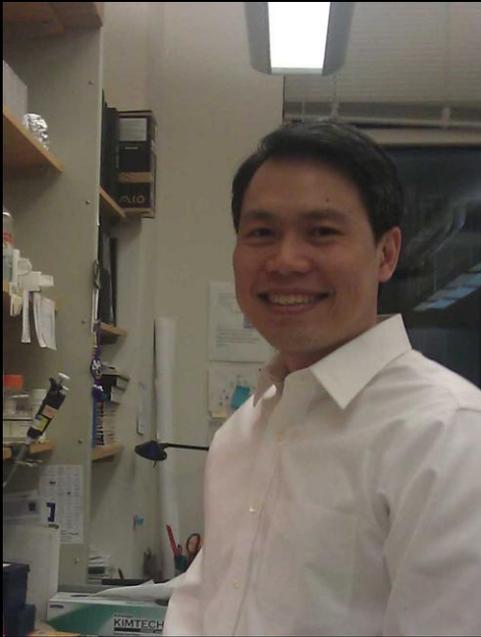


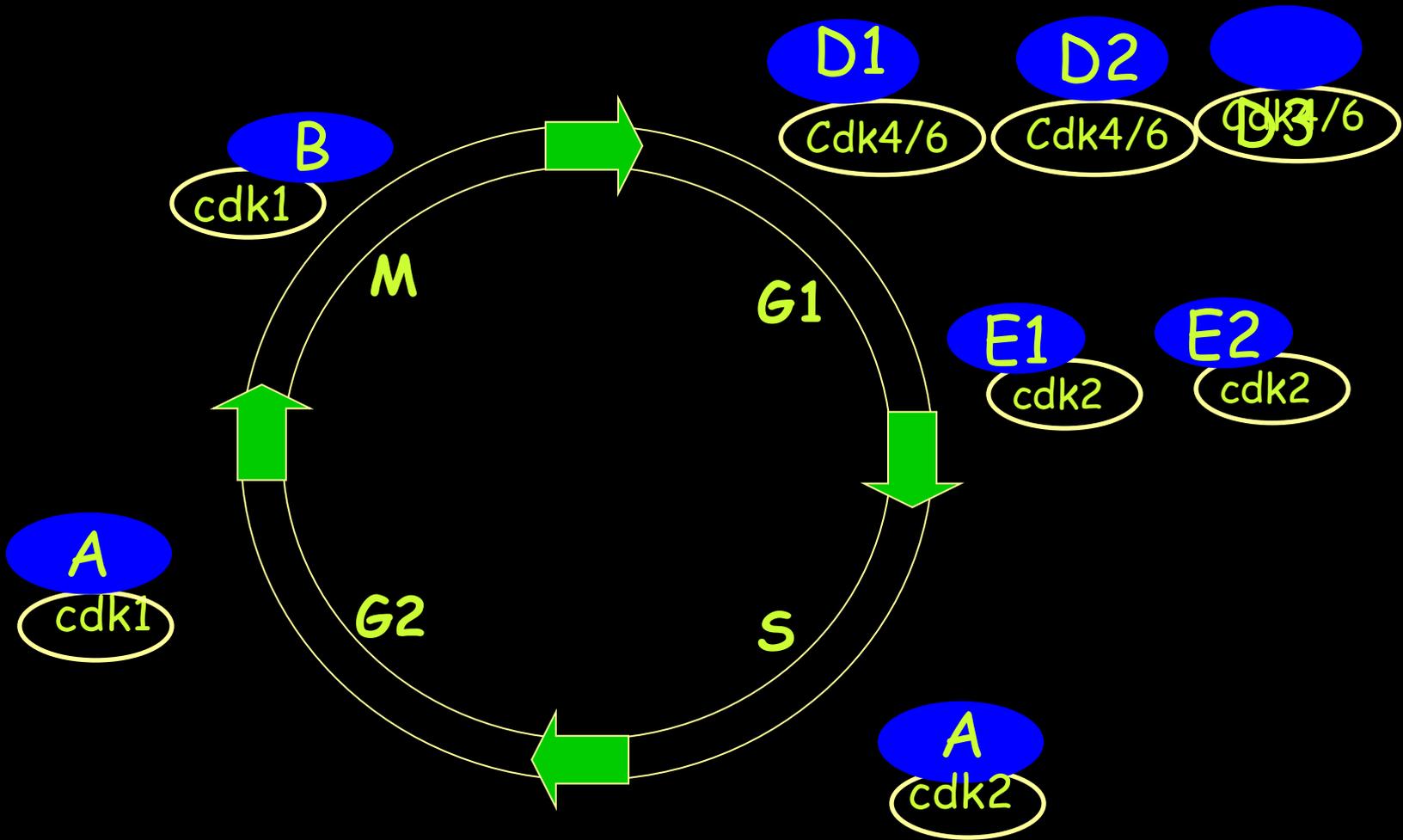
Genetic and proteomic approaches to study cell cycle machinery

