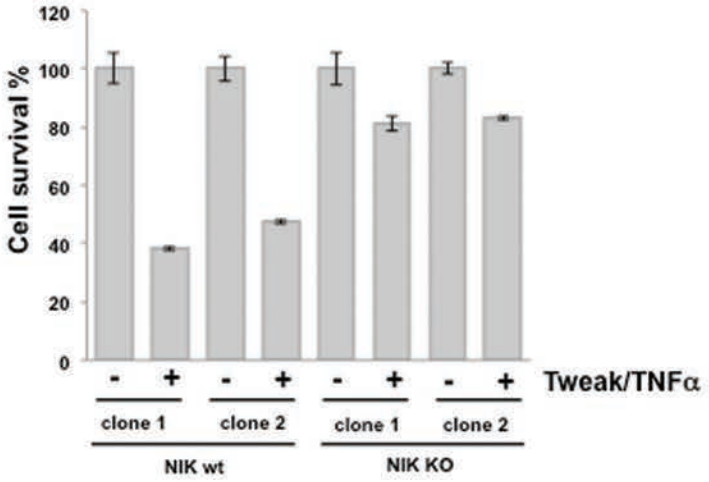
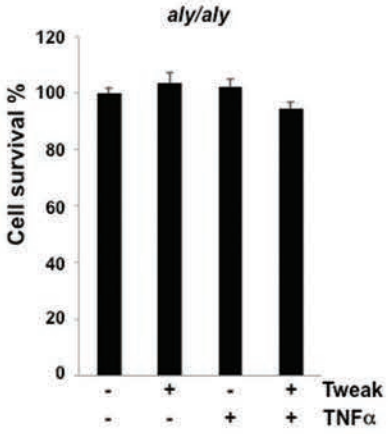
a


120
100
80
60
40
20
0
Cell survival %
- + - + - + - +
Tweak/TNFα
clone 1
clone 2
clone 1
clone 2
NIK wt
NIK KO

