

Supplementary Information

Free D-amino acids produced by commensal bacteria in the colonic lumen

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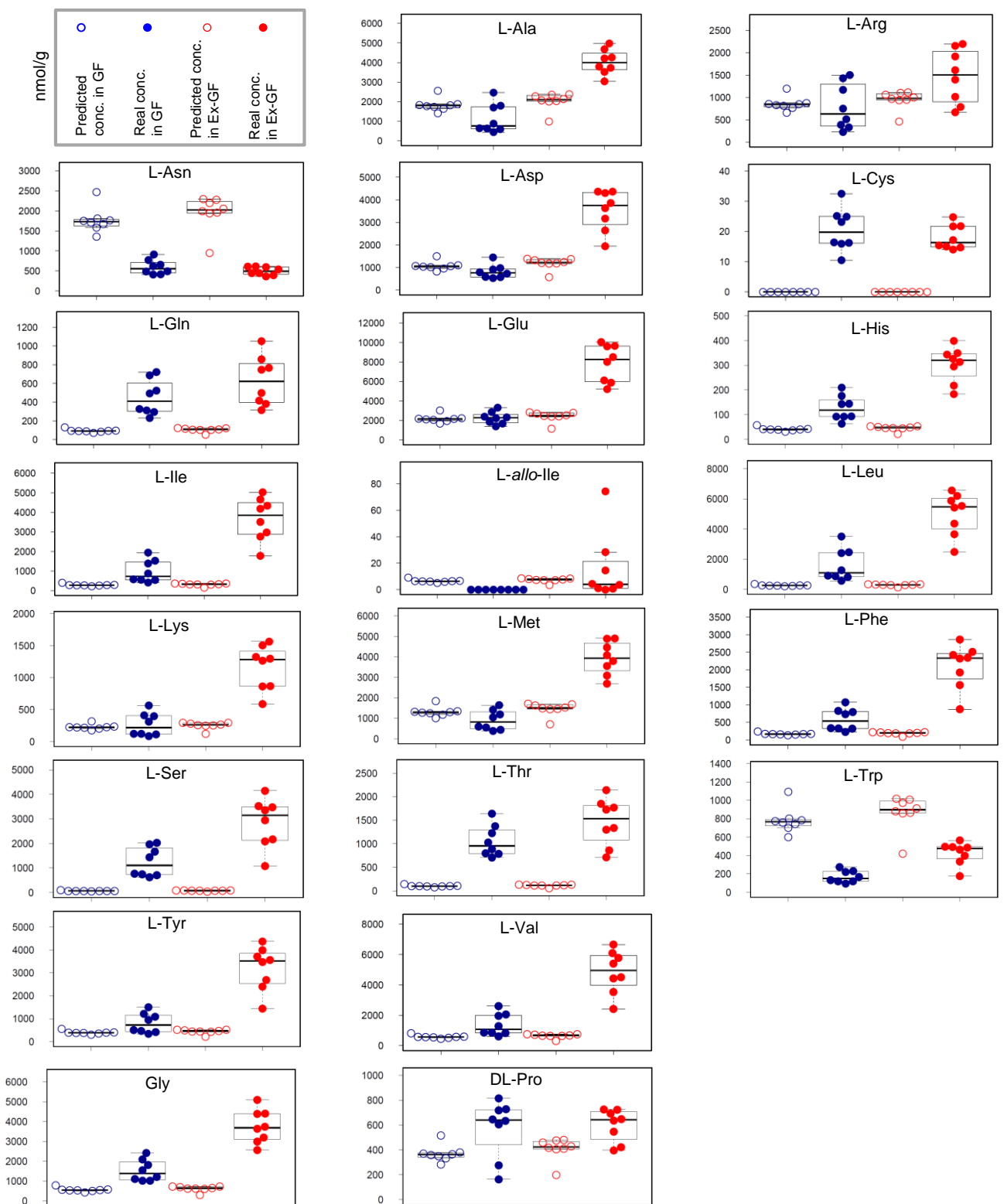
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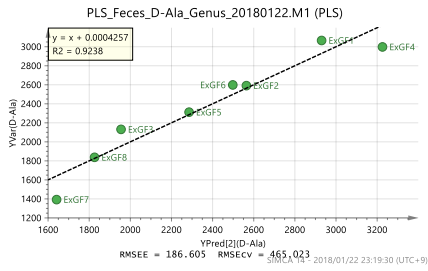


