

## Additional file 1: Supplemental Tables S1-S7

Additional file 1: Table S1. MO sequences, injection doses, and total embryo numbers analyzed for heart looping and gene expression

Gene	MO sequence 5' 3'	MO's function	Blocking site	Knockdown efficacy analysis	Dose (ng) for heart looping analysis	Numbers for heart looping analysis	Dose (ng) for gene expression analysis	Numbers for pitx2 expression analysis	Numbers for lefty2 expression analysis
<i>numb</i>	GGAAACTCTGCCGTAGCTTATTCAT	translation blocking	ATG	western blot	8	258	8	134	128
<i>pacrg</i>	TAGCCAAAGGTTCAAAGGTTCTCAT	translation blocking	ATG	western blot	8	333	8	138	120
<i>tctn2</i>	TTCGCTCAAAGTGGACTTACCAACA	splice blocking	E111	western blot	8	235	8	136	157
<i>dnah10</i>	AGATGAGAATCACGCACATGGTTGA	splice blocking	E5I5	reverse transcription PCR	8	285	8	161	155
<i>mf115</i>	CTACCAGCGACACGGACTCACCG	splice blocking	E111	reverse transcription PCR	2	230	2	168	152
<i>cfap46/ ttc40</i>	CTTAGTGAACAGTGTTTTGACGTAC	splice blocking	E111	reverse transcription PCR	8	227	8	134	174
<i>galnt11</i>	GCCATTATCAGGCCCTCGCTGATGA	translation blocking	ATG	western blot	8	253	4	97	139
<i>Standard control</i>	CCTCTTACCTCAGTTACAATTTATA	—	/	—	8	337	8	158	161

**Additional file 1: Table S2. Specific primers and vector used to produce genes' whole-length mRNA**

<b>Gene</b>	<b>Primer F</b>	<b>Primer R</b>	<b>Length (bp)</b>	<b>Restriction Enzyme Digestion</b>	<b>Vector</b>
<i>galnt11</i>	CGGGATCCTCACCAGCGAGTAACGG	CCGCTCGAGCGTCAGACCAAGGCTTCA	2060	BamHI, XhoI	pSC2+
<i>pacrg</i>	CGGGATCCGAGTTTTGTTTCTGTTTATCGG	CCGCTCGAGCAGAAATAAGAAGTGTAACATAAGGT	853	BamHI, XhoI	pSC2+
<i>numb</i>	AGCGAATTCATGAATAAGCTG	TCCCTCGAGTTATAGTTCAATCTCAAA	1857	BamHI, XhoI	pSC2+
<i>tctn2</i>	CGGAATTCTCCAGGCGAGTGTGACATACG	CCGCTCGAGCCTTTAGCCAGAGCCTCAGAATAAG	1512	EcoRI, XhoI	pSC2+
<i>mf115</i>	CGGGATCCAAAACATGGCGGAGGCTGCT	CCGCTCGAGTCAGAAGGACCATCTCTCCG	938	BamHI, XhoI	pSC2+

**Additional file 1: Table S3. Antisense RNA probes conducted for whole mount in situ hybridization**

Gene	Primer	Length (bp)	vector	Linearizing	Promoter
<i>pacrg</i>	F: ATGAGAACCTTTGAACCTTTGGCTA R: GTTGAGAAGGCAGGACTCGTAGGTGGG	696	pGEM-T easy	Sall	T7
<i>numb</i>	F: TGAAGACGGCGGGTAAGA R: TGGTGAGGAGGCTGGTTT	860	pGEM-T easy	SacII	SP6
<i>galnt11</i>	F: GAGTCAAGTGCGTAAAGGC R: CACATCCAAATCCGAAAA	849	pGEM-T easy	Sall	T7
<i>tctn2</i>	F: GTACAGAGGACCCAGAT R: AAAGCATAGTTCAATCG	871	pGEM-T easy	SpeI	T7
<i>dnah10</i>	F: TGCGCGGTGTCAGTTTC R: TGTGATTTGTGTGTGCC	1140	pGEM-T easy	Sall	T7
<i>rnf115</i>	F: TGTAAGGGTGAAGTGAG R: GCGGTTGAGAAAAGTAG	1034	pGEM-T easy	NcoI	SP6
<i>cfap46</i>	F: TTGAAGGATGAAAACCC R: TGAGCACAGCACAGGAC	1031	pGEM-T easy	Sall	T7
<i>pitx2</i>	F: TCTCCTTGCTCTCGGCT R: CTTGTTCTGGGATTCG	1288	pGEM-T easy	SpeI	T7
<i>lefty2</i>	F: TCAGCGTCTTGTGTTTCG R: TCAGTGGGGATTTGGGG	872	pGEM-T easy	Sall	T7

