Supplemental data

Supplemental materials and methods

RNA extraction and RT-PCR

Total cellular RNA was isolated using the RNeasy kit (Qiagen) according to the manufacturer's instructions. The first-strand cDNA was generated by reverse transcription of 5 μg total RNA using oligo(dT) oligonucleotides. PCR was performed with the following specific primers 5'-GAGAAGGTGGACAATTGCAG-3' and 5'-TCAATGCCTTCTCCATACCA-3' for Bim (Bim_{EL}, Bim_L and Bim_S isoforms) and 5'-GATTAGCGATGATGAACCAGGT-3' and 5'-AGTTAAAGTTGAGAGATCATCTC-3' for HPRT.

PCR products were resolved by electrophoresis in 1.8% agarose gels, transferred onto nylon membranes (Amersham Hybond-N^+) and detected by southern blotting.

Supplemental figures

Titles and Figure legends

Figure S1: DEF2 mutation interferes with the normal splicing of the *Bim* gene and thereby inhibits expression of Bim_L and Bim_S.

(a) Expression of Bim isoforms and b-actin (loading control) in thymocytes and splenocytes of two wildtype and two ΔDEF2 mice was analyzed by Western blotting. Two different exposures of the immunoblot are shown. (b) The presence of the mRNA species encoding Bim_{EL}, Bim_L, Bim_S and HPRT in thymocytes and splenocytes of wildtype or ΔDEF2 mice was analyzed by RT-PCR.

Figure S2: Hematopoietic cell composition in Bim mutant mice.

(a-b) The percentage of lymphocytes (a) and neutrophils (b) in blood from wildtype, ΔDEF2, Bim_{EL}-only and Bim_L-only mice were determined using an ADVIA blood analyzer. (c) The numbers of platelets (10^6/mL) in blood from wildtype, ΔDEF2, Bim_{EL}-only and Bim_L-only mice were measured by using an ADVIA blood analyzer. Data represent mean (+/-SEM) of at

least n=20 mice per genotype (p>0.05). (d) The percentages of B cells and $CD4^+$ as well as $CD8^+$ T cells in lymph nodes of wildtype and the various Bim mutant mice were determined by FACS analysis after staining with antibodies to the cell surface markers B220, CD4 and CD8. Data represent mean (+/-SEM) of at least n=14 per genotype. (p> 0.05). (e) The numbers of $CD4^+CD8^+$, $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes were determined by FACS analysis. Data represent mean (+/-SEM) of at least n=13 per genotype (p> 0.05). (f) The numbers of B cells and $CD4^+$ as well as $CD8^+$ T cells in spleens of wildtype, ΔDEF2, Bim_{EL}-only and Bim_L-only mice were determined by FACS analysis (see above). Data represent mean (+/-SEM) of at least n=13 mice per genotype (p> 0.05).

Figure S3: Sensitivity of $CD4^+CD8^+$ thymocytes to different apoptotic stimuli.

$CD4^+CD8^+$ thymocytes from wildtype, ΔDEF2, Bim_{EL}-only and Bim_L-only mice were FACS sorted and treated with PMA (10 ng/mL) (a), Taxol (1 μg/mL) (b) or the BH3 mimetic ABT-737 (1 μM) (c). Cell survival was assessed daily by PI staining and FACS analysis. Data represent mean (+/-SEM) of n=3 to 5 independent mice per genotype. (p> 0.05).

Figure S4: *In vitro* activation of wildtype, DDEF2, Bim_{EL}-only and Bim_L-only T lymphocytes.

Same experiments as shown in Figure 6. The absolute numbers of cells are shown to illustrate the normal mitogen induced proliferation of the T lymphocytes from the various mutant strains of mice.

Figure S5: *In vitro* activation of wildtype, DDEF2, Bim_{EL}-only and Bim_L-only B lymphocytes.

