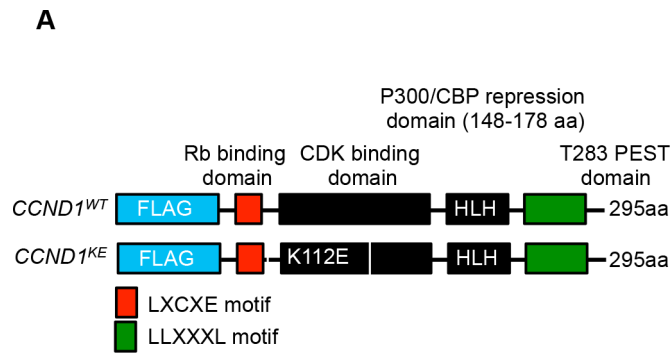
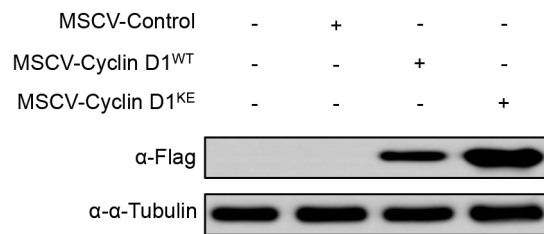


SUPPLEMENTARY FIGURES AND TABLES

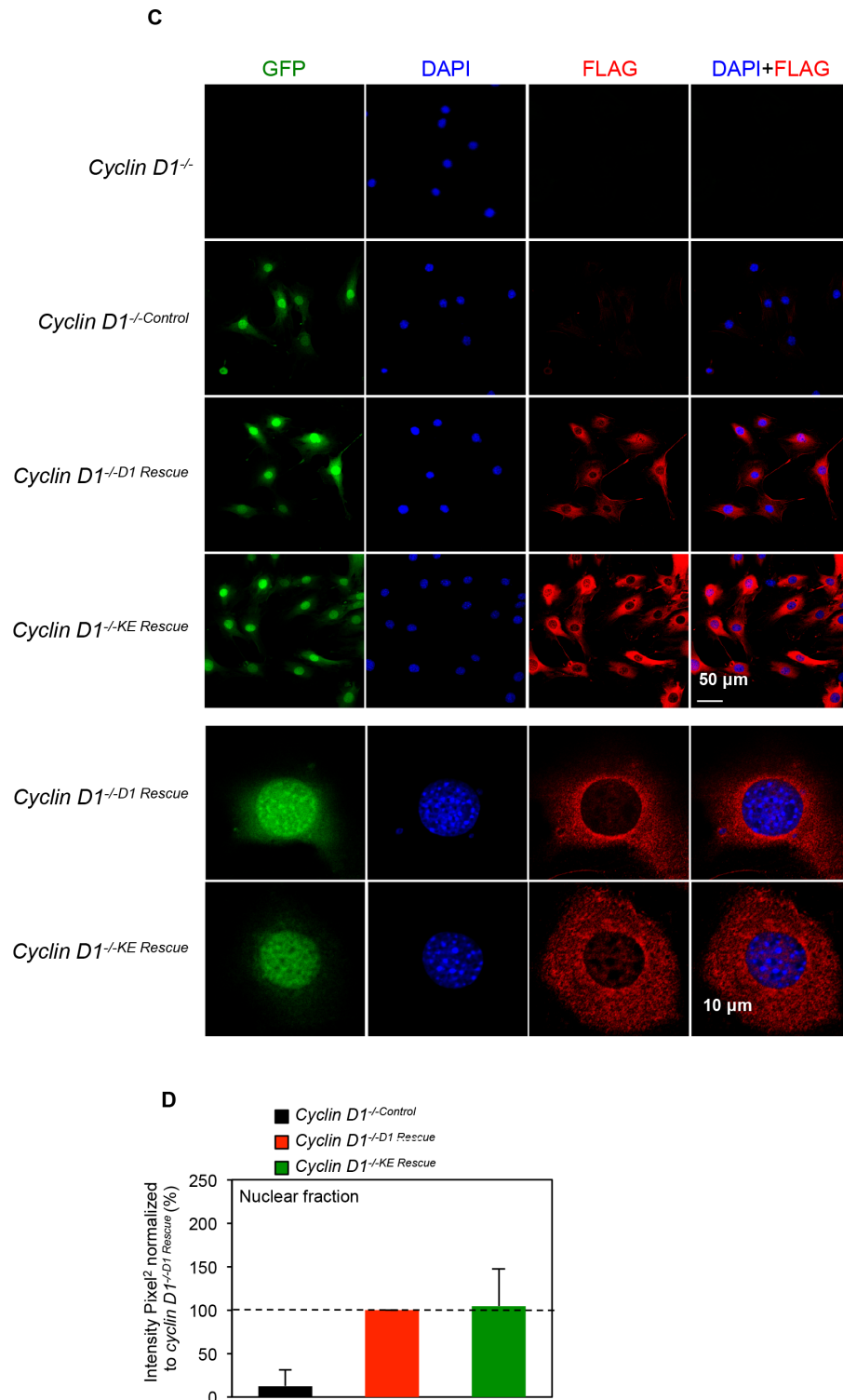


**B**

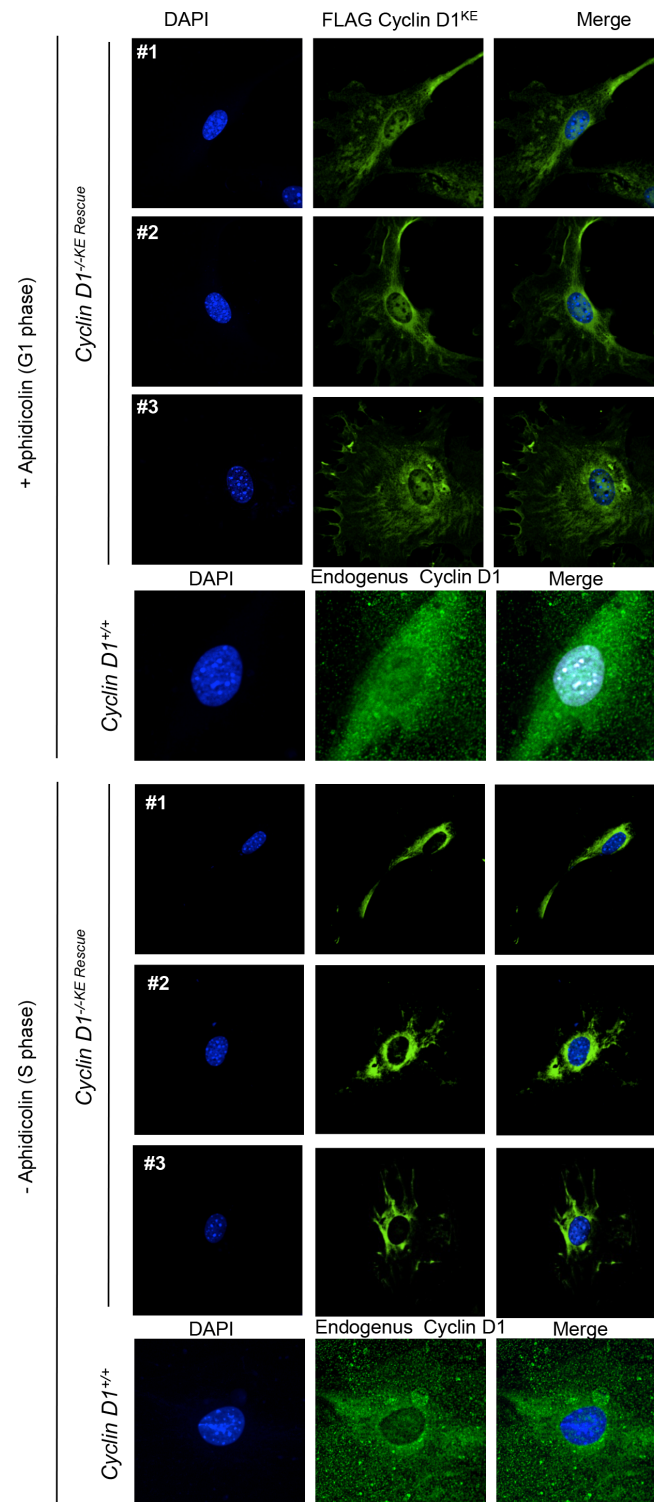


**Supplementary Figure 1: Structure of Cyclin D1<sup>WT</sup> and cyclin D1<sup>KE</sup> vectors and comparison of their abundance in rescued 3T3 cells. (A)** Schematic representation of Cyclin D1<sup>WT</sup> and cyclin D1<sup>KE</sup> proteins. **(B)** Western blot analysis on randomly cycling *cyclin D1*<sup>-/-</sup> and *cyclin D1*<sup>-/-Control</sup>, *cyclin D1*<sup>-/-D1 Rescue</sup> and *cyclin D1*<sup>-/-KE Rescue</sup> cells using anti-FLAG and anti- $\alpha$  Tubulin.