**Additional file 1: Table S4. The frequency of each candidate CNV in normal Chinese individuals and non-heterotaxy patients with developmental delay/intellectual disability**

<b>ID</b>	<b>chromosome-position</b>	<b>Type</b>	<b>Size (kbp)</b>	<b>Frequency of NCI</b>	<b>Frequency of DD/ID</b>
5	chr4:104,554,264-105,123,728	Internal dup	569.464	0	0
5	chr6:26,019,198-26,227,973	Genic dup	208.775	0	0
6	chr7:151,816,201-151,967,676	Internal dup	151.475	1	1
7	chr1:145,625,128-145,927,662	Genic del	302.534	0	0
7	chr4:48,936,820-49,093,788	Genic dup	156.968	1	1
10	chr5:115,247,380-115,683,172	Genic dup	435.792	0	0
16	chr6:54,138,106-54,277,341	Genic dup	139.235	0	0
18	chr12:173,786-356,461	Genic dup	182.675	0	0
20	chr12:123,357,010-124,310,519	Genic dup	953.509	0	0
20	chr19:47,308,130-47,418,258	Genic dup	110.128	0	0
26	chr11:60,408,411-60,465,698	Genic del	57.287	0	0
31	chr10:6,254,055-6,374,584	Internal dup	120.529	0	0
34	chr4:93,875,432-93,988,049	Genic del	112.617	0	0
39	chr8:47,398,661-48,407,568	Genic dup	1008.907	0	0
40	chr14:73,620,299-73,786,493	Genic dup	166.194	0	0
43	chr4:101,476,709-101,668,938	Genic del	192.229	0	0
59	chr2:157,170,397-157,315,649	Internal dup	145.252	0	0
59	chr6:163,549,870-163,842,358	Genic dup	292.488	0	0
59	chr9:16,826,417-16,931,236	Internal dup	104.819	0	0
63	chr3:158,198,274-158,256,949	Genic del	58.675	0	0
63	chr10:134,358,785-134,921,135	Genic dup	562.35	0	0

NCI: normal Chinese individuals; DD: developmental delay; ID: intellectual disability; Genic del: deletion of at least one coding exon; Genic dup: full duplication of at least one gene; Internal dup: duplication of internal exons

