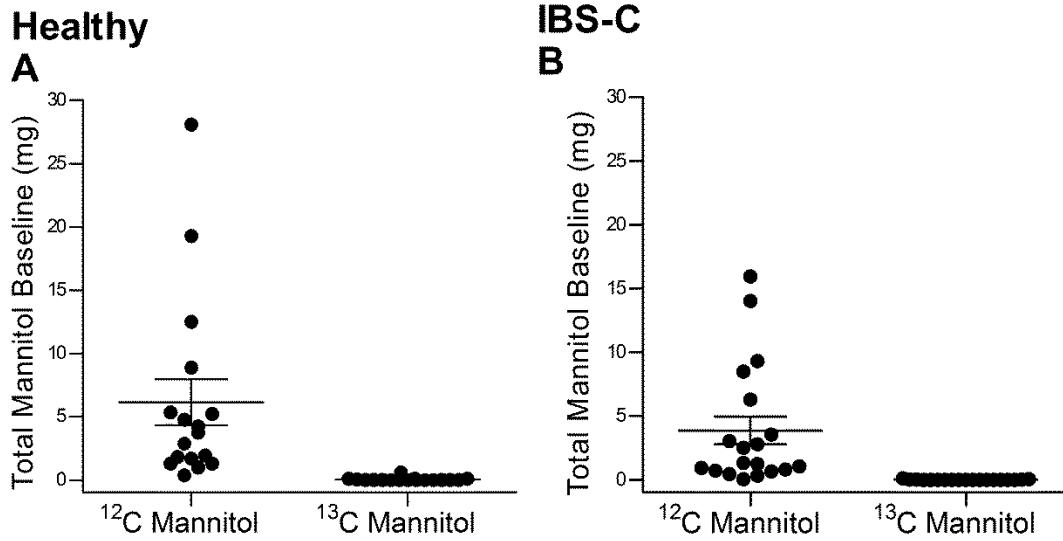


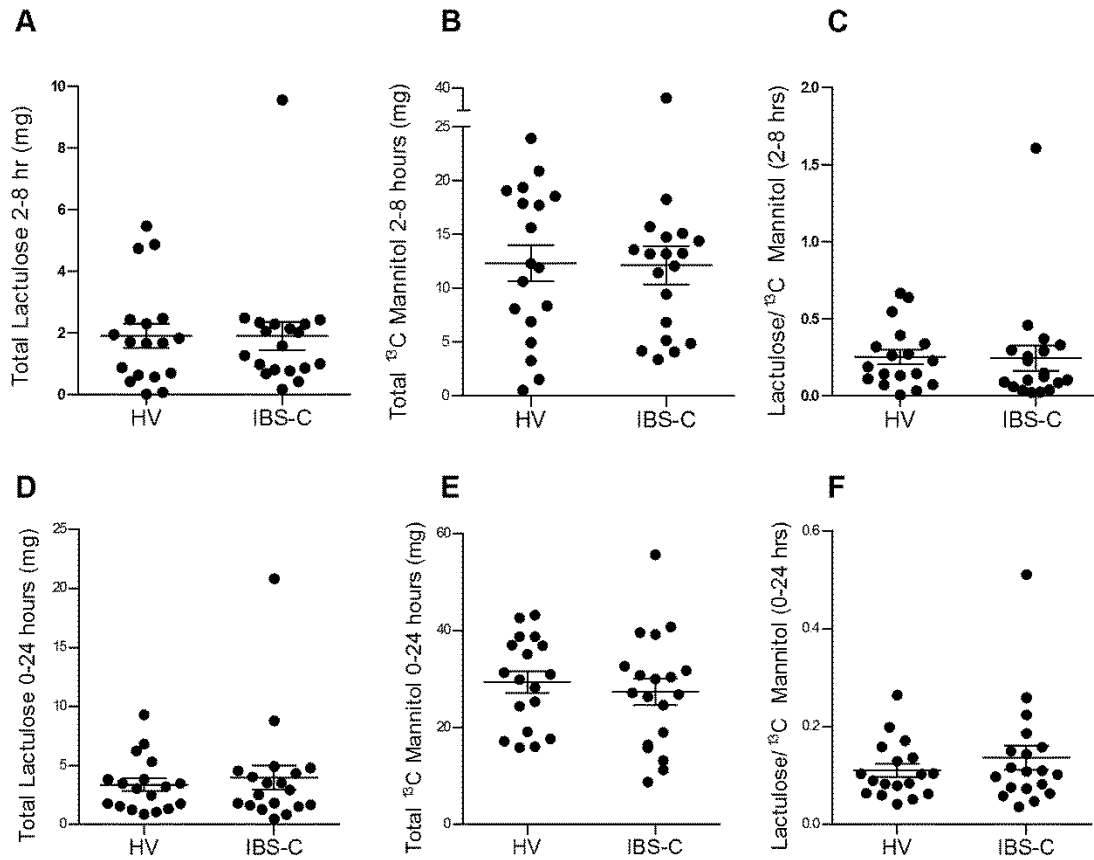
## SUPPLEMENTARY FIGURES

**Supplementary Figure 1:** Baseline mannitol excretion. (A)  $^{12}\text{C}$  mannitol vs  $^{13}\text{C}$  mannitol in healthy volunteers (B) Corresponding values for IBS-C patients.



