1 **Supplementary Material**

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3 **Tryptophan metabolism is inversely regulated in the tumor and blood**

4 **of patients with glioblastoma**

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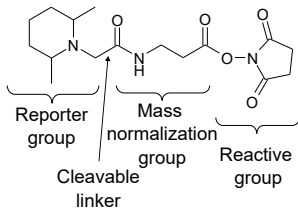
44

Figure S1

A
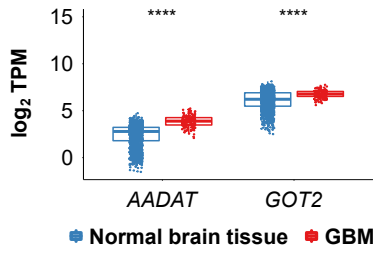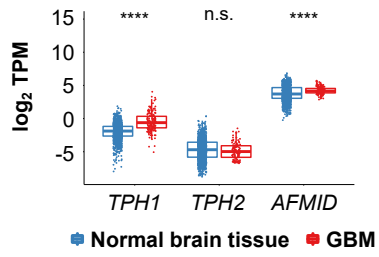
B

45   **Figure S1. Trp metabolism-associated genes are upregulated in glioblastoma, related to Figures 1 and**

46   **2.**

47   **(A)** Boxplot representation of the expression of select Trp metabolism-associated enzymes in normal brain

48   tissue (blue) (GTEx data) and in glioblastoma (GBM) tissue (red) (TCGA data) represented as $\log_2$

49   transcripts per million ($\log_2$ TPM) (Wilcoxon rank-sum test, **** $P < 0.0001$, n.s. not significant). CCBL1

50   (KAT I) and CCBL2 (KAT III) were not expressed in the TCGA data.

51   **(B)** Chemical structure of TMT® reagents.

52   Abbreviations: AADAT: alpha-aminoadipate aminotransferase; AFMID: arylformamidase; CCBL: cysteine

53   conjugate beta lyase; GBM: glioblastoma; GOT2: glutamate oxaloacetate transaminase 2; GTEx: Genotype-

54   Tissue Expression; KAT: kynurenine aminotransferase; TCGA: The Cancer Genome Atlas; TMT®: tandem

55   mass tag; TPH1/2: tryptophan hydroxylase 1/2; TPM: transcripts per million; Trp: tryptophan.

Figure S2

Bevacizumab

MR (95% CI)
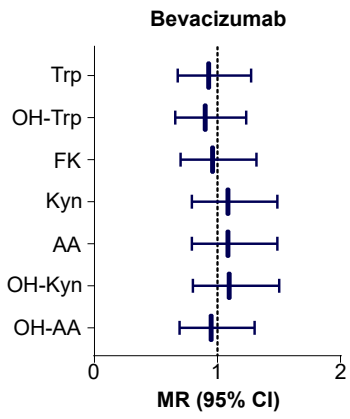
56   **Figure S2. Bevacizumab treatment does not influence metabolite levels in peripheral blood of patients,**

57   **related to Figure 3.**

58   Forest plot depicting the association of metabolite levels in peripheral blood of patients ($n = 42$) with the

59   cumulative dose of bevacizumab received prior to blood draw. Mean ratio (MR) and 95% confidence

60   intervals (CI) are shown.

