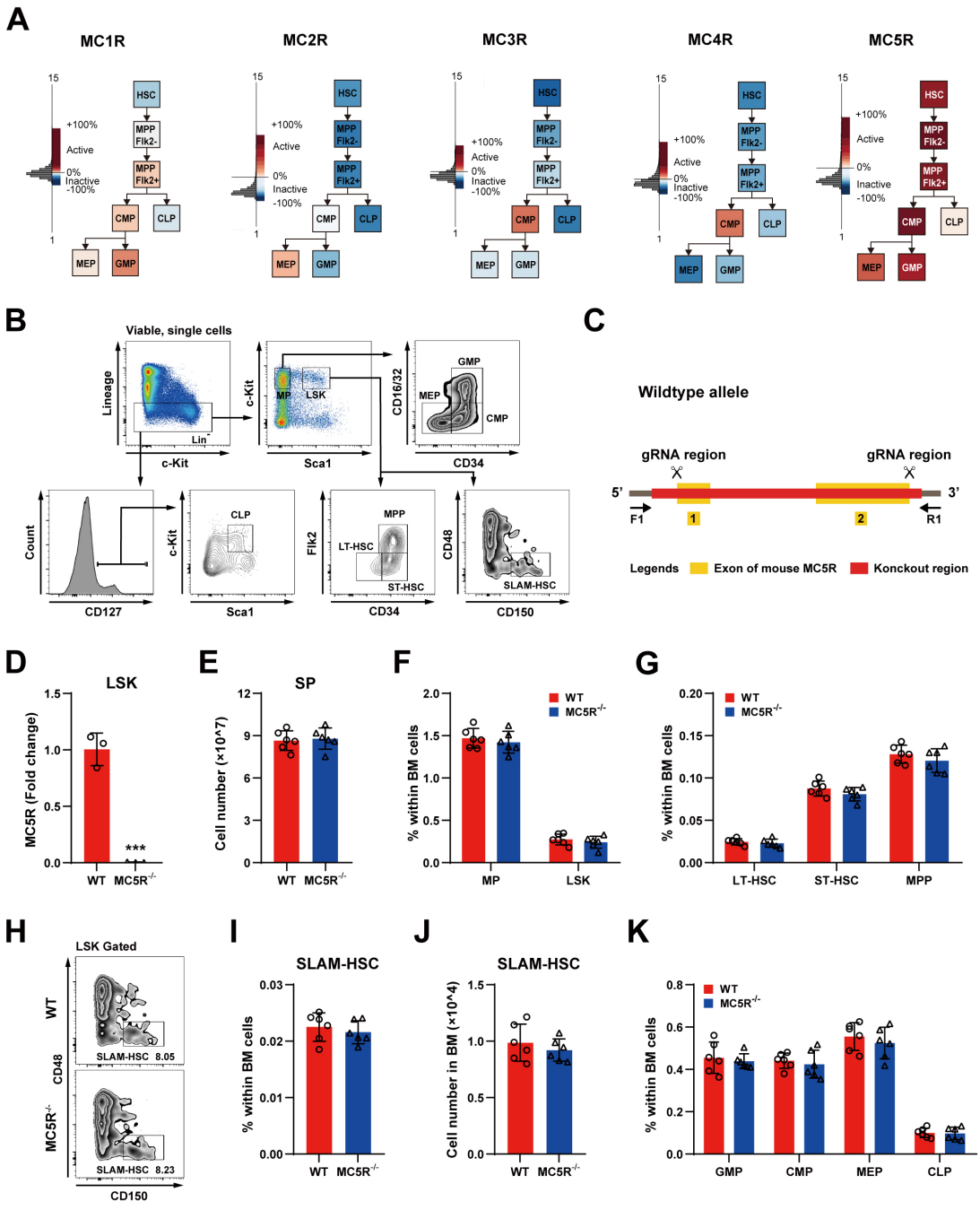
