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Supplemental information

Therapeutic efficacy of rscAAVrh74.miniCMV.*LIPA* gene therapy in a mouse model of lysosomal acid lipase deficiency

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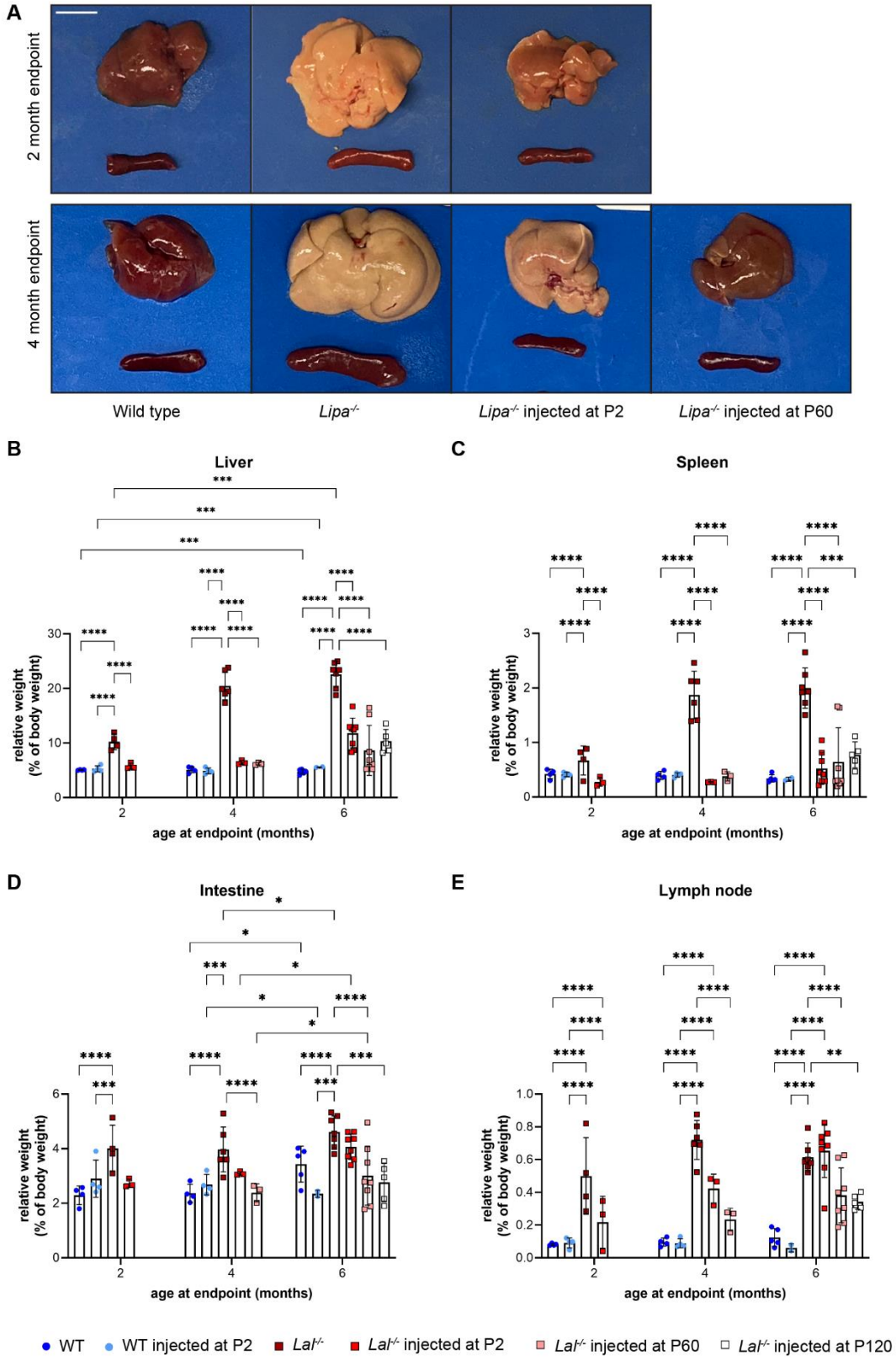

Figure S1. Organ morphology and weight at study endpoints.

(A) Gross morphology of liver and spleen at 2 months and 4 months of age. Scale bar = 1 cm. **(B-D)** Relative weights of the liver, spleen, intestine, and lymph node at 2, 4, and 6 months of age. All data represented as mean ± SD (n=3-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using two-way ANOVA with Tukey's post-hoc test.

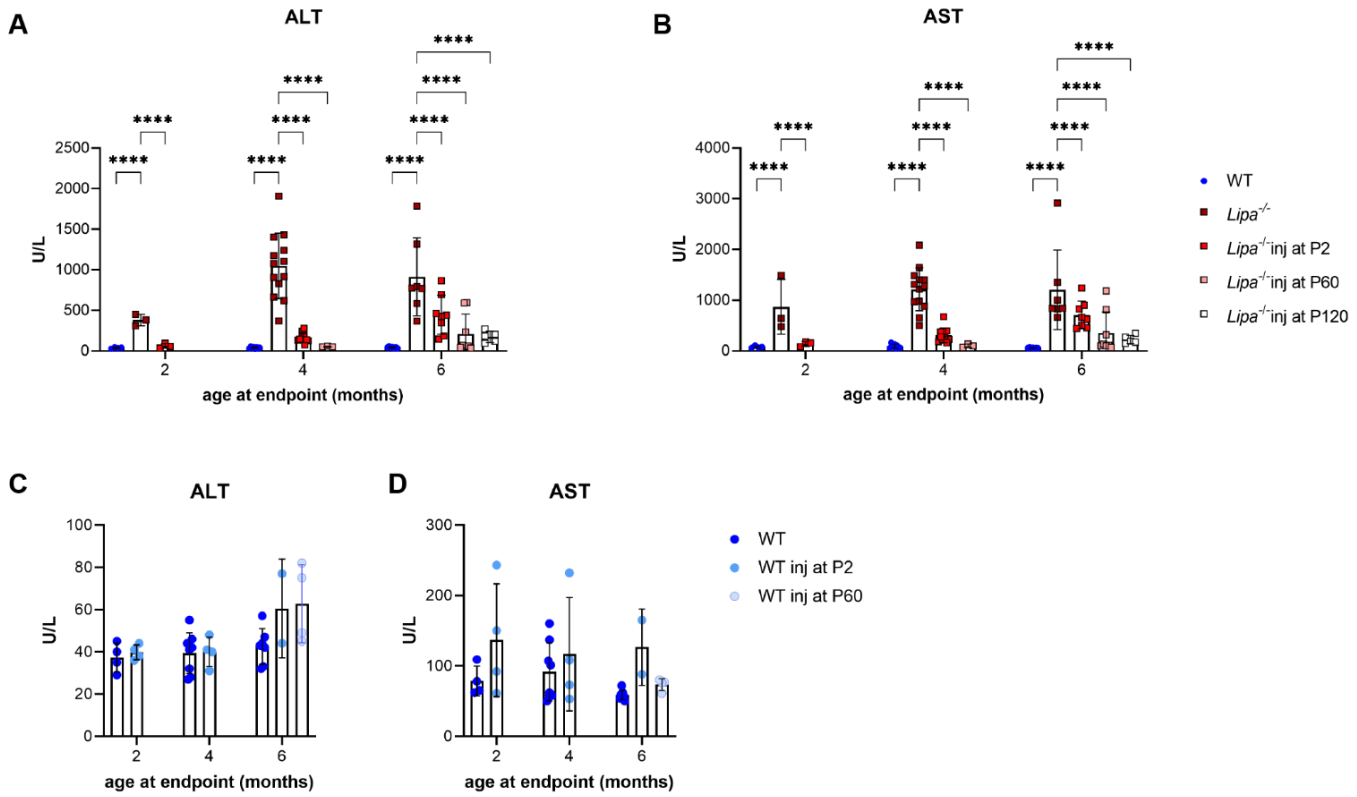

Figure S2. Serum transaminase levels at 2, 4, and 6 months of age.

