

## SUPPLEMENTAL FIGURES

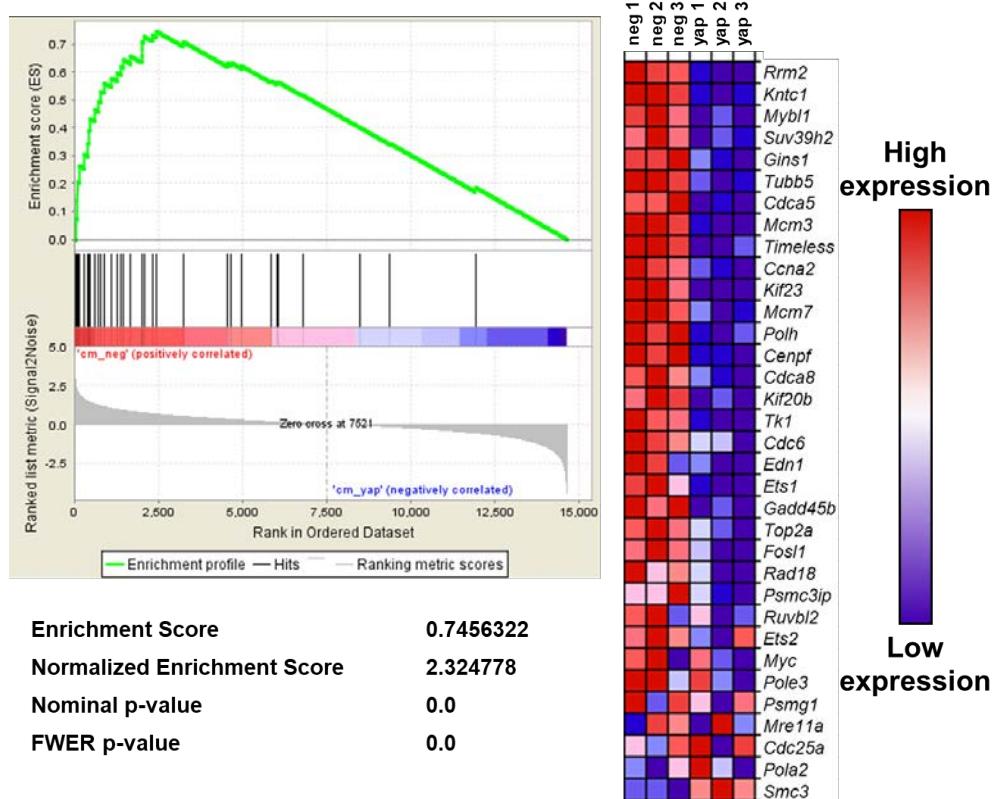
### *Ilgl1* wild-type protein

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EVHSFNLPGRPGIESTSATRVTVMLLNLSCLNLLALGTEGGGVHFELPTLTLLNKSL  
FQDEIMQSVPEEYKCGKSLGPVESLQEHPKDSDKILIGYSRGLVVLWDLSSRHVDNL  
FLGKQQLESLVWERSGNSFVSSHSDGGYMVAWSNNPCTHDPSSTIPIYGPFPCKA  
VNKILWRTTESGAPLVLFSGGMPRASYGDRHCLTILQDSSHVTLDFTSRVIDFFTIH  
CTDTEKDFDEPSALVVLLEEELVVIDLQMPGWPTVPAPYLAPLHSSAITCSCHISNV  
PPKLWERVISAGQQQCPLQNYENWPICGGKNLAPDPKQKEELLTGEDGTVRFWDAS  
GVSLKPLYKLSTASIFQTDCEHNDSLTQAGEEEWPPFRKVGCDFPYSDDPRLGIQKI  
SLCKYSGKLVVAGTAGQVIVMVLGDEKSDHMI DVATVDLLQDREGFTWKGHDRLLPK  
SGSVVFAPGFQPVVLVQCLPPAAVTAVTLHAEWNLIAFGTSHGFLFDYHRRSPVLA  
RCTLHPNDSLAMEGPLSRVKSLLKSLRQSFRRIRKSRVSGKKRVVNSPTSKVQEAN  
AHLADHDAEVTPVQRRIEPRSADDLSGVVRCLCFADTFLRDGTHHGPTMWAGTNSG  
SVYAYALDVPSQEKFSEQSVEALLGKEIQLMHRAPVVSIAVLDGRGNPLPEPYEVSR  
DLAKAPDMQGSHSVLIASEEQFKVFLPKVSAKTFKLTAHEGCRVRKVALANFTSV  
SSEDYSENALVCLTNMGDIHMFSVPALRPQVRYDCVRKEDISGIASCVFTKTGQGFY  
LISPSEYERFSLSARVITEPLCGVDVERPHDVS AHSSTTLPPQANGTHKSQAESKS  
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ITEEDGRSAGILIN

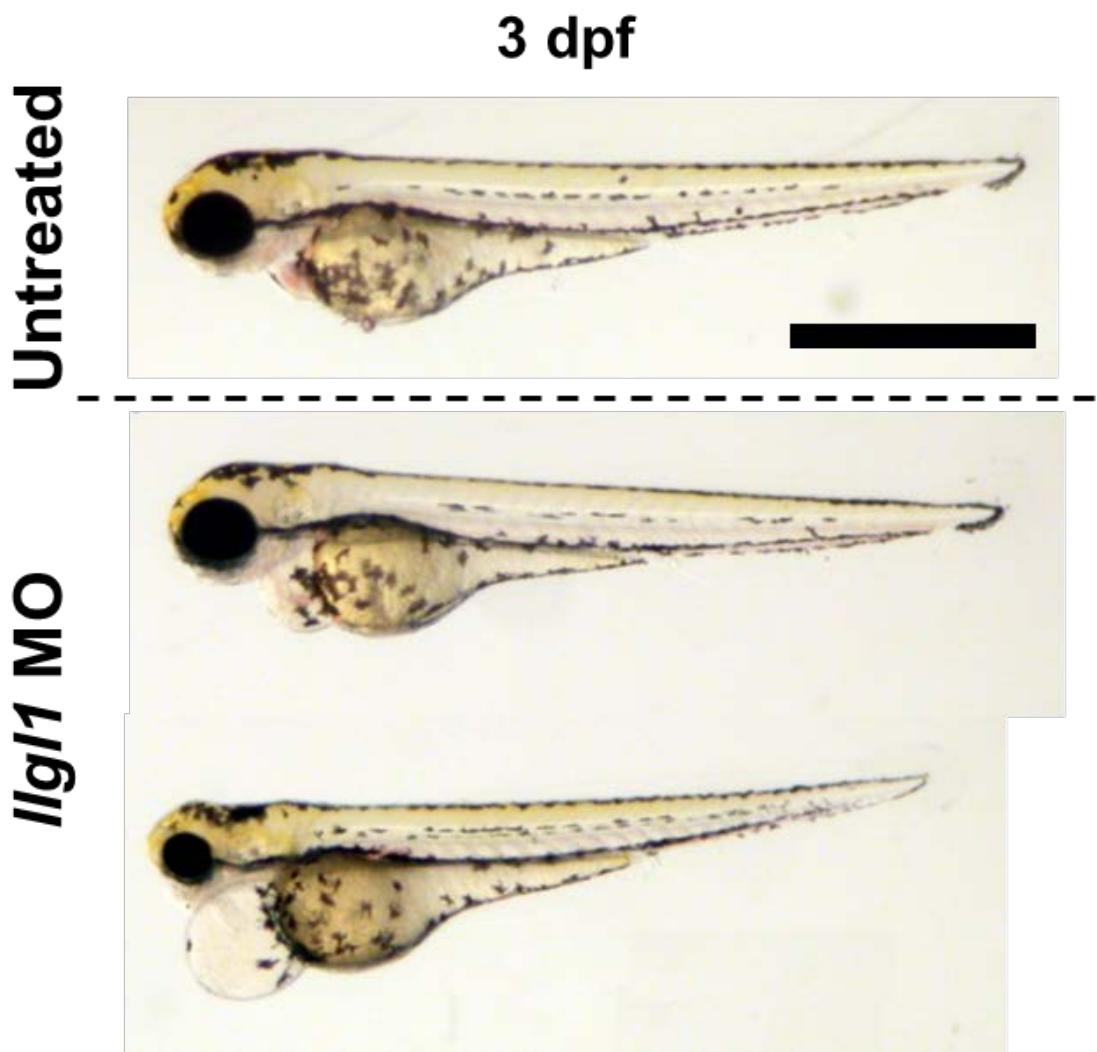
### *Ilgl1<sup>mw83</sup>* (c. 55\_64del) product

