Supplementary Material

Conjugation of hydrophobic moieties enhances potency of antisense oligonucleotides in the muscle of rodents and non-human primates

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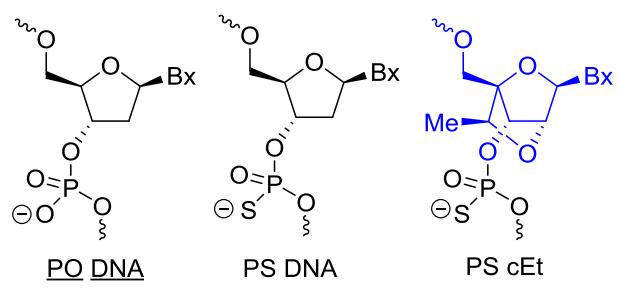
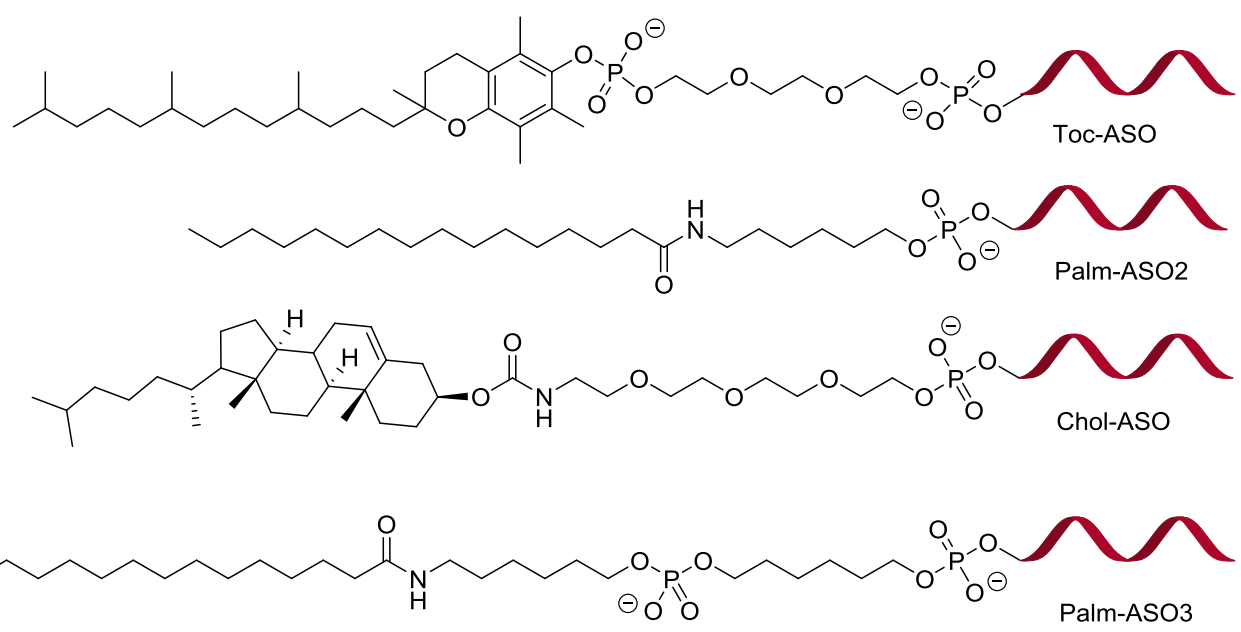
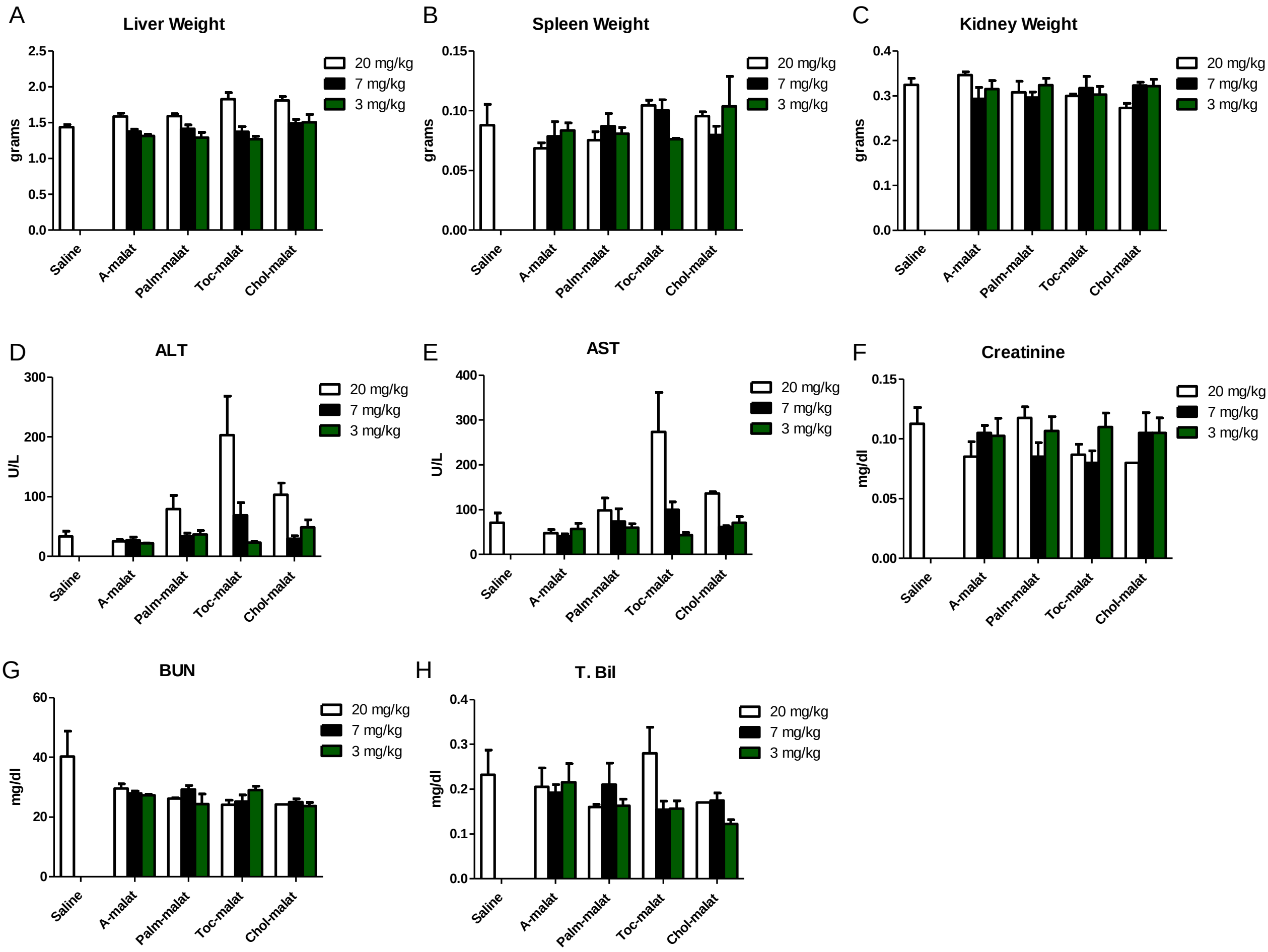
ASO	Sequence (5’ to 3’)	MW calculated	MW found	UV purity
A-malat	GCATTCTAATAGCAGC-A647	7355.4	7354.8	98.2 %
Toc-malat-647	Toc-TCAGCATTCTAATAGCAGC-A647	8108.2	8107.7	97.7 %
Palm-malat-647	Palm-TCAGCATTCTAATAGCAGC-A647	7821.0	7820.5	98.5 %
Chol-malat-647	Chol-TCAGCATTCTAATAGCAGC-A647	8085.3	8085.0	99.0 %
Toc-malat	Toc-TCAGCATTCTAATAGCAGC	7040.0	7038.8	99.6 %
Palm-malat	Palm-TCAGCATTCTAATAGCAGC	6752.8	6751.4	93.9 %
Chol-malat	Chol-TCAGCATTCTAATAGCAGC	7017.1	7016.2	97.6 %
Palm-dmpk1	Palm-HA-TCAACAATAAATACCGAGG	6780.8	6780.9	95.2 %
Palm-dmpk2	Palm-HA-ACAATAAATACCGAGG	5812.0	5811.6	96.2 %
Palm-dmpk3	Palm-HA-HDO-ACAATAAATACCGAGG	6040.3	6039.3	98.3 %
Chol-dmpk	Chol-TCAACAATAAATACCGAGG	7045.1	7044.0	98.4 %
Toc-dmpk	Toc-TCAACAATAAATACCGAGG	7068.1	7067.3	96.9 %

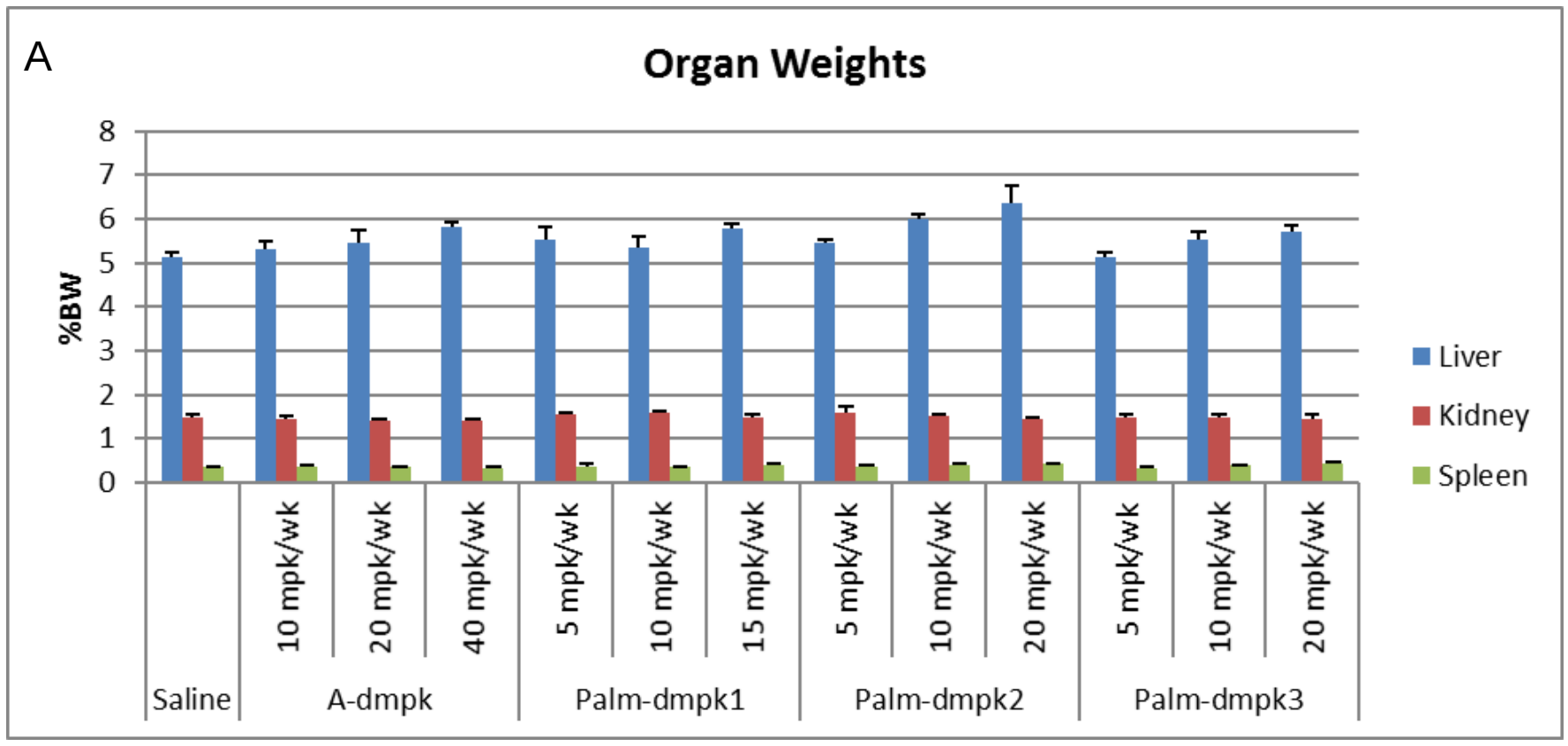
Supplementary Figure S1. Analytical data for ASOs. cEt, DNA, All ASOs are fully PS-modified except underlined nucleotides are PO, Palm=palmitic acid, Chol=cholesterol, Toc=tocopherol, A647-Alexa647, HDO=hexanediol.

