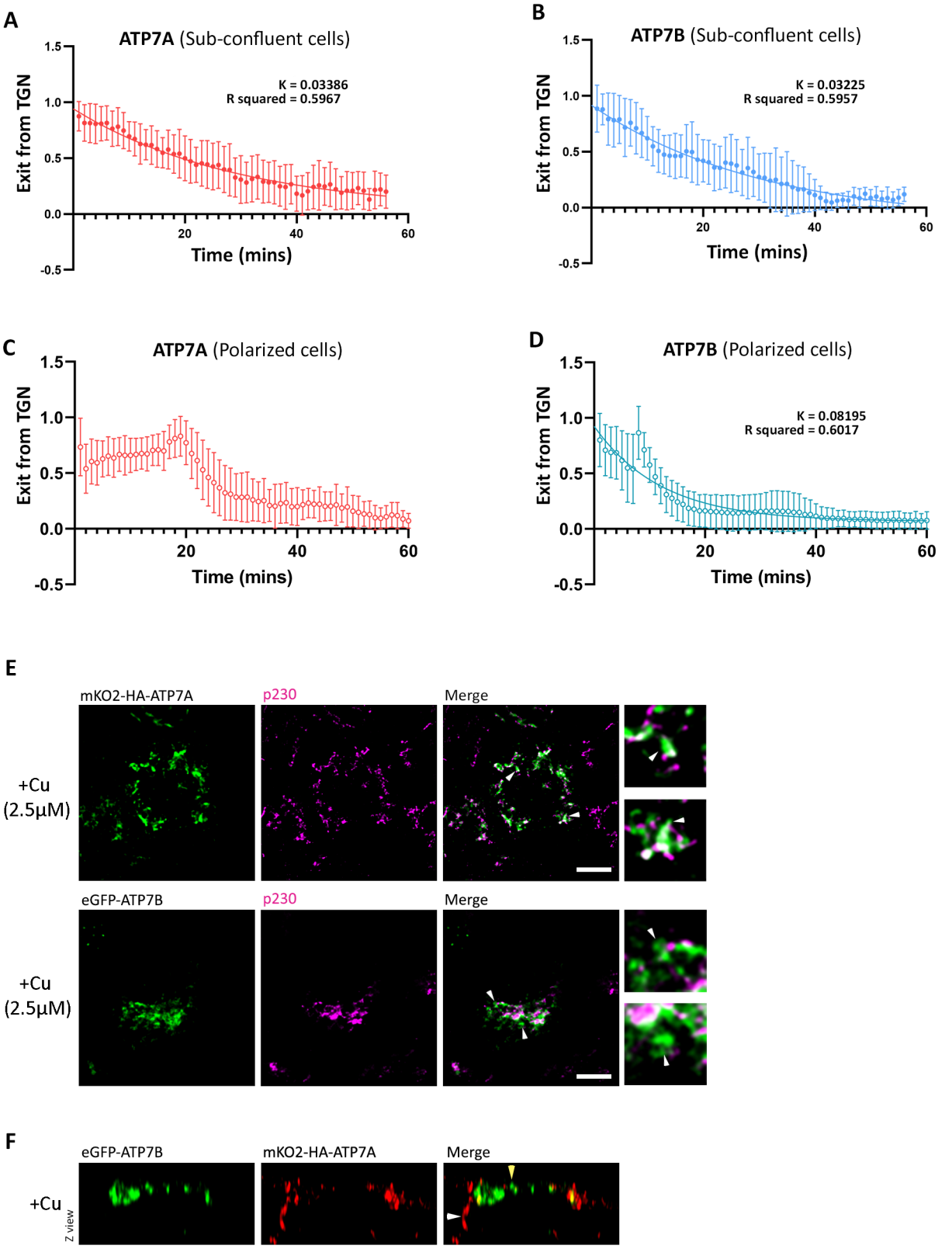


Fig. S1. (A) Fitted curve of decreased pixel count in sub-confluent MDCK cells transfected with mKO2-HA-ATP7A showing its exit rate in response to copper (i.e., k value = 0.03386). (B) Fitted curve of decreased pixel count in sub-confluent MDCK cells transfected with eGFP-ATP7B showing its exit rate in response to copper (i.e., k value = 0.03225). (C) Curve of decreased pixel count in polarized MDCK cells transfected with mKO2-HA-ATP7A showing its exit rate in response to copper. (D) Fitted curve of decreased pixel count in polarized MDCK cells transfected with eGFP-ATP7B showing its exit rate in response to copper (i.e., k value = 0.08195). (E) Representative confocal images of CuCl2 (2.5μM; 30mins) treated cells transfected with either mKO2-HA-ATP7A or eGFP-ATP7B and co-stained with p230. (Arrowheads indicate TGN exited proteins) (F) Confocal images showing co-transfected mKO2-HA-ATP7A and eGFP-ATP7B under copper-treated conditions. Yellow arrow denotes ATP7B localizing at the apical surface. White arrows mark ATP7A at the basolateral surface. Scale bar, 5μm.

