Fig. S1. Two related igf-1 receptors are expressed in zebrafish fins. (A) Quantification of expression levels of igf-1ra and igf-1rb normalized to ubiquitously expressed β-actin reference gene in amputated fins at 0, 24 and 72 hpa (error bars indicate the s.e.m.; n=3 samples prepared from 10-15 fins). The normalized gene expression (NGE) values were calculated with the Pfaffl method and multiplied by a factor of 10,000. (B-D) In situ hybridization of fin sections at 72 hpa using control sense probe (B), antisense igf-1ra probe (C) and antisense igf-1rb probe (D). Both receptors appear to be ubiquitously expressed. Higher levels of expression are detected in the blastema. Scale bar: 50 µm.

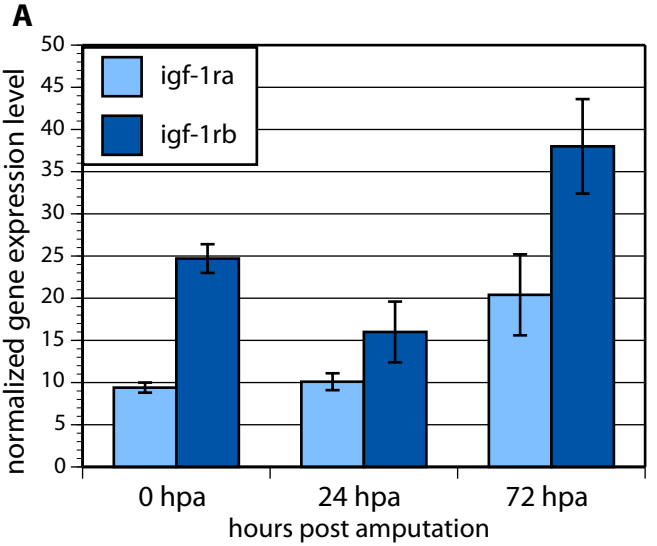
Fig. S2. NVP-AEW541 and NVP-ADW742 block Akt phosphorylation in zebrafish fins. Western blots for phospho-Akt (p-Akt) and total-Akt (t-Akt) were prepared from regenerating fins at 24 hpa treated with 0.05% DMSO (control), 5 µM NVP-AEW54 or 5 µM NVP-ADW742. A marked decrease of p-Akt is observed after treatment with NVP-AEW541 and NVP-ADW742. t-Akt was used as a loading control.

Fig. S3. The effect of IGF-1R signaling on the wound epidermis. (A-F) Hematoxylin-Eosin stained fin sections. (A-C) At 30 hpa, the basal layer of the wound epidermis of the control fin (A) appears more ordered and aligned than in fin treated with NVP-AEW541 (B) or NVP-ADW742 (C). (D-F) Drug-shift experiment: 30 hours at normal conditions and 24 hours with treatment. The architecture of the wound epidermis appears similar in control (D) and inhibitor-treated (E,F) fins. (G,H) Drug-shift experiment (30 hours at normal conditions and 24 hours with treatment). Sections of regenerates stained with active-Caspase-3 antibody in green and a nuclear marker DAPI in blue. Control fins (G) and ADW742-treated fins (H) contain low amounts of active-Caspase-3-positive cells. (I) Quantification of active-Caspase-3 in control and inhibitor-treated fins in the drug-shift experiment. n=6; P<0.01. Scale bars: 50 µm.

Fig. S4. Inhibition of IGF signaling increases cell apoptosis in uninjured epidermis. (A-C) Hematoxylin-Eosin stained rays of uninjured fins that were treated with 0.05% DMSO (A), NVP-AEW541 (B) or NVP-ADW742 (C) do not display differences in morphology of the epidermis. (D-F) Sections of uninjured fins stained with active-Caspase-3 antibody in green and with the nuclear marker DAPI in blue. IGF-1R inhibitor-treated fins (E,F) contain a few more active-Caspase-3-positive cells in the epidermis than in the control (D). (G) Quantification of active-Caspase-3 in uninjured fins treated with IGF-1R inhibitors for 3 days. n=6; P<0.01. Scale bars: 50 µm.

