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Supplemental Information

Low-Dose Liver-Targeted Gene Therapy for Pompe Disease Enhances Therapeutic Efficacy of ERT via Immune Tolerance Induction

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Supplemental Table 1: Ranking of IgG Responses

	Total IgG		IgG1		IgG2b		IgG2c	
Group	[IgG]	Rank	[IgG1]	Rank	[IgG2b]	Rank	[IgG2c]	Rank
1	1.0	2	1.9	3	1.0	2	2.2	3
1	0.8	2	0.8	2	1.0	3	1.8	3
1	8.9	3	24.2	3	5.6	3	8.9	3
1	19.8	3	66.4	3	3.1	3	2.1	3
1	3.8	3	9.5	3	1.9	3	1.8	3
1	0.5	1	0.8	2	0.0	0	1.7	3
1	0.7	2	2.4	3	0.0	0	0.0	0
1	2.8	3	9.7	3	0.0	0	0.0	0
1	31.0	3	104.9	3	0.8	2	12.5	3
2	0.9	2	1.1	3	0.7	2	2.6	3
2	0.0	0	0.0	0	0.3	1	0.6	2
2	0.3	1	0.0	0	0.6	2	2.9	3
2	0.4	1	0.0	0	0.6	2	2.2	3
2	0.1	1	0.0	0	0.5	1	1.5	3
2	0.0	0	0.0	0	0.0	0	0.0	0
2	0.0	0	0.0	0	0.0	0	0.1	0
2	0.5	2	1.0	2	0.9	2	2.0	3
2	0.4	1	0.8	2	0.7	2	1.1	3
3	0.0	0	0.0	0	0.0	0	0.9	3
3	0.0	0	0.0	0	0.3	1	0.1	2
3	0.0	0	0.0	0	0.1	0	0.0	0
3	1.7	3	3.5	3	0.7	2	2.1	0
3	12.2	3	38.3	3	1.2	3	13.9	3
3	16.4	3	2.4	3	12.6	3	66.3	3
3	0.0	0	0.0	0	0.3	1	2.3	3
3	0.0	0	0.0	0	0.0	0	0.0	3
3	0.0	0	0.0	0	0.0	0	0.0	0

Figure S1: Sex influences liver depot gene therapy and ERT. Mice described in Figure 1 were treated with either a weekly injection of rhGAA (ERT, 20 mg/kg; n=10), or a single injection of AAV2/8-LSPhGAApA (AAV, $8x10^{11}$ vg/kg; n=10). Differences between male and female mice were detected for (A) liver GAA activity, (B) diaphragm glycogen content, and (C) anti-GAA IgG1 detected by ELISA. Mean +/- s.d. shown. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001 from ANOVA.

