Supplementary Material

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Α	hFGF22	MRRRLWLGLAWLI	LARAPD	19
	zFgf22	MCKWTPTTAGLHFAGSAPPSYPATLVCLSI	LSLACSALGGCPPALGHDPLHAL	53
	mFgf22		LARAP	18
	hFGF22	AAGTP-SASRGPRSYPHLEGDVRWRRL	SSTHFFLRVDPGGRVQGTRWRHG	69
	zFgf22	AQGTNCSWTLERHTRSYNHLEGDVRLRRL		106
	mFgf22	GAPGGYPHLEGDVRWRRLF	SSTHFFLRVDLGGRVQGTRWRHG	60
	hFGF22	QDSILEIRSVHVGVVVIKAVSSGFYVAMNF	RGRLYGSRLYTVDCRFRERIEEN	122
	zFgf22	ADSLMEIRSVSVGVVAIKSVSTGLYLAMSH	KGTLFGSARYNPSCKFKERIEEN	159
	mFgf22	QDSIVEIRSVRVGTVVIKAVYSGFYVAMN	RGRLYGSRVYSVDCRFRERIEEN	113
	hFGF22	GHNTYASQRWRRRGQPMFLALDRRGGPRPC		170
	zFgf22	GYNTYASLRWKHRGRQMFVSLNGRGKPRRGHKARRRHPSTHFLPMLPT 2		207
	mFgf22	GYNTYASRRWRHRGRPMFLALDSQGIPRQC	GRRTRRHQLSTHFLPVLVSS	172
в	Zeb		Human chromosome	
		22	19p13.3	
		$^{21.8}$ \downarrow bsg	0.50 T	
		-hcn2		
		21.9	- BSG	
		polrmt		
			0.55 - HCN2	
		22.0 - ((02	* 47 (1970), 00 (1970) 10	
		- fgf22	– POLRMT	
		22.1 –	0.60 _ FGF22	
		(Mb)	(Mb)	

Fig. S1. Molecular analysis of zebrafish Fgf22. (A) The amino acid sequence of zebrafish Fgf22 (zFgf22) aligned with human FGF22 (hFGF22) and mouse Fgf22 (mFgf22). The numbers refer to the amino acid positions of zebrafish Fgf22, human FGF22, and mouse Fgf22. Asterisks indicate identical amino acid residues in the sequences. Dashes indicate introduced gaps. (B) Syntenic relationship between zebrafish chromosome 22 and human chromosome 19 at p13.3. Both the zebrafish *fgf22* and human *FGF22* genes are closely linked to the zebrafish and human *bsg, hcn2*, and *polrmt* genes. Mb, megabase.

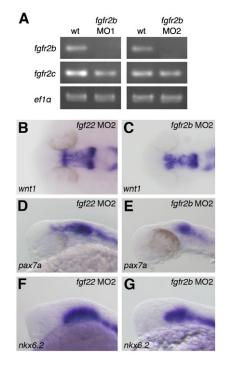
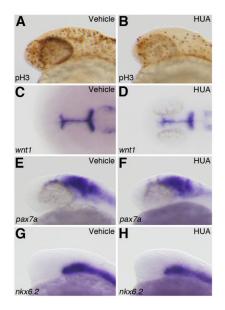


Fig. S3. Inhibition of fgf22 and fgfr2b functions in zebrafish embryos. (A) The cDNA of fgfr2b and fgfr2c was amplified from wild-type or Fgfr2b MO-injected embryonic cDNA by RT-PCR using forward and reverse primers. $efl\alpha$ cDNA was also amplified as a control. (**B**–**G**) The expression of *wnt1* (B,C), pax7a (D,E), and nkx6.2 (F,G) at 24 hpf in fgf22 MO2-injected (B,D,F), and fgfr2b MO2-injected (C,E,G) embryos.



A wt B fgf22 MO1

Fig. S2. Apoptosis in the midbrain of fgf22 morphants. At 24 hpf, apoptotic cells in the midbrain of the wild-type (A) and fgf22 MO1-injected (B) embryos were marked via TUNEL. Dorsal view with anterior to the left.

Fig. S4. Cellular proliferation does not contribute to the reduction of dorsal midbrain. (A,B) Wild-type embryos treated with 4% DMSO (A) or HUA (B) were stained using an anti-pH3 antibody. (C–H) The expression of *wnt1* (C,D), *pax7a* (E,F), and *nkx6.2* (G,H) at 24 hpf in wild-type embryos treated with 4% DMSO (C,E,G) or HUA (D,F,H).