Supplementary Information for

Amyloid beta 42 alters cardiac metabolism and impairs cardiac function in obese male mice

Liam G Hall[1,2,10], Juliane K. Czeczor[1,3,10], Timothy Connor[1], Javier Botella[1], Kirstie A. De Jong[1,4], Mark C. Renton[5], Amanda J. Genders[1,6], Kylie Venardos[1], Sheree D. Martin[1], Simon T. Bond[1,7], Kathryn Aston-Mourney[1], Kirsten F. Howlett[5], James A Campbell[8], Greg R. Collier[8], Ken R. Walder[1], Matthew McKenzie[9], Mark Ziemann[9], Sean L. McGee[1,8]

[1]Institute for Mental and Physical Health and Clinical Translation, Metabolic Research Unit, School of Medicine, Deakin University, Geelong, Australia.
[2]Department of Cellular and Physiological Sciences, Faculty of Medicine, The University of British Columbia, Vancouver, Canada.
[3]Becton Dickinson GmbH, Medical Affairs, 69126 Heidelberg, Germany.
[4]Institute of Experimental Cardiovascular Research, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany.
[5]Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, Australia.
[6]Department of Nutrition, Dietetics and Food, School of Clinical Sciences and Victorian Heart Institute, Monash University, Melbourne, Australia
[7]Baker Heart and Diabetes Institute, Melbourne, Australia.
[8]Ambetex Pty Ltd, Geelong, Australia.
[9]School of Life and Environmental Science, Deakin University, Geelong, Australia.
[10]These authors contributed equally.

This document contains 5 data tables and 7 data figures.

Corresponding author:

Professor Sean L. McGee
Metabolic Remodelling Laboratory
Metabolic Research Unit
School of Medicine
Deakin University, Waurn Ponds, Australia, 3216
Ph: +61 3 5227 2519
Fax: +61 3 5227 2945
Email: sean.mcgee@deakin.edu.au

Supplementary Table 1

Parameter	ScrAβ$_{42}$	Aβ$_{42}$	*P* value
Peak aortic flow (cm/sec)	61.9 ± 3.8	58.7 ± 1.1	0.481
Ejection time (msec)	52.5 ± 1.6	50.3 ± 2.0	0.411
Heart rate (bpm)	386 ± 24	397 ± 18	0.736
Peak E wave (cm/sec)	58.9 ± 4.9	60.6 ± 2.7	0.770
Peak A wave (cm/sec)	27.4 ± 2.4	32.0 ± 0.6	0.123
IVSd (cm)	0.126 ± 0.007	0.121 ± 0.006	0.604
LVIDd (cm)	0.346 ± 0.006	0.346 ± 0.016	0.974
LVPWd (cm)	0.108 ± 0.005	0.116 ± 0.004	0.155
IVSs (cm)	0.148 ± 0.007	0.149 ± 0.006	0.949
LVIDs (cm)	0.230 ± 0.003	0.251 ± 0.013	0.151
LVPWs (cm)	0.132 ± 0.006	0.148 ± 0.007	0.100
Estimated LV mass (mg)	163 ± 13	168 ± 14	0.822
Heart weight/tibia length (mg/mm)	7.11 ± 0.09	7.19 ± 0.17	0.751

Supplementary Table 1: Cardiac function and morphology in mice administered ScrAβ$_{42}$ or Aβ$_{42}$. IVSd, intraventricular septum thickness at diastole; LVIDd, left ventricular internal diameter at diastole; LVPWd, left ventricular posterior wall thickness at diastole; IVSs, intraventricular septum thickness at systole; LVIDs, left ventricular internal diameter at systole; LVPWs, left ventricular posterior wall thickness at systole. Data are mean ± SEM, n =10 mice/group. Groups compared by unpaired t-test, two-tailed. Source data are provided in the Source Data file.

Supplementary Table 2

Parameter	ScrAβ$_{40}$	Aβ$_{40}$	*P* value
Peak aortic flow (cm/sec)	60.4 ± 4.2	66.2 ± 5.1	0.391
Ejection time (msec)	59.3 ± 1.4	61.5 ± 1.2	0.233
Heart rate (bpm)	416 ± 12	384 ± 10	0.060
Peak E wave (cm/sec)	57.1 ± 3.6	58.1 ± 4.4	0.862
Peak A wave (cm/sec)	35.9 ± 1.8	32.4 ± 1.8	0.193
E:A ratio	1.54 ± 0.08	1.73 ± 0.10	0.153
Deceleration time (msec)	0.032 ± 0.001	0.032 ± 0.001	0.939
IVSd (cm)	0.125 ± 0.005	0.120 ± 0.006	0.498
LVIDd (cm)	0.340 ± 0.010	0.350 ± 0.012	0.528
LVPWd (cm)	0.125 ± 0.004	0.128 ± 0.003	0.533
IVSs (cm)	0.178 ± 0.007	0.174 ± 0.007	0.697
LVIDs (cm)	0.225 ± 0.012	0.226 ± 0.013	0.981
LVPWs (cm)	0.141 ± 0.004	0.152 ± 0.005	0.094
Ejection fraction (%)	71.5 ± 2.6	73.2 ± 2.1	0.602
Fractional shortening (%)	35.9 ± 2.2	36.2 ± 1.7	0.905
Estimated LV mass (mg)	217 ± 15	204 ± 11	0.502
Heart weight/tibia length (mg/mm)	9.60 ± 0.42	8.78 ± 0.24	0.112

Supplementary Table 2: Cardiac function and morphology in mice administered ScrAβ$_{40}$ or Aβ$_{40}$. IVSd, intraventricular septum thickness at diastole; LVIDd, left ventricular internal diameter at diastole; LVPWd, left ventricular posterior wall thickness at diastole; IVSs, intraventricular septum thickness at systole; LVIDs, left ventricular internal diameter at systole; LVPWs, left ventricular posterior wall thickness at systole. Data are mean ± SEM, n =12 mice/group. Groups compared by unpaired t-test, two-tailed. Source data are provided in the Source Data file.

