

Supporting Information

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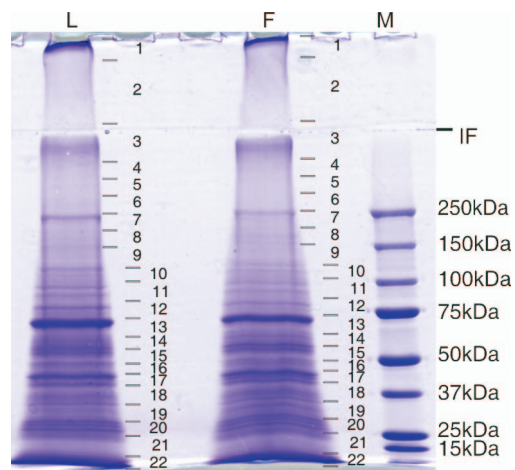


Fig. S1. PAGE of the firm and a loose mucus layers as used for proteomics identification. Loose (L) and firm (F) mucus was reduced and separated on a 4–12% SDS/PAGE and stained with Coomassie blue. A complete proteomic analysis of each lane was performed after dividing the lanes in 22 bands as indicated by the numbered lines. These bands were excised, trypsin-digested, and analyzed by nanoLC-ICR-MS/MS. The sizes of the molecular mass marker (M) are shown and the interface between stack and separating gel is indicated with IF.

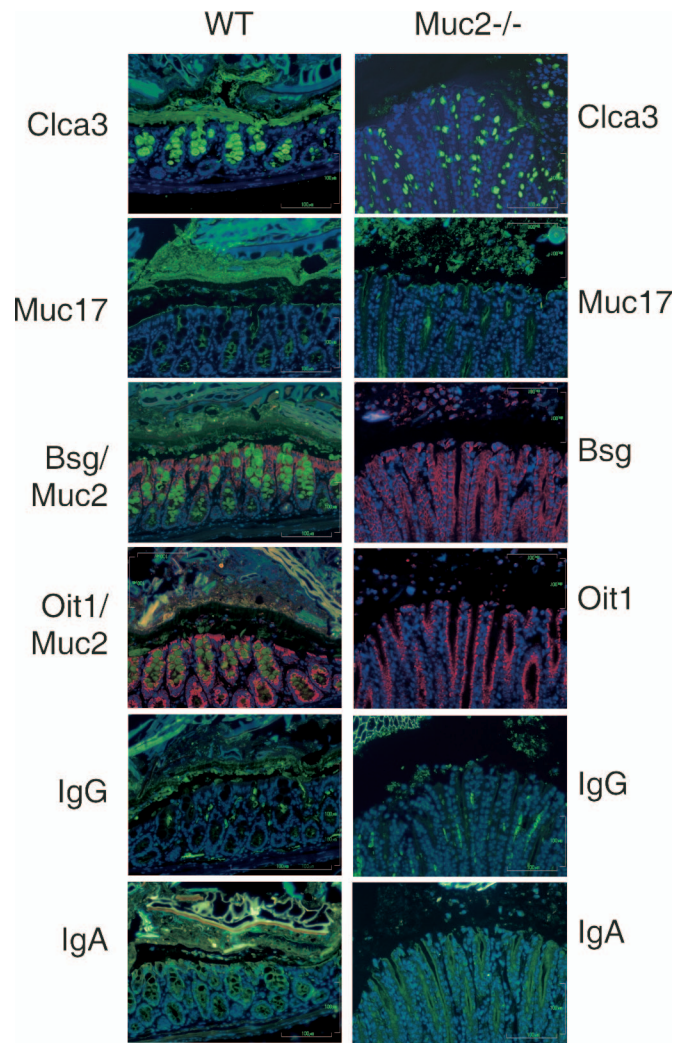


Fig. S2. Immunostaining of colon sections from WT and *Muc2*^{-/-} mice using antibodies against proteins identified in the mucus by proteomics. The antibodies used are described in Table S3. Double staining with anti-MUC2C3 was performed for Bsg and Oit1 of the WT mice. FITC-conjugated anti-rabbit and Cy3-conjugated anti-goat antibodies were used as secondary antibodies. Clca3, Muc17, Muc2, IgG, and IgA are stained green, whereas Bsg and Oit1 are stained red. The nuclear DNA is stained blue by DAPI in all pictures.

