**Supplementary material- Figure Legends**

**Fig. S1. APP structure and processing.** APP is an integral transmembrane (TM) protein that can be processed by two alternative processing pathways. (A) Non-amyloidogenic pathway. APP is cleaved by α- and γ- secretases to release the extracellular ectodomain (sAPPα), a non-toxic 3 kDa peptide (p3) and the APP intracellular domain (AICD). (B) Amyloidogenic pathway. APP is cleaved by β- and γ- secretases to release the toxic β-amyloid (Aβ) peptide, ectodomain (sAPPβ) and the AICD fragment. APP contains two copper binding sites located at the N-terminus (residues 135-175) and within the Aβ sequence (H6, H13 and H14). A zinc-binding site has also been identified at the N-terminus (residues 181-188).

**Fig. S2. Copper promotes an increase in the level of cell surface APP in SH-SY5Y.** (A) To label proteins at the cell surface, SH-SY5Y cells were incubated with NHS-SS-Biotin following copper (150μM) or copper-chelator (150 μM) treatment (3 hrs) (as previously described in (38,42)). Biotinylated proteins were analysed by western blot and APP detected using the W0-2 antibody. The molecular weight (kDa), as determined using protein standards, is indicated in the left hand side of the panels. Black arrows indicate the glycosylated forms of APP present in the total cell lysate. This western blot is representative of three independent experiments. (B) Following densitometry analysis, the level of cell surface APP was compared to that of intracellular total APP in cell lysates. As represented in the graph, there is an approximate 2-fold increase of cell surface APP following copper treatment in comparison to copper-chelator treatment (n=3, P=0.009).

**Fig. S3. Trafficking of APP is observed at lower copper concentrations for a longer incubation time.** MDCK-APP-cherry cells were incubated with media supplemented with either 50 μM copper-chelators (i) or with 25 μM copper (ii) for approximately 12 hours. Scale bar = 5 μm.

**Fig. S4. APP traffics in response to increases in copper (Cu) levels but not iron (Fe) or zinc (Zn).** MDCK-APP-cherry stable cells were incubated in growth medium supplemented with 150 μM of copper ($CuCl_2$), iron (FeCl3) or zinc ($ZnSO_4$) for 3 hours. The cellular localisation of APP-cherry following metal treatment was compared to its localisation under basal conditions. Scale bar = 5 μm.

**Fig. S5. The APP monoclonal antibody (W0-2) recognises APP-cherry following copper or copper-chelator treatment.** APP antibody W0-2 was used to detect APP in APP-cherry transfected MDCK cells following chelator (-Cu) or copper (+Cu; 150 μM) treatment. The W0-2 antibody confirmed the re-distribution of APP-cherry following increases in intracellular copper is APP specific. Scale bar =5 μm.

**Fig. S6. Copper-responsive APP trafficking is not due to an increase in copper-mediated upregulation of *APP* expression.** (A) SH-SY5Y cells were incubated in growth media supplemented with either copper-chelators (i) or copper (ii) for 3 hr. The level of APP following these treatments was measured by western blot analysis. The level of actin was analysed as a loading control. (B) SH-SY5Ycells were treated with growth media supplemented with copper-chelators (-Cu) or copper (+Cu; 150 μM) in the presence of 50 μg/ml cycloheximide, a protein synthesis inhibitor. Following 3 hr of incubation, the localisation of endogenous APP was investigated by immunofluorescence and confocal microscopy. Scale bar =10 μm.

**Fig. S7. Schematic diagram of APP antibody epitopes and secretase cleavage sites.** APP antibodies used in this study are shown in bold and recognition sites indicated by parentheses. The α-secretase (cleaves after M671), β-secretase (cleaves after K687) and γ-secretase (cleaves after either V7111 or A713) cleavage sites are also indicated. Residues in blue correspond to the Aβ(42) sequence. Histidine residues involved in copper binding are highlighted in red.

**Fig. S8. Live cell imaging of copper-stimulated APP-cherry exocytosis in MDCK cells.** MDCK cells stably expressing APP-cherry grown on coverslips were mounted to a "bioptics" live cell

imaging chamber and a sequence of images taken in basal trafficking media using an Olympus FV1000 laser scanning confocal microscope. Images were then taken at 2-minute intervals for approximately 25 minutes following the perfusion of trafficking media supplemented with 150 μM copper. The elapsed time in minutes is indicated on each frame. Scale bar = 5 μm.

