Supporting Information for

Proteasome inhibitor bortezomib stabilizes and activates p53 in hematopoietic stem/progenitors and double negative T cells *in vivo*

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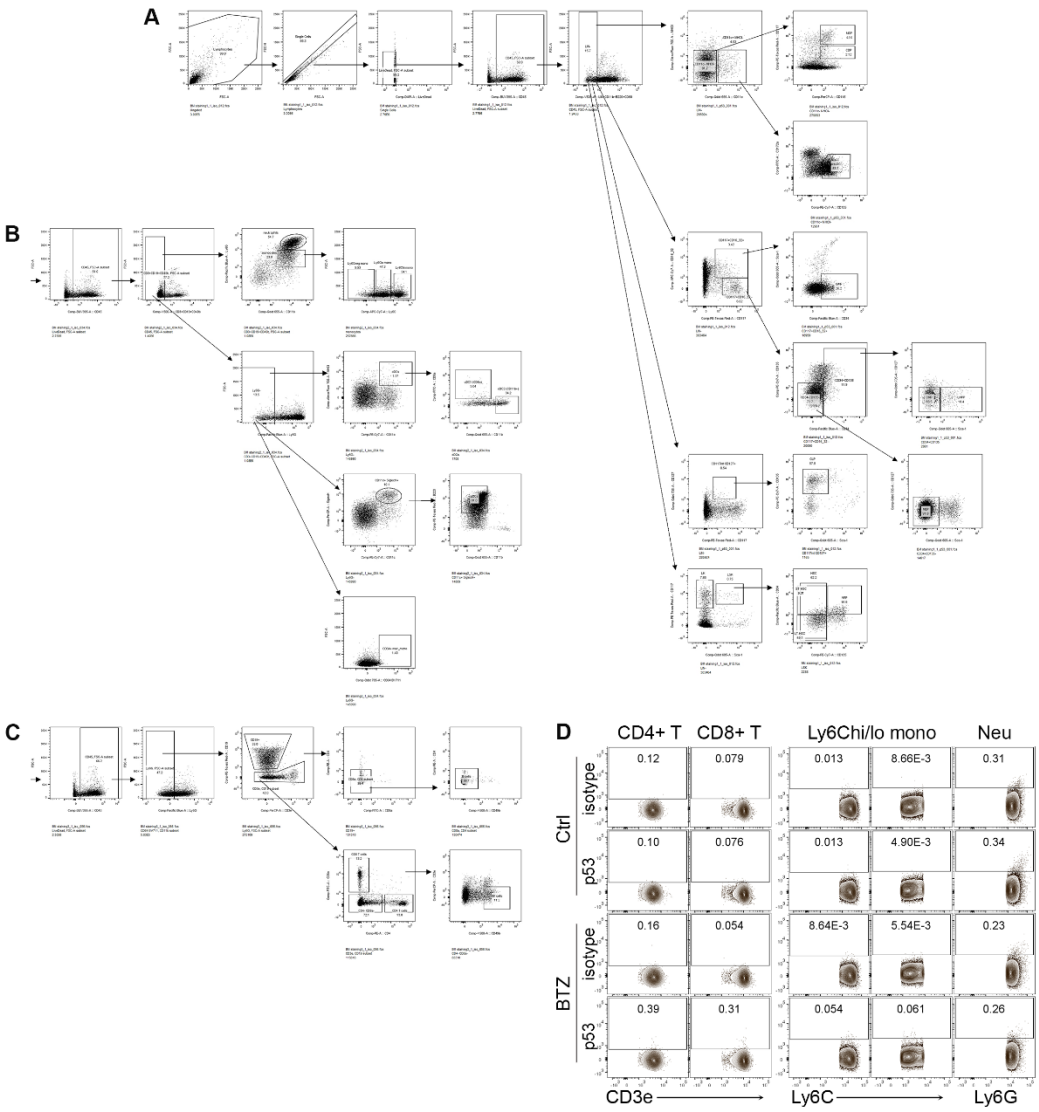


SI Appendix*, Fig. S1. Gating strategies. (A*) Gating strategies for HSPCs of bone marrow:

HSC (Lin-Sca-1^{+}CD117^{+}CD135^{-}),

MPP (Lin^{-}Sca-1^{+}CD117^{+}CD34^{+}CD135^{+}),

LMPP (Lin-Sca-1^{+}CD117^{+}CD16/32^{-}CD34^{+}CD127^{-}),

CLP (Lin^{-}Sca-1^{lo}CD117^{int}CD127^{+}CD34^{+}CD135^{+}),

GMP (Lin- Sca-1^{-}CD117^{+}CD16/32^{+}CD34^{+}),

CMP (Lin^{-} Sca-1^{-}CD117^{+}CD16/32^{-}CD34^{+}CD127^{-}),

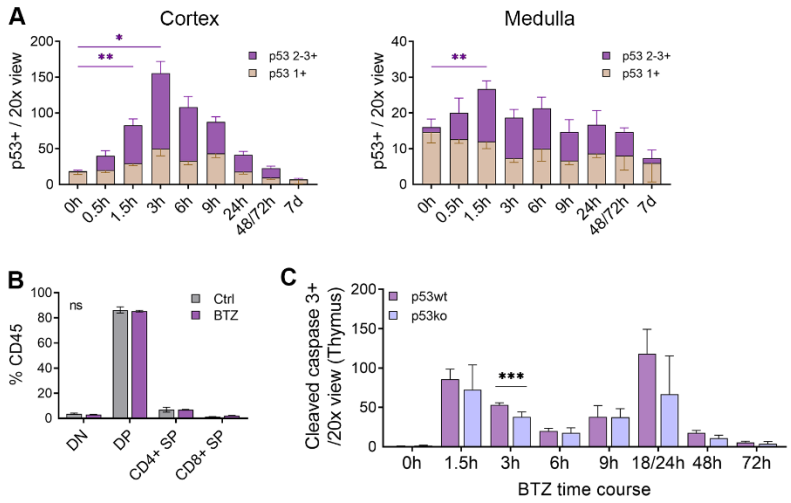
MEP (Lin^{-} Sca-1^{-}CD117^{+}CD127^{-}CD16/32^{lo}CD34^{-}),

MDP (Lin^{-}CD11c^{-}MHCII^{-}CD117^{hi}CD115^{+}),

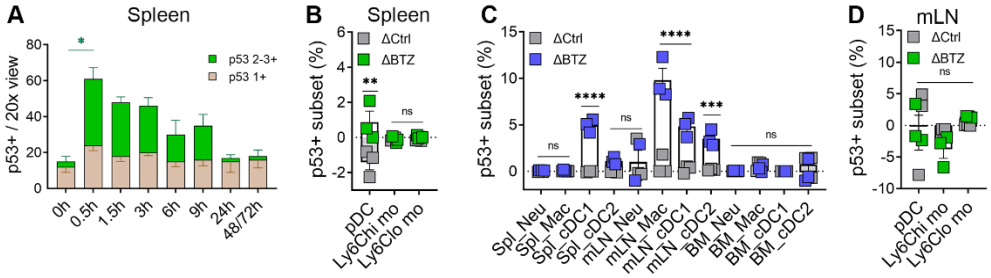
CDP (Lin^{-}CD11c^{-}MHCII^{-}CD117^{lo}CD115^{+}),

pre-DC (Lin^{-}CD11c^{+}MHCII^{-}CD172a^{lo}CD135^{+}).

