

Title

Glycolysis and oxidative stress related redox pathway upregulation along the gut-liver axis by gut microbial perturbation and host response during giardiasis in C57BL/6J mouse model

Authors

Avinash V Karpe¹, Melanie L Hutton², Steven J Mileto², Meagan L James², Chris Evans², Amol B Ghodke^{3,4}, Rohan M Shah^{1,5}, Suzanne S Metcalfe¹, Jian-Wei Liu⁶, Tom Walsh⁶, Dena Lyras^{2,†}, Enzo A Palombo^{5,†}, David J Beale^{1,†,*}

¹ Land and Water, Commonwealth Scientific and Industrial Research Organization, Ecosciences Precinct, Dutton Park, QLD 4102, Australia.

² Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, VIC 3168, Australia.

³ Queensland Alliance for Agriculture and Food Innovation, Department of Horticulture, The University of Queensland, St Lucia, QLD 4072, Australia

⁴ Health and Biosecurity , Commonwealth Scientific and Industrial Research Organization, Ecosciences Precinct, Dutton Park, QLD 4102, Australia.

⁵ Department of Chemistry and Biotechnology, School of Science, Computing and Engineering Technologies, Swinburne University of Technology, Hawthorn, VIC 3122, Australia.

⁶ Land and Water, Commonwealth Scientific and Industrial Research Organization, Agricultural and Environmental Sciences Precinct, Acton, Canberra, ACT 2601, Australia

Infection analysis and mouse strain selection

The mouse strain with optimal for infection and *Giardia* colonisation among the tested strains, was selected by conducting the pilot study. During the study period of 14 days, the mice did not show visual diarrheic effects. Also, visual inspections and weight measurements (Figure 1A) indicated that the health of the mice was not drastically affected. Swiss mice showed considerable weight gains, with an average increase of 3.02 g during study period. Body weights of C57BL/6J and BALB/C mice, although fluctuate up to 8 dpi, did not increase considerably, with an average increase on 0.45 g and 0.11 g, respectively. C57BL/6J however, showed the highest response to *Giardia* infection, with the faecal *Giardia* cyst number peaking at 6 – 7 dpi (Figure 1B).

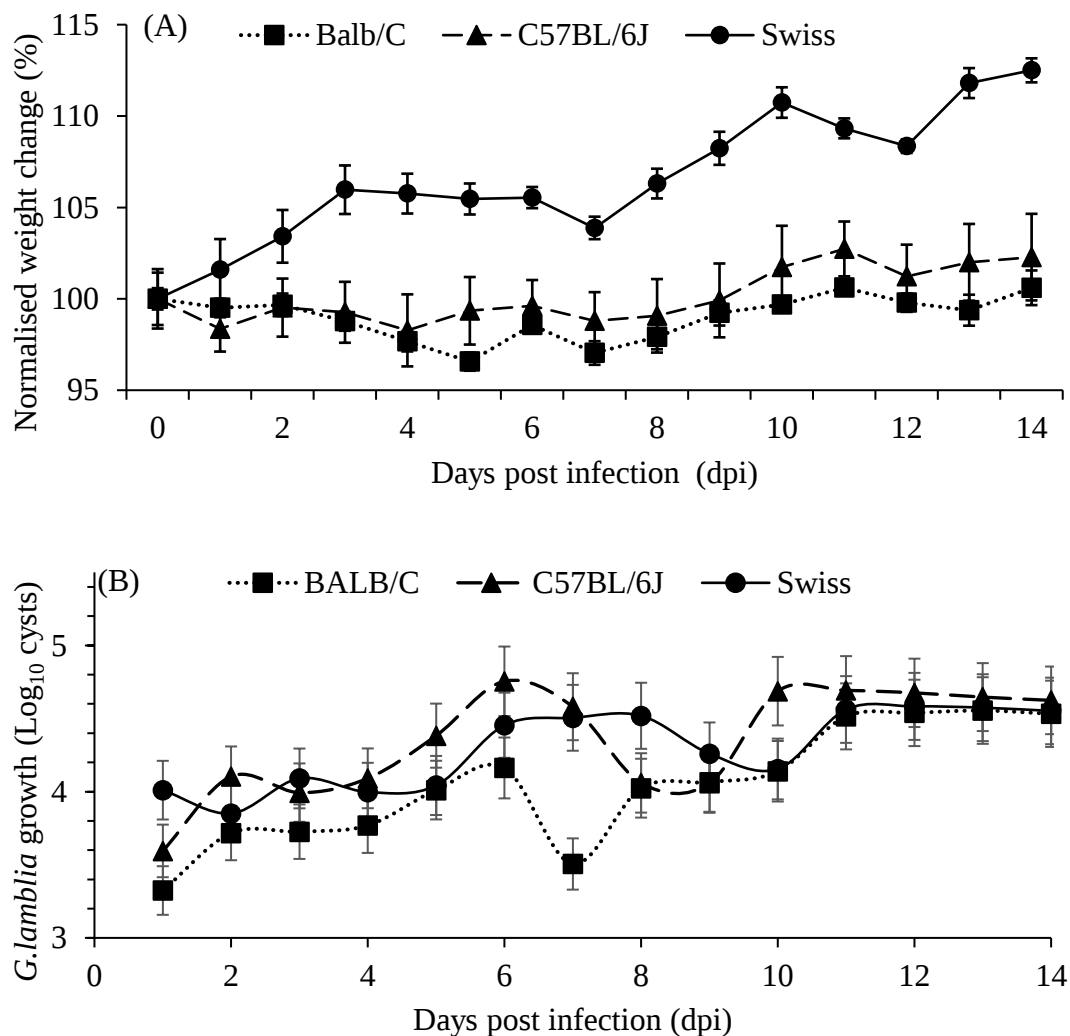


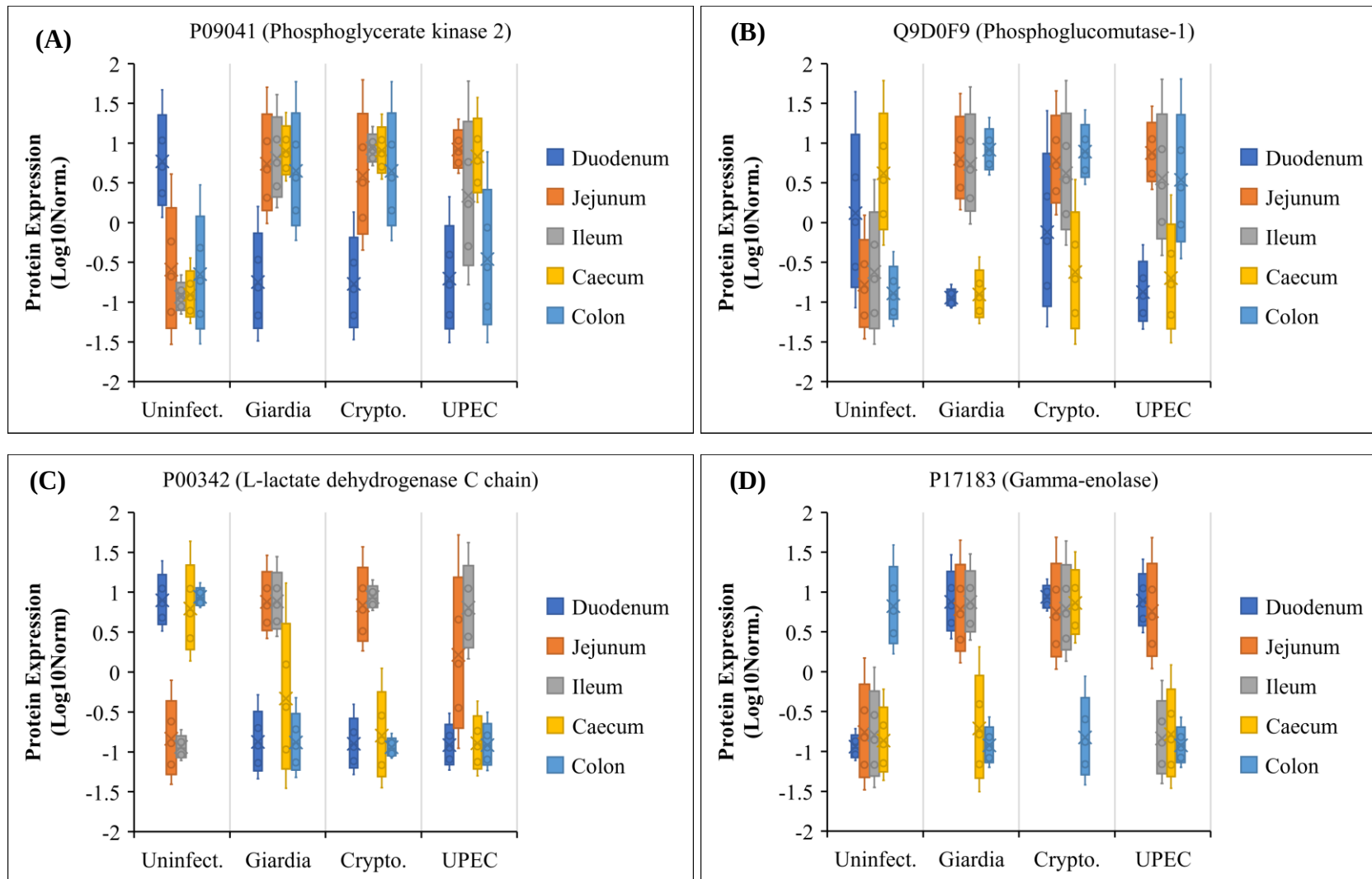
Figure S1. Growth of (A) Mice during the period of infection and (B) *G. lamblia* count during the period of infection.

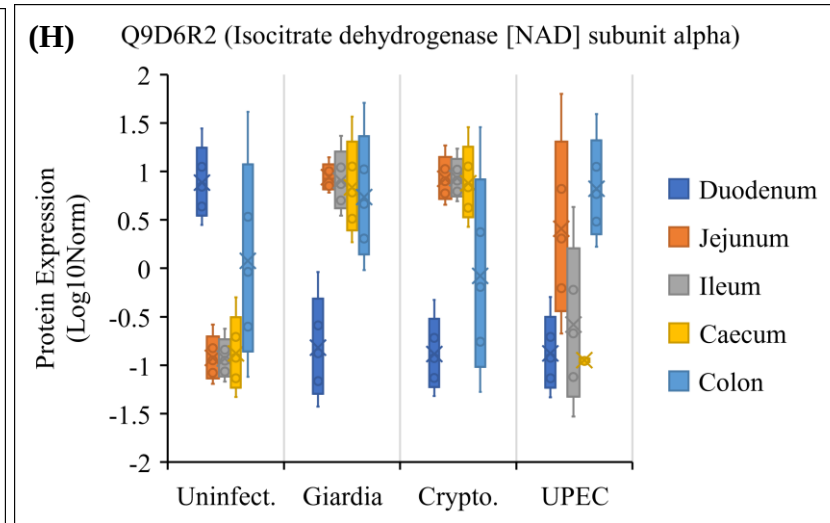
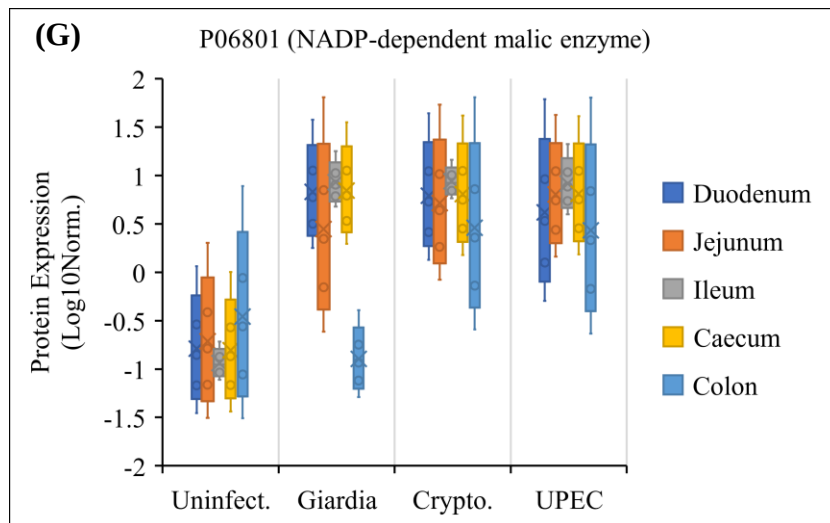
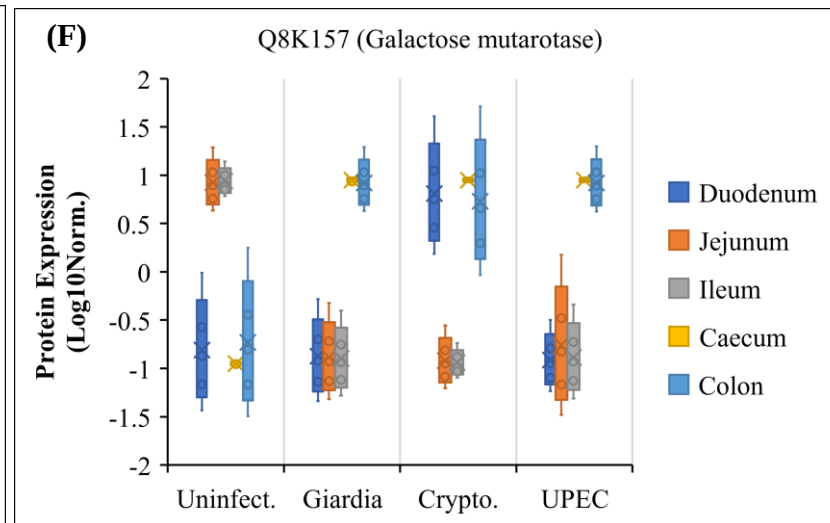
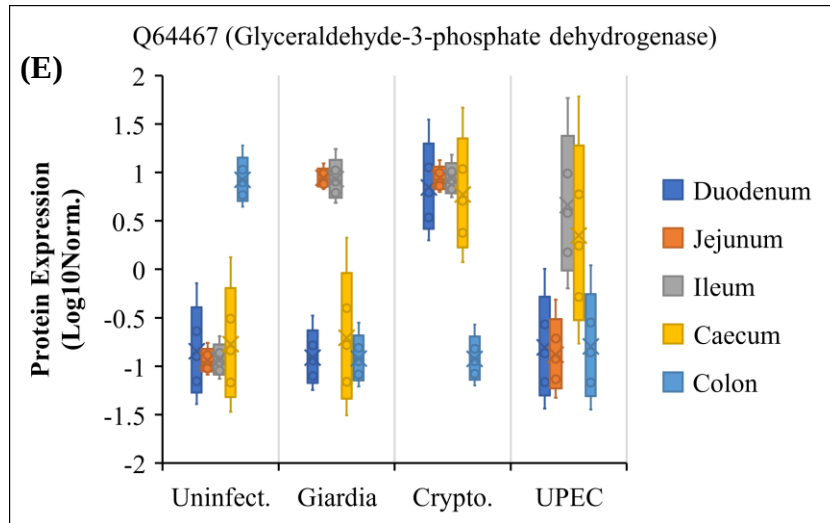
On 10 – 11 dpi, the *G. lamblia* cyst count showed a second peak before stabilising. Based on the outputs of both mice weights, *G. lamblia* cyst count and response to the infection, C57BL/6J strain was selected, with the follow-up main study shortened to 10 dpi.

Table S1. PLS-DA model fit (R^2) and predictability (Q^2) for metabolite profiles of all samples analysed for *Giardia* infection.

