Table S1. Mean Fold-Change of Expression Levels of 84 Genes Quantified Using RT^2 Profiler^{TM} Apoptosis PCR Array, Related to Figure 4

Each row corresponds to a different gene. Mean fold-change represent values obtained from n=4 biological replicates/group (MxTKO versus MxCntrl).

Gene Symbol	Mean Fold Change MxTKO/MxCntrl	Standard Deviation
Akt1	1.002425	0.104170086
Apaf1	1.0798	0.420855177
Api5	0.6384	0.089244524
Atf5	0.9321	0.161371946
Bad	0.877225	0.290208127
Bag1	0.836475	0.251702666
Bag3	0.78365	0.187213014
Bak1	0.700525	0.1746968
Bax	0.7196	0.136636983
Bcl10	2.295175	1.382199568
Bcl2	1.672275	0.43132969
Bcl2l1	2.119675	0.582835236
Bcl2l10	3.47735	2.528825566
Bcl2l2	0.797775	0.19327477
Bid	0.943925	0.351898049
Naip1	1.281825	0.753674752
Birc2	1.2755	0.247039622
Birc3	2.1974	0.568496003
Xiap	1.188125	0.301379233
Birc5	0.6318	0.56915584
Bnip2	0.99895	0.302267617
Bnip3	1.748375	0.489511907
Bnip3l	1.383525	0.548556398
Bok	2.2643	1.044107784
Card10	1.9537	0.610694187
Nod1	1.3569	0.340414732
Card6	3.73925	0.947310587
Casp1	0.888025	0.180147903
Casp12	1.256666667	0.940262702
Casp14	1.171175	0.219192693
Casp2	1.058275	0.506007862
Casp3	1.446075	0.532946477
Casp4	0.719066667	0.146430788
Casp6	1.564325	0.641774097
Casp7	1.16865	0.48793465
Casp8	1.025125	0.278321275
Casp9	1.042175	0.456053815
Cflar	1.483675	0.26879925
Cidea	1.142975	0.165256125
Cideb	0.951025	0.182413327
Cradd	1.39205	0.839907285
Dad1	0.8857	0.469494223
Dapk1	3.620825	0.993166649
Dffa	1.1438	0.679945678
Dffb	0.8782	0.244301545
Tsc22d3	2.405	0.650778822
Fadd	0.925	0.6743075

Fas	3.135475	1.2935307
Fasl	2.275425	0.787019837
Hells	0.5291	0.407944671
Il10	1.1141	0.921736608
Lhx4	1.102325	0.179788548
Ltbr	0.95645	0.173154303
Mcl1	2.863425	0.814646302
Nfkb1	1.107975	0.489015268
Nme5	4.426225	3.745419341
Nol3	3.628775	2.778984707
Pak7	1.0131	0.272984716
Pim2	2.61035	0.934315939
Polb	0.7694	0.283432276
Prdx2	1.1874	0.735440582
Pycard	1.0074	0.551907044
Ripk1	1.18705	0.200517188
Rnf7	0.719925	0.351458891
Sphk2	1.14865	0.179195152
Tnf	1.251525	0.134715095
Tnfrsf10b	1.799875	0.869962167
Tnfrsf11b	4.243225	3.336944855
Tnfrsf1a	1.742525	0.186363682
Cd40	2.39805	1.378932704
Tnfsf10	1.5235	0.225387921
Tnfsf12	1.425425	0.639290687
Cd40lg	4.699525	1.683051603
Cd70	1.277225	0.604678933
Traf1	2.406175	1.521900559
Traf2	1.62545	0.353841522
Traf3	1.312325	0.174634781
Trp53	0.946375	0.216815513
Trp53bp2	1.7321	0.247718822
Trp53inp1	3.729525	0.671401096
Trp63	1.562025	0.940506848
Trp73	1.168	0.537565098
Gusb	0.956425	0.049656287
Gapdh	0.930825	0.237991747

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of Triple-Knockout Cyclin $D1^{F/F}D2^{-/-}D3^{F/F}$ Mice