**Supplementary Material**

**Fig. S1**

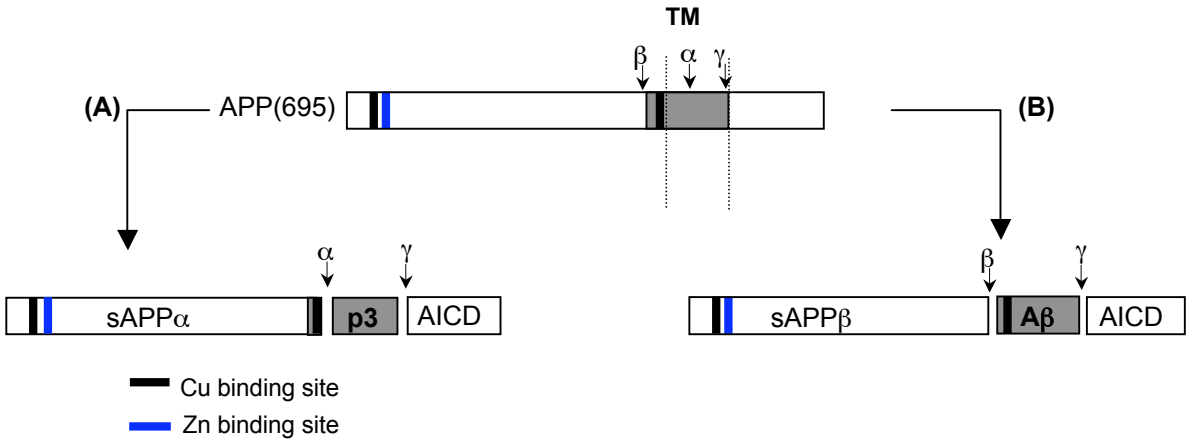


Fig. S2

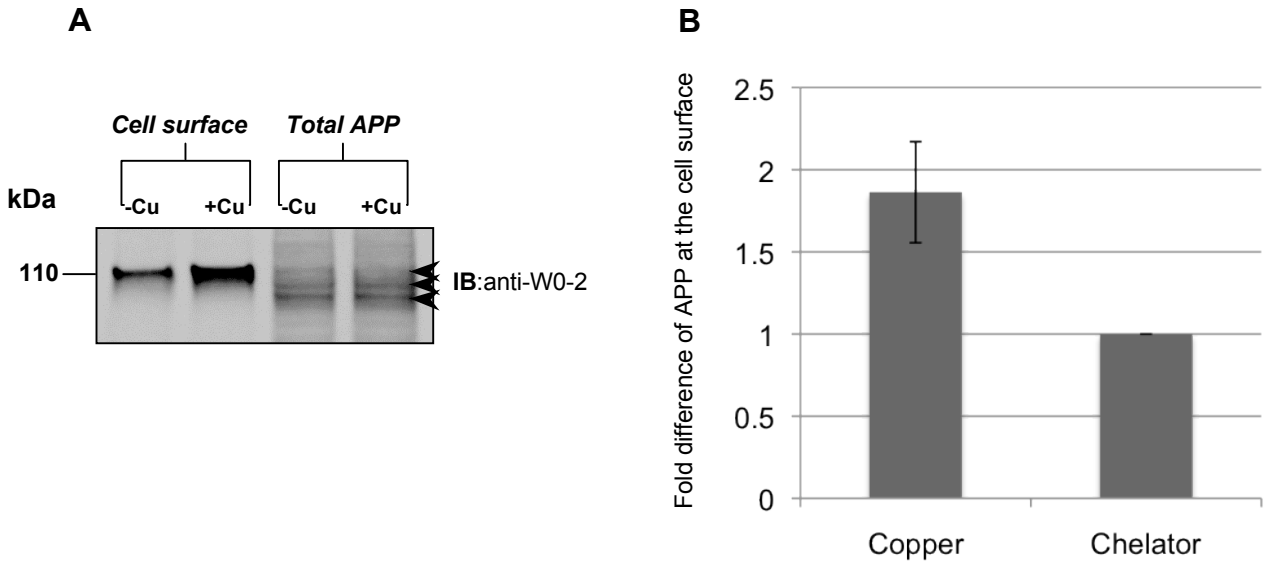

A
Cell surface
Total APP
kDa
-Cu
+Cu
-Cu
+Cu
110
IB:anti-W0-2
B
Fold difference of APP at the cell surface
2.5
2
1.5
1
0.5
0
Copper
Chelator

