Intracellular antibody-bound pathogens stimulate immune signaling via Fc-receptor TRIM21

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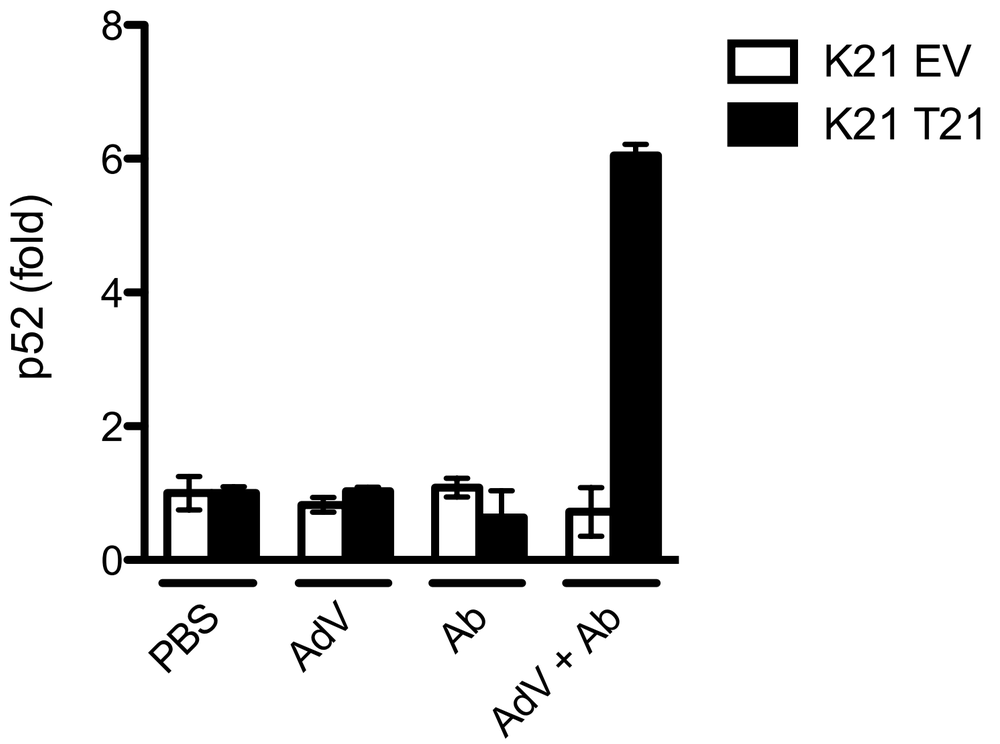
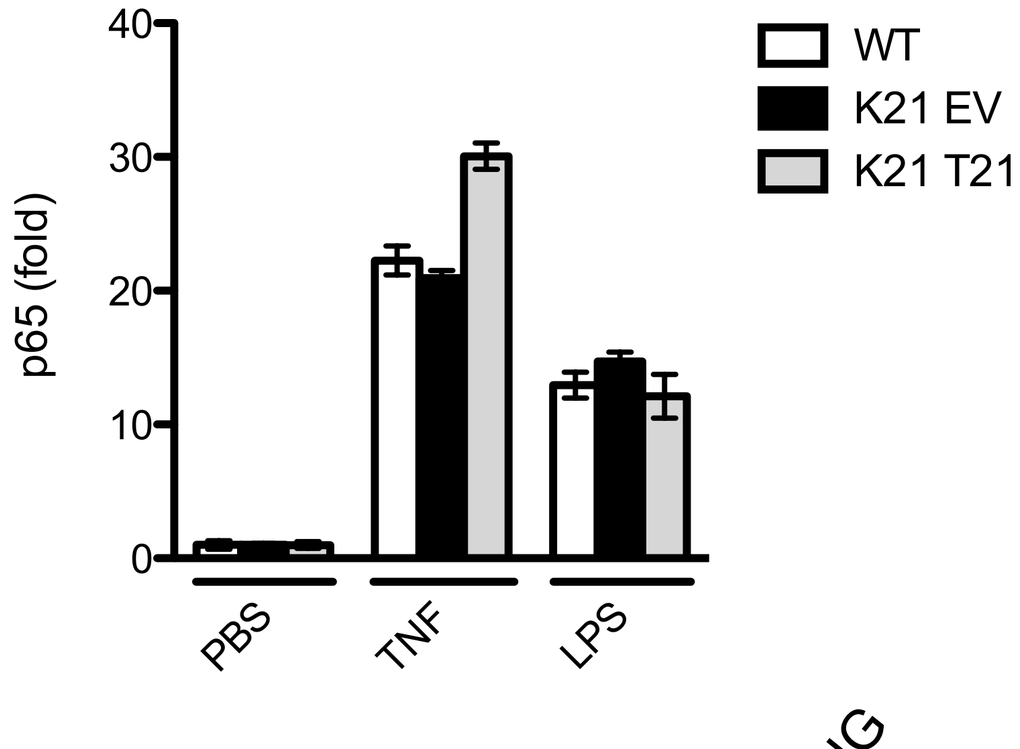
Supplemental Material