(A) Serum ALT and **(B)** AST at 2, 4, 6 month endpoints of treated *Lipa*$^{-/-}$ mice. **(C)** Serum ALT and **(D)** AST at 2, 4, and 6 month endpoints of treated WT mice. All data represented as mean ± SD (n=2-8). Statistical significance was defined as p≤0.05 (****p≤0.0001) using two-way ANOVA with Tukey's post-hoc test.

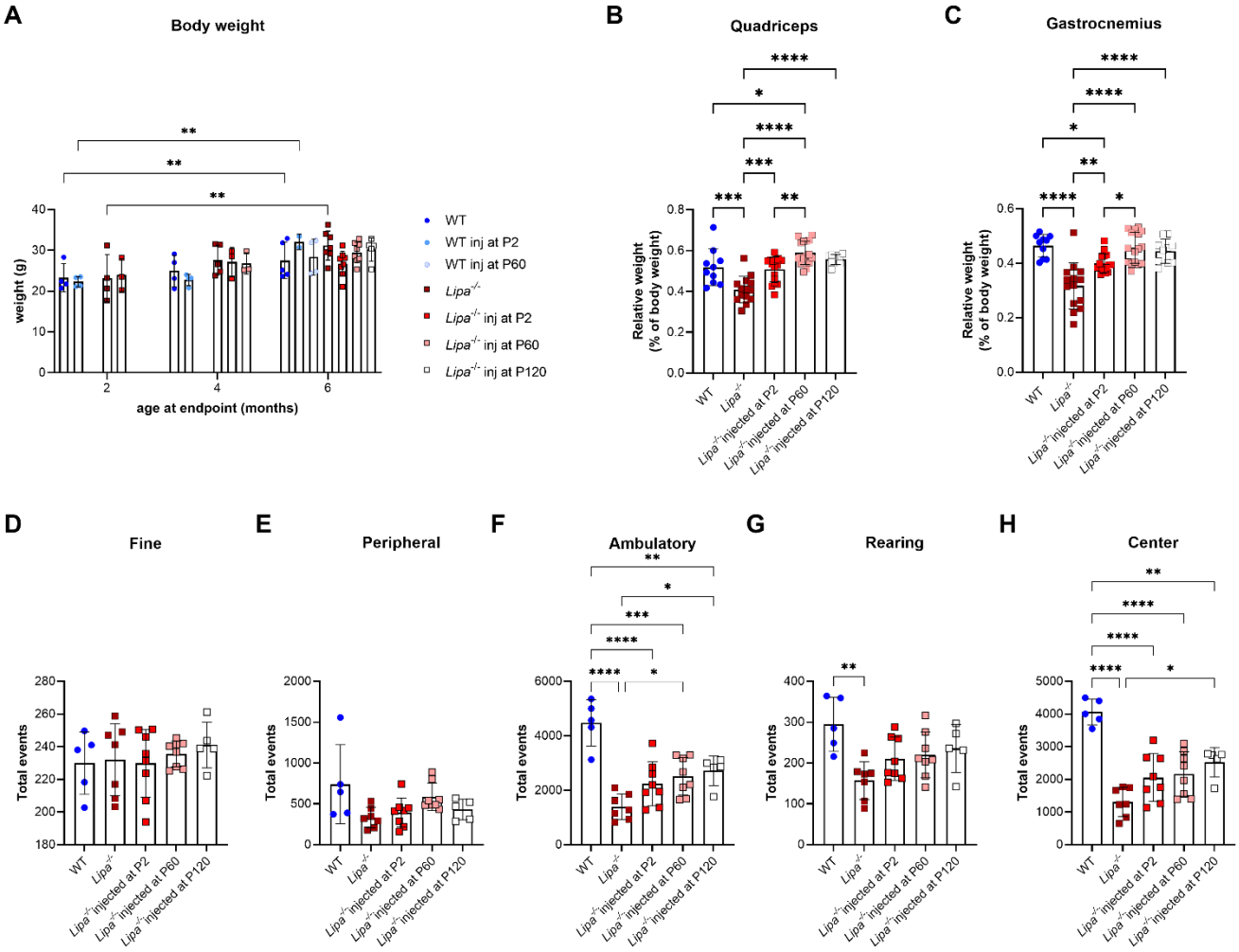

Figure S3. Muscle atrophy may contribute to ambulation differences in *Lipa*$^{-/-}$ mice.

(A) Body weight of mice at 2, 4, 6 months. **(B-C)** Relative weight of gastrocnemius muscle and quadricep muscle at 6 months. **(D-H)** Open field analysis at 6 months. All data represented as mean ± SD (n=5-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using two-way ANOVA (A) one-way ANOVA (B-H) with Tukey's post-hoc test.

