Table S1.
Haemopoietic composition of vavP-*Mcl-1*/vavP-*BCL-2* bi-transgenic mice and littermates at 6 weeks

Organ/cell type	WT	*Mcl-1*tg	*BCL-2*tg	*Mcl-1*tg/*BCL-2*tg
Peripheral blood	9.9 ± 3.9	32.4 ± 4.8***	61 ± 26***	60 ± 24***
$CD4^+$	3.4 ± 2.3	4.0 ± 2.1	5.0 ± 1.5	3.1 ± 1.1†
$CD8^+$	2.2 ± 1.8	3.8 ± 0.9	8.0 ± 4.7*	5.1 ± 2.7*
$B220^+IgM^-IgD^-$	0.61 ± 0.20	1.3 ± 0.5***	5.0 ± 5.7**	4.2 ± 2.6***
$B220^+IgM/D^+$	7.5 ± 5.5	18.8 ± 4.6**	41 ± 22**	27 ± 13**
$Mac1^+Gr1^-$	0.70 ± 0.80	0.76 ± 0.39	2.3 ± 1.3*	2.8 ± 1.5**
$Mac1^+Gr1^+$	0.48 ± 0.49	0.50 ± 0.27	1.1 ± 1.5	0.80 ± 0.79
Spleen	212 ± 51	291 ± 107	589 ± 108**	504 ± 200***
$CD4^+$	22.4 ± 3.4	25 ± 14	49.5 ± 6.5**	44 ± 17***
$CD8^+$	15.3 ± 4.0	22.4 ± 6.9*	44.1 ± 5.1**	37 ± 13***
$B220^+IgM^-IgD^-$	10.6 ± 6.1	13.7 ± 3.3	79 ± 45**	56 ± 30***
$B220^+IgM/D^+$	112 ± 27	156 ± 62	295 ± 100**	256 ± 123**
$Mac1^+Gr1^-$	3.1 ± 1.5	3.5 ± 1.2	13.9 ± 9.7**	12.7 ± 6.1***
$Mac1^+Gr1^+$	4.4 ± 1.5	3.4 ± 2.1	5.3 ± 4.0	5.9 ± 3.7
$Ter\text{-}119^+$	48 ± 30	56 ± 32	87 ± 33	94 ± 39*
LN	40 ± 23	43.9 ± 6.8	80 ± 11*	74 ± 42
$CD4^+$	14.9 ± 7.3	12.5 ± 6.2	26.4 ± 3.2*	22 ± 13†
$CD8^+$	10.2 ± 4.3	10.6 ± 0.8	23.8 ± 5.5**	19 ± 10*
$B220^+IgM^-IgD^-$	1.7 ± 1.2	1.5 ± 0.6	4.8 ± 1.7**	5.0 ± 4.5
$B220^+IgM/D^+$	22 ± 12	15.0 ± 3.2	20.5 ± 3.3	22 ± 14
BM	27.7 ± 8.0	28.6 ± 5.2	33.6 ± 5.5	30.6 ± 4.4
$Ter\text{-}119^+$	11.4 ± 5.1	9.3 ± 2.9	7.7 ± 1.5	9.2 ± 3.6
$Thy1^+$	0.77 ± 0.37	1.3 ± 0.3*	1.5 ± 0.5*	1.2 ± 0.3*
$B220^-CD43^+$	6.8 ± 2.5	5.2 ± 3.1	7.9 ± 4.3	7.4 ± 1.9
$B220^+CD43^+$	0.77 ± 0.26	0.53 ± 0.17	0.90 ± 0.25	0.94 ± 0.18
$B220^+IgM^-IgD^-$	8.4 ± 2.4	10.0 ± 5.6	9.8 ± 5.4	9.2 ± 4.6
$B220^+IgM^+IgD^-$	1.9 ± 0.8	2.1 ± 1.1	3.0 ± 1.7	2.2 ± 1.5
$B220^+IgM/D^+$	3.4 ± 1.8	5.2 ± 1.1	8.9 ± 2.7**	6.6 ± 3.3
$Mac1^+Gr1^-$	0.42 ± 0.22	0.58 ± 0.19	3.1 ± 0.9**	2.4 ± 0.6***
$Mac1^+Gr1^+$	7.0 ± 2.4	5.7 ± 1.5	5.7 ± 2.2	5.7 ± 1.3
Thymus	254 ± 85	245 ± 47	172 ± 73	284 ± 123
$CD4^-CD8^-$ (DN)	5.8 ± 2.5	8.2 ± 2.5	20.5 ± 4.7**	27.9 ± 10.3***
$CD4^+CD8^+$(DP)	199 ± 72	203 ± 38	103 ± 58*	193 ± 97†
CD4SP	23.1 ± 7.5	25.9 ± 6.6	36 ± 14	45 ± 16**
CD8SP	9.4 ± 5.0	8.5 ± 4.7	12.7 ± 3.8	17.3 ± 6.5*

Nucleated cells × 10^6, except peripheral blood cells (× 10^6/mL). Nucleated cells (mean ± SD) from 6-week-old mice: n = 5 to 8 mice per genotype. 8 WT (3F, 5M), 6 *Mcl-1*tg (2F, 4M), 5 *BCL-2*tg (5M), 8 *Mcl-1*tg/*BCL-2*tg (3F, 5M). $B220^+IgM/D^+$ indicates $B220^+$ cells that are IgM^+ and/or IgD^+.
Mann-Whitney test:
*** Significantly different from WT ($P \leq 0.001$)
** Significantly different from WT ($P \leq 0.01$)
* Significantly different from WT ($P \leq 0.05$)
† Significantly different from *BCL-2*tg ($P \leq 0.05$)

