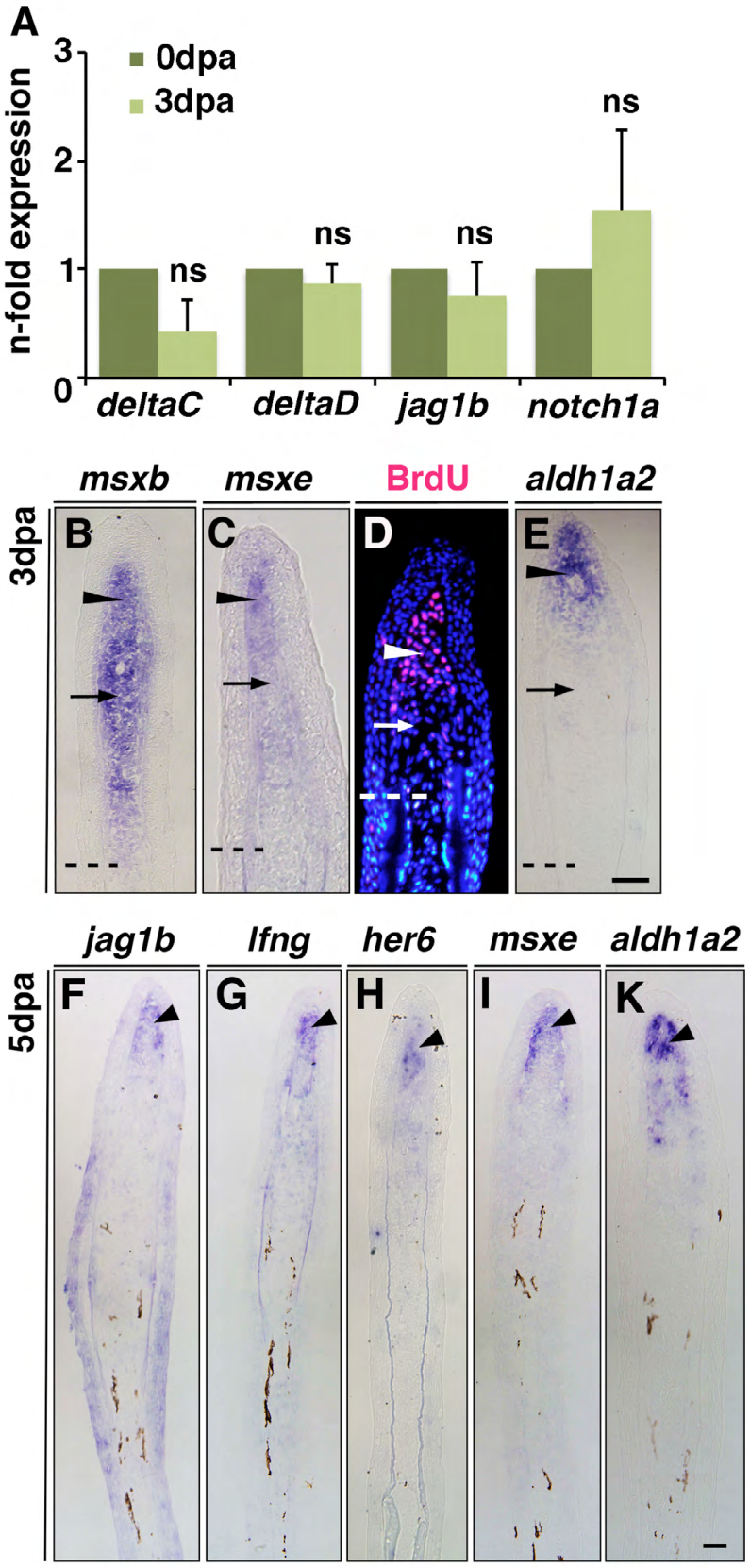

Fig. S1. Notch signalling is active in the blastema. (**A**) qPCR determination of *deltaC*, *deltaD*, *jag1b* and *notch1a* transcripts in regenerating fins at 3 dpa relative to non-amputated fins (0 dpa). (**B**,**C**,**E**) *In situ* hybridization on 3 dpa fin sections. *msxb* is expressed in the whole blastema (B) whereas *msxe* (C) and *aldh1a2* (E) expression is restricted to the distal region (arrowheads) but absent in the proximal region (arrows). (**D**) BrdU-labelled cells are densely packed in the distal region of the blastema (arrowhead), but dispersed proximally (arrow). (**F-K**) *In situ* hybridization on 5 dpa fin sections. *jag1b* (F), *lfng* (G) and *her6* (H) are expressed in the distal region of the blastema (arrowheads) but not in the more proximal differentiation zone, similar to *msxe* (I) and *aldh1a2* (K). Scale bar: 10 μm in E,K. Broken lines mark the amputation plane.

