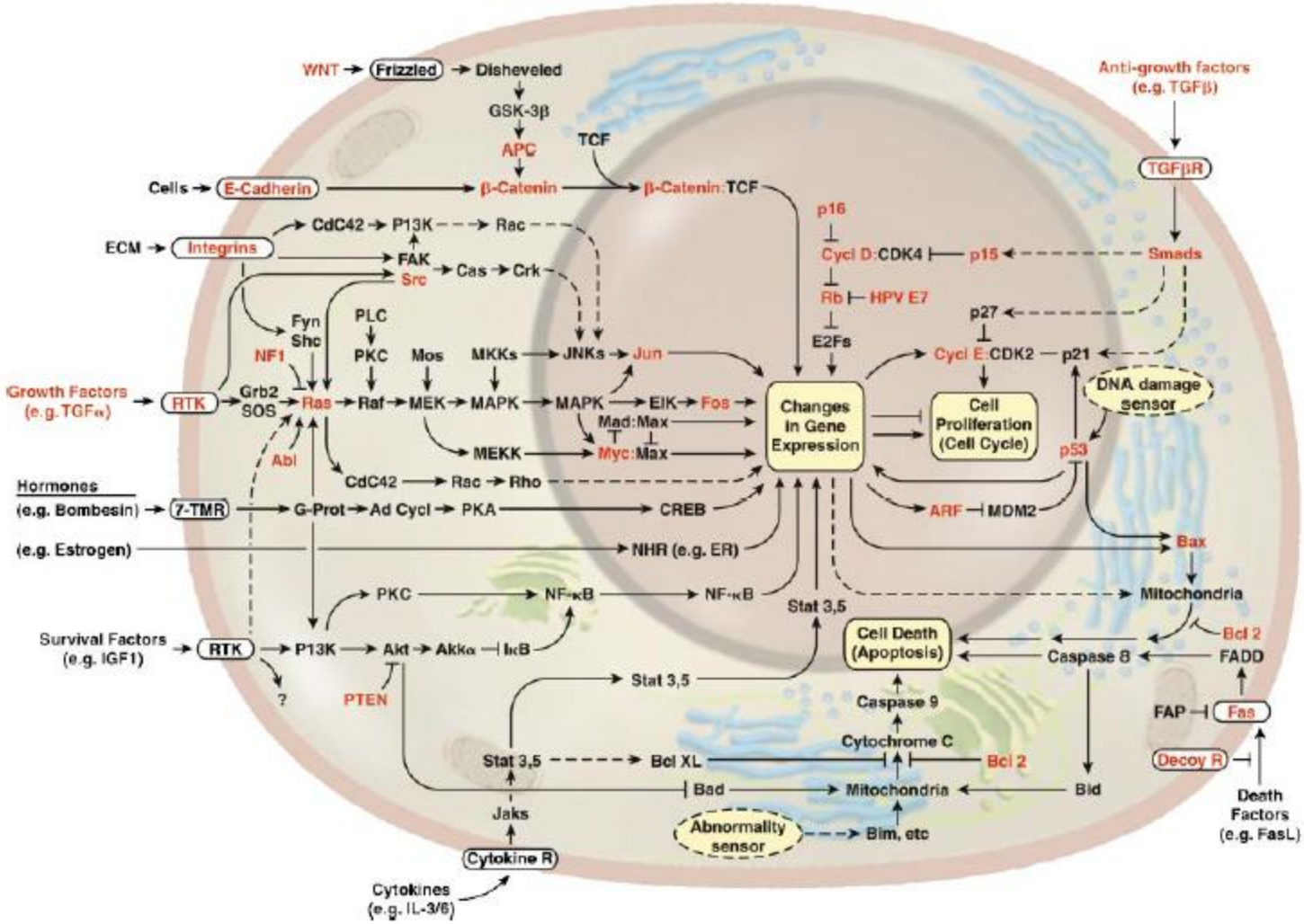
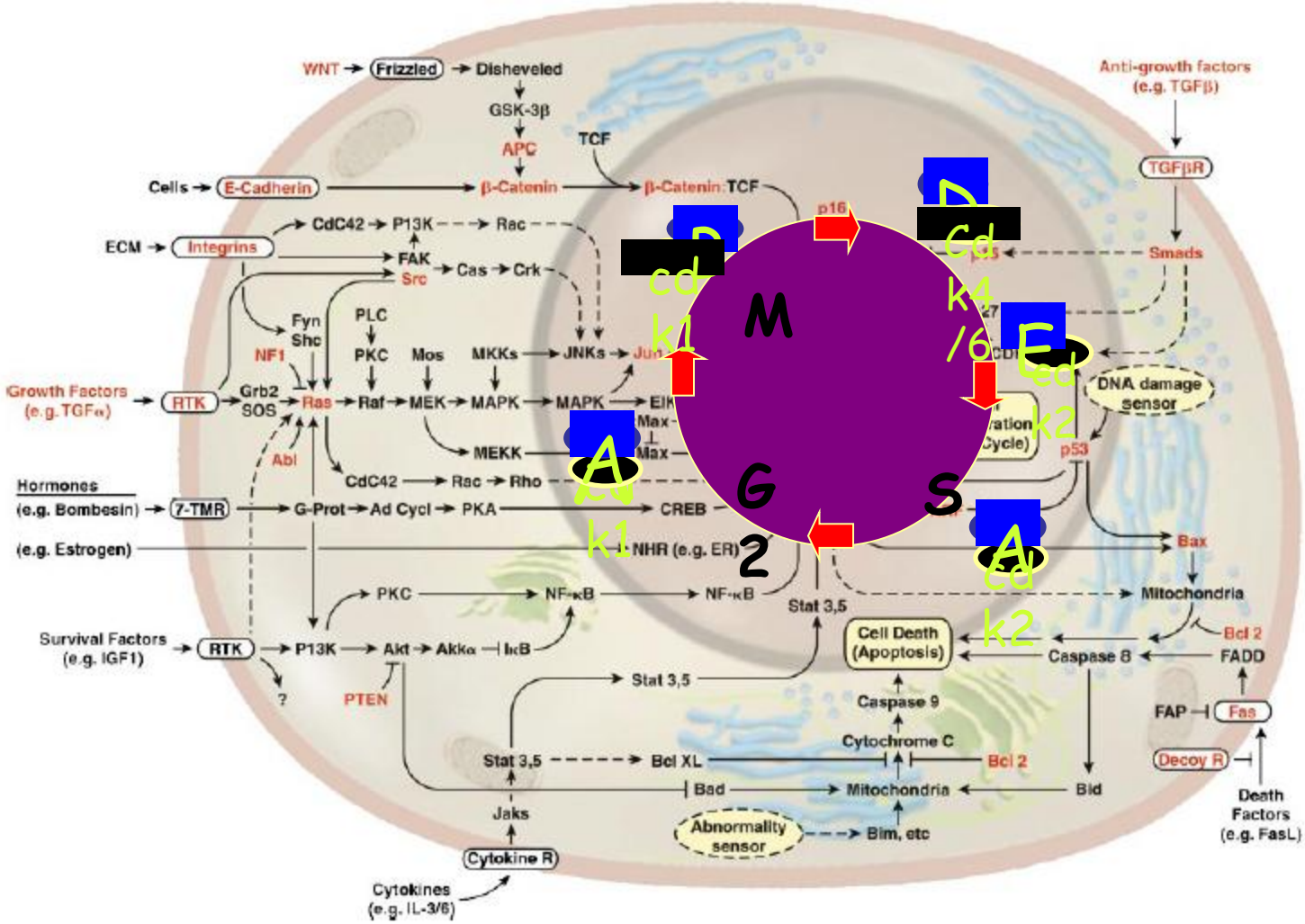
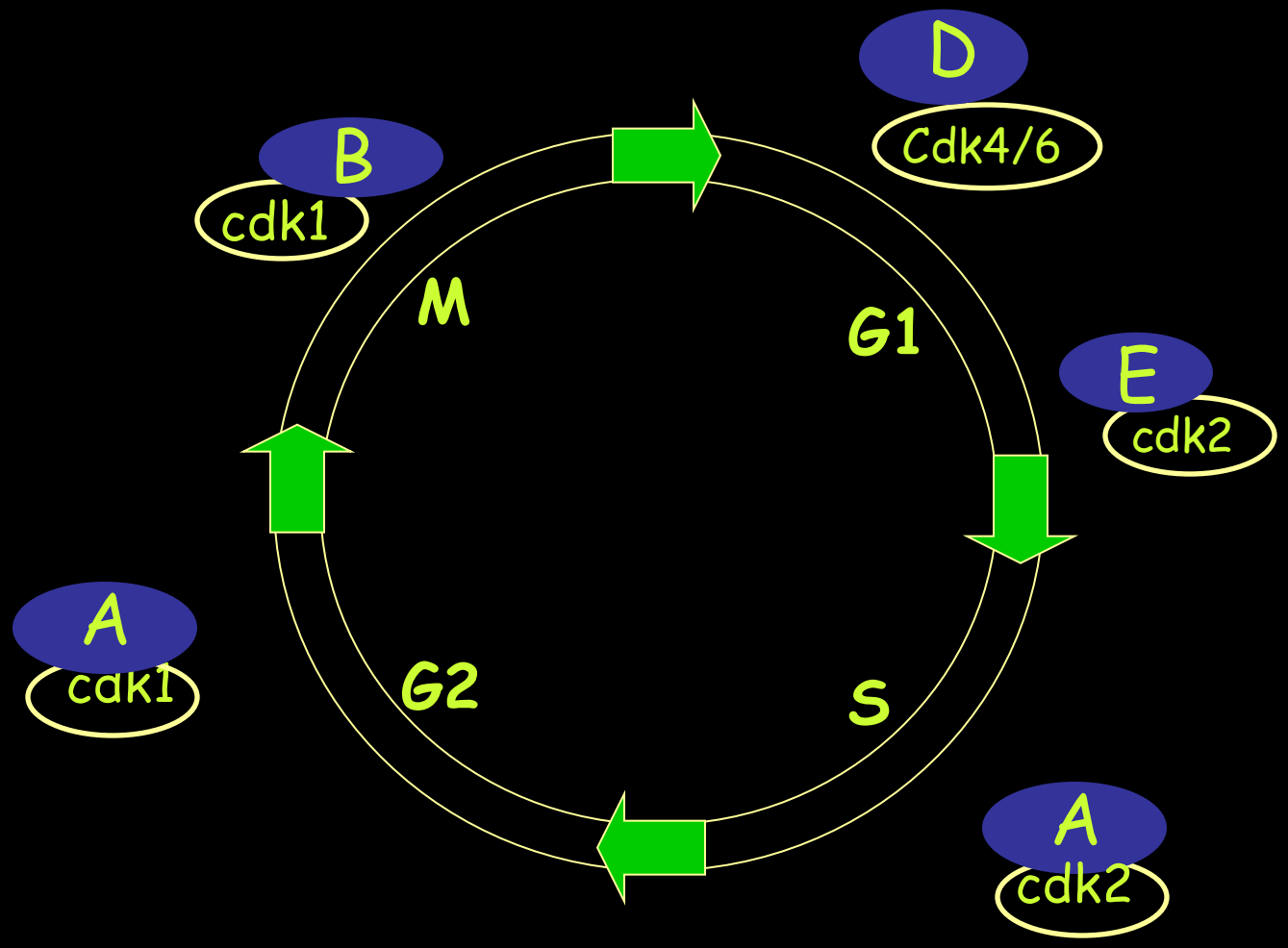
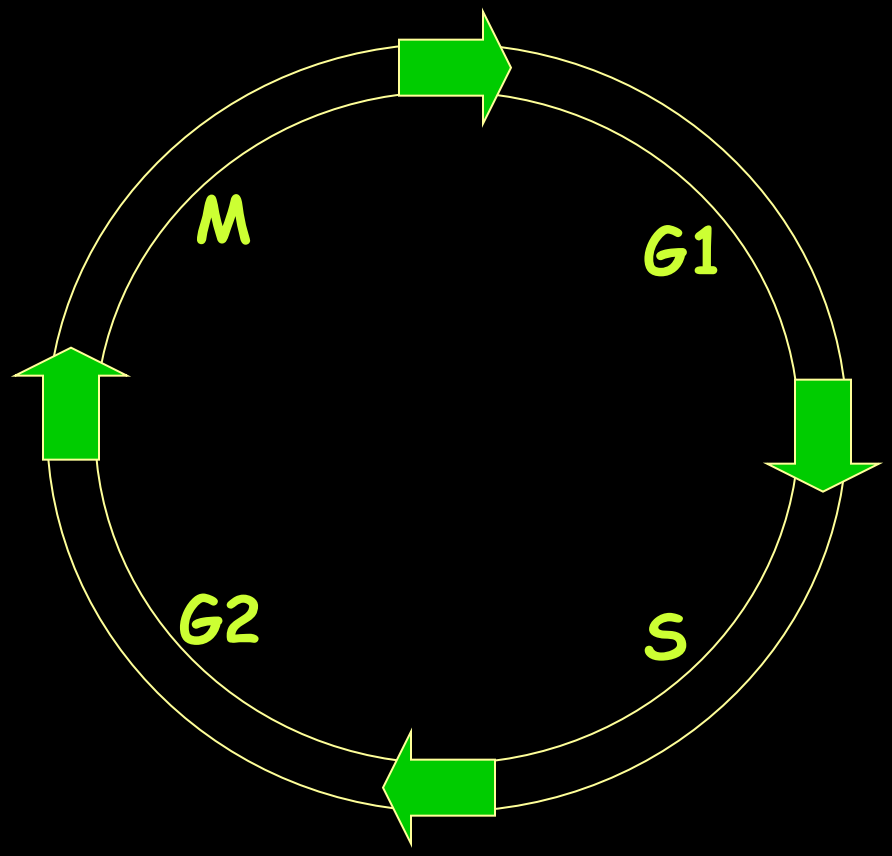
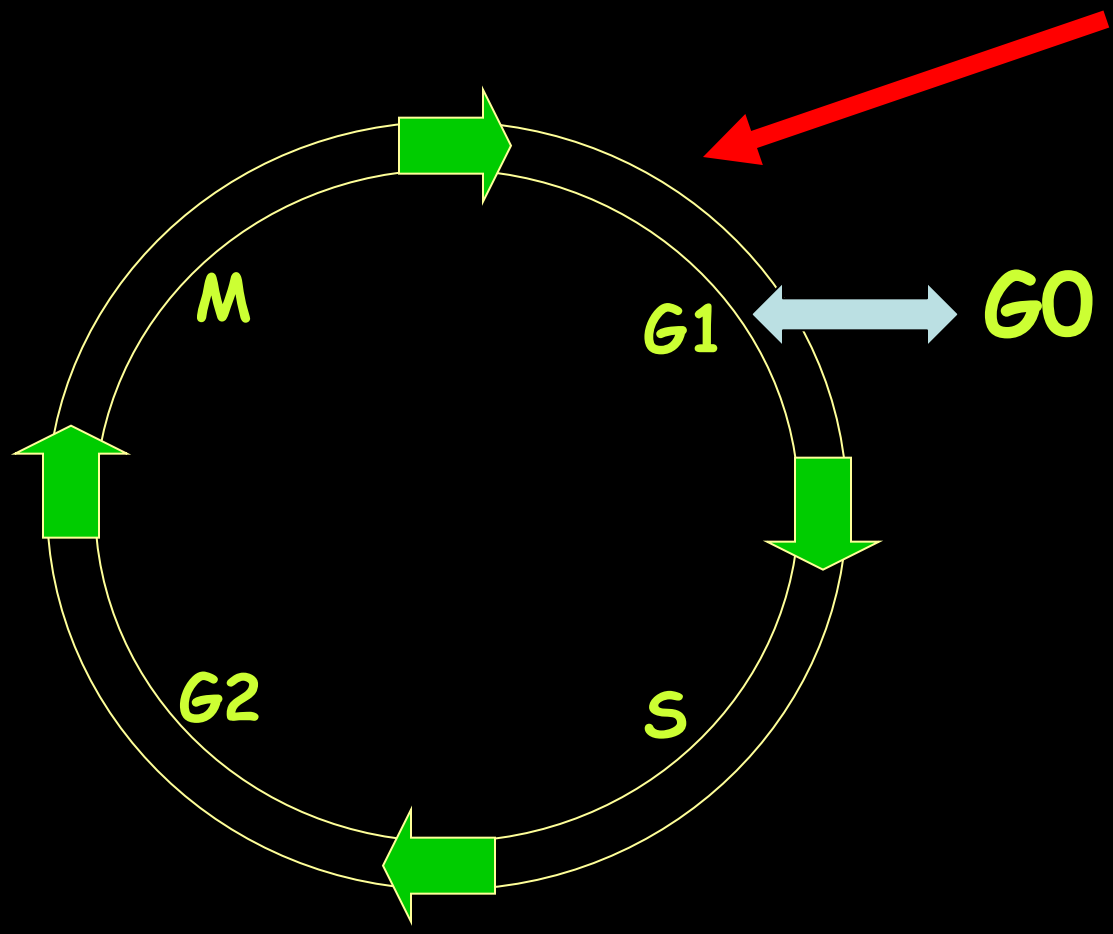

WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
β-Catenin
β-Catenin:TCF
Cells
E-Cadherin
ECM
Integrins
CdC42
P13K
Rac
FAK
Src
Cas
Crk
Fyn
Shc
PLC
PKC
NF1
Mos
MKKs
JNKs
Jun
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
MAPK
Elk
Fos
Mad:Max
Myc:Max
MEKK
Abl
CdC42
Rac
Rho
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
(e.g. Estrogen)
NHR (e.g. ER)
PKC
NF-κB
NF-κB
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
?
PTEN
Stat 3,5
Stat 3,5
Stat 3,5
Bcl XL
Bad
Jaks
Cytokine R
Cytokines
(e.g. IL-3/6)
p16
Cycl D:CDK4
p15
Rb
HPV E7
E2Fs
p27
Cycl E:CDK2
p21
Changes
in Gene
Expression
Cell
Proliferation
(Cell Cycle)
DNA damage
sensor
p53
ARF
MDM2
Bax
Mitochondria
Bcl 2
Cell Death
(Apoptosis)
Caspase 8
FADD
Caspase 9
Cytochrome C
Bcl 2
Mitochondria
Bid
Abnormality
sensor
Bim, etc
FAP
Fas
Decoy R
Death
Factors
(e.g. FasL)
Anti-growth factors
(e.g. TGFβ)
TGFβR
Smads

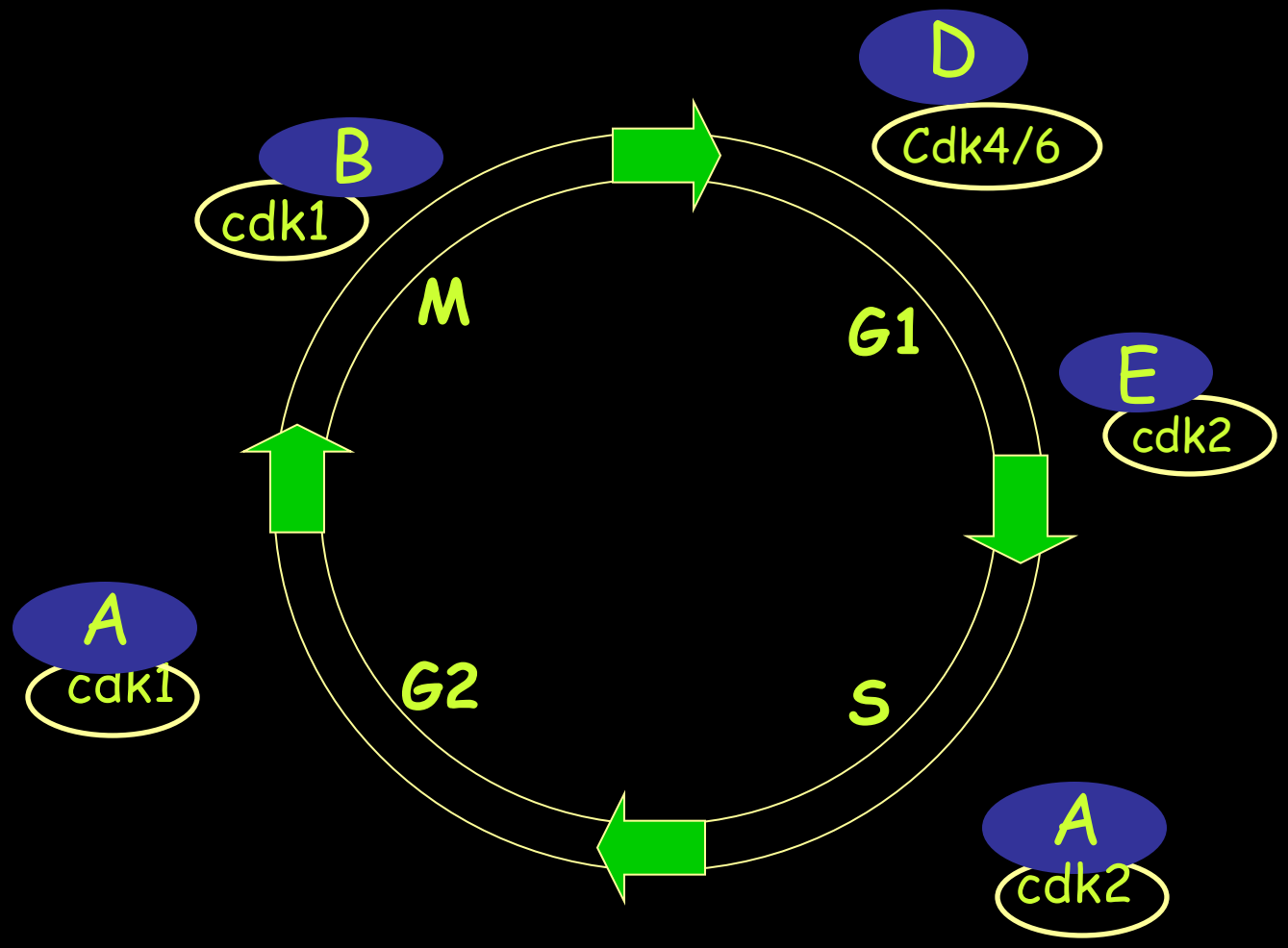

WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
β-Catenin
β-Catenin:TCF
Cells
E-Cadherin
ECM
Integrins
CdC42
P13K
Rac
FAK
Src
Cas
Crk
Fyn
Shc
PLC
PKC
NF1
Mos
MKKs
JNKs
Jun
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
Elk
Max
MEKK
Abl
CdC42
Rac
Rho
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
(e.g. Estrogen)
NHR (e.g. ER)
PKC
NF-κB
NF-κB
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
?
PTEN
Stat 3,5
Stat 3,5
Stat 3,5
Bcl XL
Bad
Jaks
Cytokine R
Cytokines
(e.g. IL-3/6)
Anti-growth factors
(e.g. TGFβ)
TGFβR
Smads
p16
p53
DNA damage
sensor
p53
Bax
Mitochondria
Bcl 2
FADD
Caspase 8
Cell Death
(Apoptosis)
Caspase 9
Cytochrome C
Bcl 2
Mitochondria
Bid
Abnormality
sensor
Bim, etc
FAP
Fas
Decoy R
Death
Factors
(e.g. FasL)
M
G
S
2
cd
k1
Cd
k4
/6
ed
k2
ed
k1
ed
k2

