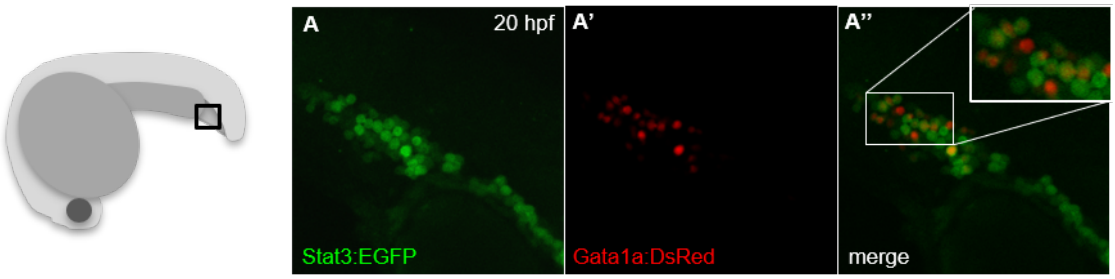
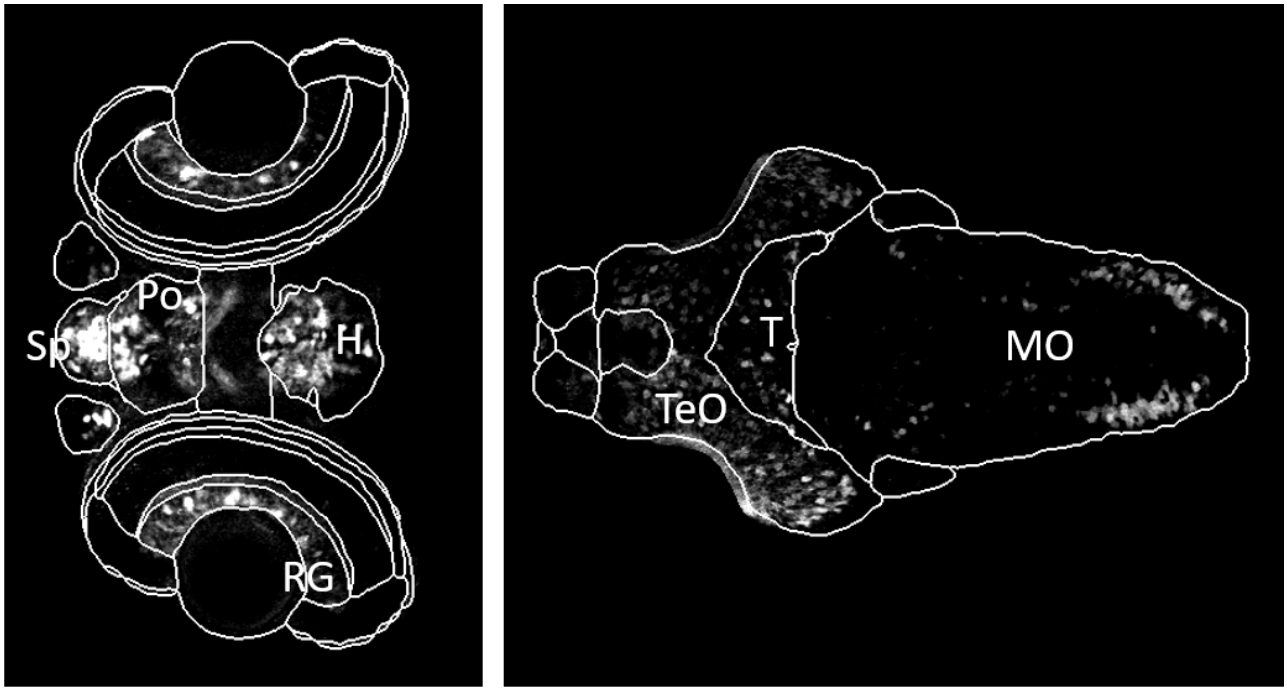


Fig. S1: Stat3 reporter is expressed in erythroid progenitor cells. A-A'': Confocal lateral view of haematopoietic tissue of double transgenic embryos obtained crossing the *Tg(7xStat3:EGFP)* (A) with *Tg(gata1a:DsRed)* (A'').



