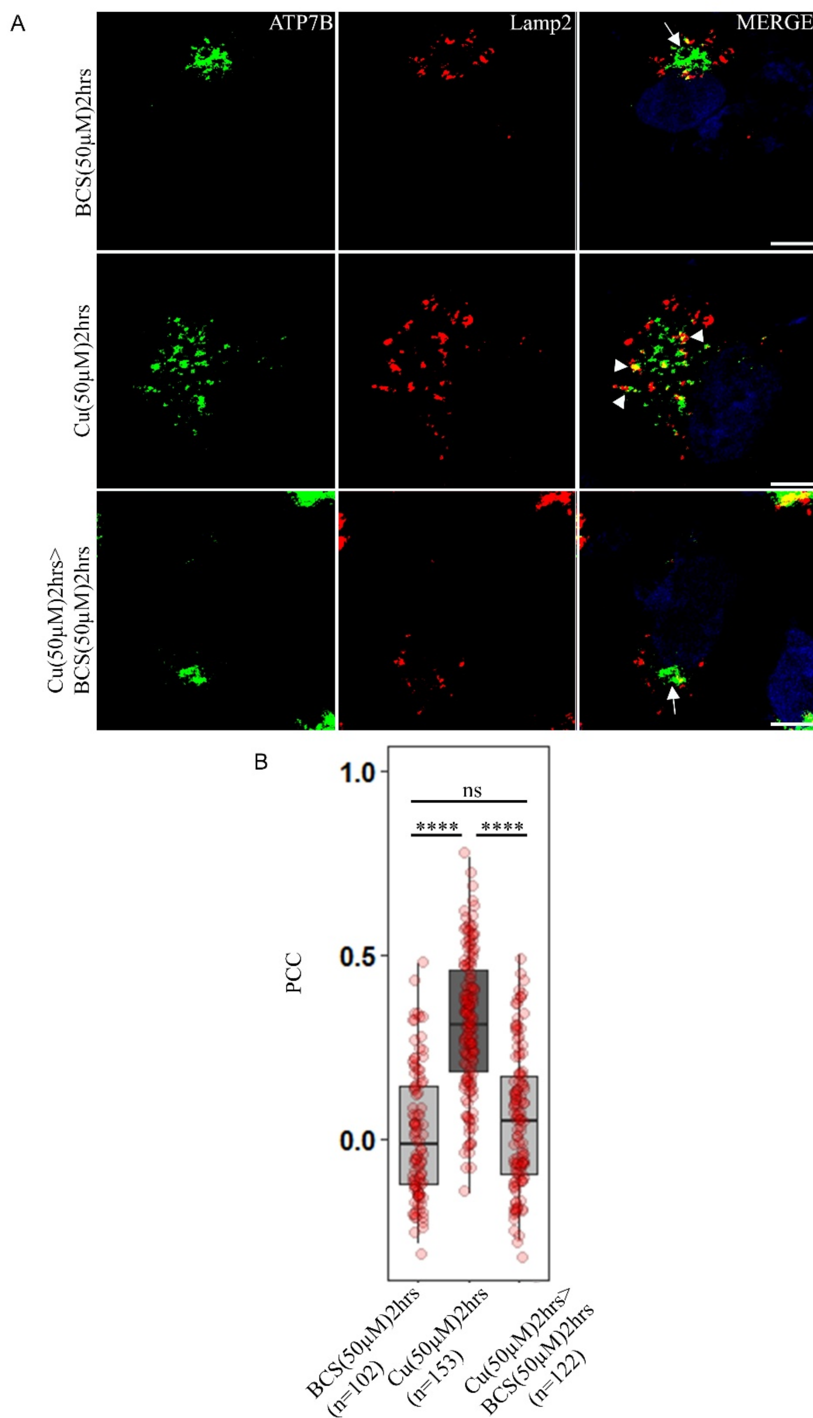

Fig. S1: Fig. S1: **ATP7B colocalizes with Lamp2 at high copper:** (A) Colocalization of ATP7B (green) with Lamp2 (red) in copper limiting, BCS (top panel) and 50µM copper (panel 2) and copper depletion post copper treatment (bottom panel). Arrowhead represents vesicularized ATP7B and arrows points at perinuclear positioned ATP7B. Scale bars represents 5µM. Blue signal represents