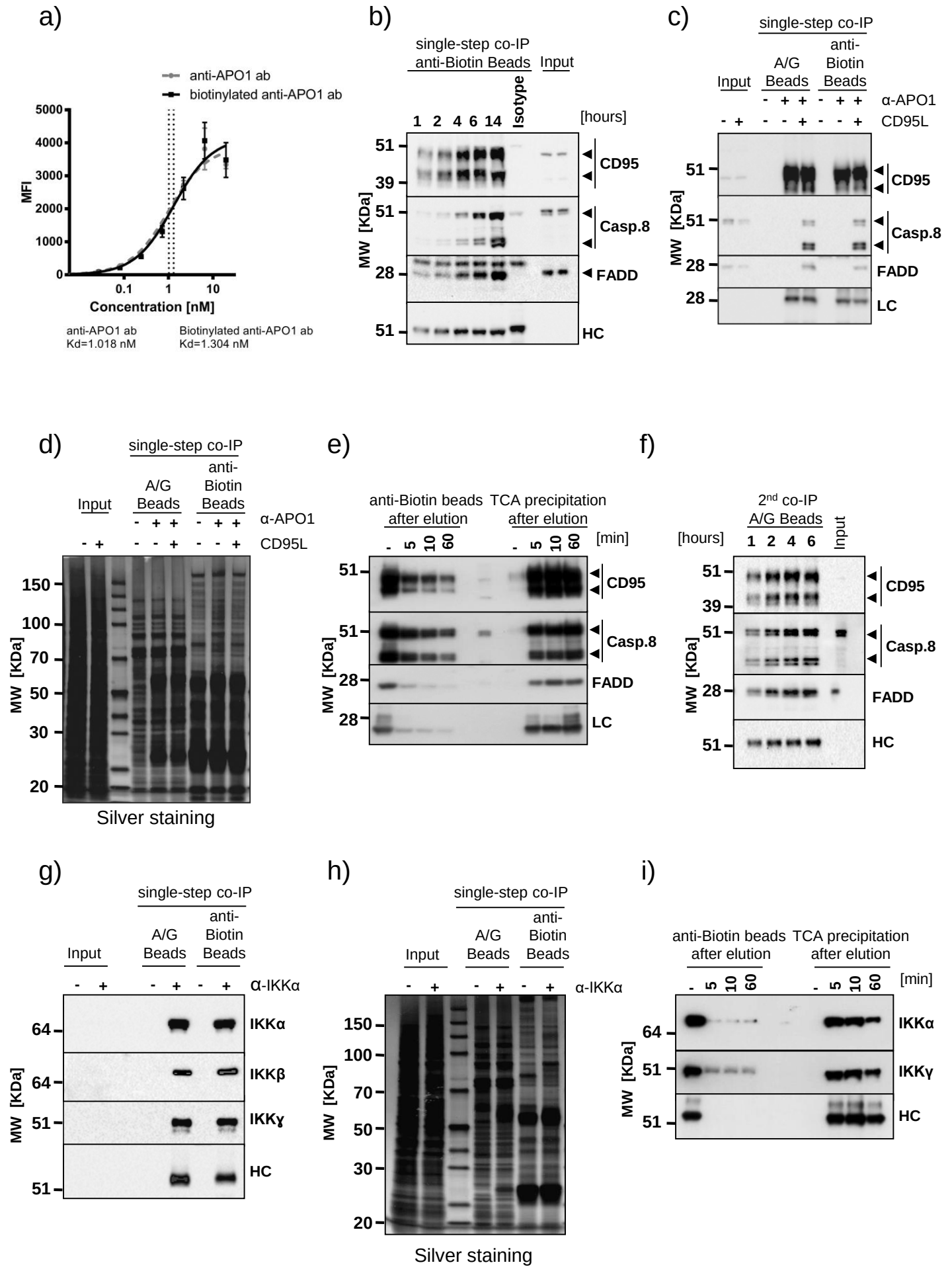
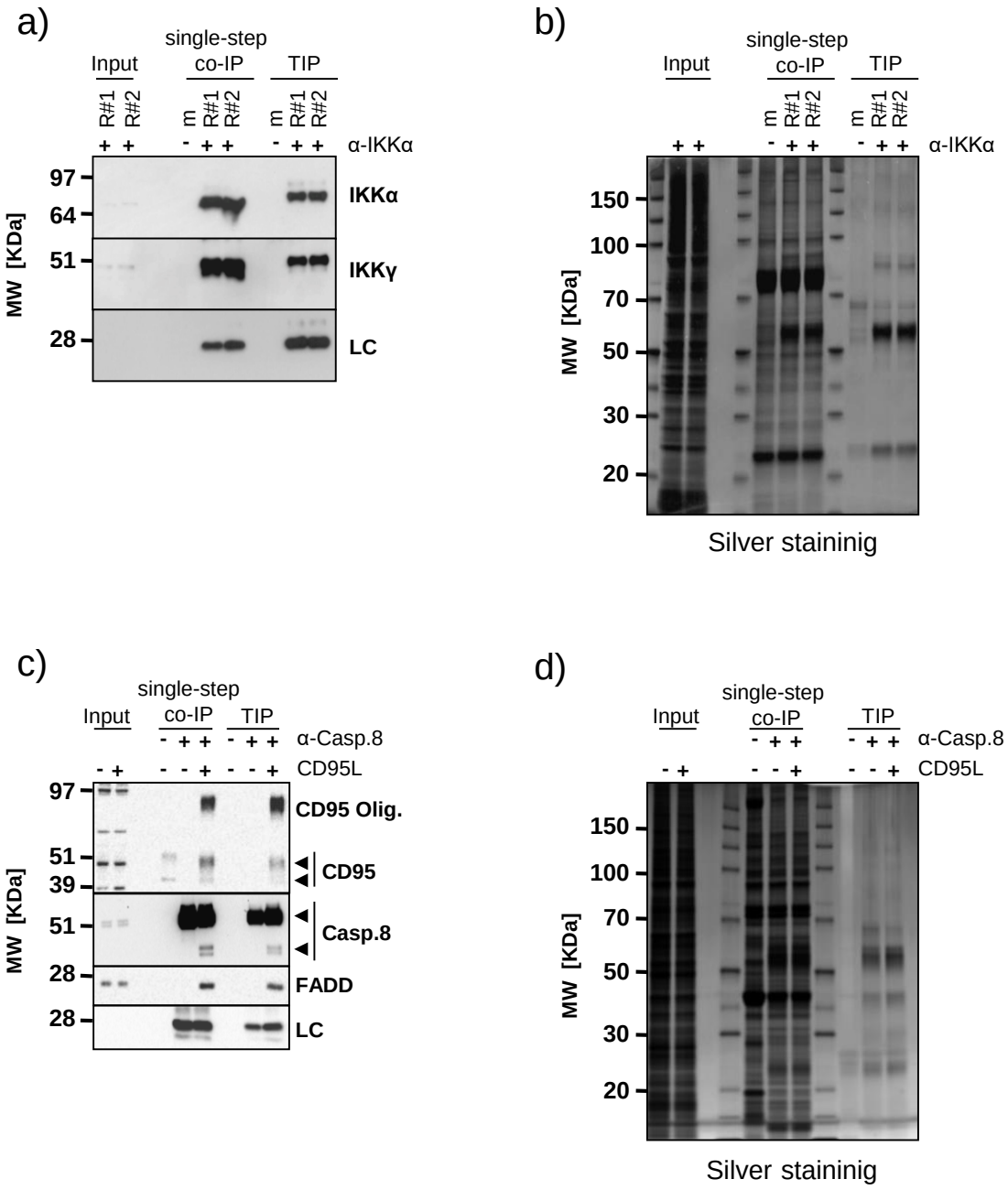
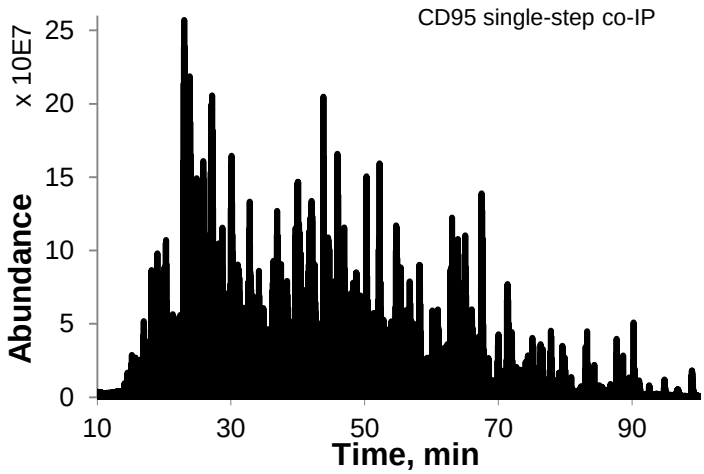
Supplementary Figure 1

