SUPPLEMENTARY MATERIAL

Direct evidence for cancer cell-autonomous extracellular protein catabolism in pancreatic tumors

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Production and purification of msAlbumin and miniaturized plasmapheresis, Related to Fig. 1.

a. Recombinant mouse serum albumin (MSA) was produced in *P. pastoris*. Supernatant from the culture was collected at 48- and 72-hours post inoculation and analyzed by SDS-PAGE and Coomassie-stain as shown. The predicted molecular weight of MSA is 69kDa.

b. [^{15}N]-MSA generated was generated in *P. pastoris*, purified, and a representative analysis of the LVQEVTDFAK tryptic peptide by LC-MS/MS is shown (This preparation corresponds to the infusate used to generate the data presented in Fig. 1).

c. To determine the extent of albumin amino acid labeling, ^{15}N-labeled MSA produced in *P. pastoris* was subjected to acid hydrolysis and amino acids assessed by GC-MS. The % ^{15}N isotopomer labeling for the indicated amino acid is shown. (Ala = alanine; Asp = aspartate; Cys = cysteine; Gln = glutamine; Glu = glutamate; Gly = glycine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Ser = serine; Thr = threonine; Val = valine).

d. Schematic representation of a miniaturized multiplexed 4-channel plasmapheresis device fabricated from PDMS as well as a schematic depicting the use of the plasmapheresis device to perform albumin exchange in mice. Blood from the carotid artery is pumped into the device using a miniaturized peristaltic pump, plasma removed, and the cellular component of blood is then re-mixed with labeled albumin and returned to the mouse via a venous catheter

e. Microscopic image of a single channel in a functioning miniaturized plasmapheresis device showing plasma skimming from arterial blood based on axial migration of red blood cells towards the center of the microchannel at Stage 1, Stage 2, and Stage 3 (see schematic in panel d). The concentrated red blood cells are then mixed with labeled albumin (right panel) prior to reinfusion into mice.

Figure S2. Labeled albumin fate in plasma, lung and muscle of WT and KP animals, Related to Fig. 1.

a. Representative plasma protein levels from a plasma exchange experiment to deliver labeled mouse serum albumin (MSA). Plasma was collected longitudinally before, during and after the plasma exchange period in WT and KP mice. The time period indicated by the double dagger corresponds to the 30-minute plasma exchange period (n = 5).

b. Enzyme linked immunoadsorption assay (ELISA) to assess MSA levels in 7-8 week old WT and KP mice, a time point when the KP mice have late stage pancreatic cancer. No significant difference (n.s.) in albumin levels was measured between WT and KP mice (WT n = 11; KP n = 7).

c. Following plasma exchange of [^{15}N]-labeled MSA for endogenous MSA in WT and KP mice, the presence of [^{15}N]-labeled MSA in tissue was determined by analysis of labeled peptides from lungs of animals with pancreatic tumors (KP) or without pancreatic tumors (WT) by LC-MS/MS. No significant difference (n.s.) in albumin levels was measured between WT and KP mice.

d. The presence of labeled free amino acids in the lungs of WT or KP mice ~12 hours after plasma exchange of [^{15}N]-labeled MSA for endogenous MSA. Labeled amino acids were determined by GC-MS.

e. Following plasma exchange of [^{15}N]-labeled MSA for endogenous MSA in WT and KP mice, the presence of [^{15}N]-labeled MSA in tissue was determined by analysis of labeled peptides from muscle of animals with pancreatic tumors (KP) or without pancreatic tumors (WT) by LC-MS/MS. No significant difference (n.s.) in albumin levels was measured between WT and KP mice.

f. The presence of labeled free amino acids in the muscle of WT or KP mice ~12 hours after plasma exchange of [^{15}N]-labeled MSA for endogenous MSA. Labeled amino acids were determined by GC-MS

(Ala = alanine; Asp = aspartate; Cys = cysteine; Gln = glutamine; Glu = glutamate; Gly = glycine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Ser = serine; Thr = threonine; Val = valine). (for panels c-f: * $p<0.05$; ** $p<0.01$, *** $p<0.001$ by unpaired t-test, n.s. differences not significant, n = 5 per genotype).

