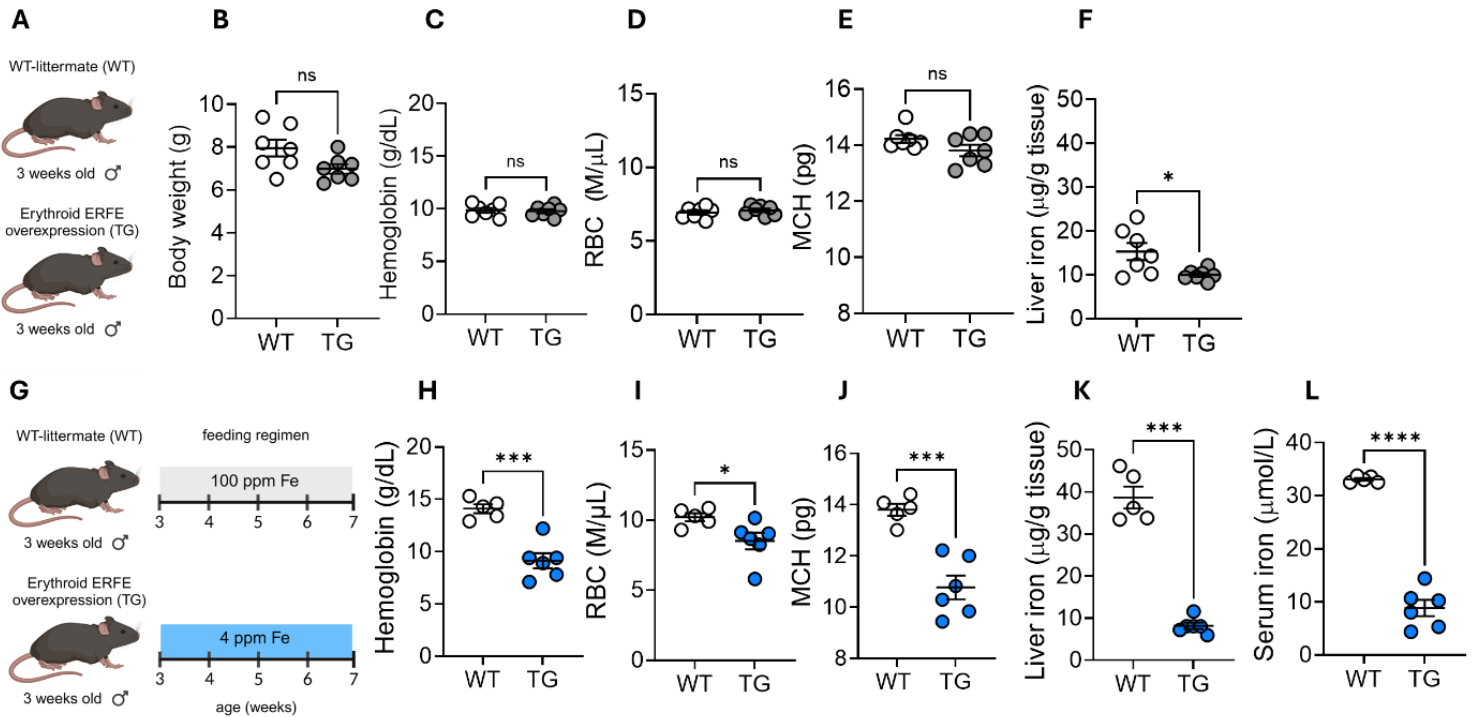
