

**Enriched environment on the intestinal mucosal barrier and brain-gut axis in rats with colorectal cancer**

**Short title:** Enriched environment on intestinal mucosal barrier and the brain-gut axis in rats with colorectal cancer

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**Abbreviated running title:** Enriched environment on the intestinal mucosal barrier of colorectal cancer

**Table S1 Variance analysis of intestinal villus length based on enlarged space, social support and cognitive stimulation**

<b>Variation</b>	<b>SS</b>	<b>MS</b>	<b>df</b>	<b>F</b>	<b>P</b>
Cognition	171.319	171.319	1	0.581	0.449
Social support	713.614	713.614	1	2.420	0.125
Enlarged space	318.297	318.297	1	1.080	0.303
C*S	0.792	0.792	1	0.003	0.959
C*E	695.874	695.874	1	2.345	0.131
S*E	68.996	68.996	1	0.232	0.632
C*S*E	64.808	64.808	1	0.215	0.644
Bias	17394.630	294.824	55		
Total	2436585.964				

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S2 Variance analysis of intestinal villus width based on enlarged space, social support, and cognitive stimulation**

<b>Variation</b>	<b>SS</b>	<b>MS</b>	<b>df</b>	<b>F</b>	<b>P</b>
Cognition	335.882	335.882	1	0.736	0.394
Social support	1.339	1.339	1	0.003	0.957
Enlarged space	2093.243	2093.243	1	4.590	0.036
C*S	1.023	1.023	1	0.002	0.962
C*E	753.929	753.929	1	1.664	0.202
S*E	804.227	804.227	1	1.775	0.188
C*S*E	439.474	439.474	1	0.969	0.329
Bias	26907.075	456.052	55		
Total	2200151.568				

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S3 Variance analysis of muscle layer thickness based on enlarged space, social support, and cognitive stimulation**

Variation	SS	MS	df	F	P
Cognition	73.893	73.893	1	0.341	0.561
Social support	263.812	263.812	1	1.218	0.274
Enlarged space	651.954	651.954	1	3.010	0.088
C*S	30.429	30.429	1	0.141	0.709
C*E	3100.261	3100.261	1	14.312	0.000
S*E	416.018	416.018	1	1.922	0.171
C*S*E	65.113	65.113	1	0.297	0.588
Bias	12563.542	216.613	58		
Total	2919011.063		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S4 Microscopic morphology of intestinal mucosa in eight groups of rats ( $\mu\text{m}$ ,  $\bar{x}\pm s$ )**

Cognition	Social support	Enlarged space	Intestinal villi	Villi width	Muscularis thickness
No	No	No	201.110 $\pm$ 11.992 <sup>ab</sup>	198.359 $\pm$ 14.695 <sup>ab</sup>	232.909 $\pm$ 9.880 <sup>ab</sup>
No	Yes	No	198.760 $\pm$ 21.406 <sup>ab</sup>	196.571 $\pm$ 39.910 <sup>ab</sup>	220.026 $\pm$ 9.303 <sup>cd</sup>
No	No	Yes	194.120 $\pm$ 22.579 <sup>ab</sup>	178.077 $\pm$ 17.036 <sup>bc</sup>	204.927 $\pm$ 20.282 <sup>fg</sup>
No	Yes	Yes	183.449 $\pm$ 22.099 <sup>cd</sup>	179.832 $\pm$ 17.878 <sup>bc</sup>	206.473 $\pm$ 13.330 <sup>ef</sup>
Yes	No	No	200.046 $\pm$ 10.138 <sup>ab</sup>	191.887 $\pm$ 11.763 <sup>bc</sup>	212.949 $\pm$ 13.920 <sup>de</sup>
Yes	Yes	No	193.116 $\pm$ 21.396 <sup>bc</sup>	179.848 $\pm$ 20.043 <sup>bc</sup>	206.991 $\pm$ 21.304 <sup>cdf</sup>
Yes	No	Yes	202.229 $\pm$ 10.342 <sup>ab</sup>	174.693 $\pm$ 18.014 <sup>cd</sup>	217.405 $\pm$ 9.965 <sup>cd</sup>
Yes	Yes	Yes	195.099 $\pm$ 10.859 <sup>ab</sup>	187.347 $\pm$ 17.604 <sup>bc</sup>	217.735 $\pm$ 14.699 <sup>bcf</sup>

Note: There is no significant difference in the right superscript with one same letter or two same letters between the two groups. There is significant difference in the right superscript with all letter differences.

