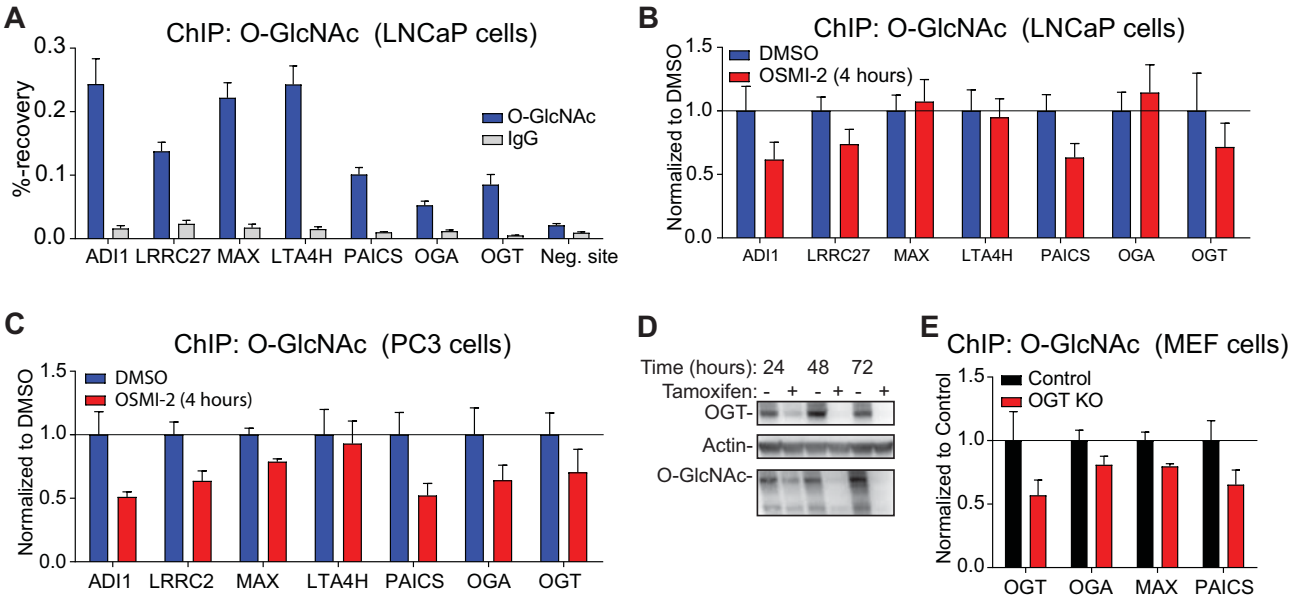
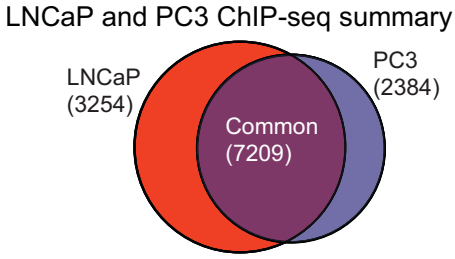
SUPPLEMENTARY TABLE LEGEND, FIGURES AND FIGURE LEGENDS

Supplementary table. Sheet 1) Primers used in this study. **Sheet 2)** Summary of read counts for ChIP-seq performed in this study. **Sheet 3)** Annotated high confidence O-GlcNAc sites on the chromatin in LNCaP cells. **Sheet 4)** Mass spectrometry data of O-GlcNAc RIME, oligo-pulldown and MYC RIME (MYC RIME is retrieved from Barfeld *et al.* [1]). **Sheet 5)** Reverse-phase protein array (RPPA) data. RPPA profiling of cells treated as indicated for 24 hours. Each condition had four biological replicates. OSMI-2 was used at 40μM dose. Control sample was set to value of 1 and other samples were normalized to this. Statistical analysis was performed using the t-test. Antibodies showing statistically significant difference between a comparison are highlighted in red ($p<0.05$). **Sheet 6)** Differentially expressed genes based on RNA-seq.



Supplementary figure 1. Validation of the O-GlcNAc ChIP-seq data using qPCR. A) Chromatin immunoprecipitation (ChIP) was performed from LNCaP cells, and isolated chromatin was used for qPCR using primers against promoter sequences of indicated genes. Primers were designed based on our ChIP-seq data and Primer3 tool. Data is represented as %-recovery. Data shown is an average of at least 3 biological replicates with SEM. **B)** LNCaP cells were treated as indicated for 4 hours with 40µM OSMI-2 and used for ChIP with O-GlcNAc-specific antibodies. Control sample was set to 1, and OSMI-2-treated sample was normalized to this. Data shown is an average of at least 3 biological replicates, and SEM is shown. **C)** PC3 cells were treated as indicated for 4 hours with 40µM OSMI-2 and used for ChIP with O-GlcNAc-specific antibody. Control sample was set to 1 and OSMI-2-treated sample was normalized to this. Data shown is an average of 3 biological replicates with SEM. **D)** Determination of time-point that shows loss of OGT with minimal toxicity in mouse embryonic fibroblast (MEF) cell line, which has been genetically engineered to enable Tamoxifen (Tam)-inducible genetic excision of OGT [2]. MEF cells were treated with 0.5µM Tam for the indicated time and analyzed using western blot. **E)** MEF cells were treated as indicated for 48 hours and used for ChIP with O-GlcNAc-specific antibody. Control sample was set to 1 and Tam-treated sample was normalized to this. Data shown is an average of 3 biological replicates with SEM.

