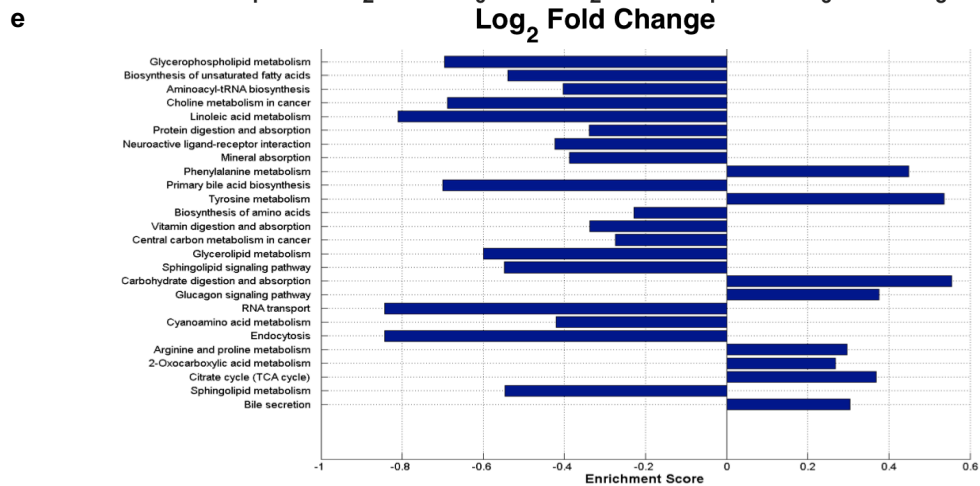
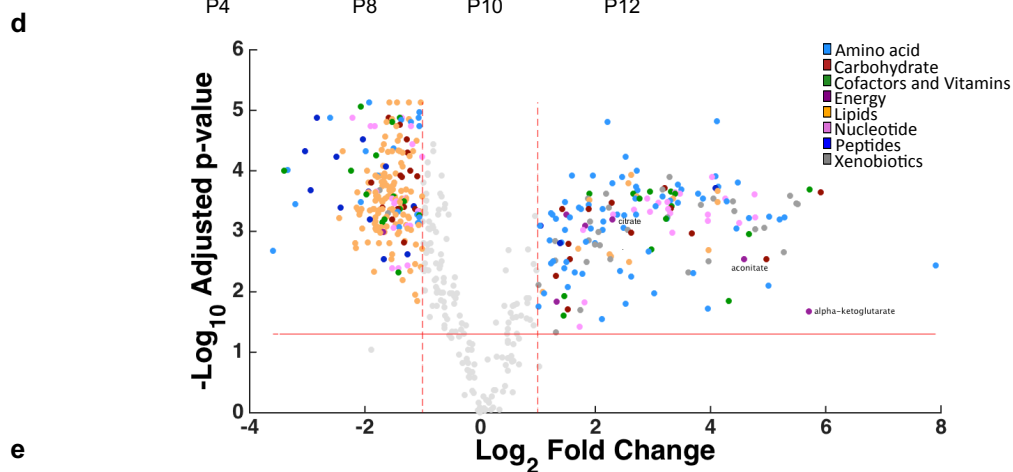
