Figure 1. Transcriptomic analysis of hiPSC-CMs identified altered genes within the HCM pathway. **(i)** Genes with transcripts of increased expression are shown in green. **(ii)** Genes with transcripts of decreased expression are shown in red.

Figure 2. Transcriptomic analysis of HCM hiPSC-CM EVs identified differential cellular expression between WT and HCM hiPSC-CM. A) Volcano plot analysis of the differential expression of total RNAs between WT and HCM hiPSC-CM EVs. A comparison of fold change (log2) and significance (-log10 p-value). RNAs significantly increased in abundance in HCM hiPSC-CM EVs (red) and in WT hiPSC-CM EVs (blue). Significance is determined as adj-p<0.05. **B)** Biological process enrichment analysis conducted using Panther to determine the number of genes per function pathway present in EVS produced from either the WT and HCM hiPSC-CMs. **C)** Detailed analysis of the catalyase activity pathways and **D)** Detailed analysis of the binding pathways accounting for the number of mRNAs found in WT and HCM hiPSC-CM EVs per pathway. Specific genes are their functions are detailed in the supplementary tables.

Figure 3. Comparison of RNAs enriched in EVs compared to their parental cell lines identified both mutation dependent and independent changes. A) Of the mRNAs enriched in EVs, 34.9% are independent of the disease mutation, whilst 65.1% are mutation dependent. **B)** Volcano plot analysis of the enriched exosome small RNA and **C)** enriched exosome total mRNA of fold change (log2) and significance (-log10 p-value). RNAs significantly increased in abundance in cells compared to EVs (red) and EVs compared to cells (blue). **(i)** Results of comparing WT hiPSC-CMs cells to WT hiPSC-CMs EVs. **(ii)** Comparison of HCM hiPSC-CMs cells to HCM hiPSC-CMs EVs. Significance is determined as adj-p<0.05. Data limited to the top 5000 hits demonstrating greatest difference in expression. **D)** Pathway enrichment analysis of the mRNAs found to be unique to either WT hiPSC-CMs EVs or HCM hiPSC-CMs EVs.

Figure 4. Altered cellular gene expression detected as a result of the presence of disease mutation and/or pacing as the stimulus. A) Breakdown of comparisons of transcripts with altered abundance detected in hiPSC-CMs as a result of the presence of the disease mutation and/or pacing stimulus. **B)** Summary of the total number of transcripts with altered abundance as a result of either the presence of the mutation and/or pacing stimulus. **C)** Biological processes regulated by changes in transcript expression mediated by pacing stimulus alone. **D)** Biological processes regulated by changes in transcript expression altered during pacing stimulus by the presence of the disease mutation.

Figure 5. Altered EVs release and small RNA cargo detected as a result of the presence of disease mutation and/or pacing as the stimulus. A) Quantification of EV release following pacing frequency stimulus. (B) Quantification of EV size following pacing frequency stimulus **C)** Small RNA with increased abundance (>2fc) in HCM hiPSC-CM EVs compared to WT hiPSC-CM when stimulated to pace. **D)** Small RNA with increased abundance (>2fc) in HCM hiPSC-CM EVs when paced compared to HCM hiPSC-CM EVs when unstimulated.

(a-b) One-way ANOVA performed with correction (Turkey). * p≤0.05, ** p≤ 0.01,*** p≤0.001 , **** p≤0.0001. (n=3 biological replicates) per assay (c-d) Bonferroni correction

Supplementary Figure 1. Characterisation of hiPSC-CMs confirms HCM phenotype. **(a)** Phenotypic analysis of HCM hiPSC-CMs compared to their isogenic WT hiPSC-CM control. **(b)** Transcriptomic analysis of hiPSC-CMs showing the GO biological processes regulated by the presence of the HCM disease mutation.

Supplementary Figure 2. Characterisation of HCM and WT hiPSC-CM EVs confirms identity EV identity and differences in size distribution. (A) Expression of surface EV marker CD9, cytosolic EV marker Alix, and non-EV Golgi structural protein GM130 in EV samples isolated from both HCM and WT hiPSCs, compared to a total cell lysate control. (B) Representative transmission electron microscopy images of EVs from HCM and WT hiPSC-CM EVs. Black arrows indicate examples of EVs in each sample. (C) Histogram representation of the size distribution of EVs isolated from each sample type (n=3 samples).

Supplementary Figure 3. List of mRNAs enriched in HCM hiPSC - CM EVs that are involved in the pathway 'catalytic activity on proteins'.

Supplementary Figure 4. List of mRNAs decreased in HCM hiPSC-CM EVs involved in the pathway 'protein binding'.

Supplementary Figure 5. List of the small RNAs with increased abundance (>2fc) in HCM hiPSC-CM EVs compared to WT hiPSC-CM EVs.