61   See also Table S11.

62   Abbreviations: AA: anthranilic acid; CI: confidence interval; FK: *N*-formylkynurenine; Kyn: kynurenine;

63   MR: mean ratio; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-

64   tryptophan; Trp: tryptophan.
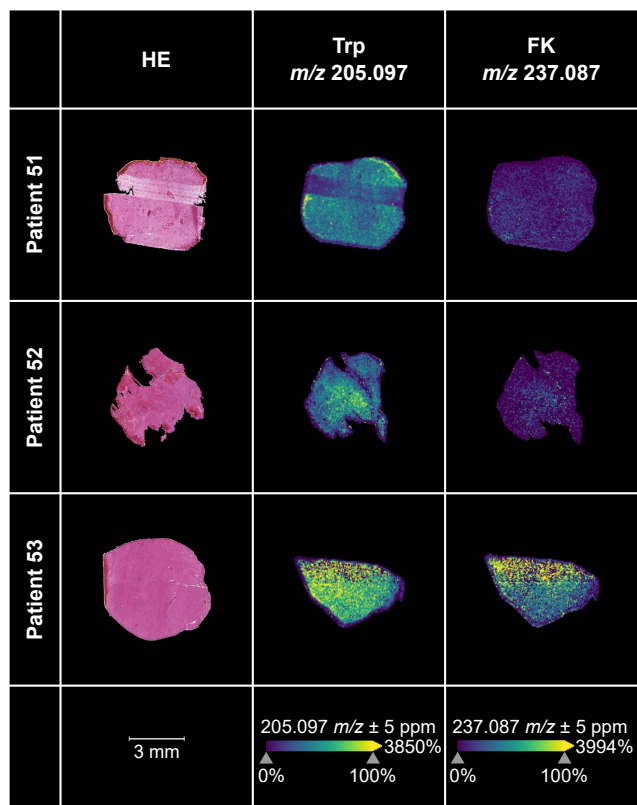
Figure S3. Trp and its metabolite FK in glioblastoma tumor tissue, related to Figure 5.

(A) MALDI MSI of Trp and FK distribution in human glioblastoma samples (middle and right column) and corresponding annotated HE-stained adjacent tissue sections (left column) (n = 3). Displ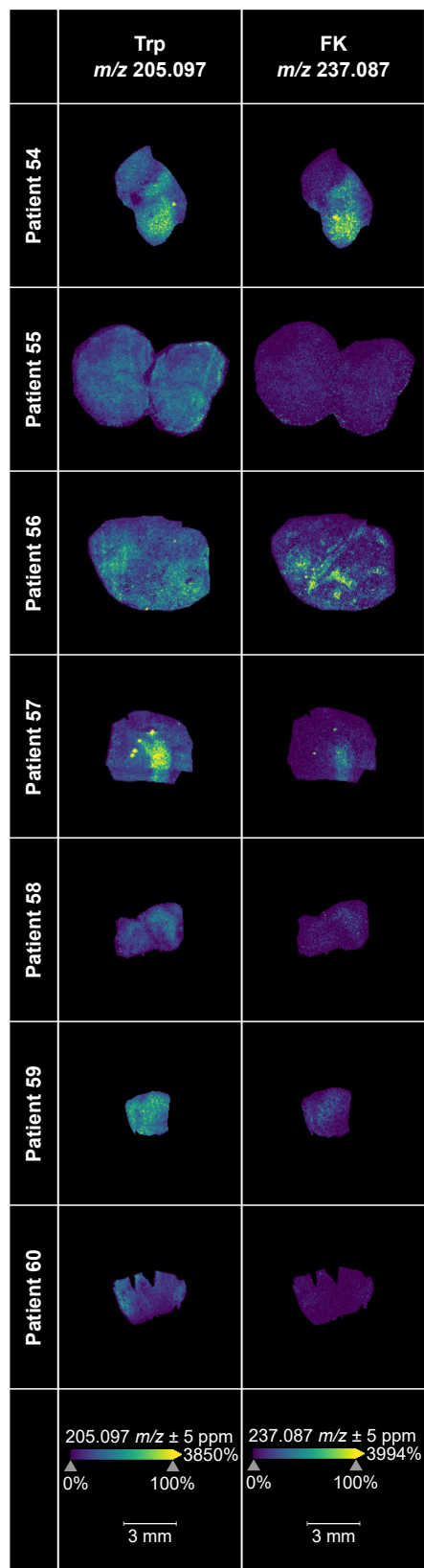ayed are Trp-D5 normalized ion density maps of Trp (m/z 205.097) and FK (m/z 237.087). Trp and FK were measured using a FT-ICR MS in positive ion mode at a raster size of 50 µm. Slides shown contain preparation artefacts. Annotations of HE-stainings: black: necrosis; red: highly vascularized tumor tissue or blood; yellow: artefacts.

