Table 1. siRNA Sequences

	Sequence 5' to 3'
LUC-S	cuuAcGcuGAGuAcuucGAdTsdT
LUC-AS	UCGAAGuACUcAGCGuAAGdTsdT
mEGLN1-S	cAAGGuAcGcAAuAAcuGudTsdT
mEGLN1-AS	AcAGUuAUUGCGuACCUUGdTsdT
m/rEGLN2-S	AGAcAAGuAucAGcuAGcAdTsdT
m/rEGLN2-AS	UGCuAGCUGAuACUUGUCUdTsdT
m/rEGLN3-S	GuAGuAAcAGuuccGGAAAdTsdT
m/rEGLN3-AS	UUUCCGGAACUGUuACuACdTsdT
rEGLN1-S	cGccAcAAGGuAcGcAAuAdTsdT
rEGLN1-AS	uAUUGCGuACCUUGUGGCGdTsdT
GFP-S	AcAuGAAGcAGcACGACuUdTsdT
GFP-AS	AAGUCGUGCUGCUUCAUGUdTsdT

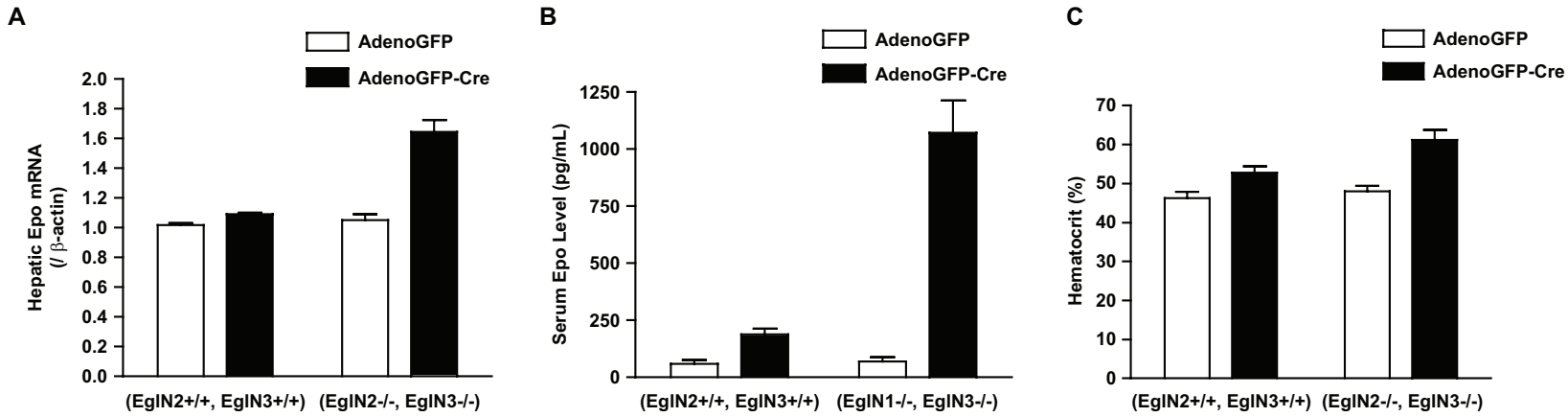
Lowercase base=2'OMe, s=phosphorothioate, m=mouse, r=rat

