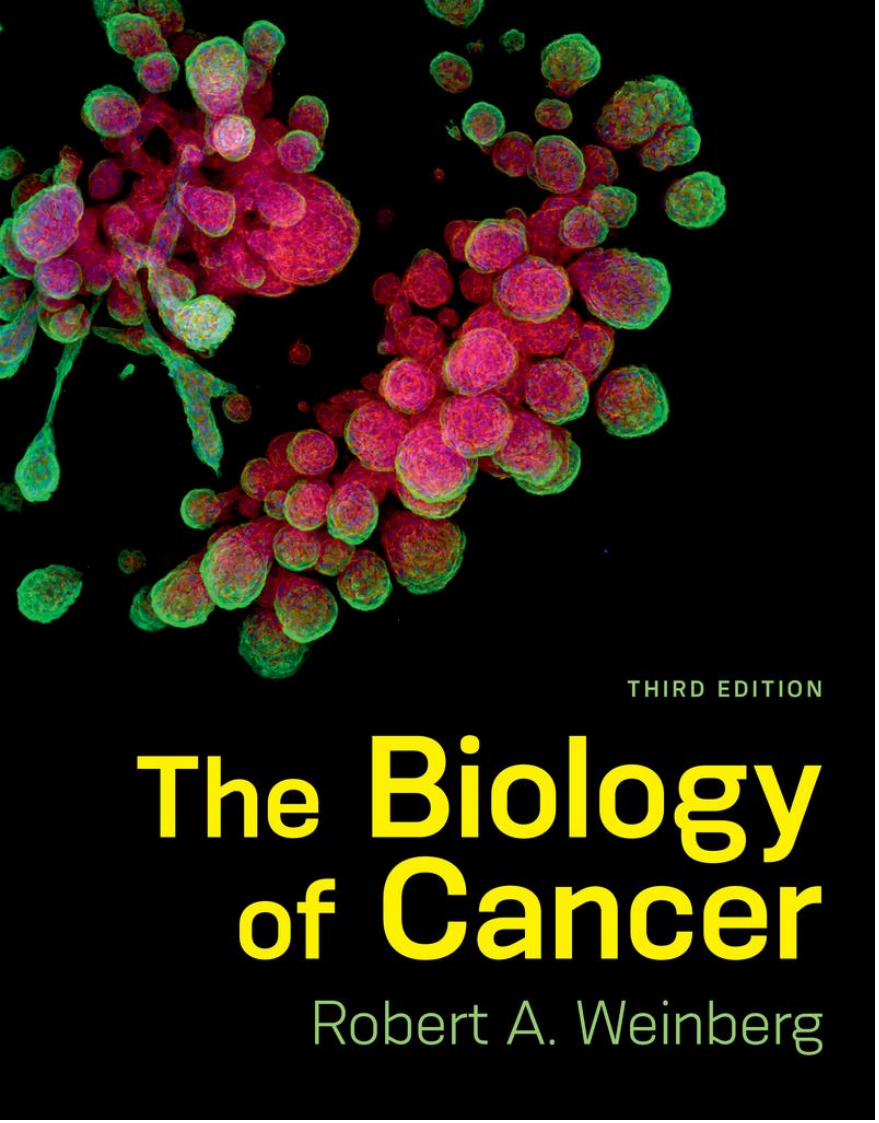


Chapter 07

TUMOR SUPPRESSOR GENES

Chapter 08

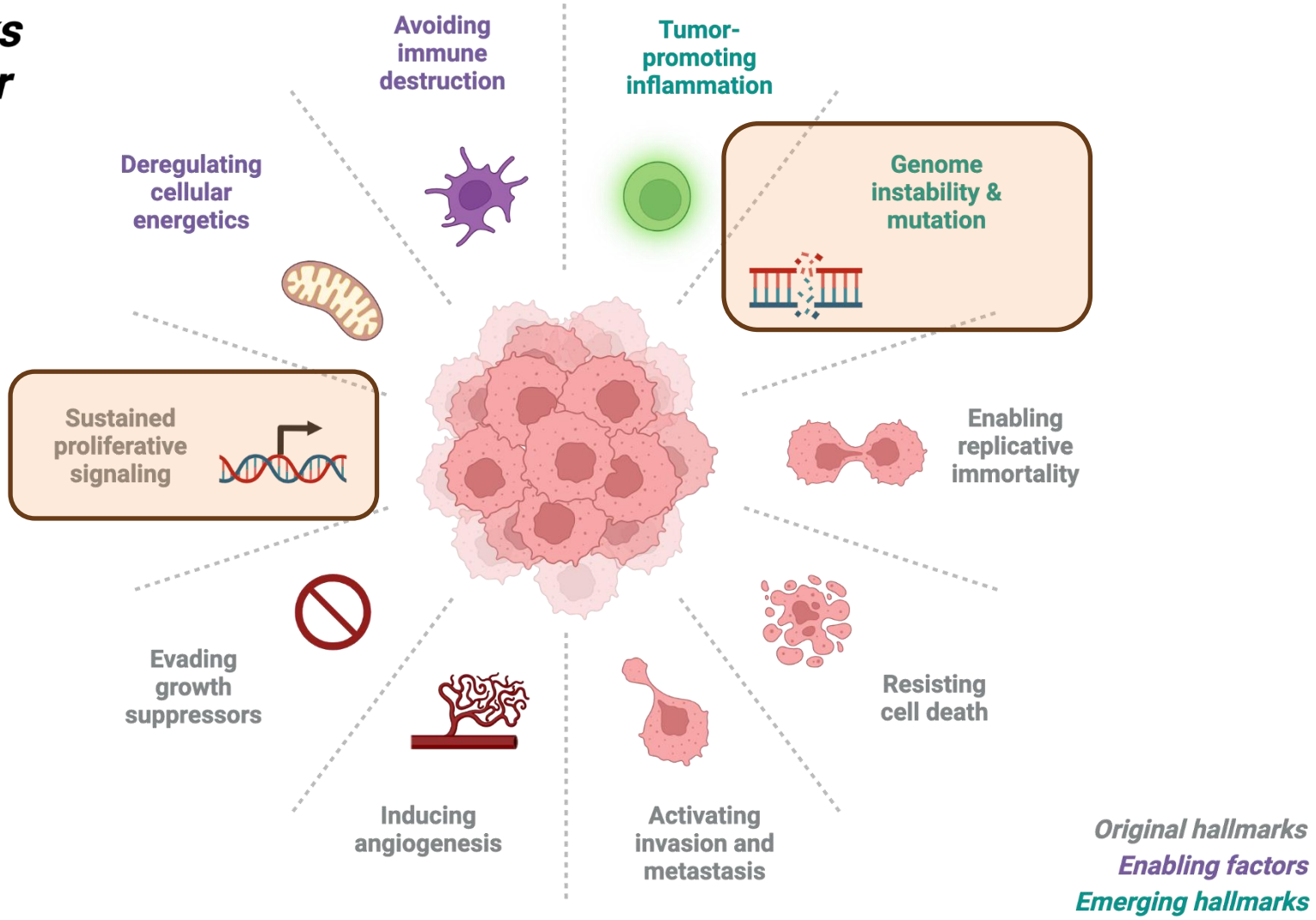
RB AND CONTROL OF THE CELL CYCLE CLOCK

Hee Won Yang (hy2602@cumc.columbia.edu)

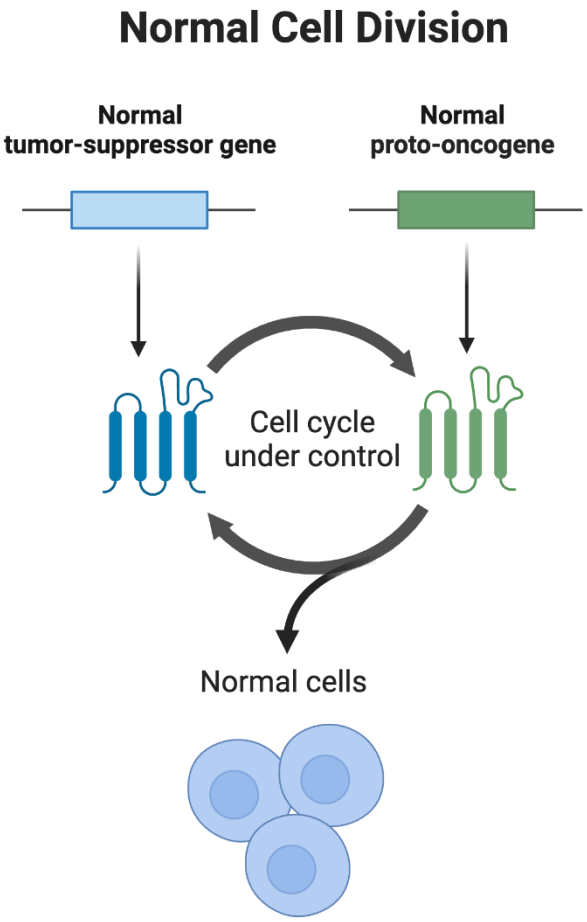
Organization of Lecture

- Proto-oncogenes vs. Tumor suppressor genes
- The retinoblastoma protein
- The p53 gene
- Targeting the Rb pathway in cancer

Hallmarks of cancer



Proto-oncogenes vs. Tumor suppressor genes



Proto-oncogenes vs. Tumor suppressor genes

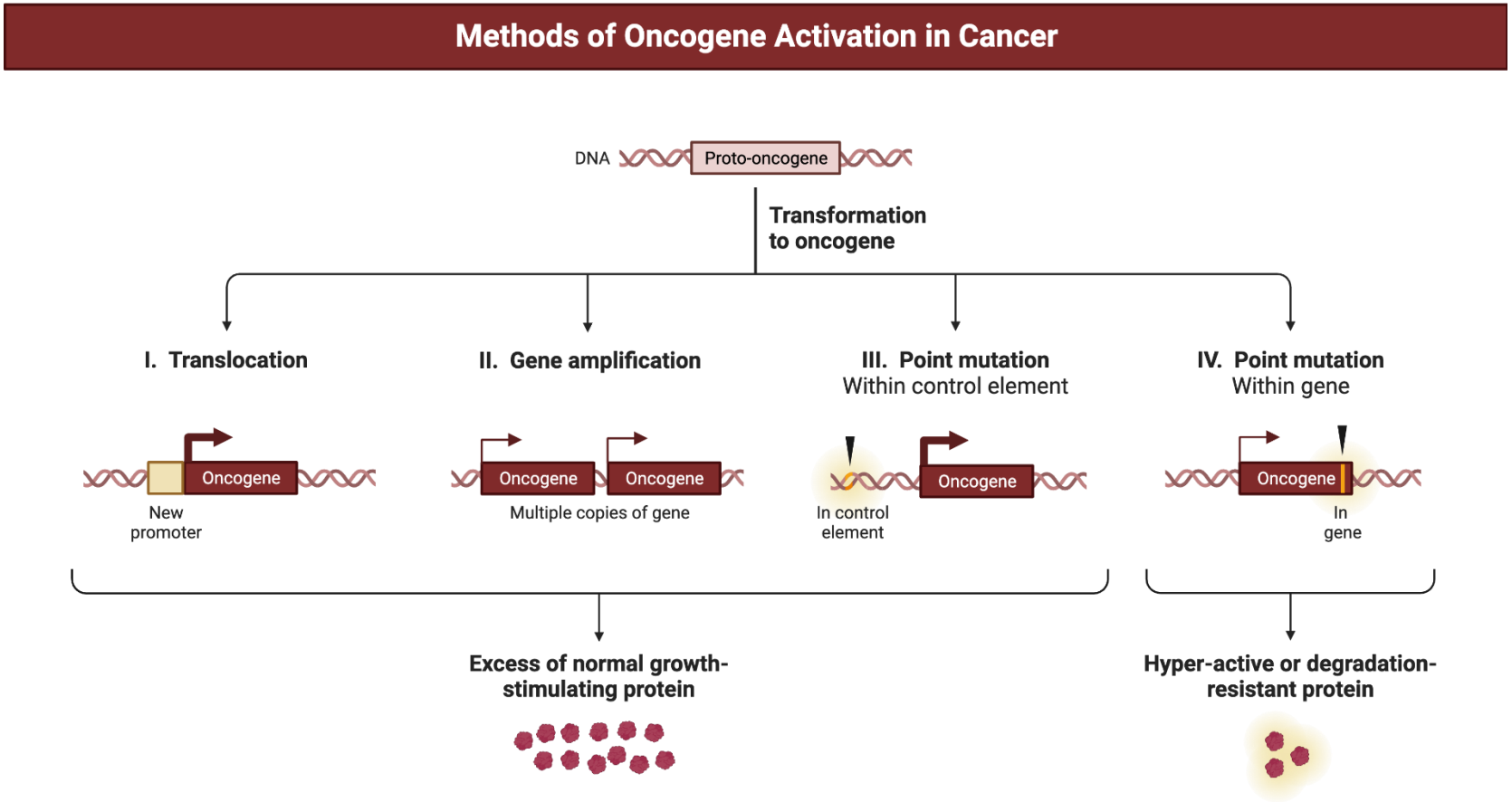
- Some are altered in a restricted set of tumor types
  - e.g., the APC (adenomatous polyposis coli) tumor suppressor in colorectal carcinoma
- Others are altered in a broad spectrum of tumor types
  - e.g., p53 tumor suppressor and the Ras proto-oncogenes
- The importance of tumor gene “pathways”
  - the Rb and p53 pathways

Proto-oncogenes vs. Tumor suppressor genes

A proto-oncogene promotes cancer when its function is malignantly activated

- An activated proto-oncogene contributes to tumorigenesis by "gain-of-function"
- Thus, an activated proto-oncogene is genetically dominant at the cellular level
  - an activated oncogene can elicit a new phenotype (tumorigenesis) even in the presence of the corresponding wild type allele

Proto-oncogenes vs. Tumor suppressor genes

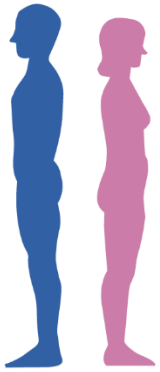


- [illegible] gene mutations, leading to gene dysregulation or overexpression
  - [illegible] gene amplification in neuroblastomas
  - c-Myc gene translocation in Burkitt's lymphoma

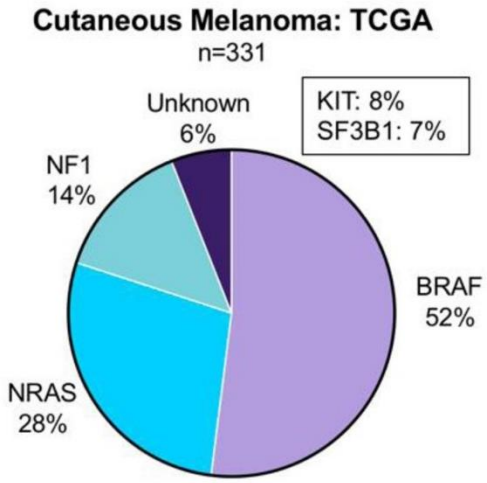
Proto-oncogene mutations in Melanoma

Estimated New Cases

Males			Females		
Prostate	288,300	29%	Breast	297,790	31%
Lung & bronchus	117,550	12%	Lung & bronchus	120,790	13%
Colon & rectum	81,860	8%	Colon & rectum	71,160	8%
Urinary bladder	62,420	6%	Uterine corpus	66,200	7%
Melanoma of the skin	58,120	6%	Melanoma of the skin	39,490	4%
Kidney & renal pelvis	52,360	5%	Non-Hodgkin lymphoma	35,670	4%
Non-Hodgkin lymphoma	44,880	4%	Thyroid	31,180	3%
Oral cavity & pharynx	39,290	4%	Pancreas	30,920	3%
Leukemia	35,670	4%	Kidney & renal pelvis	29,440	3%
Pancreas	33,130	3%	Leukemia	23,940	3%
All Sites	**1,010,310**	**100%**	**All Sites**	**948,000**	**100%**

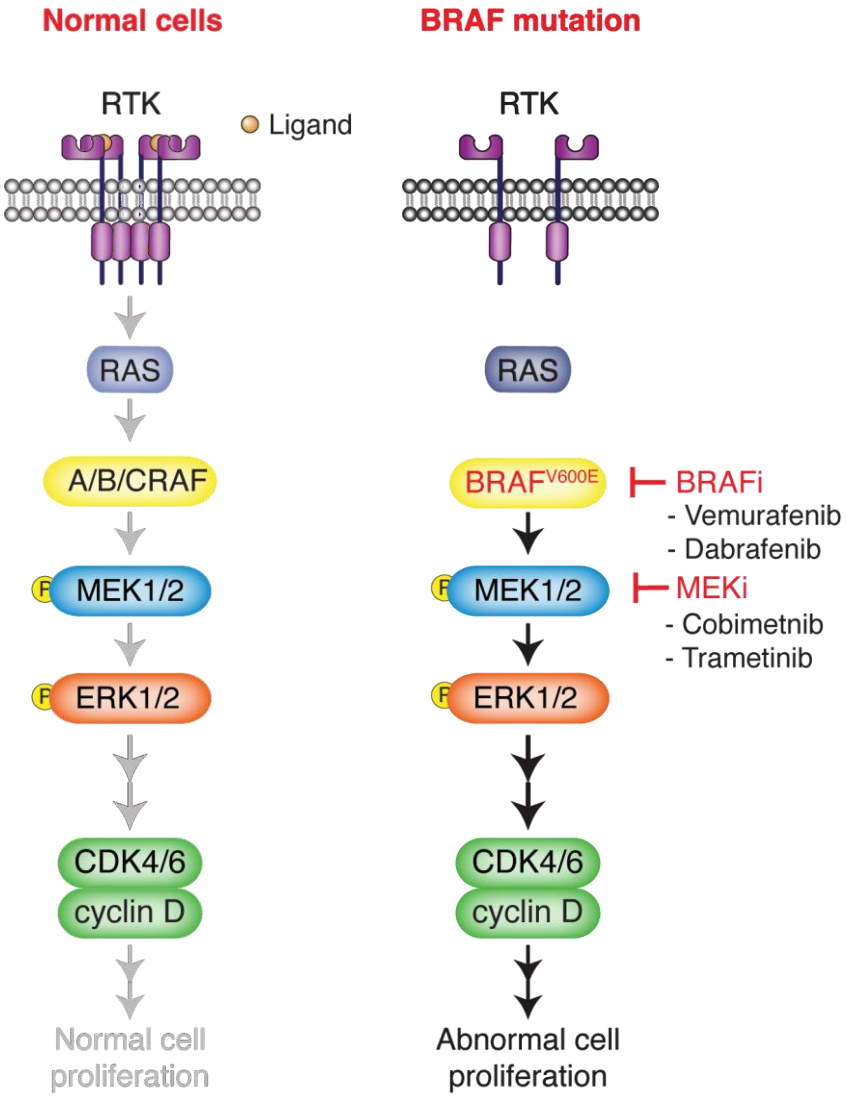


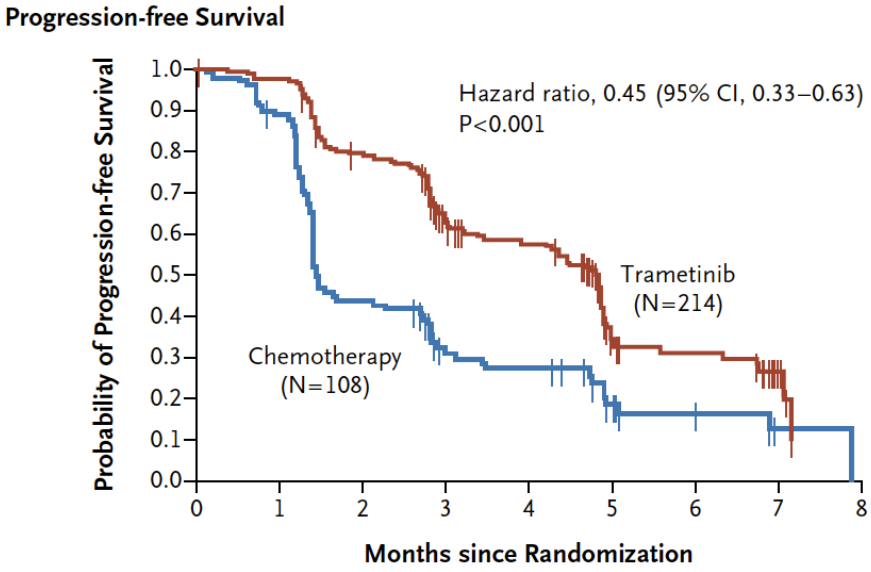
Cancer statistics, 2023



Nassar et al., 2020

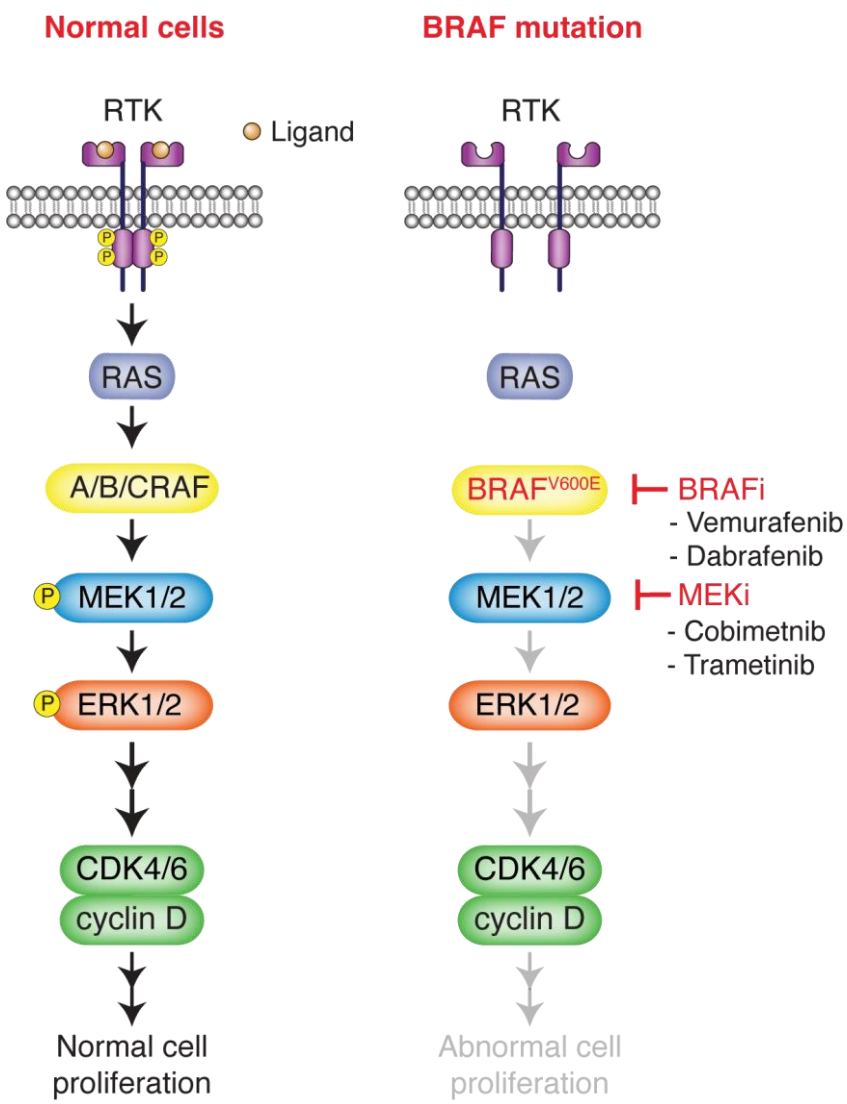
BRAF V600 mutation in melanoma cells





Flaherty et al., 2012

BRAF V600 mutation in melanoma cells



Treated with BRAF inhibitor
(Response rate >50%; Response duration: ~6 months)

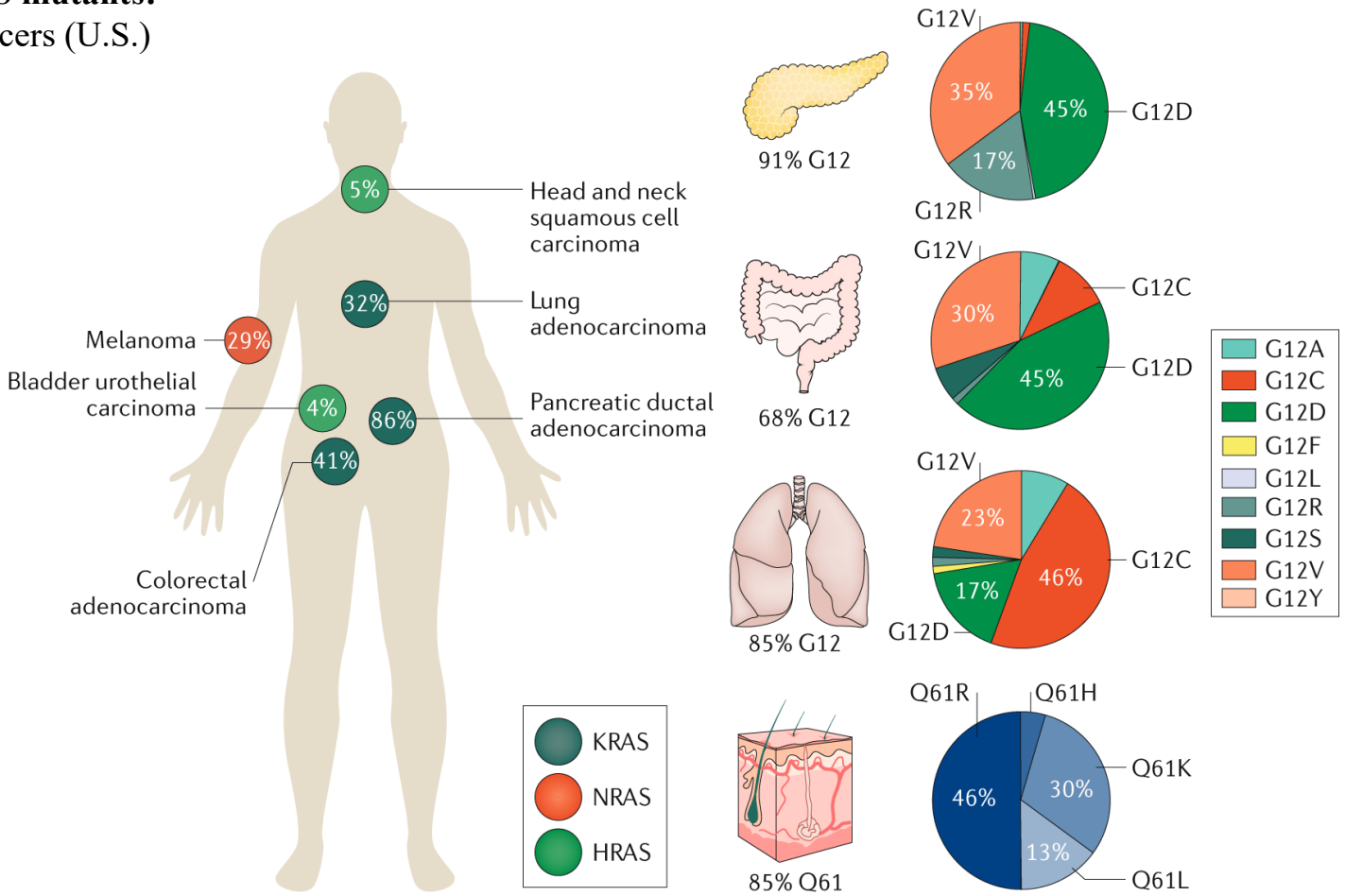
38-year-old man with BRAF-mutant melanoma

Wagle et al., 2011

About 40% of patients develop resistance to BRAF/MEK inhibitor within a year.

RAS is one of the most frequently mutated genes in human cancers

Diverse RAS mutants:
~30% of cancers (U.S.)