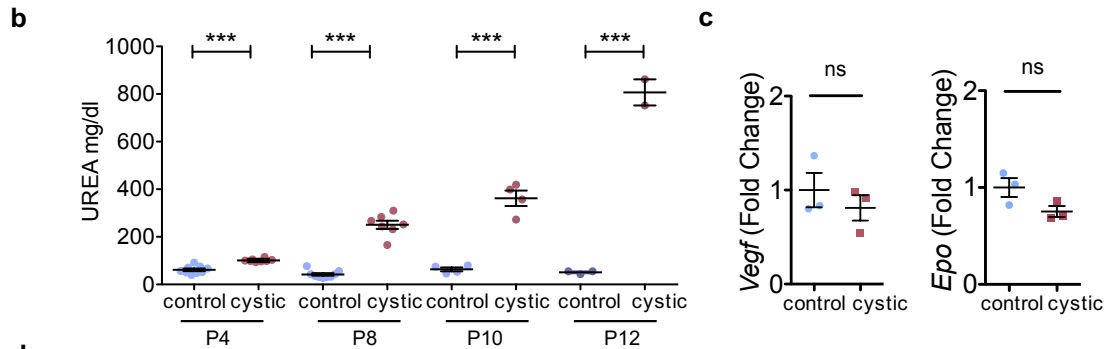
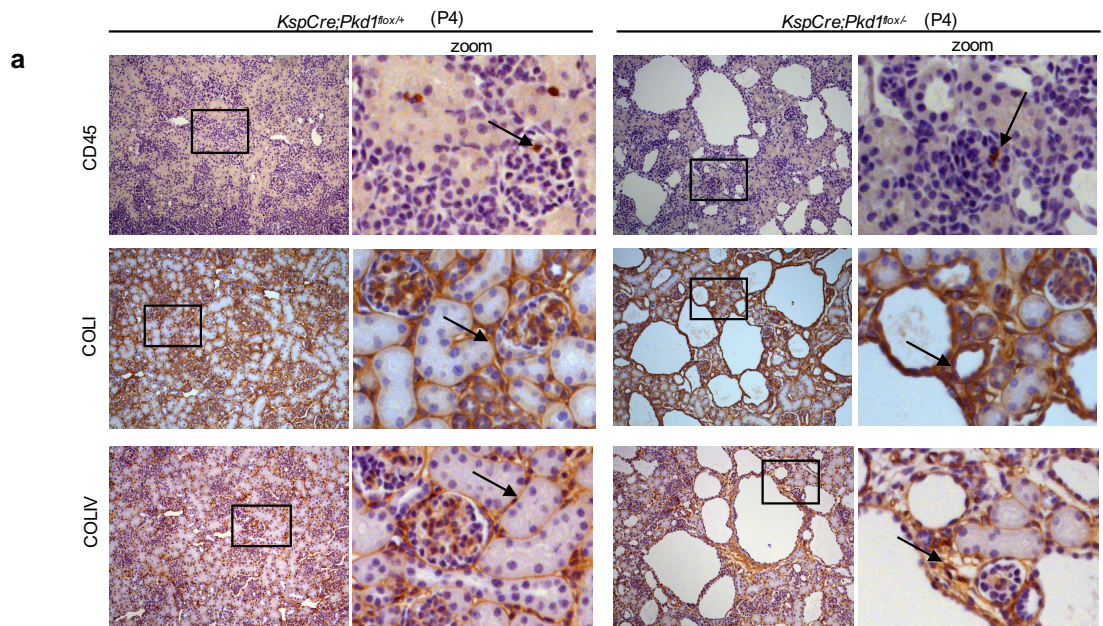
