PNAS
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Supplementary Information for

Spiny and soft-rayed fin domains in acanthomorph fish are established through a BMP-*gremlin*-*shh* signaling network

Rebekka Höch, Ralf F. Schneider, Alison Kickuth, Axel Meyer & Joost M. Woltering

Joost M. Woltering
Email:joost.woltering@uni-konstanz.de

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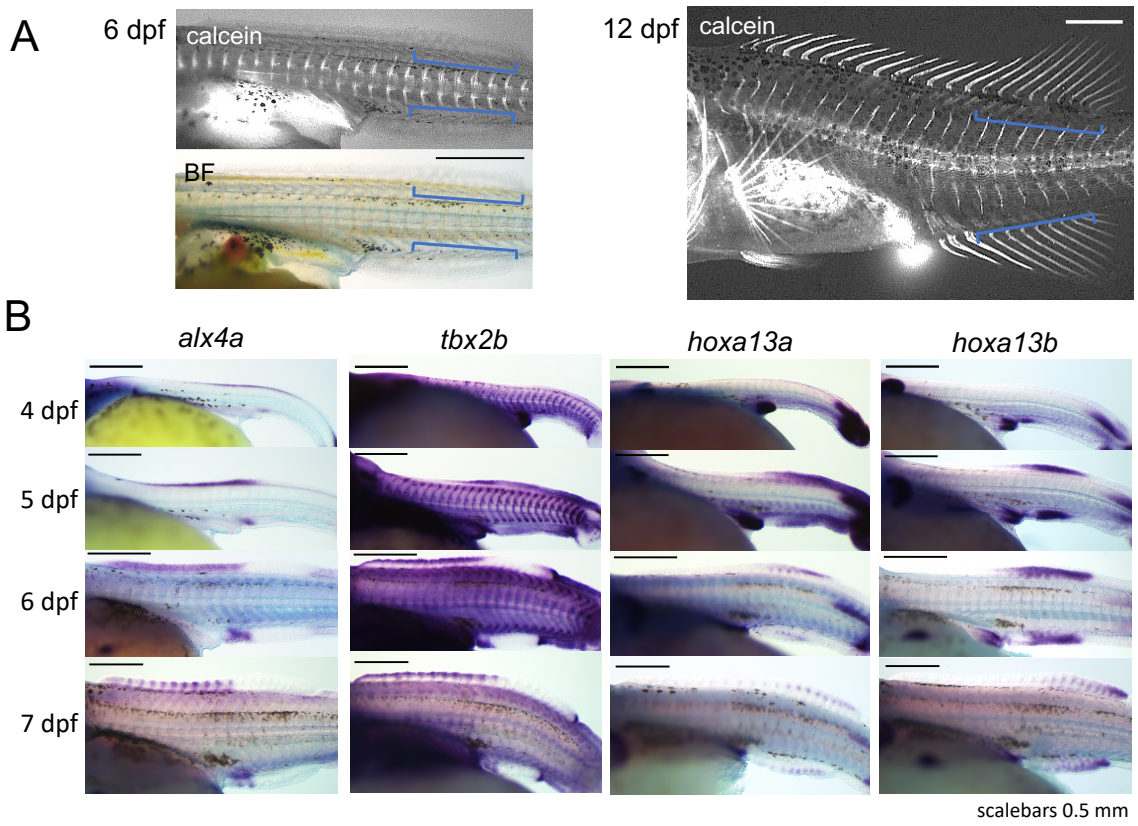

Fig. S1. Development of the soft-ray domain in comparison to vertebrae and expression of *alx4a, tbx2b, hoxa13a* and *hoxa13b* in dorsal and anal fins from 4-7 dpf.
A) Calcein staining of 12 dpf embryos with clearly differentiated spine and soft-ray characters show that the soft-ray domain (blue brackets) extends anterior for 6-7 vertebral elements from the posterior fin margin. At earlier stages (6 dpf shown) this corresponds to a width of 6-7 somites. **B**) *Alx4a* is expressed from 4 dpf onwards and is excluded from the soft-ray domain from 5 dpf. *Tbx2b* is expressed in dorsal and anal fins from 5 dpf onwards and becomes excluded from the soft-ray domain from 6 dpf onwards. *Hoxa13a* and *hoxa13b* show very similar expression dynamics. At 4 dpf these genes are expressed in the posterior trunk and become first expressed in dorsal and anal fins at 5 dpf occupying the prospective soft-ray domain. Therefore, from 5 dpf onwards the mutually exclusive expression of *alx4a* and *hoxa13a*/*hoxa13b* marks the spine to soft-ray transition, while *tbx2b* is excluded from the soft-ray domain from 6 dpf onwards. Anterior is to the left. Abbreviation: BF; bright field.

