

Fig. S1. SC in *csp*^{-/-} show delays in mitotic progression but exit mitosis with no significant increase in apoptosis

(A) Still images of time-lapse imaging in control (average of 16.67 ± 1.22 min, 6 nuclei, $n=3$ embryos) and *csp*^{-/-} (average of 98.67 ± 4.55 min, 6 nuclei, $n=3$ embryos) embryos injected with *h2b:gfp* (****, $p < 0.0001$). Arrows indicate SC nuclei from the beginning of M phase (time 0) when mitotic rounding takes place until the two nuclei split. Scale bars = 10 μ m. m, minutes.

(B) Acridine orange (AO) staining at 50 hpf in control and *csp*^{-/-} embryos within a defined region of the PLLn and spinal cord. Scale bar = 25 μ m.

(C) Quantification of the number of AO positive cells in control (average of 0.18 ± 0.08 , $n=11$ embryos) and *csp*^{-/-} (average of 0.27 ± 0.09 , $n=11$ embryos) within a defined region of the PLLn (ns, $p=0.72$).

(D) Quantification of the number of AO positive cells in control (average of 0.66 ± 0.33 , $n=6$ embryos) and *csp*^{-/-} (average of 47.83 ± 2.05 , $n=6$ embryos) within a defined region of the spinal cord (****, $p \leq 0.0001$).

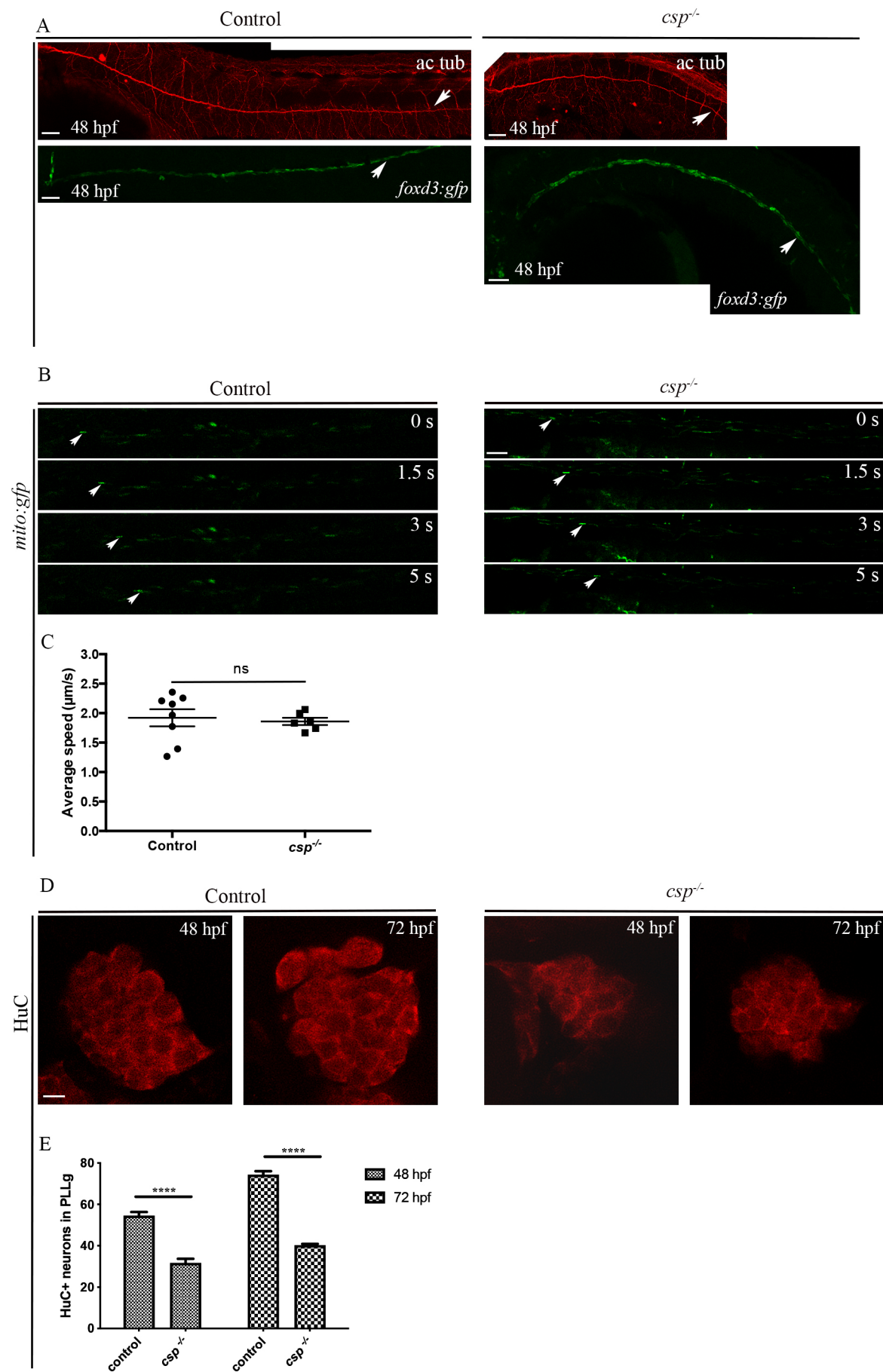


Fig. S2. Sil is not required for axonal growth nor for mitochondrial axonal transport along the PLLn but regulates the number of neurons within the PLLg

(A) Acetylated tubulin expression in a control embryo (n=12) and *csp*^{-/-}-embryo (n=13) at 48 hpf showing the PLLn nerve (arrows). Lateral views of a control (n=16) and *csp*^{-/-}-embryo (n=11) at 48 hpf showing PLLn GFP-expressing SC (arrows). Scale bars = 50 µm.