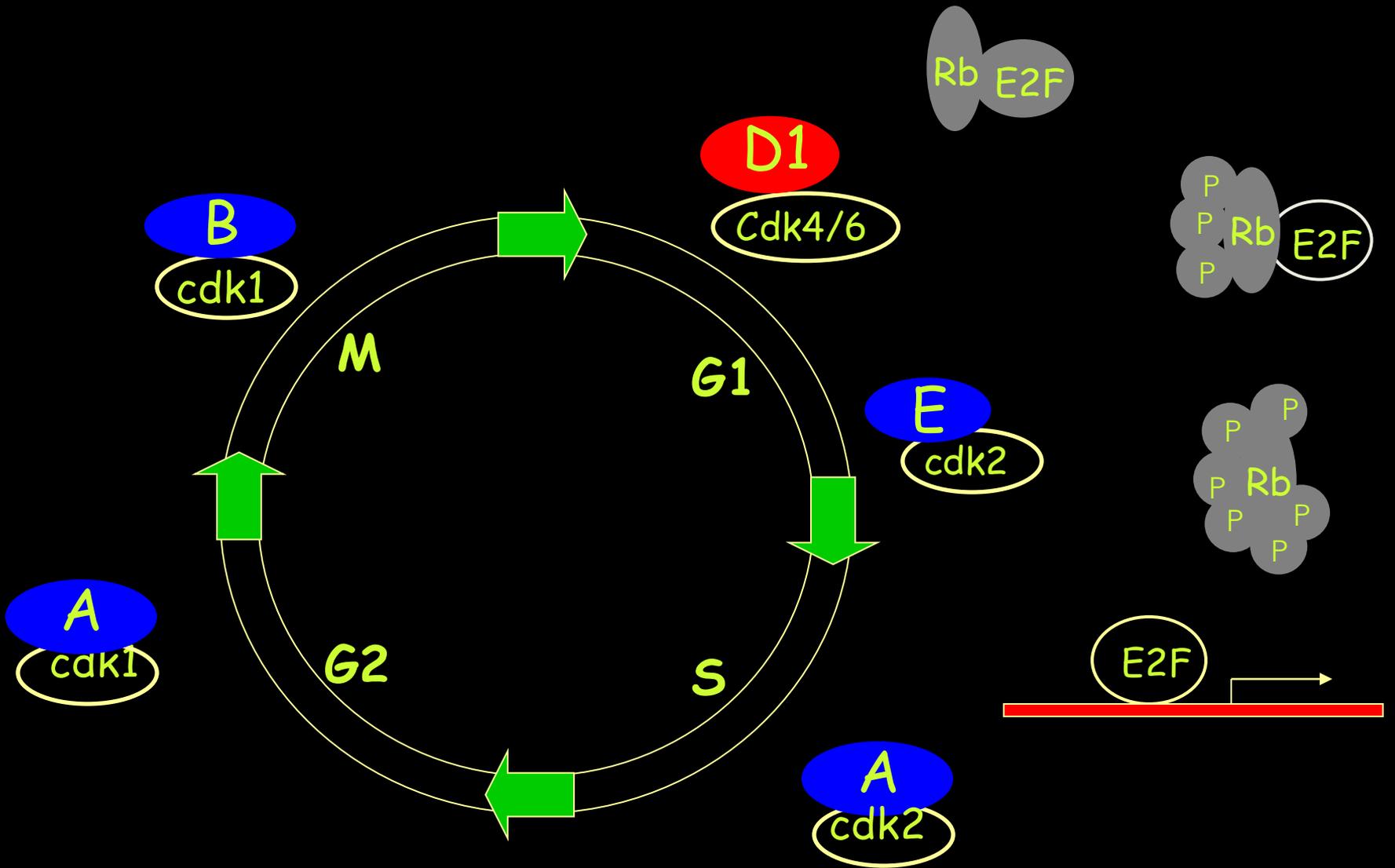


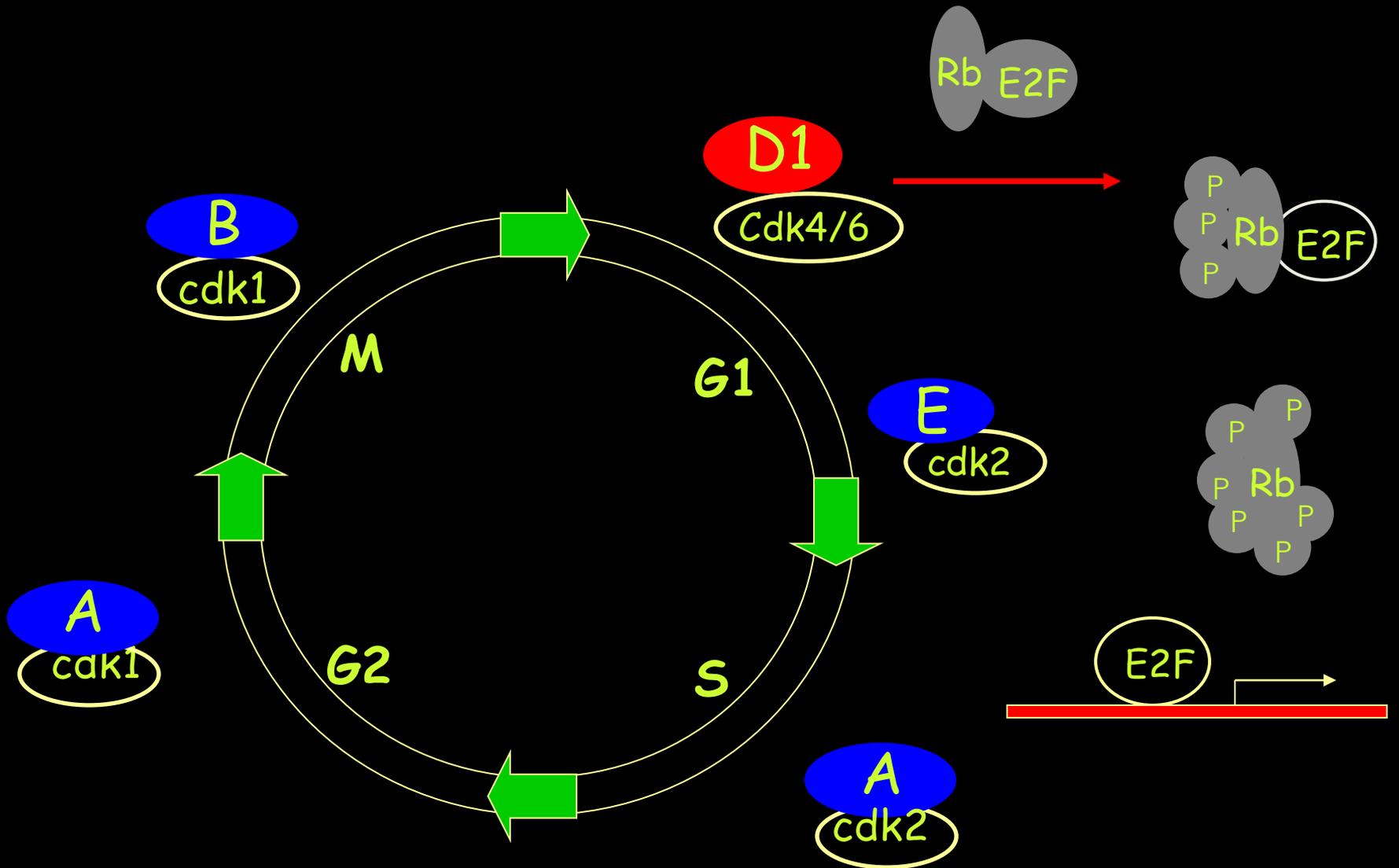
Siwanon Jirawatnatai



Frederic Bienvenu





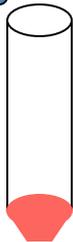
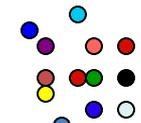


Top 10 amplified regions in 3131 cancer DNAs

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

Cells expressing tagged protein

Wildtype control cells



lysate

lysate

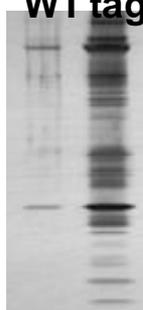
Flag

HA

HA

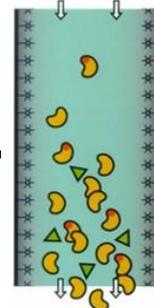
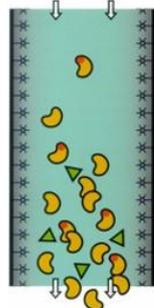
Flag

