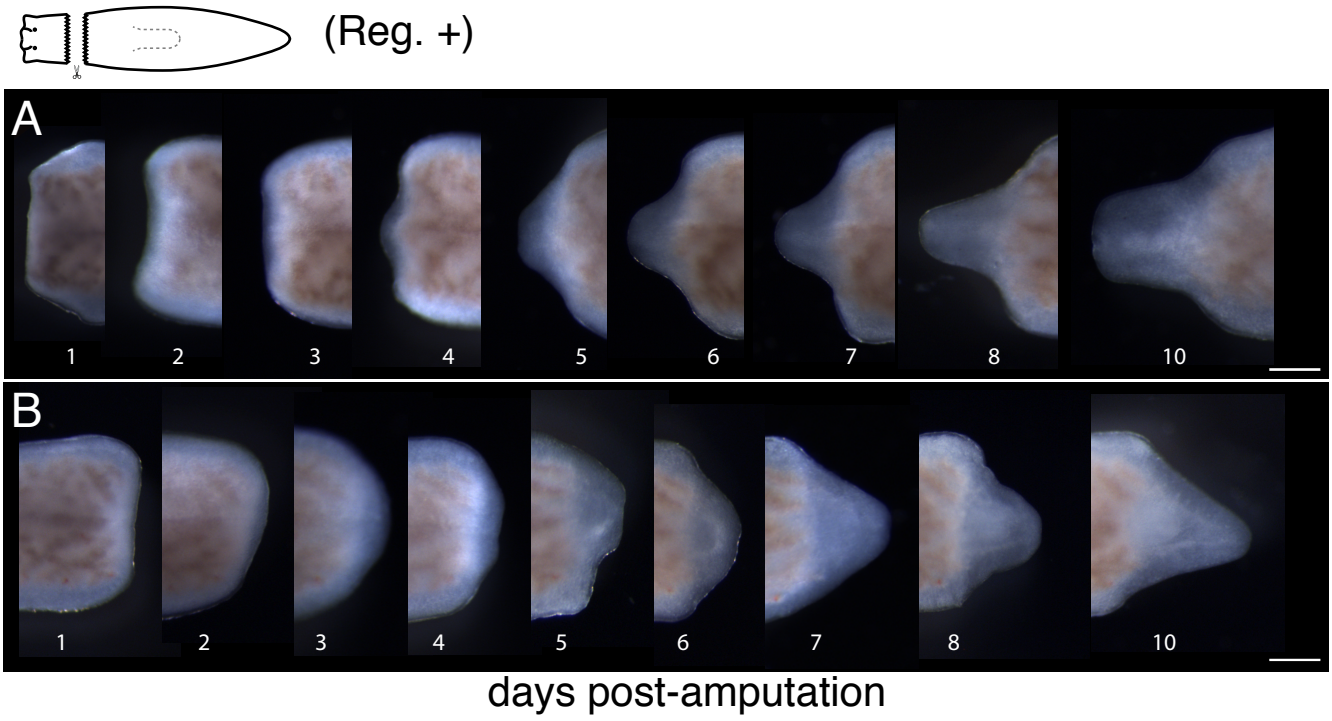
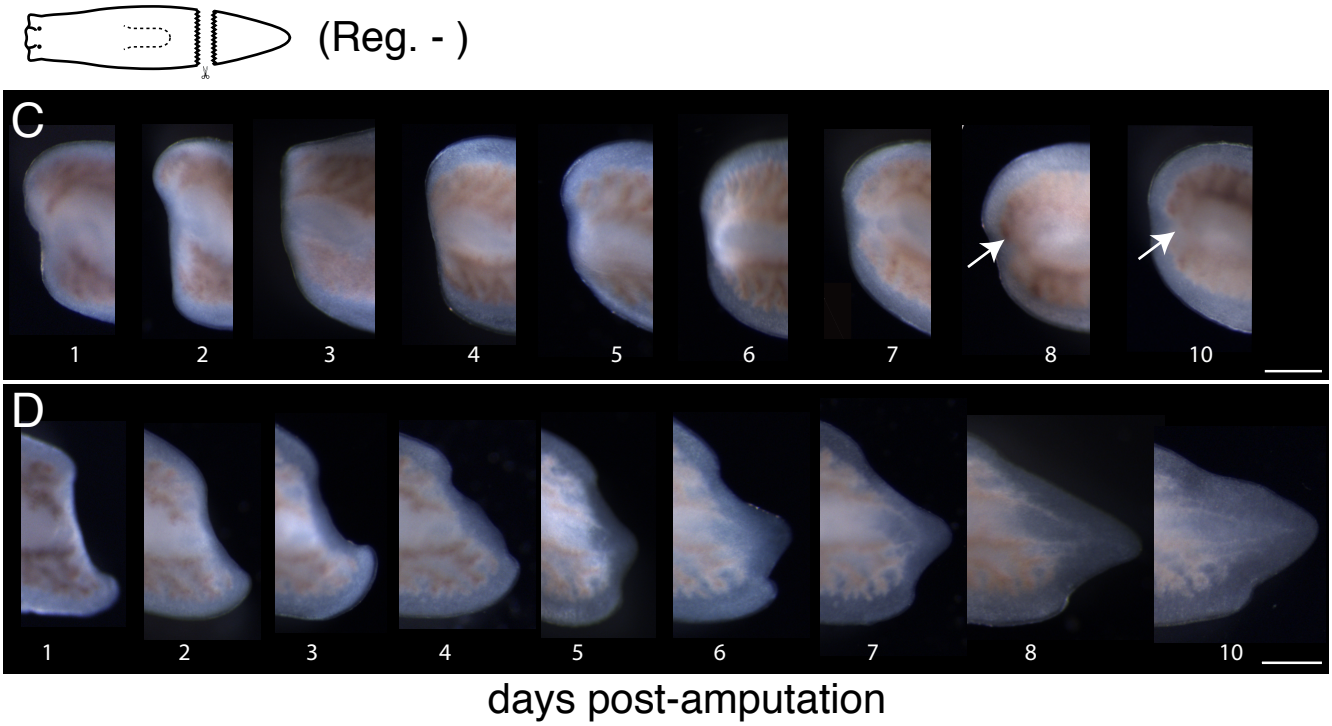
