

SUPPLEMENTARY INFORMATION FOR:

The embryological basis of subclinical hypertrophic cardiomyopathy

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SUPPLEMENTARY NOTES

Note S1. Right ventricular wall thickness and size in wildtype, HET KO and HO KO

Right ventricular (RV) free wall thickness at the 9 o'clock position was measured using linear caliper tools in OsiriX at three levels: basal, mid and apical. A total of 56 mice at E18.5 and P0 (wildtype, n = 12; HET KO n = 16, HO KO n = 28, all on a C57BL/6J background) were included in this analysis. Analysis showed that the RV basal free wall was thinner in HO KO compared to wildtype at P0 ($p = 0.037$) but other measurements were similar between groups.

SUPPLEMENTARY TABLES

Table S1. Summary of human embryo, fetal and neonatal imaging datasets.

No.	Developmental Stage	Imaging Modality	Image Resolution (μm)	Crypt (Y/N)	Data Source
1	CS20	HREM	2	0	HBDR
2	CS21	OPT ¹	20	1	HBDR
3	9 pcw	MRI	70	1	HBDR
4	19 pcw	Micro-CT	3	0	ICH UCL
5	Neonatal	MRI	340	0	ICL UCL

CS = Carnegie stage, CT = computerised tomography, HBDR = Human Developmental Biology Resource, HREM = high-resolution episcopic microscopy, ICH UCL = Institute of Child Health University College London, MRI = magnetic resonance imaging, N = neonatal, OPT = optical projection tomography, pcw = post-coital week.

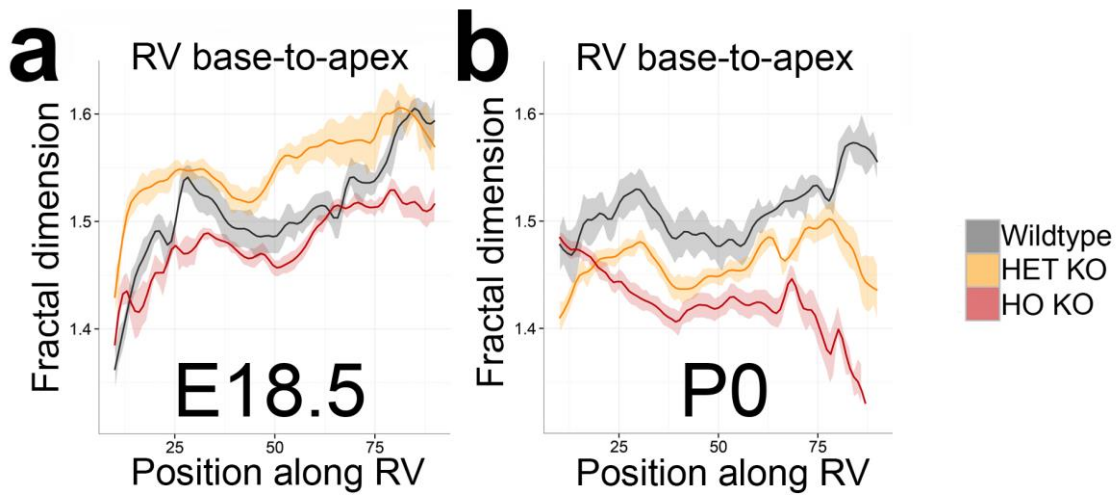
Table S2. Distribution of wildtype, HO/HET *Mybpc3*-targeted KO mice per littermate.

Littermate #	Wildtype E18.5	Wildtype P0	HET KO E18.5	HET KO P0	HO KO E18.5	HO KO P0
1	8					
2		4				
3			7			
4				9		
5					8	
6					10	
7						10
Total	8	4	7	9	18	10

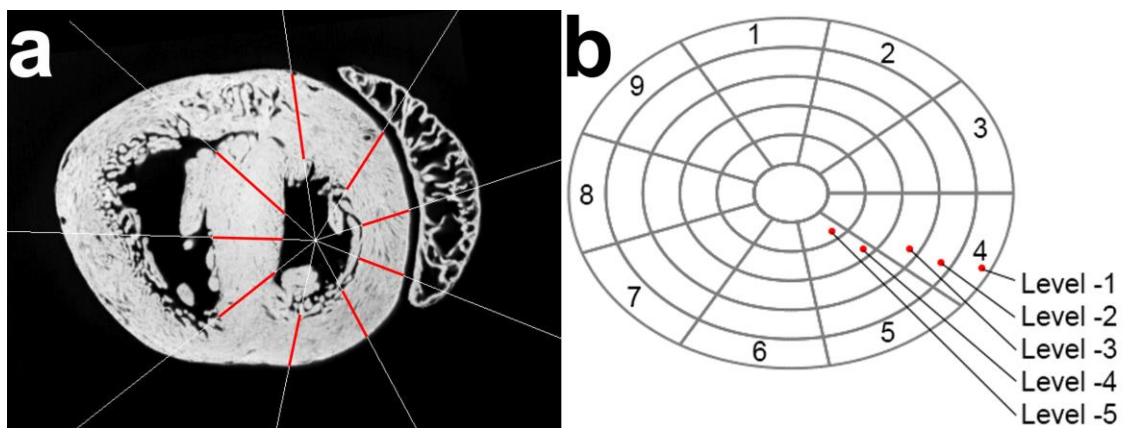
KO = knock-out, HCM = hypertrophic cardiomyopathy, HO = homozygous, HET = heterozygous.

