

Figure S1

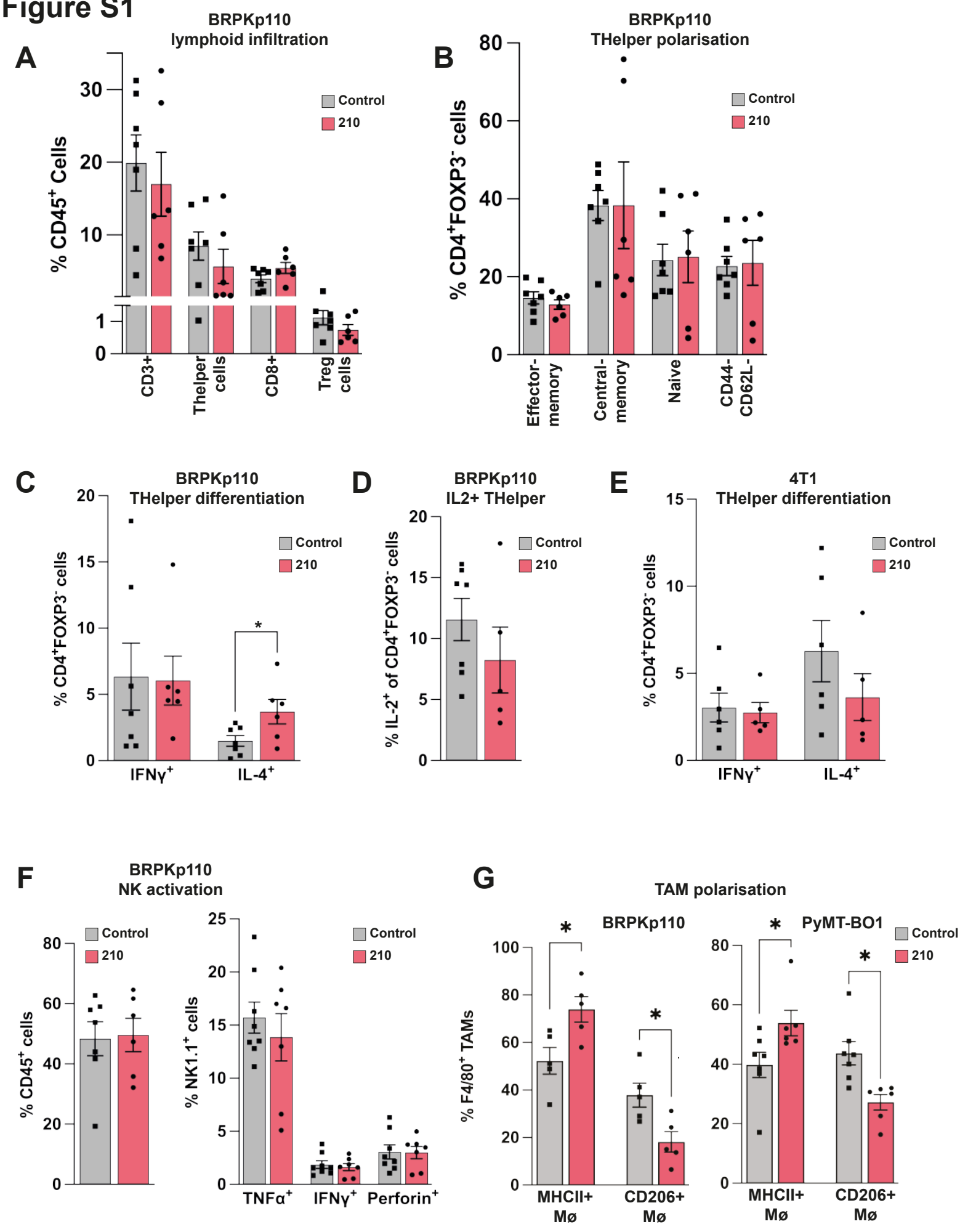
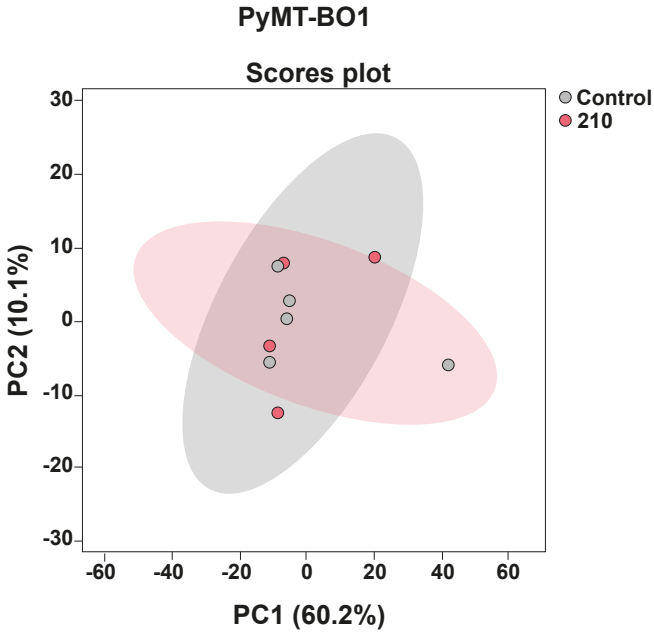
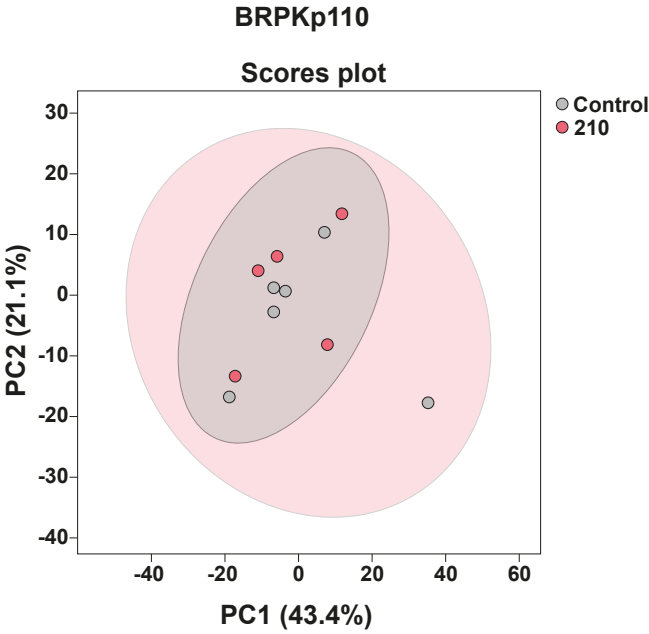
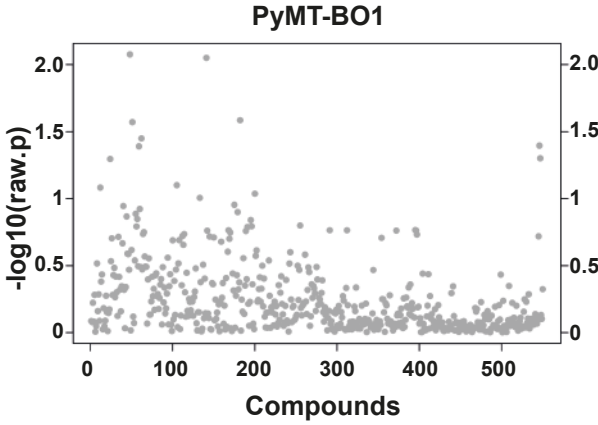
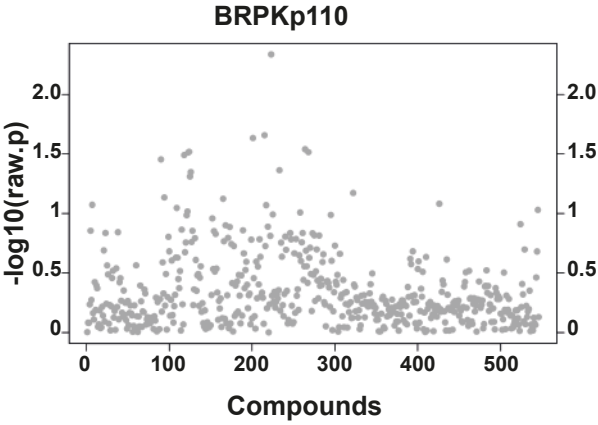


