

Fig. S1. **A)** Sirius red staining quantification at 1 and 6 months. Amount of staining is quantified in percentage of the total tissue section (mean \pm SEM). **B)** Histological sections of heart from Wild Type and Δ Mex5 mice by Sirius Red at 4 months. Immunostaining of Collagen1a1 and Collagen 3a1 in Wild Type and Δ Mex5 mice hearts at 4 months. Merge. Scale bars, 100 μ m. **C)**, **D)**, **E)**, **F)** qPCR results for fibrosis and heart damage makers (Co3a1, Vim, Tgfb1, Timp1) relative expression in Wild Type and Δ Mex5 mice hearts at 4 months.

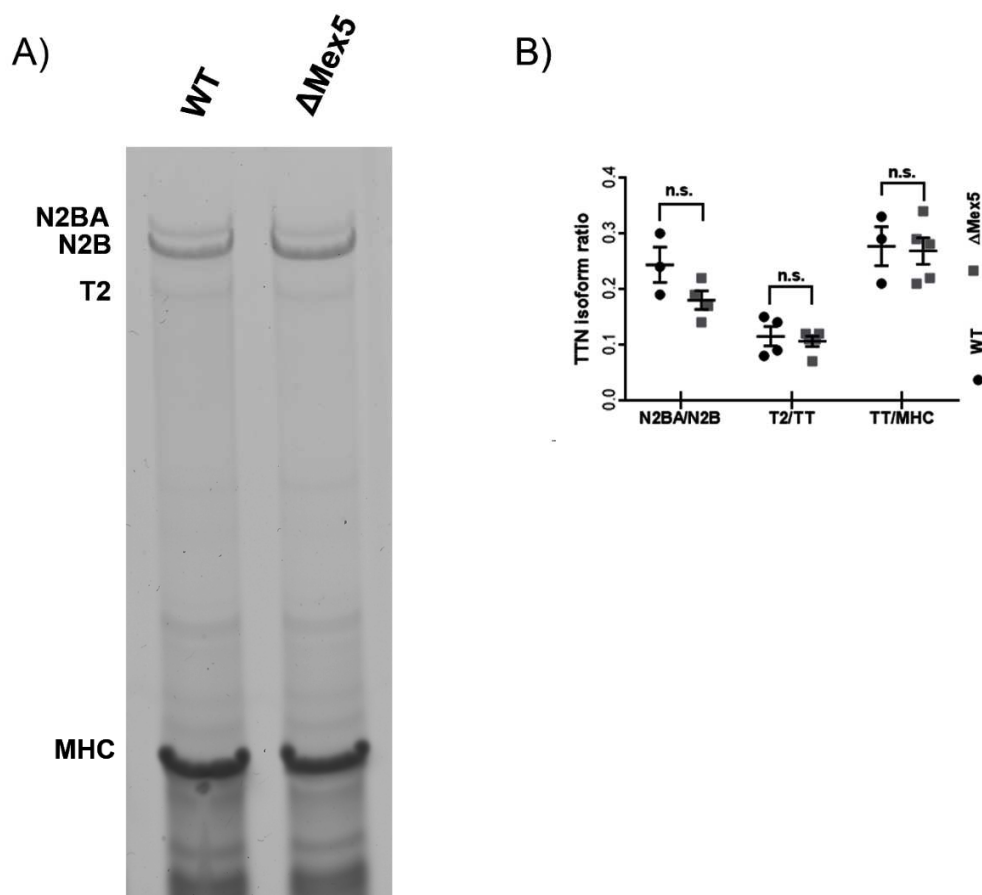


Fig. S2. **A)** Coomassie Blue gel staining on cardiac samples from Wild Type and Δ Mex5 mice at 4 months. T2 = titin degradation products. **B)** Quantification of Coomassie Blue gel. Results are presented in means \pm SEM. Unpaired Mann-Whitney test were used to compare groups.

Table S1. Echocardiographic parameters for WT and Δ Mex5 mice at different ages. Results are presented in means \pm SEM. Unpaired Mann-Whitney test were used to compare groups, * $P \leq 0.05$, ** $P \leq 0.01$.

