Wnt activity and basal niche position sensitize intestinal stem and progenitor cells to DNA damage

Si Tao[1,2,3], Duozhuang Tang[2], Yohei Morita[2], Tobias Sperka[2], Omid Omrani[2], André Lechel[4], Vadim Sakk[5], Johann Kraus[6],Hans A. Kestler[2,6], Michael Kühl[1,#], K. Lenhard Rudolph[2,#]

Supplementary Information

Figure S1. Expression of differentiation markers in intestinal stem and progenitor cells.

mRNA expression of indicated genes relative to *GAPDH* in LGR5$^{hi\text{-}high}$, LGR5$^{hi\text{-}low}$, LGR5$^{lo\text{-}high}$, and LGR5$^{lo\text{-}low}$ populations (n=3 mice). Mean values ± SEM are given.

Figure S2. Young G3 *mTerc*$^{-/-}$ mice do not lose LGR5-GFP^{+} intestinal stem and progenitor cells.

(A–D) Representative FACS plots of small intestinal crypt cells of 2-3 month old LGR5-GFP^{ki}, *mTerc*$^{+/+}$ mice and LGR5-GFP^{ki},G3 *mTerc* $^{-/-}$ mice.

Figure S3. Telomere dysfunction leads to preferential depletion of position 1 and 2 cells at the crypt bottom in between Paneth cells.

Representative pictures of HE staining of small intestinal tissue sections of 12-16 month old G3 *mTerc* $^{-/-}$ mice (D-F) and age matched *mTerc* $^{+/+}$ mice (A-C). Arrow heads and numbers indicate the positions of intestinal stem and progenitor cells (ISPCs) in the basal crypts. Scale bars: 20µm. Note the spindle shaped CBC cells in between Paneth cells are depleted in old G3 *mTerc* $^{-/-}$ mice.

Figure S4. Irradiation leads to preferential depletion of position 1 and 2 cells at the crypt bottom in between Paneth cells.

(A-C) 3 month old *mTerc* $^{+/+}$ mice were exposed to 12Gy γ-irradiation. Small intestinal tissue was collected at 24h after irradiation or from non-irradiated mice (n=4 mice per group). (A,B) Representative pictures of Msi1 staining. Arrow heads and numbers indicate ISPC positions in the crypts. Scale bar: 20µm. (C) Quantification of Msi1^{+} ISPCs at indicated positions in the crypt base. (D) 3-month old LGR5-GFP^{ki}, *mTerc* $^{+/+}$ mice were exposed to 12 Gy γ-irradiation. Small intestinal tissue was collected at 24 hours after IR. The histogram depicts quantification of GFP-staining intensity of ISPCs at indicated positions in positively stained basal crypts. Note that the staining intensity of position 4 cells is equal in irradiated and non-irradiated mice, and is significantly lower than position 1 and 2 cells of non-irradiated mice. Mean values ± SEM are given.

Figure S5. Increased sensitivity of Wnt^{hi} cells compared to Wnt^{lo} cells is not related to differences in cell cycle activity.

