

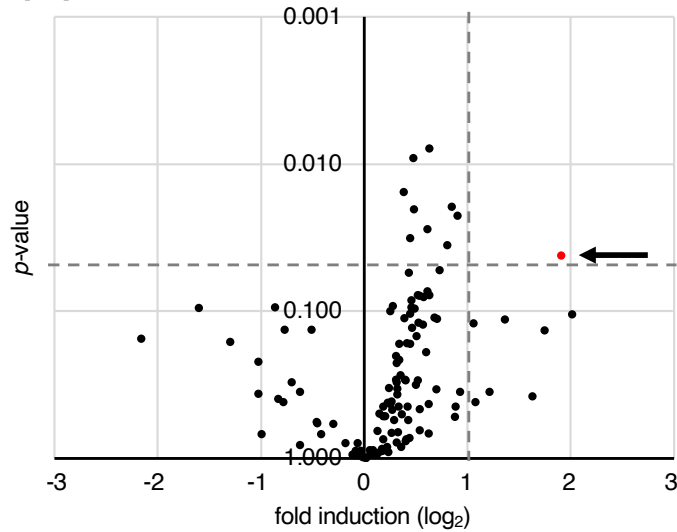
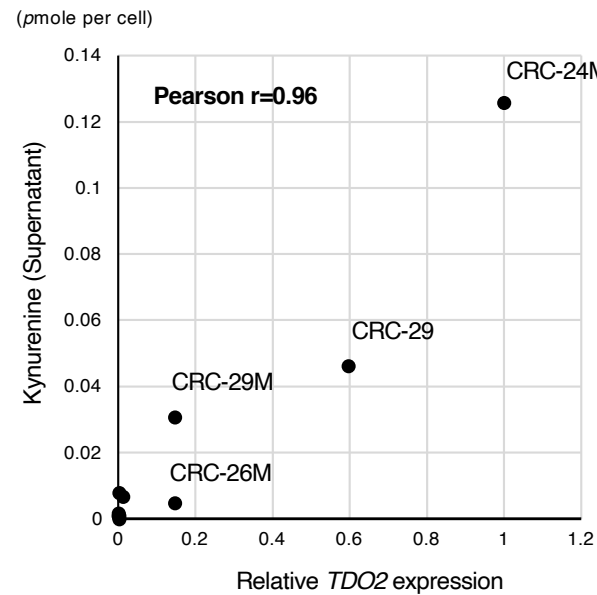
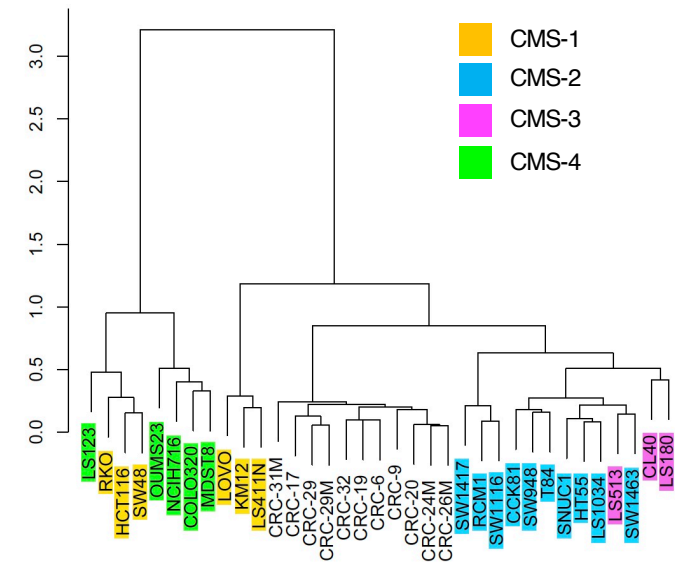
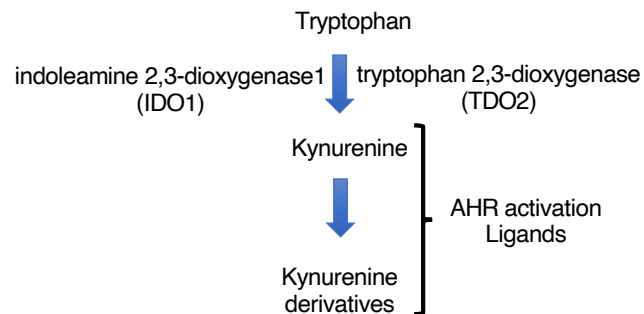
**(A)**

