Supplementary Figure Legends

Figure S1. Recording of the $G\alpha_{i2}$-activated TRPC4 and of the $G\alpha_{i3}$-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^+) to Cs^+ solution. The *horizontal bar* indicates duration of applied external cation solutions from both traces. *Dashed lines* show zero current.

Figure S2. Interaction of $G\alpha_{i3}$ 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 GTPγS stimulation. Current densities are represented by maximal current peaks (subtracted Cs^+ basal current) at -60 mV in Cs^+ solution and are indicated by means ± 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 GTPγS or $G\alpha_{i2}$.
The Δ695~724 and Δ700~728 mutants are not active, whereas the others retained partial or full activation.

Figure S4. A schematic diagram of mTRPC4β and mTRPC5 depicting their Gα interacting sites.

Figure S5. The interaction modeling of TRPC4 C-terminus (amino acids 701-720) with $G\alpha_{i2}$.
(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 GTPγS, and the red lines indicate activation by GTPγS 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 GTPγS 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^+ solution and are indicated by means ± S.E.

Figure S1

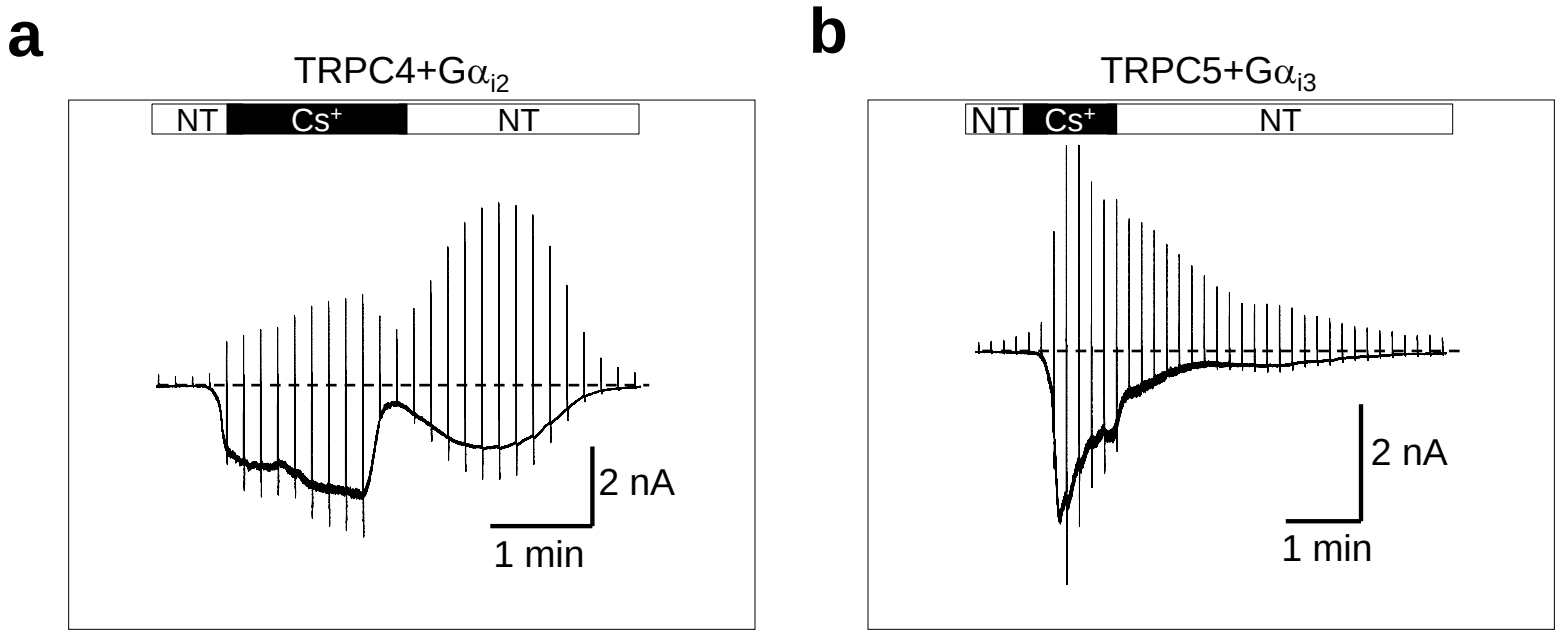


Figure S2

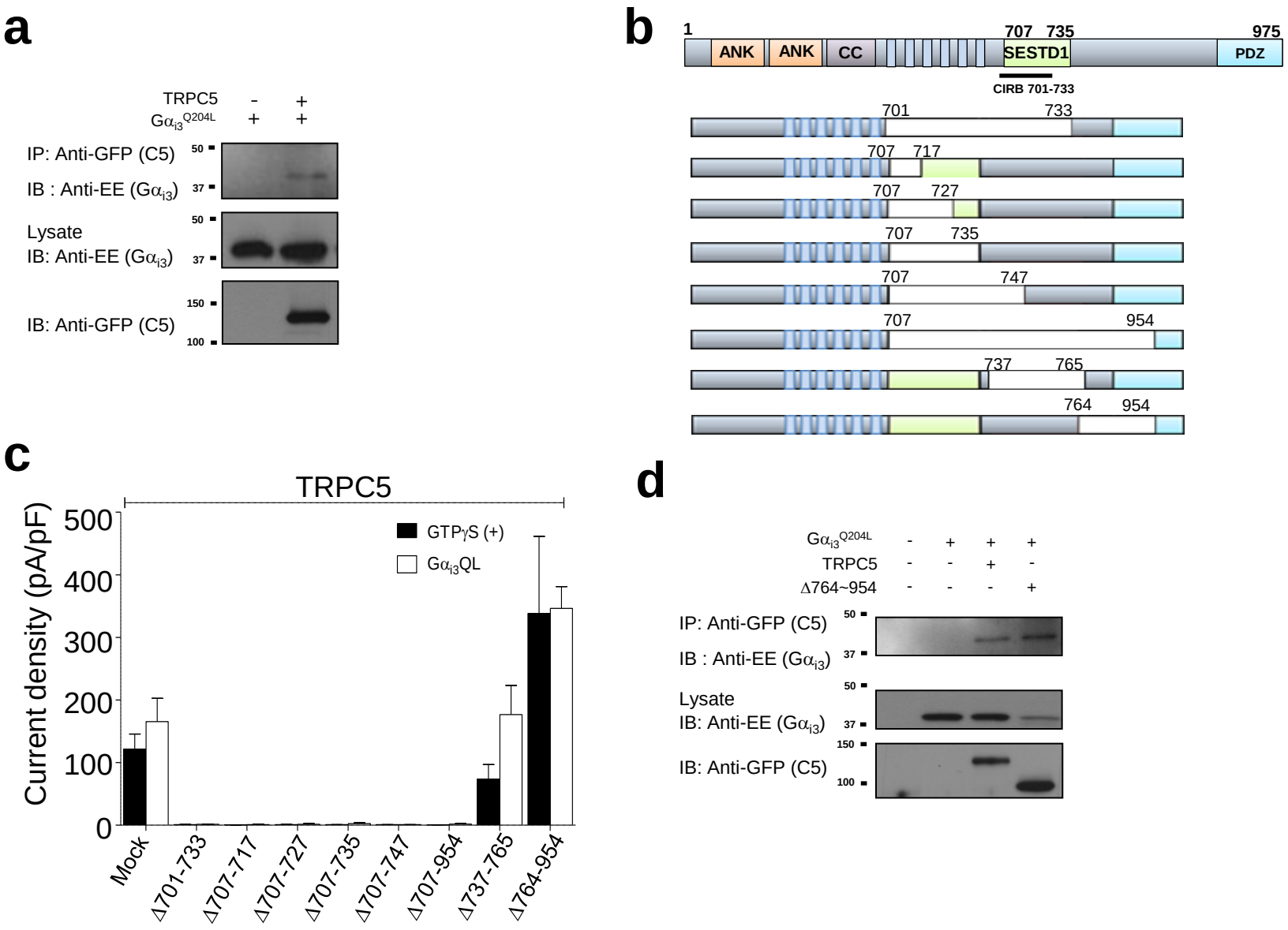

a
TRPC5 - +
Gαi3Q204L + +
IP: Anti-GFP (C5)
IB : Anti-EE (Gαi3)
Lysate
IB: Anti-EE (Gαi3)
IB: Anti-GFP (C5)
50
37
150
100
b
1
707 735
975
ANK
ANK
CC
SESTD1
PDZ
CIRB 701-733
701 733
707 717
707 727
707 735
707 747
707 954
737 765
764 954
c
TRPC5
GTPγS (+)
Gαi3QL
Current density (pA/pF)
500
400
300
200
100
0
Mock
Δ701-733
Δ707-717
Δ707-727
Δ707-735
Δ707-747
Δ707-954
Δ737-765
Δ764-954
d
Gαi3Q204L - + + +
TRPC5 - - + -
Δ764~954 - - - +
IP: Anti-GFP (C5)
IB : Anti-EE (Gαi3)
Lysate
IB: Anti-EE (Gαi3)
IB: Anti-GFP (C5)
50
37
50
37
150
100