**Additional file 1: Table S5. The function of the genes associated with the 19 rare CNV segments**

Chromosome	Gene	OMIM number	Component	Function	Ortholog with zebrafish
1q21.1	<i>RNF115</i>	—	ring finger protein 115	<b>E3 ubiquitin-protein ligase</b>	65.56
	<i>CD160</i>	604463	CD160 Molecule	MHC class I receptor activity	—
	<i>PDZK1</i>	603831	PDZ Domain Containing 1	ion transport, second messenger cascades	56.8
	<i>GPR89A</i>	612821	G Protein-Coupled Receptor 89A	voltage-gated ion channel activity	90
	<i>GPR89C</i>	—	G Protein-Coupled Receptor 89C	voltage-gated ion channel activity	90
	<i>PDZK1P1</i>	—	PDZ Domain Containing 1 Pseudogene 1	Non-Protein coding RNA	—
2q24.1	<i>NR4A2</i>	601828	Nuclear Receptor Subfamily 4 Group A Member 2	RNA polymerase II regulatory region sequence-specific DNA binding	77.18
	<i>GPD2</i>	138430	Glycerol-3-Phosphate Dehydrogenase 2	glycerol-3-phosphate dehydrogenase activity	71.47
3q25.32	<i>RSRC1</i>	613352	Arginine And Serine Rich Coiled-Coil 1	pre-mRNA splicing	56
4q22.2	<i>GRID2</i>	602368	Glutamate Ionotropic Receptor Delta Type Subunit 2	glutamate receptor activity	72.36
4q24	<i>EMCN-IT3</i>	—	Long Intergenic Non-Protein Coding RNA	Non-Protein coding RNA	—
4q24	<i>TACR3</i>	162332	Tachykinin Receptor 3	G-protein coupled receptor activity	66.05
5q23.1	<i>AP3S1</i>	601507	Adaptor Related Protein Complex 3 Sigma 1 Subunit	transporter activity	—
	<i>AQPEP</i>	—	laeverin	peptidase activity	31
	<i>LOC644100</i>	—	ADP ribosylation factor like GTPase 14 effector protein like	—	—
	<i>COMMD10</i>	616704	COMM Domain Containing 10	protein binding	60.1
6p12.1	<i>TINAG</i>	606749	Tubulointerstitial Nephritis Antigen	adhesion of proximal tubule epithelial cells	—
6p22.2	<i>HIST1H3A</i>	602810	Histone Cluster 1 H3 Family Member A	DNA binding	82.11
	<i>HIST1H4A</i>	602822	Histone Cluster 1 H4 Family Member A	DNA binding	80.26
	<i>HIST1H4B</i>	602829	Histone Cluster 1 H4 Family Member B	DNA binding	80.91
	<i>HIST1H3B</i>	602819	Histone Cluster 1 H3 Family Member B	DNA binding	—
	<i>HIST1H2AB</i>	602795	Histone Cluster 1 H2A Family Member B	DNA binding	—
	<i>HIST1H2BB</i>	602803	Histone Cluster 1 H2B Family Member B	DNA binding	—
	<i>HIST1H3C</i>	602812	Histone Cluster 1 H3 Family Member C	DNA binding	80.88

	<i>HIST1H1C</i>	142710	Histone Cluster 1 H1 Family Member C	DNA binding	72
	<i>HFE</i>	613609	Hemochromatosis	antigen binding	—
	<i>HIST1H4C</i>	602827	Histone Cluster 1 H4 Family Member C	DNA binding	100
	<i>HIST1H1T</i>	142712	Histone Cluster 1 H1 Family Member T	DNA binding	—
	<i>HIST1H2BC</i>	602847	Histone Cluster 1 H2B Family Member C	DNA binding	—
	<i>HIST1H2AC</i>	602794	Histone Cluster 1 H2A Family Member C	DNA binding	—
	<i>HIST1H1E</i>	142220	Histone Cluster 1 H1 Family Member E	DNA binding	74
	<i>HIST1H2BD</i>	602799	Histone Cluster 1 H2B Family Member D	DNA binding	—
	<i>HIST1H2BE</i>	602805	Histone Cluster 1 H2B Family Member E	DNA binding	—
	<i>HIST1H4D</i>	602823	Histone Cluster 1 H4 Family Member D	DNA binding	77.67
	<i>HIST1H3D</i>	602811	Histone Cluster 1 H3 Family Member D	DNA binding	84.31
	<i>HIST1H2AD</i>	602792	Histone Cluster 1 H2A Family Member D	DNA binding	—
	<i>HIST1H2BF</i>	602804	Histone Cluster 1 H2B Family Member F	DNA binding	—
	<i>HIST1H4E</i>	602830	Histone Cluster 1 H4 Family Member E	DNA binding	83.17
	<i>HIST1H2BG</i>	602798	Histone Cluster 1 H2B Family Member G	DNA binding	—
	<i>HIST1H2AE</i>	602786	Histone Cluster 1 H2A Family Member E	DNA binding	—
	<i>HIST1H3E</i>	602813	Histone Cluster 1 H3 Family Member E	DNA binding	83.58
6q26	<i>PACRG</i>	608427	Parkin Coregulated	<b>correlated with motile cilia during development; ubiquitin protein ligase binding</b>	91
	<i>PACRG-AS1</i>	—	PACRG Antisense RNA 1	Non-Protein coding RNA	—
	<i>DKFZp451B082</i>	—	Uncharacterized LOC401282	Non-Protein coding RNA	—
	<i>CAHM</i>	615930	Colon Adenocarcinoma Hypermethylated	Non-Protein coding RNA	—
	<i>QKI</i>	609590	QKI, KH Domain Containing RNA Binding	RNA binding, myelination	80.3
8q11.1q11.21	<i>LINC00293</i>	—	Long Intergenic Non-Protein Coding RNA 293	Non-Protein coding RNA	—
	<i>LOC100287846</i>	—	Patched 1 Pseudogene	Pseudogene	—
	<i>KIAA0146</i>	615384	KIAA0146 ortholog/Scaffolding Protein Involved In DNA Repair	DNA double-strand break (DBS) repair	49.13
9p22.2	<i>BNC2</i>	608669	Basonuclin 2	zinc ion binding	61

