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Supplementary Materials for

1-Methylnicotinamide is an immune regulatory metabolite in human ovarian cancer

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/4/eabe1174/DC1)

Tables S1 to S5

fig. S1

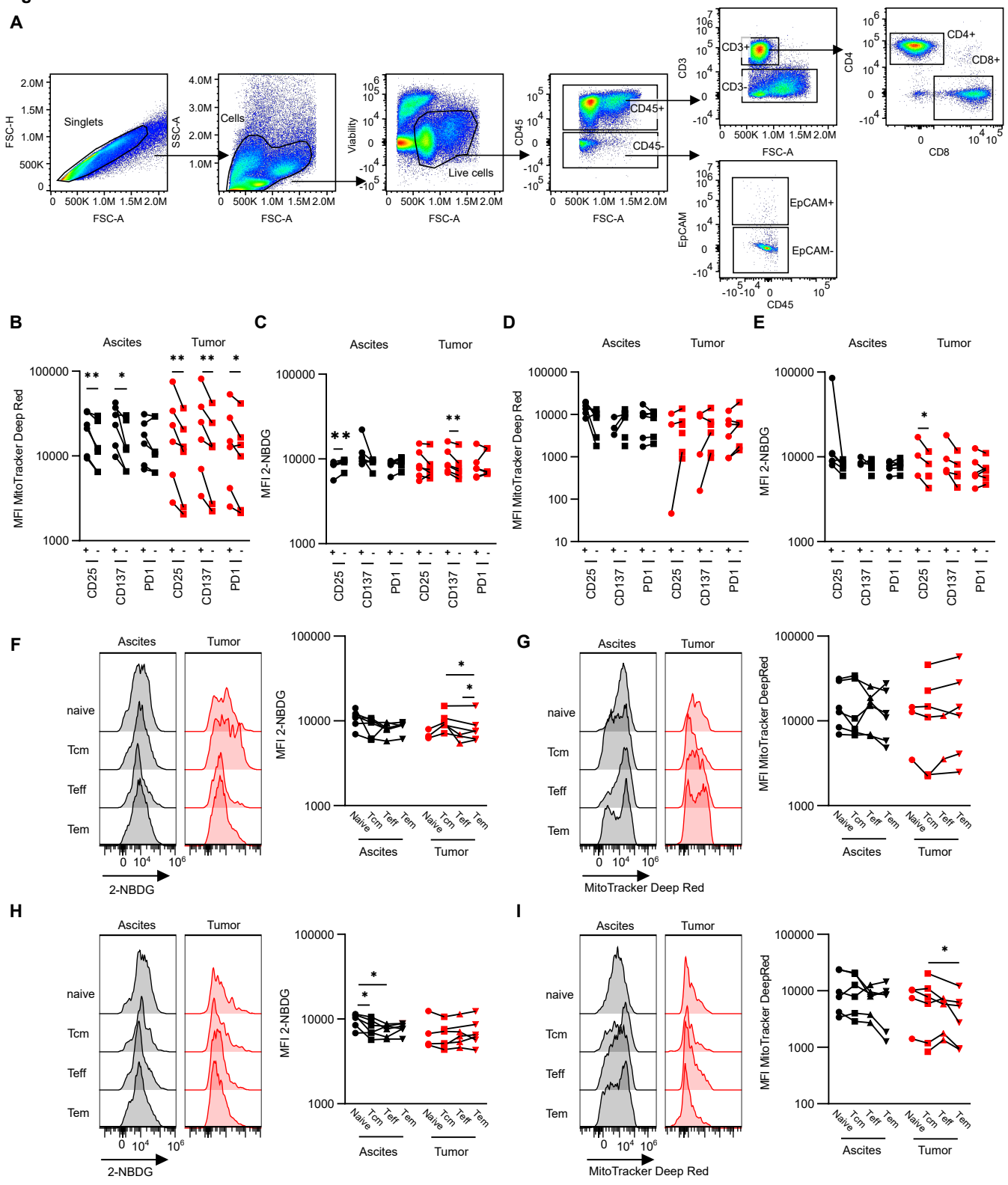

A
Singlets
Cells
Viability
Live cells
CD45+
CD45-
CD3+
CD3-
CD4+
CD8+
EpCAM+
EpCAM-
FSC-A
FSC-H
SSC-A
CD3
CD4
CD8
CD45
EpCAM
B
C
D
E
Ascites
Tumor
MFI MitoTracker Deep Red
MFI 2-NBDG
CD25
CD137
PD1
F
G
H
I
naive
Tcm
Teff
Tem
2-NBDG
MitoTracker Deep Red
MFI MitoTracker DeepRed
Naive

