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Supplemental Information

An ERK-Dependent Feedback Mechanism Prevents Hematopoietic Stem Cell Exhaustion

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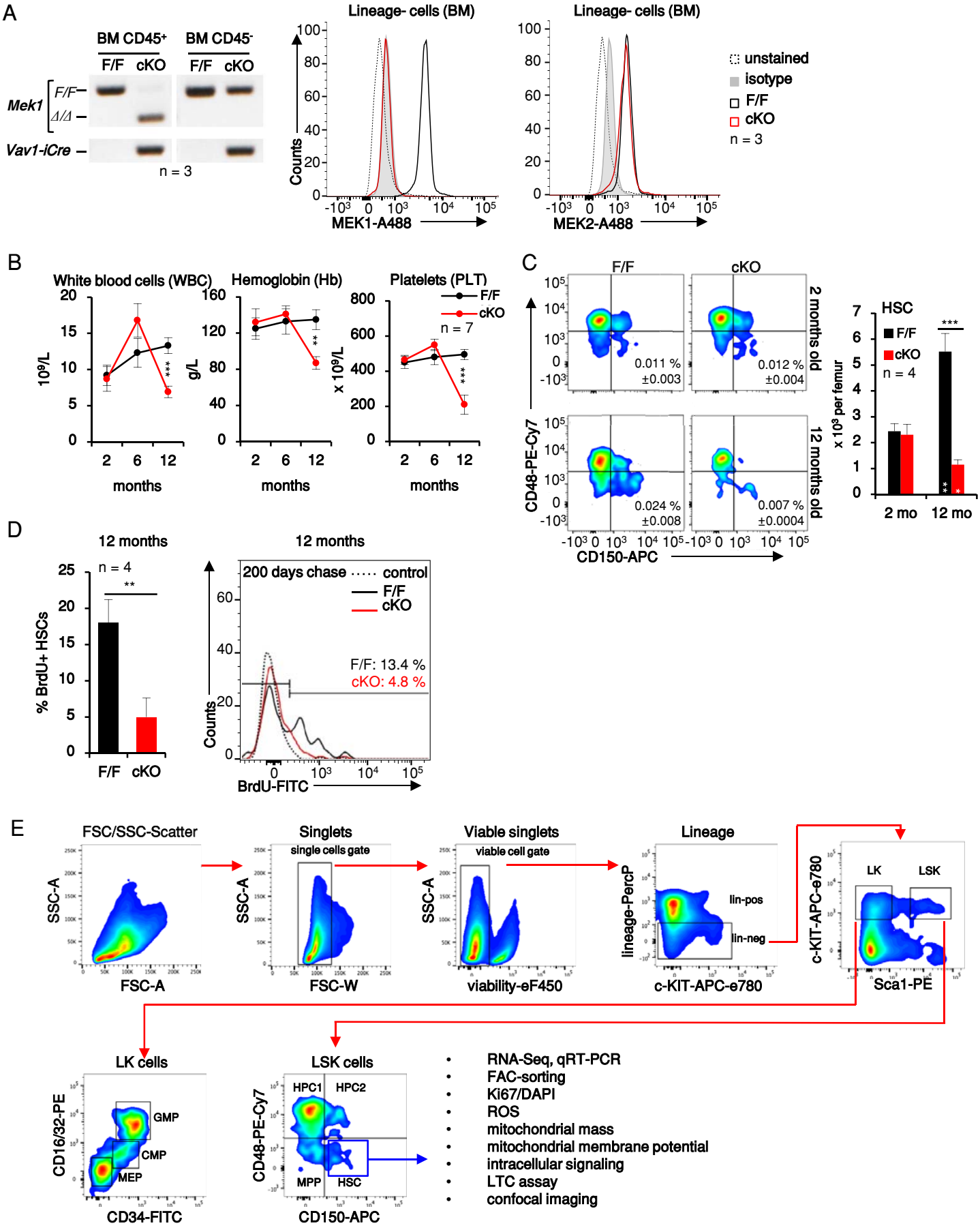

Figure S1. Related to Figure 1

MEK1 protects the hematopoietic compartment during ageing. A, Efficient MEK1 deletion in BM cells. Left, PCR genotyping of CD45+ (hematopoietic cells) and CD45- (stroma) bone marrow cells; right, FACS analysis of MEK1 and MEK2 expression in lineage- BM cells. **B**, Blood analysis of F/F and cKO mice of different ages. **C**, Frequency (representative density plots) and number of HSCs per femur in young and aged F/F and cKO mice. **D**, % BrdU+ HSCs in 12-month old mice 200 days after pulse-labelling and representative FACS histogram. **E**, Gating strategy for SLAM-marker-defined mouse HSPCs. Error bars represent the standard deviation of the mean. *P<0.05, **P<0.01, ***P<0.001. White asterisks in bar graph: comparison of young versus old animals of the same genotype.