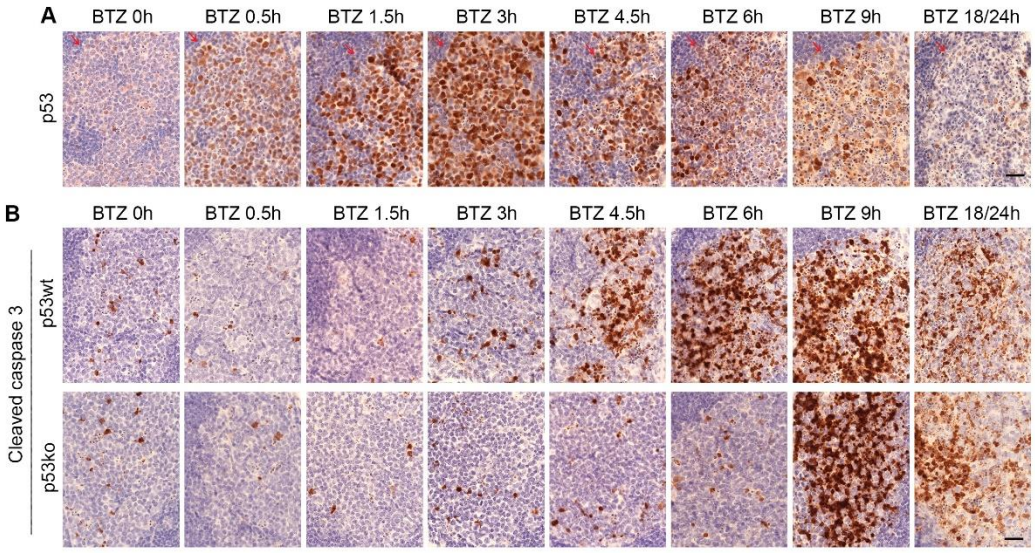
(*B* & *C*) Gating strategy for myeloid and lymphoid cell populations of bone marrow cells. (*D*) Representative FACS plots showing bone marrow cells stained for p53 or the isotype control from mice treated with Ctrl or BTZ, gated on CD4^{+} and CD8^{+} T cells, Ly6C^{hi}, and Ly6C^{lo} monocytes (mono), and neutrophils (Neu).



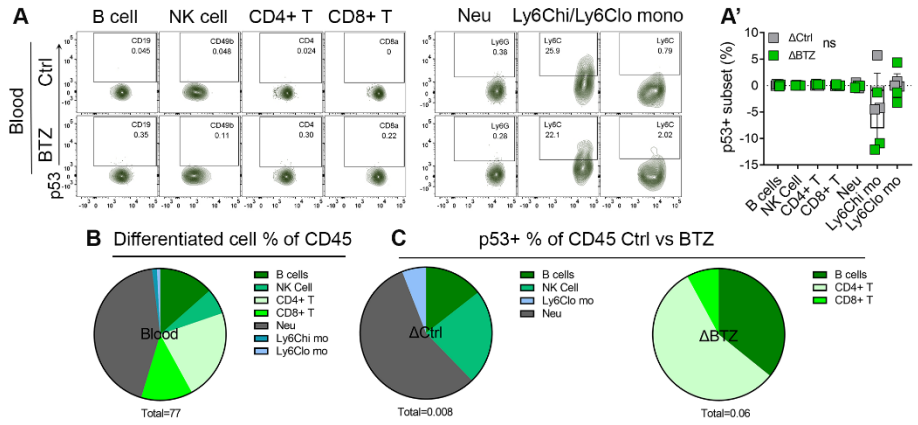
***SI Appendix*, Fig. S2. Quantification of the frequency p53^{+} subsets and cleaved caspase induction in response to bortezomib.** (*A*) Quantification of p53-positive frequencies at various time points in the cortex and medulla of the thymus from mice treated with vehicle (BTZ 0h) or BTZ. Moderate and strong p53 staining signals are classified as p53 2-3+ (in purple), mild p53 staining signals are classified as p53 1+ (in light brown). (*B*) Quantification of the frequency of p53^{+} subsets in CD45^{+} cell populations in the thymus. (*C*) Quantification of the expression of cleaved caspase 3 detected by IHC in the thymus of p53$^{+/+}$ and p53$^{-/-}$ mice at indicated time points post vehicle (BTZ 0h) or 3 mg/kg BTZ treatment (n = 3-4). The indicated *p* values were calculated by multiple unpaired t-tests using Prism. *, $P < 0.05$; **, $P < 0.001$, ***, $P < 0.0001$; ns, no significance. Error bars, mean (SEM) for each group.