A



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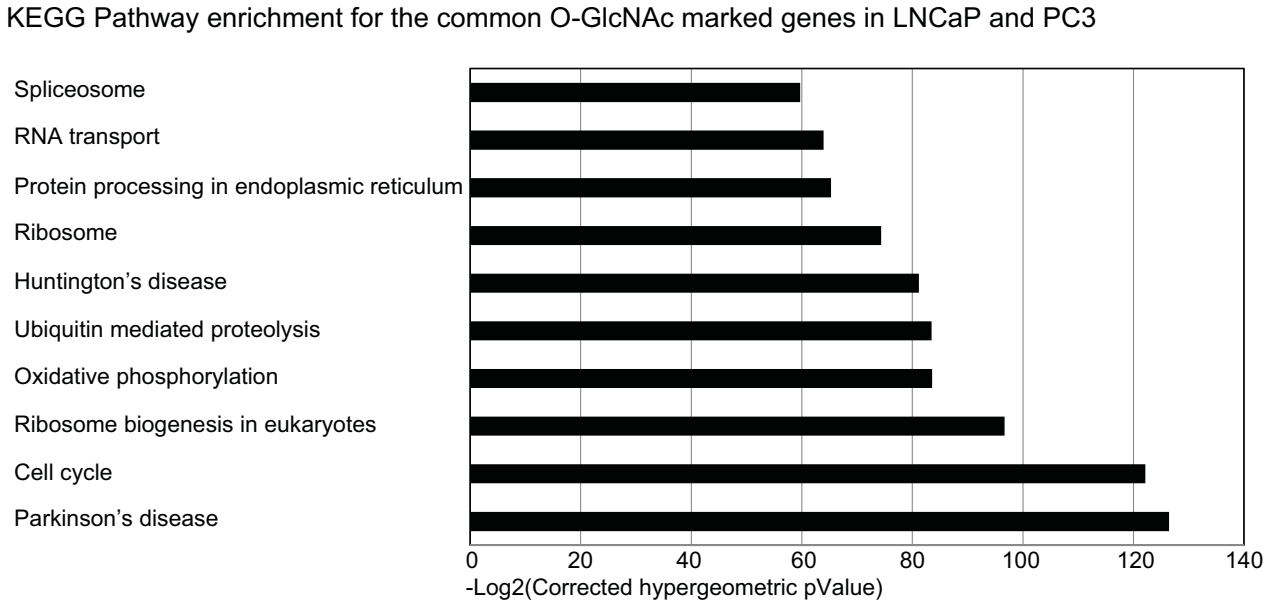