fig. S2

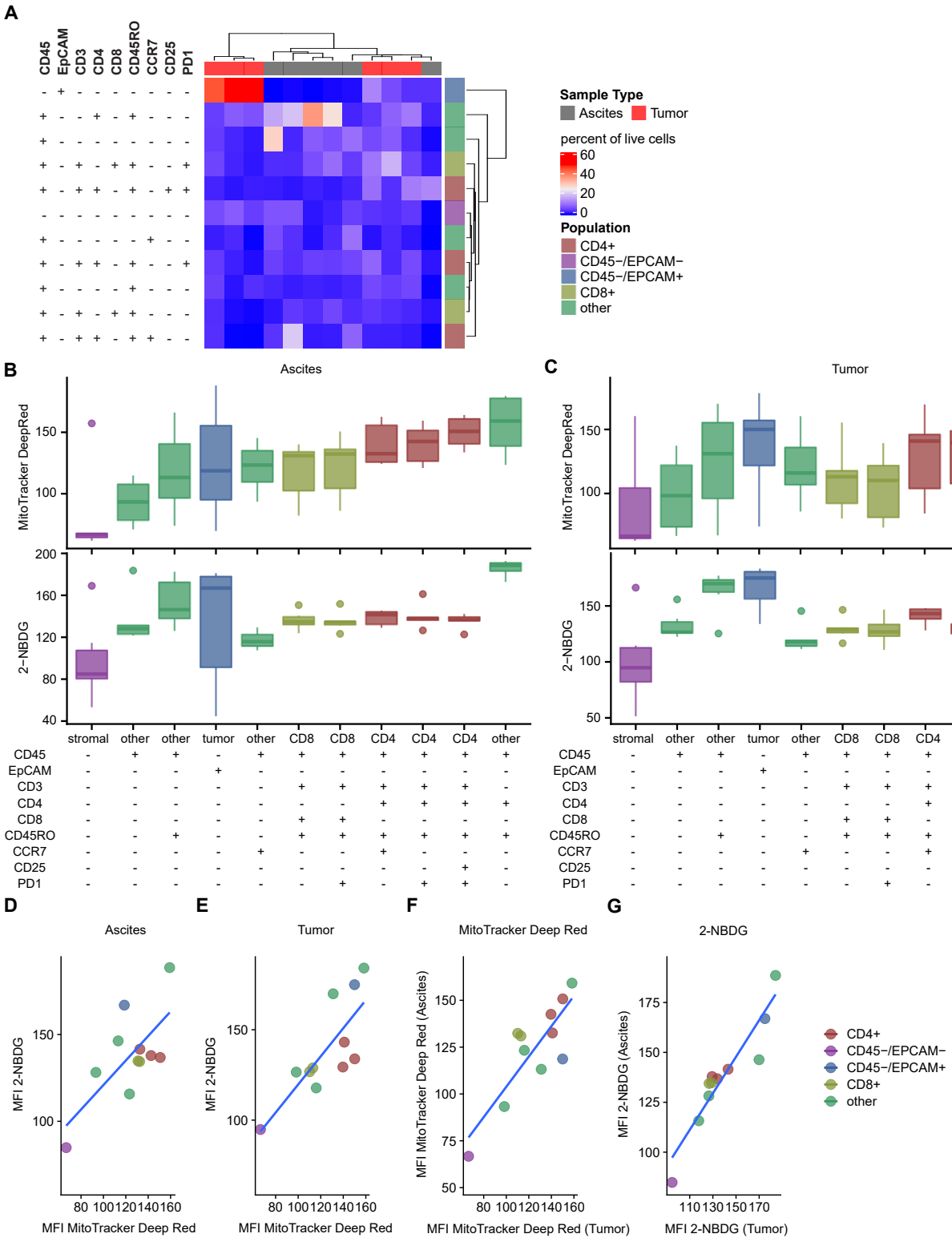

A
CD45 EpCAM CD3 CD4 CD8 CD45RO CCR7 CD25 PD1
Sample Type
Ascites Tumor
percent of live cells
60
40
20
0
Population
CD4+
CD45−/EPCAM−
CD45−/EPCAM+
CD8+
other
B
Ascites
MitoTracker DeepRed
2−NBDG
stromal other other tumor other CD8 CD8 CD4 CD4 CD4 other
CD45 - + + - + + + + + + +
EpCAM - - - + - - - - - - -
CD3 - - - - - + + + + + -
CD4 - - - - - - - + + + +
CD8 - - - - - + + - - - -
CD45RO - - + - - + + + + + +
CCR7 - - - - + - - + - - -
CD25 - - - - - - - - - + -
PD1 - - - - - - + - + + -
C
Tumor
MitoTracker DeepRed
2−NBDG
stromal other other tumor other CD8 CD8 CD4 CD4 CD4 other
CD45 - + + - + + + + + + +
EpCAM - - - + - - - - - - -
CD3 - - - - - + + + + + -
CD4 - - - - - - - + + + +
CD8 - - - - - + + - - - -
CD45RO - - + - - + + + + + +
CCR7 - - - - + - - + - - -
CD25 - - - - - - - - - + -
PD1 - - - - - - + - + + -
D
Ascites
MFI 2-NBDG
MFI MitoTracker Deep Red
E
Tumor
MFI 2-NBDG
MFI MitoTracker Deep Red
F
MitoTracker Deep Red
MFI MitoTracker Deep Red (Ascites)
MFI MitoTracker Deep Red (Tumor)
G
2-NBDG
MFI 2-NBDG (Ascites)
MFI 2-NBDG (Tumor)
CD4+
CD45−/EPCAM−
CD45−/EPCAM+
CD8+
other

