**Table S1. Cloning strategies of constructs**

| DNA constructs | PCR primers and cloning strategy used to prepare expression plasmids |
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| *pNog2-EGFP* | 1st step. Obtaining of 4172 bp DNA fragment including 5'UTR and 5'non-coding region of *Noggin2* gene by two rounds of nested PCR from *Xenopus tropicalis* genomic DNA with the following two pairs of primers: 5'-GGTTAATAAGGGCTTGCTGAAC and 5'- CTCAGGCAGATTTATCCTCTTC (26 cycles); 5'-TAACGTCGACATAGCTGCCGATCAGTAGGTC and 5'- ATCCACCGGTGATGTTCAACCCCCTTCAATG (15 cycles).<br>2nd step. Cloning into SalI/AgeI sites of pEGFP plasmid (Clontech). Checking by sequencing. |
| *pNog2-tALK4* | 1st step. Obtaining of *tALK4* cDNA fragment from gastrula first-strand total cDNA with primers: 5'-ATATACCGGTGCCACCATGGCGGAGCTACCGGCCTT and<br>5'- AATGCGGCCGCTCAGATAGTTCTCGCCACAGT.<br>2nd step. Cloning instead of *EGFP* into AgeI/NotI sites of *pNog2-EGFP*. Checking by sequencing. |
| *pNog2-tBR* | 1st step. Obtaining of *tBR* cDNA fragment from *ptBR* with primers: 5'-AATAACCGGTGCCACCATGAGAGAACGACTTTTCATTG and 5'- AATGCGGCCGCTTATTTGTAAATCCATATGATAAGA .<br>2nd step. Cloning instead of *EGFP* into AgeI/NotI sites of *pNog2-EGFP*. Checking by sequencing. |
| *pNog2-Dkk* | 1st step. Obtaining of *Dkk1* cDNA from gastrula first-strand total cDNA with primers:<br>5'- AATAACCGGTGCCACCATGGGCAGCAACATGTT and<br>5'- AATGCGGCCGCTTAGTGTCTTTGGCAAGTGTGA.<br>2nd step. Cloning instead of *EGFP* into AgeI/NotI sites of *pNog2-EGFP*. Checking by sequencing. |
| *pXanfActB-CardKate* | To construct double-cassette vector *pXanfActB-CardKate*, *Otx2* cDNA in *pXanf1-Otx2-CardKate* (Ermakova et al., 2007) was swapped by AgeI and NotI with *ActivinβB* cDNA (see below) |
| Synthetic mRNA | PCR primers and cloning strategy used to prepare DNA templates for generation of synthetic mRNA |
| *Noggin1Δ5* | 1st step. PCR from *pNogginΔ5* with forward primer 'Ng1 Δ5': 5'- ATAACCGGTGAATTCCTCCTCTGATGCAT and reverse primer 'Ng1 stop': 5'- ATTCTCGAGTCTCAGCATGAGCATTTGCA. Here and below restriction sites are underlined, start and stop codons are framed.<br>2nd step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-Noggin1Δ5*. |
| *Noggin2Δ5* | 1st step. PCR from *pBluescriptNoggin2* with forward primer 'Ng2 Δ5': 5'- ATAACCGGTAATCTAACGATCTGTAACTATTG and reverse primer 'Ng2 stop': 5'- ATTCTCGAGTTAGCATGAACACTTACACTCTG.<br>2nd step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-Noggin2Δ5.* |
| *SynNoggin1* | 1st step. PCR from *pNogginΔ5* with forward primer 'Ng1 synt 5'(Kozak site is in italics): 5'-AATTACCGGT*CGCCACC*ATGGATCATTCCCAGTGCC and 'Ng1 stop'.<br>2nd step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-SynNoggin1*. |
| *SynNoggin2* | 1st step. PCR from *pBluescrptNoggin2* with forward primer 'Ng2 synt 5'(Kozak site is in italics): 5'-AATTACCGGT*CGCCACC*ATGAAGAGGATAAATCTGC and 'Ng2 stop'.<br>2nd step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-SynNoggin2*. |
| *ΔclipNoggin1* | 1st step. Obtaining of 5' fragment of *ΔclipNoggin1* cDNA. PCR from pNogginΔ5 with 'Ng1 synt 5' and 5'-CTTCTCCTTGGGATAATGTTGGCAACCCCCTT.<br>2nd step. Obtaining of 3' fragment of Δclip *Noggin1* cDNA. PCR from pNogginΔ5 with 5'-TTGCCAACATTATCCCAAGGAGAAGGATCTTA and 'Ng1 stop'.<br>3d step. Two overlapping cDNA fragments obtained at the previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with 'Ng1 synt 5' and 'Ng1 stop' primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-ΔclipNoggin1*. |