Sample	R^2X	R^2Y	Q^2
Faeces	0.802	0.997	0.985
Duodenum wash	0.682	0.999	0.954
Jejunum wash	0.65	0.994	0.865
Ileum wash	0.611	0.996	0.915
Caecum wash	0.702	0.999	0.975
Colon wash	0.603	0.996	0.972
Serum	0.691	0.996	0.980
Lung	0.659	0.989	0.929
Liver	0.574	0.999	0.955

Host protein expression during energy pathways (Glycolysis, Citrate cycle and Glyoxylate and gluconate pathway)





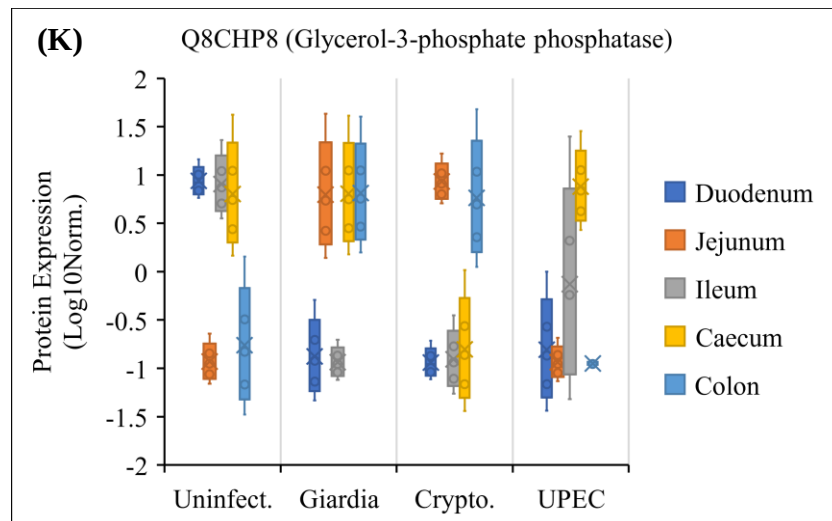
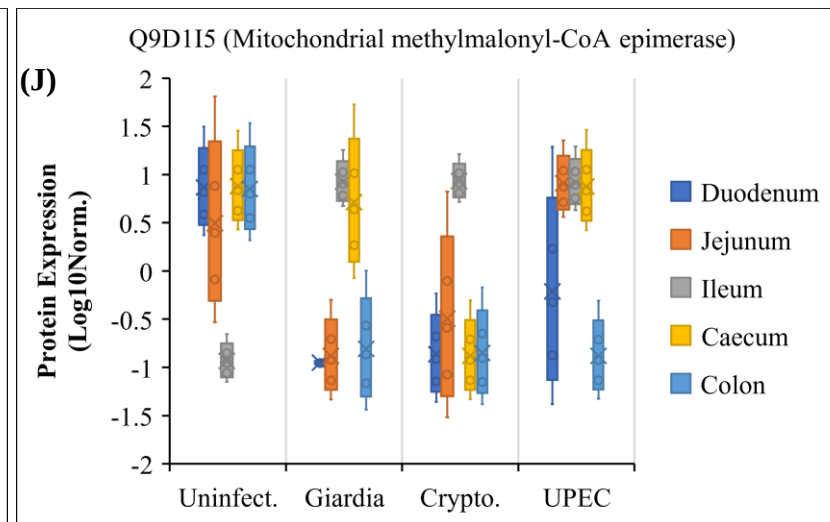
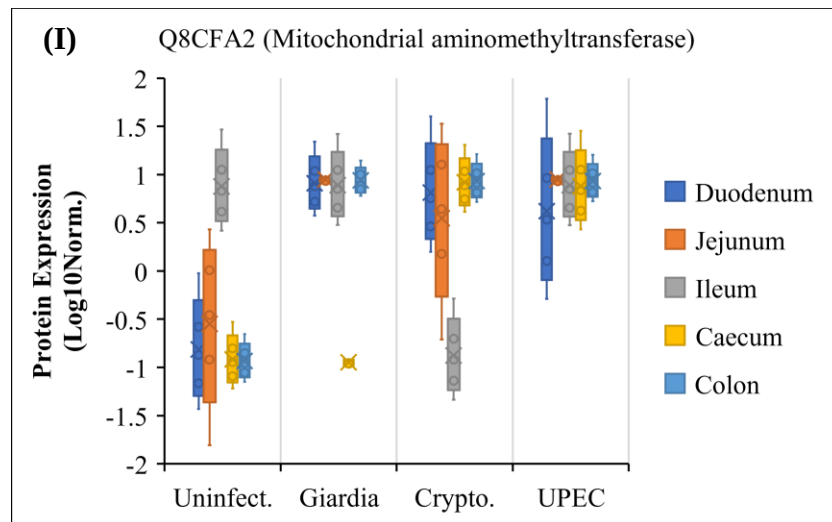
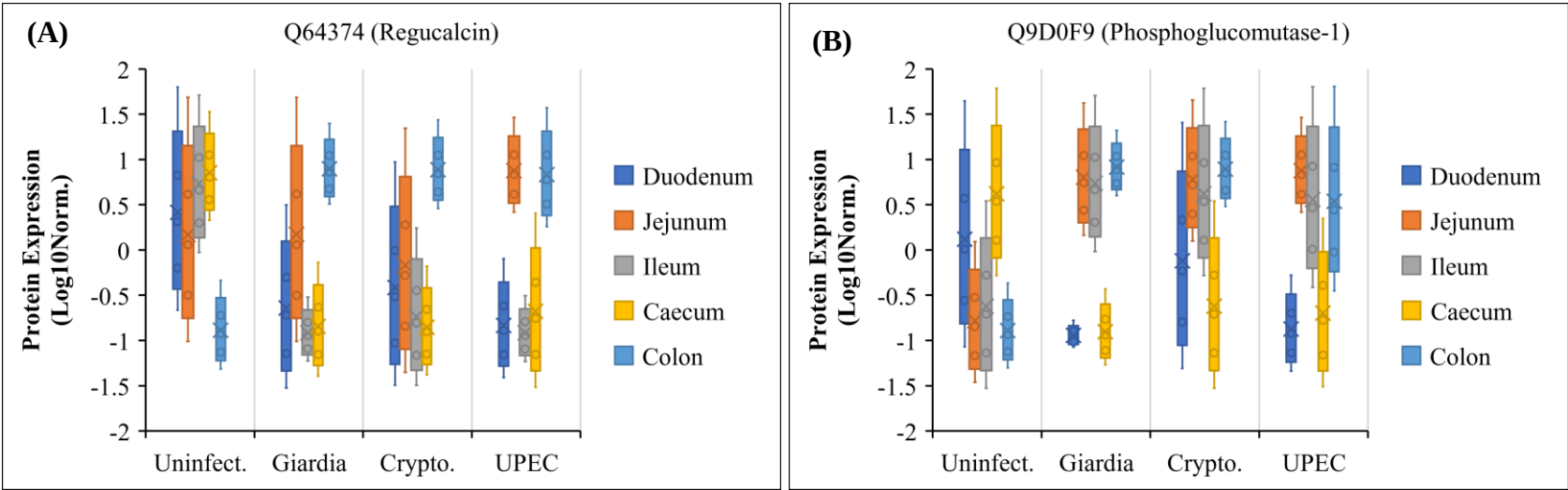
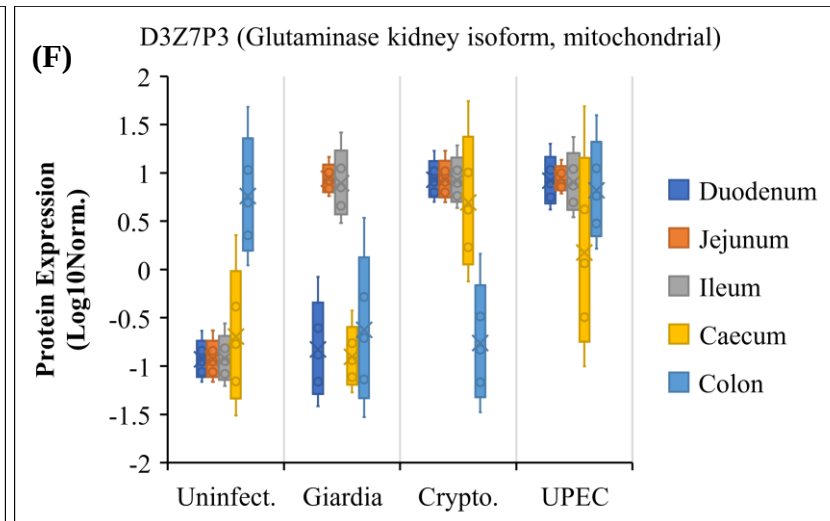
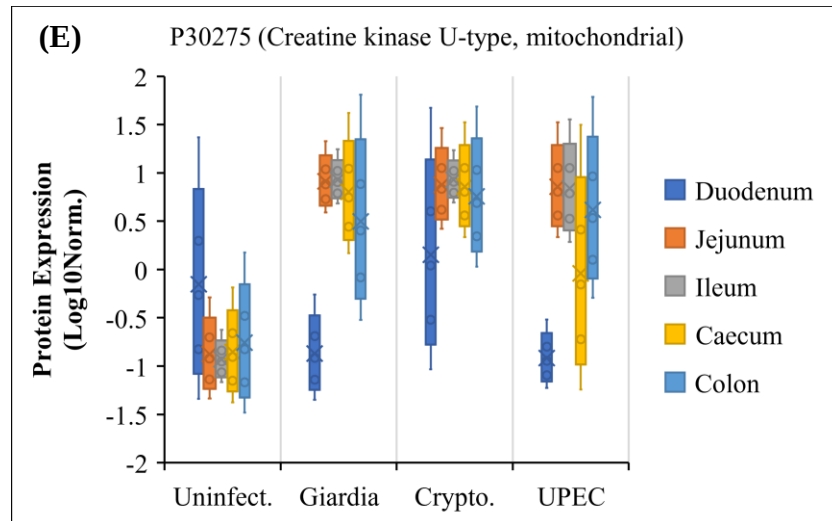
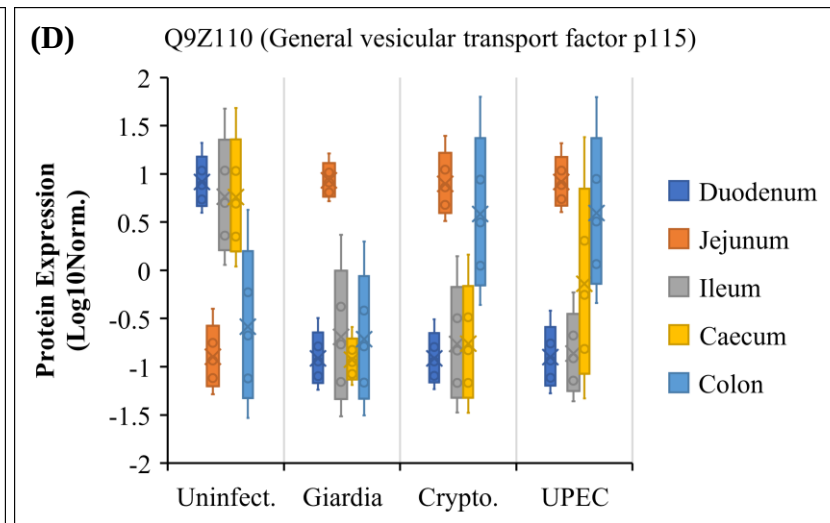
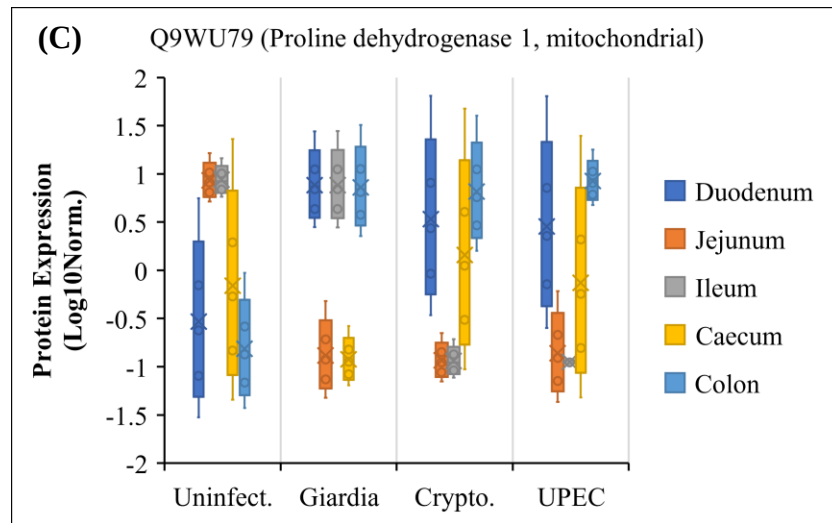


Figure S2. Log₁₀ normalised expression of major mouse proteins driving the energy metabolism pathways in the intestinal system during giardiasis, with respect to uninfected, cryptosporidiosis (positive control) and UPEC (negative control).

Pentose phosphate pathways and amino acid-sugar interconversions





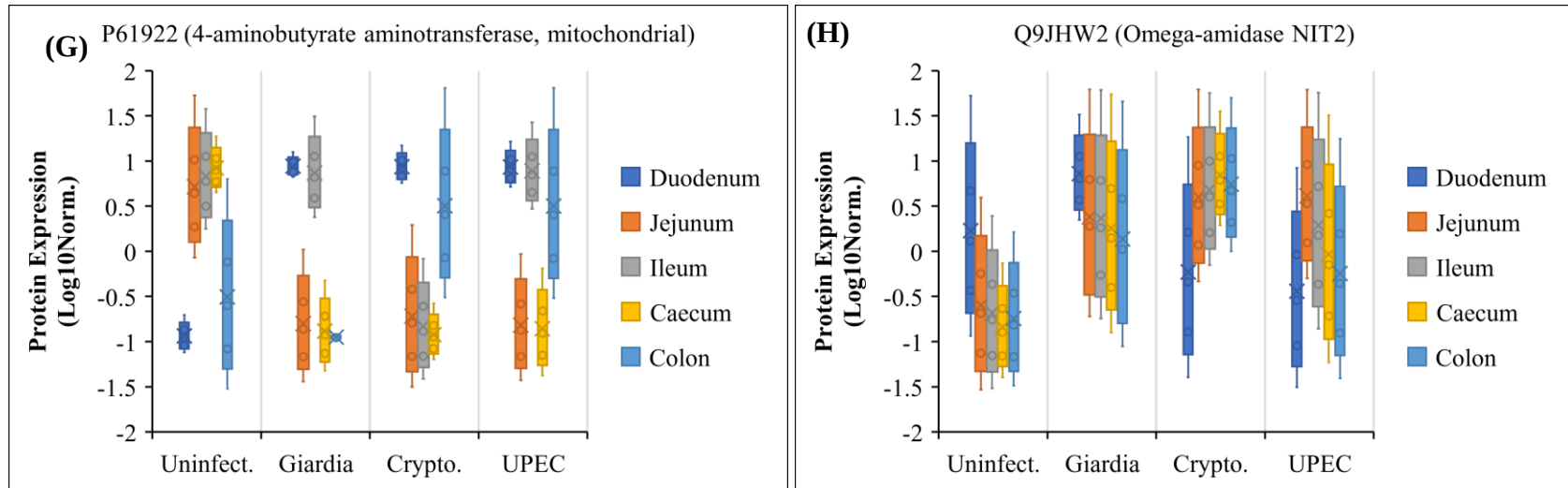
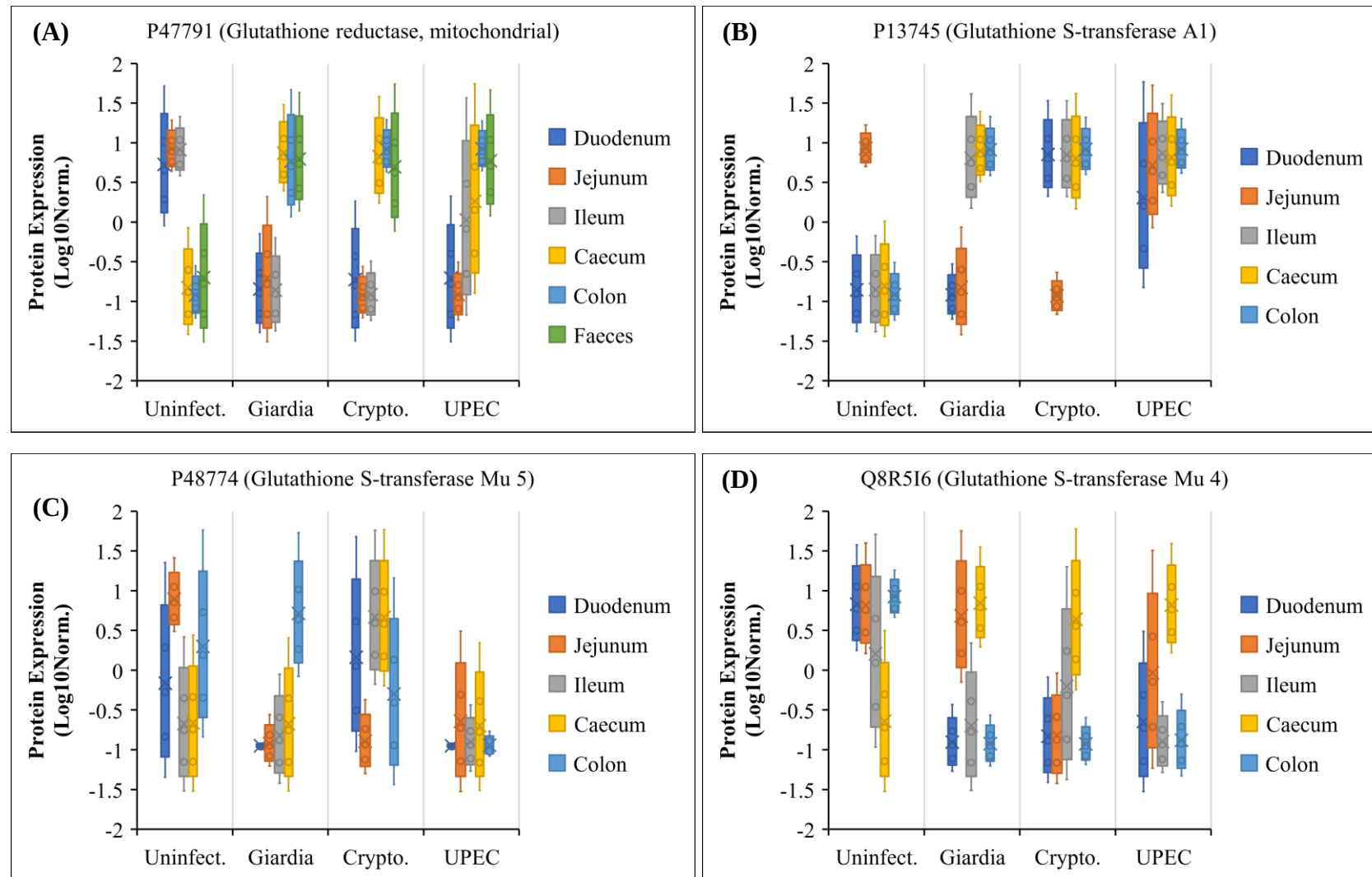
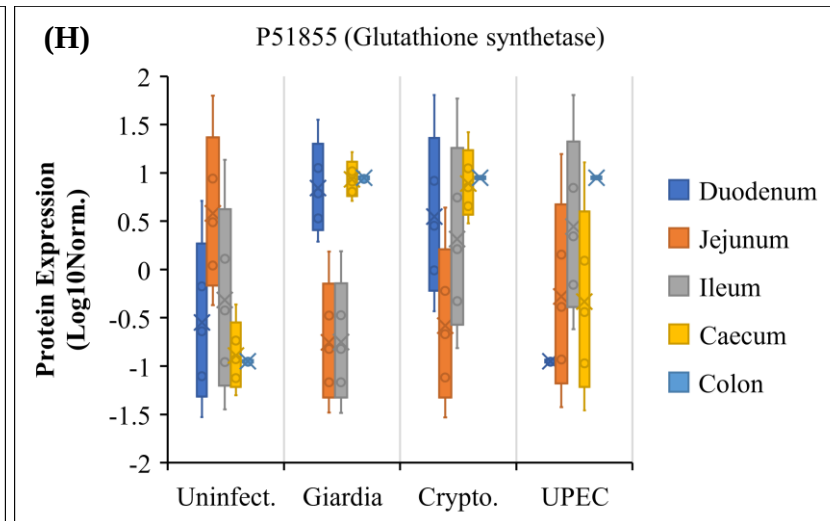
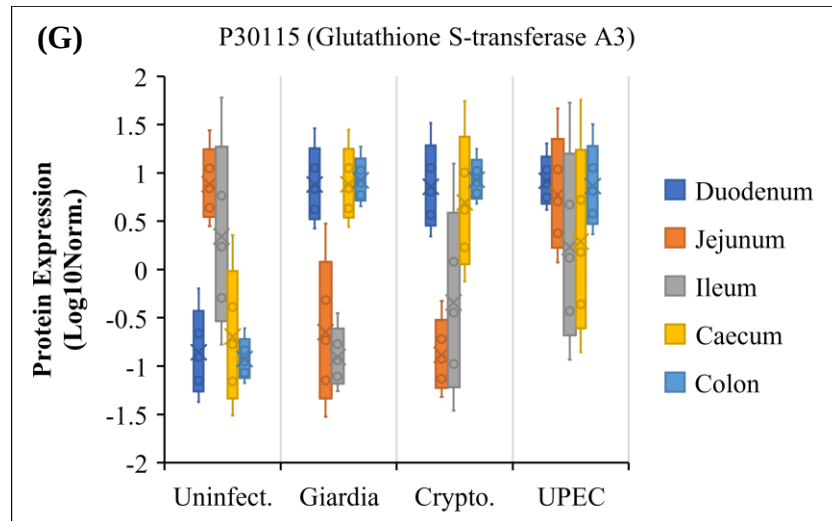
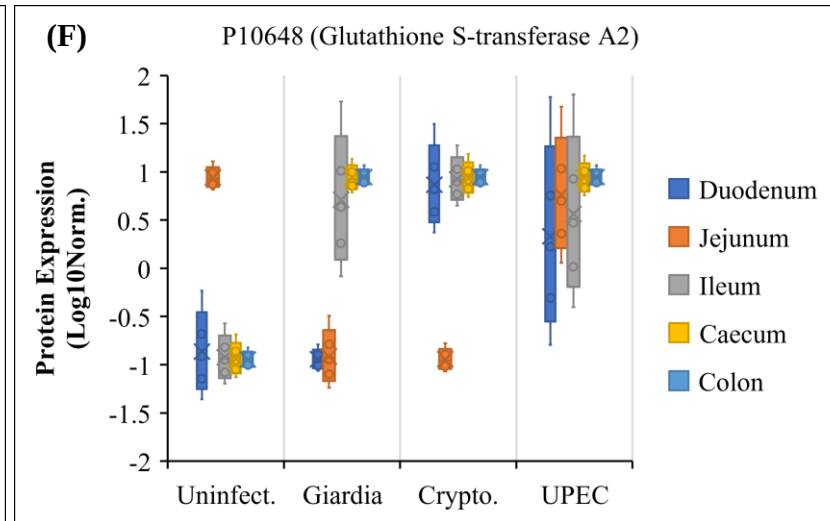
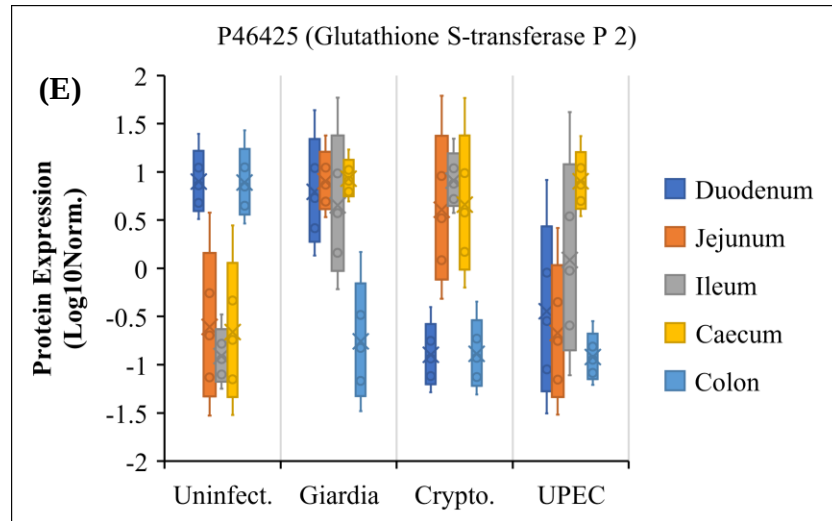


