Supporting Information: A Rapid Array-based Approach to N-glycan Profiling of Cultured Cells

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Cell Culture. Human Aortic Endothelial cells (HAEC). Male primary Human Aortic Endothelial Cells (ATCC, Manassas, VA) were grown according to manufacturer's protocols at 37°C under 5% CO_2. Cells were passaged by Versene (Thermo Fisher) and scraping. Media (Vascular Basal Cell Medium, ATCC) was prepared with Endothelial Cell Growth Kit-BBE (ATCC, PCS-100-040), including 10 mL Fetal bovine serum (FBS) (10%). For ^{15}N labeling, L-glutamine (Amide-15N, 98%+, Cambridge Isotope Laboratories, Inc) was substituted for ^{14}N glutamine in complete media. Cells were plated into cell chambers and allowed to adhere overnight prior to mass spectrometry. For ^{15}N labeling under oxidative stress, cells were plated into cell chambers, allowed to adhere overnight, followed by cultivating for one week in ^{15}N media spiked with 50 µM H_2O_2 or in ^{15}N media untreated. Media was changed daily.

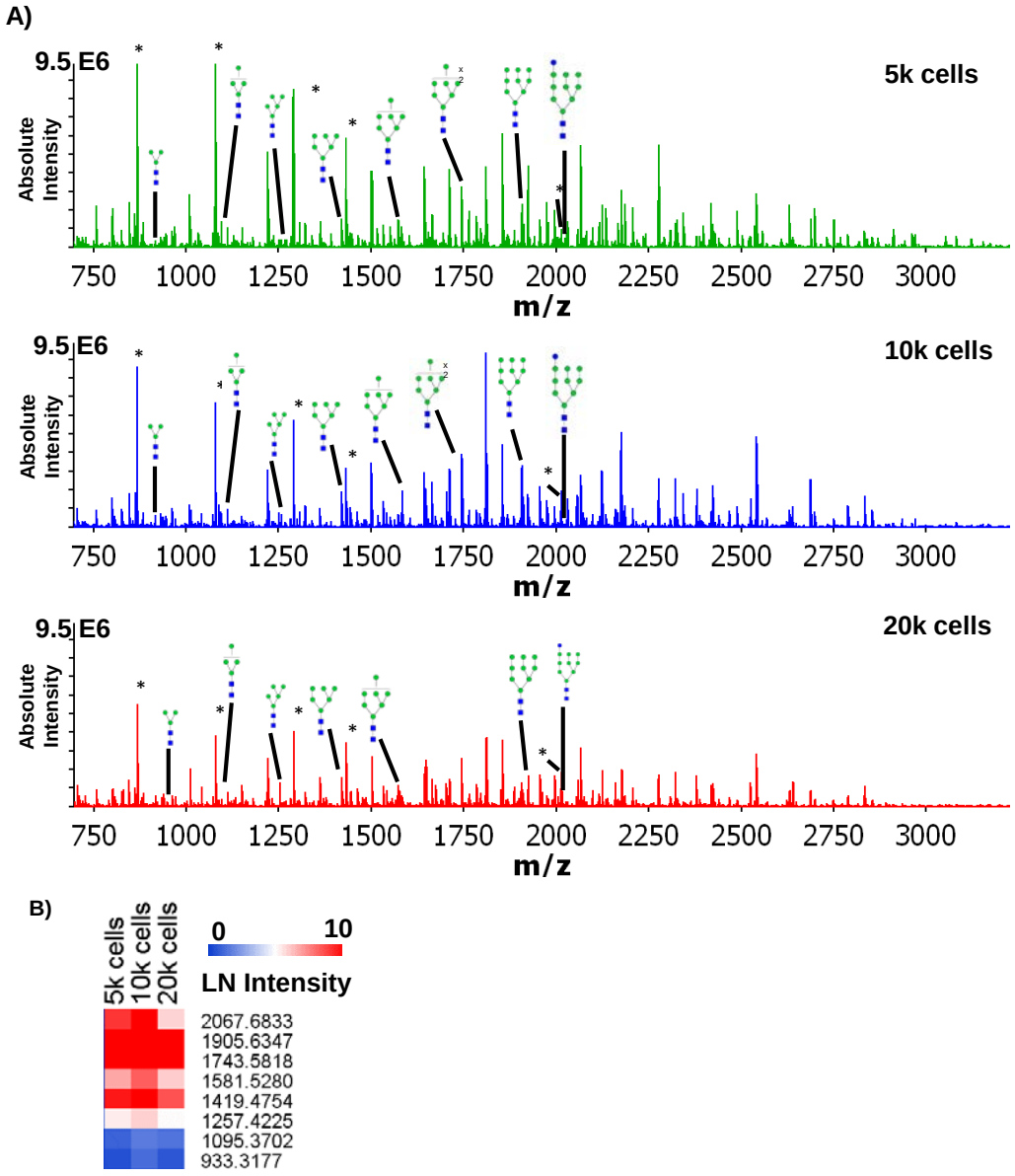
The HepG2/C3A cells are a clonal derivative of HepG2 cells derived from HepG2 hepatoblastoma cells[1]. Cells were grown as previously described for HepG2.2.15 cells[2]. Briefly, Cells were grown in RPMI containing 10% fetal bovine serume, followed by washing and culturing in serum-free media for 24 hours prior to analysis.

