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Supplemental Information

Tet2 Loss Leads to Increased Hematopoietic

Stem Cell Self-Renewal and Myeloid Transformation

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Figure S1, related to Figure 2.

Figure S2, related to Figure 3.

Figure S3, related to Figure 4.

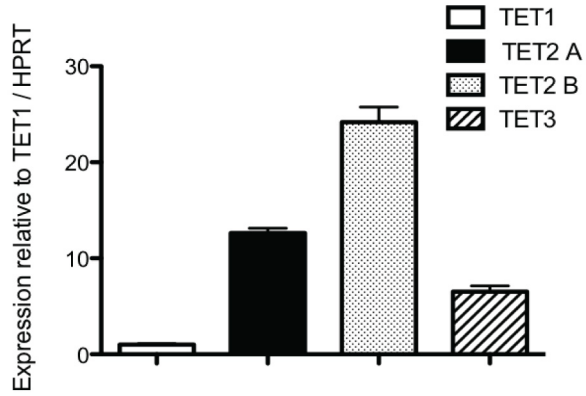
Figure S4, related to Figure 5.

Figure S5, related to Figure 6.

Figure S6, related to Figure 7.

Table S1, related to Table 1.

A



B

Clinical characteristics of *TET2*-wildtype versus *TET2*-mutant patients with CMML

Characteristic	<i>TET2</i> -wildtype (n=33)*	<i>TET2</i> -mutant (n=17)	p-value
Age	63 (31-75)	69 (54-77)	0.002
Sex (M/F)	28/5	14/3	N.S.
Ratio with splenomegaly by exam (%)	39.4	23.5	N.S.
Ratio with abnormal karyotype (%)	61.3	5.9	0.018
% BM Blasts	5 (0-19)	5 (1-16)	N.S.
Ratio with CMML-2 (%)	18.2	35.3	N.S.
% BM Monocytes	12 (1-42)	10 (1-40)	N.S.
Peripheral WBC	24.8 (2.6-99)	28.8 (2.3-87.3)	N.S.
Peripheral blood monocytosis (%)	23 (6-46)	24 (5-54)	N.S.
Hemoglobin (g/dL)	10.4 (6.1-14.1)	12 (5.1-14.8)	0.03
Platelet count ($\times 10^9/\mu\text{L}$)	72 (6-229)	72 (9-500)	N.S.

* Data displayed as median value (range in parenthesis); N.S not significant

C

TET2-mutations in de novo AML patients from ECOG E1900

Mutations	AA residue	Co-occurring mutations
FS (270)	270	
S327X	327	
K433X	433	K433X and FS (1299)
R544X	544	R544X and Q916X
R550X	550	R550X and FS (1893)
FS (586)	586	
Q622X	622	Q622X and Q1524X
Q891X	891	
FS (912)	912	
Q916X	916	R544X and Q916X
FS (921)	921	
FS (958)	958	
FS (966)	966	
W1003X	1003	
Q1021R	1021	
FS (1034)	1034	
FS (1114)	1114	
FS (1118)	1118	
H1219Y	1219	
R1261C	1261	
R1261C	1261	
R1261C	1261	
FS (1299)	1299	K433X and FS (1299)
FS (1322)	1322	
R1365H	1365	
G1369V	1369	
FS (1395)	1395	
E1405X	1405	E1405X and S1486X
FS (1439)	1439	
FS (1448)	1448	
S1486X	1486	E1405X and S1486X
Q1524X	1524	Q622X and Q1524X
H1817N	1817	
FS (1893)	1893	R550X and FS (1893)
R1896M	1896	
Y1902X	1902	
FS (1960)	1960	
P1962L	1962	

Figure S1, related to Figure 2. A) TET family gene expression in human hematopoietic cells, qPCR analysis of TET1, TET2A/B, and TET3 transcript expression in the indicated human cell populations. **B-C)** Clinical characteristics of CMML patients that carry either TET2 wild-type or mutant alleles. Error bar represents \pm SD.

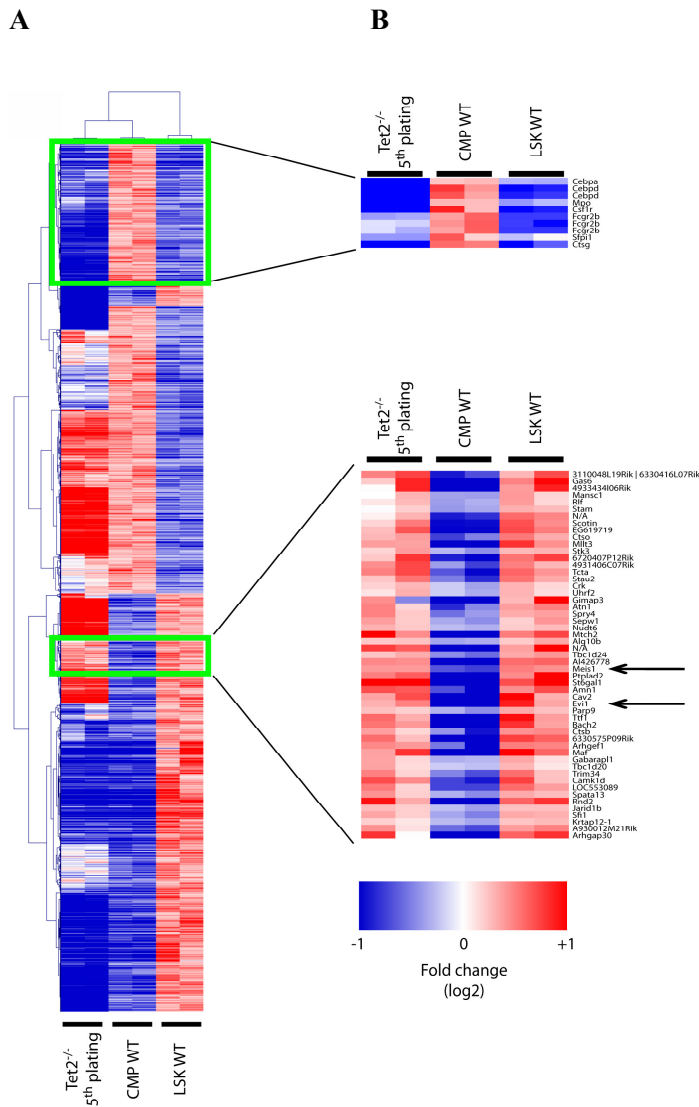
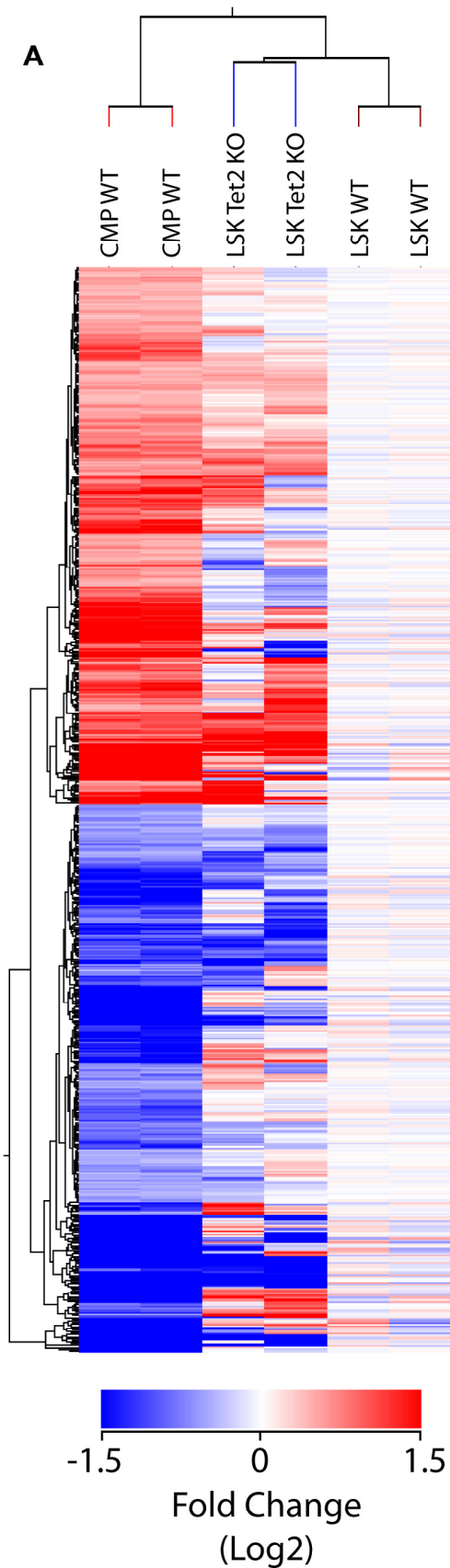
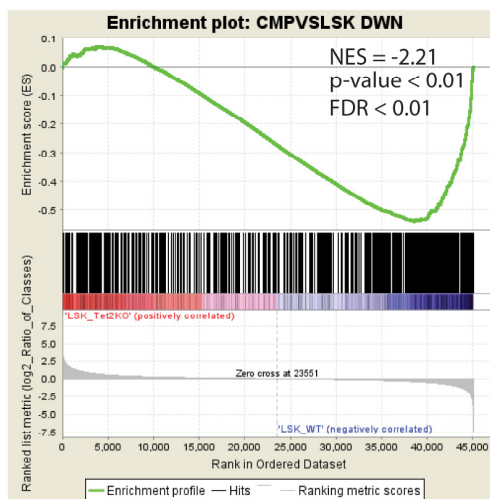
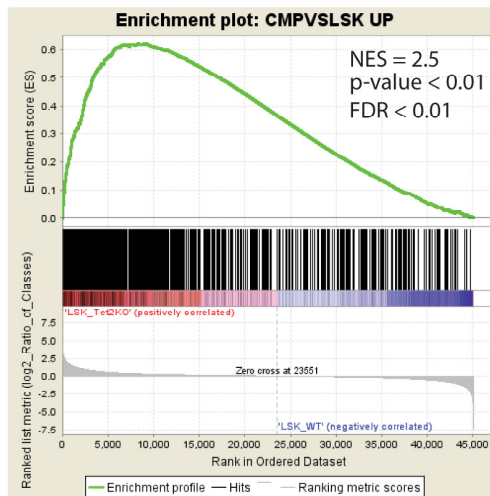