Supplementary Table 3

	Control		3D6		2-way RM ANOVA *P* value		
Parameter	**Pre**	**Post**	**Pre**	**Post**	**Tx**	**Time**	**Int.**
Peak aortic flow (cm/sec)	72.2 ± 6.9	67.9 ± 4.7	67.0 ± 6.0	65.3 ± 4.7	0.5022	0.5975	0.8217
Ejection time (msec)	48.5 ± 2.3	54.0 ± 2.2	49.4 ± 3.2	52.5 ± 2.4	0.9121	0.0969	0.6365
Heart rate (bpm)	455 ± 22	453 ± 19	437 ± 17	475 ± 22	0.9328	0.3786	0.3177
Peak E wave (cm/sec)	48.3 ± 2.7	51.0 ± 3.8	51.1 ± 4.6	49.4 ± 3.3	0.8070	0.7936	0.3434
Peak A wave (cm/sec)	28.7 ± 1.6	37.4 ± 3.5	32.7 ± 3.1	35.0 ± 2.8	0.9631	0.1365	0.1724
E:A ratio	1.69 ± 0.08	1.41 ± 0.04	1.60 ± 0.09	1.32 ± 0.04	0.1654	0.0056	0.8433
IVSd (cm)	0.116 ± 0.003	0.128 ± 0.006	0.130 ± 0.006	0.129 ± 0.006	0.2207	0.1432	0.0829
LVIDd (cm)	0.272 ± 0.012	0.307 ± 0.009	0.272 ± 0.010	0.293 ± 0.013	0.3505	0.0072	0.7772
LVPWd (cm)	0.120 ± 0.006	0.135 ± 0.008	0.130 ± 0.007	0.131 ± 0.005	0.9798	0.0013	0.2343
IVSs (cm)	0.165 ± 0.002	0.174 ± 0.006	0.168 ± 0.007	0.166 ± 0.006	0.6910	0.5835	0.3740
LVIDs (cm)	0.175 ± 0.012	0.189 ± 0.010	0.177 ± 0.011	0.195 ± 0.012	0.7513	0.1800	0.8458
LVPWs (cm)	0.137 ± 0.006	0.158 ± 0.009	0.146 ± 0.005	0.148 ± 0.010	0.9417	0.1486	0.2658
Ejection fraction (%)	75.3 ± 1.5	75.0 ± 1.8	76.1 ± 1.8	69.5 ± 2.8	0.1797	0.3923	0.5001
Fractional shortening (%)	37.7 ± 1.3	37.6 ± 1.6	37.3 ± 1.9	34.6 ± 2.4	0.7223	0.1921	0.1953

Supplementary Table 3: Cardiac function and morphology in mice fed a high fat diet and administered control or 3D6 antibodies. IVSd, intraventricular septum thickness at diastole; LVIDd, left ventricular internal diameter at diastole; LVPWd, left ventricular posterior wall thickness at diastole; IVSs, intraventricular septum thickness at systole; LVIDs, left ventricular internal diameter at systole; LVPWs, left ventricular posterior wall thickness at systole. Data are mean ± SEM, n =12 mice/group. Groups compared by two-way repeated measures ANOVA. Tx, treatment; Int, interaction. Source data are provided in the Source Data file.