		2 months	4 months	13 months
Mice number	WT	n = 7	n = 11	n = 6
	Δ Mex5	n = 6	n = 8	n = 7
Heart rate (bpm)	WT	454 \pm 19	472 \pm 24	484 \pm 23
	Δ Mex5	469 \pm 17	466 \pm 23	462 \pm 34
LV/body mass ratio	WT	4.8 \pm 0.1	4.9 \pm 0.2	4.3 \pm 0.3
	Δ Mex5	5.1 \pm 0.2	5.4 \pm 0.3	5.0 \pm 0.4
Left Ventricle Internal Diameter end Diastole (mm)	WT	4.47 \pm 0.09	4.28 \pm 0.09	4.42 \pm 0.13
	Δ Mex5	4.74 \pm 0.11	4.83 \pm 0.22 **	5.23 \pm 0.17 **
Left Ventricle Internal Diameter end Systole (mm)	WT	3.59 \pm 0.12	3.13 \pm 0.12	3.28 \pm 0.16
	Δ Mex5	4.04 \pm 0.18	3.88 \pm 0.26 **	4.24 \pm 0.21 **
LV posterior wall (mm)	WT	0.59 \pm 0.02	0.77 \pm 0.03	0.88 \pm 0.06
	Δ Mex5	0.57 \pm 0.03	0.83 \pm 0.07	0.75 \pm 0.06
Intra ventricular septum (mm)	WT	0.80 \pm 0.01	0.90 \pm 0.04	0.96 \pm 0.03
	Δ Mex5	0.78 \pm 0.03	1.00 \pm 0.05	0.88 \pm 0.05
Shortening Fraction (%)	WT	20 \pm 1	27 \pm 2	26 \pm 1
	Δ Mex5	15 \pm 2	20 \pm 3 *	19 \pm 2 *

Table S2. Measures of pressure/volumes parameters for WT and Δ Mex5 mice at 4 months. Results are presented in means \pm SEM. **EDPVR** : End Diastolic Pressure Volume Relationship. Unpaired Mann-Whitney test were used to compare groups.

Mice number	WT	n	=	5	
	Δ Mex5	n	=	5	
Heart rate (bpm)	WT	515	\pm	24	
	Δ Mex5	470	\pm	25	$p = 0.421$
End Systolic Elastance (mmHg/μL)	WT	4.08	\pm	0.66	
	Δ Mex5	2.51	\pm	0.67	$p = 0.151$
EDPVR – β (mmHg/μL)	WT	0.03	\pm	0.01	
	Δ Mex5	0.03	\pm	0.00	$p = 0.548$
End Systolic Volume (μL)	WT	26	\pm	4	
	Δ Mex5	56	\pm	11	$p = 0.032$ *
End Diastolic Volume (μL)	WT	67	\pm	5	
	Δ Mex5	91	\pm	11	$p = 0.095$
Stroke Volume (μL)	WT	40	\pm	3	
	Δ Mex5	35	\pm	1	$p = 0.151$
End Systolic Pressure (mmHg)	WT	90	\pm	4	
	Δ Mex5	89	\pm	4	$p = 0.841$
End Diastolic Pressure (mmHg)	WT	2	\pm	0	
	Δ Mex5	5	\pm	1	$p = 0.032$ *
dP/dtmin (mmHg/sec)	WT	- 7733	\pm	726	
	Δ Mex5	- 6823	\pm	554	$p = 0.310$
dP/dtmax (mmHg/sec)	WT	9155	\pm	984	
	Δ Mex5	7903	\pm	562	$p = 0.310$
Ejection Fraction (SV/EDV)	WT	61	\pm	3	
	Δ Mex5	41	\pm	5	$p = 0.016$ *

Table S3. Top 25 up regulated genes identified in RNAseq analysis of Δ Mex5 mice versus WT at 4 months. Results are sorted by absolute value of log₂FC.

