Supplemental Materials

Molecular Biology of the Cell

Grant et al.

Supplemental Figure 1. FACS data confirms the synchronicity of U2OS cells after release from thymidine or nocodazole. A-C) FACS data from select timepoints from the double thymidine arrested cells shows a population of synchronous cells. D) Thymidine-nocodazole arrested cells are synchronous after release as shown by select timepoint FACS analysis.

Supplemental Figure 2. Genes removed by spectral clustering. A) K-means clustering Power spectra of the first two principal components for each gene called as being cell cycle regulated. Blue indicates genes that passed this filtering step, red indicates genes that did not pass and were removed from further analysis. B) The expression profiles of genes that were removed due to this filtering step.

Supplemental Figure 3. The correlation scores of cell cycle regulated genes in U2OS cells show differences with HeLa cells. A) The correlation scores of all cell cycle regulated genes to the "idealized vectors" (see Figure 1) in U2OS cells after hierarchical clustering. B) The cell cycle regulated gene correlations for HeLa cells after hierarchical clustering.

Supplemental Figure 4. The Expression profiles of genes bound by FOXM1. The clustered Expression profile of the 1582 genes that are bound by FOXM1 that are not cell cycle regulated in U2OS and HeLa cells.

Supplemental Figure 5. FOXM1 binds to the promoters of previously published FOXM1 target genes. A) The FOXM1 binding profile of AURKB for each of two ChIP-seq runs. B) The FOXM1 binding profile of CCNB1 for each of the ChIP-seq runs. C) The binding profile of CCNB2 for the two FOXM1 ChIP-seq runs. D) The Profiles for PLK11 for each of the ChIP-seq runs. E) The FOXM1 binding profiles of TOP2A for both ChIP-seq runs. All binding profiles were visualized on the UCSC genome browser (http://genome.ucsc.edu/)

Supplemental Figure 6. FOXM1 ChIP-seq identifies a subset of previously published targets. A) Previously published FOXM1 targets that were confirmed by FOXM1 ChIP-seq in HeLa cells. Genes were ordered by the percent coverage of their promoters. B) FOXM1 target genes that were identified by chromatin immunoprecipitations in epithelial cell lines that were not found in this study. See Supplemental Table 4 for references. C) Displaying transcription factor binding as percent coverage of gene model regions. A. The gene model for the FOXM1 target cyclin B2 as defined by the UCSF genome browser divided into regions. Two regions of FOXM1 binding are indicated by large rectangles. B. The gene model from part A displayed as a heat map of percent coverage. In addition to the 1000bp window size depicted in part A, region sizes of 3000bp and 2000bp are also displayed in the heat map display.

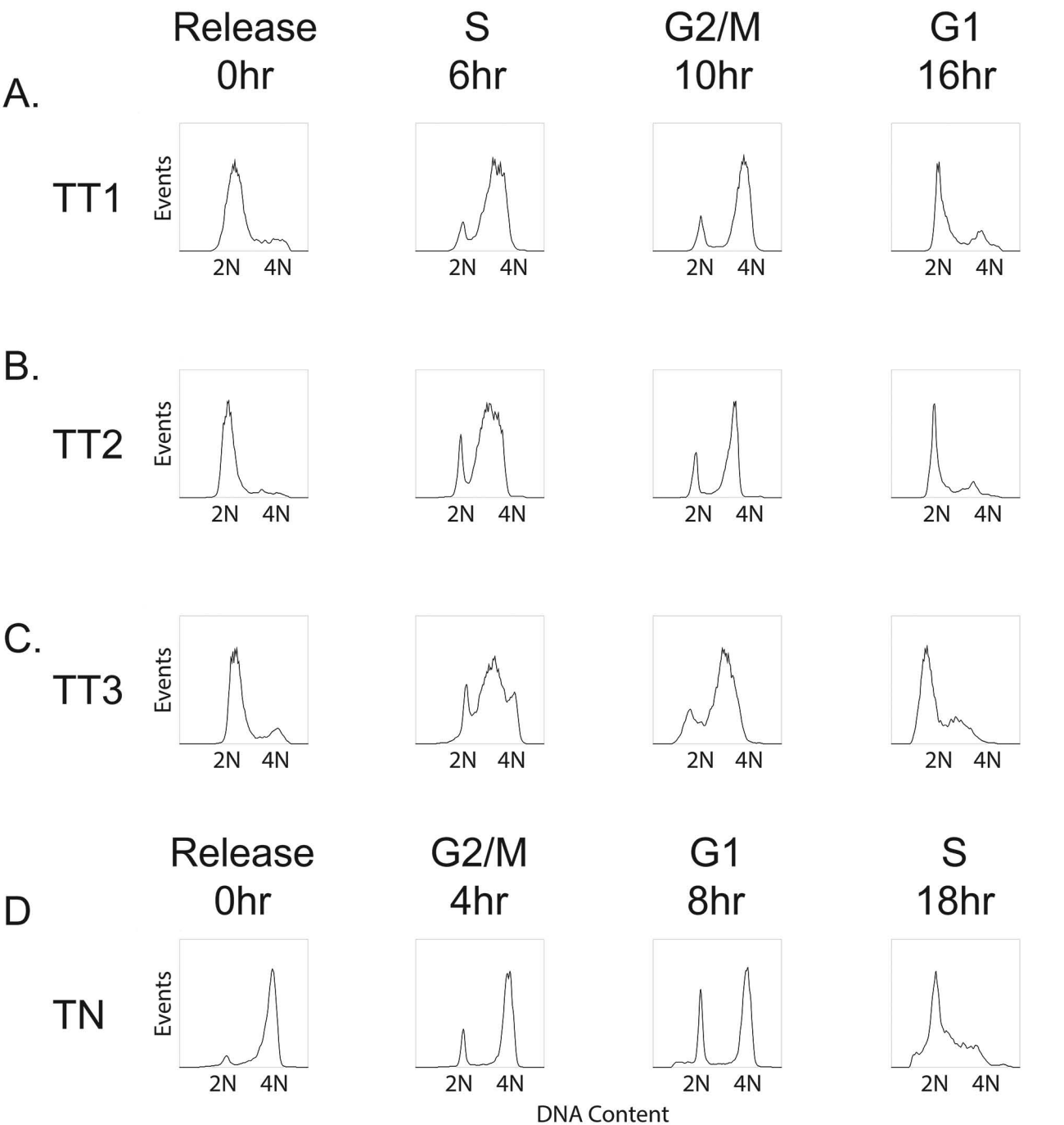
Supplemental Figure 7. The expression profiles of the 12 previously published FOXM1 targets that we did not verify by ChIP-seq. The expression profiles of the previous published chromatin immunoprecipitated FOXM1 target genes.

Supplemental Figure 8. The expression profiles of the 96 cell cycle regulated genes in Hela cells, U2OS cells, foreskin fibroblasts, and HaCaT cells. The clustered expression profiles and ENCODE data for the 96 genes that were cell cycle regulated in four independent cell types. Foreskin fibroblast and HaCat data was mean centered for display purposes (Bar-Joseph et al. 2008) (Pena-Diaz et al. 2013). Double thymidine (TT), thymidine nocodazole (TN), and Serum thymidine (S-T) synchronizations are shown. Note, the five gray bars in the HeLa datasets indicate thse genes were not included in the two time courses selected for display, but the genes were identified as cell cycle regulated in the original publication (Whitfield et al. 2002).

Supplemental Figure 9. The estimated percent false discovery rate (FDR) for the analysis using the double thymidine time courses only (grey line) or all time courses (black line). Data was randomized by rows and columns to estimate the FDR (see materials and methods).

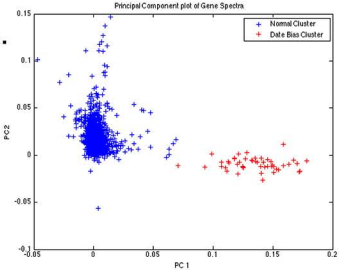
Supplemental Figure 10. Alternate color scheme for Figures 1 – 8 from the main text. Each figure showing gene expression data is presented here in a Blue / Yellow color format.

Supplemental Figure 1



Supplemental Figure 2

A.



