

## Supplementary Tables and figures:

### Figure S1. Sequence alignment of several mammalian and teleost IGFBP-5 N-domains.

Identical and similar amino acid residues are darkly and lightly shaded, respectively. Asterisks (\*) mark the residues that are critical for the different transactivation activities between zebrafish IGFBP-5a and -5b.

human IGFBP-5	SFVHCEPCDEKALSMCPPSPITGCELVKEPGCGCCMTCALAEQGSCGVYTERCAOGLRCLP RODEEKPLHALLHGRGVC
mouse IGFBP-5	SFVHCEPCDEKALSMCPPSPITGCELVKEPGCGCCMTCALAEQGSCGVYTERCAOGLRCLP RODEEKPLHALLHGRGVC
zebrafish IGFBP-5b	SFVPCPEPCDQKALSMCPPVPVGCQLVKEPGCGCCYTCALAEQGACGVYTGCTHGLRCLP RNDEEKPLHALLHGRGVC
fugu IGFBP-5b	SYVPCPEPCDQKALSMCPPVPVGCQLVKEPGCGCCLTCAISEGQACGVYTGCTQGLRCLP RSGEEKPLHALLHGRGVC
stickleback IGFBP-5b	SYVPCPEPCDQKALSMCPPVPVGCQLVKEPGCGCCLTCAISEGQACGVYTGCTQGLRCLP RSGEEKPLHALLHGRGVC
zebrafish IGFBP-5a	SFVPCPEPCDQKALSMCPPVRNGCQVKEPGCGCCLTCALEEGQACGVYTGCTGRGLRCLP RIGEEKPLHALLHGRGVC
fugu IGFBP-5a	SFVPCPEPCDQKALSMCPPVPVGCQLVKEPGCGCCLTCALEEGQACGVYTGCTGRGLRCLP RIGEEKPLHALLHGRGVC
stickleback IGFBP-5a	SYVPCPEPCDQKALSMCPPVPVGCQLVKEPGCGCCLTCALEEGQACGVYTGCTGRGLRCLP RIGEEKPLHALLHGRGVC

**Table S1. Accession numbers of the IGFBP sequences used for the phylogenetic analyses**

Protein †Species	IGFBP -1	IGFBP -2	IGFBP -3	IGFBP -4	IGFBP -5	IGFBP -6
human	NP_000587	NP_000588	NP_000589	NP_001543	NP_000590	NP_002169
bovine	NP_776979	NP_776980	NP_776981	NP_776982	NP_001098797	NP_001035585
mouse	NP_032367	NP_032368	NP_032369	NP_034647	NP_034648	NP_032370
chicken	NP_001001294	NP_990690	NP_001094504	NP_989684	-	-
frog	-	-	-	-	NP_001083938	-
zebrafish	NP_775390 NP_001091727	NP_571533 AB S30427	NP_991314	-	NP_001092224 NP_001119935	NP_001154873 NP_001154874
fugu	-	-	-	-	ENSTRUG00000013829 ENSTRUG00000004347	-
stickleback	-	-	-	-	ENSGACG00000014275 ENSGACG00000002508	-

†: human (*Homo sapiens*), bovine (*Bos taurus*), mouse (*Mus musculus*), chicken (*Gallus gallus*), frog (*Xenopus laevis*), zebrafish (*Danio rerio*), fugu (*Takifugu rubripes*), stickleback (*Gasterosteus aculeatus*).

**Table S2. NCBI or Ensembl Gene ID of the genes used for the conserved synteny analysis**

Gene †Species	IGFBP-2	IGFBP-5	TNS1	STK11IP	SLC4A3
human	3485	3488	7145	114790	6508
mouse	16008	16011	21961	71728	20536
zebrafish a	794176	795084	565181	795019	
zebrafish b	798920	403039	572346		100151543
fugu a	ENSTRUG00000013702	ENSTRUG00000013829	ENSTRUG00000013916	ENSTRUG00000013564	
fugu b	ENSTRUG00000003825	ENSTRUG00000004347	ENSTRUG00000004813		ENSTRUG00000002078
stickleback a	ENSGACG00000014280	ENSGACG00000014275	ENSGACG00000014268	ENSGACG00000014289	
stickleback b	ENSGACG00000002506	ENSGACG00000002508	ENSGACG00000002511		ENSGACG00000002501

†: human (*Homo sapiens*), mouse (*Mus musculus*), zebrafish (*Danio rerio*), fugu (*Takifugu rubripes*), stickleback (*Gasterosteus aculeatus*).

