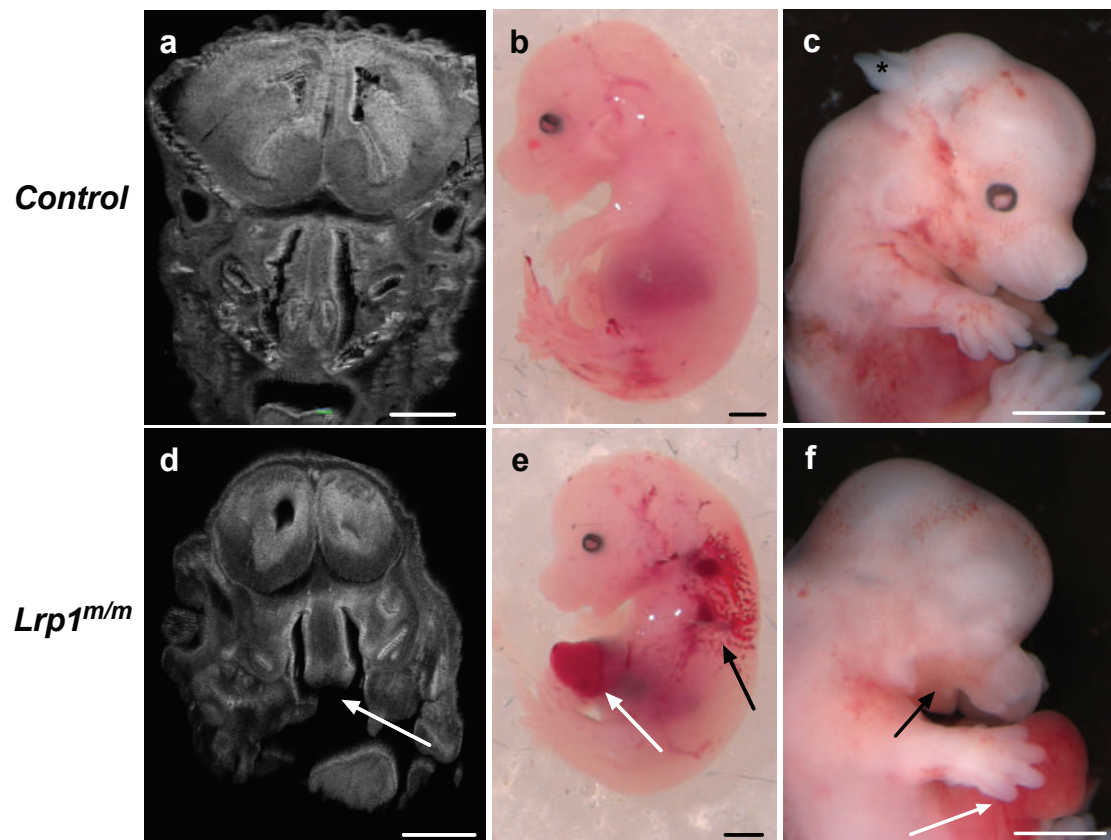


Supplementary Information



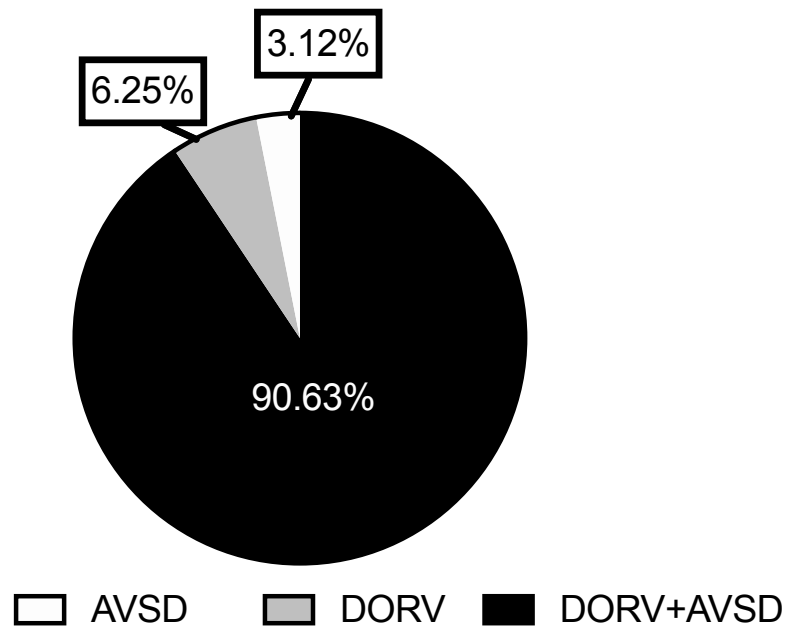
Supplementary Fig 1. Extra-cardiac phenotype of *Lrp1^{m/m}* mutants. Compared with control (a, b c) littermate, *Lrp1^{m/m}* mutant (d, e, f) demonstrated central complete cleft palate (arrow in d), telangiectasia (black arrow in e), gastroschisis (white arrow in e, f) and micrognathia (black arrow in f). Gastroschisis – liver was protruding outside the abdominal cavity through a hole in the abdominal wall. The protruding liver was not covered in a membrane (white arrow in e and f). Scale bars: 0.5mm in a and d; 1mm in b, e, f.