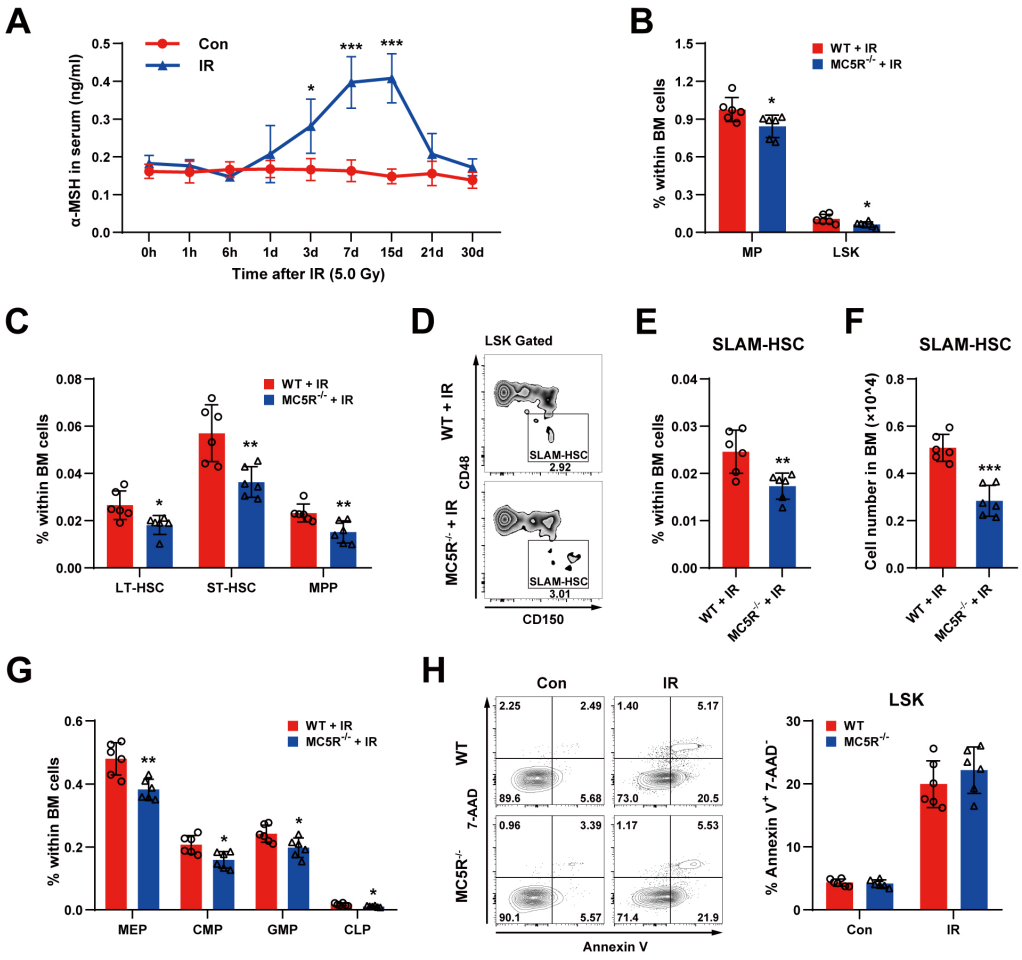
Supplementary Figure S1



