

Fig. S1. Co-staining of cardiac FUCCI expression with EdU incorporation. (A) Cells expressing the *cmlc2:mCherry-zCdt1* transgene (red) do not overlap with cells containing incorporated EdU (green). Images are representative maximum intensity projections from the same heart. DAPI, blue. Inset: High magnification of the boxed area. **(B)** A subset of cells expressing the *cmlc2:Venus-hGeminin* transgene (green) also contain incorporated EdU (red). DAPI, blue. Inset: High magnification of the boxed area. Arrowheads, co-expressing cells; dotted yellow line, outline of the heart. Note that many EdU⁺ cells in these images are not cardiomyocytes.

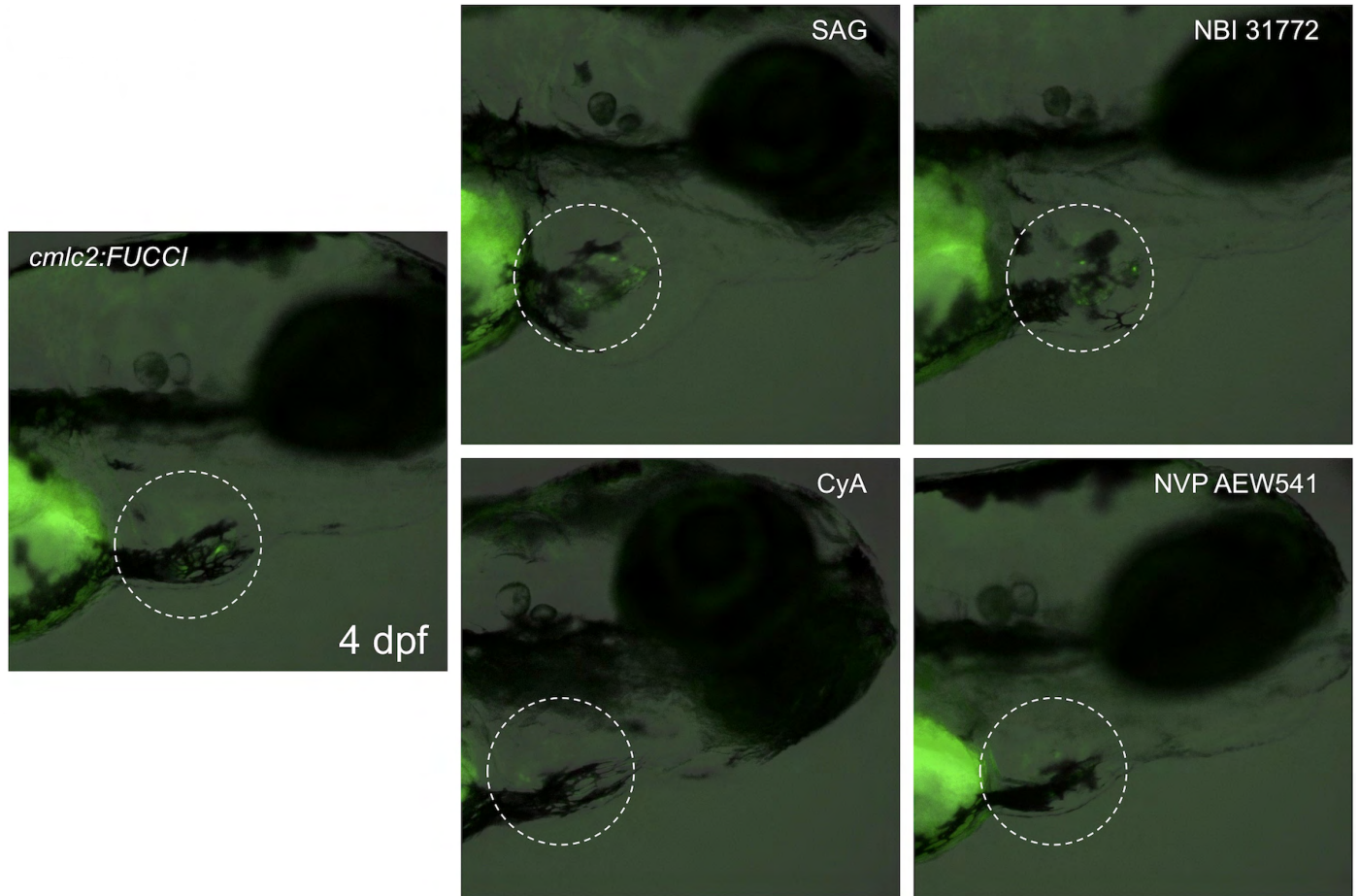


Fig. S2. Effects of drug treatment on cardiomyocyte proliferation can be visualized in live embryos with a stereomicroscope. *cmlc2:FUCCI* embryos treated with the indicated drugs from 3 to 4 dpf, exhibiting signal changes detectable via the green channel (dashed area). *cmlc2:Venus-hGeminin*, green. Bright-field image is overlaid.

