

Table S1. Patient characteristics of the discovery set

Total N=20		GI GVHD N=10	No GVHD N=10	p-value
Age (years)	Median (range)	52 (27-60)	50 (34-64)	>0.9
Disease (%)	Malignant	100% (N=10)	100% (N=10)	>0.9
	Other	0% (N=0)	0% (N=0)	
Disease status at transplant* (%)	Other/low/ intermediate risk	60% (N=6)	50% (N=5)	>0.9
	High risk	40% (N=4)	50% (N=5)	
Donor type (%)	Related donor	70% (N=7)	70% (N=7)	>0.9
	Unrelated donor	30% (N=3)	30% (N=3)	
Donor match (%)	Matched donor	100% (N=10)	90% (N=9)	>0.9
	Mismatched donor	0% (N=0)	10% (N=1)	
Conditioning regimen intensity (%)	High intensity	100% (N=10)	90% (N=9)	>0.9
	Moderate intensity	0% (N=0)	10% (N=1)	
Grade of GVHD at onset (%)	0	0% (N=0)	100% (N=10)	
	I	0% (N=0)	0% (N=0)	
	II	10% (N=1)	0% (N=0)	
	<i>Isolated upper GI GVHD</i>	10% (N=1)	0% (N=0)	
	<i>Lower GI GVHD</i>	0% (N=0)	0% (N=0)	
	III-IV	90% (N=9)	0% (N=0)	
	GI Stage 2	50% (N=5)	0% (N=0)	
	GI Stage 3	40% (N=4)	0% (N=0)	
	GI Stage 4	0% (N=0)	0% (N=0)	
Day after HCT	Median (range)	26 (7-63)	27 (14-70)	0.7

*High risk of disease status at HCT is according to Center for International Blood and Marrow Transplant Research (CIBMTR) guidelines.

Table S2. Patient characteristics of the Regensburg/Kyushu validation set

Total N=143		GI GVHD ^{†,‡} N=30	No GVHD N=53	Non- GVHD Enteritis [§] N=11	Skin GVHD N=49	p-value
Age (years)						0.22
	Median (range)	44 (15-63)	46 (24-67)	35 (15-51)	44 (19-62)	
Disease (%)						0.53
	Malignant	97% (N=29)	92% (N=49)	91% (N=10)	98% (N=48)	
	Other	3% (N=1)	8% (N=4)	9% (N=1)	2% (N=1)	
Disease status at transplant* (%)						0.09
	Other/low/intermediate risk	43% (N=13)	64% (N=34)	82% (N=9)	53% (N=26)	
	High risk	57% (N=17)	36% (N=19)	18% (N=2)	47% (N=23)	
Donor type (%)						0.96
	Related donor	23% (N=7)	23% (N=12)	27% (N=3)	20% (N=10)	
	Unrelated donor	77% (N=23)	77% (N=41)	73% (N=8)	80% (N=39)	
Donor match (%)						0.25
	Matched donor	67% (N=20)	81% (N=43)	91% (N=10)	69% (N=34)	
	Mismatched donor	33% (N=10)	19% (N=10)	9% (N=1)	31% (N=15)	
Conditioning regimen intensity (%)						0.62
	High intensity	47% (N=14)	34% (N=18)	27% (N=3)	37% (N=18)	
	Moderate intensity	53% (N=16)	66% (N=35)	73% (N=8)	63% (N=31)	
Grade of GVHD at onset (%)						
	0	0% (N=0)	100% (N=53)	100% (N=11)	0% (N=0)	
	I	0% (N=0)	0% (N=0)	0% (N=0)	61% (N=30)	
	<i>Skin Stage 1</i>	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>22%</i> (N=11)	
	<i>Skin Stage 2</i>	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>39%</i> (N=19)	
	II	63% (N=19)	0% (N=0)	0% (N=0)	39% (N=19)	
	<i>Isolated Skin Stage 3</i>	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>39%</i> (N=19)	
	<i>Isolated Upper GI Stage 1[‡]</i>	<i>20%</i> (N=6)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
	<i>Lower GI Stage 1[‡]</i>	<i>43%</i> (N=13)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
	III-IV	37% (N=11)	0% (N=0)	0% (N=0)	0% (N=0)	
	<i>Isolated Skin Stage 4</i>	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
	<i>GI Stage 2[‡]</i>	<i>17%</i> (N=5)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
	<i>GI Stage 3[‡]</i>	<i>7%</i> (N=2)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
	<i>GI Stage 4[‡]</i>	<i>13%</i> (N=4)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	<i>0%</i> (N=0)	
Day after HCT						0.07
	Median (range)	19 (8-182)	26 (14-86)	28 (14-51)	20 (11-485)	

*High risk of disease status at HCT is according to Center for International Blood and Marrow Transplant Research (CIBMTR) guidelines.

