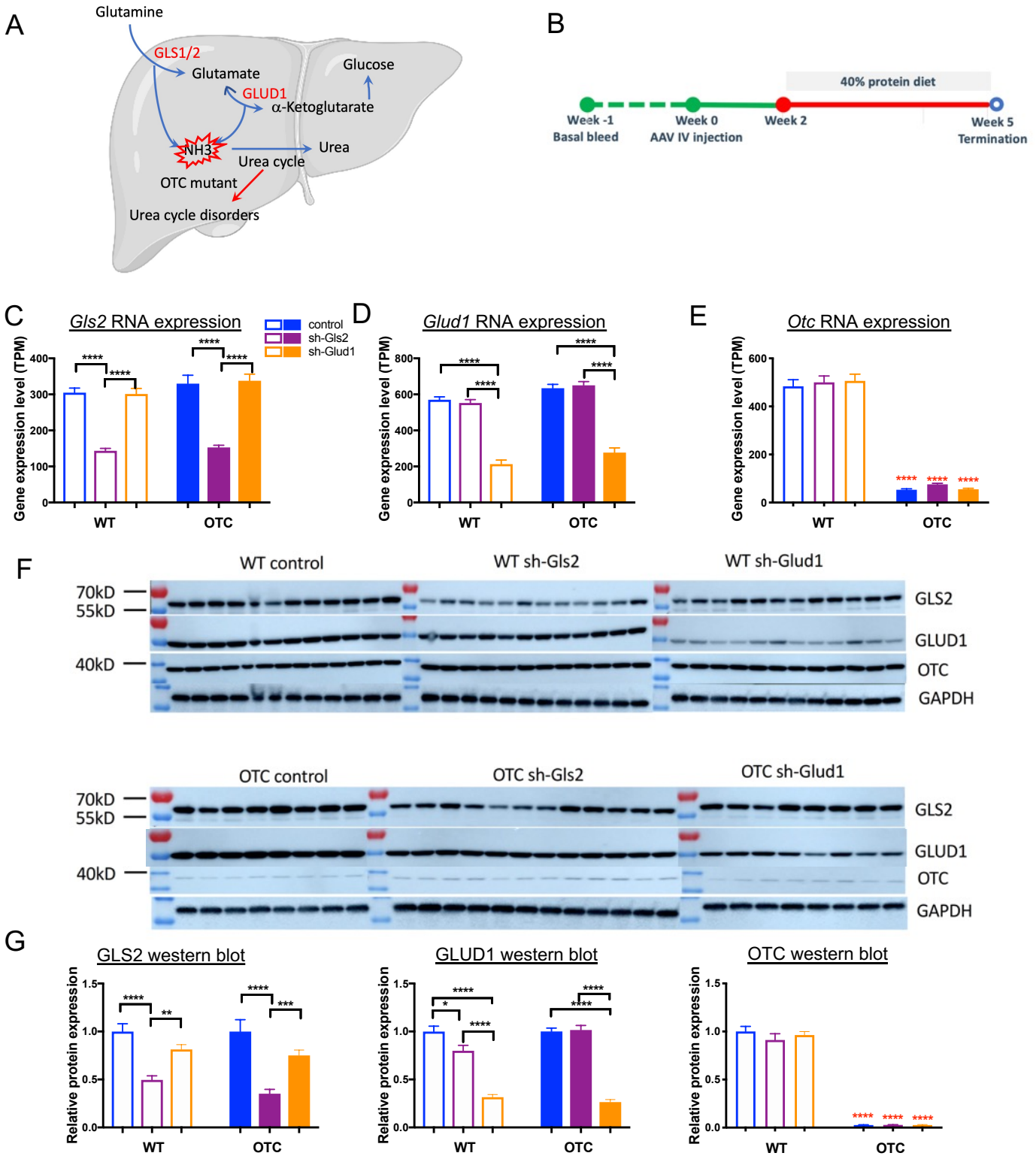


Figure 1. Hepatic *Gls2* and *Glud1* knockdown in mouse liver to target urea cycle disorders. Schematic of glutamine metabolism in the liver and mechanism of action of targeting GLS2 and GLUD1 to treat urea cycle disorders. For simplicity the conversion of glutamine to KG via the glutaminase II reaction (7) is not shown (A). Timeline of the study (B). Animals were injected AAV at week 0, were on high protein diet treatment at week 2 and were euthanized at week 5. Hepatic *Gls2* and *Glud1* knockdown at RNA and protein levels were confirmed by liver RNA-seq and western blot (C, D, F and G). Deficient *Otc* expression in OTC mice were confirmed by liver RNA-seq and western blot (E, F and G). N=8-13 per group. Data are mean ± SEM. Statistical analysis was conducted by two-way ANOVA. Comparisons were made between sh-Gls2, sh-Glud1, and control within the same genotype (black asterisks), and comparisons were made between OTC and wildtype mice among the same treatment (red asterisks). OTC protein quantification was generated from a different set of blots with a loading control for normalization between blots. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 1