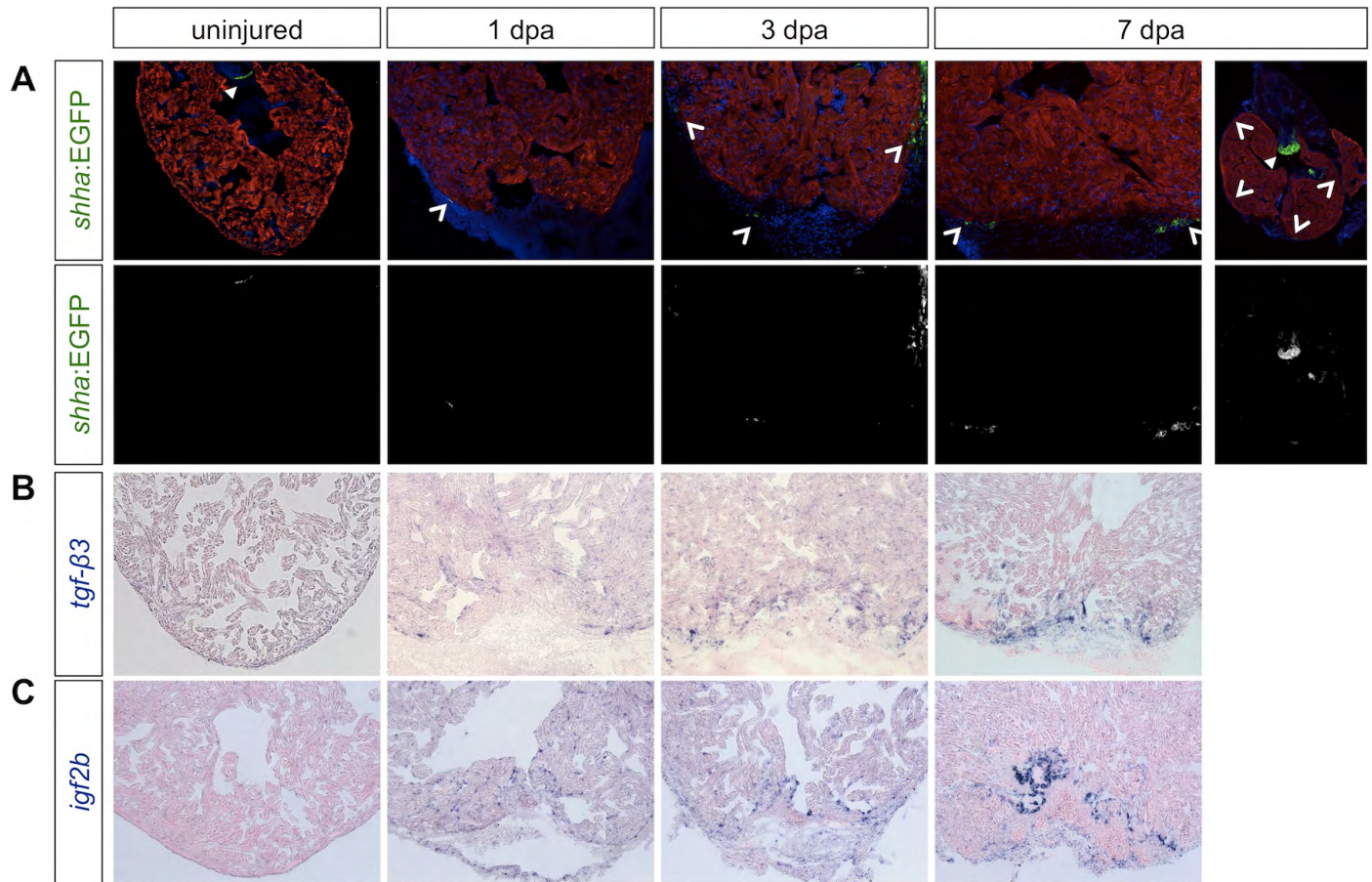
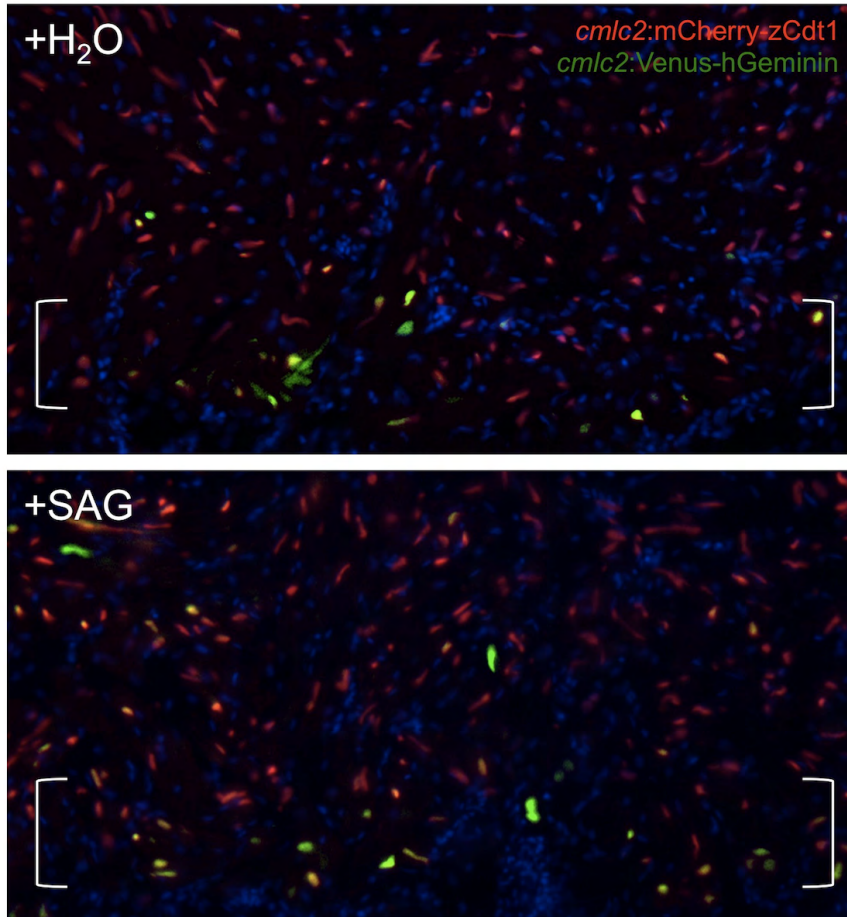