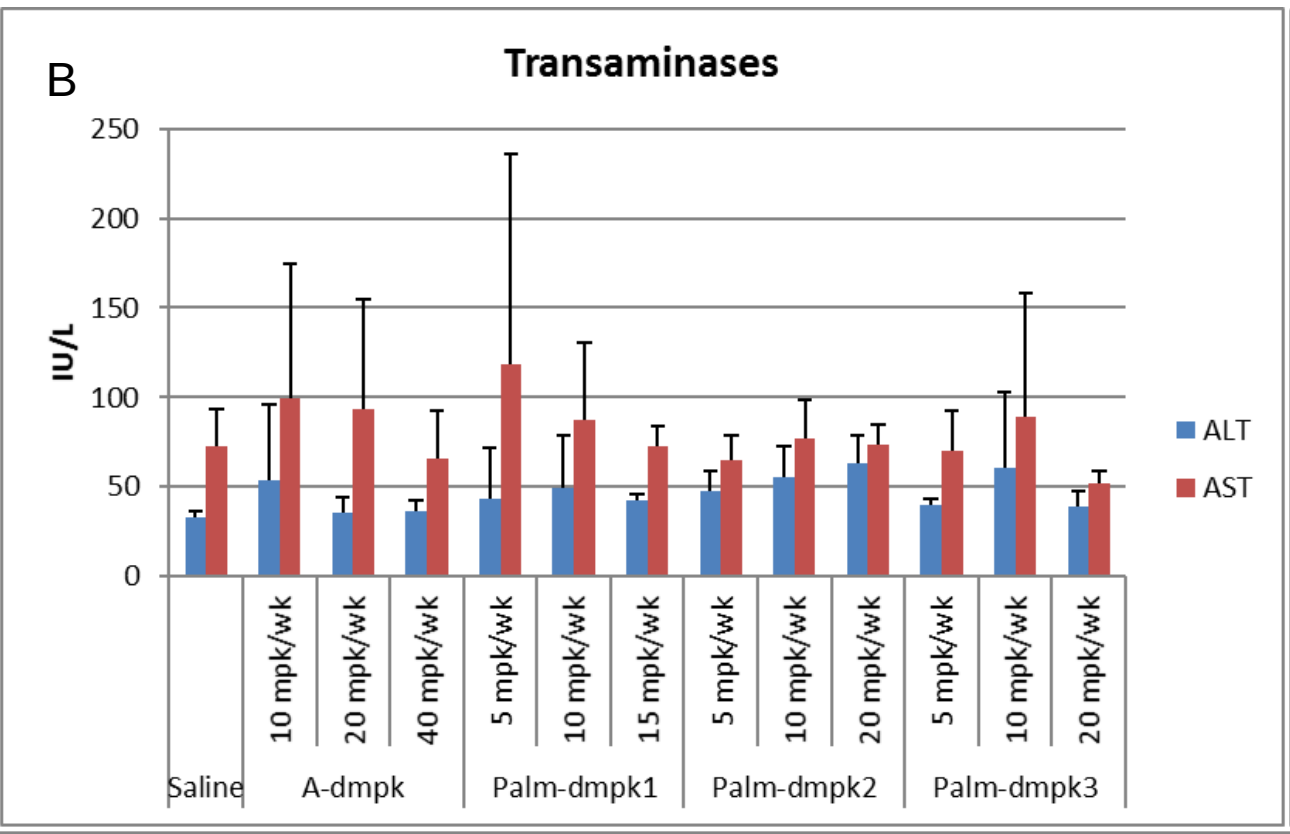


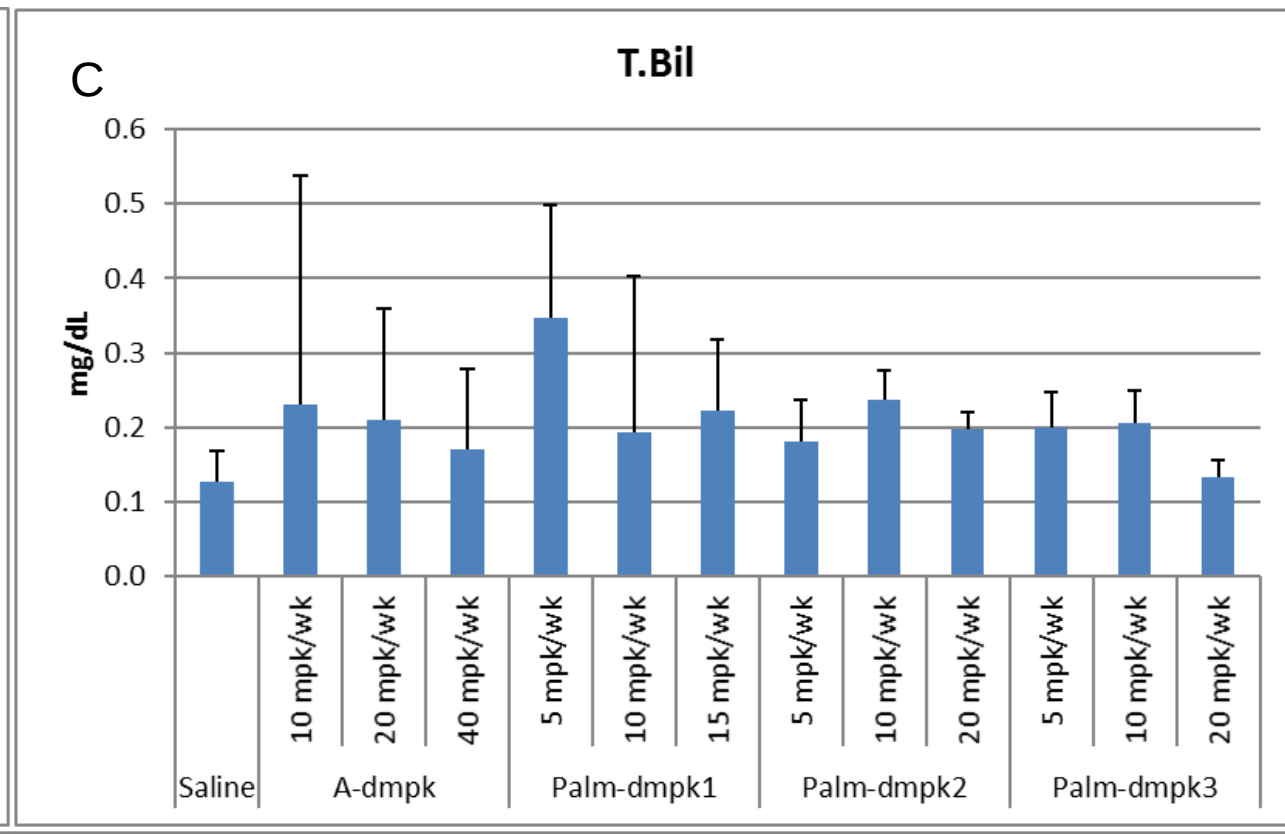


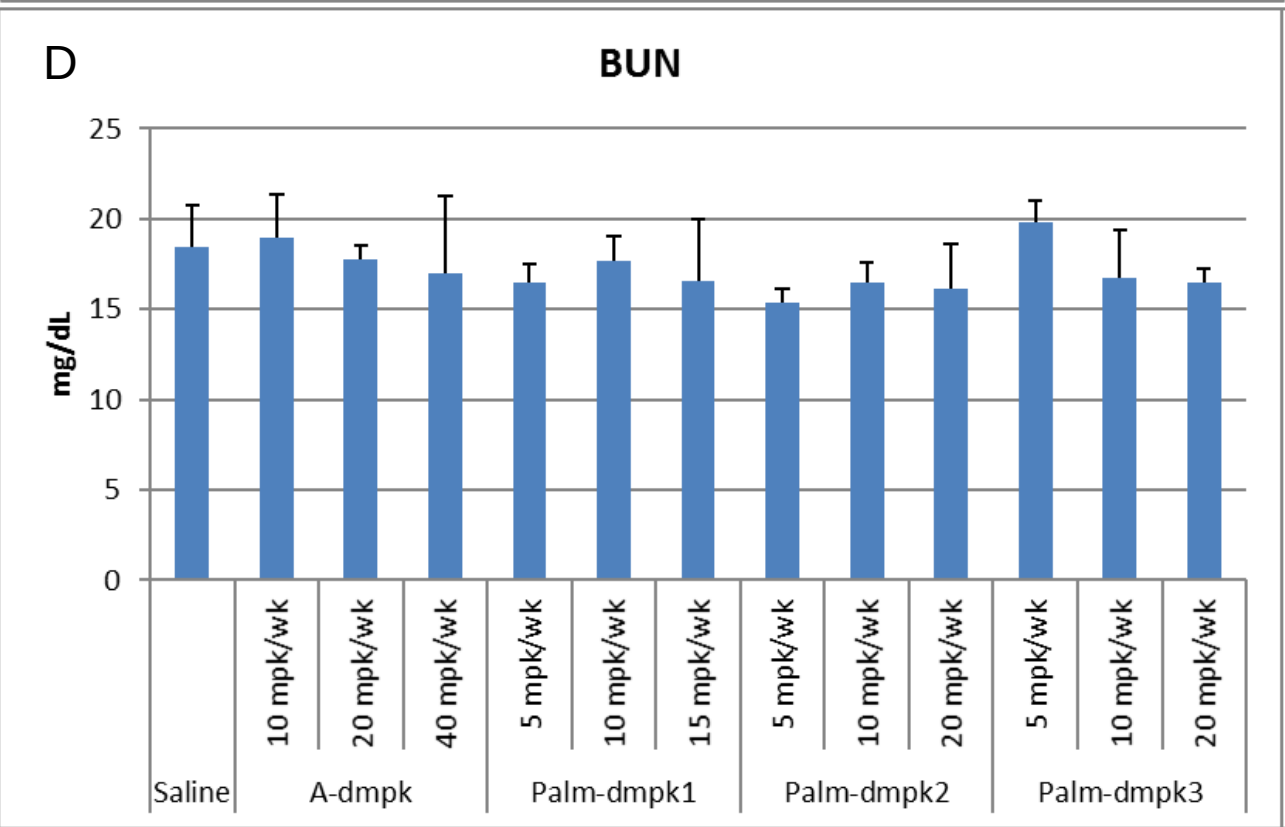


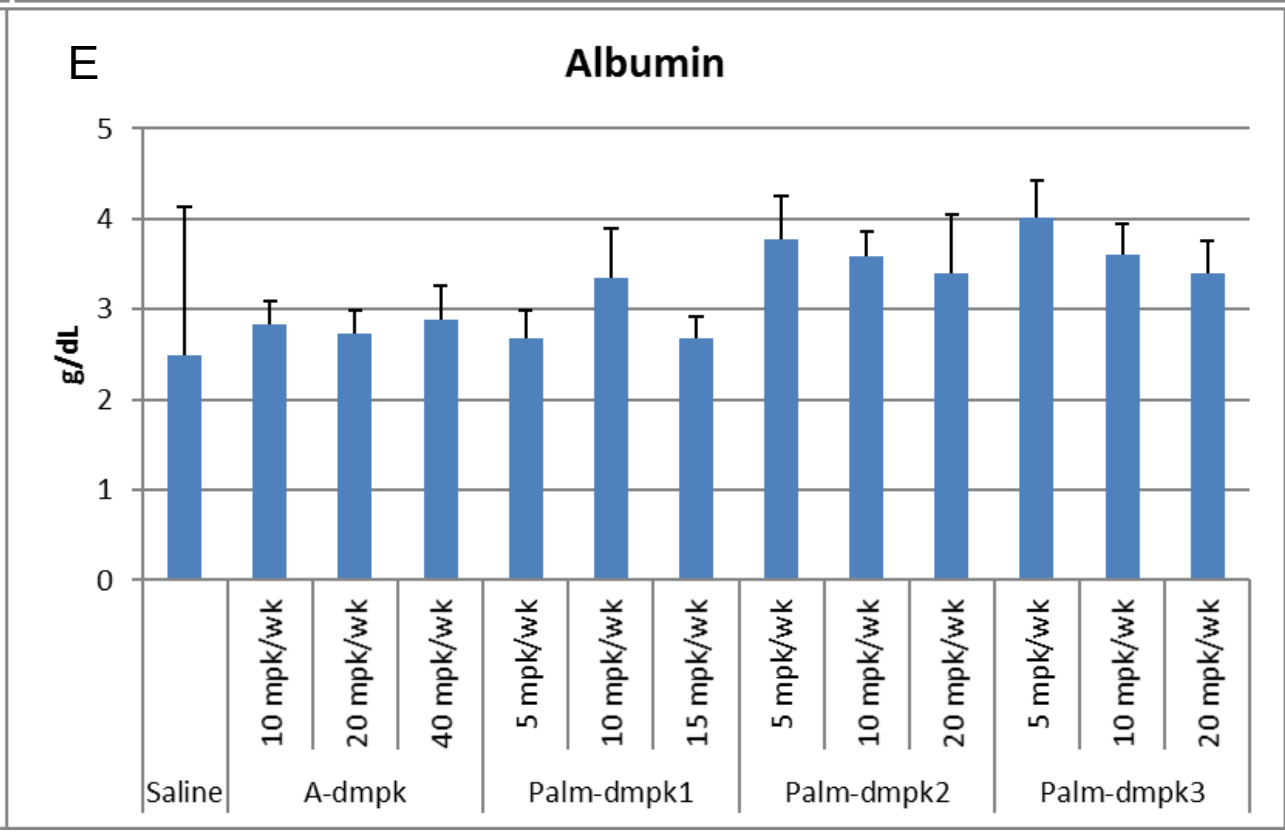
Supplementary Figure S2. Treatment of mice with ASOs A-malat, Palm-malat, Toc-malat and Chol-malat were well tolerated in mice. Mice (C57BL/6, n=4/group) were injected intravenously once weekly for two weeks with 3, 10 or 20 mg/kg of ASOs. Mice were sacrificed 48 hours after the last dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) liver (B) spleen and (C) kidney weights (D) ALT (E) AST (F) creatinine (G) Blood urea nitrogen and (H) Bilirubin. All errors are ±std.dev.



