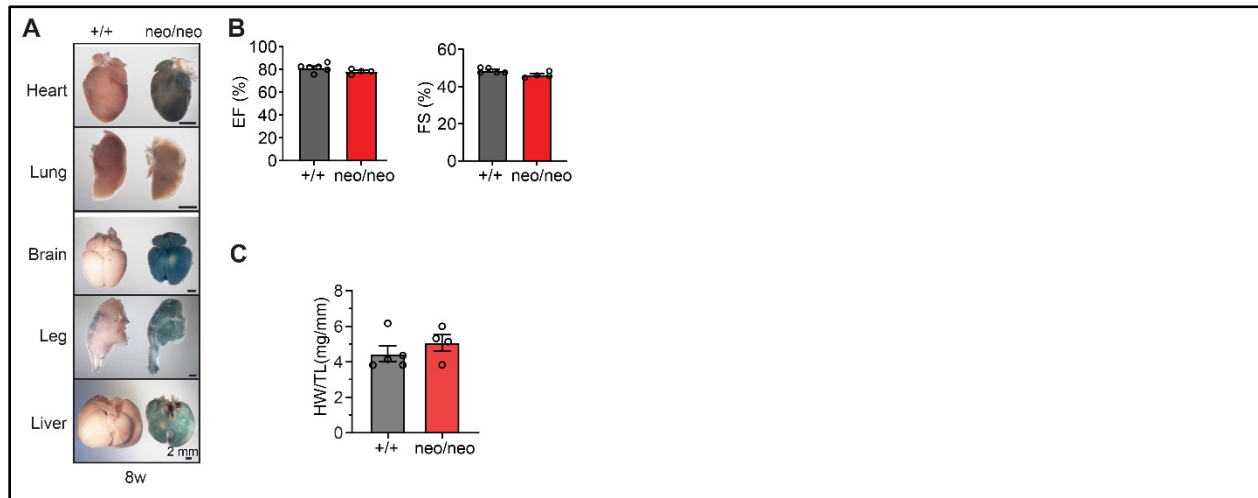


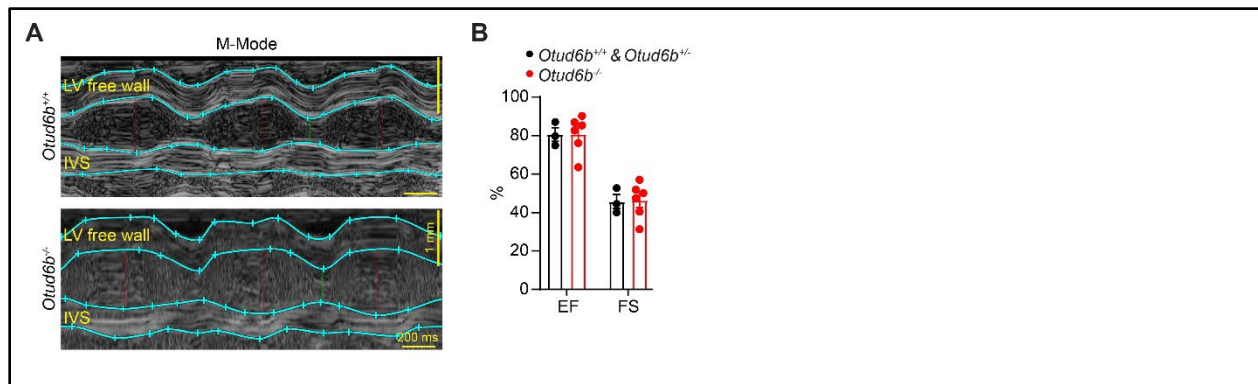
Supplementary Materials

Supplementary Figure 1. Characterization of *Otud6b*^{neo/neo} mouse model.



