Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/14/eaay4472/DC1

Supplementary Materials for

The unconventional biogenesis of Kv7.1-KCNE1 complexes

Anna Oliveras, Clara Serrano-Novillo, Cristina Moreno, Alicia de la Cruz, Carmen Valenzuela, Christian Soeller, Núria Comes, Antonio Felipe*

*Corresponding author. Email: afelipe@ub.edu

Published 1 April 2020, *Sci. Adv.* **6**, eaay4472 (2020)
DOI: 10.1126/sciadv.aay4472

The PDF file includes:

Figs. S1 to S7

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/14/eaay4472/DC1)

Movies S1 to S3

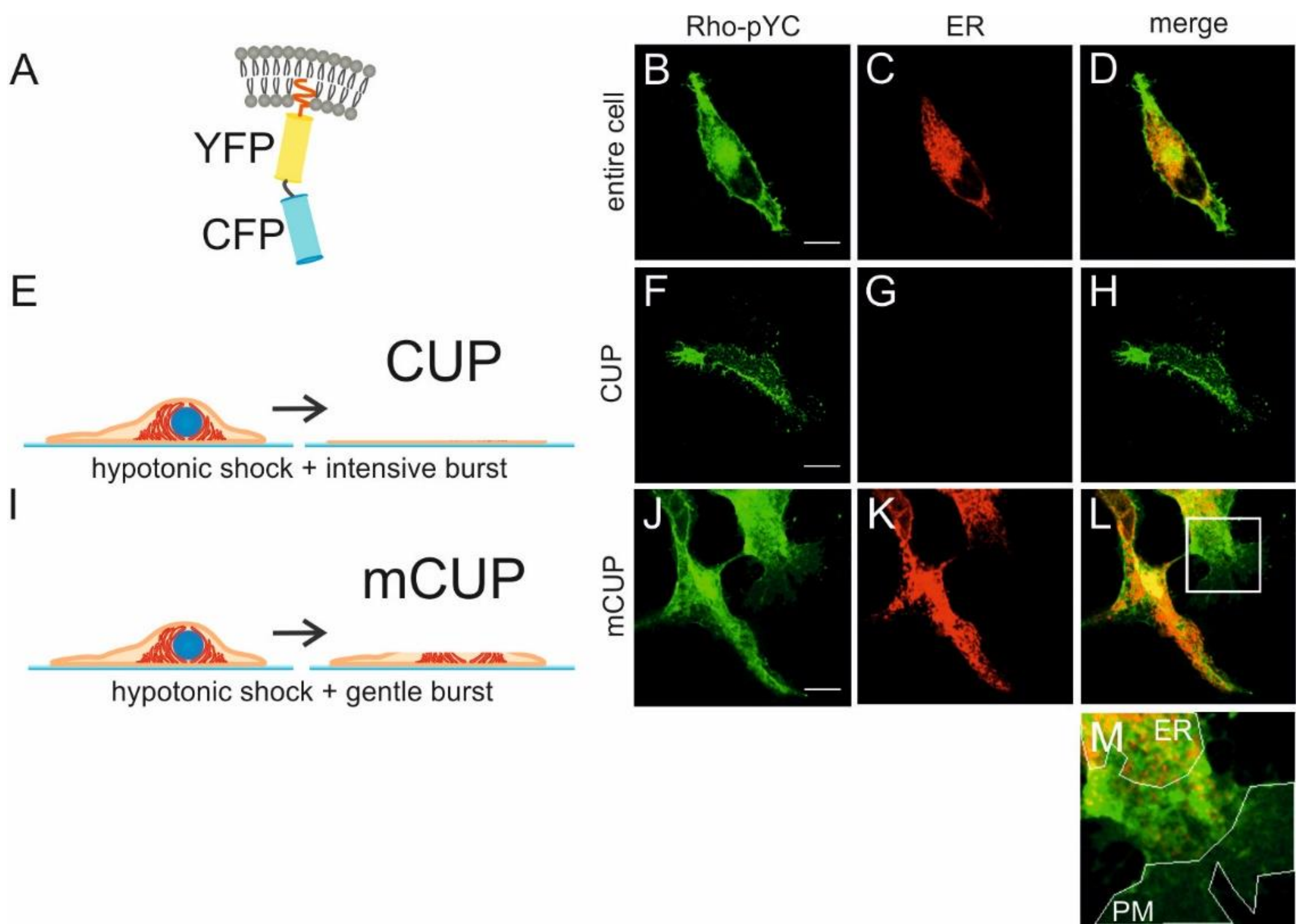
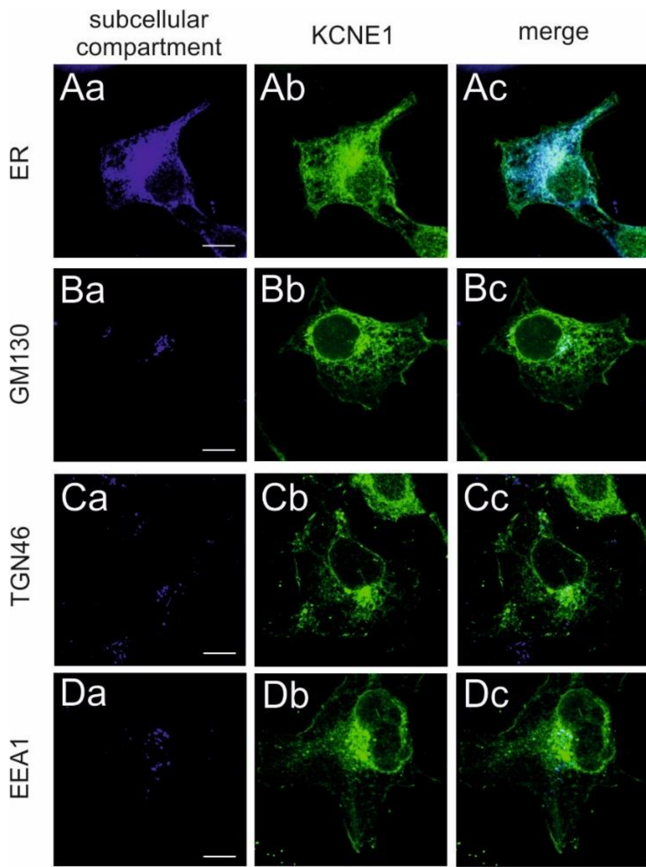
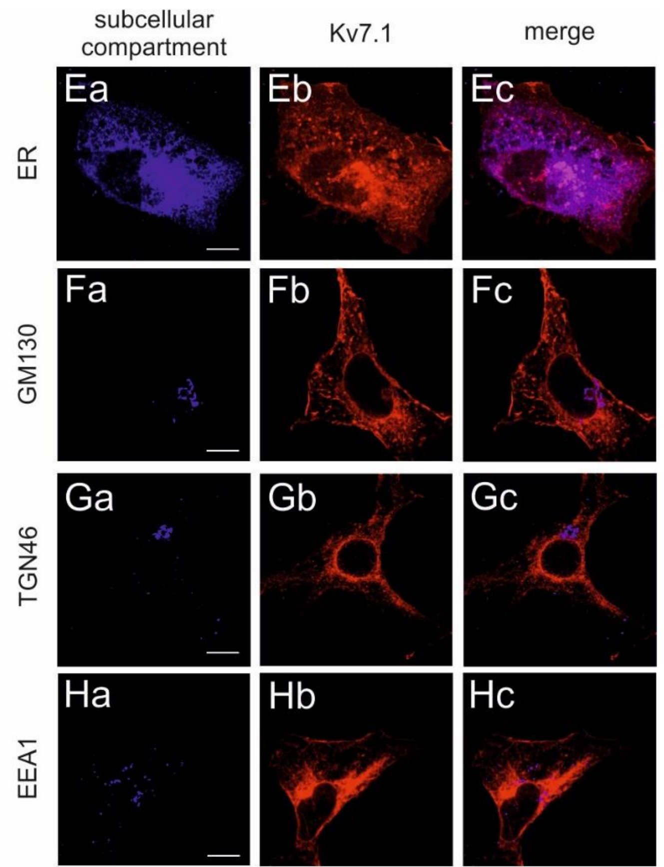