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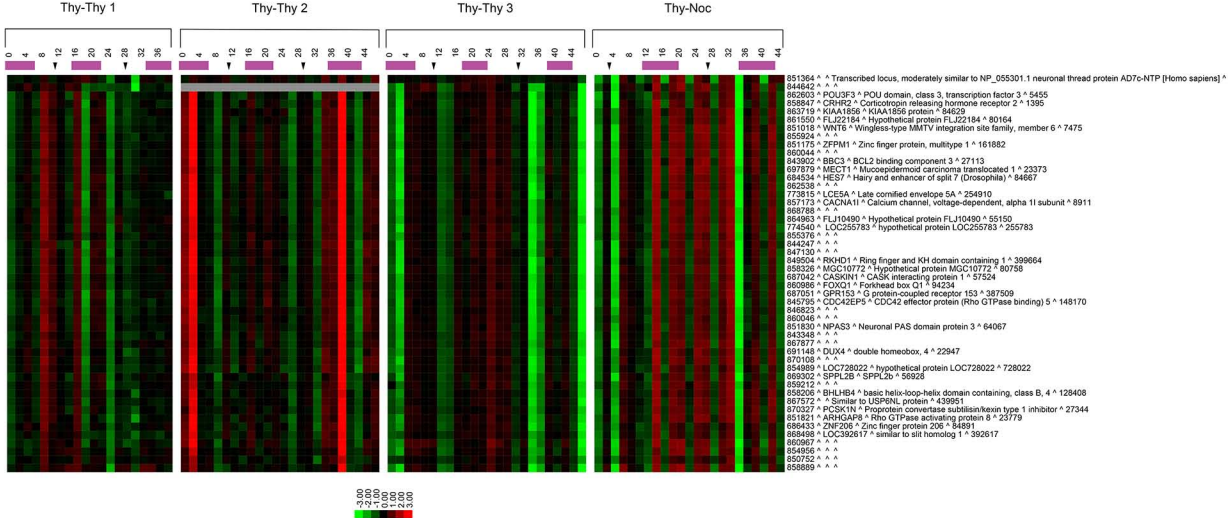
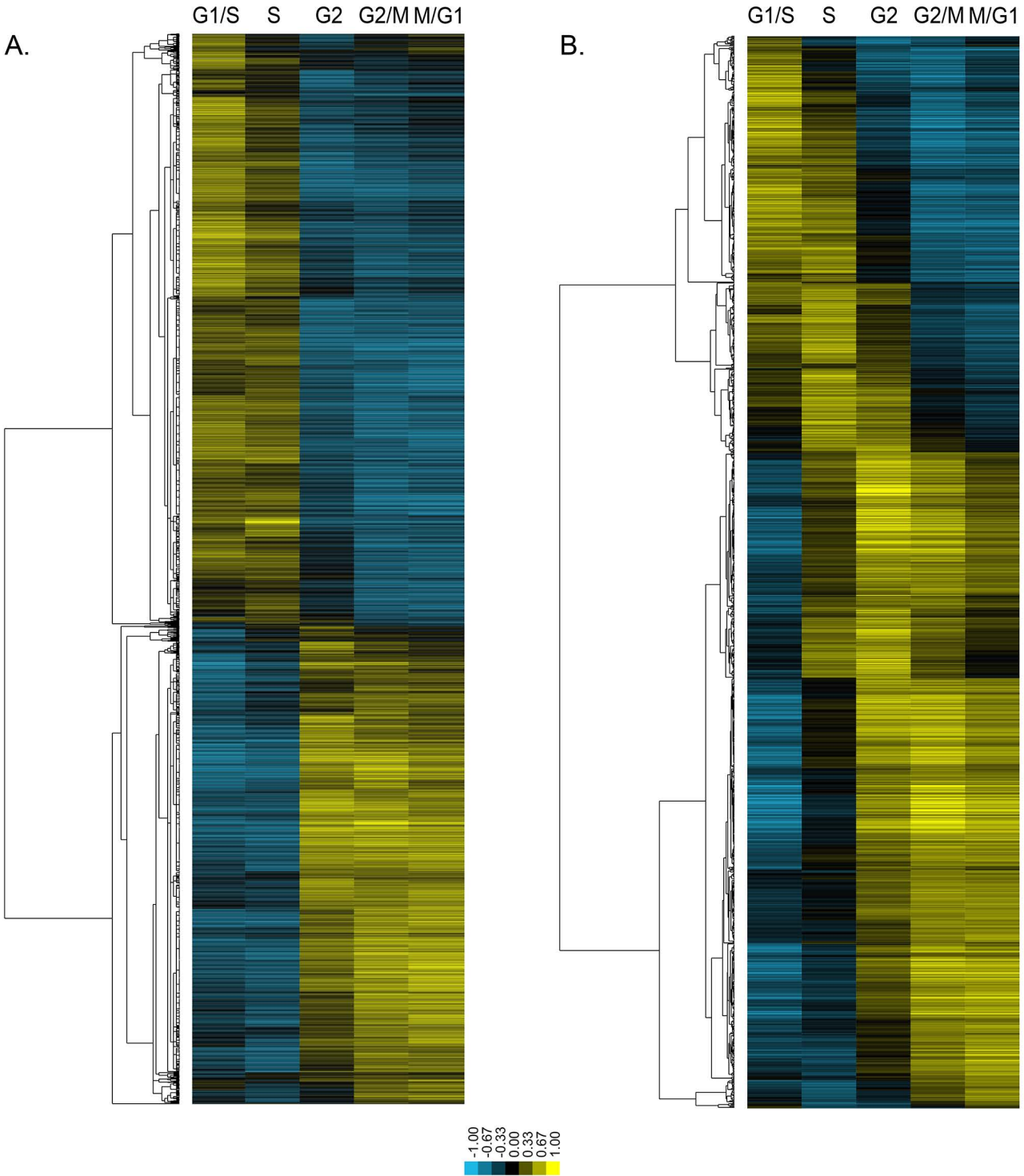
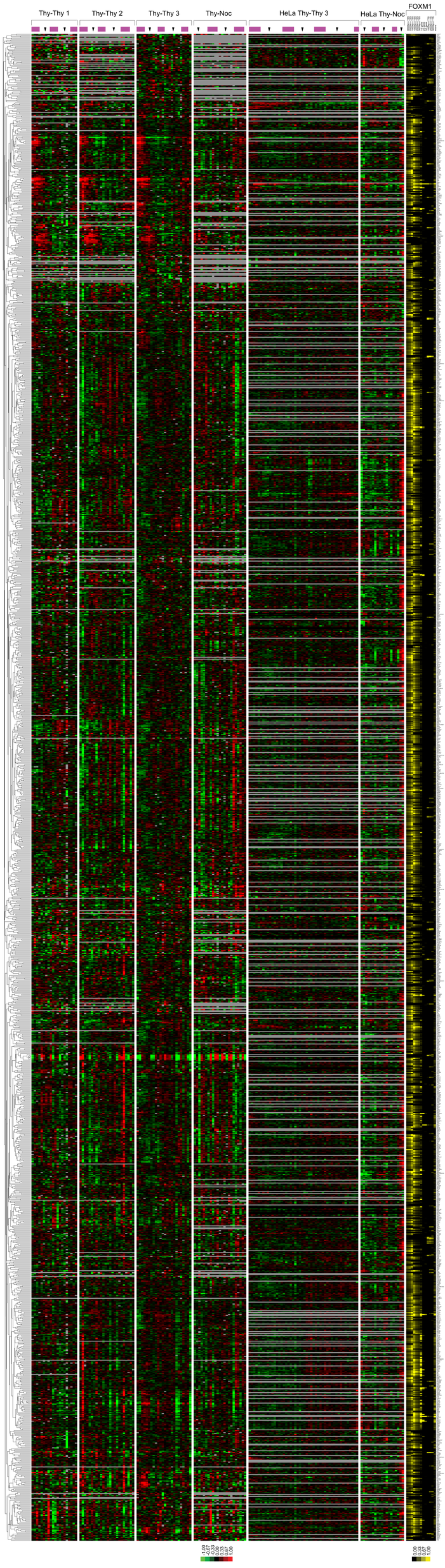


