

Fig. S1. The phenotypes of *Mir29ab1*^{-/-} mice in physiological status. (a) A schematic diagram illustrating the construction of *miR-29ab1*^{-/-} mice. (b, c) Identification of *Mir29ab1* knockout by *in situ* hybridization (b) and Q-PCR (c). Scale bar = 50 μ m. *n* = 3 mice in each group. (d) Representative images and quantification of proliferative and differentiated cells in small intestine (SI) of WT and *Mir29ab1*^{-/-} mice. Scale bar = 200 μ m. *n* = 3-6 mice in each group. Data are presented as mean \pm SD. Student's *t*-test. **P* < 0.05, ****P* < 0.001. WT represents wild type mice, KO represents *Mir29ab1*^{-/-} mice.

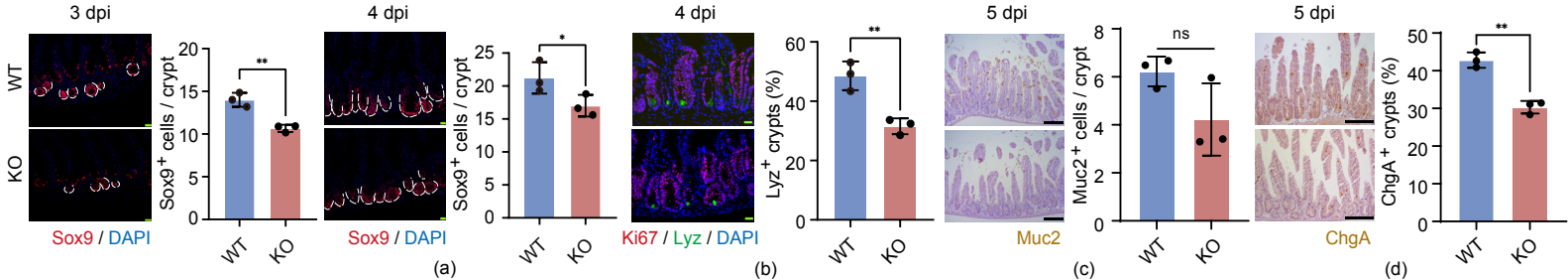


Fig. S2. *Mir29abl* deletion influences intestinal epithelial cell composition post irradiation. (a) Representative images and quantification of Sox9⁺ cells per crypt of SI from WT and *Mir29abl*^{-/-} mice 3 days and 4 days after irradiation (IR). Scale bar = 25 μ m. *n* = 3 mice in each group. (b) Representative Ki67 / Lysozyme-stained sections and quantification of Lysozyme⁺ crypt proportion of SI from WT and *Mir29abl*^{-/-} mice 4 days after IR. Scale bar = 25 μ m. *n* = 3 mice in each group. (c) Representative images and quantification of Muc2⁺ cells per crypt of SI from WT and *Mir29abl*^{-/-} mice 5 days after IR. Scale bar = 200 μ m. *n* = 3 mice in each group. (d) Representative images and quantification of ChgA⁺ cells per crypt of SI from WT and *Mir29abl*^{-/-} mice 5 days after IR. Scale bar = 200 μ m. *n* = 3 mice in each group. Data are presented as mean \pm SD. Student's *t*-test. **P* < 0.05, ***P* < 0.01. WT represents wild type mice, KO represents *Mir29abl*^{-/-} mice.

