Bowman et al., http://www.jcb.org/cgi/content/full/jcb.201512068/DC1

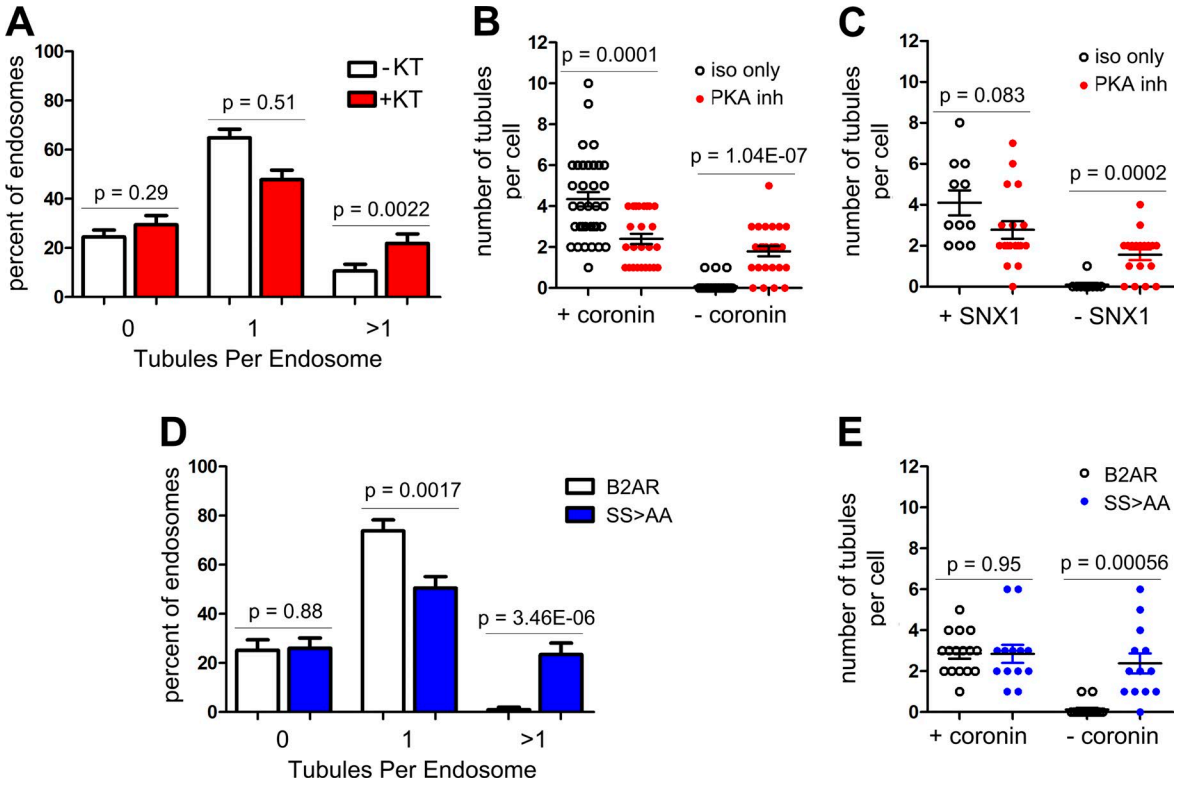

Figure S1. **PKA inhibition and B2AR SS>AA increase the number of B2AR tubules per endosome.** Related to Fig. 1. (A) Percentage of endosomes that contain zero, one, or greater than one tubule before and after PKA inhibition. Bars are the mean across 27 cells (control, –KT) and 30 cells (PKA inhibition, +KT). (B) Number of tubules per cell with or without coronin; data as in Fig. 1 C. (C) Number of tubules per cell with or without SNX1; data as in Fig. 1 E. (D) Percentage of endosomes that contain zero, one, or greater than one tubule for B2AR and SS>AA. Bars are mean across 20 cells (B2AR) and 15 cells (SS>AA). (A and D) Error bars are SEM. (E) Number of tubules per cell with or without coronin for B2AR and SS>AA; data as in Fig. 1 H. (B, C, and E) Black bars show mean and SEM.

