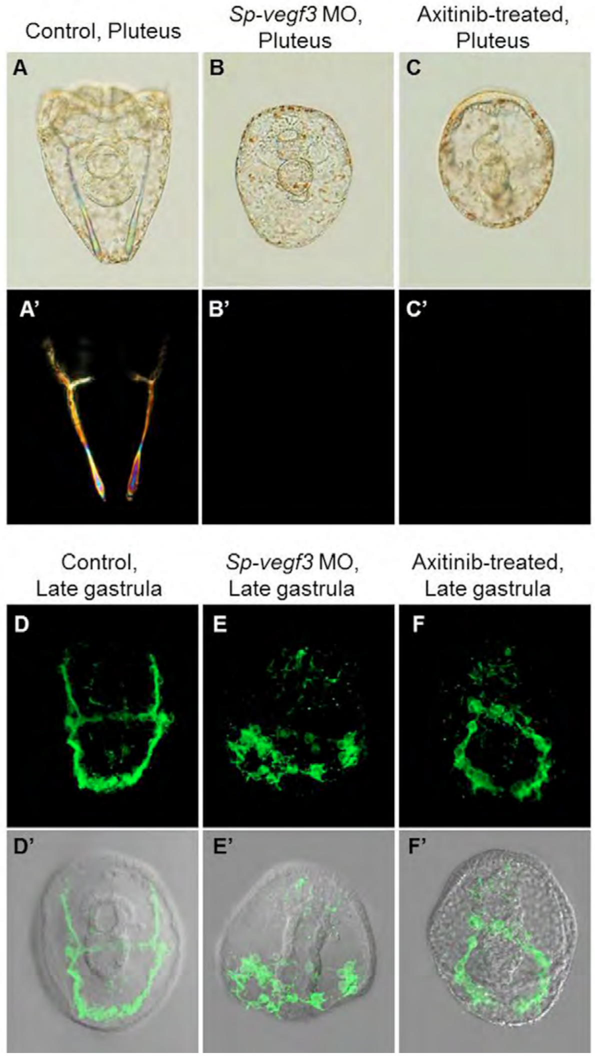
A)

```
Lv-vegf3    1 MGPSVDSKPGDRLSRVGLLD-ISGTN-VLCETTTRARDACHCSRDVADGN  48
Sp-vegf3    1 MGHSAEATPMDRLSPVGSPD-LSGTN-VLSGTSTGVRDSCKCSHYDADGR  48
Pl-vegf3    1 MGQSPEARPMDRISRVGSPDNLRGSNGPNSGTKSAARDSCQCSYLDDQAR  50
              ** *  .. * **.* **  * . *.*    . * .  **.*.**

Lv-vegf3   49 RVTERVDHAHFDKLSSTTSYPCASHATHNQVNSLSRPDLRHSRSMRTPLS  98
Sp-vegf3   49 RVFERVDRAQFTDVSSSTSYPCASHMTHNQTNSLSRPDVRHSRSRRTPSS  98
Pl-vegf3   51 RVIERVDHAHIT---TTTSHPCASHTTHTHMNSTLRPDVCHARSCRTPAS  97
              ** ****.*.      ..** ***** **.. **  ***. *.** *** *

Lv-vegf3   99 S---------SSAHHSDSVLSSQ---KATTTTTTDLLRNSHASWSLSCWF 136
Sp-vegf3   99 ATTSLSSSTSSSSSHTDSVFSSK---KAATATTPDLRRNSNASLSC-CWF 144
Pl-vegf3   98 TSSS-----SSSSSHRDSVLSSKRVTTTTTTTTPDLRRNSHASSLSCSWF 142
              .         **. * *** **.     ..*.** ** ***.**    .**

Lv-vegf3  137 SRRMYTGNIKPWTLSFMFYLFVLILSHQVESTHSVPALSR--RVDQRTNN 184
Sp-vegf3  145 SRRMYMGNITPWTLSFMFYLFVIILSHQVESTHSVPALSRRVTVEQRMRK 194
Pl-vegf3  143 SRRMYMGNIKPWTLSFIFYLFVIVLSHQVESTHSVPMLSQR-VIEERMRK 191
              ***** *** ******.*****..************ **.    ...*

Lv-vegf3  185 MNALEDYLNALSLNGTNTPRSRYFVNRSPSALNKRSYRRLGRAGSYSGSR 234
Sp-vegf3  195 VNSLEDYLNALSLNDTDTPRSRYFLNRSPSALNKRSYRRLGRAGSAGGSS 244
Pl-vegf3  192 VNSLEEYLSALSLNGTSTLRSRYYLNRSPTALNKRSYRRLGRAGSAGG-L 240
              .*.**.** ***** * * ****..****.***************  *

Lv-vegf3  235 INNAFMAKIEDERARVQCQPRDRVVDSYEELGIPRGYGDFLLPECIVVRR 284
Sp-vegf3  245 SDSAFFARLEDENARVQCQPRDRVVDSYEELGIPRGYGDFLYPECIVVRR 294
Pl-vegf3  241 QNSAFLARLEDENARVQCQPRDRVVDSYEELGIPRGYGDFLYPECIVVRR 290
                 ** *..*** **************************** ********

Lv-vegf3  285 CKQGGCCGDDQECVPSRTTNVTMNFLQVRQQIPIEIVHETVHDLECECQD 334
Sp-vegf3  295 CKQGGCCGDERECVPSRTTNITMNFLKTR----LQITREIVHDLECECQD 340
Pl-vegf3  291 CKQGGCCGDERECVPSRTTNITMNFLKTG----REITREIVKDLECECQD 336
              *********..*********.*****.        .* .* *.********

Lv-vegf3  335 KPSFCPEPVVDCPDDKVWSYSECTCKCRNRCPKPFLQDEDTCGCDCLSQD 384
Sp-vegf3  341 KPSFCPEPVVDCPDNKVWSYSECTCKCRNRCPKPFLQDEDTCGCDCLSQD 390
Pl-vegf3  337 KPSFCPEPVVDCPNNKVWSYSECTCKCRNRCPKPFLQDEDTCGCDCLSQD 386
              *************  ************************************

Lv-vegf3  385 RHCKNIYKGRRNGKLSQEECDCVRKGLCGKPPCINGAFSISDCKCINSNS 434
Sp-vegf3  391 RNCKNIYRGRRNGKLSREECDCVRKGLCGKPPCINGGFSISDCKCINSNS 440
Pl-vegf3  387 RNCKNIYRGRRNGKLSREECDCVRKGLCGYPPCINGGFSISDCKCINSNS 436
              *.*****.********.************ ****** *************
```