Supplementary Fig. S2. Quantitative comparison of L-amino acids between actual concentration in colonic content and estimated concentration when pellets reached the colon in GF mice and Ex-GF mice (nmol/g of feces). Estimated values of pellet L-AAs in the colonic lumen were calculated as follows: estimated values of D-amino acids (nmol/g) = measured concentration in pellet/[pellet solid content (%)/solid content of colonic content (%)]. L-*allo*-Thr was not detected from both colonic content and pellet.

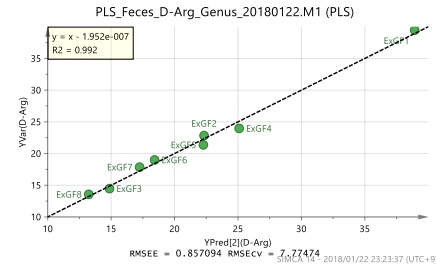


Supplementary Fig. S3. Relative abundances of colonic bacteria in Ex-GF mice by 16S rRNA gene amplicon sequencing (Class, Order, and Family). Bacteria with less than 0.1% abundance were included in others. Data of phylum and genus levels are shown in Fig. 2a.

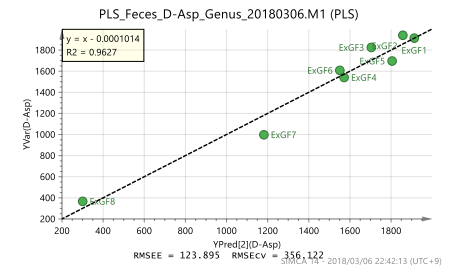
D-Ala



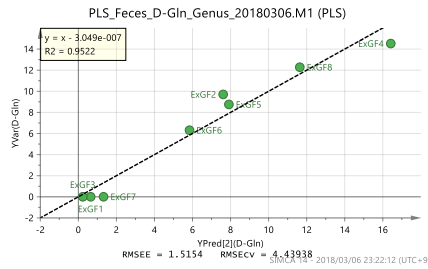
D-Arg