Fig. S3

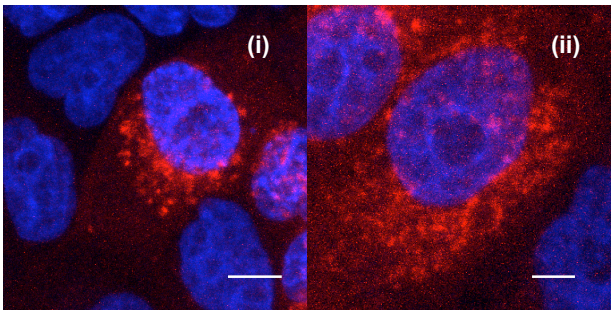

(i)
(ii)

**Fig. S4**

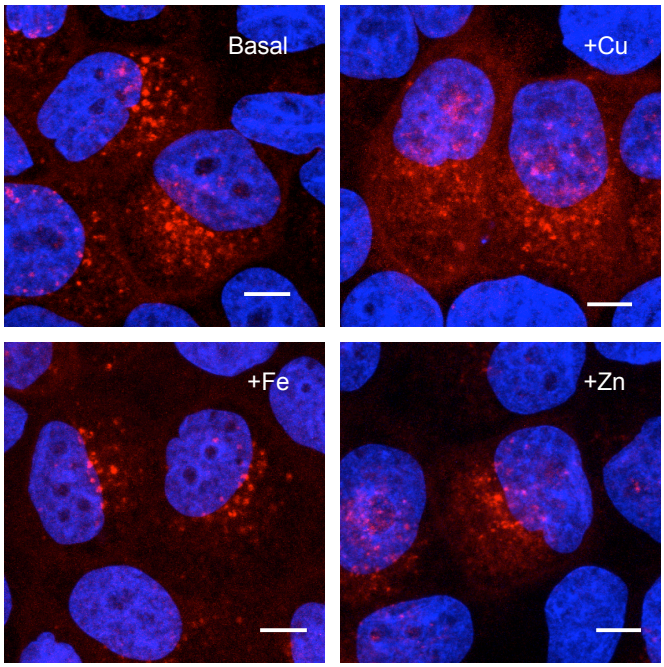


Fig. S5

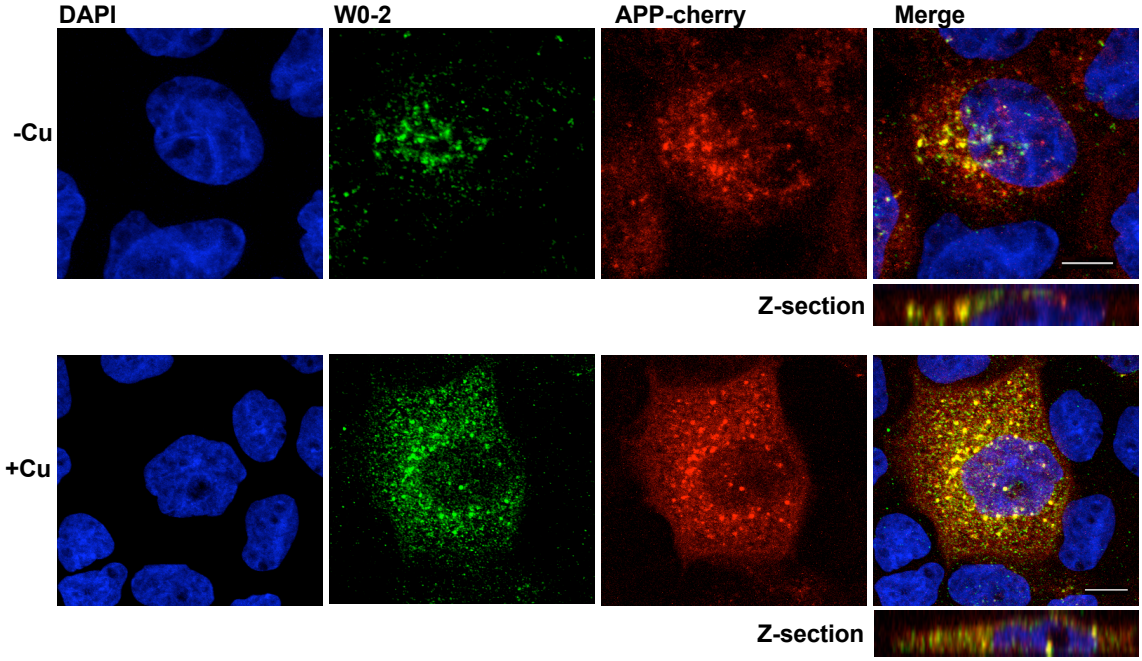
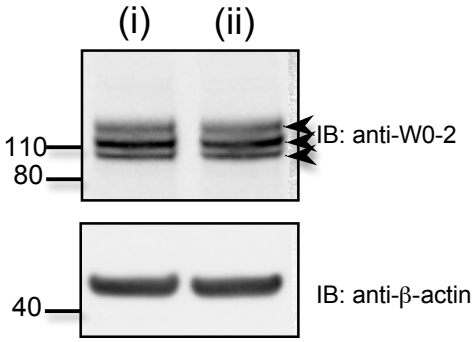


