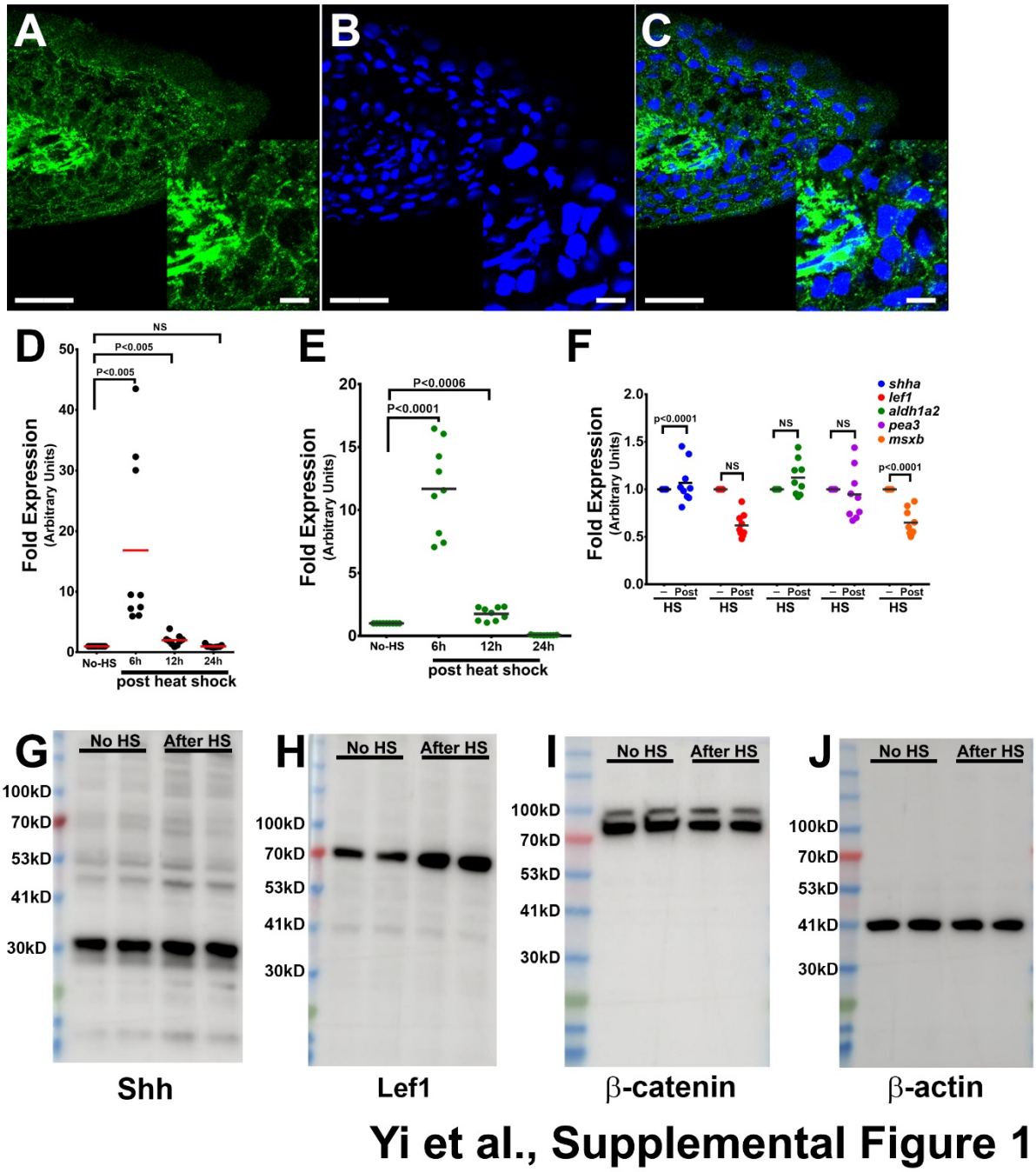