Figure S3. Log₁₀ normalised expression of major mouse proteins driving the pentose phosphate pathways and amino acid-sugar interconversions in the intestinal system during giardiasis, with respect to uninfected, cryptosporidiosis (positive control) and UPEC (negative control).

Sulphur pathways (Glutathione metabolism, Cysteine and methionine metabolism)





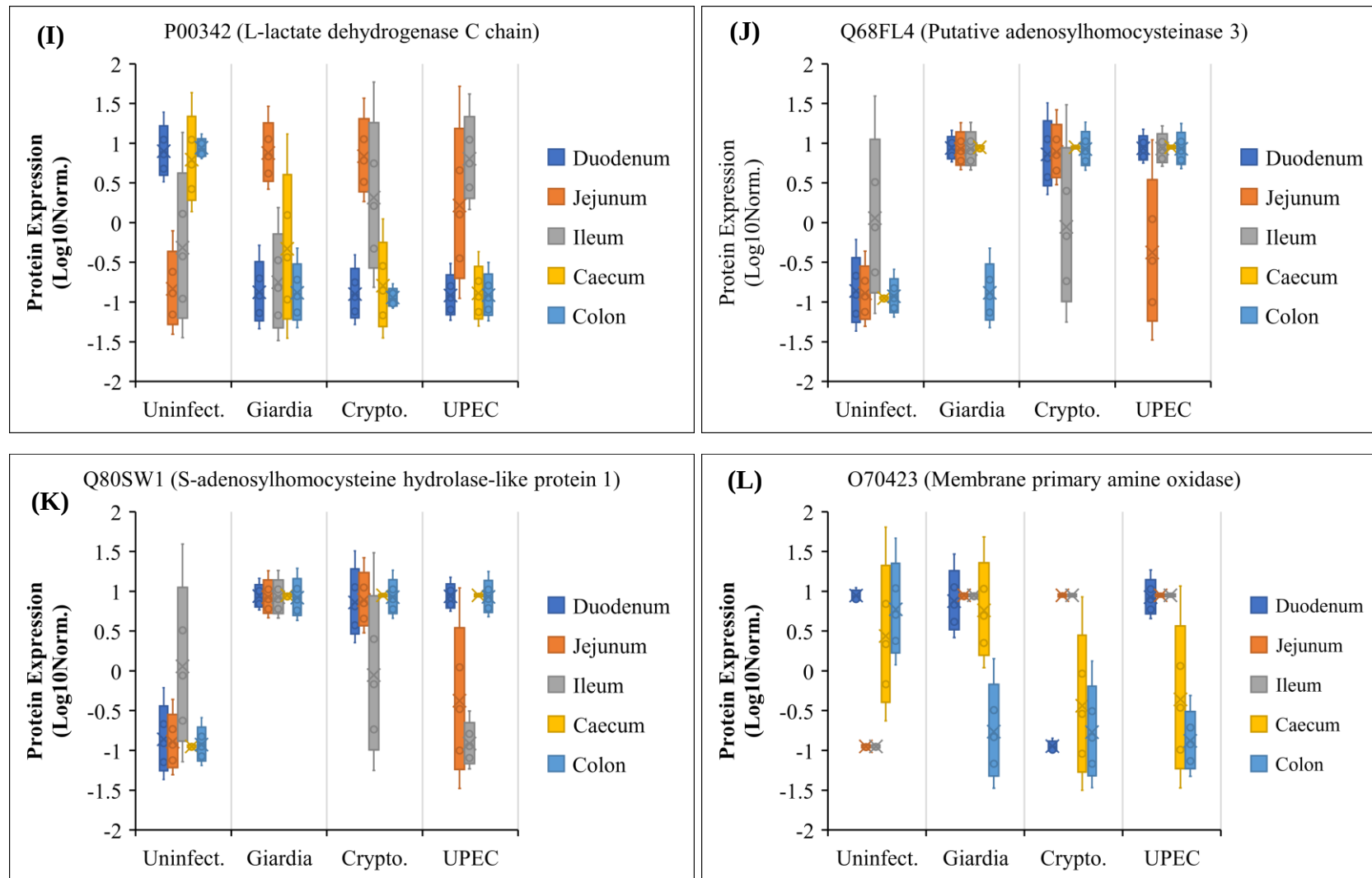
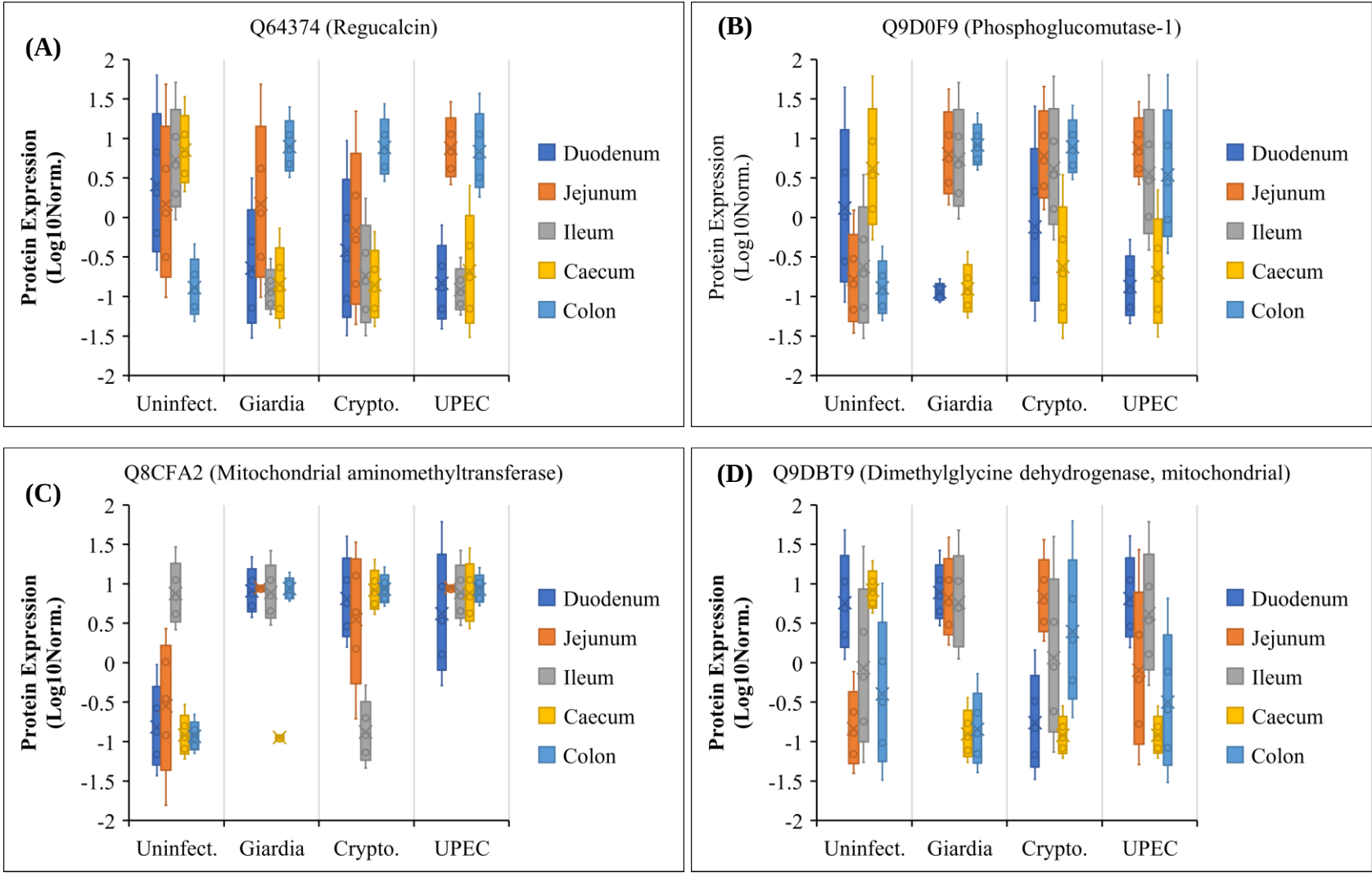
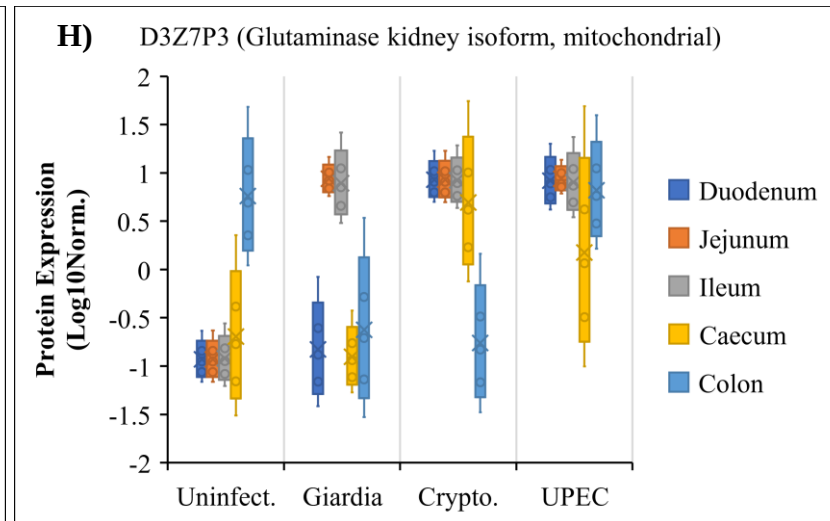
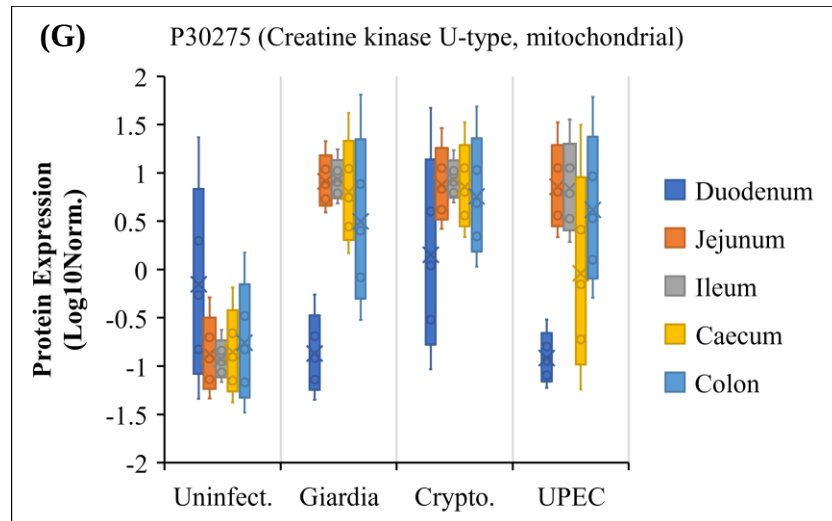
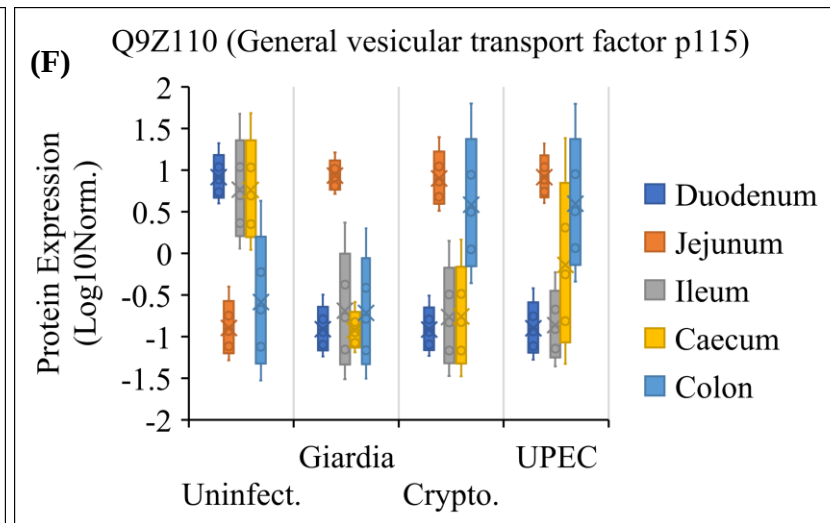
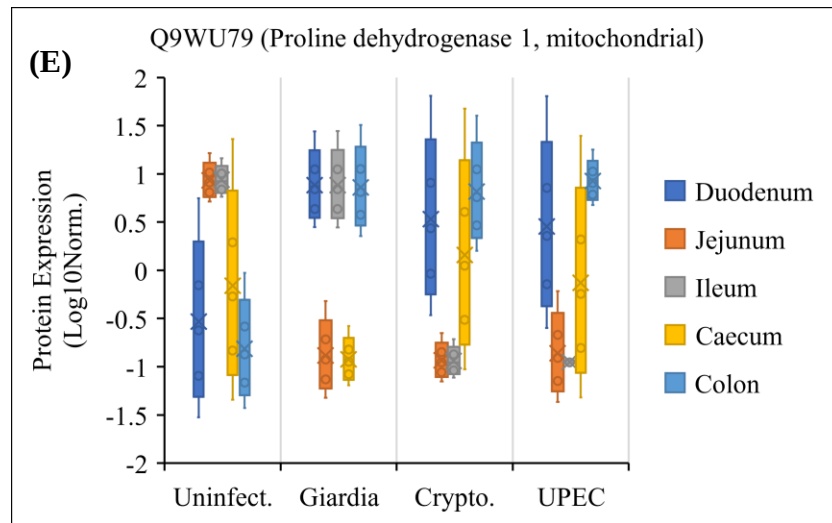


Figure S4. Log₁₀ normalised expression of major mouse proteins driving the sulphur metabolism pathways in the intestinal system during giardiasis, with respect to uninfected, cryptosporidiosis (positive control) and UPEC (negative control).

