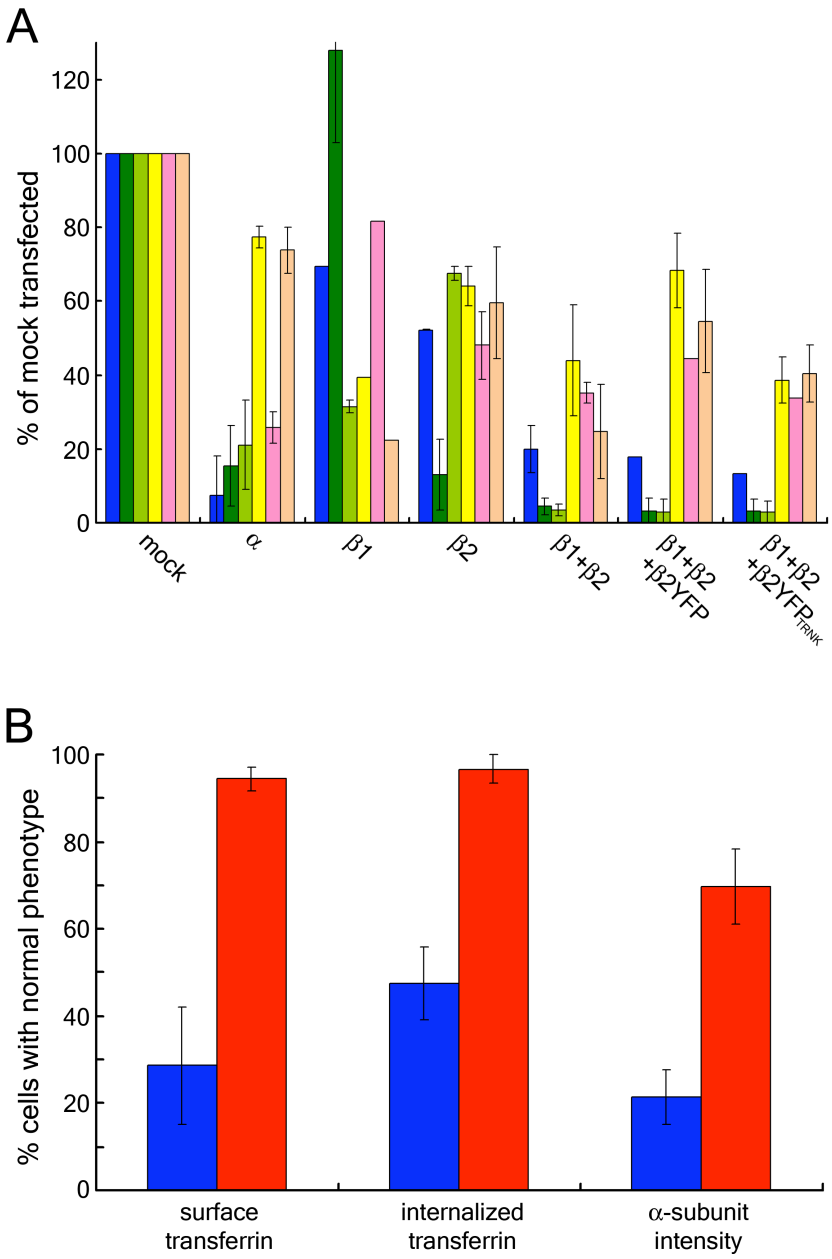
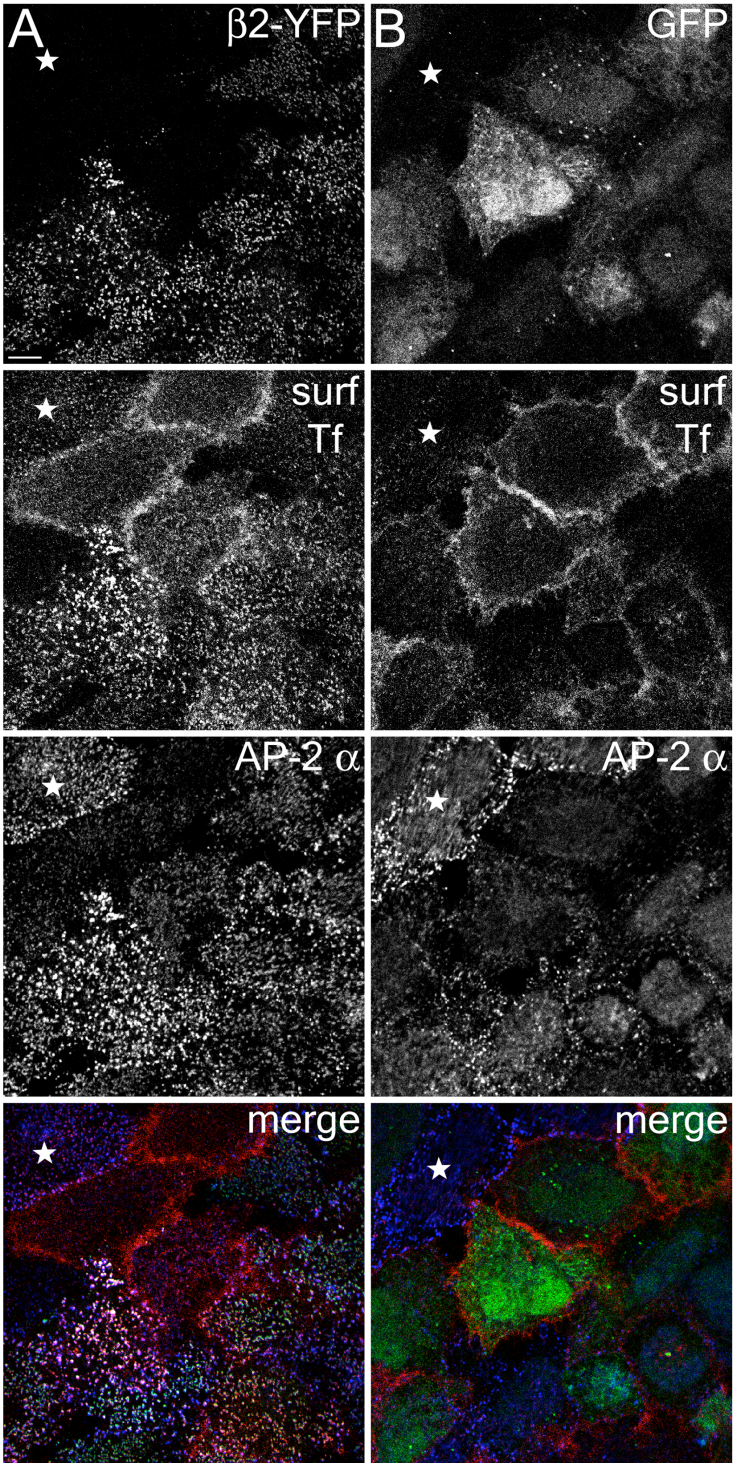
SUPPLEMENTAL DATA



Supplemental Figure S1. Quantitation of RNAi phenotypes and rescue. (A) Quantitation of band intensity of adaptor protein complex subunits on immunoblots of siRNA-treated HeLa SS6 cell lysates. Band intensities were measured in Metamorph as described in Materials and Methods, normalized to clathrin expression, and then normalized to expression in mock-treated cells. siRNA treatment and reconstitution conditions, if any, are indicated on the x-axis while the y-axis indicates level of adaptor subunit expression. The color scheme follows Fig. 4: AP-2 α-subunit, blue; AP-2 β2 subunit, forest green; AP-1 β1 subunit, lime green; AP-1 γ subunit, yellow; AP-2 μ2 subunit, pink; AP-1 μ1 subunit, salmon. (B) Quantitation of β1+β2-subunit RNAi and rescue phenotypes. Cells treated with β1+β2-subunit siRNAs and either no plasmid DNA (blue bars) or the β2-YFP (red bars) were scored for three phenotypes associated with normal AP-2 levels: surface labeled transferrin in bright punctate structures, significant transferrin internalization after 15 min, and punctate AP-2 α-subunit staining. The percentage of cells ((surface transferrin: 327 YFP-positive cells, 1067 YFP-negative cells; 15 min transferrin uptake: 78 YFP-positive cells cells, 414 YFP-negative cells; α-subunit intensity: 286 YFP-positive cells cells, 868 YFP-negative cells analyzed) expressing normal phenotypes of each condition is shown.



Supplemental Figure S2. Addition of plasmid DNA does not compromise RNAi. HeLa SS6 cells transfected with β1+β2-subunit siRNAs and either β2-YFP (A) or GFP (B) (top row, green in merge) plasmids were surface labeled with Tf568 (second row, red in merge), fixed and stained with anti-α-subunit mAb AP.6 (third row, blue in merge). Note that expression of GFP clearly does not interfere with the transferrin uptake phenotype that results from β1+β2-subunit gene silencing. The asterisk indicates a non-silenced cell in the field.