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Supplemental Information

TET2-Loss-of-Function-Driven Clonal Hematopoiesis Exacerbates Experimental Insulin Resistance in Aging and Obesity

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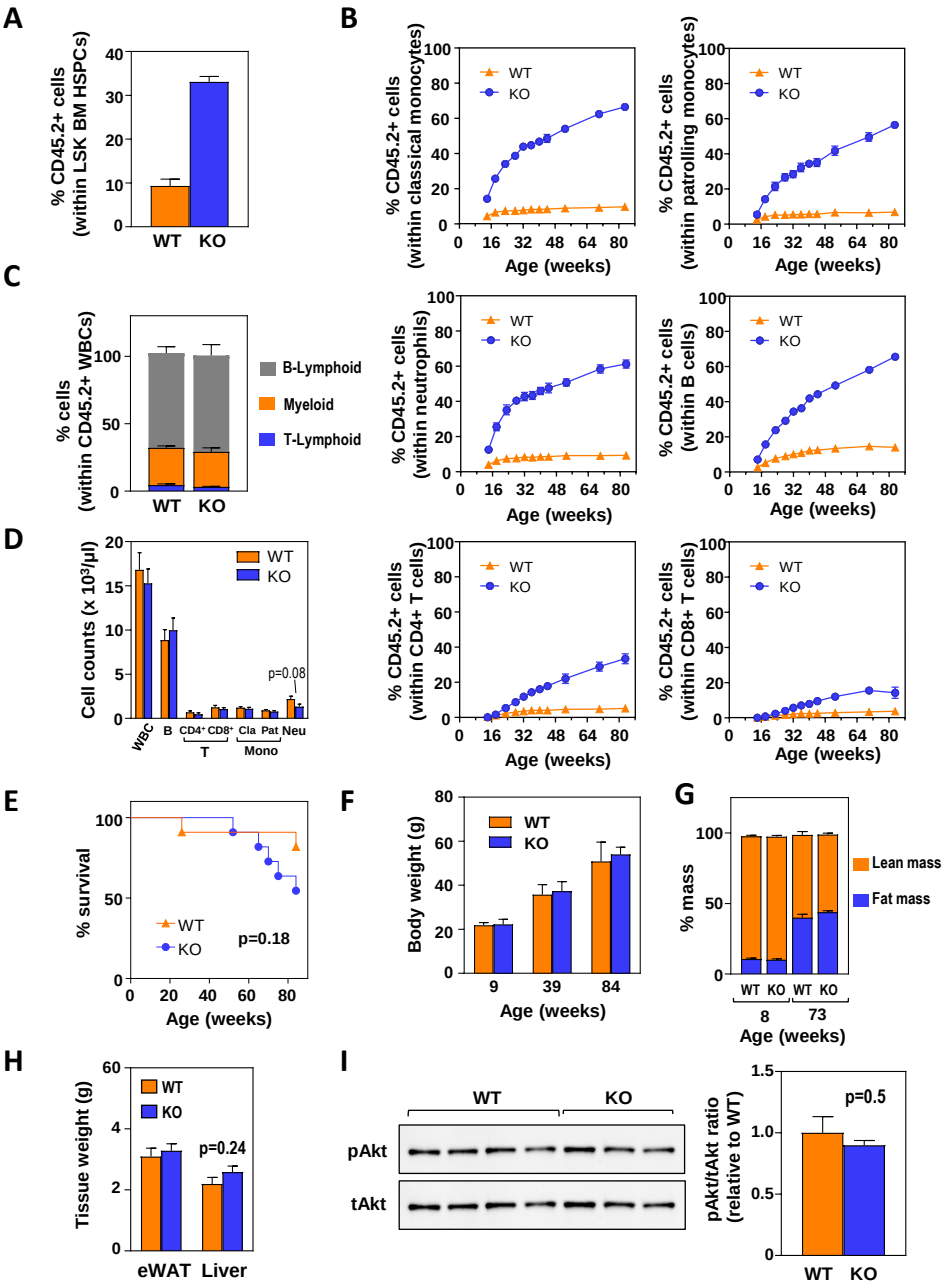
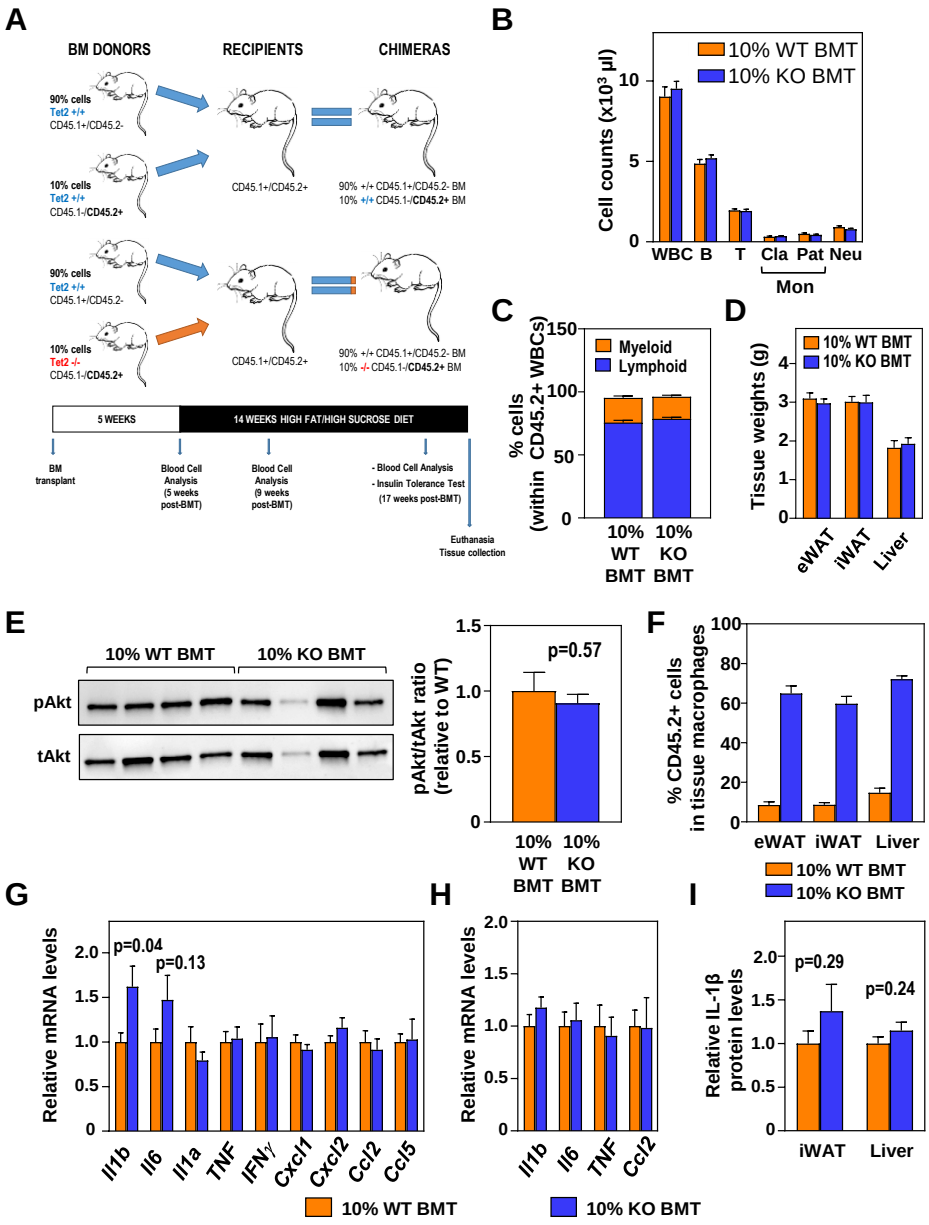
SUPPLEMENTAL MATERIAL

Gene	Forward Primer	Reverse Primer
36b4	GCTCCAAGCAGATGCAGCA	CCGGATGTGAGGCAGCAG
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Tet2 (exons 8-9)	AACCTGGCTACTGTCATTGCTC	ATGTTCTGCTGGTCTCTGTGGGAA
Il1b	TGACAGTGATGAGAATGACCT	TTGGAAGCAGCCCTTCATCT
Il6	GCTACCAAACTGGATATAATCA	CCAGGTAGCTATGGTACTCCAGAA
Ila	GCACCTTACACCTACCAGAGT	AAACTTCTGCCTGACGAGCTT
Tnf	CGGAGTCCGGGCAGG	GCTGGGTAGAGAATGGATGAA
Ifng	ACAGCAAGGCGAAAAAGGATG	TGGTGGACCACTCGGATGA
Ccl2	CAGCCAGATGCAGTTAACGC	GCCTACTCATTGGGATCATCTTG
Cxcl1	CCGAAGTCATAGCCACACTCAA	CAAGGGAGCTTCAGGGTCAA
Cxcl2	TGACTTCAAGAACATCCAGAGC	CTTGAGAGTGGCTATGACTTCTGTCT
Cxcl5	GCC GCT GGC ATT TCT GTT	GGG CAG CTT CAG CTA GAT GCT
Ccl5	CAGCAGCAAGTGCTCCAATC	CACACACTTGGCGGTTCCTT
Emr1	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Cd68	TTC TCC AGC TGT TCA CCT	GTT GCA AGA GAA ACA TGG CCC
Cd11c	ATG GAG CCT CAA GAC AGG	GGA TCT GGG ATG CTG AAA TC
Selp	CATCTGGTTCAGTGCTTTGATC	ACCCGTGAGTTATTCCATGAGT
Sele	ATGCCTCGCGCTTTCTCTC	GTAGTCCCGCTGACAGTATGC
Icam1	GTGATGCTCAGGTATCCATCCA	CACAGTTCTCAAAGCACAGCG
Vcam	TAGAGTGCAAGGAGTTCGGG	CCGGCATATACGAGTGTGAA
Irs1	CGATGGCTTCTCAGACGTG	CAGCCCGCTTGTTGATGTTG
Irs2	CTGCGTCCTCTCCCAAAGTG	GGGGTCATGGGCATGTAGC

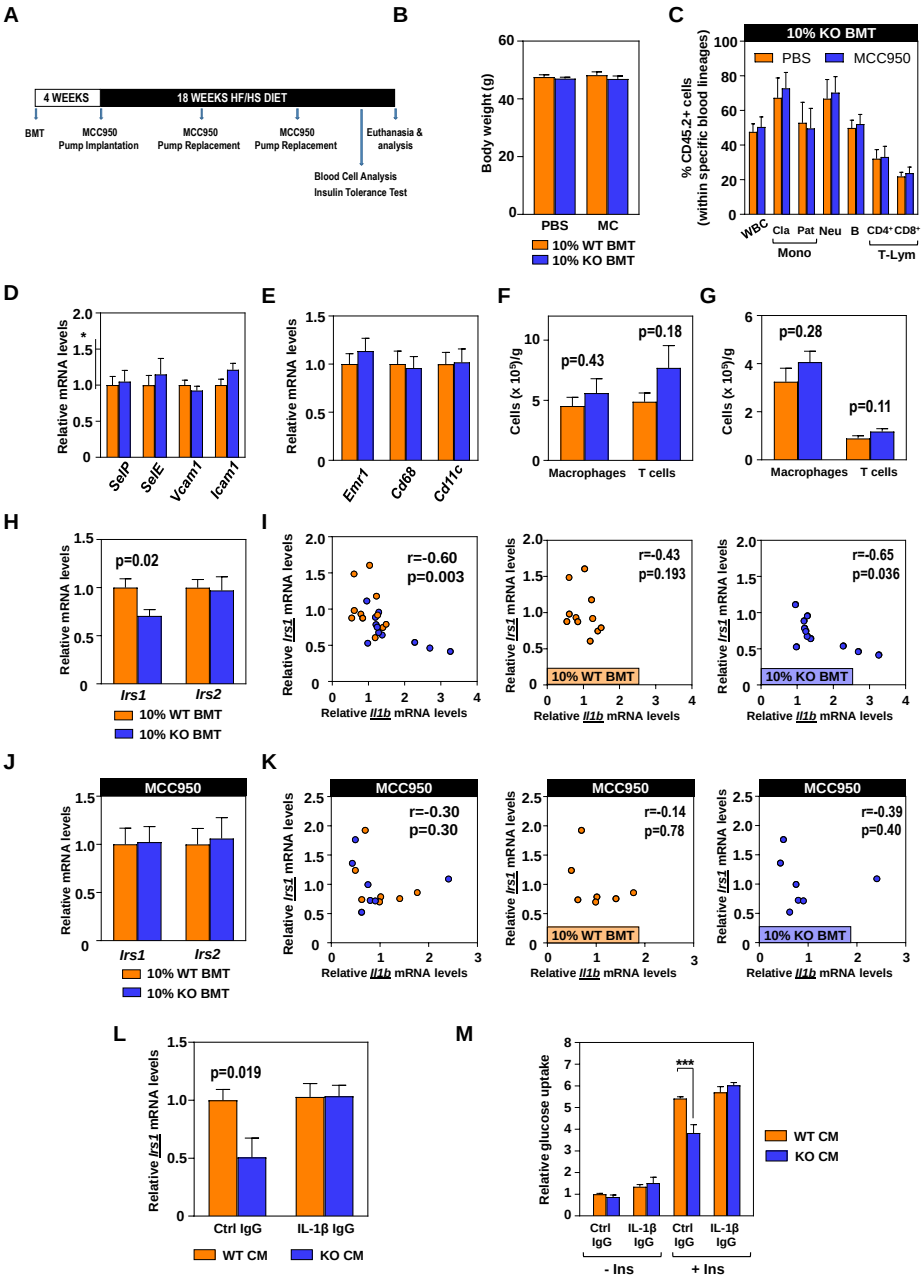
Supplemental Table (related to STAR Methods). Oligonucleotides used as primers in qPCR assays.



Supplemental Figure S1 (related to Fig.1). *Tet2*-/- (KO) or +/+ (WT) CD45.1-/CD45.2+ BM cells were delivered via adoptive transfer to non-conditioned 10 week-old CD45.1+/CD45.2- recipient mice, which were then monitored until the age of 84 weeks (~1.6 years). n=9 WT, 6 KO mice. **A.** % of CD45.2+ cells within Lineage- Sca1+ Kit+ (LSK) HSPC populations in bone marrow. **B.** % of CD45.2+ cells within various blood cell populations, evaluated by flow cytometry at various time points (Cla: classical; Pat: patrolling monocytes). **C.** % of B-lymphoid (CD115-, Ly6G-, CD3-B220+), T-lymphoid (CD115-, Ly6G-, B220- CD3+) and myeloid (CD115+ or Ly6G+) cells in CD45.2+ WBC populations at 82 weeks of age**. D**. Absolute counts of white blood cells (WBCs) and specific blood cell lineages at 82 weeks of age. **E.** Survival curves (initial sample size n=11 mice per BM genotype), compared using a Cox Mantel log-rank test. **F.** Body weight. **G.** % body fat and lean mass, assessed by magnetic resonance imaging. **H.** eWAT and liver weight. **I.** Western Blot analysis of Akt Ser473 phosphorylation in liver in *ad lib* fed mice. A representative blot is shown.