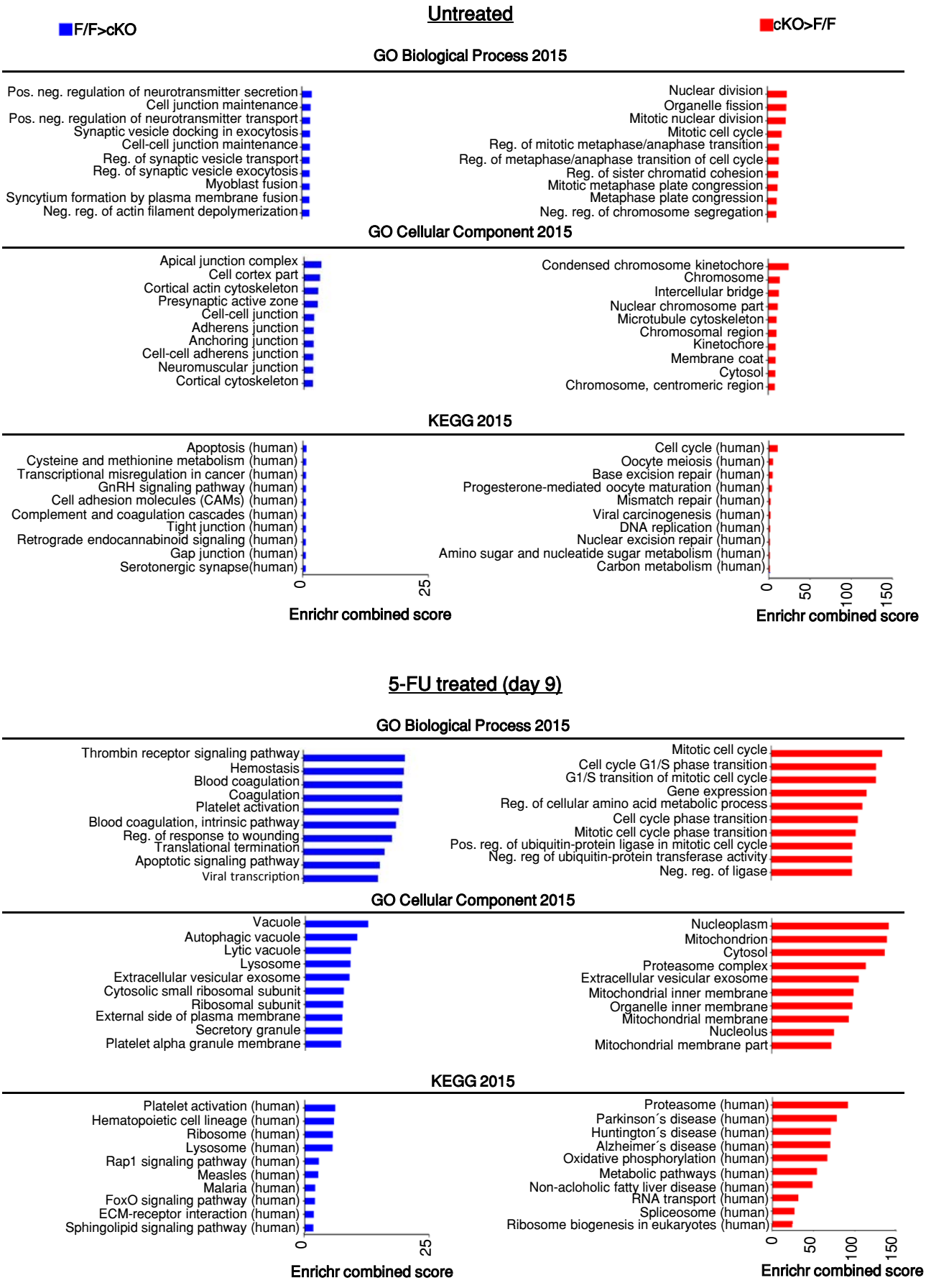

Figure S2. Related to Figure 2

Functional enrichment analysis of untreated and 5-FU treated F/F versus cKO HSCs. The top 10 terms ranked according to the combined Enrichr score (Kuleshov et al., 2016) are visualized.