Figure S2

A



B



C

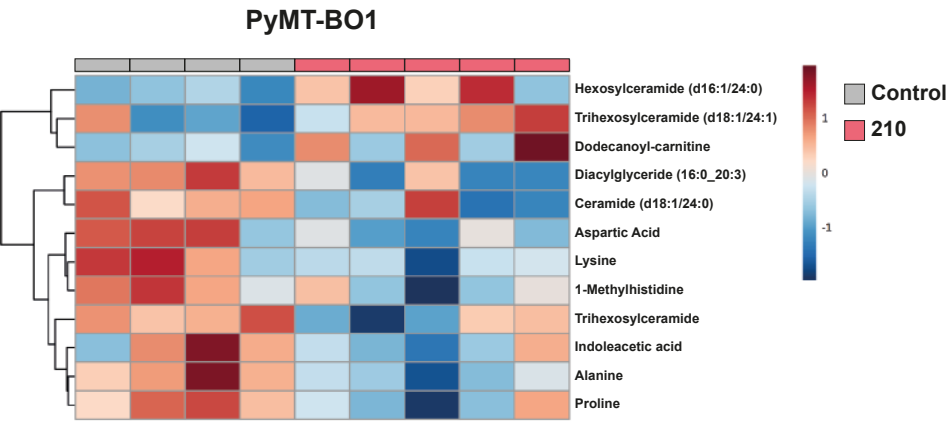
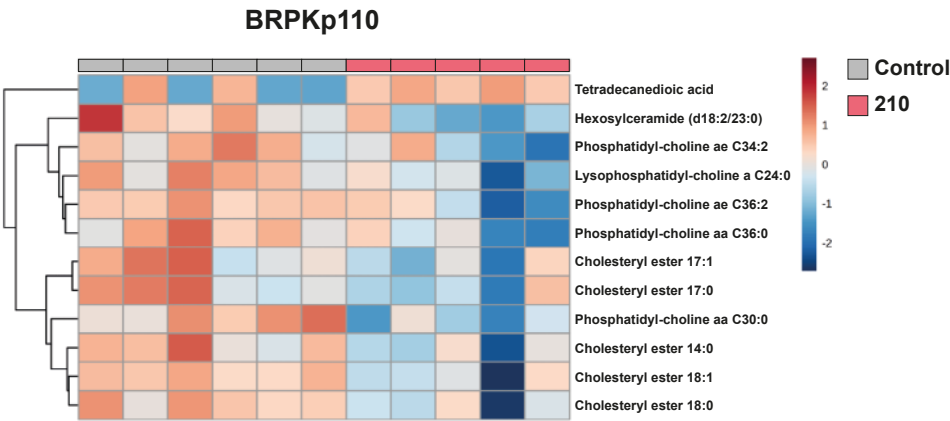
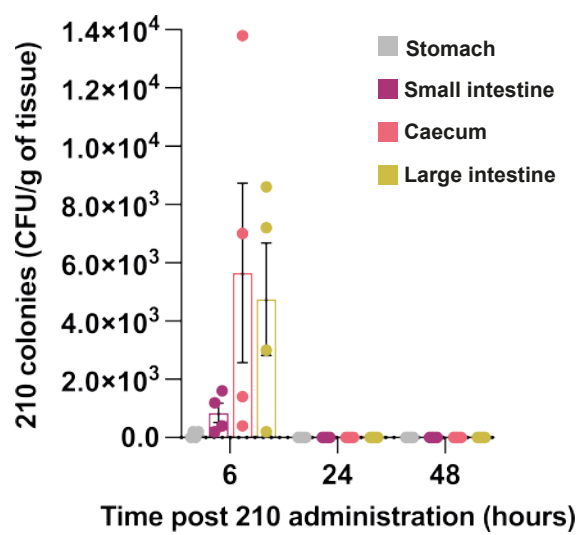
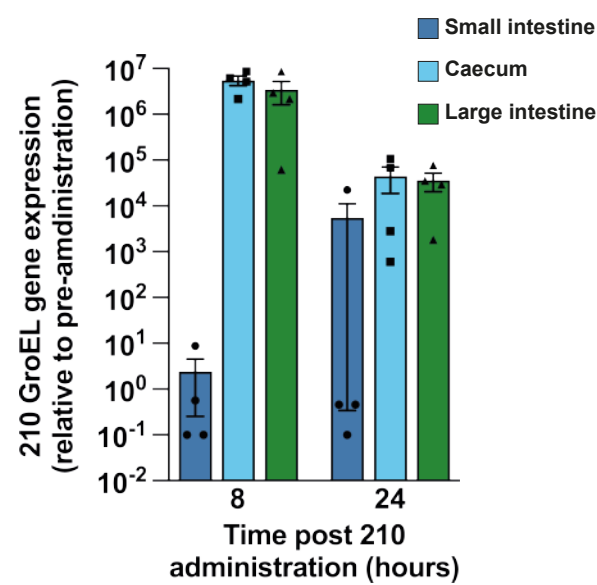


