Supporting Information

Hong and Dawid 10.1073/pnas.0812880106

VAN VAN

	10	20	30	40	50	60
H IER2	NEVCKEACRI	MTLSVWKMYH	SRIVCRGGL RL	HRSLCLSLVN	RSARELYLSA	KVEALEPEVS
M IER2	NEVCKEACRI	MTLSVWKMYH	SRIVCRGGLRL	HRSLCLSLVM	RSAREVYLSA	KVEAHCPEFP
Z IER2	NEVTAEAKCI	MVGALGKNYS	SRSCRGGLRL	HRSLLLTLVN	KSARDI YHSA	RLMSEKSCCS
	70	80	90	100	110	120
H IER2	L PAALPS	DPRLHPPREA	ESTAETATPD	GEHPFPEPMD	TCEAPT- AEE	TSACCAP- RP
M IER2	PSRRAL	DPRLHPPRED	EVAVEVASPE	AVCP- PGPMD	TCEEVLEVCE	TPALCOP- PP
Z IER2	VTEECTSHTC	EPMDTSSSTA	TPLRETSGCS	SEDGCRSGLE	GHPHPLNPAA	DKENCGPSRP
	130	140	150	160	170	180
H IER2	AKVSRKRRSS	S- LSDGGDAG	LVPSKKARLE	EKEEEEGASS	EVADRLCPPP	ACAECAFPNL
M IER2	AFVSRKRRSS	SDL SDGSDAG	LVPSKKARLE	EVECE ATS	EVPDRLCLPP	ACSECAFPNL
Z IER2	DRHSRKRRSK	T ATDSD	FI PCKKAKLE	CAEVR		G
	190	200	210	220	230	
H IER2	AFVLCRRFSG	LLNCSPAAPP	TAPPACEAKP	ACRPADSMLN	VLVRAVVAF.	
M IER2	AFVLCRRFSS	FLNCGPAVPP	PTPPTCEAKP	ACRPADNMLN	VLVRAVVAF.	
Z IER2	VI C NS-	SANCERAL DS	15	IV	PMPRTI VTF	

Fig. S1. Vertebrate ler2 Proteins. Alignment of human, mouse, and zebrafish ler2 proteins. Amino acid identity is: human vs. zebrafish, 30%; mouse vs. zebrafish, 30%; human vs. mouse 76%. No functional domain was found is ler2 through computational programs. The accession numbers are: human IER2, NM004907mouse ler2, NM010499; zebrafish ler2, EU815060.

