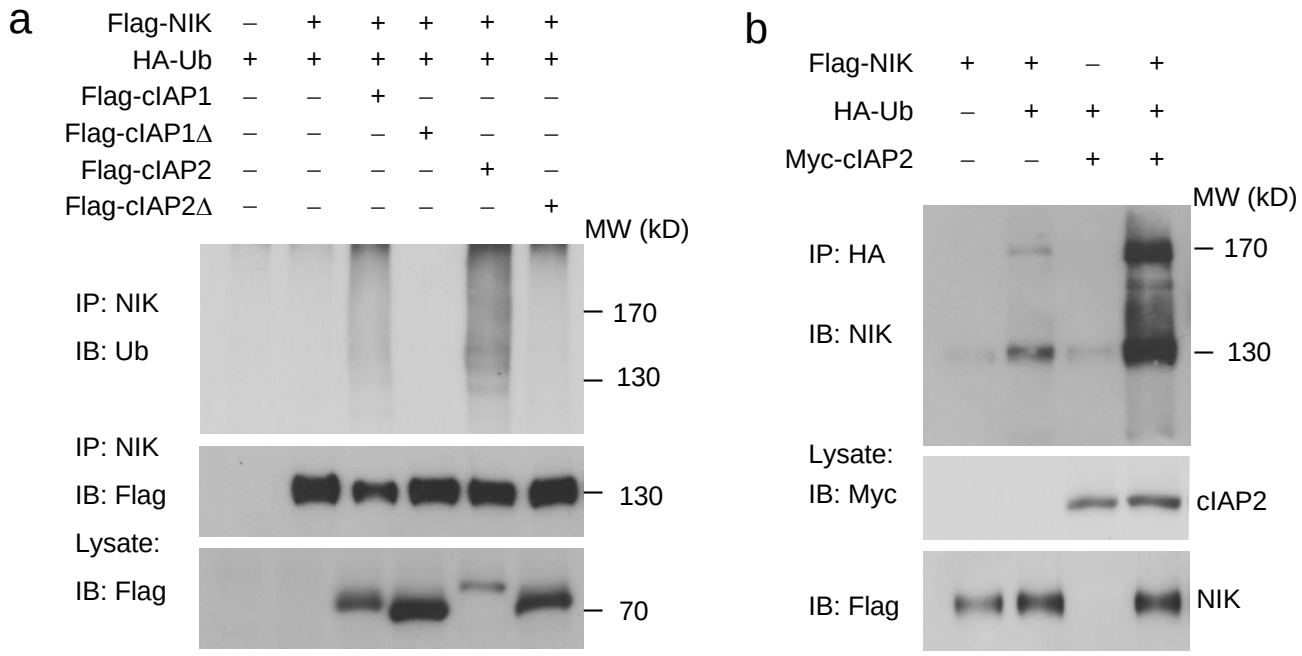
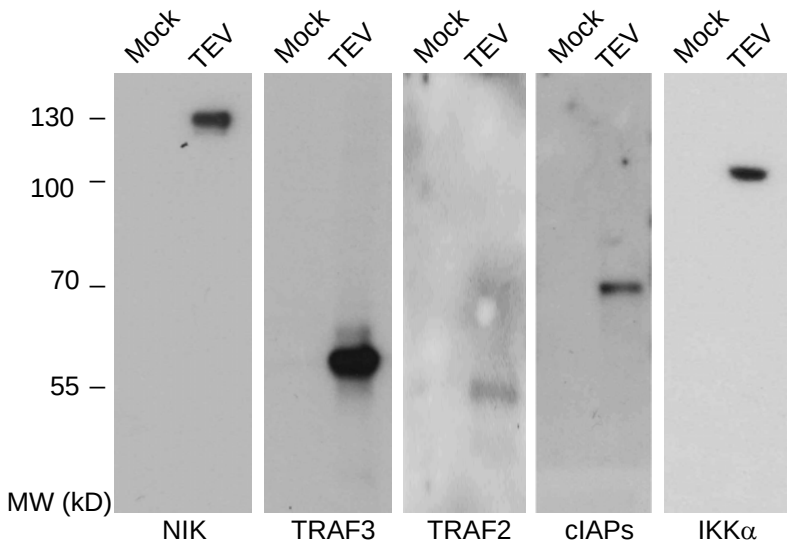
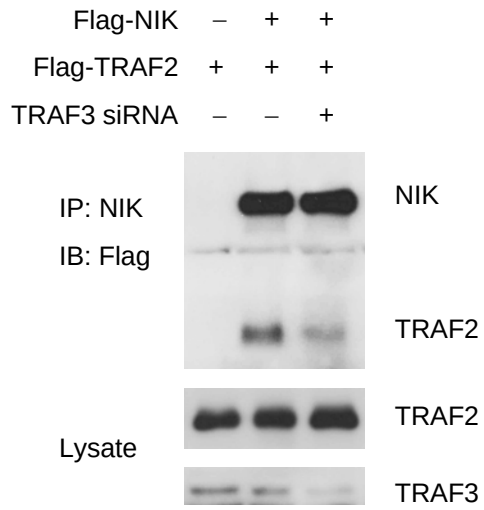