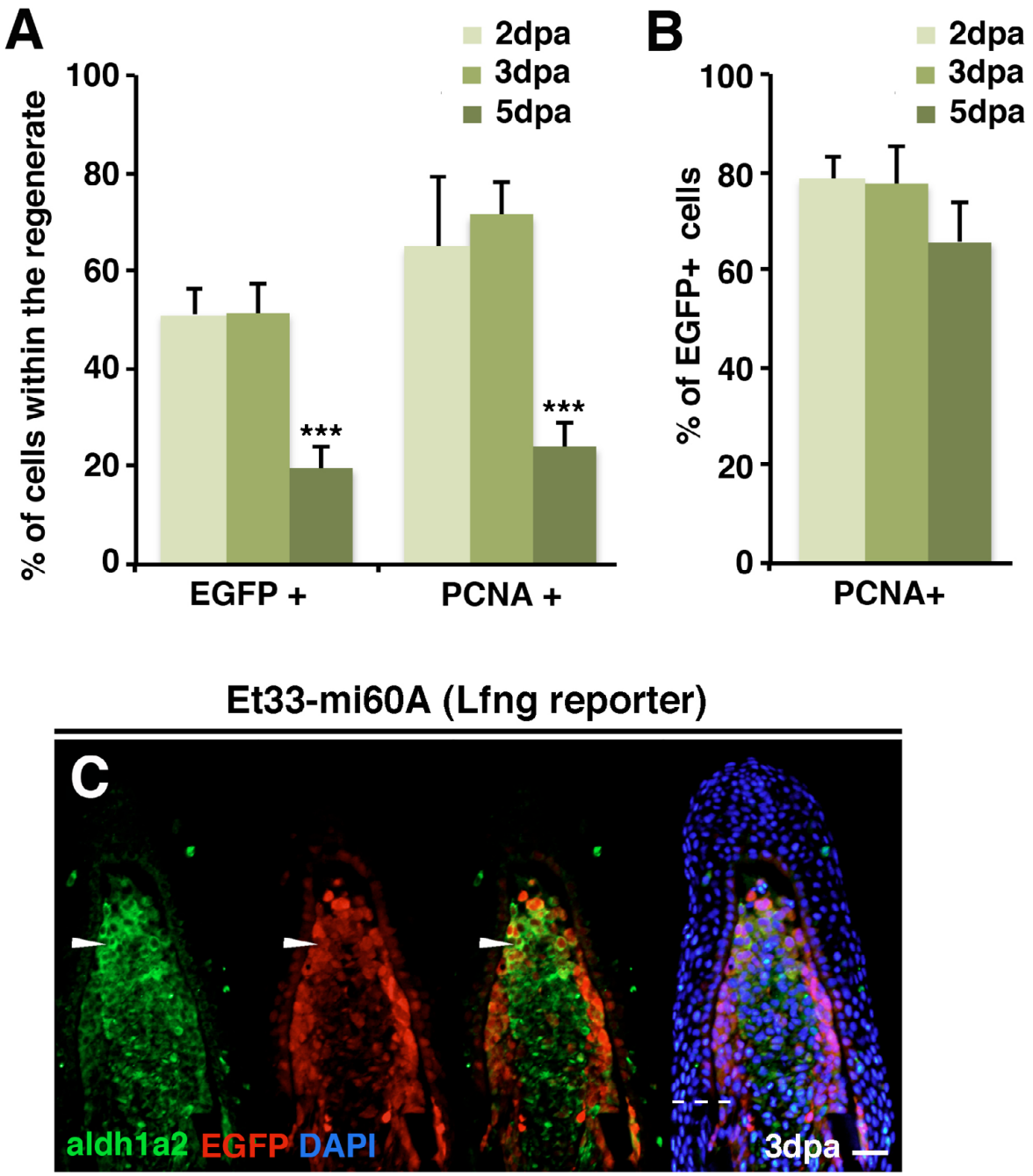

Fig. S2. Lunatic-fringe-mediated Notch signalling is activated in proliferating, *aldh1a2*-expressing cells. (**A**) Mean percentage of EGFP-expressing cells in the regenerate of *ET33-mi60A* fin sections at 2 dpa, 3 dpa and 5 dpa. ***P<0.05. (**B**) Mean percentage of EGFP$^+$ blastema cells co-labelled for PCNA in fin sections at 2 dpa, 3 dpa and 5 dpa. (**C**) Representative immunhistochemistry for EGFP and *aldh1a2* in a 3 dpa fin section. Cells are double positive in the distal region of the blastema (arrowhead). Scale bars: 100 μm in C. Broken line marks the amputation plane.

