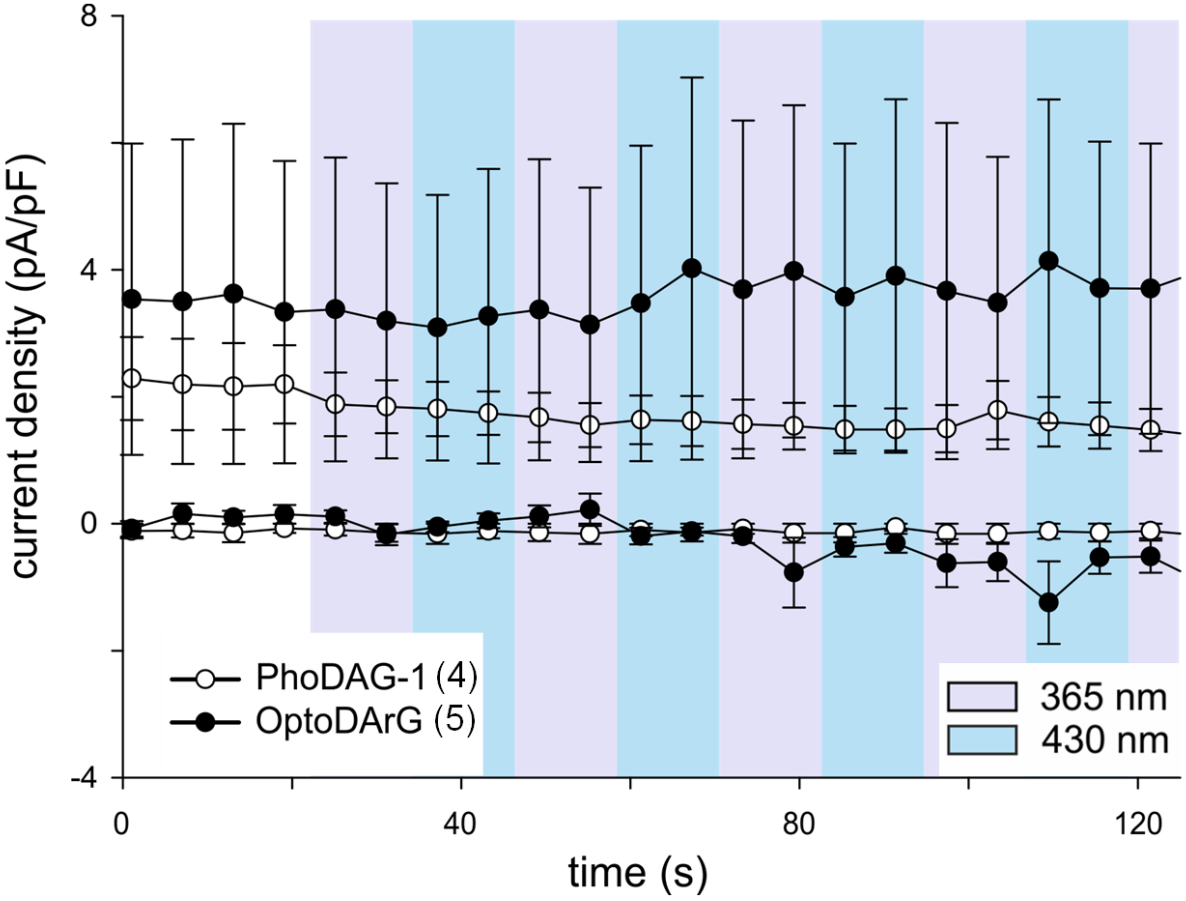
SUPPLEMENTARY INFORMATION

Supplementary Table 1: Characteristics of mutations in the TRPC3 pore domain

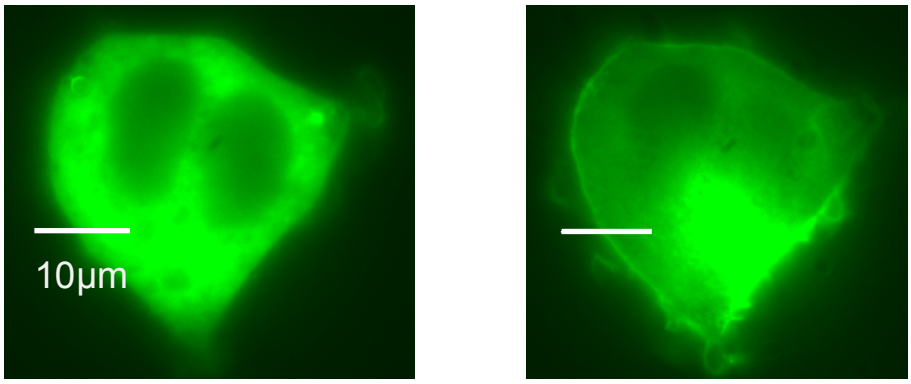
Mutation	Trafficking to the membrane	Basal activity	Agonist sensitivity	
			CCh	GSK1702934A
F618L	yes	yes	no	no
I625A	yes	no	no	no
I625G	yes	no	no	no
I625V	no	no	no	no
G652A	yes	yes	reduced	increased
G652L	yes	no	no	reduced

Supplementary Figure 1: Optical control of DAG photoswitches does not produce any significant membrane conductances in WT HEK293 cells



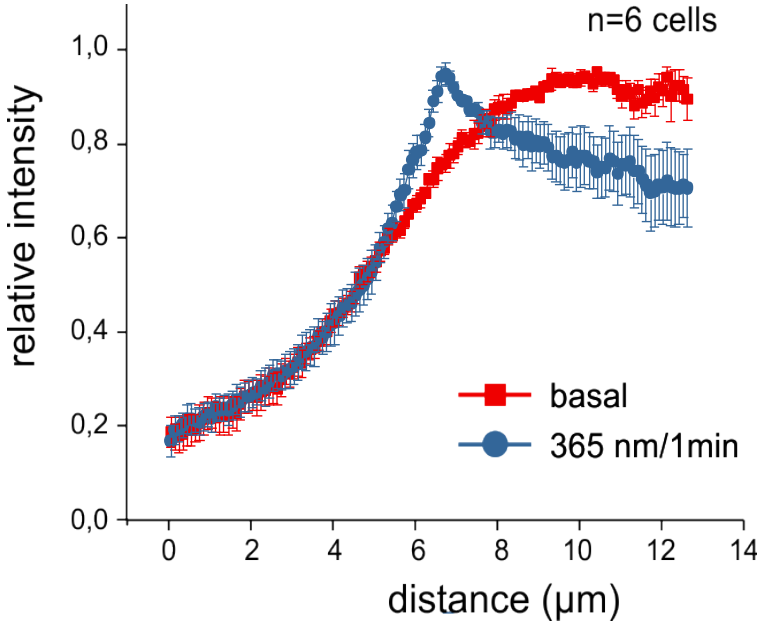
Time course of membrane conductances generated in WT HEK293, recorded at -90 mV and +70 mV during repetitive photoconversion of PhoDAG-1 (400 μM, open circles) and OptoDArG (30 μM, closed circles). UV (365 nm; violet) and blue light (430 nm; blue) irradiation are indicated, with each pulse maintained for 10 s. Mean values ± SEM are given (N of cells as indicated). Two tailed t-test or Mann-Whitney test were applied. Differences in inward- and outward currents between the two photoswitches are not statistically different.