Supplementary Figure S1. MC5R is enriched in HSPCs, but its deficiency does not disturb normal hematopoiesis. (A) The expression of MC1R, MC2R, MC3R, MC4R and MC5R in indicated HSPC populations from normal WT mice. Data were obtained from Gene Expression Commons database (https://gexc.riken.jp/models). (B) The

gating strategies for flow cytometric analysis or sorting of Lin^- cells, MPs (Lin^- $Sca1^-$ $c\text{-}Kit^+$), LSKs (Lin^- $Sca1^+$ $c\text{-}Kit^+$), LT-HSCs (Lin^- $Sca1^+$ $c\text{-}Kit^+$ $CD34^-$ $Flk2^-$), ST-HSCs (Lin^- $Sca1^+$ $c\text{-}Kit^+$ $CD34^+$ $Flk2^-$), MPPs (Lin^- $Sca1^+$ $c\text{-}Kit^+$ $CD34^+$ $Flk2^+$), SLAM-HSCs (Lin^- $Sca1^+$ $c\text{-}Kit^+$ $CD150^+$ $CD48^-$), CMPs (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD16/32^-$ $CD34^+$), GMPs (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD16/32^+$ $CD34^+$), MEPs (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD16/32^-$ $CD34^-$) and CLPs (Lin^- $CD127^+$ $Sca1^{low}$ $c\text{-}Kit^{low}$) in the BM of mice. (C) The strategy for generation of MC5R knockout mice. (D) qPCR analysis of the relative expression of MC5R in LSKs sorted from the BM of WT and $MC5R^{-/-}$ mice (n = 3). (E) Total cell number of SP in WT and $MC5R^{-/-}$ mice (n = 6). (F) Flow cytometric analysis of the percentages of MPs and LSKs in the BM of WT and $MC5R^{-/-}$ mice (n = 6). (G) Flow cytometric analysis of the percentages of LT-HSCs, ST-HSCs and MPPs in the BM of WT and $MC5R^{-/-}$ mice (n = 6). (H) Representative flow cytometric plots showing the percentage of SLAM-HSCs in the BM of WT and $MC5R^{-/-}$ mice. (I, J) Flow cytometric analysis of the (I) percentage and (J) number of SLAM-HSCs in the BM of WT and $MC5R^{-/-}$ mice (n = 6). (K) Flow cytometric analysis of the percentages of MEPs, CMPs, GMPs and CLPs in the BM of WT and $MC5R^{-/-}$ mice (n = 6). (D-G and I-K) unpaired Student's *t* test (two-tailed); ***$p < 0.001$.