**Supplementary Figure 2:** *In vivo* permeability assessment using saccharide excretion assay.

Panels (A-C) reflect 2-8 hours post-test saccharide administration and (D-F) reflect overall 0-24 hours post-test saccharide administration. (A) Cumulative lactulose excretion. (B) Cumulative  $^{13}\text{C}$  mannitol excretion. (C) Lactulose/ $^{13}\text{C}$  mannitol ratio expressed as percentage of administered dose for each volunteer. Panels D-F are corresponding cumulative excretion values and Lactulose/ $^{13}\text{C}$  mannitol ratio for 0-24 hour interval. Data are presented as mean  $\pm$  SEM. HV n=18, IBS-C n=19.



**Supplementary Table 1. Human tight junction gene expression in colonic mucosa of IBS-C patients compared to healthy controls**

Gene Name	Gene Symbol	Fold Change	p-value	95% Confidence interval
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1	0	(1.00, 1.00)
Claudin 3	CLDN3	1.3733	0.003087	(1.13, 1.62)
Symplekin	SYMPK	1.3535	0.005514	(1.06, 1.65 )
Catenin (cadherin-associated protein), beta 1	CTNNB1	1.3325	0.014354	(1.03, 1.64)
MAP/microtubule affinity-regulating kinase 2	MARK2	1.2039	0.020875	(1.03, 1.38)
Lethal giant larvae homolog 1 (Drosophila)	LLGL1	1.2506	0.021913	(1.02, 1.48)
Lethal giant larvae homolog 2 (Drosophila)	LLGL2	1.2185	0.031746	(1.02, 1.42)
Rho/rac guanine nucleotide exchange factor (GEF) 2	ARHGEF2	1.3149	0.046102	(1.03, 1.60)
Ras homolog gene family, member A	RHOA	0.8357	0.062047	(0.70, 0.97)
Claudin 5	CLDN5	1.2588	0.065328	(0.98, 1.53)
Cortactin	CTTN	1.1974	0.089768	(0.96, 1.44)
Tight junction associated protein 1 (peripheral)	TJAP1	1.1619	0.09773	(0.95, 1.37)
Protein kinase C, zeta	PRKCZ	1.1509	0.114528	(0.95, 1.35)
Phosphatase and tensin homolog	PTEN	0.8663	0.122533	(0.74, 0.99)
Tight junction protein 2 (zona occludens 2)	TJP2	1.1603	0.125545	(0.95, 1.37)
F11 receptor	F11R	1.125	0.150743	(0.97, 1.28)
Integrin-linked kinase	ILK	1.1128	0.152386	(0.97, 1.26)
T-cell lymphoma invasion and metastasis 1	TIAM1	1.2484	0.153465	(0.91, 1.59)
Cyclin-dependent kinase 4	CDK4	1.146	0.157422	(0.95, 1.35)
Claudin 1	CLDN1	0.7094	0.169881	(0.47, 0.95)
Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	RAC1	0.8873	0.223786	(0.75, 1.02)
Claudin 15	CLDN15	1.2052	0.223905	(0.86, 1.55)
Catenin (cadherin-associated protein), alpha 3	CTNNA3	0.8696	0.244078	(0.46, 1.28)
Claudin 7	CLDN7	1.1127	0.267872	(0.88, 1.34)
Immunoglobulin superfamily, member 5	IGSF5	0.8184	0.278348	(0.54, 1.10)
Cingulin	CGN	1.1086	0.33568	(0.88, 1.34)
Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	SPTAN1	1.0643	0.341253	(0.93, 1.20)
Claudin 2	CLDN2	0.8203	0.349738	(0.46, 1.18)
Crumbs homolog 3 (Drosophila)	CRB3	1.0884	0.354688	(0.90, 1.27)
Angiomotin like 1	AMOTL1	1.1195	0.356595	(0.87, 1.37)
Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	MLLT4	1.1944	0.364314	(0.82, 1.57)
Tight junction protein 1 (zona occludens 1)	TJP1	0.8556	0.374929	(0.64, 1.07)
Hypoxanthine phosphoribosyltransferase 1	HPRT1	0.912	0.381571	(0.75, 1.07)
Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)	MPP6	0.936	0.386957	(0.40, 1.47)
VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa	VAPA	0.8758	0.388812	(0.69, 1.06)
CD99 molecule	CD99	0.9402	0.390355	(0.80, 1.08)

