

Yi et al., Supplemental Figure 1

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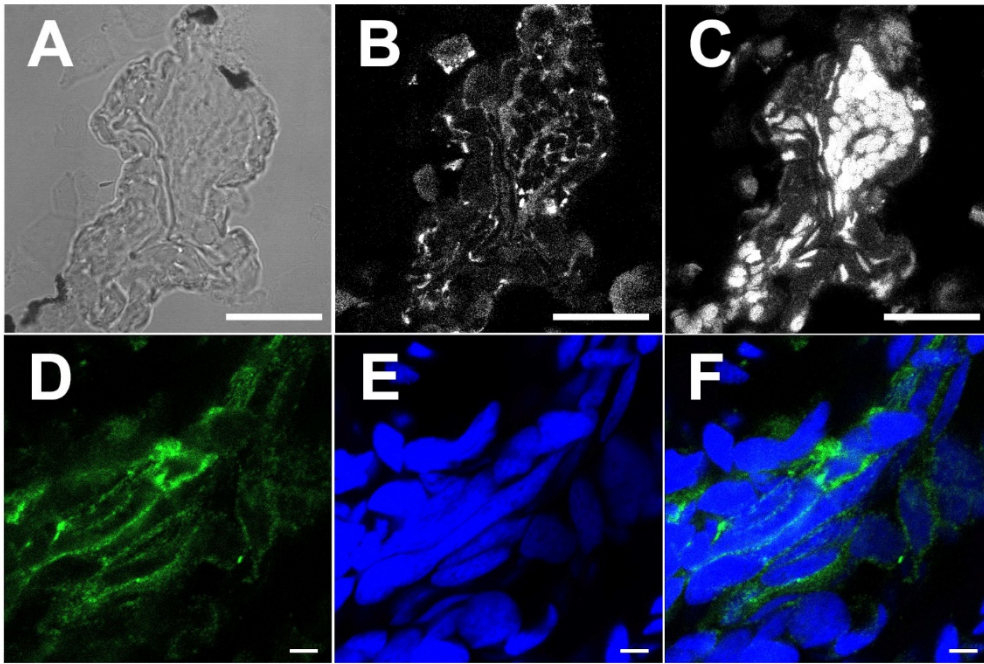
2 Supplementary Figure 1

3 (A) Confocal image of immunohistochemistry staining for GFP expression in a cross section
 4 of the distal tip of the adult fin after heat shock induction of *kcnk5b*-GFP in a fish harboring
 5 the Tg[*hsp70:kcnk5b*-GFP] transgene. The left inset is an enlarged view of the expression of
 6 the transgene in the fin. (B) Confocal image of DAPI-stained nuclei of the same confocal
 7 section in panel A. (C) Overlay of the confocal GFP and DAPI images. (D) qRT-PCR results
 8 for *kcnk5b* expression from the transgenic fish line Tg[*hsp70:kcnk5b*-GFP] at the indicated
 9 time points relative to the single heat-shock pulse. (E) qRT-PCR results for GFP expression

10 from the transgenic fish line Tg[*hsp70:kcnk5b*-GFP] at the indicated time points relative to the
11 single heat-shock pulse. (F) qRT-PCR results for the indicated genes in the caudal fin without
12 (Pre) and after (post) 24 hours after a single heat-shock pulse Tg[*hsp70:kcnk5b*-GFP]. (G)
13 Example blot for Shh expression in adult fins with or without heat-shock induction of the
14 Tg[*hsp70:kcnk5b*-GFP] transgene. (H) Example blot for Lef1 expression in adult fins with or
15 without heat-shock induction of the Tg[*hsp70:kcnk5b*-GFP] transgene. (I) Example blot for β -
16 catenin expression in adult fins with or without heat-shock induction of the Tg[*hsp70:kcnk5b*-
17 GFP] transgene. (J) Example blot for β -actin expression in adult fins with or without heat-
18 shock induction of the Tg[*hsp70:kcnk5b*-GFP] transgene.

