Supplemental Methods

Flow Cytometry – HeLa cells were synchronized by double thymidine block and released or released from nocodazol block as previously described. Cells were collected after trypsin digestion (Invitrogen) and washed in PBS. Cells were resuspended in 1 ml ice-cold PBS and fixed with ice-cold 70% ethanol (v/v) added dropwise with gentle vortexing (Slawson *et al.*, 2005). Cells were stored at -20° C until further use. Fixed cells were washed in ice-cold PBS and resuspended in PBS containing 0.1% (v/v) Igepal CA-630 (Sigma I3021) and 0.2% (w/v) propidium iodide. Cells were analyzed using a BD Bioscience FACScaliber, and data was processed using Cell Quest software.

Supplemental Figures

Figure 1 – Cell Cycle Stage after Adenoviral Infections was Confirmed by Flow Cytometry. A. 1 day after adenoviral infection cells were incubated overnight in nocodazole. Cells were washed and replated for one hour then harvested. All cells after replating show 4n amounts of DNA demonstrating M phase cells. B. Cells were harvested for flow cytometry analysis at 12 hours post double thymidine block release. OGT and OGA infected cells show a higher population of cells, which are mitotic compared to GFP control cells.

Figure 2 – Cell Cycle Stage after Inhibitor Incubation was Confirmed by Flow Cytometry. A. As before, cells were harvested one-hour post nocodazole block release. All cells were mitotic as judged by 4n amount of DNA. B. Cells were harvested for flow cytometry analysis at 12 hours post double thymidine

block release. Inhibitors were added 8 hours after release. ZM treated cells showed a large population of cells that were still mitotic compared to DMSO controls. The percent of 4n cells in the GT treated samples were similar to control cells.

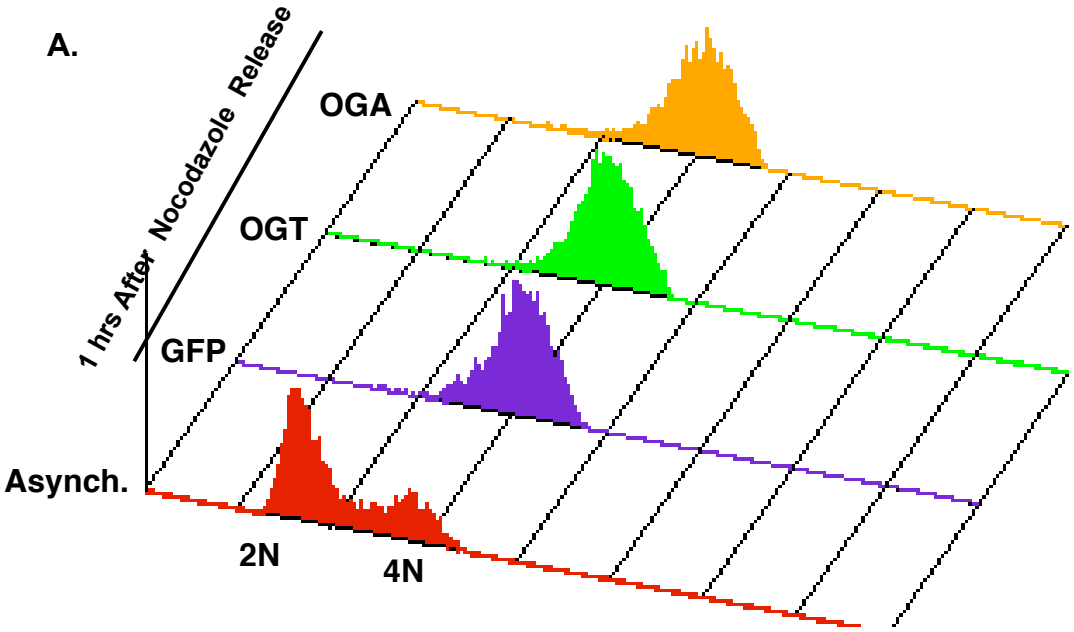
Supplemental Figure 3: Aurora B or O-GlcNAcase Inhibitor Treatment does not Alter the Signaling Complex Composition or O-GlcNAcase Cytolocolization.. Confocal staining of O-GlcNAcase and Aurora B was after double thymidine block and inhibitor treatment. O-GlcNAcase was red, Aurora B green, and DNA blue. Inhibitor treatment had no effect on O-GlcNAcase cytolocalization.