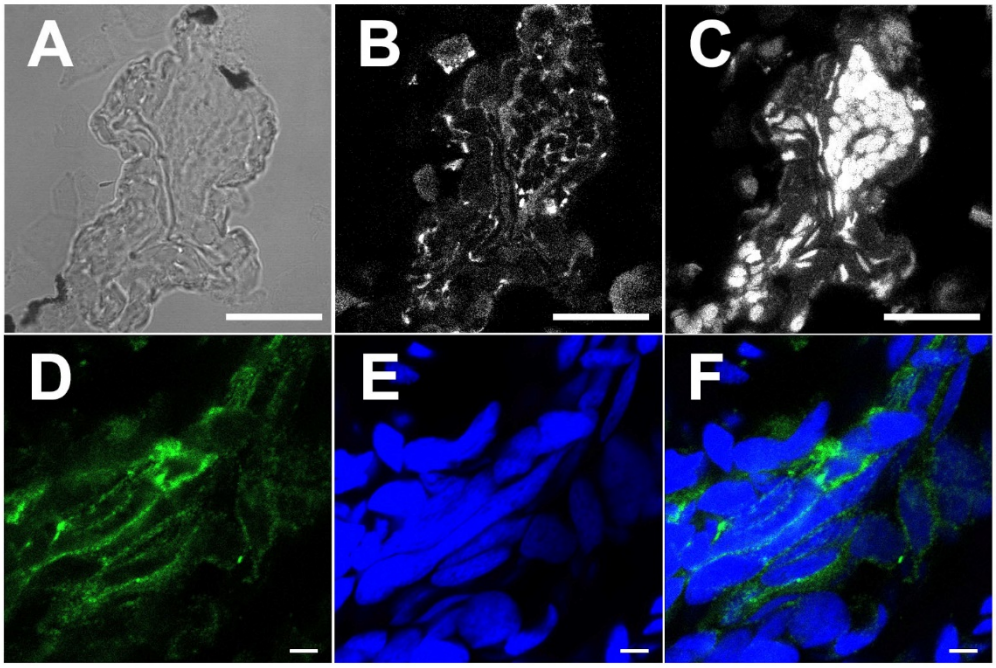