Figure S1. Characterization of cell unroofing preparations (CUPs) and modified CUPs (mCUPs). HEK-293 cells were transfected with Rho-pYC and ER-DsRed to identify the plasma membrane and ER, respectively. (A) Cartoon of Rho-pYC, a membrane-localized CFP-YFP tandem, used as a membrane marker. (B-D) Rho-pYC (green) and ER-DsRed (red) colocalization. (E) Cartoon of the protocol used to obtain CUPs. (F-H) Rho-pYC (green) and ER-DsRed (red) colocalization in CUP. Note that the ER (G) is absent. (I) Cartoon of the protocol used to obtain ER-preserved CUPs (mCUPs). (J-L) Rho-pYC (green) and ER-DsRed (red) colocalization in mCUP. (M) Magnified view of the highlighted area in mCUPs. The ER and PM are delineated with white lines. Scale bar, 10 μm.


subcellular compartment
KCNE1
merge
Aa
Ab
Ac
ER
Ba
Bb
Bc
GM130
Ca
Cb
Cc
TGN46
Da
Db
Dc
EEA1


subcellular compartment
Kv7.1
merge
Ea
Eb
Ec
ER
Fa
Fb
Fc
GM130
Ga
Gb
Gc
TGN46
Ha
Hb
Hc
EEA1

