

Supplementary Materials for
**The *Chlamydia trachomatis* Inc Tri1 interacts with TRAF7 to displace native TRAF7
interacting partners**

This PDF file includes:

Supplementary Table 1 and supplementary Fig. 1 to Fig. 5.

Table S1. Primers used in this study.

Primer	Primer Sequence (5'→ 3')	Primer Use
CH_P006	GGG GCG GCC GCT CGA GTC ATG AGC TCA GGC AAG AGT GCC CGC TA	Generate FLAG-TRAF7 (pcDNA 4/TO 3X FLAG vector)
CH_P002	CAGCGGGTTTAAACGGGCCCTTAGCAAGTCCAAACCTTCACAGTG	Generate FLAG-TRAF7 (pcDNA 4/TO 3X FLAG vector)
CH_P006	GGG GCG GCC GCT CGA GTC ATG AGC TCA GGC AAG AGT GCC CGC TA	Generate FLAG-delta WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P013	CAGCGGGTTTAAACGGGCCGTAGGAGCCTAGGATGCCC	Generate FLAG-delta WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P014	GGGGCGGCCGCTCGAGTCGACCCCTCAGCAGATCTTCAAGTG	Generate FLAG-WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P002	CAGCGGGTTTAAACGGGCCCTTAGCAAGTCCAAACCTTCACAGTG	Generate FLAG-WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P015	GGGGCGGCCGCTCGAGTCGAGATGCACGTGGCTCTGG	Generate FLAG-cc + WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P002	CAGCGGGTTTAAACGGGCCCTTAGCAAGTCCAAACCTTCACAGTG	Generate FLAG-cc + WD40 (pcDNA 4/TO 3X FLAG vector)
CH_P003	TCTCGAGCTCAAGCTTCGATGAGCTCAGGCAAGAGTG	Generate mCh-TRAF7 (pmCherry-C1 vector)
CH_P004	GATCCCGGGCCCGGTACCTTAGCAAGTCCAAACCTTCACAGTG	Generate mCh-TRAF7 (pmCherry-C1 vector)
CH_P003	TCTCGAGCTCAAGCTTCGATGAGCTCAGGCAAGAGTG	Generate mChTRAF7-delta WD40 (pmCherry-C1 vector)
CH_P016	GATCCCGGGCCCGCGGTACCGTAGGAGCCTAGGATGCCC	Generate mChTRAF7-delta WD40 (pmCherry-C1 vector)
CH_P017	TCTCGAGCTCAAGCTTCGACCCCTCAGCAGATCTTCAAGTG	Generate mCh-TRAF7 WD40 (pmCherry-C1 vector)
CH_P004	GATCCCGGGCCCGCGGTACCTTAGCAAGTCCAAACCTTCACAGTG	Generate mCh-TRAF7 WD40 (pmCherry-C1 vector)
CH_P007	CAGTGTGGTGGAATTCATGAGTTTGTGGAGATAGTGATC	Generate Tri1-Strep (pcDNA 4/TO 2X Strep)
CH_P010	CTCCCTCGAGCGGCCCAATCATTGGGAAAAATTGAGTGATAGA	Generate Tri1-Strep (pcDNA 4/TO 2X Strep)
CH_P007	CAGTGTGGTGGAATTCATGAGTTTGTGGAGATAGTGATC	Generate Tri1 1-117-Strep (pcDNA 4/TO 2X Strep)
CH_P011	CTCCCTCGAGCGGCCCAAGTCTCTGCTTATCCAAAAG	Generate Tri1 1-117-Strep (pcDNA 4/TO 2X Strep)
CH_P007	CAGTGTGGTGGAATTCATGAGTTTGTGGAGATAGTGATC	Generate Tri1 1-128-Strep (pcDNA 4/TO 2X Strep)
CH_P012	CTCCCTCGAGCGGCCCAAGTCAACGCGATTTCTTTAG	Generate Tri1 1-128-Strep (pcDNA 4/TO 2X Strep)
CH_P009	CAGTGTGGTGGAATTCATGCTTACTAGAAATTTCTGGCTATG	Generate Tri1 -84-147-Strep (pcDNA 4/TO 2X Strep)
CH_P010	CTCCCTCGAGCGGCCCAATCATTGGGAAAAATTGAGTGATAGA	Generate Tri1 -84-147-Strep (pcDNA 4/TO 2X Strep)
CH_P019	GGATCAAATTGGACCTGCTTGAAGCAAGTTCACGTCC	MEK3 mutagenesis primers (kinase dead to WT, pcDNA3.1 Myc vector)
CH_P020	GGACGTGAACCTGCTTCCAAGCAGGTCCAATTTGATCC	MEK3 mutagenesis primers (kinase dead to WT, pcDNA3.1 Myc vector)