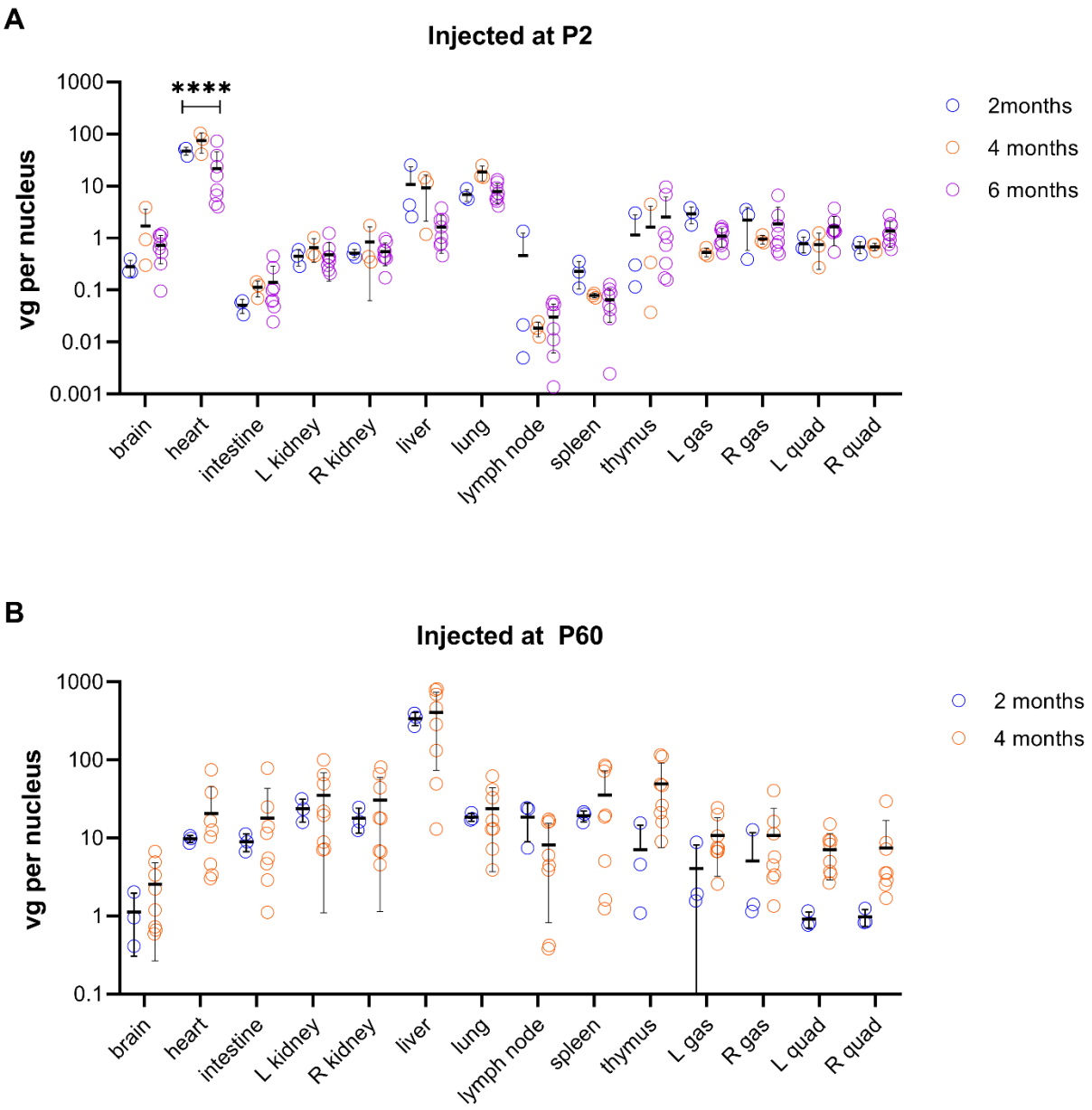

Figure S4. AAV vector genomes are relatively stable over time.

(A) Biodistribution of AAV in organs and tissues at 2-, 4-, and 6-months post-injection of *Lipa*$^{-/-}$ mice treated at P2. Change in vector genomes found in the heart is the only statistically significant change. **(B)** Biodistribution of AAV in organs and tissues at 2- and 4-months post-injection of *Lipa*$^{-/-}$ mice treated at P60. All data represented as mean ± SD (n=5-8). Statistical significance was defined as $p \leq 0.05$ (****$p \leq 0.0001$), using two-way ANOVA with Tukey's post-hoc test.

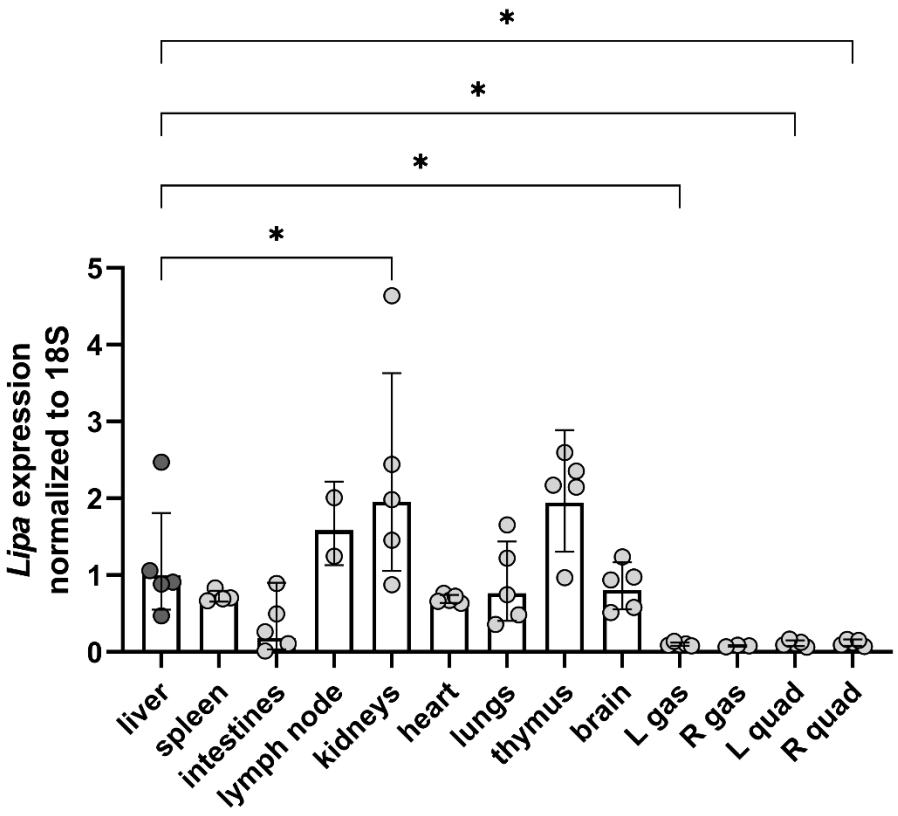

Figure S5. **Endogenous *Lipa* expression in various organs and tissues in WT mice.**

Lipa is expressed as relative to the liver. All data represented as mean ± SD (n=2-8). Statistical significance relative to liver expression was defined as p≤0.05 using one-way ANOVA with Tukey's post-hoc test.

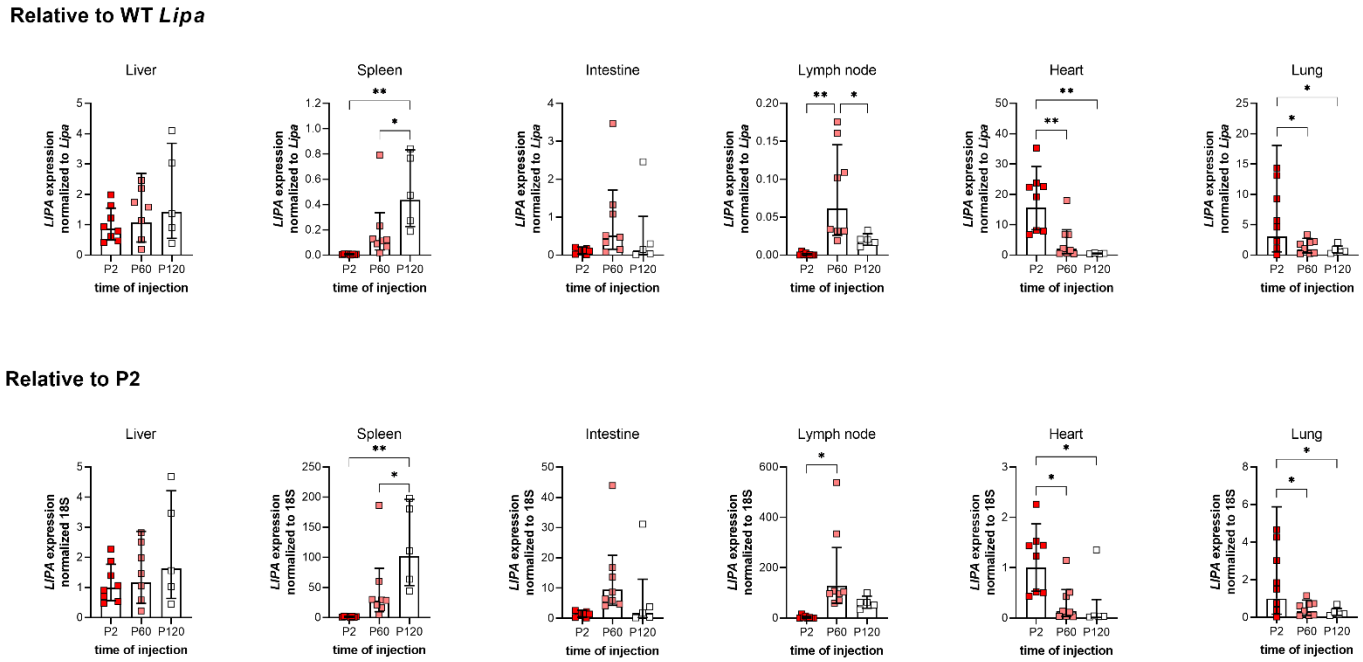

Figure S6. **RT-qPCR expression of the human *LIPA* transgene in treated mice at the 6 month endpoint.**

Expression data is presented as mRNA levels of human *LIPA* compared to the endogenous *Lipa* expression in WT, or compared to expression at when treated at P2. All data represented as mean ± SD (n=5-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01), using one-way ANOVA with Tukey's post-hoc test.

