Expanded View Figures

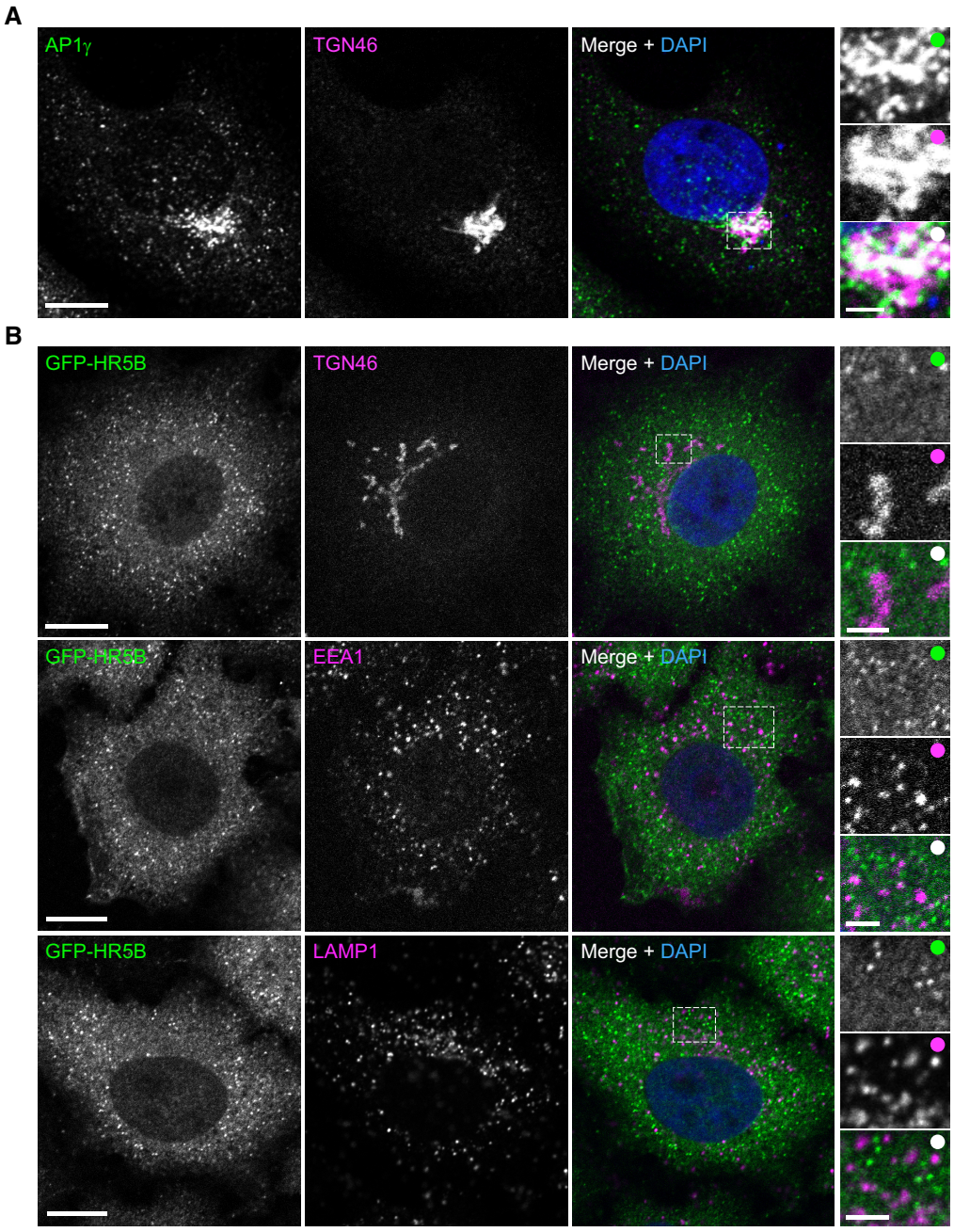

Figure EV1. Localisation of AP1γ and GFP-HEATR5B with respect to other structures in fixed human cells.

A, B Representative confocal images of wild-type (A) and stable GFP-HEATR5B (B) HeLa cells stained with antibodies to the indicated proteins (GFP signal in panel (B) was amplified with GFP antibodies). Dashed box shows area magnified in right-hand images. Panel (A) shows that AP1γ is clustered at the periphery of the TGN. Panel (B) shows that association of GFP-HEATR5B (HR5B) with TGN46, EEA1 and LAMP1 is rarely observed. Scale bars: main panels, 10 μm; insets, 2.5 μm. White circles indicate merge of magnified images.

