

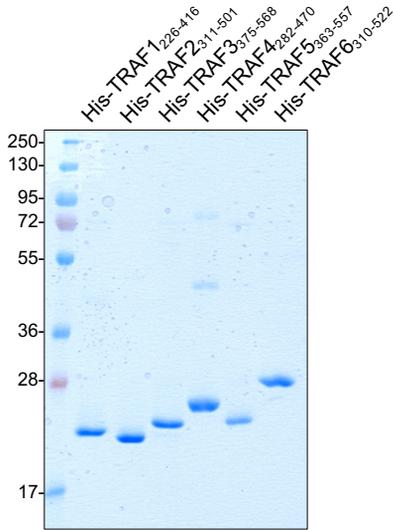
**Epstein-Barr virus-driven B cell lymphoma mediated
by a direct LMP1-TRAF6 complex**

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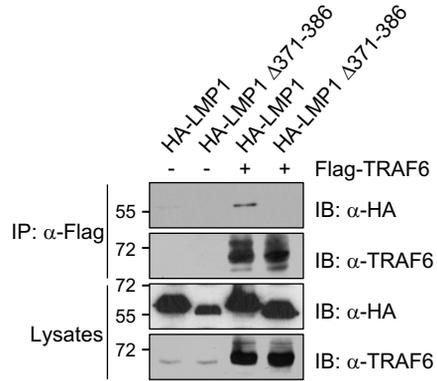
Supplementary Information

Supplementary Figure 1

a



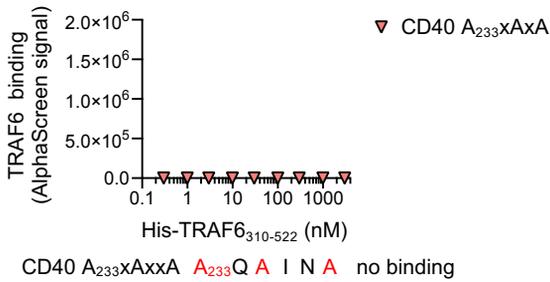
b



c

#	Protein	AA	Sequence	Mutation	T6	T2
1	CD40	230-259	KQEPQEIFPDDLPGSNTAAPVQETLHGCG	-	+	+
2	CD40	230-259	KQEPQEIFPDDLPGSNTAA AVAE LHGCG	A ₂₅₀ XAxA	+	-
3	CD40	230-259	KA EAQA IN FPDDLPGSNTAAPVQETLHGCG	A ₂₃₁ XAxA	-	+
4	CD40	244-273	GSNTAAPVQETLHGCPVTQEDGKESRISV	-	-	+
5	CD40	244-273	GSNTAA AVAE LHGCPVTQEDGKESRISV	A ₂₅₀ XAxA	-	-
6	LMP1	198-227	DDSLPHPQQATDDSGHESDSNSNEGRHLL	-	-	+
7	LMP1	198-227	DDSLPH AA ADDSGHESDSNSNEGRHLL	A ₂₀₄ XAxA	-	-
8	LMP1	371-386	-----GDDDDPHGPVQLSYD	-	+	-
9	LMP1	371-386	-----GDDDDPHGPVQL SA D	A ₃₈₄ A ₃₈₅	-	-
10	LMP1	371-386	-----GDDDDPHG AV ALAYD	A ₃₇₉ XAxA	-	-
11	LMP1	357-386	PHLPTLLLGSSGSGGDDDDPHGPVQLSYD	-	+	-
12	LMP1	357-386	PHLPTLLLGSSGSGGDDDDPHG AV ALAYD	A ₃₇₉ XAxA	-	-

