Supplemental Figure S1: **β_1 – integrins are major counterecptors of galectin-8 in the TM.** Proteins extracted from cultured TM cells were incubated with Gal8-affinity matrix. Gal8 bound proteins were eluted with β-lactose, electrophoresed on a polyacrylamide gel in the presence of SDS and identified by LC/MS/MS. A minimum of four unique, non-overlapping peptides was set as a threshold for a match. Sequences of proteins identified in the 125-kDa band (Figure 4A) are presented with matched peptides highlighted. A: integrin β_1; B: integrin α_3; C, integrin: α_5; D: integrin α_v. This experiment was performed twice, with reproducible results.

Figure S1

A 1 GEAQRESRNS RPEPTAPGPG RRAEKMNLQP IFWIGLISSV CCVFAQTDEN RCLKANAKSC GECIQAGPNC GWCTNSTFLQ
81 EGMPTSARCD DLEALKKKGC PPDDIENPRG SKDIKKNKNV TNRSKGTAEK LKPEDITQIQ PQQLVLRLRS GEPQTFTLKF
161 KRAEDYPIDL YYLMDLSYSM KDDLENVKSL GTDLMNEMRR ITSDFRIGFG SFVEKTVMPY ISTTPAKLRN PCTSEQNCTS
241 PFSYKNVLSL TNKGEVFNEL VGKQRISGNL DSPEGGFDAI MQVAVCGSLI GWRNVTRLLV FSTDAGFHFA GDGKLGGIVL
321 PNDGQCHLEN NMYTMSHYYD YPSIAHLVQK LSENNIQTIF AVTEEFQPVY KELKNLIPKS AVGTLSANSS NVIQLIIDAY
401 NSLSSEVILE NGKLSEGVTI SYKSYCKNGV NGTGENGRKC SNISIGDEVQ FEISITSNKC PKKDSDSFKI RPLGFTEEVE
481 VILQYICECE CQSEGIPESP KCHEGNGTFE CGACRCNEGR VGRHCECSTD EVNSEDMDAY CRKENSSEIC SNNGECVCGQ
561 CVCRKRDNTN EIYSGKFCEC DNFNCDRSNG LICGGNGVCK CRVCECNPNY TGSACDCSLD TSTCEASNGQ ICNGRGICEC
641 GVCKCTDPKF QGQTCEMCQT CLGVCAEHKE CVQCRAFNKG EKKDTCTQEC SYFNITKVES RDKLPQPVQP DPVSHCKEKD
721 VDDCWFYFTY SVNGNNEVMV HVVENPECPT GPDIIPIVAG VVAGIVLIGL ALLLIWKLLM IIHDRREFAK FEKEKMNAKW
801 DTGENPIYKS AVTTVVNPKY EGK

B 1 MGPGPSRAPR APRLMLCALA LMVAAGGCVV SAFNLDTRFL VVKEAGNPGS LFGYSVALHR QTERQQRYLL LAGAPRELAV
81 PDGYTNRTGA VYLCPLTAHK DDCERMNITV KNDPGHHIIE DMWLGVTVAS QGPAGRVLVC AHRYTQVLWS GSEDQRRMVG
161 KCYVRGNDLE LDSSDDWQTY HNEMCNSNTD YLETGMCQLG TSGGFTQNTV YFGAPGAYNW KGNSYMIQRK EWDLSEYSYK
241 DPEDQGNLYI GYTMQVGSFI LHPKNITIVT GAPRHRHMGA VFLLSQEAGG DLRRRQVLEG SQVGAYFGSA IALADLNNDG
321 WQDLLVGAPY YFERKEEVGG AIYVFMNQAG TSFPAHPSLL LHGPSGSAFG LSVASIGDIN QDGFQDIAVG APFEGLGKVY
401 IYHSSSKGLL RQPQQVIHGE KLGLPGLATF GYSLSGQMDV DENFYPDLLV GSLSDHIVLL RARPVINIVH KTLVPRPAVL
481 DPALCTATSC VQVELCFAYN QSAGNPNYRR NITLAYTLEA DRDRRPPRLR FAGSESAVFH GFFSMPEMRC QKLELLLMDN
561 LRDKLRPIII SMNYSLPLRM PDRPRLGLRS LDAYPILNQA QALENHTEVQ FQKECGPDNK CESNLQMRAA FVSEQQQKLS
641 RLQYSRDVRK LLLSINVTNT RTSERSGEDA HEALLTLVVP PALLLSSVRP PGACQANETI FCELGNPFKR NQRMELLIAF
721 EVIGVTLHTR DLQVQLQLST SSHQDNLWPM ILTLLVDYTL QTSLSMVNHR LQSFFGGTVM GESGMKTVED VGSPLKYEFQ
801 VGPMGEGLVG LGTLVLGLEW PYEVSNGKWL LYPTEITVHG NGSWPCRPPG DLINPLNLTL SDPGDRPSSP QRRRRQLDPG
881 GGQGPPPVTL AAAKKAKSET VLTCATGRAH CVWLECPIPD APVVTNVTVK ARVWNSTFIE DYRDFDRVRV NGWATLFLRT
961 SIPTINMENK TTWFSVDIDS ELVEELPAEI ELWLVLVAVG AGLLLLGLII LLLWKCGFFK RTRYYQIMPK YHAVRIREEE
1041 RYPPPGSTLP TKKHWVTSWQ TRDQYY

C 1 MAFPPRRRLR LGPRGLPLLL SGLLLPLCRA FNLDVDSPAE YSGPEGSYFG FAVDFFVPSA SSRMFLLVGA PKANTTQPGI
81 VEGGQVLKCD WSSTRRCQPI EFDATGNRDY AKDDPLEFKS HQWFGASVRS KQDKILACAP LYHWRTEMKQ EREPVGTCFL
161 QDGTKTVEYA PCRSQDIDAD GQGFCQGGFS IDFTKADRVL LGGPGSFYWQ GQLISDQVAE IVSKYDPNVY SIKYNNQLAT
241 RTAQAIFDDS YLGYSVAVGD FNGDGIDDFV SGVPRAARTL GMVYIYDGKN MSSLYNFTGE QMAAYFGFSV AATDINGDDY
321 ADVFIGAPLF MDRGSDGKLQ EVGQVSVSLQ RASGDFQTTK LNGFEVFARF GSAIAPLGDL DQDGFNDIAI AAPYGGEDKK
401 GIVYIFNGRS TGLNAVPSQI LEGQRAARSM PPSFGYSMKG ATDIDKNGYP DLIVGAFGVD RAILYRARPV ITVNAGLEVY
481 PSILNQDNKT CSLPGTALKV SCFNVRFCLK ADGKGVLPRK LNFQVELLLD KLKQKGAIRR ALFLYSRSPS HSKNMTISRG
561 GLMQCEELIA YLRDESEFRD KLTPITIFME YRLDYRTAAD TTGLQPILNQ FTPANISRQA HILLDCGEDN VCKPKLEVSV
641 DSDQKKIYIG DDNPLTLIVK AQNQGEGAYE AELIVSIPLQ ADFIGVVRNN EALARLSCAF KTENQTRQVV CDLGNPMKAG
721 TQLLAGLRFS VHQQSEMDTS VKFDLQIQSS NLFDKVSPVV SHKVDLAVLA AVEIRGVSSP DHVFLPIPNW EHKENPETEE
801 DVGPVVQHIY ELRNNGPSSF SKAMLHLQWP YKYNNNTLLY ILHYDIDGPM NCTSDMEINP LRIKISSLQT TEKNDTVAGQ
881 GERDHLITKR DLALSEGDIH TLGCGVAQCL KIVCQVGRLD RGKSAILYVK SLLWTETFMN KENQNHSYSL KSSASFNVIE
961 FPYKNLPIED ITNSTLVTTN VTWGIQPAPM PVPVWVIILA VLAGLLLLAV LVFVMYRMGF FKRVRPPQEE QEREQLQPRE
1041 NGEGNSET

D 1 RWGPRRRPPL LPLLLLLLPP PPRVGGFNLD AEAPAVLSGP PGSFFGFSVE FYRPGTDGVS VLVGAPKANT SQPGVLQGGA
81 VYLCPWGASP TQCTPIEFDS KGSRLLESSL SSSEGEEPVE YKSLQWFGAT VRAHGSSILA CAPLYSWRTE KEPLSDPVGT
161 CYLSTDNFTR ILEYAPCRSD FSWAAGQGYC QGGFSAEFTK TGRVVLGGPG SYFWQGQILS ATQEQIAESY YPEYLINLVQ
241 GQLQTRQASS IYDDSYLGYS VAVGEFSGDD TEDFVAGVPK GNLTYGYVTI LNGSDIRSLY NFSGEQMASY FGYAVAATDV
321 NGDGLDDLLV GAPLLMDRTP DGRPQEVGRV YVYLQHPAGI EPTPTLTLTG HDEFGRFGSS LTPLGDLDQD GYNDVAIGAP
401 FGGETQQGVV FVFPGGPGGL GSKPSQVLQP LWAASHTPDF FGSALRGGRD LDGNGYPDLI VGSFGVDKAV VYRGRPIVSA
481 SASLTIFPAM FNPEERSCSL EGNPVACINL SFCLNASGKH VADSIGFTVE LQLDWQKQKG GVRRALFLAS TQATLTQTLL
561 IQNGAREDCR EMKIYLRNES EFRDKLSPIH IALNFSLDPQ APVDSHGLRP ALHYQSKSRI EDKAQILLDC GEDNICVPDL
641 QLEVFGEQNH VYLGDKNALN LTFHAQNVGE GGAYEAELRV TAPPEAEYSG LVRHPGNFSS LSCDYFAVNQ SRLLVCDLGN
721 PMKAGASLWG GLRFTVPHLR DTKKTIQFDF QILSKNLNNS QSDVVSFRLS VEAQAQVTLN GVSKPEAVLF PVSDWHPRDQ
801 PQKEEDLGPA VHHVYELINQ GPSSISQGVL ELSCPQALEG QQLLYVTRVT GLNCTTNHPI NPKGLELDPE GSLHHQQKRE
881 APSRSSASSG PQILKCPEAE CFRLRCELGP LHQQESQSLQ LHFRVWAKTF LQREHQPFSL QCEAVYKALK MPYRILPRQL
961 PQKERQVATA VQWTKAEGSY GVPLWIIILA ILFGLLLLGL LIYILYKLGF FKRSLPYGTA MEKAQLKPPA TSDA

Supplemental Table S1: Relative Fluorescence Unit (RFU) signals of binding Gal8 to printed glycan array V3 at indicated concentrations of the lectin.