DAPI staining for nucleus. (B) Pearson's correlation coefficient of colocalization between ATP7B and Lamp2 at different copper conditions demonstrated by a box plot with jitter points is illustrated at the bottom. ($p<0.0001$). The number of cells counted to obtain the data for each condition is denoted by 'n' (for B).

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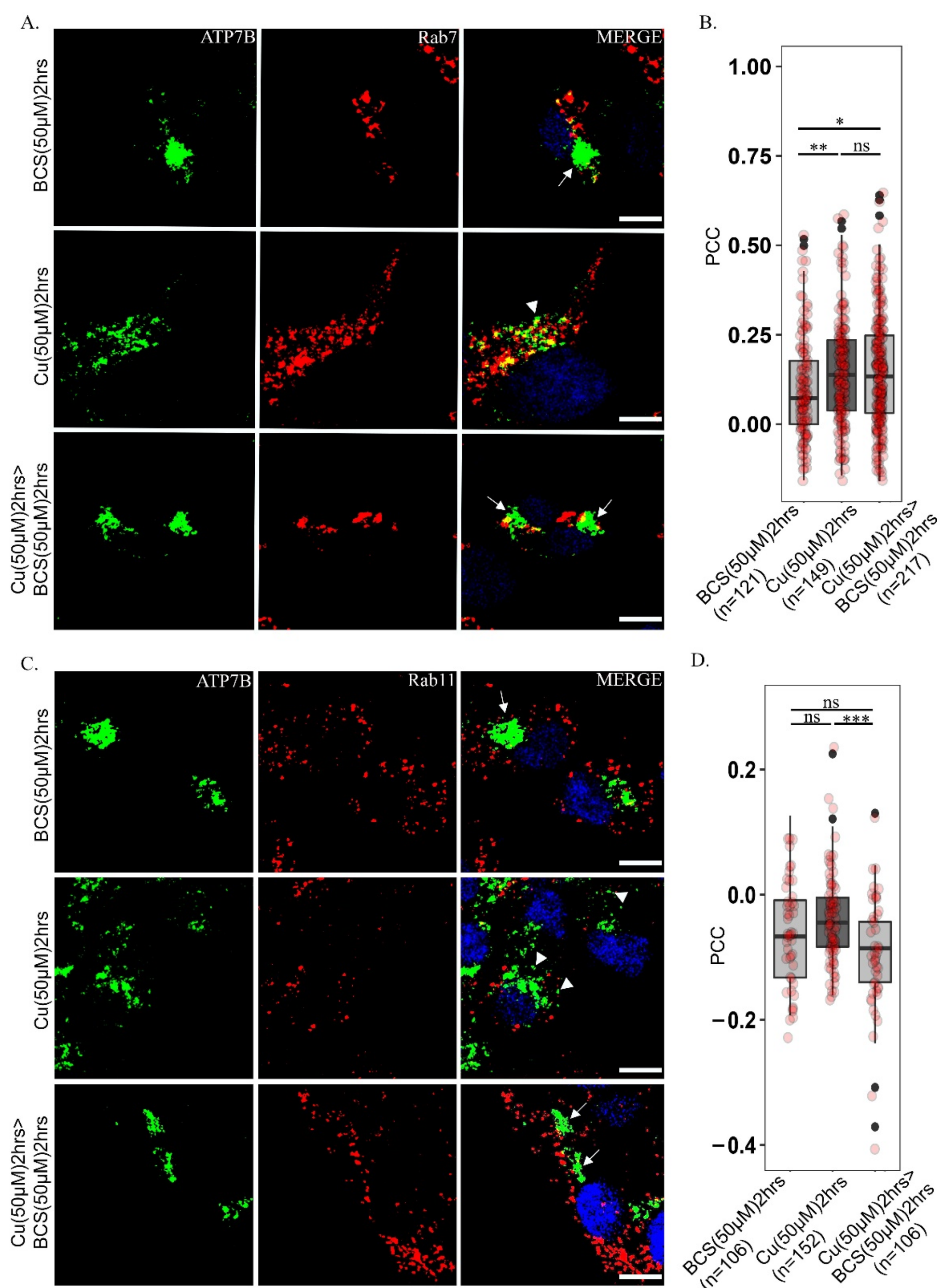

