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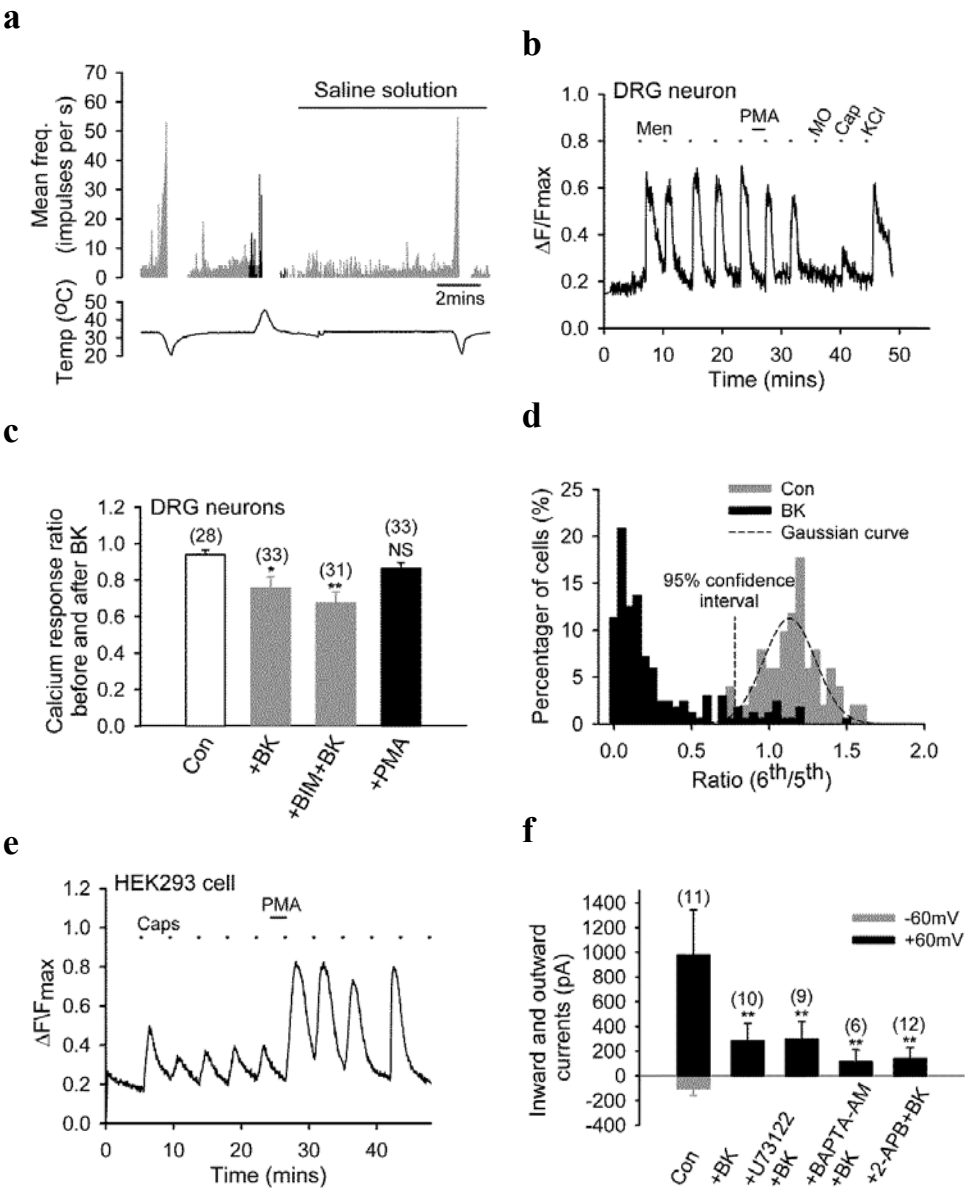