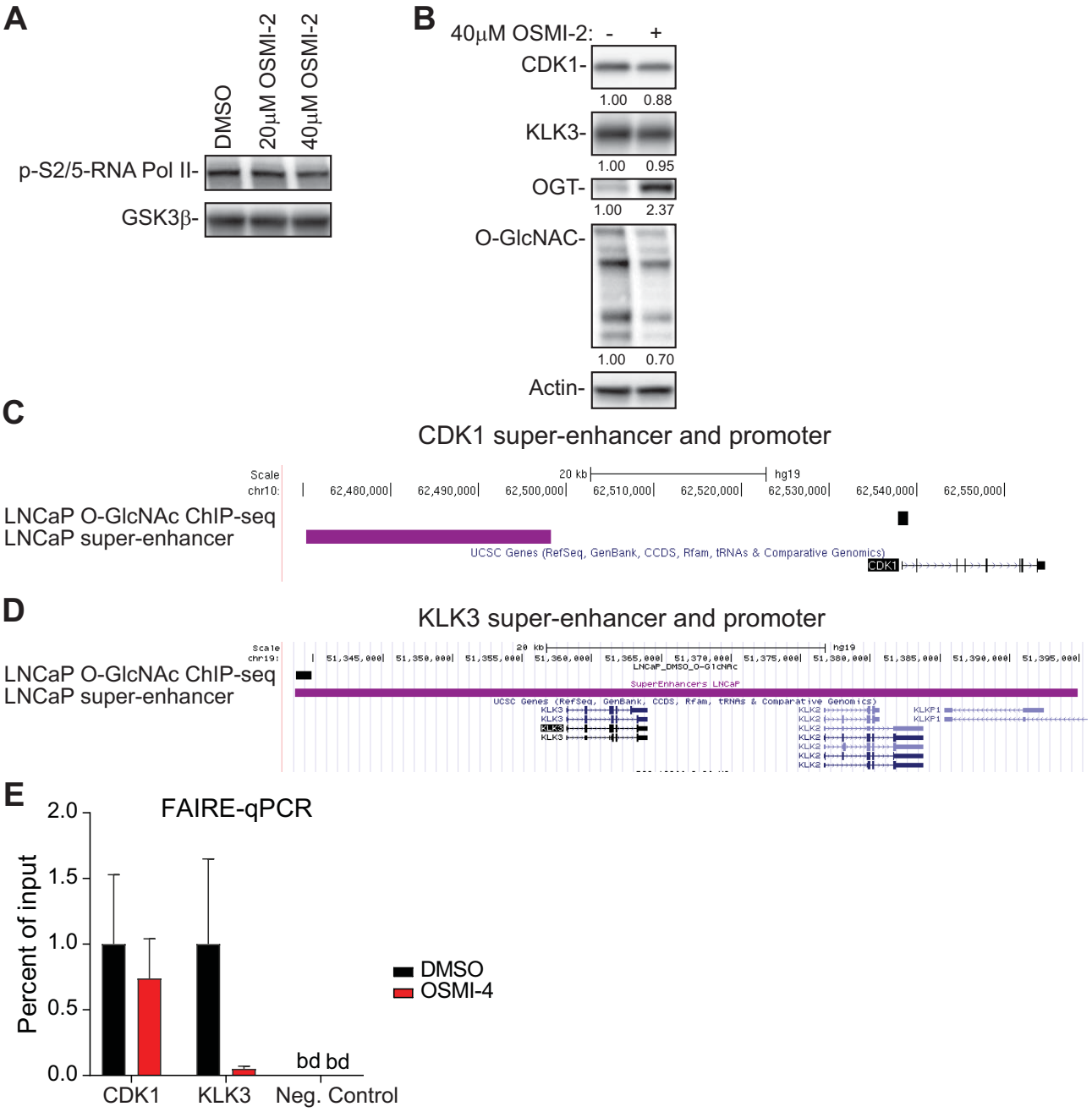
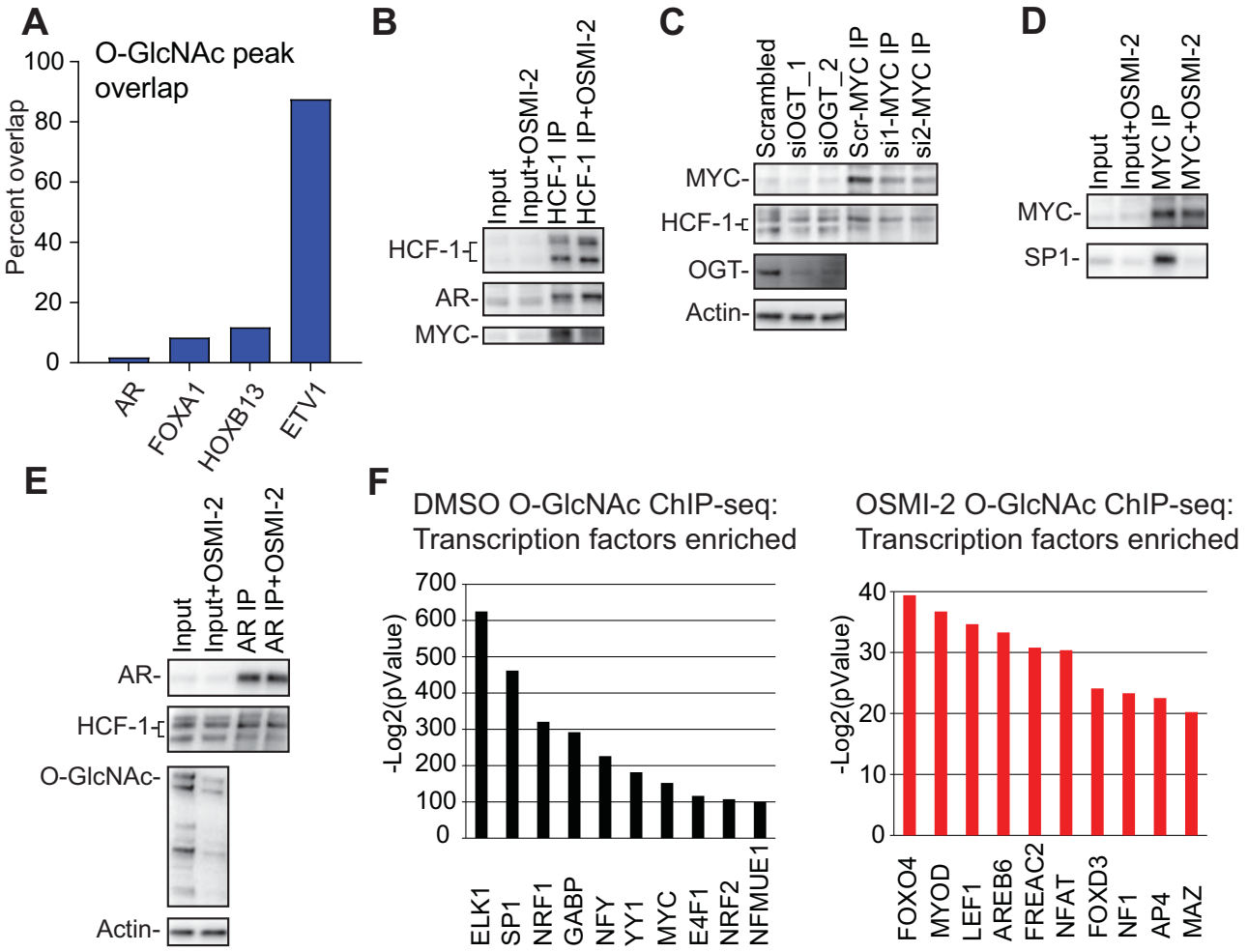


