

Writing and erasing O-GlcNAc on casein kinase 2 alpha alters the phosphoproteome

Supporting Information

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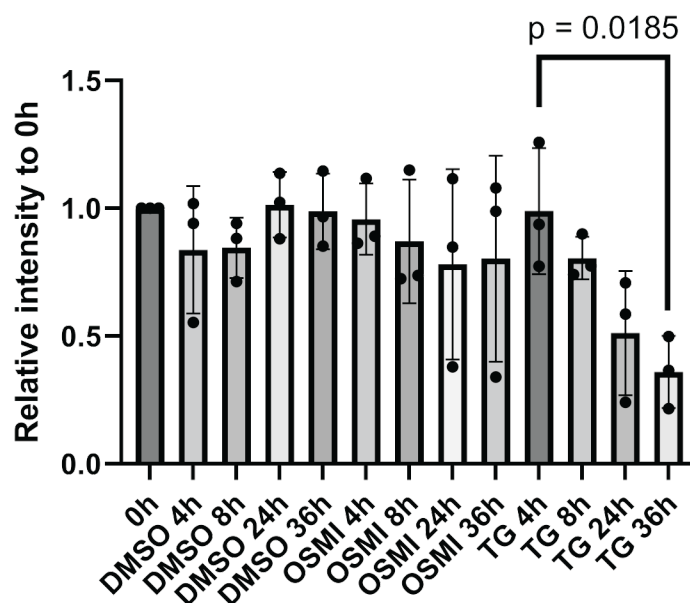
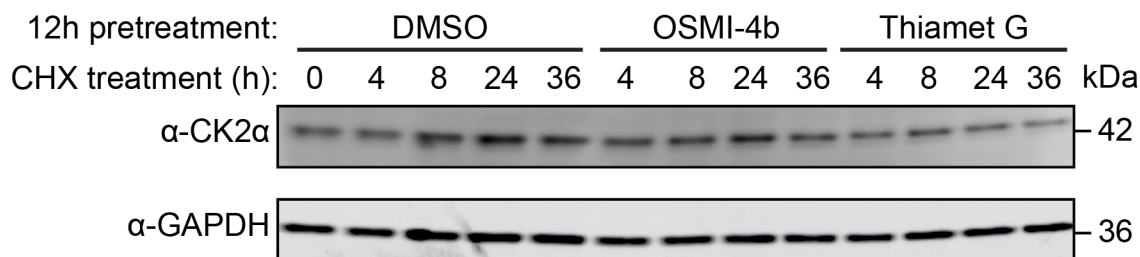
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Supplementary Figures



Supplementary Figure 1 – Endogenous CK2 α Levels Following OGT or OGA Inhibition

HEK293T cells in 6-well plates were treated with 50 μ M OSMI-4b, 100 μ M Thiamet G, or an equivalent volume of DMSO for 12 h before addition of 1X cycloheximide. Cell lysates were then collected at the notated timepoints and were separated by SDS-PAGE. CK2 α and GAPDH protein levels were measured by Western blot, and CK2 α intensity was normalized to the corresponding GAPDH intensity in ImageJ for quantification. Error bars are representative of three independent replicates. A two-tailed Student's *t*-test was used for statistical analysis.

Nanobody-OGT conditions:
(in triplicate)



Nanobody-splitOGA conditions:
(in triplicate)



Lysate preparation

Reduction/alkylation
Trypsinization
Desalting



MS sample preparation

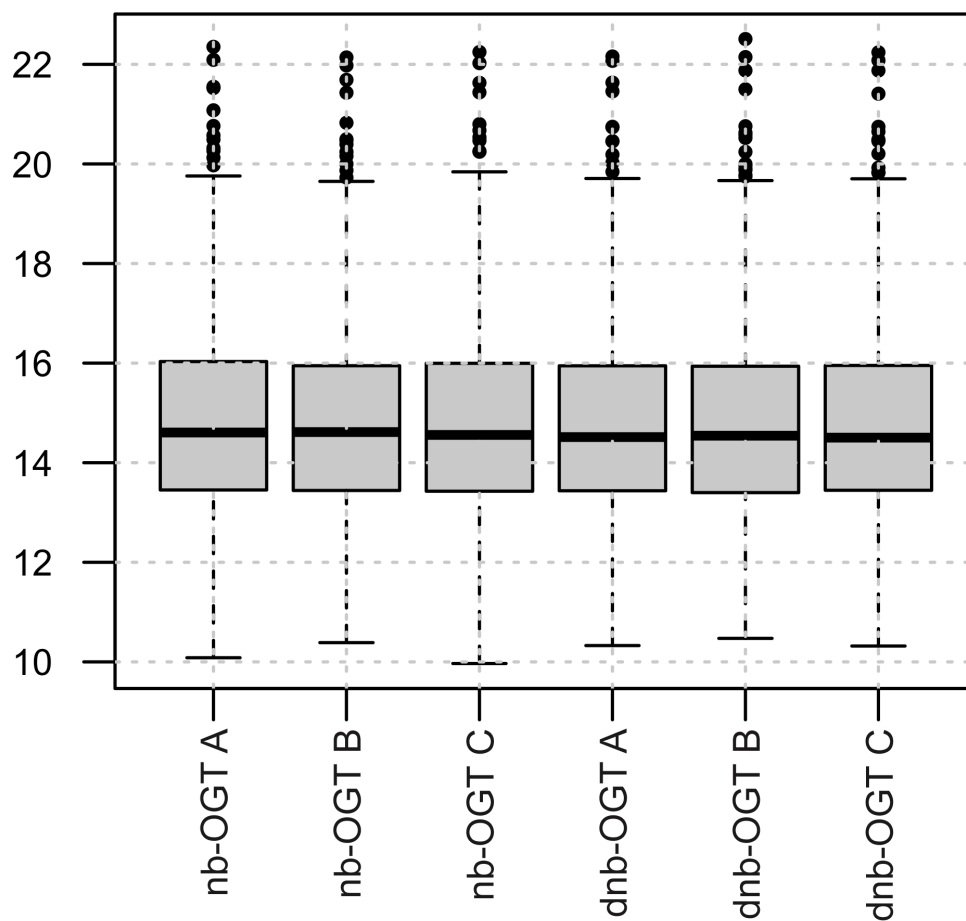
TiO₂ phosphopeptide enrichment
TMT labeling
High pH fractionation