***SI Appendix*, Fig. S3. Stabilization of p53 in the spleen and mesenteric lymph node (mLN) by bortezomib**. (*A*) Quantification of p53-positive frequencies at various time points in the spleen from mice treated with vehicle (BTZ 0h) or BTZ. Moderate and strong p53 staining signals are classified as p53 2-3+ (in green), mild p53 staining signals are classified as p53 1+ (in light brown). (*B*) Quantification of p53$^+$ subsets of the indicated splenic cells from mice treated with Ctrl (in grey) or BTZ (in green). (*C*) Comparison of the frequencies of p53$^+$ subsets in lymphoid and myeloid cells in the spleen, mLN, and bone marrow from mice treated with vehicle (in grey) or BTZ (in blue). (*D*) Quantification of p53$^+$ subsets of the indicated mLN cells from mice treated with vehicle (in grey) or BTZ (in green) (n = 3-4). The indicated *p* values were calculated by multiple unpaired t-tests of using Prism *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$; ****, $P < 0.00001$; ns, no significance. Error bars, mean (SEM) for each group.



***SI Appendix*, Fig. S4. Stabilization of p53 in Peyer's patch by bortezomib**. (*A*) IHC analysis of p53 levels in the Peyer's patch from mice at indicated time points post vehicle or 3 mg/kg of BTZ treatment (n=3-4). Red arrows point to the germinal centre. (*B*) IHC analysis and quantification of the expression of cleaved caspase 3 in the bone marrow of $p53^{+/+}$ and $p53^{-/-}$ mice at indicated time points post vehicle (BTZ 0h) or 3 mg/kg BTZ treatment (n = 3-4). Scale bars, 20 μm.



***SI Appendix*, Fig. S5. Less p53 stabilization in the blood by bortezomib**. (*A*) Representative FACS plots showing blood cells stained for p53 from mice treated with Ctrl or BTZ for 1.5h, gated on matured lymphoid and myeloid cells. (*A'*) Quantification of p53-positive frequencies of indicated mature cells in the blood from mice treated with vehicle (in grey) or BTZ (in green). (*B*) Quantification of frequencies of various CD45$^+$ blood cells from mice treated with vehicle. 77% of the total CD45$^+$ population was analyzed. (*C*) Quantification of frequencies of p53$^+$ subsets in CD45$^+$ Blood cells from mice treated with Ctrl and BTZ. ΔCtrl and ΔBTZ were calculated to remove non-specific staining (Δ = p53$^+$ frequency - isotype$^+$ frequencies). N = 3-4. The indicated P values were calculated using multiple unpaired t-tests in Prism ns, no significance. Error bars, mean (SEM) for each group.

***SI Appendix*, Table S1.** Staining panels for immune profiling

	Bone marrow only	**Bone marrow /Thymus/Spleen/Blood /Lymph node**		**Thymus**
	Panel 1	**Panel 2**	**Panel 3**	**Panel 3 Modified**
	LMPP, CMP, CLP, GMP, MDP, CDP, pre-cDC	**cDC/pDC/mono/ mac/neu**	**T/B/mac**	**T/DN**
DAPI	fixable viability dye	fixable viability dye	fixable viability dye	fixable viability dye
FITC	CD172a	CD8a	CD8a	CD8a
PE	CD115	CD103	CD4	CD4
PerCP	CD16/32	SiglecH	CD3e	CD3e
PE-Cy7	CD135-**bio**	CD11c	-	CD44
APC	**p53/iso**	**p53/iso**	**p53/iso**	**p53/iso**
APC-Cy7	Ly6C	Ly6C	-	CD25
AF700	MHCII	MHCII		
Qdot605	Sca-1	F4/80		
Qdot655	CD11c	CD11b	CD11b	CD11b
TxRed	CD117	B220	CD19	CD19
PacificBlue	CD34	Ly6G	Ly6G	Ly6G
BUV395	CD45	CD45	CD45	CD45
BV711	CD127	CD64	CD64	CD64
BV786	-	-	-	-
BV510	**LIN+CD11b+B220+CD90**	**CD3/CD19/CD49b**	CD49b	CD49b

Abbreviations: mono: monocyte; mac: macrophage; neu: neutrophil; DN double negative.