| *ΔclipNoggin2* | 1st step. Obtaining of 5' fragment of *ΔclipNoggin2* cDNA. PCR from *pBluescrptNoggin2* with 'Ng2 synt 5' and –CTGCTCCTTTGGATAAGGCTGACAGCACCCCT.<br>2nd step. Obtaining of 3' fragment of *ΔclipNoggin2* cDNA. PCR from *pBluescriptNoggin2* with 5'-CTGTCAGCCTTATCCAAAGGAGCAGGATCTGG and 'Ng2 stop'.<br>3d step. Two overlapping cDNA fragments obtained at the previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with 'Ng2 synt 5' and 'Ng2 stop' primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-ΔclipNoggin2*. |
| *misMONoggin2* | 1st step. PCR from *pBluescriptNoggin2* with 'Ng2 stop' and the mixture (ratio of 1:10 pM, respectively) of 'Ng1 synt 5' and Noggin1/Noggin2 adaptor primer 5'-GATCATTCCCAGTGCCTTGTGA*CTTTTTTGCTTTGCTTGTG* (*Noggin1* sequence is underlined, *Noggin2* sequence is in italic).<br>2nd step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-misMONoggin2.* |
| *misMOΔclipNoggin2* | The same PCR strategy was utilized as was used to generate *pCS2-misMONoggin2* except *pCS2-ΔclipNoggin2* was taken as a template for PCR.<br>Final construct: *pCS2-misMOΔclip Noggin2.* |
| *MycNoggin1Δ5* | 1st step. Obtaining of 5' fragment of *MycNoggin1Δ5* cDNA. PCR from *pNogginΔ5* with 'Ng1 Δ5' and a mixture of two reverse primers taken in ratio of 1:10 pM respectively: 5'-CAGATCCTCTTCAGAGATGAGTTTCTGCTCATAATGTTGGCAACCCCCTTG ('Ng1 Myc rev') and 5'-GAGGTCTTCCTCCGATATCAGCTTCTGTTCCAGATCCTCTTCAGAGATG ('Myc rev'). here and below Myc-tag coding sequences are underlined by dotted line.<br>2nd step. Obtaining of 3' fragment of *MycNoggin1Δ5* cDNA. PCR from *pNogginΔ5* with 'Ng1 stop' and a mixture of two forward primers taken in ratio of 1:10 pM respectively (Myc-tag coding sequences are underlined): 5'-GAGCAGAAACTCATCTCTGAAGAGG ATCTGCTGCACATCAGACCGG CT ('Ng1 Myc forw') and |

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| | 5′-GAACAGAAGCTGATATCGGAGGAAGACCTCGAGCAGAAACTCATCTCTG (‘Myc forw’).<br>3[d] step. Obtaining of cDNA encoding full *Noggin∆5* with three copies of Myc-tag epitop behind signal peptide cleavage site (see Fig. 2A). Two overlapping cDNA fragments obtained at the previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng1 ∆5’ and ‘Ng1 stop’ primers.<br>4[th] step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-MycNoggin1∆5*. |
| *MycNoggin2∆5* | 1[st] step. Obtaining of 5′ fragment of *MycNoggin2∆5* cDNA. PCR from *pBluescriptNoggin2* with ‘Ng2 ∆5’ and a mixture of two reverse primers taken in ratio of 1:10 pM respectively: 5′-CAGATCCTCTTCAGAGATGAGTTTCTGCTCATAAGGCTGACAGCACCCCTGA (‘Ng2 Myc rev’) and ‘Myc rev’.<br>2[nd] step. Obtaining of 3′ fragment of *MycNoggin2∆5* cDNA. PCR with ‘Ng1 stop’ and a mix of two forward primers taken in ratio of 1:10 pM respectively:<br>5′-AGCAGAAACTCATCTCTGAAGAGGATCTGCTCAGGCTTAGACCCTCT (‘Ng2 Myc forw’) and ‘Myc forw’.<br>3[d] step. Obtaining of cDNA encoding full *Noggin2∆5* with three copies of Myc-tag epitop behind signal peptide cleavage site (see Fig. 2A). Two overlapping cDNA fragments obtained at the previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng2 ∆5’ and ‘Ng2 stop’ primers.<br>4[th] step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2-MycNoggin2∆5*. |
