

SUPPLEMENTARY MATERIAL

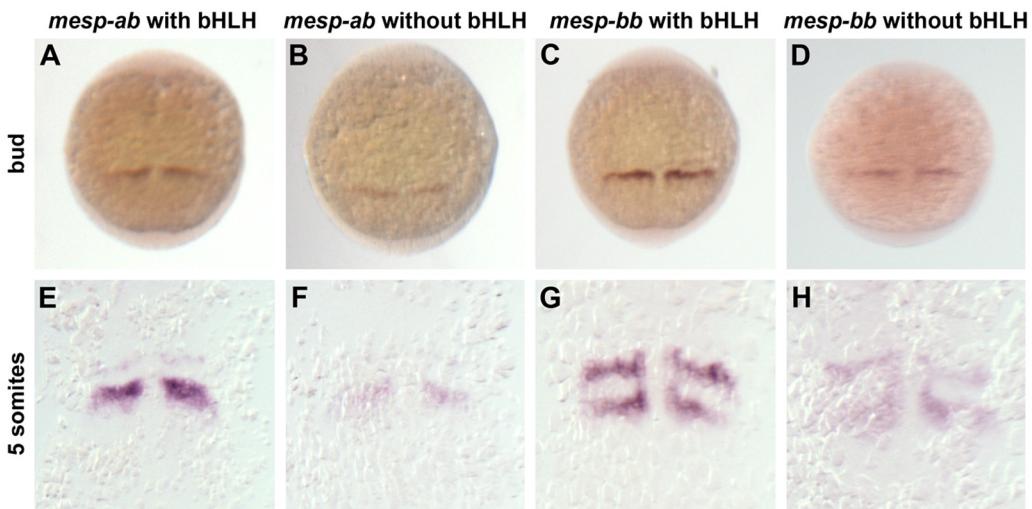
corresponding to:

Identification and expression analysis of two novel members of the Mesp family in zebrafish

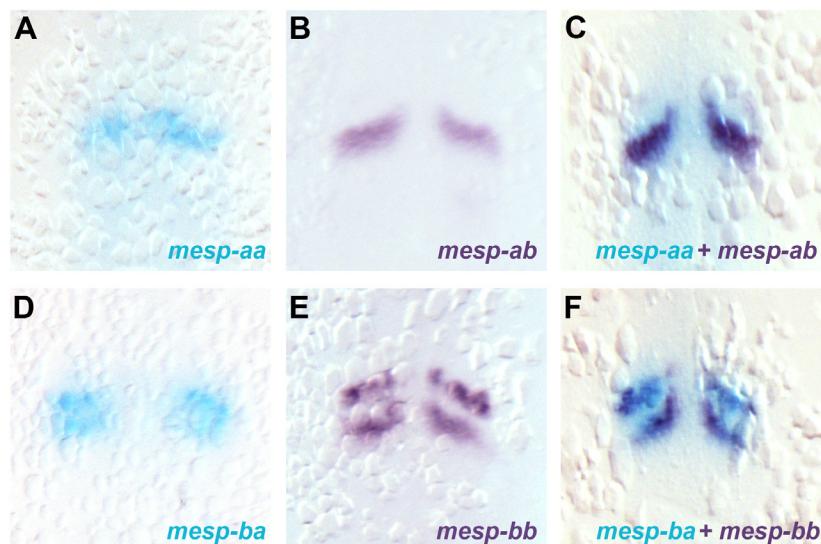
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web: <http://www.kcl.ac.uk/biohealth/research/divisions/randall/sections/signalling/wardle/index.aspx>

