

Supplementary Material

The TLR signalling adaptor TRIF/TICAM-1 has an N-terminal helical domain with structural similarity to IFIT proteins

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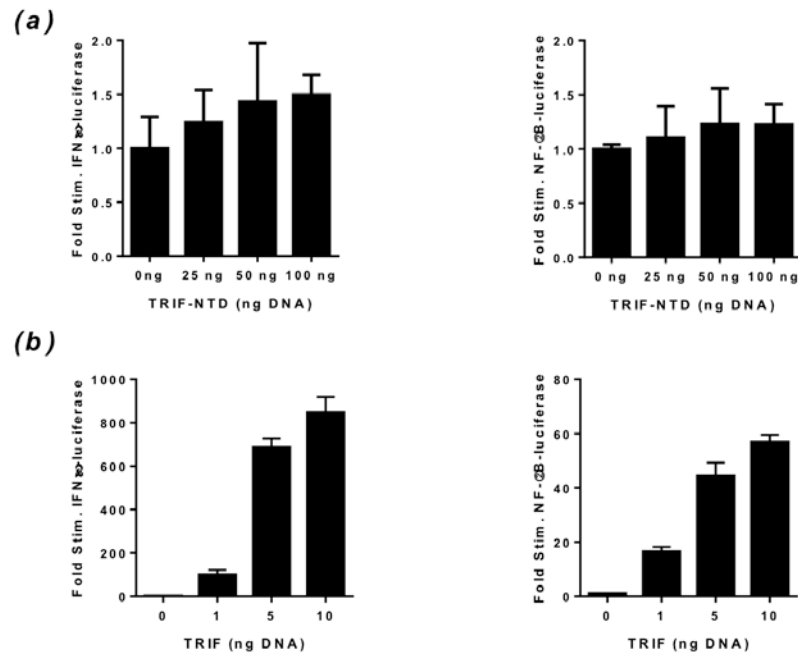


Figure S1 TRIF-NTD and wild type TRIF dose response. HEK293 cells (2×10^4 /ml) were transiently transfected for 24 h with TK-Renilla and either IFN β (left panel) or NF- κ B-luciferase (right panel) reporter constructs in conjunction with (a) TRIF-NTD (25-100 ng) or (b) wild type TRIF (1-10 ng). Readings are normalized as expressed luciferase reporter activity over constitutively expressed TK-Renilla luminescence. Results are mean \pm SEM of triplicate determinations performed in two independent experiments.