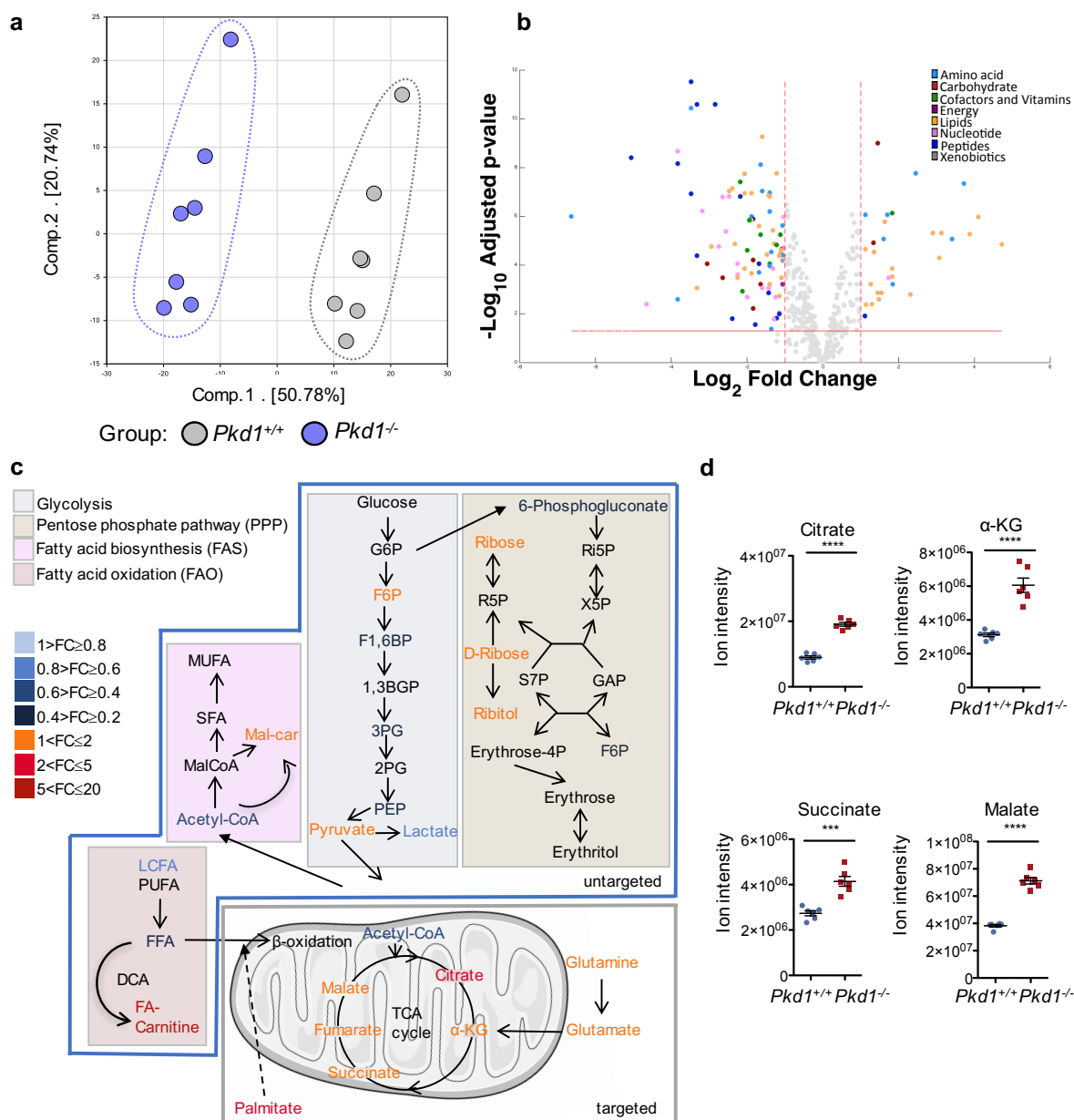
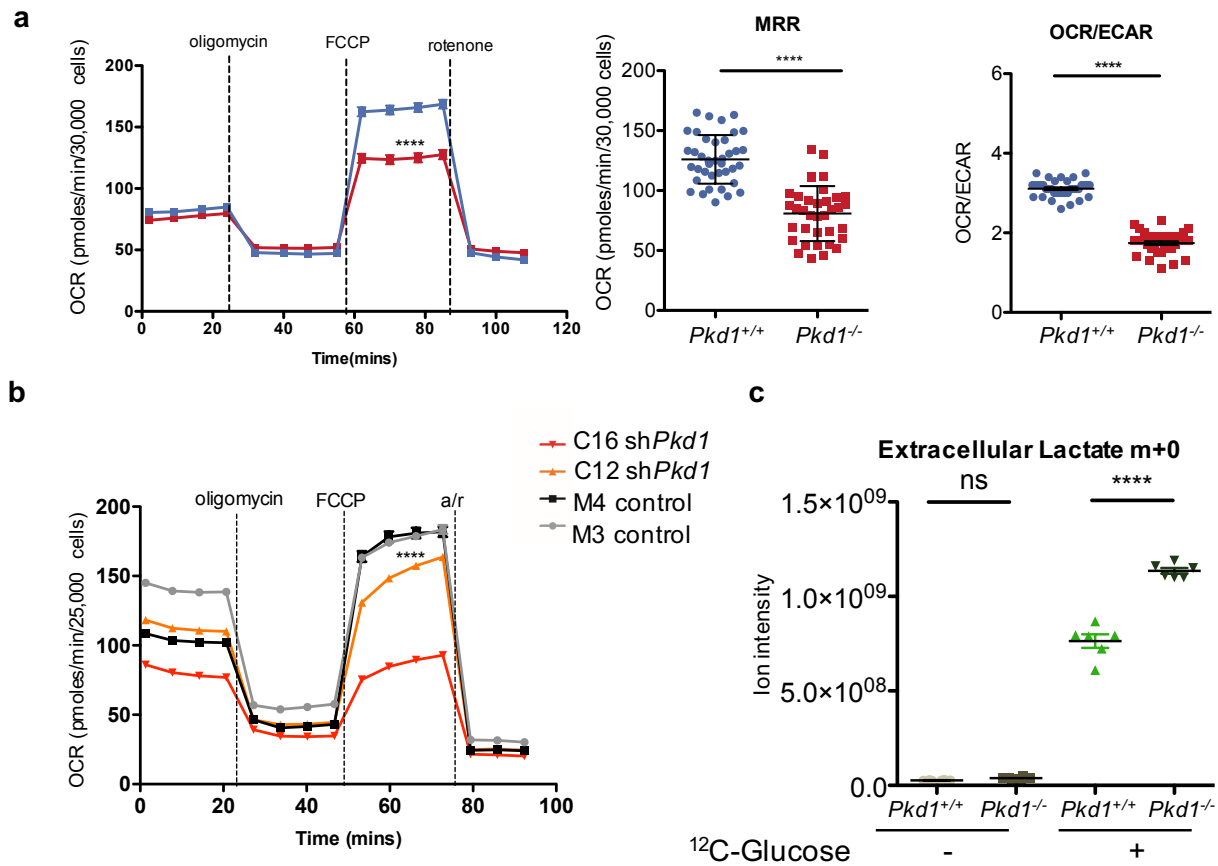