10p15.1	<i>PFKFB3</i>	605319	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3	6-phosphofructo-2-kinase activity	71.41
	<i>LOC399715</i>	—	Uncharacterized LOC399715	Non-Protein coding RNA	—
10q26.3	<i>INPP5A</i>	600106	Inositol Polyphosphate-5-Phosphatase A	inositol-polyphosphate 5-phosphatase activity	74
	<i>NKX6-2</i>	605955	NK6 Homeobox 2	DNA binding, transcription factor activity	72.99
	<i>TTC40</i>	—	Cilia And Flagella Associated Protein 46	<b>cilium movement</b>	52.04
	<i>LOC399829</i>	—	long intergenic non-protein coding RNA 1168	Non-Protein coding RNA	—
	<i>GPR123</i>	612302	G-Protein Coupled Receptor 123	transmembrane signaling receptor activity	62.25
11q12.2	<i>LINC00301</i>	—	Long Intergenic Non-Protein Coding RNA 301	Non-Protein coding RNA	—
12p13.33	<i>IQSEC3</i>	612118	IQ Motif And Sec7 Domain 3	ARF guanyl-nucleotide exchange factor activity	62
	<i>LOC574538</i>	—	Uncharacterized LOC574538	RNA Gene	—
	<i>SLC6A12</i>	603080	Solute Carrier Family 6 Member 12	neurotransmitter transporter	62
	<i>SLC6A13</i>	615097	Solute Carrier Family 6 Member 13	Sodium-dependent GABA and taurine transporter	74
12q24.31	<i>VPS37B</i>	610037	acuolar Protein Sorting 37 Homolog B	a regulator of vesicular trafficking process	60.4
	<i>ABCB9</i>	605453	ATP Binding Cassette Subfamily B Member 9	ATP binding	59.58
	<i>OGFOD2</i>	—	2-Oxoglutarate And Iron Dependent Oxygenase Domain Containing 2	oxidoreductase activity	58.36
	<i>ARL6IP4</i>	607668	ADP Ribosylation Factor Like GTPase 6 Interacting Protein 4	modulates alternative pre-mRNA splicing	44
	<i>PITPNM2</i>	608920	Phosphatidylinositol Transfer Protein Membrane Associated 2	phosphatidylinositol transporter activity	56
	<i>MIR4304</i>	—	MicroRNA 4304	RNA Gene	—
	<i>LOC100507091</i>	—	PITPNM2 antisense RNA 1	Non-Protein coding RNA	—
	<i>MPHOSPH9</i>	605501	M-Phase Phosphoprotein 9	M-phase enzymatic activities	38
	<i>C12orf65</i>	613541	Chromosome 12 Open Reading Frame 65	translation release factor	56
	<i>CDK2AP1</i>	602198	Cyclin Dependent Kinase 2 Associated Protein 1	specific inhibitor of the cell-cycle kinase CDK2	71.65
	<i>SBNO1</i>	614274	Strawberry Notch Homolog 1	molecular function	70.25
	<i>SETD8</i>	607240	SET Domain Containing (Lysine Methyltransferase) 8	methyltransferase activity	65.26