(B) Still images from time-lapse imaging in control and *csp*^{-/-} embryos injected with *mito:gfp*. Arrows point to the same mitochondria followed through time in control and *csp*^{-/-}. Scale bar = 5 µm. s, seconds.

(C) Quantification of the average speed of mitochondria along the PLLn at 50 hpf in controls (302 mitochondria, n = 8 embryos) and *csp*^{-/-}-embryos (93 mitochondria, n = 6 embryos) (ns, p=0.6976).

(D) HuC immunolabeling of the PLLg at 48 and 72 hpf in control and *csp*^{-/-} embryos. Scale bar = µm.

(E) Quantification of the number of neurons in the PLLg at 48 hpf in control (average of 54.58±5.99 neurons, n = 12) and *csp*^{-/-} (average of 31.71±5.31 neurons, n = 7) embryos and at 72 hpf in control (average of 74.33±5.88 neurons, n = 12) and *csp*^{-/-} (40.29± 1.60 neurons n = 7) embryos (****, p≤ 0.0001 ; ****, p≤ 0.0001).

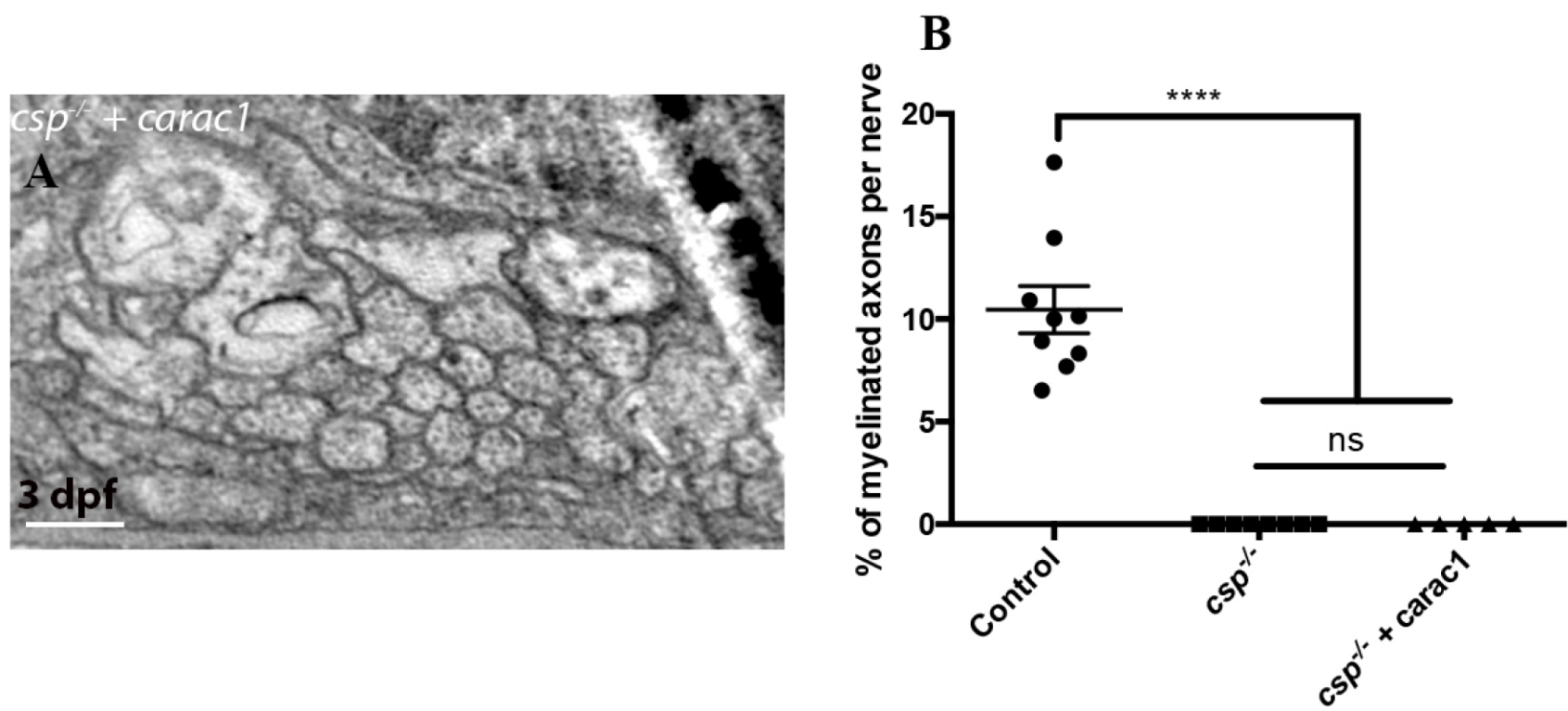
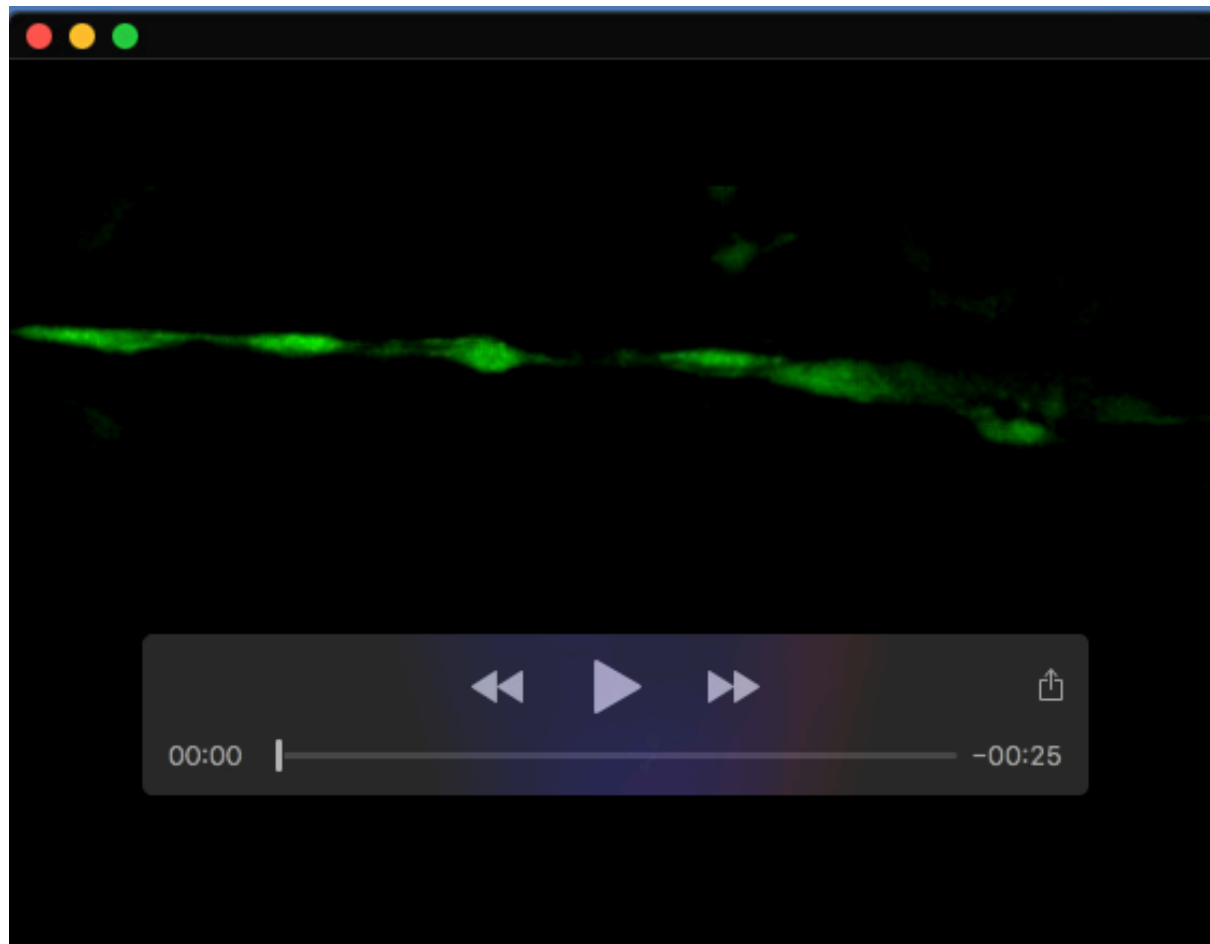


Fig. S3. Forcing Rael activity does not rescue radial sorting and myelination defects in *csp^{-/-}*

(A) TEM of a cross section of the PLLn at 3 dpf in *csp^{-/-}* embryo injected with a constitutive active form of Rael (*carac1*).

