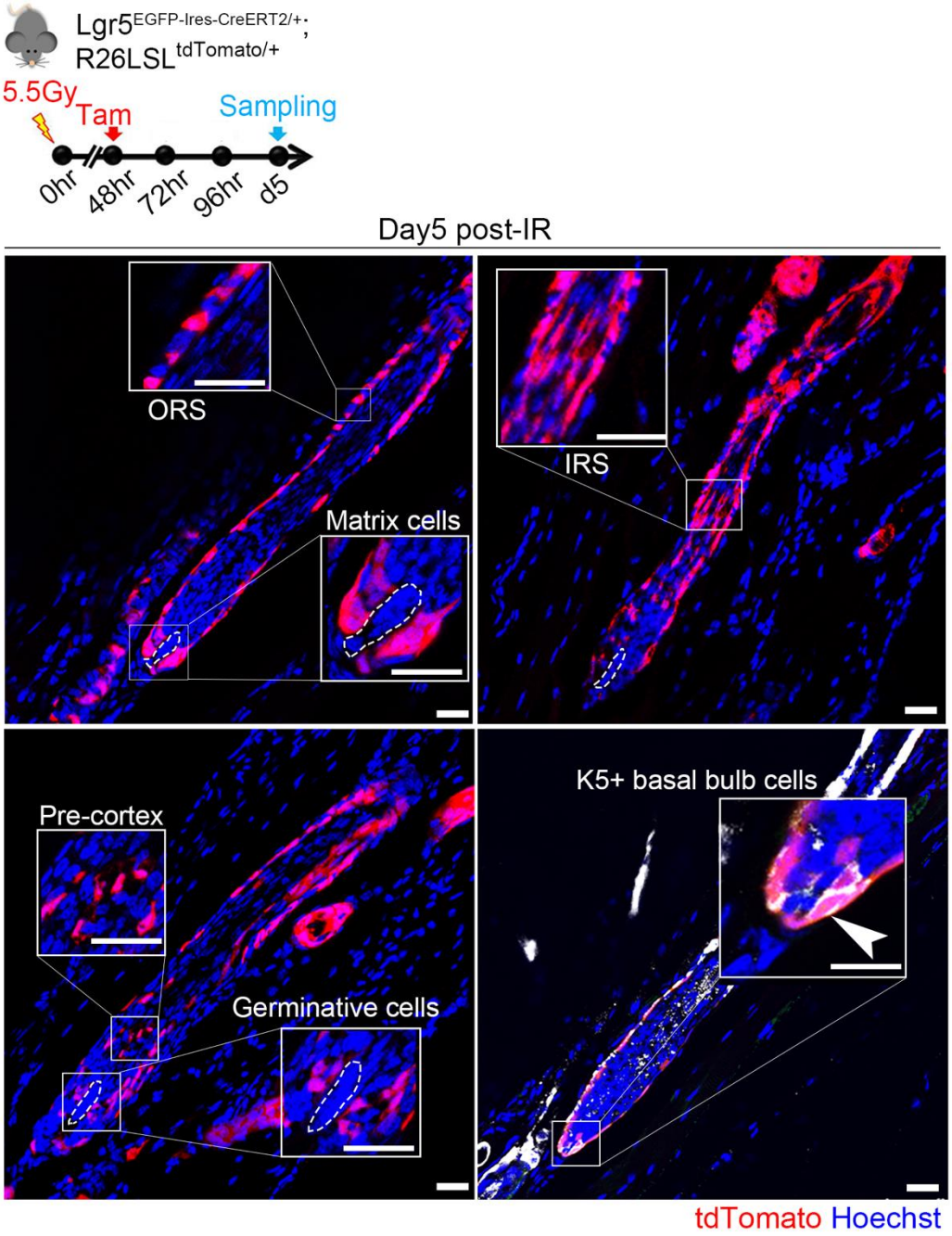


Figure S4. Lineage tracing of Lgr5^{+} cells after 5.5Gy of IR. On day 5 after IR, progeny of Lgr5^{+} cells contributed to all concentric layers of regenerated anagen HFs including ORS, matrix, IRS, pre-cortex, germinative cells and basal hair bulb cells (white arrowhead). K5 staining was shown as white color in the right lower panel. Dashed line: DP. Scale bar=75μm.