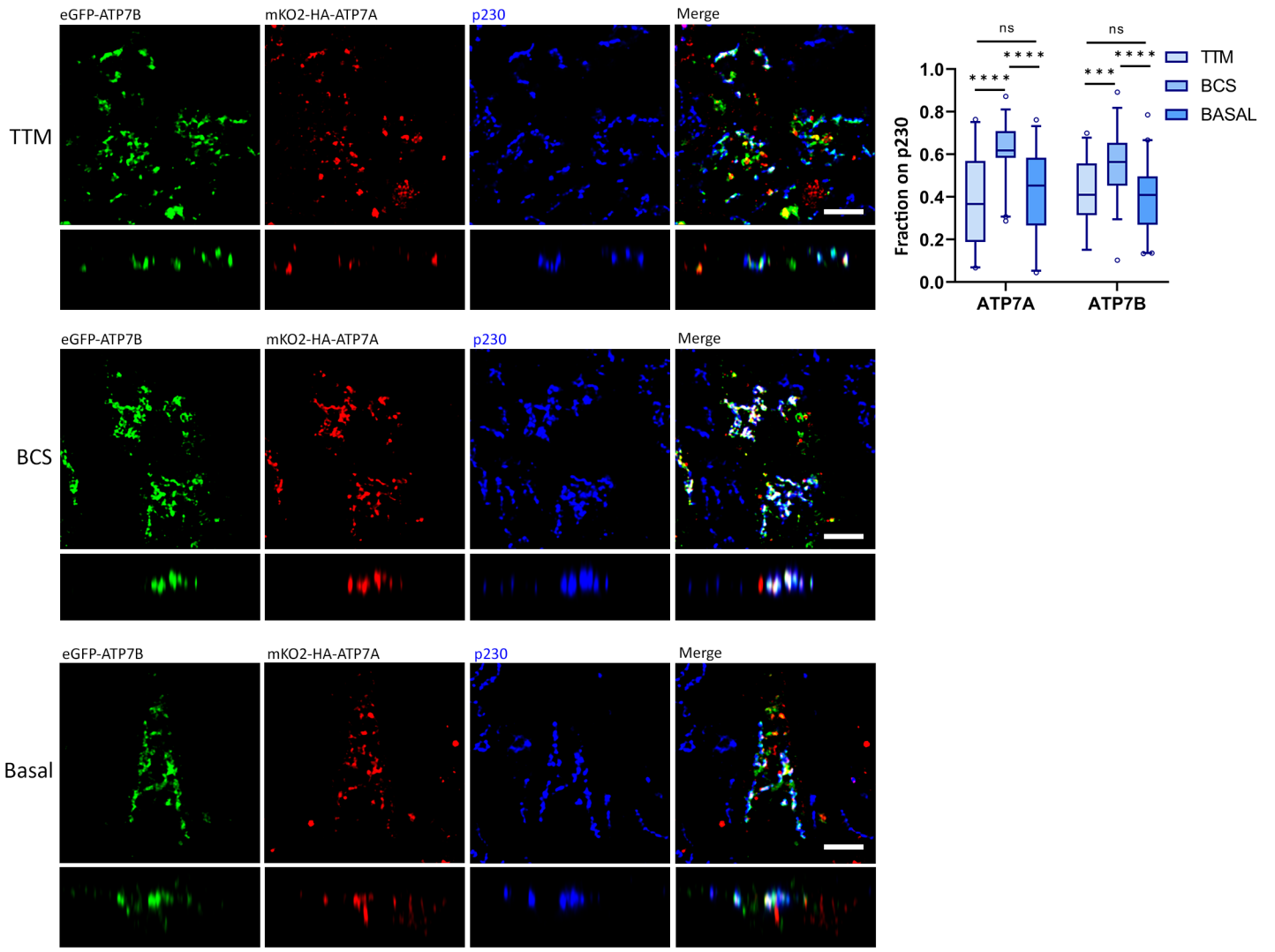
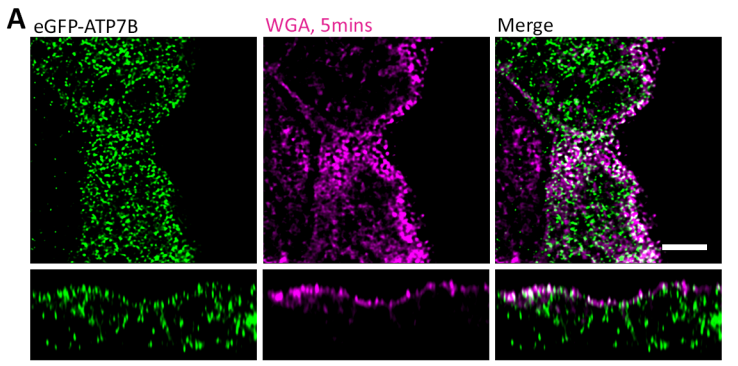
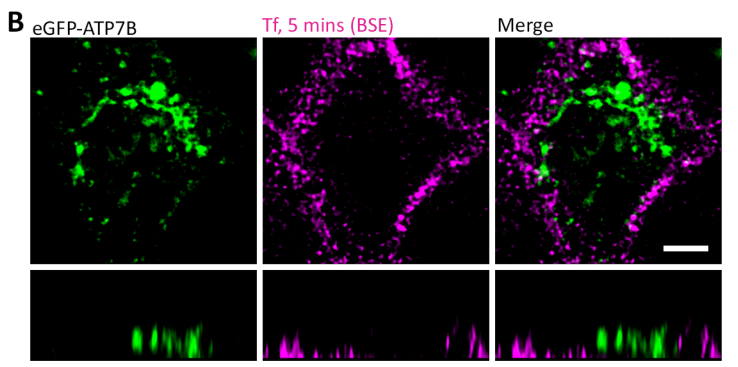
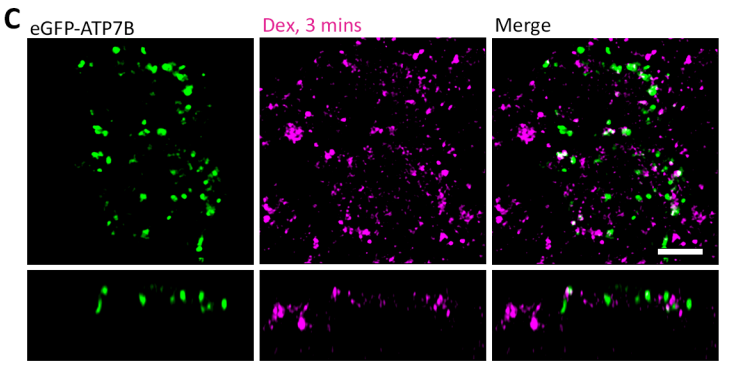


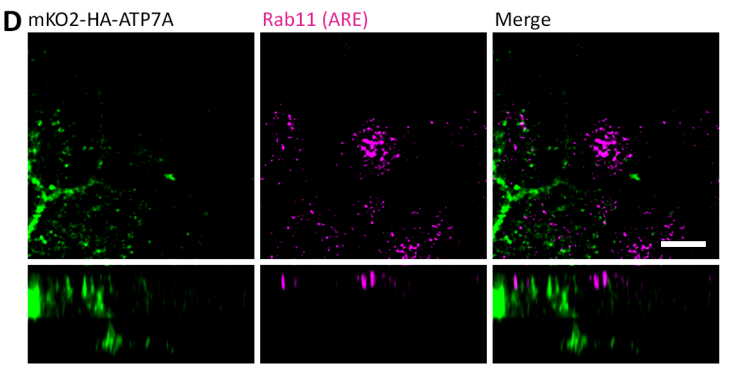
Fig. S2. Polarized MDCK cells showing localization of co-transfected mKO2-HA-ATP7A and eGFP-ATP7B with p230 under limiting copper conditions, i.e., TTM, BCS and Basal. Box-plot showing colocalization quantification (Manders' Colocalization Coefficient) of ATP7A and ATP7B with p230 under different copper conditions, i.e., TTM, BCS and Basal. The box represents the 25th to 75th percentiles, and the median in the middle. The whiskers show the data points within the range of 1.5 × interquartile range (IQR) from the first and third quartile. Sample size (N) for both ATP7A and ATP7B- TTM: 35, BCS: 39, Basal: 43. Scale bar, 5μm.

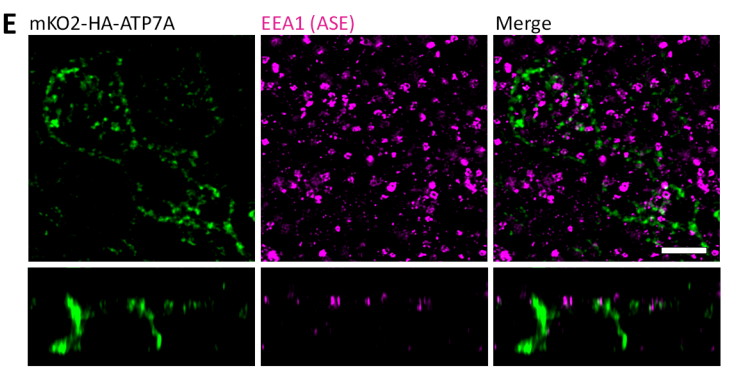
Journal of Cell Science • Supplementary information