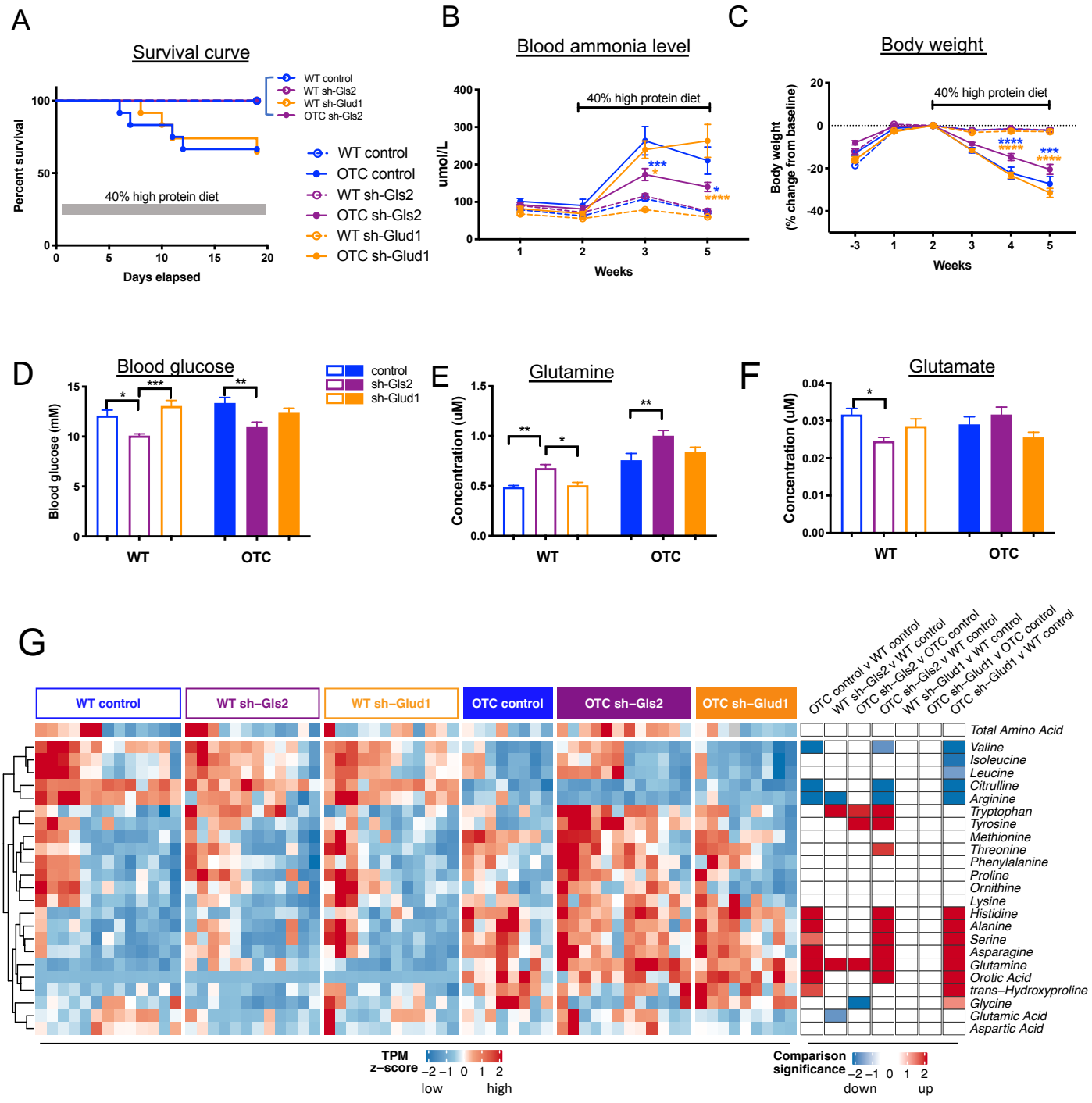


Figure 2. Hepatic *Gls2* but not *Glud1* knockdown reduces hyperammonemia and mortality in a urea cycle deficiency animal model of OTC mice on high protein diet. Survival curves (A) in OTC and wildtype (WT) mice administered IV injection of AAV shRNA *Gls2* and *Glud1* at week 0 and placed on high protein diet at week 2. Plasma ammonia levels (B), body weights (C), spot blood glucose at week 2 (D), terminal plasma glutamine/glutamate levels (E, F), terminal plasma amino acid and orotic acid concentration heatmap (G) in the treatment groups. N=8-13 per group. Data are mean ± SEM. Statistical analysis was conducted by two-way ANOVA. Comparisons were made between OTC sh-Gls2, OTC sh-Glud1 and OTC control (B, C), and between treatments within the same genotype (D-F). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Blue color indicates comparison to OTC control group and gold color indicates comparison to OTC sh-Glud1 group. (G) Heatmaps showing (left) standardized plasma concentration of each amino acid in individual mouse, grouped by genotype and treatment categories, and (right) signed significance of categorical comparisons of each amino acid. Significance is $\log_{10}$ transformed p-value from one-way ANOVA, and sign denotes direction of change. Only significances of p<0.05 are colored.