Supplementary Figure 2 | PKCα translocation upon OptoDArG activation



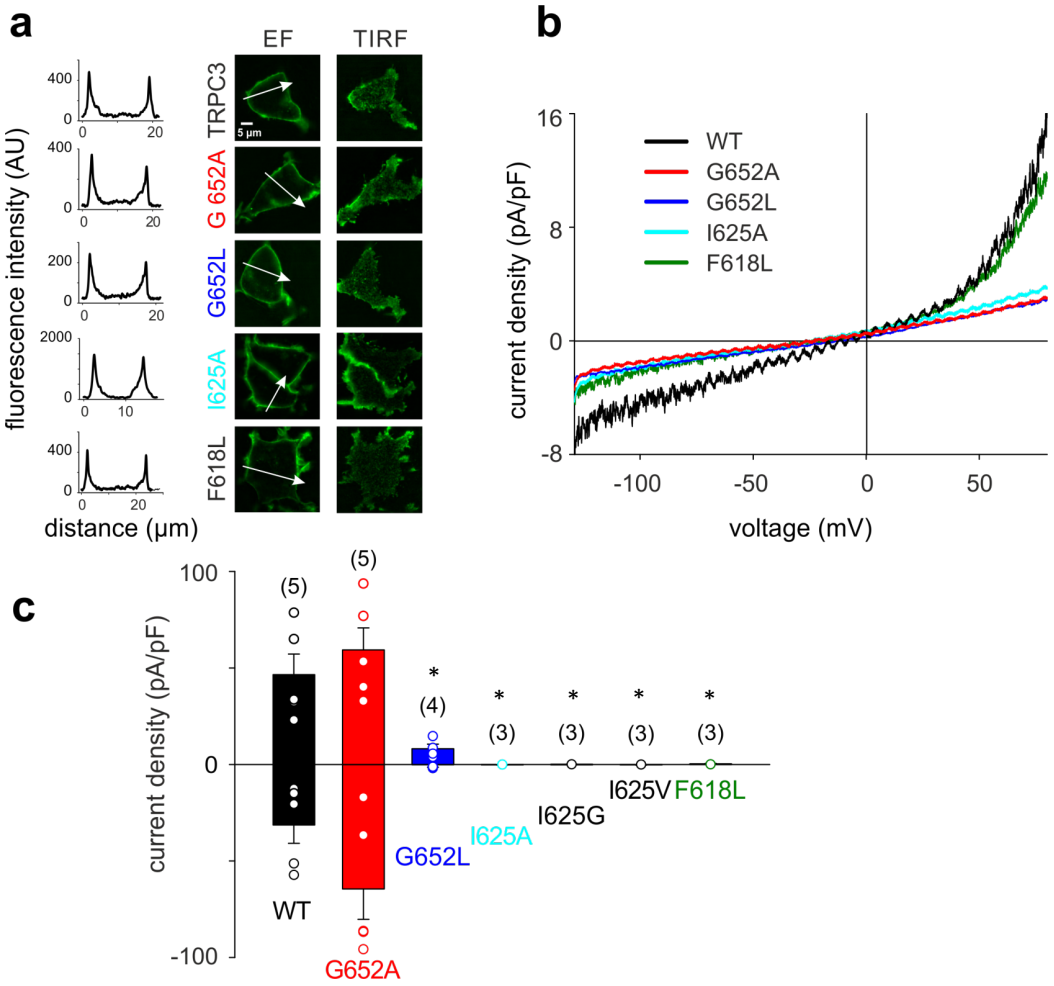
PKCα-GFP (basal)
trans-OptoDArG (10 min)

PKCα-GFP
cis-OptoDArG (365 nm/1 min)



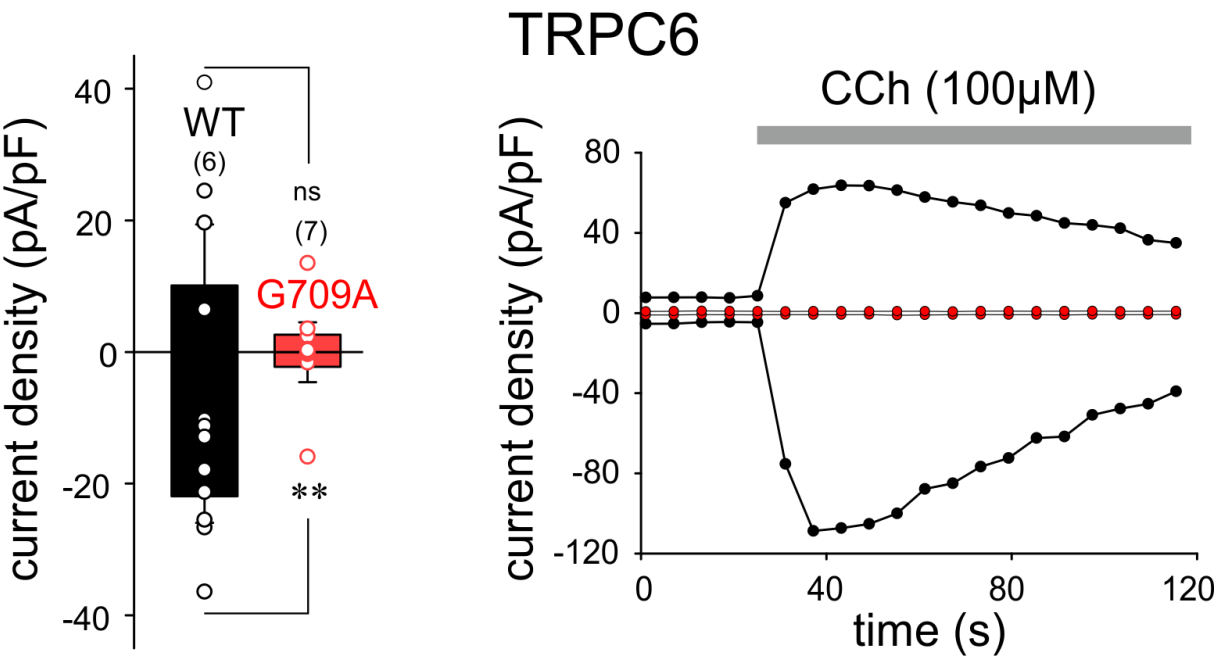
Upper left panel: Representative PKCα-EGFP fluorescence distribution in a HEK293 (epifluorescence) obtained after 10 min incubation with OptoDArG (30 µM) in the dark. PKCα-EGFP was mainly detected in the cytosol. Fluorescence in the nuclei was not observed. Upper right panel: Representative PKCα-EGFP fluorescence distribution in the same cell after 1 min of OptoDArG (30 µM) photoconvertion with UV (100% intensity). **Lower panel:** Distribution of the fluorescence intensity of PKCα along the indicated line is shown before (red) and after 1 min (blue) of OptoDArG photoconvertion with UV (100% of intensity). Peak of intensity after photoconversion corresponded to the cell boundary (plasma membrane). Mean values ± SEM are given, N = number of cells measured is indicated.