Figure S3. Device placement in autochthonous pancreatic tumors and intravital imaging of DQ-BSA in tdTomato-negative pancreatic tumors, Related to Fig 3.

a. Representative hematoxylin and eosin (H&E) staining of devices with reservoirs adjacent to tumor tissue (left, Reservoir #1) and non-tumor tissue (right, Reservoir #2). Dotted black lines indicate the diffusion distance for EIPA observed by MALDI-IMS. Scale bar, 2mm for upper panels (low magnification) and 200μm for lower panels (high magnification).

b. Devices containing DQ-BSA were implanted into the pancreas of tomato-negative KP mice with pancreatic tumors. Multiphoton imaging of DQ-BSA fluorescence in the pancreatic tissue of live mice is shown (images are representative of n = 2 mice per genotype with triplicate reservoirs). Scale bar, 50 μm.

c. Quantification of macropinocytic index of tomato-negative autochthonous KP tumors based on fluorescence from DQ-BSA as shown in a. The macropinocytic index of MIA PaCa-2 xenograft tumors based on fluorescence from DQ-BSA is shown for comparison (n=5 distinct fields were used to quantify macropinocytic index per condition).

Significance differences are noted as $P^* < 0.05$; n.s. not significant, by unpaired t-test.

Figure S4. Local depletion of amino acids by EIPA in $Kras^{G12D}$-driven pancreatic tumors and effect of EIPA on amino acid levels in cells in culture. Related to Fig 4.

a. Devices containing EIPA were implanted directly into pancreatic tumors of KP mice. The position of the device and direction of the arrow indicate orientation and direction of EIPA delivery (white circle and black arrow, respectively). 24 hours after device implantation, serial sections from the tumor were analyzed by matrix assisted laser desorption ionization coupled to imaging mass-spectrometry (MALDI-IMS). Positive ion mode was used to detect the macropinocytosis inhibitor, EIPA (EIPA diffused approximately 400-500μM), and choline, a metabolite not expected to be affected by macropinocytosis inhibition. The range of ion detection corresponding to the heat maps shown for each metabolite is included for each image; scale bar, 2mm. (images are representative of n = 2 KP mice with triplicate reservoirs).

b. Serial sections from the same tumors described in a. were examined by MALDI-IMS in negative ion mode to assess levels of the amino acids glutamine and histidine. The position of the device (red circle with black arrow showing direction of EIPA delivery) was used to orient the serial sections analyzed in negative ion mode relative to those analyzed in positive ion mode. The location corresponding to EIPA single (see a) is designated by black or white dashed line and full tissue slice is presented for orientation. The relative levels of metabolite concentration corresponding to the heat maps shown for each metabolite is included for each image; scale bar, 2mm (images are representative of n = 2 KP mice with triplicate reservoirs).

c. Pancreatic cancer cells derived from KP tumors were cultured for 6 hours in media without or with branch chain amino acids (BCAAs), in the presence or absence of 3% albumin, and in the presence of vehicle alone (DMSO) or 20μM EIPA as indicated. Relative levels of leucine and valine under each condition as determined by GC-MS are shown.