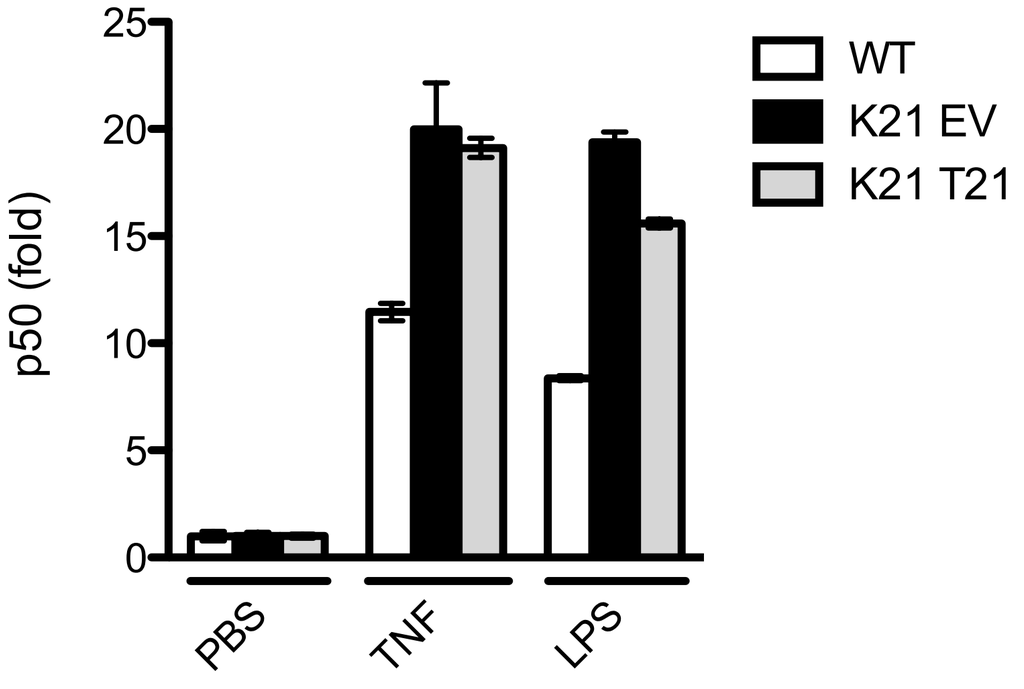
a


p52 (fold)
0
2
4
6
8
PBS
AdV
Ab
AdV + Ab
K21 EV
K21 T21

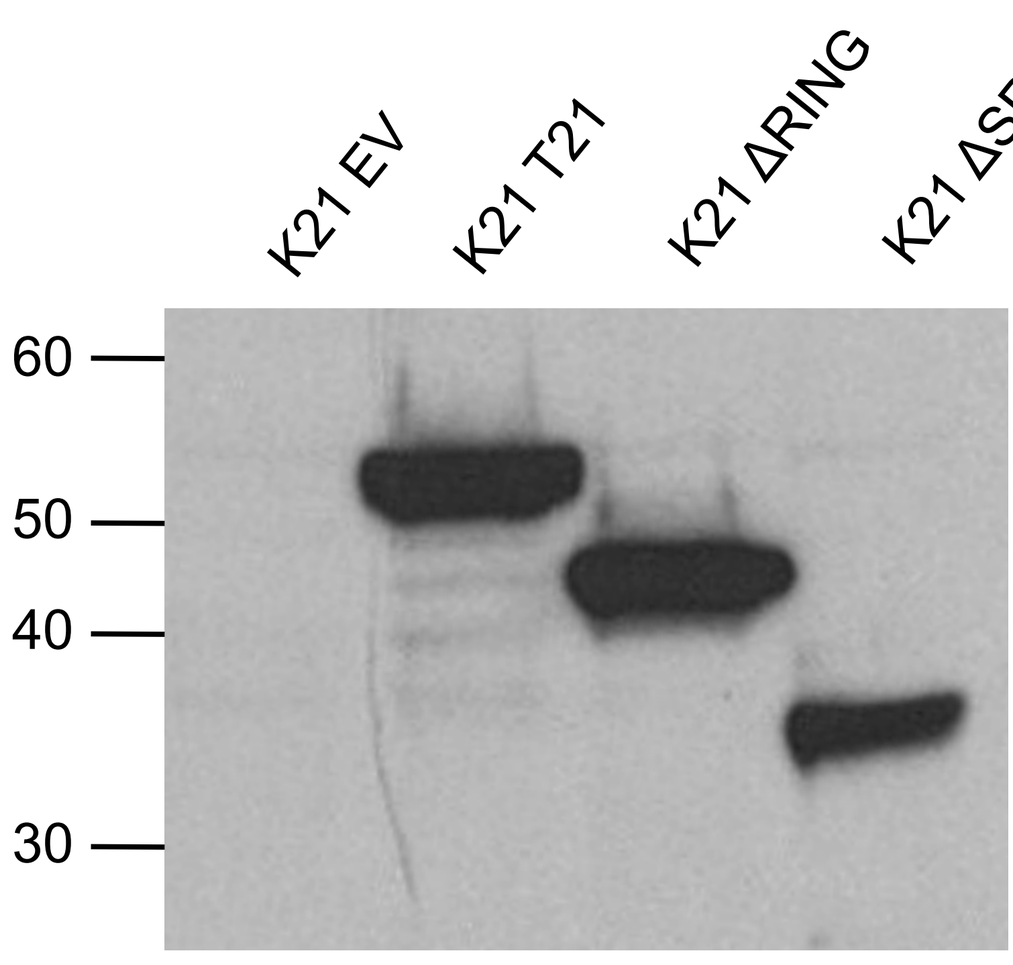
b


p65 (fold)
0
10
20
30
40
PBS
TNF
LPS
WT
K21 EV
K21 T21

c


p50 (fold)
0
5
10
15
20
25
PBS
TNF
LPS
WT
K21 EV
K21 T21

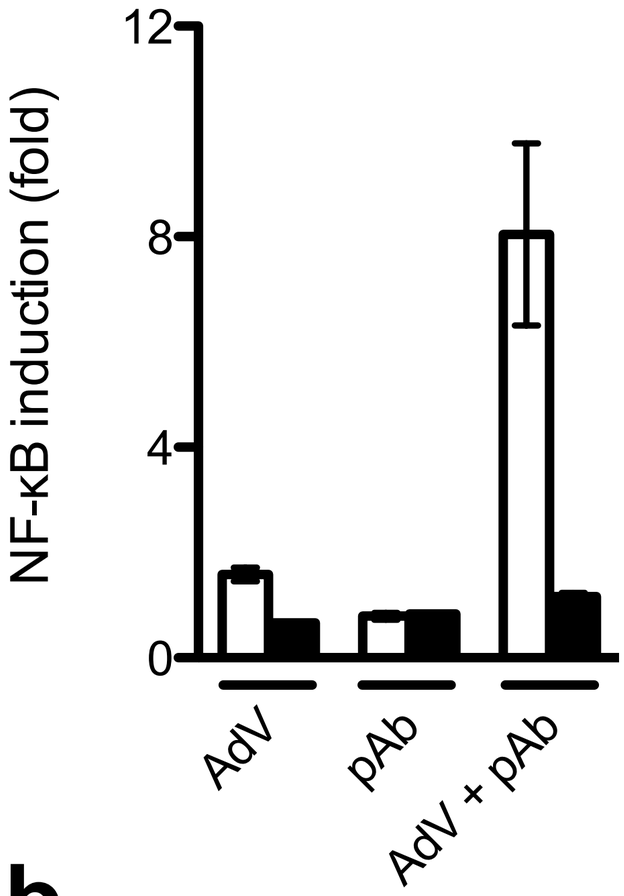
d

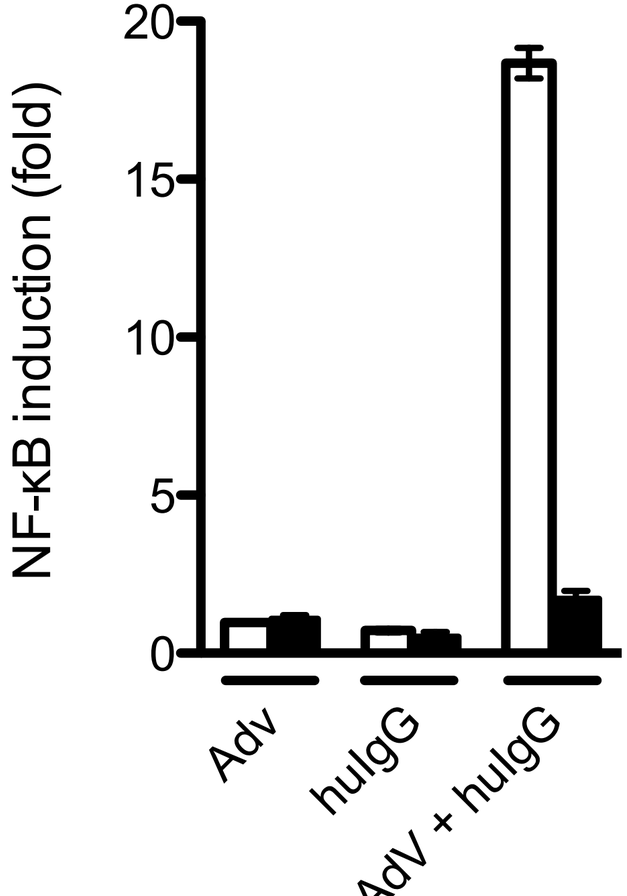

K21 EV
K21 T21
K21 ΔRING
K21 ΔSPRY
60
50
40
30

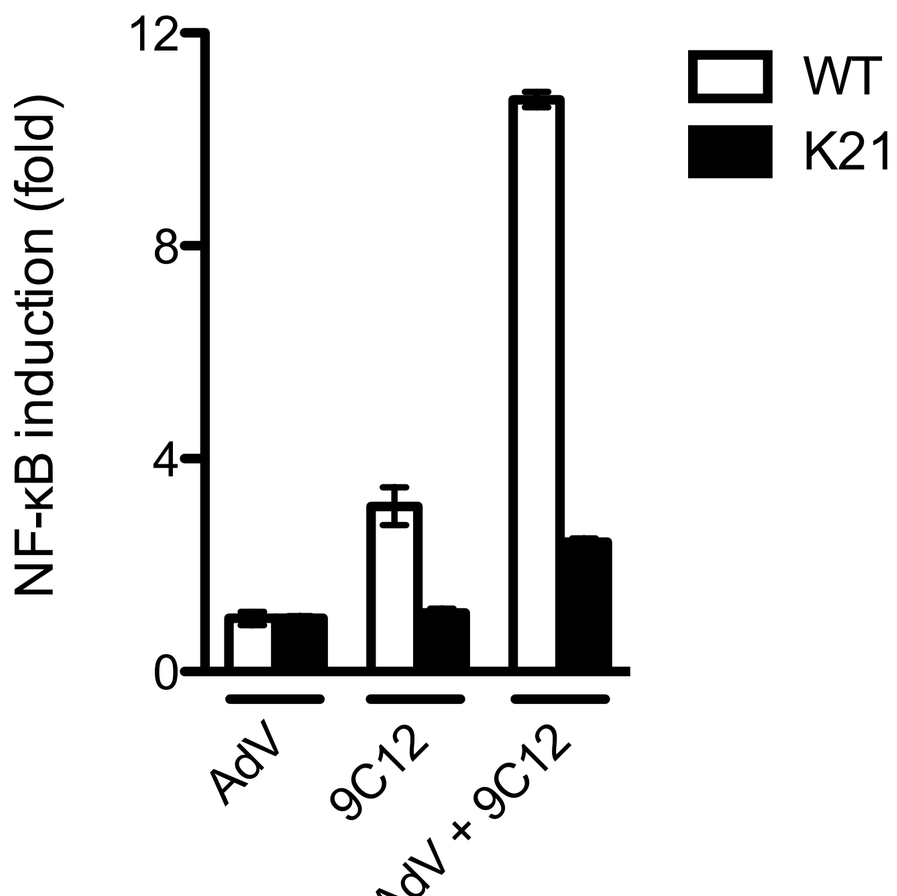
Supplementary Figure 1: TRIM21 activates p52 but is not required for constitutive activation of NF-κB.

(a) DNA binding of p52 in Trim21-deficient MEFs transduced with empty vector (K21 EV) or human TRIM21 (K21 T21) 4 h post-challenge with AdV, Ab or AdV + Ab. DNA binding of NF-κB subunits **(b)** p65 and **(c)** p50 to consensus oligonucleotides 4 h post-challenge with TNF or lipopolysaccharide (LPS). **(d)** Immunoblots for human TRIM21 from K21 cells transduced with empty vector, TRIM21 and RING and PRYSPRY domain deletions.