Supplementary Table 4

	Chow control			HFD control			HFD 3D6			Mixed effects model *P* value		
	Base-line	Pre-treat	Post-treat	Base-line	Pre-treat	Post-treat	Base-line	Pre-treat	Post-treat	Tx	Time	Int.
PAF (cm/sec)	57.8 ± 2.7	59.1 ± 4.2	61.3 ± 2.6	60.1 ± 6.3	59.3 ± 3.2	67.7 ± 5.1	58.7 ± 4.0	64.1 ± 3.0	71.8 ± 2.5	0.0955	0.0067	0.5443
ET (msec)	42.3 ± 1.9	42.3 ± 2.2	46.8 ± 2.0	41.2 ± 1.2	49.2 ± 2.4	43.8 ± 3.2	44.5 ± 1.5	46.2 ± 1.5	45.3 ± 2.6	0.6138	0.0318	0.5453
HR (bpm)	468 ± 18	491 ± 17	510 ± 23	441 ± 4	501 ± 9	556 ± 23	462 ± 10	503 ± 12	572 ± 12	0.3321	< 0.0001	0.3321
Peak E (cm/sec)	42.3 ± 3.5	51.1 ± 3.8	50.0 ± 4.1	45.2 ± 4.2	58.3 ± 4.1	53.0 ± 2.6	41.6 ± 1.4	52.3 ± 2.8	63.8 ± 3.5	0.2964	0.0003	0.2945
Peak A (cm/sec)	27.3 ± 2.2	33.5 ± 2.7	35.0 ± 1.9	24.4 ± 1.2	34.4 ± 2.4	36.2 ± 2.8	28.7 ± 1.7	32.3 ± 2.2	46.7 ± 3.8	0.0852	< 0.0001	0.0592
E:A ratio	1.62 ± 0.08	1.59 ± 0.03	1.57 ± 0.03	1.65 ± 0.05	1.67 ± 0.06	1.45 ± 0.09	1.52 ± 0.05	1.56 ± 0.07	1.41 ± 0.06	0.2417	0.1167	0.8675
IVSd (cm)	0.147 ± 0.002	0.109 ± 0.005	0.118 ± 0.004	0.135 ± 0.006	0.114 ± 0.003	0.129 ± 0.005	0.137 ± 0.008	0.125 ± 0.005	0.122 ± 0.003	0.7234	< 0.0001	0.0335
LVIDd (cm)	0.248 ± 0.011	0.314 ± 0.014	0.293 ± 0.014	0.268 ± 0.005	0.311 ± 0.015	0.308 ± 0.015	0.263 ± 0.014	0.303 ± 0.014	0.281 ± 0.009	0.4968	0.0001	0.6252
LVPWd (cm)	0.156 ± 0.008	0.136 ± 0.008	0.125 ± 0.008	0.142 ± 0.005	0.152 ± 0.007	0.137 ± 0.009	0.134 ± 0.007	0.147 ± 0.008	0.144 ± 0.007	0.6857	0.0577	0.0241
IVSs (cm)	0.189 ± 0.008	0.158 ± 0.006	0.162 ± 0.006	0.175 ± 0.008	0.169 ± 0.005	0.178 ± 0.006	0.167 ± 0.009	0.169 ± 0.003	0.173 ± 0.004	0.9053	0.0876	0.0313
LVIDs (cm)	0.160 ± 0.007	0.193 ± 0.013	0.185 ± 0.015	0.192 ± 0.010	0.192 ± 0.016	0.185 ± 0.015	0.186 ± 0.012	0.188 ± 0.014	0.172 ± 0.010	0.4420	0.6350	0.6135
LVPWs (cm)	0.171 ± 0.005	0.156 ± 0.007	0.141 ± 0.008	0.160 ± 0.006	0.174 ± 0.010	0.166 ± 0.011	0.149 ± 0.009	0.167 ± 0.008	0.157 ± 0.008	0.2567	0.2109	0.0370
EF (%)	71.0 ± 1.9	76.1 ± 2.1	74.1 ± 2.9	67.3 ± 2.6	74.9 ± 3.0	78.2 ± 2.2	66.2 ± 1.6	74.3 ± 3.1	76.7 ± 2.2	0.8585	0.004	0.5063
FS (%)	34.0 ± 1.4	39.0 ± 2.1	37.5 ± 2.6	31.8 ± 1.8	39.5 ± 3.0	40.9 ± 2.3	30.0 ± 0.9	38.3 ± 2.9	39.4 ± 2.0	0.9689	0.0005	0.6390
LV mass (mg)	152 ± 7	153 ± 9	136 ± 3	161 ± 10	164 ± 5	161 ± 8	145 ± 14	166 ± 6	153 ± 5	0.0925	0.1080	0.1357

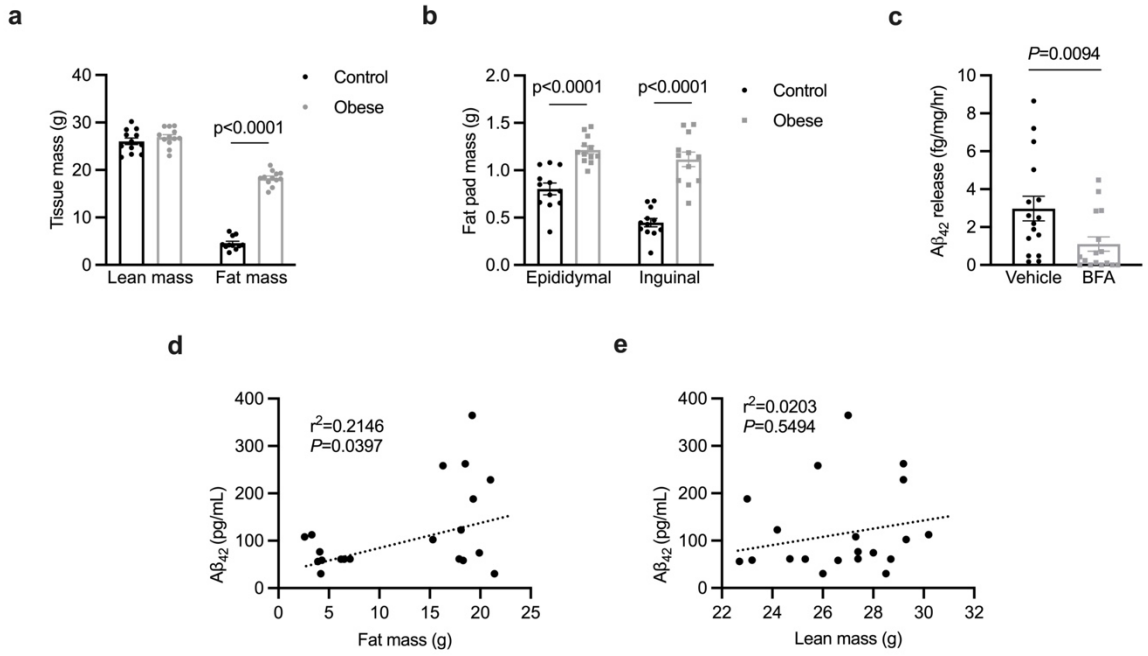
Supplementary Table 4: Cardiac function and morphology in mice fed chow or high fat diet and administered control or 3D6 antibodies. PAF, peak aortic flow; ET, ejection time; HR, heart rate; IVSd, intraventricular septum thickness at diastole; LVIDd, left ventricular internal diameter at diastole; LVPWd, left ventricular posterior wall thickness at diastole; IVSs, intraventricular septum thickness at systole; LVIDs, left ventricular internal diameter at systole; LVPWs, left ventricular posterior wall thickness at systole. EF, ejection fraction; FS, fractional shortening. Data are mean ± SEM, n =12 mice/group. Groups compared by mixed effects model. Tx, treatment; Int, interaction. Source data are provided in the Source Data file.

