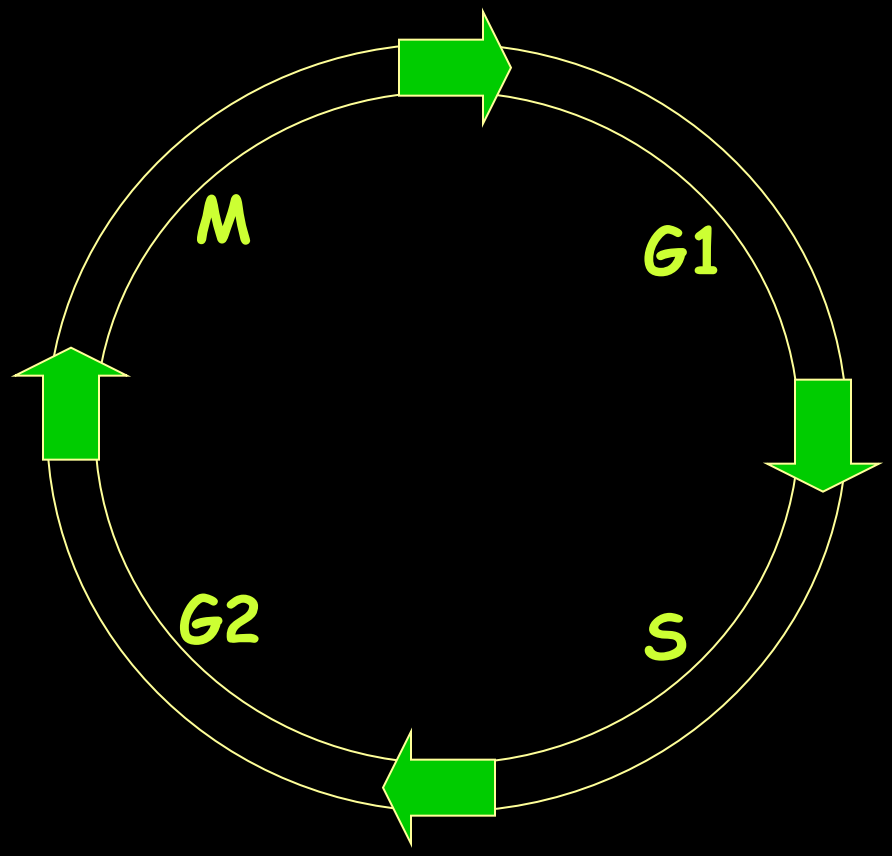
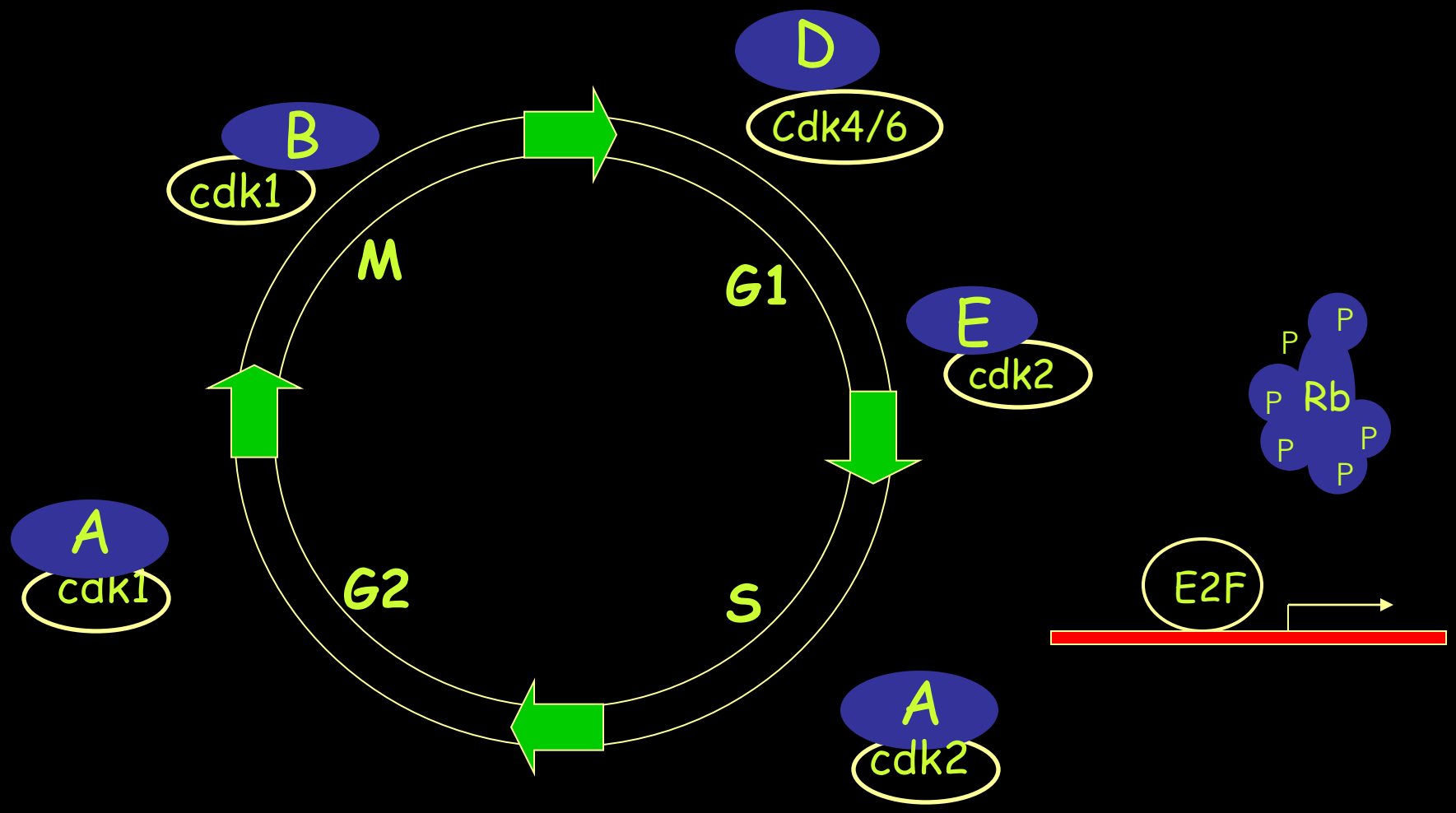
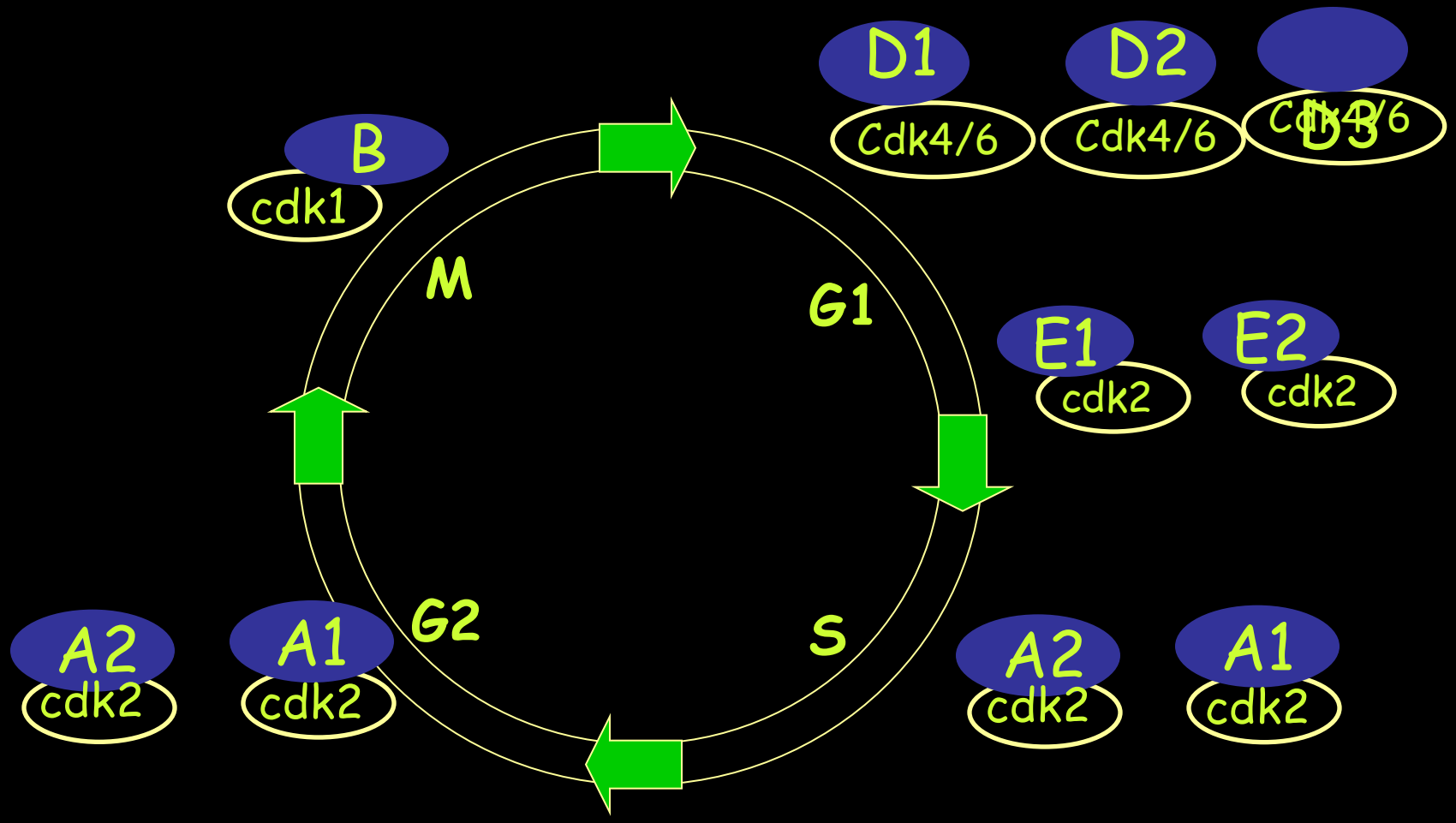
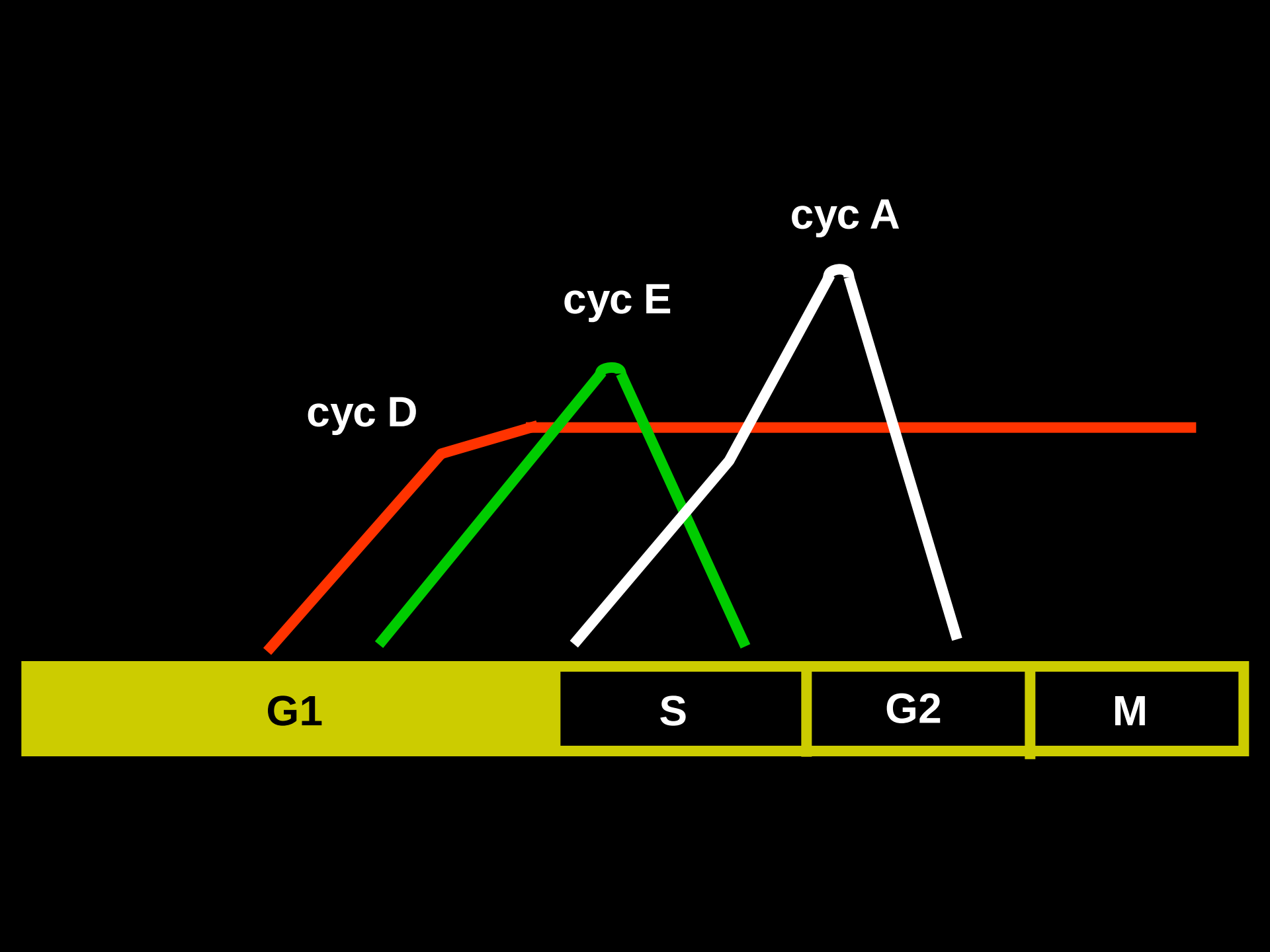
Cell cycle machinery




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


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


cyc A
cyc E
cyc D
G1
S
G2
M

E-CYCLINS

cyclin E1 and E2

associate with CDK2 (CDK3 or CDK1)

rapidly induced prior to the S phase entry

overexpression shortens G1 phase

antibodies block S phase entry

cyclin E gene on 19q12 amplified in several cancers

cyclin E overexpressed in ~25% of breast cancers

Functions for E-CYCLINS

Retinoblastoma protein (pRB) inactivation

Cell cycle progression ($p27^{Kip1}$, CDC25)

DNA replication initiation (CDC45 loading)

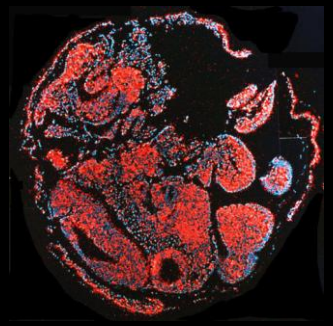
Centrosome duplication (nucleophosmin)

Histone biosynthesis (p220 NPAT)

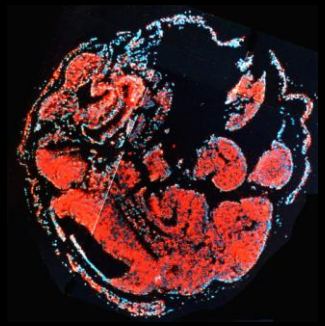
E-cyclins are absolutely essential for cell cycle progresssion