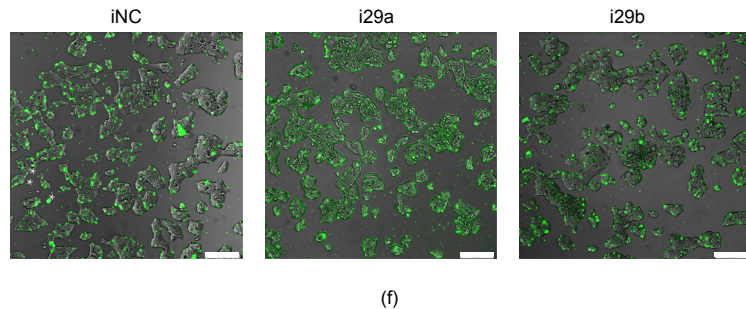
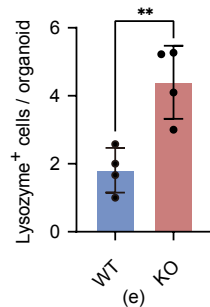
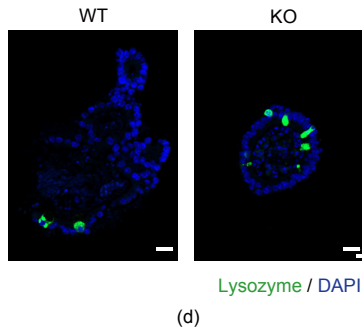
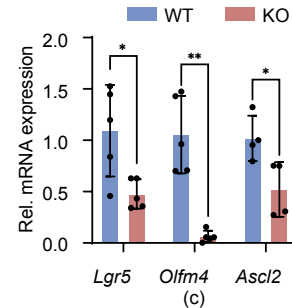
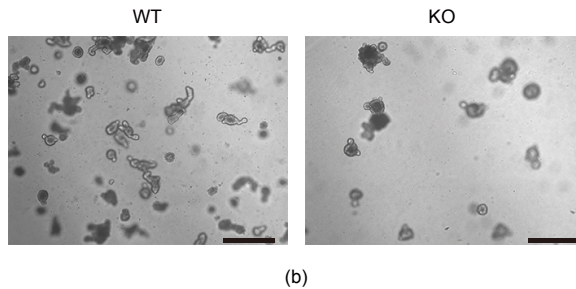
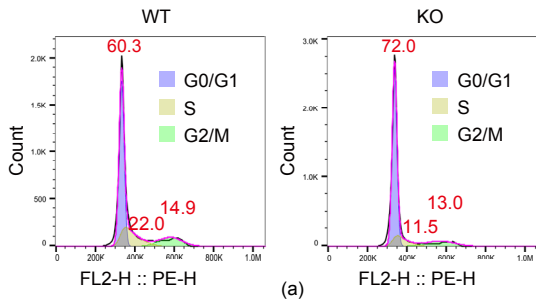
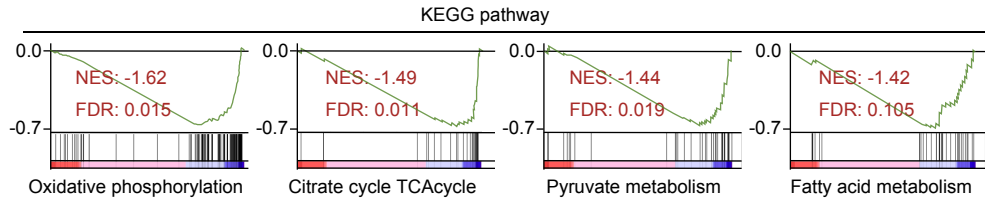
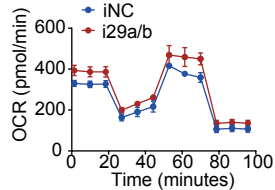


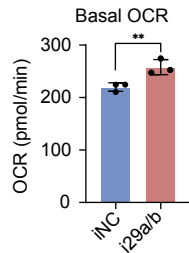
Fig. S3. Lack of miR-29a/b affects the balance between self-renewal and differentiation of ISCs. (a) Representative result of cell-cycle analysis using flow cytometry on crypts from WT and *Mir29ab1*^{-/-} mice. (b) Representative images of SI organoids in third culturing from crypts of WT and *Mir29ab1*^{-/-} mice. (c) Expression of genes related to crypt base columnar (CBC) cells in WT and *Mir29ab1*-null organoids. *n* = 5 independent experiments with organoids from different mice. (d, e) Representative images (d) and quantification (e) of Paneth cells characterized by lysozyme WT and *Mir29ab1*-null organoids. Scale bar = 200 μ m. *n* = 4 independent experiments with organoids from different mice. (f) The effect of transfection determined by efficiency of FAM-labeled inhibitors imported into cells. Scale bar = 200 μ m. Data are presented as mean \pm SD. Student's *t*-test. **P* < 0.05, ***P* < 0.01. WT represents wild type mice, KO represents *Mir29ab1*^{-/-} mice, iNC represents NC inhibitors, i29a/b represents hsa-miR-29a-3p inhibitor and hsa-miR-29b-3p inhibitor.