77 Tectum opticum (TeO)
97 Medulla oblongata (MO)
81 tegmentum (T)
7 subpallium (Sp)
45 preoptic region (Po)
48 hypothalamus (H)
59 retinal layer (RG)

Fig. S2: ***Tg(7xStat3:EGFP) reporter expression in the brain of 72-hpf larvae*****.** A-B: Single planes of *Tg(7xStat3:EGFP)* brain at 72 hpf. Images have been obtained with VIBEZ-Z software. H= hypothalamus, Sp= subpallium, Po= preoptic region, RG= retinal layer, TeO= tectum opticum, MO=medulla oblongata, T= tegumentum.

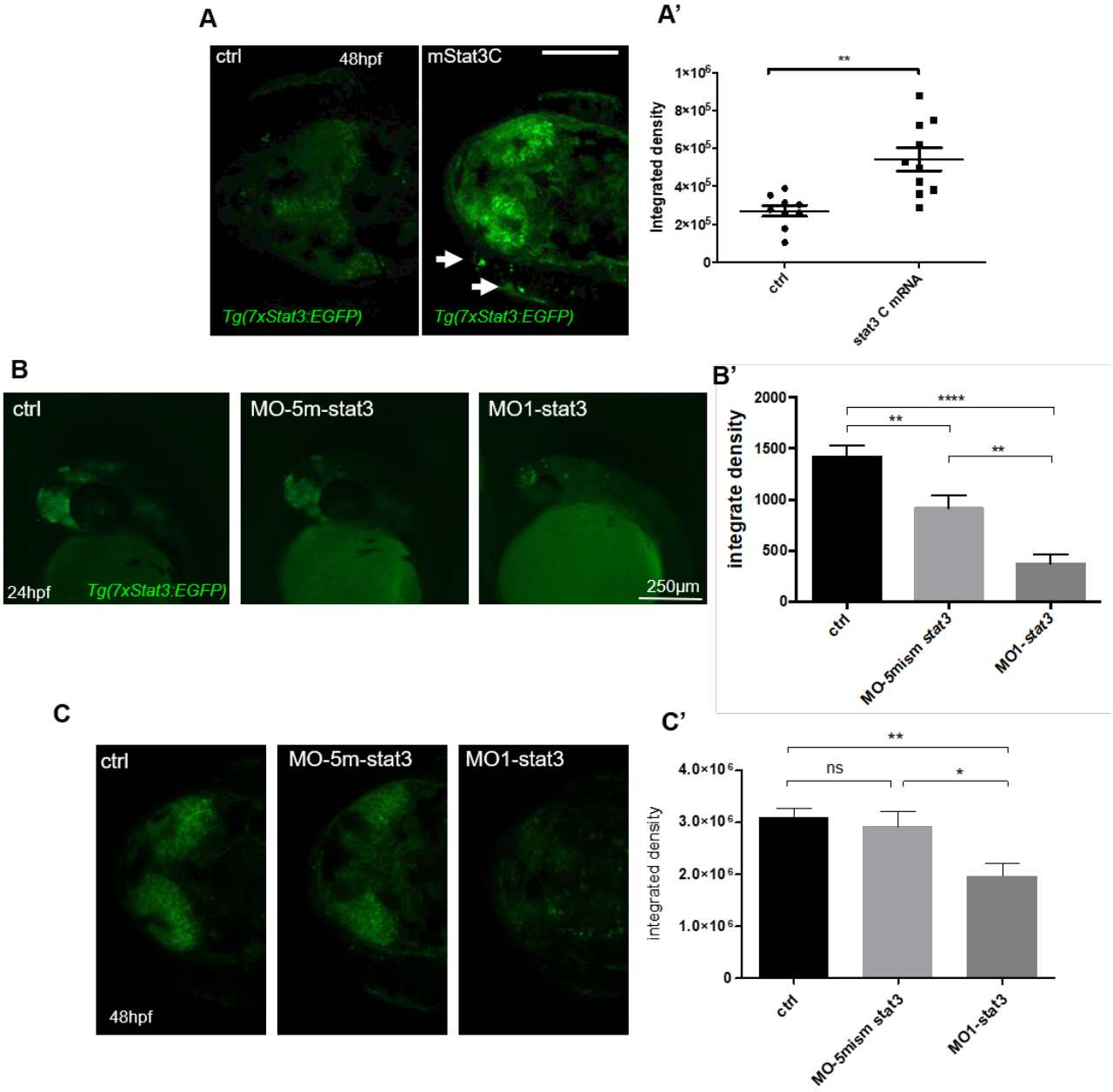


Fig. S3: *Tg(7xStat3:EGFP)* reporter line respond to silencing and overexpression of *stat3*. A: Dorsal view live image of the head of *Tg(7xStat3:EGFP*) controls (left) or m*Stat3C* mRNA injected embryos (right) at 48hpf. Arrowheads highlight ectopic fluorescent signal. Scale bar: 100µm. A': EGFP fluorescence quantification in the TeO of m*Stat3C* injected and control larvae at 48 hpf. Statistical analysis was performed by unpaired t-test (n=20); **p<0.01 B: representative pictures of 24-hpf *Tg(7xStat3:EGFP)* injected with stat3-MO1 (Liu *et al.,* 2017; Miyagi *et al.,* 2004; Yamashita *et al.,* 2002) 5-mismatch morpholino B': Quantification of EGFP fluorescence in 24-hpf embryos injected with stat3-MO1 morpholino and 5-mismatch morpholino on the reporter line. Scale bar=250µm, statistical analysis was performed by unpaired t-test on 3 biological replicates. **p<0.01, ****p<0.0001; error bars=SEM. C: dorsal view of 48-hpf *Tg(7xStat3:EGFP)* injected with stat3-MO1

and 5-mismatch morpholino. C': Quantification of EGFP fluorescence in 48-hpf *Tg(7xStat3:EGFP)* embryos injected with stat3-MO1 morpholino and 5-mismatch morpholino. *p<0.05, **p<0.01; error bars=SEM