Supplementary Table 5

Pathway	setSize	p MANOVA	p.adjust MANOVA	s.dist	s.Aβ42	s.3D6	p.Aβ42	p.3D6
TCA cycle	22	<0.0001	0.0004	0.608	-0.350	0.498	0.0045	<0.0001
Pyruvate metabolism and TCA cycle	50	<0.0001	<0.0001	0.567	-0.305	0.478	0.0002	<0.0001
Pyruvate metabolism	26	0.0002	0.0030	0.484	-0.246	0.417	0.0301	0.0002
Mitochondrial biogenesis	71	0.0009	0.0014	0.268	-0.184	0.196	0.0076	0.0044
Protein localisation	141	0.0001	0.0004	0.215	-0.146	0.158	0.0028	0.0012
Neddylation	201	<0.0001	<0.0001	0.199	-0.109	0.166	0.0079	<0.0001
Antigen processing	262	<0.0001	<0.0001	0.197	-0.075	0.182	0.0380	<0.0001
Autophagy	117	0.0037	0.0050	0.188	-0.127	0.139	0.0178	0.0097
Chromatin modifying enzymes	188	0.0006	0.0011	0.171	-0.111	0.130	0.0002	0.0022
Chromatin organisation	188	0.0006	0.0011	0.171	-0.111	0.130	0.0002	0.0022
Hemostasis	373	<0.0001	<0.0001	0.184	0.094	-0.157	0.0019	<0.0001
Platelet activation	187	<0.0001	<0.0001	0.214	0.098	-0.190	0.0208	<0.0001
Elevated platelet cytosolic-Ca2+	97	0.0002	0.0005	0.252	0.148	-0.203	0.0117	0.0005
Platelet degranulation	93	0.0001	0.0003	0.269	0.158	-0.218	0.0084	0.0003
Chondroitin sulfate metabolism	40	0.0040	0.0050	0.319	0.205	-0.244	0.0076	0.0044
Kainate receptor activation	20	0.0052	0.0060	0.439	0.339	-0.278	0.0086	0.0314
Basigin interactions	18	0.0042	0.0291	0.473	0.343	-0.326	0.0118	0.0168
Thrombin signalling through PARs	21	0.0013	0.0116	0.480	0.336	-0.343	0.0076	0.0065
Gβχ signalling through BTK	11	0.0029	0.0021	0.508	0.371	-0.346	0.00331	0.0468

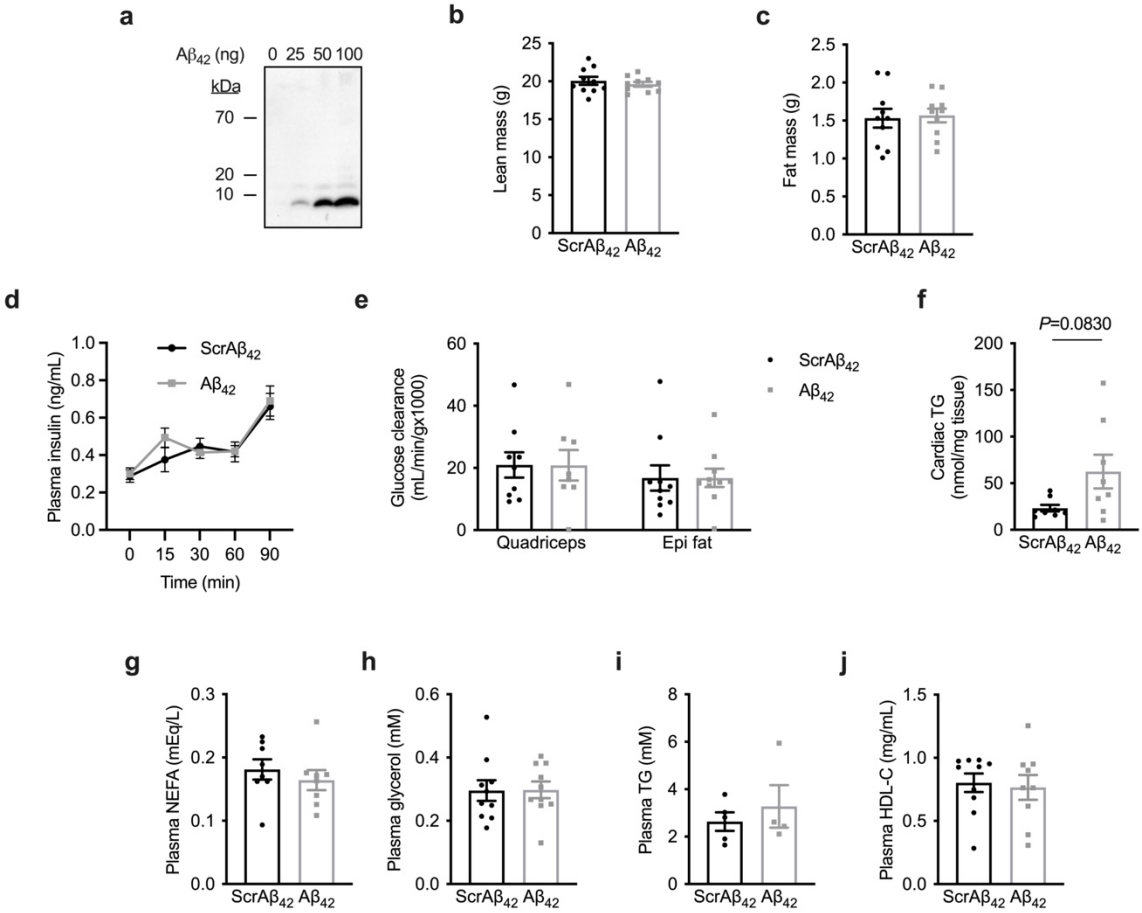
Supplementary Table 5: Reactome pathways reciprocally regulated in mice administered Aβ42 or 3D6 relative to their respective control groups, as determined by mitch. MANOVA p-values (both unadjusted and adjusted for multiple comparison testing) are for pathways in two dimensions. s.dist is the enrichment score across two dimensions. s.Aβ42 and s.3D6 are the enrichment scores for the Aβ42 and 3D6 datasets respectively. p.Aβ42 and p.3D6 indicate the significance of the pathway in the Aβ42 and 3D6 datasets respectively and have been adjusted for multiple comparisons using the FDR method. Source data are provided in the Source Data file.