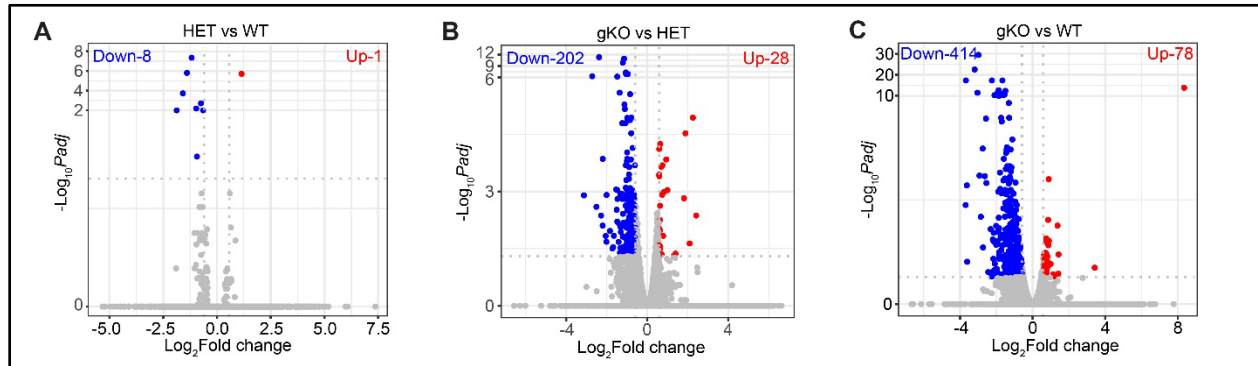
A. X-Gal staining of hearts, lungs, brains, legs and livers isolated from wild-type and *Otud6b*^{neo/neo} mouse at 8 weeks of age. **B.** Quantification of EF and FS for wild-type and *Otud6b*^{neo/neo} mouse at 24 weeks of age. **C.** Heart weight to tibial length (HW/TL). +/+ : n=5, neo/neo: n=4.

Supplementary Figure 2. Cardiac function of *Otud6b* KO embryos



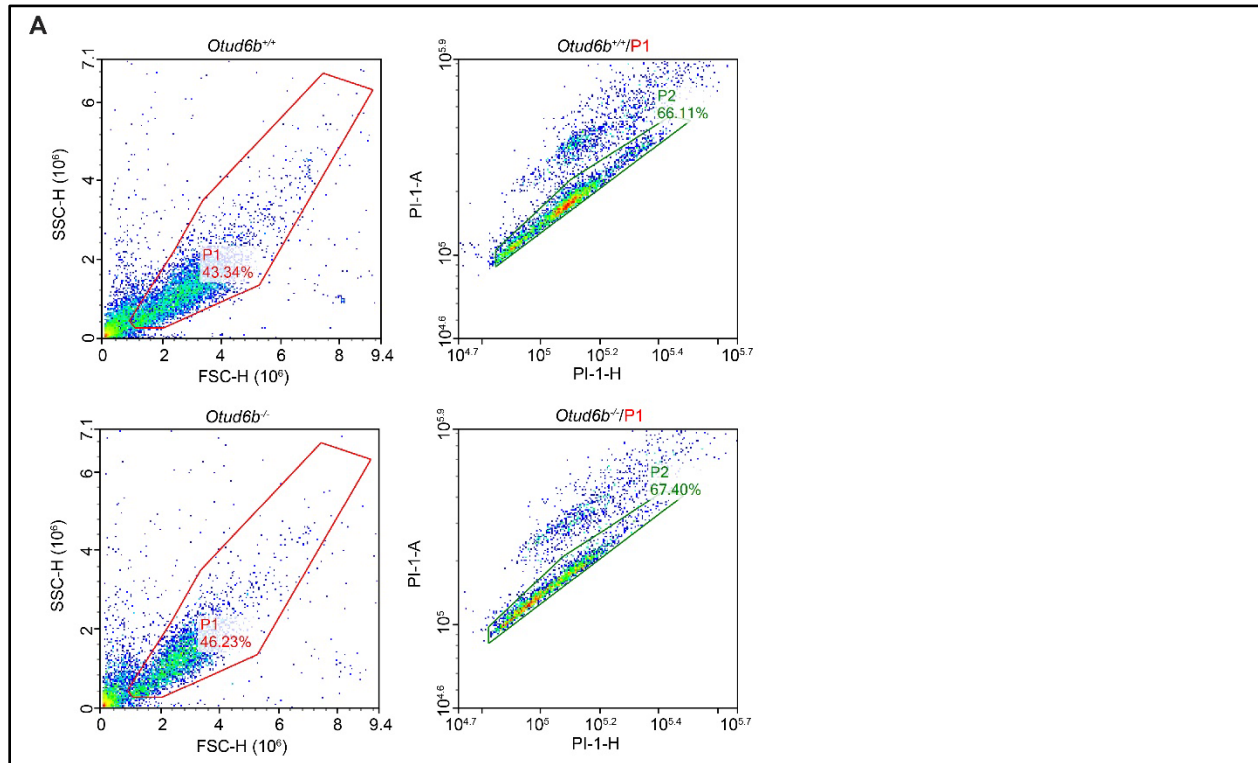
Representative M-mode images (**A**) and quantification (**B**) of echocardiograms at E19.5 embryos.