Supplementary Figure 3: Surface expression, basal activity and GSK1702934A sensitivity of TRPC3 mutants



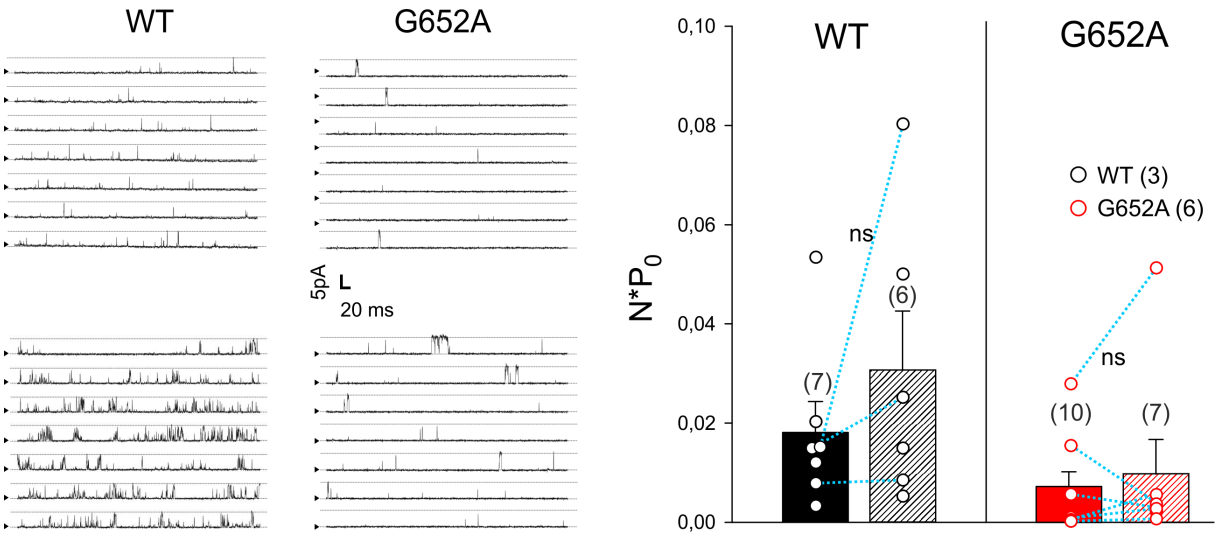
a, Epifluorescence and TIRF images of single cells expressing TRPC3 WT and TRPC3 mutants (G652A; G652L; I625A; F618L). N = number of cells measured > 3 for each of the construct. Cross-sectional profiles of the expression level of TRPC3 WT and TRPC3 mutants in the cell membrane. **b,** Representative basal current recordings in TRPC3 WT (black) and TRPC3 mutants (G652A, red; G652L, dark blue; I625A, cyan; F618L, green). **c,** Mean values of peak response induced by GSK1702934A (10 µM) in TRPC3 WT and TRPC3 mutants (G652A; G652L; I625A; I625G, I625V, F618L). Mean ± SEM, N = number of cells measured is indicated in brackets. Two tailed t-test or Mann-Whitney test were applied, * indicates significant difference at $p < 0,05$; when no p level is given, comparison with WT is not significant ($p > 0.05$). Individual values are shown for each of the data sets (circles).