Figure S3

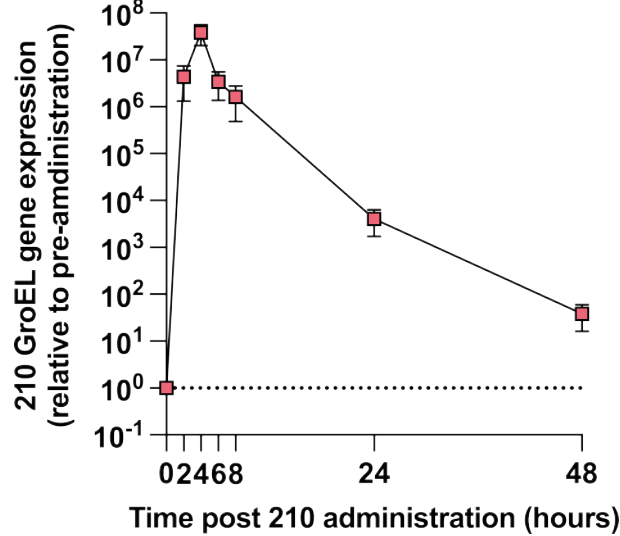
A



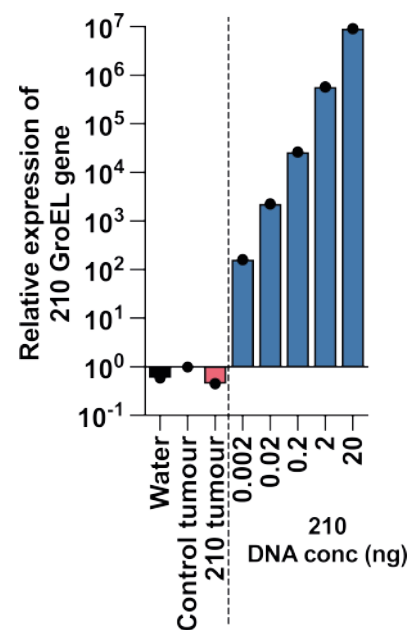
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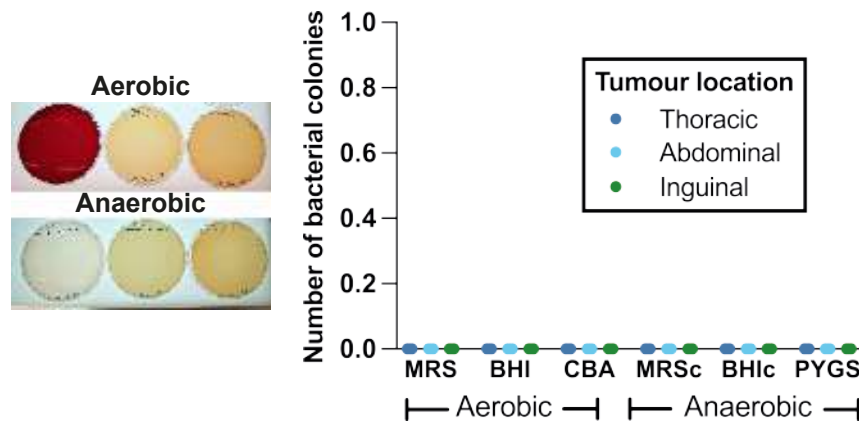
C



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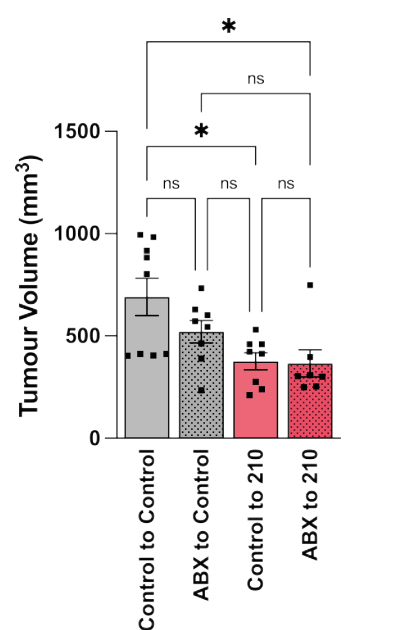
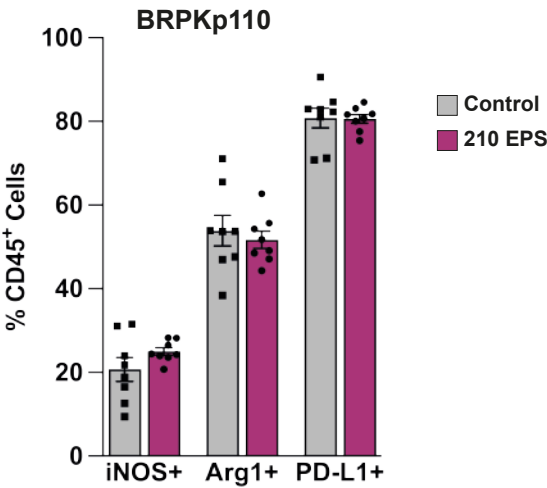
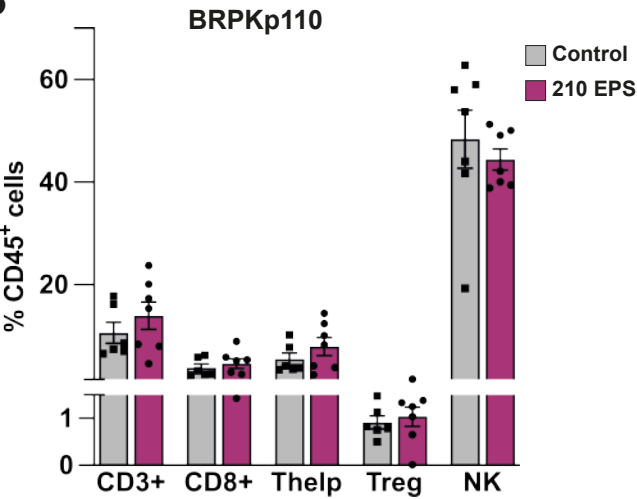


Figure S4

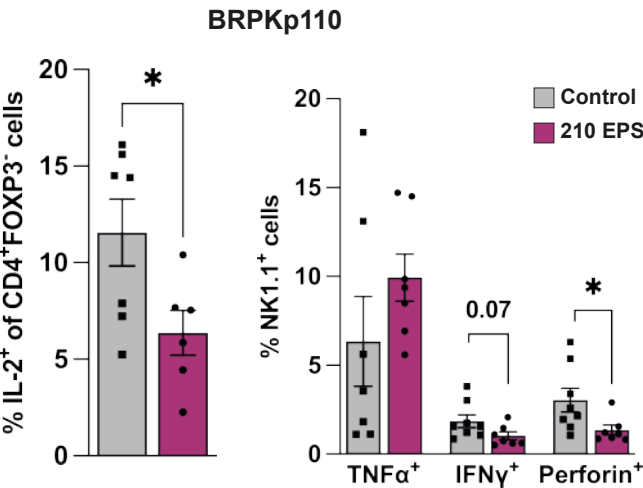
A



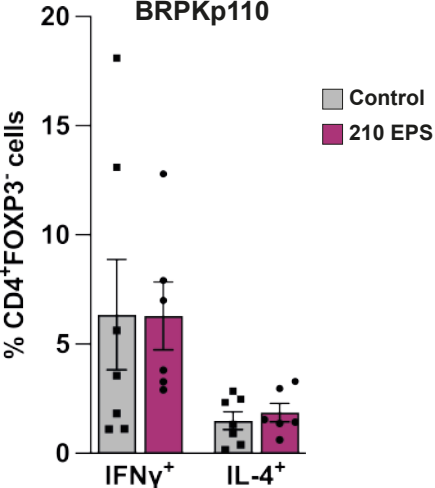
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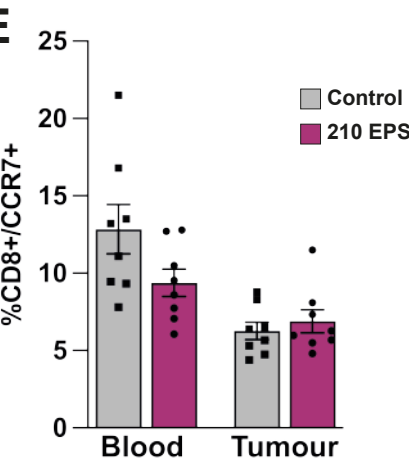
C