	10	20	30	40	50	60
Hfibp MTS	ELDI FVG	NTTLI DECVY	RLWLDGYSVT	DAVALEVRSC	I LECTGATAA	VLCSDTMDHY
Mfibp MTS	ELDI FVG	NTTLI DECVY	RLWLDGYSVN	DAVALEVRSC	I LECTGATTG	VLCSDTMDHY
XIfibp MTN	ELEVEVG	NTTLI DECVY	CLWLDGYSVS	DAVNNRLKSG	I LECTGAGAD	VLESDTMDHY
Zfibp I N I	ELEVEVG	NTTVLDEDI Y	CLVLDGHSVS	EVVRCRMDAG	VLLECEASPD	VLCSDTI DCF
	70	80	90	100	110	120
					EADLANDER OF	KI OKCTIKEDI
HIDP RIFI	HIVLERLL	HAPPKLLHGL	I FOI PPSRCA	LLI ERYYAFD	EAFVREVLOK	KLSKGTKKDL
VIEbo DTE	INLERLL	HAPPKLLHGL	I FOI PPSKGT	LLIERYYTED	EAFVREVLOK	KLSKGTKKUL
Zibb I RTF	ONCERLL	HTPPKLVGGL	LEGI PPTKGS	MLI ERYYAFU	EAFVREVLOK	KLSKGTKKUL
ZIOPIRIE	GIVCERLL	COPON VAINGL	LFGI PSEGGA	NUI ERTTEPU	SVFAREVLER	RESKEINNEL
	130	140	150	160	170	180
Hfibp DDI S	STKTCI T	LKSCRRCFDN	FKFVFKVVEE	MRGSLVDNI C	CHFLLSDRLA	RDYAAI VEFA
Mfibp DDI S	STKTGI T	LKSCRRCFDN	FKFVFKVVEE	MRGSL VDNI C	CHFLLSDRLA	RDYAAI VEFA
XIfibp DDI S	SSKTEVT	LKSCRRCFDN	FKFVFKVVEE	MRGAL VENI C	CNFLLSDKLA	RCYAAI VFFA
Zfibp1 DEVS	SAKTGI A	LKSCRRCFDN	FKFVFKVVEE	LKGPLVENI G	RHFLLSDDLA	RCYATI VFFA
	190	200	210	220	230	24
<u></u>	190	200	210	220	230	246
Hfibp NNR	FETGKKK	LCYLSFGDFA	FCAELM CNM	TLGAVDSCMD	DWDWDLDKEF	
Hfibp NNR Mfibp NNR	FETGKKK	LCYLSFCDFA LCYLSFCDFA	FCAELM CNW FCAELM CNW	TLGAVDSCMD TLGAVDSCVD	DWDWDLDKEF DWDVDLDKEF	LCDLKELKVL
Hfibp NNR Mfibp NNR Xlfibp NSR	FETGKKK FETGKKK FETGKRK	LCYLSFGDFA LCYLSFGDFA LCYLTFEDFA	FCAELM CNW FCAELM CNW TCAEHLI CNW	TLGAVDSCVD TLGAVDSCVD TLGAVD-VAD	DWDMDLDKEF DWDVDLDKEF DWDMDLDKEF	LCDLKELKVL LCDLKELKVL LCDLKDLKI L
Hfibp NNR Mfibp NNR Xlfibp NSR Zfibp 1 NSR	FETGKKK FETGKKK FETGKRK FETGKRK	LCYLSFGDFA LCYLSFGDFA LCYLTFEDFA LCYLSFCDFA	FCAELM CNW FCAELM CNW TCAEHLI CNW FCAGCLI SYW	TLGAVDSCMD TLGAVDSCVD TLGAVD-VAD TVGAVDNM E	DWDMDLDKEF DWCVDLDKEF DWCWDLDKEF DWCWDLDKEF DWCVDLEKDF	LCDLKELKVL LCDLKELKVL LCDLKDLKI L LHDLKDLKVL
Hfibp NNR Mfibp NNR Xlfibp NSR Zfibp I NSR	ETGKKK ETGKKK ETGKRK ETGKRK ETGKRK	LCYLSFGDFA LCYLSFGDFA LCYLSFGDFA LCYLSFCDFA LCYLSFCDFA	FCAELM CNW FCAELM CNW FCAELM CNW TCAEHLI CNW FCAGCLI SYW 270	TL GAVDSCMD TL GAVDSCVD TL GAVDSCVD TL GAVD- VAD TVGAVDNM E 280	230 DWDMDLDKEF DWCVDLDKEF DWDMDLDKEF DWDWDLDKEF DWCVDLEKDF 290	LCDLKELKVL LCDLKELKVL LCDLKELKVL LCDLKDLKI LHDLKDLKVL
Hfibp NNRI Mfibp NNRI Xlfibp NSRI Zfibp I NSRI	ETGKKK ETGKKK ETGKRK ETGKRK 250	LCYLSFGDFA LCYLSFGDFA LCYLSFGDFA LCYLSFCDFA LCYLSFCDFA 260	FCAELM CNW FCAELM CNW FCAELM CNW TCAEHLI CNW FCAGCLI SYW 270	TL GAVDSCMD TL GAVDSCVD TL GAVDSCVD TL GAVD- VAD TVGAVDNM E 280	230 DWDMDLDKEF DWCVDLDKEF DWDMDLDKEF DWCVDLEKDF 290	244 LCDLKELKVL LCDLKELKVL LCDLKDLKI LCDLKDLKVL 140LKDLKVL 300
Hfibp NNRI Mfibp NNRI Xlfibp NSRI Zfibp I NSRI Hfibp VAD	ETGKKK ETGKKK ETGKRK ETGKRK 250 KDLLDLH	200 LCYLSFGDFA LCYLSFGDFA LCYLSFCDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG	FCAELM CNW FCAELM CNW FCAELM CNW FCAELLI CNW FCAECLI SYW 200 KLCVFSENE	220 TL GAVDSCMD TL GAVDSCVD TL GAVD- VAD TVGAVD- VAD TVGAVDNM E 280 NFKNLSRCLV	230 DNDNDLDKEF DNEVDLDKEF DNEVDLDKEF DNEVDLEKDF 200 NVAAKLTHNK	244 LCDLKELKVL LCDLKELKVL LCDLKDLKI L LHDLKDLKVL 2000 CVRDLFVDLV
Hfibp NNR Mfibp NNR Xlfibp NSR Zfibp I NSR Zfibp I NSR Hfibp VAD Mfibp VAD	ETGKKK ETGKKK ETGKRK ETGKRK 250 KDLLDLH KDLLDLH	200 LCYLSFCDFA LCYLSFCDFA LCYLTFEDFA LCYLSFCDFA 200 KSLVCTALRC KSLVCTALRC	210 FCAELM CNW FCAELM CNW FCAELLI CNW FCAGCLI SYW 270 KLCVFSENET KLCVFSENET KLCVFSENET	TL GAVDSCMD TL GAVDSCVD TL GAVD- VAD TVGAVD- VAD TVGAVDNM E 200 NFKNLSRCLV NFKNLSRCLV NFKNLSRCLV	230 DNDNDLDKEF DNEVDLDKEF DNDNDLDKEF DNEVDLEKDF 200 NVAAKLTHNK NVAAKLTHNK NVAAKLTHNK	LCDLKELKVL LCDLKELKVL LCDLKDLKI L LHDLKDLKVL DVRDLFVDLV DVRDLFVDLV DVRDLFVDLV
Hibp NNR Mibp NNR Xlfibp NSR Zfibp I NSR Hfibp VAD Xlfibp I TD Xlfibp I TD	ETGKKK FETGKRK FETGKRK 250 KDLLDLH KDLLDLH KDLLDLH	LCYLSFGDFA LCYLSFGDFA LCYLSFGDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG KSLVCTSLRG	210 FCAELM CNW FCAELM CNW TCAEHLI CNW FCAGCLI SYW 220 KLCVFSEMEA KLCVFSEMEA KLCVFSEMEA KLCVFSEMEA KLCVFSEMEA	220 TLCAVDSCMD TLCAVDSCVD TLCAVD-VAD TVCAVDNM E 280 NFKNLSRCLV NFKNLSRCLV NFKNLSRCLV NFKNLSRALV SEKNLSRALV	230 DNDNDLDKEF DNEVDLDKEF DNDNDLDKEF DNDVDLDKEF DNEVDLEKDF 200 NVAAKLTHNK NI GSLTHNK NI GSLTHAK	244 LCDLKELKVL LCDLKELKVL LCDLKDLKVL LCDLKDLKVL UKRDLFVDLV EVRDLFVDLV EVRDLFVDLV EVRDFFI DLV