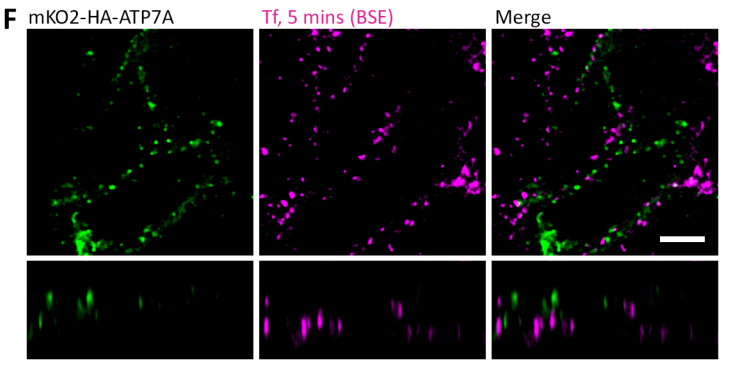
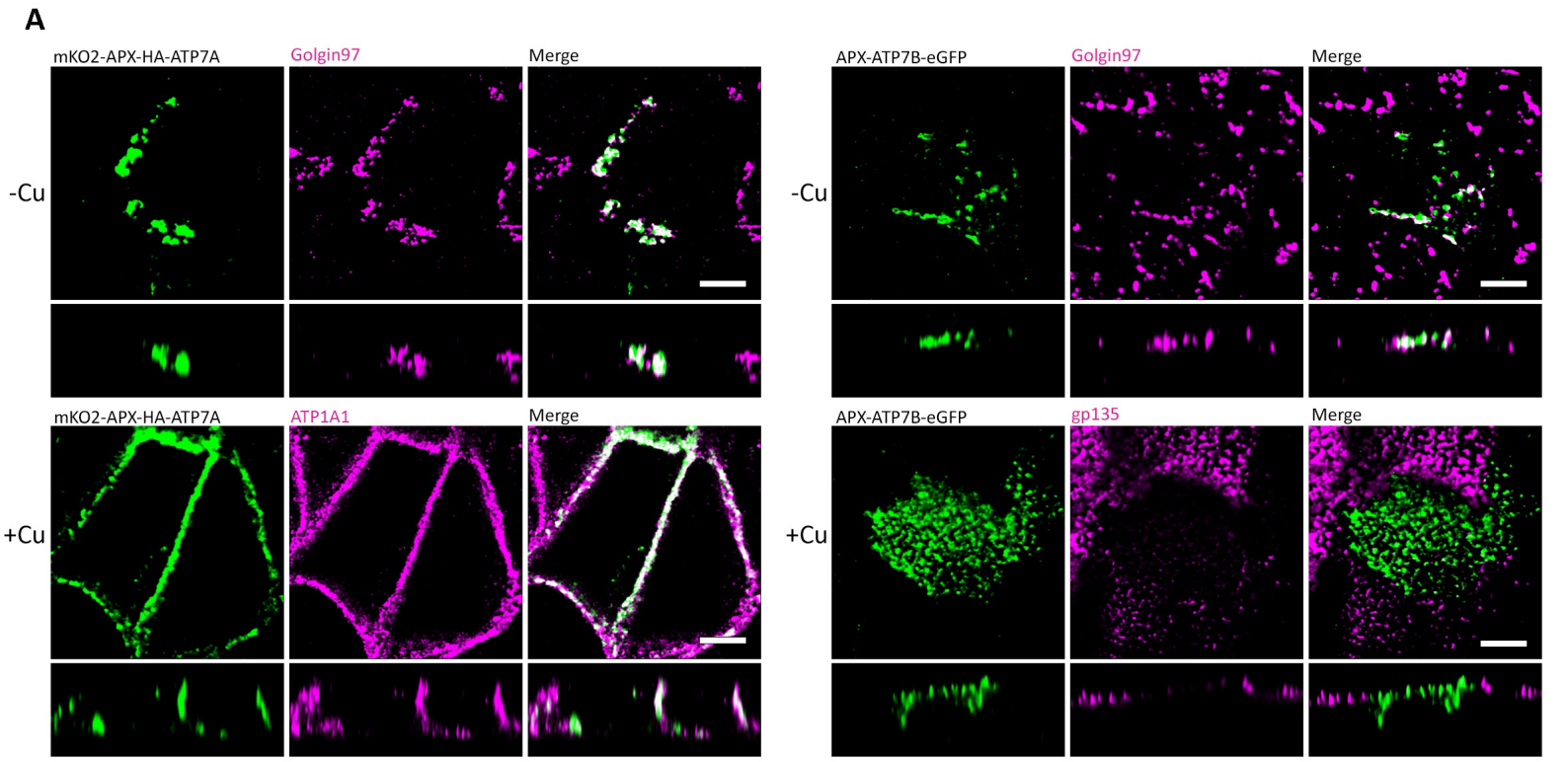


Fig. S3. (A) Confocal image of eGFP-ATP7B (green) co-stained with apically internalized WGA (5 mins) shows presence of ATP7B in ASE in response to copper. (B) Confocal image of eGFP-ATP7B (green) co-stained for BSE (magenta; 5mins Tf Internalization) shows no localization of ATP7B in BSE. (C) Confocal image of eGFP-ATP7B (green) co-stained for early endosomes (magenta; 3mins basolateral dextran uptake) shows no localization of ATP7B in basolateral early endosomes. (D) Confocal image of mKO2-HA-ATP7A (green) co-stained with Rab11 (magenta) shows no localization of ATP7A in ARE (Rab11 positive compartment). (E) Confocal image of mKO-HA-ATP7A co-stained with EEA1 shows no localization of ATP7A in ASE (EEA1 positive compartment). (F) Confocal image of mKO-HA-ATP7A co-stained for BSE shows no localization of ATP7A in Tf (5 min internalized) positive compartments. Scale bar, 5μm.



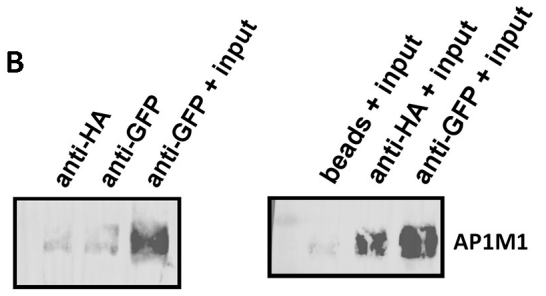


Fig. S4. (A) Polarized MDCK cells showing localization of transfected APEX2-mKO2-HA-ATP7A and APEX2-ATP7B-eGFP where they are localized at TGN in copper deprived conditions and under elevated copper levels traffic to basolateral and apical surfaces respectively, showing wild-type like phenomena. Scale bar, 5μm. (B) Controls for immuno-pulldown of mKO2-HA-ATP7A and eGFP-ATP7B which are immuno-precipitated by anti-HA and anti-GFP antibodies respectively. Isotype and protein control are shown indicating specific pulldowns.