D



E



F

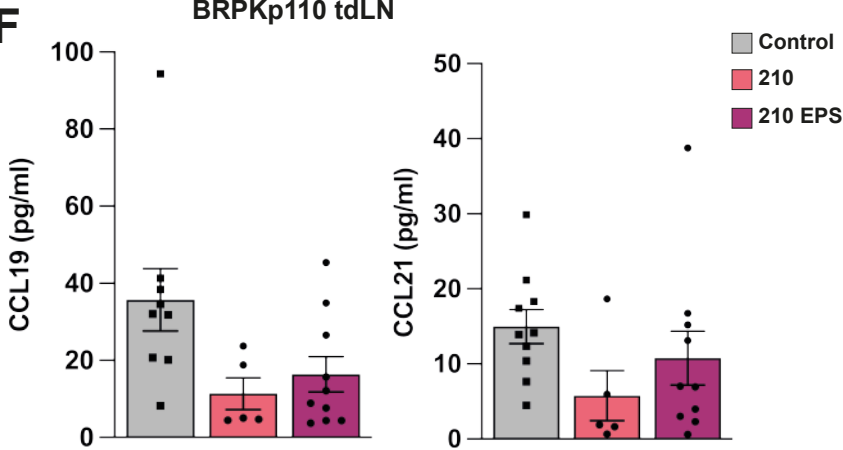


Figure S5

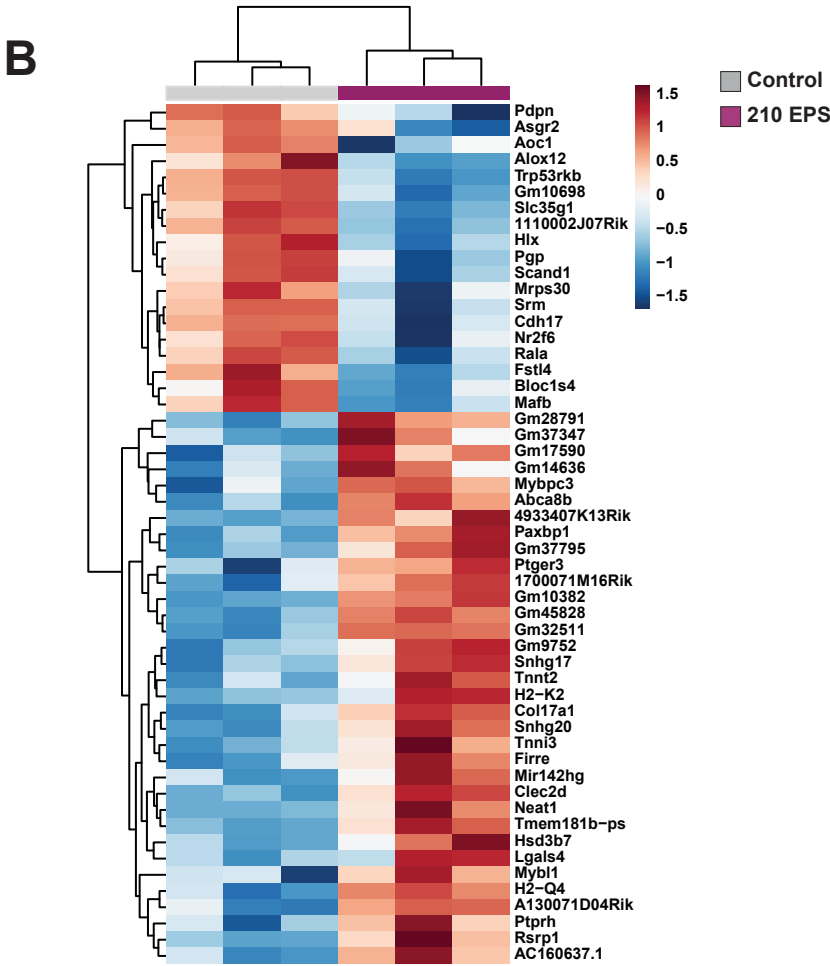
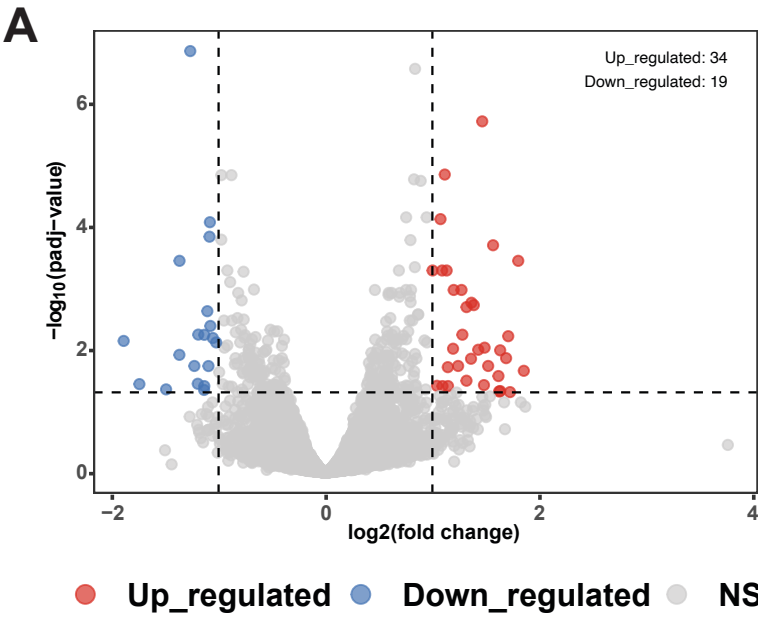


Figure S6

A

- ABC transporter

● acyltransferase

● flippase

● glycosyl hydrolase

● glycosyl transferase
- membrane spanning protein

● other function

● polymerase

● polysaccharide binding protein

● polysaccharide biosynthesis /chain length regulator
- priming glycosyl transferase