WT tag

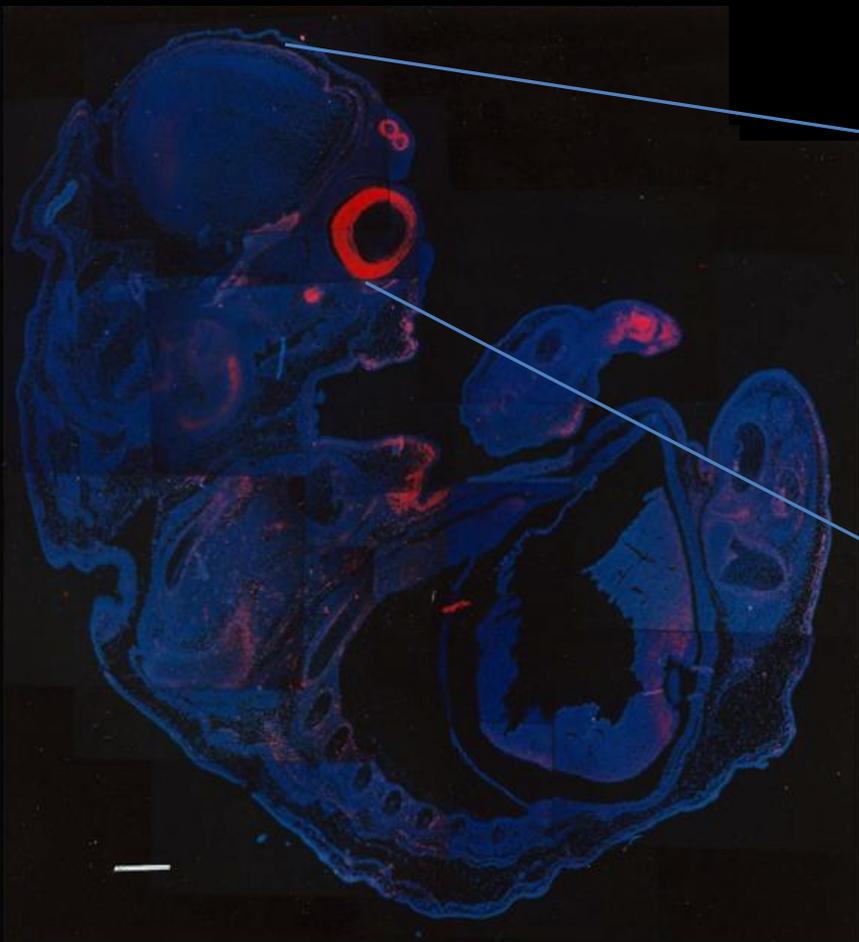


Silver stained
quality control gel

Ready for Mass Spectrometry



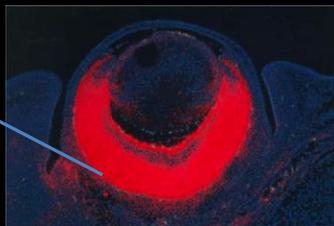
Cyclin D1 expression



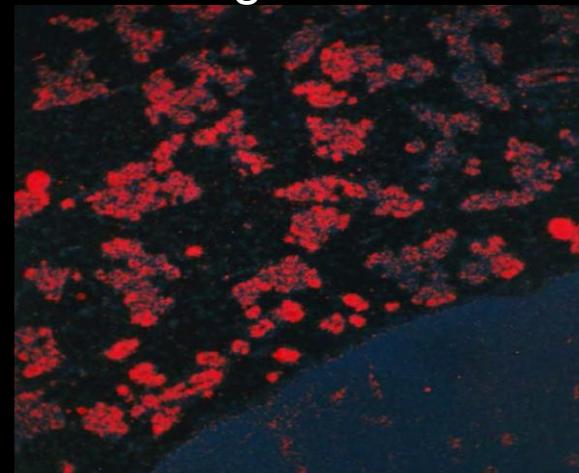
Brain



Retina

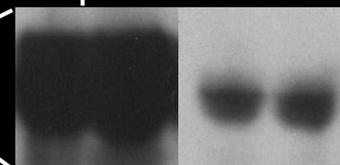


Mammary gland



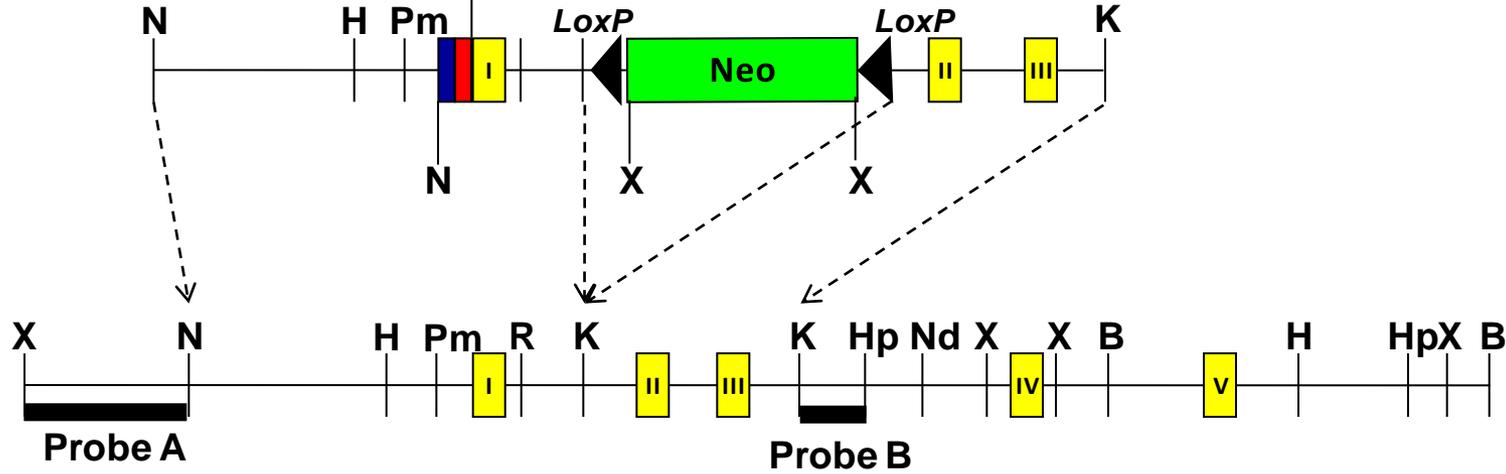
Tumor

Mam gland

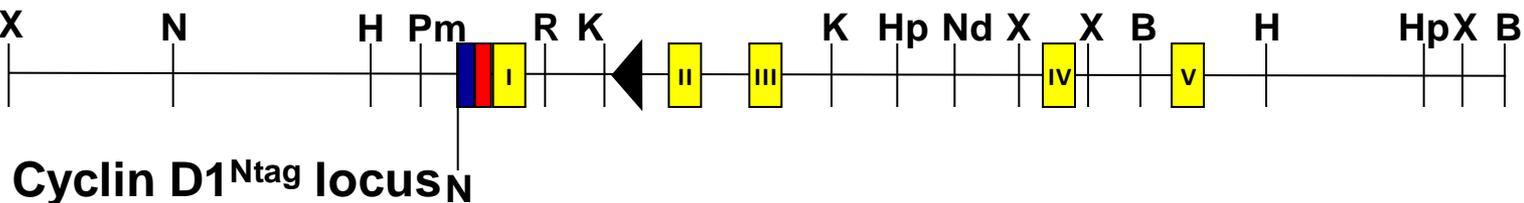
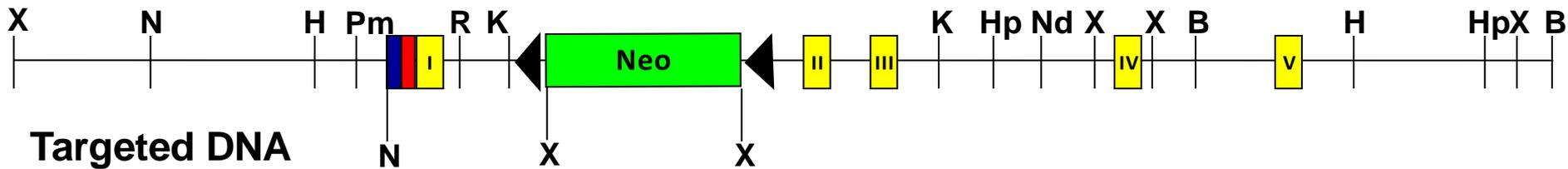


