

SUPPLEMENTARY TABLES

Table S1. List of morphological endpoints scored as a simple yes/no at 2dpf to facilitate selection of treatment groups for more in depth morphological scoring and functional phenotype analysis at 4dpf.

Endpoint	Score criteria	Score	Scored yes/no with notes
Body shape	Bent/curved	Yes/No	N/A if not dechorionated*
Somites	Poor definition	Yes/No	
Notochord	Present / absent	Yes/No	
Tail	Bent/curved	Yes/No	N/A if not dechorionated*
Heart	No heartbeat	Yes/No	
	Slow heartbeat	Yes/No	
	Swollen pericardial sac	Yes/No	
	Chambers not well defined	Yes/No	
	Missshapen	Yes/No	
Face	Optic vesicle small	Yes/No	
	Otic vesicles small	Yes/No	
	Facial oedema	Yes/No	
Neural	Irregular shape	Yes/No	Indicate fore, mid or hindbrain
Jaw/Arches	Present	Yes/No	
Body Oedema	Oedema present	Yes/No	In addition to heart or facial tissue

*Need to be hatched or dechlorinated in advance otherwise a bent body form will persist

Table S2. List of morphological endpoints scored in severity from 1 (severe) to 5 (normal) at 4dpf. Scoring criteria adapted from those of Gustafson *et al.*, (2012) and Ball *et al.*, (2014).

Endpoint	Score criteria	Score	Notes ~ scored 1 to 5 (normal)*
Body shape	Bent/curved	Yes/No	
	Body length	N/A	Standard length in mm
Somites	Poor definition	Yes/No	
	Small./ short	Yes/No	
	Missing	Yes/No	
	Cloudy	Yes/No	
Notochord	Shortened	Yes/No	
	Folded in Tail	Yes/No	
	No cellular differentiation	Yes/No	
	Wavy/Kinked	Yes/No	
	Poorly defined	Yes/No	
Tail	Kinked	Yes/No	
	Bent/Curved	Yes/No	
	Short	Yes/No	
Fins	Small	Yes/No	Indicate affected fin(s)
	Irregular Edge	Yes/No	
	Bent	Yes/No	
	Cloudy	Yes/No	
	Eroding	Yes/No	
Heart	Slow Heartbeat	Yes/No	
	No Heartbeat	Yes/No	
	Pericardial Sac-Swollen	Yes/No	
	Chambers not well defined	Yes/No	
	Missed	Yes/No	
Face	Optic Vesicle – Small	Yes/No	
	Optic Vesicle – Missed	Yes/No	
	Otic Vesicles – Small	Yes/No	
	Olfactory Region – Reduced	Yes/No	
	Olfactory Region - Not Present	Yes/No	
	Facial Oedema or Hypoplasia	Yes/No	
Neural Tube	Irregular Shape	Yes/No	Indicate irregular region(s)
	Reduced/Compressed	Yes/No	
Arches/Jaw [#]	Arches - Not Evident	Yes/No	
	Arches - Irregular Shape	Yes/No	
	Arches – Deficient	Yes/No	
	Jaw - Not Evident	Yes/No	
	Jaw - Deficient	Yes/No	Indicate irregular region(s)
	Jaw - Enlarged/ excessive	Yes/No	
	Jaw - Irregular Shape	Yes/No	
Liver	Enlarged	Yes/No	
	Not evident	Yes/No	
Yolk ball	Remnant excessive	Yes/No	

*Scoring of the jaw is variable at 4dpf and the jaw at 4dpf, will be more variable at that stage.

Table S3. Sequence of fixation and embedding steps applied to 4dpf zebrafish using an automated tissue embedder.

Solution	Duration	Temperature (°C)	Vacuum
70% isopropanol	60min	30	on
90% isopropanol	60min	30	on
95% isopropanol	60min	30	on
100% isopropanol	60min	30	on
100% isopropanol	60min	30	on
100% isopropanol	60min	30	on
Xylene	60min	30	on
Xylene	60min	30	on
Xylene	60min	30	on
Wax	80min	62	on
Wax	80min	62	on
Wax	80min	62	on

Table S4. Sequence of H&E staining steps applied to 4dpf zebrafish using an automated stainer.

Solution	Duration
Histoclear	5min
Histoclear	5min
100% IMS	2min
90% IMS	2min
80% IMS	2min
Tap water (running)	2min
Harris Haematoxylin non-acidified	15min
Tap water (running)	2min
Acid Alcohol	5sec
Tap water (running)	30sec
Ammoniated alcohol	30sec
Tap water (running)	30sec
Eosin Y Aqueous	15sec
Tap water (running)	30sec
80% IMS	30sec
90% IMS	1min
95% IMS	1min
100% IMS	2min
100% Ethanol	2min
Histoclear	2min
Histoclear	2min

IMS: Industrial Methylated Spirit

Table S5. Summary of literature data on the structure and function of the genes identified from the *in silico* work for zebrafish-based *in vivo* investigation in the current study

OFFICIAL GENE SYMBOL (ALIASES)	GENE NAME(S)	PROTEIN STRUCTURE & FUNCTION(S)	BIOLOGICAL & PHYSIOLOGICAL FUNCTION(S)
API5 <i>(AAC-11; FIF; MIG8)</i>	Apoptosis Inhibitor 5; Anti-apoptosis clone 11, Fibroblast Growth Factor 2-Interacting Factor; Cell migration-inducing gene 8	<ul style="list-style-type: none"> Belongs to the Inhibitor of apoptosis (IAP) family characterized originally as physical inhibitors of caspases (Salvesen et al 2002) May act as a scaffold for multi-protein complexes. Contains a multiple helices, forming HEAT (Huntingtin, elongation factor 3, PR65/A, TOR)-like and ARM (Armadillo)-like repeats that mediate protein–protein interactions (Han et al 2012), The C-terminal is structurally similar to the core region of the U-box-containing ubiquitin ligase E4 protein Ufd2p (Han et al 2012) 	<p><u>Apoptosis</u></p> <ul style="list-style-type: none"> Prevents apoptosis after growth factor deprivation (Tewari et al 1997) Prevents cell death by negatively regulating E2F1 transcription factor-induced apoptosis (Morris et al 2006). Contributes to E2F1 control of the G1/S cell cycle phase transition via facilitating E2F1 recruitment onto its target promoters and thus E2F1 target gene transcription (Garcia-Jove Navarro et al 2013) Regulates Acinus (ACIN1), a protein involved in chromatin condensation and DNA fragmentation during apoptosis protecting it from caspase 3 cleavage (Rigou et al 2009) Binds to and inhibits caspase-2, a key regulator of cell death, autophagy, genomic stability and ageing (Imre et al 2017) Interacts with FGF2 (Van den Berghe et al 2000) and upregulates FGF2 signaling through a FGFR1/PKCdelta/ERK effector pathway triggering degradation of the proapoptotic molecule BIM (Noh et al 2014) Regulates miR-1 induced apoptosis (Li et al 2015) Phosphorylated by PIM2 kinase and inhibits apoptosis in hepatocellular carcinoma cells through NF-κB (nuclear factor-κB) (Ren et al 2010) Downregulated in cardiomyocytes undergoing H₂O₂-induced apoptosis (Wan et al 2016) <p><u>Tumorigenesis</u></p> <ul style="list-style-type: none"> Upregulated in human carcinomas <i>in vivo</i> (Koci et al 2012) Inhibition increases anti-cancer drug sensitivity in various cancer cells (Rigou et al 2009) Increases the metastatic capacity of tumor cells by upregulating MMP levels via activation of the Erk signaling pathway (Song et al 2015) Acts as an immune escape gene in tumors by rendering them resistant to

			<p>apoptosis triggered by tumor antigen-specific T cells (Noh et al 2014)</p> <p><u>Immune response</u></p> <ul style="list-style-type: none"> • Acts as a danger-associated molecular pattern (DAMP) and stimulate the activation of immune response. Mediates TLR4-dependent activation of antigen presenting cells (Kim et al 2017)
HSPB7 (CVHSP)	Heat Shock Protein Family B (Small) Member 7; Cardiovascular Heat Shock Protein	<ul style="list-style-type: none"> • Member of the small heat shock proteins (HSPB) family of molecular chaperones. HSPBs typically associate early with misfolded proteins (Mymrikov et al 2011) • Prevents aggregation of proteins with expanded polyglutamine (polyQ) stretches (Vos et al 2010) • Binds large sarcomeric proteins Filamin C and Titin in cardiac cells (Mercer et al 2018) 	<p><u>Cardiac function</u></p> <ul style="list-style-type: none"> • Selectively expressed in cardiac and skeletal muscle • Common genetic variants associate with human HF (Cappola et al 2010) • Modulates actin filament assembly in heart (Wu et al 2017) • Cardiac-specific inducible HSPB7 KO mice display cardiac arrhythmia with abnormal conduction, rapid HF and sudden death accompanied by a downregulation of intercalated disc proteins and enrichment in Filamin C protein aggregates (Liao et al 2017) • Global depletion of Hspb7 in zebrafish disrupts normal cardiac morphogenesis (Rosenfeld et al 2013) • Loss of HSPB7 in zebrafish or human cardiomyocytes leads to enhanced autophagic pathways (Mercer et al 2018) • Dramatically increased in the heart and blood plasma immediately after myocardial infarction (Rüdebusch et al 2017) <p><u>Tumorigenesis</u></p> <ul style="list-style-type: none"> • May act as a tumor suppressor in the p53 pathway (Lin et al 2014)
LMO2 (TTG2; RBTN2; RHOM2)	LIM Domain Only 2; Rhombotin-2	<ul style="list-style-type: none"> • Belongs to the LIM-Only group of LIM domain protein superfamily. • The LIM-fingers are distinct from the zinc-fingers of transcription factors such as <i>GATA</i>, as LIM-domain-only proteins do not directly bind to DNA (8) • Mediates transactivation via protein-protein interactions with other transcriptional factors. 	<p><u>Angiogenesis</u></p> <ul style="list-style-type: none"> • Regulates endothelial proliferation and angiogenesis <i>in vitro</i> and is required for angiogenesis and tissue healing <i>in vivo</i> (Meng et al 2016) • Promotes endothelial cell migration via modulation of Sphk1 (Sphingosine Kinase). <i>Lmo2</i> KD reduced <i>Lmo2</i>-Sphk1 gene interaction, impaired intersegmental vessels formation, and reduced cell migration (Matrone et al 2017) • Angiopoietin-2 and VE-cadherin, both major regulators of angiogenesis, are direct transcriptional targets of LMO2-complexes with bHLH transcription factors TAL1 and/or LYL1 in endothelial cells (Deleuze et al. 2007, 2012) • Regulate VEGF-induced angiogenesis and lymphangiogenesis via NRP2 (Coma et al 2013)