Figure S1 Inhibition of TRPM8 by activation of $G\alpha_q$-coupled GPCRs is independent of downstream signalling pathways. **(a)** Corneal nerve terminal firing frequency in response to cold ramps did not desensitize with successive applications (grey bars) nor following a heat ramp (black bars). Bath temperature shown below. **(b)** In a DRG neuron TRPM8 mediated calcium response activated by menthol (Men, 100mM) were little inhibited when PKC was activated with PMA compared to the effect of BK (see Fig. 2a). 1mM PMA applied for 2 minutes between 5th and 6th menthol-induced response. Calcium increases monitored with Ca-sensitive dye fluo-4. Mustard oil (MO, 50mM), capsaicin (Cap, 100nM), KCl (140mM) applied as shown. **(c)** Summary of mean ratio of peak calcium responses before and after BK treatment in all TRPM8 positive DRG neurons (i.e. those responding and not responding to BK application) from the same experiments as those shown in Fig. 2b. Inhibition by PMA was not significant (final bar). Number of neurons given above each bar. *P<0.05; **P<0.01. **(d)** Distribution of TRPM8-dependent calcium response ratios before (5th response) and after BK (6th response) in HEK293 cells transfected with TRPM8 and B2R, from experiments similar to those in Fig. 2c. Threshold ratio (dashed vertical line) derived from 95% confidence interval of control group was used to determine cells inhibited by BK (85.9% cells inhibited by BK; n_{cell}=61 for control, n_{cell}=168 for BK treated). **(e)** PMA (1mM) caused sensitization of TRPV1 response in a HEK293 cell expressing TRPV1. Capsaicin (Caps, 100nM) applied as indicated. Increase 4.23±0.58-fold (n=15). **(f)** Summary of inhibition of TRPM8 inward and outward currents (at -60mV and +60mV) by BK in similar experiments to those in Fig. 2e, f, but performed with Ca^{2+} containing bath solution, and without EGTA in pipette solution. Cells pre-treated with U73122 (2.5mM, 5mins, bar 3), BAPTA-AM (25mM, 20 mins, bar 4), or 2-aminoethoxydiphenyl borate (2-APB, 100mM, 5 mins, last bar) followed by BK (1mM, 1min). Number of experiments shown above each bar. **P<0.01. All data are mean ± SEM.

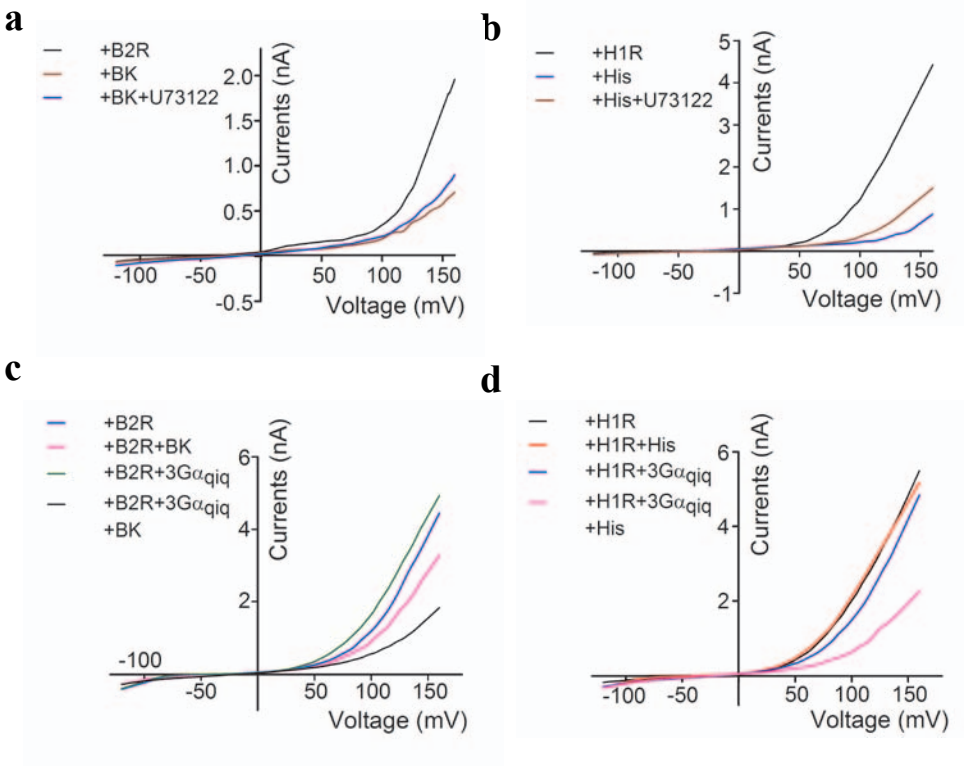

Figure S2 Inhibition of current-voltage relationship of TRPM8 by inflammatory mediators in HEK293 cells (a, b) or $G\alpha_{q/11}^{-/-}$ MEF cells (c, d) co-expressing B2R (a, c) or H1R (b, d). Same cell types as used in experiments in Fig. 2e, 2g, 5e and 5c, respectively. **(a)** TRPM8 current evoked by a voltage ramp (-120mV to +160mV, 650ms) was inhibited by BK (1mM), and the inhibition was not blocked by 2.5mM U73122 (blue trace). **(b)** Similar experiment with histamine (10mM). In this case the inhibition caused by histamine (blue curve) was partially reversed by U73122 (dark red curve). **(c, d)** Bradykinin (1mM) and histamine (10mM) have little effect on voltage-induced TRPM8 activation in $G\alpha_{q/11}^{-/-}$ MEF cells; however, bradykinin and histamine caused a large inhibition of TRPM8 after co-transfection of the $3G\alpha_{qiq}$ chimera, which does not couple to PLCb.

