Supplemental Figures

Fig. S1. Expression pattern of *scxa* and Tg(*scxa:mcherry*). (**A-C'**) *scxa* expression at 32hpf, 48hpf, and 72hpf in wild-type embryos. (**D-F'**) *mcherry* expression at 32hpf, 48hpf, and 72hpf in Tg(*scxa:mcherry*) embryos. Black arrow, *scxa* or *mcherry* expression in the pharyngeal arches. Lateral (**A-F**) and ventral (**A'-F'**) view, anterior to the left.

FIGURE S1.

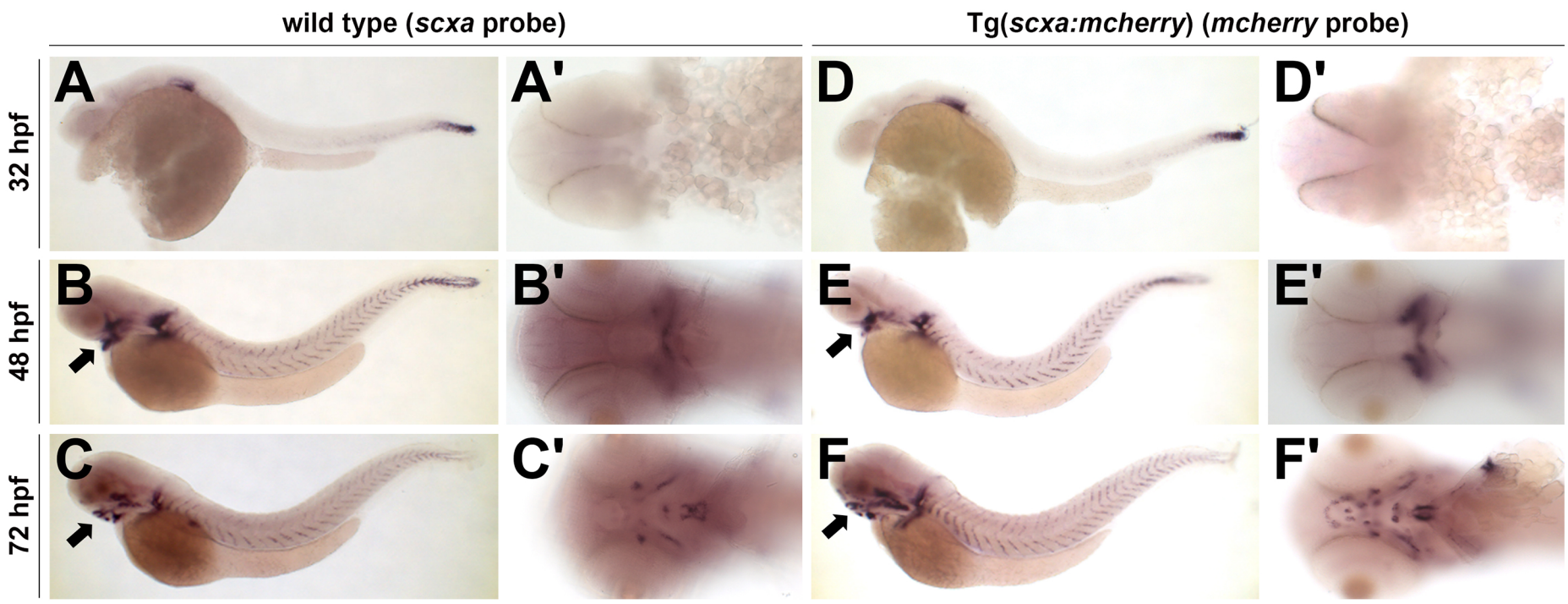


Fig. S2. Effect of statin treatment on zebrafish tendon development. (**A-F**) *scxa* expression at 56hpf upon chemical incubation from 32-56hpf. *scxa* is expressed in the ventral jaw region (arrow) of DMSO-treated embryos (**A**) and expression lost in SU5402-treated embryos (**B**), controls in the chemical screen. Lovastatin (**C**) and simvastatin (**D**), positive hits identified from the screen, caused expansion of *scxa* in the ventral craniofacial region (arrow) compared with controls. Commercially available statins that differ in their pharmacokinetics, atorvastatin (**E**) and fluvastatin (**F**), caused similar expansion of *scxa* in the ventral craniofacial region (arrow) compared with controls. (**G-L**) *scxa* expression at 56hpf upon chemical incubation from 32-56 hpf. Atorvastatin (**I-L**) caused a dose-dependent expansion of *scxa* in the ventral craniofacial region (arrow) compared with controls (**G, H**). (**M-P**) *scxa* expression at 48hpf and 56hpf upon chemical incubation from 32-48 hpf. Fluvastatin caused an expansion of *scxa* in the ventral craniofacial region (arrow) at 56hpf compared with control. (**Q, R**) *scxa* expression is not expanded at 56 hpf upon chemical incubation from 48-56 hpf. (**S-U**) *runx2a* expression at 56hpf and 72hpf upon chemical incubation from 32-56 hpf and 48-72 hpf, respectively. Atorvastatin affected the morphology of the *runx2a* expression domain at 56 hpf. At 72 hpf, treatment caused a significant reduction of *runx2a* expression in the ventral craniofacial region compared with control. (**W, X**) Pectoral fin *col1a2* expression at 74hpf upon chemical incubation from 32-56 hpf. (**Y**) Quantification of myoseptal *scxa:mcherry*$_{+}$ cells at 56 hpf and 72 hpf upon chemical incubation from 32-56 hpf and 48-72 hpf, respectively. Atorvastatin does not increase the quantity of myoseptal *scxa:mcherry*$_{+}$ cells at 56hpf or 72hpf compared with controls. (**Z-C'**) Myoseptal expression of Tg(*scxa:mcherry*) at 56hpf and 72hpf upon chemical incubation from 32-56hpf and 48-72hpf, respectively. (**D', E'**) Myoseptal expression of *col1a2* at 74hpf upon chemical incubation from 32-56hpf. Atorvastatin caused a reduction in myoseptal expression of *col1a2* compared with controls. For cell counts (**Y**), red bars indicate mean; points represent individual embryos; Mann-Whitney-Wilcoxon test. N.S, no significance. Ventral (**A-F, H, J, L, M-V**) and lateral (**G, I, K, W, X, Z-E'**) views of flat-mounted embryos, anterior to the left.

FIGURE S2.

