

Supplementary Fig. S1. Calfacilitin is conserved among different species.

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Calfacilitin      ---MGPGRAPSAALVGGSVLFGALRRAALALPRPAAVRSRPGRVWRWRNLLVSFAHSV
Human            ----MPRLLHPALPLLLGATLTFRALRRALCRLPLPVHVRADPLRTWRWHNLLVSFAHSI
Chimpanzee       ----MPRLLHPALPLLLGATLTFRALRRALCRLPLPVHVRADPLRTWRWHNLLVSFAHSI
Mouse            ----MPLLFHPAWPLLLGATLTFRALRRVLCRLPQPAHVQTDPLRTWRWHNLLVSFTHSI
Zebrafish        MDTWLNEVQKFPVLYVLCSSVLFRIHWCLOIVARPDTVTKDRWKTWKWRNLSVSLVHSL
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Calfacilitin      LAGLWALFSLWQSPELLSDIQDGYSVSGHLLVCFSSGYFIHDSLDIIFNQQRSSWEYLV
Human            VSGIWALLCVWQTPDMLVEIETAWSLSGYLLVCFSSAGYFIHDTVDIVASGQTRASWEYLV
Chimpanzee       VSGIWALLCVWQTPDMLVEIETAWSLSGYLLVCFSSAGYFIHDTVDIVTSGQTRASWEYLV
Mouse            VSGIWALLCLWQTPMLVEIETAWSASGYLLVCFSSAGYFIHDTVDIVSKQTRASWEYLV
Zebrafish        LTGTWAVACVIYYPAMVHEIHSTYTPSAYMLVVSSGYFIEDAADIVFSGHAKASWEFL
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Calfacilitin      HHAMAI SAFVSLIITGRFLVAAMLLLVEVSNIFLTIRMLLKMS-NVPSPALYEANKYVN