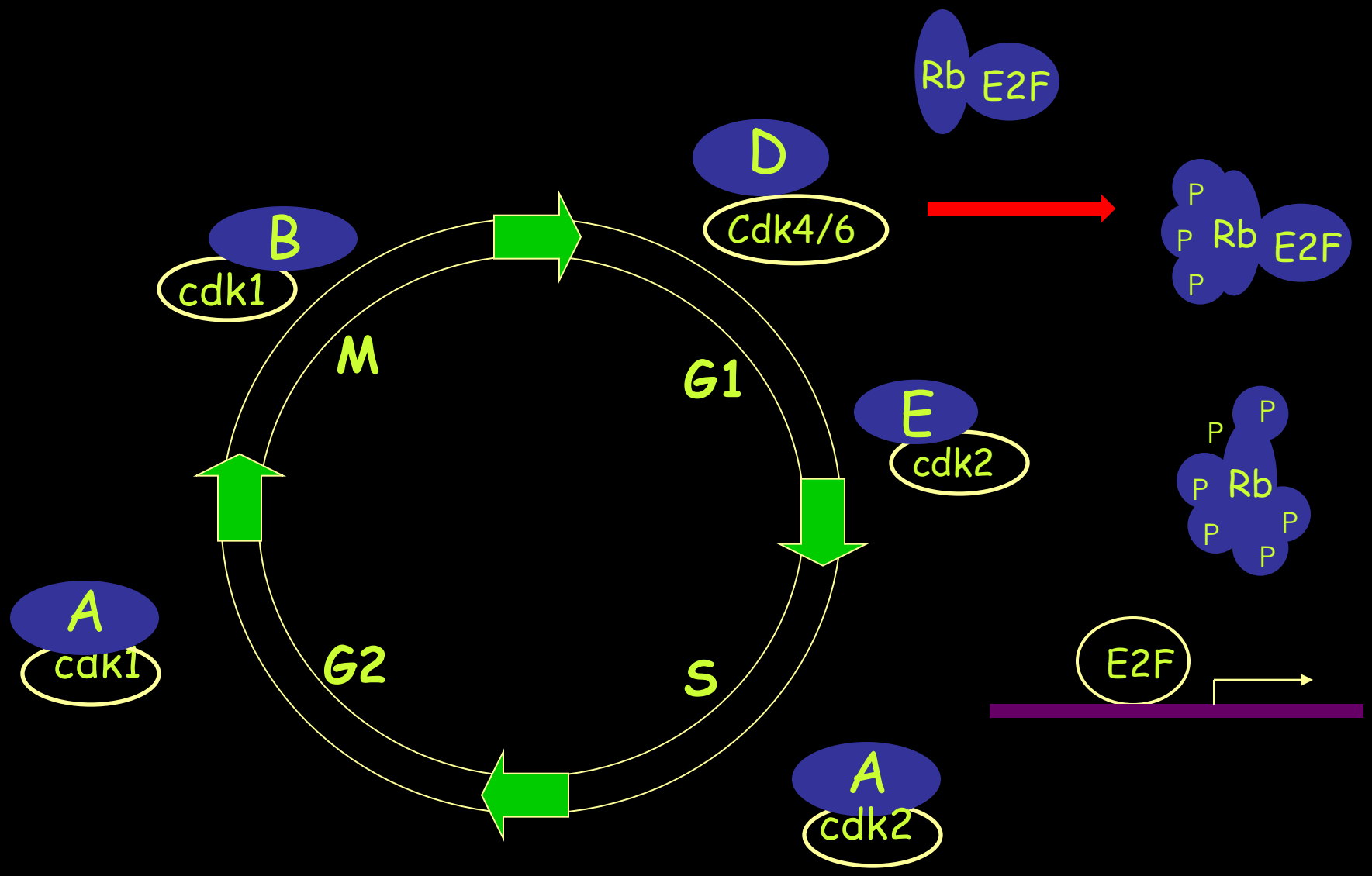

D
Cdk4/6
B
cdk1
M
G1
E
cdk2
A
cak1
G2
S
A
cdk2

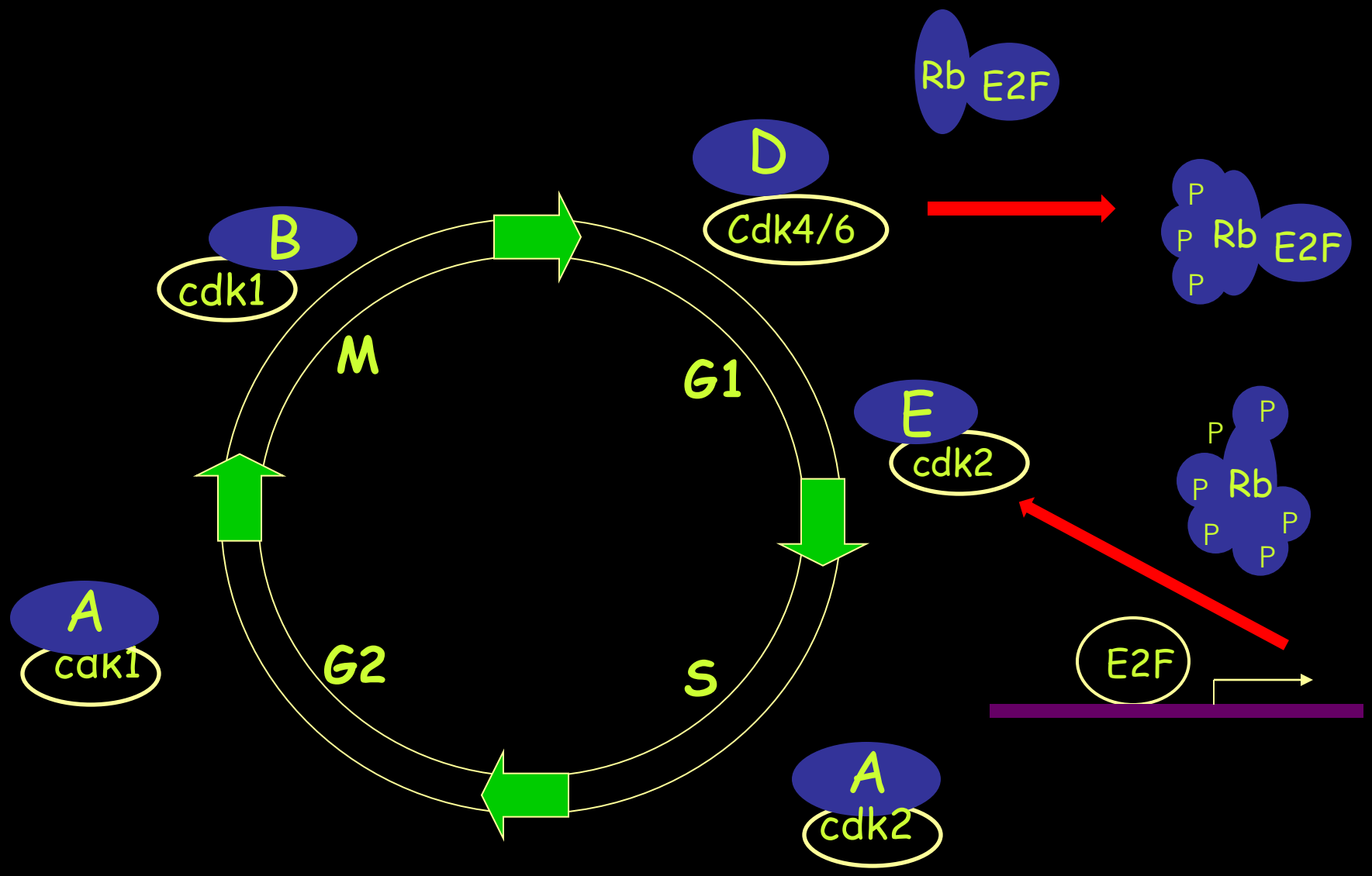

M
G1
S
G2

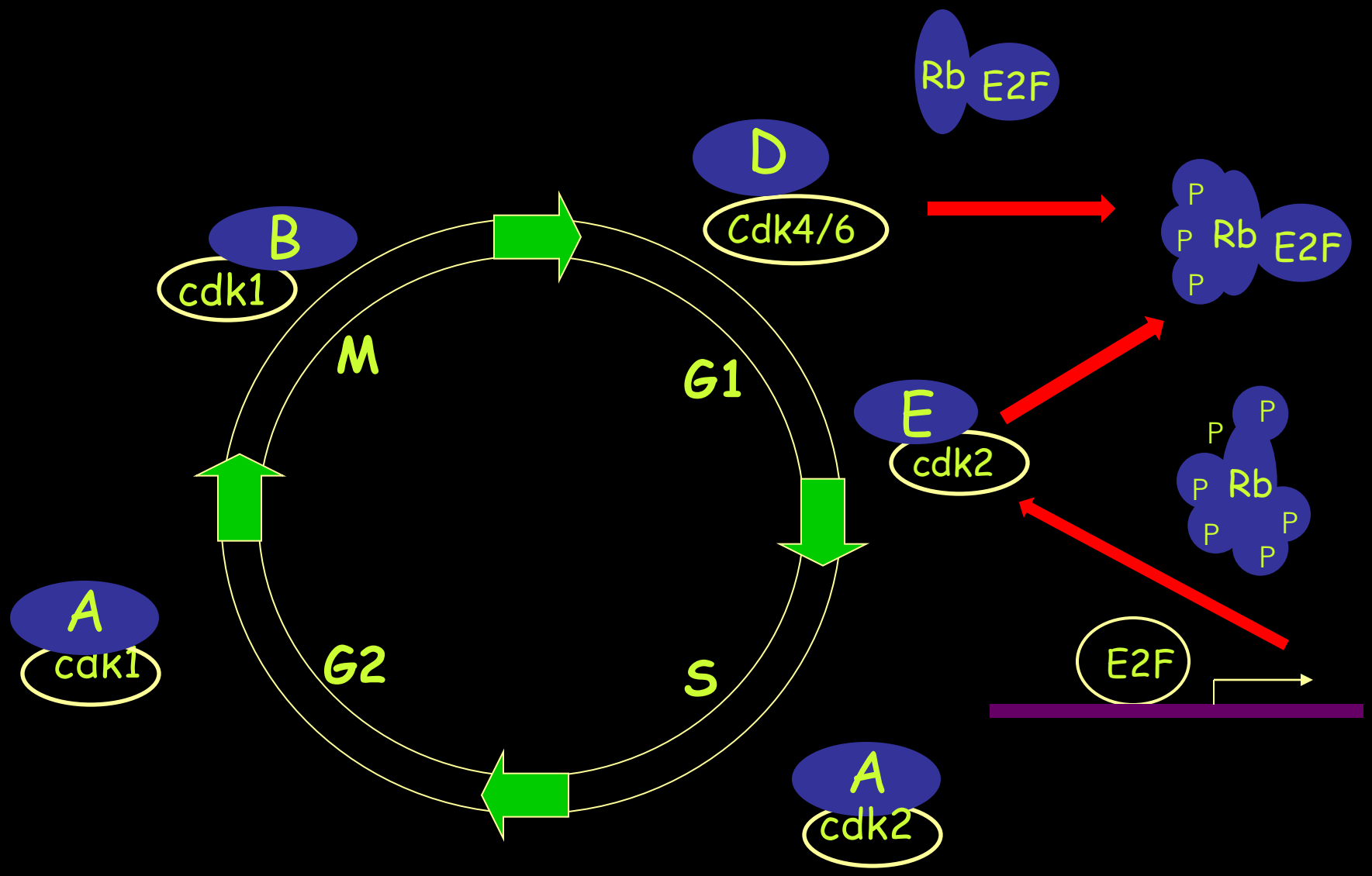
MITOGENS and ONCOGENES

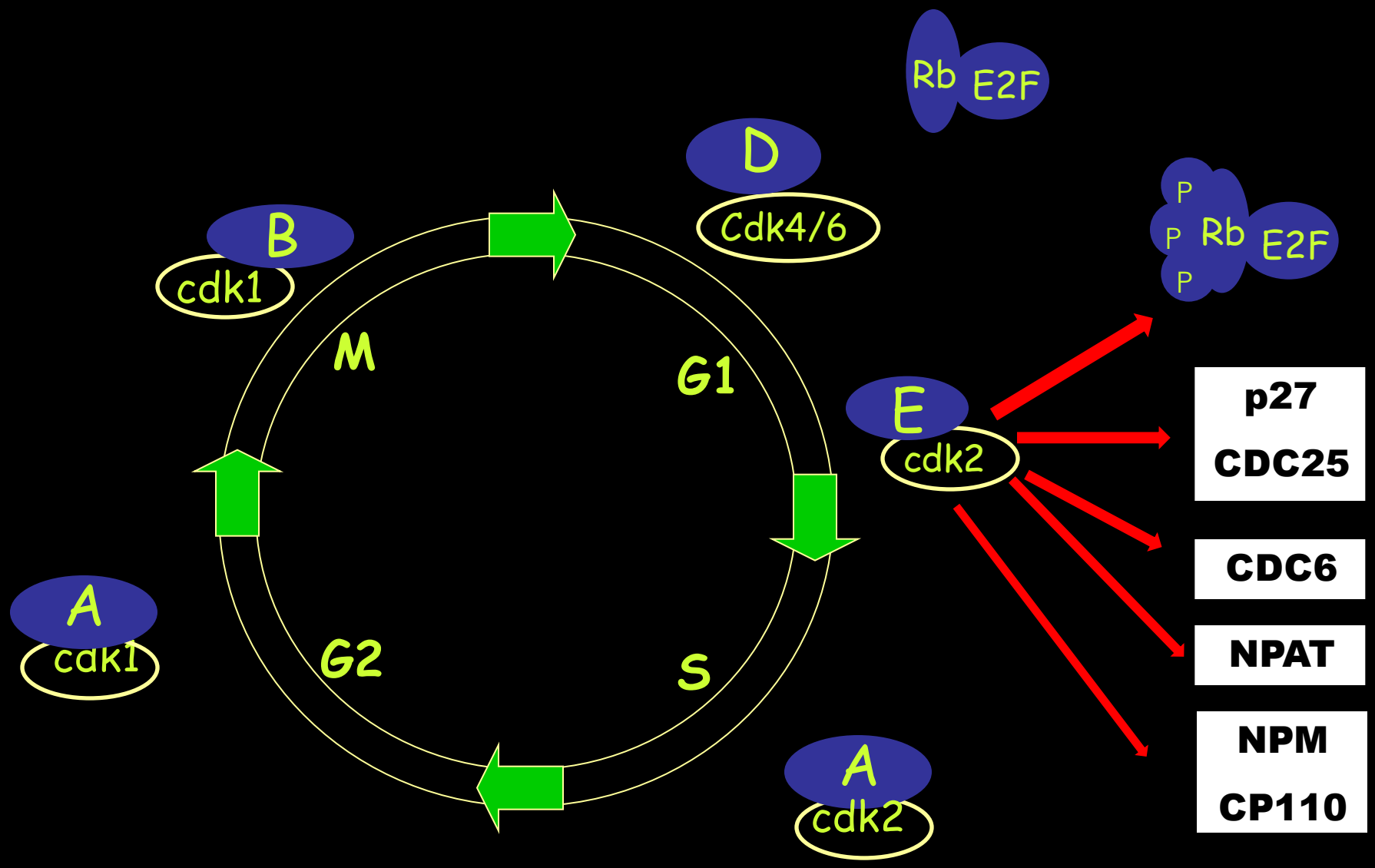



D
Cdk4/6
B
cdk1
M
G1
E
cdk2
A
cak1
G2
S
A
cdk2

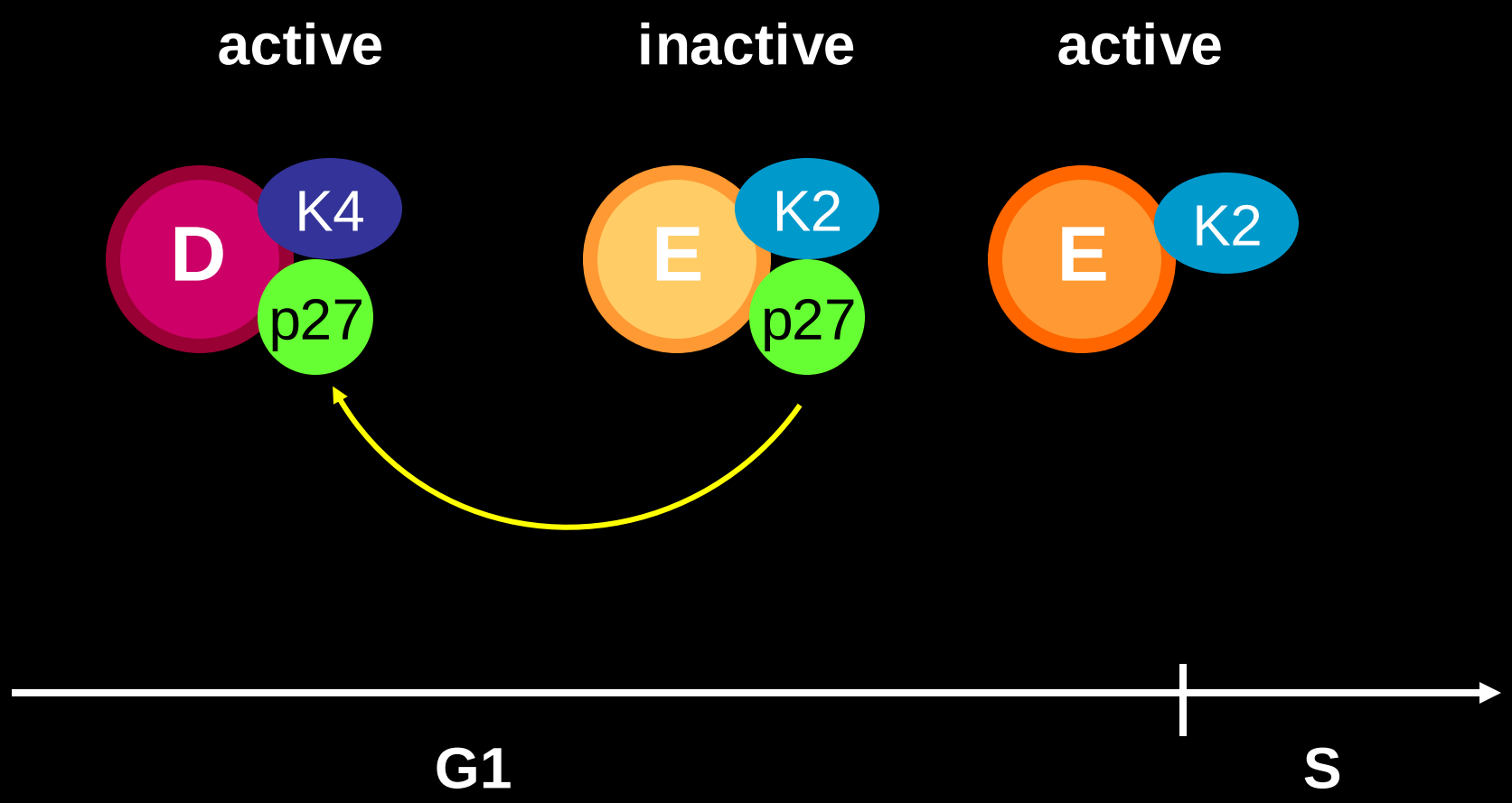

Rb
E2F
D
Cdk4/6
P
P
P
Rb
E2F
B
cdk1
M
G1
E
cdk2
P
P
P
Rb
P
P
P
A
cak1
G2
S
E2F
A
cdk2

