

## **Supporting Information**

### **Human cardiac myosin light chain 4 (MYL4) mosaic expression patterns vary by sex**

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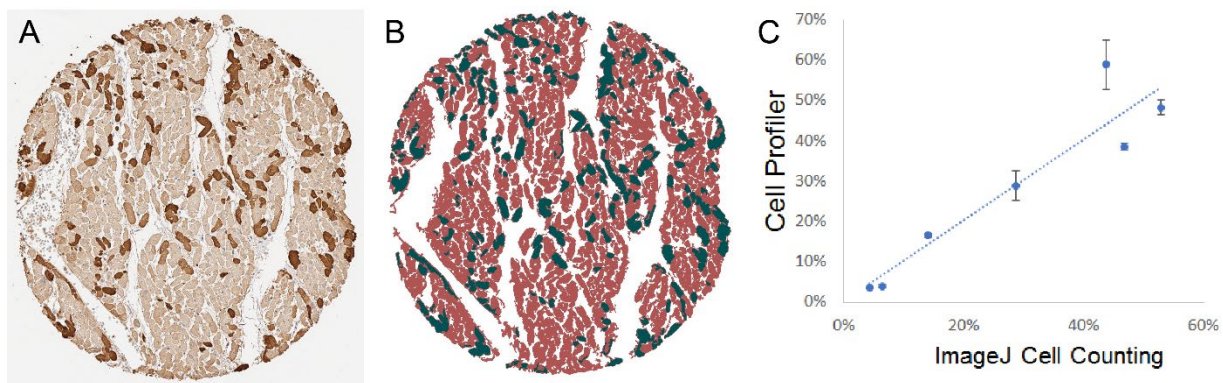
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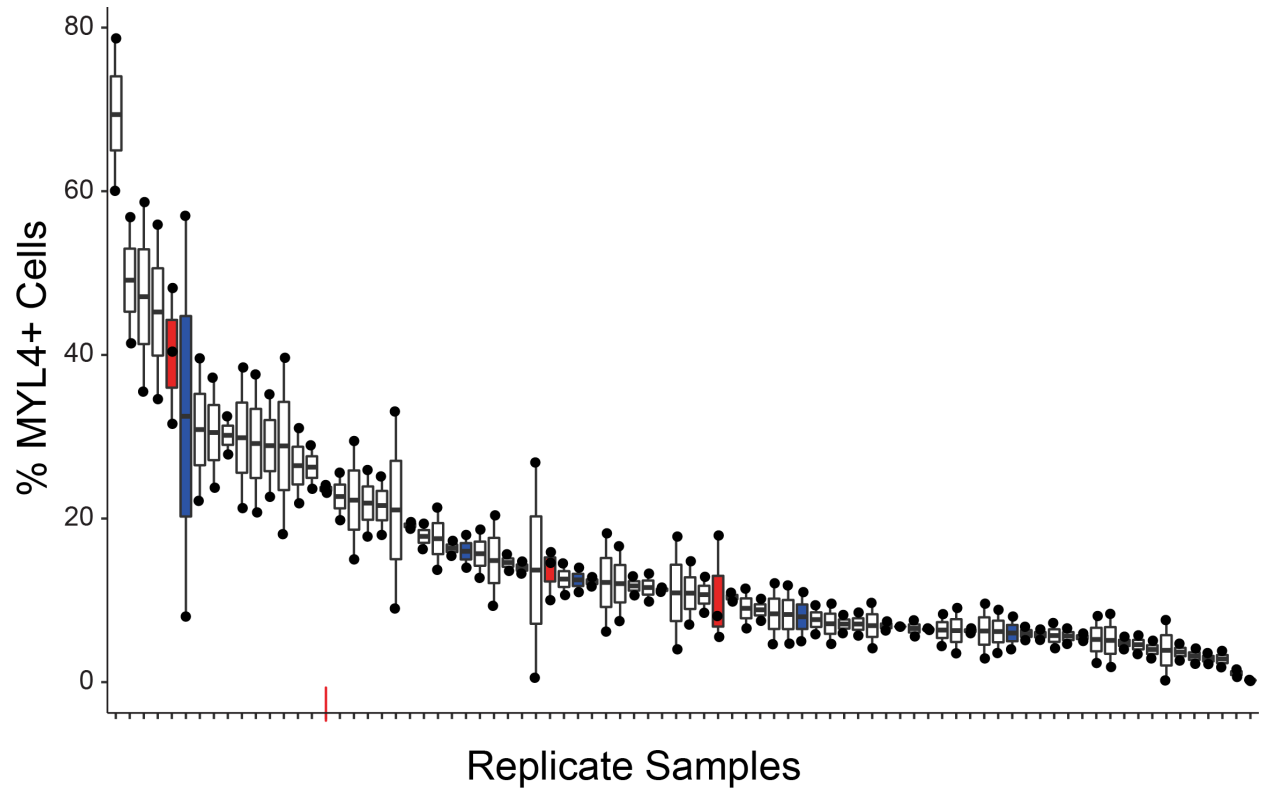
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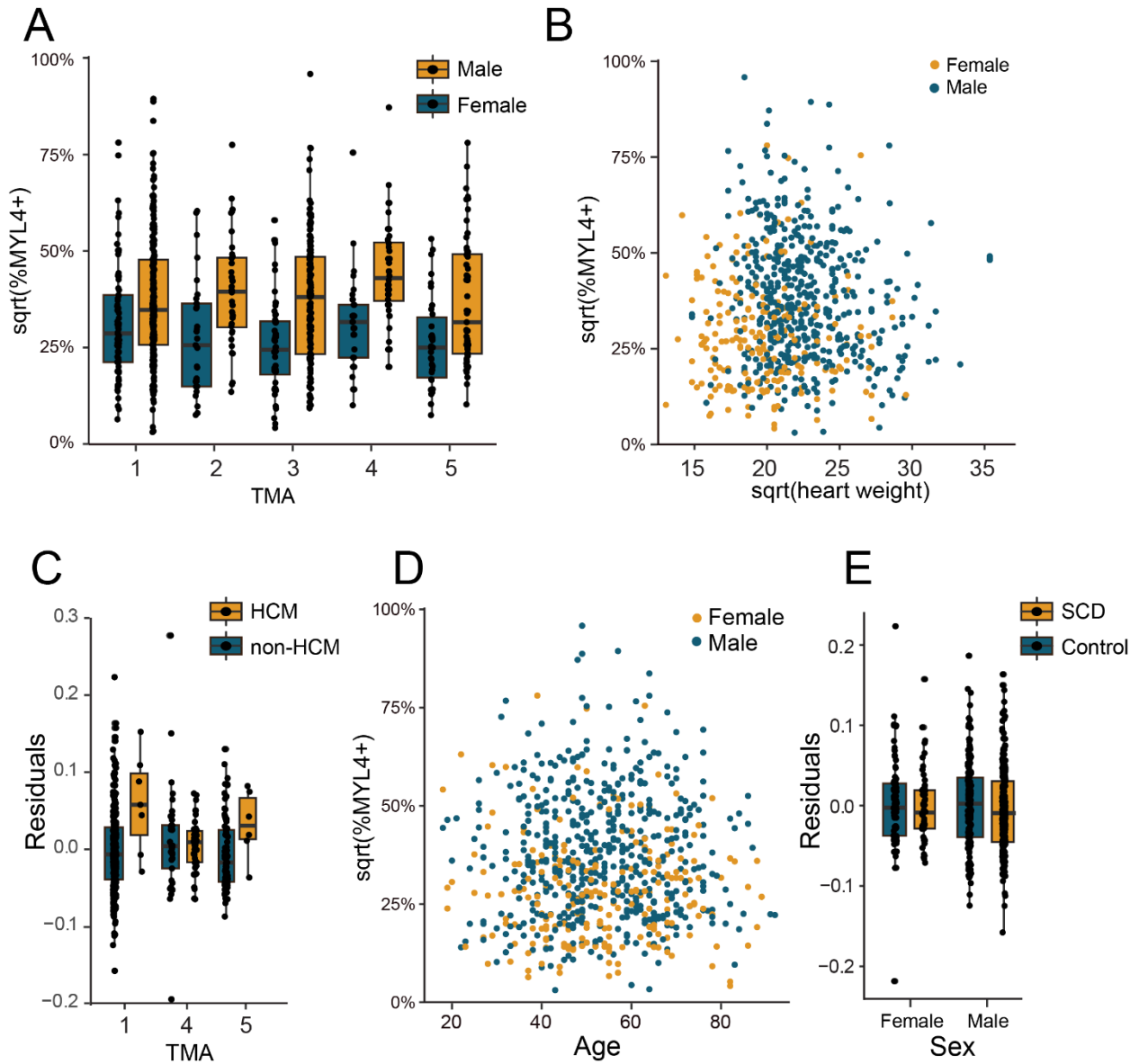
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**Figure S1: Myocyte categorization using CellProfiler and ImageJ.** A) A typical cardiac core showing MYL4+ and MYL4- cells. B) A CellProfiler mask was generated to identify all cardiomyocytes (brown) and positive staining myocytes (green) at different intensity thresholds. An optimal threshold was selected in an operator-dependent fashion. C) A correlation of semi-automated CellProfiler and manual ImageJ Cell Counting methods accurately correlated ( $r=0.94$ ) with each other on the percent of MYL4+ cells to percent of brown pigmented area.