Figure S1.

CLUSTAL 0(1.2.4) multiple sequence alignment

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Tri1 (Serovar D)  MSFVGDSVPLRSYMPEAPLVDSASKARVSCCSERIAVLALGILSILFIVTGAALFIGAGW  60
Tri1 (Serovar L2) MSFVGDSVPLRSYMPEAPLVDSASKARVSCCSERIAVLALGILSILFIVTGAALFIGAGW  60
                  *****

Tri1 (Serovar D)  TTLPMIDVVVTLVVFVGSVMLGAVLTRISSYGGEPKKVSLDRFVLENERQGFQDKQRLADI  120
Tri1 (Serovar L2) TTLPMINVVVTLVVFVGSVMLGAVLTRISGYGGEPKKVSLDRFVLENERQGFQDKQRLADI  120
                  *****;*****

Tri1 (Serovar D)  SKEEIALAKQIEEEEKAILHSIFPND      147
Tri1 (Serovar L2) SKEEIALAKQIEEEEKAILHSIFPND      147
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Fig S1.Alignment of Tri1 from L2 serovar D.

A. Clustal alignment(64) of Tri1 from *C. trachomatis* serovars D and L2. Key: * , identical residues.
 ;, highly conserved residues, ., weakly conserved residues.

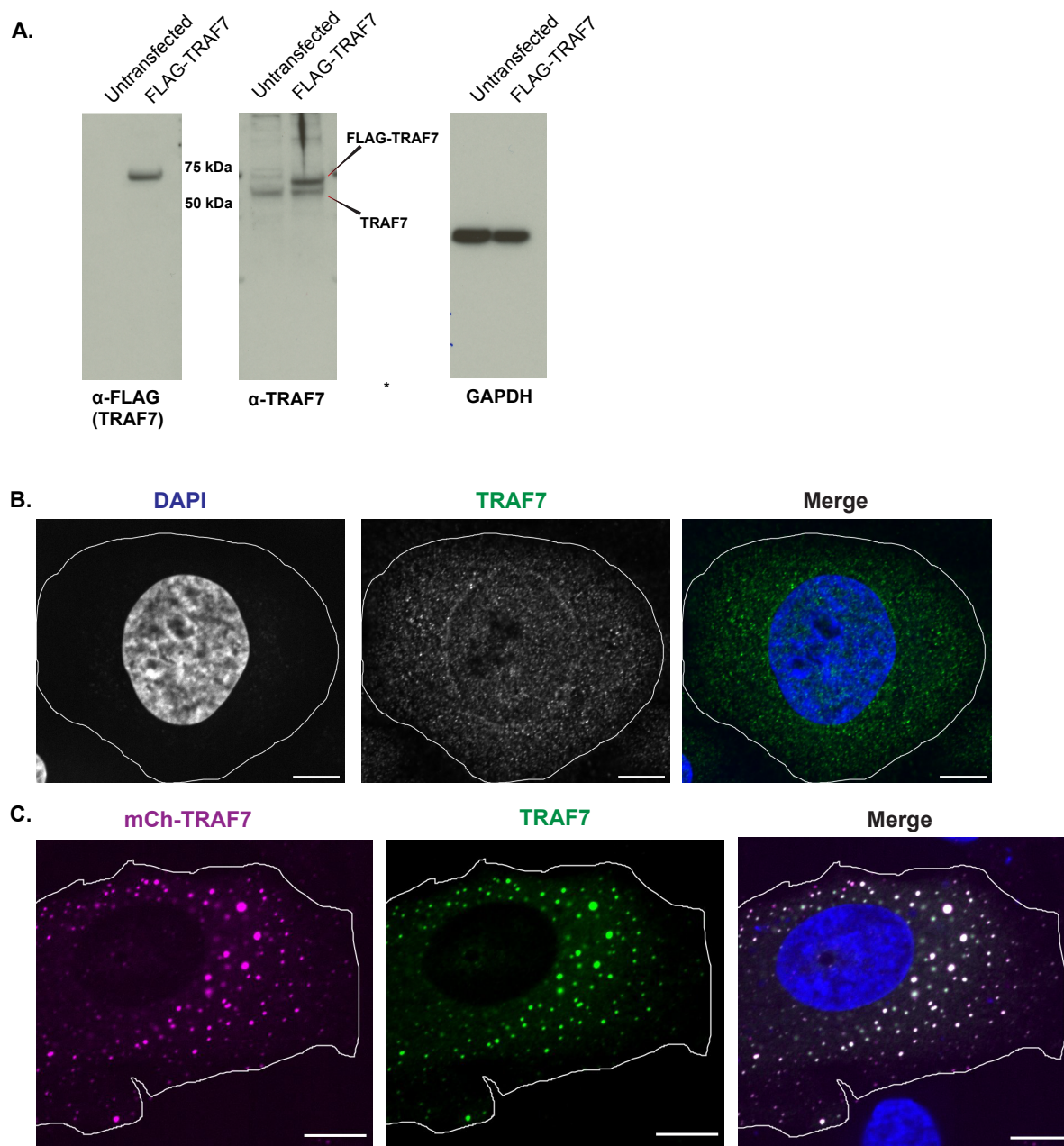


Figure S2. TRAF7 antibody detects endogenous TRAF7 and ectopically expressed TRAF7.

