Supplementary information

TRPC6 G757D loss-of-function mutation associated with focal segmental glomerulosclerosis

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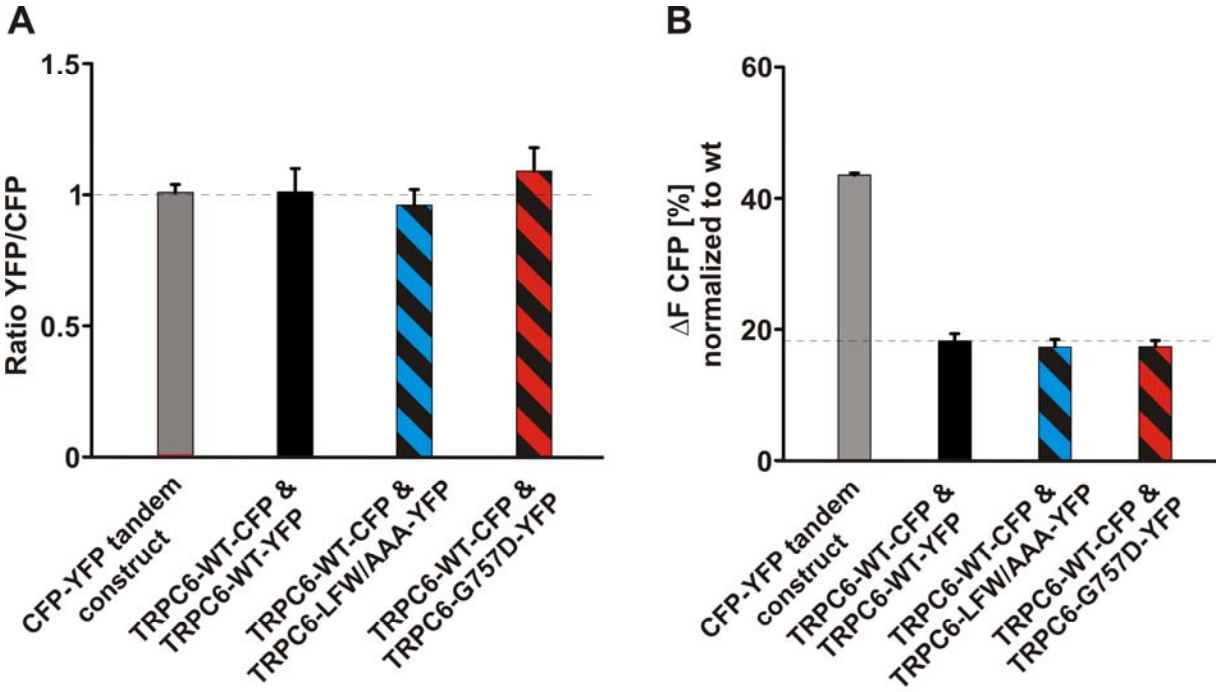

Figure S1. Optimization of fluorophore expression and fluorescence resonance energy transfer (FRET) measurements. (A) The expression ratio of TRPC6-WT-CFP and TRPC6-WT or G757D or LFW678-680AAA-YFP were optimized and iteratively adjusted by measuring the fluorescence of both fluorophores (CFP vs. YFP) as described in Method section. Shortly, fluorescent values of the fluorophores fused to TRPC6 protein were measured and compared with the fluorescence values of an intramolecularly fused CFP-YFP tandem protein allowing quantification of both expressed TRPC6 fusion proteins. With a cDNA-ratio of 1.5 g plasmid DNA coding for the CFP variant to 1 g plasmid DNA coding for the YFP variant, we obtained a ratio of fluorescence values indistinguishable from the tandem protein. Statistical analysis revealed no significant differences. Data are means ± SEM of at least two independent experiments representing in total 357 (CFP-YFP-Tandem), 78 (TRPC6 WT), 76 (TRPC6 G757D), 137 (LFW678-680AAA) transfected cells.