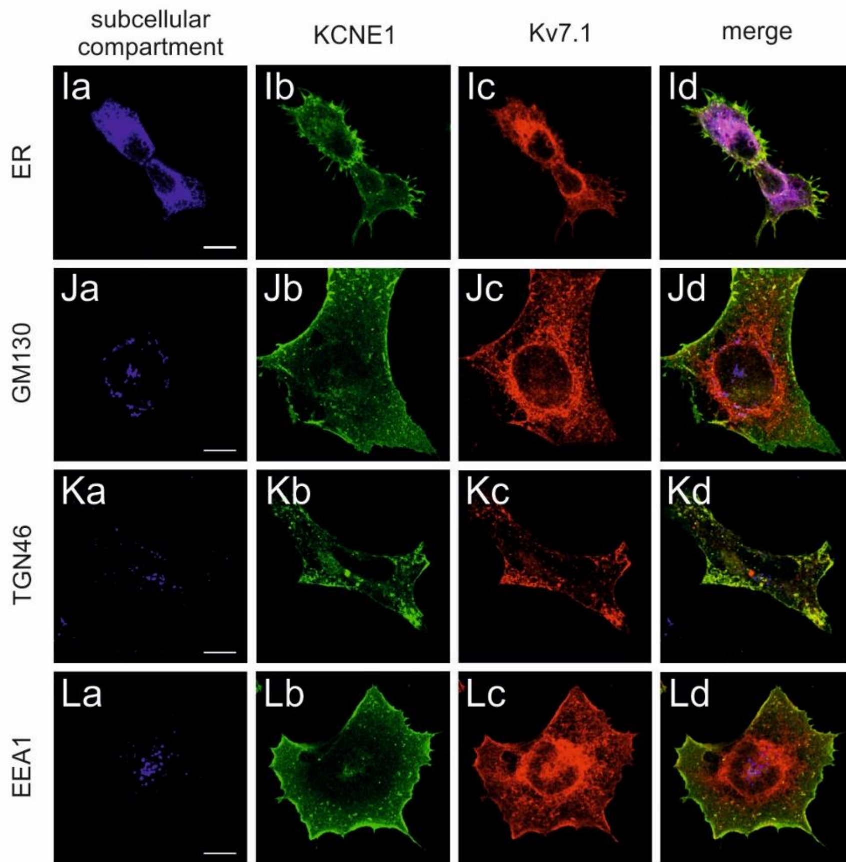

subcellular compartment
KCNE1
Kv7.1
merge
Ia
Ib
Ic
Id
ER
Ja
Jb
Jc
Jd
GM130
Ka
Kb
Kc
Kd
TGN46
La
Lb
Lc
Ld
EEA1

Figure S2. Elucidation of the secretory pathway of Kv7.1 and KCNE1. HEK-293 cells were transfected with KCNE1-YFP, Kv7.1-CFP and Kv7.1+KCNE1 and stained for different subcellular markers throughout the secretory pathway. (Aa-Dc) KCNE1 colocalization with different subcellular compartment markers from the beginning to the end of the secretory pathway. (Aa-Ac) ER (ER-DsRed); (Ba-Bc) cis-Golgi network (GM130); (Ca-Cc) trans-Golgi network (TGN46); (Da-Dc) early endosomes (EEA1). Blue, subcellular compartments; green, KCNE1-YFP. Ac, Bc, Cc and Dc, Merged images showing colocalization in cyan. (Ea-Hc) Kv7.1 colocalization with ER-DsRed (Ea-Ec), GM130 (Fa-Fc), TGN46 (Ga-Gc) and EEA1 (Ha-Hc). Blue, subcellular compartments; red, Kv7.1-CFP. Ec, Fc, Gc and Hc, Merged images showing colocalization in magenta. (Ia-Ld) Colocalization of the Kv7.1-CFP+KCNE1-YFP complex with markers of the secretory pathway. (Ia-Id), ER-DsRed; (Ja-Jd) GM130; (Ka-Kd), TGN46; (La-Ld), EEA1. Blue, subcellular compartments; green, KCNE1-YFP; red, Kv7.1-CFP. The merged images show triple colocalization in white and Kv7.1-KCNE1 colocalization in yellow. Scale bars, 10 μm.