b


aly/aly
120
100
80
60
40
20
0
Cell survival %
- + - +
Tweak
- - + +
TNFα

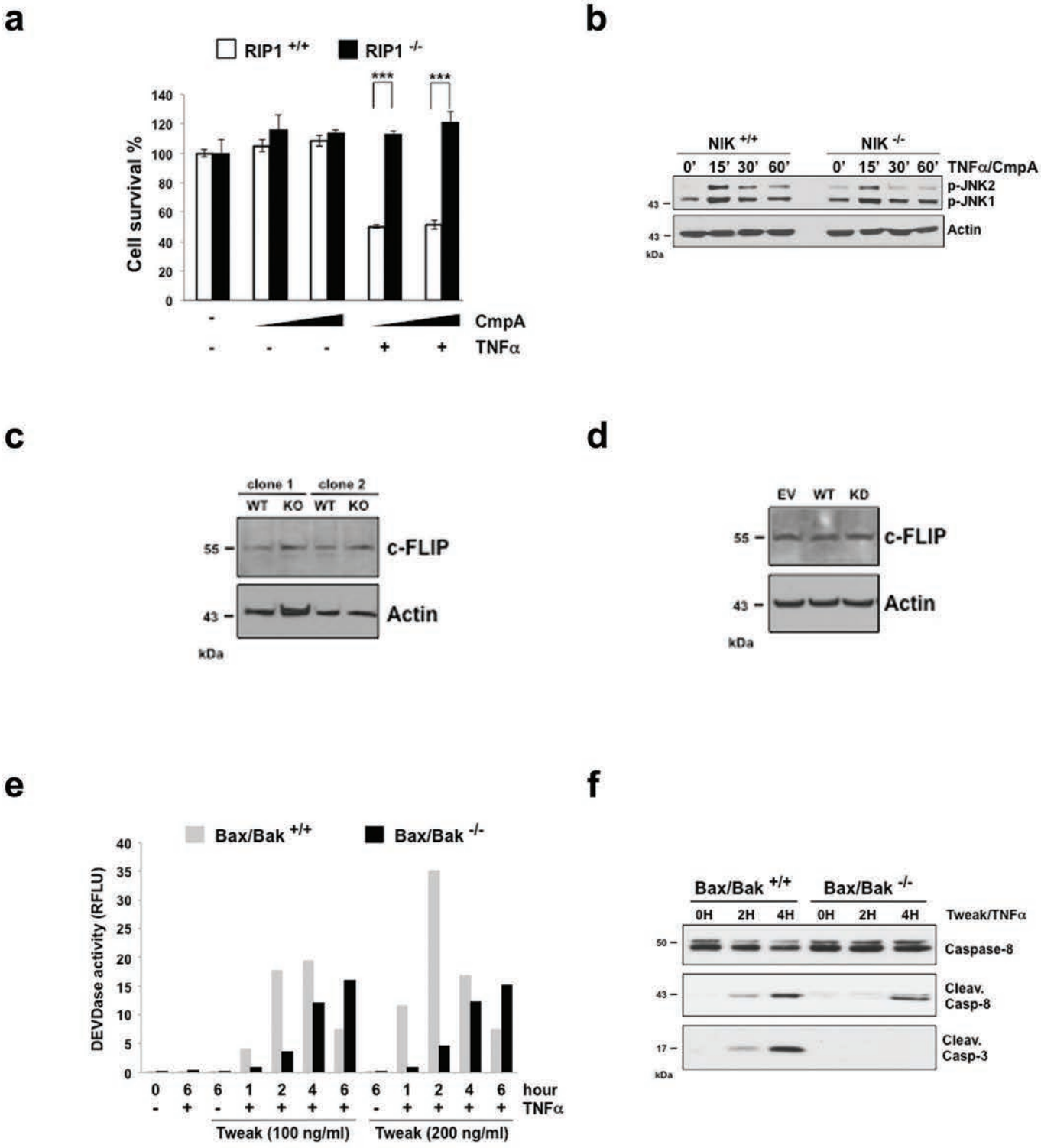
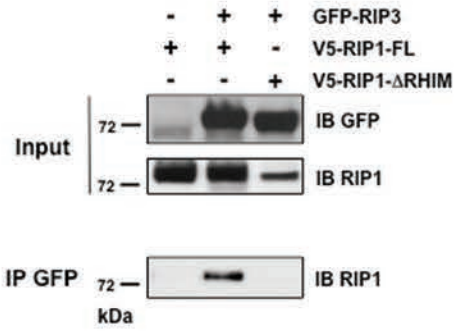
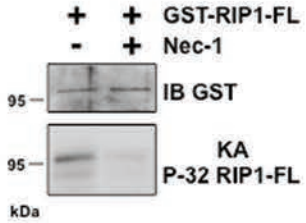

a
RIP1 +/+
RIP1 -/-
***
***
Cell survival %
140
120
100
80
60
40
20
0
-
CmpA
-
TNFα
b
NIK +/+
NIK -/-
0' 15' 30' 60'
0' 15' 30' 60'
TNFα/CmpA
p-JNK2
p-JNK1
Actin
43
43
kDa
c
clone 1
clone 2
WT KO WT KO
55
c-FLIP
43
Actin
kDa
d
EV WT KD
55
c-FLIP
43
Actin
kDa
e
Bax/Bak +/+
Bax/Bak -/-
DEVDase activity (RFLU)
40
35
30
25
20
15
10
5
0
0 6 6 1 2 4 6 6 1 2 4 6 hour
- + - + + + + - + + + + TNFα
Tweak (100 ng/ml)
Tweak (200 ng/ml)
f
Bax/Bak +/+
Bax/Bak -/-
0H 2H 4H 0H 2H 4H
Tweak/TNFα
50
Caspase-8
43
Cleav. Casp-8
17
Cleav. Casp-3
kDa