Table S2.
Haemopoietic composition of *Bak*$^{-/-}$ vavP-*BCL-2*tg mice and littermates at 12-14 weeks

Organ/cell type	WT	*Bak*$^{-/-}$	*BCL-2*tg	*Bak*$^{-/-}$*BCL-2*tg
Peripheral blood	10.0 ± 4.2	10.4 ± 3.5	56 ± 13***	74 ± 32***
CD4$^+$	1.4 ± 0.6	1.6 ± 0.5	4.0 ± 0.6***	6.4 ± 3.3***
CD8$^+$	1.1 ± 0.3	1.1 ± 0.3	5.4 ± 2.0***	8.5 ± 3.4***†
B220$^+$IgM$^-$IgD$^-$	0.14 ± 0.11	0.1 ± 0.04	5.3 ± 2.6***	8.3 ± 6.5***
B220$^+$IgM/D$^+$	5.7 ± 2.6	5.7 ± 2.5	31.6 ± 7.6***	35.3 ± 8.2***
Mac1$^+$Gr1$^-$	0.56 ± 0.36	0.39 ± 0.15	4.1 ± 2.9***	4.7 ± 1.2**
Mac1$^+$Gr1$^+$	0.10 ± 0.09	0.11 ± 0.06	0.31 ± 0.30	0.34 ± 0.16**
Spleen	89 ± 11	63 ± 20§	447 ± 130***	603 ± 160***
CD4$^+$	13.2 ± 2.0	9.8 ± 4.2	50 ± 16***	76 ± 12***††
CD8$^+$	8.1 ± 1.8	6.2 ± 1.5	36 ± 12***	56 ± 11***††
B220$^+$IgM$^-$IgD$^-$	1.5 ± 0.4	0.9 ± 0.4*	57 ± 33***	92 ± 38***†
B220$^+$IgM/D$^+$	56.5 ± 7.8	38 ± 13*	251 ± 77***	298 ± 66***
Mac1$^+$Gr1$^-$	2.8 ± 1.3	1.3 ± 1.6	11.9 ± 6.7***	16.6 ± 9.7**
Mac1$^+$Gr1$^+$	0.19 ± 0.14	0.11 ± 0.11	0.63 ± 0.58	0.94 ± 0.63*
Ter-119$^+$	6.8 ± 3.7	5.1 ± 1.5	38.3 ± 7.5***	56 ± 16***††
LN	4.9 ± 1.3	5.2 ± 2.4	21.0 ± 9.7***	21 ± 11***
CD4$^+$	1.7 ± 0.6	1.9 ± 0.9	7.1 ± 3.9***	7.2 ± 3.7***
CD8$^+$	1.2 ± 0.3	1.3 ± 0.5	7.6 ± 4.2***	7.5 ± 4.5***
B220$^+$IgM$^-$IgD$^-$	0.03 ± 0.04	0.04 ± 0.05	0.70 ± 0.54***	1.4 ± 1.2***
B220$^+$IgM/D$^+$	2.0 ± 0.5	1.9 ± 1.0	4.3 ± 1.8**	3.6 ± 1.4**
BM	18.7 ± 3.3	21.3 ± 2.9	23.5 ± 5.4	23.2 ± 6.3
Ter-119$^+$	5.5 ± 1.2	7.0 ± 1.0*	2.9 ± 0.6***	3.0 ± 0.5***
Thy1$^+$	0.54 ± 0.14	0.59 ± 0.10	1.7 ± 0.5***	1.7 ± 0.6***
B220$^-$CD43$^+$	6.4 ± 1.2	6.2 ± 1.1	6.7 ± 1.6	8.2 ± 1.1
B220$^+$CD43$^+$	0.18 ± 0.04	0.23 ± 0.07	0.34 ± 0.10**	0.35 ± 0.05**
B220$^+$IgM$^-$IgD$^-$	3.0 ± 0.7	4.0 ± 0.4	3.0 ± 0.8	3.5 ± 1.7
B220$^+$IgM$^+$IgD$^-$	1.0 ± 0.2	1.1 ± 0.3	1.5 ± 0.3**	1.0 ± 0.6†
B220$^+$IgM/D$^+$	2.2 ± 0.6	3.1 ± 0.4	6.9 ± 2.0***	6.2 ± 2.2***
Mac1$^+$Gr1$^-$	1.0 ± 0.5	1.1 ± 0.4	4.1 ± 1.6***	4.2 ± 1.3***
Mac1$^+$Gr1$^+$	5.7 ± 1.6	4.9 ± 0.8	3.6 ± 0.9**	3.6 ± 1.2*
Thymus	105 ± 30	136 ± 36	81 ± 19	79 ± 15
CD4$^-$CD8$^-$ (DN)	1.3 ± 0.4	1.7 ± 0.5	10.8 ± 1.6***	8.7 ± 1.6***†
CD4$^+$CD8$^+$ (DP)	96 ± 27	124 ± 33	48 ± 14***	50 ± 12***
CD4SP	6.1 ± 2.7	8.7 ± 2.9	13.7 ± 3.9**	13.5 ± 3.6**
CD8SP	1.6 ± 0.6	1.7 ± 0.6	8.8 ± 1.8***	7.3 ± 1.4***

Nucleated cells × 10^6, except peripheral blood cells (× 10^6/mL). Nucleated cells (mean ± SD) from 12 to 14-week-old male mice: n = 6 to 10 mice per genotype. B220$^+$IgM/D$^+$ indicates B220$^+$ cells that are IgM$^+$ and/or IgD$^+$.