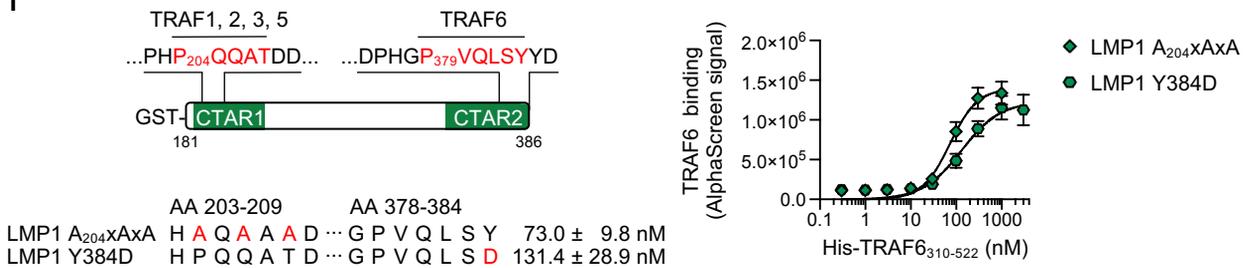
d



e

	-2	-1	0	1	2	3
LMP1 379-384	P	V	Q	L	S	Y
CD40 233-238	P	Q	E	I	N	F
RANK 344-349	P	T	E	D	E	Y
Cellular consensus	P	x	E	x	x	F/Y/D/E
Extended consensus	P	x	E/Q	x	x	F/Y/D/E

f



Supplementary Figure 1

Supplementary Figure 1. Additional information and experiments to Figure 1 (direct interaction of LMP1-CTAR2 with TRAF6).

(a) Recombinant His-TRAF preparations. Proteins are visualized by Coomassie staining after SDS-PAGE. Molecular weights are given in kilodaltons (kDa).

(b) Deletion of the sixteen C-terminal amino acids of CTAR2 abolishes the interaction of TRAF6 with LMP1 *in vivo*. HEK293 cells were transfected with Flag-TRAF6 and HA-LMP1 or the HA-LMP1 Δ 371-386 mutant. Twenty-four hours post transfection, Flag-TRAF6 was immunoprecipitated via its Flag-tag, and coprecipitating HA-LMP1 was analyzed on immunoblots via an α -HA-tag antibody. One representative result of two independent experiments is shown. Molecular weights are given in kilodaltons (kDa).

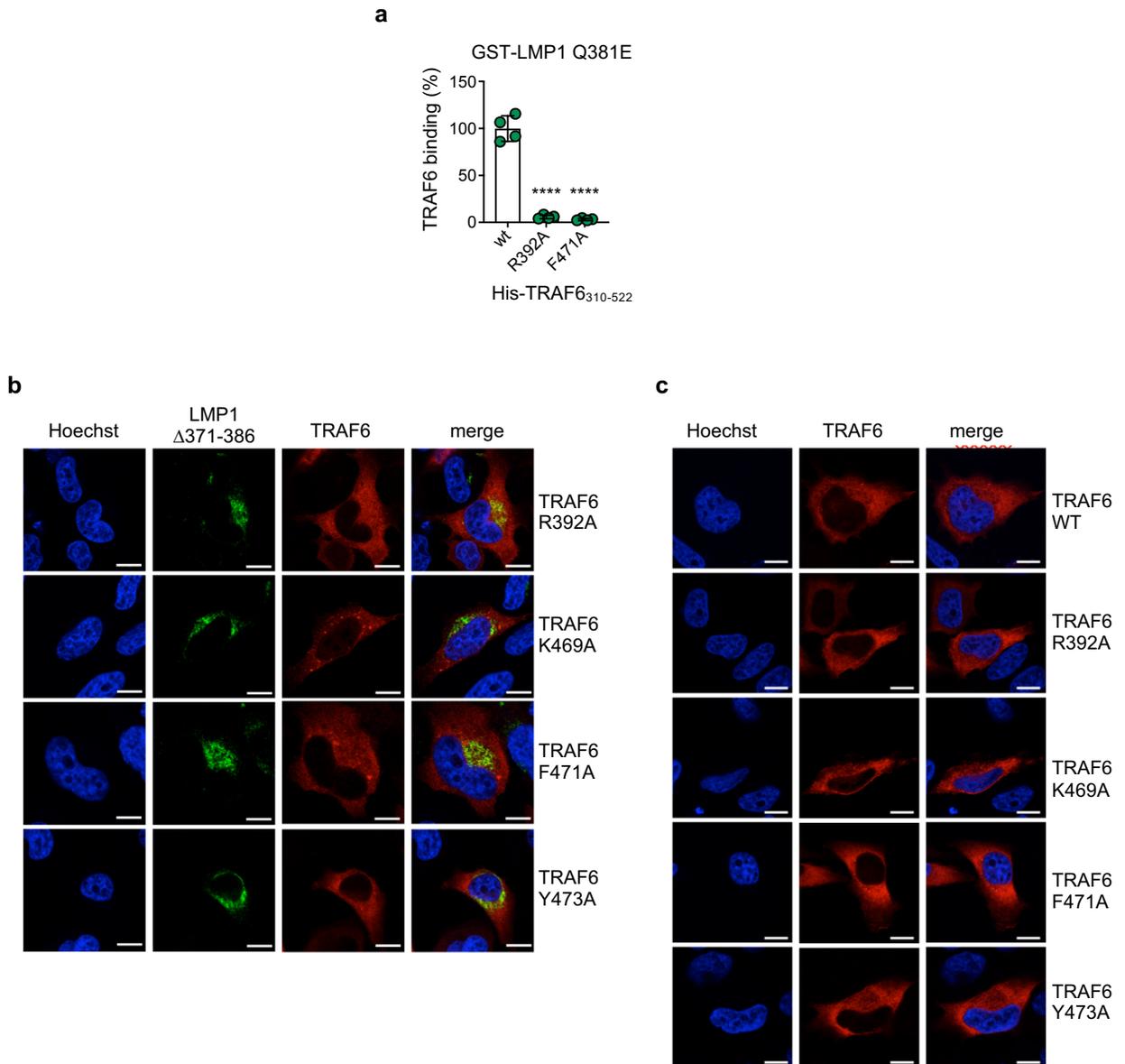
(c) Peptide sequences of the arrays shown in Figure 1c. Alanine exchanges are indicated in red. Interaction with TRAF6 or TRAF2 is indicated according to the results shown in Figure 1c.