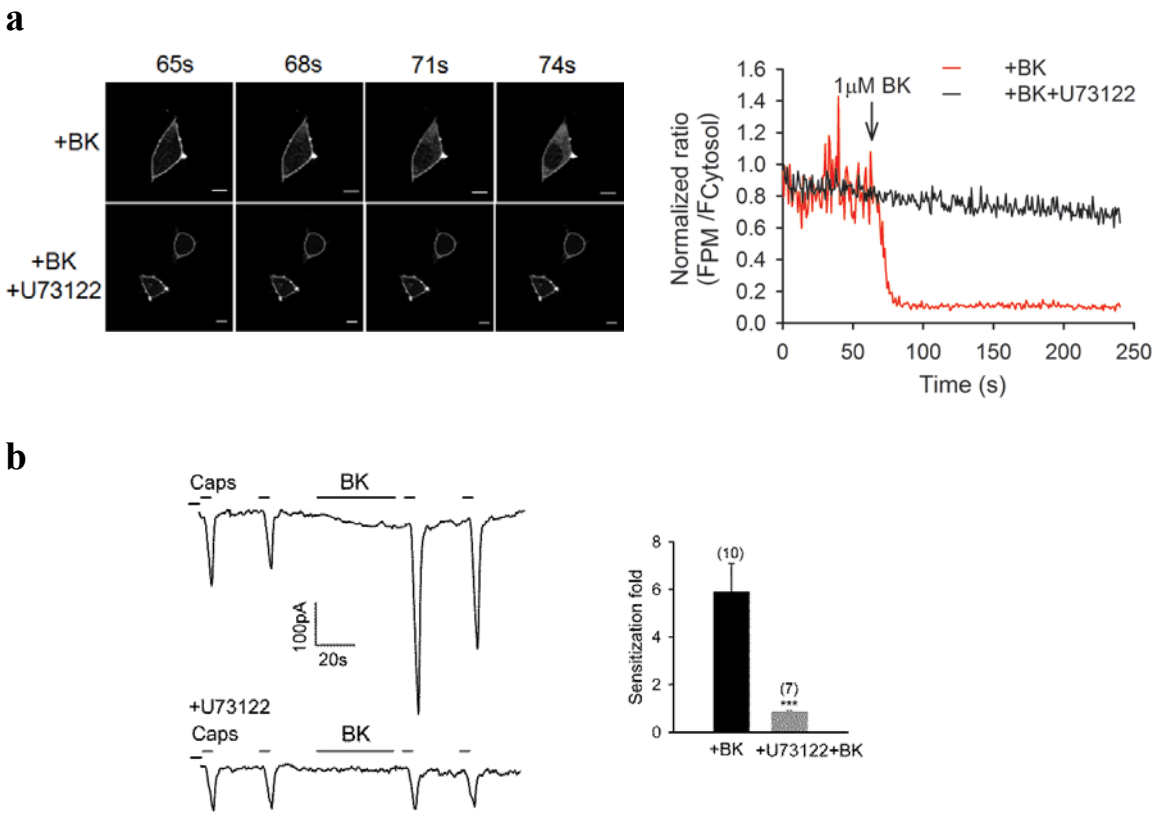

Figure S3 U73122 completely blocks BK-induced PIP_2 hydrolysis and functional sensitization of TRPV1 by BK. **(a)** HEK293 cells transfected with the bradykinin receptor B2R and Tubby-cYFP-R322H were live imaged. 1mM BK added at 65s, and Tubby translocation was observed within 10s (upper panels). U73122 (2.5mM) pre-treatment for 5 minutes completely blocked Tubby translocation (lower panels). Scale bar 10mM. Graph on right gives quantification of ratio of membrane fluorescence to that of cytosol as a function of time in the cells shown left. Arrow indicates addition of 1mM BK. **(b)** BK (1mM) sensitized TRPV1 inward current activated by 10nM capsaicin in a HEK293 cell expressing TRPV1 and B2R (top trace). U73122 (2.5mM, bottom trace) completely blocked sensitization. Zero currents indicated at left. On right is summary of similar experiments. Number of cells given above each bar, data are mean ± SEM. ***P<0.001.

