Supplemental Materials

Molecular Biology of the Cell

Kirmiz et al.

Supplemental Table 1. Figure 2C

Sample	ER-PM junction size (μm^2)	n number	p-value (two-tailed, unpaired t-test)
Kv2.2	1.98 ± 0.97	12 cells	vs. control, ****, $7.164x10^{-5}$
Kv2.1	1.69 ± 0.60	12 cells	vs. control, ****, $4.512x10^{-6}$
Kv1.4	0.49 ± 0.20	12 cells	vs. control, ns, 0.3602
control	0.57 ± 0.25	12 cells	N/A

Supplemental Table 2. Figure 2D

Sample	PM occupancy (%)	n number	p-value (two-tailed, unpaired t-test)
Kv2.2	34.9 ± 6.1	12 cells	vs. control, ****, $4.680x10^{-8}$
Kv2.1	33.0 ± 5.8	12 cells	vs. control, ****, $1.634x10^{-7}$
Kv1.4	14.7 ± 5.5	12 cells	vs. control, ns, 0.4256
control	16.4 ± 5.0	12 cells	N/A

Supplemental Table 3. Figure 2E

Sample	PCC Kv: DsRed2-ER5	n number	p-value (two-tailed, unpaired t-test)
Kv2.2	0.88 ± 0.04	15 cells	vs. Kv1.4, ****, $2.585x10^{-21}$
Kv2.1	0.87 ± 0.08	15 cells	vs. Kv1.4, ****, $5.768x10^{-19}$
Kv1.4	0.26 ± 0.08	15 cells	N/A

Supplemental Table 4. Supplemental Figure 1

Sample	PCC with GxTX	n number	p-value (ANOVA followed by Tukey's multiple comparisons test)
SEP-Kv2.1 (pHluorin)	0.85 ± 0.06	10 cells	vs. mCherry, ns, 0.5102; vs. SEC61β, *, 0.0396
SEP-Kv2.1 (mCherry)	0.86 ± 0.06	10 cells	vs. SEC61β, *, 0.0259
SEC61β	0.78 ± 0.11	10 cells	N/A

Supplemental Table 5. Figure 3C

Sample	PCC with GxTX	n number	p-value (two-tailed, unpaired t-test)
Kv2.2	0.90 ± 0.06	13 cells	vs. DsRed2-ER5, *, 0.01211
DsRed2-ER5	0.78 ± 0.14	13 cells	N/A

Supplemental Table 6. Figure 5C

Sample	Size (μm^2)	n number	p-value (two tailed, paired t-test)
Kv2.2 clusters rest	0.41 ± 0.12	7 cells	vs. +LatA, ***, 0.0007
Kv2.2 clusters +LatA	1.01 ± 0.30	7 cells	N/A
Kv2.1 clusters rest	0.48 ± 0.19	7 cells	vs. +LatA, **, 0.0019
Kv2.1 clusters +LatA	0.69 ± 0.18	7 cells	N/A

Supplemental Table 7. Figure 5D

Sample	Size (μm^2)	n number	p-value (two tailed, paired t-test)
Kv2.2-EPJ rest	0.48 ± 0.19	7 cells	vs. +LatA, **, 0.0043
Kv2.2-EPJ +LatA	0.69 ± 0.18	7 cells	N/A
Kv2.1-EPJ rest	0.64 ± 0.10	7 cells	vs. +LatA, **, 0.0039
Kv2.1-EPJ +LatA	0.98 ± 0.22	7 cells	N/A

Supplemental Table 8. Figure 5E

Sample	Number of ER:PM junctions	n number	p-value (two tailed, paired t-test)
Kv2.2-EPJ rest	104.29 ± 24.81	7 cells	vs. +LatA, *, 0.0150
Kv2.2-EPJ +LatA	74.71 ± 11.04	7 cells	N/A
Kv2.1-EPJ rest	60.43 ±- 16.69	7 cells	vs. +LatA, *, 0.0161
Kv2.1-EPJ +LatA	51.14 ± 15.74	7 cells	N/A