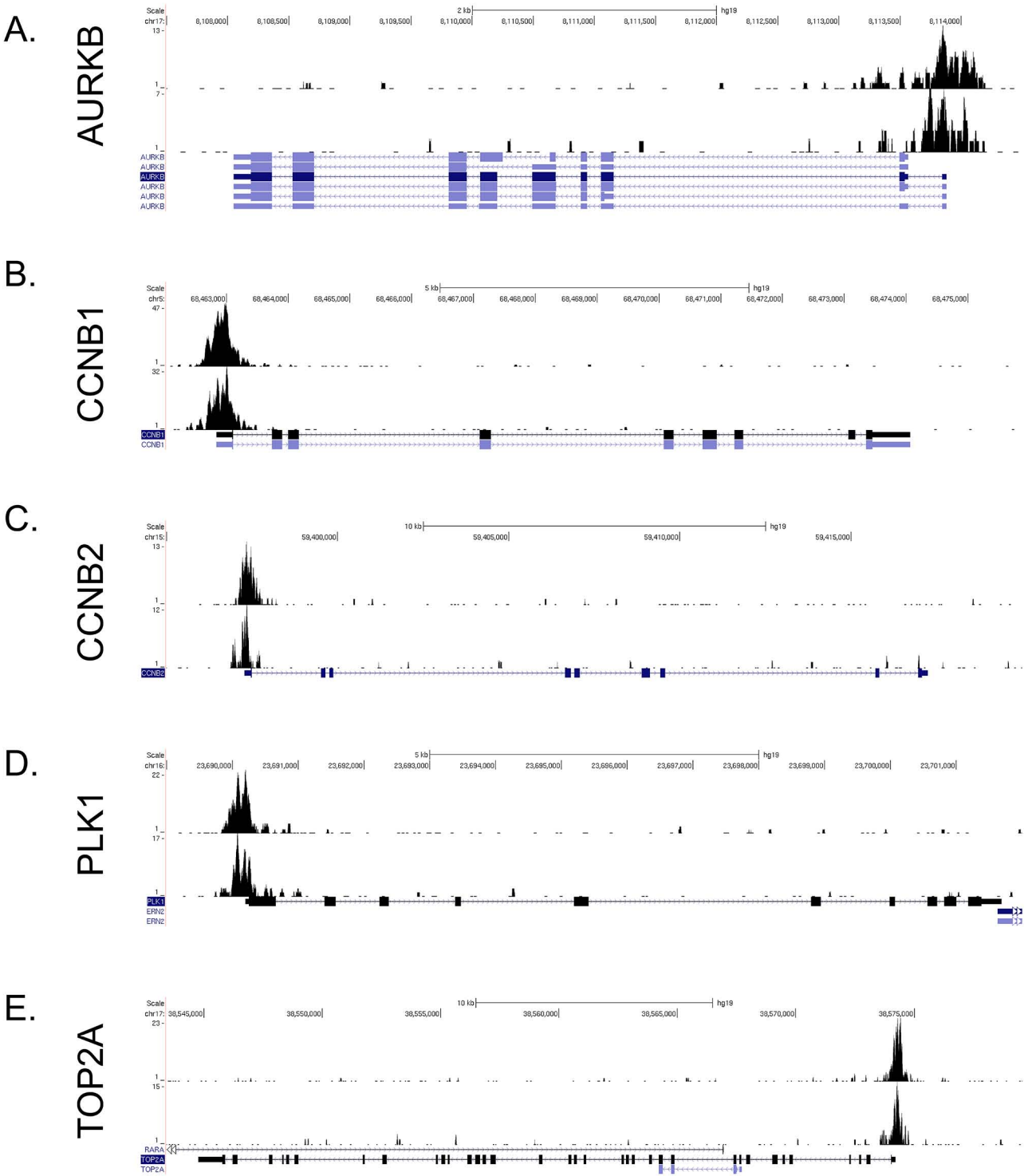
Supplemental Figure 3



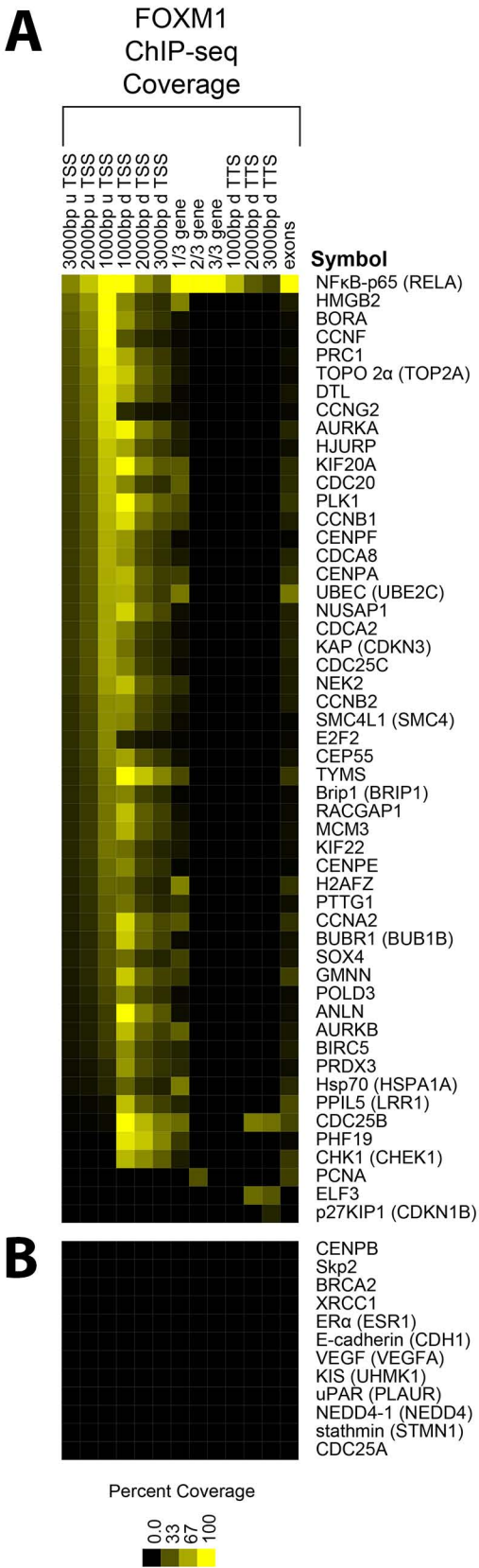
Supplemental Figure 4

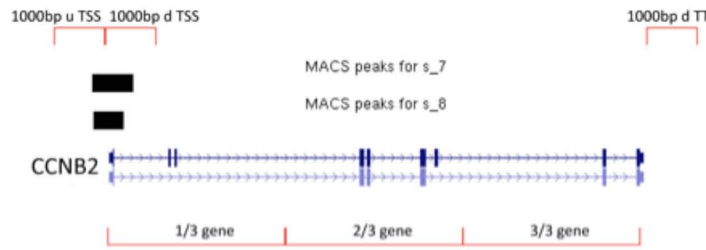


Supplemental Figure 5

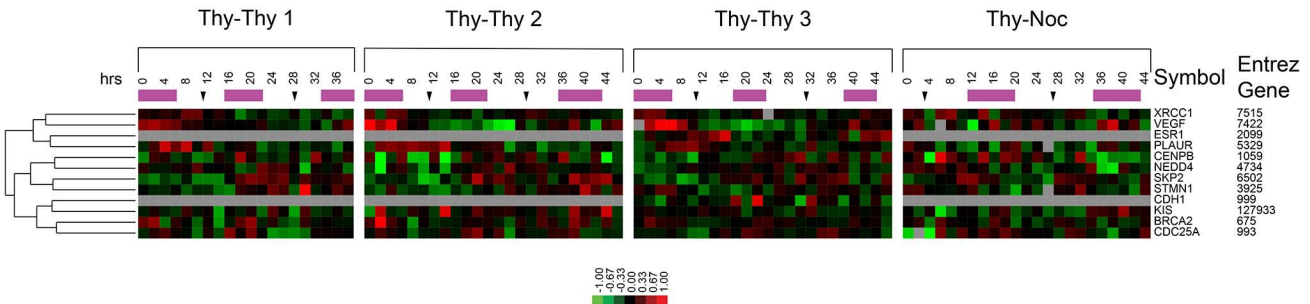


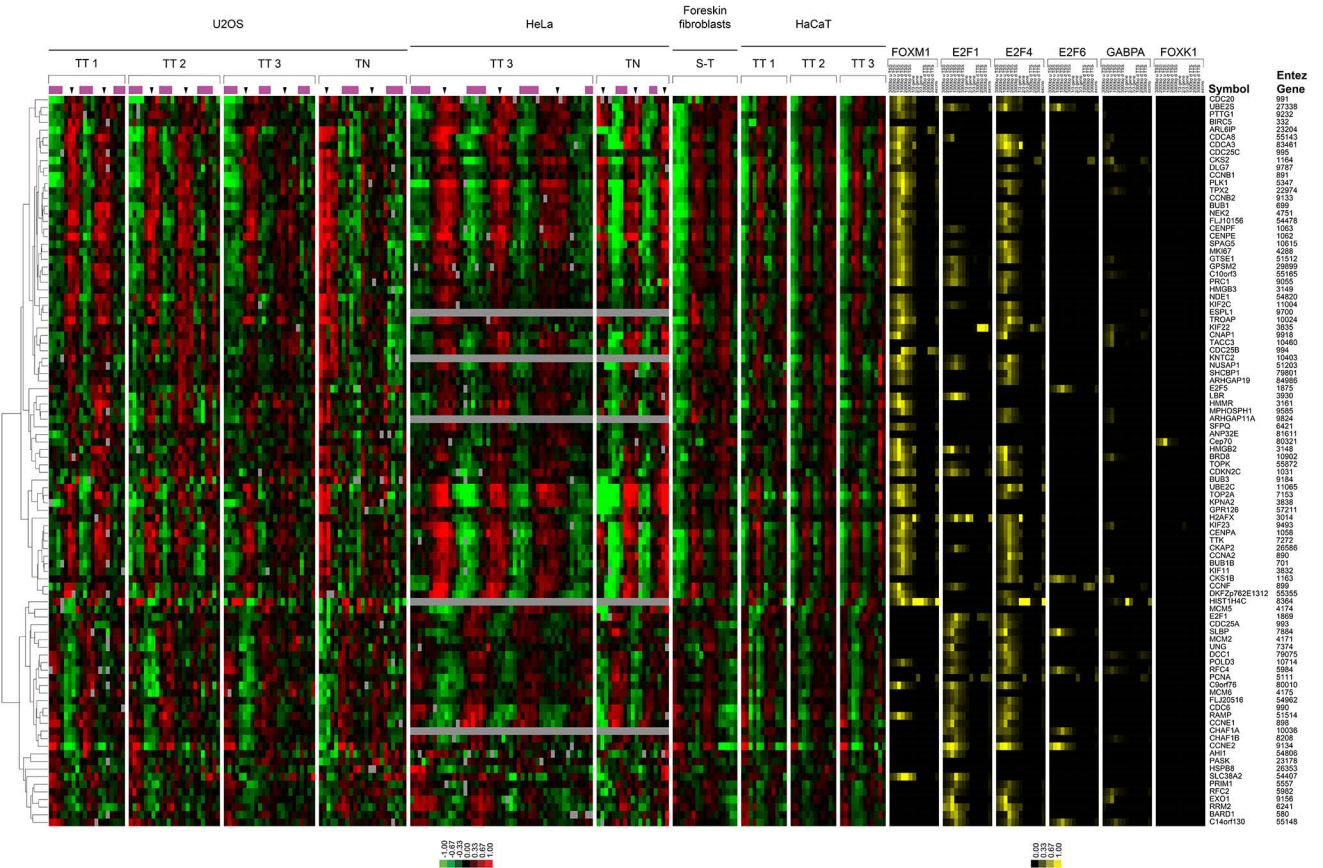
Supplemental Figure 6





Supplemental Figure 7




U2OS
HeLa
Foreskin fibroblasts
HaCaT
TT 1
TT 2
TT 3
TN
TT 3
TN
S-T
TT 1
TT 2
TT 3
FOXM1
E2F1
E2F4
E2F6
GABPA
FOXK1
Symbol
Entez Gene
CDC20 991
UBE2S 27338
PTTG1 9232
BIRC5 332
ARL6IP 23204
CDCA8 55143
CDCA3 83461
CDC25C 995
CKS2 1164
DLG7 9787
CCNB1 891
PLK1 5347
TPX2 22974
CCNB2 9133
BUB1 699
NEK2 4751
FLJ10156 54478
CENPF 1063
CENPE 1062
SPAG5 10615
MKI67 4288
GTSE1 51512
GPBM2 29899
C100rf3 55165
PRC1 9055
HMGB3 3149
NDE1 54820
KIF2C 11004
ESPL1 9700
TROAP 10024
KIF22 3835
CNAP1 9918
TACC3 10460
CDC25B 994
KNTC2 10403
NUSAP1 51203
SHCBP1 79801
ARHGAP19 84986
E2F5 1875
LBR 3930
HMMR 3161
MPHOSPH1 9585
ARHGAP11A 9824
SFPQ 6421
ANP32E 81611
Cep70 80321
HMGB2 3148
BRD8 10902
TOPK 55872
CDKN2C 1031
BUB3 9184
UBE2C 11065
TOP2A 7153
KPNA2 3838
GPR126 57211
H2AFX 3014
KIF23 9493
CENPA 1058
TTK 7272
CKAP2 26586
CCNA2 890
BUB1B 701
KIF11 3832
CKS1B 1163
CCNF 899
DKFZp762E1312 55355
HIST1H4C 8364
MCM5 4174
E2F1 1869
CDC25A 993
SLBP 7884
MCM2 4171
UNG 7374
DCC1 79075
POLD3 10714
RFC4 5984
PCNA 5111
C9orf76 80010
MCM6 4175
FLJ20516 54062
CDC6 990
RAMP 51514
CCNE1 898
CHAF1A 10036
CHAF1B 8208
CCNE2 9134
AHI1 54806
PASK 23178
HSPB8 26353
SLC38A2 54407
PRB4/1 5557
RFC2 5982
EXO1 9156
RRM2 6241
BARD1 580
C14orf130 55148
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00
0.00
0.33
0.67
1.00

