SUPPLEMENTAL FIGURES FOR

The L-type $Ca_V1.2$ Ca^{2+} channel regulates mandibular development through a calcineurin-dependent pathway

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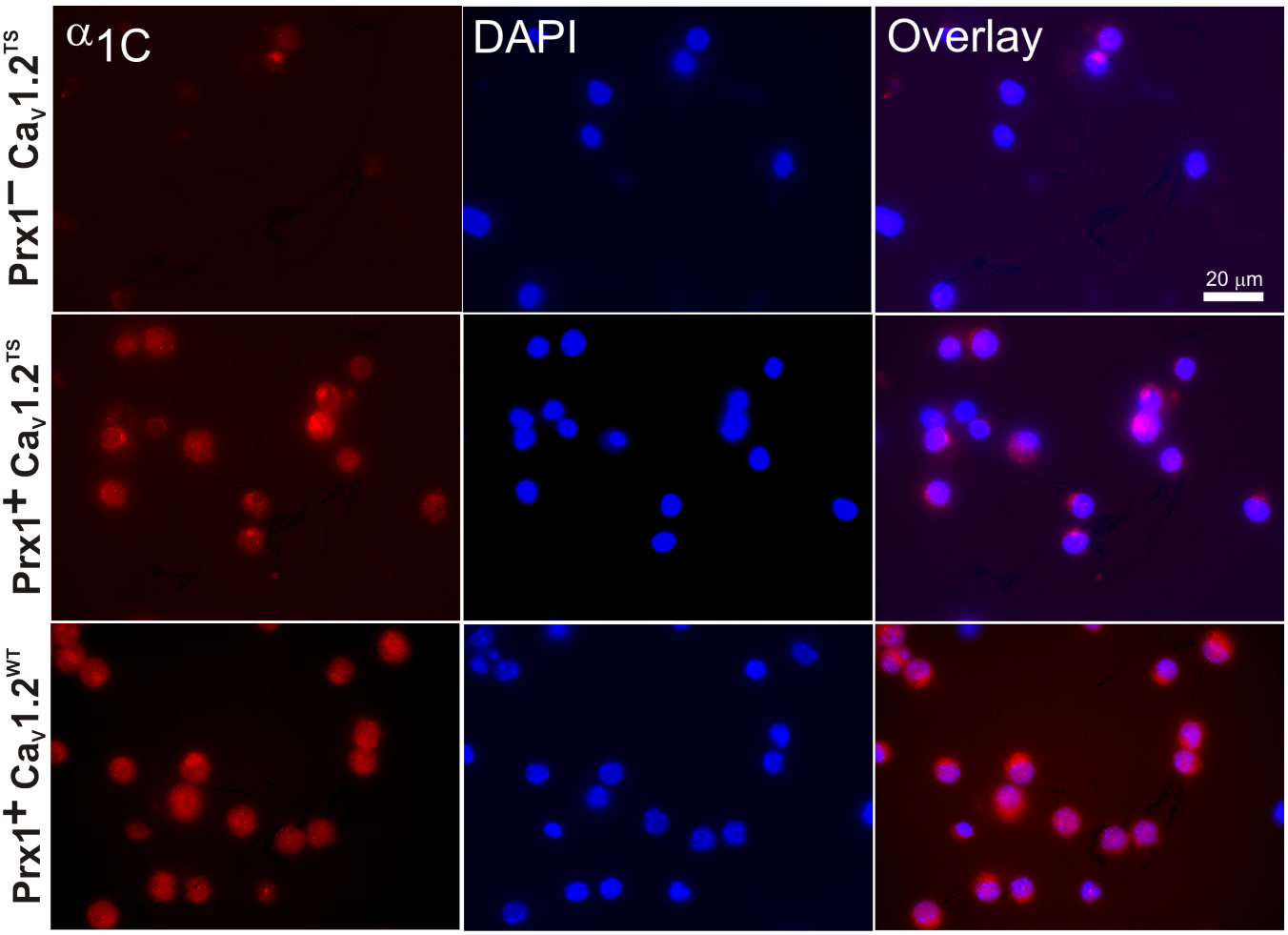
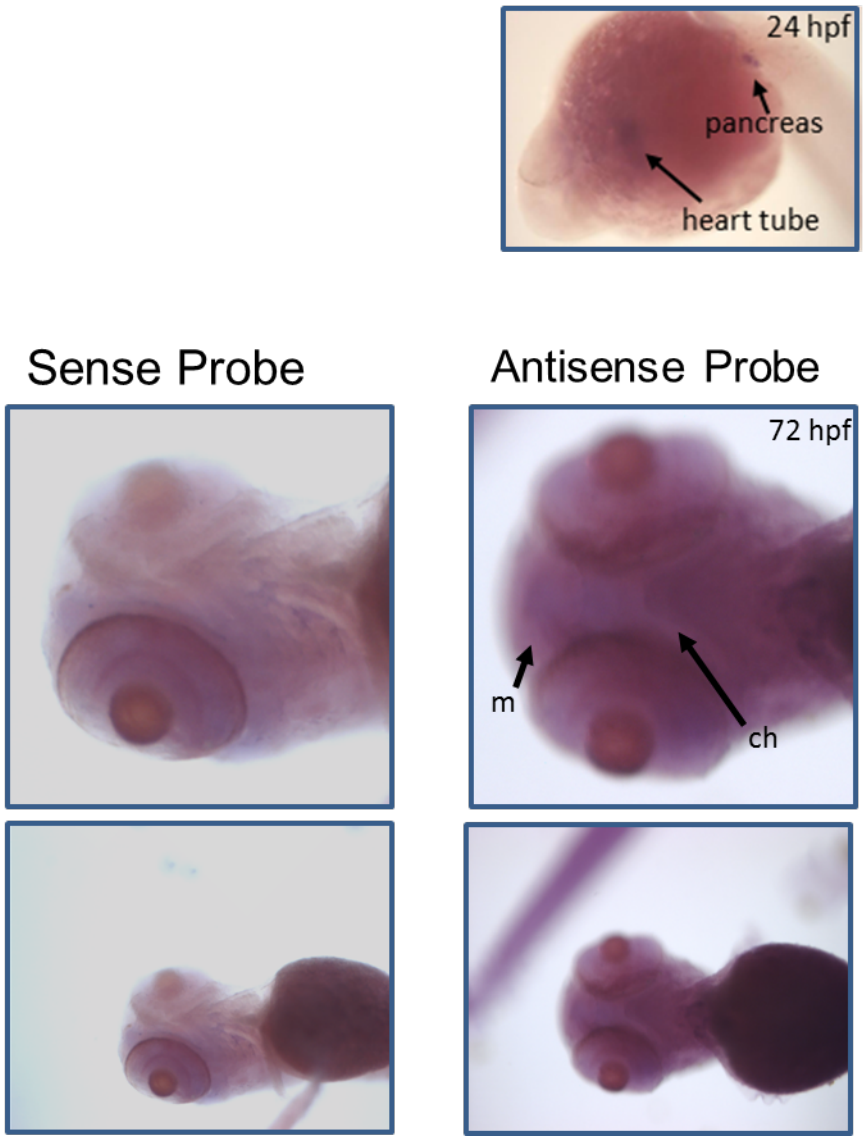
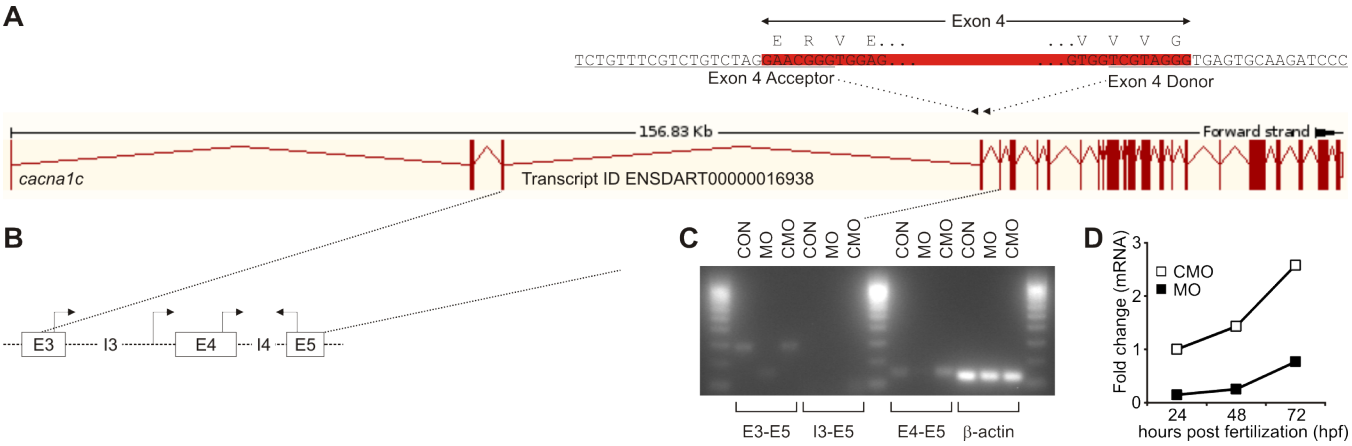