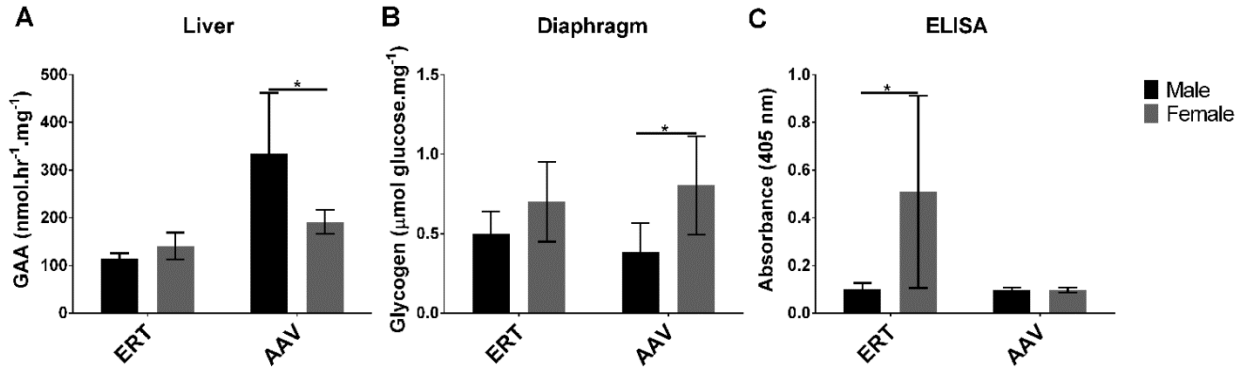

Figure S2: Sex influences response to AAV2/8-LSPhGAApA with or without simultaneous ERT. Mice described in Figure 2 were treated with the AAV2/8-LSPhGAA vector (AAV), either with or without simultaneous ERT for 4 doses (rhGAA, 20 mg/kg every 2 weeks). Number of mice per group as follows, (-) ERT: 0 (n=8), $2x10^{10}$ (n=8), $8 x10^{10}$ (n=9), $2 x10^{11}$ (n=8); (+)ERT: 0 (n=10), $2 x10^{10}$ (n=9), $8 x10^{10}$ (n=9), $2 x10^{11}$ (n=9). Differences between male and female mice were detected for biochemical correction of (A) heart, and (B) diaphragm. An independent group of untreated, age-matched GAA-KO mice (8M/5F) showed differences in biochemical correction (C), confirming that male GAA-KO had lower residual GAA activity, in comparison with females. Sex differences were detected in anti-GAA, including (D) total IgG, (E) IgG1, and (F) IgG2b. Mean +/- s.d. shown. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001 from 2 way ANOVA.