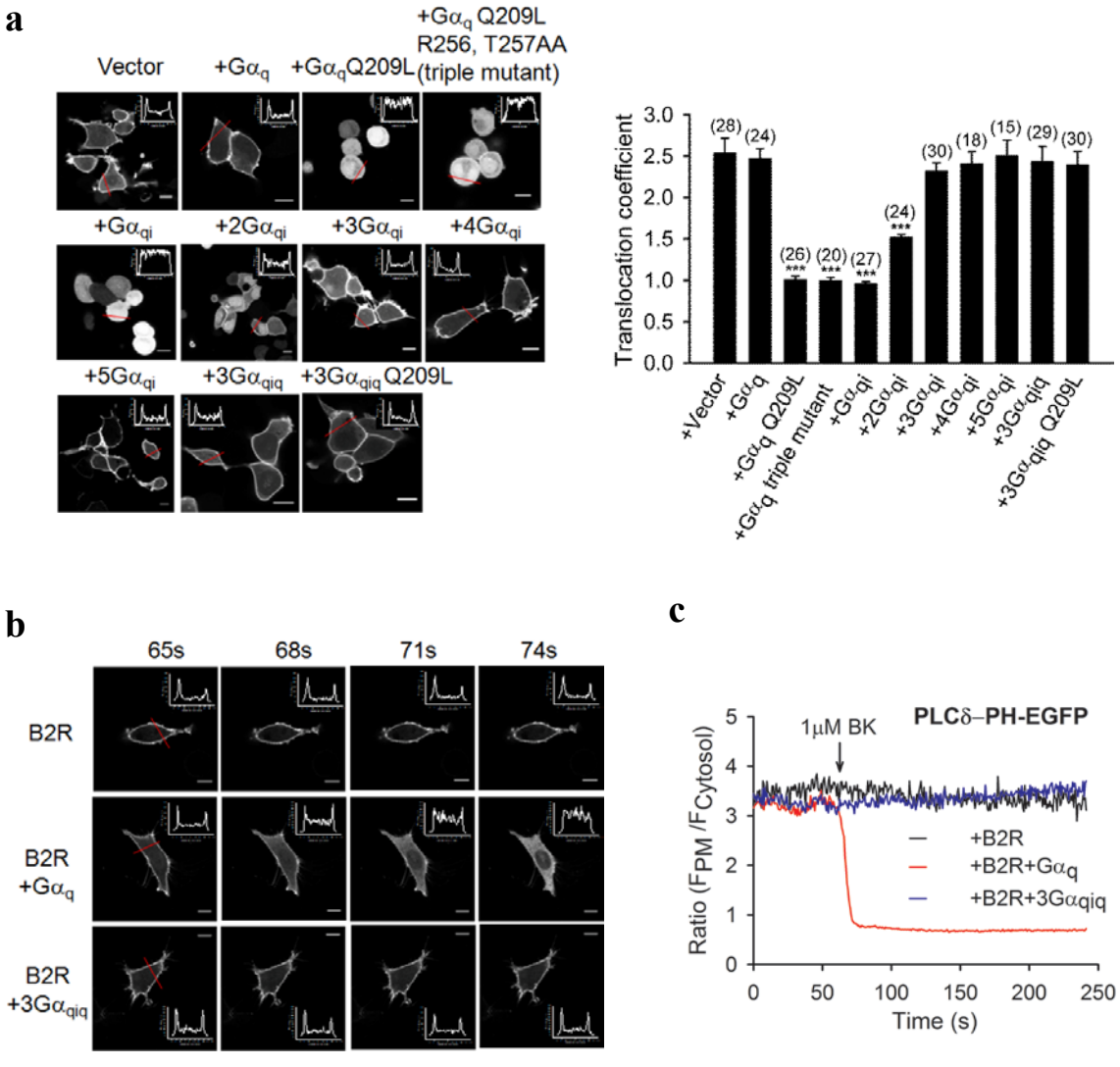

Figure S4 The effect of Ga_q chimeras on Tubby-cYFP-R332H and PLCd-PH-EGFP localization and PIP_2 hydrolysis. **(a)** Typical example images of Tubby-cYFP-R332H in HEK293 cells co-transfected with different Ga_q chimeras as indicated. Profiles of intensity across cells (indicated by red line) shown inset at top corner of the images. Ga_q Q209L, Ga_q Q209L/R256A/T257A, Ga_{qi} and $2G\alpha_{qi}$ caused PIP2 depletion and Tubby translocation, while all other chimeras lost the ability to deplete PIP2 and so to translocate the Tubby probe. Scale bar 10mM. On right is the summary of quantification of translocation coefficient (ratio of membrane peak fluorescence to that of mean fluorescence in the cytosol in line profile intensity) for each G protein. Number of cells given above each bar. Bars are mean±SEM. ***P<0.001. **(b)** Translocation of PLCd-PH-EGFP induced by BK in $G\alpha_{q/11}^{-/-}$MEF cells co-transfected with B2R and G proteins as indicated. 1mM BK was added at 65s. Scale bar 10μm. Profiles of intensity across cells (indicated by red line) shown inset at corner of the images. **(c)** Quantification of relative membrane Tubby fluorescence signal as a function of time in b. Each experiment repeated at least 4 times with similar results.