MMKFRRRQGNDPHREKICLHSIRQLNMDFQTSQLWPMIPNCISLWLLELNQGPSKY  
MVLQG

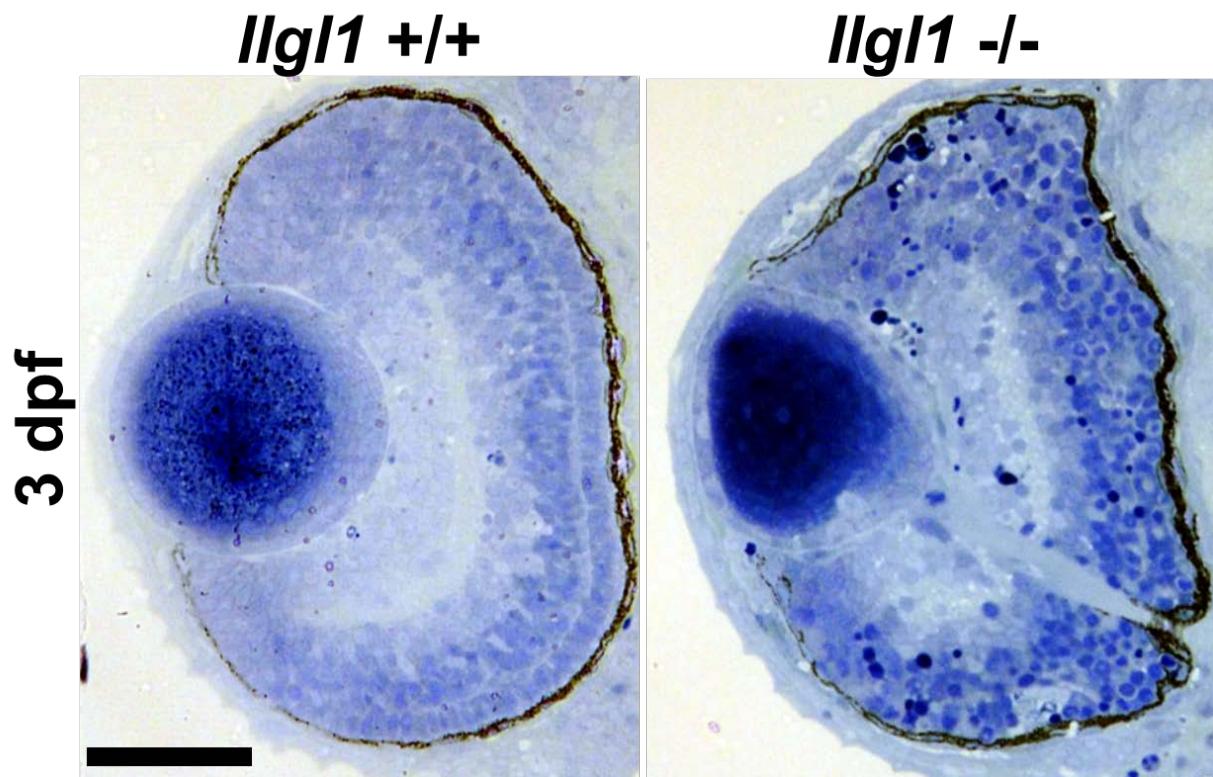
**Fig. S1. *Ilgl1* protein sequences.** Amino acid sequences for products of wildtype *Ilgl1* of *Danio rerio* and the *Ilgl1<sup>mw83</sup>* mutant allele. Yellow highlight indicates native amino acid sequence and gray indicates missense amino acid sequence.