Primary prostate adenocarcinoma (PPC1) cells were obtained from Dr. Dean Tang (Roswell Park Comprehensive Cancer Center, Buffalo, NY) and maintained in RPMI media (Cellgro, Manassas, VA) supplemented with 10% FBS (Hyclone, Logan, UT) and 1% penicillin/streptomycin (Gibco, Grand Island, NY). All tissue culture was done in a 5% $C0_2$ humidified incubator, and all cell lines were periodically verified to be free of mycoplasma with a MycoAlert PLUS kit (Lonza, Allendale, NJ). To generate Polynuclear Giant Cancer Cells (PGCCs), PPC1 cells were plated 8 X 10^5 on 100 mm plates and irradiated in a ^{137}Cs γ-irradiator (J.L Sheperd & Associates, An Fenrando, CA) to 8 Gy. Cells were then allowed to undergo endoreplication until PGCCs could be visually confirmed to be the majority of remaining adherent cells, typically about 48 hours. Irradiated cells were filtered using a 20 micrometer mesh (Pluriselect, San Diego, CA) to obtain a population further enriched in PGCCs. PPC1 cells and PGCCs were counted and plated onto chamber slides (Lab Tek II) and allowed to adhere overnight.

The 4T1 mouse mammary cells model stage IV human breast cancer cells (ATCC, Manassas, VA) were grown according to manufacturer's protocols at 37° C and 5% CO_2. Media was composed of RPMI 1640 (Corning), 10% FBS (Gibco), 2mM Glutamine (Corning), and Pen/Strep (Corning).