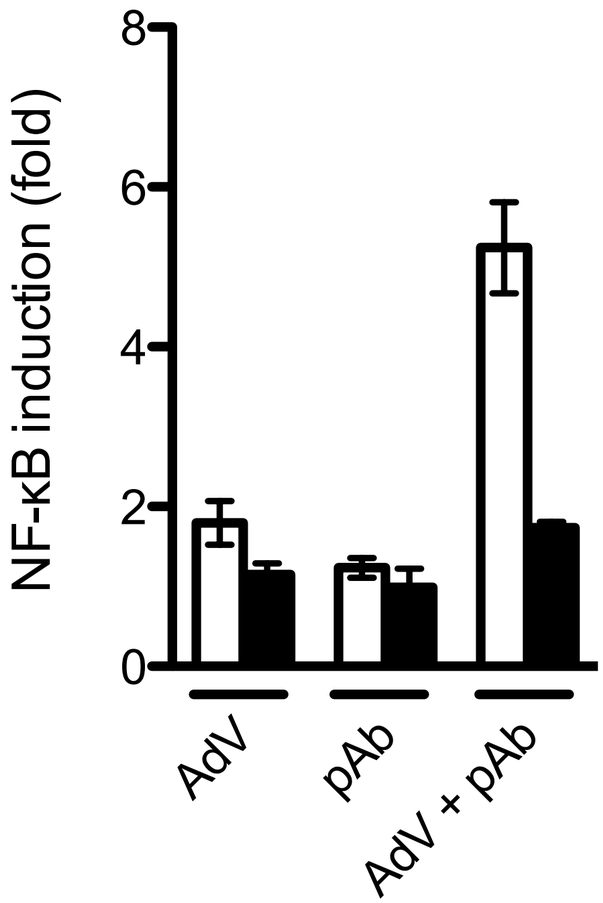
a

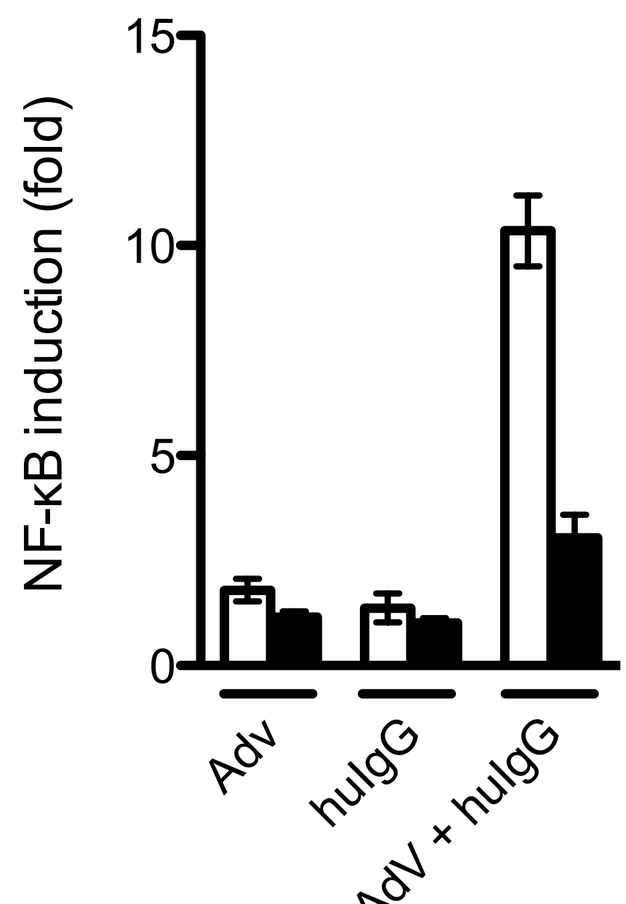

NF-κB induction (fold)
12
8
4
0
AdV
pAb
AdV + pAb


NF-κB induction (fold)
20
15
10
5
0
Adv
huIgG
AdV + huIgG


NF-κB induction (fold)
12
8
4
0
AdV
9C12
AdV + 9C12
WT
K21

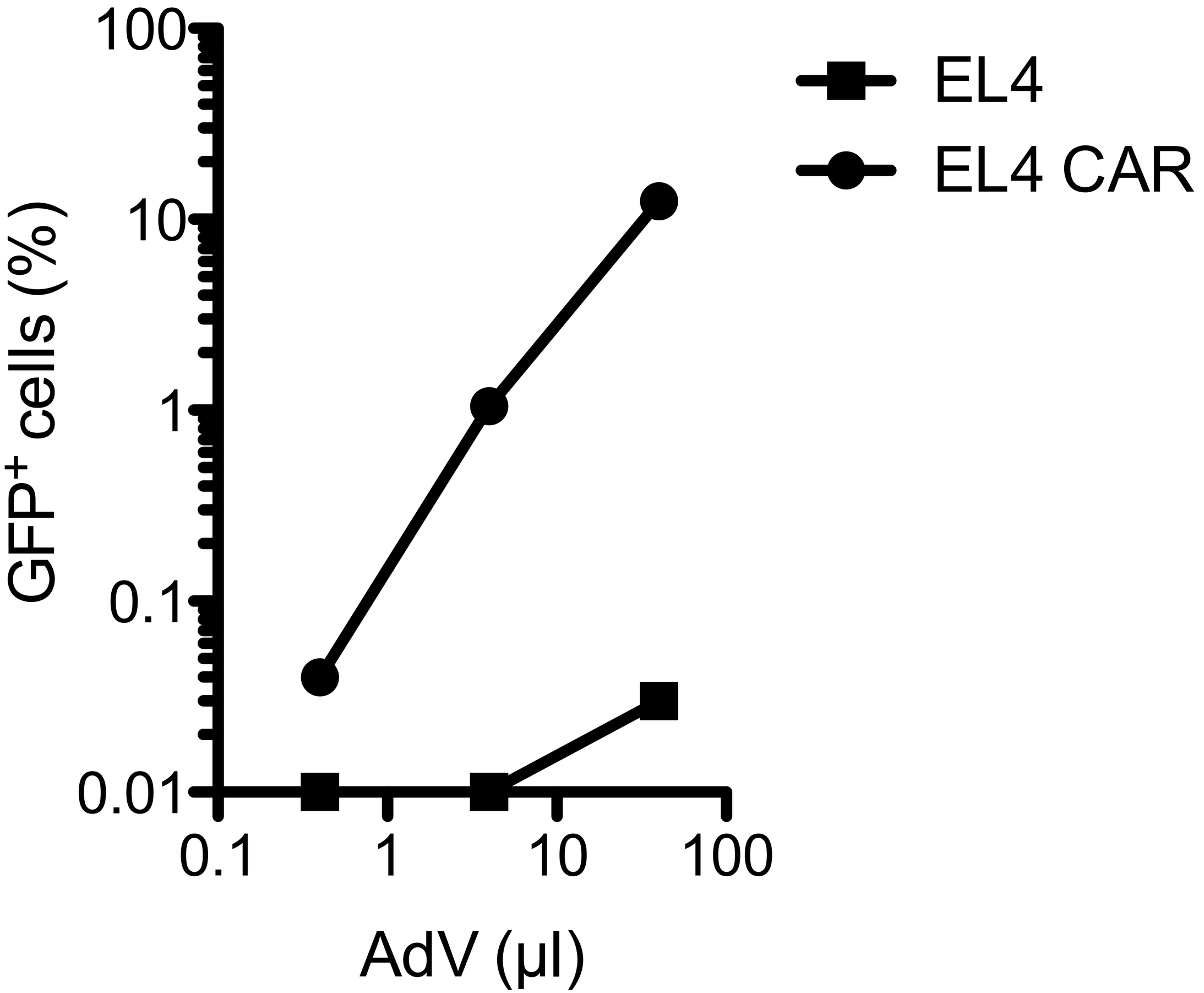
b


NF-κB induction (fold)
8
6
4
2
0
AdV
pAb
AdV + pAb


NF-κB induction (fold)
15
10
5
0
Adv
huIgG
AdV + huIgG
NC si
T21 si

Supplementary Figure 2: TRIM21 activates signalling in response to host and non-host antibody.