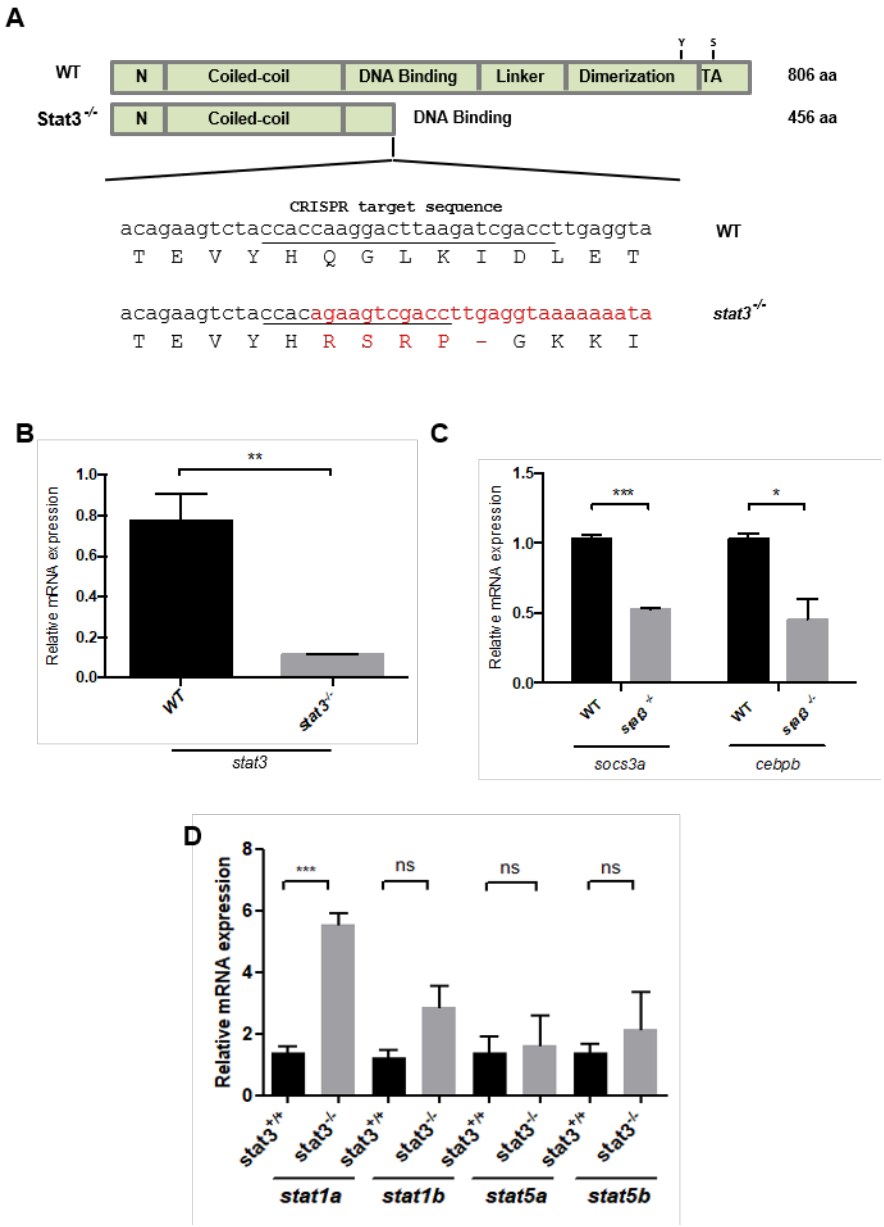


Fig. S4: *stat3*^{ia23} mutant validation. A: Schematic representation of *stat3*$^{-/-}$ mutant allele in comparison with WT. B: qRT-PCR analysis of *stat3* mRNA expression normalized on *gapdh* from WT and *stat3*$^{-/-}$ siblings at 6 dpf (p-value= 0,0073). C: qRT-PCR analysis of *socs3a* and *cebpb* Stat3 targets expression normalized on *gapdh* from WT and *stat3*$^{-/-}$ siblings at 6 dpf, *zgapdh* was used as internal control (p-values= 0,001; 0,0205); Statistical analysis was performed by unpaired t-test on 3 independent biological samples. **p<0,01; error bars=SEM. D: qRT-PCR analysis of *stat1a*, *stat1b*, *stat5a* and *stat5b* mRNA expression levels normalized on *gapdh* from *stat3*$^{+/+}$ and *stat3*$^{-/-}$ siblings at 6 dpf (***p<0.001)