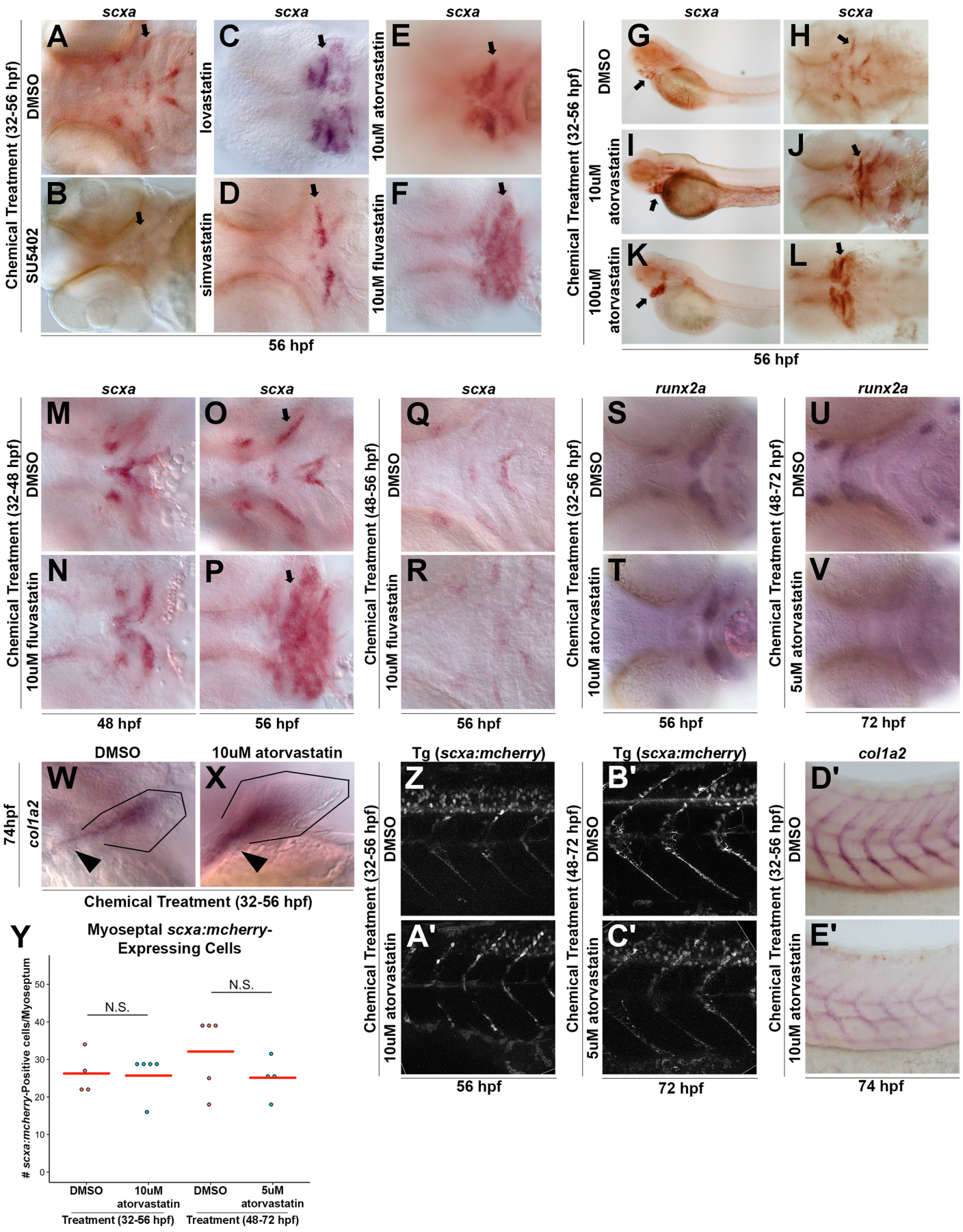


Fig. S3. Analysis of proliferation in statin-treated pectoral fin and craniofacial *scxa*-positive tendon progenitors. (**A**) Quantification of craniofacial cells expressing PH3 at 56hpf upon chemical incubation from 32-56hpf. (**B-D**) Quantification of pectoral fin cells expressing *scxa*, PH3, and co-expressing PH3 and *scxa* at 56hpf upon chemical incubation from 32-56hpf, as assessed from confocal images of embryos processed by fluorescent *in situ* hybridization and immunohistochemistry, and counterstained with Hoechst33342. (**E-H**) Craniofacial expression of *scxa* at 56hpf upon chemical incubation from 32-56hpf. *scxa* expression, compared with controls (**E**, arrow), is reduced in embryos treated with Aphidicolin (**F**, arrow**)**, and expanded in embryos treated with atorvastatin alone (**G**, arrow) and in combination with Aphidicolin (**H**, arrow). (**I**) Experimental design for counting *scxa*- and *col1a2*-expressing cells (Fig. 1L, 1N, 2I, 5O, S3B), *scxa:mcherry*-expressing cells (Fig. 1M, S2Y), PH3-positive cells (Fig. S3A, S3C), and PH3 and *scxa* co-expressing cells (Fig. 2H, S3D). (**J-K**) Example of quantification of *scxa:mcherry*-expressing cells in the pectoral fin at 56hpf upon chemical incubation from 32-56hpf, corresponding to (Fig. 1F-G). (**L-Q**) Examples of quantification of *scxa*- and PH3-expressing cells in the craniofacial region at 56hpf upon chemical incubation from 32-56hpf, corresponding to (Fig. 2D-H). For cell counts (**A-D**), red bars indicate mean; points represent individual embryos; Mann-Whitney-Wilcoxon test. N.S, no significance; ***, P<0.001; ****, P<0.0001. MIP, maximum intensity projection.

FIGURE S3.