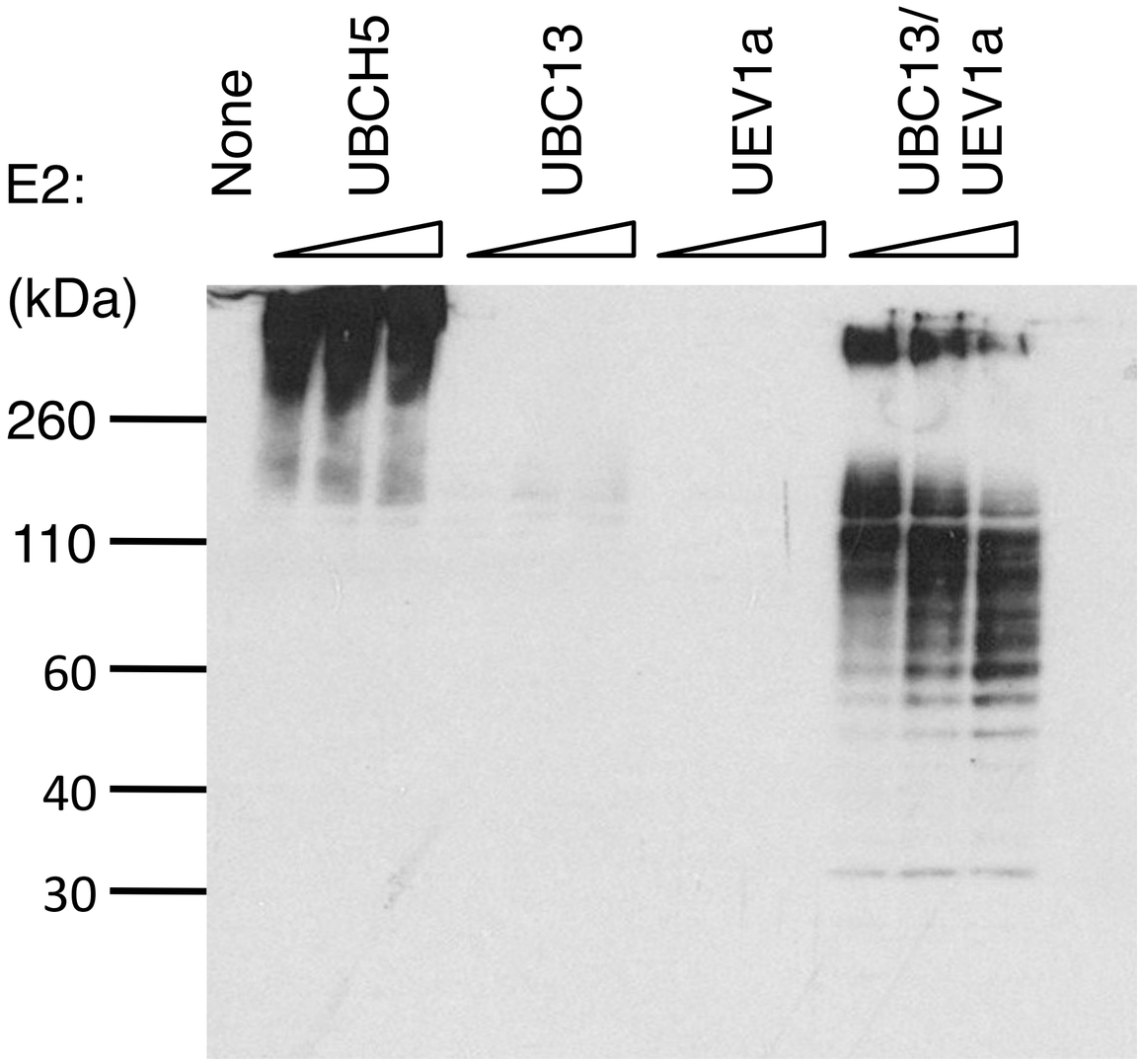
NF-κB luciferase induction in response to challenge of **(a)** wild-type (WT) and Trim21-deficient (K21) MEF and **(b)** HeLa cells treated with control (NC si) or TRIM21-directed (T21 si) siRNA. pAb, goat polyclonal antibody raised against whole adenovirus; huIgG, pooled human serum IgG; 9C12, mouse monoclonal anti-hexon.


100
10
1
0.1
0.01
GFP+ cells (%)
0.1
1
10
100
AdV (µl)
EL4
EL4 CAR

Supplementary Figure 3: Expression of CAR renders EL4 cells permissive to AdV infection.

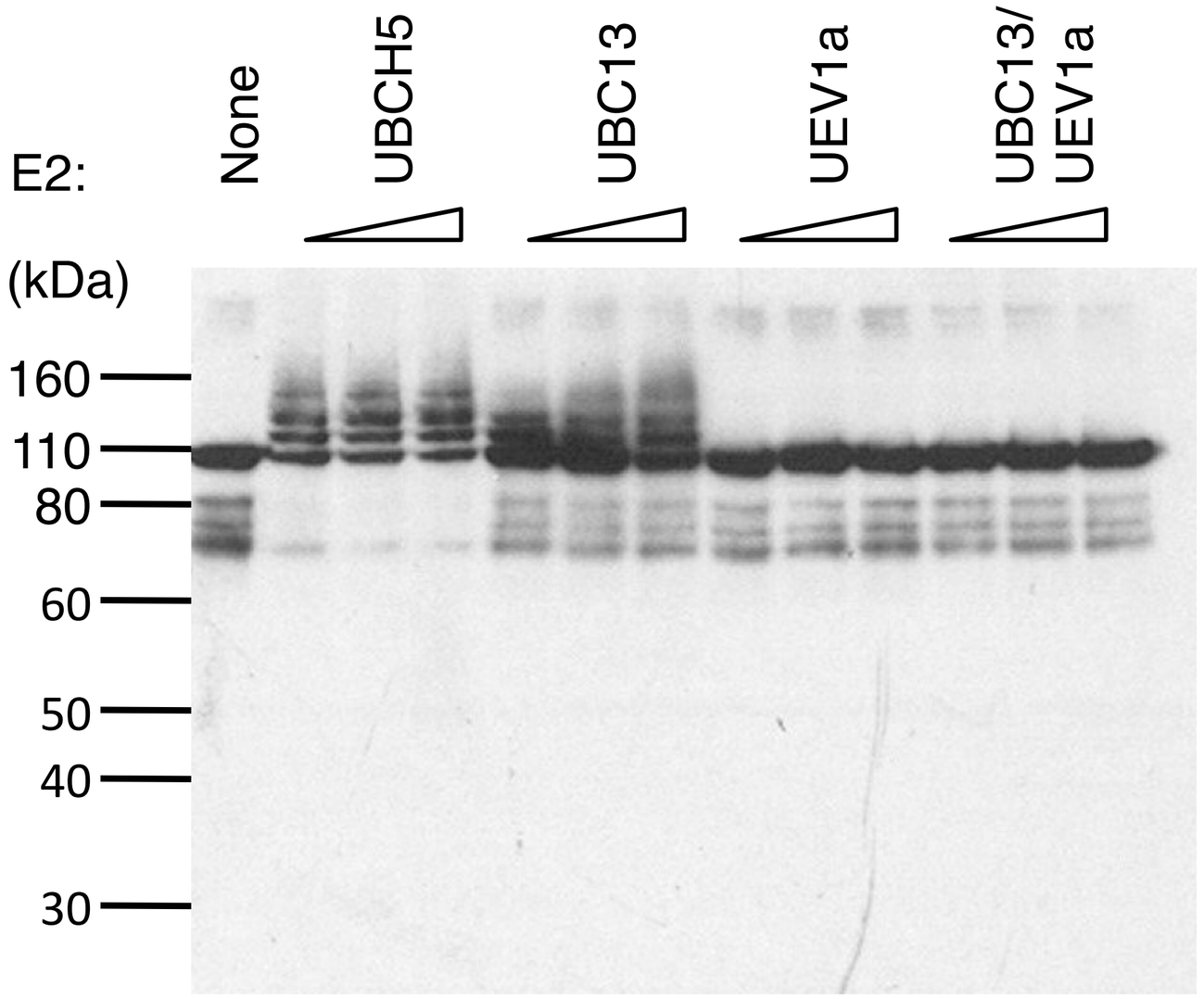
EL4 cells or EL4 cells expressing human coxsackie and adenovirus receptor were challenged with a titration of adenovirus GFP vector. GFP expression of cells was quantified by FACS 24 h post-infection.

