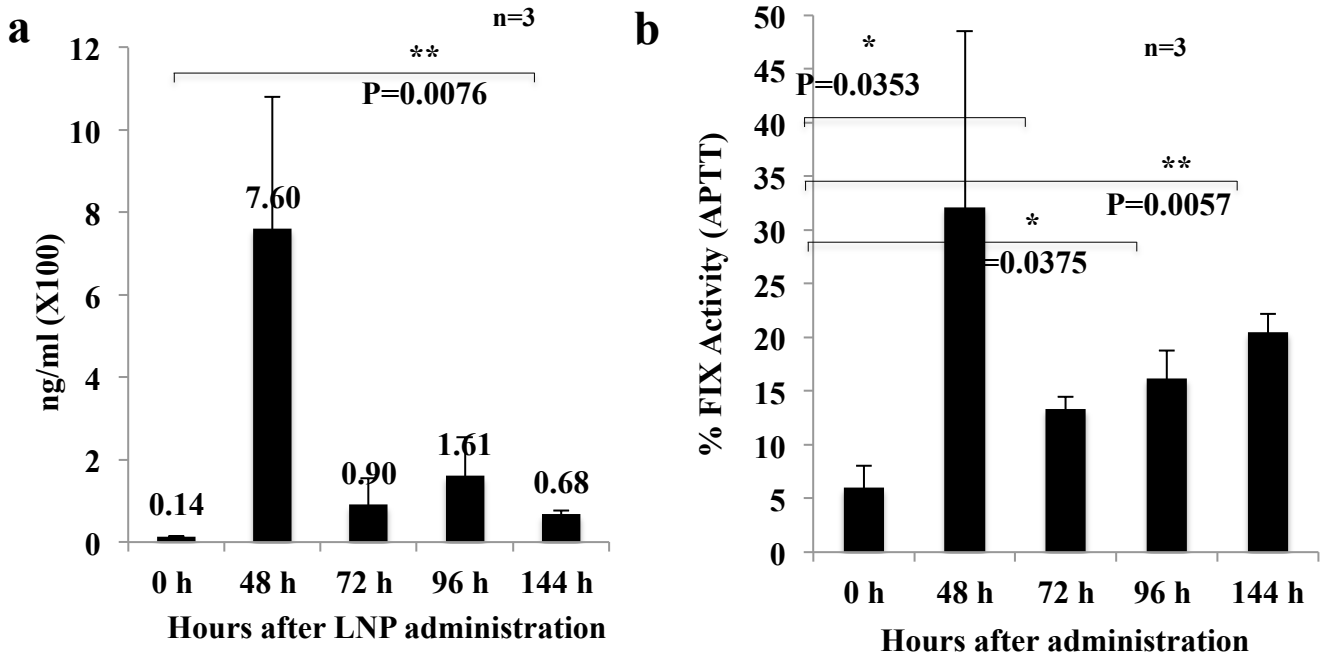
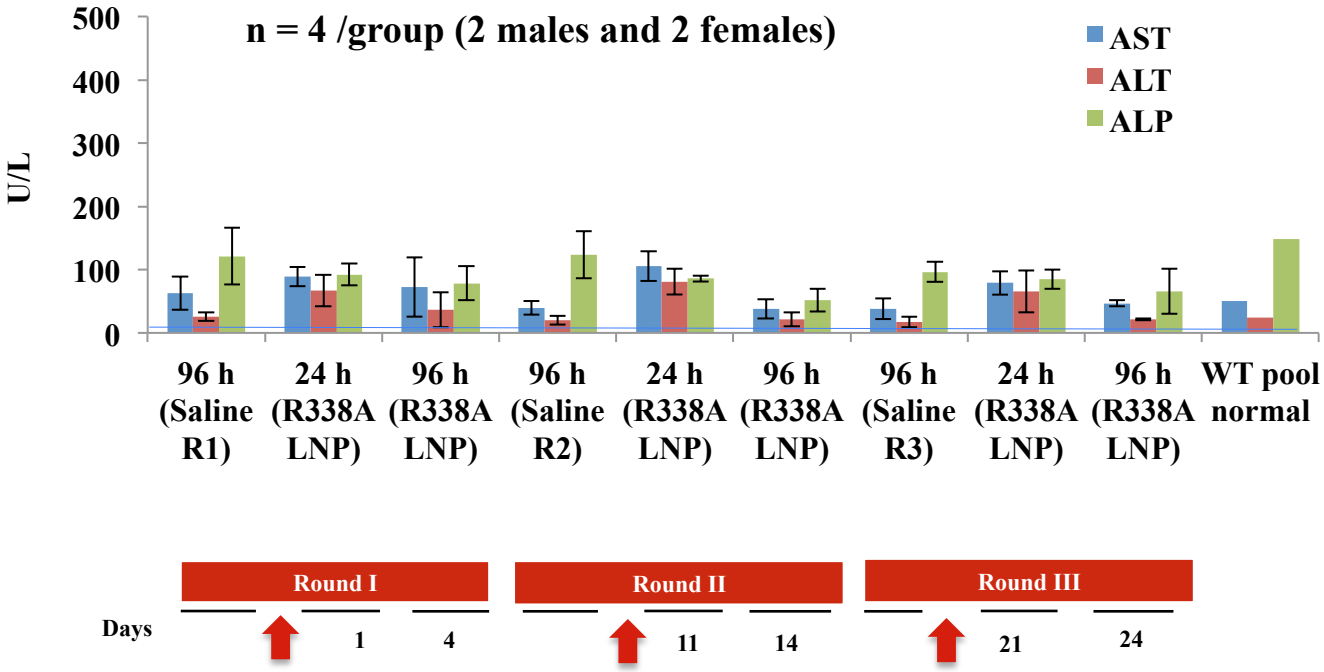
Supplementary Figures

