Supplemental Material to:

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Ambra1 knockdown in zebrafish leads to incomplete development due to severe defects in organogenesis

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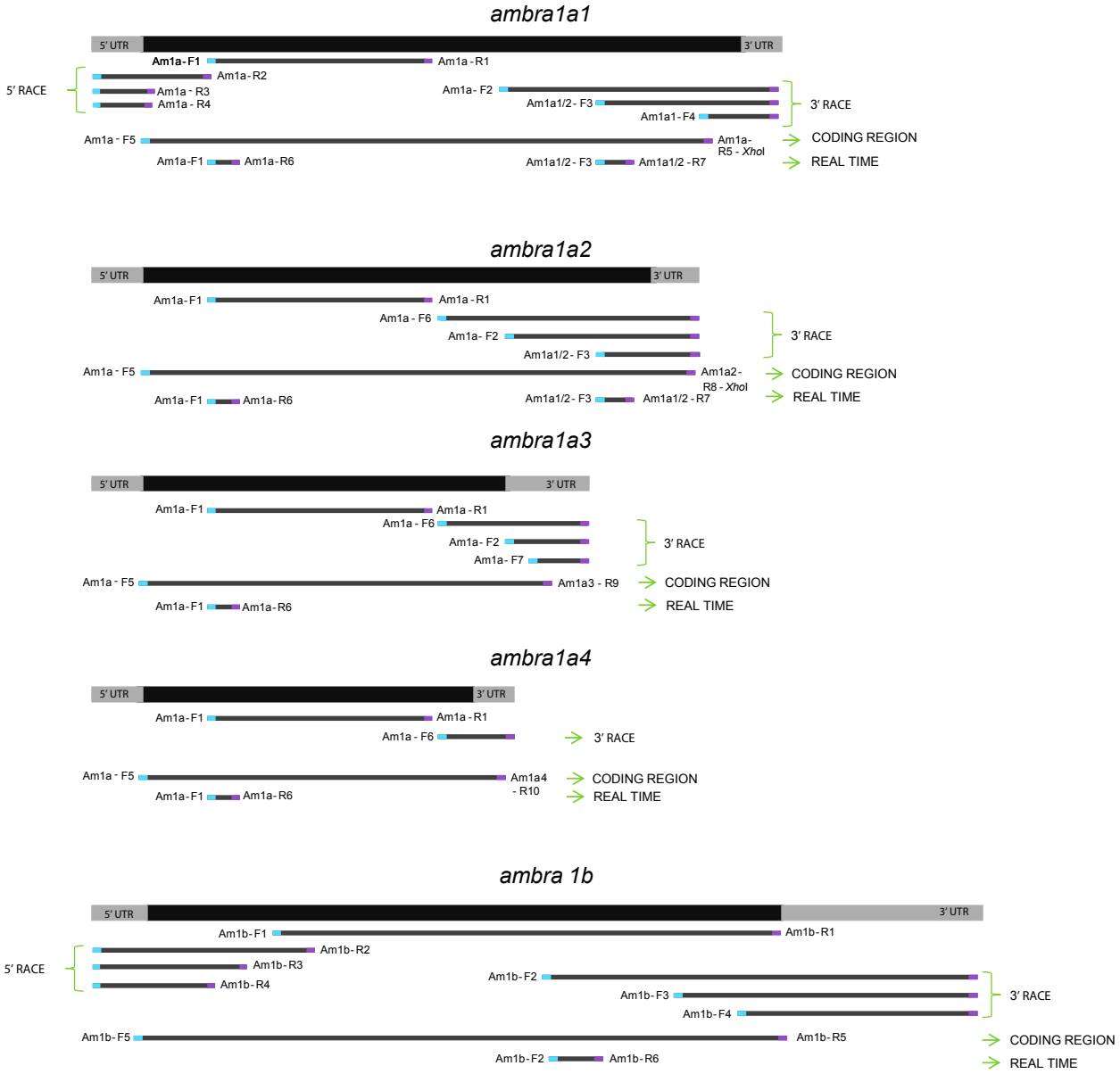
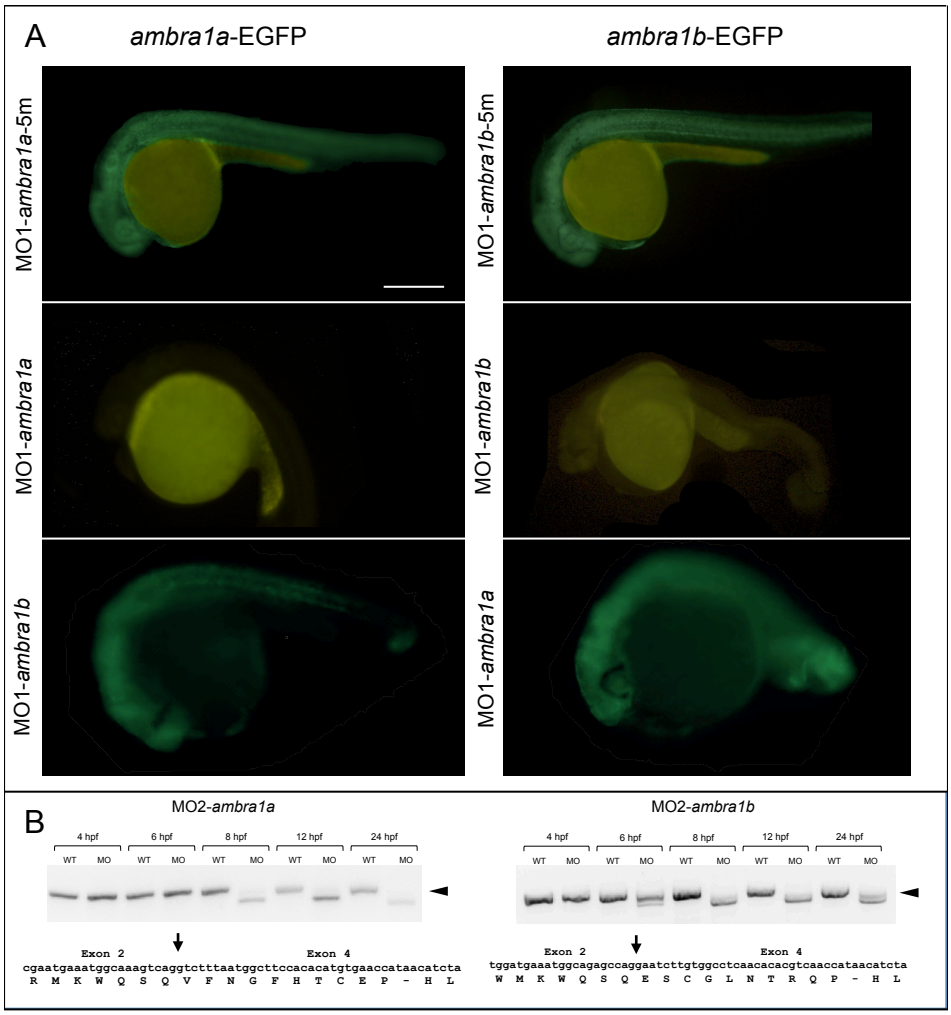