Journal of Cell Science • Supplementary information

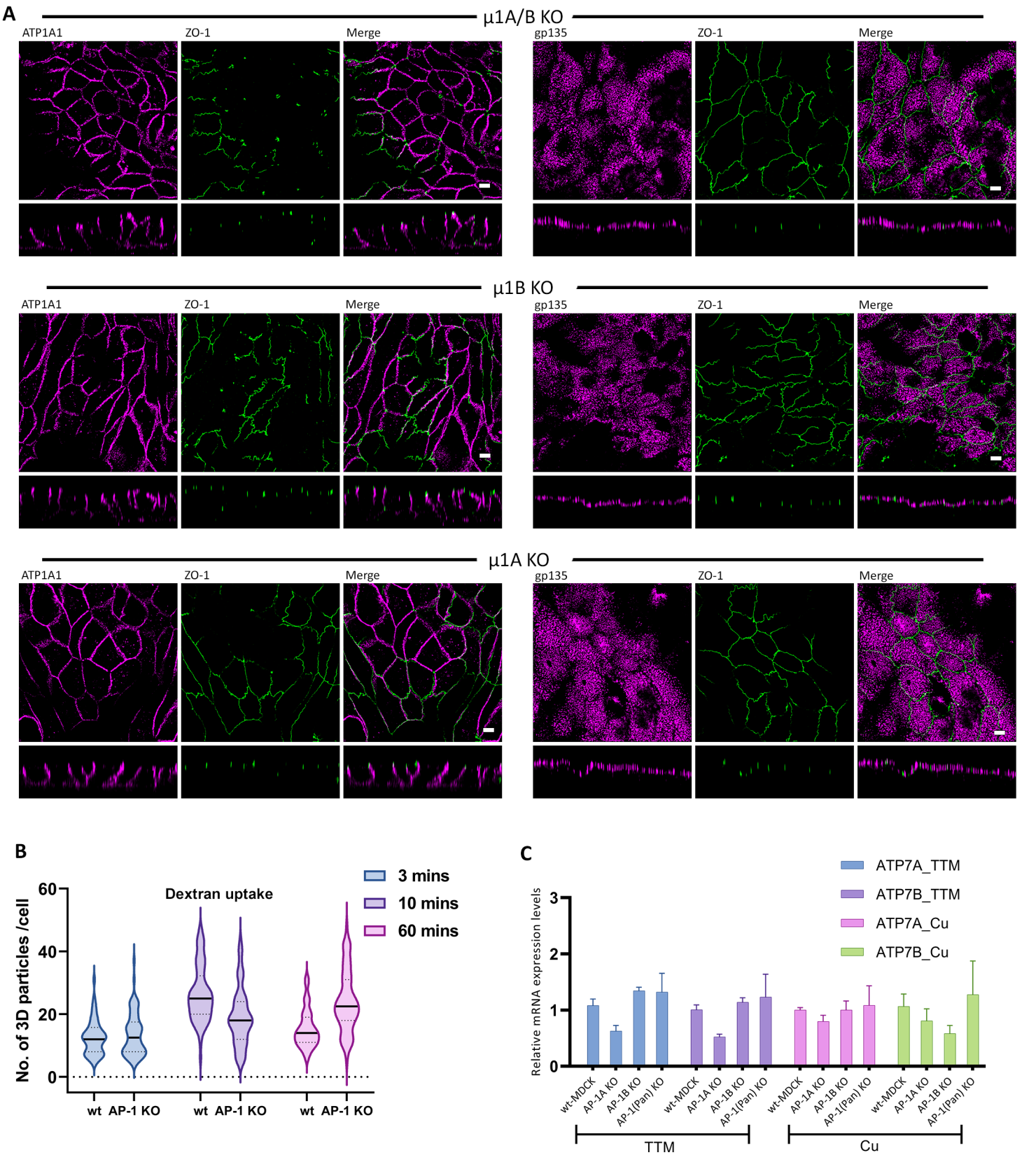
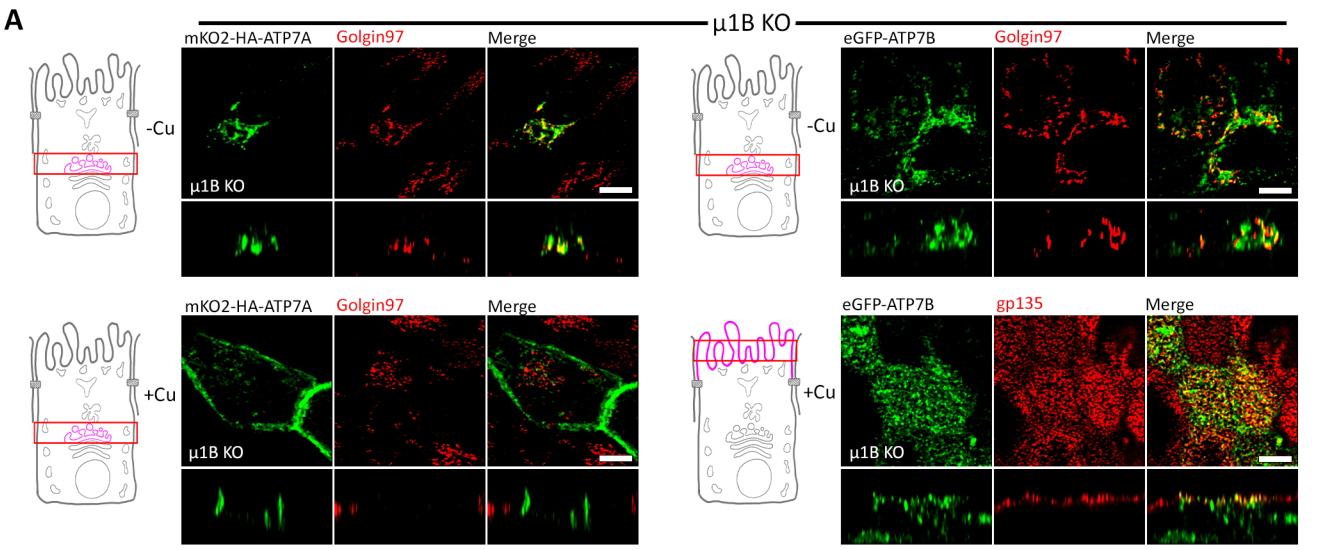
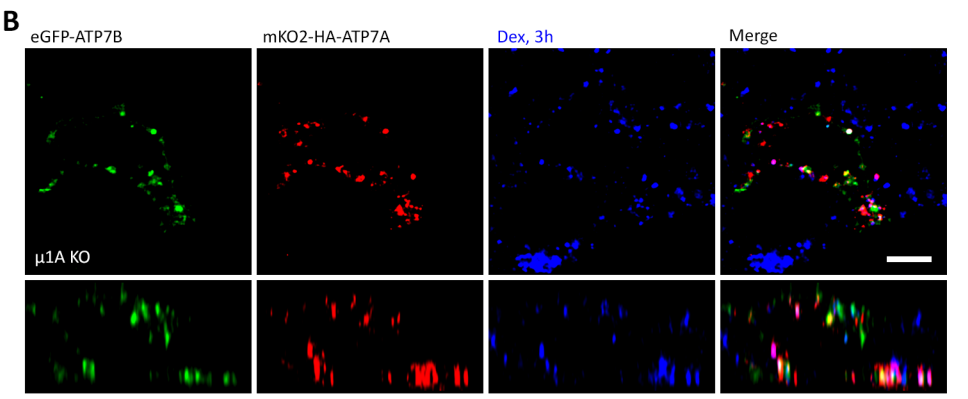
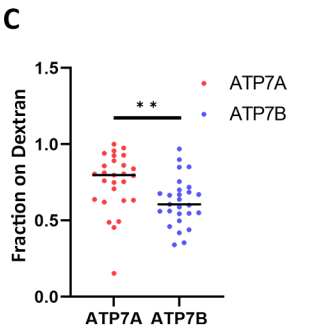


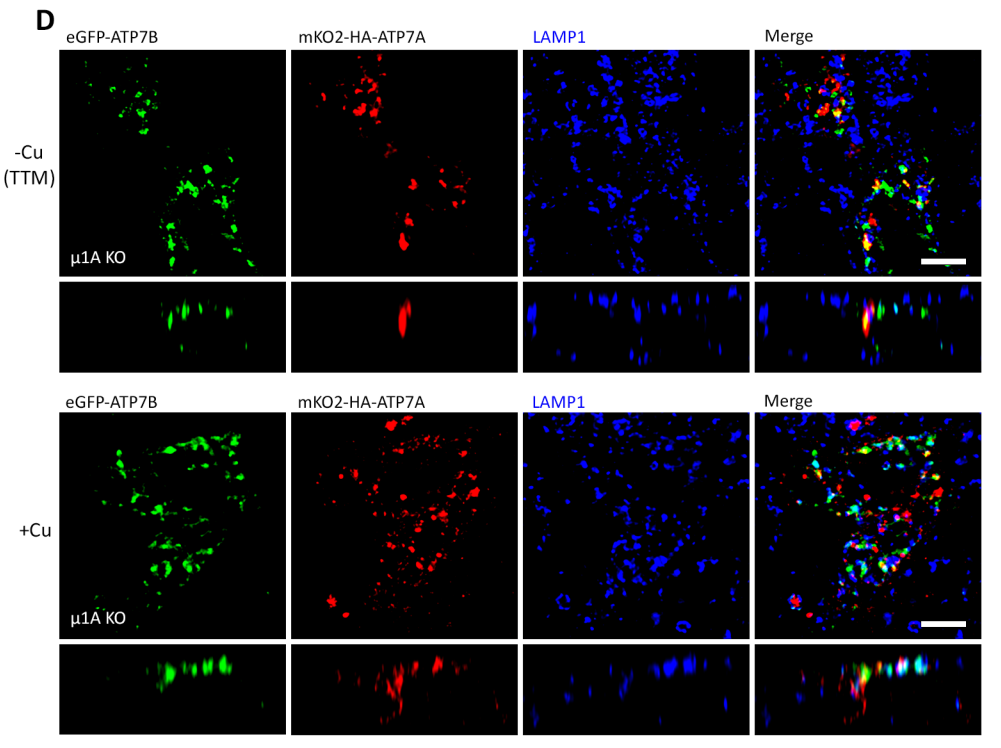
Fig. S5. (A) Images of polarized AP-1 (pan) KO, AP-1B KO, and AP-1A KO MDCK cells marked with tight junctions (stained with ZO-1) and co-stained with respective apical surface (stained with gp135) and basolateral surface (stained with ATP1A1) thus showing their proper polarization. (B) Violin-plots showing no. of 3D-particles of internalized dextran in wild-type and AP-1 (pan) KO polarized MDCK cells. Dextran was internalized for 3 mins, 10 mins and 60 mins showing endocytic endosomal populations. Sample size (N) for 3 mins- wt: 56, AP-1 KO: 93; 10 mins- wt: 79, AP-1 KO: 93; 60 mins- wt: 73, AP-1 KO: 80. (C) Relative mRNA expression levels of ATP7A and ATP7B in wild-type, AP-1A KO, AP-1B KO, and AP-1 (pan) KO MDCK cells under TTM and copper treated conditions. Scale bar, 5μm.