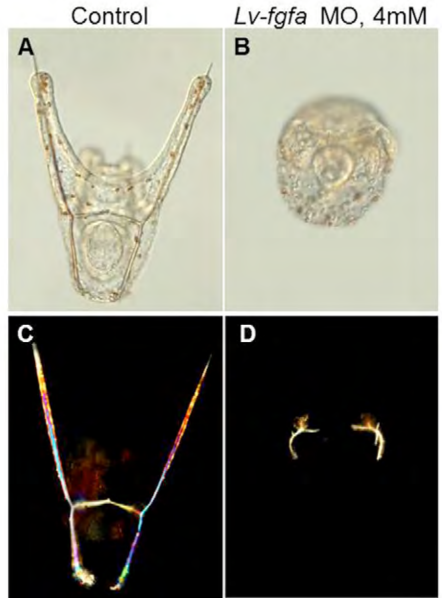
B)

```
Lv-fgfa    1 MKPKMDENYWWSSTIPASKRASHVIIIGFLCVSLAAGLSDDGGMRTRREH  50
Sp-fgfa    1 MKPKMDESCWWSSTIPASKRASHVIIIGFLCVTLAAGLSDDGGLQTRREQ  50
Pl-fgfa    1 MKAKMDESGWWSSSIPASKRASHVIIIGFLCVSLAAGLSDDGGLGTRREH  50
             ** ****  ****.******************.**********. ****.

Lv-fgfa   51 ADTRQQHHNQHQQIHTISATIRD-ADNSALLXNILASKKSASISTDSAAK  99
Sp-fgfa   51 ADTRQHHQNQ-QQPHTISATIRD-ADNSVLLHNLLATKNSASISTDSSAT  98
Pl-fgfa   51 ADTRQHHHNQ-QQLHTLSATIRDTADNSELLHNLLATKHSAQISKDSATG  99
             *****.*.** ** **.****** **** ** *.**.* ** ** **..

Lv-fgfa  100 EGHKSSNSVSNNIINQ-IKLSNITSSS-TTLSKLTASVLSRLNS-SPPSS 146
Sp-fgfa   99 G-HKSS-SLSNNIINQ-IKVSNITSSS-STISKLTASVLSRLNS-LPPSS 143
Pl-fgfa  100 H-HSSSHSNNNNNINQIIKVSNNTSSSSTTLSKLTASVLSRLNSKSPISS 148
               * ** *  ** *** **.** **** .*.************  * **

Lv-fgfa  147 SSGSNRTEQGERLHSWSPMNSDSSLQHHLRTGSQADAEPSRRVKRKASSR 196
Sp-fgfa  144 SSGSNRTERGERLHSWSPMSSDSMLEHHLRTGSQADAEPSRRVRRKASSR 193
Pl-fgfa  149 SSGLSRRDQGESLRSRSSMSANSTLPHHLRPSSQADAVPSNRVKRKASSR 198
             ***  * ..** *.* * * . * * ****  ***** ** **.******

Lv-fgfa  197 G---SPLIYNSKQPTQLFCRTNFRLAVHEDGTINGTRDNMDVYSSLYIQS 243
Sp-fgfa  194 G---STLIYNSKQPTQLFCRTNFRLAVHEDGTINGTRDNMDVYSSLYIQS 240
Pl-fgfa  199 GGNNNPLIYKAKQPTQLFCRTNFRLAVHEDGTINGTRDNMDVYSSLYIQS 248
             *     *** .*************************************

Lv-fgfa  244 QRRSIVSIKGLKSQLYVCVDDNGNLYGNRRVSRNCYFQEKLEPNFFNTYA 293
Sp-fgfa  241 QRRSIVSIKGLRSQLYVCVDDSGSLYGDTRVSRNCYFQEKLEPNFFNTYA 290
Pl-fgfa  249 QRRSIVSIKGLRSQLYVCVNDDGDLYGANRVSRNCYFQEKIEPNFFNTYA 298
             ***********.******* * * ***  ***********.*********

Lv-fgfa  294 YKMPDSTNKRRR-HRTLFLSINKYGESRIAKVRTQKKAQFIPLVPPTELL 342
Sp-fgfa  291 YKMPDSTSKRRR-HRTLFLSINKYGESRIAKVRTQKKAQFIPLVPPEELL 339
Pl-fgfa  299 YKMPDSTNKRRRRKHTPFLSINKYGESRIAKVRTQKKAQFIPLVPPEELL 348
             ******* ****  ..* ***************************** ***
```