Supplemental Table 9. Figure 5F

Sample	PCC Kv2:DsRed2-ER5	n number	p-value (two tailed, paired t-test)
Kv2.2 rest	0.84 ± 0.052	10 cells	vs. +LatA, ns, 0.2505
Kv2.2 +LatA	0.85 ± 0.055	10 cells	N/A
Kv2.1 rest	0.82 ± 0.058	10 cells	vs. +LatA, ns, 0.4408
Kv2.1 +LatA	0.81 ± 0.064	10 cells	N/A

Supplemental Table 10. Figure 6B and C

Sample	PCC with Kv2.2	MOC with Kv2.2	n number	PCC vs. MOC, p-value (two-tailed, paired t-test)
E-Syt2	0.52 ± 0.27	0.92 ± 0.045	17 cells	****, 6.229×10^{-6}
E-Syt3	0.69 ± 0.19	0.98 ± 0.034	17 cells	****, 5.690×10^{-6}
JP2	0.84 ± 0.13	0.99 ± 0.015	17 cells	***, 1.057×10^{-4}
JP4	0.90 ± 0.071	0.98 ± 0.013	18 cells	****, 7.894×10^{-5}
STIM1	0.79 ± 0.13	0.94 ± 0.031	21 cells	****, 2.070×10^{-6}
STIM2a	0.76 ± 0.051	0.95 ± 0.025	19 cells	****, 1.1×10^{-14}
STIM2β	0.82 ± 0.10	0.97 ± 0.016	17 cells	****, 2.949×10^{-6}

Supplemental Table 11. Figure 6F and G

Sample	PCC with Orai1	n number	p-value (two tailed, paired t-test)
STIM1 Rest	0.17 ± 0.26	12 cells	N/A
STIM1 +Thap	0.61 ± 0.19	12 cells	vs. Rest, ***, 0.0005092
Kv2.2 Rest + STIM1	0.21 ± 0.26	12 cells	N/A
Kv2.2 +Thap + STIM1	0.39 ± 0.19	12 cells	vs. Rest, *, 0.02501
Kv2.2 Rest no STIM1	0.018 ± 0.29	16 cells	N/A
Kv2.2 +Thap no STIM1	0.32 ± 0.31	16 cells	vs. Rest, ****, 0.00003238

Supplemental Table 12. Figure 7C

Sample	PCC Kv2: CB5-FKBP	n number	p-value (two tailed, paired t-test)
Kv2.2 Rest	0.86 ± 0.055	7 cells	N/A
Kv2.2 +Rap	0.19 ± 0.18	7 cells	vs. Rest, ****, 0.00006706
Kv2.2 +Rap +LatA	0.14 ± 0.12	7 cells	vs. +Rap, ns, 0.3099

Supplemental Table 13. Figure 8B and C

Sample	Current at +40 mV (nA)	n number	p-value (two-tailed, unpaired t-test)
GFP-Kv2.2	5.88 ± 2.31	7 cells	vs. GFP, ***, 0.000762
GFP-Kv2.2 P412W	0.70 ± 0.31	5 cells	vs. GFP, ns, 0.123
GFP-Kv2.1	9.98 ± 4.93	9 cells	vs. GFP, ***, 0.000399
GFP-Kv2.1 P404W	0.57 ± 0.27	6 cells	vs. GFP, ns, 0.287
GFP	0.42 ±0.16	7 cells	N/A

Supplemental Table 14. Figure 8F and G

Sample	Cluster size (μm^2)	n number	p-value (two-tailed, unpaired t-test)
GFP-Kv2.2 P412W	0.76 ± 0.91	3 cells	vs. GFP-Kv2.2, ns, 0.0637
GFP-Kv2.2	0.99 ± 1.14	3 cells	N/A
GFP-Kv2.1 P404W	0.59 ± 0.84	3 cells	vs. GFP-Kv2.1, ns, 0.9441
GFP-Kv2.1	0.59 ± 0.79	3 cells	N/A