Supplementary Figure 4 | TRPC6 (G709A) displays a similar phenotype as the TRPC3 G652A mutant



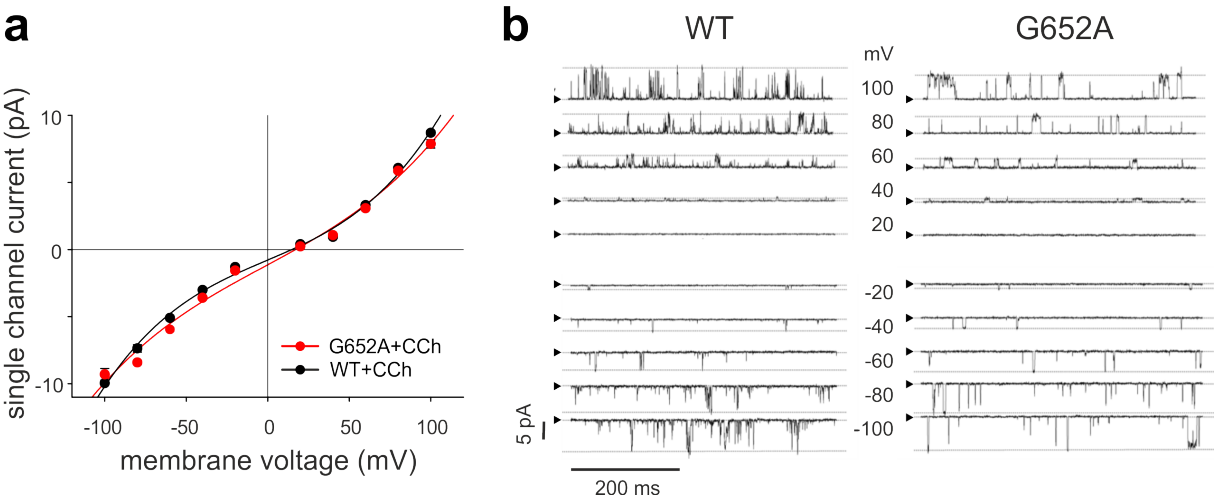
Left panel: Histogram displaying mean current densities of CCh-stimulated (100 µM) HEK293 cells, expressing TRPC6-WT (black) and G709A (red) channels. Mean ± SEM, N = number of cells measured is indicated. Two tailed t-test or Mann-Whitney test were applied, ** indicates significant difference at $p < 0{,}01$; ns = not significant ($p > 0.05$). Individual values of responses are given for each of the data sets (circles). **Right panel:** Representative time courses for current activation by CCh (100 µM, left panel) in TRPC6-WT (black) and G709A (red) mutant channels.

Supplementary Figure 5: Open probability chart for TRPC3-WT and G652A mutant channels expressed in HEK293