a

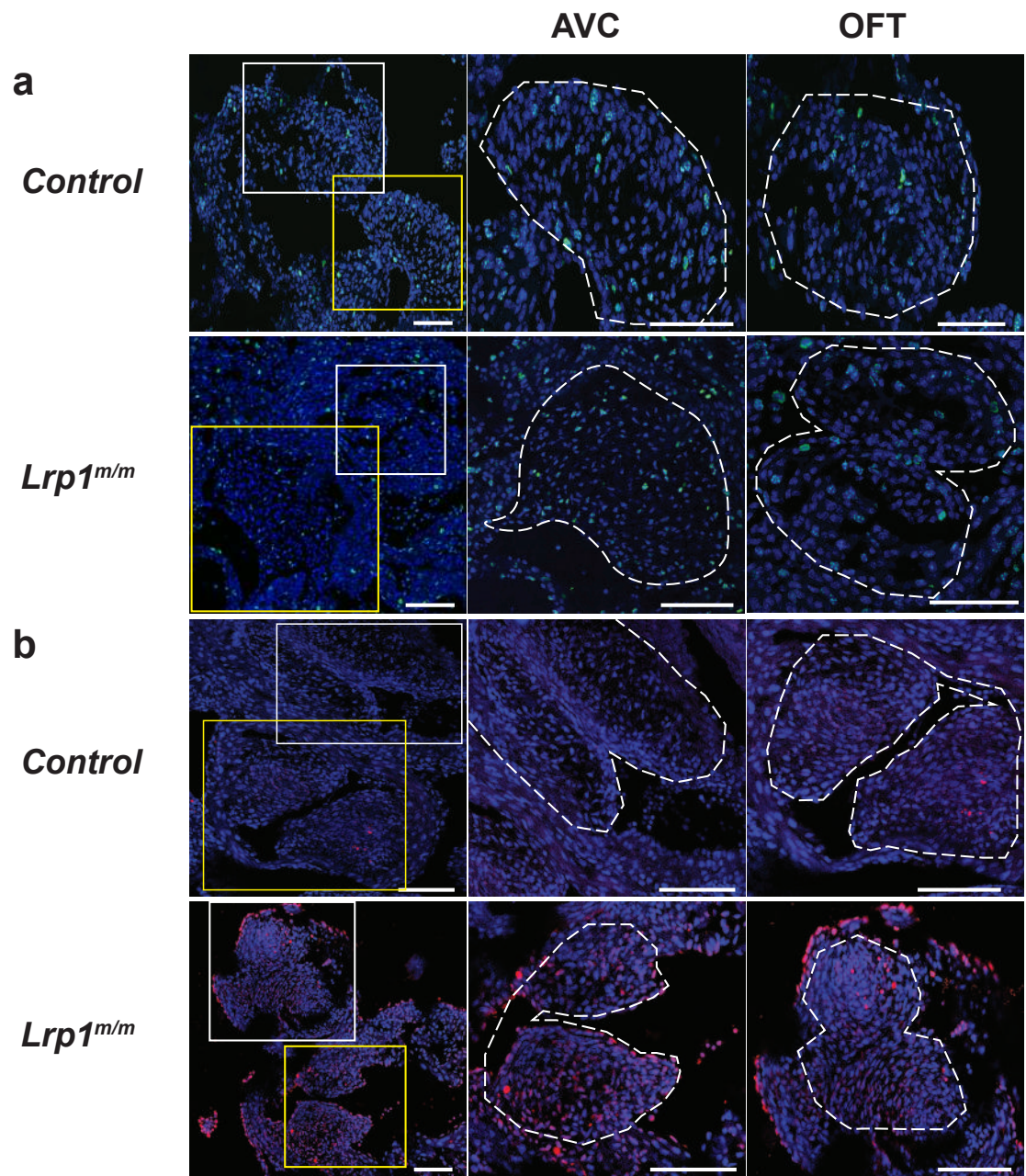
Cardiac Phenotypes of *Lrp1^{m/m}*

| Cardiac phenotypes N=32 | DORV+AVSD 90.63% (N=29) | DORV 6.25% (N=2) | AVSD 3.12% (N=1) |
|----------------------------------|-------------------------------|------------------------|------------------------|
| Unbalanced AVSD (LV dominant) | 34.5% (N=10) | 0 | 100% (N=1) |
| IAA type B | 10.3% (N=3) | 0 | 0 |
| Hypoplastic transverse arch | 6.9% (N=2) | 0 | 0 |
| Right arch | 3.5% (N=1) | 0 | 0 |