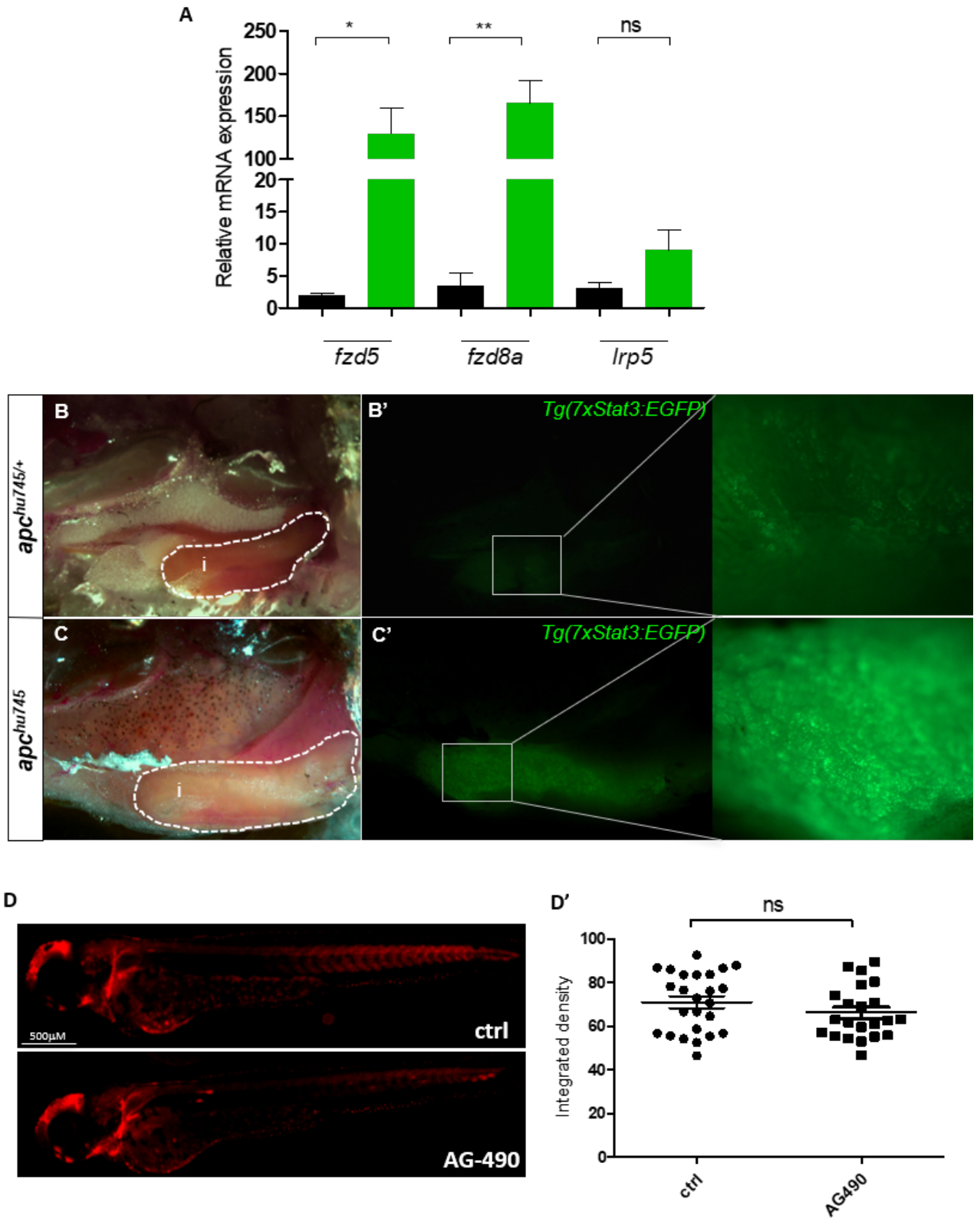


Fig. S5: Stat3 pathway is strongly activated in zebrafish *apc*-driven tumor. A: qRT-PCR analysis of *fzd5*, *fzd8a* and *lrp5* on EGFP-positive and EGFP-negative cells sorted from adult intestines. B-C'': *In vivo* EGFP expression is ectopic in 12 mpf *Tg(7xStat3:EGFP)/apc^{hu745}* hyperplastic intestine (B-B') with respect to *Tg(7xStat3:EGFP)/ apc$^{hu745/+}$* siblings (B-B'). C-C': representative pictures of 3-dpf

Tg(7xTCF-Xla.Siam:nlsmCherry)^{ia5} reporter larvae treated with DMSO and 80 µM AG-490 from 8-72 hpf (D); quantification of *Tg(7xTCF-Xla.Siam:nlsmCherry)*^{ia5} reporter larvae fluorescence (D'). Statistical analysis was performed by unpaired t-test. ns=not significant.