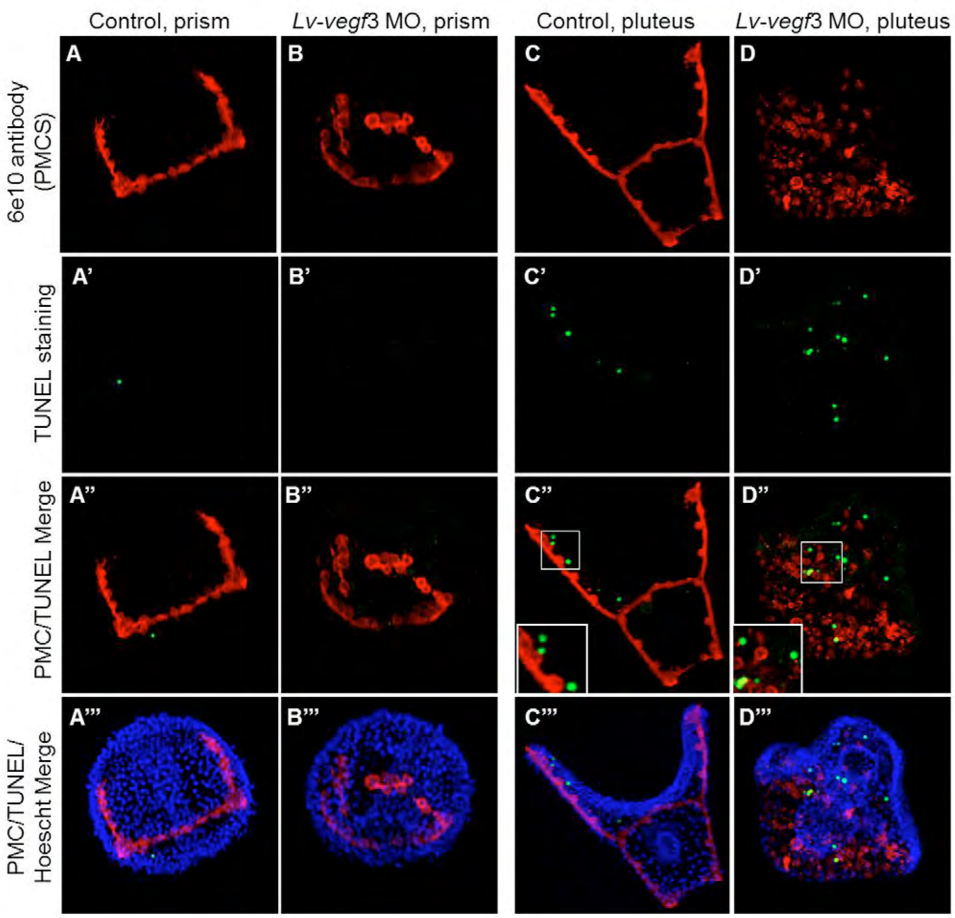
Supplementary Fig. 1: Analysis of VEGF3 and FGFA proteins in *L. variegatus*, *S. purpuratus* and *P. lividus*. ClustalW alignment of the of VEGF3 (A) and FGFA (B) protein sequences in *L. variegatus*, *S. purpuratus* and *P. lividus* show that VEGF3 proteins are 70% identical (A) and FGFA proteins are 80% identical among the three species of sea urchin. Asterisks show identical amino acids, and dashes show conserved amino acids.



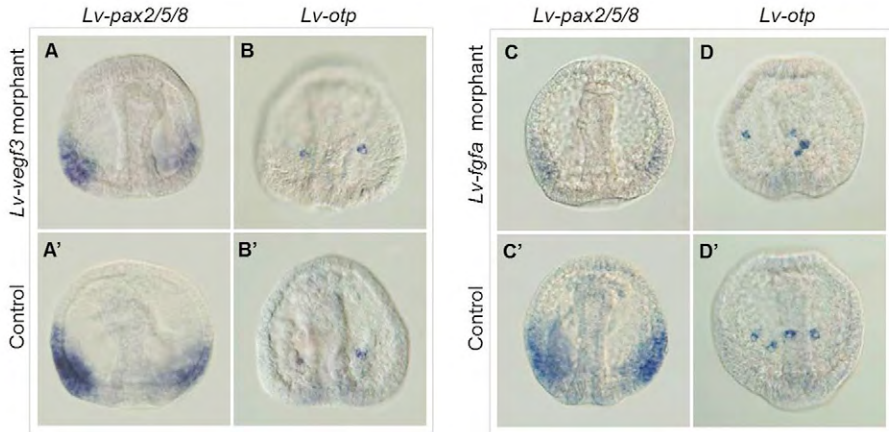
Supplementary Fig. 2: Perturbation of VEGF signaling in *S. purpuratus* inhibits PMC migration and skeletogenesis. DIC (A-C) and polarized light (A′-C′) images of *S. purpuratus* control embryo (A, A′), *vegf3* morphant embryo (B, B′) and embryo treated with 50n M axitinib from the 2-cell stage (C, C′) show that skeletogenesis is inhibited in *Sp-vegf3* morphants and axitinib-treated embryos. Fluorescence (D-F), and merged images with DIC (D′-F′) of control embryo (D, D′), *Sp-vegf3* morphants (E, E′) and axitinib-treated embryos (F, F′) at the late gastrula stage show by 6a9 immunostaining that PMC migration is perturbed in *Sp-vegf3* morphants and axitinib-treated embryos.



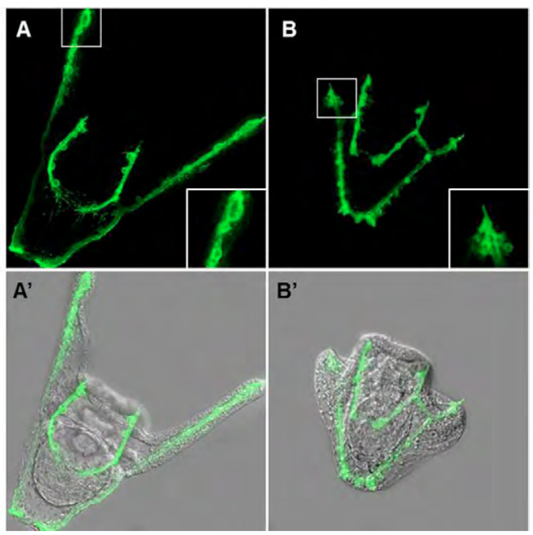
Supplementary Fig. 3: Knockdown of *Lv-fgfa* using a translation-blocking MO leads to the formation of truncated skeletal elements. DIC (A-B) and polarized light (C-D) images of control embryo (A, C) and embryo injected with 4 mM *Lv-fgfa* translation-blocking MO (B, D) show that shortened skeletal elements form in *Lv-fgfa* morphants.



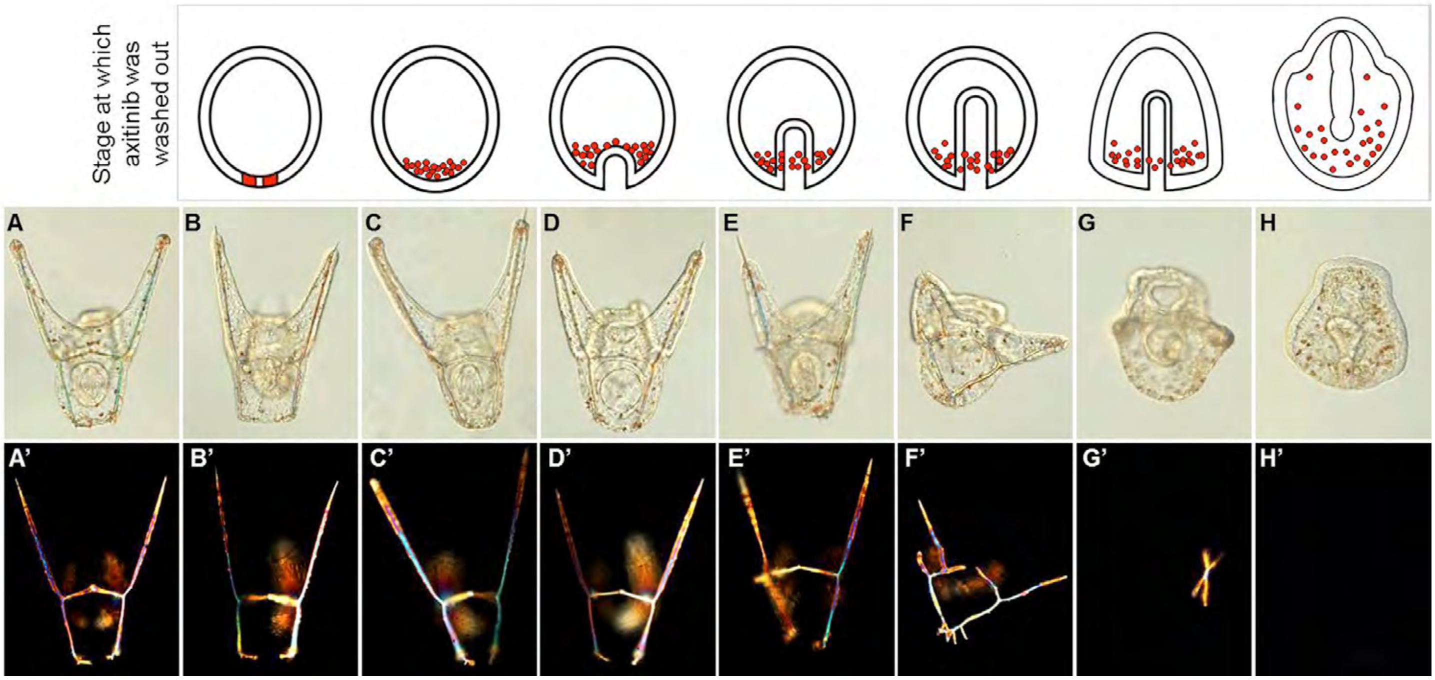
Supplementary Fig. 4: PMCs in *Lv-vegf3* morphants are not undergoing apoptosis. Fluorescence images of 6e10 antibody labeled-PMCs (A-D), TUNEL staining (A′-D′), 6e10/TUNEL merged images (A”-D”) and 6e10/TUNEL/Hoechst merged images (A”’-D”’). Though there is a general increase in the number of apoptotic cells from the prism stage (A-A”’, B-B”’) to the pluteus stage (C-C”’, D-D”’) in both controls (A-A”’, C-C”’) and *Lv-vegf3* morphant embryos (B-B”’, D-D”), there is no apparent correlation between the position of apoptotic cells and PMCs, either in control embryos or in *Lv-vegf3* morphants (C” and D”, inserts).