(d) Absent His-TRAF6₃₁₀₋₅₂₂ interaction with GST-CD40 A₂₃₃xAxxA in AlphaScreen PPI assays. Data are mean values \pm SD of three independent experiments. Source data in the Source Data file.

(e) Sequence alignment of the TRAF6 binding sequences of LMP1, CD40, and RANK with the earlier described cellular TRAF6 consensus PxExxF/Y/D/E⁴⁶, compared to the extended TRAF6 binding consensus PxE/QxxF/Y/D/E, which emerged from our present study.

(f) His-TRAF6₃₁₀₋₅₂₂ interaction with the GST-LMP1 A₂₀₄xAxxA and Y384D mutants. AlphaScreen PPI experiments. K_D values are given. Data are mean values \pm SD of four independent experiments. Curve fitting: Prism, one site-specific binding with hill slope. Source data in the Source Data file.

Supplementary Figure 2



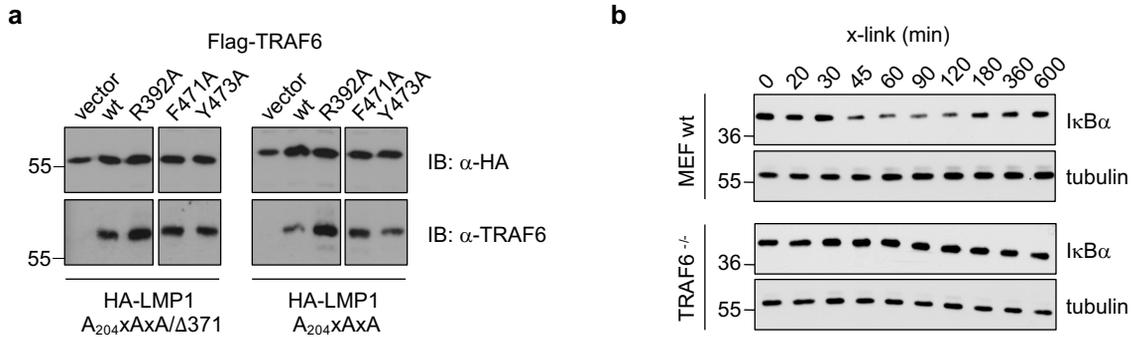
Supplementary Figure 2. LMP1 Q381E interaction with TRAF6 and additional controls to Figure 2e.

(a) The TRAF6₃₁₀₋₅₂₂ mutants R₃₉₂A and F₄₇₁A fail to interact with LMP1 Q381E. AlphaScreen PPI experiments. Data are mean values \pm SD of four independent experiments. Statistics: one-way ANOVA. p-values: ****p \leq 0.0001. Source data in the Source Data file.

(b) TRAF6 interacts with the sixteen C-terminal amino acids of CTAR2, as demonstrated by confocal microscopy. Neither TRAF6 wild-type nor any of the TRAF6 mutants colocalized with the LMP1 deletion mutant Δ 371-386 in HeLa cells. Scale bar: 10 μ m.