Fig S3

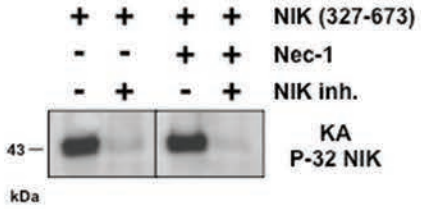
a



b



c



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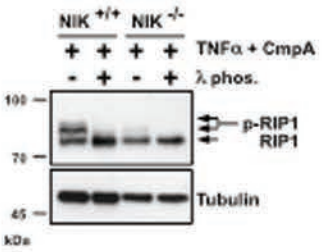
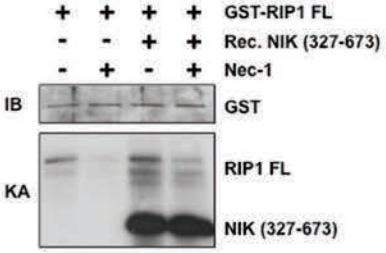


e



f

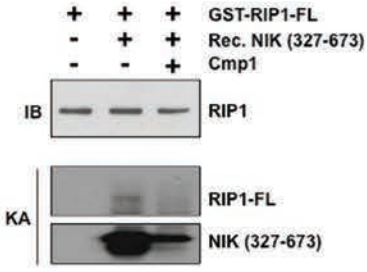
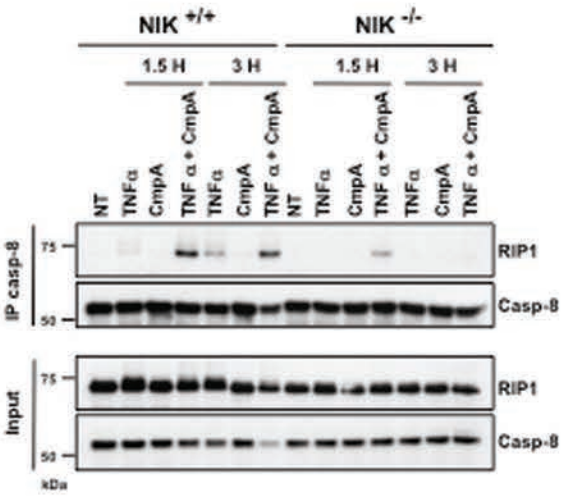
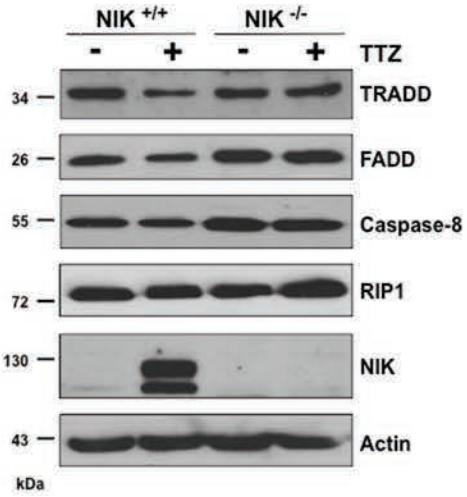


Fig S4

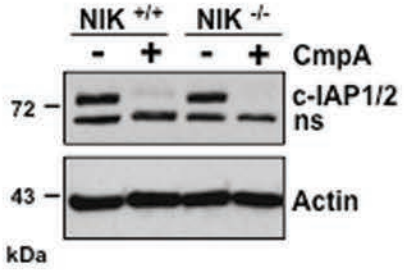
a


NIK +/+
NIK -/-
1.5 H
3 H
1.5 H
3 H
NT
TNFα
CmpA
TNF α + CmpA
TNFα
CmpA
TNF α + CmpA
NT
TNFα
CmpA
TNF α + CmpA
TNFα
CmpA
TNF α + CmpA
IP casp-8
75
50
RIP1
Casp-8
Input
75
50
RIP1
Casp-8
kDa