Glycan number on the array	Glycan name	Gal8 Concentration (ug/ml)															
		0.01		0.05		0.1		0.2		0.5		1		2		5	
		Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.	Avg. RFU	Std Dev.
1	Neu5Acα2-8Neu5Acα-Sp8	468	398	78	15	137	59	192	162	311	225	218	88	463	215	753	337
2	Neu5Acα2-8Neu5Acβ-Sp17	140	14	200	48	159	45	146	37	283	116	275	81	472	74	675	167
3	Neu5Acα2-8Neu5Acα2-8Neu5Acβ-Sp8	161	78	118	34	118	38	89	46	485	384	178	57	168	63	418	172
4	Neu5Gcβ2-6Galβ1-4GlcNAc-Sp8	124	59	127	58	131	107	201	51	211	43	157	72	256	43	230	39
5	Galβ1-3GlcNAcβ1-2Manα1-3(Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19	131	54	38	14	99	11	138	52	155	70	168	31	223	94	285	58
6	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	135	39	121	62	166	94	120	28	171	34	139	25	212	94	303	85
7	α-D-Gal–Sp8	997	539	663	518	544	270	661	299	495	201	525	230	1091	192	1911	517
8	α-D-Glc–Sp8	95	39	127	10	332	322	193	80	258	135	514	156	1105	468	2687	1199
9	α-D-Man–Sp8	149	124	66	24	129	69	111	25	133	28	315	120	248	144	508	294
10	α-GalNAc–Sp8	62	22	88	48	246	236	158	11	110	65	89	56	97	28	413	163
11	α-L-Fuc–Sp8	108	22	103	58	92	34	204	91	124	34	283	131	296	233	235	78
12	α-L-Fuc–Sp9	205	157	98	72	578	643	80	32	150	59	222	104	366	162	1092	369
13	α-L-Rhα–Sp8	7712	815	7116	1003	6775	502	6334	361	6464	1668	5957	919	5511	3631	8353	755
14	α-Neu5Ac–Sp8	276	97	128	55	756	396	74	12	295	81	388	106	544	141	401	113
15	α-Neu5Ac–Sp11	121	47	137	8	143	60	118	34	129	37	241	68	299	35	936	245
16	β-Neu5Ac-Sp8	173	66	120	25	135	40	99	47	738	742	261	49	359	128	678	283
17	β-D-Gal–Sp8	318	118	332	85	307	187	521	29	576	151	421	101	977	136	1524	514
18	β-D-Glc–Sp8	42	51	179	127	152	71	128	52	405	130	441	130	256	40	849	246
19	β-D-Man–Sp8	606	608	295	314	197	100	209	67	320	218	228	65	1164	312	2168	365
20	β-GalNAc–Sp8	104	27	136	83	150	76	117	41	320	46	402	222	264	121	956	114
21	β-GlcNAc–Sp0	209	109	62	51	86	79	129	34	145	65	135	40	168	55	504	296
22	β-GlcNAc–Sp8	112	33	89	75	1203	1072	264	258	371	201	177	36	1504	1318	7047	2906
23	β-GlcN(Gc)-Sp8	169	94	261	66	85	30	147	78	59	10	158	87	420	186	226	195
24	$(Gal\beta1\text{-}4GlcNAc\beta)_2$-3,6-GalNAcα–Sp8	87	21	98	26	343	240	125	61	204	89	165	76	318	110	2397	1048
25	GlcNAcβ1-3(GlcNAcβ1-4)(GlcNAcβ1-6)GlcNAc-Sp8	165	35	144	23	129	44	186	73	207	88	230	69	437	304	481	124
26	[3OSO3][6OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp0	784	347	723	17	1061	595	634	65	1149	512	1448	427	2219	1480	7059	1897
27	[3OSO3][6OSO3]Galβ1-4GlcNAcβ-Sp0	290	89	251	52	173	57	229	67	194	58	183	36	251	63	428	74
28	[3OSO3]Galβ1-4Glcβ-Sp8	384	77	1221	262	1274	785	5027	865	4018	771	7985	4472	25164	22564	56244	11783
29	[3OSO3]Galβ1-4(6OSO3)Glcβ–Sp0	2594	1262	15762	1798	24314	12977	46522	11291	21238	13034	44440	12381	42093	1359	47043	3102
30	[3OSO3]Galβ1-4(6OSO3)Glcβ–Sp8	5253	421	13422	1341	33041	4643	41743	8069	34744	4232	34310	2734	41137	2938	40979	3295
31	[3OSO3]Galβ1-3(Fucα1-4)GlcNAcβ–Sp8	83	31	136	37	190	61	154	50	296	86	290	121	253	99	921	190
32	[3OSO3]Galβ1-3GalNAcα–Sp8	505	183	1004	222	5893	829	3354	279	8199	1875	5757	1178	15995	3744	44650	3776
33	[3OSO3]Galβ1-3GlcNAcβ–Sp8	507	221	1609	307	5193	2521	5566	963	12247	3062	5909	1093	18578	4499	41230	6447
34	[3OSO3]Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	116	109	112	58	245	93	106	23	174	22	153	55	308	165	324	157
35	[3OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp8	887	164	2697	468	6169	1508	9191	872	18380	5499	31206	3739	40549	13155	41072	5505
36	[3OSO3]Galβ1-4GlcNAcβ–Sp0	162	77	302	95	653	223	534	120	1020	425	949	521	2351	796	16561	5667
37	[3OSO3]Galβ1-4GlcNAcβ-Sp8	160	36	212	101	332	105	473	93	520	108	841	115	2117	227	8099	1874
38	[3OSO3]Galβ–Sp8	370	97	103	21	335	386	143	96	406	211	525	192	506	285	1340	181
39	[4OSO3][6OSO3]Galβ1-4GlcNAcβ-Sp0	631	47	532	117	708	156	700	180	660	364	802	119	1151	29	1772	162
40	[4OSO3]Galβ1-4GlcNAcβ-Sp8	163	99	130	98	160	97	175	65	530	519	859	872	676	418	1084	440
41	6-H_2PO_3Manα–Sp8	385	213	532	255	249	174	387	186	315	68	682	417	436	192	1367	522
42	[6OSO3]Galβ1-4Glcβ–Sp0	214	185	93	21	247	124	162	138	211	56	275	105	469	130	715	451
43	[6OSO3]Galβ1-4Glcβ–Sp8	109	40	179	18	381	97	228	114	326	87	324	29	769	300	2693	1443
44	[6OSO3]Galβ1-4GlcNAcβ–Sp8	145	44	180	83	378	360	141	40	255	165	257	116	257	83	949	378
45	[6OSO3]Galβ1-4[6OSO3]Glcβ-Sp8	356	61	184	86	561	93	166	30	622	181	1180	107	983	373	3058	1330
46	NeuAcα2-3[6OSO3]Galβ1-4GlcNAcβ–Sp8	150	84	123	49	1970	1726	201	98	464	265	482	157	642	739	1323	514
47	[6OSO3]GlcNAcβ–Sp8	242	101	301	120	447	205	239	80	656	98	548	89	684	132	2068	646
48	9NAcNeu5Acα-Sp8	92	11	142	31	638	396	261	231	434	325	362	77	317	127	941	617
49	9NAcNeu5Acα2-6Galβ1-4GlcNAcβ-Sp8	65	16	178	69	268	107	205	71	226	36	207	70	229	72	534	157

50	Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13	83	8	71	29	103	21	152	74	166	28	156	58	408	542	333	175
51	GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13	90	24	96	55	140	59	67	37	208	100	126	40	263	44	504	220
52	Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13	94	42	55	6	185	132	184	80	128	56	266	63	283	161	597	196
53	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13	87	21	77	54	483	251	125	39	205	54	267	143	163	25	266	32
54	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp8	164	83	423	238	295	87	189	235	187	7	260	210	233	49	583	422
55	Fucα1-2Galβ1-3GalNAcβ1-3Galα-Sp9	294	43	131	55	110	43	171	78	124	78	140	19	287	172	516	281
56	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp9	548	30	621	21	859	303	705	64	785	88	886	92	899	53	1246	184
57	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ–Sp8	164	65	160	43	108	25	166	76	159	46	132	59	168	56	523	89
58	Fucα1-2Galβ1-3GalNAcα–Sp8	104	49	90	47	351	135	185	59	222	33	175	81	538	205	1906	445
59	Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-Sp0	118	9	180	95	176	87	196	49	217	39	257	82	529	215	1510	211
60	Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-Sp9	218	51	133	44	563	323	185	120	390	117	351	43	784	100	1922	786
61	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ–Sp10	1222	35	1180	117	1748	226	1757	95	2093	175	2714	353	11618	1977	30782	3206
62	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ–Sp8	1498	69	1745	84	1521	367	1705	195	1929	425	2798	445	5284	3618	34073	18292
63	Fucα1-2Galβ1-3GlcNAcβ–Sp0	174	34	233	73	489	96	556	80	666	221	1529	385	1983	688	6450	2449
64	Fucα1-2Galβ1-3GlcNAcβ–Sp8	113	59	87	45	301	68	339	92	174	239	950	570	1723	265	3550	2786
65	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	599	526	181	111	338	240	129	45	192	44	227	27	297	79	932	628
66	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	233	91	169	32	237	33	132	50	186	23	257	48	402	159	752	464
67	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ–Sp0	103	89	61	24	178	70	98	12	247	119	285	124	413	34	3744	2330
68	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	135	55	181	100	322	103	216	128	141	18	158	45	505	209	486	89
69	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc–Sp0	245	77	162	48	354	84	499	124	1149	201	1028	322	2581	616	27991	3264
70	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	174	37	367	55	1634	446	885	199	2394	705	1936	923	7934	1165	43846	1537
71	Fucα1-2Galβ1-4GlcNAcβ–Sp0	249	114	101	27	223	97	151	45	431	304	457	71	278	103	1061	226
72	Fucα1-2Galβ1-4GlcNAcβ–Sp8	128	47	76	18	239	42	166	22	564	165	333	65	676	48	3681	1490
73	Fucα1-2Galβ1-4Glcβ–Sp0	442	79	504	142	688	142	923	99	1236	57	1110	586	2963	1490	9819	3932
74	Fucα1-2Galβ–Sp8	500	77	627	29	500	87	413	129	460	315	627	138	864	159	715	372
75	Fucα1-3GlcNAcβ-Sp8	293	23	241	55	272	67	297	14	270	31	439	122	849	156	1784	229
76	Fucα1-3GlcNAcβ-Sp8	424	59	418	84	235	119	300	28	614	185	669	160	896	154	1486	231
77	Fucα1-4GlcNAcβ–Sp8	1371	1297	331	220	515	353	178	67	5821	6471	1755	1720	2588	3633	12196	12456
78	Fucβ1-3GlcNAcβ-Sp8	123	37	82	5	94	48	97	40	176	35	170	91	254	71	1287	662
79	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ-Sp0	344	74	943	96	10991	1623	3214	254	19935	1754	49337	10475	48735	9565	44160	1442
80	GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	138	47	182	130	298	96	203	56	262	131	236	77	266	32	507	190
81	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-Sp0	235	58	1071	84	4813	1604	4128	341	22759	5334	37610	16924	46730	10235	48513	1880
82	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ–Sp8	391	152	1124	69	4529	1438	4819	597	20600	3434	37793	6448	46251	9645	46301	1845
83	GalNAcα1-3(Fucα1-2)Galβ1-4Glcβ-Sp0	372	85	1062	131	3230	455	3713	407	20662	3328	34096	1867	37882	1104	46599	2602
84	GalNAcα1-3(Fucα1-2)Galβ–Sp8	133	56	116	13	276	55	234	110	250	30	341	100	726	614	575	250
85	GalNAcα1-3GalNAcβ–Sp8	294	135	491	25	658	86	358	125	1103	540	972	92	1099	232	1721	307
86	GalNAcα1-3Galβ–Sp8	384	71	443	62	520	57	336	27	533	135	690	214	1049	410	1172	194
87	GalNAcα1-4(Fucα1-2)Galβ1-4GlcNAcβ-Sp8	214	88	212	94	153	21	139	38	506	251	463	45	1879	1630	2067	1108
88	GalNAcβ1-3GalNAcα–Sp8	104	91	117	31	129	123	210	57	222	128	162	16	496	169	1554	708
89	GalNAcβ1-3(Fucα1-2)Galβ-Sp8	299	355	112	48	165	43	147	42	210	59	329	118	732	237	1615	650
90	GalNAcβ1-3Galα1-4Galβ1-4GlcNAcβ-Sp0	780	28	708	85	506	246	885	49	824	105	881	71	947	84	999	123
91	GalNAcβ1-4(Fucα1-3)GlcNAcβ-Sp0	138	30	179	116	97	40	185	56	178	30	139	27	285	151	383	131
92	GalNAcβ1-4GlcNAcβ–Sp0	1189	1116	447	400	97	29	154	88	188	48	245	60	309	96	2656	1414
93	GalNAcβ1-4GlcNAcβ–Sp8	45	36	97	14	153	68	169	57	316	72	279	98	504	126	2046	488
94	Galα1-2Galβ–Sp8	398	89	369	185	347	95	448	114	509	112	616	228	817	364	1520	823
95	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ-Sp0	1398	322	5848	929	16933	5801	19120	2642	40214	5551	38794	1393	42448	2397	46231	639
96	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	129	18	121	42	181	78	161	68	423	91	247	67	517	489	1189	553
97	Galα1-3(Fucα1-2)Galβ1-4GlcNAc-Sp0	1135	161	3883	481	13778	5694	18278	2300	47343	11829	40787	4546	44996	443	47463	662
98	Galα1-3(Fucα1-2)Galβ1-4Glcβ-Sp0	4156	785	13998	1363	49770	15118	47012	12222	34394	1511	37604	3058	47223	5069	48329	2536
99	Galα1-3(Fucα1-2)Galβ–Sp8	271	157	237	45	242	65	165	49	470	91	338	107	632	590	942	533
100	Galα1-3(Galα1-4)Galβ1-4GlcNAcβ-Sp8	192	13	146	47	145	56	263	101	239	149	237	69	308	144	532	167
101	Galα1-3GalNAcα-Sp8	66	22	78	25	141	45	104	6	168	69	125	44	101	73	330	200
102	Galα1-3GalNAcβ–Sp8	147	47	176	46	160	27	262	136	250	52	312	92	377	142	450	177
103	Galα1-3Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	111	42	86	29	152	54	145	73	133	33	326	190	491	50	933	431