Journal of Cell Science • Supplementary information









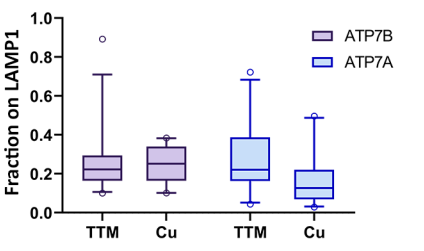
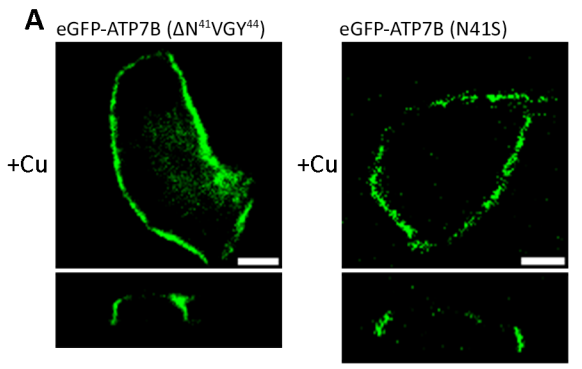
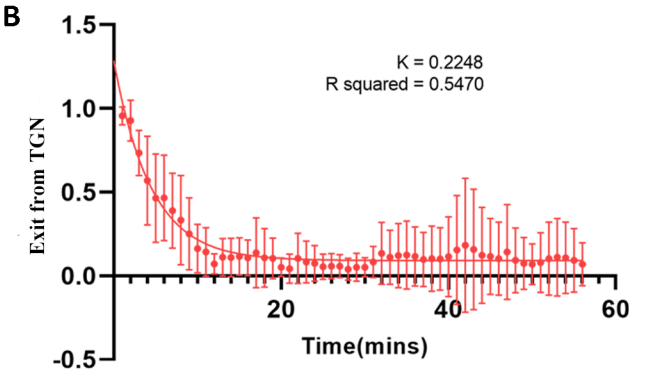
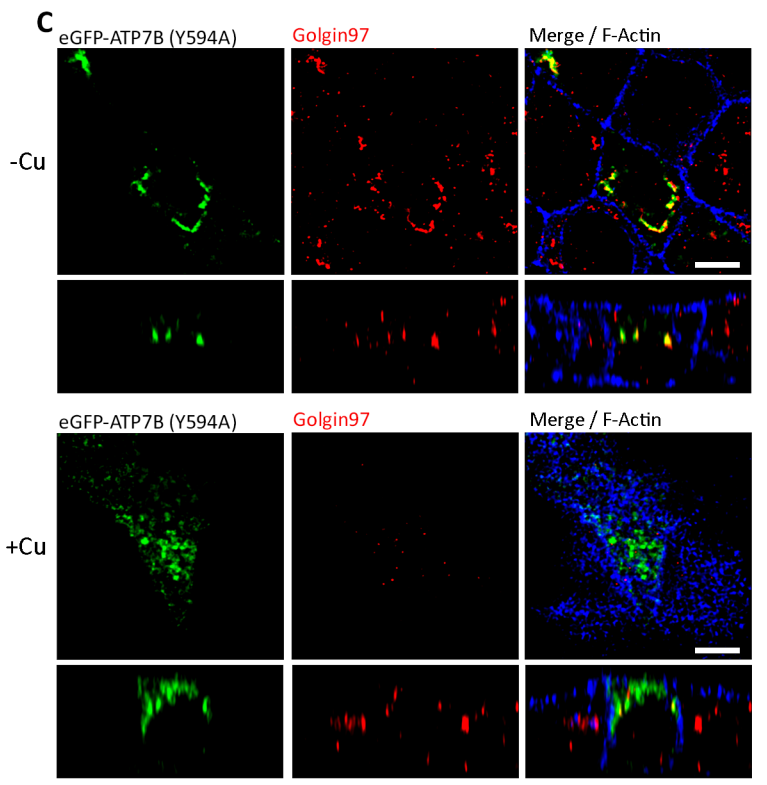


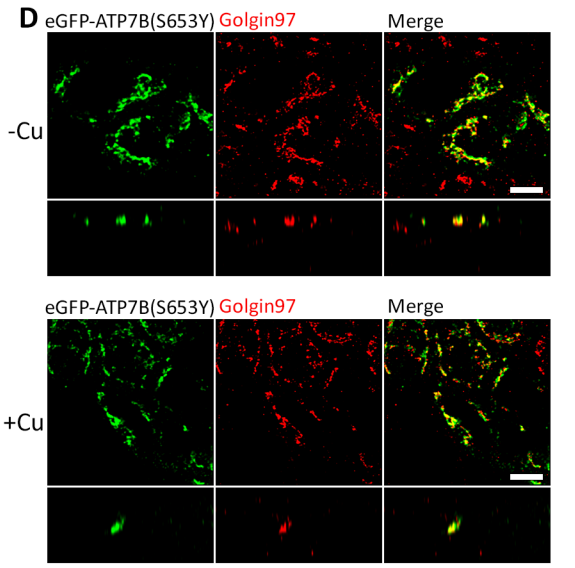
Fig. S6. (A) Confocal image of cells either transfected with mKO2-HA-ATP7A or GFP-ATP7B in AP-1B KO MDCK cells (i.e., μ1B KO cells). Under copper deprived as well as copper-treated conditions both the ATPases exhibit wild-type-like phenomena. (B) Confocal images of polarized AP-1A KO MDCK cells co-transfected with mKO2-HA-ATP7A and eGFP-ATP7B and stained with internalized dextran for 3 hours. (C) Plot showing Manders' Colocalization Coefficient (MCC) of (B), i.e., ATP7A and ATP7B with Dex, 3h. (D) Confocal images of polarized AP-1A KO MDCK cells co-transfected with mKO2-HA-ATP7A and eGFP-ATP7B and stained with LAMP1 in copper deprived and copper treated conditions. (E) Plot showing Manders' Colocalization Coefficient (MCC) of (D) i.e., ATP7A and ATP7B with LAMP1 under TTM and copper treatment. Sample size (N) for both ATP7A and ATP7B at TTM: 28, Cu: 21. Scale bar, 5μm.

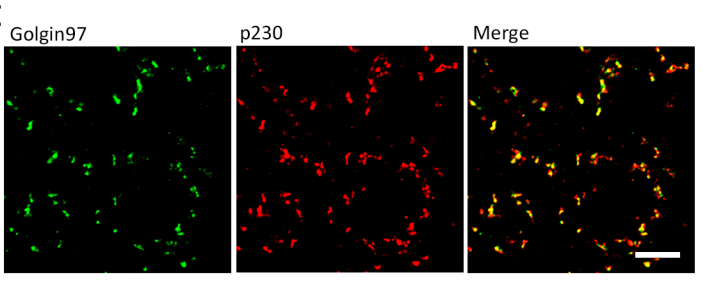
Journal of Cell Science • Supplementary information

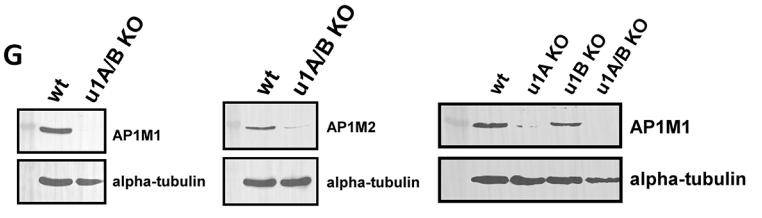












F

AP-1A KO sequenced for AP1M1

```
u1A_KO       251 TCTCCATCAGGATAGGCATGAAGTGCTCCACCTCCGACATGTCCACGTCA 300
                 ||||||||||||||||||||||||||||||||||||||||||||||||||.||||.
seq_AP1M1    248 TCTCCATCAGGATAGGCATGAAGTGCTCCACCTCCGACATGTCCCCGTCG 297

u1A_KO       301 CCGCGG-TAATTCCGGCAGATGAGCACCTTGCCC-TTTAGGTCCAGCACG 348
                 ||||||  |||||||||.|||||||.|||||||  |||||||||.|||||
seq_AP1M1    298 CCGCGGTTAATTCCGGCGGATGAGCCCCTTGCCCTTTTAGGTCCGGCACG 347

u1A_KO       349 TAGACGGCGCTGGCGGACATGACGGCTGCGGGAAGCCTCGGCAGCTGCCG 398
                 |||||||||||||.|||||||||||||||||.|.|||||||.|||||||||
seq_AP1M1    348 TAGACGGCGCTGGGGGACATGACGGCTGCGGAAGGCCTCGCCAGCTGCCG 397
```