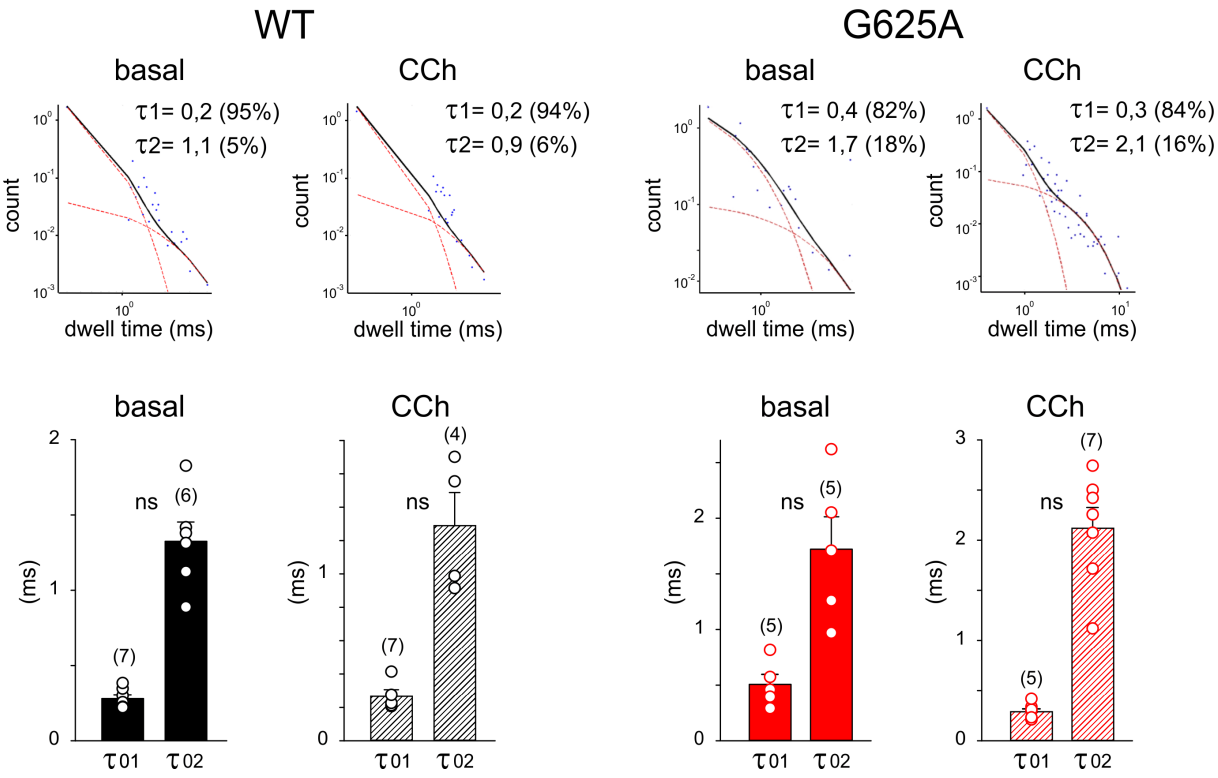
Left panel: Representative, consecutive unitary current traces (closed state is indicated) recorded from HEK293 cells expressing either wild type TRPC3 or G652A mutant activated by carbachol (100 µM). Data are derived from single cell-attached patches at a membrane potential of +80 mV. **Right panel:**Mean values ± SEM of the open probability for TRPC3-WT vs. G652A mutant channels under basal- (filled bars) and CCh-(dashed bars) stimulated conditions. Change in the open probability upon stimulation with CCh (100 µM) for individual measurements is denoted with blue dashed lines. N of cells is indicated. Two tailed t-test or Mann-Whitney test were applied, ns = not significant ($p>0.05$). Individual values of responses are indicated for each of the data sets (circles).

Supplementary Figure 6: Unitary current characteristics of the G652A mutation in comparison to TRPC3 WT