104	Galα1-3Galβ1-3GlcNAcβ-Sp0	118	40	111	65	392	173	261	114	200	126	213	77	695	399	4781	995
105	Galα1-3Galβ1-4GlcNAcβ–Sp8	78	14	55	19	291	280	301	83	415	183	355	271	751	346	7964	1686
106	Galα1-3Galβ1-4Glcβ–Sp0	556	785	214	116	1136	469	410	112	1025	432	634	102	1725	674	14961	8976
107	Galα1-3Galβ–Sp8	116	28	336	104	386	121	527	130	407	210	808	105	753	563	1646	1399
108	Galα1-4(Fucα1-2)Galβ1-4GlcNAcβ-Sp8	225	37	169	41	257	44	197	121	252	17	322	87	252	46	405	87
109	Galα1-4Galβ1-4GlcNAcβ–Sp0	578	157	426	54	440	95	234	109	665	426	642	177	841	362	1147	422
110	Galα1-4Galβ1-4GlcNAcβ–Sp8	558	46	422	81	2245	1851	579	103	796	72	905	149	978	82	1600	558
111	Galα1-4Galβ1-4Glcβ–Sp0	737	319	742	94	487	293	582	90	759	193	622	219	1125	307	2004	487
112	Galα1-4GlcNAcβ–Sp8	171	40	309	40	222	75	307	73	488	223	394	201	584	74	1792	482
113	Galα1-6Glcβ-Sp8	1141	618	2244	41	2033	215	1697	98	1614	176	2226	255	1534	459	1374	1202
114	Galβ1-2Galβ–Sp8	261	113	192	75	313	67	363	132	381	110	630	309	829	340	1426	559
115	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	141	24	217	49	215	90	162	30	258	36	351	87	251	58	585	298
116	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	1779	1531	567	633	145	49	128	17	172	82	357	80	218	37	1289	254
117	Galβ1-3(Fucα1-4)GlcNAc–Sp0	118	19	210	106	167	79	111	49	233	144	159	64	210	46	396	223
118	Galβ1-3(Fucα1-4)GlcNAc–Sp8	425	337	227	98	204	62	131	35	193	155	397	114	459	153	838	413
119	Galβ1-3(Fucα1-4)GlcNAcβ–Sp8	145	51	290	191	287	30	156	112	228	91	282	42	341	195	417	104
120	Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Sp8	105	56	111	51	143	87	143	32	339	189	454	402	1052	214	1396	417
121	Galβ1-3(GlcNAcβ1-6)GalNAcα-Sp8	225	114	114	65	272	254	117	35	227	39	325	95	795	228	2014	387
122	Galβ1-3(Neu5Acα2-6)GalNAcα-Sp8	75	24	113	27	209	16	118	27	214	44	196	16	223	91	464	264
123	Galβ1-3(Neu5Acβ2-6)GalNAcα-Sp8	146	65	90	41	206	72	100	22	470	151	423	142	441	150	1078	770
124	Galβ1-3(Neu5Acα2-6)GlcNAcβ1-4Galβ1-4Glcβ-Sp10	391	66	251	81	571	124	496	100	632	137	797	119	1915	187	1461	239
125	Galβ1-3GalNAcα-Sp8	139	37	117	50	254	85	108	68	169	71	400	158	801	224	712	339
126	Galβ1-3GalNAcβ–Sp8	187	61	340	52	263	29	640	102	435	288	1104	256	2764	1258	3077	1709
127	Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp0	828	177	880	87	1008	165	874	73	980	61	1289	138	1486	150	2013	478
128	Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-Sp0	1799	1185	472	467	440	85	358	109	855	378	731	352	1725	335	18122	10245
129	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ–Sp8	421	424	194	16	204	53	219	44	743	165	426	100	879	268	7272	1890
130	Galβ1-3Galβ–Sp8	268	78	162	44	461	52	285	49	404	115	491	173	1782	663	6399	1182
131	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	108	45	98	35	213	29	135	47	290	100	110	62	348	104	3621	332
132	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ–Sp10	1243	76	1279	297	1569	126	1622	196	2454	343	1766	174	2695	135	23845	6415
133	Galβ1-3GlcNAcβ–Sp0	79	58	107	69	359	278	168	90	323	124	259	130	457	237	2450	521
134	Galβ1-3GlcNAcβ–Sp8	108	39	142	23	79	49	107	28	151	68	328	273	396	275	1611	305
135	Galβ1-4(Fucα1-3)GlcNAcβ–Sp0	165	81	138	67	396	237	88	24	469	468	381	182	226	69	514	279
136	Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	47	16	113	41	168	76	81	52	86	17	173	84	300	135	1071	720
137	Galβ1-4(Fucα1-3)GlcNAcβ1-4Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	260	101	162	56	126	16	168	45	727	848	206	66	294	45	788	81
138	Galβ1-4(Fucα1-3)GlcNAcβ1-4Galβ1-4(Fucα1-3)GlcNAcβ1-4Galβ1-4(Fucα1-3)GlcNAcβ–Sp0	119	23	116	25	207	33	251	87	331	93	357	84	1496	552	3122	969
139	Galβ1-4[6OSO3]Glcβ–Sp0	212	61	682	128	824	500	1557	346	2500	689	2572	683	14963	10214	7755	5854
140	Galβ1-4[6OSO3]Glcβ–Sp8	431	171	430	108	636	510	1456	113	740	702	2632	594	10378	2737	14040	4513
141	Galβ1-4GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-Sp8	241	73	509	57	836	97	1268	112	1633	172	5314	906	41158	1134	44984	1239
142	Galβ1-4GalNAcβ1-3(Fucα1-2)Galβ1-4GlcNAcβ-Sp8	348	65	340	52	169	31	373	97	223	127	319	81	301	35	533	211
143	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	71	36	113	44	300	17	263	26	440	42	993	102	1316	109	1863	251
144	Galβ1-4GlcNAcβ1-3GalNAcα–Sp8	73	7	65	19	96	30	84	39	357	424	374	160	1419	943	2028	664
145	Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	138	37	232	187	262	93	273	133	379	61	388	83	475	96	2654	163
146	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ–Sp0	167	30	1065	87	3262	687	3033	341	8775	1416	6183	803	44497	10892	46294	1513
147	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ–Sp0	341	106	798	100	2646	144	3418	368	12446	4069	13151	4367	35016	3643	46848	3270
148	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ–Sp0	2484	255	10761	1930	38853	16934	33517	2863	53834	10825	48378	10407	45937	1346	44991	2961
149	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ–Sp8	2751	596	8277	882	39022	7170	39702	2491	46009	10401	39630	1907	44337	2383	50875	4340
150	Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα–Sp8	231	130	114	43	190	43	228	37	522	95	221	70	1484	237	2350	1037
151	Galβ1-4GlcNAcβ1-6GalNAcα–Sp8	112	34	191	70	143	82	118	78	208	56	297	100	340	143	721	302
152	Galβ1-4GlcNAcβ–Sp0	177	23	91	32	160	130	132	53	159	53	267	107	223	92	648	247
153	Galβ1-4GlcNAcβ–Sp8	149	53	97	54	103	26	275	111	193	50	529	62	636	77	1624	287
154	Galβ1-4Glcβ–Sp0	148	36	78	86	91	19	234	94	246	82	279	189	523	211	640	91
155	Galβ1-4Glcβ–Sp8	173	88	105	9	261	91	232	143	369	131	521	116	1302	272	3395	1079
156	GlcNAcα1-3Galβ1-4GlcNAcβ-Sp8	316	71	279	102	311	116	468	96	394	64	697	233	715	357	1240	600
157	GlcNAcα1-6Galβ1-4GlcNAcβ-Sp8	378	32	279	30	426	144	326	47	424	106	617	160	550	316	602	186
158	GlcNAcβ1-2Galβ1-3GalNAcα–Sp8	549	164	955	71	1149	304	1042	40	760	384	980	523	1166	334	2609	395

159	GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα–Sp8	41	11	78	12	71	48	119	88	105	41	207	20	150	35	809	343
160	GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAcβ–Sp8	75	51	83	72	188	112	92	34	297	121	162	56	329	40	326	190
161	GlcNAcβ1-3GalNAcα–Sp8	95	22	197	72	171	67	74	5	123	82	215	37	176	24	476	190
162	GlcNAcβ1-3Galβ-Sp8	148	101	75	21	225	126	94	16	282	34	322	83	244	95	740	281
163	GlcNAcβ1-3Galβ1-3GalNAcα-Sp8	81	20	72	19	185	80	186	57	372	71	550	200	1358	342	3106	1367
164	GlcNAcβ1-3Galβ1-4GlcNAcβ–Sp0	126	65	98	63	119	24	97	26	390	70	551	362	670	289	1425	547
165	GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp8	79	25	115	21	91	35	113	50	173	58	140	83	674	76	550	240
166	GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	230	56	620	83	1401	124	2119	253	2981	436	8257	1592	40694	4990	43377	3392
167	GlcNAcβ1-3Galβ1-4Glcβ–Sp0	179	54	171	56	292	26	281	100	478	119	551	238	1388	365	2547	645
168	GlcNAcβ1-4MDPLys	1818	52	1926	61	1999	232	1244	24	1810	208	2571	394	2173	70	3631	611
169	GlcNAcβ1-4(GlcNAcβ1-6)GalNAcα-Sp8	306	81	249	27	397	124	255	71	421	108	538	87	627	77	915	130
170	GlcNAcβ1-4Galβ1-4GlcNAcβ-Sp8	700	51	1049	28	1520	214	1075	63	1318	302	1646	403	1193	272	1550	138
171	(GlcNAcβ1-4)6β-Sp8	183	51	158	30	144	26	175	35	163	31	181	111	338	133	482	103
172	(GlcNAcβ1-4)5β-Sp8	280	241	244	107	230	60	131	63	230	50	215	58	446	67	459	213
173	GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ–Sp8	487	111	413	48	345	156	383	98	322	79	484	97	649	104	1483	271
174	GlcNAcβ1-6(Galβ1-3)GalNAcα–Sp8	264	161	67	25	165	51	153	21	576	518	295	191	1002	341	1486	416
175	GlcNAcβ1-6GalNAcα–Sp8	87	43	71	40	147	35	128	40	77	31	162	48	282	129	354	179
176	GlcNAcβ1-6Galβ1-4GlcNAcβ-Sp8	216	190	63	13	137	42	97	24	220	73	336	120	246	62	345	182
177	Glcα1-4Glcβ–Sp8	149	23	125	20	91	56	228	56	317	16	376	41	561	233	755	95
178	Glcα1-4Glca–Sp8	184	25	138	53	139	64	109	54	132	96	179	62	247	144	225	95
179	Glcα1-6Glcα1-6Glcβ-Sp8	993	139	925	86	912	204	1063	62	930	62	1167	117	1031	152	1222	157
180	Glcβ1-4Glcβ-Sp8	2700	1341	5267	548	6338	606	2976	2276	3885	1550	5498	785	5511	503	5349	192
181	Glcβ1-6Glcβ-Sp8	2339	879	2101	255	1772	136	2544	216	803	557	2151	189	2341	242	4667	537
182	G-ol-Sp8	172	106	93	37	169	63	142	77	351	166	377	199	348	150	1691	303
183	GlcAa-Sp8	211	106	181	77	151	106	265	253	241	82	454	245	1114	183	2425	563
184	GlcAβ-Sp8	135	69	237	121	287	202	376	179	648	371	631	47	712	231	2471	1222
185	GlcAβ1-3Galβ-Sp8	175	62	321	163	250	52	147	66	163	78	493	166	301	131	508	389
186	GlcAβ1-6Galβ-Sp8	469	114	454	75	742	244	398	135	519	267	486	299	753	420	1268	483
187	KDNα2-3Galβ1-3GlcNAcβ–Sp0	182	40	701	109	1724	653	2366	506	5963	2240	5978	2578	14257	3510	44417	12734
188	KDNα2-3Galβ1-4GlcNAcβ–Sp0	507	352	160	44	273	79	251	93	1074	240	935	234	2576	648	11962	2381
189	Manα1-2Manα1-2Manα1-3Manα-Sp9	154	33	194	62	237	57	274	39	299	31	273	114	226	61	452	114
190	Manα1-2Manα1-3(Manα1-2Manα1-6)Manα-Sp9	298	13	206	64	242	102	137	51	155	96	233	128	273	115	456	140
191	Manα1-2Manα1-3Manα-Sp9	202	77	196	124	147	33	153	74	218	69	356	127	489	173	632	128
192	Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	191	96	141	53	214	35	204	85	793	354	670	235	821	200	1740	1190
193	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα2Manα2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	1479	921	160	73	184	31	221	209	248	160	193	41	387	114	702	234
194	Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-3(Manα1-2Manα1-6)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	102	20	114	43	286	163	211	33	267	80	314	197	519	212	1200	528
195	Manα1-3(Manα1-6)Manα–Sp9	307	197	92	20	177	52	110	51	159	55	299	108	212	52	693	521
196	Manα1-3(Manα1-2Manα1-2Manα1-6)Manα-Sp9	142	17	147	46	238	71	187	47	349	143	410	181	439	119	555	241
197	Manα1-6(Manα1-3)Manα1-6(Manα2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	104	19	214	100	229	73	115	91	171	94	353	198	221	106	550	391
198	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4 GlcNAcβ-Sp12	72	19	125	35	725	624	72	25	580	464	146	38	442	82	292	119
199	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	576	441	191	94	404	160	206	60	386	100	265	21	651	147	3356	983
200	Manβ1-4GlcNAcβ-Sp0	188	73	134	15	110	63	93	20	224	109	458	96	378	121	372	90
201	Fucα1-3(Galβ1-4)GlcNAcβ1-2Manα1-3(Fucα1-3(Galβ1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20	103	49	59	14	172	93	119	81	275	101	121	48	179	37	1038	275
202	Neu5Acα2-3Galβ1-3GalNAcα-Sp8	243	117	596	59	2303	573	2244	180	6722	216	8226	3039	42462	5863	43932	8377
203	NeuAcα2-8NeuAcα2-8NeuAcα2-8NeuAcα2-3(GalNAcβ1-4)Galβ1-4Glcβ-Sp0	2205	1907	500	410	93	37	347	271	197	84	133	35	288	146	682	165
204	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ-Sp0	89	12	153	43	161	91	93	34	211	50	199	42	413	245	858	377
205	Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ–Sp0	382	252	435	37	2376	270	1576	76	3030	737	2727	842	7792	2192	42666	2912
206	Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ–Sp0	92	52	116	21	320	212	168	40	137	109	107	56	325	210	1216	1281
207	Neu5Acα2-8Neu5Acα2-8Neu5Acα-Sp8	132	39	104	63	96	124	98	38	223	44	301	138	405	286	957	836
208	Neu5Acα2-3(6-O-Su)Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	101	30	90	55	760	698	163	55	554	690	160	35	285	61	227	128
209	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ-Sp0	136	81	125	30	147	28	175	100	210	70	317	103	225	37	453	130
210	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ-Sp8	287	56	91	44	1182	1646	89	45	211	173	218	57	357	101	285	18
211	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ–Sp0	105	63	93	24	149	110	171	84	165	56	169	42	408	160	1329	531