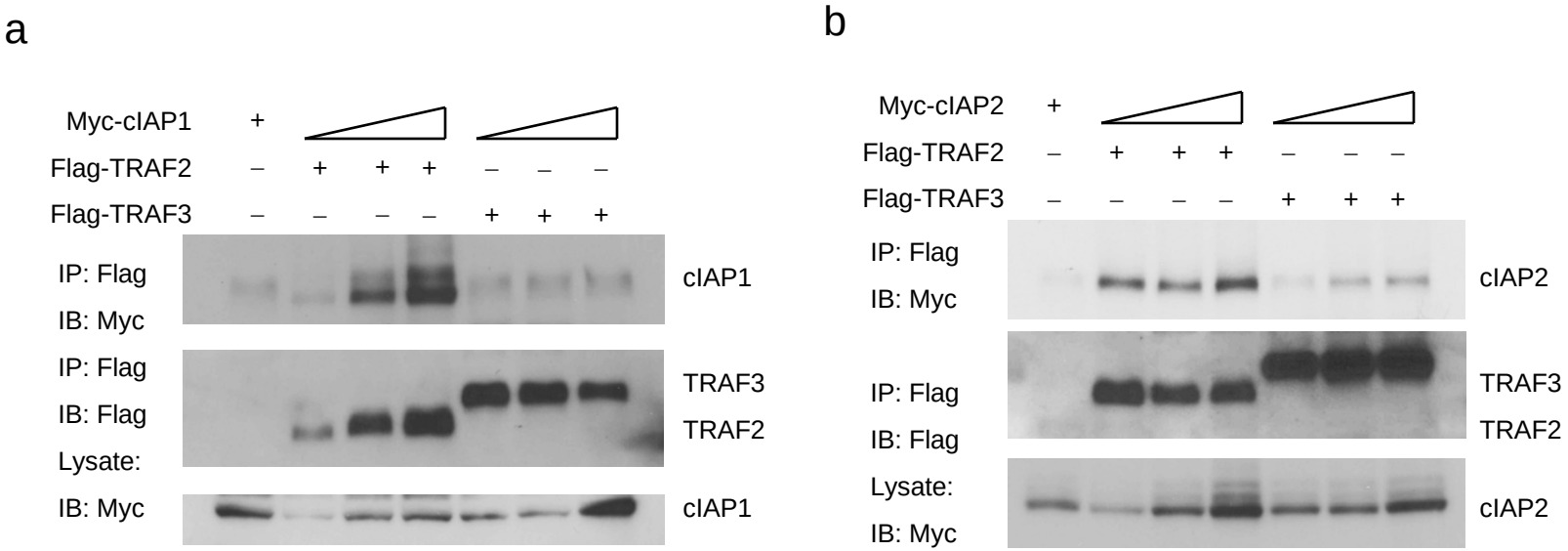
Supplementary Figure 1. cIAP1 and cIAP2 promote ubiquitination of NIK. (**a**) HEK 293T cells were transiently cotransfected with Flag-NIK and HA-Ub alone or together with Flag-tagged cIAP1, cIAP1Δ, cIAP2, or cIAP2Δ . Cells were incubated in the presence of the proteasomal inhibitor MG132 for the final 4 hrs. Ubiquitination of NIK was detected by immunoprecipitation with anti-NIK antibody followed by immunoblot analysis against HA. (**b**) HEK 293T cells were transiently cotransfected with Flag-NIK and HA-Ub alone or with Myc-cIAP2. Ubiquitination of NIK was detected by immunoprecipitation with anti-HA antibody followed by immunoblot analysis against NIK. Data are representative of at least three independent experiments.