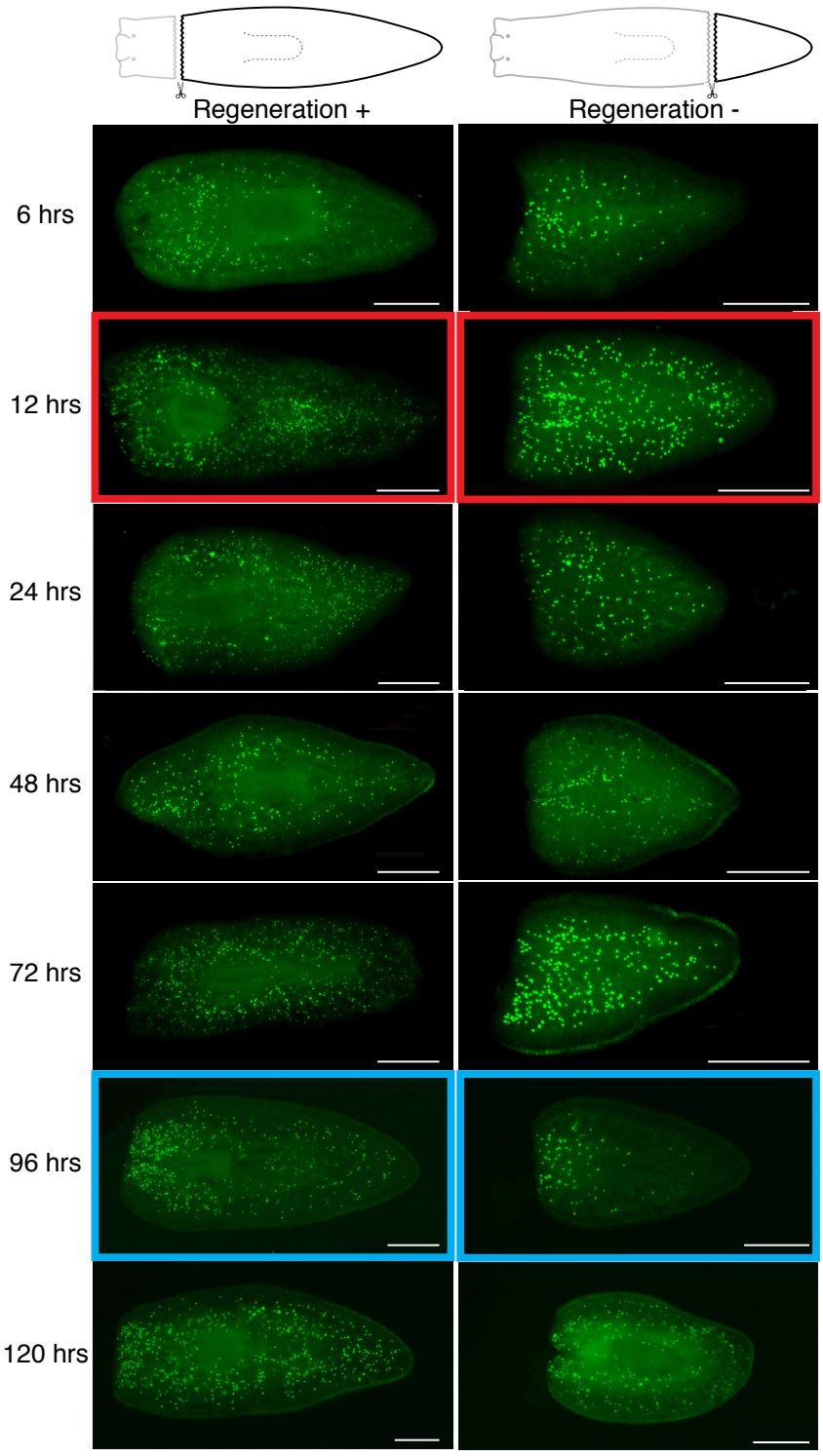
Supplemental Data