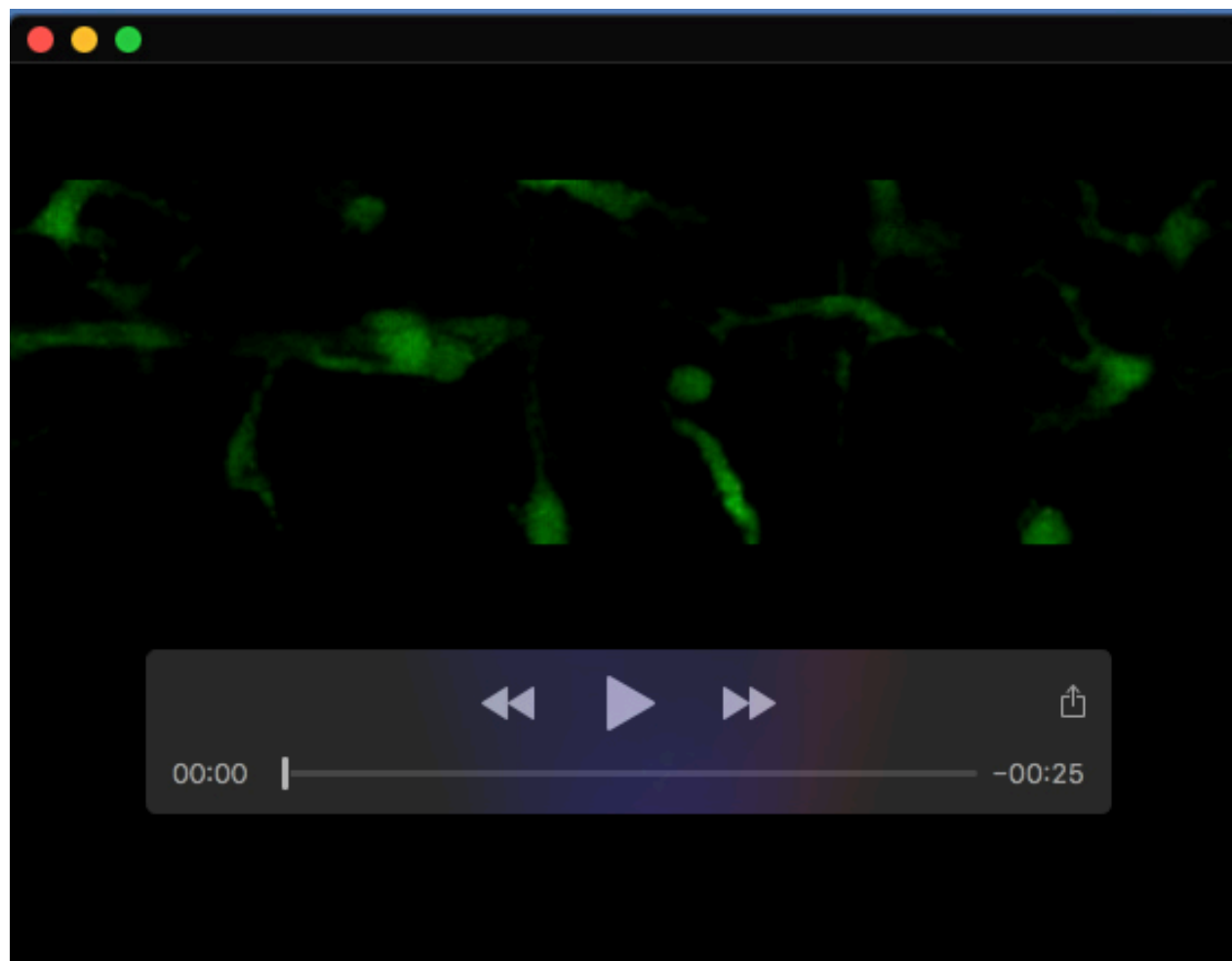
Scale bar 0.5 μ m.

(B) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.5 ± 1.13), *csp^{-/-}* (average of 0) and *csp^{-/-}* injected with *carac1* (average of 0) (****, $p \leq 0.0001$, ns, $p \geq 0.999$).



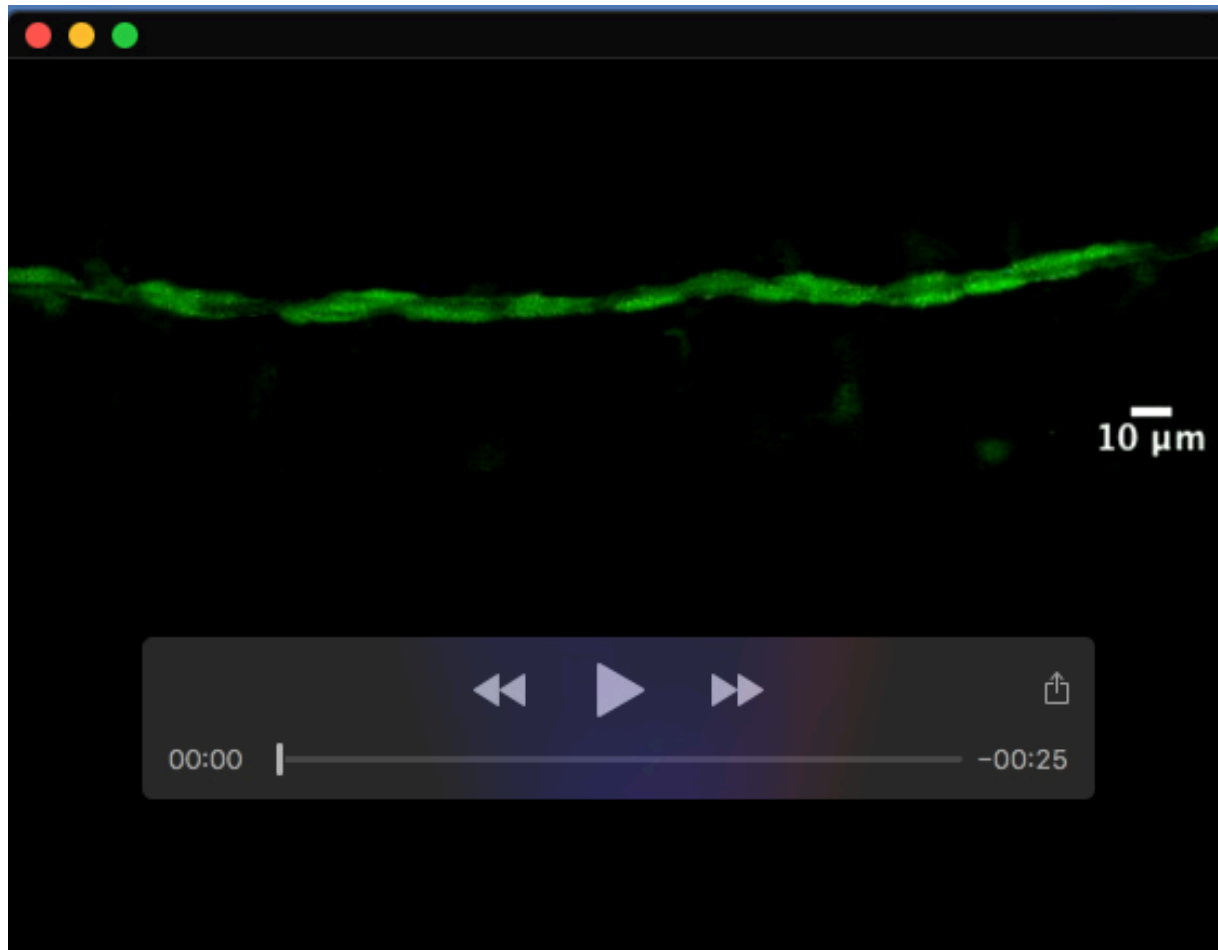
Movie 1. Real-time imaging of SC in *Tg(foxd3:gfp)* embryo at 28 hpf