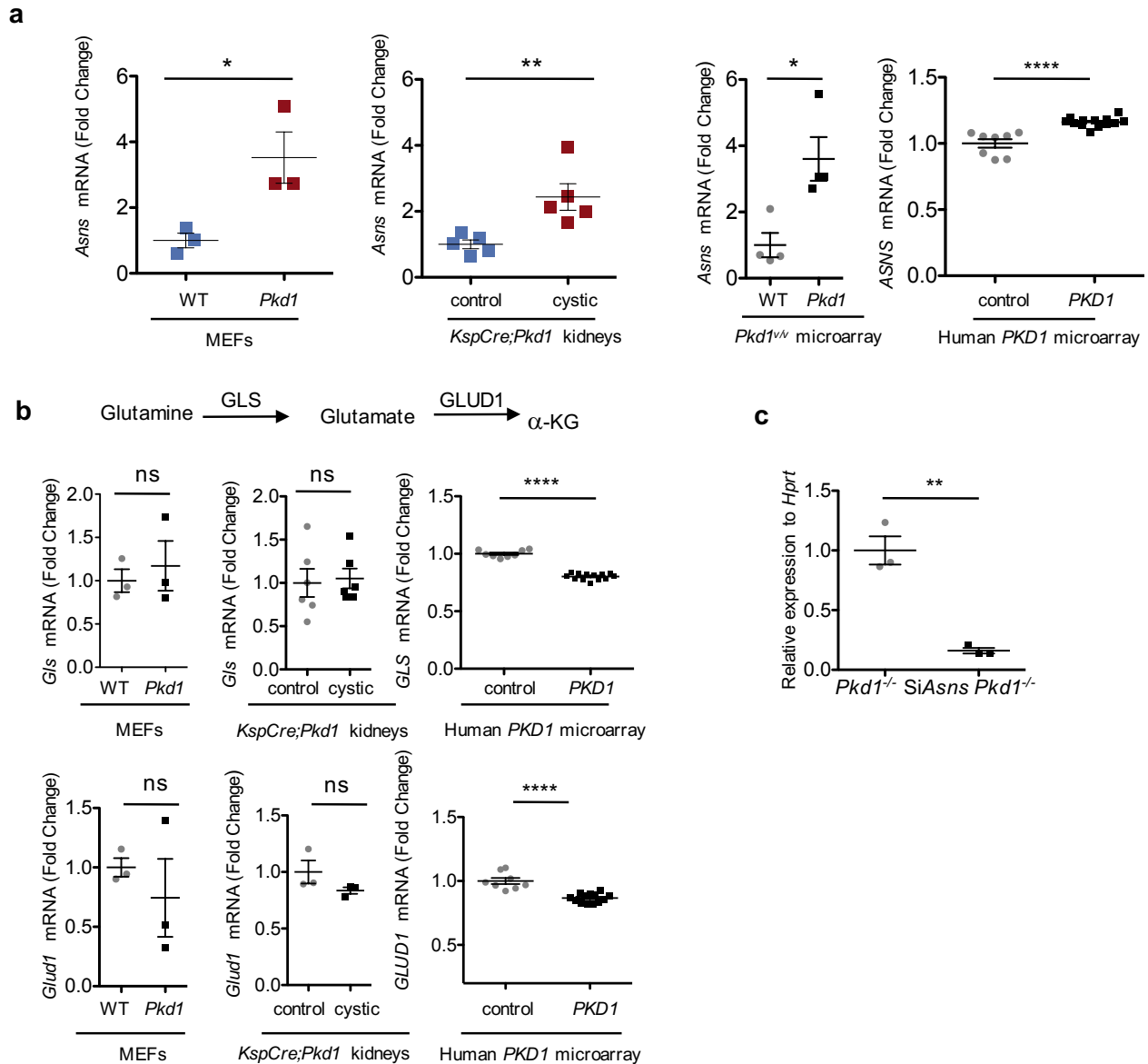
Supplementary figure 1. a Representative immunohistochemistry of cystic kidneys and control kidneys (P4) against CD45, Collagen I (COLI), Collagen IV (COLIV), respective markers of inflammation and fibrosis, showing no inflammation and only very mild fibrosis. Panels on the left and on the right show higher magnification. Bar represents 100µm. Immunohistochemistry are representative of two independent experiments. **b** Blood urea nitrogen (BUN) in mg/dL showing higher levels of BUN in cystic mice when compared to littermate controls collected at P4, P8, P10 and P12. Dot blot shown as mean standard error bars as SEM of two to nine independent biological replicates, **c** *Epo* and *Vegf* mRNA expression was assessed by quantitative RT-PCR, normalised to HPRT. No significant increase was detected for *Epo* and *Vegf* in the cystic kidneys when compared to the controls. Representative of three independent experiments. **d** Volcano plot of the 550 named metabolites profiled from mice kidneys. 384 metabolites exhibited significant changes (adjusted p value < 0.05, absolute fold change > 2) when comparing cystic kidneys with control kidneys. 171 metabolites exhibited a significant increase whilst 213 metabolites exhibited a significant decrease. Paired Student's *t*-tests were used to calculate statistical significance, and p values were corrected by using the Benjamini-Hochberg procedure (statistical analysis for all the 550 named metabolites can be found in **Supplementary Data 1**. Differentially abundant metabolites of different categories have been individually colour coded. **e** KEGG-Pathways Based Enrichment Analysis applied to the list of metabolites deriving from non-target global metabolomics performed on the *KspCre;Pkd1^{fllox/-}* kidneys and ranked in descending order according to the p values. It reveals 26 mouse pathways with a statistically significant ($p \leq 0.05$) enrichment score. Particularly, 8 pathways are enriched for upregulated metabolites whilst 18 are enriched for downregulated ones. Mean $\pm$ SEM were indicated, n.s., not significant ($p \geq 0.05$), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. *t*-test for **c** and ANOVA for **b**. Data in **d** and **e** were obtained from eight independent biological replicates.