Supplementary Figure 1

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

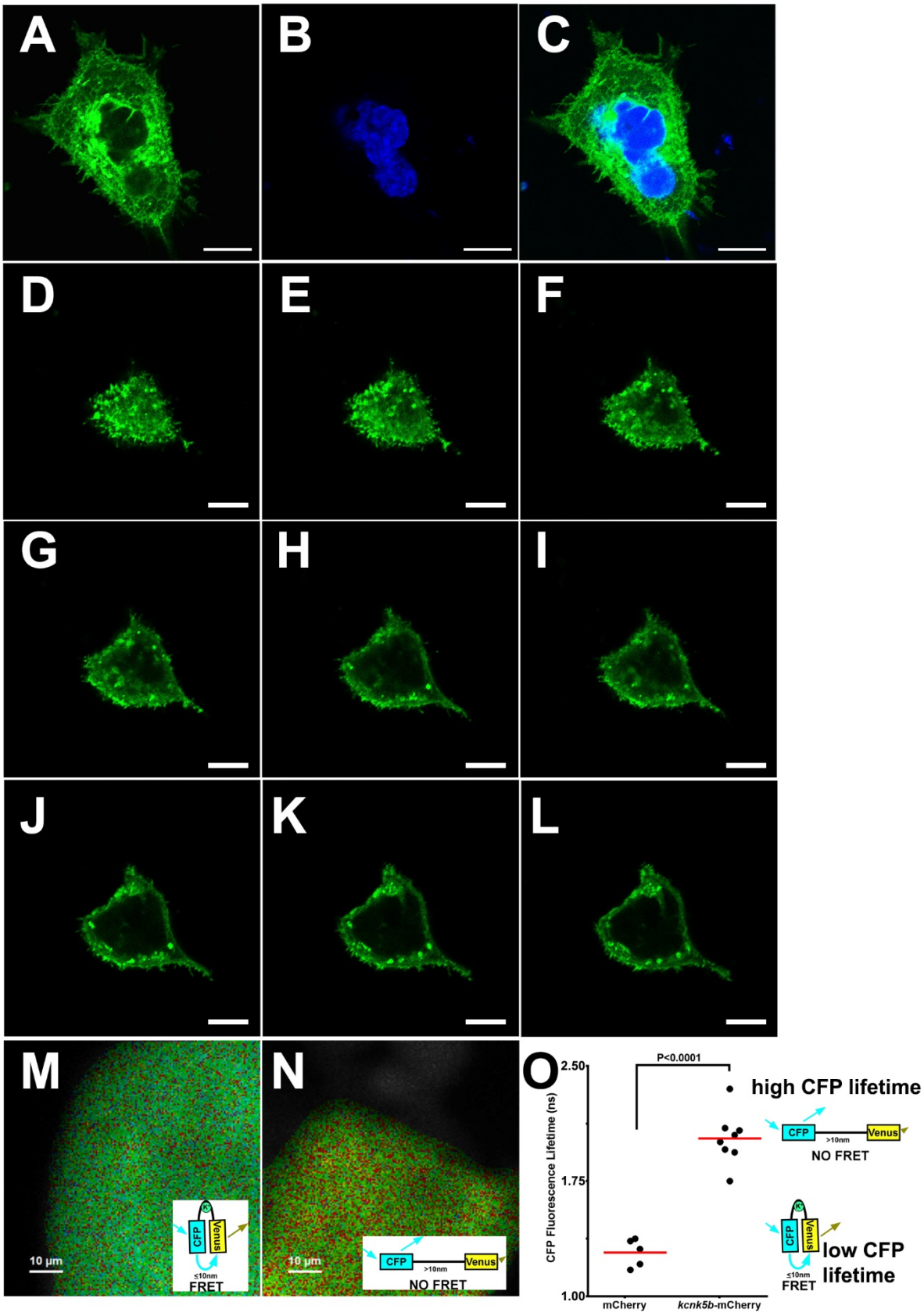
from the transgenic fish line Tg[*hsp70*:*kcnk5b*-GFP] at the indicated time points relative to the single heat-shock pulse. (**F**) qRT-PCR results for the indicated genes in the caudal fin without (Pre) and after (post) 24 hours after a single heat-shock pulse Tg[*hsp70*:*knck5b*-GFP]. (**G**) Example blot for Shh expression in adult fins with or without heat-shock induction of the Tg[*hsp70*:*kcnk5b*-GFP] transgene. (**H**) Example blot for Lef1 expression in adult fins with or without heat-shock induction of the Tg[*hsp70*:*kcnk5b*-GFP] transgene. (**I**) Example blot for β-catenin expression in adult fins with or without heat-shock induction of the Tg[*hsp70*:*kcnk5b*-GFP] transgene. (**J**) Example blot for β-actin expression in adult fins with or without heat-shock induction of the Tg[*hsp70*:*kcnk5b*-GFP] transgene.