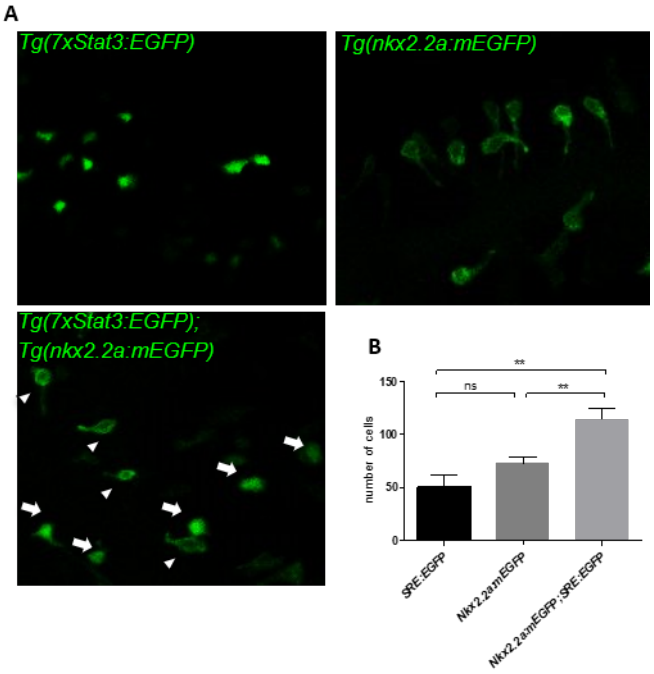


Fig. S6: EGFP positive cells of *Tg(7xStat3:EGFP)* zebrafish line are not secretory cells. A: representative prictures of *Tg(7xStat3:EGFP)*, *Tg(nkx2.2a:mEGFP)* and *Tg(7xStat3:EGFP)/Tg(nkx2.2a:mEGFP)* 6-dpf larvae intestine. B: number of EGFP-positive cells measured in intestines of *Tg(7xStat3:EGFP)*, *Tg(nkx2.2a:mEGFP)* and *Tg(7xStat3:EGFP)/Tg(nkx2.2a:mEGFP)* 6-dpf. Arrows indicate *Tg(nkx2.2a:mEGFP)* positive cells; arrowheads indicate *Tg(7xStat3:EGFP)* positive cells. **p<0.01, ns=not significant; error bars=SEM.

Gene	Forward primer sequence	Reverse primer sequence
zstat3	TGCCACCAACATCCTAGTGT	GCTTGTTTGCACTTTTGACTGA
zgapdh	GTGGAGTCTACTGGTGTCTTC	GTGCAGGAGGCATTGCTTACA
zcebpb	CCAAAAGTAACGGGCGACAC	ATCTTCCCTTACCTGACGGC
zsocs3	GGAAGACAAGAGCCGAGACT	GCGATACACACCAAACCCTG
egfp	ACGTAAACGGCCACAAGTTC	AAGTCGTGCTGCTTCATGTG
zstat1a	GCAGCTCAAGAAACTCCTGG	AAAGGTCTCTGCAGTTGGGT
zstat1b	CGAGTGGAAGAAGAGACAGC	GCTGGCCCCTTCCTAGATTT
zstat5a	TGACCCGAGAAGCTAACACC	GTATGTCCAGTCCTCCCT
zstat5b	TGAGGAAACAGCAAACCGTG	GCTGCTGAGTCAAGTGTTCA
zil6	CGTAAAGAGTCTCCTTGGCG	GGTTTGAGGAGAGGAGTGCT
zgp130	TGCTGGAGTGGGTGAATGAA	GGCTTGGTTACTGGTGTTCC
zjak2a	CTTCGAGAGTCAGGAGCCC	CTGAAGCTTCTTCACCGCC
zjak2b	ACGTATTGTGATTTCGCGGA	ACAAAAGACAAGGCCTGCAT
zsox9b	CTCGGCAAACTCTGGAGACT	GCGCATTGGTGGAGATCTG
zagr2	GCACAGACATACGAGGAAGC	GGAGACAAGTGCTTATCTGTG
zpept1	GATTGCTTTGGGAACAGGAGG	GATGGGTGTGATGAGAGTGG
zfabp2	GCTGCCCATGACAACCTG	CGTGTCTCCCTCTATGACC
znotch2	GACGAATGCATCTCCAGTGC	GCAGCAGCCACAGCAACC
zpcna	CCTTGGCACTGGTCTTTGAA	GGCACACGAGATCATGACAG
zcyclinD1	CCAACTTCCTCTCGCAAGTC	TGGTCTCTGTGGAGATGTGC
zfzd5	CCTAACTGTGCACTGCCTTG	ATTTGAAGCGCTCCATGTCG
zfzd8a	TGCAATCGGGAGTATGACGT	CTCGTTTCCCCACTTCATGC
zlrp5	TTCTCGGAGGGCCTGATTTT	TTGTCTCCGAGTCAGTCCAG

Tab. 1: list of primer used for Real Time qPCR.