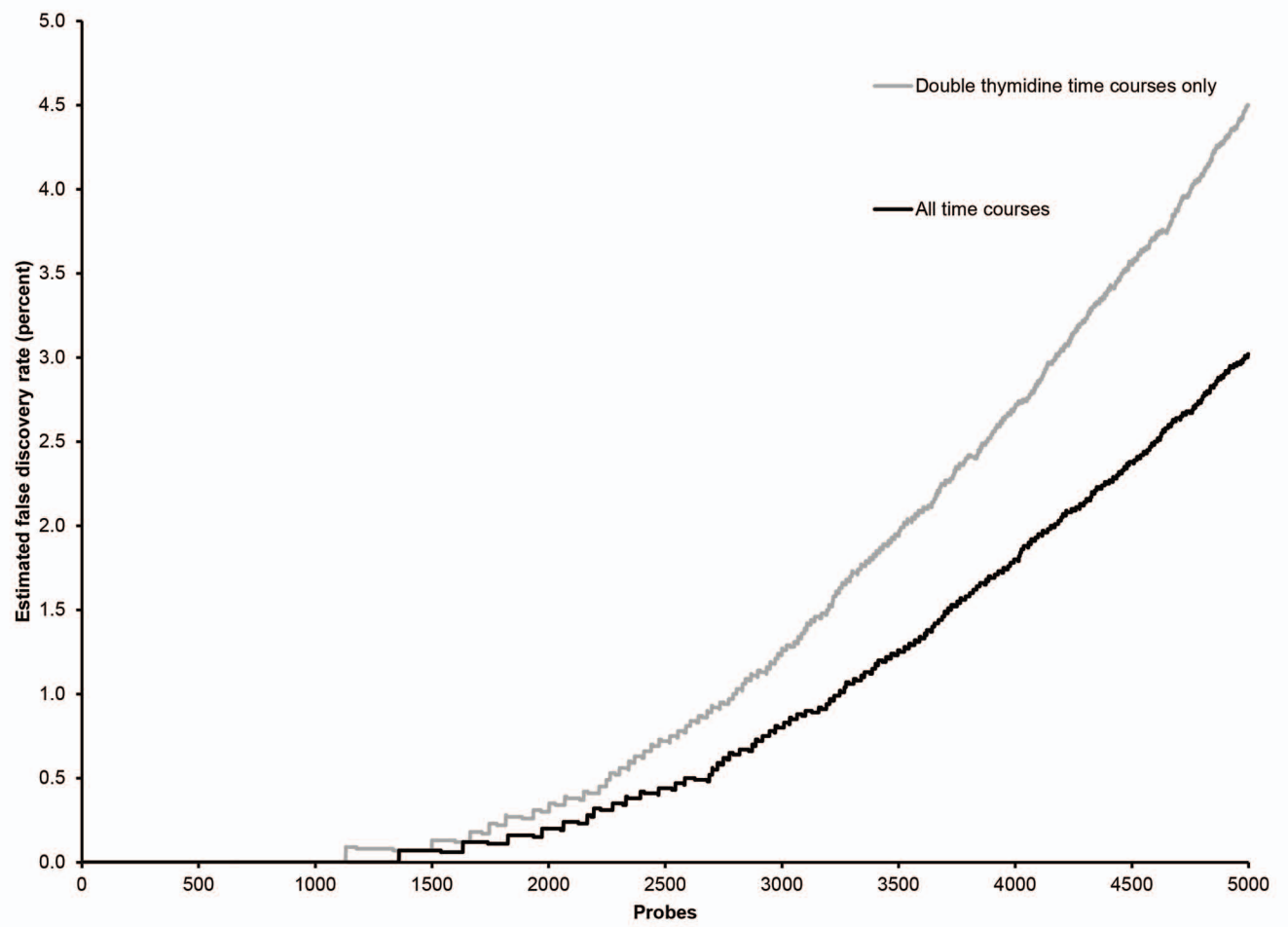
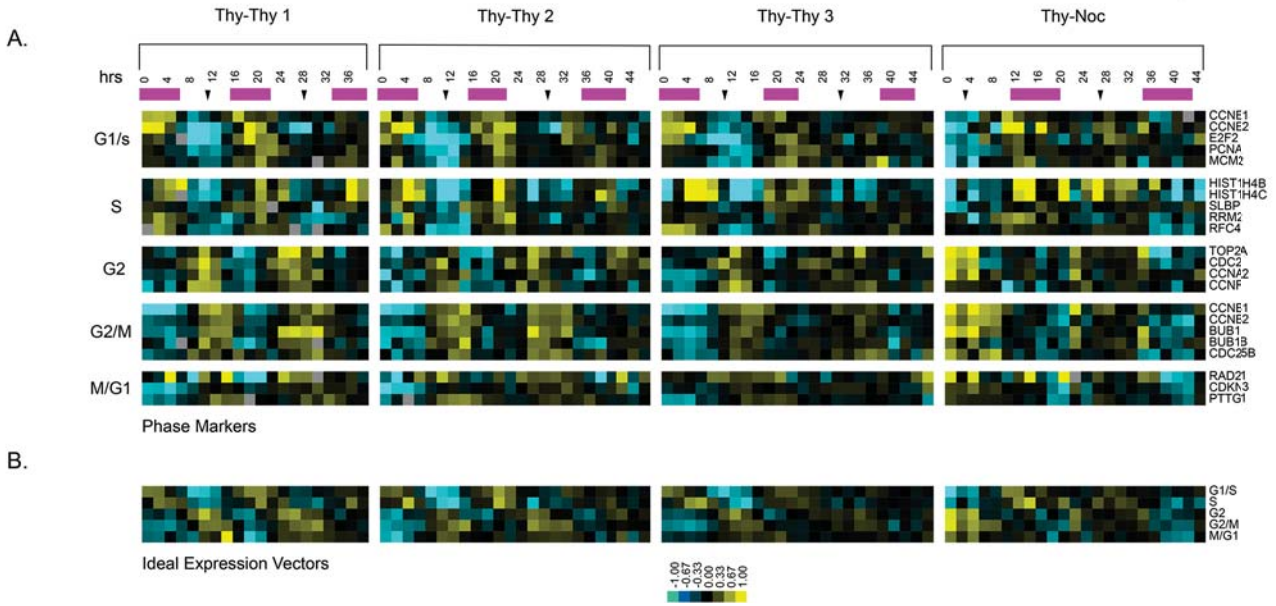

Double thymidine time courses only
All time courses
Estimated false discovery rate (percent)
Probes
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Figure 1


A.
Thy-Thy 1
Thy-Thy 2
Thy-Thy 3
Thy-Noc
hrs
G1/s
S
G2
G2/M
M/G1
CCNE1
CCNE2
E2F2
PCNA
MCM2
HIST1H4B
HIST1H4C
SLBP
RRM2
RFC4
TOP2A
CDC2
CCNA2
CCNF
CCNE1
CCNE2
BUB1
BUB1B
CDC25B
RAD21
CDKN3
PTTG1
Phase Markers
B.
G1/S
S
G2
G2/M
M/G1
Ideal Expression Vectors
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00