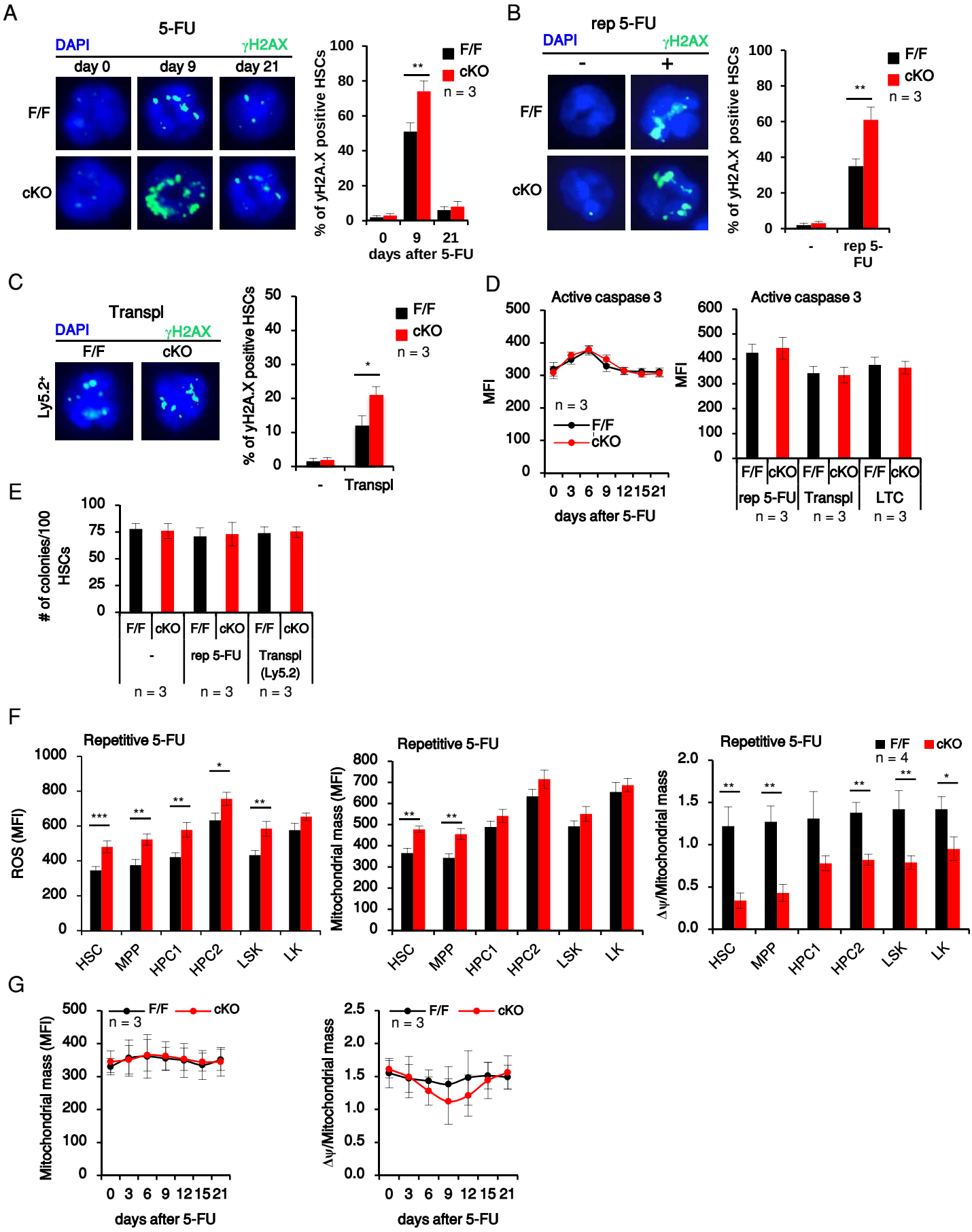

Figure S3. Related to Figure 3

DNA-damage, apoptosis, senescence, ROS levels and mitochondrial parameters in BM stem/progenitor cells from mice exposed to 5-FU or transplantation. A-C, Hematopoietic stress increases DNA damage (left panels show representative images of γH2A.X nuclear staining, the right panels a quantification of the results), but does not induce apoptosis (**D,** measured as active caspase 3) or senescence (**E,** measured as the % of HSCs able to form colonies from a single cell) in HSCs. **F,** ROS levels (left), mitochondrial mass (center), and Δψ per mitochondrial mass (right) in lineage-negative BM cells from mice exposed to rep 5-FU. Gating strategy in Figure S1E. **G**, Mitochondrial mass (left) and Δψ per mitochondrial mass (right) in HSCs recovering from a single 5-FU injection. Error bars represent the standard deviation of the mean.*P<0.05, **P<0.01, ***P<0.001.

