Supplementary material

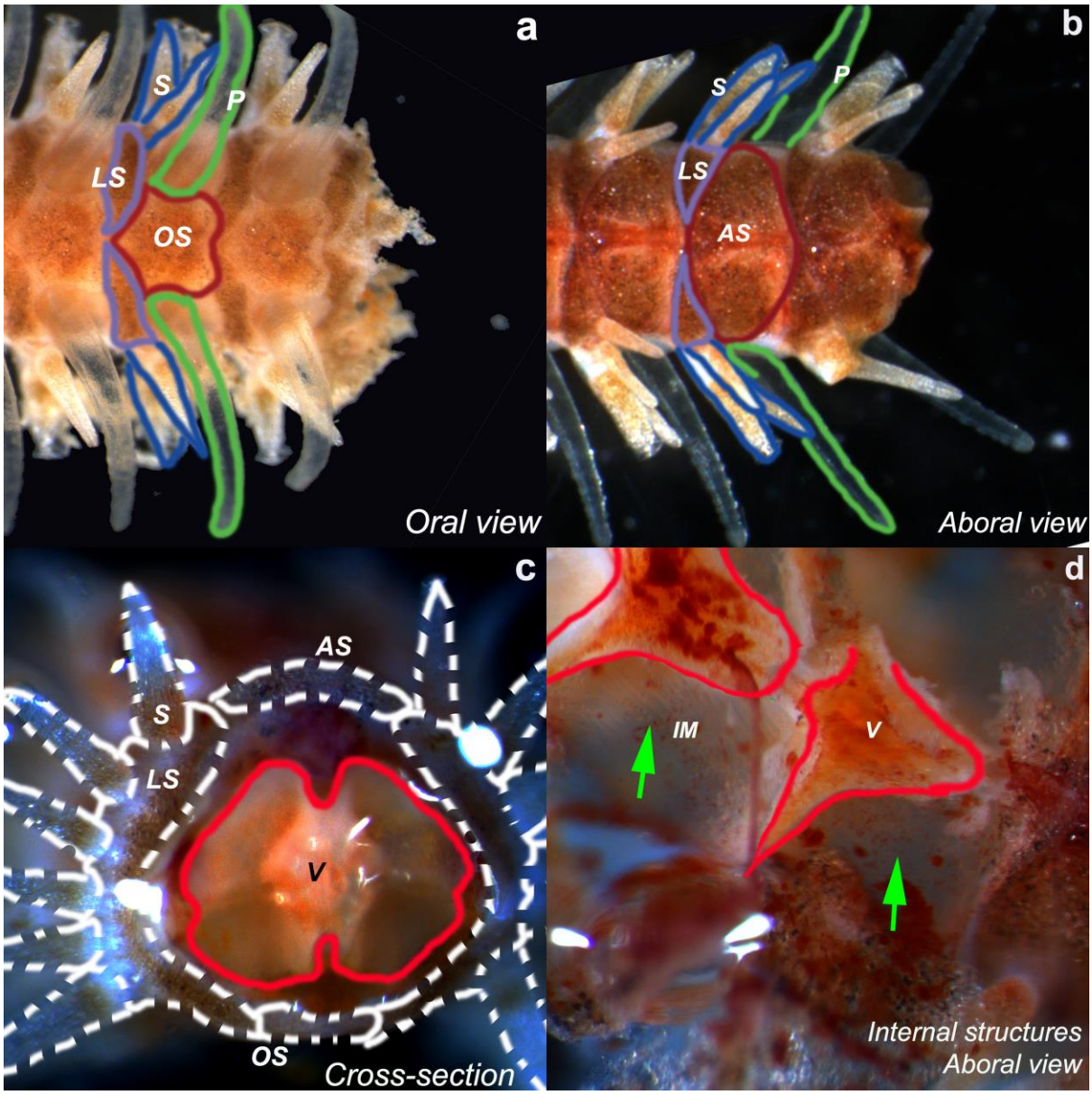

Figure S1: Anatomy of a non-regenerating brittle star arm showing skeleton and muscle structures. a) Oral view of the arm showing the position of the oral shield, lateral shields, podia and spines. b) Aboral view of the arm showing the aboral shield, lateral shields, podia and spines. c) Cross-section through the adult non-regenerating arm showing the position of the vertebra, oral and aboral shields, lateral shields and spines. d) Aboral view of the internal structures of the arm showing the vertebra and intervertebral muscles. *OS – oral shield, AS – aboral shield, LS – lateral shields, S – spines, P – podia, V – vertebra, IM – intervertebral muscles. Green arrows – intervertebral muscles.*