Gene name	log ₂ FC	pValue	Mean WT	Mean Δ Mex5
Spp1	6.60	5.3E-128	6.65	2162.74
Cilp	4.77	4.7E-278	109.77	3357.31
Ltbp2	4.74	3.0E-174	68.03	2206.18
Gpnmb	4.68	1.6E-97	19.98	764.57
Sprr1a	4.33	1.4E-36	1.39	222.75
Tnc	4.28	3.0E-33	38.66	4363.96
Timp1	3.98	5.7E-52	24.25	625.54
Col12a1	3.82	2.1E-60	50.08	989.43
Col8a2	3.74	3.3E-40	10.59	247.08
Sfrp2	3.62	2.1E-55	30.20	501.17
Thbs4	3.61	3.8E-121	66.85	919.77
Ptn	3.40	1.3E-35	17.60	290.91
Postn	3.35	1.7E-21	414.51	12040.11
Mfap4	3.31	4.0E-57	35.05	428.05
Piezo2	3.27	2.9E-31	13.45	209.01
Col3a1	3.22	1.7E-61	3682.59	40685.43
Col14a1	3.20	9.5E-116	207.54	2081.22
Ctss	3.19	3.0E-59	162.81	1773.00
Trem2	3.16	2.9E-28	17.93	259.21
Atp6v0d2	3.15	2.7E-17	0.67	58.07
Apol7d	3.15	5.2E-32	41.67	541.35
Lgals3	3.10	7.0E-19	35.14	747.38
Mpeg1	3.09	8.8E-23	143.04	2205.83
Dkk3	3.01	2.8E-32	27.12	299.63
P4ha3	2.96	1.6E-14	0.33	54.09

Table S4. Top 25 down regulated genes identified in RNAseq analysis of Δ Mex5 mice versus WT at 4 months. Results are sorted by absolute value of log₂FC.

Gene name	log ₂ FC	pValue	Mean WT	Mean Δ Mex5
Osbp16	-1.76	2.3E-21	515.48	140.16
Zfp619	-1.58	1.9E-08	103.02	28.98
Pde11a	-1.56	2.7E-04	21.94	1.85
Lgals4	-1.49	0.001	561.89	85.54
Mylk4	-1.42	1.6E-21	2880.01	1036.29
Rtn4r	-1.40	7.2E-06	75.68	23.33
Ano5	-1.39	1.4E-05	80.70	24.81
Sv2c	-1.39	2.5E-04	38.48	10.09
Pah	-1.35	0.002	19.93	3.24
Kcnv2	-1.32	1.3E-07	279.05	100.09
mt-Ts1	-1.29	0.002	333.81	84.07
Pop4	-1.26	1.6E-07	138.11	52.85
Wnk2	-1.24	3.6E-21	1894.07	782.52
Cln1	-1.18	5.5E-10	256.20	106.65
Whrn	-1.16	8.3E-10	398.94	168.86
Pzp	-1.16	0.006	32.33	9.46
Celsr3	-1.16	5.7E-06	107.32	43.91
Clec18a	-1.13	0.008	34.03	10.35
Lrrc52	-1.11	0.001	73.90	28.82
mt-Ti	-1.09	5.0E-23	4813.41	2232.44
Ctla4	-1.08	0.002	59.32	23.36
Apob	-1.08	0.015	22.66	1.83
Lmtd1	-1.06	0.015	26.52	8.54
Gal3st3	-1.05	8.7E-06	236.70	106.10
mt-Tm	-1.04	5.9E-07	2037.16	939.00

Table S5. Top 25 up regulated GO biological processes identified in RNAseq analysis of ΔMex5 mice versus WT at 4 months.