Fig. S2: **Colocalization of ATP7B with late (Rab7) and mid (Rab11) endosomal markers at different copper levels:** (A) Colocalization of ATP7B (green) with late endosome marker, Rab7 (red) in copper limiting, BCS (top panel) and 50uM copper (panel 2) and copper depletion post copper treatment (bottom panel). Scale bars represents 5μM. Blue signal represents DAPI staining for nucleus. (B) Pearson's correlation coefficient of colocalization between ATP7B and Rab7 at different copper conditions demonstrated by a box plot with jitter points. (C) Colocalization of ATP7B (green) with recycling endosomal marker, Rab11 (red) in different copper conditions (D) Pearson's correlation coefficient of colocalization between ATP7B and Rab11 at different copper conditions demonstrated by a box plot with jitter points. (*p<0.1, **p<0.01, ***p<0.001). The number of cells counted to obtain the data for each condition is denoted by 'n' (for B &D)

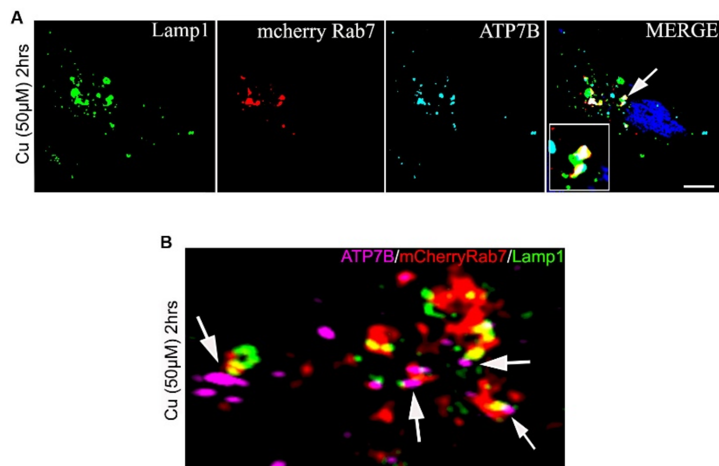

Fig. S3: **ATP7B is preferentially located in Rab7-Lamp1 positive endosomes in high copper**: (A) Colocalization of ATP7B (cyan) with lysosomal marker, lamp1 (green) and late endosome marker, mCherry Rab7 (red) at 50μM copper. The fig in the inset is representative of the region marked with an arrow. (B) 3D representation of Structured Illumination Microscopy (SIM) image of same with 100nm resolution. ATP7B is marked in magenta, Lamp1 in green and mCherry Rab7 in red. Arrow represents triple merging. Blue signal represents DAPI staining for nucleus. Scale bars represents 5μM