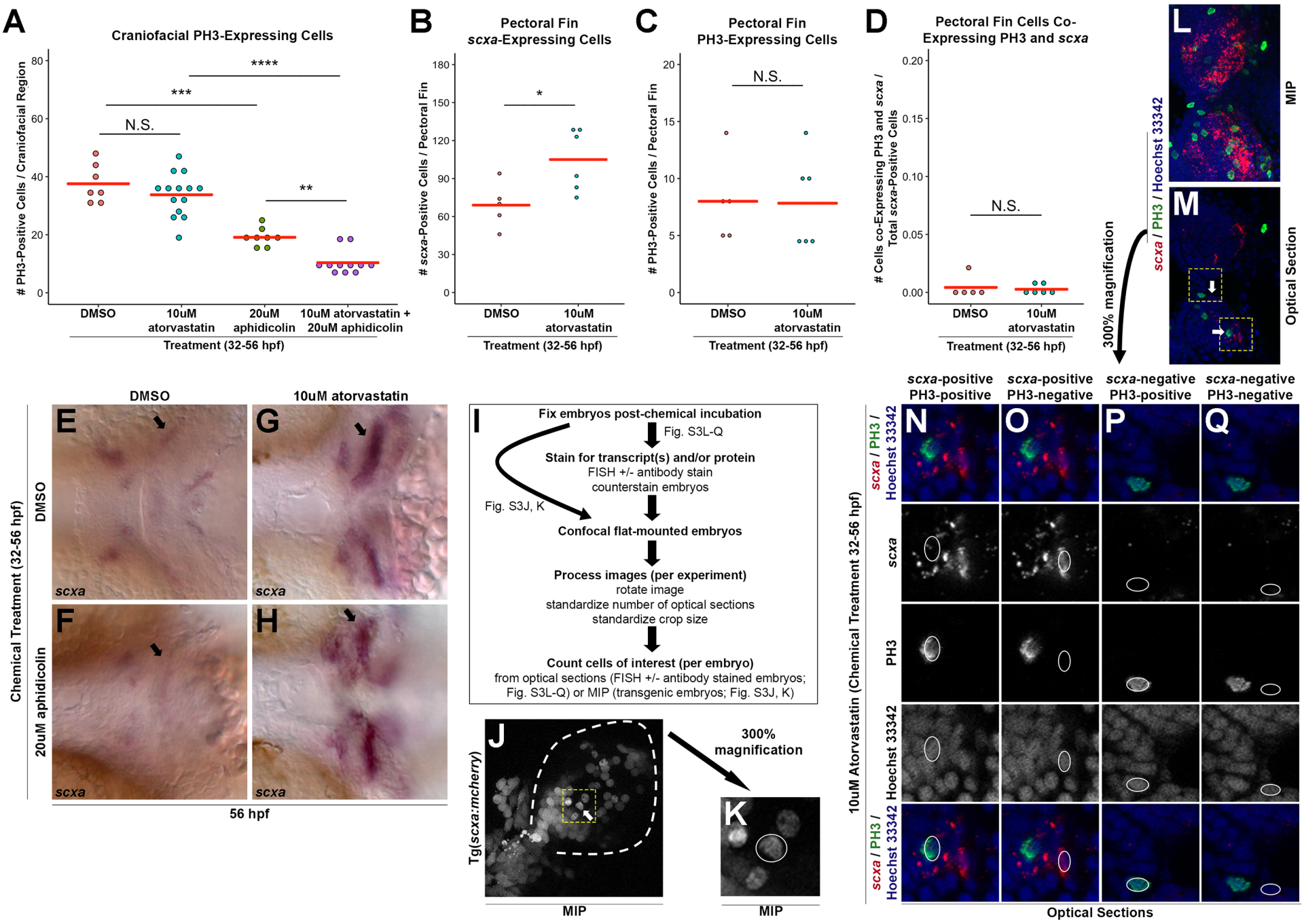


Fig. S4. Effect of statin on pectoral fin musculoskeleton and CNC origin of expanded craniofacial *scxa* cells. (**A-H**) Expression of *sox9a*, *col2a1*, *sox10:eGFP*, and *myod1* at 56hpf i the pectoral fin upon chemical incubation from 32-56hpf. At 56hpf, atorvastatin caused a s alteration in the expression of *sox9a* (**A, B**), *col2a1* (**C, D**), *sox10:eGFP* (**E, F**), and *myod1* **H**). **(I-L)** Expression of Kaede and *scxa* in Tg(*sox10:kaede*) embryos at 56hpf upon chemi incubation from 32-56hpf. Co-localization of Kaede and *scxa* at 56hpf is observed in virtua *scxa*+ domains of DMSO- and atorvastatin-treated embryos (white arrow). Yellow-colored boxe mark the craniofacial domain in the maximum-intensity projection (**I, K**) that is magnified i corresponding optical section (**J, L**). Ventral (**I-L**) and lateral (**A-H**) views of flat-mounted embryos, anterior to the left. MIP, maximum intensity projection.

FIGURE S4.