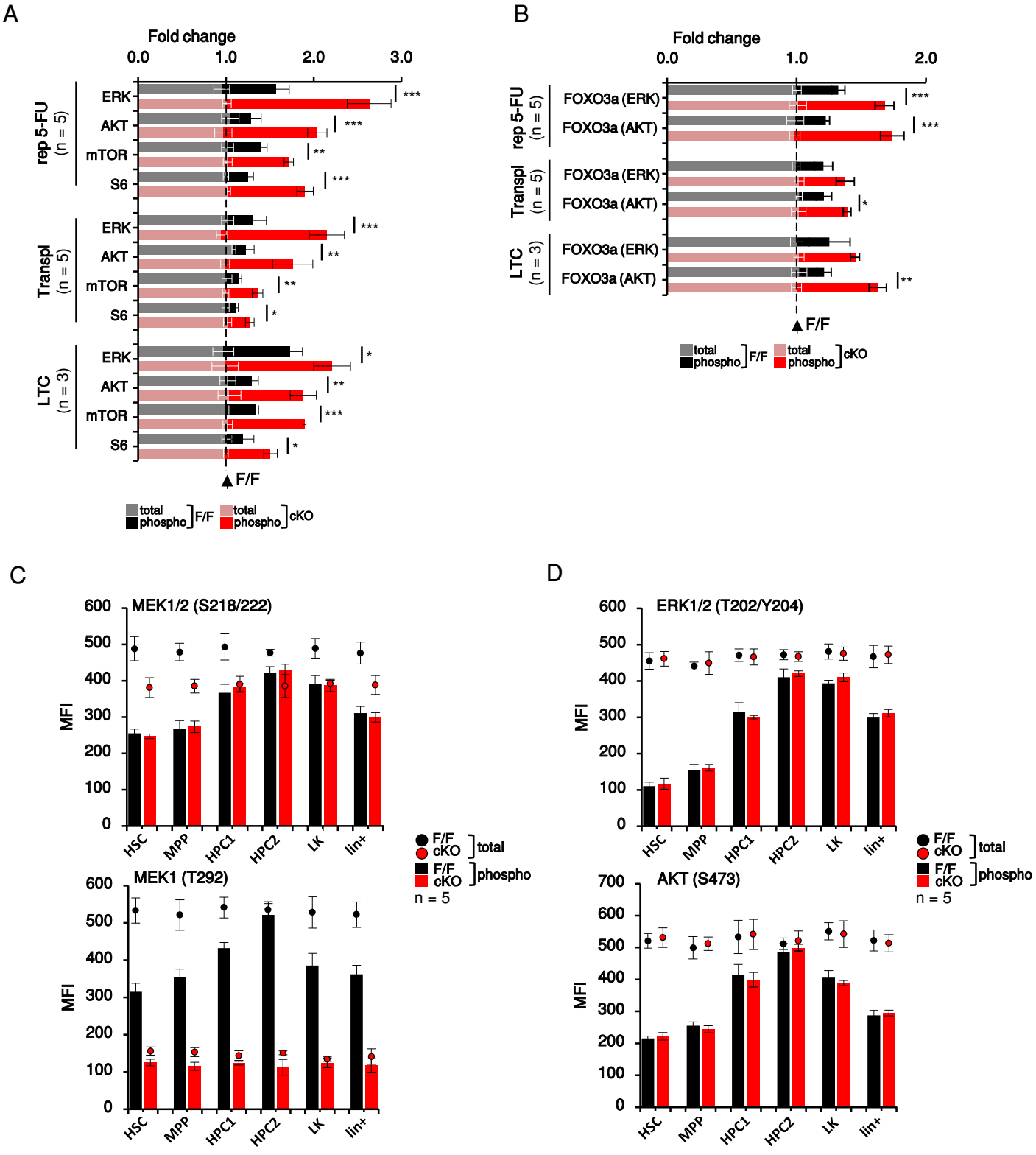

Figure S4. Related to Figure 4

Signaling events in hematopoietic stem/progenitor cells. A, Activation of ERK, AKT, mTOR and S6 and **B**, phosphorylation of FOXO3A in HSCs undergoing chronic stress. Data represent fold change relative to HSCs from untreated F/F mice. **C-D**, Expression and/or phosphorylation of MEK, ERK and AKT in hematopoietic stem and progenitor cells from untreated mice. Gating strategy in Figure S1E. Error bars represent the standard deviation of the mean. *P<0.05, **P<0.01, ***P<0.001.