| *wtMycNoggin1* | The same PCR-based strategy was utilized as was used for preparing *MycNoggin1∆5* cDNA (see above), except *pNoggin A3* plasmid and different forward primer was used for obtaining of 5′ fragment of *wtMycNoggin1* cDNA: (Ng1 wt5′) 5′- ATAACCGGTTAATAAATCCTAAGTAGCCAGA.<br>Final construct: *pCS2-wtMyc Noggin1*. |
| *wtMycNoggin2* | The same PCR-based strategy was utilized as was used for preparing *MycNoggin1∆5* cDNA (see above), except *pBluescript-wtNoggin2* and different forward primer was used for obtaining of 5′ fragment of *wtMycNoggin2* cDNA: (Ng2 wt5′) 5′- ATAACCGGTTGATTCTGCCTTACTTACTGACACA.<br>Final construct: *pCS2-wtMyc Noggin2*. |
| *SynMycNoggin1* | 1[st] step. PCR from *pCS2-MycNoggin1∆5* with primers ‘Ng1 synt 5′ and ‘Ng1 stop’.<br>2[nd] step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2- SynMycNoggin1*. |
| *SynMycNoggin2* | 1[st] step. PCR from *pCS2-Myc Noggin2∆5* with primers ‘Ng2 synt 5′ and ‘Ng2 stop’.<br>2[nd] step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI of pCS2 plasmid; checking by sequencing.<br>Final construct: *pCS2- SynMycNoggin2*. |
| *Myc∆clipNoggin 1* | The same PCR strategy was utilized as was used to generate *MycNoggin1∆5*, except ‘Ng1 synt 5′ primer was taken instead of ‘Ng1 ∆5’ and 5′-GAGCAGAAACTCATCTCTGAAGAGGATCTGCCCAAGGAGAAGGATCTTA (‘∆-clip-Ng1 forw’) was taken instead of ‘Ng1 Myc forw’.<br>Final construct: *pCS2-Myc∆clip Noggin1*. |
| *Myc∆clipNoggin 2* | The same PCR strategy was utilized as was used to generate *MycNoggin2∆5*, except ‘Ng2 synt 5′ primer was taken instead of ‘Ng2 ∆5’ and 5′-GAGCAGAAACTCATCTCTGAAGAGGATCTGCCCAAGGAGAAGGATCTTA (‘∆-clip-Ng1 forw’) instead of ‘Ng2 Myc forw’.<br>Final construct: *pCS2-Myc∆clip Noggin2*. |
| *BMP4* | 1[st] step. Obtaining of cDNA encoding full BMP4. PCR from *Xenopus laevis* gastrula first strand cDNA with primers full BMP4 forward: 5′-AATTGGATCCGCCACCATGATTCCTGGTAACCGAA and stop BMP4 reverse: 5′-AATCTCGAGTCAACGGCACCCACACCCTTCCA.<br>2[nd] step. The obtained cDNA fragments was cloned into pCS2 plasmid either by BamHI and XhoI and correct clone was selected by sequencing.<br>Final construct: pCS2-BMP4. |
| *Xnr2* | 1[st] step. Obtaining of cDNA encoding full Xnr2. PCR from *Xenopus laevis* gastrula first strand cDNA with primers full Xnr2 forward: 5′-AATTGAATTCGCCACCATGGCAAGCCTAGGAGTCATC and stop Xnr2 reverse: 5′-AATCTCGAGTCAGTTACATCCACACTCATCCA.<br>2[nd] step. Cloning of the obtained cDNA fragments was cloned into pCS2 plasmid either by EcoRI and XhoI and correct clone was selected by sequencing. Final construct: pCS2-Xnr2. |
| *Xnr4* | 1[st] step. Obtaining of cDNA encoding full Xnr4. PCR from *Xenopus laevis* gastrula first strand cDNA with primers full Xnr4 forward: 5′-AATTGGATCCGCCACCATGCATCTATACTTTTACTGTCT and stop Xnr4 reverse: 5′-AATCTCGAGTCACTGGCAGCCACACTCTTC.<br>2[nd] step. The obtained cDNA fragments was cloned into pCS2 plasmid either by BamHI and XhoI and correct clone was selected by sequencing.<br>Final construct: pCS2-Xnr4. |