ID	metastasis	meta site	sample	age	sex	location	TNM	Stage	Differentiation
CRC-9	-	-	primary	57	M	Rectum	T3N0M0	IIA	Mod
CRC-17	-	-	primary	76	M	Sigmoid	T3N2M0	IIIC	Well to mod
CRC-19	-	-	primary	48	F	Rectum	T2N0M0	I	Well
CRC-20	-	-	primary	60	M	Left	T3N0M0	IIA	Mod
CRC-6	+	liver	primary	41	F	Right	T4N2M1	IV	Mod
CRC-29	+	liver	primary	56	F	Sigmoid	T3N0M1a	IVA	Well
CRC-32	+	liver	primary	53	F	Rectum	T4aN2bM1a	IVA	Mod to well
<b>CRC-29M</b>	<b>+</b>	<b>liver</b>	<b>liver meta</b>	<b>56</b>	<b>F</b>	-	-	-	-
CRC-31M	+	liver	liver meta	80	F	-	-	-	-
<b>CRC-24M</b>	<b>+</b>	<b>liver</b>	<b>liver meta</b>	<b>56</b>	<b>M</b>	-	-	-	-
CRC-26M	+	liver	liver meta	79	M	-	-	-	-

**(E)**

■ stopgain SNV  
■ frameshift deletion & insertion  
■ nonsynonymous SNV  
■ homologous deletion

pathway	Gene	primary (w/o liver meta)				primary (w. liver meta)			liver meta			
		CRC-9	CRC-17	CRC-19	CRC-20	CRC-6	CRC-29	CRC-32	CRC-29M	CRC-31M	CRC-24M	CRC-26M
WNT	APC	48.3 48.6	100.0		99.2	63.9 29.3	92.6	65.5 33.9	92.5	49.3		100.0
	FBXW7	100.0								49.9		
	CTNNB1				41.1							
	ARID1A										33.1	
RTK/RAS/PI3-K/AKT	KRAS	29.5		64.6		67.5						
	TSC1				48.6							
	BRAF							50.5				
p53	TP53			99.9		99.8	100.0	99.6	99.9	99.6		99.9
	ATM	47.2	99.9									
TGF- $\beta$	SMAD4											
	FGFR1											
others	NOTCH3						23.3		20.0			32.0
	NT5C2											67.0
	NTRK2									30.0		
	NTRK3							59.1				

**(B)****(D)****(F)****(C)****Figure S1**

**FIGURE S1** Systematic Screening of Charged Metabolites that are Upregulated in Metastatic Spheroids. A, Clinical data of colorectal cancer from which spheroids shown in this paper were derived. Note that only CRC-29 and CRC-29M are derived from the same patients. B, Volcano plot of the relative ratio and  $p$ -value of quantified value of anionic metabolites from non-metastatic spheroids vs. metastatic spheroids. Amounts of the metabolites were measured by CE-TOF-MS in biological triplicate. The position of Octanoate is shown by the arrow. C, Explanation for the TDO2/IDO1-Kynurenine pathway. D, Correlation between Kynurenine production and *TDO2* expression. Association of L-kynurenine in supernatants and *TDO2* shown Figures 1C and 1D are presented. Pearson correlation coefficient was shown. E, Genomic alterations detected in the spheroids shown in (A). Percentages of allele frequency of the mutation are also shown. F, Clustering analyses of cancer spheroids and cell lines based on RNA-seq data. Prediction of CMS for the CRC spheroid cells by hierarchical clustering. The spheroid cells used in this study are stratified by hierarchical clustering together with the reference cells with pre-determined CMS subtypes <sup>1</sup>. Hierarchical clustering is performed using Ward's method. CMS subtypes of each cell line are shown by the indicated colors.

1 Linnekamp JF, Hooff SRV, Prasetyanti PR, et al. Consensus molecular subtypes of colorectal cancer are recapitulated in in vitro and in vivo models. *Cell Death Differ.* 2018; 25: 616-633.