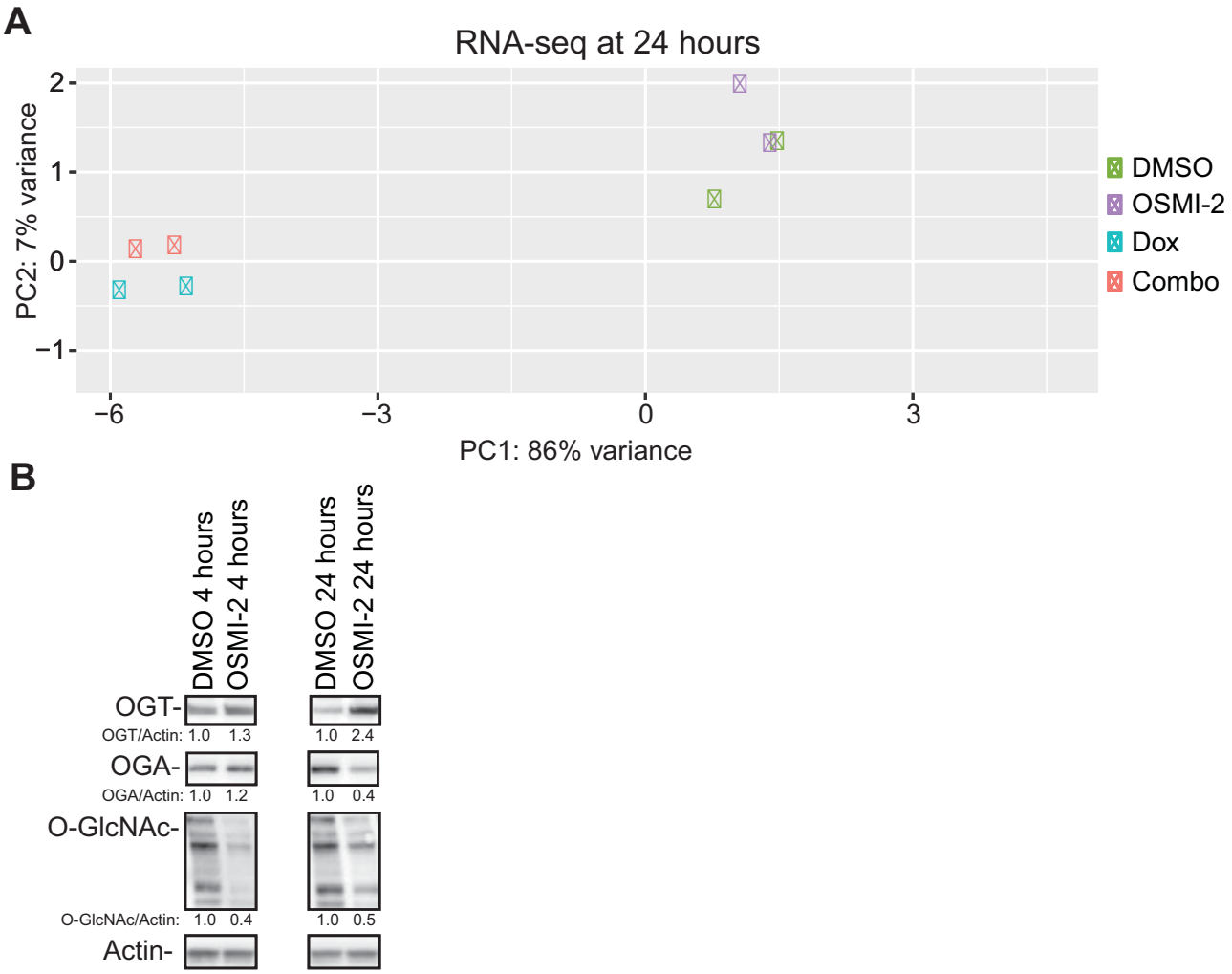
Supplementary figure 2. O-GlcNAc ChIP-seq in LNCaP and PC3 cells. A) Overlap of LNCaP and PC3 O-GlcNAc ChIP-seq data. The data shown represents overlap of three biological replicate samples for both cell lines. **B)** The genes that have a nearby chromatin O-GlcNAc mark in both LNCaP and PC3 cells were subjected to Kyoto Encyclopedia of Genes and Genome (KEGG)-pathway analysis using the Genecodis tool [3]. The top ten pathways based on the -Log2(Corrected hypergeometric pValue) are shown.