a



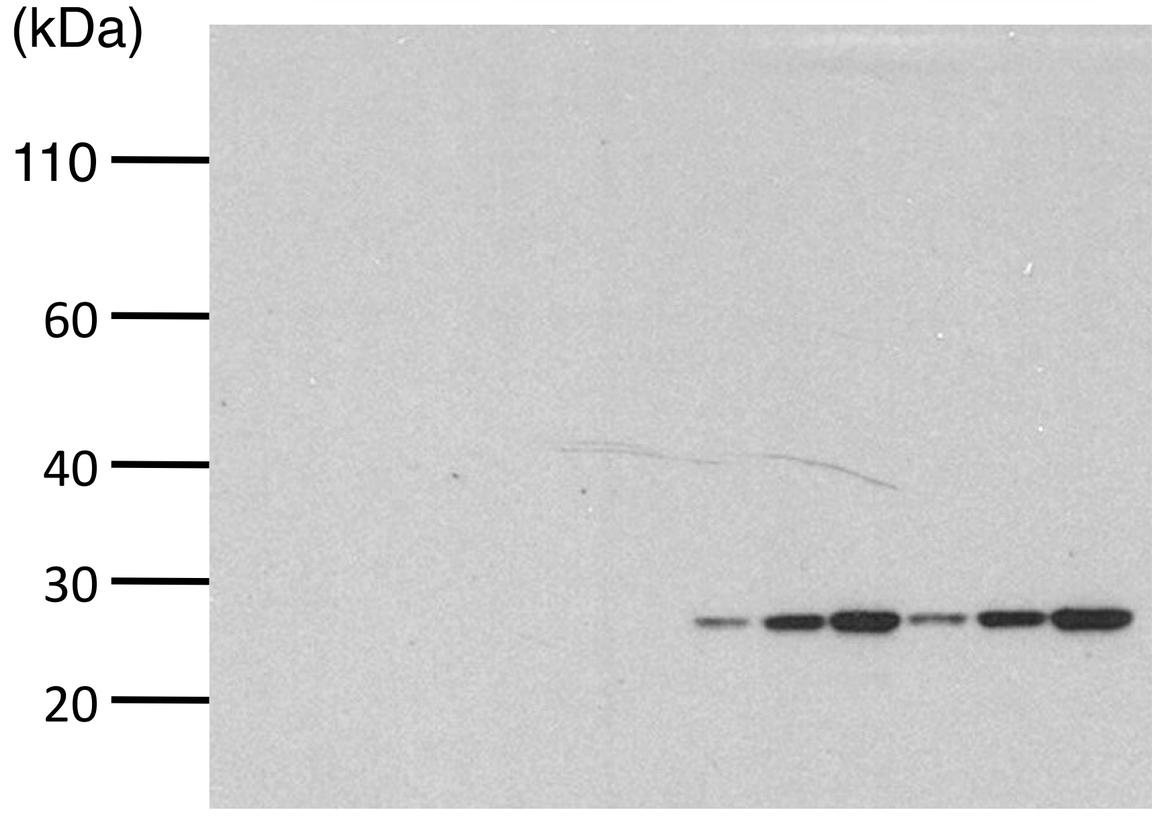
IB: Ub

b



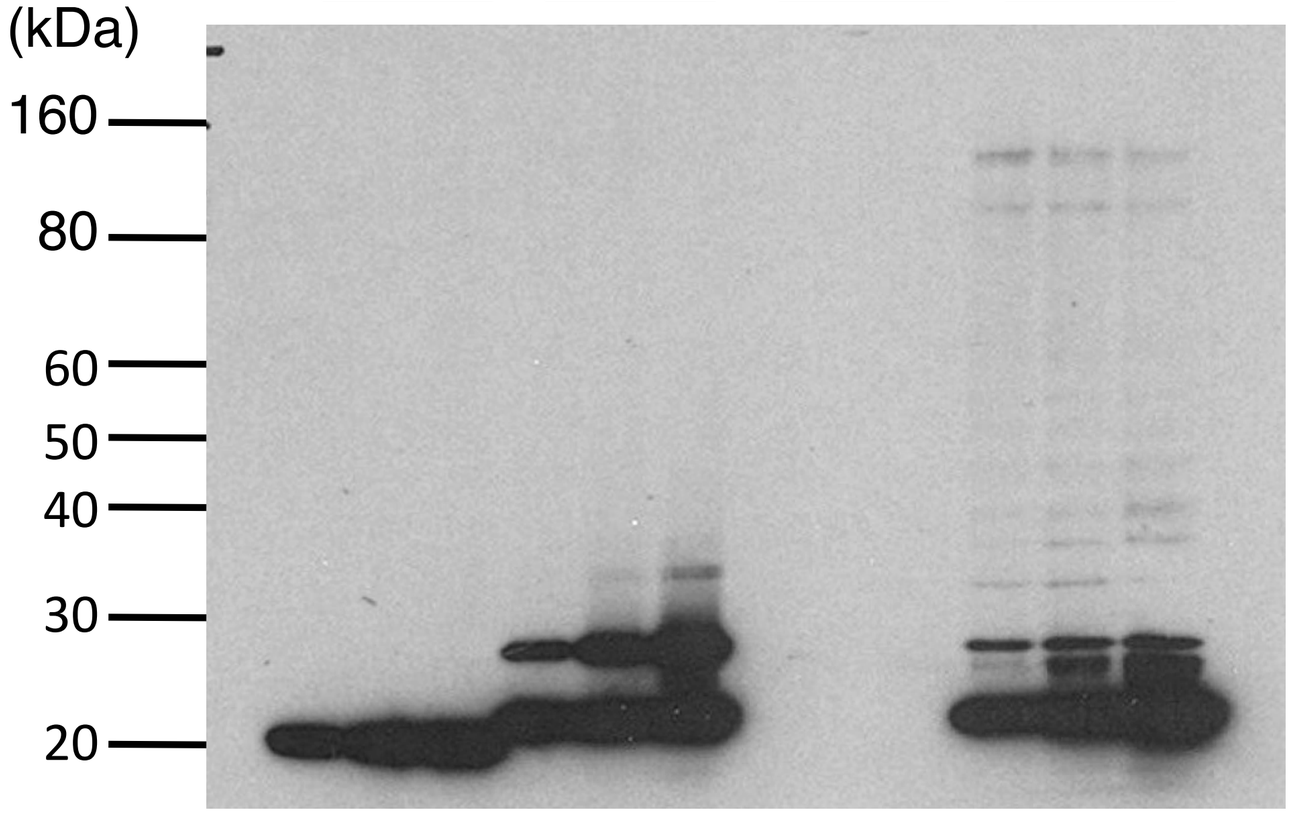
IB: T21

c



IB: UEV1a

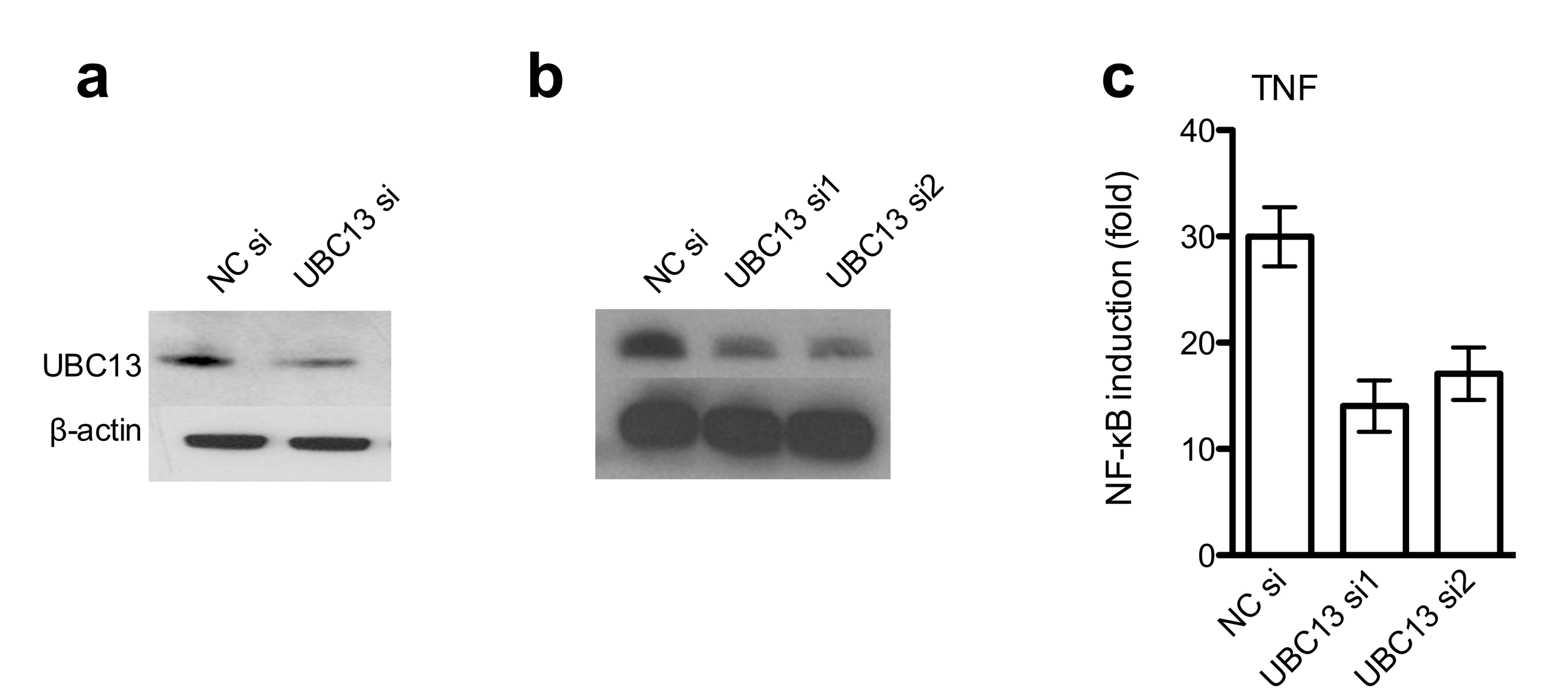
d



IB: UBC13

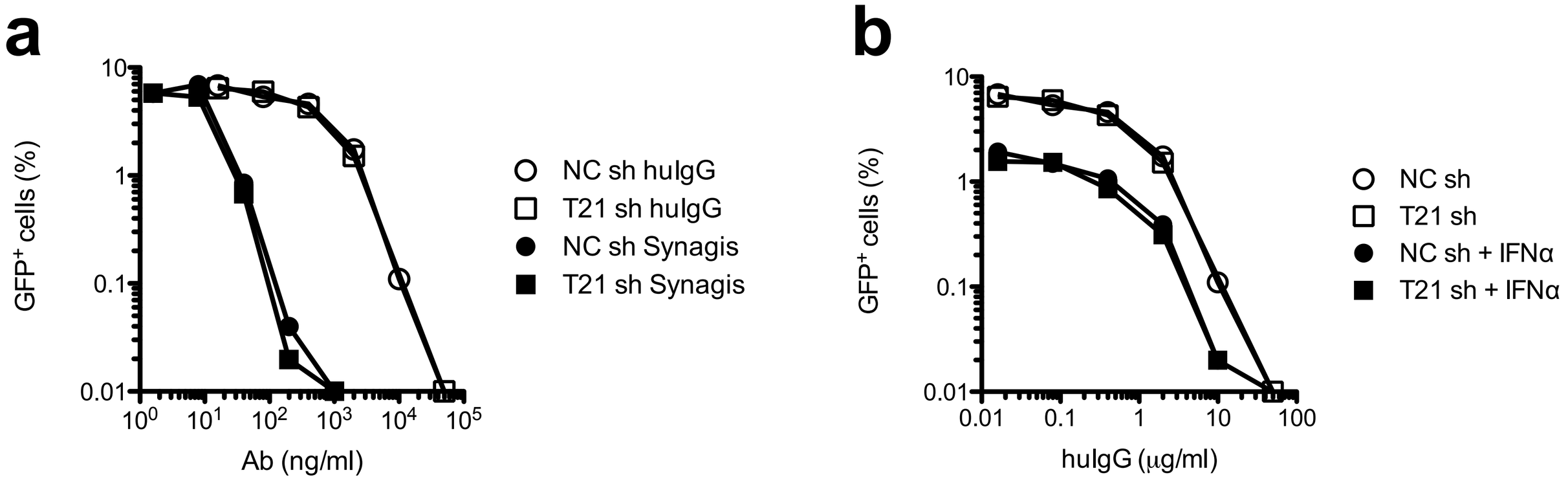
Supplementary Figure 4: E2 enzymes remain unmodified by TRIM21 K63 ubiquitin chain synthesis.

In vitro ubiquiylation reactions with titrations of E2 enzymes UBCH5, UBC13, UEV1A and UBC13/UEV1A with TRIM21. Immunoblots for **(a)** ubiquitin (Ub), **(b)** TRIM21 (T21), **(c)** UEV1a and **(c)** UBC13.


a
NC si
UBC13 si
UBC13
β-actin
b
NC si
UBC13 si1
UBC13 si2
c
TNF
NF-κB induction (fold)
40
30
20
10
0
NC si
UBC13 si1
UBC13 si2

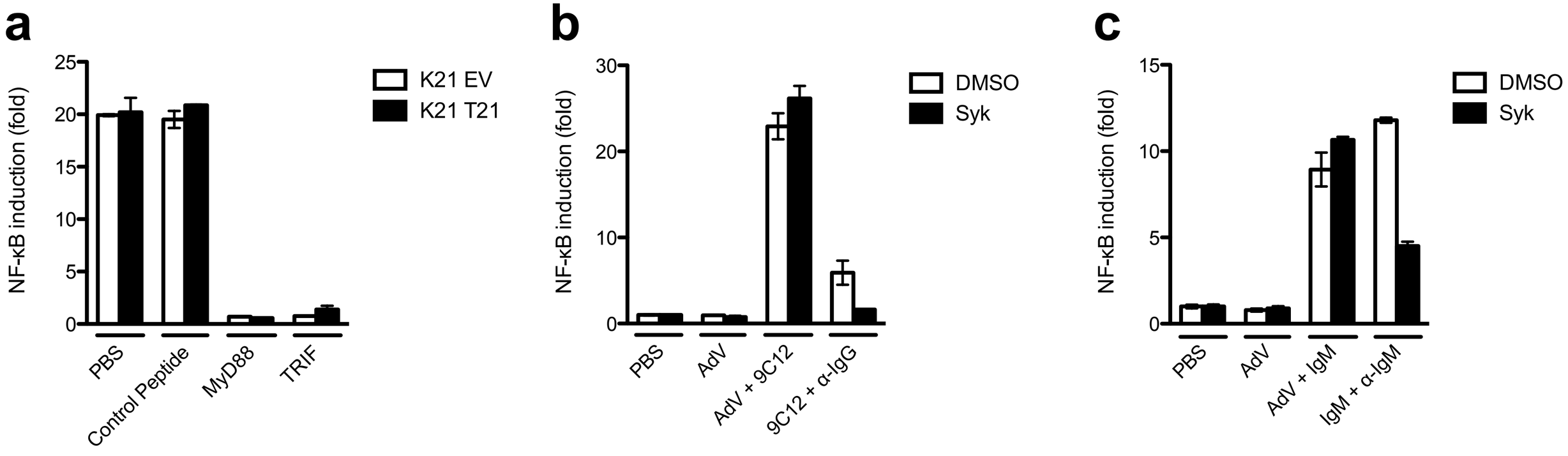
Supplementary Figure 5: Knockdown of UBC13 reduces TNF NF-κB signalling.