Nitrogen metabolism pathways





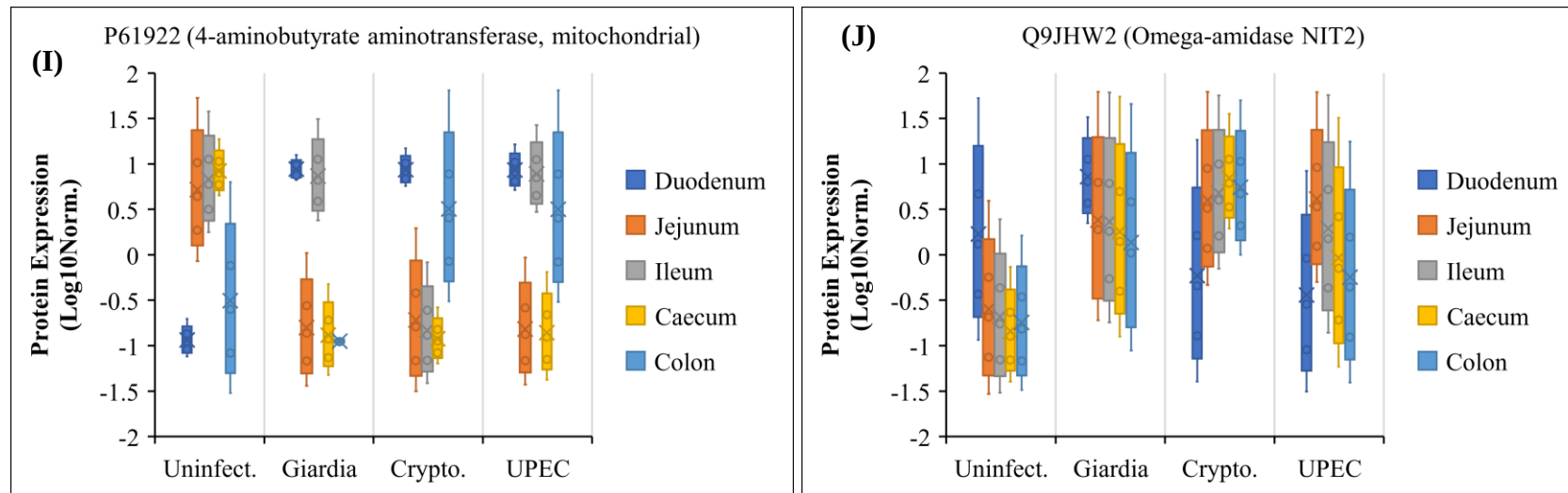


Figure S5. Log₁₀ normalised expression of major mouse proteins driving the nitrogen metabolism pathways in the intestinal system during giardiasis, with respect to uninfected, cryptosporidiosis (positive control) and UPEC (negative control).

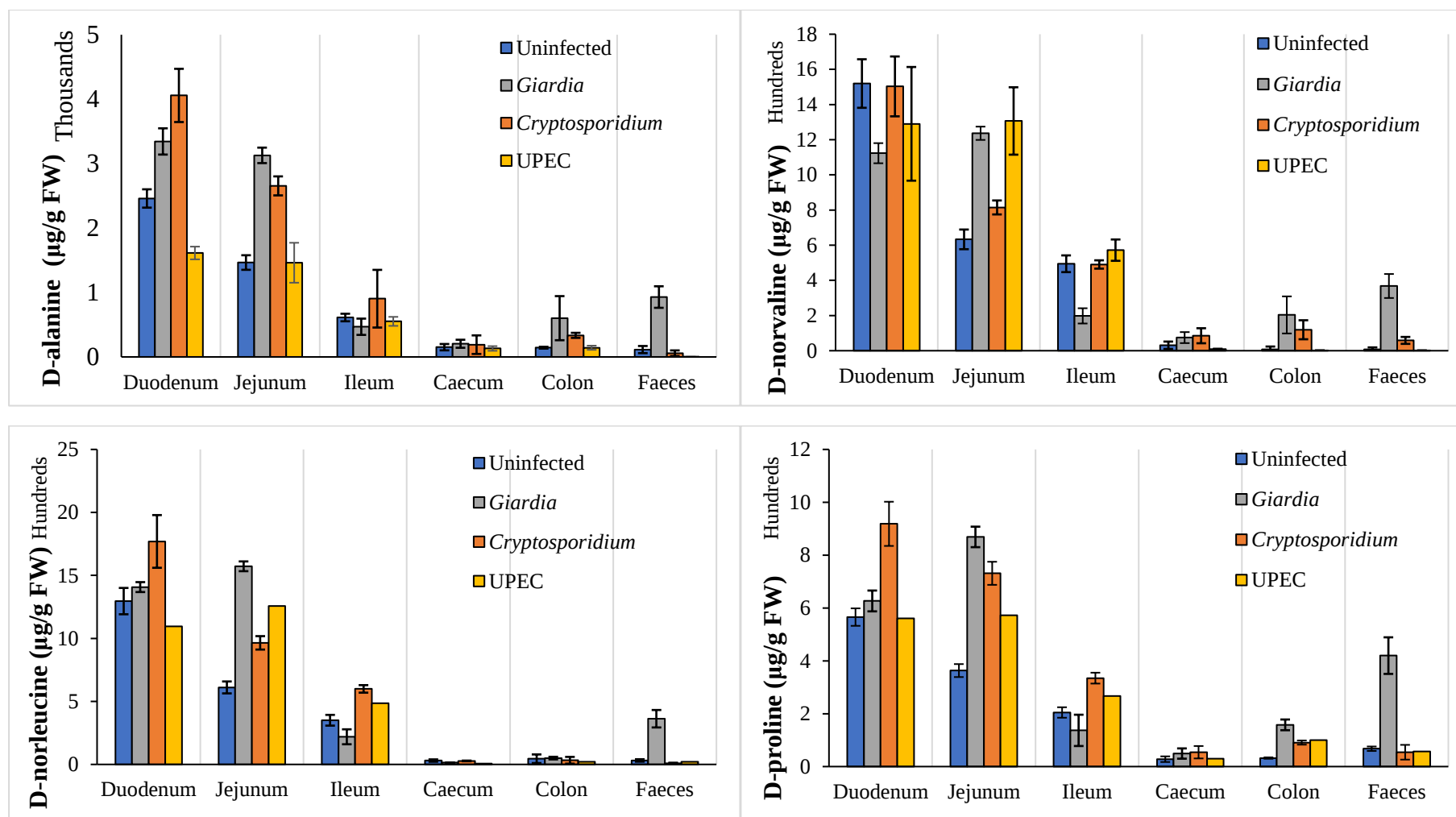


Figure S6. Accumulation of major D-amino acids throughout the mice gut during various infections. Error bars represent the standard deviation across experimental replicates (n = 5).

Table S2. Differential expression of major metabolic pathways in the mouse gut during cryptosporidiosis, giardiasis and UPEC infection in infected mice with respect to the uninfected mice. The values are represented as the normalised Log₂Fold changes, subjected to pareto scaling (n = 5). The terms refer to as C = *Cryptosporidium*, G = *Giardia*, U = UPEC.

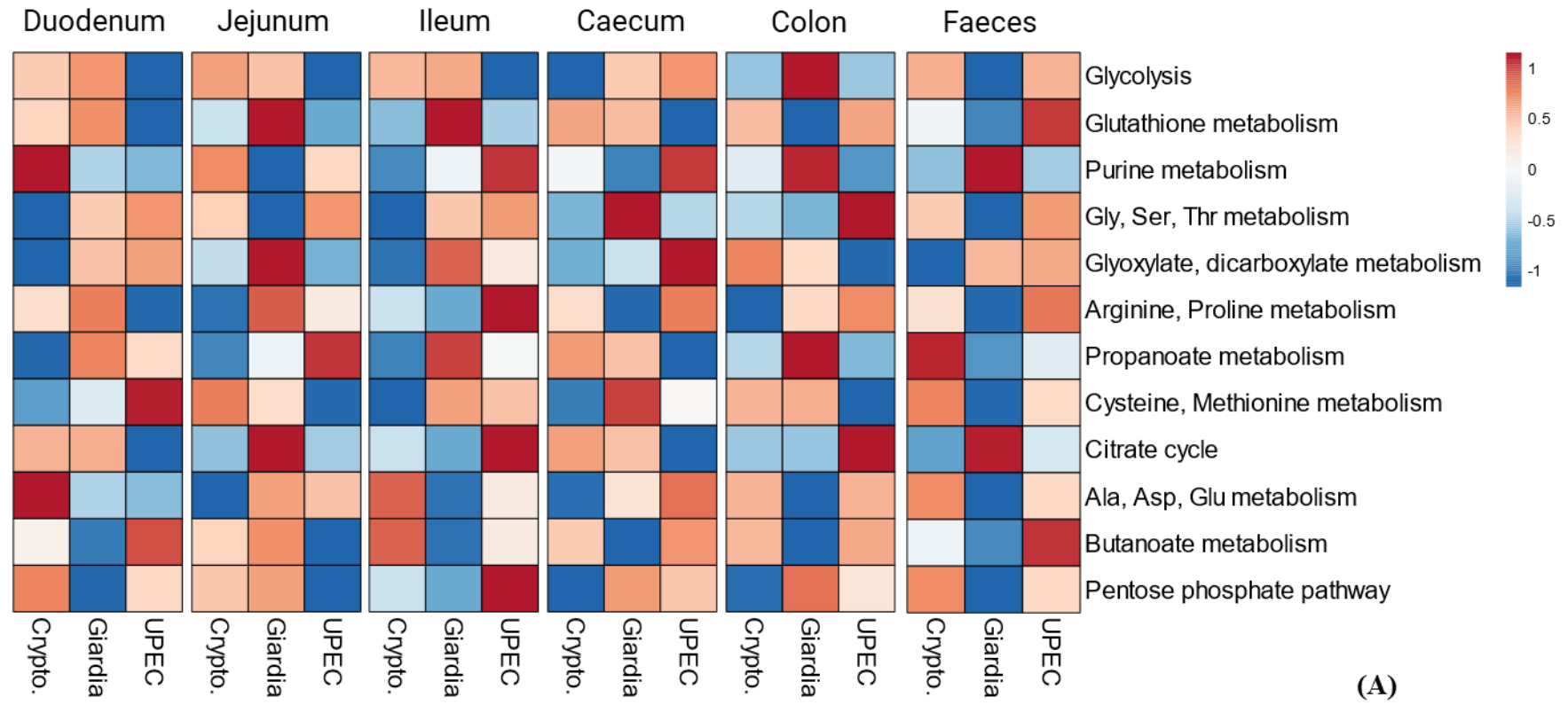
Pathway	Metabolites↓	Duodenum			Jejunum			Ileum			Caecum			Colon		
	Infections →	C	G	U	C	G	U	C	G	U	C	G	U	C	G	U
Glutathione metabolism	Putrescine	-1.35	-0.29	-2.33	0.25	0.31	-0.53	2.01	1.87	0.67	-1.63	6.77	1.10	-0.91	0.67	-1.48
	Glycine	0.25	-0.34	0.11	0.74	1.45	1.53	3.21	3.10	2.06	1.06	-2.61	1.87	-3.22	-0.58	-1.26
	Dehydro-ascorbate	-1.13	-0.07	-1.10	0.44	-0.04	-0.49	-0.14	-0.14	0.33	11.11	10.41	1.06	0.16	1.20	-0.45
	Ascorbate	0.05	-0.76	-1.12	0.41	1.15	0.50	-2.18	0.83	-3.18	-1.34	0.29	-4.38	10.67	9.68	9.34
	Cysteine	-0.84	-0.36	-2.23	1.05	2.01	0.96	-0.29	1.25	3.80	-9.80	3.13	-7.09	0.63	1.27	1.16
	Glutathione	0.30	-0.24	-0.01	-0.08	-0.14	-0.55	2.89	2.35	1.77	-10.00	-10.19	1.52	0.40	2.40	1.46
	Glutamate	0.38	0.07	0.08	-1.24	-0.07	-1.40	0.46	0.49	0.23	0.06	-0.29	-0.45	0.31	-0.02	0.23
Glycolysis	Glucose	-0.05	-0.35	-0.56	0.29	0.34	-0.68	-0.89	-0.52	-0.78	0.16	-2.70	-0.21	1.05	1.33	0.002
	Lactate	0.05	-0.07	-0.23	0.23	0.13	0.03	0.60	0.52	-0.80	-12.73	-3.01	-1.57	-0.78	1.29	0.83
	Glucose-6P	0.59	0.40	0.37	-0.05	-1.01	-1.13	1.34	1.13	0.27	10.19	2.05	7.96	-1.69	-0.18	-1.39
	Glyceraldehyde-3P	-0.38	-0.25	-0.44	0.38	0.21	-0.28	0.94	-0.63	0.97	10.41	1.23	1.95	-0.32	1.56	0.07
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
Pentose phosphate pathway	Glucose	-0.05	-0.35	-0.56	0.29	0.34	-0.68	-0.89	-0.52	-0.78	0.16	-2.70	-0.21	1.05	1.33	0.002
	Glucose-6P	0.59	0.40	0.37	-0.05	-1.01	-1.13	1.34	1.13	0.27	10.19	2.05	7.96	-1.69	-0.18	-1.39
	Glyceraldehyde-3P	-0.38	-0.25	-0.44	0.38	0.21	-0.28	0.94	-0.63	0.97	10.41	1.23	1.95	-0.32	1.56	0.07
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
	Gluconolactone	-0.23	-0.56	-0.80	-0.30	-0.89	-1.47	2.07	1.86	0.64	11.70	0.69	7.70	1.82	1.32	1.01
	Ribose-5P	0.70	0.20	-0.27	0.78	0.36	0.57	1.04	0.45	0.15	-2.33	-0.48	-0.27	-0.68	0.48	-0.80
	Erythrose-4P	0.16	-0.14	-0.39	0.28	0.27	-0.63	1.61	1.15	-0.30	3.20	-1.85	5.75	0.08	0.75	1.00
	Glyceraldehyde	-0.38	-0.25	-0.44	0.38	0.21	-0.28	0.94	-0.63	0.97	10.41	1.23	1.95	-0.32	1.56	0.07
	Glycerate	0.07	-0.21	0.07	0.37	0.55	-0.10	0.54	1.14	1.42	-2.94	-0.56	0.97	-1.13	1.80	0.79
	Gluconate	-0.23	-0.56	-0.80	-0.30	-0.89	-1.47	2.07	1.86	0.64	11.70	0.69	7.70	1.82	1.32	1.01

Arginine and proline metabolism	Putrescine	-1.35	-0.29	-2.33	0.25	0.31	-0.53	2.01	1.87	0.67	-1.63	6.77	1.10	-0.91	0.67	-1.48
	5-aminovalerate	0.61	0.60	0.92	-0.91	-1.27	-2.19	1.74	1.59	1.46	3.78	3.13	1.38	0.33	2.06	1.96
	Urea	-0.38	-0.20	-0.35	0.92	0.52	0.43	1.03	0.66	0.33	-0.45	0.96	-9.27	0.54	1.61	-1.39
	Creatinine	0.12	-0.06	-0.15	-0.20	-0.06	-0.30	0.54	-0.32	0.84	-12.90	-4.52	7.85	-0.13	-0.53	-0.19
	5-Amino-pentanoate	-1.46	-0.31	-2.99	0.65	0.41	-0.54	0.40	0.74	-6.82	10.72	-3.99	-9.91	-1.27	-0.66	-1.59
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
	Proline	0.20	0.58	1.11	-0.69	-1.09	-0.52	0.64	2.07	1.43	11.04	10.63	8.04	1.19	1.71	0.84
	Glutamate	0.38	0.07	0.08	-1.24	-0.07	-1.40	0.46	0.49	0.23	0.06	-0.29	-0.45	0.31	-0.02	0.23
Cysteine and methionine metabolism	Aspartate	1.44	0.33	1.35	0.95	0.46	-0.12	2.15	2.01	0.59	5.93	-5.59	5.17	-0.95	-0.16	-1.49
	Alanine	0.65	-0.20	-0.63	0.51	0.09	-1.11	-5.89	-2.71	0.30	0.49	5.25	4.69	0.69	0.93	-0.24
	Serine	0.35	-0.02	0.41	-0.27	0.02	-0.40	-4.38	-8.76	-0.42	-11.82	2.80	-6.96	-11.37	0.57	-0.80
	Methionine	0.48	-0.18	0.92	0.22	0.22	0.01	0.58	0.85	-0.60	-0.39	10.34	1.27	-0.66	1.29	0.50
	Cysteine	-0.84	-0.36	-2.23	1.05	2.01	0.96	-0.29	1.25	3.80	-9.80	3.13	-7.09	0.63	1.27	1.16
	Glutathione	0.30	-0.24	-0.01	-0.08	-0.14	-0.55	2.89	2.35	1.77	-10.00	-10.19	1.52	0.40	2.40	1.46
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
Citrate Cycle	Succinate	0.19	-0.35	-0.31	0.48	-1.35	-0.67	0.91	1.74	1.42	10.93	0.66	1.05	0.37	2.09	1.80
	Fumarate	0.22	0.00	-0.34	0.18	0.00	-0.50	0.10	-0.96	-0.22	-2.71	-0.71	1.52	-0.97	1.43	0.13
	cis-Aconitate	0.16	0.82	0.51	-0.25	-0.24	-1.12	4.51	3.73	2.05	1.02	1.67	-0.99	1.09	2.97	2.37
	Citrate	-0.17	-0.20	-0.87	-0.38	0.80	0.11	2.33	1.94	2.41	-2.82	-2.51	-0.08	9.42	10.32	9.62
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
	Oxoglutarate	0.19	0.47	-1.00	0.34	0.14	-0.01	0.30	0.27	0.38	-7.93	0.11	2.07	-0.82	0.36	-0.28
Alanine, aspartate and glutamate metabolism	Aspartate	1.44	0.33	1.35	0.95	0.46	-0.12	2.15	2.01	0.59	5.93	-5.59	5.17	-0.95	-0.16	-1.49
	Alanine	0.65	-0.20	-0.63	0.51	0.09	-1.11	-5.89	-2.71	0.30	0.49	5.25	4.69	0.69	0.93	-0.24
	Succinate	0.19	-0.35	-0.31	0.48	-1.35	-0.67	0.91	1.74	1.42	10.93	0.66	1.05	0.37	2.09	1.80
	Fumarate	0.22	0.00	-0.34	0.18	0.00	-0.50	0.10	-0.96	-0.22	-2.71	-0.71	1.52	-0.97	1.43	0.13
	Glutamine	-0.32	-1.47	-0.28	-1.14	-0.67	-1.44	0.90	2.02	0.43	-0.50	-0.68	8.56	6.34	6.95	7.55
	Asparagine	-0.18	-0.32	-1.40	-0.20	0.36	0.24	0.92	0.89	0.34	-2.92	-0.11	3.37	0.20	1.73	0.98
	Citrate	-0.17	-0.20	-0.87	-0.38	0.80	0.11	2.33	1.94	2.41	-2.82	-2.51	-0.08	9.42	10.32	9.62
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26

