Supplementary Figure Legends

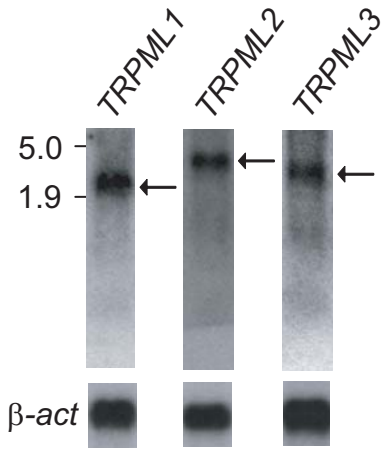
FIGURE S1. Coexpression of native TRPML mRNAs in a human cell line. A Northern blot containing RNA (0.5 μg/lane) prepared from a human T98G glioma cell line was probed with the indicated *TRPML* DNAs. The positions of two RNA size markers are indicated to the left. The *arrows* show the bands corresponding to TRPML1 (2.0 kb), TRPML2 (3.1 kb) and TRPML3 (2.8 kb). The same blots were reprobed with a β-actin (β-act) DNA probe.

Figure S2. LysoTracker-Red staining of untransfected HEK293 cells. Confocal images of four different samples of untransfected HEK293 cells that were previously loaded with LysoTracker-Red (100 nM), fixed and viewed at 568 nm.

FIGURE S3. Comparison of the subcellular distribution of TRPMLs with markers for various intracellular organelles. (A-B) Confocal images of HEK293 cells co-transfected with TRPML1-HA and ER-YFP. The cells were stained with anti-HA primary antibodies and Alexa568-conjugated secondary antibodies and viewed at the following excitation wavelengths: (A) 568 nm, TRPML1-HA, red; (B) 488 nm, ER-YFP, green. (C) Merge of A and B. (D-F) Same as A-C, but in HEK293 cells co-transfected with TRPML2-HA and ER-YFP. (G) Merged confocal image of HEK293 cells co-transfected with vectors encoding TRPML1-HA and GFP-Golgin160 and stained with anti-HA primary antibodies, Alexa568-conjugated secondary antibodies and viewed at excitation wavelengths of 568 nm (TRPML1-HA, red) and 488 nm (GFP-Golgin160, green). (H-I) Same as G, but in HEK293 cells expressing GFP-Golgin and either TRPML2-HA or TRPML3-HA respectively. (J) Merged confocal image of HEK293 cells transfected with a vector encoding TRPML1-YFP and stained with anti-Rab5 primary antibodies, Alexa568-conjugated secondary antibodies and viewed at excitation wavelengths of 568 nm (Rab5, red) and 488 nm (TRPML1-HA, green). (K-L) Same as J, but in HEK293 cells transfected with TRPML2-YFP and TRPML3-YFP respectively. (M) Merged confocal image of HEK293 cells co-transfected with vectors encoding TRPML1-YFP and Rab7-HA and stained with anti-HA primary antibodies, Alexa568-conjugated secondary antibodies and viewed at excitation wavelengths of 568 nm (Rab7-HA, red) and 488 nm (TRPML1-YFP, green). (N-O) Same as M, but in HEK293 cells co-transfected with TRPML2-YFP and TRPML3-YFP respectively. (P-R) Same as (M-O), but cells were stained with anti-Rab8 primary antibodies. The scale bar represents 200 μm.