**Table S5 Analysis of variance of occludin based on enlarged space, social support, and cognitive stimulation**

Variation	SS	MS	df	F	P
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Cognition	6.250	6.250	1	7.520	0.008
Social support	10.523	10.523	1	12.661	0.001
Enlarged space	0.052	0.052	1	0.062	0.804
C*S	0.237	0.237	1	0.274	0.603
C*E	0.237	0.237	1	0.274	0.603
S*E	0.066	0.066	1	0.076	0.784
C*S*E	2.317	2.317	1	2.760	0.102
Bias	49.036	0.831	55		
Total	855.000		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S6 Occludin level in eight groups of colorectal cancer rats ( $\bar{x}\pm s$ )**

Cognition	Social support	Enlarged space	Occludin
No	No	No	2.714±0.756 <sup>de</sup>
No	Yes	No	3.875±0.835 <sup>bc</sup>
No	No	Yes	3.000±0.756 <sup>cde</sup>
No	Yes	Yes	3.250±0.886 <sup>cd</sup>
Yes	No	No	3.500±0.756 <sup>cd</sup>
Yes	Yes	No	4.125±1.246 <sup>abd</sup>
Yes	No	Yes	3.250±0.707 <sup>de</sup>
Yes	Yes	Yes	4.500±1.195 <sup>ab</sup>

Note: There is one or two identical letters in the right sign, which is not statistically significant. The alphabet of the right label is completely different, and there is a statistical significance for the difference.

**Table S7 Variance analysis of plasma IL-10 level based on enlarged space, social support, and cognitive stimulation**

Variation	SS	MS	df	F	P
cognition	187968.974	187968.974	1	398.299	0.000
social support	177827.509	177827.509	1	376.810	0.000
enlarged space	27024.789	27024.789	1	57.265	0.000
C*S	88754.994	88754.994	1	188.069	0.000

C*E	35709.177	35709.177	1	75.666	0.000
S*E	25547.066	25547.066	1	54.133	0.000
C*S*E	3263.151	3263.151	1	6.914	0.011
Bias	25956.095	471.929	55		
Total	1245710.280				

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S8 Variance analysis of plasma IL-6 level based on enlarged space, social support, and cognition simulation**

Variation	SS	MS	df	F	P
Cognition	11753.962	11753.962	1	37.305	0.000
Social support	15009.914	15009.914	1	47.639	0.000
Enlarged space	2854.897	2854.897	1	9.061	0.004
C*S	50264.140	50264.140	1	159.530	0.000
C*E	250.569	250.569	1	0.785	0.379
S*E	161.213	161.213	1	0.505	0.480
C*S*E	1076.659	1076.659	1	3.526	0.066
Bias	18274.467	315.077	58		
Total	3762488.228		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S9 Variance analysis of plasma TNF- $\alpha$  based on enlarged space, social support, and cognition**

Variation	SS	MS	df	F	P
Cognition	9685.064	9685.064	1	172.524	0.000
Social support	8045.435	8045.435	1	143.317	0.000
Enlarged space	6346.036	6346.036	1	9.310	0.000
C*S	538.452	538.452	1	113.045	0.003

C*E	8009.906	8009.906	1	142.684	0.000
S*E	2560.892	2560.892	1	45.618	0.000
C*S* E	31.752	31.752	1	0.561	0.457
Bias	3143.692	56.137	56		
Total	800163.976		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S10 Plasma cytokines level in eight groups of colorectal cancer rats (0. 1pg/ml,  $\bar{x}\pm s$ )**

