Dynamic but discordant alterations in zDHHC5 expression and palmitoylation of its substrates in cardiac pathologies

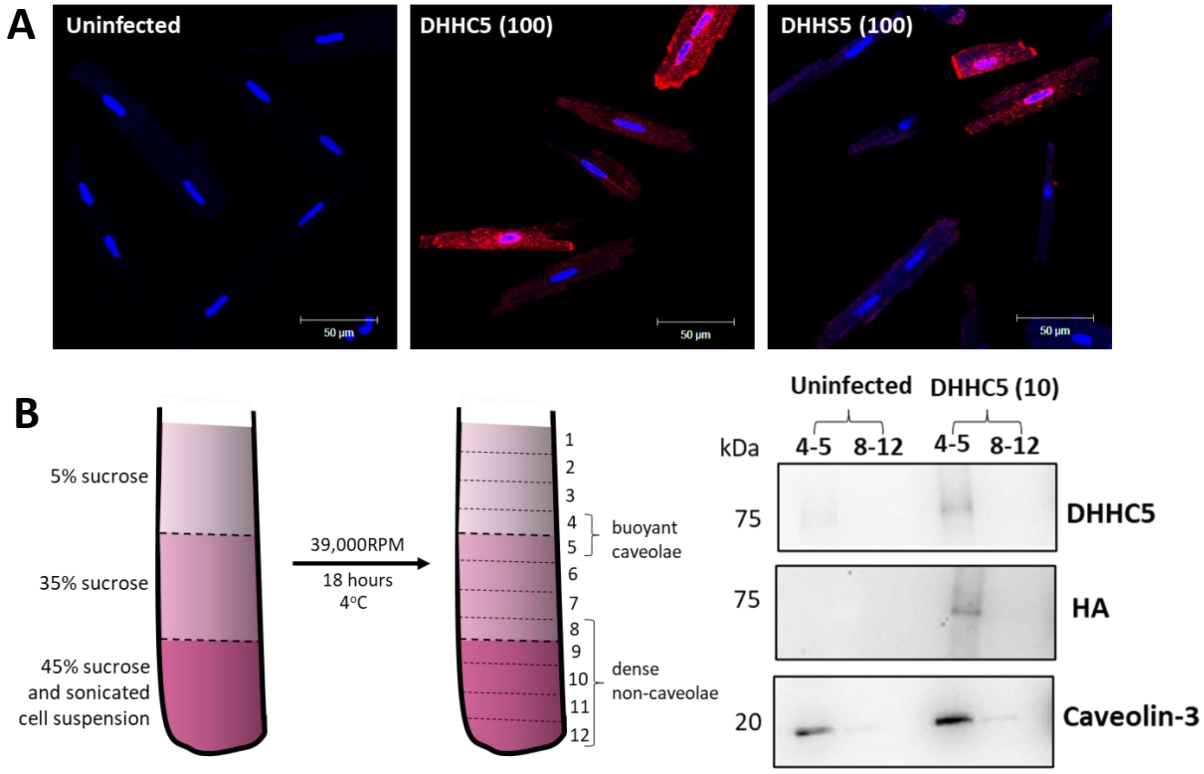
Alice Main[1], Andri Bogusalvskyii[2], Jacqueline Howie[1], Chien-wen Kuo[1], Aileen Rankin[1], Francis L. Burton[1], Godfrey L. Smith[1], Roger Hajjar[3], George S. Baillie[1], Kenneth S. Campbell[4], Michael J. Shattock[2], William Fuller[1*]

[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, G12 8QQ
[2]School of Cardiovascular and Metabolic Medicine and Sciences, King's College London, London, SE1 7EH
[3]Flagship Pioneering, Cambridge, MA 02142
[4]Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY40506