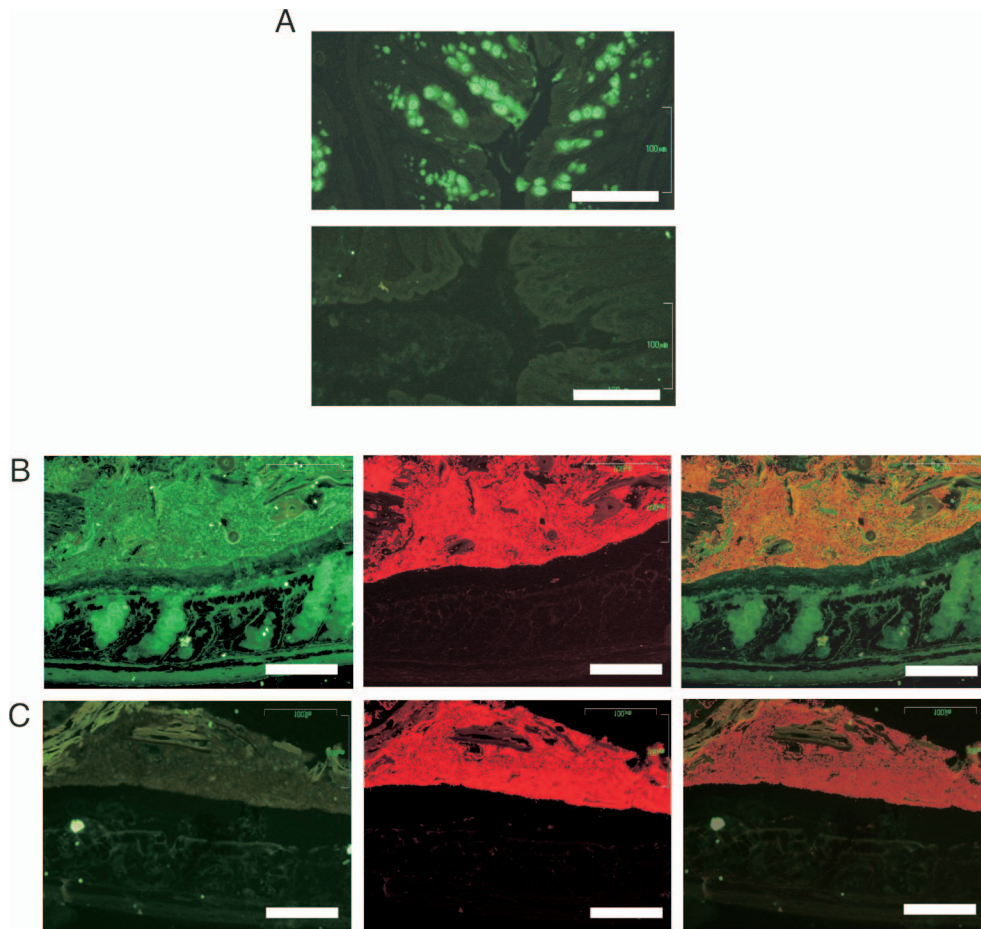


Fig. S3. Peptide block of the anti-MUC2C3 antisera. (A) Adjacent colon sections were immunostained with the anti-MUC2C3 antisera and FITC-conjugated secondary antibody (*Upper*) or pretreated with 10 μg of the immune-specific peptide per section for 30 min, abolishing the staining (*Lower*). (B) Immunostaining with anti-MUC2C3 antisera and FITC-conjugated (green, *Left*) secondary antibody combined with FISH using the EUB338-Alexa Fluor 555 probe (red, *Center*) and the composite of these (*Right*). (C) Peptide block of the anti-MUC2C3 antisera before staining preformed as in B. (Scale bars, 100 μm .)

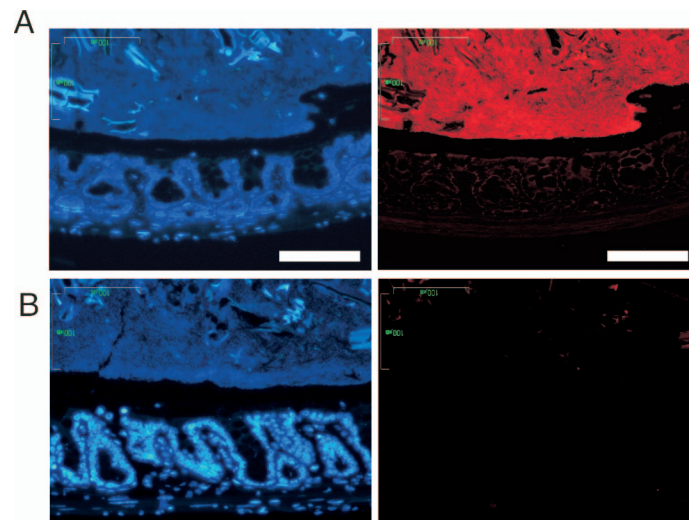


Fig. 54. Controls for *in situ* hybridization for bacterial 16S rRNA. (A) DAPI staining of epithelial cell nuclear DNA and bacterial DNA (Left) and stained with FISH using the EUB338-Alexa Fluor 555 probe (red, Right). Sections are from the distal colon. Double staining is seen in bacteria. (B) DAPI staining of epithelial cell nuclear DNA and bacterial DNA (Left) and stained with FISH using the negative control NON-EUB-Alexa Fluor 555 probe (5'-CGACGGAGGGCATCCTCA-3') (red, Right). Staining was performed as in A. (Scale bars, 100 μm .)