Supplementary Figure 1



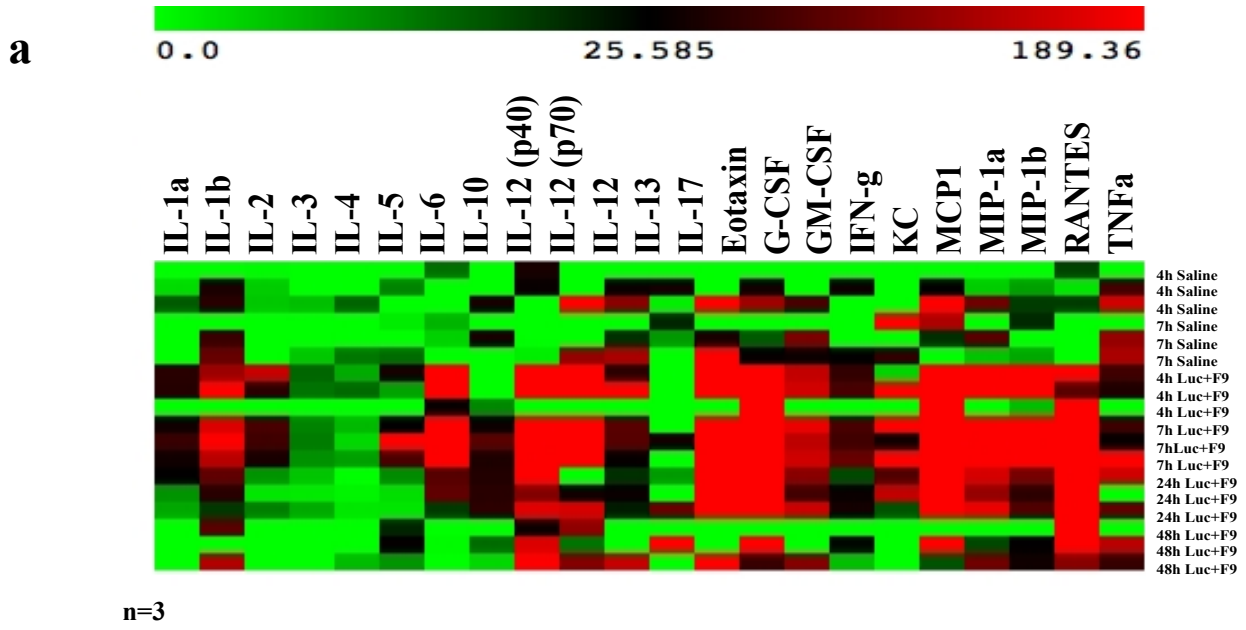
Supplementary Figure 2

a



Liver function test in animals treated with R338A FIX mRNA-LNPs repeatedly: Animals treated thrice at 4 mg/kg with LUNAR LNPs over a 40-day period showed normal liver function with no additional toxicity. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase Level Test (ALP) tests were done as an indicator of liver function.