Supplementary Figure 1



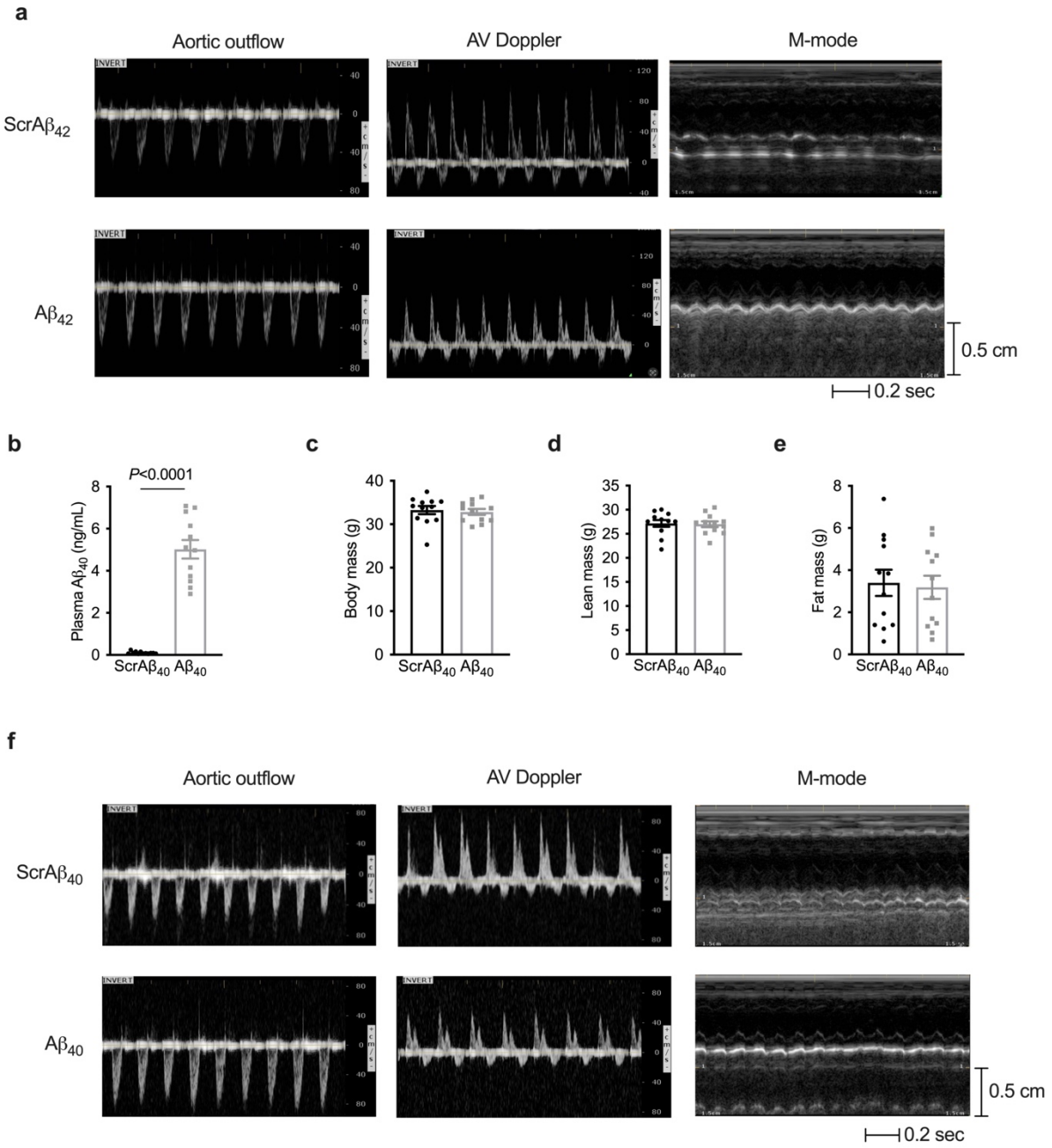
Supplementary Figure 1: $A\beta_{42}$ release by adipose tissue and body composition correlations with plasma $A\beta_{42}$. a, lean and fat mass in control and obese mice (n=12/group). **b,** fat pad mass in control and obese mice (n=12/group). **c,** relative release of $A\beta_{42}$ from adipose tissue exposed to vehicle or Brefeldin A (BFA; Mann-Whitney test, U=73; n=16/group). **d**, correlation between fat mass and plasma $A\beta_{42}$ (Pearsons correlation coefficient test; n= 20 data points). **e**, Correlation between lean mass and plasma $A\beta_{42}$ (Pearsons correlation coefficient test; n= 20 data points). All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 2