fig. S3

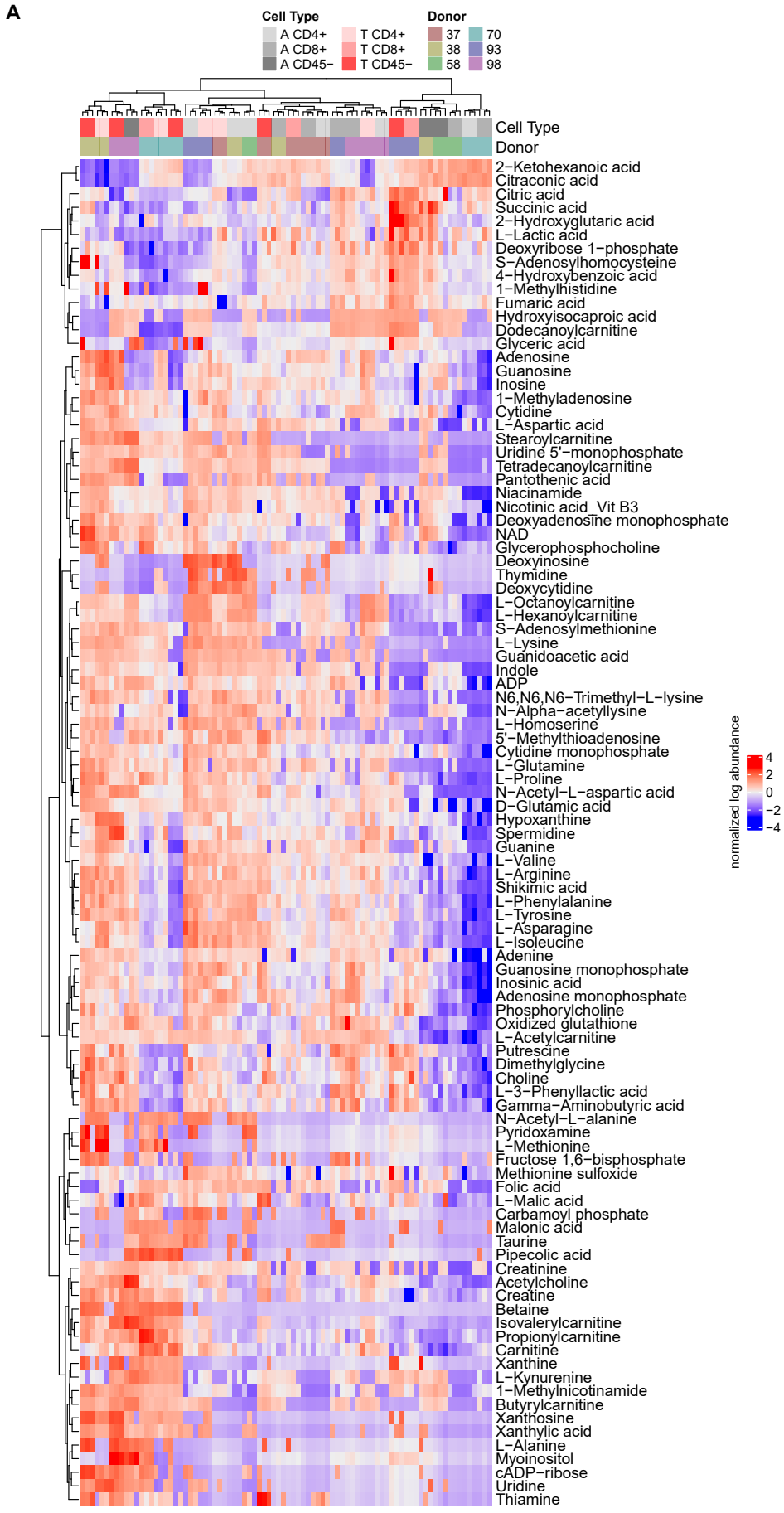

A
Cell Type
A CD4+
A CD8+
A CD45−
T CD4+
T CD8+
T CD45−
Donor
37
38
58
70
93
98
Cell Type
Donor
2−Ketohexanoic acid
Citraconic acid
Citric acid
Succinic acid
2−Hydroxyglutaric acid
L−Lactic acid
Deoxyribose 1−phosphate
S−Adenosylhomocysteine
4−Hydroxybenzoic acid
1−Methylhistidine
Fumaric acid
Hydroxyisocaproic acid
Dodecanoylcarnitine
Glyceric acid
Adenosine
Guanosine
Inosine
1−Methyladenosine
Cytidine
L−Aspartic acid
Stearoylcarnitine
Uridine 5'−monophosphate
Tetradecanoylcarnitine
Pantothenic acid
Niacinamide
Nicotinic acid_Vit B3
Deoxyadenosine monophosphate
NAD
Glycerophosphocholine
Deoxyinosine
Thymidine
Deoxycytidine
L−Octanoylcarnitine
L−Hexanoylcarnitine
S−Adenosylmethionine
L−Lysine
Guanidoacetic acid
Indole
ADP
N6,N6,N6−Trimethyl−L−lysine
N−Alpha−acetyllysine
L−Homoserine
5'−Methylthioadenosine
Cytidine monophosphate
L−Glutamine
L−Proline
N−Acetyl−L−aspartic acid
D−Glutamic acid
Hypoxanthine
Spermidine
Guanine
L−Valine
L−Arginine
Shikimic acid
L−Phenylalanine
L−Tyrosine
L−Asparagine
L−Isoleucine
Adenine
Guanosine monophosphate
Inosinic acid
Adenosine monophosphate
Phosphorylcholine
Oxidized glutathione
L−Acetylcarnitine
Putrescine
Dimethylglycine
Choline
L−3−Phenyllactic acid
Gamma−Aminobutyric acid
N−Acetyl−L−alanine
Pyridoxamine
L−Methionine
Fructose 1,6−bisphosphate
Methionine sulfoxide
Folic acid
L−Malic acid
Carbamoyl phosphate
Malonic acid
Taurine
Pipecolic acid
Creatinine
Acetylcholine
Creatine
Betaine
Isovalerylcarnitine
Propionylcarnitine
Carnitine
Xanthine
L−Kynurenine
1−Methylnicotinamide
Butyrylcarnitine
Xanthosine
Xanthylic acid
L−Alanine
Myoinositol
cADP−ribose
Uridine
Thiamine
normalized log abundance
4
2
0
−2
−4
B
Adenosine (log)
Ascites
Tumor
*
CD4+
CD8+
CD45-
CD4+
CD8+
CD45-
C
L−Kynurenine (log)
Ascites
Tumor
*
CD4+
CD8+
CD45-
CD4+
CD8+
CD45-