Propanoate and Butanoate metabolism	Oxoglutarate	0.19	0.47	-1.00	0.34	0.14	-0.01	0.30	0.27	0.38	-7.93	0.11	2.07	-0.82	0.36	-0.28
	Glutamate	0.38	0.07	0.08	-1.24	-0.07	-1.40	0.46	0.49	0.23	0.06	-0.29	-0.45	0.31	-0.02	0.23
	Succinate	0.19	-0.35	-0.31	0.48	-1.35	-0.67	0.91	1.74	1.42	10.93	0.66	1.05	0.37	2.09	1.80
	Lactate	0.05	-0.07	-0.23	0.23	0.13	0.03	0.60	0.52	-0.80	-12.73	-3.01	-1.57	-0.78	1.29	0.83
	Methylmalonate	0.62	0.71	0.27	-0.54	-0.28	-0.42	1.50	1.45	0.49	-1.50	9.57	-6.84	-2.05	1.65	-0.01
	2-hydroxy- butyrate	-1.25	0.78	-0.26	-1.80	-0.16	-0.31	-0.42	0.06	-1.58	-0.68	-1.23	1.24	1.46	1.54	0.45
	Fumarate	0.22	0.00	-0.34	0.18	0.00	-0.50	0.10	-0.96	-0.22	-2.71	-0.71	1.52	-0.97	1.43	0.13
	Pyruvate	-0.76	-0.50	-0.62	-1.34	-1.39	-1.59	7.19	7.44	8.77	1.30	6.29	7.99	-0.37	-1.63	-2.26
	Oxoglutarate	0.19	0.47	-1.00	0.34	0.14	-0.01	0.30	0.27	0.38	-7.93	0.11	2.07	-0.82	0.36	-0.28
	Glutamate	0.38	0.07	0.08	-1.24	-0.07	-1.40	0.46	0.49	0.23	0.06	-0.29	-0.45	0.31	-0.02	0.23
	D-malate	0.32	0.12	-0.26	0.37	0.39	-0.50	1.34	1.24	0.21	-6.09	-6.86	0.00	-0.21	-1.27	-0.85
	Maleate	0.32	0.12	-0.26	0.29	0.39	-0.57	1.47	1.15	0.08	1.71	1.58	0.87	-1.10	0.01	0.43

Table S3. Differential expression of major metabolic pathways in the mouse extra-gut organs during cryptosporidiosis, giardiasis and UPEC infection in infected mice with respect to the uninfected mice. The values are represented as the normalised Log₂Fold changes, subjected to pareto scaling (n = 5).

Pathway	Serum			Liver		
	Crypto.	Giardia	UPEC	Crypto.	Giardia	UPEC
Glycolysis	-0.51	1.27	-0.76	0.85	1.08	-1.92
Glutathione metabolism	-0.32	3.68	-3.36	0.26	-2.68	2.42
Purine metabolism	0.81	-1.24	0.42	1.22	1.37	-2.59
Gly, Ser, Thr metabolism	0.91	-2.21	1.30	1.27	-0.39	-0.89
Glyoxylate, dicarboxylate metabolism	0.41	-1.35	0.95	1.34	0.12	-1.46
Arginine, proline metabolism	-1.89	2.33	-1.14	0.82	0.55	-1.37
Propanoate metabolism	0.49	1.01	-1.50	1.03	-1.85	0.81
Cysteine, methionine metabolism	0.79	1.05	1.85	1.93	-1.73	-0.21
Citrate cycle	-0.94	1.18	-0.24	-0.21	0.86	-0.65
Ala, Asp, Glu metabolism	-1.21	0.20	1.00	1.22	-0.44	-0.78
Butanoate metabolism	0.29	-0.52	0.24	0.15	-0.60	0.45
Pentose phosphate pathway	-0.34	0.73	-0.39	0.66	-0.90	0.24
Phenylalanine metabolism	0.89	-2.39	1.51	-0.46	0.05	0.41
Val, Leucine, Isoleucine biosynthesis	0.42	1.22	-1.64	-0.07	1.09	-1.02
Pyruvate metabolism	0.46	-0.94	0.48	0.71	1.27	-1.97



(A)

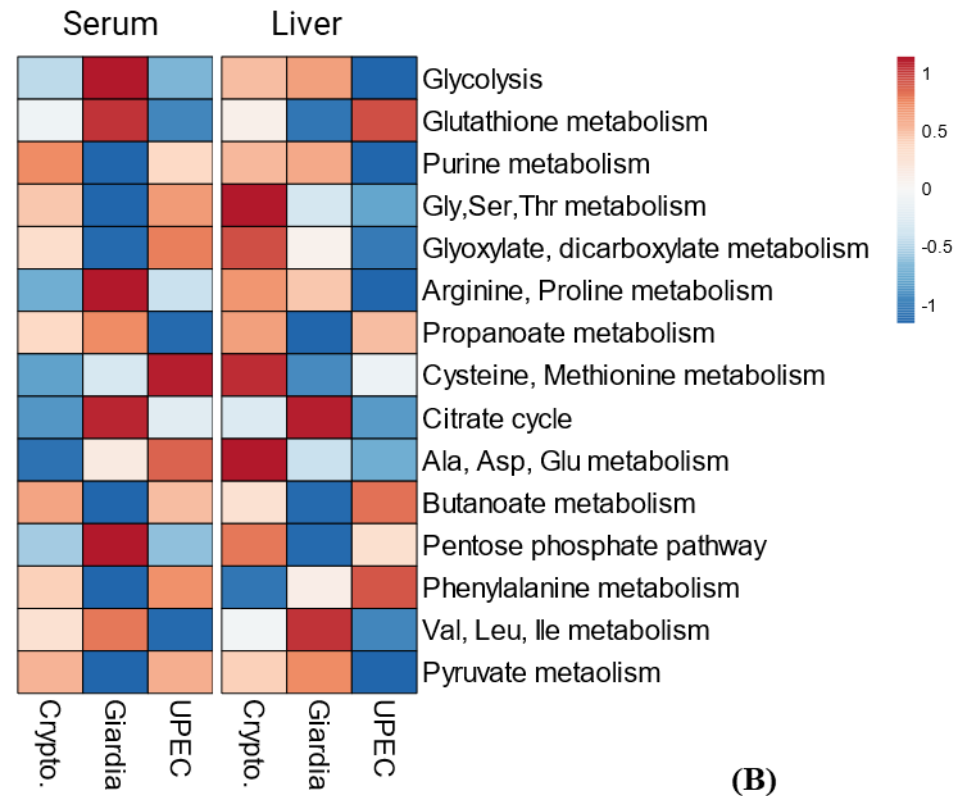


Figure S7. The heatmap represents relative upregulation (red) and downregulation (blue) of major metabolic pathways in (A) Intestine and faeces and, (B) Serum and liver, during cryptosporidiosis, giardiasis and UPEC infections in the infected mice with respect to the uninfected mice. The individual boxes indicate the relative impact as determined by the integrated proteomics-metabolomics analysis. The heatmaps were designed using Clustvis web tool.

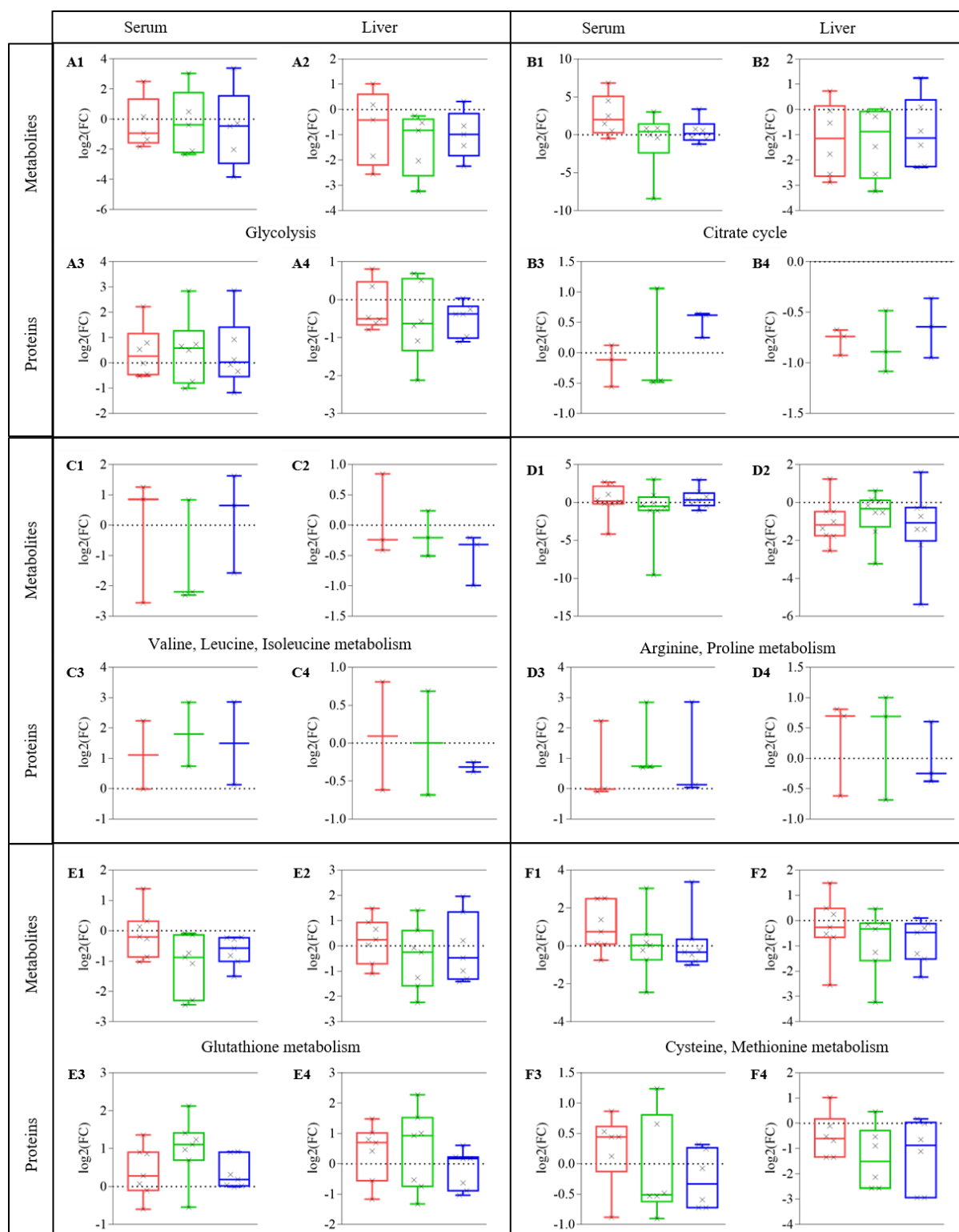


Figure S8. The comparative metabolite and protein expressions driving the key metabolic pathways in serum and liver during cryptosporidiosis (red), giardiasis (green) and UPEC (blue). The individual metabolites and proteins contributing to these expressions are presented in Table S4 and S5.

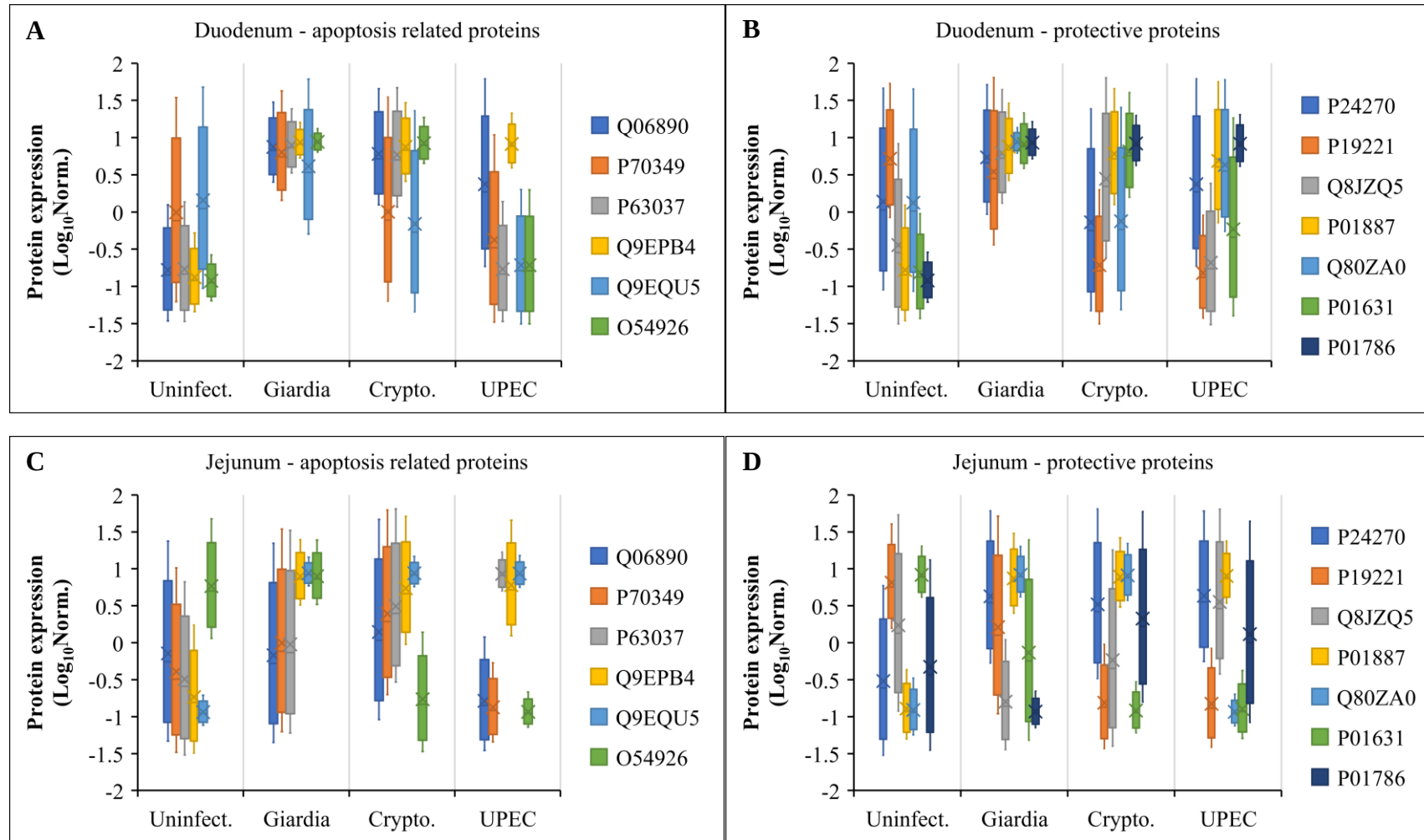
Table S4. Major metabolites contributing to the most significant metabolic pathways in serum and liver during various infections with respect to the uninfected mice.