Cognition	Social support	Enlarged space	Plasma IL-10	Plasma IL-6	Plasma TNF- $\alpha$
No	No	No	15.531 $\pm$ 12.010 <sup>a</sup>	235.893 $\pm$ 9.629 <sup>d</sup>	144.020 $\pm$ 5.575 <sup>a</sup>
No	Yes	No	72.657 $\pm$ 5.631 <sup>b</sup>	257.052 $\pm$ 32.726 <sup>bcd</sup>	104.283 $\pm$ 6.009 <sup>f</sup>
No	No	Yes	47.636 $\pm$ 10.249 <sup>c</sup>	247.968 $\pm$ 7.506 <sup>bcd</sup>	130.274 $\pm$ 13.864 <sup>b</sup>
No	Yes	Yes	52.949 $\pm$ 21.079 <sup>c</sup>	277.986 $\pm$ 8.897 <sup>bc</sup>	113.168 $\pm$ 4.794 <sup>bde</sup>
Yes	No	No	82.996 $\pm$ 8.88 <sup>b</sup>	259.817 $\pm$ 13.083 <sup>ab</sup>	92.279 $\pm$ 3.427 <sup>e</sup>
Yes	Yes	No	319.220 $\pm$ 43.224 <sup>d</sup>	184.530 $\pm$ 9.223 <sup>e</sup>	61.350 $\pm$ 1.687 <sup>g</sup>
Yes	No	Yes	48.592 $\pm$ 15.478 <sup>c</sup>	282.330 $\pm$ 13.767 <sup>ab</sup>	120.778 $\pm$ 10.247 <sup>bc</sup>
Yes	Yes	Yes	175.373 $\pm$ 28.575 <sup>f</sup>	182.798 $\pm$ 25.833 <sup>e</sup>	118.168 $\pm$ 6.859 <sup>cd</sup>

Note: There is no significant difference in the right superscript with one same letter or two same letters between the two groups. There is significant difference in the right superscript with all letter differences.

**Table S11 Variance analysis of IL-10 level in intestinal mucosa based on enlarged space, social support, and cognition**

Variation	SS	MS	df	F	P
Cognition	114833.162	114833.162	1	99.787	0.000
Social support	163688.289	163688.289	1	142.241	0.000
Enlarged space	40150.947	40150.947	1	34.890	0.000
C*S	257693.097	257693.097	1	223.929	0.000
C*E	280.419	280.419	1	0.240	0.626
S*E	34487.790	34487.790	1	29.969	0.000
C*S*E	8.282	8.282	1	0.007	0.934
Bias	65594.397	1150.779	55		

Total 24595017.75 63

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S12 Variance analysis of IL-6 level in intestinal mucosa based on enlarged space, social support, and cognition**

Variation	SS	MS	df	F	P
Cognition	25264402.41	25264402.41	1	11673.269	0.000
Social support	524281.491	524281.491	1	242.241	0.000
Enlarged space	74397.066	74397.066	1	34.375	0.000
C*S	816352.196	816352.196	1	377.191	0.000
C*E	13620.791	13620.791	1	6.293	0.015
S*E	77841.013	77841.013	1	35.966	0.000
C*S*E	26955.975	26955.975	1	12.455	0.001
Bias	119036.243	2164.295	55		
Total	27279066.55		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S13 Variance analysis of the level of TNF- $\alpha$  in the intestinal mucosa based on enlarged space, social support, and cognition**

Variation	SS	MS	df	F	P
Cognition	63721.562	63721.562	1	351.579	0.000
Social support	37031.081	37031.081	1	204.316	0.000
Enlarged space	33888.557	33888.557	1	186.978	0.000
C*S	2369.945	2369.945	1	13.076	0.001
C*E	10790.318	10790.318	1	59.535	0.000
S*E	43901.698	43901.698	1	242.225	0.000

C*S*E	11200.847	11200.847	1	61.800	0.000
Bias	9968.410	181.244	55		
Total	4179460.763		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S14 Cytokines levels in the intestinal mucosa in eight groups of rats with colorectal cancer (0.01 pg/ml,  $\bar{x}\pm s$ )**