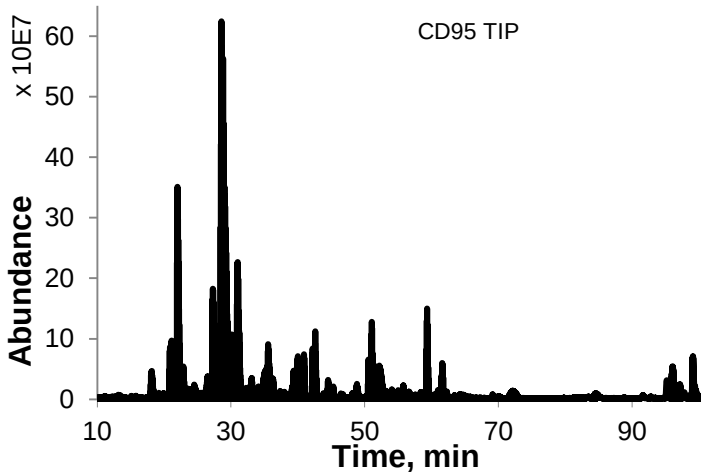



a)
single-step
Input
co-IP
TIP
R#1
R#2
m
α-IKKα
MW [KDa]
97
64
51
28
IKKα
IKKγ
LC
b)
single-step
Input
co-IP
TIP
α-IKKα
MW [KDa]
150
100
70
50
30
20
Silver staininig
c)
single-step
Input
co-IP
TIP
α-Casp.8
CD95L
MW [KDa]
97
51
39
51
28
28
CD95 Olig.
CD95
Casp.8
FADD
LC
d)
single-step
Input
co-IP
TIP
α-Casp.8
CD95L
MW [KDa]
150
100
70
50
30
20
Silver staininig

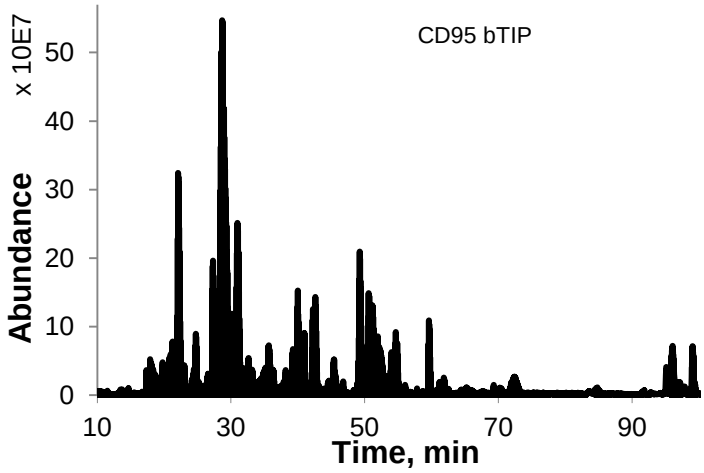
a)


CD95 single-step co-IP
x 10E7
Abundance
25
20
15
10
5
0
10
30
50
70
90
Time, min

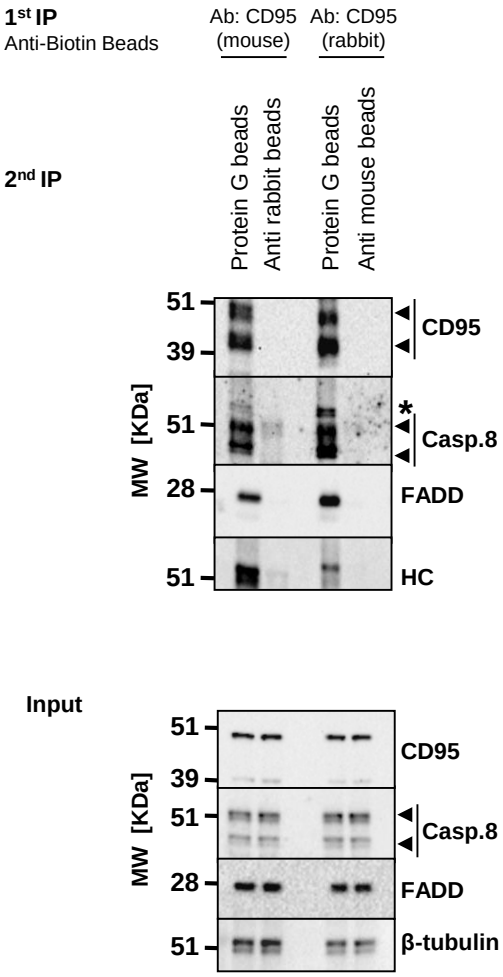
b)