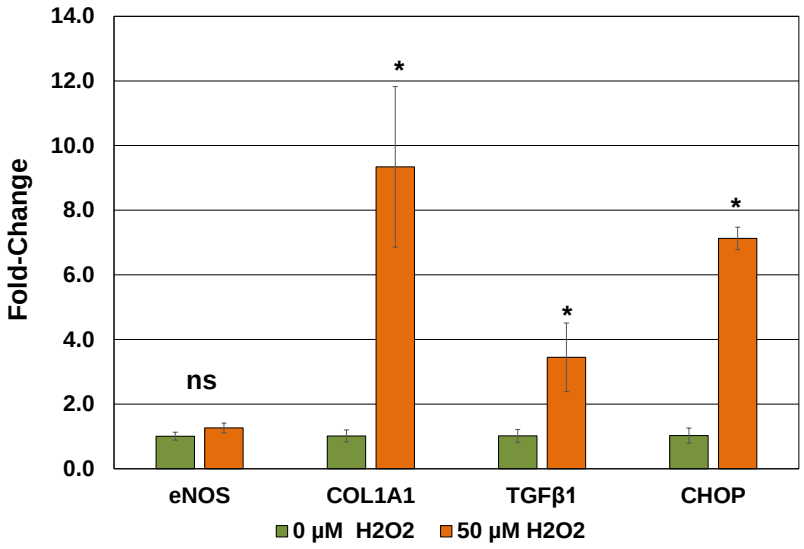
References

1. Sells, M. A.; Chen, M. L.; Acs, G., Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. *Proceedings of the National Academy of Sciences* **1987,** 84, (4), 1005-1009.
2. Norton, P. A.; Comunale, M. A.; Krakover, J.; Rodemich, L.; Pirog, N.; D'Amelio, A.; Philip, R.; Mehta, A. S.; Block, T. M., N-linked glycosylation of the liver cancer biomarker GP73. *Journal of Cellular Biochemistry* **2008,** 104, (1), 136-149.



Supplemental Figure 1. Signal suppression at 20k cell counts per chamber for HAEC. A) Cell specific signal decreases as cell count increases, likely due to ion suppression. B) evaluation of mannose series by cell counts. At 20K cell counts, Man9, Man 7 and Man 6 are decreased. We report that cell counts should be determined per cell type.

Supplemental Figure 2



*; t-test p-value ≤0.001

Supplemental Figure 2. In primary human aortic endothelial cells, unfolded protein response (CHOP) increases with potential activation of endothelial mesenchymal transition (TGFβ1, COL1A1) after 24 hours of oxidative stress (OS) treatment at 50 µM H_2O_2 compared to untreated cells. Lack of significant change in eNOS demonstrates functional status of the endothelial cells is not affected by OS dosing.

Supplemental Table 1. Common N-glycans found in each cell type. Media contributions have been subtracted.