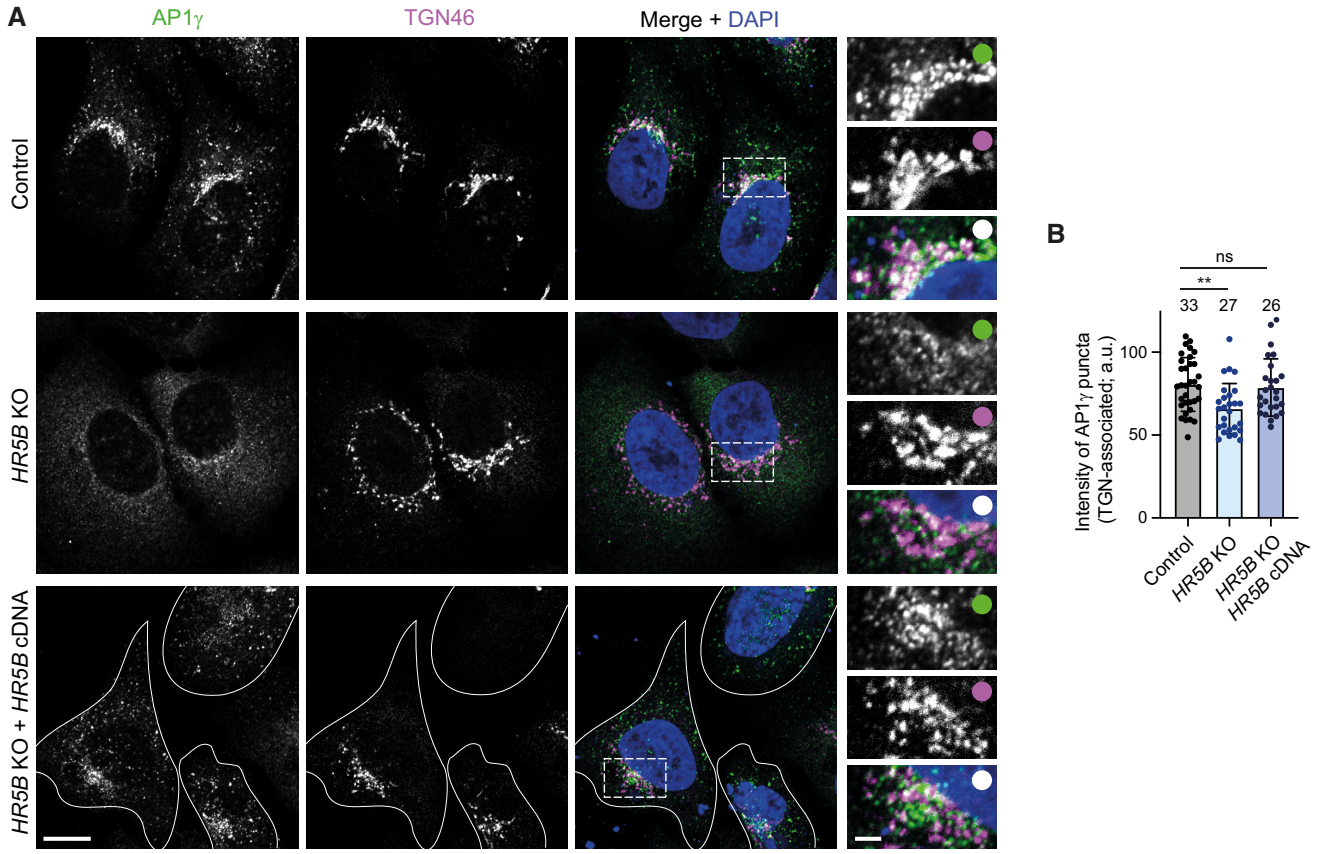

Figure EV2. Disruption of HEATR5B partially impairs association of AP1γ with the TGN.

A Representative confocal images of control (parental) U2OS cells, *HR5B* KO U2OS cells and *HR5B* KO U2OS cells transfected with a GFP-HR5B expression plasmid stained with antibodies to AP1γ and TGN46. For illustrative purposes, the *HR5B* KO cells shown are amongst those with a strong reduction in TGN-associated AP1γ signal. Dashed box shows area magnified in right-hand images. White outlines show mutant cells that express GFP-HR5B (as assessed by imaging the GFP channel). Scale bars: (A) 10 μm; (A) insets: 2.5 μm. White circles indicate merge of magnified images.

B Quantification of mean intensity of TGN-associated AP1γ puncta. Circles indicate values from individual cells, with columns and error bars representing mean ± SD. Number of cells analysed is shown above columns. Statistical significance was evaluated with a one-way ANOVA test with Dunnett's multiple comparisons correction: $^{**}P < 0.01$.