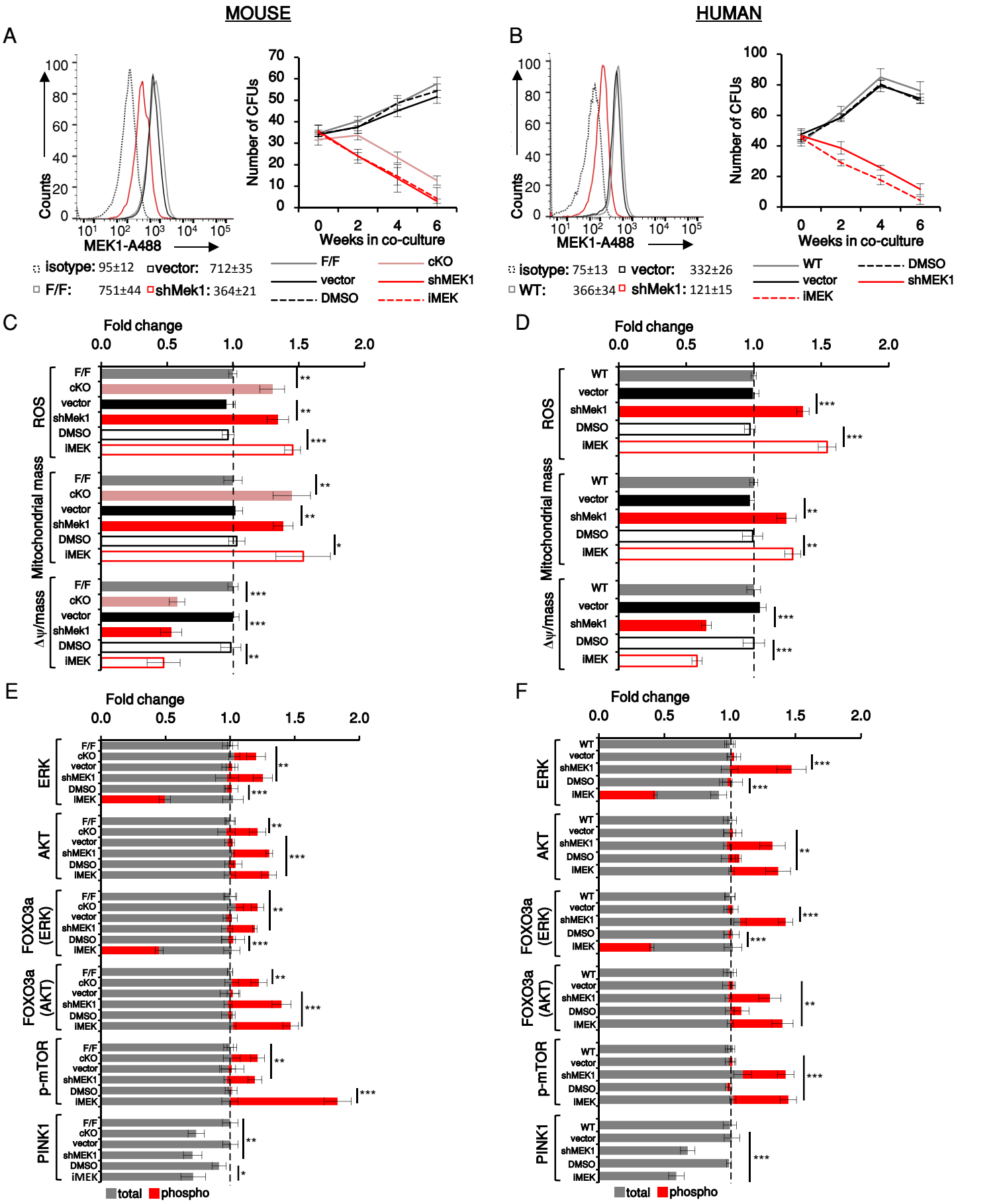

Figure S5. Related to Figure 5

MEK1 silencing and MEK inhibition phenocopy the MEK1-cKO in mouse and human HSCs. A, B MEK1 silencing in purified HSCs (left panels; mean fluorescence intensity (MFI) values ± s.d. are shown below the histograms, n=3) and CFUs derived from HSCs in LTC assays (right panels) upon MEK1 silencing or MEK inhibition (iMEK = U0126). **C, D** ROS levels, mitochondrial mass, and Δψ per