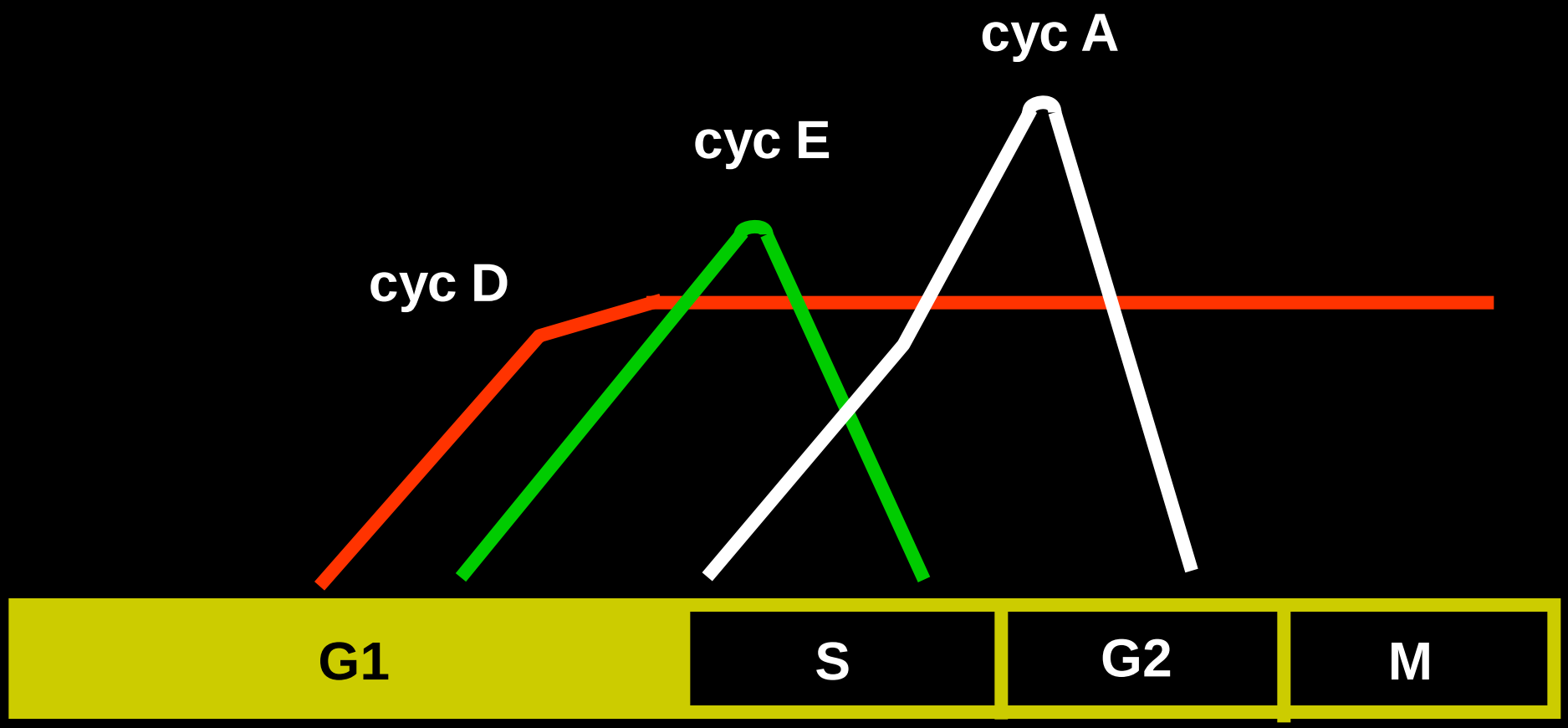

Rb
E2F
D
Cdk4/6
P
P
P
Rb
E2F
B
cdk1
M
G1
E
cdk2
P
P
P
P
P
P
Rb
A
cak1
G2
S
E2F
A
cdk2

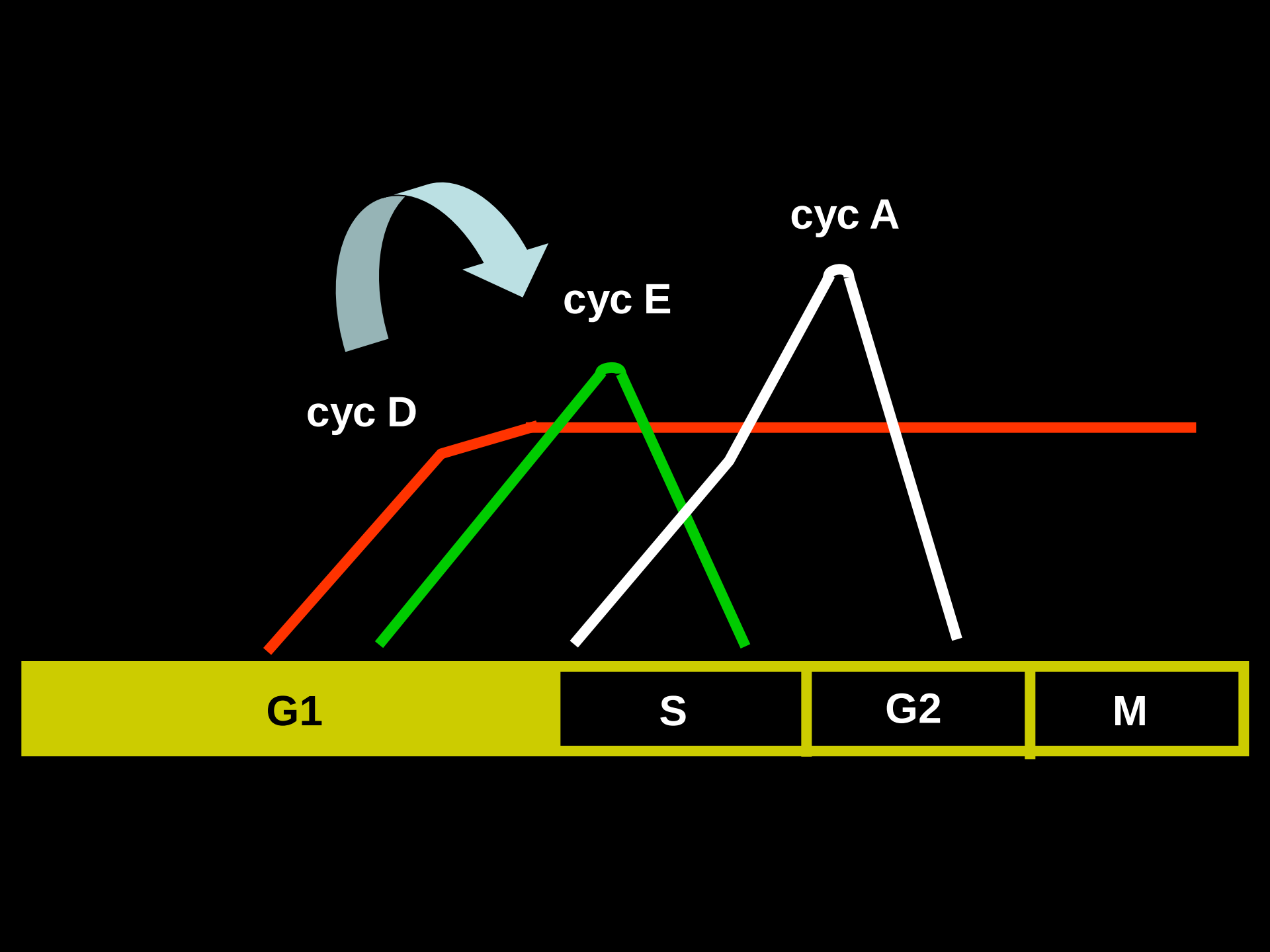

Rb
E2F
D
Cdk4/6
P
P
P
Rb
E2F
B
cdk1
M
G1
E
cdk2
P
P
P
Rb
P
P
P
A
cak1
G2
S
E2F
A
cdk2

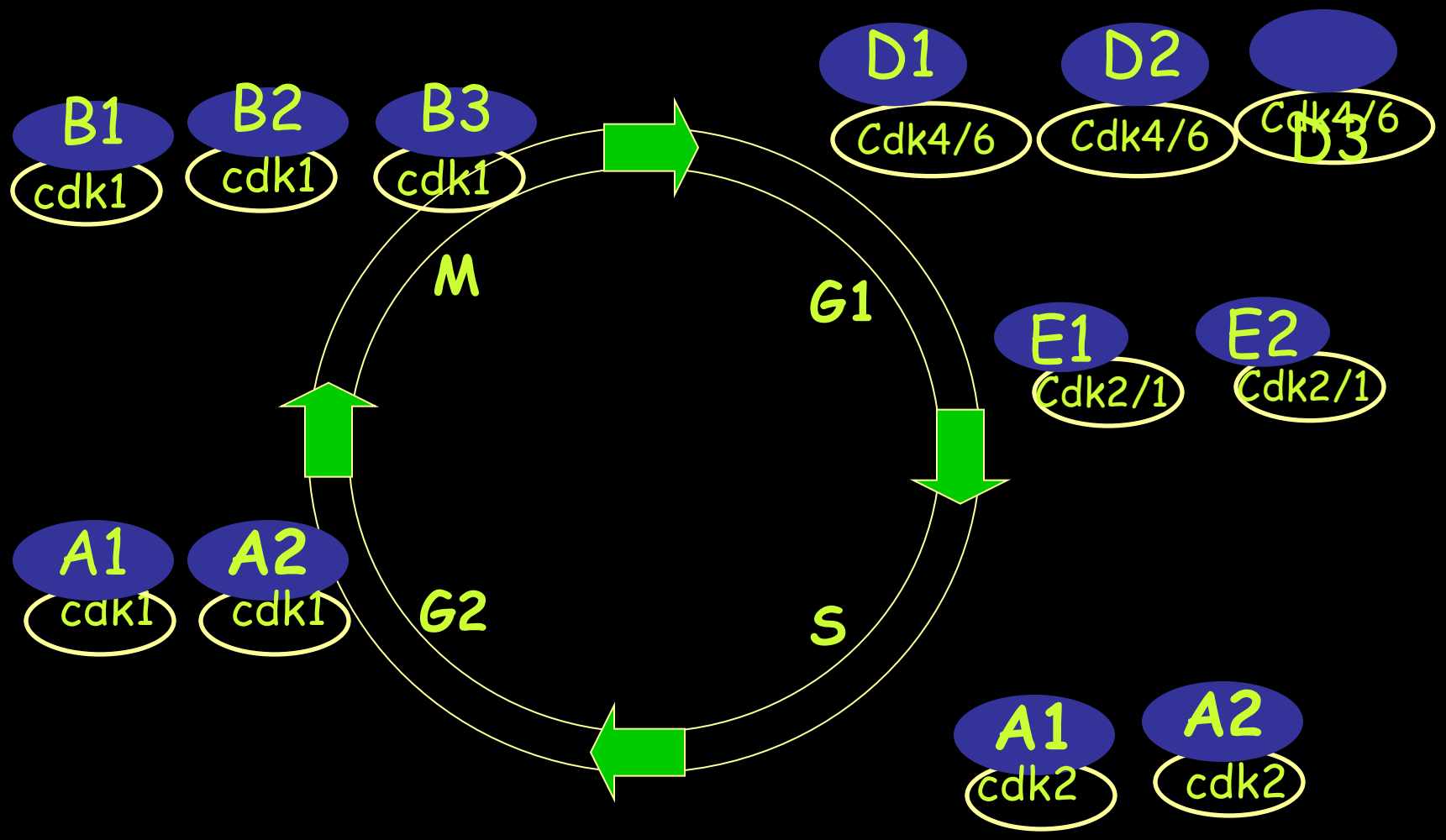

Rb
E2F
D
Cdk4/6
P
P
P
Rb
E2F
B
cdk1
M
G1
E
cdk2
p27
CDC25
CDC6
NPAT
NPM
CP110
A
cak1
G2
S
A
cdk2

INHIBITOR TITRATION

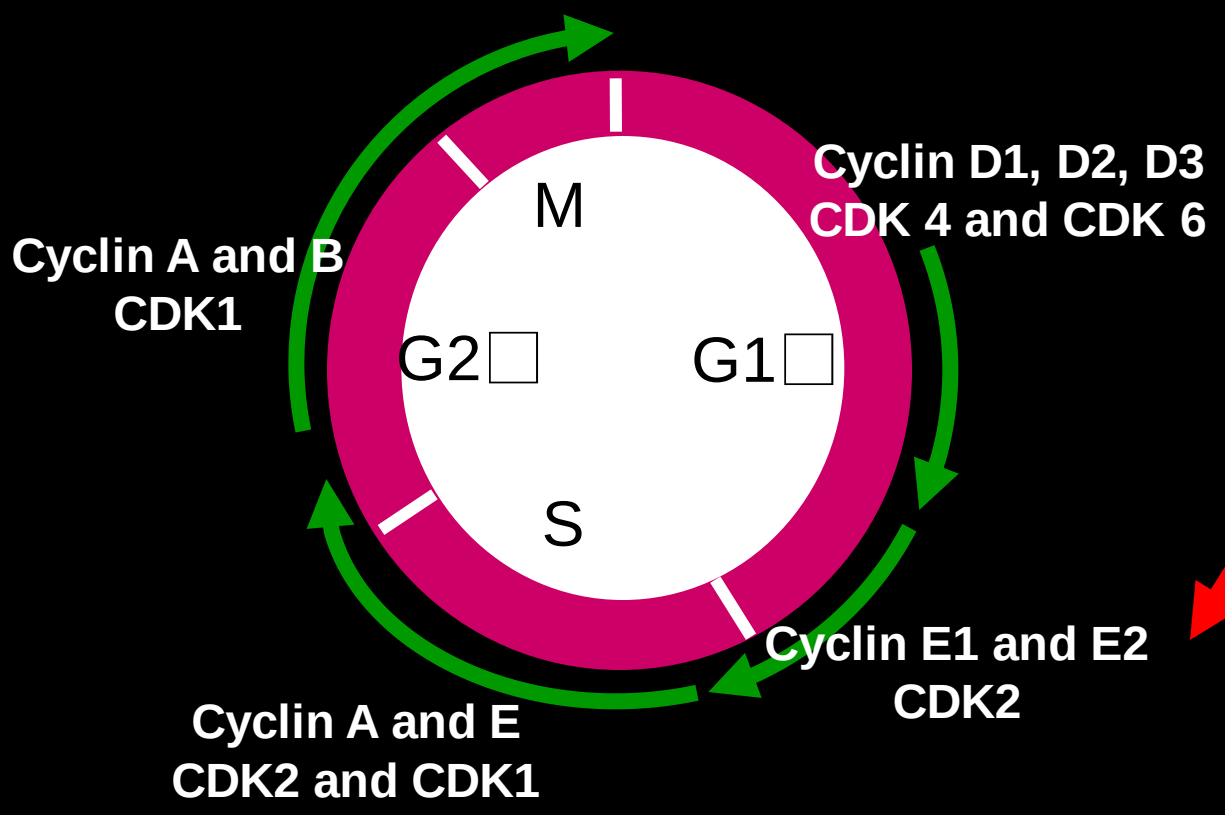



cyc A
cyc E
cyc D
G1
S
G2
M


cyc A
cyc E
cyc D
G1
S
G2
M


B1
cdk1
B2
cdk1
B3
cdk1
D1
Cdk4/6
D2
Cdk4/6
Cdk4/6
D3
M
G1
E1
Cdk2/1
E2
Cdk2/1
A1
cdk1
A2
cdk1
G2
S
A1
cdk2
A2
cdk2

MITOGENS and ONCOGENES

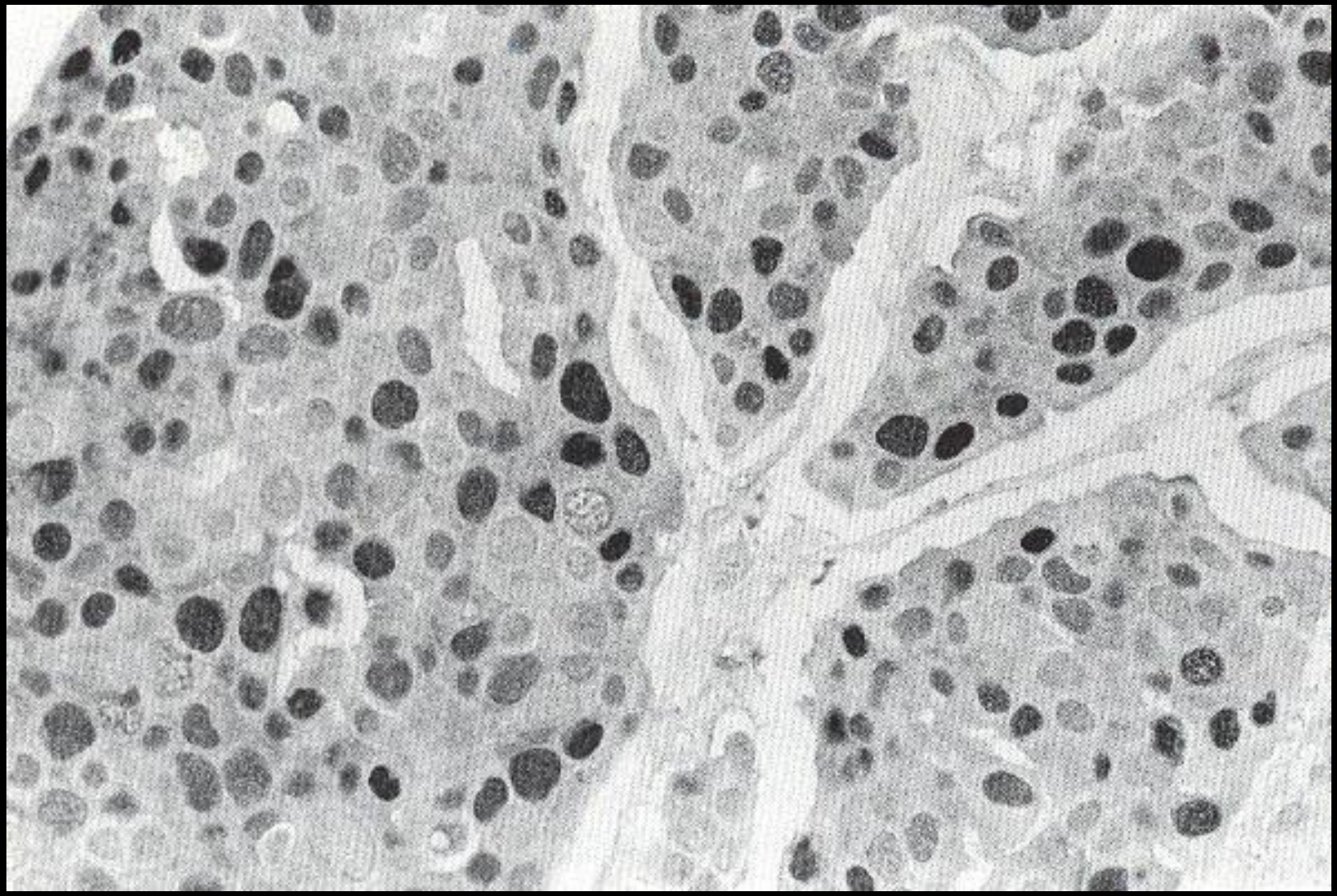


Top 10 amplified regions in 3131 cancer DNAs

Rank	Location	Gene	q-value
1)	8q	*MYC*	8e-121
2)	**11q**	***CCND1***	**4e-108**
3)	17q	*ERBB2*	3e-92
4)	**12q**	**CDK4**	**2e-72**
5)	14q	*NKX2-1*	2e-51
6)	12q	*MDM2*	9e-49
7)	7p	*EGFR*	1e-48
8)	1q	*MCL1*	1e-39
9)	8p	*FGFR1*	6e-36
10)	12q	*KRAS*	1e-36
16)	5p	*TERT*	2e-16

Beroukhim, Mermel et al., *Nature* 2010

Cyclin D1 overexpression in breast cancer



Pubmed search, April 12, 2011

Cyclin D1: 13,744 papers (~477 this year)

Cyclin D: 9,711 papers

Cyclin D1 and Breast Cancer

Cyclin D1 is located at chromosome 11q13, which is amplified in 15-20% of primary breast cancers

Cyclin D1 protein is overexpressed in about 50%of human mammary carcinomas

MMTV-Cyclin D1 transgenic mice develop mammary adenocarcinomas

D1

D2

D3

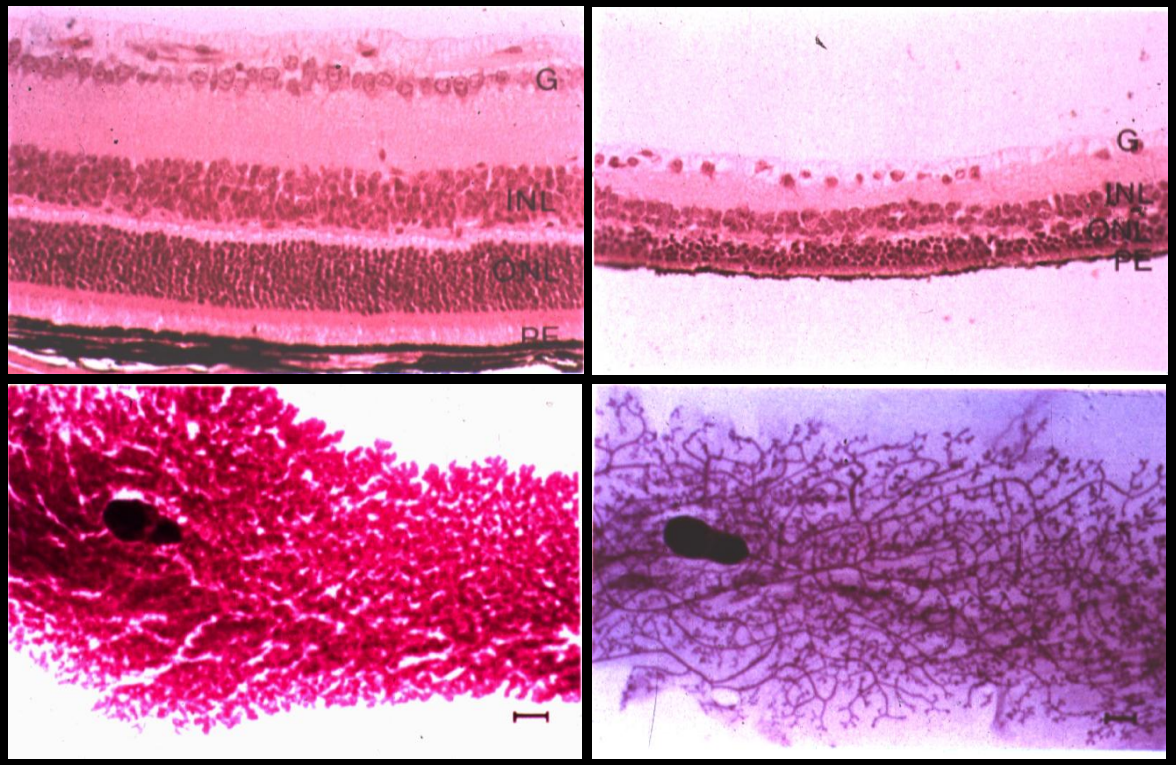
~~D1~~

D2

D3

Cyclin D1-/- mice are viable

wt **D1-/-**



Cyclin D1-deficient mice

develop relatively normally

Cyclin D1 might be be a good target in breast cancers

Oncogenic signals

↓

Cyclin D1

↓

Abnormal cell proliferation

↓

Tumor formation

Oncogenic signals

↓

~~**Cyclin D1**~~

↓

Abnormal cell proliferation?