Cognition	Social support	Enlarged space	Intestinal mucosal IL-10	Intestinal mucosal IL-6	Intestinal mucosal TNF- $\alpha$
No	No	No	585.794 $\pm$ 24.291 <sup>b</sup>	611.023 $\pm$ 34.438 <sup>c</sup>	611.023 $\pm$ 34.438 <sup>d</sup>
No	Yes	No	606.090 $\pm$ 4.447 <sup>b</sup>	685.244 $\pm$ 82.659 <sup>b</sup>	267.575 $\pm$ 13.453 <sup>b</sup>
No	No	Yes	585.700 $\pm$ 10.96 <sup>b</sup>	738.215 $\pm$ 44.489 <sup>b</sup>	376.464 $\pm$ 9.515 <sup>c</sup>
No	Yes	Yes	513.676 $\pm$ 6.51 <sup>c</sup>	754.517 $\pm$ 30.851 <sup>b</sup>	260.665 $\pm$ 13.766 <sup>b</sup>
Yes	No	No	546.845 $\pm$ 8.792 <sup>c</sup>	700.201 $\pm$ 45.962 <sup>b</sup>	700.201 $\pm$ 45.962 <sup>c</sup>
Yes	Yes	No	824.469 $\pm$ 61.249 <sup>a</sup>	401.466 $\pm$ 48.049 <sup>d</sup>	191.134 $\pm$ 8.317 <sup>a</sup>
Yes	No	Yes	539.782 $\pm$ 49.659 <sup>bc</sup>	851.341 $\pm$ 36.479 <sup>a</sup>	272.180 $\pm$ 20.814 <sup>b</sup>
Yes	Yes	Yes	722.183 $\pm$ 48.441 <sup>a</sup>	329.044 $\pm$ 21.750 <sup>c</sup>	185.211 $\pm$ 12.180 <sup>a</sup>

Note: There is no significant difference in the right superscript with one same letter or two same letters between the two groups. There is significant difference in the right superscript with all letter differences.

**Table S15 Variance analysis of the SIgA level in the intestinal mucosa based on enlarged space, social support, and cognitive simulation**

Variation	SS	MS	df	F	P
Cognition	12.046	12.046	1	14.709	0.000
Social support	8.850	8.850	1	10.806	0.02
Enlarged space	0.000	0.000	1	0.000	1.000
C*S	0.966	0.966	1	1.222	0.274
C*E	8.850	8.850	1	10.806	0.002
S*E	2.237	2.237	1	2.831	0.098
C*S*E	2.317	2.317	1	3.039	0.087
Bias	47.500	0.819	55		
Total	724.000		63		



Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S16 SIgA level in eight groups of rats with colorectal cancer (  $\bar{x} \pm s$  )**

Cognition	Social support	Enlarged space	SIgA
No	No	No	1.714±0.951 <sup>e</sup>
No	Yes	No	3.000±0.926 <sup>bc</sup>
No	No	Yes	3.250±1.165 <sup>bc</sup>
No	Yes	Yes	3.000±0.756 <sup>bc</sup>
Yes	No	No	3.500±0.535 <sup>bc</sup>
Yes	Yes	No	4.500±0.756 <sup>a</sup>
Yes	No	Yes	2.750±1.035 <sup>cd</sup>
Yes	Yes	Yes	3.75±0.707 <sup>ab</sup>

Note: There is no significant difference in the right superscript with one same letter or two same letters between the two groups. There is significant difference in the right superscript with all letter differences.

**Table S17 Variance analysis of CRF levels in the hypothalamus based on enlarged space, social support, and cognitive stimulation**

Variation	SS	MS	df	F	P
Cognition	121537.698	121537.698	1	221.197	0.000
Social support	19270.448	19270.448	1	35.072	0.000
Enlarged space	14499.563	14499.563	1	26.389	0.000
C*S	31587.652	31587.652	1	57.489	0.000
C*E	6.912	6.912	1	0.013	0.911
S*E	11962.188	11962.188	1	21.771	0.000
C*S*E	10839.627	10839.627	1	19.728	0.000
Bias	30219.980	549.454	55		
Total	947944.477		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors.