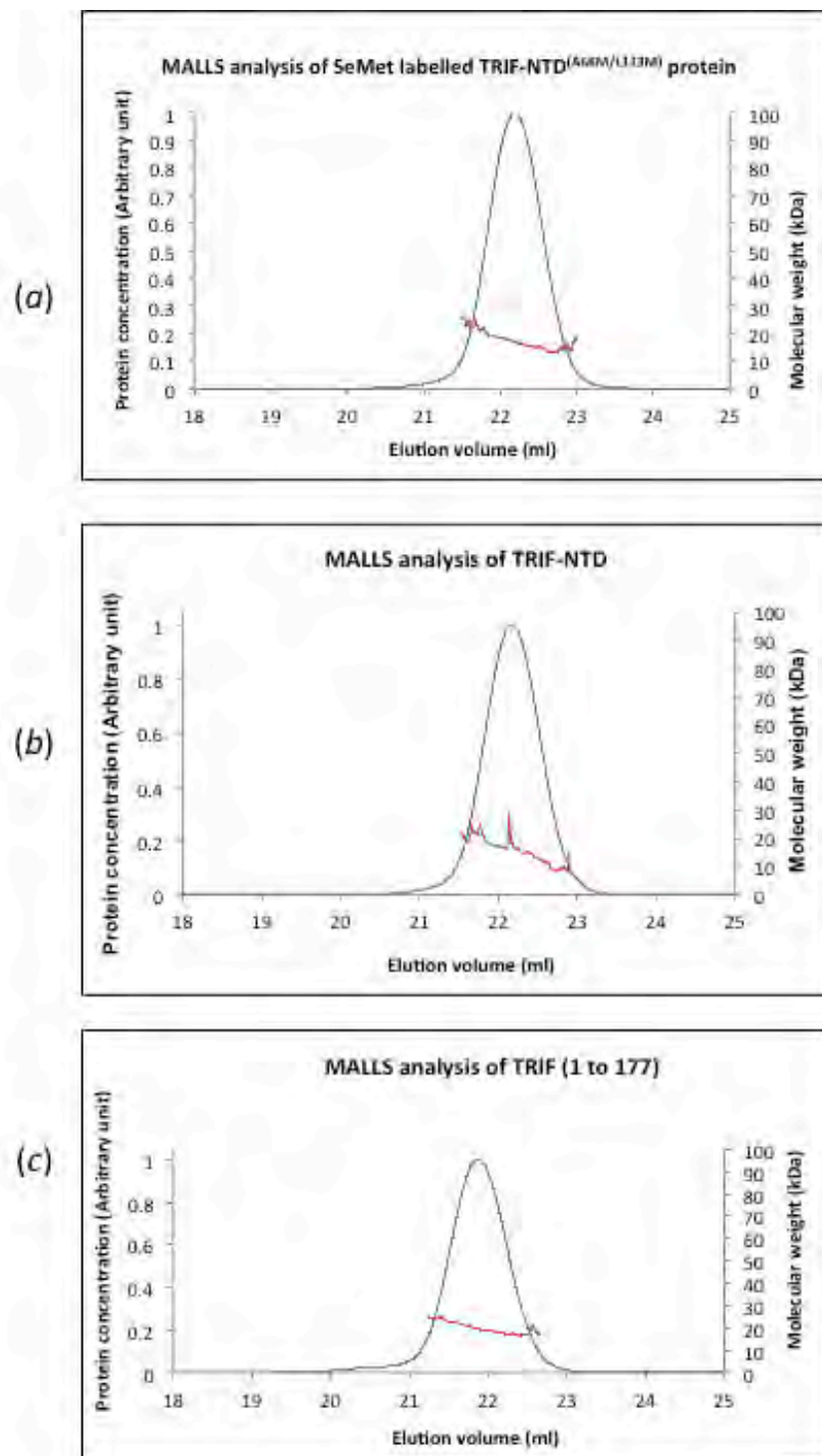


Figure S2 Multi-angle laser light scattering (MALLS) analysis of different TRIF variants. Blue line: elution profile; red line: molecular weight (MW) distribution profile (kDa). (a) MALLS analysis of SeMet-labelled TRIF-NTD^{A66M/L113M}. The theoretical and measured MWs correspond to 17.18 kDa and 19.50 ($\pm 14.8\%$) kDa, respectively. (b) MALLS analysis of wild-type TRIF-NTD. The theoretical and measured MWs correspond to 17.00 kDa and 17.00 ($\pm 14.1\%$) kDa, respectively. (c) MALLS analysis of TRIF (residues 1 to 177). The theoretical and calculated MWs of correspond to 19.42 kDa and 19.00 ($\pm 4.4\%$) kDa, respectively.