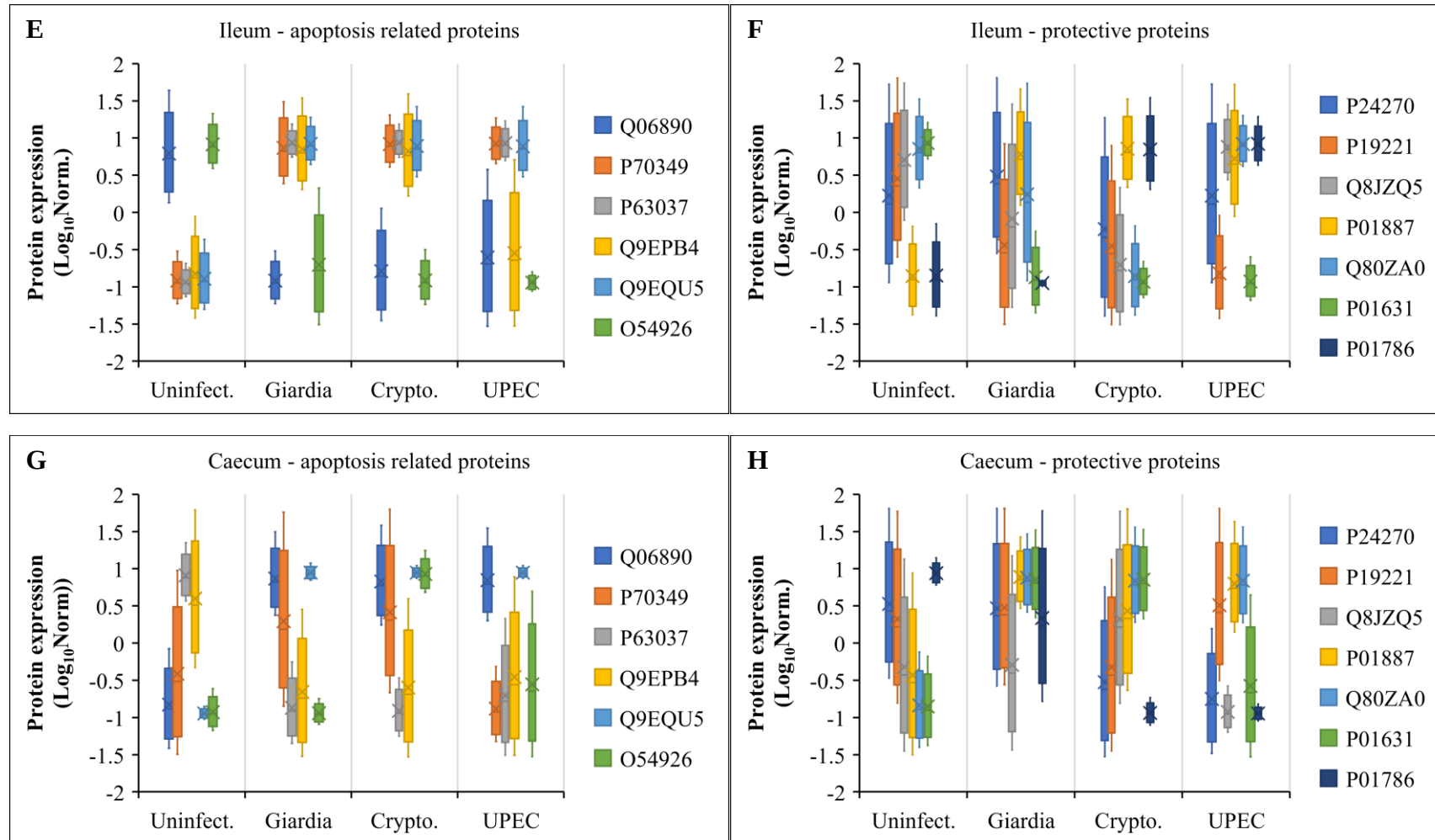
Pathway	Metabolites	Serum (Log ₂ Fold change)			Liver (Log ₂ Fold change)		
		Crypto	Giardia	UPEC	Crypto	Giardia	UPEC
Glycolysis	Glucose	-0.93	-0.39	-0.47	1.02	-0.26	-1.42
	Lactate	-1.35	0.49	-0.27	0.19	-0.52	0.33
	Glucose-6P	-1.82	-2.12	-2.02	-1.84	-2.02	-0.98
	Glyceraldehyde-3P	0.16	-2.34	-3.84	-0.41	-0.83	-0.65
	Pyruvate	2.49	3.04	3.37	-2.55	-3.23	-2.24
Citrate cycle	Succinate	4.51	0.88	0.57	-0.04	-0.28	-0.85
	Fumarate	6.83	0.00	-1.23	0.72	0.02	1.25
	Cis-acotinate	0.52	-8.41	-0.29	-1.76	-2.55	-2.28
	Citrate	1.48	0.83	0.76	-2.87	-1.46	-1.40
	Pyruvate	2.49	3.04	3.37	-2.55	-3.23	-2.24
	Oxoglutarate	-0.46	-0.36	-0.53	-0.53	-0.10	0.10
Valine, leucine, isoleucine metabolism	Valine	-2.56	-2.30	1.62	0.84	0.24	-0.21
	Methylmalonate	0.85	0.83	0.64	-0.24	-0.51	-0.99
	Isoleucine	1.25	-2.20	-1.58	-0.41	-0.20	-0.32
Arginine and proline metabolism	Putrescine	0.31	-1.08	0.28	-1.01	0.61	-1.41
	2-oxo-4-hydroxy-5-aminovalerate	-4.17	-9.55	-0.18	-0.49	-1.54	-1.41
	Urea	-0.19	-1.06	-1.08	-1.36	-0.52	-0.72
	Creatinine	0.09	-0.10	0.36	1.24	0.14	1.60
	5-aminopentanoate	-0.14	-0.64	-0.46	-1.73	-0.52	-0.29
	Pyruvate	2.49	3.04	3.37	-2.55	-3.23	-2.24
	Proline	2.68	0.95	0.76	-1.77	0.03	-5.38
	Glutamate	1.08	-0.38	1.40	-0.49	-0.17	-0.26
Glutathione metabolism	Putrescine	0.31	-1.08	-0.28	-1.10	0.61	-1.41
	Glycine	-0.26	-2.29	-1.51	0.93	-2.24	0.21
	Dehydroascorbate	-1.02	-0.09	-0.22	0.04	-0.08	-0.99
	Ascorbate	-0.86	-0.88	-0.58	0.66	1.41	1.96
	Cysteine	1.38	-2.45	-1.02	1.48	-1.59	-1.31
	Glutathione	0.13	-0.74	-0.82	0.25	-1.26	-0.48
	Glutamate	1.08	-0.38	1.40	-0.49	-0.17	-0.26
Cysteine, Methionine metabolism	Aspartate	2.51	0.60	-0.46	-0.52	-0.10	0.10
	Alanine	0.09	0.18	0.35	-0.26	-0.16	-0.12
	Serine	-0.76	0.02	-0.22	0.49	0.47	-0.30
	Methionine	0.74	-0.22	-0.33	-0.66	-0.33	-1.52
	Cysteine	1.38	-2.45	-1.02	1.48	-1.59	-1.31
	Glutathione	0.13	-0.74	-0.82	0.25	-1.26	-0.48
	Pyruvate	2.49	3.04	3.37	-2.55	-3.23	-2.24

Table S5. Major protein expressions driving the metabolic pathways in serum and liver during various infections with respect to the uninfected mice.

Pathway	Serum (Log ₂ Fold change)				Liver (Log ₂ Fold change)			
	Protein (Uniprot ID)	Crypto	Giardia	UPEC	Protein (Uniprot ID)	Crypto	Giardia	UPEC
Glycolysis	O70250	1.65	0.99	2.30	P05064	0.35	0.51	0.04
	P15327	0.76	0.76	1.38	P47740	0.81	0.69	-0.25
	P05064	-0.45	-0.74	-0.33	Q9D0F9	-0.41	-0.96	-1.03
	P47740	-0.01	0.74	0.12	Q9DBJ1	-0.47	-0.57	-0.38
	Q9DBJ1	0.79	0.50	0.93	P16125	-0.52	-2.13	-1.11
	P16125	0.53	0.66	-0.07	Q9DBF1	-0.51	-0.45	-0.63
	O08749	-0.92	-0.53	-0.78	P00329	-0.72	-0.55	-0.94
	P16858	-0.64	-0.65	-0.74	P17182	-0.29	-0.61	-0.43
	P09041	0.02	-0.60	0.04	P28474	-0.79	-1.08	-0.98
	Q9JII6	-0.65	0.60	0.72				
	P28474	-0.52	-1.01	-1.18				
	P06745	-0.17	0.63	-0.27				
	Q9JLJ2	2.23	2.85	2.86				
Citrate cycle	P14152	0.12	-0.49	0.25	P54071	-0.77	-0.88	-0.93
	Q60597	0.06	-0.94	-0.08	P08249	-0.41	-0.58	-0.25
	Q9WUM5	-0.11	-0.45	0.62	P14152	-0.68	-0.89	-0.64
	O08749	-0.92	-0.53	-0.79	Q9D6R2	0.62	1.25	0.29
	Q8K2B3	-0.52	0.59	0.19	P28271	0.04	-0.54	-0.86
	Q91V92	-0.56	1.06	0.65	Q9WUM5	-0.74	-1.09	-0.36
	O88844	0.04	0.57	0.08	P97807	-0.11	-0.59	-0.32
					Q91V92	-0.93	-0.48	-0.95
Valine, Leucine, Isoleucine metabolism					Q9Z2I9	-0.85	-0.85	-0.86
	Q9JHI5	-0.21	0.58	-0.48	Q91ZA3	-0.26	-0.76	-0.35
	P47740	-0.01	0.74	0.12	G3X982	-1.24	-1.22	-1.05
	P50136	-2.52	-1.77	-1.31	P47740	0.81	0.69	-0.25
	O08749	-0.92	-0.53	-0.79	P38060	-0.22	-0.68	-0.01
	Q8QZS1	-0.97	-0.47	-2.46	Q9DBF1	-0.51	-0.45	-0.63
	Q99MR8	1.71	1.72	0.87	O08756	0.21	0.75	-0.18
	Q8QZT1	0.11	-0.67	0.07	Q8BMS1	-0.60	-0.75	-0.76
	Q8VCH0	-0.28	-0.58	0.59	P61922	-0.53	-0.64	-0.55
	Q9JLJ2	2.23	2.85	2.86	Q8BH95	-0.37	-0.52	-0.13
					P47738	-0.38	-0.59	-0.67
					Q61425	-0.41	-0.46	-0.30
					Q9JLJ2	-0.62	-0.68	-0.38
					Q921H8	-0.83	-0.75	-0.52
Arginine, proline metabolism	P47740	-0.01	0.74	0.12	P47740	0.81	0.69	-0.25
	A2AS89	-0.16	2.57	0.22	Q61176	1.37	1.76	1.07
	O08691	0.89	1.05	-0.26	Q9Z110	-1.13	-0.46	-0.69

Glutathione metabolism	Q9D1A2	0.08	-0.56	1.18	Q9DBF1	-0.51	-0.45	-0.63
	P30275	0.16	1.57	0.32	Q9DCU9	0.54	0.44	-0.97
	Q9CPY7	-0.10	0.70	0.04	P29758	-0.32	-0.63	-0.39
	P05201	0.28	1.62	0.99	P07310	0.39	0.90	1.47
	Q9JLJ2	2.23	2.85	2.86	Q9CPY7	0.69	1.00	0.60
					P05202	-0.91	-0.75	-0.87
					P47738	-0.38	-0.59	-0.67
					Q9JLJ2	-0.62	-0.68	-0.38
	Q64471	0.07	1.11	0.19	P47791	0.42	0.93	0.18
	O70325	-0.73	-0.83	-0.08	P54071	-0.77	-0.88	-0.93
	Q9CPY7	1.36	2.13	0.92	P10649	-0.60	-0.91	-0.91
	P21765	0.28	1.41	-0.01	Q8R5I6	-1.17	-1.33	-0.89
	P97494	-0.59	-0.55	0.02	P19639	-1.46	-2.21	-0.76
	P97449	0.21	0.78	-0.76	O35660	-0.35	-1.77	0.27
	O88844	-0.10	0.70	0.04	Q9DCD0	-0.14	0.71	0.32
Cysteine, Methionine metabolism	P30115	0.91	0.97	0.90	P46412	0.64	1.37	0.37
	P51855	0.87	1.24	0.32	P11352	0.80	1.52	-1.04
	P13745	1.04	0.71	0.84	Q64471	-0.56	-0.75	-0.63
	Q64471	0.04	0.57	0.08	Q91VS7	0.17	-1.31	-0.36
	O70325	0.15	1.79	-0.16	Q9DCM2	-0.77	-0.54	-1.23
	Q9CPY7	1.72	1.40	0.14	Q80W21	-0.35	-1.77	0.27
	P21765	0.95	4.26	-1.67	P15626	-0.29	-0.65	-0.88
					Q9CPY7	0.69	1.00	0.60
					Q8K010	0.74	0.73	-0.07
					P21765	1.48	2.28	0.23
					P97494	1.02	-0.53	0.17
					Q60928	1.25	1.12	0.71
	P47791	0.44	-0.53	-0.72	Q91WT9	-0.79	-0.70	-0.92
	Q9CQU0	-0.17	-0.47	-0.03	P06151	-0.35	-0.69	-0.91
	Q8R5I6	0.53	0.66	-0.07	Q8VBT2	-0.50	-1.14	-0.73
	P11352	0.12	-0.49	0.25	P16125	-0.52	-2.13	-1.11
	Q80SW1	1.29	1.50	-0.14	P08249	-0.41	-0.58	-0.25
	Q91X83	0.44	-0.65	0.92	P14152	-0.68	-0.89	-0.64
	P16125	0.44	-0.53	-0.72	Q80SW1	-1.34	-2.56	-2.94
	P14152	0.28	1.62	0.99	Q68FL4	-1.34	-2.56	-2.94
	P52196	-0.88	-0.90	-0.59	P05202	-0.91	-0.75	-0.87
	Q3THS6	0.87	1.24	0.32	Q99J99	-0.10	0.46	0.00
	Q68FL4	1.72	1.40	0.14	P97494	1.02	-0.53	0.17
					P50247	-0.60	-0.88	-0.68
					Q8VCN5	-0.67	-0.68	-0.42





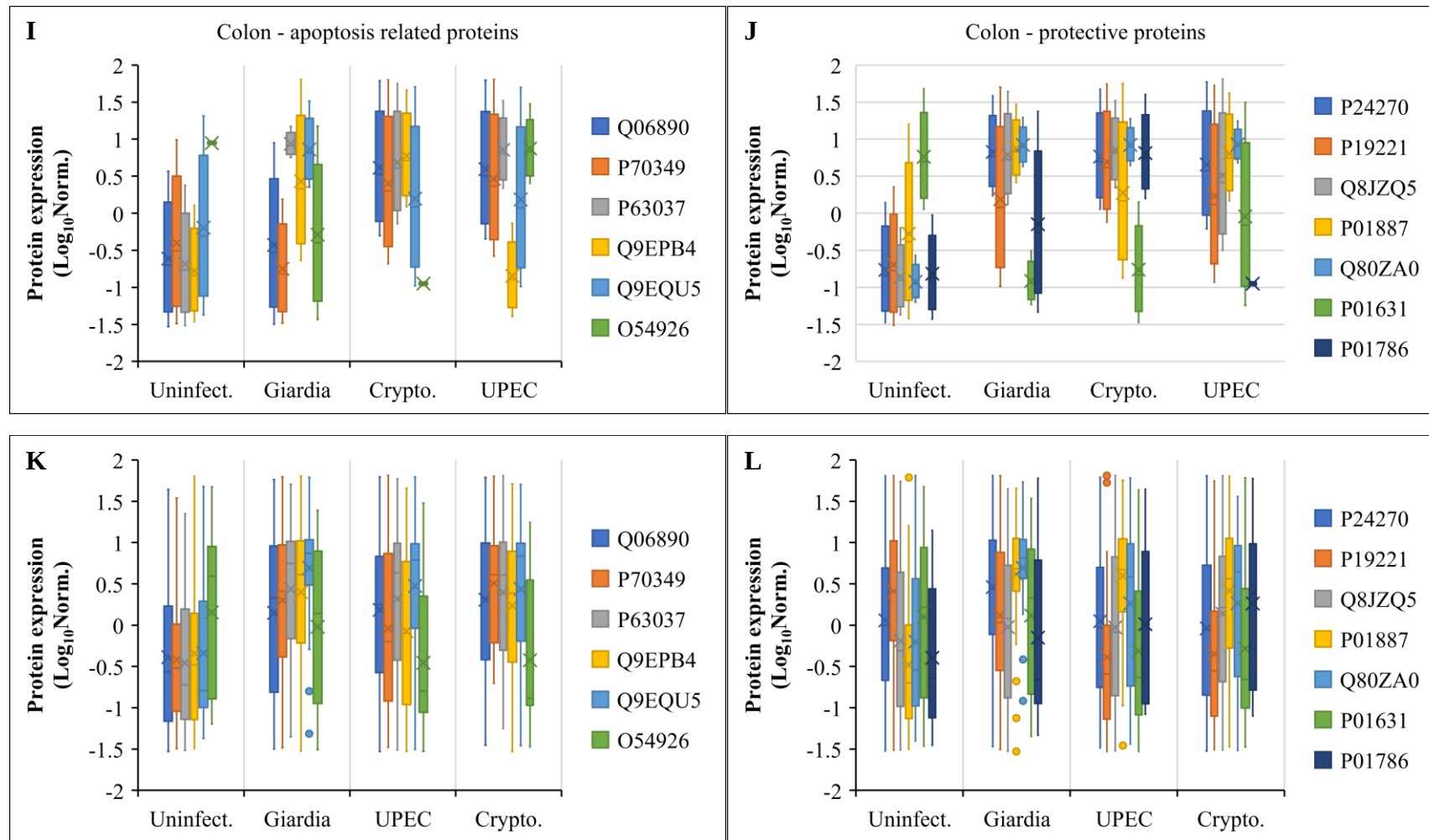


Figure S9. Log_{10} normalised expression of proteins involved in apoptosis process and cellular protection during various infections in the mouse gut sections of (A and B) Duodenum, (C and D) Jejunum, (E and F) Ileum, (G and H) Caecum and (I and J) Colon. Furthermore, an overall inter-species comparison is shown in (K) and (L).

