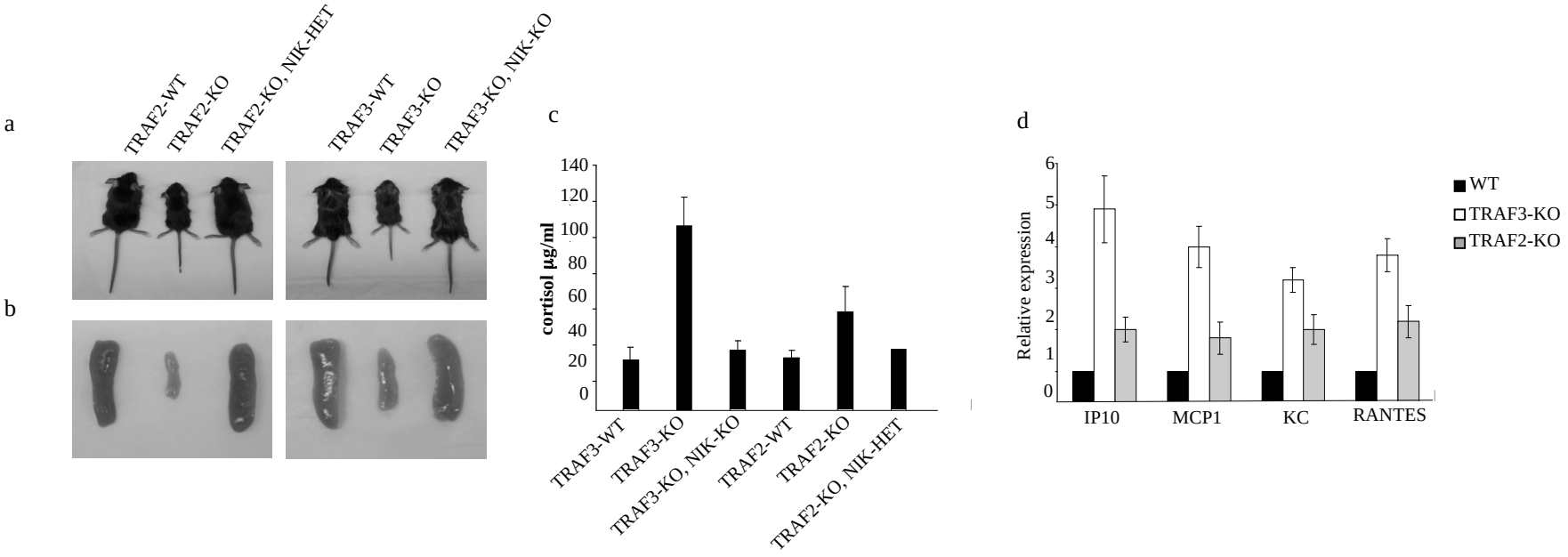
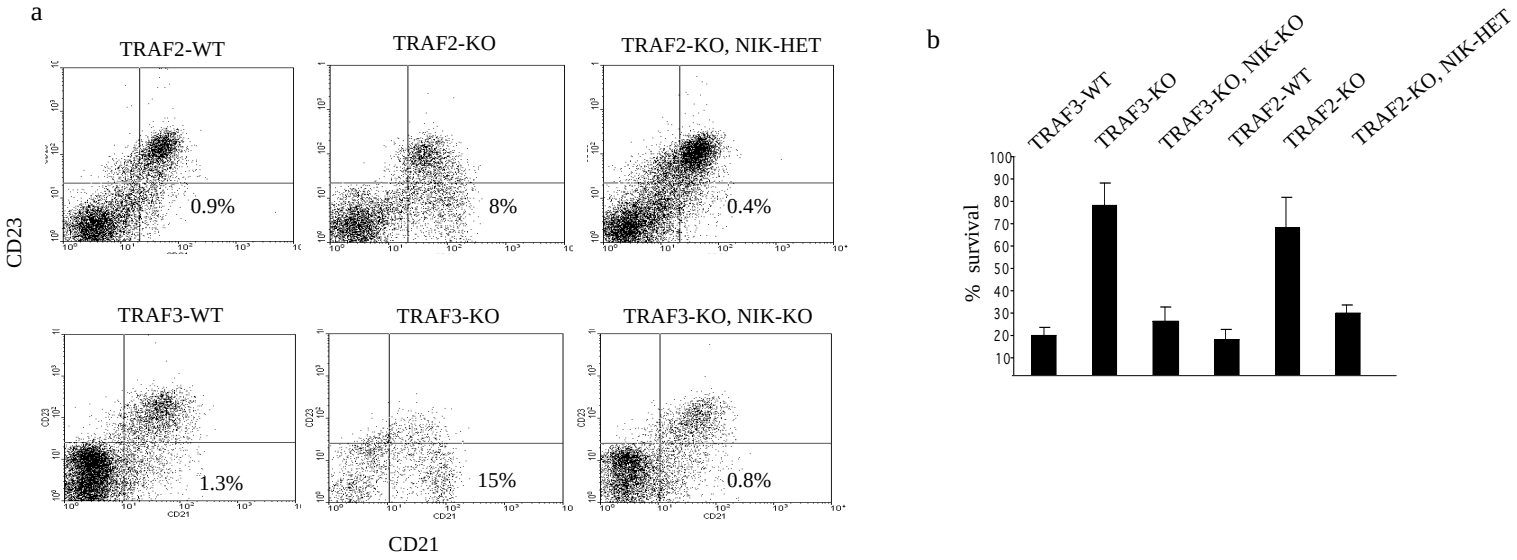
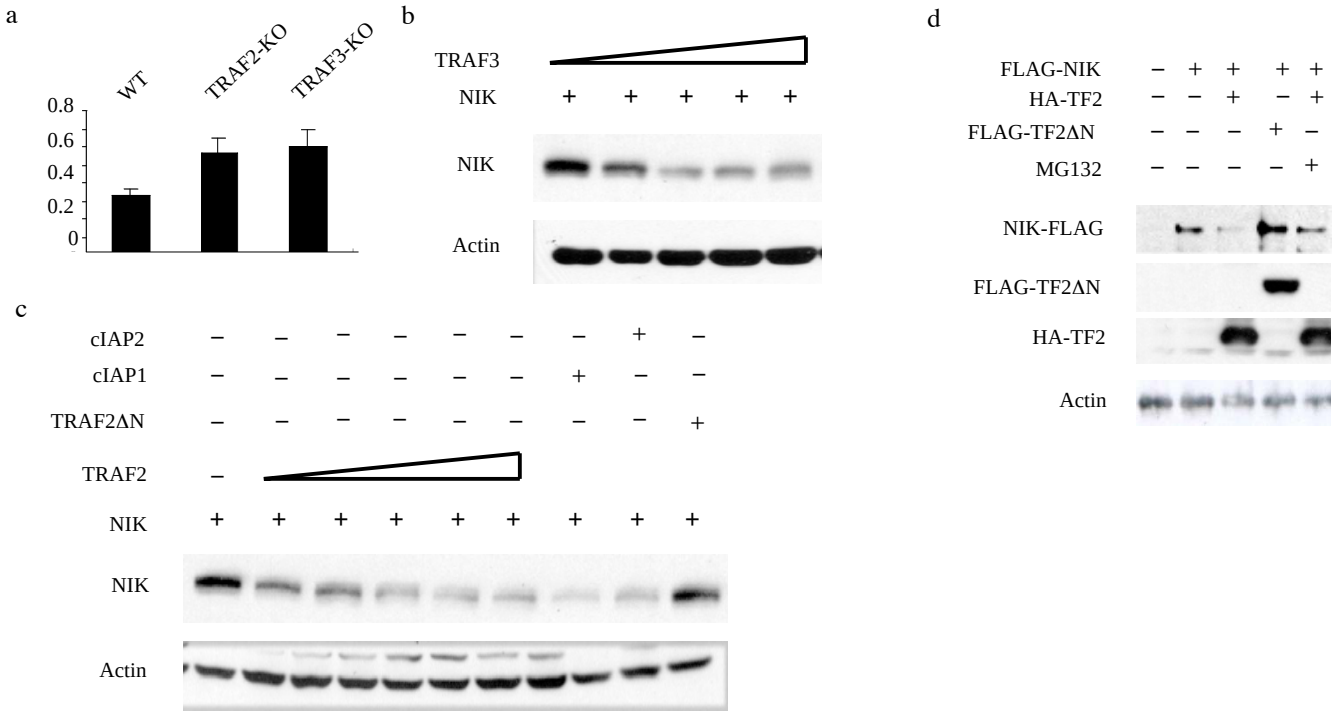


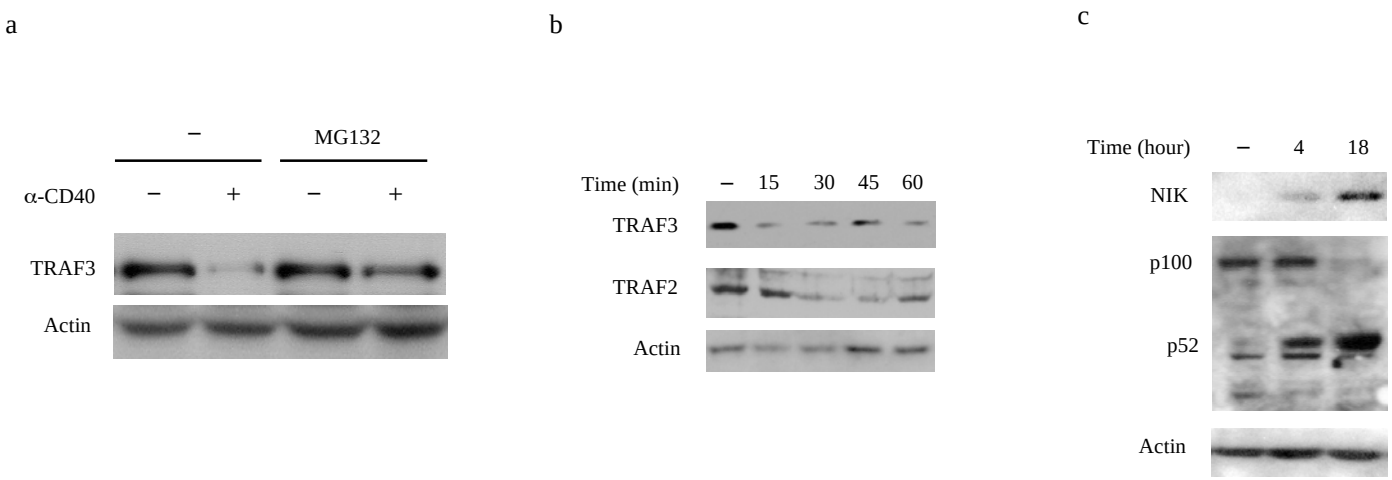
Supplementary Fig.1. Postnatal lethality, Splenic atrophy and serum cortisol levels in *Traf2*$^{-/-}$ and *Traf3*$^{-/-}$ mice are due to uncontrolled NIK signaling. (**a**) Mice of the indicated genotypes were photographed on postnatal day 15 (P15). Deletion of NIK in *Traf2*$^{-/-}$ or *Traf3*$^{-/-}$ mice prevents their runted stature (n = 4). (**b**) Spleens from mice of the indicated genotypes were removed on P15 and photographed. Deletion of NIK in *Traf2*$^{-/-}$ or *Traf3*$^{-/-}$ mice prevents splenic atrophy (n = 4). (**c**) Serum cortisol levels in the indicated mouse strains were measured by ELISA at P15 in at least 2 animals. (**d**) Total RNA was extracted from WT, *Traf2*$^{-/-}$ and *Traf3*$^{-/-}$ MEFs and the indicated cytokine mRNA levels were analyzed by quantitative RT-PCR. Results are averages ± s.d (n = 3). WT, wild-type; TRAF2-KO, *Traf2*$^{-/-}$; TRAF3-KO, *Traf3*$^{-/-}$; NIK-KO, *Map3k14*$^{-/-}$; NIK-HET, *Map3k14*$^{+/-}$.