A 28 hpf embryo expressing GFP in SC; the control embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents two hours of continuous real-time imaging.



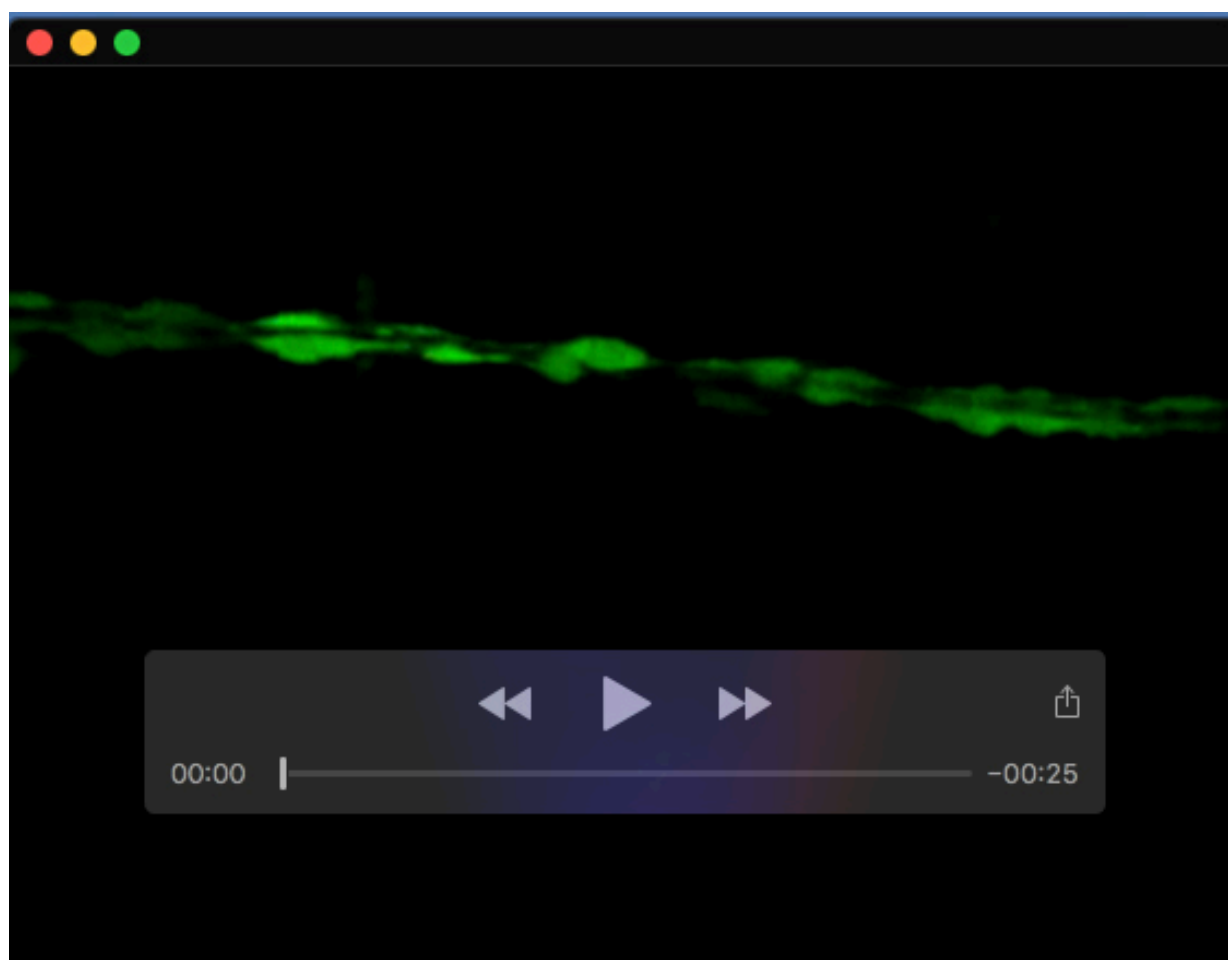
Movie 2. Real-time imaging of SC in *Tg(foxd3:gfp)/csp^{-/-}* embryo at 28 hpf.