↓

Tumor formation?

Does the absence of cyclin D1 protect cyclin D1 -/- mice from breast cancers?

Are cyclin D1-/- mice resistant to breast cancers?

Cross D1-/- mice

with MMTV-oncogene mice

Ras
ErbB2/Neu
Myc
Wnt

MMTV-oncogene
D1+/+

↓

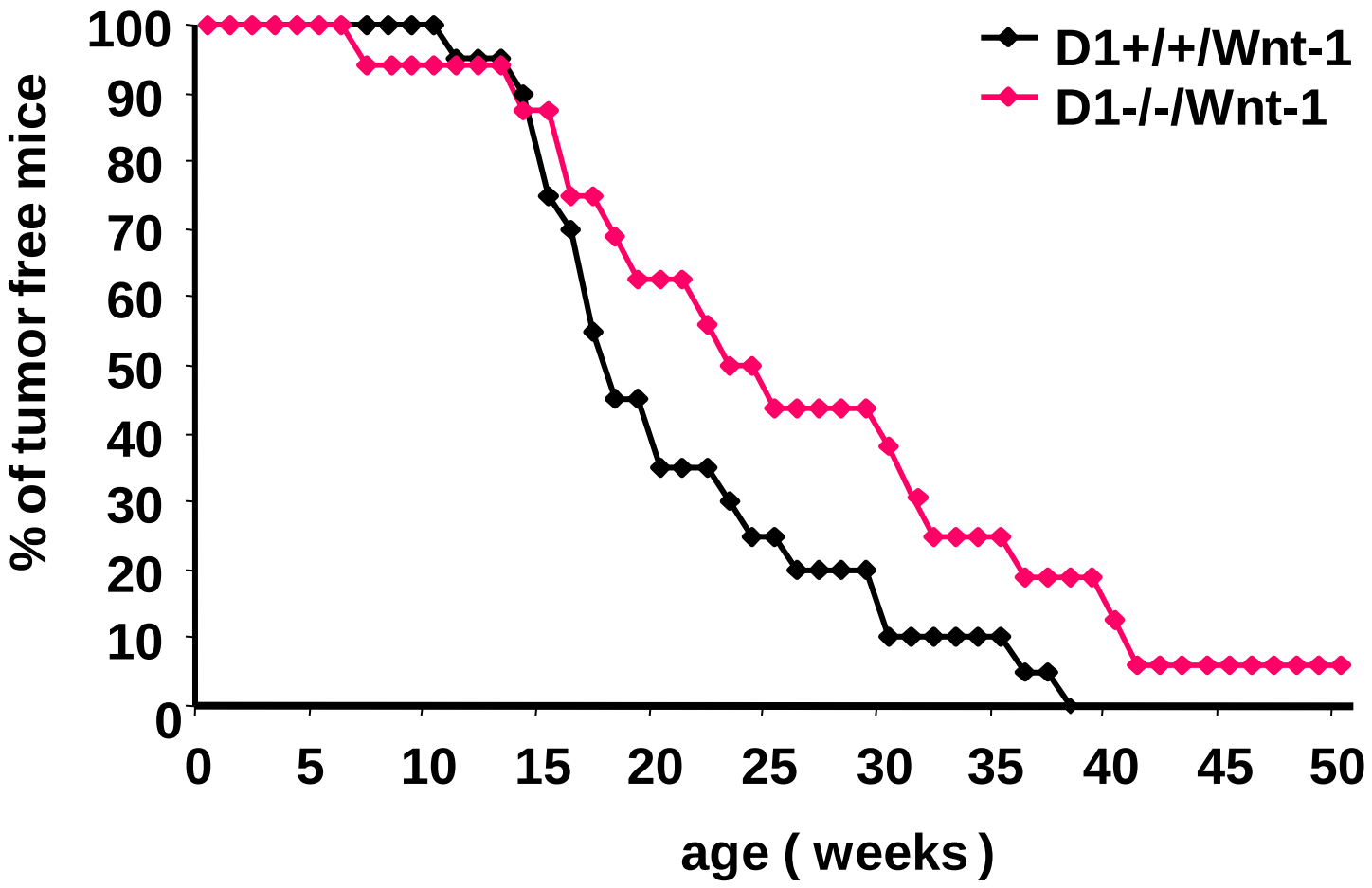
Tumors

MMTV-oncogene
D1-/-

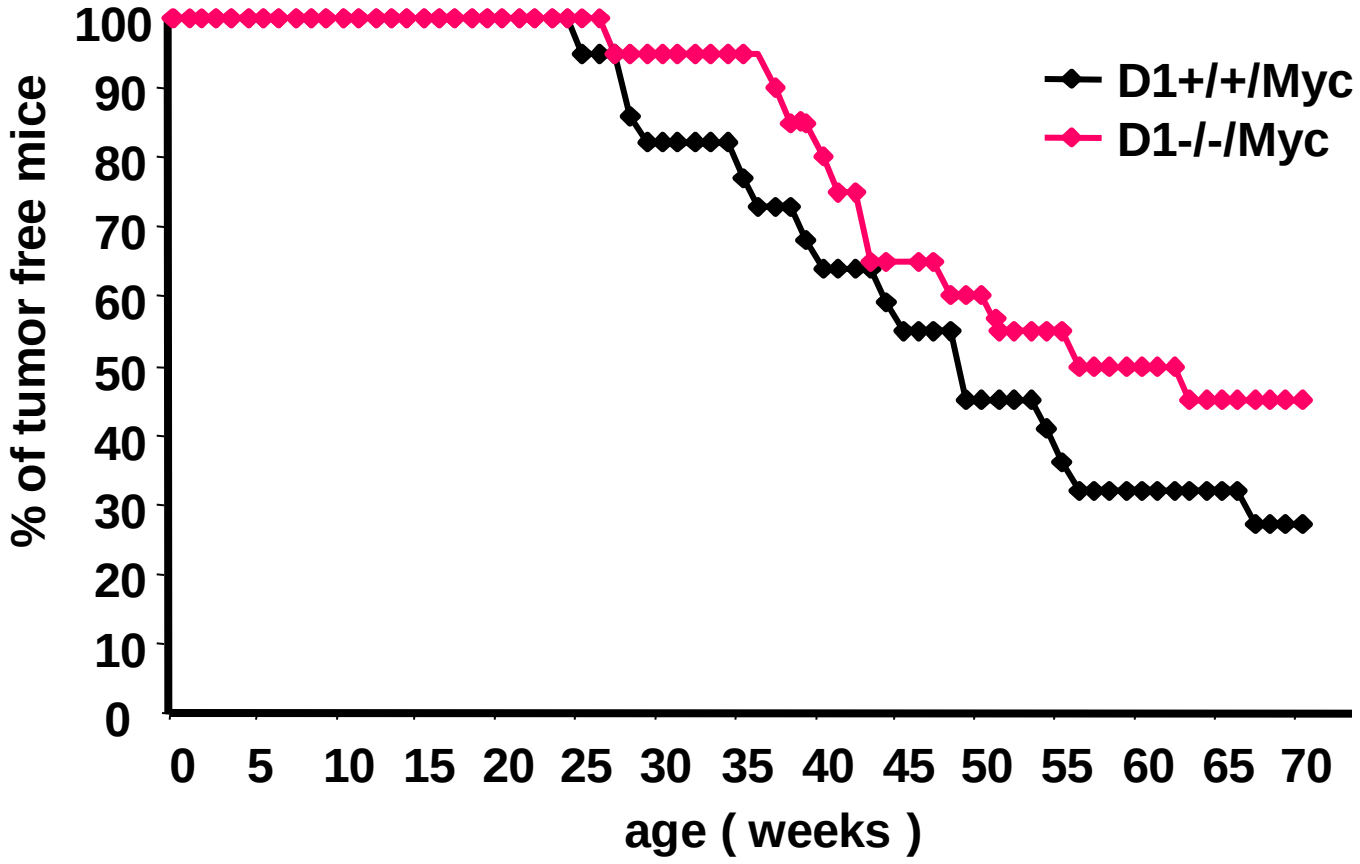
↓

?

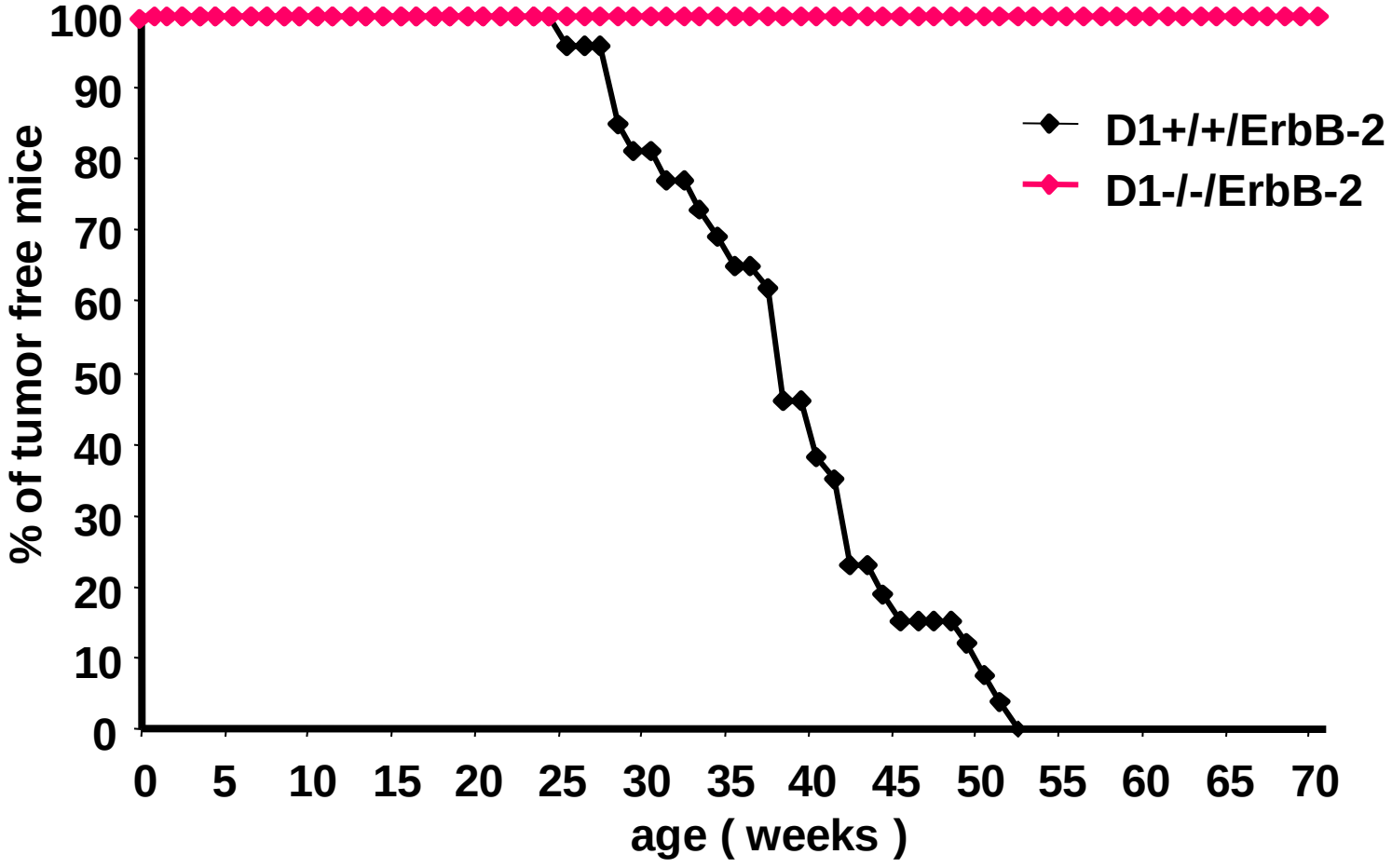
The occurrence of breast tumors in MMTV-Wnt-1 mice



The occurrence of breast tumors in MMTV-Myc mice



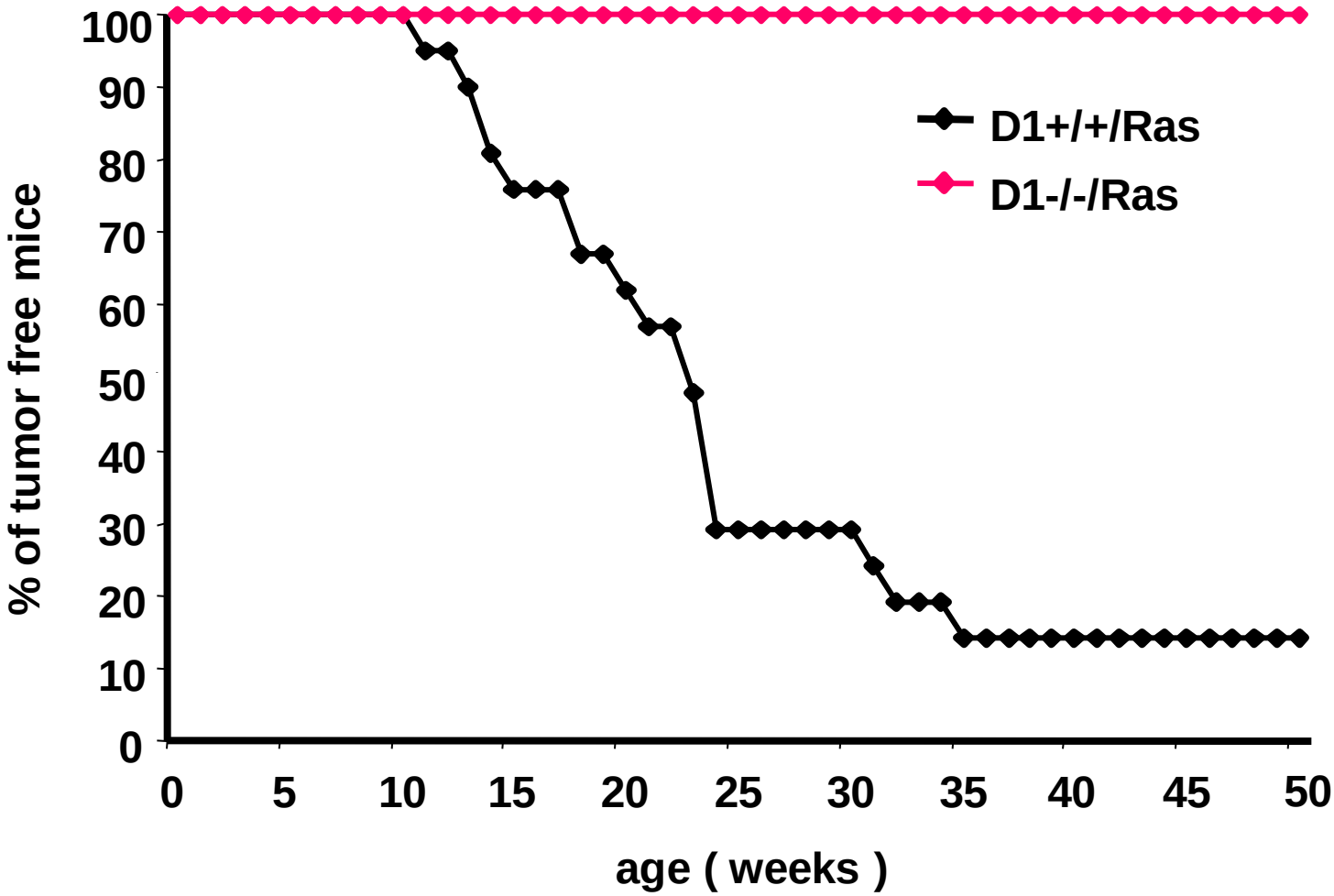
The occurrence of breast tumors in MMTV-ErbB-2 mice

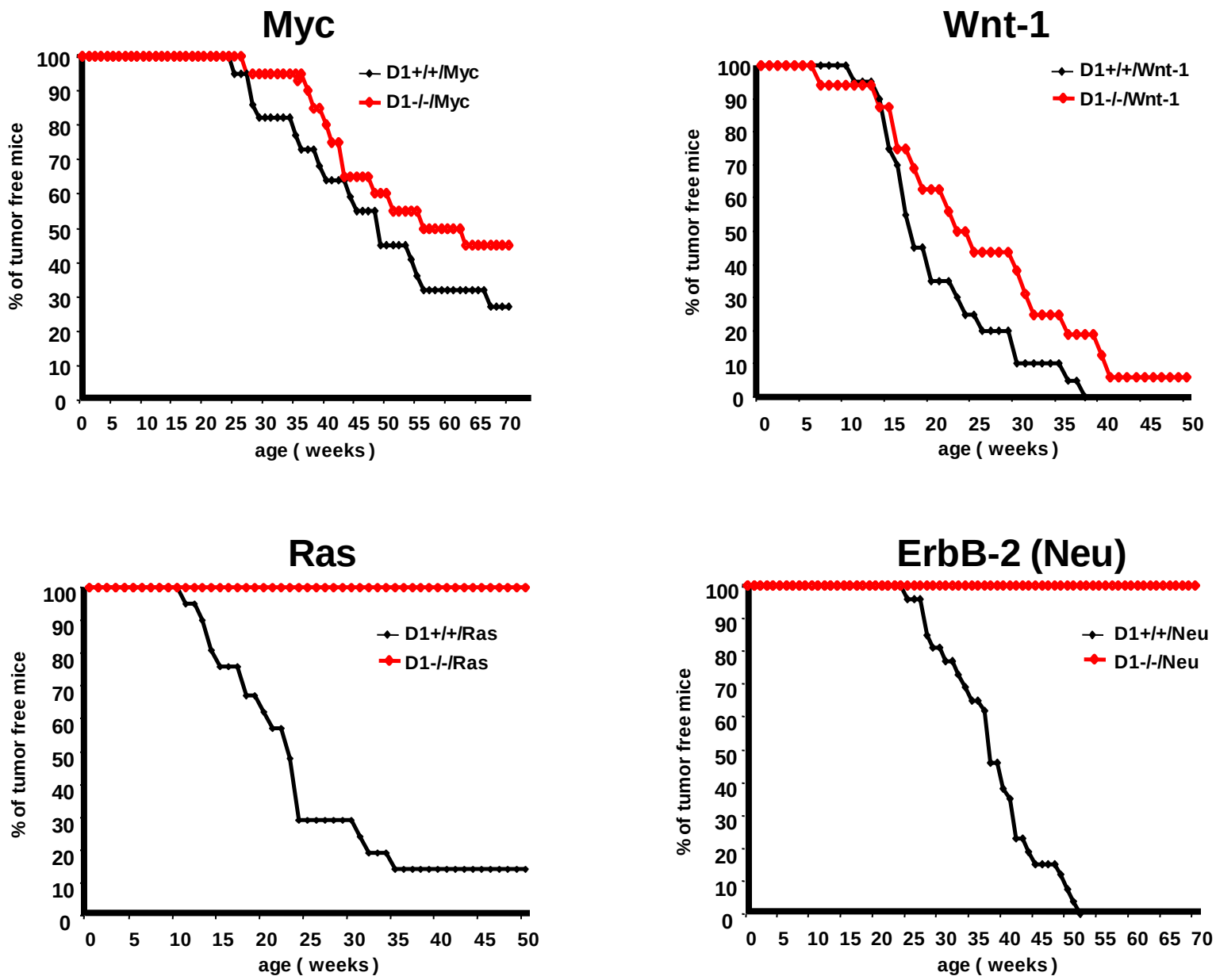


ErbB2

Ras

The occurrence of breast tumors in MMTV-Ras mice




Myc
D1+/+/Myc
D1-/-/Myc
% of tumor free mice
age (weeks)
Wnt-1
D1+/+/Wnt-1
D1-/-/Wnt-1
% of tumor free mice
age (weeks)
Ras
D1+/+/Ras
D1-/-/Ras
% of tumor free mice
age (weeks)
ErbB-2 (Neu)
D1+/+/Neu
D1-/-/Neu
% of tumor free mice
age (weeks)