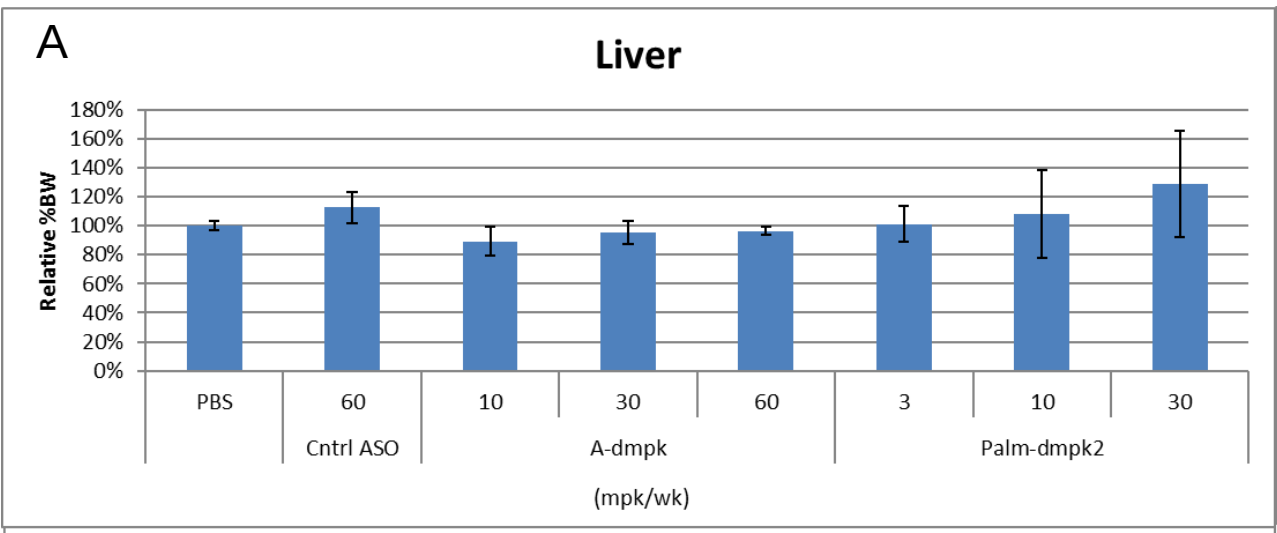


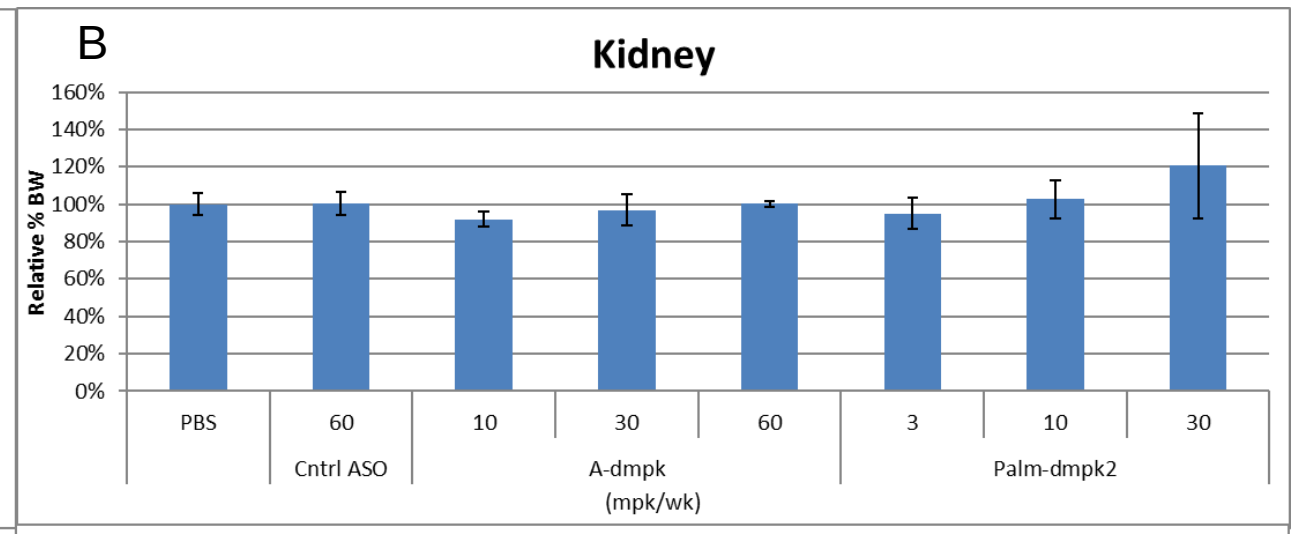


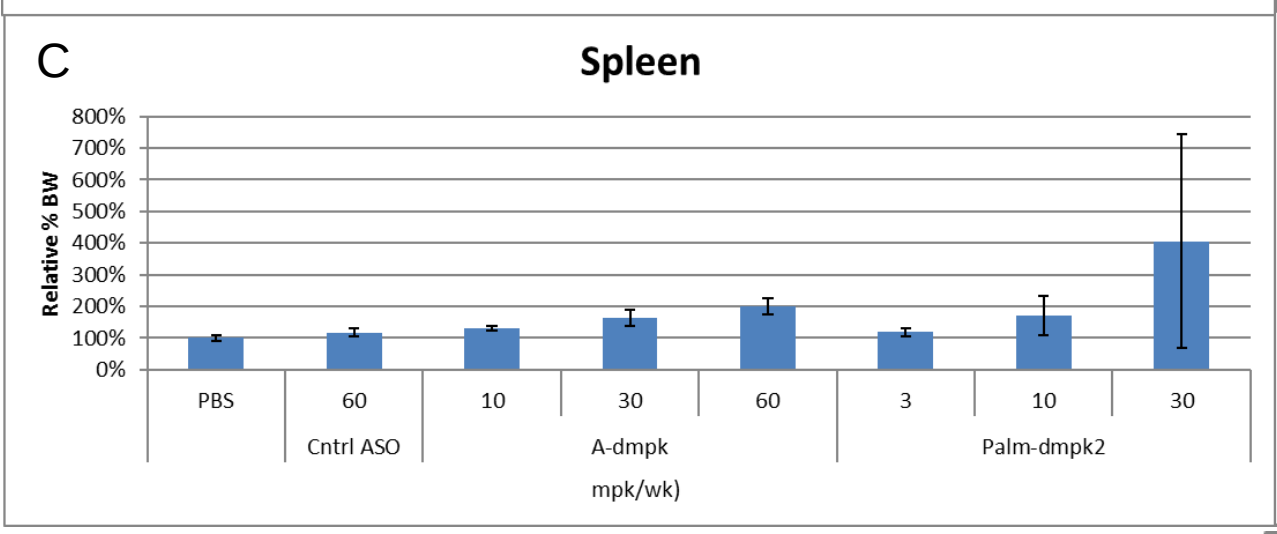


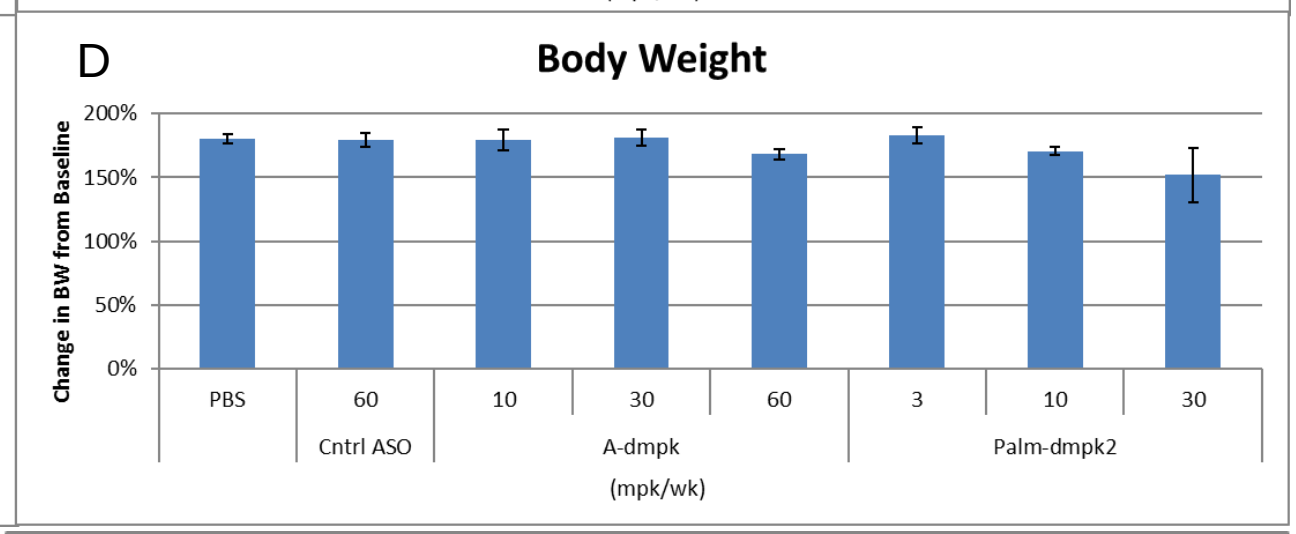


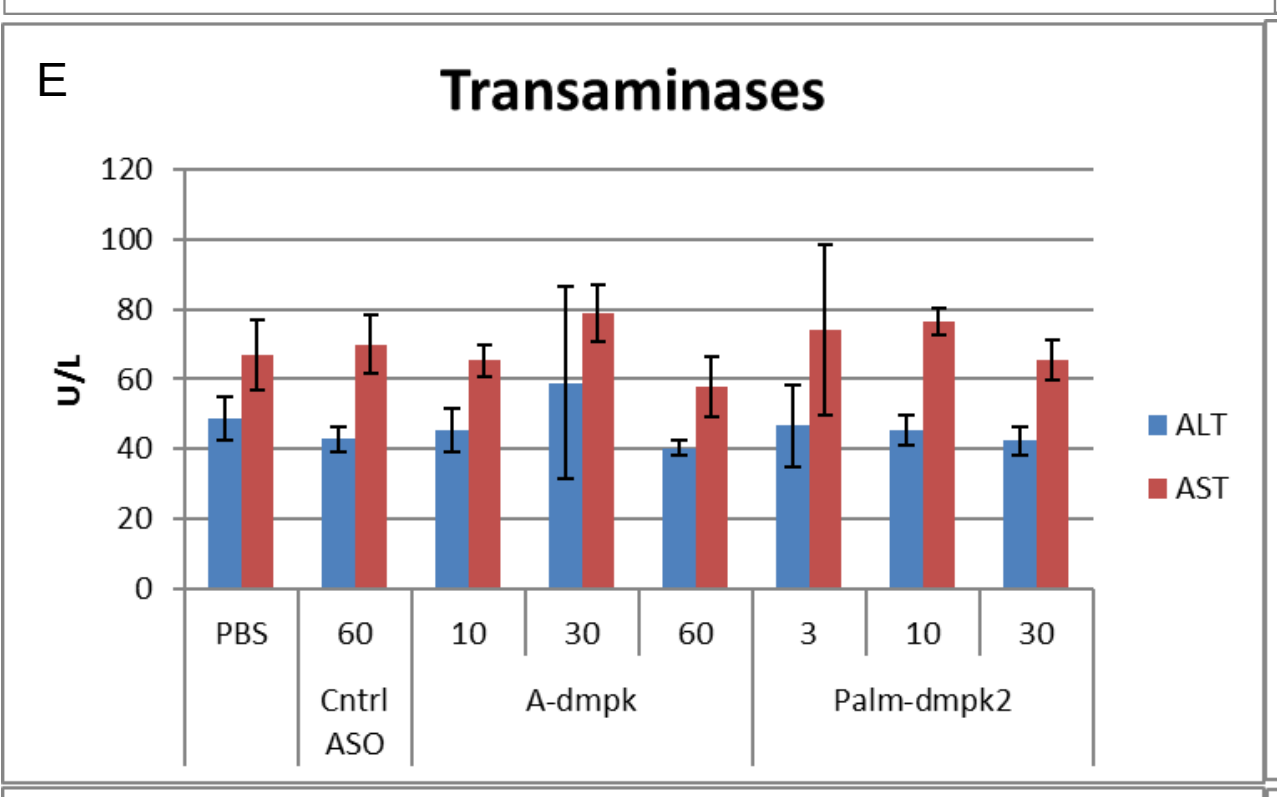
Supplementary Figure S3. Palmitate conjugated ASOs targeting DMPK mRNA were well tolerated in mice. Mice (Balb-c, n=4/group) were injected subcutaneously once weekly for 4 weeks with 10, 20 or 40 mg/kg of ASO. Mice were sacrificed 48 hours after the last ASO dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) organ weights (B) ALT or AST (C) Bilirubin (D) Blood urea nitrogen (BUN) or (E) Albumin. All errors are ±std.dev.