Figure S3

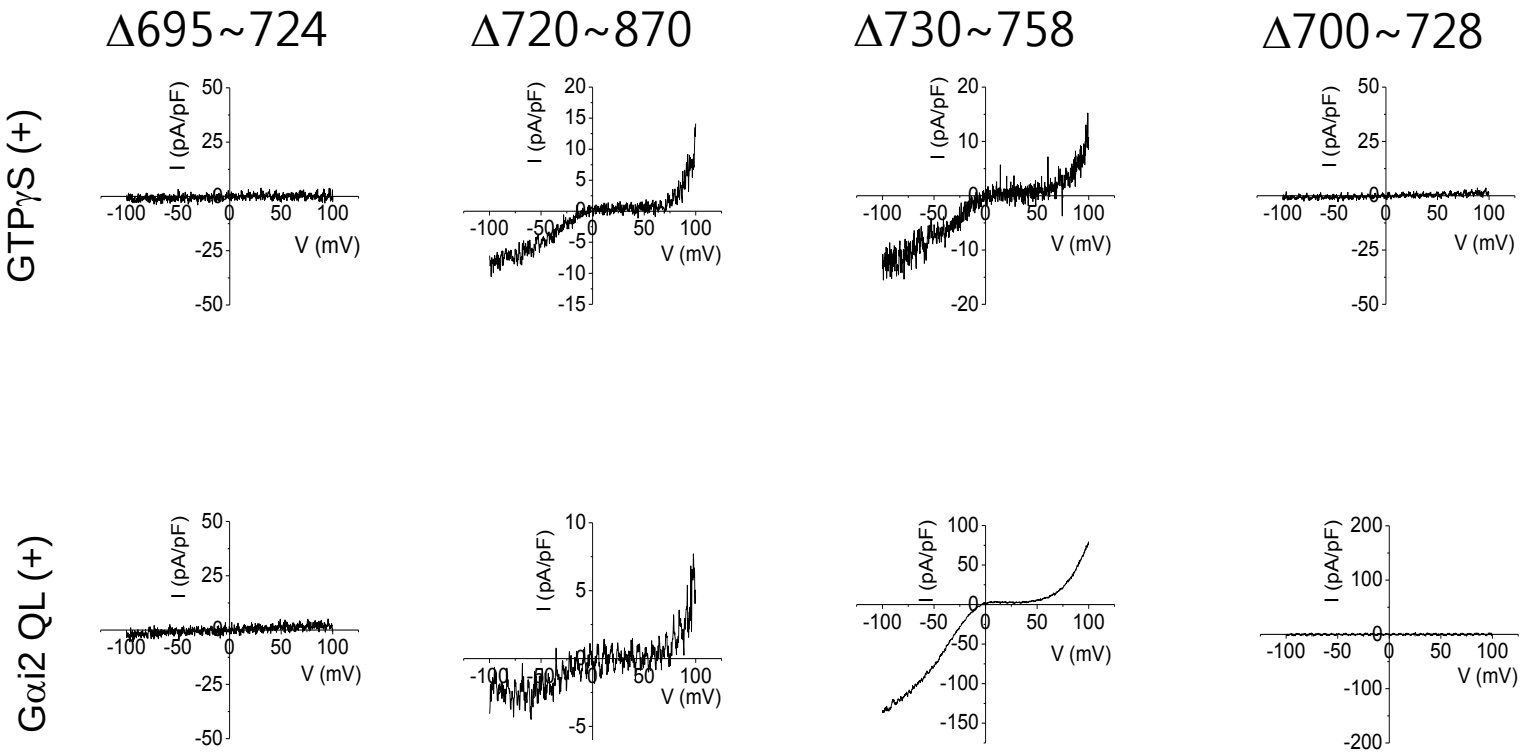
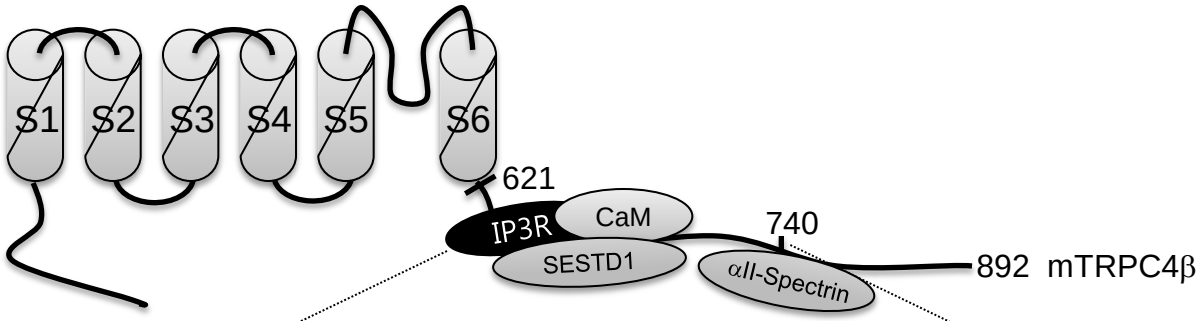


