

## **Supplementary Information**

### **Chromogranin A regulates gut permeability *via* the antagonistic actions of its proteolytic peptides**

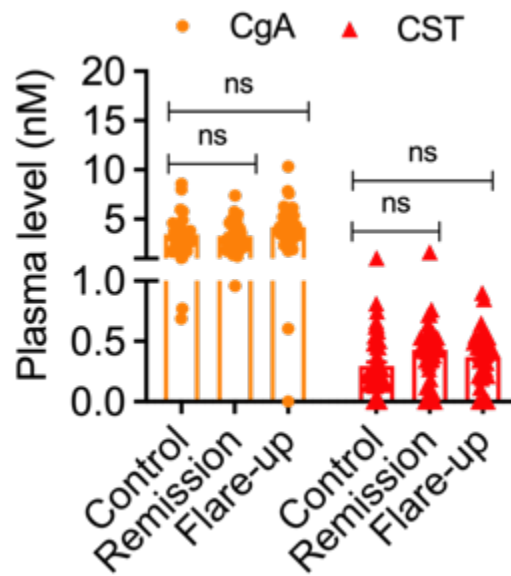
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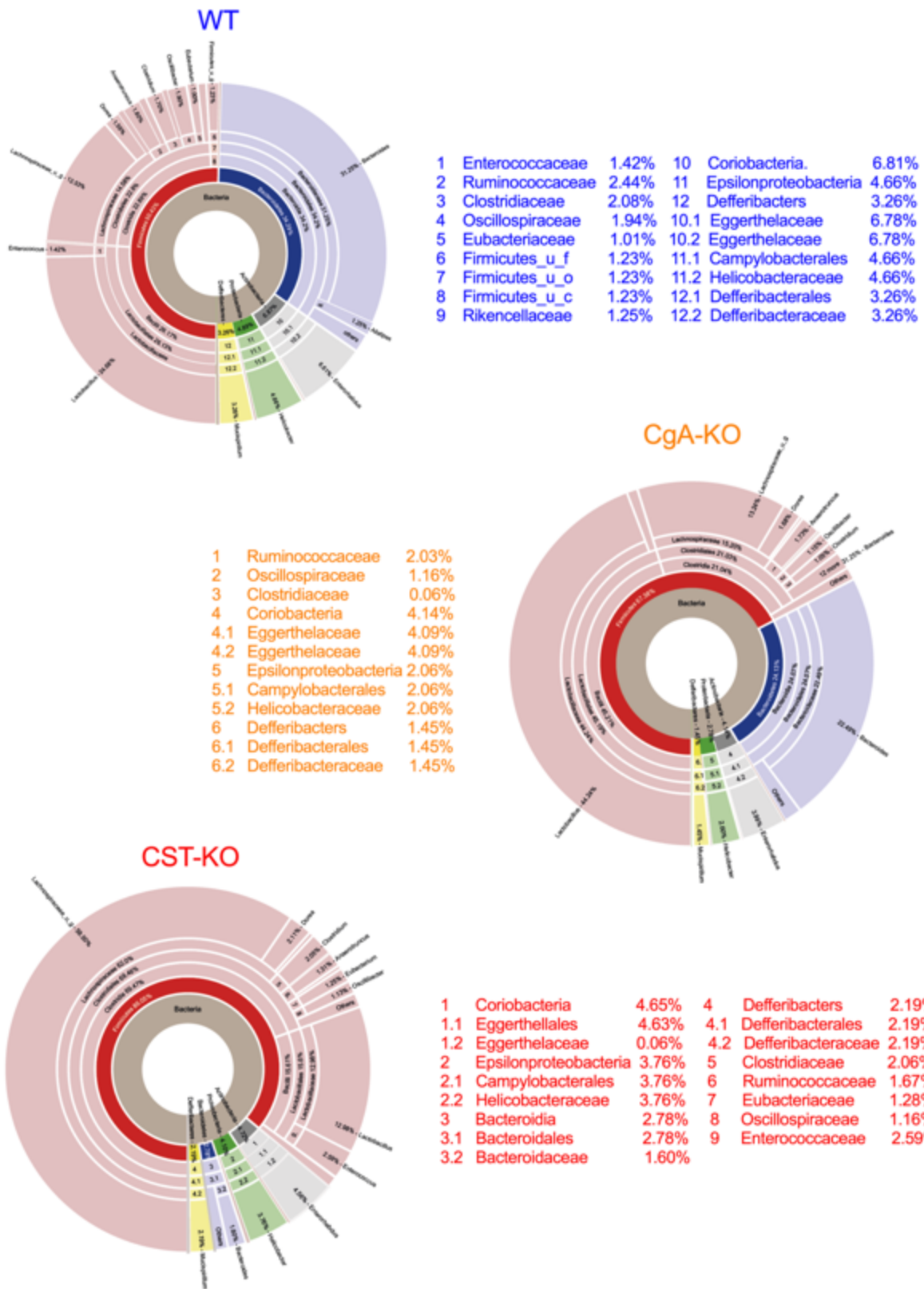
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**Sup. Fig. 1. CgA and CST levels in plasma of ulcerative colitis patients.** CgA and CST levels in EDTA-plasma of healthy donors (Control) and Ulcerative Colitis patients (N=101) in remission or flare-up (2-way ANOVA). Ns, not significant.