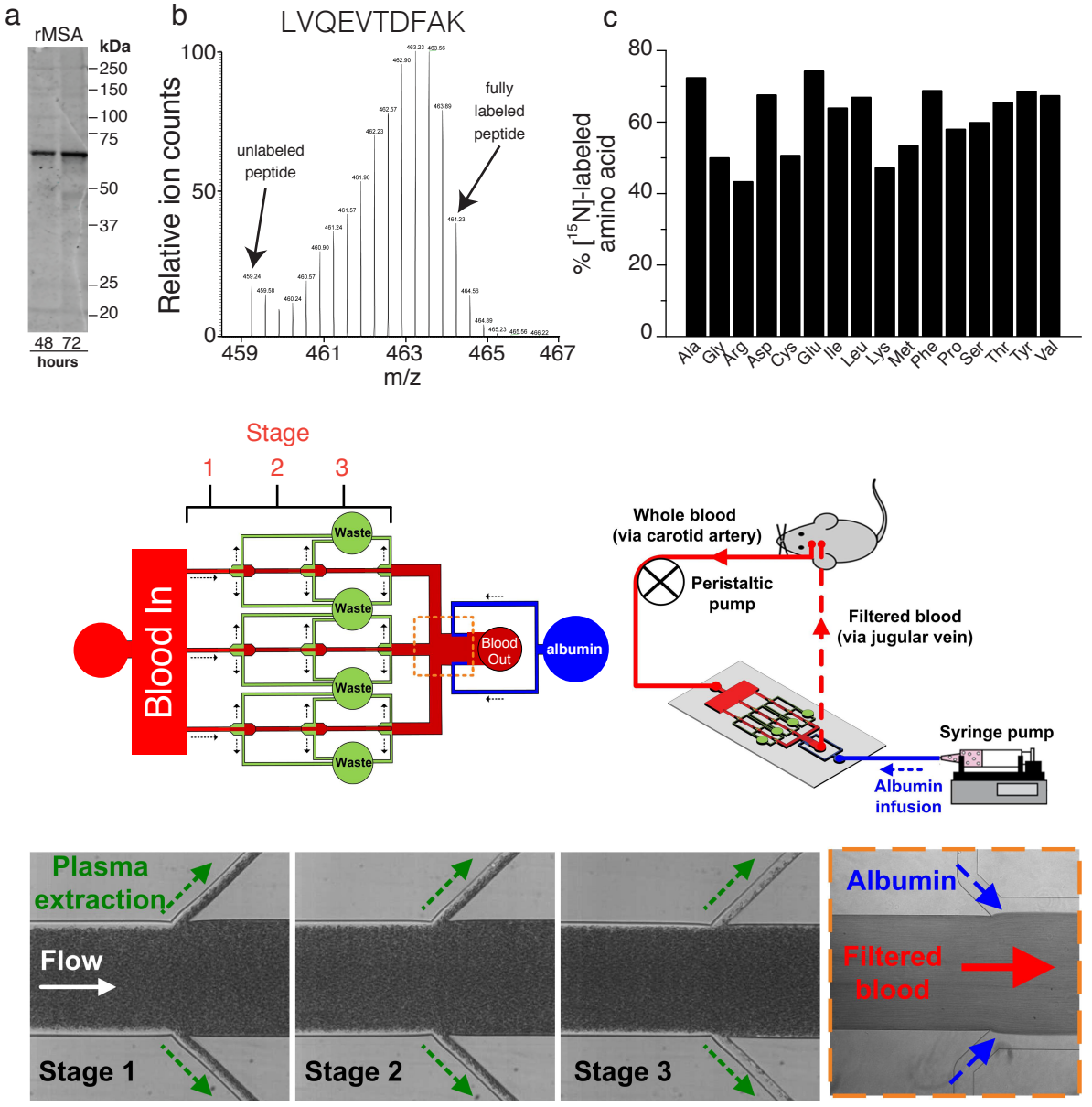
d. Pancreatic cancer cells derived from KP tumors were cultured for 6 hours in media containing uniformly labeled ^{15}N and ^{13}C-labeled amino acids in the presence of vehicle alone (DMSO) or

20μM EIPA as indicated. Total intracellular amino acid levels for each condition as determined by GC-MS are shown (n = 3 individual wells of a 6-well dish, repeated twice).