fig. S4

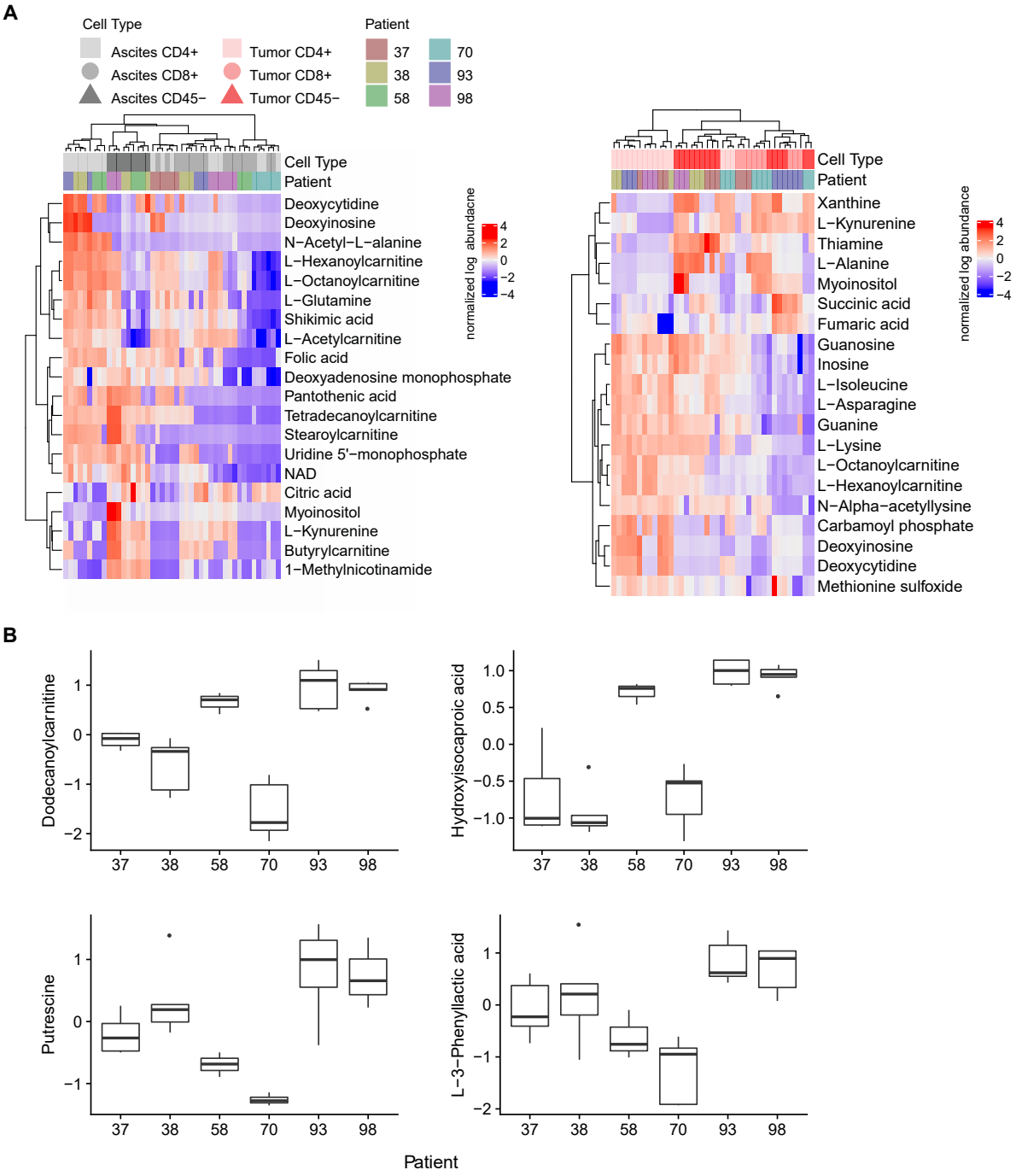