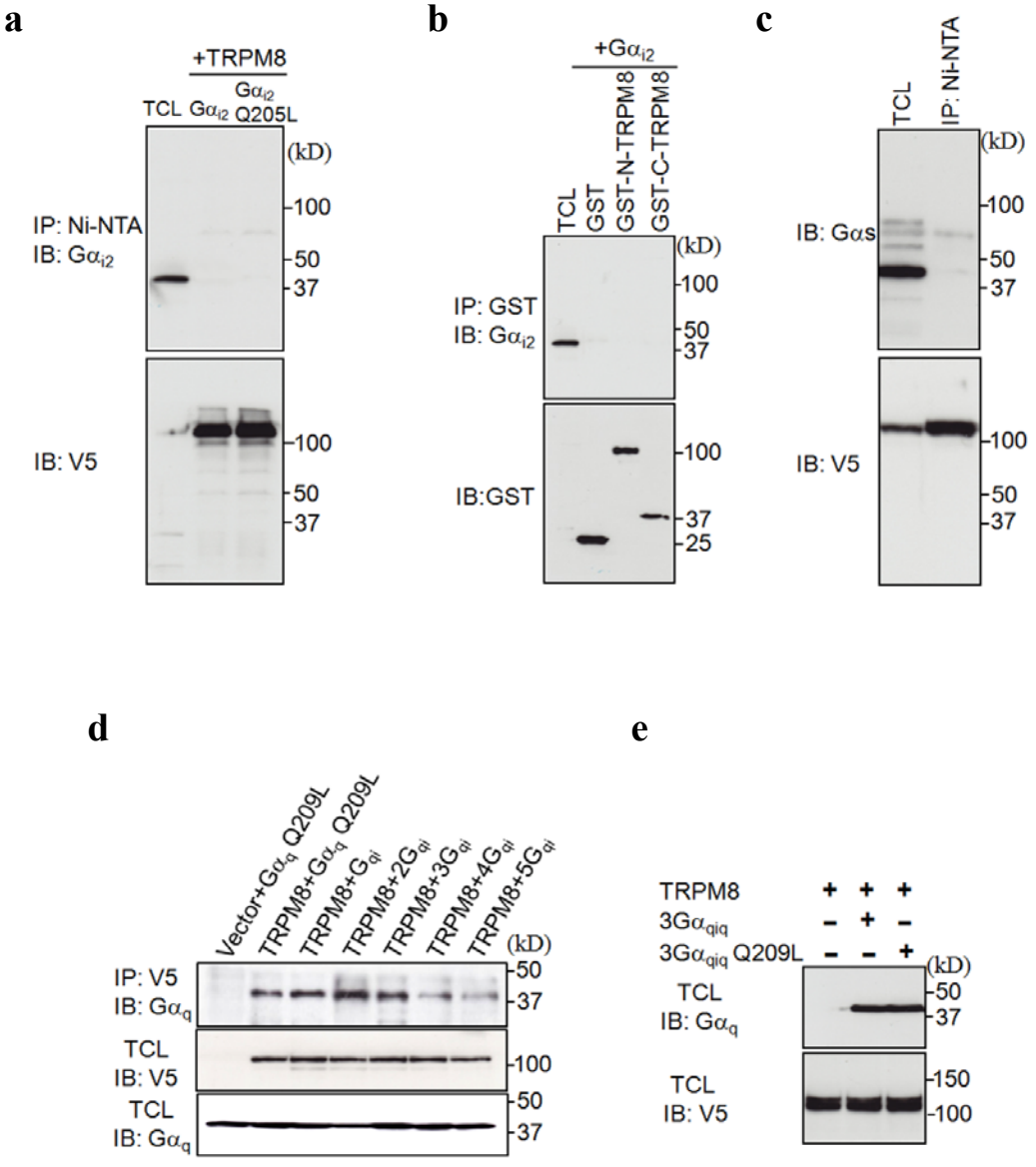

Figure S5 Interaction of TRPM8 with Ga protein subunits and $G\alpha_q$ chimeras. **(a)** TRPM8 shows no significant binding to $G\alpha_{i2}$. Experiments carried out under same conditions that had shown binding of $G\alpha_q$ to TRPM8 (see Fig. 6a-d). TRPM8 pulled down by Ni-NTA beads from HEK293 cells expressing TRPM8-V5-His and $G\alpha_{i2}$, and probed with anti-$G\alpha_{i2}$ (top blot) followed by detection of TRPM8 in stripped blot with anti-V5 (bottom blot). First lane is total cell lysate (TCL); last two lanes are pull down samples. Molecular weight shown on right, same for all other blots. **(b)** GST-coupled TRPM8 N and C terminals used to pull down $G\alpha_{i2}$ in experiments similar to those in Fig. 6d. No significant binding to $G\alpha_{i2}$ found. **(c)** TRPM8 shows no significant binding to $G\alpha_s$. TRPM8 was pulled down from HEK293 cell lysate expressing TRPM8-V5-His and $G\alpha_s$. $G\alpha_s$ detected by anti-$G\alpha_s$ (top blot) followed by anti-V5 for TRPM8 (bottom blot). No significant association found. **(d)** Interaction of TRPM8 with different $G\alpha_q$ chimeras. TRPM8 was immunoprecipitated by anti-V5 from lysate of HEK293 cells expressing TRPM8-V5 together with different $G\alpha_q$ chimeras. Co-precipitated $G\alpha_q$ chimeras were probed by anti-$G\alpha_q$ antibody (top blot). Expression of TRPM8 and $G\alpha_q$ in total cell lysate (TCL) for each