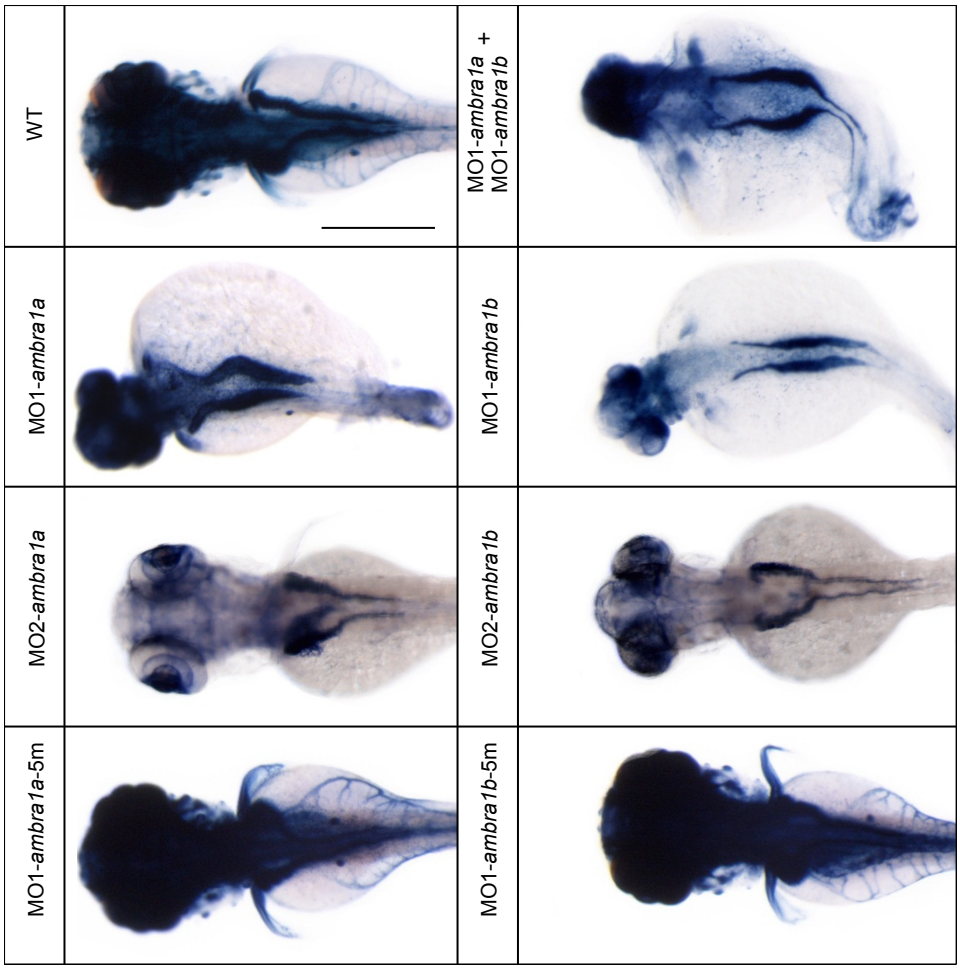
S-Fig. 1. Schematic representation of the cloning strategy for *ambra1a1, a2, a3, a4* and *ambra1b* zebrafish cDNAs. Primers used are indicated.