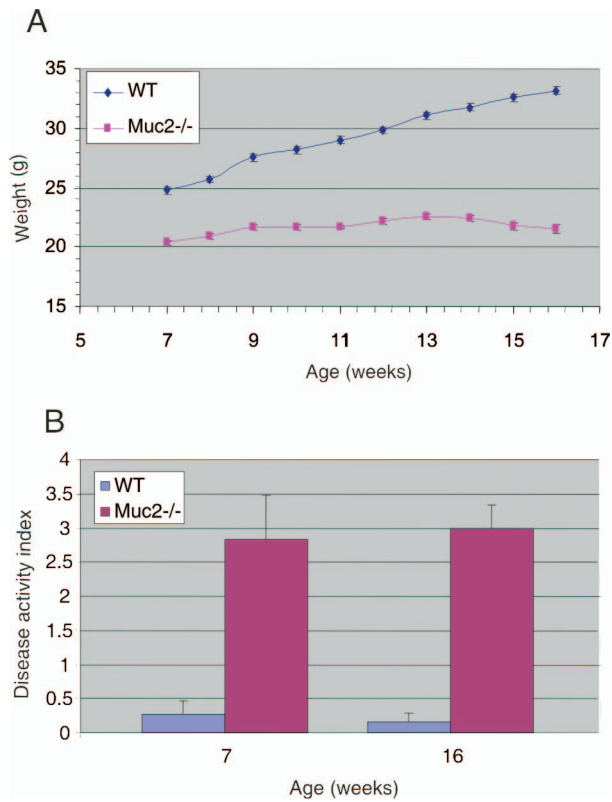


Fig. S5. Weight development and disease activity index of WT and Muc2^{-/-} mice. (A) The weight was observed over time for Muc2^{-/-} mice and compared with WT. The groups included five male mice, and data are presented as mean \pm SEM. (B) The same animal groups as in A were also compared by scoring diarrhea and blood in stool, and the disease activity index was calculated (1). Data from weeks 7 and 16 are presented as mean \pm SEM.