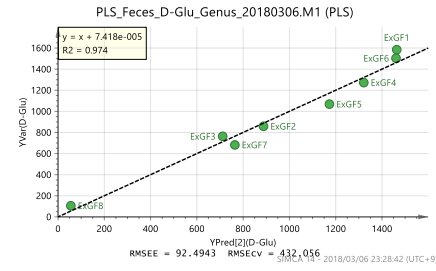
D-Asp



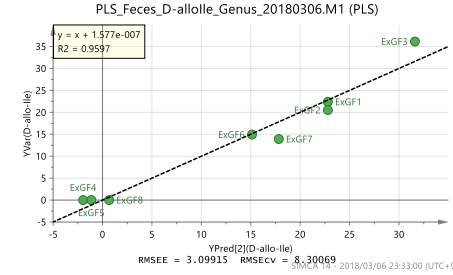
D-Gln



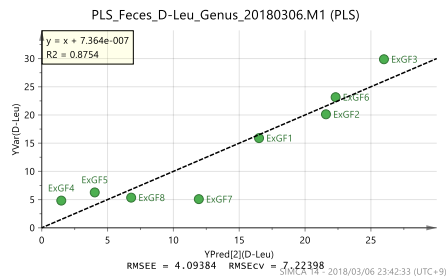
D-Glu



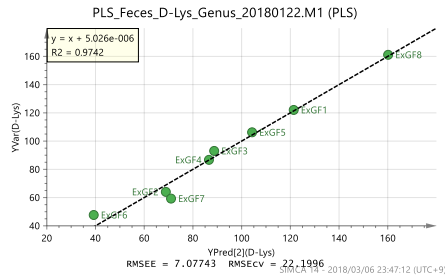
D-allo-Ile



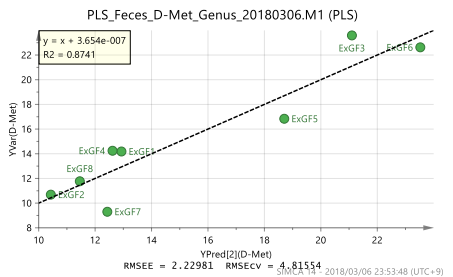
D-Leu



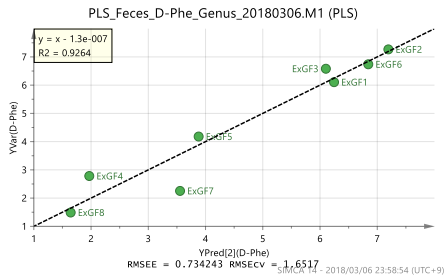
D-Lys



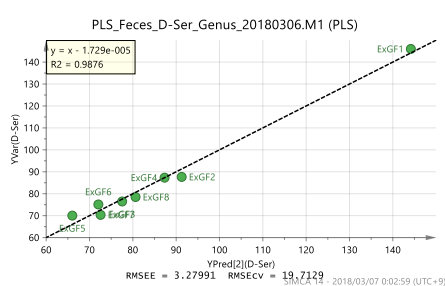
D-Met



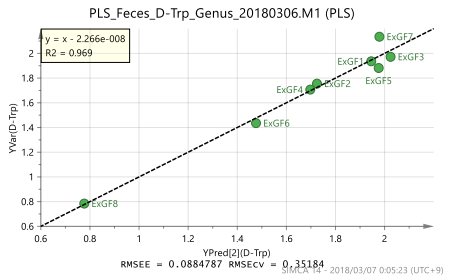
D-Phe



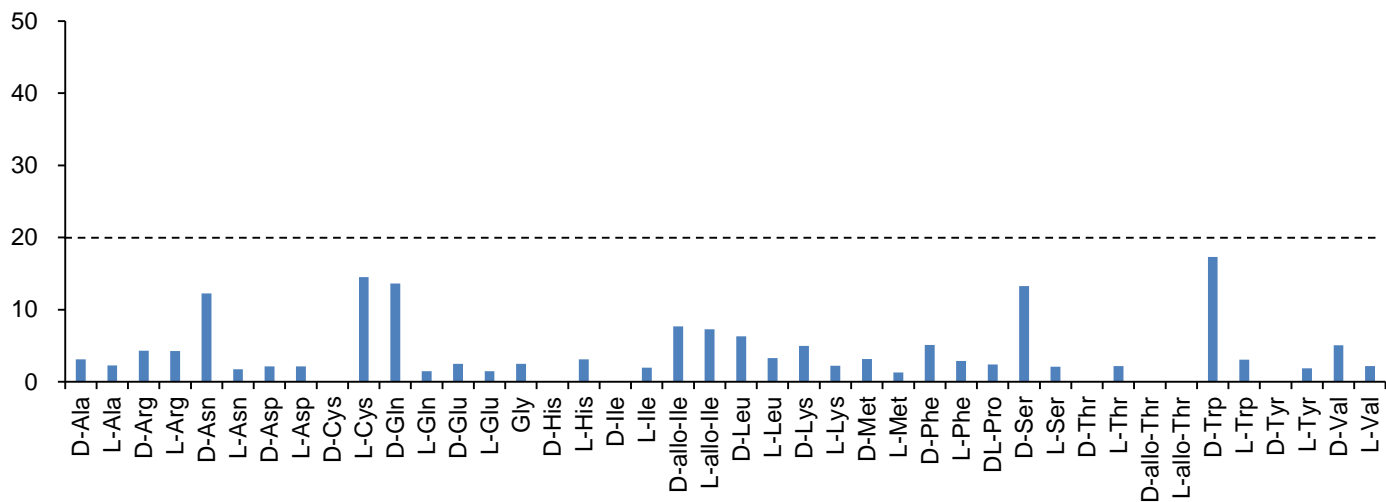
D-Ser



D-Trp



Supplementary Fig. S4. The PLS regression lines of 12 D-amino acids that were produced by intestinal bacteria



Supplementary Fig. S5. Repeatability of peak area (% Relative standard deviation)

Extract of feces from conventional mice (n = 8) were mixed to prepare the pooled quality check samples. Thirty-five chiral AAs were detected from feces, and good repeatability (RSD of the peak area ratio of AAs to ¹³C₆-L-Phe < 20%) was observed in all chiral amino acids.

