Expanded View Figures

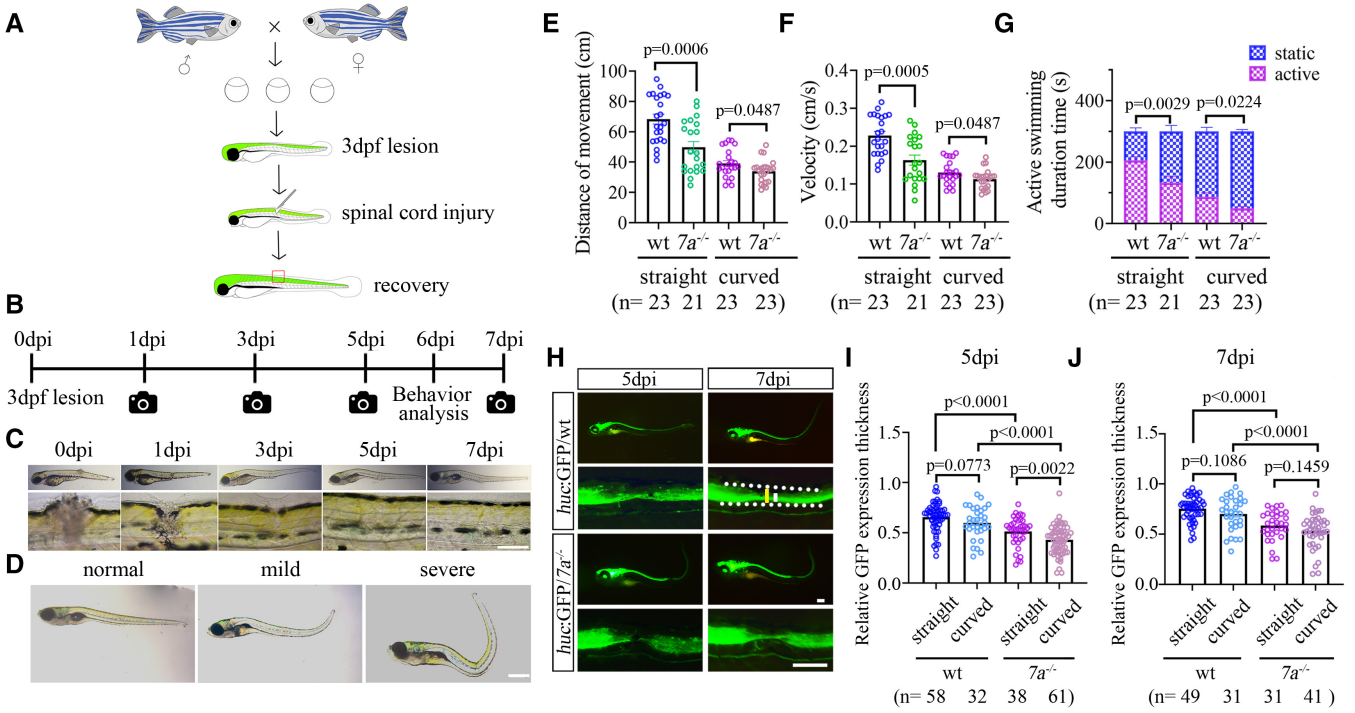

Figure EV1. Strategies of examining spinal cord regeneration after SCI.

A Diagram showing that transgenic zebrafish larva was injured at the trunk spinal cord at 3 dpf and recovered later during development.

B Timeline of imaging and behavior analysis of zebrafish larvae after SCI at 3 dpf.

C Representative images showing the recovery of a wild-type zebrafish larva injured at 3 dpf. Bottom images showing the enlarged views of the lesion sites.

D Representative images showing the external phenotypes of three categories of larvae at 6 dpi.

E Dot plots showing the swimming distance of each larva in different groups as indicated at a duration of 300 s.

F Dot plots showing the swimming velocity of wild-type and mutant larvae as indicated.

G Bar graph showing active swimming time in wild-type and mutant larvae in different groups as indicated at a duration of 300 s.

H Representative images showing GFP expression in wild-type or *rassf7a* mutants carrying Tg(*huc:GFP*) transgene at different time points after SCI. Bottom rows show the magnified views near the lesion sites. The white dashed border represents the entire spinal cord near the lesion sites. Long line in yellow indicates measurement of spinal cord thickness. Short line in white indicates the measurement of GFP fluorescence thickness.

I, J Dot plots showing relative thickness of GFP fluorescence at the lesion sites of wild-type or mutant larvae at 5 dpi (I) and 7 dpi (J) in different groups as indicated.