Human            HHVMAMGAFFSGIFWSSFVGGVLTLLVEVSNIFLTIRMMMKIS-NAQDHLLYRVNKYVN
Chimpanzee       HHVMAMGAFFSGIFWSSFVGGVLTLLVEVSNIFLTIRMMMKIS-NAQDHLLYRVNKYVN
Mouse            HHVMAMGAFFSGIFWKRFVGGVLTLLVEVSNIFLTLRMMMKIN-NAQDLLLYKVNKYIN
Zebrafish        HHVLVLWCFLYAVFTHQYVAGAVVALFVEVNSVFLHTRLLLNLAKVAHSSLIYTVNKVLN
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Calfacilitin      LVMYFARLAPQVYLTWYFVRYVEVQGGAFMANLLLLDAMILMYFSRLLRSDFPFLR
Human            LVMYFLFRLAPQAYLTHFFLRYVNQRTLGTFLLGILLMLDVMIIYFSRLLRSDFCP---
Chimpanzee       LVMYFLFRLAPQAYLTHFFLRYVNQRTLGTFLLGILLMLDVMIIYFSRLLRSDFCP---
Mouse            LVMYFLFRLAPQAYLTKFFLQYAGQRTLGTFLLAILLMLDLMIIIYFSRLLRSDFCP---
Zebrafish        VVTYVTFRLGAQFYLTWYLYHYSSLDYALYFLITMMLNIMILIYFYRLIRSDFFTKRR
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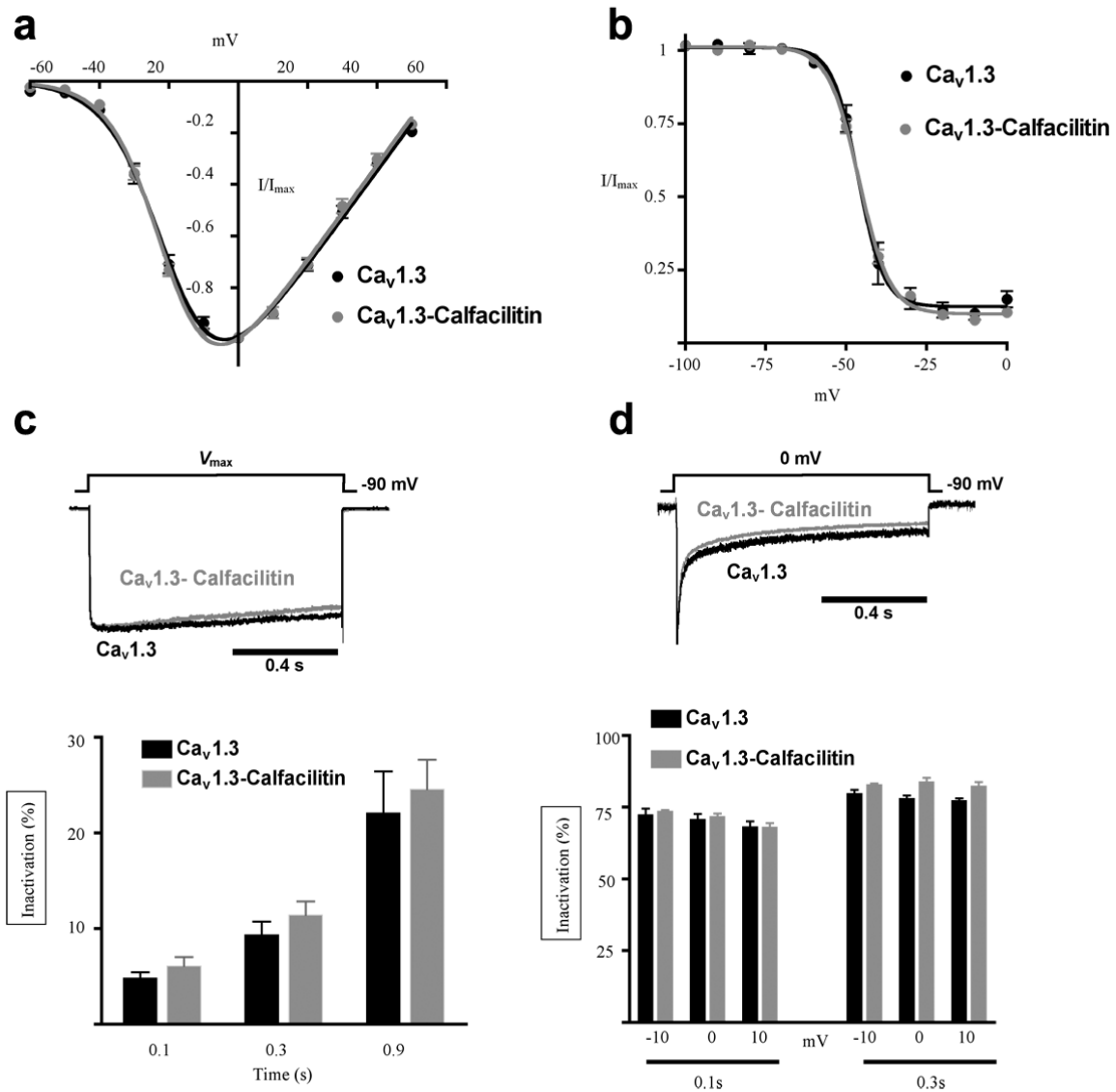
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Calfacilitin      KGSVGRDVDGEKFLID
Human            -EHVPPKQHKDKFLTE
Chimpanzee       -EHVPPKQHKDKFLTE
Mouse            -ERAPRRQQKDKFLTE
Zebrafish        IQNGIQKLAAD-----
                  :   :