Amanda R. M., *et al*., 2020

Proto-oncogenes vs. Tumor suppressor genes

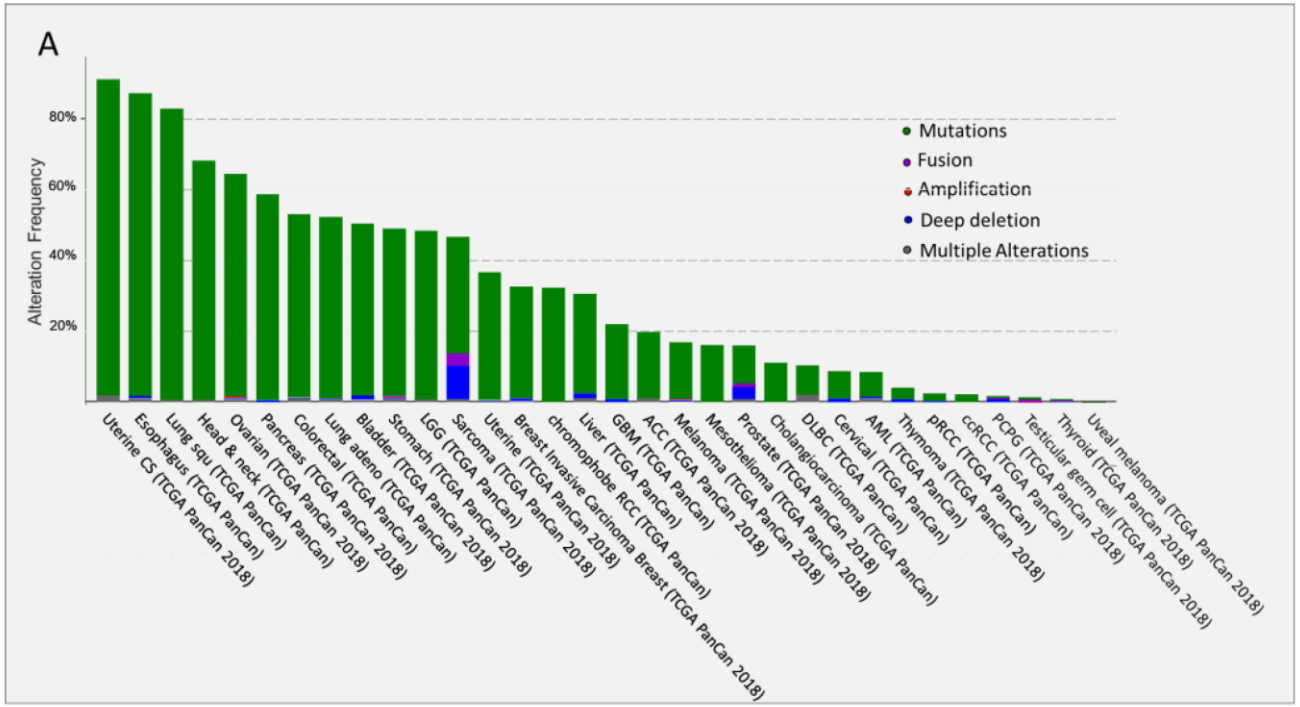
A tumor suppressor gene promotes cancer when its function is malignantly inactivated

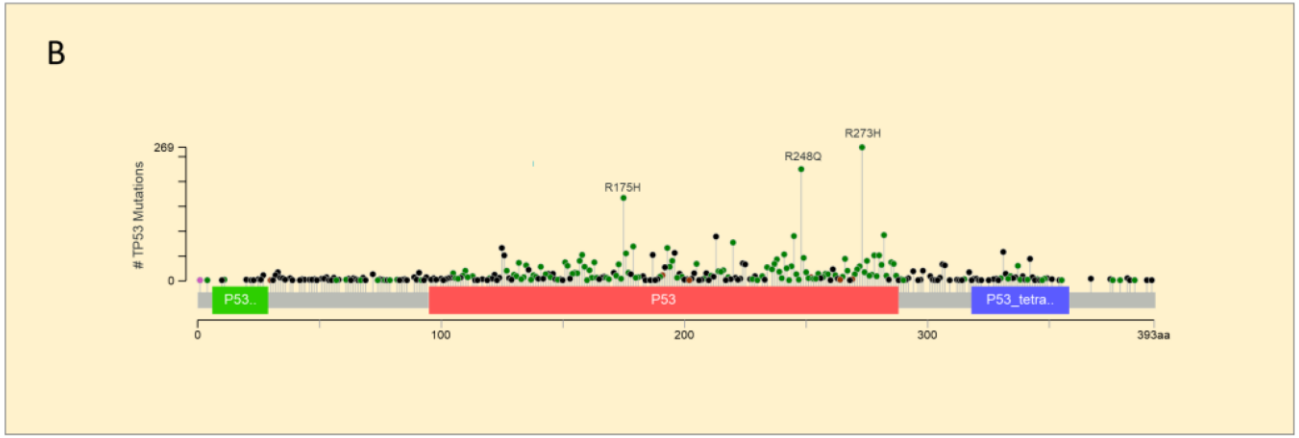
- A tumor suppressor contributes to tumorigenesis by "loss-of-function"
- In most instances, an inactivated tumor suppressor gene is genetically recessive at the cellular level.
  - It will not promote tumorigenesis in diploid cells unless the other (wildtype) allele is also lost or inactivated
  - Some exceptions:
    - dominant-negative p53 mutations
    - “haploinsufficient” tumor suppressor genes

Proto-oncogenes vs. Tumor suppressor genes

- In this lecture we will focus on…
  - the retinoblastoma susceptibility (Rb) gene
  - the p53 tumor suppressor gene
- Genetic properties
- Biochemical functions of their protein products
- the p53 and Rb tumor suppressor “pathways”

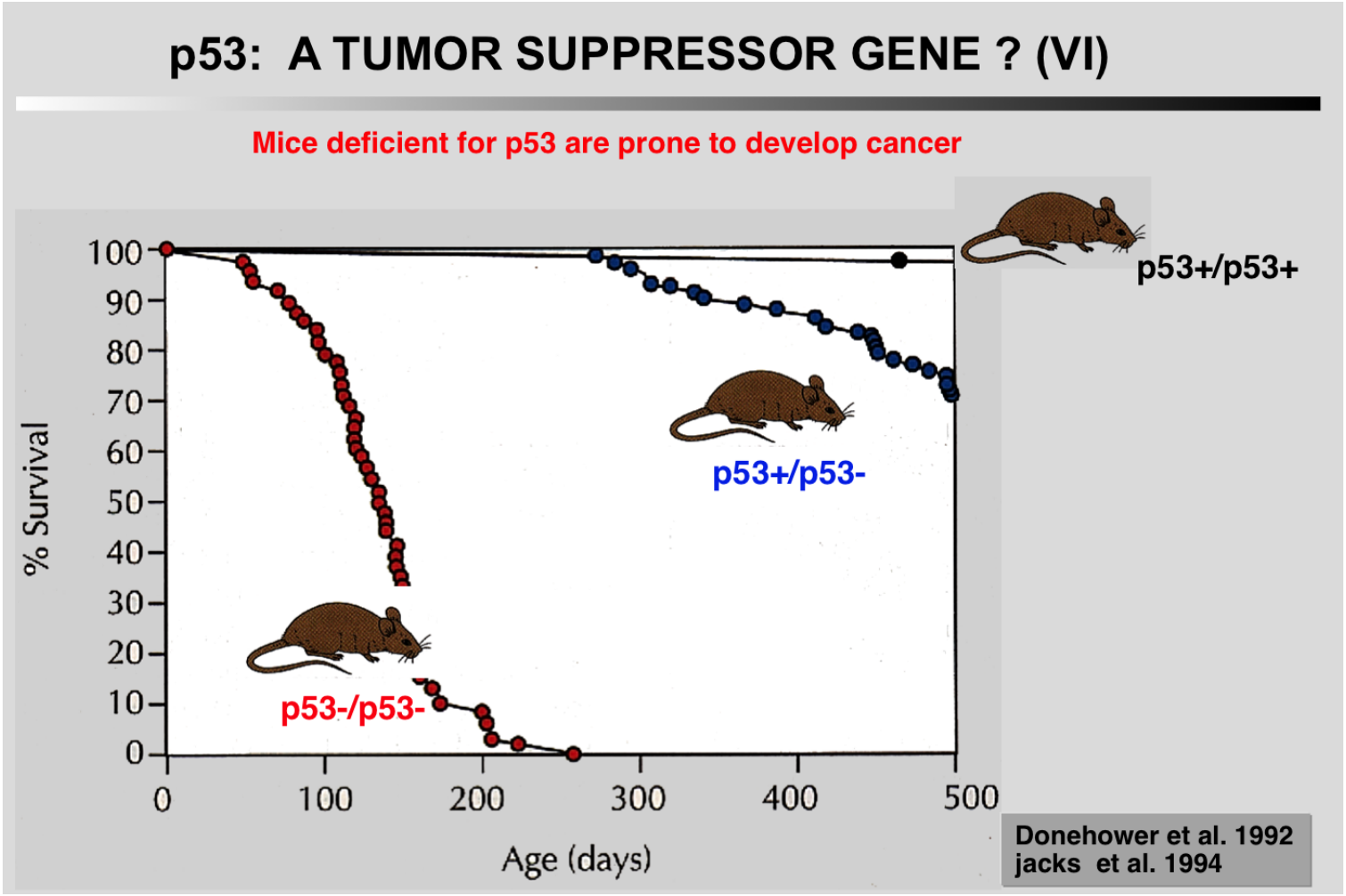
p53 is the most frequently mutated gene in cancer





Pitolli et al., 2017

The p53 gene

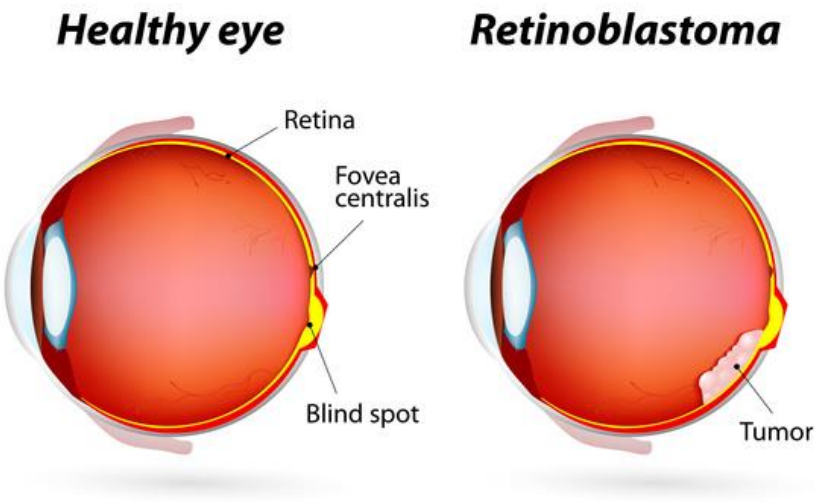


The TP53 web site (http://p53.fr/)

Retinoblastoma cancer

- Retinoblastoma is a rare type of eye cancer that primarily affects young children, usually before the age of 5.

- It originates in the retina, the light-sensitive lining on the inside of the eye.

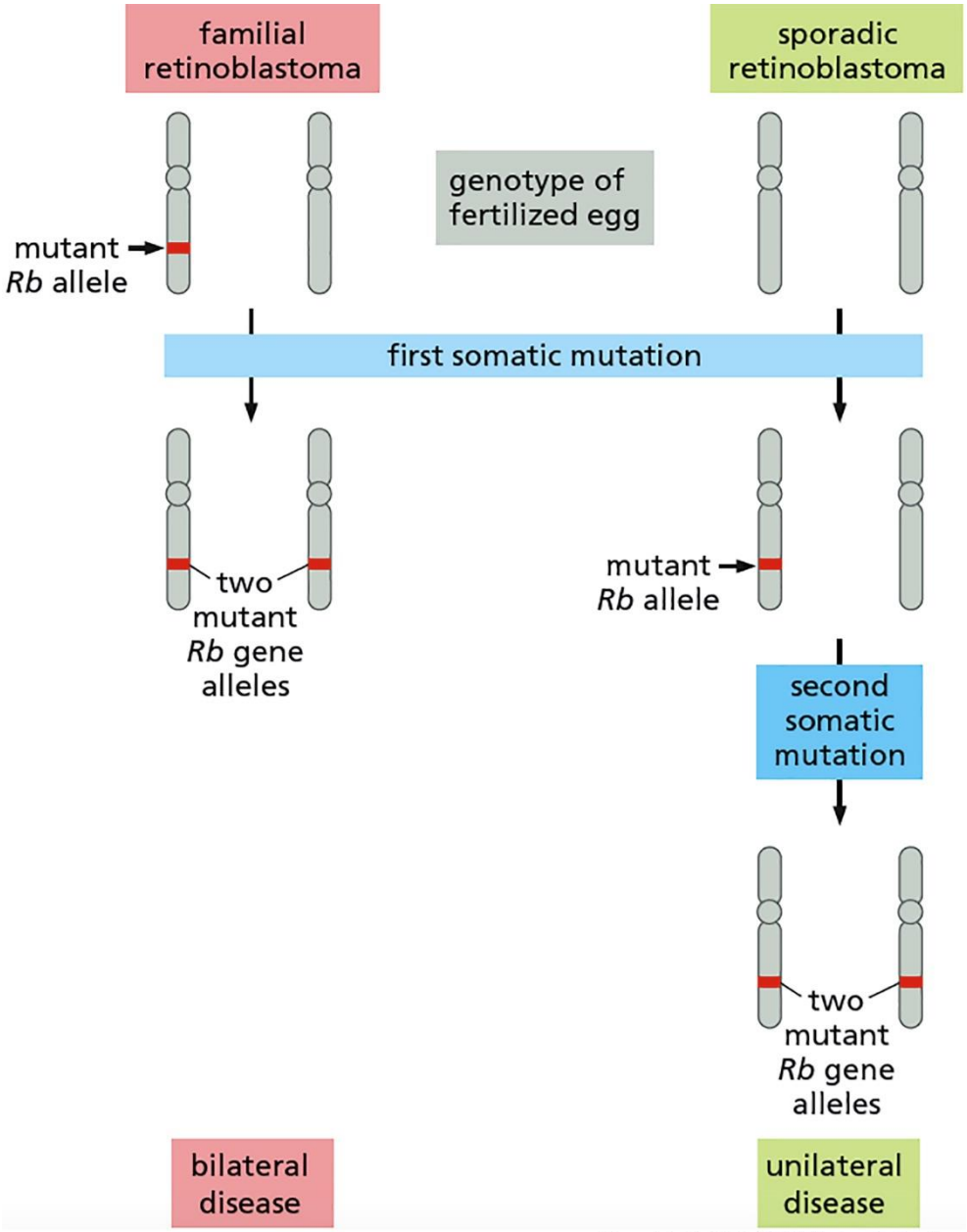


The Retinoblastoma (Rb) gene

- Cytogenetic abnormalities of chromosome 13:
  - interstitial deletions of variable length
  - always involve material from chromosome band 13q14
  - Sporadic patients: deletions in tumor cells only
  - Familial patients: deletions in both tumor & normal cells

- Is Rb susceptibility due to genetic loss at 13q14?

 If so, then the two mutations required for retinoblastoma might represent inactivation of <u>both</u> alleles of a single gene at 13q14



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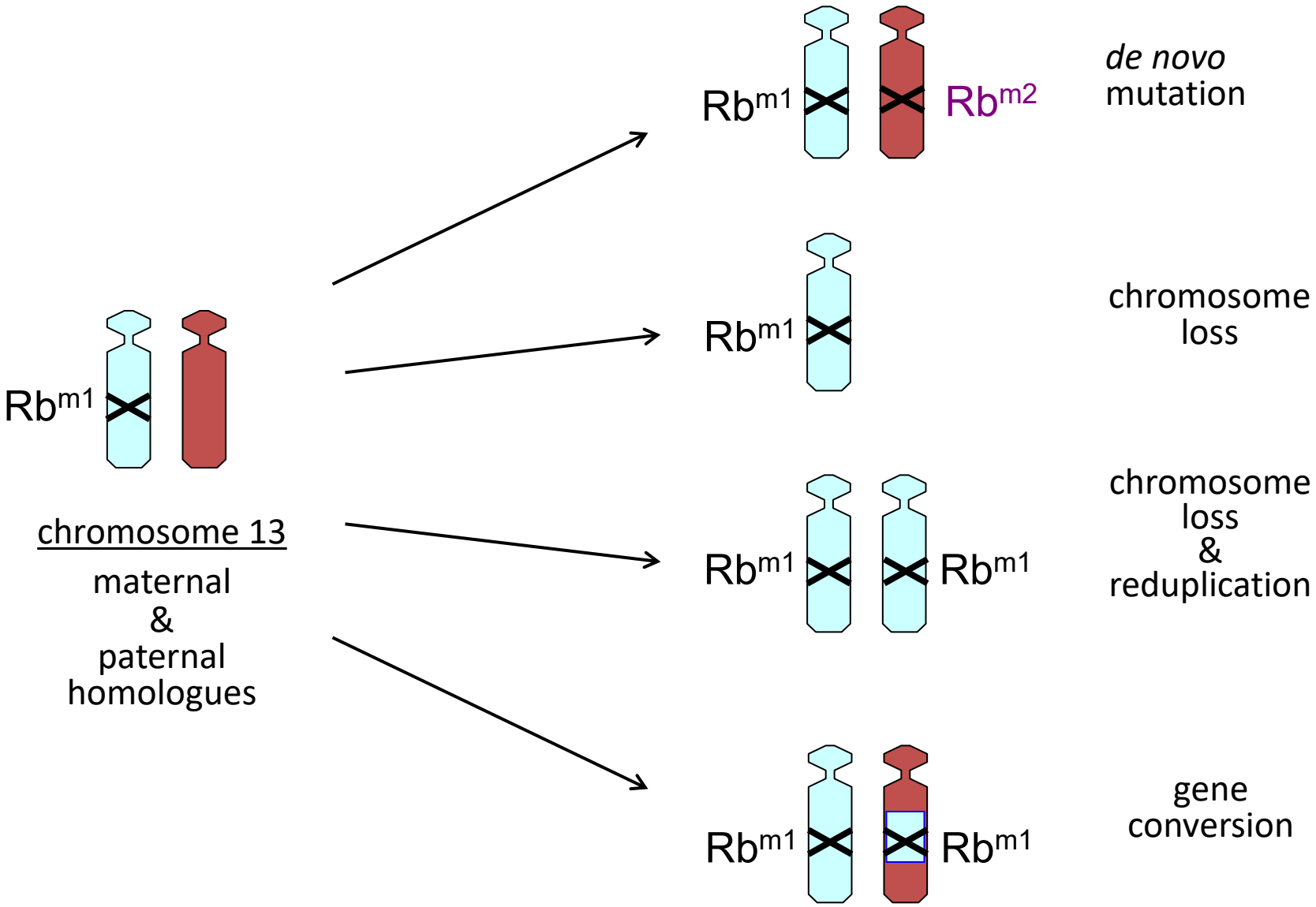
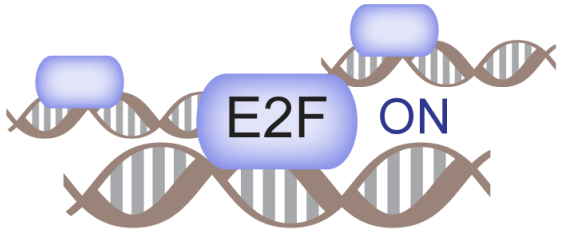
Mechanisms for inactivation of the second Rb allele in familial patients



E2F transcription factors

- The E2F family is a group of transcription factors that play an important role in regulating the cell cycle.
- G1/S transition is mediated by the E2F family of transcription factors
- E2F binds the promoters of genes required for cell cycle progression (G1/S transition and S phase).



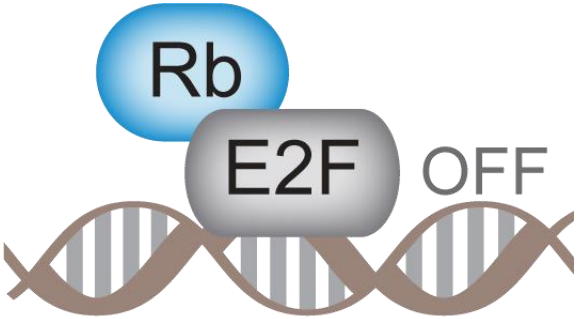
Expression of cell-cycle proteins

Some S phase genes regulated by E2F

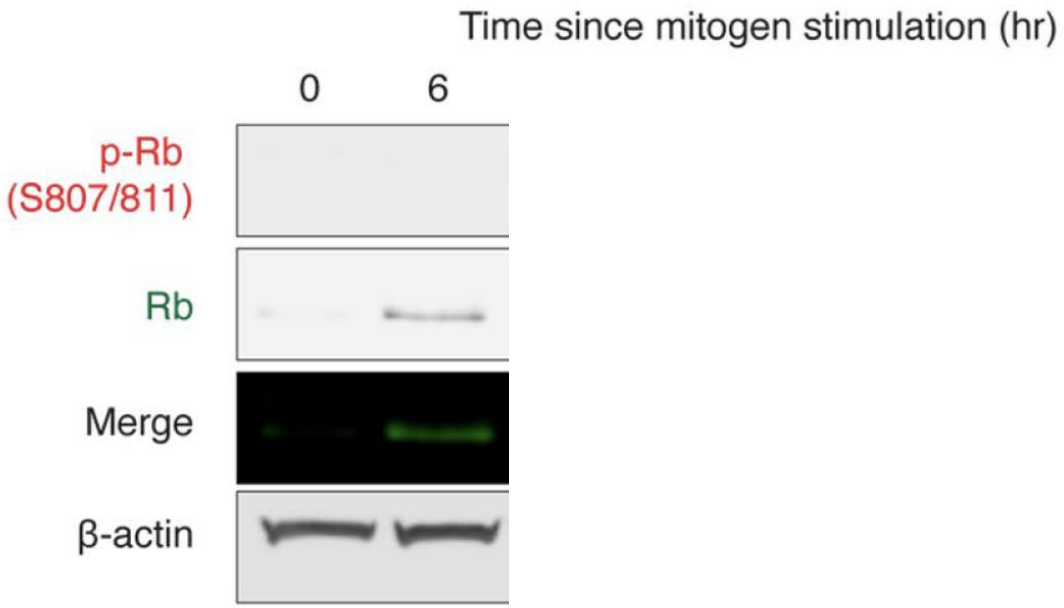
S phase gene	Function
• thymidine kinase	nucleotide synthesis
• DHFR (dihydrofolate reductase)	" "
• DNA polymerase α	DNA replication
• ORC1	" "
• histone H2A	chromosome assembly
• cyclin E	cell cycle progression
• cyclin A	" " "

Quiescent cells

- Hypophosphorylated Rb binds promoter-bound E2F
- Rb inactivates transcription by E2F
- S phase genes are repressed
- G1/S transition is blocked



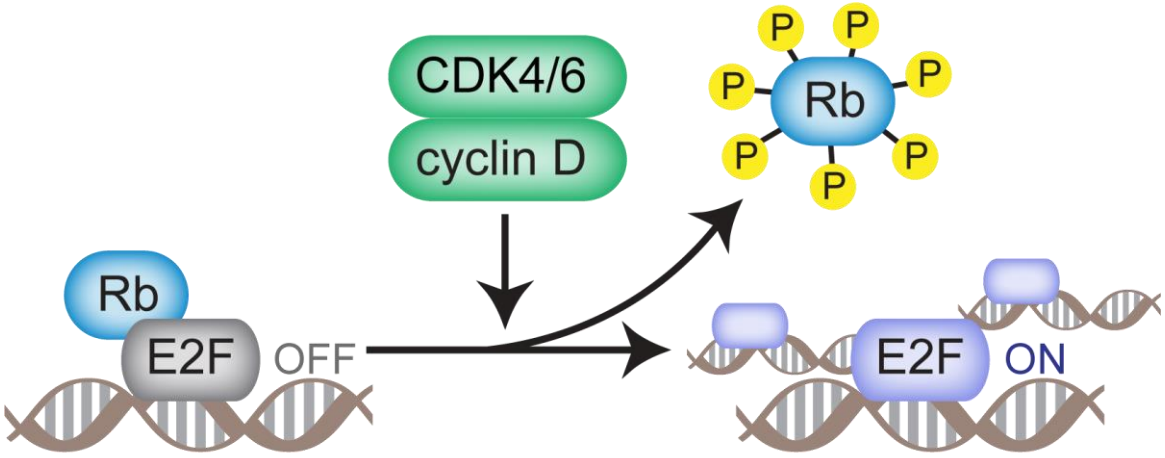
Rb is regulated by phosphorylation



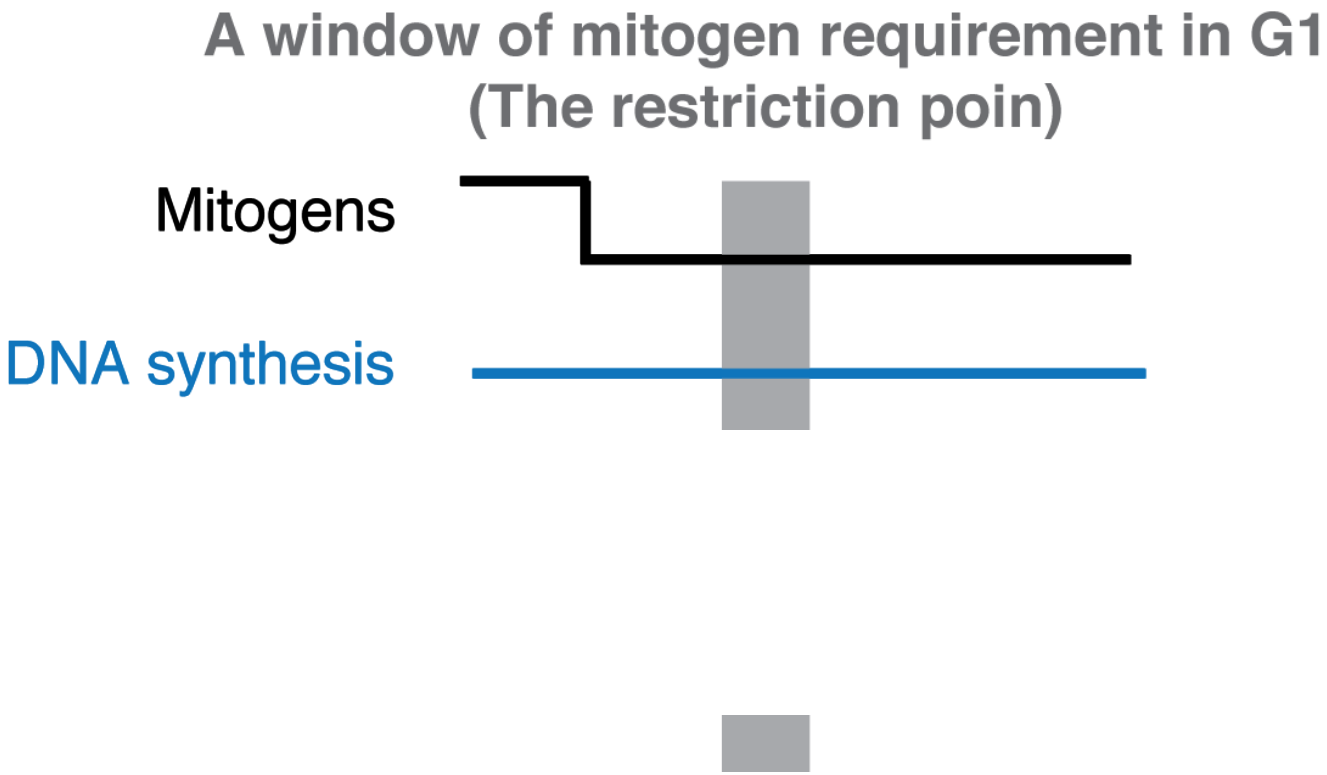
Kim et al., 2022

CDK4/6 phosphorylates Rb, liberating E2F

- CDK4/6-cyclin D phosphorylates Rb
- Hyperphosphorylated Rb dissociates from E2F
- E2F activates transcription of S phase genes
- Cells enter S phase

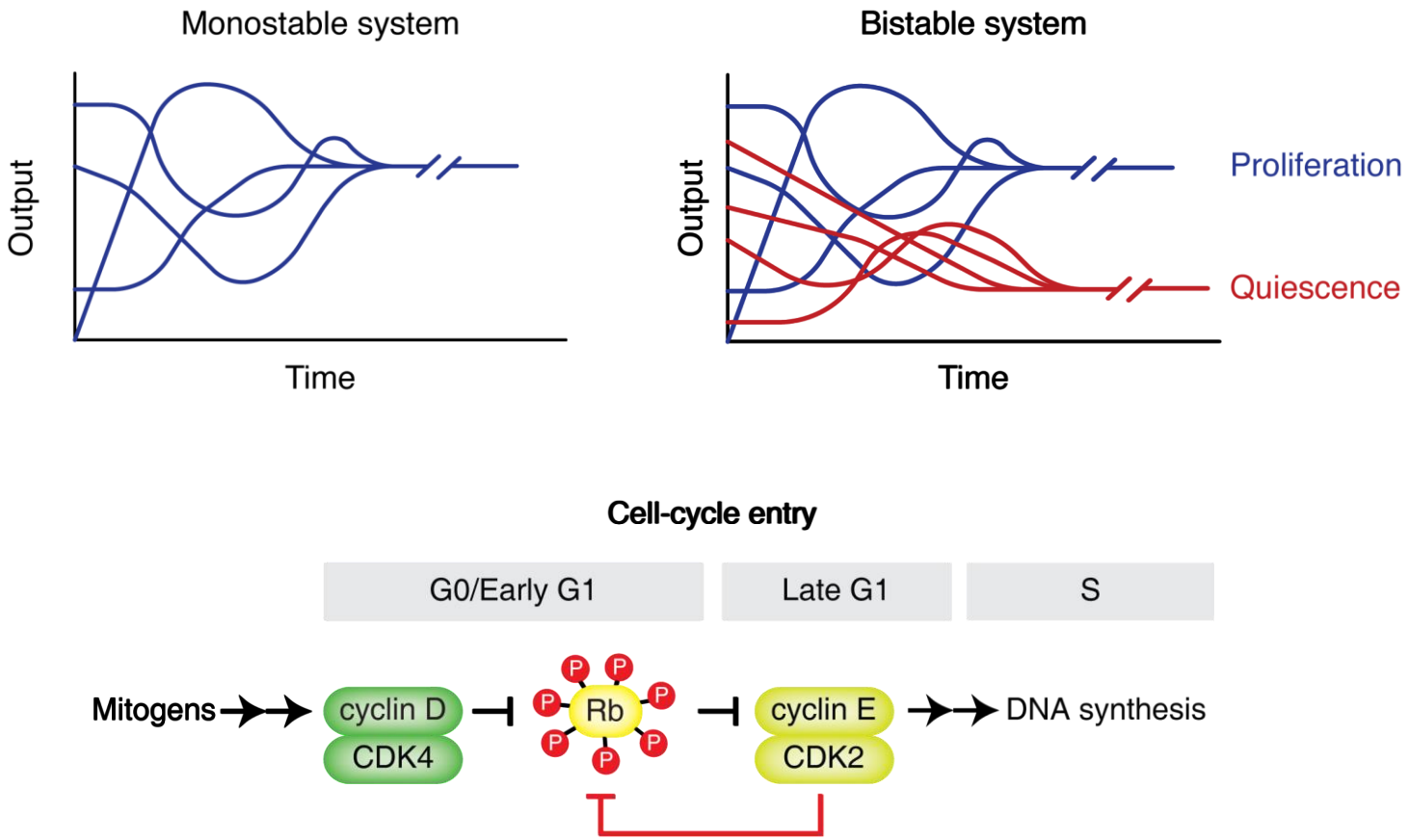


The G1/S checkpoint in cell-cycle entry



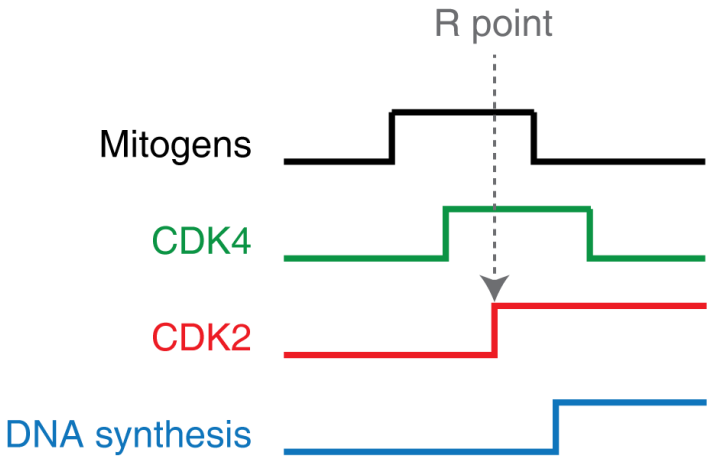
Pardee et al., 1974

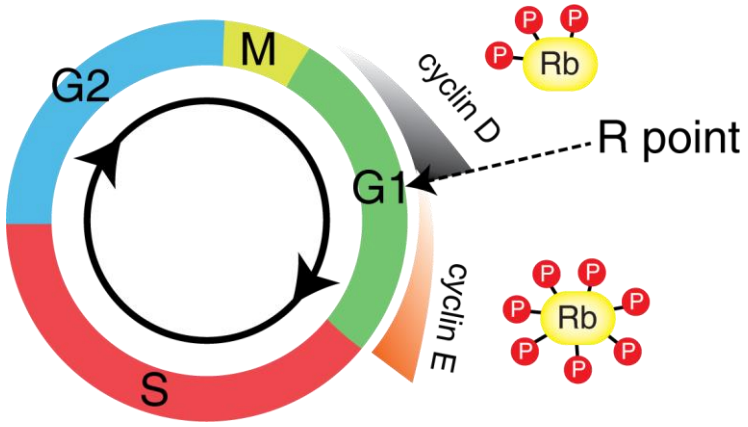
Bistability in cell-cycle entry?