Membrane associated guanylate kinase, WW and PDZ domain containing 1	MAGI1	1.0797	0.419367	(0.89, 1.27)
Calcium/calmodulin-dependent serine protein kinase (MAGUK family)	CASK	1.0488	0.446084	(0.92, 1.17)
Crumbs homolog 1 (Drosophila)	CRB1	0.781	0.455534	(0.49, 1.07)
Protein kinase C, iota	PRKCI	1.0606	0.462337	(0.89, 1.23)
Junctional adhesion molecule 2	JAM2	0.9113	0.479692	(0.68, 1.14)
Claudin 17	CLDN17	0.8086	0.479909	(0.48, 1.14)
Actinin, alpha 4	ACTN4	0.6686	0.488735	(0.00001, 1.39)
Par-6 partitioning defective 6 homolog beta (C. elegans)	PAR6B	0.9145	0.500649	(0.65, 1.18)
Casein kinase 2, alpha 1 polypeptide	CSNK2A1	2.0198	0.501709	(0.00001, 4.99)
Claudin 9	CLDN9	1.2135	0.523375	(0.78, 1.65)
Claudin 6	CLDN6	1.0752	0.560416	(0.59, 1.56)
Ash1 (Absent, Small, or Homeotic)-like (Drosophila)	ASH1L	1.0553	0.566243	(0.87, 1.24)
Claudin 11	CLDN11	1.0619	0.59513	(0.65, 1.47)
Occludin	OCLN	0.924	0.636917	(0.76, 1.09)
Ribosomal protein, large, P0	RPLP0	1.0493	0.643085	(0.84, 1.26)
Claudin 18	CLDN18	1.1697	0.654061	(0.69, 1.65)
Actinin, alpha 2	ACTN2	0.8465	0.660237	(0.53, 1.16)
Claudin 14	CLDN14	0.8456	0.666315	(0.54, 1.15)
InaD-like (Drosophila)	INADL	0.7529	0.667988	(0.13, 1.37)
Claudin 12	CLDN12	0.9517	0.675643	(0.75, 1.16)
Sterile alpha motif and leucine zipper containing kinase AZK	ZAK	1.0737	0.685611	(0.68, 1.47)
Spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	SPTA1	0.8574	0.691454	(0.55, 1.16)
Casein kinase 2, alpha prime polypeptide	CSNK2A2	1.0252	0.698409	(0.87, 1.18)
Claudin 16	CLDN16	0.8602	0.710186	(0.55, 1.17)
Par-3 partitioning defective 3 homolog (C. elegans)	PAR3	1.0201	0.712725	(0.87, 1.17)
Catenin (cadherin-associated protein), alpha 2	CTNNA2	0.9643	0.727251	(0.59, 1.34)
Claudin 4	CLDN4	0.9503	0.742827	(0.72, 1.18)
Cold shock domain protein A	YBX3	1.0203	0.749401	(0.87, 1.17)
Actinin, alpha 3	ACTN3	0.8911	0.769684	(0.58, 1.20)
Multiple PDZ domain protein	MPDZ	1.068	0.771069	(0.76, 1.38)
Junctional adhesion molecule 3	JAM3	1.0234	0.775487	(0.80, 1.24)
Tight junction protein 3 (zona occludens 3)	TJP3	0.9779	0.777915	(0.71, 1.24)
SMAD specific E3 ubiquitin protein ligase 1	SMURF1	1.0236	0.779616	(0.87, 1.18)
Actinin, alpha 1	ACTN1	1.0326	0.81302	(0.84, 1.22)
Actin, beta	ACTB	0.9735	0.82783	(0.81, 1.14)
Beta-2-microglobulin	B2M	3.0683	0.830729	(0.00001, 10.33)
Platelet/endothelial cell adhesion molecule	PECAM1	1.3879	0.854852	(0.27, 2.50)
Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	GNAI1	0.9765	0.860181	(0.78, 1.17)
Cell division cycle 42 (GTP binding protein, 25kDa)	CDC42	1.065	0.872952	(0.64, 1.49)
Erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	EPB41	0.98	0.8753	(0.80, 1.16)
Intercellular adhesion molecule 2	ICAM2	1.0491	0.885135	(0.68, 1.42)

Membrane associated guanylate kinase, WW and PDZ domain containing 2	MAGI2	0.9355	0.885355	(0.62, 1.25)
Intercellular adhesion molecule 1	ICAM1	0.8027	0.886545	(0.28, 1.33)
Claudin 8	CLDN8	0.9378	0.892385	(0.63, 1.24)
Casein kinase 2, beta polypeptide	CSNK2B	0.9859	0.905917	(0.84, 1.13)
Catenin (cadherin-associated protein), alpha 1	CTNNA1	0.9877	0.925351	(0.82, 1.16)
Claudin 19	CLDN19	0.9898	0.932433	(0.66, 1.32)
Spectrin, beta, erythrocytic	SPTB	0.9962	0.934065	(0.85, 1.15)
Claudin 10	CLDN10	0.9277	0.941097	(0.59, 1.26)
Endothelial cell adhesion molecule	ESAM	1.0263	0.954875	(0.76, 1.29)
Par-6 partitioning defective 6 homolog alpha ( <i>C. elegans</i> )	PARD6A	0.8829	0.971967	(0.44, 1.32)
Hematopoietic cell-specific Lyn substrate 1	HCLS1	0.7904	0.972109	(0.25, 1.33)
Membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)	MPP5	0.9934	0.993602	(0.83, 1.15)