Mann-Whitney test:

*** Significantly different from WT ($P \leq 0.001$)

** Significantly different from WT ($P \leq 0.01$)

* Significantly different from WT ($P \leq 0.05$)

†† Significantly different from *BCL-2*tg ($P \leq 0.01$)

† Significantly different from *BCL-2*tg ($P \leq 0.05$)

Table S3.
Lymphoid cells in spleen and thymus of *Bax*$^{-/-}$vavP-*BCL-2*tg mice and littermates at 12 weeks

Organ/cell type	WT	*Bax*$^{-/-}$	*BCL-2*tg	*Bax*$^{-/-}$*BCL-2*tg
Blood	9.5 ± 2.9	11.9 ± 2.4	67 ± 22***	67 ± 29***
Spleen	150 ± 30	160 ± 27	728 ± 208***	741 ± 119***
$CD4^+$	20.3 ± 6.0	23.3 ± 3.7	96 ± 31***	100 ± 20***
$CD8^+$	14.6 ± 4.4	18.3 ± 3.7	67 ± 27***	69 ± 17***
CD4/CD8 ratio	1.4 ± 0.3	1.3 ± 0.1	1.5 ± 0.3	1.5 ± 0.3
$CD19^+IgM/D^+$	85 ± 20	93 ± 13	360 ± 86***	358 ± 83***
$CD19^+IgM^-IgD^-$	3.2 ± 0.9	3.8 ± 1.3	100 ± 39***	107 ± 31***
$CD19^+Fas^+PNA^+$	3.6 ± 2.2	5.4 ± 3.3	94 ± 40***	101 ± 43***
Thymus	124 ± 38	185 ± 34**	115 ± 32	151 ± 47
$CD4^-CD8^-TCR\beta^-$	3.8 ± 1.9	6.5 ± 1.7*	4.3 ± 1.6	6.0 ± 3.2*
$CD4^+CD8^+$	107 ± 30	160 ± 34**	75 ± 24*	99 ± 37
CD4SP	9.3 ± 4.5	12.4 ± 4.0	20.1 ± 7.2**	26.5 ± 5.5***
CD8SP	2.9 ± 2.0	4.4 ± 1.6*	8.6 ± 3.7***	10.8 ± 3.5***
$CD4^-CD8^-TCR\beta^+$	0.9 ± 0.5	1.4 ± 0.8	6.8 ± 2.7***	9.1 ± 2.9***

Nucleated cells × 10^6, except peripheral blood cells (× 10^6/mL). Nucleated cells (mean ± SD) from 12-week-old (n = 9-10) mice. $B220^+IgM/D^+$ indicates $B220^+$ cells that are IgM^+ and/or IgD^+.
Mann-Whitney test:
*** Significantly different from WT ($P \leq 0.001$)
** Significantly different from WT ($P \leq 0.01$)
* Significantly different from WT ($P \leq 0.05$)

Table S4.
Lymphoid cells in spleen and thymus of *Bak*$^{-/-}$vavP-*BCL-2*tg mice and their littermates during maturation

6-8 wk	**WT**	***Bak*$^{-/-}$**	***BCL-2*tg**	***Bak*$^{-/-}$*BCL-2*tg**
Blood	10.3 ± 2.6	11.2 ± 2.4	59 ± 16*	53.5 ± 6.2*
Spleen	181 ± 34	139 ± 28*	571 ± 150**	444 ± 102**
$CD4^+$	29.6 ± 5.9	19.1 ± 4.0*	61 ± 18**	45.3 ± 8.8**
$CD8^+$	17.9 ± 2.7	13.5 ± 2.8*	49 ± 13**	36.8 ± 5.4**
CD4/CD8 ratio	1.6 ± 0.1	1.4 ± 0.2	1.3 ± 0.1***	1.2 ± 0.1**
$CD19^+IgM/D^+$	103 ± 20	79 ± 13	299 ± 79**	236 ± 61**
$CD19^+IgM^-IgD^-$	4.9 ± 3.0	4.7 ± 1.2	85 ± 27**	72 ± 36**
$CD19^+Fas^+PNA^+$	2.1 ± 0.6	1.0 ± 0.04	28 ± 20	10.3 ± 0.2
Thymus	237 ± 47	175 ± 54	267 ± 46	220 ± 47
$CD4^-CD8^-TCR\beta^-$	6.1 ± 1.5	5.0 ± 3.0	8.3 ± 2.5	6.8 ± 3.3
$CD4^+CD8^+$	203 ± 42	149 ± 46	167 ± 36	139 ± 30*
CD4SP	17.3 ± 5.0	12.9 ± 4.0	43.7 ± 7.0**	38.4 ± 9.8**
CD8SP	8.9 ± 1.9	7.5 ± 3.2	23.2 ± 5.1**	15.0 ± 4.3**†
$CD4^-CD8^-TCR\beta^+$	1.4 ± 0.3	0.91 ± 0.39	24.3 ± 7.2**	20.2 ± 4.3**