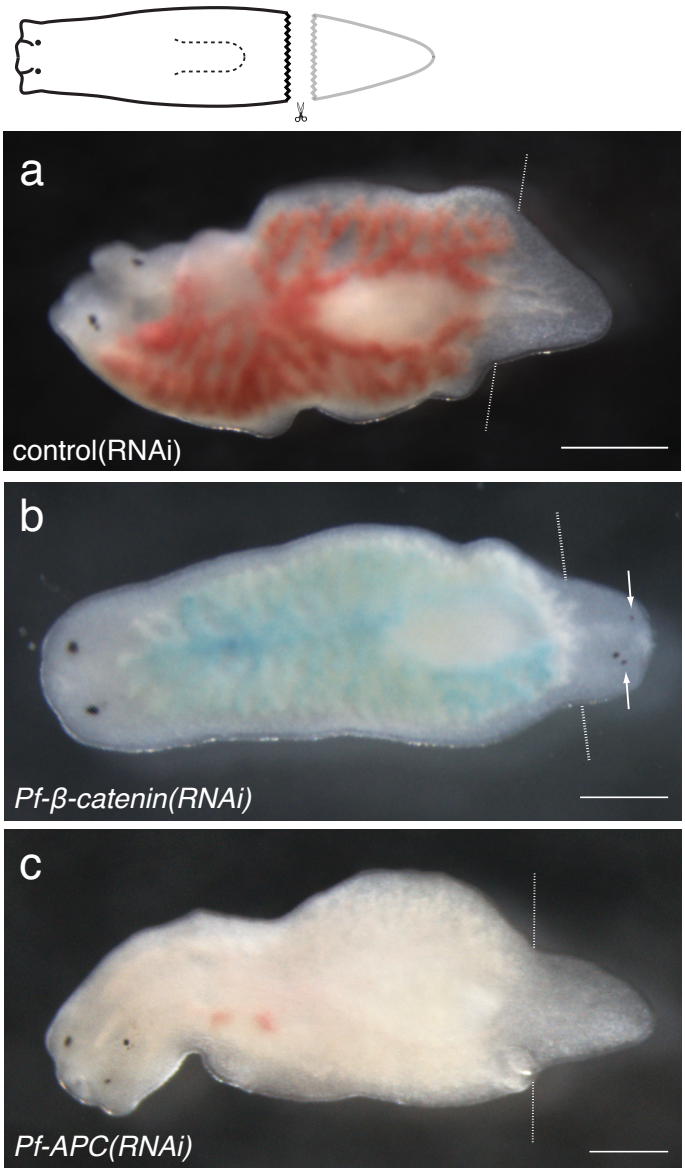




Supplemental Figure 1 | *Procotyla fluviatilis* has limited regenerative abilities. Animals fail to regenerate anterior structures when amputated in posterior body regions. (a) Tail regeneration over 10 days following amputation in Reg+ tissues. (b) Complete head regeneration 10 days after amputation in Reg+ tissues. (c) Tail regeneration occurs in posterior-facing wounds after amputation in Reg- tissues. (d) Head regeneration fails to occur when amputated in Reg- tissues posterior to the pharynx. White arrows indicate repatterning of gut branches. Cartoons represent site of respective amputation. Scale bars, 250 μm. Anterior is to the left in all panels.



Supplemental Figure 2 | Neoblast proliferation following amputation in Reg+ and Reg- tissues. Phosphohistone H3 (Ser 10) immunostaining reveals patterns of cell division after anterior amputation. Red boxes indicate time point with a systemic increase in cell division. Blue boxes indicate time point with a localized increase in cell division near the wound site. Anterior is to the left in all panels. Scale bars, 500 µm. Note that regeneration occurs more slowly in *P. fluviatilis* when compared to other planarian species, such as *Schmidtea mediterranea* and *Dugesia japonica*; therefore mitotic peaks appear somewhat delayed.



Supplemental Figure 3 | Regeneration of *P. fluviatilis* (RNAi) animals after amputation. (a) Normal tail regeneration occurs at posterior-facing wounds 15 days after amputation in control(RNAi) animals. (b) *Pf-β-catenin1(RNAi)* animals regenerate a second head at posterior-facing wounds within 15 days after amputation. White arrows indicate regenerated eyespots. (c) Normal tail regeneration occurs at posterior-facing wounds within 15 days after amputation in *Pf-APC(RNAi)* animals. Dashed lines represent amputation planes. Cartoon represents site of amputation. Anterior is to the left in all panels. Scale bars, 500 μm.

Beta-catenin [E value = 0.0]

```
Pf-Bcat          1 M------NEQL---ISMNSPQSDDVLIDKTSFTRSWQQNQYLIDSGINSALETHTHSVSS
Sm-Bcat1         1 M------MNESM---NIVNSPLADEFINDKTNPTRTWQQNQYLIDSGINSALESHSHSVSS
Dm-Armadillo     1 MSYMPAQNRTMSHNNQYNPPDLPPMVSAKEQ-TLMWQQNSYLGDSGIHSGAVTQVPSLSG

Pf-Bcat         52 RH----------------GYDEMDSEDQNKK--DWKCLSNI----------NINDGFPNDI
Sm-Bcat1        53 KH----------------GYDDMDSEDQSKNLNEWKYPSNT----------NANDGLSNDI
Dm-Armadillo    60 KEDEEMEGDPLMPDLDTGFPQNFTQDQVDDMNQQLSQTRSQRVRAAMFPETLEEGIEIPS

Pf-Bcat         85 CMLTPDSPGSNICGRQGGEAM--ADVHSTSGYDT-GRTDCDKEFGLDIDKLEAEGAIPDL
Sm-Bcat1        88 CMLTPDTPGSTSCLRHGGEGM--ADVSLSACYDNKDENNRNPDFGIDIDKIEAESAIPDL
Dm-Armadillo   120 TQFDPQQPTAVQRLSEPSQMLKHAVVNLINYQD---------------DAELATRAIPEL

Pf-Bcat        142 VKLIMDEDDAVVINEATMMVFQLSKSDAI-DALINSKEMISCIIEAMNKADHADAVRFLA
Sm-Bcat1       146 IKLINDEDDAVVINEATMMVFQLSKTDAI-DAIINSKEMISCMIEAMNKAEHAEAVRFLA
Dm-Armadillo   165 IKLLNDEDQ-VVVSQAAMMVHQLSKKEASRHAIMNSPQMVAALVRAISNSNDLESTKAAV

Pf-Bcat        201 GAIYNMSQKKYGLKVIPEANVIPCLVKLLGFSMESVLFYAITTLHNLLLYQEGGKEAVRL
Sm-Bcat1       205 GAIYNMSQKKNGLKIIPEANVIPCLVKLLGFSMESVLFYAITTLHNLLLYQDGGKEAVRS
Dm-Armadillo   224 GTLHNLSHHRQGLLAIPKSGGIPALVKLLSSPVESVLFYAITTLHNLLLHQDGSKMAVRL

Pf-Bcat        261 SSCIPKLVTLLQKNNIKFLTICTDCLQILAFNHQPSKIEILKNGGPLHLIHIIKNYDYEK
Sm-Bcat1       265 SGCIPKMVALLQKNNIKFLTICTDCLQILAFNHQPSKLEILKHGGPLHLIHIIKSYDYEK
Dm-Armadillo   284 AGGLQKMVTLLQRNNVKFLAIVTDCLQILAYGNQESKLIILASGGPNELVRIMRSYDYEK

Pf-Bcat        321 LLWTATRVLKVLSVCSANKPIIIREGGIEALTNILYHTIQRNSTVTPYHEEQTNENNESH
Sm-Bcat1       325 LLWTATRVLKVLSVCSANKPVIIREGGMDALTNILYHTIQKNTNVEPYPQVLPAENSESY
Dm-Armadillo   344 LLWTTSRVLKVLSVCSSNKPAIVDAGGMQALAMHL--------GNMSP-------------

Pf-Bcat        381 PQFSQRLLHNCLWTLRNLSDAATRIN-FDHLLKVLVQIL----------------------
Sm-Bcat1       385 PHFSQRLLHNCLWTLRNLSDAATRLN-PDHLLKVLVQILMNAFNSFQRQSAHIDTNVITC
Dm-Armadillo   384 -----RLVQNCLWTLRNLSDAATKVEGLEALLQSLVQVLGST-----------DVNVVTC
```

