Supplementary Tables

Table S1. Differential gene expression on the transcriptional and translational level as assessed by DESeq2 (FDR = 0.05). Green depicts down- and blue indicates up-regulated genes in TTNtv animals.

		log 2 Fold Change TTNtv / WT				Adj. P-value TTNtv vs WT			
		RNA-seq		Ribo-seq		RNA-seq		Ribo-seq	
Ensembl ID	**Gene Symbol**	**TTNtvA**	**TTNtvZ**	**TTNtvA**	**TTNtvZ**	**TTNtvA**	**TTNtvZ**	**TTNtvA**	**TTNtvZ**
ENSRNOG00000000164	Lamp2	0.01	0.03	0.18	0.18	0.028	n.s.	n.s.	n.s.
ENSRNOG00000001522	Metap1d	-0.08	-0.06	-0.03	0.04	n.s.	1.2E-06	n.s.	n.s.
ENSRNOG00000003189	Cited1	0.14	0.33	0.11	0.19	n.s.	0.011	n.s.	n.s.
ENSRNOG00000003284	Epn3	0.29	0.41	0.04	0.06	n.s.	0.002	n.s.	n.s.
ENSRNOG00000003626	Atp5h	-0.01	0.03	-0.08	0.05	0.002	0.001	n.s.	n.s.
ENSRNOG00000004078	Eno3	-0.23	-0.18	-0.39	-0.27	n.s.	n.s.	5.3E-06	0.024
ENSRNOG00000005670	Art4	0.43	0.40	0.39	0.49	5.6E-04	2.2E-03	n.s.	0.003
ENSRNOG00000006509	Srgap3	0.25	0.31	-0.02	0.20	n.s.	0.028	n.s.	n.s.
ENSRNOG00000007545	Angptl4	0.09	0.25	0.03	0.30	n.s.	0.016	n.s.	n.s.
ENSRNOG00000007674	Nacad	0.27	0.36	0.18	0.35	n.s.	0.013	n.s.	n.s.
ENSRNOG00000009691	Lrrn2	0.45	0.44	0.21	0.17	1.7E-04	3.1E-04	n.s.	n.s.
ENSRNOG00000011076	Ank2	-0.02	-0.08	0.23	0.33	n.s.	n.s.	n.s.	0.016
ENSRNOG00000011491	Dnajc13	-0.04	-0.04	0.08	0.17	n.s.	0.008	n.s.	n.s.
ENSRNOG00000014933	Dnttip1	0.03	-0.03	0.01	-0.01	3.3E-03	0.000	n.s.	n.s.
ENSRNOG00000015852	Arhgap35	0.02	-0.04	0.14	0.08	n.s.	0.016	n.s.	n.s.
ENSRNOG00000015912	Ptdss2	-0.06	-0.10	0.06	0.06	n.s.	0.003	n.s.	n.s.
ENSRNOG00000016483	Myo16	0.49	0.64	0.37	0.45	3.4E-05	5.8E-10	n.s.	n.s.
ENSRNOG00000016892	Nr2f6	-0.05	-0.23	-0.10	-0.03	n.s.	0.028	n.s.	n.s.
ENSRNOG00000017460	Imp3	0.00	-0.12	0.01	0.01	n.s.	0.003	n.s.	n.s.
ENSRNOG00000017503	Ppargc1b	-0.14	-0.24	-0.17	-0.22	0.001	0.001	n.s.	n.s.
ENSRNOG00000017786	Acta1	-0.42	-0.39	-0.53	-0.41	n.s.	n.s.	0.003	n.s.
