Bechler et al., http://www.jcb.org/cgi/content/full/jcb.200908105/DC1

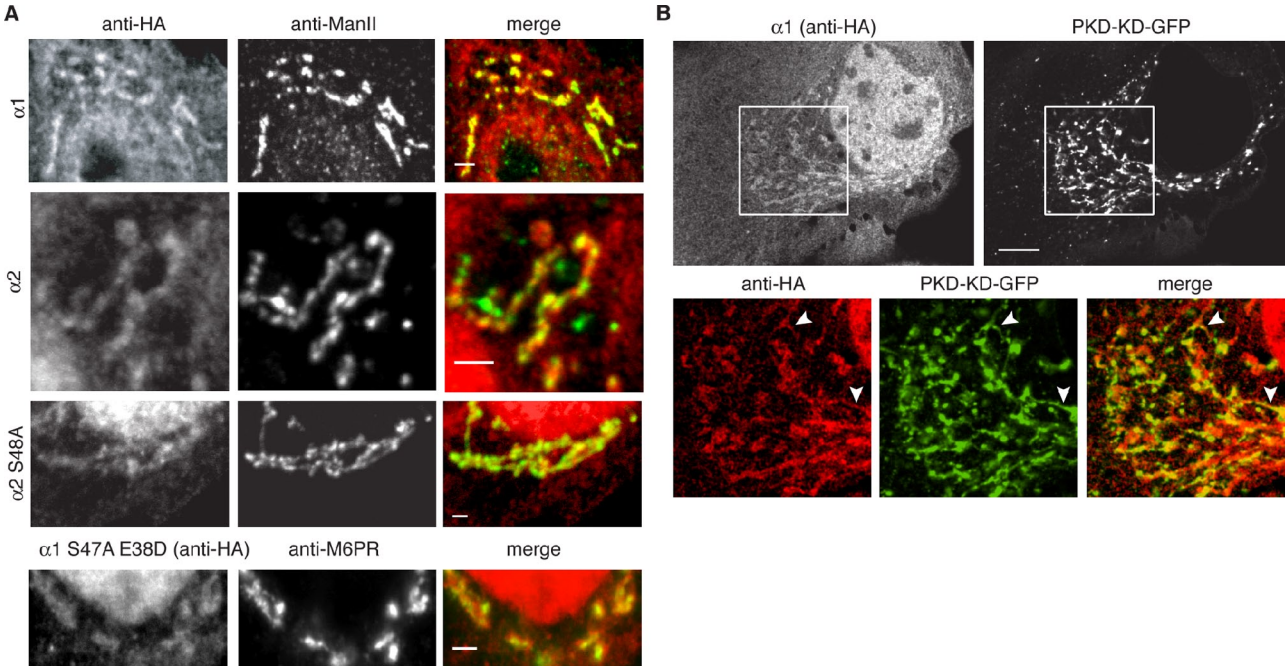

Figure S1. **PAFAH Ib α1 and α2 localize to multiple Golgi cisternae, independent of catalytic activity or LIS1 binding.** (A) Confocal stacks of BTRD cells mildly overexpressing HA tagged wild-type or catalytically inactive (S48A) α2 were labeled with *medial* Golgi marker ManII. Wide-field fluorescence of BTRD cells overexpressing low levels of α1 S47A E38D (HA) show localization with the TGN (M6PR). (B) Confocal stacks of BTRD cell overexpressing HA tagged α1 PKD-KD-GFP shows α1 localizes to a subset of TGN tubules (arrowheads). Bars: A = 2 µm, B = 10 µm.