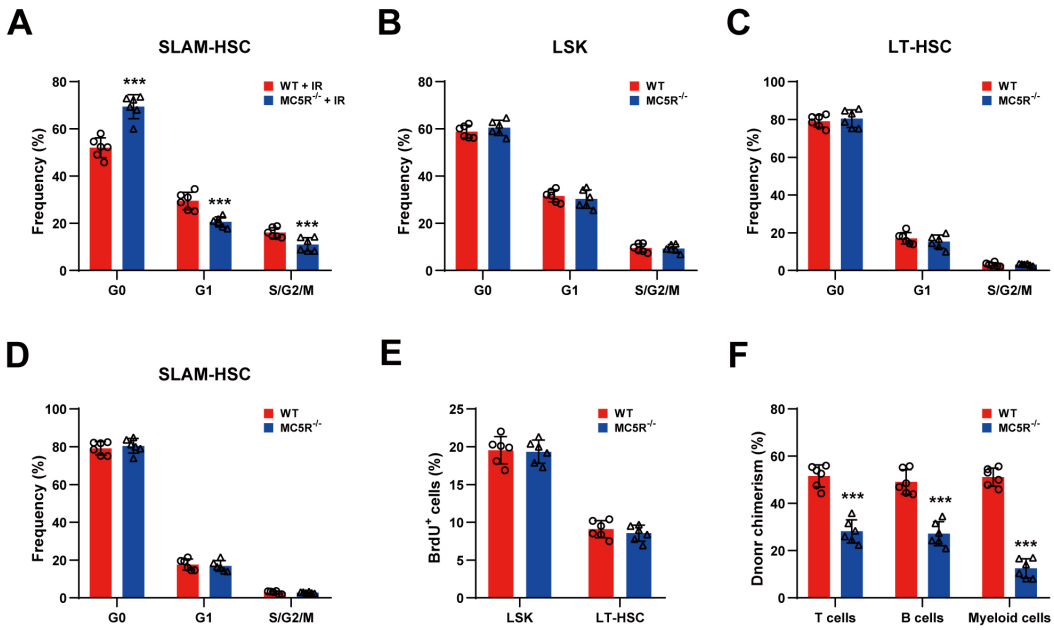
Supplementary Figure S2



Supplementary Figure S2. Loss of MC5R inhibits hematopoietic recovery after radiation injury. (A) ELISA analysis of α-MSH level in the serum of WT mice at indicated time after 5.0 Gy TBI (n = 5). Unirradiated WT mice were served as controls (n = 5). Con, Control; IR, Irradiation. (B) Flow cytometric analysis of the percentages of MPs and LSKs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 6). (C) Flow cytometric analysis of the percentages of LT-HSCs, ST-HSCs and MPPs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 6). (D) Representative flow cytometric plots showing the percentage of SLAM-HSCs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI. (E, F) Flow cytometric

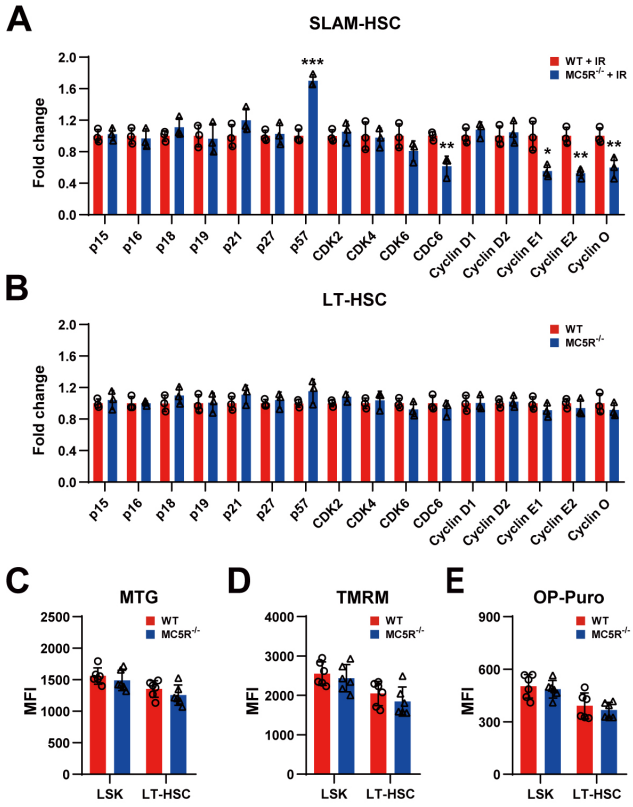
analysis of the (E) percentage and (F) number of SLAM-HSCs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 6). (G) Flow cytometric analysis of the percentages of GMPs, CMPs, MEPs and CLPs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 6). (H) The apoptosis of LSKs in the BM of WT and MC5R$^{-/-}$ mice at steady-state or 15 days after 5.0 Gy TBI (n = 6). Representative flow cytometric plots are shown in the left. (A-C and E-H) unpaired Student's *t* test (two-tailed); *p < 0.05, **p < 0.01, ***p < 0.001.