CD95 TIP
x 10E7
Abundance
60
50
40
30
20
10
0
10
30
50
70
90
Time, min

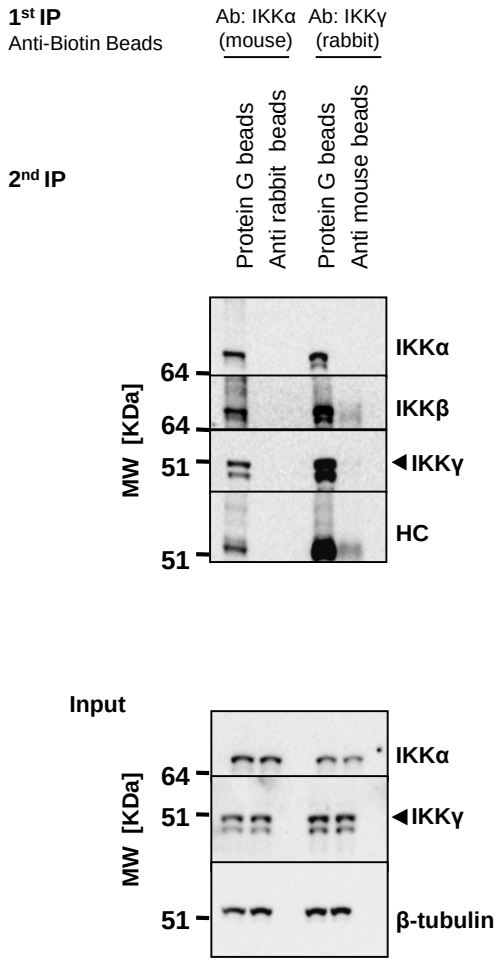
c)


CD95 bTIP
x 10E7
Abundance
50
40
30
20
10
0
10
30
50
70
90
Time, min

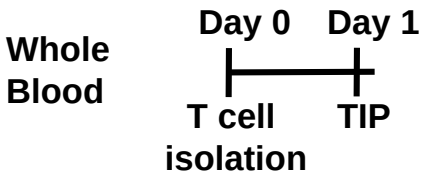
a)


1st IP
Anti-Biotin Beads
Ab: CD95 (mouse)
Ab: CD95 (rabbit)
2nd IP
Protein G beads
Anti rabbit beads
Protein G beads
Anti mouse beads
MW [KDa]
51
39
51
28
51
CD95
*
Casp.8
FADD
HC
Input
51
39
51
28
51
CD95
Casp.8
FADD
β-tubulin

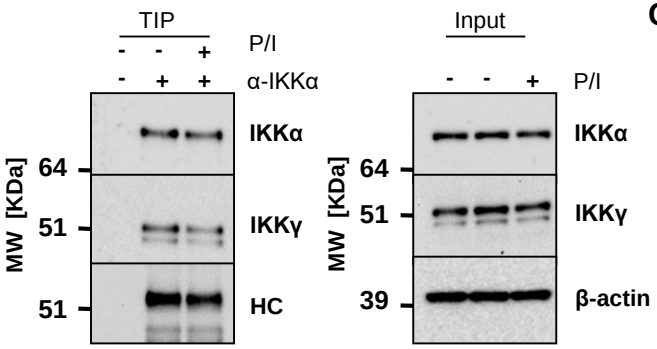
b)


1st IP
Anti-Biotin Beads
Ab: IKKα (mouse)
Ab: IKKγ (rabbit)
2nd IP
Protein G beads
Anti rabbit beads
Protein G beads
Anti mouse beads
MW [KDa]
64
64
51
51
IKKα
IKKβ
IKKγ
HC
Input
64
51
51
IKKα
IKKγ
β-tubulin