Relative intensity shown per cell line

Lowest | Intermediate | Highest

	Name	Theoretical Mass	m/z +/- 0.003%	PPM	HAEC	HepC3A	4T1	PPC1	PGCC
Mannose	Hex10HexNAc2 + 1Na	2067.6866	2067.6872	-3.0E-07	1.35	2.27	3.00	1.88	3.51
	Hex9HexNAc2 + 1Na	1905.6338	1905.6323	7.9E-07	5.57	5.04	6.18	2.48	4.15
	Hex8HexNAc2 + 1Na	1743.5810	1743.5840	-1.7E-06	4.47	1.75	3.70	2.17	4.59
	Hex7HexNAc2 + 1Na	1581.5282	1581.5331	-3.1E-06	2.07	2.36	1.71	0.72	1.37
	Hex6HexNAc2 + 1Na	1419.4754	1419.4792	-2.7E-06	1.37	1.70	1.51	0.94	1.58
	Hex5HexNAc2 + 1Na	1257.4226	1257.4240	-1.1E-06	0.03	1.21	0.80	0.36	0.59
	Hex4HexNAc2 + 1Na	1095.3698	1095.3704	-5.5E-07	0.07	0.37	0.14	0.08	0.07
	Hex3HexNAc2 + 1Na	933.3170	933.3176	-6.4E-07	0.06	0.37	0.11	0.03	0.04
	Hex2HexNAc2 + 1Na	771.2642	771.2652	-1.2E-06	0.02	0.18	0.07	0.01	0.02
Bi-, Tri- Tetra, No Fucose	Hex3HexNAc3 + 1Na	1136.3964	1136.3990	-2.2E-06	0.00	0.66	0.21	0.24	0.21
	Hex4HexNAc3 + 1Na	1298.4492	1298.4504	-9.5E-07	0.01	1.47	0.37	0.60	0.47
	Hex5HexNAc3 + 1Na	1460.5020	1460.5063	-2.9E-06	0.09	1.08	0.64	0.24	0.31
	Hex4HexNAc4 + 1Na	1501.5286	1501.5316	-2.0E-06	0.00	0.72	0.46	0.27	0.24
	Hex3HexNAc5 + 1Na	1542.5552	1542.5542	6.5E-07	0.03	2.49	0.16	0.04	0.07
	Hex5HexNAc4 + 1Na	1663.5814	1663.5843	-1.7E-06	0.00	7.54	2.47	6.15	5.61
	Hex4HexNAc5 + 1Na	1704.6080	1704.6107	-1.6E-06	0.10	1.80	0.36	0.04	0.08
	Hex5HexNAc5 + 1Na	1866.6608	1866.6630	-1.2E-06	0.00	2.04	0.77	0.78	0.71
	Hex6HexNAc5 + 1Na	2028.7136	2028.7109	1.3E-06	0.00	12.41	0.21	14.69	0.00
	Hex3HexNAc6 + 1Na	1745.6345	1745.6374	-1.6E-06	0.38	2.12	0.43	0.13	0.23
	Hex5HexNAc6 + 1Na	2069.7402	2069.7372	1.5E-06	0.32	1.37	0.78	0.19	0.34
Bi-, Tri- Tetra, With Fucose	Hex3dHex1HexNAc2 + 1Na	1079.3749	1079.3725	2.2E-06	0.05	0.33	0.13	0.04	0.08
	Hex4dHex1HexNAc2 + 1Na	1241.4277	1241.4263	1.2E-06	0.03	0.17	0.20	0.04	0.06
	Hex3dHex1HexNAc3 + 1Na	1282.4543	1282.4530	1.1E-06	0.28	0.41	0.26	0.15	0.29
	Hex4dHex1HexNAc3 + 1Na	1444.5071	1444.5062	6.2E-07	1.08	0.72	0.82	0.41	0.58
	Hex3dHex1HexNAc4 + 1Na	1485.5337	1485.5340	-1.9E-07	0.16	0.58	0.54	0.23	1.03
	Hex4dHex1HexNAc4 + 1Na	1647.5865	1647.5915	-3.0E-06	1.73	1.05	1.71	1.13	1.67
	Hex3dHex1HexNAc5 + 1Na	1688.6131	1688.6136	-3.0E-07	0.14	0.64	0.60	0.18	0.60
	Hex5dHex1HexNAc4 + 1Na	1809.6393	1809.6430	-2.1E-06	12.62	2.45	3.35	3.49	5.05
	Hex4dHex1HexNAc5 + 1Na	1850.6659	1850.6687	-1.5E-06	0.10	2.68	1.52	0.37	0.90
	Hex3dHex1HexNAc6 + 1Na	1891.6925	1891.6932	-3.5E-07	0.10	1.23	1.55	0.14	0.37
	Hex5dHex2HexNAc4 + 1Na	1955.6972	1955.6921	2.6E-06	3.22	0.70	1.44	1.20	1.75
	Hex5dHex1HexNAc5 + 1Na	2012.7187	2012.7224	-1.8E-06	1.24	1.47	2.55	2.17	2.50
	Hex5dHex3HexNAc4 + 1Na	2101.7551	2101.7515	1.7E-06	1.44	1.54	0.88	0.30	0.43
	Hex5dHex2HexNAc5 + 1Na	2158.7766	2158.7786	-9.3E-07	0.77	0.63	1.13	1.95	1.48
	Hex6dHex1HexNAc5 + 1Na	2174.7715	2174.7715	-5.1E-09	6.71	1.07	2.96	2.04	3.24