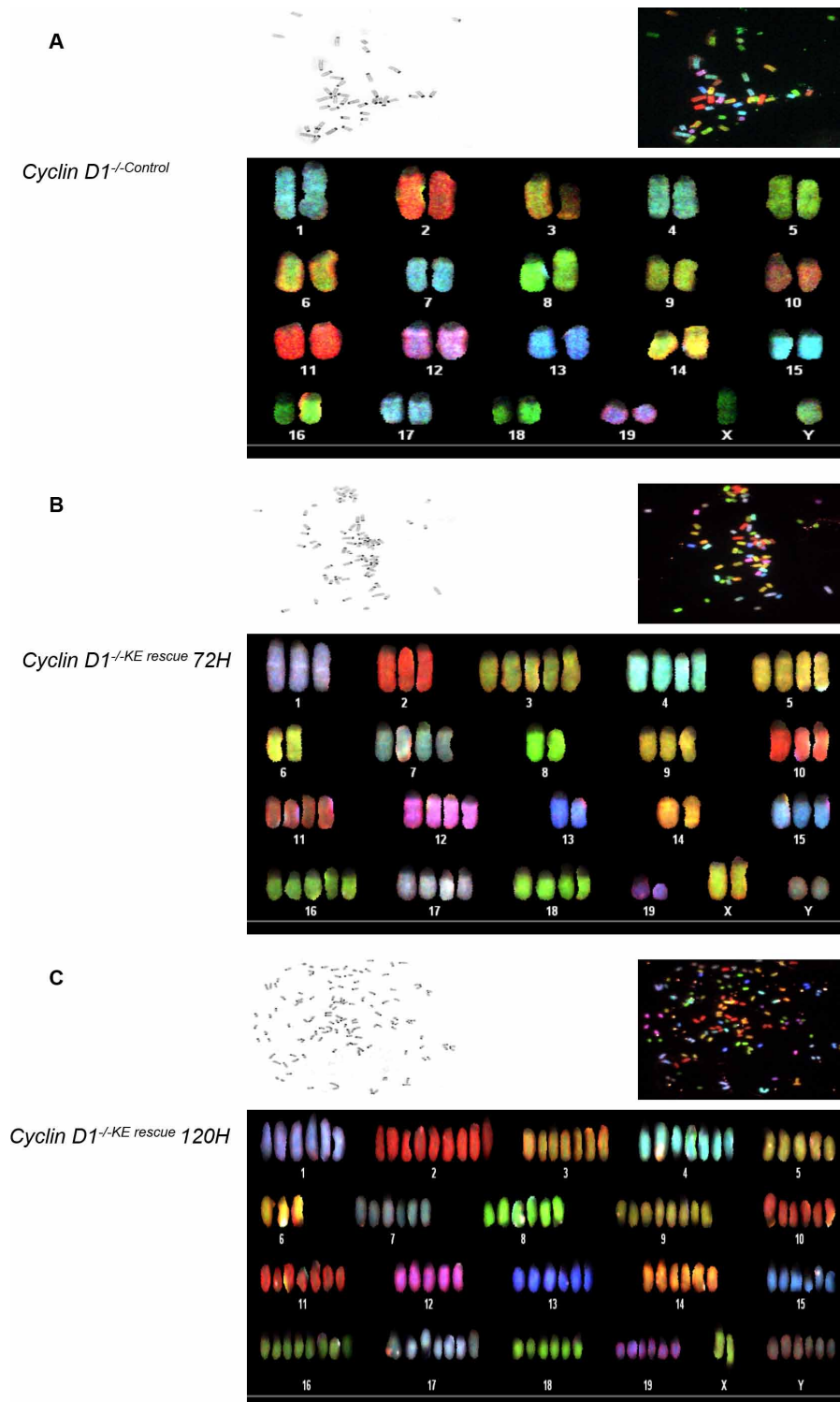
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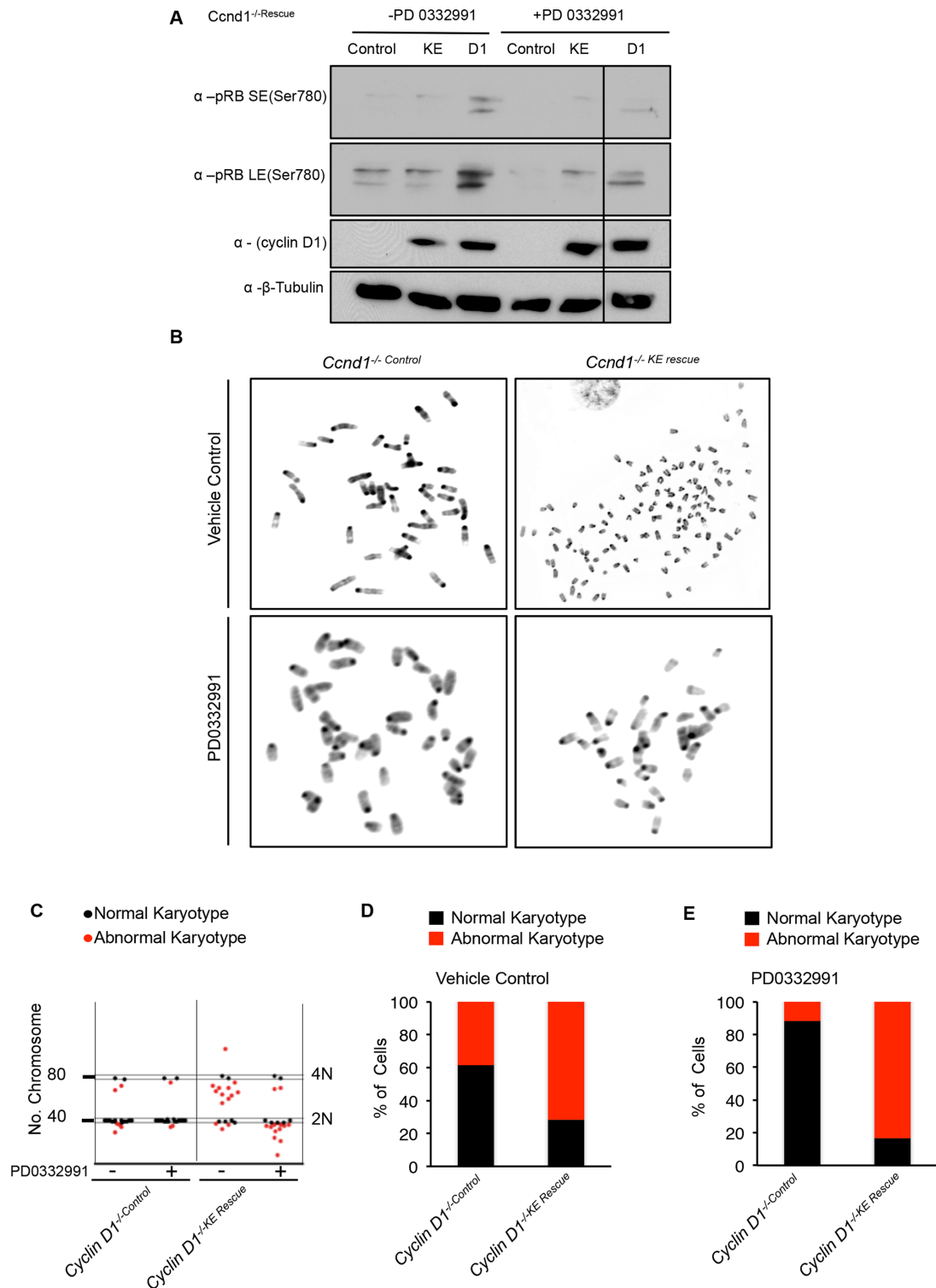
**Supplementary Figure 1: (Continued) Structure of Cyclin D1<sup>WT</sup> and cyclin D1<sup>KE</sup> vectors and comparison of their abundance in rescued 3T3 cells. (C)** Representative images of *cyclin D1<sup>-/-Control</sup>*, *cyclin D1<sup>-/-D1 Rescue</sup>* or *cyclin D1<sup>-/-KE Rescue</sup>* 3T3 fibroblast cells stained for DAPI (blue) and anti-FLAG (red) to the N-terminal FLAG-tag of cyclin D1 to discern subcellular distribution by IHC, scale bars 50  $\mu$ m (upper panels) and 10  $\mu$ m (lower panels). **(D)** Average fluorescent intensity of anti-FLAG signal in *cyclin D1<sup>-/-Control</sup>*, *cyclin D1<sup>-/-D1 Rescue</sup>* *cyclin D1<sup>-/-KE Rescue</sup>* 3T3 cells ( $n = 25$  cells) within the nuclear compartment. Signal intensity from nuclear compartment normalized to cyclin D1<sup>WT</sup>. No statistical difference was observed between cyclin D1<sup>WT</sup> or cyclin D1<sup>KE</sup> localization in the two lines.