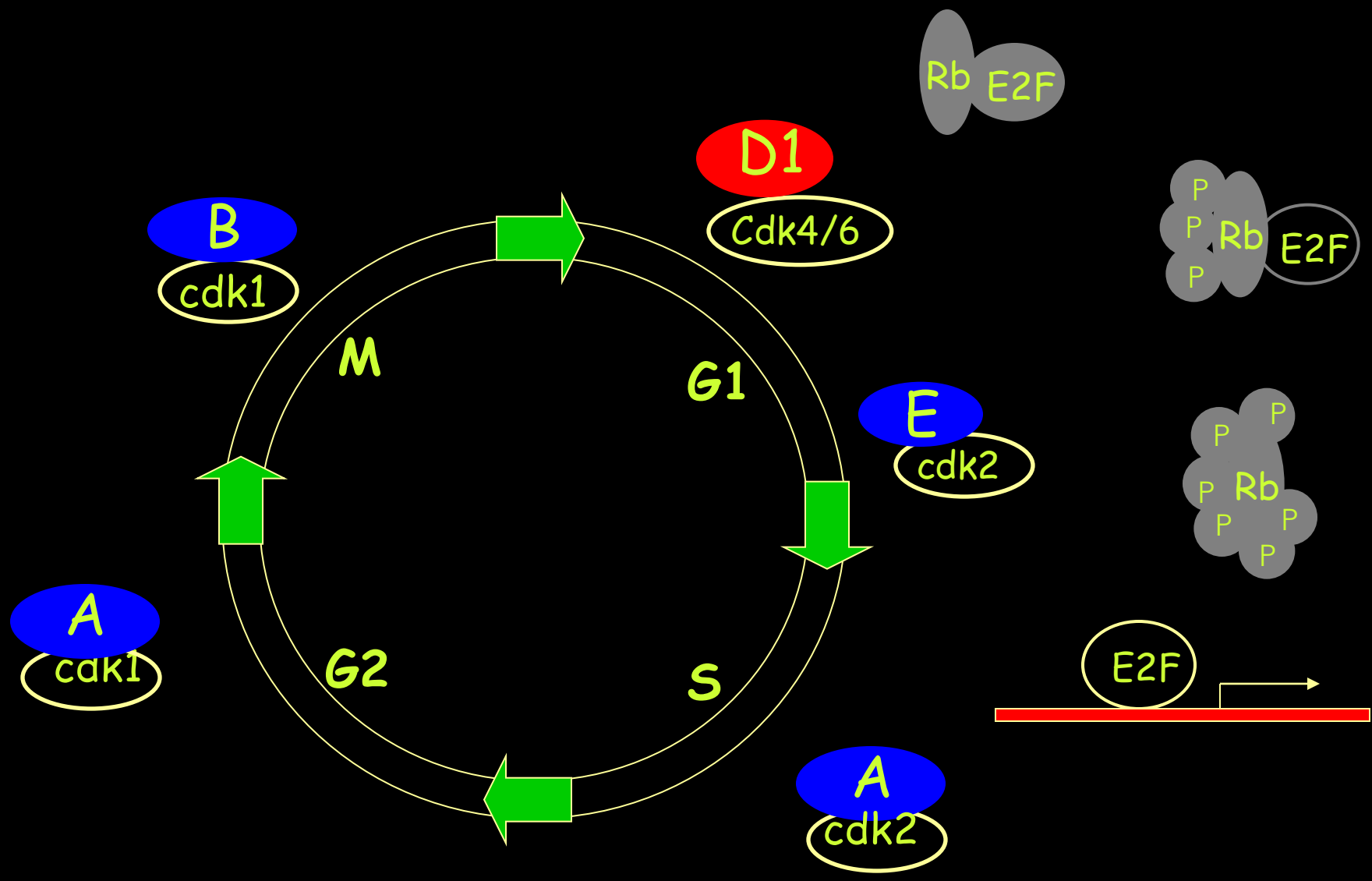
Salivary gland tumor incidence

	$D1^{-/-}$/MMTV-Ras	$D1^{+/+}$/MMTV-Ras
Ratio of mice with tumors	3/18 (17%)	1/21 (4.8%)
Total number of tumors	3	1

Breast cancer incidence

	D1$^{-/-}$ /MMTV-Ras	D2$^{-/-}$/MMTV-Ras	D3$^{-/-}$/MMTV-Ras
Ratio of mice with tumors	**0/18**	**6/11**	**8/13**
Total number of tumors	**0**	**20**	**25**

Cyclin D1 function is required for breast cancer initiation


Rb
E2F
D1
Cdk4/6
P
P
P
Rb
E2F
B
cdk1
M
G1
E
cdk2
P
P
P
P
P
P
Rb
A
cak1
G2
S
E2F
A
cdk2

CDK4$^{-/-}$ mice

Hiroaki Kiyokawa

Cross CDK4-/- mice

with MMTV-oncogene mice ErbB2/Neu

MMTV-oncogene
CDK4+/+

↓

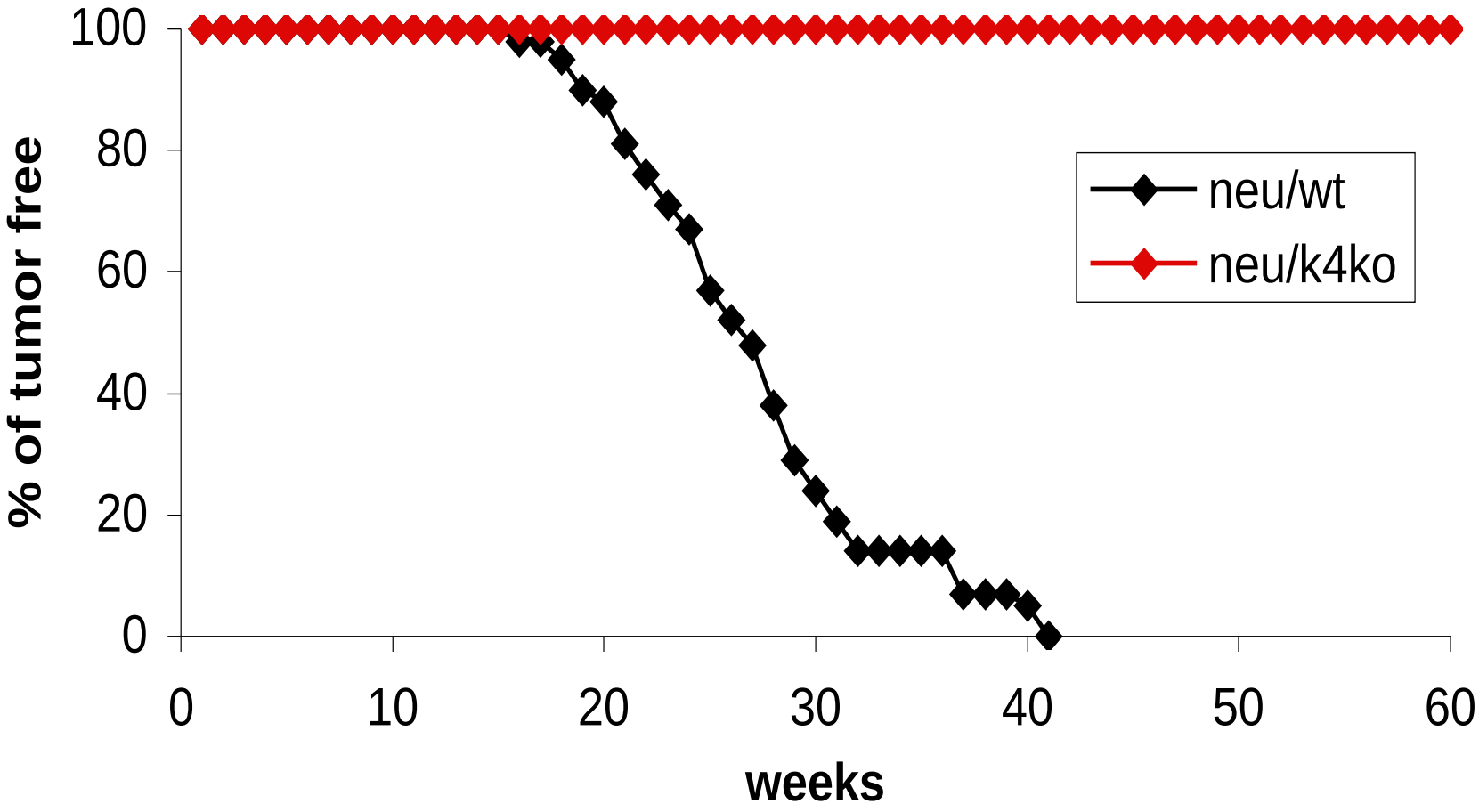
Tumors

MMTV-oncogene
CDK4-/-

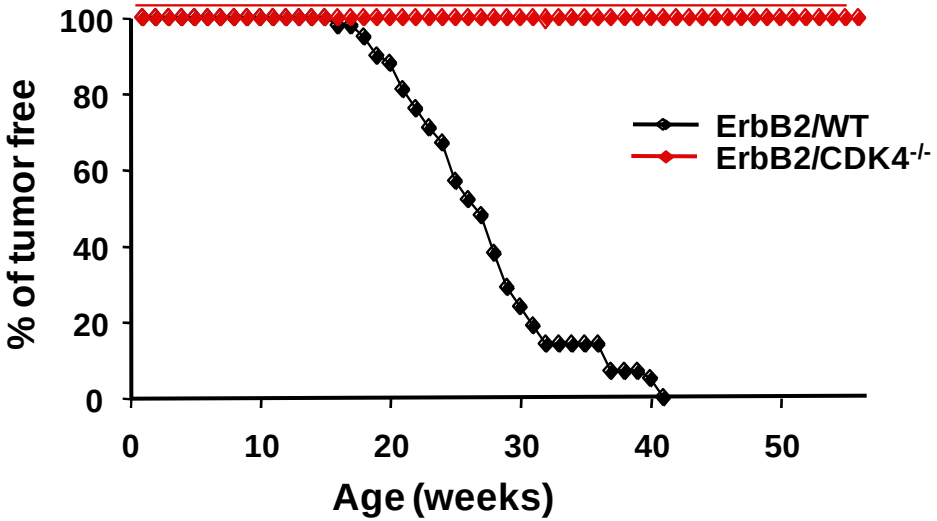
↓

?

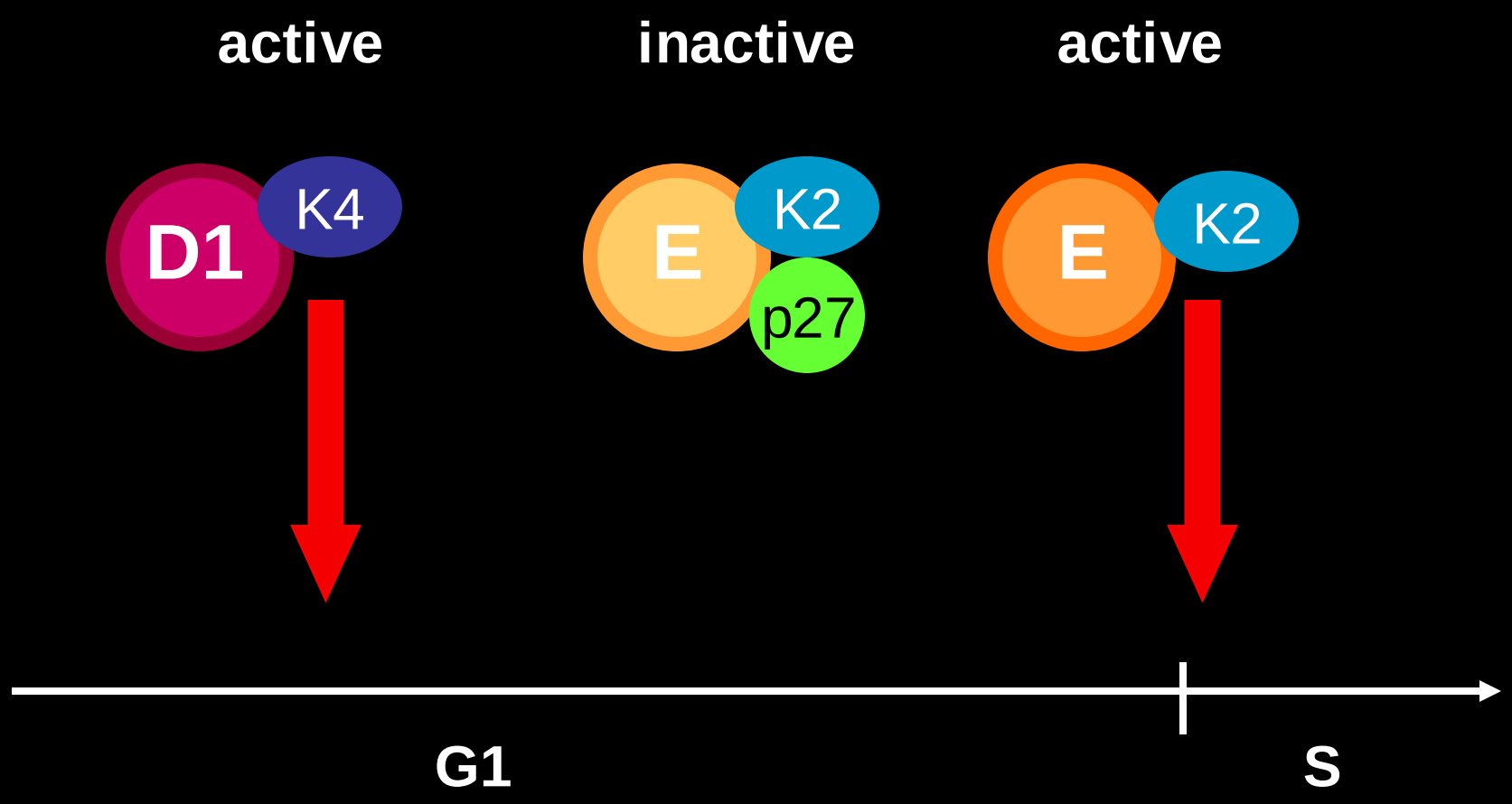
Breast cancer resistance of CDK4-null mice

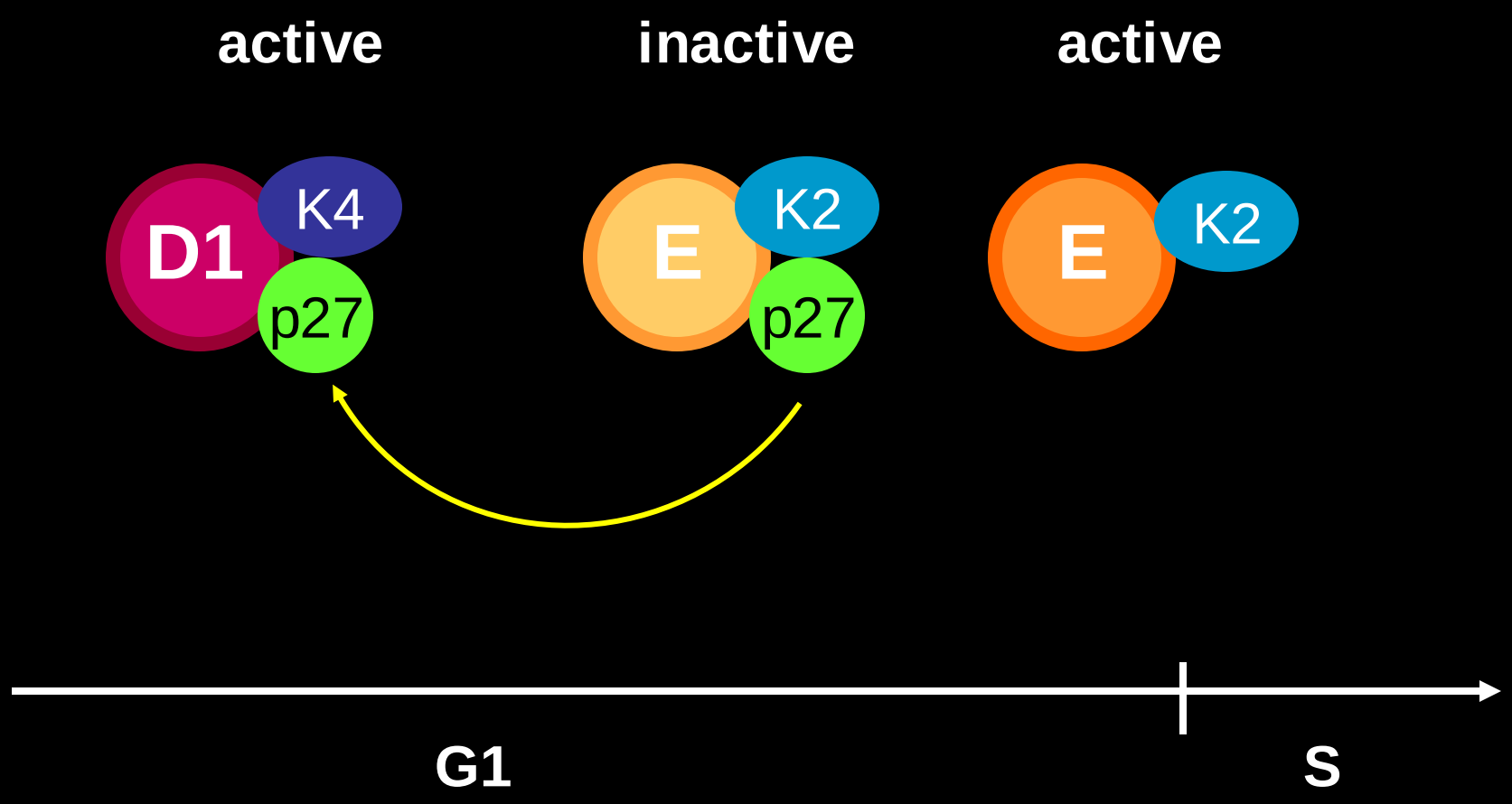


Breast cancer resistance of CDK4-null mice

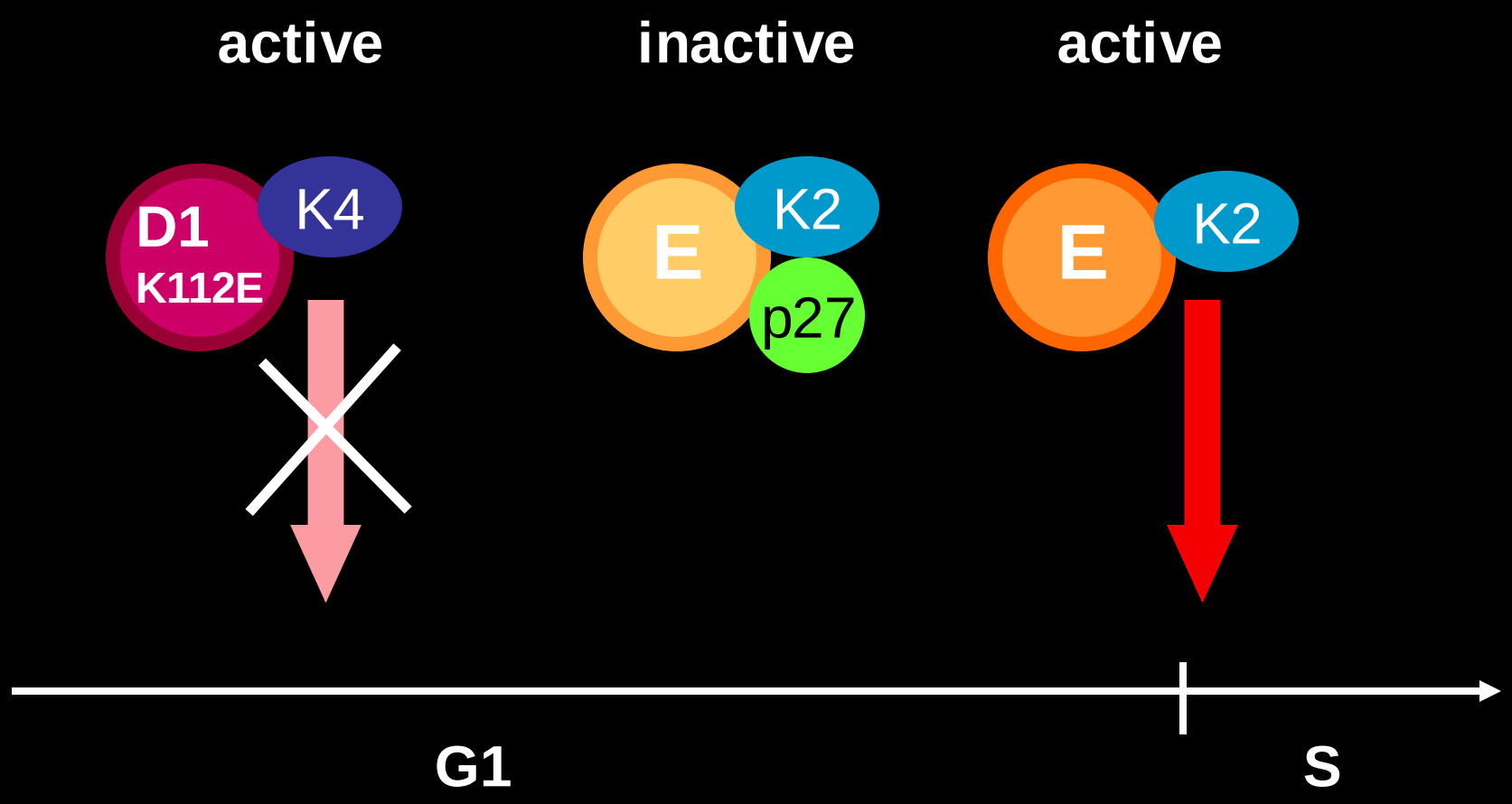


	CDK4+/+	CDK4-/-
Fraction of mice with tumors	42/42	0/38
Total number of tumors	139	0