A 28 hpf embryo expressing GFP in SC; the *csp^{-/-}* embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four hours of continuous real-time imaging.



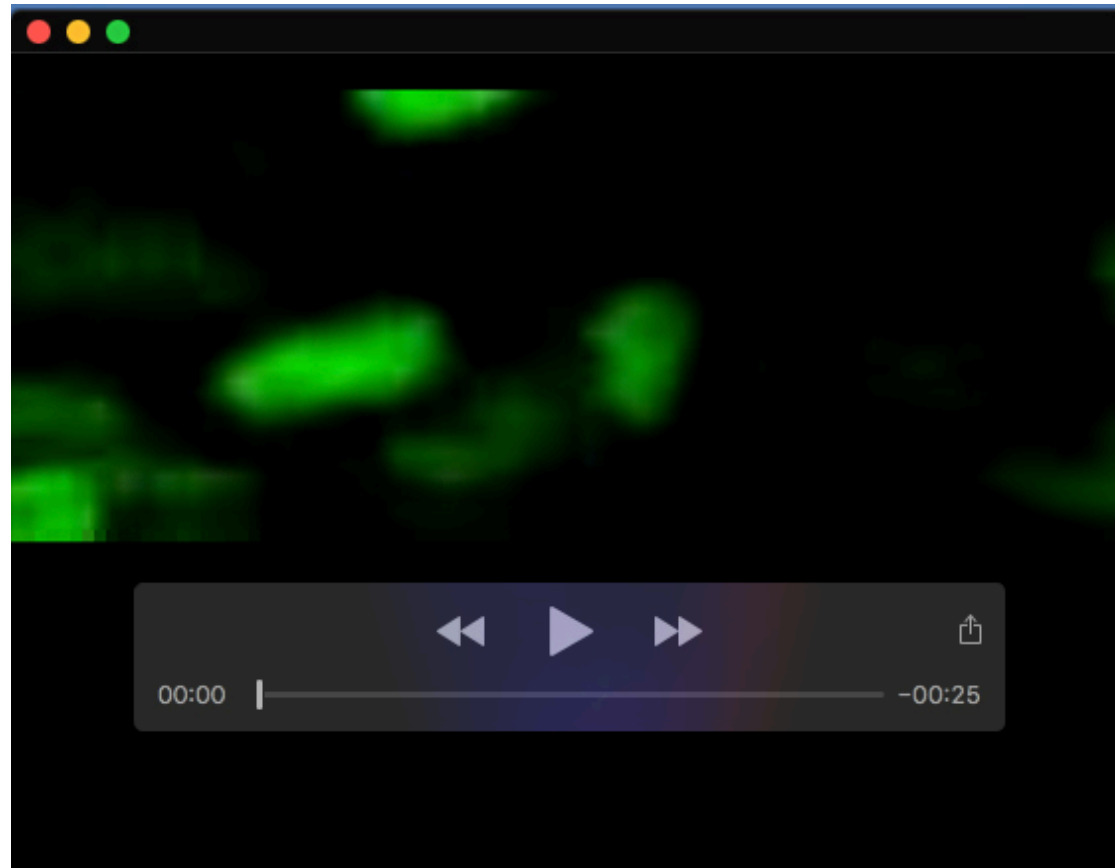
Movie 3. Real-time imaging of SC in *Tg(foxd3:gfp)* embryo at 48 hpf.

A 48 hpf embryo expressing GFP in SC; the control embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four and a half hours of continuous real-time imaging.