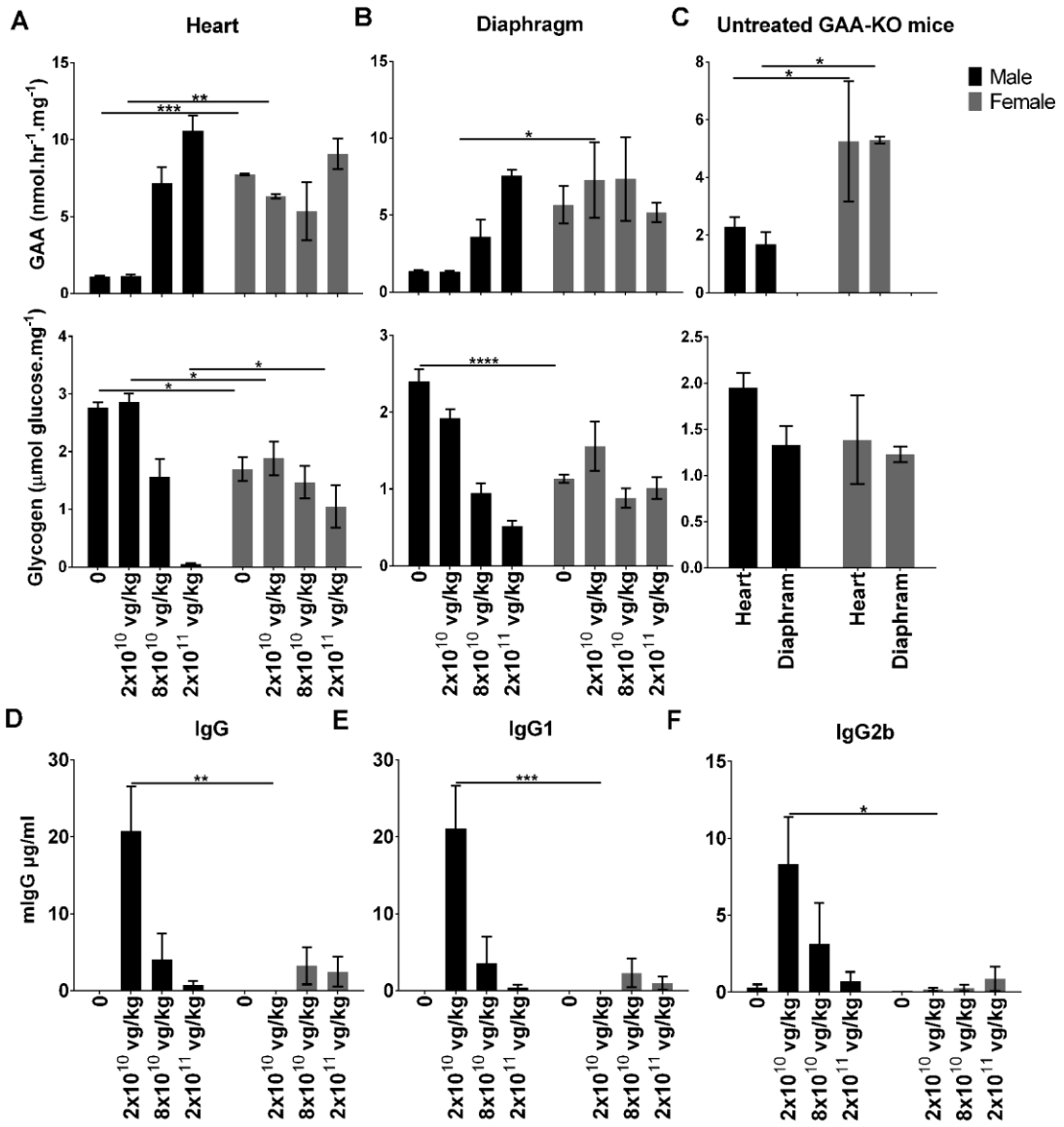

Figure S3: Sex influences differences in IgG levels in a dose-dependent manner. IgG data from mice depicted in Figure 3 were reanalyzed to detect differing responses between male and female mice. Histograms represent significant differences that were demonstrated. (A) Total IgG, (B) IgG1, and (C) IgG2b following AAV vector administration at the indicated dosages. (D) IgG2b following AAV vector administration at the indicated dosages followed by ERT. Statistical analysis has been performed by comparison of identically treated groups of male and female mice using multiple t-tests ($p<0.05$, (+)ERT vs (-)ERT). Mean +/- s.d. shown. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$ from multiple t-tests.

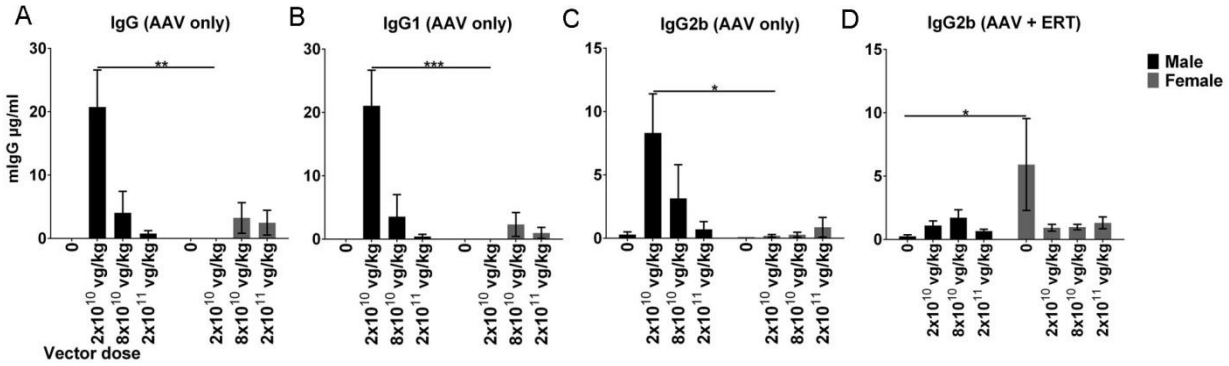

Figure S4: Sex influences long-term clearance of glycogen from the heart, diaphragm, and skeletal muscle. Data from mice depicted in Figure 4 were reanalyzed to detect differing responses between male and female mice. Biochemical correction from (A) heart, (B) diaphragm, and (C) quadriceps. Mean +/- s.d. shown. *=p<0.05, **=p<0.01, ***=p<0.001, and ****=p<0.0001, per 2 way ANOVA.

