Supplemental Materials and Methods

Generation of *Recql4^{fl/fl}* mice

The targeting vector was generated using BAC clones from the C57BL/6J RPCI-23 BAC library and was transfected into TaconicArtemis C57BL/6N Tac ES cell line. Gene targeting was performed in the TaconicArtemis C57BL/6N Tac ES cell line. Exons 9 and 10 were flanked by LoxP sites (size of loxP-flanked region: 1.0 kb) with a positive selection marker (Puromycin resistance - Puro^R) flanked by F3 sites inserted into intron 8. Homologous recombinant clones were isolated using positive (Puro^R) and negative (Thymidine kinase) selection. Positive clones were selected and expanded and screened by Southern blot for correct targeting events. A correctly targeted ES line was used to generate chimeras. Chimerism was measured in chimeras (G0) by coat color contribution of ES cells to the BALB/c host (black/white). Highly chimeric mice were bred to strain Flp-deleter C57BL/6 females (C57BL/6-*Tg^{(CAG-Flpe)2 Arte}*) (1). Once Flp recombination was confirmed the Flpe transgene was bred off the *Recql4^{fl/fl}* line. Genotyping was performed by PCR using primers: 3303_41: CACTCTAGAAGAGGGAGTCAGATGG and 3303_42: ACAGCAACAGAACAGCAACTACG to yield products of 165bp for the WT and 325bp for the floxed allele. The deleted allele was detected by adding the primer CGCGCGAAAGCTGAGGAGTT to primers 3303_41 and 3303_42 to generate a 256bp product for the excised allele. All PCR products were sequence verified. We thank J Hartner (TaconicArtemis, Cologne, Germany) for advice on targeting strategy.

Germline deletion of Recql4 was achieve by crossing *Recql4^{fl/fl}* mice with *TNAP*-Cre (provided by K Matthaei, Australian National University, Canberra, Australia (2)).

Histology

Tissue was collected and fixed immediately in 2-4% fresh paraformaldehyde. Immunohistochemistry was performed on paraffin sections of tibiae. Antigen-retrieval was undertaken using 10mM Citrate buffer pH 6.0 at 95C for 10 min. After endogenous peroxidase and non-specific protein block [3% H_2O_2, 10% FCS, 10% Normal Horse Serum], anti-cleaved caspase 3 mAb (0.65µg/ml, Cell Signaling) was incubated overnight at 4°C. After secondary pAb (Polyclonal Swine Anti-Rabbit Immunoglobulins/Biotinylated Swine F(ab')2 Dako) and streptavidin horseradish peroxidase incubation (SA-HRP, Dako), tyramide signal amplification (TSA, Perkin-Elmer) was used, followed again by SA-HRP incubation. Staining was developed with DAB+ [3'3' Diamino benzidine, Sigma) and briefly counterstained with Mayer's hematoxylin.

Immunoblot

Rabbit polyclonal antibodies against murine Recql4 were generated by GL Biochem (Shanghai) Ltd. The antibody that showed specific activity against over-expressed Flag-tagged mRecql4 was used for western blotting. This antibody was raised against a peptide fragment spanning amino acids 74-93 (sequenced used QEPSCWGPHLSRAATQNTQS).

Western blots were performed on tumour material derived from *Osx*-Cre *p53$^{fl/fl}$Recql4$^{+/+}$* and *Osx*-Cre *p53$^{fl/fl}$Recql4$^{fl/fl}$* mice (to be described in detail elsewhere).

In vitro B and T cell assay after retroviral complementation

LKS+ were isolated as previously described (3). Cells were cultured for 48 hours, infected as described in retroviral complementation section and then after 48 hours placed into B or T cell culture. For B or T cell cultures, 20,000 OP9 or OP9-DL1 cells/ml were plated in a 24-well plate 48 hours before infected LKS+ were added and cultured as described (4).

Flow Cytometric Analysis of Intracellular γH2AX (Phospho-Histone H2A.X^{Ser139}) and Cell Cycle

T and B cell precursors were collected from the co-cultures then fixed with 2% paraformaldehyde for 20 mins at RT. Cells were permeabilized with ice-cold methanol on ice, washed with PBS/BSA and resuspended in Alexa Fluor 647™ Rabbit anti-γH2AX antibody clone 20E3 (Cell Signaling). Cell were washed, resuspended in PBS containing 20 μg/ml Propidium Iodide (PI) and 100 μg/ml RNase A and analyzed by flow cytometry. Results were analyzed with FlowJo software Version 9.0 (Treestar). γH2AX foci were visualized on a per cell basis using a laser scanning cytometer (Compucyte).

Analysis of Recql4 expression in murine megakaryocytes

Raw CEL files downloaded from GEO (GSE6593) were processed in GenePattern using ExpressionFileCreator v7 (method = RMA, quantile normalization = yes, background correct = yes, compute present absent calls = yes, normalization method = median scaling). Probeset 1422922_at (RECQL4) was not detected above background in any sample.

Raw CEL files downloaded from GEO (GSE49664) were processed in GenePattern using NormalizeAffymetrixST v 2.2 (algorithm = RMA, probesets = core, controls = include, chip = Affymetrix_MoGene_1_0_st_v1.na33.2.mm9.transcript.chip). As P/M/A calls are not available we define a gene to be detected if its expression is greater that the median of the control probes, plus 1 s.d. Probeset 10430071 (Recql4) was detected in 3/4 HSC, 3/4 ERY and 0/4 MEG samples.