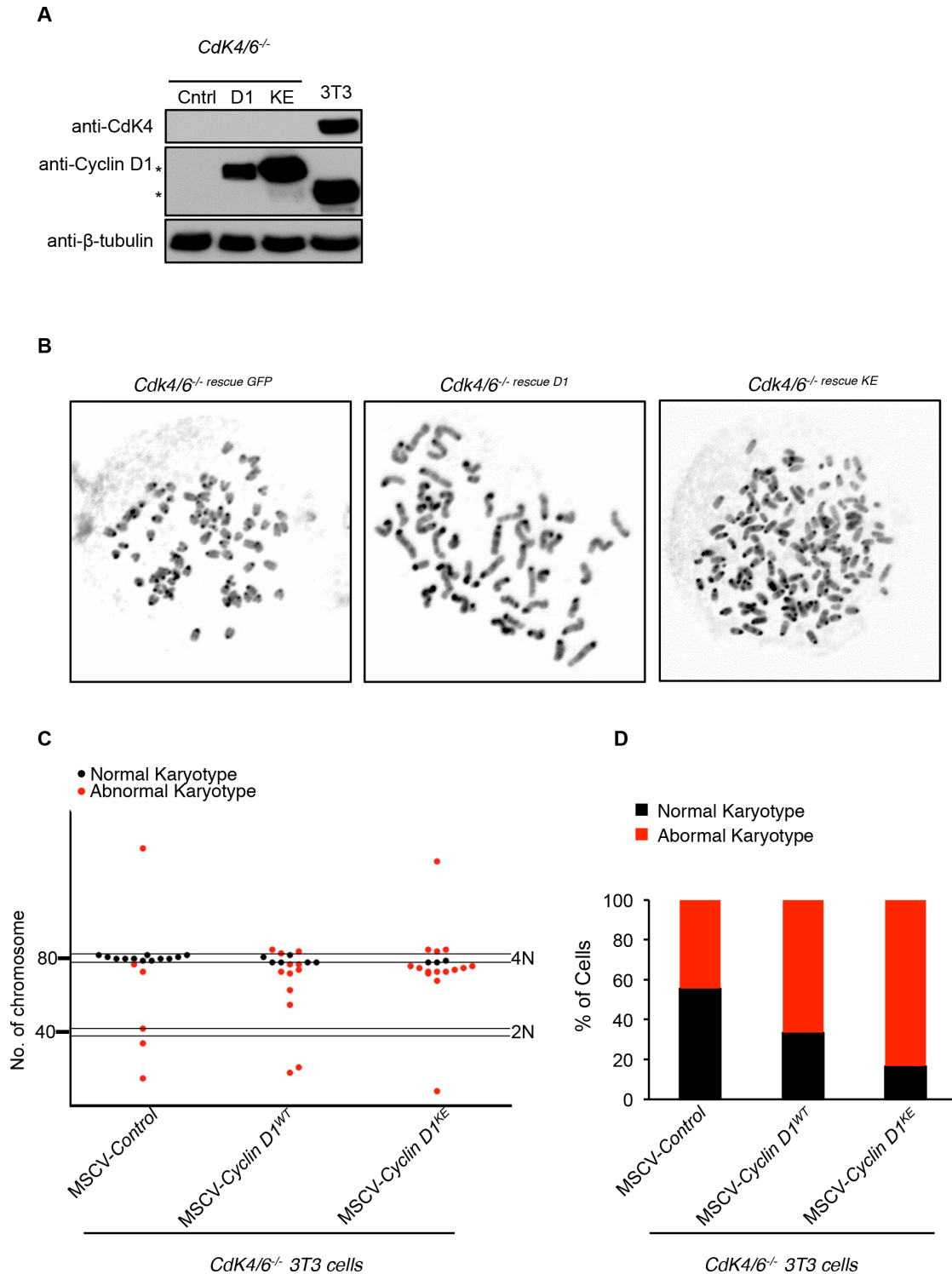
**Supplementary Figure 2: Compartmentalization of exogenous cyclin D1<sup>KE</sup> mimics endogenous cyclin D1 during transition from G1 to S phase.** Three representative images of *cyclin D1<sup>-/-</sup>KE Rescue* 3T3 fibroblast cells and one wild type 3T3 fibroblast cell arrested in G1 phase by treating with aphidicolin (+Aphidicolin (G1 phase)) for 16 hrs then released for 6 hours into S phase (-Aphidicolin (S phase)). Cells stained for DAPI (blue) and anti-FLAG (green) for exogenous cyclin D1<sup>KE</sup> and anti-cyclin D1 for endogenous cyclin D1.



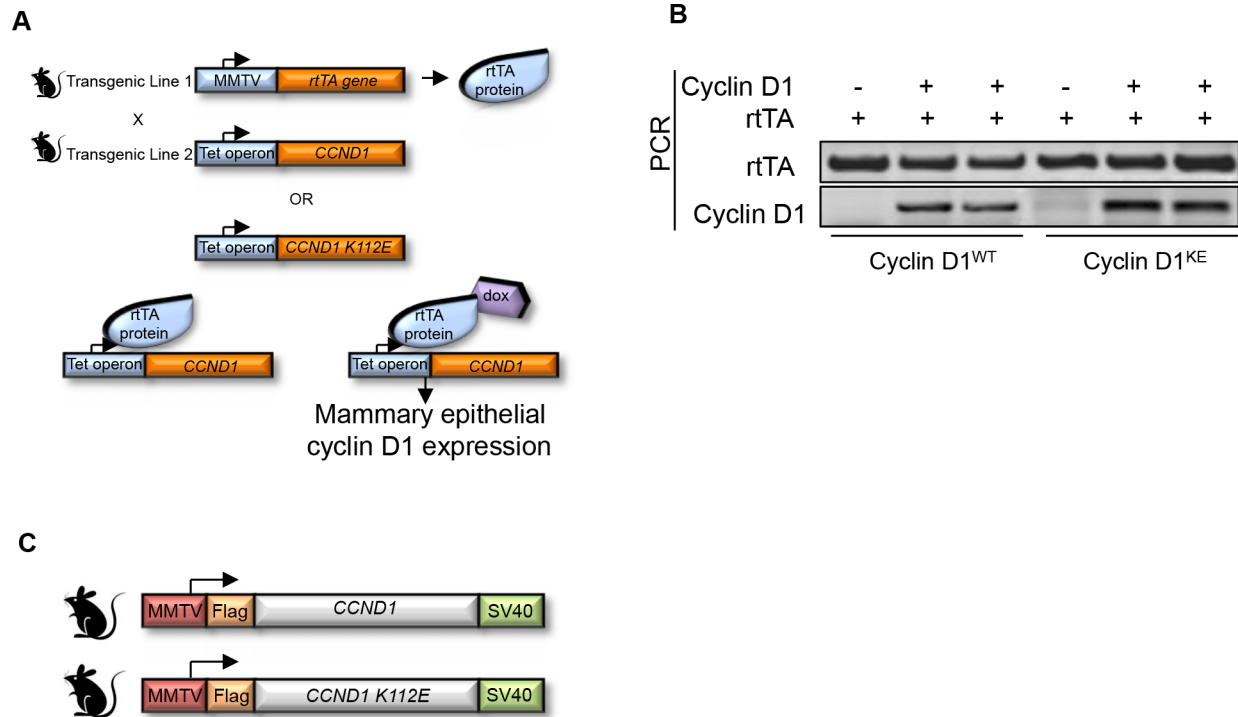
**Supplementary Figure 3: Enlarged representative metaphases from Spectral karyotyping (SKY).** Representative metaphases from Spectral karyotyping (SKY) on MEFs of *cyclin D1<sup>-/-</sup>-Control* at 72 hours (P6) (**A**), *cyclin D1<sup>-/-</sup>-KE<sup>Rescue</sup>* at 72 hours (P6) (**B**) and *cyclin D1<sup>-/-</sup>-KE<sup>Rescue</sup>* at 120 hours (**C**). Each panel contains the following images: inverted 4',6-diamidino-2-phenylindole (DAPI) image of the metaphase (top left corner), raw spectral image of the metaphase (top right) and classified metaphase of the same metaphase (lower panel).