(a-d) Purified B lymphocytes ($2x10^6$ cells/mL) from wildtype, ΔDEF2, Bim_{EL}-only and Bim_L-only mice were left untreated or stimulated with anti-IgM Fab_2 antibody fragments (10 μg/mL) plus anti-CD40 mAbs (5 μg/mL) in medium containing saturating concentrations of recombinant mouse IL-2, IL-4 and IL-5 for 24, 48 and 120 h. The percentages and numbers of

activated ($CD25^{+}FSC^{hi}$) (a and c) and surviving ($PI^{-}FSC^{hi}$) T cells (b and d) were measured after 24, 48 and 120 h of culture. Data represent mean (+/-SEM) of at least 3 to 4 mice per genotype ($p > 0.05$).

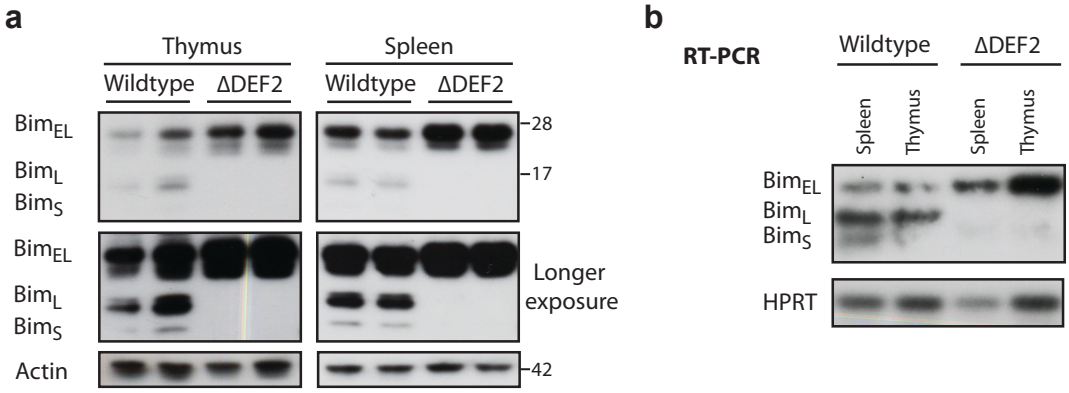
a
Thymus
Spleen
Wildtype
ΔDEF2
Wildtype
ΔDEF2
Bim$_{EL}$
Bim$_L$
Bim$_S$
28
17
Bim$_{EL}$
Bim$_L$
Bim$_S$
Longer exposure
Actin
42
b
RT-PCR
Wildtype
ΔDEF2
Spleen
Thymus
Spleen
Thymus
Bim$_{EL}$
Bim$_L$
Bim$_S$
HPRT

Figure S1

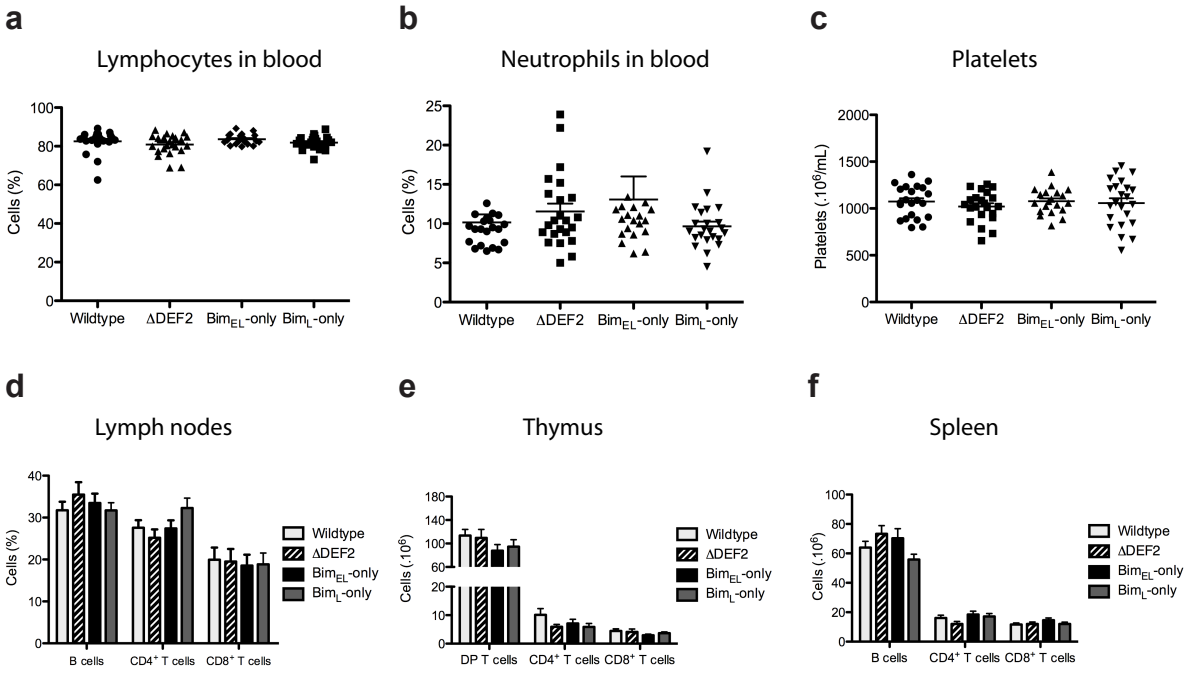
a
Lymphocytes in blood
Cells (%)
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
b
Neutrophils in blood
Cells (%)
c
Platelets
Platelets (.10^6/mL)
d
Lymph nodes
Cells (%)
B cells
CD4$^+$ T cells
CD8$^+$ T cells
e
Thymus
Cells (.10^6)
DP T cells
f
Spleen