	Hex5dHex2HexNAc6 + 1Na	2361.8560	2361.8520	1.7E-06	0.04	0.38	0.44	0.12	0.20
	Hex6dHex1HexNAc6 + 1Na	2377.8509	2377.8472	1.6E-06	2.31	0.04	1.75	0.38	0.78
	Hex6dHex3HexNAc5 + 1Na	2465.8669	2465.8590	3.2E-06	2.51	1.40	0.80	0.12	0.31
	Hex7dHex1HexNAc6 + 1Na	2539.9037	2539.9037	-4.3E-09	16.10	0.04	4.75	3.06	4.99
	Hex7dHex2HexNAc6 + 1Na	2685.9616	2685.9644	-1.0E-06	6.23	0.52	1.02	0.00	0.00
	Hex7dHex1HexNAc7 + 1Na	2742.9831	2742.9768	2.3E-06	0.00	0.36	0.23	0.00	0.00
	Hex7dHex3HexNAc6 + 1Na	2832.0195	2832.0158	1.3E-06	2.03	1.20	0.53	0.01	0.00
	Hex8dHex1HexNAc7 + 1Na	2905.0359	2905.0437	-2.7E-06	0.76	0.55	1.19	0.28	0.15
	Hex9dHex1HexNAc8 + 1Na	3270.1681	3270.1643	1.2E-06	0.00	1.28	0.78	0.00	0.10
Sialic acid containing N-glycans	Hex2dHex1HexNAc3NeuAc1 + 2Na	1433.4867	1433.4907	-2.8E-06	0.04	0.17	0.17	0.00	0.00
	Hex5HexNAc3NeuAc1 + 1Na	1913.6503	1913.7137	-3.3E-05	0.00	0.24	0.61	1.01	0.74
	Hex4dHex1HexNAc4NeuAc1 + 1Na	1938.6819	1938.6814	2.4E-07	0.15	0.93	1.04	1.27	1.04
	Hex5HexNAc4NeuAc1 + 1Na	1954.6768	1954.6826	-3.0E-06	0.27	1.09	0.65	0.32	0.28
	Hex5HexNAc4NeuAc1 + 2Na	1976.6666	1976.6703	-1.9E-06	0.00	0.53	1.94	4.29	3.21
	Hex5dHex1HexNAc4NeuAc1 + 1Na	2100.7347	2100.7309	1.8E-06	2.38	1.67	1.53	0.80	1.10
	Hex5dHex1HexNAc4NeuAc1 + 2Na	2122.7245	2122.7221	1.1E-06	5.17	0.97	1.09	0.69	0.94
	Hex4dHex1HexNAc5NeuAc1 + 1Na	2141.7613	2141.7622	-4.0E-07	0.00	1.62	1.48	2.13	1.48
	Hex5HexNAc4NeuAc2 + 1Na	2245.7722	2245.7706	7.3E-07	0.00	0.13	0.87	0.23	0.17
	Hex5dHex2HexNAc4NeuAc1 + 2Na	2268.7824	2268.7875	-2.3E-06	1.39	1.99	1.64	0.54	0.59
	Hex4dHex2HexNAc5NeuAc1 + 1Na	2287.8192	2287.8258	-2.9E-06	0.71	1.22	1.43	0.25	0.29
	Hex5dHex1HexNAc5NeuAc1 + 1Na	2303.8141	2303.8074	2.9E-06	0.07	0.70	0.54	0.71	0.18
	Hex6HexNAc5NeuAc1 + 1Na	2319.8090	2319.8065	1.1E-06	0.44	2.99	1.97	5.14	3.71
	Hex5dHex1HexNAc5NeuAc1 + 2Na	2325.8039	2325.8036	1.1E-07	0.07	0.34	0.56	0.26	0.24
	Hex6HexNAc5NeuAc1 + 2Na	2341.7988	2341.7938	2.1E-06	0.00	1.44	3.39	11.64	6.46
	Hex5dHex1HexNAc4NeuAc2 + 1Na	2391.8301	2391.8358	-2.4E-06	0.00	1.00	2.64	1.58	1.68
	Hex7HexNAc6 + 1Na	2393.8458	2393.8457	2.9E-08	1.90	0.04	3.15	0.91	1.28
	Hex5dHex2HexNAc5NeuAc1 + 2Na	2471.8618	2471.8573	1.8E-06	0.10	0.41	0.86	0.54	0.48
	Hex6HexNAc5NeuAc2 + 2Na	2632.8942	2632.8885	2.1E-06	0.00	0.87	1.24	3.16	1.32
	Hex6HexNAc6NeuAc2 + 2Na	2835.9736	2835.9790	-1.9E-06	4.26	0.33	0.80	0.00	0.09
	Hex6HexNAc5NeuAc3 + 1Na	2901.9998	2901.9935	2.2E-06	0.00	0.42	2.94	2.15	1.02
	Hex6HexNAc5NeuAc3 + 2Na	2923.9896	2923.9941	-1.5E-06	0.00	0.75	4.29	1.79	0.61
	Hex7dHex3HexNAc6NeuAc1 + 2Na	3145.1047	3145.1127	-2.5E-06	0.80	1.84	0.99	0.06	0.23
	Hex7dHex2HexNAc7NeuAc1 + 1Na	3180.1364	3180.1355	2.8E-07	0.00	1.12	2.12	0.02	0.00
	Hex7dHex2HexNAc6NeuAc2 + 1Na	3268.1524	3268.1560	-1.1E-06	0.00	1.28	1.19	0.00	0.00
	Hex7dHex2HexNAc6NeuAc2 + 2Na	3290.1422	3290.1414	2.4E-07	0.00	0.51	0.64	0.07	0.01