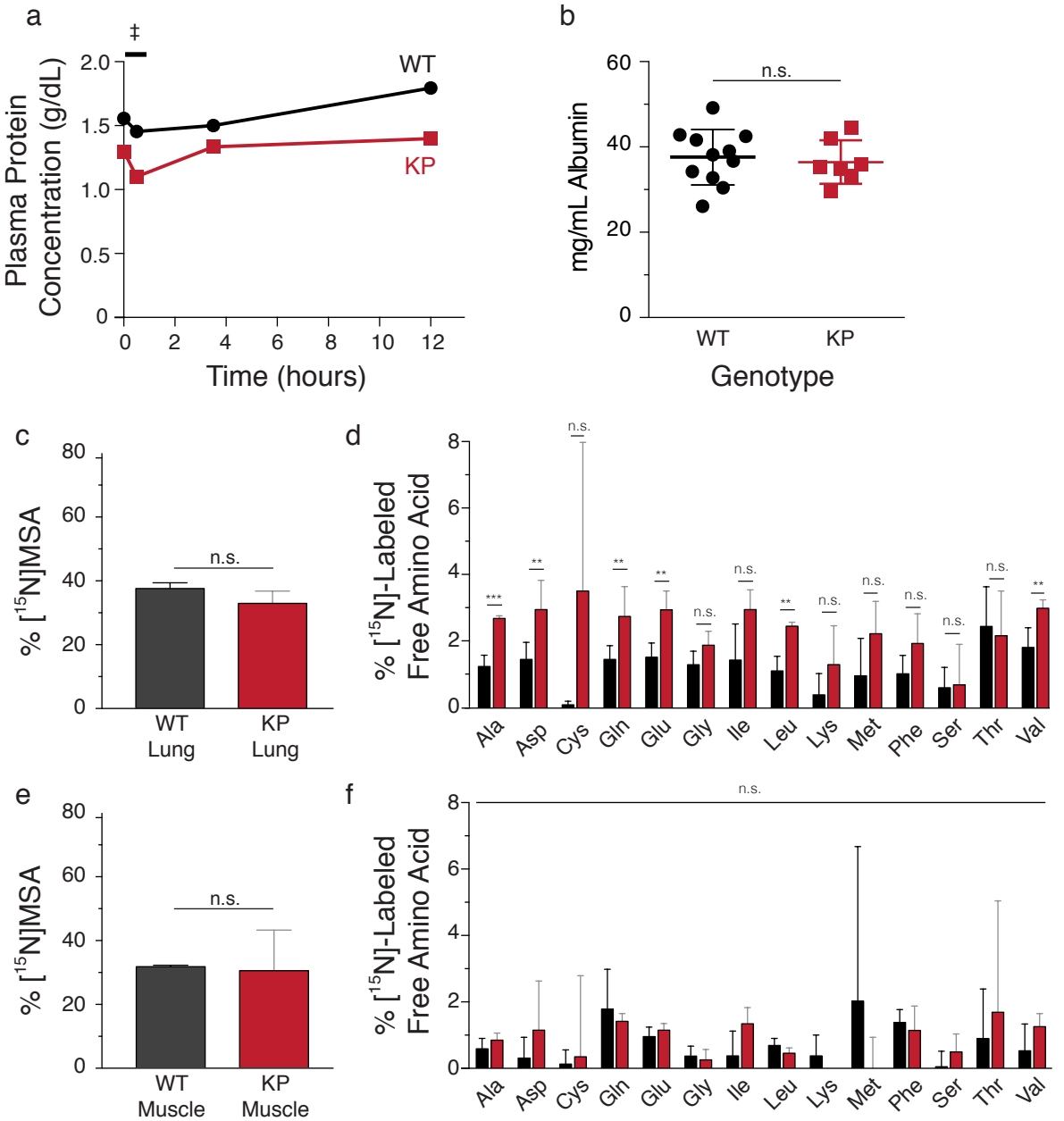
e. Pancreatic cancer cells derived from KP tumors were cultured for 6 hours in media containing uniformly labeled ^{15}N and ^{13}C-labeled amino acids in the presence of vehicle alone (DMSO) or 20μM EIPA as indicated. Total intracellular levels of ^{15}N-labeled amino acids for each condition as determined by GC-MS are shown (n = 3 individual wells of a 6-well dish, repeated twice).

f. Pancreatic cancer cells derived from KP tumors were cultured for 6 hours in media containing uniformly labeled ^{15}N and ^{13}C-labeled amino acids in the presence of vehicle alone (DMSO) or 20μM EIPA as indicated. Total intracellular levels of ^{13}C-labeled amino acids for each condition as determined by GC-MS are shown (n = 3 individual wells of a 6-well dish, repeated twice).

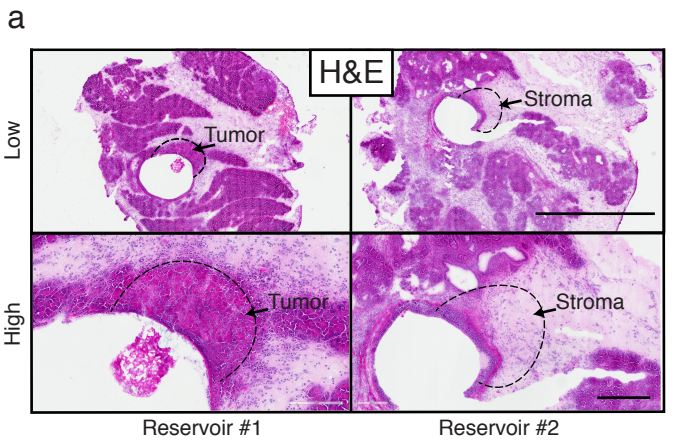
For all panels, significance differences are noted as $P^* < 0.05$ by unpaired t-test.

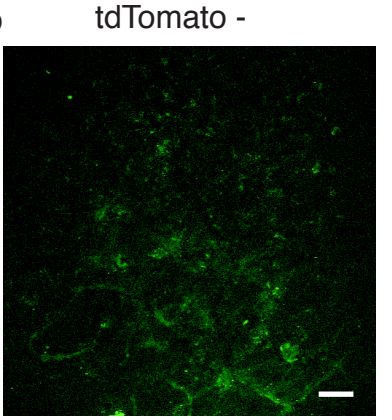


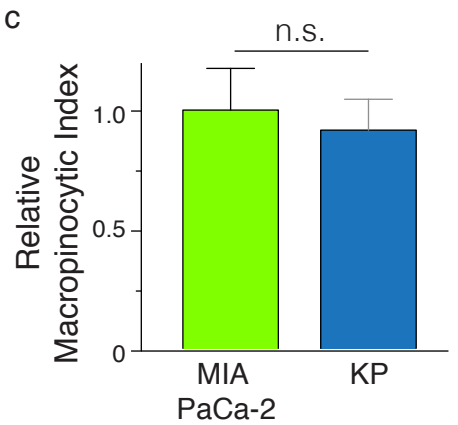
Davidson and Jonas Supplementary Figure 1