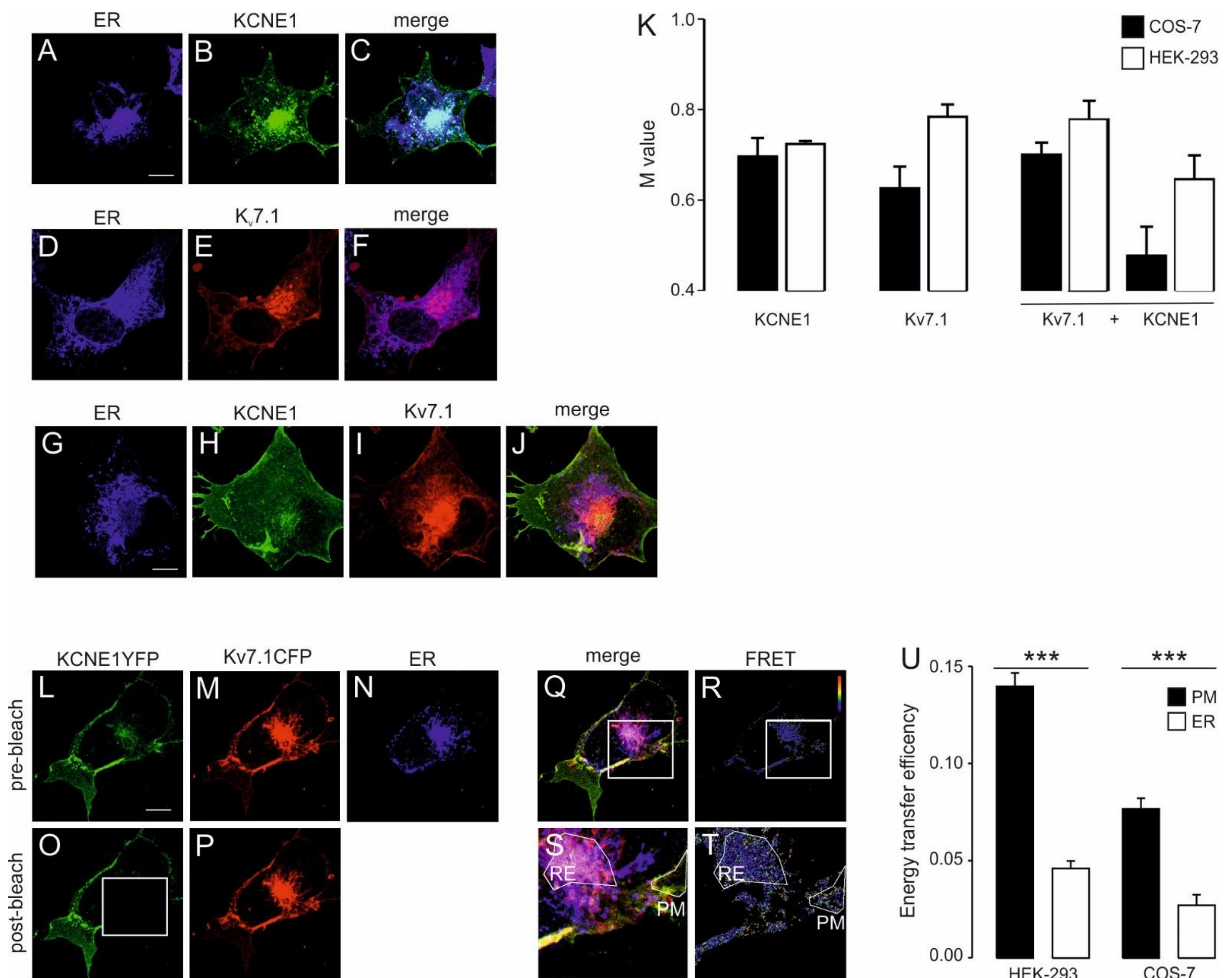


Figure S3. The trafficking and association of the Kv7.1-KCNE1 complex is also recapitulated in COS-7 cells. COS-7 cells were transfected with ER-DsRed, KCNE1-YFP and Kv7.1-CFP. (A-J) Colocalization of KCNE1 (A-C), Kv7.1 (D-F) and Kv7.1+KCNE1 (G-J) with the ER. (A, D and G) ER-DsRed, blue; (B and H) KCNE1-YFP, green; (E and I) Kv7.1-CFP, red. (C, F and J) Merged images showing colocalization between green and blue in cyan (C); colocalization between blue and red in magenta (F); triple colocalization among blue, green and red in white; and partial colocalization between green and red in yellow (J). (K) Manders' coefficient (M) colocalization analysis. The colocalization of KCNE1, Kv7.1 and Kv7.1+KCNE1 with the ER was compared between COS-7 (black) and HEK-293 cells (white) (n=9-40). The bars represent the mean±SEM. (L-U) Representative results of Kv7.1-CFP and KCNE1-YFP FRET experiments in mCUPs of COS-7 cells. (L-N) Prebleaching images of KCNE1-YFP (L), Kv7.1-CFP (M) and ER-DsRed (N). (O and P) Postbleaching images. The bleached area is highlighted with a white square. (Q) Merged image of L-N. (S) Magnified view of the bleached area in Q identifying specific areas in the PM and ER. (R and T) FRET ratio image and corresponding magnified view of the bleached area. The calibration bars range from 0.8 (blue) to 1.4 (red). Scale bar, 10 µm. (U) FRET measurements in the PM (black) or the ER (white) within the same cell. The bars represent the mean±SEM. *** $p<0.001$ for Kv7.1-KCNE1 FRET efficiency in the PM *vs* the ER (n= 10, Student's *t* test).