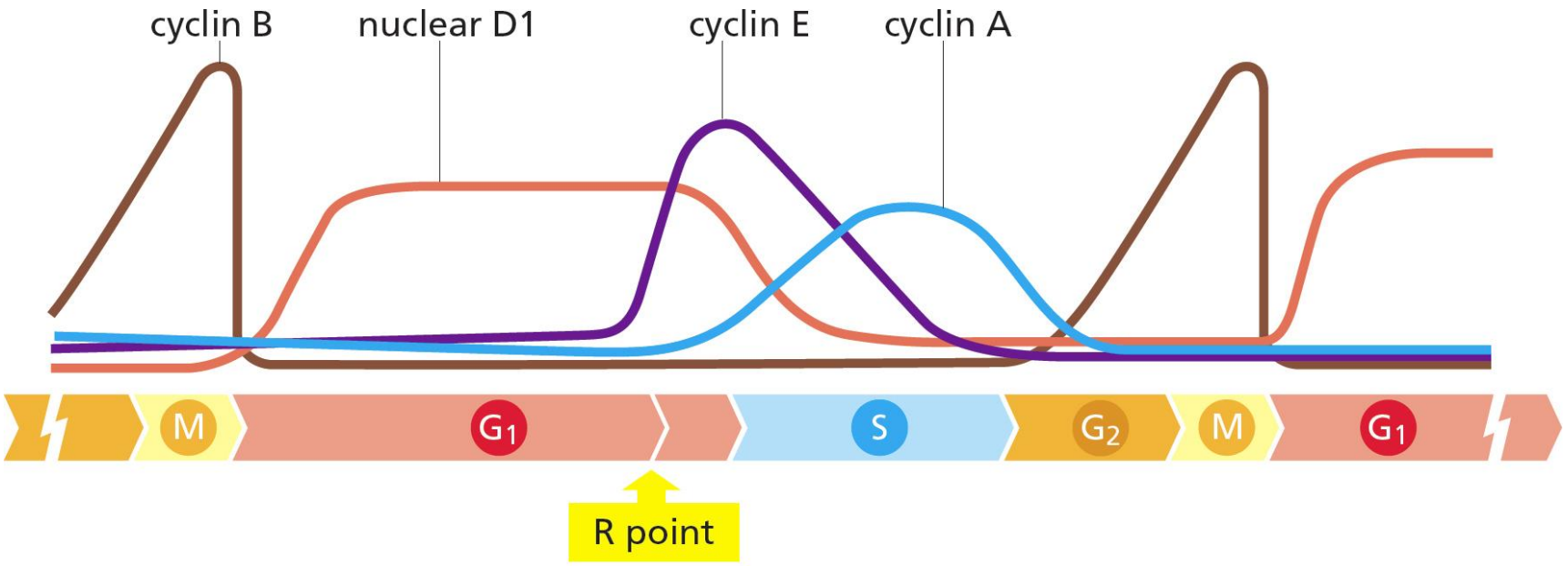
Hinds et al., 1992; Lundberg et al., 1998; Harbour et al., 1999; Yao et al., 2011;

Bistability in cell-cycle entry?





Fluctuation of cyclins



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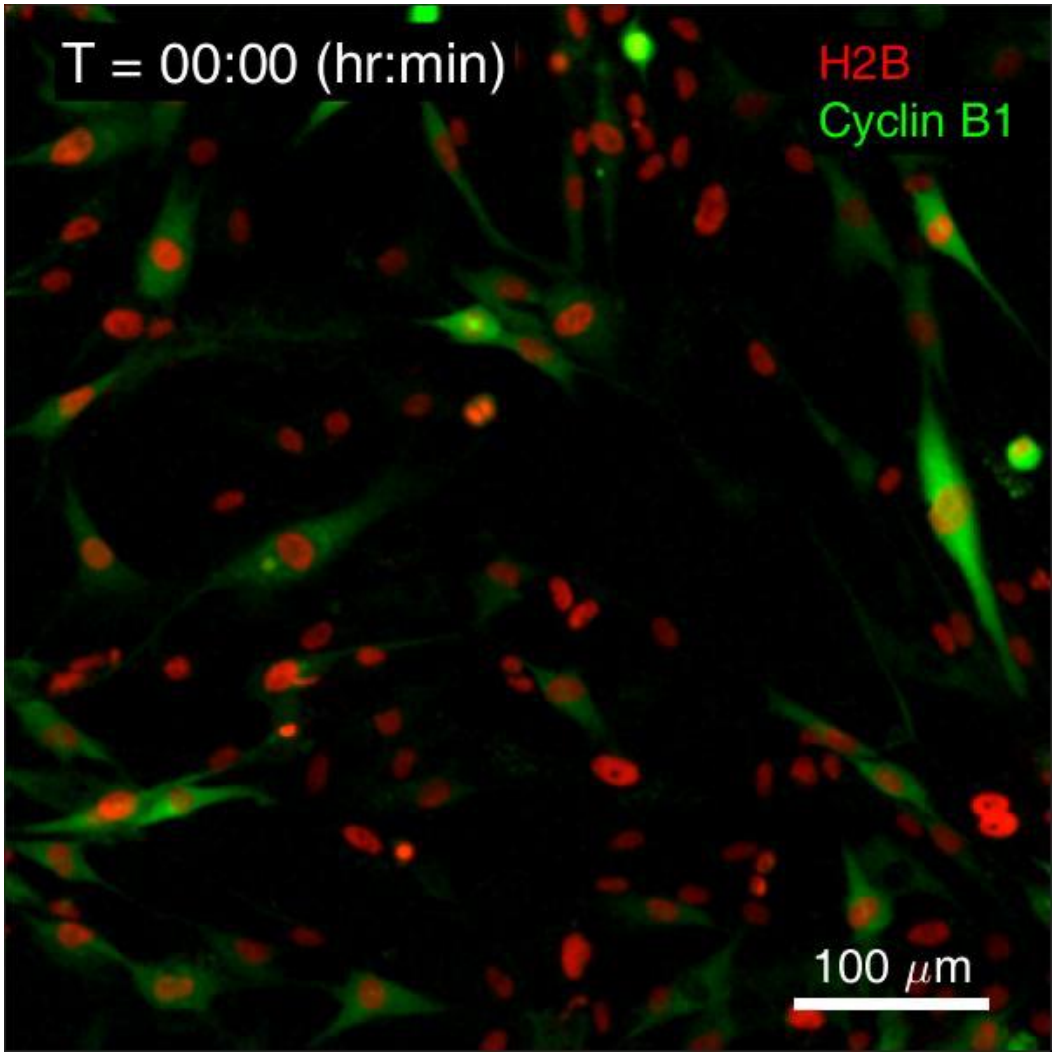
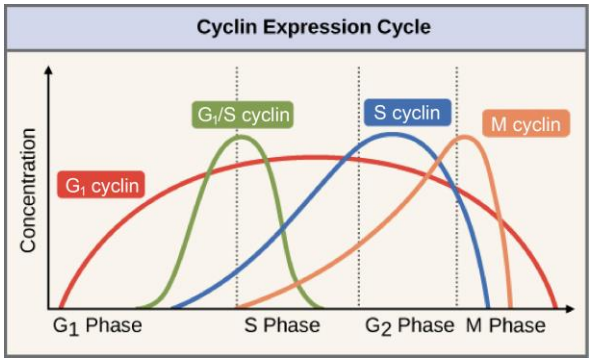
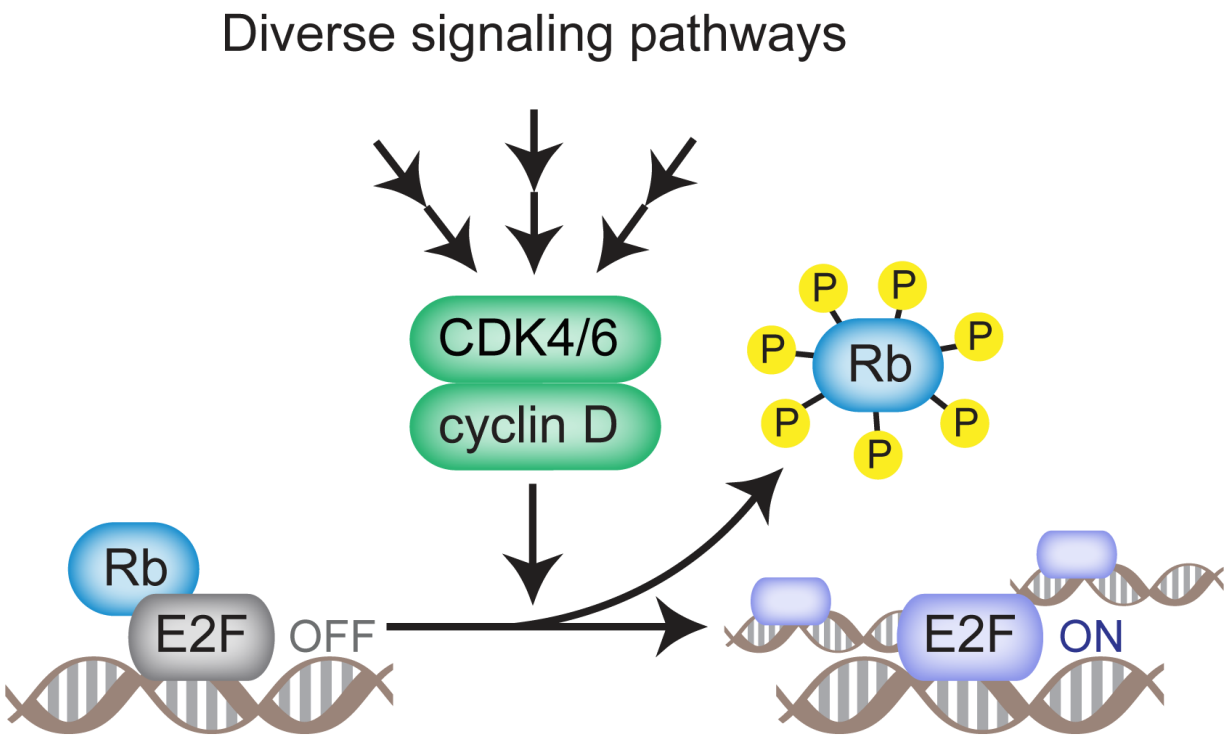
Cyclin B protein expression



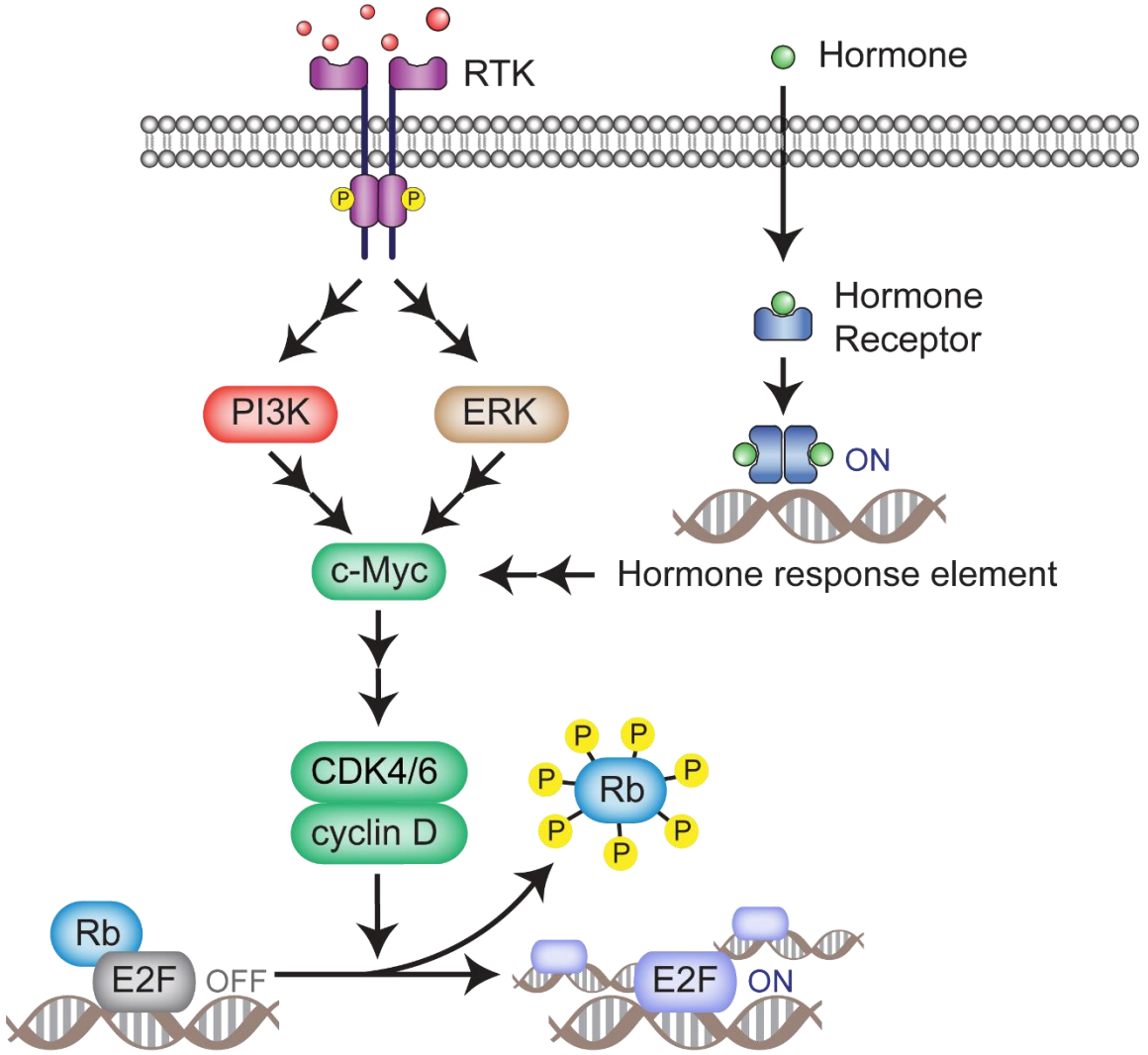


From “Control of the cell cycle: Figure 2”

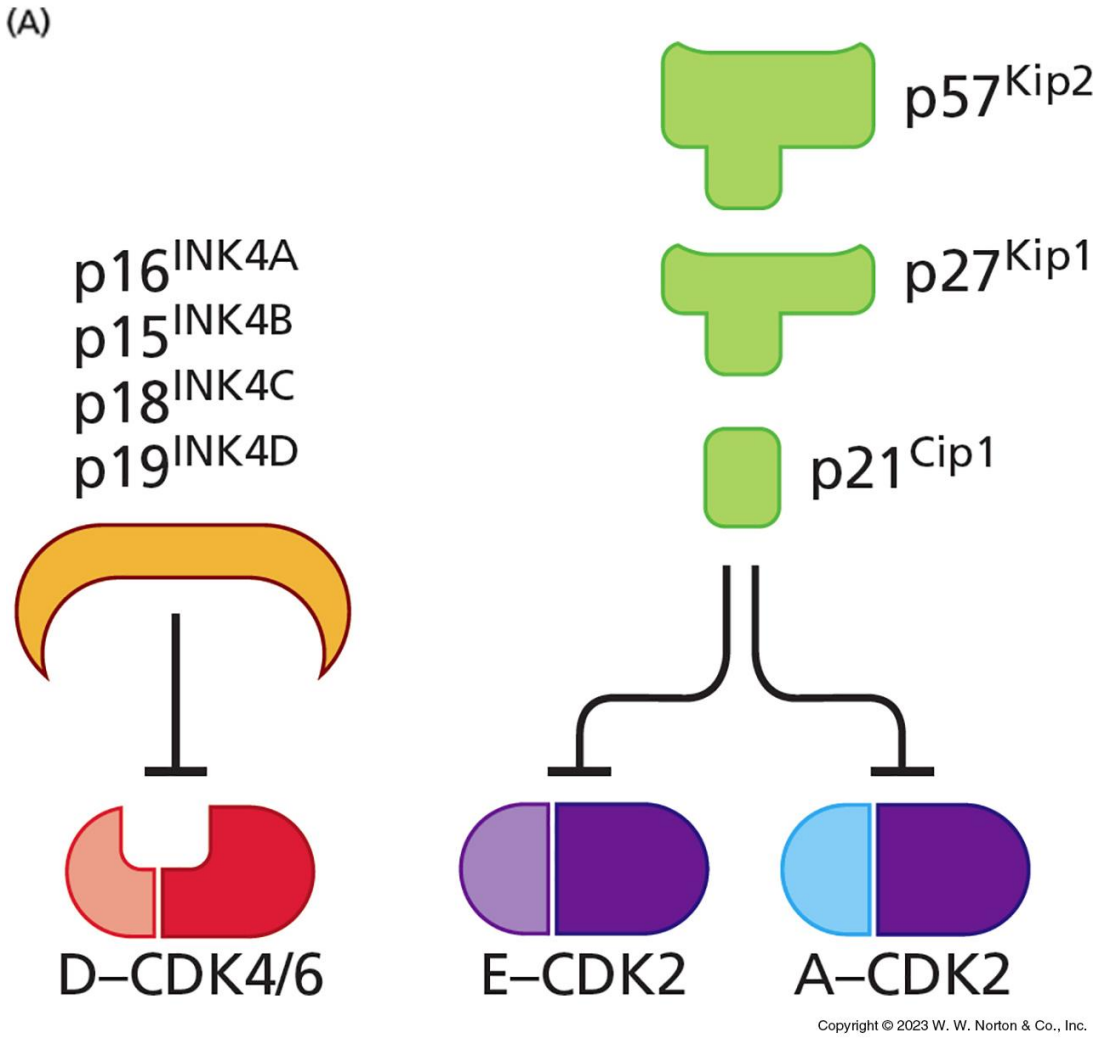
Diverse signaling pathways regulate CDK4/6 activity



Diverse signaling pathways regulate CDK4/6 activity



CDK inhibitor proteins



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CDK inhibitor proteins

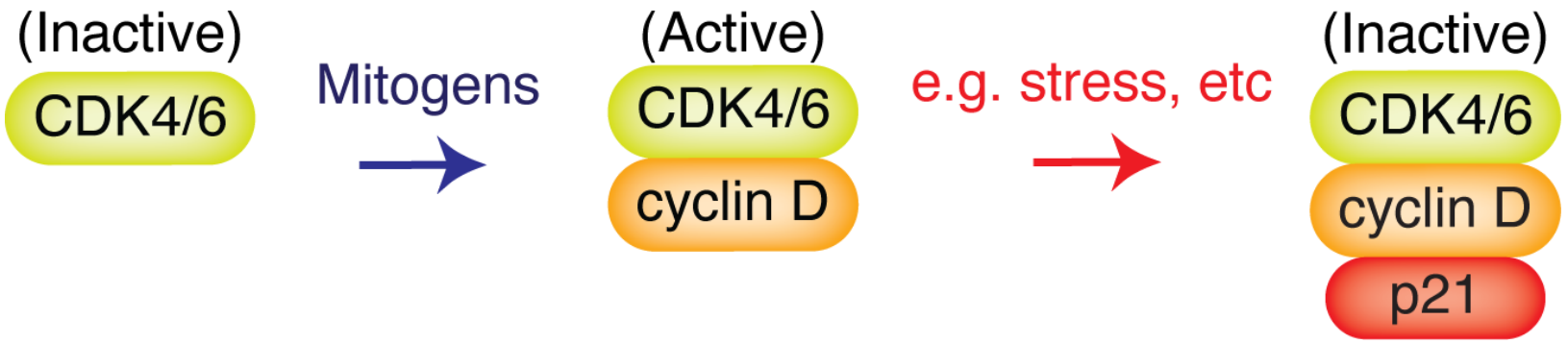
INK4 (Inhibitors of CD**K4) Family:**

- p15, p16, p18, p19
- These proteins are composed of multiple ankyrin repeats and bind only to CDK4/6

CDK inhibitor proteins

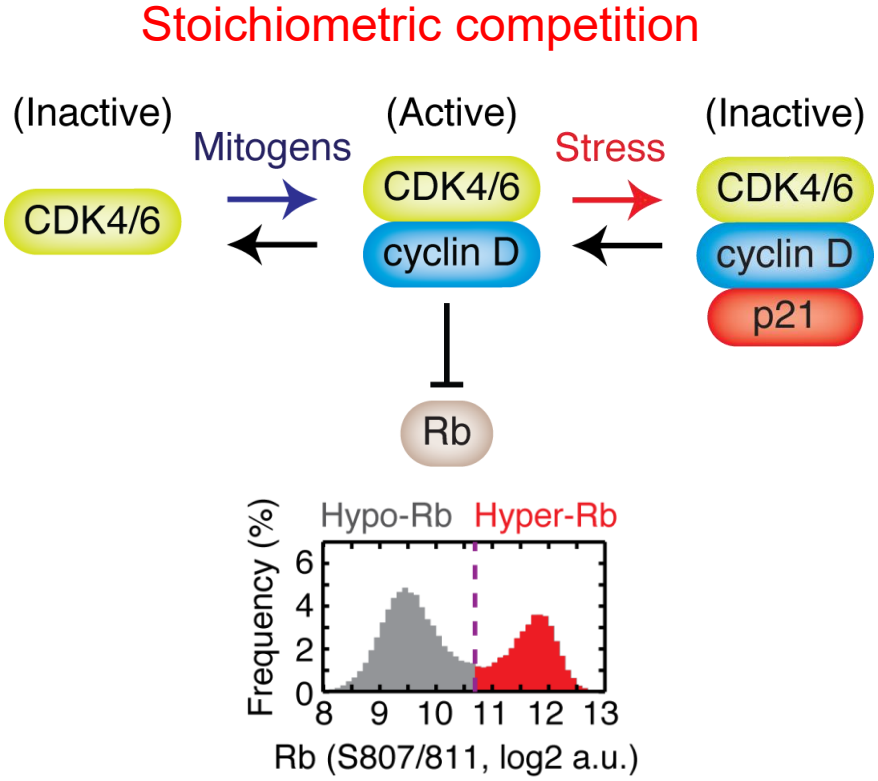
CIP/KIP (CDK interacting protein/Kinase inhibitory protein) Family:

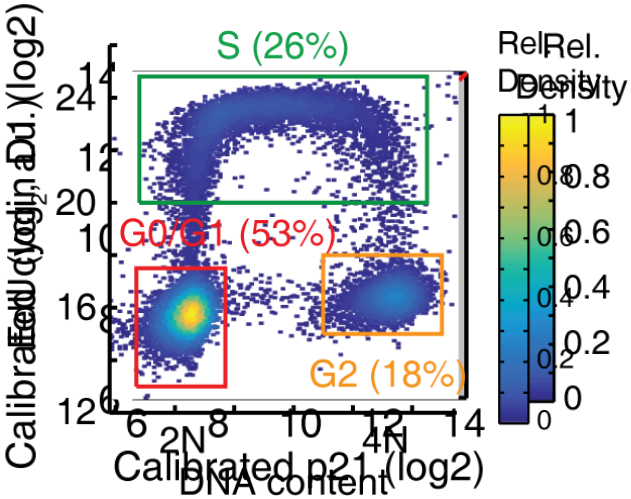
- p21, p27, p57
- Members of the CIP/KIP family bind to and inhibit the active cyclin/CDK complex