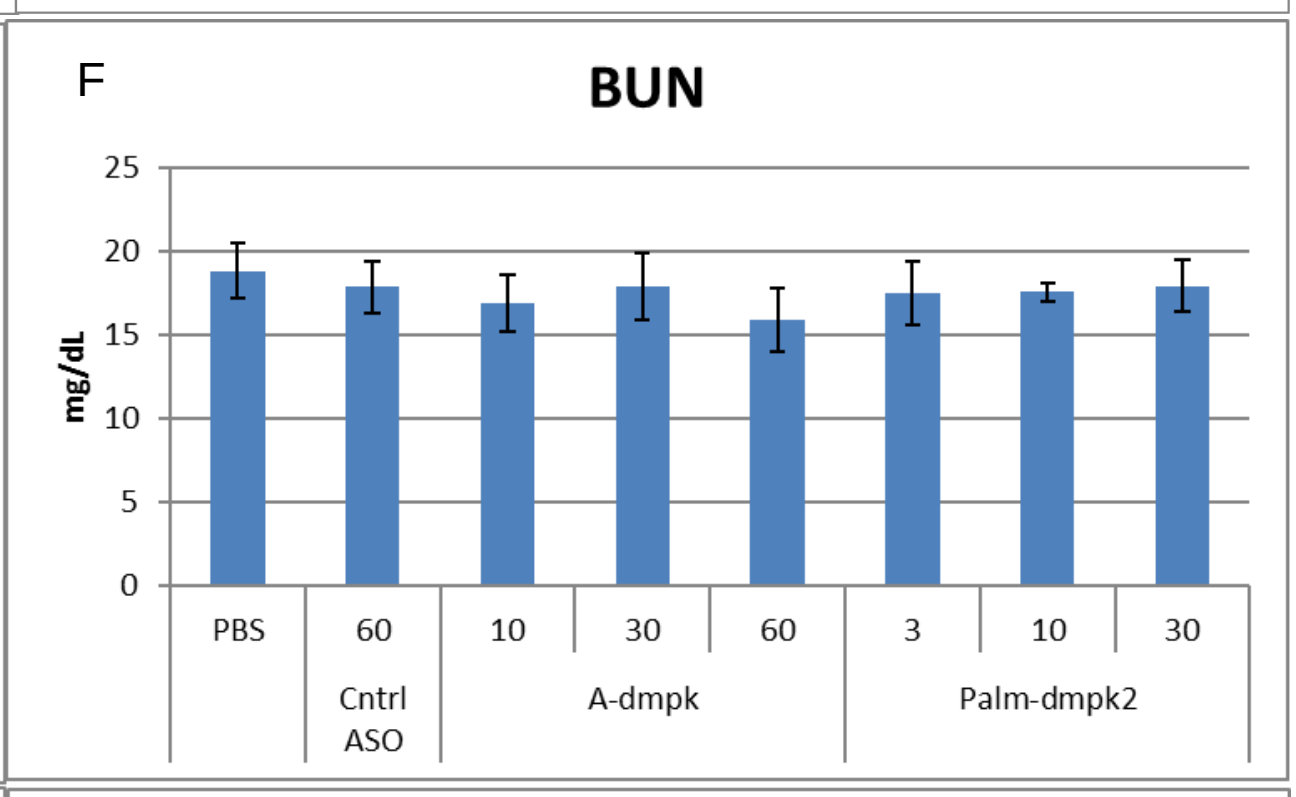


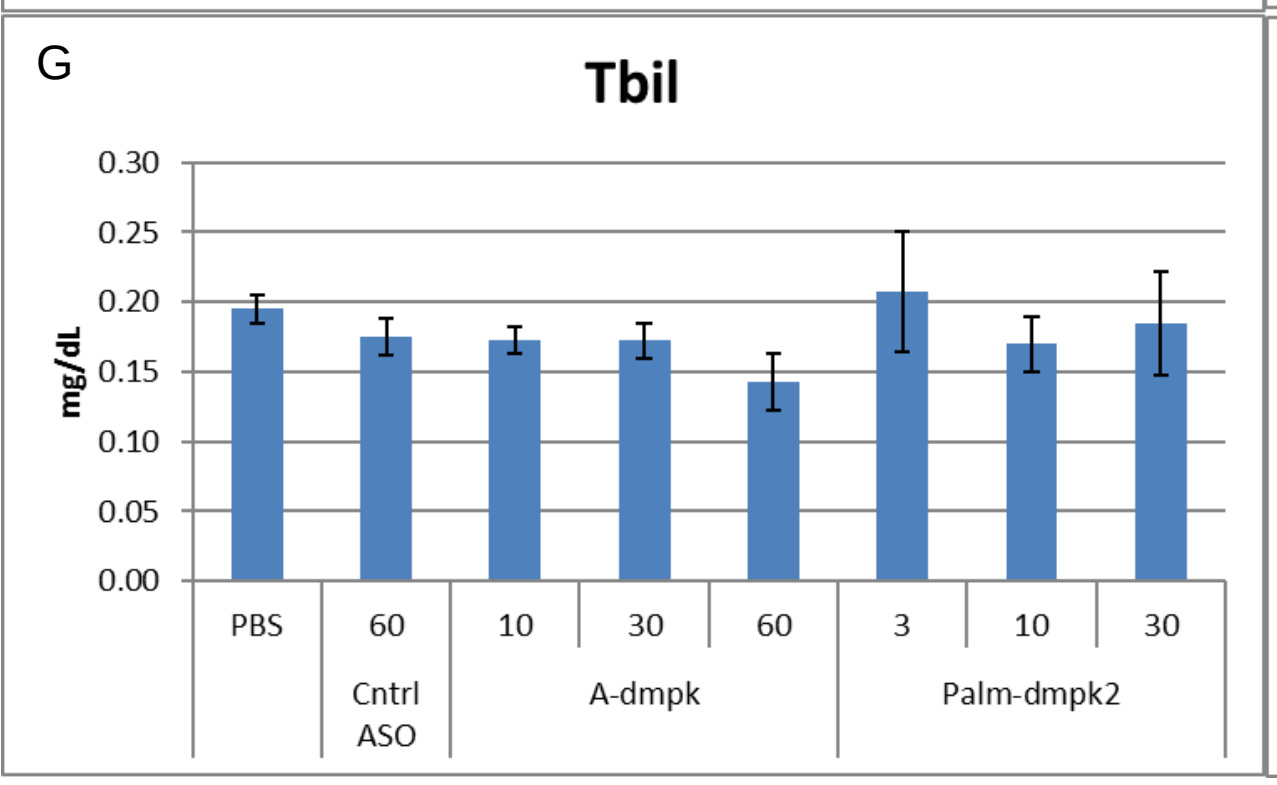


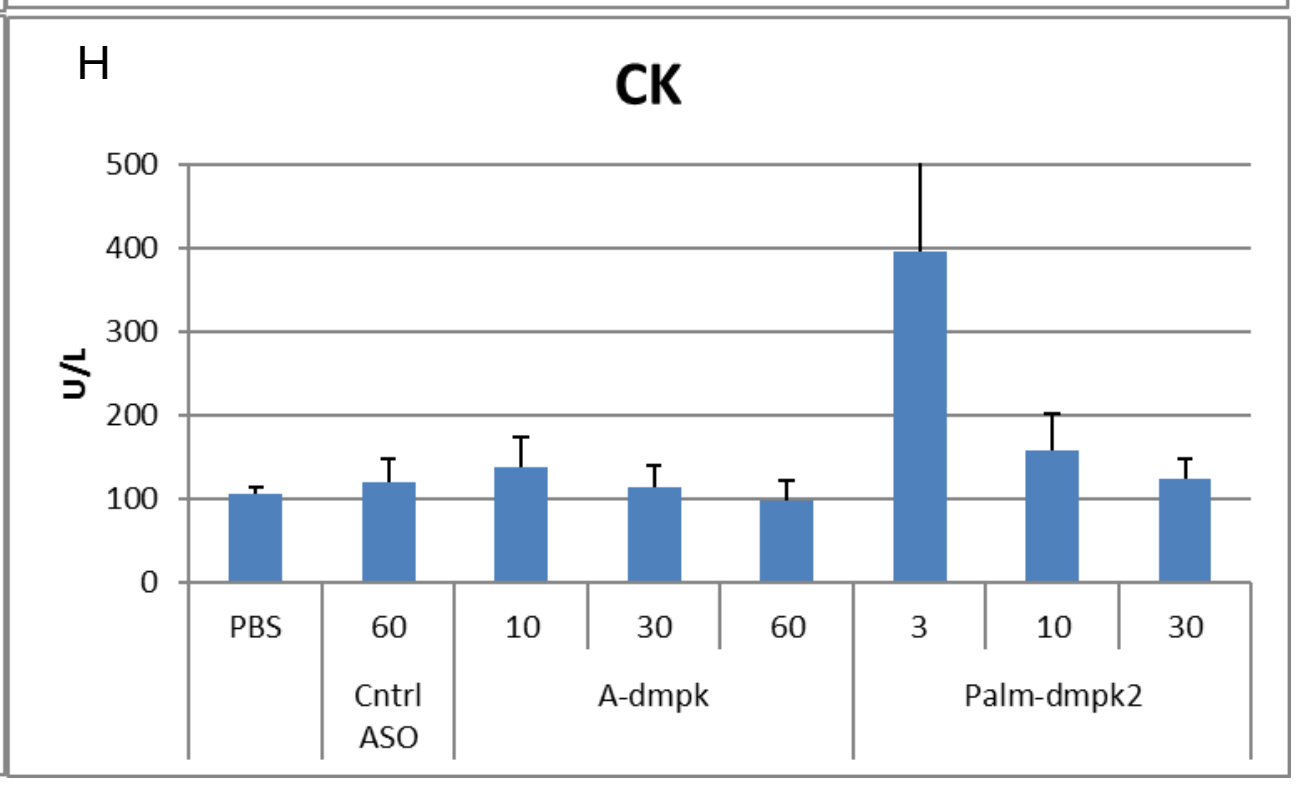




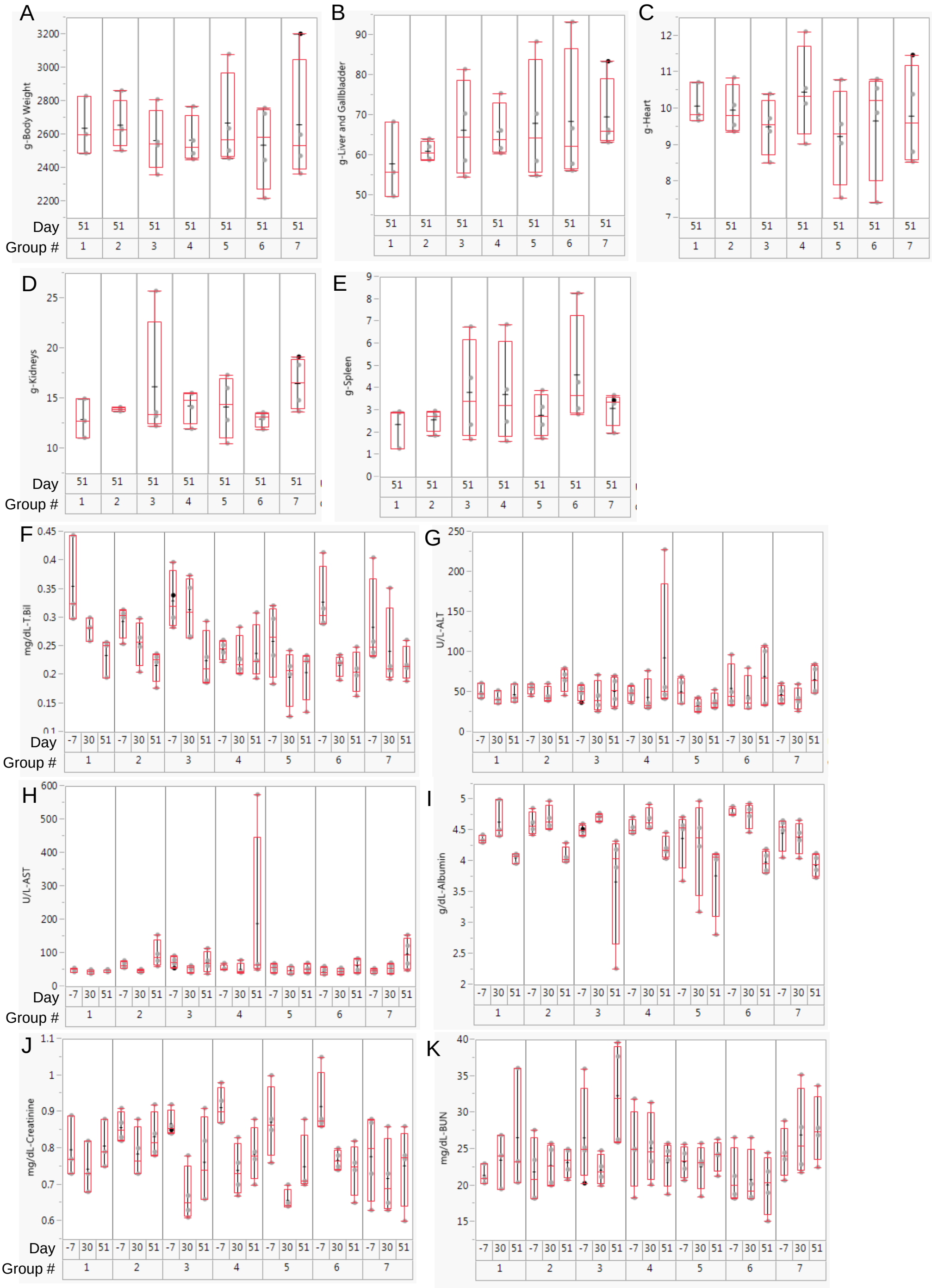




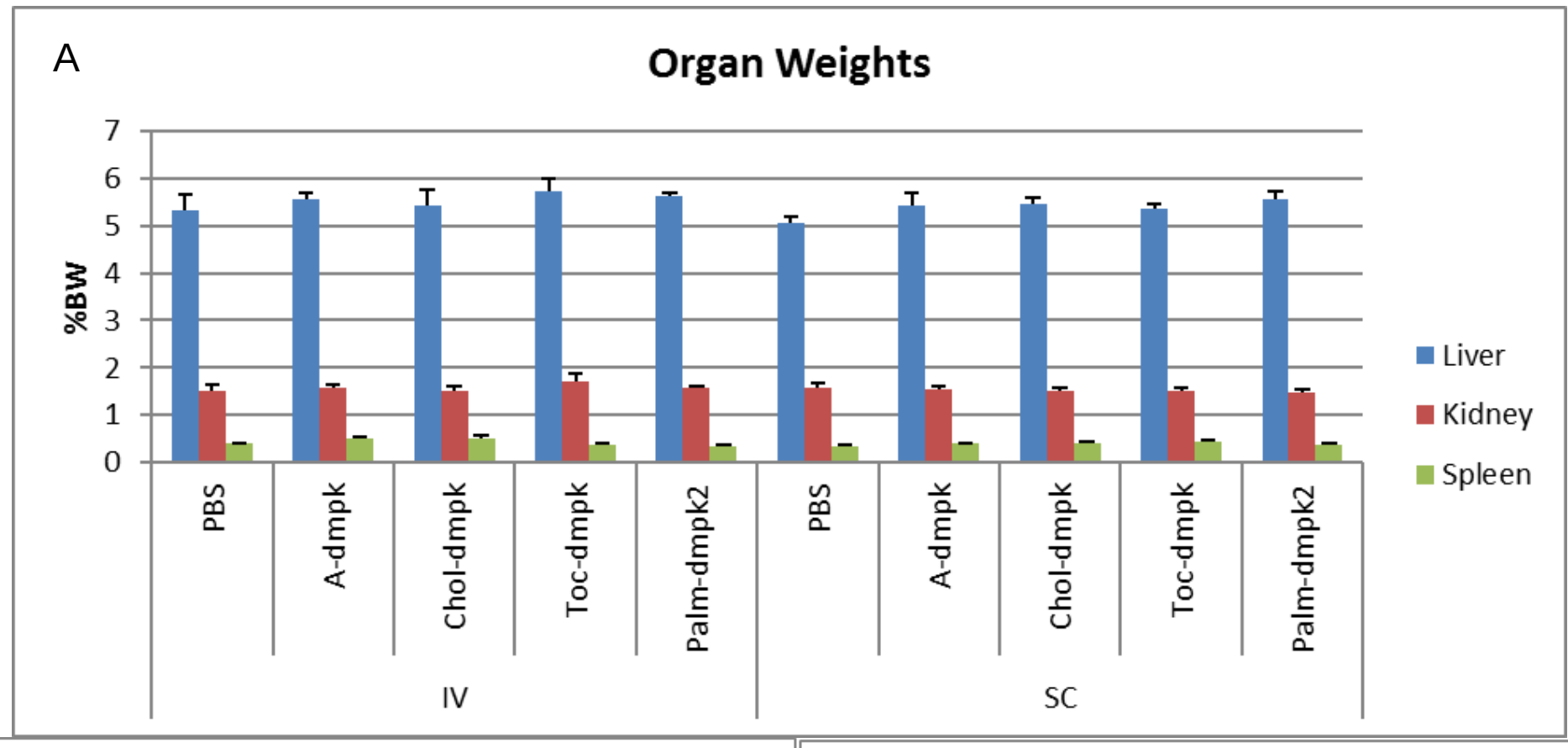


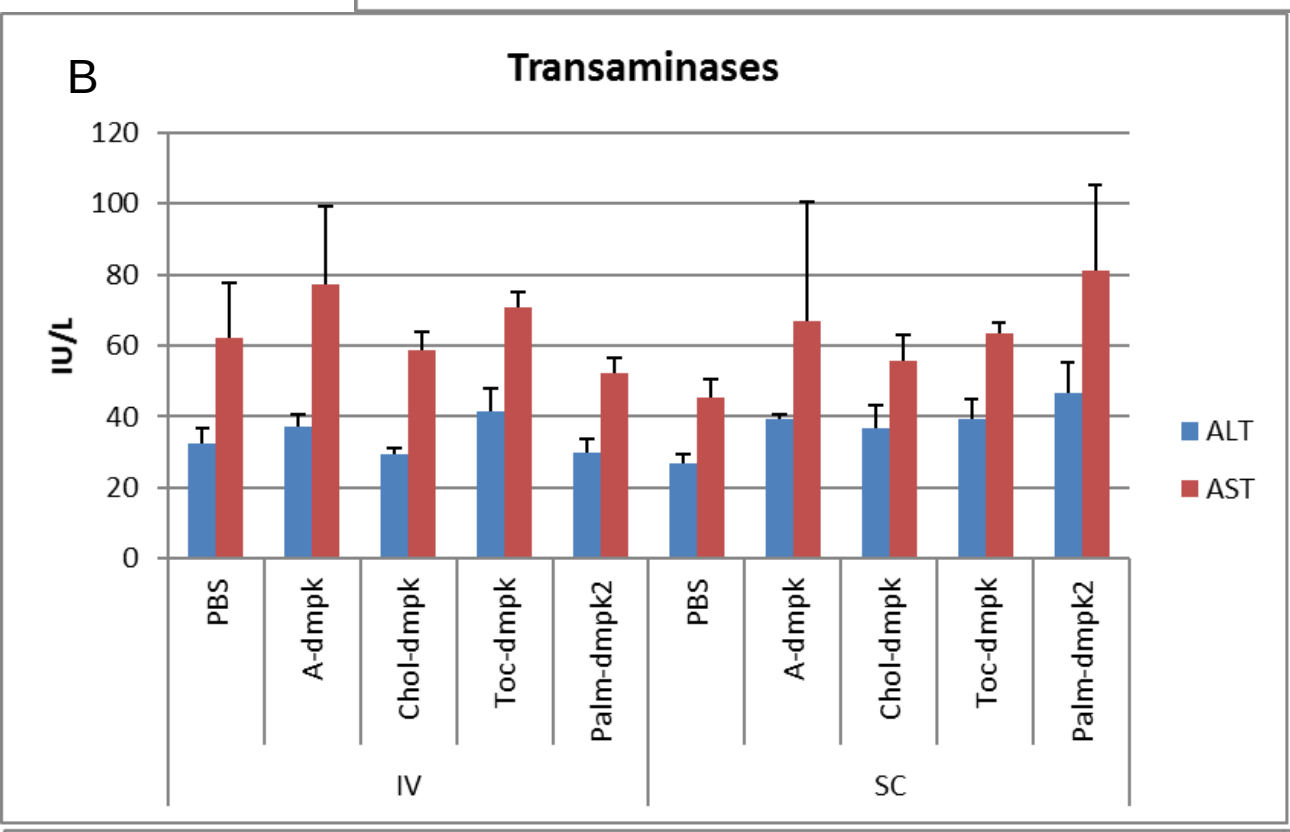


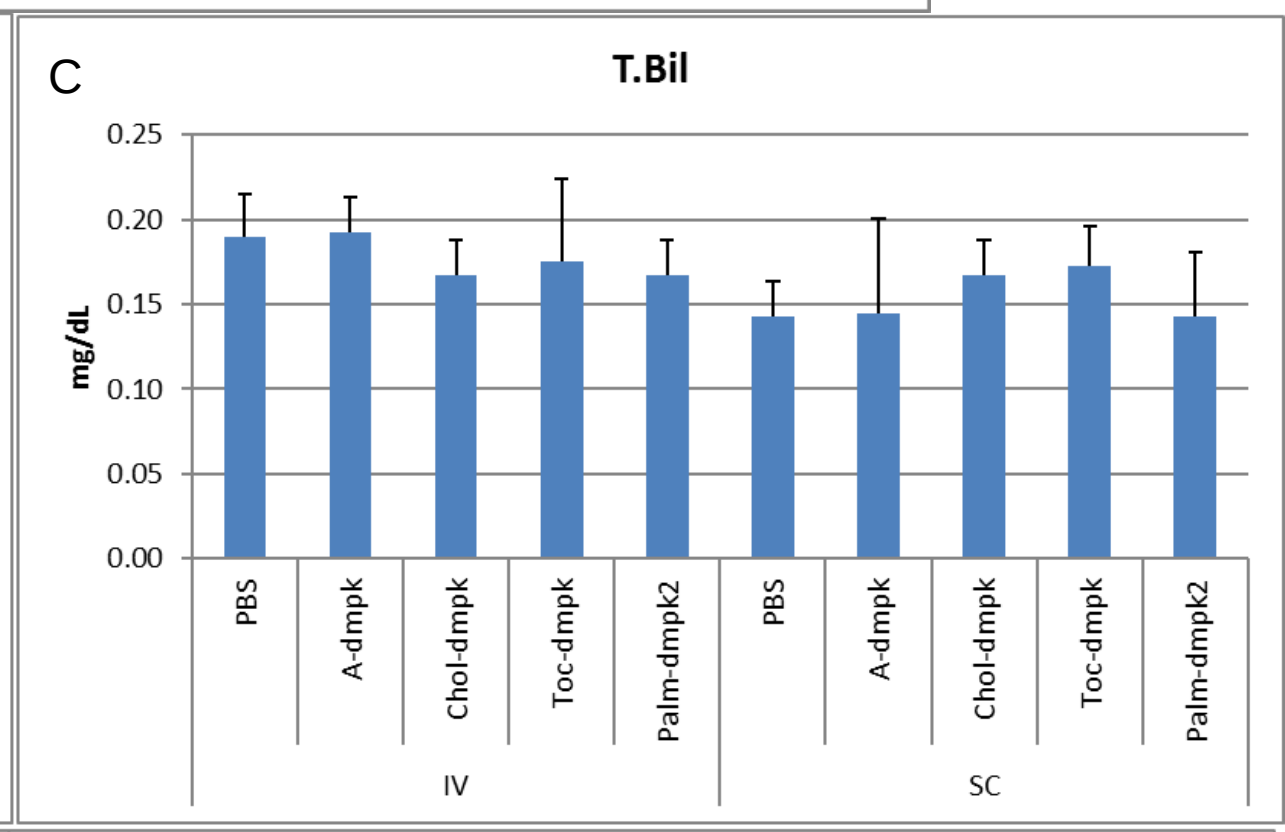
Supplementary Figure S4. Palmitate conjugated ASOs targeting DMPK mRNA were well tolerated in rats. Rats (SD, n=4/group) were injected subcutaneously once weekly for 3 weeks with 10, 30 or 60 mg/kg of ASO. Mice were sacrificed 48 hours after