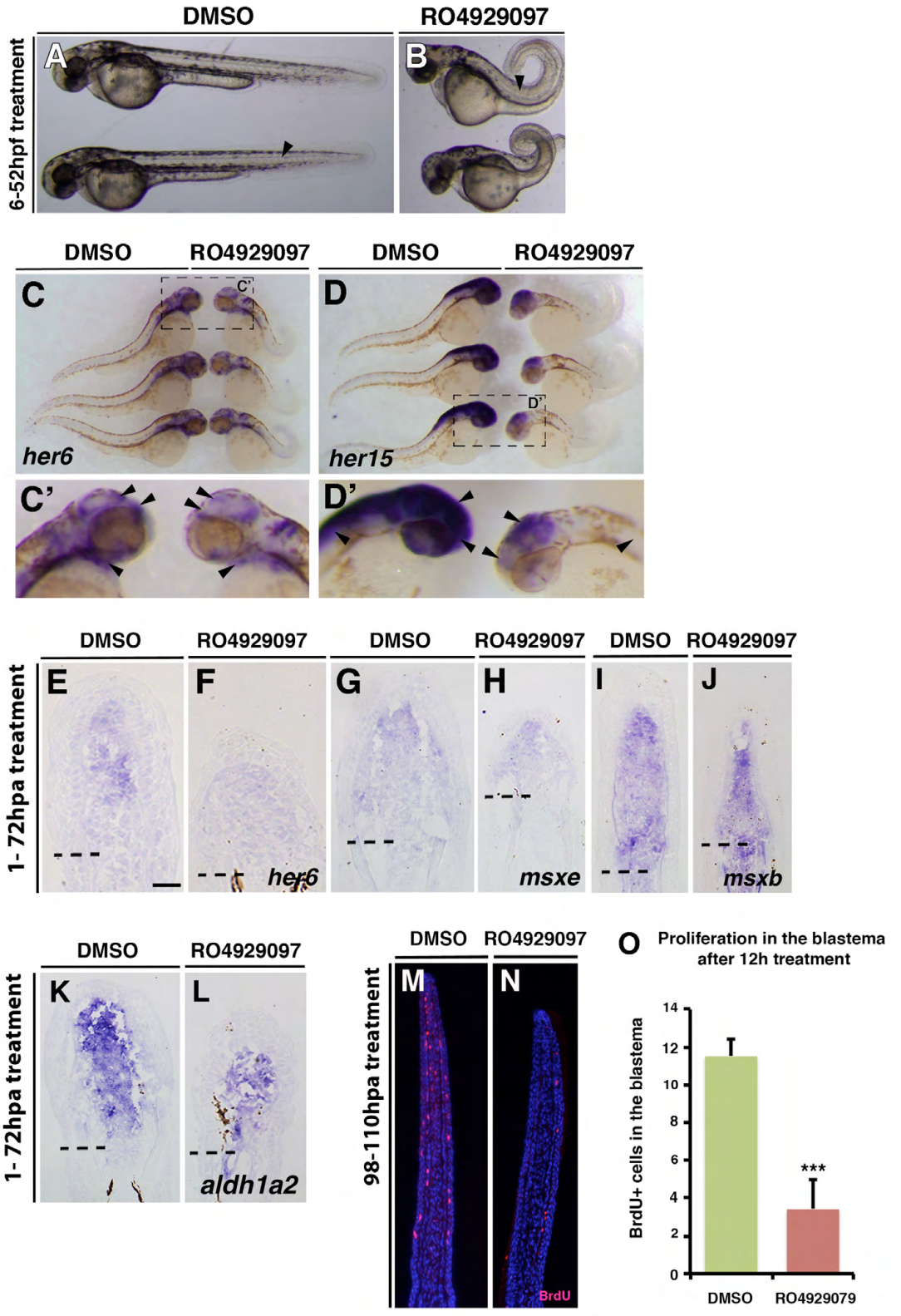

Fig. S3. RO929097 treatment leads to Notch signalling knockdown in embryos and regenerating fins and reduces proliferation. (**A-D**) Embryos treated with either DMSO or 10 µM RO929097 from 6 to 52 hpf. RO929097-treated embryos show defects in somitogenesis (arrow) and a looped tail (B). (C,D) Whole-mount *in situ* hybridization: *her6* gene expression is reduced in the brain and the gill mesenchyme of RO929097-treated embryos (arrowheads). *her15* gene expression is reduced in the brain and spinal cord of RO929097-treated embryos (arrowheads). (**E-L**) In situ on fin sections after 72 hours of DMSO or 10 µM RO929097 treatment. *her6* gene expression is reduced in RO929097-treated fins (E,F), but *msxe* (H), msxb (J) and *aldh1a2* (L) expression seems to be unchanged in RO929097-treated compared with DMSO-treated fins (G,I,K). (**M-O**) Anti-BrdU-stained fin sections and quantification of $BrdU^+$ cells within the distal most 300 µm of the mesenchyme. RO4929097-treated fins (n=4) (N) exhibit fewer BrdU-labelled cells than DMSO-treated fins (M) (n=4). ***P<0.001.