Hibp NNR Mibp NNR Xlfibp NSR Zfibp I NSR Hfibp VAD Mfibp VAD Xlfibp I TD Zfibp I VND	ETGKKK FETGKRK FETGKRK 250 KDLLDLH KDLLDLH KDLLDCH	200 LCYLSFGDFA LCYLSFGDFA LCYLSFGDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG KSLVCTSLRG KSLVCGLRG	FCAELM CNM FCAELM CNM TCAELLI CNM TCAELLI CNM FCAGCLI SYM KLCVFSEMEA KLCVFSEMEA KLSVFHENDS KI KVFNENEA	TLGAVDSCMD TLGAVDSCVD TLGAVD-VAD TVGAVD-WA NFKNLSRGLV NFKNLSRGLV NFKNLSRALV SFKNLSRALV	210 DNDNDLDKEF DNEVDLDKEF DNEVDLEKEF DNEVDLEKEF NVAAKLTHNK NVAAKLTHNK NI GTSLTHNK NI ASKLTHAK	244 LCDLKELKVL LCDLKDLKVL LHDLKDLKVL CVRDLFVDLV CVRDLFVDLV CVRDLFVDLV CVRDFFI DLV
Hfibp NNR Mfibp NNR Xlfibp NSR Zfibp I NSR Hfibp VAD Mfibp VAD Xlfibp I TD Zfibp I VND	190 FETGKKK FETGKRK FETGKRK FETGKRK KDLLDLH KDLLDLH KDLLDCH KDLLDCH	200 LCYLSFCDFA LCYLSFCDFA LCYLFEDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG KSLVCTSLRG KSLVCCSLRG	PCAELM CNM FCAELM CNM FCAELL CNM FCAGCLI SVM FCAGCLI SVM KLCVFSENET KLCVFSENET KLSVFHENDS KI KVFNENEA 310	TL GAVDSCVD TL GAVDSCVD TL GAVD VAD TVGAVDNI E NFKNLSRGLV NFKNLSRGLV NFKNLSRALV SFKNLSRALV	230 DMDNDLDKEF DMCVDLDKEF DMCVDLEKDF DMCVDLEKDF 299 NVAAKLTHNK NI GTSLTHNK NI GTSLTHNK NI ASKLTHAK	LCDL KEL KVL LCDL KEL KVL LCDL KEL KVL LCDL KDL KVL LCDL KDL KVL LVDL KDL KVL DVRDL FVDL V DVRDL FVDL V DVRDL FI DL V DVRDL FI DL V 380
Hibp NNR Mibp NNR Xifibp NSR Zfibp1 NSR Hibp VAD Xifibp 1 TD Zfibp1 VND	100 FETGKKK FETGKRK FETGKRK 250 KDLLDLH KDLLDLH KDLLDCH KDLLDCH KDLLDCH	200 LCYLSFCDFA LCYLSFCDFA LCYLFFDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG KSLVCTALRG KSLVCTSLRG KSLVCTSLRG KSLVCGLRG L100 HVPLSLVRFF	FCAELM CNW FCAELM CNW TCAEHLI CNW FCAECLI SYW FCACCLI SYW FCACCLI SYW KLCVFSENET KLSVFHENDS KI KVFNEMEA MUCYSASVHS	TL GAVDSCMD TL GAVDSCVD TL GAVDSCVD TL GAVD-VAD TVGAVDNM E 210 NFKNLSRCLV NFKNLSRCLV NFKNLSRALV SFKNLSRALV 200 FKNLSRALV 200	DWDNDLDKEF DWDNDLDKEF DWDNDLDKEF DWDNDLDKEF DWEVDLEKDF NVAAKLTHNK NI GTSLTHNK NI ASKLTHAK NI ASKLTHAK	LCDL KEL KVL LCDL KEL KVL LCDL KEL KVL LCDL KDL KVL HCL KOL KVL IIII EVRDL FVDLV EVRDL FVDLV EVRDL FI DLV EVRDL FI DLV IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
Hibp NNR Mibp NNR Xifibp NSR Zfibp 1 NSR Hibp VAD Xifibp 1 TD Zfibp 1 VND Hibp EKF	LIDCHK FETCKKK FETCKRK FETCKRK FETCKRK 250 KOLLDLH KOLLDLH KOLLDCH KOLLDCH KOLLDCH KOLLDCH KOLLDCH KOLLDCH KOLLDCH	LCYLSFCDFA LCYLSFCDFA LCYLSFCDFA LCYLSFCDFA Z00 KSLVCTALRC KSLVCTALRC KSLVCTSLRC KSLVCGCLRC Z00 HWPLSCVRFF	FCAELM CNW FCAELU CNW FCAELU CNW FCAELU CNW FCAECLI SWW 270 KL CVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET KL SVFSEMET	220 TLGAVDSCMD TLGAVDSCVD TLGAVD-VAD TVGAVDNM E 280 NFKNLSRCLV NFKNLSRALV SFKNLSRALV SFKNLSRALV 200 FKNLSRALV 200	230 DMCMDLDKEF DMCMDLDKEF DMCMDLDKEF DMCVDLEKDF 300 NVAAKLTHNK NI GTSLTHNK NI GTSLTHNK NI GTSLTHNK DRYMGTLRGC DRYMGTLRGC	LCDL KEL KVL LCDL KEL KVL LCDL KEL KVL LCDL KDL KVL LCDL KDL KVL UVRDL FVDL V EVRDL FVDL V EVRDL FVDL V EVRDL FVDL V EVRDL FI DL V LLRL YHD
Hibp NNR Mibp NNR Xifibp NSR Zfibp I NSR Hibp VAD Xifibp I TD Zfibp I TD Zfibp I VND Hibp EKF	FETCKKK FETCKKK FETCKRK FETCKRK FETCKRK FETCKRK CLLDLH KDLLDLH KDLLDCH KDLLDCH KDLLDCH KDLLDCH KDLLDCH KDLLDCH FPCRSD VEPCRSD FPCKAA	LCYLSFCDFA LCYLSFCDFA LCYLSFCDFA LCYLSFCDFA KSLVCTALRG KSLVCTALRG KSLVCTALRG KSLVCTALRG KSLVCGLRG HVPLSEVRFF HVPLSEVRFF HVPLSEVRFF	FCAELM CNW FCAELM CNW FCAELL CNW FCAELL CNW FCAGCLI SYW RLCVFSENET KLSVFHENDS KI KVFNENEA SW LSVFHENDS KI KVFNENEA SW LSCYSASVHS LSCYSASVHS LSCYSASRA	220 TL GAVDSCVD TL GAVDSCVD TL GAVDSCVD TV GAVDNM E 220 TVGAVDNM E 220 TVGA TVGA TVGA TVGA TVGA TVGA TVGA TVGA	230 DMCMDLDKEF DMCMDLDKEF DMCMDLDKEF DMCVDLEKDF 240 NVAAKLTHNK NI GTSLTHNK NI GTSLTHNK NI GTSLTHNK DRYNGTLRGC DRYNGTLRGC DRYNGTLRGC	LCDL KELKVL LCDL KELKVL LCDL KDLKVL LCDL KDLKVL LCDL KDLFVDLV CVRDLFVDLV CVRDLFI DLV EVRDLFI DLV BILLRLYHD LLRLYHN