Supplementary Figure S3



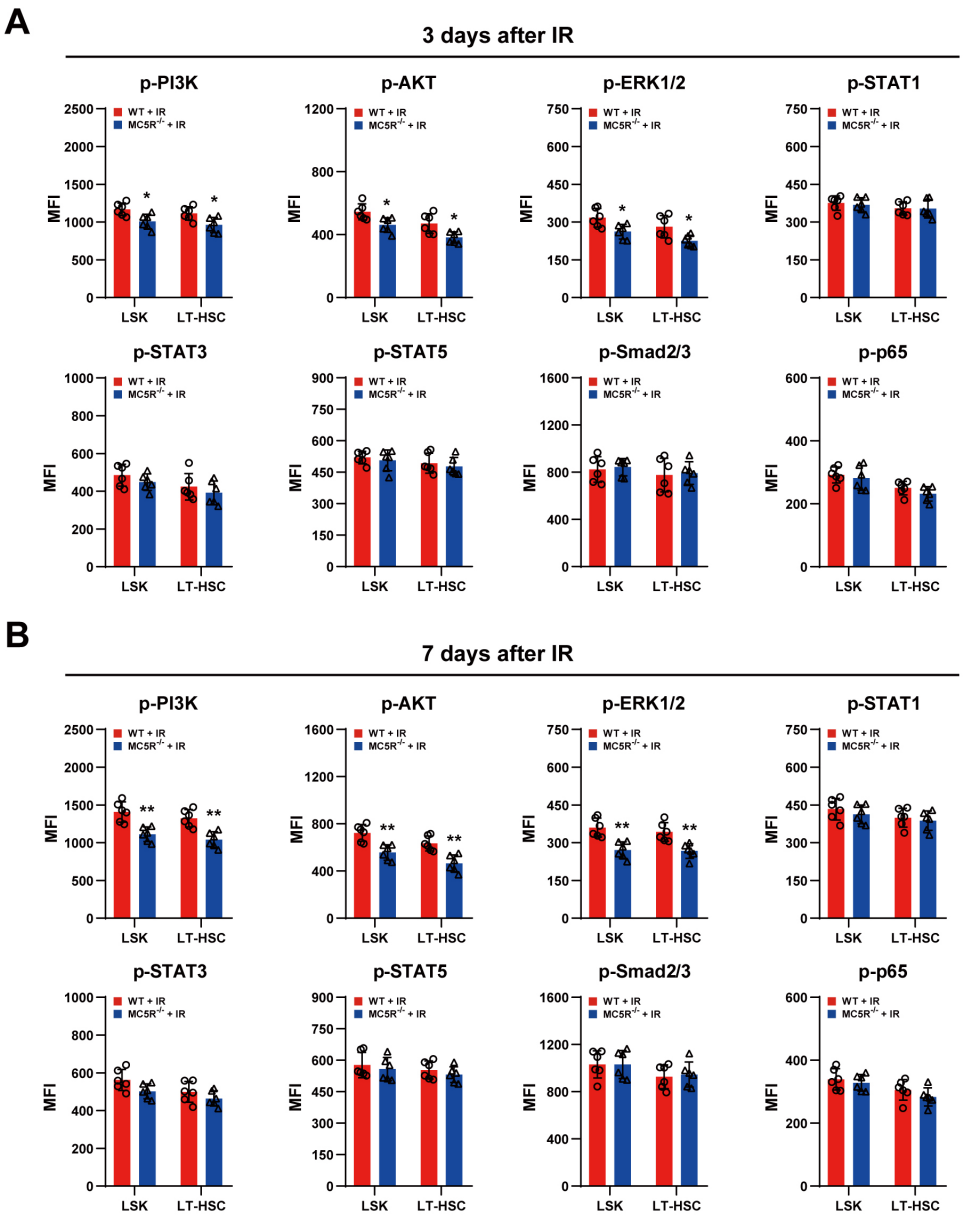
Supplementary Figure S3. MC5R knockout has no effect on the proliferation of HSPCs under normal conditions. (A) Cell cycle analysis of SLAM-HSCs in the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 6). (B-D) Cell cycle analysis of (B) LSKs, (C) LT-HSCs and (D) SLAM-HSCs in the BM of WT and MC5R$^{-/-}$ mice (n = 6). (E) Flow cytometric analysis of the percentage of BrdU^{+} cells in LSKs and LT-HSCs from the BM of WT and MC5R$^{-/-}$ mice (n = 6). (F) The chimerism levels of T cells, B cells and myeloid cells in PB of the recipient mice at 16 weeks after competitive BMT (n = 6). (A-F) unpaired Student's *t* test (two-tailed); ***p < 0.001.