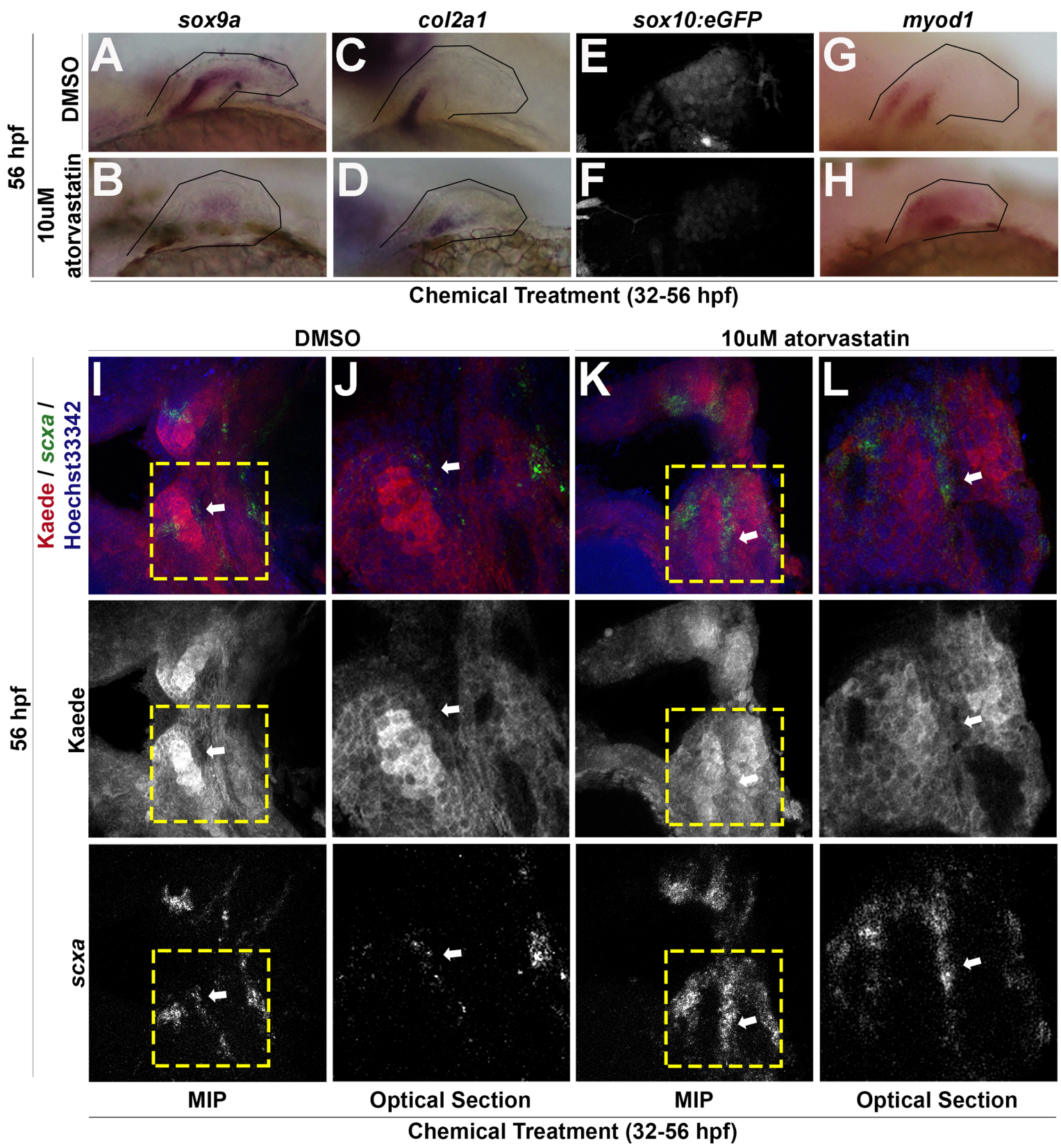


Fig. S5. Effect of statin on pharyngeal arch patterning. Craniofacial expression of markers of craniofacial patterning and associated with characterized signaling pathways. (**A-P**) Expression at 48hpf upon chemical incubation from 32-48hpf. (**A, B**) Atorvastatin caused expansion of *scxa* expression compared with controls. Atorvastatin did not drastically alter expression of (**C-F**) anterior-posterior (*hoxa2b*, *hoxb2a*) or (**G-J**) dorsal-ventral (*hand2*, *bapx1*) arch polarity markers compared with controls. Atorvastatin did not cause expansion in expression of markers associated with (**K-M**) Hedgehog (*shha, patched1*) and (**N**) Bmp (*bmp4*) signaling, compared with controls. (**O-R**) Craniofacial expression upon chemical incubation starting at 32hpf. Atorvastatin did not cause expansion in expression of markers associated with TGFβ (*tgfbr2a, tgfbi*) signaling at (**O, P**) 48hpf or (**Q**) 56hpf compared with controls. (**R**) Atorvastatin caused a reduction in *gsc* expression compared with controls at 56hpf, likely due to developmental delay, but its spatial expression in arch 1-2 was appropriate. (**S-X**) Craniofacial expression at 48hpf upon chemical incubation from 32-48hpf. Atorvastatin did not cause expansion in expression of markers associated with (**S, T**) FGF (*pea3*) or (**U-X**) Wnt (*fzd7a*, *fzd7b*) signaling compared with controls. (**Y-B'**) Expression of Tg(*6xTcf/LefBS-miniP:d2eGFP*) at 56hpf and 72hpf upon chemical incubation from 32-56hpf and 48-72hpf, respectively. Atorvastatin did not cause expansion of the *d2eGFP* reporter of Wnt/β-catenin-mediated Tcf transcriptional activity at 56hpf or 72hpf compared with controls. Ventral (**B, D, F, H, J, L, Q, R, T, V, X-Z, A', B'**) and lateral (**A, C, E, G, I, K, M-P, S, U, W**) views of flat-mounted embryos, anterior to the left.