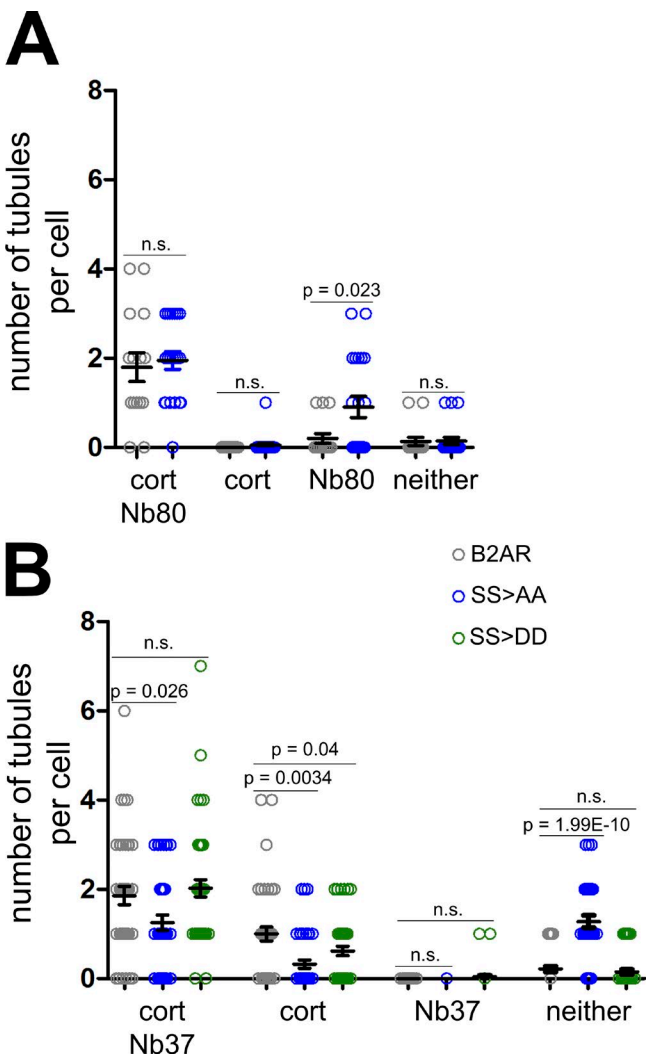

Figure S2. **B2AR, SS>AA, and SS>DD recruit Nb37 exclusively to ASRT tubules.** Related to Fig. 2. (A) Number of tubules per cell that contain the ASRT marker, cortactin, Nb80, or neither marker for B2AR and SS>AA. Nb80 is recruited to both ASRT and non-ASRT tubules. Data as in Fig. 2 C. (B) Number of tubules per cell that contain the ASRT marker, cortactin, Nb37, or neither marker for B2AR, SS>AA, and SS>DD. Data as in Fig. 2 F. Nb37 is recruited only to ASRT tubules. Data and sample size as in Figs. 2 and 3. n.s., not significant. Bars denote mean and SEM.