1. Cooper HS, Murthy SNS, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69:238–249.

Table S1. Peptides identifying mucins in the loose and firm mucus layers

Peptide*	Observed mass, Da	Charge	Loose		Firm	
			MS/MS [†]	Score [‡]	MS/MS [†]	Score [‡]
Muc2						
NHVCSTWGFHYK	556.92 [§]	3	2	30	1	9
TFDGDVYR	486.72	2	9	40	11	37
FPGLCDYNAFASDCR	606.6 [§] ;861.35 [¶]	3;2	1	16	4	65
DSYKEFAVHLK	446.23	3	3	35	4	51
DDTIYLTHK	553.28	2	1	24		
LAVVNGAMVSTPHYSSGLLIEK	768.08	3	1	4	1	35
AGLSLMVNR	532.27	2	4	61	2	56
EDALMVELDSR	647.3	2	2	60	2	60
INKPEVQCEDPEAVQEPESCSSEHR	956.42 [¶]	3			3	42
LLTSAAFEDCQTR	780.38 [§] ;520.59 [§] ;756.36 [¶]	2;3;2	15	90	17	76
VPVESYVR	474.76	2	14	49	21	49
GGACECSTLAEFSR	820.86 [§] ;547.58 [§] ;772.83 [¶]	2;3;2	11	72	11	71
QCSHAGGRPENWR	518.90 [¶]	3			1	6
TASLCPK	412.72 [§]	2	6	32	2	30
DLPCPETCALEGGSHITTFDGKK	834.39 [§] ;845.06 [¶]	3;3			3	15
KFTFHGDCYYVLTG	609.63 [§]	3			1	28
FTFHGDCYYVLTG	566.94 [§]	3	3	21	2	21
TVVLLTDDK	502.29	2	2	32		
TVVLLTDDKK	566.33	2	8	40	11	45
RSETPFAR	482.25	2	8	40	4	38
SETPFAR	404.2	2	13	39	12	38
CHLAVDPTEYYKR	567.28 [§] ;551.27 [¶]	3;3	1	49	1	18
CHLAVDPTEYYK	772.37 [§] ;515.25 [§]	2;3	9	44	11	38
GVMLWGWR	510.76	2	1	29	1	34
SLSEGDSSHCLK	640.8 [§] ;427.45 [§] ;616.78 [¶] ;411.52 [¶]	2;3;2;3	8	48	8	50
GFAPVEGCGCPDHTFMDEK	755.98 [§]	3	1	5	2	11
CSCYHHGLYLEAGDVILR	753.7	3			1	4
LQCTQVK	462.75 [§] ;438.73 [¶]	2;2	9	29	7	37
LIGHTCQYPK	632.83 [§] ;422.22 [§] ;608.81 [¶] ;406.21 [¶]	2;3;2;3	16	33	15	28
GGCVEEDKPCIHNK	901.88 [¶] ;601.59 [¶]	2;3	1	38	6	47
DLYSSGESIK	549.77	2	10	33	11	34
GRWECTR	482.72 [¶]	2	1	15	2	26
IFIGGTELK	489.29	2	17	37	20	37
QLEEGHHVPYITR	789.90;526.94	2;3	7	28	12	51
GTVCGLCGNFDDQTK	884.39 [§] ;589.93 [§] ;836.36 [¶]	2;3;2	10	37	10	75
GTVCGLCGNFDDQTKNDFTR	834.71 [§] ;802.69 [¶]	3;3			4	43
DHMMVTSSELDVFNQSWK	627.62	3	1	21	3	23
EASTCPDVSHNPDPCSLNPHR	829.37 [§] ;797.35 [¶]	3;3			4	49
EASTCPDVSHNPDPCSLNPHRR	849.38 [¶]	3			4	39
AEACVFWR	543.76 [§] ;519.74 [¶]	2;2	8	40	7	49
SFETCR	424.19 [§] ;400.17 [¶]	2;2	11	29	9	25
CPEDRIPIYDEDLK	849.39 [§]	2	1	19	2	16
CPEDRIPIYDEDLKK	860.91;889.42 [¶] ;593.29 [¶]	2;2;3	5	44	12	45
CGCYIEDTR	423.85 [§]	3	1	18	1	16
CGCYIEDTRYPPGGSVPTDEICK	892.06 [¶]	3			6	54
YPPGGSVPTDEICK	784.37 [§] ;523.25 [§]	2;3	8	36	3	24
IECHPDEGK	566.76 [§] ;542.74 [¶]	2;2	3	26	4	41
ENGGDRETFTHVCSAPEDICR	1290.05 [¶] ;860.37 [¶]	2;3	1	47	3	60
ETFTHVCSAPEDIECR	682.97 [§] ;975.92 [¶] ;650.95 [¶]	3;2;3	5	17	7	57
LSWEELGQK	545.28	2	11	64	14	64
SGDFELIK	454.74	2	19	59	22	59
AVMHSNIPLDQLGQIVVCNK	767.40 [§] ;1126.58 [¶] ;751.39 [¶]	3;2;3	3	46	20	79
EVGLVCK	426.74 [§] ;402.72 [¶]	2;2	13	21	15	23
EVGLVCKNEDQEIGGIIPMR	774.39 [§] ;758.38 [¶]	3;3			6	39
NEDQEIGGIIPMR	744.36;496.58	2;3	27	65	28	61
HETQEVQIK	556.29	2	11	48	10	47
MMPIEVEVQVNK	724.86	2	2	54	6	68
QLVALPYK	466.28	2	5	28	6	32
QLVALPYKK	530.33	2	2	19	2	20
ISYNGLSFSIR	628.84	2	1	44	2	49
FAPGYDVCVK	602.30 [§] ;401.87 [§] ;578.28 [¶]	2;3;2	8	16	10	24
TCGCVGPDNVPR	476.56 [§] ;666.3 [¶]	3;2	3	36	4	50
EFGEHFEDCK	498.21 [§]	3	3	20	3	17
EGSGIVCQPK	590.30 [§] ;566.28 [¶]	2;2	7	39	6	50
AERPTCLLGFEVK	784.42 [§] ;523.28 [§]	2;3	2	30	6	32
GVCVHQNAEYQPGSPVYSNK	761.36 [§]	3	5	45	2	50
QQYIILKPGEIHK	522.97	3	3	6	2	8
VPCSAVVMK	571.29 [§] ;547.27 [¶]	2;2	8	11	7	38
Muc3(17)						
CQCTSLFYGPR	694.81 [¶]	2	1	66	1	65
Muc13						
SVSSAIETAIK	553.31	2			2	54

Peptides from the LC-MS/MS identified mucins in loose and firm mucus from AgPAGE separation. Data from nine alcian blue-stained bands from three mucus samples are merged. All matched peptides not better identified in other proteins were included for muc.

*Oxidized methionins are italicized.

[†]No. of MS/MS spectra.

[‡]Highest individual peptide score observed.

[§]C modification: S-pyridyletyl.

[¶]C modification: carbamidomethyl.

^{||}This is the ortholog of human MUC17, annotated as Muc3 in mouse databases.

Table S2. Identified proteins that are either known to be secreted or that have a large extracellular domain found in the loose and firm mucus layers; proteins known to be present in plasma and all intracellular proteins are excluded