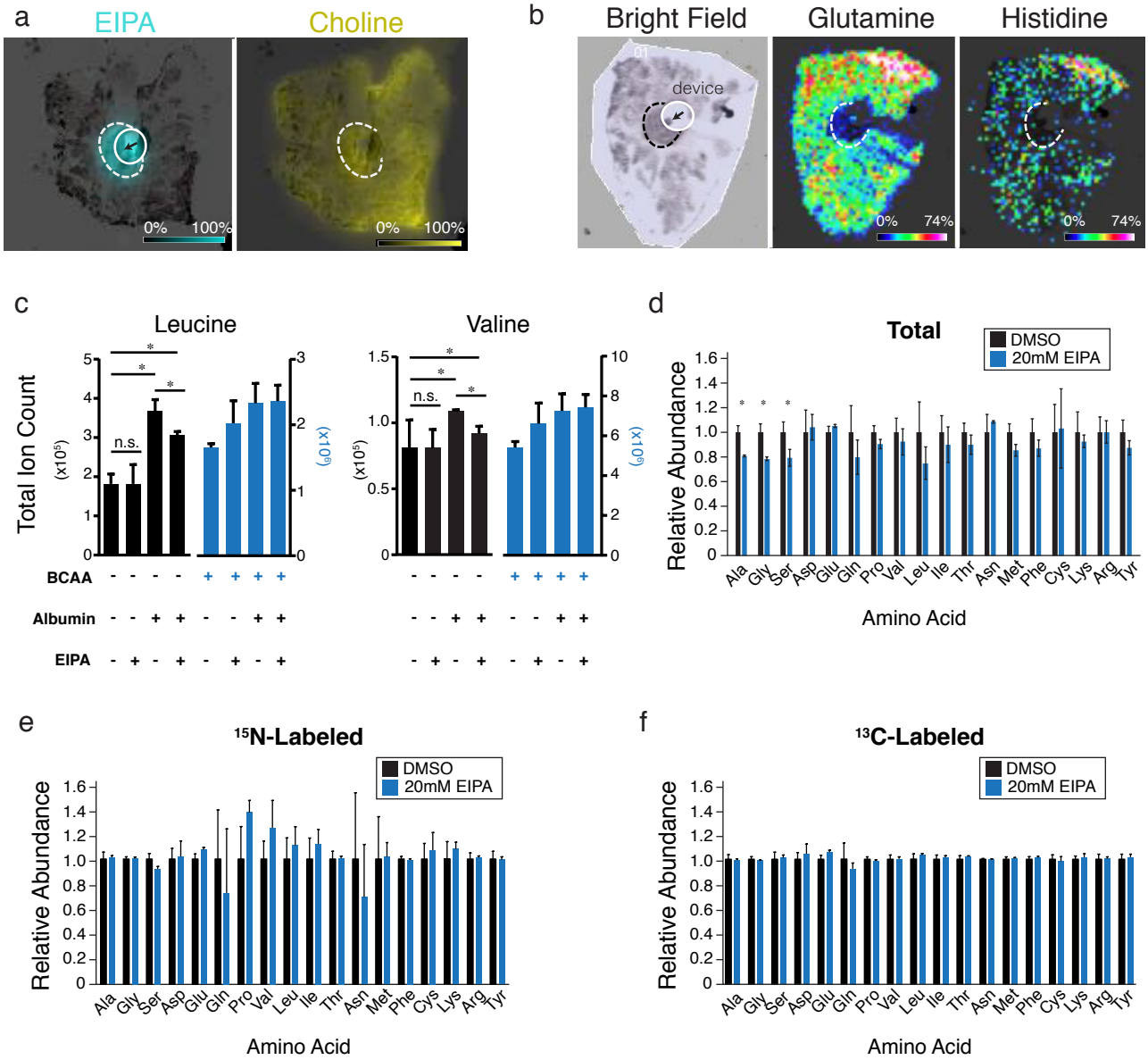
Davidson and Jonas Supplementary Figure 2







Davidson and Jonas Supplementary Figure 3



Davidson and Jonas Supplementary Figure 4