Supplementary Figure 3



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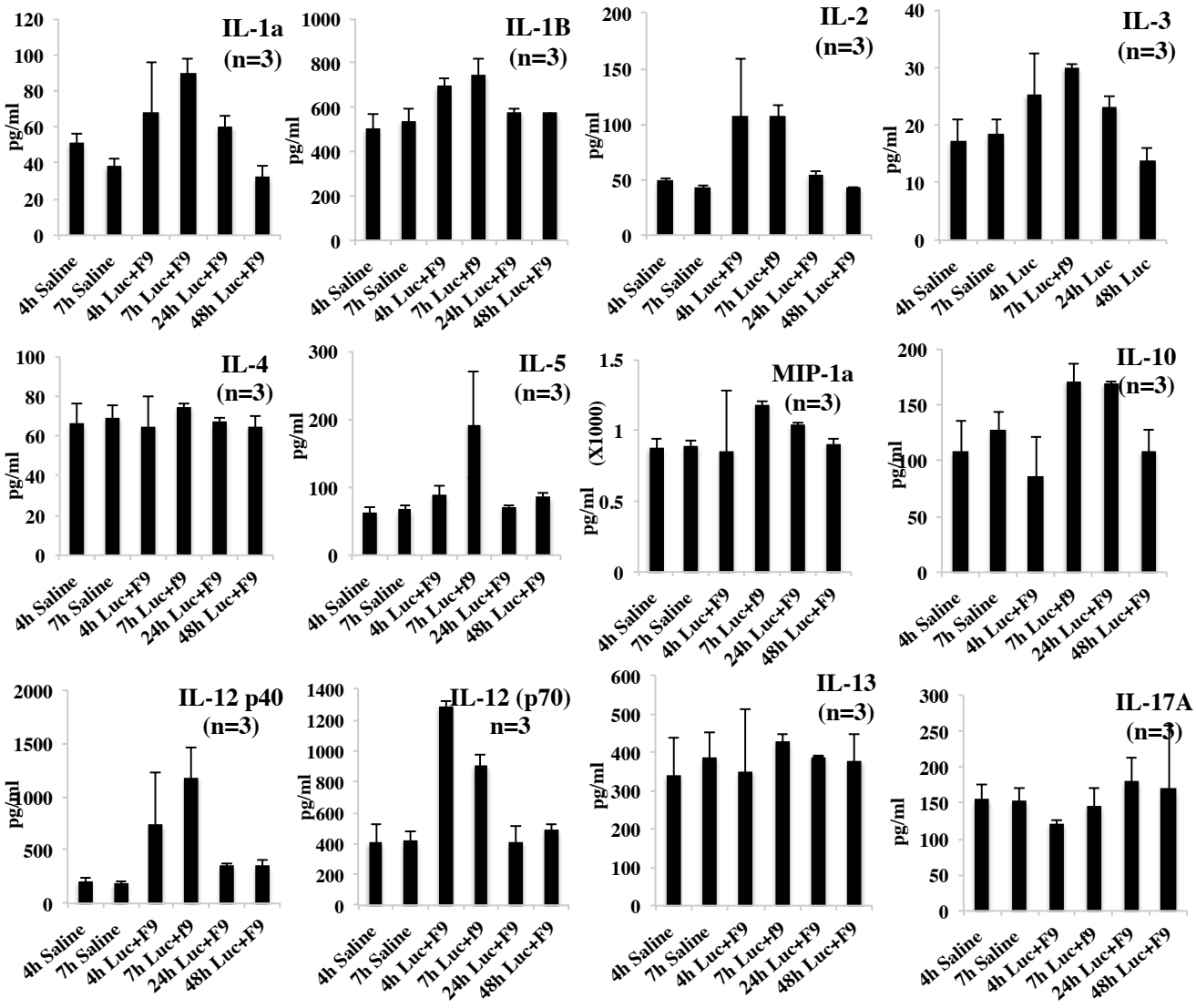
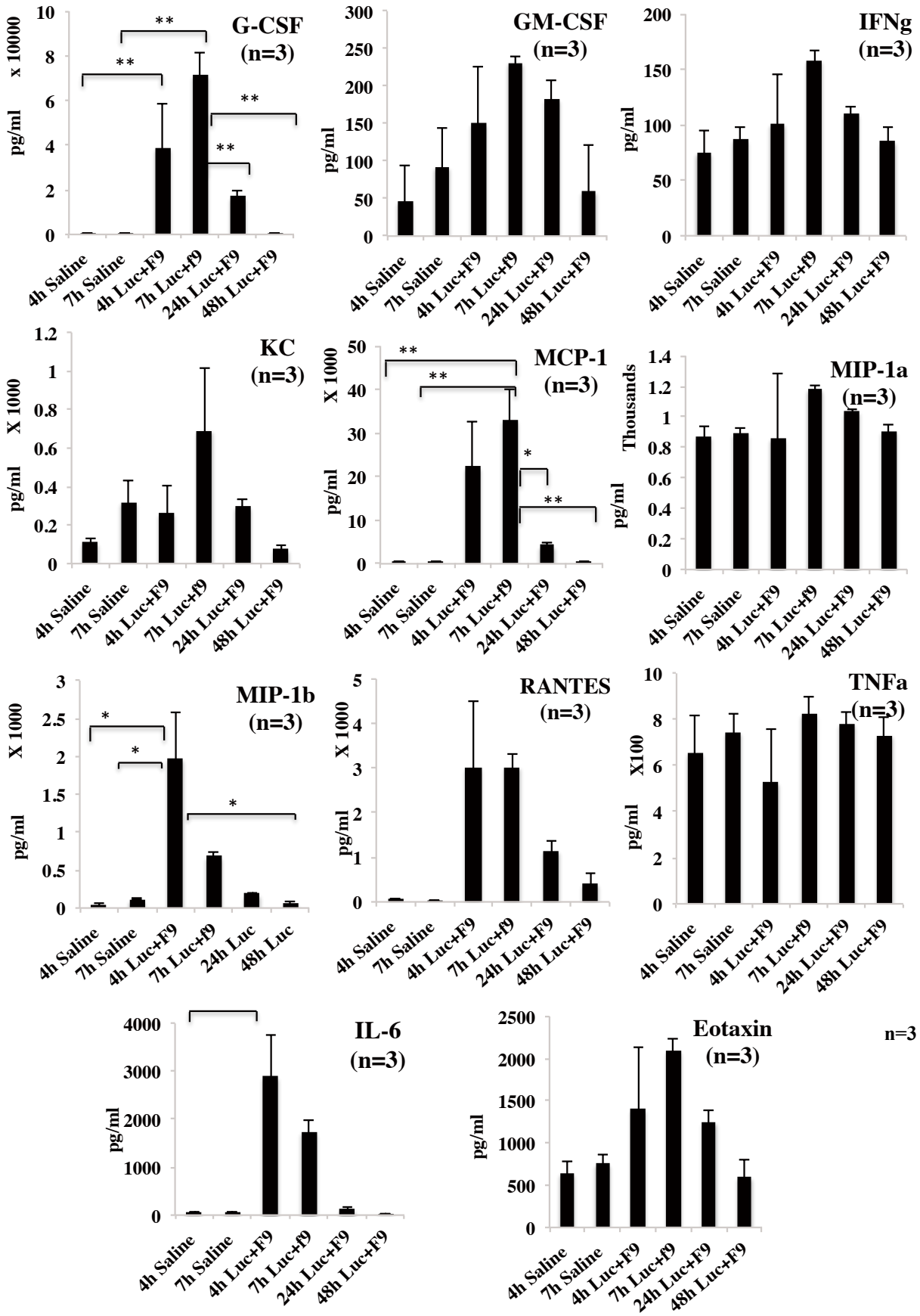


Supplementary Figure 3b (Contd.)



Systemic delivery of Factor IX messenger RNA for protein replacement therapy

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Figure Legends for Supplementary Figures:

Supplementary Figure 1:

a) ELISA for measuring levels of circulating hFIX in $FIX^{-/-}$ mice intravenously administered with hFIX mRNA: LUNAR™ LNP formulation at a dose of 4 mg/kg. A students T-test was used to compare individual groups and a p value less than 0.05 was considered significant.
b) FIX activity in these animals was measured by an APTT clotting assay. As can be seen, at a dose of 4 mg/kg, the FIX mRNA: LUNAR™ LNP treatment was able to significantly improve the clotting efficiency of these animals until 6 days post-treatment. A two-tailed students T-test was used to compare individual groups and a p value less than 0.05 was considered significant.

Supplementary Figure 2:

a) $FIX^{-/-}$ animals were re-dosed thrice with LUNAR™-R338A FIX mRNA LNPs at 4 mg/kg dose or saline over a 30-day period and their serum was assayed for AST/ALT and ALP activity to evaluate liver function. As can be seen, there was no elevation in these indicators of liver toxicity when compared to WT mouse serum thus suggesting that the LUNAR™ formulation is safe *in vivo* even after multiple administrations.

Supplementary Figure 3:

a) A single cohort of immune-competent, $FIX^{-/-}$ (hemophilic) mice were treated with LUNAR™–mRNA LNPs repeatedly when they were 6, 8 and 20 weeks old at doses of 2, 4 and 4 mg/kg respectively. The underlying premise was that repeated administration of the LNPs over a long time period will allow the development of innate and adaptive immune responses to the LUNAR™ LNPs. We estimated that a 3 to 4-month interval allowed sufficient time for any adaptive immune response. At the end of this period, we injected the animals for the third and final time with our mRNA: LUNAR™ LNP formulation and analyzed the systemic levels of pro-inflammatory cytokines in the system using the Biorad multiplex assay. Fold changes in cytokine expression levels were obtained from the multiplex assay and

analyzed using MeV (Multiple Experiment Viewer) to identify any global changes.

b) Fold changes in the levels of individual cytokines upon re-dosing were then analyzed individually by Graphpad Prism. As can be seen, despite repeated administrations and an ample opportunity for the animals to mount an adaptive immune response, there was no change in the levels of many of the critical pro-inflammatory cytokines like IFN-ϒ, TNF-α etc. Modest elevations in the levels of IL-6, MIP1-β and RANTES were seen at seven hours but these rapidly returned to their baseline levels suggesting no long-term inflammatory response to the LUNAR^{TM} LNP-mRNA formulation.