the last ASO dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) liver (B) kidney (C) spleen (D) body weights (E) ALT or AST (F) Blood urea nitrogen (BUN) (G) Bilirubin (H) Creatinine kinase. All errors are ±std.dev.

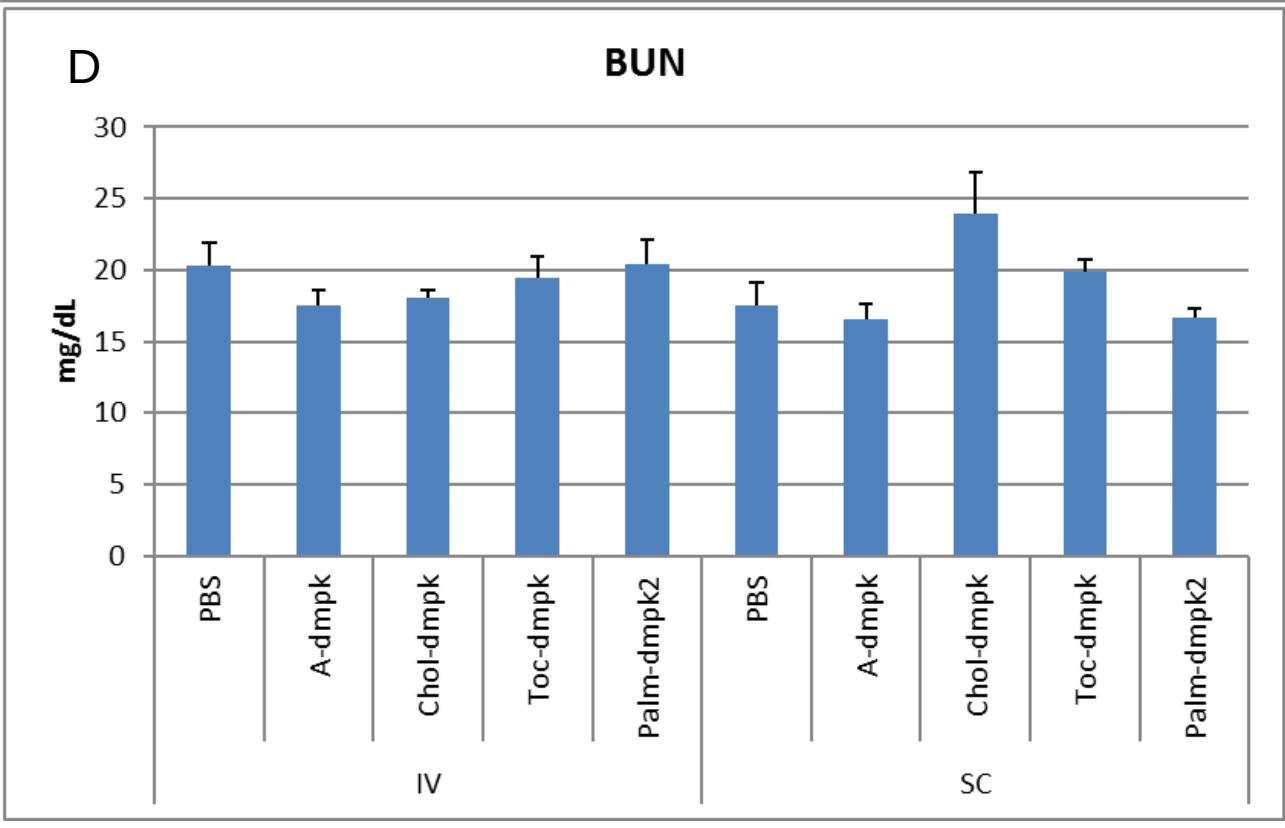


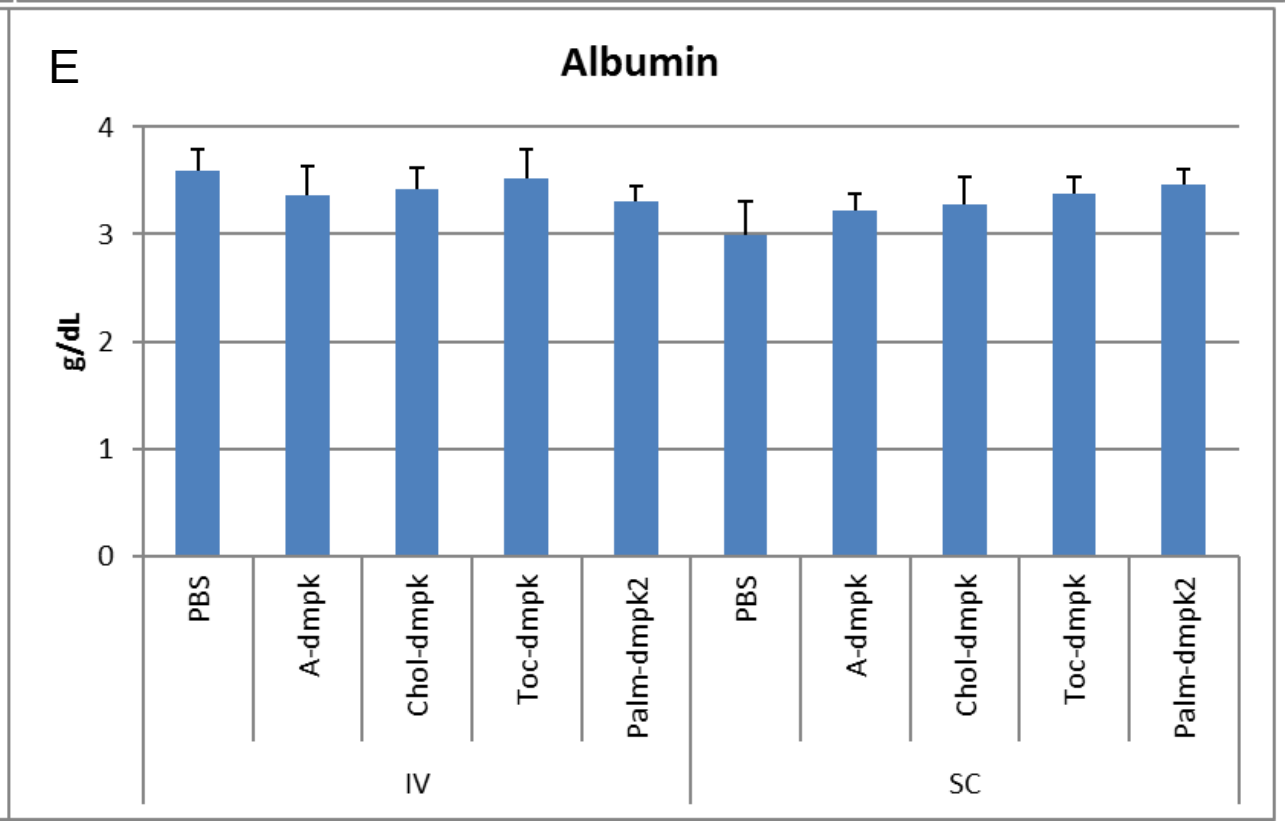
Supplementary Figure S5. Palmitate conjugated ASOs targeting DMPK mRNA were well tolerated in monkeys. Cynomolgus monkeys (n=4/group) were injected subcutaneously with 10 doses of A-dmpk or Palm-dmpk2 over 7 weeks. Monkeys were sacrificed 48 hours after the last ASO dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) Body (B) liver (C) heart (D) kidney (E) spleen weights. ASO treatment did not cause appreciable changes in (F) Bilirubin (G) ALT (H) AST (I) Albumin (J) Creatinine and (K) Blood urea nitrogen as measured on days -7, 30 and 51. Groups1 represents saline, Groups 2, 3,4 represent animals treated with 10, 20 or 40 mg/kg of A-dmpk, Groups 5, 6 and 7 represent animals treated with 10, 20 and 40 mg/kg of Palm-dmpk2. Each data point represents an animal.