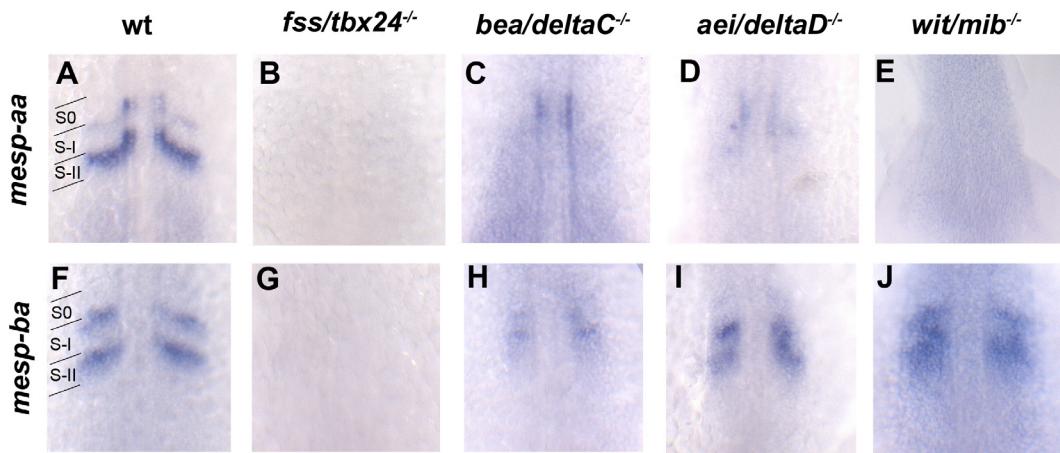
full text corresponding to this material is available at: <http://dx.doi.org/10.1387/ijdb.113447sc>



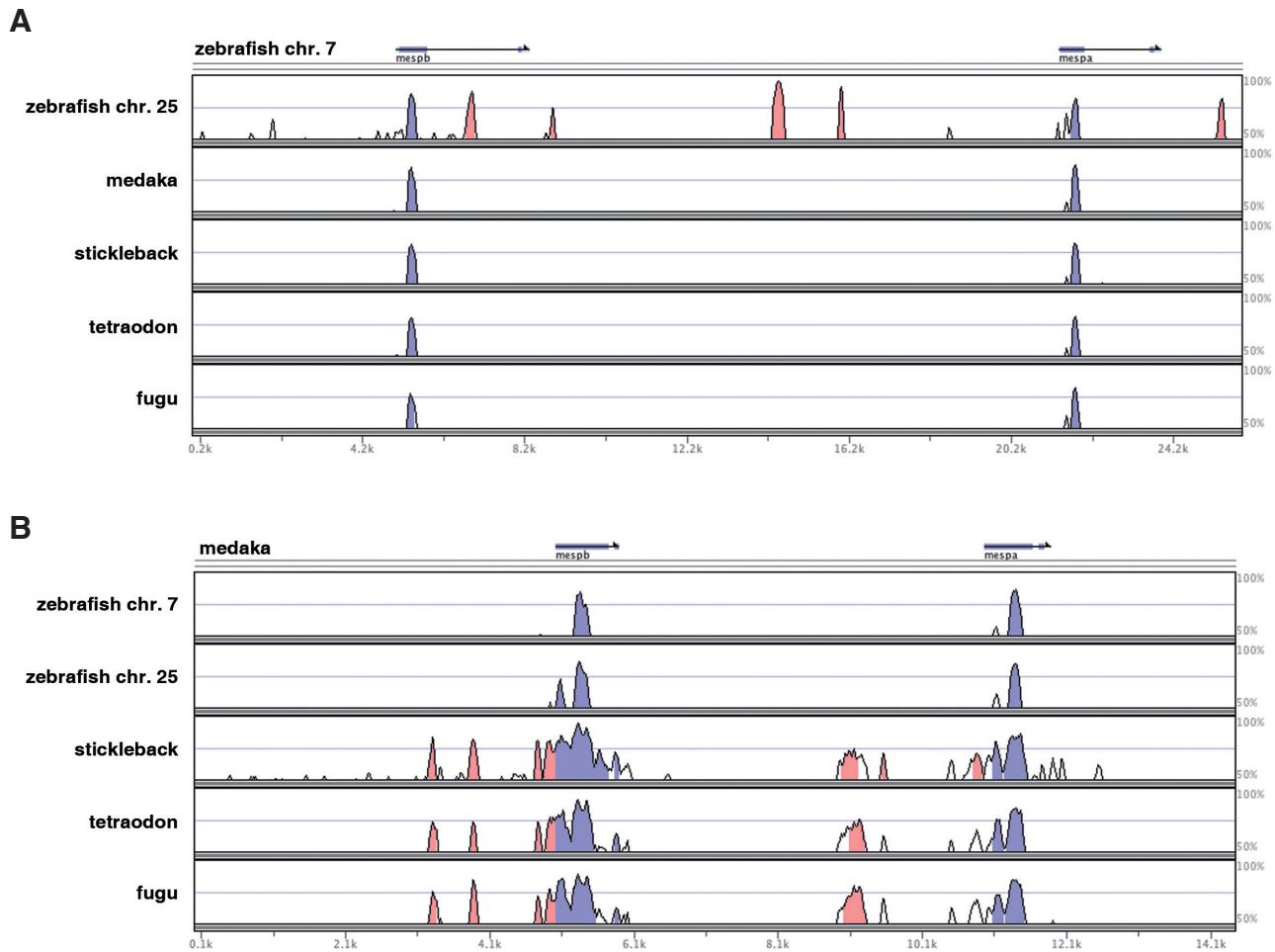
Supplementary Fig. S1. Comparison of *mesp-ab* and *mesp-bb* expression using *in situ* probes for full-length RNA (containing bHLH sequence) or for the 3' end of RNA (lacking bHLH sequence). Expression of *mesp-ab* and *mesp-bb* at bud stage (A-D) or 5-somite stage (E-H) shows spatial and temporal expression is the same for all probes. However expression of the probes lacking the bHLH sequence is weaker (compare A,C,E,G with B,D,F,H).