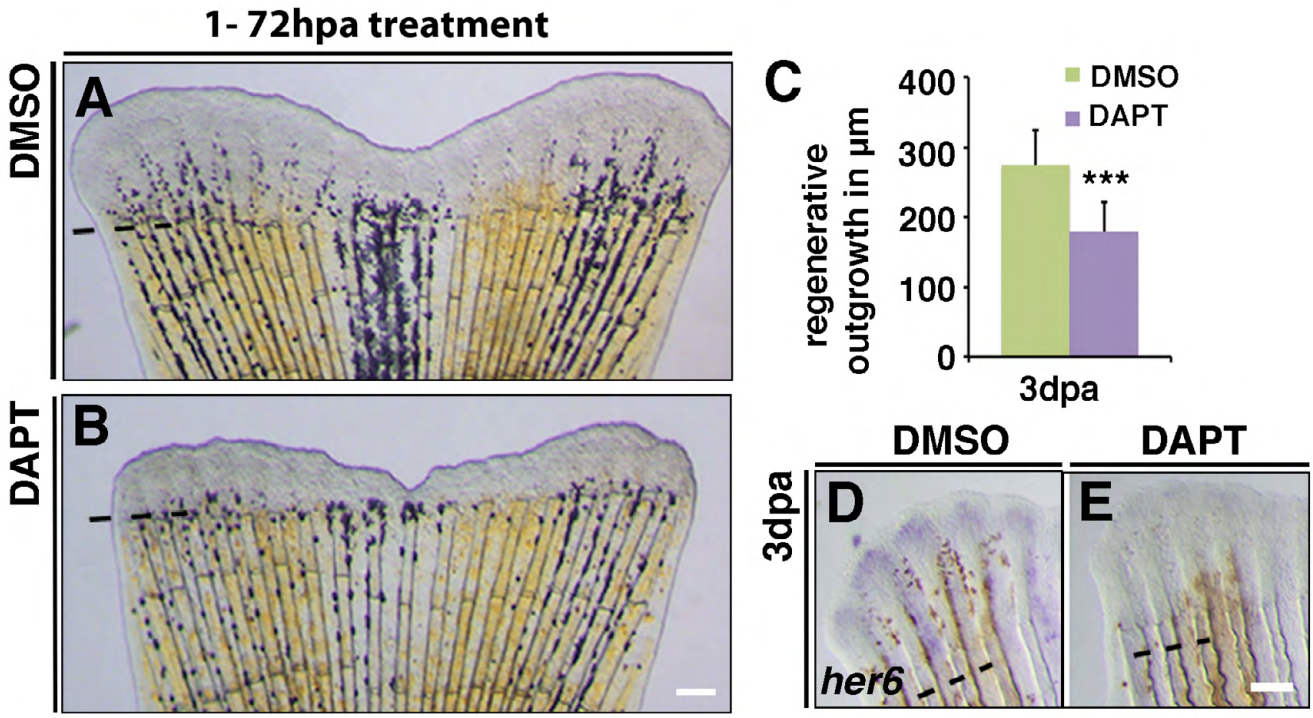

Fig. S4. DAPT treatment leads to Notch signalling downregulation in regenerating fins and reduces regenerative outgrowth. (**A**,**B**) Juvenile fish treated with 50 µM DMSO or DAPT for 3 days. Fin regeneration is impaired by DAPT treatment. (**C**) Mean length of fin regenerates; DAPT treatment (n=10) decreased regenerate length compared with DMSO-treated fins (n=6); ***P<0.005. (**D**,**E**) *her6* whole-mount *in situ* hybridization reveals Notch activity in DMSO-treated fins (D) but not in DAPT-treated fins (E).