Supplemental Table 2: Comparison of ^{15}N incorporation between untreated cells and treated cells. N-glycans are represented by composition.
Peak data is intensity data.

					Untreated			Treated 50 µM H_2O_2				
Composition	No.^{15}N Labels	Theor. m/z	Observed m/z	PPM	AVG	STDEV	%CV	AVG	STDEV	%CV	Treat / Untreated Ratio	p-value
Hex6HexNAc2 + 1Na	2	1421.4695	1421.4694	1.1E-07	16338	2536	15.5	56718	8145	14.4	3.47	3.94E-08
Hex4dHex1HexNAc3 + 1Na	3	1447.4982	1447.4960	1.5E-06	22477	2288	10.2	37080	8299	22.4	1.65	9.75E-04
Hex7HexNAc2 + 1Na	2	1583.5223	1583.5328	-6.6E-06	25911	2649	10.2	63877	9740	15.2	2.47	9.35E-07
Hex4dHex1HexNAc4 + 1Na	4	1651.5746	1651.5754	-4.8E-07	18558	2361	12.7	41946	7891	18.8	2.26	6.96E-06
Hex5HexNAc4 + 1Na	4	1667.5695	1667.5684	6.6E-07	46398	3761	8.1	88646	14911	16.8	1.91	1.71E-05
Hex8HexNAc2 + 1Na	2	1745.5751	1745.5798	-2.7E-06	22351	2629	11.8	60933	7198	11.8	2.73	1.51E-08
Hex5dHex1HexNAc4 + 1Na	4	1813.6274	1813.6224	2.8E-06	166742	16243	9.7	546409	108570	19.9	3.28	1.01E-06
Hex9HexNAc2 + 1Na	2	1907.6279	1907.6234	2.4E-06	57948	6973	12.0	122544	11775	9.6	2.11	1.03E-07
Hex6HexNAc5 + 1Na	5	2033.6988	2033.6969	9.3E-07	9937	810	8.1	30094	4356	14.5	3.03	4.72E-08
Hex5dHex1HexNAc4NeuAc1 + 2Na	5	2127.7096	2127.7147	-2.4E-06	34826	3650	10.5	58041	10673	18.4	1.67	3.90E-04
Hex6dHex1HexNAc5 + 1Na	5	2179.7567	2179.7615	-2.2E-06	34716	3083	8.9	236240	30561	12.9	6.80	7.80E-10
Hex6dHex1HexNAc5NeuAc1 + 2Na	6	2493.8389	2493.8459	-2.8E-06	26443	3646	13.8	53449	8185	15.3	2.02	6.59E-06