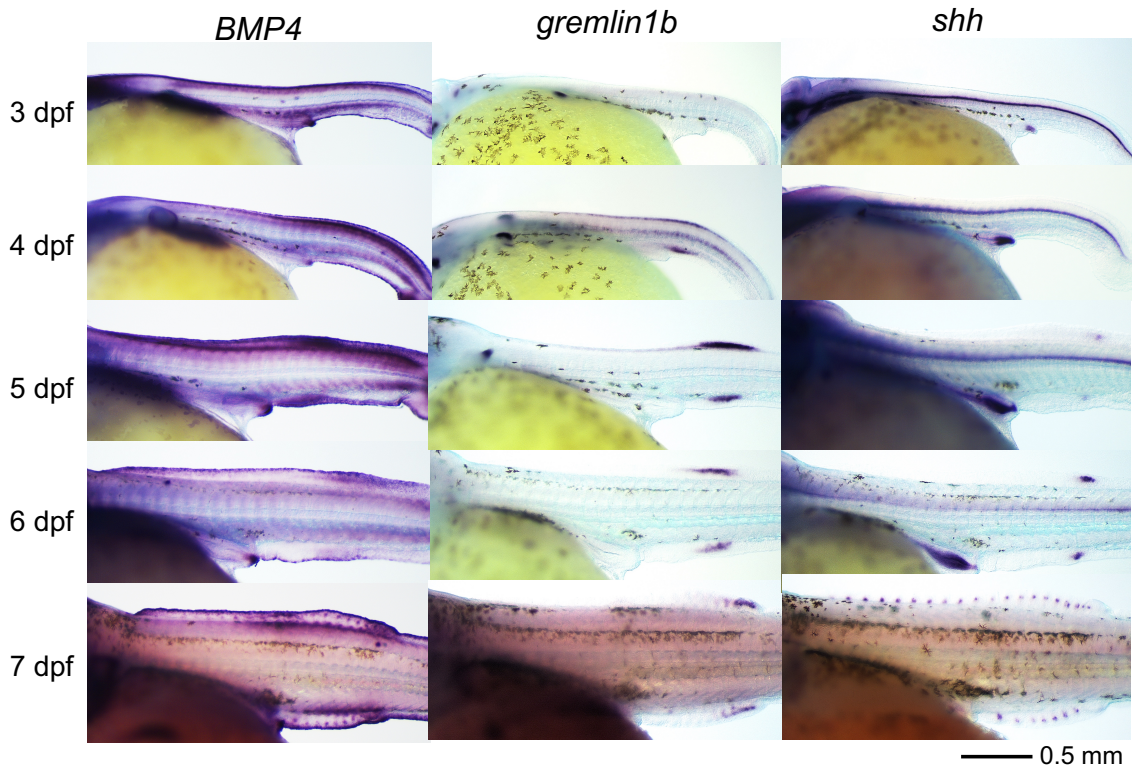

Fig. S2. Expression of *BMP4*, *gremlin1b* and *shh* in dorsal and anal fins from 3-7 dpf. BMP4 is expressed from 3 dpf throughout dorsal and anal fin primordia. From 5-7 dpf there appears a bias towards expression in the posterior fin overlapping with *gremlin1b* – possibly reflecting the feedback loop between the genes. *Gremlin1b* become expressed at 4 dpf and its initial expression extends anterior of the putative soft-ray domain. During 5 dpf expression becomes restricted to a posterior domain corresponding to approximately the length of five somites. This domain regresses posteriorly during 6 dpf and at 7 dpf becomes restricted to the posterior most fin rays. Strong *shh* expression is observed in the notochord from 3- 6 dpf running along the length of the embryo. Expression in a ZPA in dorsal and anal fins is first detected at 5 dpf and is strongest at 6 dpf. By 7 dfp the ZPA expression has disappeared and *shh* is now expressed in the tips of the forming fin elements (soft-rays as well as spines). Anterior is to the left.

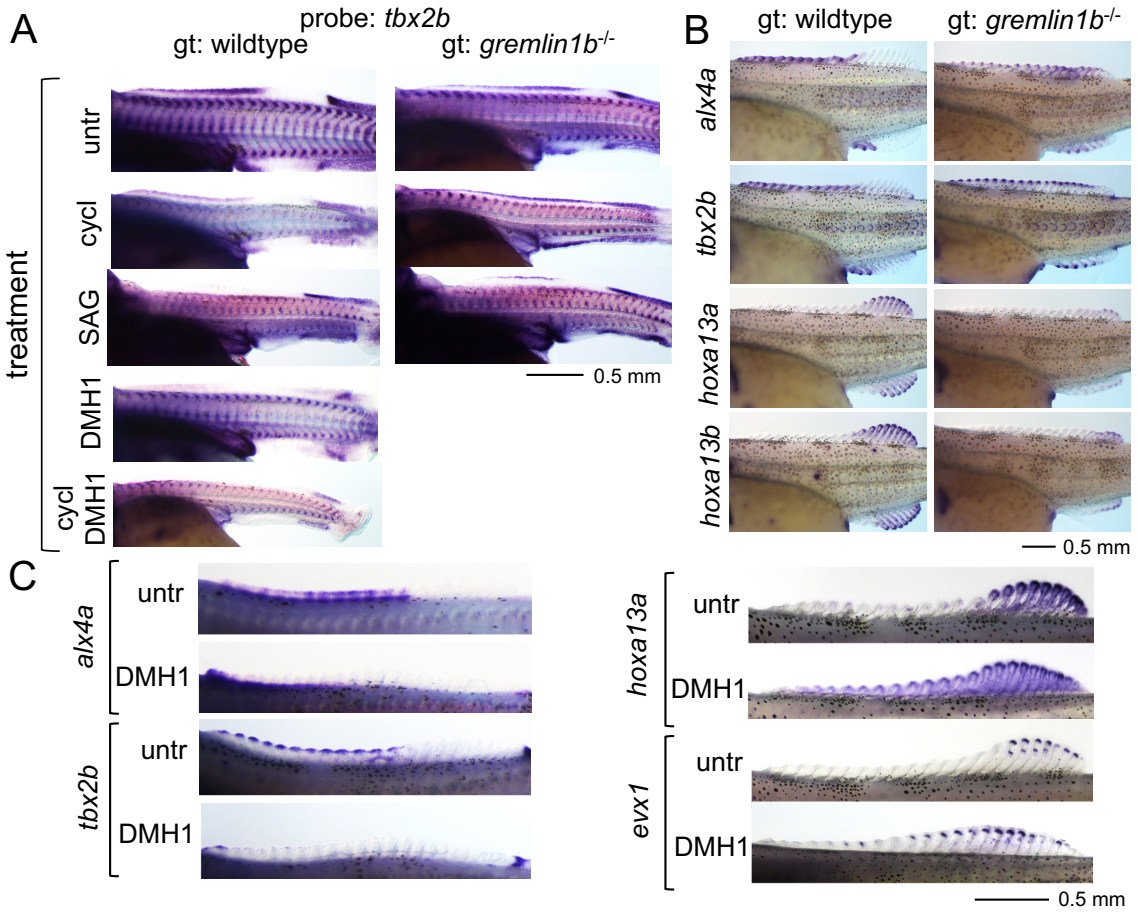

Fig. S3. Expression of spine and soft-ray markers in WT, *gremlin1b*$^{-/-}$ and DMH1 treated embryos.