FIGURE S5.

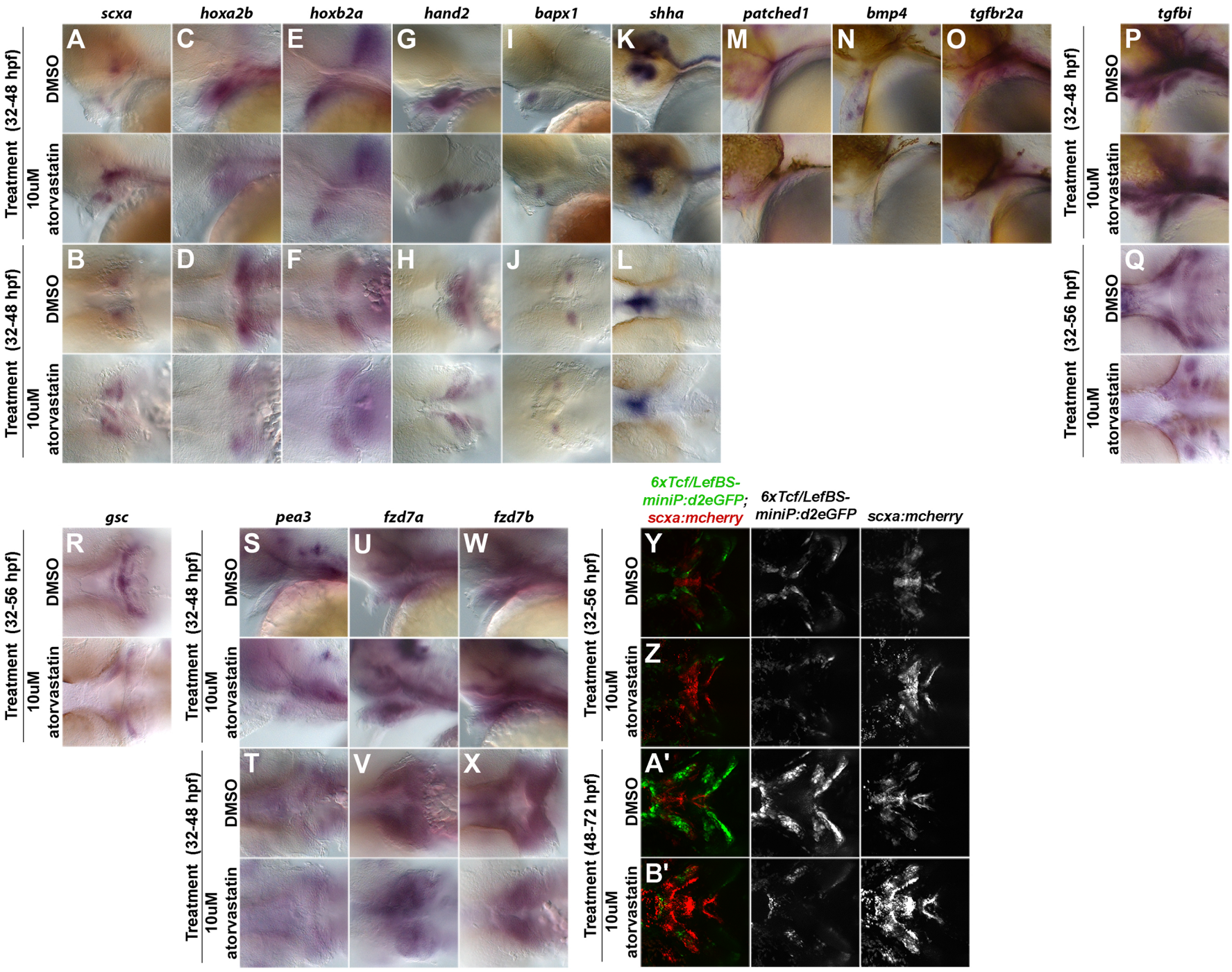