FIGURE S4. TRPML3 fluorescence intensity similar if expressed alone or in combination with organellar markers or other TRPML proteins. HEK293 cells were transfected were with plasmids expressing the following proteins and viewed by epifluorescence. (A) TRPML3-HA; (B) TRPML3-HA and ER-YFP; (C) TRPML3-HA and LAMP3-YFP; (D) TRPML3-HA and TRPML1-MYC; and (E) TRPML3-HA, TRPML1-MYC and LAMP3-YFP. The cells were stained with either rabbit anti-HA primary antibodies alone (A-C) or with both rabbit anti-HA antibody and mouse anti-MYC antibodies (D-E). The cells were subsequently stained with anti-rabbit Alexa568-conjugated secondary antibodies (A-C, and E) or with both anti-rabbit Alexa568- and anti-mouse Alexa488-conjugated secondary antibodies (D). The cells were imaged at normal ambient temperature using an Axiovert 135 TV microscope (Zeiss) with a Plan-APOCHROMAT 40x oil immersion objective (N.A. 1.4) (Zeiss) and a CoolSnap HQ CCD camera (Photometrics) at the excitation wavelengths of 568 nm (TRPML3-HA) and 488 nm (ER-YFP, LAMP3-YFP and TRPML1-MYC). Only the cells that showed fluorescence at both wavelengths were used for analysis (except when TRPML3-HA was expressed alone). For the analysis, each cell that showed fluorescence at the two wavelengths was selected by choosing a region of interest (ROI) around it. Subsequently, the average TRPML3-associated fluorescence intensity of this cell was determined by measuring the average fluorescence intensity of the ROI