(c) Distribution of TRAF6 and the indicated TRAF6 mutants in HeLa cells in the absence of LMP1. Scale bar: 10 μ m.

Supplementary Figure 3

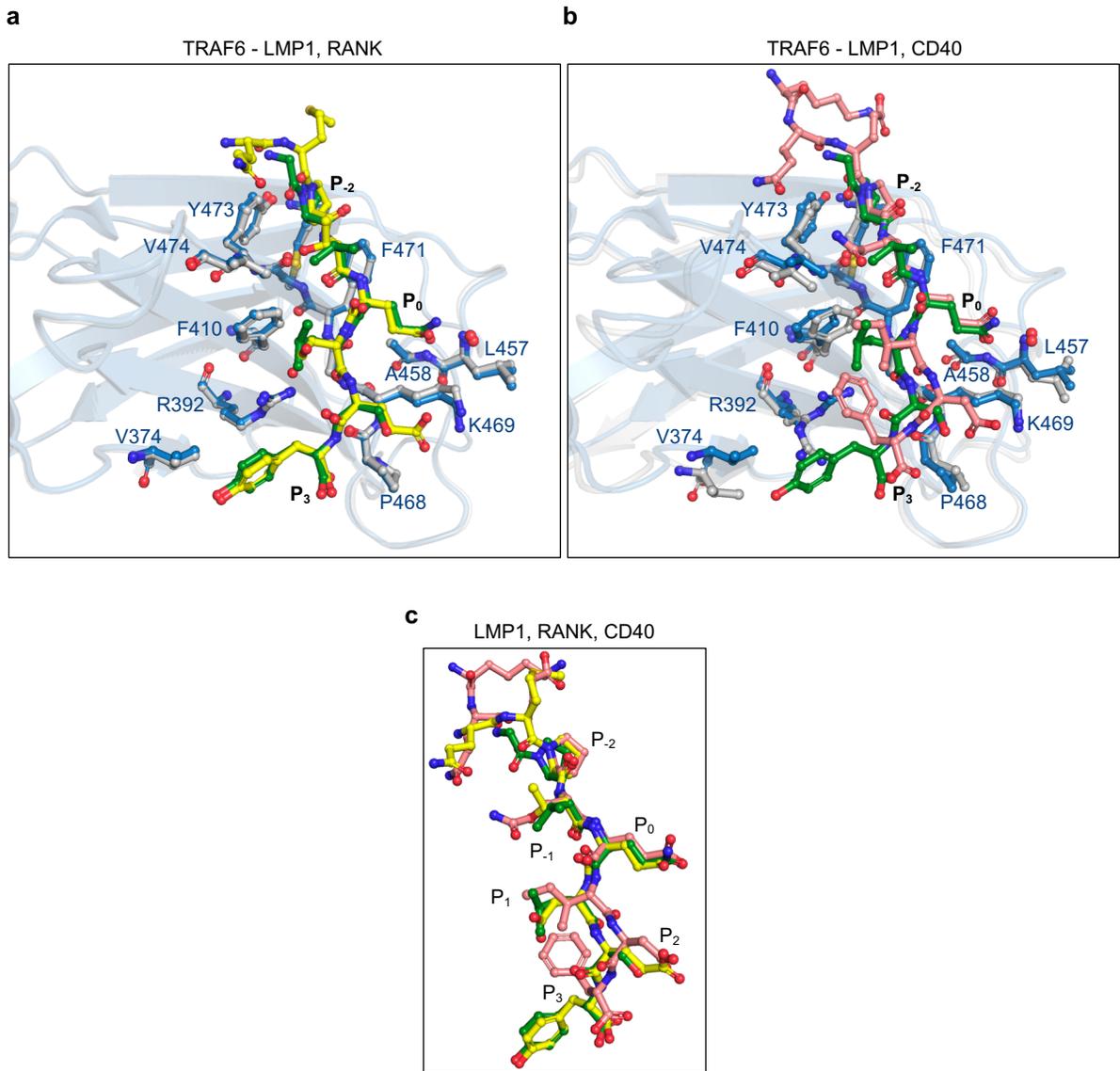


Supplementary Figure 3. Additional controls for the TRAF6 rescue experiments shown in Figure 3a.