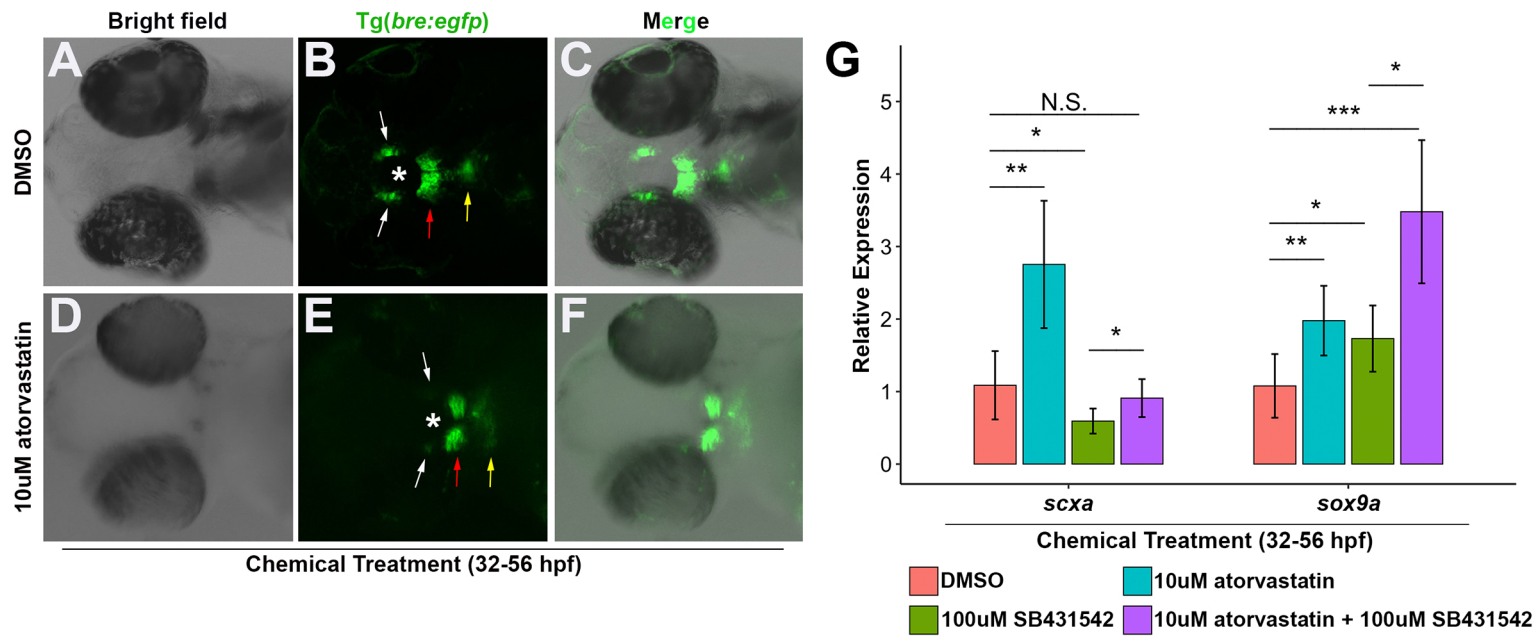