Supplementary Fig. S2. Overlap of *mesp-aa/ab* and *mesp-ba/bb* expression. (A-C) *In situ* of *mesp-aa* (pale blue) and *mesp-ab* (purple) at 12 somite stage shows that the expression of these genes overlap. (D-F) *In situ* of *mesp-ba* (pale blue) and *mesp-bb* (purple) at 12-somite stage shows that the expression of these genes overlap.



Supplementary Fig. S3. Expression of *mesp-aa* and *mesp-ba* in segmentation mutants. (A-E) Wild-type, (B-G) *fss/tbx24^{-/-}*, (C-H) *bea/deltaC^{-/-}*, (D,I) *aei/deltaD^{-/-}*, (E,J) *wit/mib^{-/-}* embryos at the 8-somite stage hybridized with *mesp-aa* (A-E) and *mesp-ba* (F-J) in situ probes. S0, S-I and S-II mark the position of presumptive somites. All embryos were flat-mounted and are shown in dorsal views, anterior to the top.



Supplementary Fig. S4. MVISTA analysis comparing the regions 5kb upstream of *mesp-b* to 2kb downstream of *mesp-a* of (A) zebrafish chr. 7 *mesp* locus to zebrafish chr. 25, medaka, stickleback, tetraodon and fugu *mesp* loci or (B) medaka *mesp* locus to zebrafish chr. 7, zebrafish chr. 25, stickleback, tetraodon and fugu *mesp* loci. Exons are shown in blue, while conserved non-coding sequence is shown in pink. The bHLH in the first exon shows high conservation between all species. In intronic and intergenic sequence small regions of conservation can be seen between the two zebrafish loci, but not the other fish (A). Regions of conservation between medaka, stickleback, tetraodon and fugu, but not zebrafish, can be found upstream of *mesp-b* and *mesp-a* (B).

SUPPLEMENTARY TABLE S1

SEQUENCES USED IN THIS STUDY, INCLUDING FULL-LENGTH *MESP-AB* AND *MESP-BB* SEQUENCES

SUPPLEMENTARY TABLE S1 (CONTINUED)

SUPPLEMENTARY TABLE S1 (CONTINUED)

SUPPLEMENTARY TABLE S1 (CONTINUED)