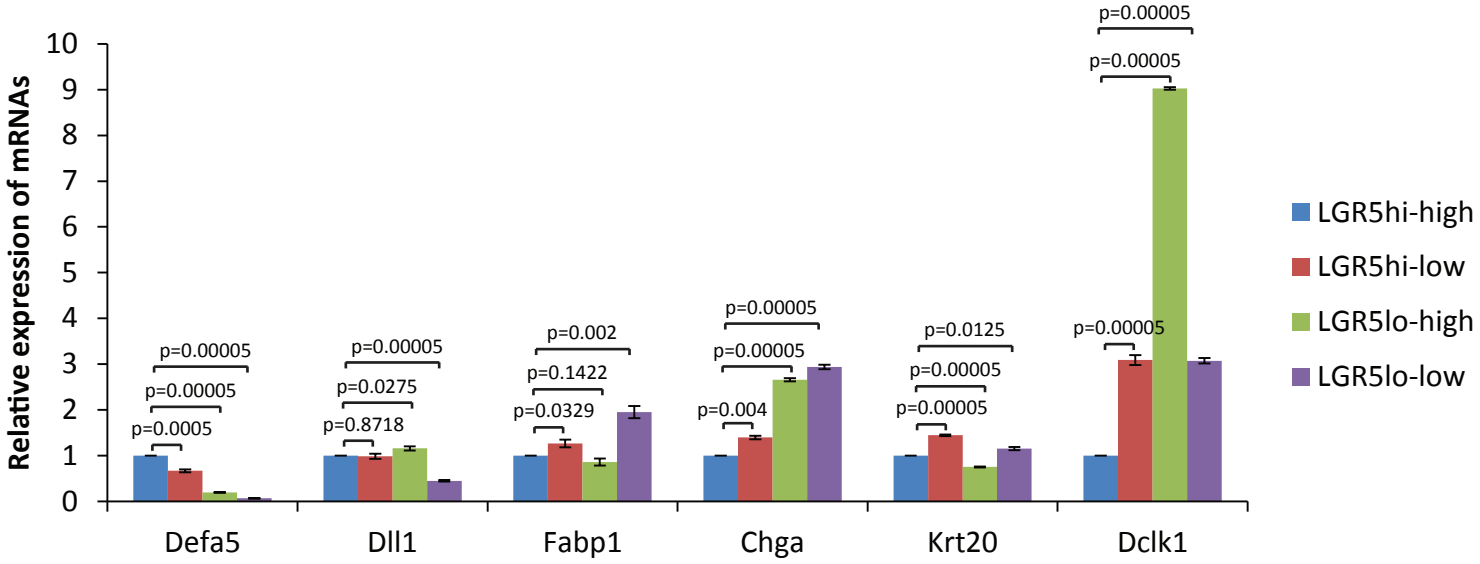
(A-D) Immunofluorescence staining of PCNA and Ki67 on small intestinal sections of 3-month old *mTerc*$^{+/+}$ mice (n=4 mice per group). (A, B) Representative pictures. Arrow heads and numbers indicate positions of the ISPCs in the crypt base. Scale bar: 20µm. (C, D) Percentage of PCNA or Ki67 positive ISPCs at indicated positions in basal crypts. (E-I) 2-3 month old LGR5-GFP^{ki}, *mTerc*$^{+/+}$ mice were exposed to 12 Gy γ-irradiation. Small intestinal tissue was collected at indicated time points and analyzed by flow cytometry (n=4-10 mice per group). (E-H) Representative FACS plots. (I) Quantification of cell cycle profiles of LGR5^{hi} and LGR5^{lo} cells at indicated time points as determined by flow cytometry analysis. Mean values ± SEM are given. NIR=non-irradiated; IR=irradiated.

Figure S6. Original Western blots related to Fig. 6 C.

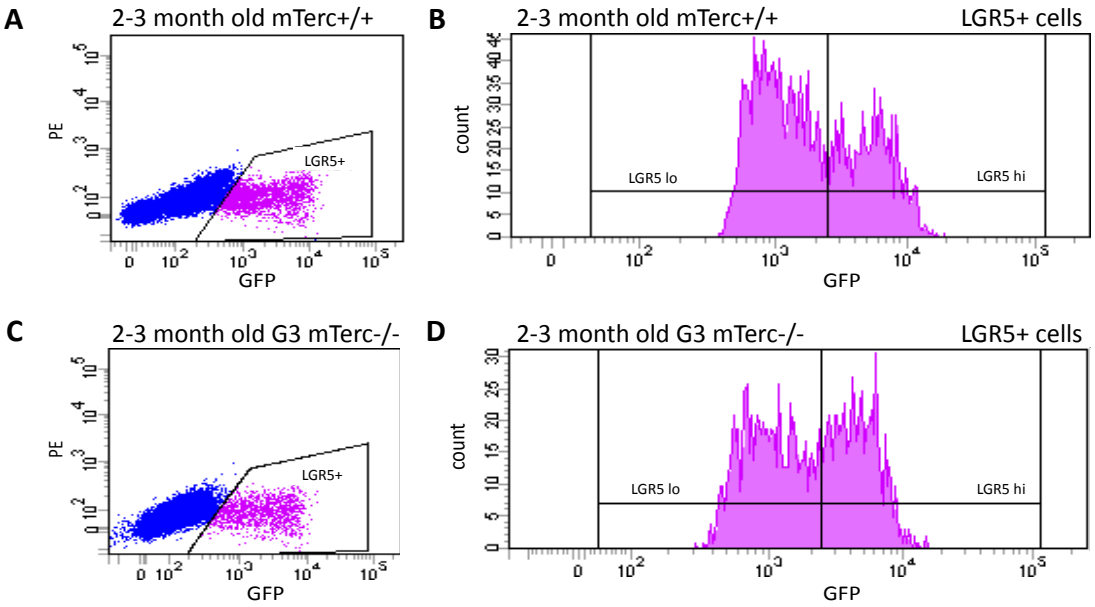
LGR5^{hi} and LGR5^{lo} cells were freshly isolated from 3-month old LGR5-GFP^{ki} mice 6 h after 12Gy γ-irradiation (IR) or from non-irradiated (NIR) mice (n=3 mice per group). Original scan of representative Western Blots were shown. Red boxes indicate areas used for Fig. 6 C.

