

**Supplementary Figure 2.** Phylogenetic footprinting of the known BMP-responsive enhancer (BRE) of *msx1* and of the *in silico* predicted BREs of *msx2* (A), *gata2* and *gata3* (B), *vox*, *vent*, *bmpr2*, *tlx2* and *tbx2* (C). Aligned are the currently available genomic sequences of *Homo* (h), *Mus* (m), *Rattus* (r), *Canis* (c), *Gallus* (g), *Xenopus* (x), *Danio* (d), *Tetraodon* (t) and *Fugu* (f). The SBE and bre7 motifs are highlighted in blue and red respectively. A BRE composed of GCCG and SBE motifs was reported in the proximal enhancer (PE; MacKenzie *et al.*, 1997) of *msx1* (Alvarez Martinez *et al.*, 2002). Multi-species alignment demonstrates the evolutionary conservation of the enhancer and the SBE motif in it, but not of the GCCG element. However, a fully conserved bre7 motif is present close to the SBE instead. The same two elements, phylogenetically conserved and with similar spacing, are also found upstream of the *msx2* gene. Therefore, we propose that BMP responsiveness of *msx* genes is due to a conserved cluster of SBE and bre7 motifs in their 5' flanking region. The observed similar organisation of the BMP-responsive enhancers of *msx1* (experimentally proven) and *msx2* (*in silico* predicted) is in line with their similar expression patterns and redundant functions. The predicted BRE of *msx2* is located downstream of the recently published mouse *msx2* Lef1/Smad-dependent enhancer (Hussein *et al.*, 2003), which is not phylogenetically conserved (data not shown). For the mouse *tlx2* promoter, a 1.6 kb fragment was found BMP-responsive (Tang *et al.*, 1998) and the enhancer activity was recently mapped to the fragment -1443/-1072 (Xiao *et al.*, 2003). This segment contains the *in silico* predicted BRE shown in panel C.

Alvarez Martinez, C.E., Binato, R., Gonzalez, S., Pereira, M., Robert, B. and Abdelhay, E. (2002) Characterization of a Smad motif similar to Drosophila mad in the mouse Msx 1 promoter. *Biochem Biophys Res Commun* **291**: 655-662.

Hussein, S.M., Duff, E.K. and Sirard, C. (2003) Smad4 and beta-catenin co-activators functionally interact with lymphoid-enhancing factor to regulate graded expression of Msx2. *J Biol Chem* **278**: 48805-48814.

MacKenzie, A., Purdie, L., Davidson, D., Collinson, M. and Hill, R.E. (1997) Two enhancer domains control early aspects of the complex expression pattern of Msx1. *Mech Dev* **62**: 29-40.

Tang, S.J., Hoodless, P.A., Lu, Z., Breitman, M.L., McInnes, R.R., Wrana, J.L. and Buchwald, M. (1998) The Tlx-2 homeobox gene is a downstream target of BMP signalling and is required for mouse mesoderm development. *Development* **125**: 1877-1887.

Xiao, C., Shim, J.H., Kluppel, M., Zhang, S.S., Dong, C., Flavell, R.A., Fu, X.Y., Wrana, J.L., Hogan, B.L. and Ghosh, S. (2003) Ecsit is required for Bmp signaling and mesoderm formation during mouse embryogenesis. *Genes Dev* **17**: 2933-2949.

**A** *msx1* (-2.8 kb, PE)

h  
m  
r  
c  
g  
x  
t  
f

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CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTAGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACACATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT
CCTTTGATCGGCCGCGCTGGCGCCTTAAAAACAACATC.CTCGTCTGCCTATTAGCGCGGCTGAGGGAATCGTGACAGATTGTTT

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h  
m  
r  
g  
x  
d  
t  
f

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GGAGCCCGCCTTTC..ATCTGCCCGCGCCTGGCGCCTGCCCG.AAGCCAGGCCCGTCTAC
GGAGCCCGCCTTTC..ATCTGCCCGCGCCTGGCGCCTACCCG.CAGCCCTGGCCCGTCTAC
GGAGCCCGCCTTTC..ATCTGCCCGCGCCTGGCGCCTACCCG.CAGCCCTGGCCCGTCTAC
GGAGCTTCCTTTC..ATCTGCCCGCTCCTGGCGCCTGCAAAAAGCCCTCAGTTTGTCTGC
GGAGCTTCCTTTC..ATCTGCCCGCTCCTGGCGCCTGCAAAAAGCCCTCAGTTTGTCTGC
GGTAGCTTCCTTTTTCATCTTCCGAGACTGGCGCCTTCCAA...AAGAGGCTTGTCTGC
GGTATCTTCTTTTTCATCTGCAGCCTGGCGCCTTCCCA...AACAACAATTGTCTGC
GGTCTCTGCTTTTTCATCTGCAGCCTGGCGCCTTCCCA...AACAACAATTGTCTGC