Gene Set ID	set Size	NES	pValue adjusted
GO_EXTRACELLULAR_STRUCTURE_ORGANIZATION	319	2.087	2.40E-34
GO_LEUKOCYTE_MIGRATION	330	2.014	1.24E-28
GO_REGULATION_OF_CELL_ACTIVATION	437	1.890	1.69E-25
GO_MYELOID_LEUKOCYTE_MEDIATED_IMMUNITY	451	1.835	2.95E-22
GO_LEUKOCYTE_DIFFERENTIATION	401	1.856	3.71E-22
GO_ADAPTIVE_IMMUNE_RESPONSE	273	1.966	3.07E-20
GO_POSITIVE_REGULATION_OF_IMMUNE_RESPONSE	480	1.773	2.07E-19
GO_WOUND_HEALING	412	1.800	2.07E-19
GO_POSITIVE_REGULATION_OF_CYTOKINE_PRODUCT ION	356	1.844	5.70E-19
GO_T_CELL_ACTIVATION	354	1.834	8.88E-19
GO_POSITIVE_REGULATION_OF_CELL_ACTIVATION	264	1.938	2.31E-18
GO_POSITIVE_REGULATION_OF_CELLULAR_COMPON ENT_MOVEMENT	476	1.740	5.31E-18
GO_CELL_CHEMOTAXIS	226	1.956	2.30E-17
GO_MYELOID_LEUKOCYTE_MIGRATION	158	2.090	2.30E-17
GO_PHAGOCYTOSIS	227	1.960	4.18E-17
GO_REGULATION_OF_INFLAMMATORY_RESPONSE	275	1.894	4.25E-17
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_EXTE RNAL_STIMULUS	397	1.767	4.95E-17
GO_POSITIVE_REGULATION_OF_CELL_ADHESION	330	1.823	9.15E-17
GO_REGULATION_OF_LYMPHOCYTE_ACTIVATION	333	1.821	1.86E-16
GO_SISTER_CHROMATID_SEGREGATION	169	2.028	3.91E-16
GO_LEUKOCYTE_CHEMOTAXIS	168	2.019	1.08E-15
GO_TAXIS	485	1.701	1.47E-15
GO_REGULATION_OF_LEUKOCYTE_MIGRATION	167	2.015	1.50E-15
GO_MITOTIC_SISTER_CHROMATID_SEGREGATION	146	2.063	1.81E-15
GO_RESPONSE_TO_BACTERIUM	410	1.726	2.76E-15

Table S6. Top 25 down regulated GO biological processes identified in RNAseq analysis of ΔMex5 mice versus WT at 4 months.

Gene Set ID	set Size	NES	pValue adjusted
GO_MITOCHONDRIAL_RESPIRATORY_CHAIN_COMPLEX_ASSEMBLY	99	-3.478	1.77E-22
GO_RESPIRATORY_ELECTRON_TRANSPORT_CHAIN	110	-3.234	1.15E-20
GO_AEROBIC_RESPIRATION	82	-3.338	5.52E-19
GO_MITOCHONDRIAL_ELECTRON_TRANSPORT_NADH_TO_UBIQUINONE	52	-3.340	6.52E-19
GO_ATP_SYNTHESIS_COUPLED_ELECTRON_TRANSPORT	92	-3.376	2.84E-18
GO_NADH_DEHYDROGENASE_COMPLEX_ASSEMBLY	63	-3.317	8.40E-18
GO_MITOCHONDRIAL_TRANSLATIONAL_TERMINATION	89	-3.233	8.09E-17
GO_TRANSLATIONAL_TERMINATION	102	-3.126	3.07E-15
GO_TRICARBOXYLIC_ACID_CYCLE	32	-3.001	5.23E-11
GO_INNER_MITOCHONDRIAL_MEMBRANE_ORGANIZATION	51	-2.865	1.50E-10
GO_MITOCHONDRIAL_TRANSMEMBRANE_TRANSPORT	96	-2.680	5.44E-10
GO_FATTY_ACID_BETA_OXIDATION	64	-2.747	3.49E-09
GO_MONOCARBOXYLIC_ACID_CATABOLIC_PROCESS	108	-2.469	2.76E-08
GO_CRISTAE_FORMATION	34	-2.803	4.41E-08
GO_ATP_SYNTHESIS_COUPLED_PROTON_TRANSPORT	25	-2.678	1.55E-07
GO_FATTY_ACID_CATABOLIC_PROCESS	93	-2.421	2.94E-07
GO_2_OXOGLUTARATE_METABOLIC_PROCESS	14	-2.475	1.06E-06
GO_CELLULAR_AMINO_ACID_CATABOLIC_PROCESS	91	-2.279	4.26E-06
GO_FATTY_ACID_BETA_OXIDATION_USING_ACYL_COA_DEHYDROGENASE	11	-2.383	5.31E-06
GO_PROTEIN_TRANSMEMBRANE_IMPORT_INTO_INTRACELLULAR_ORGANELLE	33	-2.521	1.49E-05
GO_LIPID_OXIDATION	92	-2.199	1.89E-05
GO_THIOESTER_METABOLIC_PROCESS	82	-2.209	2.03E-05
GO_MITOCHONDRIAL_RNA_METABOLIC_PROCESS	43	-2.407	2.39E-05
GO_MITOCHONDRIAL_ELECTRON_TRANSPORT_UBIQUINOL_TO_CYTOCHROME_C	13	-2.336	2.81E-05
GO_AEROBIC_ELECTRON_TRANSPORT_CHAIN	20	-2.410	2.85E-05