SUPPLEMENTARY FIGURES

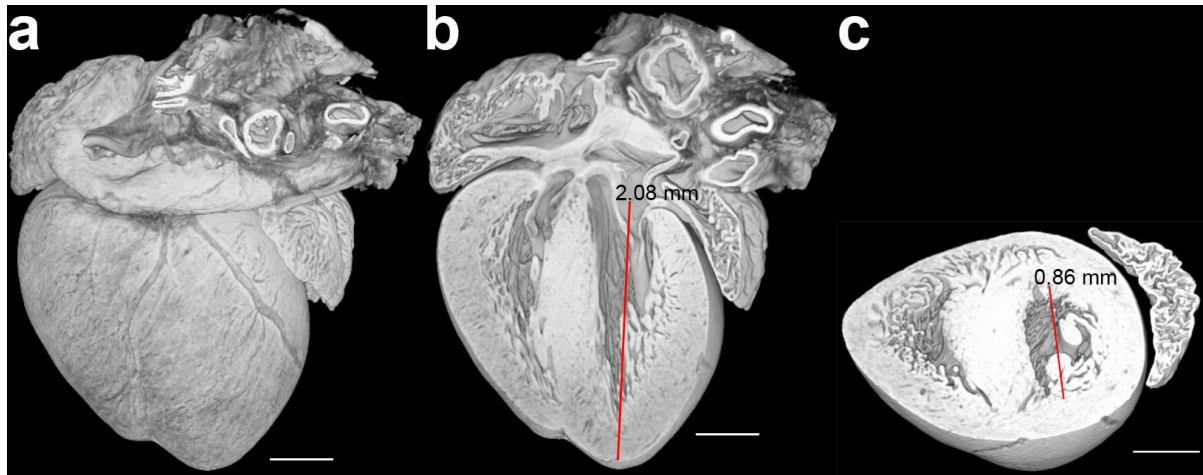
Supplementary Figure 1 Compaction data for the right ventricle in wildtype, HET KO and HO KO. Solid lines represent group means. Ribbons represent upper and lower 95% confidence limits. Trabeculation in the right ventricle (RV) was measured using fractal analysis² in wildtype, HET KO and HO KO mice (n = 56 at E18.5 [a] and P0 [b]; wildtype, n = 12; HET n = 16, HO n = 28). We show how at E18.5 (a) the RV of HET KO is more noncompacted compared to both wildtype and HO KO ($p < 0.0001$ both, by ANCOVA). By contrast the RV of HO KO was less noncompacted than wildtype and HET KO across both stages ($p < 0.0001$ all).



Supplementary Figure 2 Semi-automated method for wall thickness measurement of HREM data. (a) From the user-defined cardiac centroid 9 radial spokes are emanated at 40° intervals, transecting the LV walls from which linear caliper measurements are then extracted. Trabeculae were manually excluded from the wall thickness measurement. Where the moderator band or right ventricular septomarginal trabeculum were recognized, these were excluded from septal wall thickness measurements in line with established guidance.³ (b) Radial grids were applied at 5 levels from LV base to apex generating a total of 45 myocardial segments (9 per level) that were then rendered into bullseye plots using MATLAB®. Segments 1, 6, 7, 8 and 9 were counted as ‘septal’ and segments 2, 3, 4 and 5 as ‘lateral’ for each of the 5 ventricular levels