ENSRNOG00000018610	Pde6d	0.05	-0.04	0.05	0.02	1.8E-08	0.003	n.s.	n.s.
ENSRNOG00000019028	Znrf2	-0.06	-0.13	-0.04	0.00	n.s.	0.003	n.s.	n.s.
ENSRNOG00000019094	Gstm2	0.06	0.04	0.03	-0.03	n.s.	0.016	n.s.	n.s.
ENSRNOG00000020263	Atp1a3	0.52	0.34	0.99	0.71	n.s.	n.s.	n.s.	1.8E-06
ENSRNOG00000020373	Dap3	-0.04	-0.01	-0.14	-0.12	n.s.	0.008	n.s.	n.s.
ENSRNOG00000020417	Gsk3a	-0.03	-0.07	-0.09	0.00	0.001	0.003	n.s.	n.s.
ENSRNOG00000020641	RGD1560789	-0.16	-0.32	-0.21	-0.08	n.s.	0.034	n.s.	n.s.
ENSRNOG00000020650	Slc17a7	0.49	0.53	0.31	0.34	3.4E-05	2.5E-06	n.s.	n.s.
ENSRNOG00000020915	Setd2	-0.05	-0.12	-0.11	-0.10	n.s.	0.001	n.s.	n.s.
ENSRNOG00000021095	Fxyd3	0.40	0.33	0.25	0.26	6.5E-04	1.6E-02	n.s.	n.s.
ENSRNOG00000021242	Adam33	0.27	0.48	0.09	0.18	n.s.	0.000	n.s.	n.s.
ENSRNOG00000022178	Dcdc5	-0.25	-0.35	-0.19	-0.28	n.s.	0.015	n.s.	n.s.
ENSRNOG00000025518	Lrrc16b	0.19	0.30	-0.05	0.16	n.s.	0.001	n.s.	n.s.
ENSRNOG00000027959	Ppp1r12c	-0.13	-0.13	-0.25	-0.45	n.s.	n.s.	n.s.	0.001
ENSRNOG00000028498	Wdr1	-0.05	-0.02	-0.05	-0.04	n.s.	0.016	n.s.	n.s.
ENSRNOG00000029662	Wdfy4	0.51	0.45	0.32	0.39	1.5E-07	1.4E-05	n.s.	n.s.
ENSRNOG00000030285	Epha3	-0.01	0.16	0.23	0.42	n.s.	n.s.	n.s.	0.016
ENSRNOG00000031890	Ncam1	0.36	0.37	0.38	0.43	n.s.	n.s.	0.014	0.001
ENSRNOG00000033893	Cacna1h	0.34	0.36	0.24	0.30	0.034	0.008	n.s.	n.s.
ENSRNOG00000039582	RGD1561161	0.19	0.15	0.28	0.37	n.s.	n.s.	n.s.	0.039
ENSRNOG00000040201	Atp6ap1l	0.28	0.38	0.22	0.34	n.s.	0.001	n.s.	n.s.
ENSRNOG00000042245	Dcaf7	-0.06	-0.12	0.03	-0.03	0.034	n.s.	n.s.	n.s.
ENSRNOG00000045992	Tlr8	-0.02	-0.06	0.40	0.42	n.s.	n.s.	n.s.	0.029
ENSRNOG00000046468	Ptgfr	0.31	0.49	0.41	0.57	n.s.	n.s.	n.s.	0.001
ENSRNOG00000046772	Fam83c	0.26	0.35	0.11	0.08	n.s.	0.016	n.s.	n.s.
ENSRNOG00000050145	Ahnak	0.08	0.01	0.32	0.35	n.s.	n.s.	n.s.	0.030
ENSRNOG00000050841	Ache	0.11	0.36	0.13	0.28	n.s.	0.003	n.s.	n.s.