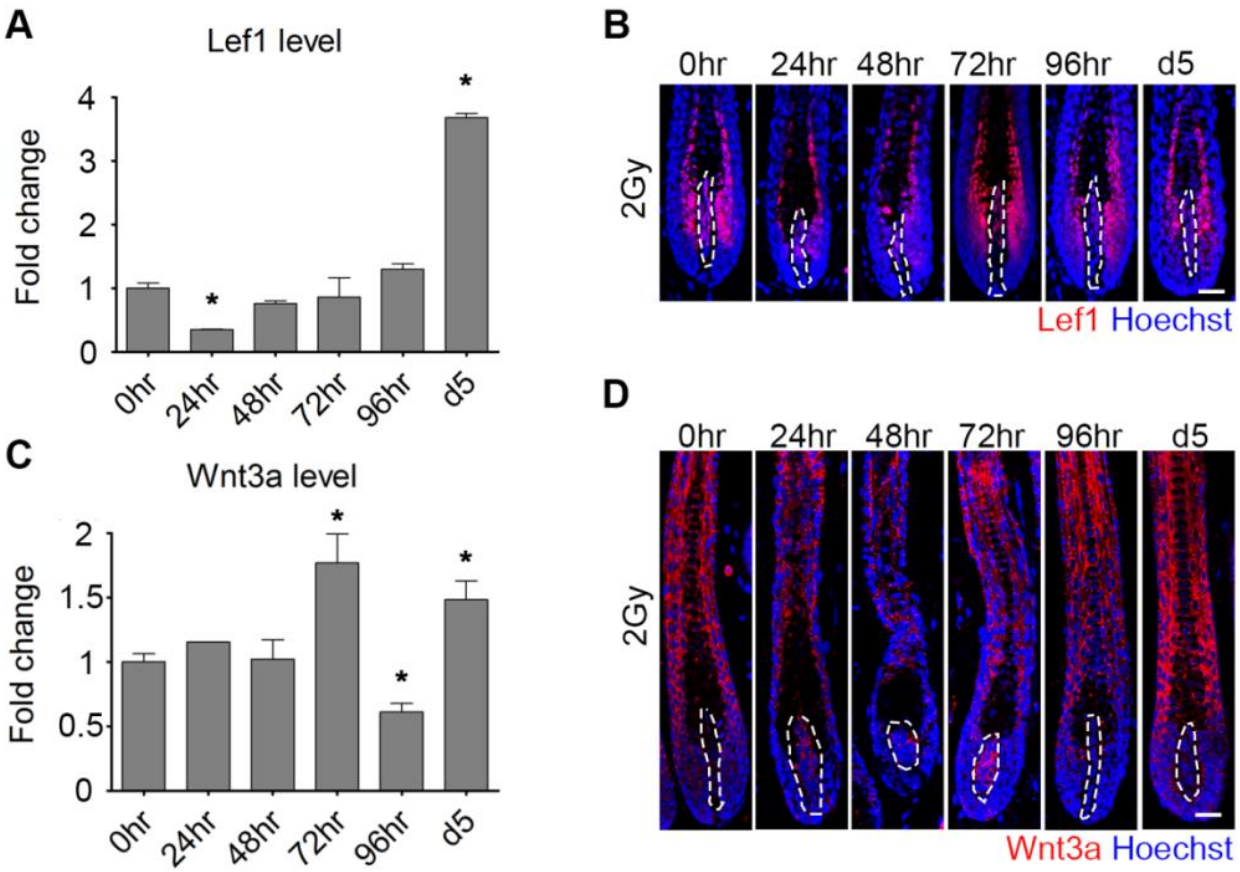


Figure S5. WNT signaling after 2Gy of IR. (A) (B) Quantitative RT-PCR and immunofluorescence for Lef1 expression after 2Gy of IR. (C) (D) Quantitative RT-PCR and immunofluorescence for Wnt3a expression after 2Gy of IR. Dashed line: DP. * $p<0.05$, compared to 0hr. Error bars represent mean ± S.E.M. Scale bar=25μm.