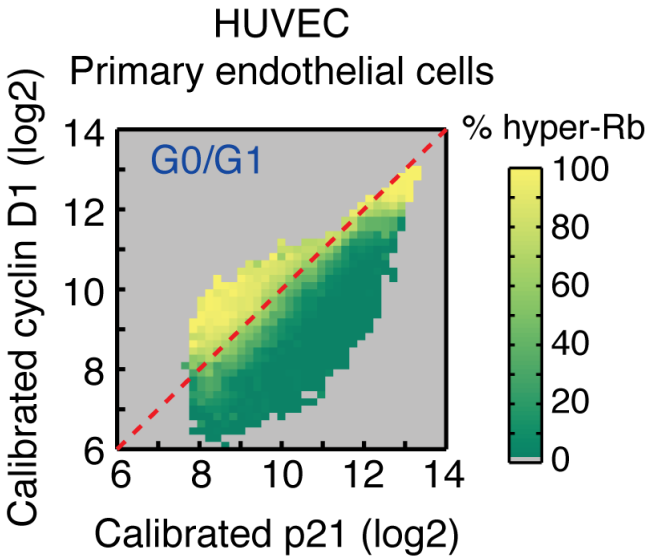


Regulation of CDK4/6 by cyclin D and p21

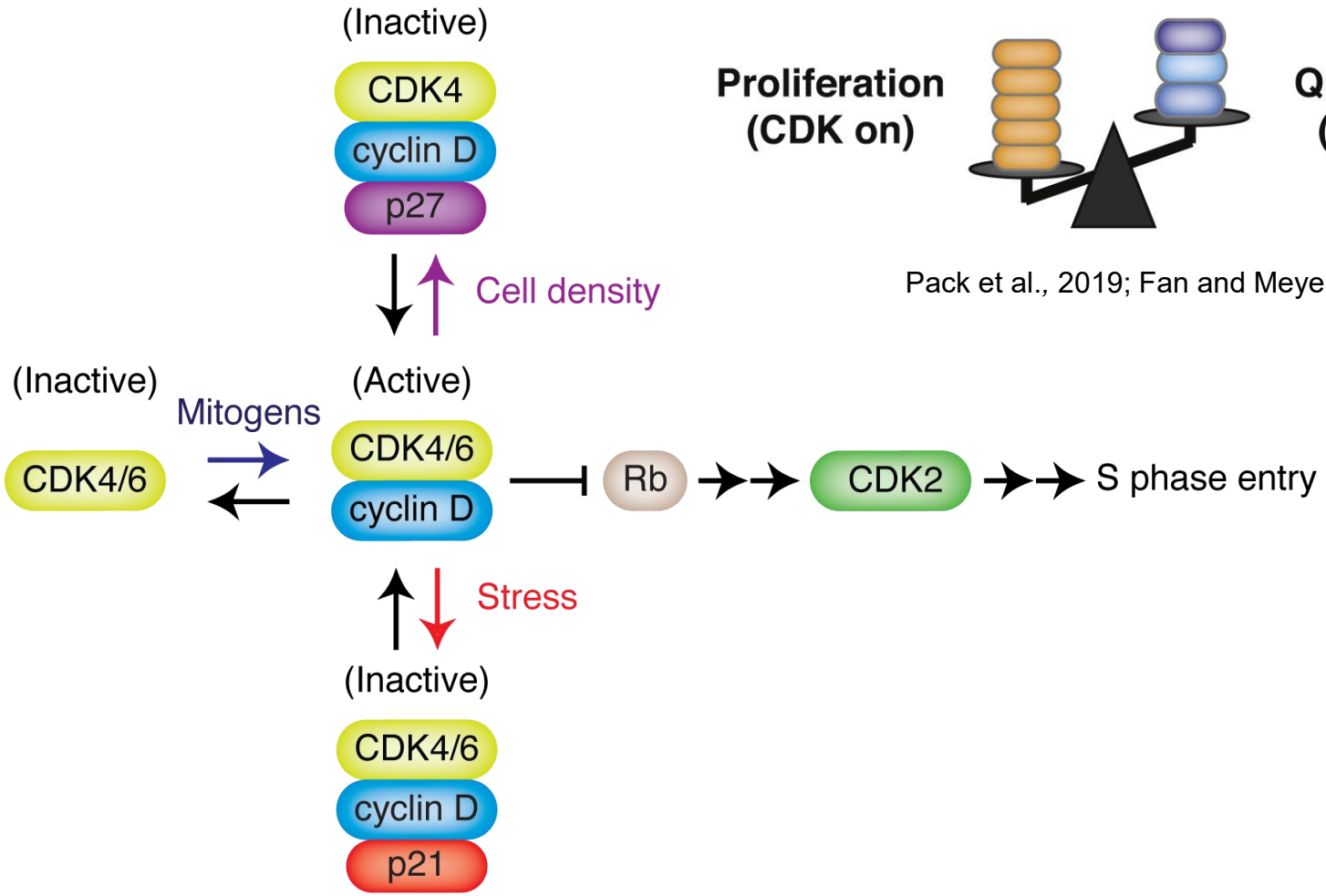
Yang et al. 2017





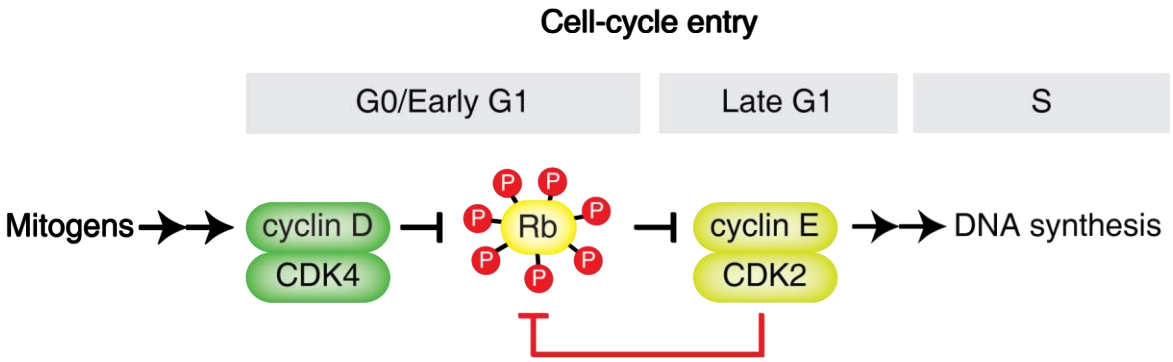


Competing mitogen and stress signaling control cell-cycle entry

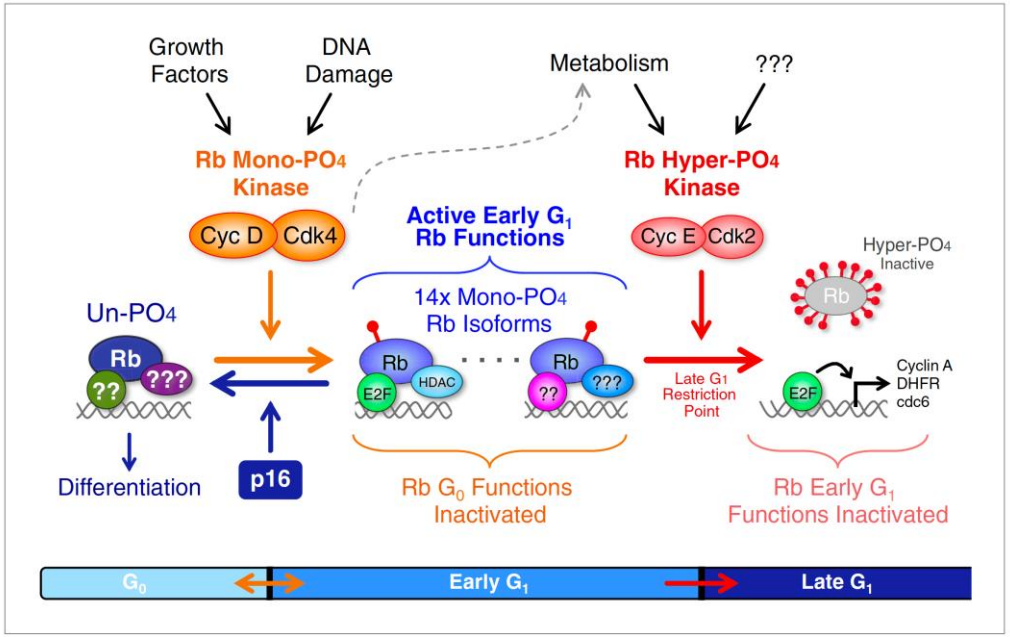


Pack et al., 2019; Fan and Meyer., 2021

The role of CDK4/6 in cell-cycle entry



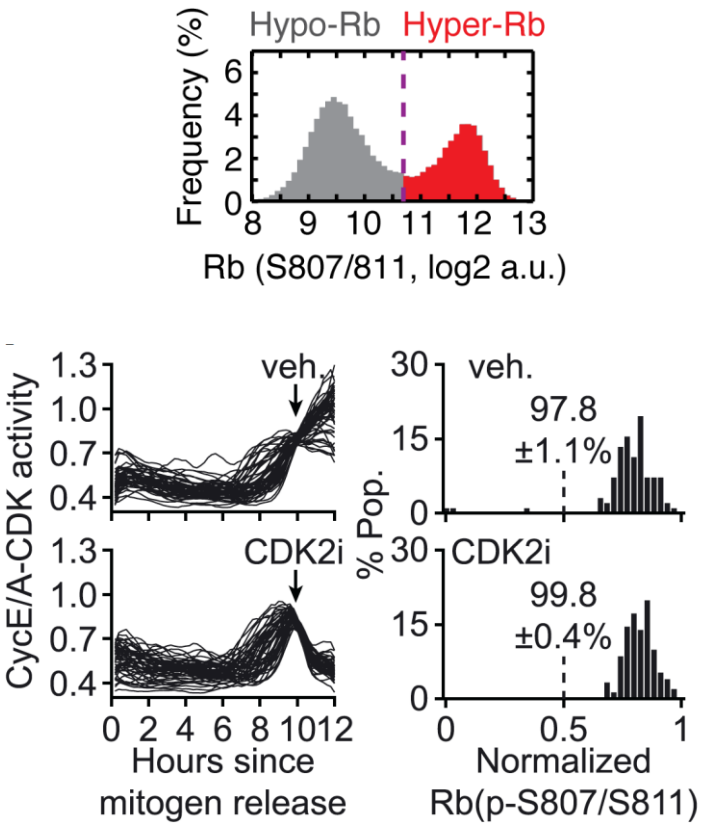
Hinds et al., 1992; Lundberg et al., 1998; Harbour et al., 1999; Yao et al., 2011;

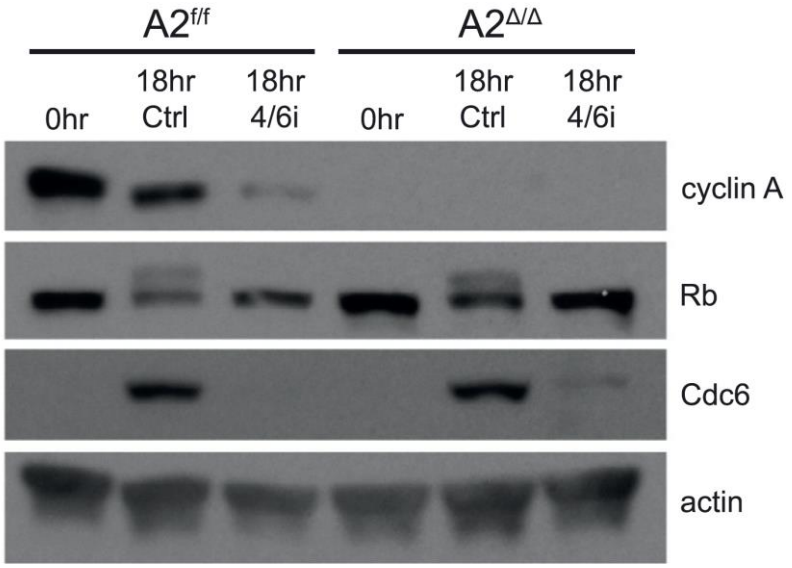


Narasimha et al., 2014

The role of CDK4/6 in cell-cycle entry

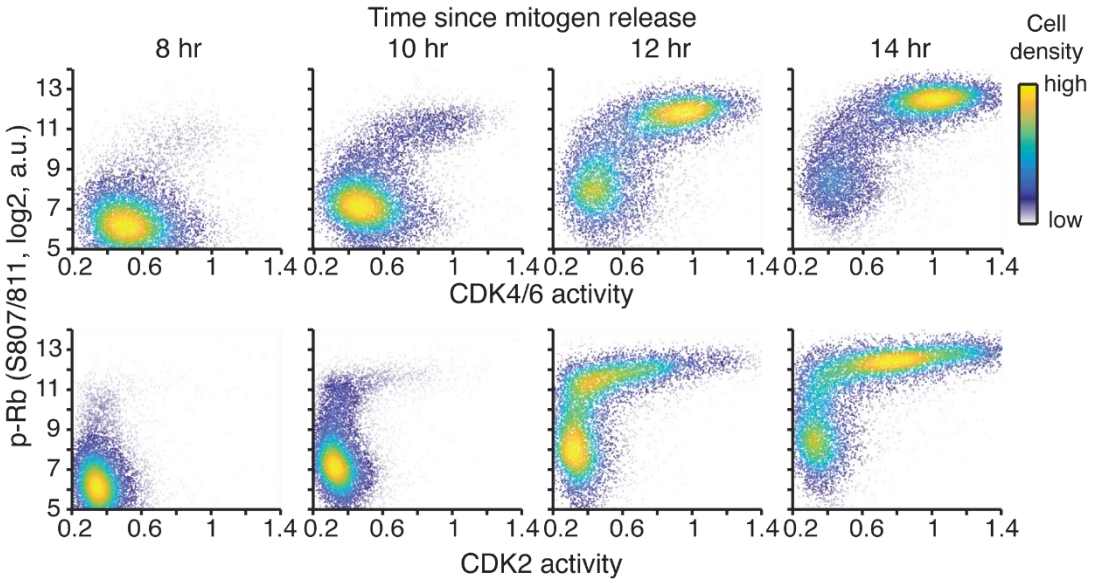
Chung et al., 2019

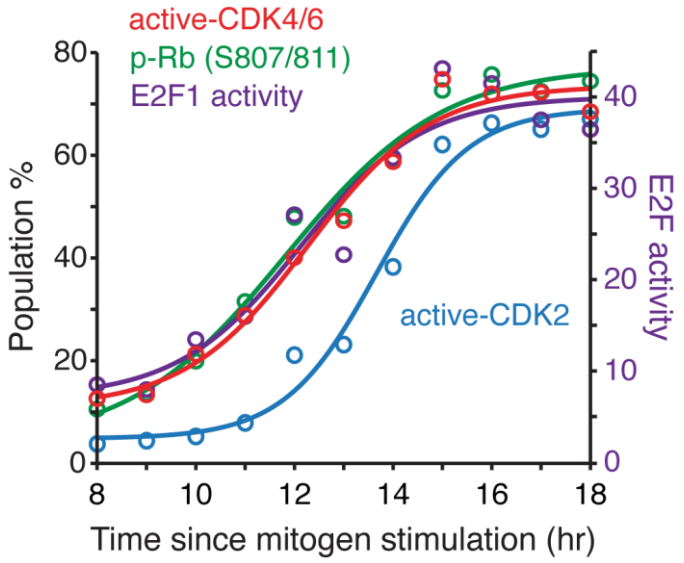




The role of CDK4/6 in cell-cycle entry

Kim et al., 2022





Direct inactivation of Rb in tumors

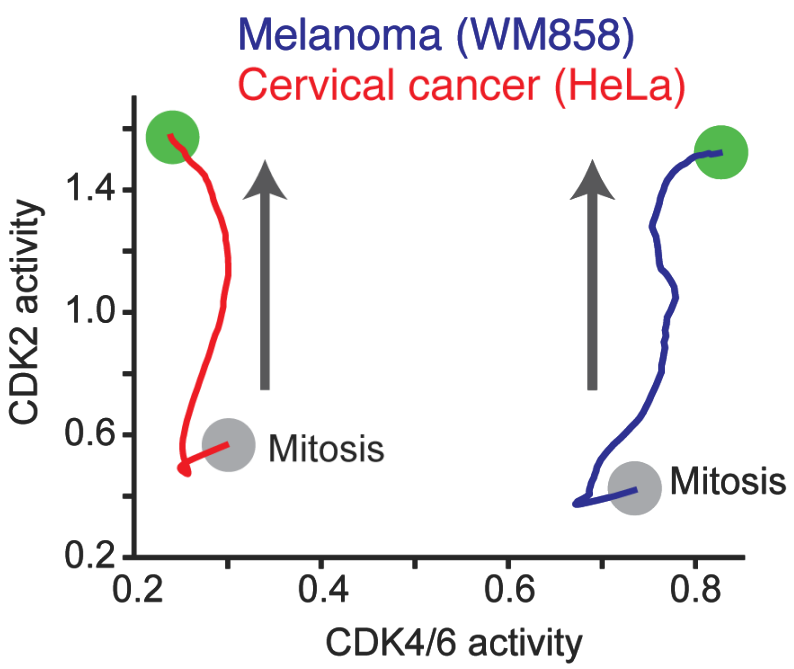
- Rb gene deletion (occurs in retinoblastoma)
- point mutations in the Rb pocket (in retinoblastoma)
- occupancy of the Rb pocket by early proteins of DNA tumor viruses
  - human papilloma virus (HPV), the main etiological agent of human cervical carcinomas
  - HPV encodes two proteins required for tumorigenesis
  - E7 binds the pocket of hypophosphorylated Rb
  - Deregulation of E2F (and the G1/S transition)

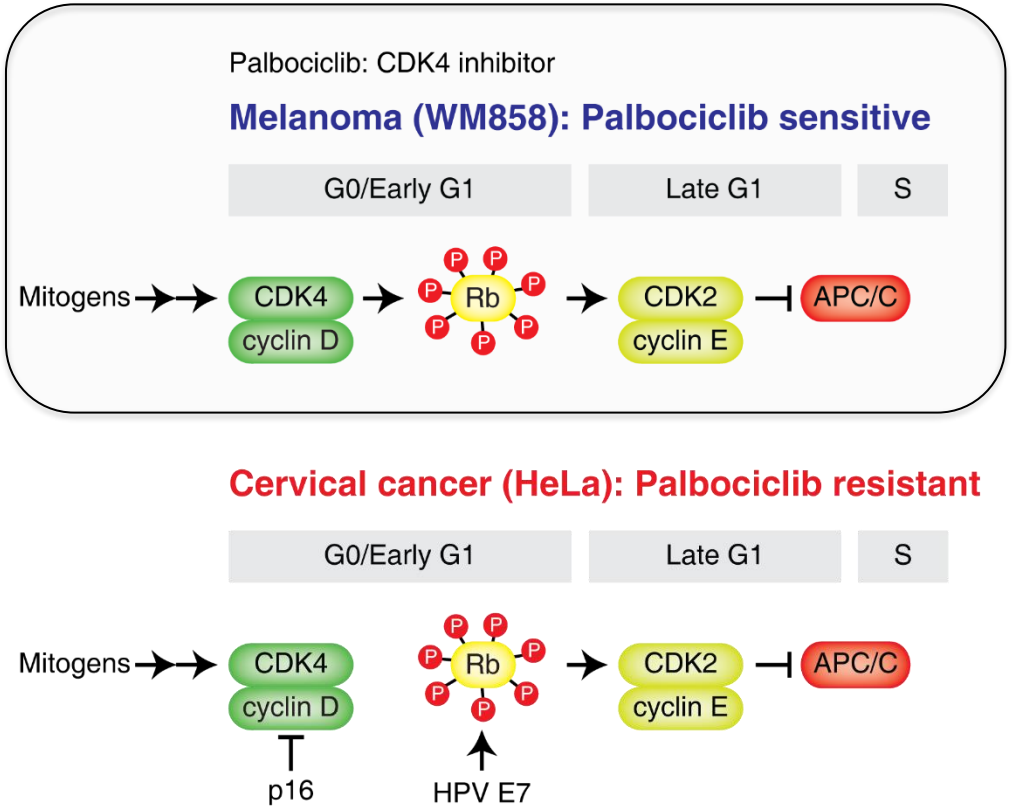
Indirect inactivation of Rb in tumors

- overexpression of cyclin D1
  - breast cancer, B cell lymphoma
- loss of p16, an inhibitor of Cdk4
  - many human cancers
- inherited point mutation in Cdk4 that renders it insensitive to inhibition by p16
  - familial melanoma

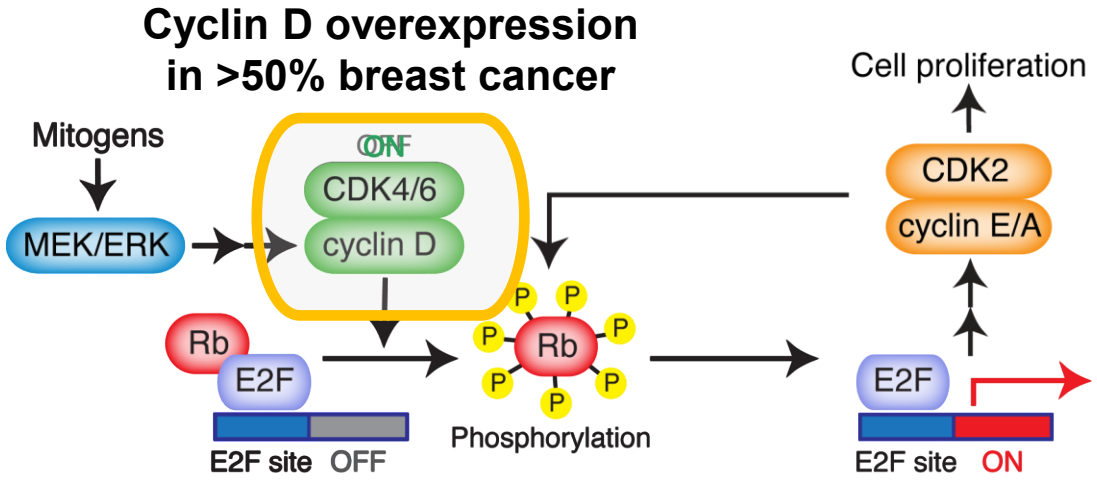
» Inactivation of the Rb pathway occurs in many human tumors!

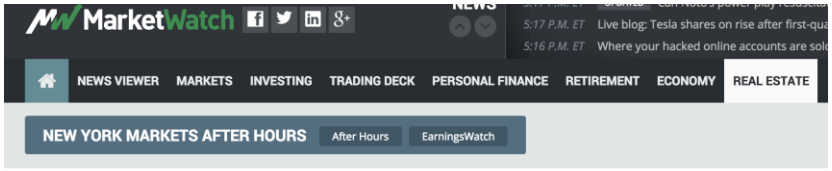
Rb-dependent and -independent cell-cycle entry





CDK4/6 is a promising target in breast cancer



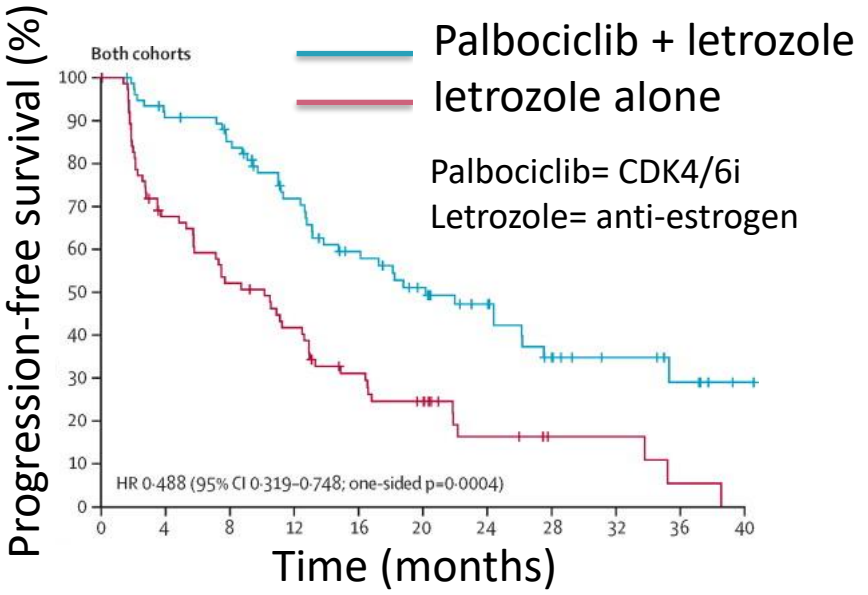


PRESS RELEASE

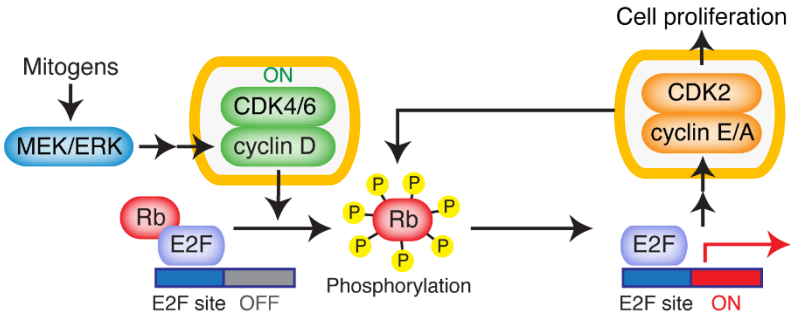
Pfizer Announces PALOMA-3 Trial For IBRANCE® (Palbociclib) Stopped Early Due To Efficacy Seen In Patients With HR+, HER2- Metastatic Breast Cancer Whose Disease Has Progressed Following Endocrine Therapy

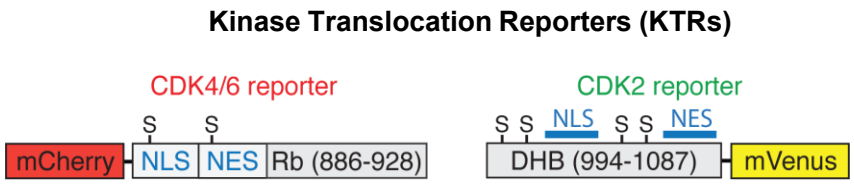
Published: Apr 15, 2015 8:00 a.m. ET

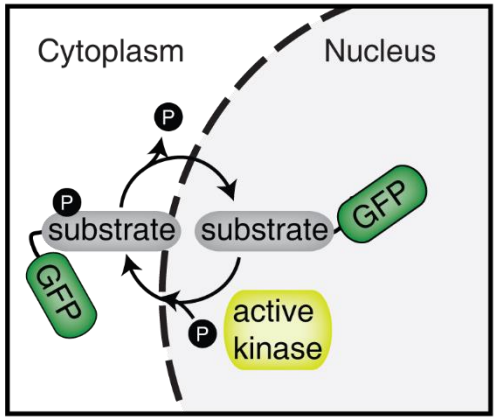
Finn et al., 2015

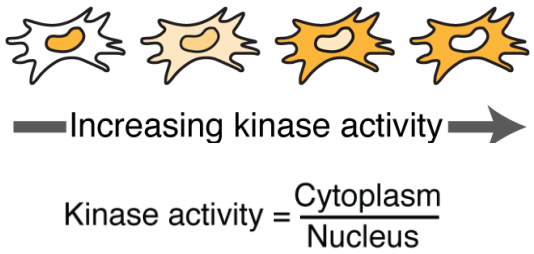


Live-cell sensor to monitor individual cell proliferation









Hahn et al., 2009 Regot et al., 2014

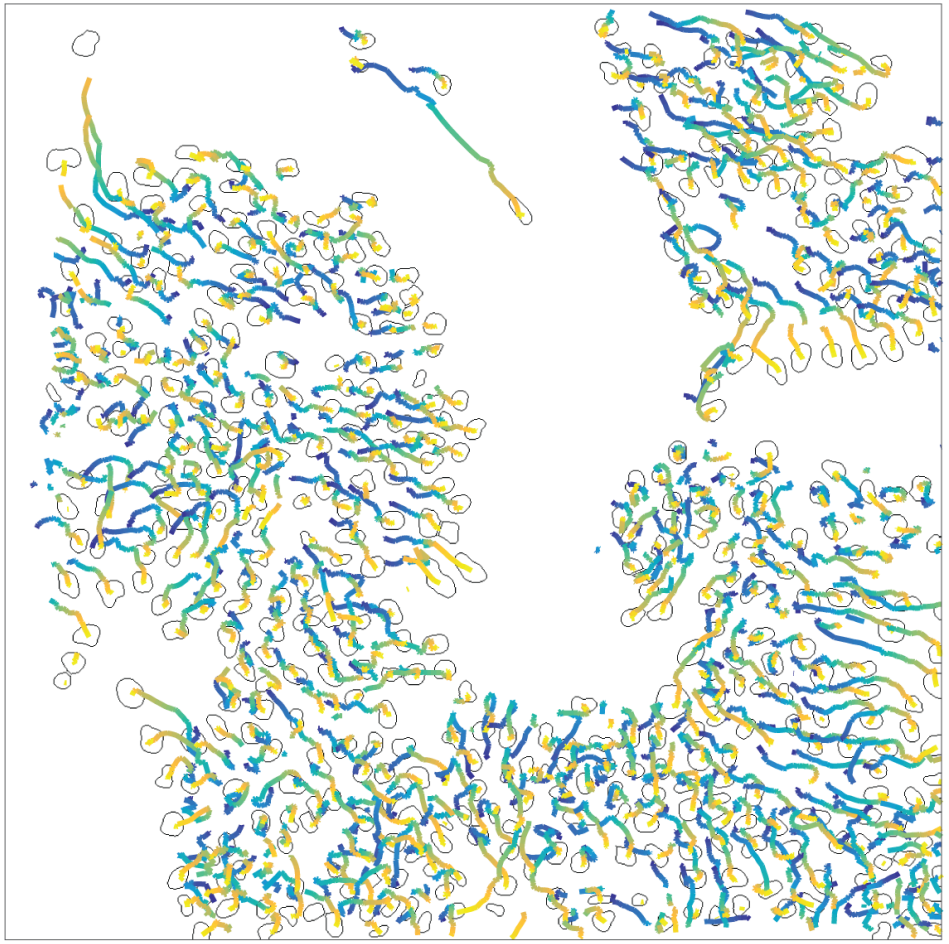
Spencer et al., 2013

Yang*, Cappell*, Jaimovich* et al., 2020

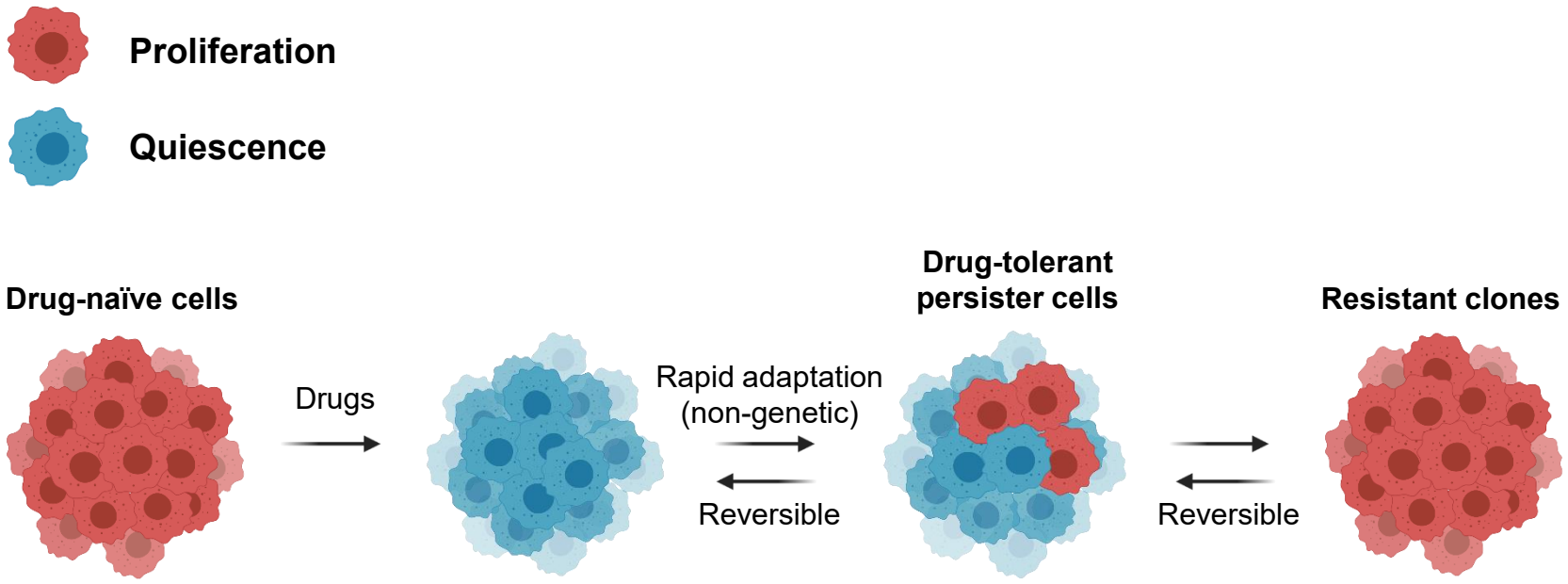
Individual cell tracking

Live cell imaging in
Highthroughput manner

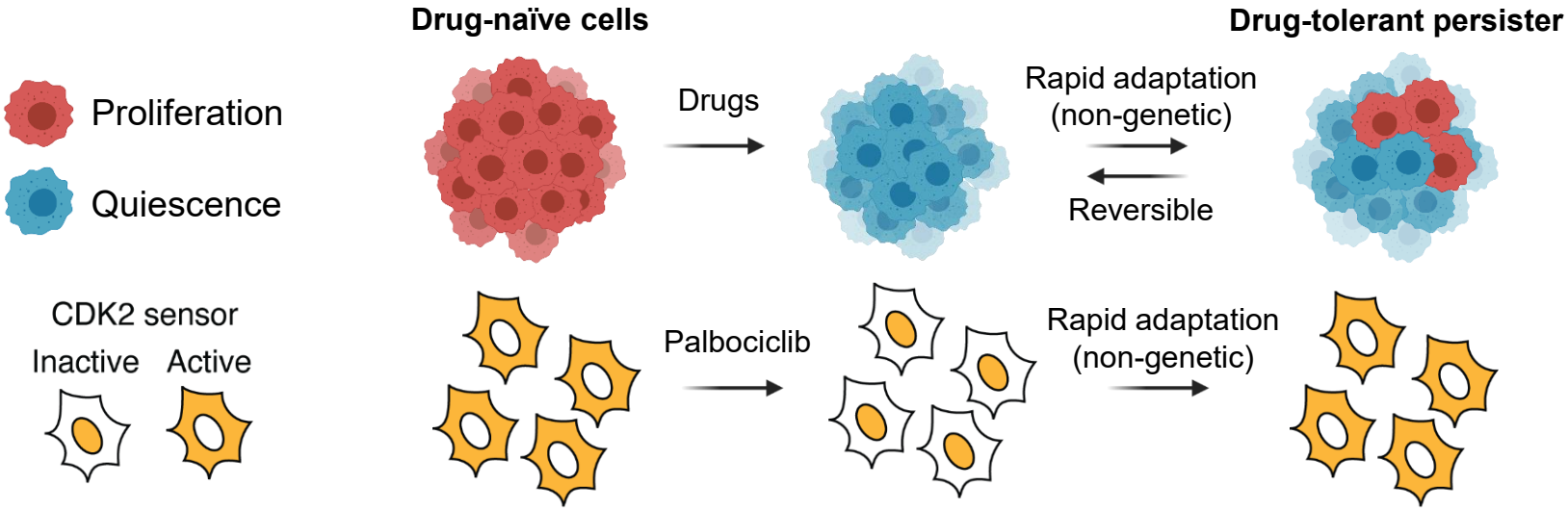
Cell tracking & Classification of cell behavior