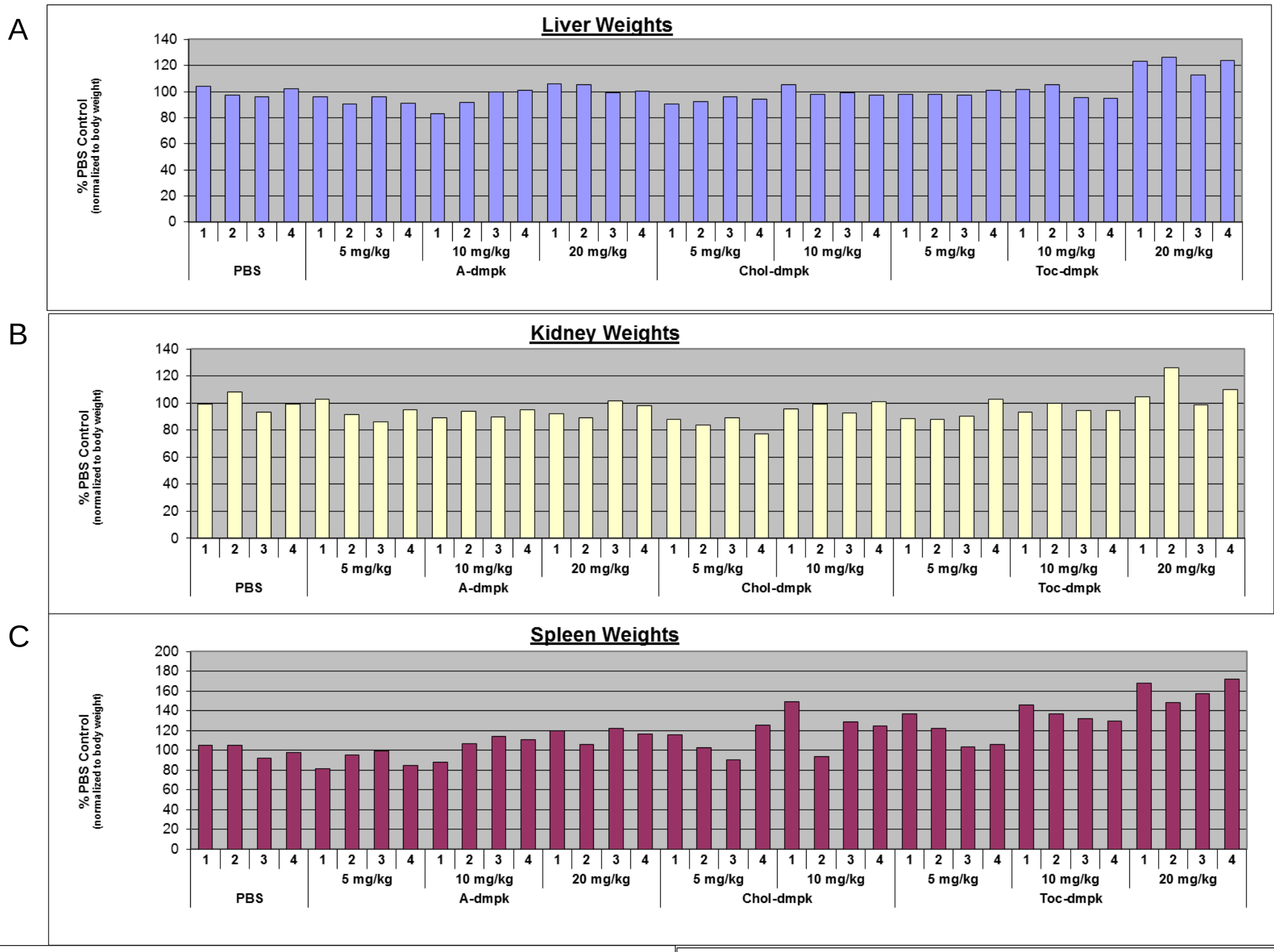


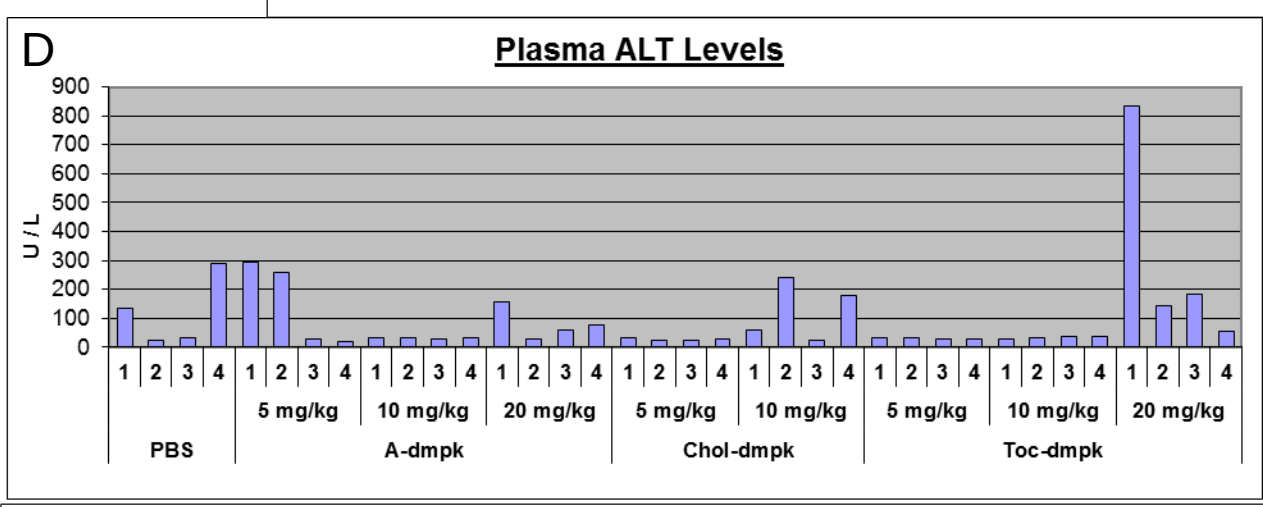


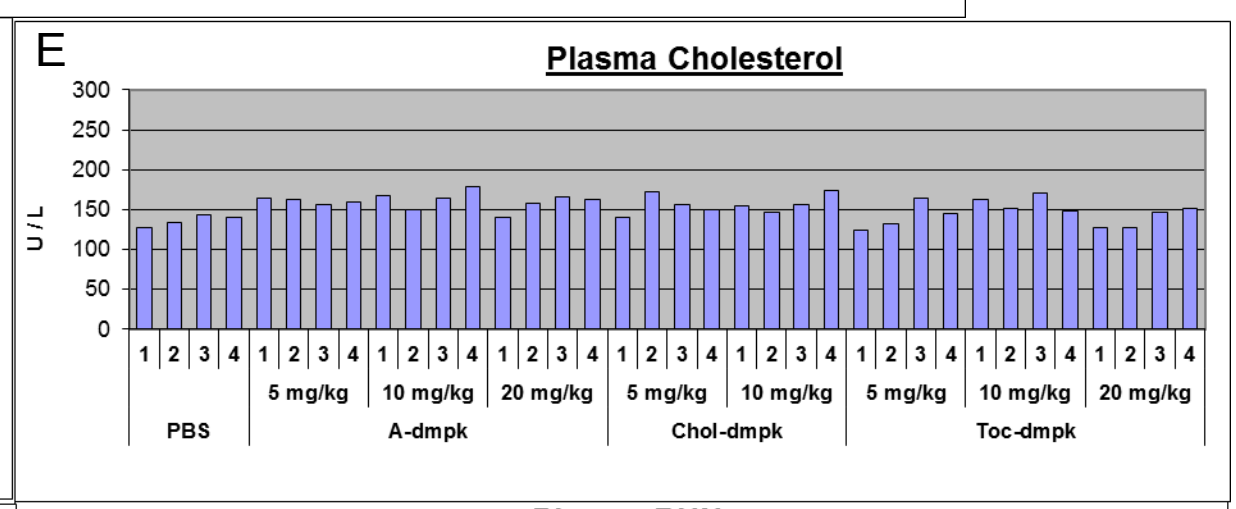


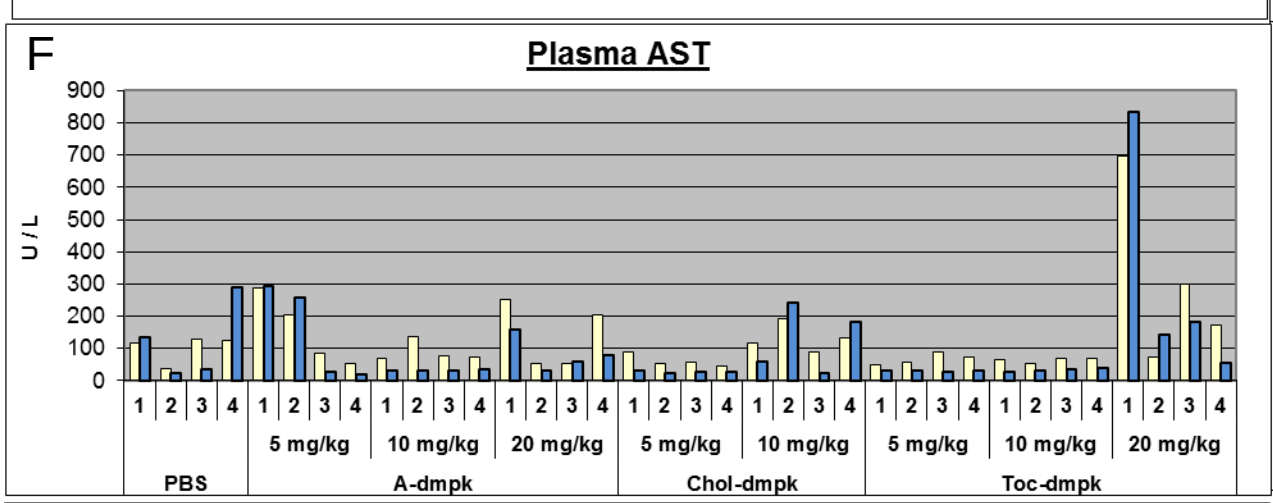


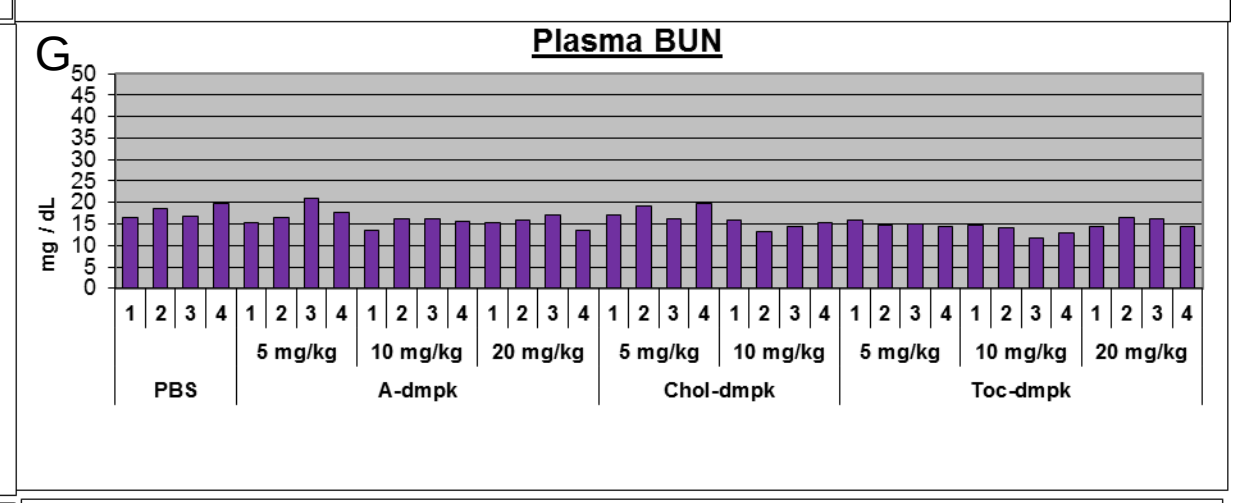
Supplementary Figure S6. Palmitate, tocopherol and cholesterol conjugated ASOs were well tolerated after subcutaneous or intravenous injections in mice. Mice (Balb-c, n=4/group) were injected once weekly for 4 weeks with 10 mg/kg of A-dmpk, Palm-dmpk2, Toc-dmpk or Chol-dmpk by subcutaneous or intravenous injections. Mice were sacrificed 48 hours after the last dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) organ weights (B) ALT or AST (C) Bilirubin (D) Blood urea nitrogen (BUN) or (E) Albumin. All errors are ±std.dev.

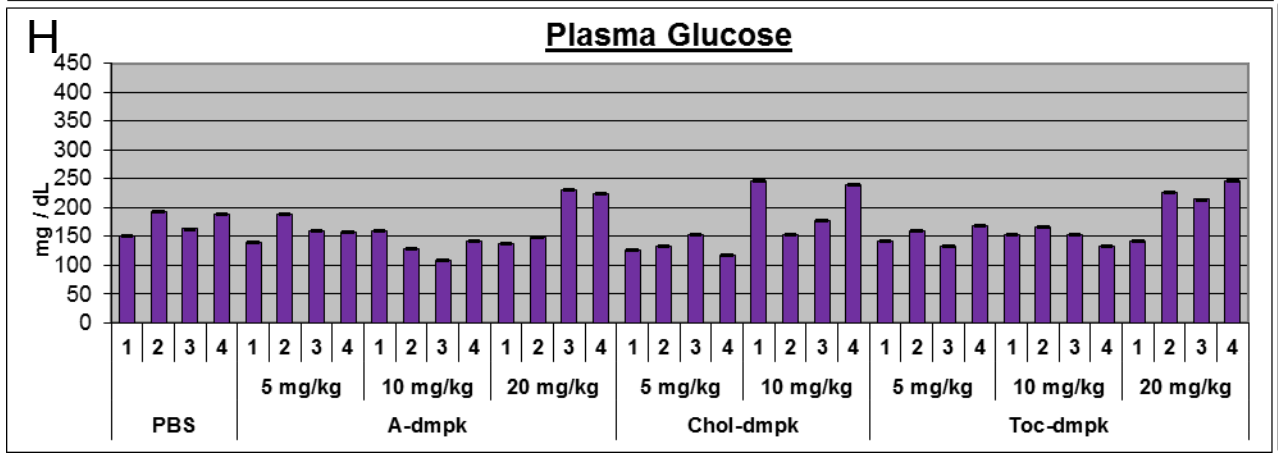


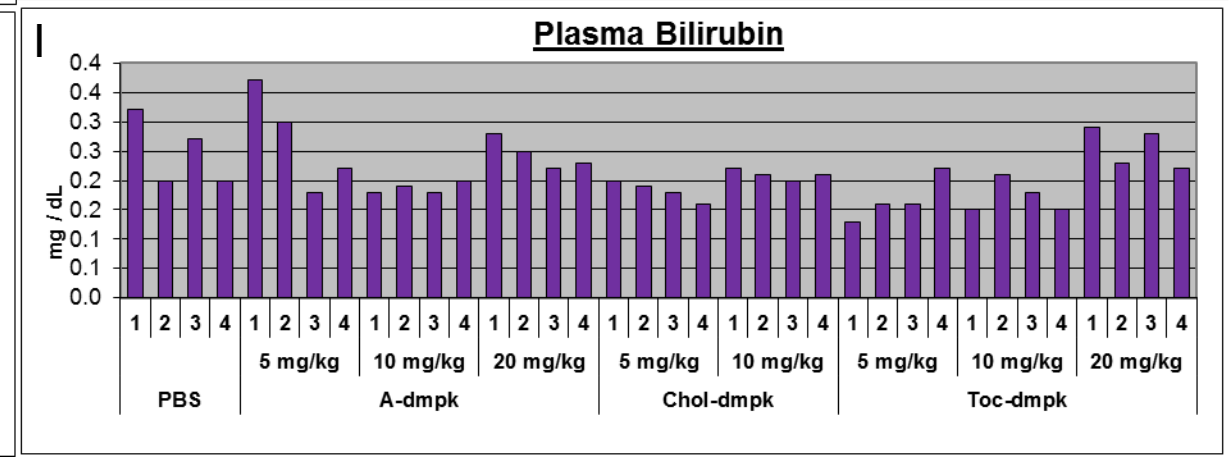




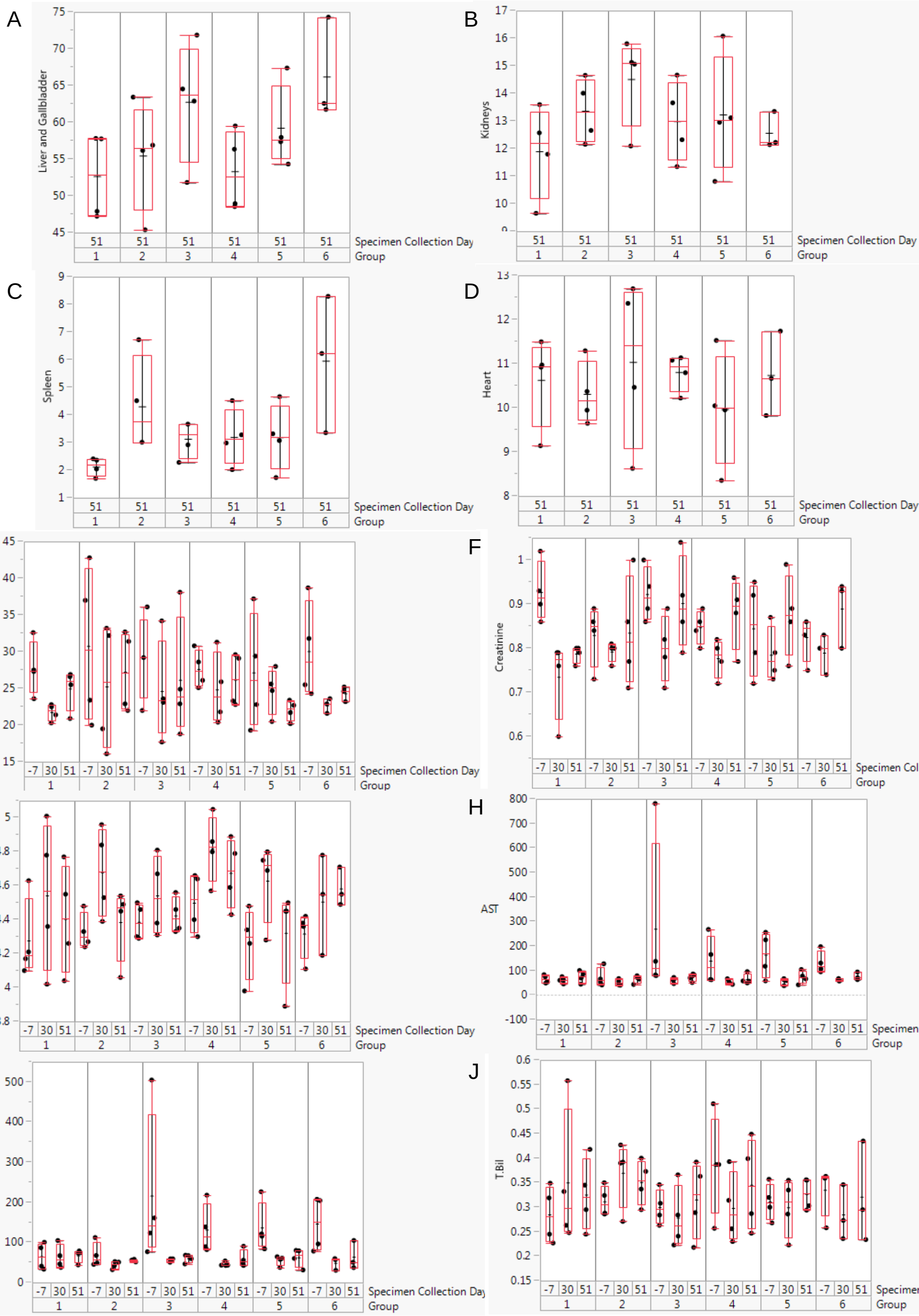








Supplementary Figure S7. Tocopherol conjugated ASO was well tolerated after multiple doses in mice but the cholesterol ASO was toxic at higher doses. Mice (Balb/c, n=4/group) were injected intravenously with 5, 10 or 20 mg/kg of A-dmpk, Chol-dmpk or Toc-dmpk once weekly for four weeks. All mice treated