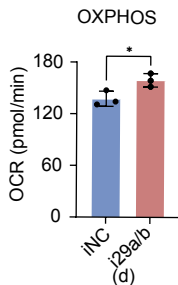
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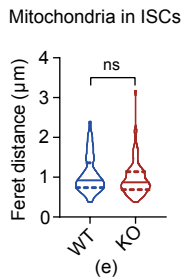
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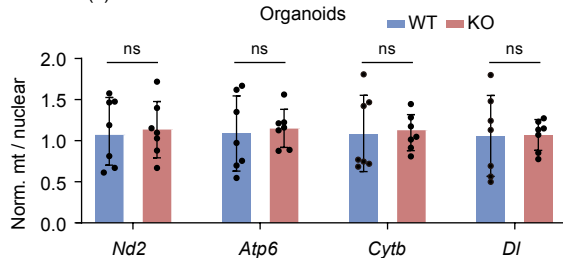
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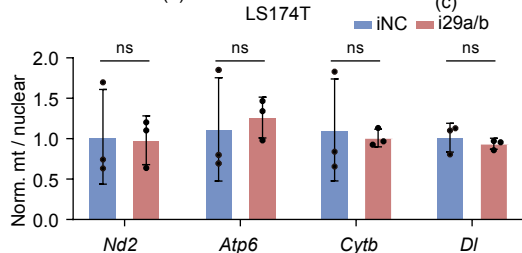
(d)



(e)



(f)



(g)

Fig. S4. Effect of *Mir29ab1* deletion on mitochondrial energy metabolism of ISCs. (a) Encyclopedia of Genes and Genomes (KEGG) pathways related to metabolism analysis by gene set enrichment analysis (GSEA) of crypts from WT and *Mir29ab1*^{-/-} mice. *n* = 3 mice in each group. (b-d) Mitochondrial stress test (Seahorse) of LS174T cells transfected with NC inhibitor and miR-29a/b inhibitors (b), as well as basal oxygen consumption rate (OCR) (c) and OCR associated with oxidative phosphorylation (OXPHOS) (d). *n* = 3 independent experiments. (e) Average Feret distance of mitochondria in ISCs determined by electron microscopy of mitochondria in SI from WT and *Mir29ab1*^{-/-} mice. *n* = 118 (WT) and 112 (*Mir29ab1*^{-/-}) mitochondria from 3 mice in each group. (f) Mitochondrial DNA copy number quantified by Q-PCR on total DNA of organoids from WT and *Mir29ab1*^{-/-} mice. Different passages of organoids from at least 3 mice for *n* = 7. (g) Mitochondrial DNA copy number quantified by Q-PCR on total DNA of LS174T cells treated with or without miR-29a/b inhibition. *n* = 3 independent experiments. Data are presented as mean ± SD. Student's *t*-test. **P* < 0.05, ***P* < 0.01. WT represents wild type mice, KO represents *Mir29ab1*^{-/-} mice, iNC represents NC inhibitors, i29a/b represents hsa-miR-29a-3p inhibitor and hsa-miR-29b-3p inhibitor.