C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S18 CRF levels in the hypothalamus of rats with colorectal cancer (pg/g,  $\bar{x} \pm s$ )**

Cognition	Social support	Enlarged space	Hypothalamus CRF
No	No	No	49.848±16.843 <sup>de</sup>
No	Yes	No	41.360±27.504 <sup>e</sup>
No	No	Yes	82.209±15.375 <sup>bc</sup>
No	Yes	Yes	71.068±19.448 <sup>cd</sup>
Yes	No	No	67.355±24.792 <sup>cd</sup>
Yes	Yes	No	201.041±27.216 <sup>a</sup>
Yes	No	Yes	150.908±15.755 <sup>a</sup>
Yes	Yes	Yes	176.903±33.155 <sup>a</sup>

Note: There is no significant difference in the right superscript with one same letter or two same letters between the two groups. There is significant difference in the right superscript with all letter differences.

**Table S19 Variance analysis of intestinal mucosal ghrelin by enlarged space, social support, and cognitive stimulation**

Variation	SS	MS	df	F	P
Cognition	219307143.6	219307143.6	1	153.017	0.000
Social support	32744658.59	32744658.59	1	22.847	0.000
Enlarged space	429139.762	429139.762	1	0.299	0.586
C*S	40211093.43	40211093.43	1	28.056	0.000
C* E	22667295.17	22667295.17	1	15.816	0.000
S*E	224979921.7	224979921.7	1	156.975	0.000
C*S* E	73414857.01	73414857.01	1	51.224	0.000
Bias	78827268.55	1433223.065	55		
Total	3.931E+10		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S20 Variance analysis of ghrelin level in the hypothalamus by enlarged space, social support, and cognition**

Variation	SS	MS	df	F	P
Cognition	235442289.9	235442289.9	1	28.149	0.000
Social support	207031266.6	207031266.6	1	24.752	0.000
Enlarged space	53329777.92	53329777.92	1	6.376	0.014
C*S	93253153.84	93253153.84	1	11.149	0.001
C*E	112672393.7	112672393.7	1	13.471	0.001
S*E	183872.158	183872.158	1	0.022	0.884
C*S*E	21779208.09	21779208.09	1	2.634	0.110
Bias	476580074.9	476580074.9	55		
Total	1.931E+10		63		

Note: SS is stdev square, MS is mean square, df is degree freedom. C is cognition, S is social support, E is enlarged space. C\*S is the interaction of cognition and social support factors. C\*E is the interaction of cognition and enlarged space factors. S\*E is the interaction of social support and enlarged space factors. C\*S\*E is the interaction of cognition, social support and enlarged space factors.

**Table S21 Intestinal and hypothalamic ghrelin secretion(  $\bar{x} \pm s$  )**

Cognition	Social support	Enlarged space	Intestinal mucosa Ghrelin	Hypothalamus Ghrelin
No	No	No	20448.714±859.189 <sup>h</sup>	14567.429±1654.631 <sup>cd</sup>
No	Yes	No	26236.875±1269.350 <sup>de</sup>	14633.375±2783.172 <sup>cd</sup>
No	No	Yes	25357.375±762.349 <sup>ef</sup>	14275.375±2485.560 <sup>d</sup>
No	Yes	Yes	19257.000±1479.306 <sup>h</sup>	16520.500±3246.074 <sup>bcd</sup>
Yes	No	No	23544.750±1153.422 <sup>g</sup>	17444.500±3824.842 <sup>bc</sup>
Yes	Yes	No	28209.500±749.865 <sup>ab</sup>	24772.000±3409.338 <sup>a</sup>
Yes	No	Yes	26532.875±665.471 <sup>cd</sup>	14191.250±2321.198 <sup>d</sup>
Yes	Yes	Yes	27953.500±1977.875 <sup>bc</sup>	18989.625±253.985 <sup>b</sup>

Note: There is one or two identical letters in the right sign, which is not statistically significant. The alphabet of the right label is completely different, and there is a statistical significance for the difference.