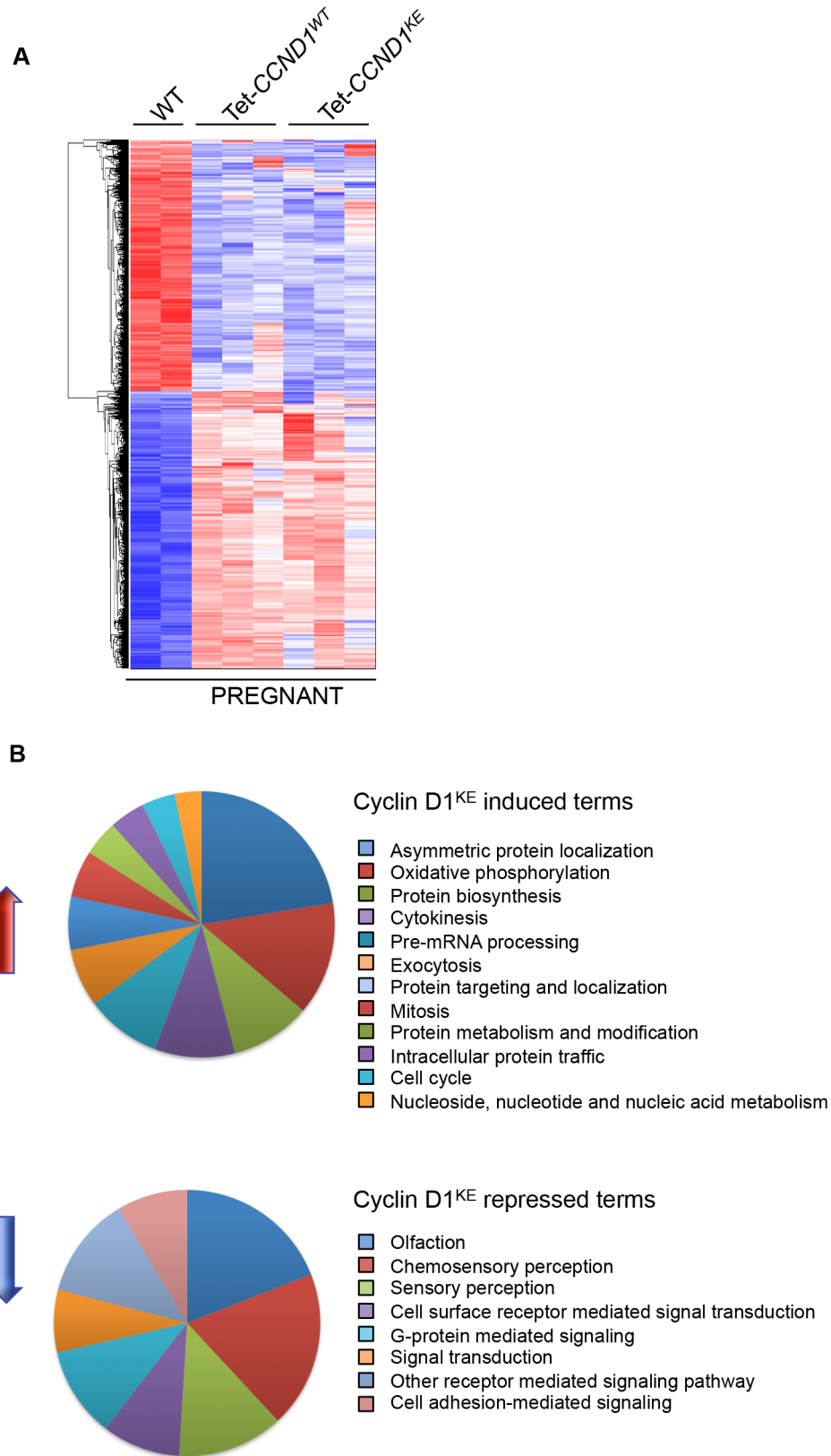
**Supplementary Figure 4: Antagonist treatment of *cyclin D1*<sup>-/-D1 Rescue</sup> and *cyclin D1*<sup>-/-KE Rescue</sup> MEF cells does not abrogate induction of aneuploidy. (A)** Western blot demonstrating phosphorylation of RB (S780) and exogenous cyclin D1 abundance in *cyclin D1*<sup>-/-Control</sup>, *cyclin D1*<sup>-/-KE Rescue</sup>, MEF cells and *cyclin D1*<sup>-/-D1 Rescue</sup> 3T3 fibroblast cells treated with vehicle control or 1 μM CDK4/6 antagonist-PD0332991 for 72 hrs **(B)** Representative metaphases from *cyclin D1*<sup>-/-Control</sup> and *cyclin D1*<sup>-/-KE Rescue</sup> MEF cell treated with control vehicle or CDK4/6 antagonist-PD0332991 for 72 hours. Metaphases were stained with DAPI and images converted to gray scale. **(C)** Scatter plots of chromosome numbers for *cyclin D1*<sup>-/-Control</sup> and *cyclin D1*<sup>-/-KE Rescue</sup> MEF cells treated with vehicle control or PD0332991. The parallel lines represent expected deviation from normal at 2N and 4N (+/- 2 chromosome) ( $n = 18$  metaphases per group). **(D)** Bar graphs showing the number of normal and abnormal karyotypes comparing *cyclin D1*<sup>-/-Control</sup> and *cyclin D1*<sup>-/-KE Rescue</sup> MEF cells at 72 hours from (D) vehicle treated and **(E)** PD0332991 treated MEF cells.