(a) Expression levels of recombinant HA-LMP1 and Flag-TRAF6 proteins for the NF- κ B rescue experiments shown in Figure 3a. Representative results.

(b) Absent canonical NF- κ B activation by NGFR-LMP1 in TRAF6^{-/-} MEFs, which had been transduced with NGFR-LMP1. NGFR-LMP1 activity was induced by antibody cross-linking for different times and I κ B α levels were analyzed by immunoblotting. Tubulin served as the loading control. Representative blots are shown.

Supplementary Figure 4



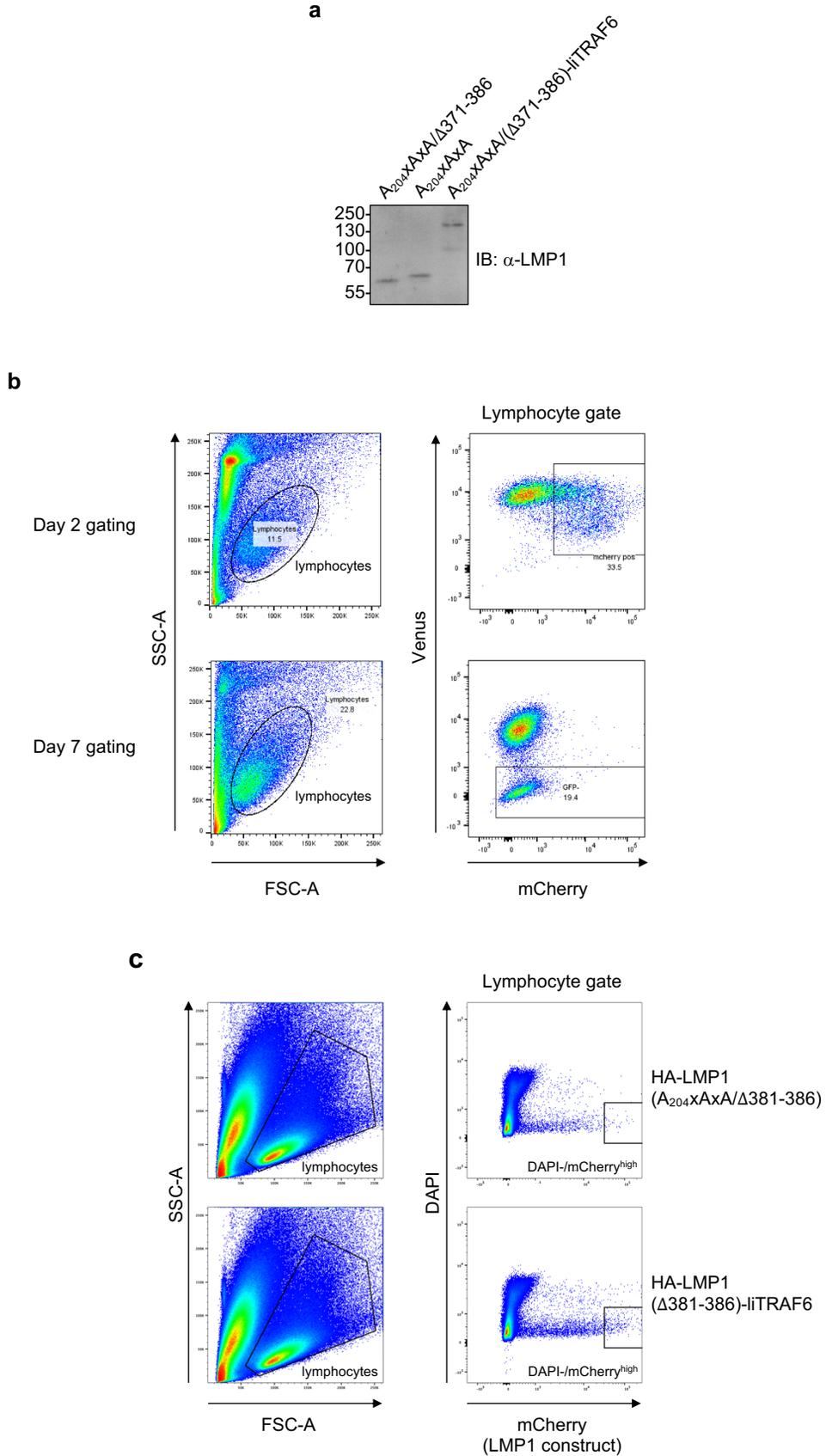
Supplementary Figure 4. Structure of an LMP1 Peptide compared to RANK and CD40 peptides on the surface of TRAF6.