A) In untreated embryos, *tbx2b* at 6 dpf is strongly expressed in the future spine domain and absent from the future soft-rays. Treatment with DMH1 or SAG expands the *tbx2b* negative territory anteriorly suggesting an expansion of the soft-ray domain. The expansion in DMH1 treatments happens independently of *shh* signaling as it also occurs in DMH1/cyclopamine double treatments. Treatment with cyclopamine alone does not affect the extent of spine and soft-ray territories noticeably. The *tbx2b* domain becomes posteriorly expanded in *gremlin1b*$^{-/-}$ embryos but leaves a small posterior *tbx2b* negative domain. This latter domain disappears in cyclopamine treated *gremlin1b*$^{-/-}$ embryos suggesting that it arises in a *shh* dependent manner. Treatment of *gremlin1b*$^{-/-}$ with SAG expands the *tbx2b* negative domain anteriorly, indicating that BMP inhibition by *gremlin1b* and *shh* signaling are synergistically but independently acting upstream of the *tbx2b* expression domain in the spiny fin. *Tbx2b* expression domains were observed on a minimum of 4/4 embryos per treatment for wildtype fish and 3/3 embryos per treatment for *gremlin1b*$^{-/-}$ embryos. **B**) Expression of spine genes *alx4a* and *tbx2b*, and soft-ray genes *hoxa13a* and *hoxa13b* in wildtype and *gremlin1b*$^{-/-}$ embryos at 9 dpf. *Gremlin1b*$^{-/-}$ embryos show posterior expansion of *alx4a* and *tbx2b* while at the same time *hoxa13a* and *hoxa13b* expression becomes posteriorly reduced, altogether visualizing the posterior shift of the spine to soft-ray boundary in absence of *gremlin1b*. Expression domains were observed on a minimum of 2/2 9 dpf embryos analyzed per probe per genotype. **C**) BMP inhibition by DMH1 leads to anterior reduction of the expression of spine genes *alx4a* and *tbx2b* and anterior expansion of *hoxa13a* and *evx1*, showing an anterior shift of the spine to soft-ray boundary (here in 8 dpf embryos). Expression domains were observed on a minimum of 3/3 8 dpf embryos per probe per treatment. Anterior is to the left. Abbreviations: gt; genotype.

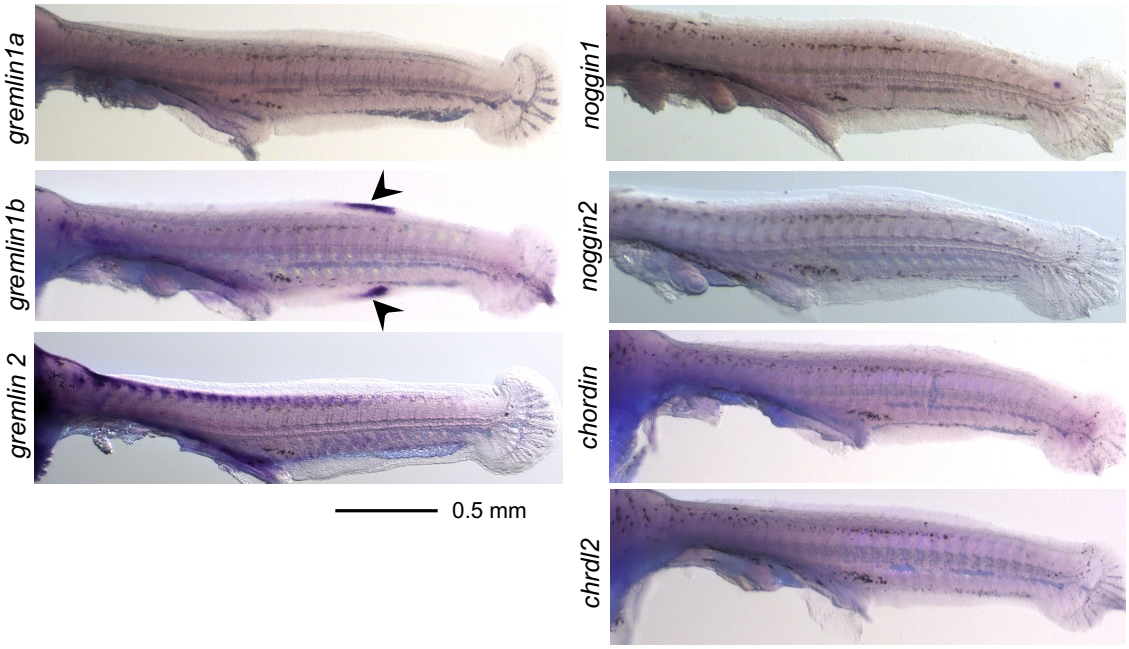

Fig. S4. Expression of secreted BMP inhibitors in the dorsal and anal fins of *A. burtoni.* *In situ* hybridization for *gremlin1a*, *gremlin1b*, *gremlin2*, *noggin1*, *noggin2*, *chordin*, *chrdl2* (*chordin-like-2*) at 6 dpf. Only *gremlin1b* is expressed in the median fins. Anterior is to the left.