Fig. S3. Complete time course panel of ligand expression. (A) *shh* regulatory sequences drive EGFP in the valves of uninjured hearts, and is upregulated in patchy areas (arrowheads) of the epicardium at 1 and 3 dpa. By 7 dpa, expression is detected in epicardial cells within the injury, as well as more globally in the epicardium surrounding the heart. Top: *shh*:EGFP, green; Myosin heavy chain, red; DAPI, blue; notched arrowheads, *shh*:EGFP expression; filled arrowheads, valve expression. Bottom: *shh*:EGFP expression. (B) *tgfb3* expression is detectable at low levels in the myocardium by 1 and 3 dpa and localizes to the injury site by 7 dpa. (C) After injury, the ligand *igf2b* is expressed in endocardial cells, and at lower levels in epicardial tissue, near the injury site.

A *cmlc2:FUCCI*



B

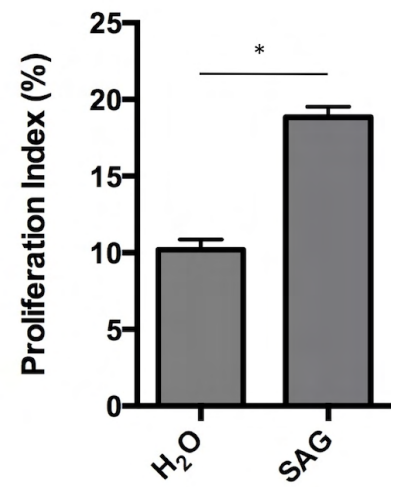


Fig. S4. Cardiomyocyte proliferation can be visualized in adult *cmlc2:FUCCI* hearts. (A) Treatment with SAG (2.5 μ M) from 6 to 7 days after resection injury increased levels of Venus-hGeminin⁺ cardiomyocytes. *cmlc2:mCherry-zCdt1*, red; *cmlc2:Venus-hGeminin*, green. Brackets indicate injury site. (B) Quantification of the effects of SAG treatment, using *cmlc2:FUCCI* fish. $n=6$, mean \pm s.e.m. * $P<0.00001$, Student's *t*-test.