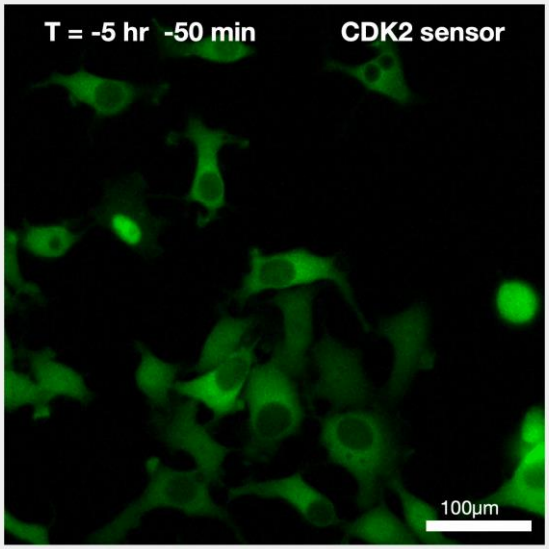


Drug-tolerant persister cells



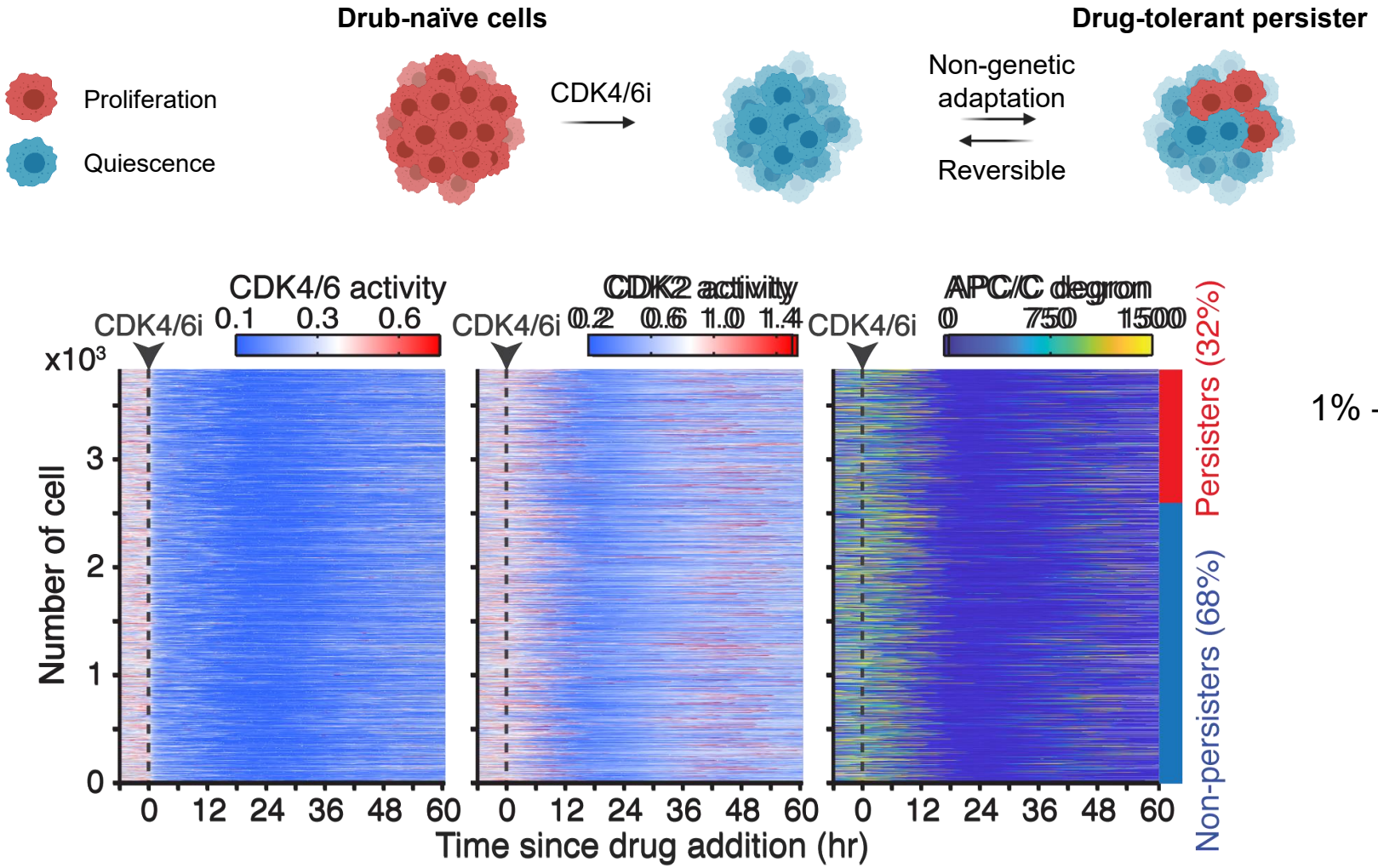
Drug-tolerant persister cells





HR^{+}/HER2^{-} MCF7 cells

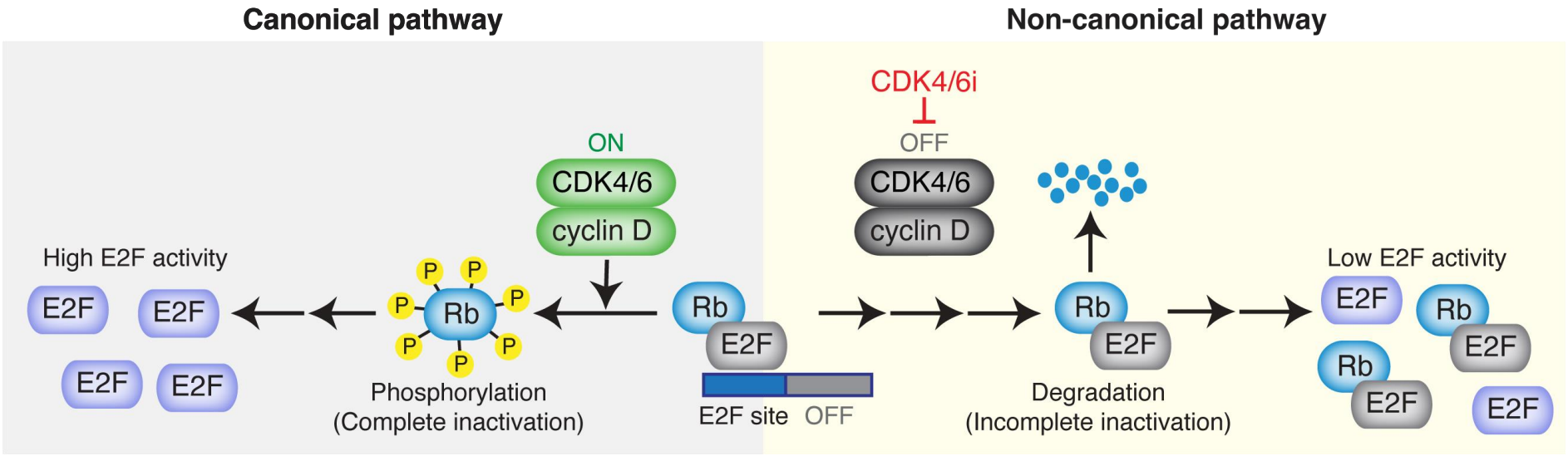
Drug-tolerant persister cells



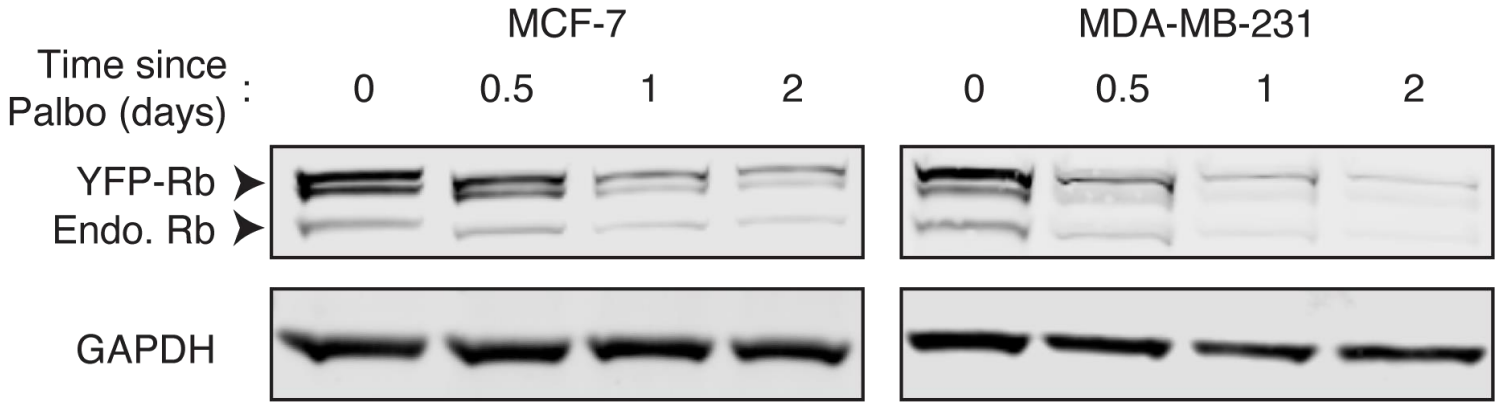
1% – 30%

Alternative pathway for Rb inactivation

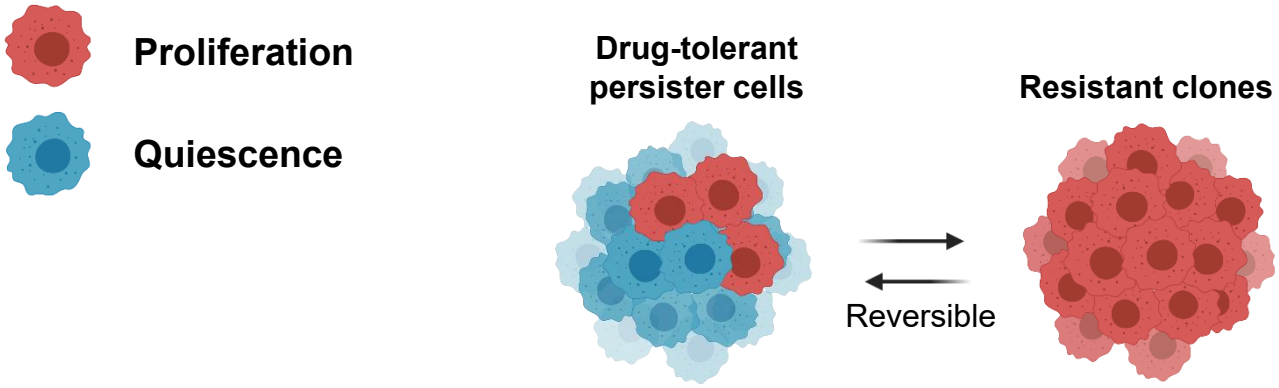
Kim et al. 2023; Zhang* and Kim* et al. 2023

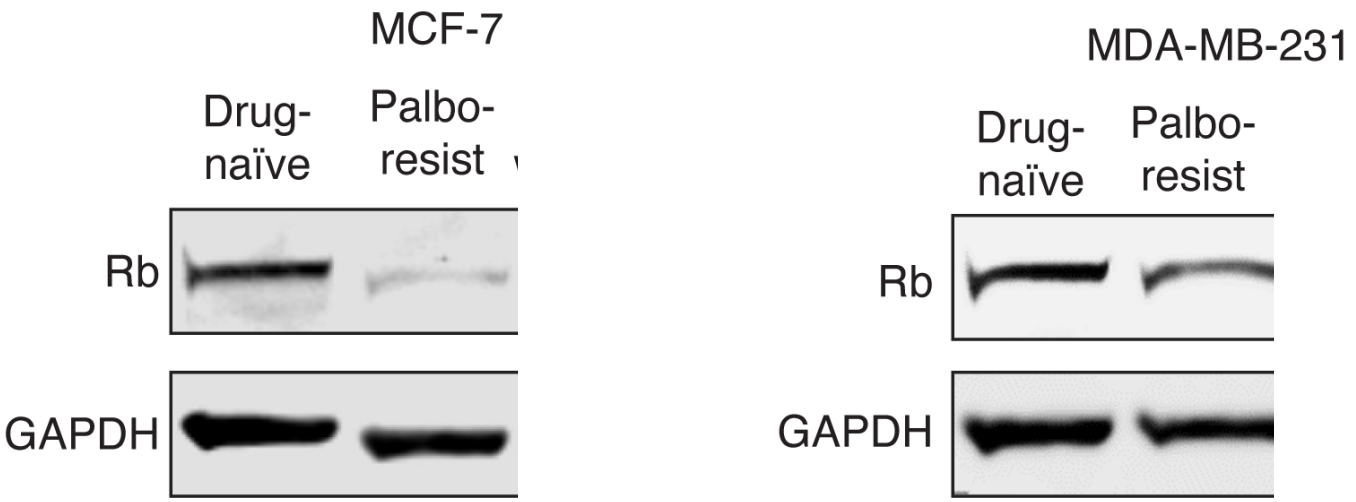


CDK4/6 inhibitor treatment results in Rb-protein reductions



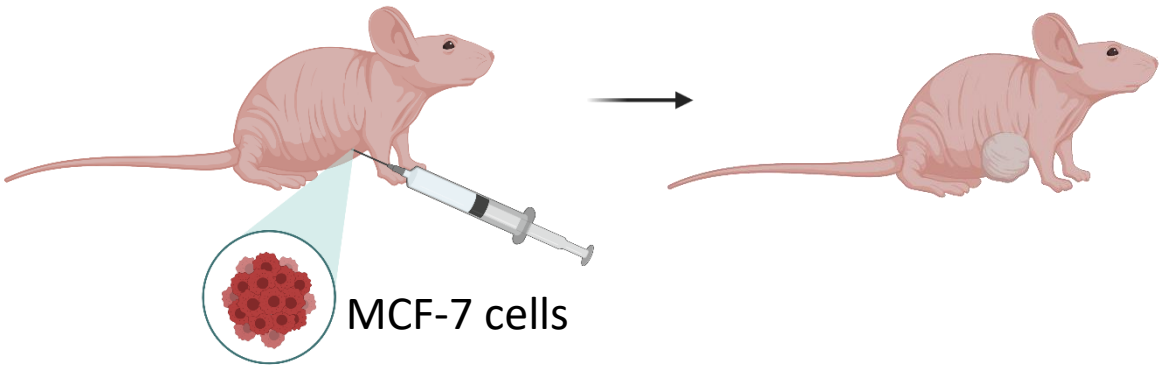
Rb-protein reductions are reversible in established CDK4/6i resistance

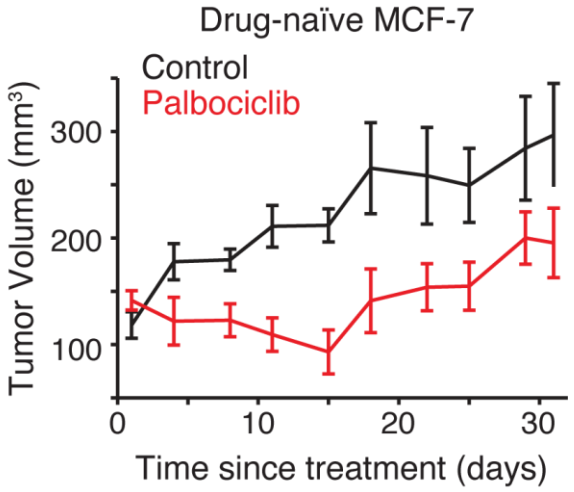


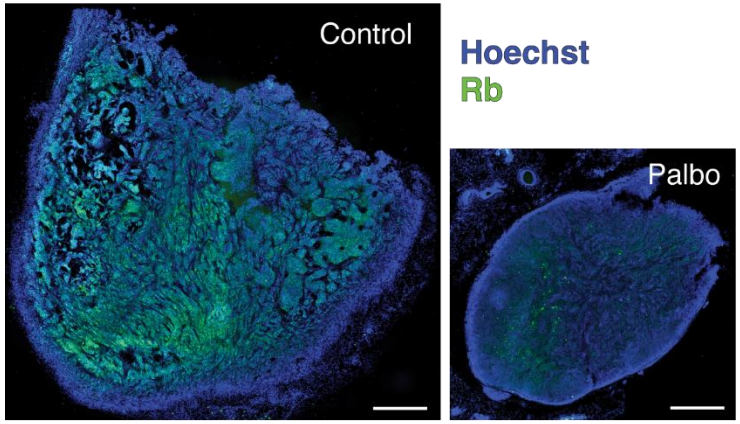


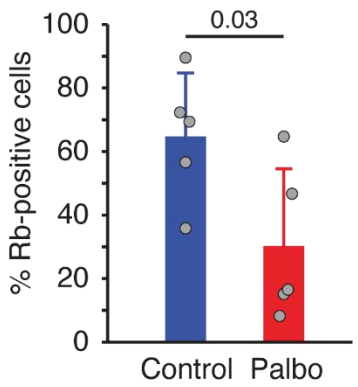
Reduced Rb protein levels in a breast cancer mouse model

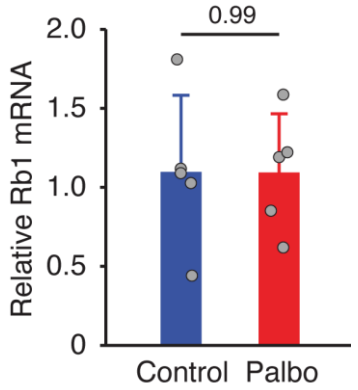
Minah Kim (CUIMC)