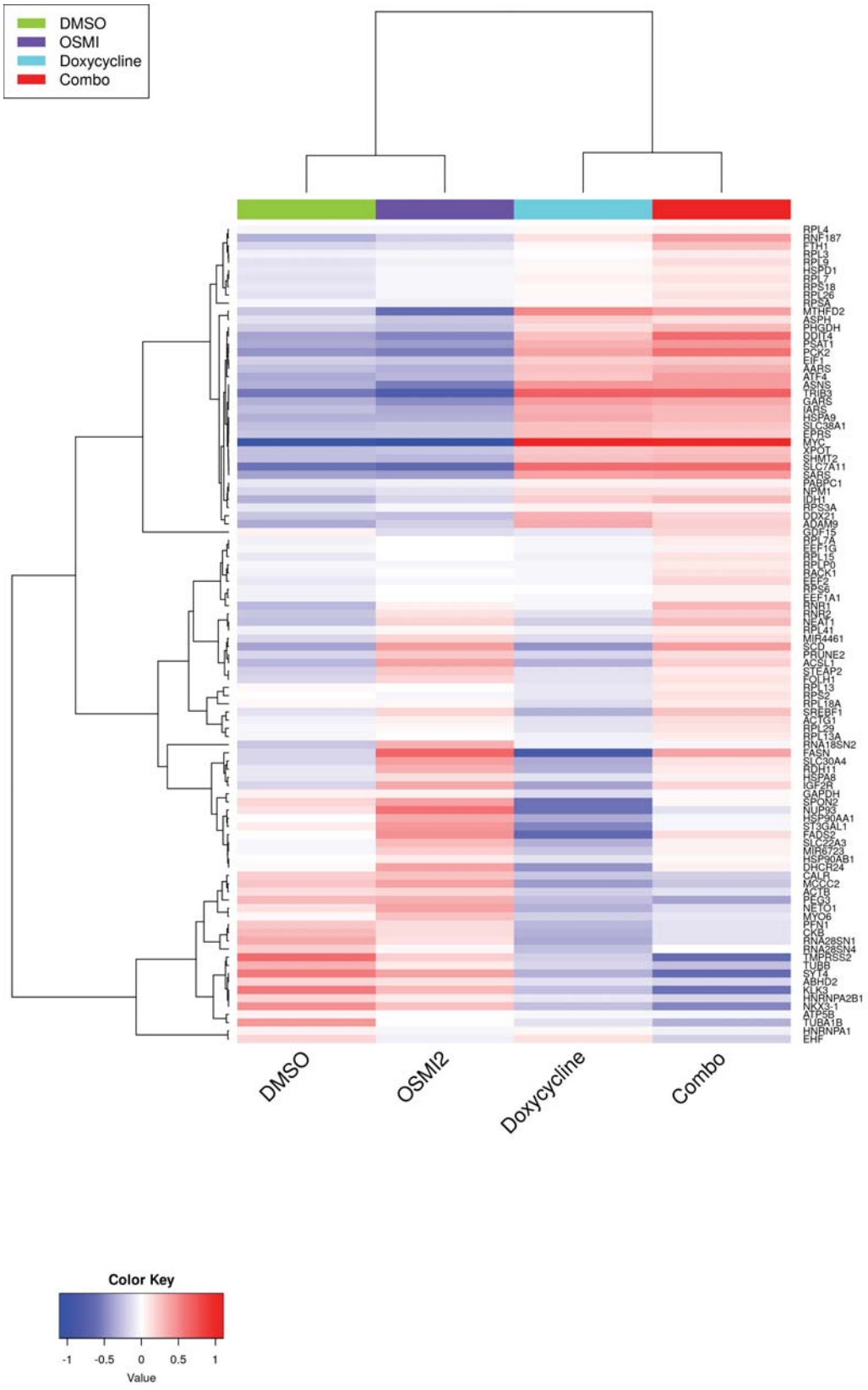
Supplementary figure 3. O-GlcNAc ChIP-seq on genes associated with super-enhancers in LNCaP cells. A, B) LNCaP cells were treated as indicated for 4 hours (**A**) or 24 hours (**B**), protein lysates isolated and western blot was performed against antibodies of interest. Densitometry was used to determine the abundance of the indicated proteins. **C, D)** University of California, Santa Cruz (UCSC) Genome Browser view of the selected chromatin regions showing LNCaP O-GlcNAc ChIP-seq (reported in this study) and LNCaP super-enhancer (reported previously [4]). **E)** The effect of 20μM OSMI-4 on chromatin compaction in CDK1 and KLK3 super-enhancers and a control region reported previously [5]. Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE)-qPCR was used to evaluate chromatin compaction. The data shown is an average of two biological replicates with SEM (bd= below detection).