		<p>Hematopoiesis/Erythropoiesis</p> <ul style="list-style-type: none"> • Homozygous inactivation in mice led to the complete absence of yolk sac erythropoiesis and early embryonic lethality (Warren et al. 1994) • Acts with TAL1/SCL to regulate erythropoiesis (Valge-Archer et al 1994) <p>Tumorigenesis</p> <ul style="list-style-type: none"> • Attenuates tumor growth by targeting the Wnt signaling pathway in breast and colorectal cancer (Liu et al 2016) • Regulator of neo-vascularization of tumors (Yamada et al. 2002) • Acts as a proto-oncogene in T cells (Mao et al 1997). Recurrent findings of interstitial deletions and translocations involving <i>LMO2</i> in T cell leukaemias.
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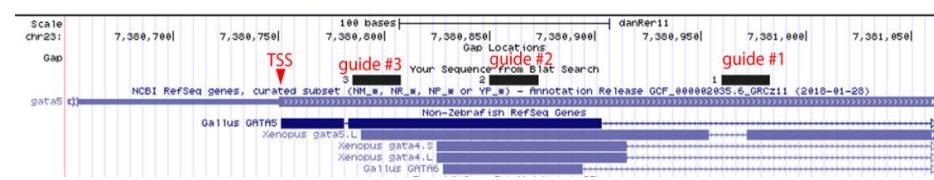
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SUPPLEMENTARY FIGURES

A

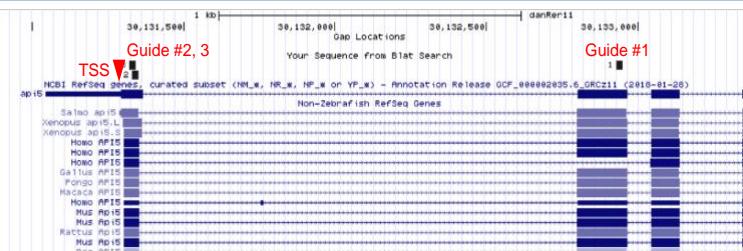
gata5

gRNA	Target sequence (5'-3')
g#1	GATAACTCTCGTCAACCCGG
g#2	GAGCATGGCTGGTACAGAGTGG
g#3	TCCCGGAATCGTGTGCGTAGGGG



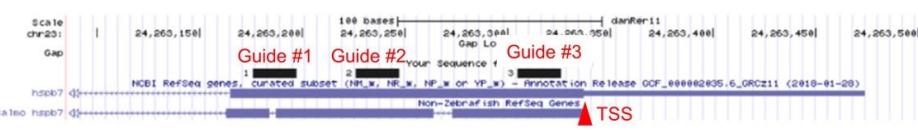
api5

gRNA	Target sequence (5'-3')
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g#2	CATCCTCGCCGACGCCAAAGAGG
g#3	CGGGCAGGGATGCCATAGTTACGG



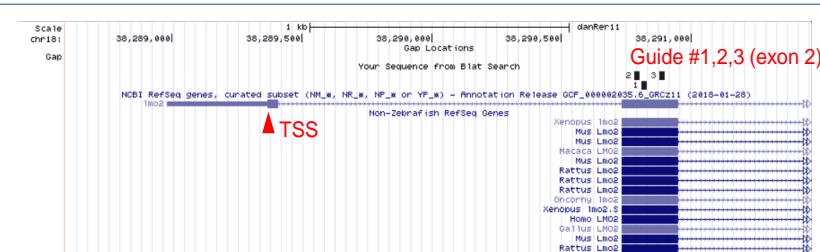
hspb7

gRNA	Target sequence (5'-3')
g#1	CATGTGTCCGAAGGATGCT <u>TTGG</u>
g#2	CCATACATGGAGAAGAGGCCGAGG
g#3	CAATTCTTCTGCCTATCGATCGG



Imo2

gRNA	Target sequence (5'-3')
g#1	CGATGGCCTTCAGAAAGAACGG
g#2	GGCGGGTGTCAAGCAGAGCATCGG
g#3	TCCCTTGCAACTGCCGTAACGCCG



B

gRNA	Target sequence (5'-3')	Efficiency score ^a	On-target score ^b	Off-target score ^b	Number of off-targets for mismatches (MMs)			
					0 MM	1 MM	2 MMs	3 MMs
gata5-g#1	GATAACTCTCGTCAACCCGG	70	62	94	0	0	0	0
gata5-g#2	GAGCATGGCTGGTACACGAGTGG	69	52	95	0	0	0	0
gata5-g#3	TCCCGGAATCGTGTGCGTAGGGG	62	23	99	0	0	0	0
api5-g#1	CGCACAGCTCGATCTGTGAGG	62	8	87	0	0	0	0
api5-g#2	CATCTCGCCGACGCCAAAGAGG	58	45	96	0	0	0	0
api5-g#3	CGGCGAGGATGCCATAGTTACGG	49	34	96	0	0	0	0
hspb7-g#1	CATGTGTCCGAAGGATGCTTG	37	40	93	0	0	0	0
hspb7-g#2	CCATACATGGAGAAGAGCCGAGG	66	68	91	0	0	0	1
hspb7-g#3	CAATTCTCTGCCTATCGATCGG	62	67	87	0	0	0	1
lmo2-g#1	CGATGGCCTTCAGAAAAGAGCGG	63	41	71	0	0	0	0
lmo2-g#2	GGGGGGTGTCAAGCAGAGCATCGG	55	19	81	0	0	0	0
lmo2-g#3	TGCGTCCCCACCTCCCCTAAGCGG	62	63	96	0	0	0	0

PAM sequences (NGG) are underlined.

^a Scores were obtained using CHOPCHOP (<https://chopchop.cbu.uib.no/>). Setting: Doench *et al.* (2016) *Nature Biotechnology*, 34, p184–191.

^b Scores were obtained using IDT CRISPR-Cas9 gRNA checker (<https://www.idtdna.com/Cas9Checker>)

Fig. S1. gRNA design for each of the 4 candidate genes. **Panel A)** For each gene in turn, the left hand table shows the sequence of each designed gRNA (g#1,2,3 encompassed all three gRNAs in one injection), and the right hand image shows the position of each of these gRNA sequences within the coding exons of each gene. The translation start site is indicated by TSS and the adjoining arrow, and other species orthologues are shown for comparison. Original images obtained using the UCS genome browser (<https://genome.ucsc.edu>). **Panel B)** Table showing the CHOPCHOP scores for efficiency, target specificity, and mismatches detected for each gRNA designed.

A

	n-number (scored)	Larvae lethality	Larvae unhatched	Larvae abnormal	Body shape	Somites	Notochord	Tail	Fins	Heart	Face	Neural tube	Arches/jaws	Group mean length (mm)	Swim bladder not inflated	Swim bladder not evident	Yolk ball - remnant excessive	Liver - not evident	Liver -enlarged	Abdomen distended/yolk sac edema
Un-injected	10	0%	0%	40%	0%	0%	0%	0%	0%	40%	0%	0%	0%	3.65	0%	0%	0%	0%	0%	
Cas 9 only	20	0%	0%	20%	0%	0%	0%	0%	0%	20%	0%	0%	0%	3.58	50%	0%	0%	0%	0%	
<i>gata5 g#1,2,3 only</i>	10	0%	0%	10%	0%	0%	0%	0%	0%	10%	0%	0%	0%	3.75	0%	0%	0%	0%	0%	
<i>gata5 g#1 + Cas9</i>	10	0%	0%	100%	10%	50%	0%	0%	40%	100%	60%	60%	80%	3.31	100%	60%	0%	60%	0%	
<i>gata5 g#1,2,3 + Cas9</i>	20	0%	0%	100%	0%	80%	20%	0%	90%	100%	85%	90%	95%	3.15	100%	85%	0%	25%	0%	

	% Cardiac specific abnormalities						
	Slow Heartbeat	No Heartbeat	Pericardial Sac-Swollen	Chambers not well defined	Misshapen	Small	Enlarged
Un-injected	0%	0%	40%	0%	0%	0%	0%
Cas 9 only	0%	0%	20%	0%	0%	0%	0%
<i>gata5 g#1,2,3 only</i>	0%	0%	10%	0%	0%	0%	0%
<i>gata5 g#1 + Cas9</i>	0%	0%	100%	60%	60%	60%	0%
<i>gata5 g#1,2,3 + Cas9</i>	0%	5%	100%	40%	85%	85%	0%

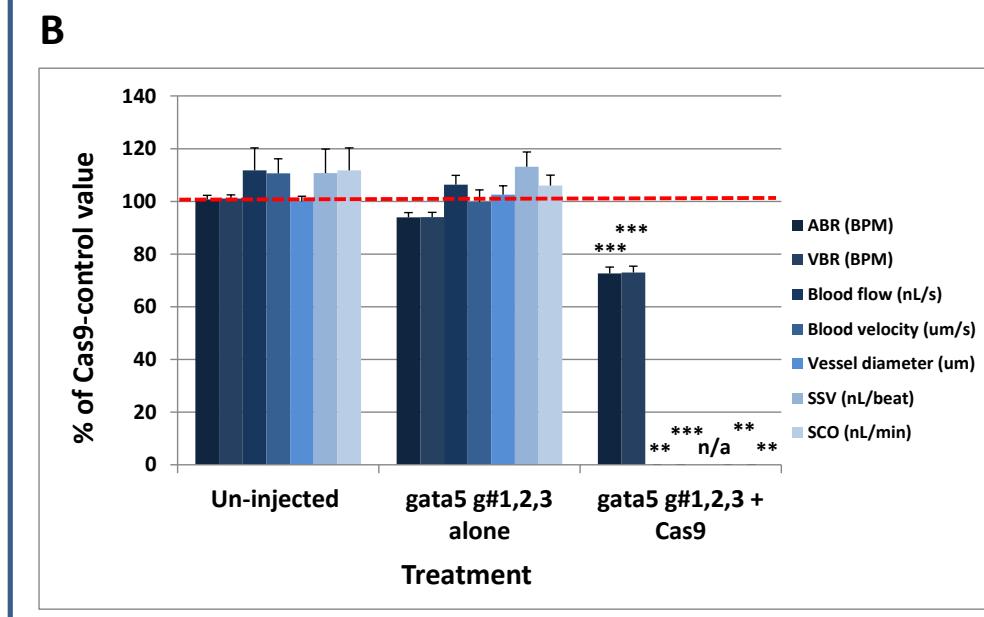


Fig. S2. Comparison of the data from various control treated 4dpf larvae. The control groups assessed were un-injected, Cas9 only injected, *gata5 g#1,2,3* only injected and *gata5 g#1,2,3 + Cas9* injected (positive control). **Panel A)** Summary of the morphological data obtained from these animals including general and heart specific endpoints. Data are shown as the % incidence of abnormalities under each category, with shades from white (0%) through to red (100%) providing an indication of the proportion of animals exhibiting a malformation within that category. Note the low incidence of abnormalities in each treatment except for the positive control group. **Panel B)** Summary of the cardiovascular function data obtained from these animals. Data are shown as the mean % change versus the Cas9-only injected group (100% indicated by the red dashed line), \pm SEM, n=9-10. **signifies a statistically significant difference versus the Cas9 control at $p<0.01$, and *** at $p<0.001$ (1-way ANOVA and Tukey's HSD tests or Kruskal-Wallis and Dunn's tests). Vessel diameter measurements not possible where no blood flow (n/a).