	<i>RILPL2</i>	614093	Rab Interacting Lysosomal Protein Like 2	cellular protein transport	61.46
	<i>SNRNP35</i>	—	Small Nuclear Ribonucleoprotein U11/U12 Subunit 35	RNA binding	69.25
	<i>RILPL1</i>	614092	Rab Interacting Lysosomal Protein Like 1	cellular protein transport	71.17
	<i>MIR3908</i>	—	MicroRNA 3908	RNA Gene	—
	<i>TMED2</i>	—	Transmembrane P24 Trafficking Protein 2	vesicular protein trafficking	80.15
	<i>DDX55</i>	—	DEAD-Box Helicase 55	ATP-binding RNA helicase	69.03
	<i>EIF2B1</i>	606686	Eukaryotic Translation Initiation Factor 2B Subunit Alpha	translation initiation factor	77
	<i>GTF2H3</i>	601750	General Transcription Factor IIH Subunit 3	protein kinase activity, damaged DNA binding	71
	<i>TCTN2</i>	613846	Tectonic Family Member 2	<b>hedgehog signaling transduction, regulate ciliogenesis</b>	48.17
	<i>ATP6V0A2</i>	611716	ATPase H <sup>+</sup> Transporting V0 Subunit A2	ATPase binding	67
	<i>DNAH10</i>	605884	Dynein Axonemal Heavy Chain 10	<b>microtubule motor activity, ATPase activity</b>	67.65
14q24.2	<i>PSEN1</i>	104311	Presenilin 1	endopeptidase activity	70.82
	<i>PAPLN</i>	—	Papilin, Proteoglycan Like Sulfated Glycoprotein	metalloendopeptidase activity	54.68
	<i>NUMB</i>	603728	NUMB, Endocytic Adaptor Protein	<b>suppress notch activity, protein binding</b>	65.28
19q13.32	<i>SNAR-E</i>	—	Small ILF3/NF90-Associated RNA E	RNA Gene	—
	<i>AP2S1</i>	602242	Adaptor Related Protein Complex 2 Sigma 1 Subunit	contributes to clathrin adaptor activity	85.68



**Additional file 1: Table S6. The bioinformatics information on the variant of *LEFTY1* in the patient with CNV of *TTC40* (*CFAP46*)**

ID	Gene	Mutation site	Amino acid change	Exonic Function	SIFT score	SIFT pred	PP2 HDIV score	PP2 HDIV pred	Mutation Taster score	Mutation Taster pred
63	<i>LEFTY1</i>	c. 841 A > G	p.Trp281Arg	nonsynonymous	0	Del	0.957	Dam	1	Dis

PP2: Polyphen2; pred: predicting; Del: Deleterious; Dam: Probably damaging; Dis: disease\_causing

**Additional file 1: Table S7. The bioinformatics information on the variants of candidate genes**

ID	Gene	Mutation site	Amino acid change	Exonic Function	SIFT score	SIFT pred	PP2 HDIV score	PP2 HDIV pred	Mutation Taster score	Mutation Taster pred
10	<i>DNAH10</i>	c.G11888A	p.R3963H	nonsynonymous	0	Del	1	Dam	1	Dis
21	<i>DNAH10</i>	c.T12026C	p.L4009P	nonsynonymous	0	Del	1	Dam	1	Dis
79	<i>DNAH10</i>	c.C10075T	p.L3359F	nonsynonymous	0	Del	1	Dam	1	Dis
93	<i>RNF115</i>	c.G799A	p.V267I	nonsynonymous	0.16	Tol	0.1	Ben	1	Dis
5	<i>TCTN2</i>	c.C91T	p.P31S	nonsynonymous	0	Del	1	Dam	0.999	Dis
101	<i>NUMB</i>	c.C518T	p.A173V	nonsynonymous	0.12	Tol	1	Dam	1	Dis

PP2: Polyphen2; pred: predicting; Del: Deleterious; Tol: tolerated; Dam: Probably damaging; Ben: benign; Dis: disease\_causing