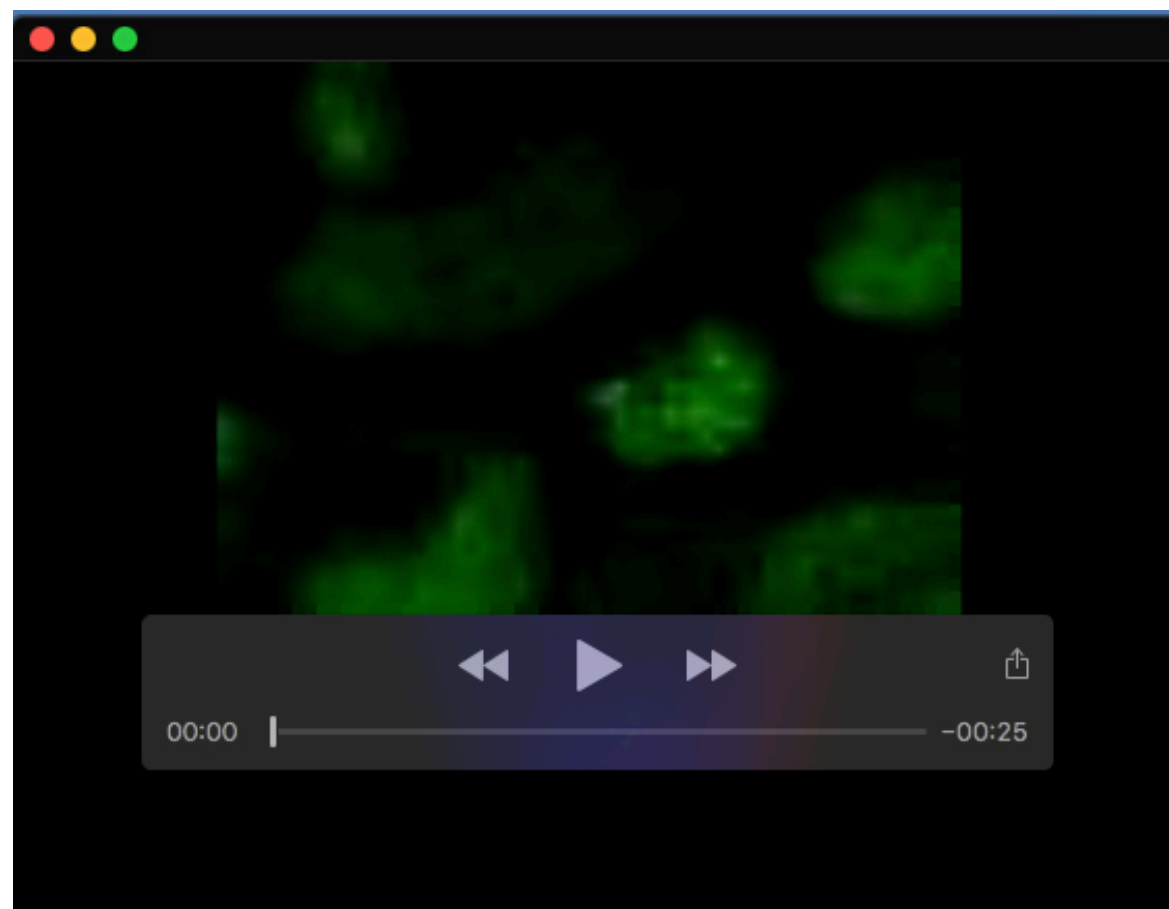
Movie 4. Real-time imaging of SC in *Tg(foxd3:gfp)/csp^{-/-}* embryo at 48 hpf.

A 48 hpf embryo expressing GFP in SC; the *csp^{-/-}* embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four and a half hours of continuous real-time imaging.



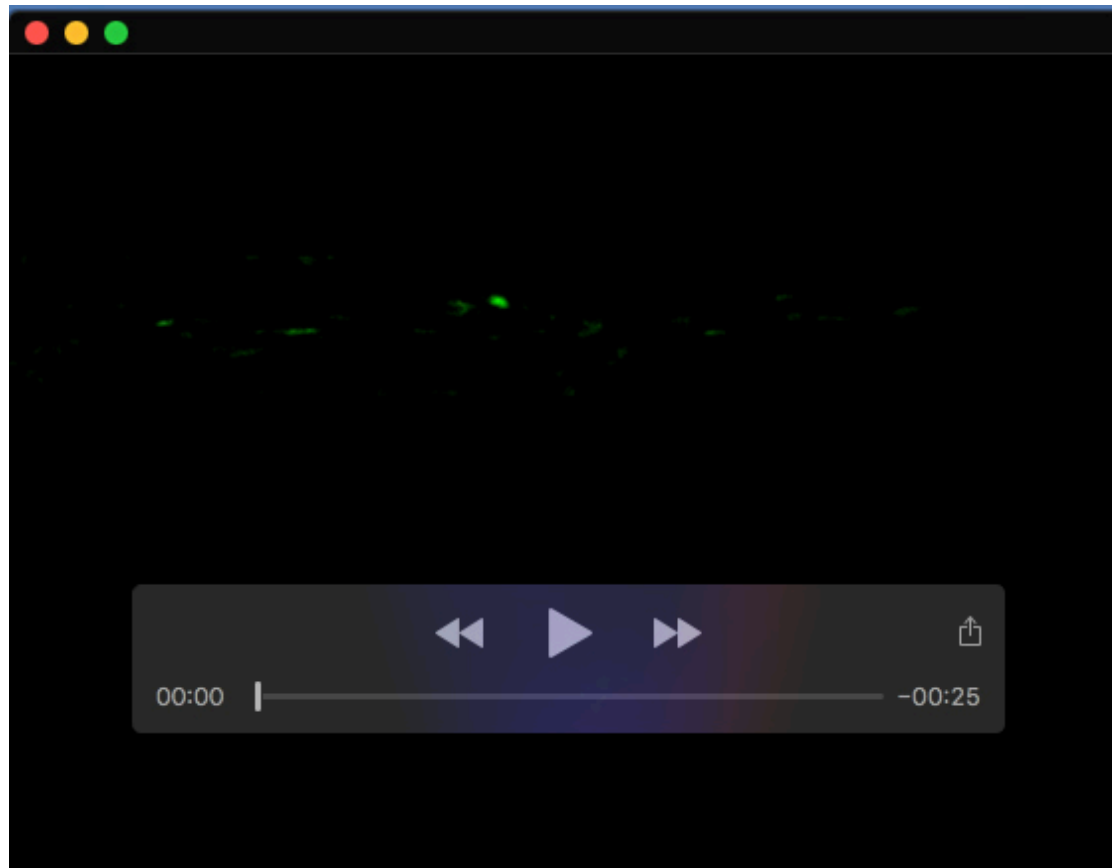
Movie 5. Real-time imaging of SC nuclei in control at 48 hpf.

