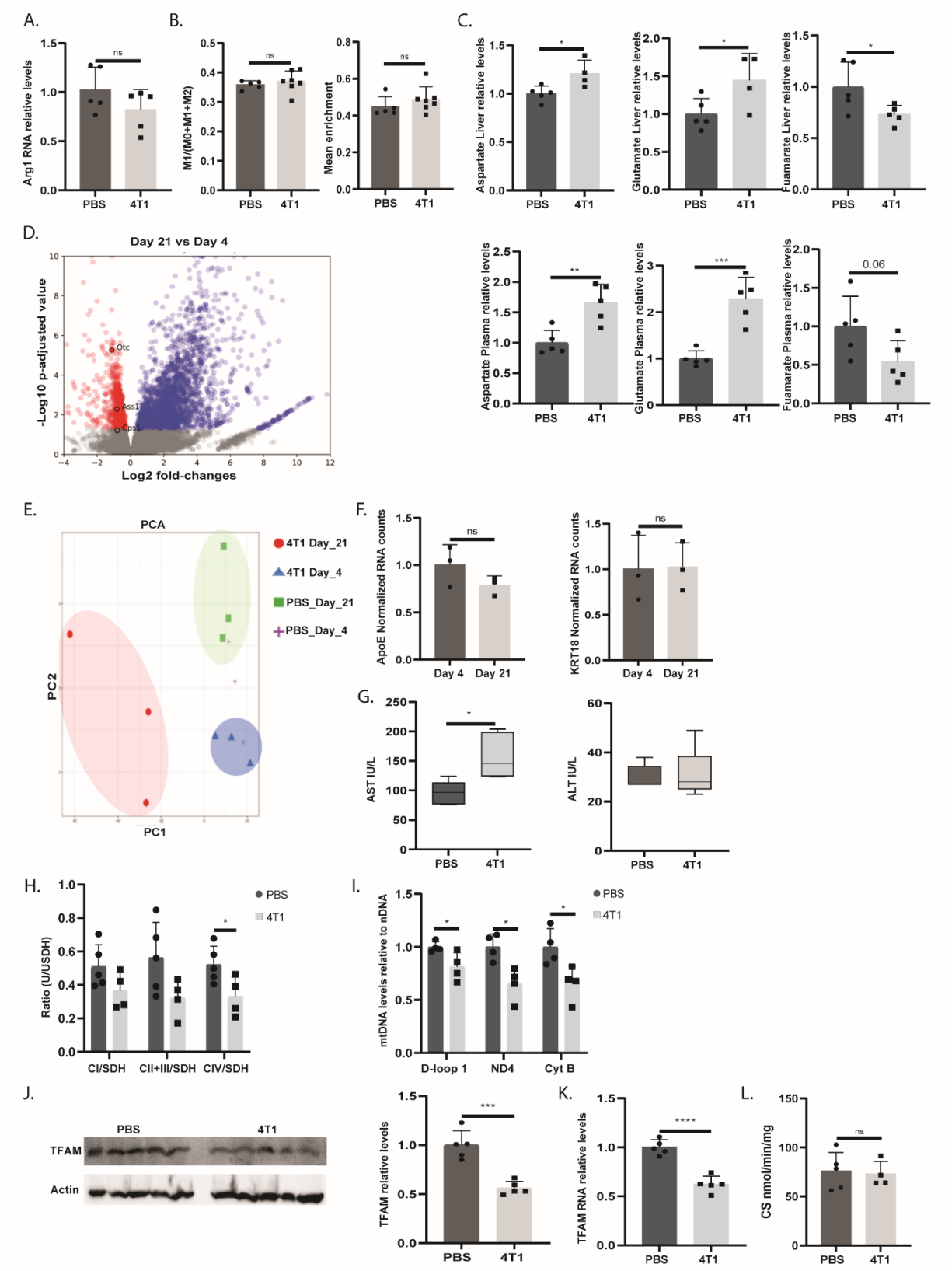
Supplementary Figure S1: Global Changes in liver metabolism during early BC carcinogenesis



Supplementary Figure 1: Global changes in liver metabolism during early BC carcinogenesis. A. RT-PCR of livers from BC-bearing mice demonstrate decreased RNA expression of the UC enzyme Arginase I on day 21 of cancer progression (n = 5 in each group, student T-Test). **B.** Left panel: Percent labeling of plasma Glutamine in infused 4T1 tumor-bearing or PBS controls following 5 hours of infusion. Right panel: The mean isotopic content of Glutamine found in the plasma of infused 4T1 tumor-bearing or PBS control mice, following 5 hours infusion, generated using IsoCor. **C.** Elevated levels of aspartate, glutamate, and decreased levels of fumarate, in livers (Upper panel) and plasma (Lower panel) of BC-bearing mice compared to livers of WT-PBS injected mice. UC intermediate levels were measured with gas chromatography-mass spectrometry (GC–MS), amino acid analyzer (AAA), and liquid chromatography-mass spectrometry (LC-MS) (GC–MS, n = 5, AAA WT n = 5, 4T1 n = 4, LC-MS n = 5, student T-Test), P values for Liver: Asp = 0.027, Glu = 0.049, Fum = 0.045. Pvalues for Plasma: Asp = 0.004, Glu = 0.0005. **D.** A Volcano plot displaying a decreased expression of the UC enzymes OTC, ASS1, and CPS1 on day 21 compared to day 4 in BC-bearing mice. **E.** Principal component analysis (PCA) plot indicates a significantly different gene expression profile for hepatocytes from BC-bearing mice on day 21 compared to all other experimental groups. **F.** No change in the RNA expression of liver-specific genes between day4 and 21. Normalized RNA counts for APOE (Left) and KTR18 (Right) in hepatocytes in day4 and day 21 showed no difference in RNA levels between the time points. **G.** Left panel-plasma measurements of AST demonstrate significantly increased levels in BC-bearing mice in comparison to WT-PBS injected mice (n = 5, student T-Test) P = 0.011. Right panel- no significant changes were found for ALT. **H.** The enzymatic activities of respiratory chain complexes were measured at 37°C by standard spectrophotometric methods as described in the methods and in [51]. Rotenone-sensitive NADH-CoQ-reductase (CI), succinate-cytochrome C- reductase (CII+CIII), and cytochrome c-oxidase (CIV). The activities were divided by the activity of succinate dehydrogenase, Complex II (SDH), and expressed as activity(micromol/min/mg) ratio (U/U) (WT n = 5, 4T1 n = 4, student T-Test) P = 0.039. **I.** qPCR of livers from 4T1 BC mice demonstrates a decrease in mtDNA copy number compared to WT-PBS injected mice (n = 4, student T-Test) P = 0.038, 0.012, 0.026 (respectively).**J.** Left panel-Western blots demonstrating decreased protein expression levels of TFAM in livers of BC-bearing mice compared to WT-PBS injected mice (n = 5, student T-Test). P = 0.0003. Right panel- quantification of TFAM western band intensity. **K.** RT-PCR of livers from 4T1 BCmice demonstrates a decreased expression of TFAM compared to WT-PBS injected mice (n =5, student T-Test) P <0.0001. L. No differences in the levels of citrate synthase in mitochondria from the livers of BC mice compared to WT-PBS injected mice (WT n = 5, 4T1 n = 4).