Yi et al., Supplemental Figure 2

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

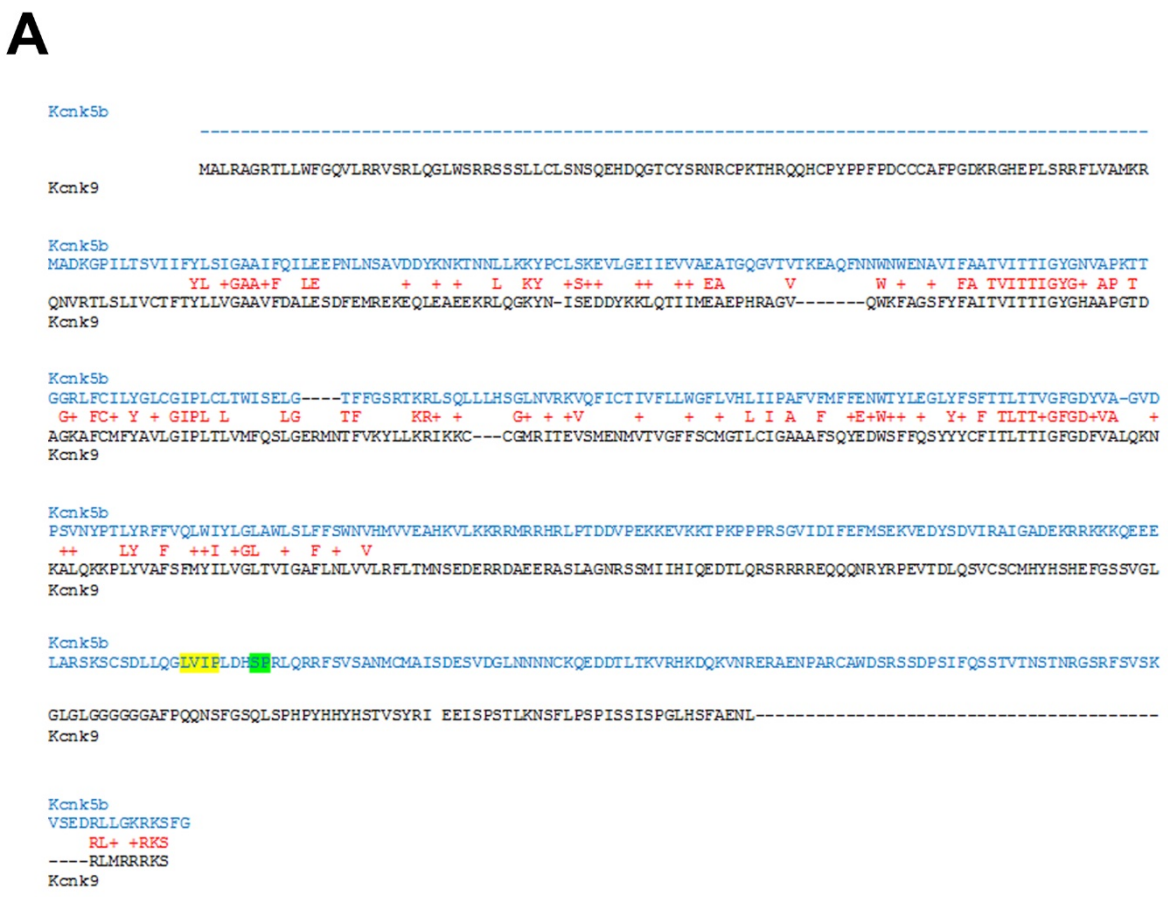
(**A**) Representative bright field cross section through the mid-section of heat-shocked transgenic zebrafish larva. (**B**) Immunohistochemical staining for GFP of the same cross section in panel A. (**C**) DAPI staining of the same cross section in panel A. (**D**) Representative confocal image of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