12 wk	**WT**	***Bak*$^{-/-}$**	***BCL-2*tg**	***Bak*$^{-/-}$*BCL-2*tg**
Blood	9.9 ± 2.2	9.3 ± 2.5	42 ± 11**	38 ± 13**
Spleen	146 ± 34	161 ± 17	642 ± 109**	763 ± 132**
$CD4^+$	20.2 ± 3.8	23.6 ± 2.3	79.2 ± 8.0**	91 ± 18**
$CD8^+$	15.1 ± 4.7	17.7 ± 2.1	58 ± 15**	67.4 ± 8.1**
CD4/CD8 ratio	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.3	1.3 ± 0.2
$CD19^+IgM/D^+$	85 ± 11	92.2 ± 9.2	332 ± 58**	362 ± 58**
$CD19^+IgM^-IgD^-$	2.6 ± 0.6	4.2 ± 1.8*	78 ± 20**	106 ± 43**
$CD19^+Fas^+PNA^+$	2.3 ± 0.7	2.7 ± 1.5	47 ± 20**	66 ± 31**
Thymus	115 ± 15	124 ± 28	81 ± 15**	72 ± 15**
$CD4^-CD8^-TCR\beta^-$	4.5 ± 1.6	5.0 ± 1.8	3.7 ± 0.7	3.1 ± 0.7*
$CD4^+CD8^+$	97 ± 12	104 ± 23	45 ± 11**	43 ± 10**
CD4SP	8.4 ± 1.8	8.9 ± 2.4	15.4 ± 2.2**	13.3 ± 2.0**
CD8SP	3.7 ± 1.3	4.7 ± 1.0	9.4 ± 1.0**	7.1 ± 1.7**†
$CD4^-CD8^-TCR\beta^+$	1.0 ± 0.3	1.0 ± 0.3	7.7 ± 1.3**	5.4 ± 1.3**†

24 wk	**WT**	***Bak*$^{-/-}$**	***BCL-2*tg**	***Bak*$^{-/-}$*BCL-2*tg**
Blood	5.9 ± 1.2	6.2 ± 2.7	33.5 ± 9.0***	26.8 ± 9.1***
Spleen	162 ± 29	157 ± 19	762 ± 168***	841 ± 123***
$CD4^+$	21.8 ± 3.7	23.3 ± 4.5	108 ± 24***	108 ± 10***
$CD8^+$	16.7 ± 2.1	18.4 ± 4.0	68 ± 17***	85 ± 24***
CD4/CD8 ratio	1.3 ± 0.2	1.3 ± 0.2	1.6 ± 0.4	1.4 ± 0.4
$CD19^+IgM/D^+$	99 ± 18	91 ± 12	299 ± 79***	283 ± 71***
$CD19^+IgM^-IgD^-$	3.7 ± 1.1	3.2 ± 0.8	186 ± 81***	225 ± 70***
$CD19^+Fas^+PNA^+$	4.6 ± 3.2	4.0 ± 1.1	161 ± 76***	213 ± 66***
Thymus	105 ± 13	97 ± 14	68 ± 12***	55.3 ± 6.1***†
$CD4^-CD8^-TCR\beta^-$	4.1 ± 0.9	2.7 ± 0.8*	4.0 ± 1.4	2.7 ± 0.9*
$CD4^+CD8^+$	90 ± 12	85 ± 12	42.5 ± 8.9***	36.9 ± 4.3***
CD4SP	7.2 ± 1.7	6.1 ± 1.8	12.4 ± 3.1**	8.4 ± 2.0†
CD8SP	3.4 ± 1.0	2.6 ± 0.8	4.9 ± 0.9*	4.0 ± 0.9
$CD4^-CD8^-TCR\beta^+$	0.84 ± 0.25	0.71 ± 0.15	3.65 ± 1.4***	3.3 ± 1.1***

Nucleated cells × 10^6, except peripheral blood cells (× 10^6/mL). Nucleated cells (mean ± SD) from male mice at 6-8 weeks (n = 6 - 7, except data for $CD19^+Fas^+PNA^+$ cells n = 2 - 4), 12 weeks (n = 6 - 7), and 24 weeks (n = 7 - 8).
Mann-Whitney test:
*** Significantly different from WT ($P \leq 0.001$)
** Significantly different from WT ($P \leq 0.01$)
* Significantly different from WT ($P \leq 0.05$)
† Significantly different from *BCL-2*tg ($P \leq 0.05$)

Table S5.
Analysis of GFP-positive populations in bone marrow chimaeras

	9 weeks			12 weeks		
	$B220^+$	RBC	platelets	RBC	platelets	MEGs
GFP	98 ± 0.6	94 ± 2.2	93 ± 2.5	95 ± 2.5	90 ± 3.0	75 ± 1.8
GFP/BCL-2	9 ± 1.2	26 ± 2.5	36 ± 6.1	19 ± 3.6	24 ± 6.0	22 ± 3.2

Percentage of the indicated cell types that were GFP-positive was determined by FACS analysis of blood at 9 weeks post-reconstitution and of blood (RBC, platelets) and bone marrow (megakaryocytes) at 12 weeks post-reconstitution. Data represents mean ± SD; n = 6 at 9 weeks and n = 3 at 12 weeks.