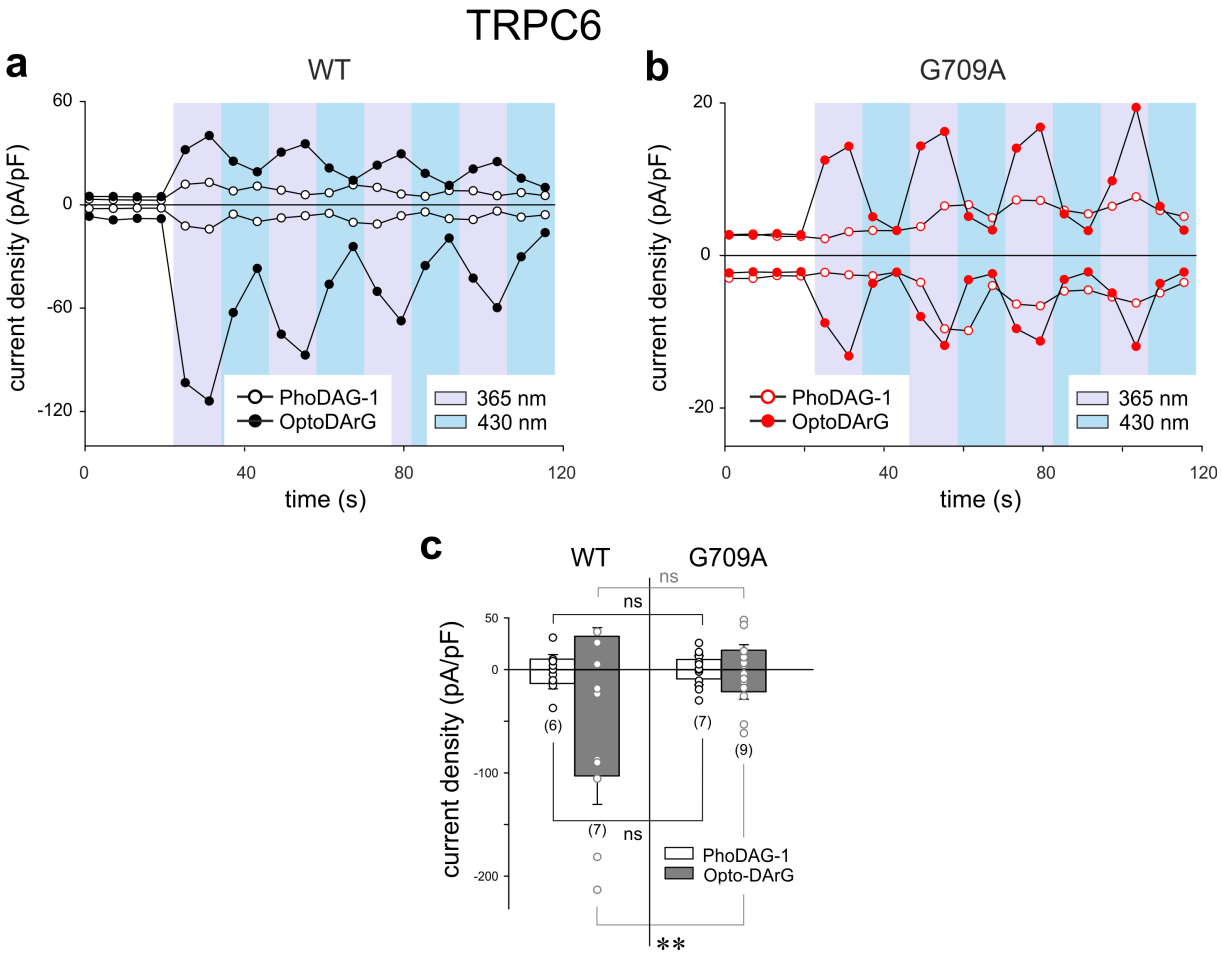
a, Unitary current to voltage (*I-V*) relationships of TRPC3 WT (black) and G652A (red) activated by CCh (100 µM). Data points represent the mean (± SEM*), N = number of cells/patches measured at each voltage > 6, *note that symbol size exceeds error bars at most voltages. Lines are least-squares fit to a third-order polynomial relationship. Data were not corrected for liquid junction potentials. **b,** Representative single-channel currents of CCh (100 µM)-induced TRPC3 WT and G652A responses at various membrane potentials ranging from 100 to -100 mV in cell-attached configuration. Closed channel state is indicated.

Supplementary Figure 7: Open time distributions for the G652A mutation in comparison to TRPC3 WT at basal and stimulated conditions