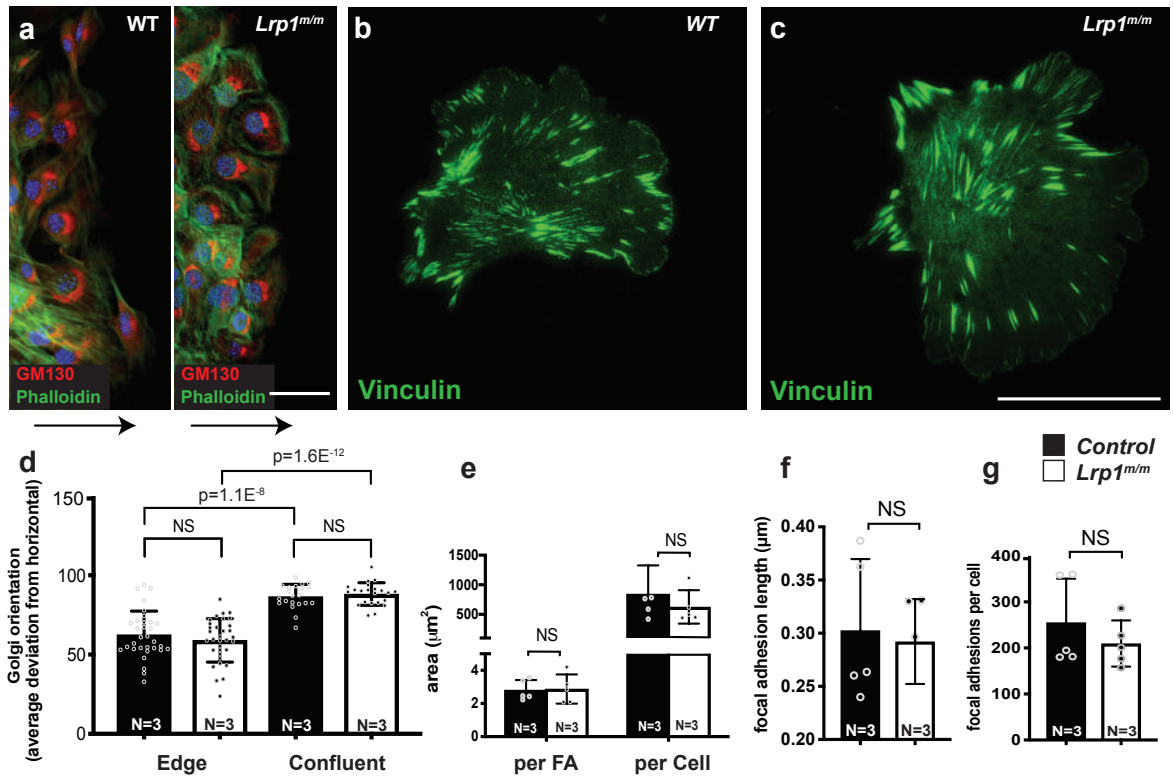
b



Supplementary Fig 2.(a) Table 1 demonstrates the distribution of different cardiac phenotype of *Lrp1^{m/m}* mutants. (b) Pie chart of the distribution of cardiac phenotype of *Lrp1^{m/m}* mutants with the majority of mutants exhibiting DORV and AVSD.



Supplementary Fig 3.(a) Cell proliferation rate was determined using the ratio of positive PH3 cells/total cushion cells in AVC and OFT cushion PH3 staining. (b) TUNEL assay shows apoptotic cells (red) in atrioventricular cushion and outflow tract cushion of E10.5 control (*Lrp1^{+/+}*) and *Lrp1^{m/m}* mutants. The magnified pictures of AVC are from the white box from the right panel and the magnified pictures of OFT are from the yellow box from the right panel. Scale bars: 100 μ m.



Supplementary Fig 4. (a) Analysis of the orientation of the Golgi labeled with anti-Giantin antibody (red) and phalloidin (green). (d) Quantitative measurement of the Golgi orientation demonstrated no difference between the control and *Lrp1^{m/m}* mutant, neither close to the wound edge nor away from the wound edge in confluent areas. (b,c) MEF cells were stained for Vinculin, and images