Supplemental Table 15. Figure 8H and I

Sample	PCC with GxTX	n number	p-value (two-tailed, unpaired t-test)
GFP-Kv2.2 P412W	0.88 ± 0.050	10 cells	vs. GFP-Kv2.2, ns, 0.1531
GFP-Kv2.2	0.91 ± 0.039	10 cells	N/A
GFP-Kv2.1 P404W	0.91 ± 0.055	10 cells	vs. GFP-Kv2.1, ns, 0.0863
GFP-Kv2.1	0.94 ± 0.030	10 cells	N/A

Supplemental Table 16. Figure 9C

Sample	ER-PM junction size (μm^2)	n number	p-value (ANOVA followed by Tukey's multiple comparisons test)
GFP-Kv2.2	1.36 ± 0.65	10 cells	vs. control, *, 0.01308
GFP-Kv2.2 P412W	1.45 ± 0.81	10 cells	vs. Kv2.2 ns, 0.9998; vs. control, **, 0.004206
GFP-Kv2.2A S605A	0.40 ± 0.11	10 cells	vs. control, ns, 0.9990
GFP-Kv2.1	1.38 ± 0.79	10 cells	vs. control, *, 0.01048
GFP-Kv2.1 P404W	1.26 ± 0.49	10 cells	vs. Kv2.1, ns, 0.9989; vs. control, *, 0.04155
GFP-Kv2.1 S586A	0.47 ± 0.081	10 cells	vs. control, ns, >0.9999
Control	0.52 ± 0.19	10 cells	N/A

Supplemental Table 17. Figure 9D

Sample	PM coverage (%)	n number	p-value (ANOVA followed by Tukey's multiple comparisons test)
GFP-Kv2.2	29.8 ± 7.0	10 cells	vs. control, ****, $5.507x10^{-5}$
GFP-Kv2.2 P412W	29.4 ± 6.4	10 cells	vs. Kv2.2, ns, >0.9999; vs. control, ***, 0.0001027
GFP-Kv2.2 S605A	12.1 ± 4.8	10 cells	vs. control, ns, 0.8047
GFP-Kv2.1	30.1 ± 7.0	10 cells	vs. control, ****, $3.3938x10^{-5}$
GFP-Kv2.1 P404W	28.6 ± 6.8	10 cells	vs. Kv2.1, ns, 0.9977; vs. control, ***, 0.0002994
GFP-Kv2.1 S586A	15.4 ± 2.6	10 cells	vs. control, ns, >0.9999
Control	15.9 ± 6.4	10 cells	N/A

Supplemental Table 18. Figure 9E

Sample	PCC with DsRed2-ER5	n number	p-value (ANOVA followed by Tukey's multiple comparisons test)
GFP-Kv2.2	0.86 ± 0.04	12 cells	N/A
GFP-Kv2.2 P412W	0.88 ± 0.04	12 cells	vs. GFP-Kv2.2, ns, 0.9943
GFP-Kv2.2 S605A	0.44 ± 0.14	12 cells	vs. GFP-Kv2.2, ****, $<1x10^{-15}$ vs. GFP-Kv2.2 P412W, ****, $<1x10^{-15}$
GFP-Kv2.1	0.86 ± 0.06	12 cells	N/A
GFP-Kv2.1 P404W	0.86 ± 0.07	12 cells	vs. GFP-Kv2.1, ns, >0.9999
GFP-Kv2.1 S586A	0.26 ± 0.17	12 cells	vs. GFP-Kv2.1, ****, $<1x10^{-15}$ vs. GFP-Kv2.1 P404W, ****, $<1x10^{-15}$