(B) MALDI MSI of Trp and FK distribution in human glioblastoma samples (n = 7). Displayed are Trp-D5 normalized ion density maps of Trp (m/z 205.097) and FK (m/z 237.087). Trp and FK were measured using a FT-ICR MS in positive ion mode at a raster size of 50 µm. Samples without available adjacent HE-stained slide are shown.

See also Table S16.

Abbreviations: FK: *N*-formylkynurenine; FT-ICR MS: Fourier-transform ion cyclotron resonance mass spectrometer; HE: hematoxylin-and-eosin; MSI: mass spectrometry imaging; Trp: tryptophan; Trp-D5: deuterated tryptophan.

A

B
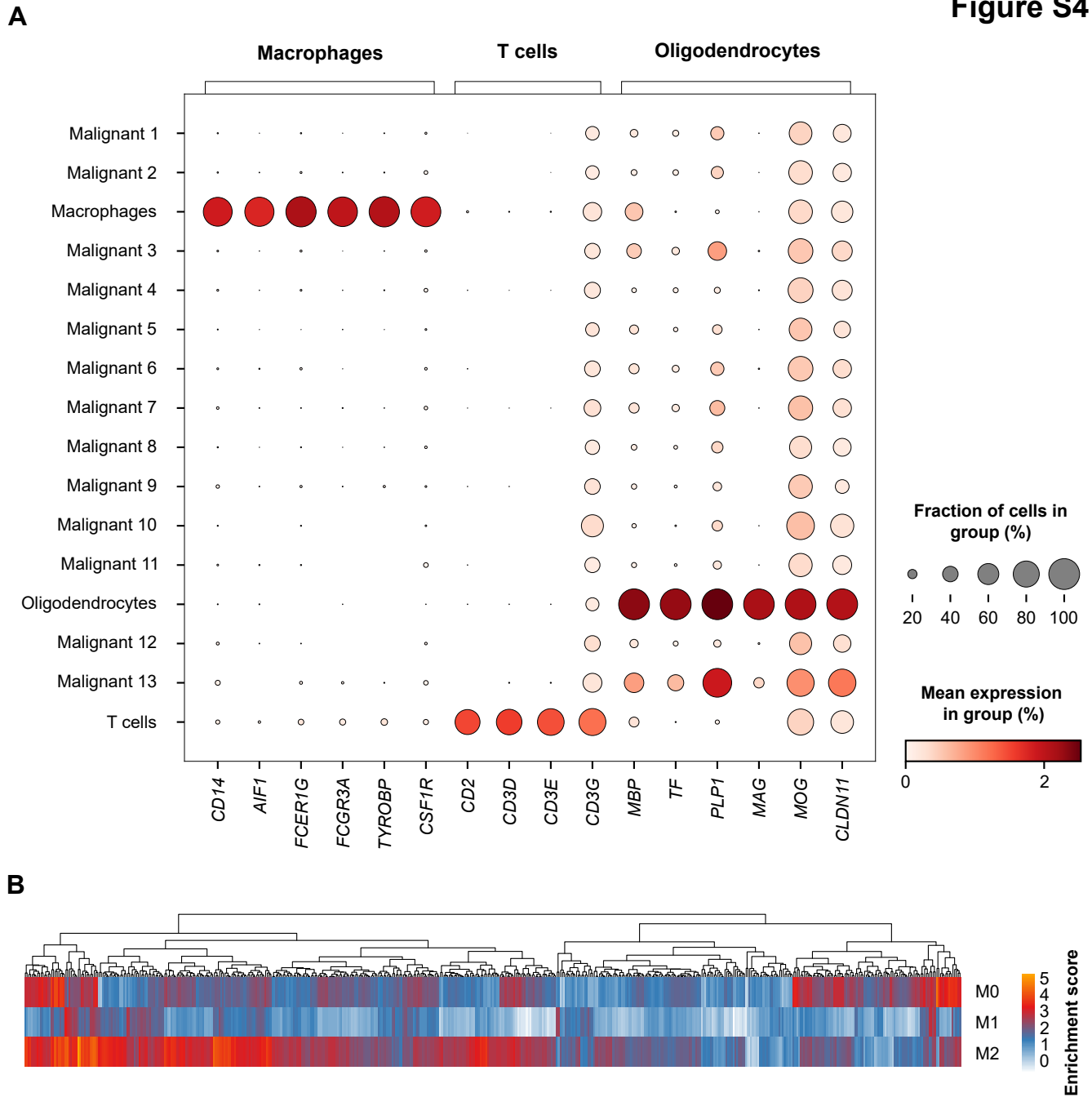
80 **Figure S4. Expression of marker genes used to characterize the non-malignant cell populations,**