mitochondrial mass in mouse (**C**) and human (**D**) HSCs after 6 weeks in LTC. **E, F** Intracellular signaling (ERK, AKT, FOXO3A, mTOR) and PINK1 expression levels in the same HSCs (n=3). Data in C-F represent fold change relative to untreated HSCs (dotted line). Error bars represent the standard deviation of the mean. *P<0.05, **P<0.01, ***P<0.001 F/F versus cKO or DMSO versus iMEK.

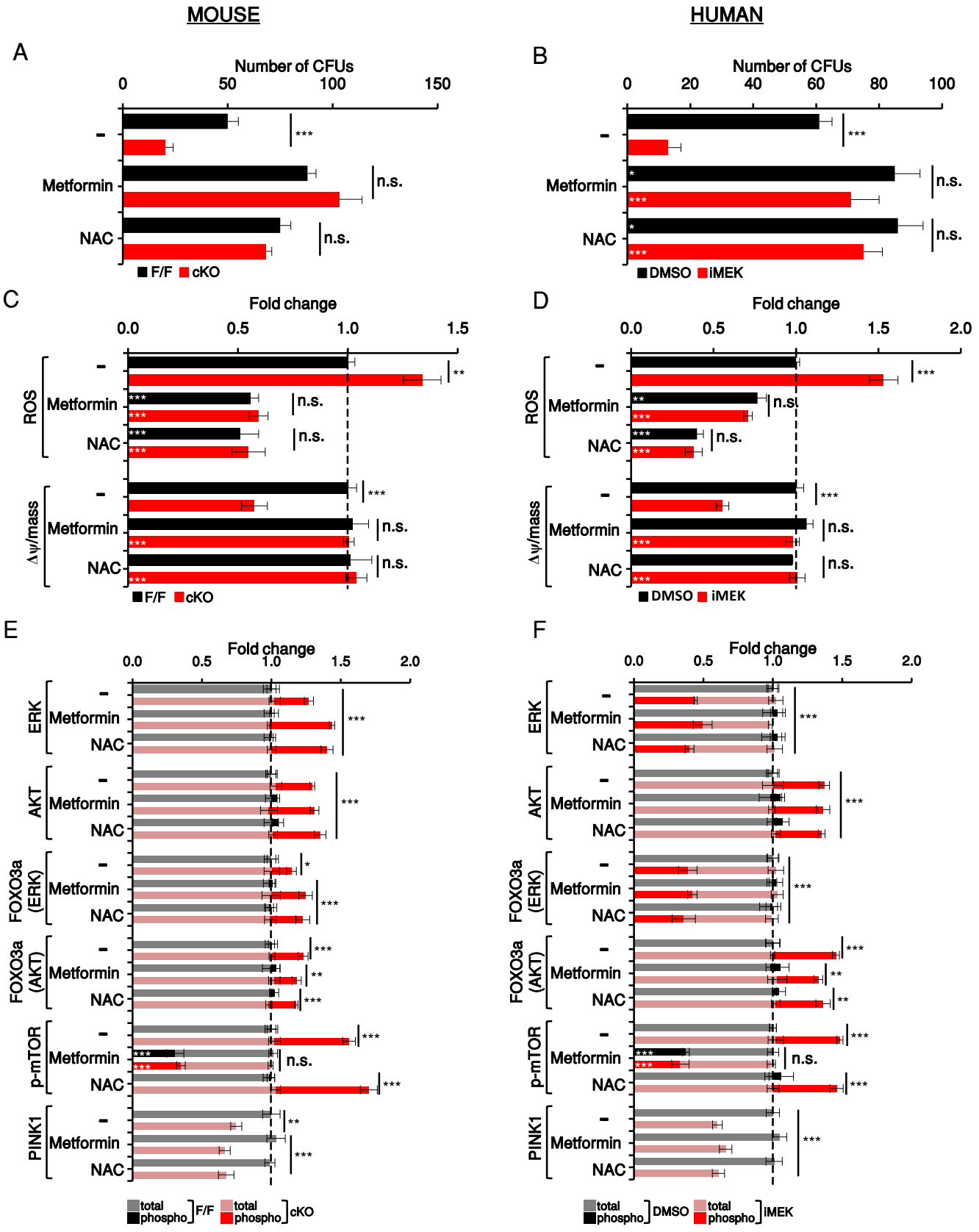

Figure S6. Related to Figure 5.