EXPRESSION OF CYCLINS E1 AND E2

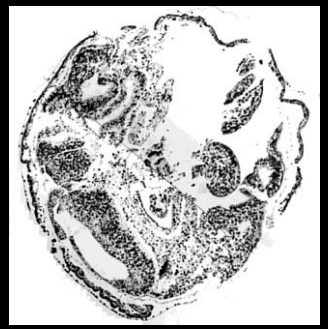
cyclin E1



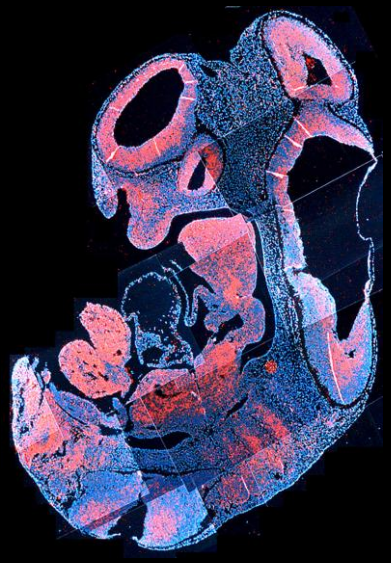
cyclin E2

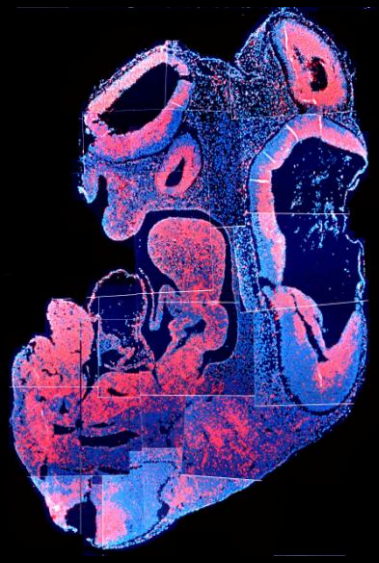


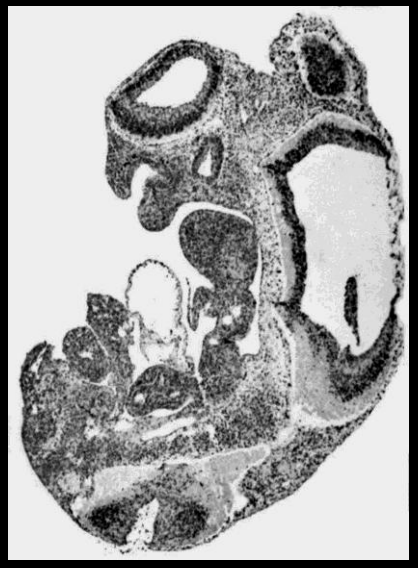
BrdU



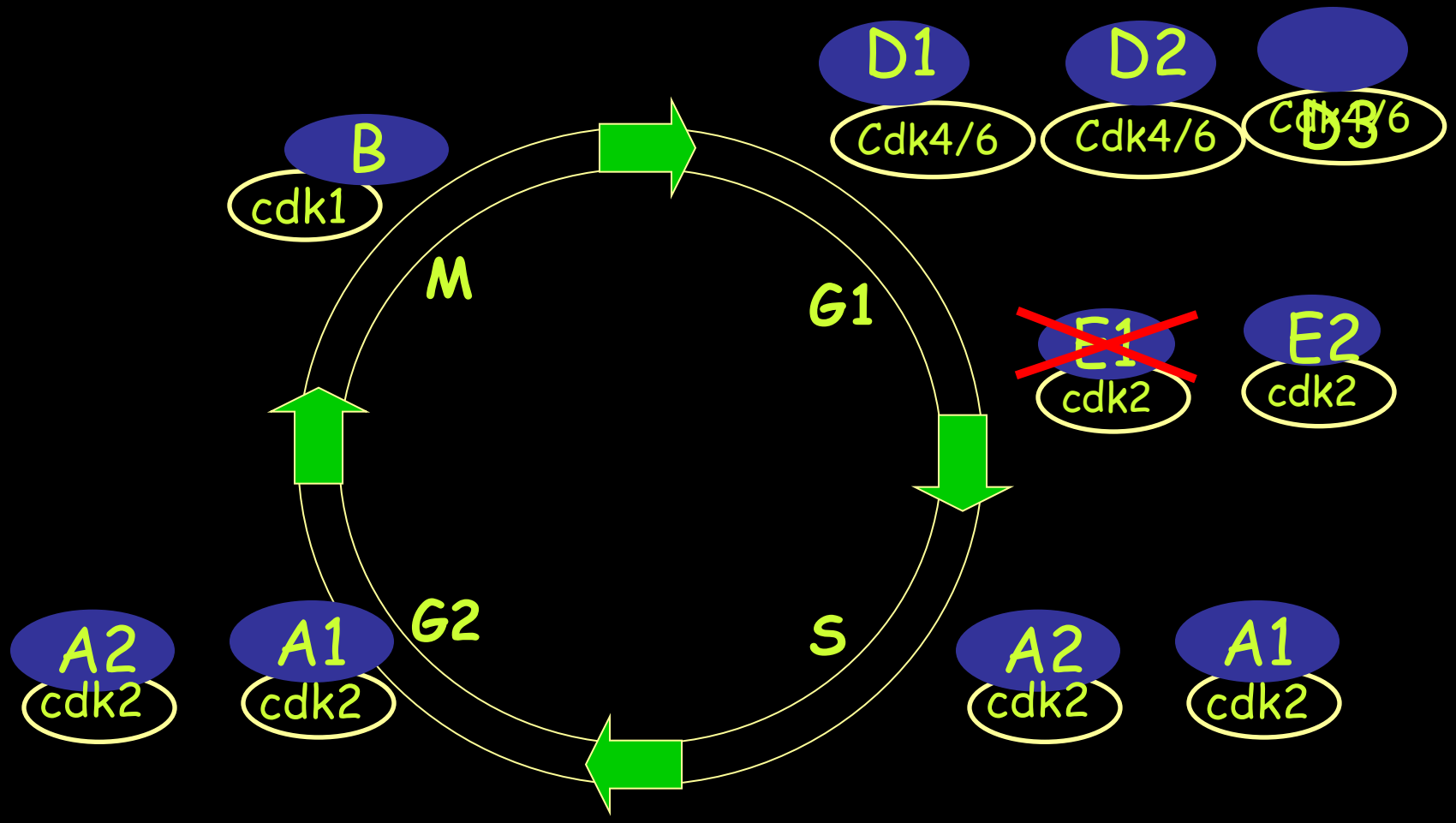
E9.5



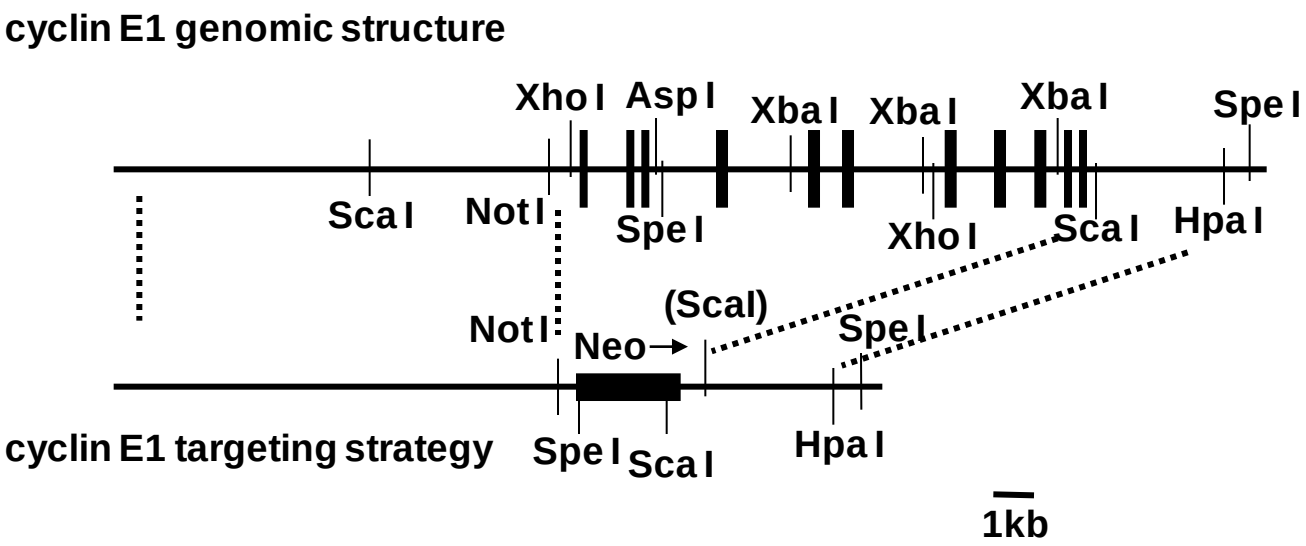


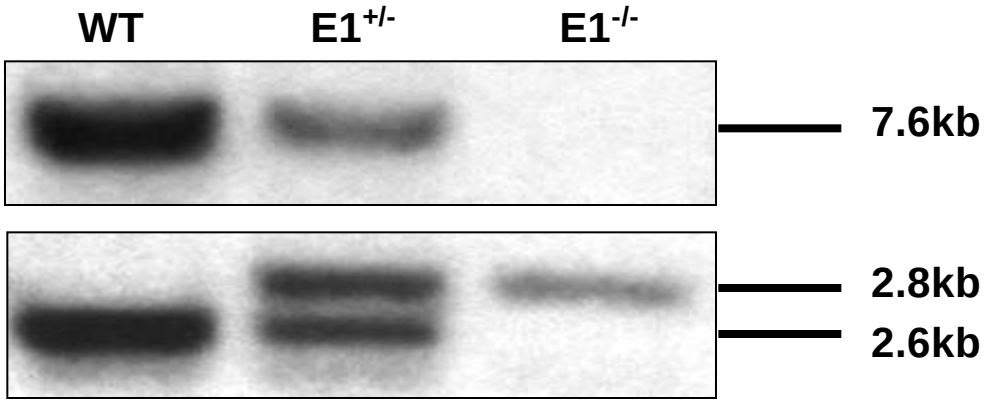


E11.5


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

Cyclin E1 knockout

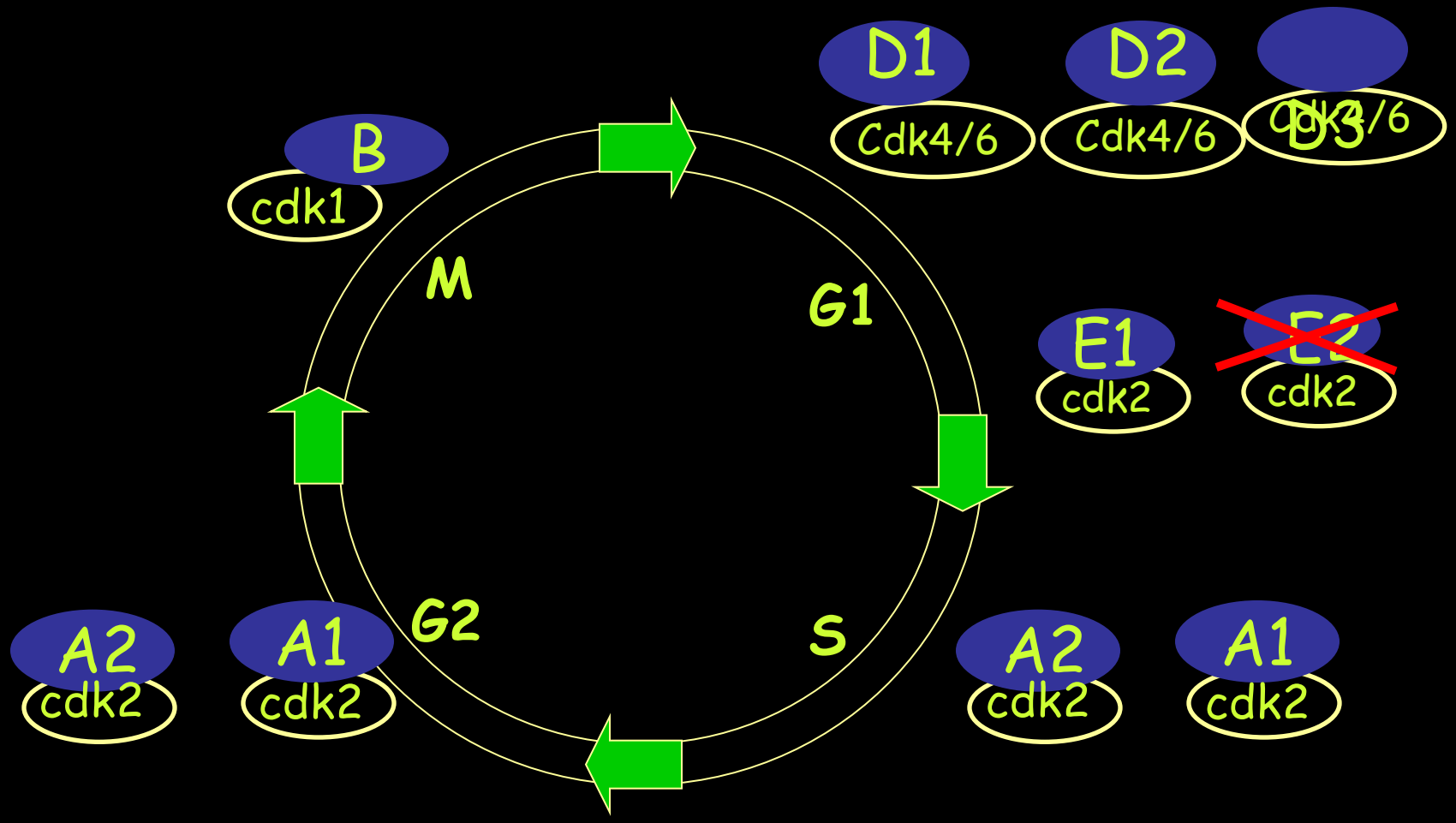




Cyclin E1$^{-/-}$ mice

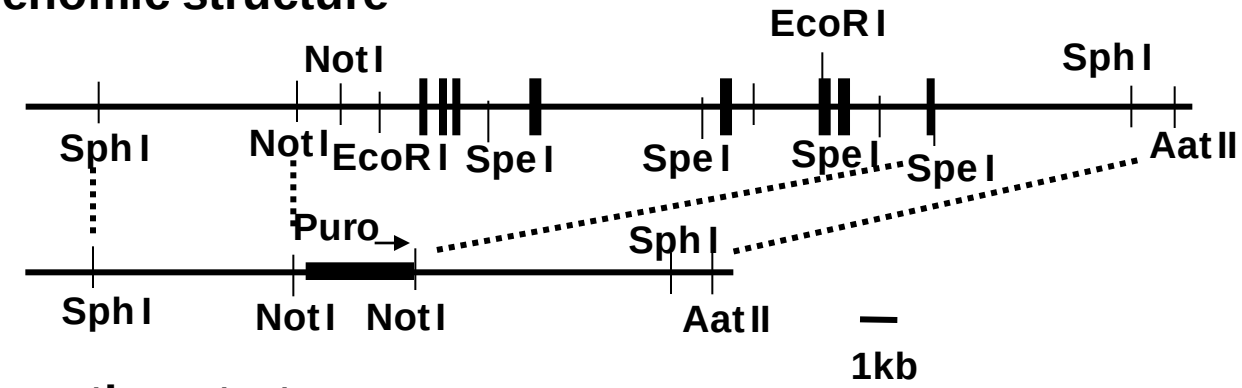
viable

develop normally

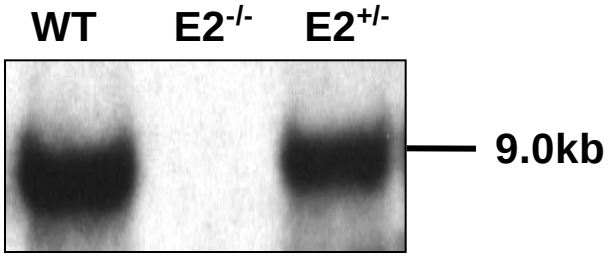

D1
Cdk4/6
D2
Cdk4/6
Cdk4/6
B
cdk1
M
G1
E1
cdk2
cdk2
G2
S
A2
cdk2
A1
cdk2
A2
cdk2
A1
cdk2

Cyclin E2 knockout

cyclin E2 genomic structure



cyclin E2 targeting strategy

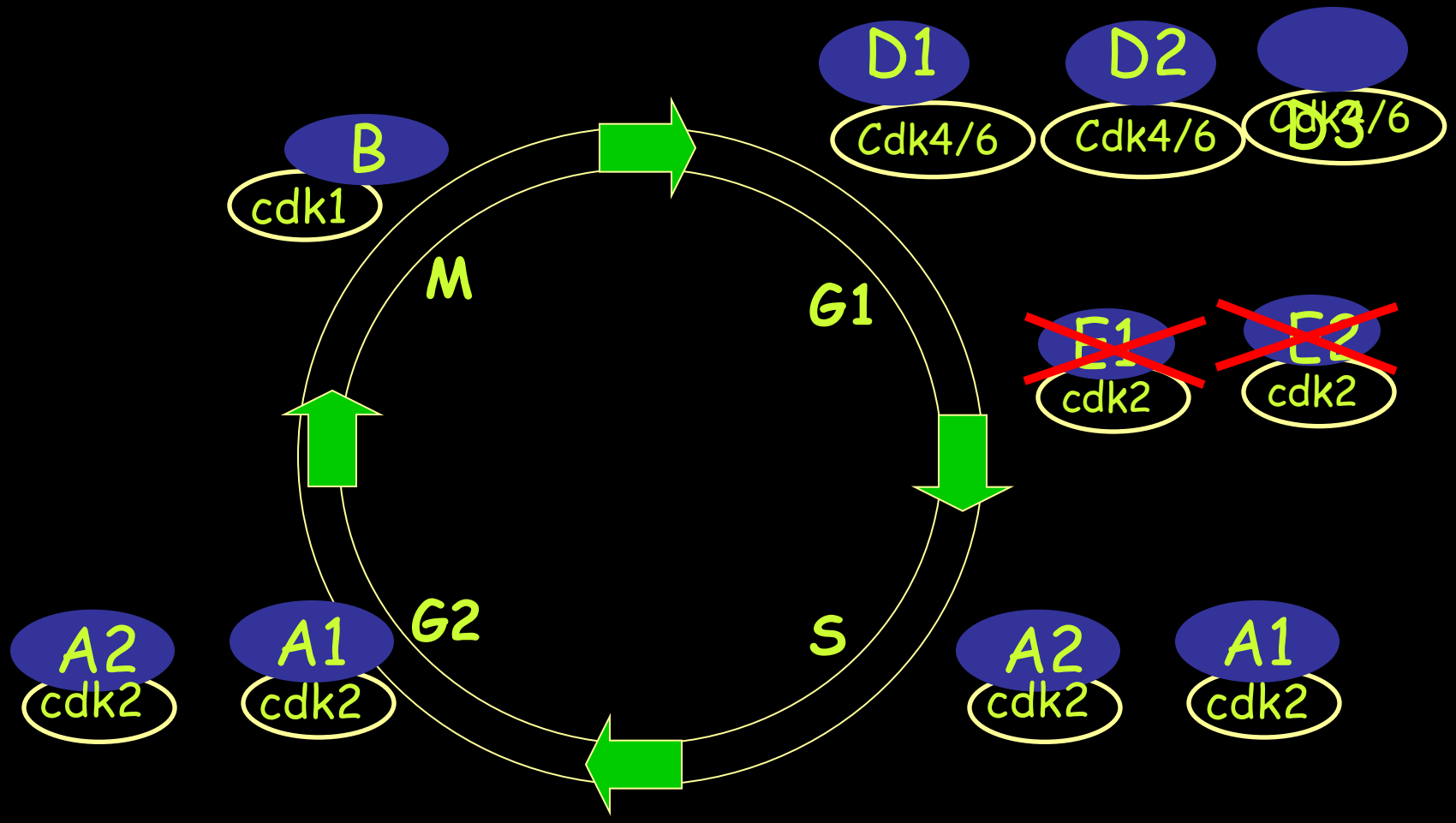


with Bruno Amati

Cyclin E2$^{-/-}$ mice

viable

some males sterile
testicular hypoplasia
reduced sperm counts


D1
Cdk4/6
D2
Cdk4/6
Cdk4/6
B
cdk1
M
G1
E1
cdk2
E2
cdk2
A2
cdk2
A1
cdk2
G2
S
A2
cdk2
A1
cdk2

Cyclin $E1^{-/-}E2^{-/-}$

Embryonic lethal

Cyclin $E1^{-/-}E2^{-/-}$ embryos

	Observed	Expected
E10.25	7/111	7/111
E10.75	8(13)/168	16/168
E11.5	3(6)/166	18/166
E12.5	(4)/82	10/82