***SI Appendix*, Table S2.** Actual percentage of cell subsets and p53+ cells in the bone marrow

18 cell types	Cell subset % in Ctrl	ΔCtrl p53+%	ΔBTZ p53+%
HSC	0.0430	0.00009	0.00274
MPP	0.0190	-0.00008	0.00221
LMPP	0.0163	-0.00005	0.00110
CLP	0.0327	-0.00009	0.00239
GMP	0.0947	-0.00005	0.02137
CMP	0.2533	0.00029	0.07823
MEP	0.6933	0.00060	0.34312
MDP	0.0095	-0.00002	0.00413
CDP	0.0293	0.00000	0.00747
Pre-DC	0.1933	-0.00057	-0.00083
cDC	0.0980	0.00043	-0.00009
pDC	0.9400	0.00100	-0.00175
Mono	11.1333	0.00137	0.00563
Neu	33.9667	-0.00014	0.01474
B cell	24.2333	-0.00333	0.24700
NK cell	1.8300	-0.00018	0.11205
CD4+ T	0.3800	-0.00012	0.00103
CD8+ T	1.0300	-0.00065	0.00268

Abbreviations: mono: monocyte; neu: neutrophil; DN double negative; Ctrl: control; BTZ: bortezomib.

***SI Appendix*, Table S3.** Actual percentage of cell subsets and p53+ cells in the spleen

Spleen	Cell subset % in Ctrl	ΔCtrl p53+%	ΔBTZ p53+%
B cell	62.2333	-0.01300	0.49200
NK Cell	2.0433	0.00075	0.01804
CD4+ T cell	10.5900	0.00227	0.12471
CD8+ T cell	7.2100	0.00152	0.08469
Neu	2.3733	-0.00157	0.00152
Mac	0.2833	-0.00004	0.00016
pDC	0.4133	-0.00720	-0.00067
cDC1	0.2867	-0.00014	0.01193
cDC2	0.7167	-0.00072	0.00665
Ly6Chi mono	0.4567	-0.00070	-0.00092
Ly6Clo mono	0.4967	-0.00060	0.00006

Abbreviations: neu: neutrophil; mac: macrophage; mono: monocyte; Ctrl: control; BTZ: bortezomib.

***SI Appendix*, Table S4.** Actual percentage of cell subsets and p53+ cells in the lymph node

Lymph node	Cell subset % in Ctrl	ΔCtrl p53+%	ΔBTZ p53+%
B cells	47.7000	-0.00431	0.39433
NK Cell	0.4167	0.00046	0.01304
CD4+ T	21.7000	0.00819	0.39065
CD8+ T	16.4333	0.00439	0.30526
Neu	0.0230	0.00055	0.00028
Mac	0.0089	0.00006	0.00071
pDC	0.1173	0.00678	0.00500
cDC1	0.1493	0.00055	0.00462
cDC2	0.4767	0.00110	0.01457
Ly6Chi mono	0.1253	0.00367	0.00000
Ly6Clo mono	0.2633	0.00033	0.00329

Abbreviations: neu: neutrophil; mac: macrophage; mono: monocyte; Ctrl: control; BTZ: bortezomib.

***SI Appendix*, Table S5.** Reagents and Antibodies for IHC

Antibodies for IHC	**Company**	**Cat No.**
p53 antibody (Rabbit anti-mouse)	Home-made (p53Lab)	N/A
p21antibody	Santa Cruz	sc-6246
Puma antibody	Cell Signaling	#4976
Cleaved caspase 3 antibody	Cell Signaling	#9661
Reagents	**Company**	**Cat No.**
Bovine serum albumin	Sigma-Aldrich	A2058 or A7906
collagenase IV	Sigma-Aldrich	C5138
non-sterile mouse serum	Sigma-Aldrich	M5905
non-sterile rat serum	Sigma-Aldrich	R9759
DAPI	Biolegend	D1306
AbC total compensation capture beads	Biolegend	A10497
fixable viability Dye eFluor 455 (UV)	Biolegend	65-0868-14
Fix/Perm concentrate	Biolegend	00-5123-43
Fix/Perm diluent reagent	Biolegend	00-5223-56
DNAseI	Roche	10104159001
RBC Lysis Buffer	Roche	11814389001
TRIzol reagent	Ambion Life Technologies	15596018