Fig. S5. In situ hybridization of fin sections with lef1 antisense probe. Drug-shift experiment (30 hours at normal conditions and 24 hours with treatment). (A) In control regenerate, lef1 transcript is detected in the basal layer of the wound epidermis, except the apical part, and in the distal blastema. (B) lef1 transcript is downregulated in the NVP-AEW541-treated fins.

Fig. S6. IGF signaling is required for the progression of normally initiated regeneration. (A-I) Bright-field images of fins in a drug-shift experiment: 30 hours at normal conditions and 5 days with treatment. (A-C) Fins before inhibitor treatment at 30 hpa. (D-F) Fins after additional 5 days exposure to 5 µM AEW541 (E) and 5 µM ADW742 (F) reveal impaired regeneration in comparison with control treated with 0.05% DMSO (D). (G-I) Higher magnifications of the fin regenerates reveal aberrant differentiation of the skeletal rays and weaker pigmentation after inhibitor treatment (H,I) in comparison with control (G). (J) Quantification of the outgrowth size after the drug-shift experiment. n=3; *, P<0.001.


A
igf-1ra
igf-1rb
normalized gene expression level
50
45
40
35
30
25
20
15
10
5
0
0 hpa
24 hpa
72 hpa
hours post amputation

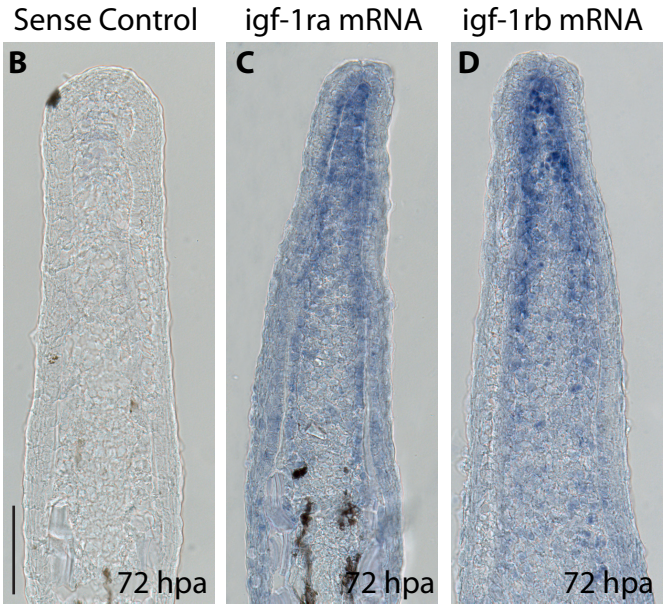

Sense Control
igf-1ra mRNA
igf-1rb mRNA
B
C
D
72 hpa
72 hpa
72 hpa

Fig 1