Table 2. 5'RACE

primers

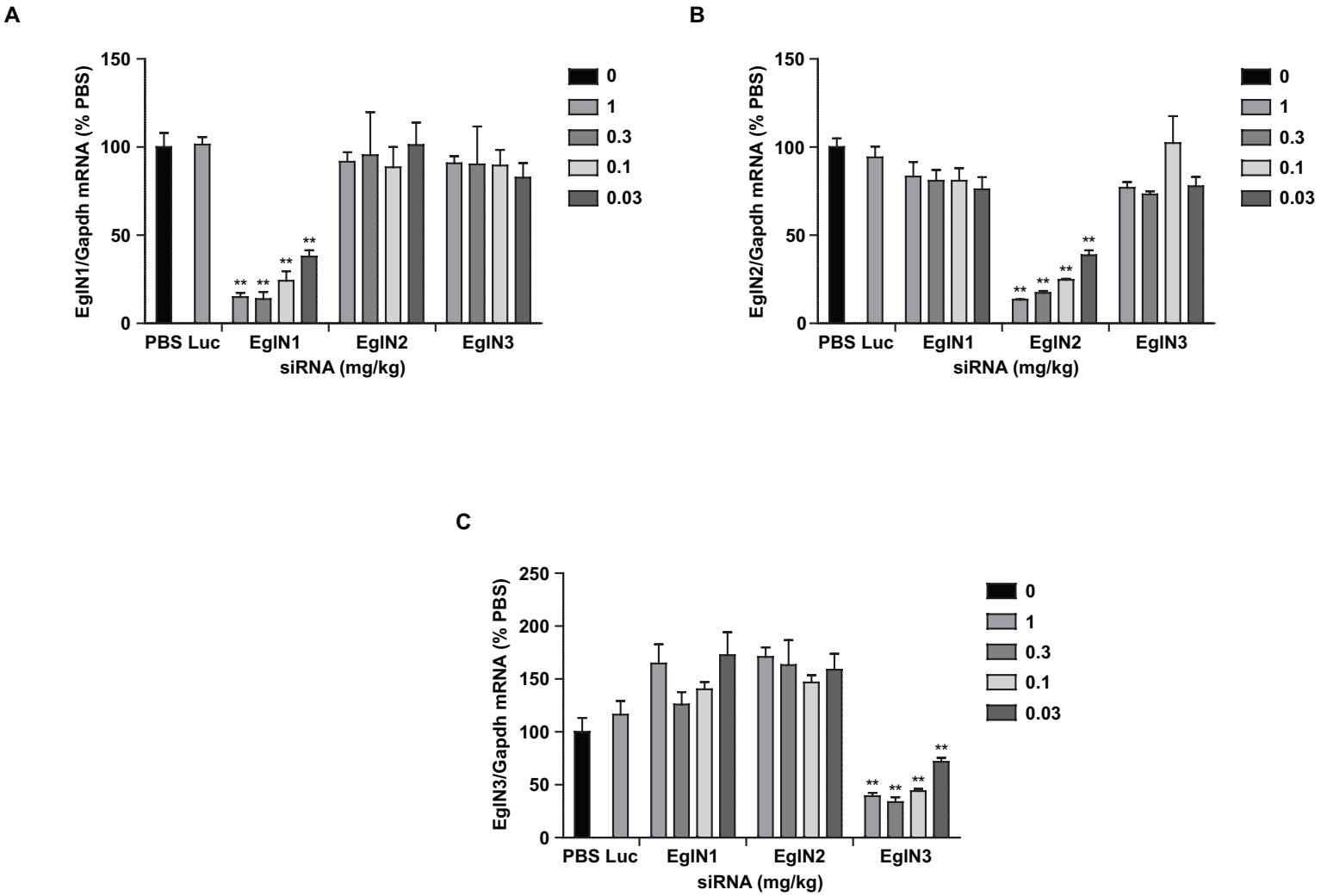
	Sequence 5' to 3'
Adaptor oligo	**CGACTGGAGCACGAGGACACTGACATGG**
Nested adaptor oligo	**GGACACTGACATGGACTGAAGGAGTAG**
EGLN1 GSP	**AGAGATGAAATGAACTCAGTTAGGTGACAGGTCTG**
EGLN1 PCR Round 1	**TTGTTTCGTGTCCAGATGGAAAAGCTACTCTCCTC**
EGLN1 PCR Round 2	**GGCTTGAGTTCAACCCTCACACCTTTCTCACCTG**
EGLN2 GSP	**TATTTCTTGGCTGGCAGAACCTCCATAC**
EGLN2 PCR Round 1	**CAGACAGTGGCAGCCCAGTCCATACACTG**
EGLN2 PCR Round 2	**CAGCAGAGGTCTCTCCTTGTTGCTCCTCAGTG**
EGLN3 GSP	**GATGTGGAAGAACTCCAATAGCTCTGAGGTC**
EGLN3 PCR Round 1	**CAGTGCTGAATTACCAGGAAGCTTTCTATCCTCTG**
EGLN3 PCR Round 2	**GCAAGAAAACATGAAGTACCACAAACAAG**