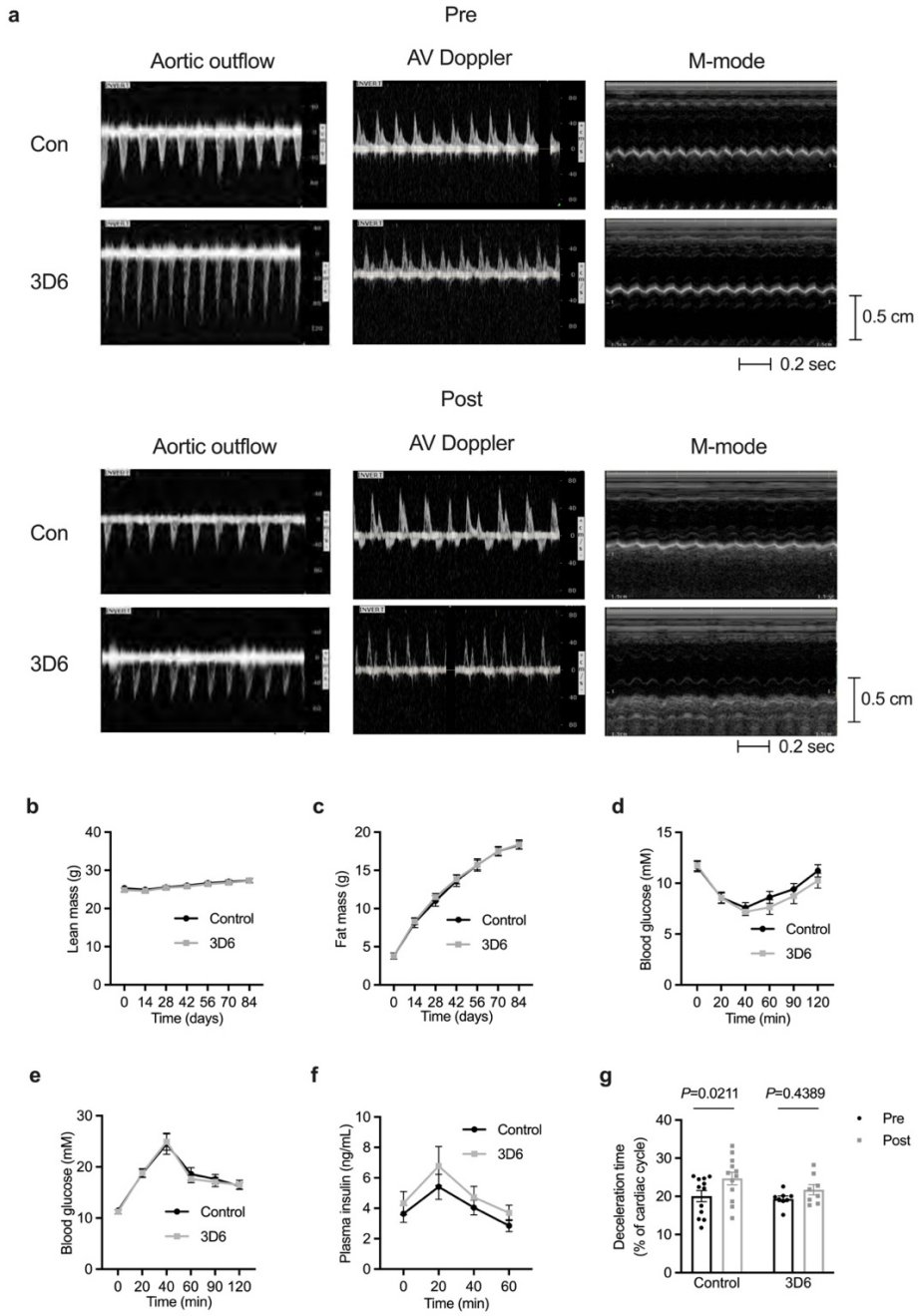
Supplementary Figure 2: Characterisation of mice administered ScrAβ$_{42}$ or Aβ$_{42}$. a, immunoblotting of increasing amounts of recombinant Aβ$_{42}$ preparations. **b,** lean mass (n=10/group); **c**, fat mass (n=10/group); **d**, plasma insulin during a glucose tolerance test (n=9 and 10/group respectively); **e** glucose clearance by the quadriceps skeletal muscle (n=9 and 8/group respectively) and epididymal fat pad (n=10/group); **f**, ^{14}C-glucose incorporation into cardiac lipids (n=8/group); **g**, plasma non-esterified fatty acids (NEFA; n=8/group); **h**, plasma glycerol (n=10/group); **i**, plasma triglycerides (TG; n=5 and 4/group respectively), and; **j**, plasma high-density lipoprotein cholesterol (HDL-C; n=10 and 9/group respectively) in mice administered ScrAβ$_{42}$ or Aβ$_{42}$. All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 3