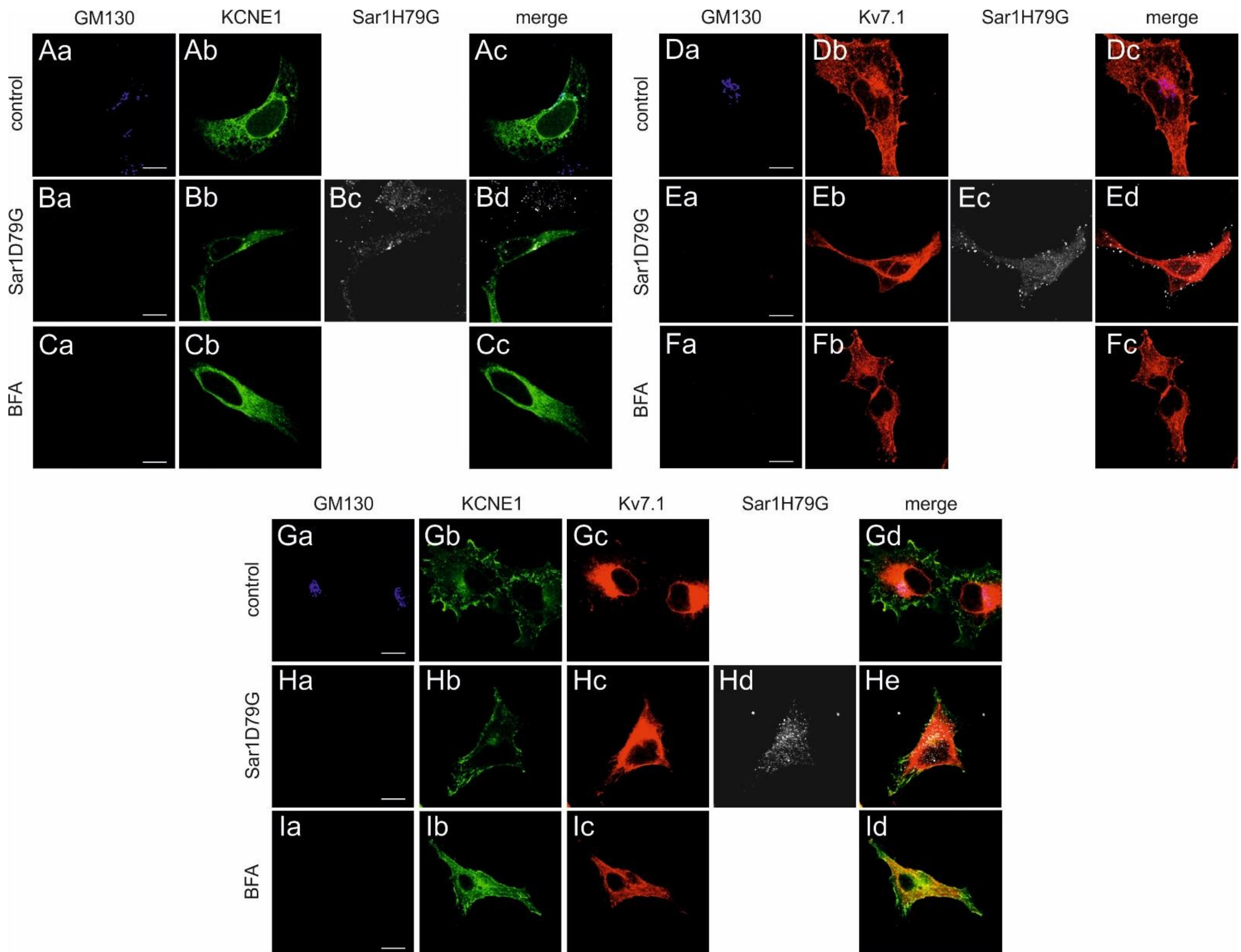


Figure S4. Disruption of ER-to-Golgi anterograde trafficking by Sar1H79G and brefeldin A treatment. HEK-293 cells were transfected with KCNE1-YFP, Kv7.1-CFP, Kv7.1-CFP+KCNE1-YFP and HA-Sar1H79G. In addition, some cells were treated with 5 μg/ml BFA for 4 h. GM130 staining was used to evaluate Golgi integrity. (Aa-Cc), KCNE1 subcellular localization. (Aa-Ac) Control conditions. KCNE1-YFP localization is shown in green (Ac), and colocalization with the cis-Golgi marker (GM130) is shown in blue (Aa). (Ac) The merged image shows colocalization in cyan. (Ba-Bd) Sar1H79G disrupted the Golgi. (Ba) GM130; (Bb) KCNE1; (Bc) Sar1H79G; (Bd) merge. (Ca-Cc) Effects of BFA on the Golgi. (Ca) GM130; (Cb) KCNE1-YFP; (Cc) merged image. Note that while KCNE1 was partially detected at the membrane surface in Ac, treatment with either Sar1H79G or BFA, while disrupting the Golgi (Ba and Ca), impaired KCNE1 surface expression (Bd and Cc). (Da-Fc) Kv7.1 subcellular localization. (Da-Dc) Control conditions. (Ea-Ed) Sar1H79G expression disrupted the Golgi. (Fa-Fc) BFA treatment collapsed the Golgi. Kv7.1-CFP, red; cis-Golgi network (GM130), blue. The merged image shows colocalization in magenta. Note that neither Sar1H79G nor BFA modified Kv7.1 surface expression. (Ga-Id) Kv7.1-KCNE1 complexes reached the PM in a COPII-independent manner and bypassed the Golgi upon Sar1H79G and BFA treatment. (Ga-Gd) Kv7.1 and KCNE1 colocalization with the cis-Golgi marker in control conditions. (Ga) GM130, blue; (Gb) KCNE1-YFP, green; (Gc) Kv7.1-CFP, red. (Gd), the merged image shows triple colocalization in white. (Ha-He) Kv7.1-KCNE1 upon Sar1H79G transfection. (Ha) GM130; (Hb) KCNE1-YFP; (Hc) Kv7.1-CFP; (Hd) Sar1H79G; (He) merge. (Ia-Id) Kv7.1-KCNE1 upon BFA treatment. (Ia) GM130; (Ib) KCNE1-YFP; (Ic) Kv7.1-CFP; (Id) merged image. Colocalization of KCNE1-YFP and Kv7.1-CFP is shown in yellow. Scale bar, 10 μm.