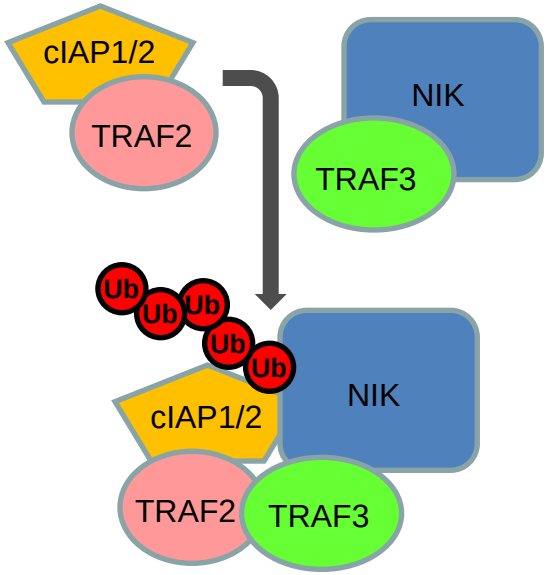
Supplementary Figure 2. Tandem affinity purification of the NIK regulatory complex in MEFs. *Map3k14*$^{-/-}$ MEFs stably transfected with TAP-NIK were lysed in mRIPA buffer after 4 hr of MG132 treatment. Lysates were incubated with anti-Flag M2 beads for 2 hr. The beads were washed with lysis buffer and TEV-protease cleavage buffer (10 mM Tris-HCl (pH 7.5), 100 mM NaCl and 0.2% NP-40). Bound proteins were mock treated (Mock) or eluted with 40 μg TEV-protease (TEV). The TEV-protease cleavage products were then incubated with calmodulin sepharose (Amersham) in the presence of 2 mM $CaCl_2$ at 4 °C for 1 hr. The TAP-NIK protein complex was then eluted from calmodulin sepharose by boiling in 1 × SDS sample buffer and followed by immunoblot analysis of the indicated proteins. Data are representative of at least three independent experiments.