Generation of N-tagged knock-in mice

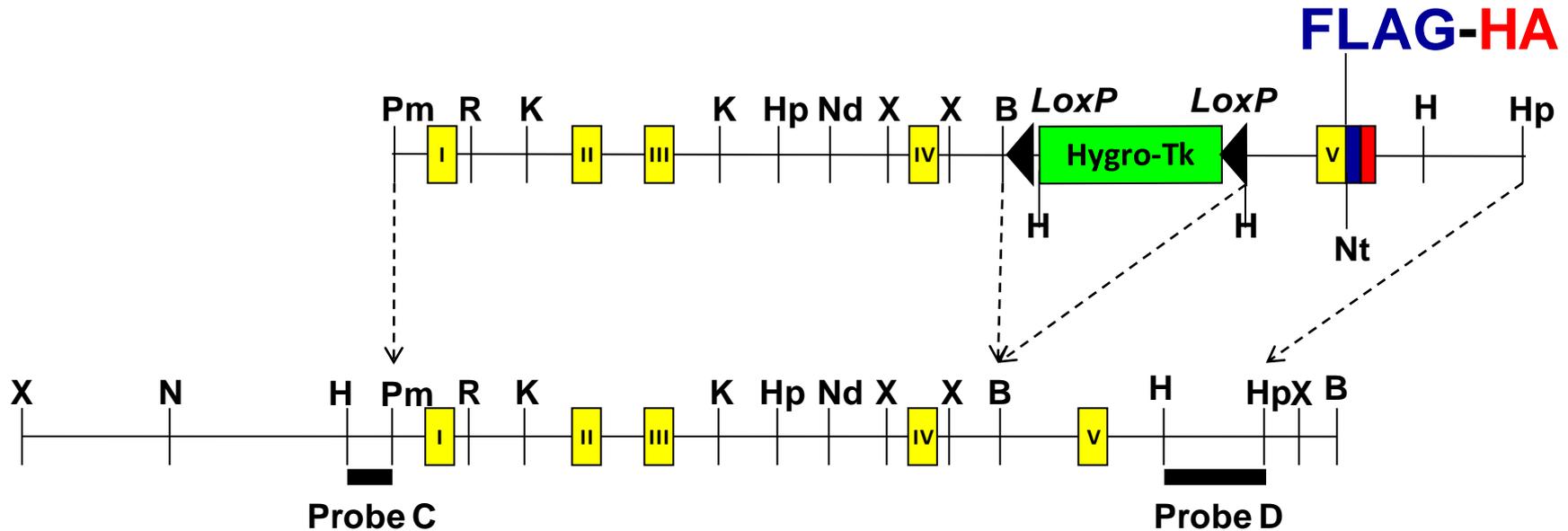
FLAG-HA



Cyclin D1 genomic locus



Generation of C-tagged knock-in mice



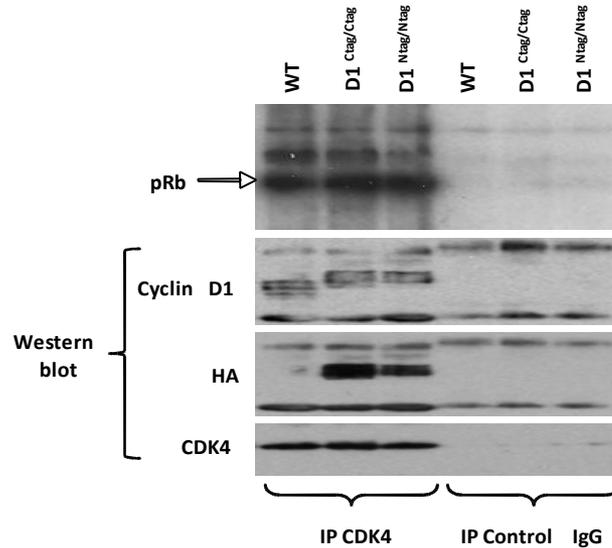
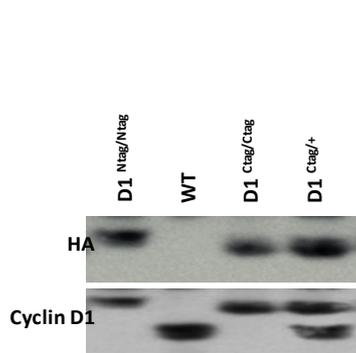
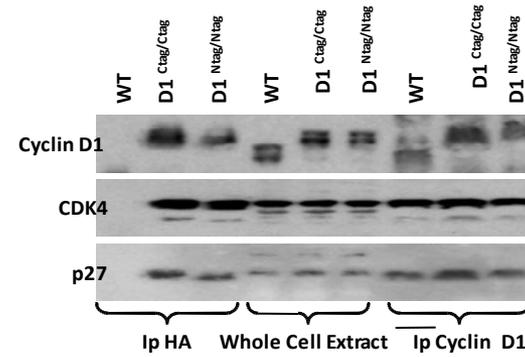
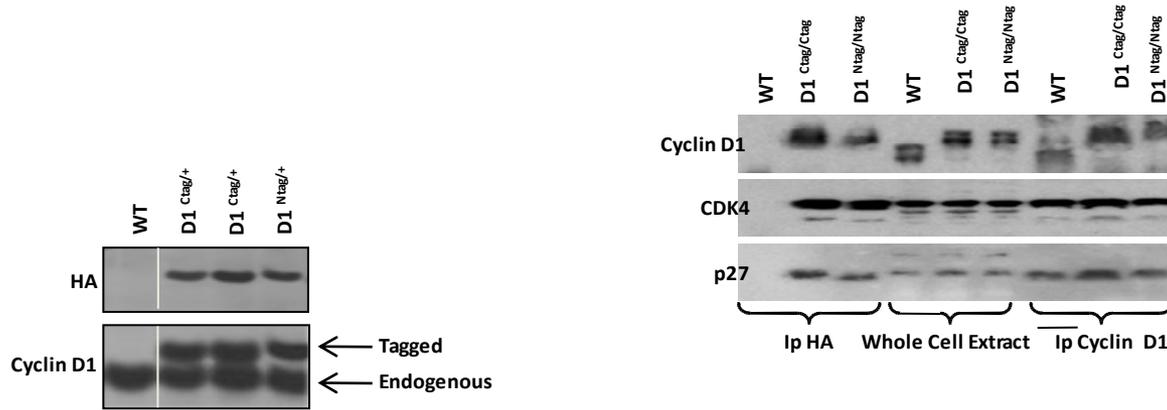
Cyclin D1 genomic locus

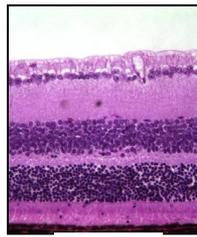
Targeted DNA

Cyclin D1^{Ctag} locus

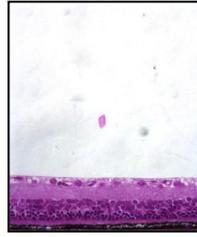
B

Tagged cyclin D1 behaves like the wild-type protein

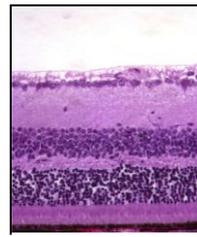




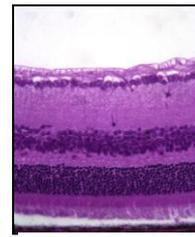
WT



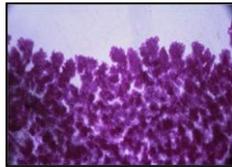
D1^{-/-}



D1^{Ctag/Ctag}



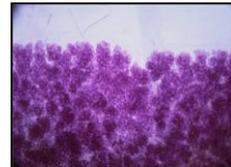
D1^{Ntag/Ntag}



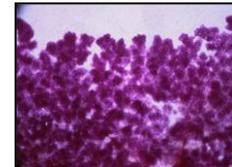
WT



D1^{-/-}



D1^{Ctag/Ctag}



D1^{Ntag/Ntag}

△



WT



D1^{-/-}D2^{-/-}



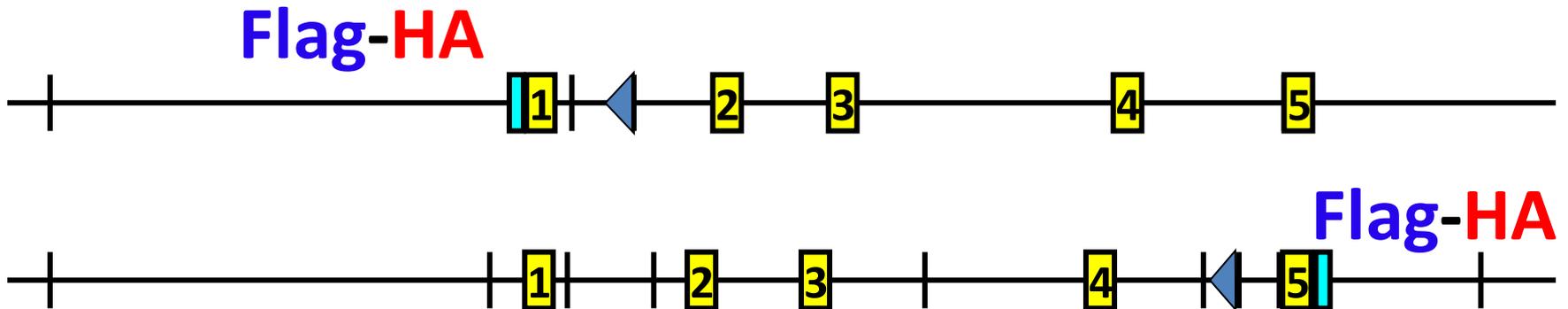
D1^{Ctag/Ctag}D2^{-/-}

Generation of N-Tag/C-Tag “hybrid” animals

$D1^{Ntag/Ntag} \times D1^{Ctag/Ctag}$

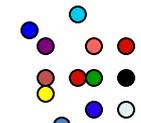


$D1^{Ntag/Ctag}$



Cells expressing tagged protein

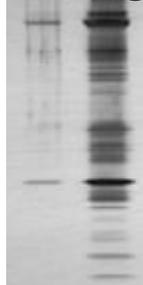
Wildtype control cells



lysate

lysate

WT tag



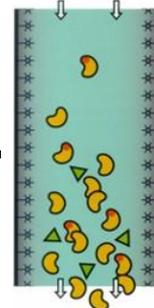
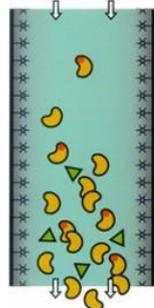
Silver stained
quality control gel