APC [E value = 0.0]

```
Sm-APC           1 DNNSNNSMLRKRNFHSYPDDYDSDTSEKPLDYSRQFGDTDEYMVYPTPIIPNQNDQNPVT
Pf-APC           1 SN------FKKRNFHSYPEDYDSDNSEKPLDYSRQFGDTDEYMTYPAPILLNQNEQSQIN
Dm-APC           1 GKSDSTKYSTKSEGAKNPFEIVTPTEEQPIDYSMK------YMEHK----PNSSKTFE--

Sm-APC          61 TQNNAFRNIINIPPPT---IKEKPKVYATEGTPVQCGSRMSPLSEEAIEDDVSNA----F
Pf-APC          55 PQVNIFRNIINIPPPT---IKEKPKVYATEGTPIQCGSRMSPLSEELEEEDAMNVGASQY
Dm-APC          49 ---------IDLDQPTDFSARYKERRSAQTAQP-ELKSETNEIRSKELQLTKSSSATELR

Sm-APC         114 NPIPVLLEESCCYSVSKKPGLEKIEERVP--SEIRENYPSTPVSTDTSALKLNKSV----
Pf-APC         112 NVMQIIHEDACCFPVSTKPELEKSEDRAN--SEIREMFSTIFVSNDNN--KSNKCV----
Dm-APC          99 NSPGLVAVSAAKQKIATETETETAERPINYCEEGTPGSFSRFDSLNSLTEKPEKCMPPKT

Sm-APC         168 --KFGSEPPHI----------IQDTPLMFSRSSSVGSL---DSFDTHSPHSSVQSEYSRR
Pf-APC         164 --KFESEPPHI----------IQDTPLMFSRSSSVESL---DSFETHSPHSSVQSEYSRR
Dm-APC         159 PTKTAVLPVHVDGNTPQNIDSALETPLMFSRRSSMDSLVGDDETVACEDNGSVISEYSRM

Sm-APC         213 TSEVVSPSELPDSPSSEIESKSPTKTAHKTASNKSRNSVSPSYSSSLSIDDNVSSTGNGT
Pf-APC         209 TSEAVSPCDLPDSPGSDMYHRSDQKSIQTT---KSKNSNSMSYSSSVSIDDNIS-TGNSN
Dm-APC         219 QSGVISPSELPDSPTQSM-PQSPRRDRKVSTQN---NLDTPEQKPSTVFEDKLNRFHVEH

Sm-APC         273 NNAAILQECIQSGMP---YSKHSEKLSKSLKSVNDKYVDNCETTISYAIEGTPFNYSTKA
Pf-APC         265 NSSAILQKCIESGMPPISKSSGSNKSSKLMIKTNGRFIDELETAVCYAVEDTEYNYSTKA
Dm-APC         275 TPAAFSCATSLSNLSMMDDSNANAIRGQRGNDING----NGDAPRSYCTEDTTAVLS-KA

Sm-APC         330 SSLSNISIPDPLEIKDQSDTSNKEQSSSS-SD-SEEQSELLSEIIQSAMPKGLNANTTLS
Pf-APC         325 SSLSDISISDPLNNKTKTCDLNKSSSSSSCSD-SEEHSELNSEIIQSAMPKGINGTSTFA
Dm-APC         330 PSNSDLSI---LSIPNDLNANEAQPVPAPRADVTGMDTRMPAEDAISKMRCGGNALPSYL

Sm-APC         388 LVHPPVN--FIQTDGVTASNTNCSTFITMSAMQYNPCYPAVVPHKSNKVQIENNSDANLV
Pf-APC         384 SIQPAVN--YAQVEPTNISN-NCSTFVTLSNIEYTESYPMIAPHKAIKSQIDNNAESNMS
Dm-APC         387 PVSDEMSKYYVEDSPCTPSVISGLSHLTVGSAKAGP---------VLKLPMRTAEEAQAP

Sm-APC         446 KFPQFYYCDIDSPKQYGVEGTPNSLSQRDSICSETNLMIKVSSD-IYSKPPGAPP--KPP
Pf-APC         441 KLSQFFYCDIDSPRQYVVEGTPNSLSQRDSICSESNLLLKNSIETLLVKPSLVPP--KPP
Dm-APC         438 KLP---------PRRSAVQGDAEPRLPPKKSDSLSSLSMDSDDDCNLLSQAIAAGSCRPQ

Sm-APC         503 KRTTSTLSSVP-----------------------------------------------------
Pf-APC         499 KRTTSTLSSVPEKATSLHMNEDDSNQEDLHVYMTENTPADYSRASPISHSLLDTPSLHET
Dm-APC         489 PSGASTSSSLANASTSTLCRENGQS-----------------------------------
```