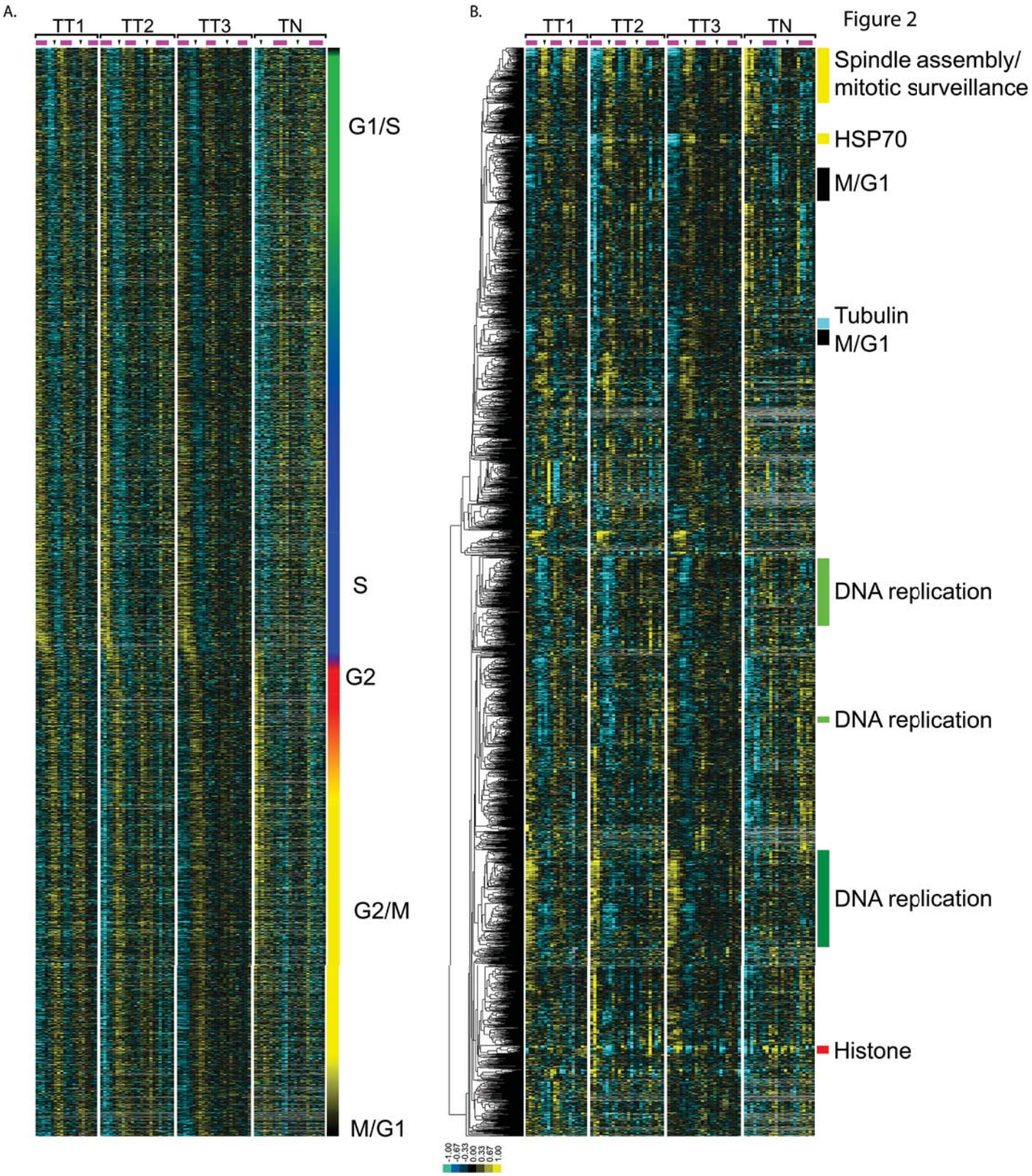
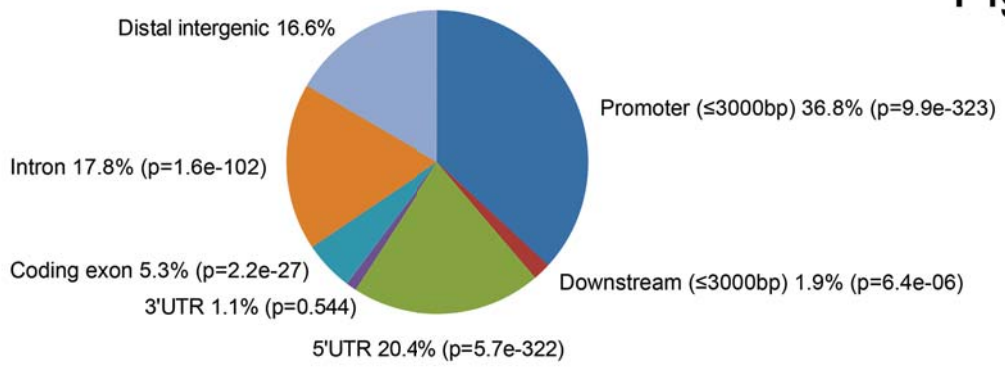

A.
TT1
TT2
TT3
TN
G1/S
S
G2
G2/M
M/G1
B.
TT1
TT2
TT3
TN
Spindle assembly/
mitotic surveillance
HSP70
M/G1
Tubulin
M/G1
DNA replication
DNA replication
DNA replication
Histone
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00

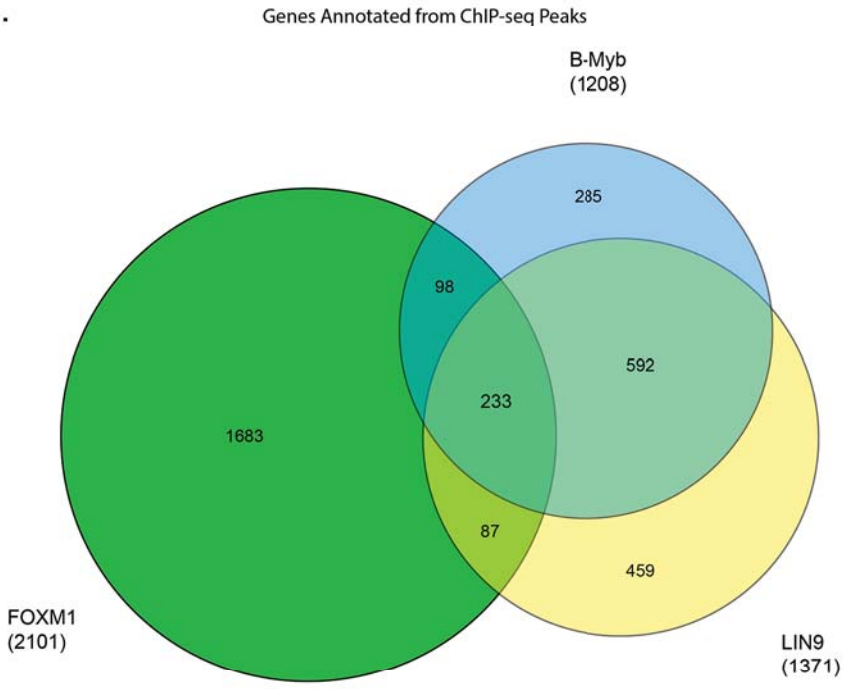
Figure 2

Figure 3

A.



B.

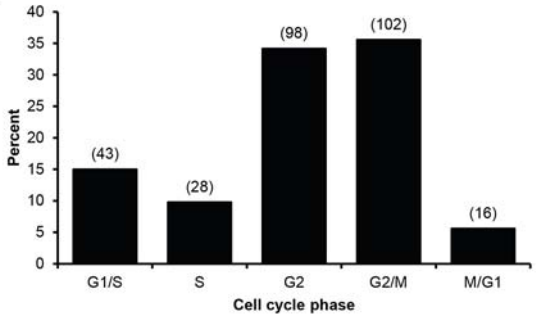


DAVID Analysis

FOXM1 only		
BP	1. Translation	3.49E-46
	2. Translational elongation	1.32E-27
CC	1. Intracellular organelle lumen	3.04E-51
	2. Ribonucleoprotein complex	8.86E-51
FOXM1 and B-Myb		
BP	1. Cell Cycle	5.35E-06
	2. Mitotic Cell Cycle	1.47E-05
CC	1. Intracellular non-membrane-bounded organelle	4.81E-09
CC	2. Non-membrane-bounded organelle	4.81E-09
FOXM1, B-Myb, and LIN9		
BP	1. M phase	3.26E-39
	2. Nuclear Division	3.81E-38
CC	1. Spindle	7.95E-28
	2. Chromosome	2.51E-27
FOXM1 and LIN9		
BP	1. Cell Division	6.36E-05
	2. M phase	8.12E-04
CC	1. Chromosome	7.29E-04
	2. Chromosomal part	1.26E-03
B-Myb only		
BP	1. Regulation of growth	1.60E-02
	2. Interphase of mitotic cell cycle	2.09E-02
CC	1. Centrosome	1.84E-02
	2. Cytosol	2.11E-02
B-Myb and LIN9		
BP	1. Actin cytoskeleton organization	8.18E-05
	2. Actin filament-based process	1.90E-04
CC	1. Cytoskeleton	2.94E-05
	2. Actin cytoskeleton	2.96E-05
LIN9 only		
BP	1. Cell adhesion	9.56E-04
	2. Biological adhesion	9.73E-04
CC	1. Cytoskeleton	1.11E-04
	2. Cell-cell junction	3.48E-04

Gene Ontology Functional Annotation

C.



D.