Supplementary Figure 3. Transcriptome analysis for *Otud6b* KO.



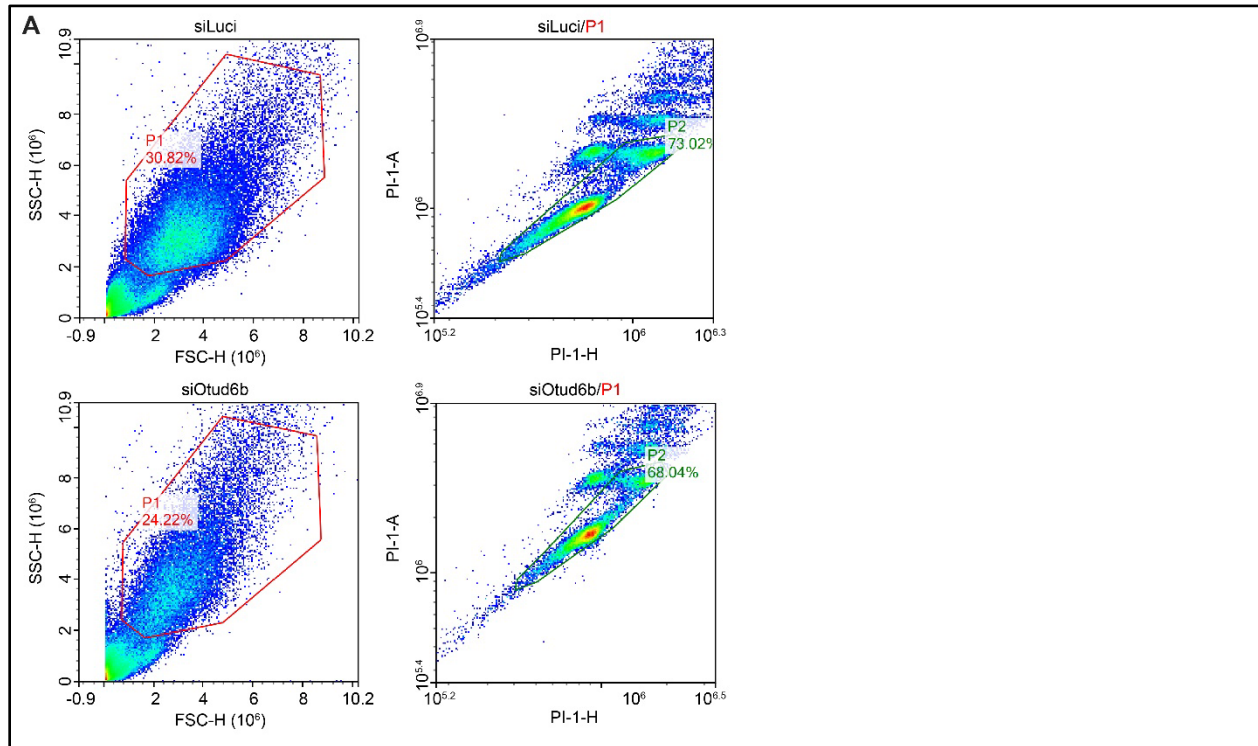
Volcano plot of differentially expressed genes in *Otud6b*^{+/-} compared with *Otud6b*^{+/+} (A), *Otud6b*^{-/-} compared with *Otud6b*^{+/-} (B), *Otud6b*^{-/-} compared with *Otud6b*^{+/+} (C).

Supplementary Figure 4. Representative flow cytometry plots of embryonic cardiomyocytes.