Figure S2, related to Figure 3. *Tet2*^{-/-} pro-myeloblasts with the ability to serially replate in vitro share gene expression signature with CMP progenitors and upregulate specific self-renewal regulators. A) Unsupervised heat-map focusing on similarities between CFU5 cells and wild-type CMP progenitors. Overall, 865 probe-sets follow similar expression patterns between these two cell types. **B)** Heat-map showing regions where CFU5 shares similarities with LSK cells. 574 probe-sets show similar patterns in these two cell populations. On the right hand panels, detailed heat maps are showing genes shared between CFU5 and LSK. Upper panel focuses on putative regulators of self-renewal (i.e. *Meis1*, *Evi1*); Lower panel focuses on regulators of myeloid differentiation (*Cebpa*, *Mpo*, etc).

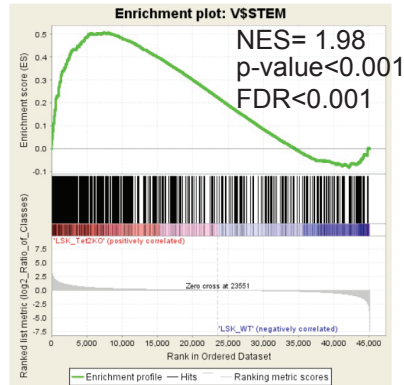
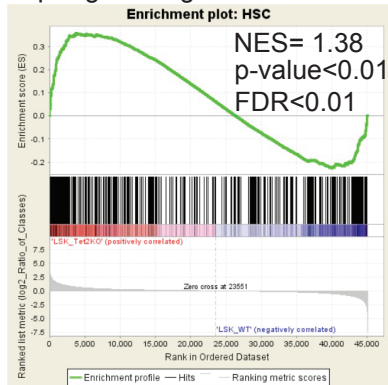


B

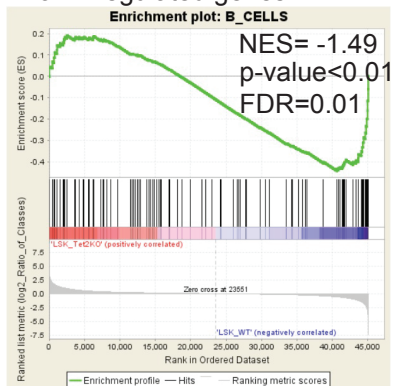


C

Up-regulated genes:



Down-regulated genes:



gene expression datasets obtained at:

<http://franklin.imgen.bcm.tmc.edu/loligag/lf.php>

Ng, SY et al., *Immunity*. 2009 Apr 17;30(4):493-507. Epub 2009 Apr 2.