nou darake [E value = 1e-118]

```
Pf-ndk           1 FIVKAIDIHSKQFEIDCQSRD---SGPCFLVPSL-TNEPEYIVEENQKIAMDCRAVMNSS
Sm-ndk-like5     1 FIVKAIDVTSKQFDLDCKSVE---DGPCFLVSSI-DNDFIFEVDEGQKVTFDCRAIVNST
DJ-noudarake     1 FNVEIIDSLQERLCAEKNPKDSKLDPPCFVDPQMRSGKITIRKEVGSTVKLPCDAI----

Pf-ndk          57 V-KDPVTYSWKRMASTESYEMKYKNSANNDGLRNTDQLLDVVPPIRKVVPNTVDDMSKWT
Sm-ndk-like5    57 TNKNQPTYNWSKVKITQSYHIK--QSLTNTGYKKSNQMLEMVPKIDKSQIDSISDMSQWG
DJ-noudarake    57 -GSPQLKYLWLTGKTIANW------IQNVEGTR-------------------------G

Pf-ndk         116 SIVMFEKVTKDMDGFYQCVASSSTHSIKRTFRLKIREIKSMNAIEGPQYQYIQTAVGSTQ
Sm-ndk-like5   115 SMVTLDKVTKEMDGLYRCSVSTTEPRISRQYRLRVKEAKSSSSIEGPLVQNISVTMGSSH
DJ-noudarake    84 PVITIRNLKSHYTGQYTCQVRNKAGSLNYTYQLIVTDPSNNS----PKI----LTDLSNY

Pf-ndk         176 TLYCK---SFS--------KP--KWGKKLDINDETP-ESEEIIKWGGSRYKMLQGSLDLE
Sm-ndk-like5   175 LLTCKATNSLS--------KP--KWGKKIEKNELGPVDLTGIIKWENSQYKILQGSLDIE
DJ-noudarake   136 TFRSKENISISVEIECECKEPIIKWLKRVDNFDSSNKEQSNGLVLPNAKDSE-KGELYVT

Pf-ndk         222 HRANGGSVK--DSKYYMNNLKLENLQTSQSGLYVCTVLT-SSTL---EHKYVNLTVEAGP
Sm-ndk-like5   225 HMNNGGMMN---NAKYYVNNLKLENIGKADLGTYICSVLTQSNSP---ETKYVYIDLKPG-
DJ-noudarake   195 -LDTGVSTKRIQPNLFKTLLNMESVETQQSGKYVVMAINKIVNLKNFEYKVVYITIEPD-

Pf-ndk         276 GYSDSSKKSNNFPIYIAVPTTVFIICLATIIYCLIHR------RMRQKANFKNRVARGTT
Sm-ndk-like5   279 GVKESSK-----FPLYAIGPIAVFVICVATVLYCLIRR------KVHQGNNFKNRSGRGPA
DJ-noudarake   253 --KDAIGIKNNLVVYIAVPMVILASSLGVIIYCCFRRHSSGYGRSASASRSSHRCANGSA

Pf-ndk         330 VTTTNSSSGNGSYSGSKNTSNPRHHMLTTTTSSSKGSQNQFSLKNEDAIMPLFTEEPKRM
Sm-ndk-like5   329 IPTTNTSSANGSSSGSRNASNPRQHMLPTSTVSSKTFQTQYVSSNE------YPTNPSST
DJ-noudarake   311 LP----SLTNGSNTGSEKLKSPS----TTSTGMVVGY-------------------NP---
```

sFRP1 [E value = 7e-103]

```
Dj-sFRPA         1 IQHESIEEAAQHSRVWIGLVNTQCHIDIRKPICCLYAPVCISNQRIQKVPPCRELCVTVK
Sm-sFRP1         1 IQHESIEEAAQHSKVWMGLVNTQCHVDIKKPICSLYAPVCISNQRNQKVPPCRELCVTVK
Pf-sFRP1         1 IQHEYLDETVQHSKVWIGLVNTQCHEDIKKFLCSLYAPVCINNMRVQKVPPCHELCISVK

Dj-sFRPA        61 ESCLPSMKMYGPDWPTIMKCSKPPQFQNSLCIPRTIVPEKNCDLCIKNPSYEVIANRFCL
Sm-sFRP1        61 ESCLPSMKMYGPDWPTIMKCSKPPQFKNSLCIPKTVVPGKKCDLCMKIASYEVIANRFCL
Pf-sFRP1        61 EACLPSMKLYGPEWPNIMKCSLPPSVRSALCIPRTVNKEKKCRACVKAASYEIMANKFCL

Dj-sFRPA       121 SPIVIRAKIKRIIPIGSKEMQIILQKKSKPLKFSANFSQNDLNLRLKCNCTNLKFKQRAL
Sm-sFRP1       121 SPIVIRAKIKRIIPTSGNAIQIILQKKSKPLKFQANFSQNALELNLKCNCTNLKFKQRAL
Pf-sFRP1       121 SHMVIRAKIKKLPPY-----------------------------------------------
```