Flag

HA

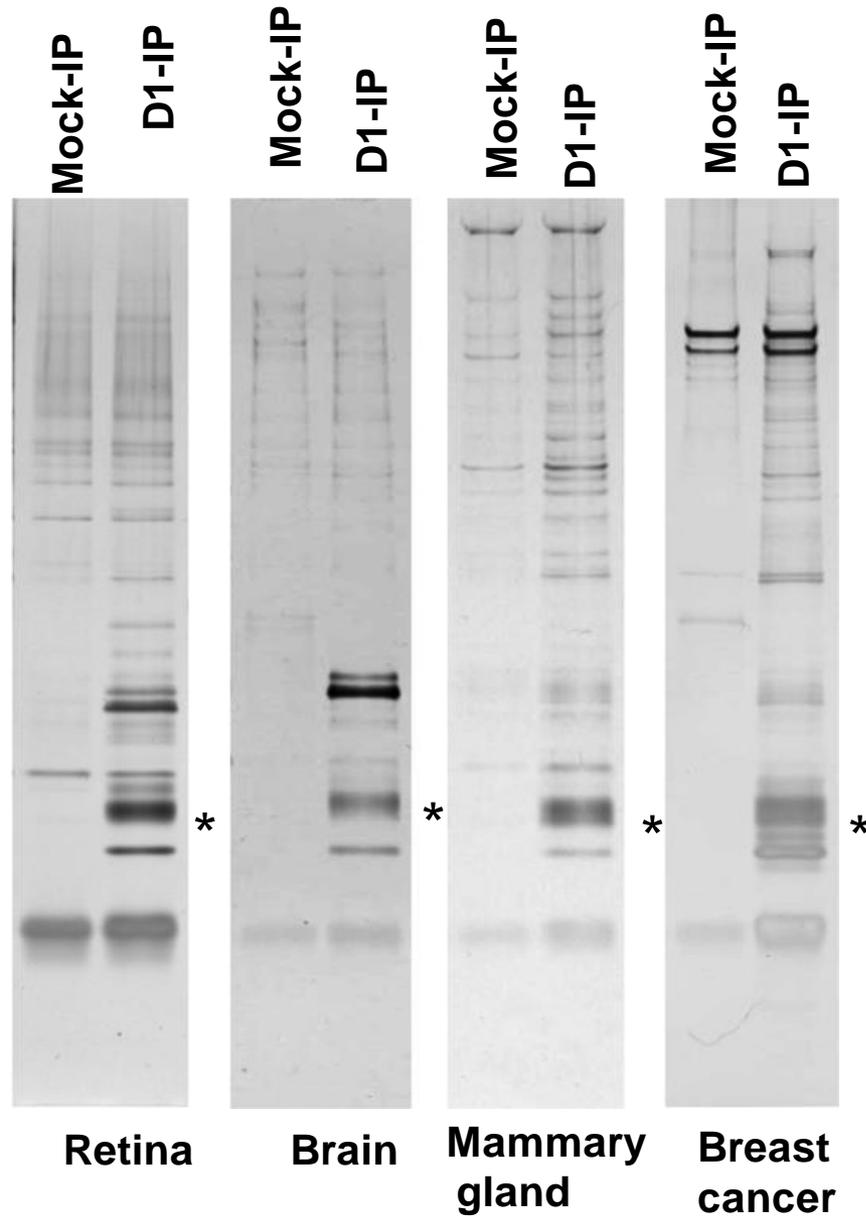
HA

Flag



Ready for Mass Spectrometry

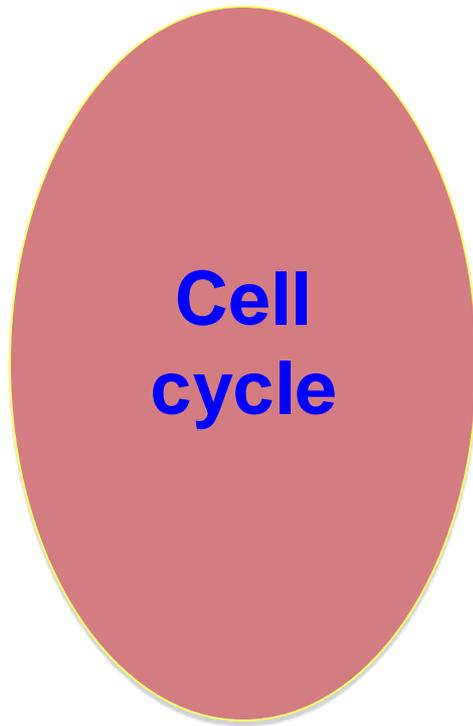
Purification products



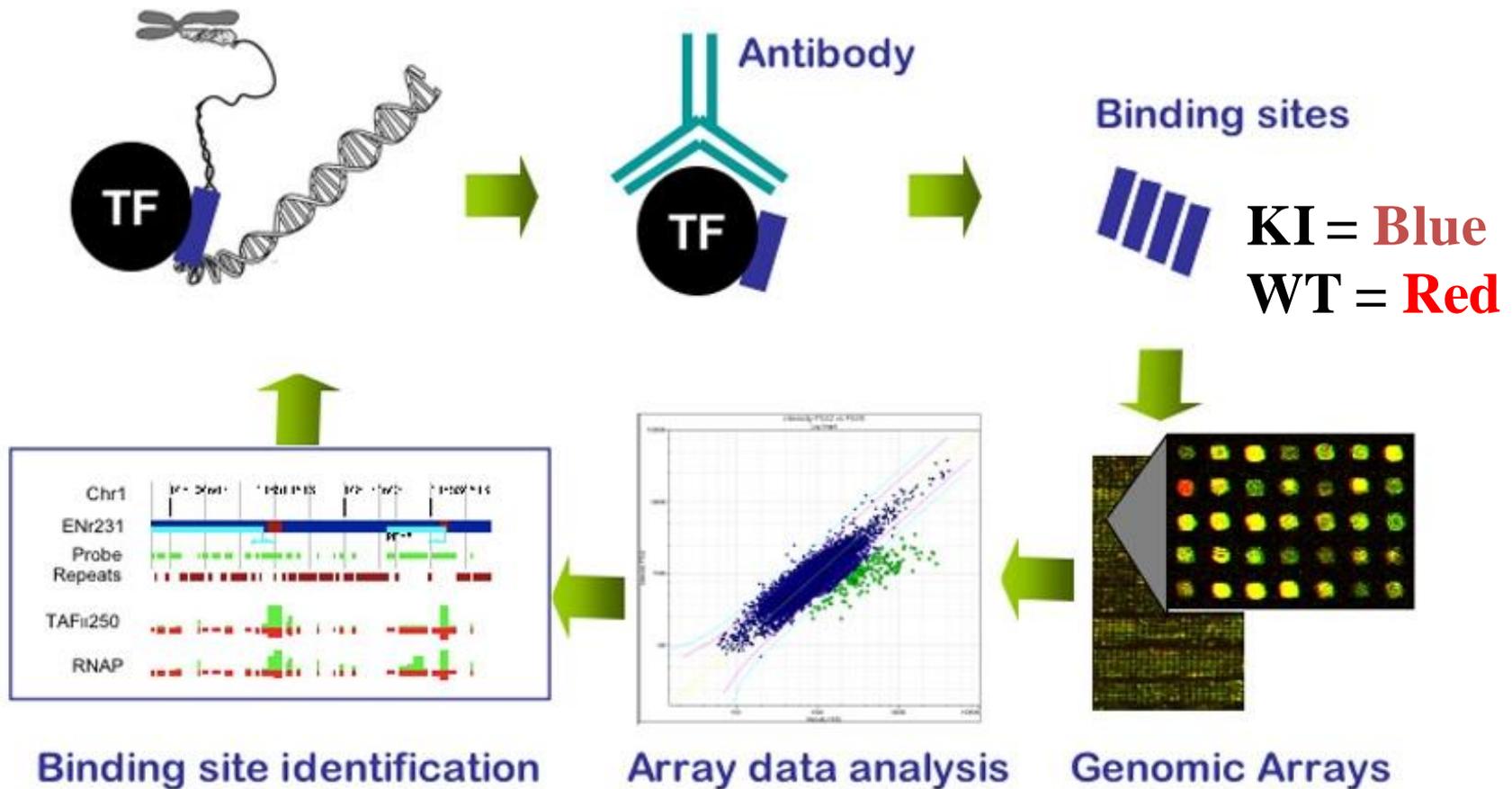
Siwanon Jirawatnotai

Josh Elias

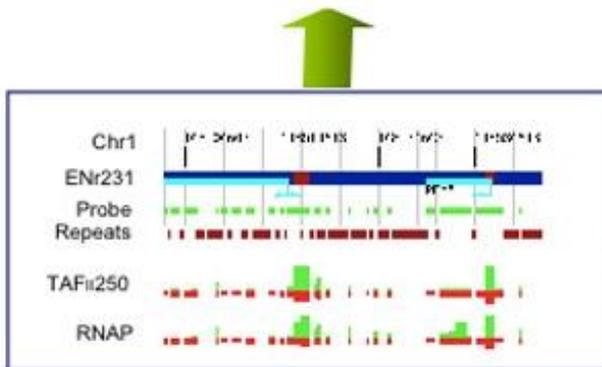
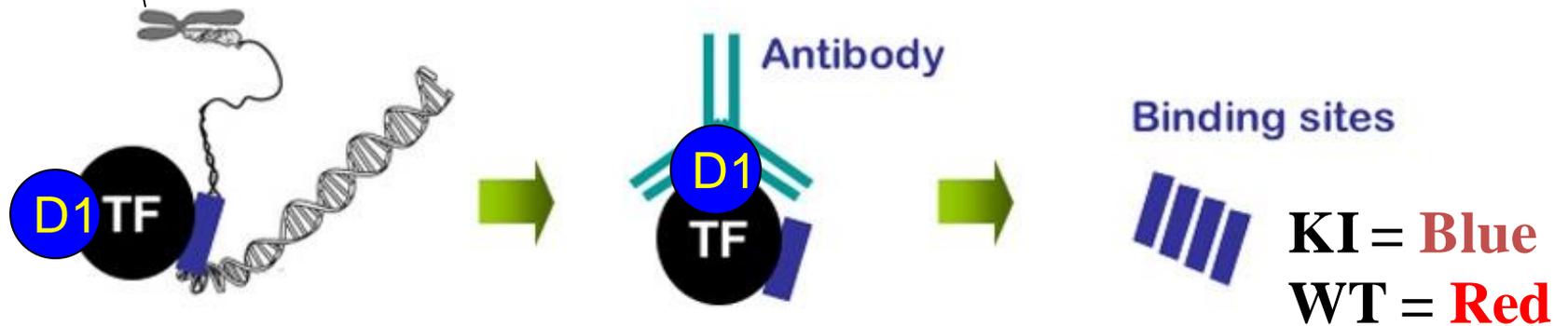
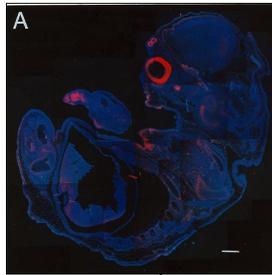
Gene Ontology analyses of interactors



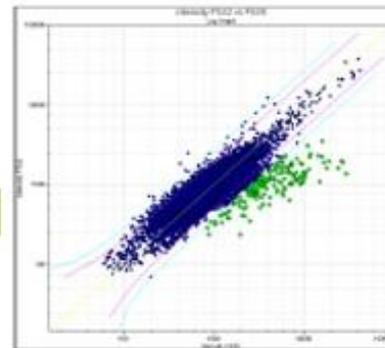
ChIP-on-chip identification of DNA targets