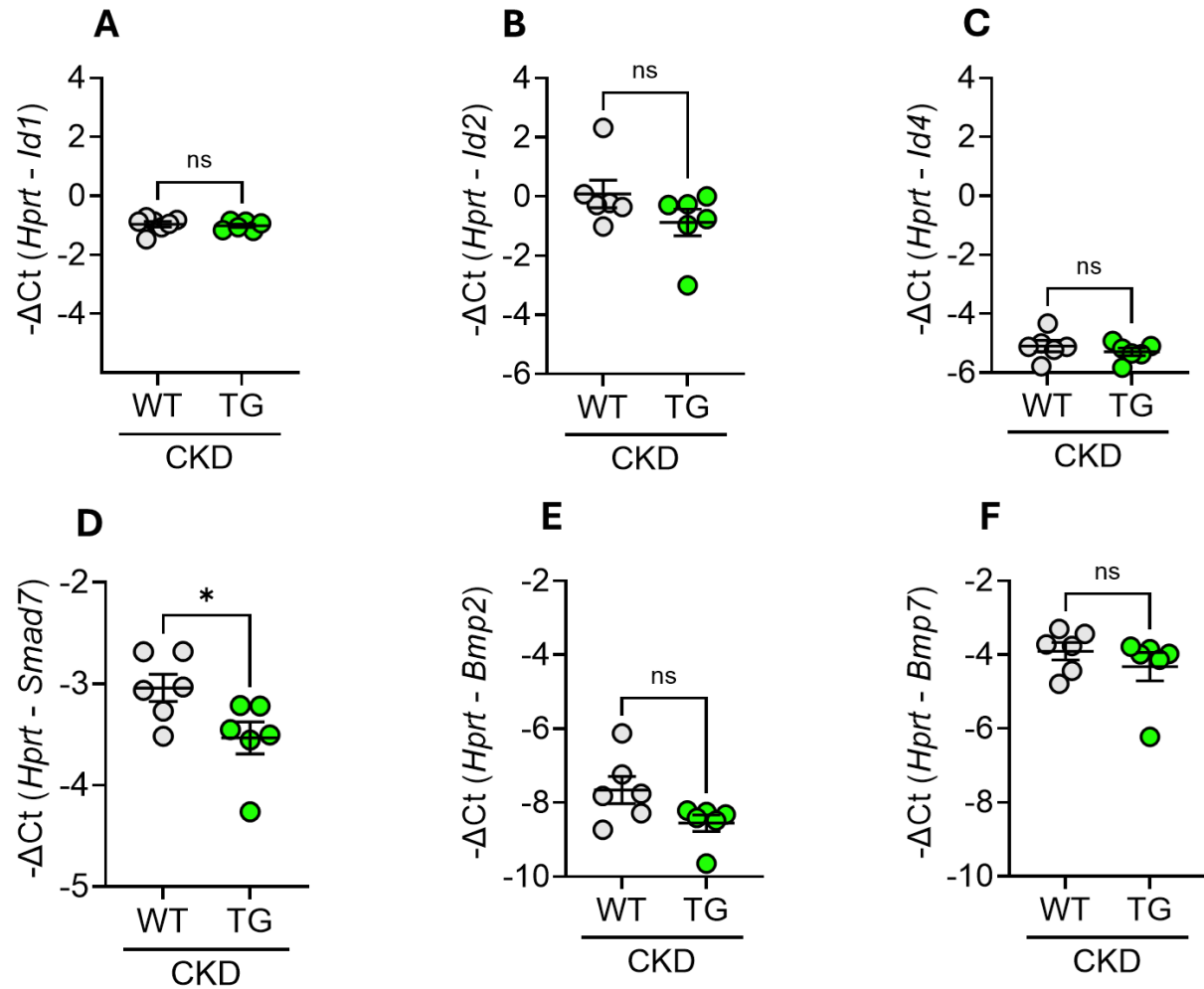