212	NeuAcα2-3(NeuAcα2-3Galβ1-3GalNAcβ1-4)Galβ1-4Glcβ-Sp0	1020	241	1252	157	3824	967	4921	816	16820	1951	13217	6699	49503	9476	40068	2247
213	Neu5Acα2-3(Neu5Acα2-6)GalNAcα–Sp8	77	9	95	11	165	41	155	102	114	71	229	74	382	185	648	499
214	Neu5Acα2-3GalNAcα–Sp8	181	75	130	55	330	117	104	48	402	326	339	131	981	120	1441	224
215	Neu5Acα2-3GalNAcβ1-4GlcNAcβ-Sp0	999	936	340	377	336	75	204	35	199	82	187	87	455	88	2121	276
216	Neu5Acα2-3Galβ1-3(6OSO3)GlcNAc-Sp8	1007	326	3379	1254	3810	4291	11290	2117	14176	16693	13349	12674	22784	16557	22590	22561
217	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ–Sp8	272	131	104	87	128	42	130	78	195	92	184	51	157	31	1006	405
218	NeuAcα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ Sp0	572	91	576	35	761	142	676	65	698	80	706	68	809	40	3968	446
219	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ-Sp8	87	32	141	62	124	50	133	17	104	47	126	83	337	169	1696	304
220	Neu5Acα2-3Galβ1-3[6OSO3]GalNAcα-Sp8	172	14	744	32	2301	689	2094	149	9948	6768	5967	3525	26685	22019	44361	11498
221	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα–Sp8	113	54	154	25	316	190	431	243	899	219	510	96	1744	364	17137	4944
222	Neu5Acα2-3Galβ-Sp8	210	132	162	24	825	940	84	41	250	191	195	106	306	249	579	308
223	NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp0	655	79	1220	155	2304	1455	5398	502	6928	1694	8752	5057	34152	13183	39230	15070
224	NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	856	182	2485	145	10422	3876	10323	740	33304	12359	27353	11284	37432	6248	40089	5710
225	Neu5Acα2-3Galβ1-3GlcNAcβ–Sp0	220	83	630	114	1851	923	2180	278	4839	1834	8804	2778	43379	12842	43444	13249
226	Neu5Acα2-3Galβ1-3GlcNAcβ–Sp8	305	128	465	92	1395	532	2302	91	2874	488	8689	1087	30527	3727	32893	6444
227	Neu5Acα2-3Galβ1-4[6OSO3]GlcNAcβ-Sp8	331	135	1206	80	2250	495	3855	517	5112	487	15696	5113	38827	6131	43229	1182
228	Neu5Acα2-3Galβ1-4(Fucα1-3)(6OSO3)GlcNAcβ–Sp8	128	56	123	55	138	30	261	115	423	167	436	171	801	386	1589	1651
229	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ–Sp0	116	13	123	12	197	53	141	32	373	85	335	75	611	230	818	83
230	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ–Sp0	1535	1902	329	156	151	77	300	262	150	50	168	53	449	88	2544	527
231	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ–Sp8	120	46	58	13	91	40	55	15	122	33	222	68	127	46	414	214
232	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ-Sp8	254	185	104	23	41	14	115	67	203	91	321	144	153	74	768	231
233	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp8	177	18	128	21	389	152	218	60	375	49	243	69	319	99	1340	293
234	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc-Sp0	158	37	388	100	776	43	1304	168	3459	1021	2520	681	5923	2062	43541	4171
235	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ–Sp0	754	115	2980	116	12889	3685	11202	1038	40552	3150	28611	9557	49274	4644	47391	2857
236	Neu5Acα2-3Galβ1-4GlcNAcβ–Sp0	100	40	167	35	241	81	444	44	825	124	984	387	1782	202	14728	982
237	Neu5Acα2-3Galβ1-4GlcNAcβ–Sp8	308	186	172	46	650	242	339	87	1141	239	1467	343	2439	258	17163	3818
238	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	135	44	186	26	225	108	512	165	587	144	1421	466	5295	448	6344	2024
239	Neu5Acα2-3Galβ1-4Glcβ–Sp0	326	95	847	115	1086	219	4095	325	2934	611	9700	3027	27499	9037	35383	1780
240	Neu5Acα2-3Galβ1-4Glcβ–Sp8	1832	141	2949	287	5140	1322	5170	465	4651	590	12320	4529	38993	9422	41996	5979
241	Galβ1-4GlcNAcβ1-2Manα1-3(Fucα1-3(Galβ1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20	75	61	107	56	113	69	72	24	153	50	183	69	99	37	872	447
242	Neu5Acα2-6GalNAcα–Sp8	2042	1761	163	78	87	16	352	388	124	45	158	94	185	78	621	251
243	Neu5Acα2-6GalNAcβ1-4GlcNAcβ-Sp0	245	84	59	24	118	88	60	13	138	62	171	73	150	76	293	129
244	Neu5Acα2-6Galβ1-4[6OSO3]GlcNAcβ-Sp8	312	228	237	144	346	94	163	48	292	124	278	164	324	104	875	189
245	Neu5Acα2-6Galβ1-4GlcNAcβ–Sp0	116	30	119	39	215	74	96	8	188	55	445	234	1152	677	3191	1700
246	Neu5Acα2-6Galβ1-4GlcNAcβ–Sp8	166	78	252	55	136	137	112	30	128	36	284	41	246	35	619	270
247	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	102	73	116	19	873	408	280	81	249	70	213	61	502	257	1071	328
248	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	182	44	553	97	1887	461	1943	257	5637	1483	6161	2568	12442	1297	54832	5608
249	Neu5Acα2-6Galβ1-4Glcβ–Sp0	276	50	690	38	2072	386	1524	199	8167	2760	4812	871	26783	4537	53285	1230
250	Neu5Acα2-6Galβ1-4Glcβ–Sp8	105	64	93	30	71	57	182	88	561	437	196	80	587	407	1177	1086
251	Neu5Acα2-6Galβ–Sp8	178	61	151	41	94	21	193	25	163	49	361	137	461	249	569	409
252	Neu5Acα2-8Neu5Acα-Sp8	166	29	100	34	73	28	201	108	145	48	201	53	282	171	611	382
253	Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ–Sp0	604	155	2339	177	8832	3526	9374	1614	13871	8387	41932	15376	40265	5447	42387	1784
254	Neu5Acβ2-6GalNAcα–Sp8	98	58	91	30	128	80	202	73	131	65	164	59	224	75	470	194
255	Neu5Acβ2-6Galβ1-4GlcNAcβ-Sp8	304	157	73	12	118	62	155	70	115	21	231	86	187	49	1392	1719
256	Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp21	77	18	77	21	478	335	119	75	191	48	216	91	159	34	271	39
257	Neu5Gcα2-3Galβ1-3(Fucα1-4)GlcNAcβ-Sp0	149	46	175	52	464	304	164	25	290	77	299	76	451	101	5080	2305
258	Neu5Gcα2-3Galβ1-3GlcNAcβ-Sp0	260	74	561	125	1921	374	2313	552	8376	4042	10813	5157	16359	5170	47997	9751
259	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0	102	31	98	53	787	809	173	77	288	56	286	170	512	214	1640	457
260	Neu5Gcα2-3Galβ1-4GlcNAcβ–Sp0	126	56	127	70	285	79	316	90	781	346	710	96	1456	166	12400	1631
261	Neu5Gcα2-3Galβ1-4Glcβ–Sp0	349	327	937	104	3128	1775	2513	228	6697	2137	5909	2048	19128	7330	59440	4642
262	Neu5Gcα2-6GalNAcα–Sp0	110	29	133	91	120	34	226	38	295	121	285	144	133	32	314	127
263	Neu5Gcα2-6Galβ1-4GlcNAcβ–Sp0	142	109	60	32	118	80	114	24	128	65	182	104	165	67	900	236
264	Neu5Gcα–Sp8	91	34	155	82	167	86	180	100	261	138	188	127	186	120	707	232

265	[3OSO3]Galβ1-4(Fucα1-3)(6OSO3)Glc-Sp0	295	29	299	64	329	51	436	155	1250	697	844	131	3220	738	7021	4215
266	[3OSO3]Galβ1-4(Fucα1-3)Glc-Sp0	106	13	120	12	265	74	198	51	218	144	650	150	427	79	889	341
267	[3OSO3]Galβ1-4[Fucα1-3][6OSO3]GlcNAc-Sp8	335	190	282	91	595	251	540	116	916	410	760	288	2180	638	9650	4906
268	[3OSO3]Galβ1-4[Fucα1-3]GlcNAc-Sp0	229	98	128	32	96	112	151	45	237	94	263	52	432	302	309	146
269	Fucα1-2[6OSO3]Galβ1-4GlcNAc-Sp0	132	26	124	36	148	69	187	49	170	30	243	182	275	152	294	69
270	Fucα1-2Galβ1-4[6OSO3]GlcNAc-Sp8	288	90	207	71	588	338	572	162	926	355	874	172	5065	2046	7253	1559
271	Fucα1-2[6OSO3]Galβ1-4[6OSO3]Glc-Sp0	204	56	235	84	291	128	261	52	579	84	402	66	811	215	1355	198
272	Fucα1-2-(6OSO3)-Galβ1-4Glc-Sp0	199	88	262	34	346	69	236	19	254	125	322	192	448	246	815	318
273	Fucα1-2-Galβ1-4[6OSO3]Glc-Sp0	265	81	841	147	1871	1035	2441	378	2145	839	5283	675	20879	11147	26190	4452
274	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ-Sp0	2195	1595	144	10	280	45	130	54	251	75	259	120	163	23	474	116
275	Galβ1-3-(Galβ1-4GlcNacβ1-6)GalNAc-Sp14	179	68	205	68	172	86	245	69	129	110	250	66	557	87	635	263
276	Galβ1-3(GlcNacβ1-6)GalNAc-Sp14	238	379	163	49	258	178	145	72	373	161	222	68	568	326	1794	541
277	Galβ1-3-(Neu5Aa2-3Galβ1-4GlcNacβ1-6)GalNAc-Sp14	193	128	233	54	514	265	614	74	1641	734	737	170	1690	259	20603	7089
278	Galβ1-3GalNAc-Sp14	210	165	174	65	193	35	117	59	136	55	236	80	234	14	1328	92
279	Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	86	64	195	57	113	22	226	110	492	30	184	33	705	95	9891	3241
280	Galβ1-4[Fucα1-3][6OSO3]GlcNAc-Sp0	86	15	172	81	166	51	319	153	244	87	387	160	252	73	663	357
281	Galβ1-4[Fucα1-3][6OSO3]Glc-Sp0	122	27	152	112	656	344	144	72	242	30	345	113	363	130	729	100
282	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ-Sp0	118	15	111	41	214	49	161	38	168	17	223	104	341	151	586	173
283	Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	456	142	2957	355	7178	3167	9780	302	23304	9296	18020	4385	37164	4412	43220	2722
284	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	972	79	2917	377	13583	1265	15424	1587	42202	4455	41343	2049	45073	2117	46369	3025
285	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	250	152	139	54	517	275	487	81	1020	252	886	280	5870	897	10227	2094
286	[3OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp0	634	177	2231	394	6254	924	9619	2213	12764	3052	32428	4465	48808	9487	46394	8328
287	[3OSO3][4OSO3]Galβ1-4GlcNacβ-SpSp0	7994	628	10291	539	12439	3328	9074	503	11824	1624	10552	750	9513	1840	10635	1956
288	[6OSO3]Galβ1-4[6OSO3]GlcNacβ-Sp0	266	71	149	65	173	116	317	105	424	340	452	129	1007	186	1927	355
289	6-H2PO3Glcβ-Sp10	370	161	697	56	254	136	674	114	578	286	782	431	1015	432	1019	517
290	Galα1-3(Fucα1-2)Galβ–Sp18	211	52	229	52	143	50	195	48	260	65	339	77	244	14	356	56
291	Galα1-3GalNAcα-Sp16	128	143	85	24	63	37	116	46	122	100	141	74	194	41	166	81
292	Galβ1-3GalNAcα-Sp16	1039	1260	126	76	196	83	277	195	278	117	353	167	521	161	1180	439
293	Galβ1-3(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAc–Sp14	183	91	121	18	228	35	190	42	233	38	347	57	221	31	1009	110
294	Galβ1-3Galβ1-4GlcNAcβ-Sp8	225	158	142	75	1111	282	291	81	780	102	609	110	1828	619	16356	4438
295	Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	127	45	145	23	609	595	150	69	359	59	236	22	372	70	2453	685
296	Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc-Sp0	110	42	92	18	101	23	79	18	272	121	278	110	354	102	1575	564
297	Galβ1-4GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAc-Sp0	603	553	105	88	91	21	123	24	249	180	184	100	426	146	1313	439
298	Galβ1-4GlcNAcα1-6Galβ1-4GlcNAcβ-Sp0	112	53	203	68	137	41	125	11	136	48	185	20	375	43	493	247
299	Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ-Sp0	311	257	95	56	177	89	109	25	398	314	245	135	258	180	568	300
300	GalNAcα-Sp15	82	13	151	93	216	65	276	158	246	93	236	38	296	130	787	449
301	GalNAcα1-3(Fucα1-2)Galβ–Sp18	132	57	190	51	230	123	174	67	409	25	346	105	285	161	530	142
302	GalNAcβ1-3Galβ-Sp8	77	16	149	39	420	187	377	81	559	335	1585	806	3540	1513	3975	2148
303	GlcAβ1-3GlcNAcβ-Sp8	5216	248	5952	204	5335	417	4785	321	4474	1438	7164	1498	5836	873	7391	614
304	GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	2663	2397	251	113	211	61	222	159	138	19	154	46	555	46	468	169
305	GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	108	41	52	44	209	137	100	62	98	9	199	111	182	31	398	146
306	GlcNAcβ1-3Man-Sp10	166	70	138	57	266	110	316	131	647	68	681	132	1636	662	2027	923
307	GlcNAcβ1-4GlcNAcβ-Sp10	129	69	155	53	715	648	218	71	1202	987	631	256	2061	2968	1997	1042
308	GlcNAcβ1-4GlcNAcβ-Sp12	167	30	114	28	233	40	195	109	223	42	298	139	539	196	577	236
309	HOOC(CH3)CH-3-O-GlcNAcβ1-4GlcNAcβ-Sp10	733	414	604	16	715	118	395	89	784	111	745	105	688	134	809	328
310	Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	115	35	109	53	91	34	157	39	134	50	113	45	220	126	224	81
311	Manα1-6Manβ-Sp10	56	18	114	58	171	78	125	62	269	294	205	110	259	101	672	530
312	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ-Sp10	143	30	91	24	58	21	103	41	111	49	240	63	227	121	239	198
313	Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-6(Manα1-3)Manα1-6)Manα-Sp9	281	102	105	30	221	95	124	32	243	105	207	56	281	25	248	139
314	Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6)Manα-Sp9	146	78	178	51	256	167	189	44	251	136	325	210	200	33	255	76
315	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAc–Sp14	109	26	400	32	883	303	1398	64	1013	123	4531	1096	7615	1655	9264	2263
316	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAc-Sp14	1704	1141	327	181	301	61	349	80	425	88	523	113	543	399	2735	515
317	Neu5Acα2-3Galβ1-3GalNAc–Sp14	149	40	337	166	931	278	1007	116	2725	513	1331	165	3708	1461	45352	11643

