

SUPPLEMENTAL MATERIAL

Supplemental Methods

rtPCR

Total RNA was isolated from adult female flies using a Qiagen RNeasy Mini kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. cDNA was prepared from 1 µg of total DNase-treated RNA by incubating the RNA with Oligo-dT primers at 65°C for 10 min and then placing the reaction on ice for 5 min. Second strand synthesis was performed using Roche Expand RT (Roche Products Limited, Welwyn Garden City, UK). Quantitative PCR was then performed using 10 µL reaction volumes in 384-well format in a Roche LightCycler 480 Intron-spanning primers were used to determine the relative concentration of *Drosophila SPARC* (left, CGACATCGATGAGAACGAAG; right, TCGCGCTCAATATCCTTGAT), using *Actin5C* as a reference control. Quantified data are presented at the mean (+/-SEM) of six independent samples from wild type and *SPARC*-mutant flies.

Imaging the adult heart

Adults (2-3 week old unless stated otherwise) were anaesthetised with Flynap (Carolina Biological Supply Company, Burlington, NC, USA), dissected and hearts stained as described previously^{1,2}. For some experiments, vital dyes were used to identify functional nephrocytes or test their endocytic function (wheat germ agglutinin at 1µg / mL for 15 minutes or 50 µg / mL 10 kDa fluorescently labelled dextran for 0-30 minutes). Semi-intact preparations were then washed three times, fixed for 20 minutes with 1% formaldehyde and co-stained with antibodies (and then the relevant secondary antibodies) or Hoechst to visualise DNA and then imaged.

Epifluorescence microscopy of adult fly tissues

Semi-intact preparations were washed three times, fixed for 20 minutes with 1% formaldehyde, permeabilised with 0.1% TritonX-100 in phosphate buffered saline and co-stained with phalloidin (to visualise the actin cytoskeleton of the heart) and antibodies to the nephrocyte endocytosis protein Amnionless. To identify the

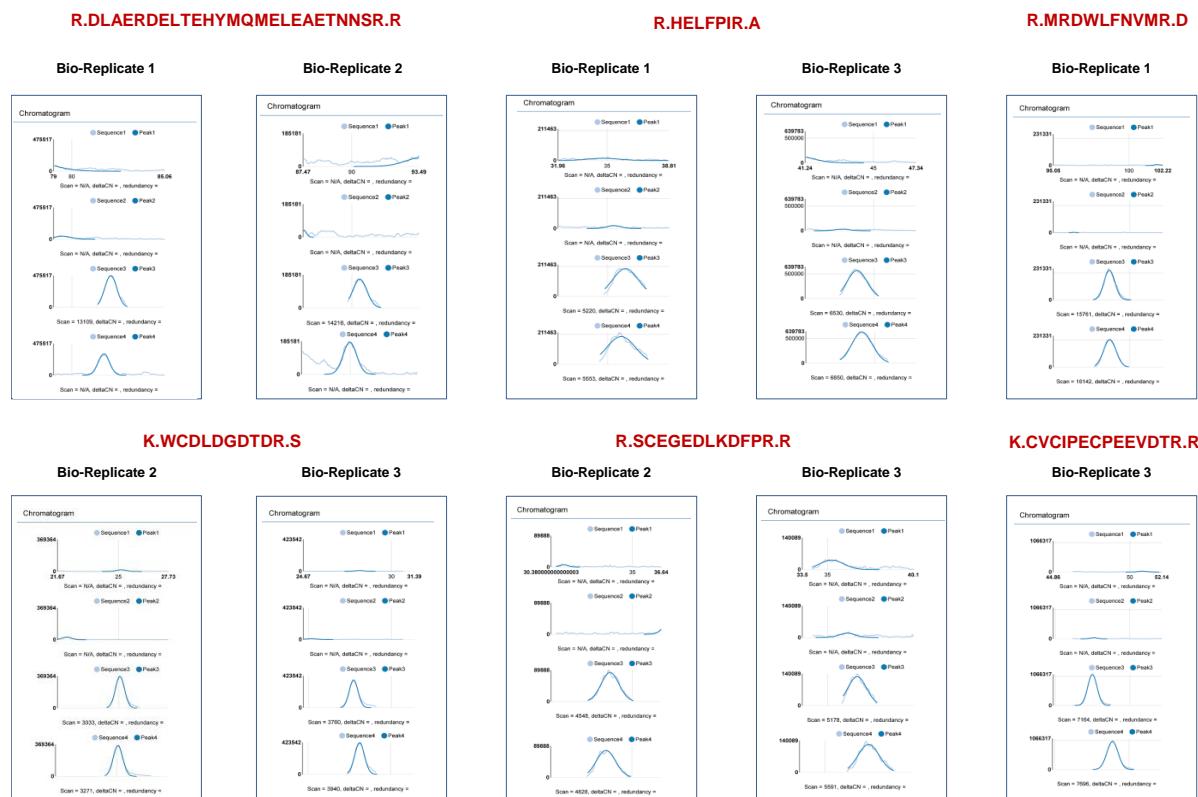
garland cells Hoechst 33342 was used to visualise DNA (garland cells having a binucleate nuclear morphology and distinct anatomical location at the interface between the oesophagus and paraventriculus). Fluorescence microscopy of flies was performed using a Zeiss LSM780 coupled to Zen image analysis software (Carl Zeiss, Welwyn Garden City, UK). Phase images were captured on a Zeiss Axiolab and images captured with an ORCA-ER CCD camera (Hamamatsu Photonics KK, Japan; Welwyn Garden City, UK) coupled to Openlab 4.1 (Improvision, Coventry, UK). Images were coloured, contrast enhanced and overlaid using Photoshop CS3. All micrographs were collected using the same microscope settings and image alterations, which were limited to contrast and brightness enhancement.