were processed to increase contrast and quantify size and number of focal adhesions. Particles less than $0.45 \mu\text{m}^2$ were excluded from quantification. (e,f, g) Quantitative measurement demonstrated no difference between control and *Lrp1^{m/m}* mutant in the area per focal adhesion, area per cell, length of focal adhesion and number of focal adhesions per cell. Scale bars: $50 \mu\text{m}$.

Supplementary Table 1. 10 homozygous coding mutations were identified from line 1554

| Gene | Chr | Mutation Position | Ref | Alt | Mutation |
|-------------|------------|--------------------------|------------|------------|--------------------|
| Ccdc53 | 10 | 87664630 | A | G | c.A125G:p.N42S |
| Ern2 | 7 | 129321282 | C | T | c.G928A:p.V310M |
| Hsp90aa1 | 12 | 111930377 | T | C | c.A2039G:p.E680G |
| Lrp1 | 10 | 126978069 | A | G | c.T12694C:p.C4232R |
| Mettl9 | 7 | 128195689 | C | A | c.C445A:p.L149I |
| Rfx6 | 10 | 51431681 | T | A | c.T48A:p.N16K |
| Tbc1d12 | 19 | 38957237 | C | T | c.C953T:p.T318I |
| Trhde | 10 | 114004196 | A | G | c.T1577C:p.I526T |
| Trmt5 | 12 | 74383712 | C | A | c.G658T:p.V220F |
| Zfp784 | 7 | 4987997 | C | T | c.G163A:p.V55I |