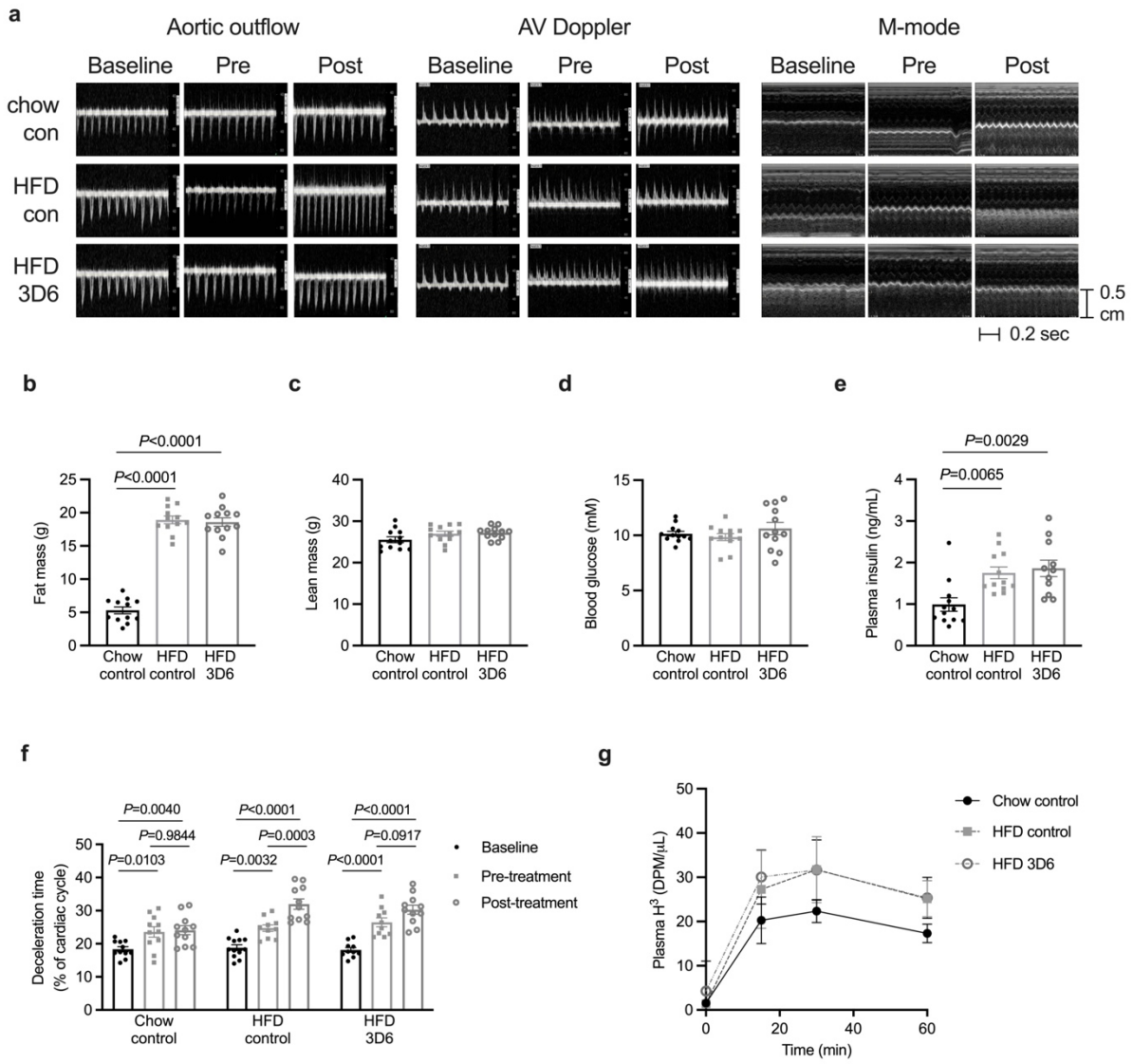
Supplementary Figure 3: Characterisation of mice administered ScrAβ42 or Aβ42 and ScrAβ40 or Aβ40. a, representative echocardiography images of mice administered ScrAβ42 or Aβ42. **b**, plasma Aβ40 60 min after Aβ40 administration (n=12/group). **c**, body weight (n=12/group); **d**, lean mass (n=12/group); **e**, fat mass (n=12/group), and; **f**, representative echocardiography images in mice administered ScrAβ40 or Aβ40. All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 4



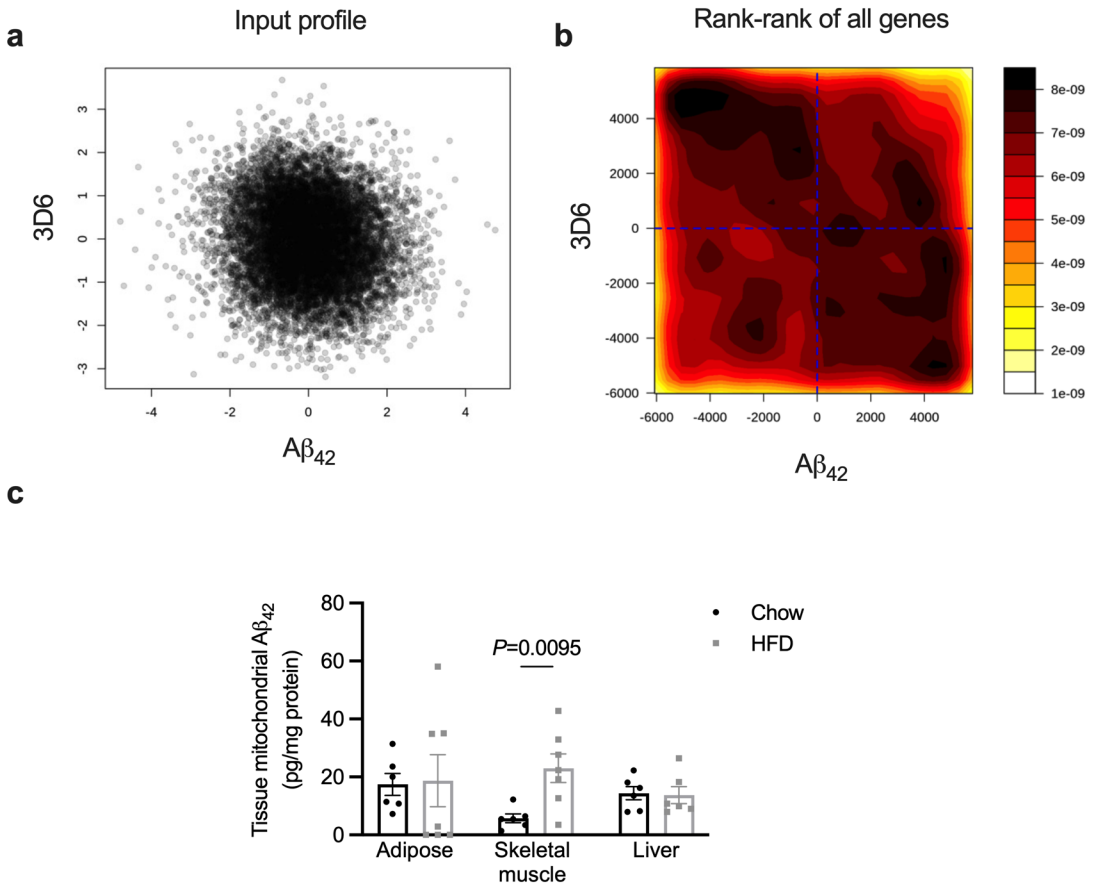
Supplementary Figure 4: Characterisation of mice fed a high fat diet and administered control or 3D6 antibodies. a, representative echocardiography images; **b**, lean mass (n=12/group); **c**, fat mass (n=12/group), **d**, blood glucose during the insulin tolerance test (n=12/group); **e**, blood glucose during the glucose tolerance test (n=11 and 12/group respectively); **f**, plasma insulin during the glucose tolerance test (n=9 and 10/group respectively), and; **g**, cardiac deceleration time expressed as a percentage of the cardiac cycle (mixed effects model (time $P = 0.0140$, $F(1,18) = 7.407$) with Sidak's repeated measures test *P.*adjusted; n=12 and 8/group respectively) pre- and post-treatment in mice fed a high fat diet and administered control and 3D6 antibodies. All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 5