Figure S2

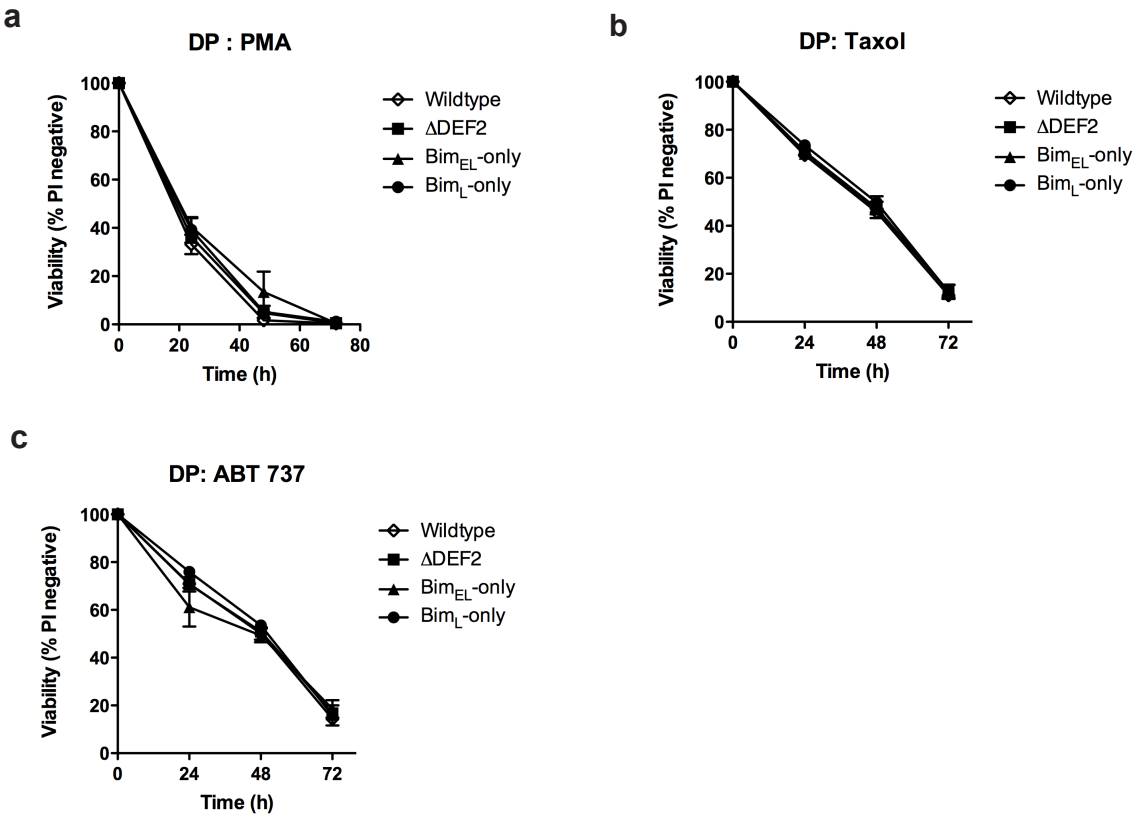
a
DP : PMA
Viability (% PI negative)
Time (h)
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
b
DP: Taxol
Viability (% PI negative)
Time (h)
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
c
DP: ABT 737
Viability (% PI negative)
Time (h)
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only