Figure S1



untransfected HEK 293 cells

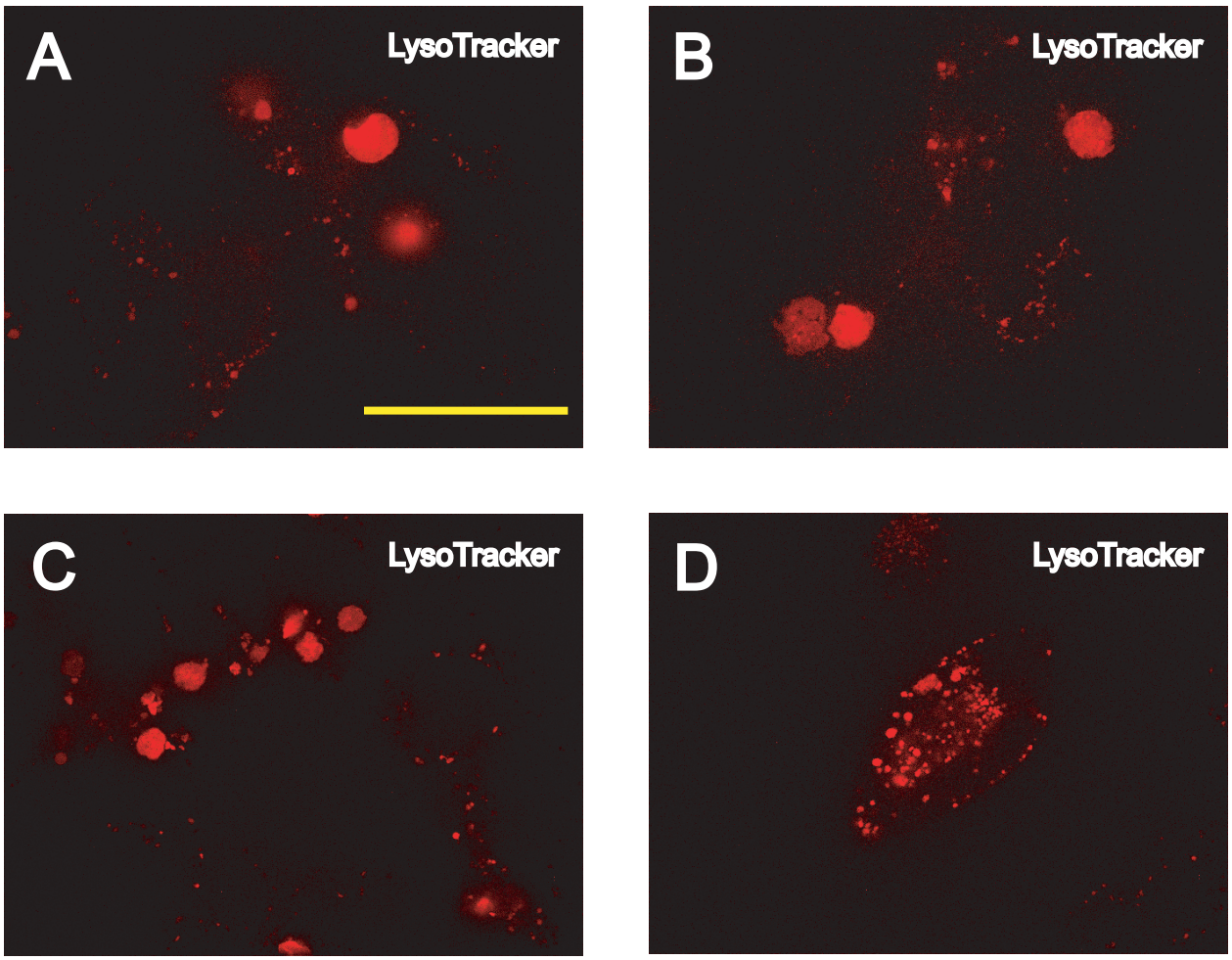
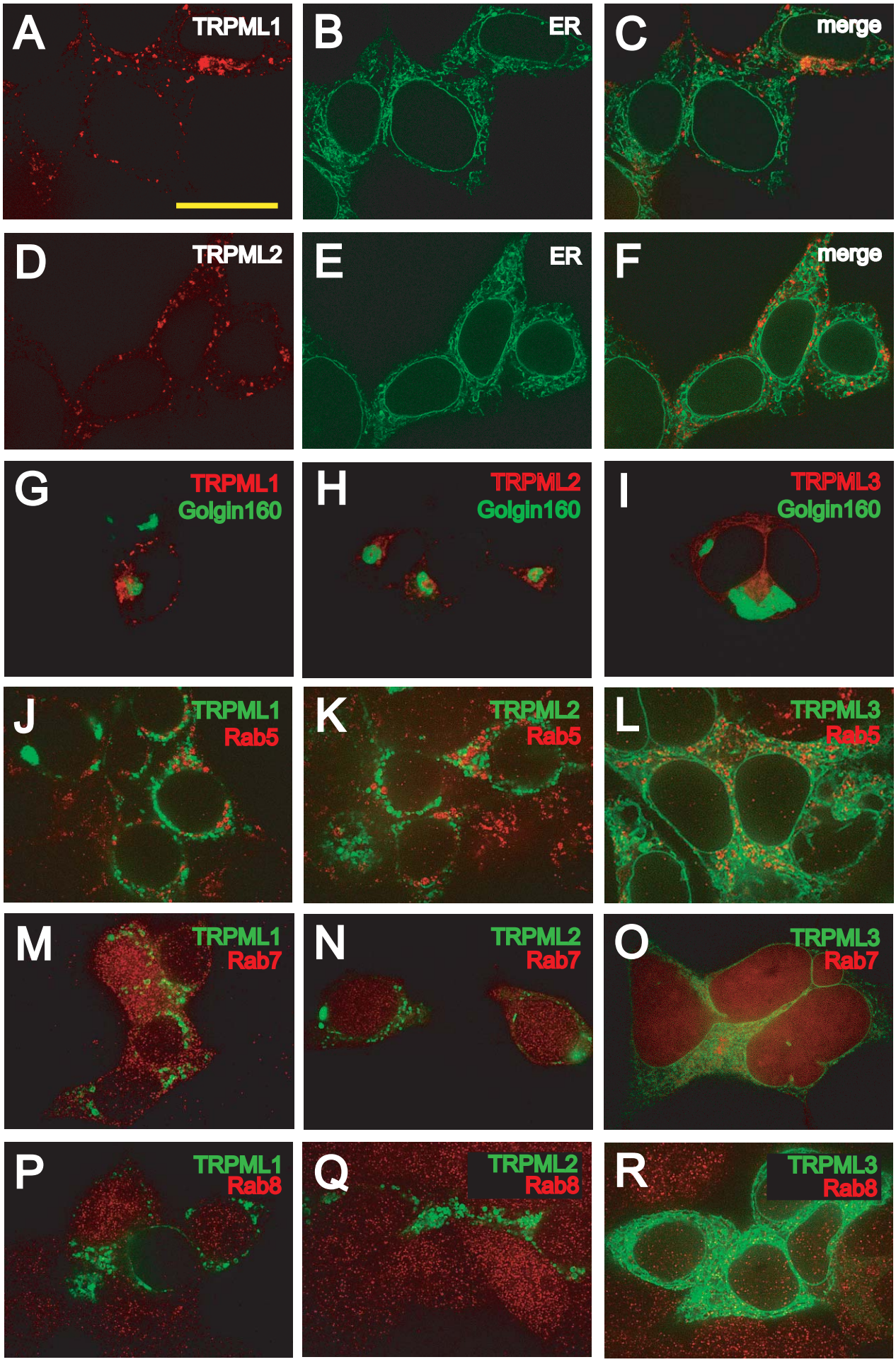