## Supplemental Figure and Data

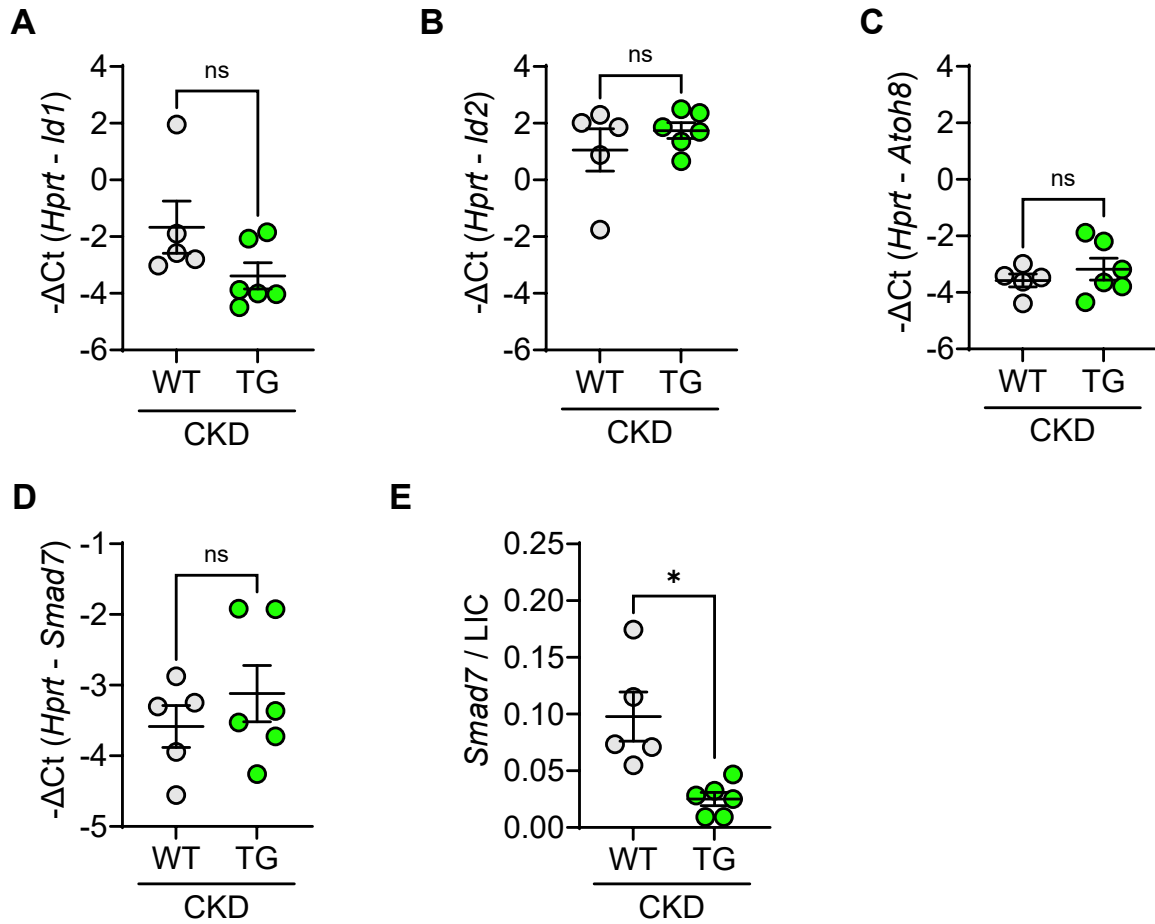


**Figure S1. Experimental design development.** (A) WT and TG (n= 7 mice/group) mice at weaning (3 weeks of age), (B) body weight, (C) hemoglobin concentrations, (D) RBC count, (E) MCH, (F) liver iron concentration (LIC). (G) At weaning (3 weeks of age), WT and TG (n=5-6/group) mice were placed on 100 ppm Fe diet and 4 ppm Fe diet respectively, and analyzed after 4 weeks for (H) hemoglobin concentration, (I) RBC count, (J) MCH, (K) liver iron concentration (LIC) and (L) serum iron concentration. Data are mean  $\pm$  SEM, analyzed by unpaired-*t*-test with Welch's correction (two-tailed). \*\*\**P* < .001; \**P* < .05; ns = non-significant.