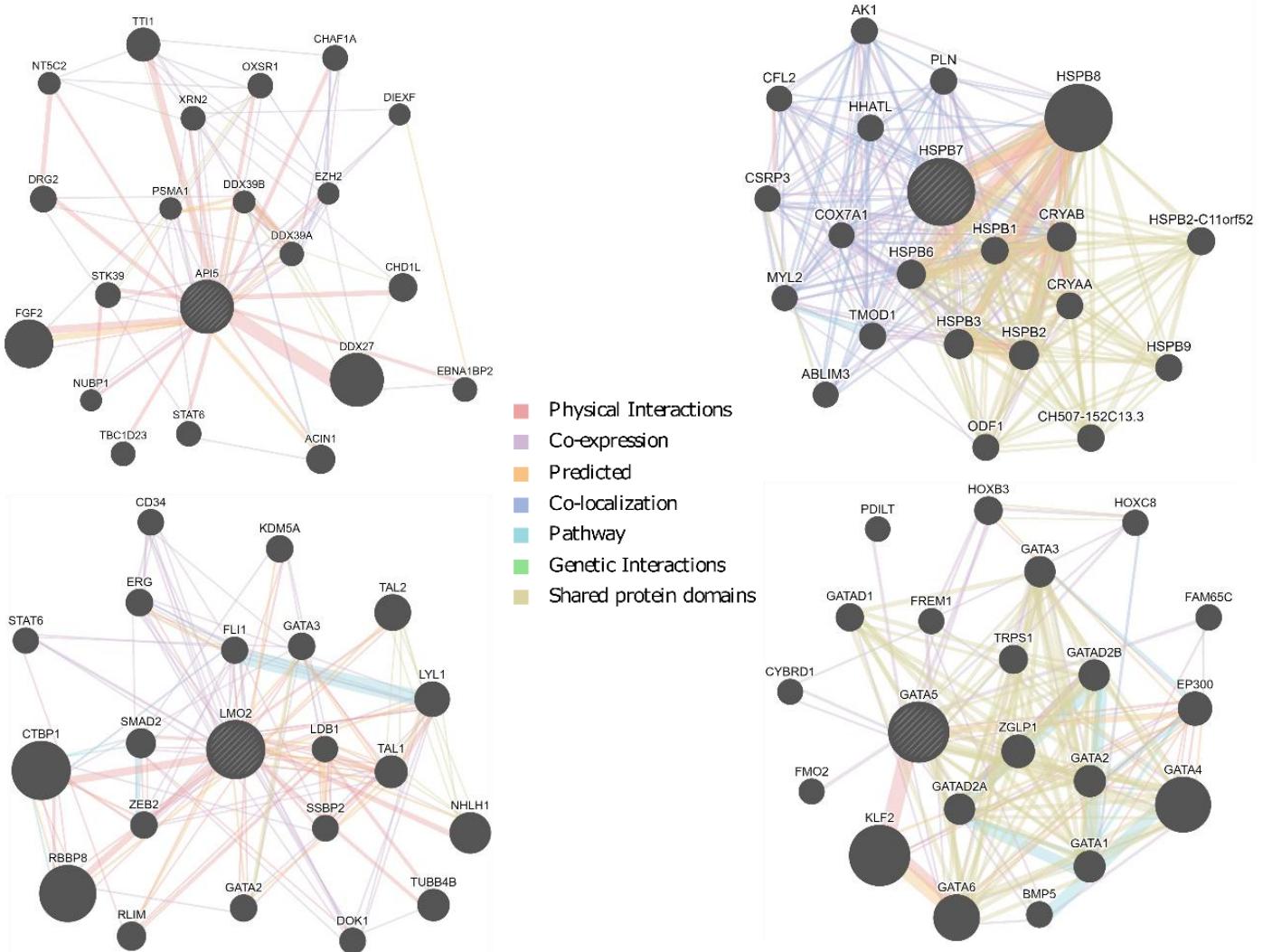
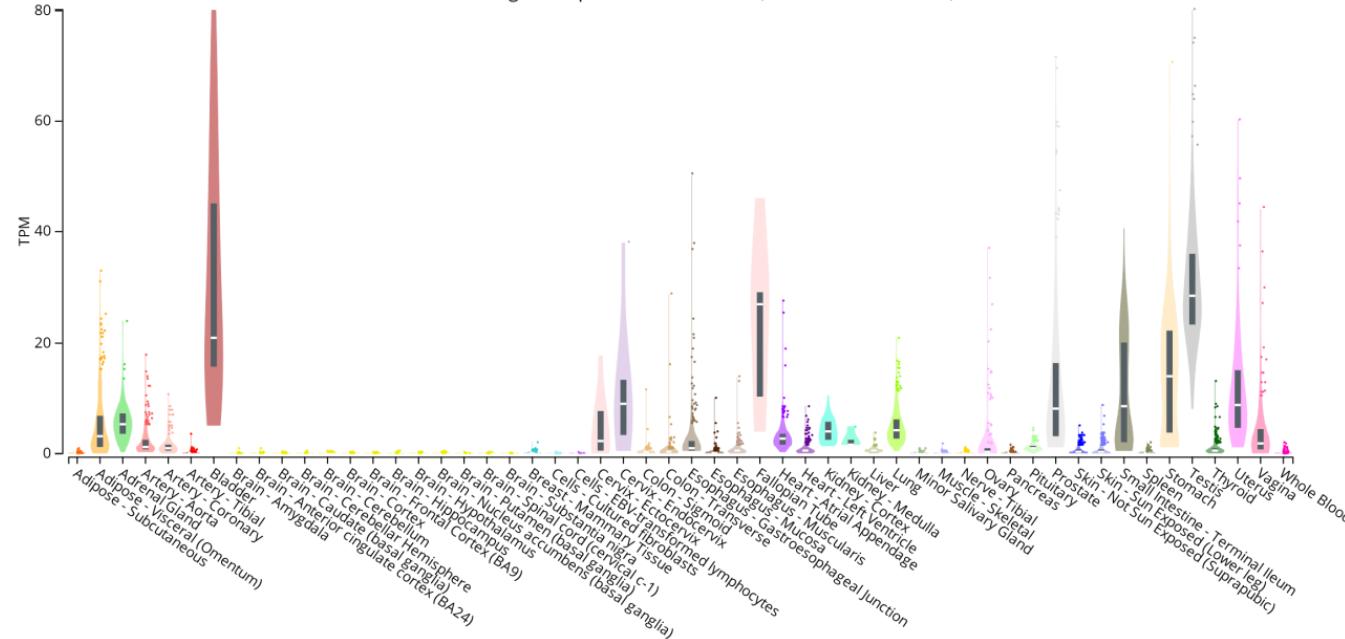


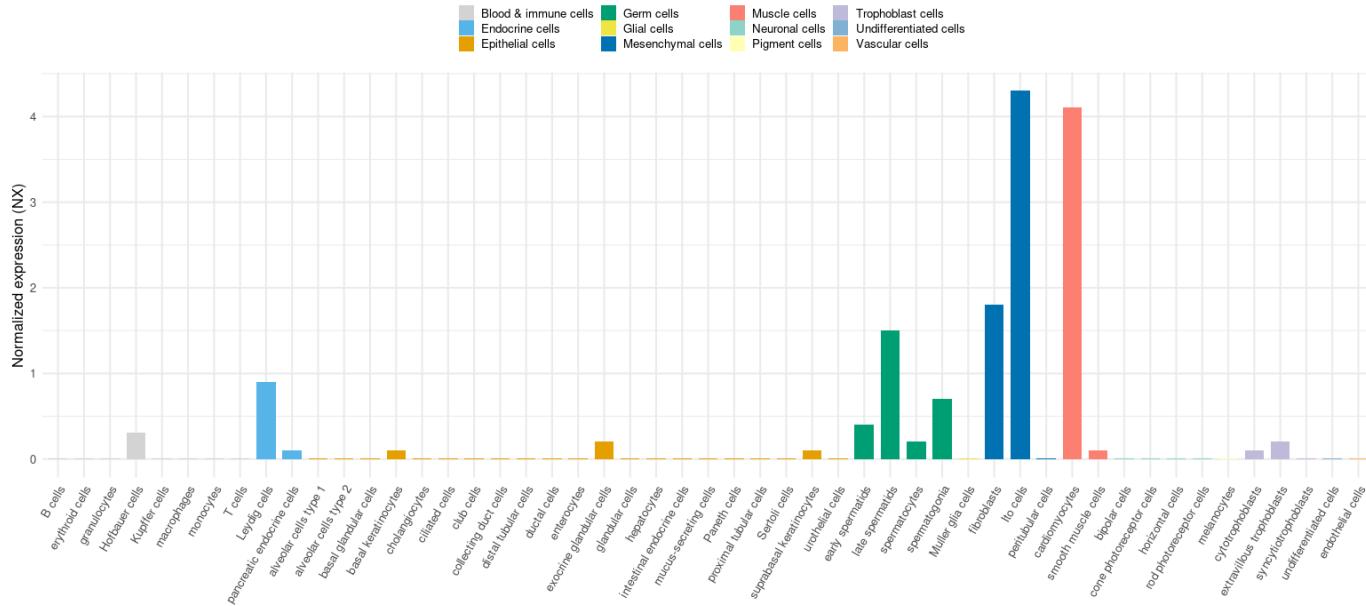
Fig. S3. GeneMANIA network analysis using *API5*, *HSPB7*, *LMO2* and *GATA5* as input genes. GeneMANIA uses very large set of functional association data including protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity (Warde-Farley *et al.*, 2010. *Nucleic Acids Res*, 38, W214-20). Colour of network edges indicate evidence types.

i) GATA5 - Enhanced in testis, bladder, stomach and female reproductive organs

Bulk tissue gene expression for GATA5 (ENSG00000130700.6)