***SI Appendix*, Table S6.** Antibody list for flow cytometry

Antibodies for FACS	Clone	Conjugate/ Fluorochrome	Company	Catalog No.
anti-mouse CD45	30-F11	BUV395	BD Bioscience	564279
anti-mouse CD49b	HMa2	BV510	BD Bioscience	740133
anti-mouse CD49b	HMa2	FITC	Biolegend	103503
anti-mouse CD3	17A2	FITC	eBioscience	11-0032-82
anti-mouse Ly-6G/Ly-6C	RB68C5	FITC	Biolegend	108405
anti-mouse CD19	1D3/CD19	FITC	Biolegend	152404
anti-mouse B220	RA3-6B2	FITC	Biolegend	103205
anti-mouse CD150	TC15-12F12.2	PE	Biolegend	115903
anti-mouse CD115	AFS98	PerCP-eFluor 710	eBioscience	46-1152-80
anti-mouse CD135	A2F10	Biotin	eBioscience	13-1351-85
anti-mouse CD16/32	93	APC-Cy7	Biolegend	101328
anti-mouse Sca-1/Ly6A/E	D7	BV785	Biolegend	108139
anti-mouse CD48	HM48-1	BV510	Biolegend	103443
anti-mouse CD11b	M1/70	FITC	Biolegend	101205
anti-mouse CD135	A2F10	PE	eBioscience	12-1351-82
anti-mouse CD43	eBioR2/60	Biotin	eBioscience	13-0431-85
anti-mouse B220	RA3-6B2	APC-Cy7	Biolegend	103224
anti-mouse CD24	M1/69	BV605	Biolegend	101827
anti-mouse CD19	eBio1D3	eFluor 450	eBioscience	48-0193-82
anti-mouse CD127	A7R34	Brilliant Violet 711	Biolegend	135035
anti-mouse CD3	17A2	Brilliant Violet 786	BD Bioscience	564010
anti-mouse CD8a	53-6.7	FITC	Biolegend	100705
anti-mouse CD4	RM4-5	PE	Biolegend	100511
anti-mouse CD3e	145-2C11	PerCP-Cy5.5	Biolegend	100327
anti-mouse/human CD44	IM7	PE-Cy7	Biolegend	103029
anti-mouse CD25	PC61	APC-Cy7	Biolegend	102025
anti-mouse CD117	2B8	PE-CF594	BD Bioscience	562417
anti-mouse CD34	SA376A4	BV421	Biolegend	152207
anti-mouse CD19	1D3	BV510	BD Bioscience	562956
anti-mouse Ly-6G	1A8	BV510	Biolegend	127633
anti-mouse CD11b	M1/70	BV510	Biolegend	101245
Streptavidin		PE-Cy7	Biolegend	405206
anti-mouse CD172	P84	FITC	Biolegend	144005
anti-mouse NK1.1	PK136	FITC	eBioscience	11-5941-85
anti-mouse CD115	AFS98	Alexa 488	eBioscience	53-1152-80
anti-mouse CD127	A7R34	FITC	eBioscience	11-1271-82
anti-mouse CD3e	145-2C11	FITC	eBioscience	11-0031-82
anti-mouse SiglecH	551	PerCP-Cy5.5	Biolegend	129613