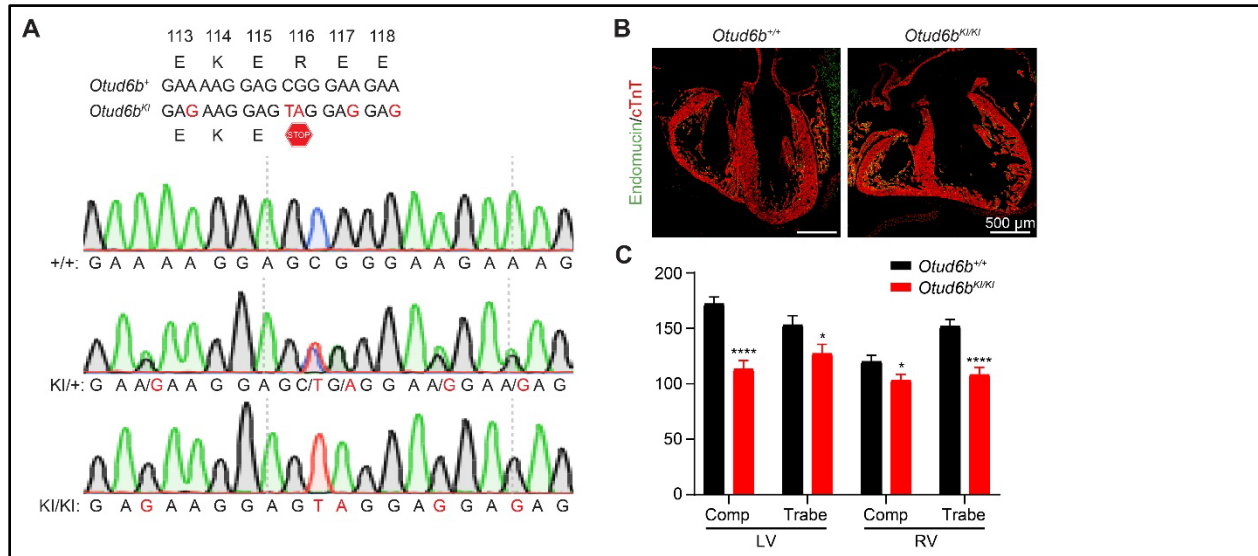
A. Representative plots of flow cytometry for cell cycle assay in embryonic cardiomyocytes.

Supplementary Figure 5. Representative flow cytometry plots of neonatal rat ventricular cardiomyocytes (NRVCs).



A. Representative plots of flow cytometry for cell cycle assay in NRVCs.

Supplementary Figure 6. Characterization of *Otud6b*^{R116*} mouse model.



A. Sanger sequencing results for *Otud6B*^{+/+}, *Otud6B*^{KI/+} and *Otud6B*^{KI/KI} embryos. **B.** Heart sections stained with Endomucin (green), cTnT (red) in E18.5 embryos. **C.** Quantification of compact layer and trabecular layer. Data are presented as mean $\pm$ SEM. Unpaired t-test was performed in C. * $P < 0.05$, **** $P < 0.0001$.

Major Resources Table

Primer

Otud6b_5P_F (Genotyping)	GATTCACCTCTCTGCCTCCC
Otud6b_5P_R (Genotyping)	TGCTATGTTCCCAGCACCTC
LacZ_2_F (Genotyping)	ATCACGACGCGCTGTATC
LacZ_2_R (Genotyping)	ACATCGGGCAAATAATATCG
Otud6b-F (qPCR)	GAGAATCAACCACCTCGGATTT
Otud6b-R (qPCR)	CAGCCTCTGCTATCCTTTCTTC
Ms-Hprt-F (qPCR)	GGCCAGACTTTGTTGGATTG
Ms-Hprt-R (qPCR)	CGCTCATCTTAGGCTTTGTATTG

Antibody

OTUD6B	ABCLONAL A14511
p53	Cell signaling 32532
GAPDH	Cell signaling 5174
Endomucin	Santa Cruz sc-65495
Troponin T	DSHB CT3
Ki67	Abcam ab15580
Aurora A	Cell signaling 14475
Aurora B	Cell signaling 3094
Cyclin D1	Cell signaling 2978
Cyclin D2	Cell signaling 3741
Cyclin D3	Cell signaling 2936
Cyclin E1	Cell signaling 20808
Cyclin A2	Cell signaling 4656
Cyclin B1	Cell signaling 12231
CDK1	Cell signaling 28439
CDK2	Cell signaling 2546