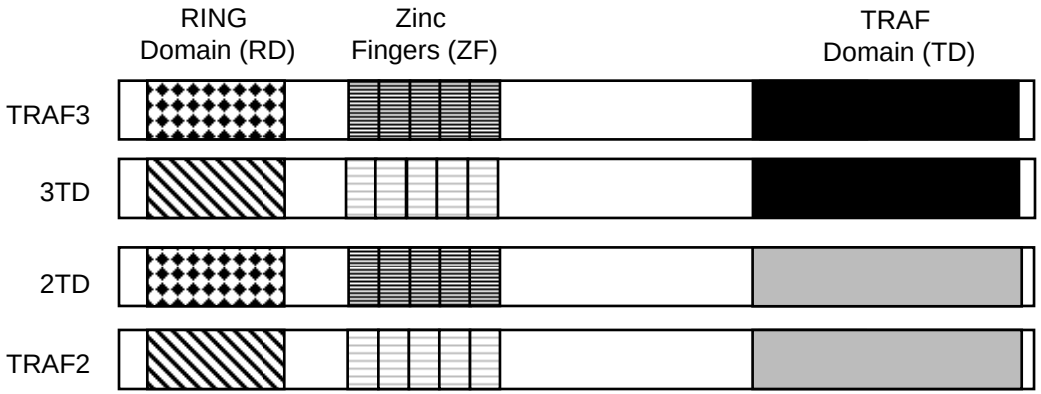
Supplementary Figure 3. Interaction of overexpressed TRAF2 with NIK requires endogenous TRAF3. HEK 293T cells were transiently co-transfected with Flag-NIK and Flag-TRAF2 in the presence or absence of Stealth siRNA against human TRAF3 (Invitrogen). Cell Lysates were incubated with anti-NIK antibody followed by protein-G sepharose and immunoprecipitates were analyzed by immunoblot as indicated. Data are representative of at least three independent experiments.



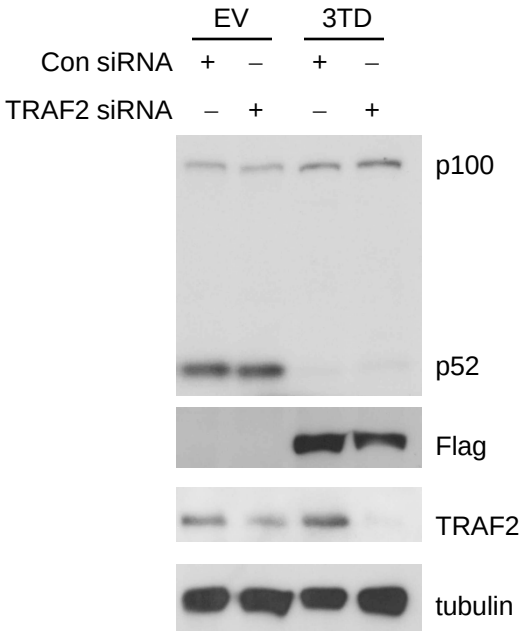
Supplementary Figure 4. cIAP1 and cIAP2 preferentially interact with TRAF2. (**a**,**b**) Flag-TRAF2 or Flag-TRAF3 was co-expressed with titrations of Myc-cIAP1 (**a**) or Myc-cIAP2 (**b**) in HEK 293T cells. Lysates were incubated with anti-Flag M2 beads and cIAP-TRAF complexes were detected by immunoblot analysis against Myc. Data are representative of at least three independent experiments.



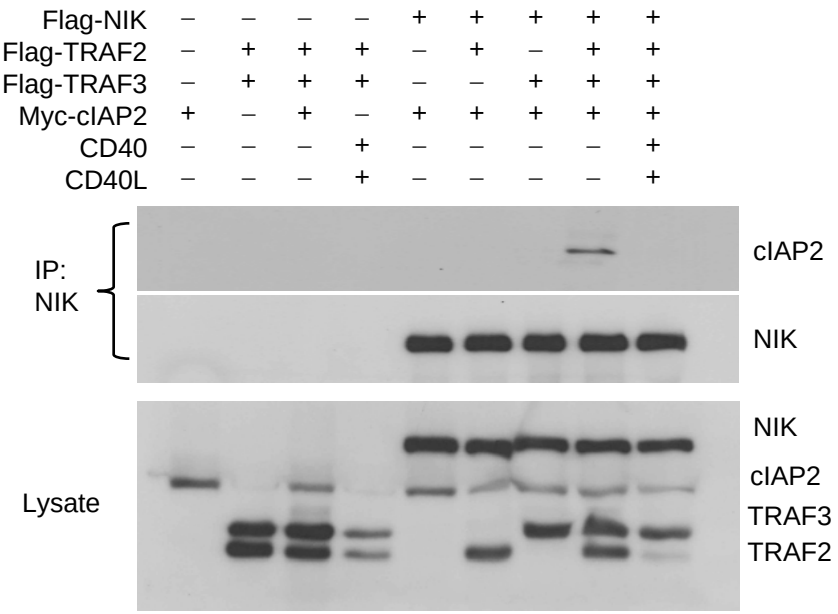
Supplementary Figure 5. A model for assembly of the NIK regulatory complex. In parallel, TRAF2 binds cIAP1 and cIAP2 with high affinity while TRAF3 binds NIK with high affinity. Heterodimerization of TRAF2 and TRAF3 permits cIAP1 and cIAP2 contact with NIK and initiation of NIK polyubiquitination and degradation.