ii) GATA5 - Enhanced in Hepatic stellate (Ito) cells and Cardiomyocytes



*iii) GATA5 – Generally weak expression.
Highest in Cardiomyocytes (atrial)*

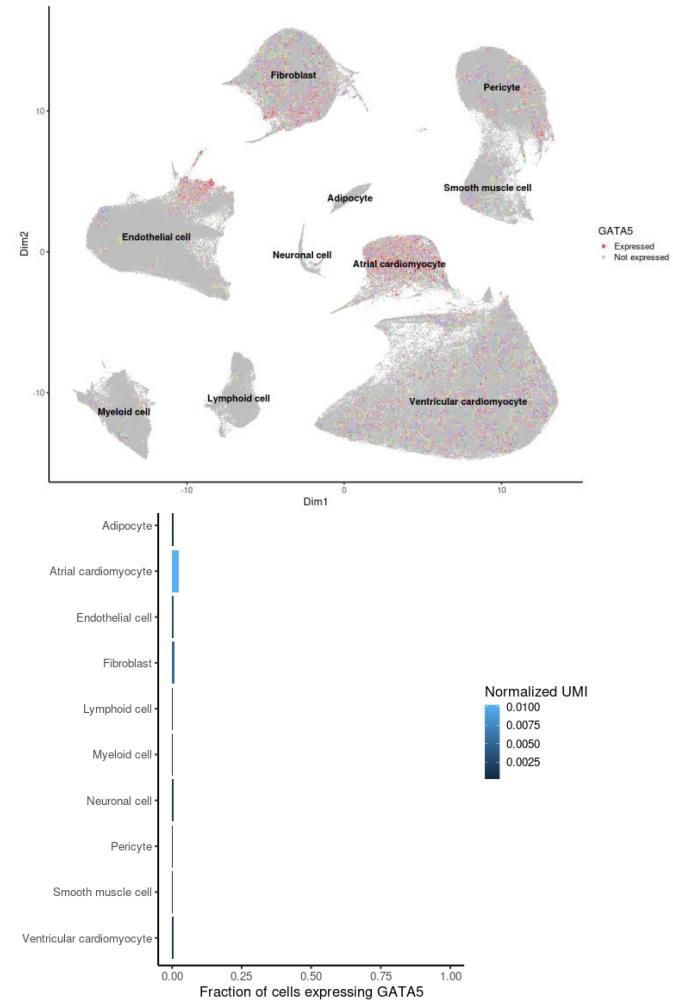
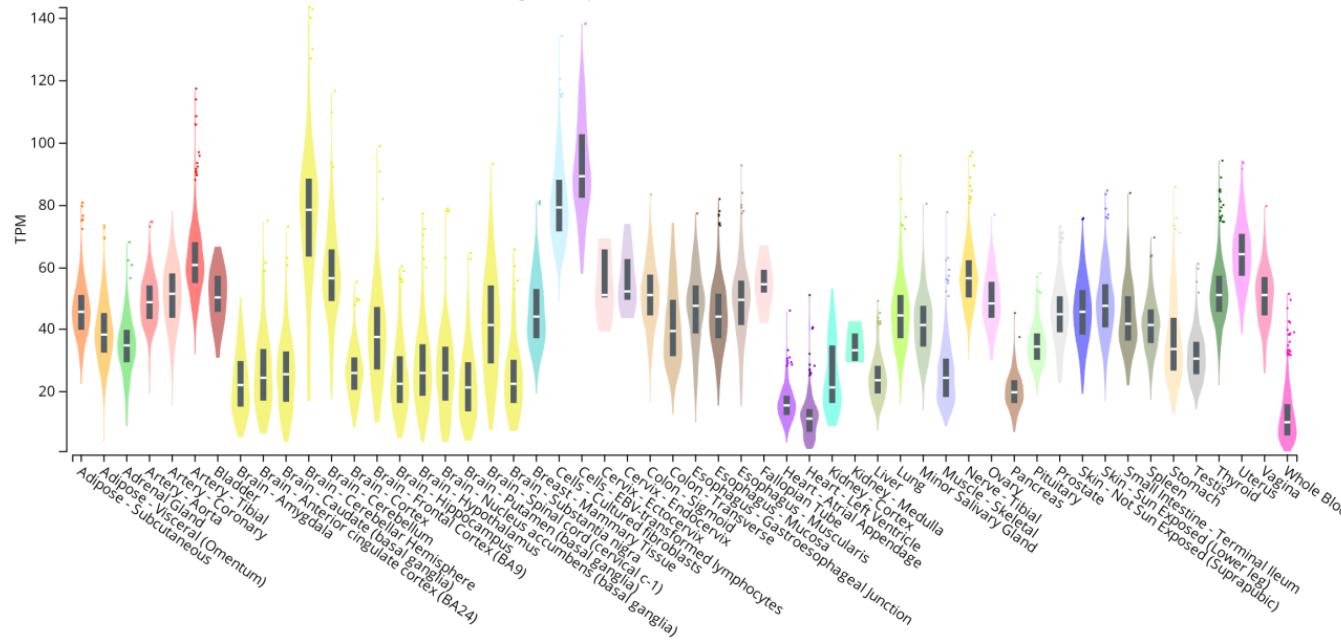


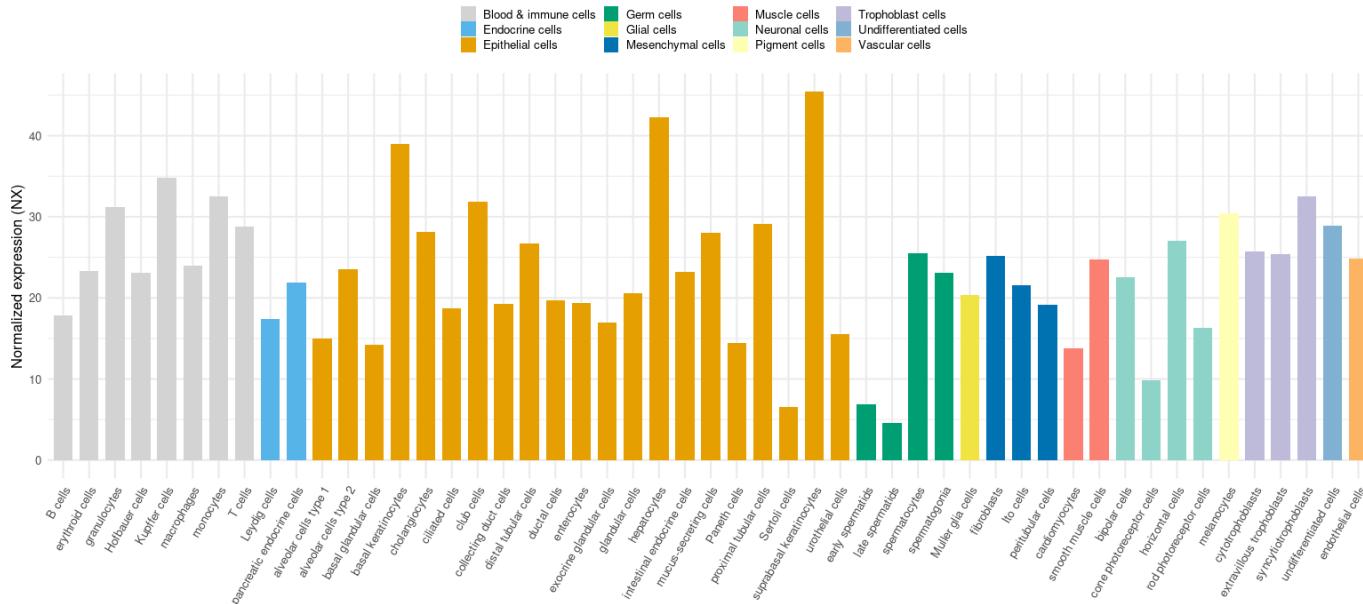
Fig. S4A. Transcriptomics data analysis on GATA5.
Data shown are the tissue and single cell type mRNA expression levels based on RNA sequencing data sets from the **i)** GTExPortal (<https://gtexportal.org/home/>), **ii)** the Human Protein Atlas (<https://www.proteinatlas.org/>) and **iii)** the Heart Cell Atlas (<https://www.heartcellatlas.org/>).

i) API5 – Ubiquitous. Low tissue specificity

Bulk tissue gene expression for API5 (ENSG00000166181.12)



ii) API5 – Ubiquitous. Low cell type specificity



iii) API5 – Ubiquitous. Low cell type specificity

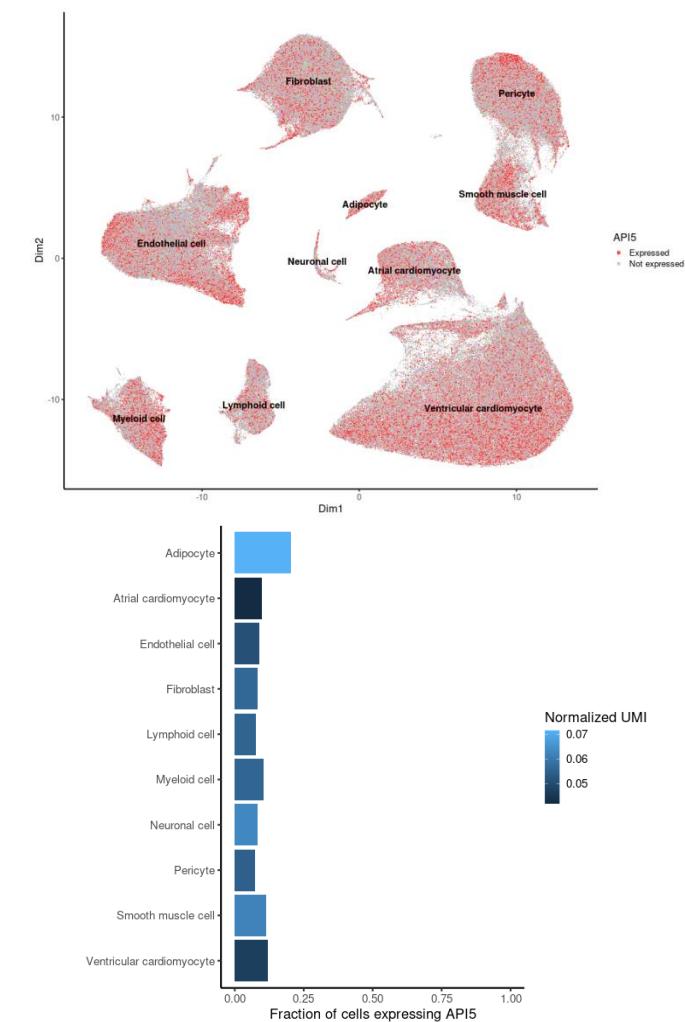
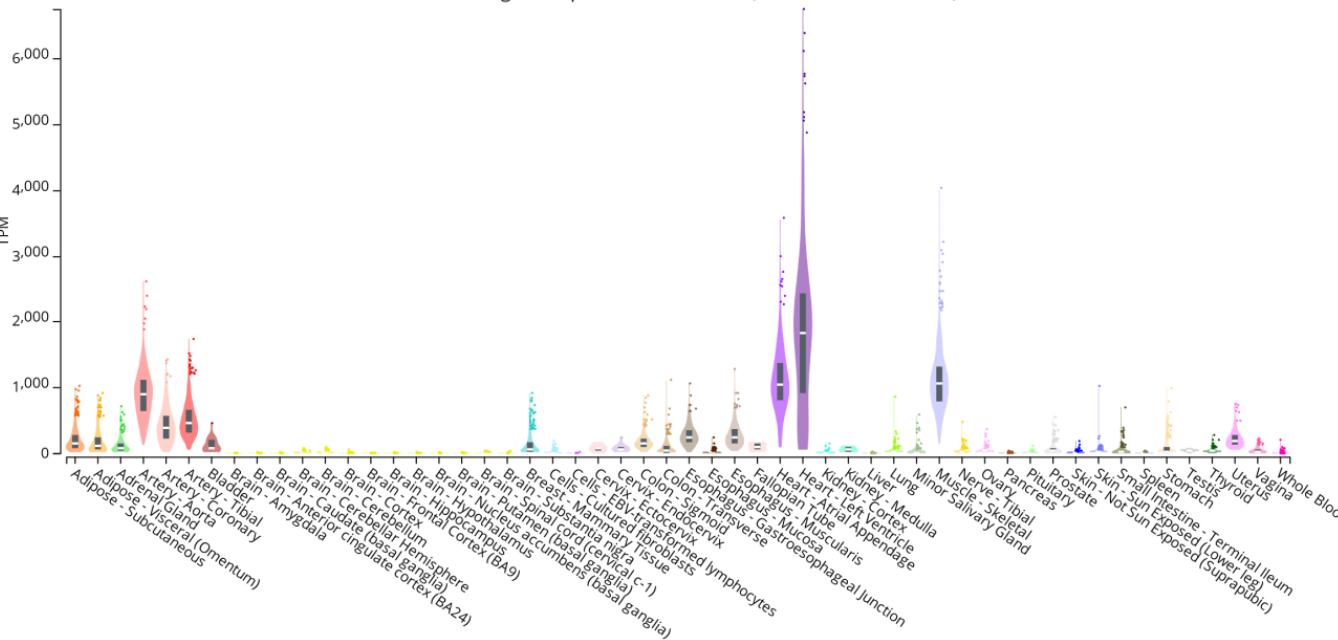


Fig. S4B. Transcriptomics data analysis on API5.
Data shown are the tissue and single cell type mRNA expression levels based on RNA sequencing data sets from the **i**) GTExPortal (<https://gtexportal.org/home/>), **ii**) the Human Protein Atlas (<https://www.proteinatlas.org/>) and **iii**) the Heart Cell Atlas (<https://www.heartcellatlas.org/>).