Supplemental Figure 1



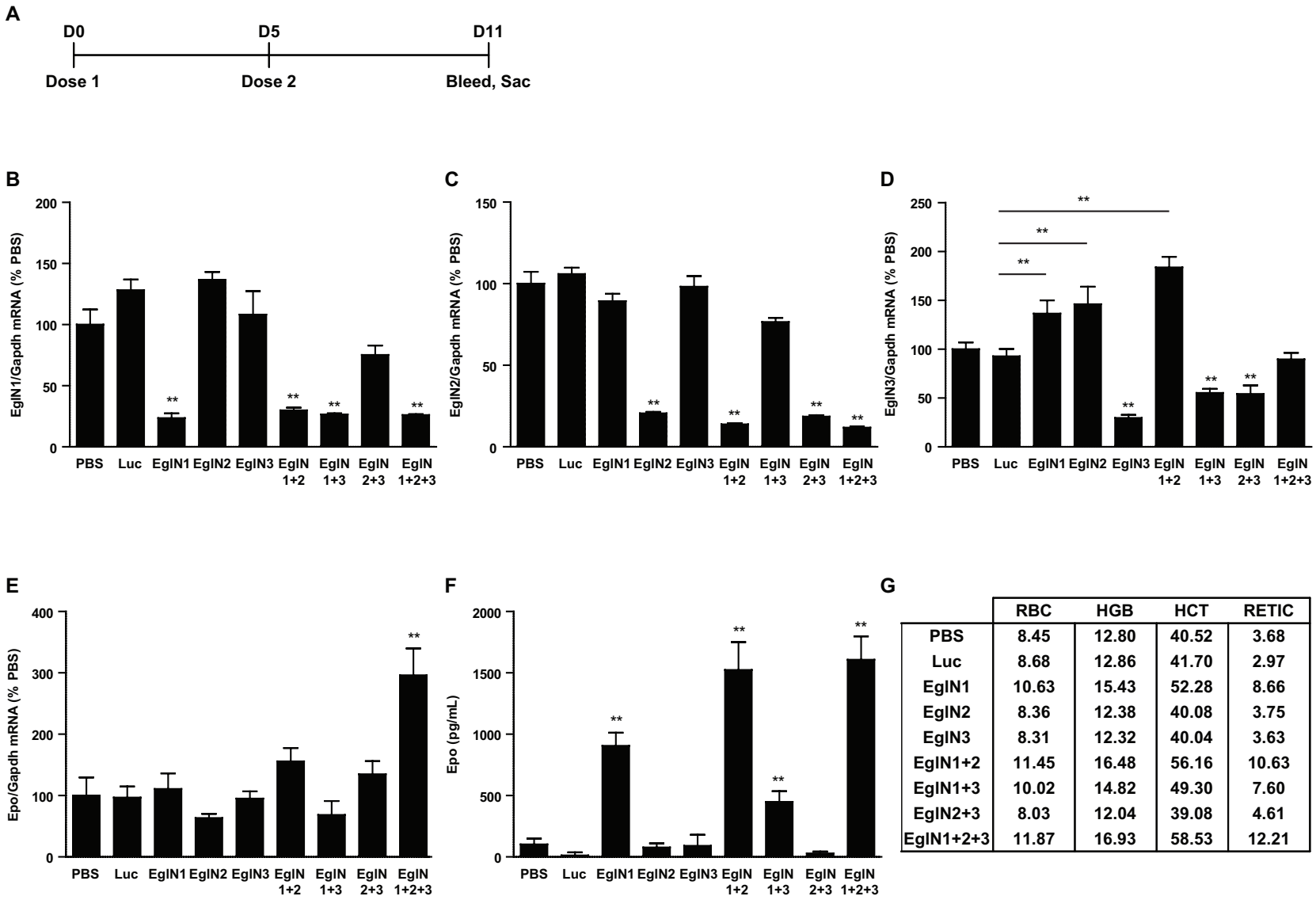
Supplemental Figure 1. Control of hepatic EPO production by EglN1 and its paralogs. Hepatic mRNA, serum EPO, and Hematocrit values in *EglN1 f/f* mice with the indicated EglN2 and EglN3 genotypes two weeks after receiving adenoviruses encoding a GFP-Cre fusion protein or GFP alone by tail vein injection.

Supplemental Figure 2



Supplemental Figure 2. Validation of EgIN siRNAs. *EgIN1*, *EgIN2*, and *EgIN3* hepatic mRNA levels in mice 72 hours after receiving increasing amounts of LNPs containing the indicated siRNAs by tail vein injection (0.03, 0.1, 0.3, or 1 mg/kg). LUC = firefly luciferase. mRNA levels were normalized to GAPDH and then to the corresponding value for PBS-treated mice. n=3. Error Bars represent 1 std. dev. **P<0.01.