Data information: *P* values for unpaired Student's *t*-test (E-F, J), unpaired Mann–Whitney test (I) and Two-way ANOVA with Bonferroni's multiple comparisons test (G) are indicated. Data are shown as mean ± S.E.M. Each data point represents an individual fish. Scale bars: 500 μm in (C, D), 250 μm in (H).

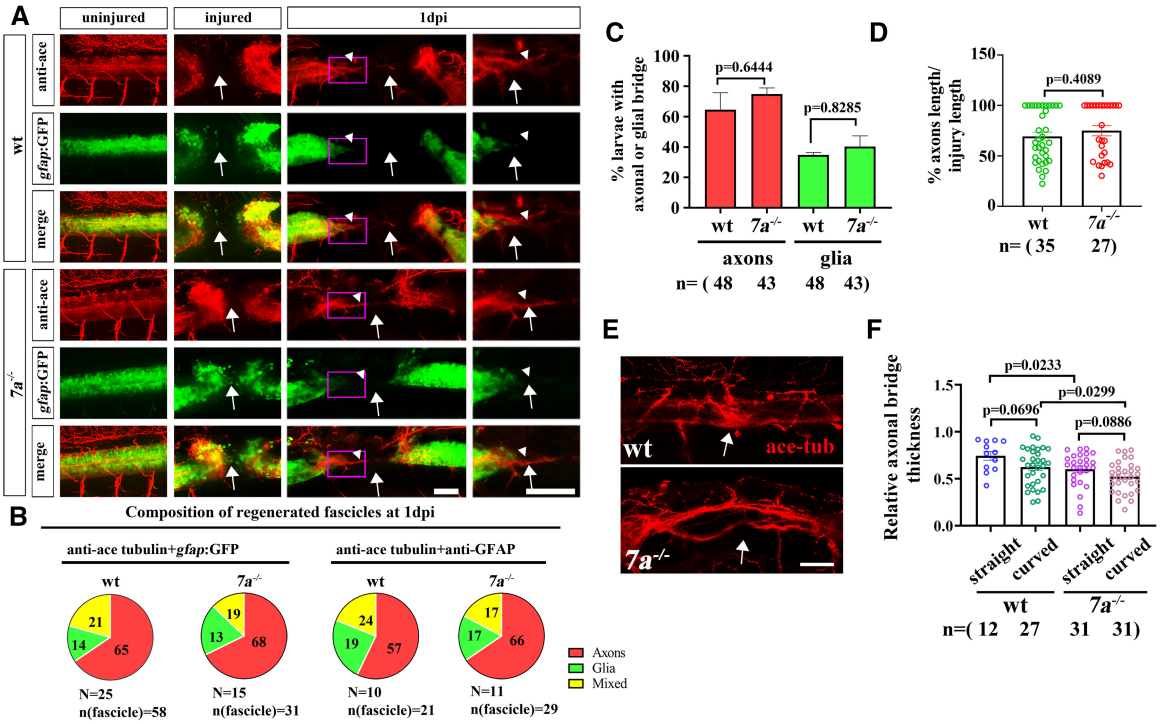

Figure EV2. Axonal regeneration in *rassf7a* mutants after SCI.

A Representative confocal images showing the regrowth of axons (anti-acetylated Tubulin) and astroglia-like processes (*gfap:GFP*) at 1 dpi. These images show examples of axonal-only fascicles (arrowheads). Arrows indicate injury sites. High magnification images of boxed areas are shown on the right.

B Quantification of fascicle composition at 1 dpi of wild-type and mutant larvae. The glial processes were visualized with either GFP transgene or GFAP antibody staining.

C Bar graph showing the percentages of wild-type and *rassf7a* mutant larvae with axonal or glial processes at 1 dpi.

D Relative axonal bridge length of wild-type and *rassf7a* mutants at 1 dpi.

E Representative images showing axonal bridges at 5 dpi in wild-type and *rassf7a* mutants visualized with anti-acetylated Tubulin antibody.

F Dot plots showing relative axonal bridge thickness at the lesion sites of wild-type or mutant larvae at 5 dpi in different groups as indicated.

Data information: *P* values for a Fisher's Exact test (C), unpaired Mann–Whitney test (D) and unpaired Student's *t*-test (F) are indicated. Data are shown as mean ± S.E.M. Each data point represents an individual fish. Scale bars: 50 μm in (A, E).

Figure EV3. Neuronal differentiation defects in *rassf7a* mutants after SCI.