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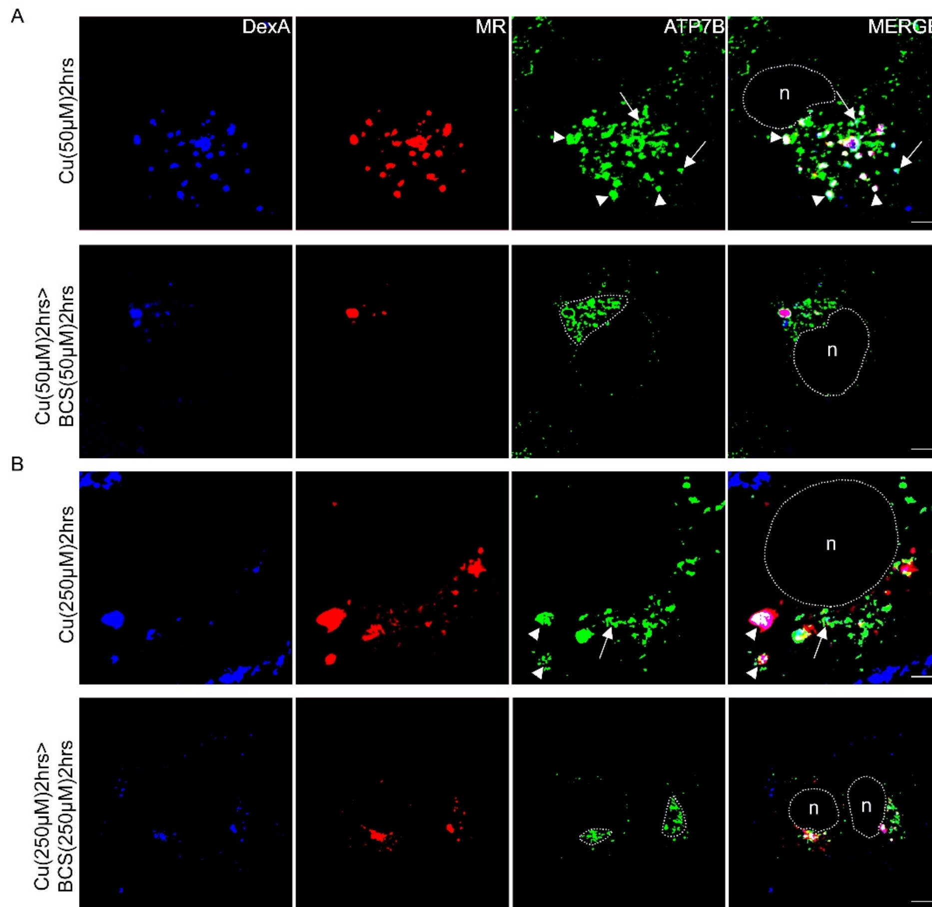

Fig. S4: **ATP7B shows preferential localization at acid-hydrolase positive endolysosomes at high copper:** Split channel illustration of Fig2 displaying colocalization of ATP7B (green), Alexa-647-Dextran (blue) and Magic Red, MR (red) in (A) 50µM copper (upper panel) and 50µM > 50µM BCS (lower panel) and (B) 250µM copper (upper panel) and 250µM > 250uM BCS (lower panel). Arrowhead: ATP7B in active endolysosomes and Arrow: ATP7B in storage lysosomes

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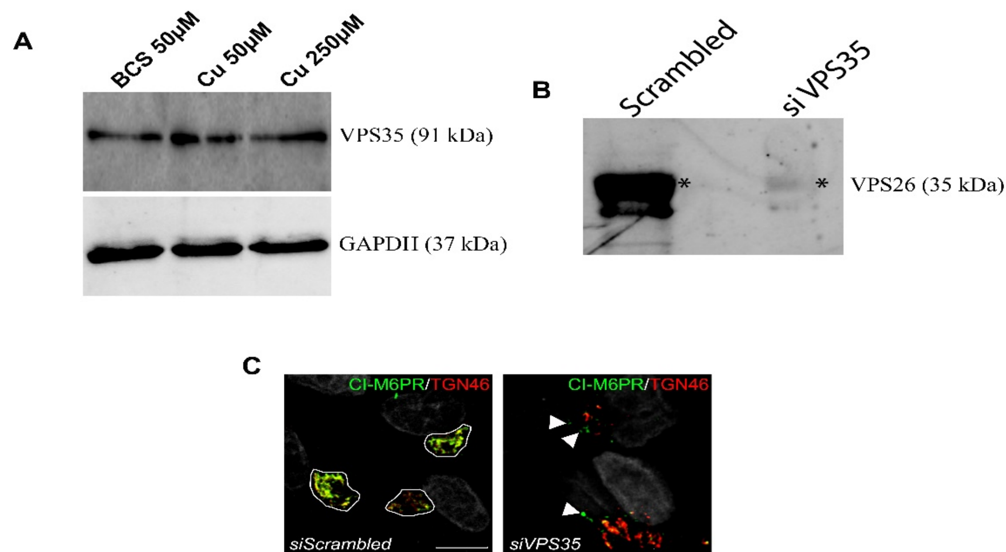