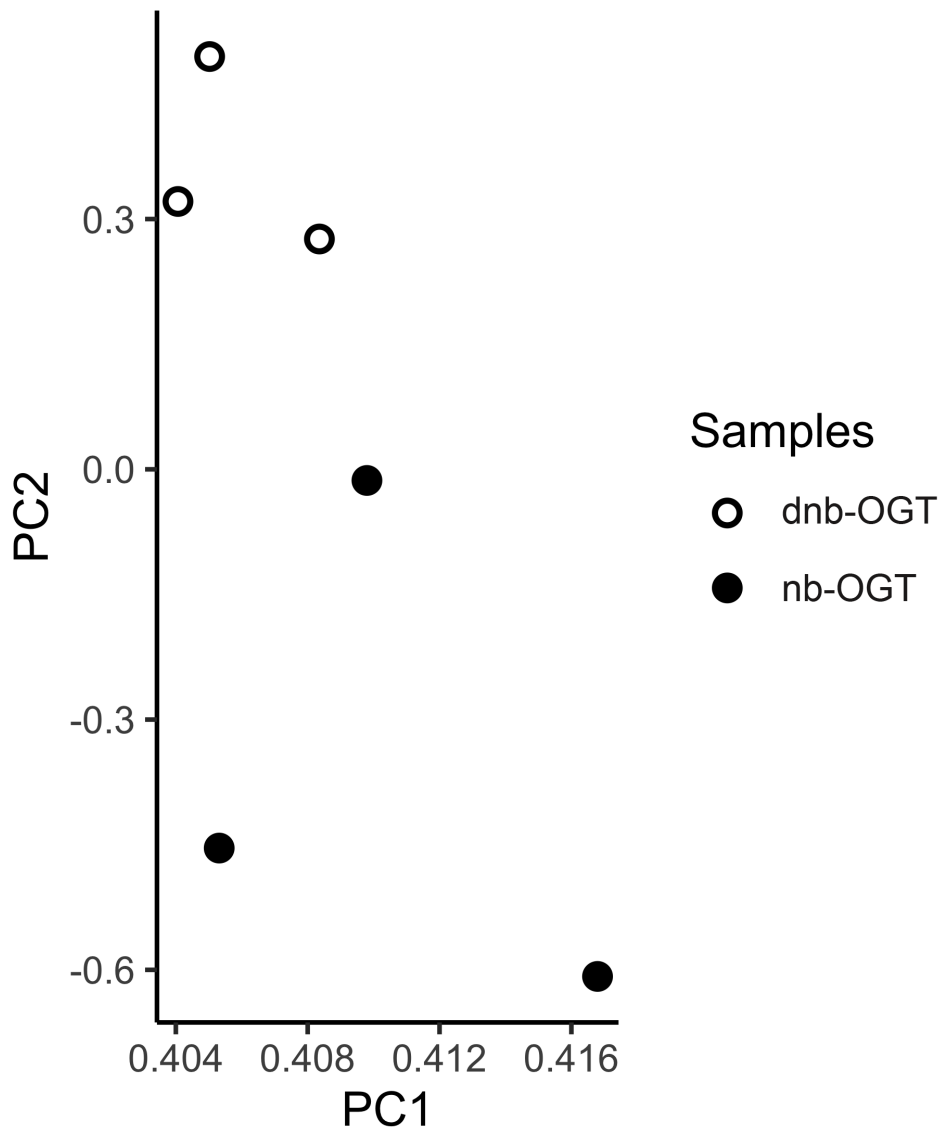
LC-MS/MS & Data Analysis

Supplementary Figure 2 – Phosphoproteomics Workflow

Cells were lysed by adding 1 mL of lysis buffer containing phosphatase inhibitors. Protein concentrations were determined by BCA assay. Reduction and alkylation were performed as previously described. S-trap digestion was done according to the manufacturer's instructions resulting in 0.8 mg tryptic peptides/sample. Samples were desalted and dried. The enrichment of phosphopeptides was performed by TiO₂ Phosphopeptide Enrichment Kit. Briefly, approximately 0.8 mg of tryptic peptides were resuspended in 150 μL of Binding/Equilibration buffer. The suspended peptide sample was added to a pre-equilibrated TiO₂ spin tip and centrifuged at 1000 × g for 5 minutes. The spin tip was then washed with 20 μL of Binding/Equilibration/Wash Buffer for three times, and eluted by 50 μL of Elution buffer for two times. The eluates were evaporated to near dryness and subjected to the TMT-labeling. For each sample, 10 μL the corresponding amine-based TMT 10-plex reagents (10 μg/μL) was added and reacted for 1 h at room temperature. The reactions were quenched with 2 μL 5% hydroxylamine solution and combined. The combined mixture was concentrated to dryness. High-pH fractionation (ThermoFisher Scientific) was done according to the manufacturer's instructions resulting in 6 fractions.