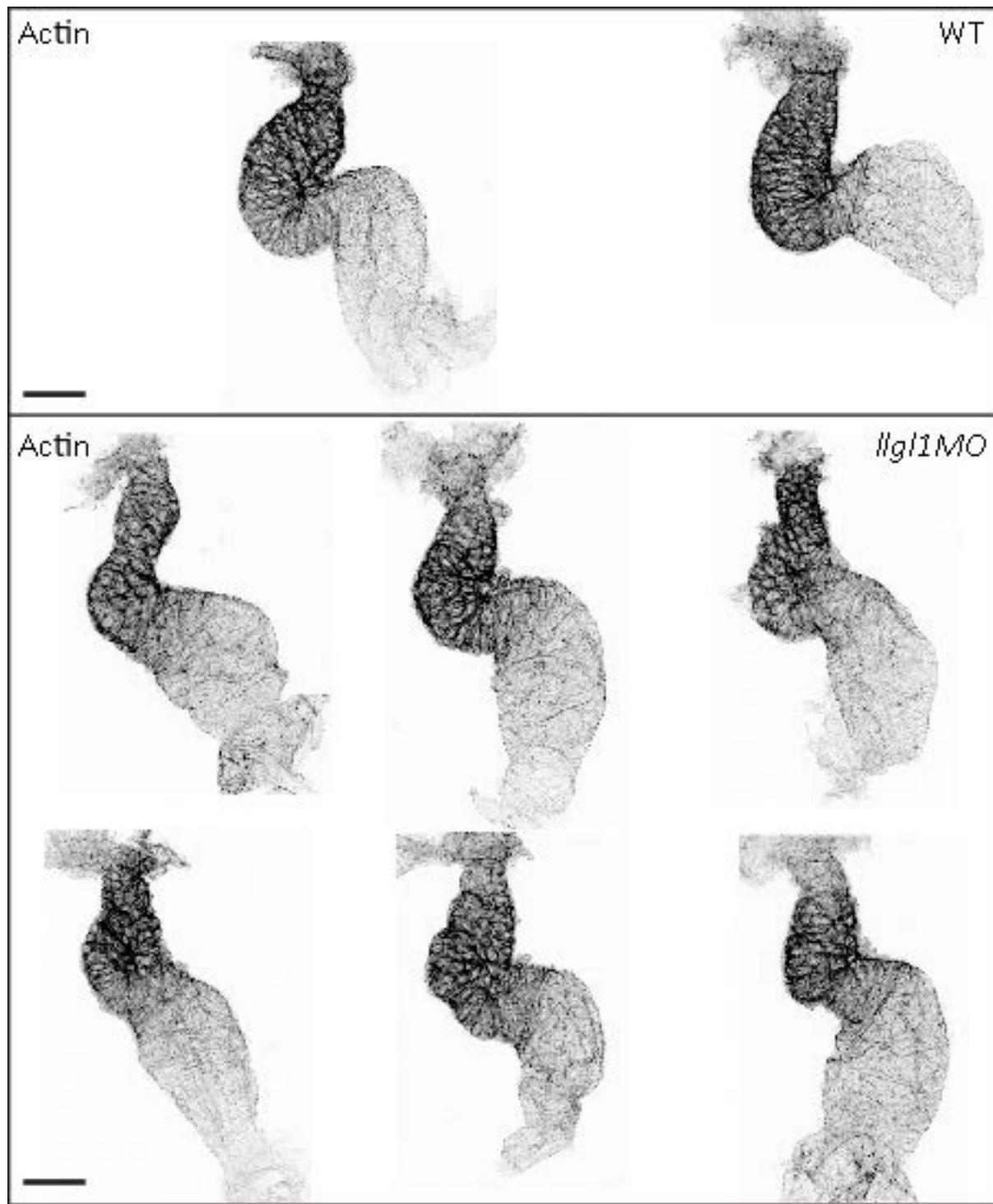
**Fig. S2. Assessment of YAP/WWTR1-TEAD mediated positive regulators of growth upon Yap siRNA knockdown in neonatal rat cardiomyocytes.** GSEA for YAP/WWTR1-TEAD target genes involved in proliferation utilizing RNAseq data from cultured rat cardiomyocytes. GSEA was performed on data previously generated by Flinn et al., (2019) and available at GSE112464 of the Gene Expression Omnibus.



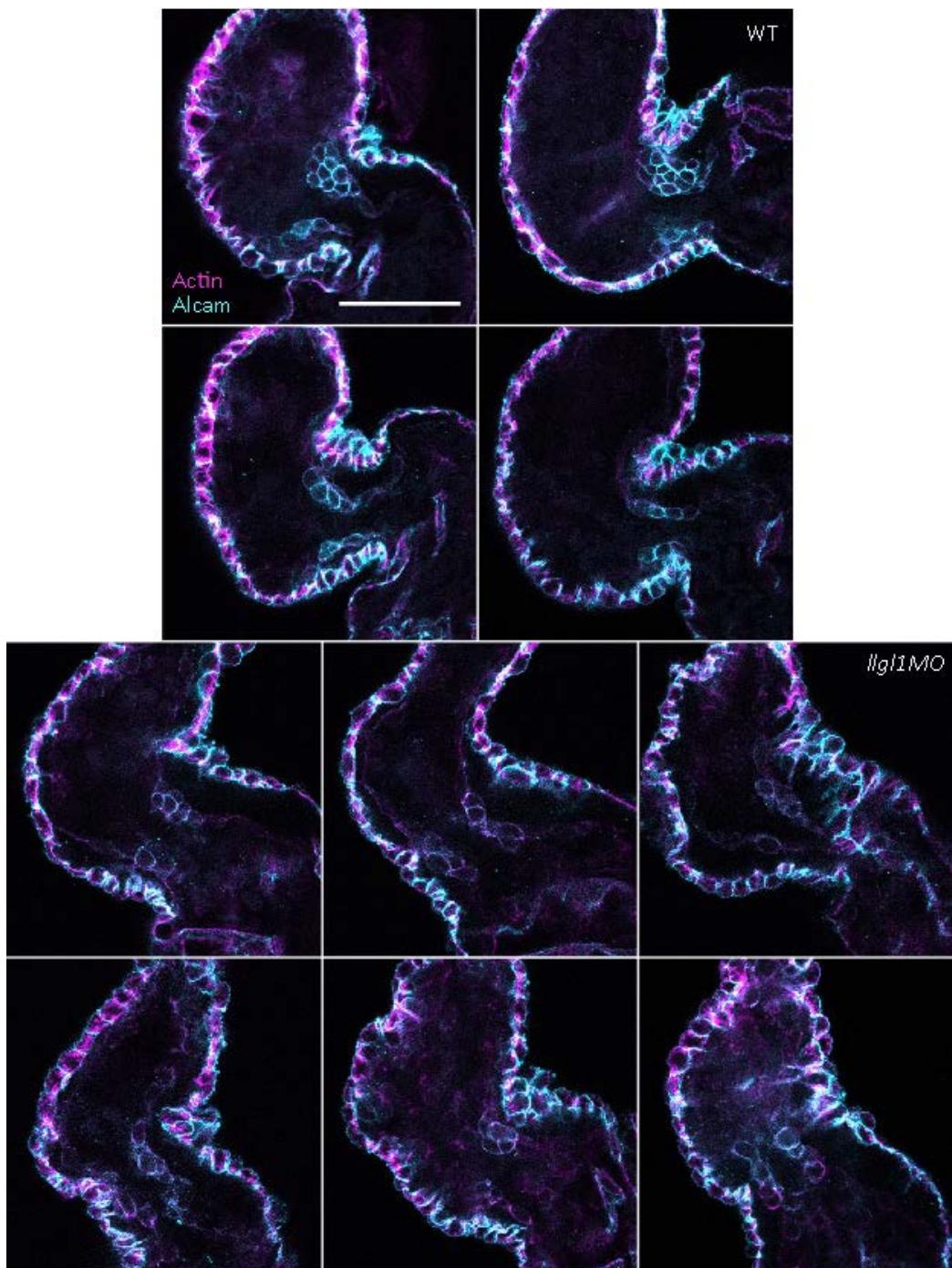
**Fig. S3. Pericardial effusion in *Ilgl1* morphants.** 3 dpf embryos displaying variable expressivity of phenotype. Scale bar: 1 mm.