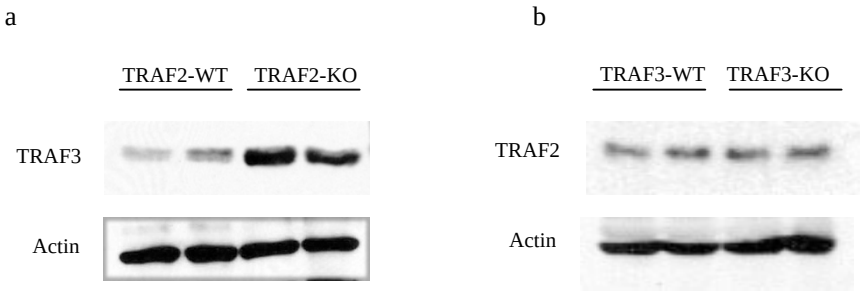
Supplementary Fig. 2. B-cell abnormalities in *Traf2*$^{-/-}$ and *Traf3*$^{-/-}$ mice are due to uncontrolled NIK signaling (**a**). Splenocytes from the indicated mice were analyzed by flow cytometry after staining with CD23 and CD21 specific antibodies. Numbers indicate the percentages of MZB cells. (**b**) Splenic B-cells from the indicated mice were purified and their survival was analyzed after 24 hrs in culture by trypan blue exclusion. Results are average $\pm$ s.d (n = 3). WT, wild-type; TRAF2-KO, *Traf2*$^{-/-}$; TRAF3-KO, *Traf3*$^{-/-}$; NIK-KO, *Map3k14*$^{-/-}$; NIK-HET, *Map3k14*$^{+/-}$.



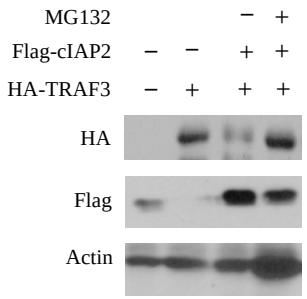
Supplementary Fig. 3. TRAF2, TRAF3, cIAP1 and cIAP2 induce NIK degradation. (**a**) Total RNA from WT, *Traf2*$^{-/-}$ and *Traf3*$^{-/-}$ MEFs was extracted and NIK mRNA levels were analyzed by quantitative RT-PCR. (**b-d**) HEK293T cells were co-transfected with the indicated expression vectors (TF2 = TRAF2). Where indicated, cells were treated with 20 mM MG-132 for 6 hrs to inhibit proteasome activity starting 18 hrs after transfection. NIK amounts were examined by immunoblotting using anti-Flag. WT, wild-type; TRAF2-KO, *Traf2*$^{-/-}$; TRAF3-KO, *Traf3*$^{-/-}$



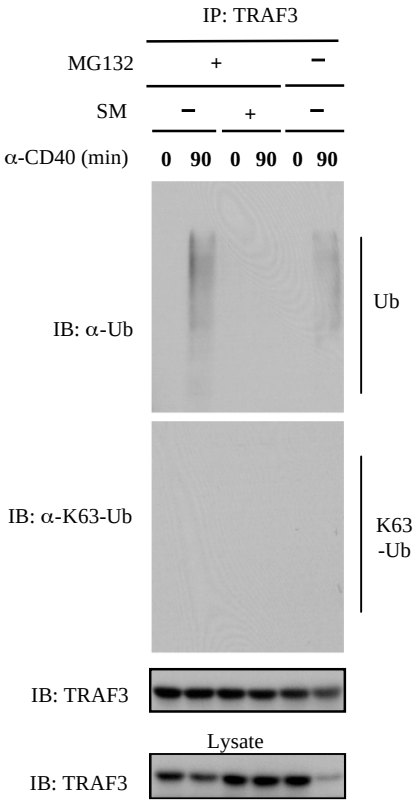
Supplementary Fig. 4. **CD40 signaling induces TRAF3 degradation and NIK accumulation**. (**a**) A20 cells were stimulated for 30 min with anti-CD40 in the presence or absence of MG-132 and TRAF3 levels were analyzed by immunoblotting . (b) Splenic B-cells were purified and cultured in the presence of anti-CD40 for the indicated times and the amounts TRAF3 and TRAF2 were analyzed by immunoblotting. (**c**) Splenic B-cells were cultured in the presence or absence of anti-CD40 for the indicated time points and NIK and NF-κB2-p100 and p52 levels were analyzed by immunoblotting.