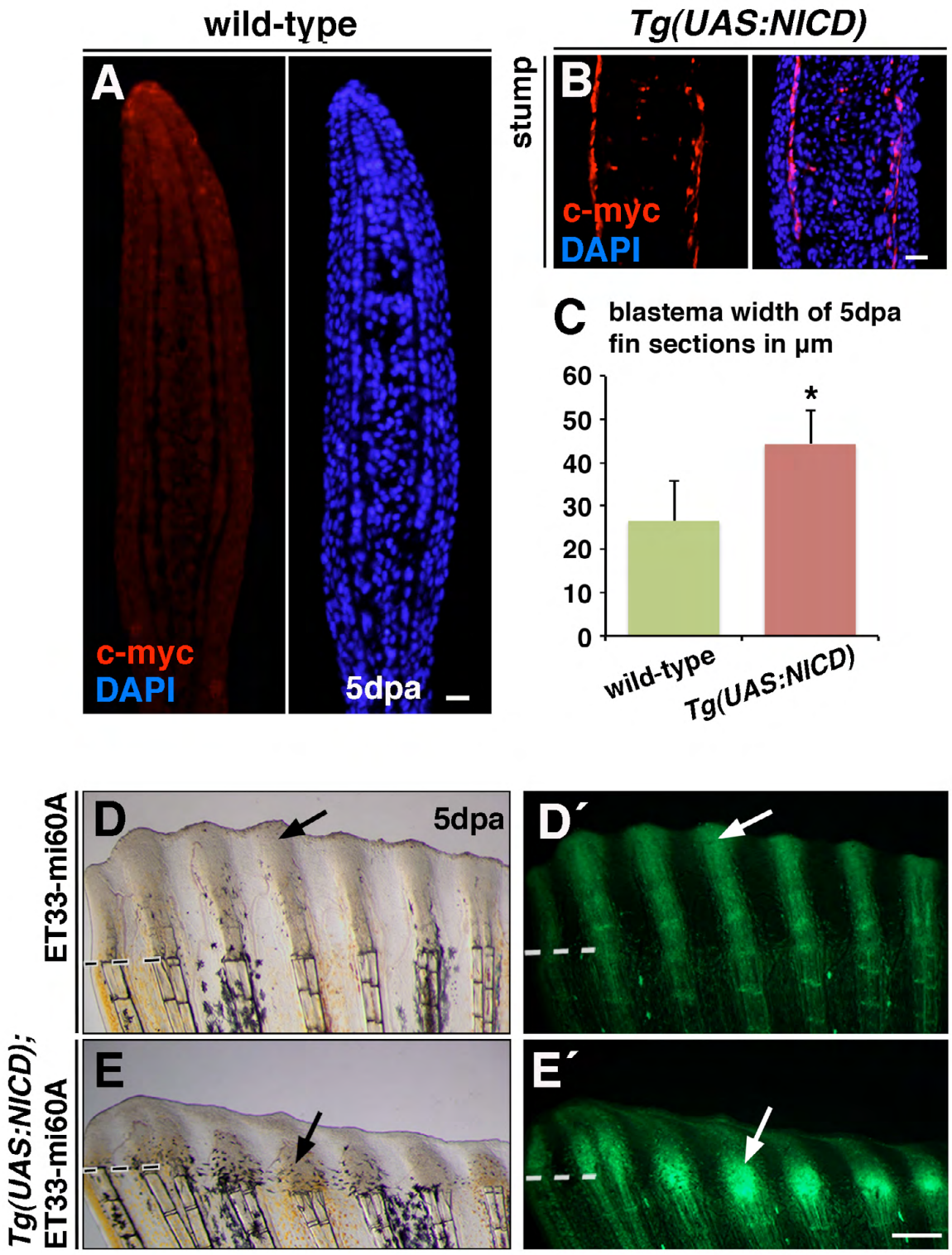

Fig. S5. NICD overexpression leads to increased blastema width and increases EGFP expression in *Tg(UAS:NICD);Et33-mi60A* fish. (**A**,**B**) Immunohistochemistry for Myc does not label wild-type fin sections (A), whereas Myc-NICD expression is detected in peripheral cells, most likely osteoblasts, and cells within the ray in the fin stump of *Tg(UAS:NICD)* fish (B). (**C**) Mean blastema width of wild-type and *Tg(UAS:NICD)* fin sections at 5 dpa. ***$P<0.05$. (**D-E′**) *Tg(UAS:NICD)* fish were crossed with *Et33-mi60A* fish and the *Tg(UAS:NICD);Tg Et33-mi60A* fish were exposed to a series of heat shocks during 5 days of regeneration. EGFP expression is increased in *Tg(UAS:NICD);ET33-mi60A* fish at 5 dpa (E′) compared with *ET33-mi60A* fish. Scale bars: 10 µm. Broken lines mark the amputation plane.