**Supplementary Figure 5: Cyclin D1 induces aneuploidy in *cdk4/6<sup>-/-</sup>* 3T3 cells.** (A) Western blot demonstrating *cdk4* and exogenous cyclin D1 abundance in *cdk4/6<sup>-/-</sup>* 3T3 fibroblast cells transduced with MSCV-Control, MSCV-Cyclin D1<sup>WT</sup> and MSCV-Cyclin D1<sup>KE</sup>. 3T3 wild type fibroblast cells represent positive control for *cdk4* abundance. (B) Representative metaphases from *cdk4/6<sup>-/-</sup>/control*, *cdk4/6<sup>-/-</sup>/cyclin D1<sup>WT</sup>* and *cdk4/6<sup>-/-</sup>/cyclin D1<sup>KE</sup>* 3T3 fibroblast cells. Metaphases were stained with DAPI and images converted to gray scale. (C) Scatter plots of chromosome numbers for *cdk4/6<sup>-/-</sup>/control*, *cdk4/6<sup>-/-</sup>/cyclin D1<sup>WT</sup>* and *cdk4/6<sup>-/-</sup>/cyclin D1<sup>KE</sup>* 3T3 fibroblast cells. The parallel lines represent expected deviation from normal at 2N and 4N (+/- 2 chromosome) (*n* = 15 metaphases per group). (D) Bar graph showing the number of normal and abnormal karyotypes comparing *cdk4/6<sup>-/-</sup>/control*, *cdk4/6<sup>-/-</sup>/cyclin D1<sup>WT</sup>* and *cdk4/6<sup>-/-</sup>/cyclin D1<sup>KE</sup>* 3T3 fibroblast cells at 72 hours.

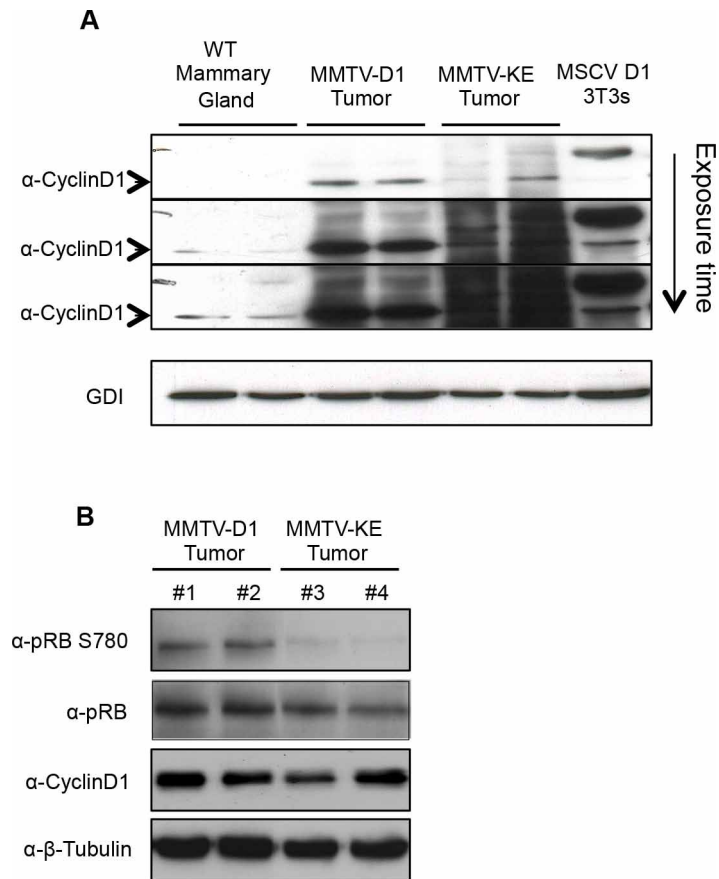


**Supplementary Figure 6: Overview of Tet-*CCND1*<sup>WT</sup> and Tet-*CCND1*<sup>KE</sup> mice.** (A) Schematic depiction of the tet-inducible mammary gland specific cyclin D1<sup>WT</sup> and cyclin D1<sup>KE</sup> transgenic lines. (B) Genotyping of Tet-*CCND1*<sup>WT</sup>, Tet-*CCND1*<sup>KE</sup>, and MMTV-rtTA transgenic mice. The cyclin D1<sup>WT</sup> portion of figure has been published previously<sup>20</sup>. (C) Schematic depiction of the mammary gland specific MMTV-*CCND1*<sup>WT</sup> and MMTV-*CCND1*<sup>KE</sup> transgenic lines.