OXPHOS or ROS inhibition promote HSC expansion and rescue the mitochondrial defects of MEK1-deficient or MEK-inhibited HSCs. F/F and cKO HSCs (**A**) and human DMSO or iMEK treated HSCs (**B**) were treated with Metformin or NAC for 6 weeks in LTCs. A,B CFUs, **C,D** ROS levels and Δψ per mitochondrial mass and E,F intracellular signaling were determined in these cells. Data in C-F represent fold change relative to F/F HSCs (dotted line; n=3). Error bars represent the standard deviation of the mean.*P<0.05, **P<0.01, ***P<0.001. White asterisks in bar graphs: comparison of untreated versus Metformin or NAC treated cultures.

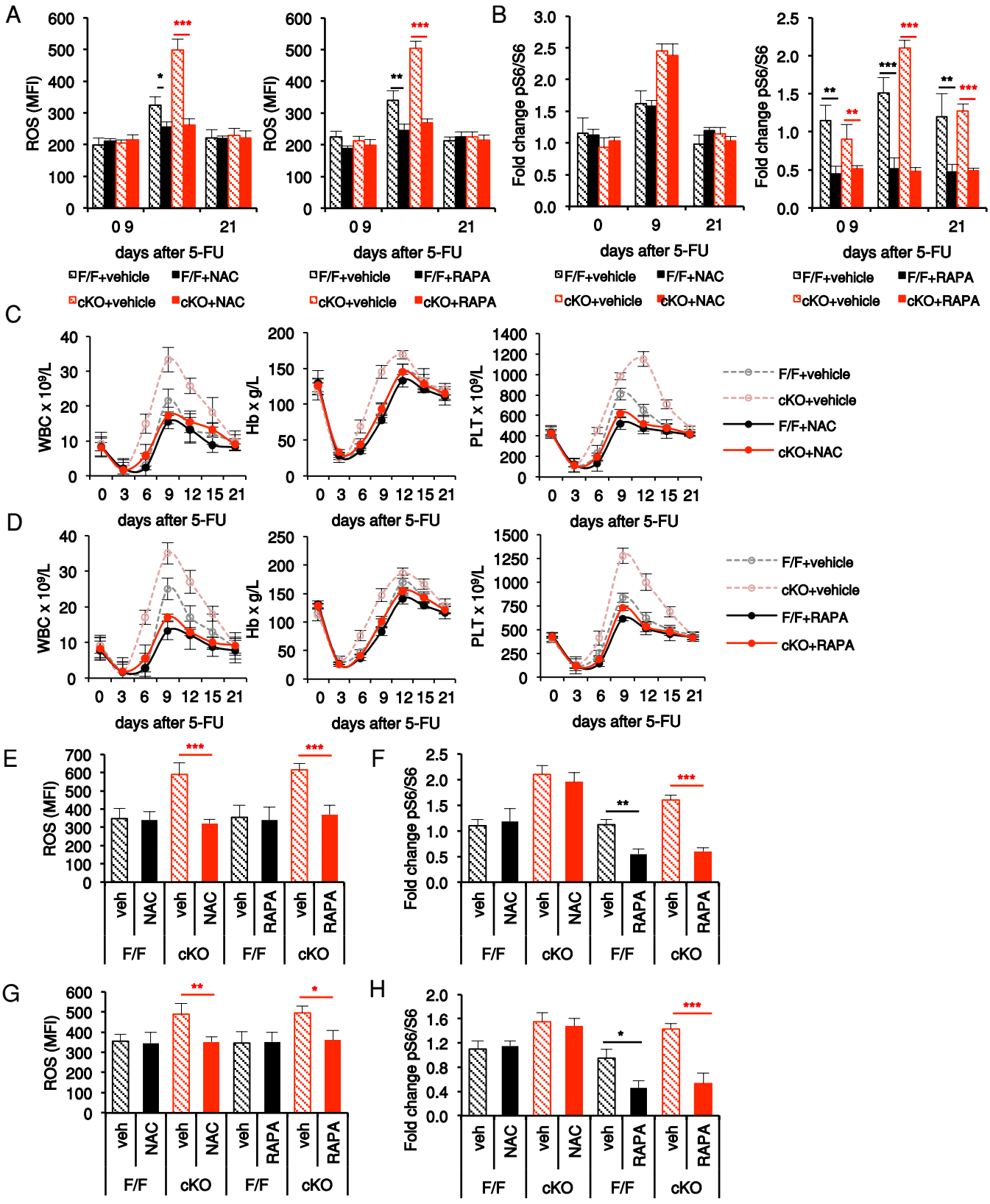

Figure S7. Related to Figure 6.
Impact of NAC and Rapamycin on ROS production and S6 phosphorylation during HSC activation in vivo. HSC ROS and S6 phosphorylation levels (**A,B**) and peripheral blood parameters (WBC, white blood cells; Hb, hemoglobin; PLT, platelets; **C,D**) in vehicle, NAC, or Rapamycin-treated F/F and MEK1-cKO mice recovering from a single 5-FU injection. **E-H**, ROS and S6 phosphorylation levels in the HSCs of vehicle- NAC, or Rapamycin-treated F/F and MEK1-cKO mice subjected to chronic 5-FU treatment (**E,F**) or in F/F and cKO HSCs transplanted in lethally irradiated recipient mice treated with NAC or Rapamycin (**G,H**) as indicated. Error bars represent the standard deviation of the mean.*P<0.05, **P<0.01, ***P<0.001 comparing vehicle versus NAC or RAPA treatment of the same genotype.