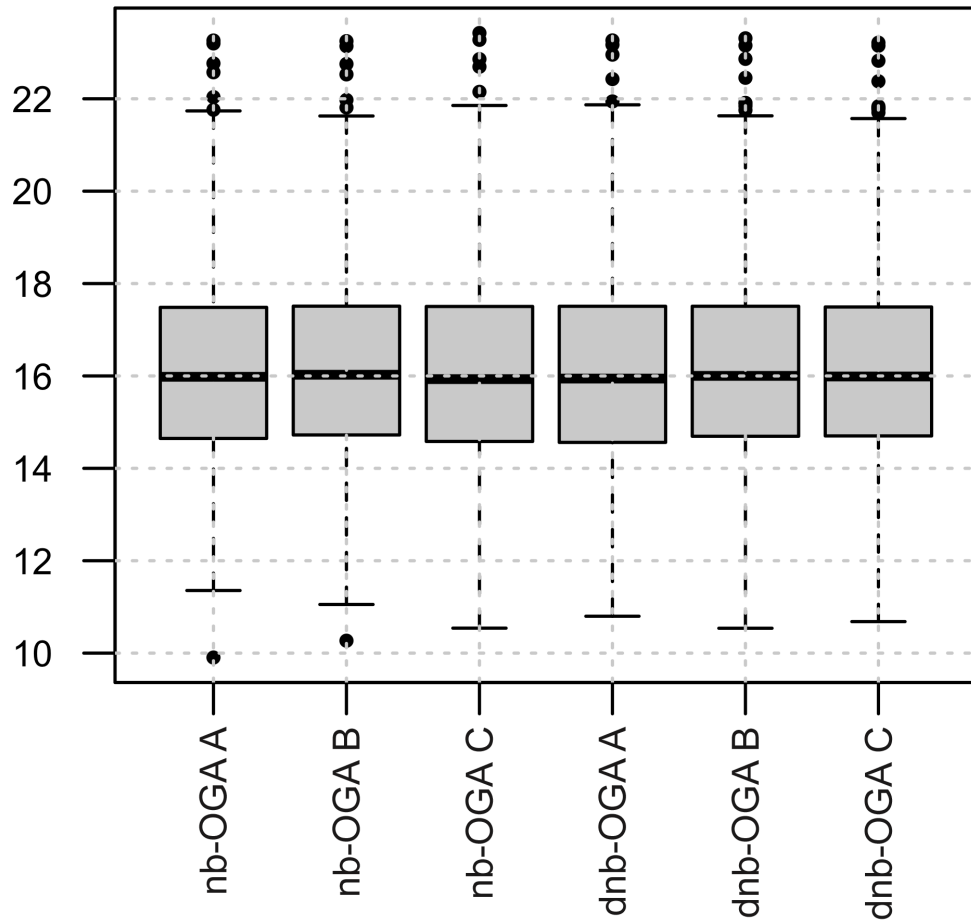


Supplementary Figure 3 – Nanobody-OGT Phosphoprotein Level Normalization Box Plot
 Normalization of TMT channels during protein-level analysis of nanobody-OGT phosphoproteomics experiment. Each channel represents GFP-FLAG-CK2 α -EPEA co-transfected with active or inactive nanobody-OGT. nb-OGT = active nanobody-OGT, dnb-OGT = inactive nanobody-OGT.