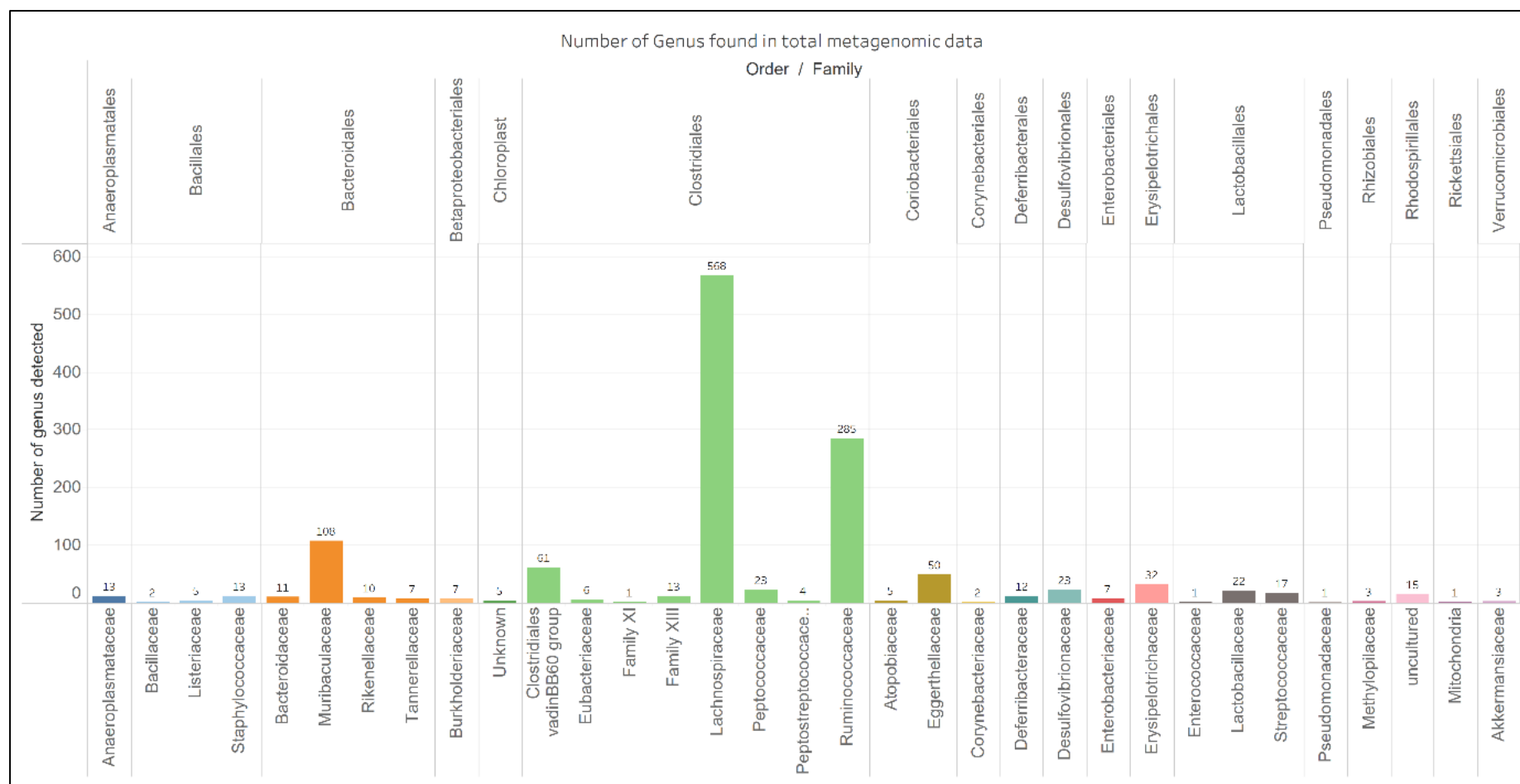


Figure S10. – Number of genres detected for each bacterial family. *Clostridiales* showed highest number of genera, followed by *Bacteroidales*. *Lachnospiraceae*, *Ruminococcaceae* and *Muribaculaceae*, in the mouse gut system during giardiasis

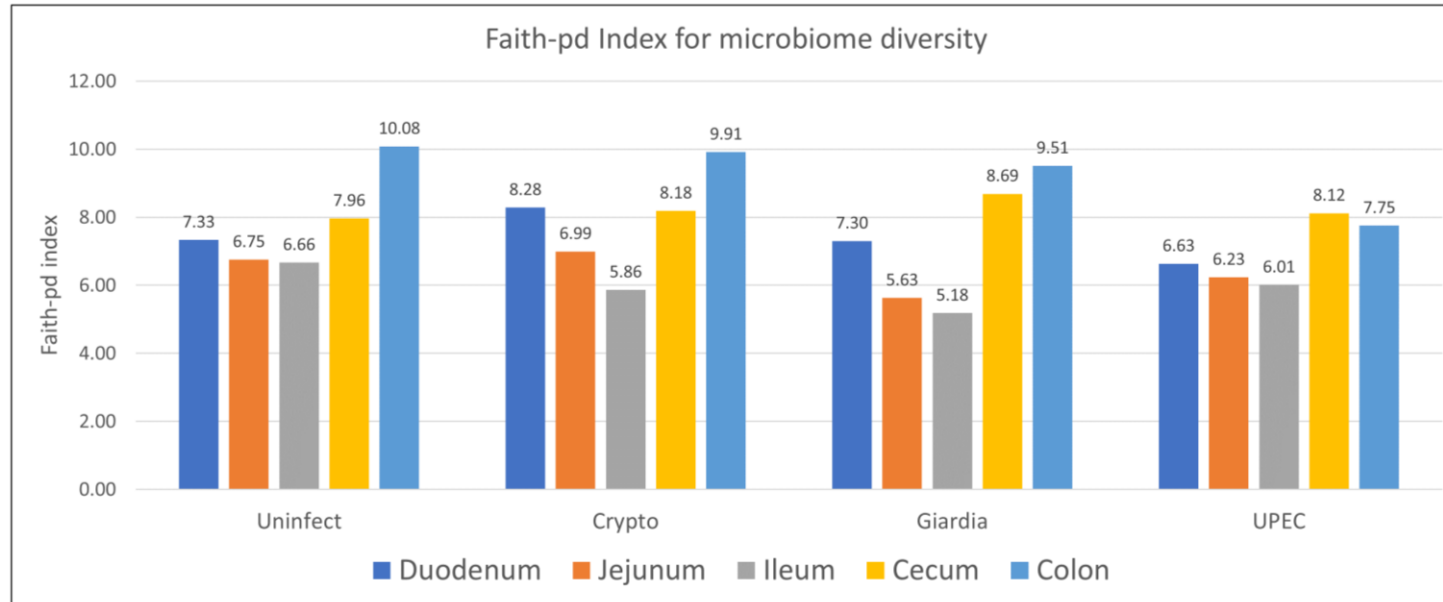


Figure S11. – Faith-pd index indicating overall microbial index detected in parts of the gut after specific diet treatment. Ileum was found most affected part of the gut showing decrease in the overall diversity during cryptosporidiosis and giardiasis.

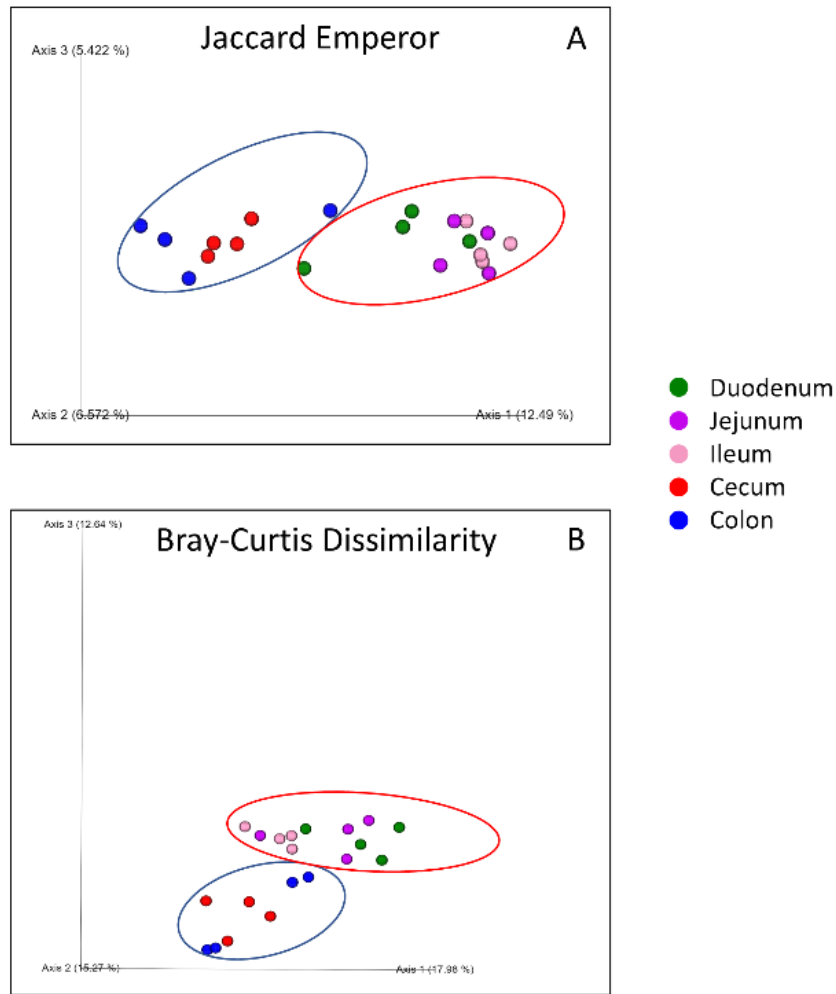
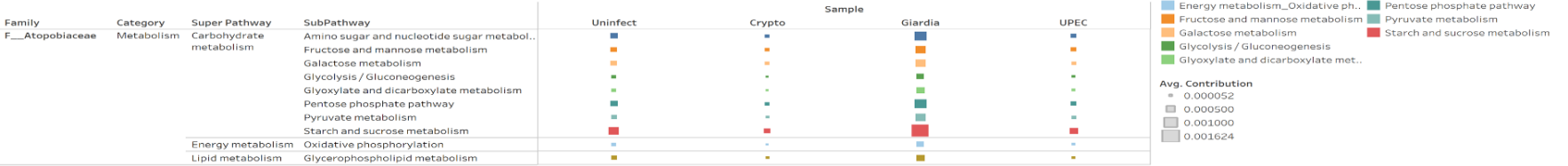
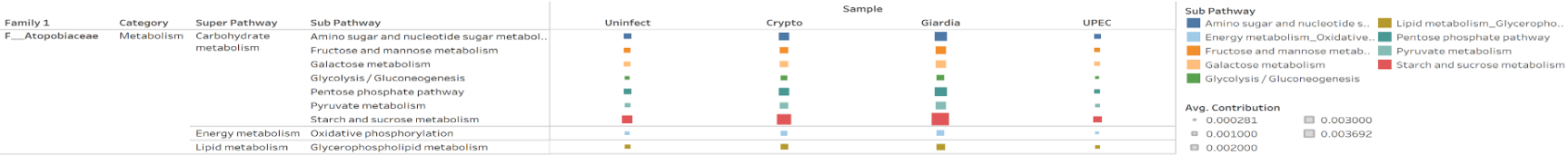


Figure S12. Jaccard Emperor (A) and Bray-Curtis Dissimilarity PCoA plots showing dissimilarity between small intestine (Duodenum, Jejunum and Ileum) and large intestine (Cecum and Colon). Axis 1 represents maximum dissimilarity followed by axis 2 and axis 3 between samples.

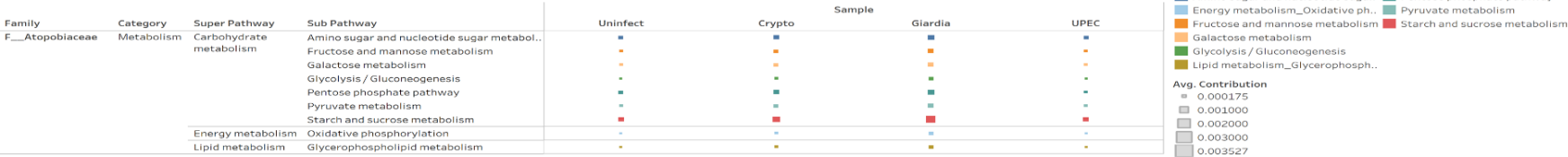
Duodenum



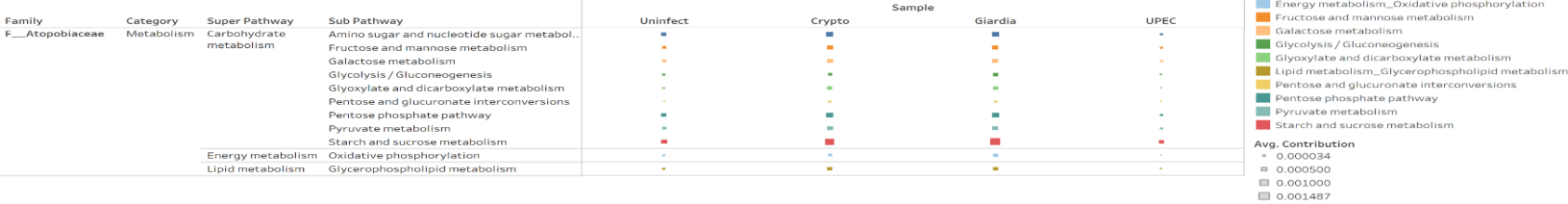
Jejunum



Ileum



Cecum



Colon

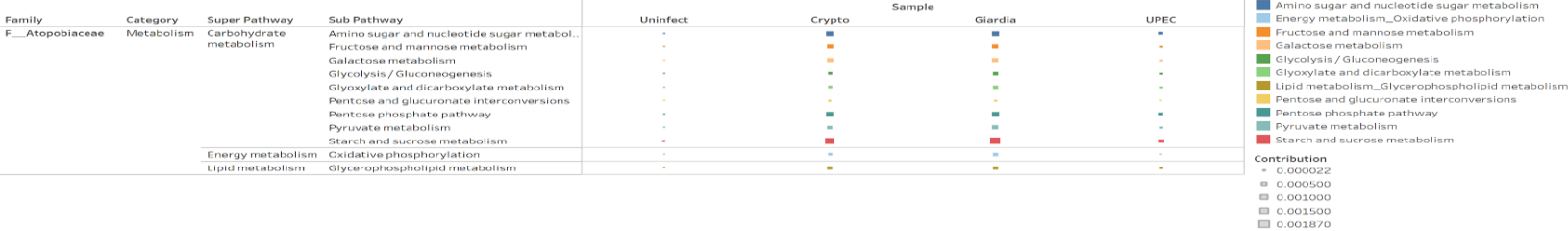
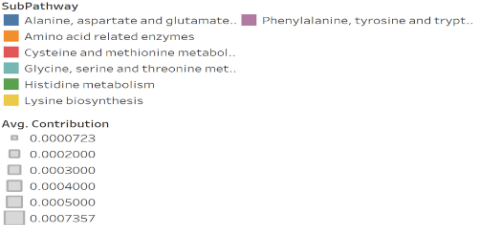
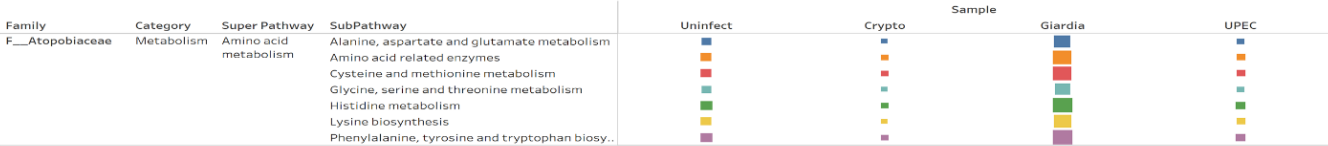
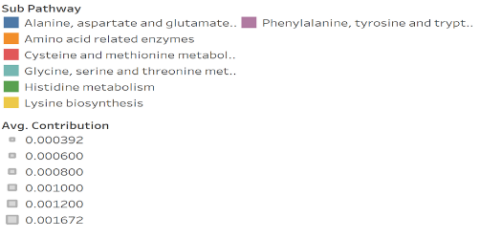
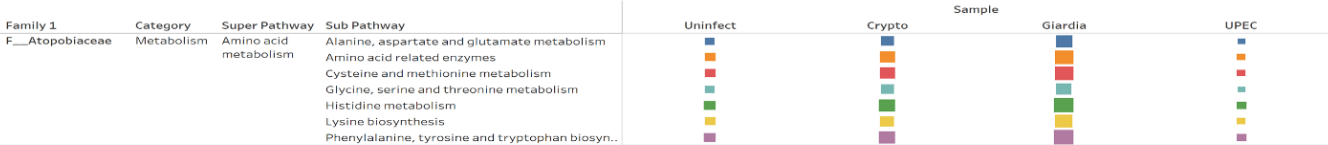


Figure S13. Functional analysis for *Atopobiaceae* family to predict its effect on **carbohydrate metabolism, energy metabolism and lipid metabolism** during cryptosporidiosis and giardiasis.