Figure 2

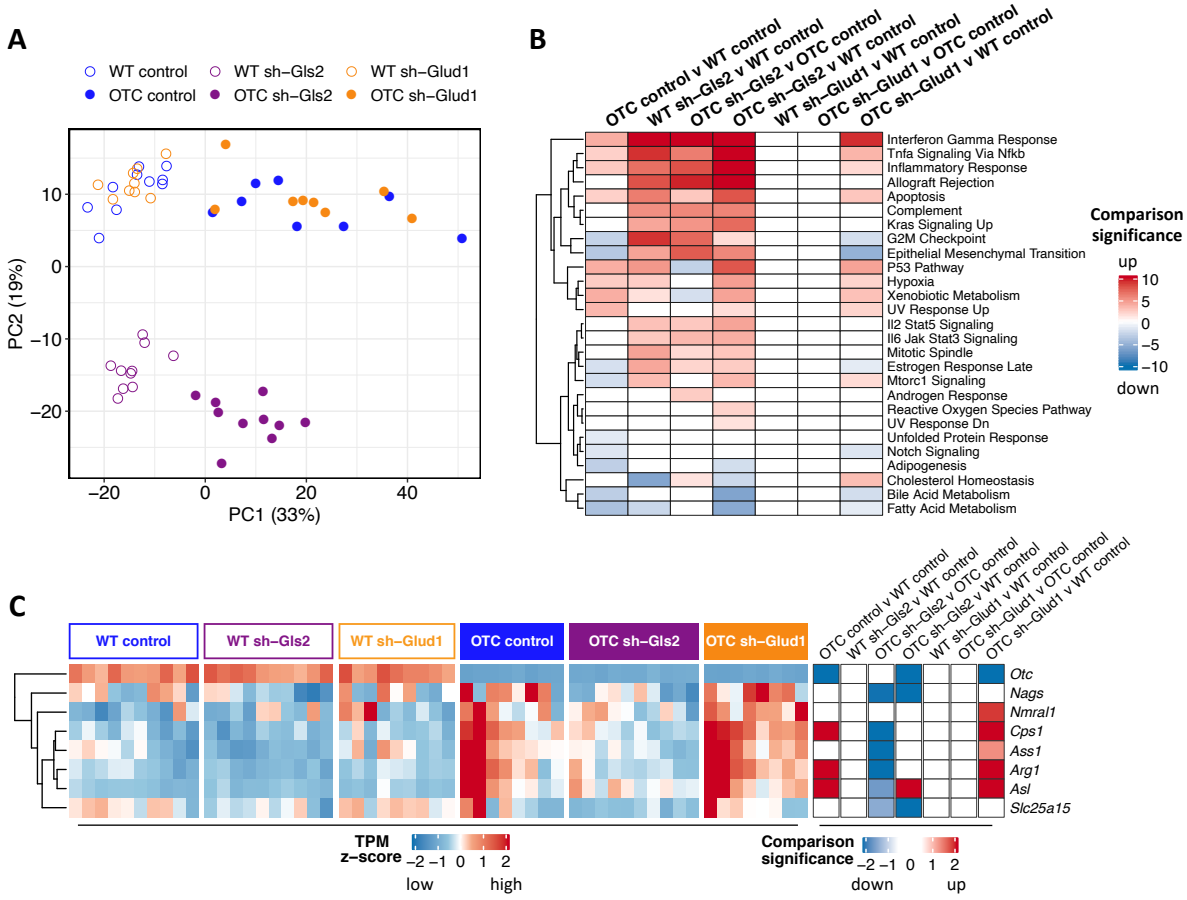


Figure 3. Liver RNA-seq analysis with hepatic *Gls2* and *Glud1* knockdown in OTC and wildtype mice. (A) Principal component analysis plot of liver gene expression in mice. (B) Enrichment of differentially expressed genes in hallmark gene sets in categorical comparisons. Significance is $\log_{10}$ transformed p-value from Fisher's exact test, and sign indicates whether enriched genes are up-regulated or down-regulated in the comparison. P-values were multiple-comparison adjusted. Only significances of $p<0.05$ are colored. (C) Heatmaps showing (left) standardized TPM of each urea cycle gene in individual mice, grouped by genotype and treatment categories, and (right) signed significance of categorical comparisons of each gene. Significance is $\log_{10}$ transformed p-value from DESeq2, and sign denotes direction of change. P-values were multiple-comparison adjusted. Only significances with $p<0.05$ are colored.