Supplemental Figure 3

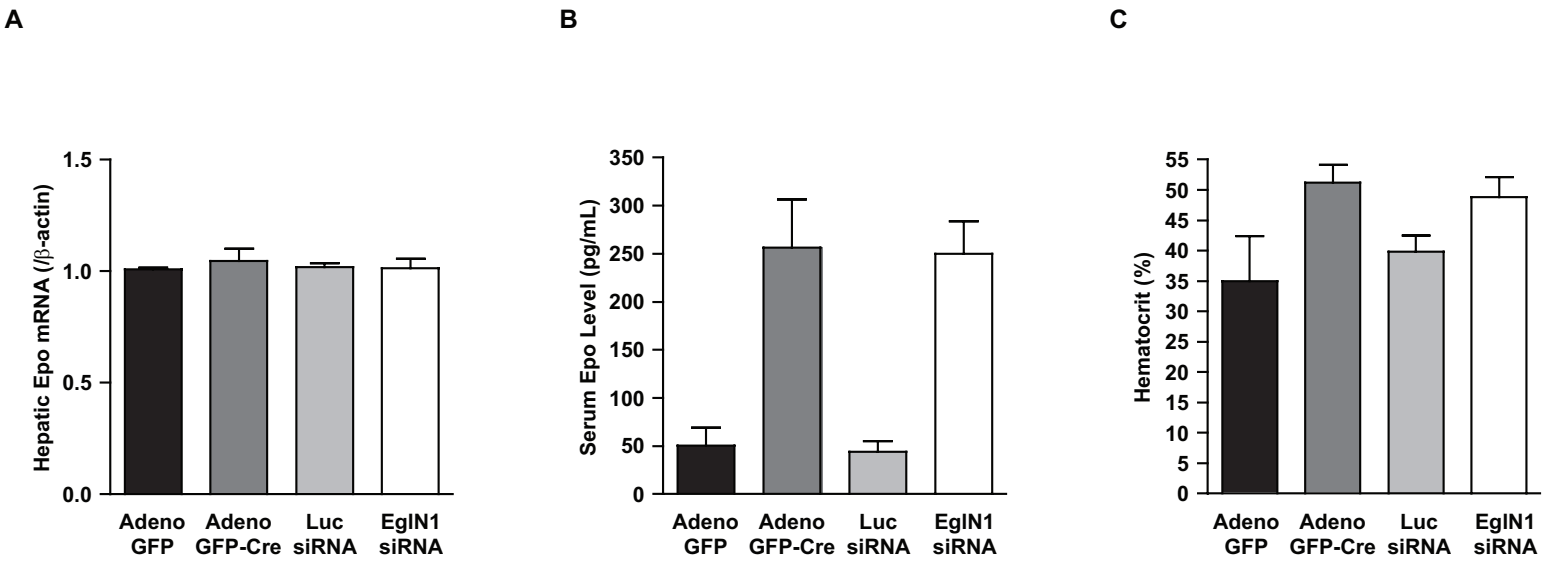


G

	RBC	HGB	HCT	RETIC
PBS	8.45	12.80	40.52	3.68
Luc	8.68	12.86	41.70	2.97
EglN1	10.63	15.43	52.28	8.66
EglN2	8.36	12.38	40.08	3.75
EglN3	8.31	12.32	40.04	3.63
EglN1+2	11.45	16.48	56.16	10.63
EglN1+3	10.02	14.82	49.30	7.60
EglN2+3	8.03	12.04	39.08	4.61
EglN1+2+3	11.87	16.93	58.53	12.21