[†]Including 6 patients with isolated upper GI GVHD and 24 with lower GI ± upper GI GVHD.

[‡]With or without other GVHD target organ involvement.

[§]Including 8 patients with isolated upper GI non-GVHD enteritis and 3 patients with lower GI ± upper GI non-GVHD enteritis.

Table S3. Causes of non-GVHD enteritis in the Regensburg/Kyushu validation set

Non-GVHD lower GI enteritis +/- upper GI symptoms: N=3	
C. difficile infection	33% (N=1)
Diarrhea; biopsy negative	33% (N=1)
Diarrhea; no biopsy, spontaneously resolved	33% (N=1)
Non-GVHD upper GI enteritis without diarrhea: N=8	
Nausea/vomiting; biopsy negative	75% (N=6)
Nausea/vomiting; no biopsy, spontaneously resolved	12% (N=1)
CMV gastritis	13% (N=1)

Table S4. ELISA assay parameters

	STD Curve Range	Dilution Factor	CV%*	ULOD	LLOD
REG3a	100 – 1.6 ng/ml	1/10	5.91	1.80 ± 0.13	0.04 ± 0.08
IL-2Ra	2000 – 31.2 pg/ml	Undiluted	2.59	1.11 ± 0.29	0.03 ± 0.02
TNFR1	800 – 12.5 pg/ml	1/25	4.23	1.64 ± 0.36	0.05 ± 0.03
Elafin	2000 – 31.2 pg/ml	1/20	6.46	2.26 ± 0.63	0.16 ± 0.05
HGF	4000 – 62.5 pg/ml	1/2	2.35	1.96 ± 0.60	0.07 ± 0.11
IL-8	200 – 3.1 pg/ml	1/6	7.13	1.86 ± 0.76	0.03 ± 0.04

*CV calculated on 3rd highest standard concentration; CV= (standard deviation/mean)*100.