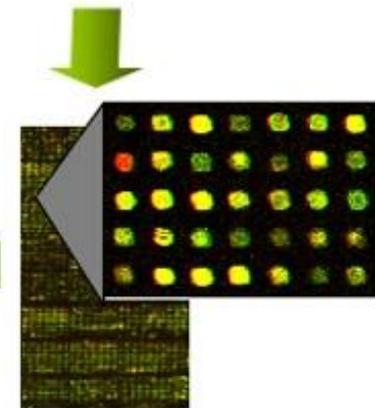
ChIP-on-chip identification of DNA targets



Binding site identification



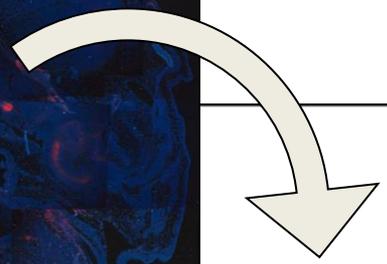
Array data analysis



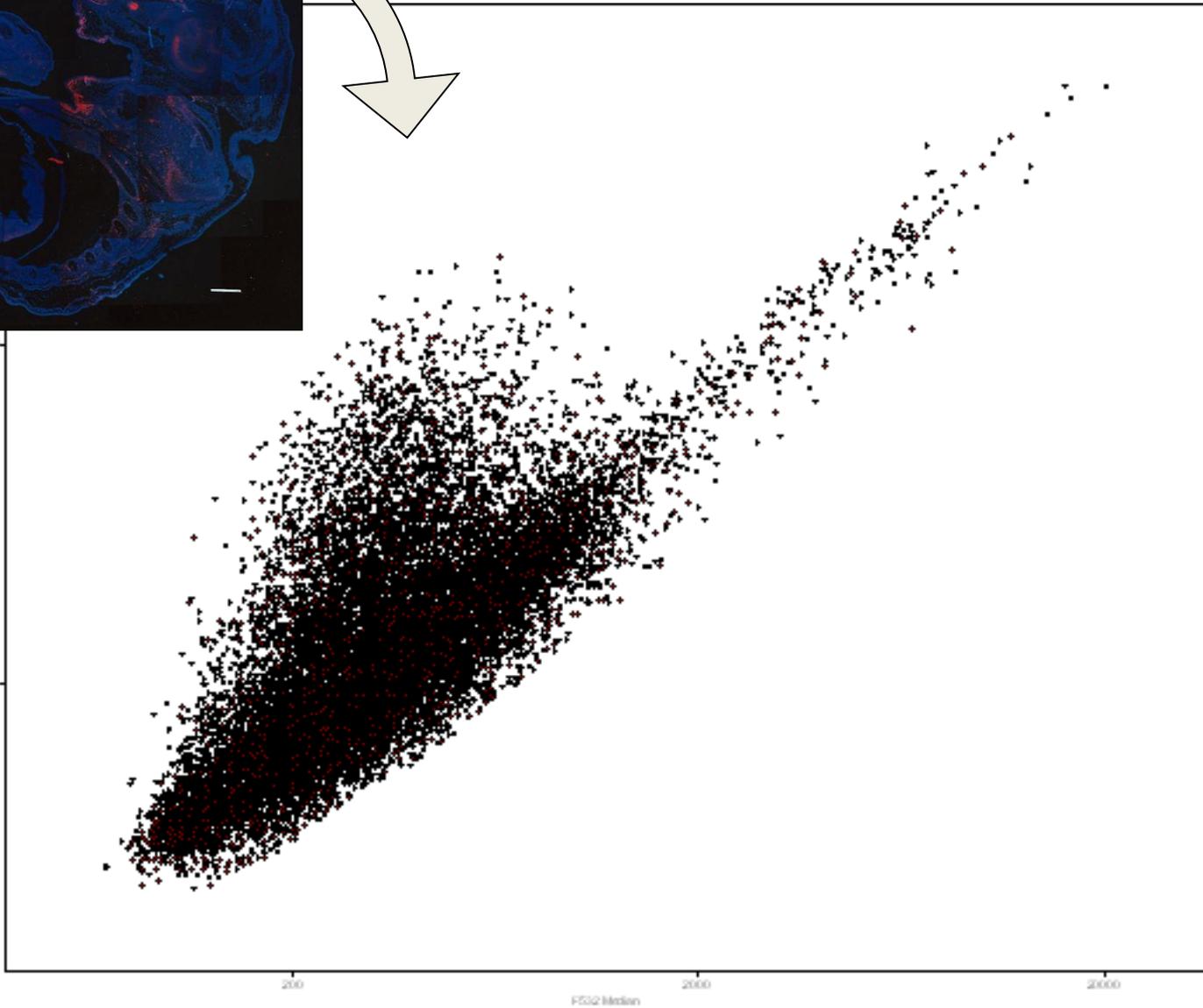
Genomic Arrays

A

Retinal ChIP-chip



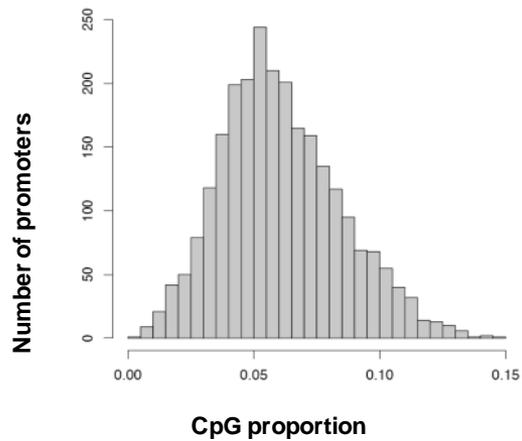
KI Monoclonal Flag IP



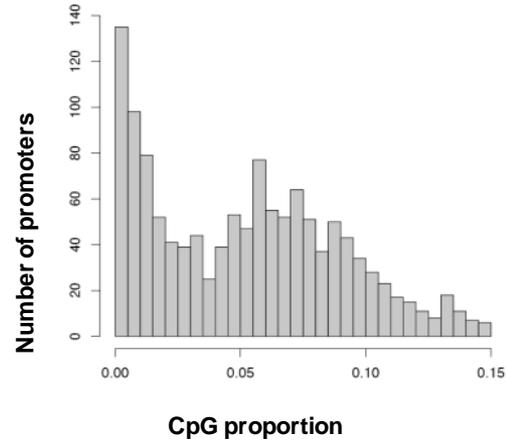
WT Monoclonal Flag IP