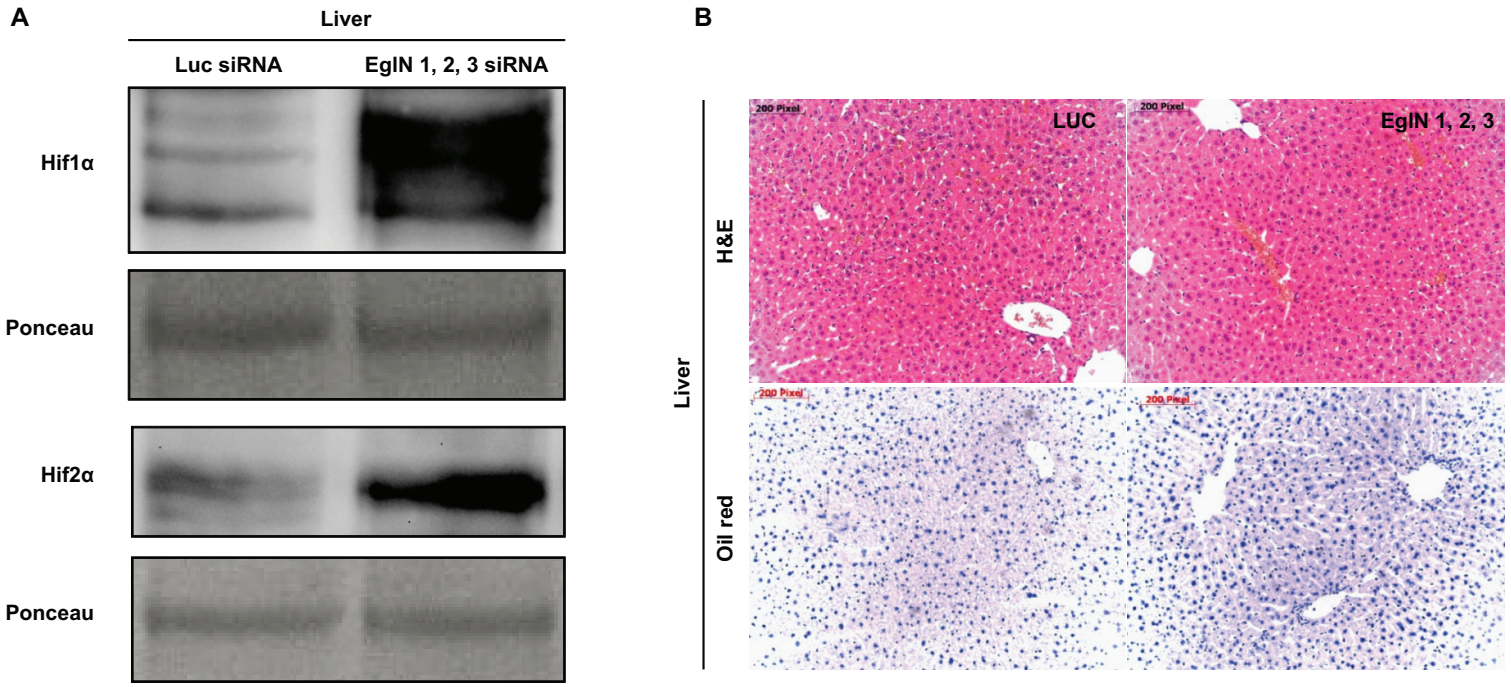
Supplemental Figure 3. Combinatorial effects of EglN siRNAs on hepatic EPO production. *(*A) Dosing Schema. (B-E). Hepatic *EglN* (B-D) and *EPO* (E) mRNA levels for mice treated with combinations of siRNA nanoparticles targeting the indicated EglN family members (0.5 mg/kg per EglN family member). mRNA levels were normalized to GAPDH and then to the corresponding value for PBS-treated mice. (F and G). Serum EPO (F) and hematologic (G) values for mice treated as above. n=3. Error Bars represent 1 std. dev. **P<0.01