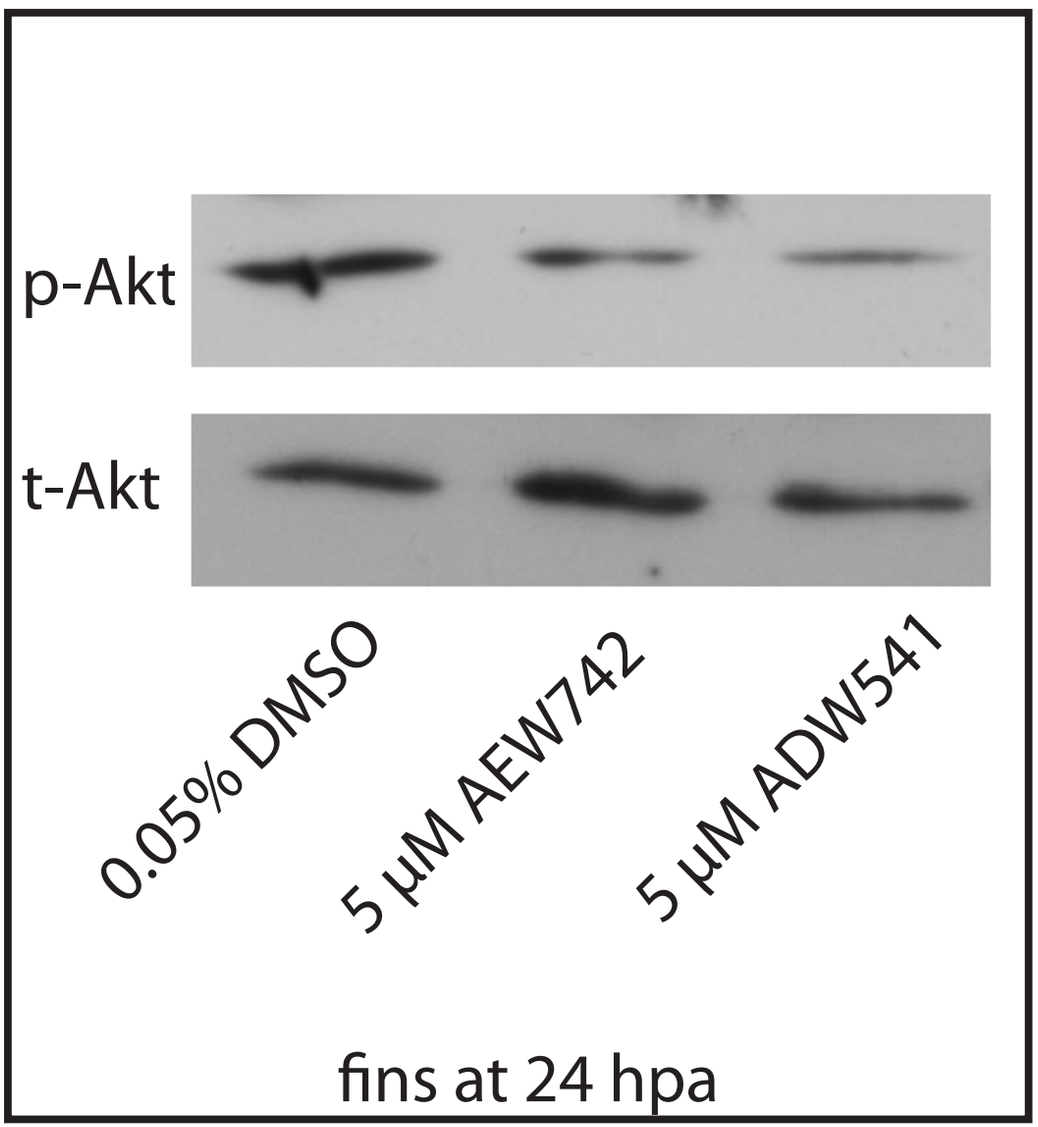


Fig 2

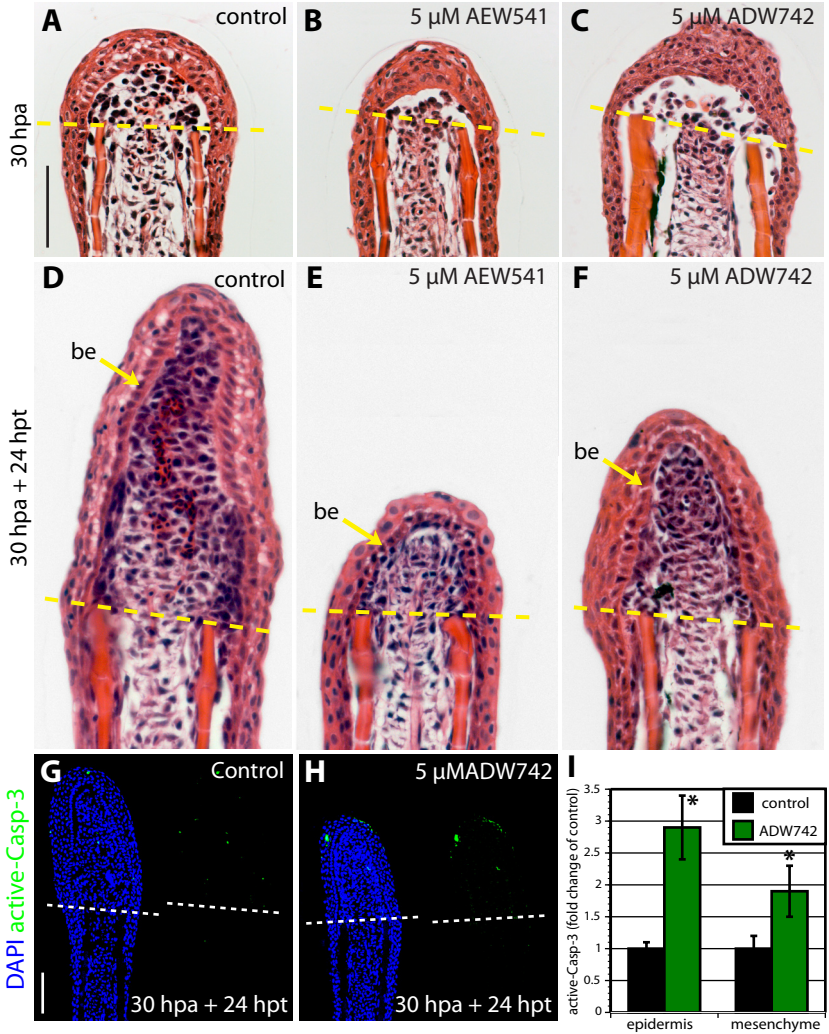
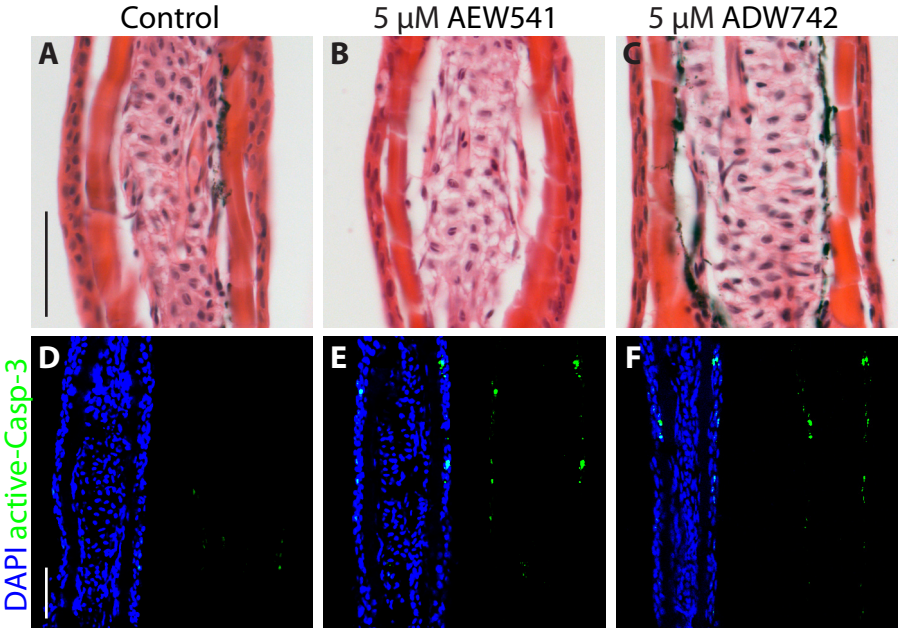

A
control
B
5 μM AEW541
C
5 μM ADW742
30 hpa
D
control
E
5 μM AEW541
F
5 μM ADW742
be
be
be
30 hpa + 24 hpt
G
Control
H
5 μMADW742
I
DAPI active-Casp-3
30 hpa + 24 hpt
30 hpa + 24 hpt
control
ADW742
active-Casp-3 (fold change of control)
3.5
3
2.5
2
1.5
1
0.5
0
*
*
epidermis
mesenchyme

Fig 3


Control
5 μM AEW541
5 μM ADW742
A
B
C
D
E
F
DAPI active-Casp-3

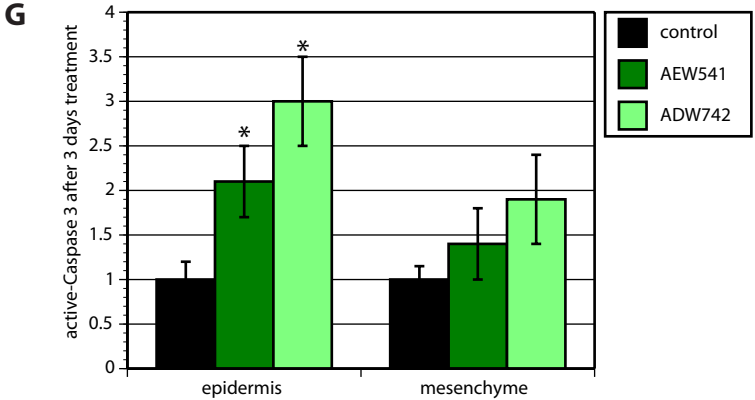

G
active-Caspase 3 after 3 days treatment
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
epidermis
mesenchyme
control
AEW541
ADW742