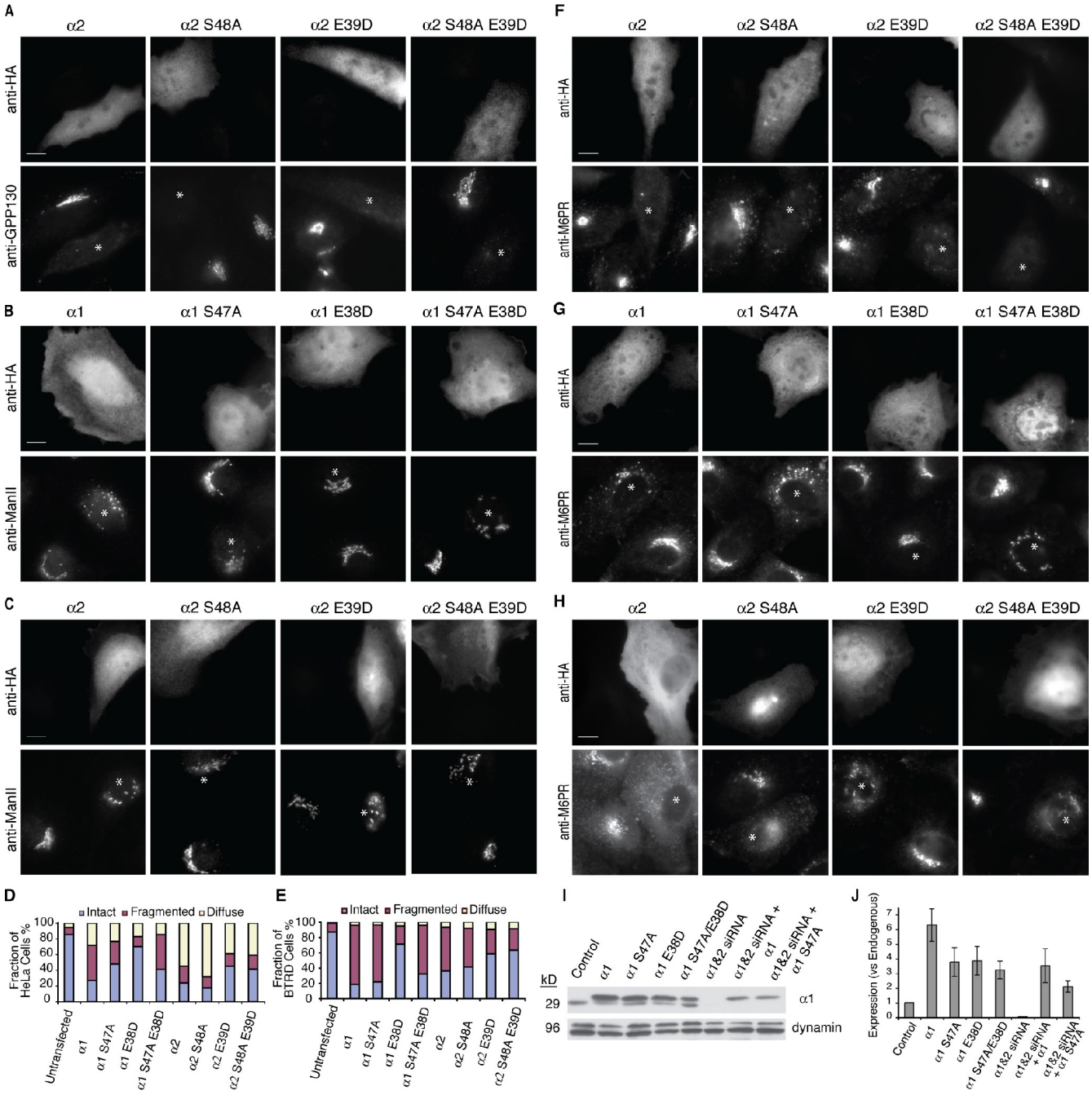

Figure S2. **PAFAH Ib α1 and α2 disrupt Golgi and TGN structure.** (A) Wide-field fluorescence images showing HeLa cells overexpressing α2 (HA, *) wild type or mutants disrupt Golgi (GPP130) structure as compared with untransfected cells. (B and C) Overexpression of α1 or α2 (HA, *) and indicated mutants, except α1 E38D, disrupt Golgi (ManII) structure, as compared with neighboring untransfected BTRD cells. (D and E) The severity of Golgi disruption was quantified in untransfected and overexpressing cells (as indicated). Intact, fragmented or diffuse Golgi were counted; $n = 3$. (F) Wide-field fluorescence images of HeLa cells overexpressing indicated constructs of α2 (HA, *) disrupt TGN (M6PR) structure in comparison to untransfected cells. (G and H) Overexpression of the indicated α1 or α2 (HA, *) constructs, except α1 E38D, disrupts TGN (M6PR) structure in BTRD cells. (I) Western blot of α1 in control cells, cells overexpressing α1 and mutants, α1 and α2 siRNA-treated cells, or α1 and α2 knockdown cells expressing an RNAi-resistant α1 or α1 S47A. (J) Relative expression levels of α1 when knocked down or overexpressed. Bars = 10 μm.