Supplementary Figure S4



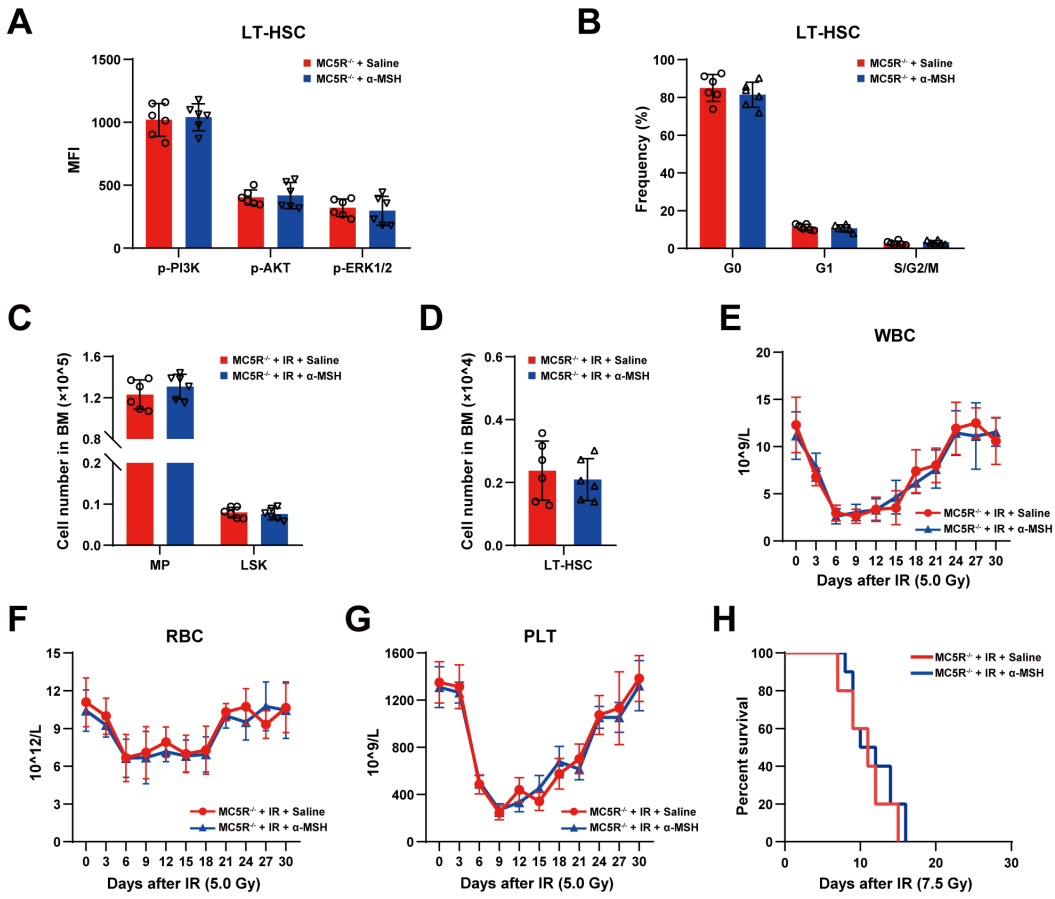
Supplementary Figure S4. MC5R deficiency leads to reduced HSPC activation after irradiation but not at steady-state. (A) qPCR analysis of the relative expression of cell cycle-associated genes in SLAM-HSCs sorted from the BM of WT and MC5R$^{-/-}$ mice at 15 days after 5.0 Gy TBI (n = 3). (B) qPCR analysis of the relative expression of MC5R in LT-HSCs sorted from the BM of WT and MC5R$^{-/-}$ mice (n = 3). (C) Flow cytometric analysis of mitochondrial mass in LSKs and LT-HSCs from the BM of WT and MC5R$^{-/-}$ mice by MTG staining (n = 6). (D) Flow cytometric analysis of mitochondrial membrane potential in LSKs and LT-HSCs from the BM of WT and MC5R$^{-/-}$ mice by TMRM staining (n = 6). (E) Flow cytometric analysis of the protein synthesis rate in LSKs and LT-HSCs from the BM of WT and MC5R$^{-/-}$ mice by OP-Puro incorporation analysis (n = 6). (A-E) unpaired Student's *t* test (two-tailed); *p < 0.05, **p < 0.01, ***p < 0.001.