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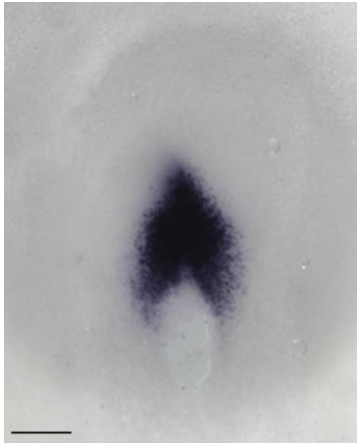
Sequence alignment of the predicted protein sequence of Calfacilitin with its putative orthologues in other species. Chick Calfacilitin has been submitted to GenBank and assigned accession number GQ504719. Orthologues: Zebrafish: AAH83250.1; Mouse: NP_080984; Chimpanzee: XP_511362.1; Human: BC014072.1. Shaded areas correspond to the predicted transmembrane domains, which are conserved.

Supplementary Fig. S2. Lack of effect of calfacilitin on $Ca_v1.3$ channels.



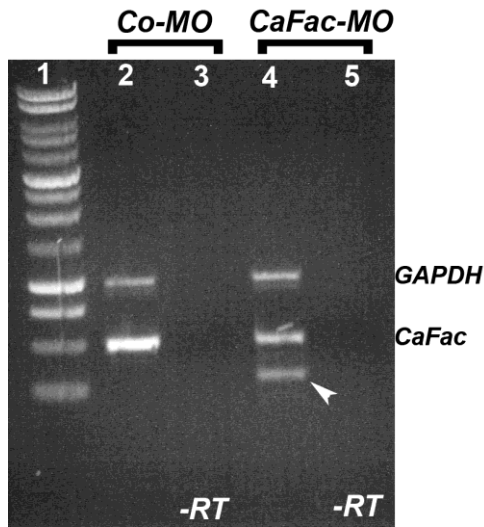
a. Normalized $I-V$ curves for I_{Ca} . $V_{0.5}$ ($Ca_v1.3$) = -19.36 ± 0.64 mV ($n=7$). $V_{0.5}$ ($Ca_v1.3$ -Calfacilitin) = -20.0 ± 0.53 mV ($n=5$). $P > 0.05$ (Student's t -test). **b.** Steady-state inactivation properties. $V_{0.5}$ ($Ca_v1.3$) = -46.3 ± 0.64 mV ($n=7$). $V_{0.5}$ ($Ca_v1.3$ -Calfacilitin) = -45.9 ± 0.3 mV ($n=7$). $P > 0.05$ (Student's t -test). **c.** Representative I_{Ba} during depolarizations to V_{max} and percentage of I_{Ba} inactivation. No difference was found between the presence and absence of calfacilitin. $N_{cell}=7$ ($Ca_v1.3$). $N_{cell}=5$ ($Ca_v1.3$ -Calfacilitin). **d.** Representative I_{Ca} and percentage I_{Ca} inactivation during depolarizations to -10, 0 and 10 mV at 0.1 and 0.3 s after peak current. There is no difference between the presence and absence of calfacilitin. $N_{cell}=7$ ($Ca_v1.3$). $N_{cell}=7$ ($Ca_v1.3$ -Calfacilitin). Error bars in all panels correspond to the standard error of the mean.

Supplementary Fig. S3. Nicardipine treatment does not affect expression of *Chordin*.



Embryo treated with Nicardipine as shown in Fig. 5c, after in situ hybridisation with the organizer marker *Chordin*. This embryo is at stage 4⁺ and expression is confined, as in normal embryos, to the organizer and emerging head process (dark blue signal). Scale bar: 100µm.

Supplementary Fig. S4. Morpholino directed against an exon-intron junction of Calfacilitin causes exon-skipping and generates a truncated form.



Embryos were electroporated with Control (Co-MO, Lanes 2-3) or Calfacilitin (CaFac-MO, Lanes 4-5) morpholinos, the latter targeting a splice junction. The products were amplified by RT-PCR. Lane 1, size markers (Promega 1kb ladder; the lowest four markers correspond to 253, 500, 750 and 1000 bp respectively, from bottom to top). Lanes 3 and 5 contain the same samples as 2 and 3 but Reverse Transcriptase (RT) was omitted as a control. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was used as a loading control. The Morpholino causes a decrease in the Calfacilitin (CaFac) band and appearance of a smaller band corresponding to a truncated version caused by exon skipping (arrowhead). Note that electroporation targets a mosaic of cells therefore each sample is a mixture of targeted and normal cells; this can account for the remaining full-length Calfacilitin in the CaFac-MO sample.