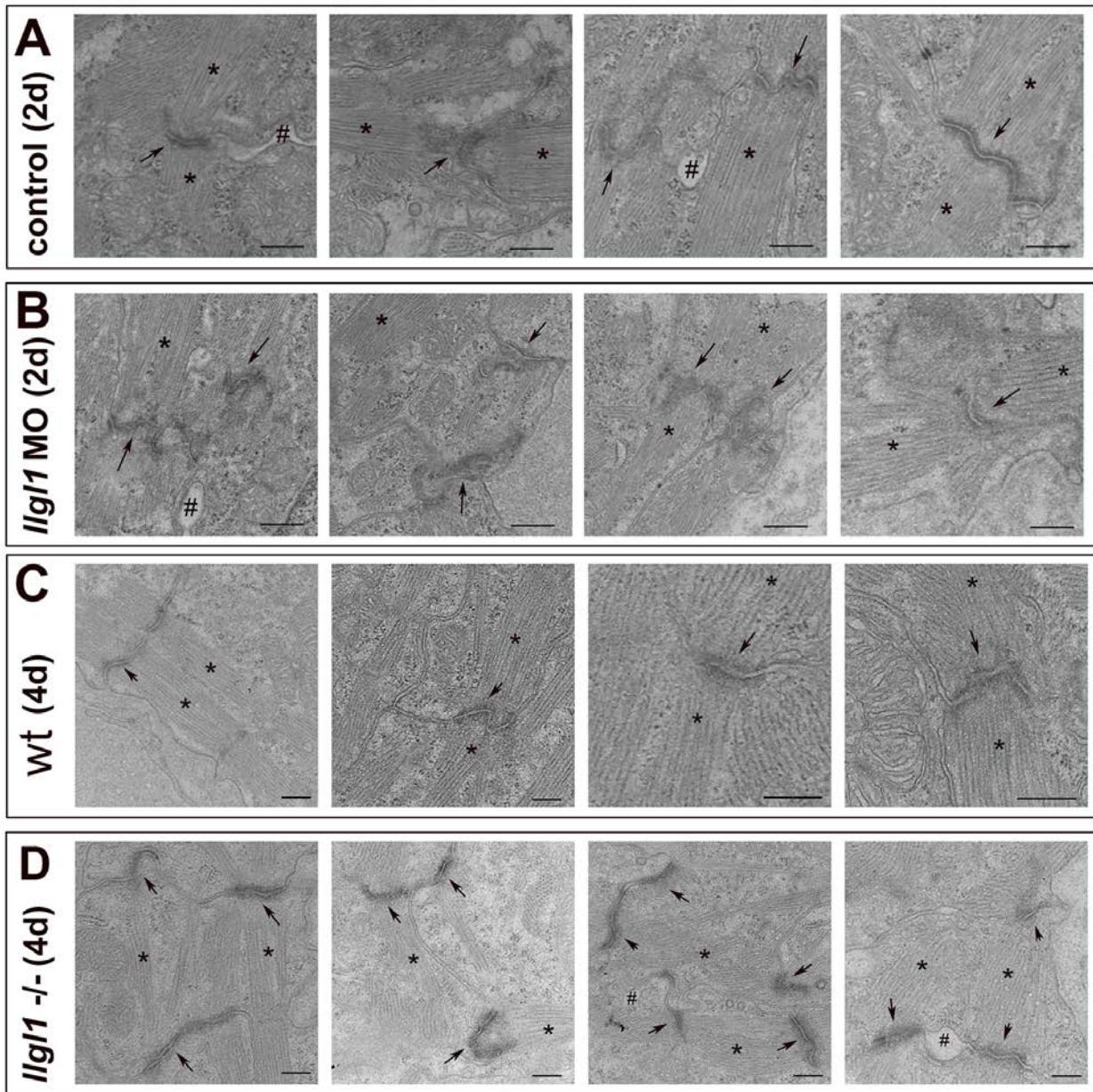
**Fig. S4. Eye of *Ilgl1*<sup>-/-</sup> embryos show disrupted retinal development.** Representative images of retinal histology from 3 dpf zebrafish. Embryos were derived from the *Ilgl1*<sup>mw8</sup> line. Light micrographs of epon-embedded sections stained with toluidine blue. Scale bar: 50  $\mu$ m.



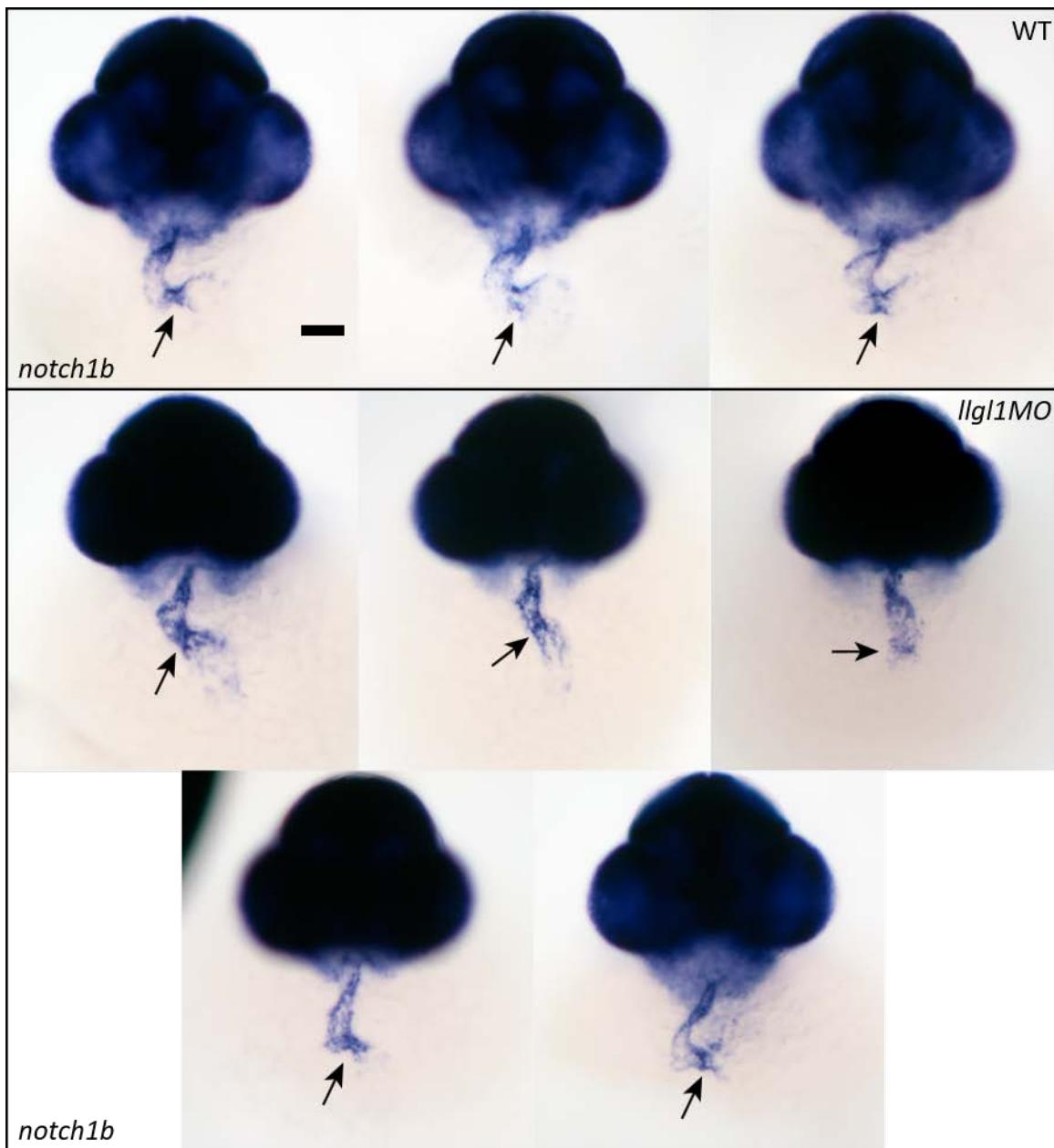
**Fig. S5. Depletion of *Llgl1* results in impaired cardiac looping.** Actin staining of 48 hpf embryos comparing uninjected controls to *llgl1* morphants. Scale bar: 50  $\mu$ m.