Table S2. Metabolite measurements of acylcarnitines from WT and TTNtv rat hearts.

Acylcarnitine	Concentration (pmol/mg tissue)								TTNtv/WT Ratio	TTNtv/WT *P* value
	WT (n=5)		TTNtvA (n=5)		TTNtvZ (n=5)		TTNtv			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
C2	394.91	43.55	528.12	104.03	297.18	86.89	412.65	137.78	1.04	0.717
C3	4.36	0.99	5.76	1.68	4.84	1.84	5.30	1.80	1.22	0.213
C4	8.99	1.78	11.11	1.26	7.79	1.65	9.45	2.23	1.05	0.693
C5:1	0.56	0.11	0.50	0.08	0.61	0.18	0.55	0.14	0.99	0.966
C5	1.26	0.43	1.24	0.42	1.78	0.90	1.51	0.69	1.20	0.404
C4-OH	8.02	2.43	9.98	2.85	8.20	5.40	9.09	4.21	1.13	0.544
C6	3.16	0.92	2.45	0.80	1.40	0.58	1.93	0.71	0.61	0.036
C5-OH/C3-DC	3.67	0.51	4.23	0.66	3.08	0.53	3.66	0.82	1.00	0.971
C4-DCC6-OH	10.77	1.78	13.32	2.02	9.63	2.12	11.47	2.63	1.07	0.553
C8	1.14	0.43	0.87	0.36	0.53	0.23	0.70	0.25	0.61	0.081
C5-DC	0.49	0.05	0.50	0.05	0.41	0.05	0.45	0.07	0.92	0.226
C8-OH/C6-DC	0.38	0.08	0.41	0.14	0.20	0.10	0.30	0.16	0.80	0.235
C12:1	0.54	0.20	0.49	0.17	0.33	0.09	0.41	0.11	0.76	0.235
C12	1.91	0.71	1.54	0.61	0.95	0.49	1.25	0.46	0.66	0.110
C12:1-OH	0.09	0.02	0.08	0.03	0.05	0.02	0.06	0.02	0.68	**0.041**
C12-OH/C10-DC	0.12	0.03	0.12	0.03	0.07	0.04	0.10	0.04	0.78	0.139
C14:2	0.70	0.32	0.55	0.30	0.47	0.22	0.51	0.19	0.73	0.270
C14:1	2.93	0.97	2.68	0.79	1.95	0.80	2.31	0.68	0.79	0.251
C14	7.54	2.77	5.97	2.56	4.61	1.68	5.29	1.36	0.70	0.147
C14:2-OH	0.41	0.16	0.30	0.18	0.33	0.21	0.31	0.15	0.75	0.271
C14:1-OH	1.48	0.47	1.20	0.54	0.81	0.31	1.00	0.34	0.68	0.090
C14-OH/C12-DC	0.80	0.13	0.74	0.13	0.63	0.28	0.69	0.20	0.86	0.219
C16:2	1.42	0.61	1.24	0.50	1.11	0.39	1.17	0.29	0.83	0.431
C16:1	7.94	2.46	6.81	1.84	4.85	2.55	5.83	2.02	0.73	0.142
C16	39.33	12.07	29.09	11.32	21.63	9.28	25.36	7.62	0.64	0.059
C16:2-OH	0.50	0.13	0.38	0.15	0.32	0.12	0.35	0.10	0.70	0.069
C16:1-OH/C14:1-DC	1.71	0.46	1.48	0.46	1.28	0.59	1.38	0.42	0.81	0.213
C16-OH	2.11	0.35	2.11	0.22	1.94	0.93	2.03	0.65	0.96	0.760
C18:3	2.29	1.04	1.35	1.05	1.05	0.24	1.20	0.31	0.52	0.077
C18:2	17.85	8.08	11.51	7.94	9.49	2.42	10.50	2.42	0.59	0.111
C18:1	39.98	13.59	29.46	12.27	20.50	10.26	24.98	8.64	0.62	0.068
C18	12.03	3.78	6.56	4.63	6.02	1.85	6.29	1.55	0.52	**0.025**
C18:2-OH/C16:2-DC	0.94	0.32	0.85	0.25	1.02	0.57	0.93	0.39	1.00	0.993
C18:1-OH/C16:1-DC	2.62	0.59	2.58	0.37	2.27	1.20	2.43	0.84	0.93	0.618
C18-OH/C16-DC	0.71	0.20	0.60	0.19	0.58	0.25	0.59	0.17	0.83	0.265
C20:4	1.72	0.64	0.92	0.82	0.85	0.36	0.89	0.35	0.51	**0.040**
C20:3	0.51	0.17	0.32	0.20	0.31	0.13	0.32	0.10	0.62	0.067
C20:2	0.71	0.19	0.65	0.13	0.70	0.48	0.68	0.33	0.95	0.794
C20:1	1.37	0.37	1.20	0.29	1.19	0.71	1.20	0.49	0.88	0.468
C20	0.31	0.09	0.24	0.08	0.20	0.11	0.22	0.08	0.71	0.098
C20:1-OH/C18:1-DC	0.32	0.08	0.34	0.03	0.29	0.16	0.32	0.11	1.00	0.985
C20-OH/C18-DC	0.15	0.06	0.11	0.06	0.08	0.02	0.09	0.03	0.60	0.094
C22:4	0.12	0.04	0.07	0.06	0.07	0.05	0.07	0.05	0.59	0.076
C22:1	0.13	0.04	0.14	0.03	0.12	0.05	0.13	0.04	1.04	0.798
C22	0.11	0.03	0.15	0.02	0.07	0.01	0.11	0.05	1.04	0.825

Table S3. Metabolite measurements of glycolysis intermediates from WT and TTNtv hearts.

Metabolite	Concentration (nmol/g tissue)								TTNtv/WT	
	WT (n=6)		TTNtvA (n=6)		TTNtvZ (n=6)		TTNtv			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Ratio	*P* value
Dihydroxyacetone phosphate	*176*	*64*	*183*	*49*	*172*	*46*	*177*	*46*	*1*	*0.96*
Fructose 1,6-diphosphate	*271*	*129*	*297*	*138*	*299*	*127*	*298*	*126*	*1.1*	*0.68*
Fructose 6-phosphate	*349*	*156*	*380*	*152*	*409*	*167*	*394*	*153*	*1.1*	*0.57*
Glucose 1-phosphate	*117*	*50*	*125*	*49*	*132*	*54*	*129*	*49*	*1.1*	*0.63*
Glucose 6-phosphate	*1460*	*715*	*1605*	*709*	*1773*	*805*	*1689*	*728*	*1.2*	*0.54*
Lactic acid	*13744*	*2540*	*15430*	*2501*	*14835*	*3163*	*15132*	*2736*	*1.1*	*0.31*
Phosphoenolpyruvic acid	*10*	*2*	*10*	*2*	*11*	*1*	*11*	*2*	*1.1*	*0.43*

Table S4. TTNtvs in expressed exons (PSI>15%) in healthy individuals who underwent cardiac MRI in the Digital Heart Project.