318	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	149	70	105	32	228	28	160	51	240	96	253	168	473	142	4183	341
319	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	93	26	146	60	517	471	152	25	183	35	228	33	347	29	751	83
320	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	131	44	130	24	178	68	156	25	147	14	241	56	289	42	467	254

Galectin-8 Promotes Cytoskeletal Rearrangement in Trabecular Meshwork Cells through Activation of Rho Signaling

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Running Head: **Galectin-8 Activates Rho Signaling in TM Cells**

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Purpose: The trabecular meshwork (TM) cell-matrix interactions and factors that influence Rho signaling in TM cells are thought to play a pivotal role in the regulation of aqueous outflow. The current study was designed to evaluate the role of a carbohydrate-binding protein, galectin-8 (Gal8), in TM cell adhesion and Rho signaling. **Methods:** Normal human TM cells were assayed for Gal8 expression by immunohistochemistry and Western blot analysis. To assess the role of Gal8 in TM cell adhesion and Rho signaling, the cell adhesion and spreading assays were performed on Gal8-coated culture plates in the presence and the absence of anti-β_1 integrin antibody and Rho and Rho-kinase inhibitors. In addition, the effect of Gal-8-mediated cell-matrix interactions on TM cell cytoskeleton arrangement and myosin light chain (MLC) phosphorylation was examined. **Results:** We demonstrate here that Gal8 is expressed in the TM and a function-blocking anti-β_1 integrin antibody inhibits the adhesion and spreading of TM cells to Gal8-coated wells. Cell spreading on Gal8 substratum was associated with the accumulation of phosphorylated myosin light chain and the formation of stress fibers that was inhibited by the Rho inhibitor, C3 transferase, as well as by the Rho-kinase inhibitor, Y27632. **Conclusion:** The above findings present a novel function for Gal8 in activating Rho signaling in TM cells. This function may allow Gal8 to participate in the regulation of aqueous outflow.

INTRODUCTION

Primary Open Angle Glaucoma (POAG) is a major cause for irreversible blindness. Factors that lead to the development of POAG are not yet fully known. It is clear, however, that elevated intraocular pressure is a major causal risk factor[1]. Elevation in intraocular pressure is due to the dysfunction of outflow pathway tissues resulting in inadequate clearance of aqueous humor. Trabecular meshwork (TM) cell-matrix adhesion is crucial for the maintenance of the outflow pathway. In the short term, experimental procedures that cause loss of TM cell contact with the beams lead to a sharp increase in aqueous outflow[2-4]. In the long term, beams denuded of cells (typical of POAG eyes) tend to collapse on one another, blocking the outflow channels[5]. In recent years, a large body of research in the field of POAG has focused on the role of Rho signaling in the regulation of outflow facility through regulation of the TM actin cytoskeleton. The emerging paradigm is that the inhibition of Rho signaling leads to elevation in outflow facility, while induction of Rho signaling leads to increased resistance to outflow[2, 6-8]. In cultured TM cells, inhibitors of the Rho signaling cascade cause TM cell rounding, loss of stress fibers and focal adhesions and retraction of cell processes[6, 9]. In perfused human and animal eyes, inhibitors of Rho signaling cause TM cell rounding and detachment from the beams concomitant with a marked elevation in outflow facility[2, 4]. In nonocular studies, a carbohydrate-binding protein, galectin-8 (Gal8), has been shown to form high-affinity interactions with integrins, modulate cell-matrix interactions, and promote cell spreading by activating PI3K and the small GTPases, Ras and Rac[10-12]. Little is known about the role of the carbohydrate-mediated recognition systems in TM cell adhesion and signaling. In a recent study, we have observed that TM cells adhere to Gal8 substratum and that β_1 integrins derived from TM cells bind to Gal8 in a carbohydrate-dependent fashion[13]. The role of Gal8 in the regulation of Rho signaling that modulates stress fiber formation and focal adhesion assembly has, thus far, not been investigated in any cell type. In the current study, we underscore for the first time the function of Gal8 in modulating the Rho signaling pathway. We demonstrate here that: (i) TM cells adhere and spread on Gal8 coated wells in serum-free media; (ii) β_1 integrin function-blocking antibody inhibits the adhesion and spreading of TM cells on Gal8-coated wells; (iii) cells adhered to Gal8 accumulate phosphorylated myosin light chain (MLC), and accumulation of MLC is associated with stress fiber formation that is abolished by the presence of either the Rho inhibitor, C3 transferase, or the Rho-kinase (ROCK) inhibitor, Y27632. These data lead us to propose that Gal8 promotes cytoskeletal rearrangement in TM cells through interaction with β_1 integrins leading to activation of the Rho/ROCK/MLC signaling pathway.

MATERIALS AND METHODS

Immunohistochemical Detection of Galectin-8 in Human TM

Human eyes with no known history of eye disease were obtained from the National Disease Research Interchange (Philadelphia, PA) within 24 h postmortem. The eyes were bisected by an equatorial incision and the anterior segment of the eye was cut into four quadrants. Tissues were fixed in formaldehyde (4% , 3 hr, 25ºC) and were then processed for paraffin embedding. For immunostaining, longitudinal tissue sections (5 µm) were deparaffinized and sequentially treated with a basic pH antigen retrieval reagent (R&D systems, Minneapolis, MN), normal horse serum (R&D systems), goat anti-human Gal8 (2 hr, 37ºC, R&D Systems; the goat antibody is specific for Gal8 and it does not recognize other galectins including Gal3 or Gal1[*]), biotinylated anti-goat IgG (1 hr, 25ºC, R&D Systems), a freshly prepared solution of avidin-biotin-complex (R&D Systems' Cell and Tissue Staining Kit, 30 min, 25ºC) and a diaminobenzidine/H_2O_2 reagent (R&D

systems). Control sections were processed the same way except that either the step involving incubation with the primary antibody was omitted or primary antibody was substituted with nonimmune goat serum (R&D systems).

Preparation of Human TM Cell Cultures

Normal human cadaver eyes (donor age 71-89 years) were obtained from the Central Florida Lions Eye Bank (Tampa, FL). All eyes were enucleated within 6 hr postmortem and tissues were explanted for culture within 24 hours postmortem. TM cells were grown in tissue culture as described previously [14]. Cultures were propagated in DMEM supplemented with 10% FBS and cells were used at third to fourth passage. Consistent with published studies, these cells expressed aquaporin1 and CD44, and, when treated with Dexamethasone, markedly upregulated the expression of myocilin. (Not Shown, expression levels of CD44 and myocilin were detected by Western blot and RT-PCR analyses, respectively, and the expression of aquaporin1 was detected in a microarray study using 48.5 Illumina HEEBO oligo microarrays [Microarrays, Inc. Nashville, TN]).

RT-PCR Amplification of Galectin-8

Confluent cultures of TM cells from two different donors were subjected to RNA extraction using the RNEasy RNA extraction kit (Qiagen, Valencia, CA). On-column DNase (Qiagen) digestion was performed during RNA purification to avoid any DNA contamination. Reverse transcription was performed on total RNA (1 μg) with random primers (Invitrogen) in the presence or the absence of reverse transcriptase (RT) (Super Script II, Invitrogen). Primer Sequences: 5’ ccc/tgt/tct/ctt/gag/ctt/cg 3’ and 5’ cac/tgg/gga/agg/agt/tgt/gt 3’, were chosen for an expected product size of 191 bp. PCR products were electrophoresed on 1% agarose gel, and sequenced at the Tufts University Core Facility.

Quantitative RT-PCR

To assess the abundance of Gal8 expression in TM, the expression level of Gal8 was compared with that of GAPDH by quantitative RT-PCR. Quantitative RT-PCR was performed using the Mx4000 real-time PCR machine (Stratagene, La Jolla, CA). Briefly, cDNA was synthesized from 25 ng total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR was carried out in triplicates using inventory gene-specific primers (Gal8:HS00180706; GAPDH: HS99999905, Applied Biosystems) and the TaqMan Universal PCR Master Mix containing ROX as a passive reference. Reactions performed in the absence of template served as negative controls. Fluorescent signals were recorded once per cycle with a detector corresponding to FAM. To normalize the non-PCR related fluctuations between wells, each fluorescent reporter signal was measured against the ROX (internal reference dye) signal. Amplification plots showing the increase in the FAM fluorescence with each cycle of PCR (ΔRn) were generated and threshold cycle values (C_t) were calculated for all samples. The C_t value represented the PCR cycle number at which the fluorescence was detectable above a threshold based on the variability of the baseline data during the first 15 cycles. All C_t values were obtained in the exponential phase.

Western Blot Analysis

To detect the expression of Gal8 in TM cells, 0.6 ml of cold radioimmunoprecipitation (RIPA) buffer containing a protease inhibitor cocktail (Roche Diagnostic, Mannheim, Germany) was added to washed confluent cell cultures. Since Gal8 is a carbohydrate binding protein with high affinity towards β-lactose, a β-lactose affinity chromatography assay was used. After 30 min on ice, cell extracts were clarified by centrifugation and were incubated with Sepharose beads conjugated with β-lactose (EY labs, San Mateo, CA) for 1 hr at 4ºC. Following the incubation period, the beads were washed with Triton X-100, eluted first with a non-competing sugar, sucrose towards which Gal8 has no affinity (100mM in PBS-Triton), and then with β-lactose (100mM in PBS-Triton). Eluates were dialyzed and then analysed by SDS-PAGE. Protein blots were stained with Ponceau S (Sigma) and were then processed for immunostaining using goat anti-human Gal8 as a primary antibody (R&D Systems, 1 hr, 25ºC), peroxidase-labeled anti-goat IgG (Vector Labs) as a secondary antibody, and a chemiluminescence detection system (Perkin Elmer, Boston, MA).

Preparation of Recombinant Human Glutathione-S-Transferase Tagged Galectin-8

Recombinant human glutathione-S-transferase (GST) tagged Gal8, was produced and purified as previously described[12] with a few modifications. Lysates of bacteria expressing GST-Gal8 (2L culture) were chromatographed on a β-lactose-conjugated Sepharose column (EY Labs; 1 ml bed volume). After allowing Gal8 to bind to the affinity matrix, the gel bed was washed first with wash buffer I (20 ml of 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.2 mM PMSF, 4 mM β-mercaptoethanol) and then with 10 ml of wash buffer II (PBS containing 0.2 mM PMSF, 4 mM β-mercaptoethanol). GST-Gal8 was eluted from the column with 10 ml wash buffer II containing 100mM β-lactose. Fractions containing the lectin were dialyzed against PBS containing 2% glycerol and 4 mM β-mercaptoethanol and stored at -80ºC.

Quantification of Cell Adhesion and Spreading

To assess the adhesion and spreading of TM cells onto different substrates, 96-well microtiter plates were coated with Gal8 (30 μg/ml), human fibronectin from placenta (Sigma) (30 μg/ml), and poly-L-lysine (Sigma) (100 μg/ml). Gal8 was prepared as described above. Confluent TM cultures were dissociated with freshly prepared 5 mM EDTA (Invitrogen) in Ca^{2+} free PBS (Invitrogen) and plated in serum-free DMEM on microtiter plates coated with Gal8 and other substrates described above (50,000 cells/well, 3 wells/group). Following incubation (4hr, 37ºC), cells were washed with PBS and stained with Giemsa stain (Diff Quik stain set, Dade Behring, Newark, DE). Plates were scanned with an Epson Perfection 4490 scanner and approximate cell density was assessed using image-analysis software (Gene Tools, Synoptics, Cambridge, England). Micrographs were taken on a Nikon Eclipse TE200 inverted microscope (Nikon, Melville, NY) equipped with a Spot camera (Diagnostic Instruments, Sterling Heights, MI). Ten random fields of each experimental condition were photographed, and spread cells were counted manually. One thousand cells were counted at each condition and the percent spread cells was calculated. Statistical significance was calculated with an unpaired student *t*-test. To assess the involvement of β_1-integrins in the adhesion and spreading of TM cells on Gal8, cells were incubated in the presence of JB1A, a function-blocking monoclonal mouse antibody raised against human β_1-integrin (Chemicon, Temecula, CA). The JB1A antibody binds to a specific regulatory epitope (amino acids: 82 to 87) on β_1 integrin that is distinct from the RGD binding site (amino acids: 140-164)[15]. The antibody binding to this epitope locks the integrin in an inactive conformation and does not allow it to be activated regardless of the nature of the ligand presented[16]. This antibody has been shown to interfere with the adhesion of various cell types to a number of ECM substrates including fibronectin and collagen I [17-21].