Supplementary Figure 6. Schematic of TRAF2-TRAF3 Chimeras. 3TD, consisting of the TRAF3 TRAF Domain and the Ring and Zinc Finger Domains of TRAF2, is predicted to bind NIK, cIAP1, and cIAP2 as strongly as TRAF3 and TRAF2, respectively. 2TD, consisting of the TRAF2 TRAF Domain and the Ring and Zinc Finger Domains of TRAF3, is predicted to bind NIK, cIAP1, and cIAP2 as weakly as TRAF2 and TRAF3, respectively.



Supplementary Figure 7. 3TD rescues constitutive p100 to p52 processing in the absence of TRAF2. *Traf3*$^{-/-}$ MEFs reconstituted with empty pBABE-Flag vector (EV) or pBABE-Flag-3TD (3TD) were transiently transfected with control or Stealth siRNA against murine TRAF2 (Invitrogen). Forty-eight hours post transfection, cells were lysed in 1 × SDS lysis buffer and the lysates were then subjected to immunoblot analysis with the indicated antibodies. Data are representative of at least three independent experiments.

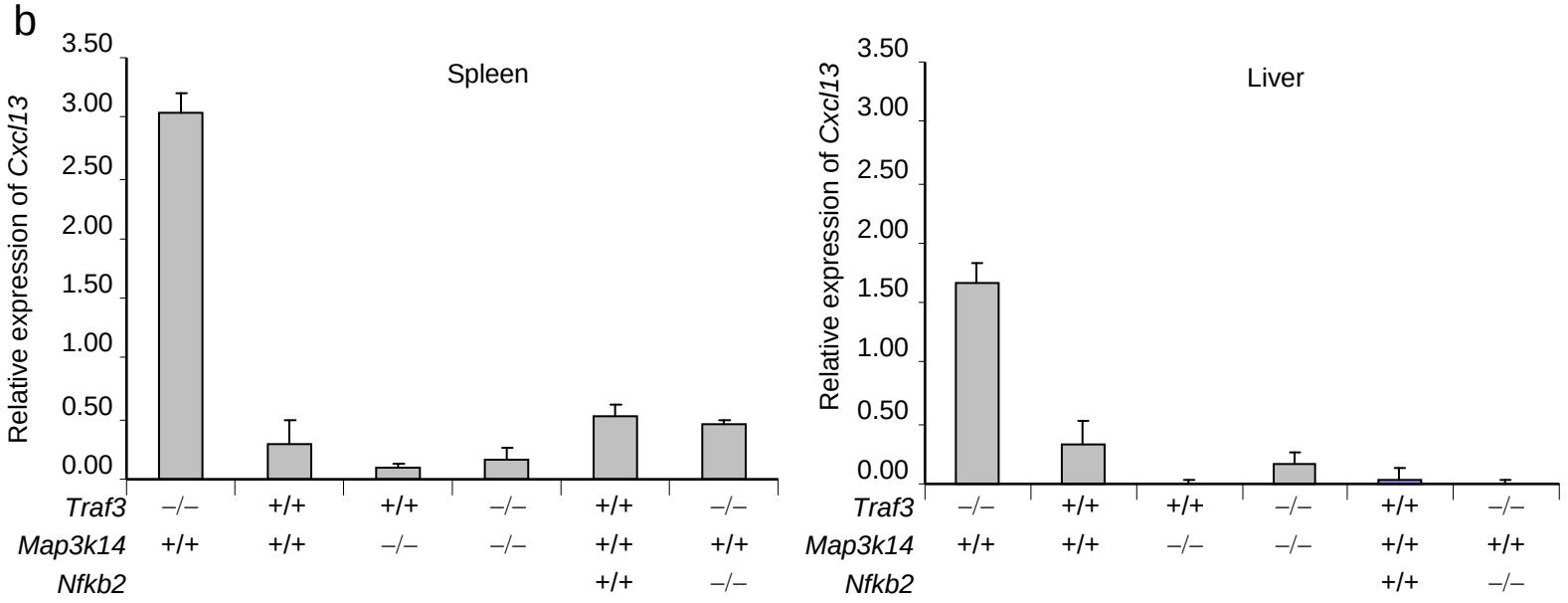


Supplementary Figure 8. CD40 signaling causes disassociation of the NIK-cIAP complex in HEK 293T cells. NIK was co-expressed with Myc-cIAP2 in the presence or absence of TRAF2, TRAF3 or both TRAF2 and TRAF3 together with or without CD40 and CD40L as indicated. Lysates were incubated with anti-NIK antibody and NIK-cIAP2 association was detected by immunoblot analysis against Myc. Data are representative of at least three independent experiments.