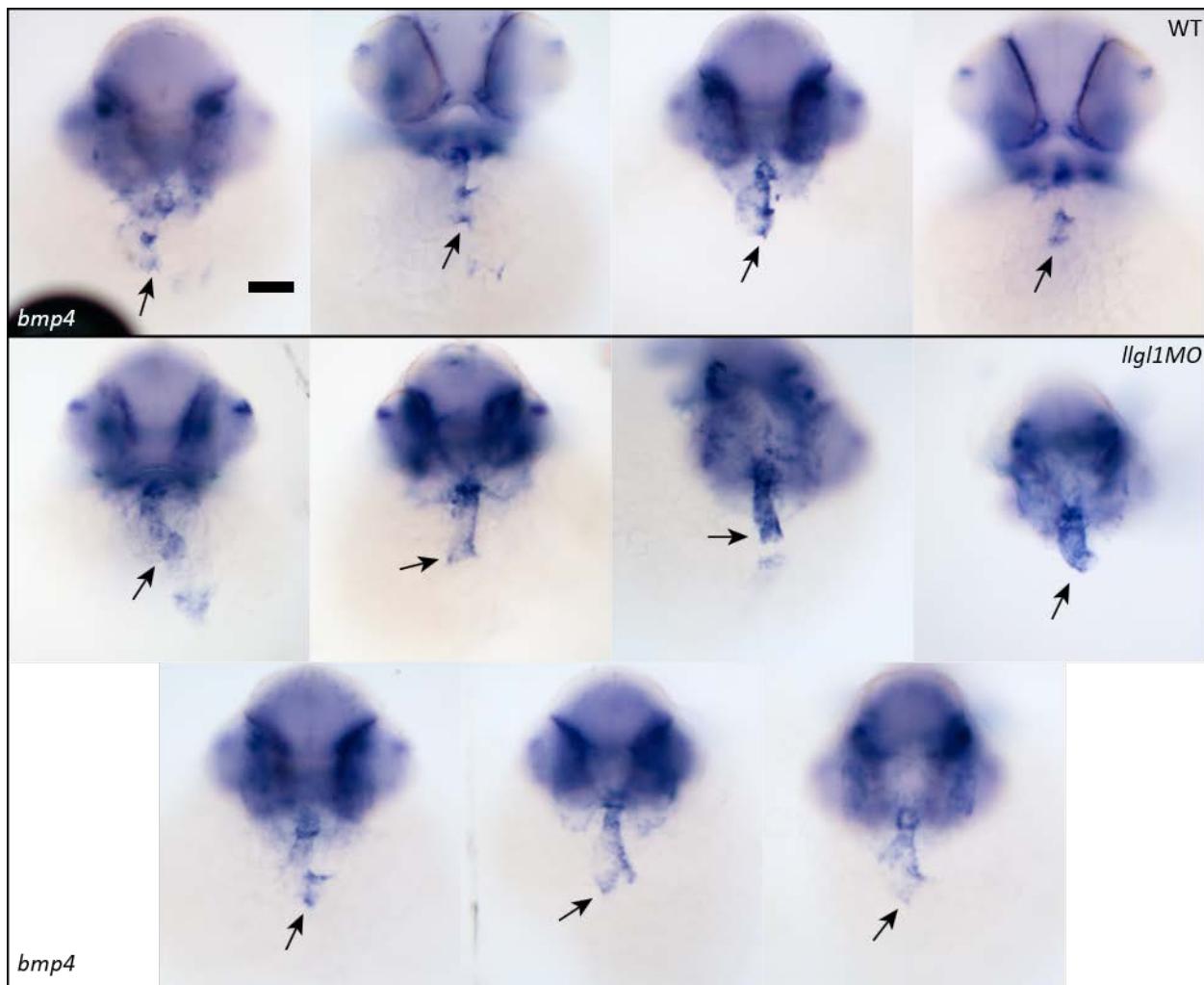
**Fig. S6. Depletion of *Llgl1* results in dysmorphic valve leaflets.** Additional images depicting AVC in *llgl1* morphants and uninjected control 48 hpf embryos. Staining was performed using rhodamine phalloidin for actin and anti-Alcam. Scale bar: 50  $\mu$ m.