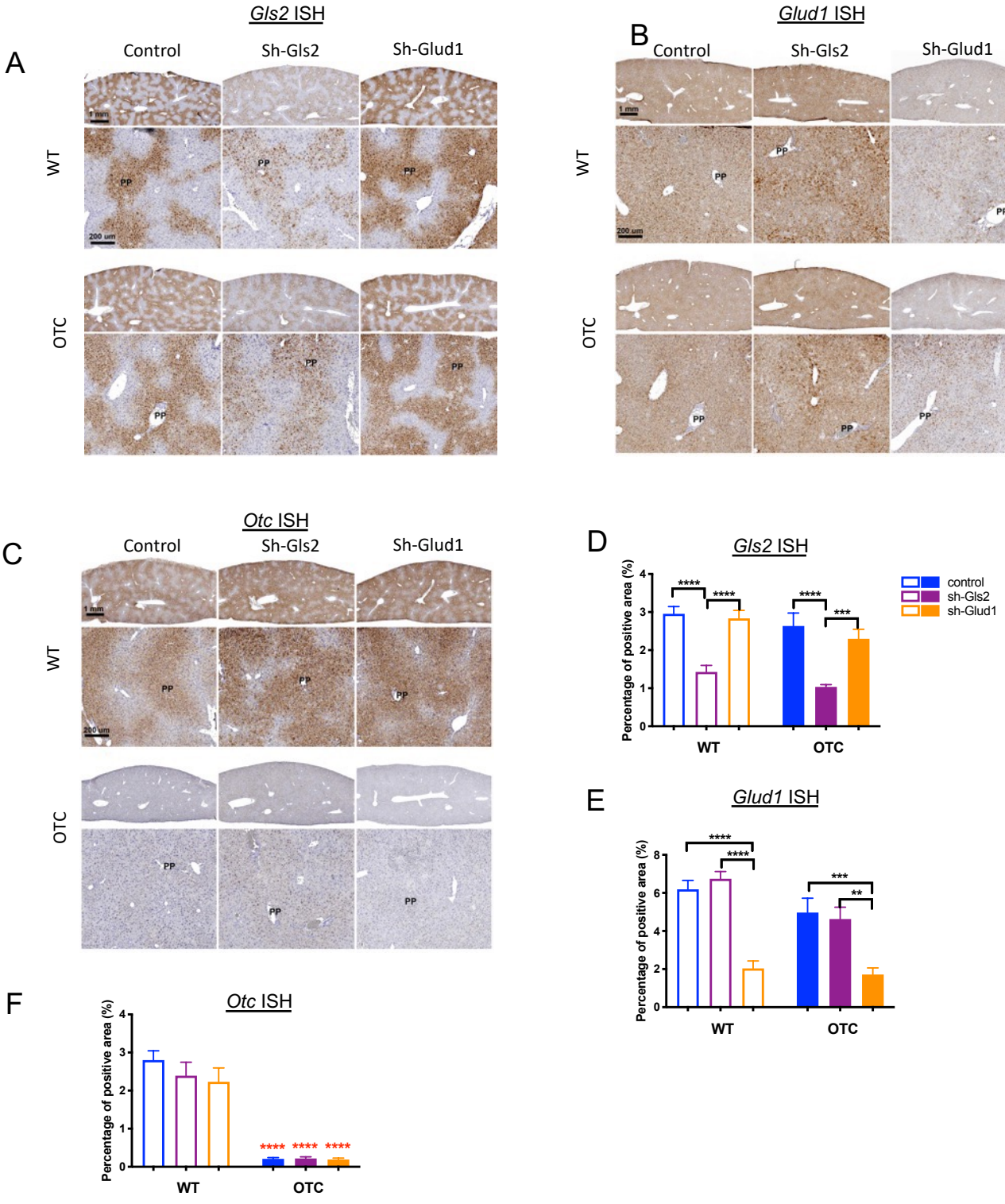