(a) Overlay of the *in silico* model of LMP1 (green) and the crystal structure of RANK (PDB 1LB5, yellow) in complex with TRAF6 (blue with LMP1, gray with RANK).

(b) Overlay of the *in silico* model of LMP1 (green) and the crystal structure of CD40 (PDB 1LB6, salmon) in complex with TRAF6 (blue with LMP1, gray with CD40).

(c) Overlay of the LMP1 (green), RANK (yellow), and CD40 (salmon) peptides in complex with TRAF6.

Supplementary Figure 5



Supplementary Figure 5

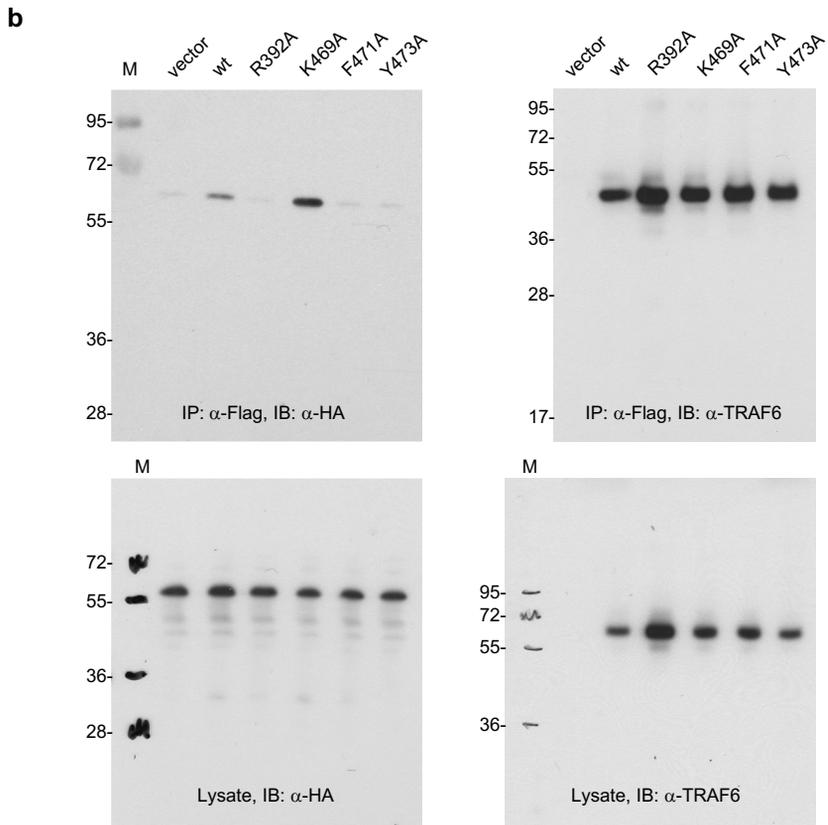
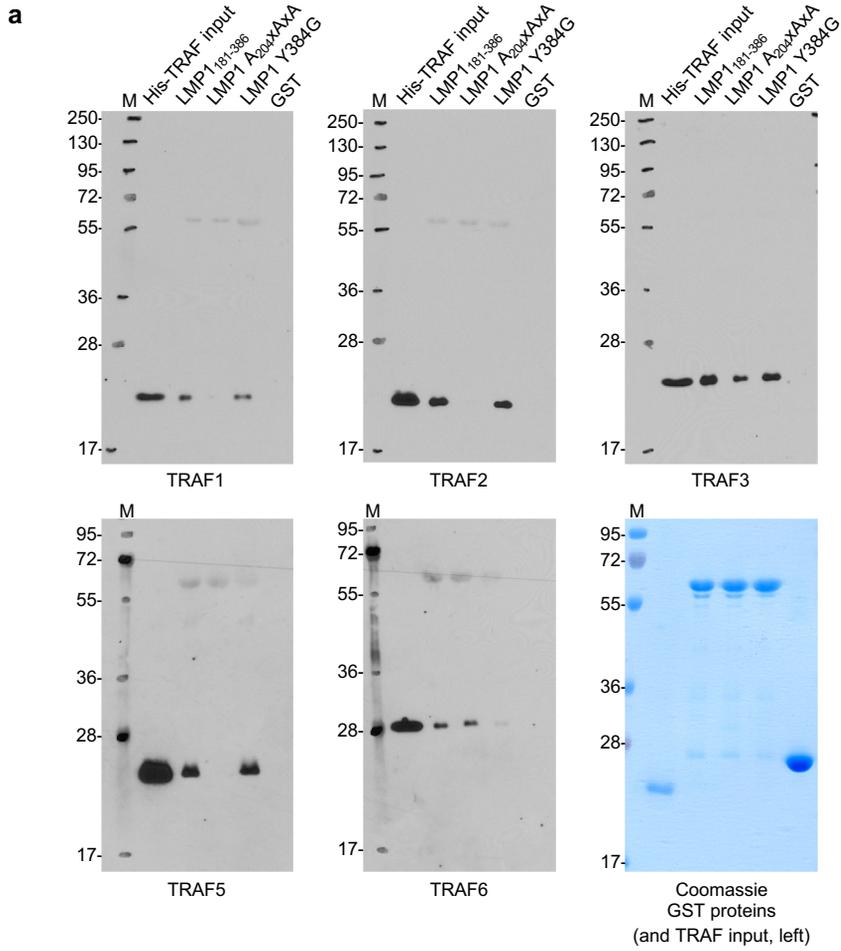
Supplementary Figure 5. Protein expression of the LMP1-TRAF6 fusion protein and gating strategies.