Supplementary figure 4. Identification of transcription factors regulated by OGT. A) O-GlcNAc ChIP-seq consensus overlap with AR, FOXA1, HOXB13 and ETV-1 ChIP-seq data. Data for these transcription factors was downloaded and reanalyzed (accession numbers: GSE28126, GSE30624, GSM1716764 and GSM1145322, respectively). **B)** OSMI-2 treatment disrupts the interaction between HCF-1 and MYC but does not affect the interaction between HCF-1 and AR. LNCaP cells were kept in androgen-deprived media for 3 days, treated with 40μM OSMI-2 for 4 hours and used for immunoprecipitation (IP). **C)** Knockdown of OGT decreases interaction between MYC and HCF-1. Knockdown of OGT was performed in LNCaP cells for 3 days and cell lysates were used for MYC-immunoprecipitation. Samples were analyzed using western blot. **D)** OSMI-2 treatment disrupts the interaction between MYC and SP1. LNCaP cells were treated with 40μM OSMI-2 for 4 hours and used for immunprecipitation. Samples were analyzed using western blot. **E)** OSMI-2 treatment does not affect the interaction between AR and HCF-1. LNCaP cells were treated with 40μM OSMI-2 for 4 hours and used for immunprecipitation. Samples were analyzed using western blot. **F)** Transcription factors that potentially regulate O-GlcNAc marked genes. Genes associated with O-GlcNAc peaks specifically in DMSO-treated (left) and specifically in 40μM OSMI-2 treated (right) PC3 cells was identified using Genecodis [6]. The top ten transcription factors are shown based on the -Log2(Corrected hypergeometric pValue).



Supplementary figure 5. Overexpression of MYC (Dox) has the predominant effect on mRNA levels based on RNA-seq. A) RNA-seq 24 hours after MYC overexpression (Doxycycline, Dox) and 40μM OSMI-2 treatment. Principal component analysis of the RNA-seq data. **B)** Treatment with 40μM OGT inhibitor OSMI-2 decreases total-O-GlcNAc levels and leads to increased expression of OGT and downregulation of OGA as determined using western blot. Densitometry was used to determine the abundance of the indicated proteins.



Supplementary figure 6. Heatmap presentation of the 100 most affected genes by MYC overexpression and OGT inhibition. Initially, all genes were ranked by standard deviation across all samples then the top 100 genes are used in hierarchical clustering. The data is centered by subtracting the average expression level for each gene. The average linkage was used with the distance matrix is 1-r, where r is Pearson's correlation coefficient.

A

Reactome pathway enrichment for mRNAs downregulated by OSMI-2+Dox combination

Pathway name	Entities found	Entities total	p-value	FDR
Cell Cycle	28	682	5.55E-16	1.5E-13
Cell Cycle Checkpoints	17	279	1.65E-12	2.23E-10
Mitotic G1-G1/S phases	14	173	4.57E-12	3.08E-10
Cell Cycle, Mitotic	22	570	4.6E-12	3.08E-10
G1/S Transition	13	150	1.22E-11	6.57E-10
G2/M Checkpoints	13	154	1.68E-11	7.56E-10
Activation of ATR in response to replication stress	8	39	1.76E-10	6.68E-9
S Phase	12	179	1.41E-9	4.55E-8
DNA Replication	11	141	1.52E-9	4.55E-8
Activation of E2F1 target genes at G1/S	7	43	1.23E-8	2.94E-7

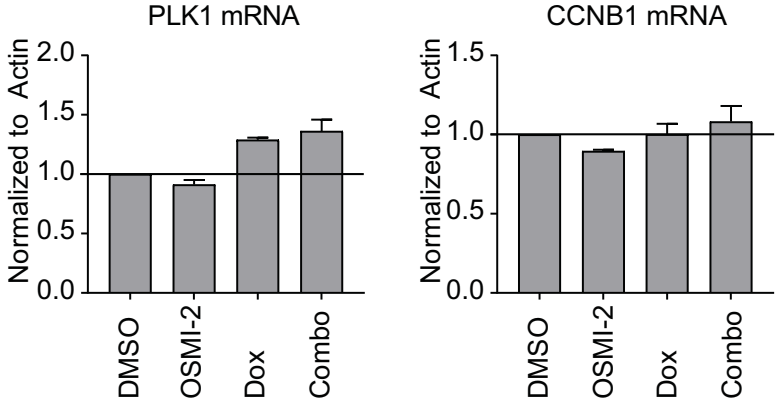
B

Reactome pathway enrichment for proteins affected by MYC-overexpression and antagonized by OSMI-2