**Fig. S6**

A


(i)
(ii)
110
80
IB: anti-W0-2
40
IB: anti-β-actin

B

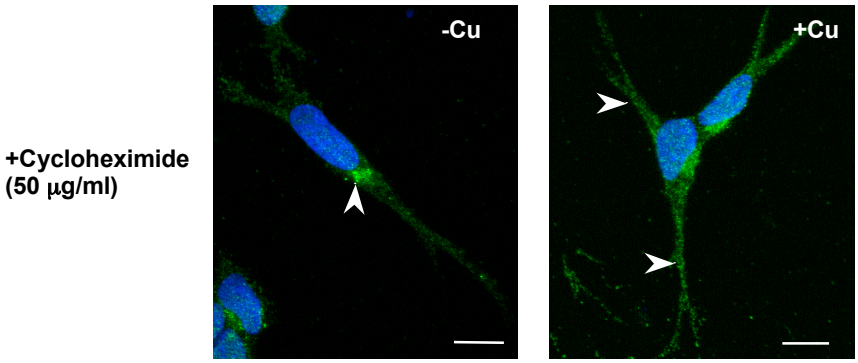

+Cycloheximide
(50 μg/ml)
-Cu
+Cu

**Fig. S7**

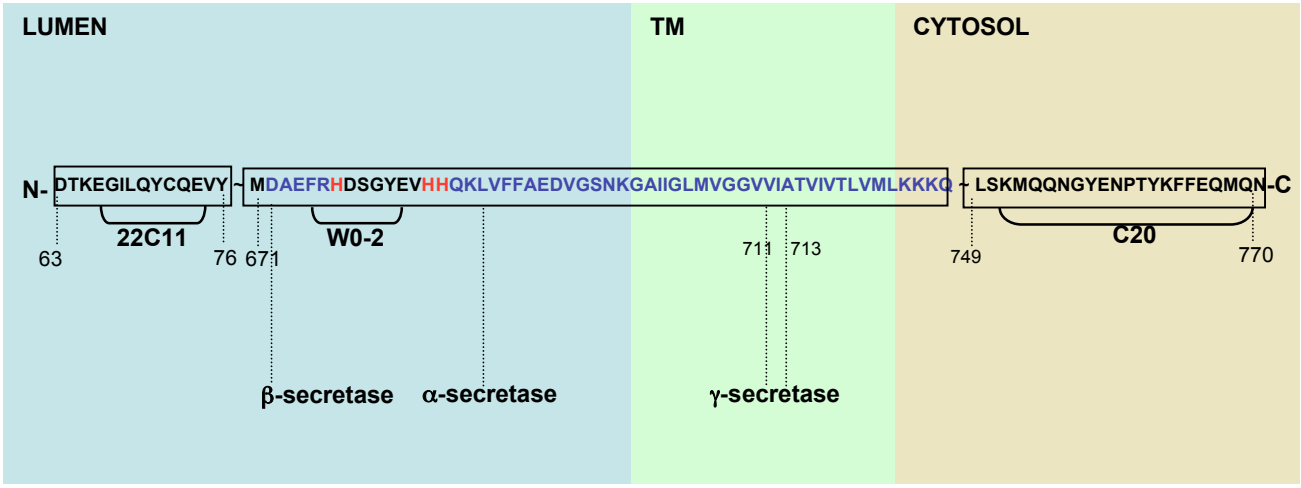



**Fig. S8- see attached Quicktime movie file**