i) HSPB7 - Enriched in enriched heart muscle and skeletal muscle

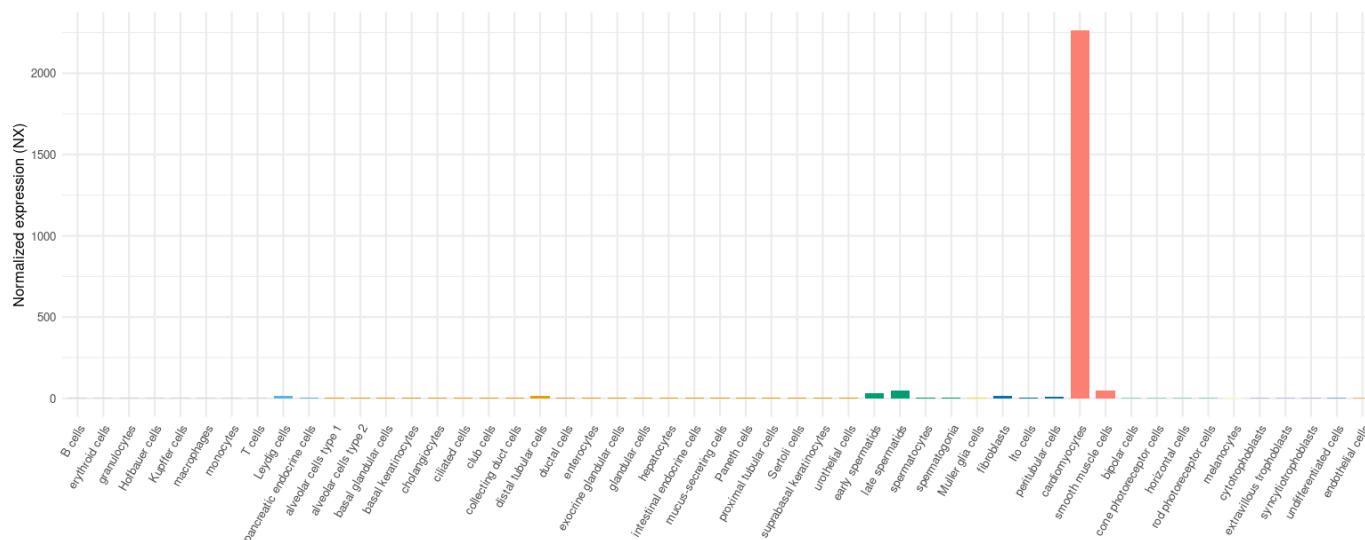
Bulk tissue gene expression for HSPB7 (ENSG00000173641.17)



ii) HSPB7 - Enriched in Cardiomyocytes

Legend:

- Blood & immune cells: Blue
- Germ cells: Green
- Muscle cells: Red
- Trophoblast cells: Purple
- Endocrine cells: Yellow
- Gliai cells: Yellow
- Neuronal cells: Teal
- Undifferentiated cells: Light Blue
- Epithelial cells: Orange
- Mesenchymal cells: Dark Blue
- Pigment cells: Yellow
- Vascular cells: Orange



iii) HSPB7 - Enriched in Cardiomyocytes

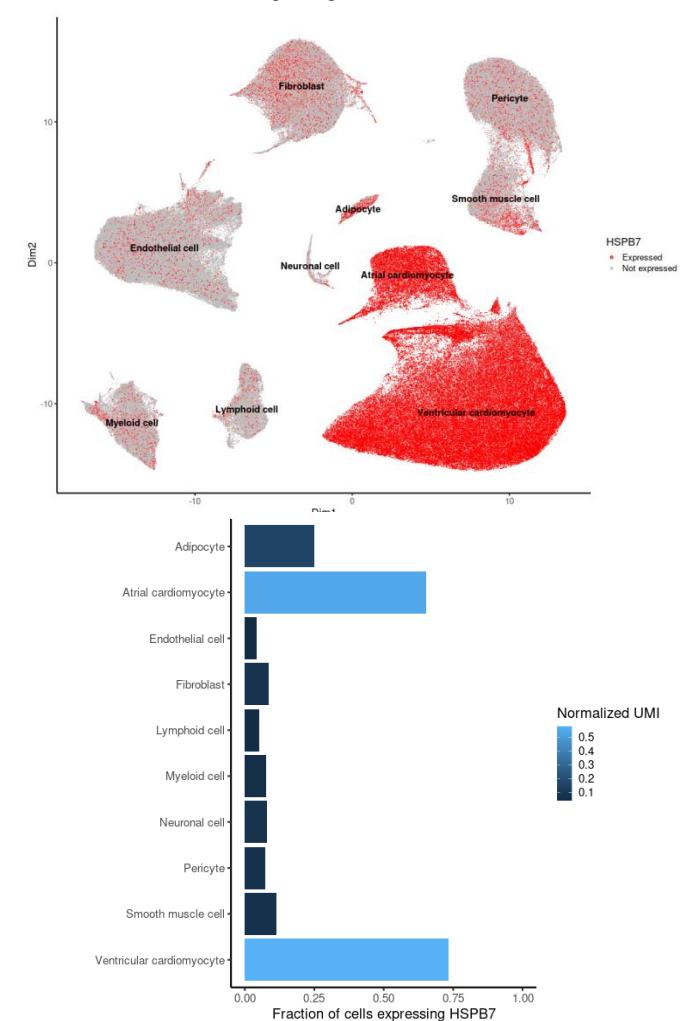
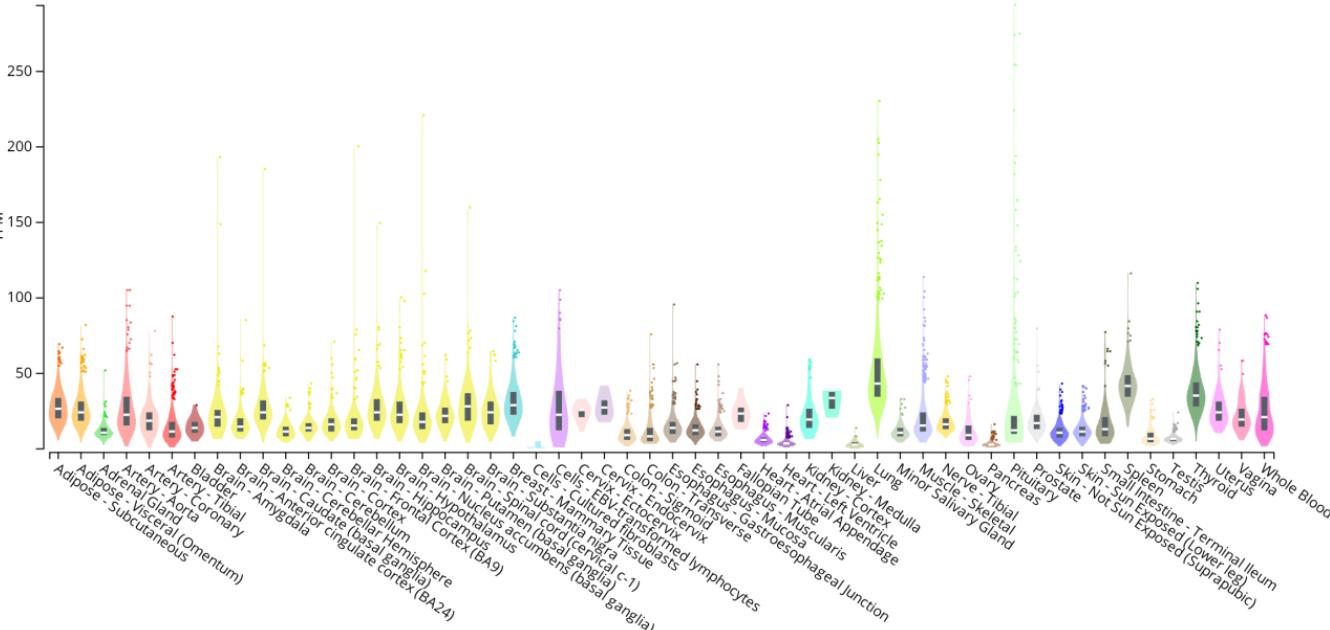


Fig. S4C. Transcriptomics data analysis on HSPB7.

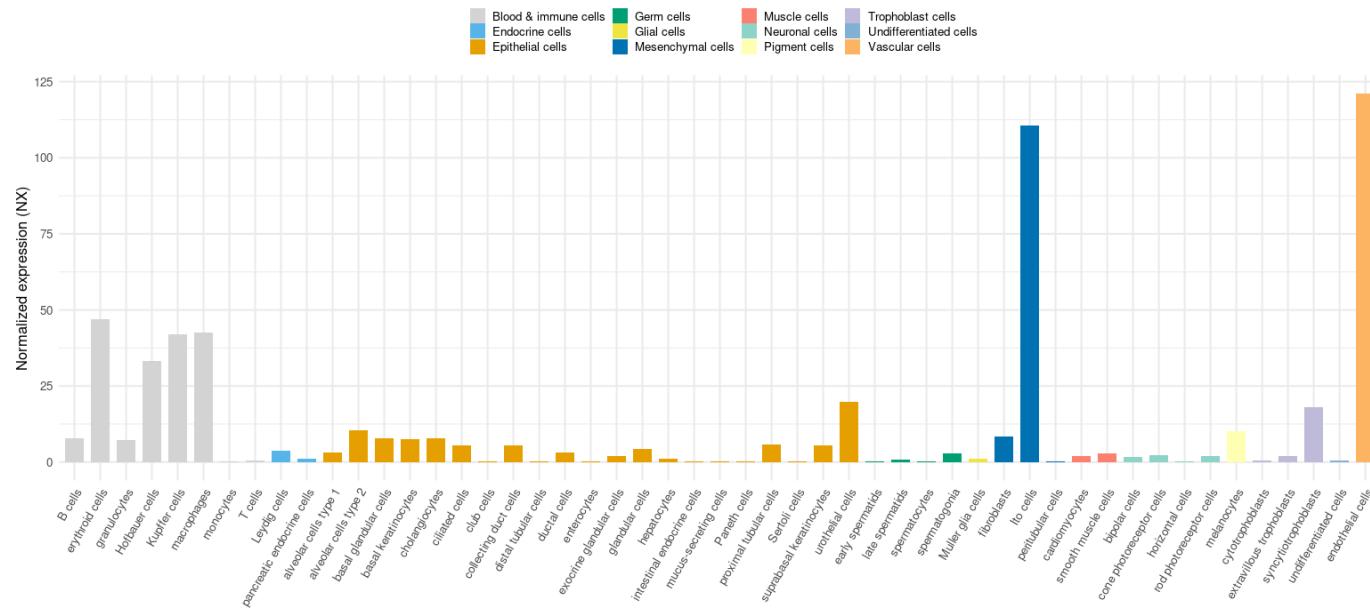
Data shown are the tissue and single cell type mRNA expression levels based on RNA sequencing data sets from the **i)** GTExPortal (<https://gtexportal.org/home/>), **ii)** the Human Protein Atlas (<https://www.proteinatlas.org/>) and **iii)** the Heart Cell Atlas (<https://www.heartcellatlas.org/>).