A. Lysates prepared from a HeLa cell line that only expresses TRAF7 isoform 2 (see materials and methods) that were either untransfected or transfected with FLAG-TRAF7 were immunoblotted with the indicated antibodies. Antibodies to FLAG and TRAF7 detect similar sized proteins in the transfected lysates, which correspond with the predicted size of FLAG-TRAF7 (~78 kDa). The faster migrating band seen in the immunoblots probed with anti-TRAF7 represents endogenous TRAF7 isoform 2 (~67 kDa). GAPDH serves as a loading control. B. HeLa cells that were transfected with mCh-TRAF7 for 24 hrs were fixed and stained with anti-TRAF7 and DAPI and imaged by fluorescence confocal microscopy. B. Endogenous TRAF7 localizes to both the nucleus and cytosol. Cell membrane is outlined. Shown are Single z-slices. Scale bar = 10 μ m.

Uncropped blots corresponding to Fig. 1

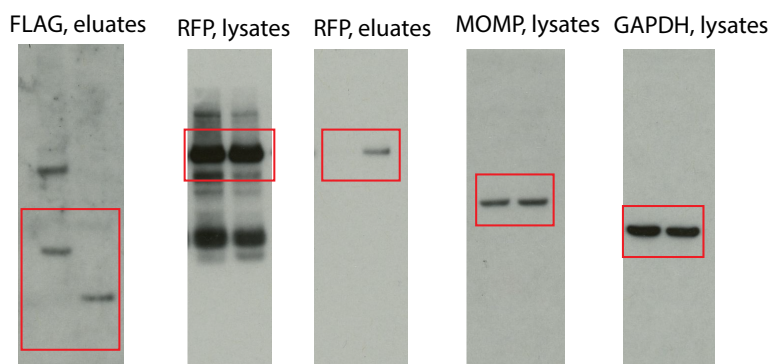


Fig S3. Uncropped immunoblots corresponding to Figure 1.