Fig. 52. Alignment of Fibp Amino Acid Sequence. Zebrafish Fibp1 is 70% identical to human, mouse, and *Xenopus* Fibp1, *Xenopus* and human or mouse are 77–78% identical, whereas human and mouse are 97% identical. Computational analysis programs did not reveal functional domains in Fibp1. The accession numbers of Fibp1 family members are: human (AF171946); mouse (NM021438); *Xenopus* (BC097711); zebrafish (BC085535).

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Fig. S3. Expression Pattern of *ier2*. (*A*) Lateral view of *ier2* expression at 3.5hpf. *ier2* expression can be detected only after the midblastula transition (MBT). (*B*) Lateral view of 2-color in situ hybridization of *ier2* (blue) and the ventral marker *vent* (red) at 4hpf. Arrowhead points to *ier2* expression at the dorsal side. (C) Lateral view of *ier2* expression at 7.5hpf in the marginal region. (*D*) Dorsal view of notochord and marginal region expression of *ier2* at 9hpf. (*E*–G) Lateral (*E*) and dorsal views (*F* and *G*) of *ier2* expression pattern of wt (*E* and *F*) and *ace* (*G*) embryos at the 3-somite stage. Arrowheads in *G* indicate loss of *ier2* expression in *ace* mutant. (*H*) Lateral view of 2-color in situ hybridization of *ier2* (blue) and *f* grad *G*) of *ier2* expression pattern of wt (*E* and *F*) and *ace* (*G*) embryos at the 5-somite stage. Arrowheads in *G* indicate loss of *ier3* expression in *ace* mutant. (*H*) Lateral view of 2-color in situ hybridization of *ier2* (blue) and *fg* (red) at the 5-somite stage. (*I*–L) Lateral (*I*, *K*, and *L*) and dorsal view (*J*) of *ier2* expression in the head (*I* and *J*) and trunk (*K* and *L*) region at 36hpf. Yellow arrows in *I* indicate pharyngeal expression of *ier2*. Arrow in *K* points to blood vessel. b, brachial arch; e, eye; f, forebrain; h, hyoid arch; m, mandibular arch; n, notochord; ov, otic vesicle; pec, pectoral fin; th, tail bud.