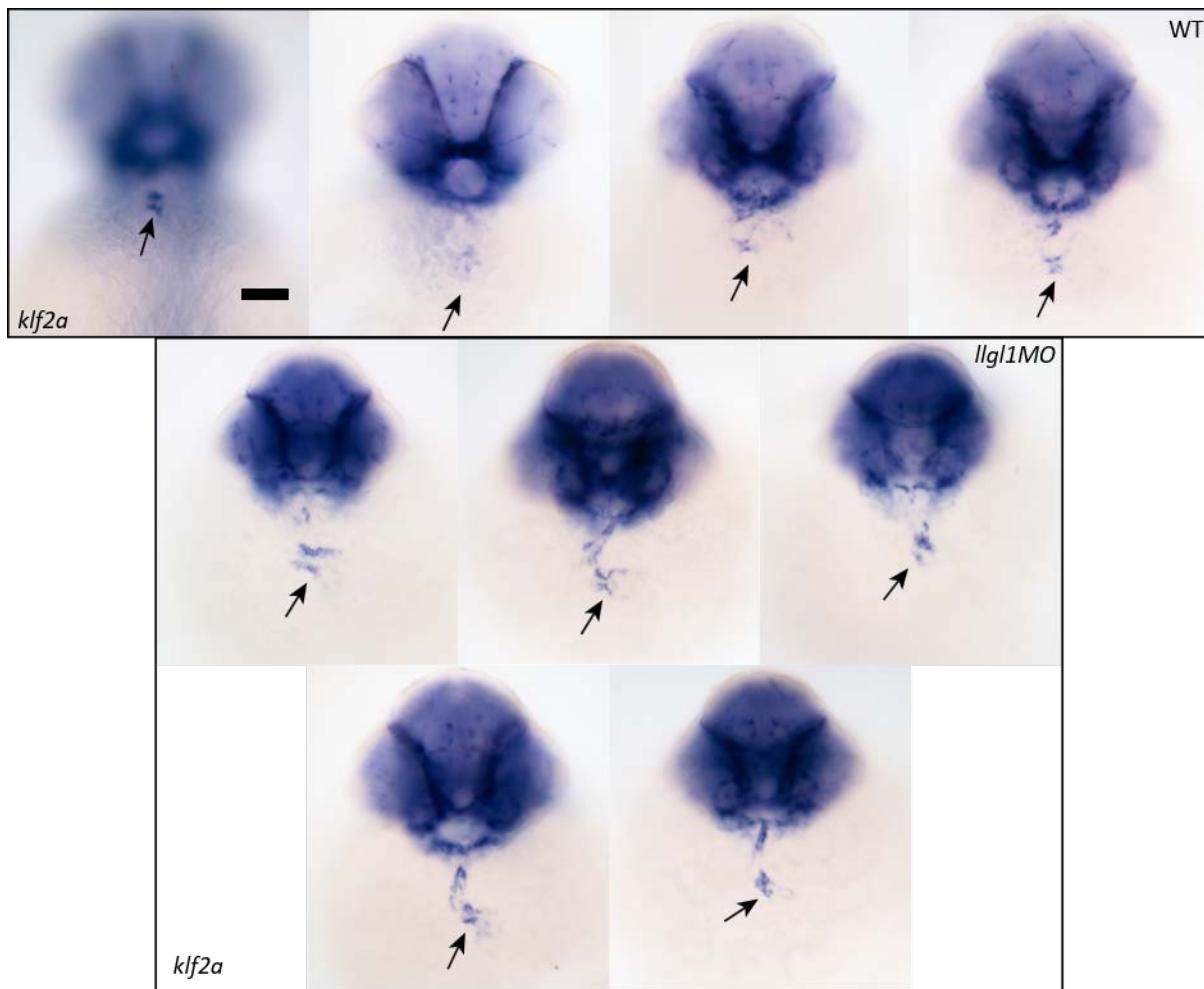
**Fig. S7. Additional images ultrastructural images of 2dpf and 4dpf control and *Ilg1* deficient cardiomyocytes.** (A) Control and (B) *Ilg1* morphant 2 dpf cardiomyocytes. (C) Wild-type and (D) *Ilg1*<sup>-/-</sup> mutant 4 dpf cardiomyocytes. For all images labeling indicates electron dense intercalated discs (arrows), sarcomeres (asterisks), and regions lacking cell adhesion (hashtags). Scale bars: 500 nm.



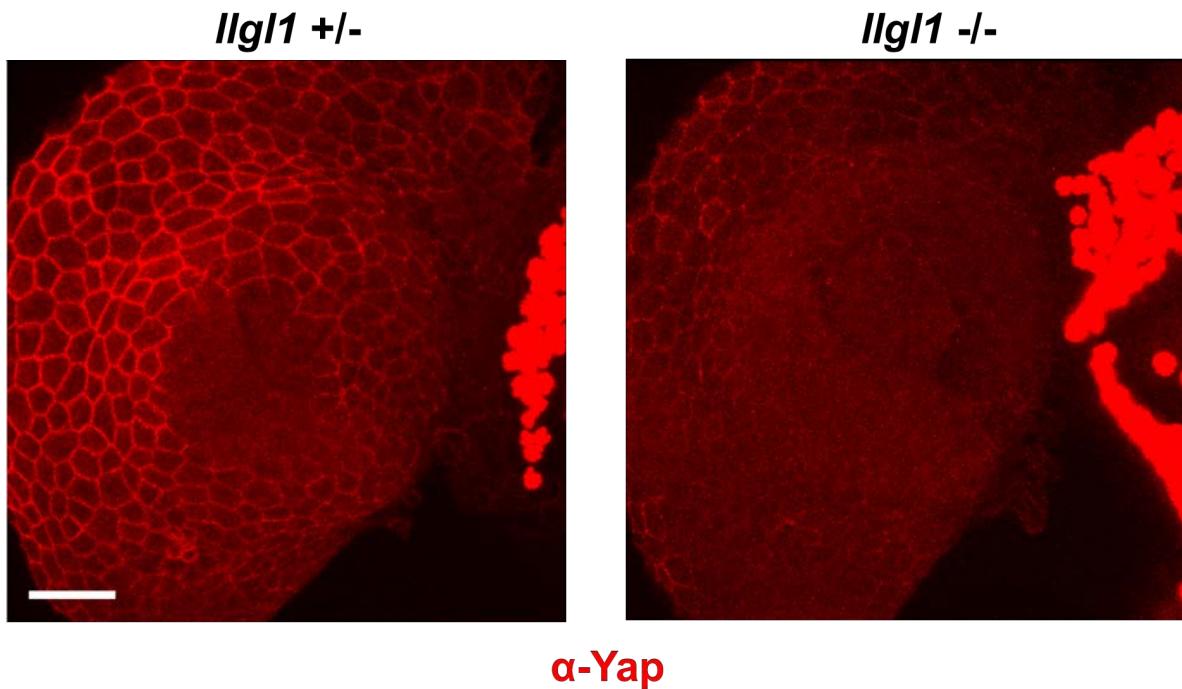
**Fig. S7. Expression of *notch1b* in *Llg1* morphants.** Additional images depicting *in situ* hybridization of *Llg1* morphants and uninjected control 48 hpf embryos. Arrows denote AVC. Scale bar: 100  $\mu$ m.