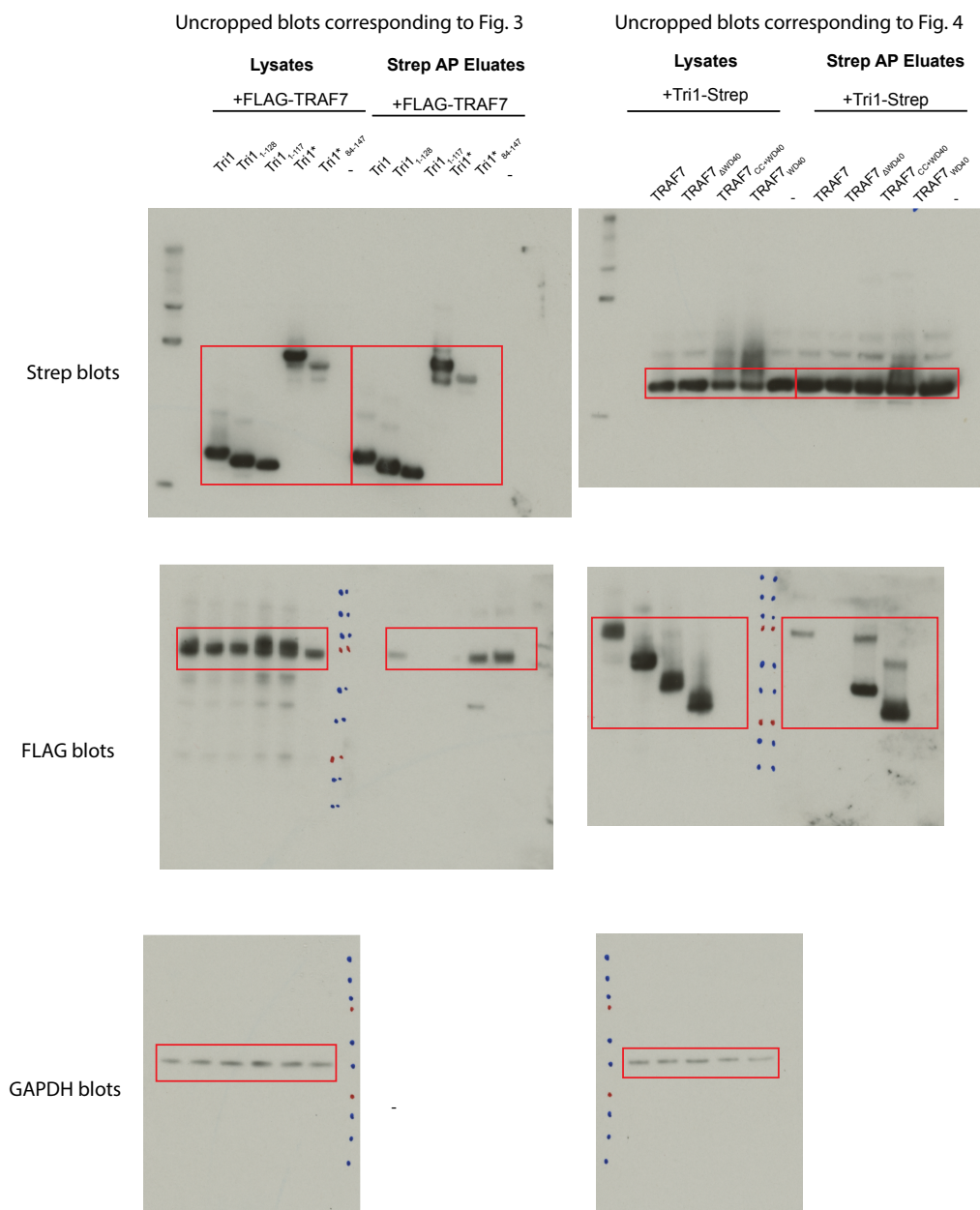
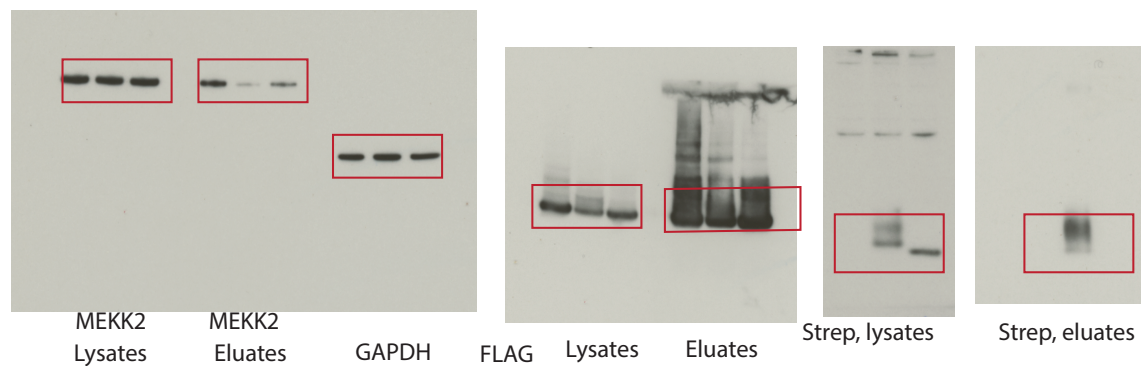
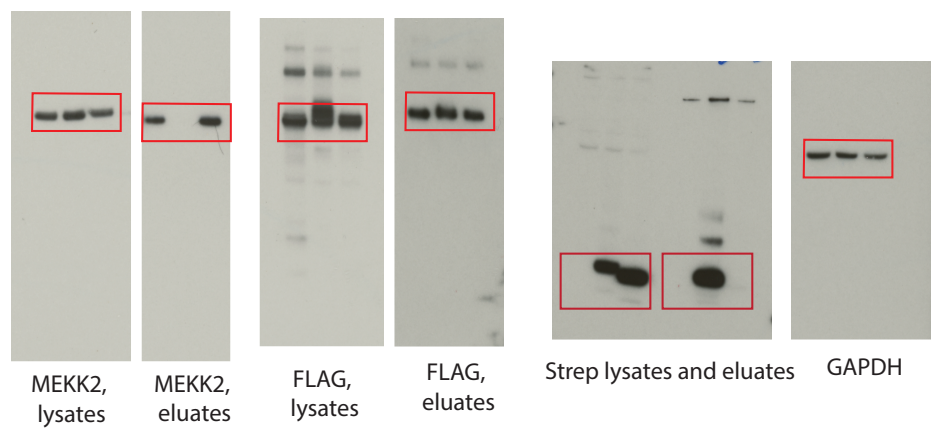