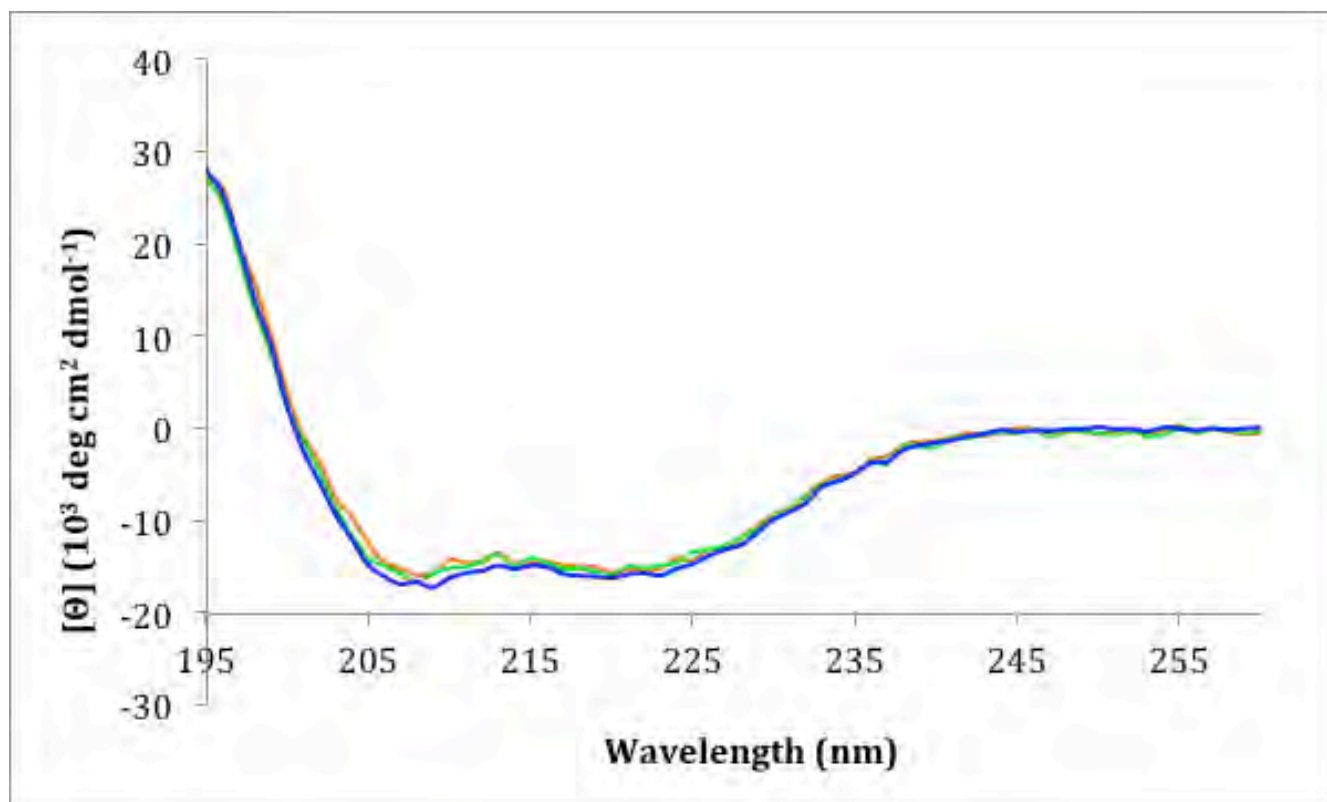


Figure S3 Circular dichroism spectra of different TRIF variants. TRIF-NTD, orange; SeMet-labelled TRIF-NTD^{A66M/L113M}, green; TRIF-NTD (residues 1-177), blue.