Individual channels of Fig 3A showing the localization of $BrdU^+$ cells (red) near the lesion sites in wild-type and *rassf7a* mutants carrying different transgenes. Arrows represent lesion sites. Scale bars: 20 μm.

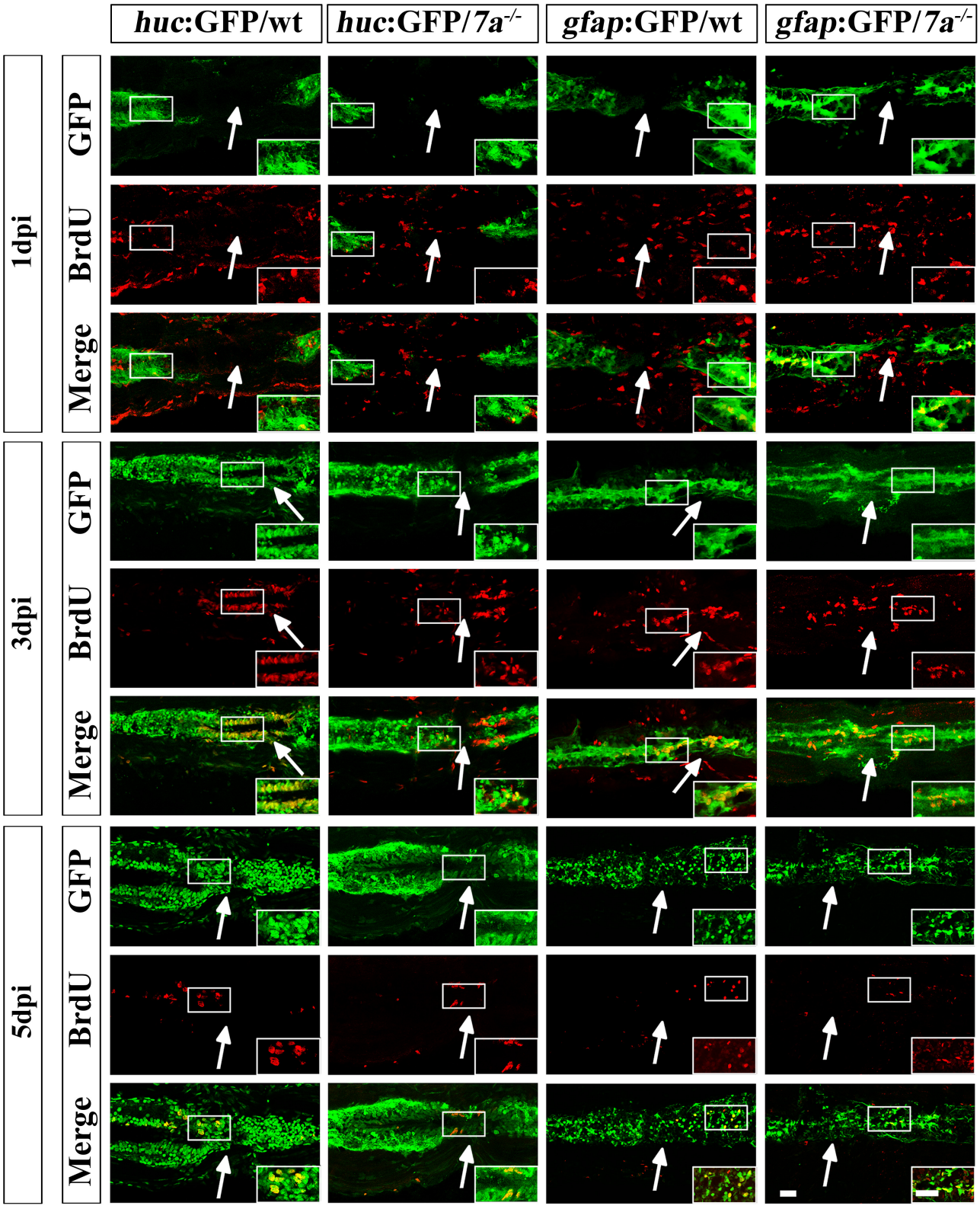

Figure EV3.