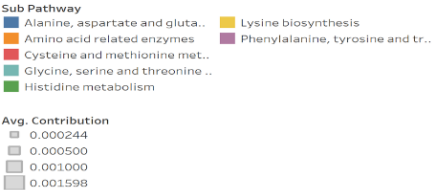
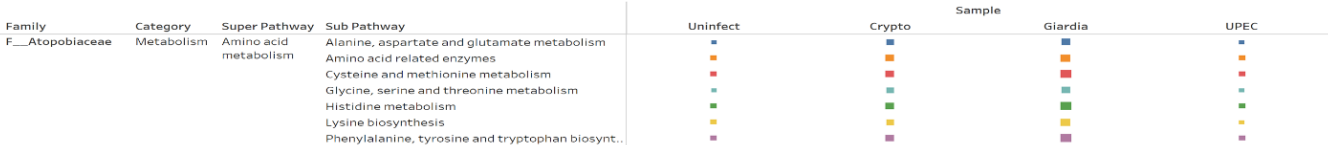
Duodenum



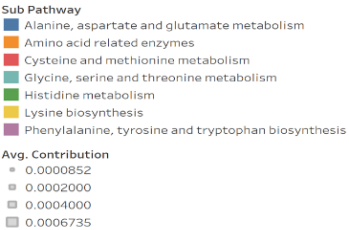
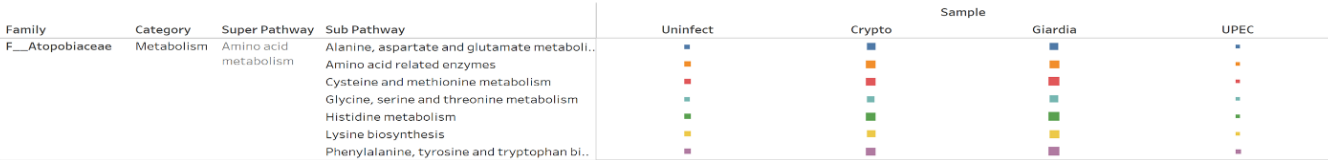
Jejunum



Ileum



Cecum



Colon

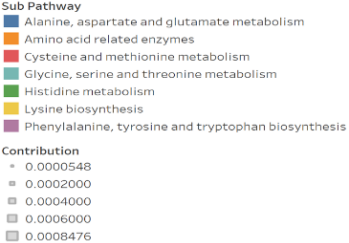
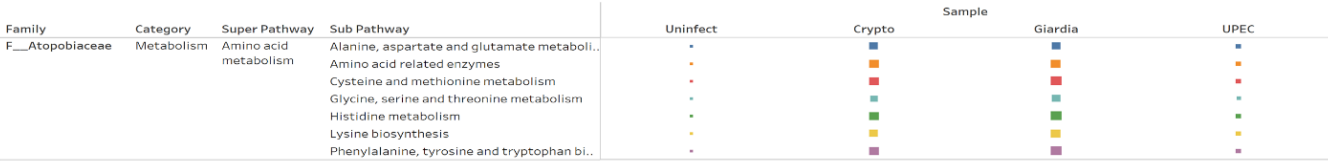
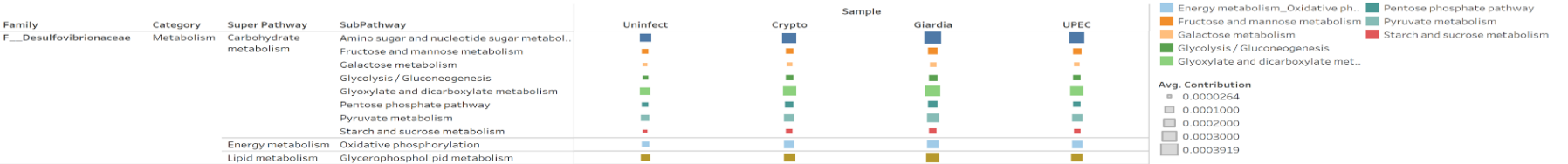
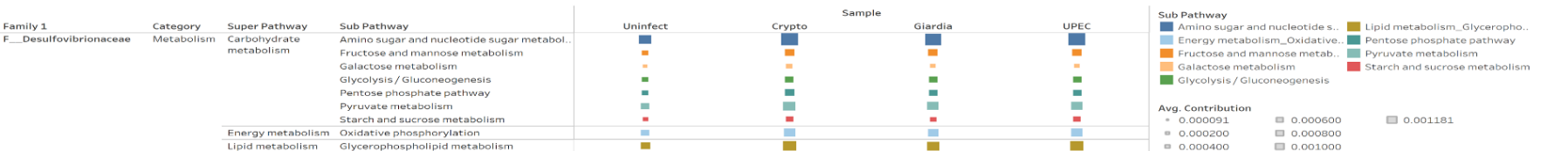


Figure S14. – Functional analysis for *Atopobiaceae* family to predict its effect on **amino acid metabolism** during cryptosporidiosis and giardiasis.

Duodenum



Jejunum



Ileum



Cecum

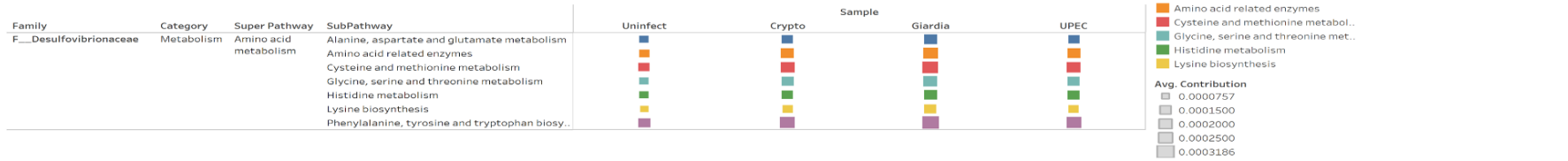


Colon

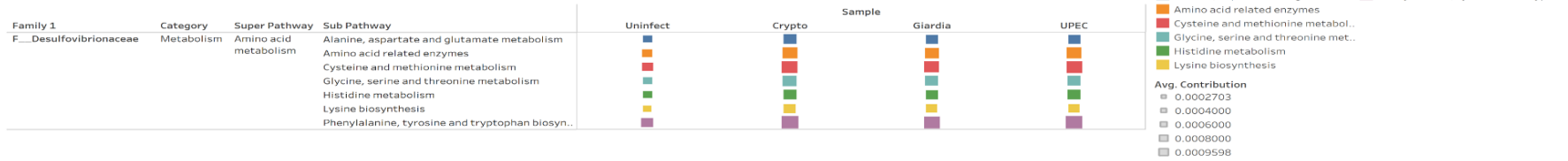


Figure S15. Functional analysis for *Desulfovibrionaceae* family to predict its effect on **carbohydrate metabolism, energy metabolism and lipid metabolism** during cryptosporidiosis and giardiasis.

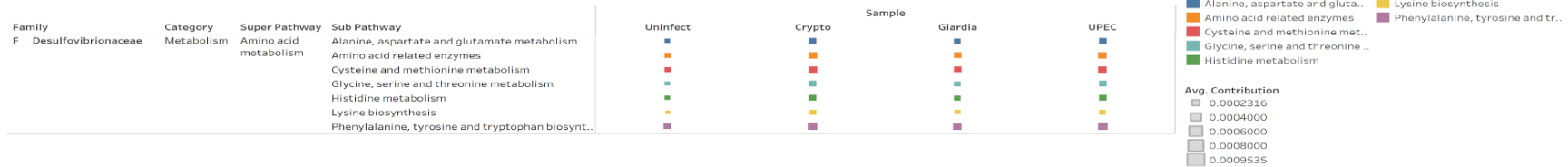
Duodenum



Jejunum



Ileum



Cecum



Colon

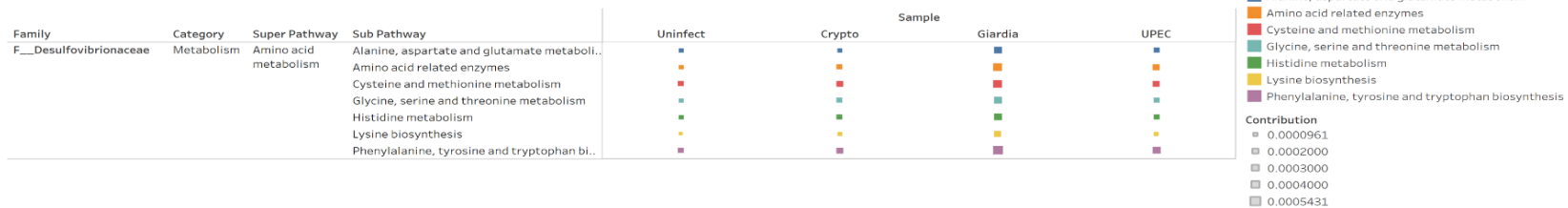
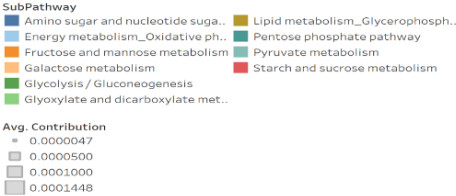
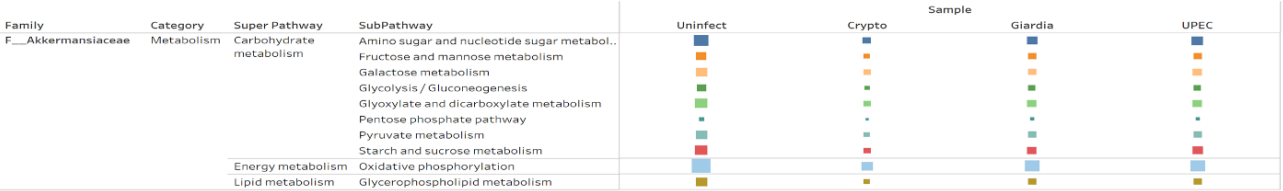
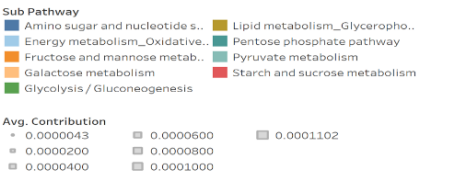
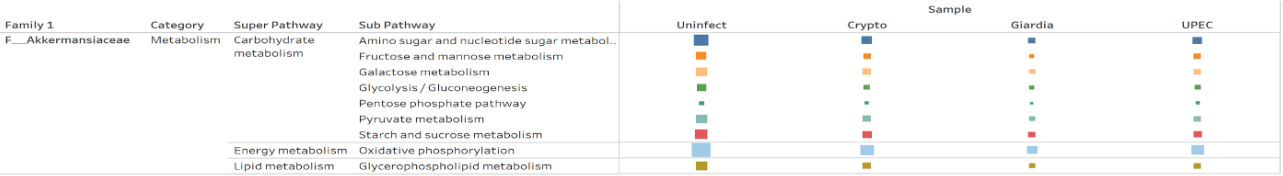


Figure S16. Functional analysis for *Desulfovibrionaceae* family to predict its effect on **amino acid metabolism** during cryptosporidiosis and giardiasis.

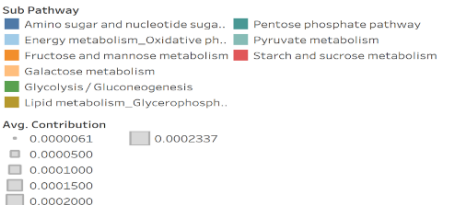
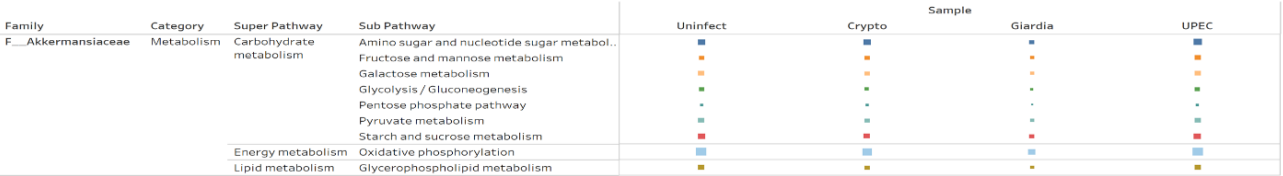
Duodenum



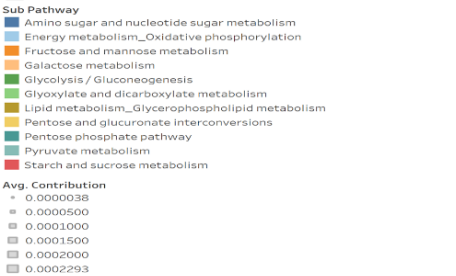
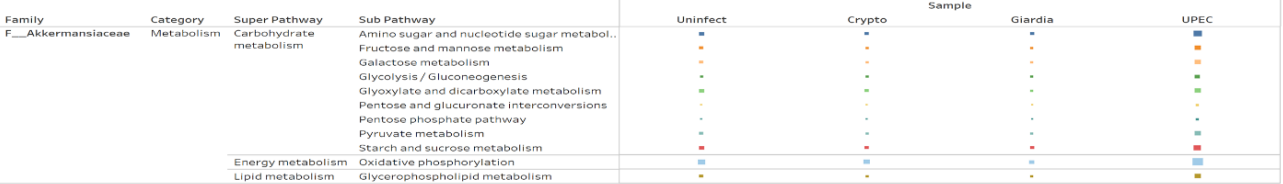
Jejunum



Ileum



Cecum



Colon

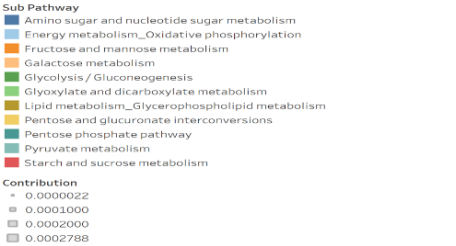
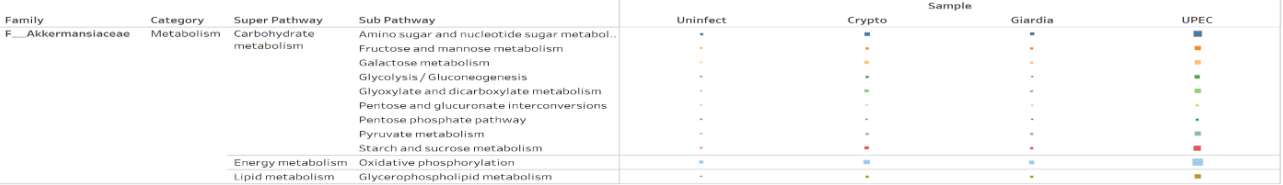
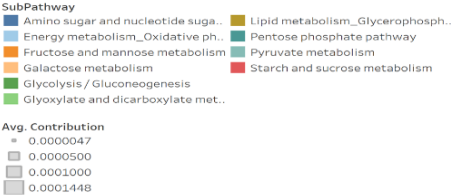
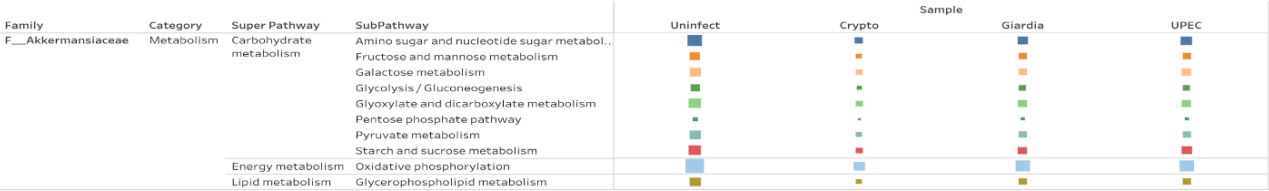
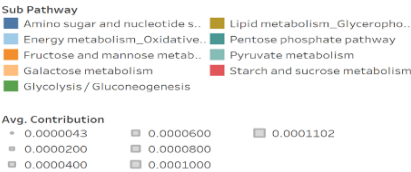
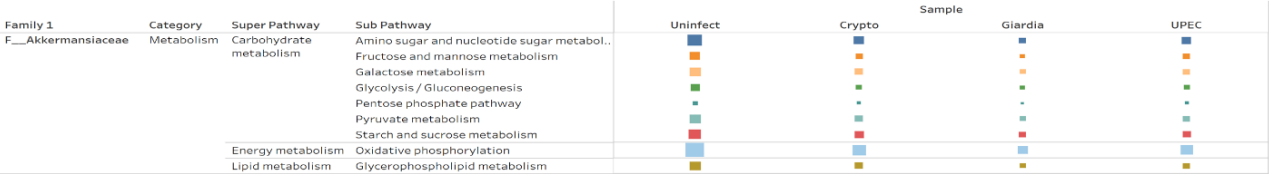


Figure S17. Functional analysis for *Akkermansiaceae* family to predict its effect on **carbohydrate metabolism, energy metabolism and lipid metabolism** during cryptosporidiosis and giardiasis.

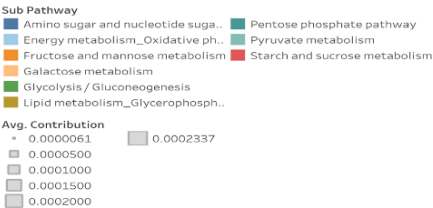
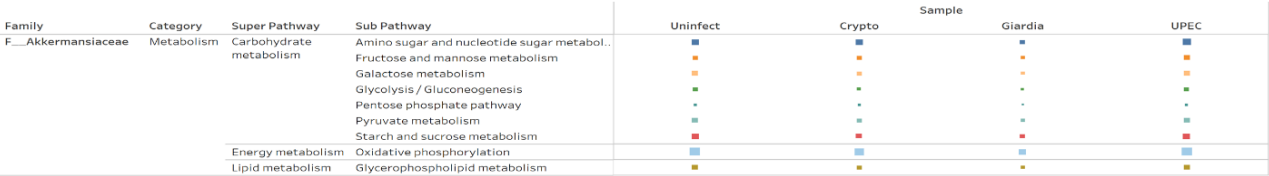
Duodenum



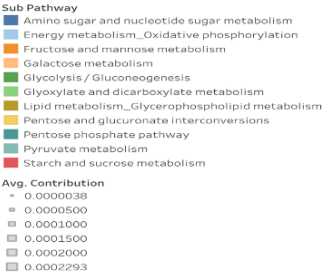
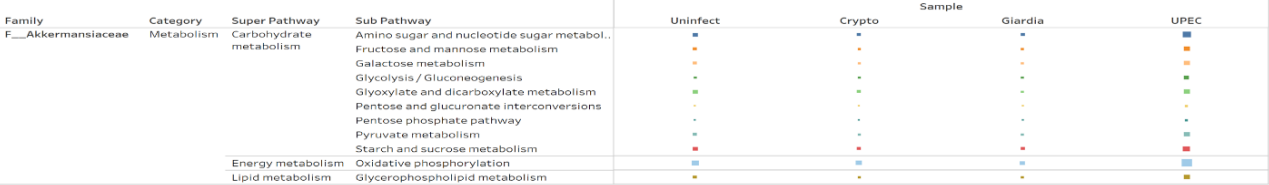
Jejunum



Ileum



Cecum



Colon

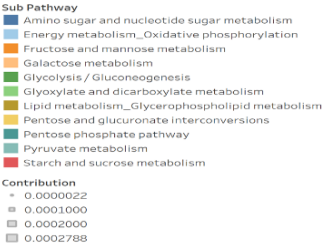
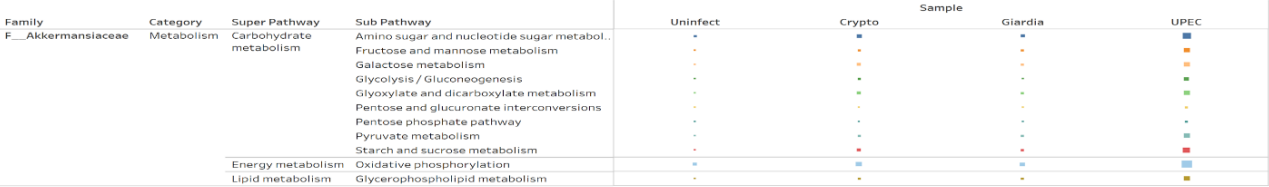


Figure S 18. Functional analysis for *Desulfovibrionaceae* family to predict its effect on **amino acid metabolism** during cryptosporidiosis and giardiasis.