Supplementary Table S1. Concentration of D-amino acid and L-amino acids in the pellet (nmol/g)

	Mean	SD		Mean	SD
D-Ala	286.3	9.4	L-Ala	5772.3	288.6
D-Arg	8.2	0.5	L-Arg	2695.1	15.4
D-Asn	22.1	1.3	L-Asn	5555.2	53.5
D-Asp	366.5	17.8	L-Asp	3369.6	130.4
D-Cys	ND		L-Cys	ND	
D-Gln	ND		L-Gln	296.4	9.6
D-Glu	90.7	0.4	L-Glu	6849.4	42.7
D-His	ND		L-His	130.0	8.1
D-Ile	ND		L-Ile	895.1	49.8
D- <i>allo</i> -Ile	4.4	0.3	L- <i>allo</i> -Ile	20.6	1.6
D-Leu	9.4	0.3	L-Leu	832.8	53.0
D-Lys	7.2	1.1	L-Lys	716.7	49.2
D-Met	4510.3	452.4	L-Met	4138.4	401.5
D-Phe	7.8	0.2	L-Phe	540.0	21.8
D-Ser	ND		L-Ser	205.9	41.5
D-Thr	ND		L-Thr	316.8	14.7
D- <i>allo</i> -Thr	ND		L- <i>allo</i> -Thr	ND	
D-Trp	4.5	0.3	L-Trp	2466.2	139.1
D-Tyr	ND		L-Tyr	1253.8	50.1
D-Val	19.3	0.8	L-Val	1826.7	77.4
			DL-Pro	1162.8	38.2
			Gly	1747.7	64.6

ND: Not detected

Pellets were measured in triplicates

Supplementary Table S2. Solid contents of mice colonic contents

Mice	Solid %	Mice	Solid %
GF1	29.656	ExGF1	32.886
GF2	28.766	ExGF2	33.481
GF3	29.412	ExGF3	38.250
GF4	22.804	ExGF4	36.862
GF5	30.407	ExGF5	34.564
GF6	41.477	ExGF6	15.909
GF7	26.625	ExGF7	38.596
GF8	28.105	ExGF8	32.538
Pellet	93.451		

Supplementary Table S3. Intestinal absorption and bacterial production of each D-amino acid.

	Intestinal absorption	Bacterial production
D-Ala	+	+
D-Arg	weak	+
D-Asn	weak	-
D-Asp	+	+
D-Cys	?	-
D-Gln	?	+
D-Glu	+	+
D-His	?	weak
D-Ile	?	-
D- <i>allo</i> -Ile	+	+
D-Leu	+	+
D-Lys	+	+
D-Met	+	+
D-Phe	+	+
D-Ser	?	+
D-Thr	?	-
D- <i>allo</i> -Thr	?	-
D-Trp	-	+
D-Tyr	?	-
D-Val	+	-

+, positive; -, negative; weak, Quantities of absorption/production are low.; ?, absorption/production cannot be judged by this study.

Supplementary Table S6. Recovery of isotope-labeled L-amino acids (%) by the high-sensitivity LC-MS/MS chiral AA analysis

Isotope-labeled L-AAs	Recovery rate (%)
L-Ala	98.6
L-Arg	89.5
L-Asp	80.3
L-Glu	89.9
Gly	93.2
L-His	65.3
L-Ile	97.1
L-Leu	98.2
L-Lys	77.7
L-Met	93.7
L-Phe	99.2
DL-Pro	96.9
L-Ser	91.4
L-Thr	95.3
L-Tyr	98
L-Val	100

Good recovery (80–100%) was obtained for 14 D-AAs except L-His and L-Lys from feces.