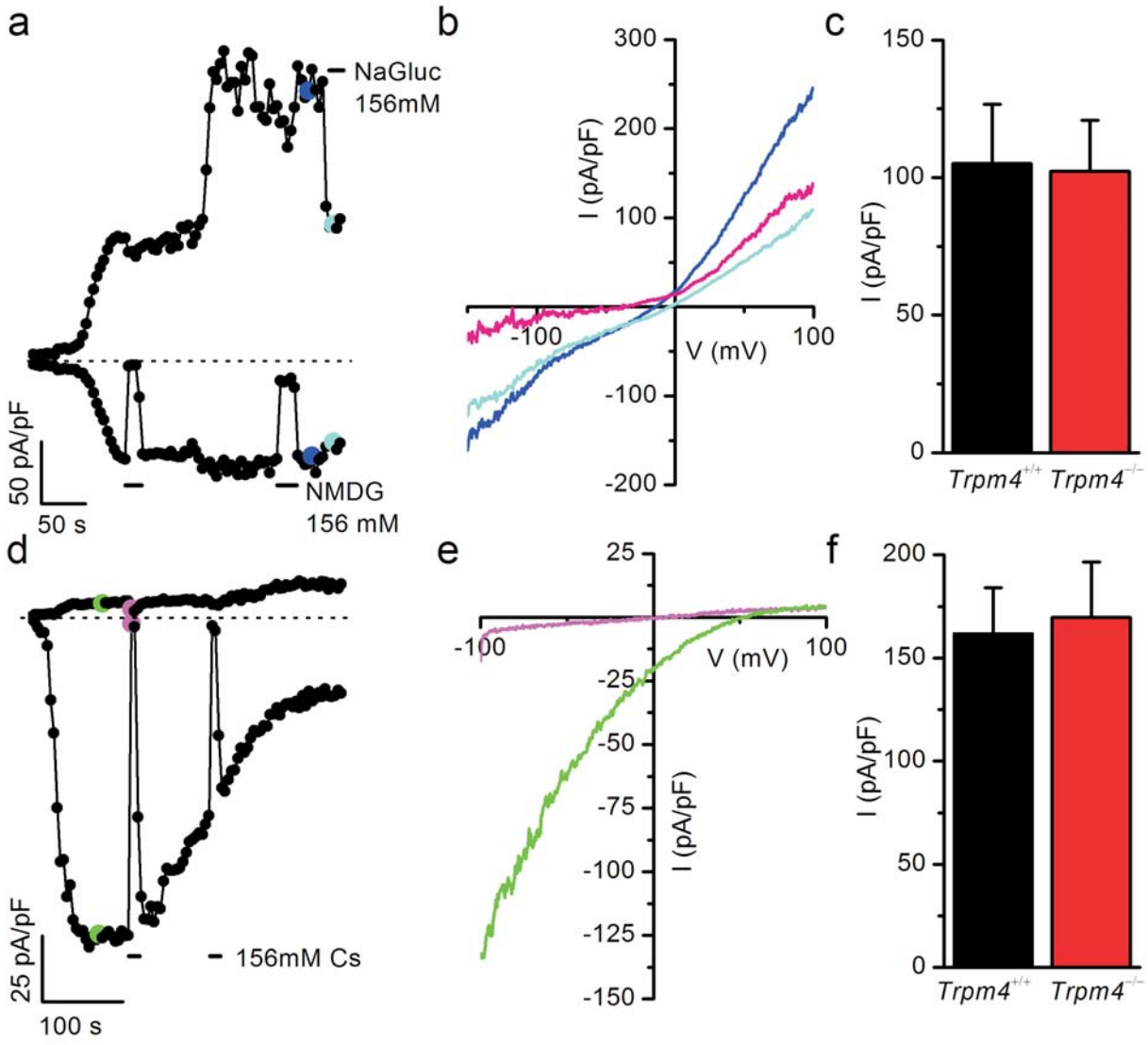
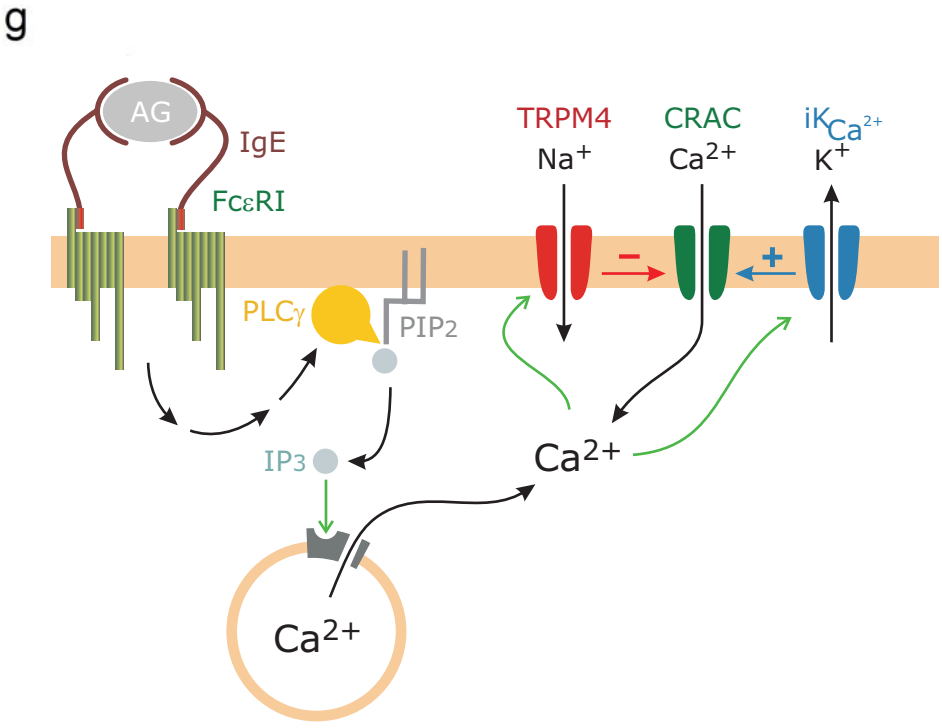