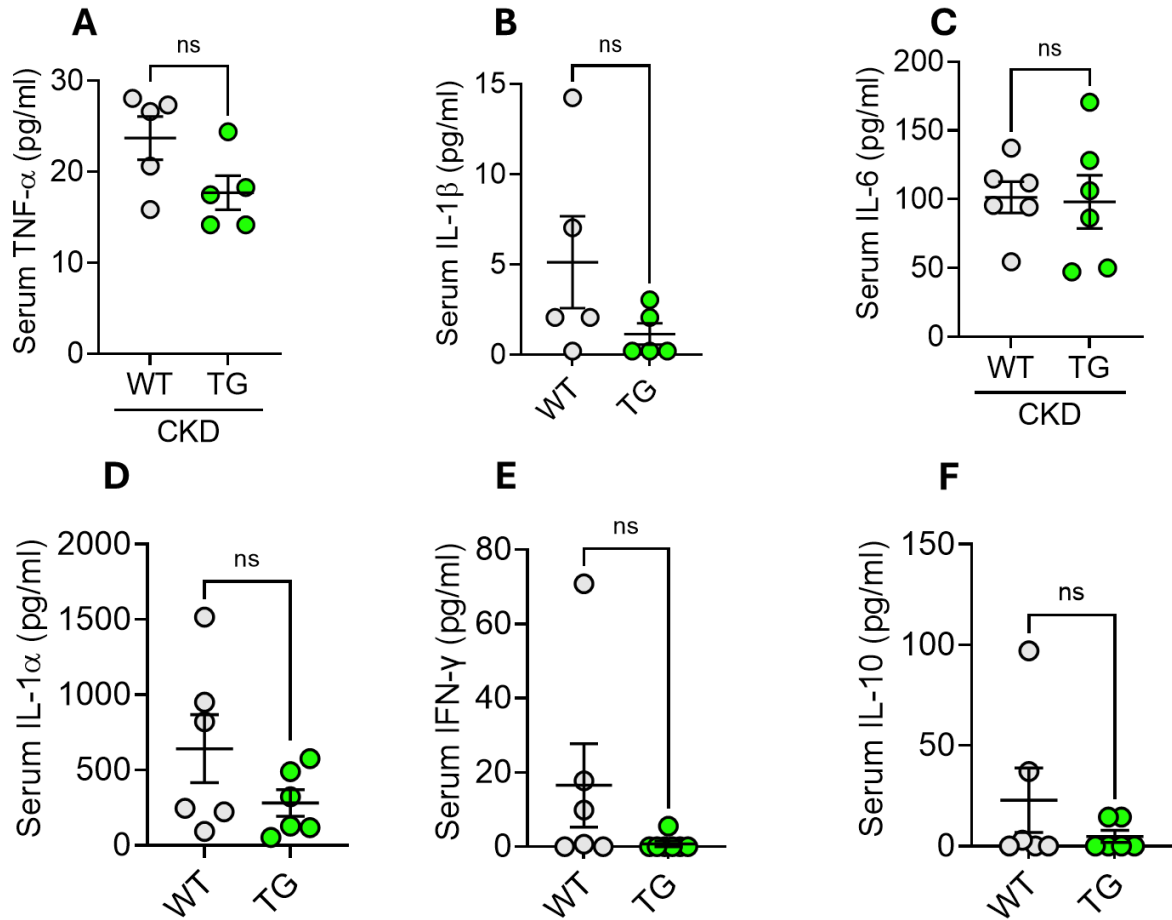


**Figure S2. ERFE augmentation results in unchanged or mildly suppressed kidney BMP signaling in adenine-induced CKD.** WT and TG mice from Figure 1G (n=6-7 mice/group) were analyzed by qPCR of kidney tissue for (A) *Id1*, (B) *Id2*, (C) *Id4*, (D) *Smad7*, (E) *Bmp2* and (F) *Bmp7*. Data are mean  $\pm$  SEM, analyzed by unpaired-*t*-test with Welch's correction (two-tailed).