AC*	Protein name	Gene	Mass, Da	Length, amino acids	Loose1			Loose 2			Loose 3			Firm 1			Firm 2			Firm 3		
					Pep [†]	Uni [‡]	Cov [§]	Pep [†]	Uni [‡]	Cov [§]	Pep [†]	Uni [‡]	Cov [§]	Pep [†]	Uni [‡]	Cov [§]	Pep [†]	Uni [‡]	Cov [§]	Pep [†]	Uni [‡]	Cov [§]
gil6753010	Anterior gradient 2	<i>Agx2</i>	19,979	175	3	3	26	4	4	29	3	3	25	6	6	42	6	6	42	6	6	42
gil8567336	Ca-activated Cl channelβ3	<i>Clca3</i>	101,003	913	30	30	38	29	29	28	22	22	27	22	22	32	25	25	32	25	25	32
gil50882459	Ca-activated Cl channelβ6	<i>Clca6</i>	924	924	6	6	7	4	4	7	2	2	2	2	2	2	2	2	2	2	2	2
gil148692210	IgG Fc binding protein	<i>Fcgbp</i>	156,416	1,398	13	13	12	18	18	11	10	10	8	14	14	13	17	17	16	17	17	16
gil3831564	γ-Glutamyl hydrolase	<i>Ggh</i>	35,465	315				2	2	9				3	3	12	5	5	19			19
gil473961	Immunoglobulin light chain	<i>Ig</i>	22,983	206	4	4	1	23														
gil4930001	Chain A, Fab–Fab complex	<i>Ig</i>	24,147	214	5	1	26															
gil62027409	IgH protein [<i>Mus musculus</i>]	<i>Igh</i>	53,801	485				6	6	17	3	3	8									
gil18043871	IgH protein	<i>Igh</i>	54,034	484	6	6	23	3	1	8												
gil16323017	Immunoglobulin alpha heavy	<i>Igh</i>	46,927	344	6	6	23	4	4	17	4	4	17	4	4	17	2	2	9			
gil37589305	IgH protein	<i>Igh</i>	53,646	485	3	3	10			5	2	1	5									
gil26330510	IgH protein	<i>Igh</i>	45,216	406	3	3	10															
gil15637214	IgG1 light chain	<i>Igl</i>	22,319	200	4	4	1	24														
gil99032605	Heavy and light chain variable	<i>Ighl</i>	31,730	291				2	1	9												
gil90898	Igκ chain V region	<i>Igk</i>	11,881	108	2	1	16															
gil13359417	Antibody κ light chain	<i>Igk</i>	23,749	213	4	4	2	26														
gil83405792	Igκ C protein	<i>Igk</i>	26,203	235	3	1	15															
gil110434	Igκ chain V region	<i>Igk</i>	24,556	219	4	4	23	3	3	17	2	2	10	2	2	8						
gil6678740	Lumican	<i>Lum</i>	38,824	338	2	2	5	2	2	7												
mMuc2	Muc2	<i>Muc2</i>	310,735	2,700	20	20	8	21	21	9	9	9	3	17	17	8	11	11	4			
gil127531	Major urinary proteins 11 and 8	<i>Mup</i>	17,762	151	11	11	82															
gil22164770	Oncoprotein-induced transcript 1	<i>Oit1</i>	25,256	223	3	3	20	4	4	30	2	2	9	2	2	9	3	3	13			
gil13384888	Mucosal pentraxin	<i>Ptx</i>	24,721	219	3	3	20	4	4	30	4	4	30	3	3	20	4	4	30			
gil1703832	Arginyl aminopeptidase	<i>Rnpep</i>	73,149	650	5	5	9	7	7	15	5	5	10	6	6	11	4	4	8			
gil201102	Seminal vesicle secretory protein IV	<i>Sx4</i>	11,957	108				2	2	25	2	2	44	4	4	8	4	4	9			
gil23943876	Zymogen granule membrane protein 16	<i>Zg16</i>	18,411	167	5	5	37	4	4	32	4	4	32	4	4	45	5	5	52			
mMuc3(17)	Muc3(17)	<i>Muc3(17)</i>	109,960	1,024	3	3	4	3	3	4	3	3	4	3	3	4	4	4	(1)			(1)
gil7209584	Vomeroglandin	<i>Dmbt1</i>	219,223	1,957	5	5	3	6	6	4	2	2	1			(1)						(1)
gil458422	Polymeric immunoglobulin receptor	<i>Pigr</i>	86,678	771	5	5	7	5	5	8												
gil12963609	Quiescin Q6 sulfhydryl oxidase 1	<i>Qsox1</i>	64,220	568	2	2	3	2	2	3	2	2	3	2	2	3	3	3	7			
mMuc13	Muc13	<i>Muc13</i>	60,085	573	6	6	13	6	6	13	5	5	11	5	5	10	3	3	6	3	3	7
gil2808470	Basigin	<i>Bsg</i>	30,539	275	2	2	5	6	6	14	2	2	8	5	5	5	5	5	14			
gil55151	Uvomorulin	<i>Cdh1</i>	78,611	711	2	2	5	6	6	14	2	2	4	3	3	6	5	5	14			
gil9790073	Cadherin 17	<i>Cdh17</i>	92,157	827	12	12	19	16	16	25	18	18	26	7	7	11	19	19	33			
gil399117	CEACAM 1	<i>Ceacam1</i>	57,407	521	3	3	8	2	2	6	3	3	5	3	3	33	22	22	34			
gil6754180	Hephaestin	<i>Heph</i>	130,736	1,157							2	2	1									
gil21312502	Mucin-like protocadherin	<i>Mupcdh</i>	73,068	669	3	3	4	5	5	11	4	4	6	2	2	3	2	2	4	3	3	3
gil9992880	Nicastrin	<i>Ncstrn</i>	79,292	708				3	3	5	2	2	2	2	2	3	2	2	4	2	2	3
gil85662397	Protocadherin 24	<i>Pcdh24</i>	143,399	1,308	2	2	2	4	4	3	6	6	5	2	2	2	2	2	2	2	2	2
gil193008	Tumor-associated Ca signal transducer 1	<i>Tacst1</i>	35,778	314	3	3	18															
gil82802624	Maltase-glucoamylase	<i>Mgam</i>	26,5920	2,379				3	3	1	4	4	1			(1)						(1)