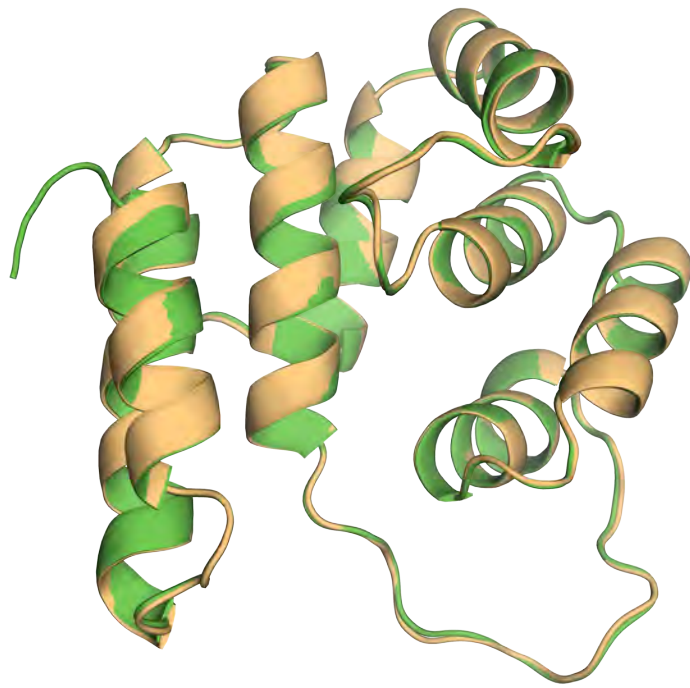
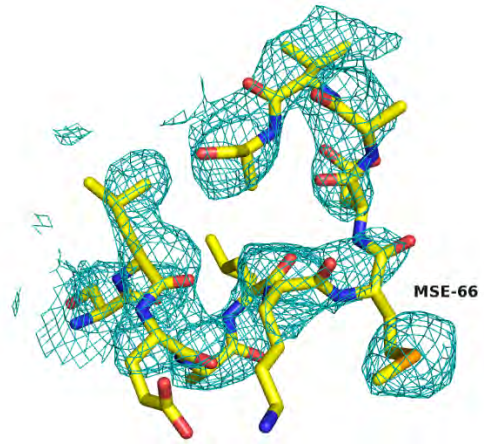
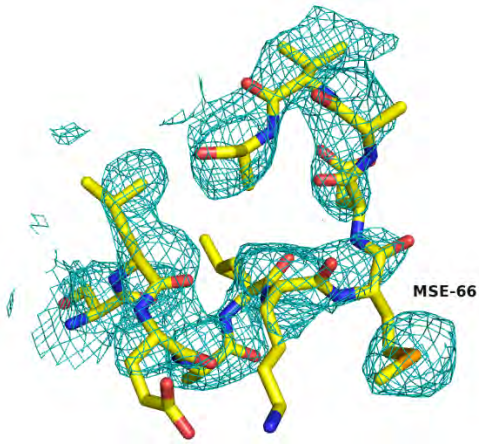
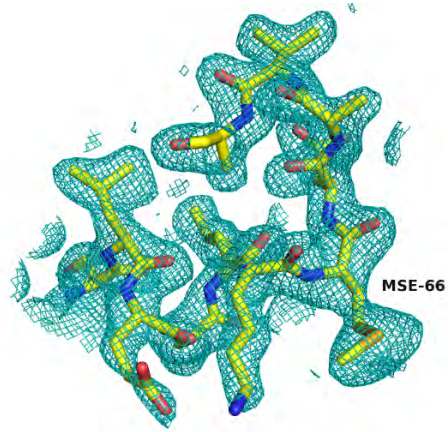
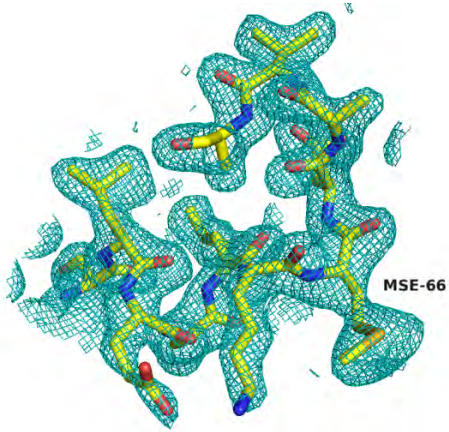


Figure S4 Superposition of the structures of wild-type TRIF-NTD (green) and TRIF-NTD^{A66M/L113M} (light orange).

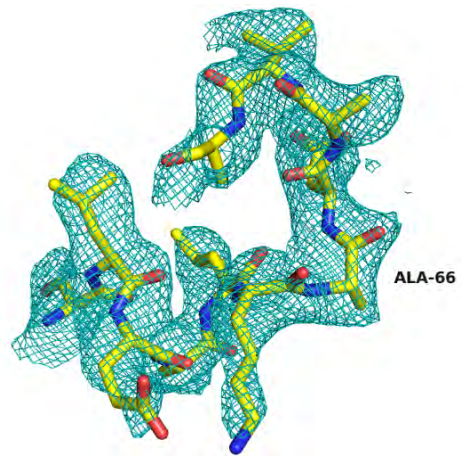
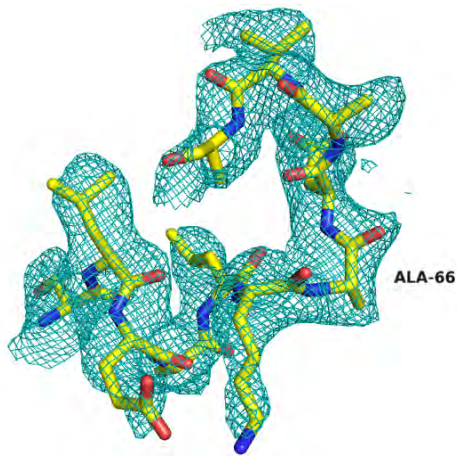
(a)