(a) Expression levels of recombinant HA-LMP1 A₂₀₄XAxA/ Δ 371-386-liTRAF6 in MEF TRAF6^{-/-}. Representative results. Molecular weights are given in kilodaltons (kDa).

(b) Gating strategy for the flow cytometric analysis shown in Figures 6b and 6c.

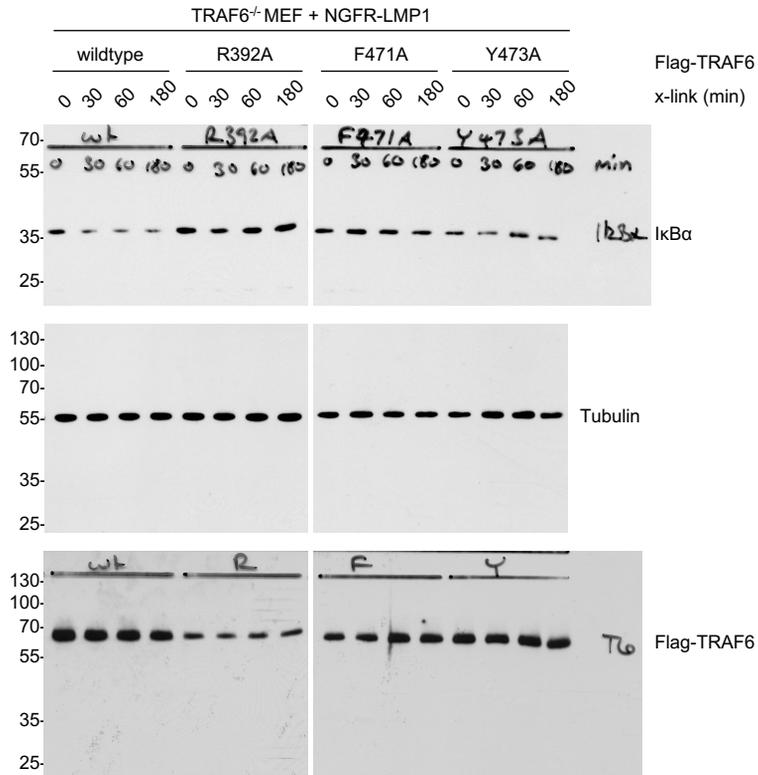
(c) Gating strategy for the flow cytometric analysis shown in Figure 7c.

Supplementary Figure 6



Supplementary Figure 6

C



Supplementary Figure 6. Uncropped images of immunoblots

(a) Uncropped images of immunoblots shown in Figure 1b.

(b) Uncropped images of immunoblots shown in Figure 2d.

(c) Uncropped images of immunoblots shown in Figure 3c.

Molecular weights are given in kilodaltons (kDa). M, molecular weight marker.