Figure 2 - Figure Supplement 1

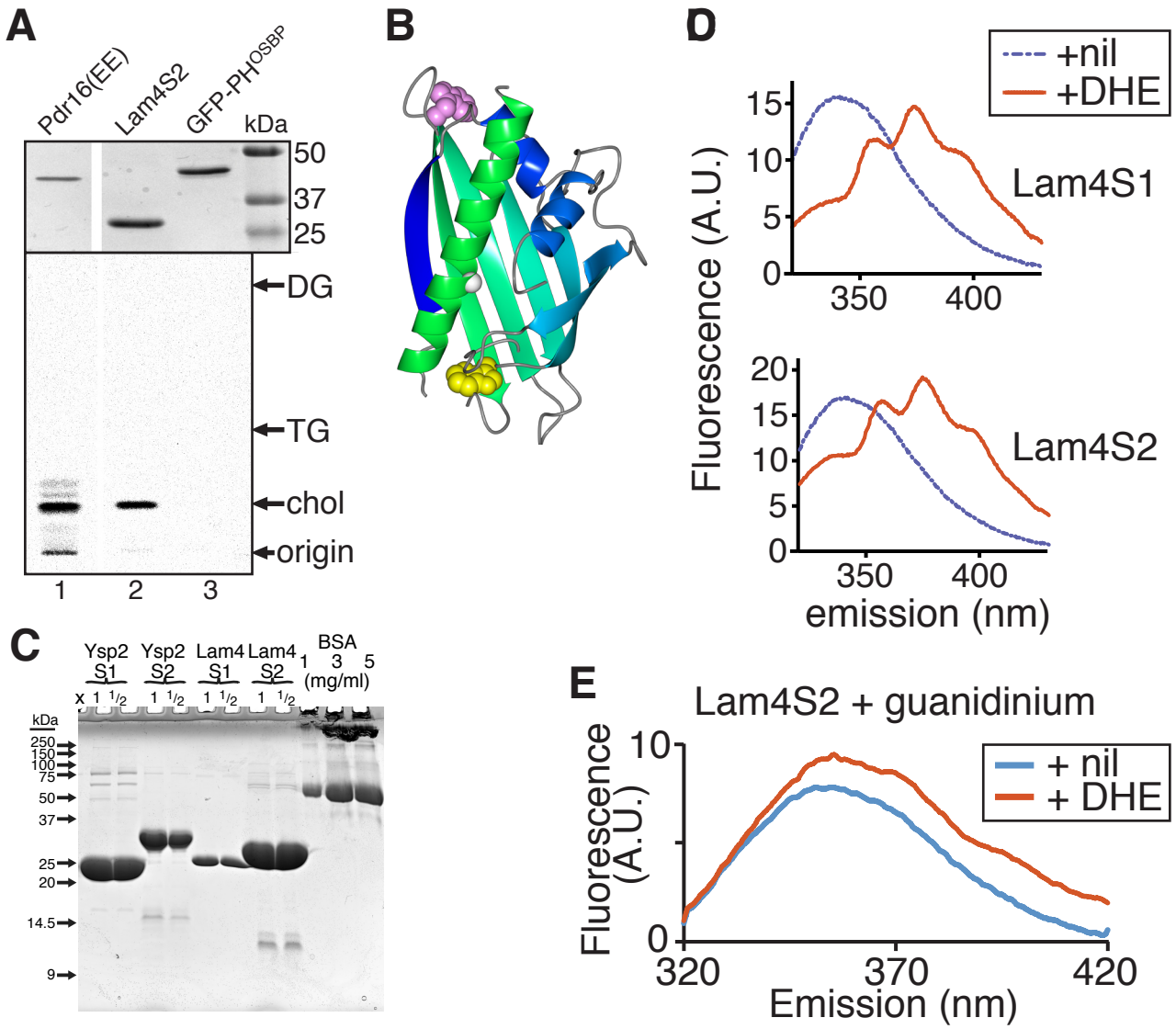

Figure 2–Figure Supplement 1: Lipid binding properties of StART-like domains

A. Lam4S2 binding to cellular lipids (see Fig. 2A). TOP: His6-tagged proteins 1. Pdr16(EE) (Holic *et al.*, 2014), 2. Lam4S2, 3. GFP-PH^{OSBP} were re-isolated after incubation with radiolabelled cells, separated by SDS-PAGE and stained by Coomassie (MW markers indicated). BOTTOM: Bound lipids that ran near the solvent front in the first TLC (Fig 2A) were scraped and re-separated to distinguish between cholesterol and other neutral lipids triacyl- and diacyl-glycerol (TG and DG). **B.** Lam4S2 (966-1136) was modelled using SAM-T08 (Karplus, 2009), colored in a spectrum from N-terminus (blue) to C-terminus (green). Also shown: two conserved tryptophans either inside (yellow) or outside (pink) the pocket, a conserved glycine in the C-terminal helix (white = G1205 in Ysp2p, G1119 in Lam4p). **C.** Stock solution of BSA and aliquots of the indicated purified StART-like domains (x1 and x1/2 volume compared to BSA) were run on a 15% SDS-PAGE gel that was stained by Coomassie. **D.** FRET between Lam4S1 or Lam4S2 and DHE. Tryptophan fluorescence (excitation at 295 nm) with purified protein either on its own or incubated with DHE. **E.** Tryptophan emission spectrum of Lam4S2 with and without DHE (as in panel C) but in the presence of 7M guanidinium to denature the protein. This shows that DHE is only slightly fluorescent when stimulated at 295 nm.