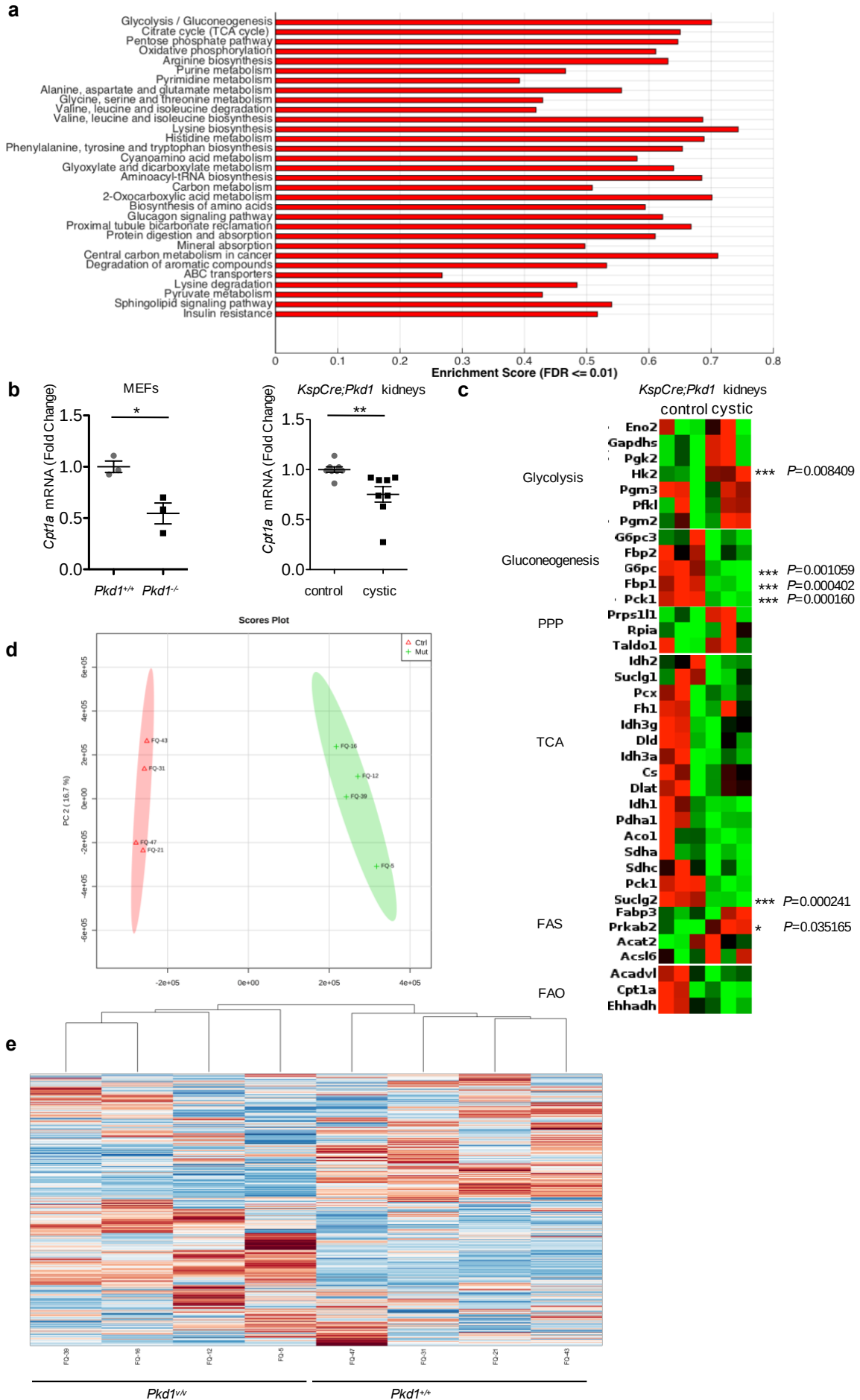
Supplementary Figure 2. **a** Principal component analysis (PCA) applied to the metabolites identified showed good separation between *Pkd1*^{+/+} and *Pkd1*^{-/-} cells. **b** Volcano plot of the metabolites profiled from *Pkd1*^{+/+} and *Pkd1*^{-/-} cells. 122 metabolites exhibited significant changes (adjusted *P* value < 0.05, absolute fold change > 2) when comparing *Pkd1*^{+/+} with *Pkd1*^{-/-} cells. 31 metabolites exhibited a significant increase whilst 91 metabolites exhibited a significant decrease. Paired Student's *t*-tests were used to calculate statistical significance, and *P* values were corrected by using the Benjamini-Hochberg procedure. Differentially abundant metabolites of different categories have been individually colour coded. **c** Significant metabolites were individually colour coded according to the pathway classification. Scheme of the glycolysis, pentose phosphate pathway, fatty acid oxidation and fatty acid biosynthesis *Pkd1*^{+/+} and *Pkd1*^{-/-} cells. Colour corresponds to the fold changes between *Pkd1*^{-/-} and *Pkd1*^{+/+} cells, orange-red labelled ones correspond to the metabolites more abundant, whereas blue labelled ones correspond to the metabolites less abundant in *Pkd1*^{+/+} compared *Pkd1*^{-/-} cells. Untargeted methodology is shown in the blue box whereas the targeted are shown in the grey box. **d** Levels of TCA intermediates citrate, α-KG, succinate and malate were assessed by LC-MS in *Pkd1*^{+/+} and *Pkd1*^{-/-} MEFs showing that they are significantly higher more abundant in the mutant cells compared to the controls. Dot plots showing means, SEM. *n.s.*, not significant (*P* ≥ 0.05), * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; **** *P* < 0.0001. *t*-test for **d** and six technical replicates for **d**. All graphs represent the data of seven technical replicates of one experiment.