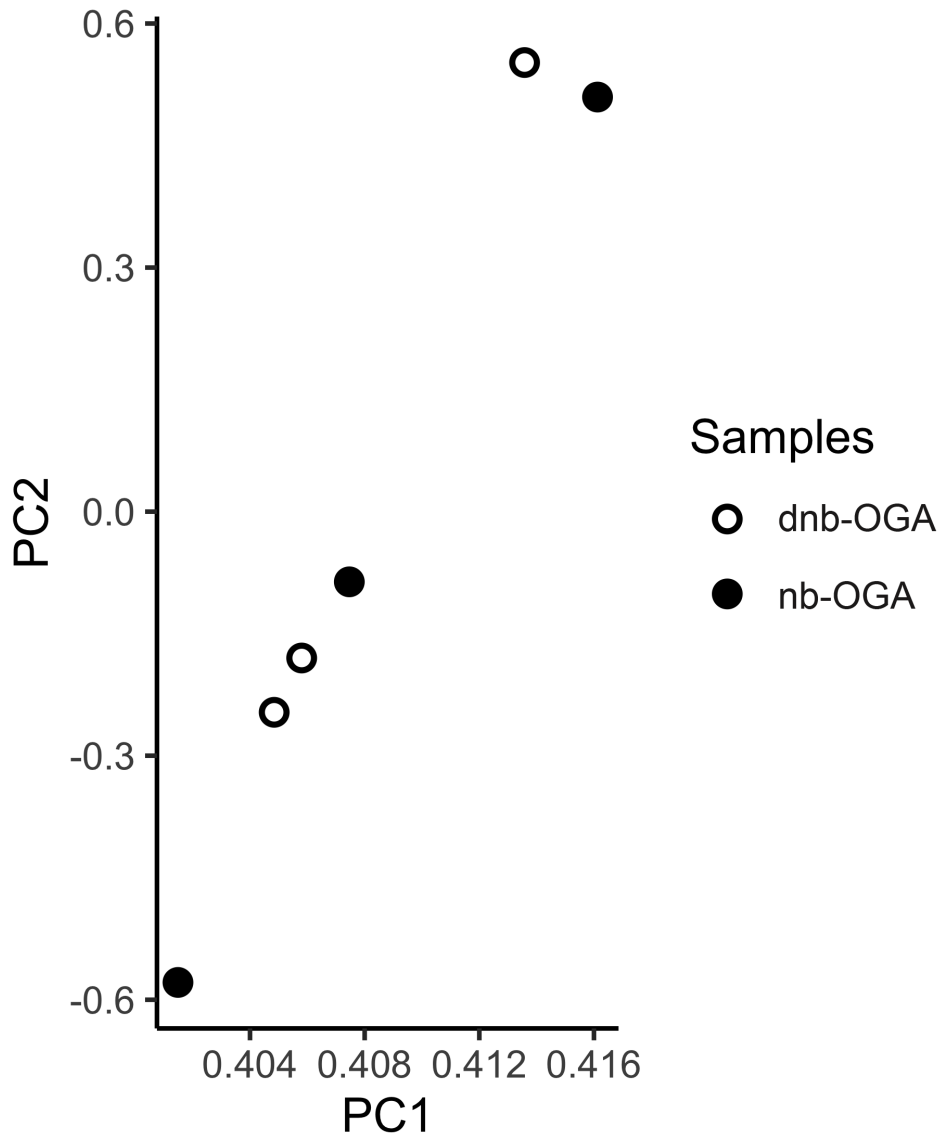
Supplementary Figure 4 – Nanobody-OGT Phosphoprotein Level Principal Component Analysis

Principal component analysis of nanobody-OGT protein level data indicating independent clustering between the control (inactive nanobody-OGT co-transfection) and treatment (active nanobody-OGT co-transfection). ■ nb-OGT = active nanobody-OGT, ○ dnb-OGT = inactive nanobody-OGT.



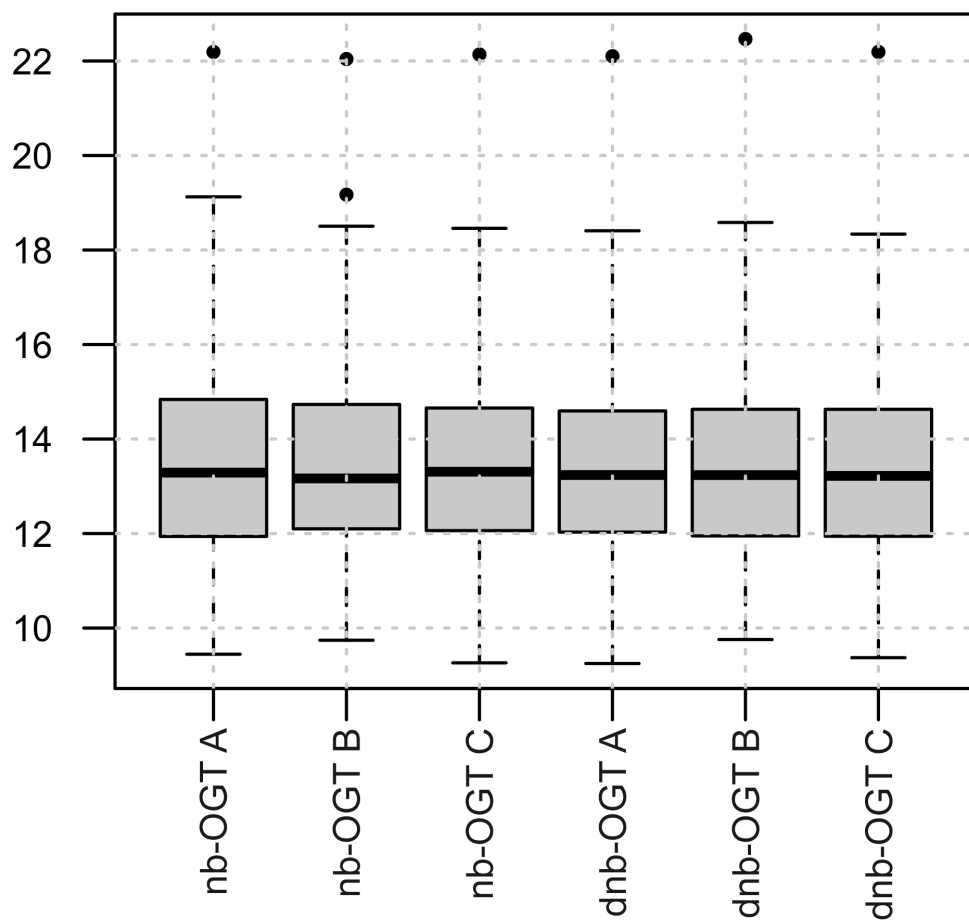
Supplementary Figure 5 – Nanobody-splitOGA Phosphoprotein Level Normalization Box Plot

Normalization of TMT channels during protein-level analysis of nanobody-splitOGA phosphoproteomics experiment. Each channel represents GFP-FLAG-CK2 α -EPEA co-transfected with active or inactive nanobody-splitOGA. nb-OGA = active nanobody-splitOGA, dnb-OGA = inactive nanobody-splitOGA.