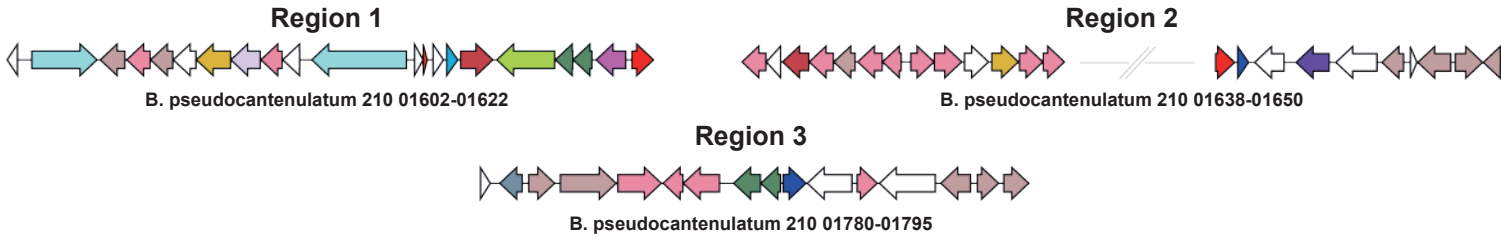
● protein tyrosine phosphatase

● rhamnose precursors biosynthesis

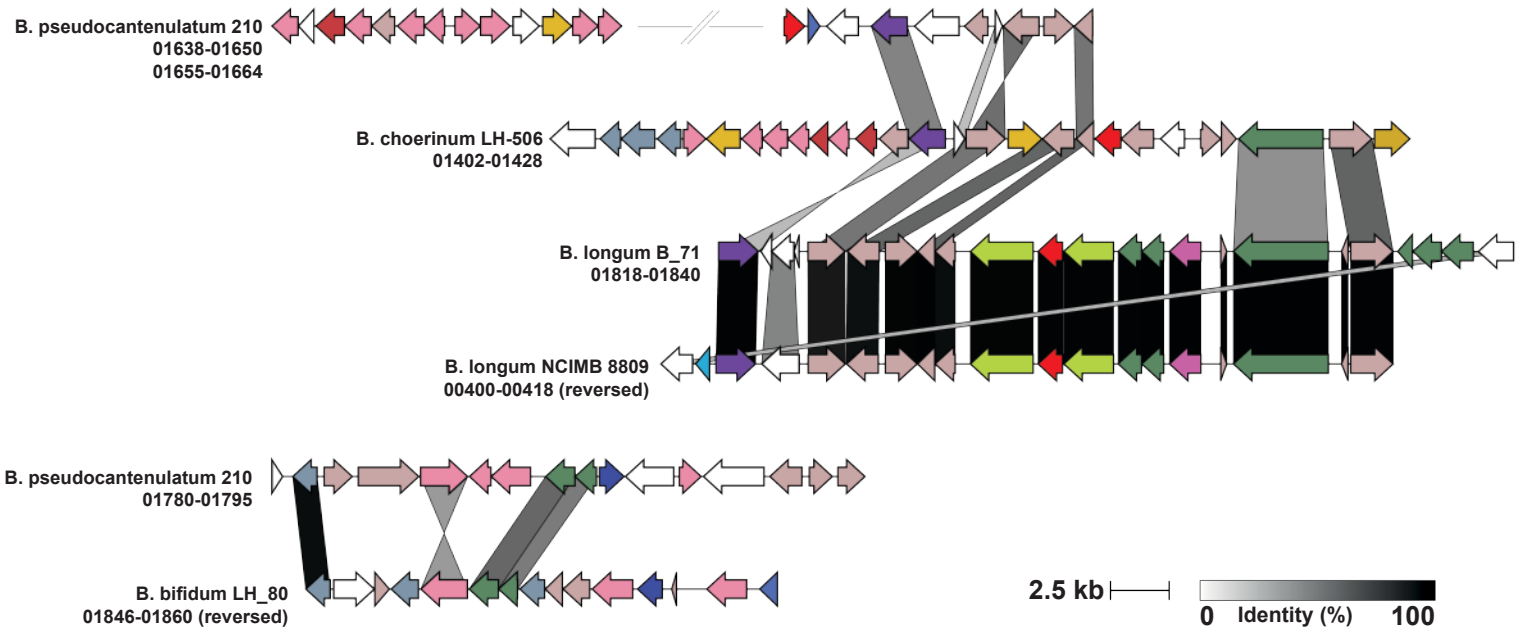
● solute-binding protein

● transcriptional regulator
- transposase

○ unknown function



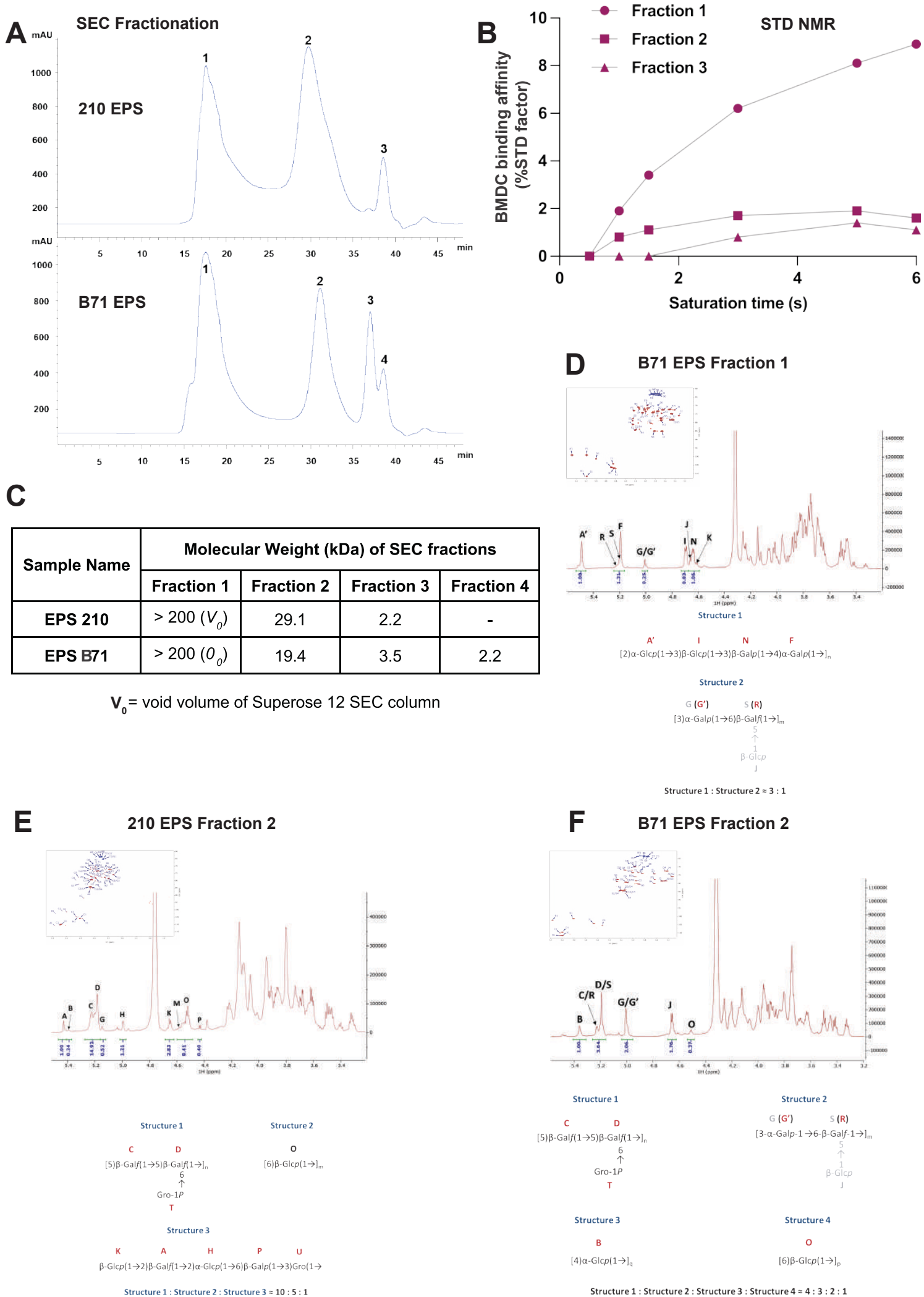
B



Bar graph showing the effect of 210 EPS, DSM EPS, and B71 EPS on the release of endotoxin (LPS) from *E. coli* cells. The y-axis represents '% CD release' (0-80) and the x-axis represents 'EPS dose' (1µg, 10µg, 100µg). A legend indicates: Unstimulated (grey), 210 EPS (pink), DSM EPS (pink with diagonal lines), and B71 EPS (blue). Error bars represent standard deviation.