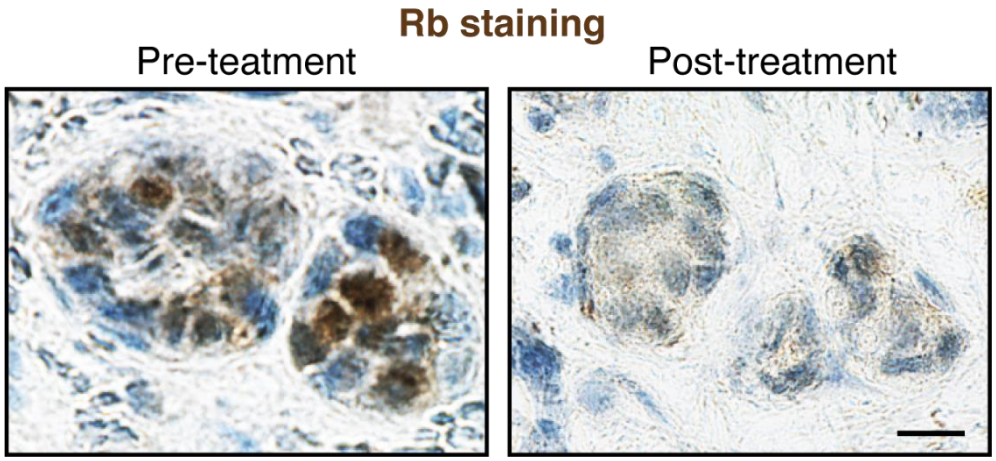


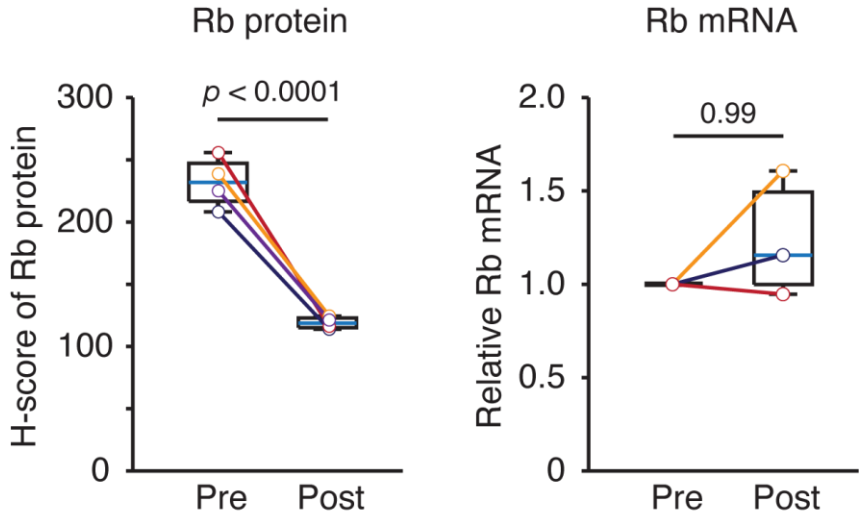




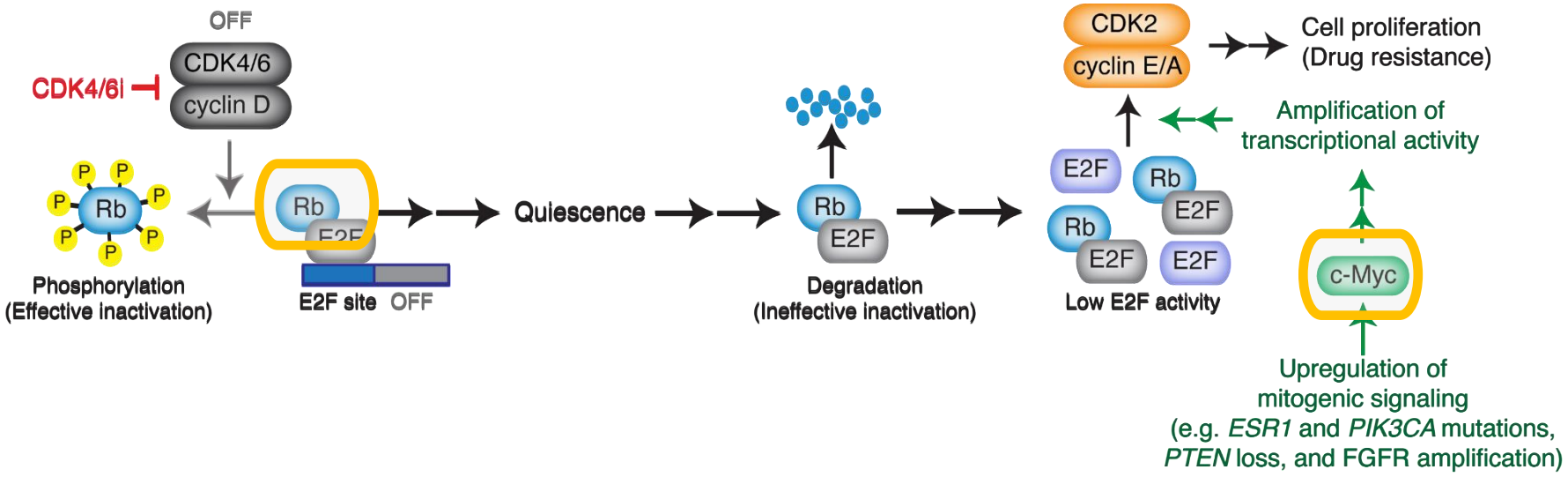


Rb-protein reduction in patient samples



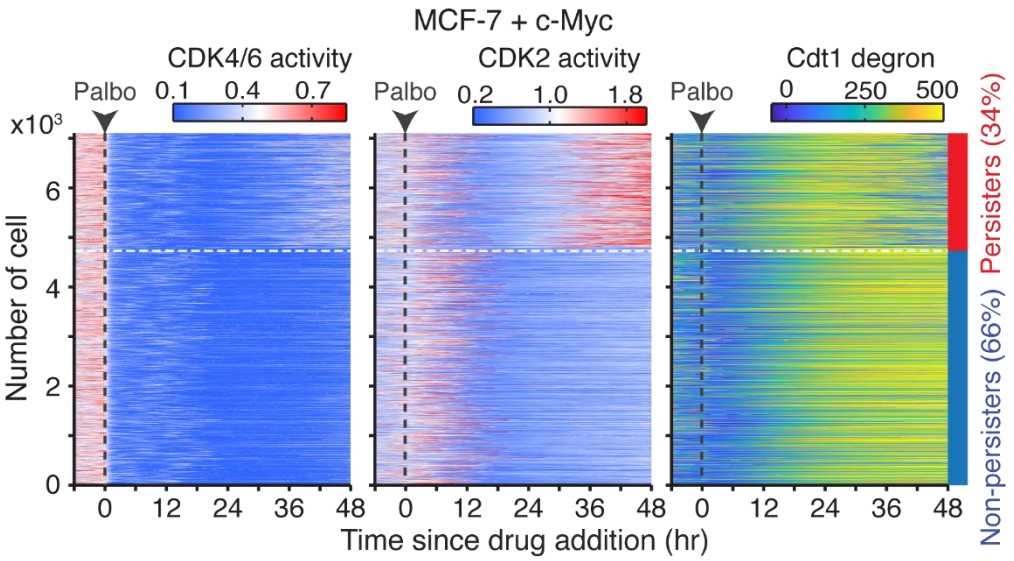


Sequential regulation of E2F activation

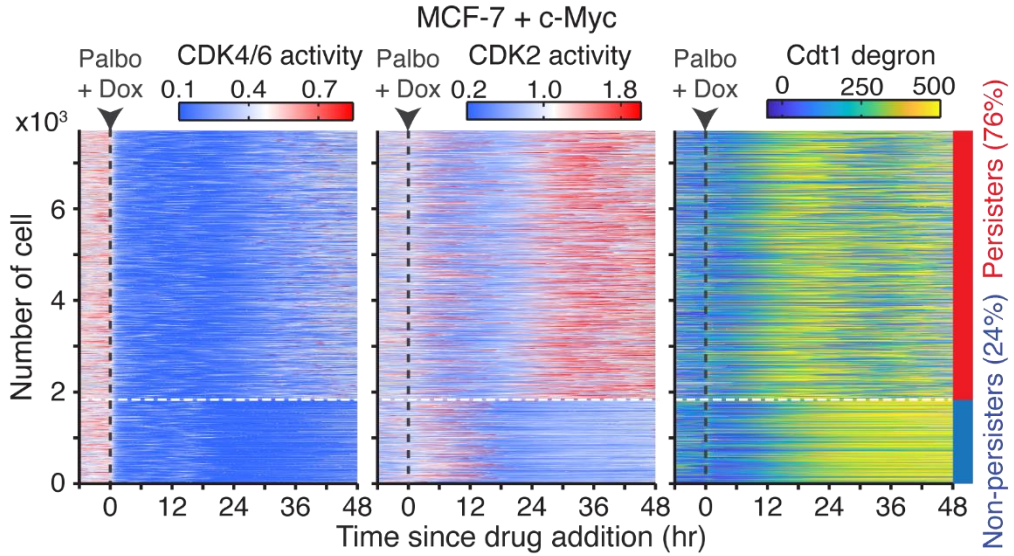


Induction of c-Myc expression facilitates CDK4/6i-tolerant persisters

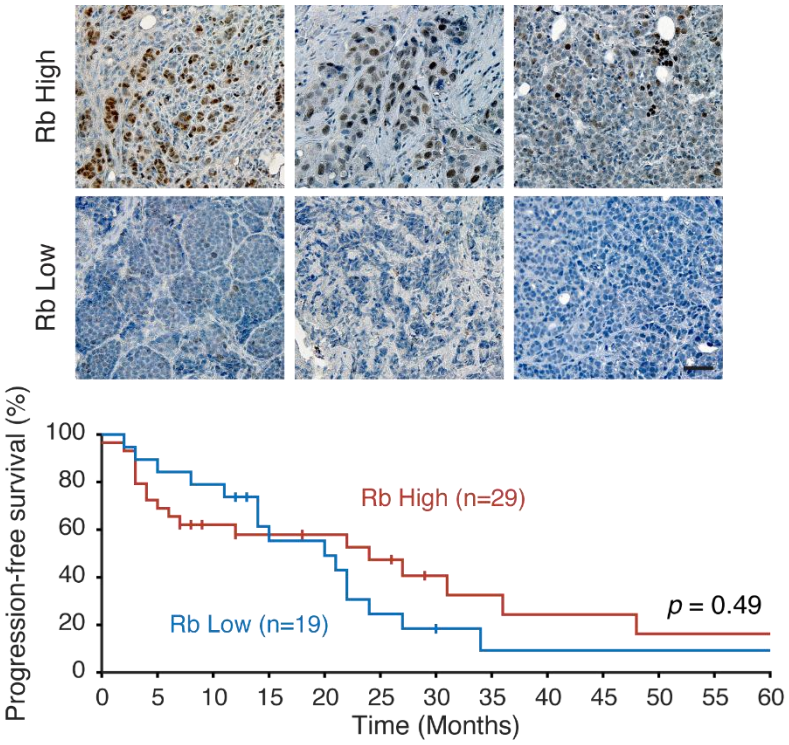
Control

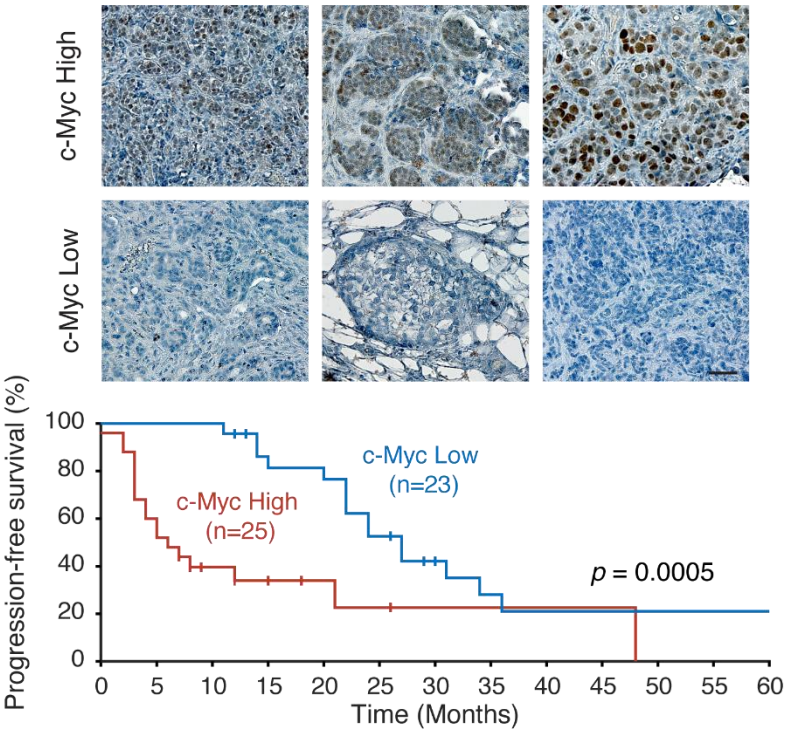


c-Myc induction

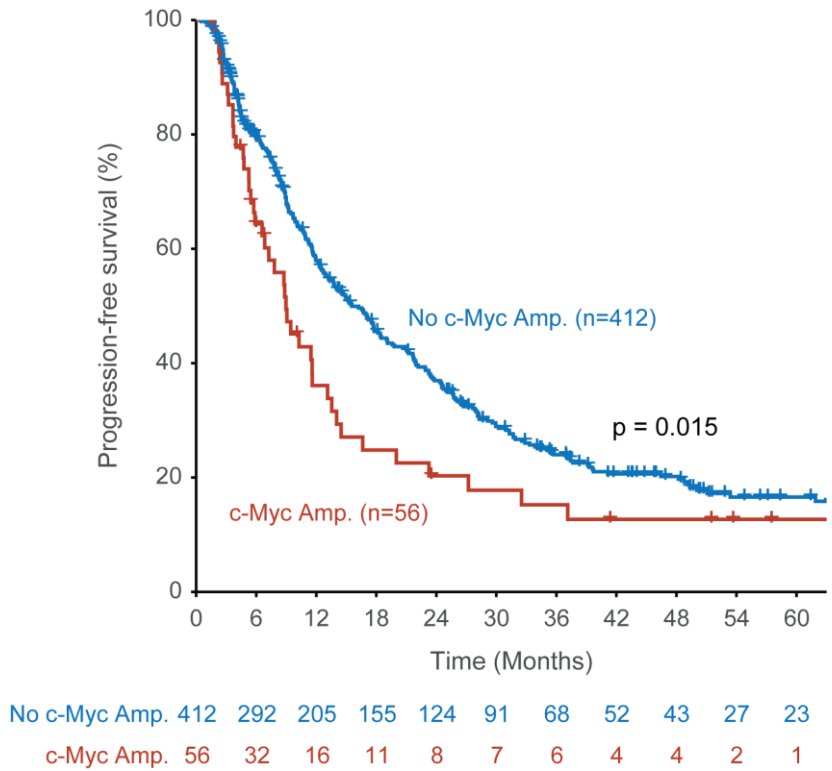


Rb and c-Myc levels in pre-treatment samples and PFS





c-Myc amplification status in pre-treatment samples and PFS

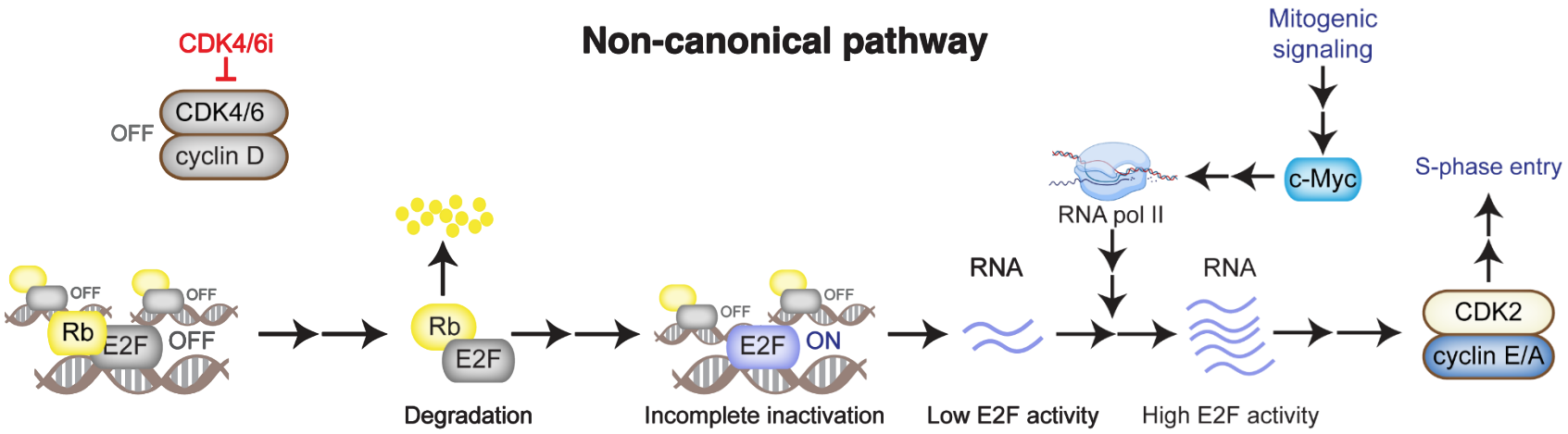


Adjusted by hormone therapy alone

Sarat Chandarlapaty (MSKCC)
Pedram Razavi (MSKCC)
Anton Safonov (MSKCC)

Basic cell-cycle regulation

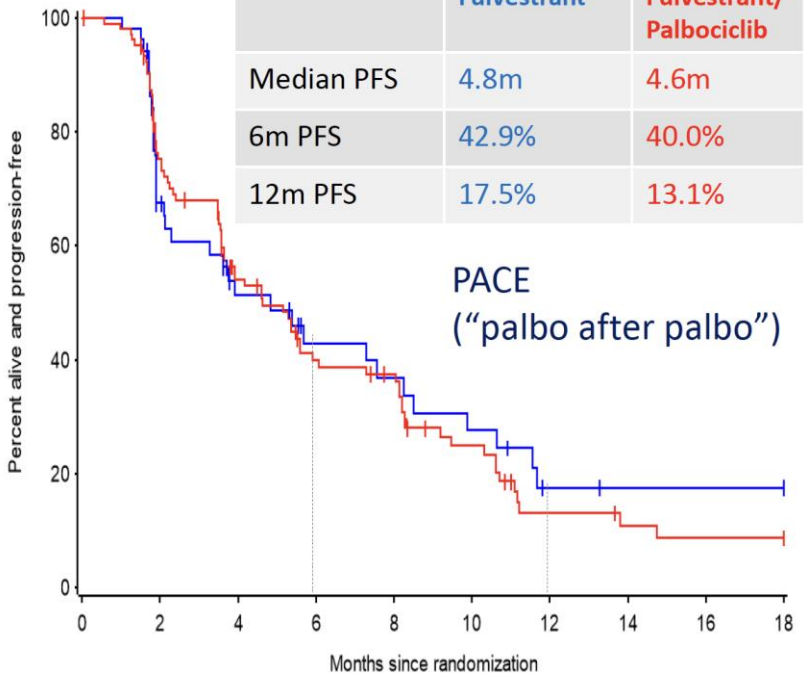
Kim et al. 2023; Zhang et al. 2023;



Continuing CDK4/6i beyond progression?

PACE and MAINTAIN clinical trials

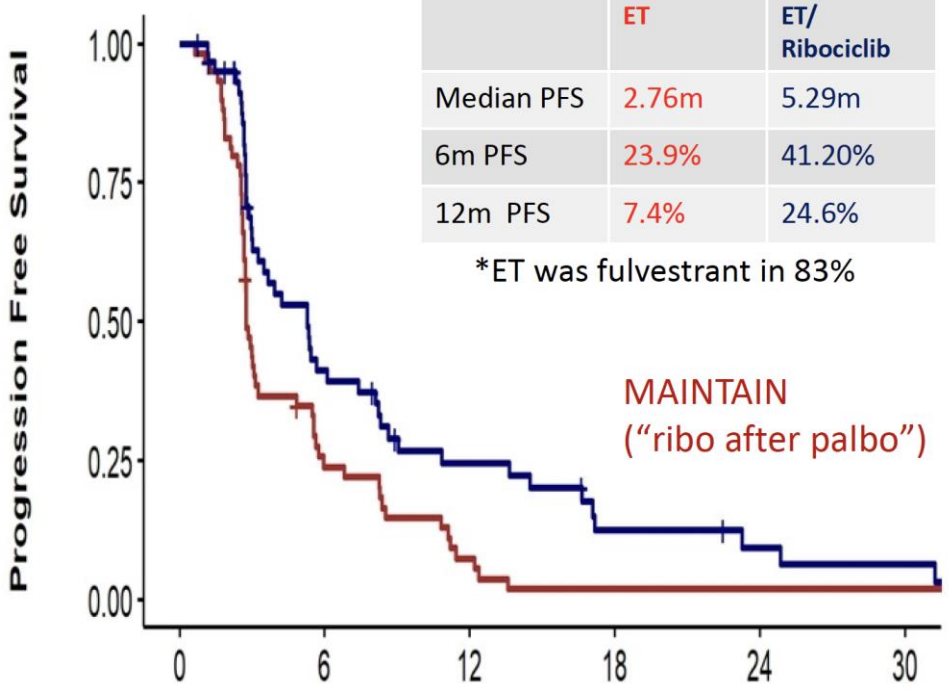
	Fulvestrant	Fulvestrant/ Palbociclib
Median PFS	4.8m	4.6m
6m PFS	42.9%	40.0%
12m PFS	17.5%	13.1%



PACE
("palbo after palbo")

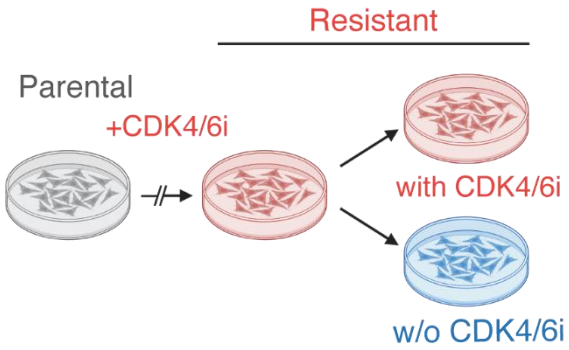
	ET	ET/ Ribociclib
Median PFS	2.76m	5.29m
6m PFS	23.9%	41.20%
12m PFS	7.4%	24.6%

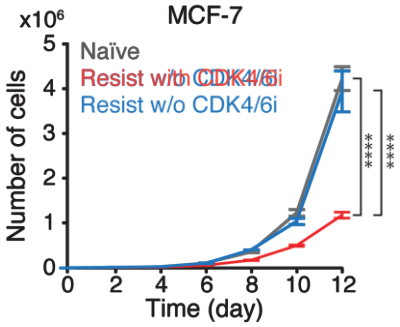
*ET was fulvestrant in 83%

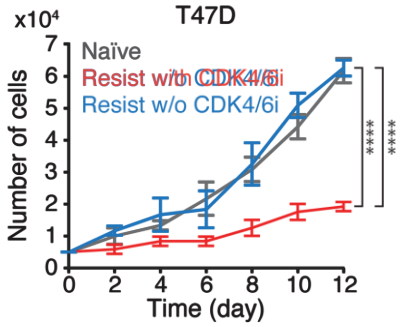


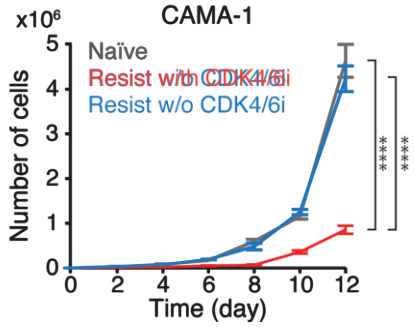
MAINTAIN
("ribo after palbo")

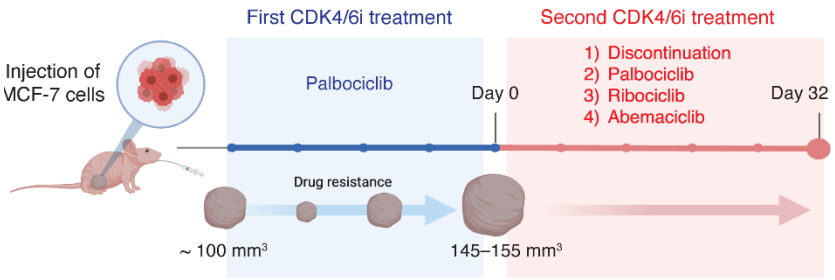
The benefits of CDK4/6i maintenance beyond disease progression

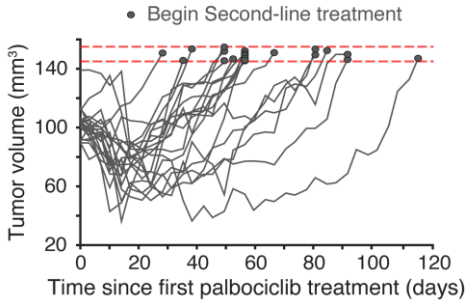


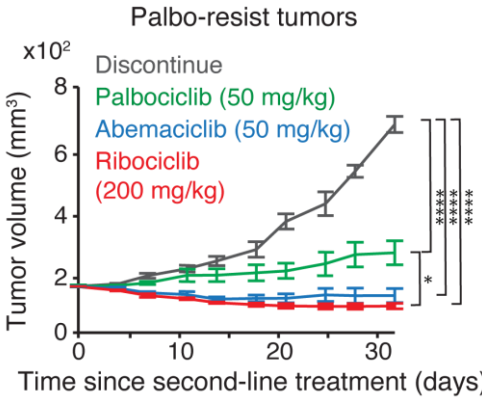




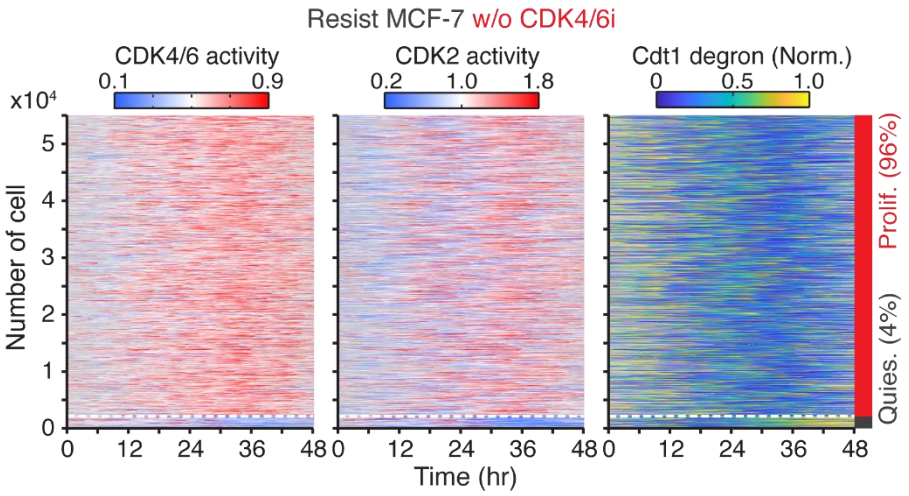


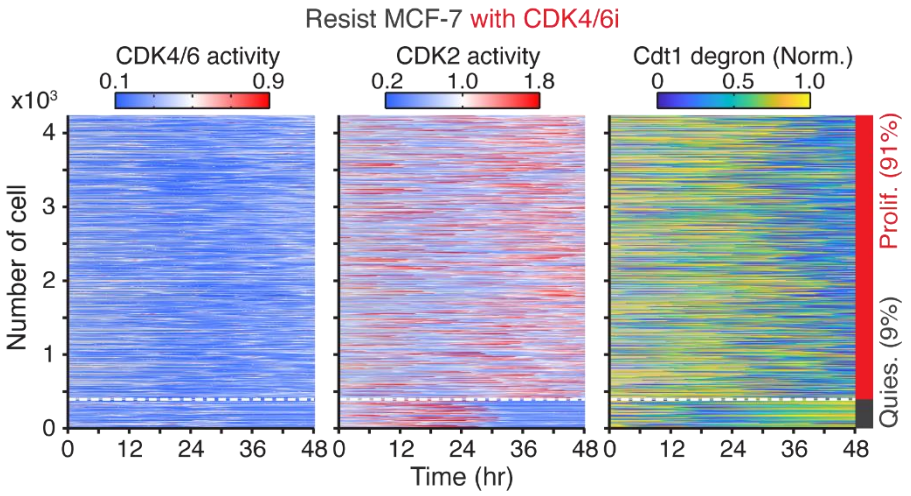


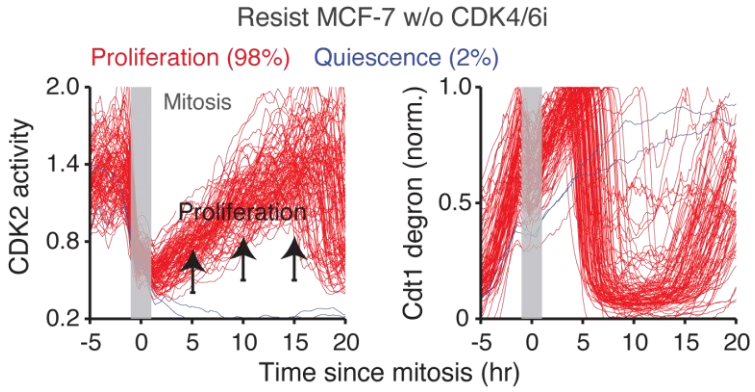


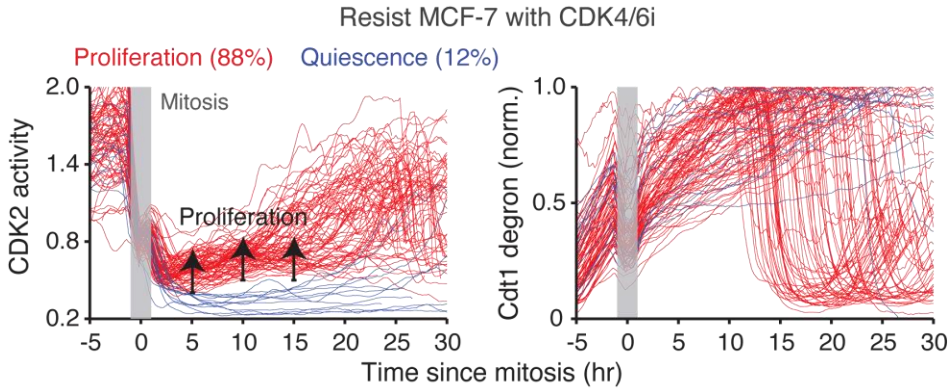


The benefits of CDK4/6i maintenance beyond disease progression

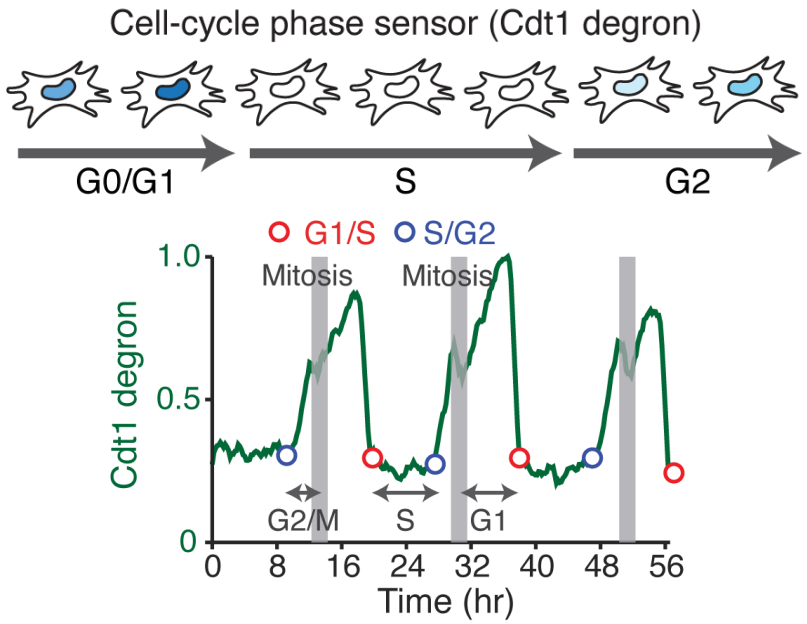


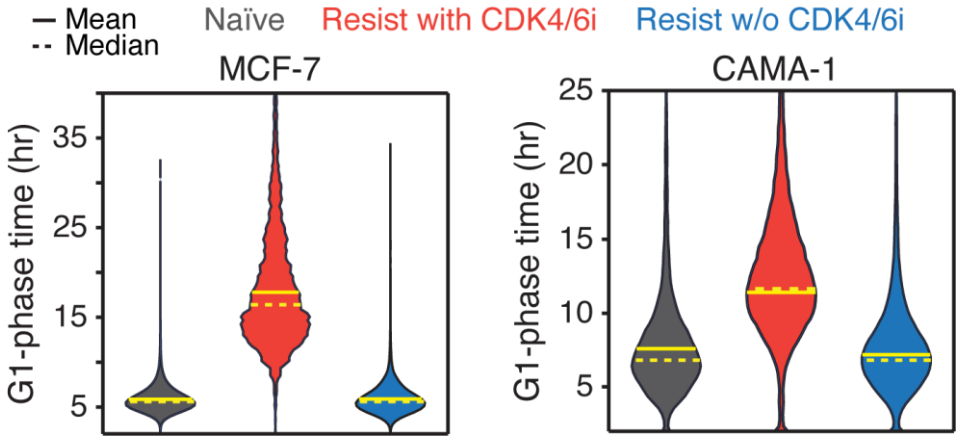


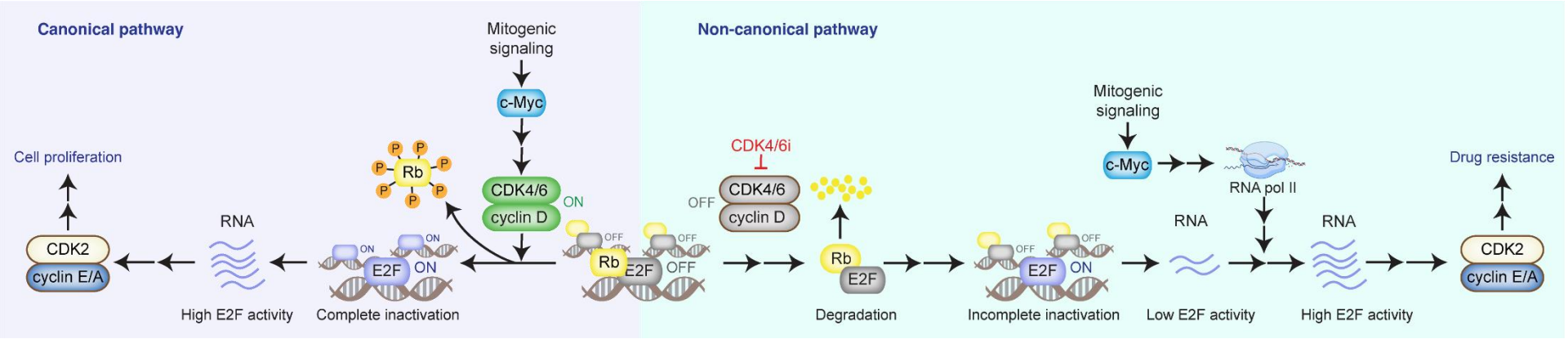




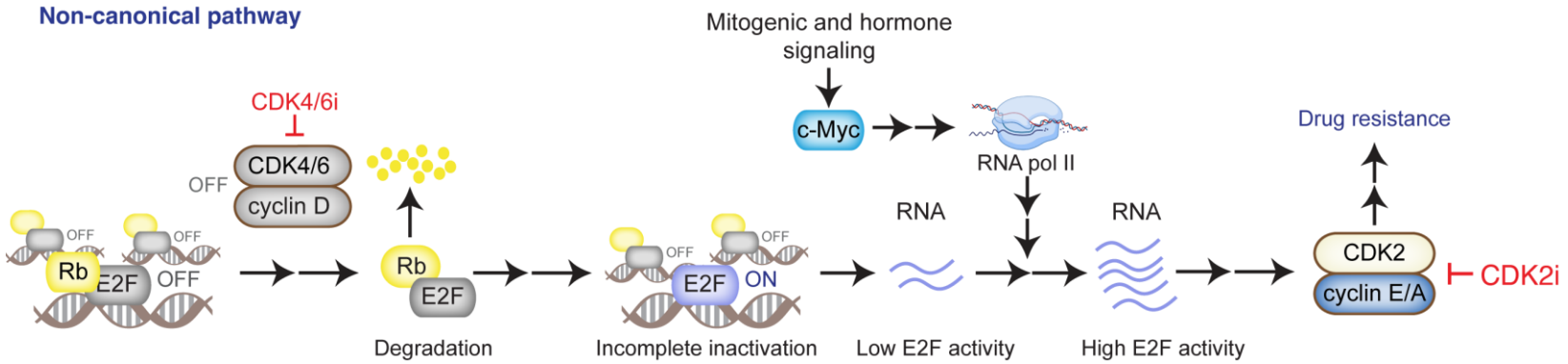
The benefits of CDK4/6i maintenance beyond disease progression

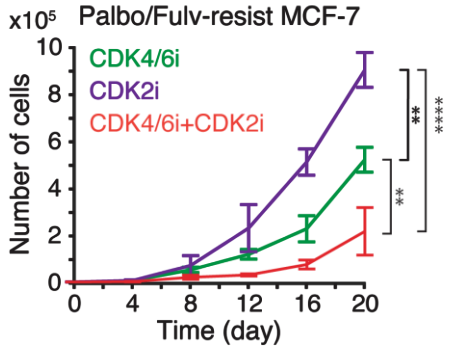


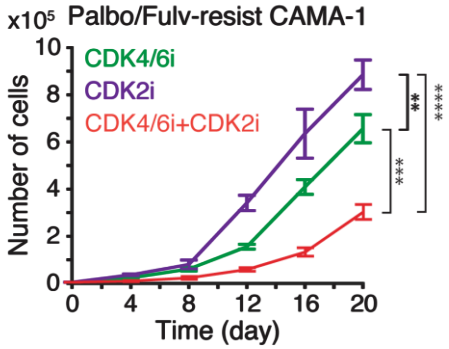


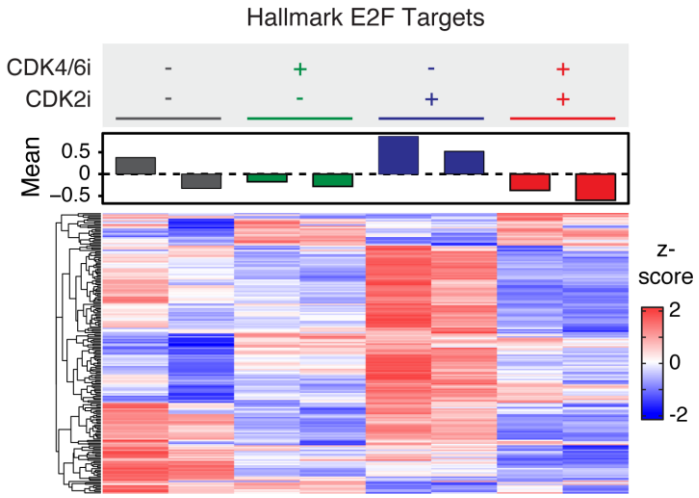


The benefits of CDK4/6i maintenance beyond disease progression









The role of CDK4/6 beyond the restriction point

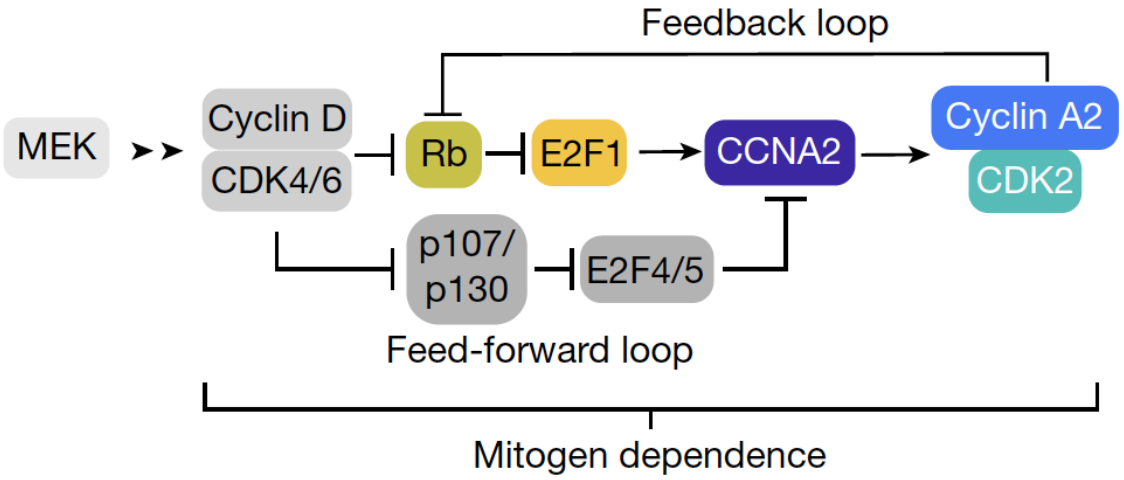
Article

Loss of CDK4/6 activity in S/G2 phase leads to cell cycle reversal

https://doi.org/10.1038/s41586-023-06274-3
Received: 9 September 2022

James A. Cornwell[1], Adrijana Crncec[1], Marwa M. Afifi[1], Kristina Tang[1], Ruhul Amin[1] & Steven D. Cappell[1]✉