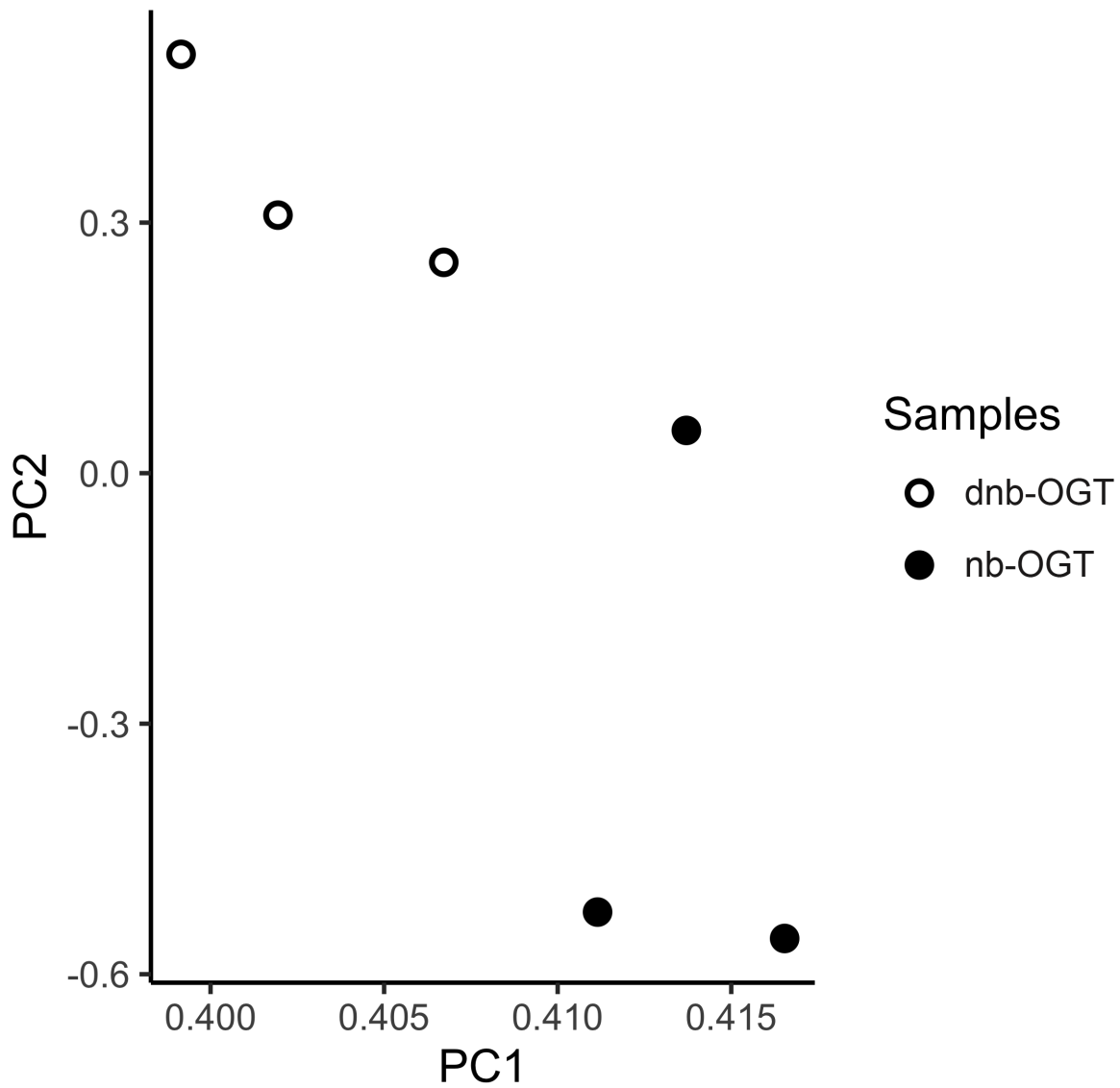
Supplementary Figure 6 – Nanobody-OGA Phosphoprotein Level Principal Component Analysis

Principal component analysis of nanobody-splitOGA protein level data indicating clustering between the control (inactive nanobody-splitOGA co-transfection) and treatment (active nanobody-splitOGA co-transfection). nb-OGA = active nanobody-splitOGA, dnb-OGA = inactive nanobody-splitOGA.