Figure 4 – Increased Vimentin Filament Staining in Cells with Altered GlcNAcylation. Cells were synchronized by double thymidine block and released as before. Vimentin, pSer55, and pSer71, were stained green while DNA was red. OGT over-expression and inhibitor treatment (ZM and GT) all caused an increase in filament staining near the cleavage furrow compared to control cells. All treatments had little effect in the pattern of staining by the phosphorylation specific antibodies.

Slawson, C., Zachara, N.E., Vosseller, K., Cheung, W.D., Lane, M.D., and Hart, G.W. (2005). Perturbations in O-linked beta-N-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. J Biol Chem *280*, 32944-32956.

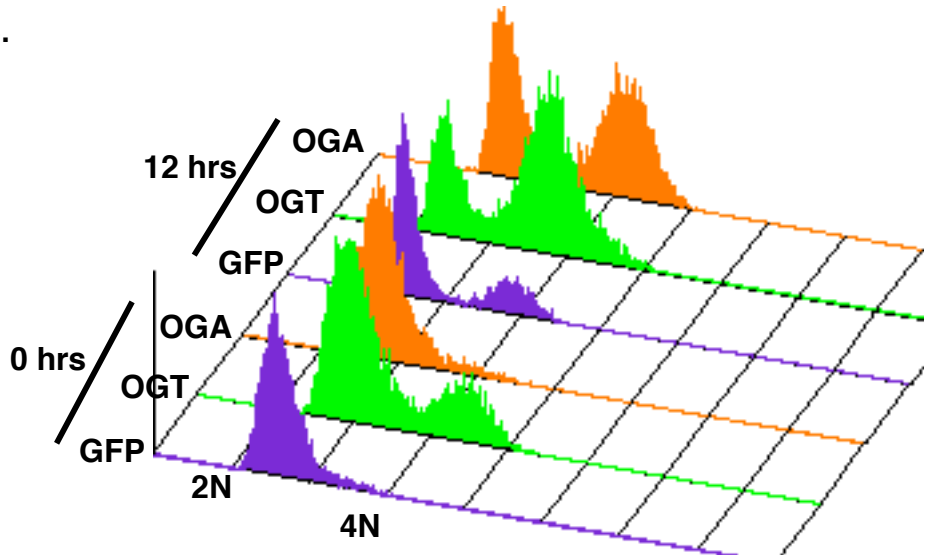
Supplemental 1

A.



Sample	%2N	%2+N	%4N
Asynch.	39.55	46.5	15.57
GFP	1.58	20.19	78.34
OGT	0.74	22.45	76.79
OGA	0.89	19.93	79.23