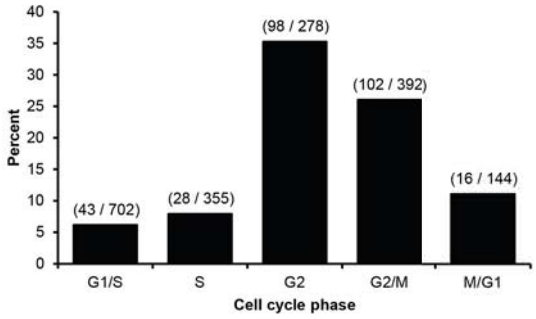


Figure 4

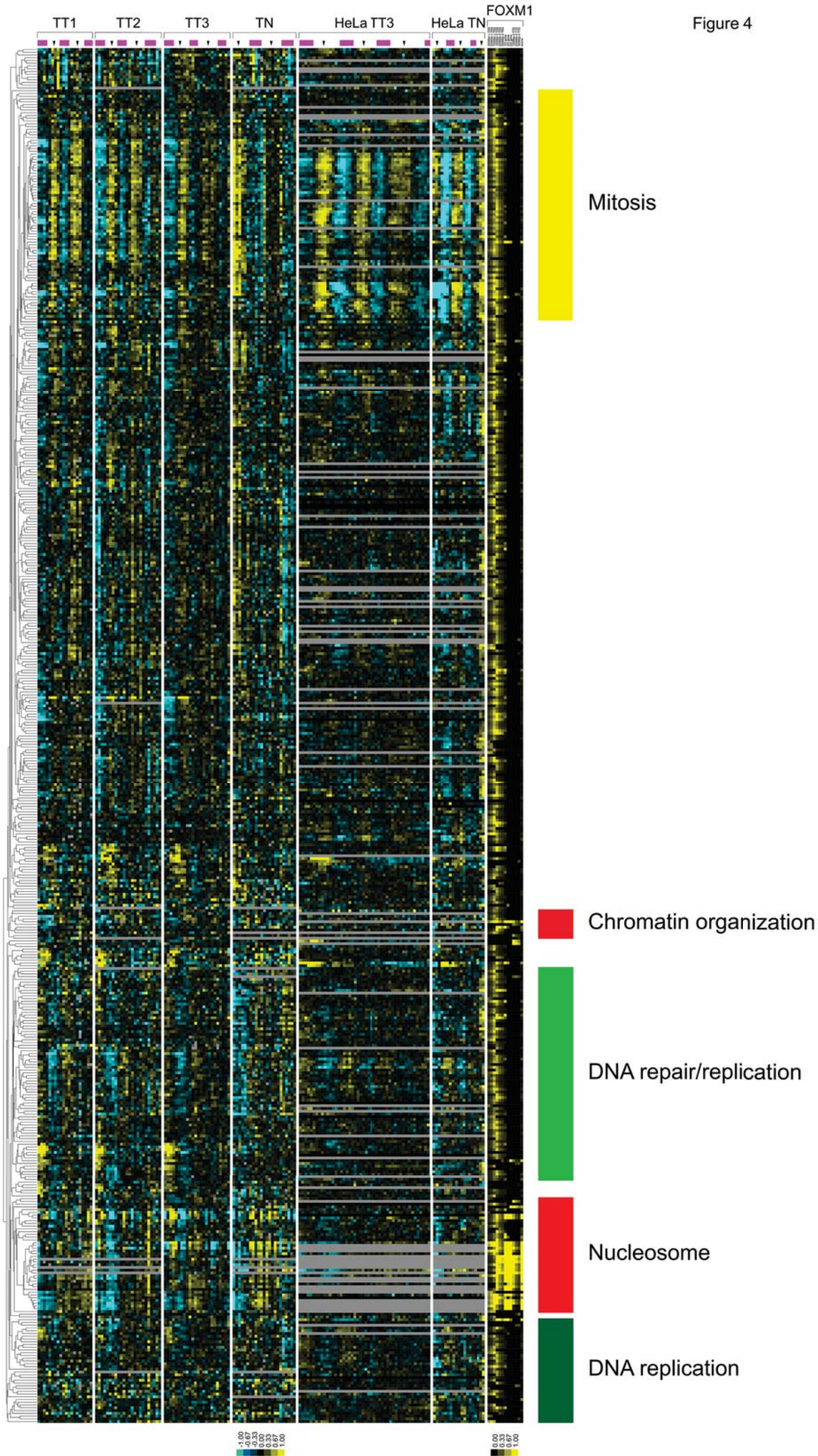
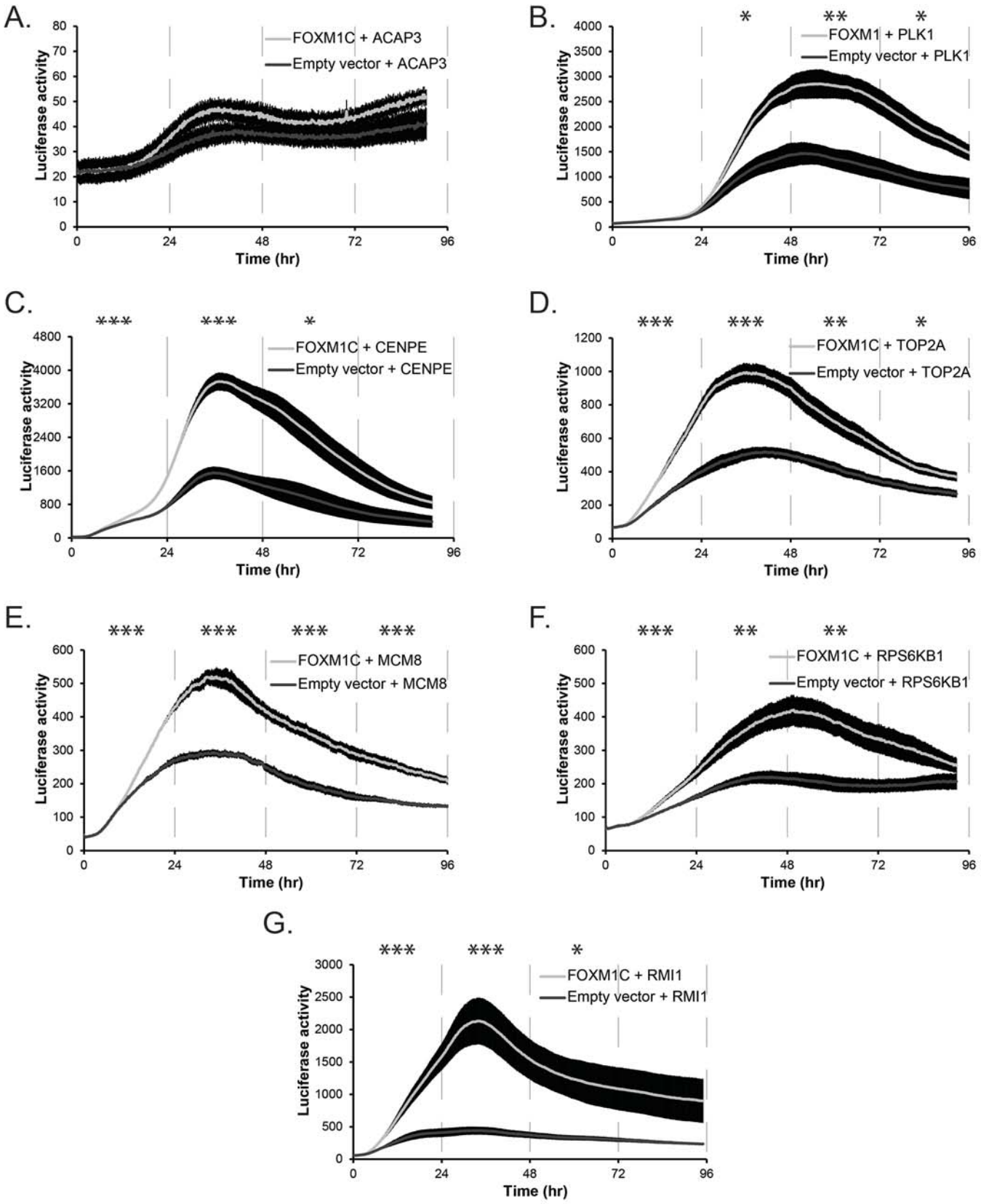

TT1
TT2
TT3
TN
HeLa TT3
HeLa TN
FOXM1
Mitosis
Chromatin organization
DNA repair/replication
Nucleosome
DNA replication

Figure 5


A.
FOXM1C + ACAP3
Empty vector + ACAP3
Luciferase activity
Time (hr)
B.
FOXM1 + PLK1
Empty vector + PLK1
C.
FOXM1C + CENPE
Empty vector + CENPE
D.
FOXM1C + TOP2A
Empty vector + TOP2A
E.
FOXM1C + MCM8
Empty vector + MCM8
F.
FOXM1C + RPS6KB1
Empty vector + RPS6KB1
G.
FOXM1C + RMI1
Empty vector + RMI1