(B) FRET efficiencies as measure of the vicinity and interaction of proteins were measured in co-transfected HEK293 expressing TRPC6-WT (C-terminally fused to CFP) and TRPC6 WT, TRPC6 LFW678-680AAA (LFW/AAA) or TRPC6 G757D (each C-terminally fused to YFP). Statistical analysis revealed no significant differences; the values of TRPC6WT-CFP & TRPC6-WT-YFP correspond to published values (Hofmann et al., PNAS 2002). Data are means ± SEM of at least three independent experiments representing in total 247 (CFP-YFP-Tandem), 87 (WT / 1:1), 113 (G757D & WT / 1:1), 59 (LFW/AAA & WT / 1:1) transfected cells

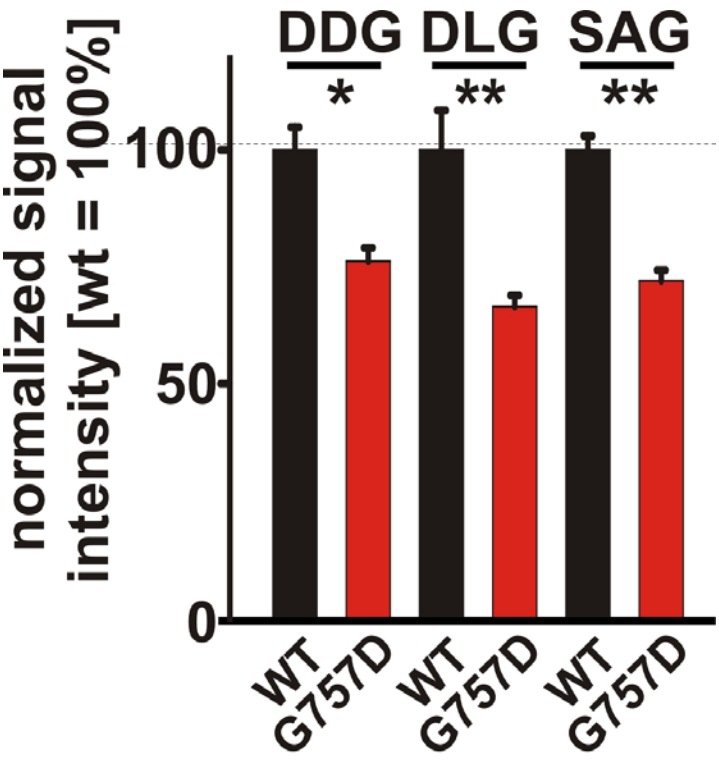

Figure S2. Effect of diacylglycerol analogues on WT TRPC6 and TRPC6 G757D Ca^{2+} entry.

Response to different diacylglycerol analogues were measured in TRPC6 WT and TRPC6 G757D expressing HEK293 cells. Statistical analysis of data obtained during at least three independent experiments is presented as bar graphs. The graph shows the responses of the cells after application of 1,2-Didecanoyl-*sn*-glycerol (DDG): 536 and 715 (WT/G757D) cells; 1,2-Dilauroyl-*sn*-glycerol (DLG): 581 and 623 (WT/G757D) cells; 1-Stearyl-2-Arachidonoyl-*sn*-glycerol (SAG): 459 and 581 (WT/G757D) cells. The amplitudes were determined as difference between peak and baseline Ca^{2+} levels relative to WT TRPC6 signals (*** $P \leq 0.001$).