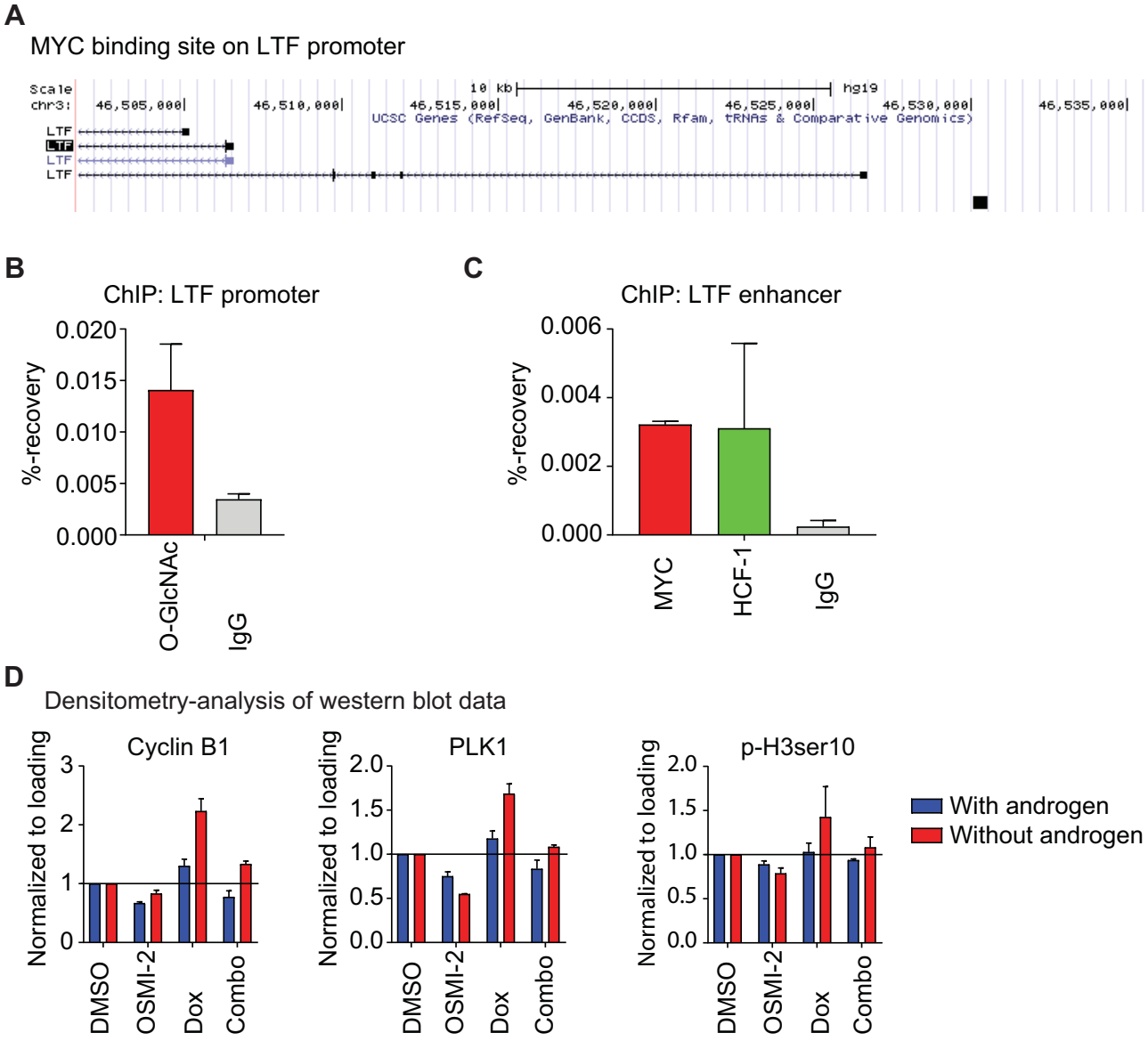
Pathway name	Entities found	Entities total	p-value	FDR
Polo-like kinase mediated events	6	23	0,002	3.93E-9
Cyclin A/B1/B2 associated events during G2/M transition	6	32	0,002	1.4E-8
Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex	4	15	0,001	3.28E-6
G2/M Transition	7	212	0,015	2.1E-5
Mitotic G2-G2/M phases	7	214	0,015	2.1E-5
G2/M DNA replication checkpoint	3	7	0,001	2.1E-5
G2/M DNA damage checkpoint	5	81	0,006	3.52E-5
G2/M Checkpoints	6	154	0,011	3.52E-5
Cell Cycle	10	682	0,049	5,00E-05
Cell Cycle Checkpoints	7	279	0,02	6.13E-5