81 **related to Figure 6.**

82 **(A)** Bubble plot heatmap showing the mean expression of the select marker genes [7] used to characterize

83 the non-malignant Louvain cell clusters of the scRNA-seq dataset (GSE131928). The red color intensity is

84 directly proportional to the expression level of a marker gene in a specific cell population. The size of the

85 circle denotes the fraction of cells expressing the marker gene.

86 **(B)** Heatmap showing the BPA scores of the macrophage signatures of the subsets M0, M1 and M2 in the

87 scRNA-seq dataset (GSE131928).

88 Abbreviation: BPA: biological process activity; scRNA-seq: single cell RNA-sequencing.

89   **Table S1. Transition parameters for Trp, related to Material and Methods.**

90   Transition parameters used to measure Trp. The ion masses of parent and products after fragmentation are

91   given, together with their respective collision energy (CE) values and the S-lens radio frequency levels. For

92   each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	434.26	126.13	29	172
TMT® 127	434.26	127.13	29	172
TMT® 128	434.26	128.13	29	172
TMT® 129	434.26	129.14	29	172
TMT® 130	434.26	130.14	29	172
TMT® 131	434.26	131.14	29	172
Fragment 188	434.26	188.07	28	172
Abbreviations: CE: collision energy; TMT®: tandem mass tag; Trp: tryptophan.				

93

94   **Table S2. Transition parameters for Kyn, related to Material and Methods.**

95   Transition parameters used to measure Kyn. The ion masses of parent and products after fragmentation are

96   given, together with their respective collision energy (CE) values and the S-lens radio frequency levels. For

97   each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	438.25	126.13	35	173
TMT® 127	438.25	127.13	35	173
TMT® 128	438.25	128.13	35	173
TMT® 129	438.25	129.14	35	173
TMT® 130	438.25	130.14	35	173
TMT® 131	438.25	131.14	35	173

Fragment 146	438.25	146.06	28	173
Abbreviations: CE: collision energy; Kyn: kynurenine; TMT®: tandem mass tag.				

Table S3. Transition parameters for FK, related to Material and Methods.

Transition parameters used to measure FK. The ion masses of parent and products after fragmentation are given, together with their respective collision energy (CE) values and the S-lens radio frequency levels. For each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	466.25	126.13	30	150
TMT® 127	466.25	127.13	30	150
TMT® 128	466.25	128.13	30	150
TMT® 129	466.25	129.14	30	150
TMT® 130	466.25	130.14	30	150
TMT® 131	466.25	131.14	30	150
Fragment 174	466.25	174.05	30	150
Abbreviations: CE: collision energy; *N*-formylkynurenine; TMT®: tandem mass tag.				

Table S4. Transition parameters for OH-Trp, related to Material and Methods.

Transition parameters used to measure OH-Trp. The ion masses of parent and products after fragmentation are given, together with their respective collision energy (CE) values and the S-lens radio frequency levels. For each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	450.25	126.13	30	150
TMT® 127	450.25	127.13	30	150
TMT® 128	450.25	128.13	30	150

Analyte				
TMT® 129	450.25	129.14	30	150
TMT® 130	450.25	130.14	30	150
TMT® 131	450.25	131.14	30	150
Fragment 175	450.25	175.08	30	150
Abbreviations: CE: collision energy; OH-Trp: hydroxy-tryptophan; TMT®: tandem mass tag.				

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Table S5. Transition parameters for OH-Kyn, related to Material and Methods.