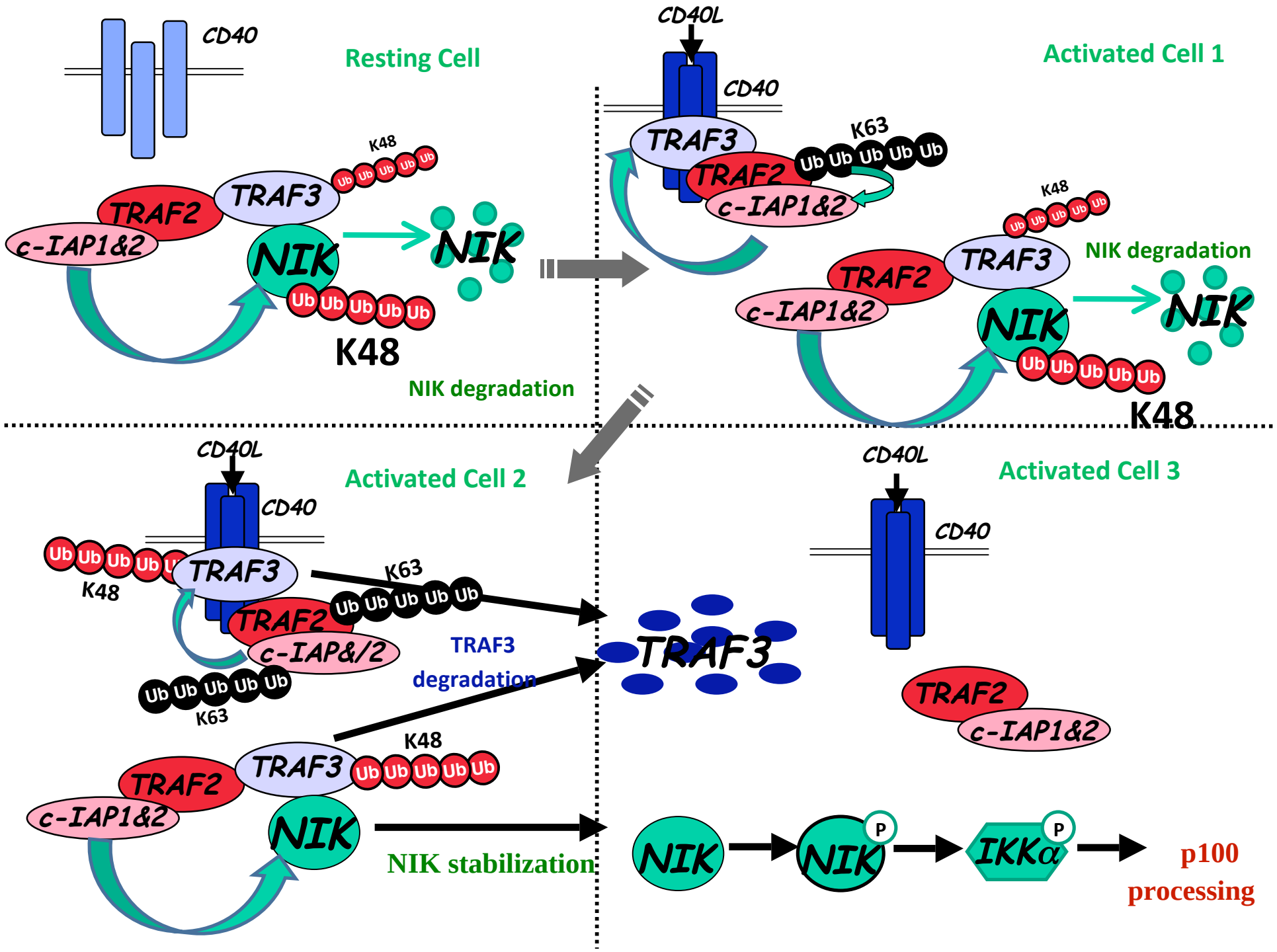
Supplementary Fig. 5. Elevated TRAF3 expression in TRAF2-deficient MEFs (**a,b**). Total cell lysates of WT, *Traf2*$^{-/-}$ and *Traf3*$^{-/-}$ MEFs were prepared and TRAF2 and TRAF3 amounts were analyzed by immunoblotting. The two lanes for each cell type represent duplicate experiments. WT, wild-type; TRAF2-KO, *Traf2*$^{-/-}$; TRAF3-KO, *Traf3*$^{-/-}$



Supplementary Fig.6. cIAP2 induces proteasome dependent TRAF3 degradation. CD40-expressing HEK293T cells were transfected with expression vectors for Flag-cIAP2 and HA-TRAF3 as indicated and cultured in the presence or absence of MG-132 and the amounts of TRAF3 as well as cIAP2 were analyzed by immunoblotting.



Supplementary Fig. 7. Endogenous TRAF3 undergoes K48-linked polyubiquitination upon CD40-stimulation. Splenic B-cells were purified and stimulated for the indiacted time with anti-CD40 in the presence or absence of Smac mimic (SM) and MG-132. TRAF3 was immunoprecipitated and its ubiquitination was analyzed by immunoblotting using anti-ubiquitin and K63-specific anti-ubiquitin. Recovery of TRAF3 as well as its levels in the lysates was examined by immunoblotting with anti-TRAF3.


CD40
Resting Cell
TRAF2
TRAF3
c-IAP1&2
NIK
K48
Ub
NIK
K48
NIK degradation
CD40L
CD40
Activated Cell 1
TRAF3
TRAF2
