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online data

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[1743], Gln[1832], Ala[1836], Leu[1840] and Leu[1919]) are indicated by arrows.

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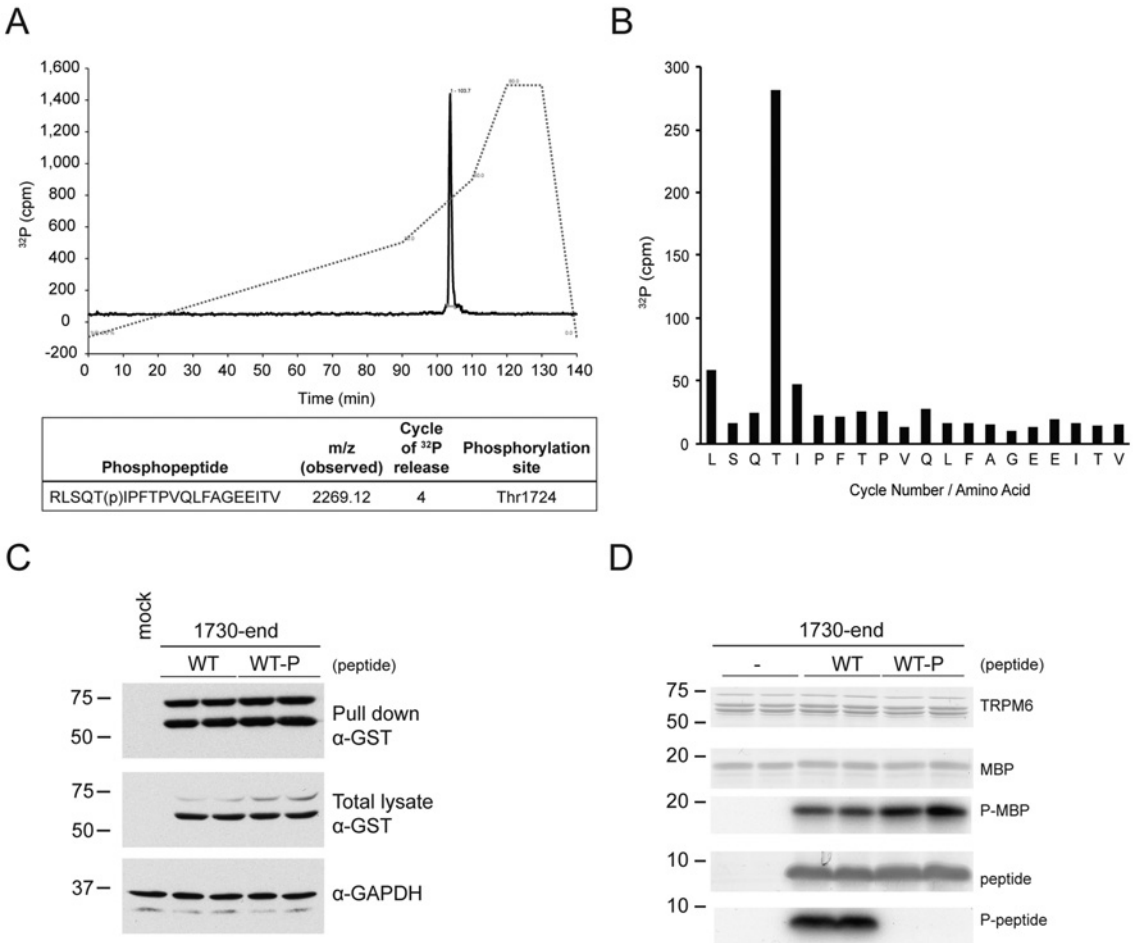

Phosphopeptide	m/z (observed)	Cycle of ^{32}P release	Phosphorylation site
RLSQT(p)IPFTPVQLFAGEEITV	2269.12	4	Thr1724

Figure S2 Identification and characterization of phosphosite Thr^{1724} 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 μM). 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_{18} 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 ^{32}P radioactivity by Cerenkov counting. A major ^{32}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^{1724} 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 (top panel). Total cell extracts were immunoblotted 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, followed by immunoprecipitation and subjected to a kinase assay using 30 μM 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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