Supplemental Table 19. Supplementary Figure 2C

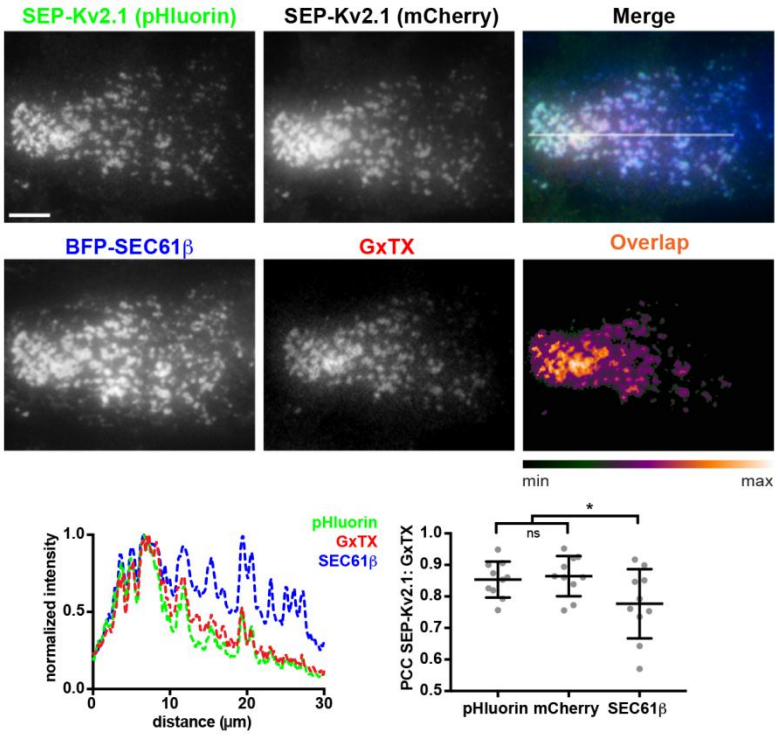
Sample	ER-PM junction velocity (μm/sec)	n number	p-value (ANOVA followed by Tukey's multiple comparisons test)
GFP-Kv2.2	0.069 ± 0.019	3 cells	vs. control, ****, $1.358x10^{-11}$

GFP-Kv2.2 P412W	0.067 ± 0.018	3 cells	vs. Kv2.2, ns, 0.9796; vs. control, $1.038x10^{-12}$
GFP-Kv2.1	0.062 ± 0.015	3 cells	vs. control, ****, $7.15x10^{-13}$
GFP-Kv2.1 P404W	0.066 ± 0.017	3 cells	vs. Kv2.1, ns, 0.8041; vs. control, ****, $8.49x10^{-13}$
Control	0.093 ± 0.020	3 cells	N/A