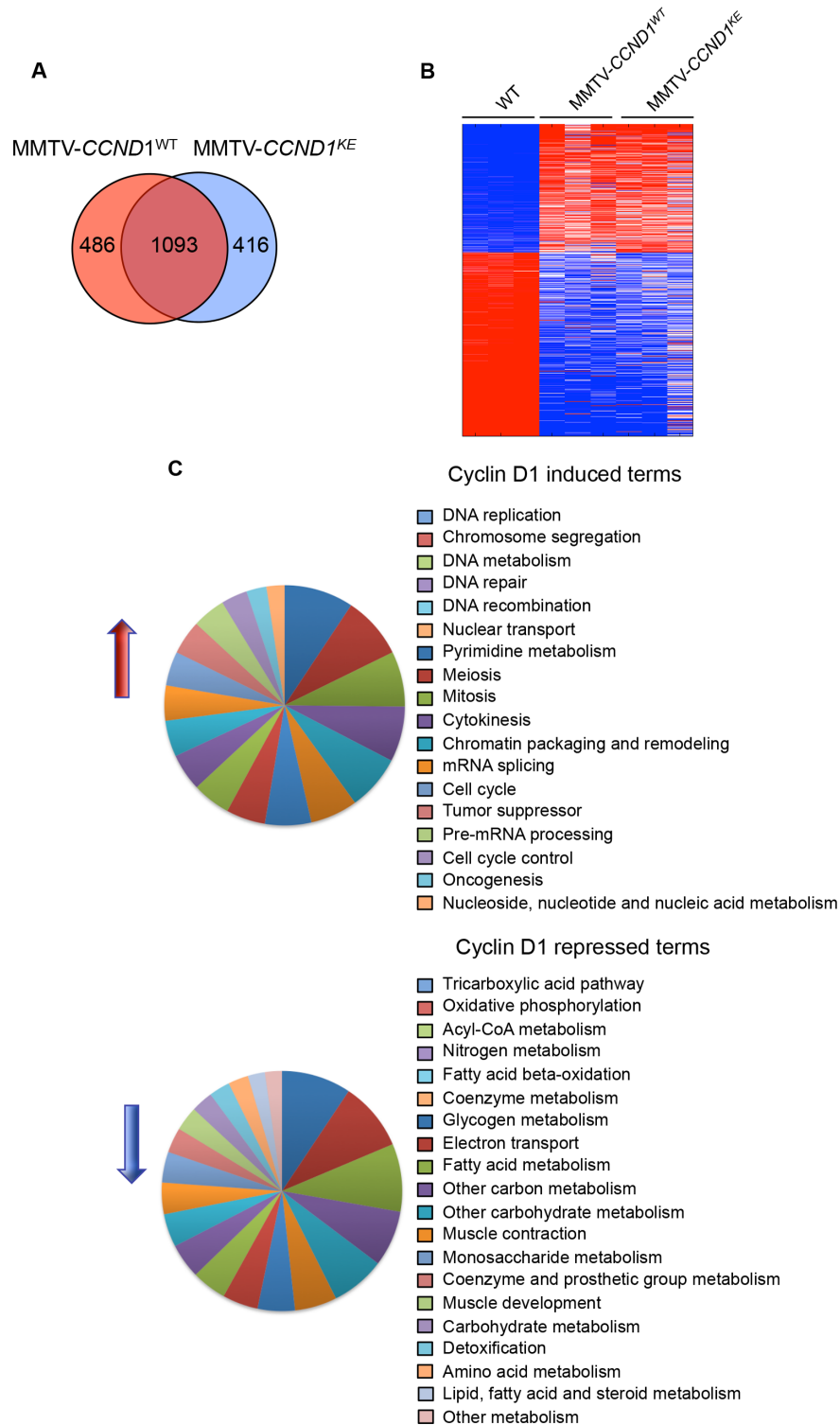


**Supplementary Figure 7: Microarray analysis of Tet-CCND1<sup>WT</sup> and Tet-CCND1<sup>KE</sup> mouse mammary glands.** (A) Hierarchical clustering of a superset of genes significantly differentially regulated between Tet-CCND1<sup>WT</sup> vs control and Tet-CCND1<sup>KE</sup> vs control mammary gland. (B) Pie chart representation of pathway analysis based on gene ontology (PANTHER-DAVID) of cyclin D1<sup>KE</sup> induced and repressed genes charted using an enrichment score.





**Supplementary Figure 8: Relative abundance of cyclin D1 and pRB phosphorylation status in mammary gland tumors derived from MMTV-*CCND1*<sup>WT</sup> and MMTV-*CCND1*<sup>KE</sup> mice.** Western blot demonstrating (A) cyclin D1 abundance WT mammary gland compared to tumors from MMTV-*CCND1*<sup>WT</sup> and MMTV-*CCND1*<sup>KE</sup> mice. MSCV-cyclin D1<sup>WT</sup> from 3T3 fibroblast cells used as a control; migration slightly retarded due to 3xFLAG. The WT mammary gland and MMTV-D1 tumor lanes have been published previously. (B) Western blot analysis of pRB S780 phosphorylation status in tumors from MMTV-*CCND1*<sup>WT</sup> and MMTV-*CCND1*<sup>KE</sup> mice.



**Supplementary Figure 9: Microarray analysis of MMTV-CCND1<sup>WT</sup> and MMTV-CCND1<sup>KE</sup> mouse mammary tumors. (A)** Venn diagram representing genes differentially regulated by MMTV-CCND1<sup>WT</sup> ( $n = 3$ ) and MMTV-CCND1<sup>KE</sup> ( $n = 3$ ) and visualized by **(B)** Hierarchical clustering of 1995 significantly regulated genes ( $P \leq 0.01$  and fold change  $> 2$ ). **(C)** Pie chart representation of functional annotation clustering (DAVID) of cyclin D1<sup>KE</sup> induced and repressed terms based on percentage enrichment score of the top hits.