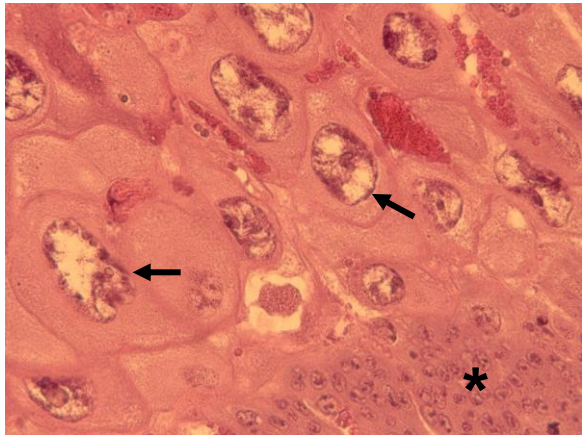
CYCLIN $E1^{-/-}E2^{-/-}$ EMBRYOS

Control $E1^{-/-}E2^{-/-}$

day E 10.75

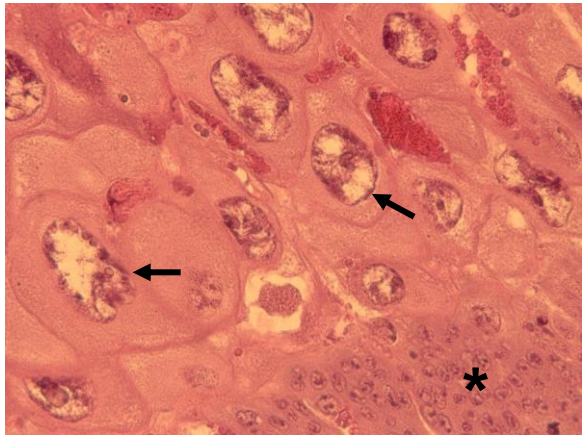
Phenotype of trophoblast giant cells

WT

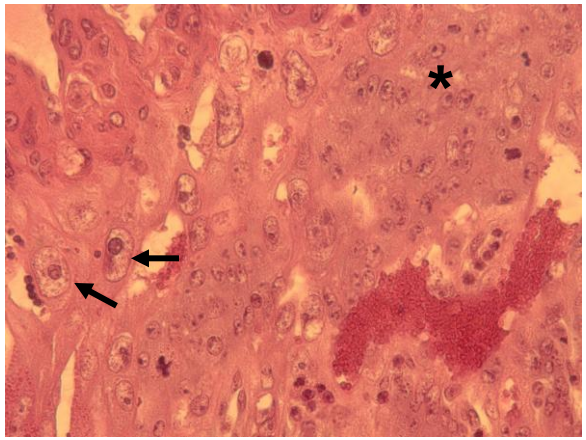


Phenotype of trophoblast giant cells

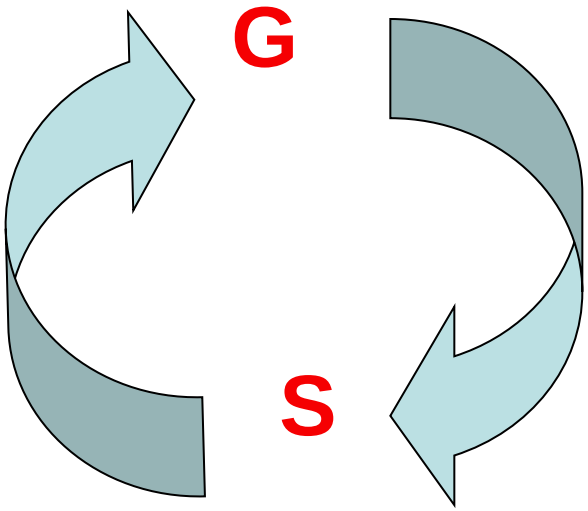
WT



$E1^{-/-}E2^{-/-}$



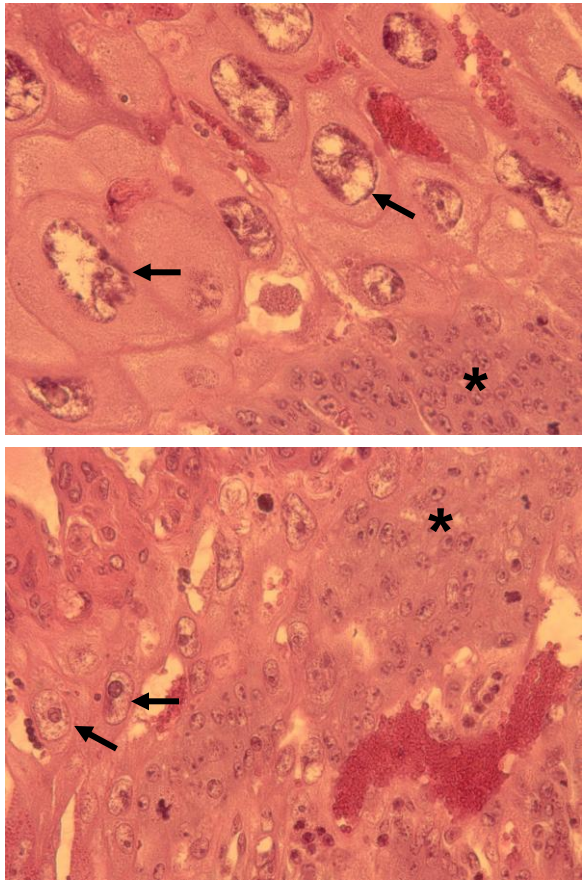
ENDOREPLICATION

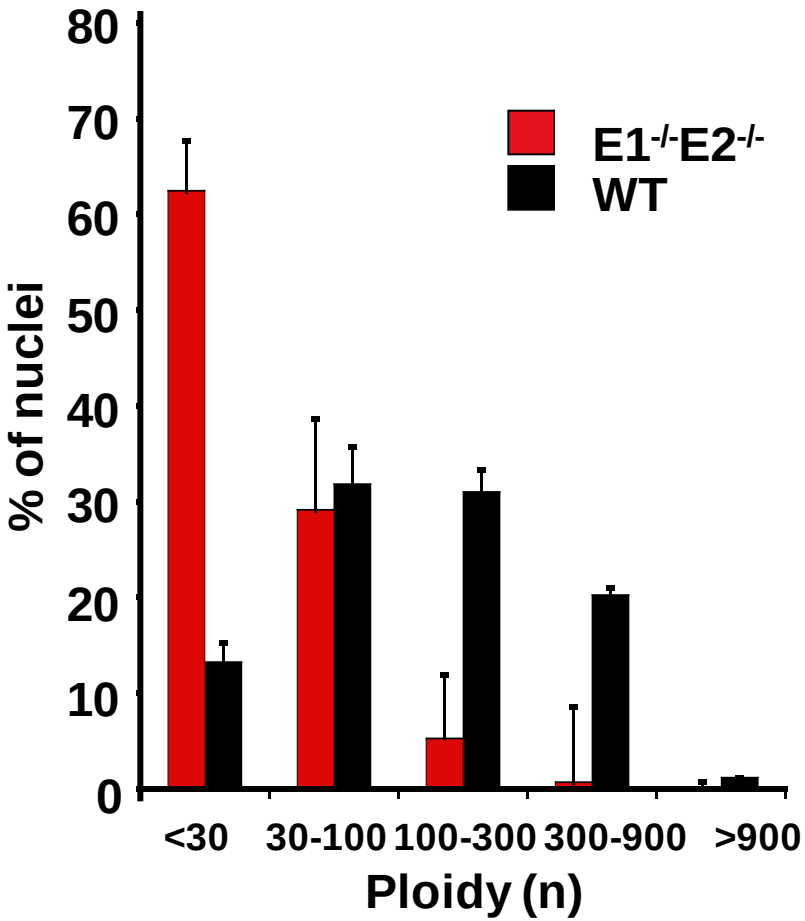


Phenotype of trophoblast giant cells

WT

E1$^{-/-}$E2$^{-/-}$





Cyclin $E1^{-/-}E2^{-/-}$ mice

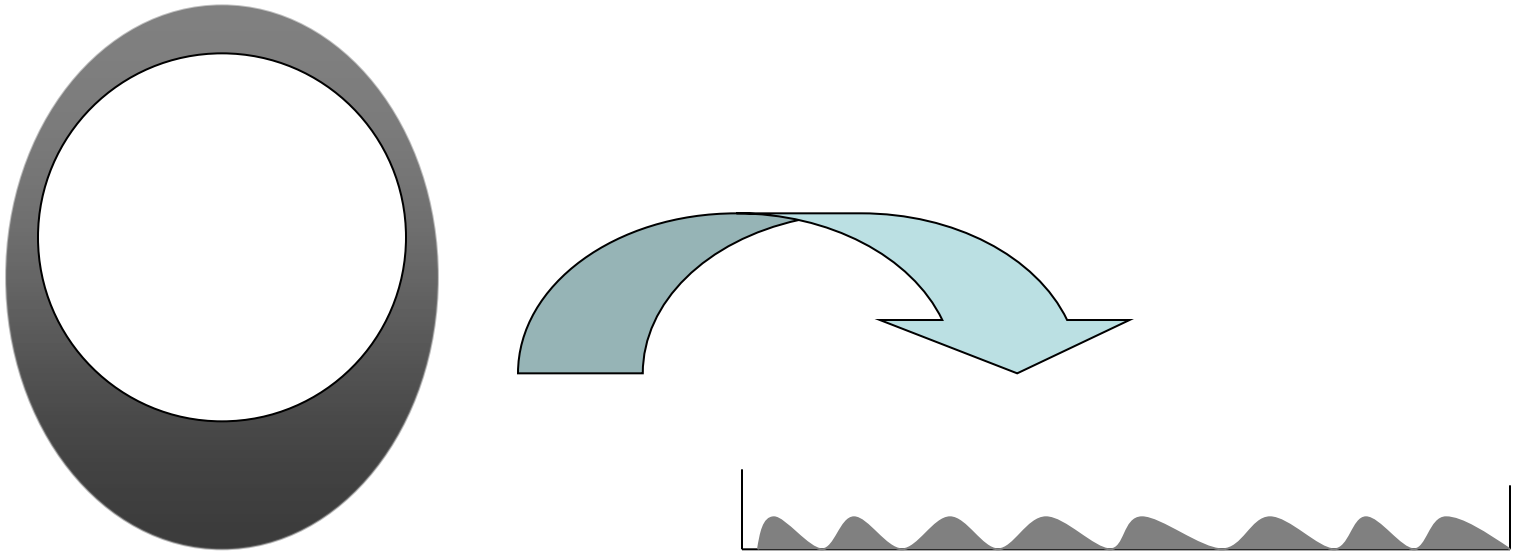
E9.0 - E10.75

Normal embryo development

Abnormal placental development
Paucity of trophoblast giant cells

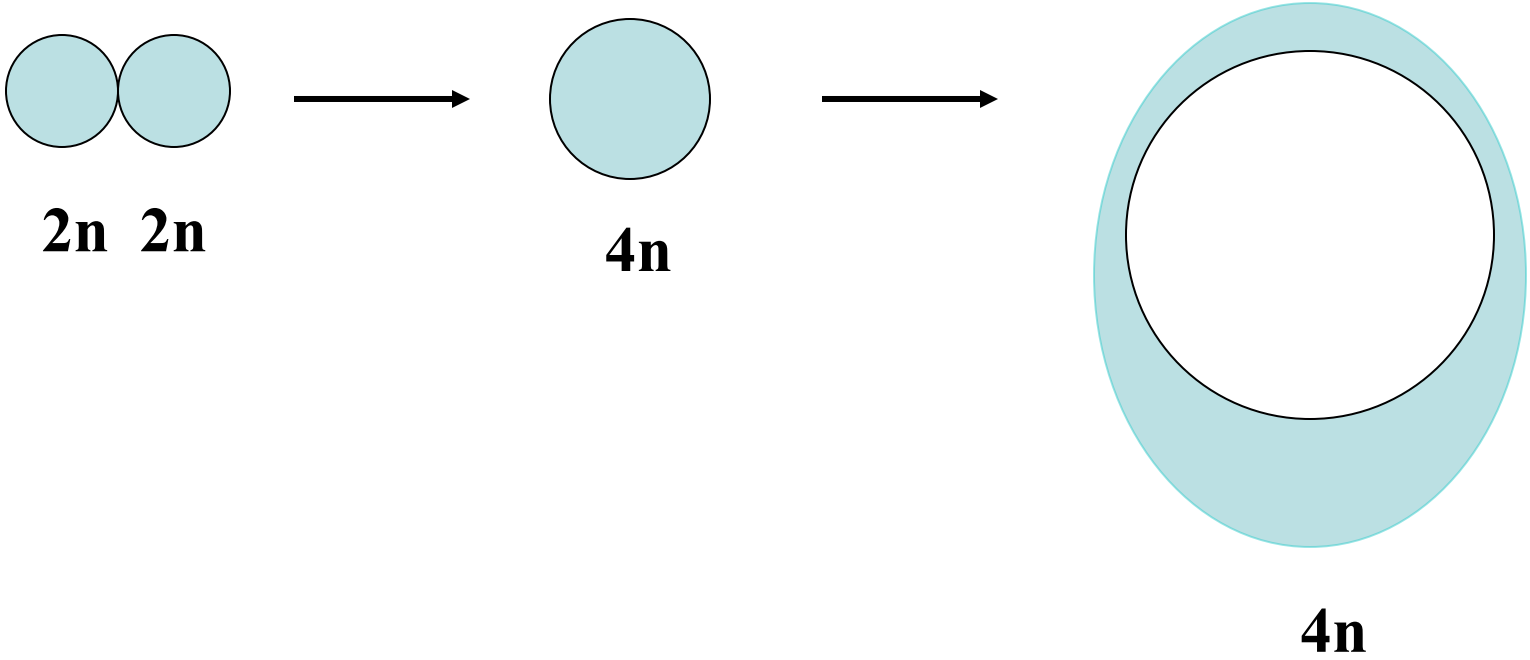
Endoreplication problem

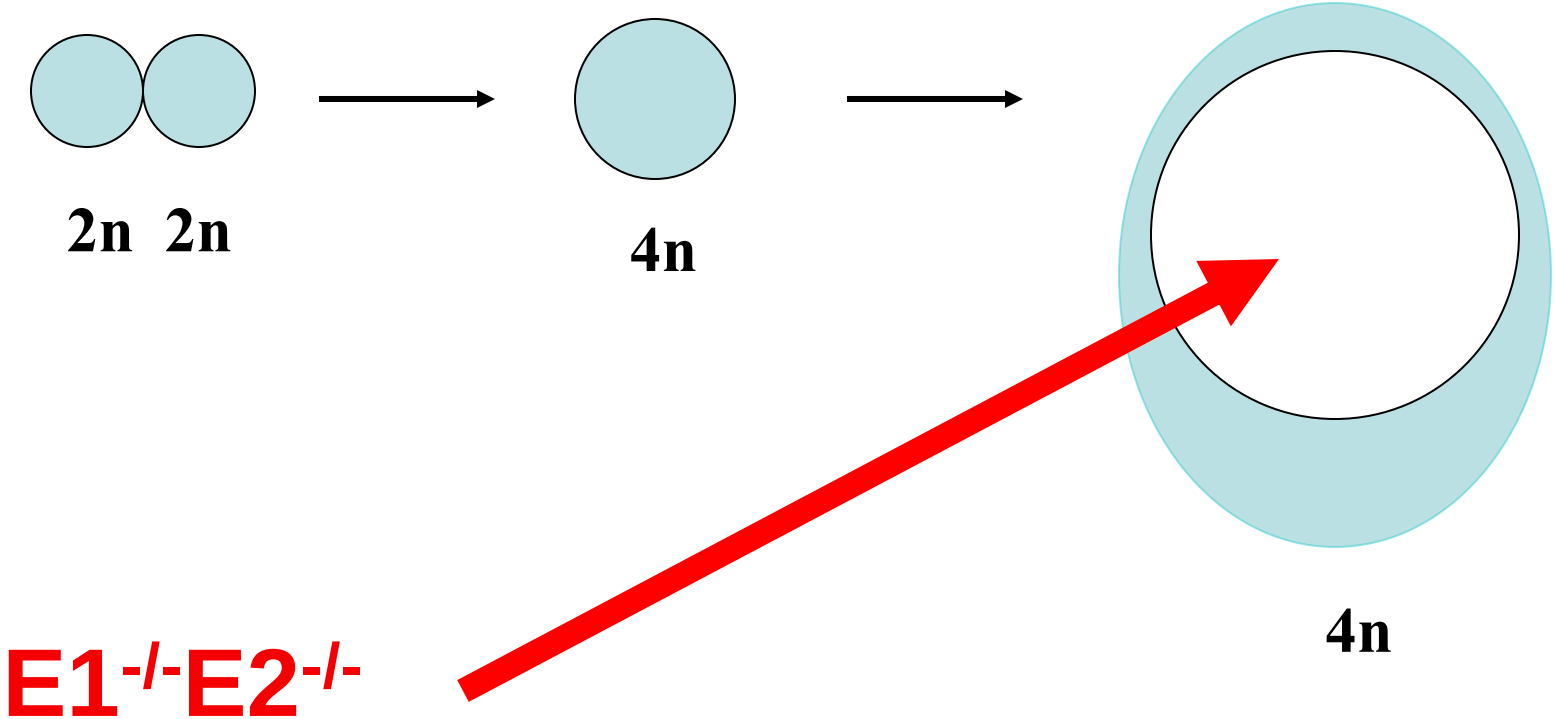
Mate $E1^{+/-}$ $E2^{+/-}$ mice



$E1^{-/-}$ $E2^{-/-}$ blastocysts

$E1^{-/-}$ $E2^{-/-}$ ES cells


2n 2n
4n
4n

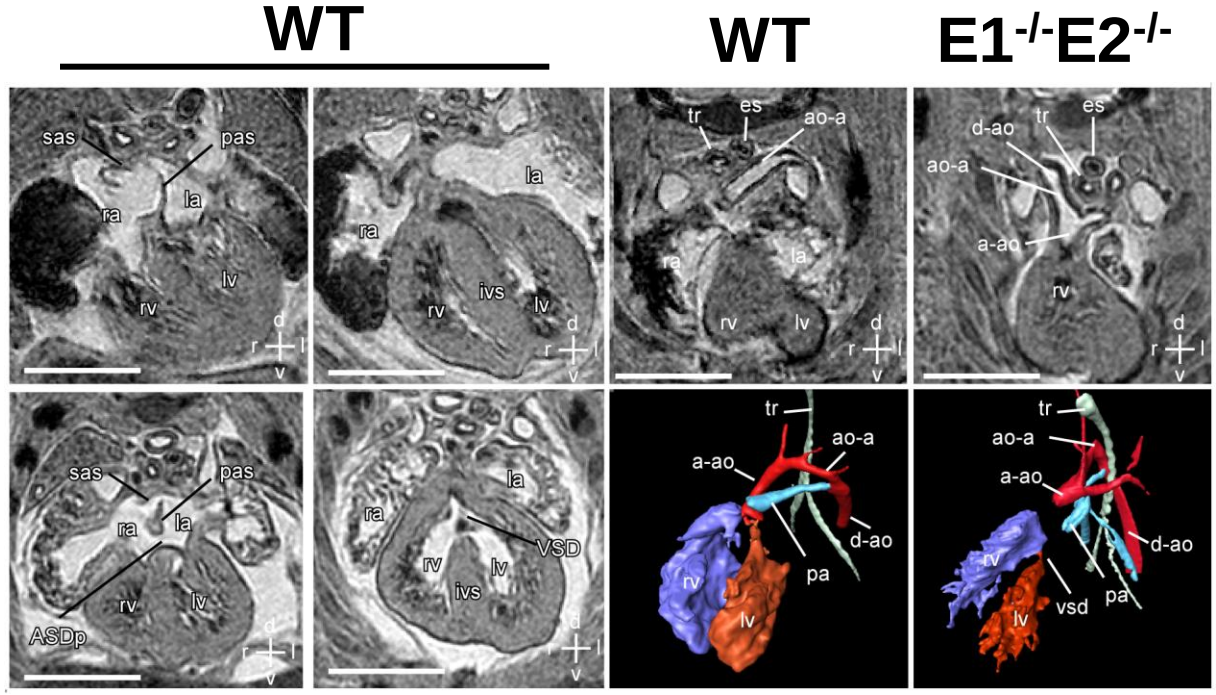


$E1^{-/-}E2^{-/-}$ ES Cells

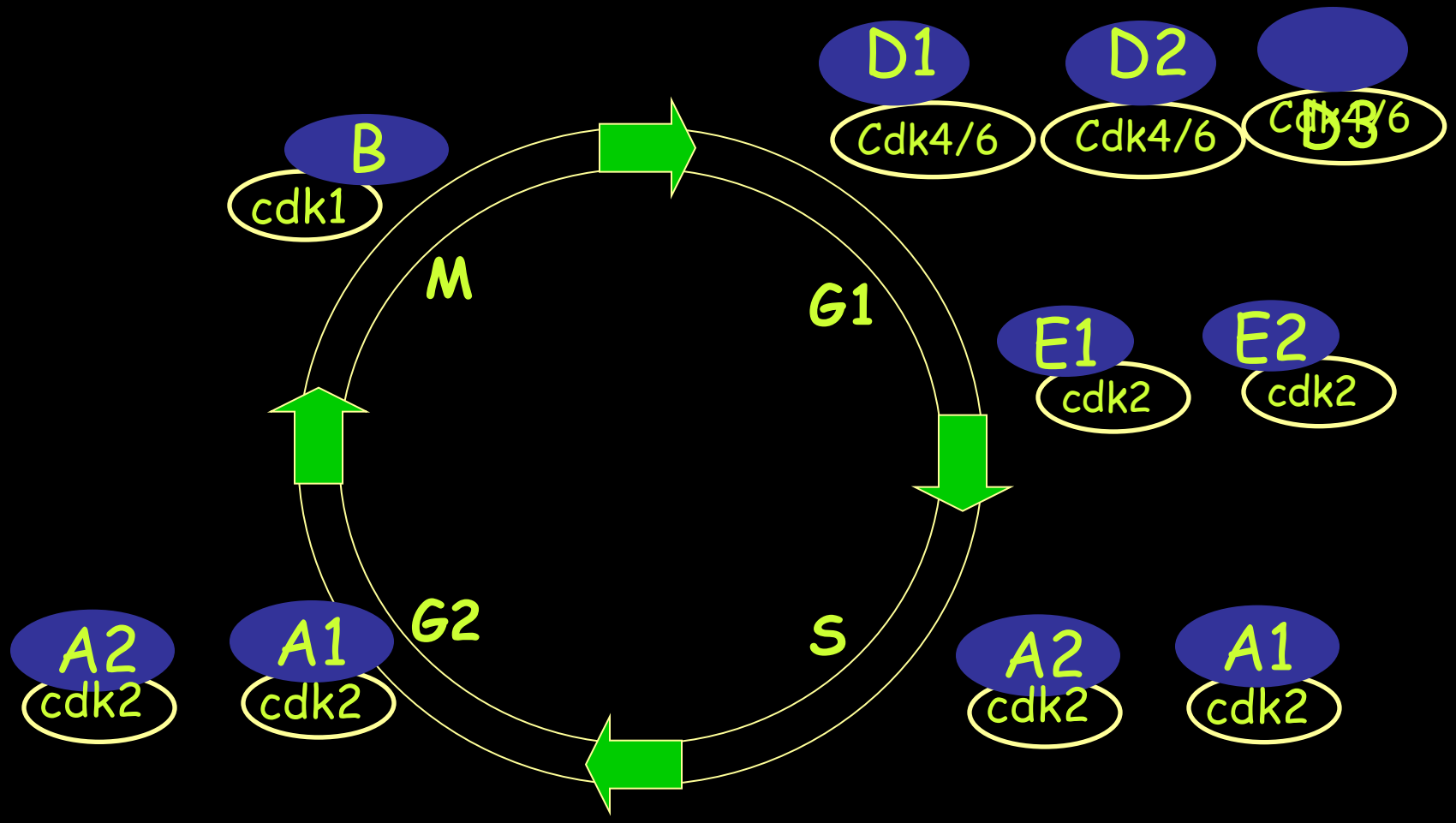
“Rescued” cyclin E-null neonates

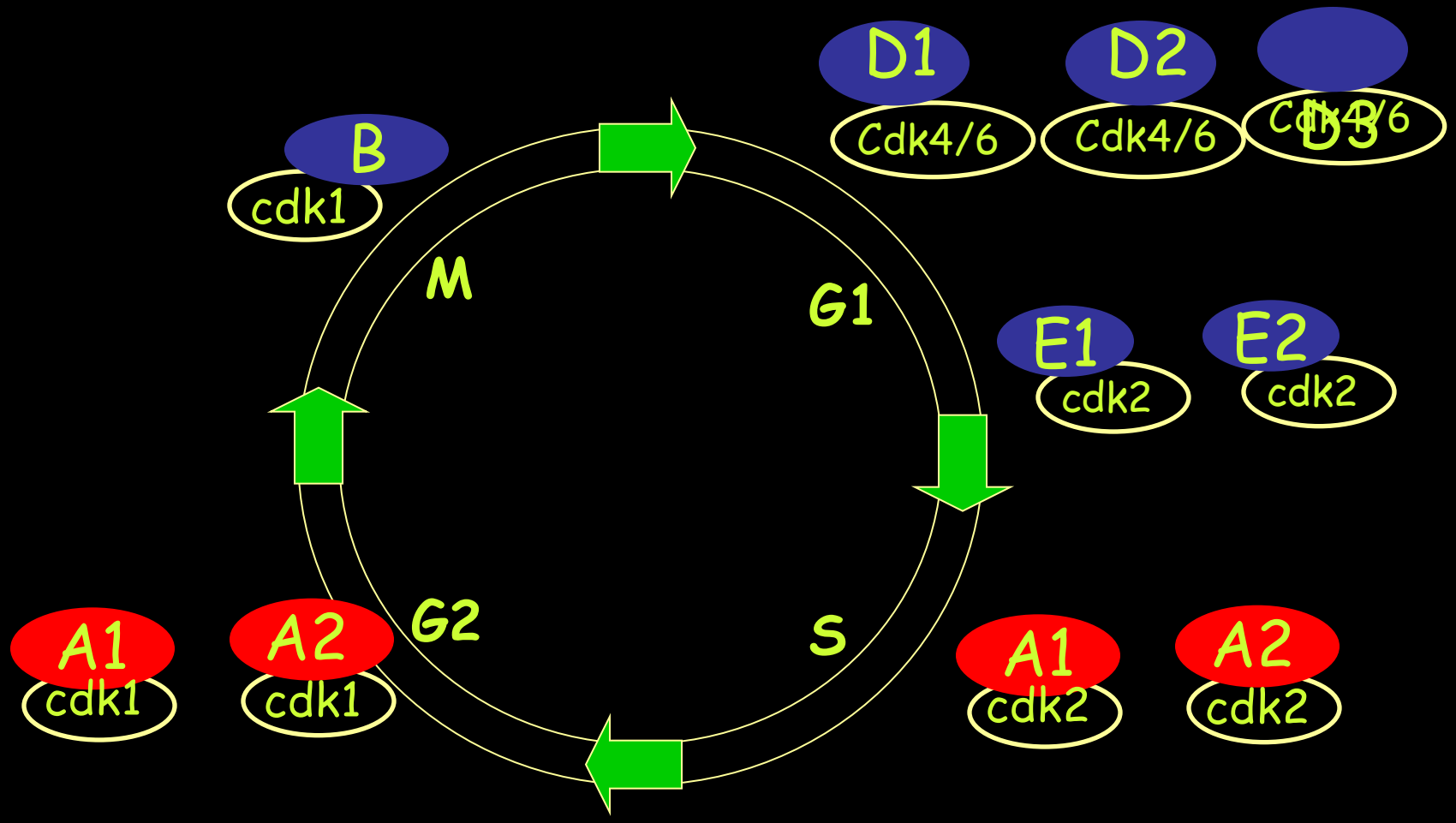
WT **E1$^{-/-}$E2$^{-/-}$**

Cardiac abnormalities in cyclin E-null neonates



Cyclin E is largely dispensable for development


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


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

Role of cyclin A in the cell cycle

- Regulate DNA synthesis

  induce the initiation of DNA replication

  prevent re-replication

- Plays a role in centrosome duplication

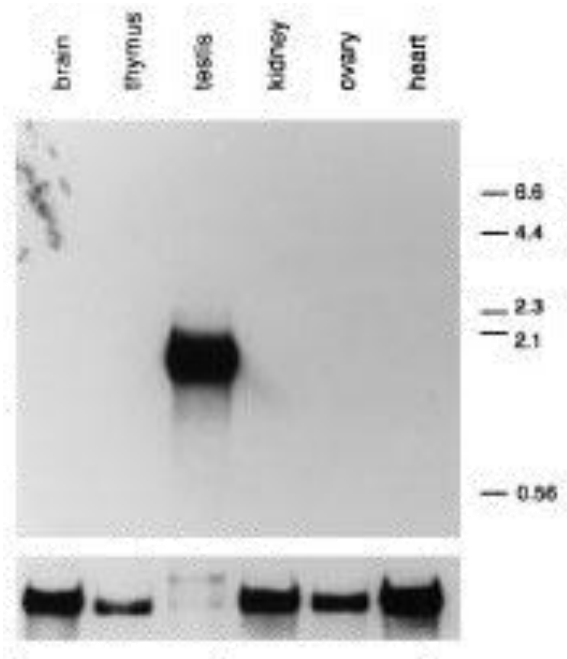
- Entry into mitosis

A1

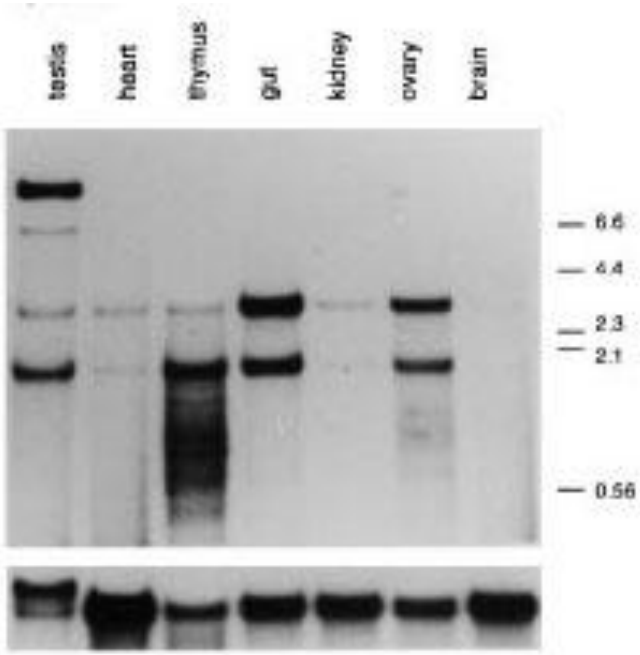
A2

Expression pattern of cyclins A1 and A2

A1

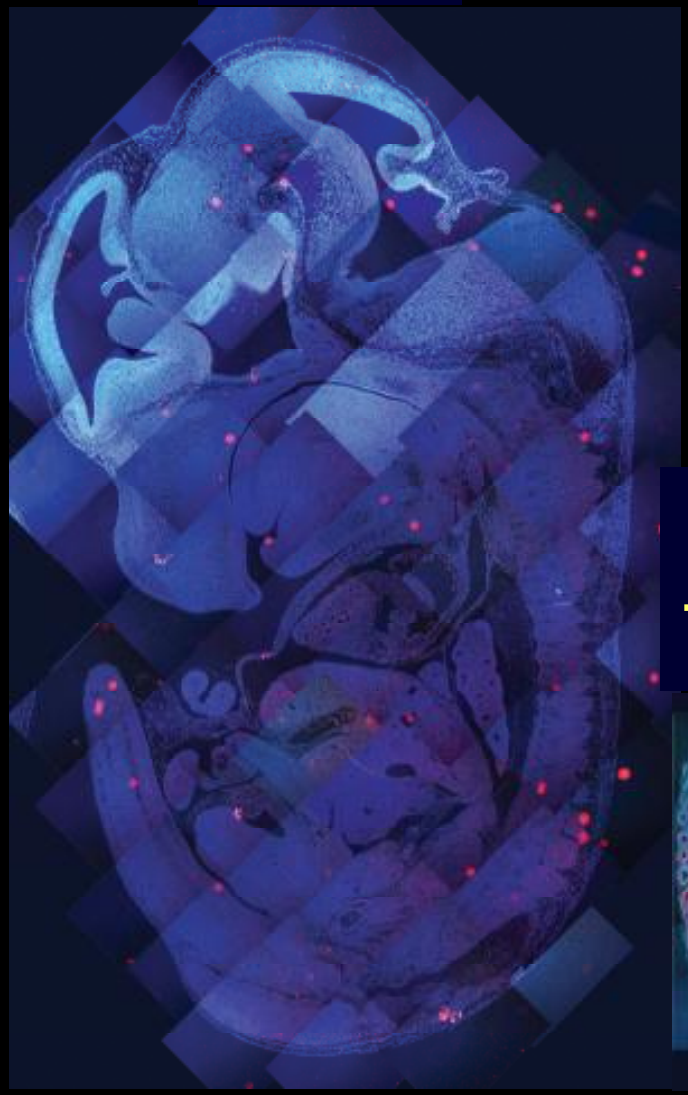


A2



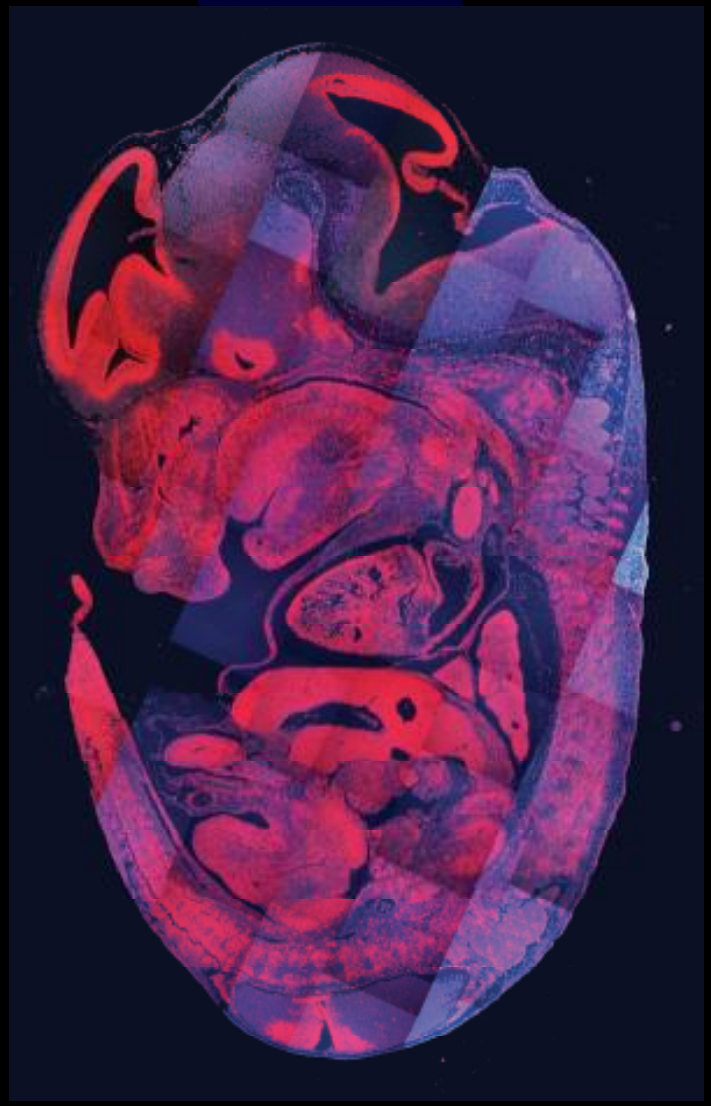
C. Sweeney et al., 1996

A1



A1
Testis

A2



“cyclin A2 is an essential component of all embryonic and somatic cell cycles in mammals”

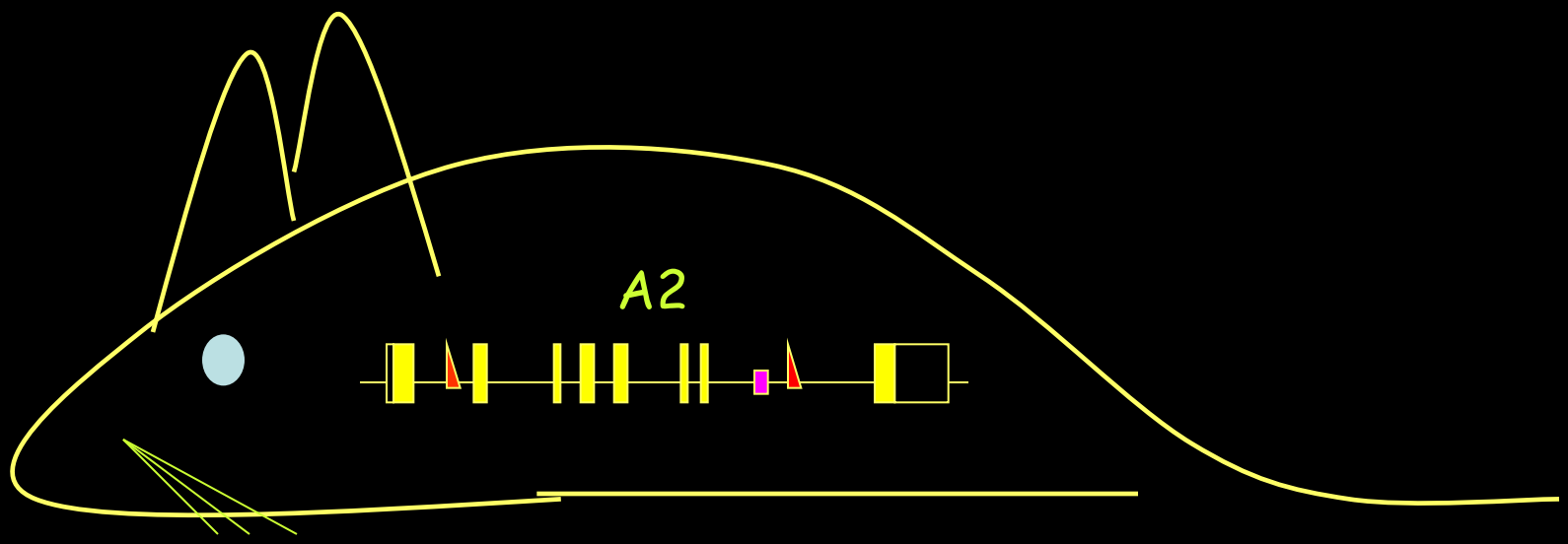
“there are essentially two types of cyclins: those that are essential components of the cell cycle engine [cyclins A and B], and those involved in cell type specific regulation of the cycle [G1 cyclins]”

Consequence of cyclin A2 deletion in mice

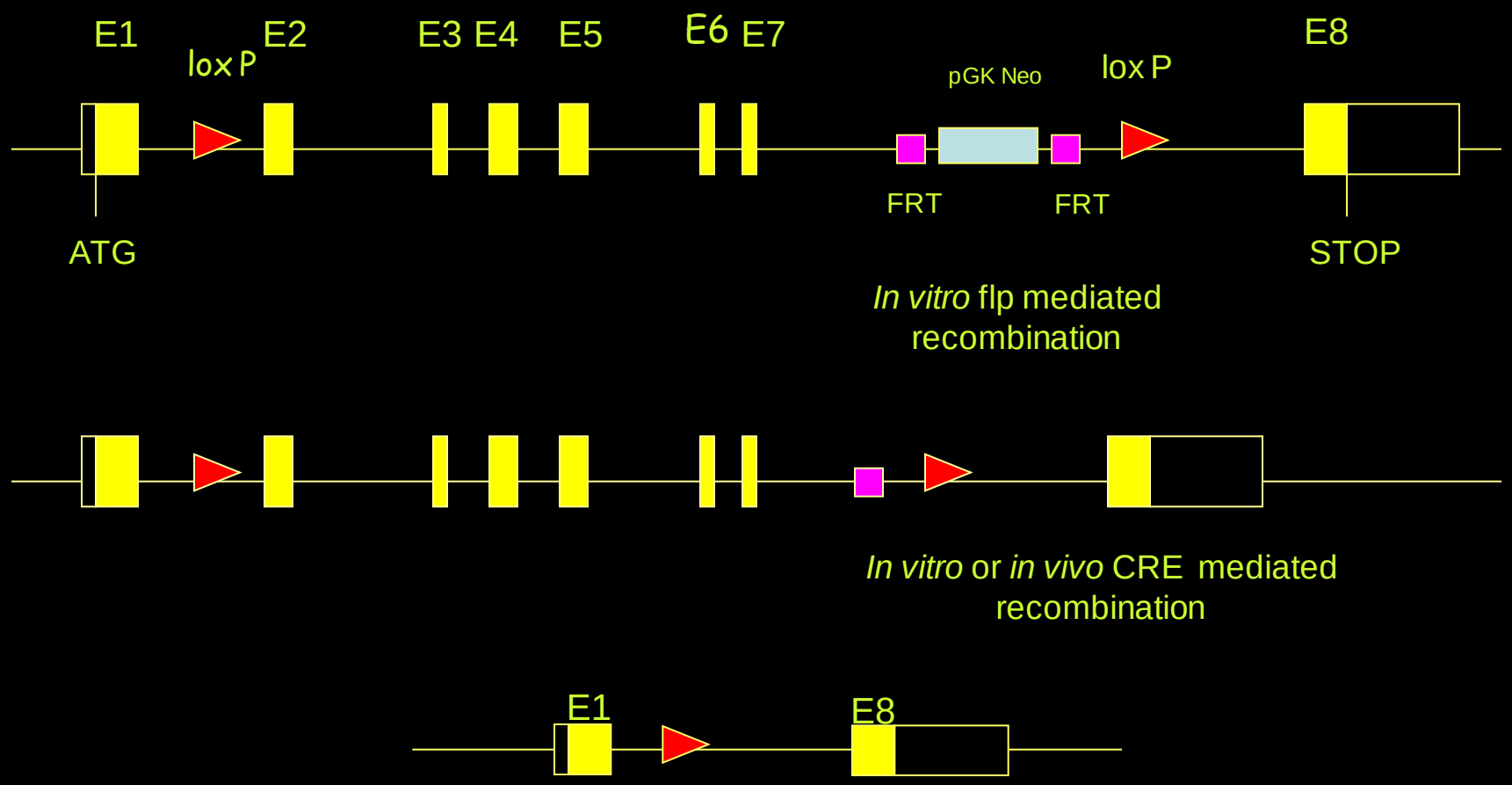
Ccna2 -/- mouse fail to develop past 5.5 days pc