Fig. S6. Analysis of BMP and TGFβ signaling in the craniofacial region of statin treated embryos. (**A-F**) *egfp* expression in Tg(*bre:egfp*) embryos at 56hpf upon chemical incubation from 32-56hpf. Atorvastatin did not expand expression of the BMP reporter line Tg(*bre:egfp*) but altered spatial expression of *egfp* around the mouth and posterior pharyngeal arch region, compared to controls. White asterisk, presumptive mouth opening; white arrow, maxillary process; red arrow, mandibular process; yellow arrow, posterior pharyngeal arch region. (**G**) Inhibition of TGFβ signaling (SB431542) reduced *scxa* and increased *sox9a* at 56hpf upon 32-56hpf incubation, compared with DMSO controls. The combination of statin treatment and inhibition of TGFβ signaling amplified the increase in *sox9a* expression and rescued *scxa* expression to levels comparable to that of the DMSO controls. N=3, head region, Welch's 2-tailed t-test. (**H-S'**) Expression of Phospho-Smad3 in Tg(*col2a1a:eGFP; scxa:mcherry*) embryos at 56hpf upon chemical incubation from 32-56hpf. Atorvastatin altered the pattern of Phospho-Smad3 staining compared to controls (compare cyan arrow in **K'** and **Q'**), but was not co-expressed with *scxa:mcherry*. White arrow, Phospho-Smad3 staining in a lateral region near the palatoquadrate cartilage; cyan arrow, Phospho-Smad3 staining in a medial region near Meckel's cartilage; red arrow, *scxa:mcherry*+ tendon cell; green arrow, *col2a1a:eGFP*+ palatoquadrate cartilage. MIP, Maximum Intensity Projection. Ventral (A-F, H-S') views of live (A-F) and fixed (H-S') embryos, anterior to the left. N.S, no significance; *, P<0.05; **, P<0.01; ***, P<0.001.

FIGURE S6.