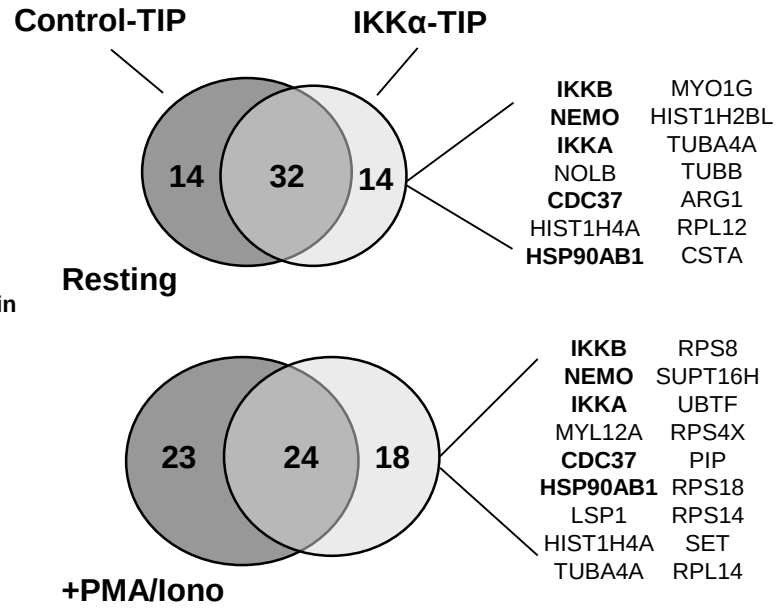
a)


Whole Blood
Day 0
Day 1
T cell isolation
TIP

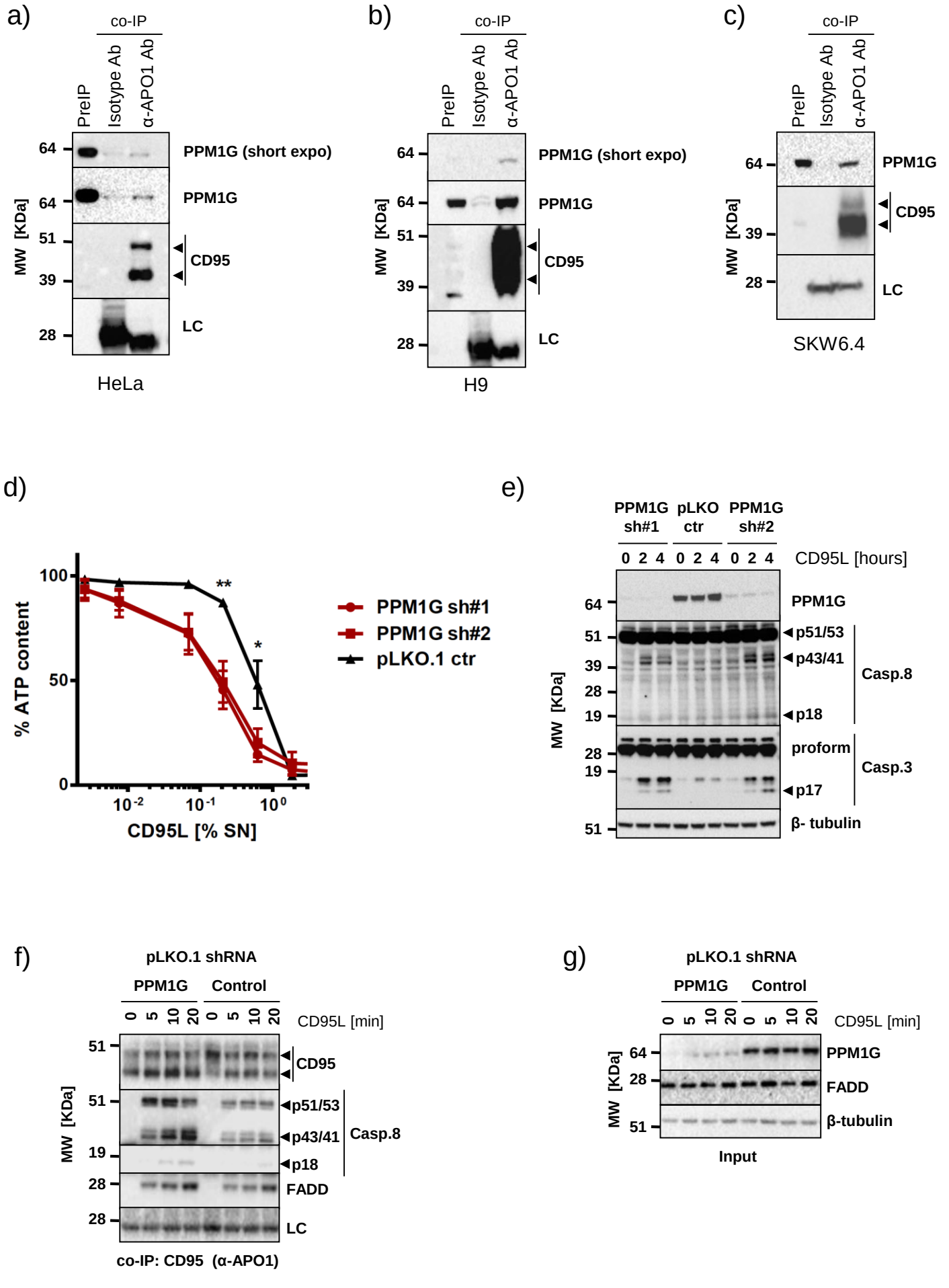
b)