(Murphy at al., 1997)

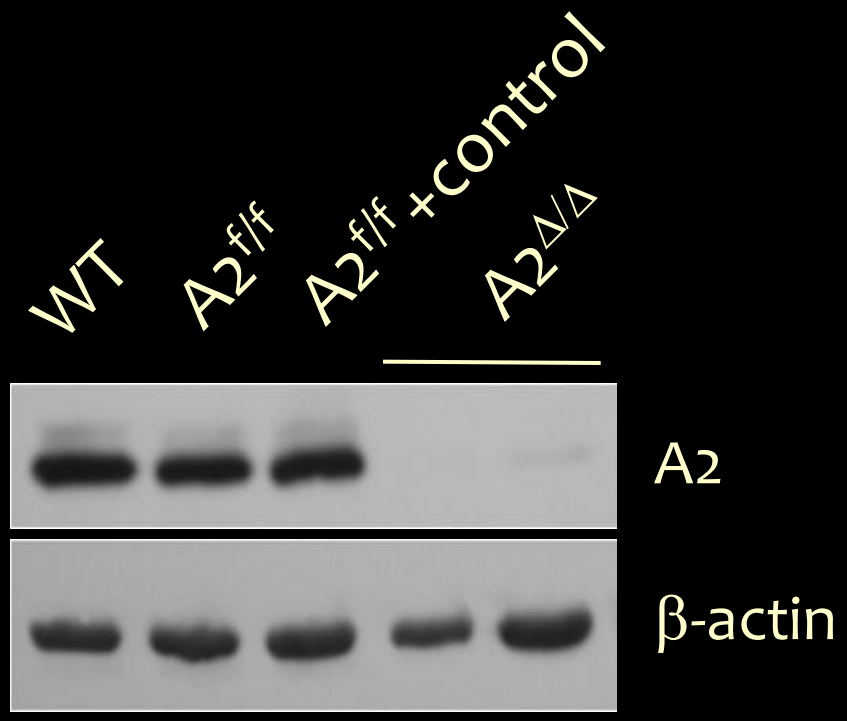
Conditional knockout of cyclin A2



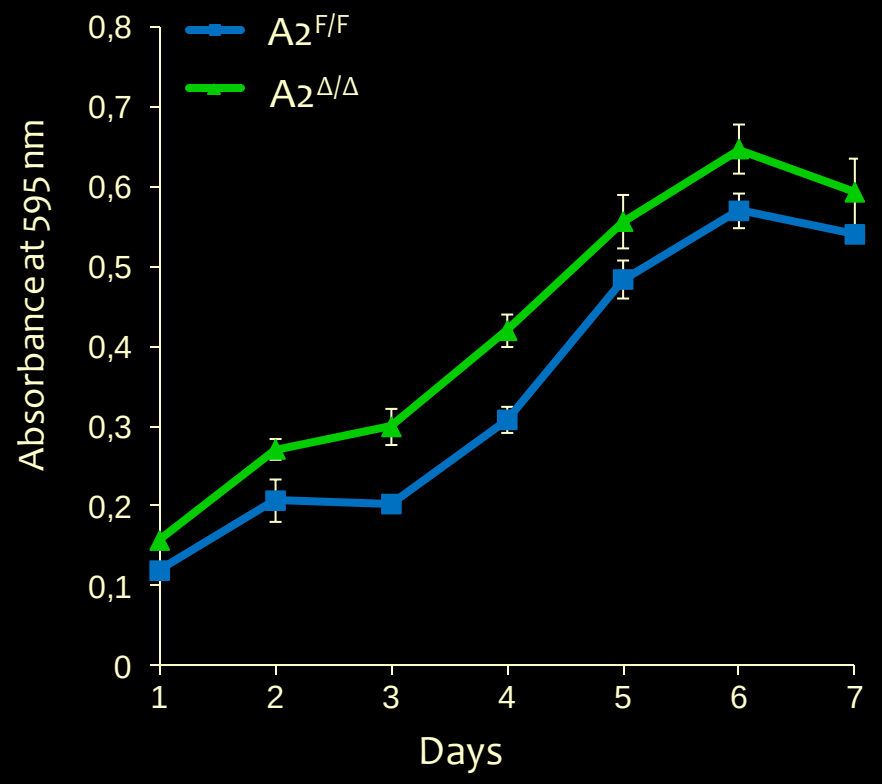
Cyclin A2 conditional construct



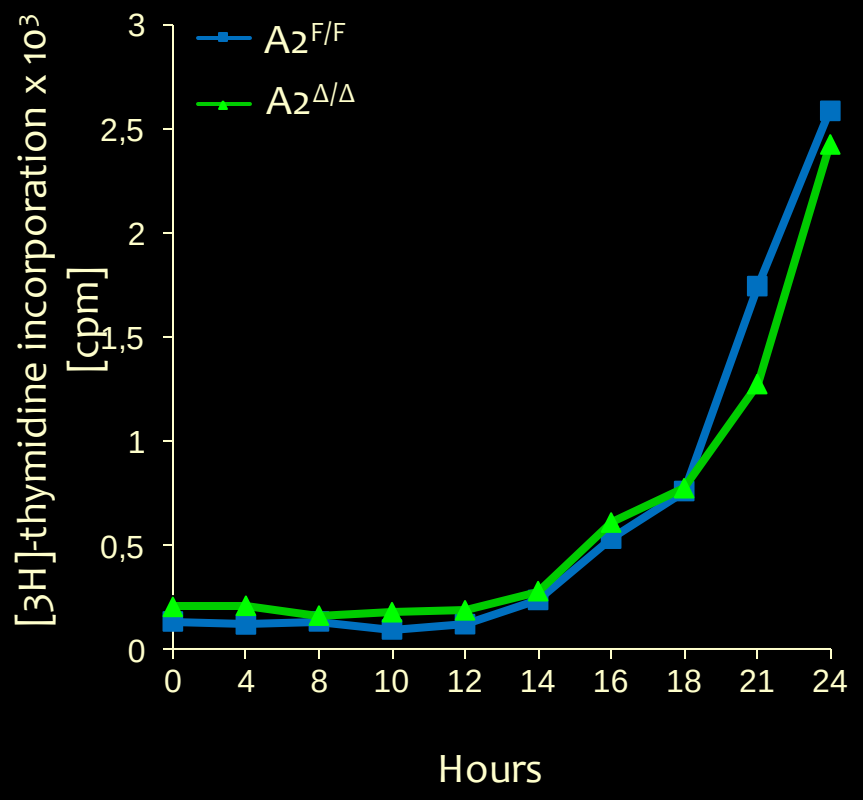
Cyclin A2 expression level after Cre mediated recombination



In vitro proliferation of cyclin A deficient fibroblasts

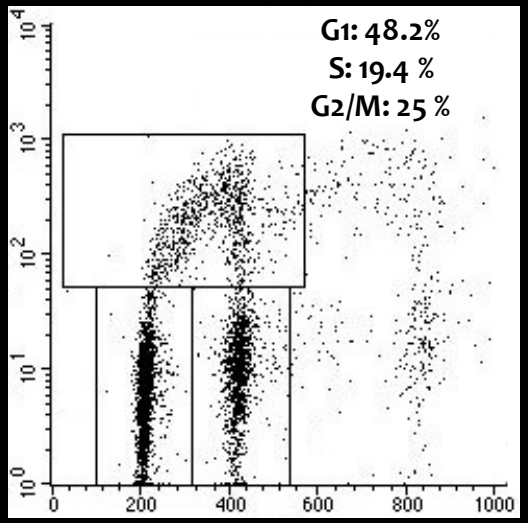


Re-entry of cell cycle by cyclin A deficient fibroblasts

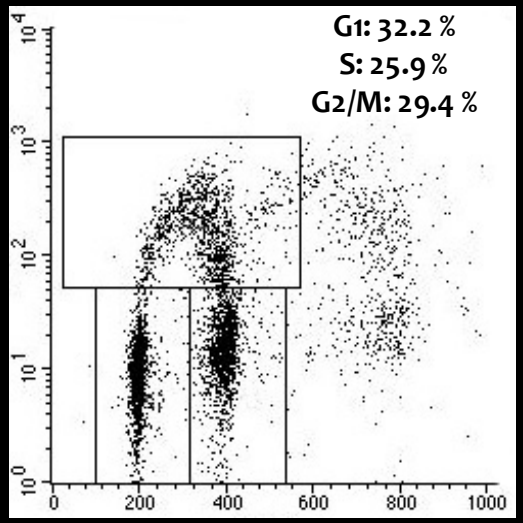


Cell cycle profile of MEFs lacking cyclin A2

$A2^{F/F}$



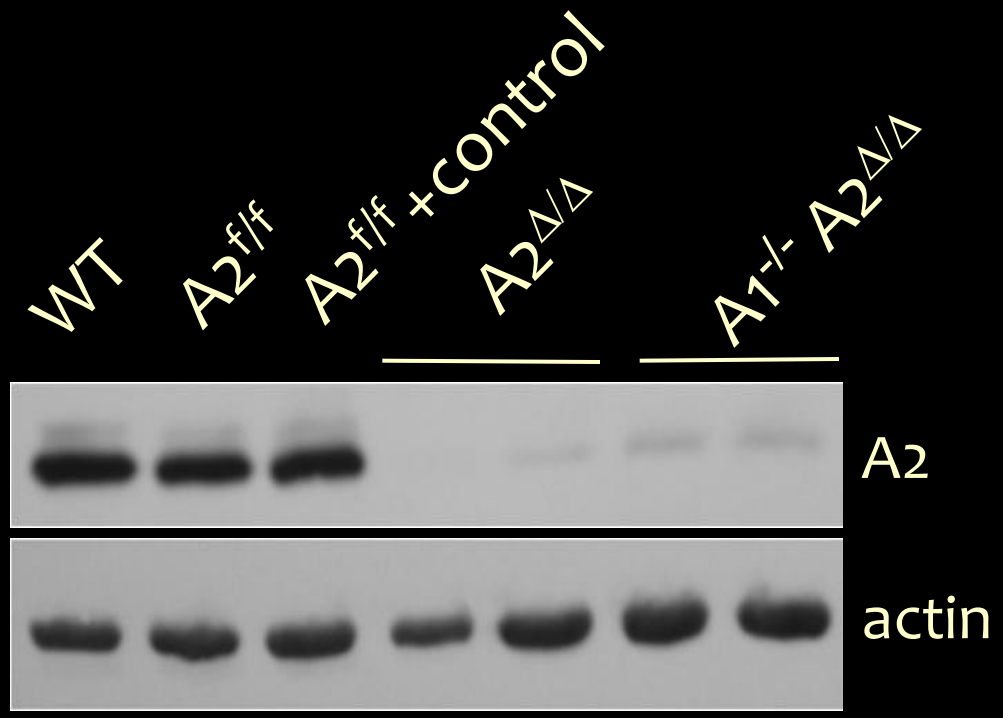
$A2^{\Delta/\Delta}$



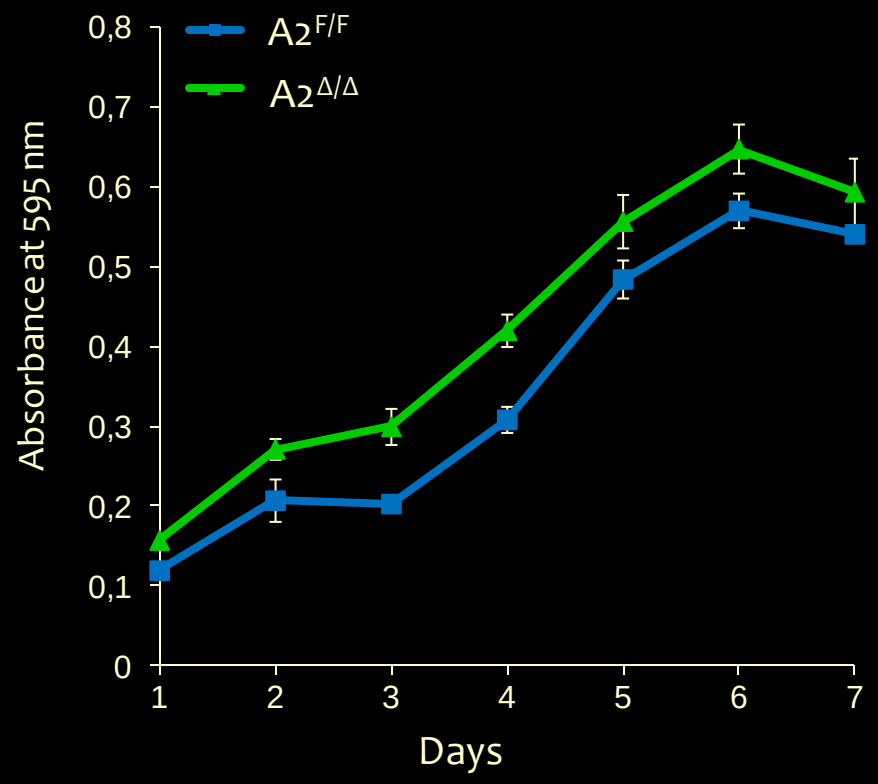
$A1^{-/-} \times A2^{F/F}$

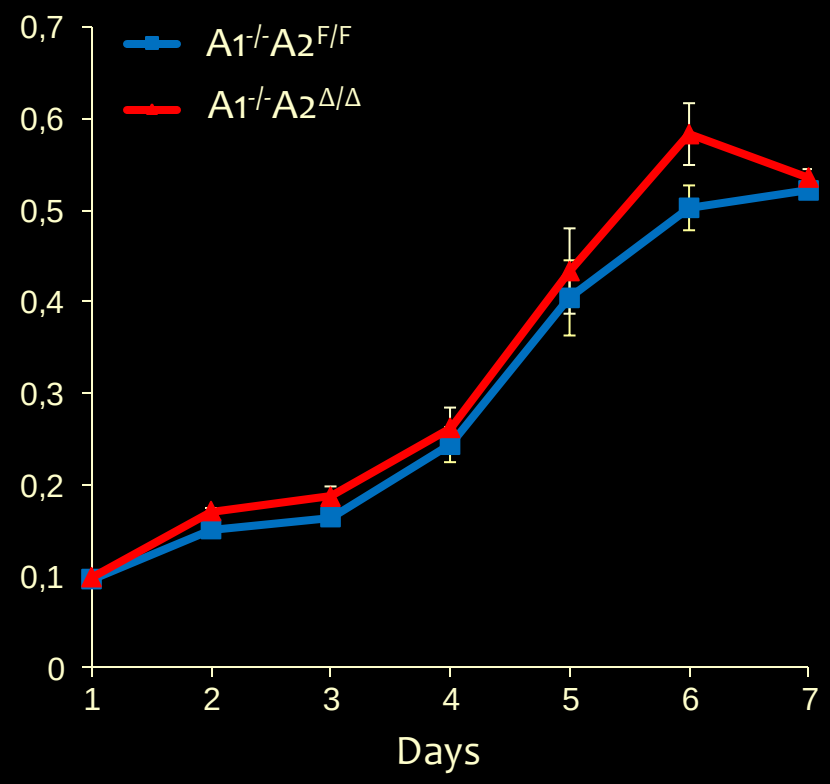
D. Wolgemuth

Cyclin A2 expression level after Cre mediated recombination

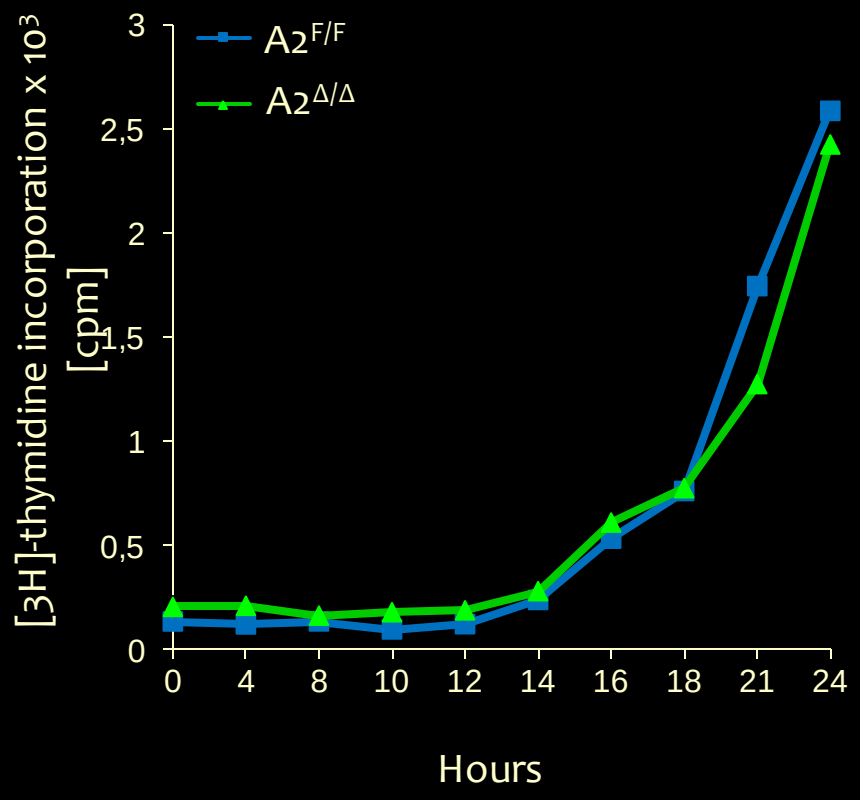


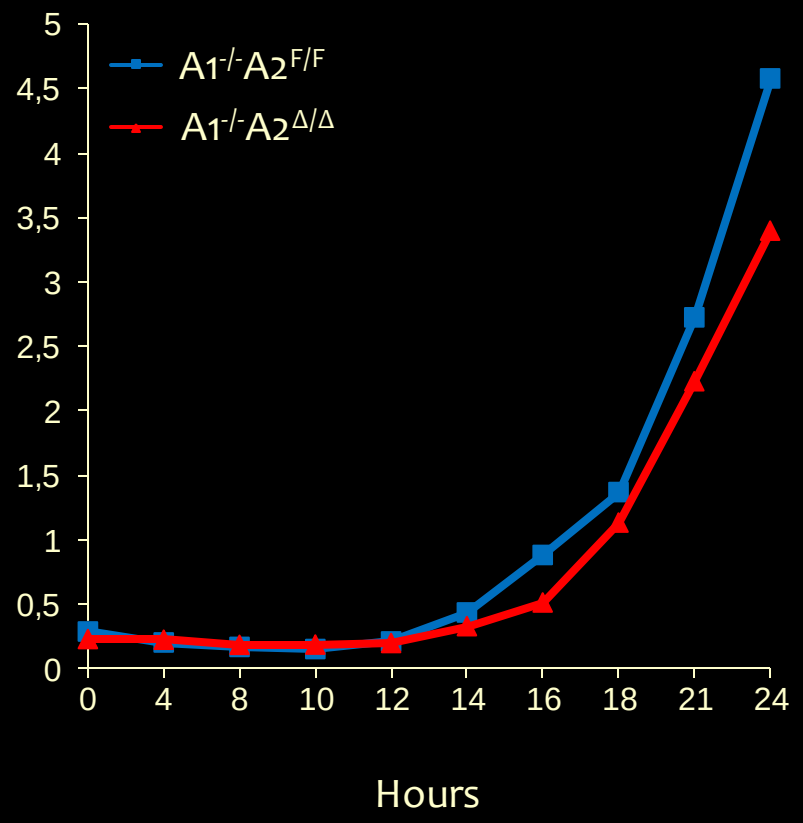
In vitro proliferation of cyclin A deficient fibroblasts





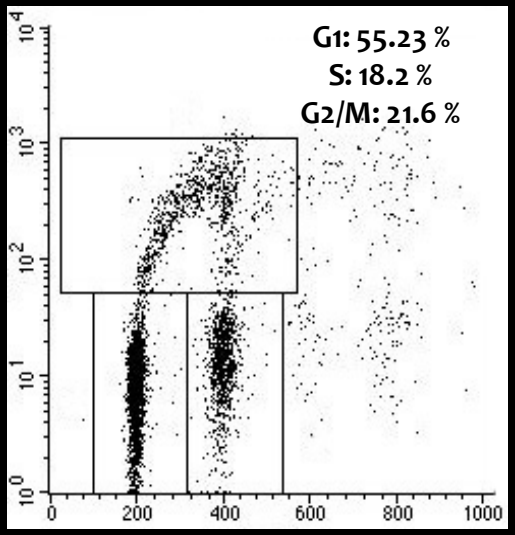
Re-entry of cell cycle by cyclin A deficient fibroblasts



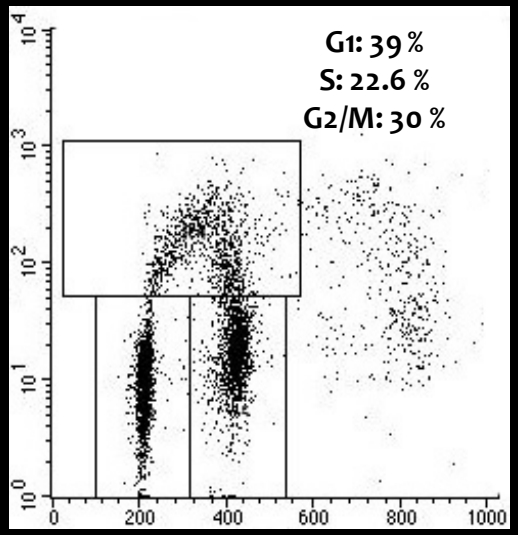


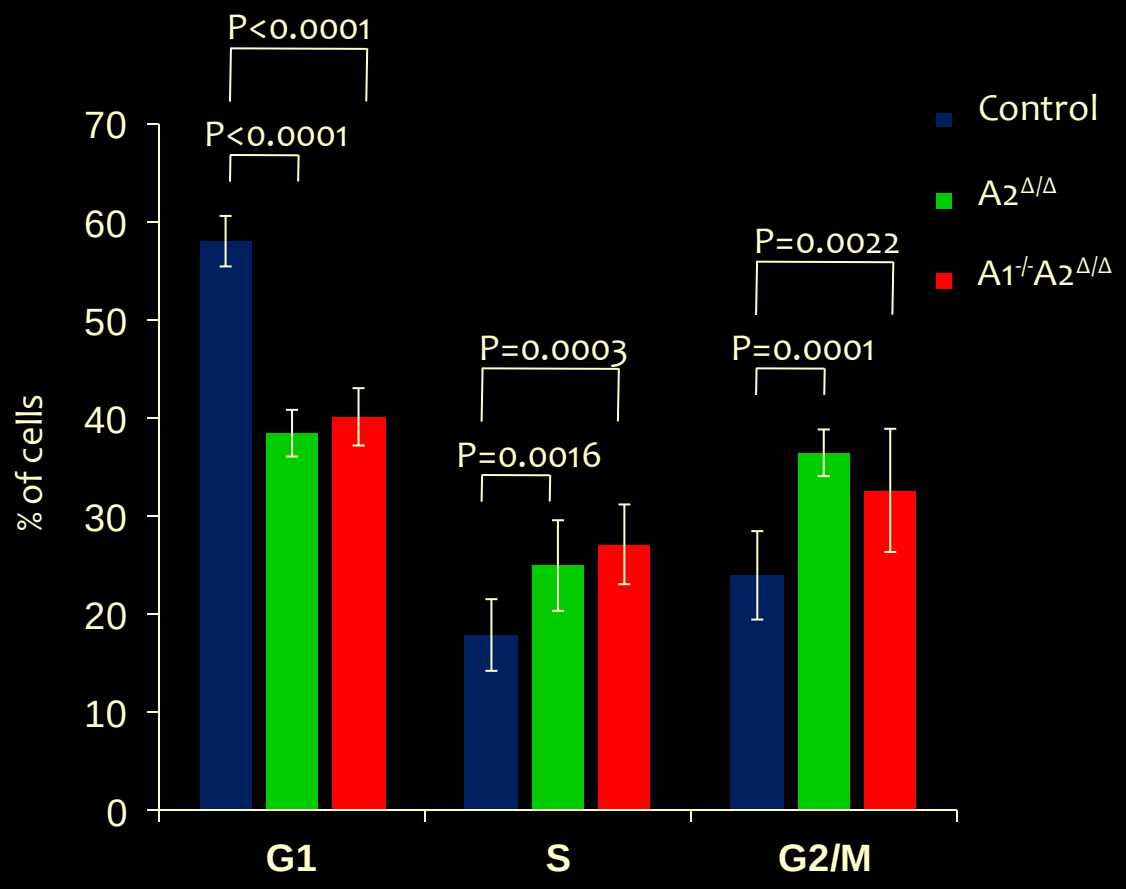
Cell cycle profile of MEF lacking cyclins A1 & A2