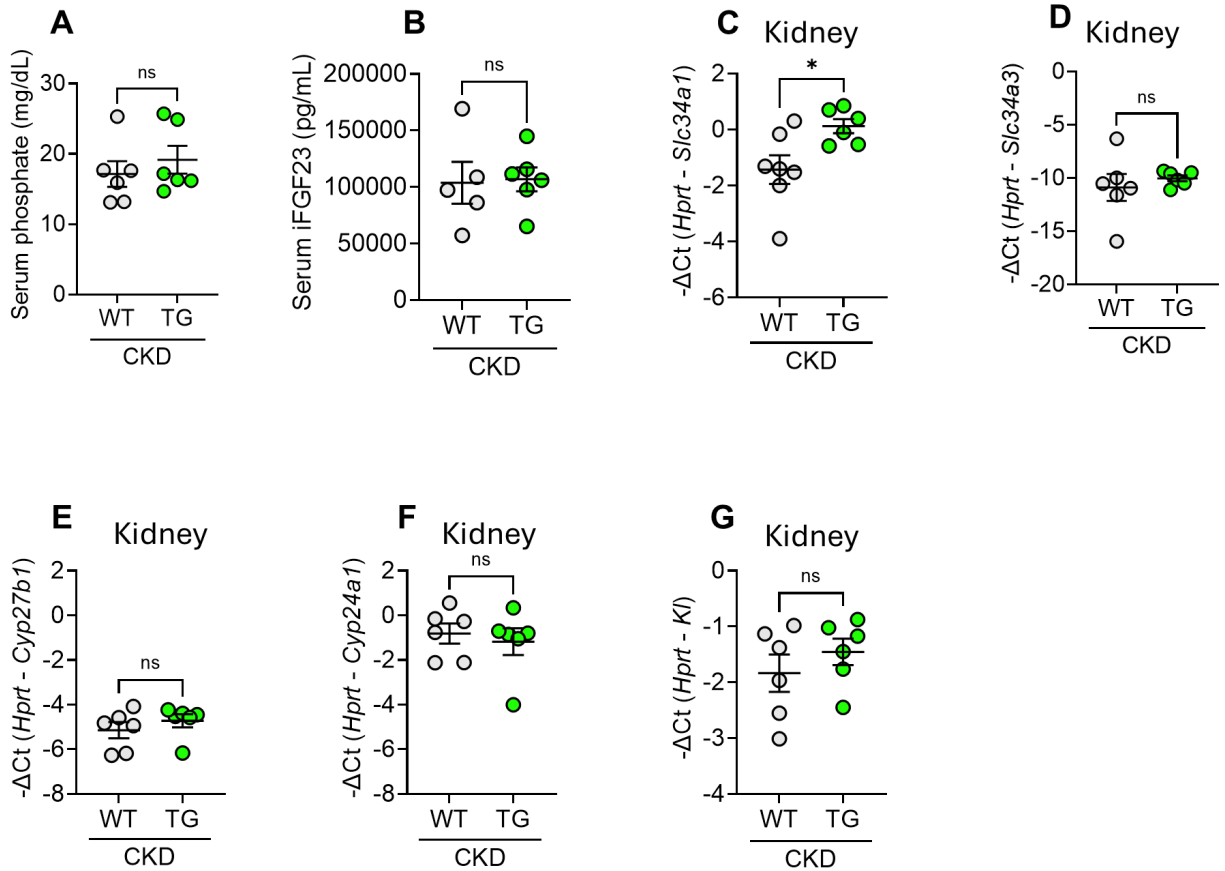
\*P < .05; ns = non-significant.



**Figure S3. ERFE augmentation results in unchanged or mildly suppressed liver BMP signaling in adenine-induced CKD.** WT and TG mice from Figure 1G (n=5-6 mice/group) were analyzed by qPCR of liver tissue for (A) *Id1*, (B) *Id2*, (C) *Atoh8*, (D) *Smad7*, (E) liver *Smad7* mRNA/LIC. Data are mean  $\pm$  SEM, analyzed by unpaired-*t*-test with Welch's correction (two-tailed). \**P* < .05; ns = non-significant.



**Figure S4. ERFE augmentation does not impact systemic inflammation in adenine-induced CKD.** WT and TG mice from Figure 1G (n=6-7 mice/group) were analyzed by serum 32-plex cytokine/chemokine assay for (A) TNF $\alpha$ , (B) IL-1 $\beta$ , (C) IL-6, (D) IL-1 $\alpha$ , (E) IFN $\gamma$ , and (F) IL-10. Data are mean  $\pm$  SEM, analyzed by unpaired-*t*-test with Welch's correction (two-tailed). ns = non-significant.



**Figure S5. ERFE augmentation does not alter markers of mineral metabolism in adenine-induced CKD.** WT and TG mice from Figure 1G (n=6-7 mice/group) were analyzed. Measured parameters include (A) serum phosphate, (B) serum intact iFGF23, and qPCR analysis of kidney tissue for (C-G) *Slc34a1* and *Slc34a3* (encoding sodium-dependent phosphate transporters 2A and 2C), *Cyp27b1* and *Cyp24a1* (encoding the enzymes that respectively generate and break down the active form of vitamin D, 1,25-dihydroxyvitamin D<sub>3</sub>) and *Kl* (encoding Klotho, the renal coreceptor for iFGF23). Data are mean  $\pm$  SEM, analyzed by unpaired-*t*-test with Welch's correction (two-tailed). \**P* < .05; ns = non-significant.