AP-1 (pan) KO sequenced for AP1M1

```
u1A_B_KO     208 CAGGATGGGCGACAGCATCCCTTCCTCCTCCTTCTCCATCAGGATAGGCA 257
                 ||||||||||||||||||||||||||||||.||||||||||||||||||||
seq_AP1M1    200 CAGGATGGGCGACAGCATCCCTTCCTCCTACTTCTCCATCAGGATAGGCA 249

u1A_B_KO     258 TGAAGTGCTCCACCTCCGACATGTCCACGTCACCGCGG-TAATTCCGGCA 306
                 ||||||||||||||||||||||||||||||||||.|||| ||||||||||||
seq_AP1M1    250 TGAAGTGCTCCACCTCCGACATGTCCACGTCGCCGCGGTTAATTCCGGCA 299

u1A_B_KO     307 GATGAGCACCTTGCCCTTTAGGTCCAGCACGTAGACGGCGCTGGCGGACA 356
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||
seq_AP1M1    300 GATGAGCACCTTGCCCTTTAGGTCCAGCACGTAGACGGCGCTGGCGGACA 349
```

AP-1B KO sequenced for AP1M2

```
u1B_KO       257 AAAATGCACCCGGCCATGGCTCAGTAGGGGGGCCAGGGCGCCCTCCTCTT 306
                 ||||||||.|||||||||||||||||||||||||||||||||||||||||||
seq_AP1M2    252 AAAATGCAGCCGGCCATGGCTCAGTAGGGGGGCCAGGGCGCCCTCCTCTT 301

u1B_KO       307 CCCGCTGCATGAGCAGAGGCATGAAGTGCTCAATCTCACTCATGGCCACG 356
                 ||||||
seq_AP1M2    302 CCCGCT------------------------------------------- 307

u1B_KO       357 TCGCCCTTGTAGTTGCGGCTGATCAGGGGCTTGCCCTTGACGTCGAGGAT 406
                                  |.|||||     ||||||||||||||||||||
seq_AP1M2    308 -----------------GATGATC-----CTTGCCCTTGACGTCGAGGAT 335

u1B_KO       407 GAAGACAGCCGAGGCG    422
                 ||||||||||||||||
seq_AP1M2    336 GAAGACAGCCGAGGCG    351
```

AP-1 (pan) KO sequenced for AP1M2

```
u1A_B_KO     251 CCACAGAAAATGCACCCGGCCATGGCTCAGTAGGGGGGCCAGGGCGCCCT 300
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||
seq_AP1M2    247 CCACAGAAAATGCACCCGGCCATGGCTCAGTAGGGGGGCCAGGGCGCCCT 296

u1A_B_KO     301 CCTCTTCCCGCTGCATGAGCAGAGGCATGAAGTGCTCAATCTCACTCATG 350
                 ||||||||||||
seq_AP1M2    297 CCTCTTCCCGCT------------------------------------ 308

u1A_B_KO     351 GCCACGTCGCCCTTGTAGTTGCGGCTGATCAGGGGCTTGCCCTTGACGTC 400
                                     |.|||||     ||||||||||||||||
seq_AP1M2    309 ----------------------GATGATC-----CTTGCCCTTGACGTC 330

u1A_B_KO     401 GAGGATGAAGACAGCCGAGGCG    422
                 |||||||||||||||||||||
seq_AP1M2    331 GAGGATGAAGACAGCCGAGGCG    352
```

Journal of Cell Science • Supplementary information

Fig. S7. (A) Confocal images of ΔN41VGY44 and N41S of ATP7B mutants shows their basolateral localization in copper-treated conditions. (B) Fitted curve of decreased pixel count of Δ9aa GFP-ATP7B mutant in response to copper showing its dispersion and the exit rate (i.e., k value = 0.2248). (C) Confocal images of Y594A-ATP7B mutant shows wildtype-like copper-mediated trafficking behaviour. (D) Confocal image of S653Y-ATP7B mutant unable to exit TGN in copper-treated condition. (E) Confocal images of polarized MDCK cells stained for Golgin97 and p230 shows colocalization which indicates that they mark the same TGN compartment(s). (F) Sequence alignment of AP1M1 and AP1M2 PCR products from cDNA of AP-1A, AP-1B and AP-1(pan) KO cells show insertions/mismatch/deletions highlighted in yellow, indicating proper knockouts. (G) Immunoblot of AP1M1 and AP1M2 in wild-type and AP-1 knockout MDCK cells. The blots were stripped and re-probed with α tubulin as loading control. Scale bar, 5μm.

Table S1.

Available for download at
https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.261258#supplementary-data

Table S2.

Available for download at
https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.261258#supplementary-data

Table S3.

Available for download at
https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.261258#supplementary-data

Journal of Cell Science • Supplementary information

Table S4. Primers used for ATP7B mutants.

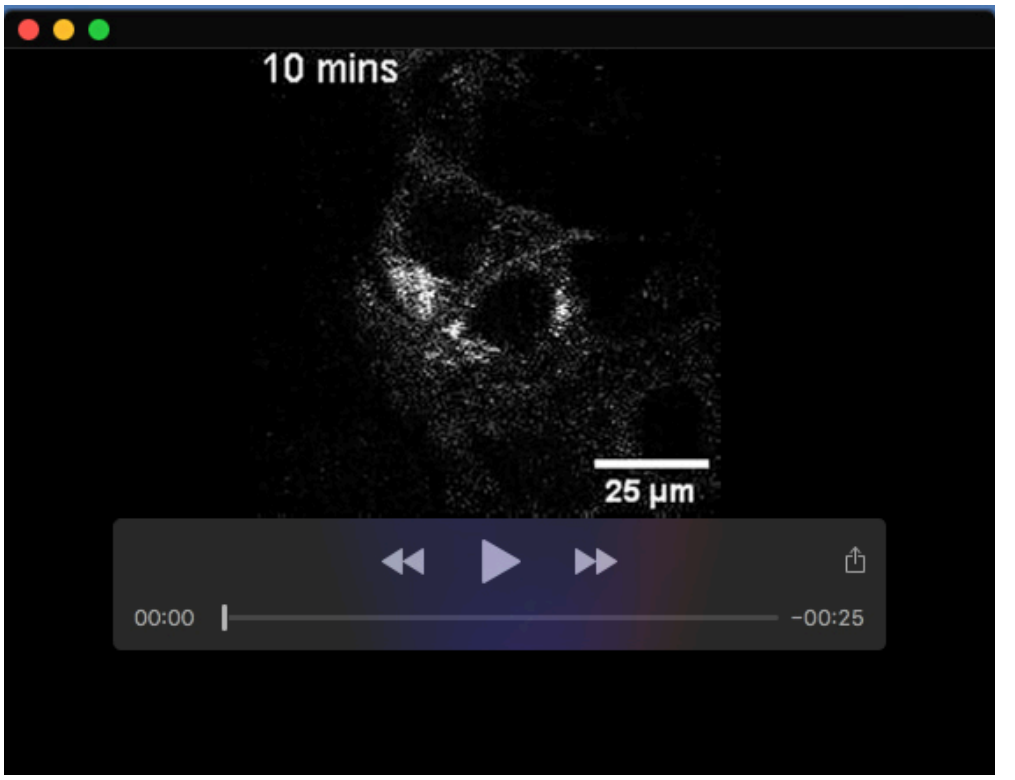
Mutants	Forward Primer	Reverse Primer
$\Delta F^{37}\text{-}E^{45}$	5'GGTGGTCTGGATGGCCTG3'	5'ACTCTTCTTCATTGCTGGTTCC3'
$\Delta^{41}NVGY^{44}$	5'GAAGGTGGTCTGGATGGC3'	5'GTCAAAAGCAAAACTCTTCTTCATTG3'
N41S	5'TTTTGCTTTTGACAGTGTTGGCTATGAAG3'	5'CATAGCCAACACTGTCAAAAGCAAAACTC3'
LL1455AA	5'CGCGAATGGCAGGGATGAGGAG	3'GCCGCAGACCACTTGTCCCCATC
Y1376A	5'GCTCAAGTGCGCTAAGAAGCCTGACCTG3'	5'TGCAGGGATGAGAGCACC3'
Y594A	5'TGGCATCACTGCTGCCTCCGTTG3'	5'TTTGTCCTCGTGAGTTTG3'
S653Y	5'TGGAAGAAGTATTTCCTGTGC3'	5'CTGCTTTATTTCCATCTTG3'

Table S5. Primers used for reverse transcription PCR.