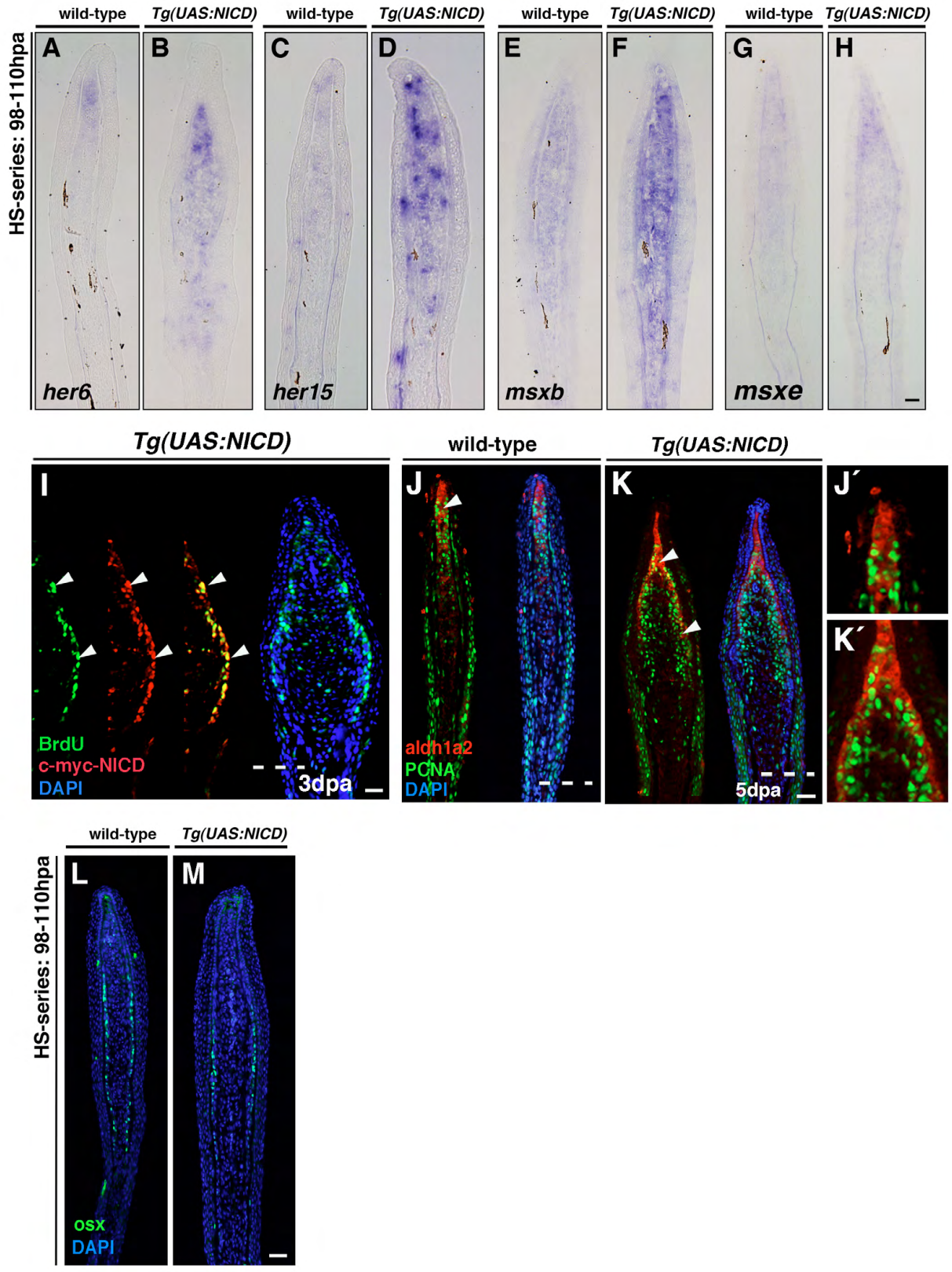

Fig. S6. Notch signalling pathway overactivation leads to increased blastema marker expression and higher proliferation. (**A-H**) *In situ* hybridization of fins after a 12-hour heat-shock period (98-110 hpa). *her6*, *her15*, *msxb* and *msxe* expression is present in the distal region of the blastema in wild-type fins (A,C,E,G). Gene expression is stronger and expanded proximally in *Tg(UAS:NICD)* fish (B,D,F,H). (**I**) Double immunohistochemistry reveals co-labeling of Myc-NICD and BrdU in many cells (arrowheads). (**J-K′**) Immunohistochemistry of PCNA and *aldh1a2* on 5 dpa fin sections. Double labelled cells (J′,K′) are restricted to the distal region of the wild-type fin (J, arrowhead) but is expanded proximally in *Tg(UAS:NICD)* fins at 5 dpa (K, arrowheads). (**L**,**M**) Immunohistochemistry against Osx of fins after a 12-hour heat-shock period (98-110 hpa). Scale bars: 10 μm in H,I,K,M. Dashed lines indicate the amputation plane.