Table S5. GI GVHD candidate biomarkers identified by IPAS

IPI*	Gene Name	Gene description	RATIO (mean)	#Events	Preferential GI expression [†]	Suitable antibodies [‡]
IPI00032214	BRD1	Bromodomain-containing protein 1.	35.5	1	No	No
IPI00012549	PCDHGA11	Isoform 2 of protocadherin gamma a11 precursor.	34.0	1	No	No
IPI00738813	OXR1	Oxidation Resistance Protein 1	25.1	1	No	No
IPI00456604	FAM19A1	Family with sequence similarity 19, member A1 precursor	12.0	1	No	No
IPI00060310	PLD4	Phospholipase d4.	11.8	1	No	No
IPI00100668	GBA2	Isoform 1 of non-lysosomal glucosylceramidase.	11.7	1	No	No
IPI00010295	CPN1	Carboxypeptidase N catalytic chain precursor	8.9	2	Yes	No
IPI00010779	TPM4	Isoform 1 of tropomyosin alpha-4 chain.	8.4	1	No	Yes
IPI00410143	CENPM	Isoform 2 of centromere protein m.	7.7	2	No	No
IPI00305698	GGCX	Vitamin k-dependent gamma-carboxylase.	7.6	1	No	No
IPI00012011	CFL1	Cofilin, non-muscle isoform	7.5	9	No	No
IPI00059279	EXOC4	Exocyst complex component 4	7.5	2	No	No
IPI00020687	SPINK1	Pancreatic secretory trypsin inhibitor precursor	7.4	4	Yes	No
IPI00022417	LRG1	Leucine-rich alpha-2-glycoprotein precursor.	7.2	1	No	Yes
IPI00005822	CDC23	Cell division cycle protein 23.	7.0	2	No	No
IPI00009822	SRP54	Signal recognition particle 54 kDa protein	7.0	4	No	No
IPI00008274	CAP1	Adenylyl cyclase-associated protein 1	6.1	2	No	No
IPI00009143	ADAMTS5	ADAM metalloproteinase with thrombospondin type 1 motif, 5 precursor	5.1	1	No	Yes
IPI00292950	SERPIND1	Heparin cofactor II precursor	4.8	7	No	Yes
IPI00036578	ADAMTS12	ADAM metalloproteinase with thrombospondin type 1 motif, 12 preproprotein	4.4	1	No	No
IPI00299155	PSMA4	Proteasome subunit alpha type 4.	4.3	2	No	No
IPI00216691	PFN1	Profilin-1	4.2	3	No	No
IPI00290420	HPGD	15-hydroxy prostaglandin dehydrogenase.	4.1	1	No	No
IPI00304922	LSMD1	LSM domain containing 1	4.0	1	No	No
IPI00477868	LAMA5	Laminin, alpha 5	3.8	1	No	No
IPI00414467	COLEC12	Nurse cell scavenger receptor 2	3.8	1	No	No
IPI00011155	ASGR2	Splice Isoform 1 of Asialoglycoprotein receptor 2	3.7	1	No	No
IPI00294615	FBLN5	Fibulin-5 precursor	3.5	1	No	No
IPI00376787	EZH2	Enhancer of zeste 2 isoform a.	3.3	1	No	No
IPI00293276	MIF	Macrophage migration inhibitory factor	3.3	2	No	Yes
IPI00006971	CD248	Tumor endothelial marker 1	3.3	2	No	No
IPI00184019	PILRA	Paired immunoglobulin-like receptor alpha	3.2	1	No	No
IPI00019372	PRG1	Secretory granule proteoglycan core protein precursor	3.2	1	No	No
IPI00018136	VCAM1	Splice Isoform 1 of Vascular cell adhesion protein 1 precursor	3.1	11	No	No
IPI00022585	AKAP1	Isoform 1 of a kinase anchor protein 1, mitochondrial precursor.	3.0	1	No	No
IPI00239077	HINT1	Histidine triad nucleotide-binding protein 1	2.9	1	No	No
IPI00166197	PALLD	Palladin	2.9	1	Yes	No
IPI00009027	REG1A	Lithostathine 1 alpha precursor	2.9	3	Yes	No
IPI00298547	PARK7	Protein DJ-1	2.9	2	No	No
IPI00218288	SEC24D	Sec24-related protein D	2.8	2	No	No
IPI00010341	PRG2	Eosinophil granule major basic protein precursor	2.8	5	No	No
IPI00299977	PHPT1	14 kDa phosphohistidine phosphatase	2.8	1	No	No
IPI00004656	B2M	Beta-2-microglobulin precursor	2.8	48	No	Yes
IPI00030154	PSME1	Proteasome activator complex subunit 1.	2.6	3	No	No
IPI00328257	APIB1	Isoform a of ap-1 complex subunit beta-1.	2.6	1	No	No
IPI00291866	SERPING1	Plasma protease C1 inhibitor precursor	2.6	60	No	No
IPI00001458	KNTC1	Kinetochore-associated protein 1.	2.6	1	No	No
IPI00002436	CNOT4	Isoform 5 of ccr4-not transcription complex subunit 4.	2.6	1	No	No
IPI00027848	MRC1	Macrophage mannose receptor 1 precursor.	2.6	1	No	No
IPI00006717	CCL16	Small inducible cytokine A16 precursor	2.6	1	No	Yes
IPI00022429	ORM1	Alpha-1-acid glycoprotein 1 precursor	2.5	192	No	Yes
IPI00296713	GRN	Splice Isoform 1 of Granulins precursor	2.5	1	No	Yes
IPI00022200	COL6A3	Alpha 3 type VI collagen isoform 1 precursor	2.4	1	No	No
IPI00419585	PPIA	Peptidyl-prolyl cis-trans isomerase A	2.4	2	No	No
IPI00022284	PRNP	Major prion protein precursor	2.4	1	No	Yes
IPI00032292	TIMP1	Metalloproteinase inhibitor 1 precursor	2.3	9	No	Yes
IPI00022418	FN1	Splice Isoform 1 of Fibronectin precursor	2.2	224	No	Yes
IPI00414283	FN1	Fibronectin 1 isoform 4 preproprotein.	2.2	36	No	Yes

IPI00029039	REG3A	Regenerating islet-derived protein 3 alpha precursor	2.2	4	Yes	Yes
IPI00005769	FANCG	Fanconi anemia group g protein.	2.2	1	No	No
IPI00164104	DLEC1	Isoform 1 of deleted in lung and esophageal cancer protein 1.	2.2	1	No	No
IPI00008148	GFRA1	Isoform 1 of gdnf family receptor alpha-1 precursor.	2.1	1	No	No
IPI00103636	WFDC2	Splice Isoform 2 of WAP four-disulfide core domain protein 2 precursor	2.1	2	No	No
IPI00376005	EIF5A	Isoform 2 of eukaryotic translation initiation factor 5a-1.	2.1	1	No	No
IPI00026941	PRSS23	Serine protease 23 precursor	2.1	2	No	No
IPI00025155	FSTL3	Follistatin-related protein 3 precursor.	2.1	1	No	Yes
IPI00013831	CD48	B-lymphocyte activation marker BLAST-1 precursor	2.1	1	No	No
IPI00295339	SELP	P-selectin precursor	2.1	1	No	Yes
IPI00760855	TMEM110	Transmembrane protein 110	2.0	3	No	No
IPI00030144	PPIAL4	Peptidyl-prolyl cis-trans isomerase.	2.0	1	No	No
IPI00479186	PKM2	Pyruvate kinase 3 isoform 1 variant	2.0	2	No	No
IPI00015029	PTGES3	Telomerase-binding protein p23	2.0	3	No	No
IPI00029623	PSMA6	Proteasome subunit alpha type 6	2.0	2	No	No
IPI00219018	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase, liver	2.0	3	No	No

* **IPI**: International Protein Index

† **Preferential GI expression**: proteins expressed in GI tract but not in skin, bone marrow or lymphoid tissue, as referred by gene ontology, human protein atlas and literature search

‡ **Suitable antibodies**: Established antibody pairs for ELISA screening.