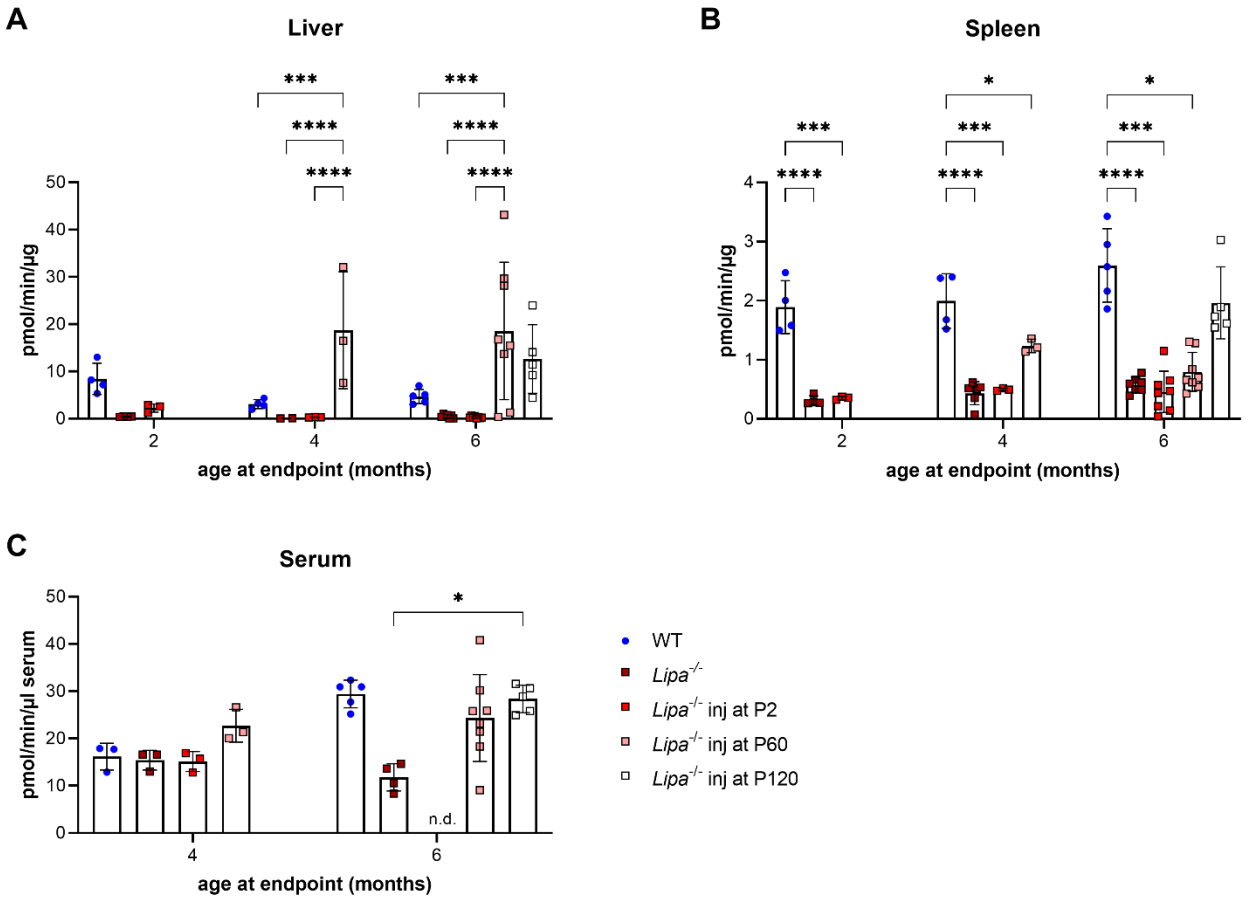

Figure S7. Lysosomal acid lipase activity is reduced in *Lipa*$^{-/-}$ mice at all timepoints.

Lysosomal acid lipase activity in **(A)** liver, **(B)** spleen, and **(C)** serum at 2, 4 and 6 months. Treatment at later time points (P60 and P120) result in restored enzyme activity in the liver. All data represented as mean ± SD (n=5-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using two-way ANOVA with Tukey's post-hoc test. n.d. = not determined due to insufficient sample.

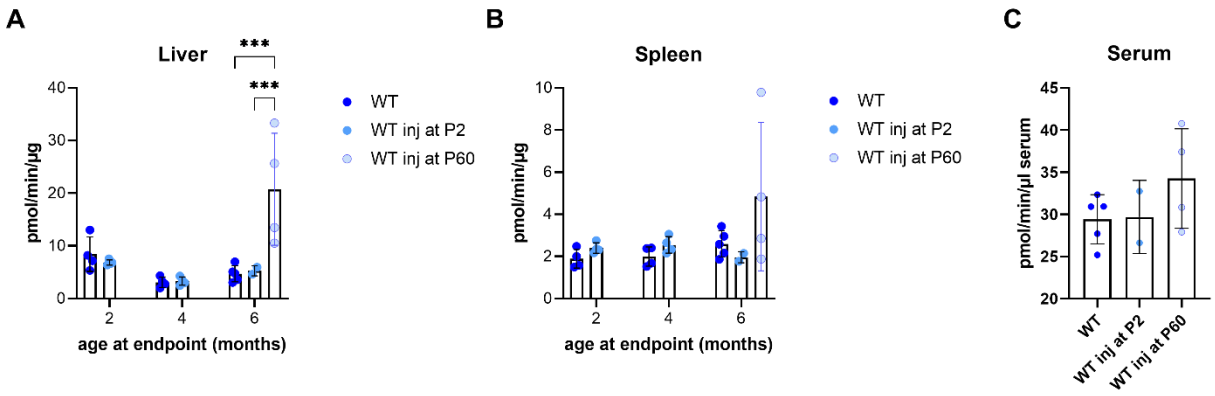

Figure S8. Lysosomal acid lipase activity in treated WT mice.

(A) Similar to treatment of *Lipa*$^{-/-}$ mice, only treatment at P60 resulted in statistically significant increase in LAL activity in liver. **(B)** Spleen LAL activity did not change with treatment in WT mice. **(C)** At the 6 month endpoint, serum LAL activity did not significantly change with treatment. Statistical significance was defined as $p \leq 0.05$ (***$p \leq 0.001$), using two-way ANOVA (A, B) or one-way ANOVA (C) with Tukey's post-hoc test.