References.

1. Catterson JH, Heck MM, Hartley PS. Fermitins, the orthologs of mammalian kindlins, regulate the development of a functional cardiac syncytium in *drosophila melanogaster*. *PLoS One*. 2013;8:e62958.
2. Park SK, Venable JD, Xu T, Yates JR. A quantitative analysis software tool for mass spectrometry-based proteomics. *Nature Methods*. 2008;5:319-322.

Supplemental Figure S1.

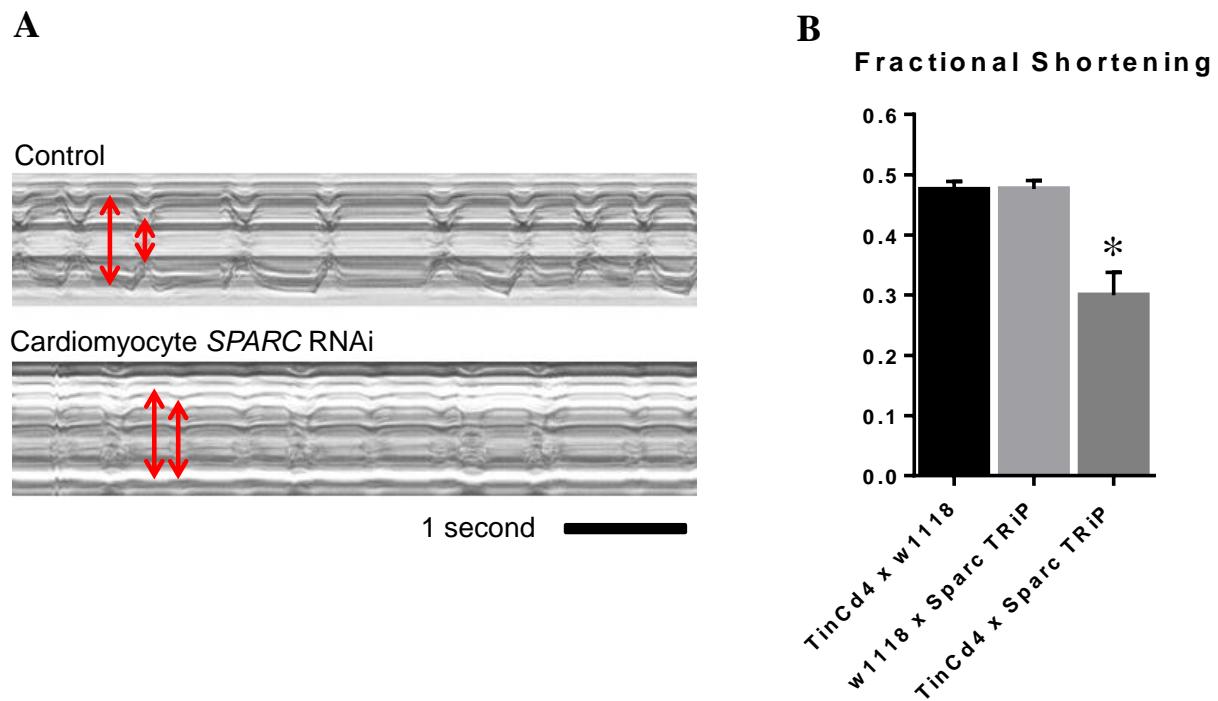
SPARC peptide peak areas. Chromatograms for identified SPARC peptides are shown. In each panel the upper two chromatograms show peptide peaks from two technical replicates of a sample from the wild type (w^{1118}) hemolymph and the lower two chromatograms correspond to two technical replicates from a sample of the mutant ($dKlf15^{NN}$) hemolymph. In each case the peptide is detected in the mutant but not the wild type hemolymph.



Supplemental Figure S2.

Effect of *SPARC* knock-down on heart function.