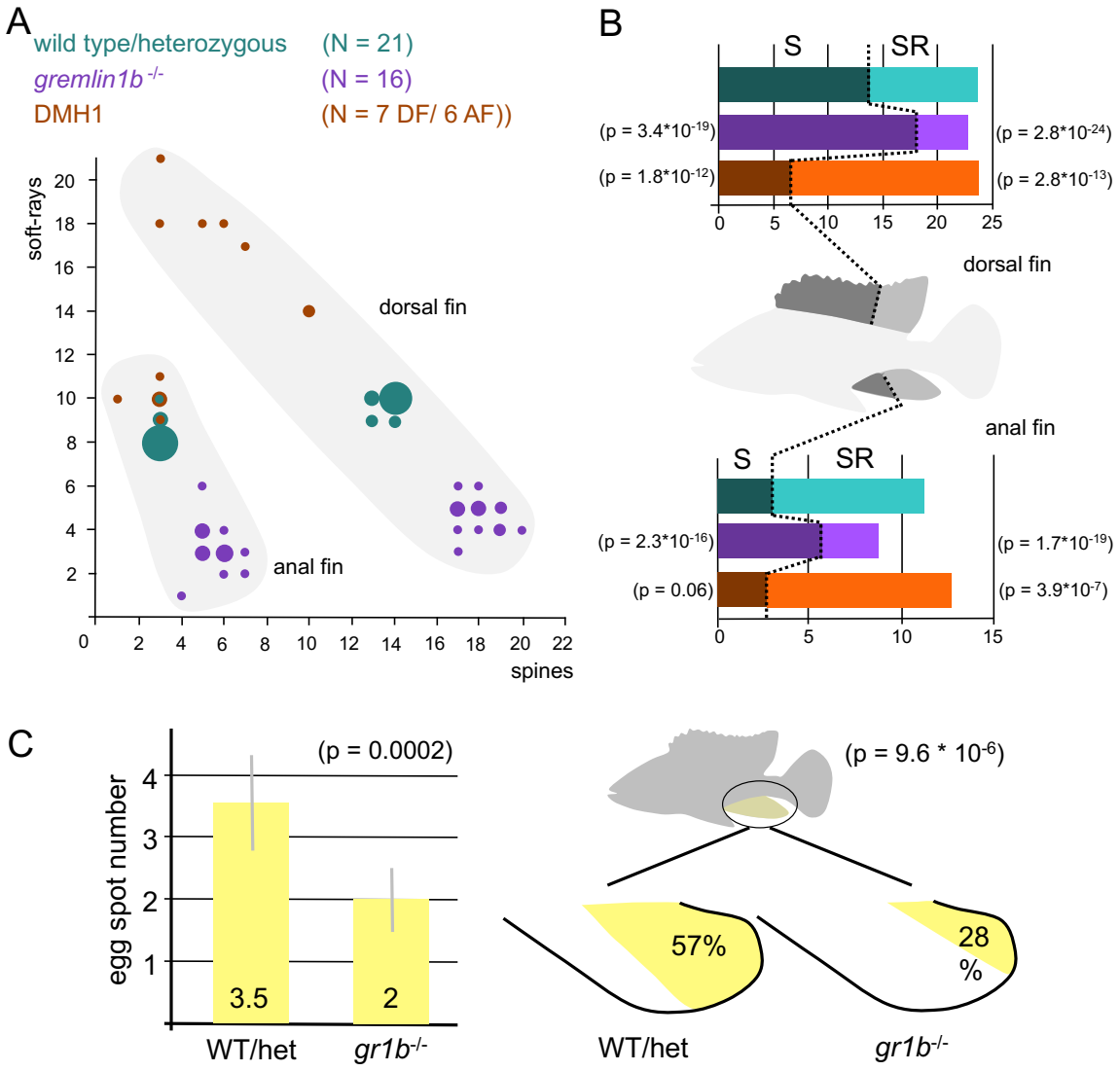

Fig. S5. Quantitative analysis of spine and soft-ray phenotypes.
A) Scatter morphoplot of spine and soft-ray counts for individual wild-type/heterozygous, *gremlin1b*$^{-/-}$ and DMH1 treated fish at approximately 3 months of age. Point size is proportional to the number of individuals. **B**) Average number of spines and soft-rays for the same fish as in panel **A** depicted as bar graphs for dorsal and anal fins. P-values indicate the significance between spines (S) and soft-rays (SR) with the wildtype/heterozygous fish. The number of DMH1 individuals analyzed for anal fin formula is 6 because in one specimen the number of soft-rays could not be reliably determined due to damage of the anal fin. **C**) Left: bar graphs visualizing average number of egg spots in wildtype/heterozygous males (3.5) and *gremlin1b*$^{-/-}$ (2) males. Right: average percentage of the anal fin, as measured along the anterior-posterior fin base, over which egg spots are distributed in WT (57%) and *gremlin1b*$^{-/-}$ (28%) males. Abbreviations: S; spines, SR; soft-rays, WT; wildtype, het; heterozygous, *gr1b*; *gremlin1b*. Anterior is to the left.