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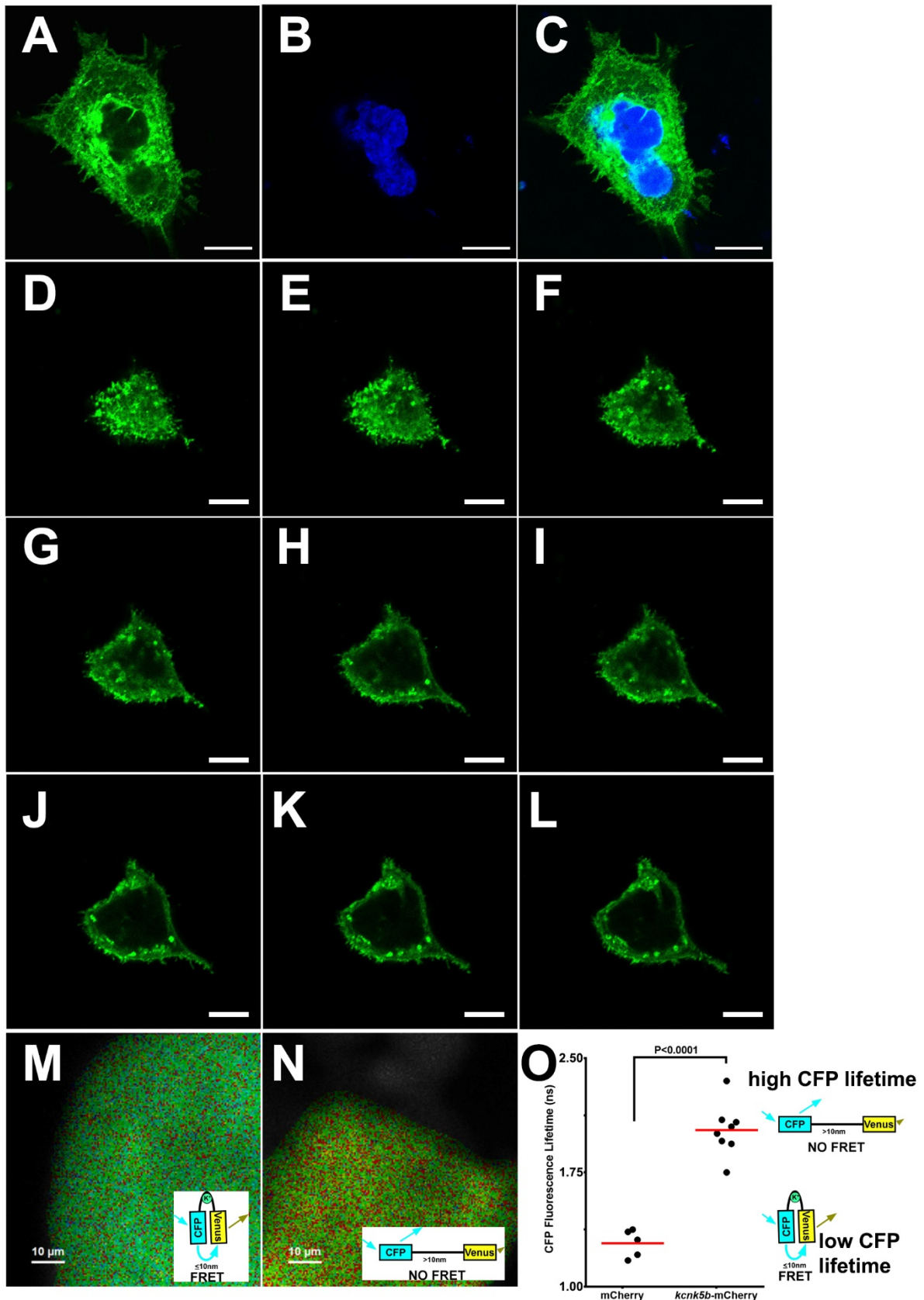
21 Yi et al., Supplemental Figure 2

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22 **Supplementary Figure 2: Expression of Kcnk5b-GFP in transgenic Tg[hsp70:kcnk5b-**
23 **GFP] larva after single heat shock.**

24 (A) Representative bright field cross section through the mid-section of heat-shocked transgenic
25 zebrafish larva. (B) Immunohistochemical staining for GFP of the same cross section in panel
26 A. (C) DAPI staining of the same cross section in panel A. (D) Representative confocal image
27 of immunohistochemical staining for GFP in heat-shocked transgenic zebrafish larva showing
28 membranous staining of Kcnk5b-GFP. (E) DAPI staining of the same confocal section in panel
29 D. (F) Merged image of panels D and E. Scale bars equal 40 μm (A-C) and 5 μm (D-F).