| *FlagActivinB* | 1[st] step. Obtaining of double-stranded cDNA fragment encoding three Flag epitops. Annealing 5′-TAAGTCGACTACAAAGACGATGATGACAAAGATTACAAGGATGACGACG and 5′-TAACTCGAGTTTGTCATCATCGTCTTTGTAGTCCTTATC*GTCGTCATCC* (complementary sequences are in italics), filing of nested ends by Klenow fragment of DNA polymerase, restriction by SalI and XhoI.<br>2[nd] step. Cloning of the obtained cDNA in XhoI site of *pSP64-ActivinB*. The resulting construct had a sequence encoding for three Flag-tag epitops located posterior to the ActivinβB pre-proregion cleavage site (cleavage site is framed, Flag equences are underlined):…RGLDYKDDDDKDYKDDDDKDYKDDDDKLECDG…Importantly, in the resulting plasmid (*pSP64-Flag- ActivinB*), the region encoding for mature ActivinβB was flanked from 3′-end by XhoI site which allowed us to generate Flag-<br>tagged chimeric constructs, composed of the pre-proregion of ActivinβB and the mature region of any desired TGF-beta factor.<br>Final construct: *pSP64-FlagActivinB*. |
| *FlagADMP* | 1[st] step. Obtaining of cDNA fragment encoding for mature part of ADMP. PCR from Xenopus laevis gastrula first strand cDNA with primers mature ADMP forward: 5′-ATTCTCGAGTCAGTAGAAGAAGATGGACAA and stop ADMP reverse: 5′-ATAGAATTCTTAGTGGCACCCGCAGCT.<br>2[nd] step. The obtained cDNA fragment was cloned into *pSP64-FlagActivinB* plasmid by XhoI and EcoRI instead of the |

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| | fragment encoding mature ActivinB and checked by sequencing.<br>Final construct: *pSP64-FlagADMP*. |
| *FlagBMP4* | 1st step. Obtaining of cDNA fragment encoding for mature part of BMP4. PCR from *pCS2-BMP4* with primers mature BMP4 forward: 5′-AATCTCGAGCAGAGACCCCGTAAAAAAAAC and stop BMP4 reverse.<br>2nd step. The obtained cDNA fragment was cloned into *pSP64-FlagActivinB* plasmid by XhoI and EcoRI (blunted) instead of the fragment encoding mature ActivinB and checked by sequencing.<br>Final construct: *pSP64-FlagBMP4*. |
| *FlagXnr2* | 1st step. Obtaining of cDNA fragment encoding for mature part of Xnr2. PCR from *pCS2-Xnr2* with primers mature Xnr2 forward: 5′-TAACTCGAGATTGTCATGAACACCATCCCTTC and stop Xnr2 reverse.<br>2nd step. The obtained cDNA fragment was cloned into *pSP64-FlagActivinB* plasmid by XhoI and EcoRI (blunted) instead of the fragment encoding mature ActivinB and checked by sequencing.<br>Final construct: *pSP64-FlagXnr2*. |
| *FlagXnr4* | 1st step. Obtaining of cDNA fragment encoding for mature part of BMP4. PCR from *pCS2-Xnr4* with primers mature Xnr4 forward: 5′- ATACTCGAGTTTAAGGAACATGTTATGGGT and stop Xnr4 reverse.<br>2nd step. The obtained cDNA fragment was cloned into *pSP64-FlagActivinB* plasmid by XhoI and EcoRI (blunted) instead of the fragment encoding mature ActivinB and checked by sequencing.<br>Final construct: *pSP64-FlagXnr2*. |
| *XWnt8* | 1st step. Obtaining of cDNA fragment encoding for mature part of BMP4. PCR from *pCSKA-XWnt8* first strand cDNA with primers XWnt8 forward: 5′-AATTGGATCCGCCACCATGCAAAACACCACTTTGTTCATC and XWnt8 reverse: 5′-ATGCATGCTCGAGTCATCTCCGGTGGCCTCT.<br>2nd step. The obtained cDNA fragment was cloned into pCS2 plasmid by BamHI and XhoI and checked by sequencing.<br>Final construct: *pSP64-Xwnt8*. |
| *Xwnt8-Flag* | 1st step. Obtaining of *XWnt8* cDNA deprived of 3′-terminal stop-codon by PCR from *pCSKA-XWnt8* with primers XWnt8 forward and XWnt8 reverse: 5′-ATGCATGCTCATGATTCTCCGGTGGCCTCT.<br>2nd step. The obtained cDNA fragment was cloned by EcoRV/BamHI (blunted) and NcoI/PagI sites into pCS4- 3Flag plasmid (gift from Dr Asashima) and checked by sequencing.<br>Final construct: *pSP64-Xwnt8-Flag*. |