Fig. S5: (A) **Immunoblot of VPS35 in different Copper conditions:** Upper panel shows abundance of VPS35 remain unchanged in all copper conditions. Lower panel shows the same for GAPDH, as a control for cytosolic proteins. (B) **Immunoblot of VPS26 in siVPS35 HepG2:** siRNA mediated knockdown of Vps35 in HepG2 cells shows downregulation of its core partner VPS26 as compared to its control. (*) denotes the VPS26 protein. (C) **Knockdown of VPS35 abrogates recycling of CI-M6PR:** Colocalization of CI-M6PR (green) and TGN46 (red) in cells transfected with siRNA against VPS35 or scrambled siRNA (control). Vesicular puncta of CI-M6PR was noted in addition to its TGN localization in VPS35 KD cells, as against its complete localization in TGN in control cells

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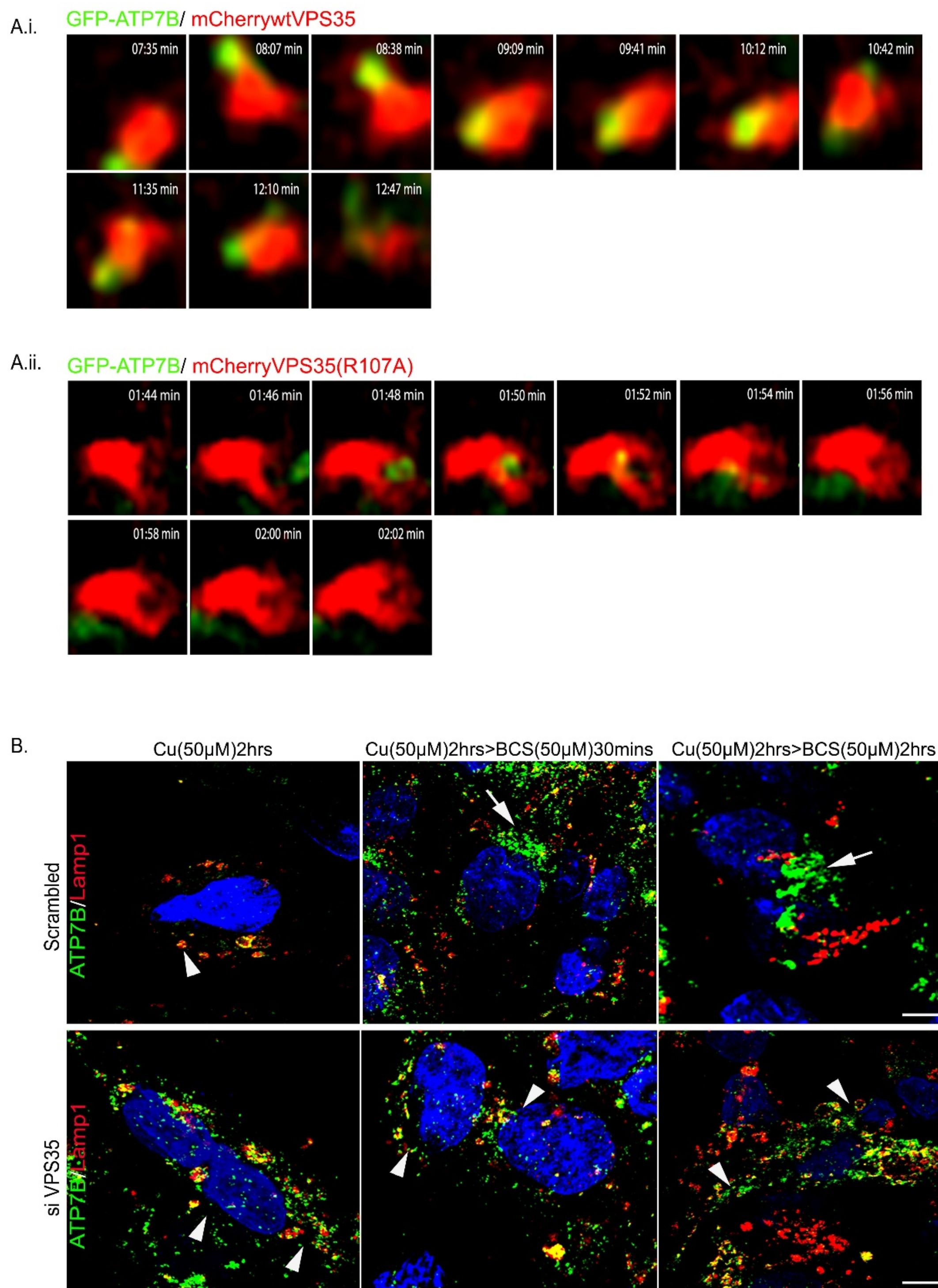