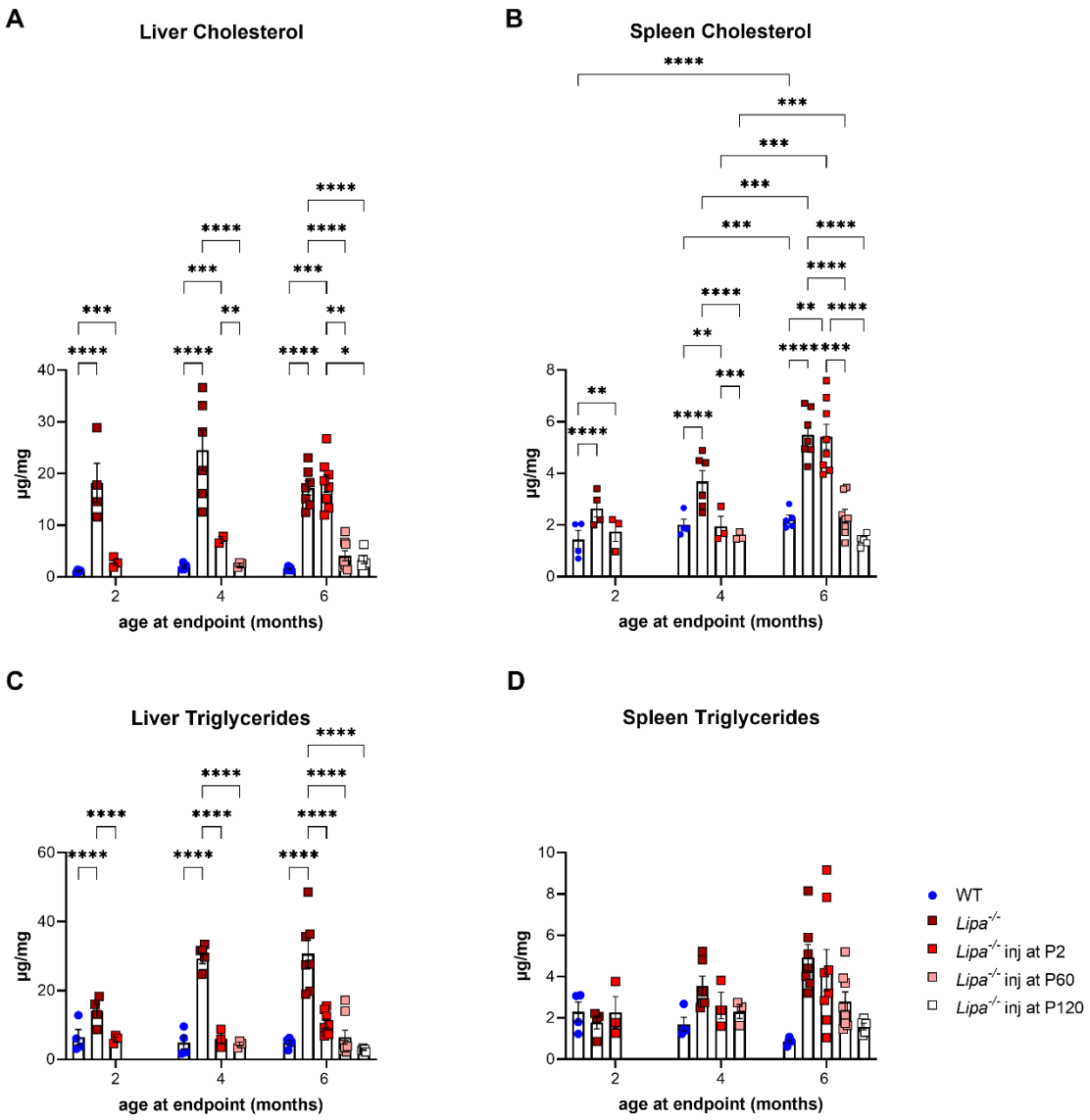

Figure S9. Cholesterol and triglyceride content at 2, 4, 6 months of age.

(A-B) Cholesterol content in (A) liver and (B) spleen at 2, 4, 6 months. **(C-D)** Triglyceride content in (C) liver and (D) spleen at 2, 4, 6 month endpoints. All data represented as mean ± SD (n=3-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using two-way ANOVA with Tukey's post-hoc test.

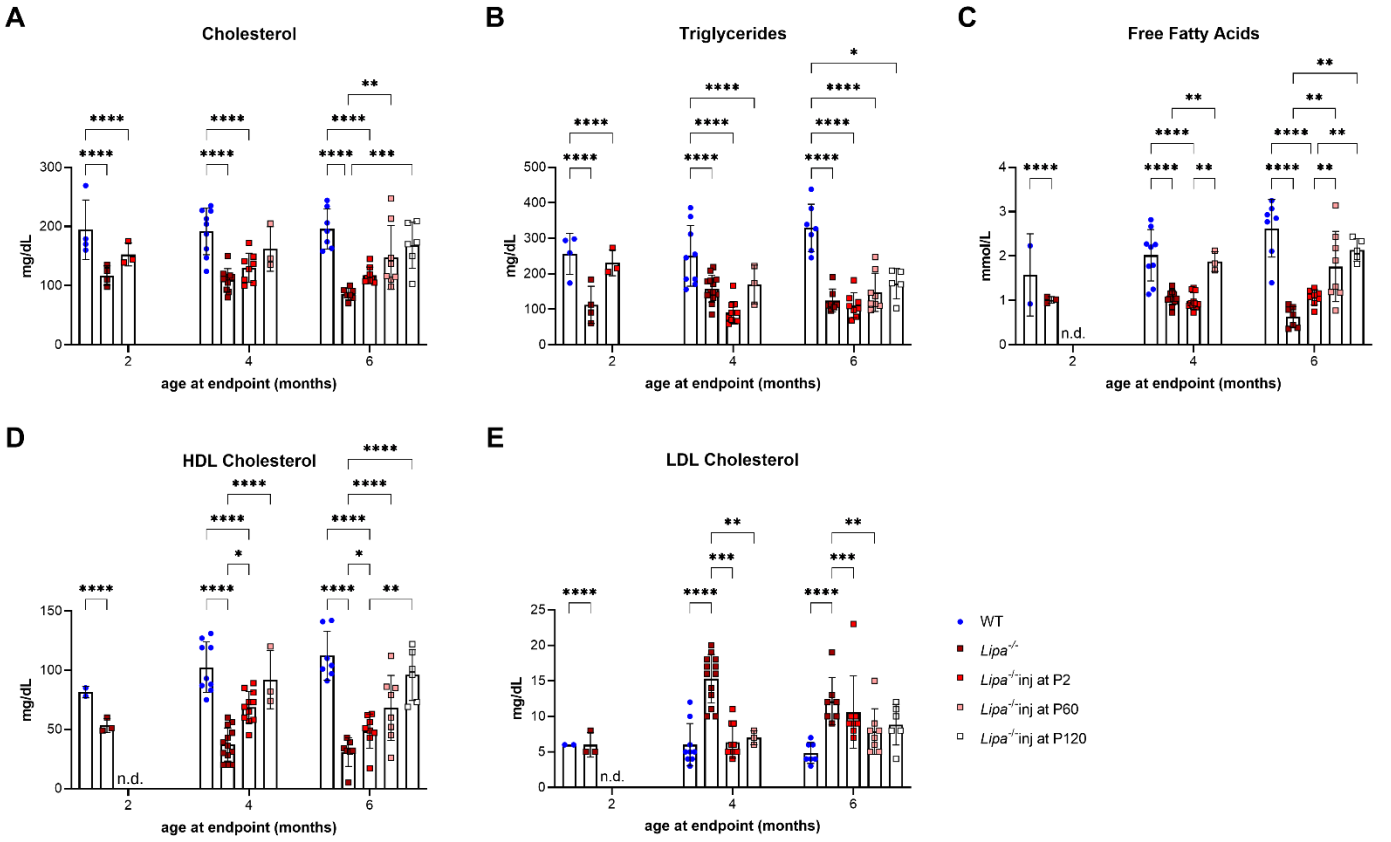

Figure S10. Serum lipid panel at 2, 4, and 6 months.