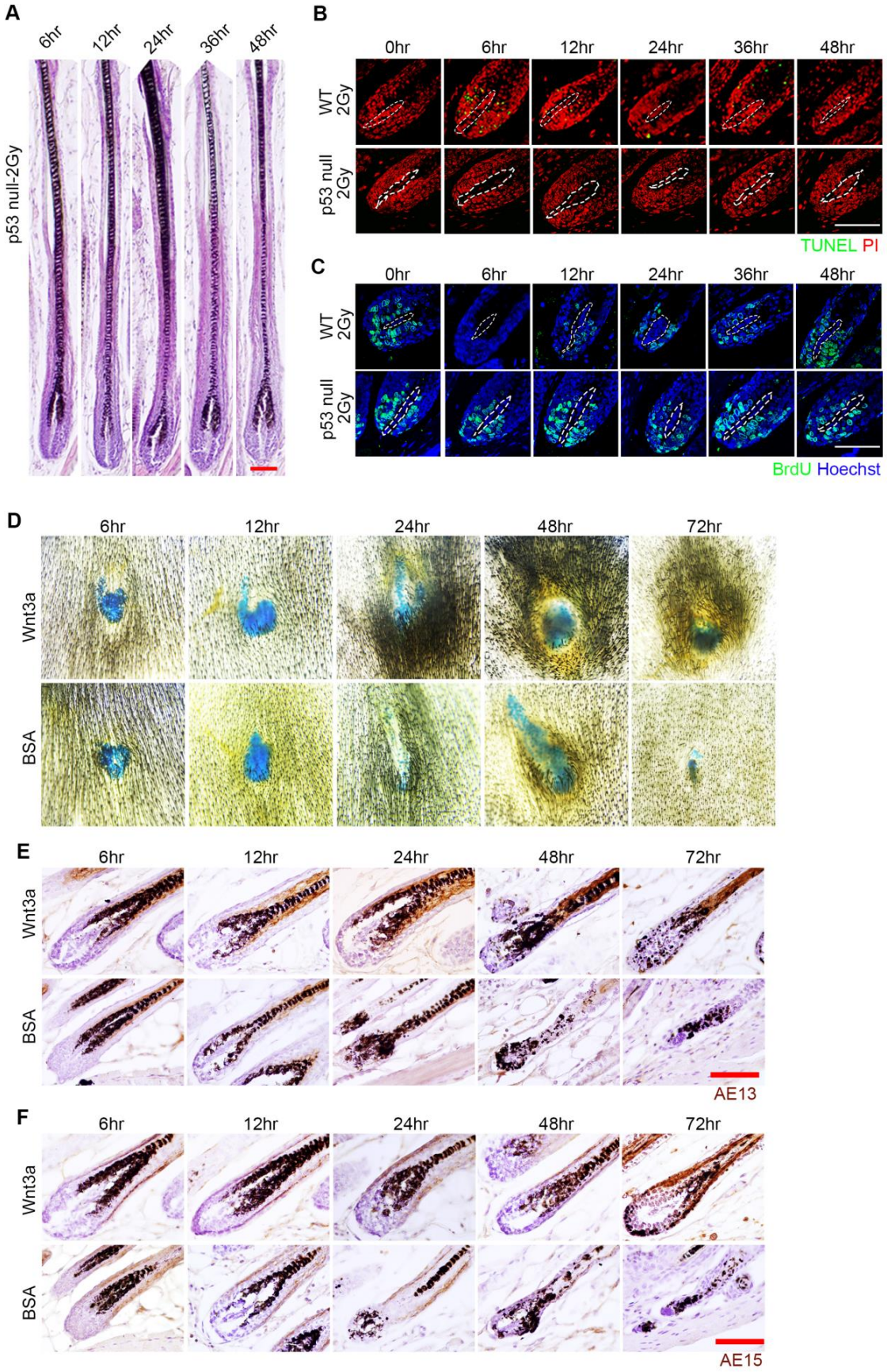


Figure S6. Histology, apoptosis and cell proliferation in *p53* null mice after 2Gy of IR, and the effect of Wnt3a treatment on HF differentiation. (A) Histology. HF dystrophy was not induced in *p53* null mice. (B) Apoptosis detected by TUNEL staining. Apoptosis of matrix cells was not

induced by IR in *p53* null mice. (C) Cell proliferation mapped by BrdU pulse labeling. Matrix cell proliferation was not suppressed by IR in *p53* null mice. (D) Skin samples from Wnt3a- and BSA-treated experiments at indicated time points. Skin samples were immersed in xylene for better visualization of hair growth. Local delivery of Wnt3a-soaked beads (blue beads) reduced hair loss. (E) Hair cortex recognized by immunohistochemistry for AE13. (F) IRS recognized by immunohistochemistry for AE15. Better preservation of differentiation toward hair cortex and IRS was observed after Wnt3a treatment. Dashed line: DP. WT: wild type. Scale bar=75μm.