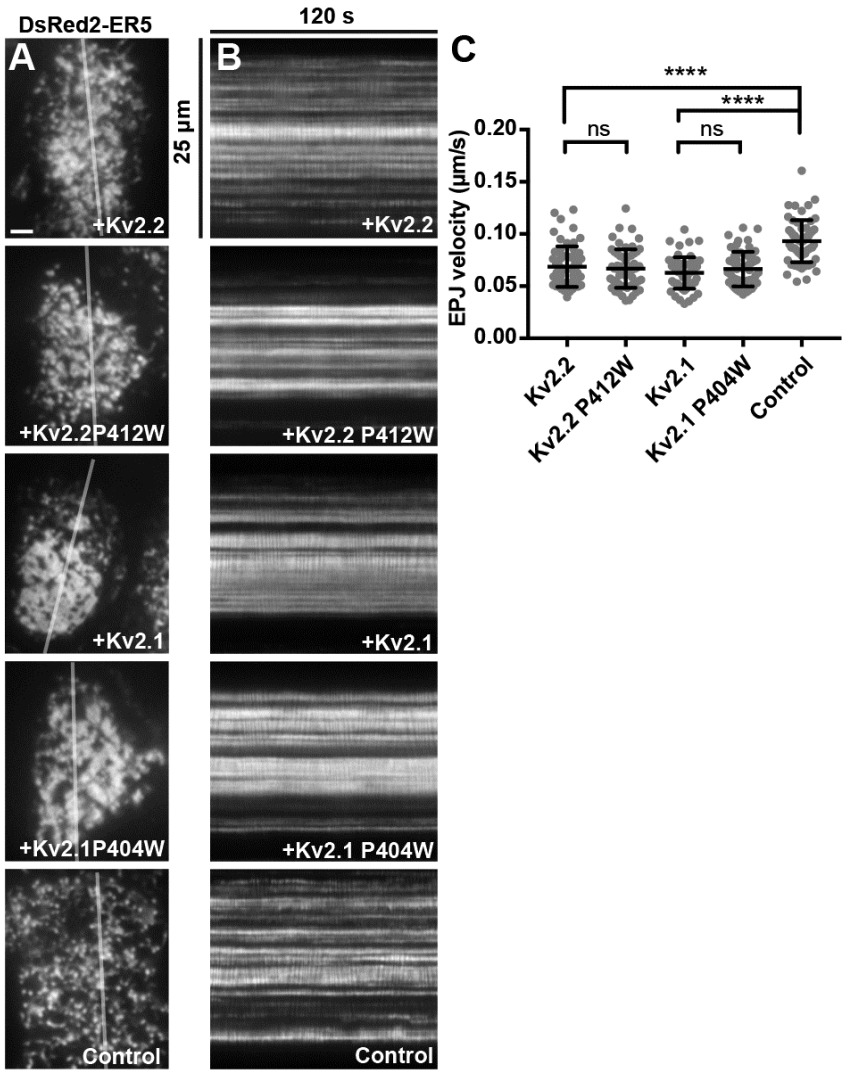
Supplemental Table 20. Figure 11F

Sample	RyR cluster size (μm^2)	n number	p-value (two tailed, unpaired t-test)
WT	0.22 ± 0.012	3 animals	N/A
Kv2.1 KO	0.21 ± 0.0075	3 animals	vs. WT, ns, 0.0884
Kv2.2 KO	0.22 ± 0.012	2 animals	vs. WT, ns, 0.5506
Kv2 dKO	0.17 ± 0.039	3 animals	vs. WT, *, 0.0199

Supplemental Figure Legends.



Supplemental Figure 1. Cell surface SEP-Kv2.1 at ER-PM junctions can be efficiently labeled with GxTX-633. Top panels. TIRF images of live HEK293T cells coexpressing SEP-Kv2.1 and BFP-SEC61β and surface labeled with GxTX-633. The merged image shows SEP-Kv2.1 (pHluorin), BFP-SEC61β, and GxTX-633. Scale bar is 5 μm and holds for all panels. Heat map shows overlap of SEP-Kv2.1 (pHluorin) and GxTX-633 pixels. Bottom left panel shows the normalized fluorescence intensity values across the line scan depicted by the white line in the merged image. Graph on bottom right shows PCC values between GxTX and SEP-Kv2.1 or BFP-SEC61β. Bars are mean ± SD. See Supplemental Table 4 for values and statistical analyses.



Supplemental Figure 2. Both wild-type and nonconducting Kv2 channel mutants stabilize ER-PM junctions in HEK293T cells. (A) TIRF images of DsRed2-ER5 expressed in live HEK293T cells with and without coexpression of wild-type and mutant Kv2 channel isoforms as labeled. Scale bar is 2.5 µm and holds for all panels. (B) Kymographs of DsRed2-ER5 mobility from regions indicated by the lines in the adjacent panels. (C) Graph of ER-PM junction (EPJ) velocity (as reported by DsRed2-ER5 in TIRF). Bars are mean ± SD. See Supplemental Table 19 for values and statistical analyses.