Peptides Level												
IPI	Z	Time	PreMass	CalMass	dMppm	Expect	q3L	q3H	NRatio	Prob	Peptide Sequence	dbHits
IPI00029039	2	1626.3	1686.83	1686.81	11	0.098	0	329190	9999	0.97	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1729.8	1686.83	1686.81	10	0.054	0	108078	9999	1	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	2	1730.1	1686.83	1686.81	14	0.029	0	67488	9999	1	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1641.6	1686.83	1686.81	13	0.28	0	5991	9999	0.97	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	2	1609.4	1687.84	1686.81	15	0.085	0	25367	9999	0.86	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1885.1	1687.83	1686.81	8	0.64	3852	6344	1.6	0.91	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1500.9	1686.83	1686.81	14	0.1	11429	23797	2.0	0.99	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1597.4	1686.83	1686.81	14	0.39	0	5007	9999	0.99	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	3	1836.6	1687.83	1686.81	11	2.2	740	1279	1.7	0.81	NPSTISSPGHC[CysH]ASLSR	1
IPI00029039	2	2462	1310.6	1310.59	8	0.017	0	341159	9999	1	SWTDADLAC[CysH]QK	1
IPI00029039	2	2565.4	1310.61	1310.59	12	0.012	24969	103841	4.0	1	SWTDADLAC[CysH]QK	1
Big Quant Level												
IPI	Chr	Length	MW	GMean	Events	StDev	TTest	Pvalue				
IPI00029039	2	175	19395	2.2	4	0.43	3.6	0.0343				

IPI: International Protein Index; Z: charge, PreMass: Precursor Mass; CalMass: Calculated Mass; dMppm: fractional delta mass in part per million; Expect: Expected value; q3L: quantified Light value; q3H: quantified Heavy value; Nratio: Normalized ratio; Prob: Peptide Probability; dbHits: number of indistinguishable hits in IPI database; GMean: geometric mean.

Isolated skin GVHD	339
Isolated GI GVHD*	118
GI GVHD plus skin GVHD†	79

* Including 9 patients with liver GVHD
† Including 13 patients with liver GVHD

Table S8. Positive (PPV) and negative (NPV) predictive values for GI GVHD of plasma REG3α concentrations at the onset of diarrhea		
Cutoff	PPV	NPV
151 ng/ml (50 %-ile*)	95%	32%
100 ng/ml (42%-ile*)	95%	35%
57 ng/ml (25 %-ile*)	92%	44%
28 ng/ml (10 %-ile*)	84%	50%

* %-ile of REG3 α concentration in patients with lower GI GVHD at onset.

Table S9. Causes of 1-year mortality in lower GI GVHD patients (N=97)	
Non-relapse mortality	79% (N=77)
Acute GVHD	65% (N=50)
Infection/sepsis	12% (N=9)
Chronic GVHD	12% (N=9)
Graft failure	3% (N=2)
Multiple organ failure	1% (N=1)
SOS	1% (N=1)
Intracranial hemorrhage	1% (N=1)
Unknown	5% (N=4)
Relapse mortality	21% (N=20)

Table S10. GI GVHD patients by number of risk factors (N=140)	
0 risk factors	31 (22%)
1 risk factor	49 (35%)
High REG3 α	27 (19%)
GI GVHD onset stage 2-4	21 (15%)
Histologic grade 4	1 (1%)
2 risk factors	46 (33%)
High REG3 α /GI GVHD onset stage 2-4	28 (20%)
High REG3 α /Histologic grade 4	8 (6%)
GI GVHD onset stage 2-4/Histologic grade 4	10 (7%)
3 risk factors	14 (10%)

Figure S1. Identification of REG3 α through discovery phase proteomics. MS/MS of the identified peptide, REG3 α . B_n or y_n denotes the fragment ion generated by cleavage of the peptide bond after the nth amino acid containing either the peptide N terminus (b series) or the C terminus (y series), respectively. The identified b and y ions and all fragment ion (m/z) values are indicated in the table. C* denotes cysteine residues modified by acrylamide containing three ¹³C atoms. The identified peptide sequence location is highlighted in red within the protein sequence.