Transition parameters used to measure OH-Kyn. The ion masses of parent and products after fragmentation

are given, together with their respective collision energy (CE) values and the S-lens radio frequency levels.

For each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	342.21	126.13	35	133
TMT® 127	342.21	127.13	35	133
TMT® 128	342.21	128.13	35	133
TMT® 129	342.21	129.14	35	133
TMT® 130	342.21	130.14	35	133
TMT® 131	342.21	131.14	35	133
Fragment 190	342.21	190.05	25	133
Abbreviations: CE: collision energy; OH-Kyn: hydroxy-kynurenine; TMT®: tandem mass tag.				

113

Table S6. Transition parameters for AA, related to Material and Methods.

Transition parameters used to measure AA. The ion masses of parent and products after fragmentation are

given, together with their respective collision energy (CE) values and the S-lens radio frequency levels. For

each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens

Analyte	Parent	Product	CE	S-lens
TMT® 126	367.22	126.13	26	138
TMT® 127	367.22	127.13	26	138
TMT® 128	367.22	128.13	26	138
TMT® 129	367.22	129.14	26	138
TMT® 130	367.22	130.14	26	138
TMT® 131	367.22	131.14	26	138
Fragment 120	367.22	120.04	32	138
Abbreviations: AA: anthranilic acid; CE: collision energy; TMT®: tandem mass tag.				

Table S7. Transition parameters for OH-AA, related to Material and Methods.

Transition parameters used to measure OH-AA. The ion masses of parent and products after fragmentation

are given, together with their respective collision energy (CE) values and the S-lens radio frequency levels.

For each metabolite the six TMT® reporter ions and a metabolite specific fragment were monitored.

Analyte	Parent	Product	CE	S-lens
TMT® 126	383.21	126.13	26	142
TMT® 127	383.21	127.13	26	142
TMT® 128	383.21	128.13	26	142
TMT® 129	383.21	129.14	26	142
TMT® 130	383.21	130.14	26	142
TMT® 131	383.21	131.14	26	142
Fragment 136	383.21	136.04	27	142
Abbreviations: CE: collision energy; OH-AA: hydroxy-anthranilic acid; TMT®: tandem mass tag.				

124 **Table S8. CV values of the MS/MS measurements of Trp and its metabolites, related to Material and**

125 **Methods.**

126 Representation of the mean coefficient of variation (CV) values and their SD (SD (CV)) for the different

127 metabolites in the MS/MS measurements.

Metabolite	CV mean [%]	SD (CV) [%]
Trp	2.7	1.6
OH-Trp	2.4	2.0
FK	1.8	1.6
Kyn	2.4	1.6
AA	2.9	3.5
OH-Kyn	3.8	3.6
OH-AA	5.8	4.4
Abbreviations: AA: anthranilic acid; CV: coefficient of variation; FK: *N*-formylkynurenine; Kyn: kynurenine; MS/MS: tandem mass spectrometry; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; SD: standard deviation; Trp: tryptophan.		

128

129 **Table S9. Overview of the characteristics of the study cohort for serum metabolite measurements,**

130 **related to Figure 3.**

	Patients	**Controls**
n	43	43
Median age in years	54.11	54.34
Sex	F: 19 M: 24	F: 19 M: 24
IDH status	WT: 33 Unknown: 10	
Prior bevacizumab treatment	yes: 29 no: 14	
Abbreviations: F: female; IDH: isocitrate dehydrogenase; M: male; WT: wild type.		

131

Table S10. Detailed patient characteristics of the study cohort for serum metabolite measurements, related to Figure 3.

