### **Supplementary Figure Legends**

### Figure S1. Recording of the Ga<sub>i2</sub>-activated TRPC4 and of the Ga<sub>i3</sub>-activated TRPC5 currents.

(A) Representative current trace of TRPC4 co-expressed with  $G\alpha_{i2}$  showing that  $G\alpha_{i2}$  activates the TRPC4 current. (B) Representative current trace of TRPC5 co-expressed with  $G\alpha_{i3}$  showing that  $G\alpha_{i3}$ activates TRPC5. Both current traces are recorded at -60 mV in external solution changed from NT (Normal tyrode: Na<sup>+</sup>) to Cs<sup>+</sup> solution. The *horizontal bar* indicates duration of applied external cation solutions from both traces. Dashed lines show zero current.

#### Figure S2. Interaction of Ga<sub>i3</sub> with the C-terminus of TRPC5.

(A) TRPC5 co-immunoprecipitates with  $G\alpha_{i3}$ . HEK cells were transfected with  $G\alpha_{i3}$  alone or  $G\alpha_{i3}$ with TRPC5-GFP and were used to test co-IP of TRPC5 and  $G\alpha_{i3}$ . TRPC5-GFP immunoprecipitated by GFP antibody and  $G\alpha_{i3}^{Q205L}$  (containing internal epitope of EE) and was stained by EE-epitope antibody. (B) A schematic of GFP-fused mTRPC5 and mTRPC5 deletion mutants. (C) Summary of the effects of  $G\alpha_{i3}^{Q205L}$  on current by TRPC5 deletion mutants in the presence and absence of GTPyS stimulation. Current densities are represented by maximal current peaks (subtracted Cs<sup>+</sup> basal current) at -60 mV in Cs<sup>+</sup> solution and are indicated by means  $\pm$  S.E. (D) TRPC5 (764-954) is not required for interaction with  $G\alpha_{i3}$ .

#### Figure S3. I-V curves of TRPC4 deletion mutants activated by GTPyS or Ga<sub>i2</sub>.

The  $\Delta 695 \sim 724$  and  $\Delta 700 \sim 728$  mutants are not active, whereas the others retained partial or full activation.

#### Figure S4. A schematic diagram of mTRPC4 $\beta$ and mTRPC5 depicting their G $\alpha$ interacting sites.

### Figure S5. The interaction modeling of TRPC4 C-terminus (amino acids 701-720) with Ga<sub>i2</sub>.

(A) Alignment of  $G\alpha_i$  and  $G\alpha_{q/11}$ . Nine sites where the  $G\alpha_i$  family has negative charges but the  $G\alpha_{q/11}$ family does not are boxed in green. Among the nine sites of  $G\alpha_{i2}$ , only Asp252 belongs to areas of interaction with other proteins.

Figure S6. The CIRB region of TRPC5 is critical for channel activation by  $G\alpha_{i3}$ . (A) Representative *I-V* curves of mTRPC5 currents with and without  $G\alpha_{i3}^{Q205L}$ . The black lines indicate the absence of GTP $\gamma$ S, and the red lines indicate activation by GTP $\gamma$ S infusion in patch pipette. Panels (B, C) Summary of the effects of  $G\alpha_{i3}^{Q205L}$  on current by mTRPC5 and mTRPC5 deletion mutants in the presence and absence of GTPyS stimulation. The mTRPC5 CIRBm1 mutant was changed by substituting Arg718, Lys722, and Arg723 with alanine. Another mutant (CIRBm2) was substituted at CIRB residues (I717D/L720E/V721A). The CBII mutant is formed by deleting Pro828 to Asn854. All current densities are represented by maximal current peaks at -60 mV in Cs<sup>+</sup> solution and are indicated by means  $\pm$  S.E.









