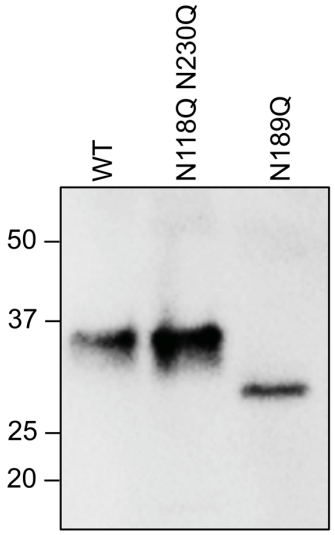

Figure S1. Tspan15 contains a single glycosylation site, **related to Figure 1.** Tspan15 contains three predicted N-linked glycosylation sites. Wild-type FLAG-tagged Tspan15 and predicted glycosylation site mutants were expressed in Expi293 cells. The anti-FLAG western blot shows that there is no change in migration on the gel with the N118Q/N230Q double mutant compared to wild-type Tspan15. The N189Q mutant has a clear change in migration position with a sharpened band due to loss of the glycan.

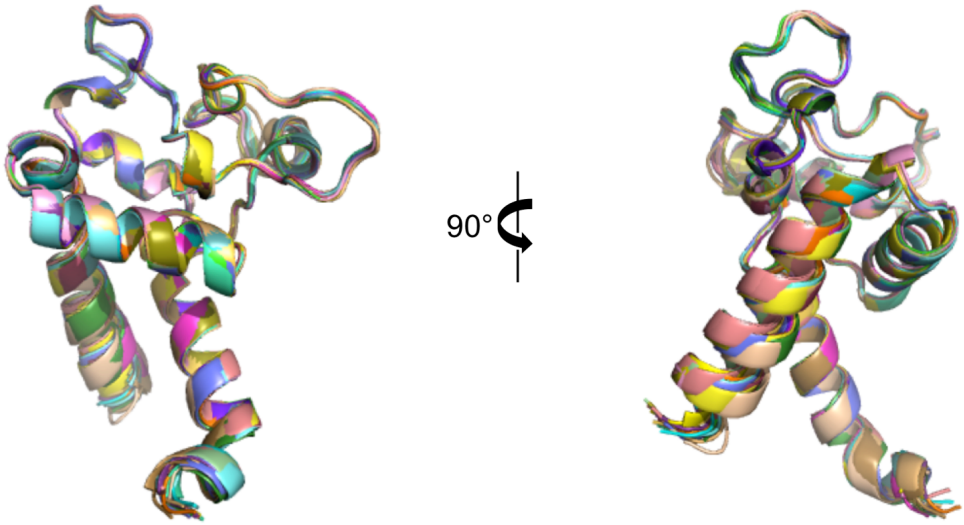

Figure S2. Alignment of all 24 copies of Tspan15 LEL in the asymmetric unit, related to Figure 1. Each copy of Tspan15 LEL is aligned and displayed in cartoon representation in a different color.