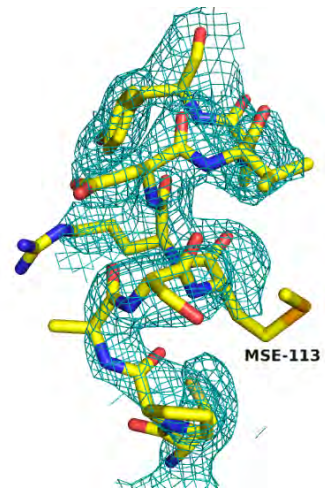
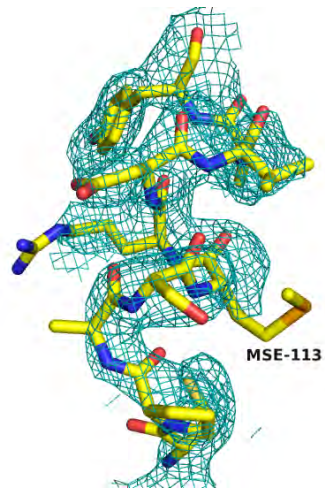
(b)



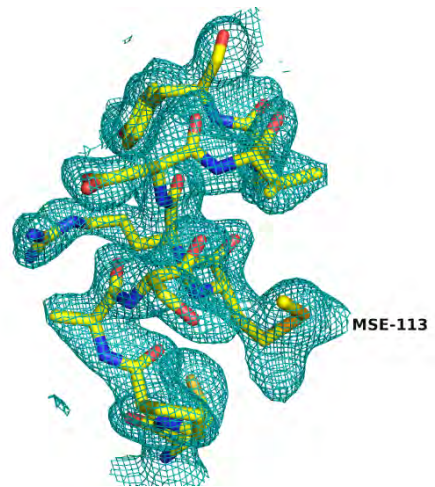
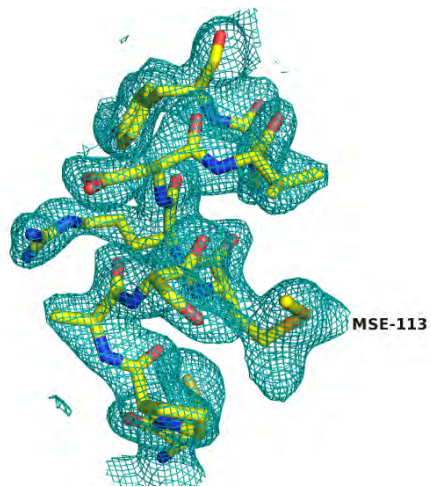
(c)



(d)



(e)



(f)

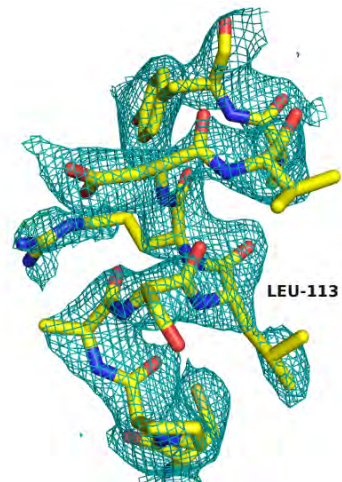
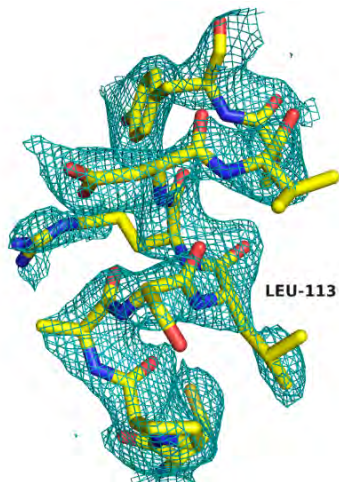