Figure S3


A
TRPML1
B
ER
C
merge
D
TRPML2
E
ER
F
merge
G
TRPML1
Golgin160
H
TRPML2
Golgin160
I
TRPML3
Golgin160
J
TRPML1
Rab5
K
TRPML2
Rab5
L
TRPML3
Rab5
M
TRPML1
Rab7
N
TRPML2
Rab7
O
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P
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Rab8
Q
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Rab8
R
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Rab8

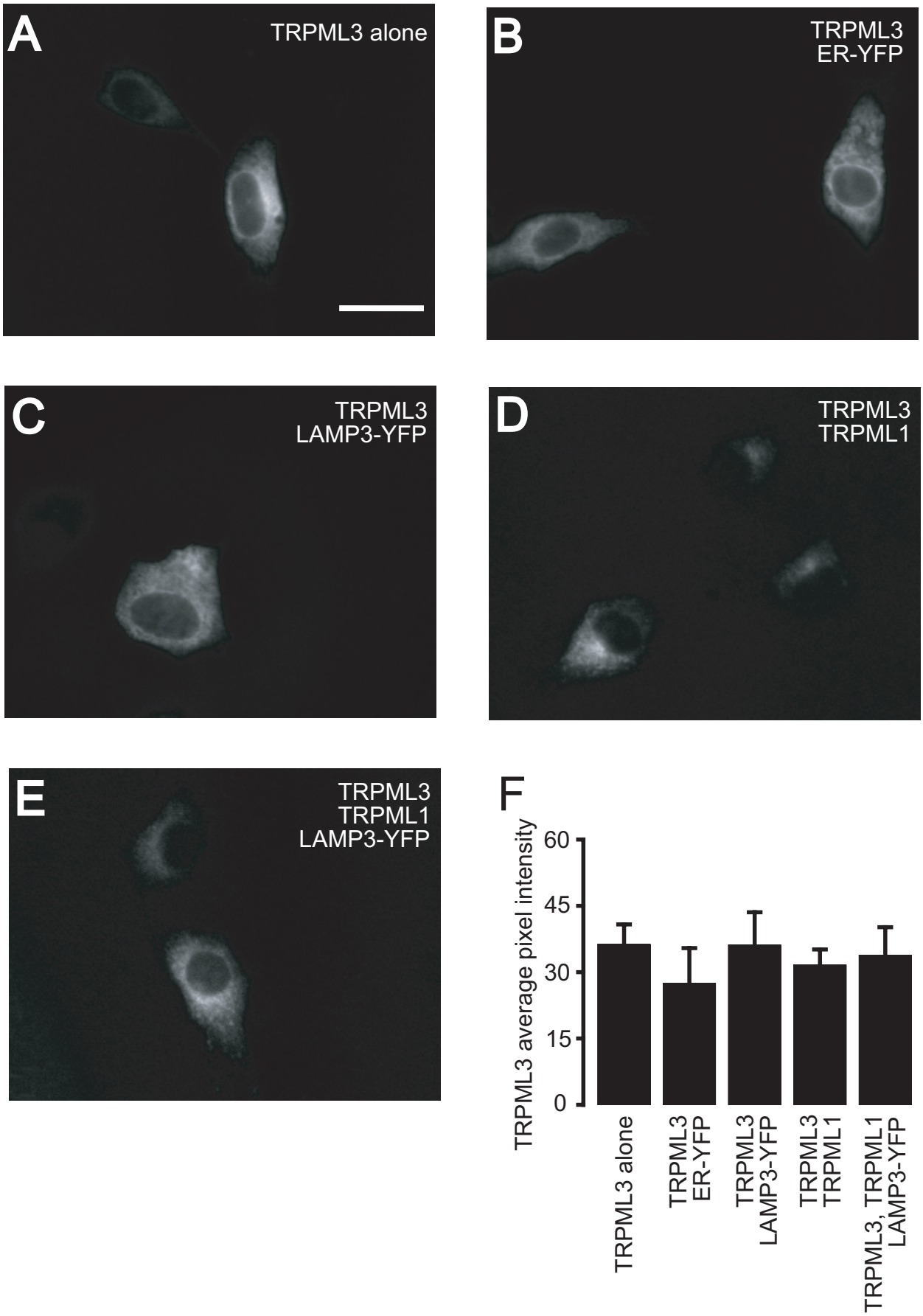
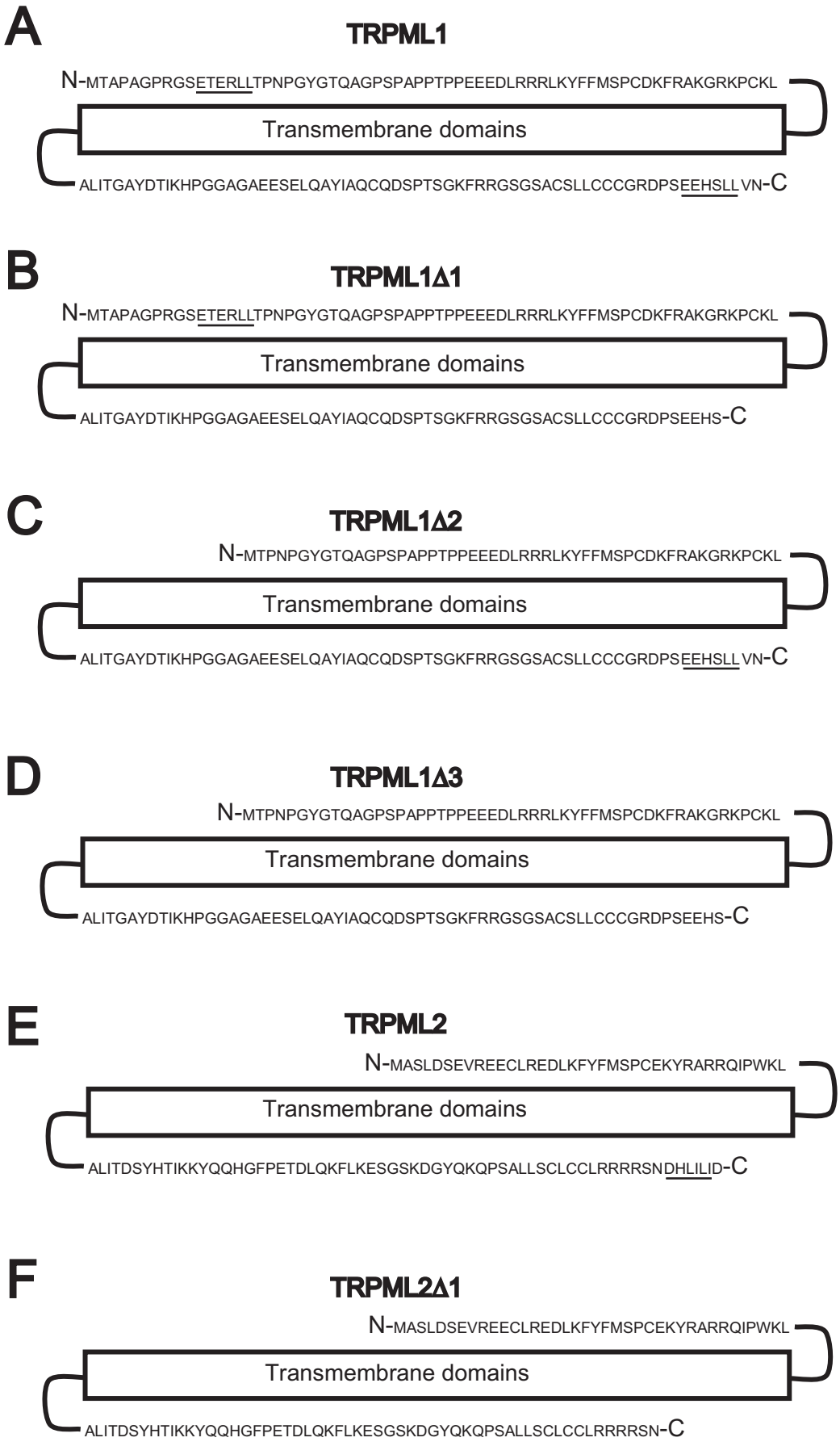