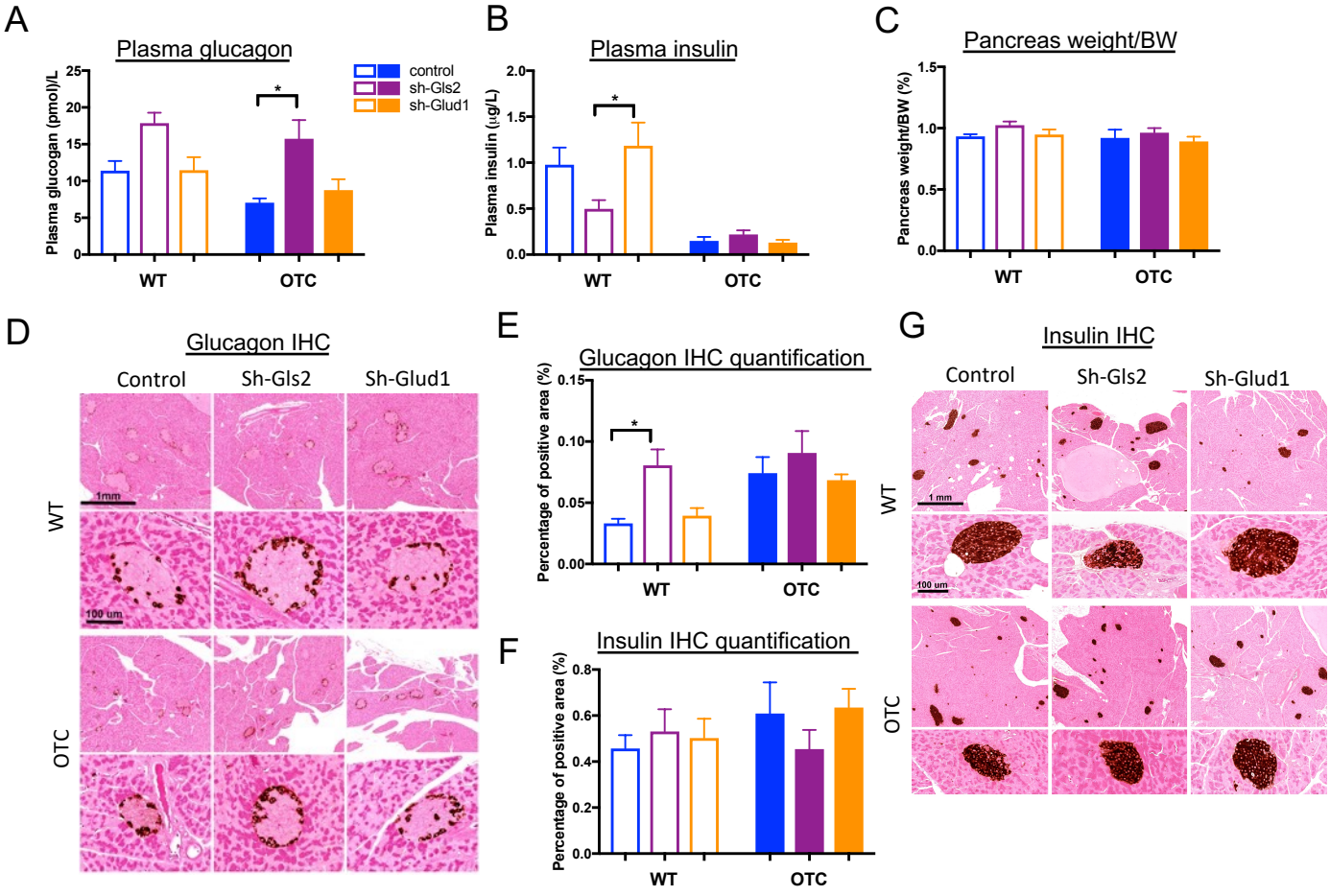