Additional Data table S1 (separate Excel file). Related to Figure 2.
Full list of genes expressed in wild-type and cKO HSC isolated from untreated or 5-FU-treated mice. The list is the basis for the analysis shown in Fig. 2 and S2.

Table S2

List of qRT-PCR primers used in this study. Related to STAR Methods.

qRT-PCR primer	Sequence
Actb forward	5′-CGCCACCAGTTCGCCATGGA-3′
Actb reverse	5′-TACAGCCCGGGGAGCATCGT-3′
Catalase forward	5′-GTCCAGTGCGCTGTAGATGTG-3′
Catalase reverse	5′-CCTCCTCATTCAACACCTTTGTG-3′
Ndufb6 forward	5′-TCGCTGTTTCTCATGTGCTT-3′
Ndufb6 reverse	5′-TCTCCAGTCTCCAGAATTGTATCA-3′
Cox5a forward	5′-TGCCTGGGAATTGCGTAAAGGGATG-3′
Cox5a reverse	5′-TCAAGGCCCAGCTCCTCTGGA-3′
Cyc1 forward	5′- AGCCTACAAGAAAGTTTGCCTAT-3′
Cyc1 reverse	5′-TCTTCTTCCGGTAGTGGATCTTGGC-3′
Sdhb forward	5′-GCTGCGTTCTTGCTGAGACA-3′
Sdhb reverse	5′-ATCTCCTCCTTAGCTGTGGTT-3′
Atp5o forward	5′-ACTCGGGTTTGACCTACAGC-3′
Atp5o reverse	5′-GGTACTGAAGCATCGCACCT-3′
Sod2 forward	5′-TTAACGCGCAGATCATGCA-3′
Sod2 reverse	5′- GGTGGCGTTGAGATTGTTCA-3′
Pink1 forward	5′-GCGAAGCCATCTTAAGCAAA-3′
Pink1 reverse	5′-TGGGACCATCTCTGGATCTT-3′
Cdk4 forward	5′-AACAAGTCCCCACCTCTCCT-3′
Cdk4 reverse	5′-TCAGGGAGGGAAGAAGACAG-3′

Ccnd1 forward	5′-GTTCATTTCCAACCCACCCTC-3′
Ccnd1 reverse	5′-AGAAAGTGCGTTGTGCGGTAG-3′
Ccnb1 forward	5′-AAAGGGAAGCAAAAACGCTAGG-3′
Ccnb1 reverse	5′-TGTTCAAGTTCAGGTTCAGGCTC-3′
Cdkn2c forward	5′-GGCTGTCCGTTTCACTATCA-3′
Cdkn2c reverse	5′-TTTTGAAGGATTTGGCTGCT-3′
Cdkn1a forward	5′-TCCACAGCGATATCCAGACA-3′
Cdkn1a reverse	5′-GGACATCACCAGGATTGGAC-3′