## Supplementary Tables and figures:

**Table S3. Oligonucleotide primers used for plasmid construction in this study**

vector	Target region	Primer name	Primer sequence
pcDNA3.1(-)/ myc-His A	zebrafish IGFBP-5a ORF	IGFBP-5a XhoI F	5'-GTCTCGAGACCATGCTGCTAAGTTTGTATGCT-3'
		IGFBP-5a HindIII R	5'-CAAAGCTTTTCGTTGTTGTTGCTGTG-3'
pcDNA3.1(-)/ myc-His A	zebrafish IGFBP-5b ORF	IGFBP-5b XhoI F	5'-GTCTCGAGACCATGGCTCTTCTTGTGCTGGGTACA-3'
		IGFBP-5b HindIII R	5'-CAAAGCTTCTCGTTGTTGTTGTTGCTGTTCTCC-3'
pcDNA3.1(-)/ myc-His A	human IGFBP-5 ORF	IGFBP-5 XhoI F	5'-GTCTCGATATGGTGTGCTCACCCGCG-3'
		IGFBP-5 HindIII R	5'-GTAAGCTTCTCAACGTTGCTGCTGTC-3'
pCS2+/EGFP	zebrafish IGFBP-5a ORF	IGFBP-5a BamHI F	5'-GTGGATCCACCATGCTGCTAAGTTTGTATGCT-3'
		IGFBP-5a ClaI R	5'-CAATCGATGTTCTGTTGTTGTTGCTGTG-3'
pCS2+/EGFP	zebrafish IGFBP-5b ORF	IGFBP-5b BamHI F	5'-GTGGATCCACCATGGCTCTTCTTGTGCTGGGTACA-3'
		IGFBP-5b ClaI R	5'-CAATCGATGCTCGTTGTTGTTGTTGCTGTTCTCC-3'
pBIND	zebrafish IGFBP-5a N-domain	IGFBP-5a BamHI F	5'-CGGGATCCCAGCGTCGTTCTGTCAT-3'
		IGFBP-5a NotI R	5'-ATAAGAATGCGGCCGCTCAGCAAACCTTTGCCGTA-3'
pBIND	zebrafish IGFBP-5b N-domain L15M	IGFBP-5bN L15M F	5'-GCGATCAGAAGGCATGTCCATGTGCTCC-3'
		IGFBP-5bN L15M R	5'-GGAGGACACATGGACATCGCTTCTGATCGC-3'
pBIND	zebrafish IGFBP-5b N-domain P22R	IGFBP-5bN P22R F	5'-GTCCTCCGGTCCGGGTGGGCTGTC-3'
		IGFBP-5bN P22R R	5'-GACAGCCACCCGGACCCGAGGAC-3'
pBIND	zebrafish IGFBP-5b N-domain H56R	IGFBP-5bN H56R F	5'-GTACATGCACACGCGGGTGCCTG-3'
		IGFBP-5bN H56R R	5'-CAGCGCAGCCCGCTGTGCATGTAC-3'
pBIND	zebrafish IGFBP-5b N-domain N64I	IGFBP-5bN N64I F	5'-CTGCCGCGCATCGCGGAGGAGAAG-3'
		IGFBP-5bN N64I R	5'-CTTCTCTCGCCGATGCGCGGACAGG-3'
pBIND	zebrafish IGFBP-5b N-domain E8A/D11S/E43L	IGFBP-5bN E8A/D11S F	5'-GTACCGTGCAGCCGTCAGTCAAGAGCGC-3'
		IGFBP-5bN E8A/D11S R	5'-GCGCCTTCTGACTGCACGGCGCACCGGTAC-3'
		IGFBP-5bN E43L F	5'-GCGCTCTGGCGCTGGGGCAGGCGTG-3'
		IGFBP-5bN E43L R	5'-CACGCCTGCCCGACGCGCCAGAGCGC-3'
pBIND	zebrafish IGFBP-5a N-domain R22P	IGFBP-5aN R22P F	5'-GTCCTCCGGTCCGAACGGGTGTACAG-3'
		IGFBP-5aN R22P R	5'-CTGACACCCGTTCCGACCCGAGGAC-3'
pBIND	zebrafish IGFBP-5a N-domain R56H	IGFBP-5aN R56H F	5'-CCGGTACATGTGGACACGGACTGCGATGCC-3'
		IGFBP-5aN R56H R	5'-GGCATCGCAGTCCGTGTCCACATGTACCGG-3'
pBIND	zebrafish IGFBP-5a N-domain R56Q	IGFBP-5aN R56Q F	5'-CGGTACATGTGGACAAGGACTGCGATGCC-3'
		IGFBP-5aN R56Q R	5'-GGCATCGCAGTCCGTTGTCCACATGTACCG-3'
pBIND	zebrafish IGFBP-5b N-domain H56Q	IGFBP-5bN H56Q F	5'-GTACATGCACACAGGGGCTGCGCTGC-3'
		IGFBP-5bN H56Q R	5'-GCAGCGCAGCCCTGTGTGCATGTAC-3'
pBIND	zebrafish IGFBP-5b N-domain H56Q/G52E	IGFBP-5bN H56Q/G52E F	5'-GGTGTACACCGAGACATGCACACAGGGGCTGCGCTG-3'
		IGFBP-5bN H56Q/G52E R	5'-CAGCGCAGCCCTGTGTGCATGTCTCGGTGACACC-3'
pBIND	zebrafish IGFBP-5b N-domain H56Q/G52E/Q12E	IGFBP-5bN Q12E F	5'-GCCGTGCGATGAGAAGGCCTCTCC-3'
		IGFBP-5bN Q12E R	5'-GGAGAGCGCCTTCTCATCGCACGGC-3'

**Table S4. Sequence identities between zebrafish IGFBP-5a/-5b and IGFBPs in human and mouse**

Protein † S pecies	IGFBP -1	IGFBP -2	IGFBP -3	IGFBP -4	IGFBP -5	IGFBP -6
human	28/29	26/24	36/37	30/32	<b>47/52</b>	28/29
mouse	29/28	27/27	36/41	30/31	<b>48/53</b>	27/29

†: human (*Homo sapiens*), mouse (*Mus musculus*).