Cornwell et al. 2023



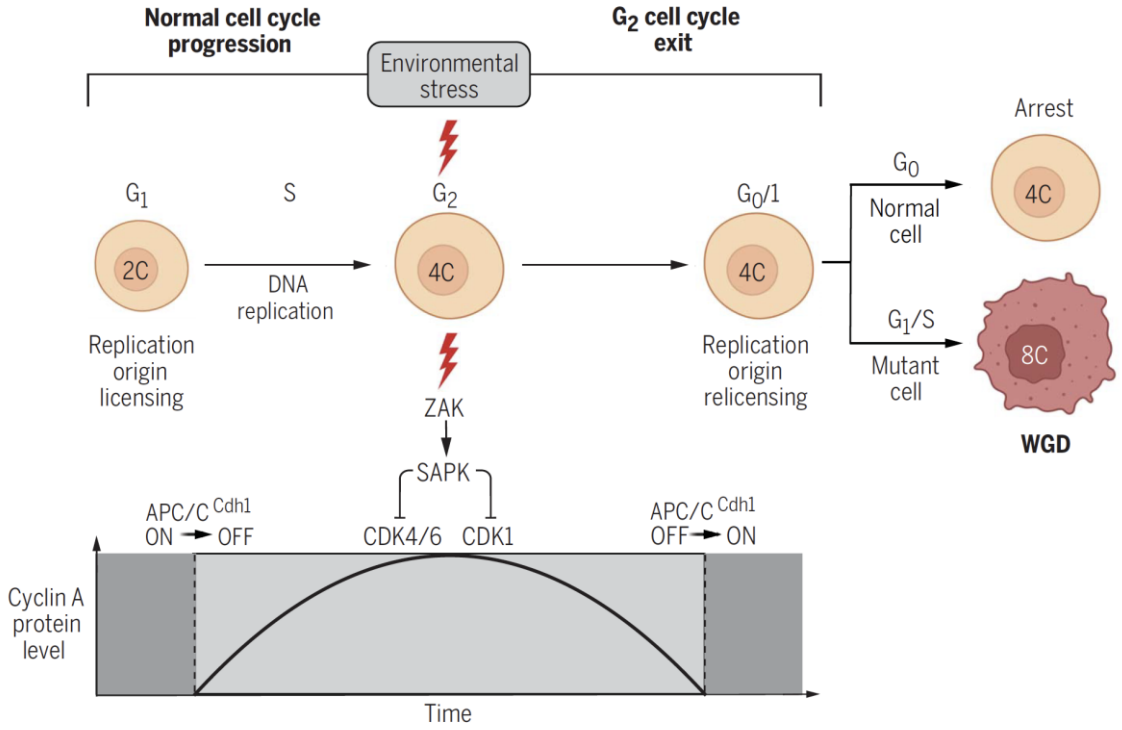
The role of CDK4/6 beyond the restriction point

CELL CYCLE

CDK4/6 activity is required during G_2 arrest to prevent stress-induced endoreplication

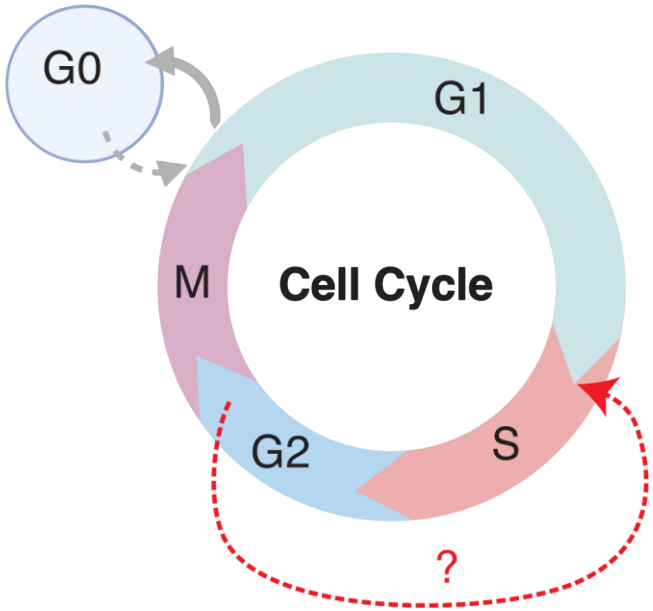
Connor McKenney, Yovel Lendner, Adler Guerrero Zuniga, Niladri Sinha, Benjamin Veresko, Timothy J. Aikin, Sergi Regot*

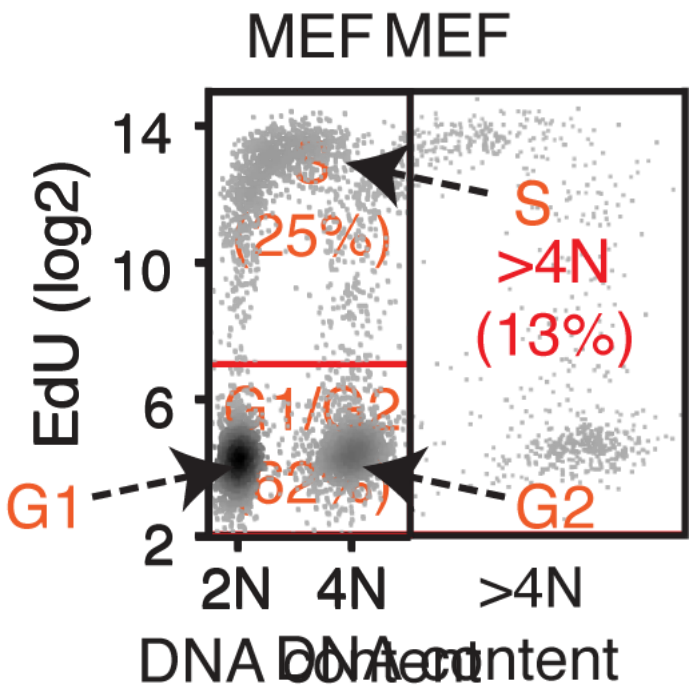
McKenney et al. 2024



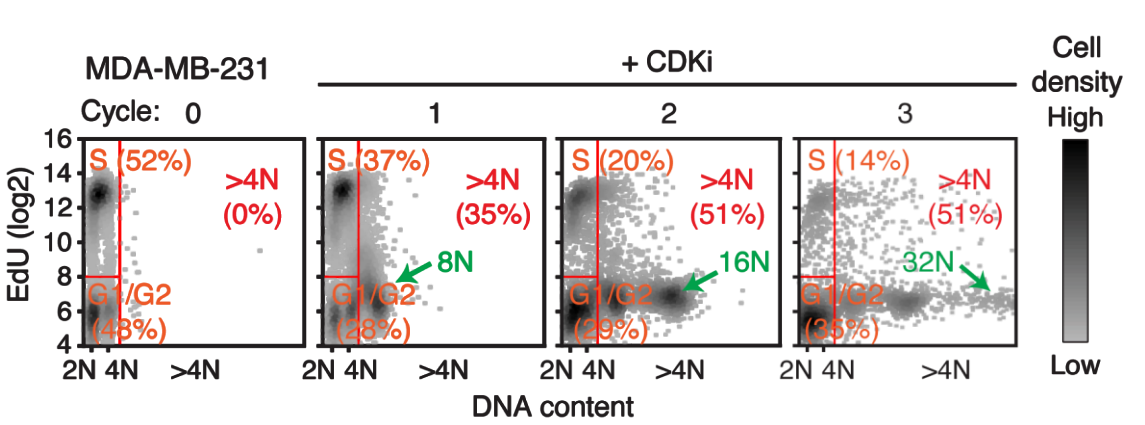
Whole-genome duplication

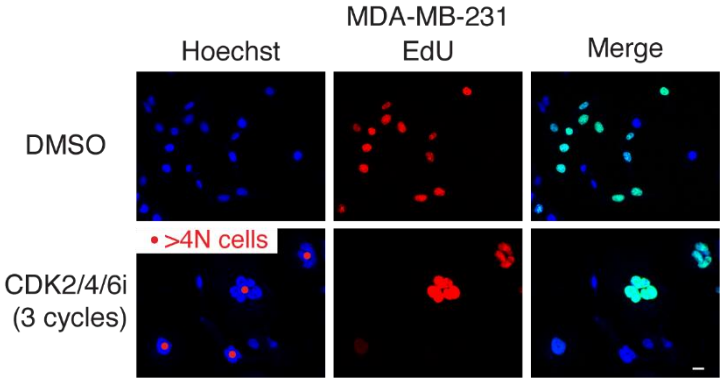
Kim et al. 2025

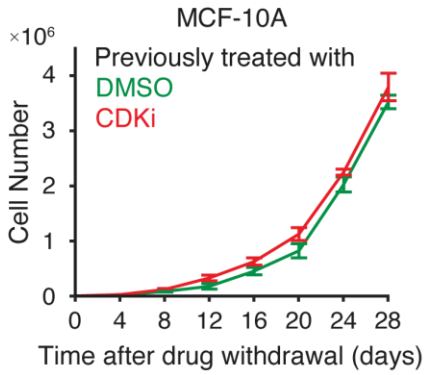


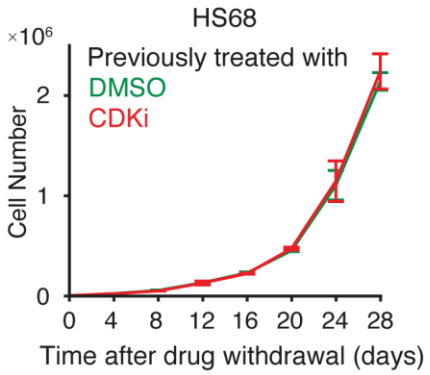


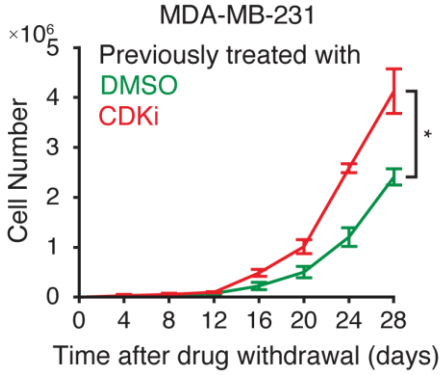
Consequences of WGD

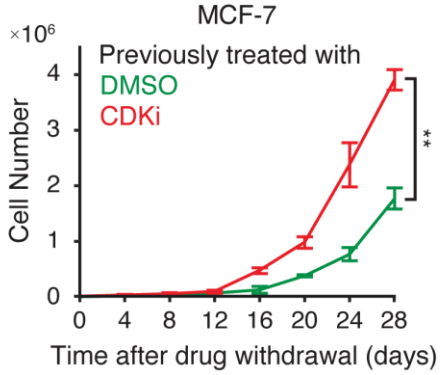




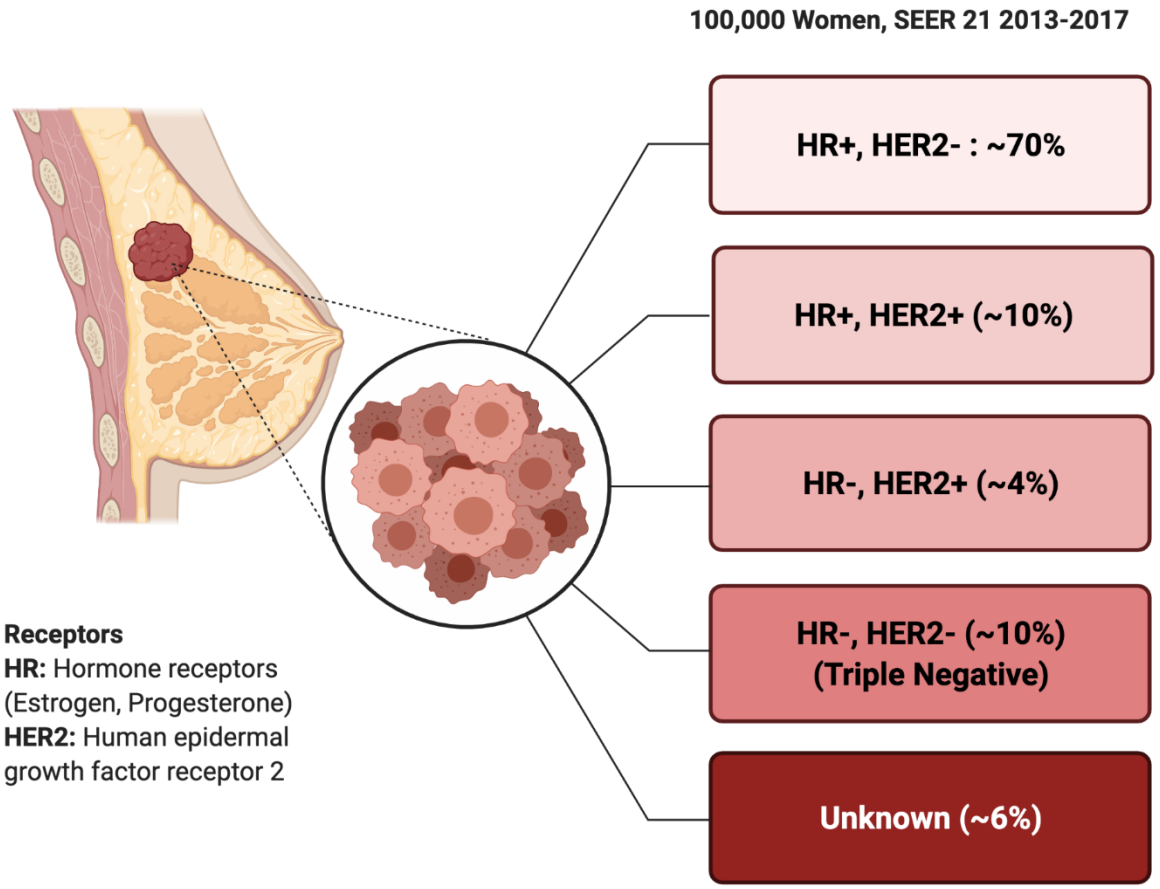




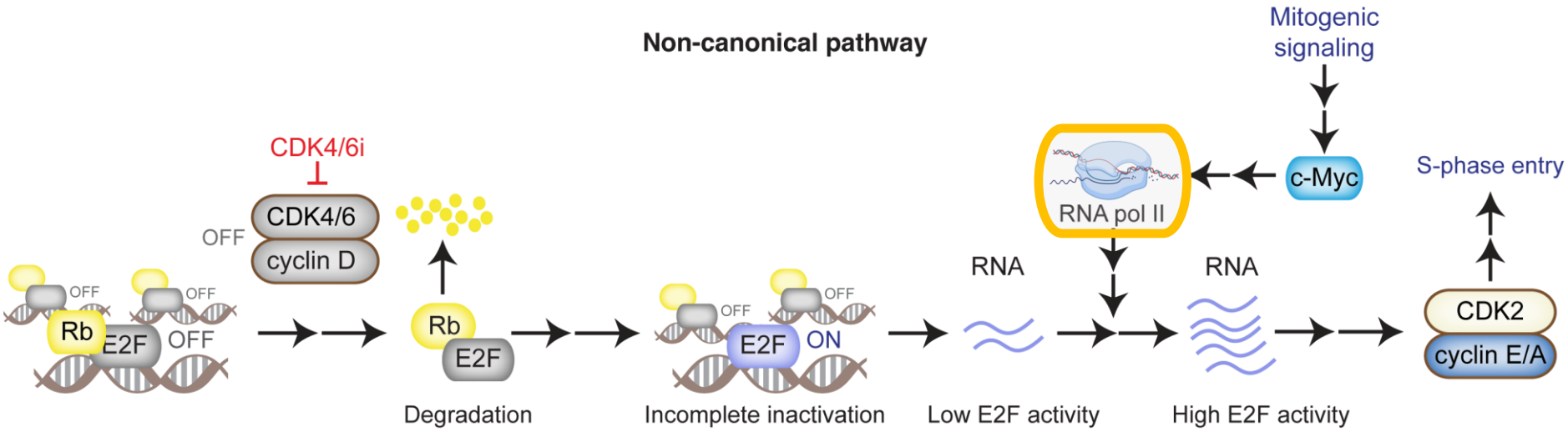




Targeted therapeutic strategies for TNBC

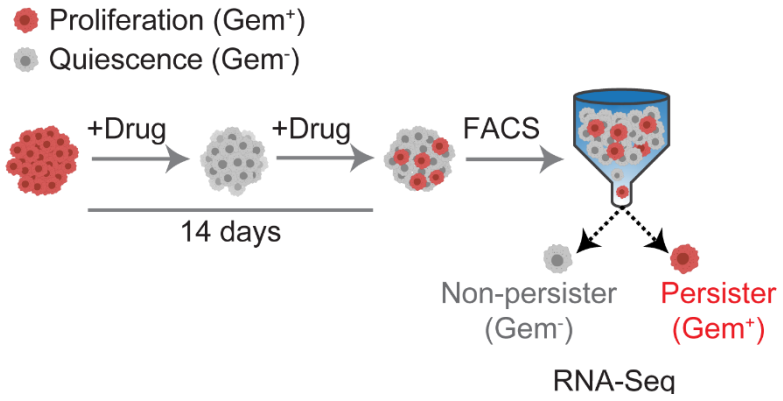


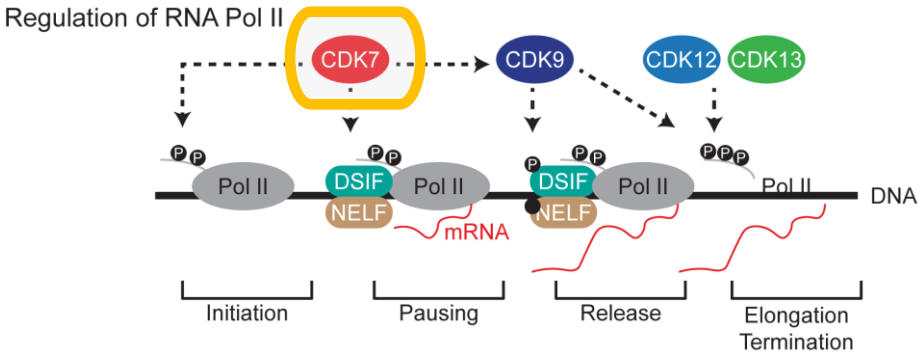
Targeting RNA polymerase II activity

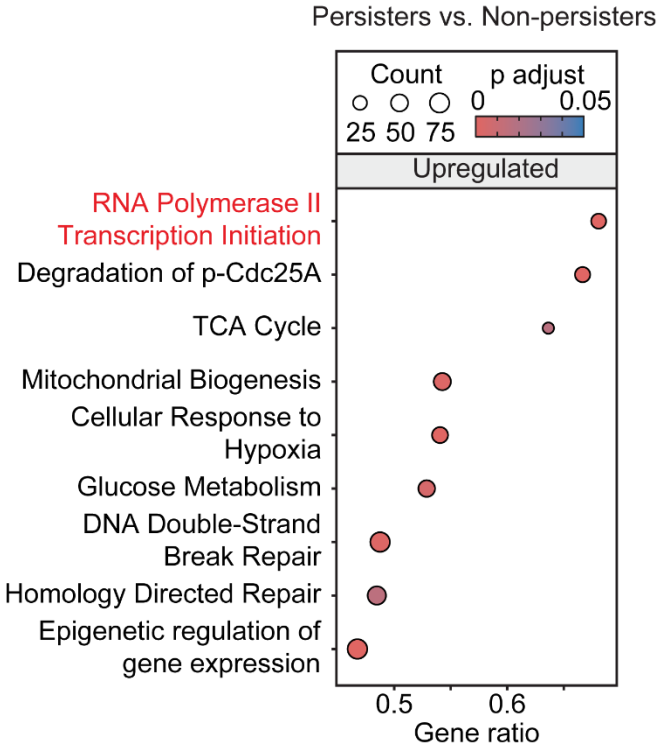


Lin et al., 2012; Nie et al., 2012; Rahl et al., 2010;

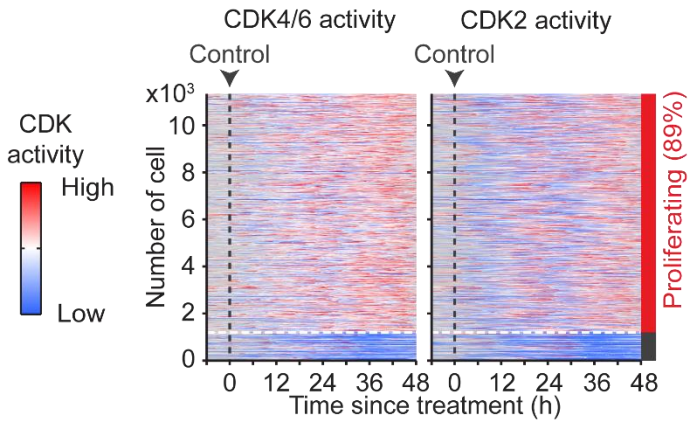
Targeting RNA polymerase II activity by CDK7 inhibitors

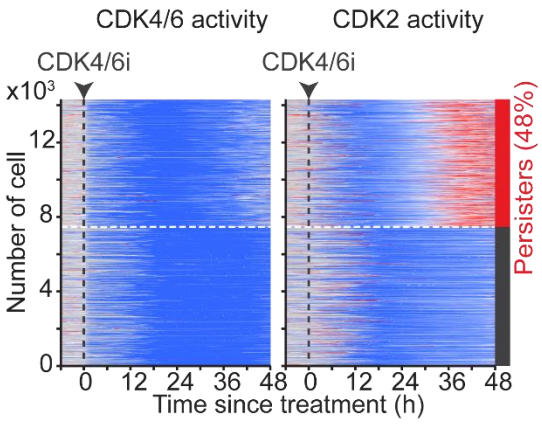


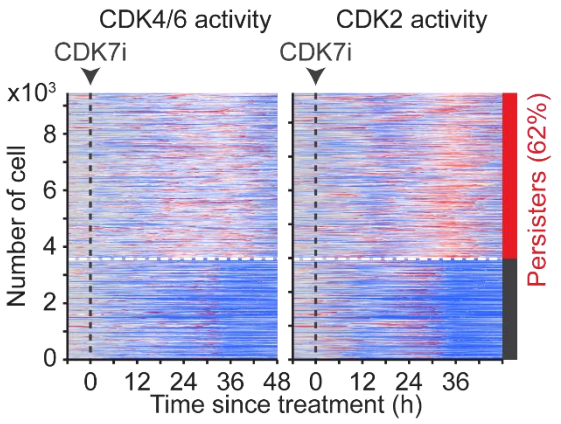


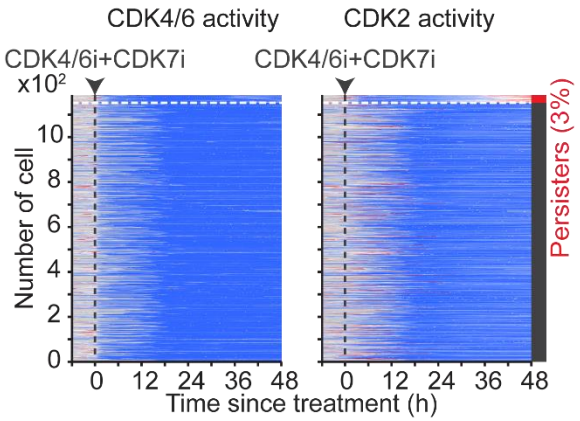


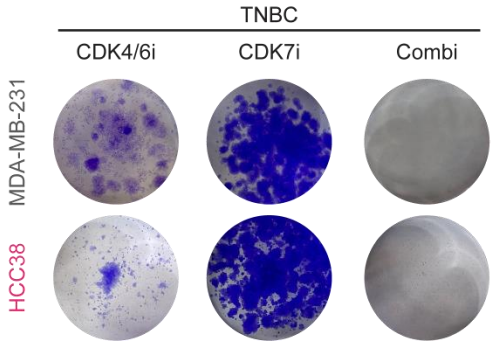
Dual targeting CDK4/6 and CDK7 activity (TNBC cell lines)

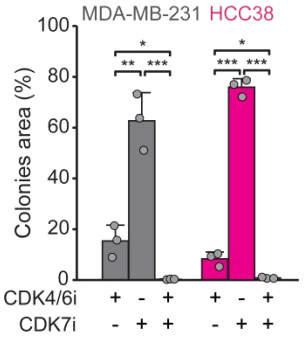




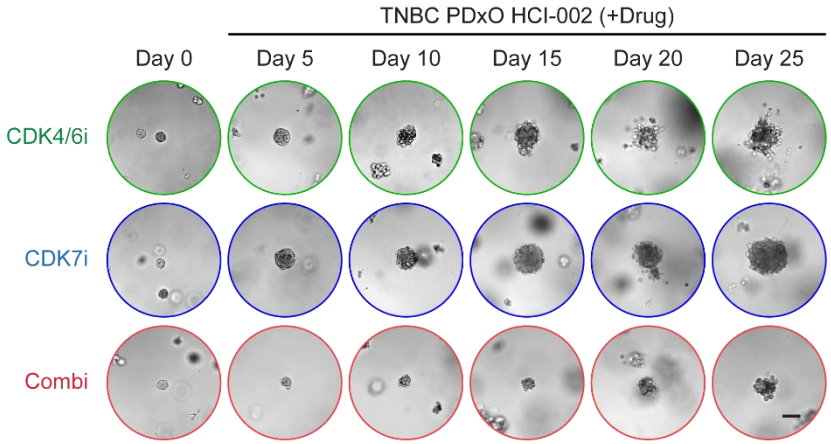


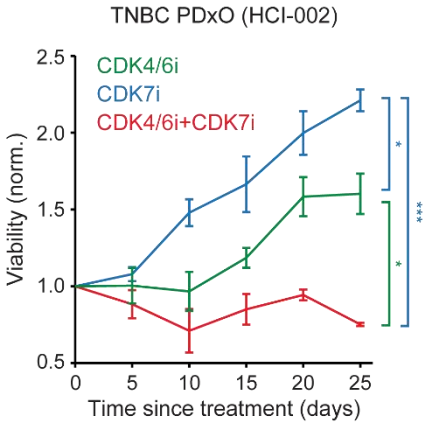


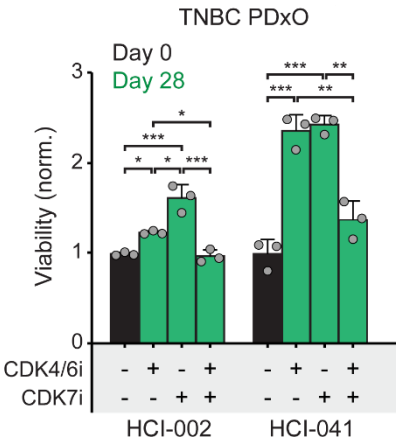




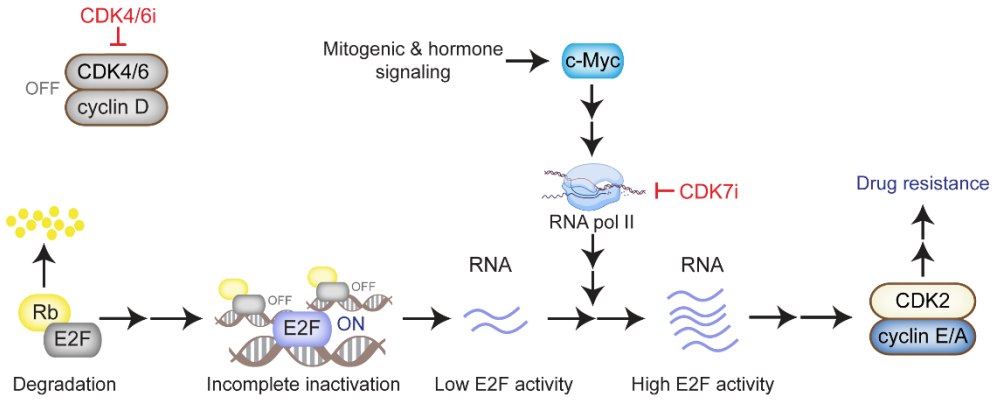
Dual targeting CDK4/6 and CDK7 activity (patient-derived organoids)