Table S1. Oligonucleotides used for site-directed mutagenesis

Mutation	direction	sequence
85_88dupAYMF	sense	aggttagctaatcgaggaccagcatacatgtttGCATACATGTTTagtg atcgctccacaagcctatctata
85_88dupAYMF	antisense	tagataggcttgtggagcgatcactAAACATGTATGCaaaca tgtatgctggtcctcgattagctaacctt
G109S	sense	ctttttggatgcagctgaatatTCGaacatcccagtggtgcggaagat
G109S	antisense	atcttccgcaccactgggatgttCGAatattcagctgcatccaaaaag
N110H	sense	ctttttggatgcagctgaatatggtCacatcccagtggtgcggaagatgtt
N110H	antisense	aacatcttccgcaccactgggatgtGaccatattcagctgcatccaaaaag
P112Q	sense	ctttttggatgcagctgaatatggtaaTatTcAagtggtgcggaagatgttagaaga
P112Q	antisense	tcttctaacatcttccgcaccactTgAatAttaccatattcagctgcatccaaaaag
N125S	sense	ttagaagaatgccactcactcaGcgttaactgtgtggattacatg
N125S	antisense	catgtaatccacacagttaacgCtgagtgagtggcattcttctaa
M132T	sense	caacgttaactgtgtggattacaCCggTcagaatgccctacagttggcagt
M132T	antisense	actgccaactgtagggcattctgAccGGtgtaatccacacagttaacgttg
N143S	sense	gccctacagttggcagtggcTaGCgagcatctggaaattacaga
N143S	antisense	tctgtaatttccagatgctcGCtAgccactgccaactgtagggc
R175Q	sense	ctagctattagtaaaggttatgttcAgattgtggaagcaattctcagtcat
R175Q	antisense	atgactgagaattgcttccacaatcTgaacataacctttactaatagctag
H218L	sense	gatgaagatgggacacggttctcccTtgatgtgactccaatcattctggct
H218L	antisense	agccagaatgattggagtcacatcaAgggagaaccgtgtcccatcttcatc
S270T	sense	cagaagcatgactcgtttagccacAcGCgTtctaggattaatgcctataaaggcc
S270T	antisense	ggcctttataggcattaatcctagaAcGCgTgtggctaaacgagtcatgcttctg
R360H	sense	gaaacgctccagagtggtgatcaTggCcAcccaaatctcagccgtttaaaact
R360H	antisense	agttttaaacggctgagatttgggTgGccAtgatcaccactctggagcgtttc

L395A	sense	acttctctccatttggtatgagaatGCttctggtttacgacagcagacaatgg
L395A	antisense	ccattgtctgctgtcgtaaaccagaaGCattctcataccaaatggagagaagt
A404V	sense	tctggtttacgacagcagacaatggTggtcaagttccttgtggtccttgct
A404V	antisense	agcaaggaccacaaggaacttgaccAccattgtctgctgtcgtaaaccaga
G757D	sense	tctggttttcctactttgaggaggAcagaacacttcctgtacccttcaatct
G757D	antisense	ttgaagggtacaggaagtgttctgTcctcctcaaagtaggaaaaccagagtt
L780P	sense	ctgttttatctcttactgaagcCtaaaaaatggatttctgagctgtt
L780P	antisense	aacagctcagaaatccatttttttaGgcttcagtaagagataaaacag
Q889K	sense	aggggaactgaaggaaattaagAaggaTatctcaagtctccgctatgaact
Q889K	antisense	agttcatagcggagacttgagatAtcctTcttaatttccttcagttcccct
R895C	sense	gcaggacatctcaagtctcTgctatgaactccttgaaga
R895C	antisense	tcttcaaggagttcatagcAgagacttgagatgtcctgc
R895L	sense	attaagcaggacatctcaagtctccTctatgaactccttgaagaaaaatct
R895L	antisense	agatttttcttcaaggagttcatagAggagacttgagatgtcctgcttaat
E897K	sense	gcaggacatctcaagtctccgctatAaGctTcttgaagaaaaatctcagaatacag
E897K	antisense	ctgtattctgagatttttcttcaagAagCtTatagcggagacttgagatgtcctgc
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LFW678-680AAA	sense	cttcacaacagttgaagagagttttaagacaGCTGCcGCggctatatttggacttt ctgaagtgaaatcagtggtcatc
LFW678-680AAA	antisense	gatgaccactgatttcacttcagaaagtccaaatatagccGCgGCAGCtgtctt aaaactctcttcaactgttgtgaag