**Sup. Fig. 2: detailed microbial composition of WT, CgA-KO and CST-KO mice.** Graphs show complete microbiota composition in cecum of wild-type (WT), CgA-KO and CST-KO mice. Circles from inside to outside show kingdom, phylum, class, order, family and genus

**Supplementary Table S1. Patient characteristics.<sup>1</sup>**

	Healthy controls (n = 50)	CD remission (n = 49)	CD flare (n = 40)	UC remission (n = 51)	UC flare (n = 50)
<b>Female</b> (n, %)	41 (82%)	32 (65%)	25 (51%)	24 (47%)	23 (46%)
<b>Age</b> (average, range)	43 (21-68)	49 (22-71)	46 (20-72)	51 (20-76)	49 (24-75)
<b>Disease duration</b> (average, range)	-	21 (8-51)	18 (7-48)	19 (6-46)	19 (3-51)
<b>Montreal</b> (n, %)	-	L1 2 (4%)	0 (0%)	E1 8 (16%)	2 (4%)
		L2 21 (43%)	18 (37%)	E2 20 (39%)	14 (28%)
		L3 24 (49%)	28 (57%)	E3 22 (41%)	34 (68%)
		L2 + L4 0 (0%)	2 (4%)		
		L3 + L4 2 (4%)	1 (2%)		
<b>IBD medication (%)</b>					
None	50 (100%)	9 (18%)	14 (29%)	7 (14%)	1 (2%)
Mesalazine preparations		9 (18%)	5 (10%)	36 (71%)	39 (78%)
Corticosteroids		1 (2%)	6 (12%)	0 (0%)	7 (14%)
Immunosuppressives		26 (53%)	14 (29%)	20 (39%)	15 (30%)
Biologicals		12 (24%)	16 (33%)	4 (8%)	3 (6%)
<b>TNF<math>\alpha</math> plasma levels</b> (nM) (average, range)	5.9 (0-21.9)	2.6 (0-15.8)	5.2 (0-26.1)	3.4 (0-33.8)	5.5 (0-45.6)

<sup>1</sup>Characteristics of the patients at time of inclusion. As an indication for the extent of disease, the location has been scored according to the Montreal classification. For CD; L1 is ileal disease, L2 is colonic disease, L3 is ileocolonic disease, and L4 is isolated upper gastro-intestinal tract. For UC; E1 is distal to the rectosigmoid junction (proctitis), E2 is distal to the splenic flexure (left-sided) and E3 is proximal to the splenic flexure (extensive). Medication entails therapeutic categories in use for IBD; other therapies are not included. Some patients use more than one drug which all have been scored separately. Biologicals comprised anti-TNF- $\alpha$  compounds except for one patient in the CD flare group. CD: Crohn's disease, IBD: inflammatory bowel disease, UC: ulcerative colitis, TNF- $\alpha$ : tumor necrosis factor alpha. No correlation was observed between Chromogranin A and TNF- $\alpha$  levels (Pearson's correlation:  $r = 0.12$ ,  $p = 0.08$ ). Also no correlation was observed between Chromogranin A and TNF- $\alpha$  levels when plotted per disease group (healthy control, UC and CD remission and flare-up; Table S2).