Figure S7. Instructed modification of canonical Wnt/β-catenin signaling activity.

(A) mRNA expression of *Axin2* relative to *GAPDH* in isolated LGR5^{hi} and LGR5^{lo} cells from cultured crypts of 2-3 month old LGR5-GFP^{ki} mice at indicated time points after γ-irradiation with 6Gy (IR) or non-irradiated (NIR) controls (n=4 independent experiments). (B-E) Crypts of 2-3 month old LGR5^{ki} mice were cultured, and were exposed to the indicated treatment on day 9 of culture. 16 h after treatment, cells were harvested to check for cell cycle and Wnt activity. (n=4 independent experiments per group.) (B, C) mRNA expression of *Axin2* was measured in relation to *GAPDH* mRNA expression by qPCR. (B) Inhibition of Wnt signaling was achieved by adding DKK1 to the culture medium or reducing R-spondin by 50% (50% Rspo). *Axin2* mRNA expression level was normalized to 1 in control cells.(C) Activation of Wnt signaling was achieved by treatment with 6-BIO. Me-BIO served as control. *Axin2* mRNA expression level was normalized to 1 in Me-BIO treated cells.(D,E) Flow cytometry analysis of cell cycle profiles of LGR5^{+} cells from non-irradiated crypt organoid cultures exposed to the indicated treatments. (F) 2 month old LGR5-GFP^{ki}, *mTerc*$^{+/+}$ mice were i.v. injected with anti-LRP6 antibody or IgG which serves as a vehicle control. LGR5^{hi} cells of small intestinal epithelial cells were FACS purified 12 h after injection. mRNA expression of *Axin2* was measured in relation to *GAPDH* mRNA expression by qPCR. *Axin2* mRNA expression level was normalized to 1 in control group.Results are displayed as mean ± SEM.



Tao et al., Supplementary Figure S1.