Gene	Forward Primer	Reverse Primer
ATP7A	5'CTTGTTGTGAGAGGAATGACGTGTGCC3'	5'CAAGTGGCTTGCTGACCGATCCTTC3'
ATP7B	5'GCTCTTTGTGTTCATTGCCCTGGGG3'	5'CCCGGGGACCACCTTGATGACATC3'
GAPDH	5'CCTGCCGCCTGGAGAAAGC3'	5'TGGAAGAGTGGGTGTCACTGTTG3'

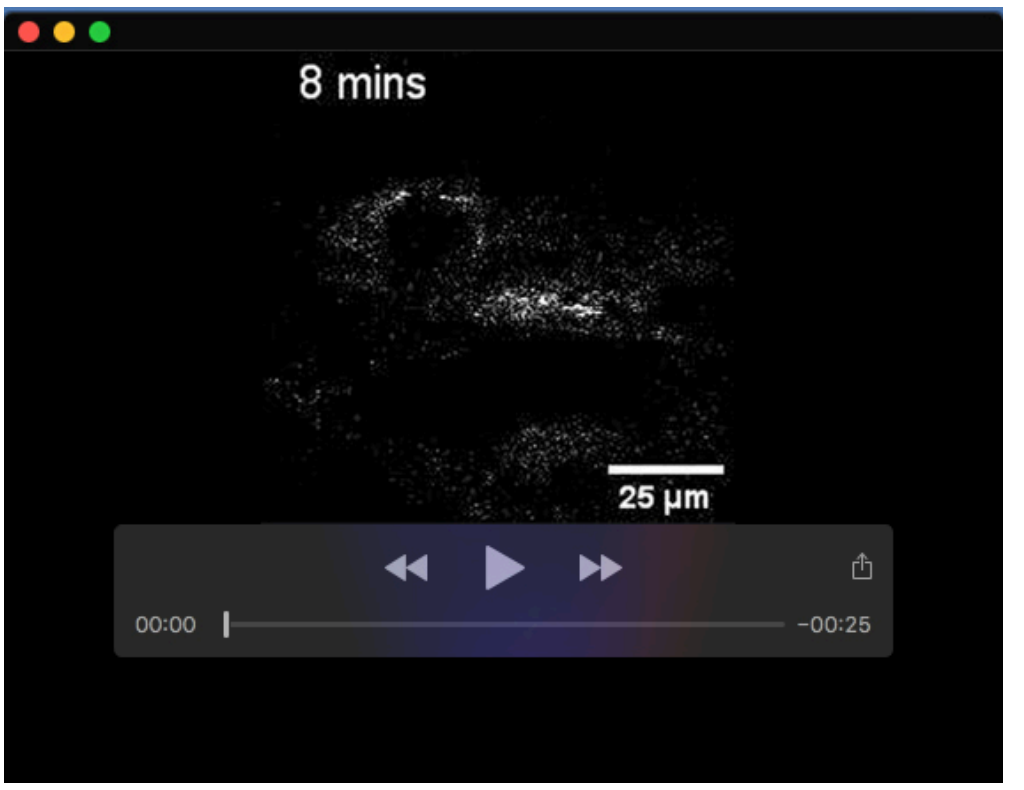
Table S6. Gradient between solution A (5 % acetonitrile-water containing 0.1 % formic acid) and solution B (95 % acetonitrile in water containing 0.1% formic acid) at the flow rate of 300 nl/min.

Time (min)	% sol. B
0	3
41	25
48	40
54	95
60	95



Movie 1. Representative video of dispersion rate of ATP7A from Golgi in response to copper in sub-confluent MDCK cells.

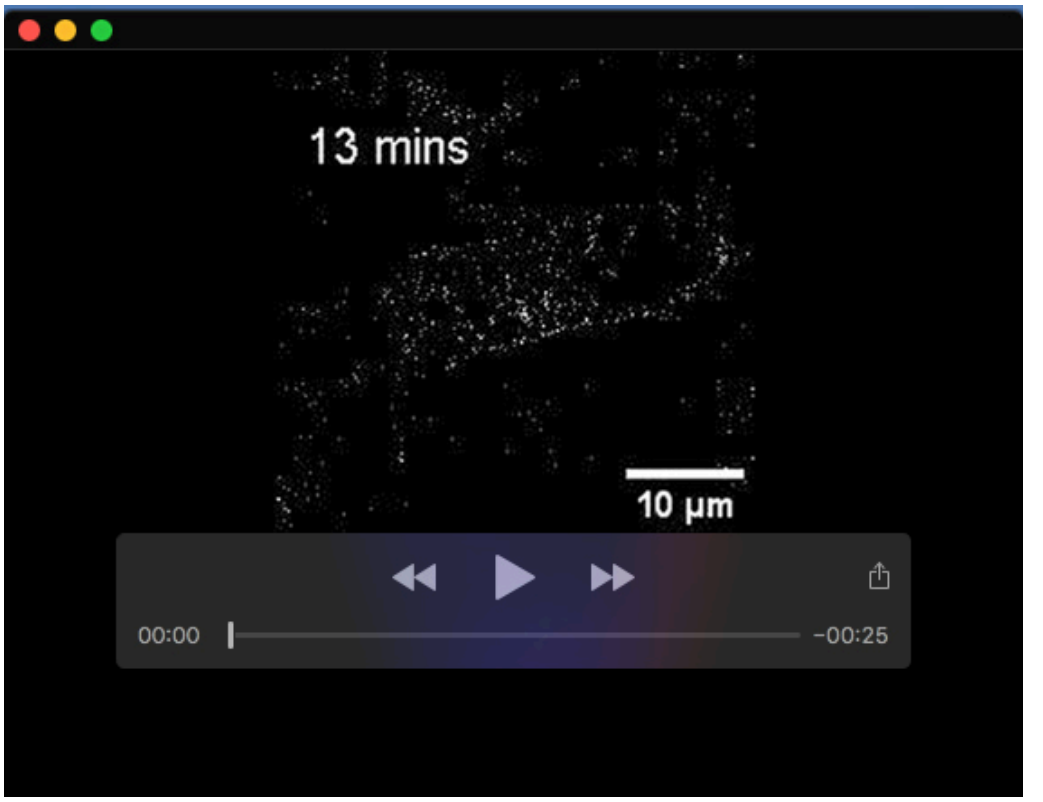
MDCK cells transfected with mKO2-HA-ATP7A were plated on glass bottom confocal dishes. Images of sub-confluent MDCK cells treated with 50µM $CuCl_2$ were captured at an interval of 60 seconds for 1 hour. Pixels having 95% of the signals under basal conditions were considered as Golgi, the disappearance of which represents the Golgi-exit rate.



Movie 2. Representative video of dispersion rate of ATP7B from Golgi in response to copper in sub-confluent MDCK cells.