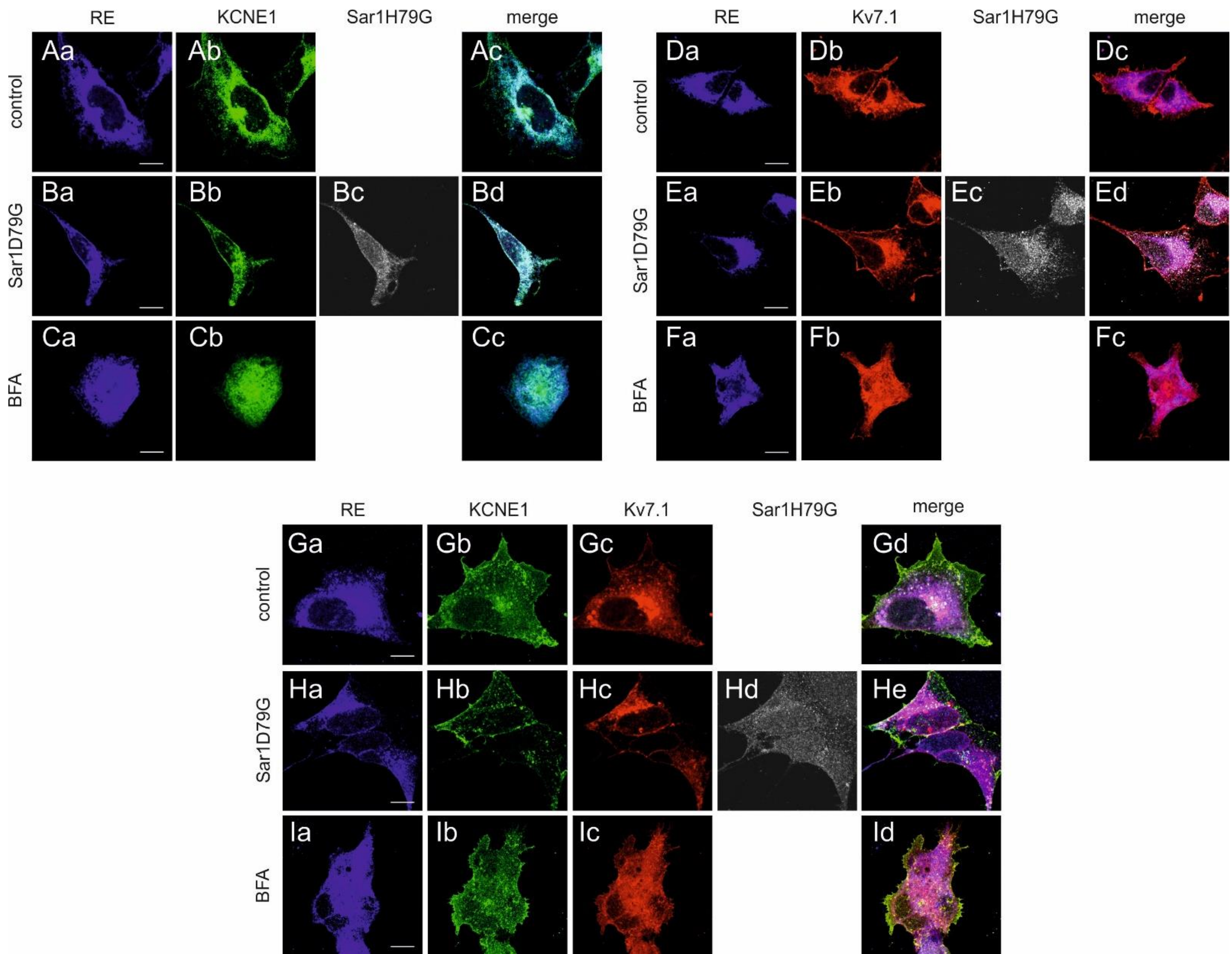


Figure S5. Kv7.1 and Kv7.1-KCNE1 complexes reach the cell surface independently of COPII vesicles and bypass the Golgi. HEK-293 cells were transfected with KCNE1-YFP, Kv7.1-CFP, Kv7.1-CFP+KCNE1-YFP and HA-Sar1H79G. In addition, some cells were treated with 5 µg/ml BFA for 4 h. ER staining was performed by transfection with ER-DsRed (blue). (Aa-Cc) KCNE1 and ER colocalization upon Golgi disruption. (Aa-Ac) Control experiment. (Aa) ER; (Ab) KCNE1-YFP, green; (Ac), merge, cyan. (Ba-Bd) Effects of Sar1H79G expression on KCNE1. (Ba) ER; (Bb) KCNE1-YFP; (Bc) Sar1H79G; (Bd) Merge image of Ba-Bc. (Ca-Cc) BFA treatment. (Ca) ER; (Cb) KCNE1-YFP, green; (Cc) merge. Note that while a notable amount of KCNE1 reached the plasma membrane in Ac, almost no KCNE1 was detectable at the cell surface in Bd and Cc. (Da-Fc) Kv7.1 trafficking upon blockade of ER-Golgi transport. (Da-Dc) Control conditions. Kv7.1-CFP, shown in red (Db), colocalized with the ER, shown in blue (Da). (Dc) Merge, magenta. (Ea-Ed) Sar1H79G expression. (Ea) ER in blue; (Eb) Kv7.1-CFP; (Ec) Sar1H79G; (Ed) merge of Ea-Ec. (Fa-Fc) Golgi bypass upon BFA treatment. (Fa) ER, blue; (Fb) Kv7.1-CFP, red; (Fc) merged image. Note that Kv7.1 reached the cell surface upon blockade of ER-Golgi transport. (Ga-Id) Kv7.1-KCNE1 complexes reach the PM in a COPII-independent manner, bypassing the Golgi. (Ga-Gd) Kv7.1 and KCNE1 colocalization with the ER compartment in control conditions. (Gd) Merge: triple colocalization is shown in white, and partial colocalization of green and red is shown in yellow. (Ha-He) Sar1H79G expression blocks ER-Golgi transport. (Hd) Sar1H79G expression; (He) merged image. (Ia-Id) BFA treatment; (Id) merge: triple colocalization is shown in white, and partial colocalization of green and red is shown in yellow. In all panels: ER, blue; KCNE1, green; Kv7.1, red. Scale bar, 10 µm. Note that, unlike Aa-Cc, KCNE1 reached the plasma membrane in the presence of Kv7.1 regardless of whether Sar1H79G or BFA was present.