S-Fig. 2. Validation of the ATGMOs and SPLICMOs mediated knockdown of *ambra1* genes. (A) Control experiments to verify the ATGMOs-mediated knockdown of *ambra1a* and *ambra1b*. *ambra1a*- and *ambra1b*-EGFP mRNAs were coinjected with ATGMOs as indicated inside each image, and embryos were examined for the presence of EGFP fluorescence at 24 hpf. All the embryos are lateral view, anterior to the left. Bar= 200 µm. (B) RT-PCR analysis with cDNA of 4, 6, 8, 12 and 24 hpf embryos (controls and SPLICMOs-injected) confirmed deletion of exons 3 in the *ambra1a* and *b* transcripts. The arrow-head indicates the residual wild-type transcripts at the different stages. Under the agarose gel, sequences of the misspliced *ambra1a* and *b* transcripts show the loss of exon 3 (arrow) and the introduction of a premature stop codon.



S-Fig. 3. Alkaline phosphatase staining showing well-organized sub-intestinal vessels (SIVs) in WT larvae at 3 dpf. In *ambra1*-MOs-injected larvae SIVs are reduced or absent and pattern of intersegmental vessels in the trunk and tail are also less defined. Bar= 200 μm. Sv=sub-intestinal vessels.



Supplementary Table 1. Exons and introns size of *Mus musculus* and *Danio rerio ambra1* genes.

	1° ex	1° intr	2° ex	2° intr	3° ex	3°intr	4° ex	4° intr	5° ex	5° intr	6° ex	6°intr	7° ex	7° intr	8° ex	8° intr	9° ex	9°intr	10° ex	10° intr	11° ex	11° intr	12° ex	12°intr	13° ex	13° intr	14° ex	14° intr	15° ex	15°intr	16° ex	16°intr	17° ex	17° intr	18° ex	18° intr	19° ex
Mus musculus Ambra1	201	36070	254	350	59	570	178	1030	180	1820	65	1310	1459	31260	87	4920	182	14250	79	710	100	50360	113	9770	189	1030	155	13520	137	9710	92	1020	197	5720	878		
Danio rerio ambra1a1	206	3010	233	2168	59	167	184	113	173	986	67	97	1373	7293	87	4736	183	2268	78	917	101	19177	111	20026	189	3132	155	4999	140	3466	111	3226	209	21056	132	3025	733
Danio rerio ambra1a2	206	3010	233	2168	59	167	184	113	173	986	67	97	1373	7293	87	4736	183	2268	78	917	101	19177	111	20026	189	3132	155	4999	140	3466	111	3226	507				
Danio rerio ambra1a3	206	3010	233	2168	59	167	184	113	173	986	67	97	1373	7293	87	4736	183	2268	78	917	340																
Danio rerio ambra1a4	206	3010	233	2168	59	167	184	113	173	986	67	97	1373	7293	87	4736	548																				
Danio rerio ambra1b	266	801	217	1462	59	104	184	233	173	437	67	115	1463	2937	87	275	183	163	81	2285	101	103	111	115	189	412	155	2050	140	4475	99	95	203	2009	123	3125	1802

Supplementary Table 2. Effects of different dosages of morpholinos on the percentages of dead fish and of normal and abnormal phenotypes calculated from the number of surviving prelarvae at 3 dpf.

MO1-*ambra1a*	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	161	12±6	141	50±3	50±3
10.3 ng/embryo	328	17±2	271	31±9	69±9
15.5 ng/embryo	354	24±12	269	30±6	70±6
20.6 ng/embryo	350	25±17	260	10±8	90±8
25.8 ng/embryo	278	47±6	147	4±8	96±8
MO2-*ambra1a*	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	183	3±1	176	96±3	4±3
10.3 ng/embryo	152	3±4	147	89±2	11±2
15.5 ng/embryo	151	6±1	142	87±2	13±2
18.5 ng/embryo	269	19±7	218	64±6	36±6
20.6 ng/embryo	173	22±7	136	67±4	33±4
25.8 ng/embryo	185	47±6	99	71±5	29±5
MO1-*ambra1a*-5m	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	164	1±2	162	99±0	1±0
10.3 ng/embryo	304	14±7	269	94±3	6±3
15.5 ng/embryo	321	11±12	277	93±5	7±5
20.6 ng/embryo	294	36±5	182	92±3	8±3
25.8 ng/embryo	245	30±5	164	82±9	18±9
MO1-*ambra1b*	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	159	5±4	151	64±4	36±4
10.3 ng/embryo	195	2±2	189	61±7	39±7
15.5 ng/embryo	298	4±1	286	32±7	68±7
20.6 ng/embryo	317	29±9	226	16±8	84±8
25.8 ng/embryo	231	36±16	150	14±7	86±7
MO2-*ambra1b*	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	163	7±1	153	87±2	13±2
10.3 ng/embryo	174	6±3	165	74±7	26±7
15.5 ng/embryo	271	7±1	253	45±3	55±3
20.6 ng/embryo	161	13±10	141	22±2	78±2
25.8 ng/embryo	180	30±7	127	38±3	62±3
MO1-*ambra1b*-5m	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
8.2 ng/embryo	161	1±1	160	98±1	2±1
10.3 ng/embryo	218	3±1	209	91±3	9±3
15.5 ng/embryo	286	7±5	267	92±3	8±3
20.6 ng/embryo	318	7±2	295	83±7	17±7
25.8 ng/embryo	287	13±6	249	77±8	23±8
MO1-*ambra1a* + MO1-*ambra1b*	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
1/2 ng/embryo	276	55±8	128	24±3	76±3
1/4 ng/embryo	283	23±4	215	26±4	74±4