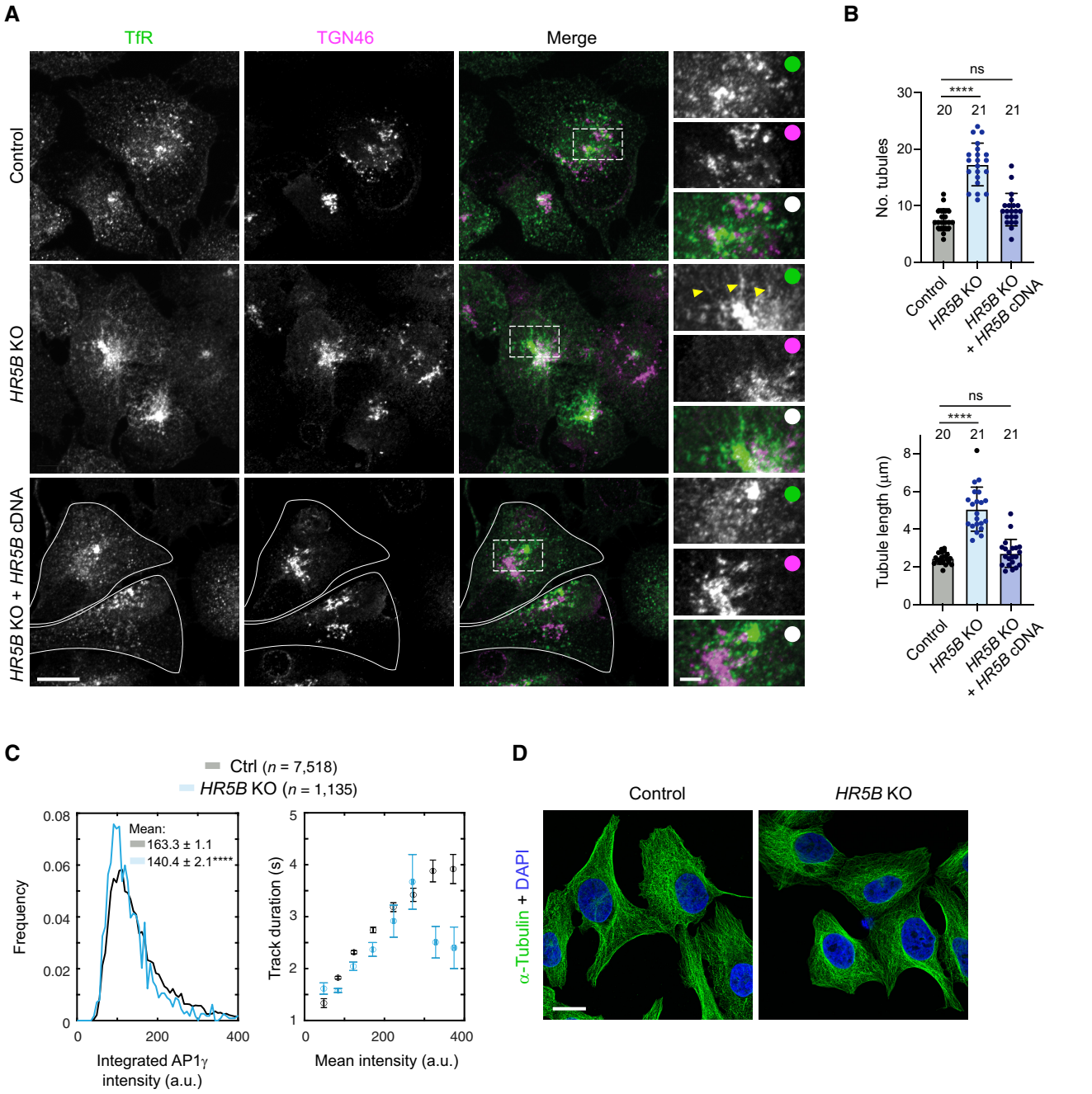

Figure EV3. Supplementary data on *HEATR5B* KO phenotypes.

A Representative confocal images of control (parental) U2OS cells, *HR5B* KO U2OS cells and *HR5B* KO U2OS cells transfected with a GFP-HR5B expression plasmid stained with antibodies to Transferrin receptor (TfR), which marks the recycling compartment in association with the TGN, and TGN46. Dashed box shows area magnified in right-hand images. Arrowheads show examples of tubulation in *HR5B* KO cells. White outlines show mutant cells that express GFP-HR5B (as assessed by imaging the GFP channel). White circles indicate merge of magnified images.