Supplementary Figure 3. **a** OCR and ECAR were measured using the XF Extracellular Flux Analyzer (Seahorse Bioscience) on primary mouse embryonic fibroblasts isolated from $Pkd1^{+/+}$ or $Pkd1^{\Delta C/\Delta C}$ embryos at E11.5. Dot plots showing means, MRR calculated from the 12th measurement (after subtraction of the 15th measurement), OCR/ECAR from basal measurement, 2nd time point. **b** sh*Pkd1* (C16, C12) cells compared to scrambled controls (M3, M4). **c** Extracellular lactate. Levels of unlabelled M+0 extracellular lactate were measured in $Pkd1^{-/-}$ and $Pkd1^{+/+}$ cells in the presence (+) or in the absence (-) of ^{12}C -glucose. Mean $\pm$ SEM were indicated, n.s., not significant ($p \geq 0.05$), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. *t*-test for **a** and **b**, ANOVA for **c**. Representative graphs of at least two independent experiments made from 33 to 38 (**a**) and from 20 (**b**) replicate wells. One experiment made with six technical replicates is represented in **c**.



Supplementary Figure 4. a Dot plots showing means fold change, SEM, mRNA levels of *Asns* in *Pkd1*^{-/-} compared to *Pkd1*^{+/+} cells normalised to *Hprt*. *n*=3, three technical replicates of at least 3 independent experiments. Dot plots showing means fold change, SEM, mRNA *Asns* levels measured in control compared to cystic kidneys normalised to *Hprt*. *n*=5, two technical replicates of at least 2 independent experiments. Microarrays from *Pkd1*^{V/V} P10 kidneys and human-derived microarrays of PKD patients samples showing upregulation of *ASNS*. **b** Quantitative RT-PCR of the genes coding for GLS or GLUD1, two key enzymes involved in glutamine usage (top scheme) normalised to *Hprt* shows that they are not increased in *Pkd1*^{-/-} MEFs, cystic kidneys and human-derived microarray of PKD patients compared to the relative controls. **c** mRNA levels of *Asns* in *Pkd1*^{-/-} compared to *Pkd1*^{-/-} (mock) cells. Graphs represents. Mean $\pm$ SEM were indicated, *n.s.*, not significant ($P \geq 0.05$), * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$; **** $P < 0.00001$. *t*-test used for (a, b and c). (a and b) four independent biological replicates for *Pkd1*^{V/V} animal model, eight (normal and minimal cyst) and 13 independent biological replicates for large, medium, small cyst for human *PKD1* microarrays (a and b). Graphs in b corresponding to the MEFs are representative of means of data of two independent experiments made on three experimental replicates and for the mice they are representative of five independent biological replicates (b) and three independent biological replicates (c).