Detection of Cytoskeletal Changes in Human TM Cells Adhered to Galectin-8

Eight-chamber glass slides were coated with Gal8 (30 μg/ml), human fibronectin from placenta (Sigma) (30 μg/ml), and poly-L-lysine (Sigma) (100 μg/ml). Confluent TM cultures were dissociated with EDTA and were plated on Gal8-coated slides (10,000 cells/ chamber in serum-free DMEM). Following 4 hr incubation at 37ºC, cells were washed, fixed and stained with rhodamine-labeled phalloidin as previously described [9]. To assess the involvement of Rho signaling in stress-fiber formation in cells adhered to Gal8, assays were performed in the presence of the ROCK inhibitor, Y27632 (5-20 μM) (Sigma), or the Rho specific inhibitor, C3 transferase from *clostridium botulinum* (1-2 μg/ml; Cytoskeleton, Denver, CO). This enzyme is a highly specific inhibitor of Rho that does not affect other Rho family members such as Rac or cdc42. It is a ribosyltransferase that by modifying the Asn 41 residue of Rho, renders it biologically inactive [22]. To assess the cytotoxicity of C3 transferase and Y27632, cells were stained with ethidium homodimer III (Biotium Inc. Hayward, CA) and Hoechst 33342 (Biotium) according to the manufacturer's instructions. As opposed to Hoechst 33342, which is cell permeable and can stain DNA in live cells, ethidium homodimer III is not cell permeable, and, therefore, can only stain DNA in necrotic cells whose membrane is disrupted. Experiments were carried out in triplicates. Random fields of each experimental condition were photographed and dead cells were counted manually. Tert butyl hydroperoxide (tBH, 3.5 mM), a potent cytotoxic reagent, was used as a positive control in this assay.

Detection of Myosin Light Chain Phosphorylation in TM Cells Adhered to Galectin-8

In an effort to further confirm the involvement of Rho signaling in stress fiber formation in TM cells adhered to Gal8, confluent TM cell cultures were dissociated with EDTA and plated on Gal8, fibronectin or poly-L-lysine-coated dishes. After 4 hour incubation (37ºC), phosphorylated proteins were isolated using a phosphoprotein affinity chromatography kit (PhosphoProtein purification kit, Qiagen), according to the manufacturer's instructions and were subjected to SDS-PAGE. Protein blots of the gels were immunostained using a rabbit anti-MLC (16hr, 4ºC; Cell Signaling Technology, Danvers, MA) as a primary antibody and peroxidase-labeled anti-rabbit IgG (Vector Labs) as a secondary antibody. Relative band intensity was quantified by the image analysis software, Image J, provided by the National Institutes of Health (Bethesda, MD). Immunoblot band intensity among different samples was normalized based on Ponceau S band intensity for the purpose of inter-sample comparisons.

RESULTS

Galectin-8 is Expressed in Human TM Tissue and in Cultured TM Cells

Multiple techniques including RT-PCR, Western blot and immunohistochemical staining were used to detect Gal8 expression in TM. In immunohistochemical staining, anti-Gal8 reacted strongly with the TM cells on beams (Figure 1A i, arrows) and with TM cells in the juxtacanalicular region (Figure 1A i, arrowheads). Some staining was observed in the ECM in all parts of the tissue and in the wall of Schlemm's canal (Figure 1A i, *SC*). No staining was observed when sections were not exposed to a primary antibody (Figure 1A, ii) or exposed to nonimmune goat IgG (Not Shown). To assess the expression of Gal8 in human cultured TM cells, RT-PCR experiments were performed on total RNA preparations from two different donors. All RNA preparations produced the expected size Gal8 (191bp) product (Figure 1Bi). In all cases, when reaction mixtures lacked RT, no components were amplified (Figure 1B i). Bands were isolated from the gel and sequenced. The product was found by BLAST analysis to be 98% identical to the published Gal8 cDNA sequence [11]. To assess the abundance of Gal8 expression in TM, the expression level of Gal8 was compared with that of GAPDH by quantitative RT-PCR. The qPCR experiments were performed in triplicates on whole RNA extracts from cultured TM cells derived from two different donors. In each case, a robust Gal8 signal was detected (Ct: 37.37 and 33.38 for Gal8 and GAPDH, respectively) (Figure 1B ii). To verify Gal8 protein expression, TM cell lysates were incubated with β-lactose conjugated beads, proteins bound to the beads were eluted first with sucrose, a non-competing sugar, and then with β-lactose, a competing sugar. Both the lactose eluate (Figure 1B iii, right panel, lane *L)* and the unfractionated total extract (Figure 1B iii, right panel, lane *T*) contained a major anti-Gal8-reactive 36-kDa band, which is the published molecular weight for human Gal8 [11]. This band was absent from the sucrose eluate as well as from the unbound fraction (Figure 1B iii, right panel, lanes *S* and *UB*). Many other protein bands in both the total extract and unbound fractions that were detected by Ponceau S staining (Figure 1B iii, left panel, lanes *UB* and *T*) were not stained with anti-Gal8, thereby attesting to the high specificity of the antibody. No bands were visible in control immunoblots that were not exposed to primary antibody (Not Shown).

TM Cell Adhesion and Spreading on Gal8 is Mediated by β_1 Integrins

In a recent study, we have observed that TM cells adhere to and spread on Gal8 substratum and that β_1 integrins derived from TM cells bind to Gal8 in a carbohydrate-dependent fashion[13]. Because β_1 integrins are known to play a central role in cell adhesion and spreading[19, 20, 23], we sought to determine whether the adhesion and spreading of TM cells to Gal8 is mediated by β_1 integrins. For this, we conducted the cell adhesion assay in the presence and the absence of a function-blocking anti-β_1 integrin antibody (JB1A). Anti-β_1 integrin antibody inhibited cell adhesion to Gal8 by about 45%, similar to the extent of inhibition of cell adhesion to fibronectin (Figure 2A). The antibody had no effect on cell adhesion to poly-L-lysine and control IgG inhibited cell adhesion to Gal8 by only 7% (Figure 2A). Microscopic examination revealed that anti-β_1 integrin antibody abolished cell spreading on Gal8-coated wells (80% reduction of cell spreading), whereas control IgG had no such effect (Figure 2B). These data suggest that TM cell adhesion and, in particular spreading on Gal8, is mediated by one or more β_1 integrins.

Galectin-8 Promotes Rearrangement of Cytoskeleton and Modulates Rho Signaling in TM Cells

Having established that Gal8 modulates TM cell spreading, it was of interest to determine whether the lectin has the capacity to influence the structure of cytoskeleton and Rho signaling. To determine whether Gal8 promotes rearrangement of the actin cytoskeleton, TM cells incubated on slides coated with Gal8 were stained with rhodamine-labeled phalloidin. Slides coated with human fibronectin and poly-L-lysine served as positive and negative controls, respectively. Cells incubated on Gal8 for 30 min appeared round and showed membrane protrusions (Figure 3a, red arrow). Following 1 hr incubation, cells began to spread and the actin cytoskeleton showed punctate staining, suggesting the presence of short actin bundles (Figure 3b, green arrow). At 2 hr, initial stress fibers were seen (Figure 3c, white arrow) and by 4 hr the cells were fully spread and contained stress fibers aligned along the longitudinal axes of the cells (Figure 3d). A very similar transition from rounded cells with punctate actin staining to spread cells with stress fibers was observed in cells adhered to fibronectin, albeit with a different timeline, as cells presented cortical stress fibers by 1 hr and fully developed, longitudinally arranged stress fibers appeared by 2 hr (Figure 3 i-l). In contrast, cells adhered to poly-L-lysine retained their rounded shape and punctate actin staining up to 4 hr incubation in serum-free medium (Figure 3 e-h). These results suggest that the interaction between integrins and Gal8 which leads to cell spreading is indeed mediated through rearrangement of the actin cytoskeleton and formation of stress fibers. To determine whether stress fiber formation in TM cells adhered to Gal8 is mediated by Rho GTPase; we conducted the cytoskeletal rearrangement assay in the presence of C3 transferase. Cells incubated on Gal8 for 4 hr, showed fully developed stress fibers (Figure 4A a). When cells were incubated on Gal8-coated glass slides in the presence of C3, the formation of actin stress fibers was significantly reduced (Figure 4A, b and c). Cells were spread, but only a few isolated stress fibers formed, and actin staining became predominantly punctate, suggesting short bundles.

A key regulator of actin cytoskeleton is the small GTPase Rho[24-26]. One of the effector proteins of Rho is the Rho-kinase, also known as Rho associated coiled coil kinase (ROCK). To assess the involvement of ROCK in the formation of stress fibers in

TM cells adhered to Gal8, we conducted the cytoskeletal rearrangement assay in the presence of a well characterized ROCK inhibitor, Y27632. At all concentrations tested (5-20 μM), in the presence of Y27632, no stress fibers formed in TM cells adhered to Gal8. Instead, all actin staining in the cells was in the form of punctate staining, suggesting the presence of short bundles (Figure 4A d-f). Despite these profound effects, neither Y27632 nor C3 transferase were cytotoxic to TM cells (Figure 4B). Together, these results suggest that Gal8 promotes stress fiber formation in TM cells through activation of Rho signaling and that the pathway for this effect runs through ROCK. Activated ROCK promotes the accumulation of phosphorylated MLC which, in turn, promotes the formation of stress fibers [27]. To find whether MLC is phosphorylated in TM cells adhered to Gal8; we analyzed the phospho-MLC content of the cells by isolating phosphoproteins and immunoblotting the phosphorylated fraction with a MLC antibody. TM cells adhered to Gal8 showed a time-dependent accumulation of phosphorylated MLC (Figure 5A *top right* and *bottom left* panels). The extent of accumulating phosphorylated MLC on Gal8-coated wells was similar to that seen in cells adhered to fibronectin (Figure 5, *bottom right* panel). Together, these data suggest a novel activity for Gal8 involving induction of the Rho/ROCK/MLC pathway leading to cell spreading and stress fiber formation.

DISCUSSION

Perturbation of the outflow of the aqueous humor leads to elevation in intraocular pressure, which is one of the major causal risk factors for vision loss in POAG [1]. The dynamic structure of the TM cells actin cytoskeleton with its focal adhesions is an important determinant of outflow facility. Reagents that disrupt the actin cytoskeleton such as cytochalasins, lantrunculin and H-7, cause elevation in outflow facility as well as loss of TM cell contact with the beams and neighboring cells [28-31]. Our data support the hypothesis that Gal8 can regulate TM actin cytoskeleton. TM cell adhesion to Gal8 was followed by cell spreading and actin cytoskeleton rearrangement, showing gradual actin polymerization into stress fibers over time in adhered cells. A key regulator of actin cytoskeleton is the small GTPase Rho[24-26]. Activated (GTP-bound) Rho interacts with ROCK and activates its kinase activity. This in turn, phosphorylates and inactivates MLC phosphatase, and also phosphorylates MLC directly. The phosphorylation of MLC leads to rearrangement of cytoskeleton and stress fiber formation [27]. Recent studies have shown that phosphorylation of MLC in TM cells is dependent on activation of the Rho/ROCK signaling pathway [8, 9]. In the current study, we demonstrate for the first time that Gal8 can modulate Rho GTPase signaling. Our findings that spreading of TM cells on Gal8 is associated with the accumulation of phosphorylated MLC and that inhibitors of Rho as well as of ROCK attenuate TM cell spreading and stress fiber formation, suggest that Gal8 has the ability to participate in the regulation of the Rho/ROCK/MLC signaling pathway in TM cells. Rho signaling has been intimately linked to the regulation of outflow facility. Specific inhibitors of ROCK, Y27632[6, 7] and H1152[8] have been shown to increase outflow facility in whole monkey and porcine eyes in a time- and dose-dependent manner. Likewise, in a perfused human eye, dominant negative RhoA [2] and dominant negative ROCK[4] caused a marked increase in outflow facility, TM cell rounding and detachment from the beams. In cultured TM cells, delivery of a dominant negative RhoA[2] and dominant negative ROCK[4] caused disruption of the actin cytoskeleton, TM cell rounding, loss of stress fibers and focal adhesions.

The current study has focused on characterization of Gal8 effects on normal TM cells. The relevance of these effects to the pathogenesis of POAG needs to be investigated further. The LGALS8 gene encodes six different isoforms of Gal8, resulting from alternative splicing and the use of multiple polyadenylation signals[32]. Different isoforms differ in the length of their hinge peptide [32]. It has been shown that isoforms with a short hinge peptide are severely impaired in their biological activity, they are profoundly less capable of promoting cell adhesion and of inducing outside-in signaling[33]. In a recent study conducted in our lab[34], glycogene expression patterns were compared between normal and glaucomatous TM tissues, using a specialized glycogene microarray, GLYCOv2 (Consortium for Functional Glycomics). While this study revealed no differences in Gal8 expression levels between normal and glaucomatous TM tissues, the Gal8 probes used in the GLYCOv2 microarray were not designed to detect the alternatively spliced isoforms of Gal8 with the different hinge peptide lengths. Thus, one cannot eliminate the possibility that normal and glaucomatous TM may express distinct isoforms of Gal8 resulting in different effects on the tissues. A different regulatory mechanism may involve not Gal8 itself but its countereceptors. TM cells treated with dexamethasone show elevated expression levels of α_v integrin[35] which we previously found to bind to Gal8[13]. It is possible that glaucomatous TM cells bearing more countereceptors for Gal8 are more susceptible to its effects.