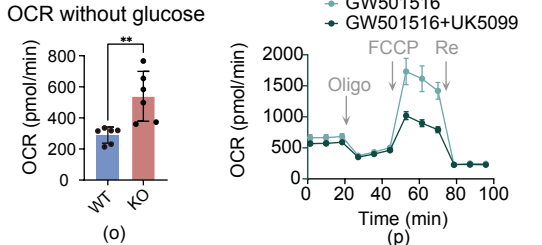
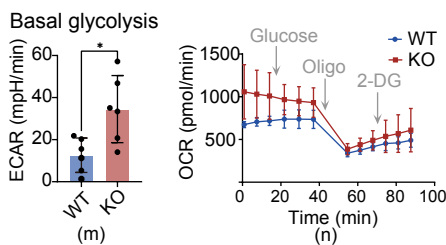
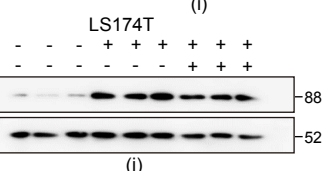
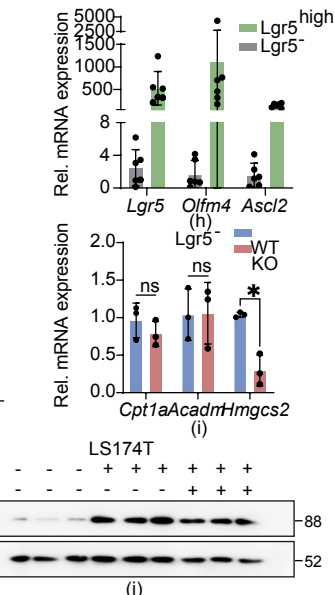
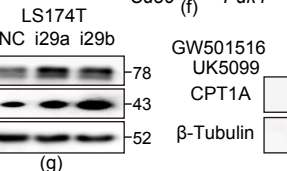
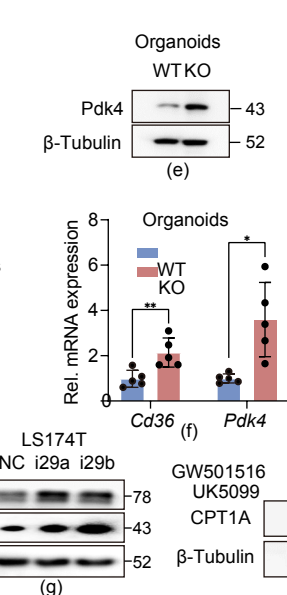
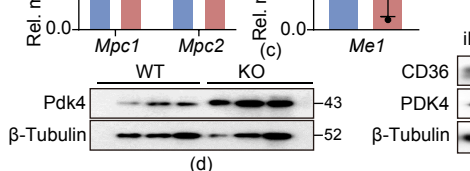
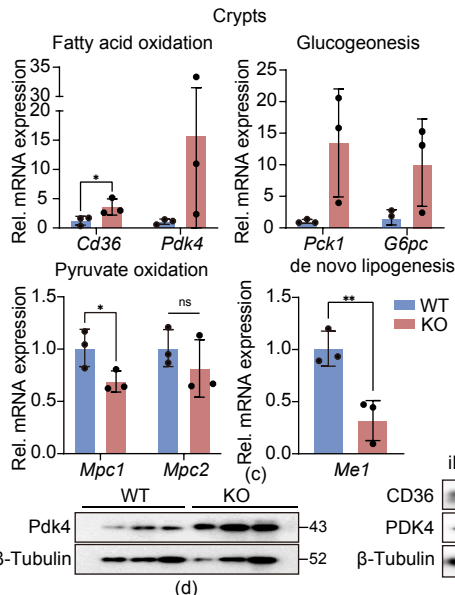
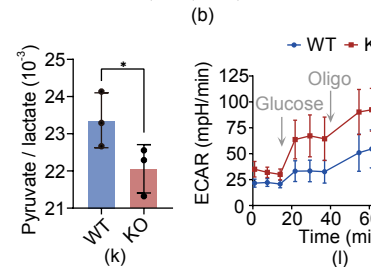
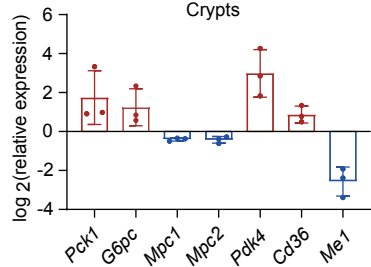
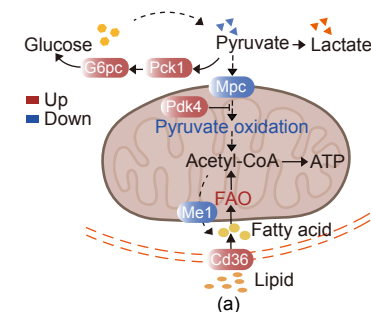


Fig. S5. Fatty acid oxidation activity changes with *Mir29ab1* deletion or miR-29a/b inhibition. (a) A diagram illustrating the enzymes related to mitochondrial metabolism that were changed upon *Mir29ab1* deletion. (b) Metabolism-related genes differentially expressed in crypts from WT and *Mir29ab1*^{-/-} mice based on RNA-sequencing. *n* = 3 mice in each group. (c) Changes of metabolism-related genes in crypts from WT and *Mir29ab1*^{-/-} mice analyzed by Q-PCR. *n* = 3 mice in each group. (d-f) Induction of fatty acid oxidation (FAO)-related genes or proteins in deficiency of miR-29a/b in SI crypts (d, *n*=4) and organoids (e, f, different passages of organoids from at least 3 mice for *n* = 5). (g) Representative immunoblots of FAO-related proteins in LS174T cells with or without inhibition of miR-29a/b for 48 h. (h) Q-PCR analysis of CBC markers in Lgr5^{high} and Lgr5^{negative} cells sorted from mice. *n* = 6, samples combined from 15 different mice. (i) Q-PCR analysis of FAO-related genes in Lgr5^{negative} cells from WT and *Mir29ab1*^{-/-} mice. *n* = 3, samples combined from 7 (*Mir29ab1*^{-/-}) or 8 (WT) mice. (j) Representative immunoblots of the key FAO-related protein in LS174T cells with treatment of 1 μM GW501516 or 10 μM UK5099 coupled 1 μM GW501516. *n* = 3 independent experiments. (k) Pyruvate/lactate ratio in crypts from WT and *Mir29ab1*^{-/-} mice were measured. *n* = 3 mice in each group. (l, m) Glycolytic capacity of organoids from WT and *Mir29ab1*^{-/-} mice measured by glycolysis stress test (Seahorse) with basal extracellular acidification rate (ECAR) determined. Organoids from at least 3 mice for *n* = 6. Results were confirmed in at least 3 independent experiments. (n, o) OCR without glucose of organoids from WT and *Mir29ab1*^{-/-} mice was determined by Seahorse. Organoids from at least 3 mice for *n* = 6. Results were confirmed in at least 3 independent experiments. (p) OCR measured by mitochondrial stress test (Seahorse) in LS174T cells treated with GW501516 alone or GW501516 together with UK5099. Data are presented as mean ± SD. Student's *t*-test. **P* < 0.05, ***P* < 0.01. WT represents wild type mice, KO represents *Mir29ab1*^{-/-} mice, iNC represents NC inhibitors, i29a/b represents hsa-miR-29a-3p inhibitor and hsa-miR-29b-3p inhibitor.

