Supp. Table S1. Mutagenic primers used to generate HA-tagged variant *ZIC3* expression constructs

Variant	Forward Primer Sequence	Reverse Primer Sequence
p.Asp6GlufsX32 (c.18_19delinsA)	5’-ACCCATGACGATGCTCCTGGAAGAGGCCCGCAGTTCCCTGGGC-3’	5’-GCCCAGGGAACTGCGGCCTCTTCCAGGAGCATCGTCATGGGT-3’
p.Gly17Cys (c.49G>T)	5'-GGGCTGGGAGTGTGCAGCTTCGGCG-3'	5'-CGCCGAAGCTGCACACTCCCAGCCC-3'
p.Ala50ProfsX9 (c.148delG)	5'-CACGCCGCCGCCGCCCCGCCGCCGCCGCTGCC-3’	5'-GGCAGCGGCGGCGGCGGGGCGGCGGCGGCGTG-3’
p.Ala53dup (c.157_159dupGCC)	5’-ACTCAACCCACGCCGCCGCCGCCGCCGCCGCCGCCGCCGCTGCCTTCAAGCTGAGC-3’	5'-GCTCAGCTTGAAGGCAGCGGCGGCGGCGGCGGCGGCGGCGGCGTGGGTTGAGT-3’
p.Ala52_Ala53dup (c.154_159dupGCCGCC)	5’-ACTCAACCCACGCCGCCGCCGCCGCCGCCGCCGCCGCTGCCGCCGCCTTCAAGCTGAGC-3’	5’-GCTCAGCTTGAAGGCGGCGGCAGCGGCGGCGGCGGCGGCGGCGGCGTGGGTTGAGT-3’
p.Ser109Cys (c.326C>G)	5'-ACGGTGGCGCTGCCTGTGCCGCCT-3'	5'-AGGCGGCACAGGCAGCGCCACCGT -3'
p.Glu155X (c.G463T)	5'-GCTGGCATCCCCTAGCCCCCTAGCT-3'	5'-AGCTAGGGGGCTAGGGGATGCCAGC-3'
p.Tyr199delfsX19 (c.595-610del TACCGCCCAGTGGCCA)	5'-GGCCGTGCTGACCCAGCCCGCGC-3'	5'-GCGCGGGCTGGGTCAGCACGGCC-3'
p.Ser252X (c.755C>A)	5'-ATCAAGCAGGAGCTGTAGTGCAAGTGGATCGAC-3'	5'-GTCGATCCACTTGCACTACAGCTCCTGCTTGAT-3'

Variant	**Forward Primer Sequence**	**Reverse Primer Sequence**
p.Glu291GlyfsX53 (c.866dupG)	5'-GCATGTGGGGGGCCCGGAGCAGAA-3'	5'-TTCTGCTCCGGGCCCCCCACATGC-3'
p.His318Asn (c.952C>A)	5'-AGTACAAACTGGTCAACAACATCCGAGTGCACACG-3'	5'-CGTGTGCACTCGGATGTTGTTGACCAGTTTGTACT-3'
p.Arg350Gly (c.1048A>G)	5'-CTCAAGATCCACAAGGGGACCCACACAGGTG-3'	5'-CACCTGTGTGGGTCCCCTTGTGGATCTTGAG-3'
p.Ser402Pro (c.1204T>C)	5'-ACGCACCCGAGCCCCCTGCGCAAAC-3'	5'-GTTTGCGCAGGGGGCTCGGGTGCGT-3'
p.Ala447Gly (c.1340C>G)	5'-CACTAAAACCCCTTCTGGAGTTCAAACTAGCACCA-3'	5'-TGGTGCTAGTTTGAACTCCAGAAGGGGTTTTAGTG-3'

Supp. Table S2. Phenotypic summary of *ZIC3* variant carriers relative to the 440 patient heterotaxy and CHD cohort

	Heterotaxy Cohort		**ZIC3 Mutation Cohort**		**Non-Mutation Cohort**		
Overall Phenotype:	**n**	**% (n/440)**	**n**	**% (n/15)**	**n**	**% (n/425)**	**p-value[1]**
Situs Inversus totalis	47	10.68	1	6.67	46	10.82	NS, p = 1.000
Heterotaxy	248	56.36	8	53.33	240	56.47	NS, p = 0.7895
Isolated CHD	117	26.59	5	33.33	112	26.35	NS, p = 0.5570
VACTERL-spectrum	28	6.36	1	6.67	27	6.35	NS, p = 1.000
Abdomen/gastrointestinal:							
Abdominal Situs Inversus	129	29.32	3	20.00	126	29.65	NS, p = 0.5688
Bile duct hypoplasia/biliary atresia	7	1.59	0	0.00	7	1.65	NS, p = 1.000
Gallbladder abnormalities	10	2.27	0	0.00	10	2.35	NS, p = 1.000
Malrotation of the gut	93	21.14	2	13.33	91	21.41	NS, p = 0.7472
Asplenia	112	25.45	3	20.00	109	25.65	NS, p = 0.7696
Polysplenia	46	10.45	1	6.67	45	10.59	NS, p = 1.000
Multilobated spleen	1	0.23	0	0.00	1	0.24	NS, p = 1.000
HJ bodies	1	0.23	0	0.00	1	0.24	NS, p = 1.000
Imperforate anus	14	3.18	0	0.00	14	3.29	NS, p = 1.000
Renal anomalies	24	5.45	0	0.00	24	5.65	NS, p = 1.000
Liver anomalies	24	5.45	1	6.67	23	5.41	NS, p = 0.5749
Skeletal/limbs							
Vertebral anomalies	33	7.50	0	0.00	33	7.76	NS, p = 0.6165
Rib anomalies	14	3.18	0	0.00	14	3.29	NS, p = 1.000
Scoliosis	12	2.73	0	0.00	12	2.82	NS, p = 1.000
Limb defects	7	1.59	1	6.67	6	1.41	NS, p = 0.2169
Cardiac position:							
Levocardia	323	73.41	11	73.33	312	73.41	NS, p = 1.000
Mesocardia	14	3.18	0	0.00	14	3.29	NS, p = 1.000
Dextrocardia	103	23.41	4	26.67	99	23.29	NS, p = 0.7588
Atria:							
Common atrium	13	2.95	0	0.00	13	3.06	NS, p = 1.000
Atrial isomerism	9	2.05	0	0.00	9	2.12	NS, p = 1.000
ASD	132	30.00	6	40.00	126	29.65	NS, p = 0.3982
AV canal	98	22.27	3	20.00	95	22.35	NS, p = 1.000
Ventricles:							
Single ventricle	67	15.23	3	20.00	64	15.06	NS, p = 0.7115
VSD	176	40.00	8	53.33	168	39.53	NS, p = 0.2960
Vessels:							

SVC abnormality	83	18.86	1	6.67	82	19.29	NS, p = 0.3226
IVC abnormality	93	21.14	1	6.67	92	21.65	NS, p = 0.2113
TAPVR	63	14.32	1	6.67	62	14.59	NS, p = 0.7066
PAPVR	20	4.55	2	13.33	18	4.24	NS, p = 0.1446
Great arteries/conotruncal:							
Aortic arch abnormalities	87	19.77	2	13.33	85	20.00	NS, p = 0.7455
d-TGA	116	26.36	5	33.33	111	26.12	NS, p = 0.5546
l-TGA	40	9.09	1	6.67	39	9.18	NS, p = 1.000
DILV	17	3.86	1	6.67	16	3.76	NS, p = 0.4517
DORV	114	25.91	2	13.33	112	26.35	NS, p = 0.3730
PA	71	16.14	6	40.00	65	15.29	**S, p = 0.0215**
PS	78	17.73	2	13.33	76	17.88	NS, p = 1.000
AS	11	2.50	0	0.00	11	2.59	NS, p = 1.000
CoA	28	6.36	0	0.00	28	6.59	NS, p = 0.6129
TOF	9	2.05	0	0.00	9	2.12	NS, p = 1.000
BAV	5	1.14	0	0.00	5	1.18	NS, p = 1.000

[1] p-values for statistical significance ($p<0.05$) represent comparisons between *ZIC3* mutation and non-mutation cohorts by two-tailed Fischer's Exact tests using Graphpad Instat v.3 software. NS = non-significant, S = significant.

Abbreviations: AS = aortic stenosis; ASD = atrial septal defect; CAVC = complete atrioventricular canal; BAV = bicuspid aortic valve; CHD = congenital heart disease; CoA = coarctation of the aorta; d-TGA = dextro-transposition of the great arteries; DILV = double inlet right ventricle; DORV = double outlet right ventricle; HJ bodies = Howell-Jolly bodies; IVC = inferior vena cava; l-TGA = levo-transposition of the great arteries; PA = pulmonary atresia; PAPVR = partial anomalous pulmonary venous return; PS = pulmonic stenosis, including sub, valvar, and supravalvar but not branch PS; SVC = superior vena cava; TAPVR = total anomalous pulmonary venous return; TOF = Tetralogy of Fallot; VACTERL = vertebral, anal, cardiac, tracheo-esophageal, renal, radial, limb; VSD = ventricular septal defect