A
TRPML3 alone
B
TRPML3
ER-YFP
C
TRPML3
LAMP3-YFP
D
TRPML3
TRPML1
E
TRPML3
TRPML1
LAMP3-YFP
F
TRPML3 average pixel intensity
60
45
30
15
0
TRPML3 alone
TRPML3
ER-YFP
TRPML3
LAMP3-YFP
TRPML3
TRPML1
TRPML3, TRPML1
LAMP3-YFP

Figure S5


A
TRPML1
N-MTAPAGPRGSETERLLTPNPGYGTQAGPSPAPPTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPCKL
Transmembrane domains
ALITGAYDTIKHPGGAGAEESELQAYIAQCQDSPTSGKFRRGSGSACSLLCCCGRDPSEEHSLLVN-C
B
TRPML1Δ1
N-MTAPAGPRGSETERLLTPNPGYGTQAGPSPAPPTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPCKL
Transmembrane domains
ALITGAYDTIKHPGGAGAEESELQAYIAQCQDSPTSGKFRRGSGSACSLLCCCGRDPSEEHS-C
C
TRPML1Δ2
N-MTPNPGYGTQAGPSPAPPTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPCKL
Transmembrane domains
ALITGAYDTIKHPGGAGAEESELQAYIAQCQDSPTSGKFRRGSGSACSLLCCCGRDPSEEHSLLVN-C
D
TRPML1Δ3
N-MTPNPGYGTQAGPSPAPPTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPCKL
Transmembrane domains
ALITGAYDTIKHPGGAGAEESELQAYIAQCQDSPTSGKFRRGSGSACSLLCCCGRDPSEEHS-C
E
TRPML2
N-MASLDSEVREECLREDLKFYFMSPCEKYRARRQIPWKL
Transmembrane domains
ALITDSYHTIKKYQQHGFPETDLQKFLKESGSKDGYQKQPSALLSCLCCLRRRRSNDHLILID-C
F
TRPML2Δ1
N-MASLDSEVREECLREDLKFYFMSPCEKYRARRQIPWKL
Transmembrane domains
ALITDSYHTIKKYQQHGFPETDLQKFLKESGSKDGYQKQPSALLSCLCCLRRRRSN-C

(at 588 nm) using the Photoshop image analysis software (Adobe). An ROI in the close vicinity of the cell was subtracted as background. (F) Shown are the average fluorescence intensities of several individual cells (n=3 independent transfections per condition, with over 10 cells from each plate) at 588 nm. The mean TRPML3 fluorescence under these conditions did not show statistically significant differences when expressed alone or with the other proteins ($p>0.1$).

FIGURE S5. Sequences of N- and C-termini of TRPML1, TRPML2 and trafficking mutants. (A) TRPML1. (B) TRPML1Δ1. (C) TRPML1Δ2. (D) TRPML1Δ3. (E) TRPML2 (F) TRPML2Δ1. Di-leucines motifs are underlined in (A-C) and (E).