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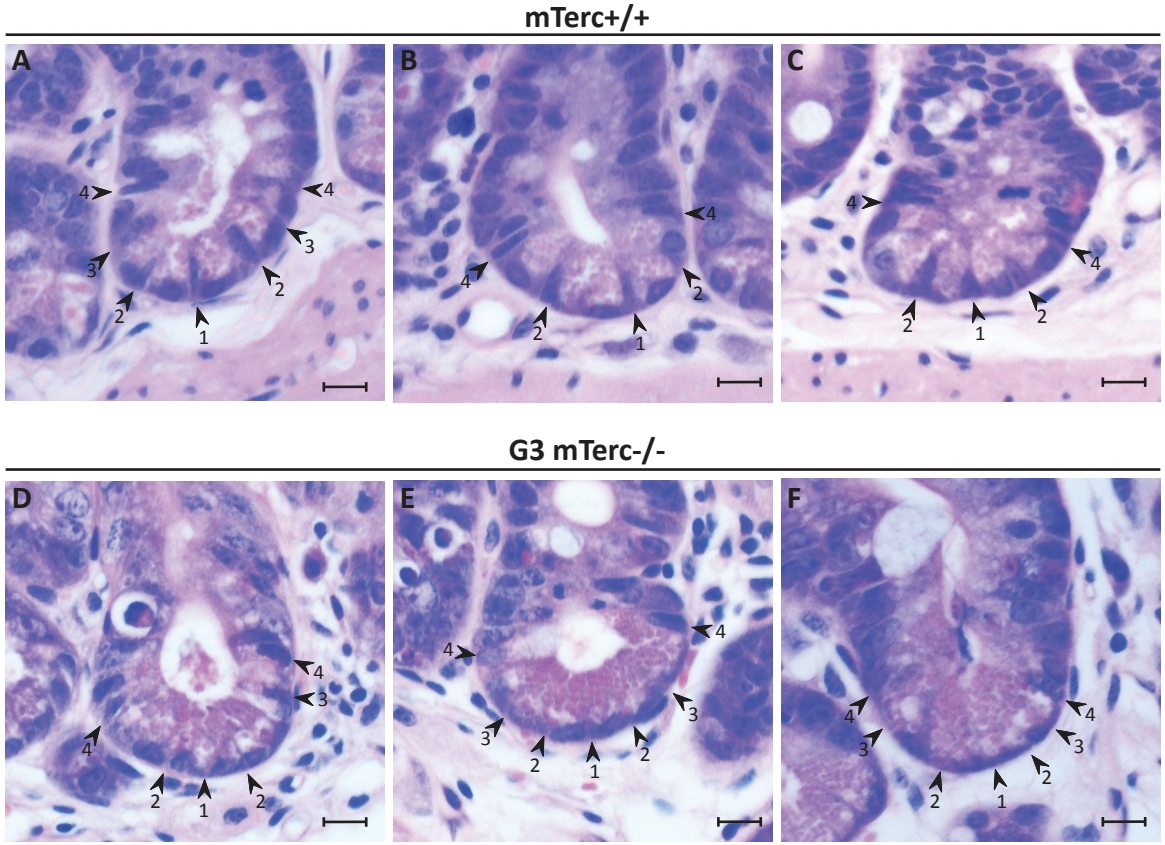