D

Upregulated genes in CMP Vs. LSK

Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1415719 s at	Armc1	1416864 at	Surf6	1418060 a at	Mapk7	1419508 at	Ripk1
1415732 at	Bat5	1416873 a at	Cdk2	1418074 at	St6galnac4	1419594 at	Ctsq
1415828 a at	Serp1	1416875 at	Parvg	1418090 at	Plvap	1419606 a at	Tnnt1
1415857 at	Emb	1416893 at	Fam107b	1418096 at	Dok3	1419608 a at	Mia1
1415925 a at	Nup62	1416947 s at	Acaa1a /// Aca	1418171 at	LOC10004503	1419609 at	Ccr1
1415926 at	Nup62	1416988 at	Msh2	1418202 a at	Wiz	1419610 at	Ccr1
1415960 at	Mpo	1416989 at	Vps53	1418340 at	Fcer1g	1419639 at	Efnb2
1415987 at	Hdlbp	1417061 at	Slc40a1	1418451 at	Gng2	1419669 at	Prtn3
1416022 at	Fabp5	1417093 a at	Gtf2h4	1418601 at	Aldh1a7	1419821 s at	Idh1
1416146 at	Hspa4	1417099 at	Ftsj1 /// LOC1	1418624 at	LOC10004518	1419838 s at	Plk4
1416147 at	Hspa4	1417229 at	Capn1	1418655 at	B4galnt1	1420023 at	Etf1
1416155 at	Hmgb3	1417235 at	Ehd3	1418826 at	Ms4a6b	1420377 at	St8sia2
1416208 at	Usp14	1417294 at	Akr7a5	1418920 at	Cldn15	1420385 at	Gna14
1416213 x at	Surf4	1417324 at	Mast2	1418937 at	Dio2	1420472 at	Mtpn
1416234 at	Lrrc59	1417389 at	Gpc1	1418939 at	Hlx	1420501 at	Dnajc1
1416271 at	Perp	1417446 at	Slc12a4	1418956 at	Tssk6	1420518 a at	IgSF9
1416312 at	Rars	1417561 at	Apoc1	1418982 at	Cebpa	1420520 x at	Eral1
1416348 at	Men1	1417588 at	Galnt3	1419013 at	Gpatch1	1420563 at	Gria3
1416358 at	Mfsd10	1417629 at	Prodh	1419050 at	1110002H13Ri	1420572 at	Ms4a3
1416360 at	Snx18	1417630 at	Mknk1	1419130 at	Adat2	1420629 a at	Dnaja3
1416511 a at	Cdc42ep4	1417728 at	Mbd3	1419262 at	Acad8	1420653 at	Tgfb1
1416619 at	4632428N05R	1417841 at	Pxmp2	1419271 at	Pax6	1420699 at	Clec7a
1416714 at	Irf8	1417854 at	Map2k5	1419353 at	Dpm1	1420776 a at	Auh
1416779 at	Sdpr	1417878 at	E2f1	1419396 at	Arid3a	1420804 s at	Clec4d
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1421186 at	Ccr2	1422548 at	Dhdds	1423324 at	Pnn	1424622 at	Hsf1
1421187 at	Ccr2	1422549 at	Arl2	1423465 at	Frrs1 /// LOC1	1424658 at	Taok1
1421188 at	Ccr2	1422557 s at	Mt1	1423519 at	Fam108c	1424663 at	BC017647
1421302 a at	Gna15	1422572 at	Rhog	1423543 at	Swap70	1424674 at	Slc39a6
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1421340 at	Map3k5	1422703 at	Gyk	1423587 a at	Exosc10	1424745 at	Agxt2l2
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1421408 at	IgSF6	1422886 a at	Clk4	1423612 at	Clp1	1424841 s at	Rbks
1421410 a at	Pstpip2	1422903 at	Ly86	1423643 at	Ddx39	1425045 at	Jmjd7
1421411 at	Pstpip2	1422928 at	Ela2	1423706 a at	Pgd	1425091 at	Arrrs2
1421702 at	Rdh1	1422947 at		1423720 a at	Sar1a	1425145 at	Il1r1
1421768 a at	Homer1	1422948 s at	Hist1h4a /// Hs	1423755 at	Zcchc8	1425157 x at	Tspan33
1421815 at	Epdr1	1423024 at	Sh2d1b1	1423843 at	Lrrc61	1425227 a at	Atp6v0a1
1421853 at	Psen1	1423120 at	Ide	1423976 at	4930453N24R	1425250 a at	Slc14a2
1421898 a at	Mr1	1423138 at	Wdr4	1424030 at	Grhl1 /// LOC1	1425349 a at	Myef2
1421901 at	Eif2ak1	1423143 at	Ctbbp4	1424114 s at	Lamb1-1	1425356 at	Zfp142
1421906 at	Med1	1423178 at	Abi1	1424222 s at	Rad23b	1425408 a at	2610034M16R
1421947 at	Gng12	1423181 s at	Clns1a	1424229 at	Dyrk3	1425472 a at	Lmna
1421950 at	Pfdn2	1423186 at	Tiam2	1424317 at	Slc25a19	1425523 at	Rbm25
1421968 a at	Nipa2	1423233 at	Cebpd	1424330 at	Serp1	1425531 at	Znhit1
1421972 s at	Hcfc1	1423238 at	Itgb1bp2	1424353 at	Lrrppc	1425532 a at	Bin1
1422433 s at	Idh1	1423246 at	Erp44	1424385 at	5830417110Ri	1425564 at	Rest
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1425753 a at	Ung	1426727 s at	100039405 ///	1427974 s at	Cacna1d	1431166 at	Chd1
1425773 s at	Nmnat1	1426736 at	Gspt1	1428065 at	Slc44a2	1431188 a at	Tom1
1425811 a at	Csrp1	1426751 s at	Nup107	1428069 at	Cdca7	1431701 a at	Pdzk1
1425837 a at	Ccrn4l /// LOC	1426755 at	Ckap4	1428095 a at	C2cd2l	1431706 at	Ipo5
1425888 at	Klra17	1426761 at	Kdm1	1428100 at	Sfrs1	1431830 at	Zfp329
1426055 a at	Pigg	1426979 at	Mlxip	1428114 at	Slc14a1	1431848 at	Angptl2
1426104 at	Mapk14	1426997 at	Thra	1428117 x at	Dynl1	1432006 at	Ap2a2
1426209 at	Strn4	1427039 at	Epn1	1428146 s at	Acaa2	1432526 a at	Snf8
1426212 s at	Tmem161a	1427046 at	Grhl2	1428216 s at	Tomm7	1433450 at	Cdk5r1
1426214 at	Imp4	1427059 at	Tmem184b	1428244 at	Larp1	1433639 at	Fam117a
1426356 at	6330578E17R	1427090 at	Zbed4	1428331 at	2210016F16Ri	1433753 x at	Eral1
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1426449 a at	Pja1	1427381 at	Irg1	1428803 at	Acot6	1433880 at	Dnajc11
1426455 at	Sdcccag10	1427391 a at	Col12a1	1428862 at	Ttc17	1433883 at	Tpm4
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1436189 at	Nqo2	1438139 at	Arhgap28	1439778 at	Cables1	1441291 at	Abl1	
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1443002 at	Zfr	1444998 at		1446637 at	EG666539	1453366 at	Tdrkh	
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1455308 at	Ano6	1456482 at	Pik3r3	1457780 at	Stx11	1459896 at	Pogk	
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1455437 at	BC033915	1456871 a at	Phf20i1	1458214 at	Zfp786	1460067 at	Ccr2	
1455528 at		1456896 at	6720462K09R	1458255 at		1460085 at		