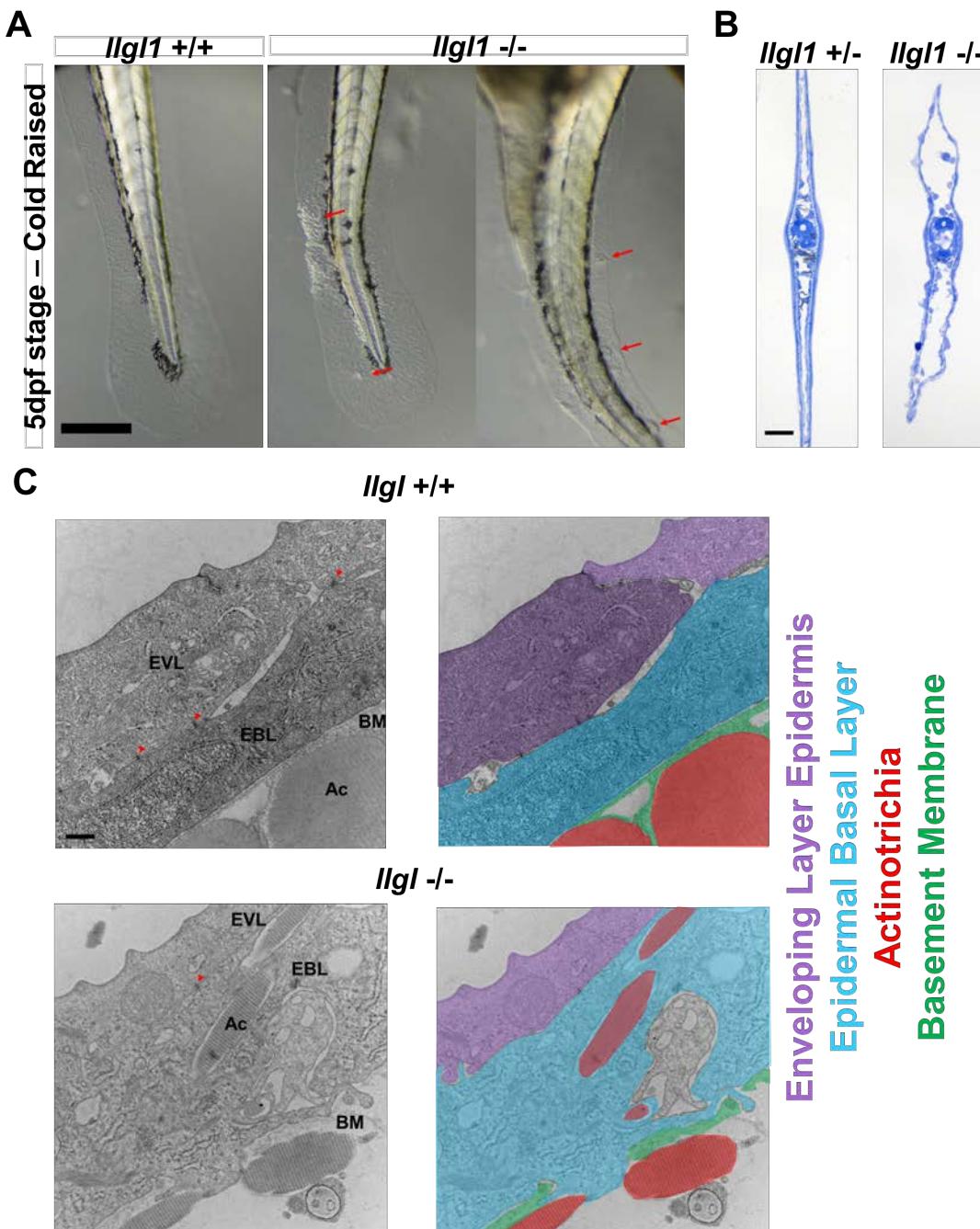
**Fig. S8. Expression of *bmp4* in *Llgl1* morphants.** Additional images depicting *in situ* hybridization of *Llgl1* morphants and uninjected control 48 hpf embryos. Arrows denote AVC. Scale bar: 100  $\mu$ m.



**Fig. S9. Expression of *klf2a* in *llgl1* morphants.** Additional images depicting *in situ* hybridization of *llgl1* morphants and uninjected control 48 hpf embryos. Arrows denote AVC. Scale bar: 100  $\mu$ m.



**Fig. S10. Loss of *Iigl1* results in depletion of Yap from the cell cortex of the epidermis.**  
Representative confocal z-stack images of anti-Yap immunostaining in 2 dpf zebrafish head and eye. Scale bar: 50  $\mu$ m.



**Fig. S11. Loss of *Llgl1* causes epidermal abnormalities in cold raised embryos.** (A) Representative images of zebrafish tail blistering in embryos raised at 20.5°C to the 5 dpf stage. Red arrows denote blistering along the fins in *llgl1*<sup>-/-</sup> embryos. Scale bar: 300 µm (B) Representative images of tail cross sections from 5 dpf staged zebrafish. Light micrographs of epon-embedded sections stained with toluidine blue. Scale bar: 50 µm (C) Representative electron micrographs depicting fin epidermal layers in 5 dpf staged embryos and pseudocolored schematic depicting various cells and structures. Note the lack of hemidesmosome formation in *llgl1*<sup>-/-</sup> mutants (red arrow). Enveloping epidermal layer (EVL), epidermal basal layer (EBL), basement membrane (BM), actinotrichia (Ac). Scale bar: 500 nm.

**Table S1. TALEN Sequence**

Llgl1 TALEN sequence:	TGACCCTCATCGTAAAAAAATCAAGCAGGATCTGTTGCATTCAATAAGGTA
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**Table S2. siRNA reagents**

siRNA Reagent	Target	Source
MISSION® siRNA Universal Negative Control #1 - SIC001-10NMOL	Negative Control	Sigma
MISSION® siRNA Universal Negative Control #2 - SIC002-10NMOL	Negative Control	Sigma
SASI_Rn01_00114054	<i>Yap1</i>	Sigma
SASI_Rn02_00203876	<i>Yap1</i>	Sigma
SASI_Rn01_00081640	<i>Llgl1</i>	Sigma
SASI_Rn02_00268906	<i>Llgl1</i>	Sigma
SASI_Rn02_00254243	<i>Llgl2</i>	Sigma
SASI_Rn02_00254244	<i>Llgl2</i>	Sigma