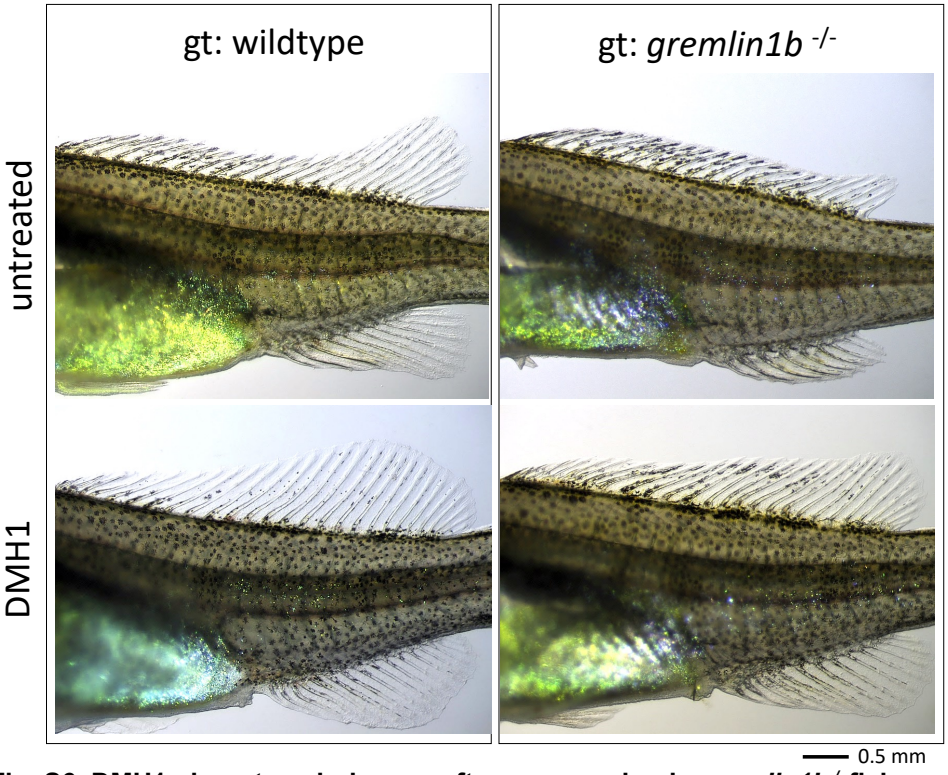

Fig. S6. DMH1 phenotype induces soft-ray expansion in *gremlin1b*$^{-/-}$ fish.
WT and *gremlin1b*$^{-/-}$ fish at 13 dpf treated with the BMP inhibitor DMH1. *Gremlin1b*$^{-/-}$ fish show an anterior expansion of the soft-ray domain, similar as that occurs in WT fish. The rescue experiment was observed on 5/5 *gremlin1b*$^{-/-}$ embryos treated with DMH1. Abbreviations: gt; genotype. Anterior is to the left.

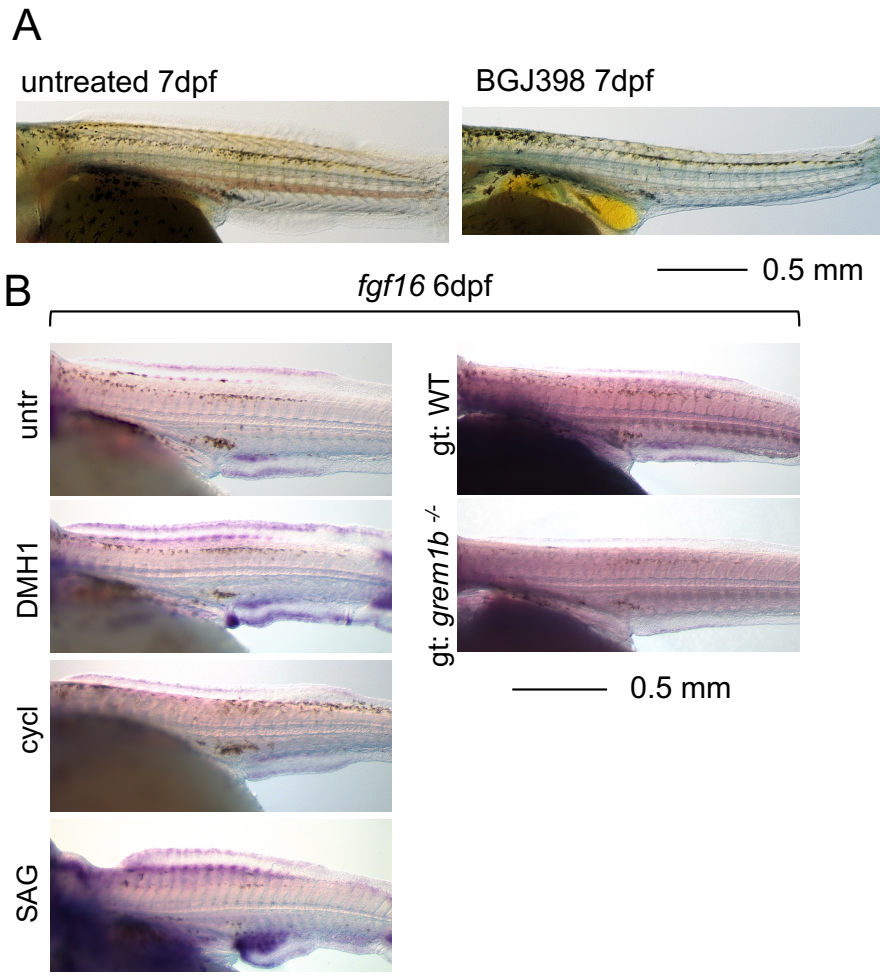

Fig. S7. Effects of FGF inhibition on fin development and expression of *fgf16* in *A. burtoni.* **A**) Treatment with the FGF signaling inhibitor BGJ398 results in absence of fin outgrowth, affecting spiny and soft-ray parts of the dorsal and anal fins (N=9/9 embryos treated with BGJ398). **B**) *Fgf16* is expressed in the distal dorsal and anal fins at 6 dpf and overlaps with both spiny as well as soft-ray fin domains. Expression of additional candidate ectodermal FGF species *fgf8a*, *fgf8b* and *fgf4* was also investigated but their expression was not detected in the median fins using *in situ* hybridization. DMH1 and SAG treatment leads to moderate upregulation of *fgf16*, whereas treatment with cyclopamine or loss of *gremlin1b*$^{-/-}$ results in reduction of *fgf16* expression. *Fgf16* expression domains were observed on a minimum of 6/6 embryos per treatment and on 2/2 *gremlin1b*$^{-/-}$ embryos. A separate sibling control WT embryo is shown for the *gremlin1b*$^{-/-}$ experiment. Anterior is to the left.

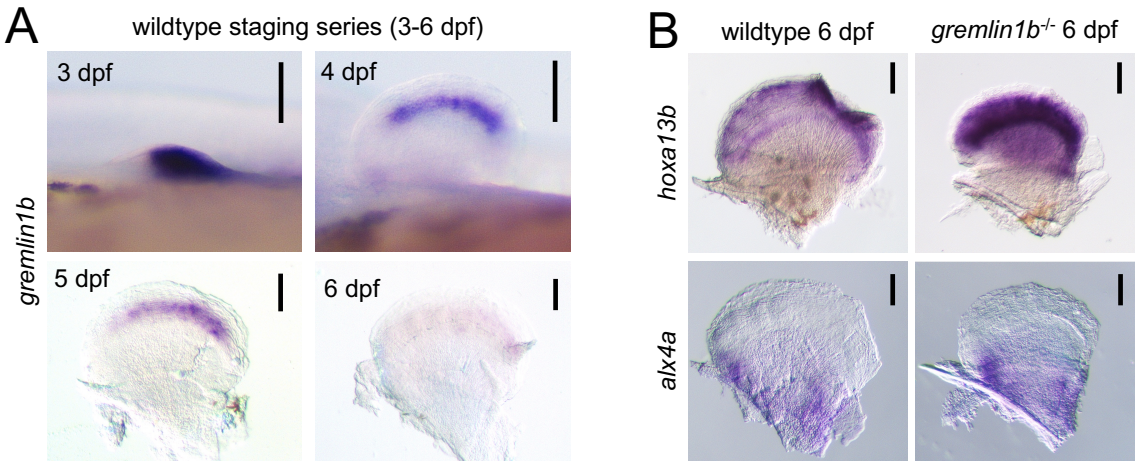

Fig. S8. Loss of *gremlin1b* does not affect anterior-posterior expression of *hoxa13b* or *alx4a* in pectoral fins of *A. burtoni.*