Supplementary Figure 5. a KEGG-Pathways Based Enrichment Analysis applied to the list of metabolites deriving from *in silico* simulation of increased glycolysis ranked according to the DFA algorithm. Only human pathways for which at least five metabolites are present in our mathematical model have been considered. Picture shows that 31 pathways result in an enrichment scores having $FDR \leq 0.01$, where FDR has been computed by applying Benjamini-Hochberg procedure. **b** Quantitative RT-PCR of genes encoding *Cpt1a* show decreased in *Pkd1*^{-/-}, MEFs and cystic kidneys compared to controls. **c** Clustergram performed non-supervised hierarchical clustering of the entire dataset displays a heat map with co-regulated genes across groups (cystic Vs. controls) for Glycolysis, Gluconeogenesis, PPP, TCA, FAS and FAO. The *P* values are calculated based on a Student's t-test of the replicate $2^{\Delta(-\Delta C_T)}$ values for each gene in the control group and treatment groups, and *P* values less than 0.05 are indicated in red. **d** Principal component analysis applied to the genes in microarrays data from *Pkd1*^{V/V} animal model at P10 shows good separation between mutant versus control. **e** Hierarchical clustering analysis applied to the genes in microarrays data from *Pkd1*^{V/V} animal model at P10 shows separation between mutant versus control. Graphs in **b** are representative of means of data of three independent experiments made on three experimental (MEFs) or biological replicates (Mice).