Figure S1

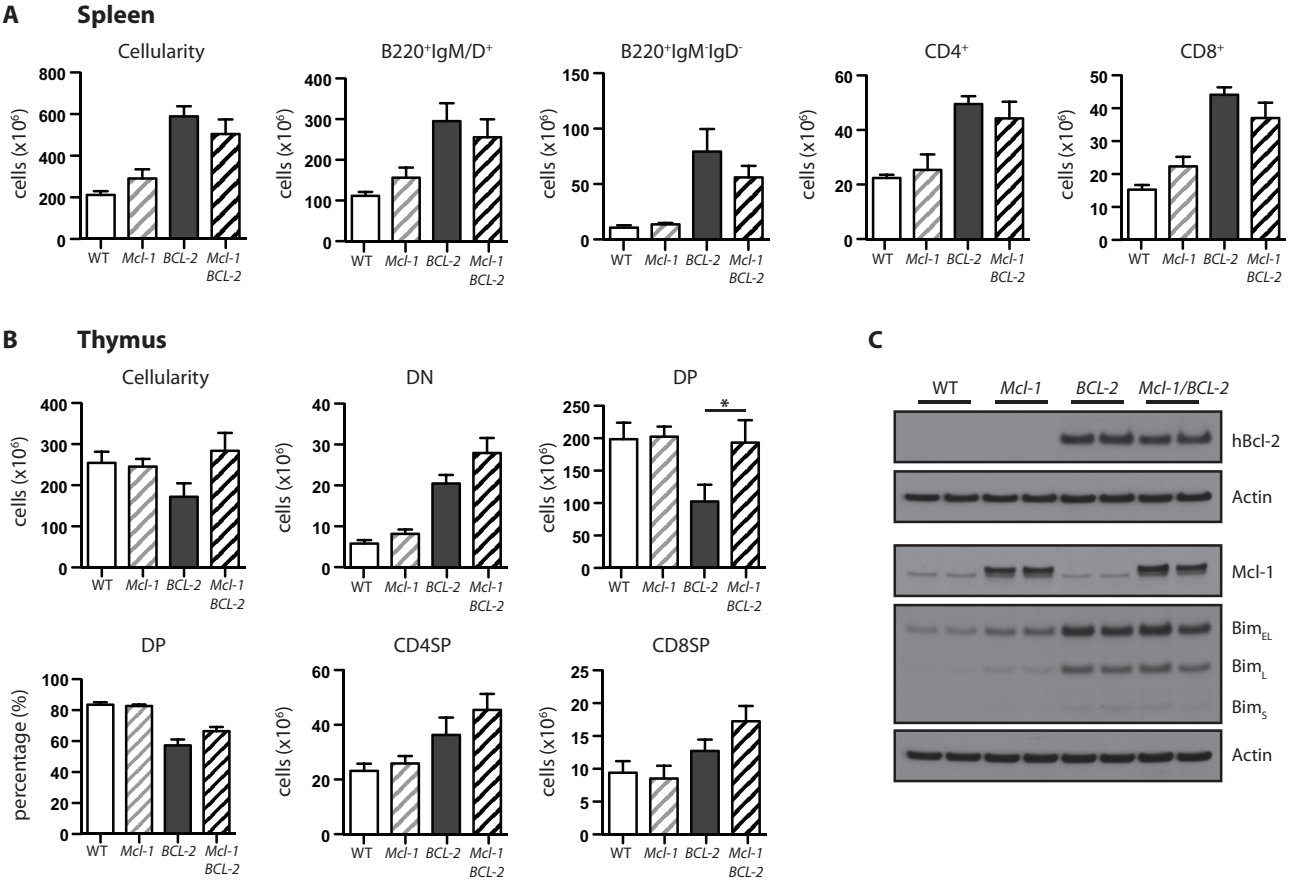

Figure S1. Overexpression of Mcl-1 does not alter the phenotype of *BCL-2*tg mice. Enumeration of total leukocytes and indicated lymphoid populations in the (A) spleen and (B) thymus of 6-8 week-old mice (n=5-8 per genotype. WT, white; *Mcl-1*tg, grey hatched; *BCL-2*tg, dark grey; *Mcl-1*tg/*BCL-2*tg, black hatched). Bars represent mean ± SEM; see also Supplementary Table S1. Statistical significance is shown only for *BCL-2*tg vs *Mcl-1*tg/*BCL-2*tg; **P*<0.05, Mann-Whitney test. Cell composition was determined by cell surface staining with fluorochrome-conjugated monoclonal antibodies followed by flow cytometry. $B220^+IgM/D^+$ indicates $B220^+$ cells that are IgM^+ and/or IgD^+. (C) Expression of the indicated Bcl-2 family proteins in FACS-sorted DP ($CD4^+CD8^+$) thymocytes determined by western blot analysis of cells from two independent mice for each genotype. The *Mcl-1* transgene is N-terminally FLAG-tagged and therefore slightly larger than the endogenous protein.