Figure S3

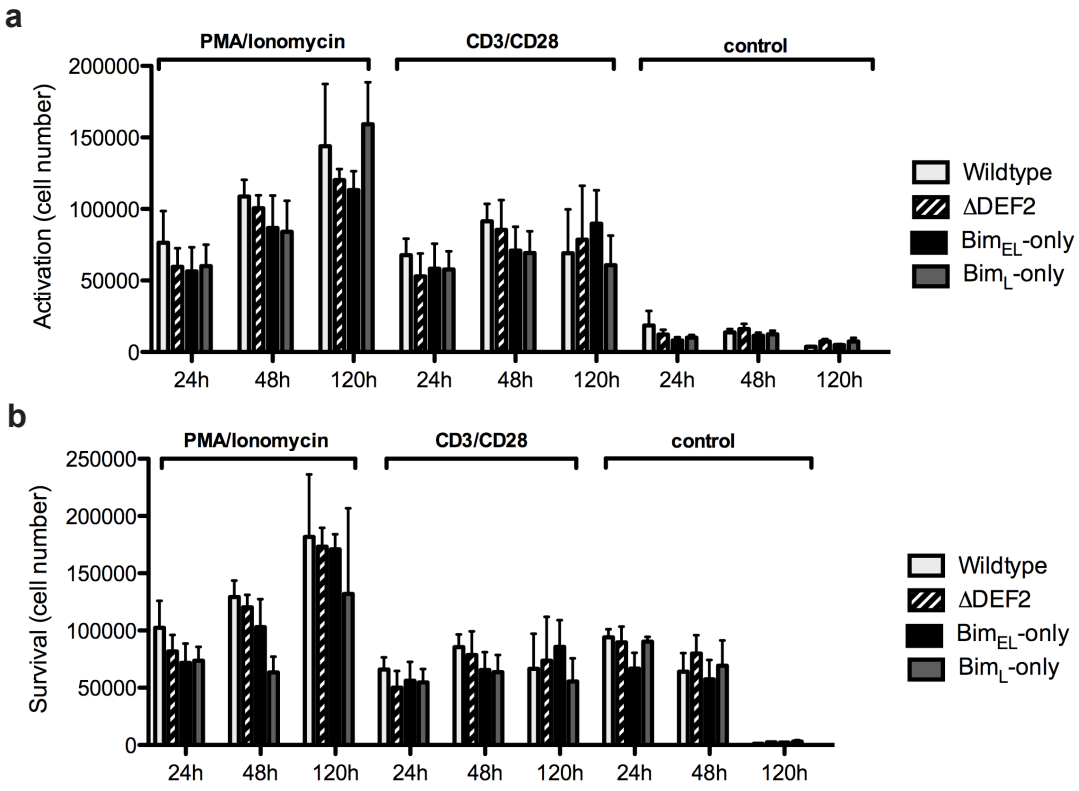
a
PMA/Ionomycin
CD3/CD28
control
Activation (cell number)
200000
150000
100000
50000
0
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
b
PMA/Ionomycin
CD3/CD28
control
Survival (cell number)
250000
200000
150000
100000
50000
0
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only