A) During pectoral fin development (3-6 dpf) *gremlin1b* is expressed in a central domain resembling the expression in developing mouse limbs (1, 2). **B**) In *gremlin1b*$^{-/-}$ pectoral fins expression of *hoxa13b* and *alx4a* resembles that in wildtype embryos. (Expression domains were observed on 4/4 fins analyzed for each probe per genotype). 5 dpf and 6 dpf fins were dissected and flat mounted. Anterior is to the left.

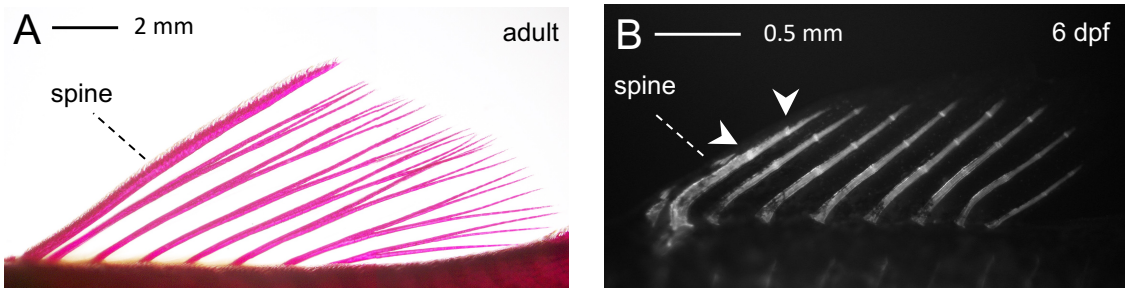

Fig. S9. Early segmentation of the anterior fin spine in South American catfish *Ancistrus* sp.
A) *Ancistrus* catfish have a dorsal fin that is restricted to the anterior part of the trunk. This fin consists of posterior soft-rays and a single anterior spine. **B**) This spine initially develops as a soft-ray with segments (arrowheads) that is indistinguishable from the posterior soft-rays. Anterior is to the left.

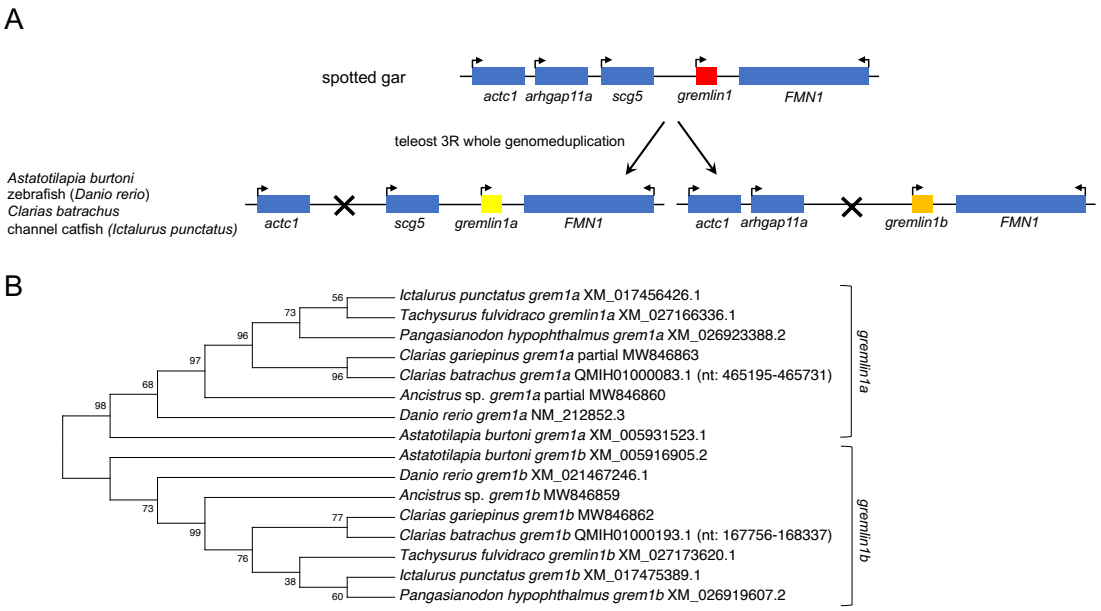

Fig. S10. Identification of *gremlin1* ohnologs.
A) The ancestral actinopterygian condition (as present in spotted gar) is the presence of a single *gremlin1* ortholog. The *gremin1a* and *gremlin1b* ohnologs arose during the teleost 3R whole genome duplication. The microsyntenic region of *gremlin1a* is characterized by loss of *ahrgap11a* whereas the microsyntenic region containing *gremlin1b* lost *scg5*. **B)** A maximum likelihood tree with 500 bootstrap pseudoreplicates, generated with MEGAX (3) from MUSCLE-aligned *gremlin1* protein sequences from catfish (*Clarias gariepinus*, *Clarias batrachus*, *Ancistrus* sp., *Ictalurus punctatus*, *Pangasianodon hypophthalmus* and *Tachysurus fulvidraco*), zebrafish and *Astatotilapia burtoni*, confirms the inferred *gremlin1a* and *gremlin1b* clades. GenBank accession numbers as well as genomic regions for *Clarias batrachus* are indicated. Percentage of replicate trees supporting each node are indicated. Abbreviation; nt: nucleotide.