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### a

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
GNAi1 GNAi3 GNAi2 GNAo GNAq GNA11 Consensus	HTLESI MTLESH	HGCTLSAE HGCTLSAE HGCTVSAE HGCTLSAE HACCLSEE HACCLSDE HgCLISAE	DKAAVERSK DKAAVERSK DKAAAERSK ERAALERSK AKEARRIND VKESKRINA •kaa•ersk	MIDRNLREDGEK MIDRNLREDGEK MIDKNLREDGEK AIEKNLKEDGIS EIERQLRRDKRD EIEKQLRRDKRD .I#k#LreDg	AAREYKLL AAKEYKLL AAREYKLL AAREYKLL ARRELKLL ARRELKLL ARRELKLL Aar #yKLL	LLGAGESGKS LLGAGESGKS LLGAGESGKS LLGAGESGKS LLGTGESGKS LLGTGESGKS LLGAGESGKS	TIYKQHKIIFEA TIYKQHKIIFED TIYKQHKIIFED TIYKQHKIIFED TIYKQHKIIFGS TFIKQHRIIFGA TIFKQHRIIFGA	G 'SEEECKO G 'SEDECKO G 'SEEECRO G 'SGEDYKO G 'SDEDKRO G 'SEEDKRO G 'SEEDKRO G 'SEEDKRO	QYKAYYSNTJ QYKYYYSNTJ QYRAYYYSNTJ QYRAYYYSNTJ GFTKLYYQNIF GFTKLYYQNIF GFTKLYYQNIF 3%YYSNCJ	QSTIALIRAN QSTIALIRAN QSTALIRAN QSTAALVKAN QSLAALVRAN TAMQAMIRAN TAMQAMIRAN qs.,Ai!rAN	IGRLKIDF GDS IGRLKIDF GEA IGNLQIDF ADP IDTLGIEY GDK IDTLKIPY KYE Ittlkity Kye	CRFDDA:QLF URFDDA:QLF SRFDDA:QLF CRKADA:QLF CRKADA:MYC UNKANA:LTF CNKANA:LTF	VLAGAAEE-GI VLAGSAEE-GI ALSCTAEEQGI DVYSRMEDTEI REVDVEKVSI REVDVEKVT E	FN TAEL VI TPEL VL PDDL PF SAEL RF ENP' TF EHQ' • f • • • •
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
GNAi1 GNAi3 GNAi2 GNAo GNAq GNA11 Consensus	AGVIKR AGVIKR SGVIRR LSAMMR VDAIKS VSAIKT	LHKDSGVQ LHRDGGVQ LHADHGVQ LHADHGVQ LHADPGIQ LHNDPGIQ LHEDPGIQ LH.D.G!Q	ACFNRSREY ACFSRSREY ACFGRSREY ECFNRSREY ECYDRRREY ECYDRRREY eC%, RSREY	QLNDSAAYYLND QLNDSASYYLND QLNDSAAYYLND QLNDSAAYYLND QLNDSAKYYLDS QLSDSTKYYLND QLSDSAKYYLTD QLSDSAKYYLND	LDRIAQPN LDRISQSN LERIAQSD LDRIGAAD LDRYADPA YDRIATLG 1#R!a	YIPTQQDYLR YIPTQQDYLR YIPTQQDYLR YQPTEQDILR YYLPTQQDYLR YLPTQQDYLR YLPTQQDYLR Y_PT#QD!LR	TRYKTTGIVETH TRYKTTGIVETH TRYKTTGIVETH TRYKTTGIVETH VRYPTGIEYPI VRYPTTGIEYPI VRYPTTGIEYPI ŁRYKTTGI!ELH	TFKDLHFN TFKDLYFN TFKDLHFN TFKNLHFF DLQSYIFF DLENIIFF CLENIIFF	KHFDVGGQRSE KHFDVGGQRSE KHFDVGGQRSE RLFDVGGQRSE RHVDVGGQRSE RHVDVGGQRSE *\$fDVGGQRSE	RKKHIHCFEC RKKHIHCFEC RKKHIHCFEC RKKHIHCFEL RRKHIHCFEN RKKHIHCFE RKKHIHCFE	VTAIIFCVAL VTAIIFCVAL VTAIIFCVAL IVTAIIFCVAL IVTSINFLVAL IVTSINFLVAL VTSINFLVAL	SDYDLVLAE SDYDLVLAE SAYDLVLAE SGYDQVLHE SEYDQVLVES SEYDQVLVES S,YDQVLVES	JEEMNRMHESMI JEEMNRMHESMI JEEMNRMHESMI JETTNRMHESLI JONENRMEESKI JONENRMEESKI 14., NRMhES,	KL FDSI KL FDSI KL FDSI HL FDSI AL FRTI AL FRTI ↓L FdSI
	261	270	280	290	300	310	320	330	340	350	36062			*
GNAi1 GNAi3 GNAi2 GNAo GNAq GNA11 Consensus	CNNKHI CNNKHI CNNKHI CNNKFI ITYPHI ITYPHI cnnkui	TDT SIILF TEI SIILF TDT SIILF IDT SIILF QNS SVILF QNS SVILF .#L S!ILF	LNKKDLFEE LNKKDLFEE LNKKDLFEE LNKKDLFGE LNKKDLLEE LNKKDLLED LNKKDLLED	KIKKSPLTICYP KIKRSPLTICYP KITHSPLTICFP KIKKSPLTICFP KIMYSHLVDYFP KILYSHLVDYFP KILYSHLVDYFP	EYAGSNT EYTGSNT EYTGANK EYTGANK EYTGPNT EYDGPQRI EFDGPQRI E%LGP#.	EER 1-AYIQC EER 1-AYIQC DER 1-SYIQS EDA 1-SYIQS EDA 1-AYIQA AQA 1REFILK AQA 1REFILK #A 1XIq.	QFEDLNKRKDIK QFEDLNRRKDIK KFEDLNKRKDIK QFESKN-RSPNKI MFYDLNPDSD-K MFYDLNPDSD-K .FedlN.rsd.Ka	I THFTCF I THFTCF I THFTCF I CHMTCF I CHMTCF I SHFTCF I SHFTCF I SHFTCF	ATDTKNYQFY ATDTKNYQFY ATDTKNYQFY ATDTNNIQYY ATDTENIRFY ATDTENIRFY ATDTENIRFY ATDT_N!qfy	DAY TOYIIKA DAY TOYIIKA DAY TOYIIKA DAY TOYIIKA DAY TOYIIKA AAY KOTILQL AAY KOTILQL (dAY CD,Ii,r	INLKDCGLF INLKECGLY INLKDCGLF INLRGCGLY INLKEYNLY INLKEYNLY INLKEYNLY			