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*msx2*  
(-3.3 kb)

*gata3*  
(-0.1 kb)

**B**

h  
m  
r  
g  
x  
d  
t  
f

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CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATACCTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA
CCTCCTAAAGTTGCGTCGCGCCACAGCTGTCTGCGAAACACTGAGCTGCCTGGCGCGGCTCTGATAGTTTTCAGAAAGAAATGCATTCCTCTGTAACAAAA

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h  
m  
r  
g

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TGCGCGGCCCGCTTCATGTCTGTGCAGGAGTCGGCAGCTGGCGCCAGGGCGGCCGAGGATGCAGAGGGG
TGCGCGGCCCGCTTCATGTCTGTGCAGGAGTCGGCAGCTGGCGCCAGGGCGGCCGAGGATGCAGAGGGG
TGCGCGGCCCGCTTCATGTCTGTGCAGGAGTCGGCAGCTGGCGCCAGGGCGGCCGAGGATGCAGAGGGG
TGCGCGGCCCGCTTCATGTCTGTGCAGGAGTCGGCAGCTGGCGCCAGGGCGGCCGAGGATGCAGAGGGG

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*gata2*  
(+5.5 kb)

**C** *voX* (-0.2 kb)

d  
t  
f

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ATTAG.GCCTAATGGCTCGTTATTGAAGTCTTTACATATCCTGTGAGTGTGTGAAGCCCTGGCGCCAGCTTGTCTGTGAGAA
ATTAGCGGTTAATGGCTCGTTATTGAAGTCTTTACATATCCTGTGAGTGTGTGAAGCCCTGGCGCCAGCTTGTCTGTGAGAA
ATTAGGGGTTAATGGCTCGTTATTGAAGTCTTTACATATCCTGTGAGTGTGTGAAGCCCTGGCGCCAGCTTGTCTGTGAGAA

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*bmpr2*  
(-1.8 kb)

h  
m  
r  
c

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TGACGTGCTTGGCGCCATGATCGGAAGTCTGGACTGTAATCTTTAGCTGCGAGGTGGAAAAATGCGTGGGAGGGCGATCTTT
TGACGTGCTTGGCGCCATGATCGGAAGTCTGGGTTTAATCTTTAACTGCGAGGTGGAAAAATGCGTGGGAGGGCGATCTTT
TGACGTGCTTGGCGCCATGATCGGAAGTCTGGGTTTAATCTTTAACTGCGAGGTGGAAAAATGCGTGGGAGGGCGATCTTT
TGACGTGCTTGGCGCCATGATCGGAAGTCTGGTGTTAATCTTTAGCTGCGAGGTGGAAAAATGCGTGGGAGGGCGATCTTT

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*tlx2*  
(-1.2 kb)

h  
m  
r  
c

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GCCCAGTCTACCGGGCTCAGAGGCGCCAGGGCCCCCCCAGGGGCGGGGCGGGCCGGGGAAAGTAATGTAATTCCG
GCCCAGTCTACCGGGCTCAGAGGCGCCAGGGCCCCCCCAGGGGCGGGGCGGGCCGGGGAAAGTAATGTAATTCCG
GCCCAGTCTACCGGGCTCAGAGGCGCCAGGGCCCCCCCAGGGGCGGGGCGGGCCGGGGAAAGTAATGTAATTCCG
GCCCAGTCTACCGGGCTCAGAGGCGCCAGGGCCCCCCCAGGGGCGGGGCGGGCCGGGGAAAGTAATGTAATTCCG

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*tbx2*  
(-2.9 kb)

h  
m  
r  
f

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GTCTGCGGGACCTGGCCGATGCGAAGTGACAGGGGCCGAGCTTTGTTGTGGAGCCTCGGCCGCTGGCGCCAGCCGCCC
GTCTGCGGGTCCCGCCGATGCGAAGTGACAGGGGCCGAGCTTTGTTGTGGAGCCTCGGCCGCTGGCGCCAGCCGCCC
GTCTGCGGGTCCCGCCGATGCGAAGTGACAGGGGCCGAGCTTTGTTGTGGAGCCTCGGCCGCTGGCGCCAGCCGCCC
GTCTTTCAGAACTGTGAGCCTGTCTTTTGTCTCCATTTTGTCTGCTGGGCTCCG...CCTGGCCAGCCAGCCGCCC

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