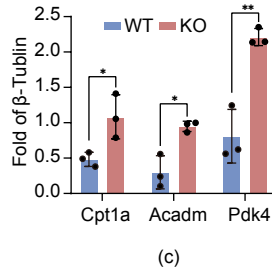
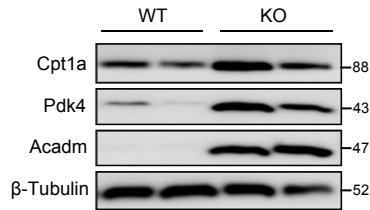
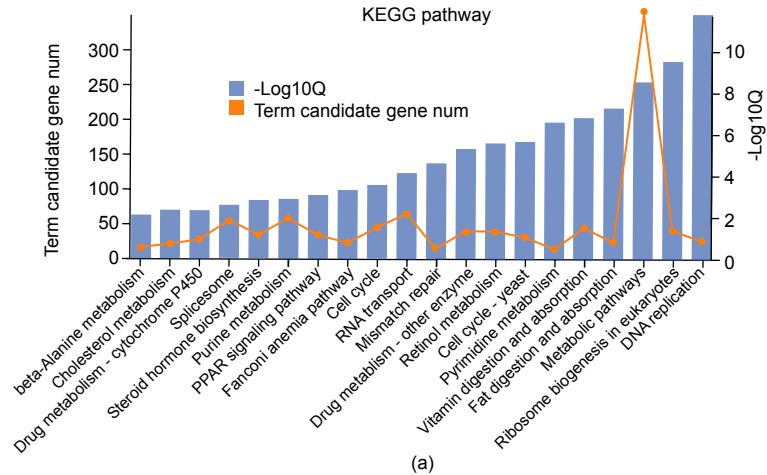
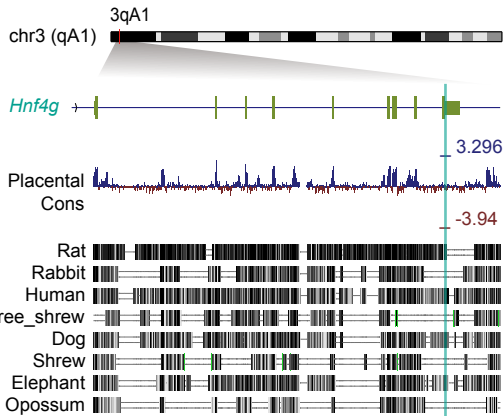
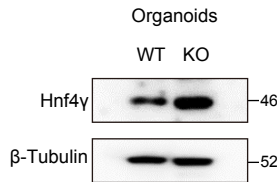


Fig. S6. Deletion of *Mir29abl* promotes fatty acid oxidation of regenerated crypts after irradiation. (a) KEGG pathway analysis of RNA-sequencing data on crypts from WT and *Mir29abl*^{-/-} mice 3 days after IR. *n* = 3 mice in each group. (b, c) Representative immunoblots (b) and quantification (c) of FAO-related proteins in crypts of WT and *Mir29abl*^{-/-} mice after IR injury. *n* = 3 mice in each group. Data are presented as mean ± SD. Student's *t*-test. **P* < 0.05, ***P* < 0.01. WT represents wild type mice, KO represents *Mir29abl*^{-/-} mice.