Table S7. M10 Binding partner values obtained by RNAseq analysis of Δ Mex5 mice versus WT at 4 months.

Gene name	Protein	log2FC	pValue	Mean WT	Mean Δ Mex5
Capn3	Calpain3	0.19	0.636	92.67	107.19
Cmya5	Cardiomyopathy associated protein 5	-0.58	4.0E-04	37053.21	24367.82
Obscn	Obscurin	-0.44	0.001	44418.89	32389.48
Obsl1	Obscurin like-1	-0.27	0.028	2143.24	1770.10
Synm	alpha-synemin	0.32	0.112	285.49	360.00

Table S8. Genes with a role in Ca²⁺-signaling. Values obtained by RNAseq analysis of Δ Mex5 mice versus WT at 4 months.

Gene name	Protein	log2FC	pValue	Mean WT	Mean Δ Mex5
Atp2a2	Serca2	-0.58	1.6E-06	266940.42	177612.55
Cacna1c	Dihydropyridine receptor	-0.25	0.057	10077.29	8462.90
Jph2	Junctophilin 2	-0.42	0.001	7420.52	5525.69
Pln	Phospholamban	-0.45	0.015	74465.34	53850.42
Ryr2	Ryanodine receptor 2	-0.42	2.5E-05	95617.29	71024.99

Table S9. Antibodies used for Immunohistochemistry and Western Blots.

Primary antibody target	Source	Experiment	Dilution
α-actinin	sigma A7811	IHF	1:20
Cmya5	abcam ab75351	IHF	1:25
Cmya5	NovusBio NBP1-77117	WB	1:1000
Collagen1	abcam ab6308	IHF	1:100
Collagen3	abcam ab7778	IHF	1:100
Dhpr	abcam ab58552	IHF	1:50
Dhpr	Alomone ACC-003	WB	1:500
GAPDH	Sigma PLA0302	WB	1:2000
Junctophilin-2	Santa-Cruz sc-377086	WB	1:1000
Obscurin	Atlas antibody HPA019497	IHF	1:100
Obscurin	Milipore ABT160	WB	1:1000
Ryr2	abclonal A0298	IHF WB	1:200 1:500
Serca2	Abclonal A1097	WB	1:1000
Synemin	Atlas antibody HPA040066	IHF WB	1:50 1:500
Titin is7	(Charton et al., 2016)	IHF WB	1:2 1:2000
Titin M8M9	Myomedix	IHF WB	1:75 1:2000
Titin M10	(Hackman et al., 2008)	IHF WB	1:75 1:2000
Titin Z1Z2	Abnova H00007273-M06	WB	1:2000

Table S10. Taqman probes used for qRT-PCR.

Gene	Taqman probe
Atp2a2	Mm01201431_m1
Cacna1c	Mm01188822_m1
Cmya5	Mm01282681.m1
Colla1	Mm00801666_g1
Col3a1	Mm00802300_m1
Fn1	Mm01256744_m1
Jph2	Mm00517621_m1
Myh6	Mm00440359_m1
Myh7	Mm01319006_g1
Nppa	Mm01255747_g1
Obscn	Mm01341542_g1
Ryr2	Mm00465877_m1
Sym	Mm00809202_s1
TgfB1	Mm01178820_m1
Timp1	Mm01341361_m1
Ttn	Mm00621005_m1
Vim	Mm01333430_m1

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