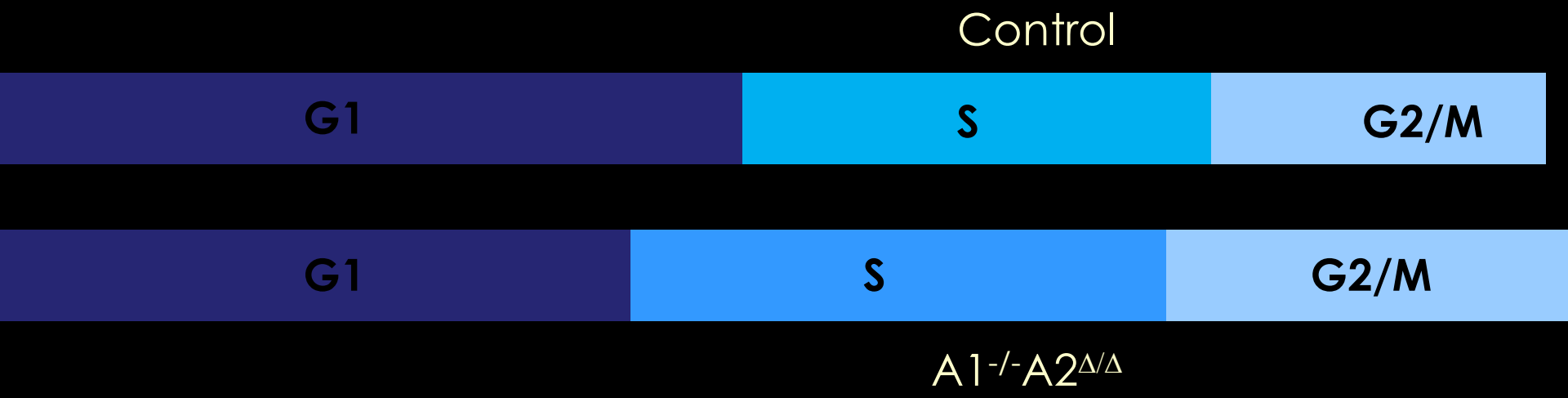
$A1^{-/-}$ $A2^{F/F}$



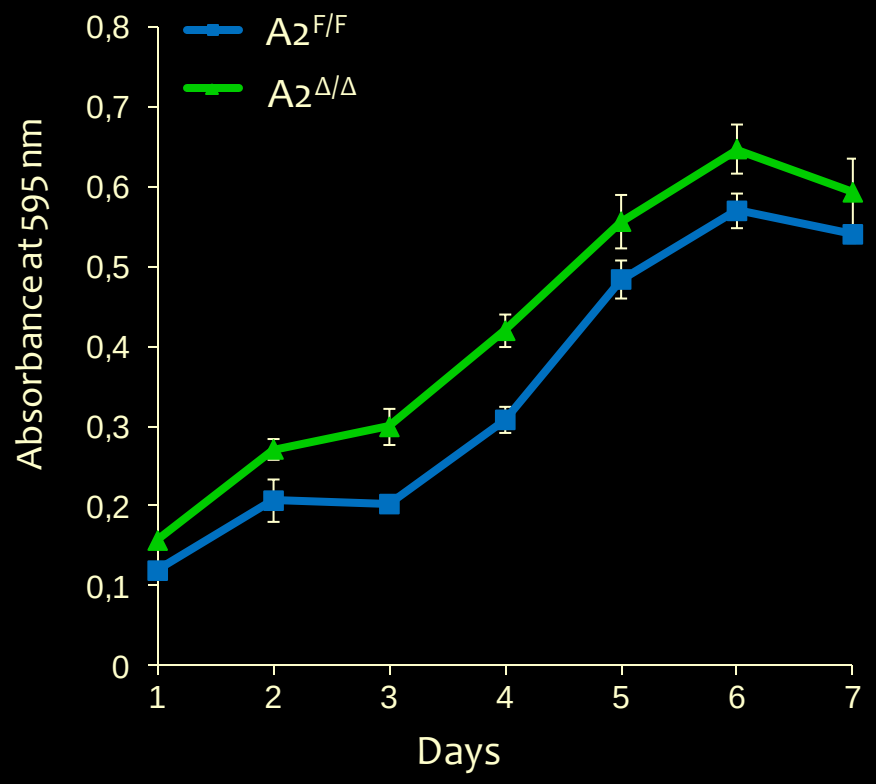
$A1^{-/-}$ $A2^{\Delta/\Delta}$

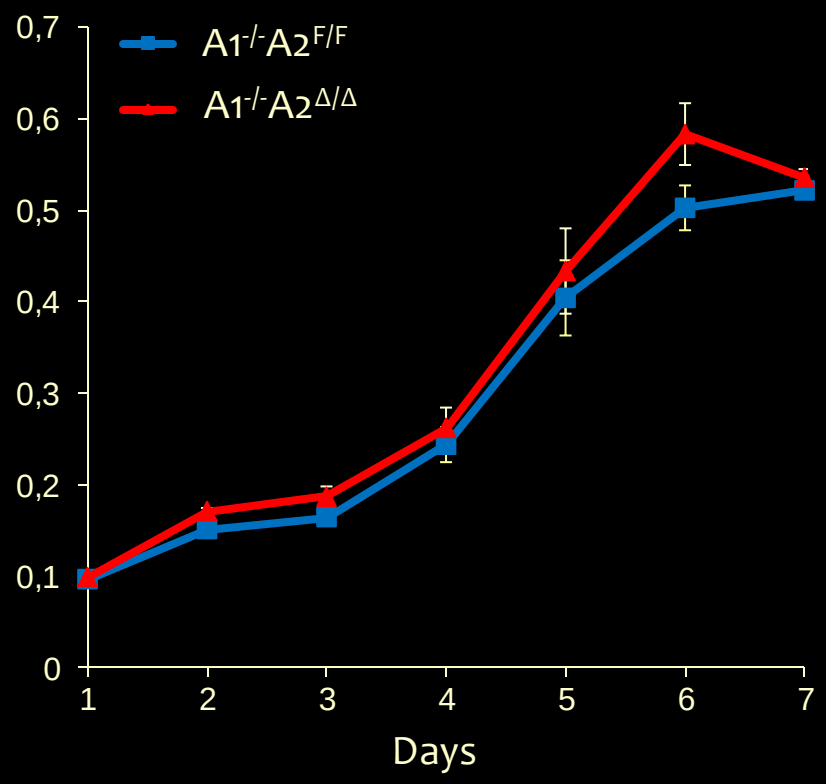


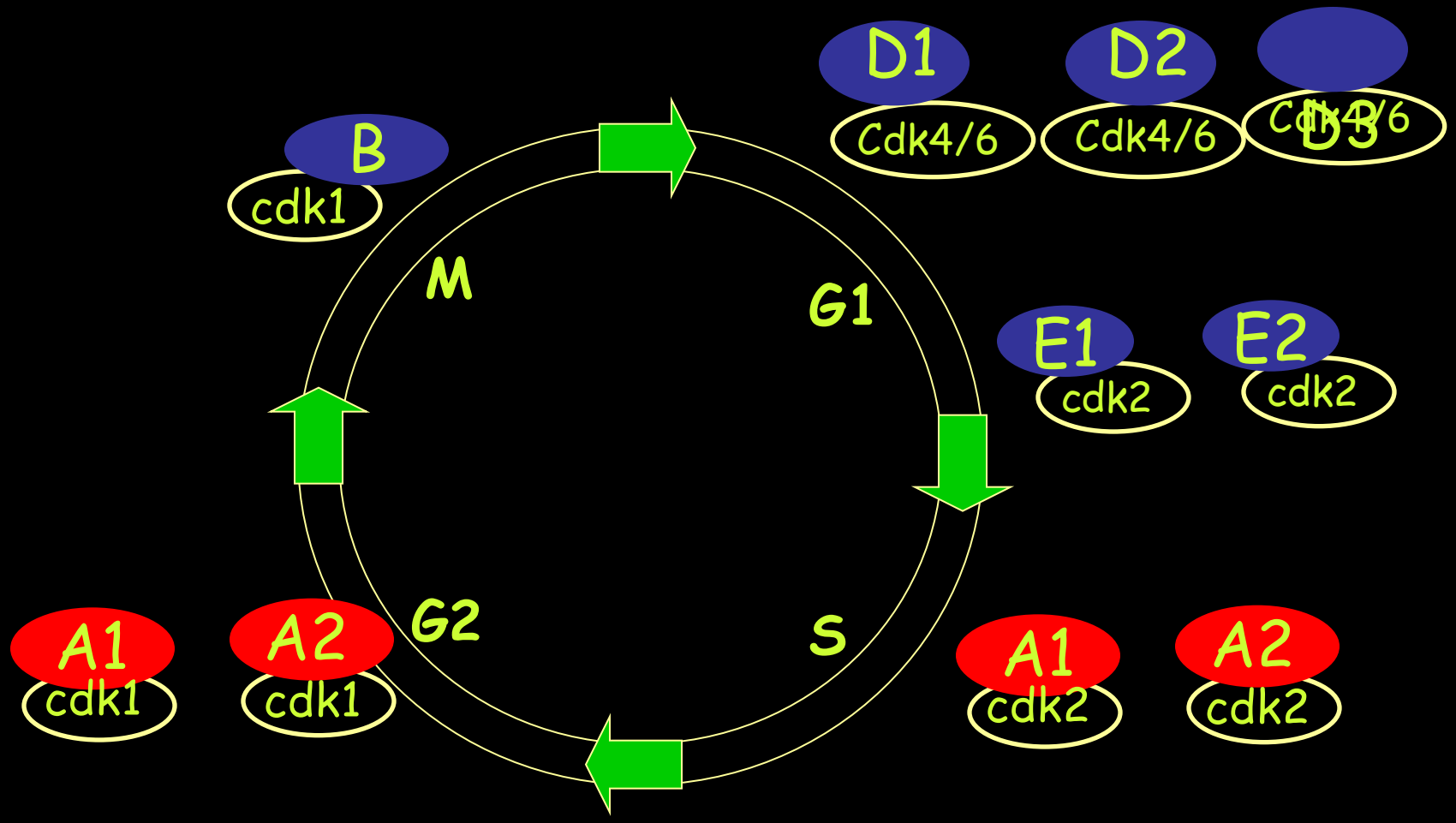

P<0.0001
P<0.0001
P=0.0003
P=0.0016
P=0.0022
P=0.0001
70
60
50
40
30
20
10
0
% of cells
G1
S
G2/M
Control
A2Δ/Δ
A1-/-A2Δ/Δ


Control
G1
S
G2/M
G1
S
G2/M
A1-/-A2Δ/Δ

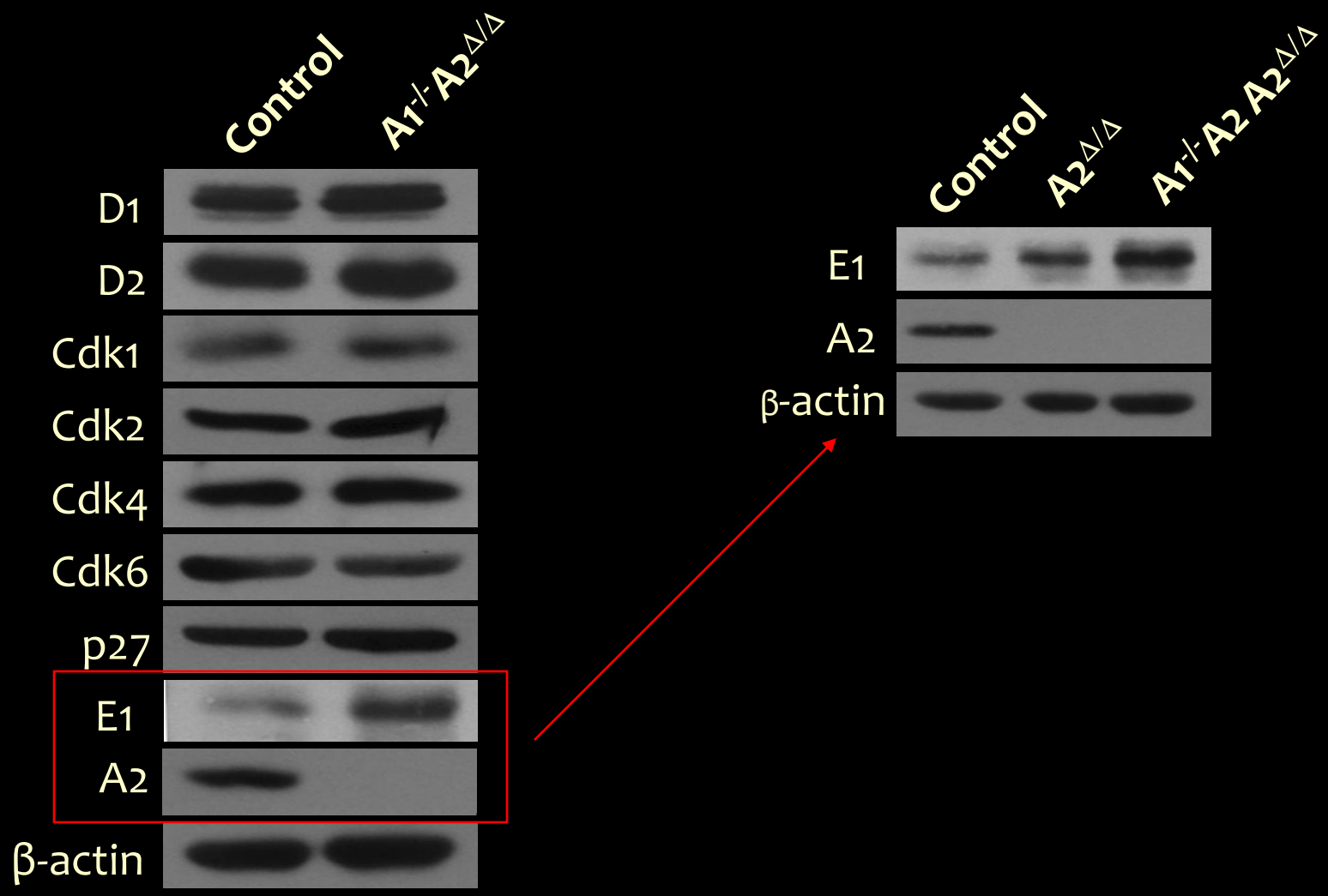
In vitro proliferation of cyclin A deficient fibroblasts

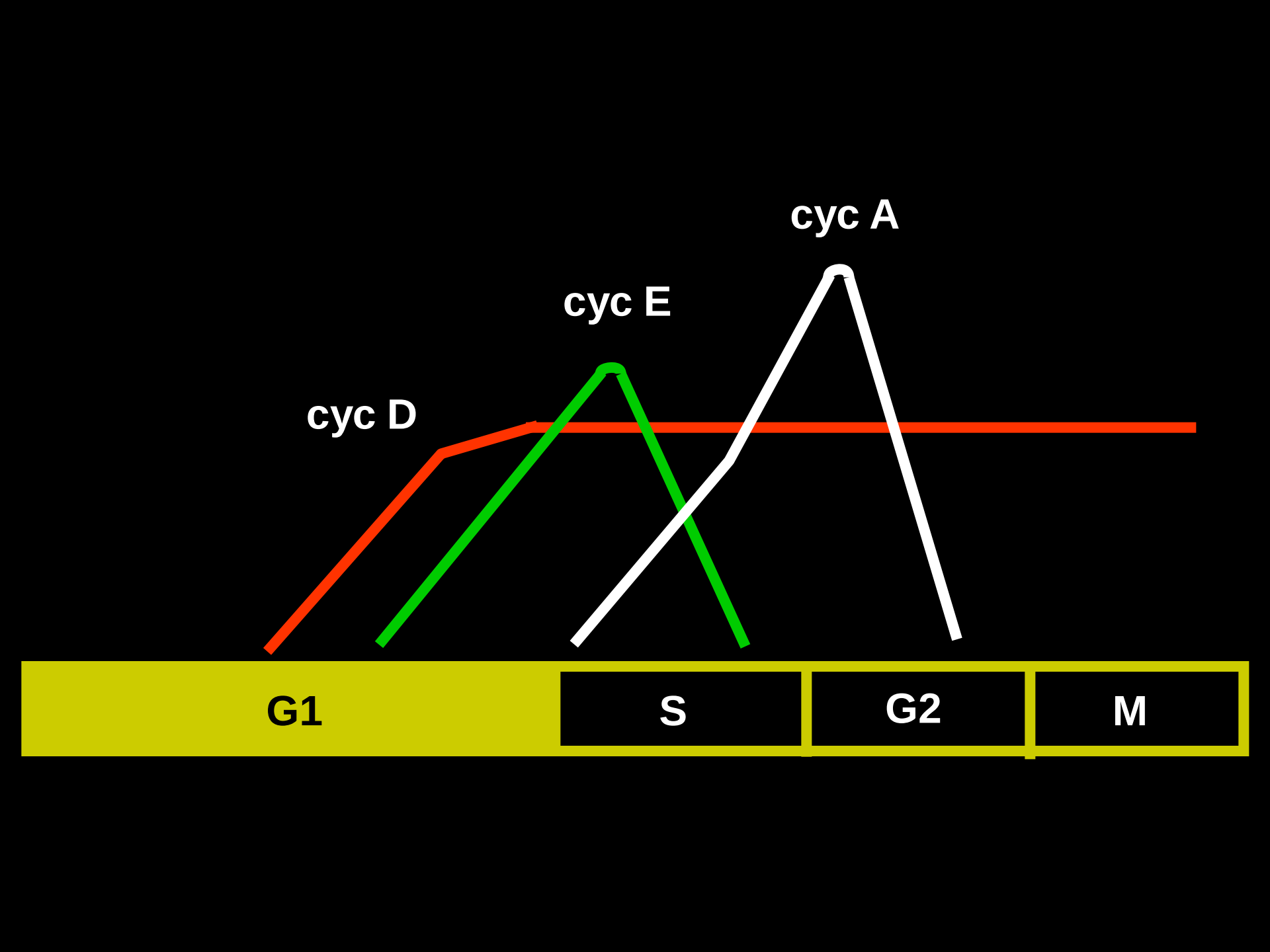




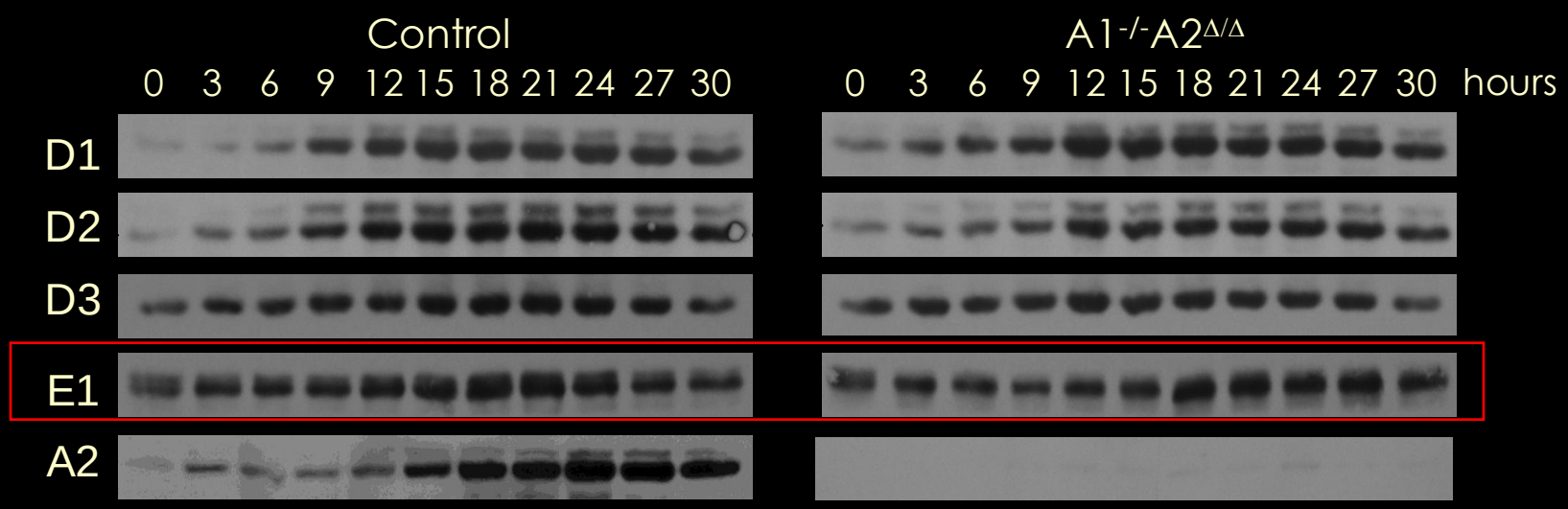

D1
Cdk4/6
D2
Cdk4/6
Cdk4/6
D3
B
cdk1
M
G1
E1
cdk2
E2
cdk2
A1
cdk1
A2
cdk1
G2
S
A1
cdk2
A2
cdk2

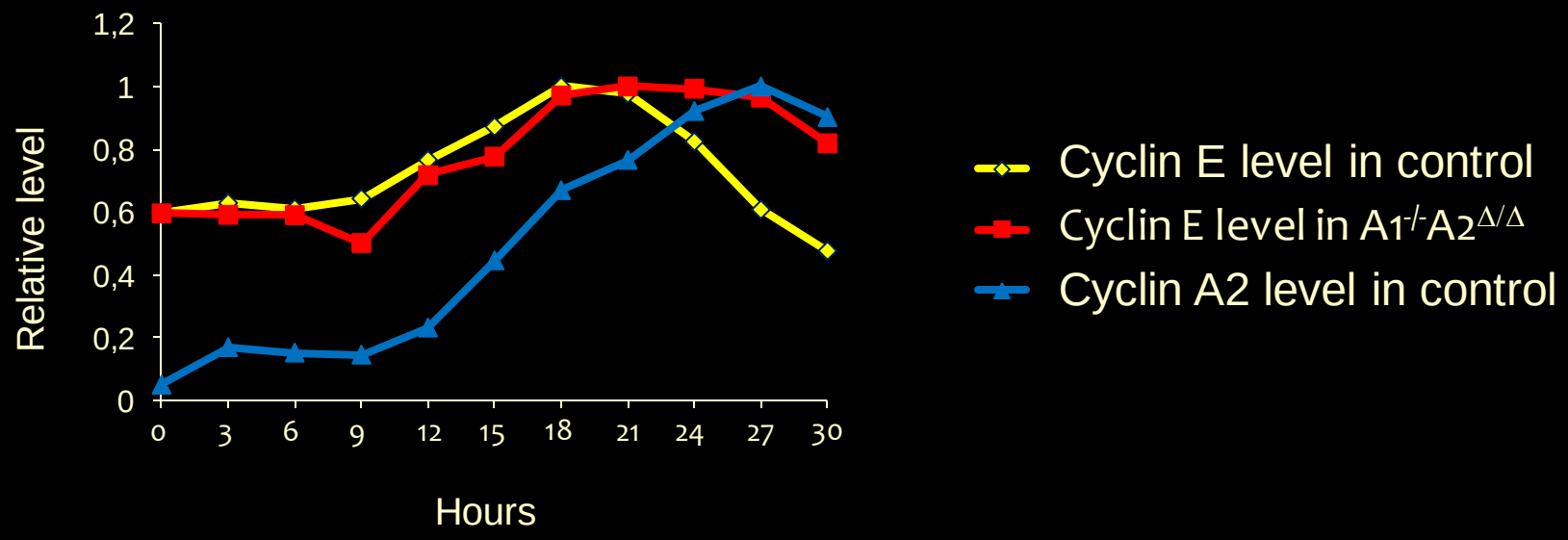
The levels of cell cycle regulators in cyclin A-deficient fibroblasts