Figure S5: Stereo-views (wall-eyed) of electron density maps for the crystals of TRIF-NTD and TRIF-NTD^{A66M/L113M}. (a) Stereo-view of the experimental electron density map of SeMet-labelled TRIF-NTD^{A66M/L113M} in the vicinity of residue 66 (Met66), generated using the program *AutoSol* after density modification (contoured at 1 sigma). (b) Stereo-view of the electron density map of SeMet-labelled TRIF-NTD^{A66M/L113M} in the vicinity of residue 66 (Met66) after refinement. (c) Stereo-view of the electron density map of wild-type TRIF-NTD in the vicinity of residue 66 (Ala66) after refinement (coefficients 2Fo-Fc, contoured at 1 sigma). (d) Stereo-view of the experimental electron density map of SeMet-labelled TRIF-NTD^{A66M/L113M} in the vicinity of residue 113 (Met113), generated using the program *AutoSol* after density modification (contoured at 1 sigma). (e) Stereo-view of the electron density map of SeMet-labelled TRIF-NTD^{A66M/L113M} in the vicinity of residue 113 (Met113) after refinement (coefficients 2Fo-Fc, contoured at 1 sigma). (f) Stereo-view of the electron density map of wild-type TRIF-NTD in the vicinity of residue 113 (Leu113) after refinement (coefficients 2Fo-Fc, contoured at 1 sigma).

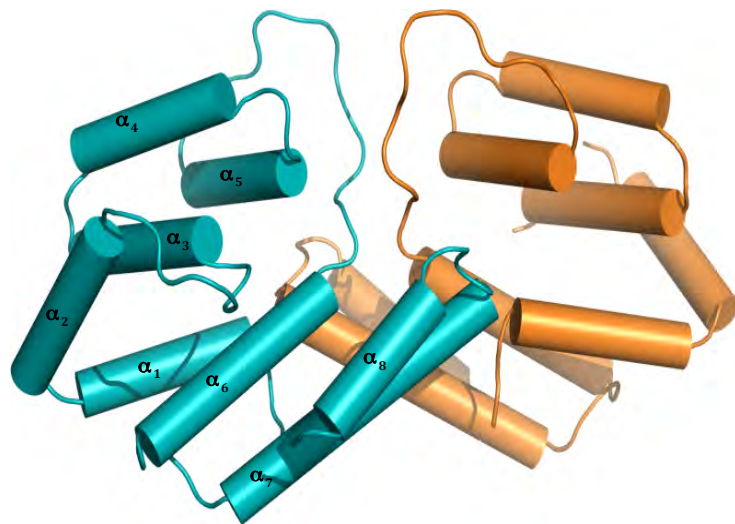


Figure S6 Cylinder representation of the TRIF-NTD dimer observed in the crystals. TRIF-NTD monomers are highlighted in orange and teal.