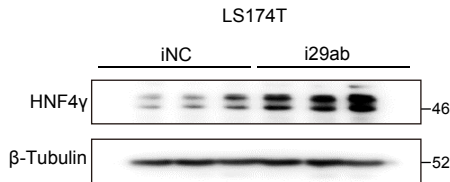
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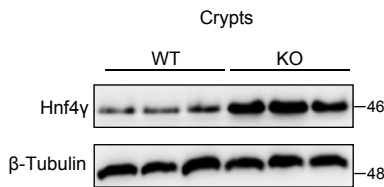
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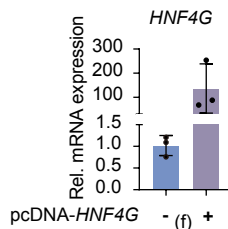
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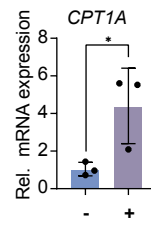
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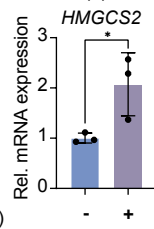
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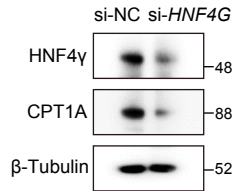
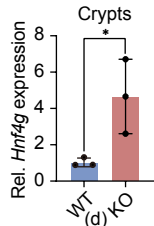
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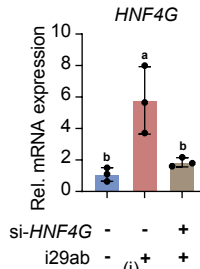
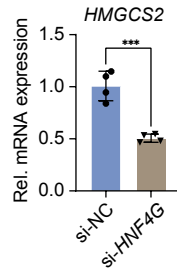
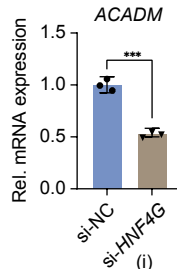
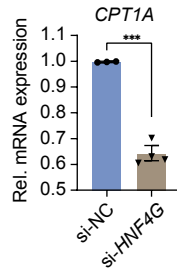
(g)



(g)



(h)



(i)

(j)

Fig. S7. Intervention of HNF4 γ is associated with miR-29a/b inhibition. (a) The position of *Hnf4g* and the conservation among various species of the binding sites of miR-29a/b-3p in the 3'-UTR of *Hnf4g* displayed in UCSC GRCm38/mm10 dataset. (b) Representative immunoblots of Hnf4 γ in WT and *Mir29abl*-null SI organoids. *n* = organoids from 3 mice in each group. (c) Representative immunoblots of HNF4 γ in LS174T cells treated with or without miR-29a/b inhibitors for 48 h. *n* = 6 independent experiments. (d, e), Q-PCR (d) and western blot analysis (e) of *Hnf4g*/Hnf4 γ expression in crypts from WT and *Mir29abl*^{-/-} mice 3 days after IR treatment. *n* = 3 mice in each group. (f) Q-PCR analysis of *HNF4G* in LS174T cells 24 h after transfection with or without pcDNA-*Hnf4g*. *n* = 3 independent experiments. (g) Q-PCR analysis of FAO-related genes in LS174T cells with or without *HNF4G* overexpression. *n* = 3 independent experiments. (h, i) Representative immunoblots (h) and Q-PCR (i) analysis of FAO-related protein or gene expression with or without *HNF4G* siRNA-mediated knockdown in LS174T cells for 24 h (gene) and 48 h (protein). *n* = 3-4 independent experiments. (j) Q-PCR analysis of *HNF4G* expression in LS174T cells treated with miR-29a/b inhibitors or with miR-29a/b inhibitors and si-*HNF4G*. *n* = 3 independent experiments. Data are presented as mean \pm SD. Student's *t*-test. **P* < 0.05, ****P* < 0.001 by Student's *t*-test (d, f, g, i) or Duncan's post hoc test following one-way ANOVA (j). a, b represents significant differences (*P* < 0.05). WT represents wild type mice, KO represents *Mir29abl*^{-/-} mice, iNC represents NC inhibitors, i29a/b represents hsa-miR-29a-3p inhibitor and hsa-miR-29b-3p inhibitor.