B Quantification of number and length of TfR-positive, TGN-associated tubules. Circles indicate values from individual cells, with columns and error bars representing mean ± SD. Number of cells analysed is shown above columns. Statistical significance was evaluated with a one-way ANOVA test with Dunnett's multiple comparisons correction. ****$P < 0.0001$.

C Quantification of AP1σ1-RFP particle intensity (left) and track duration versus mean particle intensity (right) in image series of control and *HR5B* KO live U2OS cells. n = number of particles (from 31 control and 17 KO U2OS cells). Errors represent SEM. In left-hand panel, a shift in the distribution of intensity values to the left in mutant cells indicates dimmer fluorescence (statistical significance was evaluated with a Mann–Whitney U test. ****$P < 0.0001$).

D Representative confocal images of control (parental) and *HR5B* KO U2OS cells stained with α-tubulin antibodies, showing no overt difference in the architecture of the microtubule cytoskeleton.

Data information: Scale bars: (A) 15 μm; (A) insets, 3 μm; (D) 20 μm.

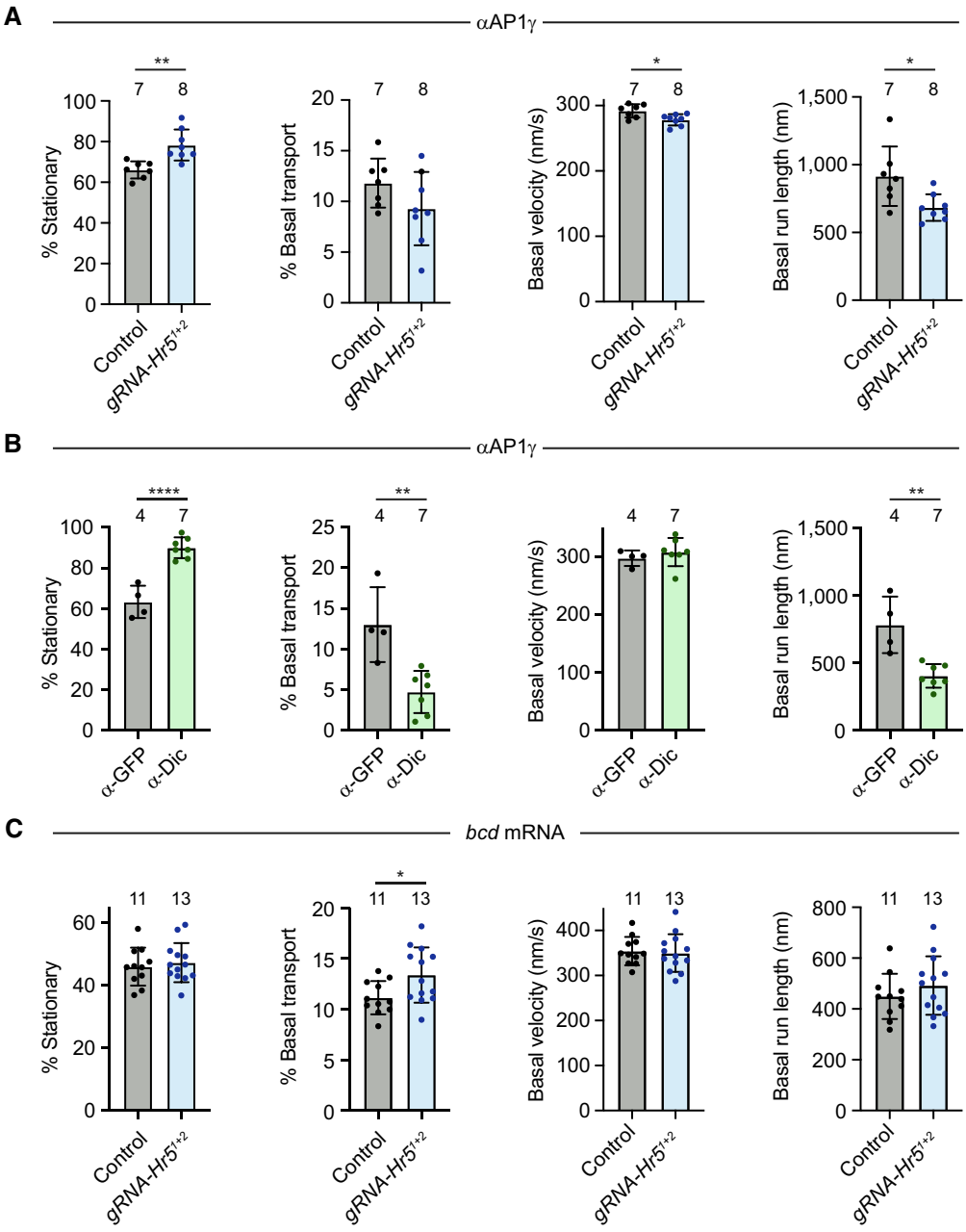

Figure EV4. Quantification of frequency of stationary events and basal transport in AP1γ and bcd mRNA motility assays.