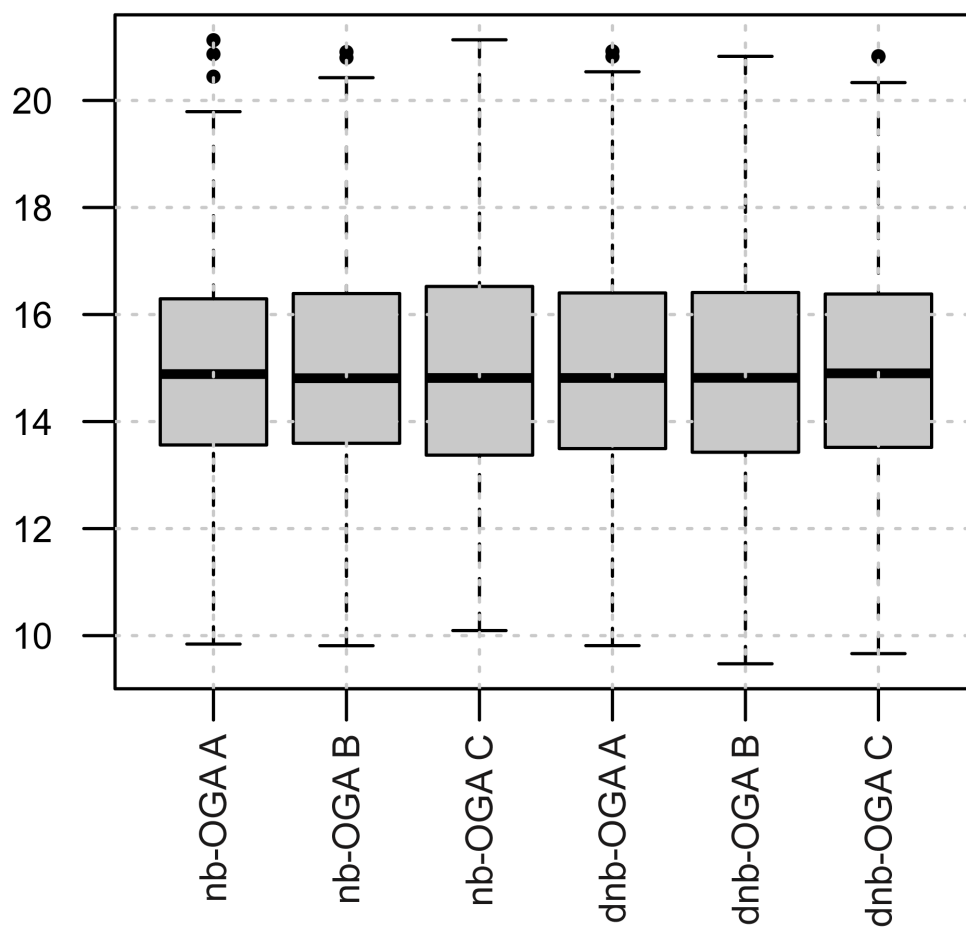
Supplementary Figure 7 – Nanobody-OGT Phosphosite Level Normalization Box Plot

Normalization of TMT channels during site-level analysis of nanobody-OGT phosphoproteomics experiment. Each channel represents GFP-FLAG-CK2 α -EPEA co-transfected with active or inactive nanobody-OGT. nb-OGT = active nanobody-OGT, dnb-OGT = inactive nanobody-OGT.