wnt11-1 [E value = 2e-73]

```
Pf-wnt11-1       1 CHGVSGSCANEICPRQLRRLDDVVLLKKLKDSYITAKYVTSGRNGILYTD-------QNV
Sm-wnt11-1       1 CHGVSGSCINKICPRQLRRLDDEELLKMIKDYYIRAKHVTMEDDNKLYPTHFITTGLQKE

Pf-wnt11-1      54 ALKETELAFMEHSPDYCEPEPKMGSIGTLNRQCETTNLTQADTYHCNNMCCGRGFRTSIE
Sm-wnt11-1      61 PLKTTDLAFIEHSSDYCEPDPSAGSIGTQGRQCETVNVTLSKTNHCSNMCCGRGYREVME

Pf-wnt11-1     114 EETVKCKCSIDQYFSVKCKLCTKINEYKFCL
Sm-wnt11-1     121 KETVQCRCELVDVYIVKCKQCIQINIRKFCL
```

wnt11-2 [E value = 8e-120]

```
Sm-wnt11-2       1 PENQELERFSPLECRFNNLNHGLQKRQTKFCLSLQHSYAMHGVLRASKAAVFYCMKTFQD
Dj-wnt11-2       1 ----------------------------------------------------KAAVFYCMKTFQD
Pf-wnt11-2       1 PEIHELKAFSEVECHFISTNHGLRQRQARFCLSKQHKFAMHGILRGAKAAVYYCMKTFQD

Sm-wnt11-2      61 RRWNCSSAERLPYIGKDLESGKKLIWFHGYIFQSGTQEQAVVHAPSSASLLFEIARRCAQ
Dj-wnt11-2      14 RRWNCSSAERLPYIGKDLESG--------------TQEQAIVHAPSSASLLFEISRRCAQ
Pf-wnt11-2      61 RRWNCSSTERLPHVGKDLQLG--------------TQEQAVVHAPASSSVLYEIARRCAQ

Sm-wnt11-2     121 NKMAHCSCGTSNPTANQEITQEKLLPAGCPDNVEIGLEYVRKFTGYHKLGRKK-SKEKPR
Dj-wnt11-2      60 NKMAHCSCGPSGPNTNKDIIQEKLIPAGCPDNIEIGLEYIKKFTGYHKLGRKKLKKRKPN
Pf-wnt11-2     107 NKISYCSCGNSDQSQSEENSQRRLMPAGCPDNVDVGIKYVKKFTGYHKSGRKPKKK----

Sm-wnt11-2     180 KEKFSFYVAEKRAKMQKKLKKIQRLNGHNYEAGLRIQLDNQKIHCKCHGVSGGCNQRICY
Dj-wnt11-2     120 RENLNYYVVDKQAAMQKKLKTINRLNGHNYEAGLKIQMENQKQHCKCHGVSGACTQRICY
Pf-wnt11-2     163 ----------LQKVQDDKVKRIRIINGHNYKAGIMIQLENQRTHCKCHGVSGSCAQRVCY

Sm-wnt11-2     240 RQVRRLDDDVLLNVLRKQYLSAKYVDILSDDSLVAADPFGGSE-ERPKPISQTDLVFTEH
Dj-wnt11-2     180 RQIRRLDDEELHTILRKQYLAAKYVEIQSDDTLVAVDPFGGSD-ESAKPVSSKDLVFIEH
Pf-wnt11-2     213 RQLRRLDDDVLLNVLRKQYLTAKFVEISADNKLYIGNPFETQEGPTLKYLSNTDLAFIEH

Sm-wnt11-2     299 SPDFCEPDPTTGSVGTHGRVCKTENTSKTSTEHCTNMCCGRGHRTVLEQKRTKCGCFIDD
Dj-wnt11-2     239 SPDYCEPDTSTGSLGTHGRKCKMDNVSKTSTEHCTNMCCGRGYRMLLEQLRTKCGCRLDE
Pf-wnt11-2     273 SPDFC----VGGSFGGLLR-------------------------------------------

Sm-wnt11-2     359 KPKIQCDDCYSNVKTFYCL
Dj-wnt11-2     299 -PKIYCDDCFSNVKTYYCL
Pf-wnt11-2     288 -------NSWQTLQDRECE
```

Supplemental Figure 4 | Sequence alignments of characterized *P. fluviatilis* genes. *Pf-β-catenin1, Pf-APC, Pf-ndk, Pf-sFRP1, Pf-wnt11-1*, and *Pf-wnt11-2* were aligned to the corresponding planarian (Smed, *Schmidtea mediterranea*; Dj, *Dugesia japonica*) and/or insect (Dm, *Drosophila melanogaster*) homologs. Homology was based on sequence similarity via BLAST. All *P. fluviatilis* protein sequences were deduced from cDNA sequences. The expectation value (e-value) shown corresponds to the top reciprocal BLAST hit obtained for each gene to platyhelminth sequences in the NCBI nr protein database.