sample was detected by anti-V5 (middle blot) and anti-$G\alpha_q$ (bottom blot), and was similar in all cases. **(e)** $3G\alpha_{qiq}$ chimera and Q209L mutant do not affect TRPM8 expression. HEK293 cells co-transfected with cDNAs as indicated, expression of $3G\alpha_{qiq}$ chimera and TRPM8 protein detected by anti-$G\alpha_q$ (top blot) and anti-V5(bottom blot), respectively.

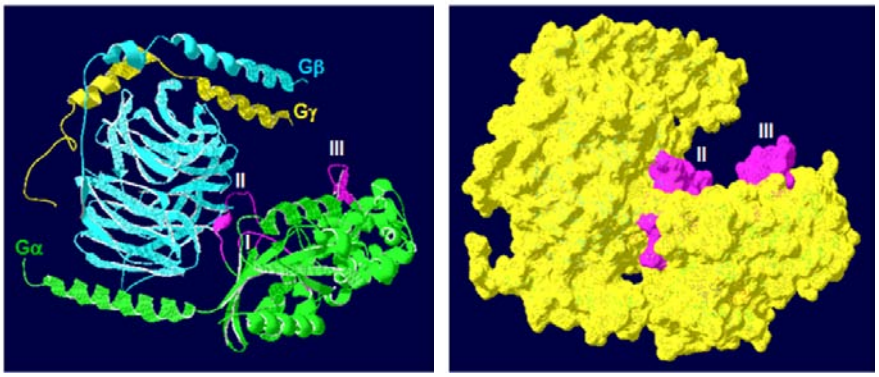

Figure S6 Structure of $G\alpha_q\beta g$ modelled by homology modelling based on the crystallized structure of $G\alpha_i\beta g$ (Protein Data Bank code, 1GG2)[1] by using Swiss-Pdb viewer 4.0 software together with Swiss online modelling software. $G\alpha_q$ protein in green, Gb in blue and Gg in yellow. Switch I, II and III regions of $G\alpha_q$ in pink. On right is molecular surface representation. Surfaces of Switch II and III regions shown in pink. Surface of Switch I region is at the back and cannot be seen in these orientations. Switch III region is largely free to be accessed by effectors and is identified as the TRPM8 binding region (see Fig.6f). The activated form of $G\alpha_q$ has a similar structure in the Switch III region[2] (Protein Data Bank code, 2BCJ), which would also be free to engage TRPM8. Thus based on our current knowledge of the structure of $G\alpha_q$, the Switch III region is available to be accessed and engaged by effectors such as TRPM8 in both the active and inactive configurations.