**Supplementary Table S2. Correlation matrix patient characteristics.<sup>1</sup>**

	<b>CgA (nM)</b>	<b>CST (nM)</b>	<b>TNF<math>\alpha</math> (nM)</b>
<b>CgA (nM)</b>	1.000	0.221	0.127
<b>CST (nM)</b>	0.221	1.000	-0.002
<b>TNF<math>\alpha</math> (nM)</b>	0.127	-0.002	1.000
<b>Disease status</b>	0.196	0.298	0.038
<b>Sex</b>	0.020	-0.147	-0.111
<b>Age</b>	-0.006	-0.008	-0.066
<b>Years since diagnosis</b>	-0.143	0.006	0.027
<b>Montreal classification</b>	0.030	-0.034	0.057
<b>Clinical symptoms</b>	0.147	-0.052	0.250
<b>Medication use</b>	-0.079	-0.118	0.093
<b>Mesalazine preparations</b>	-0.009	-0.136	0.003
<b>Corticosteroids</b>	0.079	0.077	0.102
<b>Immunosuppressives</b>	-0.062	0.016	-0.028
<b>Biologicals</b>	0.059	-0.086	0.064
<b>anti-TNF<math>\alpha</math> therapy</b>	0.069	-0.051	0.030

<sup>1</sup>Correlation matrix of all patient characteristics (removed endoscopy and pathology due to many absent values, and healthy control subset as most factors were not determined). CgA and CST correlated strongest with disease status (CD and UC remission and flare-up). All characteristics were categorical (yes/no or female/male) except for age, years since diagnosis and CgA, CST and TNF- $\alpha$  levels. A log transformation was performed when a characteristic was not normally distributed.

**Supplementary Table S3. Primer sequences for RT-qPCR experiments.**

Gene	Gene ID	Forward primer (5'-3')	Reverse primer (3'-5')
<i>Arg1</i>	11846	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
<i>Ccl2</i>	20296	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
<i>Cdh1</i>	12550	AGGAAATGCACCCCTCCAAT	AATCGGCCAGCATTCTG
<i>Cldn2</i>	12738	CAACTGGTGGGCTACATCCTA	CCCTTGAAAAGCCAACCG
<i>Clec10a</i>	17312	TGAGAAAGGCTTTAAGAAGTGGG	GACCACCTGTAGTGATGTGGG
<i>Clec7a</i>	56644	GACTTCAGCACTCAAGACATCC	TTGTGTCGCCAAAATGCTAGG
<i>Ctnna1</i>	12385	ACTTTGATGTCAGAAGCAGGACC	CACCTGTTCTGCAATCTTTGCTTT
<i>Ctnnb1</i>	12387	AAGGAAGCTTCCAGACATGC	AGCTTGCTCTCTTGATTGCC
<i>Ctsl</i>	13039	ATCAAACCTTTAGTGCAGAGTGG	CTGTATTCCCCGTTGTGTAGC
<i>Cxcl1</i>	14825	GCTTGAAGGTGTTGCCCTCAG	AAGCCTCGCACCATTCTTG
<i>Dsg2</i>	13511	CGTGGTTGAAGGCATTCTTTTC	TAGCTGCTTGACCAGTGTCTT
<i>Emr1</i>	13733	TTGTACGTGCAACTCAGGACT	GATCCCAGAGTGTGATGCAA
<i>F11r</i>	16456	TCTCTTCACGTCTATGATCCTGG	TTTGATGGACTCGTTCTCGGG
<i>lfng</i>	15978	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCTCTC
<i>Il10</i>	16153	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<i>Il4</i>	16189	GGTCTCAACCCCAAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
<i>Itgam</i>	16409	ATGGACGCTGATGGCAATACC	TCCCCATTACGTCTCCA
<i>Itgax</i>	16411	CTGGATAGCCTTTCTCTGCTG	GCACACTGTGTCGGAACCTCA
<i>Marveld2</i>	218518	GGGTCGCAAGGCACCTTTAAT	ACCTTCTCAGAATACGTCCGC
<i>Mrc1</i>	17533	CTCTGTTTCAAGTATTGGACGC	CTCTGTTTCAAGTATTGGACGC
<i>Ocln</i>	18260	TTGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
<i>Plg</i>	18815	TGCAGTGGAGAAAAGTATGAGGG	AGGGATGTATCCATGAGCATGT
<i>S100a9</i>	20202	ATACTCTAGGAAGGAAGGACACC	TCCATGATGTCATTTATGAGGGC
<i>Tgfb1</i>	21803	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
<i>Tjp1</i>	21872	GCCGCTAAGAGCACAGCAA	TCCCCACTCTGAAAATGAGGA
<i>Tnfa</i>	21926	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG