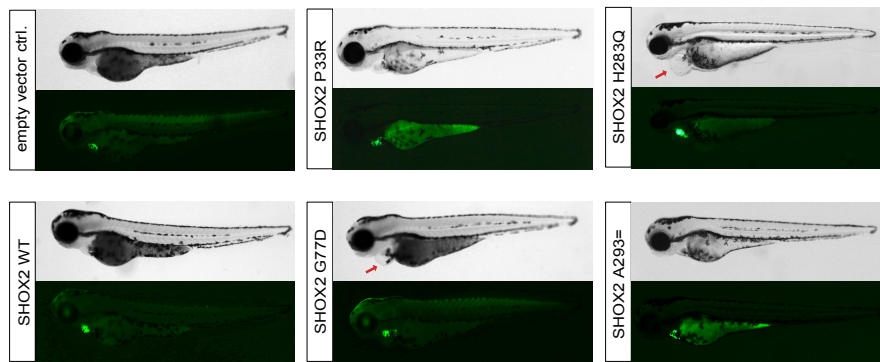


			p. P33R		p. G77D		
Human	1	MEELTAFVSKSFDQKVKEKKEAITREVLESG	R	LRGAKEPTGCT-EAGRDRSSPAVRAAGGGGGGGGGGGGGGG	G	77	
Chimpanzee	1	MEELTAFVSKSFDQKVKEKKEAITREVLESG	R	LRGAKEPTGCT-EAGRDRSSPAVRAAGGGGGGGGGGGGGGG	G	77	
Pig	1	MEELTAFVSKSFDQKVKEKKEAITREVLESG	R	LRGAKEPTGYA-EPGRDRSSPAVRAAGGGGGGGGGGGGGGG	G	79	
Mouse	1	MEELTAFVSKSFDQKVKEKKEAITREVLESG	R	LRGAKEP-GCV-EPGRDRSSPAVRAAGGGGGAGGGGGGG-GGGGGG	G	77	
Rat		-----		-----			
Chicken	1	MEELTAFVSKSFDPKAKEKELITYREVLESG	R	LRGAREPGGAAAEFGRDETGSPA		56	
Zebrafish	1	MEELTAFVSKSFDQKVKEKKEIVTYREVLETS	R	VRN-RES--LSADPNREISSIT		53	
Frog	1	MEELTAFVSKSFDQKIKKKEKEMITYREVLESG	R	ARG-KEP-GCG-EGARED	G	62	
		p. G81E					
Human	78	VGGGGAGGGAGGGRS	FVRE	LDMGAAERSREP	SGSPRLTEGRRKPTKAEVQATLLLPGEAFRFLVSP	PELKD	155
Chimpanzee	78	VGGGGAGGGAGGGRS	FVRE	LDMGAAERSREP	SGSPRLTEGRRKPTKAEVQATLLLPGEAFRFLVSP	PELKD	155
Pig	80	AGGGGAGGGAGGGRS	FVRE	LDMGAAERSREP	SGSPRLTE-----	VSP	133
Mouse	78	AGGGGAGGGAGGGRS	FVRE	LDMGAAERSREP	SGSPRLTE-----	VSP	131
Rat	1	-----	MGA	ERSREP	SGSPRLTE-----	VSP	33
Chicken	57	-----	GR	AGGGRSPP	REPDAAAADRAADAATPKLSD-----	VSP	103
Zebrafish	54	-----	RSG	VRSSPV	READMLASERSRDSSSPKLTD-----	GNTDM	99
Frog	63	-----	G	GGGGARS	FVLELDL-SVERIRESGSPKLTE-----	VSP	111
		p. R194X					
Human	156	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	235
Chimpanzee	156	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	235
Pig	134	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	213
Mouse	132	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	211
Rat	34	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	113
Chicken	104	DEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	183
Zebrafish	100	DETQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	179
Frog	112	EEGQTKIKQRSRTNFTLEQLNELERLDFETHYPD	AFMR	RELS	QLGLSEARVQVWFQNRRAKCRKQENQLHKG	VLI	191
				p. H283Q	p. A293=		
Human	236	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	TPLS	FQVQALQDSAVAHHHHHPHLA	AHAPY	315
Chimpanzee	236	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	TPLS	FQVQALQDSAVAHHHHHPHLA	AHAPY	315
Pig	214	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	TPLS	FQVQALQDSAVAHHHHHPHLA	AHAPY	293
Mouse	212	SQFEACRVAPYVNVGALRMPFQQD	-----	VQ	AQALQDSAVAHHHHHPHLA	AHAPY	279
Rat	114	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	TPLS	FQVQAHVQDSAVAHHHHHPHLA	AHAPY	193
Chicken	184	SQFEACRVAPYVNVGALRMPFQQD	-----	VQ	AQALQDSAVAHHHHHPHLA	AHAPY	249
Zebrafish	180	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	PPSF	FQVQALQDSAVAHHHHHPHLA	AHAPY	259
Frog	192	SQFEACRVAPYVNVGALRMPFQQD	SHCNV	TPLS	FQVQALQDSAVAHHHHHPHLA	AHAPY	271
Human	316	DSASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		355
Chimpanzee	316	DSASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		355
Pig	294	DSASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		333
Mouse	280	DSASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		319
Rat	194	DSASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		233
Chicken	250	ESASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		289
Zebrafish	260	ESASAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		299
Frog	272	ETATAASVAAAAAAKTTSKNSSI	IADRL	LKAKK	HAAALGL		311

Suppl. Fig. 1. Multiple sequence alignment of SHOX2 protein among different species. Novel identified variants are highlighted in red, while the previously reported variants are depicted in grey.



Suppl. Fig. 2. Fluorescence imaging of cardiac-specific overexpression of SHOX2 mutants in zebrafish. Bright field (upper image) and corresponding fluorescence imaging (lower image) of cardiac-specific overexpression of SHOX2 mutants compared to SHOX2 WT (wildtype) and empty vector control show pericardial edema (red arrow) for p.G77D and p.H283Q but not for p.P33R and p.A293= 72hpf.