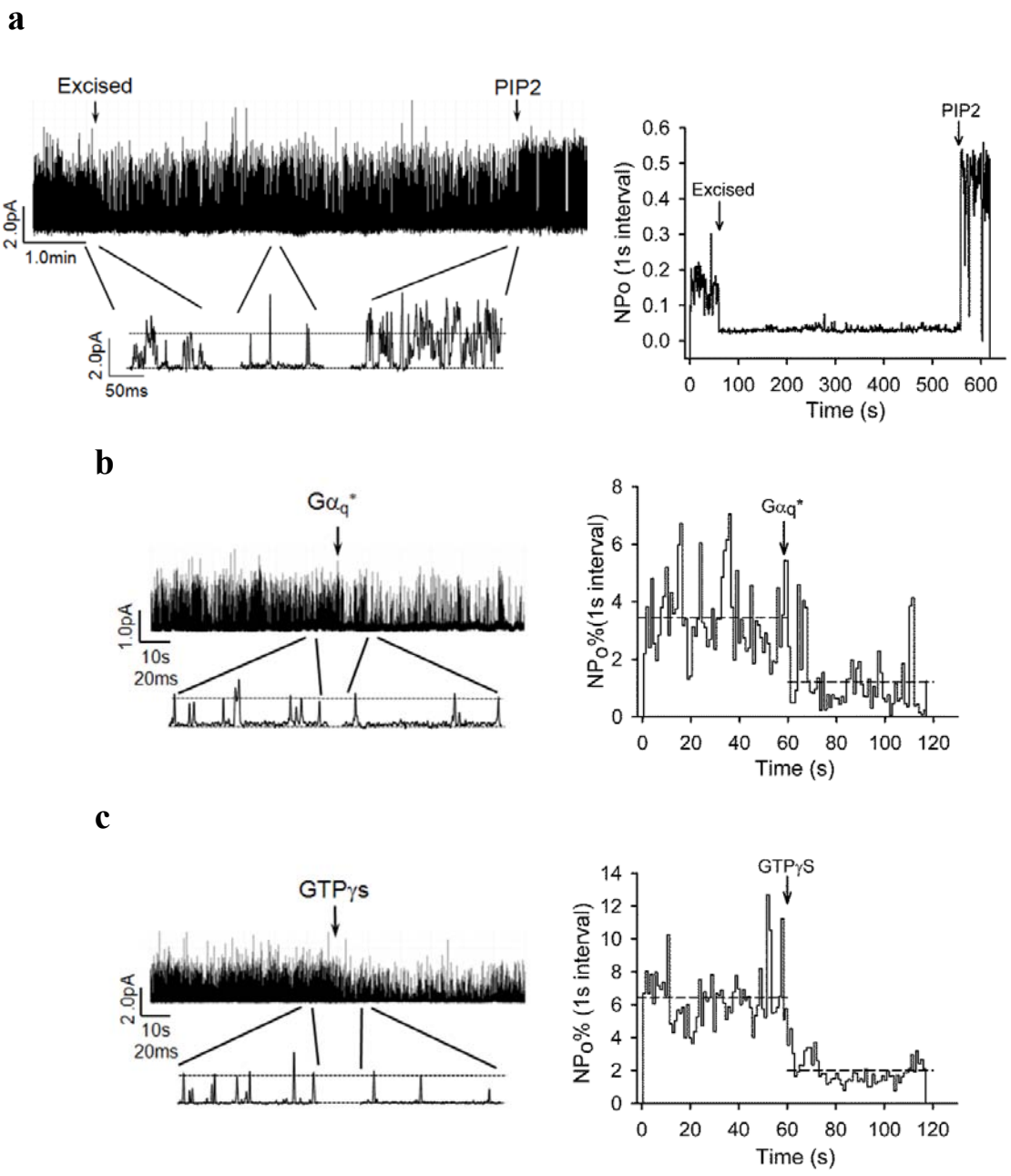

Figure S7 Effect of DiC8-PIP_2, activated $G\alpha_q$ and GTPγS on TRPM8 channels excised from HEK293 cells expressing TRPM8. **(a)** Example trace of TRPM8 channel activity (+40mV) in cell attached mode, following excision and with addition of DiC8-PIP_2 (50mM), as indicated by arrows. Sections of traces shown below at higher time resolution. Right: real time quantification of NP_o. On cell NP_o = 0.15±0.0054; after excision NP_o = 0.032±0.00028; after DiC8-PIP_2 addition NP_o = 0.43±0.015. TRPM8 channels typically maintain constant low level of activity over 6 mins following excision. **(b, c)** Left: typical examples of channel activity at +40mV in already run-down patches excised from HEK cells expressing TRPM8 after addition of 50nM $G\alpha_q$* ($G\alpha_q$ pre-incubated with GTPγS, b) or 100mM GTPγS (c). Arrows indicate time of addition of $G\alpha_q$* or GTPγS. Sections of traces are shown below at higher time resolution. Right: real time quantification of NP_o; dashed lines give mean NP_o over indicated time periods. NP_o before $G\alpha_q$*, 0.034 ± 0.0018; after $G\alpha_q$*, 0.012 ± 0.0016; P<0.001. NP_o before GTPγS, 0.064±0.0022; after GTPγS, 0.02 ± 0.0012; P<0.001.