Figure S2

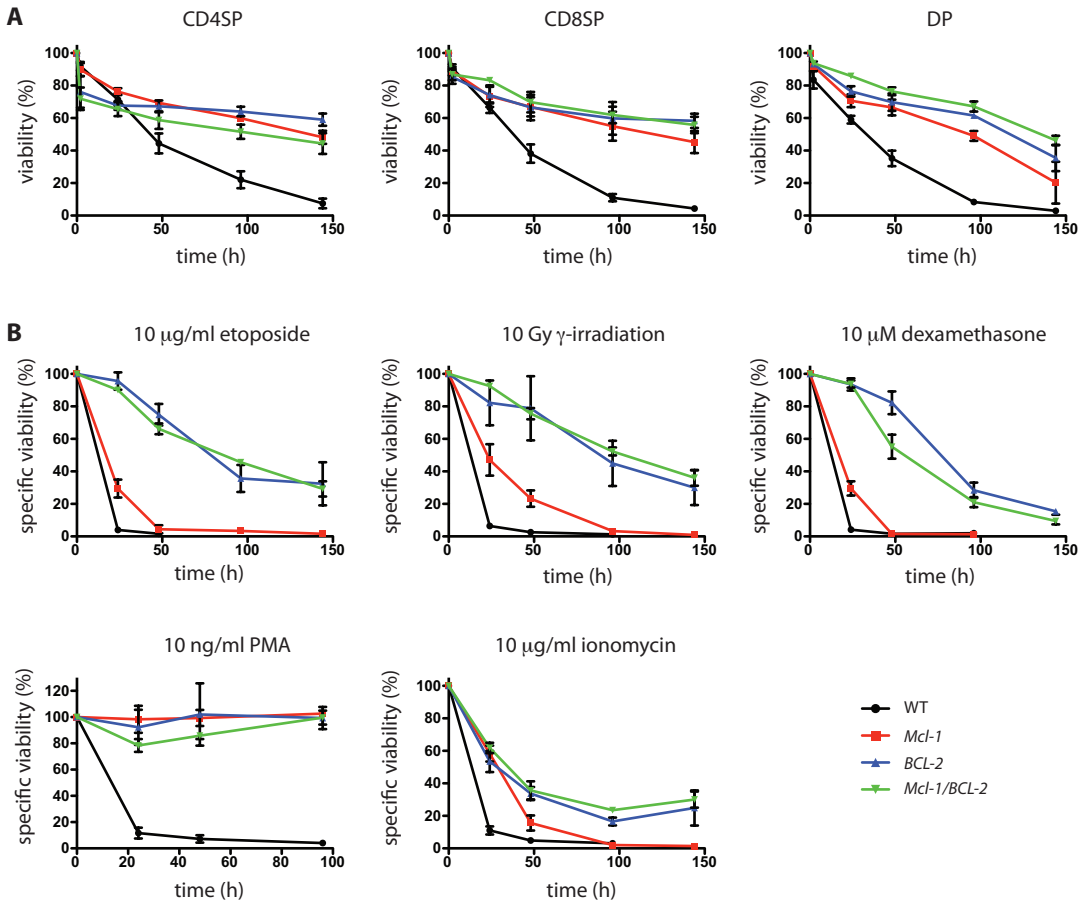

Figure S2. Elevated Mcl-1 expression provides no additional survival advantage to *BCL-2*tg thymocytes. (A) Sorted SP and DP thymocytes from WT, *Mcl-1*tg, *BCL-2*tg and *Mcl-1*tg/*BCL-2*tg mice were cultured *in vitro* for 7 days (A) without cytokines and viability determined as indicated below. (B) Sorted DP thymocytes were cultured in the presence of the indicated concentrations of etoposide, dexamethasone, PMA, ionomycin or after subjection to 10 Gy γ-irradiation. Cell viability was determined by PI and annexin V staining followed by flow cytometry; specific viability was calculated relative to the viability of untreated cells at each time point. WT, black (n = 4-8 mice); *Mcl-1*tg, red (n = 3-5), *BCL-2*tg, green (n = 3-4), *Mcl-1*tg/*BCL-2*tg, blue (n = 7), except for DP thymocytes stimulated with PMA where n=3 for WT and n=2 for other genotypes. Values represent mean ± SEM. Using the Student's t-test no significant differences between *BCL-2*tg and *Mcl-1*tg/*BCL-2*tg were observed.

Figure S3

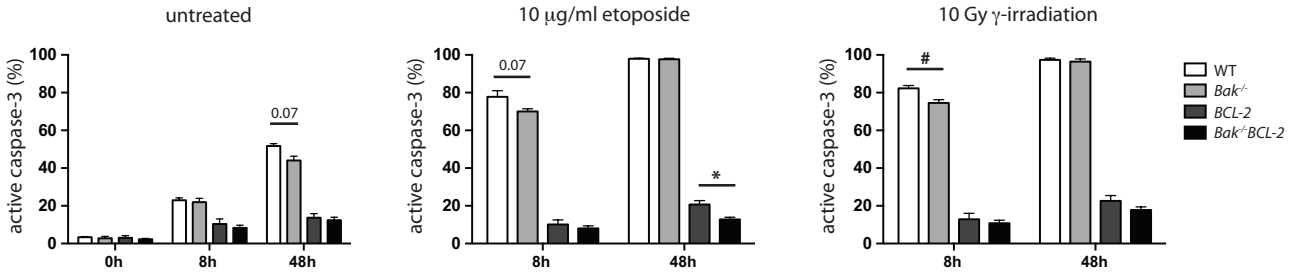

Figure S3. Loss of Bak provides thymocytes with a modest protection from apoptosis both alone and when *BCL-2* is overexpressed. Sorted DP thymocytes from WT, *Bak*$^{-/-}$, *BCL-2*tg and *Bak*$^{-/-}$*BCL-2*tg mice were cultured in medium lacking cytokines (untreated), or in the presence of 10 μg/mL etoposide, or following treatment with 10 Gy γ-irradiation. Apoptotic cells were identified at 0, 8 and 48 hours by intracellular staining for active caspase-3 and analysis by flow cytometry. The percentage of active caspase-3 positive (apoptotic) cells (mean ± SEM) are shown; n = 4 mice for each genotype. Statistical significance is only indicated for *BCL-2*tg vs *Bak*$^{-/-}$*BCL-2*tg, **P*<0.05, and *WT* vs *Bak*$^{-/-}$, #*P*<0.05, Student's t-test.