Figure S2. REG3 α concentrations in the discovery set. Plasma concentrations of REG3 α were measured by ELISA in the 20 individual samples of the discovery set, and are presented as scatter plots with lines for means.

Figure S3. ROC curves for two independent validation sets. ROC curves comparing plasma REG3 α concentrations in patients with diarrhea caused by GVHD (N=162) and not caused by GVHD (N=42). University of Michigan validation set (thick line), AUC=0.80; Regensburg/Kyushu validation set (dashed line), AUC=0.84.

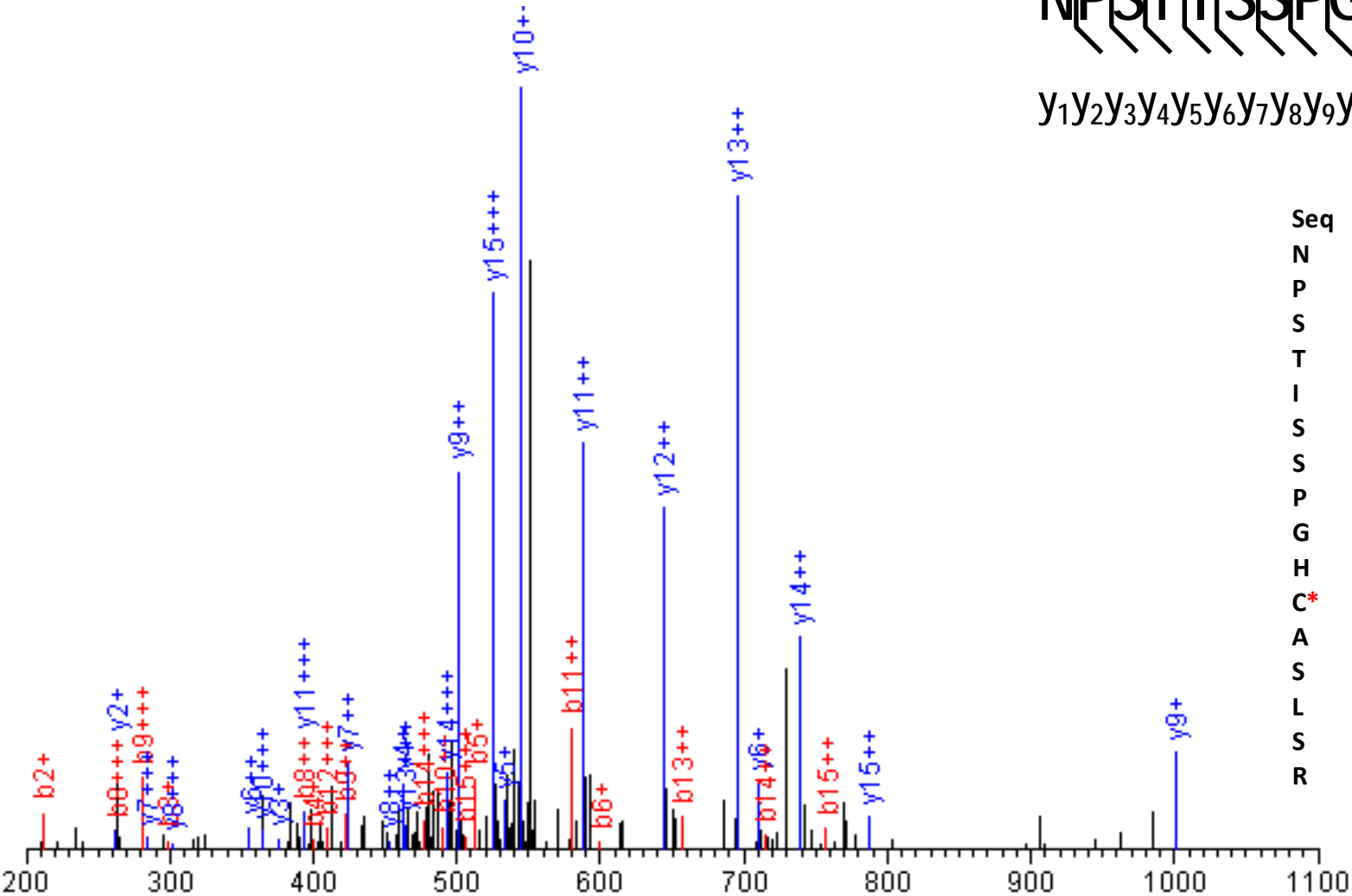
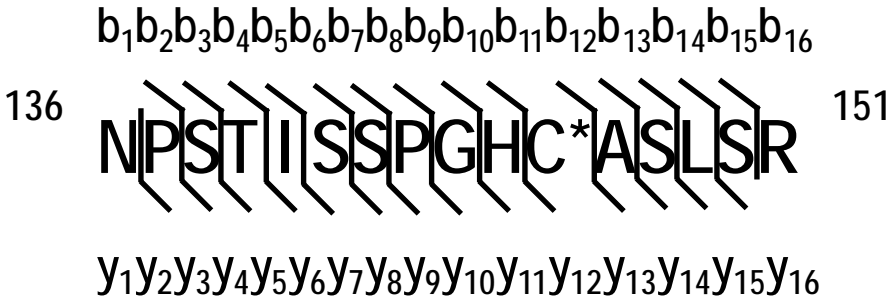
Figure S4. ROC curve for patients with less than 1 liter of stool per day. ROC curve comparing plasma REG3 α concentrations in patients with diarrhea less than 1000 ml/day caused by GVHD (N=80) and not caused by GVHD (N=29); AUC=0.81.