a. Control

GGATCCTAATACGACTCACTATAGGGAGATTTAGGTGACACTATAGAAGTGACCTTAGGTCCTCGAG
TTTCTACTGTATCGACCTGCAGACTGGCTGTGTATAAGGGAGCCTGACATTTATATTCCCCAGAACAT
CAGGTTAATGGCGTTTTTGATGTCATTTTCGCGGTGGCTGAGATCAGCCACTTCTTCCCCGATAACG
GAGACCGGCACACTGGCCATATCGGTGGTCATCATGCGCCAGCTTTCATCCCCGATATGCACCACC
GGGTAAAGTTCACGGGAGACTTTATCTGACAGCAGACGTGCACTGGCCAGGGGGATCACCATCCGT
CGCCCGGGCGTGTCAATAATATCACTCTGTACATCCACAAACAGACGATAACGGCTCTCTCTTTTATA
GGTGTAAACCTTAAACTGCATTTCACCAGTCCCTGTTCTCGTCAGCAAAAGAGCCGTTCATTTCAATA
AACCGGGCGACCTCAGCCATCCCTTCCTGATTTTCCGCTTTCCAGCGTTCGGCACGCAGACGACGG
GCTTCATTCTGCATGGTTGTGCTTACCAGACCGGAGATATTGACATCATATATGCCTTGAGCAACTGA
TAGCTGTCGCTGTCAACTGTCACTGTAATACGCTGCTTCATAGCACACCTCTTTTTGACATACTTCGG
GTATACATATCAGTATATATTCTTATACCGCAAAAATCAGCGCGCAAATACGCATACTGTTATCTGGCT
TTTAGTAAGCCGGATCCACGCGATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCAT
TAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCA
GCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATT
GGCCACGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCA
ATAAACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAG
AAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAA
ACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGGAATT
CCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGGATAAAACTTGTGCTTATTTTT
CTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTG
ACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATT
TTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGAT
CTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTT
GGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCCGTC
ACAGGTATTTATTCGGCGCAAAGCGGCCGCGACCTAAGGTCTCCCTTTAGTGAGGGTTAATTCTCCC
TATAGTGAGTCGTATTAGGATCC

b. *Pf-wnt1*

TAACTGGAACAATCCCGTCTTCGTAAATCATCTGCGGATCACAAGATGGTGGCATCTTTATAAATTGC
GAATTACCCCTGATTTCCCAACTAATTCTTTACAATACGCTTTCACCAGACATCAATGGCACCAGTTTT
ATAGTTTTGATCCGATTCGTCGTCAGTTATATTCGAGACAAAGTAACTTCAATTGGAAAAACGATACC
AATATCCCGGCAAATAAAGATCTGATTCAAATAACTCTTGAAGCTGCTAGAAAAGGAATATTCACATG
TCATCAGCAATTTGCAACCAGTAGATGGAACTGTCCAACTCCAAATCTCAACAATCCGTCAGCGTTAT
TATTCGGTGATATTACGTTGAAAGGTCTTCCTGAAACTGCGTTCATTTATGCCATGTTAAGTGCTAGTT
TAGTTCAAACAGTGGCCGAAGCTTGTTCAAATCGATTACCTCATTGTCCTTGTAATAACAAAGGAAGG
ACGACGCAAAACAAATGG

c. *Pf-wnt11-1*

GCAGGTCTTGAGATAATGAAACGAAGTTGGAAGTCTAAATGCAAGTGTCACGGAGTAAGTGGAAGTT
GTGCCAATGAAATCTGCTTCCGACAGCTTCGAAGACTTGATGATGTCGTTCTACTCAAGAAACTCAAA
GATAGCTACTTGACTGCAAAATACGTAACTTCTGGAAGAAATGGGATTTTATATACTGATCAAAATGTT
GCTCTGAAAGAAACAGAGTTAGCTTTTATGGAGCATTCTCCTGATTATTGTGAACCAGAACCAAAAAT
GGGATCAATTGGAACTCTGAATAGACAATGTGAAACGACGAATCTAACTCAGGCGGACACTTATCAT
TGTAACAACATGTGCTGTGGAAGAGGATTCCGAACATCGATAGAAGAAGAAACTGTCAAATGCAAAT
GCAGTTTAGATCAGTATTTCTCCGTTAAGTGTAAATTGTGCACTAAAATAAATGAATATAAATTTTGTC
TGTAAAATTCATGTTGCATTTTCGCTCAACAAACTGACATTTACTTTGTGATAGACTCGTACGAA

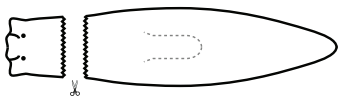
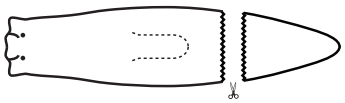
d. *Pf-wnt11-2*

CATGAGTATCATGGTCCCAGACTATGGATCTACCTCATCATACTATCTTCCGTGGTCAGCCTCCACCA
ATCCATCAACTGGTTAGGCATCTCTCAGTACAACCGACCCGAAATTCACGAATTGAAGGCATTCTCTG
AGGTCGAATGTCATTTCATCAGTACCAACCATGGTCTAAGACAACGCCAGGCGAGATTTTGCCTTAG
CAAACAACATAAATTCGCAATGCACGGTATTCTGAGGGGGGCCAAGGCAGCAGTGTATTACTGCATG
AAAACCTTCCAGGATCGACGGTGGAATTGCTCCTCAACCGAGAGACTGCCACACGTAGGCAAAGAT
CTCCAGCTTGGTACCCAGGAACAAGCAGTGGTACATGCTTTTGCCAGTTCTTCAGTGTTGTACGAAA
TTGCGAGAAGATGTGCCCAGAATAAAATTTCTTATTGCTCTTGTGGGAATTCCGACCAAAGTCAGAGC
GAGGAAAATTCTCAGCGTCGTCTTATGTTTGCAGGGTGTCCTGACAATGTAGATGTGGGGATTAAGT

Supplemental Figure 5 | Nucleotide sequences used for RNAi. DNA sequences used as templates for dsRNA synthesis for (a) Control, (b) *Pf-wnt1*, (c) *Pf-wnt11-1*, (d) *Pf-wnt11-2*, (e) *Pf-β-catenin1* and (f) *Pf-APC* RNAi treatments.