i) LMO2 - Ubiquitous. Low tissue specificity

Bulk tissue gene expression for LMO2 (ENSG00000135363.11)



ii) LMO2 - Enhanced in Hepatic stellate (Ito) cells, Endothelial cells, Erythroid cells, Kupffer cells, Microglial cells



iii) LMO2 - Enriched in Endothelial cells and Myeloid cells

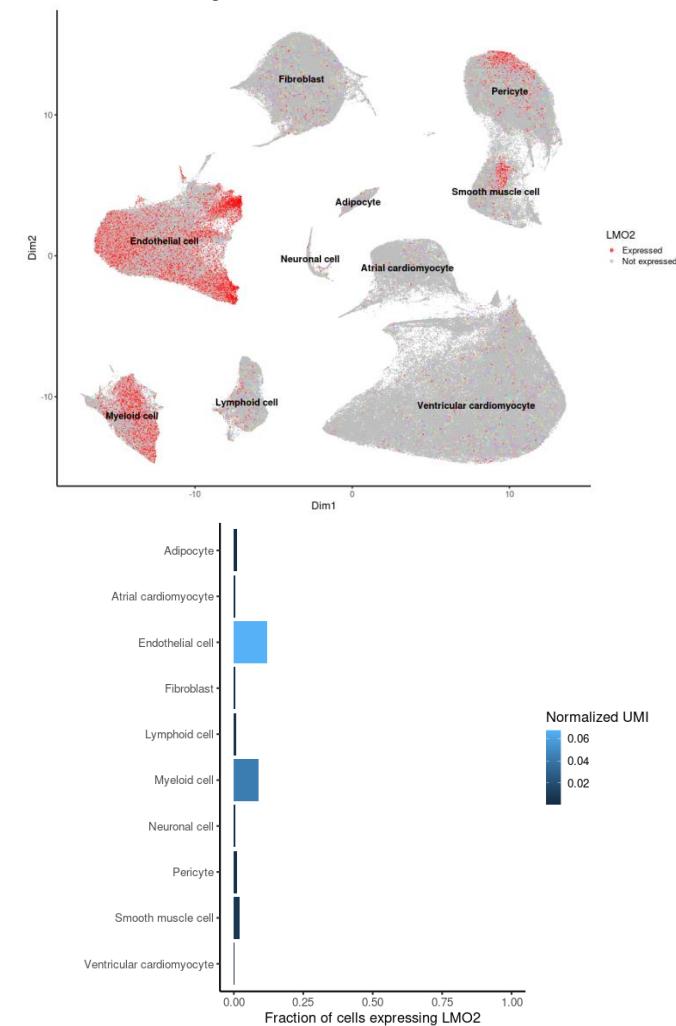


Fig. S4D. Transcriptomics data analysis on LMO2.

Data shown are the tissue and single cell type mRNA expression levels based on RNA sequencing data sets from the **i)** GTExPortal (<https://gtexportal.org/home/>), **ii)** the Human Protein Atlas (<https://www.proteinatlas.org/>) and **iii)** the Heart Cell Atlas (<https://www.heartcellatlas.org/>).

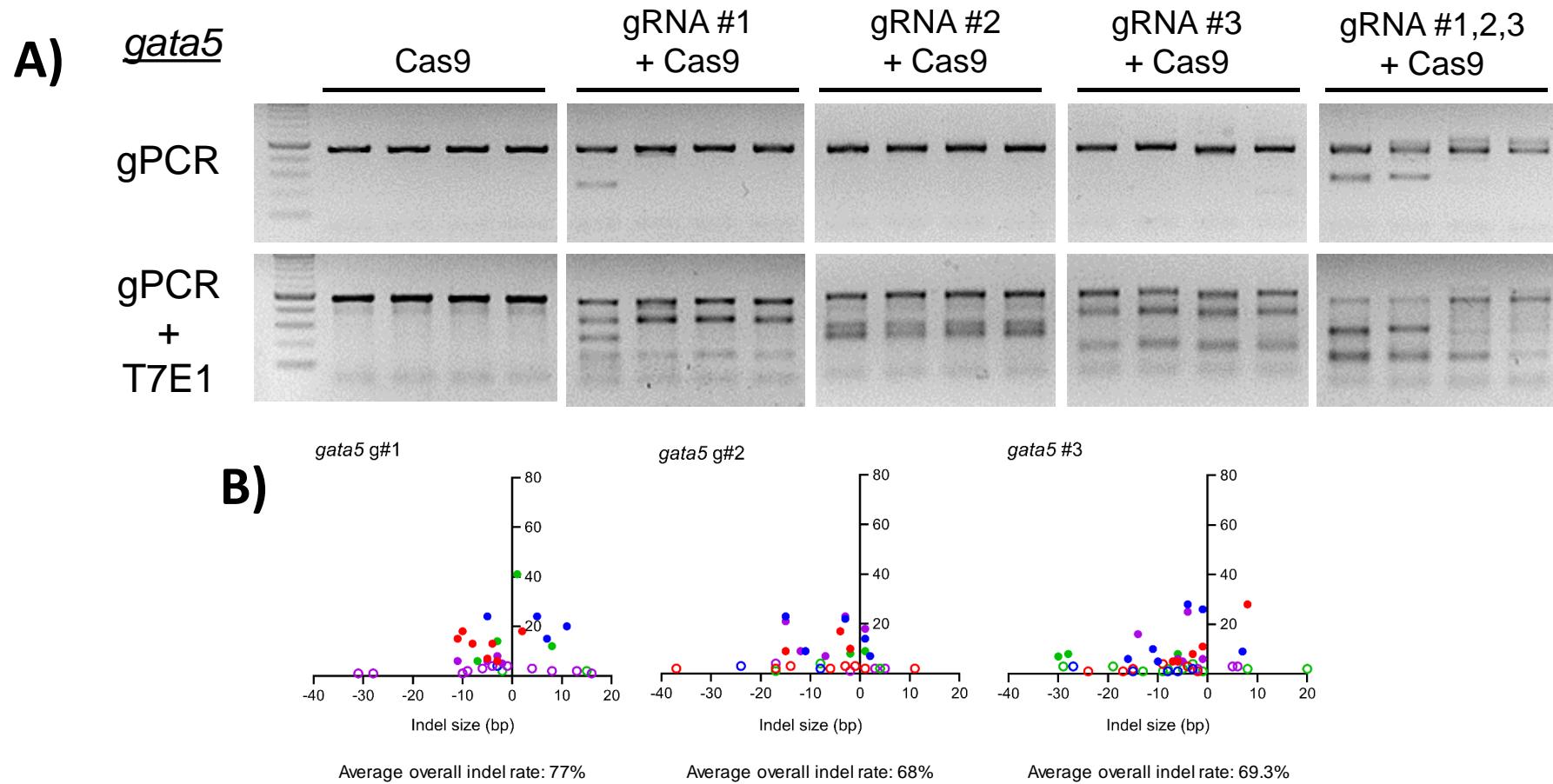


Fig. S5. Measures of mutation efficiency after injection of *gata5* g#1, g#2, and g#3 with Cas9 individually or in combination. Panel A) Data shown left to right (top), genomic DNA PCR products from 4 larvae subjected to injection of Cas9 protein alone, *gata5* gRNA #1 + Cas9, *gata5* gRNA #2 + Cas9, *gata5* gRNA #3 + Cas9, and *gata5* gRNA #1,2,3 + Cas9. Note the presence of extra bands, particularly after injection of the *gata5* gRNA #1,2,3 + Cas9 indicating the presence of large deletions in the *gata5* gene. Data shown left to right (bottom), are the same samples after T7 endonuclease I assay resulting in the cleavage of heteroduplex DNA. The presence of multiple bands in all gRNA + Cas9 samples indicates the presence of multiple small deletions in the *gata5* gene, which are not visible after injection of the Cas9 protein alone. Collectively these data that 16/16 (100% across all guide combinations) animals showed mutation in the presence of gRNAs + Cas9, but zero in the Cas9-only control group animals (4/4 or 100%). These data also support the use of this approach to rapidly indicate effective mutagenesis in each of the candidate genes as part of the higher throughput work flow used to support early target validation. **Note:** the data for the Cas9 control, *gata5* gRNA #1 + Cas9 and *gata5* gRNA #1,2,3 + Cas9 are also shown in Fig. 2 of the main manuscript. Panel B) Scatter plot graphs in each panel show the indel size and frequency in the PCR products from the three individual gRNAs used for *gata5* + Cas9 group (n=4) per gene assessed using ICE. Data points with same colour indicate the indels identified in the same individual embryo within the group. The size ranges of deletions between gRNA target sites are shaded. Indels with less than 5% frequency are presented by open circles.

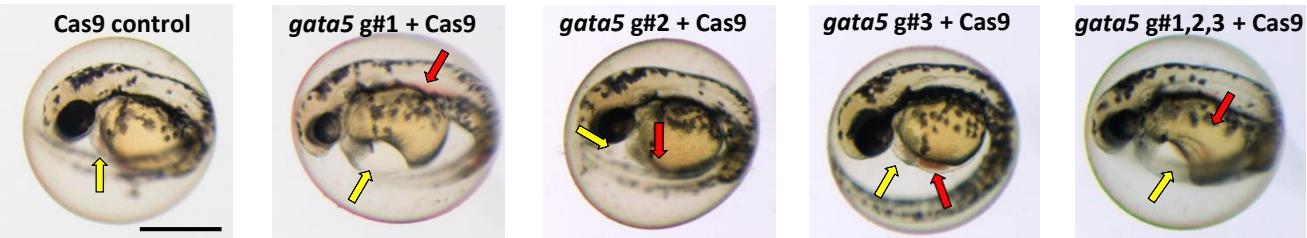
A)

	n-number (scored)	% Lethality (by 2dpf)	Malformations at 2 days post fertilisation (2dpf)									
		% Larvae Abnormal	Body Shape	Somites	Notochord	Tail	Heart	Face	Neural Tube	Arches/Jaws	Abdomen	dilated/yolk oedema
Cas9	110	0%	3%	0%	2%	2%	0%	3%	2%	2%	1%	1%
<i>gata5</i> g#1 + Cas9	95	0%	68%	0%	0%	0%	0%	68%	0%	0%	0%	65%
<i>gata5</i> g#2 + Cas9	57	0%	18%	0%	0%	0%	0%	18%	0%	0%	0%	7%
<i>gata5</i> g#3 + Cas9	60	0%	18%	0%	0%	0%	0%	18%	0%	0%	0%	17%
<i>gata5</i> g#1,2,3 + Cas9	102	2%	87%	0%	3%	1%	0%	87%	3%	1%	0%	94%

Aii

	n-number (scored)	% Abnormal	Heart-specific malformations at 2dpf				
			Slow Heartbeat	No Heartbeat	Pericardial Sac-Swollen	Chambers not well defined	Missshapen
Cas9	110	3%	0%	0%	3%	1%	2%
<i>gata5</i> g#1 + Cas9	97	54%	3%	10%	31%	2%	37%
<i>gata5</i> g#2 + Cas9	57	18%	0%	0%	18%	0%	0%
<i>gata5</i> g#3 + Cas9	60	18%	0%	0%	17%	0%	3%
<i>gata5</i> g#1,2,3 + Cas9	100	87%	19%	8%	73%	10%	53%