Patient number	MGMT promotor status	EGFR amplifi-cation	Evidence for PTEN loss	Timespan x between last surgery and blood draw [years]	Previous RT and TMZ treat-ment	Previous second line therapy with nitrosourea	Previ-ous ICB	Bevacizumab treatment before blood draw
1	n.a.	n.a.	n.a.	$1.0 < x \leq 1.5$	yes	no	no	yes
2	methylated	n.a.	n.a.	$1.5 < x \leq 2.5$	yes	yes	yes	yes
3	n.a.	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	no	yes	no
4	n.a.	n.a.	n.a.	$1.5 < x \leq 2.5$	yes	yes	no	yes
5	unmethylated	not amplified	yes	$0.5 < x \leq 1.0$	yes	no	no	yes
6	n.a.	n.a.	n.a.	$1.5 < x \leq 2.5$	yes	yes	no	yes
7	n.a.	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	yes	no	yes
8	unmethylated	amplified	no	$0.5 < x \leq 1.0$	yes	yes	yes	yes
9	n.a.	n.a.	no	$1.0 < x \leq 1.5$	yes	no	no	yes
10	methylated	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	no	no	yes
11	methylated	not amplified	n.a.	$0.5 < x \leq 1.0$	yes	no	no	yes
12	unmethylated	not amplified	yes	$1.0 < x \leq 1.5$	yes	yes	no	yes
13	n.a.	n.a.	n.a.	$1.0 < x \leq 1.5$	yes	no	no	yes
14	unmethylated	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	no	no	yes
15	n.a.	n.a.	n.a.	$1.5 < x \leq 2.5$	yes	yes	no	yes
16	unmethylated	not amplified	yes	$0.5 < x \leq 1.0$	yes	yes	no	yes
17	unmethylated	amplified	yes	$1.0 < x \leq 1.5$	yes	yes	no	yes
18	unmethylated	not amplified	n.a.	$0.5 < x \leq 1.0$	yes	no	no	yes
19	methylated	amplified	no	$1.0 < x \leq 1.5$	yes	yes	no	no
20	unmethylated	not amplified	n.a.	$1.0 < x \leq 1.5$	yes	no	no	no

21	unmethylated	n.a.	n.a.	$0.5 < x \leq 1.0$	yes	yes	no	no
22	methylated	amplified	n.a.	$1.0 < x \leq 1.5$	yes	yes	no	yes
23	n.a.	n.a.	n.a.	$0 < x \leq 0.5$	yes	no	no	yes
24	n.a.	n.a.	n.a.	$1.0 < x \leq 1.5$	yes	yes	no	yes
25	methylated	n.a.	no	$0 < x \leq 0.5$	yes	yes	no	yes
26	methylated	not amplified	yes	$1.5 < x \leq 2.5$	yes	no	no	yes
27	unmethylated	n.a.	yes	$1.0 < x \leq 1.5$	yes	no	no	yes
28	unmethylated	n.a.	n.a.	$1.0 < x \leq 1.5$	only RT	no	no	no
29	n.a.	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	yes	no	yes
30	methylated	n.a.	n.a.	$0 < x \leq 0.5$	yes	no	no	no
31	n.a.	n.a.	n.a.	$1.5 < x \leq 2.5$	yes	no	no	no
32	methylated	n.a.	n.a.	$2.5 < x \leq 6.5$	yes	no	no	yes
33	methylated	not amplified	yes	$0 < x \leq 0.5$	yes	no	no	no
34	methylated	n.a.	n.a.	$0 < x \leq 0.5$	yes	yes	no	no
35	unmethylated	amplified	yes	$0 < x \leq 0.5$	yes	no	no	no
36	methylated	not amplified	n.a.	$0.5 < x \leq 1.0$	yes	no	no	no
37	n.a.	n.a.	no	$1.0 < x \leq 1.5$	yes	yes	no	yes
38	methylated	amplified	yes	$0 < x \leq 0.5$	yes	yes	no	yes
39	methylated	not amplified	yes	$0 < x \leq 0.5$	yes	no	no	no
40	methylated	n.a.	n.a.	$0 < x \leq 0.5$	yes	no	no	no
41	unmethylated	not amplified	yes	$0 < x \leq 0.5$	yes	yes	no	yes
42	n.a.	n.a.	n.a.	$0.5 < x \leq 1.0$	yes	no	no	no
43	unmethylated	not amplified	no	$0 < x \leq 0.5$	yes	no	no	yes

Abbreviations: EGFR: epidermal growth factor receptor; ICB: immune checkpoint blockade; MGMT: O^6-methylguanine-DNA methyltransferase; n.a.: not applicable, clinical data not available; PTEN: phosphatase and tensin homolog; RT: radiotherapy; TMZ: temozolomide.