Fig 4

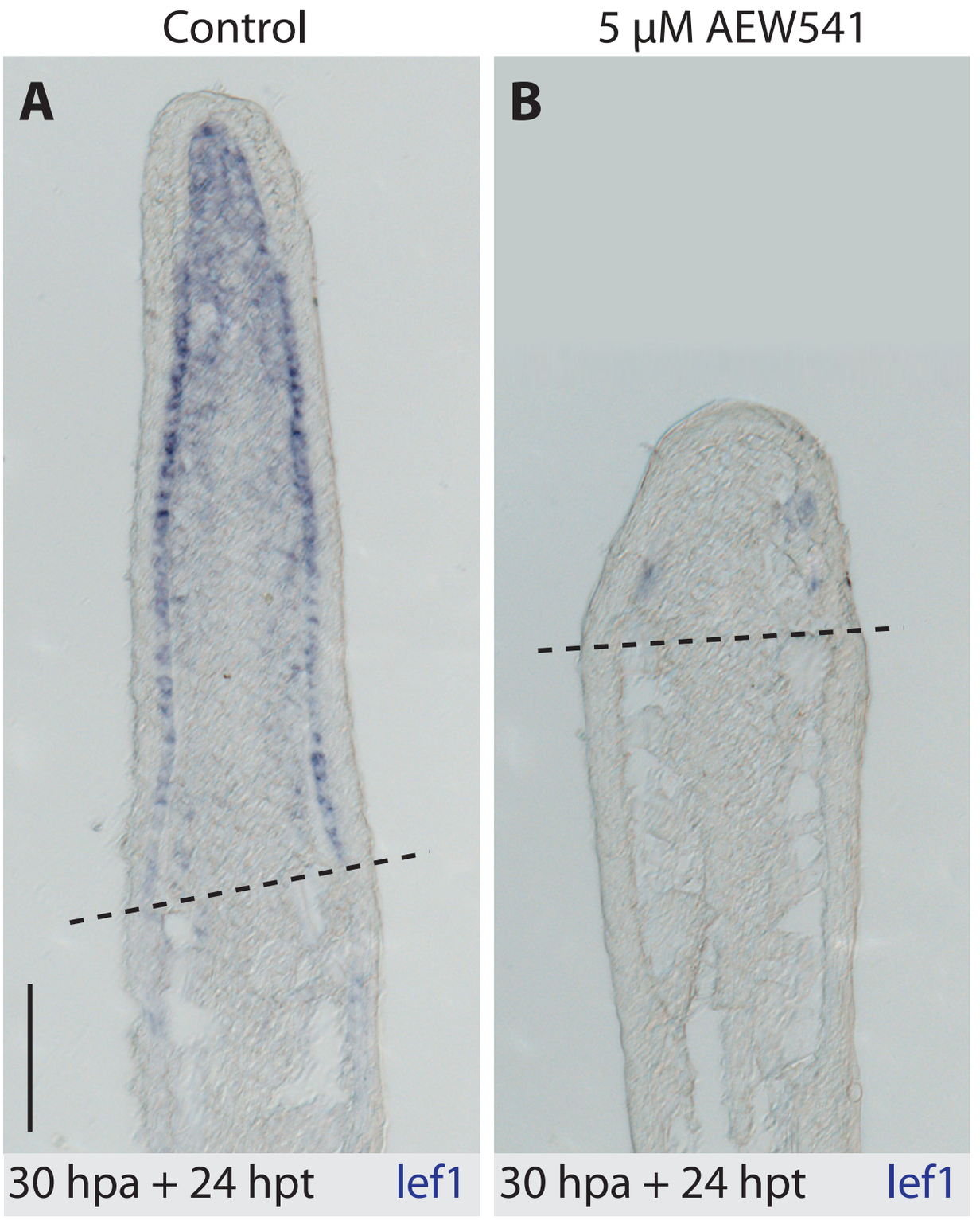


Fig 5

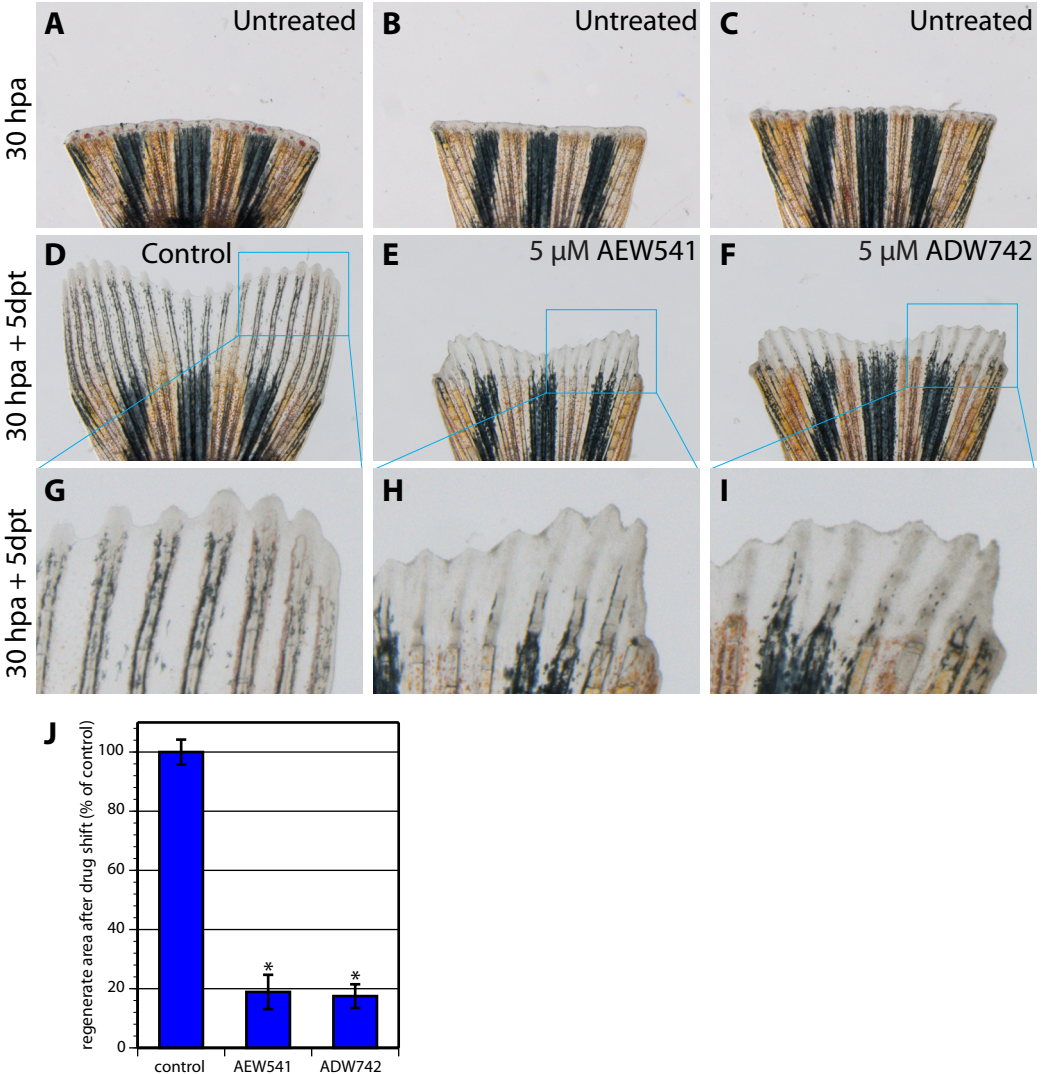

A
Untreated
B
Untreated
C
Untreated
30 hpa
D
Control
E
5 µM AEW541
F
5 µM ADW742
30 hpa + 5dpt
G
H
I
30 hpa + 5dpt
J
regenerate area after drug shift (% of control)
100
80
60
40
20
0
*
*
control
AEW541
ADW742

Fig 6