SUPPLEMENTARY TABLE S2

COMPARISON OF ZEBRAFISH MESP-AA, MESP-AB, MESP-BA, MESP-BB WITH OTHER MESP-RELATED PROTEINS

	% identity			
	Full length protein		bHLH domain	
	huMESP1	huMESP2	huMESP1	huMESP2
Mesp-aa	25.4	18.9	74.1	72.2
Mesp-ab	26.5	27.4	83.3	81.5
Mesp-ba	29.6	24.9	75.5	73.5
Mesp-bb	31.1	23.9	79.6	77.6

	% identity over full-length protein							
	Medaka mesp-a	Medaka mesp-b	Fugu mesp-a	Fugu mesp-b	Tetraodon mesp-a	Tetraodon mesp-b	Stickleback mesp-a	Stickleback mesp-b
Mesp-aa	38.5	36.3	35.3	33.3	38.9	34.3	39.8	36.2
Mesp-ab	38.1	33.6	37.2	31.9	35.7	32.5	39	31.7
Mesp-ba	35.4	41	35	40.5	35.1	40.8	34.3	40.4
Mesp-bb	34.6	42	34.2	41	37.9	39.9	33.7	40.3

	% identity over full length protein		
	Mesp-aa	Mesp-ab	Mesp-ba
Mesp-aa			
Mesp-ab	41.2		
Mesp-ba	38.9	32.3	
Mesp-bb	32.7	32.2	42.2

	% identity over bHLH domain (protein sequence)		
	Mesp-aa	Mesp-ab	Mesp-ba
Mesp-aa			
Mesp-ab	87.0		
Mesp-ba	85.7	85.7	
Mesp-bb	83.7	85.7	88.9

	% identity over bHLH domain (cDNA sequence)		
	Mesp-aa	Mesp-ab	Mesp-ba
Mesp-aa			
Mesp-ab	75.3		
Mesp-ba	80.2	71.7	
Mesp-bb	76.4	73.9	82.0

The table shows a high level of sequence identity over the bHLH domain but little over the whole protein sequence. Table shows pairwise identity (from CLUSTALW2 alignment) of full-length proteins or bHLH domains (54 amino acids; PF00010).

SUPPLEMENTARY TABLE S3

PCR PRIMERS USED IN THIS STUDY

Primer name	Primer sequence 5'-3'
1. Primers used to isolate <i>mespab</i> and <i>mespbb</i> from cDNA	
mespabF2	AGCATTCACTCAAGCTCCAG
mespabR2	GGGGTTTAATTTAAAAAAAGACAAT
mespbbF	ATGGACGCATCATCTCCTTTC
mespbbR	TCATCCCCAGAACACTGGAG
2. Primers used in 5' and 3' RACE	
5' RACE Primer	GGCCACCGCGTCGACTAGTACGGIIGGGIIGGGIIG
mespab RACE GSP1	GCAGGGAAACATATAA
mespab RACE GSP2	GCTGATTGCGCTTCATTCC
3' RACE adaptor primer	GGCCACCGCGTCGACTAGTACTTTTTTTTTTTTTT
5' and 3' RACE abridged primer	GGCCACAGCGTCGACTAGTAC
mespbb 5' RACEGSP1	GTTGTAGCCCTGATAT
mespbb 5' RACEGSP2	CGTTGGCCAACGGGAGCTA
3. Primers used for bHLH minus <i>in situ</i> probes (in conjunction with T7 or SP6)	
mespaa-bHLH	GTGAAGATGTGGAGATTG
mespab-bHLH	CTGAGGTGCCAATTATGAG
mespb-a-bHLH	GTGAAGAAGAGCTGAGCTAC
mespb-b-bHLH	CGGAAGAGTCTTGTGCAAG
4. Primers used in qPCR	
qPCR mespaaF2	GCAGGACGAGGTTATCAG
qPCR mespaaR2	GGGAATGAATGGAAATCAC
qPCR mespbbF2	TTATGAGATGTGCTGCTCTG
qPCR mespabR2	GCTGTTCTCCATCTGTTCTC
qPCR mespa2	TGTGATGGACAGTATGAGGA
qPCR mespb2	GAACGAGATGATAGCCAAAG
qPCR mespabF2	CTCAAACCACTGCTATTCC
qPCR mespabF2	TCTGAGCTGGAATTAAAGGA