Figure 4. Liver *Gls2, Glud1* and *Otc* mRNA distribution in the liver. Liver representative RNA-ISH images of *Gls2* (A), *Glud1* (B), or *Otc* (C) and respective quantifications of RNA-ISH positive area percentages (D, E, F) in all groups (N = 8-13 per group). Top panel has the same magnification and scale bar indicates 1 mm. Bottom panel has the same magnification and scale bar indicates 200 µm. Data are mean ± SEM. Statistical analysis was conducted by two-way ANOVA. Comparisons were made between sh-Gls2, sh-Glud1, and control within the same genotype (black asterisks), and comparisons were made between OTC and wildtype mouse among the same treatment (red asterisks). ***p*<0.01, ****p* <0.001, *****p* <0.0001.

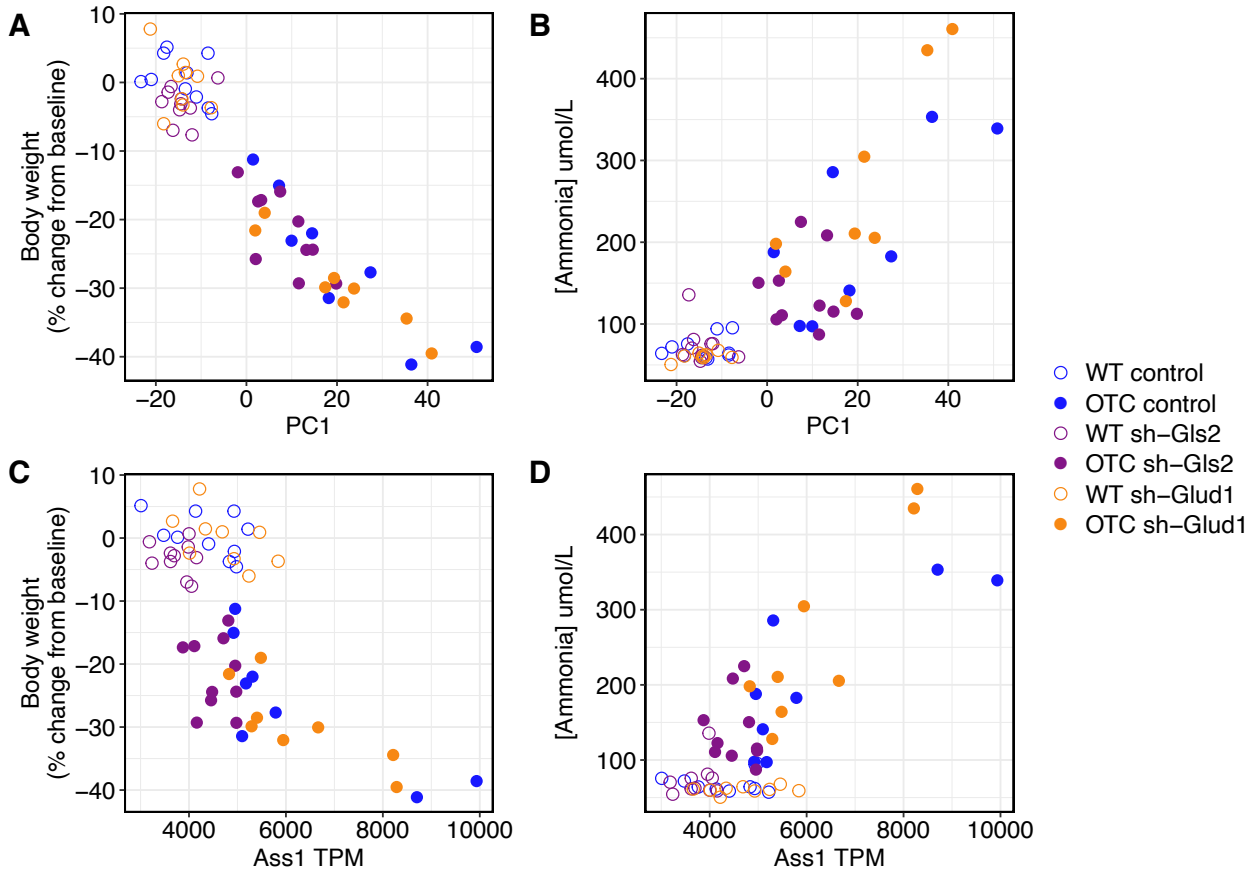
Figure 4

Suppl Fig 1



Supplemental Figure 1. *Gls2* knockdown induces increased plasma glucagon level but not alpha-cell hyperplasia in OTC mice. Terminal plasma glucagon (A), insulin (B), and pancreas weights (C) of OTC and wildtype (WT) mice administered IV injection of AAV shRNA *Gls2* and *Glud1* at week 0, placed on high protein diet at week 2, and terminated at week 5. Representative immunohistochemistry images of pancreas section stained for glucagon or insulin (D, G). Percentage of glucagon and insulin IHC positive area were quantified (E, F). N=8-13 per group. Top panel has the same magnification and scale bar indicates 1 mm. Bottom panel has the same magnification and scale bar indicates 100 μm. Data are mean ± SEM. Statistical analysis was conducted by two-way ANOVA. Comparisons were made between treatments within the same genotypes. *p <0.05.

Suppl Fig 2



Supplemental Figure 2. Physiological parameters correlated with liver gene expression. Relationship across individual mouse of (A, C) terminal body weight and (B, D) terminal plasma ammonia concentration with (A, B) the first principal component and with (C, D) liver *Ass1* gene expression.