Databasesearches using the Mascot software in NCBI nonredundant (October 11, 2007, 5,539,440 sequences) and mucin (www.medkern.gu.se/mucinbiology) databases are combined. Identification: One peptide at 99% significance level (individual peptide score cutoff = 47) and one supporting peptide at 95% significance level (individual peptide score cutoff = 40). Proteins identified in only loose or firm were further analyzed for peptides with ion score cutoff = 25 (marked by parentheses). Multiple entries for the same protein were combined, and identification in two of three analyses of at least one sample (loose or firm) was required for inclusion. All identified proteases are presented in Table S4.

*Accession number in the NCBI protein database.
[†]Pep is the number of peptides identified with individual ion score cutoff >40 ($P > 0.05$).
[‡]Uni is the number of unique peptides identified for the protein. The + indicates identification of the protein in other species; (,) number of peptides identified with ion score = 25 (analyzed for samples where listed proteins were not identified at $P > 0.05$).
[§]Cov is the sequence coverage of the peptides for the identified protein sequence.

Table S3. Analysis of localization, function, and expression of proteins identified in the loose and firm mucus by proteomics as shown in Table S2

AC*	Protein name	Gene	Loc [†]	GO term (function, biological process) [‡]	Gene expr [§]	Hpa	Ab	Ab staining**
gi 6753010	Anterior gradient 2	<i>Agr2</i>	SEC	Cl channel activity, hydrogen ion transporting ATPase activity, rotational mechanism, chloride transport	LI, SI	n.d.	-	
gi 8567336	Ca-activated Cl channel β 3	<i>Clca3</i>	SEC	Cl channel activity, ligand-gated ion channel activity, chloride transport	LI, SI	n.d.	AbCam46512	GC, M, Lum
gi 50882459	Ca-activated Cl channels	<i>Clca6</i>	SEC	Cl channel activity, ligand-gated ion channel activity, chloride transport	LI, SI	No ortholog	-	
gi 148692210	IgG Fc binding protein	<i>Fcgbp</i>	SEC	Binding of sperm to zona pellucida, cell adhesion	LI, SI	LI, SI	-	
gi 3831564	γ -Glutamyl hydrolase	<i>Ggh</i>	SEC	γ -Glutamyl hydrolase activity	LI	n.d.	-	
gi 473961	Immunoglobulin light chain	<i>Igl</i>	SEC		LI, SI	n.d.	INV_A11001 (IgG)	LPC, M, Lum
gi 4930001	Chain A,Fab-Fab complex	<i>Igl</i>	SEC		LI, SI	n.d.	BD 559354 (IgA)	EC, M, Lum
gi 62027409	IgH protein	<i>Igh</i>	SEC		LI, SI	n.d.	-	
gi 18043871	IgH protein [<i>Mus musculus</i>]	<i>Igh</i>	SEC		LI, SI	n.d.	-	
gi 16323017	IgH protein	<i>Igh</i>	SEC		LI, SI	n.d.	-	
gi 37589305	Immunoglobulin- α heavy	<i>Igh</i>	SEC		LI, SI	n.d.	-	
gi 26330510	IgH protein	<i>Igh</i>	SEC		LI, SI	n.d.	-	
gi 15637214	IgG1 light chain	<i>Igl</i>	SEC		LI, SI	n.d.	-	
gi 99032605	Heavy And light chain variable	<i>Ighl</i>	SEC		LI, SI	n.d.	-	
gi 90898	Igk chain V region	<i>Igk</i>	SEC		LI, SI	n.d.	-	
gi 13359417	antibody kappa light chain	<i>Igk</i>	SEC		LI, SI	n.d.	-	
gi 83405792	Igk-C protein	<i>Igk</i>	SEC		LI, SI	n.d.	-	
gi 110434	Igk chain V region	<i>Igk</i>	SEC		LI, SI	n.d.	-	
gi 6678740	Lumican	<i>Lum</i>	SEC		LI	n.d.	-	
mMuc2	Mucin2	<i>Muc2</i>	SEC	Protein binding, apoptosis, induction of apoptosis, negative regulation of cell migration, negative regulation of cell proliferation	n.d.	LI, SI	anti-MUC2C3	GC, M, Lum
gi 127531	Major urinary proteins 11 and 8	<i>Mup</i>	SEC	Negative regulator of insulin secretion	n.d.	No ortholog	-	