Cyclin $D1^{F/F}$ and $D3^{F/F}$ mice (Choi et al., 2012) were intercrossed with cyclin $D2^{-/-}$ mice (Sicinski et al., 1996) giving rise to cyclin $D1^{F/F}D2^{-/-}D3^{F/F}$ animals. Genotyping and deletion analysis of floxed/deleted cyclin D1 and D3 alleles were performed with primers (see list of primer sequences below) D1F-5', D1F-3', D1-Δ for the cyclin D1 locus, and D3F-5', D3F-3', D3F-Δ for the cyclin D3 locus, by pre-denaturation of genomic DNA at 94°C 5 min, followed by 33 cycles of amplification: 94°C 30 sec, 60°C 30 sec, 72°C 30 sec and a final extension step at 72°C for 10 min. The PCR products were of the following sizes: $D1^{wt}$ = 180bp, $D1^{F}$ = 420bp, $D1^{\Delta}$ = 476bp; $D3^{wt}$ = 210bp, $D3^{F}$ = 500bp, $D3^{\Delta}$ = 450bp. Genotyping of cyclin $D2^{-/-}$ mice was performed as described (Sicinski et al., 1996). Wild-type mice (CD45.1) used in bone marrow transplantations were purchased from the Jackson Laboratory. All mice were housed under specific pathogen free conditions. Animal procedures were performed according to protocols approved by the Animal Care and Users Committee at DFCI.

Bone Marrow Reconstitution

For non-competitive reconstitution assay, $5x10^6$ bone marrow cells from $CD45.2^+$, MxTKO or MxCntrl donor mice were intravenously injected into lethally irradiated $CD45.1^+$ recipients (1200 rads in two doses of 600 rads, 4 h apart). Recipient mice were maintained on antibiotic (Baytril) for 2 weeks. Six weeks after the transplantation, mice were treated five times with pI-pC (to delete D-cyclins in MxTKO cells), analyzed for the presence of $CD45.2^+$ peripheral blood cells and observed for survival.

Flow Cytometry and Hematologic Analyses

Six to 10 weeks old Mx-Cre mice (TKO or control) were injected 1-3 times with pI-pC and peripheral blood analyses were performed on MASCOT Hemavet 850 Counter (CDC Technologies). Methylcellulose assays (M3434) were performed

according to manufacturer's specifications (Stemcell Technologies) and scored after 12 days of incubation at 37ºC in 5% CO_2. For lineage detection, freshly isolated bone marrow cells were first stained with a cocktail of biotinylated anti-mouse antibodies against Mac-1α (CD11b), Gr-1 (Ly-6G/C), Ter119 (Ly-76), CD3, CD4, CD8a (Ly-2) and B220 (CD45R) (BD Biosciences) followed by detection with streptavidin conjugated to pacific orange (Invitrogen) or directly to individual fluorochrome-conjugated antibodies. SLAM-HSC (referred in the text as hematopoietic stem cells, HSC) or HSC-enriched progenitor population LKS ($Lin^-c^-kit^+Sca^+$, referred in the text as hematopoietic stem/progenitor cells, HSPC) were analyzed based on lineage markers and antibodies to the following: c-Kit, (BD Biosciences), CD48 and CD150 (BioLegend) and Sca-1 (Invitrogen) (Kiel et al., 2005). For congenic strain determination, anti-CD45.1-FITC and anti-CD45.2-PE antibodies (BD Biosciences) were used. For apoptosis analysis of HSC and HSPC we used AnnexinV-FITC and 7-AAD (BD Biosciences). All sorting and data collection was performed on LSRII, Facs Aria, Fortessa and Facscan (BD Biosciences).

Cell cycle analysis and quiescence (G_0) evaluation of HSPC was performed as previously described (Wilson et al., 2008). Briefly, bone marrow cells were stained for HSPC markers as described above. Subsequently, cells were fixed and permeabilized using Cytofix/Cytoperm kit (from BD) and stained with 4',6-diamidino-2-phenylindole (DAPI) and a FITC conjugated anti-Ki-67 antibody (BD Bioscience). G_0 cells were gated based on their DNA content (2N) and low/negative Ki-67 expression. This population was previously shown to correspond to a rarely dividing population as demonstrated by H2B-GFP staining (Wilson et al., 2008; Foudi et al., 2009). For the detection of active caspase 3 in G_0 HSPC, a PE-conjugated anti-cleaved caspase 3 antibody was used (BD Biosciences) in combination with the Ki-67 and DAPI staining in fixed and permeabilized HSPC.

Apoptosis Array and Gene Expression Analysis

For the apoptosis pathway array, bone marrows were harvested 48 h after pI-pC injection from either MxTKO or MxCntrl mice (n=4 biological replicates per group). Total RNA was prepared using Qiagen RNeasy kit and 2 μg of RNA were used to prepare cDNA using SABiosciences-Qiagen first strand kit. The cDNA was then analyzed using RT^2 Profiler^{TM} Apoptosis PCR SArray (Qiagen) run on 384-well format Applied Biosystems, (ABI) 7900HT machine. Data was analyzed using PCR Array Data Analysis web-based portal (Qiagen). For other analyses of bone marrow, cDNA was synthesized using Applied Biosciences Two-step RT kit/2X SYBRgreen master mix and run on ABI 7300 PCR machine. To quantify transcripts in hematopoietic stem cells or hematopoietic stem/progenitor cells, HSC or HSPC were stained and flow sorted as described above from MxTKO and MxCntrl mice. For detection of cyclin D1, D2 and D3 transcripts, HSC were flow-sorted from wild-type C57BL/6 mice (from the Jackson Laboratory) as above. cDNA template from these cells was amplified using Nugen Amplification kit. The ΔΔ-Ct method was applied to determine relative mRNA expression using GAPDH mRNA levels for normalizing cDNA levels. Quantitative PCR primer sequences are provided below.

Promoter Constructs and Reporter Gene Assays

The FasL promoter construct was previously described (Nakamura et al., 2000); Fas promoter was cloned as described (Anders et al., 2011). To remove the E2F1 binding fragment, we deleted DNA sequences between nt – 1617-1707 (relative to transcription start site) from the human Fas promoter (corresponding to nt – 1693-1793 in the mouse promoter). E2F1 non-DNA binding and transactivation-deficient mutant E2F1(132)(1-374) was as described (Hsieh et al., 1997). In Figures 6H and S5G, U2OS cells were transfected with Lipofectamine 2000 (Invitrogen) in 24-well plates. Cells were lysed 30 h post-transfection and reporter assays performed with the Dual Luciferase Reporter Assay System (Promega). In Figure S5G, luciferase activity was normalized to percentage of GFP expressing cells.

Chromatin Immunoprecipitation (ChIP)

HSPC were sorted from wild-type C57BL/6 mice (from the Jackson Laboratory) as described above, and ~50,000 cells were used per experiment, n=3 biological replicates. ChIP analysis was performed using MAGnify™ Chromatin Immunoprecipitation System (Life Technologies) according to manufacturers specifications. E2F1 antibodies SC-193x (Santa Cruz) and 05-379 (Millipore) were used for E2F1 ChIP analysis. Enrichment of genomic regions bound by E2F1 across the Fas promoter versus non-bound sequences (negative region) was quantified by qPCR. Sequences of primers used for control ChIP (p107, cyclin A2, E2F1) have been previously described (Rayman et al., 2002).

Mitochondrial Membrane Potential Assays

For mitochondrial membrane potential assays, MxTKO and MxCntrl mice were injected with a 1-3 doses of pI-pC. Bone marrow cells were harvested after 2, 4 and 6 days and stained with cationic dye, JC-1, using Mitoprobe JC-1 Assay Kit (Life Technologies) and analyzed by FACS. As a positive control, MxCntrl bone marrow cells were treated with 25-100 μM carbonyl cyanide 3-chlorophenylhydrazone (CCPP) for 10 min and analyzed in parallel.

Gene/Primer Name	Primer sequences
D1F-5’	5' - GAG TTT TCC GGG TGC GTT - 3'
D1F-3’	5' - CTG TGG TGT CGC TGA CA - 3'
D1-Δ	5' - GGC AGT AGC AAG ATC TGT TA- 3'
D3F-5’	5' - CTG CGT TCT GTC CCT TTC CTT - 3'
D3F-3’	5' - CGC GAT AGA CAC AGG AAC CA - 3'
D3-Δ	5' - CCA GAC TGG AGC CAG AGA TAA - 3'
D1_Fwd	5’-TGT TAC TTG TAG CGG CCT GTT G-3’
D1_Rev	5’-CCG GAG ACT CAG AGC AAA TCC-3’
D2_Fwd	5’-CAC GAC TTC ATT GAG CAC ATC CT-3’
D2_Rev	5’-GCG GAT CAG GGA CAG CTT CT-3’
D3_Fwd	5’-TGC CAA AAC GCC CCA GTA C-3’
D3_Rev	5’-CGG GAT GCC CGA AGG A-3’
Bax.1_Fwd	5’-GATCAGCTCGGGCACTTTAG- 3'

