SUPPLEMENTAL DATA--SUMMARY

1. Detailed methods: **Non-targeted metabolomic analysis**

2. Supplemental Table 1: averaged tumor + kidney weights at sacrifice for each animal

3. Supplemental Table 2: $R^2$ and $Q^2$ values of the first 3 latent components for distinguishing cancer and sham surgery mice based on the metabolome in the tissue, serum and urine

4. Supplemental Table 3: all identified metabolites in each matrix:
   a. tissue
   b. serum
   c. urine

5. Supplemental Table 4: Variable Importance in Projection (VIP) data for Tissue, Serum and Urine

6. Supplemental Fig. 1: The PPAR-α agonist Wy-14,643 decreases tryptophan and increases nicotinamide levels *in vitro*.
   Caki-1 cells were incubated for 24 h with Wy-14,643 at the concentrations indicated. Subsequently the cells were harvested and analyzed by GC-TOF as indicated in Materials and Methods. Authentic standards were used for the targeted metabolite analysis. *p<0.05 compared to control.

7. Supplemental Fig. 2: The IDO inhibitor 1-MT increases tryptophan levels *in vitro*.
   ACHN cells were incubated for 24 h with 1-MT at the concentrations indicated. Subsequently the cells were harvested and analyzed by GC-TOF as indicated in Materials and Methods. Authentic
standards were used for the targeted metabolite analysis.
SUPPLEMENTAL DATA

1. Detailed methods: **Non-targeted metabolomic analysis**

   *Sample extraction:* The samples were extracted using an automated MicroLab STAR® system (Hamilton Company, Salt Lake City, UT) in 400 μl of methanol, containing the recovery standards. The samples were then separated into three equal aliquots for analysis in three independent platforms as described below.

   *Instrumentation platforms:* The platform consisted of three platforms: ultra-high performance liquid chromatography/tandem mass spectrometry (UHLC/MS/MS²) optimized for basic species, UHLC/MS/MS² optimized for acidic species, and gas chromatography/mass spectrometry (GC/MS). The samples destined for GC/MS analysis were dried under vacuum desiccation for a minimum of 24 h and then derivatized under dried nitrogen using bistrimethyl-silyl-trifluoroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp was from 40° to 300° C in a 16 minute period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. UPLC/MS was carried out using a Waters Acquity UHPLC (Waters Corporation, Milford, MA) coupled to an LTQ mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) equipped with an electrospray ionization source. Two separate UHPLC/MS injections were performed on each sample: one optimized for positive ions and one for negative ions [**Evans**]. Chromatographic separation followed by full scan mass spectra was carried out to record retention time, molecular weight (m/z) and MS/MS² of all detectable ions presented in the samples.

   Quality is monitored by several processes: Process blank samples are interspersed throughout the instrument run, and these are used to reject various artifactual peaks arising from solvent impurities, plastic tubing, etc. Process reproducibility is assessed in two ways. First, a set of 10 injection and retention standards are spiked into each sample immediately before injection. These standards are monitored to assess the reproducibility of instrument performance. Detection of instrument drift is
cause for rejection of the day run. The median relative standard deviation for these 30 compounds across all platforms in the present study was 0.07, 0.04, and 0.08 for tumor tissue, serum, and urine, respectively. Secondly, a set of six technical replicate samples per day (created by pooling aliquots of the various study samples for each matrix) are interspersed among study samples and analyzed in the same manner as study samples. Monitoring variation of all the matrix biochemicals which are detected in each of the technical replicate samples allows the assessment of the overall process reproducibility, including the extraction, recover, and analysis steps. The median relative standard deviation for all these biochemicals across all platforms was 0.12, 0.12, and 0.10 for tissue, serum, and urine, respectively. No significant carry-over of compounds occurs between sample runs. Ionization efficiencies and extraction efficiencies for individual compounds are assumed to be relatively consistent within each matrix type, and no cross-matrix comparisons are made. The only valid comparisons are those for individual compounds across study sample groups within a matrix. The retention index method is referenced within Evans, et al.

**Metabolite identification:** Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries. Libraries were constructed as described in Evans, et al, by analysis of authentic standards (currently >2500 compounds) on all three platforms at eight concentrations. As described in the methods section, primary spectra (molecular ion m/z, adducts, in-sources fragments), MS/MS spectra, and retention index for all compounds are retained in an Oracle database and are used for compound matching against raw data using a proprietary matching algorithm. All automated calls are confirmed by human inspection. This library allowed the rapid identification of metabolites in the experiment with high confidence. For ions that were not covered by the standards, additional library entries were added based on their unique ion signatures (chromatographic and mass spectral) and also by virtue of their recurrent nature among samples. With the exception of glutathione species, no claim is made that relies on absolute
quantitation. Metabolomic studies are designed to measure relative biological effects within a study structure; significance is based on the direction and magnitude of changes relative to a control group. For more detailed analysis focused on absolute concentrations of specific metabolites, targeted and quantitative analyses would be useful, including the use of NMR.

Comparison across matrices: Cross-sample normalization is based on constant starting sample amount, such as extraction at a fixed sample mass/extraction solvent volume ratio for tissue samples, and a constant volume per sample for liquid samples. It is not valid to make cross-tissue comparisons, as the data are relative quantitation; the only relevant comparisons are made for individual compounds within the structure of the study design, i.e. their relative concentrations between study groups within a matrix. Any cross-matrix comparisons must be limited to post-statistical inferences, such as the direction and magnitude of changes within the biological context of the study design.