Supplementary Table 1: Primer Sequences

	forward	reverse
<i>Epo</i>	CATCTGCGACAGTCGAGTTCTG	CACAACCCATCGTGACATTTTC
<i>Vefg</i>	CTGTGCAGGCTGCTGTAACG	GTTCCCGAAACCCTGAGGAG
<i>Fasn</i>	TTGCTGGCACTACAGAATGC	AACAGCCTCAGAGCGACAAT
<i>Cpt1a</i>	AGTGGCCTCACAGACTCCAG	GCCCATGTTGTACAGCTTCC
<i>Glud1</i>	CCCAACTTCTTCAAGATGGTGG	AGAGGCTCAACACATGGTTGC
<i>Gls</i>	TTCGCCCTCGGAGATCCTAC	CCAAGCTAGGTAACAGACCCT
<i>Asns</i>	GGTTTTCTCGATGCCTCCTT	TGTGGCTCTGTTACAATGGTG
<i>Hprt</i>	TTATGTCCCCCGTTGACTGA	ACATTGTGGCCCTCTGTGTG

Supplementary Table 2: Abbreviations**Abbreviations:**

α -KG, alpha-ketoglutarate	2PG, 2-phosphoglycerate
1,3BPG, 1,3-bisphosphoglycerate	3PG, 3-phosphoglycerate
BUN, blood urea nitrogen	PPP, pentose phosphate pathway
DCA, dicarboxylic acids	PUFA, polyunsaturated fatty acid
FA-Carn, fatty acyl-carnitine	R5P, ribose 5-phosphate
FAS, fatty acid synthesis	Ri5P, ribulose 5-phosphate
FAO, fatty acid oxidation	SFA, saturated fatty acids
FI, Fold Induction	SPF, specific pathogen free
FFA, free fatty acids	S7P, sedoheptulose 7-phosphate
F6P, fructose 6-phosphate	X5P, xylulose 5-phosphate
F1,6BP, fructose 1,6-bisphosphate	
G6P, glucose 6-phosphate	
GAP, glyceraldehyde 3-phosphate	
Gln, glutamine	
Glu, glucose	
LCFA, long chain fatty acids	
LC-MS, liquid chromatography coupled to mass spectrometry	
MalCoA, malonyl-CoA	
Mal, Malonate	
MS, mass spectrometry	
MUFA, monounsaturated fatty acids	
OAA, oxaloacetate	
OCR, oxygen consumption rate	
OxPhos, oxidative phosphorylation	
PEP, phosphoenolpyruvate	
6PG, 6-phosphogluconate	

Supplementary Methods

Blood Urea Nitrogen (BUN)

Analysis of serum samples was assessed by ILab Aries, a bench-top analyzer that can perform photometry, turbidimetry, and potentiometry tests (Instrumentation Laboratory, Werfen Group, Milan, Italy). BUN was detected using kits and controls supplied by ILab Aries. Standard controls were run before each determination to monitor the precision throughout the experiment, and the values obtained for controls were always within the expected ranges.

Immunohistochemistry

For immunohistochemistry (IHC), formalin-fixed paraffin-embedded consecutive sections (4 μ m) were dewaxed and hydrated through graded decrease alcohol series. Stained for anti CD45 antibody (BD Biosciences) and anti-Collagen IV Antibody (abcam) as per manufacturer instructions. Antigen unmasking with Citrate Buffer pH 6.0, any endogenous peroxidase activity was quenched with 3% peroxidase water for 20 min at Room Temperature (RT). Antibodies dilution of 1:400, 1hr RT, and developed with Rat on Mouse HRP-Polymer (Biocare Medical,) or EXPOSE Rabbit specific HRP/DAB detection IHC kit (abcam). Immunostaining, DAB substrate chromogen was applied to sections for 5 min at RT and counterstained with Mayer's hematoxylin, dehydrated and mounted with Eukitt (BioOptica). External positive and negative controls were run simultaneously. Images were acquired using a Zeiss AxioImager M2m with AxioCam MRc5.

PCR Arrays

RNA was isolated from snap-frozen kidneys using RNeasy Plus Mini kit (Qiagen) following the manufacturer's instructions. In total, 1 μ g total RNA was subjected to single-stranded cDNA using the RT2 first strand kit (QIAGEN). The cDNA was used in the Mouse Glucose Metabolism (PAMM-006ZF) and Fatty Acid Metabolism (PAMM-007Z) RT² Profiler PCR arrays according to the manufacturer's instructions and assay on a LightCycler 480 Instrument (Roche). Data were analysed using the RT² Profiler program supplied by QIAGEN and normalized to the geometric mean of the housekeeping genes, actin-beta, glyceraldehyde-3-phosphate dehydrogenase, and heat shock protein 90kda for PAMM-006ZF and beta-actin and heat shock protein for PAMM-007Z.