Figure S5. Albumin concentrations by severity of lower GI GVHD diarrhea. (A) Serum albumin correlation by clinical lower GI GVHD stage. Stage 1 (N=67) versus stage 2-4 (N=73), p=0.005. (B) Serum albumin concentrations and histologic grade. Histologic grade 1-3 (intact mucosa, N=107) versus grade 4 (denuded mucosa; N=33), p=0.04.

Figure S6. Correlation of REG3 α concentrations at onset of lower GI GVHD correlate with eventual maximum GVHD severity. Plasma REG3 α concentrations in patients with lower GI GVHD at onset (y-axis) are compared between patients with maximum GVHD severity of grade 2 (N=49) and patients who eventually developed maximum grade 3-4 GVHD (N=113), p<0.001.

Figure S7. 1-Year NRM for lower GI GVHD patients by other risk factors and risk factor combinations. (A) 1-year NRM by Clinical GI GVHD stage 2-4 (solid line) versus stage 1 (dashed line); 61% vs. 33%, respectively; p<0.001. (B) 1-year NRM by histologic grade 4 GI GVHD (solid line) versus histologic grade 1-3 (dashed line); 73% vs. 40%, p<0.001. (C) 1 year NRM for patients classified by number of risk factors at GVHD onset, using onset REG3 α concentration (high risk > 151 ng/ml) and clinical stage (high risk = stage 2-4). 0 (purple, NRM=27%); 1 (red, NRM=40%); 2 (blue, NRM=75%). 0 vs. 1, p=0.1; 1 vs. 2, p<0.001. (D) 1 year NRM for patients classified by number of risk factors at GVHD onset, using onset REG3 α concentration (high risk > 151 ng/ml) and histologic grade (high risk = grade 4). 0 (purple, NRM=31%); 1 (red, NRM=49%); 2 (blue, NRM=82%). 0 vs. 1, p=0.02; 1 vs. 2, p<0.001.

Figure S1



Seq #	b	y	#
N 1	115.1118		16
P 2	212.2285	1574.7163	15
S 3	299.3067	1477.5996	14
T 4	400.4117	1390.5214	13
I 5	513.5712	1289.4164	12
S 6	600.6494	1176.2569	11
S 7	687.7276	1089.1787	10
P 8	784.8443	1002.1005	9
G 9	841.8962	904.9838	8
H 10	979.0373	847.9319	7
C* 11	1156.2228	710.7908	6
A 12	1227.3016	533.6053	5
S 13	1314.3798	462.5265	4
L 14	1427.5392	375.4483	3
S 15	1514.6174	262.2889	2
R 16	-	175.2107	1

REG3A, IPI00029039, Q06141

N-terminus MLPPMALPSV SWMLLSCLML LSQVOGEEPO RELPSARIRC PKGSKAYGSH CYALFLSPKS
 WTDADLACQK RPSGNLVSVL SGAEGSFVSS LVKSIGNSYS YWIGLHDPT QGTEPNGEGW
 EWSSSDVMNY FAWER**NPSTI SSPGHCASLS** RSTAFLRWKD YNCNVRLPYV CKFTD - C-terminus