**Table S3. qRT-PCR primer sequences**

<i>Yap</i>	Forward	ACCATAAGAACAAAGACCACATCC	<i>Rattus norvegicus</i>
<i>Yap</i>	Probe	TGGTCATGGCAAAACGAGGGTCA	<i>Rattus norvegicus</i>
<i>Yap</i>	Reverse	CTTCACTGGAGCACTCTGAG	<i>Rattus norvegicus</i>
<i>Tbp</i>	Forward	Rn01455656_m1 from ThermoFisher	<i>Rattus norvegicus</i>
<i>Tbp</i>	Probe	Rn01455656_m1 from ThermoFisher	<i>Rattus norvegicus</i>
<i>Tbp</i>	Reverse	Rn01455656_m1 from ThermoFisher	<i>Rattus norvegicus</i>
<i>Llgl1</i>	Forward	ACAAGATTCTGTGGCGGAG	<i>Rattus norvegicus</i>
<i>Llgl1</i>	Probe	ACTGTGTGAGTGTACTGCAGGC	<i>Rattus norvegicus</i>
<i>Llgl1</i>	Reverse	ACGGGAGGTGAAGTCTAGG	<i>Rattus norvegicus</i>
<i>Llgl2</i>	Forward	ACAGAGAAAAGATTGAAGCCCG	<i>Rattus norvegicus</i>
<i>Llgl2</i>	Probe	ACAGGTCTCGTCAGAACGCTCTACT	<i>Rattus norvegicus</i>
<i>Llgl2</i>	Reverse	CATATGCAAACACAGCTCCG	<i>Rattus norvegicus</i>
<i>llgl1</i>	Forward	CAAGTGCTTGGCCTATGA	<i>Danio rerio</i>
<i>llgl1</i>	Reverse	CGATCTCCCAAAGGTGAATC	<i>Danio rerio</i>
<i>llgl2</i>	Forward	CTTAACCGCTGTGTCCATAC	<i>Danio rerio</i>
<i>llgl2</i>	Reverse	ACTCAAACGCCCTCTCT	<i>Danio rerio</i>
<i>ctgfa</i>	Forward	CTGCACAGCCAGAGATG	<i>Danio rerio</i>
<i>ctgfa</i>	Reverse	CACTCCCCAGGCACCTT	<i>Danio rerio</i>
<i>cyr61</i>	Forward	CCGTGTCCACATGTACATGGG	<i>Danio rerio</i>
<i>cyr61</i>	Reverse	GGTGCATGAAAGAAGCTCGTC	<i>Danio rerio</i>
<i>clu</i>	Forward	GTCGCAAGTTGGTGAGAAATACC	<i>Danio rerio</i>
<i>clu</i>	Reverse	CTCCTTCATCTCCTGAGCCATC	<i>Danio rerio</i>
<i>fn1b</i>	Forward	CAGTACTGTACAGTCAGGGGAAGC	<i>Danio rerio</i>
<i>fn1b</i>	Reverse	CACGACCGTTGTCATTACAGCC	<i>Danio rerio</i>
<i>hbegfa</i>	Forward	CAAGCAAGGTGCATATAATGTGG	<i>Danio rerio</i>
<i>hbegfa</i>	Reverse	CTGCCAAACAAACACGGTCAC	<i>Danio rerio</i>
<i>lats2</i>	Forward	CTCCGAGAGATCCGCAAGTC	<i>Danio rerio</i>
<i>lats2</i>	Reverse	CACGTACAATCTGTTAGTGTG	<i>Danio rerio</i>
<i>pawrl</i>	Forward	GAACAAAGACCTTGCTGAAAGTG	<i>Danio rerio</i>
<i>pawrl</i>	Reverse	CACTTCCACAATCAAAGCGTCC	<i>Danio rerio</i>
<i>eef1a1l1</i>	Forward	TCTCTCAATCTGAAACTTATCAATCA	<i>Danio rerio</i>
<i>eef1a1l1</i>	Reverse	AACACCCAGGCGTACTTGAA	<i>Danio rerio</i>

**Table S4. Antibody information**

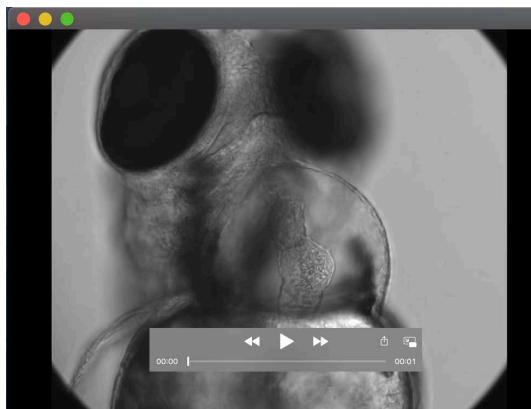
Antibody	Use	Manufacturer	Lot #	Concentration Used
Anti-Yap (4912S)	epidermis IHC and western blotting	Cell Signaling	3	1:400
Anti-Yap (ab81183)	cardiomyocytes IHC	Abcam		1:200
Anti-Tropomyosin (CH1)	cardiomyocyte IHC	Developmental Studies Hybridoma Bank		1:400
Anti-Alcam (zn-8)	cardiomyocyte IHC	Developmental Studies Hybridoma Bank		1:25
Anti- $\gamma$ -Tubulin	western blotting	Sigma-Aldrich		1:400
Alexa Fluor 488 goat anti-mouse	IHC	Life Technologies	1858182	1:200
Alexa Fluor 568 donkey anti-rabbit	IHC	Life Technologies	182664	1:200
IRDye 680RD donkey anti-mouse	western blotting	Licor	C71201-15	1:15000
IRDye 800CW donkey anti-rabbit	western blotting	Licor	C70918-03	1:15000

**Table S5. Adult genotype data from *Ilgl1*<sup>+/-</sup> x *Ilgl1*<sup>-/-</sup> crosses.**

Cross	Age (months)	<i>Ilgl1</i> +/ -	<i>Ilgl1</i> -/-
Cross 1	4	19	11
Cross 2	4	9	1
Cross 3	4	18	12
Cross 4	15	4	1
Cross 5	14	6	2
Cross 6	20	2	4
	Total	58	31



**Movie 1. 78 hpf */lgf1<sup>+-</sup>* zebrafish embryo**



**Movie 2. 78 hpf */lgf1<sup>-</sup>* zebrafish embryo**



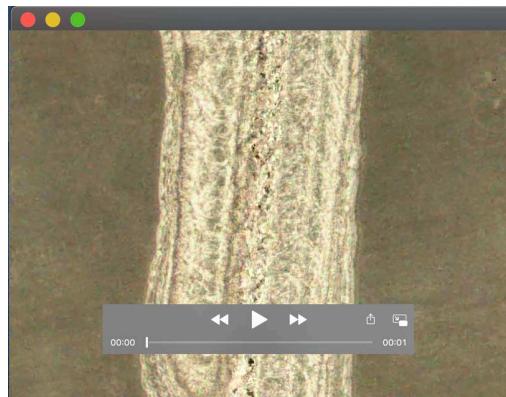
**Movie 3. 99 hpf */lgf1<sup>+-</sup>* zebrafish embryo**



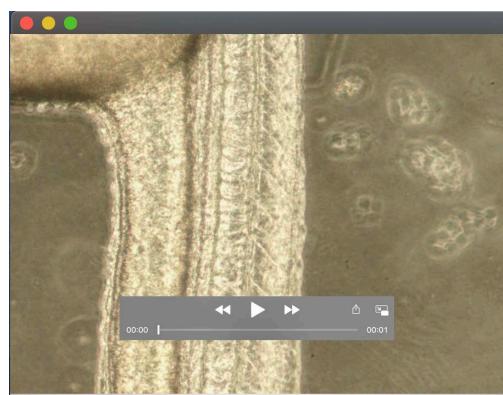
**Movie 4. 99 hpf */lgf1*<sup>-/-</sup> zebrafish embryo with moderate pericardial effusion**



**Movie 5. 99 hpf */lgf1*<sup>-/-</sup> zebrafish embryo with severe pericardial effusion**



**Movie 6. Blood flow in 30 hpf control zebrafish embryo**



**Movie 7. Blood flow in 30 hpf */lgf1* morpholino treated zebrafish embryo**