TIP
P/I
α-IKKα
IKKα
IKKγ
HC
MW [KDa]
64
51
51
Input
P/I
IKKα
IKKγ
β-actin
MW [KDa]
64
51
39

c)


Control-TIP
IKKα-TIP
14
32
14
Resting
IKKB MYO1G
NEMO HIST1H2BL
IKKA TUBA4A
NOLB TUBB
CDC37 ARG1
HIST1H4A RPL12
HSP90AB1 CSTA
23
24
18
+PMA/Iono
IKKB RPS8
NEMO SUPT16H
IKKA UBTF
MYL12A RPS4X
CDC37 PIP
HSP90AB1 RPS18
LSP1 RPS14
HIST1H4A SET
TUBA4A RPL14

Supplementary Figure 6



Supplementary Figure legends:

Figure S1: Validation of the first co-immunoprecipitation and elution efficiency

(a) Saturation binding curves and Kd values of biotinylated (black line) and non-biotinylated (grey dashed line) anti-CD95/FAS (α-APO1) antibodies on BJAB cells (anti-APO1 ab Kd: 1.018 nM±0.169; biotinylated anti-APO1 ab Kd: 1.304 nM±0.266).

(b) $5x10^7$ BJAB cells were stimulated with CD95L for 20 min and single-step co-IP was performed with biotinylated anti-CD95/FAS (α-APO1) using anti-biotin beads for the indicated hours (HC: biotinylated IgG heavy chain; arrowheads: specific signals).

(c) $5x10^7$ BJAB cells were left untreated (-) or were stimulated (+) with CD95L for 20 min and single-step co-IP was performed with biotinylated anti-CD95/FAS (α-APO1) ab and protein A/G or anti-biotin beads. Please note that Caspase 8 and FADD are only associated in CD95L-stimulated cells (LC: biotinylated IgG light chain; arrowheads: specific signals).

(d) Silver staining of the samples shown in (c).

(e) The elution of the proteins from the anti-biotin beads was determined by western blot analysis for bead-bound and eluted CD95, Caspase-8 and FADD either without (-) or after the addition of D-biotin for 5, 10, 60 min (LC: biotinylated IgG light chain; arrowheads: specific signals).

(f) After the elution, the second step of TIP (2^{nd} co-IP) was performed with protein A/G beads for the indicated hours and the bead-associated components were evaluated by western blot analysis. (HC: biotinylated IgG heavy chain; arrowheads: specific signals).

(g) The IKK complex of $1x10^7$ BJAB cells was isolated with biotinylated anti-IKKα ab and protein A/G or anti-biotin beads (single-step co-IP). The indicated proteins were detected by western blot (HC: biotinylated IgG heavy chain).

(h) Silver staining of the samples shown in (g).

(i) The elution of the IKK-complex from the anti-biotin beads was determined by western blot analysis for bead-bound and eluted IKKα and IKKγ either without (-) or after the addition of D-biotin for 5, 10, 60 min (HC: biotinylated IgG heavy chain).

Figure S2. TIP-isolation of IKKα- and Caspase 8-interacting proteins

(a) IKK-complex isolation from $1x10^7$ BJAB cells using single-step co-IP and TIP. Mock (m) indicates the beads incubated with the lysate. Shown is a representative western blot analysis for the indicated proteins (R#1 and R#2 represent two independent replicates, LC: biotinylated IgG light chain).

(b) SDS PAGE of the IKK-complex isolation described above after silver staining.