In conclusion, data presented in the current study suggest a novel function for Gal8 in activation of Rho signaling and the regulation of TM cell cytoskeleton. It lays a foundation for exploration of a possible role for Gal8 in regulation of aqueous outflow and the pathogenesis of POAG.

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FIGURE LEGENDS

Figure 1: **Galectin-8 is expressed in the Trabecular Meshwork (TM)**. **A:** paraffin sections of anterior chamber angle from a normal human eye were immunostained with anti-Gal8 antibody. (i) anti-Gal8 IgG reacted intensely with cells on the trabecular beams (arrows) and with cells in the juxtacanalicular portion of TM (arrowheads). Staining was also observed in the ECM of both portions of TM (JCT and CS) and in the wall of Schlemm's canal. (ii) no staining was observed when the sections were not exposed to the primary antibody. Sections exposed to control goat IgG were identical to those exposed to no primary antibody (not shown). *SC*- Schlemm's canal, *JCT*-

juxtacanalicular TM; *CS beams*- corneoscleral beams. Bar: 25μm. **B:** (i) *RT-PCR.* Total RNA (1.0 μg) from confluent cultures of normal human TM cells was subjected to RT-PCR. The expected 191bp fragment was amplified using Gal8- specific-primers. In each case, no components were amplified when reaction mixtures lacked reverse transcriptase (RT). (ii) *qRT-PCR.* Total RNA was subjected to Taq-Man RT-PCR using Gal8 specific primers. Original amplification plots of Gal8 and GAPDH mRNAs genes are shown (Ct 37.37 and 33.38 for Gal8 and GAPDH, respectively). N=3 for each experiment; all experiments were performed twice using TM cells from two different donors with reproducible results. (iii) *Western Blot Analysis.* Protein extracts from confluent cultures of normal human TM cells were incubated with lactogel beads and eluted first with sucrose, a non-competing sugar, and then with lactose, a competing sugar. Eluted proteins were electrophoresed, the protein blot of the gel was stained with Ponceau S and was then processed for immunostaining with goat anti-Gal8. Both the total cell extract (*T*) and the lactose eluate (L) contained a major 36-kDa anti-Gal8 reactive component. This component was not detected in the unbound fraction (*UB*) and in the sucrose eluate (*S*). Many protein bands in both the total extract and unbound fractions that were detected by Ponceau S staining were not stained with the anti-Gal8 antibody thereby attesting to the high specificity of the antibody.

Figure 2**: TM cell adhesion and spreading on Gal8 is mediated by β_1 integrins.**

A: Cell adhesion study. Normal human TM cells incubated on microtiter wells coated with Gal8, fibronectin, or poly-L-lysine (37ºC, 7% CO_2, 2 hr) in serum-free DMEM, in the presence and absence of a function-blocking anti-β1 integrin antibody or control mouse IgG. Following incubation, cells were fixed and stained with Giemsa.

Approximate cell density of adhered cells in each well, as measured by image analysis software, is shown. Note that the function-blocking anti-β_1 integrin antibody inhibited cell adhesion to Gal8 by about 50%, but that control IgG had no significant inhibiting effect. Anti-β_1-integrin antibody interfered with cell adhesion to fibronectin, but not to poly-L-lysine, a substrate to which adhesion is not integrin mediated. Cell density of a single fibronectin-coated well was set as 100% (positive control); cell density in other wells is presented as percent of positive control. **B: Cell spreading study.** Ten random fields of each experimental condition were photographed, and spread cells were counted manually. Percent spread cells for cells adhered to Gal8 in the presence of anti-integrin and control IgG is shown in panel Bi. Anti-β_1 integrin antibody inhibited cell spreading on Gal8 by about 80% while control IgG had no effect. Representative micrographs of TM cells incubated in the presence and the absence of anti-β_1-integrin antibody are shown in panel Bii. Arrows, unspread cells; arrowheads, spread cells. Mean value ± standard deviation are shown (N=3), *, $P < 0.05$ compared to incubation without antibody. This experiment was performed three times with reproducible results.

Figure 3: **Galectin-8 promotes cytoskeletal rearrangement in TM cells.** Normal human TM cells were plated on eight-chamber glass slides coated with recombinant human Gal8, fibronectin, or poly-L-lysine in serum-free DMEM for 30 min, 1, 2 and 4 hr (37ºC, 7% CO_2). Following the incubation period, cells were fixed in formaldehyde and stained with rhodamine-labeled phalloidin. After 30 min incubation on Gal8 (a), cells have adhered, but are round, and show membrane protrusions (red arrow); following 1 hr incubation (b), membrane protrusions are still present (red arrow), but cells have begun to spread, and punctate actin staining is apparent (green arrow) suggesting the formation of

short actin bundles; following 2 hr incubation (c), cells have fully spread, initial stress fibers are seen (white arrow), but punctate actin staining is still abundant (green arrow); and by 4 hr (d), the cells contain fully developed stress fibers aligned along the longitudinal axes of the cells (white arrow). Cells adhered to poly-L-lysine maintained their round shape, membrane protrusions and some punctate actin staining throughout the incubation timeline. These cells did not develop stress fibers (e-h). Cells adhered to FN (i-l), developed cortical stress fibers as early as 1hr (j) and were fully spread with longitudinally arranged fibers by 2hr (k). Blue, DAPI; Red, phalloidine. Magnification bar, 50μm.

Figure 4: **Rho and ROCK inhibitors inhibit Galectin-8 induced stress fiber formation. A:** Normal human TM cells were incubated on chamber glass slides coated with recombinant human Gal8 (37ºC, 7% CO_2, 4 hr) in the absence (a) or the presence of the Rho inhibitor, C3 transferase, at 1 μg/ml (b) or 2 μg/ml (c) ; or the ROCK inhibitor, Y27632, at 5μM (d), 10μM (e) or 20μM (f). Following the incubation period, cells were fixed in formaldehyde and stained with rhodamine-labeled phalloidin. Stress fibers were prominent in cells plated on Gal8-coated wells (a, white arrows). Note that both Y27632 and C3 transferase interfered with the formation of actin stress fibers (b-e); both inhibitors caused a shift towards predominance of punctate actin staining, suggesting short actin bundles (green arrows). Neither inhibitor had any effect on the actin cytoskeleton of cells adhered to poly-L-lysine (not shown). This experiment was performed three times with reproducible results. **B:** *Rho and ROCK inhibitors are not cytotoxic to TM cells:* Cells were incubated on glass slides coated with recombinant

human Gal8 in the absence (a) or the presence of: Y27632 at 20μM (b), or C3 transferase at 2 μg/ml (c) or tert-butyl hydroperoxide (tBH) at 3.5 mM (d), which served as a positive control for cytotoxicity. Following the incubation period, cells were washed and stained with ethidium homodimer III (red) which stains dead cells and Hoechst 33342 (blue) which stains nuclei of both living and dead cells. In the l*eft panel* are representative micrographs from each group showing no significant cell death in the presence of Y27632 (b) or (c) C3 transferase and significant cell death in the presence of tBH (d). Random fields of each experimental condition were photographed, and dead cells were counted manually. Percent dead cells for cells adhered to Gal8 in the presence of the different inhibitors are shown in the *right panel*. Mean values ± standard deviation are shown (N=3).

Figure 5: **Galectin-8 promotes phosphorylation of myosin light chain.** Normal human TM cells were incubated on Gal8-coated 100-mm dishes for 0.5, 1, 2 and 4 hr. Following incubation, cells were lysed, and protein extracts were subjected to affinity chromatography using a phosphoprotein affinity column. Bound fraction was electrophoresed on SDS-polyacrylamide gel and gel blots were stained with Ponceau S (*Top Left*) and were then processed for immunostaining with anti-myosin light chain antibody (*Top Right*). Phosphoproteins isolated from cells incubated on fibronectin and poly-L-lysine for 4 hr served as positive and negative controls respectively. The expected MLC band of 20-kDa appeared in the phosphorylated fraction of all cell lysates. Approximate band intensity was quantified by image analysis software and normalized to Ponceau S staining. In *lower left panel* the accumulation of phosphorylated MLC over

time in TM cells adhered to Gal8 is plotted. In *lower right panel* is a comparison of phosphorylated MLC content in cells adhered to different substrates following 4 hr incubation. Note that by 4 hr, MLC phosphorylation is similar in cells adhered to Gal8 and to fibronectin. Lys, poly-L-lysine; FN, human fibronectin.

Figure 1

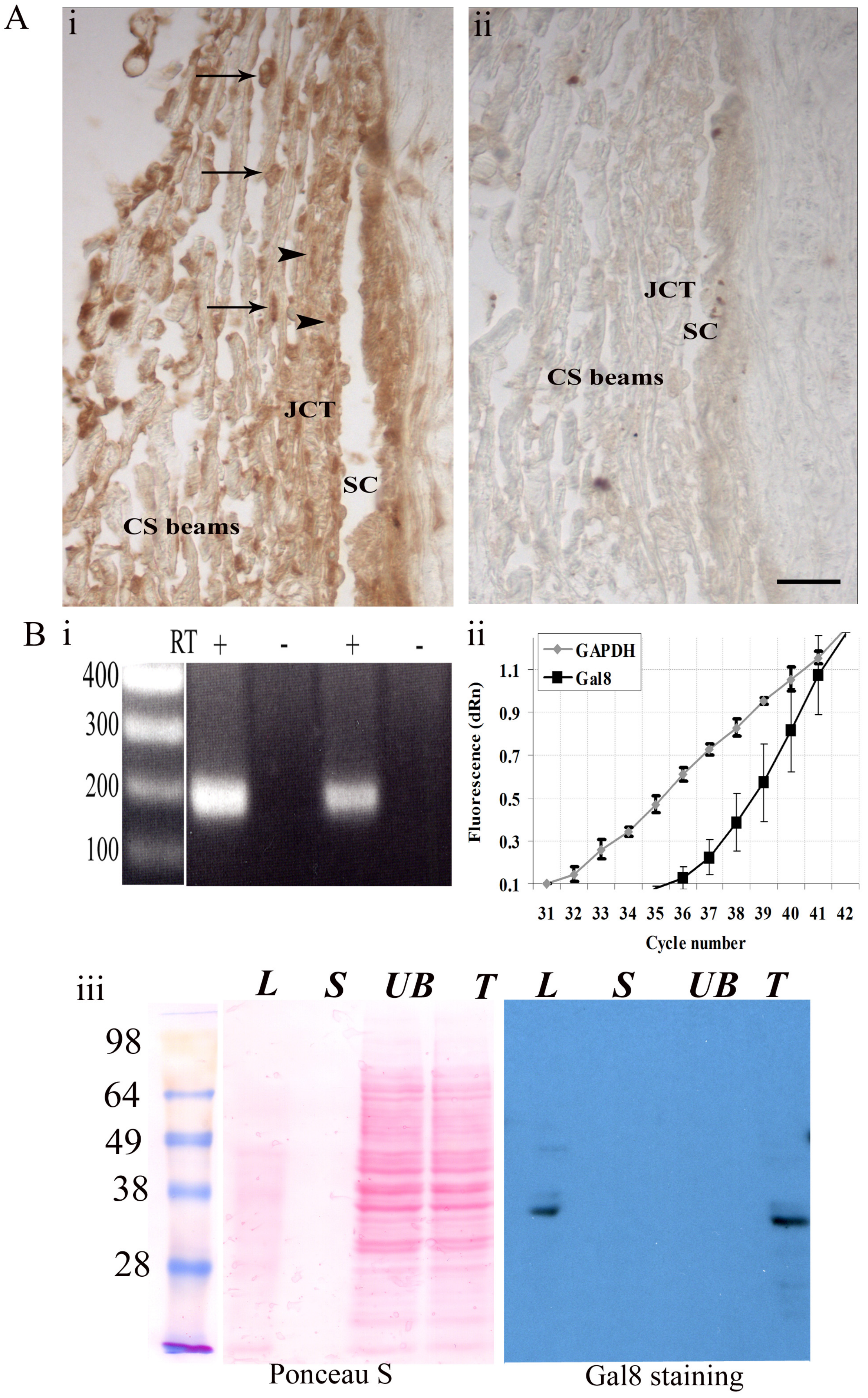

A
i
CS beams
JCT
SC
ii
JCT
SC
CS beams
B i
RT + - + -
400
300
200
100
ii
GAPDH
Gal8
Fluorescence (dRn)
0.1
0.3
0.5
0.7
0.9
1.1
31 32 33 34 35 36 37 38 39 40 41 42
Cycle number
iii
L S UB T
98
64
49
38
28
Ponceau S
L S UB T
Gal8 staining