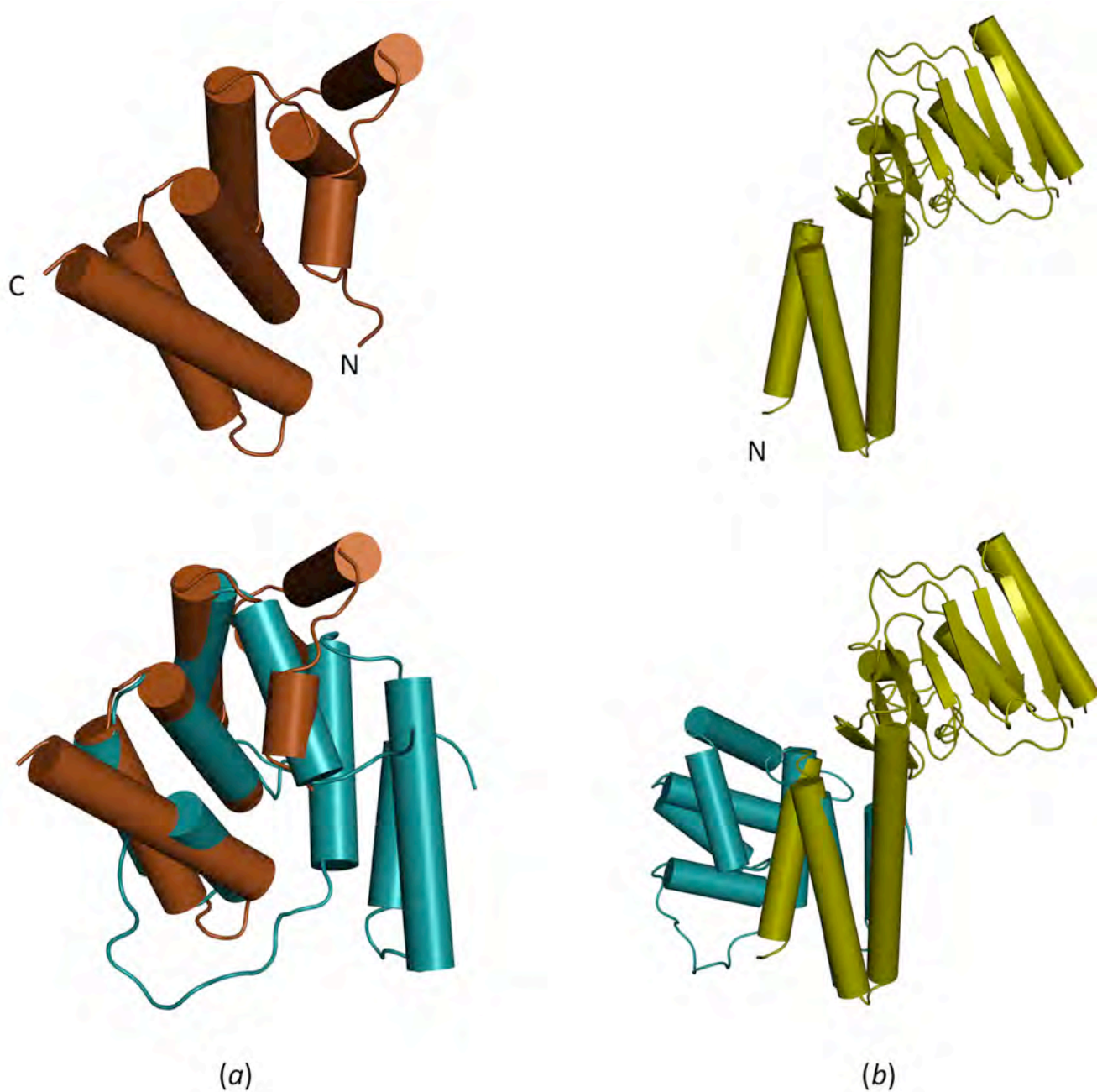


Figure S7 Structural alignment of TRIF-NTD with structurally similar proteins. (a) Crystal structure of yeast Fis1 (mitochondrial fission protein 1, PDB ID 2PQR) highlighted in brown (top). Superposition of TRIF-NTD (cyan) with yeast Fis1 (bottom). (b) Crystal structure of human MIT (microtubule-interacting and trafficking) domain containing protein 1 (PDB ID 2YMB) highlighted in deep olive (top). Superposition of TRIF-NTD (cyan) with human MIT domain containing protein 1 (bottom).