References:

1. Buchholz, F., Angrand, P.O., and Stewart, A.F. 1998. Improved properties of FLP recombinase evolved by cycling mutagenesis. *Nat Biotechnol* 16:657-662.
2. Lomeli, H., Ramos-Mejia, V., Gertsenstein, M., Lobe, C.G., and Nagy, A. 2000. Targeted insertion of Cre recombinase into the TNAP gene: excision in primordial germ cells. *Genesis* 26:116-117.
3. Walkley, C.R., Fero, M.L., Chien, W.M., Purton, L.E., and McArthur, G.A. 2005. Negative cell-cycle regulators cooperatively control self-renewal and differentiation of haematopoietic stem cells. *Nat Cell Biol* 7:172-178.
4. Smeets, M.F., Chan, A.C., Dagger, S., Bradley, C.K., Wei, A., and Izon, D.J. 2013. Fli-1 overexpression in hematopoietic progenitors deregulates T cell development and induces pre-T cell lymphoblastic leukaemia/lymphoma. *PLoS One* 8:e62346.

AnnexinV and 7AAD data from Day 14 post tamoxifen in indicated populations within the bone marrow
All animals fed tamoxifen containing chow for 14 days
Control = R26-CreERT2 ki/+ Recql4fl/+ (n=3); KO = R26-CreERT2 ki/+ Recql4fl/fl (n=4)
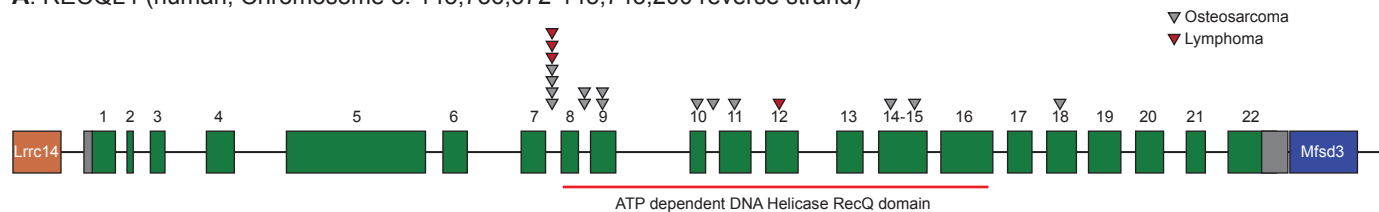Data expressed as mean +/- sem

Population	Annexin V+/7AAD-		Fold difference	T test	Annexin V+/7AAD+		Fold difference	T test	AnnexinV-/7AAD+		Fold difference	T test
	Control	Recql4fl/fl	(KO/WT)		Control	Recql4fl/fl	(KO/WT)		Control	Recql4fl/fl	(KO/WT)	
LT-HSC (LKS+CD150+CD105+)	21.0 +/- 4.4	13.8 +/- 0.77	0.65	0.114	21.5 +/- 2.8	21.1 +/- 4.35	0.98	0.946	9.06 +/- 3.9	19.2 +/- 3.0	1.99	0.104
MPP (LKS+CD150-CD105-)	7.2 +/- 0.8	4.8 +/- 0.85	0.68	0.118	14.7 +/- 1.2	21.3 +/- 2.86	1.45	0.119	12.4 +/- 3.5	26.6 +/- 1.91	2.15	0.012
GMP	2.3 +/- 0.4	2.8 +/- 0.6	1.22	0.541	24.7 +/- 1.6	22.6 +/- 3.71	0.91	0.667	14.4 +/- 4.0	20.0 +/- 1.68	1.38	0.21
MkP	4.7 +/- 0.3	4.4 +/- 0.87	0.95	0.842	24.9 +/- 0.6	25.2 +/- 3.56	1.01	0.942	20.0 +/- 4.9	29.4 +/- 2.4	1.47	0.084
Pre-MegE	5.6 +/- 0.9	4.7 +/- 0.89	0.84	0.519	11.9 +/- 2.6	16.1 +/- 3.23	1.35	0.383	5.5 +/- 1.7	18.2 +/- 2.44	3.31	0.011
Pre-CFU-E	12.1 +/- 1.0	11.2 +/- 2.19	0.92	0.753	1.3 +/- 0.8	1.5 +/- 0.54	1.18	0.819	2.8 +/- 1.4	2.1 +/- 0.46	0.74	0.3
CFU-E	11.3 +/- 2.3	9.3 +/- 2.09	0.82	0.545	4.9 +/- 0.5	5.8 +/- 1.74	1.16	0.718	5.1 +/- 1.9	8.7 +/- 2.4	1.7	0.32
Granulocytes	5.3 +/- 0.7	7.6 +/- 0.77	1.42	0.92	1.9 +/- 0.3	3.4 +/- 0.32	1.79	0.023	2.1 +/- 0.3	3.1 +/- 0.49	1.49	0.172
Monocytes/Macrophages	18.6 +/- 3.7	20.8 +/- 2.28	1.12	0.616	4.4 +/- 0.9	6.7 +/- 1.31	1.54	0.233	3.8 +/- 0.4	4.2 +/- 0.41	1.11	0.527
Erythroid												
CD71+ Ter119+	5.6 +/- 0.9	6.6 +/- 1.6	1.19	0.436	1.71 +/- 0.1	3.0 +/- 0.38	1.74	0.039	2.4 +/- 0.7	2.6 +/- 0.56	1.1	0.783
CD71 mid Ter119+	7.7 +/- 1.4	8.6 +/- 0.98	1.13	0.578	6.5 +/- 1.5	10.0 +/- 1.49	1.52	0.172	2.9 +/- 0.6	3.5 +/- 0.59	1.2	0.532
CD71- Ter119+	1.2 +/- 0.0	1.2 +/- 0.1	0.97	0.743	0.4 +/- 0.1	0.5 +/- 0.11	1.19	0.651	0.2 +/- 0.0	0.2 +/- 0.03	1.15	0.645
B lymphoid												
Mature B (B220+IgM+)	22.6 +/- 2.8	18.4 +/- 1.96	0.81	0.258	16.7 +/- 3.5	21.2 +/- 1.24	1.27	0.226	1.9 +/- 0.4	2.1 +/- 0.54	1.14	0.733
PreProB	17.6 +/- 2.2	18.2 +/- 2.27	1.03	0.537	7.0 +/- 0.6	8.0 +/- 1.09	1.13	0.537	1.0 +/- 0.3	1.6 +/- 0.45	1.6	3.42
ProB	4.89 +/- 0.1	5.7 +/- 0.89	1.18	0.441	1.4 +/- 0.1	3.3 +/- 0.49	2.4	0.022	1.0 +/- 0.2	2.0 +/- 0.52	2.09	0.165
PreB	25.9 +/- 0.9	27.4 +/- 2.55	1.06	0.629	14.5 +/- 0.4	21.2 +/- 1.46	1.46	0.012	14.5 +/- 0.4	1.9 +/- 0.47	1.38	0.427
T lymphoid												
CD4+ CD8-	13.8 +/- 1.0	21.7 +/- 2.7	1.57	0.069	5.6 +/- 0.3	7.1 +/- 1.05	1.26	0.309	4.4 +/- 0.4	4.2 +/- 0.52	0.97	0.872
CD4- CD8+	6.2 +/- 0.5	7.7 +/- 0.56	1.25	0.147	15.5 +/- 2.2	24.9 +/- 3.96	1.61	0.148	9.8 +/- 0.6	10.6 +/- 0.63	1.08	0.495