EPS dose	Unstimulated	210 EPS	DSM EPS	B71 EPS
1µg	~28	~48	~50	~45
10µg	~28	~62	~60	~50
100µg	~28	~68	~60	~58

Figure S8



Supplementary Figure Legends

Figure S1. The *B. pseudocatenulatum* 210-induced immune phenotype is not primarily associated with gross changes in lymphoid infiltration but is accompanied by tumour-associated macrophage repolarisation. (A) Quantification of lymphoid cell infiltration in BRPKp110 primary tumours following 210 administration. $n = 6-7$. (B) Quantification of T helper cell differentiation states in BRPKp110 tumours. $n = 6-7$. (C) Quantification of IFN γ ⁺ and IL-4⁺ CD4⁺FOXP3⁻ T helper cells in BRPKp110 tumours. $n = 6-7$. (D) Quantification of IL-2⁺ CD4⁺FOXP3⁻ T helper cells in BRPKp110 tumours. $n = 6-7$. (E) Quantification of IFN γ ⁺ and IL-4⁺ CD4⁺FOXP3⁻ T helper cells in 4T1 tumours. $n = 5-6$. (F) Quantification of NK cell abundance and TNF α , IFN γ and perforin expression in BRPKp110 tumours. $n = 6-8$. (G) Quantification of MHCII⁺ and CD206⁺ tumour-associated macrophages in BRPKp110 and PyMT-BO1 tumours. $n = 5-7$. Statistical significance was assessed by two-tailed unpaired t test. * $P < 0.05$.

Figure S2. Administration of *B. pseudocatenulatum* 210 does not significantly alter serum metabolite levels in untargeted analyses. (A) PCoA score plots of serum metabolite profiles from BRPKp110- and PyMT-BO1-bearing animals treated with PBS control or 210; shaded ellipses indicate 95% confidence intervals. BRPKp110, $n = 5-6$; PyMT-BO1, $n = 4-5$. (B) Differential metabolite significance plots for BRPKp110 and PyMT-BO1 sera following 210 treatment. (C) Heatmaps showing the top differentially abundant serum metabolites in BRPKp110 and PyMT-BO1-bearing animals following 210 treatment. Statistical significance was assessed by two-tailed unpaired t test with FDR correction.

Figure S3. *B. pseudocatenulatum* 210 is transiently detectable after oral administration, does not colonise tumours, and acts independently of secondary commensals.

(A) Quantification of culturable 210 colonies recovered from stomach, small intestine, caecum and large intestine of germ-free monocolonised mice at the indicated timepoints. $n = 4$. (B) Quantification of *B. pseudocatenulatum* 210 GroEL gene abundance by qPCR in wild-type small intestine, caecum and large intestine following administration. $n = 4$. (C) Time-course of faecal GroEL gene abundance following 210 administration. (D) Standard curve for GroEL qPCR using serial dilutions of 210 DNA and controls. (E) Representative aerobic and anaerobic culture plates and quantification of bacterial colonies recovered from MMTV-PyMT tumours at different anatomical sites. (F) Endpoint BRPKp110 tumour volumes following antibiotic pre-treatment and subsequent supplementation with vehicle or 210. $n = 7-9$. Statistical significance was assessed by one-way ANOVA with Tukey's multiple-comparisons test. * $P < 0.05$.

Figure S4. *B. pseudocatenulatum* 210 EPS-induced anti-tumour immunity is not accompanied by major changes in alternative lymphoid effector populations or tdLN chemokines. (A)

Quantification of iNOS⁺, Arg1⁺ and PD-L1⁺ tumour-associated macrophages in BRPKp110 primary tumours following 210 EPS administration. $n = 7$. (B) Quantification of lymphoid cell infiltration in BRPKp110 primary tumours following 210 EPS administration. $n = 7$. (C) Quantification of IL-2⁺ CD4⁺FOXP3⁻ T helper cells and TNF α , IFN γ and perforin expression by NK1.1⁺ cells in BRPKp110 tumours following 210 EPS administration. $n = 6-7$. (D) Quantification of IFN γ ⁺ and IL-4⁺ CD4⁺FOXP3⁻ T helper cells in BRPKp110 tumours following 210 EPS administration. $n = 6-7$. (E) Quantification of CCR7⁺ CD8⁺ T cells in blood and tumour following 210 EPS administration. (F) Quantification of CCL19 and CCL21 levels in BRPKp110 tumour-draining lymph nodes following

1 treatment with control, 210, or 210 EPS.

2 Statistical significance was assessed by two-tailed unpaired t test. *P < 0.05.

3
4 **Figure S5. RNASeq identifies differential gene expression in BMDCs following *B.***

5 ***pseudocatenulatum* 210 EPS stimulation.** (A) Volcano plot showing differentially expressed genes
6 in BMDCs treated with 210 EPS versus PBS control. Upregulated genes are shown in red,
7 downregulated genes in blue. (B) Heatmap of differentially expressed genes in BMDCs treated with
8 210 EPS versus PBS control. Genes with adjusted P ≤ 0.05 were considered differentially expressed.

9
10 **Figure S6. Putative EPS biosynthetic loci are highly variable across *Bifidobacterium* strains used**

11 **in this study.** (A) Predicted architecture of putative EPS biosynthetic loci in *B. pseudocatenulatum*
12 210. (B) Homology maps comparing 210 loci with those of *B. choerinum* LH506, *B. longum* B71, *B.*
13 *longum* NCIMB 8809 and *B. bifidum* LH80. Gene functions were predicted from combined blastp,
14 RefSeq protein database and dbCAN3 analyses as described in Methods.