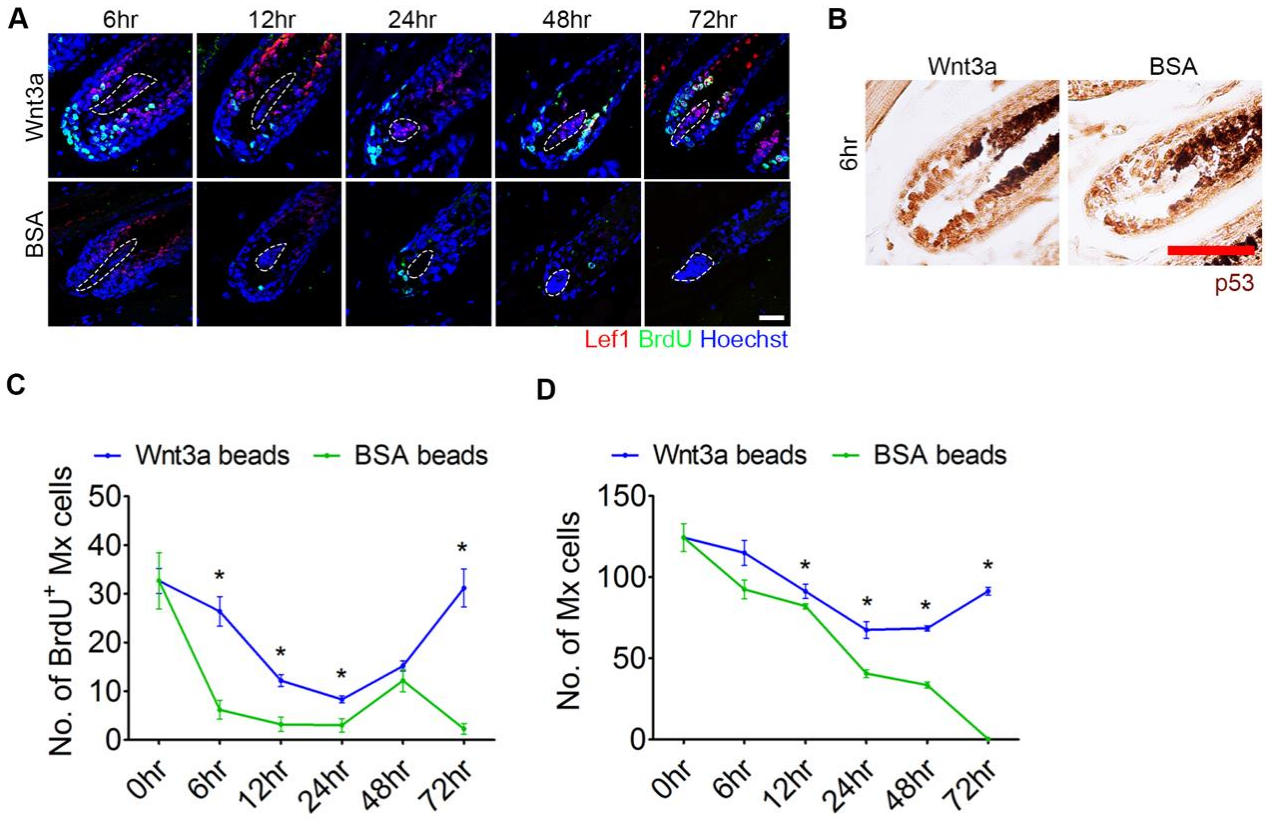


Figure S7. Effect of Wnt3a treatment on HFs after 5.5Gy of IR. (A) Immunofluorescence for Lef1. Compared to HFs of BSA-treated skin, Lef1 expression was more prominent in HFs of Wnt3a-treated skin. (B) Immunohistochemistry for p53. p53 was detected in HFs of Wnt3a- and BSA-treated skin. (C) Quantification of $BrdU^+$ matrix cells. Wnt3a treatment increased proliferative cells in the hair matrix. (D) Quantification of matrix cells. Wnt3a treatment significantly increased the number of hair matrix cells at 24hrs and 72hrs. BSA: bovine serum albumin. Dashed line: DP. Error bars represent mean ± S.E.M. Scale bar=75μm in (B) (C) (E), 25μm in (D). * $p<0.05$.