C

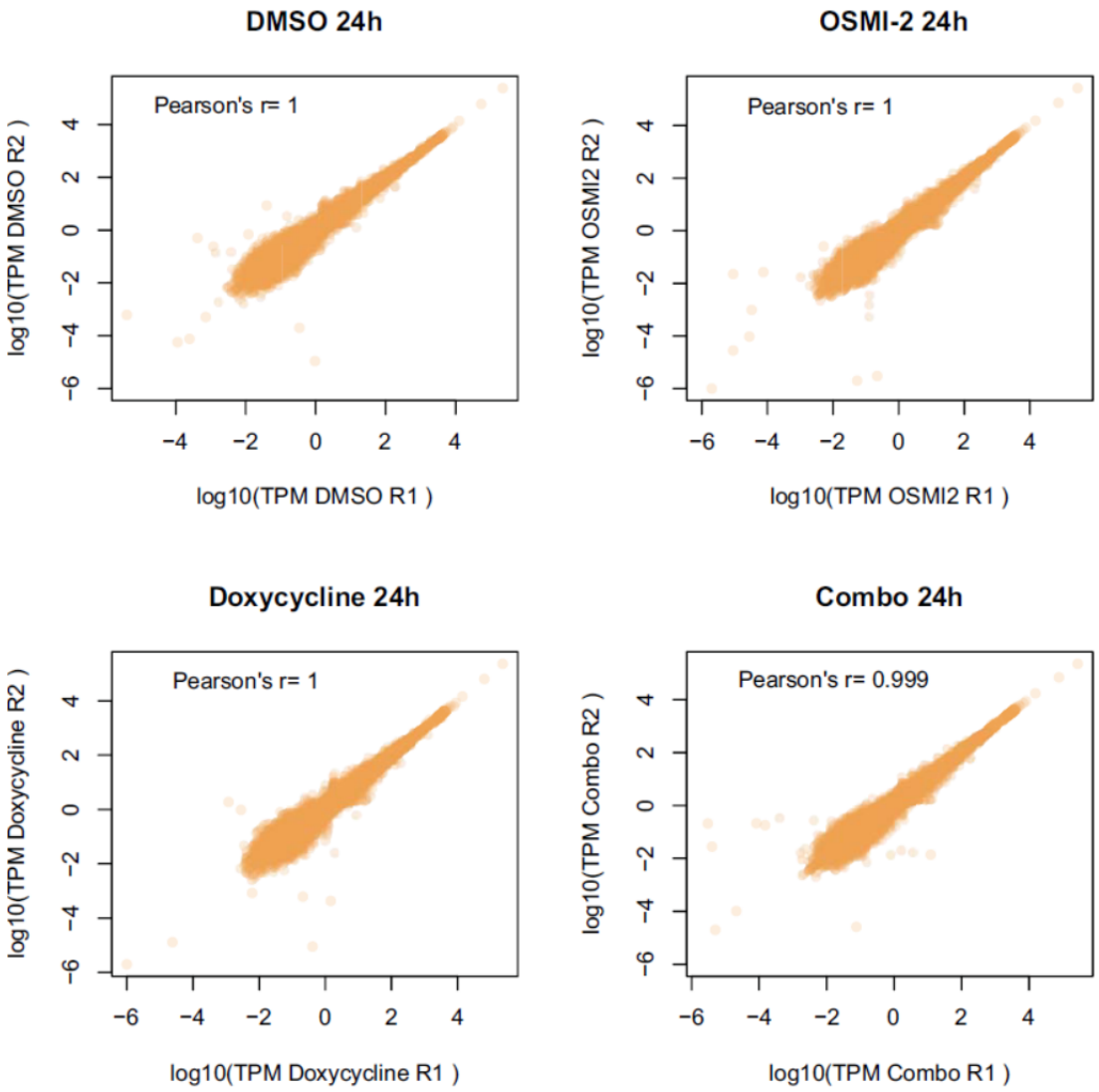
mRNA profiling of selected transcripts



Supplementary figure 7. Characterization of the RNA-seq and RPPA data. A) Processes that are enriched for the genes that were specifically downregulated by OSMI-2+Dox treatment based on RNA-seq were identified using Reactome database [7]. **B)** Processes that are enriched for protein targets of the antibodies that exhibit statistically significant change between Doxycycline and Dox+OSMI-2 treatments were identified using Reactome database [7]. **C)** Cells were treated as indicated (OSMI-2 dose: 40μM), mRNA collected and used for RT-qPCR with indicated primers. Data shown is an average of 2-3 biological replicates with SEM.



Supplementary figure 8. Validation of *LTF* promoter. A) UCSC Genome browser screenshot depicting putative MYC binding site near *LTF* gene based on the data reported by Barfeld *& al.* [1]. **B) and C)** Validation of O-GlcNAc, MYC and HCF-1 binding to the putative MYC-binding site. For MYC and HCF-1 ChIP, MYC was overexpressed in the LNCaP-MYC cell line for 4 hours prior to ChIP. **D)** OSMI-2 antagonizes MYC-induced upregulation of Cyclin B1 and PLK1 and this effect is stronger in the androgen-deprived condition. Densitometry was used to determine the intensity of the indicated proteins. Data shown is an average of 2-3 biological replicates with SEM. Example images are shown in the main **figure 4D and 4F**.



Supplementary figure 9. Correlation of the RNA-seq data between the two biological replicates.

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