Bax.1_Rev	5'-TTGCTGATGGCAACTTCAAC- 3'
Bax.2_Fwd	5'-GATGGTTCTGATCAGCTCGG- 3'
Bax.2_ Rev	5'-TGTTTGCTGATGGCAACTTC - 3'
Noxa.1_ Fwd	5'-ACTTTGTCTCCAATCCTCCG - 3'
Noxa.1_ Rev	5'-GAAGTCGCAAAAGAGCAGGA- 3'
Noxa.2_ Fwd	5'-TTGAGCACACTCGTCCTTCA- 3'
Noxa.2_ Rev	5'-GGAGTGCACCGGACATAACT- 3'
Puma.1_ Fwd	5'-TGTCGATGCTGCTCTTCTTG- 3'
Puma.1_ Rev	5'-GTGTGGAGGAGGAGGAGTGG - 3'
Puma.2_ Fwd	5'-GGTGTCGATGCTGCTCTTCT- 3'
Puma.2_ Rev	5'-GTGTGGAGGAGGAGGAGTGG- 3'
CD40L_Fwd	5'-AGC TAA AGA GAT GCA ACA AAG GAG-3'
CD40L_Rev	5'-GAC AAG GTC TTC AAA TTG CCT TCT CA-3'
E2F1_Fwd	5'-GGG AGA AGT CAC GCT ATG AAA CCT-3'
E2F1_Rev	5'-ATG TCA TAG ATG CGC CGT TTC TGC-3'
E2F2_Fwd	5'-TTC ATT TATC CTG AGC GAG TCG GA-3'
E2F2_Rev	5'-TTT GGA CTT CTT GCG GAT GAG CTG -3'
E2F3_Fwd	5'-GCT GCA GCC TCC ATG GAC AAA -3'
E2F3_Rev	5'-AAG GGT TCG TAG TGA GGA TCT GGA-3'
Fas_Fwd	5'-AGA GGT GGA AAC AAA CTG CAC CCT-3'
Fas_Rev	5'-ACA CAG TGT TCA CAG CCA GGA GAA-3
Fas deletion 90 Sense	5'-TAC CCA ACC CTT TGA CAT TAT ATG TCT CCC CAC AAA GC-3'
Fas deletion 90 Antisense	5'-GCT TTG TGG GGA GAC ATA TAA TGT CAA AGG GTT GGG TA-3'
FasL_Fwd	5'-ATC CCT CTG GAA TGG GAA GACACA -3'
FasL_Rev	5'-AGT TTC GTT GAT CAC AAG GCC ACC-3'

Chip primers	
Cycin A_Fwd	5'-TGTAAGATTCCCGTCGGGCCTTC-3'
Cycin A_Rev	5'-AGGCGGGAGGAGCGTAGAGCC-3'
E2F1_Fwd	5'-ATCGGAGC CTCCGTCGTCACA-3'
E2F1_Rev	5'-AGGCCGCGGCGAGGGCTCGAT-3'
FPR1_Fwd	5'-CTGTAATGGGATCCGACACC-3'
FPR1_Rev	5'-TCCTTGACTATCTGATAAGAGTGTTG-3'
FPR2_Fwd	5'-CAGTCTCTGTGAGGTCATGTG-3'
FPR2_Rev	5'-AAGAGGAGGAGGCAGGAA-3'
FPR3_Fwd	5'-GCCACTATATGCACTAGCTCATC-3'
FPR3_Rev	5'-TCATCCCTACATGCCAACATC-3'
Nes3_Fwd	5'-CCAAGCAGCAAGAGACGTAA-3'
Nes3_Rev	5'-GTTGTCAGCGTCCTGAAGAG-3'
p107_Fwd	5'-TTAGAGTCCGAGGTCCATCTTCT-3'
p107_Rev	5'-GGGCTCGTCCTCGAACATATCC-3'

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