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## SUPPLEMENTARY ONLINE DATA Kinase and channel activity of TRPM6 are co-ordinated by a dimerization motif and pocket interaction

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## Figure S1 Sequence alignment between human TRPM6 and mouse TRPM7

Conserved dimerization pocket motif residues (Leu<sup>1743</sup>, Gln<sup>1832</sup>, Ala<sup>1836</sup>, Leu<sup>1840</sup> and Leu<sup>1919</sup>) are indicated by arrows.

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## Figure S2 Identification and characterization of phosphosite Thr<sup>1724</sup> in the dimerization motif peptide

(A) Mapping of phosphopeptides on the dimerization motif peptide after phosphorylation by TRPM6 *in vitro*. GST-tagged TRPM6-(1700–end) was immunoprecipitated and subjected to a kinase assay including the biotinylated dimerization peptide (500  $\mu$ M). Assays were terminated by addition of LDS loading buffer, and proteins were separated by SDS/PAGE. Proteins were detected by Colloidal Coomassie Blue staining, and the stained phosphorylated dimerization motif peptide was digested with trypsin, followed by reverse-phase HPLC on a Vydac C<sub>18</sub> column equilibrated with 0.1 % trifluoroacetic acid, and the column was developed with an acetonitrile gradient (broken line). The flow rate was 0.2 ml/min and fractions (0.1 ml each) were collected and analysed for <sup>32</sup>P radioactivity by Cerenkov counting. A major <sup>32</sup>P-labelled peak was identified. (B) Solid-phase sequence analysis of the tryptic phosphopeptide RLSQT(p)IPFTPVQLFAGEEITV (residues 19–41). The peptide was phosphorylated at threonine at position 22, corresponding to Thr<sup>1724</sup> in TRPM6. (C) GST-tagged TRPM6-(1730–end) was expressed in HEK-293 cells, and lysates were subjected to a peptide pull-down, as described in the Materials and methods section of the main text, using either wild-type (WT) or phospho-wild-type (WT-P) dimerization motif peptide. The samples were analysed by immunoblotting with anti-GST antibody (middle panel), using GAPDH expression as loading control (bottom panel). (D) GST-tagged TRPM6-(1730–end) was expressed in HEK-293 cells, roteins were separated by SDS/PAGE, stained with Coomassie Blue, and MBP phosphorylation as well as peptide phosphorylation were detected by immunobletide to a kinase assay using 30  $\mu$ M of either wild-type (WT) or phospho-wild-type (WT-P) dimerization motif peptide. Proteins were separated by SDS/PAGE, stained with Coomassie Blue, and MBP phosphorylation as well as peptide phosphorylation were detected by autoradiography. Molecular masses are indicated in kDa.

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