A 48 hpf control embryo expressing GFP in SC nuclei after *h2b-gfp* mRNA injection; the embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 40 minutes of continuous real-time imaging.



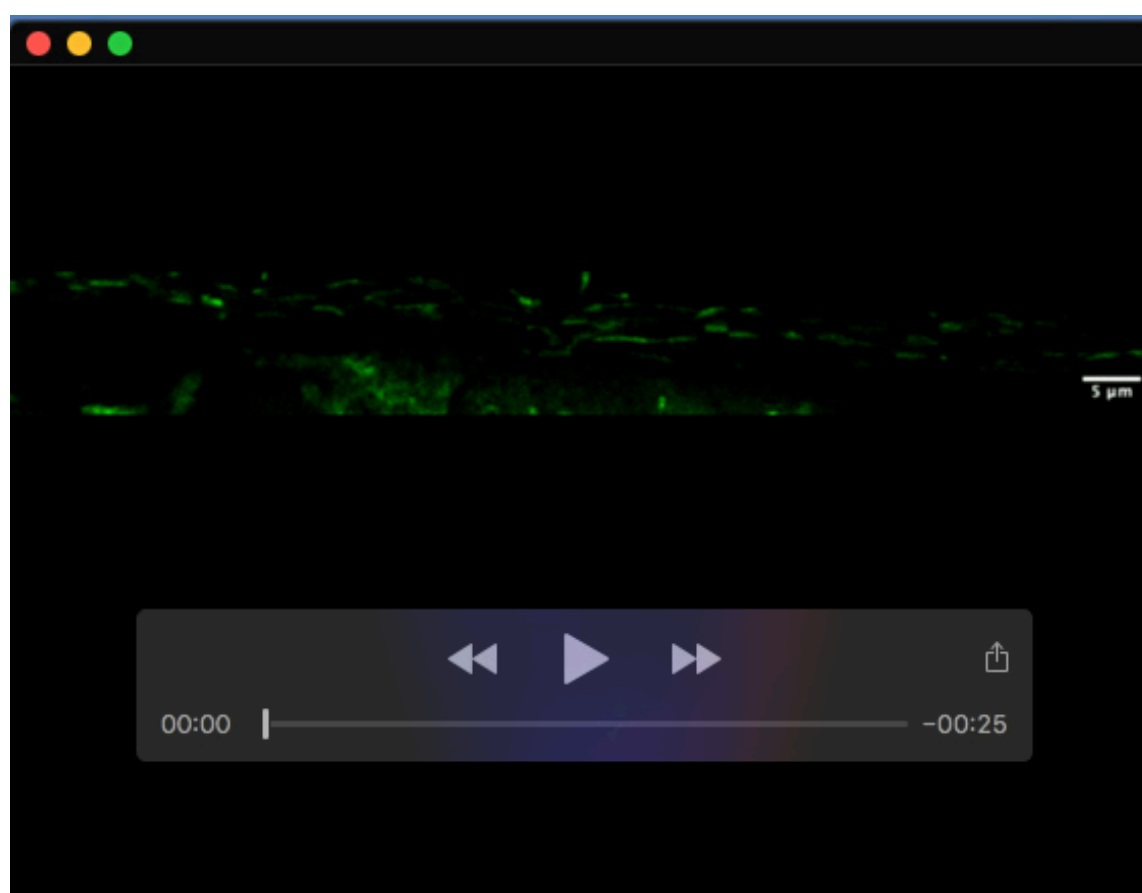
Movie 6. Real-time imaging of SC nuclei in *csp*^{-/-} at 48 hpf.

A 48 hpf *csp*^{-/-} embryo expressing GFP in SC nuclei after *h2b-gfp* mRNA injection; the embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 100 minutes of continuous real-time imaging.



Movie 7. Real-time imaging of mitochondria in a control PLLn at 48 hpf.

A 48 hpf control embryo expressing GFP in mitochondria after *mito-gfp* mRNA injection; the embryo was imaged every 120 milliseconds for several minutes by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 18 seconds of real-time continuous imaging. White arrow highlights an anterograde moving mitochondria while yellow arrow highlights a retrograde moving mitochondria.



Movie 8. Real-time imaging of mitochondria in a *csp*^{-/-} PLLn at 48 hpf.

A 48 hpf *csp*^{-/-} embryo expressing GFP in mitochondria after *mito-gfp* mRNA injection; the embryo was imaged every 120 milliseconds for several minutes by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 36 seconds of continuous real-time imaging. White arrow highlights an anterograde moving mitochondria while yellow arrow highlights a retrograde moving mitochondria.