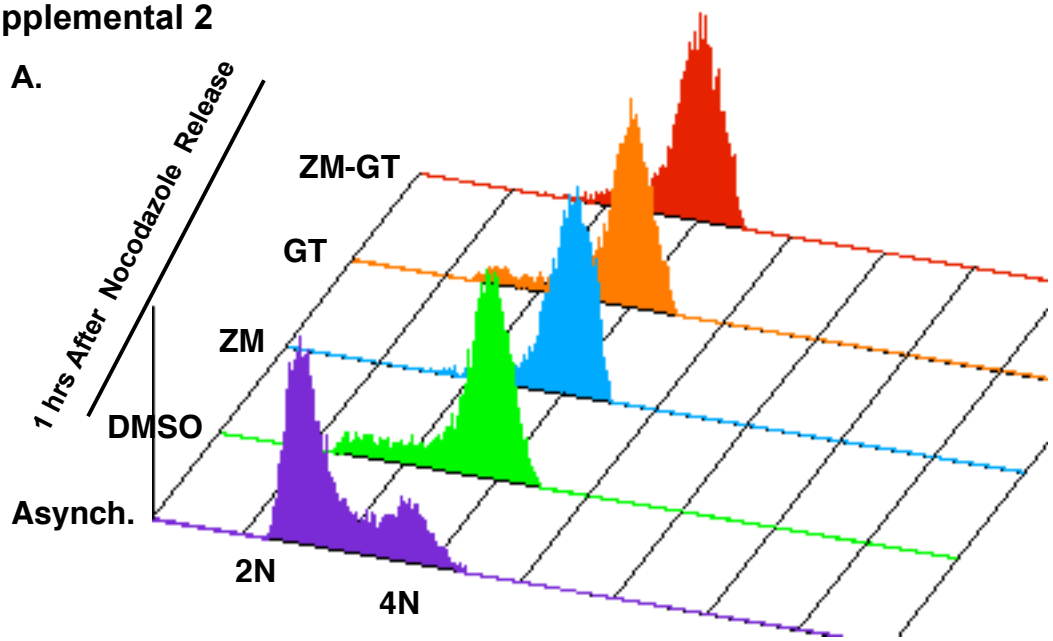
B.



Sample	%2N	%2+N	%4N
GFP 0	64.45	35.74	0.95
OGT 0	12.45	74.19	9.6
OGA 0	41.55	58.27	1.51
GFP 12	66.5	20.33	14.01
OGT 12	25.6	37.51	34.14
OGA 12	39.95	31.77	27.79

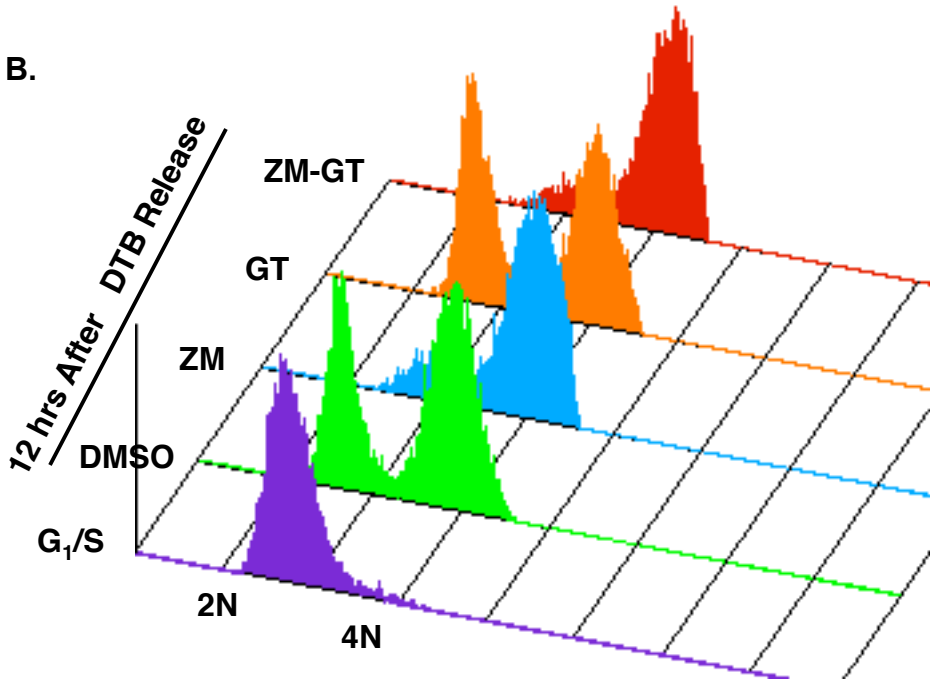
Supplemental 2

A.