Supplementary Table 3. Effects of different mRNAs coinjected with MOs on the percentages of dead embryos and of normal and abnormal phenotypes calculated from the number of surviving prelarvae at 3 dpf.

	n.	Dead (%)	n. surviving	Normal (%)	Abnormal (%)
MO1-*ambra1a* + **20ng** mRNA *ambra1a1*	164	46±9	90	39±7	61±7
MO1-*ambra1a* + **15ng** mRNA *ambra1a3*	170	51±4	83	28±5	72±5
MO1-*ambra1a* + **20ng** mRNA *ambra1a1* + **15ng** mRNA *ambra1a3*	160	30±3	112	73±7	23±7
MO1-*ambra1a* + **20ng** mRNA *ambra1b*	240	58±12	121	35±7	65±7
MO1-*ambra1b* + **20ng** mRNA *ambra1b*	329	35±5	224	73±9	27±9
MO1-*ambra1b* + **20ng** mRNA *ambra1a1*	152	24±6	105	31±6	69±6
MO1-*ambra1a* + MO1-*ambra1b* + **20ng** mRNA *ambra1a1* + **15ng** mRNA *ambra1a3* + **20ng** mRNA *ambra1b*	177	39±10	110	65±10	35±10

Supplementary Table 4. List of Primers used in this work. The recognition sequences for restriction enzymes are shown in bold italic letters.

Primer	SEQUENCE 5'-3'
Am1a-F1	CTGCTGCTCATTGCCACC (18)
Am1a-F2	GCAACGCACTCATCCGTC (18)
Am1a1/2-F3	GTCGATGTGCATTCTGATGG (20)
Am1a1-F4	CGGAGTCTTTAGCTGCAGC (19)
Am1a-F5	GAGGAAGAGTGTTTGGAGATG (21)
Am1a-F6	ACAGTCTGCCTCCTCTCG (18)
Am1a-F7	AGGAGGACTCTCAGCTGG (18)
Am1a-M-F-*Cla*I	*CC**ATCGAT**GG*CTCAGCAACAGTCTTTCGTGATGAAGCTGG (40)
Am1a-5'-F-*Xba*I	*GC**TCTAGA**GC*GGTAGCAGCAGAGGTAG (27)
Am1a-R1	CTGCTCCTCATGCTGACC (18)
Am1a-R2	GTGGCAATGAGCAGCAGC (18)
Am1a-R3	ACAAGCTGCTGCAGAACC (18)
Am1a-R4	CGCTCTCGACTGGACAGG (18)
Am1a1-R5-*Xho*I	*CCG**CTCGAG**CGG*GATAACTACTATCGCTGTTGC (33)
Am1a-R6	CGCATCTCCACACTGTCC (18)
Am1a1/2-R7	GCTGGTTCTGTGTCTGCG (18)
Am1a2-R8-*Xho*I	*CCG**CTCGAG**CGG*CGGATGGACTTCACTCAC (30)
Am1a3-R9	CGTCCTACAGTAACTTTGCAC (21)
Am1a4-R10	CTCTGGTAGAATCGTTGGC (19)
Am1a-5'-R-*Kpn*I	*CGG**GGTACC**CCG*GTTCCTCTGTCCCAGC (28)
Am1b-F1	GCATACCACGTCAGACTCG (19)
Am1b-F2	AGGTGACGGACAGTCAGC (18)
Am1b-F3	GAACACACACACCACATCC (19)
Am1b-F4	GTAGACTCTCTAGAAGCTCC (20)
Am1b-F5	GCGTGCTGCTGAGTTAGTG (19)
Am1b-M-F-*Cla*I	*CC**ATCGAT**GG*GGTGCAGGACTTAACAGCATAAATGGC (37)
Am1b-F5-*Xba*I	*GC**TCTAGA**GC*GTGCTGCTGAGTTAGTG (27)
Am1b-R1	TCTGCCATACAGGTCGTC (18)
Am1b-R1-*Xho*I	*CCG**CTCGAG**CGG*GACTATCTGCCATACAGGTCG (33)
Am1b-R2	CTGAGTTCCCTGCAGTCC (18)
Am1b-R3	AAGCCATCTCCATACTATCC (20)
Am1b-R4	CGATGAGGAGAAGCTGAGC (19)
Am1b-R5	*CCG**CTCGAG**CGG*TCCAGCACCATGCAGACC (30)
Am1b-R6	CCTACCATCACATAGCAGC (19)
Am1b-5'-R-*Kpn*I	*CGG**GGTACC**CCG*TCTGTTCTGCACAGCCA (29)
LC3-F	GAGAAGTTTTTGCCGCCTCT (20)
LC3-R	ACCTGTGTCCGAACATCTCC (20)
BECLIN-F	GGACCACTTGGAACAACT (18)
BECLIN-R	CCGAAGTTCTTCAGTGTCCATC (21)
ARP-F	CTGAACATCTCGCCCTTCTC (19)
ARP-R	TAGCCGATCTGCAGACACAC (19)
18S-F	TCGAATGTCTGCCCTATCAACT (21)
18S-R	AGACTTGCCCTCCAATGGATC (20)