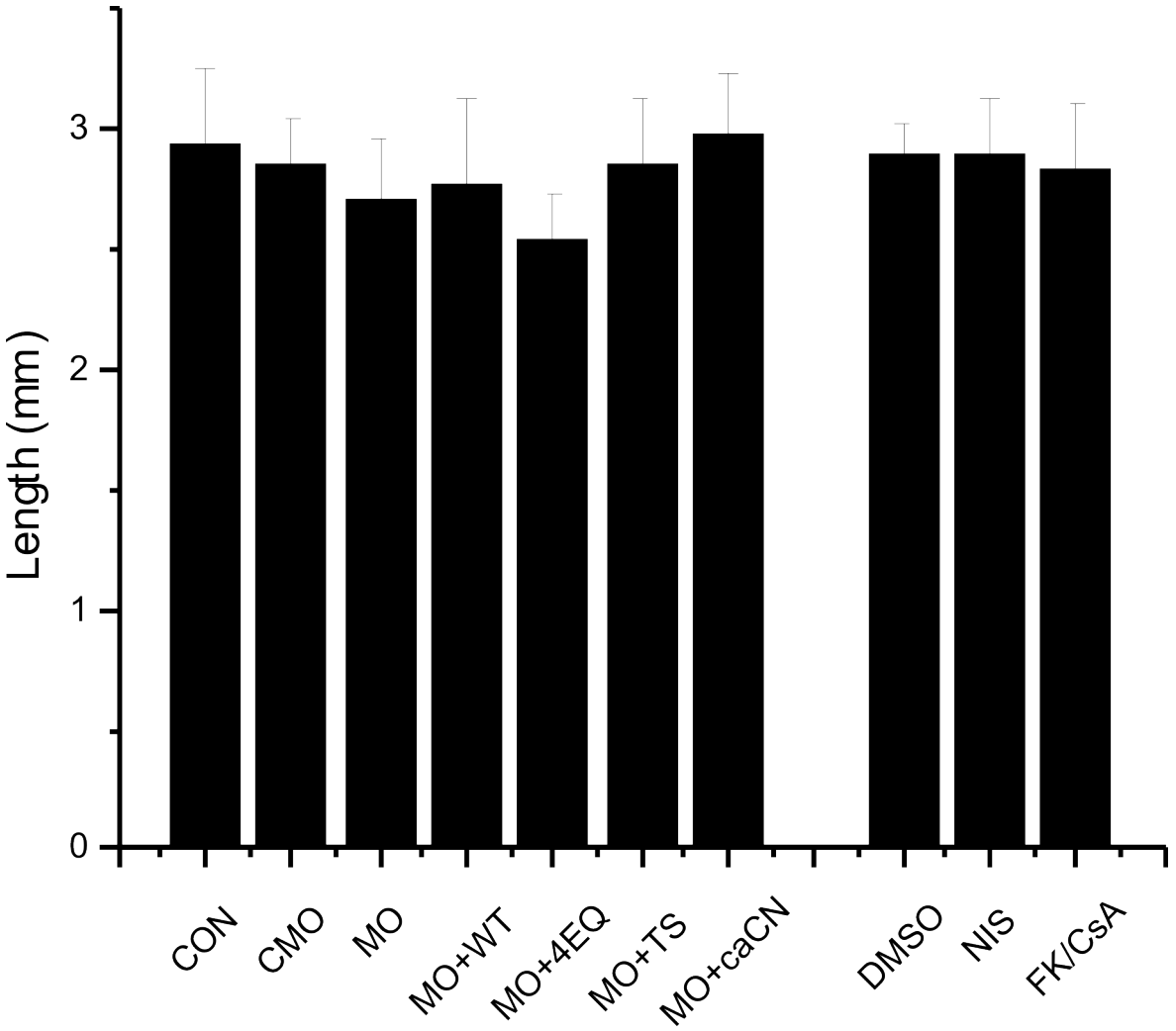
Supplemental Figure 1. ***Immunocytochemistry for α_{1C} on isolated chondrocytes from Prx1^{+}-Ca$_V$1.2^{TS}, Prx1^{+}-Ca$_V$1.2^{WT} and Prx1^{-}-Ca$_V$1.2^{TS}***. Representative images taken by an experimenter blinded to the genotype.