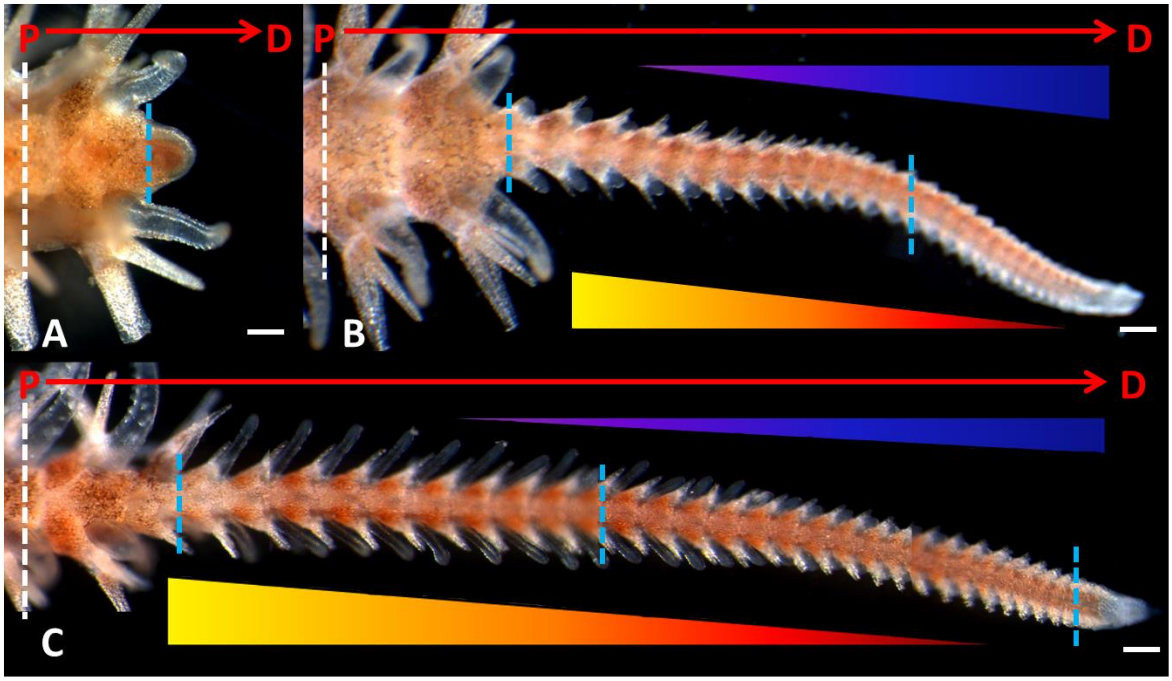

Figure S2: Oral view of the regenerating arm and amputation sites for sample collection. a) A blastema is formed distally to the site of amputation. b) As regeneration progresses into a 50% DI stage, the metameric units are added proximally with respect to the growth zone and differentiate gradually in a proximal to distal direction. There are two equal but opposing gradients acting on the regenerating arm – a gradient of differentiation (warm colour scale) and proliferation (cold colour scale). c) At the 95% differentiation stage only the distal-most segments remain proliferative and unspecified, while proximally, the segments acquire pronounced skeletal and muscle structures. The differentiation gradient remains the same however the proliferation gradient is greatly reduced at this stage. *Scale bars – 200µm*, w*hite dashed lines – site for cutting for WMISH experiments, blue dashed lines – sites for cutting for RNA extraction and QPCR. P – proximal, D – distal, warm colour scale – differentiation gradient, cold colour scale – proliferation gradient.*