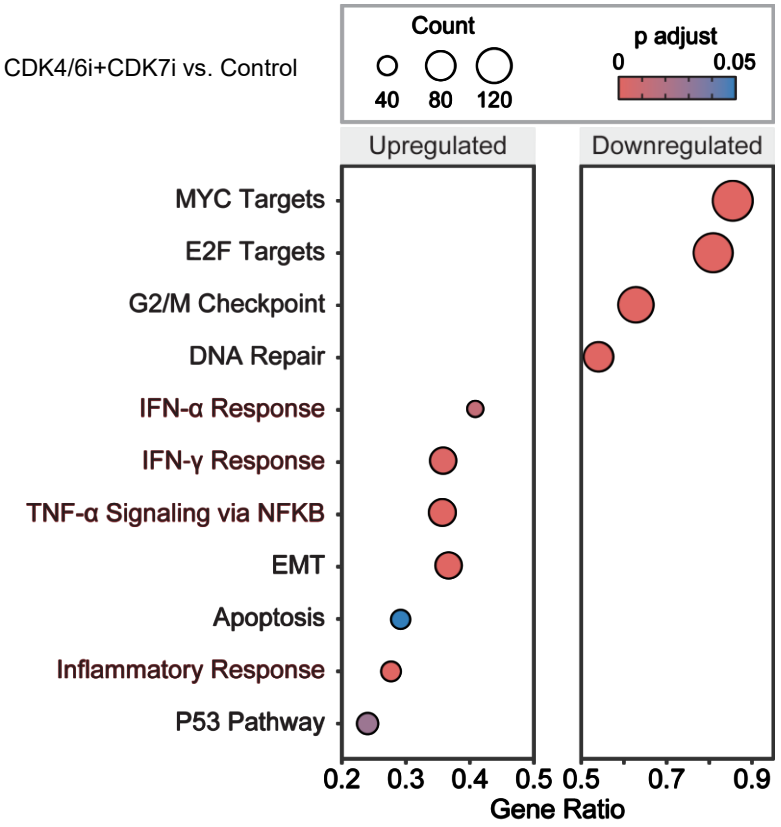






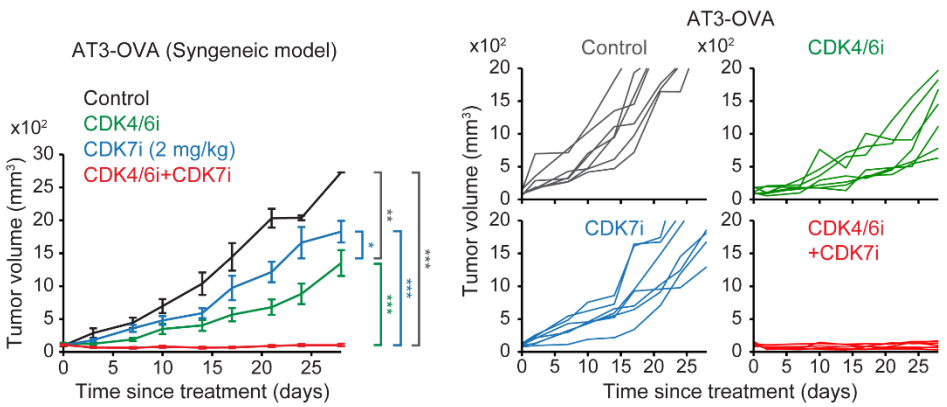
Upregulation of immune-related pathways

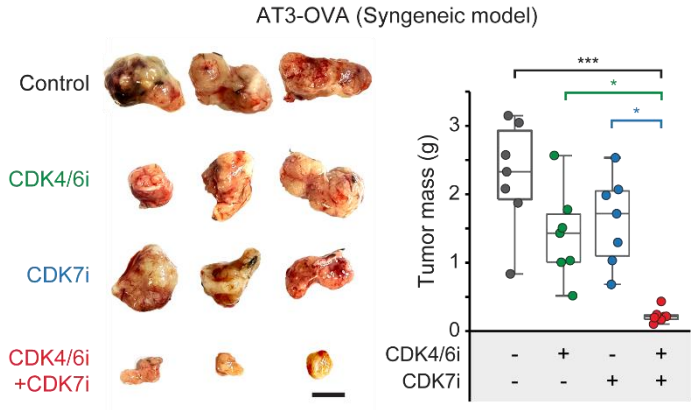


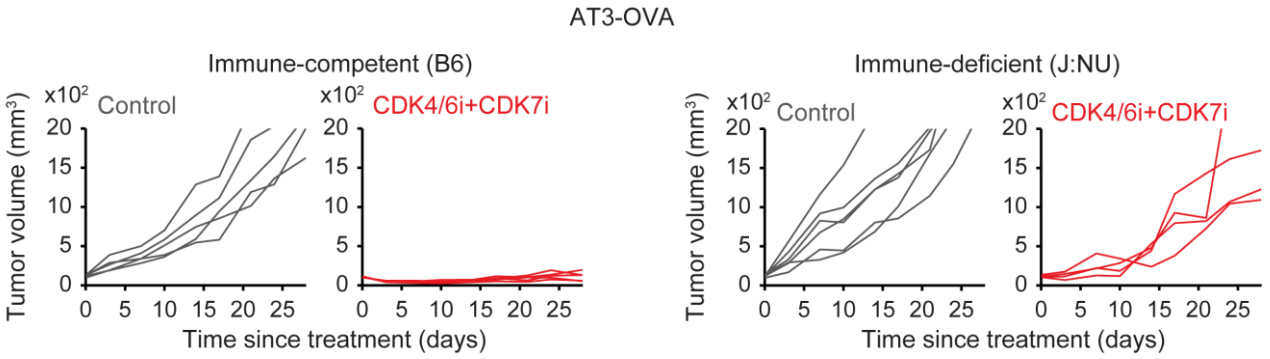


Dual targeting CDK4/6 and CDK7 activity (in vivo models)

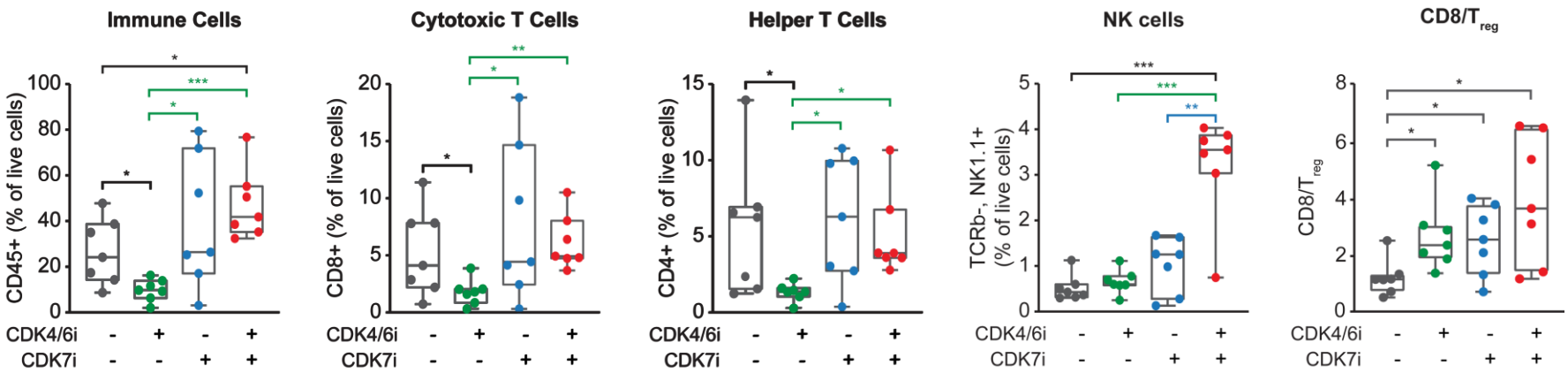
P < 0.01, ** P < 0.001, *** P < 0.0001





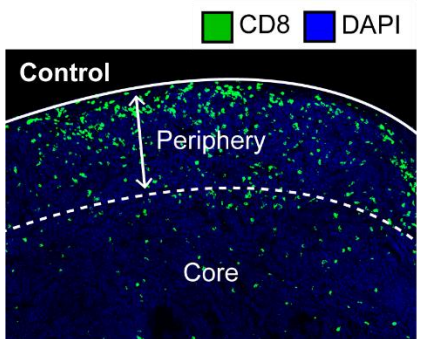


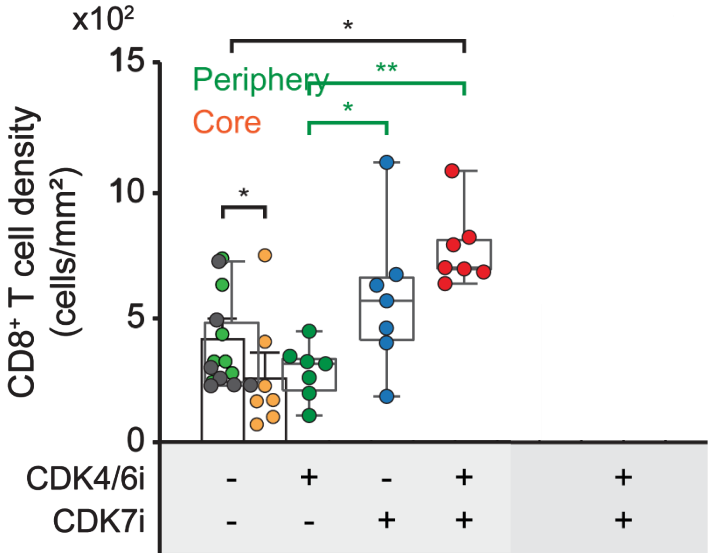
Impact on the tumor microenvironment



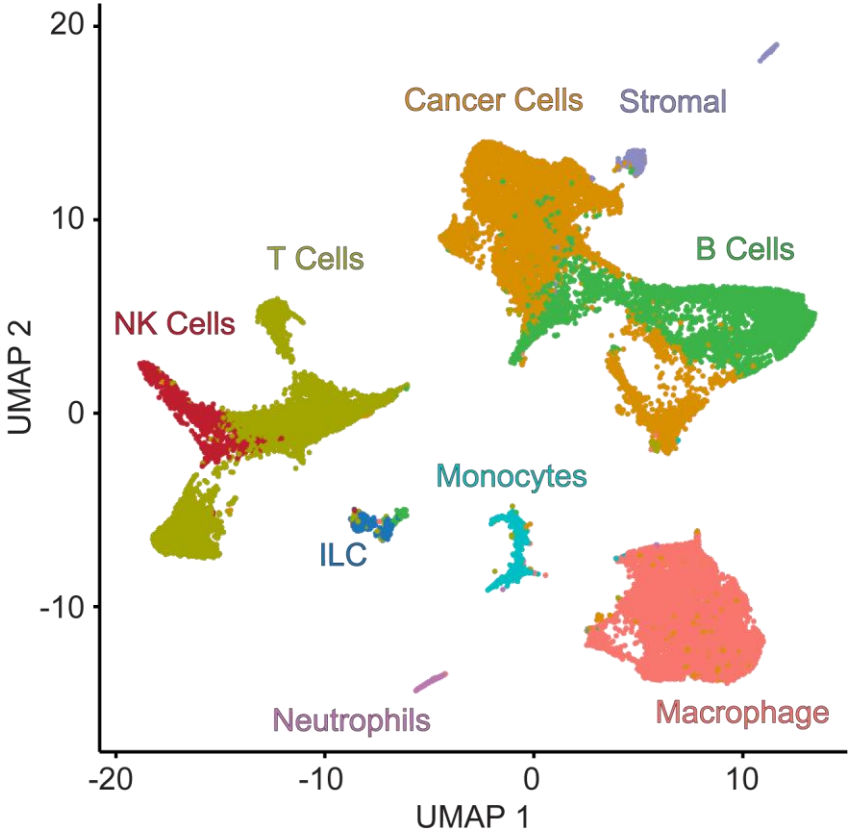
* P < 0.01, ** P < 0.001, *** P < 0.0001: one-way ANOVA

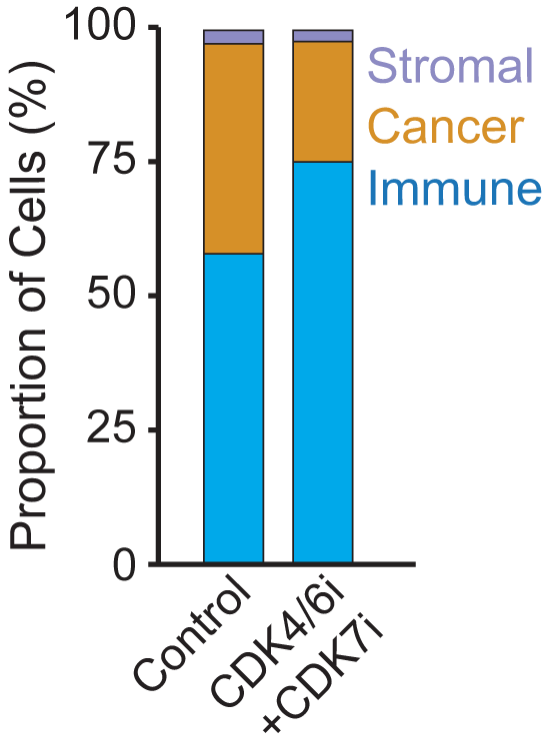
Impact on the tumor microenvironment



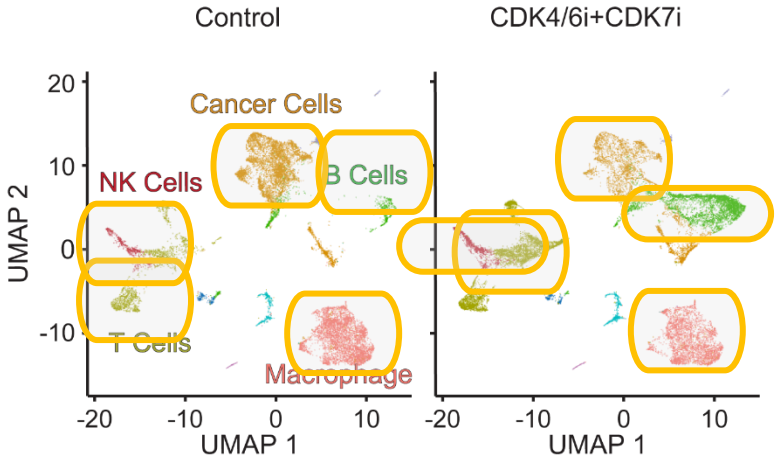


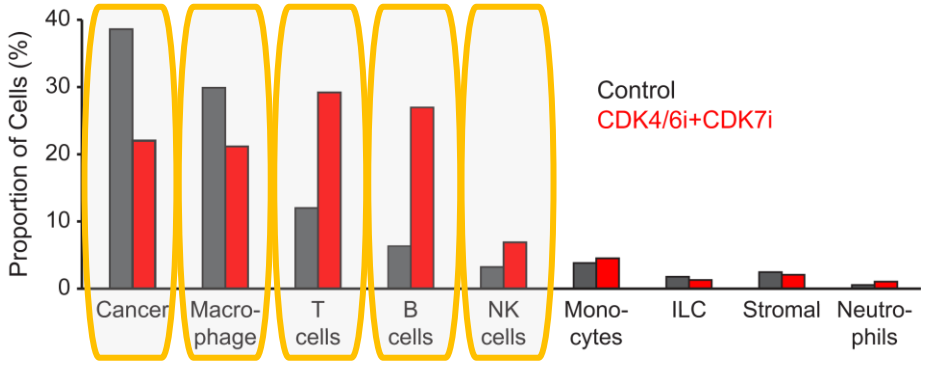
Impact on the tumor microenvironment

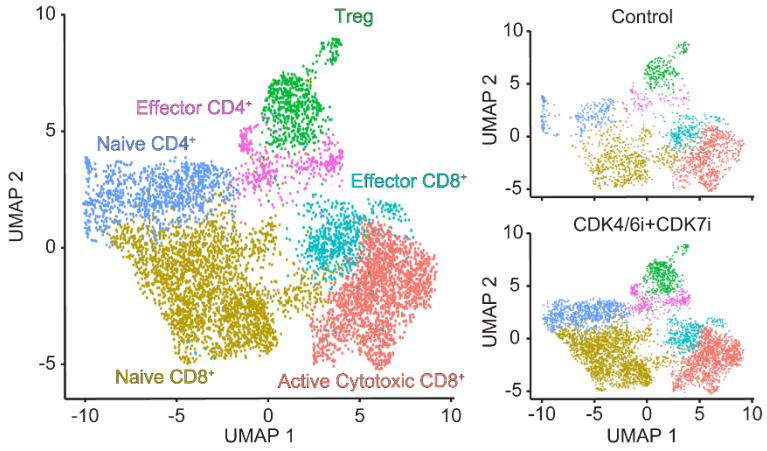


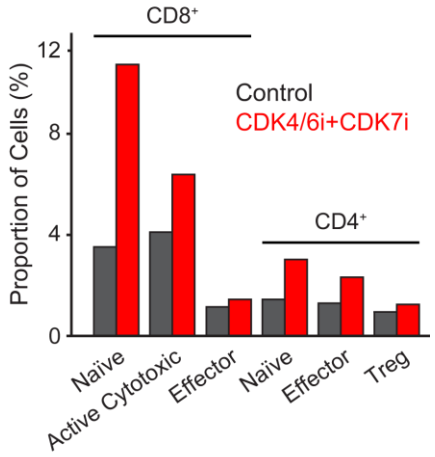


Impact on the tumor microenvironment

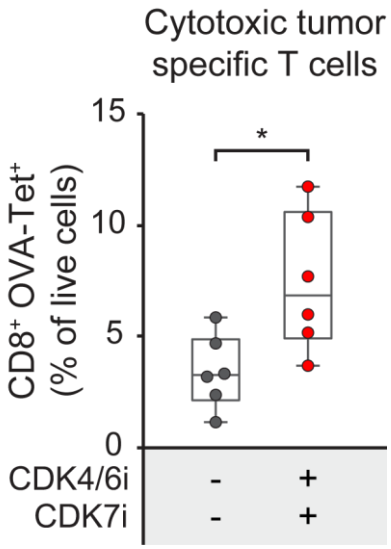


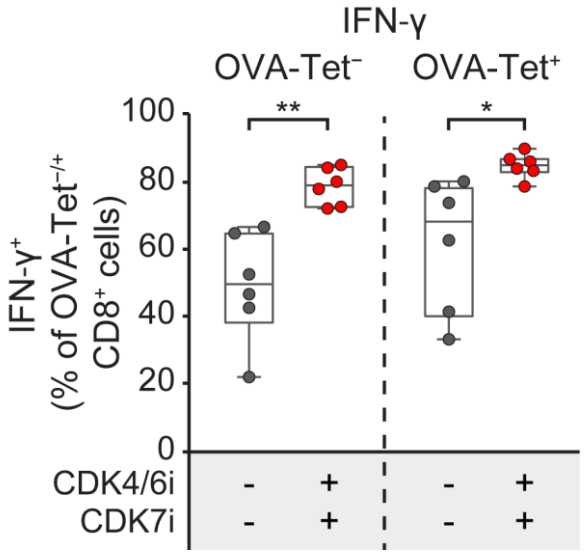


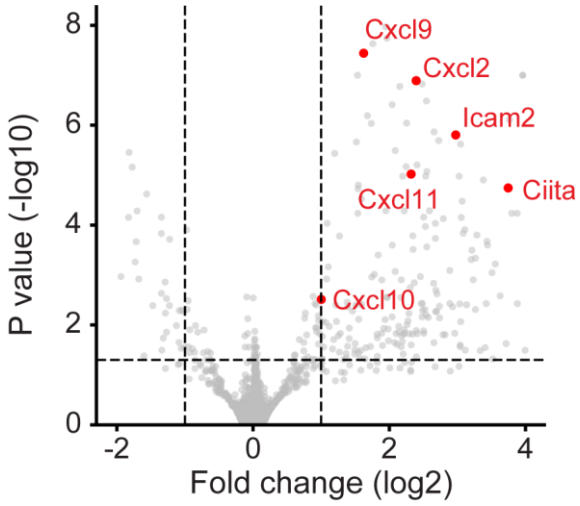




Impact on the tumor microenvironment

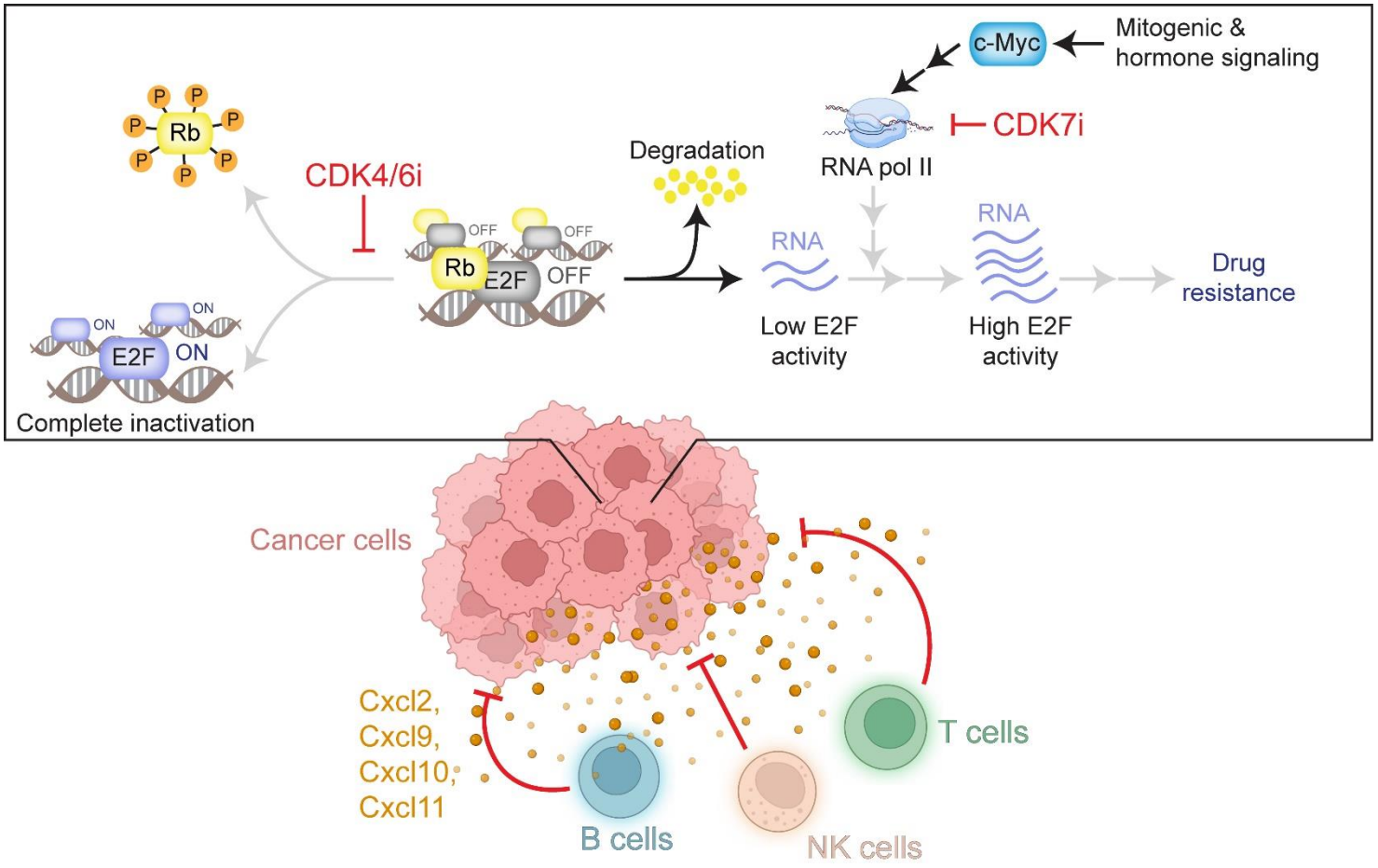




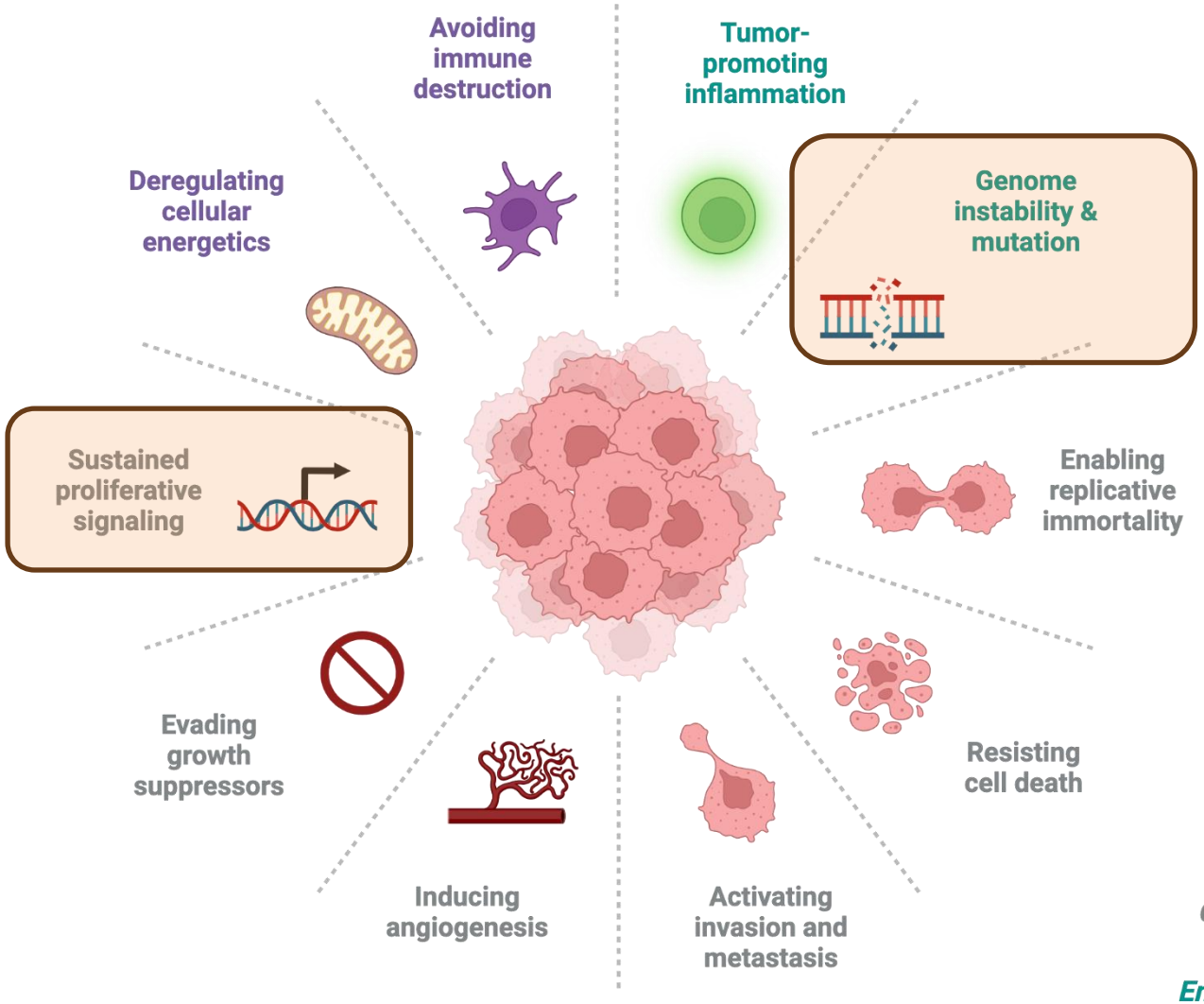


New Therapeutic strategy based on basic cell-cycle studies

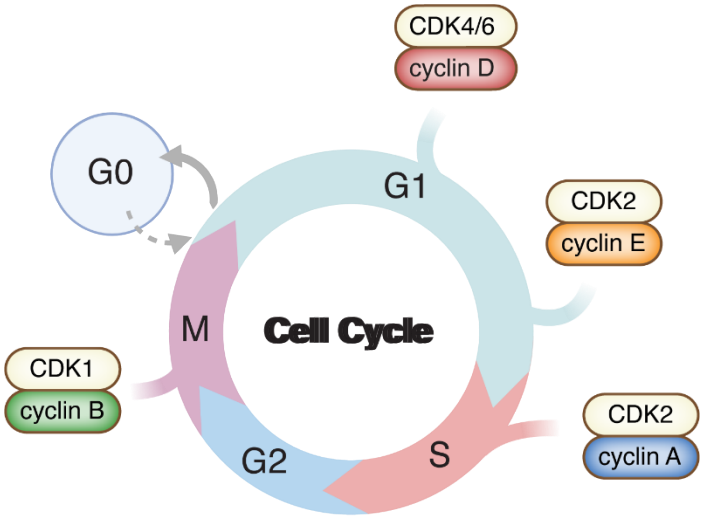
Kim* and Son* et al., 2025

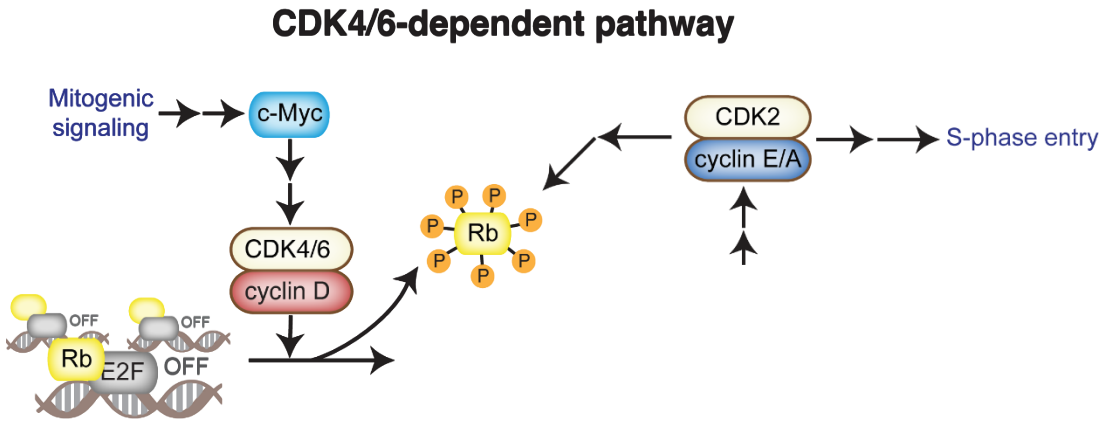


Hallmarks of cancer



Cell cycle regulation





Any questions?