a

Genotype	$CD4^+$	$CD8^+$	B cells	%IgM of $B220^+$	%CD35/21 of $B220^+$	$CD11b^+$	$Gr1^+$	NK1.1
Traf3$^{-/-}$*Map3k14*$^{-/-}$	18.9	13.2	5.8	84.0	20.7	19.2	16.3	15.1
Traf3$^{+/+}$*Map3k14*$^{+/+}$	19.7	18.8	30.4	88.3	68.3	5.9	10.0	8.3
Traf3$^{+/+}$*Map3k14*$^{-/-}$	15.0	13.0	4.9	82.6	30.5	28.3	31.5	25.8
Traf3$^{-/-}$*Nfkb2*$^{-/-}$	38.6	34.1	3.4	80.2	37.3	10.4	15.0	7.9

b



Supplementary Figure 9. Compound loss of *Map3k14* rescues TRAF3-null phenotypes. (**a**) Total splenocytes were harvested from the spleens of 8-10 week old mice of the indicated genotypes and analyzed for expression of the indicated cell surface markers by FACS (*n* = 1 per strain). (**b**) RNA was harvested from the spleen and liver of mice of the indicated genotypes. RNA was reverse transcribed for cDNA synthesis and analyzed for expression of *Cxcl13* by quantitative PCR performed in triplicate.

Gene	siRNA sequence	
Non-targeting	GGA UCC UUG ACA AUA CCA A[dT][dT]	UUG GUA UUG UCA AGG AUC C[dT][dT]
cIAP1	GCA AGU GCU GGA UUC UAU U[dT][dT]	AAU AGA AUC CAG CAC UUG C[dT][dT]
cIAP2	GCA CAA GUC CCU ACC ACU U[dT][dT]	AAG UGG UAG GGA CUU GUG C[dT][dT]
TRAF3	GAA GGU UUC CUU GUU GCA GAA UGA A	UUC AUU CUG CAA CAA GGA AAC CUU C
TRAF2	GGA CCA UGU UAG AGC AUG CAG CAA A	UUU GCU GCA UGC UCU AAC AUG GUC C

Supplementary Table. siRNA sequences for non-targeting siRNA, siRNA against murine cIAP1, murine cIAP2, human TRAF3, and murine TRAF2.