(A) Cholesterol, **(B)** Triglyceride, **(C)** free fatty acids **(D)** HDL cholesterol, **(E)** LDL cholesterol, and were measured in blood serum at 2, 4 and 6 months of age. All data represented as mean ± SD (n=5-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using two-way ANOVA with Tukey's post-hoc test. n.d. = not determined due to insufficient sample at the 2-month endpoint.

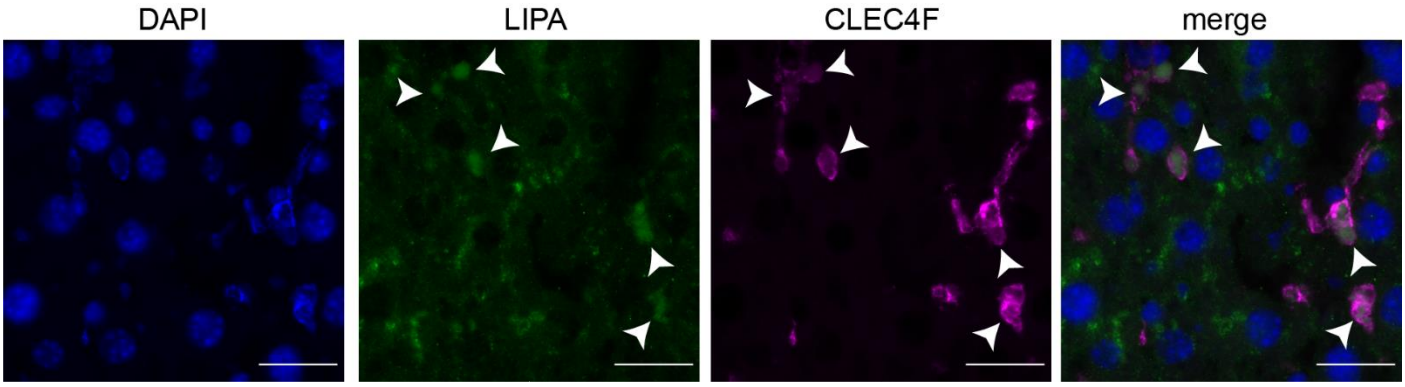

Figure S11. LIPA is expressed in hepatocytes and Kupffer cells.

Immunofluorescence staining of liver sections from WT mice treated at P60, 6 month endpoint. LIPA (green) is expressed throughout the liver. CLEC4F (magenta) is a Kupffer cell marker. The merged image shows co-localization of LIPA with CLEC45 (as indicated by arrows), as well as LIPA staining in hepatocytes (non-Kupffer cells). Scale bar = 25μm.

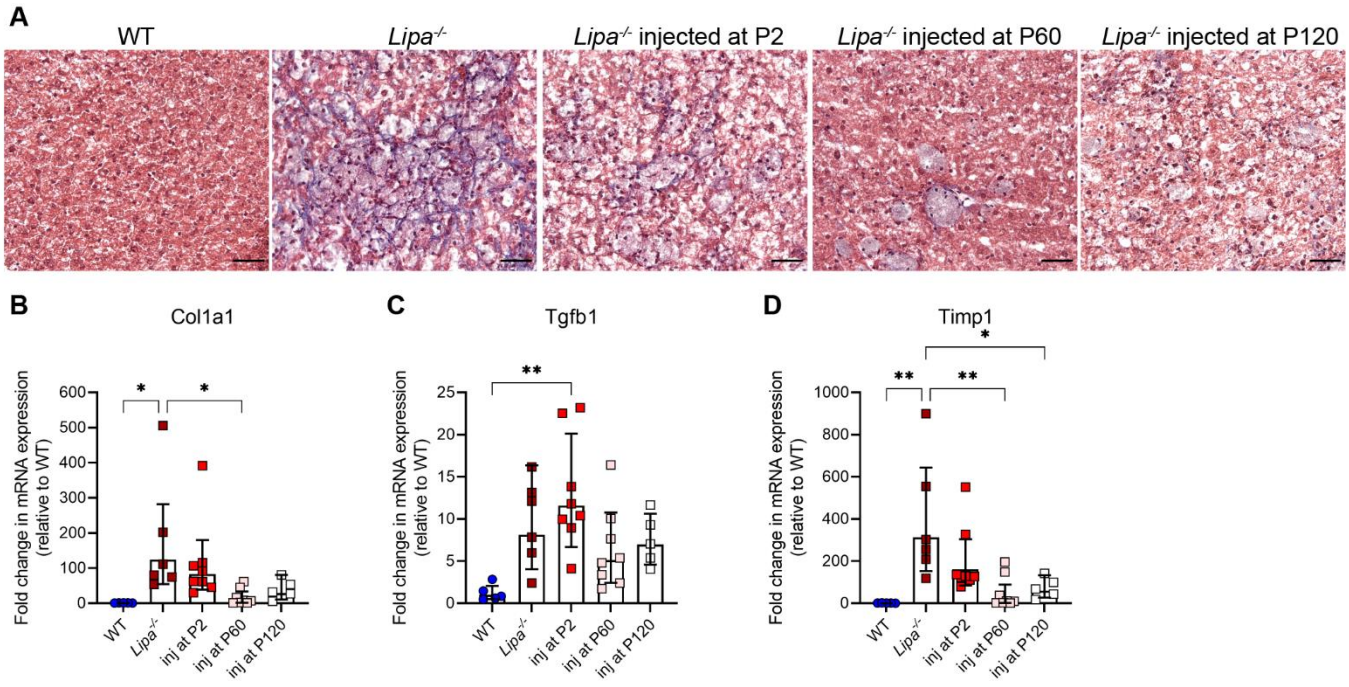

Figure S12. Fibrosis is decreased with treatment in *Lipa*$^{-/-}$ mice.

(A) Masson's Trichrome staining of liver sections at the 6 month endpoint. *Lipa*$^{-/-}$ mice show high amounts of fibrosis as evident from collagen staining (blue). Treatment at all timepoints decreases the amount of collagen staining. Scale bar = 25µm. **(B-D)** RT-qPCR was used to determine the expression of fibrosis genes (B) *Col1a1*, (C) *Tgfb1*, (D) *Timp1* in livers. All data represented as mean ± SD (n=2-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01), using one-way ANOVA with Tukey's post-hoc test.