Figure S2

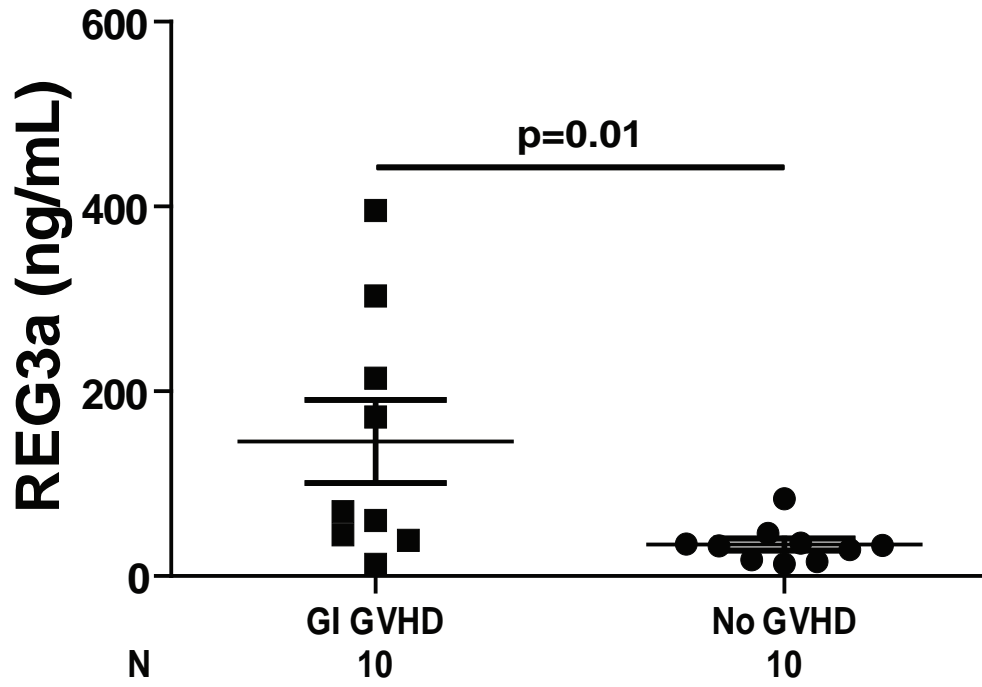


Figure S3

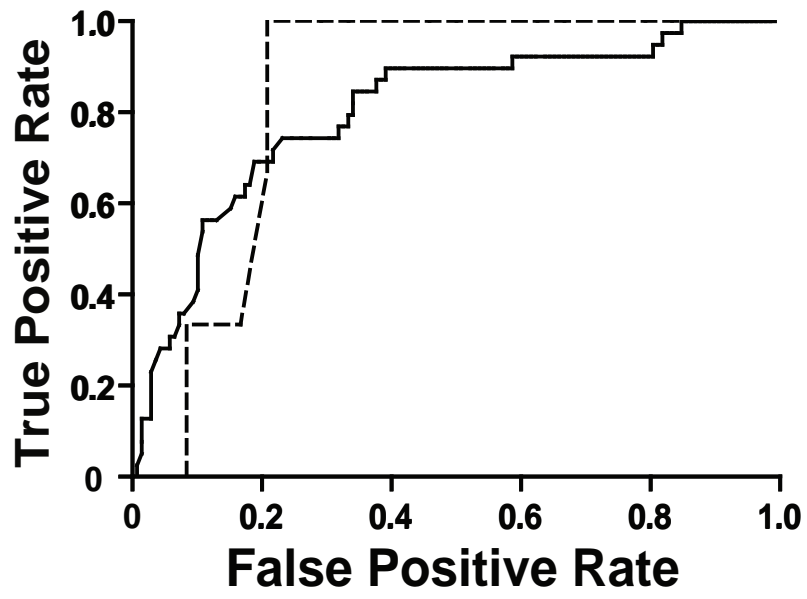


Figure S4

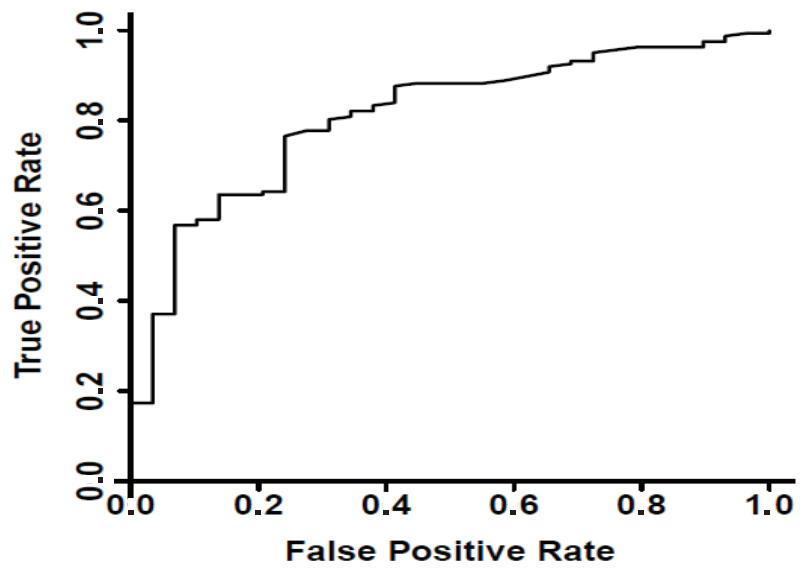
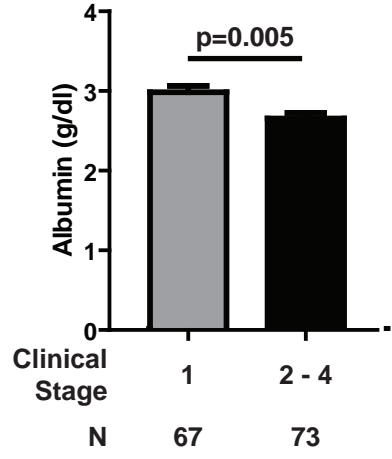