Aiii



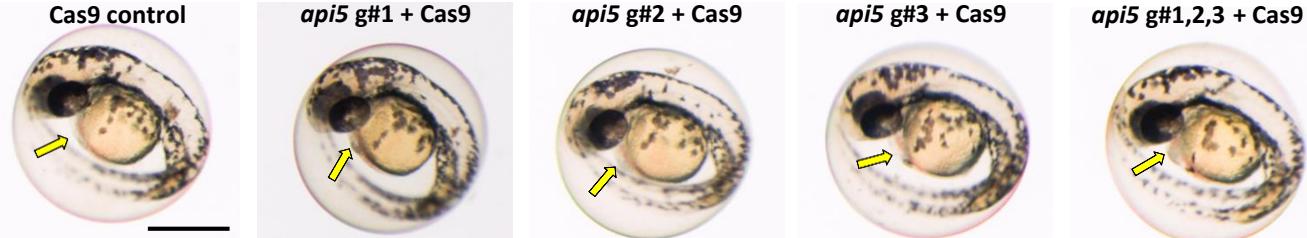
B)

	n-number (scored)	% Lethality (by 2dpf)	Malformations at 2 days post fertilisation (2dpf)									
		% Larvae Abnormal	Body Shape	Somites	Notochord	Tail	Heart	Face	Neural Tube	Arches/Jaws	Abdomen	dilated/yolk oedema
Cas9	42	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
<i>api5</i> g#1 + Cas9	44	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
<i>api5</i> g#2 + Cas9	53	19%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
<i>api5</i> g#3 + Cas9	44	2%	2%	0%	2%	2%	0%	2%	2%	2%	2%	2%
<i>api5</i> g#1,2,3 + Cas9	50	14%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Bii

	n-number (scored)	% Abnormal	Heart-specific malformations at 2dpf				
			Slow Heartbeat	No Heartbeat	Pericardial Sac-Swollen	Chambers not well defined	Missshapen
Cas9	44	0%	0%	0%	0%	0%	0%
<i>api5</i> g#1 + Cas9	43	0%	0%	0%	0%	0%	0%
<i>api5</i> g#2 + Cas9	43	0%	0%	0%	0%	0%	0%
<i>api5</i> g#3 + Cas9	43	2%	0%	2%	0%	0%	2%
<i>api5</i> g#1,2,3 + Cas9	41	0%	0%	0%	0%	0%	0%

Biii

**Fig. S6A and B. Comparison of morphological endpoints measured in 2dpf zebrafish.**

Panel A) General whole body morphological endpoints measured following injection of Cas9 alone, or injection of *gata5* gRNAs 1, 2 and 3 in isolation or combined (g#1, g#2, g#3 or g#1,2,3 respectively) all with Cas9. Data are shown as the % incidence of abnormalities under each of the morphological categories, with shades from white (0%) through to red (100%) providing an indication of the proportion of animals exhibiting a malformation within that category. **Ai:** Expansion of the heart-specific endpoints measured showing the full range of endpoints scored within this category, across the same treatments. **Aii:** Example images of typical 2dpf animals within each treatment category. The yellow arrows show the position of the pericardial membrane and the extent of pericardial oedema in that animal, and the red arrows shows regions of blood pooling; **Panel B)** As Panel A, but for *api5*. More detailed whole body morphological endpoint scoring and cardiovascular functional assessment was undertaken on the two guide combinations showing the strongest phenotypes from the 2dpf data. Scale bar shown in the first image of each panel represents 500μm.

C)

	n-number	Malformations at 2 days post fertilisation (2dpf)													
		% Lethality (by 2dpf)		% Larvae Abnormal		Body Shape	Somites	Notochord	Tail	Heart	Face	Neural Tube	Arches/Jaws	Abdomen	distended/yolk oedema
Cas9	48	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	
<i>hspb7</i> g#1 + Cas9	47	0%	2%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	
<i>hspb7</i> g#2 + Cas9	48	0%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	0%	0%	
<i>hspb7</i> g#3 + Cas9	41	0%	12%	0%	10%	7%	5%	10%	10%	10%	10%	10%	10%	10%	
<i>hspb7</i> g#1,2,3 + Cas9	48	0%	17%	10%	13%	10%	13%	10%	13%	13%	8%	13%	0%	0%	

	n-number (scored)	Heart-specific malformations at 2dpf						
		% Abnormal	Slow Heartbeat	No Heartbeat	Pericardial Sac-Swollen	Chambers not well defined	Missshapen	
Cas9	48	0%	0%	0%	0%	0%	0%	
<i>hspb7</i> g#1 + Cas9	47	2%	0%	0%	2%	0%	0%	
<i>hspb7</i> g#2 + Cas9	48	2%	0%	2%	0%	2%	2%	
<i>hspb7</i> g#3 + Cas9	41	10%	5%	0%	5%	0%	5%	
<i>hspb7</i> g#1,2,3 + Cas9	48	10%	0%	4%	10%	6%	8%	

Ciii



D)

	n-number	Malformations at 2 days post fertilisation (2dpf)													
		% Lethality (by 2dpf)		% Larvae Abnormal		Body Shape	Somites	Notochord	Tail	Heart	Face	Neural Tube	Arches/Jaws	Abdomen	distended/yolk oedema
Cas9	46	8%	7%	0%	2%	2%	2%	7%	2%	2%	2%	2%	2%	2%	
<i>lmo2</i> g#1 + Cas9	3	94%	33%	0%	33%	33%	33%	33%	33%	33%	33%	33%	33%	33%	
<i>lmo2</i> g#2 + Cas9	45	10%	91%	2%	20%	7%	11%	91%	24%	11%	9%	18%	18%	18%	
<i>lmo2</i> g#3 + Cas9	43	24%	91%	5%	12%	5%	5%	91%	14%	9%	7%	44%	44%	44%	
<i>lmo2</i> g#1,2,3 + Cas9	12	76%	92%	25%	67%	50%	58%	92%	58%	58%	42%	58%	58%	58%	

	n-number (scored)	Heart-specific malformations at 2dpf						
		% Abnormal	Slow Heartbeat	No Heartbeat	Pericardial Sac-Swollen	Chambers not well defined	Missshapen	
Cas9	46	7%	2%	0%	7%	0%	4%	
<i>lmo2</i> g#1 + Cas9	3	33%	0%	33%	33%	33%	33%	
<i>lmo2</i> g#2 + Cas9	45	91%	7%	73%	73%	11%	82%	
<i>lmo2</i> g#3 + Cas9	43	91%	2%	2%	60%	2%	81%	
<i>lmo2</i> g#1,2,3 + Cas9	12	92%	8%	17%	58%	25%	92%	

Diii



Fig. S6C and D. Comparison of morphological endpoints measured in 2dpf animals. Panel C)
General whole body morphological endpoints measured following injection of Cas9 alone, or injection of *hspb7* gRNAs 1, 2 and 3 in isolation or combined (g#1, g#2, g#3 or g#1,2,3 respectively) all with Cas9. Data are shown as the % incidence of abnormalities under each of the morphological categories, with shades from white (0%) through to red (100%) providing an indication of the proportion of animals exhibiting a malformation within that category. **Ci:** Expansion of the heart-specific endpoints measured showing the full range of endpoints scored within this category, across the same treatments. **Cii:** Example images of typical 2dpf animals within each treatment category. The yellow arrows show the position of the pericardial membrane and the extent of pericardial oedema in that animal, and the red arrows shows regions of blood pooling; **Panel D)** as Panel C) but for *lmo2*. More detailed whole body morphological endpoint scoring and cardiovascular functional assessment was undertaken on the two guide combinations showing the strongest phenotypes from the 2dpf data. Scale bar shown in the first image of each panel represents 500μm.

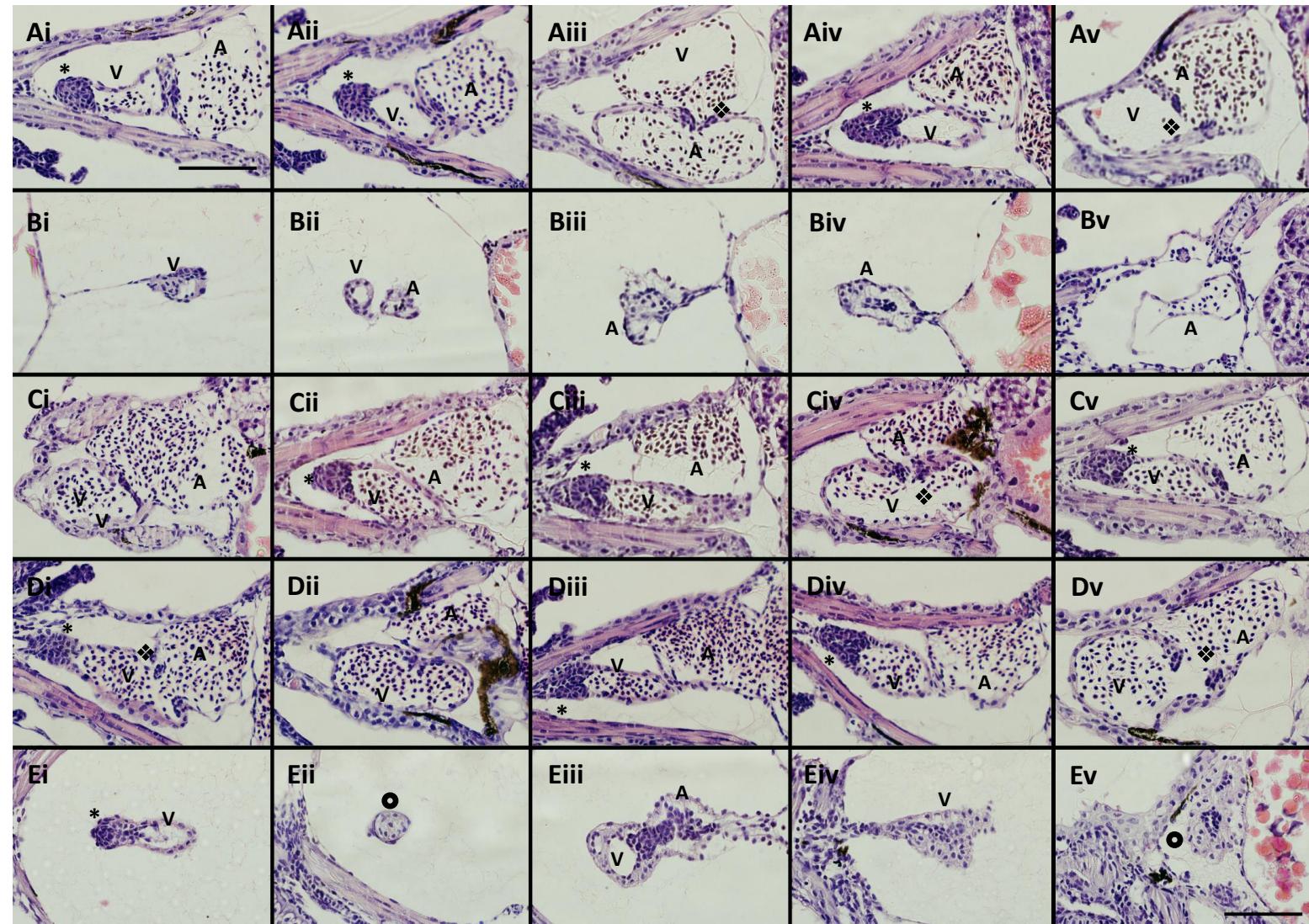
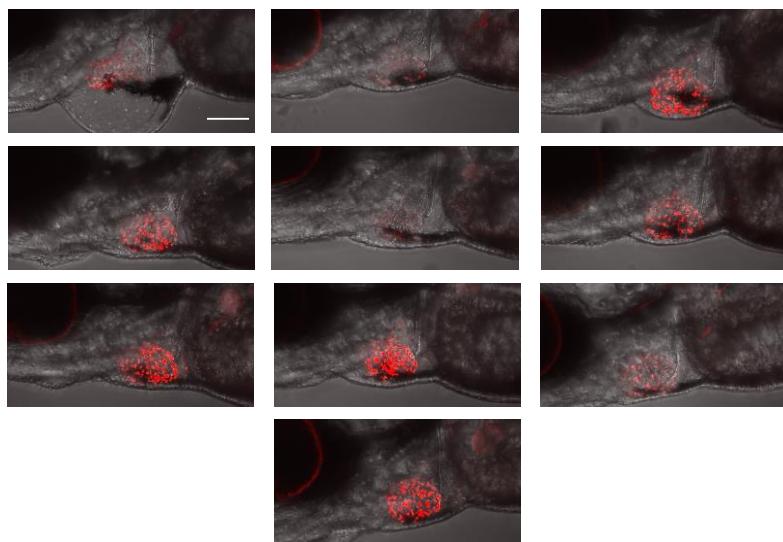
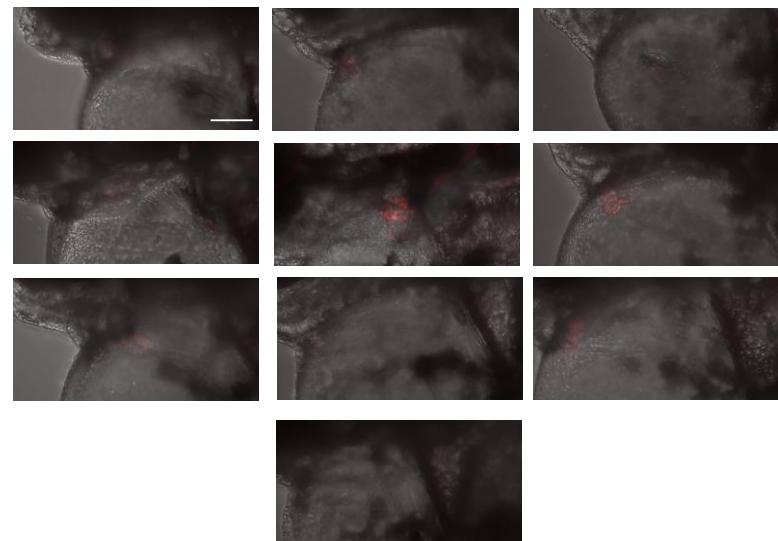