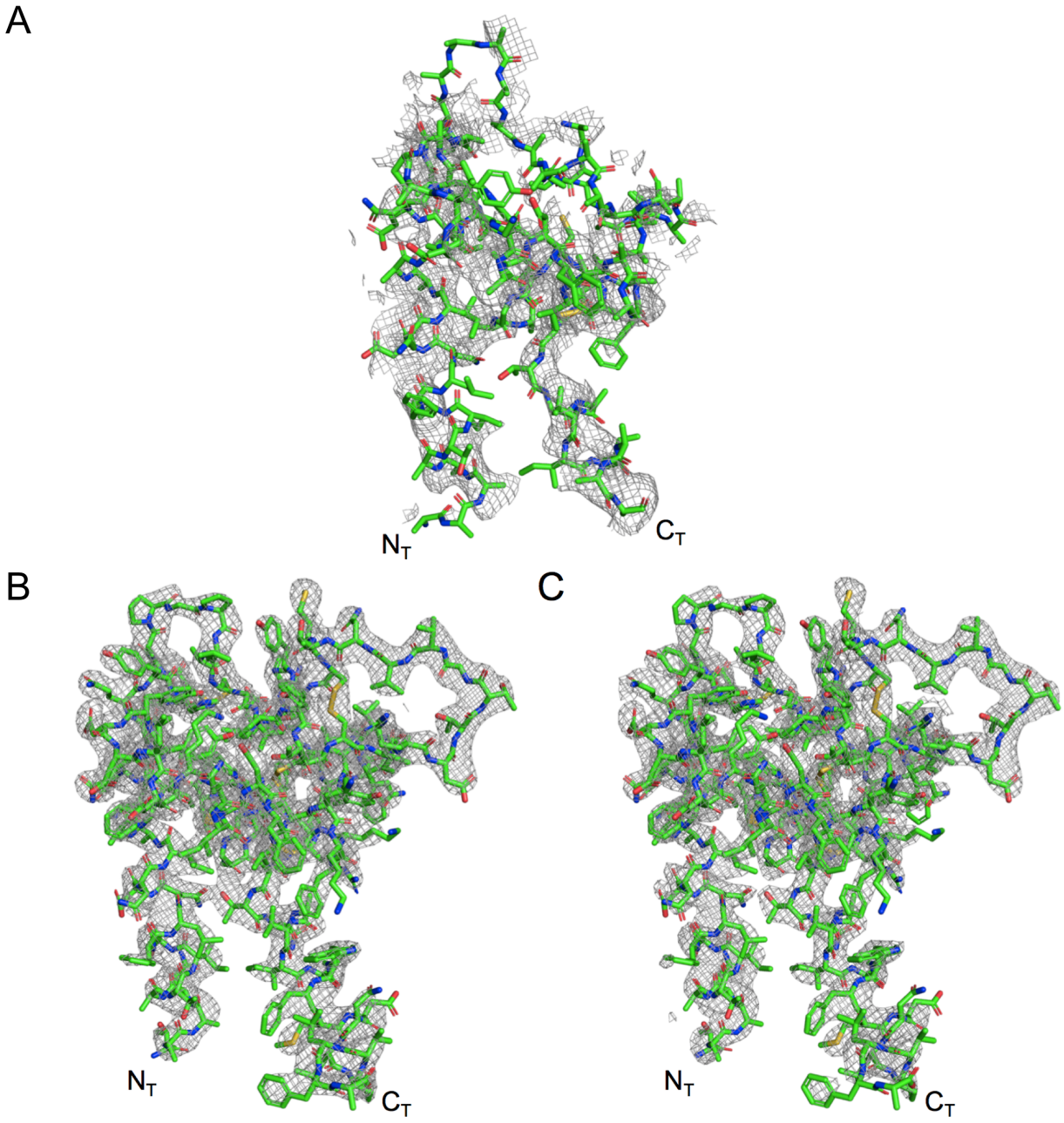

Figure S3. Representative electron density maps, related to Figure 1. (A) Simulated-annealing composite omit 2mFo-Fc map following molecular replacement with partial model (with 13 of 24 chains placed) and rigid body refinement contoured to 1.0 σ. (B) The final refined 2mFo-DFc map and (C) simulated-annealing composite omit 2mFo-Fc map, both contoured to 1.5 σ.

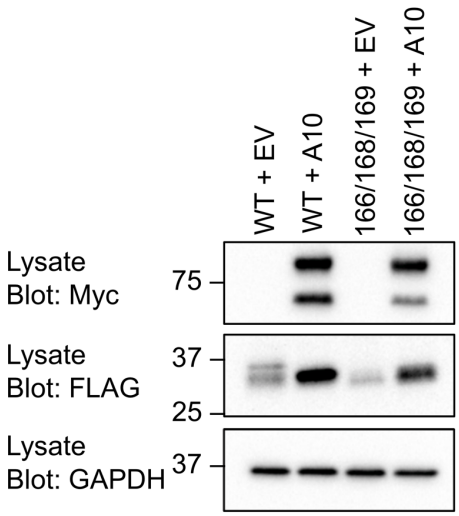

Figure S4. Tspan15 protein amount is increased by co-expression with ADAM10, related to Figure 4. HEK293T cells were co-transfected with FLAG-tagged Tspan15 or Tspan15 166/168/169 mutant and either myc-ADAM10 or empty vector. Western blots show increased abundance of both wild-type and mutant Tspan15 proteins when co-expressed with ADAM10.

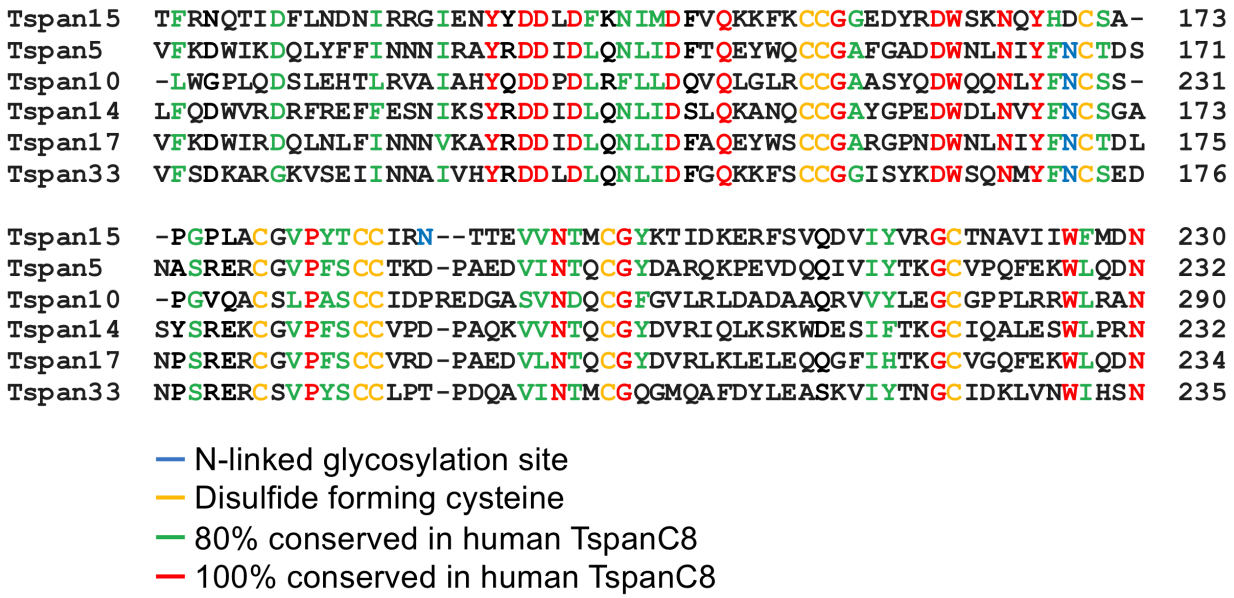

Figure S5. Sequence alignment of the LEL domain of human TspanC8 proteins, related to Figures 4 and 5. Alignment of the six human TspanC8 LEL domain sequences. Conserved TspanC8 cysteines are colored in yellow, N-linked glycosylation sites are blue, sites that are 100% conserved in the human TspanC8 proteins are red and sites that are at least 80% conserved (including conservative substitutions) are green.