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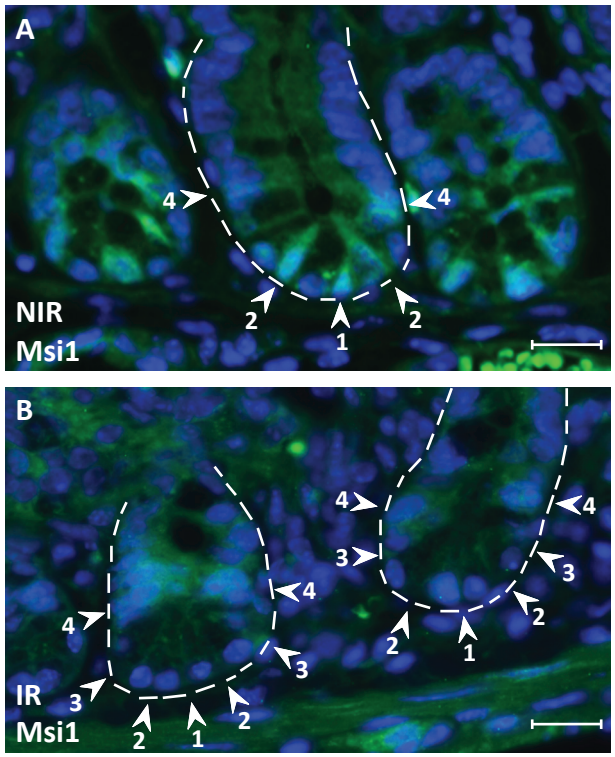