Supplementary Fig. 5: WMISH analysis of *Lv-pax2/5/8* (A, A′, C, C′) and *Lv-otp* (B, B′, D, D′) expression in *Lv-vegf3* morphants (A, B), *Lv-fgfa* morphants (C, D) and controls (A′-D′) at the late gastrula stage show that *Lv-pax2/5/8* expression is not strongly affected in *Lv-vegf3* morphants (A), while its expression is downregulated in *Lv-fgf* morphants (C). *Lv-otp* expression is not affected in either *Lv-vegf3* (B) or *Lv-fgfa* (D) morphants.



Supplementary Fig. 6: Blocking VEGF signaling at later stages does not affect the location of PMCs along the skeletal rods. Fluorescence (A, B) and merged images with DIC (A′, B′) of pluteus embryos treated with DMSO (A, A′) or axitinib (B, B′) from the late gastrula stage. PMCs are visible along the skeletal rods and at the tips of the body rods (A, B, inserts) in both control and axitinib-treated embryos.



Supplementary Fig. 7: The effects of VEGFR inhibition are reversible early in development. DIC (A-H) and polarized light (A′-H′) images of control embryo (A- A′), and embryos treated with the VEGFR inhibitor axitinib at the 2-cell stage and washed out of the drug at hatched blastula (B, B′), mesenchyme blastula (C, C′), early gastrula (D, D′), mid-gastrula (E, E′), late gastrula (F, F′), prism (G, G′) and pluteus (H, H′) stages. Embryos are able to recover and secrete skeletal elements comparable to controls if VEGFR inhibition is alleviated prior to the late gastrula stage (B′-E′), and truncated skeletal elements are secreted if the inhibitor is washed out at late gastrula or prism (F′, G′). Embryos do not form skeletal elements if axitinib is washed out at the pluteus stage (H′). Schematic diagrams indicate the stage of development at which axitinib was washed out of embryo cultures (PMCs are represented in red).