Supplemental Figure 4



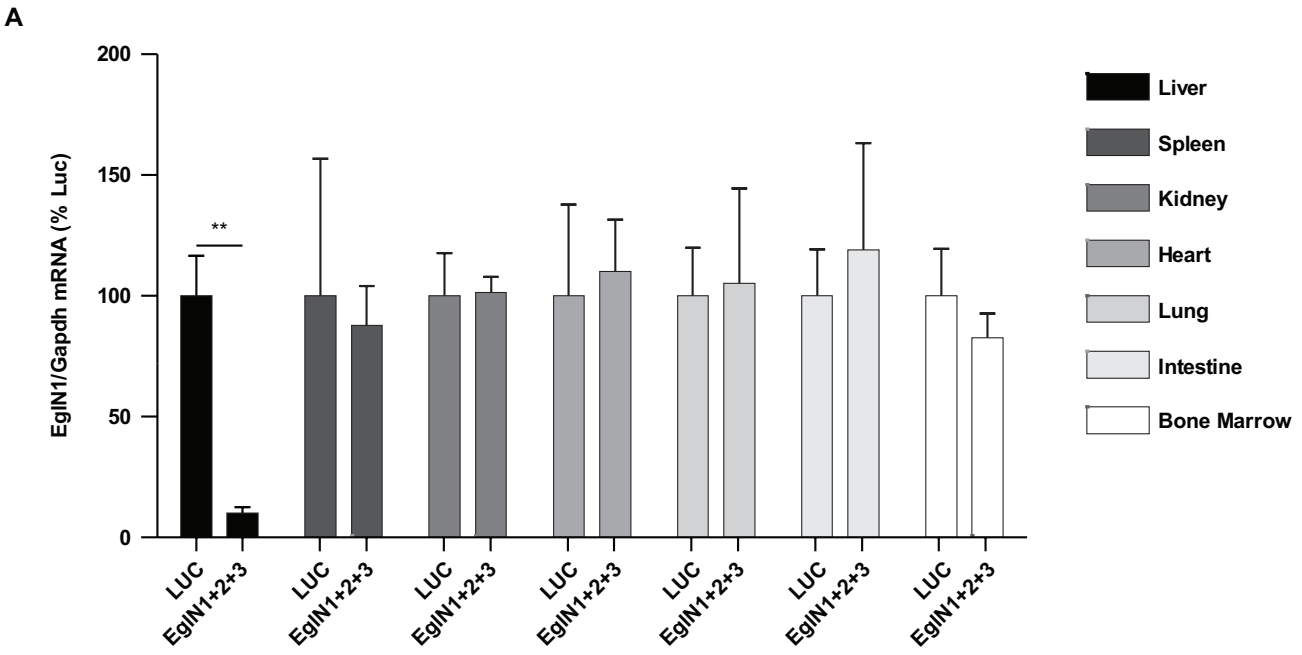
Supplemental Figure 4. Acute inactivation of EglN1 with either Cre Recombinase or siRNA induces erythropoiesis. Hepatic EPO mRNA, serum EPO, and Hematocrit values in *EglN1 f/f*; *EglN2* +/+; *EglN3*+/+ mice two weeks after receiving either an adenovirus encoding a GFP-Cre fusion protein (or GFP alone) or one dose of EglN1 siRNA LNP (or luciferase siRNA LNP) (1 mg/kg) by tail vein injection. Error bars represent 1 std. dev.

Supplemental Figure 5



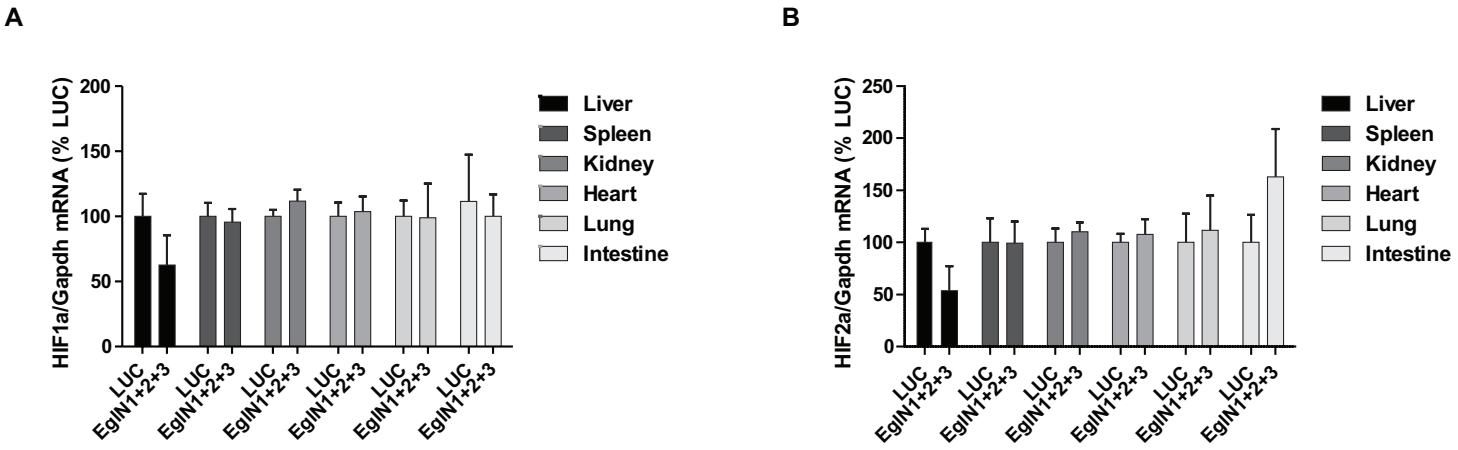
Supplemental Figure 5. Four weeks of EglN mRNA suppression with LNPs does not result in fatty liver or histological abnormalities. (A). Immunoblot analysis of liver extracts prepared from mice (day 9) that received two doses (day 0 and day 4) of LNPs targeting EglN1+EglN2+EglN3 or luciferase (1 mg/kg siRNA/dose). (B) Hematoxylin and Eosin staining (top) and oil red staining (bottom) of liver tissue sections from mice treated with 4 weekly doses (1.5 mg/kg siRNA) of LNPs targeting EglN1+EglN2+EglN3(0.5 mg/kg each)(right) or luciferase (left).