Table S1. Primers for Real-time PCR

Gene	Species	Forward primer	Reverse primer
<i>Gapdh</i>	Mouse	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Gapdh</i>	Human	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
<i>β-actin</i>	Mouse	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
<i>Lgr5</i>	Mouse	ACATTCCCAAGGGAGCGTTC	ATGTGGTTGGCATCTAGGCG
<i>Olfm4</i>	Mouse	CAGCCACTTTCCAATTTCACTG	GCTGGACATACTCCTTCACCTTA
<i>Ascl2</i>	Mouse	AAGCACACCTTGACTGGTACG	AAGTGGACGTTTGCACCTTCA
<i>Pcna</i>	Mouse	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
<i>Clu</i>	Mouse	AGCAGGAGGTCTCTGACAATG	GGCTTCCTCTAAACTGTTGAGC
<i>Anxa1</i>	Mouse	ATGTATCCTCGGATGTTGCTGC	TGAGCATTGGTCCTCTTGGTA
<i>Cldn1</i>	Mouse	GGGGACAACATCGTGACCG	AGGAGTCGAAGATTTGCACT
<i>Ocln</i>	Mouse	TTGAAAGTCCACCTCCTTACA GA	TCCCCACTCTGAAAATGAGGA
<i>Dclk</i>	Mouse	TCCACCGGAATTGAACTCGG	GGGAGCGAACAGTCTCAGA
<i>Lrig1</i>	Mouse	TTGAGGACTTGACGAATCTGC	CTTGTTGTGCTGCAAAAAGAGAG
<i>Bmi1</i>	Mouse	TTCATTGTCTTTTCCGCCCCG	AGTACCCTCCACACAGGACA
<i>Msi1</i>	Mouse	CCTCTCACGGCTTATGGGC	CTGTGGCAATCAAGGGACC
<i>Lyz1</i>	Mouse	GAGACCGAAGCACCGACTATG	CGGTTTTGACATTGTGTTCCG
<i>Ccnd1</i>	Mouse	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGTCTC
<i>Ctnnb1</i>	Mouse	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA
<i>Axin2</i>	Mouse	AACCTATGCCCCGTTTCCTCTA	GAGTGTAAGACTTGGTCCACC
<i>Cd44</i>	Mouse	CAGTATCTCCCGGACTGAGG	GCCAACTTCATTTGGTCCAT
<i>Klf4</i>	Mouse	CTGAACAGCAGGGACTGTCA	GTGTGGGTGGCTGTTCTTTT
<i>Myc</i>	Mouse	TTCCTGGTAACCGAATGCTGA	TTCCTGGTAACCGAATGCTGA
<i>Bmp4</i>	Mouse	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
<i>Tgfb</i>	Mouse	GGGGTTGTAGTGGACGAG	CGTTGTTGAAGGACGGGATAAC
<i>Atoh1</i>	Mouse	GGACGATGTTTCAGATAACCC	CCACATTGTCCTCGCAGTA
<i>Dll1</i>	Mouse	TTCCAGGCAACCTTCTCCGA	ACTGCCGCTATTCTTGTCCTC
<i>Dll4</i>	Mouse	AGGAACGCAGCTTTGACTGT	CCTGTGTGGATGAAGGTGTG
<i>Gfi1</i>	Mouse	AACCTATGCCCCGTTTCCTCTA	GAGTGTAAGACTTGGTCCACC
<i>Pck1</i>	Mouse	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCGTACTCC
<i>G6pc</i>	Mouse	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
<i>Mpc1</i>	Mouse	AGCAAGGACTTCCGGGACTAT	AAAGTCATCCGCCCACTGAT
<i>Mpc2</i>	Mouse	GCCACCTACCACCGACTCAT	CCTGCCGGGTGGTTGTAAA
<i>Pdk4</i>	Mouse	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
<i>Cd36</i>	Mouse	AGGGAGGTCGAGCTGTTCTC	GGAGTGTTCACTAAGCGGTCA
<i>Me1</i>	Mouse	GTCGTGCATCTCTCACAGAAG	TGAGGGCAGTTGGTTTTATCTTT
<i>Cpt1a</i>	Mouse	CCATGAAGCCCTCAAACAGAT C	ATCACACCCACCACCACGATA