Supplementary tables

Table S1. Antibodies used in immunostaining

Antibody	**Source**	**Catalog number**	**Dilution**
p53	Cell Signaling	#2524	1/100
AE13	Abcam	ab16113	1/200
AE15	Abcam	ab58755	1/200
Cytokeratin 5	Abcam	ab52635	1/200
β-catenin	BD	610154	1/100
BrdU	Abcam	ab6326	1/200
γ-H2AX	Millipore	#MABE205	1/100
P-cadherin	R&D Systems	AF761	1/200
Wnt3a	Abcam	Ab19925	1/200
Lef1	Cell Signaling	#2230	1/200
Cytokeratin 15	Thermo	MA1-90929	1/100
CD34	Abcam	ab8158	1/100
Cytokeratin 75	Abcam	ab76486	1/200
Sox9	Millipore	AB5535	1/500
Lgr5	Abcam	ab75732	1/100
Cleaved caspase 3	Cell Signaling	#9664	1/200

Table S2. Primer sequences for quantitative RT-PCR

Gene Symbol	Forward primer	Reverse primer
ccnb1	5’-CCTACAGTGAAGACTCCCTGCT-3’	5’-CGGCCTTAGACAAATTCTGAAC-3’
clca1	5’-AAAATGTTGCCATTTTGATTCC-3’	5’-TCCACATGCTCCTATATGTTCG-3’
sox4	5’-CCCAATTTTGCTTGAAGAGACT-3’	5’-TTCGGTCTTTAGAAGCTTTGCT-3’
sox6	5’- CCTTCCCTGACATGCATAACTC-3’	5’-GTAAGGTTGCTTCTCCTGGTTG-3’
lef1	5’-CACGGACAGTGACCTAATGC-3’	5’-ATGAGGTCTTTTGGGCTCCT-3’
wnt3a	5’-GGAATGGTCTCTCGGGAGTT-3’	5’-CTTGAGGTGCATGTGACTGG-3’
bax	5’-CCGCGTGGTTGCCCTCTTCTAC-3’	5’-TTTCCCCTTCCCCCATTCATCC-3’
noxa	5’- GAGATGCCCGGGAGAAAG -3’	5’- CTGCGAACTCAGGTGGTAGC -3’
puma	5’- GCTGTATCCTGCAGCCTTTG-3’	5’-GTGACAGGGAGGGCTGAG-3’
p21	5’- CTGAGCGGCCTGAAGATT-3’	5’-GCTAAGGCCGAAGATGGGGAAGA-3’
gapdh	5’-TCAACGACCCCTTCATTGAC-3’	5’-TTCCCATTCTCAGCCTTGAC-3’

Table S3. Pathways significantly affected in the epithelial cells after 5.5Gy of IR

KEGG analysis: 5.5Gy-24hr vs. 0hr	
Term	**P-value**
Basal cell carcinoma	3.10E-07
Hedgehog signaling pathway	5.10E-05
Pathways in cancer	6.60E-03
Wnt signaling pathway	1.10E-02
Melanogenesis	3.60E-02
Systemic lupus erythematosus	4.00E-02
Cell cycle	7.60E-02
KEGG analysis: 5.5Gy-72hr vs. 24hr	
Term	**P-value**
Ribosome	1.50E-07
Basal cell carcinoma	5.50E-06
Hedgehog signaling pathway	6.00E-04
Wnt signaling pathway	2.90E-03
Terpenoid backbone biosynthesis	1.40E-02
Arginine and proline metabolism	3.00E-02
Melanogenesis	4.00E-02
Pathways in cancer	5.50E-02

RNA-sequencing data were analyzed by DAVID Bioinformatics Resources 6.7 (https://david.ncifcrf.gov/). Pathways significantly downregulated at 24hrs after 5.5Gy of IR as compared to those at 0hr and pathways significantly upregulated at 72hrs as compared to those at 24hrs after 5.5Gy of IR. Genes with ≥ two-fold change were included for the analysis. The pathways were ranked by *p*-values.