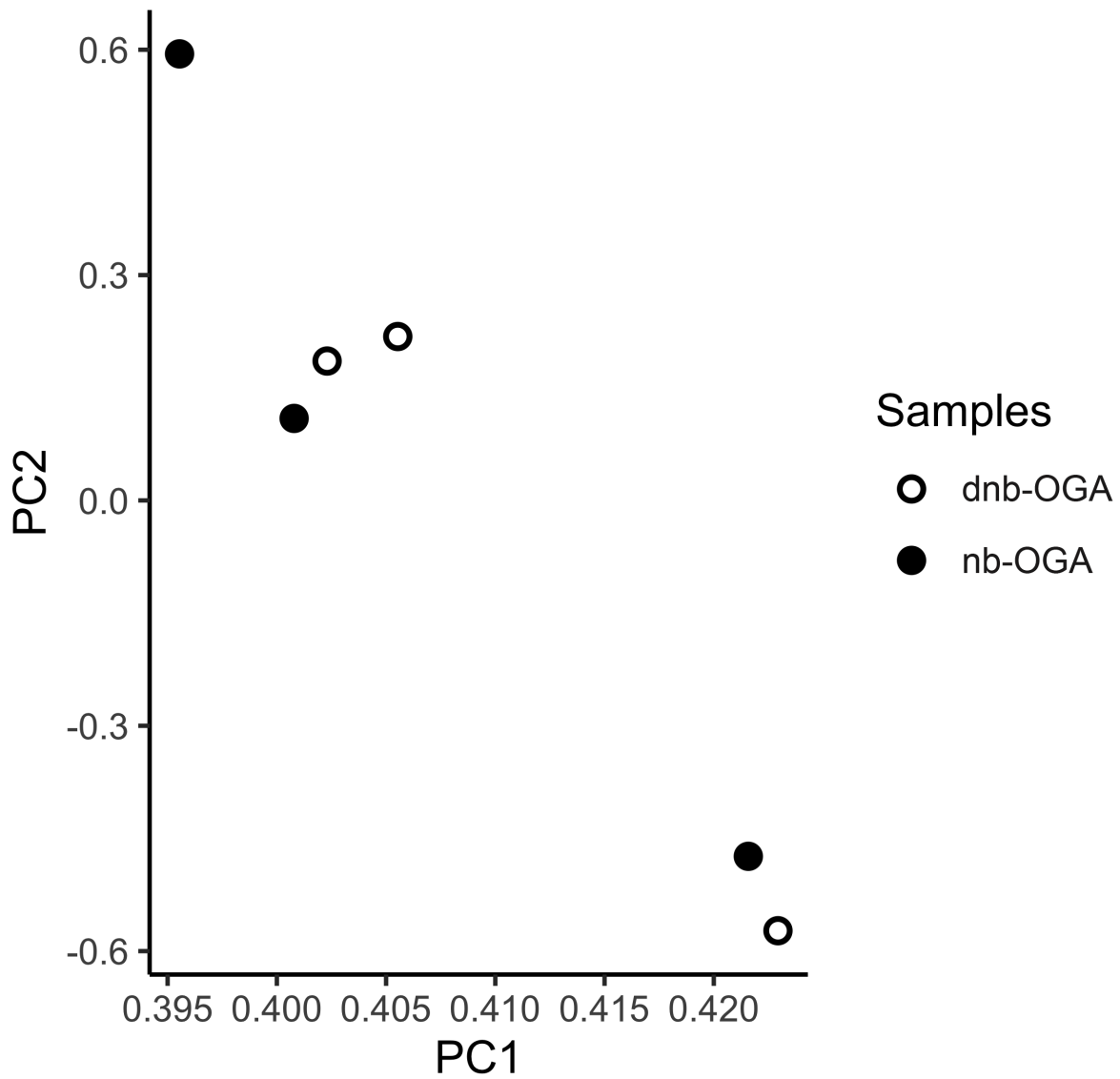


Supplementary Figure 8 – Nanobody-OGT Phosphosite Level Principal Component Analysis

Principal component analysis of nanobody-OGT phosphosite level data indicating independent clustering between the control (inactive nanobody-OGT co-transfection) and treatment (active nanobody-OGT co-transfection). nb-OGT = active nanobody-OGT, dnb-OGT = inactive nanobody-OGT.

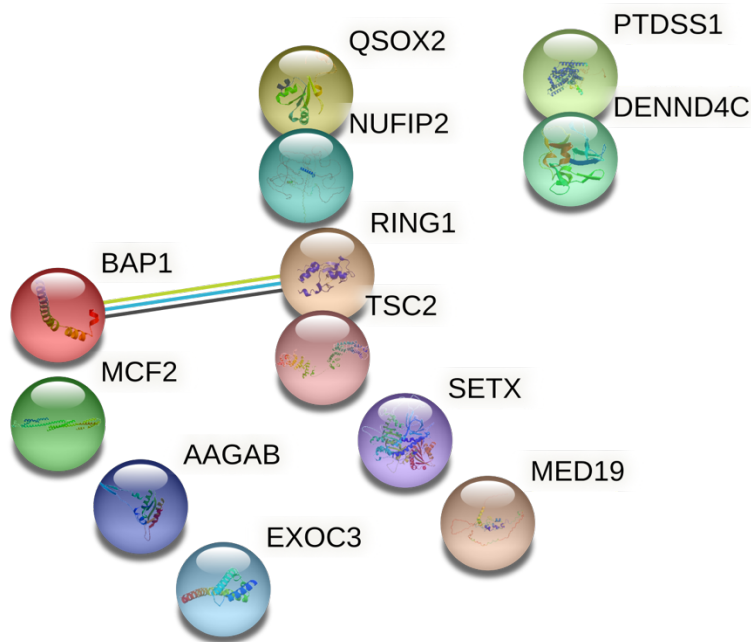


Supplementary Figure 9 – Nanobody-splitOGA Phosphosite Level Normalization Box Plot
 Normalization of TMT channels during site-level analysis of nanobody-splitOGA phosphoproteomics experiment. Each channel represents GFP-FLAG-CK2 α -EPEA co-transfected with active or inactive nanobody-splitOGA. nb-OGA = active nanobody-splitOGA, dnb-OGA = inactive nanobody-splitOGA.



Supplementary Figure 10 – Nanobody-splitOGA Phosphosite Level Principal Component Analysis

Principal component analysis of nanobody-splitOGA phosphosite level data indicating clustering between the control (inactive nanobody-splitOGA co-transfection) and treatment (active nanobody-splitOGA co-transfection). nb-OGA = active nanobody-splitOGA, dnb-OGA = inactive nanobody-splitOGA.



Supplementary Figure 11 – STRING network of phosphoproteins negatively correlated with CK2 α glycosylation

Phosphoproteins that significantly increased in enrichment in the nanobody-splitOGA condition or decreased in the nanobody-OGT condition were compiled and imported into STRING for interactome analysis. Only BAP1 and RING1 are functionally associated (with ubiquitination of histones).

Supplementary Tables

Supplementary Table 1 – MS Data (protein level hits)

