SUPPORTING INFORMATION

Optochemical dissection of T-box gene-dependent medial floor plate development

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Supplementary Movie 1. Time-lapse videomicroscopy of Tg(-2.4shha-ABC:GFP) zebrafish injected with either a ntlα MO or a combination of ntlα and spt MOs. Both brightfield and fluorescence images spanning mid-gastrulation to early somitogenesis (7 to 13 hpf) are shown. The movie images were acquired at 10-minute intervals and are shown at approximately 6 frames/second. Scale bar: 200 µm.
Supplementary Figure 1. *ntla* and *spt* MOs recapitulate single and double mutant morphological phenotypes. Tissue morphologies (24 hpf) and Ntla and Spt expression levels (10 hpf) for wildtype (a), *ntla* morphant (b), *spt* morphant (c) and *ntla; spt* morphant embryos. Embryo orientations: brightfield micrographs, lateral view and anterior left; fluorescence micrographs, dorsal view and anterior up. Scale bars: 200 µm.
Supplementary Figure 2. *ntla* and *spt* MOs recapitulate posterior MFP defects. (a) Tg(-2.4shha-ABC:GFP) embryos injected with a *ntla* MO, a *spt* MO, or a *ntla* MO/spt MO mixture. (b) Wildtype embryos injected with the designated MOs and then stained for expression of the MFP marker shhb. 24-hpf embryos are shown in the following orientations: left panels, lateral view and anterior left; right panels, dorsal view and anterior left. Scale bars: 200 µm.
Supplementary Figure 3. Hh signaling is maintained and ectopically activated in *ntla; spt* morphants. Expression of the Hh target gene *ptch2* in wildtype embryos (a) and those injected with *ntla* and *spt* MOs (b). 10-hpf embryos are shown in dorsal view, anterior up. Scale bar: 200 µm.
Supplementary Figure 4. Spatiotemporal control of Spt protein expression with a spt cMO. (a-b) Zebrafish embryos were injected with a spt cMO, either alone or in combination with a ntl a MO, and globally irradiated at the specified times. The embryos were then fixed at 13 hpf, and Spt protein was detected by immunofluorescence. (c) Localized irradiation of a 6-hpf embryo that was previously injected with caged fluorescein-conjugated dextran (cFD). A 100-µm-diameter region within the ventral margin was targeted, and the opposing embryonic shield is labeled by the arrowhead. (d) Regiospecific Spt protein knockdown in an embryo co-injected with cFD and the spt cMO, irradiated as shown in (c), and then immunostained at 10-hpf for Spt and fluorescein. Arrowheads designate the region of co-localized Spt knockdown and FD uncaging, and an embryo injected with cFD alone is shown as a comparison control. Embryo orientations: (a-b and d), posterior view and dorsal up; (c, top panel), ventral view and animal pole up; (c, bottom panel), animal pole view and dorsal up. Scale bars: 200 µm.
Supplementary Figure 5. Spt turnover upon global spt cMO photoactivation. Zebrafish embryos were co-injected with a ntlia MO/spt cMO mixture and half were irradiated globally at 6 hpf. The embryos were then fixed at the designated time points and immunostained for Spt protein. Scale bar: 400 µm.
Supplementary Figure 6. Midline morphogenetic defects in *ntla*spt morphants. Zebrafish embryos were injected with either caged fluorescein-conjugated dextran (cFD) alone or a cFD/ntla MO/spt MO mixture, and a 100-µm-diameter circular region within the shield was irradiated at 6 hpf. The embryos were then fixed at 10 hpf and immunostained for Ntla and fluorescein to label the midline and shield-derived cells, respectively. Embryo orientations: dorsal view and anterior up. Scale bar: 200 µm.