Supplementary Table 5. List of markers used in the whole-mount *in situ* hybridization analyses

Gene	Reference	GenBank cDNA reference	Vector	Endonuclease and RNA polymerase
chd	Miller-Bertoglio *et al*., 1997. [38]	AF034606	pBluescriptKS(+)	*Spe*I, T7
gsc	Schulte-Merker *et al*., 1994. [39]	NM_131017	pBS SK	*Bam*HI, T7
shha	Krauss et al., 1993. [40]	NM_131063	pCS2+	*Hind*III, T7
z-am1a1-3'-UTR	This work	XM_002667669	pGEM	*Apa*I, Sp6
z-am1b-3'-UTR	This work	XR_084457	pGEM	*Sal*I, T7

Supplementary Table 6. Summary of the ratio of abnormal phenotypes present in each class.

Deformity grade	MO1-*ambra1a*	MO1-*ambra1a* + *tp53*MO	MO2-*ambra1a*	MO1-*ambra1b*	MO1-*ambra1b* + *tp53*MO	MO2-*ambra1b*	MO1-*ambra1a* + MO1-*ambra1b*
N° of injected eggs	354	320	269	317	288	271	283
Dead (%)	24±12	21±9	19±7	29±9	13±11	7±1	23±4
N° surviving	269	250	218	226	253	253	215
Normal (%)	30±6	38±9	64±6	16±8	43±6	45±3	26±4
Tot. abnormal (%):	70 ±6	62±9	36±6	84±8	57±6	55±3	74±4
Abnormal class I - slight developmental delay - smaller eyes and otolithis - ventral curvature of the spine with misshapen tail - pericardial oedema and persistent voluminous and oedematous yolk sac - slight ventralization*	68±8*	53±14*	36±6	77±14	55±6	55±3	63±3*
Abnormal class II - smaller head, eyes and otoliths - curved or twisted tail - pericardial oedema and persistent voluminous and oedematous yolk sac - delayed pigmentation - ventralization	2±2	9±5	-	7±9	2±1	-	9±5
Abnormal class III - extensive morphological alterations with a complete derangement of the body plan	-	-	-	-	-	-	2±1