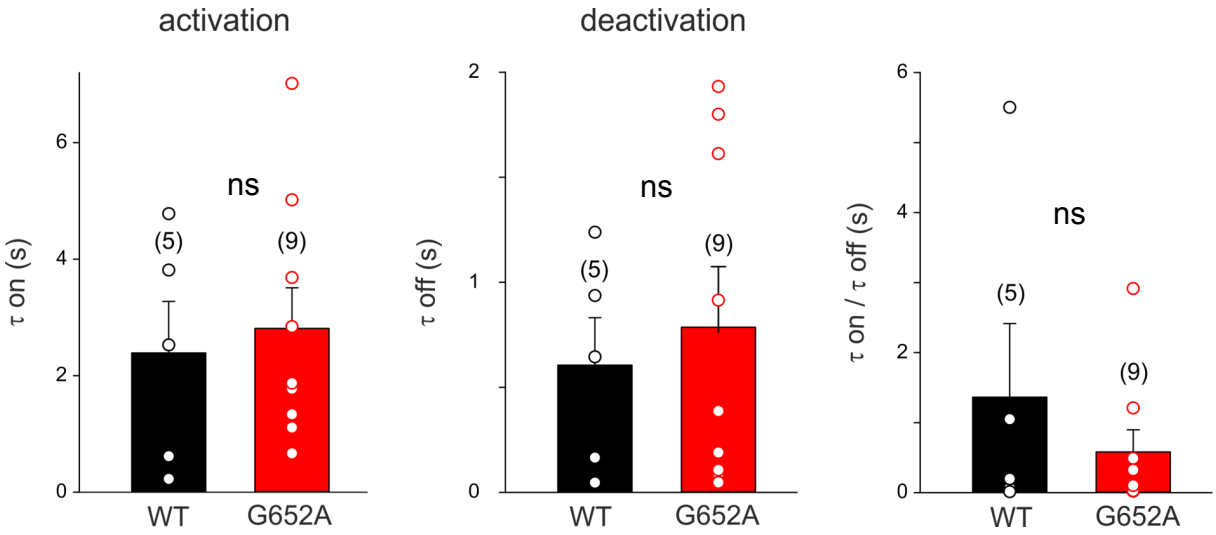
Upper panel: Open time distributions of the non-stimulated (basal) and CCh-stimulated (100 µM) TRPC3-WT and G652A channels expressed in HEK293 cells. Data are derived from single cell-attached patches at a membrane potential of +80 mV. The sum (black) of two exponential equations (red) and individual components (blue) with time constants (τ_1- τ_2) are indicated. **Lower panel:** Comparison of the time constants for TRPC3-WT and G652A mutant channels under basal- and CCh-stimulated conditions. Mean ± SEM, N = number of cells measured is indicated. Two tailed t-test or Mann-Whitney test were applied. All comparisons between basal and CCh-stimulated are not significant ($p > 0.05$). Individual values are shown for each of the data sets (circles).