Fig. S6: **Comparative dwell time analysis of ATP7B and wtVPS35 vs its mutant, VPS35 R107A**: Live-cell time-lapse high resolution deconvolution confocal microscopy shows dwell time of GFP-ATP7B with mCherry VPS35-WT to be ≈ 4 mins (Ai) and for mutant ≈ 3 seconds (Aii) in 50uM copper. Images were taken at every 1.964 s interval. (B) **VPS35 regulates retrieval of ATP7B from endolysosome to TGN:** siVPS35 treated (bottom panel) and scrambled RNA treated (upper panel) HepG2 cell shows colocalization of ATP7B (green) with Lamp1 (red). Cells treated with copper for 2h (left image), subsequent copper depletion for 30 mins (center) and 2h (right image). Arrowhead shows ATP7B located in endolysosomal vesicles marked with Lamp1 and arrows shows perinuclear ATP7B indicative of its TGN localization. Scale bars represent 5μM. Blue signal represents DAPI staining for nucleus.

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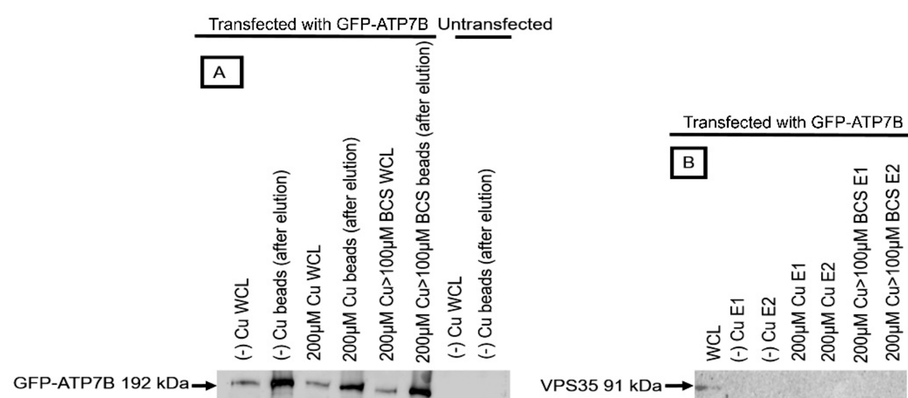