Figure S4



695

mTRPC4β RAADNL RRHHQYQEVM RNLVKRYVAA MIRAVKTEEG LTEENVKELK

CIRB 1 (695~724)

SESTD1 (700-728)

Putative interacting site of $G\alpha_{i2}$

* αII-Spectrin in hTRPC4 (730-758)

701

mTRPC5 ERHADSL IQN QHYQEVIRNLVKRYVAA MIRNSKTN EG LTEENFKELK

CIRB (701~733)

SESTD1 (707-735)

Putative interacting site of $G\alpha_{i3}$

Figure S5

a

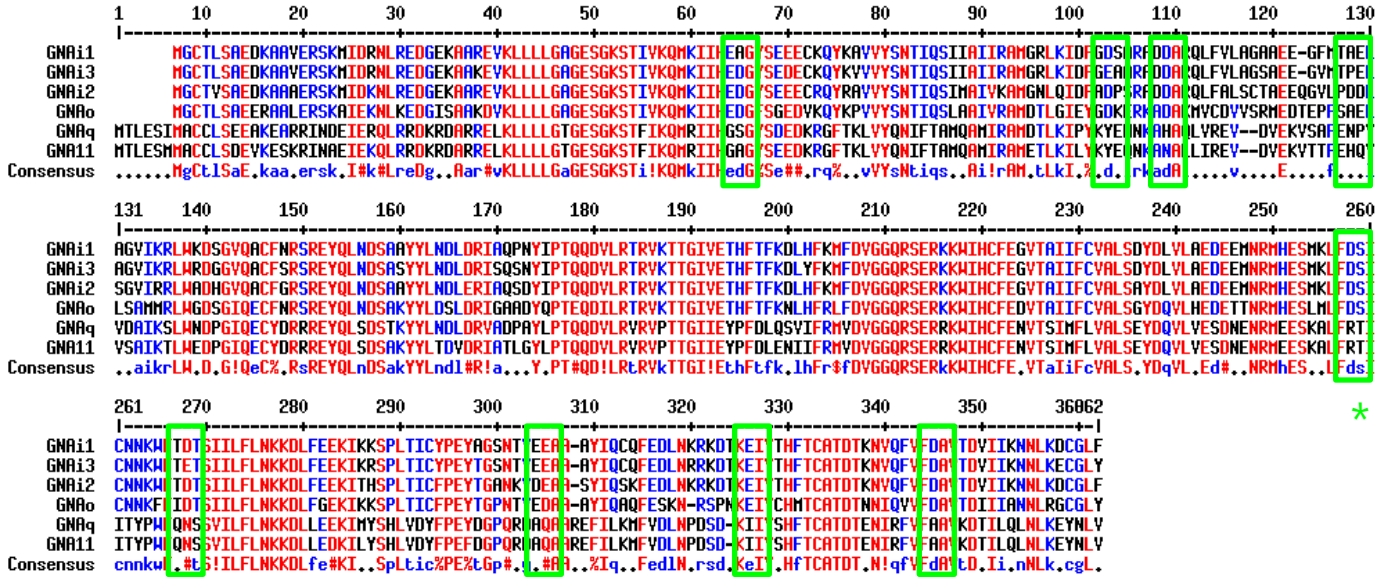
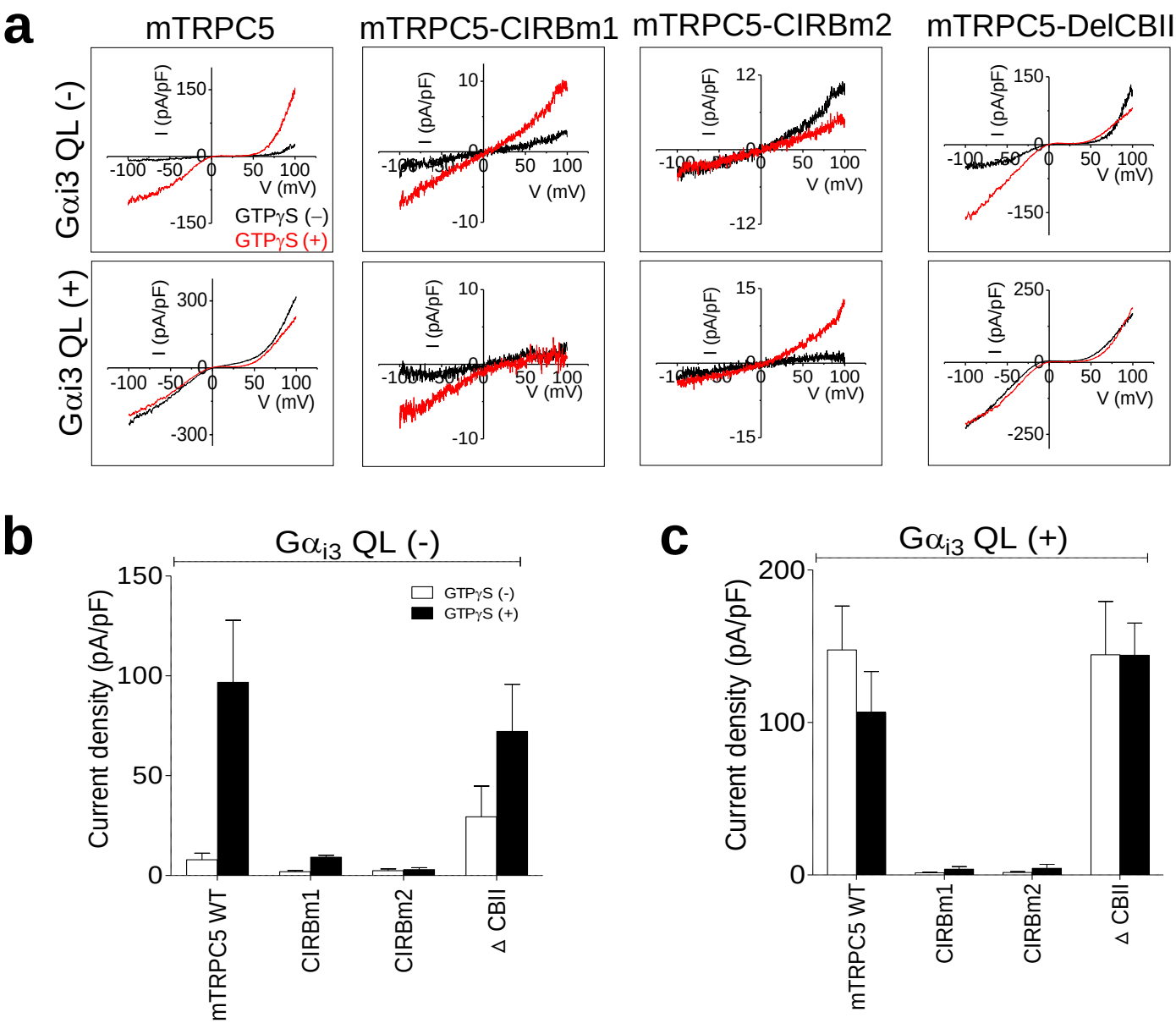


Figure S6


a
mTRPC5
mTRPC5-CIRBm1
mTRPC5-CIRBm2
mTRPC5-DelCBII
Gαi3 QL (-)
Gαi3 QL (+)
I (pA/pF)
V (mV)
GTPγS (–)
GTPγS (+)
b
Gαi3 QL (-)
c
Gαi3 QL (+)
Current density (pA/pF)
GTPγS (-)
GTPγS (+)
mTRPC5 WT
CIRBm1
CIRBm2
Δ CBII