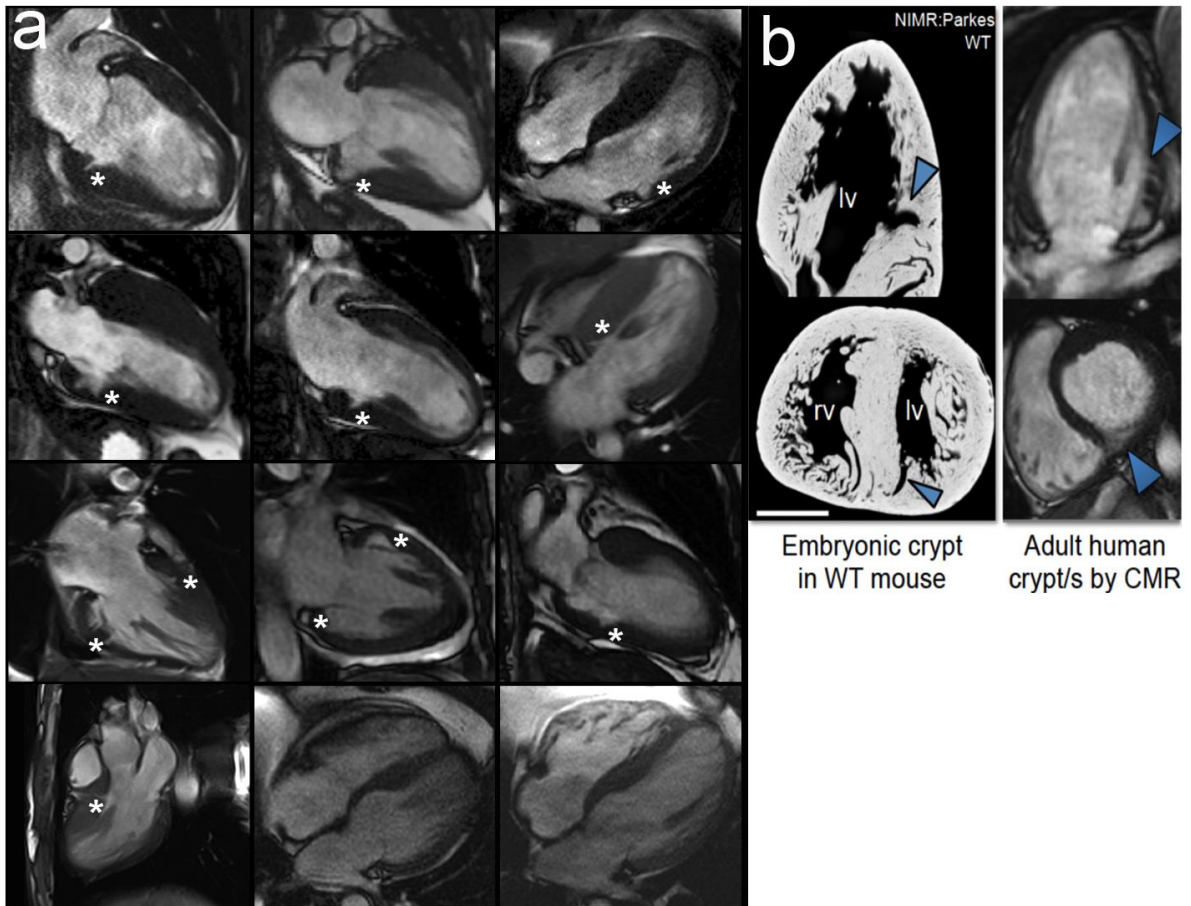


Supplementary Figure 3 Example measurements of left ventricular length and anteroposterior luminal dimensions. Example images from a wildtype mouse at E18.5. **(a)** Whole heart volume-rendered 3D reconstruction in OsiriX. **(b)** Eroded coronal image showing caliper measurement of LV length from the mitral annular plane to the epicardial surface of the LV apex. **(c)** Eroded short axis view showing caliper measurement of LV mid ventricular anteroposterior internal luminal diameter at the level of the papillary muscles. Scale bars, 0.5 mm.



Supplementary Figure 4 Crypts in adult human hearts.

(a) Cardiovascular magnetic resonance (CMR) cines pertaining to twelve adult patients with HCM caused by a truncating *MYBPC3* mutation (c.772G>A transition, NM_000256.3). Ten patients exhibited at least one crypt (*); two patients had no crypts (bottom right). The CMR protocol was approved by the institutional review boards of the respective US and EU centers. Written informed consent was obtained from all participants according to the ethics committee guidelines at the respective academic centers. All 12 patients pertaining to 10 unrelated families had consented to blood sample collection at initial evaluation and genomic DNA had been isolated from peripheral blood lymphocytes, using standard methodology. CMR was performed using 1.5 and 3-Tesla (T) magnets from different manufacturers. (b) A basal inferior crypt as seen in a long and short-axis left ventricular HREM view in wildtype (WT) mouse at E18.5 that is strikingly similar to the basal inferior crypt in a healthy adult human heart imaged by CMR. Scale bar, 0.5mm. NIMR = National Institute of Medical Research.



SUPPLEMENTARY VIDEOS

Video S1.

(a) Volumetric model of left ventricular lumen derived from HREM data of a HO KO embryonic heart (C57BL/6J background) at P0. Initially viewing each free wall side, the digital cast rotates to show the septal wall surface and also the multiple deep myocardial crypts that appear as finger-like projections (red arrows). (b) Virtual HREM image stack of the same heart, digitally eroding from base to apex, revealing the same deep myocardial crypts as in (a) but in the short-axis view.

Video S2.

Black and white 2D movie of a raw HREM stack, from base to apex, showing a different HO KO mouse heart at P0. Red arrows point to the location of three deep myocardial crypts.

Video S3.

Black and white 2D movie of a raw HREM stack, from base to apex, showing a wildtype mouse heart at P0 lacking deep myocardial crypts.

REFERENCES FOR SUPPLEMENTARY MATERIAL

1. Kerwin, J. *et al.* 3 dimensional modelling of early human brain development using optical projection tomography. *BMC Neurosci.* **5**, 27 (2004).
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3. Ho, S. Y. & Nihoyannopoulos, P. Anatomy, echocardiography, and normal right ventricular dimensions. *Heart* **92 Suppl 1**, i2–13 (2006).