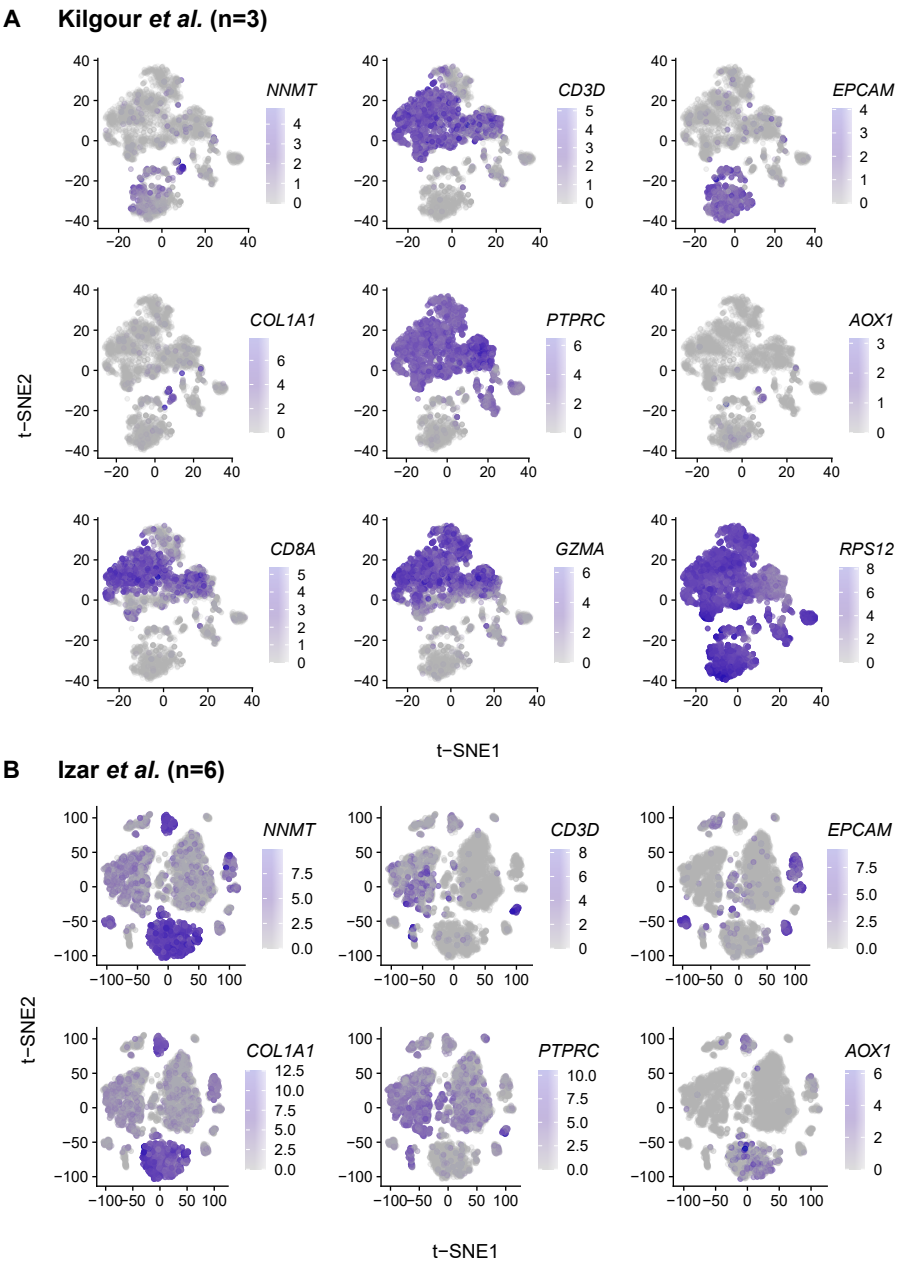