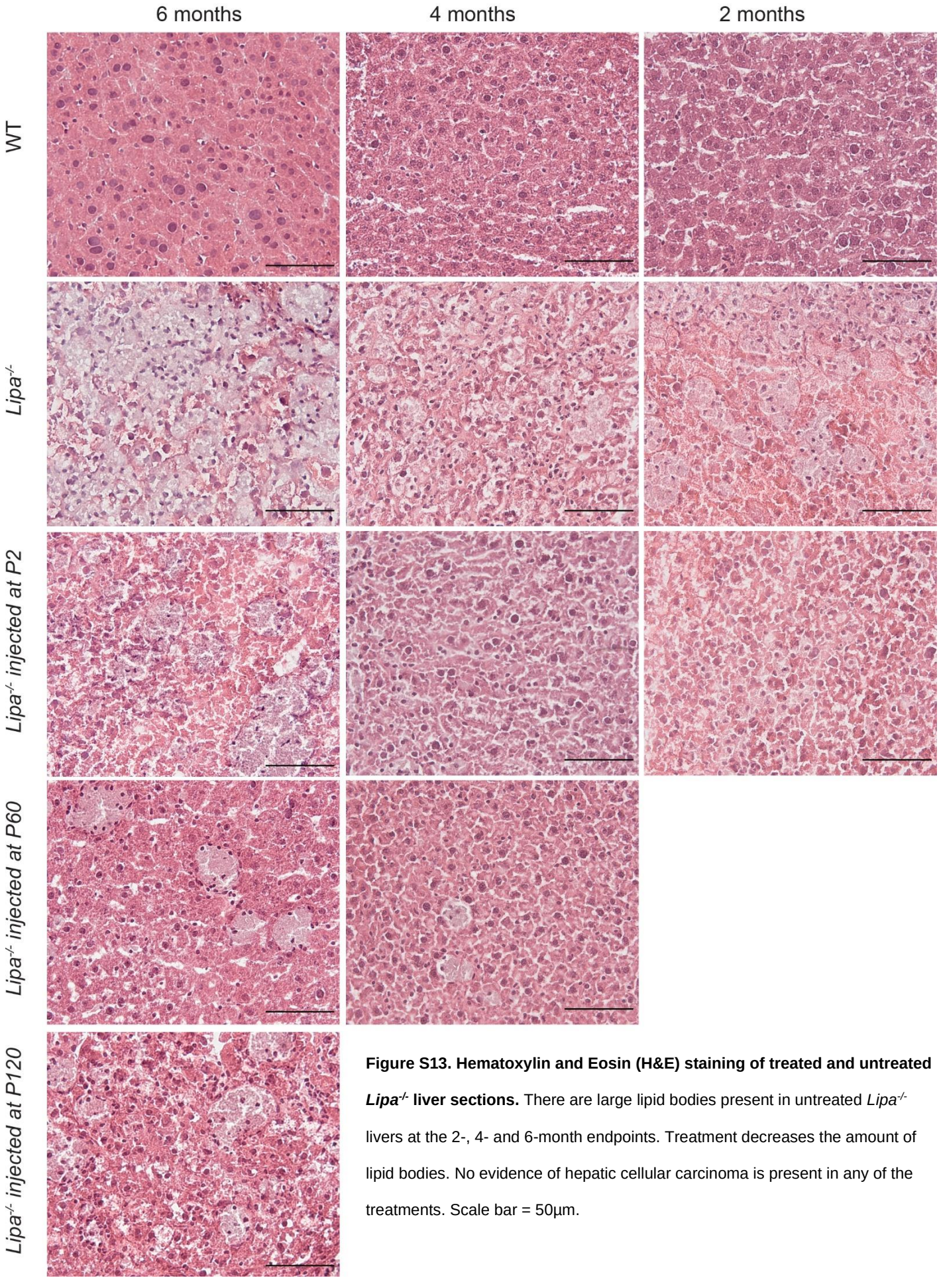

Figure S13. Hematoxylin and Eosin (H&E) staining of treated and untreated *Lipa*$^{-/-}$ liver sections. There are large lipid bodies present in untreated *Lipa*$^{-/-}$ livers at the 2-, 4- and 6-month endpoints. Treatment decreases the amount of lipid bodies. No evidence of hepatic cellular carcinoma is present in any of the treatments. Scale bar = 50µm.

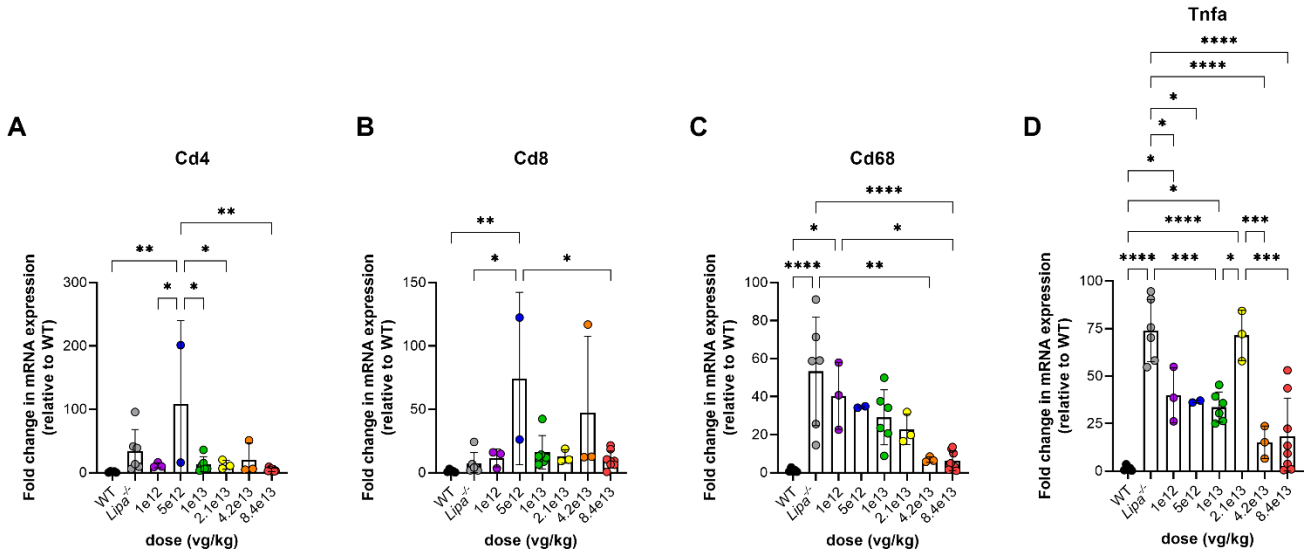

Figure S14. RT-qPCR analysis of various markers of inflammation in livers after different doses of gene therapy.

All data represented as mean ± SD (n=2-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001), using one-way ANOVA with Tukey's post-hoc test.

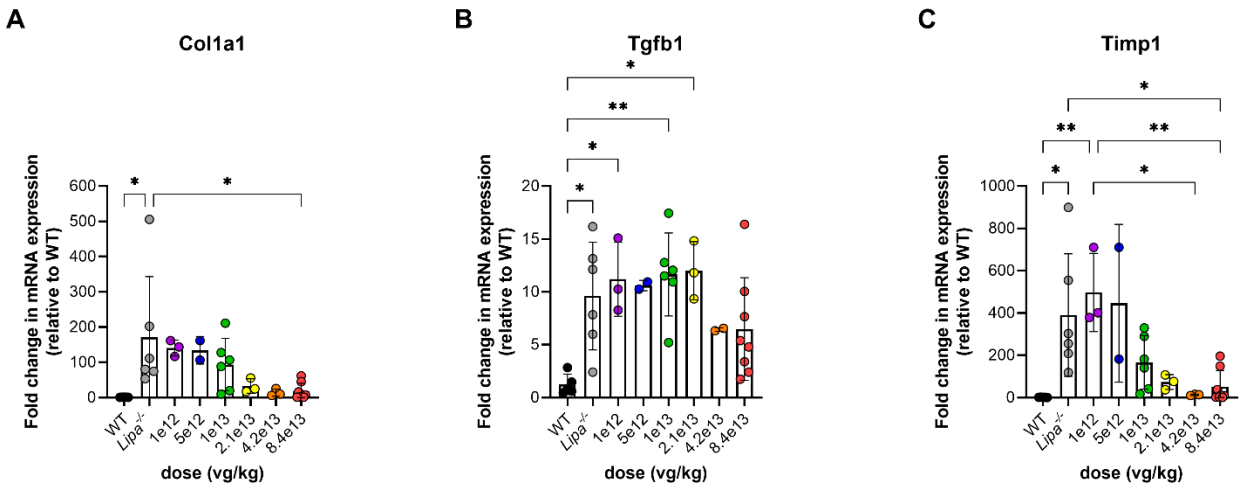

Figure S15. **RT-qPCR analysis of various markers of fibrosis after treatment with different doses.**

All data represented as mean ± SD (n=2-8). Statistical significance was defined as p≤0.05 (*p≤0.05, **p≤0.01), using one-way ANOVA with Tukey's post-hoc test.