Fig. S7: **Co-immunoprecipitation assay to determine the interaction between full length ATP7B and VPS35:** Cell was transfected with GFP-ATP7B and treated with different copper conditions as mentioned. Lysates were incubated with GFP-trap beads. Untransfected cells were used as negative control. (A) Immunoblot showing the presence of GFP-ATP7B in whole cell lysates and GFP-trap beads after elution. (B) GFP-trap immune-co-precipitated products were subjected to immunoblot as indicated. Abbreviations: *FT: Flow through; WCL: Whole cell lysate, E: Eluate.*

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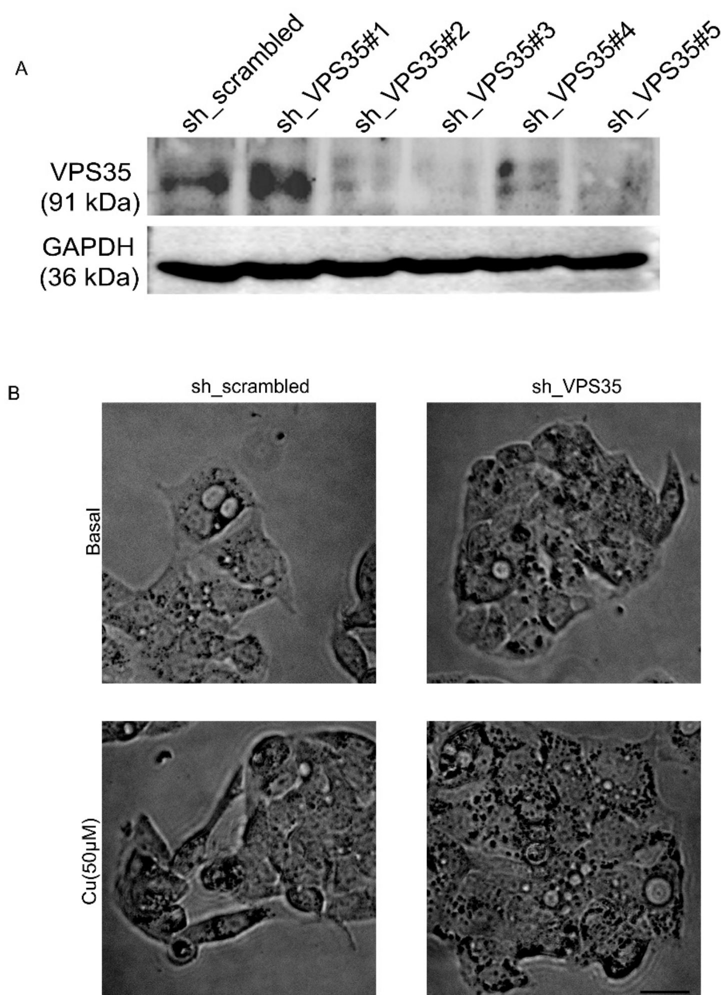
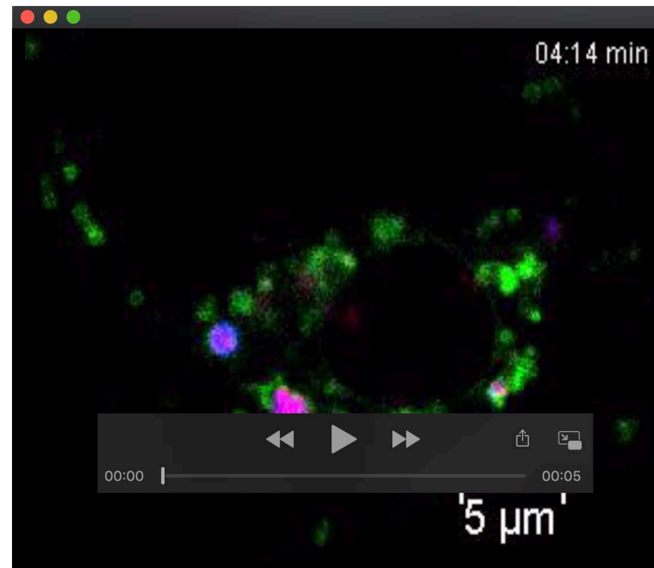
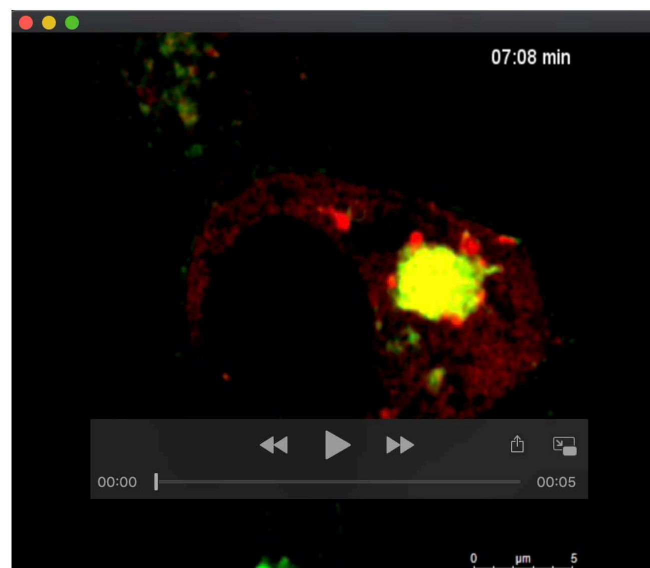