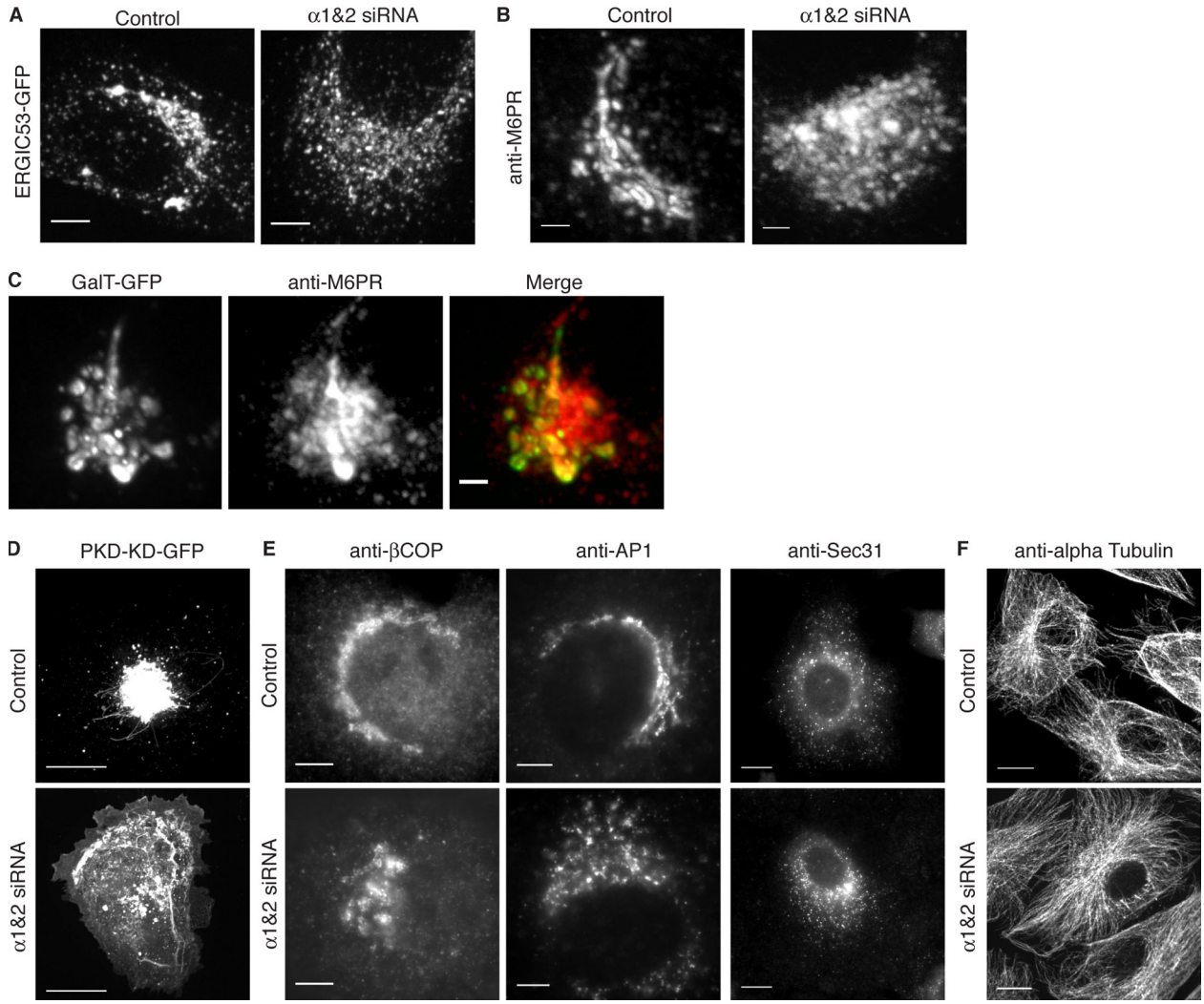

Figure S3. **The ERGIC and TGN are perturbed in α1 and α2 knockdown cells, and the TGN is associated with mini-stacks.** α1 and α2 knockdown does not affect vesicle coat protein or microtubule distribution but alters protein kinase D localization. (A) Reduced α1 and α2 levels disrupt ERGIC structure. Control or α1 and α2 siRNA-transfected BTRD cells transiently transfected with ERGIC53-GFP under a low expression promoter. (B) Knockdown of α1 and α2 in BTRD cells fragments the TGN (M6PR). (C) The TGN in α1 and α2 siRNA-transfected BTRD cells is present in mini-stacks, as seen by the overlap of M6PR (red) and expressed GalT-GFP (green) in confocal images. (D) PKD kinase-dead GFP (PKD-KD-GFP) distribution is affected by α1 and α2 siRNA knockdown, as seen by confocal microscopy. (E) The localization of vesicle proteins COPII (Sec31), COPI (β-COP), and AP1 clathrin (γ-adaptin) is unchanged by α1 and α2 knockdown. (F) Microtubules (α-tubulin) are unaffected with α1 and α2 knockdown. Bars: B and C = 2 μm; A and E (except Sec31) = 5 μm; D, Sec31 (E), and F = 10 μm.