Figure S4

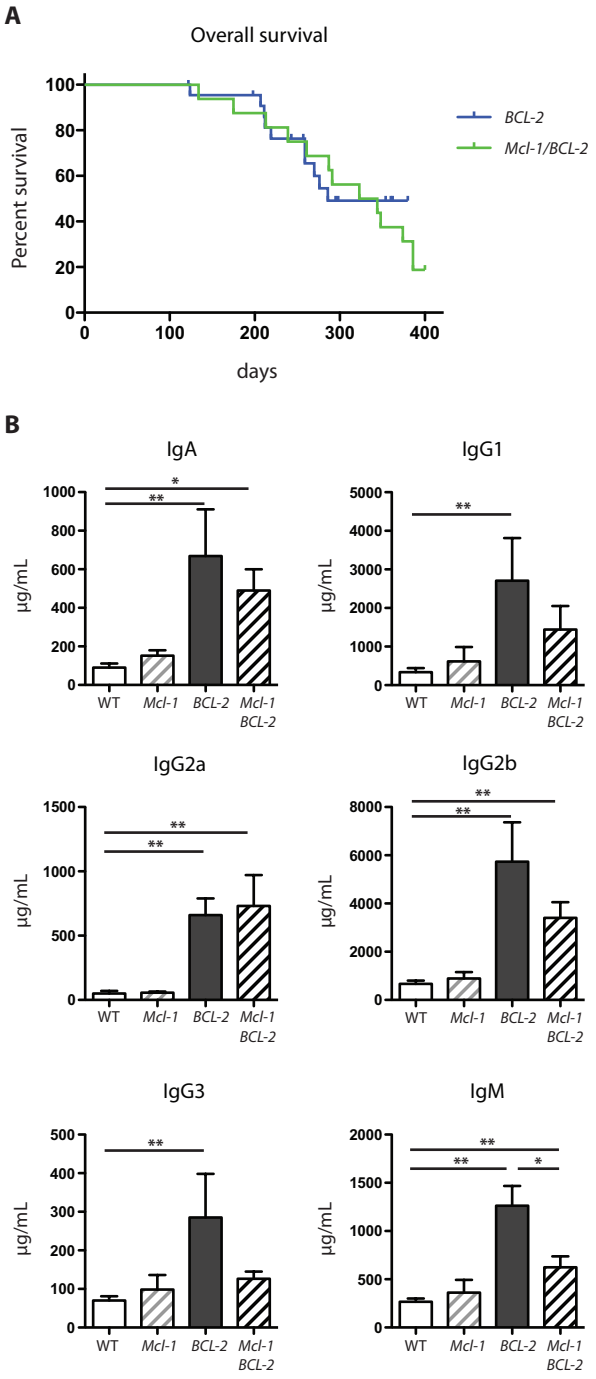

Figure S4. Autoimmune disease in *BCL-2*tg mice is not exacerbated by overexpression of Mcl-1. (A) Kaplan-Meier survival curves for *BCL-2*tg (blue) (n = 23, median survival 286 d) and *Mcl-1*tg/*BCL-2*tg (green) (n = 16, median survival 333 d). (B) *BCL-2*tg and *Mcl-1*tg/*BCL-2*tg mice have similarly high levels of serum immunoglobulin. The levels of each Ig isotype were quantified for 8

week-old female mice (n = 5-6) by ELISA. WT (white), *Mcl-1*tg (grey hatched), *BCL-2*tg (dark grey) and *Mcl-1*tg/*BCL-2*tg (black hatched) mice. Bars represent mean ± SEM; Significance is only indicated for *BCL-2*tg and *Mcl-1*tg/*BCL-2*tg versus WT, or for *Mcl-1*tg/*BCL-2*tg versus *BCL-2*tg; **P*<0.05, ***P*<0.01, Mann-Whitney test.