Supplementary Figure S5



Supplementary Figure S5. MC5R deletion impairs the PI3K/AKT and MAPK pathways but not the STATs, TGFβ and NF-κB pathways in HSPCs after irradiation. (A, B) Flow cytometric analysis of the expression of p-PI3K, p-AKT, p-ERK1/2, p-STAT1, p-STAT3, p-STAT5, p-Smad2/3 and p-p65 in LSKs and LT-HSCs in the BM of WT and MC5R$^{-/-}$ mice at (A) 3 and (B) 7 days after 5.0 Gy TBI (n = 6). (A and B) unpaired Student's *t* test (two-tailed); *p < 0.05, **p < 0.01.

Supplementary Figure S6



Supplementary Figure S6. Melanocortin promotes hematopoietic regeneration of irradiated mice via MC5R. (A) Flow cytometric analysis of the expression of p-PI3K, p-AKT and p-ERK1/2 in LT-HSCs in the BM of MC5R$^{-/-}$ mice after treated with saline or α-MSH (n = 6). (B) Cell cycle analysis of LT-HSCs in the BM of MC5R$^{-/-}$ mice after treated with saline or α-MSH (n = 6). (C, D) The numbers of MPs, LSKs and LT-HSCs in the BM of MC5R$^{-/-}$ mice treated with saline or α-MSH at 15 days after 5.0 Gy TBI (n = 6). (E-G) The counts of WBC (E), RBC (F) and PLT (G) in the PB of MC5R$^{-/-}$ mice treated with saline or α-MSH at 15 days after 5.0 Gy TBI (n = 9). (H) The survival rates of MC5R$^{-/-}$ mice treated with saline or α-MSH after 7.5 Gy TBI (n = 10). (A-G) unpaired Student's *t* test (two-tailed); (H) Log-rank test.

Supplementary Table S1

Primers for mRNA expression analysis

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
MC5R	AGAGCAGAATGGTAAATCCGATG	CTCTGAGGCGTTCAGGGTAAG
p15	CGAAGGACCATTTCTGCCACA	TGCCCATCATCATGACCTGGAT
p16	TGAATCTCCGCGAGGAAAGC	TGCCCATCATCATCACCTGAA
p18	GTGAATGTGTAGAGGTCTC	AAACAAAGCGAAAGGAAAC
p19	GAAGTGTGAGCCATCTAC	CAACAGACTGGAAACCTT
p21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
p27	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
p57	CGAGGAGCAGGACGAGAATC	GAAGAAGTCGTTCGCATTGGC
CDK2	ACTGAGACTGAAGGTGTA	TTGACGATATTAGGGTGATTA
CDK4	TGATGGATGTCTGTGCTA	TCCTGGTCTATATGCTCAA
CDK6	CTGGTGTGAGATGTTATCATT	TAGTCAGAGCAGGAAGTG
CDC6	AAAGGCCCCATGATCGTGTT	CCAATGAGCACCAATCGGGA
Cyclin D1	GTTCTAATGGAATGGATGG	CAAGCACCTCATACTACC
Cyclin D1	CATTCAGACACAGGACTT	GTATAGATGCCAAGAAGGAA
Cyclin E1	ATGGAATTGATGATGATGA	CACTTGGACATAGACATT
Cyclin E2	CACAGATGAGGTCAATACT	GCAATGAACAATGAGGTAA
Cyclin O	CCGAATCGCGCTGTAAACTG	CGTGAGGAGAAAACGGTCCA
GAPDH	CCTCGTCCCGTAGACAAAATG	TCTCCACTTTGCCACTGCAA