Table S1. Biodistribution of AAV at 6-month endpoint. Values are represented as mean vg/nucleus ± SD.

	Injected at P2 (n=8)	Injected at P60 (n=8)	Injected at P120 (n=5)
Brain	1.20±0.14	2.55±0.81	1.64±0.21
Heart	21.82±8.41	20.52±8.79	12.9412.11
Intestines	0.14±0.05	17.93±9.08	12.58±3.73
Liver	1.62±0.39	406.13±117.59	90.69±21.48
Lung	7.87±1.16	23.91±7.15	45.47±14.73
Lymph node	0.03±0.01	8.14±2.59	4.99±1.94
L kidney	0.48±0.12	34.95±11.97	33.00±6.93
R kidney	0.55±0.09	30.66±10.44	38.83±8.58
Spleen	0.07±0.02	35.66±13.19	28.31±9.92
Thymus	2.52±1.28	49.44±14.81	19.25±8.36
L gastrocnemius	1.10±0.15	10.75±2.66	6.19±3.48
R gastrocnemius	1.86±0.73	10.79±4.61	11.89±5.07
L quadricep	1.65±0.33	7.11±1.48	4.55±0.65
R quadricep	1.38±0.25	7.50±3.29	6.81±1.53

Table S2. Biodistribution of AAV at the 4-month endpoint. Values are represented as mean vg/nucleus ± SD.

	Injected at P2 (n=3)	Injected at P60 (n=3)
Brain	1.70±1.09	1.13±0.48
Heart	74.34±18.01	0.97±0.61
Intestines	0.11±0.02	8.97±1.33
Liver	9.21±4.09	340.12±37.33
Lung	18.42±3.32	18.61±1.25
Lymph node	0.02±0.00	18.55±5.58
L kidney	0.66±0.18	23.74±4.53
R kidney	0.84±0.45	17.78±3.59
Spleen	0.08±0.01	19.26±1.79
Thymus	1.63±1.44	7.09±4.36
L gastrocnemius	0.54±0.06	4.09±2.35
R gastrocnemius	0.95±0.10	5.08±3.09
L quadricep	0.74±0.29	0.91±0.13
R quadricep	0.68±0.07	0.98±0.14

Table S3. Biodistribution of AAV at the 2-month endpoint. Values are represented as mean vg/nucleus ± SD.

	Injected at P2 (n=3)
Brain	0.28±0.06
Heart	47.27±4.76
Intestines	0.05±0.01
Liver	10.73±7.29
Lung	6.85±1.01
Lymph node	0.46±0.45
L kidney	0.45±0.09
R kidney	0.51±0.05
Spleen	0.23±0.07
Thymus	1.15±0.95
L gastrocnemius	2.92±0.60
R gastrocnemius	2.23±0.95
L quadricep	0.78±0.15
R quadricep	0.67±0.10

Table S4. Biodistribution of AAV at the various doses, treated at P60, 6 month endpoint. Values represent mean vg/nucleus ± SD.

	$1.0x10^{12}$ vg/kg (n=3)	**$5.0x10^{12}$ vg/kg (n=2)**	**$1.0x10^{13}$ vg/kg (n=3)**	**$2.1x10^{13}$ vg/kg (n=3)**	**$4.2x10^{13}$ vg/kg (n=3)**	**$8.4x10^{13}$ vg/kg (n=8)**
Brain	0.03±0.01	0.32±0.12	0.23±0.06	0.77±0.17	1.46±0.62	2.55±0.81
Heart	0.18±0.03	0.87±0.33	1.80±0.65	3.70±2.38	12.51±1.55	20.52±8.79
Intestines	0.21±0.05	0.88±0.08	1.94±0.61	4.29±2.21	10.72±3.26	17.93±9.08
Liver	0.03±0.00	1.58±0.06	10.84±5.88	39.99±12.80	316.38±95.55	406.13±117.59
Lung	0.15±0.02	0.89±0.53	2.28±0.84	3.30±0.15	23.89±8.48	23.91±7.15
Lymph node	0.03±0.00	0.93±0.68	0.38±0.11	0.75±0.37	5.32±4.45	8.14±2.59
L kidney	0.11±0.02	1.49±0.67	3.98±2.15	10.94±4.56	24.38±3.99	34.95±11.97
R kidney	0.14±0.04	1.92±0.16	4.39±1.99	9.23±2.65	44.94±10.31	30.66±10.44
Spleen	0.02±0.00	0.31±0.21	1.13±0.48	3.24±1.32	33.41±12.28	35.66±13.19
Thymus	0.22±0.11	0.82±0.15	3.08±1.13	6.73±1.84	74.99±2.58	49.44±14.81
L gastrocnemius	0.12±0.02	1.47±0.96	0.92±0.20	2.66±1.21	6.97±2.76	10.75±2.66
R gastrocnemius	0.36±0.19	1.65±1.21	1.19±0.40	2.54±0.73	12.25±4.62	10.79±4.61
L quadricep	0.15±0.03	1.09±0.73	0.64±0.12	1.54±0.61	9.26±3.41	7.11±1.48
R quadricep	0.10±0.02	0.27±0.11	0.66±0.14	1.37±0.67	6.18±4.14	7.50±3.29

Table S5. Sequences of primers used in this study

		Sequence
WT – 323 bp	exon5-F	5’- CTGCATGGAGACTCACAAAGGA- 3’
	exon5-R	5’- AAGTCTTCCCTGTTCCCATGG- 3’
LAL KO – 203 bp	HPRT-F	5’- CGTCGTGATTAGCGATGATGA- 3’
	HPRT-R	5’- TCCAGCAGGTCAGCAAAGAA-3’
AAV biodistribution	Primer1	5’-TGACGTCAATGGGAGTTTGTT-3’
	Primer2	5’-ATATAGACCTCCCACCGTACAC-3’
	Probe	5’-/56-FAM/CATTGACGC/ZEN/AAATGGGCGGTAGG/3IABkFQ/-3’
Human *LIPA* expression	Primer1	5’-ACTAGAATCTGCCAGCAAGC-3’
	Primer2	5’-TCTGTGCCTTAACCGAATTCC-3’
	Probe	5’-/56-FAM/TCCCAAACC/ZEN/AGTTGTCTTCCTGCA/3IABkFQ/-3’
Mouse *Lipa* expression	Primer1	5’-ATTCTCAAGGCTGCACCATAG-3’
	Primer2	5’-CAAGCGTCCCAATTGAAGTAG-3’
	Probe	5’-/56-FAM/TTAGTCTTG/ZEN/GCTCCCGTGTTGTCTC/3IABkFQ/-3’
Eukaryotic 18S rRNA Endogenous Control		ThermoFisher Cat# 4319413E
Pre-designed Taqman Gene Expression Assays (ThermoFisher)		
Gene		**Assay ID**
Cd4		Mm00442754_m1
Cd8a		Mm01182108_m1
Cd68		Mm00839636_m1
Tnfa		Mm99999068_m1
Col1a1		Mm00801666_g1
Tgfb1		Mm01178820_m1
Timp1		Mm00441818_m1

Table S6. Complete sequence of the therapeutic cassette (miniCMV promoter-*hLIPA*-SV40 polyA) including the AAV2 ITR and truncated ITR region.

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