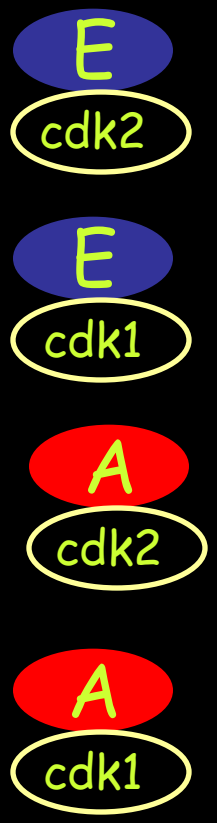

cyc A
cyc E
cyc D
G1
S
G2
M

The levels of cell cycle regulators in cyclin A-deficient fibroblasts

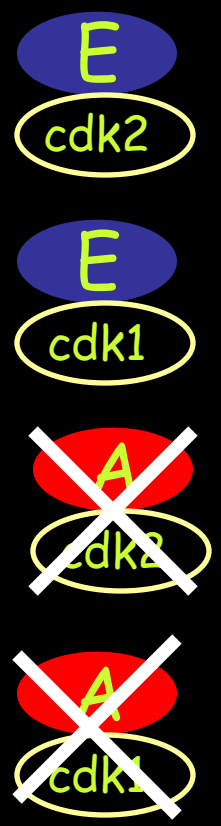


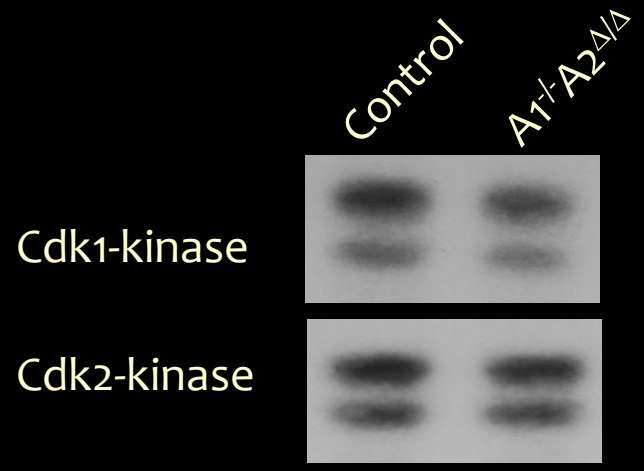


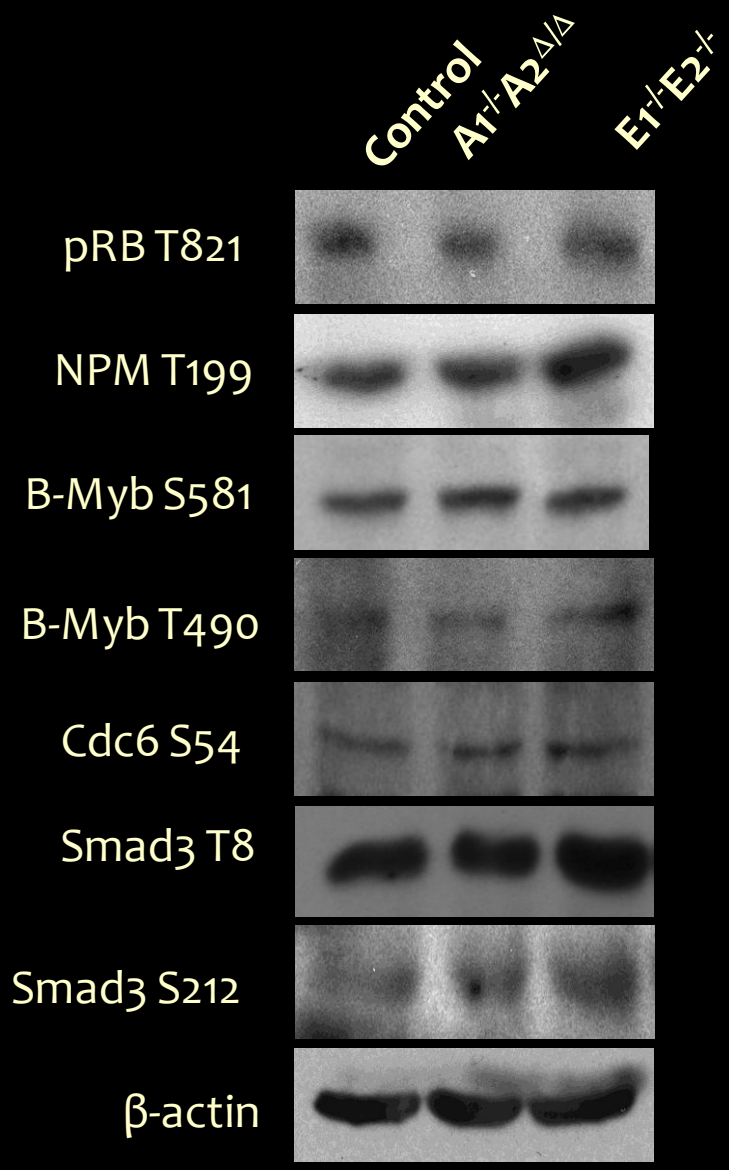
Cyclin A-Cdk and E-cdk complexes

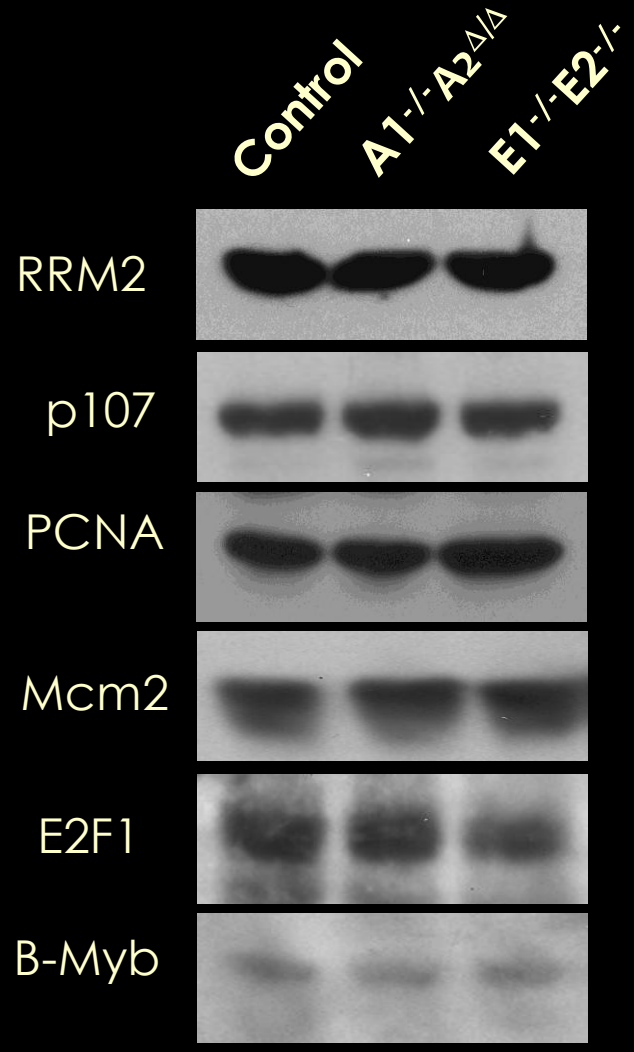


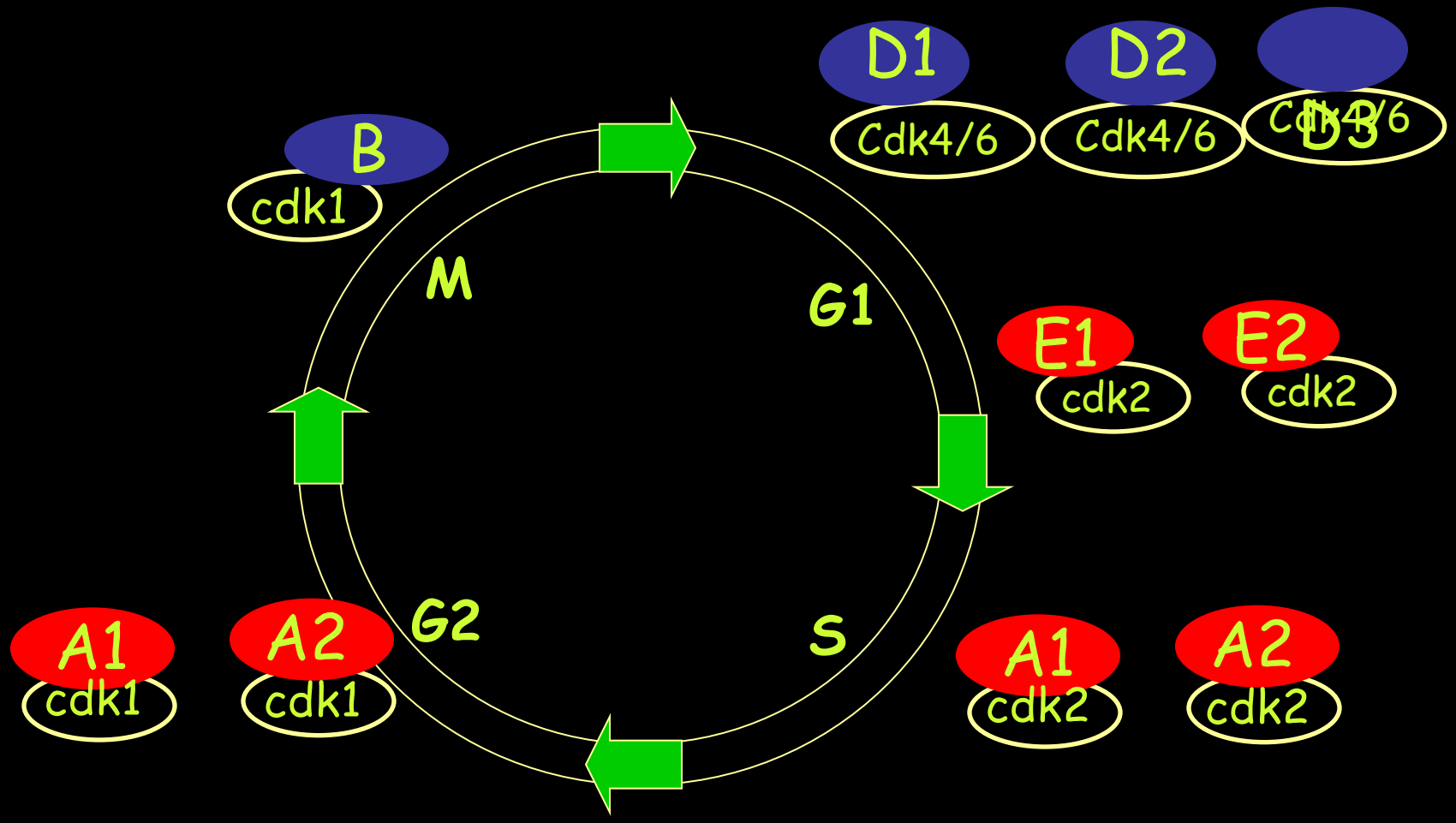
Cyclin A-Cdk and E-cdk complexes






Control
A1-/-A2Δ/Δ
E1-/-E2-/-
pRB T821
NPM T199
B-Myb S581
B-Myb T490
Cdc6 S54
Smad3 T8
Smad3 S212
β-actin