Figure S4

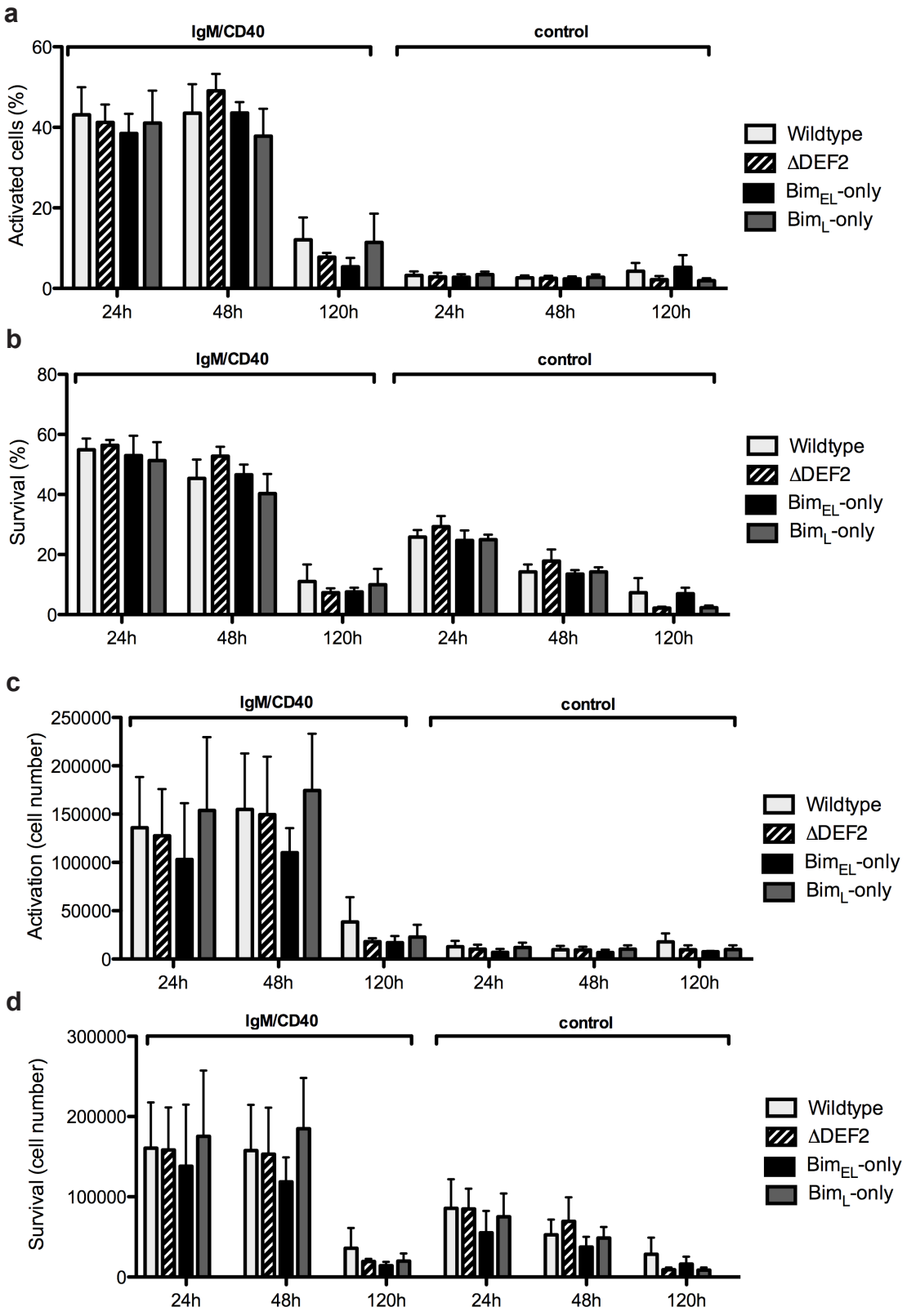
a
IgM/CD40
control
Activated cells (%)
60
40
20
0
24h
48h
120h
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
b
IgM/CD40
control
Survival (%)
80
60
40
20
0
24h
48h
120h
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
c
IgM/CD40
control
Activation (cell number)
250000
200000
150000
100000
50000
0
24h
48h
120h
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only
d
IgM/CD40
control
Survival (cell number)
300000
200000
100000
0
24h
48h
120h
24h
48h
120h
Wildtype
ΔDEF2
Bim$_{EL}$-only
Bim$_{L}$-only

Figure S5