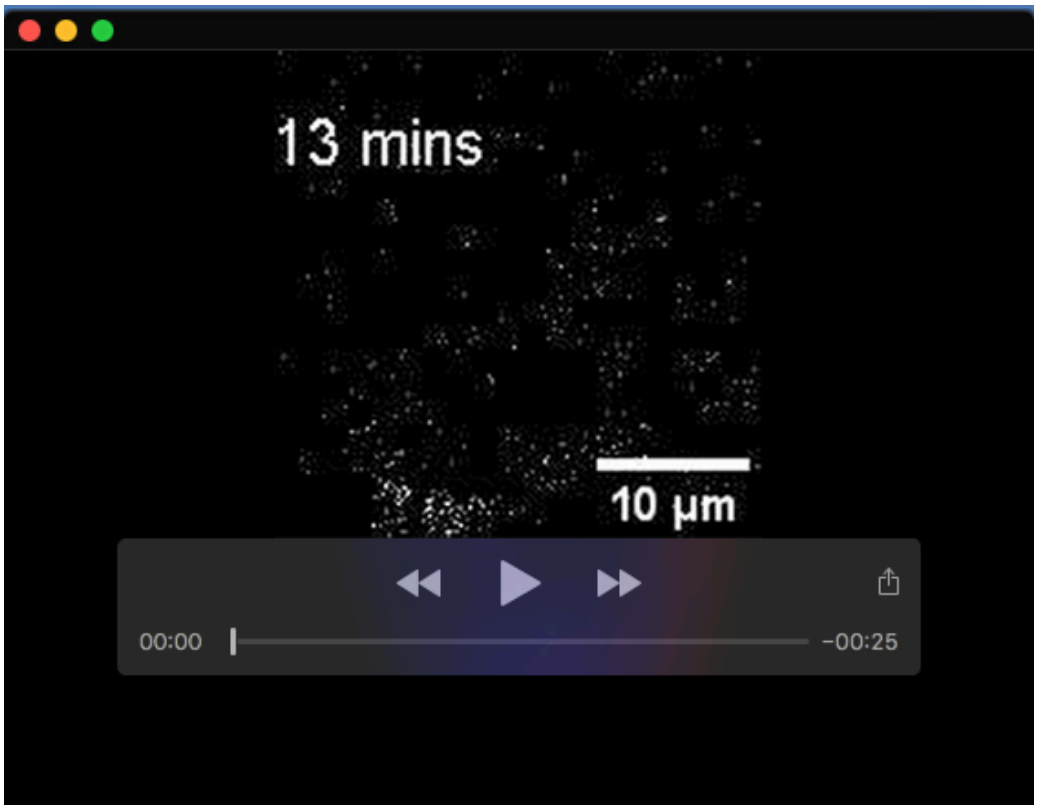
MDCK cells transfected with eGFP-ATP7B were plated on glass bottom confocal dishes. Images of sub-confluent MDCK cells treated with 50µM $CuCl_2$ were captured at an interval of 60 seconds for 1 hour. Pixels having 95% of the signals under basal conditions were considered as Golgi, the disappearance of which represents the Golgi-exit rate.

Journal of Cell Science • Supplementary information



Movie 3. Representative video of dispersion rate of ATP7A from Golgi in response to copper in polarized MDCK cells.

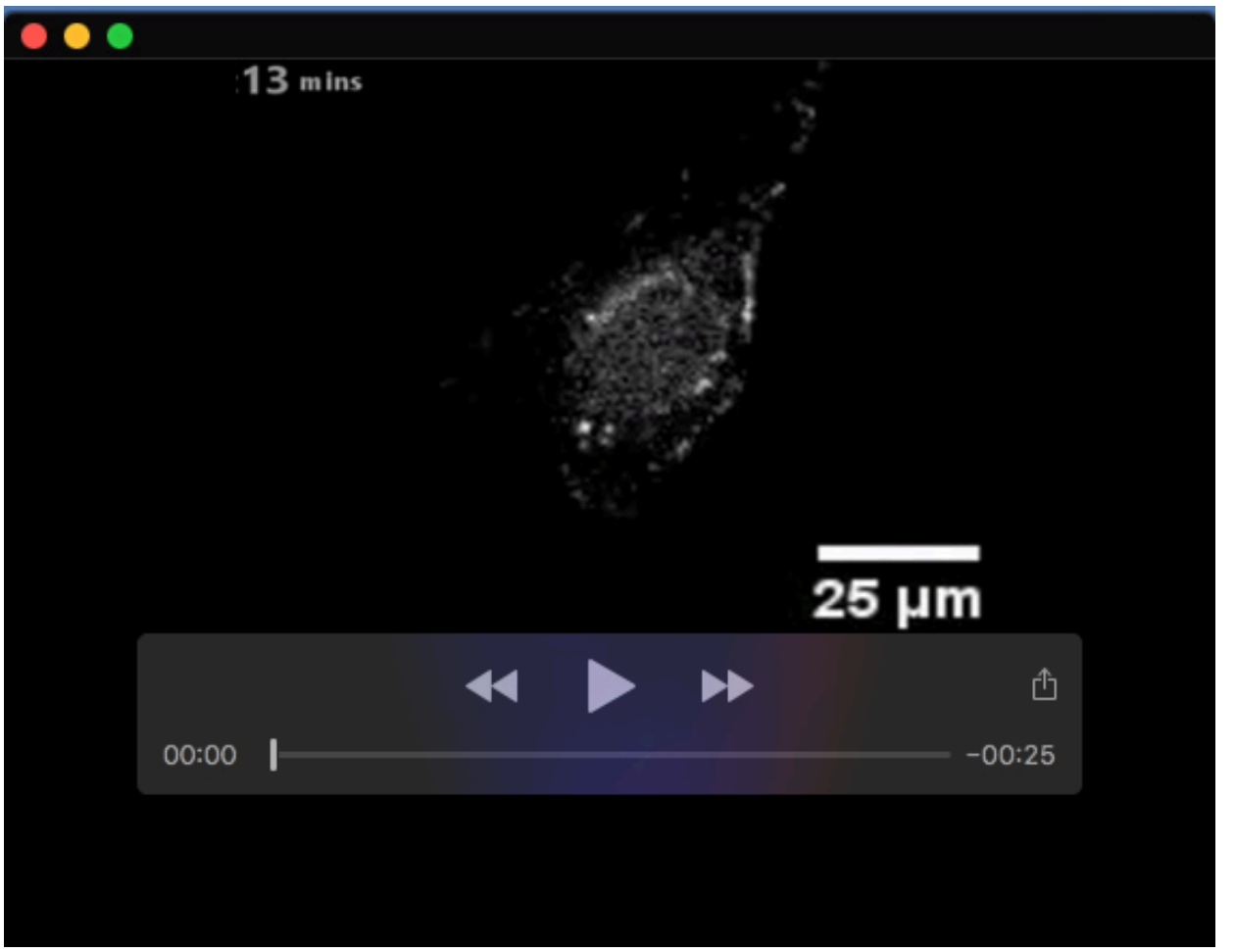
MDCK cells transfected with mKO2-HA-ATP7A were seeded on 0.4µm, 12mm inserts. Images of polarized MDCK cells treated with 50µM $CuCl_2$ were captured at an interval of 60 seconds for 1 hour. Pixels having 95% of the signals under basal conditions were considered as Golgi, the disappearance of which represents the Golgi-exit rate.



Movie 4. Representative video of dispersion rate of ATP7B from Golgi in response to copper in polarized MDCK cells.

MDCK cells transfected with eGFP-ATP7B were seeded on 0.4µm, 12mm inserts. Images of polarized MDCK cells treated with 50µM $CuCl_2$ were captured at an interval of 60 seconds for 1 hour. Pixels having 95% of the signals under basal conditions were considered as Golgi, the disappearance of which represents the Golgi-exit rate.

Journal of Cell Science • Supplementary information



Movie 5. Representative video of dispersion rate of ATP7B ΔF37-E45 mutant from Golgi in response to copper.

MDCK cells transfected with eGFP-ATP7B ΔF^{37}-E^{45} were plated on glass bottom confocal dishes. Images of sub-confluent MDCK cells treated with 50µM $CuCl_2$ were captured at an interval of 60 seconds for 1 hour. Pixels having 95% of the signals under basal conditions were considered as Golgi, disappearance of which represents the Golgi-exit rate.

Journal of Cell Science • Supplementary information