Fig. 54. *fibp1* Expression Pattern. (A) Dorsal view of maternal expression of *fibp1* at the 8-cell stage. (*B*) Lateral view of *fibp1* expression at 4hpf. (*C*) Lateral view of *fibp1* expression at 8.5hpf. A red arrowhead points to *fibp1* expression in the fore-runner cells that are precursors of Kupffer's vesicle. (*D–F*) Ubiquitous expression of *fibp1* at 3-somite and 42hpf stages (*E* and *F*). Arrow in *F* points to the notochord.

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Fig. S5. Efficiency of *ier2* and *fibp1* MOs. (*A* and *B*) *ier2* MO plus *ier2-egfp1* RNA-injected embryos completely lost the GFP signal (*b*) compared with control MO-injected embryos (*a*), as seen at the 3-somite stage. (*C* and *D*) *fibp1* MO likewise inhibited the GFP signal d, as seen at the 5-somite stage.

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Fig. S6. Dorsal-ventral axis specification in MO injected embryos. Embryos were injected with the MO shown at the top of each column, fixed at the times shown on the right, and hybridized with *bmp4* (ventral marker) or *chordin* (dorsal marker) as indicated on the left. The injection of these MOs had no substantial effect on the dorsal-ventral patterning of these embryos.

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Fig. 57. Synergism between *ier2* and *fibp1* RNA injection. Embryos were injected as indicated in the figure and photographed at the same time when control embryos were at 80% epiboly. A dosage dependent delay in epiboly is seen after RNA injection, with a synergistic effect of injection of both RNA. To the right are shown percentages of embryos for each condition that look like the embryo illustrated–thus the numbers represent normal embryos in *A*, *B*, and *D* and delayed embryos in *C*, *E*, and *F*.

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