

Supplementary Materials for
Single Amino Acid Substitutions Confer the Antiviral Activity of the
TRAF3 Adaptor Protein onto TRAF5

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Fig. S1. Binding of TRAF3 and TRAF5 to the TIMs of Cardif.

Fig. S2. Alternative confirmation of the C-terminal peptide of Cardif bound to TRAF3.

Fig. S3. The C-terminal region of the Cardif peptide is necessary for the interaction with TRAF3.

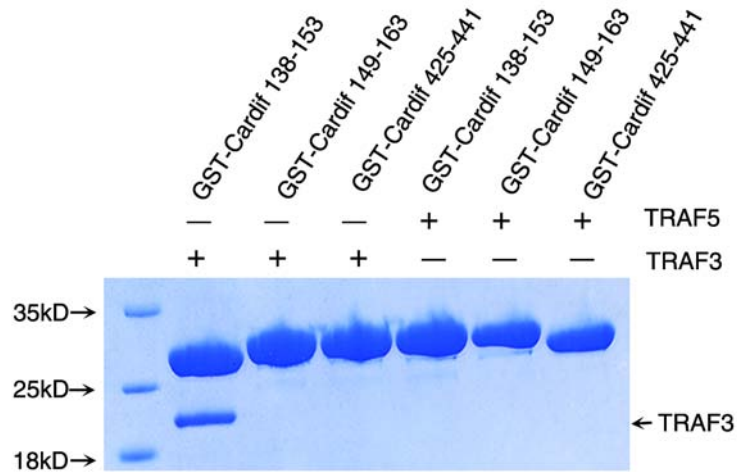


Fig. S1. Binding of TRAF3 and TRAF5 to the TIMs of Cardif. As a prelude to obtaining a TRAF3-Cardif complex, we tested the ability of the TRAF domain of TRAF3 to bind to various Cardif peptides, including 138-153 (T2BM), 151-165 (T6BM1) and 425-439 (T6BM2). GST-tagged Cardif 138-153, Cardif 151-165 and Cardif 425-439 were bound to glutathione beads. After incubation with the TRAF domains of TRAF3 (lanes 2-4) or TRAF5 (lanes 5-7), the GST beads were washed and analyzed by SDS-PAGE for TRAF-Cardif binding. These results were confirmed using different batches of proteins (N=3 independent experiments).

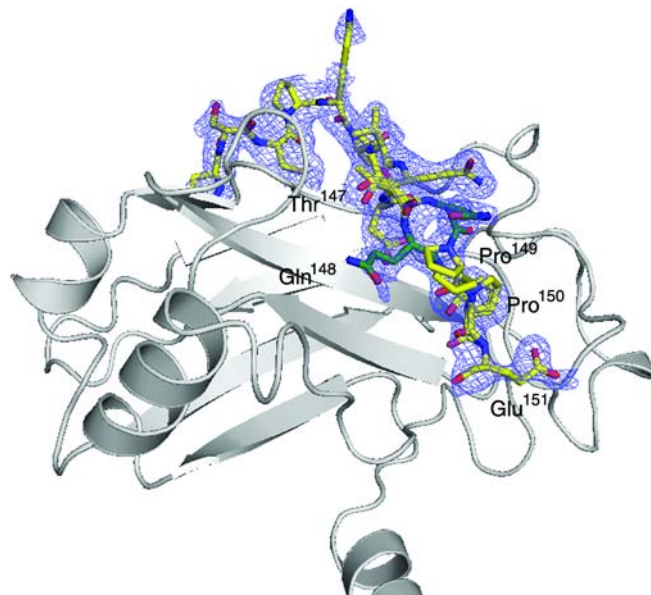


Fig. S2. Alternative confirmation of the C-terminal peptide of Cardif bound to TRAF3. The blue meshwork represents the $2F_o - F_c$ electron density map contoured at 0.7σ . TRAF3 is in gray; the Cardif peptide is in yellow. Gln¹⁴⁸ is highlighted in green. The C-terminal residues of Cardif are labeled.

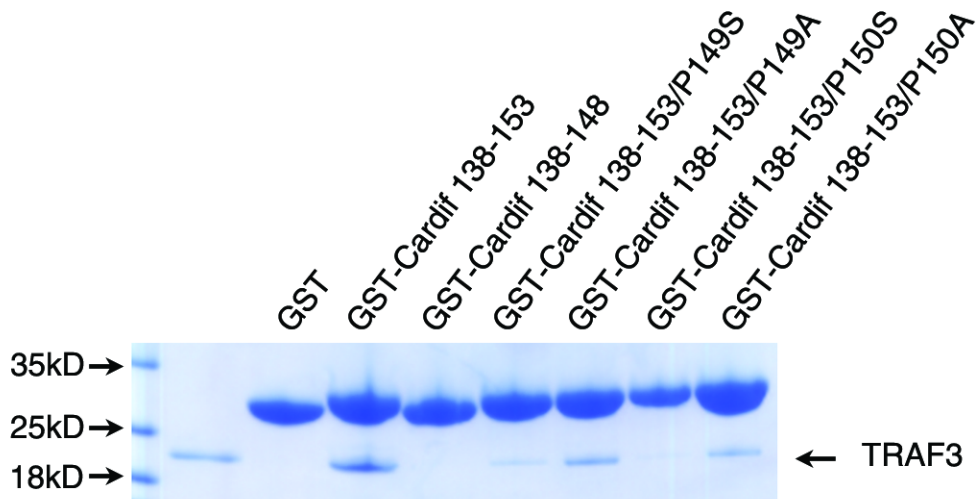


Fig. S3. The C-terminal region of the Cardif peptide is necessary for the interaction with TRAF3. The N-terminally GST-tagged Cardif peptides (residues 138-153, 138-148, P149A, P149S, P150A, P150S) were bound to glutathione beads. After incubation with TRAF3, the glutathione beads were washed and analyzed by SDS-PAGE for TRAF3-Cardif binding. These results were confirmed using different batches of proteins (N=3 independent experiments).