The function of the adult heart was analysed by high frame rate videomicroscopy (as described in the methods section of the main document). (A) The M-modes show the distance moved by the wall of the heart during a contraction (Fractional Shortening, red arrows). The control heart shows robust contraction between diastole to systole, whereas there is significantly less contraction when *SPARC* is silenced in the cardiomyocytes. (B) The graph shows the relative distance moved by the heart walls during diastole and systole (Fractional Shortening). The knock-down of *SPARC* in cardiomyocytes using the *TinCΔ4* driver (*TinCd4* x *Sparc TRiP*) had a significant impact on fractional shortening that was not seen in either of the control genotypes (*TinCd4* x *w1118* and *w1118* x *Sparc TRiP*). $n = 19$ to 29 flies per genotype. * $P < 0.001$.



tr Q4TWT4 Q4TWT4_DROME	Q4TWT4	su(r)	5	0	0		0	0	0	2	2		0	0	0.374	0.407
tr Q9VSL4 Q9VSL4_DROME	Q9VSL4	GstO2	5	0	0		0	0	0	2	2		0	0	0.374	0.407
tr Q7KUO2 Q7KUQ2_DROME	Q7KUQ2	CG9674-RB	4	1	0		0	0	0	2	1		0	0	0.197	0.407
tr O97428 O97428_DROME	O97428	cib	2	3	0		2	4	0	2	1		2	1	0.879	0.883
sp Q02645-1 HTS_DROME	Q02645	hts	1	4	0		0	0	0	2	1		0	0	0.267	0.407
sp Q9VNNT5 TRXR2_DROME	Q9VNNT5	Trxr-2	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr A1ZSU4 A1ZSU4_DROME	A1ZSU4	Cet5	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr A8DYP0 A8DYP0_DROME	A8DYP0	Unc-89	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr Q24062 Q24062_DROME	Q24062	b	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr Q9VFF0 Q9VFF0_DROME	Q9VFF0	CG3731	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr Q9VH01 Q9VH01_DROME	Q9VH01	Bruce	4	0	0		4	0	0	1	1		1	1	0.940	0.940
tr Q7V16 Q7V16_DROME	Q7V16	GstE13	3	1	0		0	0	0	1	1		0	0	0.171	0.407
sp Q95083 PSA5_DROME	Q95083	Prosalpha5	1	3	0		0	0	0	1	1		0	0	0.147	0.407
sp Q9XZJ4 PSA6_DROME	Q9XZJ4	Prosalpha1	1	3	0		0	0	0	1	1		0	0	0.147	0.407
tr Q967S0 Q967S0_DROME	Q967S0	Prat2	1	3	0		0	0	0	1	1		0	0	0.147	0.407
tr Q7KSB5 Q7KSB5_DROME	Q7KSB5	CG4390	1	3	0		0	3	0	1	1		1	1	0.694	0.703
sp Q6NN85-1 SSH_DROME	Q6NN85	ssh	0	4	0		0	0	0	1	1		0	0	0.374	0.407
sp Q9V813 MTAP_DROME	Q9V813	CG4802	0	4	0		0	0	0	1	1		0	0	0.374	0.407
sp Q9VXX8 Rl371_DROME	Q9VXX8	Rpl37a	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr A1Z8G7 A1Z8G7_DROME	A1Z8G7	Listericin	0	4	0		0	0	0	1	1		0	0	0.374	0.407
tr A8Q134 A8Q134_DROME	A8Q134	CG40625	0	4	0		0	0	0	1	1		0	0	0.374	0.407
tr B7YZX6 B7YZX6_DROME	B7YZX6	CG10600	0	4	0		0	0	0	1	1		0	0	0.374	0.407
tr B7Z076 B7Z076_DROME	B7Z076	CG6852	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr O97479 O97479_DROME	O97479	Sodh-1	0	4	0		0	0	0	1	1		0	0	0.374	0.407
tr Q8INQ3 Q8INQ3_DROME	Q8INQ3	CG11760-RB	0	4	0		0	0	0	1	1		0	0	0.374	0.407
tr Q9U9B0 Q9U9B0_DROME	Q9U9B0	gig	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr Q9VP18 Q9VP18_DROME	Q9VP18	net	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr Q9VWSS Q9VWSS_DROME	Q9VWSS	CG15040	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr Q9W137 Q9W137_DROME	Q9W137	CG4707	0	0	4		0	0	0	1	1		0	0	0.374	0.407
tr Q0E8H9 Q0E8H9_DROME	Q0E8H9	Hexo1	0	4	0		0	3	0	1	1		1	1	0.795	0.802
sp Q9NIV1 E2AK3_DROME	Q9NIV1	PEK	4	0	0		0	0	0	1	1		0	0	0.374	0.407
sp Q9V3J1-1 VATH_DROME	Q9V3J1	VhaSFD	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr Q9VIQ8 Q9VIQ8_DROME	Q9VIQ8	CoIV	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr Q9VKM3 Q9VKM3_DROME	Q9VKM3	I(2)06225	4	0	0		0	0	0	1	1		0	0	0.374	0.407
tr A1Z784 A1Z784_DROME	A1Z784	ACC	2	1	0		0	0	0	1	1		0	0	0.141	0.407
tr Q0E993 Q0E993_DROME	Q0E993	Aats-val	2	1	0		0	0	0	1	1		0	0	0.141	0.407
tr Q1RL06 Q1RL06_DROME	Q1RL06	GS	1	0	2		2	0	0	1	1		1	1	0.476	0.494