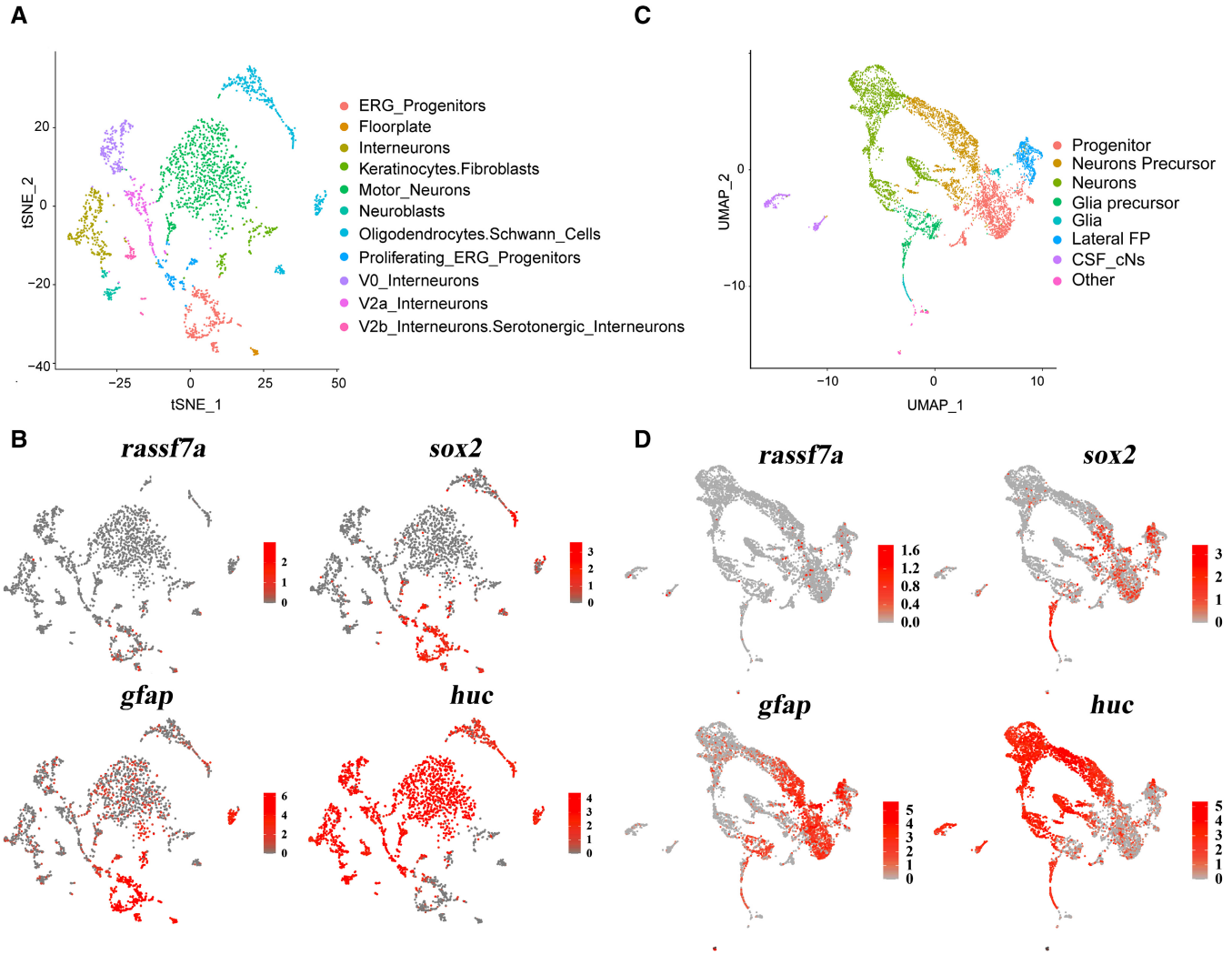

Figure EV4. Expression of *rassf7a* is enriched in the neural progenitor cells.

A A t-distributed Stochastic Neighbor Embedding (tSNE) plot of scRNA-seq dataset (Accession number: SAMEA8658904) from 4 dpf Tg(*her4.3:GFP*) zebrafish larvae.
B Gene expression patterns of *rassf7a*, *sox2*, *gfap* and *huc* on plot from (A). Color codes indicate normalized expression levels from low (gray) to high (red).
C Unsupervised UMAP of integrated scRNA-seq dataset (Accession number: GSE173350) from *olig2:EGFP* spinal cord cells obtained from 24 hpf Tg (*olig2:EGFP*) embryos.
D Gene expression patterns projected onto UMAP plot (from (C)) of *rassf7a*, *sox2*, *gfap* and *huc*.

Figure EV5. Abnormal mitotic spindle rotation of NPCs in *rassf7a* morphants.