Gene Name	PDB ID	Log ₂ (FC) OGT	P value OGT	Log ₂ (FC) OGA	P value OGA	Reported CK2 sub.?
MARCKS	P29966	1.36750281	3.8657E-07	-0.009162	0.89814825	No
ILF3	Q12906	1.19323052	0.01876606	-0.0733866	0.30002011	No
HDAC1	Q13547	1.08712459	0.00351943	-0.0990389	0.43198513	Yes ³⁰
HMGB2	P26583	1.06279556	2.0455E-05	N/A	N/A	Yes ¹⁷
HDAC2	Q92769	1.05118692	0.00777629	-0.0332643	0.6414608	Yes ³⁰
UGP2	Q16851	1.05007777	0.00600021	N/A	N/A	No
EPB41L2	O43491	1.02714016	5.6632E-06	-0.0670828	0.32753689	No
RPL27A	P46776	0.99070725	3.5807E-05	N/A	N/A	No
RPL34	P49207	0.95956758	4.3797E-05	-0.5646497	0.00012171	No
TPD52L2	O43399	0.93300518	0.00016678	0.42744399	0.00058086	No
HCFC1	P51610	0.91898783	0.00905294	-0.0628119	0.53619673	No
RPS3A	P61247	0.90641691	0.00299694	N/A	N/A	No
DDX42	Q86XP3	0.8890198	0.01237976	N/A	N/A	No
ENSA	O43768	0.87119279	9.9768E-05	-0.6537205	6.5355E-05	No
MYLK3	Q32MK0	0.83529321	0.01151515	N/A	N/A	No
MED19	A0JLT2	-0.7632699	0.00107056	0.12685194	0.53994595	No
AKT1S1	Q96B36	0.7265354	0.00116863	-0.0565795	0.60974879	No
UTP14A	Q9BVJ6	0.70578475	0.01287049	N/A	N/A	No
IQSEC1	Q6DN90	0.68529865	0.00801551	-0.1387021	0.11543139	No
NFIC	P08651	0.6787496	0.00091429	N/A	N/A	No
TBC1D4	O60343	0.67332465	0.00297638	-0.0780943	0.48076431	No
MDH1	P40925	0.63299339	0.01058569	-0.0878243	0.18815606	No
PTDSS1	P48651	-0.6310514	0.00060673	0.03246701	0.85838788	No
QSOX2	Q6ZRP7	-0.6246512	0.00211926	N/A	N/A	No
BAP1	Q92560	-0.6224754	0.01120594	N/A	N/A	No
SMARCAD1	Q9H4L7	0.60898284	0.00020408	-0.6594163	0.00011993	No
KHSRP	Q92945	0.60236907	3.8665E-05	-0.1613364	0.29451128	No
EXOC3	O60645	-0.5971443	0.0486203	N/A	N/A	No
RBM34	P42696	0.59702178	0.00036202	-0.37369	0.00385585	No
DENND4C	Q5VZ89	-0.5898665	0.00721184	-0.0054706	0.93685289	No
SETX	Q7Z333	-0.5887437	0.00054032	N/A	N/A	Yes ³¹
MCF2	P10911	-0.5832619	0.00114332	N/A	N/A	No
DAXX	Q9UER7	0.55998809	0.01803783	-0.0409057	0.53149013	Yes ³⁰
LUC7L3	O95232	0.54222196	0.00549679	N/A	N/A	No
RING1	Q06587	-0.5355431	0.01986045	-0.1315409	0.81440089	No
PFKP	Q01813	0.53464855	0.00202774	-0.6693487	3.1213E-05	No
NCBP3	Q53F19	0.53253321	0.04912922	0.08806703	0.25442596	No
AAGAB	Q6PD74	-0.5202489	0.00104418	N/A	N/A	No
RIOX2	Q8IUF8	0.51598347	0.00231079	N/A	N/A	No
ADAM22	Q9P0K1	0.50719107	0.00485886	N/A	N/A	No
RPL37	P61927	0.50432442	0.0053132	N/A	N/A	No
TFAM	Q00059	0.50214963	0.0122408	0.03561791	0.57239368	No

PABPN1	Q86U42	0.02174837	0.8726238	-1.2207843	0.00080145	No
SRSF2	Q01130	-0.1694018	0.07328998	-0.9482095	3.1687E-06	No
NUFIP2	Q7Z417	0.07818113	0.51571786	0.6055824	0.01815937	No
MOV10	Q9HCE1	N/A	N/A	-0.5864239	0.00018435	No
RBBP7	Q16576	N/A	N/A	-0.568389	0.00683292	No
PPP1R12A	O14974	0.31895842	0.03708686	-0.5415793	0.00054957	No
REEP5	Q00765	N/A	N/A	-0.5286674	7.9512E-05	No
RAD23A	P54725	-0.0433452	0.72509578	-0.5268616	0.01010218	No
TSC2	P49815	0.0960385	0.27813694	0.5065094	0.00076195	No

Supplementary Table 2 – MS Data (site level hits)

Gene Name	PDB ID	Phospho-site	Condition	Log ₂ (FC)	P value	Reported CK2 sub.?
MARCKS	P29966	S170	OGT	1.47934	6.6677E-06	No
HMGB2	P26583	T179	OGT	1.01355	0.00013414	Yes ¹⁷
HDAC1	Q13547	S343	OGT	1.06832832	0.00567005	Yes ³²
HDAC2	Q92769	S394	OGT	1.04528135	0.00861108	Yes ³²
UGP2	Q16851	Y248	OGT	1.02774156	0.00953618	No
CWC25	Q9NxE8	S218	OGT	1.04628939	0.0209202	No
ILF3	Q12906	S382	OGT	1.36850275	0.0200335	No
EPB41L2	O43491	S550	OGT	1.02099418	0.01224519	No
MDC1	Q14676	T404	OGT	2.21424469	0.03398253	Yes ⁴⁸
MDC1	Q14676	S329	OGT	-1.1436185	0.01538304	Yes ⁴⁸
NUFIP2	Q7Z417	T571/S572	OGA	1.02597445	0.00252018	No
PABPN1	Q86U42	S95	OGA	-1.1619051	0.00081583	No