15
16 **Figure S7. *B. pseudocatenulatum* DSM20438 recapitulates key immune features of 210, whereas**

17 **LH14 does not.** (A) Quantification and representative flow cytometry plots of IFNγ+TNFα+ CD8+ T
18 cells in BRPKp110 tumours following DSM20438 treatment. n = 7–8. (B) Quantification of CD8+
19 effector-memory differentiation in BRPKp110 tumours following DSM20438 treatment. n = 7–8. (C)
20 Quantification of IFNγ+ CD8+ T cells and CD8+ effector-memory differentiation following LH14
21 treatment. n = 6. (D) Quantification of circulating DCs, cDC1 and cDC2 subsets, and mature
22 (CD80+CD86+) DCs following DSM20438 treatment. n = 7–8. (E) Representative flow cytometry
23 plots of mature tumour DCs following DSM20438 treatment. (F) Quantification of CD80, CD86 and
24 MHCII expression on tumour DCs and cDC1 cells following DSM20438 treatment. n = 7–8. (G)
25 Quantification of DC, cDC1 and cDC2 frequencies in tdLNs following DSM20438 treatment. n = 7–8.
26 (H) Dose-response analysis of MHCII mean fluorescence intensity and CD80+CD86+ maturation in
27 BMDCs stimulated for 24 h with purified EPS from 210, DSM20438 or B71. Statistical significance
28 was assessed by two-tailed unpaired t test. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05.

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30 **Figure S8. SEC fractionation and NMR analysis reveal structural heterogeneity of purified 210 EPS**

31 **and B71 EPS.** (A) Size exclusion chromatography traces of purified 210 EPS and B71 EPS showing
32 three and four fractions, respectively. (B) On-cell STD NMR analysis of 210 EPS fractions 1–3,
33 showing highest binding-associated signal for fraction 1. (C) Estimated molecular weights of SEC
34 fractions from 210 EPS and B71 EPS. (D) NMR spectrum and proposed structures identified in B71
35 EPS fraction 1. (E) NMR spectrum and proposed structures identified in 210 EPS fraction 2. (F) NMR
36 spectrum and proposed structures identified in B71 EPS fraction 2.

37
38 **Supplementary Tables**

Supplementary Table 1

Species	Strain designation	Proven- ience	Host	Host gender	Geographical location	Birth mode	Birth weight (g)	Diet at sampling	Age at sampling (d)
Bifidobacterium bifidum	LH_80	in- house	Human infant	Male	Norfolk, UK	Emergency caesarean	508	Formulae	150
Bifidobacterium pseudocatenulatum	210	in- house	Human infant	Female	Norfolk, UK	Vaginal	3500	Breast milk	159
Bifidobacterium choerinum	LH_506	in- house	Barbary sheep	n/a	Norfolk, UK	n/a	n/a	n/a	n/a
B. longum subsp. longum	NCIMB 8809	public	Human infant	n/a	n/a	n/a	n/a	n/a	n/a
B. longum subsp. longum	B_71	in- house	Human infant	Male	Berkshire, UK	Vaginal	n/a	Formulae	70
Bifidobacterium pseudocatenulatum	DSM204387	public	Human infant	n/a	n/a	n/a	n/a	n/a	n/a
Bifidobacterium pseudocatenulatum	LH14	in- house	Human infant	Male	Norfolk, UK	Vaginal	3500	Breast milk	102

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Supplementary Table 2A: ^1H and ^{13}C NMR chemical shifts of sugar residues identified from 210 EPS fraction 1 NMR spectra. Values in boldface are glycosylated or substituted positions.

No.	Residue		Chemical shift (ppm)						HMBC	NOESY
			1	2	3	4	5	6		
F	4- α -Galp	^1H	5.18	3.92	4.04	4.11	4.25	3.84	J3	J3
		^{13}C	98.4	71.2	71.8	81.5	73.9	62.9		
I	4- α -GlcP	^1H	4.97	3.62	3.87	3.71	4.23		F4	F4
		^{13}C	102.6	74.3	74.1	80.6	73.3			
J	3,6- β -Galp	^1H	4.66	3.74	3.78	4.17	3.80	3.75/3.61	N3 Lac2	N3
		^{13}C	106.7	72.3	80.2	68.0	76.1	71.5		
N	3- β -Galp	^1H	4.53	3.73	3.84	4.18	3.74	3.80/3.74	I4	I4
		^{13}C	105.4	72.9	84.9	71.2	77.7	63.8		
Lac	Lactate	^1H	-	3.91	1.32				J6	J6
		^{13}C	183.2	80.5	21.1					

Supplementary Table 2B. The chemical shifts table for the minor residue identified in EPS 210 SEC fraction 1 NMR spectra whose inter-residue could not be identified. Values in boldface are glycosylated or substituted positions

No.	Residue		Chemical shift (ppm)						HMBC	NOESY
			1	2	3	4	5	6		
		SEC Peak 1								
G	3-Hex	^1H	5.14	4.13	4.54	3.95				
		^{13}C	100.6	72.0	82.3	79.2				
L	3- β -Galp	^1H	4.61	3.68	3.77	4.15				
		^{13}C	107.1	74.6	76.1	68.0				

Supplementary Table 3: Monosaccharide compositions and total carbohydrate content in EPS 210 and EPS 871 samples.

Sugar residue	EPS 210 (400 µg)		EPS 871 (400 µg)	
	Weight (µg)	mol %	Weight (µg)	mol %
Arabinose (Ara)	1.8	0.8	3.8	1.3
Rhamnose (Rha)	0.7	0.3	0.9	0.3
Fucose (Fuc)	0.3	0.1	0.3	0.1
Xylose (Xyl)	2.4	1.1	2.2	0.8
Glucuronic Acid (GlcA)	2.1	0.7	3.9	1.1
Mannose (Man)	3.9	1.5	2.1	0.6
Galactose (Gal)	182.7	67.8	186.8	55.5
Glucose (Glc)	73.8	27.4	134.2	39.9
<i>N</i> -Acetylglucosamine (GlcNAc)	1.2	0.3	1.6	0.4
Lactate-substituted Galactose	Detected	Detected	ND	ND
Total	268.8	100.0	335.9	100.0
% CHO	67.2		84.0	

ND: Not detected

Supplementary Table 4: Relative percentage of each detected linked glycosyl residue in EPS 210 and EPS 871 samples.

Glycosyl residue	Relative % area	
	Sample: EPS 210	Sample: EPS 871
t-Araf	0.2	0.2
t-Xylp	0.1	ND
2-Rhap	0.1	0.1
t-Manp	0.4	0.2
t-Glcp	2.6	7.9
t-Hexf	0.8	0.2
t-Galp	0.8	0.4
4-Araf or 5-Araf	0.3	0.3
2 Xylp + 4 Xylp	0.2	0.1
2,4-Rhap	Tr	ND
3-Glcp	ND	12.2
2-Manp	0.4	0.7
2-Glcp	0.9	13.8
2-Hexf	0.1	ND
3-Galp	26.0	26.4
3-Hexf	0.8	0.7
6-Manp	0.1	0.0
6-Glcp	3.9	0.9
4-Galp or 5-Galf	37.5	17.0
4-Glcp	19.8	8.5
6-Galf	0.6	1.9
6-Galp	0.5	0.1
3,4-Galp	1.0	0.5
3,4-Glcp	0.2	0.1
2,3-Glcp	ND	0.2
2,6-Manp	0.4	0.1
2,6 Glcp + 4,6-Glcp	0.5	0.3
4,6-Galp or 5,6-Galf	0.5	7.0
2,6-Hexf	1.4	ND
3,6-Galp	ND	0.3
Total	100	100

Abbreviations: ND: not detected; Tr: traces (<0.01%); t: terminal; Araf: Arabinofuranosyl; Arap: Arabinopyranosyl; Glcp: Glucopyranosyl; Hexf: Hexofuranosyl; Galp: Galactopyranosyl; Galf: Galactofuranosyl; Manp: Mannopyranosyl; Rhap: Rhamnopyranosyl; Xylp: Xylopyranosyl.