Immunoblots of UBC13 and β-actin in **(a)** siRNA (si)-treated MEF cells and **(b)** siRNA-treated HeLa cells. Control siRNA (NC si). **(c)** NF-κB luciferase reporter induction by TNF under conditions of UBC13 siRNA depletion in HeLa cells.


a
GFP+ cells (%)
10
1
0.1
0.01
10^0
10^1
10^2
10^3
10^4
10^5
Ab (ng/ml)
NC sh hulgG
T21 sh hulgG
NC sh Synagis
T21 sh Synagis
b
GFP+ cells (%)
10
1
0.1
0.01
0.01
0.1
1
10
100
hulgG (μg/ml)
NC sh
T21 sh
NC sh + IFNα
T21 sh + IFNα

Supplementary Figure 6: Neutralization of RSV is independent of TRIM21 and interferon.

(a) RSV infection of HeLa cells and TRIM21 siRNA-treated HeLa cells in the presence increasing concentrations of Synagis or pooled human serum IgG (hu). **(b)** RSV infection with a titration of human serum IgG on control or TRIM21 knockdown HeLa cells in the absence or presence of IFN-α.


a
NF-κB induction (fold)
25
20
15
10
5
0
K21 EV
K21 T21
PBS
Control Peptide
MyD88
TRIF
b
NF-κB induction (fold)
30
20
10
0
DMSO
Syk
PBS
AdV
AdV + 9C12
9C12 + α-IgG
c
NF-κB induction (fold)
15
10
5
0
DMSO
Syk
PBS
AdV
AdV + IgM
IgM + α-IgM

Supplementary Figure 7: Inhibition of MyD88, TRIF and Syk prevents TLR and Fc-receptor signaling.

(a) NF-κB luciferase reporter induction in response to LPS treatment in Trim21-deficient MEFs cells transduced with empty vector (K21 EV) or human TRIM21 (K21 T21) after incubation with control peptide or MyD88 and TRIF inhibitory peptides. NF-κB induction in WT MEF cells after challenge with AdV, AdV + Ab and **(b)** cross-linked monoclonal IgG (9C12) or **(c)** cross-linked IgM after treatment with solvent only (DMSO) or Syk inhibitor.