Yi et al., Supplemental Figure 3

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

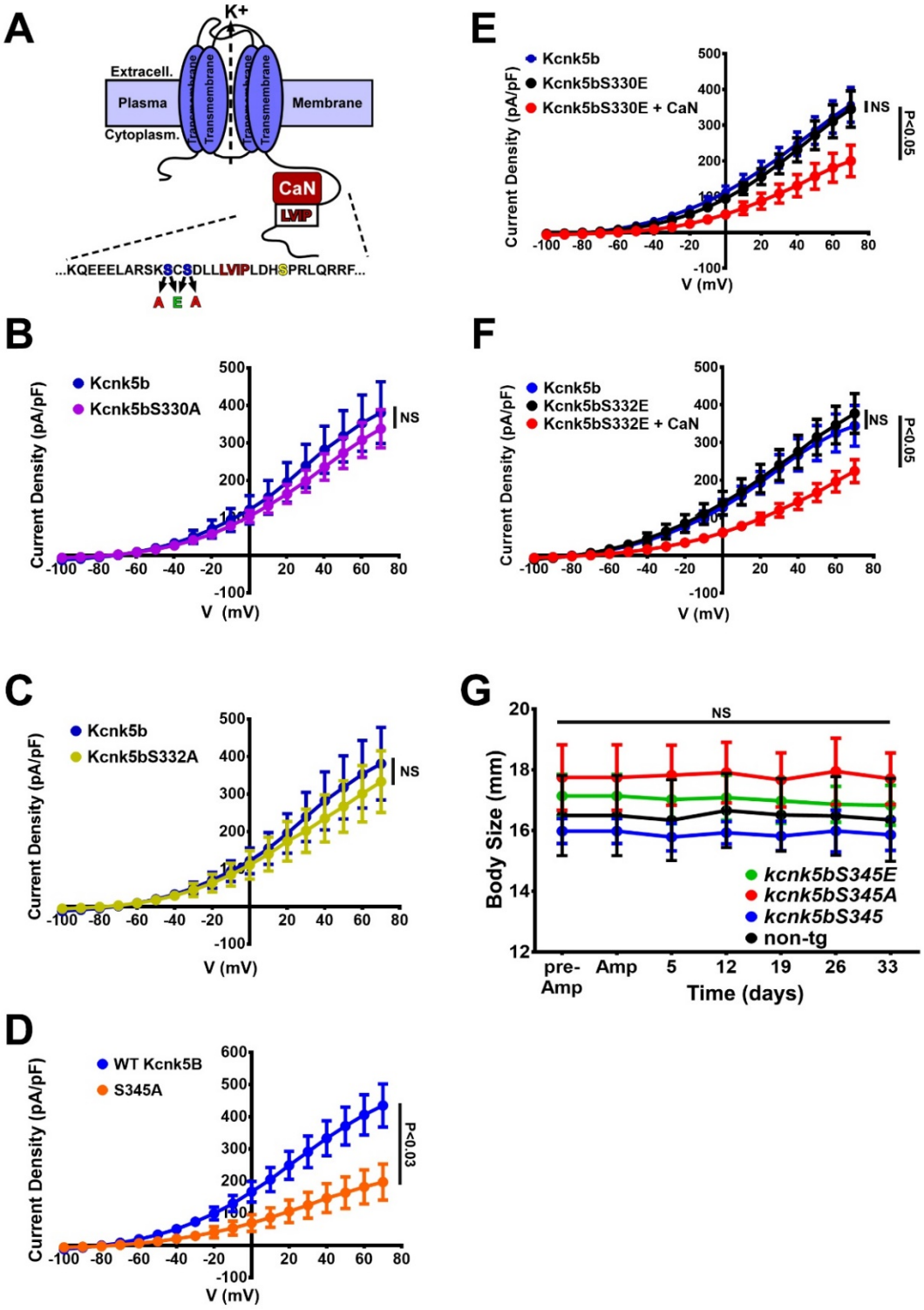
(**A**) Confocal image of through an immunocytochemical staining of a HEK293T cell transfected with zebrafish *kcnk5b*-GFP transgene (**B**) DAPI staining of the same cell in panel A. (**C**) Merged panels A and B. (**D-L**) Serial confocal cross sections for GFP in a live HEK293T cell from its surface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



Yi et al., Supplemental Figure 4

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

Comparison between zebrafish amino acid sequences from Kcnk5b (blue) and Kcnk10b (black) show conserved (red letter) and similar properties (red plus sign) amino acids. Yellow-highlighted letters indicate calcineurin binding site, and green-highlighted letters indicate the site of post-translational modification by calcineurin.



Yi et al., Suppl Fig. 5

Supplementary Figure 5: Activity measurements of Kcnk5b Serine mutant channels

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

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