Control
A1-/-A2Δ/Δ
E1-/-E2-/-
RRM2
p107
PCNA
Mcm2
E2F1
B-Myb


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

$A1^{-/-}A2^{F/F}$ $E1^{-/-}E2^{-/-}$

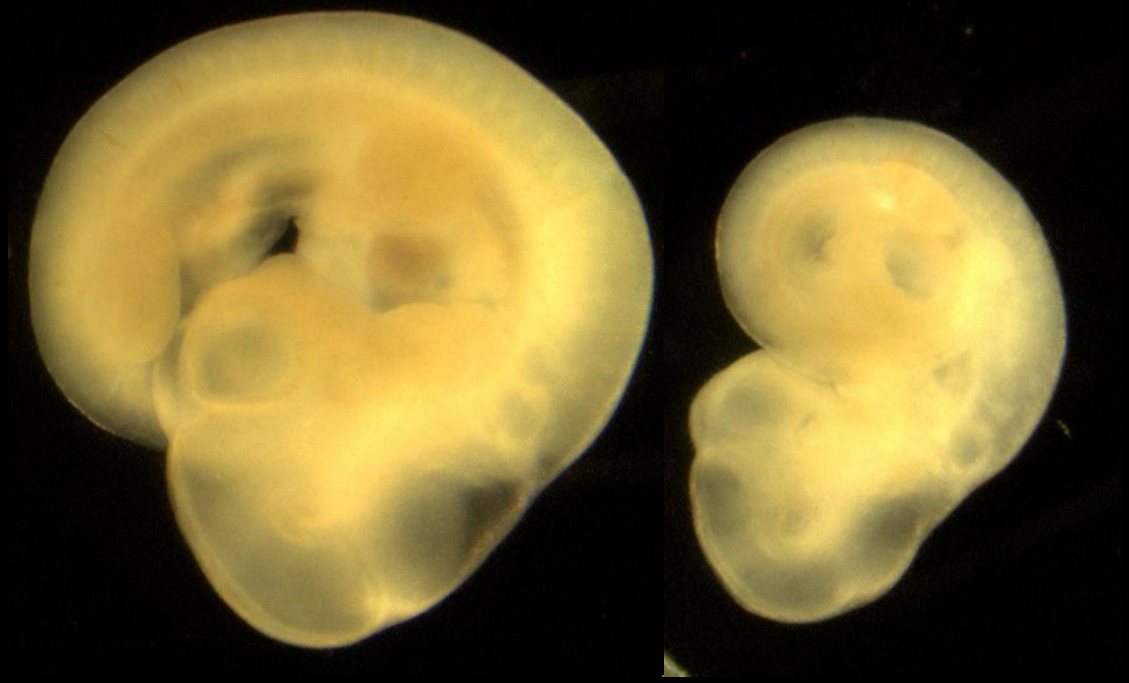
E1

E2

~~E1~~

~~E2~~

Cyclin $E1^{-/-}E2^{-/-}$



day E 10.75

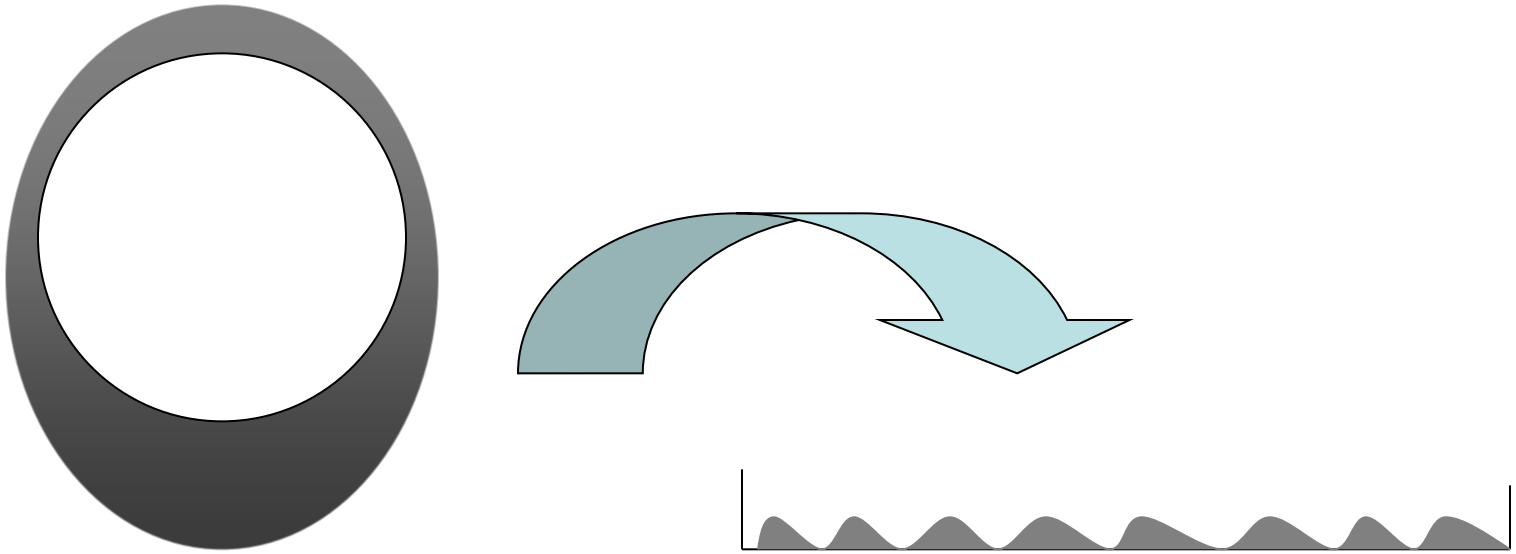
Yan Geng

CYCLIN $E1^{-/-}E2^{-/-}$ MICE

Control

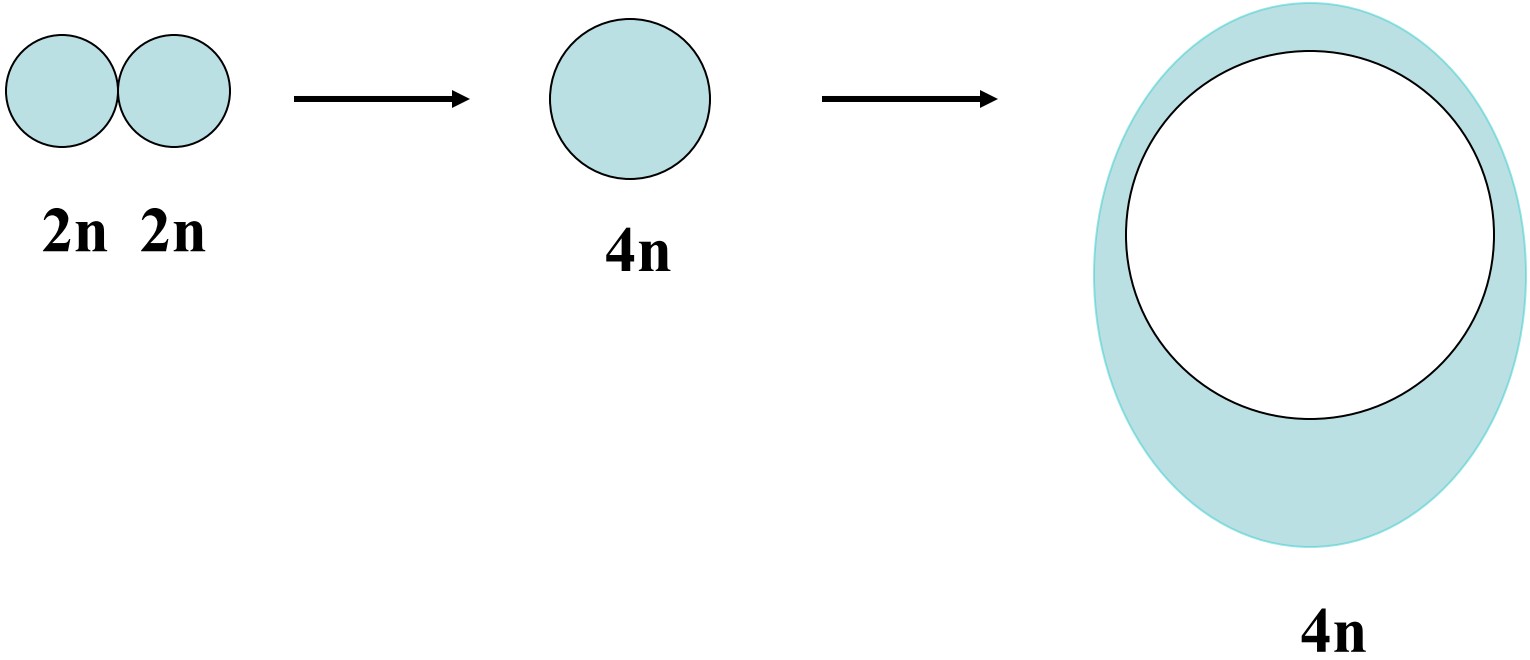
$E1^{-/-}E2^{-/-}$

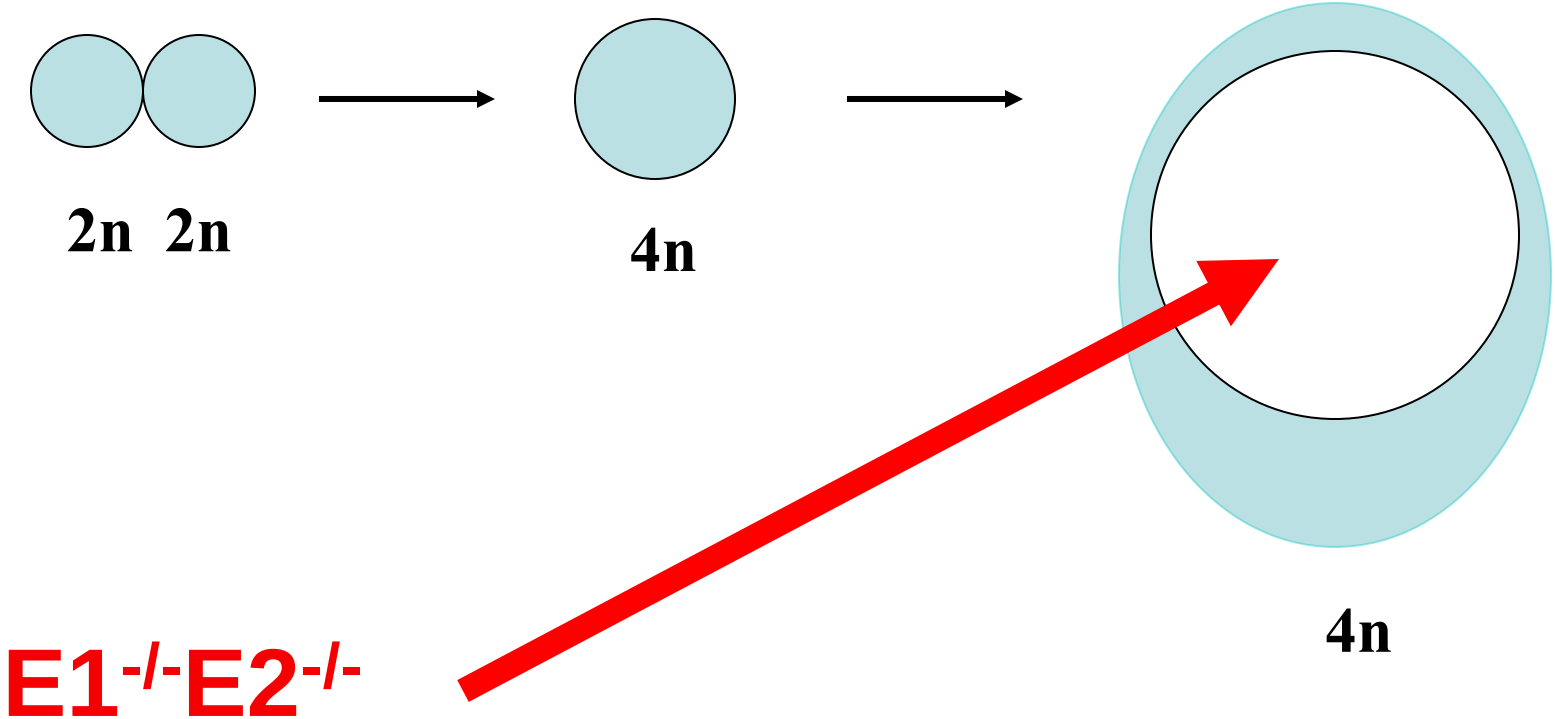
Mate $E1^{+/-}$ $E2^{+/-}$ mice



$E1^{-/-}$ $E2^{-/-}$ blastocysts

$E1^{-/-}$ $E2^{-/-}$ ES cells


2n 2n
4n
4n

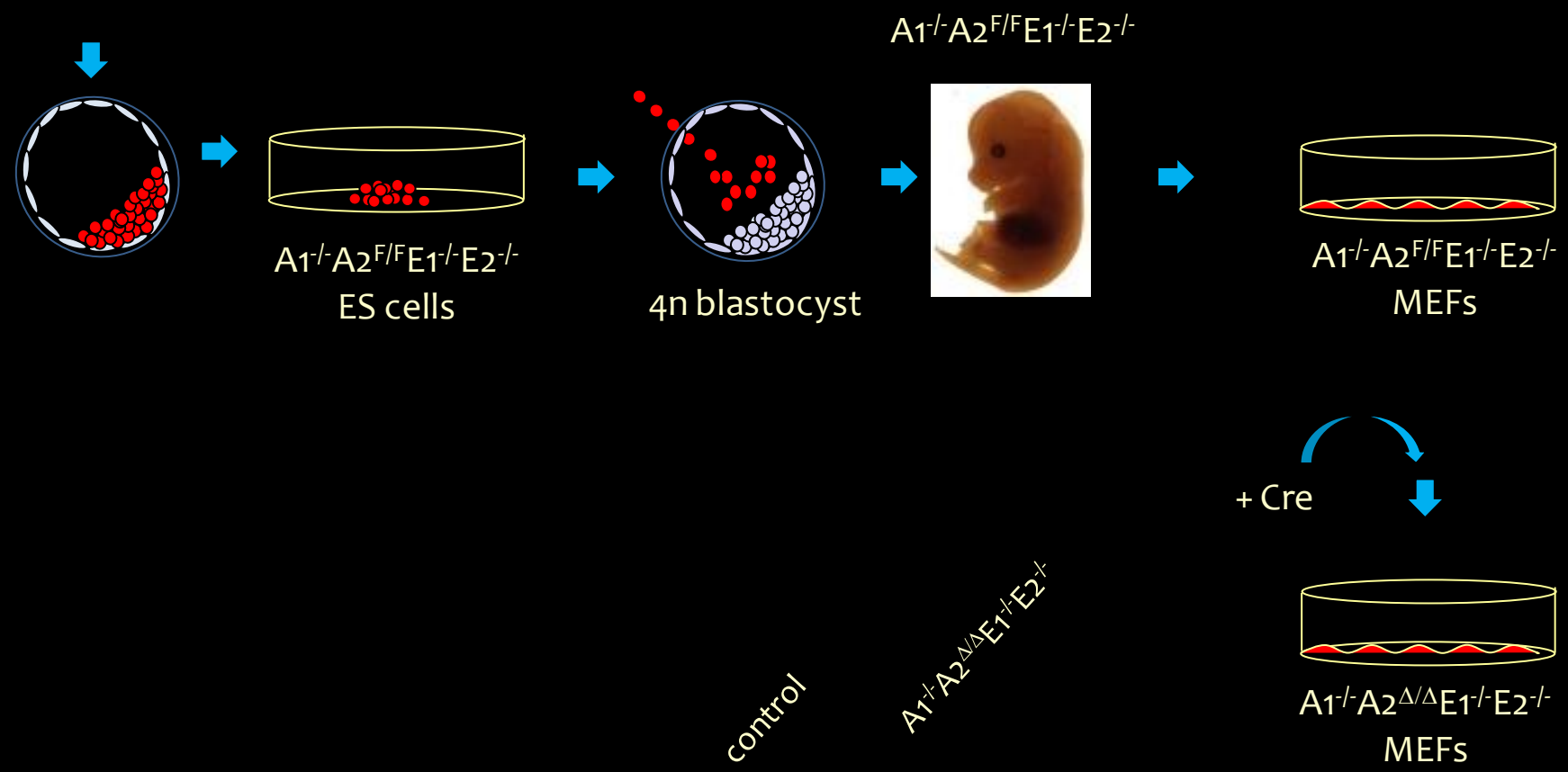


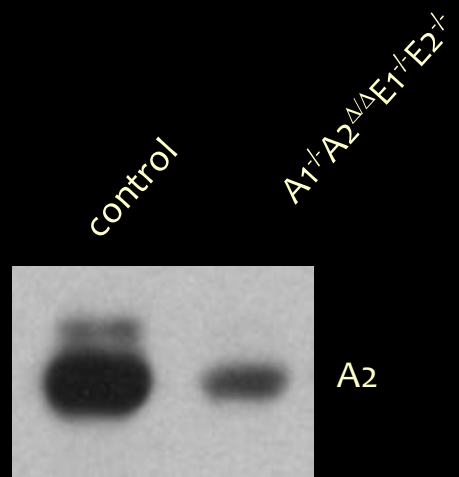
$E1^{-/-}E2^{-/-}$ ES Cells

♀ $A1^{-/-}A2^{F/F}E1^{-/-}E2^{+/-}$

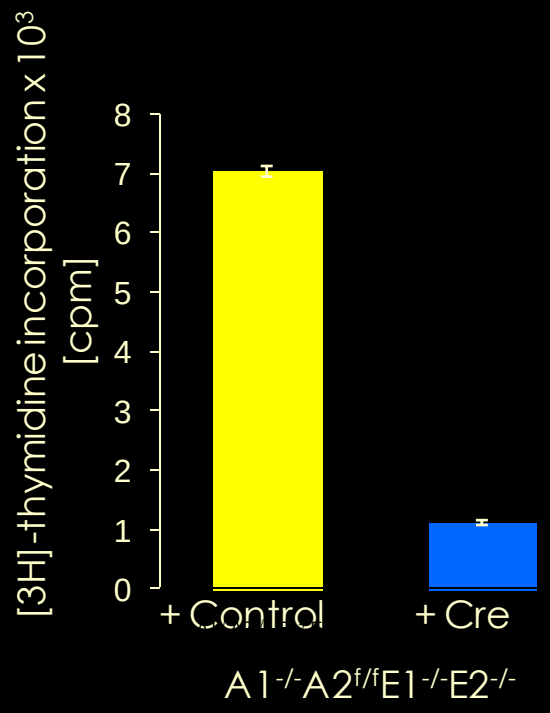
x

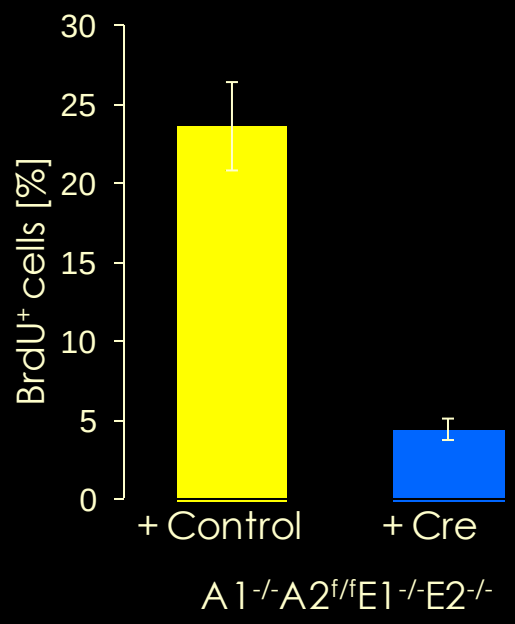
♂ $A1^{+/-}A2^{F/F}E1^{+/-}E2^{+/-}$



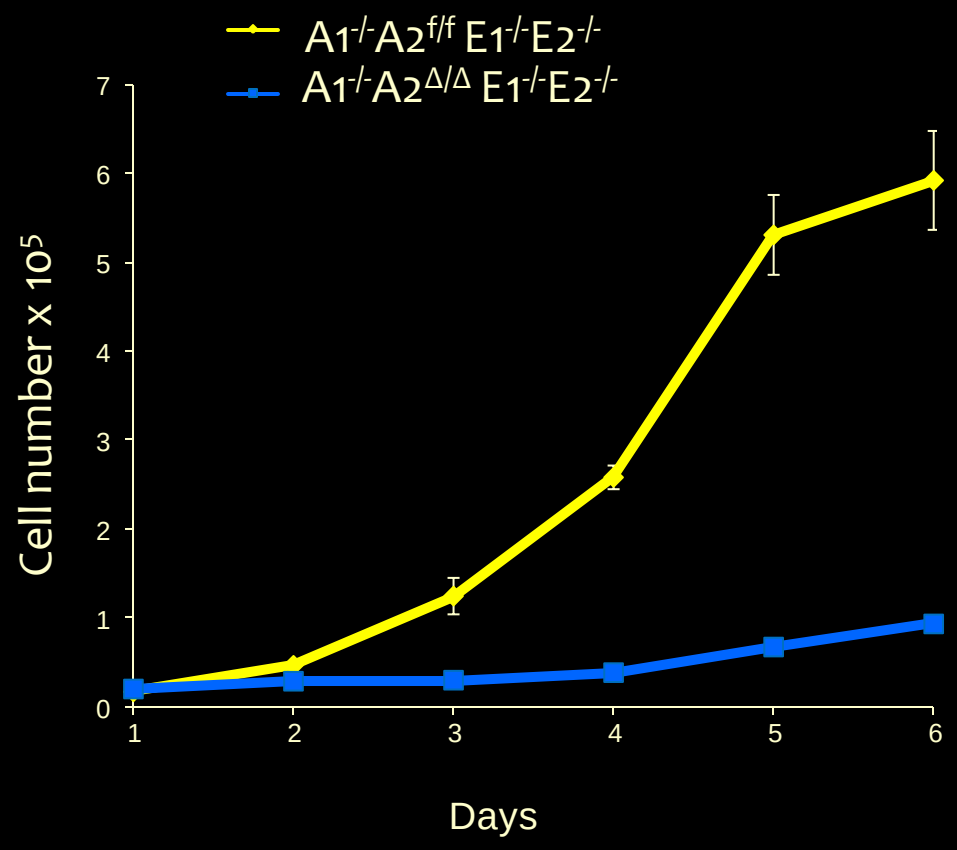


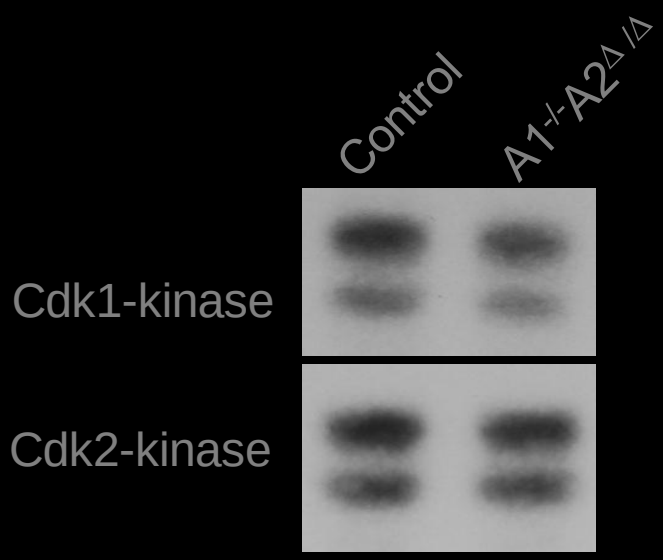
Analysis of fibroblasts lacking all A- and E-type cyclins

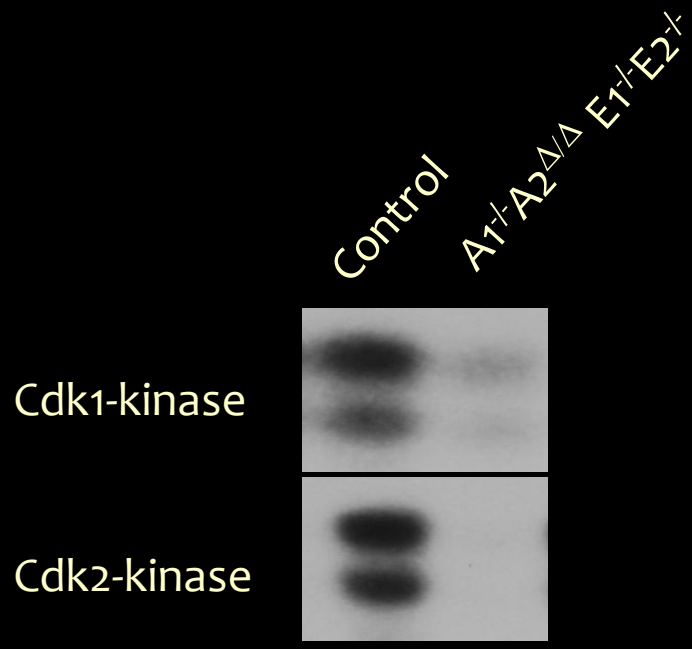


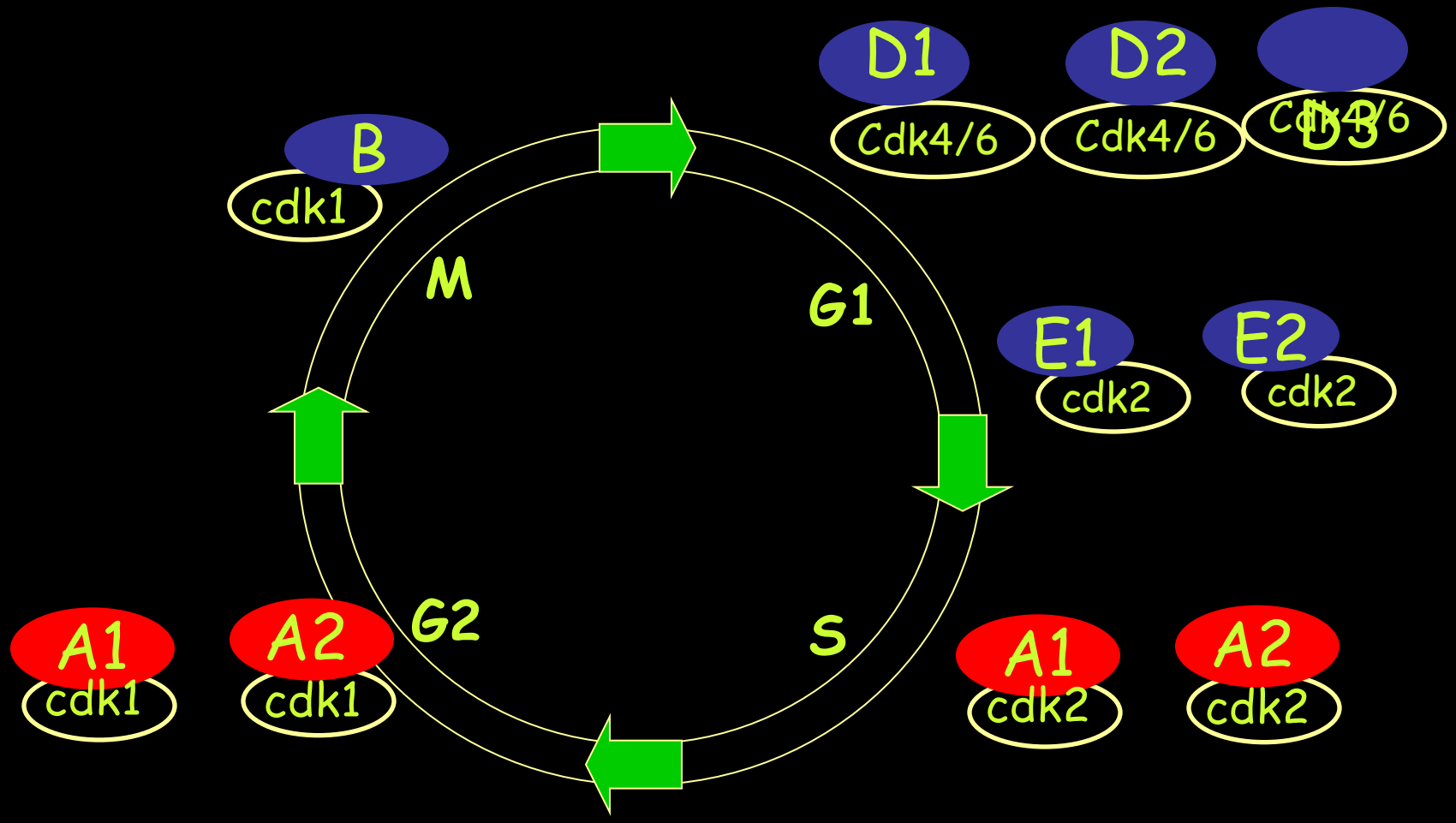


Analysis of fibroblasts lacking all A- and E-type cyclins




Control
A1-/-A2Δ/Δ
Cdk1-kinase
Cdk2-kinase


Control
A1-/-A2Δ/Δ E1-/-E2-/-
Cdk1-kinase
Cdk2-kinase


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


Debra Wolgemuth
Columbia
Hirokazu Shigematsu
Tadafumi Iino
Shin-ichi Mizuno
Koichi Akashi
DANA-FARBER
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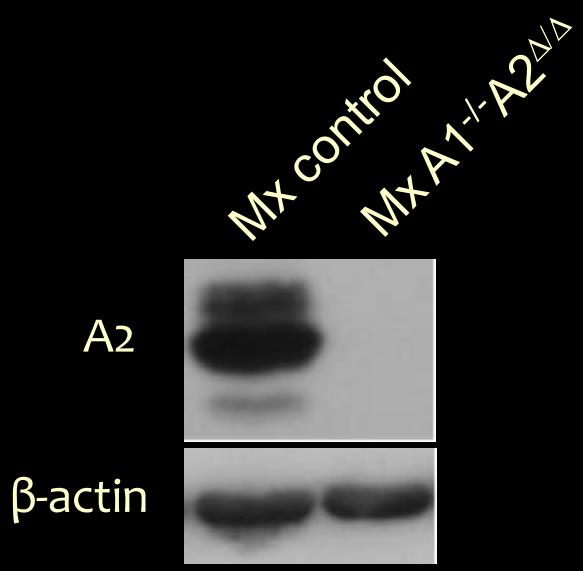
Consequence of cyclin A deletion *in vivo*

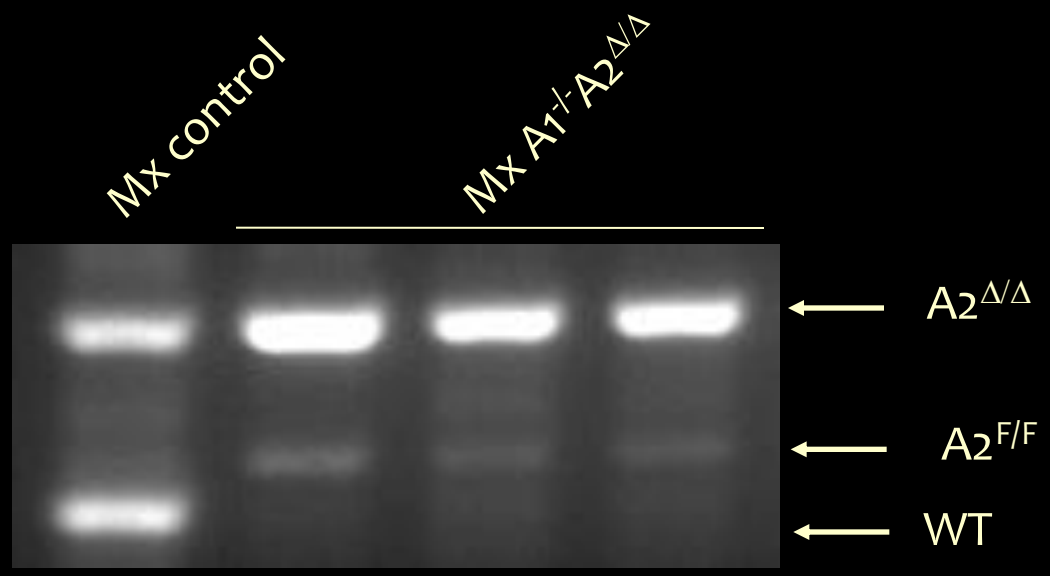
Bone marrow specific deletion

in Mx $A1^{-/-}A2^{F/F}$ mice

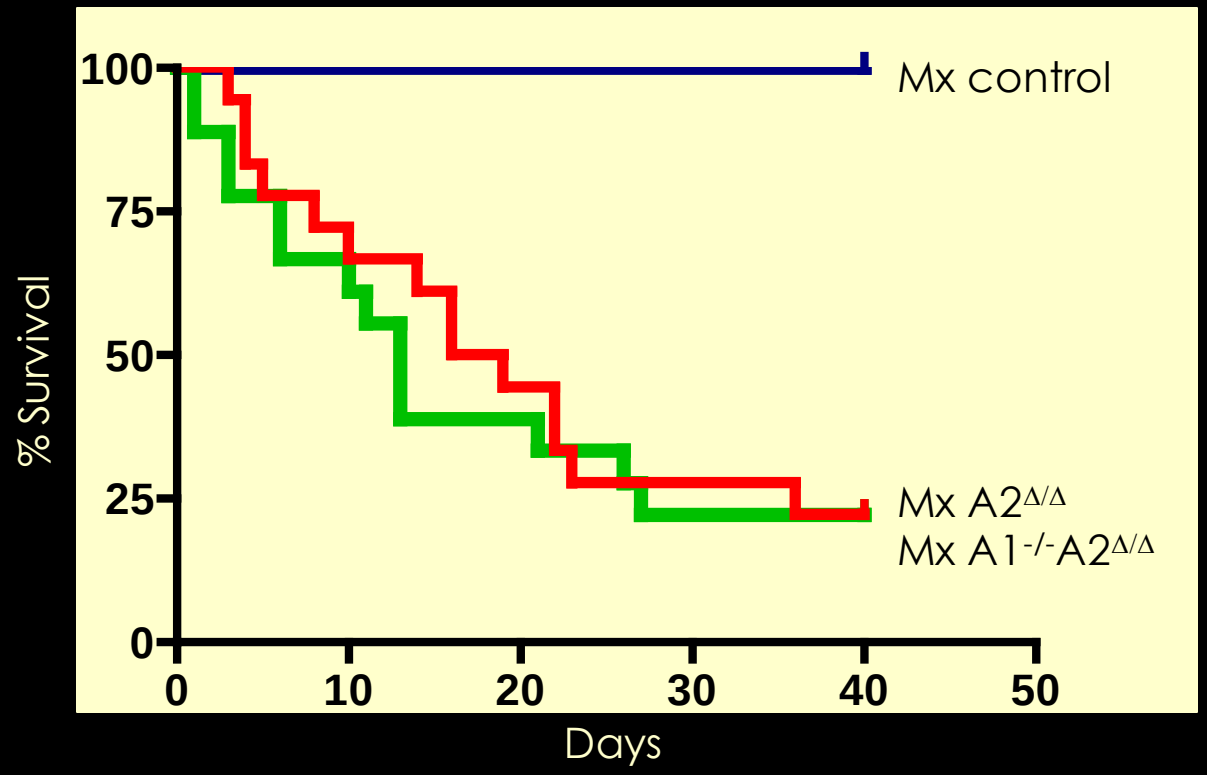
Cyclin A2 expression level in A2 $^{F/F}$ Mx1 mice