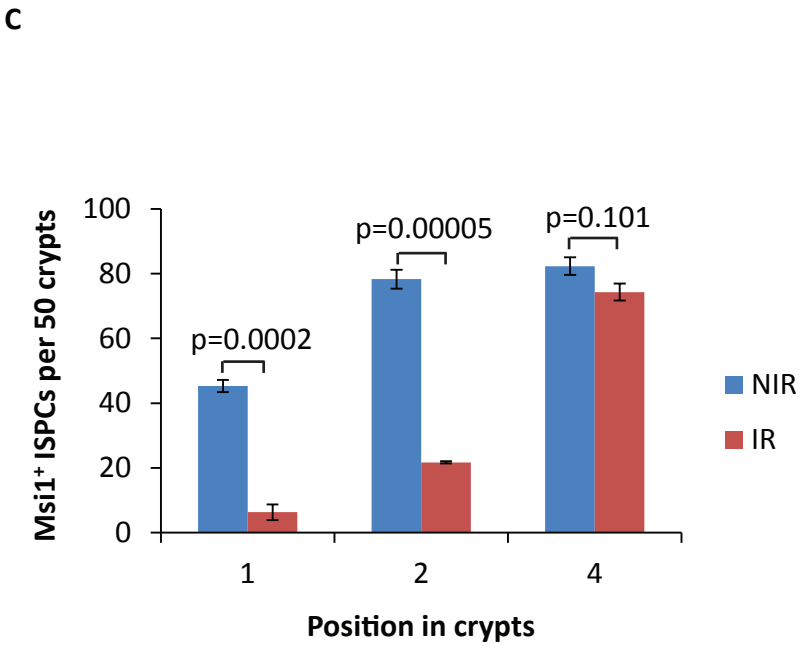
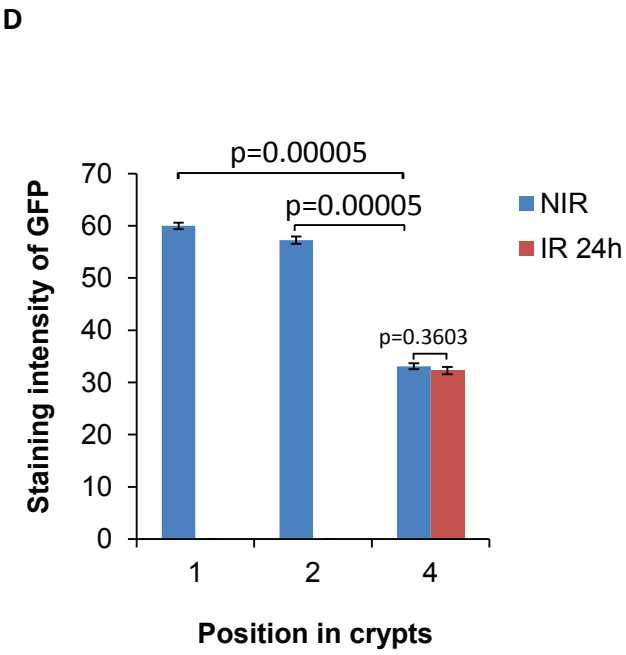
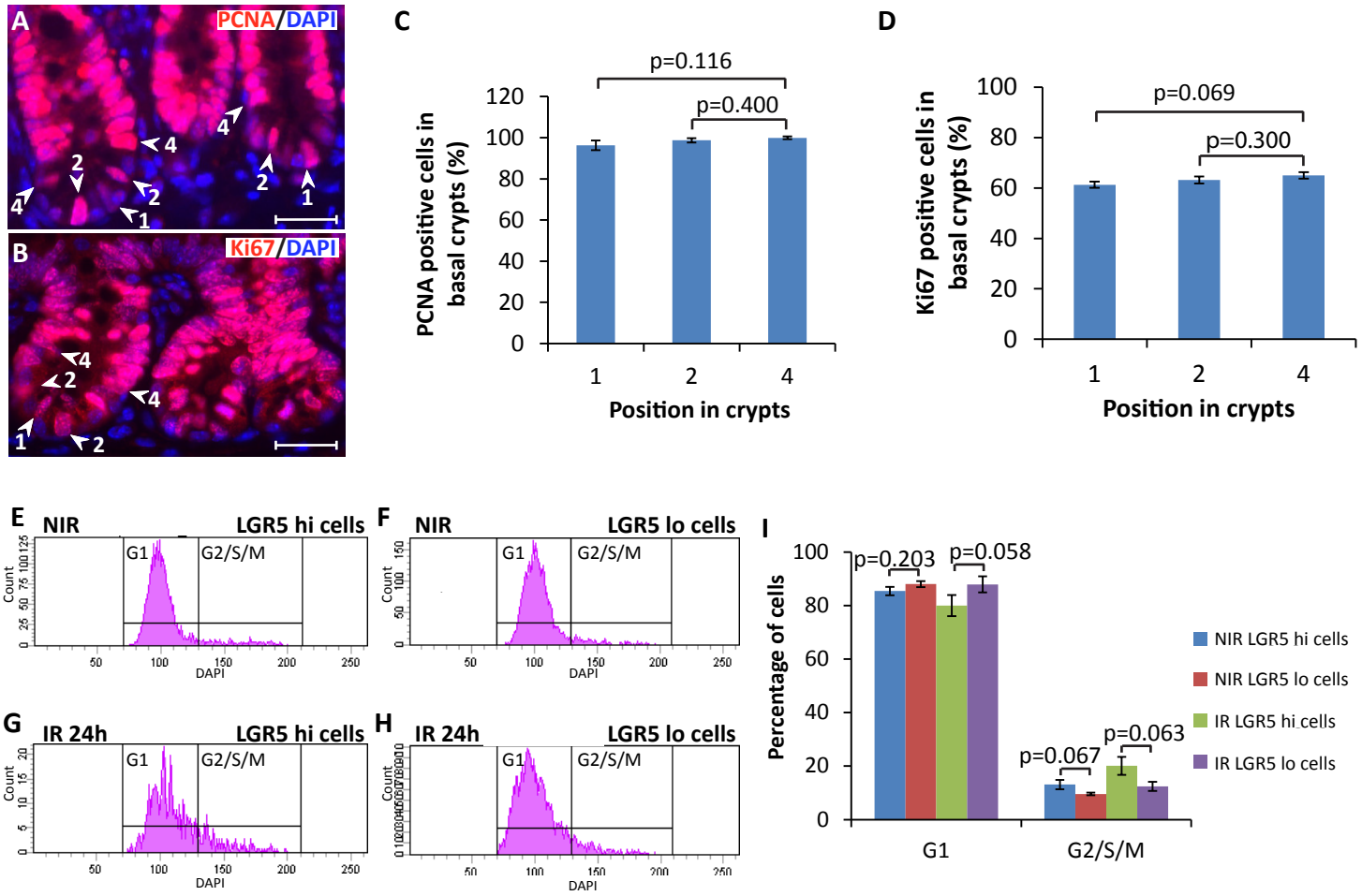