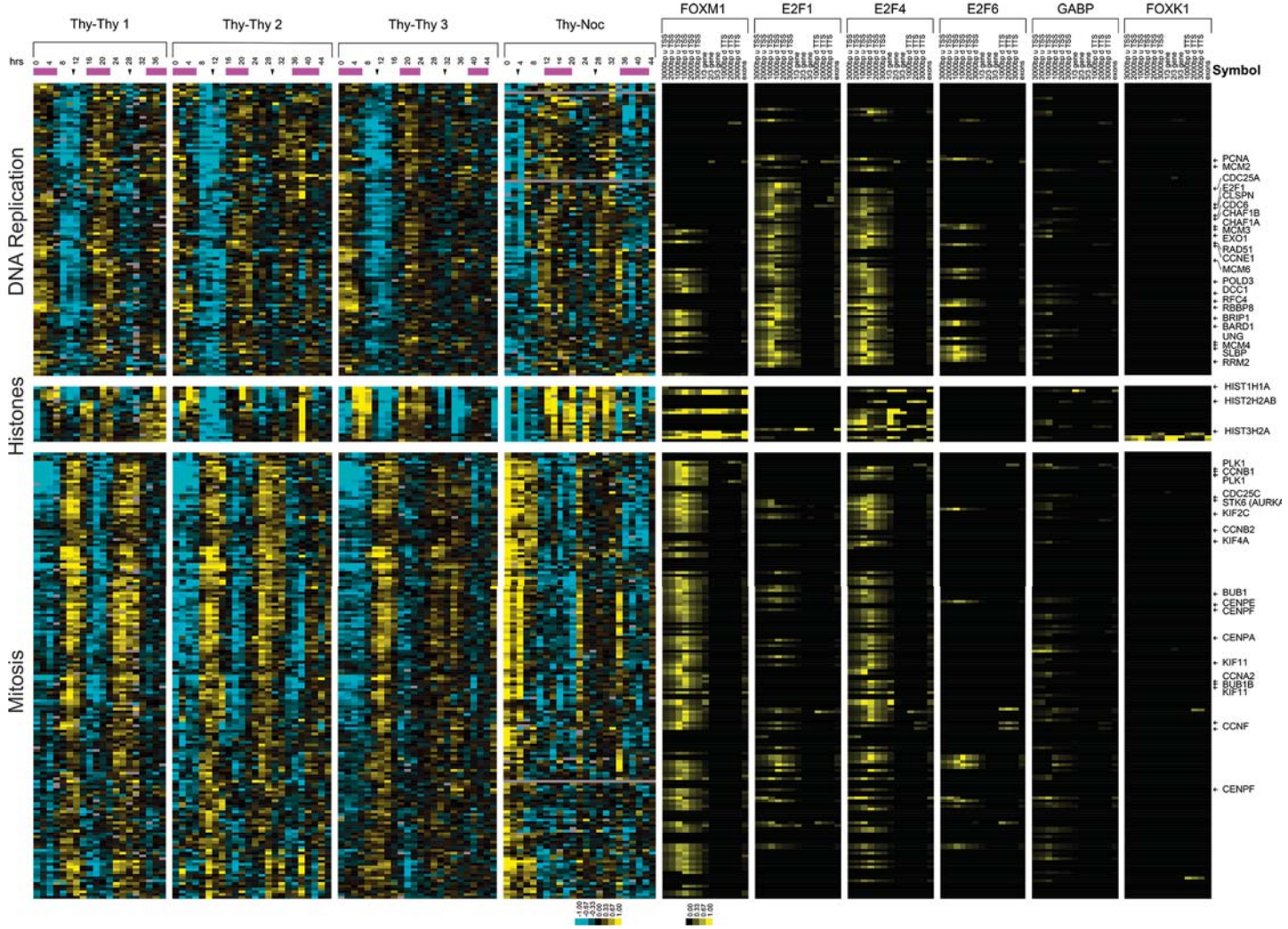

Thy-Thy 1
Thy-Thy 2
Thy-Thy 3
Thy-Noc
FOXM1
E2F1
E2F4
E2F6
GABP
FOXK1
hrs
Symbol
DNA Replication
Histones
Mitosis
PCNA
MCM2
CDC25A
E2F1
CLSPN
CDC6
CHAF1B
CHAF1A
MCM3
EXO1
RAD51
CCNE1
MCM6
POLD3
DCC1
RFC4
RBBP8
BRIP1
BARD1
UNG
MCM4
SLBP
RRM2
HIST1H1A
HIST2H2AB
HIST3H2A
PLK1
CCNB1
PLK1
CDC25C
STK6 (AURKA
KIF2C
CCNB2
KIF4A
BUB1
CENPE
CENPF
CENPA
KIF11
CCNA2
BUB1B
KIF11
CCNF
CENPF

Figure 7

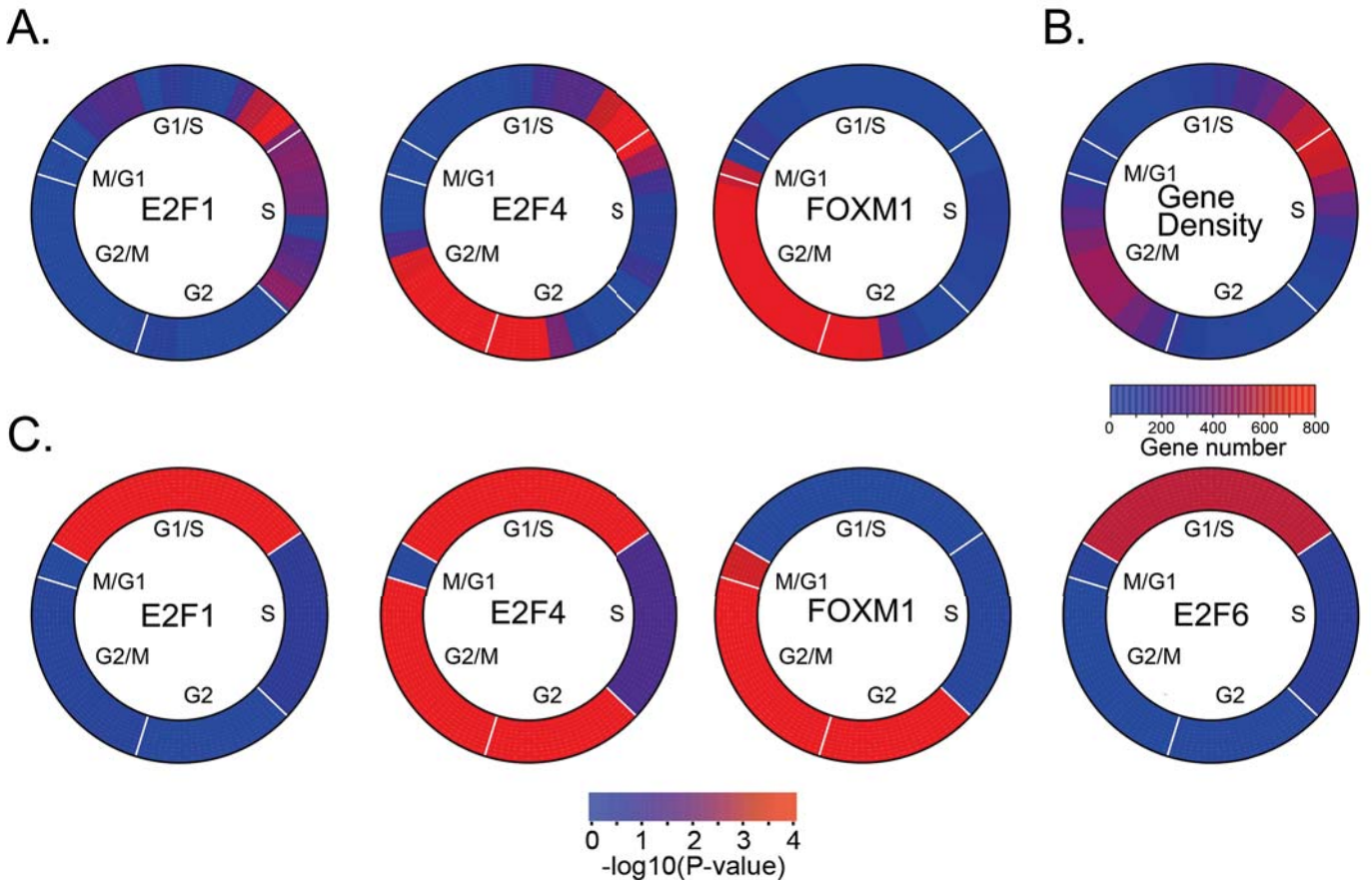
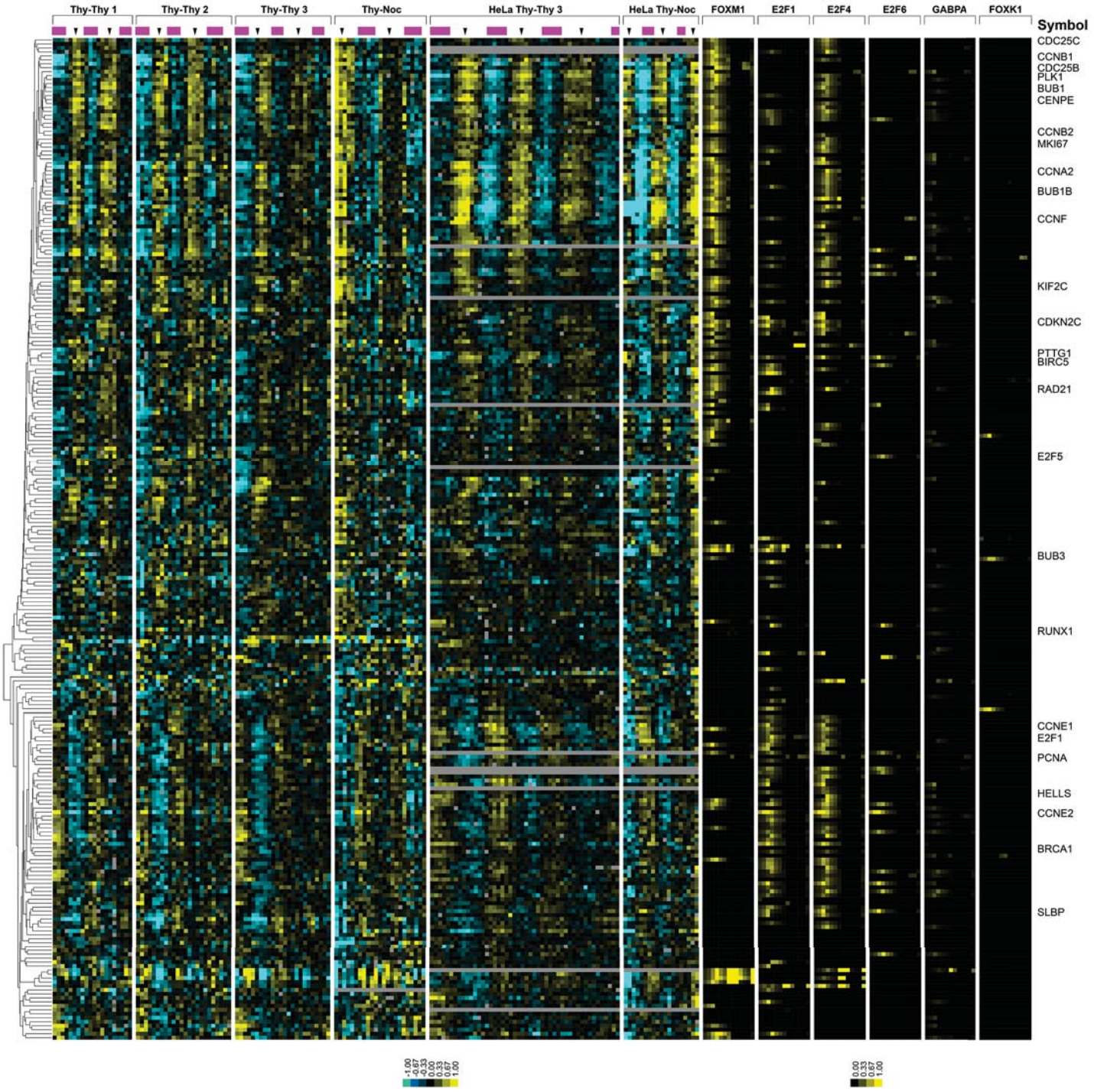