Supplemental Table 2

Figure 5 Panel All statistical tests done with Ordinary One-Way ANOVA with multiple comparison test using Prism software
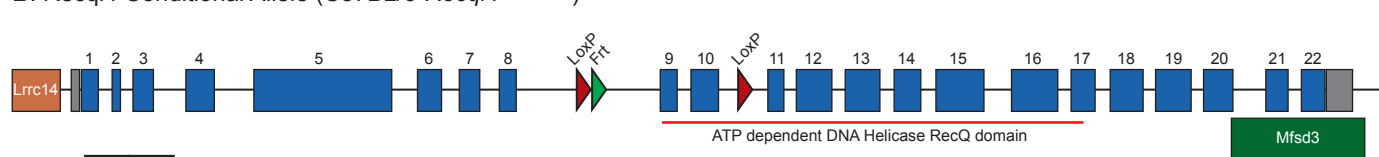
J — Day 30 WBC count in PB

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	4.493	2.373 to 6.613	Yes	***
Recql4fl/+ vs. p53fl/fl	0.8929	-1.227 to 3.013	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	3.943	1.823 to 6.063	Yes	***
Recql4fl/fl vs. p53fl/fl	-3.6	-5.992 to -1.208	Yes	**
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.55**	**-2.942 to 1.842**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	3.05	0.6584 to 5.442	Yes	*

Day 30 RBC count in PB

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	6.325	4.544 to 8.106	Yes	****
Recql4fl/+ vs. p53fl/fl	-0.025	-1.806 to 1.756	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	4.725	2.944 to 6.506	Yes	****
Recql4fl/fl vs. p53fl/fl	-6.35	-8.359 to -4.341	Yes	****
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-1.6**	**-3.609 to 0.4094**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	4.75	2.741 to 6.759	Yes	****

K — BM cellularity

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	9.208	5.738 to 12.68	Yes	****
Recql4fl/+ vs. p53fl/fl	-0.2167	-3.687 to 3.254	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	8.383	4.913 to 11.85	Yes	****
Recql4fl/fl vs. p53fl/fl	-9.425	-13.23 to -5.623	Yes	****
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.825**	**-4.627 to 2.977**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	8.6	4.798 to 12.40	Yes	****

L — LT-HSC

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	0.0001833	-0.001116 to 0.001482	No	ns
Recql4fl/+ vs. p53fl/fl	0.0007333	-0.0005656 to 0.002032	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	-0.0006417	-0.001941 to 0.0006573	No	ns
Recql4fl/fl vs. p53fl/fl	0.00055	-0.0008729 to 0.001973	No	ns
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.000825**	**-0.002248 to 0.0005979**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	-0.001375	0.002798 to 4.795e-00	No	ns

MPP

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	0.002083	-0.01915 to 0.02332	No	ns
Recql4fl/+ vs. p53fl/fl	0.003558	-0.01768 to 0.02480	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	-0.01789	-0.03913 to 0.003345	No	ns
Recql4fl/fl vs. p53fl/fl	0.001475	-0.02179 to 0.02474	No	ns
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.01998**	**-0.04324 to 0.003289**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	-0.02145	-0.04471 to 0.001814	No	ns

M — B220+IgM+ B cells

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	0.6949	0.2097 to 1.180	Yes	**
Recql4fl/+ vs. p53fl/fl	0.04517	-0.4400 to 0.5303	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	0.7489	0.3060 to 1.192	Yes	**
Recql4fl/fl vs. p53fl/fl	-0.6497	-1.210 to -0.08951	Yes	*
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**0.05397**	**-0.4701 to 0.5780**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	0.7037	0.1797 to 1.228	Yes	**

B220+IgM- B cells

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	1.414	0.2857 to 2.543	Yes	*
Recql4fl/+ vs. p53fl/fl	-0.3462	-1.475 to 0.7824	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	1.465	0.4352 to 2.496	Yes	**
Recql4fl/fl vs. p53fl/fl	-1.76	-3.064 to -0.4573	Yes	**
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**0.05116**	**-1.168 to 1.270**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	1.812	0.5926 to 3.031	Yes	**

N — CD71+Ter119+ erythroid cells in BM

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	3.497	1.847 to 5.148	Yes	***
Recql4fl/+ vs. p53fl/fl	0.5843	-1.066 to 2.234	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	3.368	1.718 to 5.018	Yes	***
Recql4fl/fl vs. p53fl/fl	-2.913	-4.721 to -1.105	Yes	**
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.1294**	**-1.937 to 1.678**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	2.784	0.9761 to 4.591	Yes	**