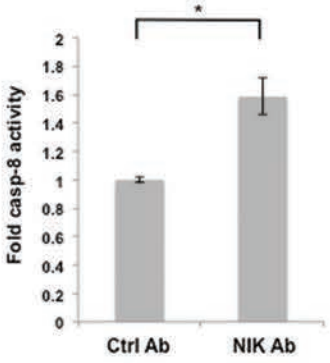
b


NIK +/+
NIK -/-
-
+
-
+
TTZ
34
TRADD
26
FADD
55
Caspase-8
72
RIP1
130
NIK
43
Actin
kDa

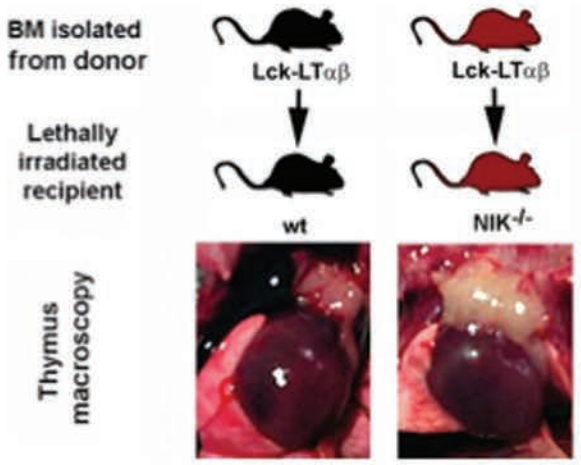
c


NIK +/+
NIK -/-
-
+
-
+
CmpA
72
c-IAP1/2
ns
43
Actin
kDa

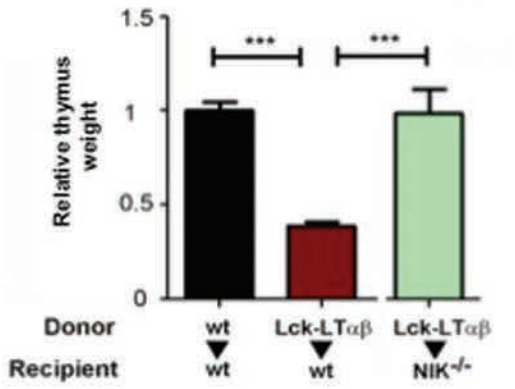
d


*
Fold casp-8 activity
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ctrl Ab
NIK Ab

a


BM isolated from donor
Lck-LTαβ
Lck-LTαβ
Lethally irradiated recipient
wt
NIK-/-
Thymus macroscopy

b


Relative thymus weight
1.5
1
0.5
0
***
***
Donor
Recipient
wt
wt
Lck-LTαβ
wt
Lck-LTαβ
NIK-/-

c

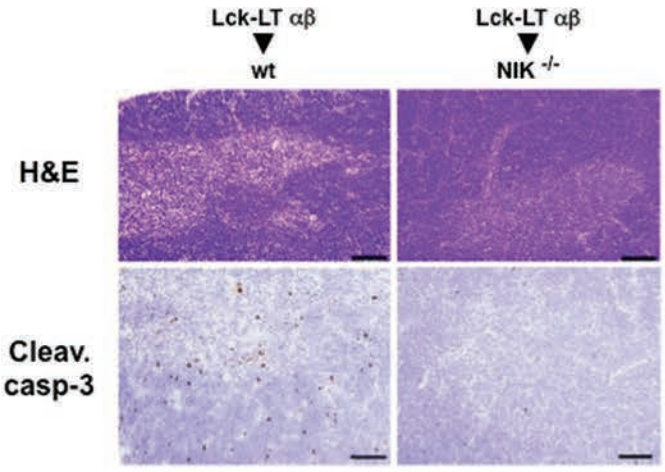
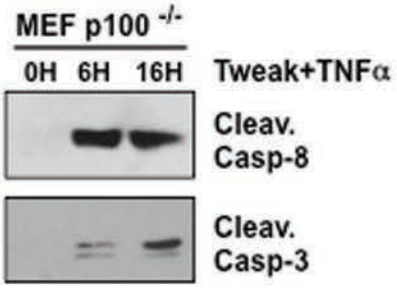
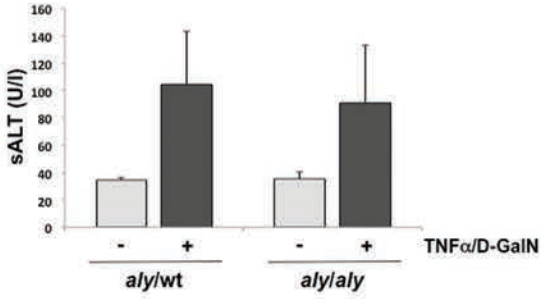