Supplemental Figure 6



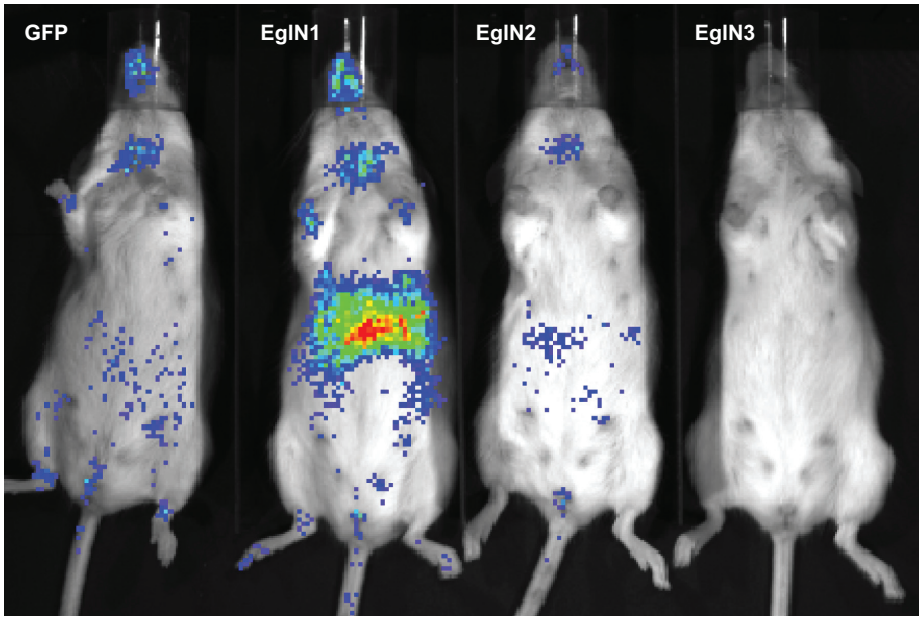
Supplemental Figure 6. EglN mRNA silencing in different tissues. *EglN1* mRNA levels in various mouse tissues 48 hours after receiving LNPs containing EglN or LUC control siRNAs by tail vein injection at 1 mg/kg dose. LUC = firefly luciferase. mRNA levels were normalized to GAPDH and then to the corresponding value for LUC-treated mice. n=3. Error Bars represent 1 std. dev. **P<0.01.