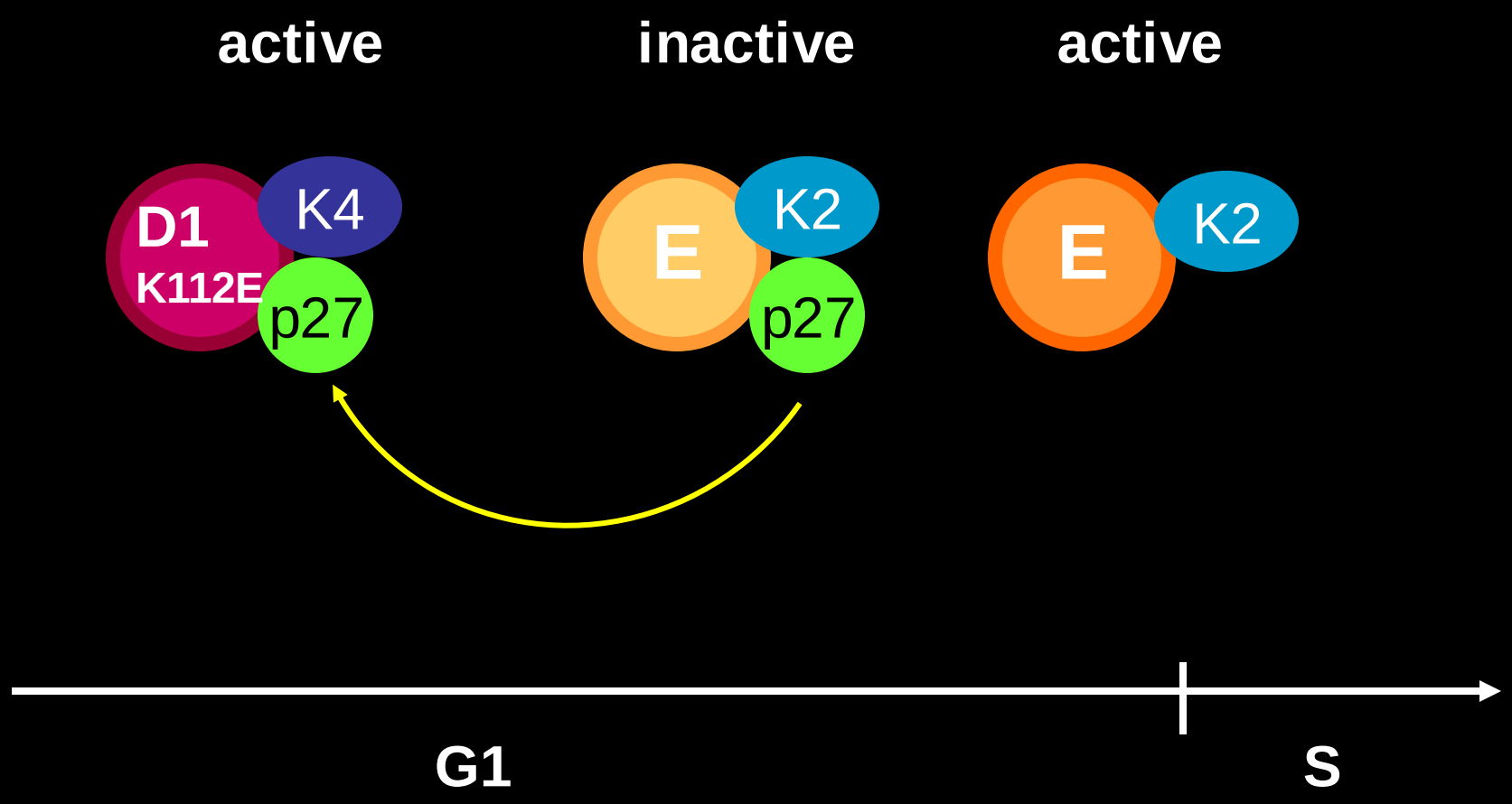

active
inactive
active
D1
K4
E
K2
p27
E
K2
G1
S


active
inactive
active
D1
K4
p27
E
K2
p27
E
K2
G1
S

Cyclin D1 K112E

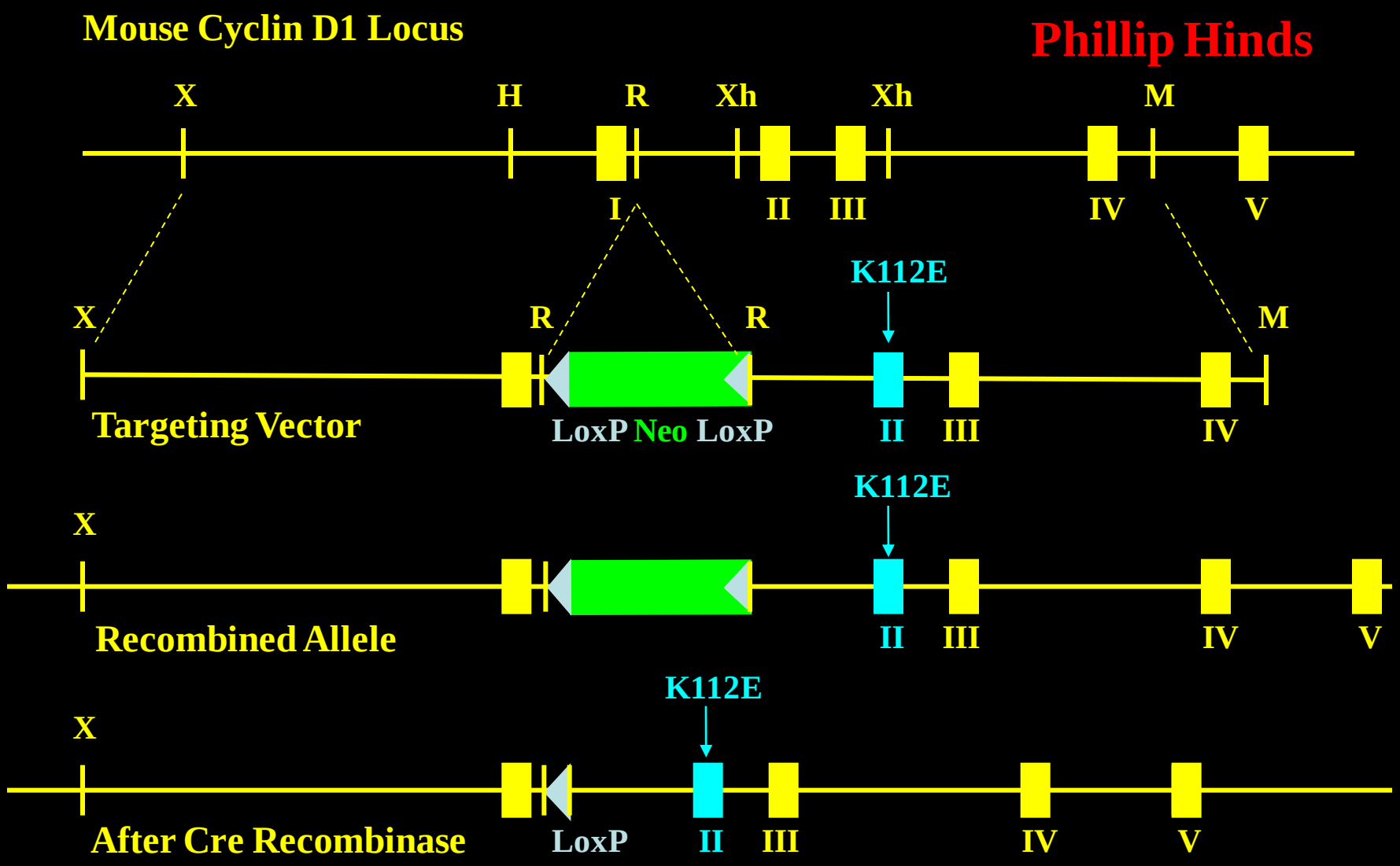


Cyclin D1 K112E



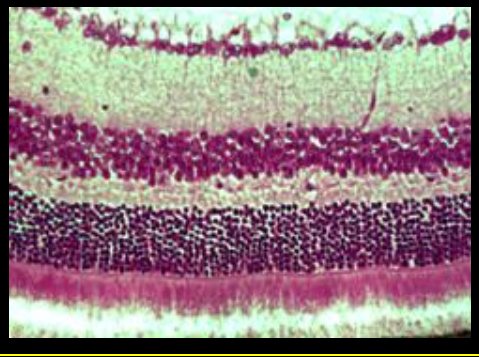
Mouse Cyclin D1 Knock-In Strategy

Mark Landis
Phillip Hinds

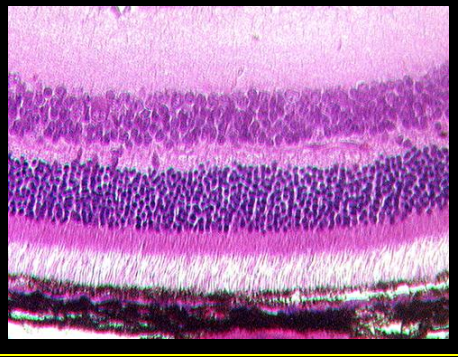


Phenotype of KE/KE mice

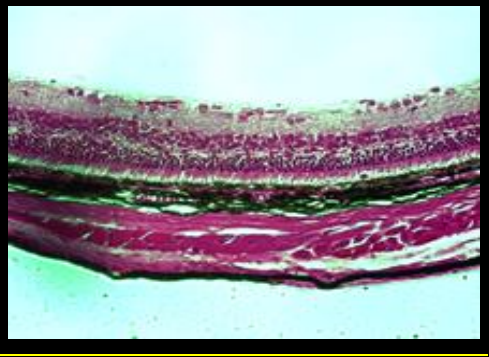
WT/WT



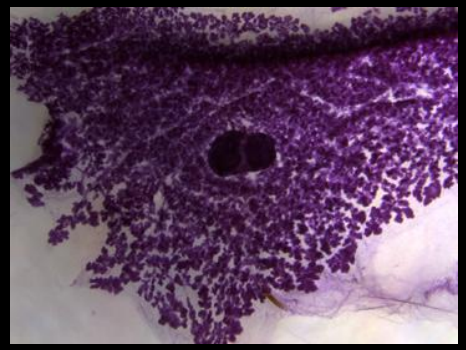
KE/KE



D1 -/-

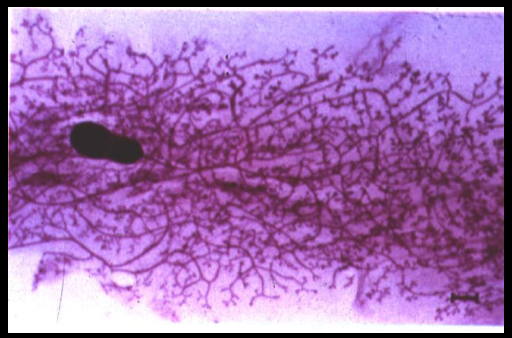


WT/WT



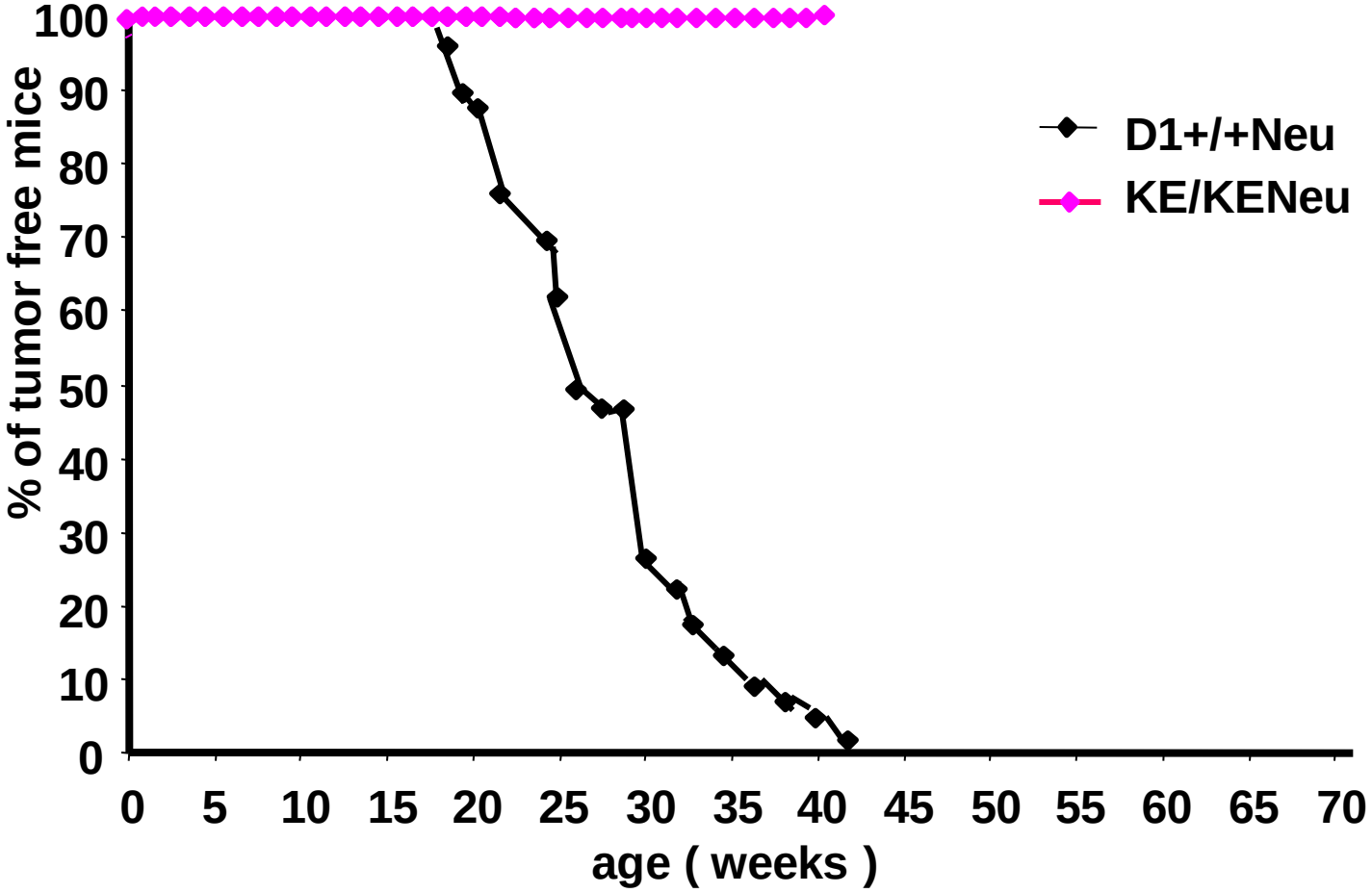
KE/KE

D1-/-

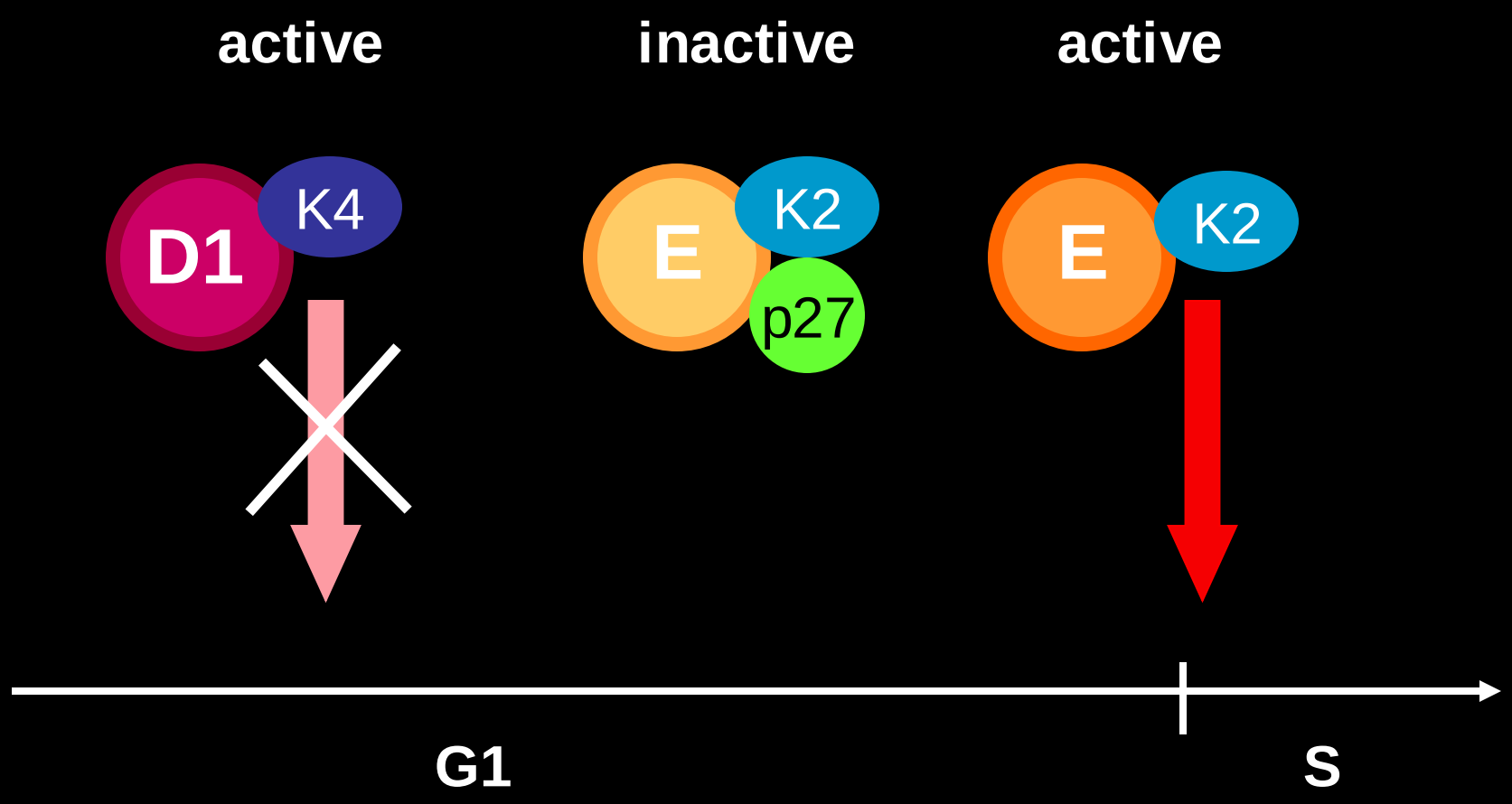


Day One Post-Partum Mammary Gland

Breast cancer resistance of KE/KE mice



Cyclin D1 kinase activity required for tumorigenesis

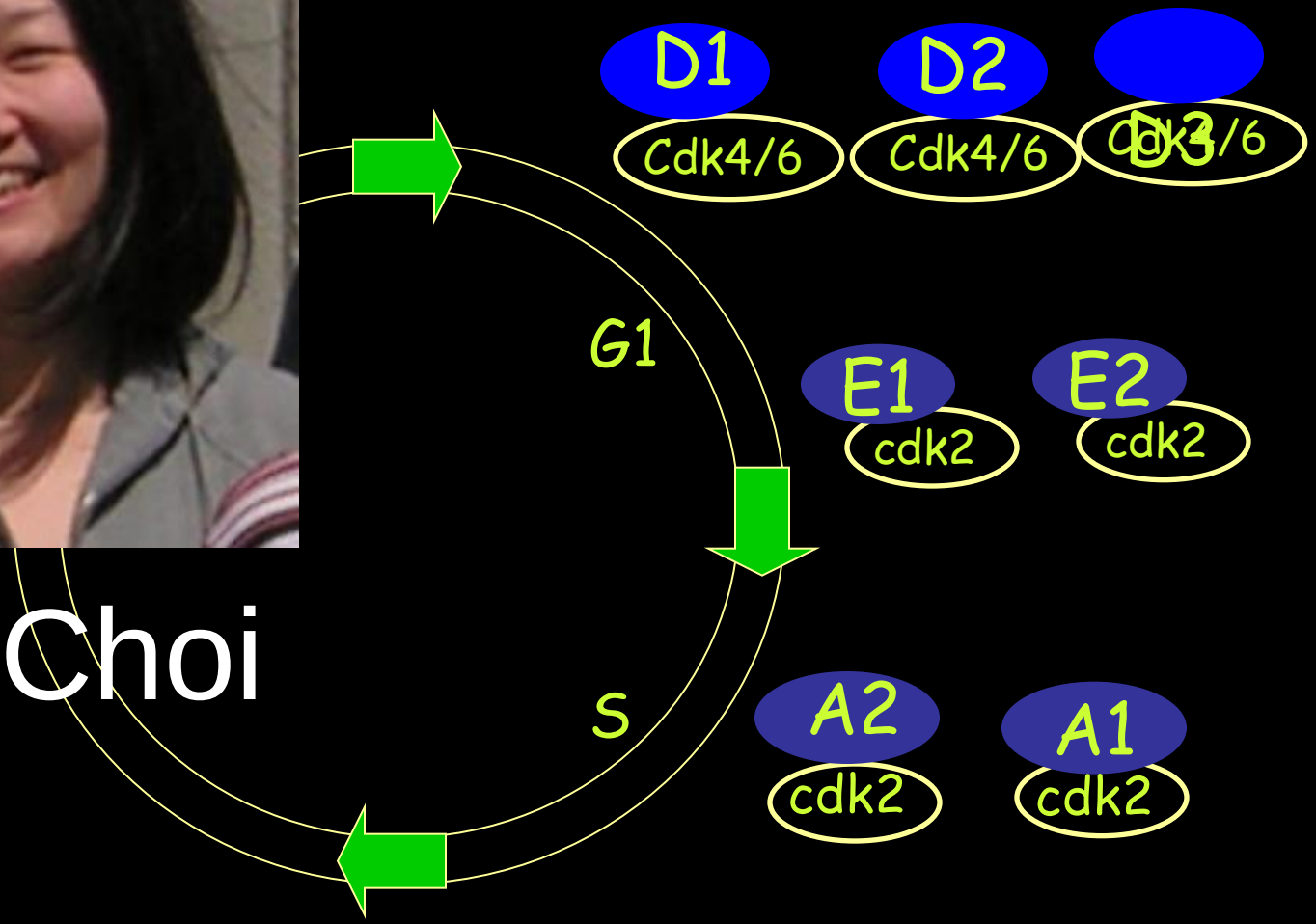


Cyclin D1-Cdk4 kinase is required for breast cancer initiation

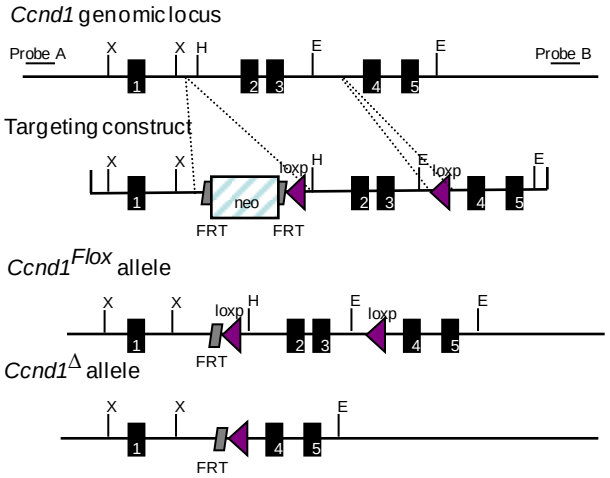
Outstanding issues:

- **Tumor initiation versus <u>maintenance</u>**
- **Consequences of an <u>acute</u> shutdown**

Yoon Choi



Conditional cyclin D1 knockout ($D1^{F/F}$) mice

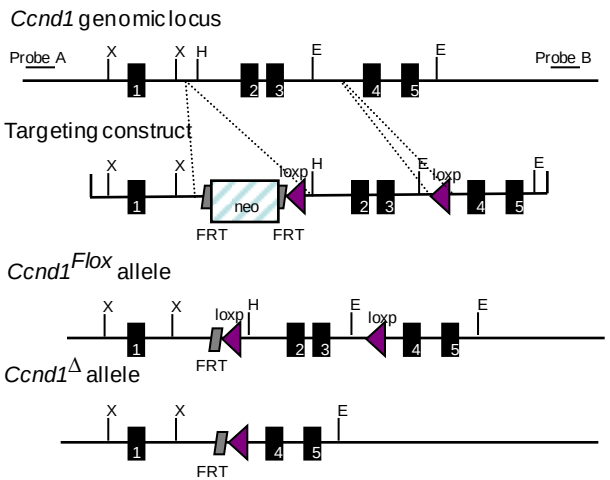


Cyclin $D1^{F/F}$ x Esr1-Cre

↓

Cyclin $D1^{F/F}$/Esr1-Cre

Conditional cyclin D1 knockout ($D1^{F/F}$) mice



Cyclin $D1^{F/F}$ x Esr1-Cre

↓

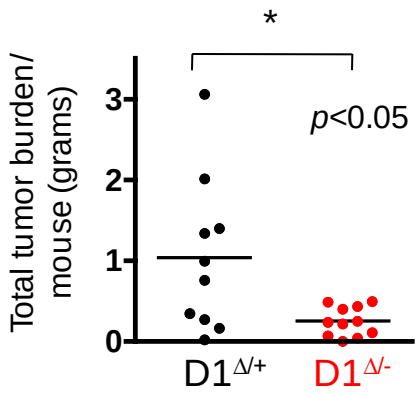
Cyclin $D1^{F/F}$/Esr1-Cre **NO ABNORMALITIES (1 year)**

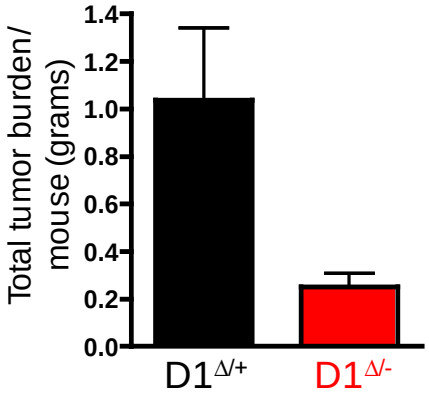
Cyclin D1$^{F/F}$/Esr1-Cre x MMTV-ErbB2

↓

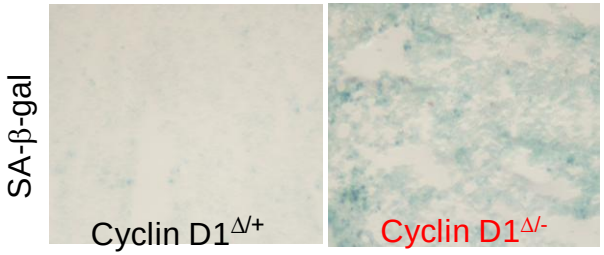
Cyclin D1$^{F/F}$/Esr1-Cre/MMTV-ErbB2

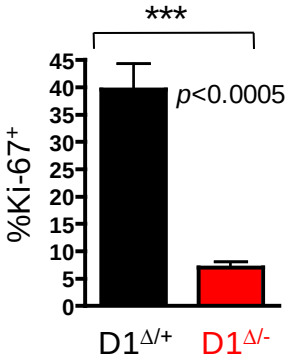
Acute ablation of cyclin D1 blocks breast cancer growth

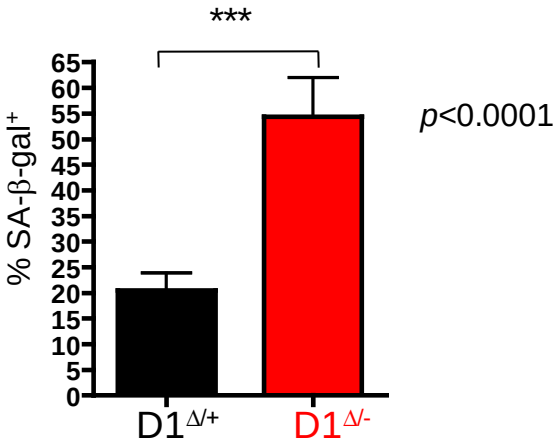




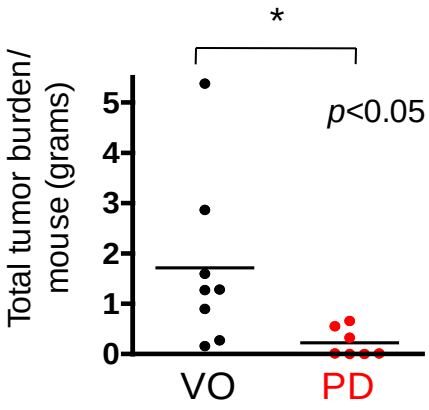
Acute ablation of cyclin D1 triggers cancer cell senescence

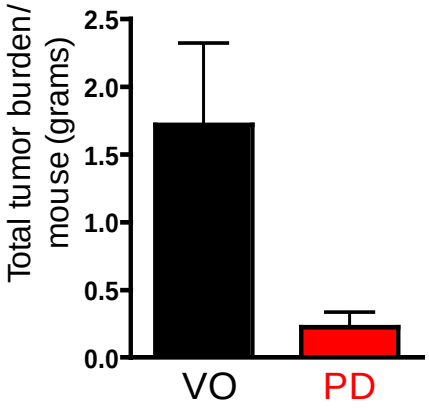




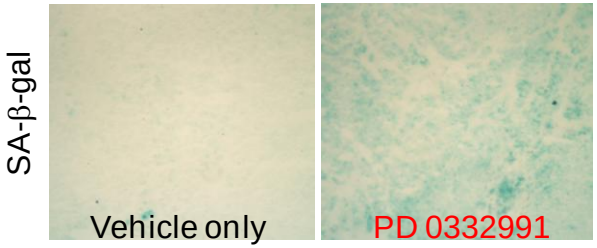


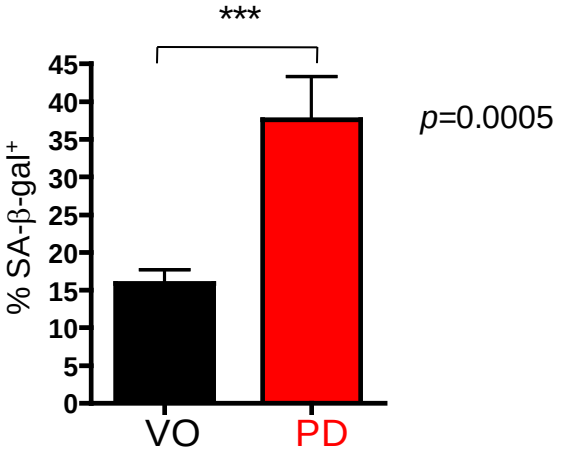
Acute inhibition of cyclin D-Cdk4/6 kinase (by PD 0332991) blocks breast cancer growth





Acute inhibition of cyclin D-Cdk4/6 kinase triggers tumor cell senescence



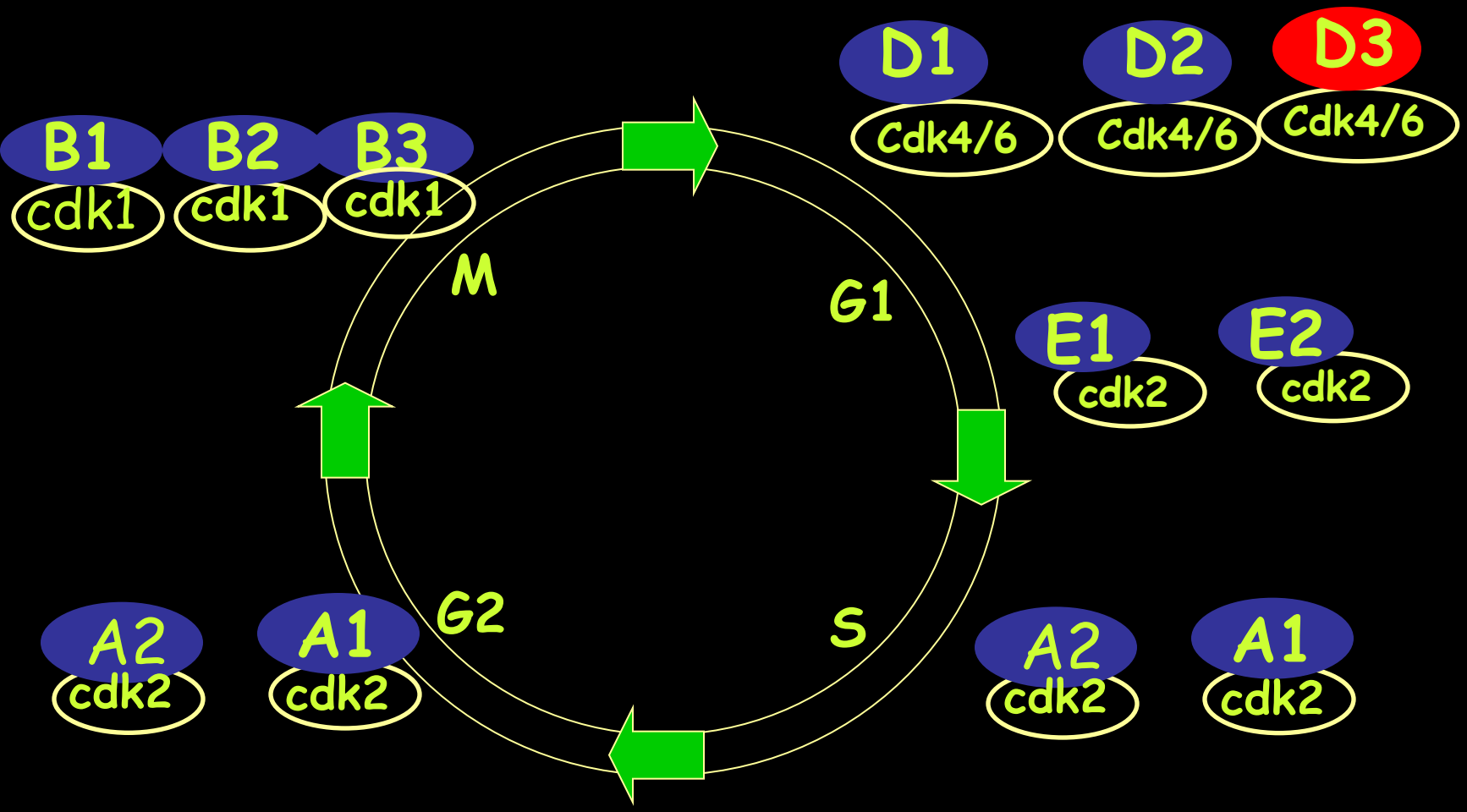


Cyclin D1-Cdk4 is required for

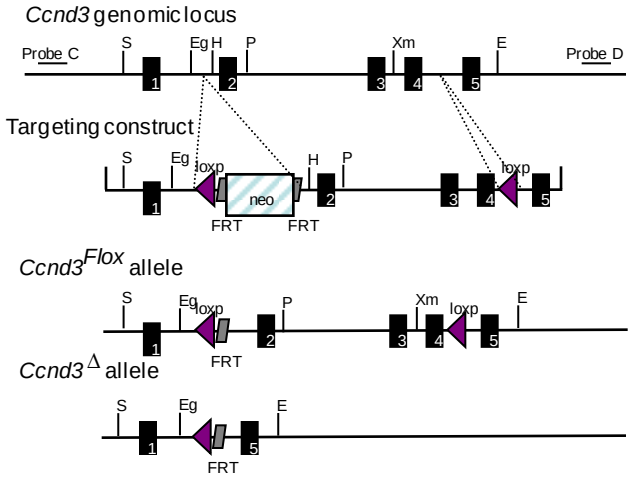
breast cancer maintenance,

but not for normal mouse physiology

Inhibition of cyclin D-Cdk4/6 triggers tumor cell senescence


D1
Cdk4/6
D2
Cdk4/6
D3
Cdk4/6
B1
cdk1
B2
cdk1
B3
cdk1
M
G1
E1
cdk2
E2
cdk2
G2
S
A2
cdk2
A1
cdk2
A2
cdk2
A1
cdk2

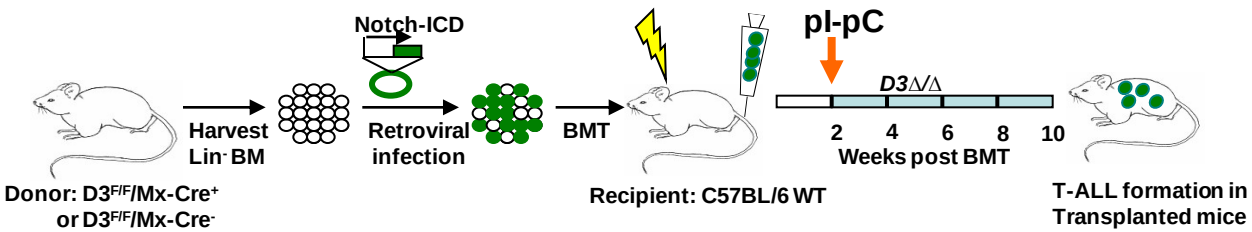
Conditional cyclin D3 knockout ($D3^{F/F}$) mice

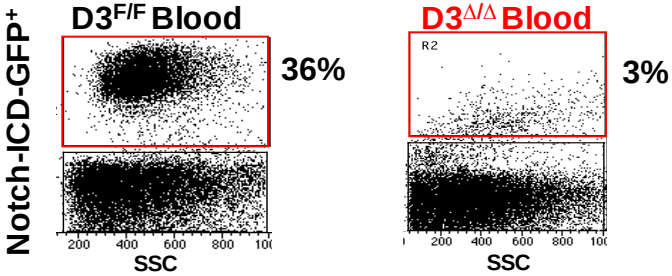


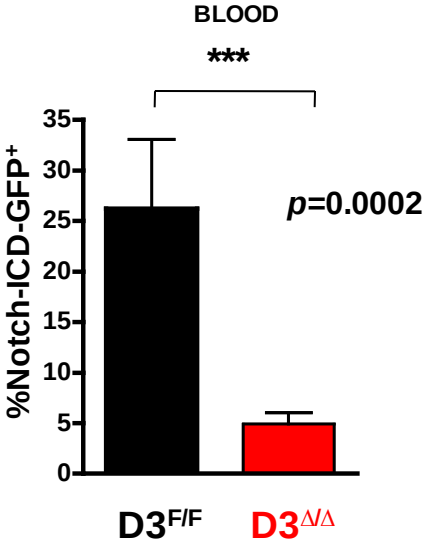
Cyclin $D3^{F/F}$/Esr1-Cre

Cyclin $D1^{F/F}$/Mx1-Cre

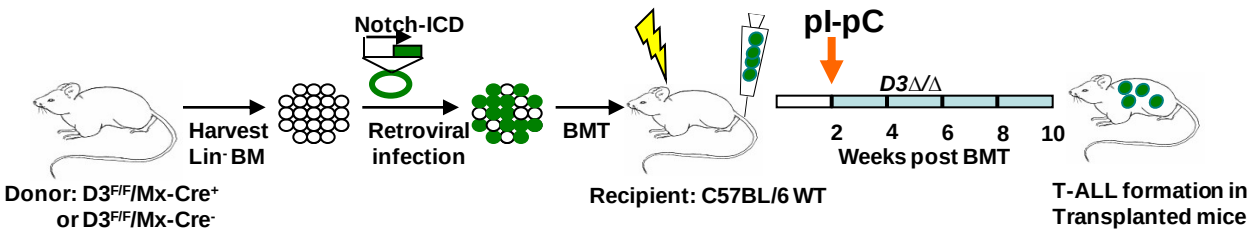
Acute ablation of cyclin D3 at an early stage of T-ALL development

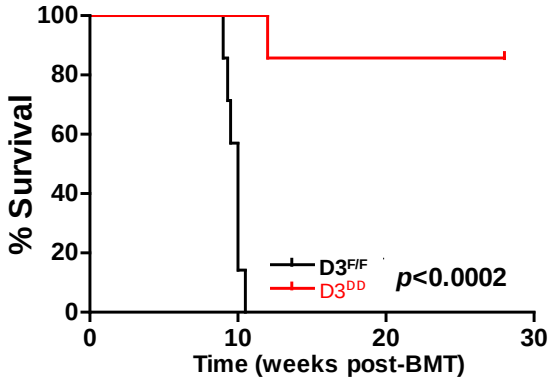




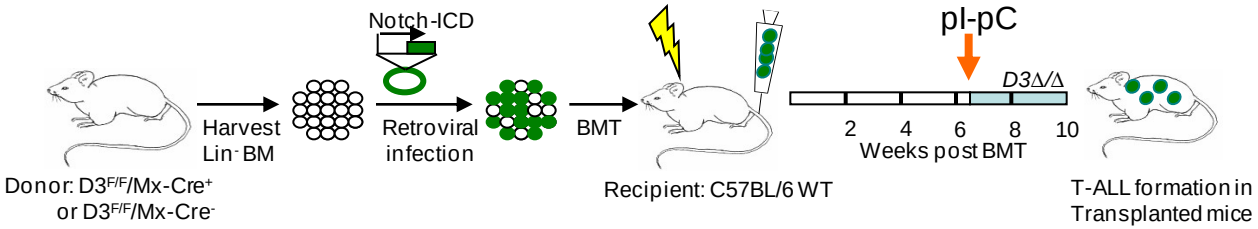


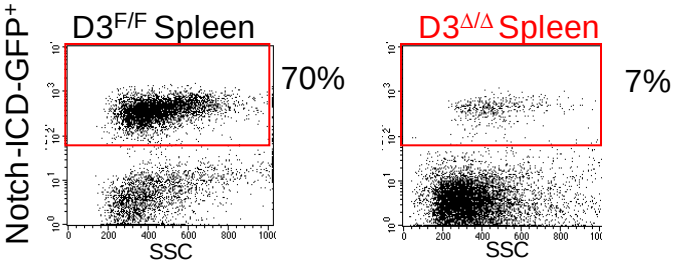
Acute ablation of cyclin D3 at an early stage of T-ALL development

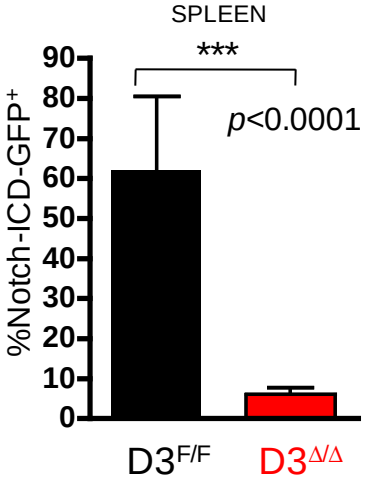




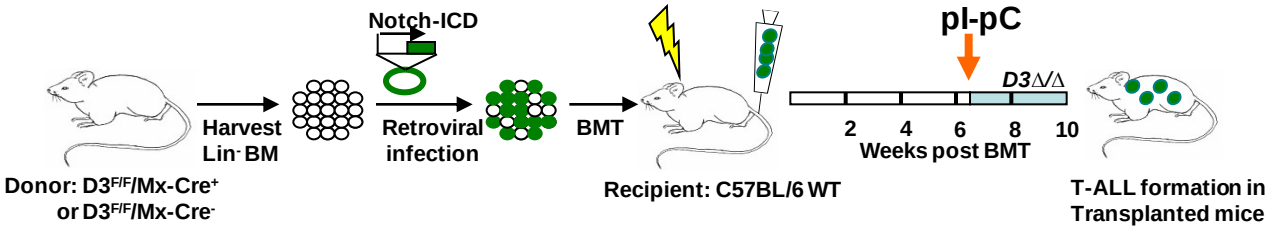
Acute ablation of cyclin D3 in tumor-bearing mice

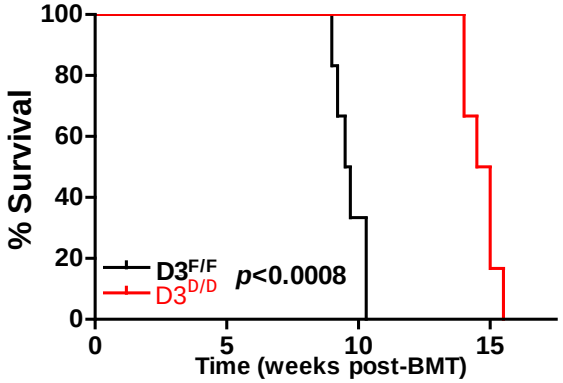






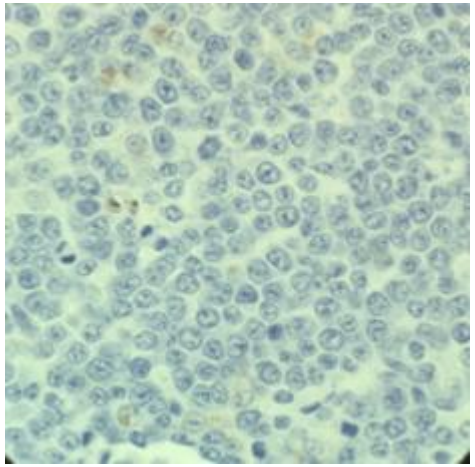
Acute ablation of cyclin D3 in tumor-bearing mice