c-IAP1&2
K63
Ub
NIK degradation
NIK
K48
Activated Cell 2
CD40L
CD40
TRAF3
K48
K63
TRAF2
c-IAP&/2
TRAF3
degradation
TRAF3
K63
K48
TRAF2
c-IAP1&2
NIK
NIK stabilization
Activated Cell 3
CD40L
CD40
TRAF2
c-IAP1&2
NIK
NIK
P
IKKα
P
p100
processing

Supplementary Fig. 8. Control of NIK turnover and alternative NF-κB signaling by TRAF2, TRAF3, cIAP1 and cIAP2.

In resting cells TRAF3 links NIK to an E3 complex containing TRAF2 and cIAP1 and cIAP2, thereby promoting cIAP1- and/or cIAP2-mediated K48-linked NIK polyubiquitination, and proteasomal degradation. There is also a low level of K48-linked polyubiquitination of TRAF3 under these conditions. Upon CD40 (or BAFF-R) engagement, the cIAP1-cIAP2-TRAF2-TRAF3 complex is recruited to the receptor, where TRAF2 undergoes K63-linked self-ubiquitination and ubiquitinates cIAP1and/or cIAP2 also through a K63-linkage. The K63-linked polyubiquitination of cIAP1 and/or cIAP2 enhances their K48-specific E3 activity towards TRAF3, resulting in TRAF3 proteasomal degradation. When TRAF3 amounts in the cell drop below a critical threshold, NIK can no longer be recruited to the cIAP1-cIAP2-TRAF2 complex. This results in stabilization and accumulation of newly synthesized NIK, whose autophosphorylation leads to IKKα activation, phosphorylation of p100 and processing of the latter.