Supplementary Figure 8 | Optical control of TRPC6 conductances expressed in HEK293 cells by DAG photoswitches



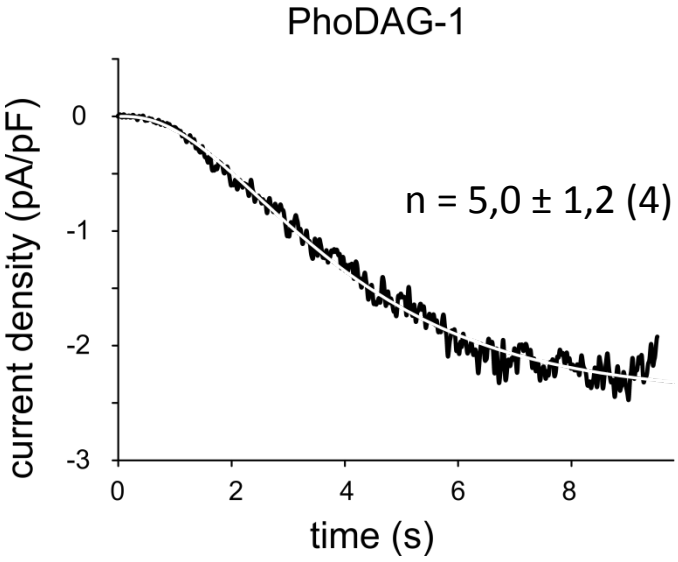
Upper panel: Representative time courses of the TRPC6-WT (black) and G709A (red) conductances recorded at -90 mV and +70 mV during repetitive photoconversion of PhoDAG-1 (400 µM, open circles) and OptoDArG (30 µM, closed circles). UV (365 nm; violet) and blue light (430 nm; blue) irradiation are indicated, with each pulse maintained for 10 s.. Mean values ± SEM is displayed below for maximum responses. **Lower panel:** Current density (at -90 mV and +70 mV; mean ± SEM, N as indicated) of the maximum responses obtained with TRPC6-WT and G709A, induced by PhoDAG-1 (400 µM, white) and OptoDArG (30 µM, grey). Mean ± SEM, N = number of cells measured is indicated. Two tailed t-test or Mann-Whitney test were applied, ** indicates significant difference at $p < 0,01$; ns = not significant ($p > 0.05$). Individual values are shown for each of the data sets (circles).

Supplementary Figure 9: Electrophysiological parameters derived by 'optical lipid-clamp' of the G652A mutation in comparison to TRPC3 WT



Time constants for activation (on) and deactivation (off) kinetics derived by 'lipid-clamp' pulses at -40 mV in HEK293 cells expressing TRPC3 WT or G652A mutant channels using OptoDArG (30 µM). Mean ± SEM, N = number of cells measured is indicated. Two tailed t-test or Mann-Whitney tests were applied, ns = not significant ($p > 0.05$). Individual values are shown for each of the data sets (circles).

Supplementary Figure 10 | Activation kinetics of TRPC3-WT channel during cis photoconversion of PhoDAG-1 (400 μM)



Representative traces showing the inward current (normalized by capacitance) induced by illumination at 365 nm and PhoDAG-1 (400 μM) at a holding potential of −40 mV in TRPC3-WT expressing HEK293 cells. The response was induced with 100% intensity of UV. Exponential fit of current activation and power (n) of power-exponential fitting (f = A*(1-exp(-x/tau))^n) are indicated.