Supplementary Table 5A: ^1H and ^{13}C NMR chemical shifts of sugar residues identified from 210 EPS fraction 2 NMR spectra. Values in boldface are glycosylated or substituted positions.

No.	Residue		Chemical shift (ppm)						HMBC	NOESY
			1	2	3	4	5	6		
A	2- β -Gal f	^1H	5.43	4.38	4.24	4.15	4.03	3.80	H2	H2
		^{13}C	110.2	91.8	78.7	85.4	72.0	63.9		
C	5- β -Gal f	^1H	5.22	4.15	4.12	4.15	3.94	3.80	D5	D5
		^{13}C	110.1	84.5	79.4	84.5	78.3	63.9		
D	5- β -Gro1P-6-Gal f	^1H	5.19	4.14	4.11	4.14	4.09	4.06	C5	C5
		^{13}C	109.8	84.3	79.3	84.3	77.2	68.0		
H	3- α -Gal p	^1H	4.99	3.96	3.96	4.12	3.99	3.74	P6	
		^{13}C	101.3	70.2	80.0	72.0	73.5	63.8		
K	β -Glc p	^1H	4.64	3.30	3.50	3.40	3.46	3.92/3.72	A2	
		^{13}C	104.5	75.8	78.5	72.4	78.5	63.6		
O	6- β -Glc p	^1H	4.51	3.33	3.50	3.45	3.62	4.21/3.86	O6	O6
		^{13}C	105.7	75.8	78.5	72.5	77.7	71.7		
P	6- β -Gal p	^1H	4.42	3.56	3.65	3.95	3.90	3.64		
		^{13}C	105.8	73.5	75.4	71.5	76.6	71.4		
T	Gro-1P	^1H	3.95/3.89	3.90	3.61/3.68				D6 (^{31}P HMBC)	
		^{13}C	69.2	73.5	64.8					
U	3-Gro	^1H	3.68/3.61	3.94	3.90/3.78					
		^{13}C	65.1	73.2	73.7					

Supplementary Table 5B: The chemical shifts table for the minor residue identified in EPS 210 SEC fraction 2, whose inter-residue could not be identified. Values in boldface are glycosylated or substituted positions.

B	2-Hex	¹ H	5.36	3.64	3.95	3.62	3.83			
		¹³ C	102.3	80.0	76.0	74.3	74.1			
G	3,4-Hex	¹ H	5.14	4.13	4.54	4.65	4.56		M3	
		¹³ C	100.6	72.0	82.3	79.6	77.7			
M	3-β-Galp	¹ H	4.57	3.62	3.78	4.11	3.72		G4	
		¹³ C	104.6	72.4	84.4	71.0				

Supplementary Table 6: ¹H and ¹³C NMR chemical shifts of sugar residues identified from B71 EPS fraction 1 NMR spectra. Values in boldface are glycosylated or substituted positions.

No.	Residue		Chemical shift (ppm)						HMBC	NOESY
			1	2	3	4	5	6		
A'	2-α-Glcp	¹ H	5.48	3.69	3.85	3.52	4.07	3.81	I3	I3
		¹³ C	99.3	78.6	74.2	72.2	74.3	63.2		
F	4-α-Galp	¹ H	5.20	3.96	4.02	4.26	4.23	3.73	A'2	A'2
		¹³ C	99.1	71.5	72.6	81.1	73.0	63.4		
G/G'	3-α-Galp	¹ H	5.01	3.96	3.90	4.12	4.02	3.74	S6	S6
		¹³ C	101.4	70.0	80.4	72.0	73.5	63.9		
I	3-β-Glcp	¹ H	4.69	3.47	3.67	3.64	3.46	3.74/3.87	N3	N3
		¹³ C	106.4	74.8	85.1	72.6	78.2	63.2		
J	β-Glcp	¹ H	4.66	3.33	3.50	3.41	3.45	3.74	S5	S5
		¹³ C	104.3	75.9	78.6	72.6	78.5	63.7		
N	3-β-Galp	¹ H	4.64	3.76	3.82	4.15	3.69	3.76/3.78	F4	F4
		¹³ C	106.9	73.4	85.0	71.0	77.5	63.3		
R	6-β-Galf	¹ H	5.23	4.21	4.08	4.12			3.93/80.06 (G'3)	
		¹³ C	111.7	84.5	79.6	86.0				
S	5,6-β-Galf	¹ H	5.20	4.20	4.26	4.21	4.26	3.75/3.96	G3	G3
		¹³ C	111.7	84.1	78.9	84.4	77.6	69.7		

Supplementary Table 7: ^1H and ^{13}C NMR chemical shifts of sugar residues identified from B71 EPS fraction 2 NMR spectra. Values in boldface are glycosylated or substituted positions.

No.	Residue		Chemical shift (ppm)						HMBC	NOESY
			1	2	3	4	5	6		
B	4- α -GlcP	^1H	5.37	3.6 4	3.83	3.6 3	3.9 5	3.81/3.8 8	B4	B4
		^{13}C	102.3	74. 3	74.0	80. 0	76. 0	63.3		
C	5- β -Galf	^1H	5.22	4.1 5	4.12	4.1 5	3.9 4	3.80	D5	D5
		^{13}C	110.1	84. 5	79.4	84. 5	78. 3	63.9		
D	5- β -Gro1P-6-Galf	^1H	5.19	4.1 3	4.11	4.1 3	4.0 9	4.06	C5	C5
		^{13}C	109.6	84. 1	79.2	84. 1	77. 2	68.0		
G/G'	3- α -GlcP	^1H	5.01	3.9 6	3.90	4.1 2	4.0 2	3.74	S6	S6
		^{13}C	101.4	70. 0	80.4	72. 0	73. 5	63.9		
J	3- β -GlcP	^1H	4.66	3.3 3	3.50	3.4 1	3.4 5	3.74/3.9 1	S5	
		^{13}C	104.3	75. 9	78.6	72. 6	78. 5	63.6		
O	6- β -GlcP	^1H	4.51	3.3 3	3.45	3.4 5	3.6 3	3.85/4.1 9	O6	O6
		^{13}C	105.7	75. 9	78.5	72. 5	77. 7	71.7		
R	6- β -Galf	^1H	5.23	4.2 1	4.08	4.1 2				G3
		^{13}C	111.7	84. 5	79.6	86. 0				
S	5,6- β -Galf	^1H	5.20	4.2 0	4.26	4.2 1	4.2 6	3.75/3.9 6		G3
		^{13}C	111.7	84. 1	78.9	84. 4	77. 7	69.7		
T	Gro-1P	^1H	3.94/3.8 9	3.8 9	3.61/3.6 7				D6 (^{31}P HMBC)	
		^{13}C	69.2	73. 5	65.0					