A.
B.
C.
G1/S
M/G1
S
G2/M
G2
E2F1
E2F4
FOXM1
Gene
Density
E2F6
0 200 400 600 800
Gene number
0 1 2 3 4
-log10(P-value)

Figure 8


Thy-Thy 1
Thy-Thy 2
Thy-Thy 3
Thy-Noc
HeLa Thy-Thy 3
HeLa Thy-Noc
FOXM1
E2F1
E2F4
E2F6
GABPA
FOXK1
Symbol
CDC25C
CCNB1
CDC25B
PLK1
BUB1
CENPE
CCNB2
MKI67
CCNA2
BUB1B
CCNF
KIF2C
CDKN2C
PTTG1
BIRC5
RAD21
E2F5
BUB3
RUNX1
CCNE1
E2F1
PCNA
HELLS
CCNE2
BRCA1
SLBP
-1.00
-0.67
-0.33
0.00
0.33
0.67
1.00
0.00
0.33
0.67
1.00

Supplemental Table 4: Previous identified FOXM1 target genes

Symbol	Entrez GeneID	Citations
NFκB-p65 (RELA)	8970	(Bao, Wang et al. 2011)
HMGB2	3148	(Huynh, Soh et al. 2011)
BORA	79866	(Alvarez-Fernandez, Halim et al. 2010)
CCNF	899	(reviewed in (Wierstra and Alves 2007))
PRC1	9055	(Huynh, Soh et al. 2011)
TOPO 2α (TOP2A)	7153	(Wang, Meliton et al. 2009)
DTL	51514	(Anders, Ke et al. 2011)
CCNG2	901	(Anders, Ke et al. 2011)
AURKA	6790	(Calvisi, Pinna et al. 2009; Lefebvre, Rajbhandari et al. 2010; Mencalha, Binato et al. 2012; Raghavan, Zhou et al. 2012; Sadasivam, Duan et al. 2012)
HJURP	55355	(Huynh, Soh et al. 2011)
KIF20A	10112	(reviewed in (Wierstra and Alves 2007)) (Davis, Lavine et al. 2010)
CDC20	991	(reviewed in (Wierstra and Alves 2007))
PLK1	5347	(reviewed in (Wierstra and Alves 2007)) (Fu, Malureanu et al. 2008; Chen, Dominguez-Brauer et al. 2009; Alvarez-Fernandez, Halim et al. 2010; Davis, Lavine et al. 2010; Anders, Ke et al. 2011; Chen, Yang et al. 2012; Ho, Wang et al. 2012; Sadasivam, Duan et al. 2012)
CCNB1	891	(reviewed in (Wierstra and Alves 2007)) (Fu, Malureanu et al. 2008; Laoukili, Alvarez et al. 2008; Park, Kim et al. 2008; Xia, Huang et al. 2009; Alvarez-Fernandez, Halim et al. 2010; Davis, Lavine et al. 2010; Nakamura, Hirano et al. 2010; Xue, Chiang et al. 2010; Chen, Yang et al. 2012; Mencalha, Binato et al. 2012; Sadasivam, Duan et al. 2012; Xue, Xiao et al. 2012)
CENPF	1063	(reviewed in (Wierstra and Alves 2007)) (Laoukili, Alvarez et al. 2008; Anders, Ke et al. 2011)
CDCA8	55143	(Davis, Lavine et al. 2010; Bergamaschi, Christensen et al. 2011)
CENPA	1058	(reviewed in (Wierstra and Alves 2007)) (Chen, Dominguez-Brauer et al. 2009; Davis, Lavine et al. 2010; Zhou, Wang et al. 2010)
UBEC (UBE2C)	11065	(Huynh, Soh et al. 2011)
NUSAP1	51203	(Huynh, Soh et al. 2011)
CDCA2	157313	(Davis, Lavine et al. 2010)
KAP (CDKN3)	1033	(reviewed in (Wierstra and Alves 2007))
CDC25C	995	(reviewed in (Wierstra and Alves 2007))
NEK2	4751	(reviewed in (Wierstra and Alves 2007)) (Laoukili, Kooistra et al. 2005; Calvisi, Pinna et al. 2009; Davis, Lavine et al. 2010)
CCNB2	9133	(reviewed in (Wierstra and Alves 2007))
SMC4L1 (SMC4)	10051	(Huynh, Soh et al. 2011)
E2F2	1870	(Anders, Ke et al. 2011)
CEP55	55165	(Gemenetzidis, Bose et al. 2009)
TYMS	7298	(Huynh, Soh et al. 2011)
Brip1 (BRIP1)	83990	(Monteiro, Khongkow et al. 2012)
RACGAP1	29127	(Sadasivam, Duan et al. 2012)

MCM3	4172	(Lefebvre, Rajbhandari et al. 2010)
KIF22	3835	(Huynh, Soh et al. 2011)
CENPE	1062	(Davis, Lavine et al. 2010)
H2AFZ	3015	(Huynh, Soh et al. 2011)
PTTG1	9232	(Lefebvre, Rajbhandari et al. 2010)
CCNA2	890	(Davis, Lavine et al. 2010) (reviewed in (Wierstra and Alves 2007))
BUBR1 (BUB1B)	701	(Lefebvre, Rajbhandari et al. 2010; Wan, Yeung et al. 2012)
SOX4	6659	(Wang, Zhang et al. 2010)
GMNN	51053	(Huynh, Soh et al. 2011)
POLD3	10714	(Anders, Ke et al. 2011)
ANLN	54443	(Huynh, Soh et al. 2011)
AURKB	9212	(reviewed in (Wierstra and Alves 2007)) (Fu, Malureanu et al. 2008; Park, Wang et al. 2008; Chen, Dominguez-Brauer et al. 2009; Davis, Lavine et al. 2010; Nakamura, Hirano et al. 2010; Zhou, Wang et al. 2010; Bergamaschi, Christensen et al. 2011; Wang and Gartel 2011; Bonet, Giuliano et al. 2012; Sadasivam, Duan et al. 2012)
BIRC5	332	(reviewed in (Wierstra and Alves 2007)) (Chen, Dominguez-Brauer et al. 2009; Dai, Pieper et al. 2010; Nakamura, Hirano et al. 2010; Ahmad, Ali et al. 2011; Bergamaschi, Christensen et al. 2011; Chen, Yang et al. 2012; Down, Millour et al. 2012)
PRDX3	10935	(Park, Carr et al. 2009)
Hsp70 (HSPA1A)	3303	(reviewed in (Wierstra and Alves 2007))
PPIL5 (LRR1)	122769	(Anders, Ke et al. 2011)
CDC25B	994	(reviewed in (Wierstra and Alves 2007)) (Kalin, Wang et al. 2008; Nakamura, Hirano et al. 2010; Zhou, Wang et al. 2010; Bergamaschi, Christensen et al. 2011; Wang and Gartel 2011; Ho, Wang et al. 2012; Mencalha, Binato et al. 2012)
PHF19	26147	(Huynh, Soh et al. 2011)
CHK1 (CHEK1)	1111	(Tan, Chen et al. 2010)
PCNA	5111	(Davis, Lavine et al. 2010; Bergamaschi, Christensen et al. 2011)
ELF3	1999	(Ustiyan, Wert et al. 2012)
p27KIP1 (CDKN1B)	1027	(reviewed in (Wierstra and Alves 2007)) (Zeng, Wang et al. 2009; Nakamura, Hirano et al. 2010; Xue, Chiang et al. 2010; Xue, Xiao et al. 2012; Zhang, Zeng et al. 2012)
CENPB	1059	(reviewed in (Wierstra and Alves 2007)) (Zhou, Wang et al. 2010; Chen, Yang et al. 2012)
Skp2	6502	(reviewed in (Wierstra and Alves 2007)) (Park, Wang et al. 2008; Calvisi, Pinna et al. 2009; Park, Kim et al. 2009; Nakamura, Hirano et al. 2010; Zhou, Wang et al. 2010; Ahmad, Ali et al. 2011; Anders, Ke et al. 2011; Chen, Yang et al. 2012; Ho, Wang et al. 2012; Mencalha, Binato et al. 2012; Zhang, Zeng et al. 2012)
BRCA2	675	(reviewed in (Wierstra and Alves 2007)) (Alvarez-Fernandez, Halim et al. 2010)
XRCC1	7515	(reviewed in (Wierstra and Alves 2007)) (Chetty, Bhoopathi et al. 2009)
ERα (ESR1)	2099	(reviewed in (Wierstra and Alves 2007))
E-cadherin (CDH1)	999	(reviewed in (Wierstra and Alves 2007)) (Zhou, Wang et al. 2010; Wierstra 2011)
VEGF (VEGFA)	7422	(Zhang, Zhang et al. 2008; Gemenetzidis, Bose et al. 2009; Li, Zhang et al. 2009; Ahmad, Wang et al. 2010; Ahmad, Ali et al. 2011; Bao, Wang et al. 2011; Chen, Yuan et al. 2011; Park, Gusarova et al. 2011; Karadedou, Gomes et al. 2012; Lynch, Ferrer et al. 2012; Xue, Xiao et al. 2012)

KIS (UHMK1)	127933	(Petrovic, Costa et al. 2008; Nakamura, Hirano et al. 2010)
uPAR (PLAUR)	5329	(Ahmad, Wang et al. 2010; Lok, Chan et al. 2011; Li, Wei et al. 2012)
NEDD4-1 (NEDD4)	4734	(Dai, Pieper et al. 2010; Kwak, Wang et al. 2012)
Stathmin (STMN1)	3925	(Carr, Park et al. 2010; Park, Gusarova et al. 2011)
CDC25A	993	(reviewed in (Wierstra and Alves 2007))

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