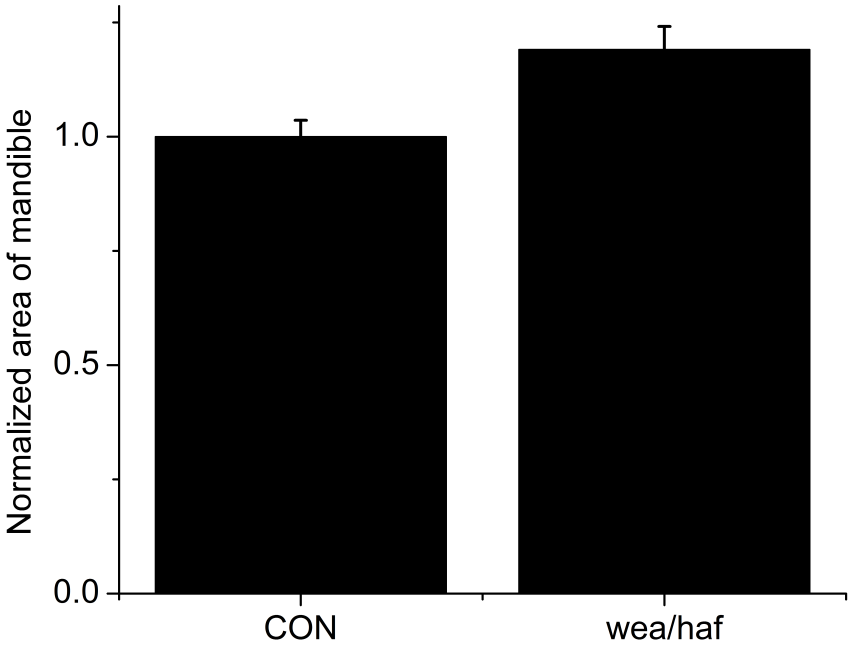
Supplemental Figure 2. *In situ* hybridization with probe against *cacna1c*. Top, antisense probe at 24 hpf shows signal in the heart tube and pancreas, consistent with Rottbauer et al. (1). Bottom, sense and antisense probes at 72 hpf, showing signal in the mandible (m) and the ceratohyal (ch) with the antisense probe only.



Supplemental Figure 3. Knockdown of *cacna1c* ($Ca_V1.2$) and affects mandible formation. *A*, map of the *cacna1c* gene and transcript is shown with the position of the two morpholinos used that target exon 4 (at the exon donor and acceptor sites). The targeted sequences are underlined. For each, in red is shown the exon sequence with the amino acid sequence above. *B*, schematic of the region between exons 3-5 and the intervening introns. The positions of the primers used for PCR are indicated. *C*, RT-PCR (oligo-dT used for cDNA synthesis) on RNA from embryos uninjected (CON), injected with a cocktail of the two $Ca_V1.2$ morpholinos (MO), or injected with a control morpholino (CMO), The primers used were exon 3-exon 5 (E3-E5); intron 3-exon 5 (I3-E5); exon 4-exon 5 (E4-E5), and β-actin. With the knockdown (MO) by the E4 primers, PCR with the E3 and E5 primers produced a 140 bp predicted product consistent with the *absence* of exon 4 and different from the 300 bp product in CMO. *D*, qRT-PCR showing efficacy of $Ca_V1.2$ knockdown.



Supplemental Figure 4. Length measurements of embryos (72 hpf) after treatment with indicated morpholinos and cRNAs or drugs. *N* = 15-32. CON, Uninjected embryos; CMO, control morpholino; MO, $Ca_V1.2$ morpholino cocktail; WT, cRNA for morpholino-resistant $Ca_V1.2^{WT}$; 4EQ, cRNA for morpholino-resistant impermeant $Ca_V1.2$; TS, cRNA for morpholino-resistant $Ca_V1.2^{TS}$; caCN, cRNA for constitutively active calcineurin; NIS, nisoldipine; FK/CSA, FK506 + cyclosporine.



Supplemental Figure 5. Normalized mandible size at 72 hpf (calculated as in Fig. 3) for double heterozygotes (*wea*;*haf*) identified by lack of atrial and ventricular activity at 48 hpf compared to embryos with a normal beating heart at 48 hpf.

Supplemental Figure 6. Normalized mandible size at 72 hpf (calculated as in Fig. 3) for offspring of the $Ca_V1.2$ null mutant *cacna1c*^{m321} displaying the *isl* or beating phenotype at 48 hpf.

Supplemental Movie 1. Non-beating, "*silent*" ventricle phenotype recorded 48 hpf after injection of $Ca_V1.2$ morpholinos.

Supplemental Movie 2. Normal ventricle phenotype recorded 48 hpf after injection of CON morpholino.

Supplemental Movie 3. Non-beating, "*silent*" ventricle phenotype recorded 48 hpf after injection of $Ca_V1.2$ morpholinos and cRNA for caCN.

REFERENCES

1. Rottbauer, W., Baker, K., Wo, Z.G., Mohideen, M.A., Cantiello, H.F., and Fishman, M.C. 2001. Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel alpha1 subunit. *Dev Cell* 1:265-275.