gi 22164770	Oncoprotein induced transcript 1	<i>Oit1</i>	SEC		LI, SI	n.d.	RD AF3027	Ep, M
gi 13384888	Mucosal pentraxin	<i>Ptx</i>	SEC		n.d.	n.d.	-	
gi 21703832	Arginyl aminopeptidase	<i>Rnpep</i>	SEC	Aminopeptidase activity, hydrolase activity, membrane alanyl aminopeptidase activity, metalloproteinase activity, proteolysis	LI, SI	n.d.	-	
gi 201102	Seminal vesicle secretory protein IV	<i>Svs4</i>	SEC		NO	No ortholog	-	
gi 23943876	Zymogen granule membrane protein 16	<i>Zg16</i>	SEC		LI, SI	n.d.	-	
mMuc3(17)	Mucins(17)	<i>Muc3(17)</i>	SEC-TM1	Blastocyst development, inner cell mass cell proliferation, positive regulation of epithelial cell differentiation	LI, SI ^{††}	n.d.	anti-Muc3(17)-S2	Ep, Mem, M, Lum
gi 7209584	Vomeroglandin	<i>Dmbt1</i>	SEC-TM1		LI, SI	n.d.	-	
gi 458422	Polymeric immunoglobulin receptor	<i>Pigr</i>	SEC-TM1	Flavin-linked sulfhydryl oxidase activity	LI, SI	LI, SI	-	
gi 12963609	Quiescin Q6 sulfhydryl oxidase 1	<i>Oxol1</i>	SEC-TM1		LI (SI)	n.d.	-	
mMuc13	Mucin13	<i>Muc13</i>	TM1	Protein binding, apoptosis, induction of apoptosis, negative regulation of cell migration, negative regulation of cell proliferation	LI, SI	n.d.	-	
gi 2808470	Basigin	<i>Bsg</i>	TM1	Cell-cell adhesion, protein homooligomerization, protein metabolic process, regulation of caspase activity	LI, SI	n.d.	RD AF772	Ep, Mem
gi 55151	Uvomorulin	<i>Cdhl1</i>	TM1	Protein binding, proton-dependent oligopeptide secondary active transmembrane transporter activity, calcium-dependent cell-cell adhesion, homophilic cell adhesion, oligopeptide transport	LI, SI	LI, (SI)	-	
gi 9790073	Cadherin 17	<i>Cdh17</i>	TM1	Positive regulation of MAP kinase activity	LI, SI	LI, SI	-	
gi 399117	CEACAM 1	<i>Ceacam1</i>	TM1	Erythrocyte differentiation, iron ion transport	LI, SI	n.d.	-	
gi 6754180	Hephaestin	<i>Heph</i>	TM1	Homophilic cell, calcium ion binding	LI, SI	n.d.	-	
gi 21312502	Mucin-like protocadherin	<i>Mucadh</i>	TM1	Protein binding	LI, SI	n.d.	-	
gi 992880	Nicastrin	<i>Ncstrn</i>	TM1	Extracellular space, integral to membrane	n.d.	n.d.	-	
gi 85662397	Protocadherin 24	<i>Pcdh24</i>	TM1	α -Glucosidase activity, amylase activity	LI, SI	LI, SI	-	
gi 193008	Tumor-associated Ca signal transducer	<i>Tacstd1</i>	TM1		LI, (LI)	SI	-	
gi 82802624	Maltase-glucoamylase	<i>Mgaim</i>	TM2				-	

*Accession number in the NCBI protein database.

[†]Localization (UniProtKB). SEC, secreted; TM1, transmembrane type1; TM2, transmembrane type2.

[‡]Gene Ontology terms for molecular function and biological process.

[§]Mouse gene expression from the GNF SymAtlas. LI, large intestine; SI, small intestine; NO, no expression; nd, not done.

^{||}Human protein expression from the Human protein atlas. LI, large intestine; SI, small intestine; NO, no expression; nd, not done.

^{||}Antibodies used for immunostaining. SC, Santa Cruz; BD, Becton Dickinson; RD, R and D; INV, Invitrogen; Anti Muc3(17)-S2 [Malmberg EK, et al. (2006) Increased levels of mucins in the cystic fibrosis mouse small intestine, and modulator effects of the Muc1 mucin expression. *Am J Physiol* 291:G203-G210].

**Result of immunostaining. Data are presented in Fig. S5. Ep, epithelial cell; GC, goblet cells; LPC, lamina propria cells; MEM, membrane-associated; M, mucus-associated; Lum, lumen.

^{††}Muc3 in GNF SymAtlas.