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Yi et al., Supplemental Figure 3

32 **Supplementary Figure 3: Expression and activity of Kcnk5b-GFP in transfected**
33 **HEK293T cells.**

34 (A) Confocal image of through an immunocytochemical staining of a HEK293T cell
35 transfected with zebrafish *kcnk5b*-GFP transgene (B) DAPI staining of the same cell in panel
36 A. (C) Merged panels A and B. (D-L) Serial confocal cross sections for GFP in a live
37 HEK293T cell from its surface (D) through to a mid-section of the cell (L) after transfection
38 with the zebrafish *kcnk5b*-GFP transgene. (M) FRET-FLIM image after measuring the life
39 time of CFP of the K⁺ FRET reporter KIRIN in a cell transfected with a control plasmid only
40 expressing mCherry. The inset image of the confirmation of the FRET reporter depicting how
41 FRET occurs. (N) FRET-FLIM image after measuring the life time of CFP of the K⁺ FRET
42 reporter KIRIN in a cell transfected with a plasmid expressing *kcnk5b*-mCherry. The inset
43 image of the confirmation of the FRET reporter depicting how FRET do not occur. (O) The
44 graphed assessments of the changes in CFP lifetime in the control mCherry-expressing cells
45 and the cells expressing *kcnk5b*-mCherry. The diagrams to the right of the graph depict the
46 conformational changes that result in the values of the lifetimes of CFP.

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A

Kcnk5b

MALRAGRILLWFGQVLRVSRRLQGLWSRRSSLLCLSNSQEHDDQGTCTYSRNRCPKTHRQQHCPYPPFPDCCCAFPGDKRGHEPLSRRLVAMKR
Kcnk9

Kcnk5b
MADKGPILTSVII FYLSIGAAI FQILEE PNLNSAVDDYKNTNNLLKYPCLSKVELGEIIEVVAEATGQGVTVTKEAQFNNWNWENAVIFAATVITTIYGNVAPKTT
YL +GAA+F LE + + + L KY +S++ ++ ++ EA V W + + FA TVITTIYGY+ AP T
QNVRTL SLIVCTFTYLLVGAAVFDALESDFEMREKEQLEAEEKRLQ GKYN-ISEDDYKQLQTIIMEAEPHRAGV-----QWKFAGSFYFAITVITTIYGHAAAPGTD
Kcnk9

Kcnk5b
GGRLFCILYGLGCIPLCLTWISLG---TFFGSRTRKRLSQQLLHSGLNVRKVVQFICTIVFLLWGFVHLII PAFVEMFFENWYILEGLYFSFTTLTIVGFGDYVA-GVD
G+ FC+ Y + GIPL L LG TF KR+ + G+ + +V + + + L I A F +E+W++ + Y+ F TLTT+GFGD+VA +
AGKAFCMFYAVLGIPLVMFQSLGERMNTFVKYLLKRIKCC---CGMRITEVSMENMVVGFSCMGILCI GAAAFSQVEDWSFFQSYYYCFITLTTIGFGDFVALQKN
Kcnk9

Kcnk5b
PSVNYPTLYRFFVQLWYIYGLAWLSLFFSWNVHMVVEAHKVLKRRMRRLRPTDDVPEKKEVKTKPKPPRSQVIDI FEFMSEKVEDYSDVIRAI GADEKRRKKQEE
++ LY F ++I +GL + F + V
KALQKKPLYVAFSFMYLVLVGLVIGAPLNLVVLRLTMSSEDEERRDAEERASLAGNRS SMI IHIQEDTLQRSRRRREQQNRNRYRPEVITLQSVCSMHHYSHEFGSSVGL
Kcnk9

Kcnk5b
LARSKSCDLLQG LVIELDH RLQRRFVSANMCMASIDESVDGLNINNCKQEDD TLTKVRHKDQKVNREANPARCAWDSRSDPSIFQSSTVTNSTNRGSRFVSK
GLGLGGGGGAFPPQNSFGSQLSPHYHHYHSTVSYRI EESISPTLKNFSLPSPISSISPLGHSFAENL-----
Kcnk9