anti-mouse CD115	AFS98	PerCP-eFluor 710	eBioscience	46-1152-80
anti-mouse B220	RA3-6B2	PerCP-Cy5.5	BD Bioscience	552771
anti-mouse Ly-6C	HK1.4	PerCP-Cy5.5	eBioscience	45-5932-82
anti-mouse SiglecH	551	PerCP-Cy5.5	Biolegend	129613
anti-mouse CD117	2B8	PerCP-Cy5.5	Biolegend	105824
anti-mouse Ly-6G	RB6-8C5	PerCP-Cy5.5	eBioscience	45-5931-80
anti-mouse Ly-6C	HK1.4	PerCP-Cy5.5	eBioscience	45-5932-82
anti-mouse CD16/32	93	PerCP-Cy5.5	eBioscience	45-0161-80
anti-mouse CD19	eBio1D3	PE	eBioscience	12-0193-83
anti-mouse CD133	13A4	PE	eBioscience	12-1331-82
anti-mouse CD45	30-F11	PE	Biolegend	103106
anti-mouse CD115	AFS98	PE	eBioscience	12-1152-81
anti-mouse F4/80	BM8	PE	Biolegend	123110
anti-mouse MHC Class II I-A/I-E	M5/114.15.2	PE	eBioscience	12-5321-81
anti-mouse CD103	2E7	PE	Biolegend	121405
anti-mouse CD19	1D3	PECF594	BD Bioscience	562291
anti-mouse B220	RA3-6B2	PE-Dazzle 594	Biolegend	103257
anti-mouse CD127	5B/199	PE-CF594	BD Bioscience	562419
anti-mouse B220	RA3-6B2	PE-CF594	BD Bioscience	562313
anti-mouse Ly-6C	HK1.4	PE-Cy7	eBioscience	25-5932-82
anti-mouse Ly-6C	HK1.4	PE-Cy7	eBioscience	25-5932-82
anti-mouse CD11c	N418	PE-Cy7	eBioscience	25-0014-82
anti-mouse CD34	RAM34	eFluor 450	eBioscience	48-0341-82
anti-mouse Sca-1/Ly6A/E	D7	Brilliant Violet 605	Biolegend	108133
anti-mouse F4/80	BM8	Brilliant Violet 605	Biolegend	123133
anti-mouse Ly-6G	1A8	Pacific Blue	Biolegend	127611
anti-mouse CD11c	N418	Brilliant Violet 650	Biolegend	117339
anti-mouse CD11b	M1/70	Brilliant Violet 650	Biolegend	101259
anti-mouse CD11c	N418	Brilliant Violet 605	Biolegend	117333
anti-mouse CD11b	M1/70	Brilliant Violet 650	Biolegend	101239
Streptavidin		Brilliant Violet 711	Biolegend	405241
anti-mouse CD64	10.1	Brilliant Violet 711	Biolegend	305041
anti-mouse CD3e	145-2C11	BV510	BD Bioscience	563024
anti-mouse B220	RA3-6B2	BV510	BD Bioscience	563103
anti-mouse CD90.2	53-2.1	BV510	BD Bioscience	740102
anti-mouse MHC Class II I-A/I-E	M5/114.15.2	Alexa Fluor 700	eBioscience	56-5321-82
anti-mouse CD45	30-F11	Alexa Fluor 700	Biolegend	103128
anti-mouse Sca-1/Ly6A/E	D7	Alexa Fluor 700	Biolegend	108142
anti-mouse Sca-1/Ly6A/E	D7	Alexa Fluor 647	Biolegend	108118
anti-mouse CD34	SA376A4	Alexa Fluor 647	Biolegend	152205
anti-mouse CD49b	DX5	APC-Cy7	Biolegend	108919

anti-mouse CD45	30-F11	APC-Cy7	BD Bioscience	557659
anti-mouse Ly-6C	HK1.4	APC-Cy7	Biolegend	128026
anti-mouse CD16/32	93	APC-Cy7	Biolegend	101328
anti-mouse CD25	M-A251	Brilliant Violet 786	BD Bioscience	563700
anti-mouse CD45	30-F11	APC-Cy7	BD Bioscience	557659
anti-mouse Sca-1/Ly6A/E	D7	APC-Fire 750	Biolegend	108145
anti-mouse B220	RA3-6B2	APC-eFluor 780	eBioscience	47-0452-82
anti-mouse CD45	30-F11	APC-Cy7	BD Bioscience	557659
anti-mouse CD135	A2F10	Biotin	eBioscience	13-1351-82
anti-mouse CD8a	53-6.7	APC	Biolegend	100711
anti-mouse p53	1C12 (IgG1)	Alexa 647	Cell Signaling	#2533
anti-mouse IgG1	MOPC-21	Alexa 647	Cell Signaling	#4843

***SI Appendix*, Table S7.** qPCR primers

Cdkn1a-F	GTCTTGCACTCTGGTGTCTG
Cdkn1a-R	GATAGAAATCTGTCAGGCTGGTC
Bbc3-F	AGCAGCACTTAGAGTCGCC
Bbc3-R	CGCTCGTACTGCGCGTTG
Gadd45a-F	AGCAGAAGACCGAAAGGATG
Gadd45a-R	CAGGCACAGTACCACGTTAT
Trp53-F	GTTATGTGCACGTACTCTCCTC
Trp53-R	CGTCATGTGCTGTGACTTCT
Ccna2-F	TCAAGACTCGACGGGTTGC
Ccna2-R	GTGAAGGCAGGCTGTTTACT