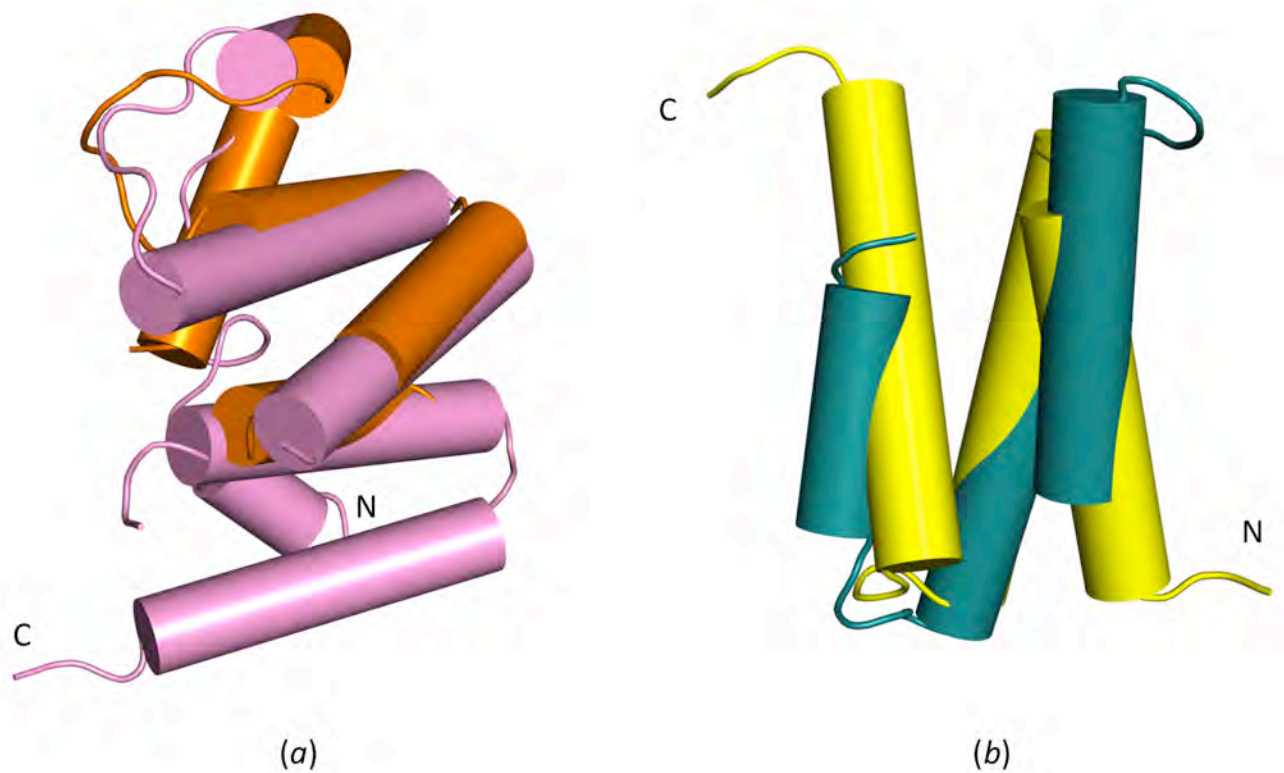


Figure S8 Superposition of TRIF-NTD with IFIT5. (a) Superposition of sub-domain 1 (helices $\alpha 1-5$) of TRIF-NTD and the N-terminal sub-domain of IFIT5 (helices 1-6). TRIF-NTD and IFIT5 are highlighted in orange and pink, respectively. (b) Superposition of TRIF-NTD sub-domain 2 (helices $\alpha 6-\alpha 8$) and the middle TPR region of IFIT5 (helices 7-9). TRIF-NTD and IFIT5 are highlighted in teal and yellow, respectively.

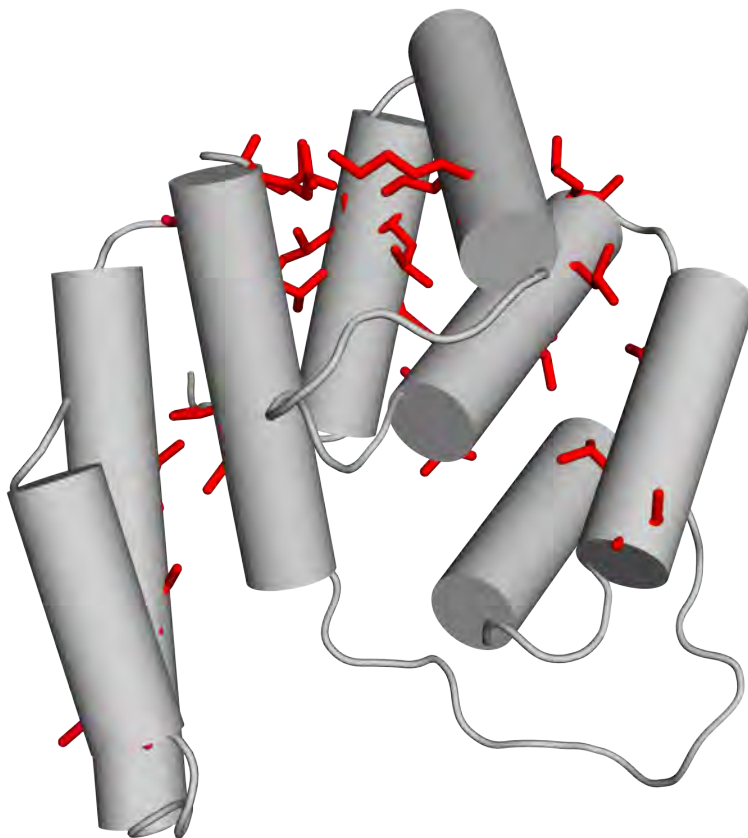


Figure S9 Cylinder representation of TRIF-NTD, with strictly conserved residues among TRIF orthologues displayed in wireframe (red).