Fig S4. Uncropped immunoblots corresponding to Figures 3 and 4.

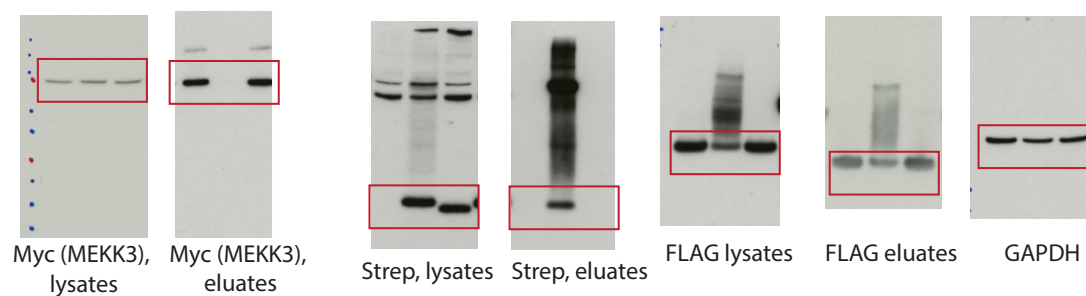
Uncropped blots corresponding to Fig. 5C



Uncropped blots corresponding to Fig. 5D



Uncropped blots corresponding to Fig. 5E

**Fig S5. Uncropped immunoblots corresponding to Figure 5.**