Supplemental Figure S2 (related to Fig.2). Competitive BMTs were used to generate mice carrying 10% *Tet2*-/- (10% KO BMT) or +/+ (10% WT BMT) hematopoietic cells, which were then fed HF/HS diet for 14 weeks. **A.** Summary of the BMT approach and the timeline of BMT and hematological and metabolic studies used to investigate the effects of TET2 loss of function-driven clonal hematopoiesis in obese mice. **B.** Absolute counts of WBCs and different blood cell lineages after 12 weeks of HF/HS diet feeding (17 weeks post-BMT, n=15 mice per BM genotype) (Cla: classical; Pat: patrolling monocytes). **C**. % of lymphoid (CD115-, Ly6G- and B220+ or CD3+) and myeloid (CD115+ or Ly6G+) cells in CD45.2+ WBC populations (n=15 mice per BM genotype)**. D.** eWAT, inguinal WAT (iWAT) and liver weight (n=11 per BM genotype). **E.** Western Blot analysis of insulin-induced Akt Ser473 phosphorylation in liver (n= 4 mice per BM genotype). **F.** % CD45.2+ cells within F4/80+ macrophage populations in matched eWAT, iWAT and liver samples (n=5 mice per BM genotype). **G, H**. qPCR analysis of transcript levels of several pro-inflammatory cytokines and chemokines in eWAT (G) and liver (H) (n=11 per BM genotype). **I**. ELISA analysis of IL-1β protein levels in iWAT and liver (n=11 per BM genotype).



Supplemental Figure S3 (related to Figs-1-3). A-C. HF/HS-diet fed 10% WT BMT and 10% KO BMT mice received a continuous infusion of MCC950 or phosphate buffered saline vehicle via subcutaneous osmotic pumps. (A) Timeline of BM transplantation, MCC950 treatment, HF/HS diet feeding and hematological and metabolic studies. (B) Body weight. (C) Percentage of CD45.2+ cells within WBCs and main blood cell lineages in 10% KO BMT mice after 17 weeks on HF/HS diet (21 weeks post-BMT), measured by flow cytometry (n=7 mice per treatment, MCC950 or PBS vehicle). **D, E.** qPCR analysis of transcript levels of endothelial adhesion molecules (D) and transcripts expressed at high levels in macrophages (E) in eWAT of obese 10% KO BMT and 10% WT BMT mice (n=11 per experimental group). **F, G.** Flow cytometry analysis of absolute numbers of F4/80+ macrophages and CD3+ T cells in eWAT of aged mice after adoptive transfer (F, n=9 WT, 6 KO) or obese mice after competitive BMT (G, n=7 10% WT BMT, n=6 10% KO BMT) mice. **H, I.** qPCR analysis of *Irs1* and *Irs2* transcript levels (H) and Spearman analysis of the correlation between *Irs1* and *Il1b* transcript levels (I) in eWAT of obese

10% KO BMT and 10% WT BMT mice (n=11 per BM genotype). **J, K.** qPCR analysis of *Irs1* and *Irs2* transcript levels (J) and Spearman analysis of the correlation between *Irs1* and *Il1b* transcript levels (K) in eWAT of MCC950-treated 10% WT BMT mice and 10% KO BMT mice after 18 weeks on HF/HS diet (n=7 per BM genotype). In I and K, left panels show the combined analysis of all mice in each experiment; central and right panels show the analysis of each BM genotype separately. **L, M.** Cultured 3T3-L1 adipocytes were treated with conditioned medium (CM) from LPS/IFNγ-primed, ceramide-treated peritoneal macrophages (isolated from *Tet2*-/- mice and WT controls) in the presence of an IL-1β-neutralizing IgG or a control IgG. Adipocyte *Irs1* transcript expression was evaluated by qPCR (L, n=6 different conditioned media per genotype). Insulin-induced glucose uptake was assessed by a bioluminescent enzymatic method (M, n=3 different conditioned media per genotype; ***p=0.0002 by 2-way ANOVA with Sidak's multiple comparisons test). Data are shown relative to WT CM/control IgG-treated samples.