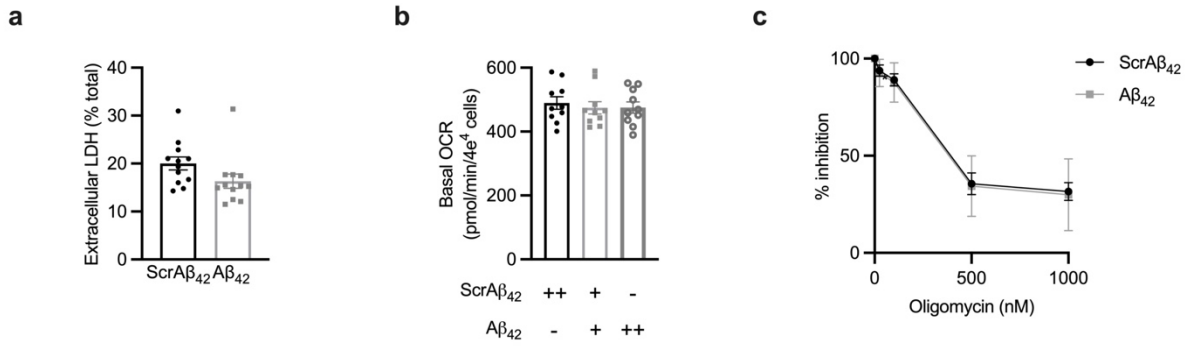
Supplementary Figure 5: Characterisation of mice fed a high fat diet and administered control or 3D6 antibodies. a, representative echocardiography images; **b**, fat mass (one-way ANOVA (P < 0.0001; F(2,33) = 177.6) with Sidak's repeated measures test P.adjusted; n=12/group); **c**, lean mass (n=12/group), **d**, fasting blood glucose (n=12/group); **e**, fasting plasma insulin (Kruskal-Wallis test (P = 0.0011; X^2 = 13.66) with Dunn's repeated measures test P.adjusted; n=12, 12 and 11/group respectively). **f**, cardiac deceleration time expressed as a percentage of the cardiac cycle (mixed effects model (time P < 0.0001, F(2,56) = 56.37; treatment P = 0.0044, F(2,32) = 6.445; interaction P = 0.0145, F(4,56) = 3.410) with Sidak's repeated measures test P.adjusted; n=12/group); **g,** plasma ^{3}H concentration in mice fed regular chow and administered control antibody, or mice fed a high fat diet (HFD) and administered control or 3D6 antibodies (n=7, 8 and 8/group respectively). All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 6



Supplementary Figure 6: Mechanisms by which Aβ$_{42}$ has its effects on the heart. a, input profile defined as differential expression score defined as the sign of the fold change multiplied by the -log10(p-value); **b**, gene ranks from bulk RNA-seq analysis of gene expression in the hearts of mice administered Aβ$_{42}$ compared with mice administered ScrAβ$_{42}$ (x-axis) and hearts of mice administered 3D6 antibody compared with mice administered control antibody (y-axis). **c,** Aβ$_{42}$ in mitochondrial fractions isolated from adipose tissue, skeletal muscle and liver of chow or HFD-fed mice (unpaired t-test; n=6 and 7/group respectively). All data are mean ± SEM. Statistical tests are two-tailed. Source data are provided in the Source Data file.

Supplementary Figure 7



Supplementary Figure 7: Effect of $A\beta_{42}$ on cardiomyocytes. a, extracellular lactate dehydrogenase (LDH) in primary neonatal ventricular cardiomyocytes (NVCM) exposed to $ScrAb_{42}$ or Ab_{42} for 48 hr (n=12 biological replicates/group). **b,** basal oxygen consumption rate (OCR) in FAO hepatocytes exposed to $ScrA\beta_{42}$ or $A\beta_{42}$ ($ScrA\beta_{42}$ at 300, 100 and 0pM and co-incubated with $A\beta_{42}$ at 0, 200 and 300pM) for 48 hrs with glucose and pyruvate as exogenous substrates (n=10 biological replicates/group). **c,** inhibition of respiration in response to increasing concentrations of oligomycin in primary NVCM exposed to $ScrA\beta_{42}$ or $A\beta_{42}$ for 48 hrs (n=5 biological replicates/group). All data are mean ± SEM. Source data are provided in the Source Data file.