Table S4. All proteins with identified proteolytic activity found by proteomics in the loose and firm mucus layers; protein identification was as in Table S2

AC*	Protein name	Gene	Loc ^t	Mass, Da	Length, amino acids	Loose1			Loose2			Loose3			Firm1			Firm2			Firm3			
						Pep [†]	Uni [§]	Cov [¶]	Pep [†]	Uni [§]	Cov [¶]	Pep [†]	Uni [§]	Cov [¶]	Pep [†]	Uni [§]	Cov [¶]	Pep [†]	Uni [§]	Cov [¶]	Pep [†]	Uni [§]	Cov [¶]	Pep [†]
gil22122789	Acylpeptide hydrolase	Apeh	IC	8,0991	717	7	7	11	7	7	12													
gil23271646	Bleomycin hydrolase	Blmh	IC	53,230	455	3	3	8	2	2	5	3	3	8										
gil476218	Interleukin-1 β converting enzyme	Casp1	IC	46,022	399	2	2	5	3	3	7	6	6	16	2	2	5	2	2	2	5	3	3	9
gil31981273	CNDP dipeptidase 2	Cndp2	IC	53,302	475	7	7	17	10	10	28	7	7	19	6	6	15	3	3	3	8	4	4	11
gil31560449	Aspartyl aminopeptidase	Dnpep	IC	52,884	473				3	3	7	4	4	11	3	3	8	4	4	4	13	2	2	4
gil3831564	γ -Glutamyl hydrolase	Ggh	SEC	35,465	315				2	2	9													
gil9989702	Kallikrein	Klk	SEC- Blood	29,495	261	6	6	31	6	6	33	8	8	38	3	3	15	2	2	2	8	3	3	17
gil31981147	Leucine aminopeptidase 3	Lap3	IC	56,647	519	4	4	8	4	4	8	3	3	6	3	3	3	3	3	3	5	2	2	4
gil68226731	Aminopeptidase puromycin sensitive	Npepps	IC	104,112	920	8	8	9	9	9	11	4	4	4	3	3	3	6	6	6	7	3	3	3
gil790470	p38-2G4	Pa2 g4	IC	38,303	340	3	3	9	5	5	15	2	2	5	4	4	11	3	3	3	8	6	6	17
gil55741460	DJ-1 protein	Par-k7	IC	20,293	189	4	4	38	5	5	41	7	7	48	5	5	5	5	5	43	3	3	22	
gil28570174	Plasma glutamate carboxypeptidase	Pgcp	SEC- Blood	51,851	470																			
gil6755152	Prolyl endopeptidase	Prep	IC	81,908	710	2	2	3	2	2	3													
gil1709759	Proteasome subunit α type 2	Psma2	IC	26,051	234				4	4	31													
gil6755196	Proteasome subunit α type 4	Psma4	IC	29,807	261	2	2	6	3	3	15	2	2	6	2	2	6							
gil7106387	Proteasome subunit α type 5	Psma5	IC	26,607	241	2	2	14	2	2	9	2	2	9	2	2	9	2	2	2	12	2	2	9
gil6755198	Proteasome subunit α type 6	Psma6	IC	27,923	246	3	3	16	5	5	25	2	2	9	2	2	8	2	2	2	9	2	2	12
gil7106389	Proteasome subunit α type 7	Psma7	IC	28,099	248	2	2	10	3	3	18	2	2	10	2	2	10	3	3	3	13	4	4	22
gil7242197	Proteasome subunit β type 1	Psmb1	IC	26,639	240							3	3	20										
gil31981327	Proteasome subunit β type 2	Psmb2	IC	23,065	201	2	2	10	2	2	10													
gil9910832	Proteasome subunit β type 2	Psmb2	IC	23,105	201							4	4	26										
gil6755202	Proteasome subunit β type 2	Psmb3	IC	23,305	205				3	3	23				2	2	16							
gil14198355	Proteasome subunit, β type 4	Psmb4	IC	29,255	264				2	2	13				2	2	15	2	2	2	15	2	2	15
gil3914434	Proteasome subunit β type 5	Psmb5	IC	23,166	209	3	3	16	3	3	16	7	7	39										
gil31982099	Proteasome subunit β type 6	Psmb6	IC	22,051	202				3	3	14	4	4	19	4	4	19	3	3	3	14	3	3	14
gil21703832	Arginyl aminopeptidase	Rnpep	SEC	73,149	650	5	5	9	7	7	15	5	5	10	6	6	11	4	4	4	8	4	4	9
gil21619359	Thimet oligopeptidase 1	Thop1	IC	78,972	687	5	5	9	4	4	7	3	3	5										
gil7305619	Ubiquitin-specific protease 5	Usp5	IC	96,909	858																			

*Accession number in the NCBI protein database.

^tLocalization (UniprotKB). IC, intracellular; SEC, secreted; Blood, plasma protein.[†]Pep is the number of peptides identified with individual ion score cutoff >40 ($P > 0.05$).[§]Uni is the number of unique peptides identified for the protein.[¶]Cov is the sequence coverage of the peptides for the identified protein sequence.