a
NaGluc
156mM
50 pA/pF
50 s
NMDG
156 mM
b
300
200
100
I (pA/pF)
-100
V (mV)
100
-100
-200
c
150
100
50
0
I (pA/pF)
Trpm4+/+
Trpm4−/−
d
25 pA/pF
100 s
156mM Cs
e
25
-100
V (mV)
100
-25
-50
-75
-100
-125
-150
I (pA/pF)
f
200
150
100
50
0
I (pA/pF)
Trpm4+/+
Trpm4−/−


g
AG
IgE
FcεRI
PLCγ
PIP2
IP3
Ca2+
Ca2+
TRPM4
Na+
CRAC
Ca2+
iKCa2+
K+
−
+

Supplementary Figure 7. (**a-f**) Ca^{2+} activated K^+ and Cl^- currents in *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ BMMCs. (**a**) Time-course of a whole cell patch clamp recording at -80 and +80mV from a *Trpm4*$^{+/+}$ mast cell. Pipette solution contained 10µM Ca^{2+}, and Na^+ instead of K^+. Voltage protocol as in **Fig. 2**. Bars specify the application of 156mM NMDG and 156mM NaGluconate (both replacing NaCl) containing bath solution, as indicated. **(b**) Representative current traces in response to a linear voltage protocol, taken at the indicated points in panel **a** (coloured points). The pink trace depicts the difference in the current in normal or 156mM NaGluconate-containing bath solution, and represents the pure Cl^- current. (**c**) Mean amplitude of the Nagluconate-sensitive current at +80mV from *Trpm4*$^{+/+}$ (*n*=25) and *Trpm4*$^{-/-}$ (*n*=28) BMMCs. *P* > 0.1. (**d**) Time course at -80 and +80mV from a *Trpm4*$^{+/+}$ mast cell. Pipette solution contained 10µM Ca^{2+}, and Na^+ instead of K^+. Extracellular solution contains 156mM KCl. Bars specify the perfusion of a 156mM CsCl containing bath solution (replacing KCl). (**e**) Representative current traces taken at the indicated times in panel **d** (colored points). (**f**) Mean amplitude of the Cs^+-sensitive current from *Trpm4*$^{+/+}$ (*n*=10) and *Trpm4*$^{-/-}$ (*n*=10) BMMCs. *P* > 0.1. (**g**) Schematic model showing the role of TRPM4 in FcεRI induced Ca^{2+} signalling in mast cells. Crosslinking of the FcεRI receptor results in activation of several signaling molecules and adaptor proteins. A major downstream target is phospholipase Cγ1, which catalyzes the hydrolysis of phosphatidylinositol(4,5)P_2 to diacylglycerol and inositol(1,4,5)P_3 (IP_3). The latter releases Ca^{2+} from intracellular Ca^{2+} stores through activation of IP_3 receptors. Upon store-depletion, store-operated Ca^{2+} influx channels (CRAC channels) are activated through an (to date) unknown mechanism which will further increase the $[Ca^{2+}]_{cyt}$. Initial activation of Ca^{2+} activated K^+ channels (iK_{Ca2+}) will hyperpolarize the membrane potential, increasing ("+") the driving force for Ca^{2+} influx through CRAC channels. Subsequent activation of Ca^{2+} activated non-selective cation channels (TRPM4) will depolarize the membrane potential, limitting ("-") the driving force for Ca^{2+} entry whereupon the Ca^{2+} signal can be terminated through the action of Ca^{2+} pumps. TRPM4 thus functions as a molecular brake on Ca^{2+} entry, and in *Trpm4*$^{-/-}$ mast cells Ca^{2+} influx is largely increased upon FcεRI activation.