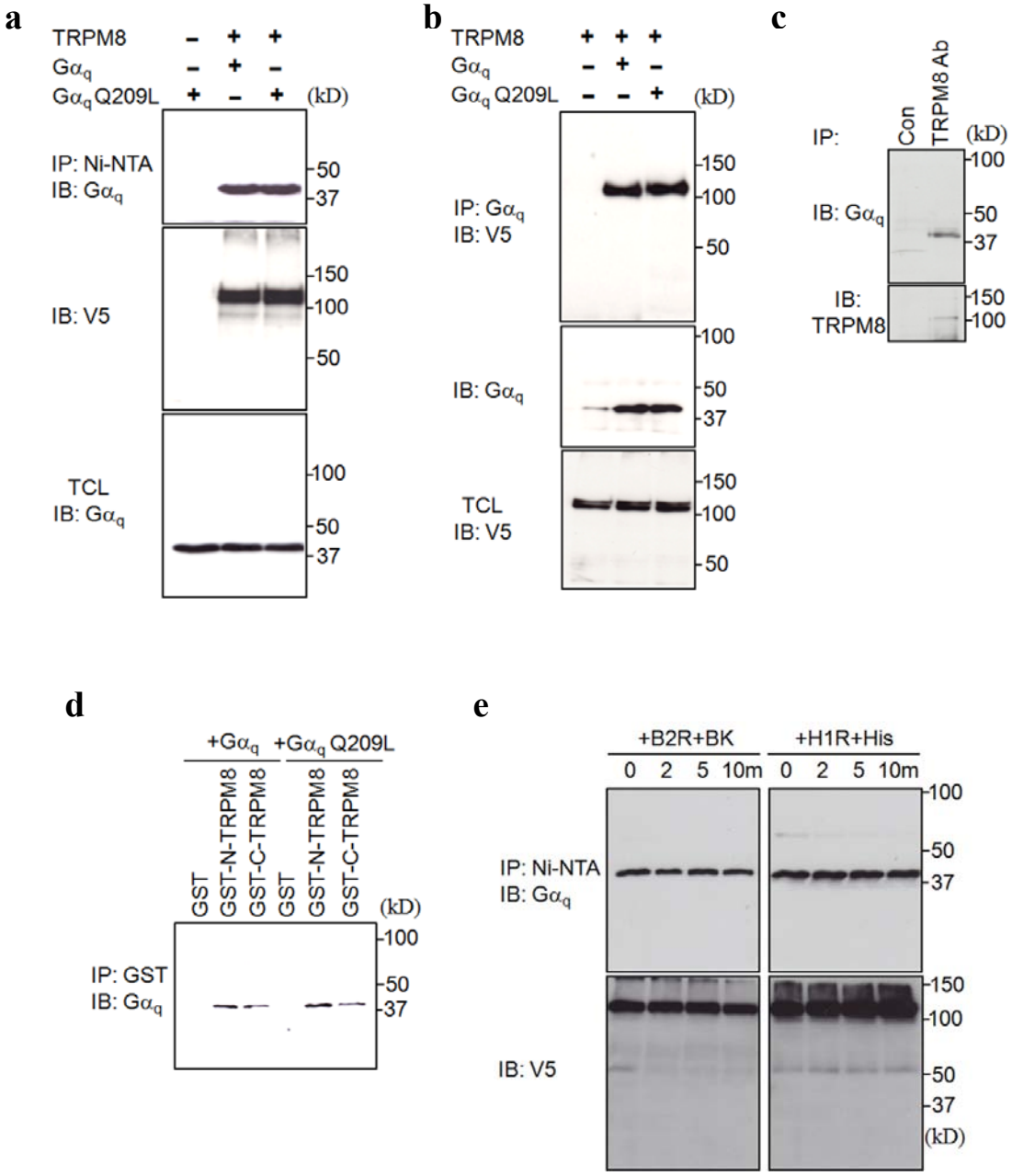

Figure S8 Full scan images of blots of Figure 6. **(a)** Fig.6a. **(b)** Fig. 6b. **(c)** Fig. 6c. **(d)** Fig.6d. **(e)** Fig. 6e.

References

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2. Tesmer, V.M., Kawano, T., Shankaranarayanan, A., Kozasa, T., & Tesmer, J.J. Snapshot of activated G proteins at the membrane: the Galphaq-GRK2-Gbetagamma complex. *Science* **310,** 1686-1690 (2005).