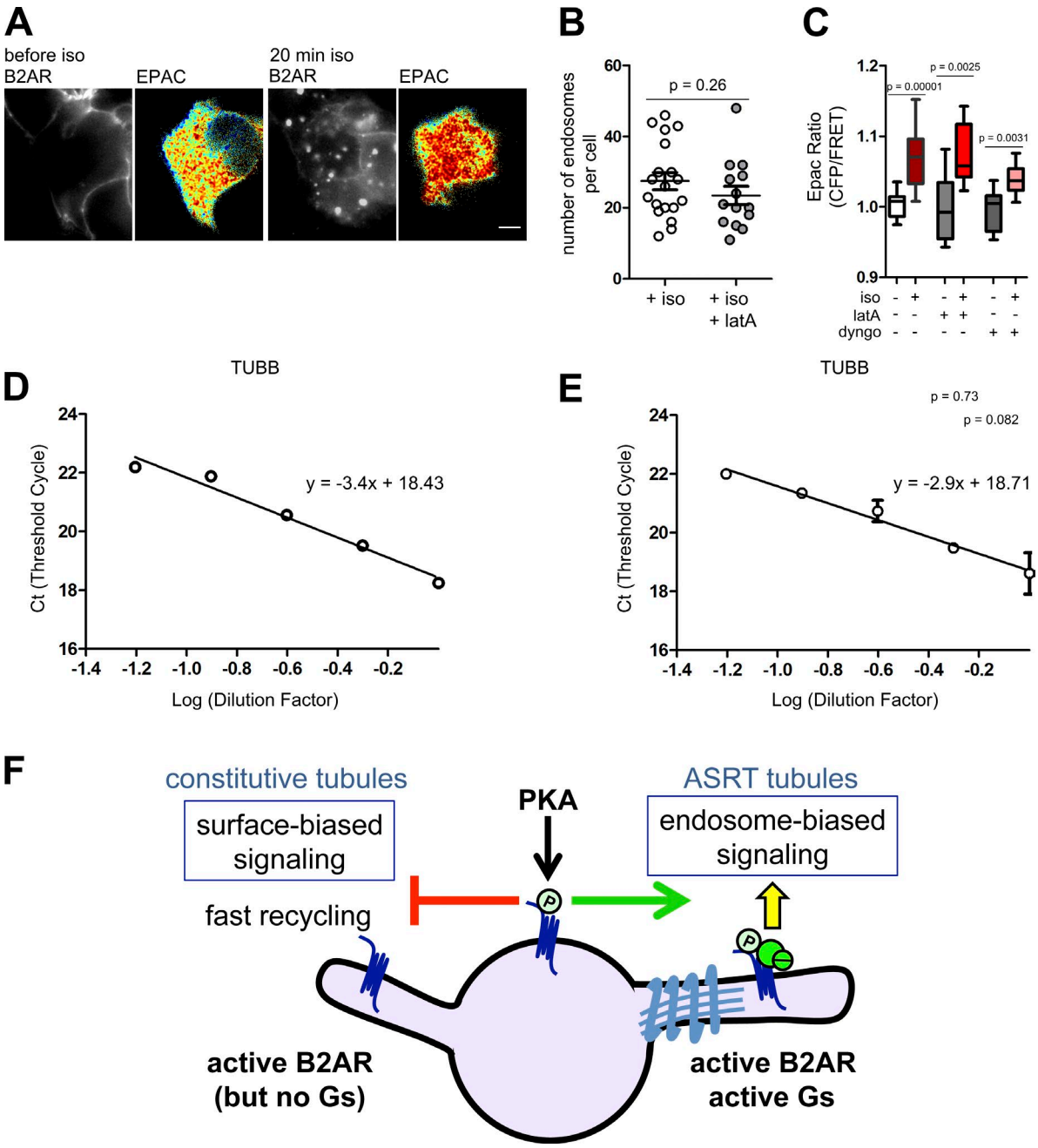

Figure S3. **LatA does not alter B2AR endocytosis or iso-induced cAMP signaling, and TUBB expression is not changed by iso treatment.** Related to Fig. 4 and Fig. 5. (A) Example images of FLAG-B2AR and Epac in HEK 293 cells before and 20 min after cotreatment with iso and latA. Bar, 10 µm. (B) Quantitation of the number of endosomes per cell in cells treated with iso only or cotreated with iso and latA. The number of endosomes per cell 20 min after iso was not significantly different from those with iso and latA cotreatment, suggesting that latA does not alter B2AR endocytosis. Bars are mean and SEM across 19 cells (iso only) and 14 cells (iso and latA). (C) Quantitation of CFP/FRET ratio across multiple B2AR cells. Addition of latA did not significantly change the amount of total cAMP produced 20 min after iso + latA addition, compared with iso addition alone. 15 min of pretreatment with Dyngo-4a reduced cAMP levels 20 min after iso addition, but Dyngo-4a alone did not alter cAMP levels compared with untreated cells. LatA only, n = 8 cells; latA + iso, n = 10 cells; Dyngo-4a only, n = 9 cells; Dyngo-4a + iso, n = 9 cells. (D) Standard curve of 1:16, 1:8, 1:4, 1:2, and 1:1 dilutions, plotted as log (dilution factor) against cycle threshold (Ct) number for TUBB in untreated FLAG-B2AR–expressing HEK 293 cells. TUBB expression increases with increased cDNA input concentration. Line shows linear regression, slope = −3.4. Error is SEM across two replicates. (E) Standard curve of dilutions and TUBB cycle threshold in FLAG-B2AR–expressing HEK 293 cells treated with iso for 2 h. TUBB expression increases with increased cDNA input. Line shows linear regression, slope = −2.9, generating similar slopes (slope to slope ratio of 1.18) between no-treatment and iso conditions, indicating that TUBB expression is independent of iso addition and a suitable reference gene for normalization of PCK1, CGA, and NR4A1 expression. Error is SEM across three separate experimental replicates. (F) Model for PKA-induced regulation of B2AR signaling from ASRT domains. PKA, activated by B2AR itself or by signaling cross talk, causes phosphorylation of the C-terminal tail of B2AR at S345 and S346 and restricts the receptor to ASRT-endosomal microdomains. B2AR activates Gαs exclusively in ASRT microdomains, although it might be in an active conformation throughout the endosome. When PKA becomes less active, B2AR sorts into constitutive (non-ASRT) tubules, where it does not couple to Gαs to produce the endosomal G protein signaling response. The red line represents inhibition of the pathway on the left, and the green line represents activation of the pathway on the right. Fast recycling from these tubules may generate a bias toward signaling from the surface.