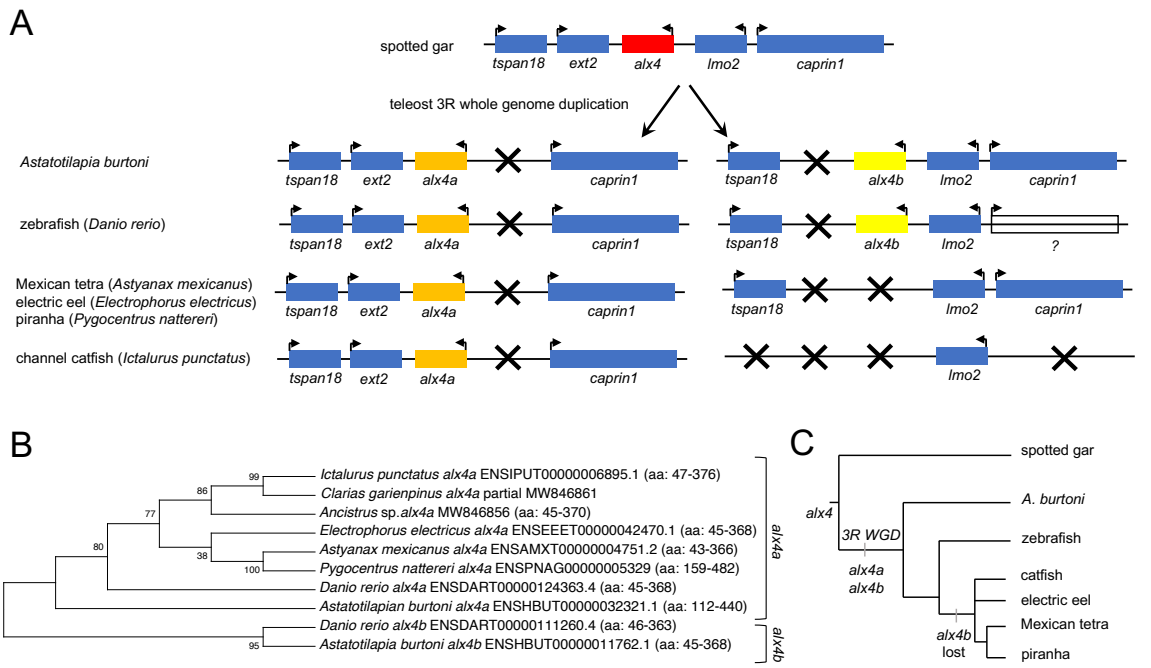

Fig. S11. Identification of *alx4 ohnologs and putative loss of alx4b in siluriformes, gymnotiformes* and *characiformes.*

A) The ancestral actinopterygian condition (as present in spotted gar) is the presence of a single *alx4* ortholog. The *alx4a* and *alx4b* ohnologs arose during the teleost 3R whole genome duplication. The microsyntenic region of *alx4a* is characterized by loss of *lmo2* whereas the microsyntenic region containing *alx4b* lost *ext2* in *A. burtoni* and zebrafish (and possibly *caprin1* in zebrafish). In the genomes of the channel catfish (*Ictalurus punctatus*) as well as its sister lineages of the Mexican tetra (*Astyanax mexicanum*), electric eel (*Electrophorus electricus*) and piranha (*Pygocentrus nattereri*) we identify only a single *alx4* ohnolog. In Mexican tetra, piranha and electric eel the microsyntenic region of *tspan18*, *lmo2* and *caprin1* provides a "ghost locus" for *alx4b*. In channel catfish this locus appears to have degenerated further and preserves only *lmo2*. **B**) A maximum likelihood tree with 500 bootstrap pseudoreplicates, generated with MEGAX (3) from MUSCLE-aligned catfish (*Ictalurus punctatus, Clarias gariepinus, Ancistrus sp.*), electric eel, Mexican tetra, piranha, zebrafish and *Astatotilapia burtoni alx4* sequences confirms that the *alx4* ohnolog preserved in catfish, electric eel. Mexican tetra and piranha is *alx4a*. **C**) Altogether this suggest loss of *alx4b* at the base of the *Siluriformes* (catfish), *Gymnotiformes* (electric eel) and *Characiformes* (Mexican tetra and piranha). The maximum likelihood tree in panel **B** was generated using sequences that were trimmed at the N-terminus aligning with the N-terminal end of the *Clarias gariepinus alx4a* sequence (amino acid sequences used in the alignment are indicated as well as GenBank or ENSEMBL accession numbers). Percentage of replicate trees supporting each node are indicated. The species tree shown in panel **C** is after the species tree provided by ENSEMBL (https://www.ensembl.org/info/about/speciestree.html). Abbreviation; aa: amino acid.