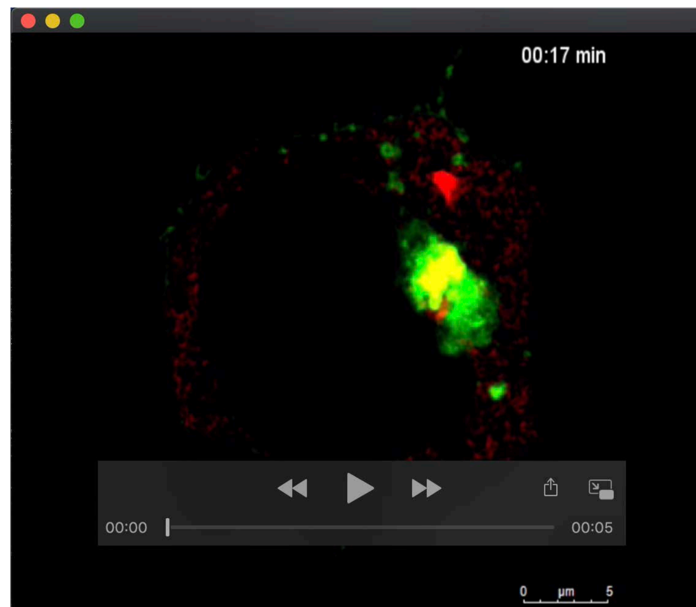
Fig. S8: (A) **shRNA mediated VPS35 knockdown:** VPS35 knockdown in HepG2 cells using lentivirus mediated delivery of 5 different shRNA targeted against 5 unique regions of the gene. shRNA #2,3 and 5 shows robust knockdowns. GAPDH is used as a control. These were subsequently used in experiments. (B) **Cell morphology using bright field images** of HepG2 cells: no apparent change in the cell morphology was noticed in cells with VPS35 expression silenced by lentiviral mediated delivery of shRNA (right images, top and bottom) or not (non-targeting shRNA) (left, top and bottom) in two different copper conditions. Scale bar represents 100 μM.



Movie 1: Time lapse imaging to record colocalization of ATP7B (green), Magic red (red) and DexA (blue) in high copper.



Movie 2A: Time lapse imaging to record colocalization of GFP-ATP7B (green) and mCherry-wt-VPS35 (red) in high copper.



Movie 2B: Time lapse imaging to record colocalization of GFP-ATP7B (green) and mCherry-R107A-VPS35 (red) in high copper.