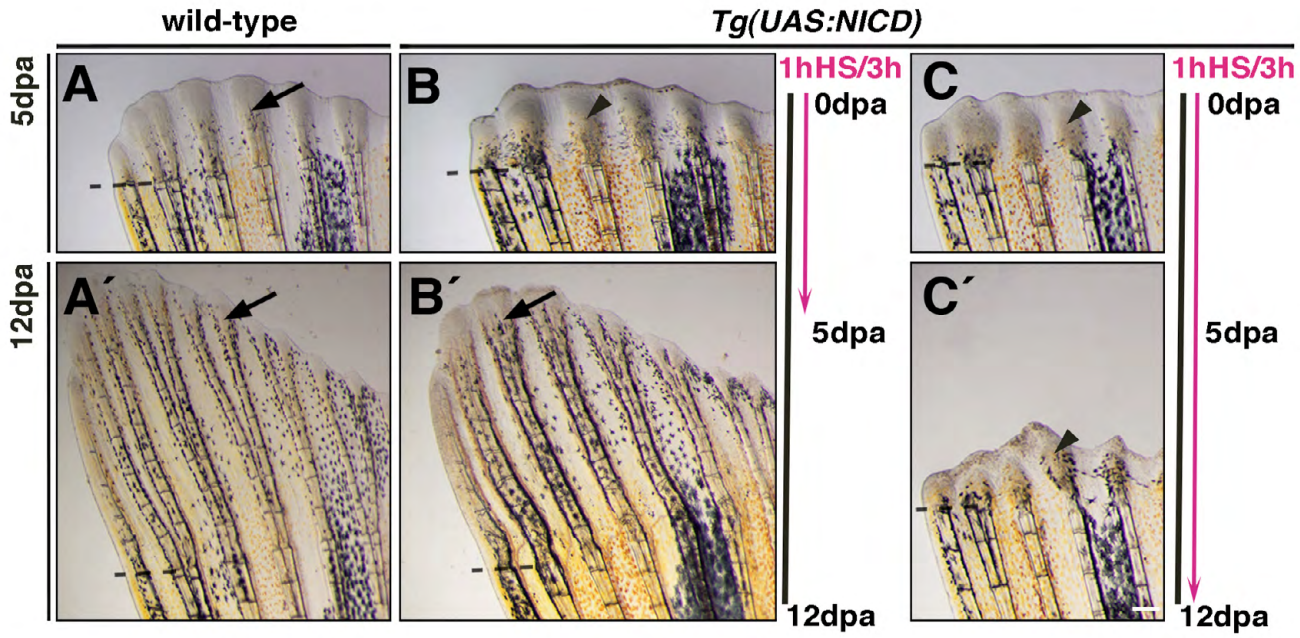

Fig. S7. NICD-induced blastema expansion is reversible. (**A-C**′) Heat-shock cycles were applied over 5 days of regeneration (A-B′) or 12 days (C,C′). Black line indicates time of regeneration. Pink line indicates time of heat-shock treatment (1 hour of heat-shock every 3 hours for 5 or 12 days). Arrows indicate regenerating radials. Arrowheads indicate expanded blastemas. Regeneration is inhibited in *Tg(UAS:NICD)* (B,C) fins but not in wild-type fins (A). Regeneration reverts to normal when heat-shock treatment is stopped (A′,B′, arrows) but the blastema remains close to the amputation plane (arrowhead) when heat-shocked continued up to 12 days (C′). Scale bars: 100 μm. Dashed lines mark the amputation plane.

Table S1. ISH probes

Probe	**Reference**
msxb	(Akimenko et al., 1995)
msxe	(Akimenko et al., 1995)
