Diacylglycerols interact with the L2 lipidation site in TRPC3 to induce a sensitized channel state

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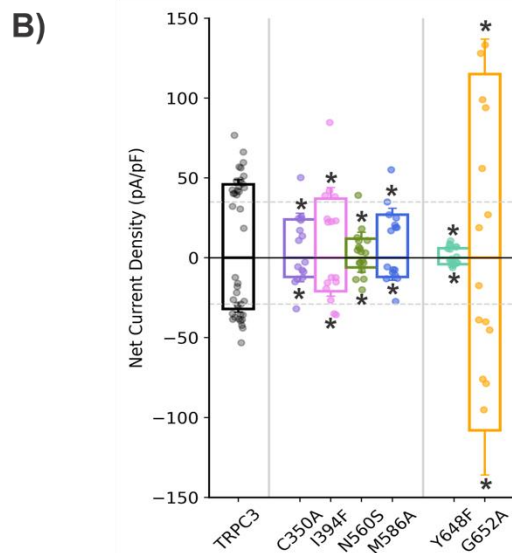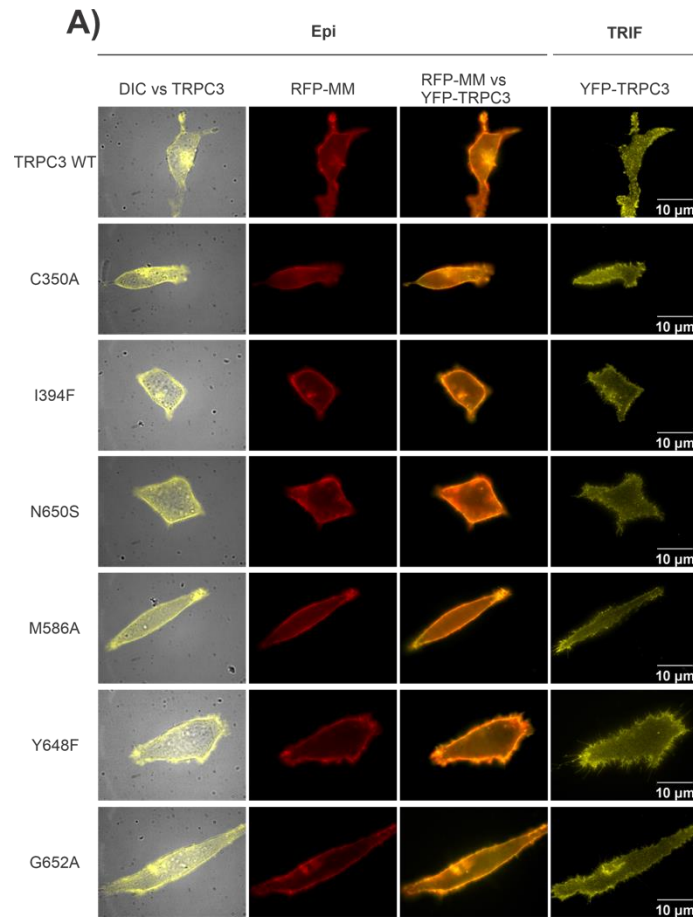
Appendix Table of Contents

Appendix Figures:

Appendix Figure S1 - Fluorescence images of WT and mutant TRPC3 membrane targeting.

A. representative epifluorescence (EF) and TIRF images of single cells expressing TRPC3 WT and TRPC3 mutants (L1 Mutants: C350A; I394F; N560S; M586A; L2 Mutants: Y648F; G652A). N = number of cells measured > 6 for each of the construct; scale bar = 10 µm.

B. Mean net current densities of GSK (10 µM)-stimulated inward and outward currents through TRPC3-WT (black) and mutant channels (color-coded as indicated) expressed in HEK293 cells. Values from individual experiments are shown for each of the columns (circles). Number of biological repetitions for each condition ≥ 6. Data are mean ± S.E.M; two-tailed t-test or Mann-Whitney test were applied between TRPC3-WT and mutants; *P < 0.05.

Constructs	1st UV ON $\tau \pm$ SEM	2nd UV ON $\tau \pm$ SEM	1st UV ON N (Power)	2nd UV ON N (Power)
TRPC3	1127 ± 250	128 ± 7	3.6 ± 0,7	2.0 ± 0,2
C350A	1130 ± 328	96 ± 20	3.6 ± 1,4	3.3 ± 0,5
I394F	1106 ± 185	113 ± 13	3.6 ± 1,6	2.1 ± 0,3
N650S	897 ± 153	106 ± 16	3.5 ± 0,5	3.3 ± 0,9
M586A	1029 ± 422	121 ± 8	3.2 ± 0,7	2.8* ± 0,3
Y648F	990 ± 292	44* ± 8	2.7 ± 0,9	9.9* ± 1,2
G652A	2918* ± 647	118 ± 3	3.6 ± 1	2.5 ± 0,5

Appendix Table S1 - Speed of current activation and power with repetitive photostimulation.

Number of biological repetitions ≥ 6. Data are mean ± S.E.M.; two-tailed t-test or Mann-Whitney test were applied between TRPC3-WT and mutants; *$P < 0.05$.

System	Lipid ratios, inner leaflet (%)	Lipid ratios, outer leaflet (%)	Length (µs)
Control	Chol (30), PC (20), PE (35), PS (15)	Chol (30), PC (60), PE (10)	20
2% DAG (SAG)	DAG (2), Chol (28), PC (20), PE (35), PS (15)	Chol (30), PC (60), PE (10)	40
10% DAG (SAG)	DAG (10), Chol (20), PC (20), PE (35), PS (15)	Chol (30), PC (60), PE (10)	20

Appendix Table S2 - Membrane systems used in this paper.

The abbreviations used in this table are: PC (phosphatidylcholine, POPC 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine), Chol (cholesterol, CHOL), PE (phosphatidylethanolamine, POPE 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine), PS (phosphatidylserine, POPS 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylserine), and DAG (diacylglycerol, specifically stearoyl-arachidonoyl glycerol, PADG stearoyl-arachidonoyl-diacylglycerol).

lb				ub
$-\infty$	$<$	A	$\leq$	0
0	$\leq$	S	$<$	$+\infty$
10	$\leq$	T	$<$	$+\infty$
1	$\leq$	P	$<$	$+\infty$
$-\infty$	$<$	K	$\leq$	0
$-\infty$	$<$	C	$<$	$+\infty$

Appendix Table S3 - Lower and upper bounds of the parameter set for curve fitting.