**Supplemental Table 1: Body and organ weights of wildtype (WT) and ERFE-TG (TG) mice receiving a 0.2% adenine diet as described in Figure 1G.**

|                           | WT + 0.2% adenine diet | TG + 0.2% adenine diet |
|---------------------------|------------------------|------------------------|
| Starting body weight (g)  | 23.7 ± 0.7             | 18.9* ± 0.8            |
| Final body weight (g)     | 16.4 ± 0.4             | 16.5 ± 0.4             |
| Wet liver weight (mg)     | 691.4 ± 32.4           | 711.5 ± 31.3           |
| Wet spleen weight (mg)    | 44.1 ± 3.5             | 54.8* ± 2.4            |
| Wet kidney weight (mg)    | 262.5 ± 12.1           | 207.3* ± 13.3          |
| Liver to body weight (%)  | 3.78 ± 0.2             | 4.32* ± 0.1            |
| Spleen to body weight (%) | 0.2 ± 0.02             | 0.32* ± 0.02           |
| Kidney to body weight (%) | 1.6 ± 0.1              | 1.2* ± 0.1             |

Values are expressed as mean ± SEM. Comparison between groups was performed in form of an unpaired-t-test with Welch's correction, N=6/group; \*p ≤ 0.05.

**Supplemental Table 2. Oligonucleotides used as sequence specific primers in qPCR analyses.**

| Gene               | Species      | Orientation        | Primer Sequence (5' – 3')                                               |
|--------------------|--------------|--------------------|-------------------------------------------------------------------------|
| <i>Acta2</i>       | Mus musculus | Forward<br>Reverse | GTC CCA GAC ATC AGG GAG TAA<br>TCG GAT ACT ACT TCA GCG TCA GGA          |
| <i>Colla1</i>      | Mus musculus | Forward<br>Reverse | ATG GAT TCC CGT TCG AGT ACG<br>TCA GCT GGA TAG CGA CAT CG               |
| <i>Col3a1</i>      | Mus musculus | Forward<br>Reverse | GAC CAA AAG GTG ATG CTG GAC AG<br>CAA GAC CTC GTG CTC CAG TTA G         |
| <i>Krt20</i>       | Mus musculus | Forward<br>Reverse | CAA CGG ATC GGA CCT GTT TG<br>AGC GCA CTT TTT CTA GGT AGT TT            |
| <i>Fn1</i>         | Mus musculus | Forward<br>Reverse | GAT GTC CGA ACA GCT ATT TAC CA<br>CCT TGC GAC TTC AGC CAC T             |
| <i>Hamp</i>        | Mus musculus | Forward<br>Reverse | GAG CAG CAC CAC CTA TCT CC<br>TTG GTA TCG CAA TGT CTG CC                |
| <i>Hprt</i>        | Mus musculus | Forward<br>Reverse | CTG GTT AAG CAG TAC AGC CCC AA<br>CGA GAG GTC CTT TTC ACC AGC           |
| <i>Il1b</i>        | Mus musculus | Forward<br>Reverse | TGC CAC CTT TTG ACA GTG ATG<br>TGA TGT GCT GCT GCG AGA TT               |
| <i>Il6</i>         | Mus musculus | Forward<br>Reverse | CTC TGG GAA ATC GTG GAA AT<br>CCA GTT TGG TAG CAT CCA TC                |
| <i>Havcr1/Kim1</i> | Mus musculus | Forward<br>Reverse | CGA GTG GAG ATT CCT GGA TGG<br>GGA CGT GTG GGA ATC TCT GG               |
| <i>Slc5a2/Sgt2</i> | Mus musculus | Forward<br>Reverse | TGG GCT GGA TAT TTG TCC CGA<br>CAA ACC GCT TCC GCA GAT ACT              |
| <i>Vegfa</i>       | Mus musculus | Forward<br>Reverse | ACA TTG GCT CAC TTC CAG AAA CAC<br>GGT TGG AAC CGG CAT CTT TAT C        |
| <i>Lcn2/Ngal</i>   | Mus musculus | Forward<br>Reverse | GTC CCC ACC GAC CAA TGC<br>ATT GGG TCT CTG CGC ATC C                    |
| <i>Gapdh</i>       | Mus musculus | Forward<br>Reverse | CCA ATG TGT CCG TCG TGG ATC T<br>GTT GAA GTC GCA GGA GAC AAC C          |
| <i>Angptl1</i>     | Mus musculus | Forward<br>Reverse | GAC AAT TCA CTC GAA CTT TCC CA<br>CCT TGT TGC CAT CTT CAG CAT T         |
| <i>Saa1</i>        | Mus musculus | Forward<br>Reverse | ACA CCA GCA GGA TGA AGC TAC T<br>GAG CAT GGA AGT ATT TGT CTG AGT        |
| <i>Epo</i>         | Mus musculus | Forward<br>Reverse | TCT ACG TAG CCT CAC TTC ACT<br>ACC CGG AAG AGC TTG CAG AAA              |
| <i>Tgfb</i>        | Mus musculus | Forward<br>Reverse | ATA CGT CAG ACA TTC GGG AAG CAG TG<br>AAT AGT TGG TAT CCA GGG CTC TCC G |
| <i>Klotho/Kl</i>   | Mus musculus | Forward<br>Reverse | TGA TGT CGT CCA ACA CGT AGG CTT<br>GCA AAG TGC TCA ACT GGC TAA GGT      |
| <i>Tnfa</i>        | Mus musculus | Forward<br>Reverse | CCC TCA CAC TCA GAT CAT CTT CT<br>GCT ACG ACG TGG GCT ACAG              |