Sample identification	Hg19 genomic start position	LRG genomic start position	Transcript effect	Protein effect	Exon number	Median exon PSI	Present in N2BA	Present in N2B	Band	Variant type	ExAC frequency
14AB03203	179629515	71015	c.9727C>T	p.Q3243X	42	100	Y	Y	I-band	Nonsense	0
14ZN01340	179595884	104646	c.17508dupA	NA	61	35	Y		I-band	Frameshift	0
14JD01896	179588844	111686	c.21142C>T	p.R7048X	74	25	Y		I-band	Nonsense	0.00002
14AL03036	179563642	136888	c.30683-1G>T	NA	113	80	Y		I-band	Splice acceptor variant	0
14EC01433	179560998	139532	c.30803-2A>G	NA	115	74	Y		I-band	Splice acceptor variant	0
14MS02961	179554624	145906	c.31763-1G>A	NA	123	78	Y		I-band	Splice acceptor variant	0.00027
14RH03039	179554624	145906	c.31763-1G>A	NA	123	78	Y		I-band	Splice acceptor variant	0.00027
14CB02200	179506963	193567	c.40558+1G>A	NA	220	100	Y	Y	I-band	Splice donor variant	0.00012
14HB02599	179495671	204859	c.44015-1G>T	NA	239	100	Y	Y	I-band	Splice acceptor variant	0
14MO01427	179486054	214476	c.45391delA	NA	247	100	Y	Y	I-band	Frameshift	0
14RS02105	179479631	220899	c.48703C>T	p.Q16235X	261	100	Y	Y	A-band	Nonsense	0
14JM01448	179444855	255675	c.67159delA	p.I22387X	319	100	Y	Y	A-band	Frameshift	0
14SM01546	179444666	255864	c.67348C>T	p.Q22450X	319	100	Y	Y	A-band	Nonsense	0
14JC01930	179404286	296244	c.98506C>T	p.R32836X	353	100	Y	Y	A-band	Nonsense	0
14TW02859	179394786	305744	c.106432G>T	p.E35478X	360	100	Y	Y	M-band	Nonsense	0

Table S5: For each cardiac phenotype two linear regression models were built to adjust for the known contribution of clinical variables. The first model was optimized to minimize the Bayesian Information Criterion. To the optimized first model, the *ttn* genotype was then added to create a second model and details of this presented here. The two models were then compared using ANOVA. BSA = body surface area; LVEDV = left ventricular end diastolic volume; LVESV = left ventricular end systolic volume; LVEF = left ventricular ejection fraction; LVM = left ventricular mass; SBP = systolic blood pressure; TTNtv = titin truncating variants.

Phenotype	Model Terms	Unstandardized Coefficient	Standardized Coefficient	Standard Error	Component P value	Full model		P value for comparison between models
						Adjusted R^2	P value	
LVEDV (ml)	(Intercept)	-3.99	0	7.46	0.59	0.58	4.57 x10^{-261}	0.03
	Age (years)	-0.54	-0.23	0.04	1.13 x10^{-31}			
	Gender (male)	13.8	0.21	1.44	3.52 x10^{-21}			
	SBP (mmHg)	0.15	0.07	0.04	0.001			
	Race	-4.64	-0.12	0.65	2.05 x10^{-12}			
	BSA (kg/m^2)	75.3	0.48	3.53	9.08 x10^{-88}			
	Activity Score	8.06	0.22	0.66	3.23 x10^{-32}			
	Heart Rate (bpm)	-0.13	-0.07	0.03	1.47 x10^{-05}			
	TTNtv	11.9	0.04	5.39	0.03			
LVESV (ml)	(Intercept)	4.58	0	4	0.25	0.43	5.4 x10^{-170}	0.015
	Age (years)	-0.29	-0.24	0.02	2.25 x10^{-30}			
	Gender (male)	9.06	0.28	0.83	1.33 x10^{-26}			
	Race	-1.8	-0.09	0.38	3.21 x10^{-06}			
	BSA (kg/m^2)	26.2	0.33	2.03	7.29 x10^{-36}			
	Activity Score	3.73	0.19	0.39	4.25 x10^{-21}			
	Heart Rate (bpm)	-0.04	-0.05	0.02	0.008			
	TTNtv	7.69	0.05	3.17	0.015			
LVEF (%)	(Intercept)	60.5	0	1.18	<1x10^{-100}	0.14	3.05 x10^{-43}	0.03
	Age (years)	0.07	0.18	0.01	4.79 x10^{-11}			
	Gender (male)	-2.95	0.27	0.28	7.93 x10^{-24}			
	SBP (mmHg)	0.04	0.12	0.01	2.12 x10^{-05}			
	Activity Score	-0.65	-0.11	0.16	3.32 x10^{-05}			
	TTNtv	-2.81	-0.05	1.29	0.03			