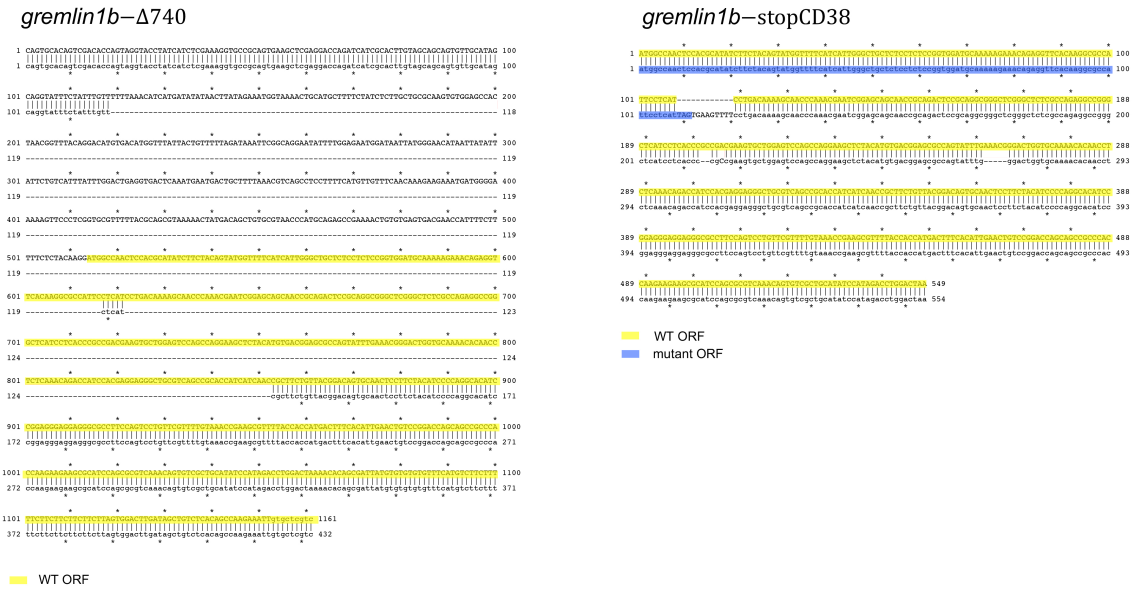

Fig. S12. *A. burtoni* CRISPR/Cas9 *gremlin1b*$^{-/-}$ alleles.
Left panel: the 740 bp deletion removes the 5` 339 bp of the *gremlin1b* open reading frame together with its 5`upstream genomic region. Right panel: the *gremlin1b*-stopCD38 mutant introduces a premature STOP codon at position 38 of the protein and results in a truncated protein lacking the C-terminal BMP inhibitory domain. In both panels the wildtype open reading frame is indicated in yellow. In the right panel the mutant open reading frame is indicated in blue. Abbreviations: WT; wildtype, ORF; open reading frame.

Table S1.

species	gene	primer FW	primer RV	Genbank ID
A. burtoni	*alx3*	ccttcagcacatttcaactggagg	acagttctgaatagcaacacgtctc	XM_005914882.2
	alx4a	cacaacggagggctcatctc	cccaagatatggctgcactg	XM_005931432.2
	alx4b‡	acgctcatctcctgctatggaaa	gagatcatagccattgattccccca	XM_005929113.2
	BMP4	gacgagcttctgtcttccgcag	ctccactaccatttcctggtag	XM_005934275.2
	chordin	tacgaggacaggtccacgtg	tggagcaggtcagtagtggac	XM_005938310.2
	chrdl2	tctgttccagatacctgctg	ttaggatttaggaaagtcac	XM_005922203.1
	evx1	tgcacgcctgaaagcatggactac	ctctggtccagtgtcacagatgag	XM_005928644.2
	fgf16	atggcagaggtcgtgagtgttc	ctctcccagtgtaagcactggtc	XM_005921241.1
	gli1	caggggagagtatgggaatg	ccttctgaatgaacaggtcttg	XM_014335964.1
	gremlin1a	atgcagacacattcagtgcagagc	ttcacagaagacaatattactgg	XM_005931523.1
	gremlin1b	cagtgcacagtcgacaccagtag	gacgagcacaatttcttggctgt	XM_005916905.2
	gremlin2	atgctgtggagaataactatccctg	agcacataaatgatgtcattcc	XM_005914226.1
	hand2	gtcatgcaccatcacgacag	ctactgcttcagttccagag	XM_005948191.2
	hoxa9a	atgtcgacatctggaacgctg	gtgtacggacagcgcttttt	EF594313.1
	hoxa11a‡	atgatggattttgacgaaagg	tgctgccgccggttttctcctc	EF594313.1
	hoxa13a‡	cctggtatggaggatttccacatg	tgacaacgtcactgctcctccgtc	EF594313.1
	hoxa13b‡	ctatgaccgcctcattactcctcc	ttgtaaagtggatttccacagag	EF594311.1
	hoxd12a‡	atggaaatgtgtgagcggaatc	cttgagcaaaagtcgtcctggag	EF594315
	noggin1	ctcgtgctctctgtaggctttgg	ctcgaacagaagacaaagtca	XM_005944766.2
	noggin2	tgtaggctttgggacggagga	ccagtctgttctcagctgtccac	XM_005946174.2
	pax9	atggagcctgccttcggtgaag	tcacagctgtggggagatagag	XM_005925986.1
	shh	cagtggcagcaaagtcagg	gattatgcacaatttgctgctc	XM_005912379.2
	tbx18	tatccccagtgaggaccatc	gtccgagaatcgaacaagaagc	XM_005917755.2
	tbx2b	ttacaaagcttggctctgtc	tcacttgggacagtctgtatttg	XM_005930856.1
Ancistrus sp.	*alx4a*	ccaaacactgaccccacaggaatg	ctgttgtgtgatcatgtagcccag	†MW846856
	gremlin1b	atgttcaggtttgtgtggctgg	cttcttaagccaaatgaatgtgag	†MW846856
	hoxa13b	gtggcgcatcgctgccttatg	gttctgaaaccagatggtcacctg	†MW846859
C. gariepinus	*alx4a*	gcaacggaggactcatctcctg*	gatggctgcactgtgttccttg	†MW846861
	gremlin1b	tcacgtgtgcagtgctcttg	atgcagcggcactgtttgac	†MW846862
	hoxa13b	gacgtgagcaagaacatggagg	gaacttattggccgcatattc	†MW846866

Primer sequences used for probe cloning.
Primer sequences used for probe cloning. The right-most column gives genbank IDs for the used genes. The *C. gariepinus alx4a* FW primer used for probe cloning (labeled *) contains one mismatch with the RNAseq transcript identified because it was designed based on putative sequence similarity before the RNAseq dataset was available. Probes for genes labeled ‡ have been described before (4, 5) and are included here for completeness. NCBI GenBank accession numbers labeled † were deposited as part of this study. The *A. burtoni alx4b* probe was referred to as "*alx4a"* in a previous publication (4). For updated *alx4* ohnology nomenclature also see **Fig. S11**.

SI References

1. B. D. Harfe *et al.*, Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell* **118**, 517-528 (2004).
2. L. Panman *et al.*, Differential regulation of gene expression in the digit forming area of the mouse limb bud by SHH and gremlin 1/FGF-mediated epithelial-mesenchymal signalling. *Development* **133**, 3419-3428 (2006).
3. S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* **35**, 1547-1549 (2018).
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