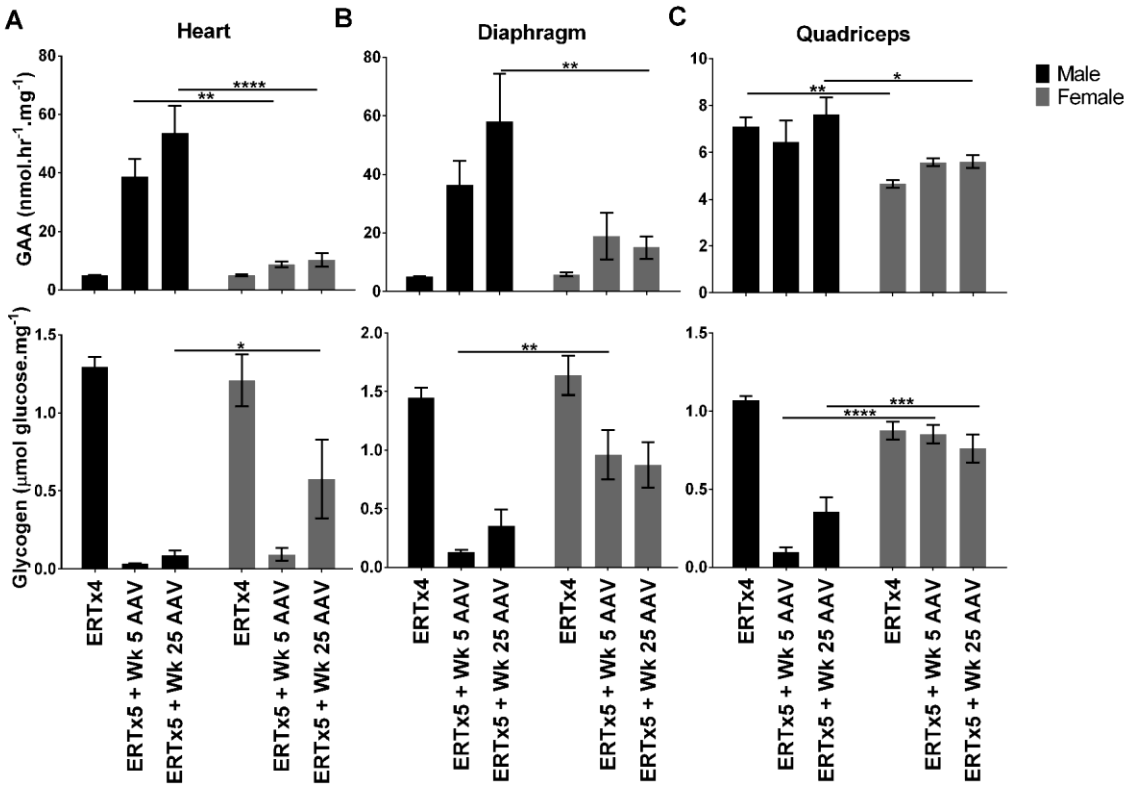

Figure S5: **Liver gene transfer eradicates humoral immune response triggered by multiple rhGAA infusions.** GAA-KO mice from the three groups were injected with 20 mg/kg of rhGAA and $8x10^{11}$ vg/kg of AAV8-LSP-hGAA as indicated in the experimental plan (Fig. 4A). Scatter plots show total IgG2b (A), IgG2c (B), and ranked IgG2b (C) and IgG2c (D), which was measured in plasma 36 weeks after vector injection and ranked by the degree of antibody production. Antibodies were ranked as follows : <0.25=0, 0.25-0.5=1, 0.51-0.75=2, 0.76-1=3, and >1=4 (Supplemental Table). The quantification of antibody isotypes has been performed using purified mouse IgG isotypes as standard. Mean +/- s.d. shown. *=p<0.05, **=p<0.01 from ANOVA.

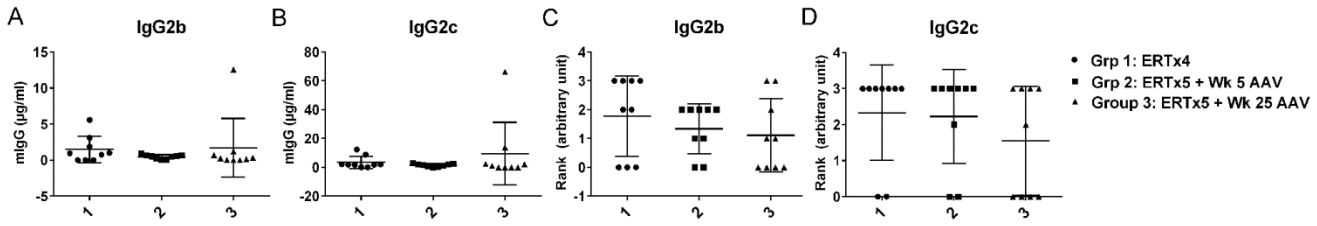

Figure S6: **IgG1 quantity correlated with titer.** GAA-KO mice from the three groups were injected with 20 mg/kg of rhGAA and $2x10^{12}$ vg/kg of AAV8-LSP-hGAA as indicated in the experimental plan (Fig. 4A). Scatter plot of IgG1 concentration versus inverse titer for male mice at Wk 36.