Kcnk5b
VSEDRLGKRKSG
RL+ +RKS
----RLMRRRKS
Kcnk9

B

MKFPTEPRKPGNWNPPVFPVQINLVPPKVKVQPGMLQSSLVQASVATMQNPM
Kcnk10a

Kcnk5b
-----MADKGPILTSVII FYLSIGAAI FQILEE PNLNSAVDDYKNTNNLLKYPCLSKVELGEIIEVVAEATGQGVTVTKEA
G +F+ LE+ D K L K+PC++ + L E+I+ +A GV+ +
GCSLPRLSVSRPASMVASMEAVADGSALLTVMKWKTVLAVFVVVAYLVAGGLVFRALQHFERYQKDSITLKKAAFLKHPCVTPDELEELIKHSVDVAVNGVSPIGDT
Kcnk10a

Kcnk5b
QFNNWNE--NAVIFAATVITTIYGNVAPKTTGGRLFCILYGLGCIPLCLTWIS---ELGFFGSRTRKRLSQQLL--HSGLNVRKVVQFICTIVFLLWGFVHLII PAF
+N+ +W+ ++ FA TVITTIYGN+AP T GG+FCILY + GIPL ++ +LGI FG ++ ++ H+ ++ K++ T++F+L G ++ + IPA
SYNSSHWDLGSSFFAGTVITTIYGNVAPKTTGGRIFCILYAFGIPLFGFLLAGVGDQLGTIPGKSIKVEKFRKRNHNSQTKIRVASTLLFI LAGCILFVTIPAI
Kcnk10a

Kcnk5b
VEMFFENWYILEGLYFSFTTLTIVGFGDYVAGVDPVNYPTLYRFFVQLWYIYGLAWLSLFFSWNVHMVVEAHKVLKRRMRRLRPTDDVPEKKEVKTKPKPPRSQV
+F E WI LE +YF TLTTVG GDYVAG + + Y YR V WI +GLA+ + S M+ + +VL K+
IFKHIEGWIGLEAIYFVITLTTVIGDYVAGGNRRIEYRKYRPLWFVILVGLAYFAAVLS---MIGDWLVL SKKTKMEVGEIKAHAAEWKANVRAELRETRRRLS
Kcnk10a

Kcnk5b
DIFEFMSEKVEDYSDVIRAI GADEKRRKKQEEELARSKSCDLLQG LVIELDH RLQRRFVSANMCMASIDESVDGININNCKQEDD TLTKVRHKDQKVNREANF
VEVHDKLQRAATIRSMERRQLGFDQRAHSLDMLSPERRAAFNSLDATNFKTSQESIDTLKNNLRLRVEQNEHRRSDPSQAYSEDNINFLGSVTKLAKRKNRDLKKN
Kcnk10a

Kcnk5b
ARCAWDSRSDPSIFQSSTVTNSTNRGSRFVSKVSEDRLGKRKSG
LDDGRKALDSFCDTPMDEKKEAEDEELEKEVNI SLTNLPLFVESPKNQNGFVPLPPQTKKEETETKLEDKEFRLQVDP
Kcnk10a