Lck-LT αβ
wt
Lck-LT αβ
NIK -/-
H&E
Cleav. casp-3

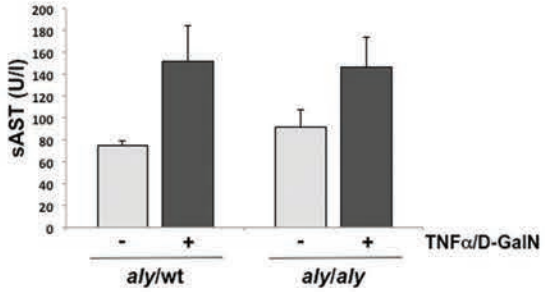
Fig S6



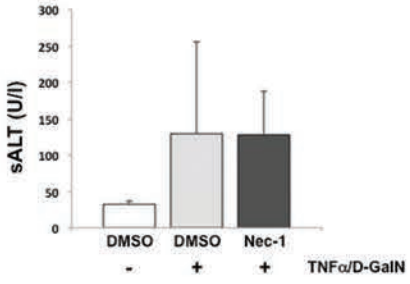
a


sALT (U/l)
160
140
120
100
80
60
40
20
0
- + - +
TNFα/D-GalN
aly/wt
aly/aly

b


sAST (U/l)
200
180
160
140
120
100
80
60
40
20
0
- + - +
TNFα/D-GalN
aly/wt
aly/aly

c


sALT (U/l)
300
250
200
150
100
50
0
DMSO DMSO Nec-1
- + +
TNFα/D-GalN

d

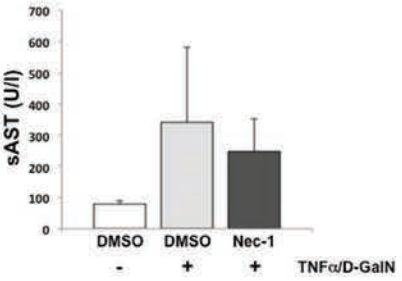

sAST (U/l)
700
600
500
400
300
200
100
0
DMSO DMSO Nec-1
- + +
TNFα/D-GalN

Figure S1: NIK-deficient cells resist to Tweak/TNFα-induced cell death

(a) Two independent clones of NIK WT and NIK KO MEFs were treated for 24 hours with Tweak (200 ng/ml) and TNFα (200 U/ml) and cell survival was measured with Cell Titer-Glo kit. (**b**) *aly*/*aly* homozygote MEFs were treated and analyzed like in (a).

Figure S2: NIK/RIP1-mediated caspase activation acts through the mitochondrial pathway

(a) RIP1 $^{+/+}$ and RIP1 $^{-/-}$ MEFs were treated for 24 hours with or without TNFα (200 U/ml) and increasing amount of the Smac mimetics CmpA (250 nM), and cell survival was measured with Cell Titer-Glo kit. (**b**) NIK $^{+/+}$ and NIK $^{-/-}$ MEFs were treated as indicated and protein extracts were analyzed by Western-blot for phosphor-JNK expression. (**c**) Independent WT and KO MEFs for NIK were tested for their expression level of c-FLIP by Western-blot. (**d**) NIK KO MEFs reconstituted with empty vector (EV), NIK WT (WT) or NIK kinase-dead (KD) were analyzed for their c-FLIP content **(e)** DEVDase activity of Bax/Bak $^{+/+}$ and Bax/Bak $^{-/-}$ MEFs treated for various time with two doses of Tweak and a fixed concentration of TNFα (200 U/ml). **(f)** Bax/Bak $^{+/+}$ and Bax/Bak $^{-/-}$ MEFs were treated with Tweak (200 ng/ml) and TNFα (200 U/ml) for different time periods. Levels of cleaved caspase-8 and cleaved caspase-3 were assessed by Western-blot. Statistical analyses: *** $P<0.001$.