1434859	at	Umps	1437142	a at	Pigo	1439444	x at	Tmed10	1448858	at	Ulk2
1435056	x at	Pofut2	1437208	at	10-Sep	1439462	x at	Tmed10	1448881	at	Hp
1435067	at	B230208H17R	1437343	x at	Atad3a	1440936	at	Serac1	1448916	at	Mafg
1435114	at	Wdhd1	1437380	x at	Pgd	1441992	at	Rab14	1448929	at	F13a1
1435140	at	lde	1437682	x at	1110004E09Ri	1447958	at		1448931	at	F2r1
1435148	at	Atp1b2	1437723	s at	Derl1	1448016	at	Sass6	1448943	at	Nrp1
1435226	at	Rnf19b	1437845	x at	Pofut2	1448019	at	Taok3	1449024	a at	Hexa
1435271	at	Irf3	1437974	a at	Hk1	1448053	at		1449062	at	Khk
1435331	at	LOC10004830	1438019	at	Ippk	1448081	at	Colq	1449074	at	1700019N12Ri
1435390	at	Exod1	1438120	x at	lrak1	1448171	at	Siah2	1449127	at	Selplg
1435476	a at	Fcgr2b	1438155	x at	Pigo	1448209	a at	Slc22a17	1449137	at	Pdha1
1435477	s at	Fcgr2b	1438164	x at	Flot2	1448225	at	Gpaa1	1449140	at	Nudcd2
1435517	x at	Ralb	1438178	x at	Atad3a	1448309	at	Ap3m1	1449181	at	Fech
1435735	x at	H47	1438188	x at	Slc25a29	1448311	at	Usp5	1449305	at	F10
1435830	a at	5430435G22R	1438627	x at	Pgd	1448377	at	Sipi	1449344	s at	2210409E12Ri
1436007	x at	Thumpd1	1438844	x at	Spata5	1448452	at	Irf8	1449356	at	Asb5
1436048	at	Exoc8	1438912	at	Hdgfrp2	1448550	at	Lbp	1449362	a at	Mink1
1436149	at	Cox5b	1438913	x at	Hdgfrp2	1448580	at	Glg1	1449392	at	Hsd17b1
1436267	a at	Frap1	1438917	x at	Nup62	1448730	at	Cpa3	1449405	at	Tns1
1436382	at	Zbtb12	1439040	at	Cenpe	1448758	at	Nrbf2	1449485	at	Ripk1
1436716	at	Ppp1r14b	1439049	at	Dph5	1448778	at	Sfrs4	1449568	at	Klb
1436771	x at	Pgd	1439074	a at	Son	1448794	s at	Dnajc2	1449642	at	
1436801	x at	Cdc42ep4	1439144	at	Cwf19i1	1448795	a at	LOC10004616	1449647	at	
1436935	x at	Clns1a	1439214	a at	Api5	1448811	at	Mrpl2	1449955	at	Cacna1f
1437044	a at	Gba	1439396	x at	Gpd1	1448828	at	Smc6	1449965	at	Mcpt8
1449968	s at	Aco110 /// Aco1	1451070	at	Gdi1	1452409	at	Gltscr2	1454932	at	Rcor1
1449996	a at	Tpm3	1451091	at	Txndc5	1452410	a at	Fes	1454963	at	E430028B21Ri
1450020	at	Cx3cr1	1451169	at	Nomo1	1452414	s at	Ccdc86	1454964	at	Rprd1a
1450099	a at	Gba	1451175	at	Spcs3	1452419	at	Heatr1	1454977	at	C2cd3
1450100	a at	Tcerg1	1451249	at	Trmt1	1452461	a at	Gnptab	1455128	x at	Tnrc6a
1450157	a at	Hmmr	1451328	at	Pcnxl3	1452580	a at	Mrpl21	1455141	at	Tnrc6a
1450168	at	Ankrd12	1451353	at	Tm6sf1	1452664	a at	Tm7sf3	1455332	x at	Fcgr2b
1450200	s at	Csf2rb /// Csf2	1451367	at	Cops6	1452720	a at	Fip11	1455405	at	Pstpip2
1450234	at	Ms4a6c	1451439	at	BC027231	1452999	at	Srnndc1	1455435	s at	Chdh
1450236	at	Foxo3	1451472	at	Ears2	1453063	at	Cltb	1455575	at	Eif4ebp2
1450297	at	Il6	1451511	at	Hibch	1453106	a at	Rnmt	1455642	a at	Tspan17
1450368	a at	Ppp3r1	1451512	s at	Hibch	1453117	at	Surf2	1455691	at	Cyp21a1
1450399	at	Psen1	1451573	a at	Stx4a	1453169	a at	Gtf2h1	1455740	at	Hnmpa1
1450422	a at	Kdelc1	1451609	at	Tspan33	1453182	a at	Smpd4	1455781	at	
1450643	s at	Acs1	1451628	a at	Ank3	1453256	at	Polr3c	1456011	x at	Acaa1a
1450695	at	Ahr	1451631	at	Rprd1a	1453470	a at	Gna13	1456431	at	Zfp64
1450718	at	Sh2b2	1451649	a at	Wdr75	1453633	a at	Rnf41	1456541	x at	Atad3a
1450751	at	Zbbp	1451745	a at	Znhit1	1453767	a at	Nt5m	1456605	at	Cebpd
1450816	at	Polg2	1451788	at	F11	1453827	at	1110035H17Ri	1456627	at	Ubqln2
1450907	at	Spcs2	1451796	s at	Hdc	1453993	a at	Bnip2	1456630	x at	Son
1450969	at	Pccb	1451804	a at	Lrrc16a	1454197	a at	Ccdc86	1457675	at	2510002D24Ri
1450983	at	Akap8	1452175	at	1810026J23Ri	1454688	x at	Tmed10	1459902	at	2700007P21Ri
1451044	at	Sip1	1452206	at	Sucla2	1454737	at	Dusp9	1459922	at	Al646383
1451054	at	Orm1	1452307	at	Cables2	1454786	at	5031439G07Ri	1459929	at	Zfp568
1451057	x at	Dnm2	1452356	at	lqcc	1454893	at	1110013L07Ri	1459999	at	Sfrs15
1460271	at	Trem3	1428949	at	Xpot	1430379	at	5830411K21Ri	1433010	at	5530400N10Ri
1460283	at	Mefv	1428950	s at	Nol8	1430564	at	Mobk1a	1433098	at	4921524M04Ri
1460330	at	Anxa3	1429024	at	Rbm20	1430569	at	Ttc9c	1433379	at	9430019H13Ri
1460360	at	Asrgl1	1429083	at	Agl	1430608	at	4930535I16Ri	1433498	at	2010005J08Ri
1460373	a at	Setd4	1429107	at	Ubr3	1430815	at	4930534B04Ri	1433759	at	Dpy19I1
1460390	at	Sorl1	1429132	at	Ube2v2	1430866	at	Ccdc41	1433766	at	C330023M02Ri
1460391	at	Ola1	1429152	at	Zkscan1	1430878	at	2210406H18Ri	1433921	s at	Dph3
1460460	a at	Gorasp2	1429216	at	Paqr3	1430892	at	1700011F03Ri	1433930	at	Hpse
1460548	a at	Eral1	1429233	at	11-Sep	1430941	at	Bbs7	1434222	at	Sipa11
1460571	at	Dicer1	1429325	at	Wdr51b	1430984	at	Azin1	1434304	s at	LOC10004760
1460653	at	Atxn2 /// LOC1	1429327	at	Sdccag1	1431053	at	Mphosph9	1434365	a at	BC055324
1460695	a at	201011101Ri	1429392	at	Wdr40a	1431066	at	Fut11	1434424	at	Mfsd7b
1419824	a at	Aasdh	1429504	at	Rnpc3	1431141	at	2610507I01Ri	1434556	at	
1428210	s at	Chuk	1429539	at	Bcl2l13	1431234	at	Itpril1	1434583	at	Tmem26
1428385	at	8-Mar	1429543	at	6230424C14Ri	1431303	at	Stk38	1434622	at	lqce
1428415	at	Rnf113a2	1429570	at	Mkl1	1431361	at	Prpc	1434638	at	
1428428	at	Abhd11	1429673	at	5830407E08Ri	1431561	a at	Dhx34	1434657	at	Gls
1428458	at	Pop1	1429743	at	6720468P15Ri	1431925	at	4933433H22Ri	1434684	at	Rin3
1428547	at	Nt5e	1429778	at	Optn	1432245	s at	Ttc9c	1434728	at	Gria3
1428677	at	Wdr73	1429792	at	9530048O09Ri	1432268	at	2310068J16Ri	1434815	a at	Mapkapk3
1428680	at	Cds1	1429802	at	Hsd17b14	1432363	at	100041434	1434845	at	
1428687	at	Zfp687	1429816	at	Armc3	1432427	at	Ndufb4	1434847	at	Cnm4
1428814	at		1429852	at	Ccdc57	1432625	at	5830487K18Ri	1435000	at	Gspt1
1428876	at	Srp72	1430138	at	Cd3eap	1432708	at		1435132	at	Disp1
1428940	at	Gnaq	1430199	at	1700007E05Ri	1432825	at	2900018N21Ri	1435171	at	2810416G20Ri

Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1460141_at	Frrs1						
1460143_at							
1460620_at	Zfp592						

Downregulated genes in CMP Vs. LSK

Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1415677 at	Dhrs1	1416418 at	Gabarapl1	1417070 at	Cyp4v3	1417749 a at	Ijp1
1415834 at	Dusp6	1416419 s at	Gabarapl1	1417071 s at	Cyp4v3	1417756 a at	Lsp1
1415871 at	Tqfbi	1416473 a at	Igdcc4	1417136 s at	Srpk2	1417787 at	Dkk1
1415919 at	Npdc1	1416514 a at	Fscn1	1417155 at	Mycn	1417806 at	Popdc2
1415943 at	Sdc1	1416521 at	Sepw1	1417160 s at	Expi	1417836 at	Gpx7
1415944 at	Sdc1	1416530 a at	LOC100045567	1417185 at	Ly6a	1417872 at	Fhl1
1415976 a at	Carhsp1	1416536 at	Mum1	1417211 a at	1110032A03Rik	1417952 at	Cyp2j6
1415997 at	Txnip	1416589 at	Sparc	1417271 a at	Eng	1418004 a at	Tmem176b
1416013 at	Pld3	1416600 a at	Rcan1	1417273 at	Pdk4	1418045 at	Inpp1
1416039 x at	Cyr61	1416607 at	4931406C07Rik	1417327 at	Cav2	1418058 at	Eitd1
1416130 at	Pmp	1416630 at	Id3	1417332 at	Rfx2	1418059 at	Eitd1
1416148 at	Laptm4b	1416803 at	Fkbp7	1417378 at	Cadm1	1418065 at	Rag2
1416183 a at	Ldhb	1416811 s at	Ctla2a /// Ctla2b	1417383 at	Entpd5	1418102 at	Hes1
1416221 at	Fstl1	1416827 at	Tbxas1	1417384 at	Entpd5	1418216 at	Ggt5
1416230 at	Rfk	1416861 at	Stam	1417399 at	Gas6	1418219 at	Il15
1416253 at	100043858 /// C	1416872 at	Tspan6	1417445 at	Ndc80	1418240 at	Gbp2
1416263 at	Abcb9	1416897 at	Parp9	1417527 at	Ap3m2	1418301 at	Irf6
1416268 at	Ets2	1416930 at	Ly6d	1417539 at	LOC100046775	1418349 at	Hbegf
1416296 at	Il2rg	1416957 at	Pou2af1	1417599 at	Cd276	1418415 at	Hoxb5
1416301 a at	Ebf1	1416969 at	Gtse1	1417601 at	Rgs1	1418486 at	Vnn1
1416302 at	Ebf1	1416978 at	Fcgrt	1417654 at	Sdc4	1418507 s at	Socs2
1416315 at	Abhd4	1416992 at	LOC100046464	1417662 at	Elk3	1418512 at	Stk3
1416361 a at	Dync1i1	1417040 a at	Bok	1417683 at	Diablo	1418609 at	Il1f6
1416401 at	Cd82	1417063 at	C1qb	1417689 a at	Pdzk1ip1	1418643 at	Tspan13
1416405 at	Bgn	1417068 a at	Ptpn1	1417702 a at	Hnmt	1418776 at	5830443L24Rik
Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1418830 at	Cd79a /// LOC100044830	1420166 at	Dntt	1421223 a at	Anxa4	1422607 at	Etv1
1418843 at	Slc30a4	1420176 x at	Igll1	1421230 a at	Msi2	1422632 at	Ctsw
1418947 at	Nek3	1420177 at	Igll1	1421240 at	Em1	1422646 at	Mga
1418984 at	Inadl	1420318 at		1421368 at	Scrt1	1422659 at	Camk2d
1419032 at	2610018G03Rik	1420382 at	Apob48r	1421377 at	Traf6	1422670 at	Rnd2
1419043 a at	liqp1	1420458 at	Tac4	1421413 a at	Pdlim5	1422711 a at	Pnck
1419103 a at	Abhd6	1420459 at	Ripply3	1421415 s at	Gcnt2	1422772 at	C1galt1
1419104 at	Abhd6	1420515 a at	Pglyrp2	1421461 at	Mpl	1422824 s at	Eps8
1419123 a at	Pdgfc	1420545 a at	Chn1	1421550 a at	Trim34	1422997 s at	Acot1 /// Acot2 /// LOC100044830
1419135 at	Ltb	1420604 at	Hesx1	1421578 at	Ccl4	1423021 s at	Insl3 /// Jak3
1419188 s at	Ccl27a	1420664 s at	Procr	1421784 a at	Efna4	1423055 at	Nsg1
1419222 at	Tbxa2r	1420682 at	Chrnbl	1421909 at	Tcf20	1423187 at	Gabarapl2
1419249 at	Pfkf1	1420772 a at	Tsc22d3	1421920 a at	Ccr9	1423231 at	Nrgn
1419339 at	Neu3	1420832 at	Qsox1	1421983 s at	Hnf4a	1423252 at	Hdgrp3
1419434 at	Slc2a10	1420859 at	Pkia	1422024 at	Fli1	1423321 at	Myadm
1419647 a at	Ier3	1420876 a at	6-Sep	1422256 at	Sstr2	1423336 at	Orc4
1419690 at	2610002M06Rik	1420928 at	St6gal1	1422411 s at	Ear1 /// Ear12 /// LOC100044830	1423356 at	Snap29
1419706 a at	Akap12	1421037 at	Npas2	1422416 s at	Vpreb1 /// Vpreb2	1423461 a at	Ubl3
1419714 at	Cd274	1421074 at	Cyp7b1	1422473 at	Pde4b	1423478 at	Prkcb
1419717 at	Sema3e	1421075 s at	Cyp7b1	1422474 at	Pde4b	1423487 at	Cript
1419722 at	Klk8	1421098 at	LOC100047840	1422498 at	Mageh1	1423499 at	Sncap
1419724 at	Edar	1421149 a at	Atn1 /// Rnu7	1422551 at	Zkscan3	1423555 a at	Ifi44
1419867 a at	Ankhd1 /// Eif4e	1421163 a at	Nfia	1422567 at	Fam129a	1423771 at	Prkcdp
1419905 s at	Hpgd	1421168 at	Abcg3	1422574 at	Mxd4	1423809 at	Tcf19
1420164 at	D7ErtD183e	1421173 at	Irf4	1422603 at	Rnase4	1423859 a at	Ptgds
Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1423986 a at	Shisa5	1424930 s at	Fam83f	1425582 a at	Emcn	1426603 at	Rnase1
1424068 at	Tcta	1424931 s at	Igl /// Igl-V1 /// LOC100044830	1425603 at	Tmem176a	1426624 a at	Ypel3
1424127 at	Eya2	1425077 at	Dnajc18	1425607 at	Hlcs	1426725 s at	Ets1
1424172 at	Hagh	1425084 at	Gimap7	1425742 a at	Tsc22d1	1426743 at	App2
1424179 at	Plekhl1	1425086 a at	Siamf6	1425802 a at	Fcrla	1426772 x at	621968 /// LOC381765 /// LOC665506
1424208 at	Ptger4	1425115 at	Rbbp6	1425813 at	Piqn	1426774 at	Parp12
1424246 a at	Tes	1425134 a at	Piqx	1425814 a at	Calcr1	1426852 x at	Nov
1424254 at	Ifitm1	1425187 at	Sei1l	1425846 a at	Caln1	1426894 s at	Fam102a
1424305 at	Igj	1425226 x at	LOC665506	1425854 x at	LOC665506	1427000 at	Hnf4a
1424335 at	Ppcdc	1425240 at	BC011426	1426010 a at	Epb4.1l3	1427001 s at	Hnf4a
1424359 at	Oplah	1425281 a at	Tsc22d3	1426060 at		1427072 at	Stard8
1424369 at	Psmf1	1425282 at	Rnf144b	1426061 x at		1427075 s at	Pcmt2
1424375 s at	Gimap4	1425293 a at	Zcchc18	1426065 a at	Trib3	1427095 at	Cdcp1
1424400 a at	Aldh1l1 /// LOC100044830	1425307 at	Dnahc1	1426159 x at	Tcrb-V13	1427102 at	Slnf4
1424416 at	Nkiras2	1425345 at	Ccdc28a	1426215 at	Ddc	1427103 at	Plekho2
1424446 at	Armc7	1425363 at	B4galnt1	1426254 at	Tm2d1	1427107 at	Slc16a11
1424448 at	Trim6	1425396 a at	Lck	1426278 at	Ifi2712a	1427143 at	Kdm5b
1424486 a at	Txnrd1	1425483 at	Tox	1426301 at	Alcam	1427171 at	Rlf
1424510 at	Nudt6	1425484 at	Tox	1426324 at	H2-D1	1427186 a at	Mef2a
1424613 at	Gprc5b	1425503 at	Gcnt2	1426348 at	Col4a1	1427216 at	Ilnz
1424660 s at	Crtc2	1425506 at	Myk	1426388 s at	Ryk	1427242 at	Ddx4
1424683 at	Fam134b	1425518 at	Rapgef4	1426417 at	Yipf4	1427284 a at	Tpa
1424733 at	P2ry14	1425519 a at	Cd74	1426566 s at	Il17re	1427329 a at	Igh-6
1424763 at	Rspn9	1425533 a at	Stau2	1426576 at	Sgms1	1427345 a at	Sult1a1
1424923_at	Serpina3g	1425553_s_at	Hip1r	1426590_at	Gfm2	1427351_s_at	Igh-6

Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1427385	s at Actn1	1428572	at Basp1 /// LOC1	1433668	at Pnrc1	1437188	at Gabbr1
1427417	at Scml4	1428585	at Actn1	1433899	x at Tsc22d1	1437279	x at Sdc1
1427419	x at Ccr9	1428879	at Pitpnc1	1434099	at	1437503	a at Shisa5
1427455	x at EG637227 /// EN	1429159	at Itih5	1434100	x at	1437540	at Mcoln3
1427558	s at Alq12	1429292	a at 2310046K01Rik	1434151	at Mettl7a1	1437724	x at Pitpnm1
1427576	at EG637227 /// Iq	1429319	at Rhoh	1434372	at AW112010	1437726	x at C1qb
1427577	x at EG637227 /// Iq	1429359	s at Rbpms	1434703	at Extl3	1437867	at
1427608	a at Tcrg-C	1429400	at Clcn5	1434735	at Hlf	1437882	s at 3110048L19Rik /// 6330416L07Rik
1427646	a at Arhgef2	1429428	at Tcf7l2	1434962	x at Ccl27a	1437889	x at Bgn
1427660	x at EG637227 /// EN	1430375	a at Ccl27a	1435176	a at Id2	1438023	at
1427666	a at Tcrb-J	1430447	a at Lair1	1435259	s at Tmem141	1438676	at Mpa2l
1427667	s at Tcrb-J	1430519	a at Cnot7	1435290	x at H2-Aa	1438939	x at Ndn
1427677	a at Sox6	1430523	s at Igl-V1	1435316	at Psma6	1439296	at Prickle3
1427792	at	1430692	a at Sel1l	1435669	at Zfp532	1439364	a at Mmp2
1427837	at Iqgk15-103	1430826	s at Gcnt2	1435758	at	1440192	at Ttc39b
1427850	x at Igh-VJ558	1431359	a at 1110007C09Rik	1436058	at Rsad2	1442169	at Vldlr
1427891	at Gimap6	1431475	a at Hoxa10	1436228	at Zranb1	1442744	at Rbm39
1427903	at Phpt1	1431591	s at 677168 /// lsg1	1436448	a at Ptgs1	1442900	at
1427920	at Phf19	1431653	at Tcrb-J	1436510	a at Lrrfp2	1447970	at Rpp30
1427934	at Lymr2	1431705	a at Mcoln2	1436570	at	1448021	at
1428003	s at Thtpa /// Zfhx2a	1431746	a at Uba3	1436780	at Ogt	1448121	at Wbp2
1428042	at ENSMUSG0000	1431890	a at Milt3	1436879	x at Afp	1448123	s at Tgfb1
1428306	at Ddit4	1432151	at Ola1	1436915	x at Laptm4b	1448147	at Tnfrsf19
1428453	at Nat12	1433508	at Klf6	1436990	s at Chchd10	1448162	at Vcam1
1428502	at Actr6	1433662	s at Timp2	1437052	s at Slc2a3	1448194	a at H19
Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol	Probe Set ID	Gene Symbol
1448233	at Prnp	1448999	at Trappc5	1449913	at Zfp2	1451024	at S1pr4
1448248	at Crk	1449008	at Tulp3	1449927	at S100a5	1451029	at Bcl2l2
1448259	at Fstl1	1449009	at Tgtp /// Tgtp2	1449988	at Gimap1	1451107	at Tbc1d22a
1448260	at Uchl1	1449028	at Rhou	1450029	s at Itga9	1451130	at Use1
1448293	at Ebf1	1449078	at St3gal6	1450057	at Sys1	1451156	s at Vldlr
1448313	at Tpp1	1449090	a at Yes1	1450078	at Nrk	1451230	a at Wbp5
1448323	a at Bgn	1449109	at Socs2	1450080	at Cxx1c	1451264	at Frmd6
1448378	at Fscn1	1449126	at Zfp90	1450082	s at Etv5	1451310	a at Ctsl
1448436	a at Irf1	1449134	s at Spic	1450138	a at Serpinb6a	1451321	a at Rbm43
1448471	a at Ctla2a	1449143	at Rtp4	1450141	at Abcg3	1451416	a at Tgm1
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1444363_at		1447101_at	Rnf122	1454104_a_at	Slc16a9	1456060_at	Maf
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1459659 at	LOC100040377						
1459847 x at	Gfra2						
1459854 s at	Dynl13						
1459866 x at	Cyflp1 /// Nipa2						
1459913 at	Tnfrsf10						
1460108 at							