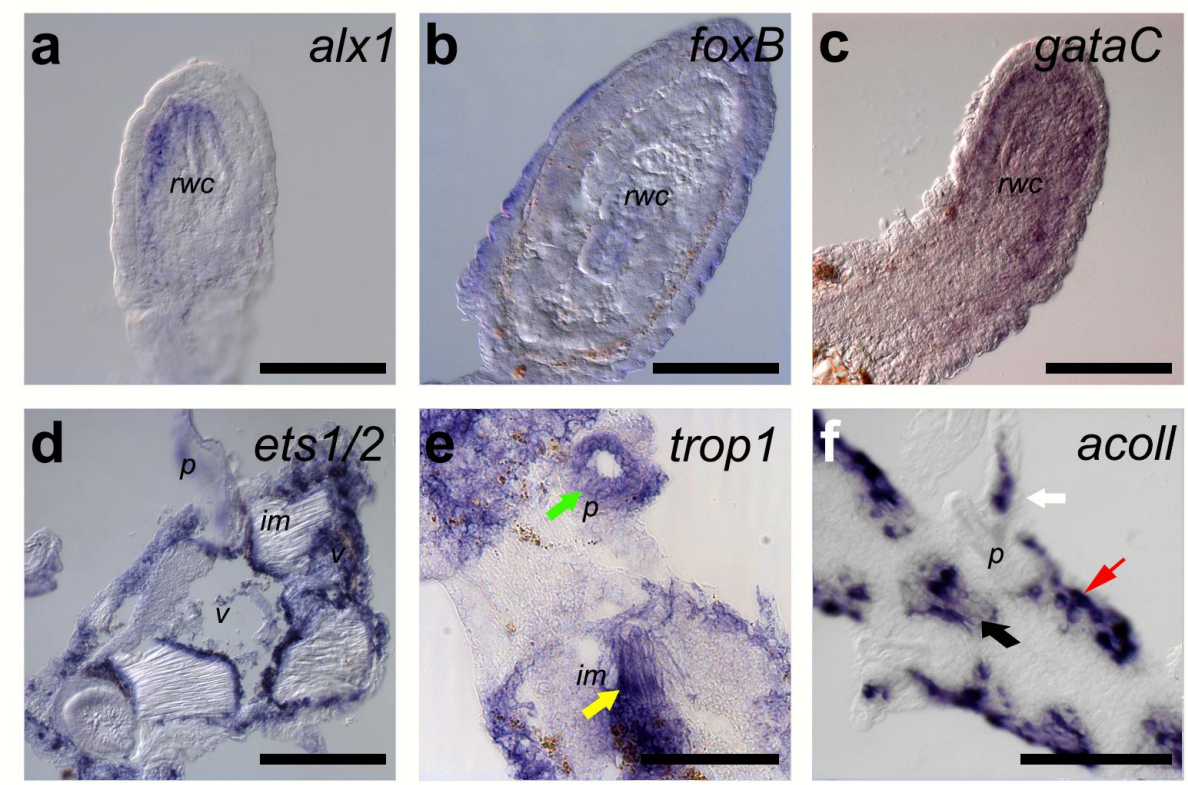

Figure S3: Supporting micrographs of longitudinal cryosections of arms after *in situ* hybridization. a,b,c) Cryosections confirm *Afi-alx1*and *Afi-gataC* are both confined to the inner layer of the blastema, in a subepithelial location, but *Afi-foxB* is expressed in the outer epithelium. Distal end of blastema is facing upwards. d,e,f) Skeletal elements interfere with keeping tissues intact after sectioning; however *Afi-ets1* expression can still be detected in the regenerating arm localized to the remaining tissue surrounding the missing vertebra. *Afi-trop1* is expressed at the base of podia (green arrow) and in the intervertebral muscles (yellow arrow). *Afi-acoll* is found in the spines (white arrow), oral arm shields (black arrow) and at the lateral arm shields (red arrow). *Scale bars: a,b,c,d – 100µm, e,f – 50µm*. *im – intervertebral muscles, p – podia, v – vertebra, rwc – radial water canal.*

Gene name	**Cloning/RACE primer sequences**	**Probe length**	**Accession number**
Afi-trop1	F: TGCGCTAGACTCCAAAGATG R: AGGGTTCTGTCGACGTCCT	820bp	JG391333
Afi-alx1	5O: CTTGCGCCATTTAGCTCTG 5I: GCCATTTAGCTCTGCGATTT	634bp	KC788414
Afi-ets1/2	5O: ACCATGGACGGATCAAACAT 5I: CGACGACTCCAGGCTGTAA	700bp	KC788415
Afi-foxB	F: AACACCCARMGNTGGCAGAA R: GATGATRTTCTCGATRGTGAA	424bp	KC788416
Afi-gataC	3O: ACCGCGTGGTTATAAGGAG 3I: GAGTAGGCCTGTGGACTGA	1019bp	KC788417
Afi-tbr	5O: TGTTTCCTGGAAGCTGTGTG 5I: CATTGTCTTTGCCCTTGTT	1127bp	KC788418

Gene name	Library clone[1]	Probe length	Accession number
Afi-*αcoll*	P2A8	3kbp	JG391435

[1](Burns et al., 2011)

Figure S4: Gene names, cloning primer sequences and NCBI Genbank accession numbers.