134

Table S11. Prior bevacizumab treatment does not influence Trp metabolite levels in serum, related to Figure 3.

Representation of the mean metabolite abundance in the sera of glioblastoma patient groups having received prior therapy with bevacizumab or not relative to reference sample depicted as $\log_2$Ratio. Group comparisons were performed using a two-tailed unpaired Student's t test, p values are given as numbers.

Metabolite	Bevacizumab treatment prior to blood draw ($n = 29$)	No bevacizumab treatment prior to blood draw ($n = 14$)	P value
Trp	-0.23929	-0.171806	0.541873
OH-Trp	-0.20438	-0.147861	0.590434
FK	-0.18088	-0.128576	0.623908
Kyn	-0.30523	-0.296833	0.943081
AA	-0.19042	-0.203277	0.861635
OH-Kyn	0.54967	0.431191	0.376339
OH-AA	-0.21835	-0.074910	0.365114
Abbreviations: AA: anthranilic acid; FK: *N*-formylkynurenine; Kyn: kynurenine; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; Trp: tryptophan.			

Table S12. MGMT promotor methylation status does not influence Trp metabolite levels in serum, related to Figure 3.

Representation of the mean metabolite abundance in the sera of glioblastoma patient groups with a methylated or unmethylated MGMT promotor relative to reference sample depicted as $\log_2$Ratio. Group comparisons were performed using a two-tailed unpaired Student's t test, p values are given as numbers.

Metabolite	methylated MGMT promotor ($n = 15$)	unmethylated MGMT promotor ($n = 14$)	P value
Trp	-0.307804	-0.23550	0.606817
OH-Trp	-0.290598	-0.17417	0.379436
FK	-0.244386	-0.19412	0.710315
Kyn	-0.318028	-0.36445	0.737927

AA	-0.269437	-0.19970	0.408155
OH-Kyn	0.552551	0.42303	0.412657
OH-AA	-0.165172	-0.06865	0.568299
Abbreviations: AA: anthranilic acid; FK: *N*-formylkynurenine; Kyn: kynurenine; MGMT: O^6-methylguanine-DNA methyltransferase; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; Trp: tryptophan.			

146

Table S13. EGFR amplification status does not influence Trp metabolite levels in serum, related to Figure 3.

Representation of the mean metabolite abundance in the sera of glioblastoma patient groups with EGFR amplification or not relative to reference sample depicted as $\log_2$Ratio. Group comparisons were performed using a two-tailed unpaired Student's t test, p values are given as numbers.

Metabolite	EGFR amplification ($n = 6$)	No EGFR amplification ($n = 12$)	P value
Trp	-0.284102	-0.36048	0.682655
OH-Trp	-0.190309	-0.33811	0.413630
FK	-0.226813	-0.31161	0.635678
Kyn	-0.333124	-0.42910	0.589516
AA	-0.264457	-0.30276	0.730573
OH-Kyn	0.526196	0.34979	0.392807
OH-AA	-0.264156	-0.17875	0.709499
Abbreviations: AA: anthranilic acid; EGFR: epidermal growth factor receptor; FK: *N*-formylkynurenine; Kyn: kynurenine; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; Trp: tryptophan.			

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Table S14. PTEN loss does not influence Trp metabolite levels in serum, related to Figure 3.

Representation of the mean metabolite abundance in the sera of glioblastoma patient groups with evidence for PTEN loss or not relative to reference sample depicted as $\log_2$Ratio. Group comparisons were performed using a two-tailed unpaired Student's t test, p values are given as numbers.

Metabolite	Evidence for PTEN loss ($n = 11$)	No evidence for PTEN loss ($n = 6$)	P value
Trp	-0.395536	-0.24675	0.429786
OH-Trp	-0.333291	-0.16009	0.360572
FK	-0.350850	-0.15310	0.272037
Kyn	-0.464462	-0.19459	0.134742
AA	-0.300172	-0.21243	0.447791
OH-Kyn	0.440620	0.64832	0.248894
OH-AA	-0.117671	-0.51601	0.064070
Abbreviations: AA: anthranilic acid; FK: *N*-formylkynurenine; Kyn: kynurenine; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; PTEN: phosphatase and tensin homolog; Trp: tryptophan.			