tcf7	(Li et al., 2009)
aldh1a2	(Grandel et al., 2002)
notch1b	(Westin and Lardelli, 1997)
jagged1b	(Zuniga et al., 2010)
her15	(Shankaran et al., 2007)
lunatic fringe	(Prince et al., 2001)
her6	This report: Forward, 5'-CATCATTGCCGCACCA-3'; Reverse, 5'-TGTGTTTAGGGCAGCGGTCAT-3'

Table S2. qPCR primers

Gene	Forward (5′-3′)	Reverse (5′-3′)
deltaC	CGCAGAAACCTCTGACCAGT	CAGTCCTCACTGATAGCGAGTC
deltaD	GTTCACCAACCCCATTCCTT	TGTGCAGCGCTTCAATAATC
jag1b	ACATGCGAGTGTCAAGAAGGT	CATGGGTTACTTTCACAATCGTT
notch1a	TGTGAATGCACCCCAGGT	GACGCACACTCGTTGATGTC
notch1b	GGGCACCTGCGTACAGAA	CAAATTCCTGCCGACCTG
lfng	TCTGTTGAGGAGGACCCATC	GCACCAAGGAGTGTCTGGAT
her6	GGCTTCGGAACACAGAAAGT	TGACCCAAGCTTTCGTTGa
her15	TCGCTCTGCTCAGAAACA	ACCACTGGCTTTCGAAT
msxb	GGTCAAACTCTTCATCTTTCACATC	TCTTGTGCTTGCGTAAGGTG
msxe	GAGCGGAGCACATGGGTA	CCGGTTGGTTTTGTGTTTTC
aldh1a2	GGGGGAAGCTACTGTTCAAAT	TCCAGAGACTCCAGGGTAGC