(c) Isolation of caspase-8 associated proteins from $1x10^7$ BJAB cells using a polyclonal goat isotype control or anti-caspase 8 ab from untreated (-) and CD95L stimulated (+) cells analyzed by western blot for the indicated proteins (LC: biotinylated goat IgG light chain; arrowheads: specific signals).

(d) SDS PAGE of the anti-caspase 8 isolations described in (c) after silver staining.

Figure S3. Chromatogram of CD95/FAS-DISC isolated by single-step co-IP, TIP and bTIP

Typical MS1 base peak chromatograms of stimulated CD95/FAS-DISC purified with anti-CD95/FAS (α-APO1) ab. Protein samples were reduced with DTT, cysteine carbamidomethylated and digested with trypsin. Then, the samples were analyzed by nanoLC-Orbitrap-MS applying 2h gradients. If compared with single-step co-IP (a), the sample complexity of TIP (b) and bTIP (c) are clearly reduced. The most abundant peak in TIP and bTIP at RT=29 min is a trypsin peptide fragment VATVSLPR with an m/z of 421.759.

Figure S4. Validation of the resins used for TIP with two independent antibodies

(a) $5x10^7$ BJAB cells were treated with CD95L for 20 min and the TIP procedure was performed with biotinylated mouse anti-CD95/FAS ab (α-APO1) (left) or with rabbit anti-CD95/FAS and biotinylated goat anti-rabbit secondary ab (right). In order to validate the specificity of the second pull down, the eluted complexes were mixed with anti-rabbit or anti-mouse beads, respectively. As control served a pull-down with protein G beads. After the precipitation, the bead-associated proteins were analyzed by western blot (HC: rabbit and mouse IgG heavy chain, respectively). The total protein lysate is shown in the lower part (arrowheads: specific signals; asterisks: non-specific signals).

(b) The IKK-complex from $5x10^7$ BJAB cells was first isolated with mouse anti-IKKα and biotinylated goat anti-mouse secondary ab (left) or rabbit anti-IKKγ and biotinylated goat anti-rabbit secondary ab (right). To test the specificity of these antibodies, the eluted complexes were incubated with anti-rabbit or anti-mouse beads. As positive control the second precipitation step was performed with Protein G

beads. The bound proteins were analyzed by western blot (HC: mouse and rabbit IgG heavy chains). The total protein lysate is shown in the lower part (arrowheads: specific signals).

Figure S5. TIP for IKKα- complex isolation from primary human $CD4^+$ T cells

(a) Scheme for the isolation and stimulation of primary human $CD4^+$ T cells.

(b) IKKα-TIP was performed with $2x10^7$ resting (-) or PMA/ionomycin stimulated (+) primary human $CD4^+$ T cells. Shown is the western blot analysis for the indicated proteins. The mock (m) served as control for resting cells (HC: IgG heavy chain).

(c) Venn diagram of the proteins identified and quantified by MaxQuant analysis comparing the isotype control-TIP and anti-IKKα-TIP in resting (-) and PMA/ionomycin treated (+) cell samples. The list shows the proteins selectively enriched in the anti-IKKa samples (ordered by LFQ values). In bold are the proteins already published to form this complex. For the full analysis see Supplementary table 6.

Figure S6. Validation of PPM1G and CD95/FAS interaction and PPM1G function

(a) Validation of the PPM1G-CD95/FAS interaction in HeLa (a), H9 (b) and SKW6.4 (c) cells. Anti-CD95/FAS co-IP was performed and shown is the western blot analysis for the indicated proteins (arrowheads: specific signals).

(d) Effect of PPM1G knockdown in H9 cells. H9 cells (T cell lymphoma) were silenced with control and two independent PPM1G shRNAs. Viability was evaluated 24 hours after CD95L treatment (**$p<0.01$; *$p<0.05$, ANOVA).

(e) The processing of caspases upon CD95L stimulation of the cells described in (b) was investigated. Shown is a representative western blot analysis for the indicated proteins (arrowheads: specific signals).

(f) Analysis of the CD95/FAS-DISC from H9 cells transduced with control or PPM1G shRNAs for 6 days before the assay. H9 cells were stimulated with CD95L for the indicated time frames and CD95/FAS was precipitated using anti-APO1 ab. Shown is a representative western blot analysis for the indicated proteins (arrowheads: specific signals).

(g) Western blot analysis for the total protein lysates used in (f).