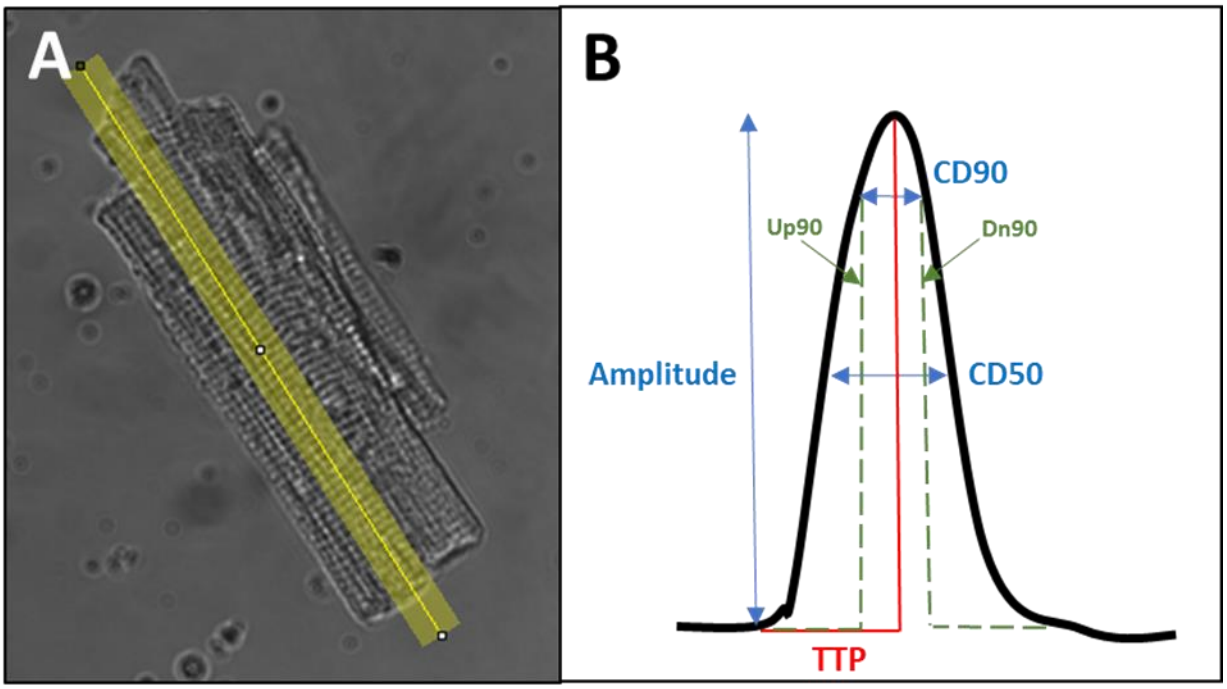
Supplementary Figures:

Human heart failure patient and organ donor details:

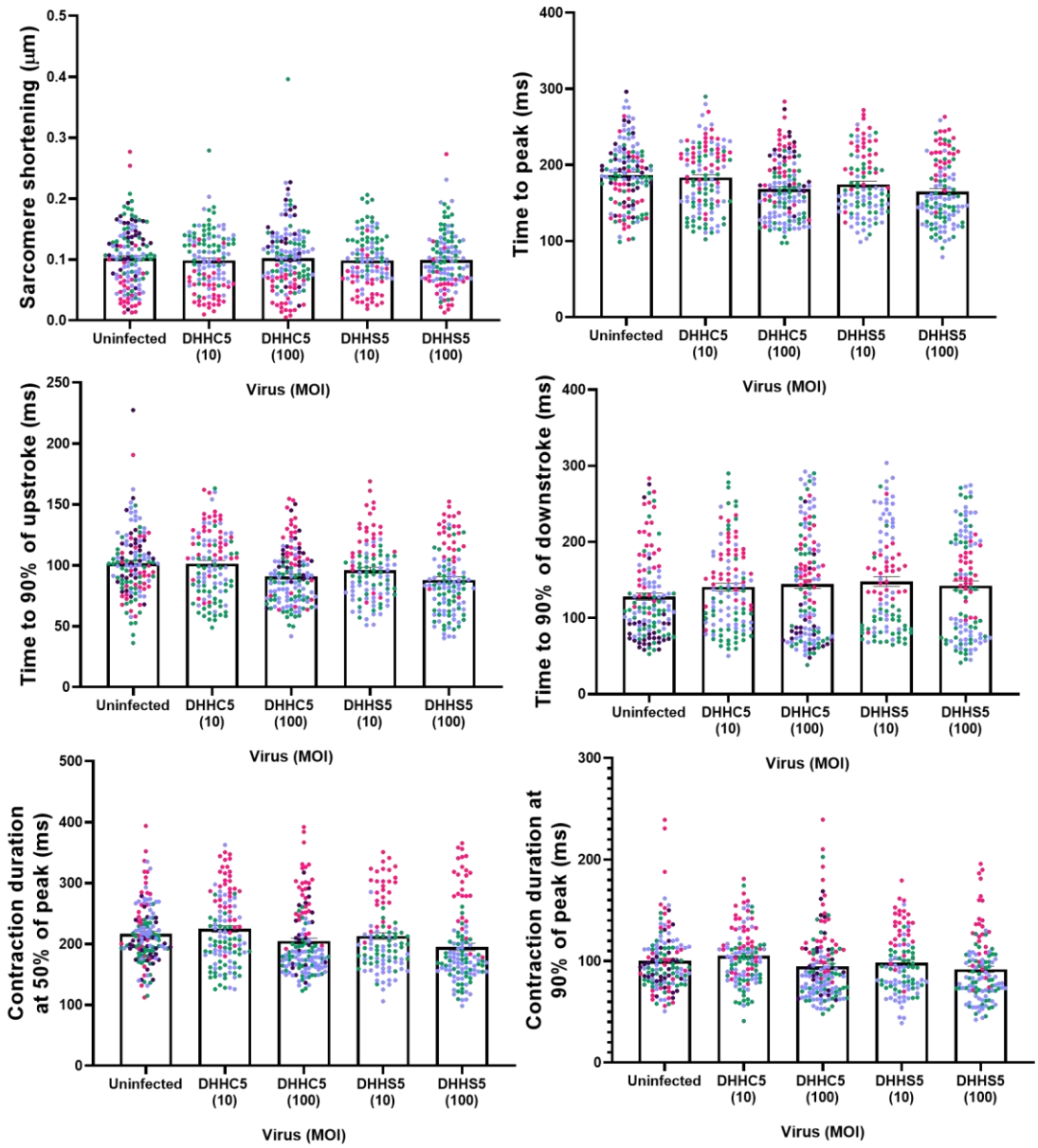
Record ID	Case type	Sex	Primary diagnosis
24713	Organ Donor	Female	N/A
2B487	Organ Donor	Male	N/A
31331	Organ Donor	Female	N/A
4B3FA	Organ Donor	Female	N/A
4D931	Organ Donor	Male	N/A
5155D	Organ Donor	Male	N/A
632FD	Organ Donor	Male	N/A
8CB30	Organ Donor	Female	N/A
B23E3	Organ Donor	Male	N/A
BC90C	Organ Donor	Female	N/A
DOF54	Organ Donor	Male	N/A
D61ZE	Organ Donor	Male	N/A
FC3CB	Organ Donor	Female	N/A
046E	Heart Failure	Male	Ischaemic cardiomyopathy
05FF7	Heart Failure	Female	Ischaemic HFrEF
14C39	Heart Failure	Male	Ischaemic cardiomyopathy
3F6DC	Heart Failure	Male	Ischaemic cardiomyopathy
58545F	Heart Failure	Male	Ischaemic cardiomyopathy s/p MI
6DB85	Heart Failure	Male	HFrEF from Ischemic cardiomyopathy
7CE52	Heart Failure	Female	Ischaemic heart failure
8296A	Heart Failure	Male	Ischaemic cardiomyopathy
8E8D8	Heart Failure	Male	Ischaemic cardiomyopathy
97CDC	Heart Failure	Male	Ischaemic cardiomyopathy
9D7E9	Heart Failure	Male	Ischaemic cardiomyopathy
AF1FF	Heart Failure	Male	Chronic systolic HF
B8BE2	Heart Failure	Male	Ischaemic heart failure
BO644	Heart Failure	Female	Ischaemic cardiomyopathy
C3B57	Heart Failure	Male	Ischaemic cardiomyopathy
CB8A5	Heart Failure	Male	Ischaemic cardiomyopathy
DA820	Heart Failure	Male	Ischaemic cardiomyopathy
EF5CB	Heart Failure	Male	Ischaemic heart failure
FE8E2	Heart Failure	Male	Chronic systolic HF