Supplementary Table 2. PCR primers used to confirm genotypes

| Name | Oligonucleotide Sequences (Forward & Reverse) |
|--|---|
| LRP1 Flox genotype primers | F: CATACCCTCTTCAAACCCCTTCCTG |
| | R: GCAAGCTCTCCTGCTCAGACCTGGA |
| 1554 LRP1 genotype primers | F: AGATGGGGTTGAAGTGGATG |
| | R: CCTTCCCATGCTCTGATGA |
| Universal Cre Recombinase genotype primers | F: GGACATGTT CAGGGATCGCCAGGCG |
| | R: GCATAACCAGTGAAACAGCATTGCTG |
| Nkx2.5 Cre specific genotype primers | Wildtype F: GAGCCTGGTAGGGAAAGAGC |
| | Mutant F: TTACGGCGCTAAGGATGACT |
| | R: GTGTGGAATCCGTCGAAAGT |
| Tie2 Cre specific genotype primers | F: CCCTGTGCTCAGACAGAAATGAGA |
| | R: CGCATAACCAGTGAAACAGCATTGC |

Supplementary Table 3. Primary Antibodies

| Primary Antibody | Type | Dilution | Manufacturer | Catalog Number |
|---------------------------|------------------------|----------|--------------------------------------|----------------|
| Anti-AP-2 α | Mouse IgG2b Monoclonal | 1:50 | Developmental Studies Hybridoma Bank | 3B5 |
| Anti-CD31 | Rat IgG2a Monoclonal | 1:250 | BD Biosciences | 557355 |
| Anti-Clathrin Heavy Chain | Mouse IgG1 Monoclonal | 1:250 | Sigma Aldrich | C1860 |
| Anti-EEA1 | Mouse IgG2a Monoclonal | 1:250 | Sigma Aldrich | E7659 |
| Anti-GM130 | Mouse IgG1 Monoclonal | 1:250 | BD Transduction Laboratories | 51-9001978 |
| Anti-Islet1 | Mouse IgG1 Monoclonal | 1:25 | Developmental Studies Hybridoma Bank | 40.3A4 |
| Anti-KDEL | Rat Monoclonal | 1:250 | Abcam | ab50601 |
| Anti-Ki67 | Rabbit Polyclonal | 1:500 | Abcam | ab15580 |
| Anti-LRP1 | Rabbit Monoclonal | 1:500 | Abcam | ab92544 |
| Anti-NFATc | Mouse IgG1 Monoclonal | 1:50 | Developmental Studies Hybridoma Bank | 7A6 |
| Anti-Periostin | Rabbit Polyclonal | 1:250 | Abcam | ab14041 |
| Anti-Phospho-Histone H3 | Mouse Monoclonal | 1:500 | Abcam | ab14955 |
| Anti-Vinculin | Mouse IgG1 Monoclonal | 1:400 | Sigma | V9131 |
| DAPI | | 1:100 | Thermo Scientific | 62248 |

Supplementary Table 4. Secondary Antibodies

| Secondary Antibody | Type | Dilution | Manufacturer | Catalog Number |
|------------------------------------|----------|----------|-------------------|----------------|
| Donkey anti-mouse Alexa Flour 488 | IgG(H+L) | 1:1000 | Invitrogen | A-21202 |
| Goat anti-mouse Alexa Flour 647 | IgG2a | 1:1000 | Invitrogen | A-21241 |
| Donkey anti-rabbit Alexa Flour 555 | Rabbit | 1:1000 | Invitrogen | A-31572 |
| Goat anti-Mouse Alexa Flour 647 | IgG1 | 1:1000 | Fisher Scientific | A-21141 |
| Goat anti-Mouse Alexa Flour 488 | IgG2b | 1:1000 | Fisher Scientific | A-21141 |
| Donkey anti-Rat Alexa Flour 488 | IgG(H+L) | 1:1000 | Fisher Scientific | A-21208 |