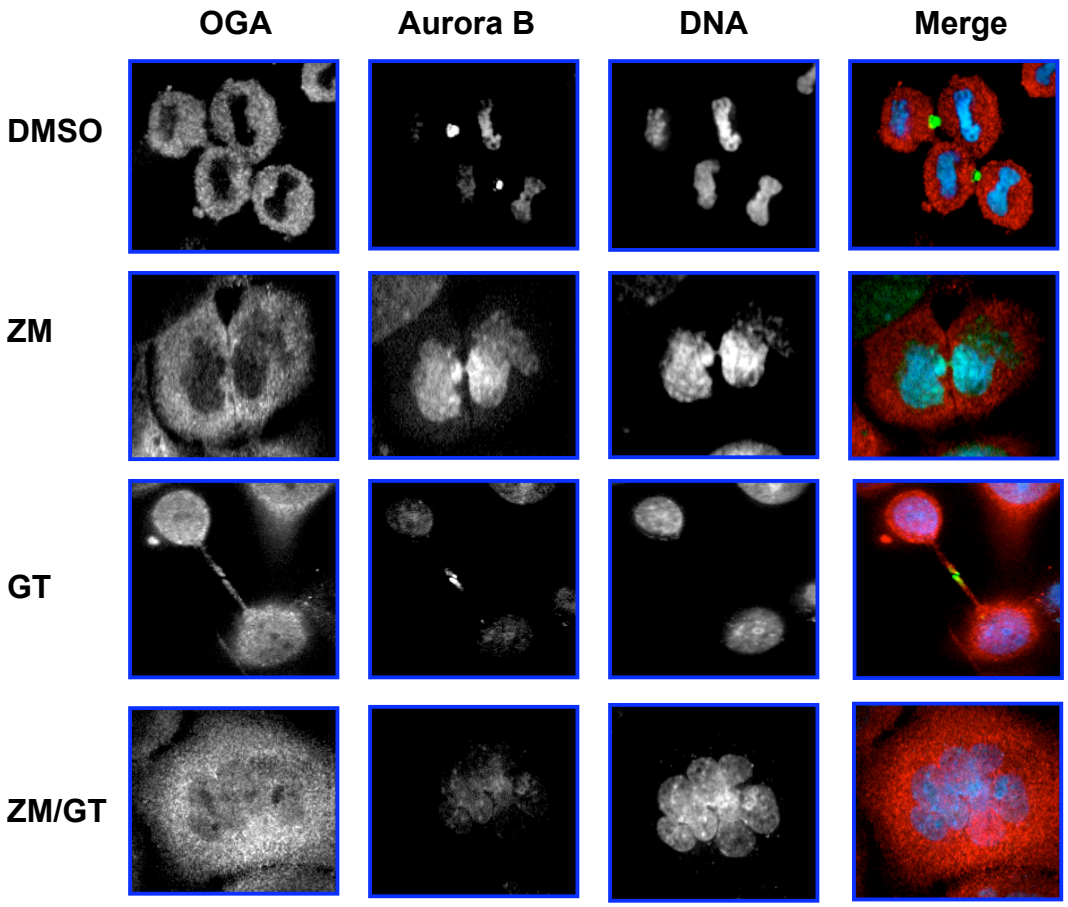
Sample	%2N	%2+N	%4N
Asynch.	46.54	40.35	14.29
DMSO	4.77	24	72.76
ZM	0.99	16.55	78.78
GT	3.99	17.54	79.42
ZM-GT	0.97	17.6	82.32

B.



Sample	%2N	%2+N	%4N
G1/S	59.9	33.75	4.29
DMSO 12	37.65	13.61	48.67
ZM 12	3.09	10.88	86.08
GT 12	35.87	12.66	50.68
ZM-GT 12	2.55	10.05	87.45

Supplemental 3



Supplemental 4