Figure S5

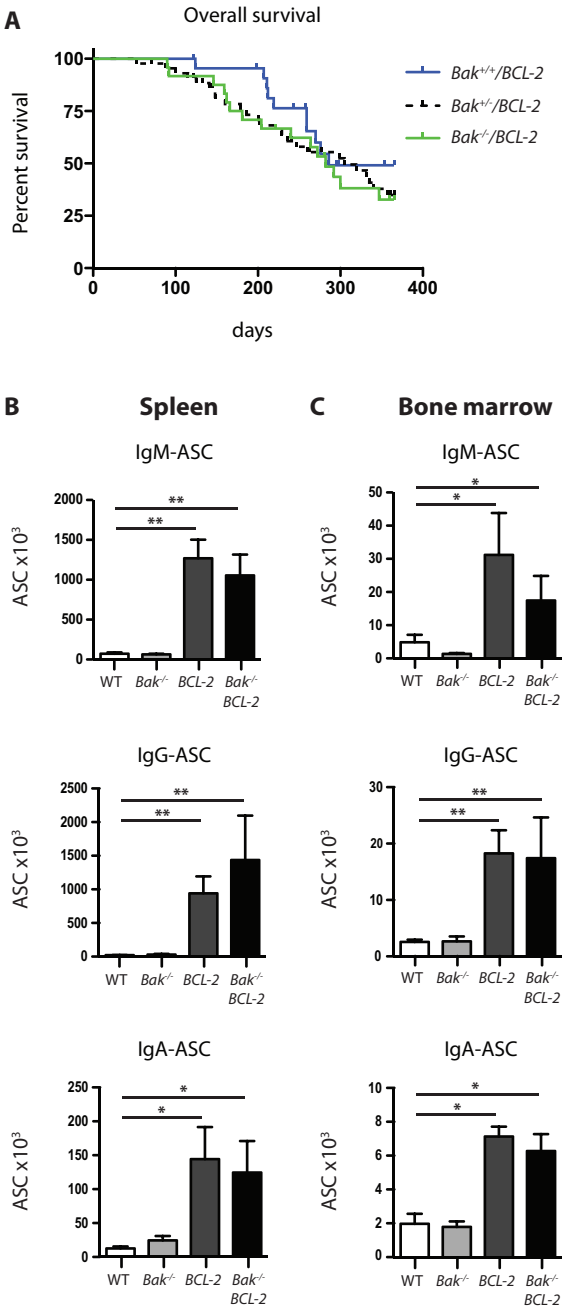

Figure S5. Loss of Bak does not alter the incidence of autoimmune disease in *BCL-2*tg mice. (A) Kaplan-Meier survival curves for *BCL-2*tg (blue) (n = 23, median survival 286 d); *Bak*$^{+/-}$*BCL-2*tg (black dashed) (n = 44, median survival 305 d); and *Bak*$^{-/-}$*BCL-2*tg (green) (n = 24, median survival 282 d) mice. (B, C) Loss of Bak does not further increase the number of antibody-secreting cells (ASC) in the spleen (B) or bone marrow (C) of *BCL-2*tg mice. ASC numbers were determined for 7-8 week-old female mice (n = 6) by ELISPOT following red cell depletion. Total ASC/organ was calculated from ASC/10^5 cells x organ cellularity. Data represent mean ± SEM; significance is

only indicated for *BCL-2*tg and *Bak*$^{-/-}$*/BCL-2*tg versus WT, or for *Bak*$^{-/-}$*/BCL-2*tg versus *BCL-2*tg; **P*<0.05, ***P*<0.01, Mann-Whitney test.

Figure S6

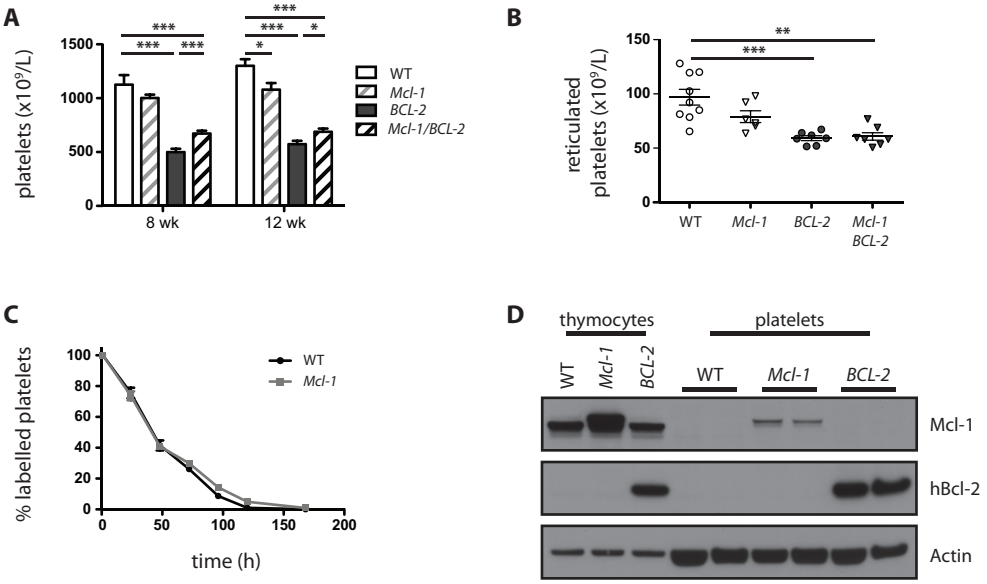

Figure S6. Overexpression of Mcl-1 does not rescue thrombocytopenia in *BCL-2*tg mice. (A) Platelet counts in WT (white), *Mcl-1*tg (grey hatched), *BCL-2*tg (dark grey) and *Mcl-1*tg/*BCL-2*tg (black hatched) mice at 8 weeks (n = 8-14) and 12 weeks (n = 6-14). Bars represent mean ± SEM, **P*<0.05, ****P*<0.001, Student's t-test. (B) *Mcl-1*tg/*BCL-2*tg mice have a reduced number of reticulated platelets, as is observed for *BCL-2*tg mice. The percentage of reticulated platelets was determined by staining with thiazole orange and APC-labelled anti-CD41, then converted to absolute platelet numbers using the ADVIA total platelet count. Data represent mean ± SEM, n = 5-9 female mice at 6 weeks. ***P*<0.01, ****P*<0.001, Student's t-test. (C) Platelet life span is not extended in *Mcl-1*tg mice. Platelets were labelled by intravenous injection of NHS-biotin and peripheral blood samples taken at the indicated times. Data represent mean ± SEM, n = 8. (D) Western blot analysis of transgenic Mcl-1 and Bcl-2 protein expression in platelets. Platelet and thymocyte lysates were prepared from WT, *Mcl-1*tg and *BCL-2*tg mice and 10µg (thymocytes) or 50µg (platelets) protein used for analysis. Platelets were isolated from two independent mice of each genotype. The low level of transgenic Mcl-1 in platelets reflects its short half-life in this anuclear cell type, due in part to rapid proteosomal degradation.[29,41]