Fig. S7. Haematoxylin and eosin stained coronal sections through the hearts of 4dpf CRISPR-mutant zebrafish. In each row are sections from 5 animals (i-v) subjected to A) Cas9 injection; B) gata5 g#1,2,3 + Cas9 injection; C) api5 g#1,2,3 + Cas9 injection; D) hspb7 g#1,2,3 + Cas9 injection and E) lmo2 g#1,2,3 + Cas9 injection. In each panel, animals are orientated with the head to the left, and viewed in the dorsal plane at a magnification of 40x (Scale bar shown in top left and bottom right images represents 200μm). On various panels, labelled structures include the ventricle (v); atrium (A); bulbus arteriosus (*); atrioventricular value (❖); and unspecified heart tissue (●).

Cas9



gata5



api5

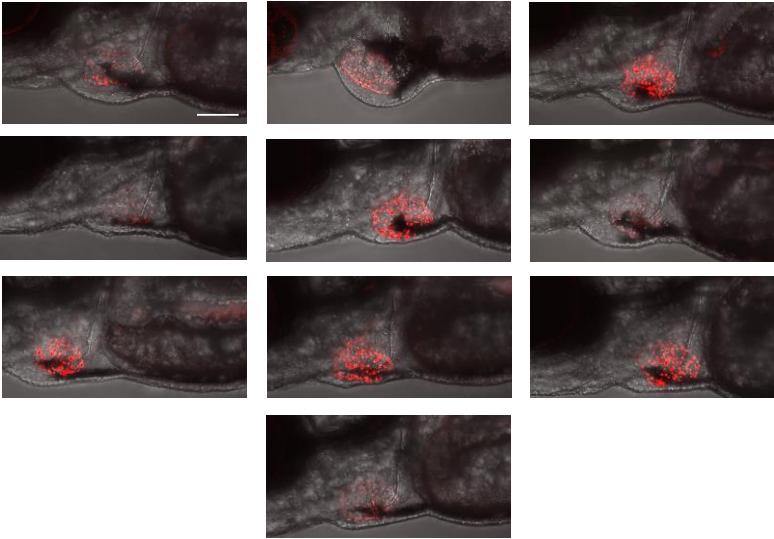
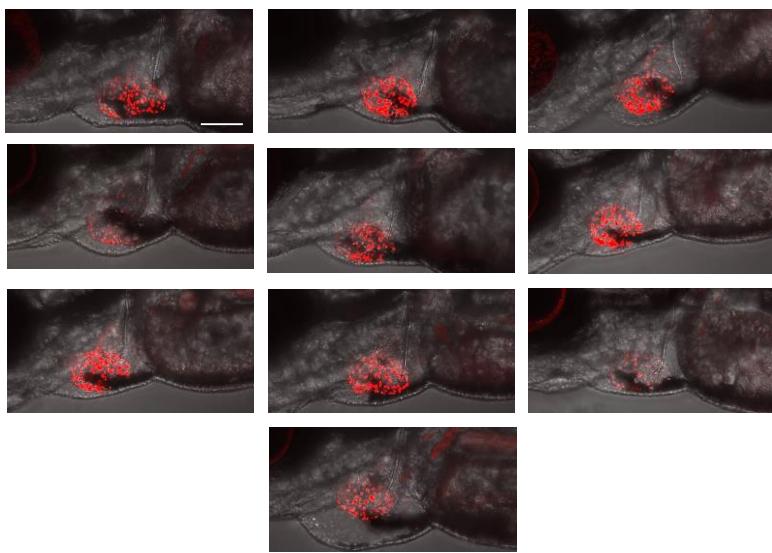
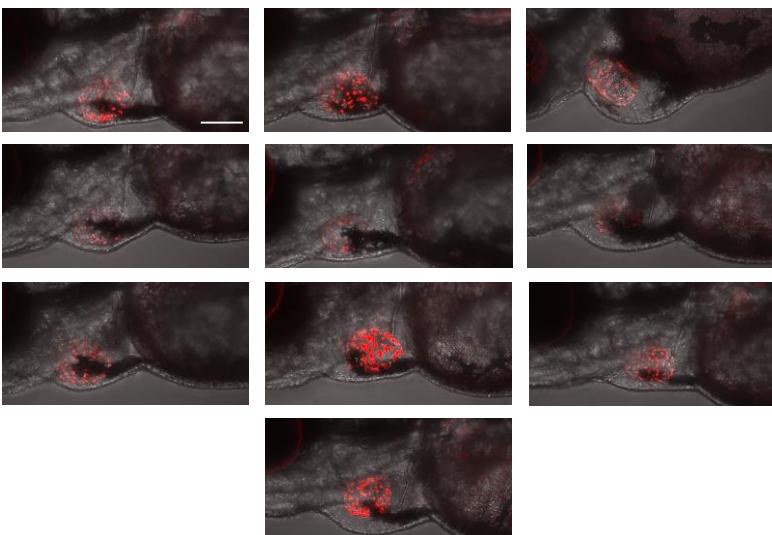


Fig. S8A. Confocal maximum intensity projection images of hearts from *cmlc2::DsRed2-nuc* mutant larvae. The images shown are the combined transmitted light and *cmlc2::DsRed2-nuc* fluorescence signals from 10 randomly sampled larvae per treatment, with cardiomyocytes fluorescing in red, especially prominently in the ventricle (all laser settings identical). The top set of images shows hearts from Cas9 injected (injection controls) and then from embryos injected with *gata5* g#1,2,3 + Cas9 (middle), and *api5* g#1,2,3 + Cas9, (bottom). Scale bar shown in the first image of each panel represents 100 μ m.

Cas9



hspb7



lmo2

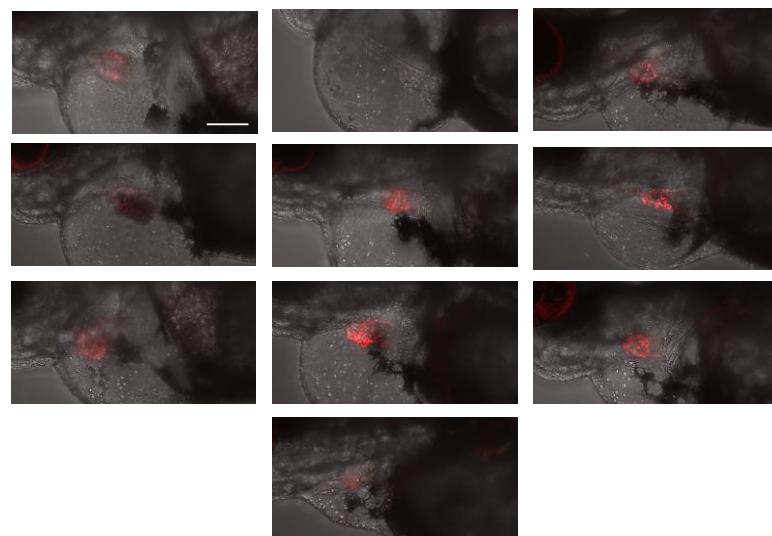


Fig. S8B. Confocal maximum intensity projection images of hearts from *cmlc2::DsRed2-nuc* mutant larvae. The images shown are the combined transmitted light and *cmlc2::DsRed2-nuc* fluorescence signals from 10 randomly sampled larvae per treatment, with cardiomyocytes fluorescing in red, especially prominently in the ventricle (all laser settings identical). The top set of images shows hearts from Cas9 injected (injection controls) and then from embryos injected with *hspb7* g#1,2,3 + Cas9 (middle) and *lmo2* g#2 + Cas9 (bottom). Scale bar shown in the first image of each panel represents 100 μm .

SUPPLEMENTARY VIDEOS

Video 1) V1 Cas9 heart beat (control)

Video 2) V2 Cas9 blood flow (control)

Video 3) V3 gata5 g#1 heart beat

Video 4) V4 gata5 g#1 blood flow

Video 5) V5 gata5 g#123 heart beat

Video 6) V6 gata5 g#123 blood flow

Video 7) V7 api5 g#1 heart beat

Video 8) V8 api5 g#1 blood flow

Video 9) V9 api5 g#123 heart beat

Video 10) V10 api5 g#123 blood flow

Video 11) V11 hspb7 g#3 heart beat

Video 12) V12 hspb7 g#3 blood flow

Video 13) V13 hspb7 g#123 heart beat

Video 14) V14 hspb7 g#123 blood flow

Video 15) V15 lmo2 g#2 heart beat

Video 16) V16 lmo2 g#2 blood flow

Video 17) V17 lmo2 g#123 heart beat

Video 18) V18 lmo2 g#123 blood flow

SUPPLEMENTARY DATASETS

Data S1) Data S1_Network Analysis.xlsx

Data S2) Data S2_Differential Expression.xlsx

Data S3) Data S3_Genetic Associations.xlsx

Data S4) Data S4_Homologies.xlsx