A
Cell Type
Ascites CD4+
Ascites CD8+
Ascites CD45−
Tumor CD4+
Tumor CD8+
Tumor CD45−
Patient
37
38
58
70
93
98
Cell Type
Patient
Deoxycytidine
Deoxyinosine
N−Acetyl−L−alanine
L−Hexanoylcarnitine
L−Octanoylcarnitine
L−Glutamine
Shikimic acid
L−Acetylcarnitine
Folic acid
Deoxyadenosine monophosphate
Pantothenic acid
Tetradecanoylcarnitine
Stearoylcarnitine
Uridine 5'−monophosphate
NAD
Citric acid
Myoinositol
L−Kynurenine
Butyrylcarnitine
1−Methylnicotinamide
normalized log abundance
4
2
0
−2
−4
Cell Type
Patient
Xanthine
L−Kynurenine
Thiamine
L−Alanine
Myoinositol
Succinic acid
Fumaric acid
Guanosine
Inosine
L−Isoleucine
L−Asparagine
Guanine
L−Lysine
L−Octanoylcarnitine
L−Hexanoylcarnitine
N−Alpha−acetyllysine
Carbamoyl phosphate
Deoxyinosine
Deoxycytidine
Methionine sulfoxide
normalized log abundance
4
2
0
−2
−4
B
Dodecanoylcarnitine
Hydroxyisocaproic acid
Putrescine
L−3−Phenyllactic acid
37
38
58
70
93
98
Patient