Before and after poly(I) poly (C) treatment





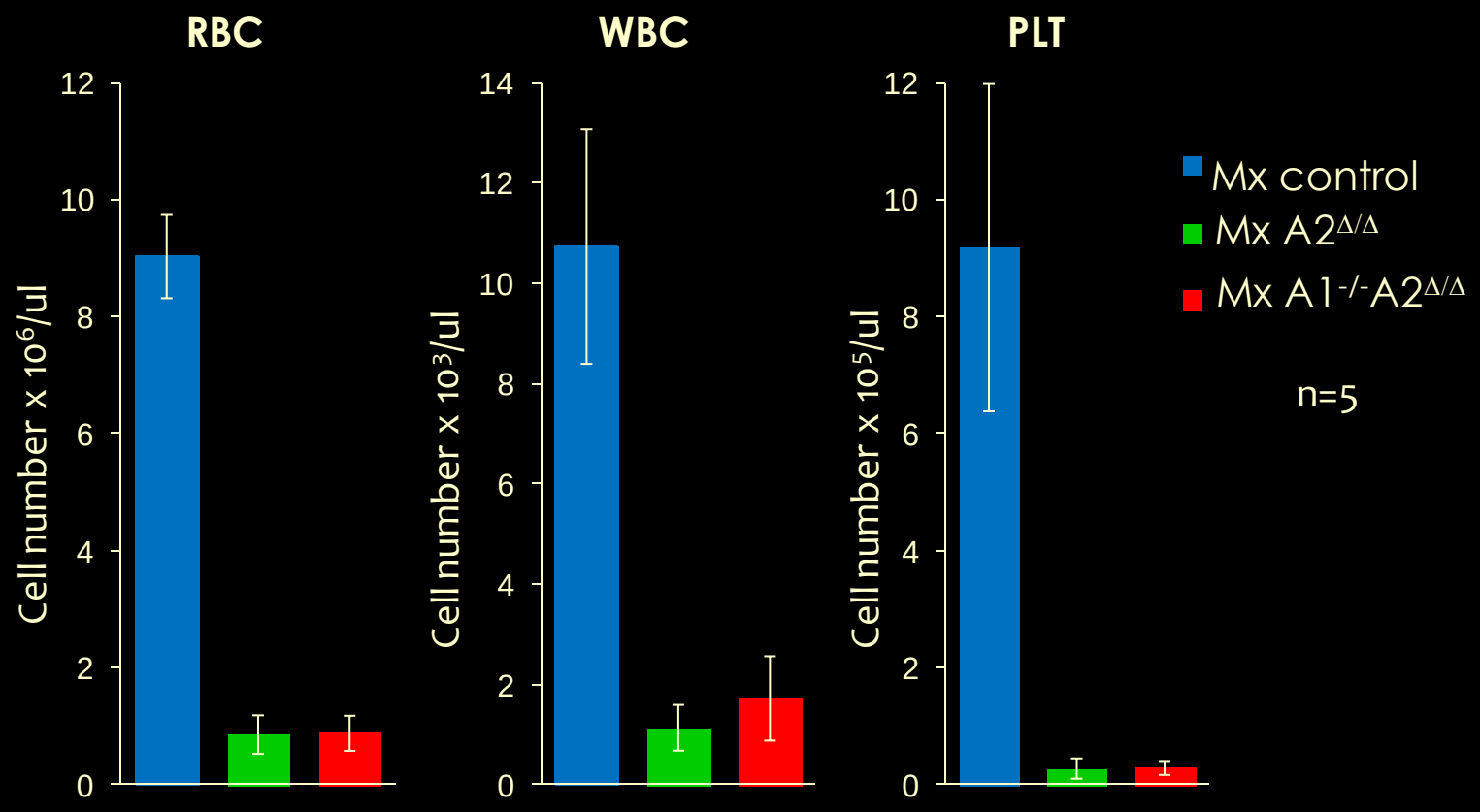
Kaplan-Meier survival graph



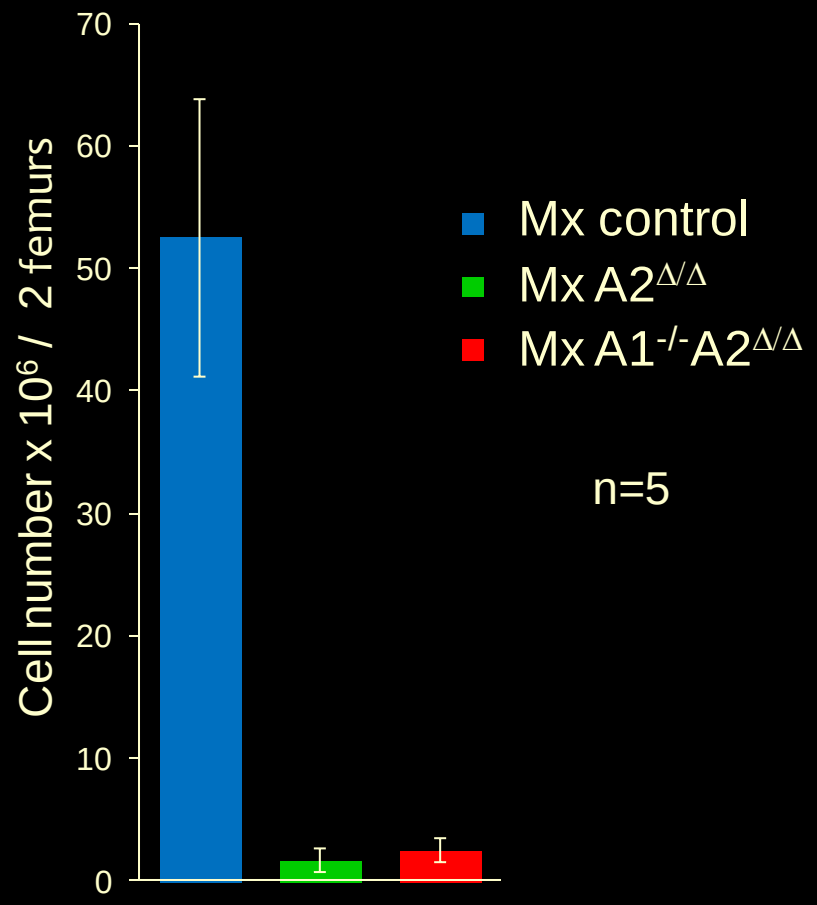
n=18

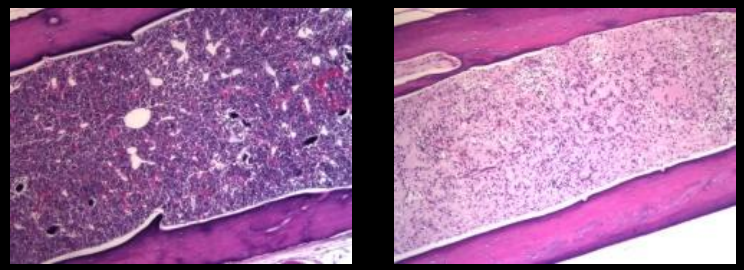
Time after 6x pIpC injection

Peripheral blood analysis



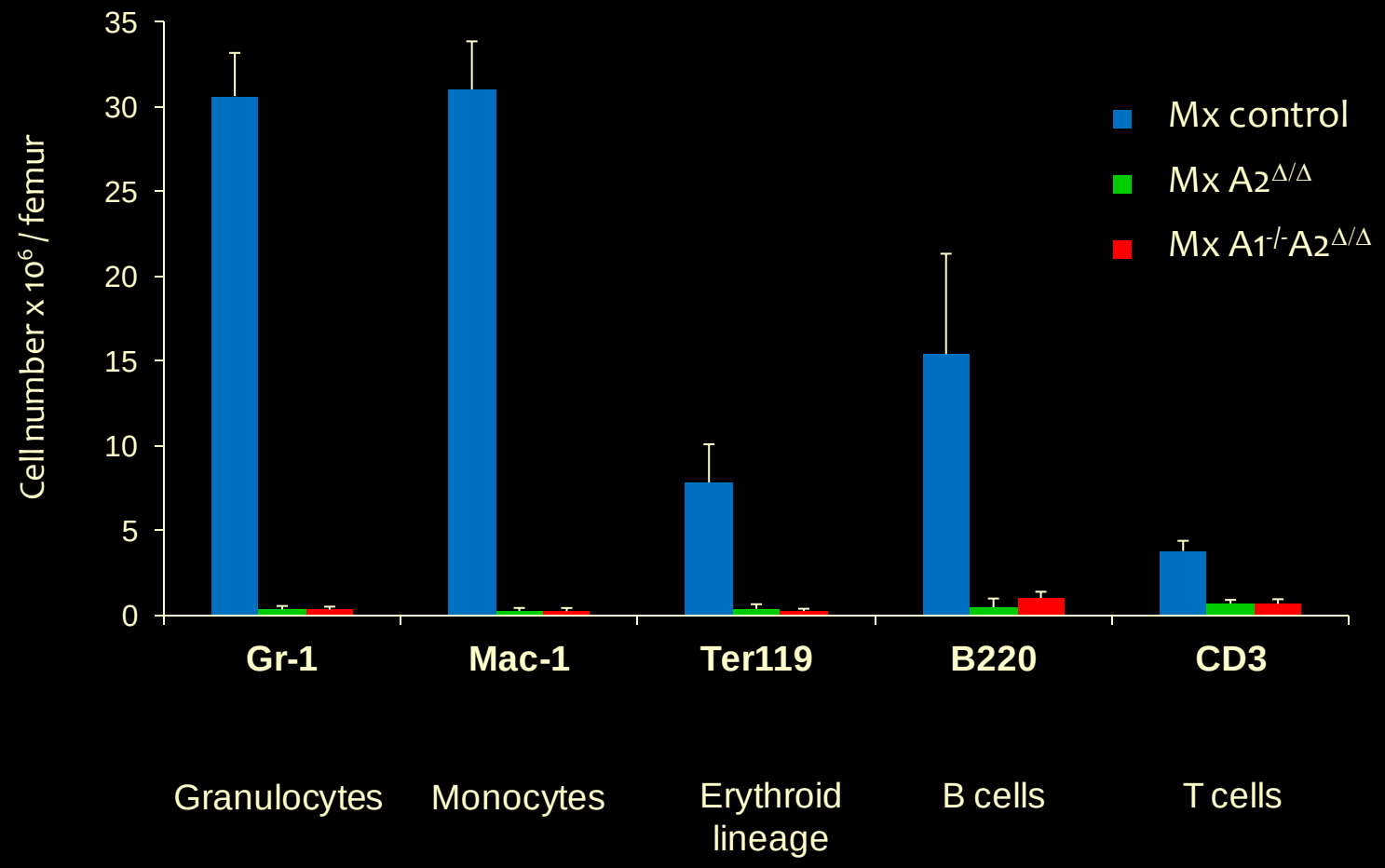
Analysis of bone marrow cells





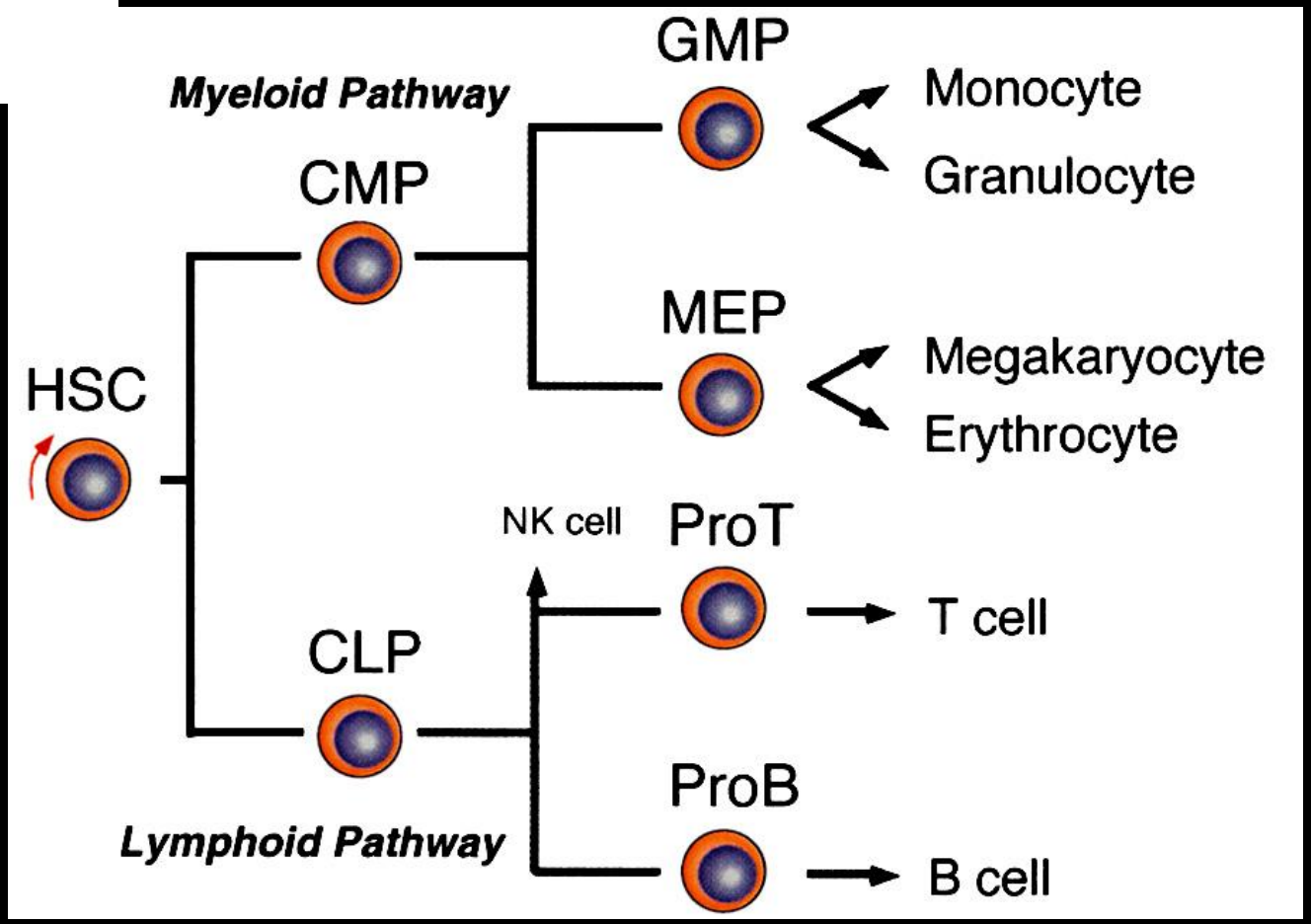
H&E

Analysis of BM subpopulations

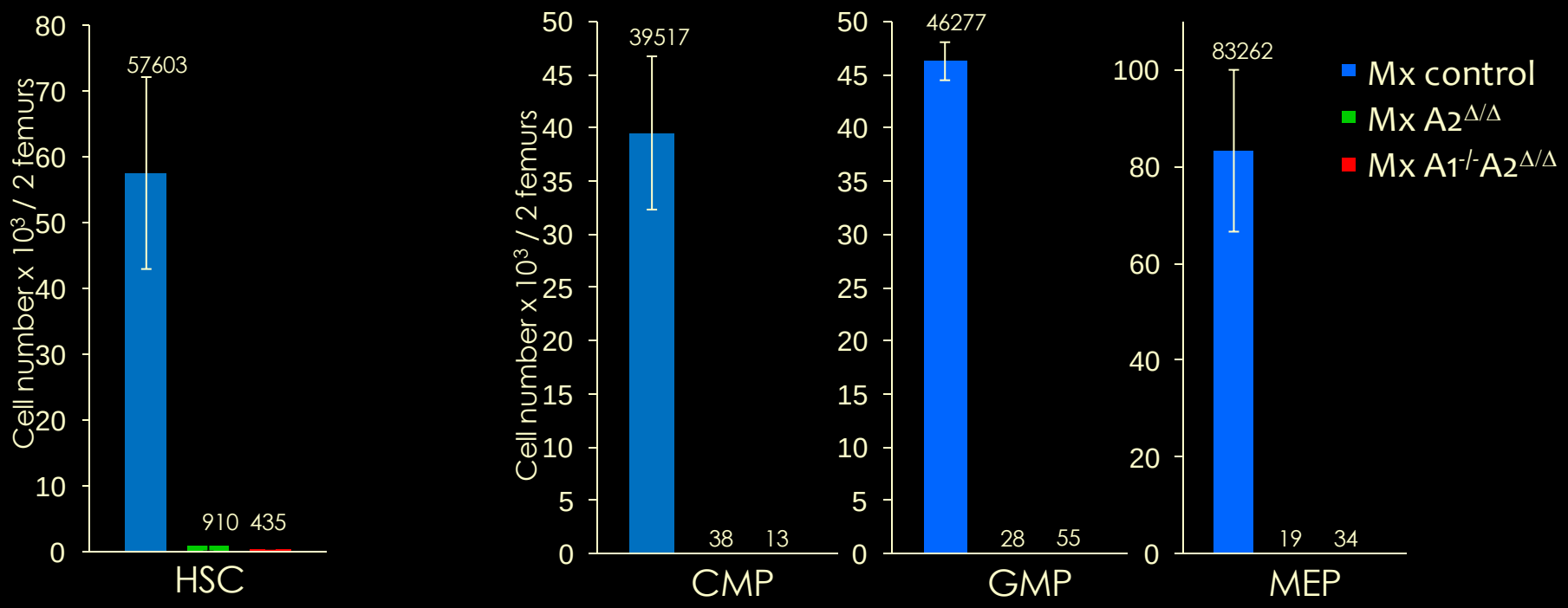


n=6

At which stage of hematopoiesis cyclin A is rate-limiting?



Analysis of hematopoetic stem cells and progenitors



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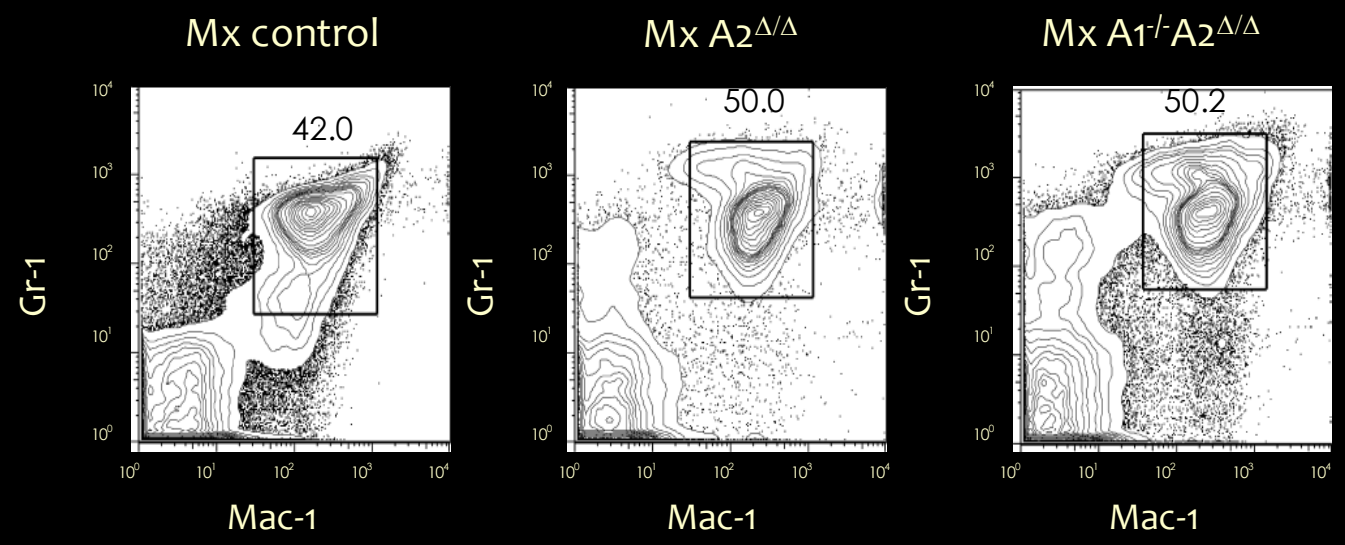
Shin-ichi Mizuno

Koichi Akashi

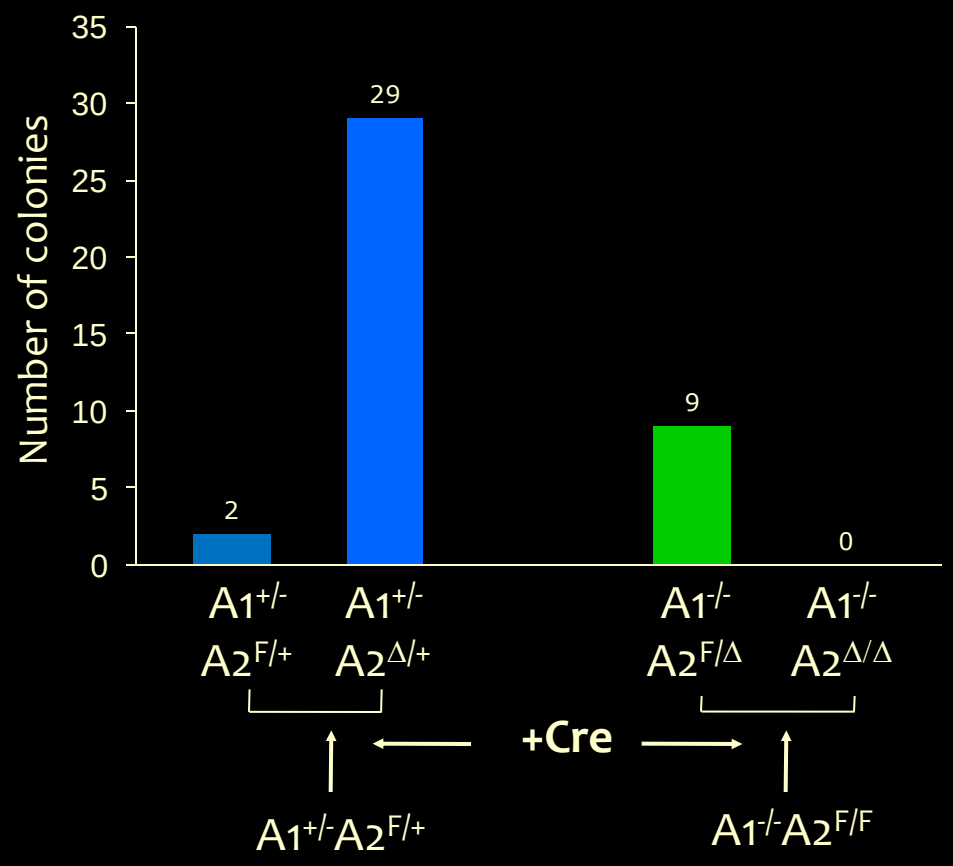
Analysis of progenitors

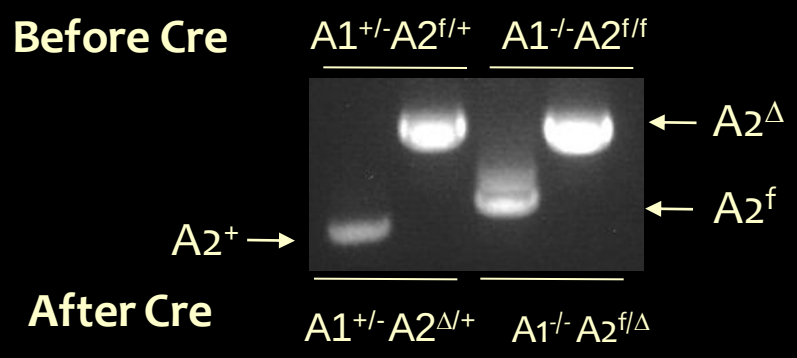


Tadafumi Iino
Shin-ichi Mizuno

Koichi Akashi

Analysis of Hematopoetic Stem Cells



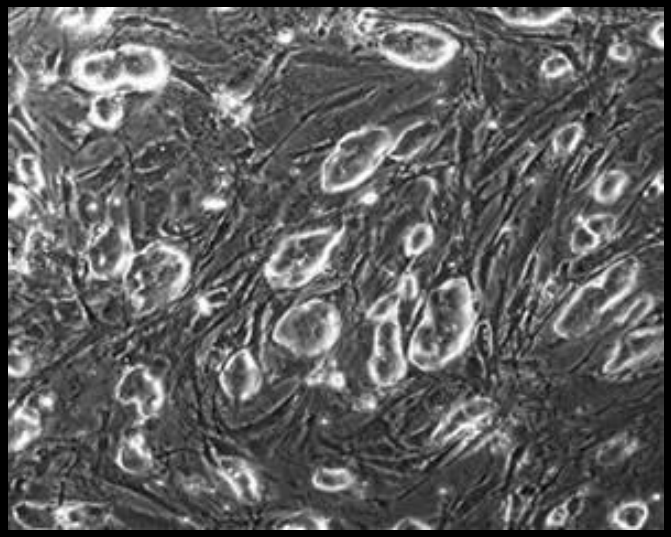


Tadafumi Iino
Shin-ichi Mizuno

Koichi Akashi

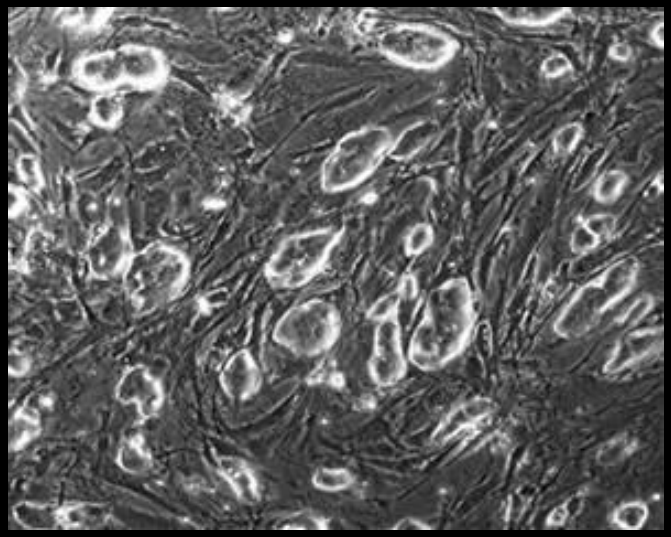
Embryonic stem cells

$A1^{-/-}A2^{F/F}$



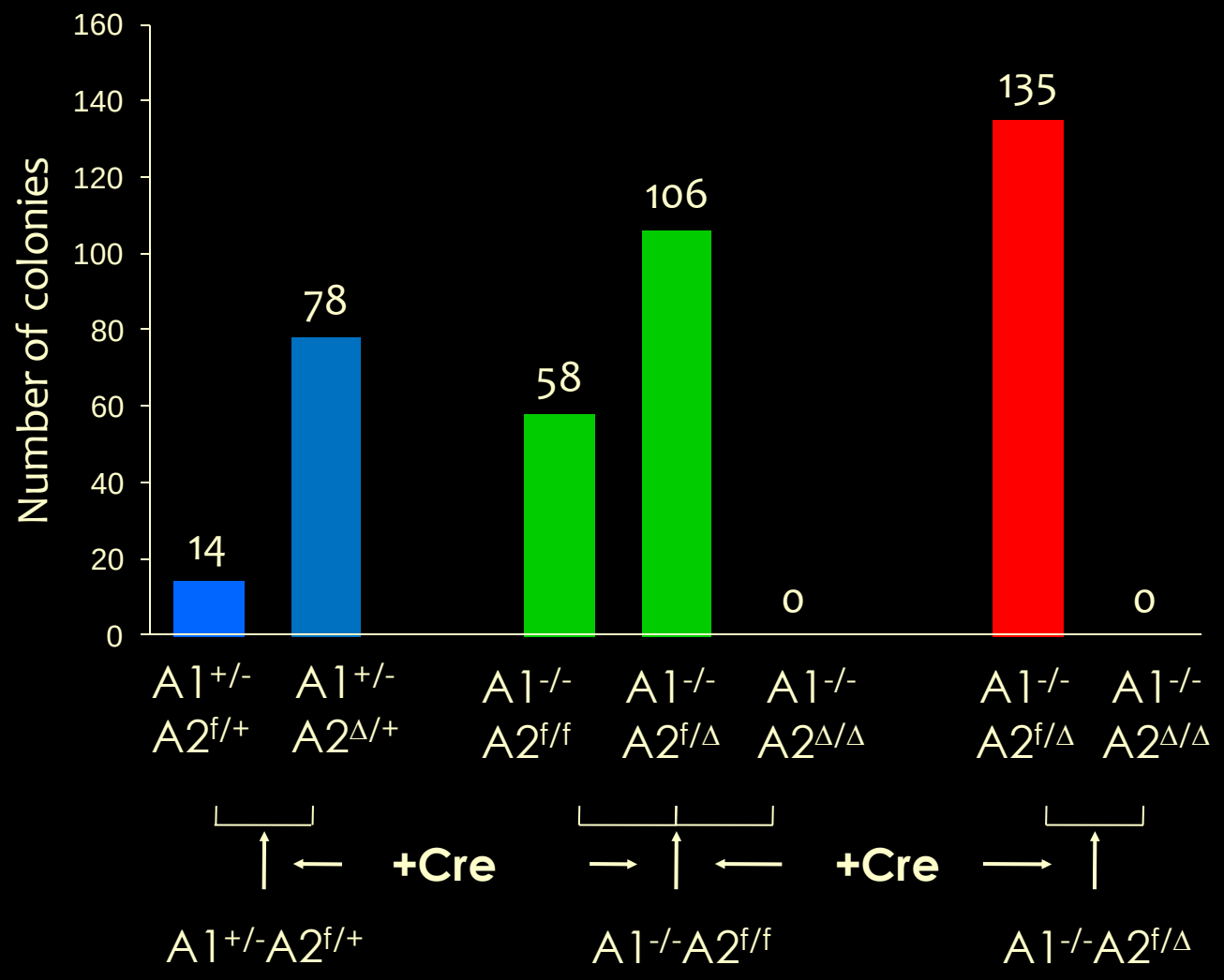
Embryonal stem cells

$A1^{-/-}A2^{F/F}$



+Cre

Analysis of embryonal stem cells



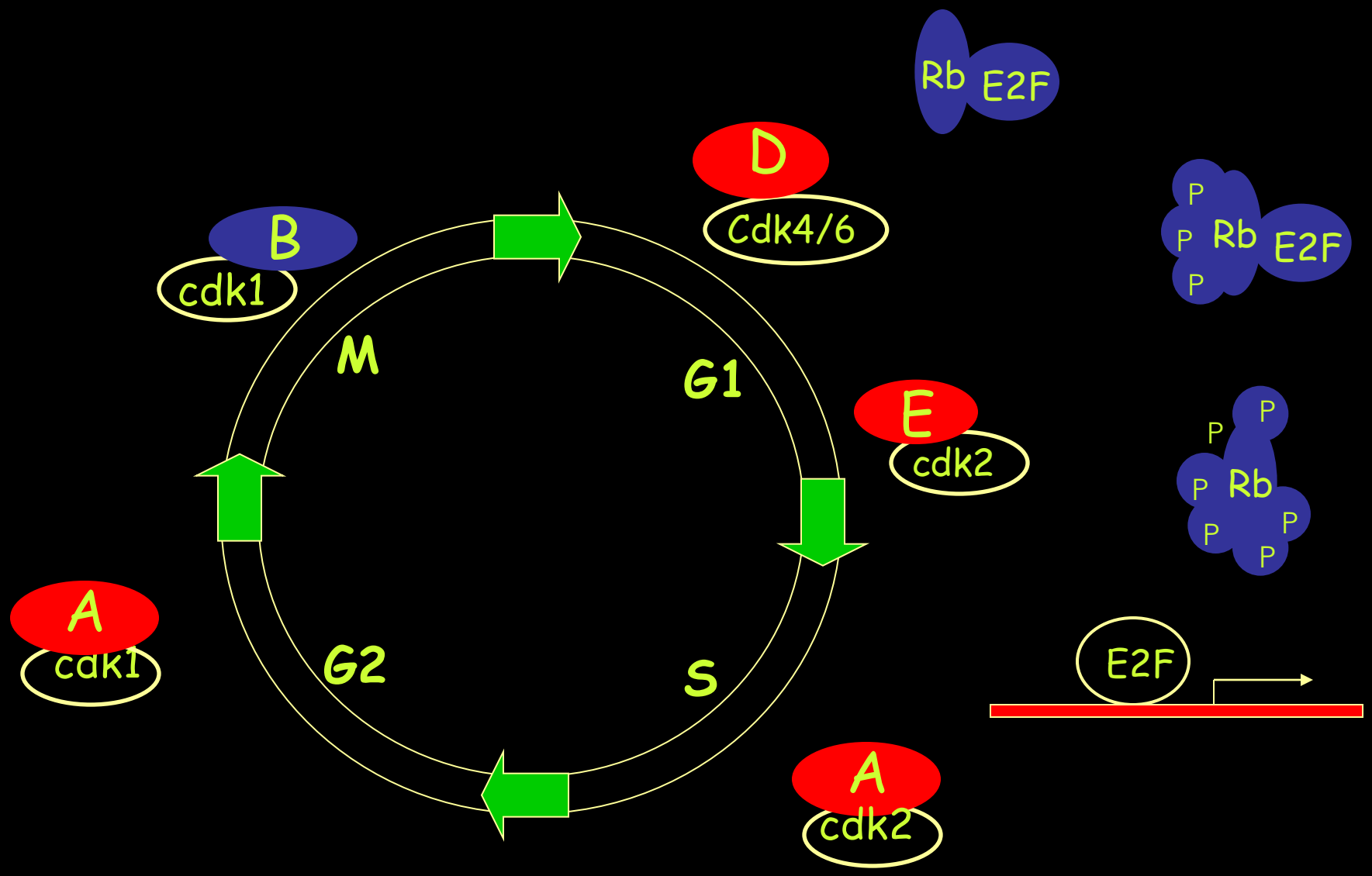
Proliferation of MEFs does NOT depend on cyclin A

but

Proliferation (survival) of HSC and ES cells critically depends on cyclin A

What is the molecular basis of this difference?

Mammalian cell cycle in stem cells

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