Figure S3, related to Figure 4. GSEA analysis showing enrichment of a “CMP-like” expression signature in *Tet2*^{-/-} LSK cells. The CMP-like signature was generated by comparing gene expression between wildtype LSK and CMP cells (see experimental procedures). **A)** Heat map showing comparative expression changes between the indicated cell populations, **B)** GSEA analysis showing enrichment of CMP genes. **C)** The stem cell signature was derived from two independent studies (see figure for references). Upper panel: enplot showing enrichment of stem cell signatures in *Tet2*^{-/-} LSKs. Lower panel: enplot showing negative correlation of a mature hematopoietic cell signature in *Tet2*^{-/-} LSKs compared to *Tet2*^{+/+} LSK. **D)** Complete list of differentially expressed genes derived from comparing the gene expression signatures of WT LSK to WT CMP.

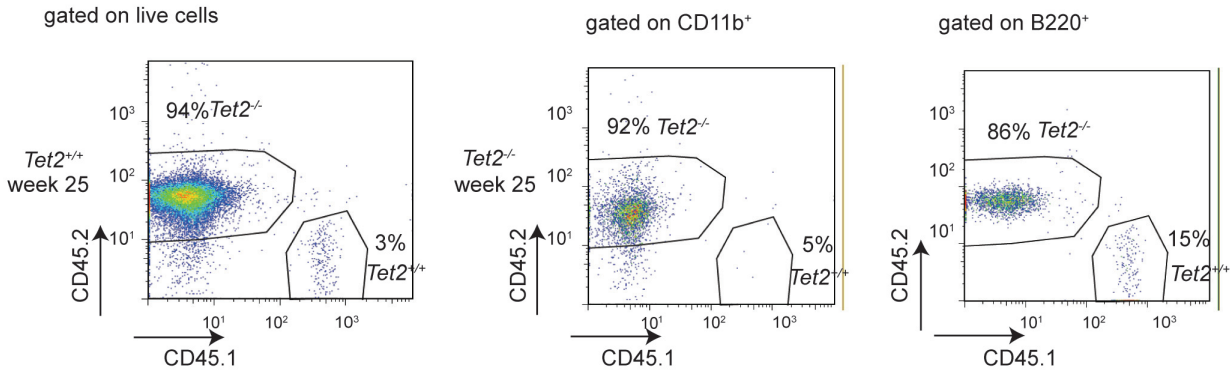


Figure S4, related to Figure 5. Peripheral blood analysis of *Tet2-VavCre* competitive transplants. Left Panel: Chimerism of CD45.1 (recipient) and CD45.2 (*Tet2*^{-/-}*VavCre*^{+/+}) in the peripheral blood of recipient animals at 25 weeks post transplant. Chimerism of CD45.1 and CD45.2 in the myeloid (middle panel) and lymphoid (right panel) lineages of recipient animals at 25 weeks.

A

4-6 weeks

MOUSE	WBC	Neutrophils	Monocytes	Lymphocytes	HCT	PLT	HGB	MCV	RDW
Control 36	4.2	0.504	0.882	2.814	36	324	120	53.1	14.9
Control 37	4.5	0.63	0.99	2.88	31.8	386	117	51	14.2
Control 38	4.7	0.705	0.893	3.102	36.1	434	120	54	13.5
Control 91	5	1.45	1.45	2.1	56.3	295	172	53.2	13.4
Control 84	4.4	1.1	1.364	1.936	58	270	190	50.4	12.8
Het 43	9	0.9	1.62	6.48	50.9	374	153	54.6	15.2
Het 71.2	3.5	0.84	1.225	1.435	58.8	276	179	53.1	13.9
Het 82	7.8	1.248	3.12	3.432	53.5	305	165	53.2	13.4
Het 87	8.1	0.729	3.726	3.645	52	385	164	51.6	13.3
KO 49	6.4	1.152	1.024	4.224	53.7	429	154	56.9	18
KO 75	3.6	0.396	2.124	1.08	55.5	256	183	48.9	13.7
KO 79	5	0.5	2.4	2.1	48.7	282	149	51.8	13.6
KO 86	8.3	1.162	3.901	3.237	54	215	167	51.9	13.3

B

20 weeks

MOUSE	WBC	Neutrophils	Monocytes	Lymphocytes	HCT	PLT	HGB	MCV	RDW
Control 1	4.4	0.66	0.66	2.596	42.8	408	145	41	13.8
Control 2	4.5	0.54	0.63	3.33	50.5	652	147	53.5	13.5
Control 3	5.5	0.55	0.385	4.565	40.9	296	140	42.5	13.6
Het 1	7.1	1.136	1.562	4.402	60.9	762	177	55.1	15.2
Het 2	10.1	1.335	3.56	4.005	47.3	396	164	46.3	13.9
Het 3	9.9	1.173	2.553	3.174	53.8	507	165	50.3	14.1
KO 1	7.2	1.08	1.584	4.536	51.5	580	161	48.9	14.4
KO 2	15.1	1.51	3.624	9.966	65.5	590	174	56.1	18.1
KO 3	7.5	1.425	2.55	3.525	46.3	432	154	47.2	14.6
KO 4	11.1	1.332	1.554	8.214	42.3	621	141	43.3	14.4
KO 5	10.9	0.872	1.635	8.393	63.2	520	178	50.9	18.8

Table S1, related to Table 1. Complete peripheral blood analysis of *Tet2*^{+/+} (Control), *Tet2*^{+/-} and *Tet2*^{-/-} mice at 4-6 weeks and 20 weeks of age. A) Manual differential counts for a subset of mice at 4-6 and 20 weeks (B) of age.