Bioinformatic analyses of promoter CpG content

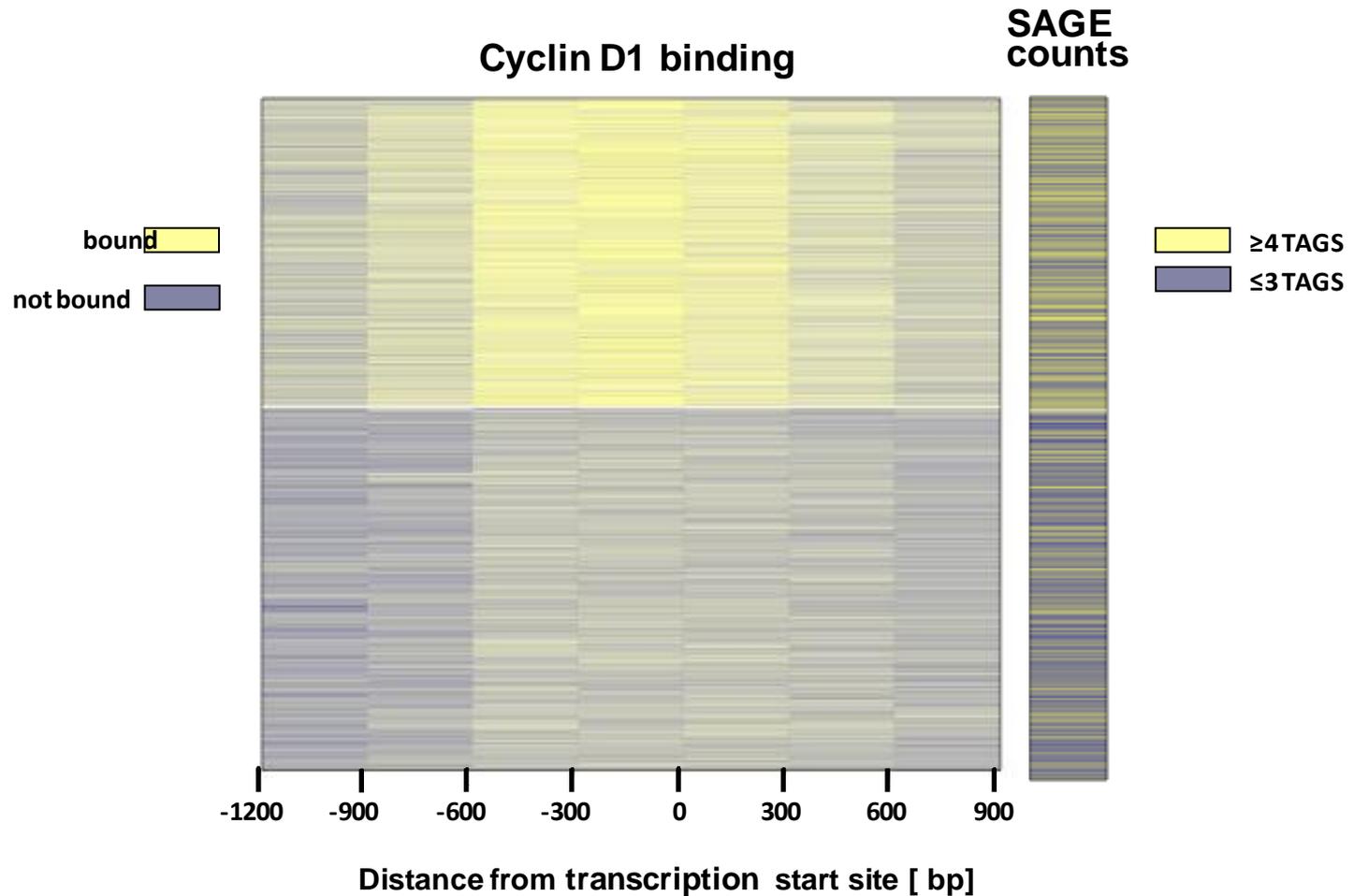
D1-bound promoters



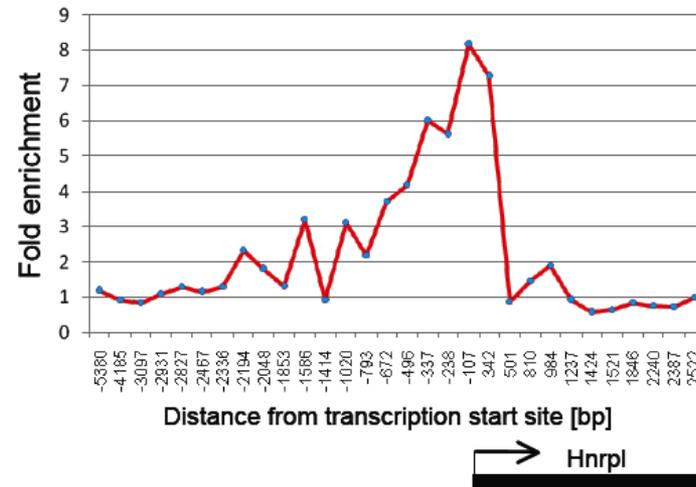
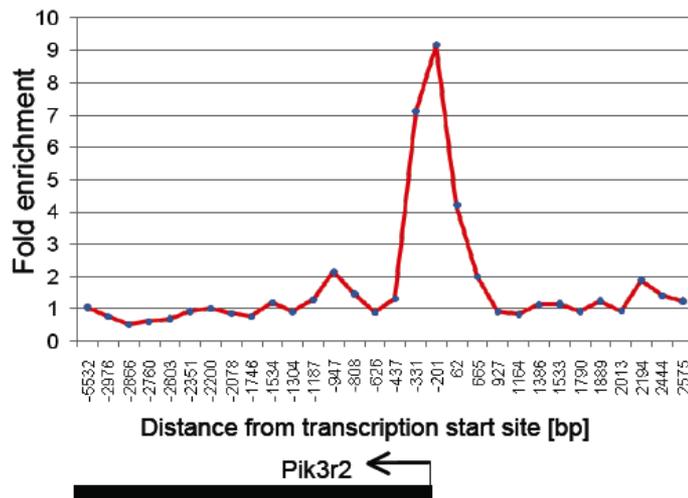
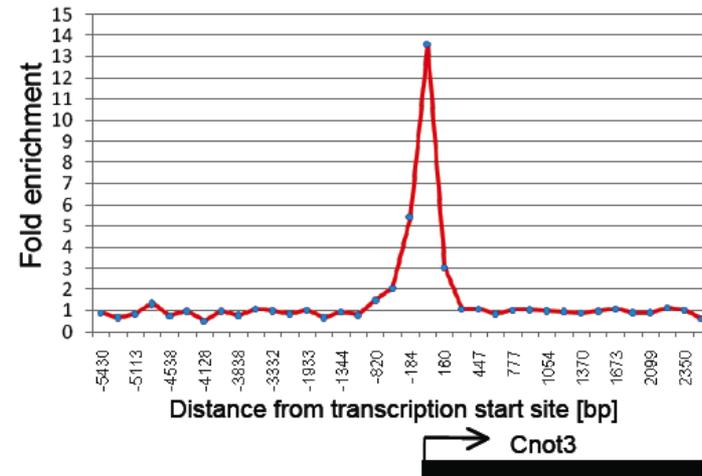
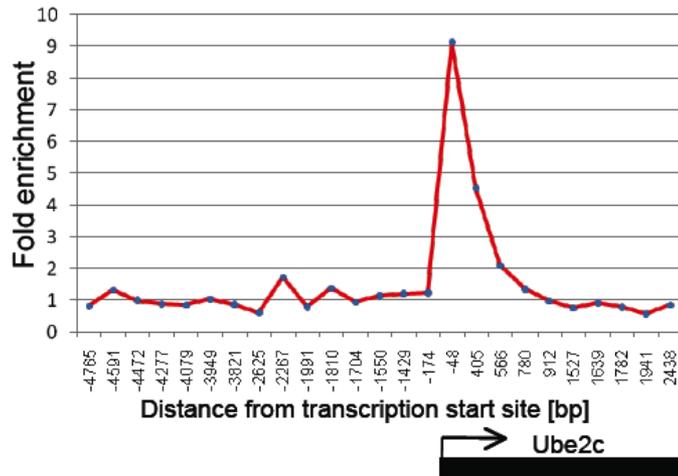
D1-not bound promoters



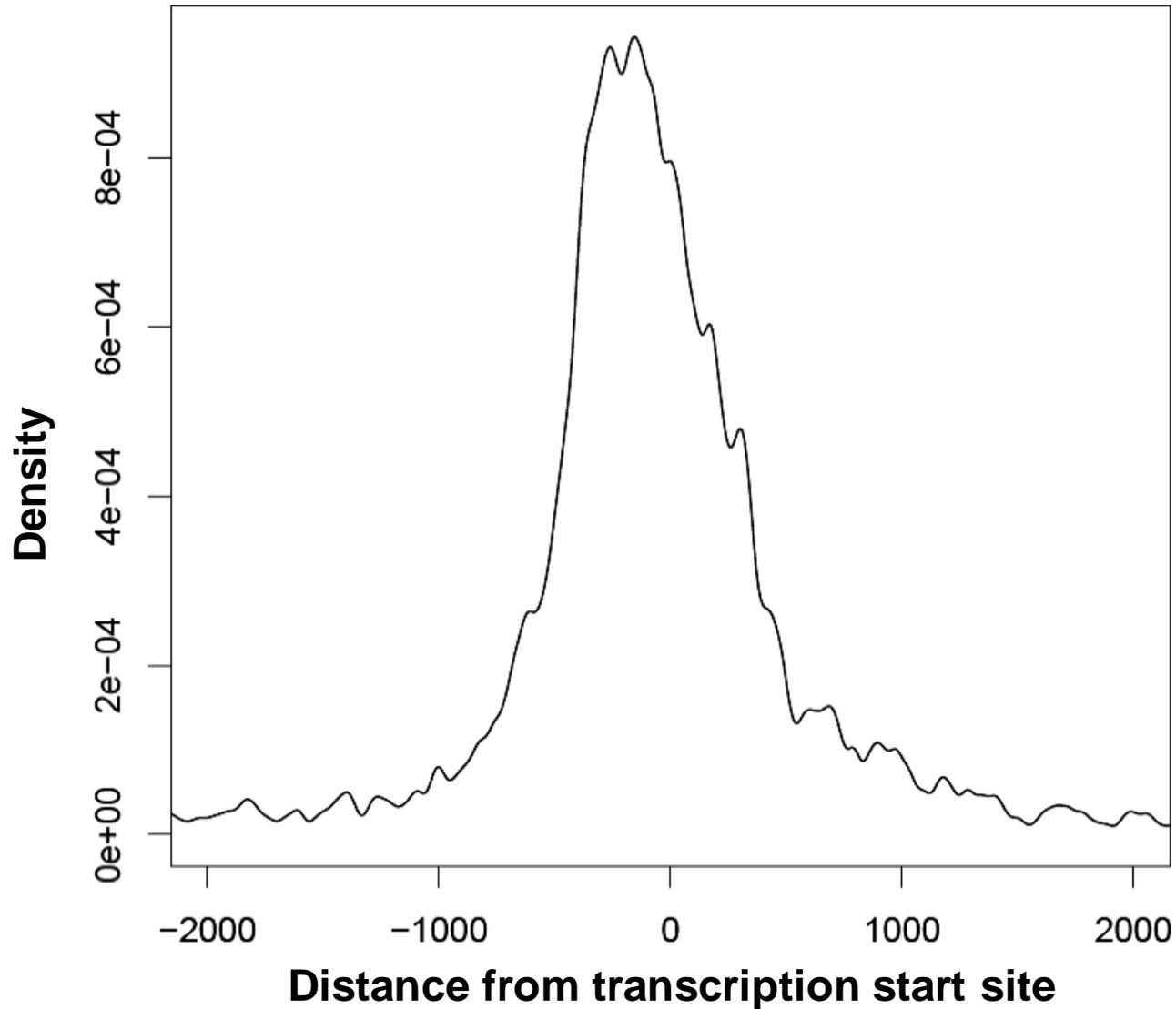
Bioinformatic analyses of SAGE libraries