Figure 2

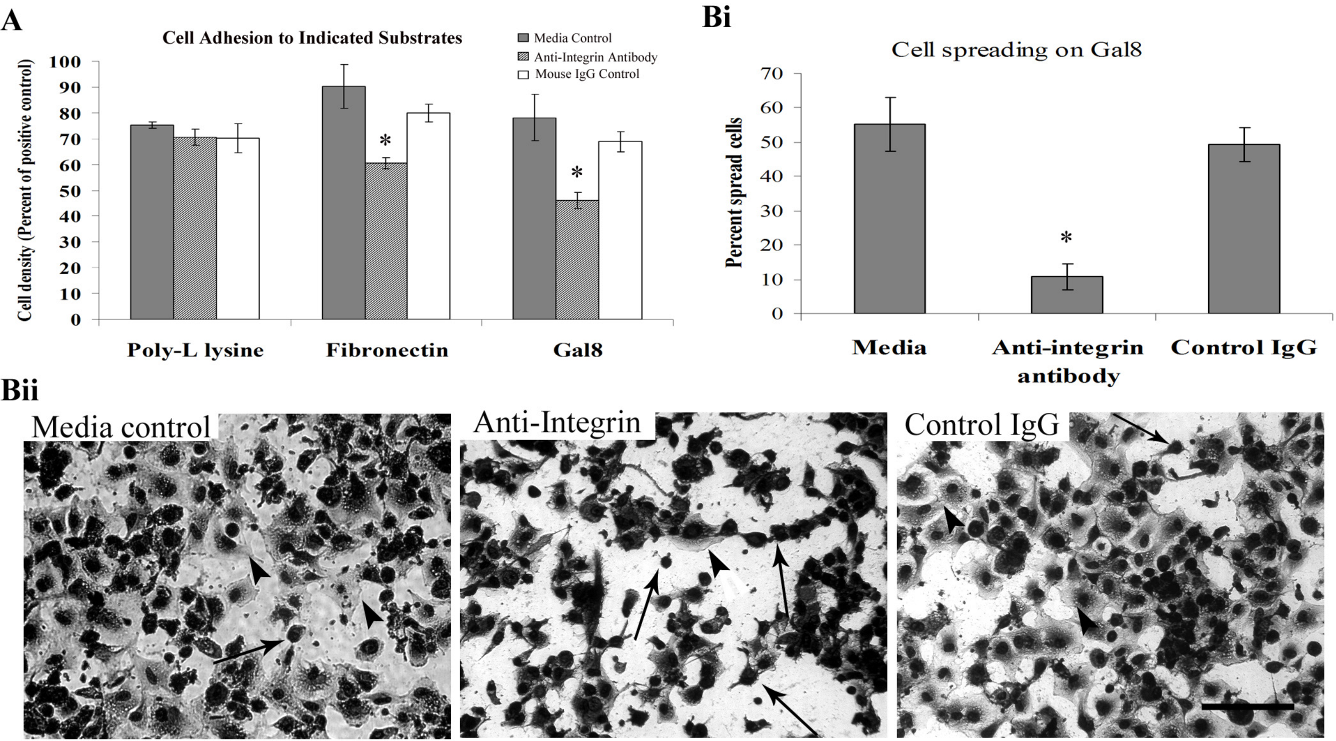

A
Cell Adhesion to Indicated Substrates
Media Control
Anti-Integrin Antibody
Mouse IgG Control
Cell density (Percent of positive control)
100
90
80
70
60
50
40
30
20
10
0
*
*
Poly-L lysine
Fibronectin
Gal8
Bi
Cell spreading on Gal8
Percent spread cells
70
60
50
40
30
20
10
0
*
Media
Anti-integrin antibody
Control IgG
Bii
Media control
Anti-Integrin
Control IgG

Figure 3

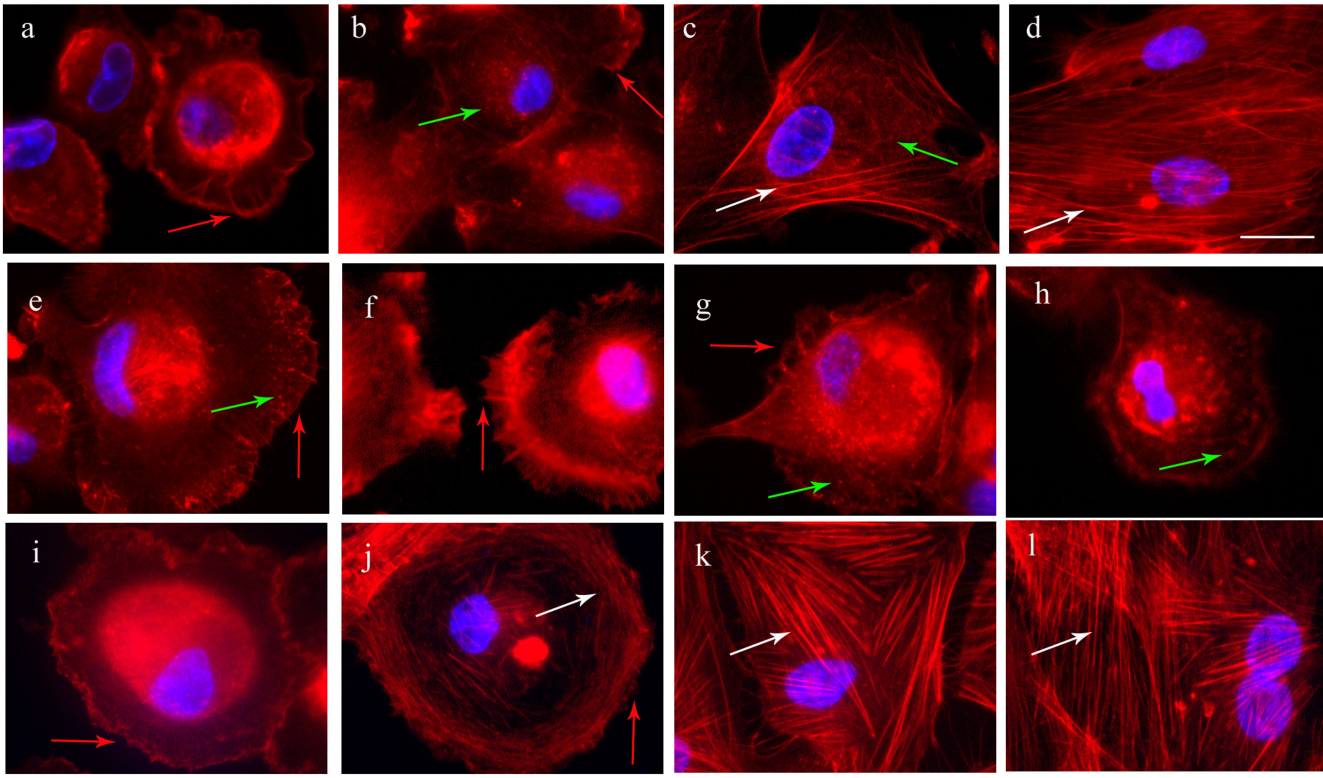
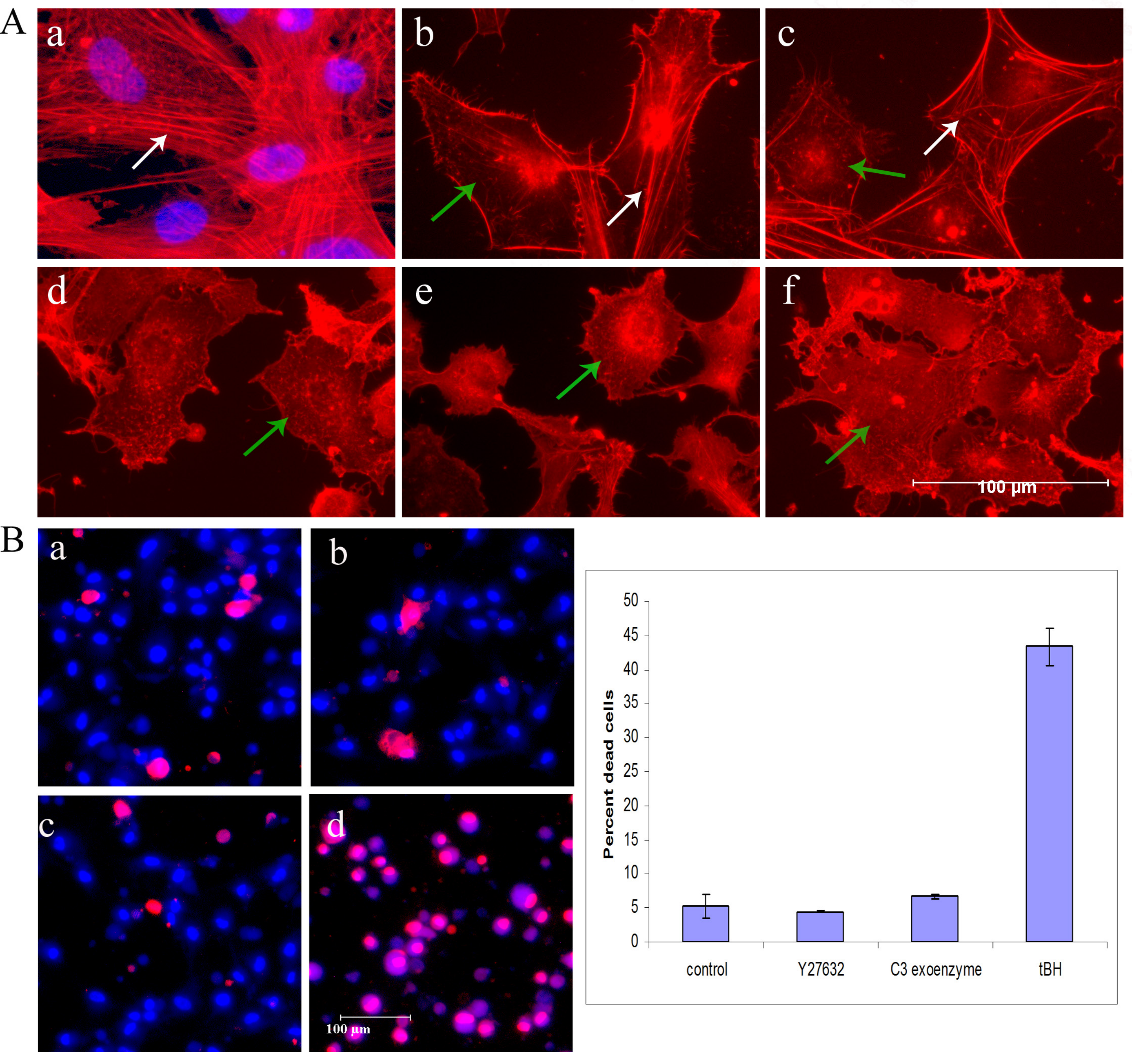
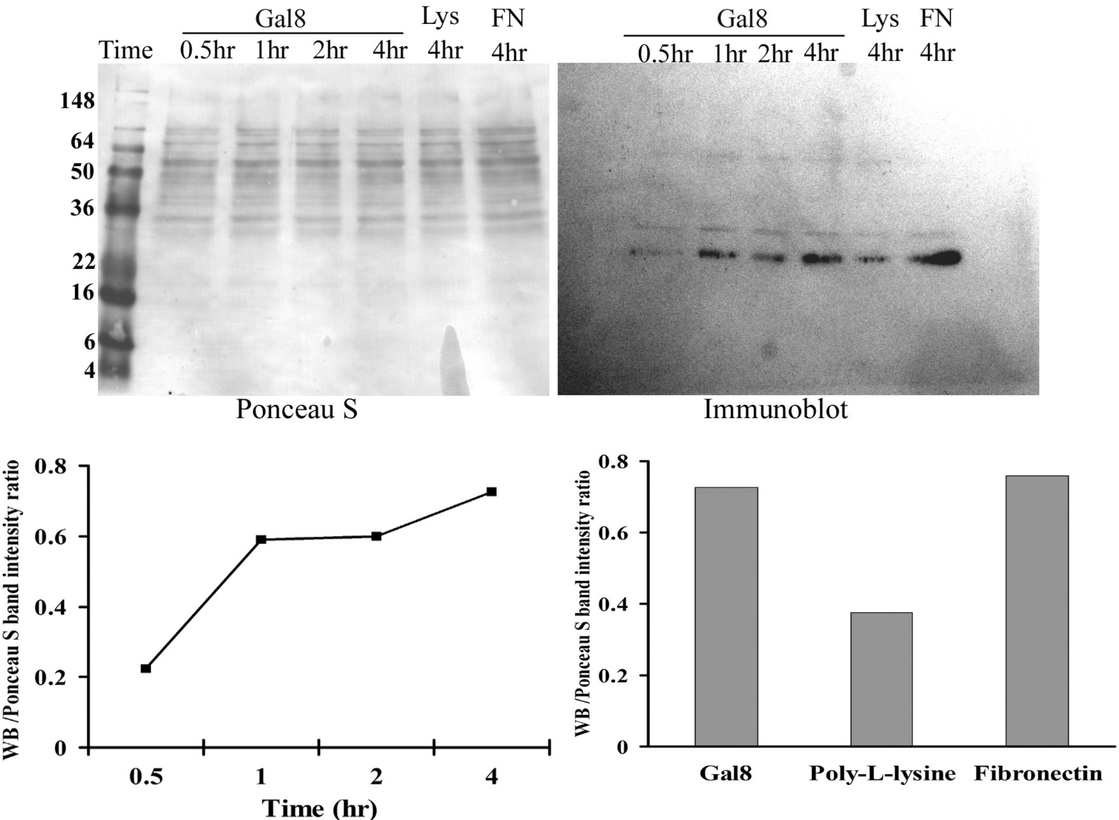


Figure 4



A
a
b
c
d
e
f
100 µm
B
a
b
c
d
100 µm
Percent dead cells
50
45
40
35
30
25
20
15
10
5
0
control
Y27632
C3 exoenzyme
tBH

Figure 5



Gal8
Lys
FN
Time
0.5hr
1hr
2hr
4hr
4hr
4hr
148
64
50
36
22
16
6
4
Ponceau S
Gal8
Lys
FN
0.5hr
1hr
2hr
4hr
4hr
4hr
Immunoblot
WB /Ponceau S band intensity ratio
0.8
0.6
0.4
0.2
0
0.5
1
2
4
Time (hr)
WB /Ponceau S band intensity ratio
0.8
0.6
0.4
0.2
0
Gal8
Poly-L-lysine
Fibronectin