Supplemental Figure 7



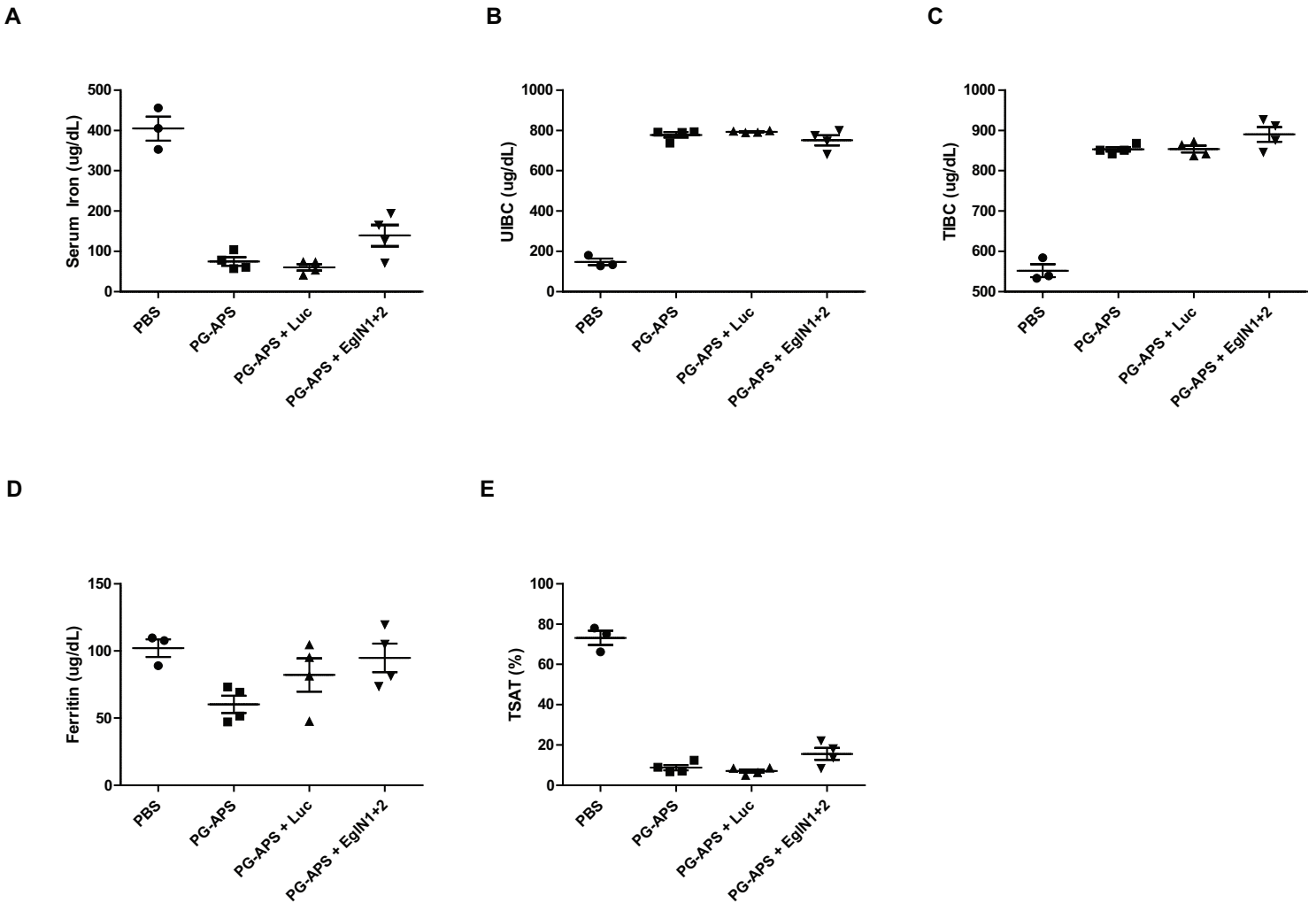
Supplemental Figure 7. HIF mRNA changes in different tissues after EglN mRNA knockdown. HIF1α (A) and HIF2α (B) mRNA in the indicated tissues of mice treated 48 hours earlier with LNPs containing EglN or LUC control siRNAs (1mg/kg) by tail vein injection. LUC= firefly luciferase. mRNA levels were normalized to GAPDH and then to the corresponding value for LUC-treated tissue. n=3. Error Bars represent 1 std. dev.

Supplemental Figure 8



Supplemental Figure 8. Non-invasive imaging of HIF stabilization after EglN1 inactivation. Bioluminescent images of HIF1α-Luc mice 72 hours after a single intravenous dose of LNPs with siRNA targeting the indicated EglN family members or, as a negative control, green fluorescent protein (GFP) (1 mg/kg).

Supplemental Figure 9



Supplemental Figure 9. Iron parameters in rat anemia of inflammation model. Serum iron (A), UIBC (B), TIBC (C), Ferritin (D) and Transferrin saturation (TSAT) (E) at day 37 after 3 weekly 1 mg/kg doses of LNPs targeting EglN1/2 or Luciferase. N=3 (PBS), N=4 (other groups). Error Bars represent 1 std. dev.