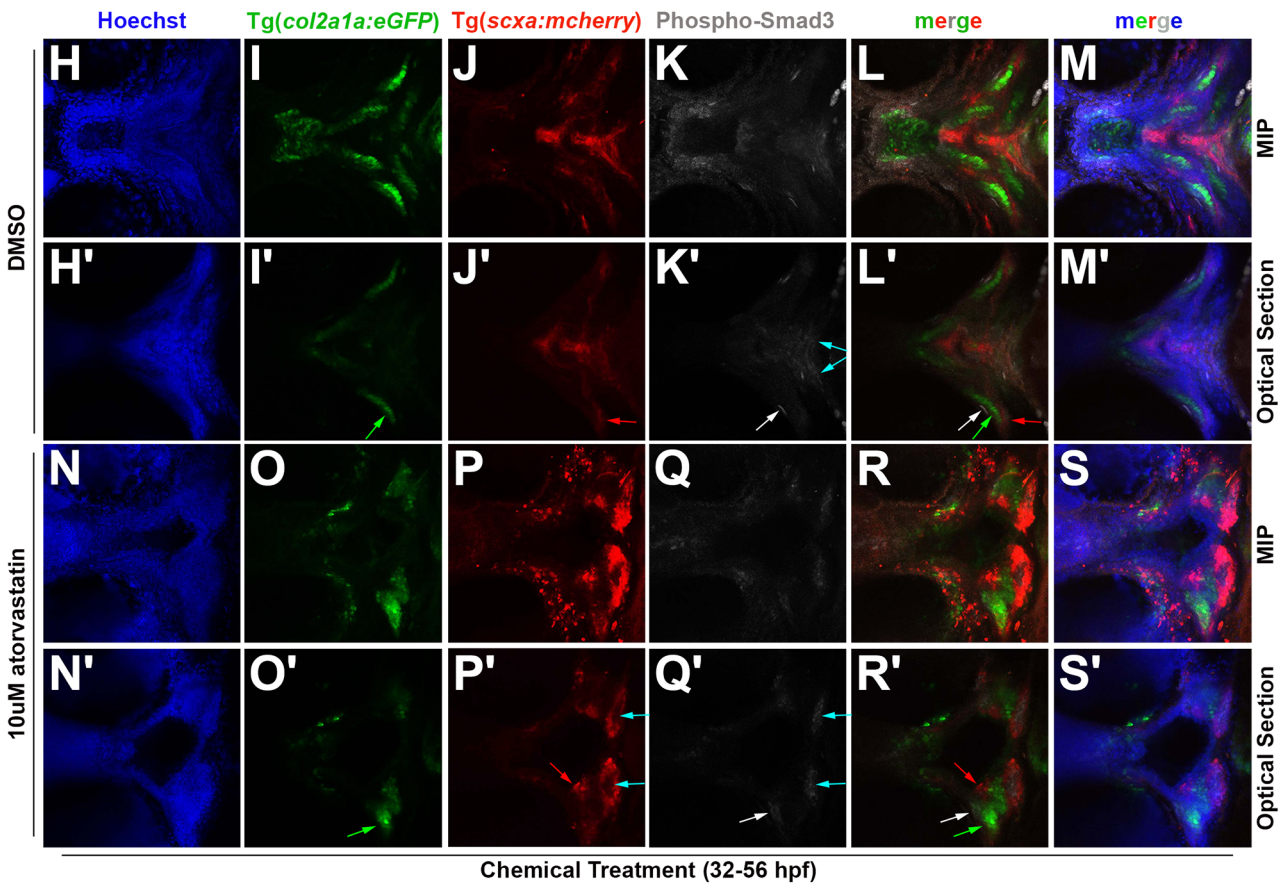


Fig. S7. Role of the mevalonate pathway in statin-mediated expansion of *scxa*-positive tendon progenitors. (**A-D**) Craniofacial expression of *scxa* at 60hpf (**A, B,** 100% mutants expanded expression, n=36) and *col1a2* at 76hpf (**C, D,** 83% mutants expanded, n=23) is expanded in the *hmgcr1b*$_{s617}$ mutants, identified by their severe pericardial edema. (**E-H**) Craniofacial expression of *scxa* is expanded at 56hpf in embryos exposed to synergistic interaction of *hmgcr1b* morpholino-mediated knockdown and chemical treatment from 32-56hpf (**H**), but not in singly chemically treated (**F**) or in MO-injected embryos (**G**). (**I-K**) Craniofacial expression of *scxa* at 56hpf, compared with controls (**I**, 100%, n=6), is expanded upon co-treatment with GGTI-286, RO48-8071, and FTI-277 following chemical incubation from 32-56hpf (**J**, 30%; n=10). This expansion in *scxa* expression phenocopies that of atorvastatin-treated embryos (**K**). Arrows indicate craniofacial domain where *scxa* is expanded in statin-treated embryos. Ventral views of flat-mounted embryos, anterior to the left.

FIGURE S7.