OBSERVED PHENOTYPE	STAGE AT WHICH AXITINIB WAS WASHED OUT															
	DMSO CONTROL		HATCHED BLASTULA		MESENCHYME BLASTULA		EARLY GASTRULA		MID-GASTRULA		LATE GASTRULA		PRISM		PLUTEUS	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
NO SKELETON	0	0	0	0	0	0	0	0	0	0	3	1.3	42	16.3	**236**	**100**
TINY SKELETAL DEPOSITS (SPECKS)	0	0	0	0	0	0	0	0	0	0	0	0	15	5.8	0	0
BRANCHED SKELETAL RUDIMENTS	2	0.9	9	3.6	5	2.3	11	4.3	5	2.1	**116**	**49.8**	**183**	**70.9**	0	0
EXTENDED RODS, WITH PROMINENT BODY AND DORSOVENTRAL CONNECTING RODS	0	0	0	0	2	0.9	1	0.4	0	0	0	O	7	2.7	0	0
SHORT PLUTEUS SKELETON	10	4.4	31	12.4	26	11.9	28	11.0	61	26.0	114	48.9	11	4.3	0	0
WILD TYPE PLUTEUS SKELETON	**214**	**94.7**	**211**	**84.0**	**185**	**84.9**	**215**	**84.3**	**169**	**71.9**	0	0	0	0	0	0
TOTAL	226	100	251	100	218	100	255	100	235	100	233	100	258	100	236	100

Supplementary Table 1: Distribution of phenotypes in axitinib washout experiments. Bold type indicates prevalent phenotype for drug washout at each stage of development.

Supplementary Table 2: List of probes used in Nanostring nCounter analyses (excel document).

Download Table S2

Supplementary Table 3: Complete table of results obtained from Nanostring nCounter analysis of changes in gene expression between control and either axitinib treated embryos or *Sp-vegf3* morphants (excel document). Table shows, for all three trials conducted, the numbers of transcripts counted in control and either axitinib treated embryos or *Sp-vegf3* morphants, the percentage changes in transcript numbers between controls and either axitinib treated embryos or *Sp-vegf3* morphants, and the average percentage changes obtained.

Download Table S3

Primer Name	Primer Sequence
Lv-fgfa forward degenerate primer	5'-CAYGARGAYGGNACNATHAAYGG-3'
Lv-fgfa reverse degenerate primer	5'-GGDATRAAYTGNGCYTTYTTYTG-3'
Lv-vegf3 forward degenerate primer	5'-CGTGTGGTGGAYTCGTACGAGGAGCTG-3'
Lv-vegf3 reverse degenerate primer	5'-GGCACTTGCAGGTRCACTCGCTG-3'
Lv-vegf3 5' RACE primer	5'-CTGCACCCGTGCCCTCTCATCCTCAA-3'
Lv-vegf3 3' RACE primer	5'-GGAGCTGGGCATCCCCAGAGGGTAT-3'
Lv-fgfa 3' RACE primer	5'-GTCGCAGAGGCGGAGTATTGTTTCCAT-3'
Lv-pax2/5/8 forward primer	5'-GATAGAATTCGCCAGCACGTTGTCGA-3'
Lv-pax2/5/8 reverse primer	5'-GCAGTCTAGATGTGGCGATATCACCC-3'
Lv-otp forward primer	5'-GGTCGAATTCATGGAGCGAACTCTAG -3'
Lv-otp reverse primer	5'- GGCATC TAGACT AAAGATTCCCATTGA -3'
Lv-fgfa forward RT-PCR primer	5'-GGTTGCATAGCTGGAGCCCAATGA-3'
Lv-fgfa reverse RT-PCR primer	5'-CCTTTTGTTGGTGCTGTCTGGCATC-3'.

Supplementary Table 4: List of primers.

MO Name	MO Sequence
Lv-fgfa splice-blocking MO	5'-TAATAAACCCTACTTACGTTTCCGT-3'
Lv-fgfa translation blocking MO	5'-GTCGCACACAGACGATGTCCAACGC-3'
Lv-vegf3 translation-blocking MO	5'-TCGACTGAAGGTCCCATCGTGCTTT-3'
Sp-vegf3 translation-blocking MO	5'-GGCTGAGTGCCCCATCGTGCTTCAA-3'
Standard control MO	5'-CCTCTTACCTCAGTTACAATTTATA-3'

Supplementary Table 5: List of morpholino antisense oligonucleotides.