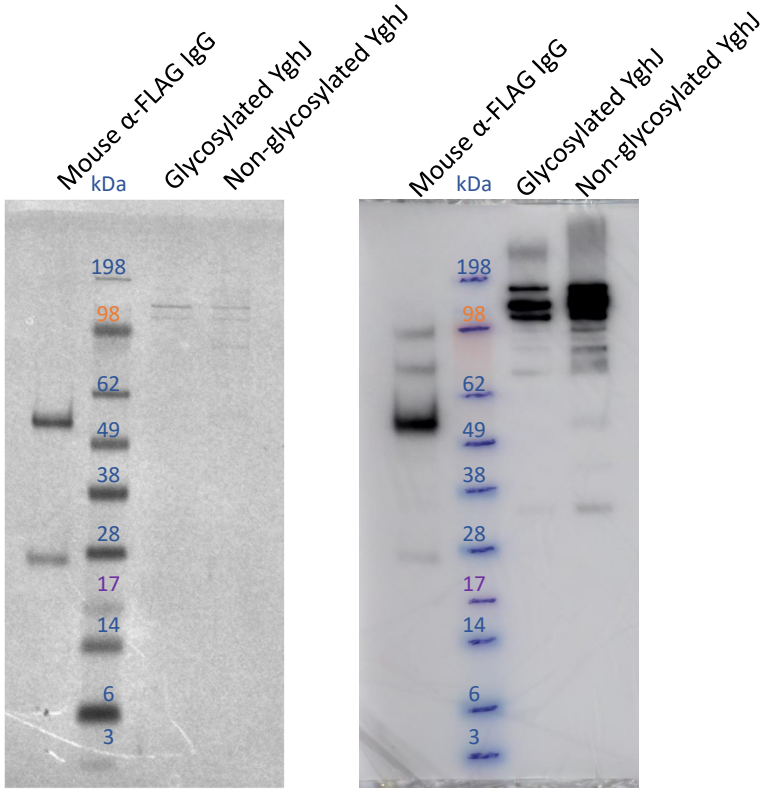
The Coomassie stained gel (left panel) in figure shows YghJ in the expected mass range and the same extent of degradation for both glycosylated and non-glycosylated YghJ (also shown in Figure 1). In the Western blot (right panel), some of the extra bands below YghJ can be observed. These bands can be assigned to monoclonal mouse α-FLAG IgG. We have discovered, that even though the anti-FLAG antibodies are covalently coupled to the agarose beads (Sigma A2220), the resin bleeds IgG during our FLAG purification protocol. The Coomassie stained gel also shows that no contaminating protein(s) can be observed in the mass range below what the Western Blot in Supplementary Figure S2 reveals i.e., 3-35 kDa.



Supplementary Figure S3: Coomassie staining and Western blot analysis of purified glycosylated and non-glycosylated YghJ as well as mouse α-FLAG IgG antibody. Left panel: 0.2ug purified glycosylated and non-glycosylated YghJ as well as 0.4ug mouse α-FLAG IgG antibody was loaded onto a PAGE gel and run under reducing conditions. Proteins were visualized using Coomassie blue (Candiano et al. Electrophoresis, 2004). Molecular weight marker (kDa) is indicated. Right panel: 0.2ug purified glycosylated and non-glycosylated YghJ as well as 0.4ug mouse α-FLAG IgG antibody was loaded onto a PAGE gel and run under reducing conditions. Western blot was used to visualize proteins. Primary mouse α-FLAG IgG antibody (diluted x50.000). Secondary mouse IgG was diluted x8.000. Molecular weight marker (kDa) is indicated.