Figure S5

A



B

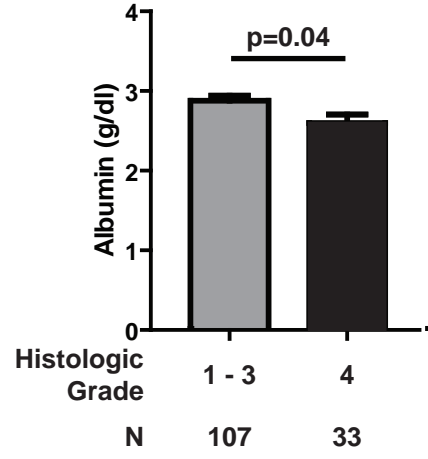


Figure S6

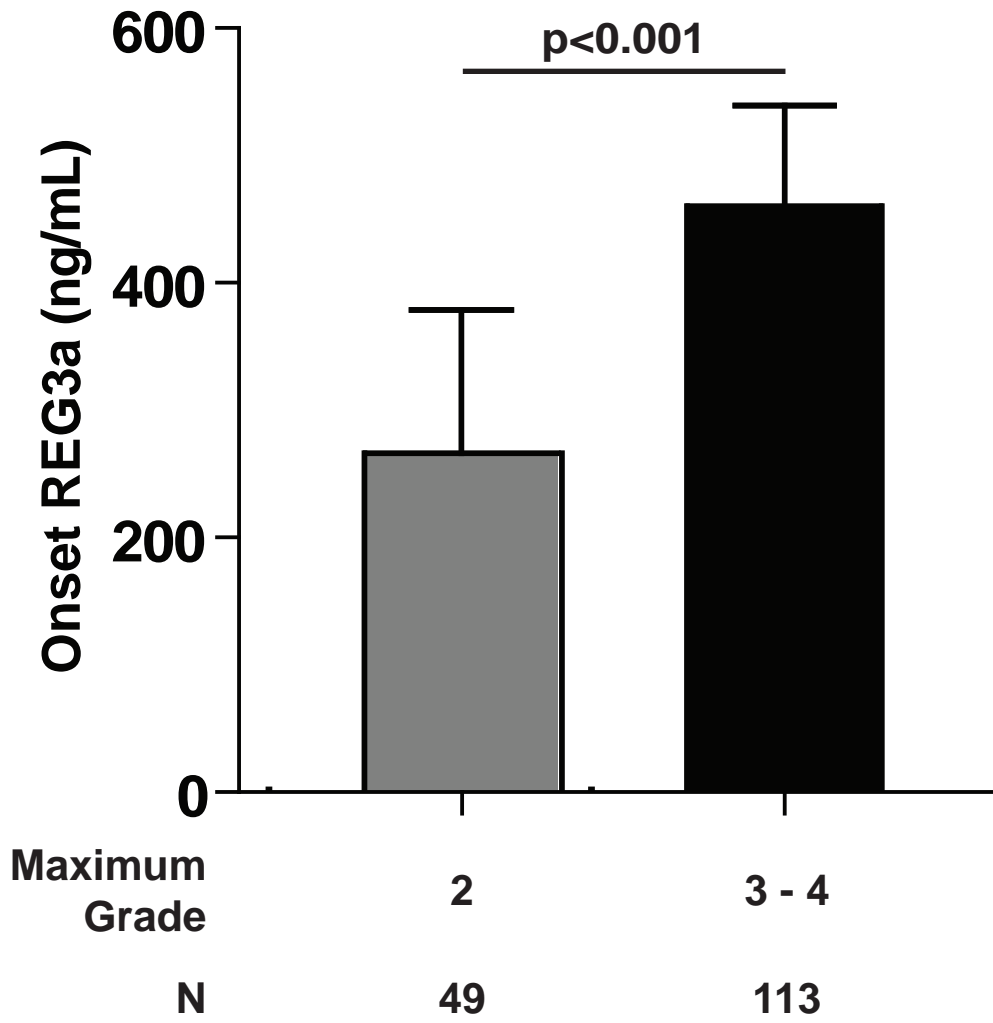


Figure S7