CD71midTer119+ erythroid cells in BM

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	0.007183	-0.6453 to 0.6597	No	ns
Recql4fl/+ vs. p53fl/fl	-0.5537	-1.206 to 0.09885	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	0.002358	-0.6502 to 0.6549	No	ns
Recql4fl/fl vs. p53fl/fl	-0.5609	-1.276 to 0.1540	No	ns
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.004825**	**-0.7196 to 0.7100**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	0.556	-0.1588 to 1.271	No	ns

CD71-Ter119+ erythroid cells in BM

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	0.5287	-0.9528 to 2.010	No	ns
Recql4fl/+ vs. p53fl/fl	-2.275	-3.757 to -0.7936	Yes	**
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	0.4507	-1.031 to 1.932	No	ns
Recql4fl/fl vs. p53fl/fl	-2.804	-4.427 to -1.181	Yes	***
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.078**	**-1.701 to 1.545**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	2.726	1.103 to 4.349	Yes	**

O — Thymus Cellularity

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	106.7	47.19 to 166.2	Yes	***
Recql4fl/+ vs. p53fl/fl	-8.775	-68.26 to 50.71	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	103.6	44.09 to 163.1	Yes	**
Recql4fl/fl vs. p53fl/fl	-115.5	-178.2 to -52.75	Yes	**
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-3.1**	**-65.80 to 59.60**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	112.4	49.65 to 175.1	Yes	***

P — Thymic subsets
CD4+

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	8.461	-8.747 to 25.67	No	ns
Recql4fl/+ vs. p53fl/fl	-7.041	-24.25 to 10.17	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	7.298	-9.910 to 24.51	No	ns
Recql4fl/fl vs. p53fl/fl	-15.5	-33.64 to 2.638	No	ns
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-1.163**	**-19.30 to 16.98**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	14.34	-3.801 to 32.48	No	ns

CD4+CD8+

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	91.66	48.37 to 134.9	Yes	***
Recql4fl/+ vs. p53fl/fl	16.94	-26.34 to 60.22	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	90.74	47.46 to 134.0	Yes	***
Recql4fl/fl vs. p53fl/fl	-74.72	-120.3 to -29.09	Yes	**
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.9165**	**-46.54 to 44.71**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	73.8	28.18 to 119.4	Yes	**

CD8+

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Recql4fl/+ vs. Recql4fl/fl	2.365	-15.15 to 19.88	No	ns
Recql4fl/+ vs. p53fl/fl	-12.53	-30.05 to 4.983	No	ns
Recql4fl/+ vs. Recql4fl/fl p53fl/fl	2.184	-15.33 to 19.70	No	ns
Recql4fl/fl vs. p53fl/fl	-14.9	-33.36 to 3.566	No	ns
Recql4fl/fl vs. Recql4fl/fl p53fl/fl	**-0.1805**	**-18.64 to 18.28**	**No**	**ns**
p53fl/fl vs. Recql4fl/fl p53fl/fl	14.72	-3.746 to 33.18	No	ns

Symbol	P value
*	0.05
**	0.01
***	0.001
****	0.0001
ns	not significant

Figure 1 Smeets et al

A. RECQL4 (human; Chromosome 8: 145,736,672-145,743,200 reverse strand)

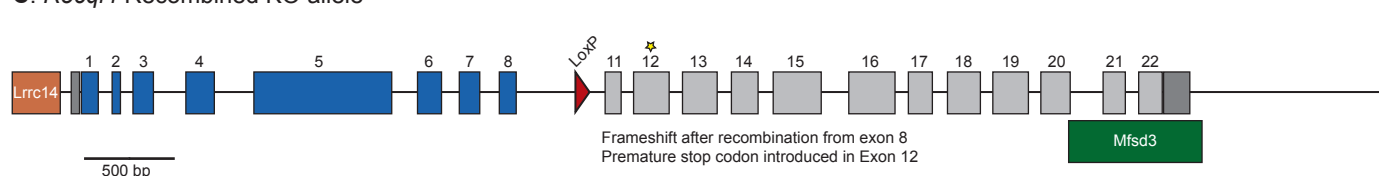ATP dependent DNA Helicase RecQ domain

Rothmund-Thomson Syndrome associated tumour mutations (RTS); adapted from Eur J Hum Genet. 2009 Feb;17(2):151-8

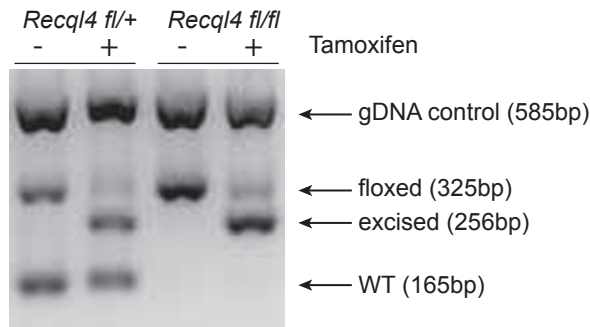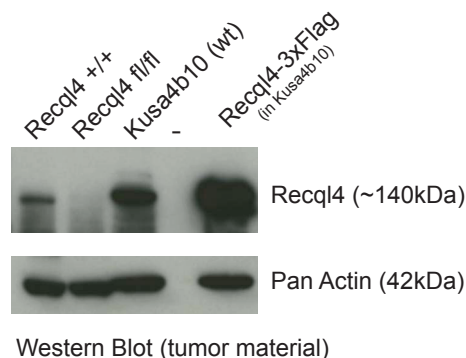**B**. Recql4 Conditional Allele (C57BL/6-*Recql4*tm2272Arte)

ATP dependent DNA Helicase RecQ domain

500 bp

+ Cre

C. *Recql4* Recombined KO allele

Frameshift after recombination from exon 8
Premature stop codon introduced in Exon 12

500 bp

D. *Rosa26*-CreER^T2

E.

Western Blot (tumor material)

Supplemental Figure 1. Outline of targeted allele and data from germ-line deleted allele analysis
A. Human Recql4 locus with mutations in RTS patients indicated
B. Mouse Recql4 locus with inserted LoxP sites indicated. Exon 9 and 10 of mouse Recql4 are flanked by LoxP elements
C. Recombined Recql4 allele after Cre exposure. Frame shift predicted to introduce a stop codon in exon 12.
D. Genomic PCR showing recombination of the floxed allele to produce the excised product of the expected size.
E. Western blot of Recql4 expression in tumours from Cre+ Recql4+/+ and Cre+ Recql4fl/fl mice as indicated. Kusa4b10 endogenous control and Recql4-3xFlag tagged expressing Kusa4b10 used as a control. Tumour model will be described elsewhere in detail.

Gene Expression Commons

https://gexc.stanford.edu/

Gene Name Search Result

On **Mouse Hematopoiesis Model** submitted by Jun Seita (Stanford University)

Reference: PubMed ID: 22815738 , GEO GSE ID: 34723

Recql4 RecQ protein-like 4
Other Name: RecQ4
NCBI Entrez Gene: 79456

1 probeset on Affymetrix Mouse Genome 430 2.0 Array

Probeset	Gene	Alignment	Dynamic-range
1422922_at	Recql4	chr15:76533990-76540978 (-), 99.62, q24.2	4.58

Recql4 by Probeset 1422922_at

Alignment: chr15:76533990-76540978 (-), 99.62, q24.2

Probeset Meta Profile

11939 Microarrays in GEO Analyzed

Dynamic-range: 4.58
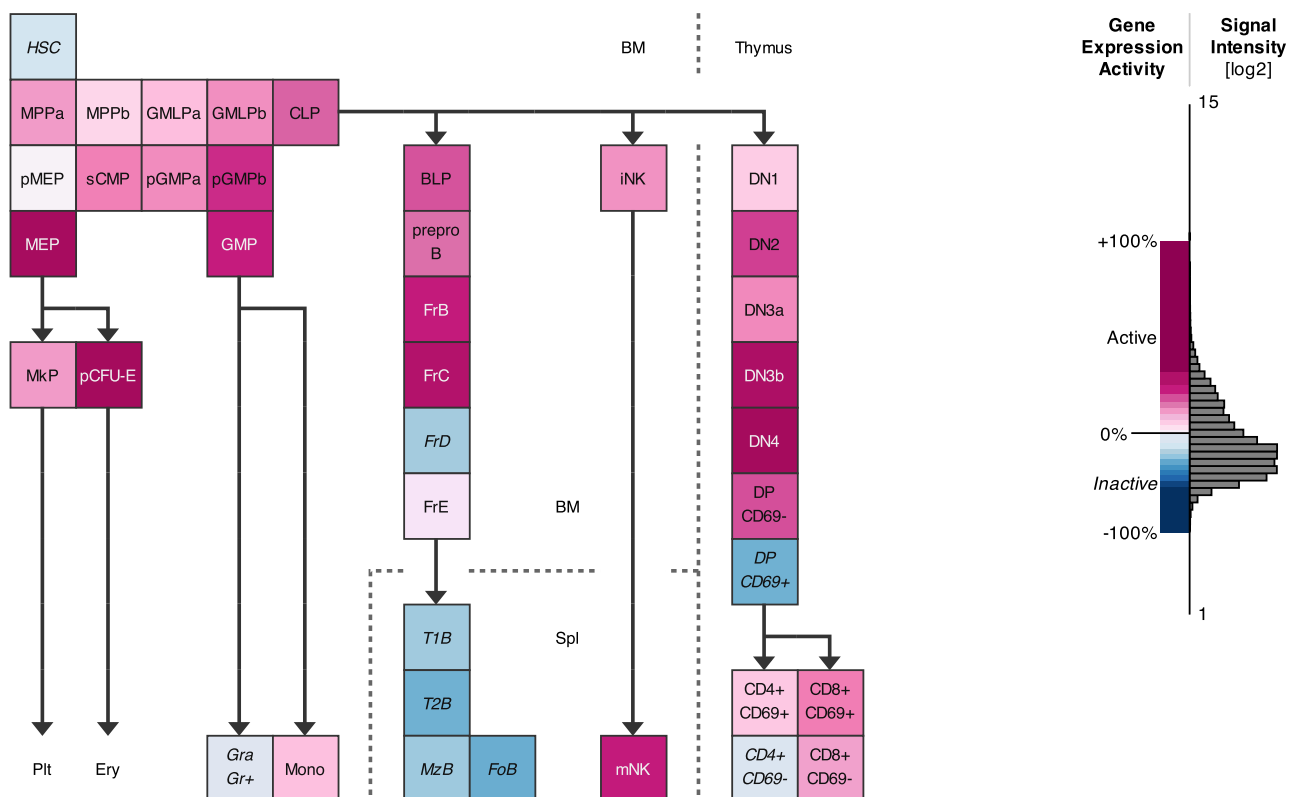
Supplemental Figure 2. Expression of Recql4 in murine hematopoietic cell subsets from Gene Expression Commons database
The expression of Recql4 in murine hematopoiesis based on data retrieved from the Gene Expression Commons database.
Blue indicates low expression, red indicates highest expression.
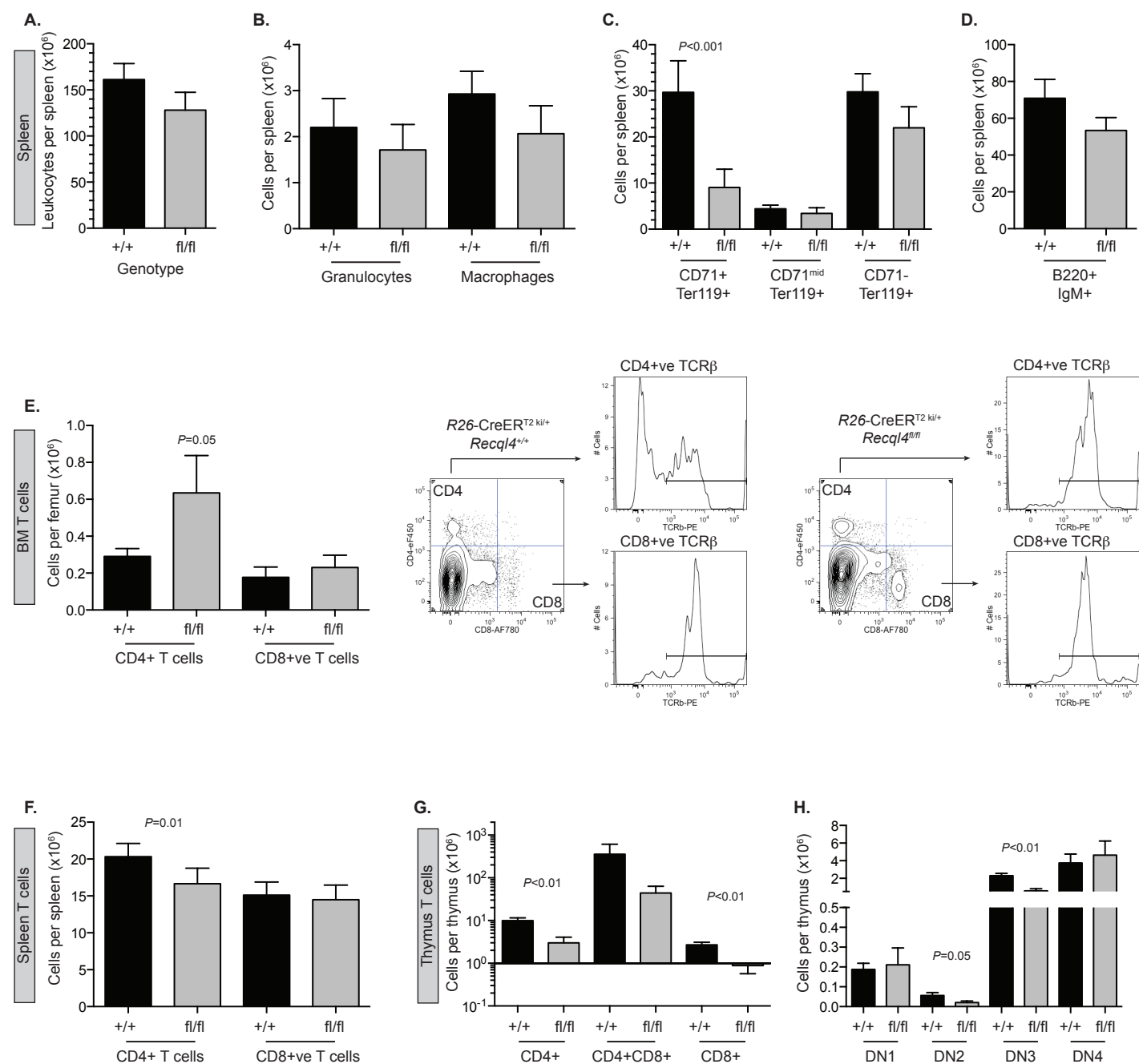
Supplemental Figure 3. Spleen, bone marrow T cell and thymus data
A. Spleen cellularity;
B. Numbers of granulocytes and macrophages per spleen;
C. Erythroid fractions based on CD71/Ter119 staining from the indicated genotype;
D. B lymphocyte number per spleen .
E. CD4 and CD8 mature T cells in the bone marrow and representative FACS plots of TCRβ staining on each population;
F. T cells per spleen;
G. Mature T cell subsets in the thymus;
H. T cell progenitors in the thymus.
Data expressed as mean ± sem with students's T test statistic. *P<0.05, **P<0.001 or P value as indicated on the figure; n>5 per genotype.
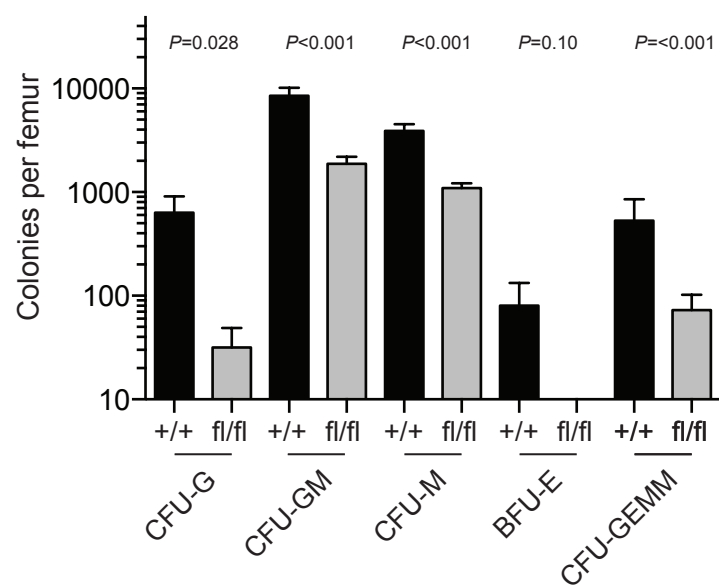
Supplemental Figure 4. Colony forming cell analysis per femur
Frequency of each colony forming cell type per femur of the indicated genotypes.
Data expressed as mean ± sem with students's T test statistic. *P<0.05, **P<0.001 or P value as indicated on the figure; n>5 per genotype.

A.
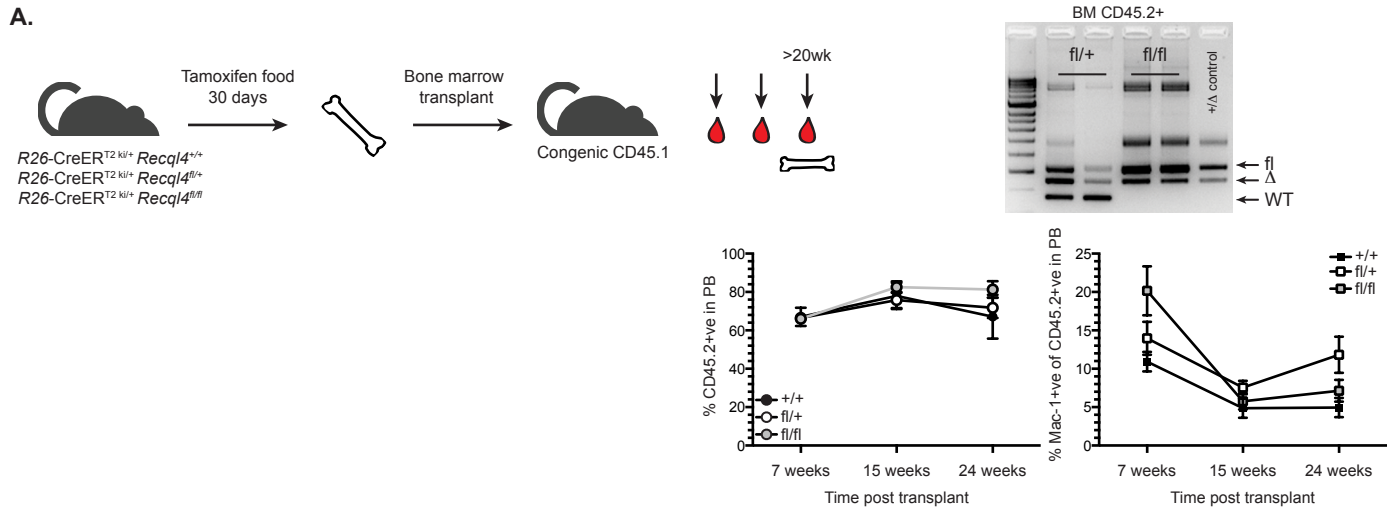
Supplemental Figure 5. Profound selection against Recql4 deletion in hematopoietic stem cells.
(**A**) Schematic of bone marrow transplant strategy. Whole bone marrow was isolated from donors of each genotype treated with tamoxifen for 30 days. Donor cells were competitively transplanted with CD45.1/CD45.2 competitor bone marrow into CD45.1 recipients. Peripheral blood total chimerism (CD45.2+ve) and myeloid chimerism (Mac-1+ve) are shown from one of two independent experiments. At end point CD45.2 cells were isolated from the bone marrow and genomic PCR performed to determine the status of Recql4; n=5 recipients per genotype per experiment; experiment performed twice.
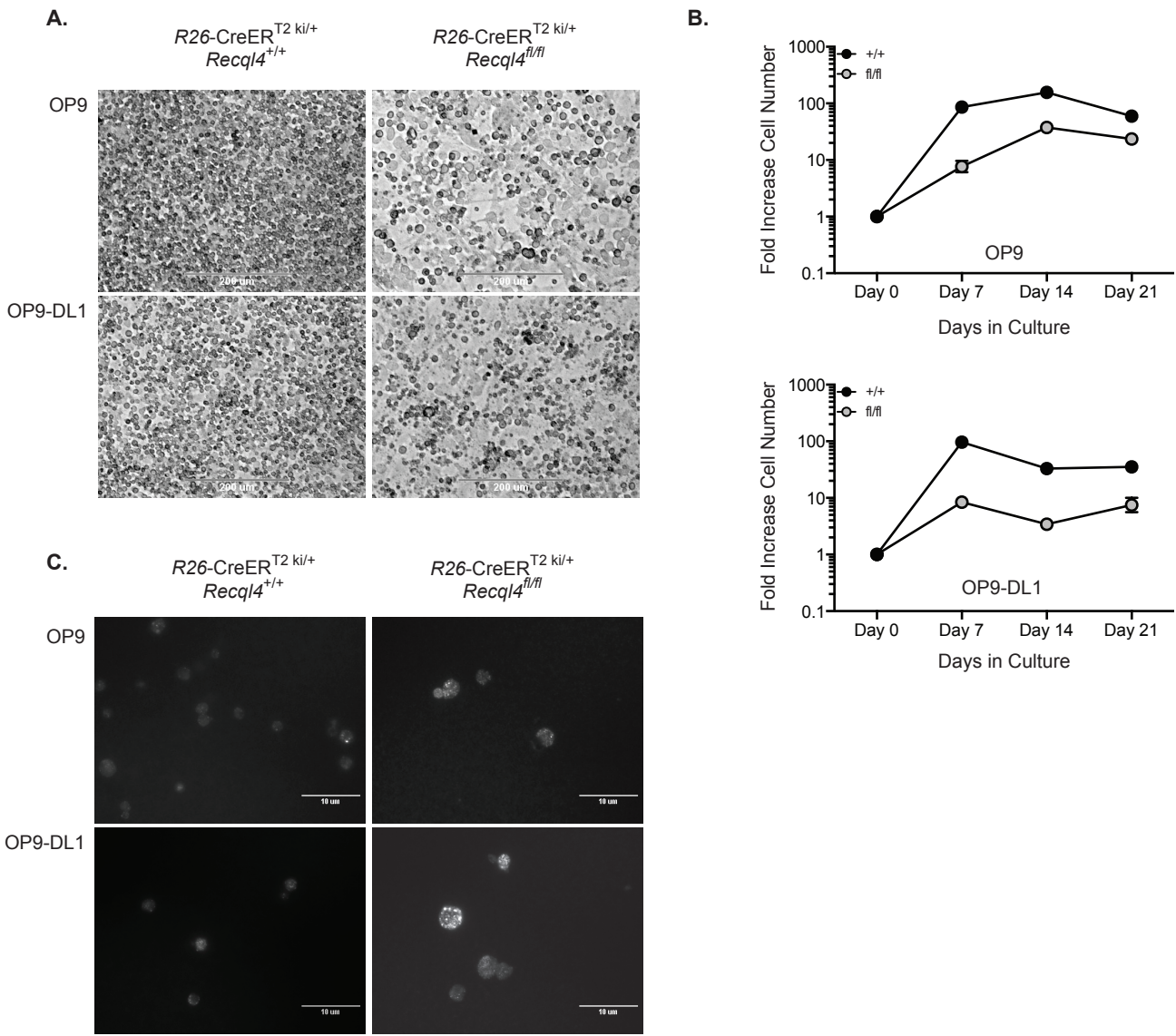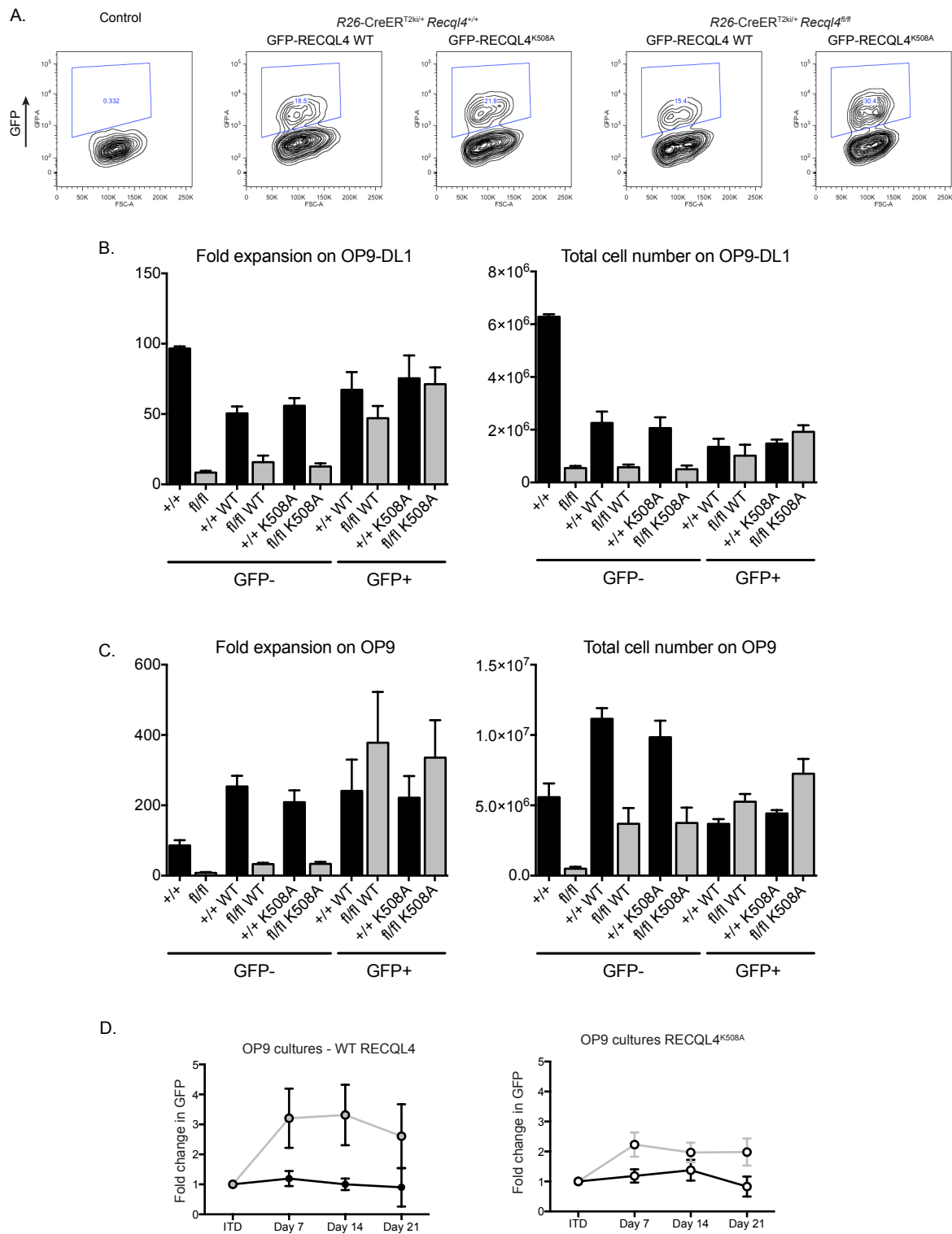
Supplemental Figure 6. OP9 and OP9-DL1 cultures
A. Representative phase contrast photos of hematopoietic cells of the indicated genotypes cultured on OP9 or OP9-DL1. Note the significantly larger cell size of the Recql4 deficient cultures.
B. Fold change in cell numbers over the period of culture for cells cultured on OP9 and OP9-DL1 as indicated
C. Images of γH2A.X foci in cells from the indicated genotype/culture system.
Scale bars as indicated.

Supplemental Figure 7. Retroviral complementation additional information
A. Representative FACS plots showing GFP expression in virally transduced cells
B. Fold expansion of cells of the indicated genotype on OP9-DL1 (T cell cultures) for GFP- (non rescued) or GFP+ (rescued)
C. Fold expansion of cells of the indicated genotype on OP9 (B cell cultures) for GFP- (non rescued) or GFP+ (rescued)
D. Fold change in GFP levels in OP9 cultures (B cells) for the EGFP-RECQL4 or EGFP-RECQL4^{K508A}