|                       |              |                    |                                                                 |
|-----------------------|--------------|--------------------|-----------------------------------------------------------------|
| <i>Id1</i>            | Mus musculus | Forward<br>Reverse | CCT AGC TGT TCG CTG AAG GC<br>CTC CGA CAG ACC AAG TAC CAC       |
| <i>Id2</i>            | Mus musculus | Forward<br>Reverse | ATG AAA GCC TTC AGT CCG GTG<br>AGC AGA CTC ATC GGG TCG T        |
| <i>Id4</i>            | Mus musculus | Forward<br>Reverse | CCG CCC AAC AAG AAA GTC AG<br>CCA GGA TGT AGT CGA TAA CGT G     |
| <i>Smad7</i>          | Mus musculus | Forward<br>Reverse | GGG CTT TCA GAT TCC CAA CTT<br>AGG GCT CTT GGA CAC AGT AGA      |
| <i>Bmp2</i>           | Mus musculus | Forward<br>Reverse | TCA AGC CAA ACA CAA ACA GC<br>AGC CAC AAT CCA GTC ATT CC        |
| <i>Bmp7</i>           | Mus musculus | Forward<br>Reverse | CAT CGT CCA GAC ACT GGT TCA C<br>TCG AAG TAG AGG ACA GAG ATG GC |
| <i>Slc34a1/NaPi2a</i> | Mus musculus | Forward<br>Reverse | GGC TCC AAC ATT GGC ACT ACC A<br>ACC ACA GTA GGA TGC CCG AGA T  |
| <i>Slc34a3/NaPi2c</i> | Mus musculus | Forward<br>Reverse | GCG GTA TTA CCA GCA ACA CCA C<br>TGT CCT CCT CTG GAG ATG CTG A  |
| <i>Cyp27b1</i>        | Mus musculus | Forward<br>Reverse | TTC GGC TTT GGC AAA CGG AGC T<br>GGC TTG ATA GGA AGA GCA CCT G  |
| <i>Cyp24a1</i>        | Mus musculus | Forward<br>Reverse | GCT CCT TCA AAA GGA CAC AGA GG<br>CGC TTG CCA CAC TTT GGT GTT G |
| <i>Fam132b/Erfe</i>   | Mus musculus | Forward<br>Reverse | ATG GGG CTG GAG AAC AGC<br>TGG CAT TGT CCA AGA AGA CA           |