fig.S5


A
Kilgour et al. (n=3)
NNMT
CD3D
EPCAM
COL1A1
PTPRC
AOX1
CD8A
GZMA
RPS12
t−SNE2
t−SNE1
B
Izar et al. (n=6)
NNMT
CD3D
EPCAM
COL1A1
PTPRC
AOX1
t−SNE2
t−SNE1

SUPPLEMENTAL FIGURE LEGENDS

fig. S1. T cell metabolism is minimally impacted by expression of activation and differentiation markers.
(**A**) Representative gating strategy for analysis by flow cytometry.
(**B-C**) Mitochondrial activity (B) and glucose uptake (C) of CD25, CD137 and PD1 +/- CD4+ T cells.
(**D-E**) Mitochondrial activity (D) and glucose uptake (E) of CD25, CD137 and PD1 +/- CD8+ T cells.
(**F-G**) Representative plot (left) and tabulated data (right) for glucose uptake (F) and mitochondrial activity (G) of naive, Tcm, Teff and Tem CD4+ T cells.
(**H-I**) Representative plot (left) and tabulated data (right) for glucose uptake (H) and mitochondrial activity (I) of naive, Tcm, Teff and Tem CD8+ T cells. p-values (*p<0.05, **p<0.01, ***p<0.001) determined by paired t-test (B-I). Lines indicate matched patients (B-I). Fluorescence Minus One (FMO); Median Fluorescence Intensity (MFI); Central memory T cells (Tcm); Effector T cells (Teff); Effector memory T cells (Tem).

fig. S2. Automated analysis of metabolism and cell type by flow cytometry.
(**A**) Heat map of cell type abundance.
(**B-C**) Glucose uptake and mitochondrial activity of cell fractions within ascites (B) and tumor (**C**). Boxplots show medians (lines), interquartile range (box hinges) and range of data (box whiskers; excepting outliers, shown as points).
(**D-E**) Correlation between glucose uptake and mitochondrial activity of cell fractions within the ascites (D) and tumor (E).
(**F-G**) Correlation of glucose uptake (F) and mitochondrial activity (G) of cell fractions between ascites and tumor. Median (per phenotype) of median (per sample) biexponential-transformed Median Fluorescence Intensity (MFI) values shown.

fig. S3. Cell-specific metabolite profiling reveals changes in relative metabolite abundance
(**A**) Heatmap of normalized metabolite abundance, with dendrograms representing Ward's clustering of Euclidean distances among samples.
(**B-C**) Relative abundance of adenosine (B) and L-kynurenine (C) measured by liquid chromatography tandem mass spectrometry (LC-MS/MS)**.** Boxplots show medians (lines), interquartile range (box hinges) and range of data up to 1.5X interquartile range (box whiskers; outliers shown as points). P-values are determined using *limma* with patient as a random effect, as described in methods (*p<0.05).

fig. S4. Changes in relative metabolite abundance across cell types within the ascites and tumor.
(**A**) Heatmap of normalized metabolite abundance, with dendrograms representing Ward's clustering of Euclidean distances among samples. Relative abundance of metabolites in the ascites (left) and tumor (right).
(**B**) Top four significantly differing metabolites across patients (all P_{adj} for F-Test of patient effect in *limma* < 0.05). Boxplots show medians (lines), interquartile range (box hinges) range of data up to 1.5X interquartile range (box whiskers; excepting outliers, shown as points).

fig. S5. Expression of population defining makers and metabolic genes within the scRNA-seq data. Expression of *NNMT, CD3D, EPCAM, COL1A1, PTPRC, AOX1, CD8A, GZMA,* and *RPS12* within ascites and tumor, shown as $\log_2$ normalized unique molecular identifier (UMI) counts. Data in (**A**) was obtained from samples from the BC Cancer Tumour Tissue Repository (n=3), and data from (**B**) was adapted from Izar *et al.* (n=6) (*16*) as described in the methods.