with Chol-dmpk died after one or two injections of 20 mg/kg ASO. Mice were sacrificed 48 hours after the last dose and organs were harvested and weighed. ASO treatment did not cause appreciable changes in (A) Liver (B) kidney (C) spleen weights (D) ALT (E) Cholesterol (F) AST (G) Blood urea nitrogen (H) glucose (I) bilirubin. Data from individual animals shown.



Supplementary Figure S8. Tocopherol conjugated ASOs targeting DMPK mRNA were well tolerated in monkeys. Cynomolgus monkeys (n=4/group) were injected subcutaneously with 10 doses of A-dmpk or Toc-dmpk over 7 weeks. Monkeys were sacrificed 48 hours after the last ASO dose and organs were harvested and weighed. All ASOs were well tolerated and did not cause appreciable changes in (A) Liver (B) kidney (C) spleen (D) heart weights. ASO treatment did not cause appreciable changes in (E) Blood urea nitrogen (F) creatinine (G) Albumin (H) AST (I) ALT and (J) Bilirubin as measured on days -7, 30 and 51. Groups1 represents saline, Groups 2 and 3 represent animals treated with 20 or 40 mg/kg of A-dmpk, Groups 4, 5 and 6 represent animals treated with 10, 20 and 40 mg/kg of Toc-dmpk. Each data point represents an animal.