Figure S3: RIP1 is a substrate of NIK but its interaction does not require the RHIM domain

(a) HEK293T cells were transfected with GFP-RIP3 and FL kinase dead RIP1-V5 or ΔRHIM RIP1-V5 expression vectors. RIP3-GFP complexes were immunoprecipitated using an anti-GFP antibody and RIP1 interactions were revealed by Western-blot with an anti-RIP1 antibody. **(b)** Specificity of recombinant full-length RIP1was assessed by *in vitro* kinase assay using RIP1 kinase inhibitor Nec-1. **(c)** Specificity of recombinant NIK kinase domain was assessed by *in vitro* kinase assay using NIK kinase inhibitor and/or RIP1 kinase inhibitor Nec-1. **(d)** NIK $^{+/+}$ and NIK $^{-/-}$ MEFs were left untreated or treated with a mix of TNFα and Smac mimetics (CmpA) and the phosphorylation status of RIP1 was analyzed by Western-blot with cell extracts treated or not with lambda phosphatase (λ phos.). (**e**) *In vitro* kinase assay with active full-length RIP1 in the presence of active recombinant NIK and the RIP1 kinase inhibitor Nec-1. (**f**) *In vitro* kinase assay with inactive full-length RIP1 in the presence of active recombinant NIK.

Figure S4: Smac mimetics/TNFα-induced complex IIb assembly is defective in NIK-deficient MEFs

(a) NIK $^{+/+}$ and NIK $^{-/-}$ MEFs were treated with CmpA and/or TNFα for the indicated time periods prior to an immunoprecipitation with an anti-caspase-8 antibody and detection of both caspase-8 and RIP1 by Western-blot. **(b)** Expression profile of death domain-containing proteins in NIK +/+ and NIK **-/-** MEFs treated with Tweak/TNFα in the presence of z-VAD-FMK (TTZ). **(c)** Level of c-IAP1/2 depletion in NIK $^{+/+}$ and NIK $^{-/-}$ MEFs treated with CmpA, ns, non-specific signal. (**d**) NIK $^{+/+}$ MEFs were treated with Tweak/TNFα/Z-VAD for 60 min. prior to immunoprecipitation with irrelevant or anti-NIK antibodies. The immunoprecipitates were subjected to *in vitro* caspase-8 activity measurement. P value was 0,02.

Figure S5: TNFR1/LTβR-induced thymus involution and cell death is blocked in NIK knock-out mice.

(a) Images of thymi from postnatal (8 weeks) BM reconstitution experiments. Lethally irradiated (1100 rad) WT and NIK $^{-/-}$ recipient mice were reconstituted with BM from Tg Lck-LTαβ mice. (**b**) Quantitative analyses of relative thymus weight of reconstitution experiments achieved in (a). (**c**) Analysis of thymic architecture (H&E staining) of the indicated post-reconstitution (5-6 weeks) experiments and cleaved caspase-3 staining. Statistical analyses: *** $P<0.001$.

Figure S6: p100-deficiency does not prevent Tweak/TNFα-induced apoptosis.

MEFs deficient for p100 treated with Tweak and TNFα displays the apoptotic cleavage of caspase-8 and caspase-3.

Figure S7: RIP1 and NIK mediate Adenoviral/TNFα-induced hepatocyte caspase-3 cleavage.

(a, b) Dosage of serum ALT and serum AST in *aly*/wt (n=3) and *aly* / *aly* (n=3) mice treated for 4 hours with D-Gal-N and TNFα. **(c, d)** Dosage of serum ALT and serum AST in WT mice treated for 4 hours with D-Gal-N and TNFα in the presence or absence of Nec-1 (n=3 for each treatment).