<i>Hnf4g</i>	Mouse	GTGTCAACTGTTTATGTGCCAT C	GTTCATTTTGCACCGCTTCTTTT
<i>Ppara</i>	Mouse	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
<i>Fasn</i>	Mouse	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
<i>Adob</i>	Mouse	GAAACCGCCTGCAAAGGATAA	GAGGGTCTCGTGGAAAAGGAT
<i>Acs15</i>	Mouse	TCCTGACGTTTGGAACGGC	CTCCCTCAATCCCCACAGAC
<i>Fabp6</i>	Mouse	CTTCCAGGAGACGTGATTGAA A	CCTCCGAAGTCTGGTGATAGTTG
<i>Fabp2</i>	Mouse	GTGGAAAGTAGACCGGAACG A	CCATCCTGTGTGATTGTCAGTT
<i>Guca2 b</i>	Mouse	AGGGTGTCTACATCAAGTACC AT	AAGGGCAAGGCTGGGTTATG
<i>Apoa4</i>	Mouse	CAACAGGCTGAAGGCTACGAT	CGATTTTTCGGAGACCTTGG
<i>Hnf4g</i>	Huma n	CCTCCTCGCTTTCAGCAAAC	CGGTCAAGGCAGCAATCATGT
<i>Cpt1a</i>	Huma n	TCCAGTTGGCTTATCGTGGTG	TCCAGAGTCCGATTGATTTTGC
<i>Acadm</i>	Mouse	AGGGTTTAGTTTTGAGTTGAC GG	CCCCGCTTTTGTTCATATTCCG
<i>Acadm</i>	Huma n	ACAGGGGTTTCAGACTGCTATT	TCCTCCGTTGGTTATCCACAT
<i>Hmgcs 2</i>	Mouse	GAAGAGAGCGATGCAGGAAA C	GTCCACATATTGGGCTGGAAA
<i>Hmgcs 2</i>	Huma n	GCCCAATATGTGGACCAAAC	GAAGCCCATACGGGTCTGG
<i>Nd2</i>	Mito	CACGATCAACTGAAGCAGCAA	ACGATGGCCAGGAGGATAATT
<i>Atp6</i>	Mito	AATTACAGGCTTCCGACACAA AC	TGGAATTAGTGAAATTGGAGTTC CT
<i>Cytb</i>	Mito	GCCACCTTGACCCGATTCT	TTCTAGGGCCGCGATAAT
<i>Dl</i>	Mito	AATCTACCATCCTCCGTGAAA CC	GCCCGGAGCGAGAAGAG

Table S2. Primers for miR-29 quantification

Primer	Application	Sequence (5' to 3')
miR-29a-3p-RT	Reverse transcription	CTCAACTGGTGTCGTGGAGTCGGCAATTCA GTTGAGTAACCGAT
miR-29b-3p-RT	Reverse transcription	CTCAACTGGTGTCGTGGAGTCGGCAATTCA GTTGAGAACACTGA
U6-RT	Reverse transcription	CGCTTCACGAATTTGCGTGTCAT
U6-F	Real-time PCR	CTCGCTTCGGCAGCACAA
U6-R	Real-time PCR	AACGCTTCACGAATTTGCGT
All-R	Real-time PCR	TCAACTGGTGTCGTGGAGT
miR-29a-3p-F	Real-time PCR	TCACGTAGCACCATCTGAA

miR-29b-3p-F	Real-time PCR	TCACGTAGCACCATTGAAA
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Table S3. Sequences for miRNA inhibitors

Name	Sequence
miR-29a-3p-inhibitor	mUmAmAmCmCmGmAmUmUmUmCmAmGmAmUmGmGmUmGmCmUmA
miR-29b-3p-inhibitor	mAmAmCmAmCmUmGmAmUmUmUmCmAmAmAmUmGmGmUmGmCmUmA
NC-inhibitor	mCmAmGmUmAmCmUmUmUmUmGmUmGmUmAmGmUmAmCmAmA

Table S4. Sequences for miRNA mimics

Name	Sense sequence (5'to 3')	Antisense sequence (5'to 3')
miR-29a-3p mimics	UAGCACCAUCUGAAAUCGGUUA	ACCGAUUUCAGAUGGUGCUAUU
miR-29b-3p mimics	UAGCACCAUUUGAAAUCAGUGUU	CACUGAUUUCAAAUGGUGCUAUU
NC mimics	UUGUACUACACAAAAGUACUG	GUACUUUUGUGUAGUACAAUU

Table S5. RNA mutant sites

Name	WT sequence (5'to 3')	Mut sequence (5'to 3')
<i>Hnf4g</i> 3'-UTR	...GGCAAGGCUUCUUCAUGGUGCU G...	...GGCAAGGCUUCUUCAACCCAAG G...

Table S6. Sequences for siRNA

Name	Species	Sense sequence (5'to 3')	Antisense sequence (5'to 3')
si- <i>Hnf4g</i>	Human	CCAGCUGUGAUGGGUGCAATT	UUGCACCCAUCACAGCUGGTT
si-NC	Human	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Movie S1. Mitochondrial membrane potential represented by JC-1 staining in crypts of WT and *miR-29ab1*-null organoids (related to Fig. 5).