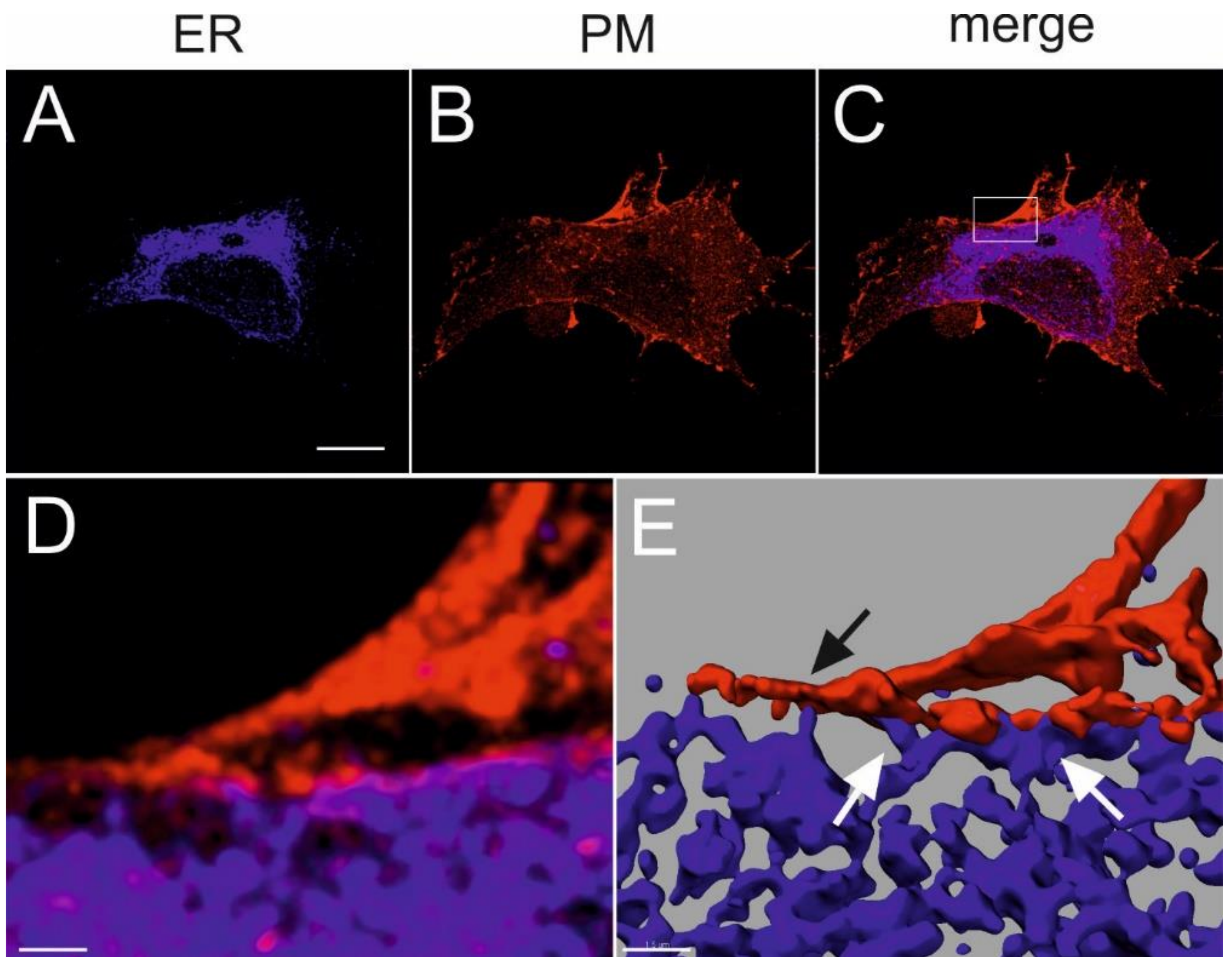


Figure S6. Identification of ER-PM junctions in HEK-293 cells. HEK-293 cells were transiently transfected with a PM marker (Akt-PH-pDsRed) and immunolabeled with calnexin, an ER-resident protein. (A-C) Maximal projection of the z stack of the whole cell volume. (A) Calnexin, blue (ER); (B) Akt-PH-pDsRed, red (PM); (C) merge. Scale bar, 10 μm. The white rectangle highlights the magnified area in D and E. (D) Magnified view from C showing PM and ER colocalization. The merged image indicates the sites for ER-PM contact in magenta. (E) Surface render of the area magnified in C. The ER appears in blue, and the PM appears in red. The black arrow points to a projection of the cortical ER juxtaposed with the PM. The white arrows denote sites where the ER marker fused with the PM. Scale bar, 1.5 μm.

