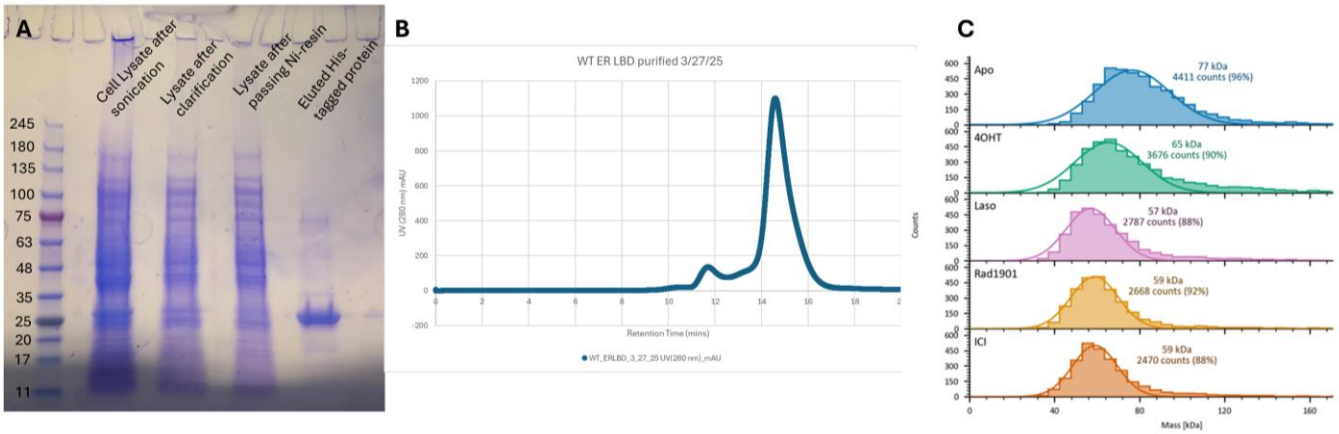

Figure 1: ER LBD sample quality. A) SDS-PAGE gel showing several steps of protein purification with the purified sample shown just before loading onto the size exclusion column. B) Size exclusion column trace showing the homogenous peak from minutes 14-16 that was concentrated and flash frozen. C) Mass photometry readings for ER LBD incubated for 1 hour with 1 mM of each ER ligand before diluting to 50 nM, demonstrating ER LBD remains a dimer and does not form aggregate species.

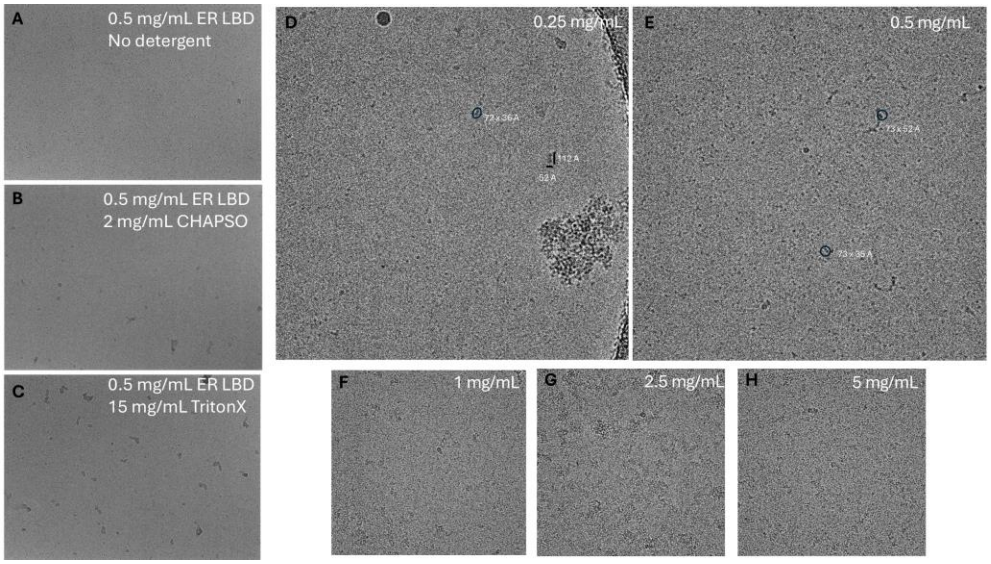

Figure 2: Grid Optimization. A-C) Optimization of sample conditions using detergents added to 0.5 mg/mL ER LBD A) no detergent. B) 2 mg/mL CHAPSO. C) 15 mg/mL TritonX. D-H) Optimization of ER LBD concentration. D) ER LBD at 0.25 mg/mL with particles circled with measurements made using ImageJ listed. E) ER LBD at 0.5 mg/mL with particles circle and dimensions listed. F) ER LBD at 1 mg/mL. G) ER LBD at 2.5 mg/mL. H) ER LBD at 5 mg/mL.

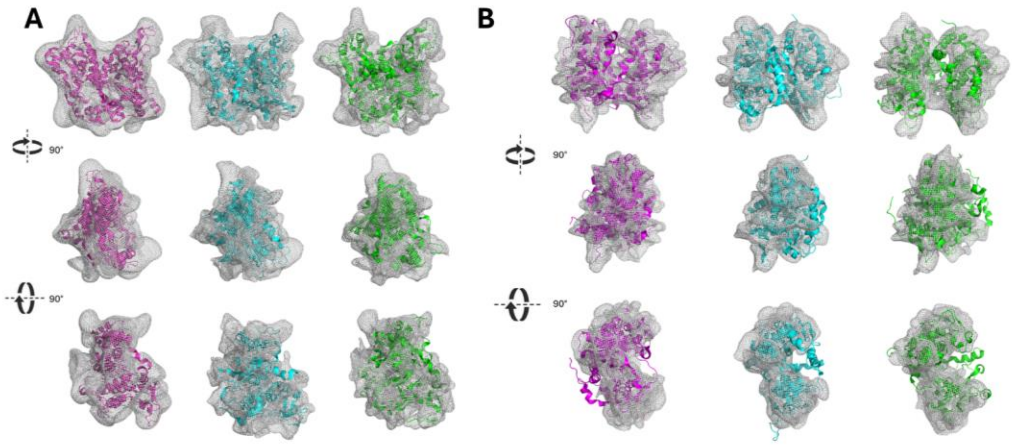

Figure 3: Preliminary 3D reconstructions of ER LBD. 3D variability analysis was performed on a consensus structure to produce 3 electron density maps (grey mesh) fit with ER LBD crystal structures using Isolde (colored in pink, cyan, and green, respectively). A) Apo ER LBD apo with a reported resolution of 4.99 Å. B) ER LBD bound to ICI with a reported resolution of 6.01 Å