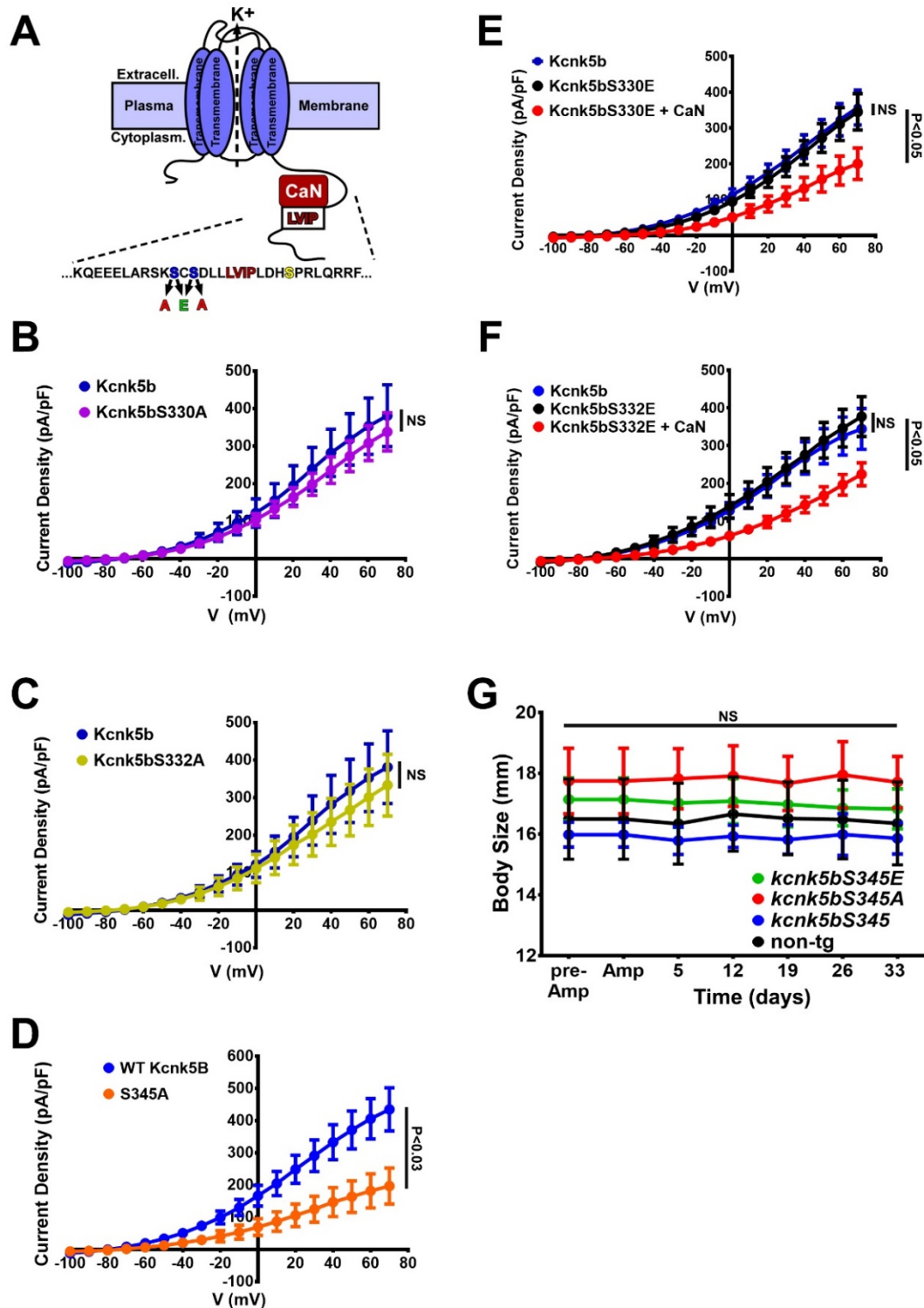
Yi et al., Supplemental Figure 4

49 **Supplementary Figure 4: Amino acid comparison of Kcnk5b with Knck9 or with**
50 **Kcnk10**

51 Comparison between zebrafish amino acid sequences from Kcnk5b (blue) and Kcnk10b
52 (black) show conserved (red letter) and similar properties (red plus sign) amino acids.

53 Yellow-highlighted letters indicate calcineurin binding site, and green-highlighted letters
54 indicate the site of post-translational modification by calcineurin.

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Yi et al., Suppl Fig. 5

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57 **Supplementary Figure 5: Activity measurements of Kcnk5b Serine mutant channels**

58 (A) Diagram of Kcnk5b channel showing proposed Serine345 calcineurin dephosphorylation
 59 site (yellow) adjacent the calcineurin-interaction site (LVIP). Two other serines (blue) were
 60 substituted with alanines or glutamic acids to mimic dephosphorylation or phosphorylation.

61 **(B)** Electrophysiology measurements of wild-type Kcnk5b (blue) and serine-to-alanine mutant
62 Kcnk5bS330A (purple). **(C)** Electrophysiology measurement of wild-type Kcnk5b (blue) and
63 serine-to-alanine mutant Kcnk5bS332A (yellow). **(D)** Electrophysiology measurement of
64 wild-type Kcnk5b (blue) and serine-to-alanine mutant Kcnk5bS345A (orange). **(E)**
65 Electrophysiology measurement of wild-type Kcnk5b (blue), serine-to-glutamic acid mutant
66 Kcnk5bS330E (black) and Kcnk5bS330E plus calcineurin (CaN) (red). **(F)** Electrophysiology
67 measurement of wild-type Kcnk5b (blue), serine-to-glutamic acid mutant Kcnk5bS332E
68 (black) and Kcnk5bS332E plus calcineurin (CaN) (red). **(G)** Body length measurements from
69 tip of the head to the base of the fin for each transgenic fish are represented as averages and
70 standard deviation.
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