A–C Quantification of the indicated parameters for AP1γ (A, B) and *bcd* mRNA (C) in embryos of control (*nos-cas9*) and *nos-cas9*, *gRNA-Hr5*$^{1+2}$ mothers (A, C) or wild-type embryos pre-injected with function-blocking Dic antibodies or control GFP antibodies (B). "% stationary" and "% basal" are the percentages of particle trajectory time that are classed as immobile or undergoing basal transport, respectively. Circles are mean values for individual embryos; columns and error bars represent means ± SD of these mean values; numbers of embryos injected is shown above columns. At least 24 particles were analysed per embryo. Statistical significance was evaluated with an unpaired two-tailed *t*-test: ****$P < 0.0001$; **$P < 0.01$; *$P < 0.05$.

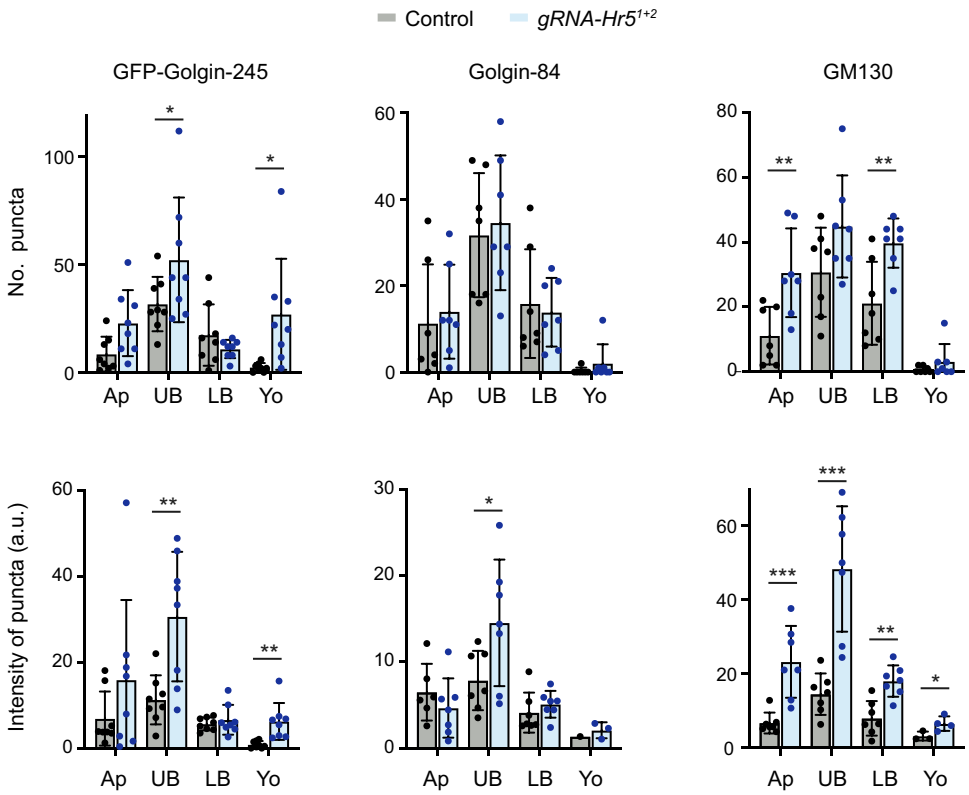

Figure EV5. Quantification of Golgi protein localisation in control and *Heatr5* mutant blastoderm embryos.

Charts show values for number of puncta and intensity of puncta for the indicated golgin proteins in different regions of the cytoplasm of embryos from control (*nos-cas9) and nos-cas9, gRNA-Hr5*$^{1+2}$ *mothers (Ap, apical to the nuclei; UB, upper basal region; LB, lower basal region; Yo, yolk). Columns and error bars show mean of values per embryo ± SD; circles show mean values for individual embryos. At least 50 puncta were analysed per embryo. Statistical significance was evaluated with an unpaired two-tailed t-test: ***P < 0.001; **P < 0.01; *P < 0.05. Consistent phenotypes were observed in two biological replicates; quantification is from a single biological replicate.*