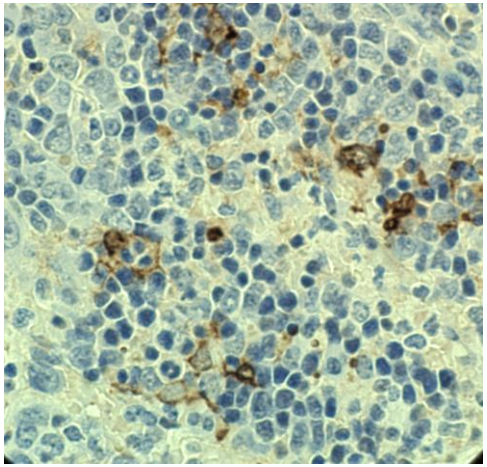
Massive tumor cell apoptosis upon acute ablation of cyclin D3

Spleen D3$^{F/F}$



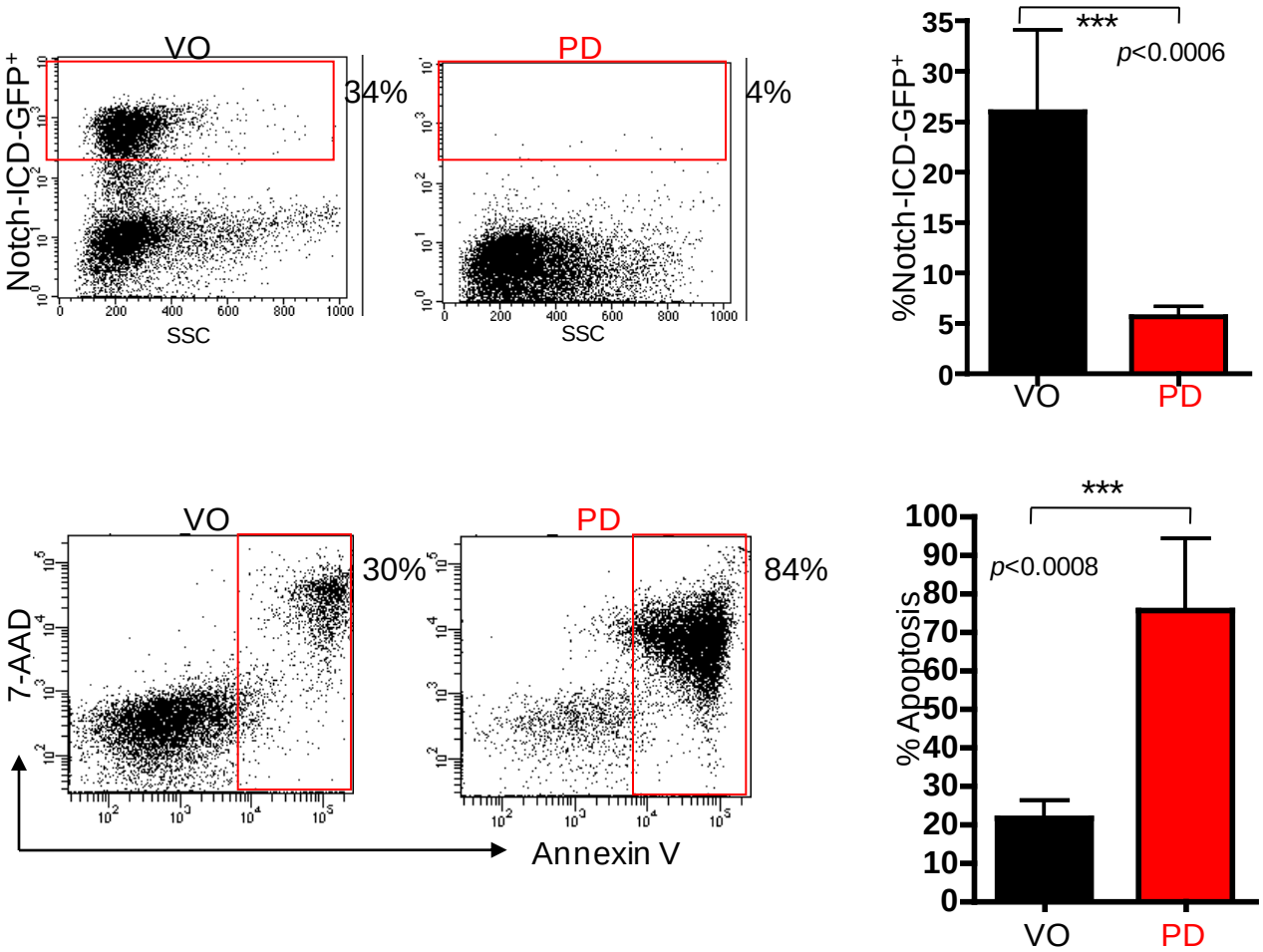
TUNEL

Spleen D3$^{\Delta/\Delta}$

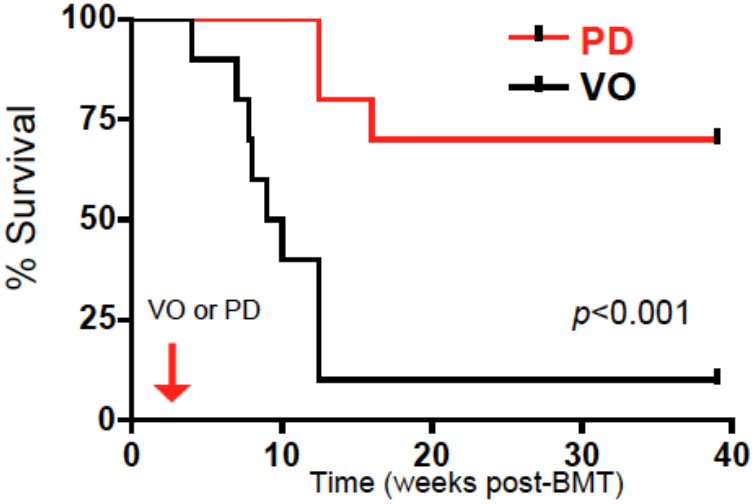


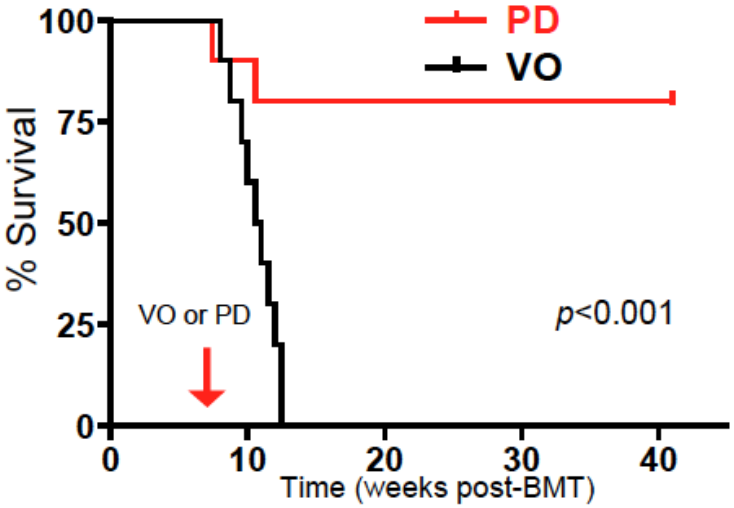
TUNEL

Massive tumor cell apoptosis upon acute inhibition of cyclin D-cdk4/6 kinase

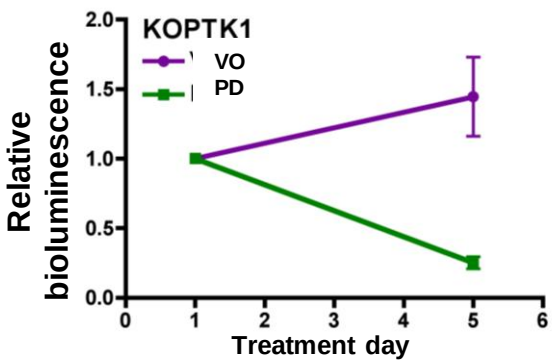


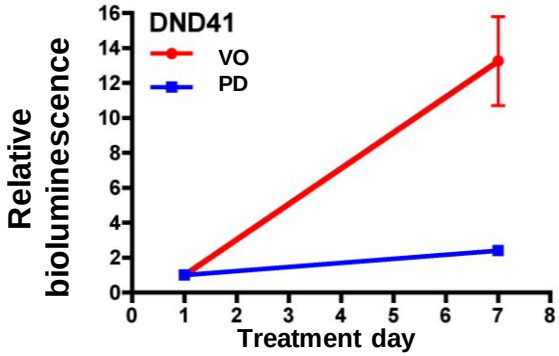
Massive tumor cell apoptosis upon acute inhibition of cyclin D-cdk4/6 kinase

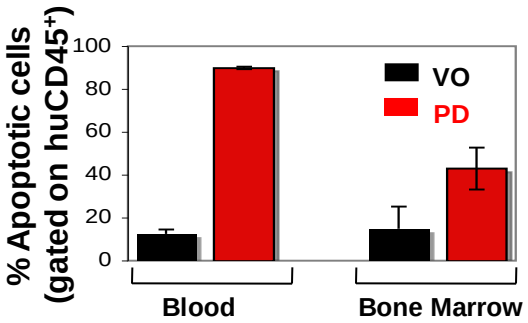


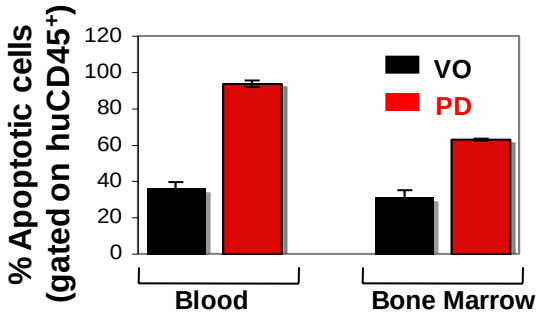


Apoptosis in human T-ALL cell lines upon cyclin D-Cdk4/6 inhibition *in vivo*

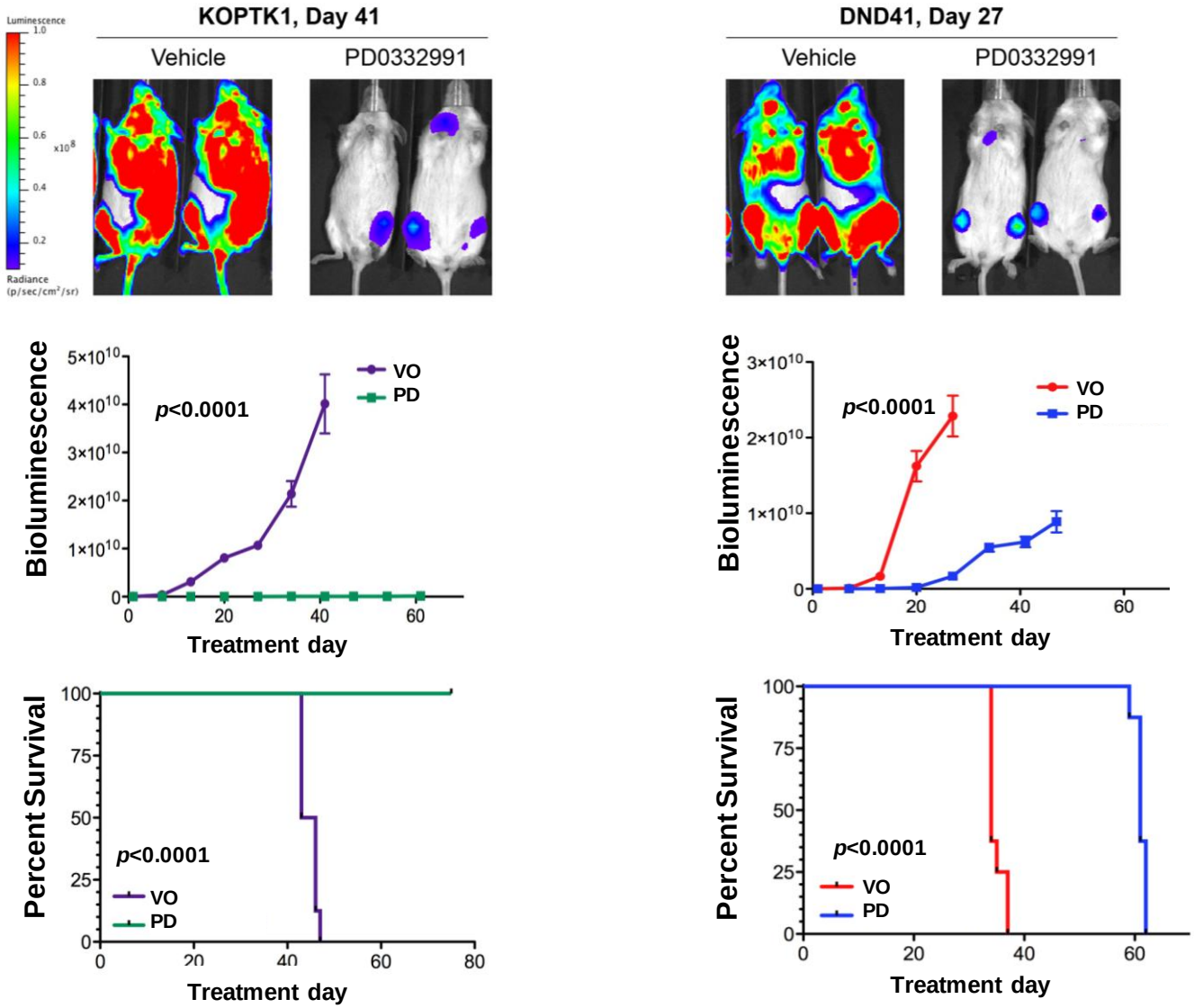








Inhibition of cyclin D-cdk4/6 kinase *in vivo*

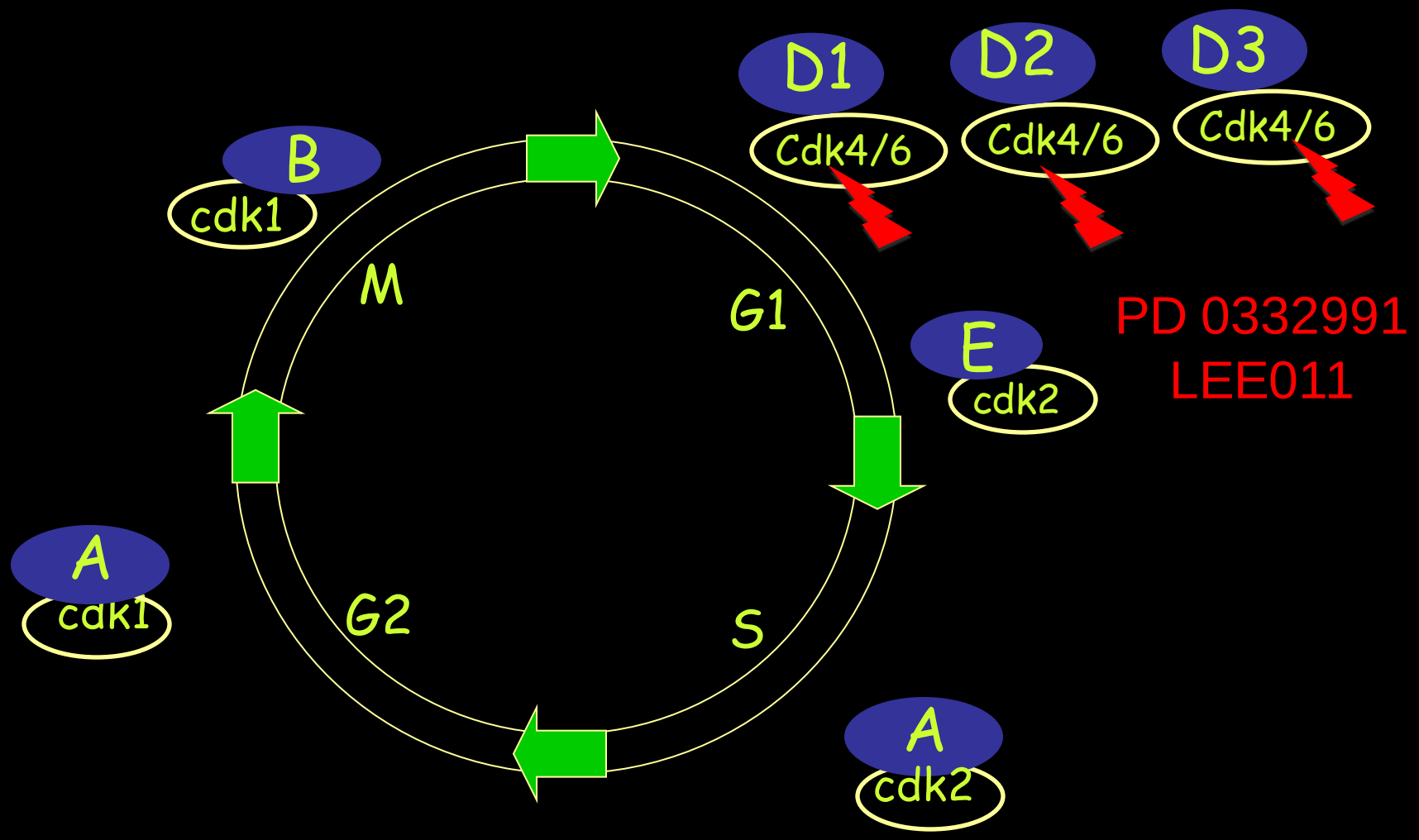


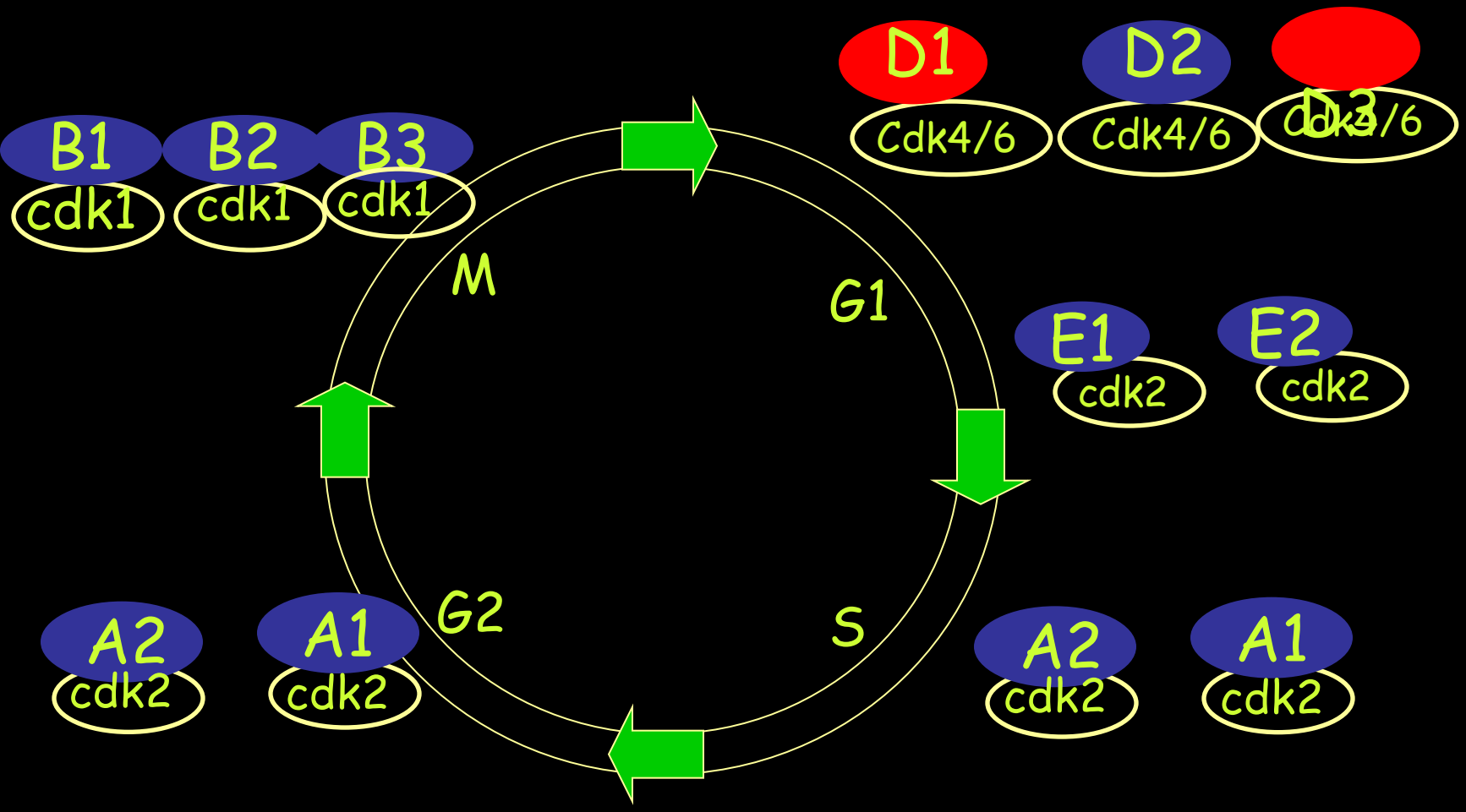
Cyclin D3-Cdk6 is required for

T-ALL maintenance,

but not for normal mouse physiology

Inhibition of cyclin D-Cdk4/6 triggers tumor cell apoptosis


D1
Cdk4/6
D2
Cdk4/6
D3
Cdk4/6
B
cdk1
M
G1
E
cdk2
PD 0332991
LEE011
A
cak1
G2
S
A
cdk2


D1
Cdk4/6
D2
Cdk4/6
D3
Cdk4/6
B1
cdk1
B2
cdk1
B3
cdk1
M
G1
E1
cdk2
E2
cdk2
G2
S
A2
cdk2
A1
cdk2
A2
cdk2
A1
cdk2

Qunyan Yu
Yoon Choi