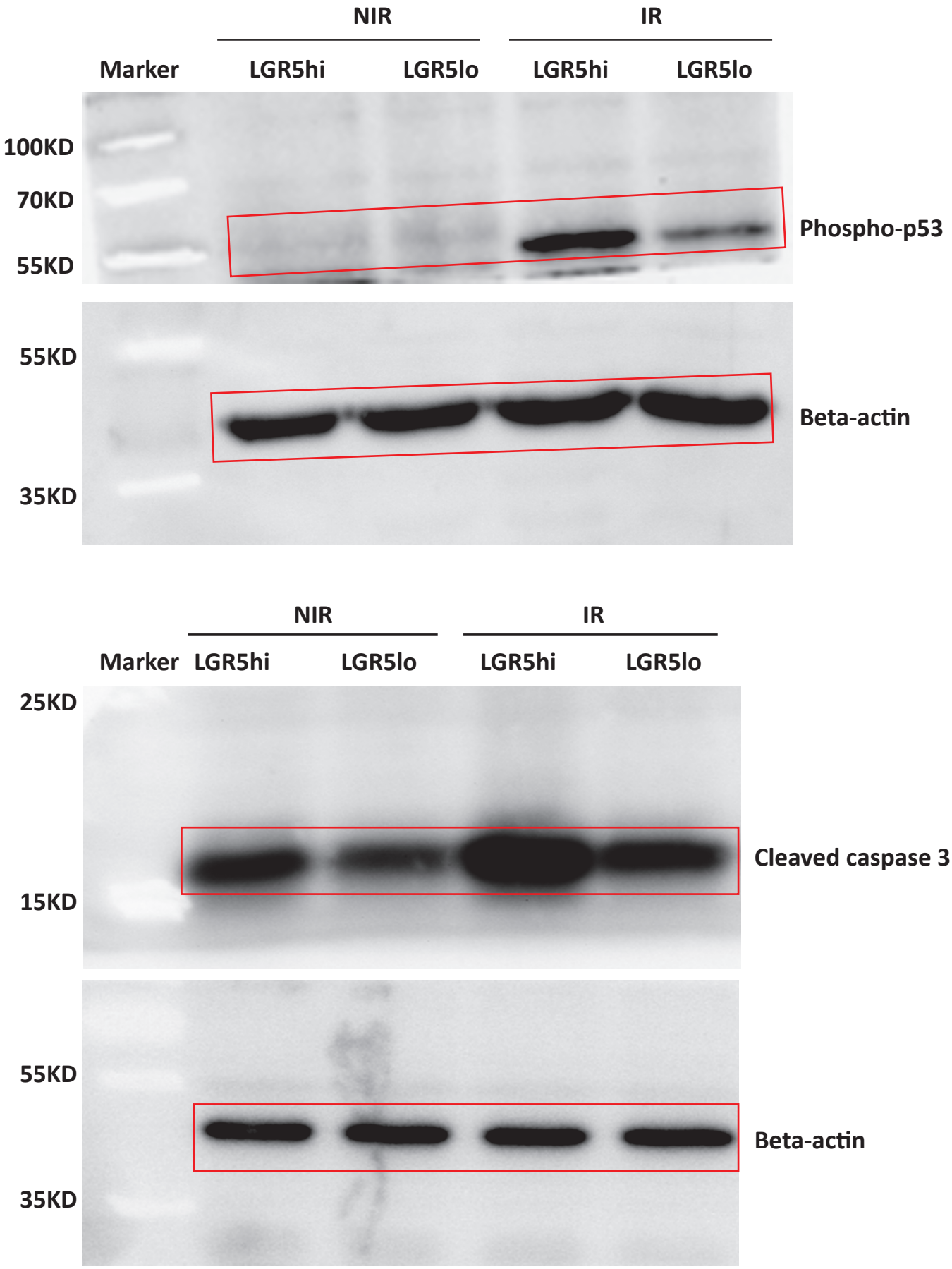




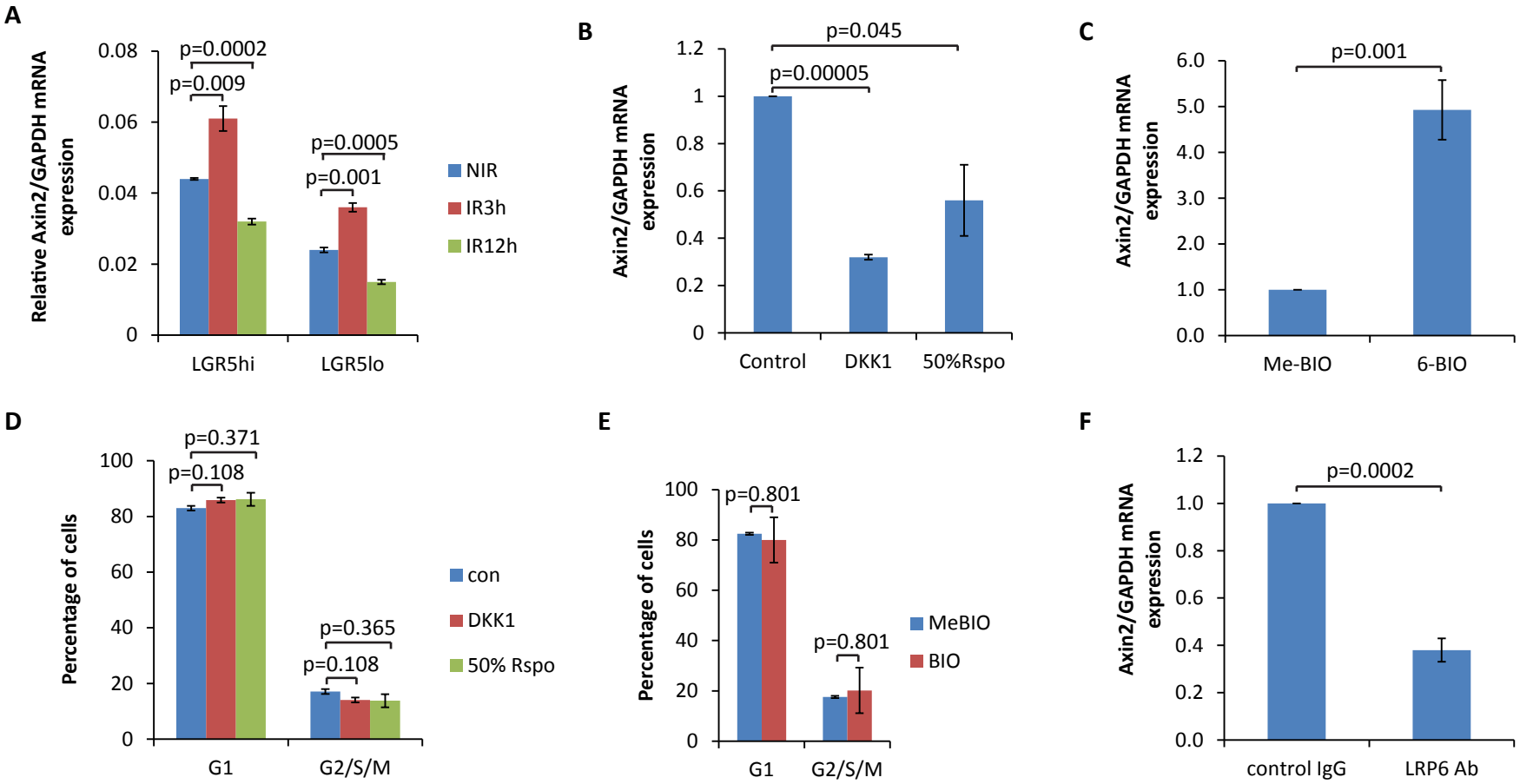
Tao et al., Supplementary Figure S4.



Tao et al., Supplementary Figure S5.



Tao et al., Supplementary Figure S6.



Tao et al., Supplementary Figure S7.

Table S1. Primer list

Gene symbol	Left primer	Right primer
Axin2	gagagtgagcggcagagc	cggctgactcgttctcct
Ascl2	gagagctaagcccgatgga	tcagtagccccctaaccaac
Bbc3 (PUMA)	tggagggtcatgtacaatctctt	gttgggctccatttctgg
Bok	agtggcaggccacatctt	ccacggaatacagggacacta
Cdkn1a (p21)	aacatctcagggccgaaa	tgcgcttggagtgatagaaa
Chga	cgatccagaaagatgatggtc	cggaagcctctgtctttcc
Dclk1	ttcaacacaggccccaag	ccttatcaagagcggtggtt
Defa5	caggctgatcctatccacaaa	ggcctccaaaggagatagaca
Dll1	gggacagaggggagaagatg	tccatgttggtcatcacacc
Fabp1	ccatgactggggaaaaagtc	gcctttgaaagttgtcaccat
Fzd1	cagcagtacaacggcgaac	gagatgggctggcagtagc
Fzd7	cgtcttcagcgtgctctaca	tcataaaagtagcaggccaaca
Krt20	agctgagacgcacctaccag	tgcgctccagagactctttc
Lef1	tcctgaaatccccaccttct	tgggataaacaggctgacct
Msi1	gaggactcagttggcagacc	ctctttcacctccccgaact
Pmaip1 (NOXA)	cagatgcctgggaagtcg	tgagcacactcgtccttcaa
Tcf4	gaaaagttcctccgggtttg	tccctgttgtagtcggcagt
Wnt10a	agccagcacgtcttgagg	gtcgttgggtgctgacct