157

158 **Table S15. Reduction of metabolite levels in glioblastoma patient sera, related to Figure 4.**

159 Representation of the $\log_2$ fold change (FC) values of metabolite level reduction in patient versus age- and

160 sex-matched control sera as depicted in Figure 4B and the corresponding reduction of metabolite levels

161 given in %.

Metabolite	Log$_2$ (FC patients vs controls)	FC (patients vs controls)	Metabolite level reduction in patients vs controls (%)	P value
Trp	-0.300	0.812	18.8	0.000261358
OH-Trp	-0.268	0.830	17.0	0.000460516
FK	-0.273	0.827	17.3	0.000558024
Kyn	-0.219	0.859	14.1	0.010150130
AA	-0.164	0.892	10.8	0.007304935
OH-Kyn	-0.042	0.971	2.9	0.686404933
OH-AA	-0.007	0.995	0.5	0.952159761
Abbreviations: AA: anthranilic acid; FC: fold change; FK: *N*-formylkynurenine; Kyn: kynurenine; OH-AA: hydroxy-anthranilic acid; OH-Kyn: hydroxy-kynurenine; OH-Trp: hydroxy-tryptophan; Trp: tryptophan; vs: versus.				

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163   **Table S16. Characteristics of tumor tissue samples analyzed with MALDI MSI, related to Figure 5**

164   **and Figure S3.**

Patient number	Primary glioblastoma	IDH status	MGMT promotor status	EGFR amplification	Evidence for PTEN loss
44	primary	WT	methylated	amplified	n.a.
45	primary	WT	methylated	not amplified	n.a.
46	primary	WT	unmethylated	not amplified	n.a.
47	primary	WT	unmethylated	not amplified	no
48	primary	WT	unmethylated	amplified	no
49	primary	WT	methylated	not amplified	n.a.
50	primary	WT	unmethylated	not amplified	no
51	primary	WT	unmethylated	not amplified	no
52	primary	WT	methylated	amplified	n.a.
53	primary	WT	unmethylated	amplified	yes
54	primary	WT	unmethylated	n.a.	yes
55	n.a.	WT	unmethylated	not amplified	no
56	primary	WT	methylated	not amplified	no
57	n.a.	n.a.	n.a.	n.a.	n.a.
58	primary	WT	methylated	not amplified	no
59	primary	n.a.	n.a.	not amplified	n.a.
60	primary	WT	methylated	not amplified	no
Abbreviations: EGFR: epidermal growth factor receptor; IDH: isocitrate dehydrogenase; MALDI MSI: MALDI mass spectrometry imaging; MGMT: O^6-methylguanine-DNA methyltransferase; n.a.: not applicable, clinical data not available; PTEN: phosphatase and tensin homolog; WT: wild type.					

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166 **Table S17. AHR activity associates with worse overall survival in glioblastoma patients, related to**

167 **Figure 6.**

168 Univariate and multivariate cox regression analysis of the effect of AHR activity and age at diagnosis on

169 overall survival in glioblastoma patients in the TCGA database.

Univariate analysis				
	Coef	**Se.coef.**	**Z**	**P value**
Age at diagnosis	9.60E-05	2.44E-05	3.930614673	8.47E-05
AHR activity	0.190344934	0.092285956	2.06255581	0.03915485
Multivariate analysis				
	Coef	**Se.coef.**	**Z**	**P value**
Age at diagnosis	9.75E-05	2.49E-05	3.912168313	9.15E-05
AHR activity	0.187806232	0.092976393	2.019934585	0.043390173
Abbreviations: AHR: aryl hydrocarbon receptor; Coef: coefficient; Se.coef.: Standard error of the coefficient; Z: Wald test z score, which is the coefficient divided by its standard error.				

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