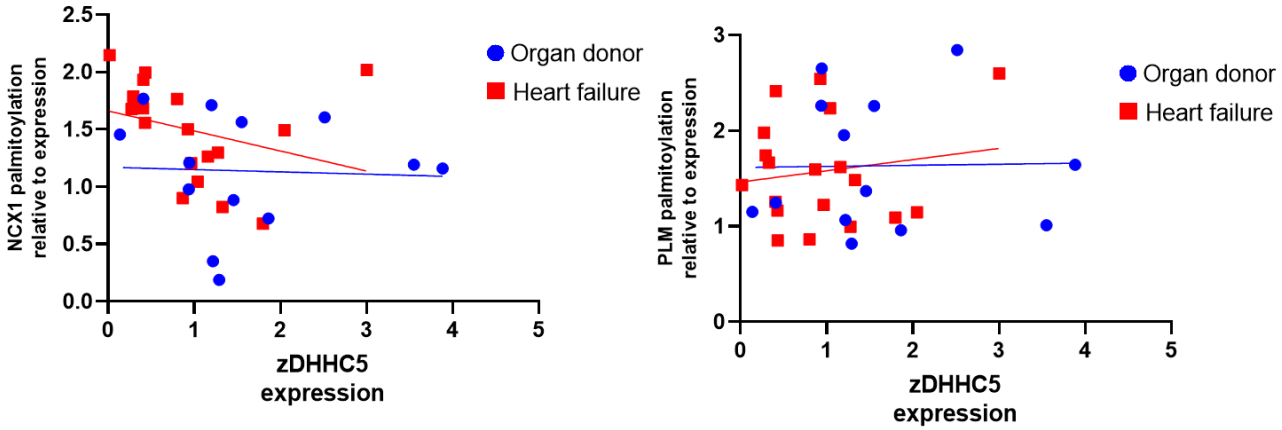
Supplementary Figure 1: Localisation of viral zDHHC5 in rabbit cardiomyocytes. **A)** Rabbit ventricular cardiomyocytes were cultured for 18-24 hours in the presence of either HA-DHHC5 virus or a HA-DHHS5 dominant negative virus at a multiplicity of infection (MOI) of 100. Viral zDHHC5 was detected using an anti-HA antibody, Alexa Fluor-546 secondary antibody and DAPI was used to stain the nuclei of the cells. Cells were visualised using a Zeiss LSM 510 META Confocal Microscope (40x objective) and 10 images per sample were captured. All cells appeared multinucleated and bright red fluorescence was observed at the intercalated discs, cell surface and perinuclear membrane in infected cells but not in uninfected controls. Representative images of the uninfected and viral samples are shown with contrast enhanced using ImageJ. Scale bar represents 50µm. **B)** Rabbit ventricular cardiomyocytes were cultured for 18-24 hours and infected with HA-DHHC5 (MOI 10). Sucrose gradient fraction was used to separate buoyant membranes containing caveolae from non-caveolae membranes and revealed both endogenous and virally expressed zDHHC5 is found in the buoyant membranes alongside Caveolin-3. N=1.



Supplementary Figure 2: CellOPTIQ® measurement of cardiac contractility. **A)** Cardiomyocyte contractility is recorded at 100fps and a section for analysis highlighted in ImageJ. **B)** An ImageJ Macro (Clydes Biosciences, Ltd) produces an average fractional shortening trace determined from changes sarcomere length over time. A number of parameters can be determined from this including amplitude of peak, time to peak (TTP), time at 90% of the upstroke (Up90) and downstroke (Dn90), and contraction duration at 90% (CD90) and 50% (CD50) of the peak.