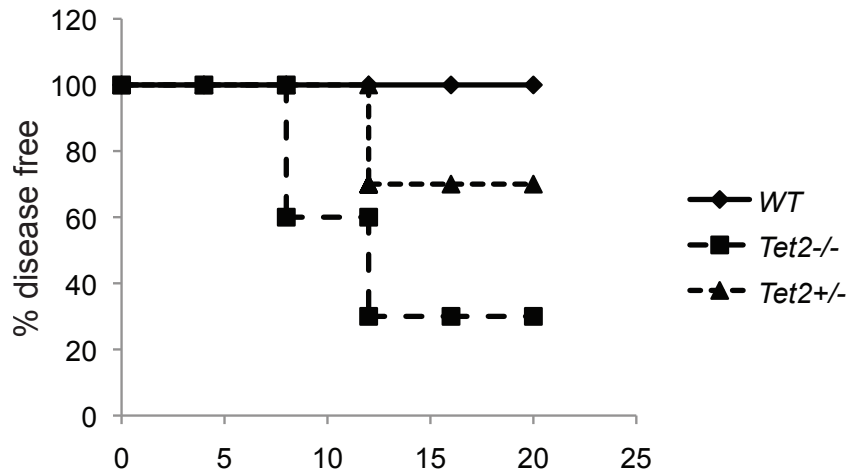
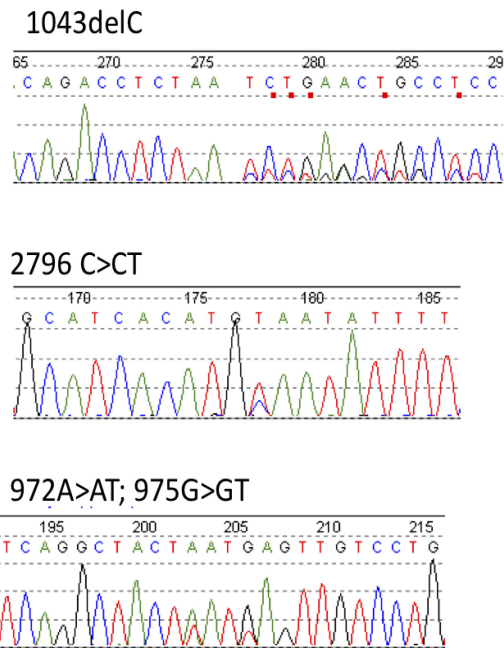


Figure S5, related to Figure 6: Kaplan-Meier curve showing the percentage of Tet2^{-/-} mice that are disease free at 20 weeks of age.

A. Genomic DNA



B. cDNA

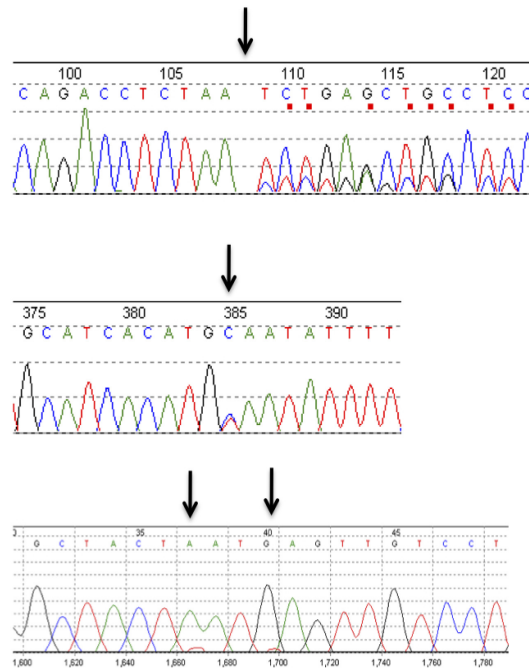


Figure S6, related to Figure 7: Expression of wild type allele in TET2 mutant patient samples. Sequencing results for paired genomic DNA (A) and cDNA (B) from patient samples with TET2 mutations.

Supplemental Experimental Procedures

Quantitative real-time PCR

RNA was isolated with the RNeasy-Plus mini kit (Qiagen). The RNA was then used as a template for cDNA synthesis using the SuperScript III Reverse Transcriptase kit according to manufacturer's protocol (Invitrogen). Quantitative real-time PCR reactions were carried out using SYBR Green I Master on a Light Cycler 480 (Roche). Relative expression was determined by the Δ/Δ CT method and normalized to the internal control, *GAPDH*. For real-time primer sequences see below.

Flow cytometry and antibodies

Flow cytometry was carried out as previously described (Thompson et al., 2008). Antibodies used for flow cytometry were as follows: anti-CD4 (L3T4), anti-CD44 (IM7), anti-Gr1 (Ly6G), anti-B220 (RA3-682), IgM (II/41), anti-IL7R (A7R34), anti-CD34 (RAM34), FC γ R(93) anti-Sca-1 (D7; all from eBioscience); anti-c-Kit (2B8), anti-Mac-1 (CD11b) (M1/70), anti-NK1.1 (PK136), anti-Ter119 (Ter119,553673), anti-CD3 (145-2C11), anti-CD19 (1D3), anti-CD8 (53-6.7), CD45.1 (A20), CD45.2 (104) and anti-CD25 (PC61; all from BD Biosciences); and anti-CD150 (TC-15-12F2.2) and CD48 (HM48-1; both from Biolegend. Both the bone marrow and spleen 'lineage cocktail' included anti-CD4, anti-CD8, anti-IL-7R, anti-Gr-1, anti-Mac-1 (CD11b), anti-NK1.1, anti-B220, and anti-Terr-119.

Genotyping

Tails were clipped from mice 1-2 weeks after birth and subsequently incubated in tail lysis buffer containing proteinase K overnight at 55 C. The next day, cell debris was pelleted by centrifugation upon the addition of NaCl. DNA was precipitated with isopropanol and pelleted by centrifugation. The DNA pellet was washed once in 70% ethanol and re-pelleted. DNA pellets dissolved in water were used for PCR analysis. Primers were designed to detect the *Tet2* WT, floxed, and recombined allele in standard PCR conditions. Both Mx-1 and Vav- specific primers were used to detect the cre recombinase alleles carried by the *Tet2* mice. For primer sequences, see below.

shRNA sequences	
Tet2 (mouse)	accgatgtcctttaggac
Tet2 (mouse)	tctatacattatagtccta
genotyping Primer Sequences	
Tet2 LoxP3R	tagagggagggggcataagt
Tet2 Flox F	aagaattgctacaggcctgc
Tet2 Flox R	ttcttagcccttgctgagc
qRT PCR Primer Sequences	
Tet1 F	acatcccacagaccgaagat
Tet1 R	ttctggggtttcactcctc
Tet2 F	agagcctcaagcaacaaaa
Tet2 R	acatccctgagagctcttg
Tet3 F	agagcctcaagcaacaaaa
Tet3 R	aaagtggttctgaggctcca
Tet1 F (human)	ccgaatcaagcggaagaata
Tet1 R (human)	cctggagatgcctctttcac
Tet2 isoform A F (human)	ggagtggtgccattcaggta
Tet2 isoform A R (human)	tgtggtggctgcttctgtag
Tet2 isoform b F (human)	cacatggcgttatccagaa
Tet2 isoform b R (human)	gctgcctagctgtctctcca
Tet3 F2 (human)	gcactccggagaagatcaag
Tet3 R2 (human)	ggagtacacgctgttcatgc

Supplemental References

Thompson, B. J., Jankovic, V., Gao, J., Buonamici, S., Vest, A., Lee, J. M., Zavadil, J., Nimer, S. D., and Aifantis, I. (2008). Control of hematopoietic stem cell quiescence by the E3 ubiquitin ligase Fbw7. *J Exp Med.*