**Supplementary Table 1: Histological analysis of tumors from wild type FVB, MMTV-Cyclin D1<sup>WT</sup> and MMTV-Cyclin D1<sup>KE</sup> Mice**

<b>WT FVB Mice (6 tumors)</b>	
Adenocarcinoma with squamous differentiation	
Papillary adenocarcinoma	
Partially necrotic secretory adenocarcinoma	
EMT-phenotype	
Complex adenoacanthoma	
EMT-phenotype	
<b>MMTV-Cyclin D1<sup>WT</sup> Mice (10 tumors)</b>	
Keratoacanthoma	
EMT-phenotype	
Keratoacanthoma	
Adenosquamous AC	
Adenosquamous AC and Spindle cell tumor	
Adenocarcinoma	
Papillary Adenocarcinoma	
Adenosquamous AC (Keratoacanthoma)	
Lobular hyperplasia and secretory adenocarcinoma	
EMT-phenotype	
<b>MMTV-Cyclin D1<sup>KE</sup> Mice (7 tumors)</b>	
Partially necrotic adenocarcinoma	
Adenocarcinoma	
Papillary adenocarcinoma	
EMT-phenotype	
Complex adenoacanthoma	
Papillary adenocarcinoma	
EMT-phenotype	

**Supplementary Table 2: Interval data from cyclin D1<sup>KE</sup> associated genomic regions generated by ChIP-Seq**

<b>Description</b>	<b>Act Regions</b>
Number of Intervals Converted	4296
Number of Intervals within -10000/+10000 bp of NCBI Genes	3494
Number of Intervals NOT within -10000/+10000 bp of NCBI Genes	802
Number of NCBI Genes with Intervals within -10000/+10000 bp	4446
Number of Intervals within Promoter Region (-7500/+2500 bp of NCBI Gene Start)	3305
Number of NCBI Genes with intervals in Promoters (-7500/2500 bp of start)	3780

**Supplementary Table 3: Full list of transcription factor binding sites identified in cyclin DIKE peak interval sequences**

Transcription Factor Name	P-value	Experimental Hits
SP1	4.70E-07	1717
RREB1	2.64E-06	1233
GABPA	2.20E-08	810
EWSR1-FLI1	9.78E-07	805
ETS1	3.82E-07	729
ELK4	7.83E-08	551
FEV	8.19E-08	521
NR2F1	2.41E-06	512
MZF1_1-4	3.06E-07	489
CTCF	1.29E-07	483
ZFX	1.90E-07	477
ELK1	8.06E-08	471
GATA2	7.40E-07	471
NFYA	6.12E-08	422
MYF	6.45E-08	418
PLAG1	5.06E-07	384
ZNF143	4.06E-07	382
EGR1	2.25E-08	378
ZEB1	1.29E-07	351
IRF1	1.85E-06	343
ARNT	6.21E-07	337
USF1	6.21E-07	337
NR4A1	1.03E-06	316
PAX5	1.50E-07	304
CREB1	9.10E-08	299
STAT3	3.15E-07	294
SPIB	3.76E-08	290
NHLH1	4.61E-07	289
ESR1	1.65E-07	274
PPARG::RXRA	6.42E-08	274
SPI1	5.73E-07	267
STAT1	1.51E-07	261
REST	5.00E-08	255
FOXD3	1.09E-07	244
MZF1_5-13	1.51E-07	233
NFIC	7.09E-08	219

(Continued)

Transcription Factor Name	P-value	Experimental Hits
MAX	1.02E-07	217
YY1	1.41E-07	204
ESR2	2.57E-07	186
MYCN	7.59E-08	176
REL	7.61E-08	174
ESRRB	7.10E-07	168
HNF4A	2.37E-07	165
RUNX1	1.80E-08	161
NFE2L1::MAFG	6.41E-07	157
FOXA1	2.92E-07	144
RXR::RAR_DR5	4.72E-08	141
HAND1::TCFE2A	1.00E-08	140
NFKB1	6.46E-08	137
ARID3A	1.07E-06	136
FOXO3	1.37E-07	136
MYC::MAX	2.33E-07	132
PAX2	1.95E-08	130
TP53	3.05E-07	121
NF-KAPPAB	2.05E-08	120
NR2E3	2.99E-08	120
IRF2	1.00E-08	118
GFI	2.75E-07	117
KLF4	4.63E-07	116
SPZ1	9.76E-08	115
RELA	7.28E-08	112
MAFB	3.95E-08	111
PPARG	1.00E-08	111
TAL1::TCF3	4.13E-02	108
MYC	6.75E-08	107
AP1	8.31E-08	106
NFE2L2	1.25E-05	103
AR	1.00E-08	101
FOXA2	1.12E-05	101
MEF2A	2.24E-07	101
E2F1	2.36E-06	99
NFATC2	1.59E-05	90
INSM1	2.47E-03	89

(Continued)

Transcription Factor Name	P-value	Experimental Hits
NR1H2::RXRA	4.96E-04	88
RORA_1	1.41E-07	88
EVI1	1.29E-02	87
SOX2	1.19E-04	87
SRY	2.12E-07	87
RXRA::VDR	7.53E-07	86
GATA3_1	1.00E-08	81
NR3C1	1.27E-07	80
GATA1	2.60E-07	78
SOX5	2.23E-06	78
NKX2-5	2.00E-06	76
FOXF2	7.68E-03	75
DDIT3::CEBPA	4.84E-04	73
MIZF	1.00E-08	72
FOXL1	1.17E-05	66
MYB	1.04E-07	66
SOX17	8.70E-03	65
HIF1A::ARNT	4.34E-04	64
HNF1A	2.39E-03	63
LHX3	1.90E-02	62
NKX3-2	8.93E-04	62
CEBPA	2.73E-08	61
PAX6	3.81E-03	59
TAL1::GATA1	4.12E-04	59
SOX9	5.15E-06	58
FOXC1	3.81E-05	55
HLF	6.66E-08	54
NOBOX	1.07E-06	54
FOXD1	2.14E-05	53
HNF1B	2.61E-06	53
RORA_2	3.55E-04	52
SRF	1.35E-04	51
T	2.91E-02	51
BRCA1	8.58E-05	48
PAX4	1.34E-04	48
PRRX2	2.62E-03	45
HOXA5	1.90E-02	43
PDX1	7.33E-03	39
HLTF	3.41E-02	35

**Supplementary Dataset 1: Full list of genes regulated by cyclin D1<sup>WT</sup> and cyclin D1<sup>KE</sup> in mammary gland of transgenic mice**

**Supplementary Dataset 2: Full gene list of the mammary tumors for MMTV-cyclin D1<sup>KE</sup> and MMTV-cyclin D1<sup>WT</sup> mice, includes list of genes common to the two sets**

**Supplementary Dataset 3: Complete list of cyclin D1<sup>KE</sup> bound genes by ChIP-Seq analysis**