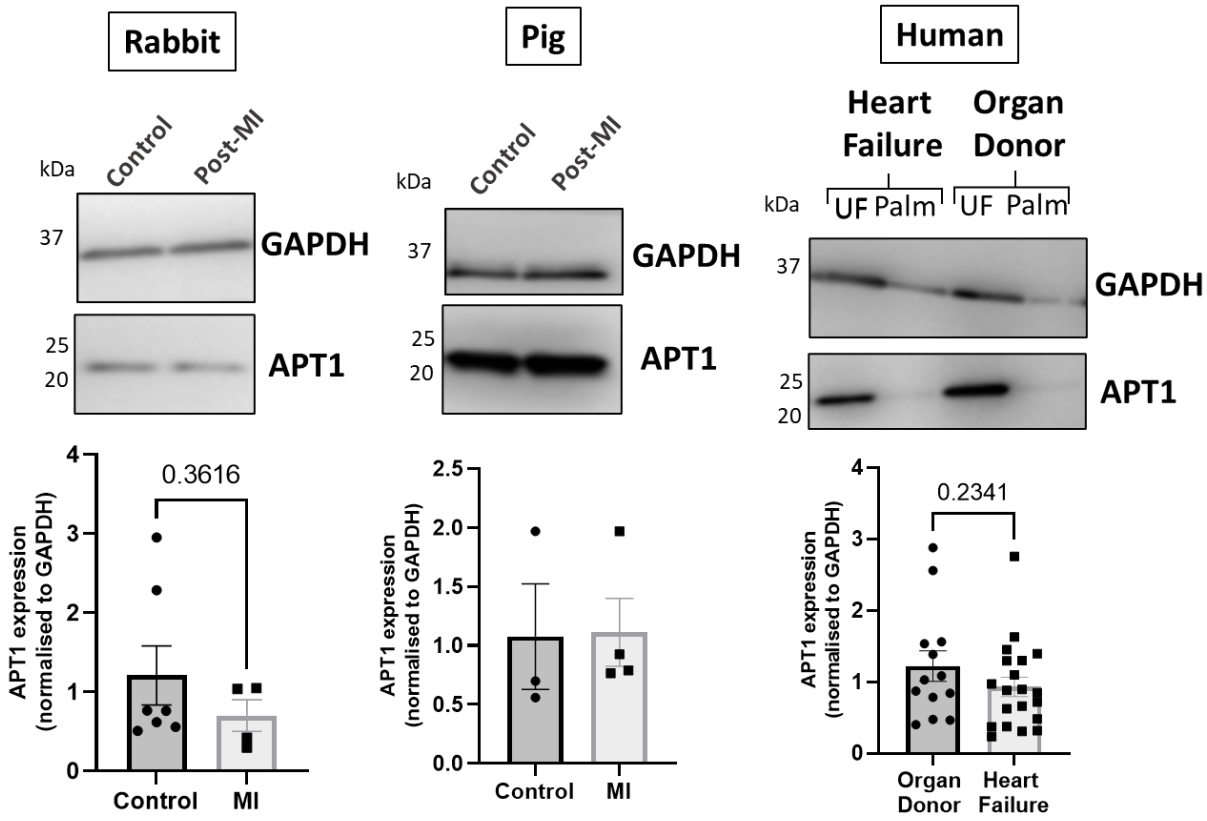


Supplementary Figure 3: zDHHC5 and zDHHS5 overexpression do not alter parameters of rabbit ventricular cardiomyocyte contractility. Rabbit septal cardiomyocyte cells were infected with zDHHC5 or zDHHS5 viruses at an MOI of 10 or 100 and cultured for 18-24 hours following which contractility recordings were taken using CellOPTIQ®, whereby cells were paced (2Hz frequency, 0.2ms duration, 40V) and 5 second recordings at 100fps were recorded. Recordings were analysed using an ImageJ macro and the contractility parameters of amplitude (sarcomere shortening), time to peak, time to 90% of peak upstroke, time to 90% of peak downstroke, contraction duration at 50% of peak and contraction duration at 90% of peak were compared across the groups. Expression of either zDHHC5 or zDHHS5 at MOI 10 or 100 had no significant effect on the parameters of contractility investigated. Data are mean ±S.E.M and average of the biological replicates were statistically compared using a one-way ANOVA with a Sidak's post-hoc test. N=3 biological replicates for S10, C100, S100 and n=4 biological replicates for uninfected and C100, with n=20-51 cells per replicate. Each colour represents cells from one biological replicate.



Substrate	Category	R^2 value	P-value
NCX1	Organ donor	0.001979	0.8853
NCX1	Heart failure	0.09111	0.2091
PLM	Organ donor	0.0003378	0.9525
PLM	Heart failure	0.02418	0.5250

Supplementary Figure 4: Linear regression analysis of DHHC5 expression compared to substrate palmitoylation. zDHHC5 expression (normalised to GAPDH) and substrate palmitoylation (NCX1 or PLM) were plotted for each sample from human organ donor (n=13, blue) or human heart failure (n=19, red) Linear regression analysis was performed and the R^2 value determined (table). There was no significant relationship between zDHHC5 expression and the level of palmitoylation of NCX1 or PLM in either group.



Supplementary Figure 5: APT1 expression in heart failure. In the rabbit model of MI-induced HF (8-weeks post-MI), a pig model of MI-induced HF with reperfusion (3-months post-MI) and samples from patients with ischaemic heart failure, APT1 expression was not significantly different to controls or organ donors. APT1 expression was normalised to loading control GAPDH. Statistical comparisons made by unpaired Student's t-test. Data are mean ±S.E.M.