| *SynNog2-Cer* | Preparing of chimeric cDNA encoding for signaling peptide of Noggin2 and mature part of Cerberus.<br>1st step. Obtaining of cDNA fragment encoding Noggn2 signal peptide by PCR from *pCS2-SynNoggin2* with primers ‘Ng2 synt 5′ and 5′-ATAAGGCTGACAGCACCCCT.<br>2nd step. Obtaining of cDNA fragment encoding mature Cerberus by PCR from *pSP35-Cer* with primers 5′-AGGGGTGCTGTCAGCCTTATCACTCAGAAGGACGAGAAAG and 5′-AATTCTCGAGTTAATGGTGCAGGGTAGTAGATGTAT (‘Cer-stop’).<br>3d step. Two overlapping cDNA fragments obtained at previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng2 synt 5′ and ‘Cer-stop’ primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI sites of pCS2 plasmid. Checking by sequencing.<br>Final construct: *pCS2-SynNog2-Cer*. |
| *SynMycNog2-Cer* | Preparing of chimeric cDNA encoding for signaling peptide of Noggin2 and mature part of Cerberus.<br>1st step. Obtaining of cDNA fragment encoding Noggn2 signal peptide by PCR from *pCS2-SynMycNoggin2* with primers ‘Ng2 synt 5′ and 5′-CAGATCCTCTTCAGAGATGAGTTTCTGCTCTAGGTCT.<br>2nd step. Obtaining of cDNA fragment encoding mature Cerberus by PCR from *pSP35-Cer* with primers 5′-TCATCTCTGAAGAGGATCTGCACTCAGAAGGACGAGAAAG and ‘Cer-stop’.<br>3d step. Two overlapping cDNA fragments obtained at previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng2 synt 5′ and ‘Cer-stop’ primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI sites of pCS2 plasmid. Checking by sequencing.<br>Final construct: *pCS2-SynMucNog2-Cer*. |
| *SynNog2-Fol* | Preparing of chimeric cDNA encoding for signaling peptide of Noggin2 and mature part of Follistatin.<br>1st step. Obtaining of cDNA fragment encoding Noggn2 signal peptide by PCR from *pCS2-SynNoggin2* with primers ‘Ng2 synt 5′ and 5′-ATAAGGCTGACAGCACCCCT.<br>2nd step. Obtaining of cDNA fragment encoding mature Follistatin by PCR from *p64TNE-XFS319* with primers 5′-AGGGGTGCTGTCAGCCTTATAATTGCTGGCTGCAGCAGTC and 5′-ATTCTCGAGTCACTTACAGTTGCAAGAT (‘Fol-stop’).<br>3d step. Two overlapping cDNA fragments obtained at previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng2 synt 5′ and ‘Fol-stop’ primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI sites of pCS2 plasmid. Checking by sequencing.<br>Final construct: *pCS2-SynNog2-Fol*. |
| *SynMycNog2-Fol* | Preparing of chimeric cDNA encoding for signaling peptide of Noggin2 and mature part of Follistatin.<br>1st step. Obtaining of cDNA fragment encoding Noggn2 signal peptide by PCR from pCS2-SynMycNoggin2 with primers ‘Ng2 synt 5′ and 5′-CAGATCCTCTTCAGAGATGAGTTTCTGCTCTAGGTCT.<br>2nd step. Obtaining of cDNA fragment encoding mature Follistatin by PCR from *p64TNE-XFS319* with primers 5′-TCATCTCTGAAGAGGATCTGAATTGCTGGCTGCAGCAGTC and ‘Fol-stop’.<br>3d step. Two overlapping cDNA fragments obtained at previous two steps were purified from non-incorporated primers, mixed, denatured, annealed and subjected to PCR with ‘Ng2 synt 5′ and ‘Fol-stop’ primers.<br>4th step. Cloning by AgeI (blunted) and XhoI into BamHI (blunted) and XhoI sites of pCS2 plasmid. Checking by sequencing.<br>Final construct: *pCS2-SynMycNog2-Fol*. |