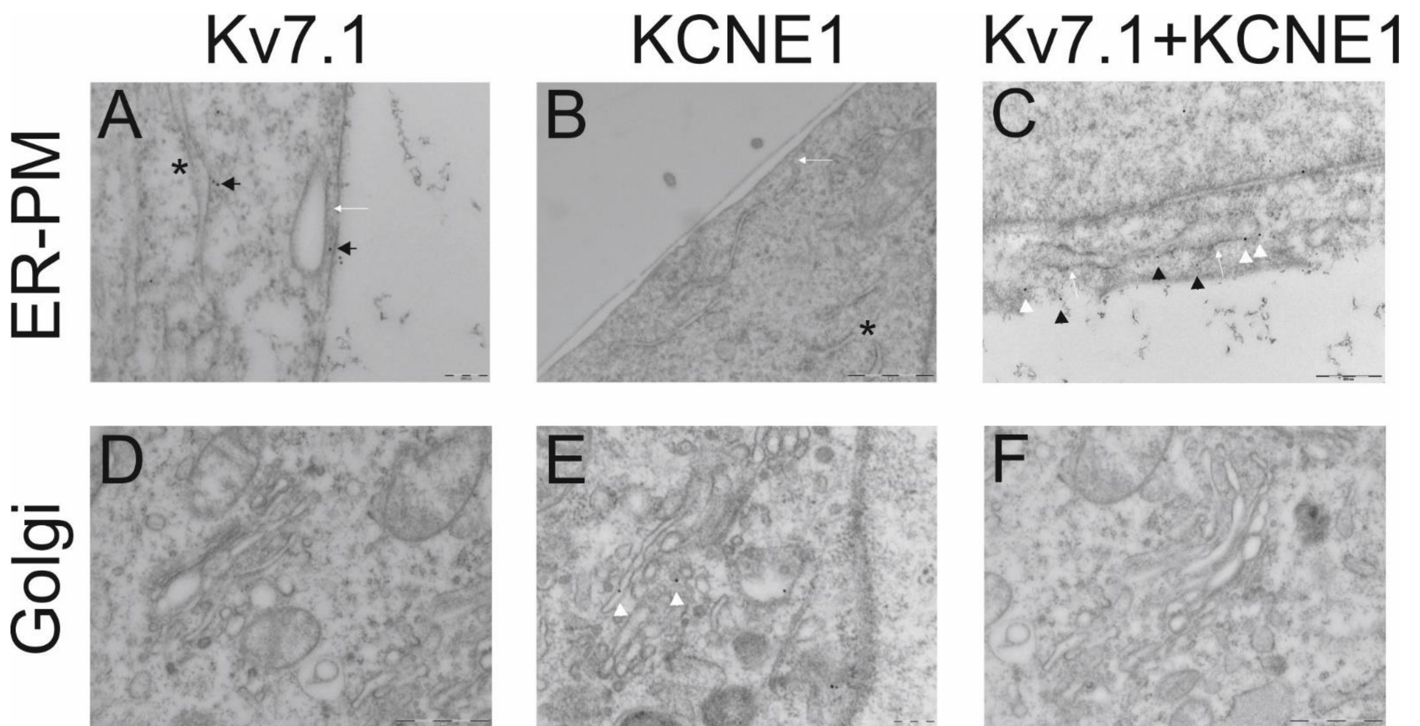


Figure S7. Kv7.1-KCNE1 complexes localize at ER-PM junctions. HEK-293 cells were transfected with Kv7.1-CFP, KCNE1-YFP and Kv7.1-CFP+KCNE1-YFP. Representative electron micrographs showing that KCNE1, which was detected in the Golgi (E), was present in ER-PM structures upon Kv7.1 coexpression (C). Top panels: Kv7.1 (A) and KCNE1 (C) in ER-PM structures. Kv7.1 (12 nm gold particles, black arrowheads) was detected in the ER (asterisk) and in ER-PM structures (white arrow). KCNE1 (B) was detected in neither the ER (asterisk) nor ER-PM structures (white arrow). KCNE1 (C, 18 nm gold particles indicated with white arrowheads) was detected in the ER-PM (white arrows) upon coexpression with Kv7.1 (12 nm particles, black arrowheads). Bottom panels: Kv7.1 (D) was absent from the Golgi. KCNE1 (white arrowheads, E) was detected in Golgi cisternae but disappeared from these structures upon coexpression with Kv7.1 (F). The bars represent 50 nm.

Supplemental video 1. Identification of ER-PM junctions in HEK-293 cells. Representative 3D maximal projection of the z stack of the whole cell volume stained for nucleus (light blue, DAPI), plasma membrane (red, Akt-PH-pDsRed) and endoplasmic reticulum (dark blue, calnexin). A close zoom in highlights ER projections contacting with PM.

Supplemental video 2. Kv7.1-KCNE1 complexes localize at tip ER projections. HEK-293 cells were transfected with Kv7.1-CFP (green) KCNE1-YFP (red) and ER-DsRed (blue, ER marker). Kv7.1 and KCNE1 subunits colocalize at discrete sites in distal ER domains.

Supplemental video 3. Kv7.1-KCNE1 complexes localize at ER-PM junctions. Multiple staining of HEK-293 cells transfected with Kv7.1-CFP (light blue) KCNE1-YFP (green) and Akt-PH-pDsRed (red, plasma membrane). Calnexin stains ER structures (dark blue). A mask of cortical ER juxtaposed with the PM (ER-PM junctions) is highlighted in pink. Note that Kv7.1-KCNE1 complexes are in close contact with pink ER-PM structures.