Expression Δ Reg+ (A-A')	↓	↑	↑	↓	↑	–	↓	–
Expression Δ Reg- (B-B')	↓	↑	↓	↑	–	↑	–	↓
Number of contigs	35	39	537	842	5928	3004	4386	970
Ratio of contigs	0.3%	0.2%	3.4%	5.3%	37.7%	19.0%	27.9%	6.2%

Supplementary Table 1 | Distribution of contigs (n=15,741) with significant changes in expression level (compared to uncut controls) after amputation in Reg+ (red) and Reg– (blue) tissues based on RNAseq.

	 Regeneration-proficient tissue		 Regeneration-deficient tissue	
RNAi Treatment	**Anterior reg.**	**Posterior reg.**	**Anterior reg.**	**Posterior reg.**
Pf-wnt1	-- (10/10)	-- (10/10)	-- (10/10)	-- (9/9)
Pf-wnt11-1	-- (9/9)	delay (10/10)	-- (10/10)	delay (9/9)
Pf-wnt11-2	-- (10/10)	delay (10/10)	-- (10/10)	delay (10/10)
Pf-wnt1 + Pf-wnt11-1 + Pf-wnt11-2	-- (10/10)	delay (8/9)	-- (10/10)	delay (9/9)
beta-catenin1	-- (9/9)	head reg. (9/9)	head reg. (64/71)	head reg. (56/64)
APC	tail reg. (5/10)	-- (9/9)	tail reg. (14/53)	-- (53/53)
control	-- (9/9)	-- (10/10)	-- (67/67)	-- (67/67)

Supplementary Table 2 | Effects of dsRNA injection on anterior and posterior regeneration following amputations in Reg+ and Reg- tissues. Dashes represent no alteration from the normal condition following amputation. Results labeled in yellow indicate rescue of regeneration in Reg- tissue.

Primers for gene amplification

Gene	Fwd primer	Rev primer
Pf-APC	5’-CAGCTCGTGCTTCTTTGAAC-3’	5’-ACACCTTCGGTTTCTCCTTG-3’
Pf-beta catenin	5’-GGGTACGATGAAATGGATTCTG-3’	5’-GTTGCAGCATCTGACAAGTTTC-3’
Pf-ndk	5’- AGTCTACGACAAAGACATGTGCAGC-3’	5’-CGCATTTGGCAAAGTGAGACCCTTC-3’
Pf-sFRP1	5’- CGGAGTGGAACAACCTCATCTACGG-3’	5’-GCTTCCACAAATCTGTTGCTCTAGCG-3’
Pf-wnt11-1	5’- AAGATTAACGGCCACAACTACG-3’	5’-CTATCGATGTTCGGAATCCTCT-3’
Pf-wnt11-2	5’-ATGAGTATCATGGTCCCAGAC-3’	5’-CGAACGGATTCCCTATGTAAAG-3’
Pf-wnt1	5’-TAACTGGAACAATCCCGTCTTC-3’	5’-GCGTCGTCCTTCCTTTGTTAT-3’

Primers for qPCR

Gene	Fwd primer	Rev primer
Pf-actin	5’-AGAGGAAGACGTTGCTGCTT-3’	5’-TGACCCATACCAACCATGAC-3’
Pf-APC	5’-TGTTCTCACGGTCAAGTTCC-3’	5’-GATCACATGGTGAGACTGCTTC-3’
Pf-beta catenin	5’-ACAGCTGAAGACATCGAGGA-3’	5’-GTGGCACTTGTTCCTGGTTT-3’
Pf-ndk	5’-TACGGATCAGTTGCTGGATG-3’	5’-AGCCATCCATATCCTTGGTG-3’
Pf-sFRP1	5’-AGCGTGCTTACCGAGTATGA-3’	5’-TGTTGACAGTTCGAGGGATG-3’
Pf-sFRP2	5’-AGGATTGGCAAAGACTCACC-3’	5’-GCGTACAAGGAGCACAGAAA-3’
Pf-wnt1	5’-AGTTCAAACAGTGGCCGAAG-3’	5’-CCATTTGTTTTGCGTCGTC-3’
Pf-wnt2	5’-CCGTCAAAGAGGAAACTCGT-3’	5’-GGATGTGTCGTTGCATTCTC-3’
Pf-wnt11-1	5’-CGCAGCAGGTCTTGAGATAA-3’	5’-AGCTGTCGGAAGCAGATTTC-3’
Pf-wnt11-2	5’-GCAATGCACGGTATTCTGAG-3’	5’-TCTCGGTTGAGGAGCAATTC-3’
Pf-wnt11-5	5’-AAATCTACCCCGGAATCAGC-3’	5’-CTGCAAGCCTTTGTGACTTG-3’

Supplemental Table 3 | Oligonucleotides used to amplify genes from *P. fluviatilis* cDNA and to conduct qRT-PCR.