A Sequences of time-lapse photographs to illustrate mitotic rotations of a single neural progenitor cell in control or *rassf7a* morphants expressing $Sox2^{+}$-GFP transgene. Anterior is to the left, dorsal is to the up.
B, C Statistical analysis of angles of mitotic spindle rotation in control or *rassf7a* morphants.
D History plots of mitotic spindle orientation in control or *rassf7a* morphants at different time points. Green dashed line indicated 45° spindle rotation.
E Bar graph showing the time of cell division in control or *rassf7a* morphants.

Data information: *P* values for unpaired Mann–Whitney test (B) and unpaired Student's *t*-test (E) are indicated. Data are shown as mean ± S.E.M. Each data point represents a dividing cell. Scale bars: 5 μm in (A).

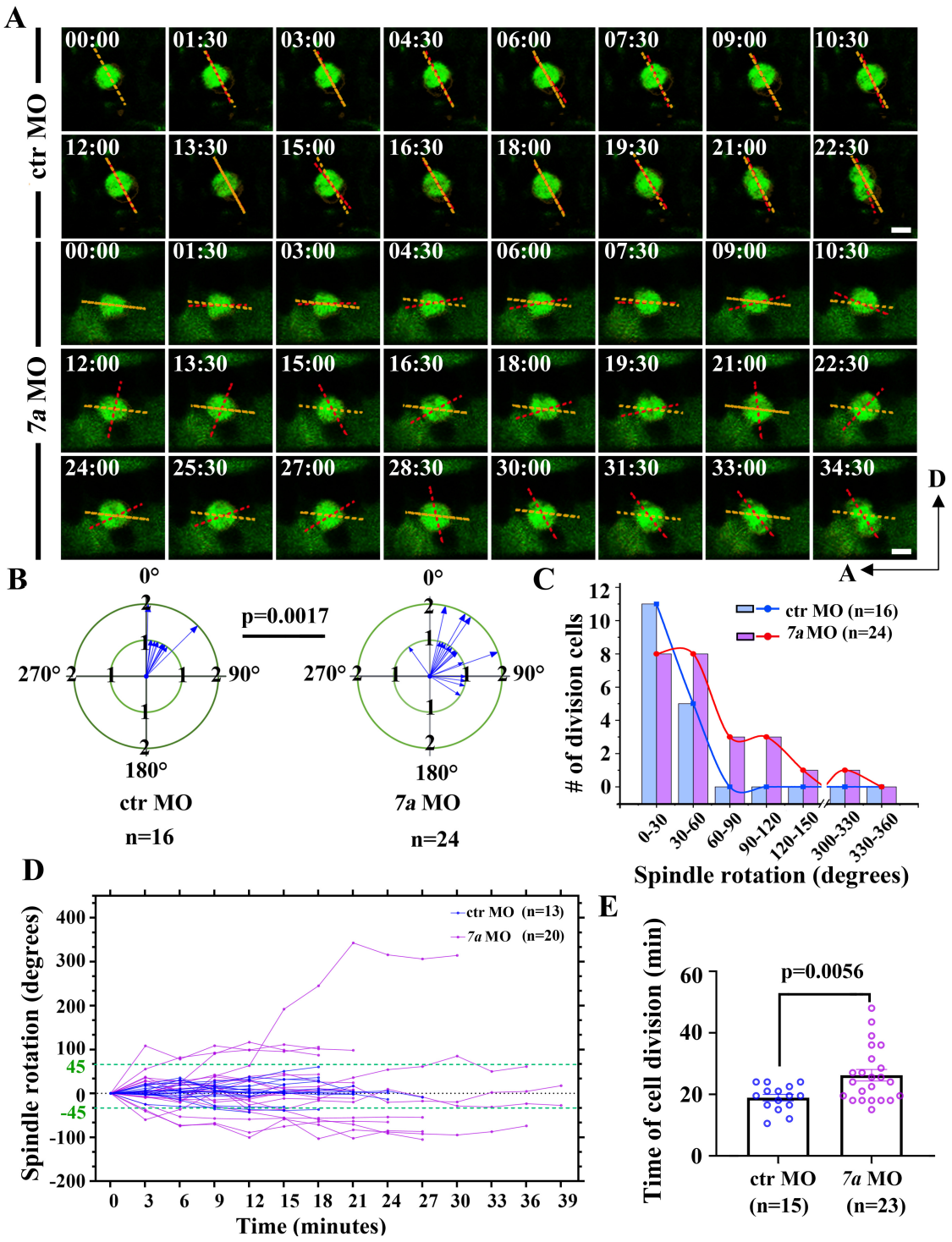

Figure EV5.