**Figure S2. Reproducibility of MYL4 percent positivity by subject.** Blue bars are septal – left ventricular comparisons and red bars are triplicate samples. The red line on the x axis indicates one of the triplicate samples.



**Figure S3. MYL4 correlations by sex, heart weight, HCM status, age and SCD status. A)** In each TMA, male MYL4 positivity was increased independently. **B)** There was a significant increase in MYL4 positivity with heart weight ( $p= 4.52e-03$ ). **C)** Across all samples, HCM MYL4 positivity was increased over all non-HCM samples, but not significantly for septal tissues on TMA 4. **D)** The percent of MYL4+ cells was not related to subject age, when age >20 years. **E)** There was no overall correlation between MYL4 positivity and SCD in men or women.

	<b>Estimate</b>	<b>Std. Error</b>	<b>t. value</b>	<b>p.z</b>
(Intercept)	0.30	0.21	14.28	0
Age	-0.00045	0.00036	-1.23	0.29
Sex	0.096	0.013	7.69	1.51e-14
HCM	0.074	0.021	3.49	4.85e-04
IHD	0.036	0.027	1.31	0.19
DCM	0.011	0.025	0.44	0.66

**Table S1.** Linear mixed model evaluating TMAs 1-5 for age, sex, HCM, IHD and DCM.