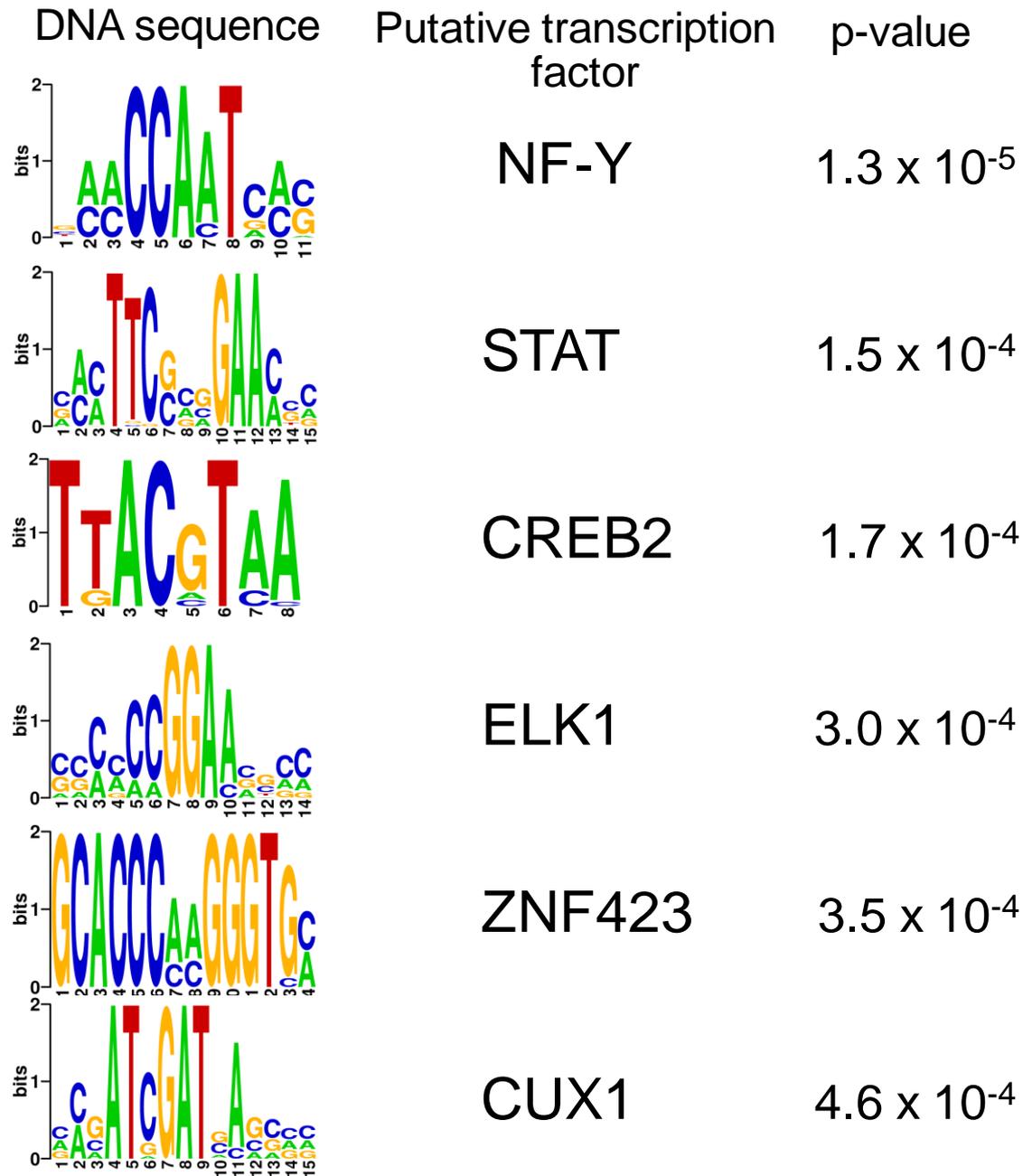


Interaction of cyclin D1 with the genome



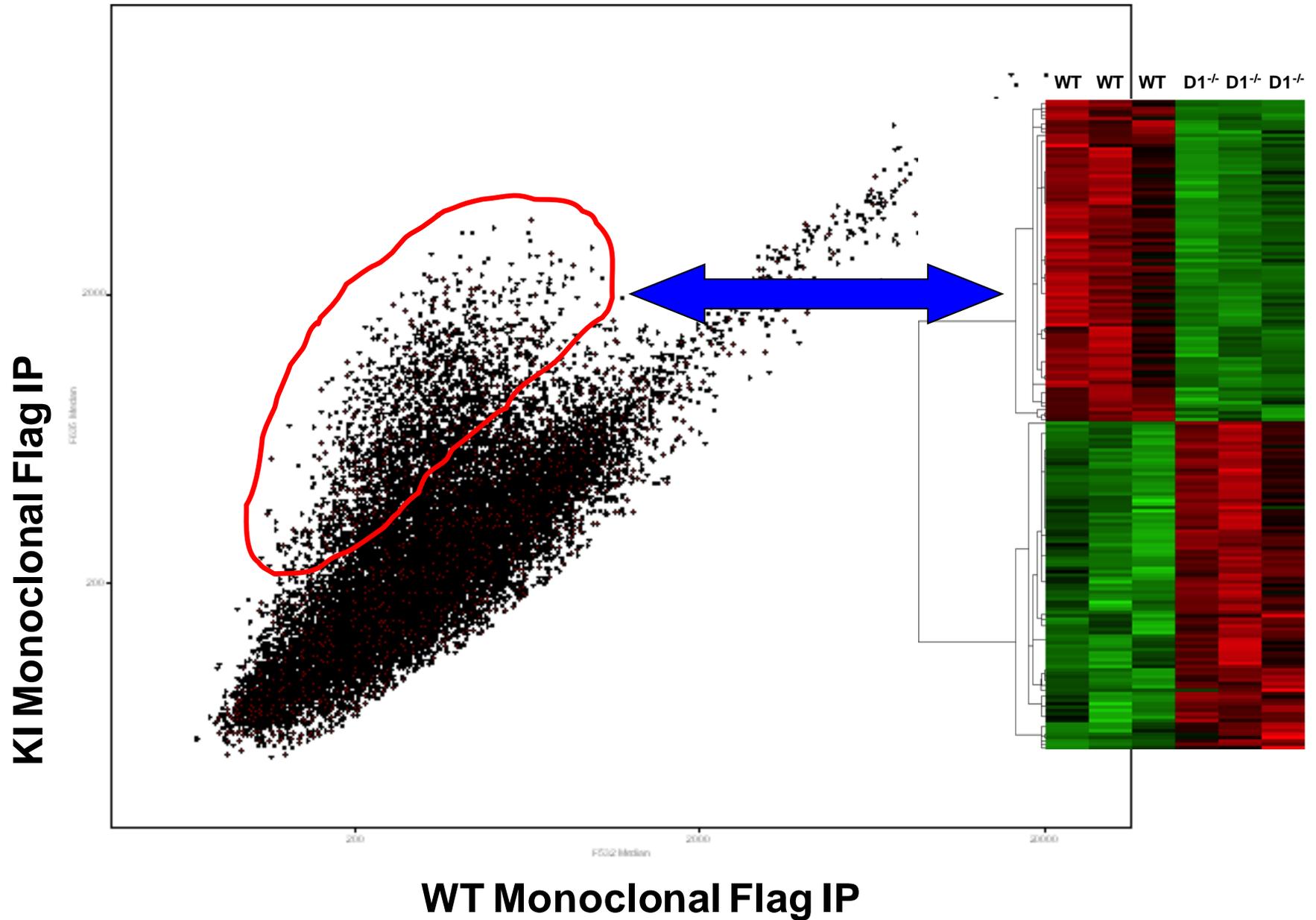
Interaction of cyclin D1 with the genome





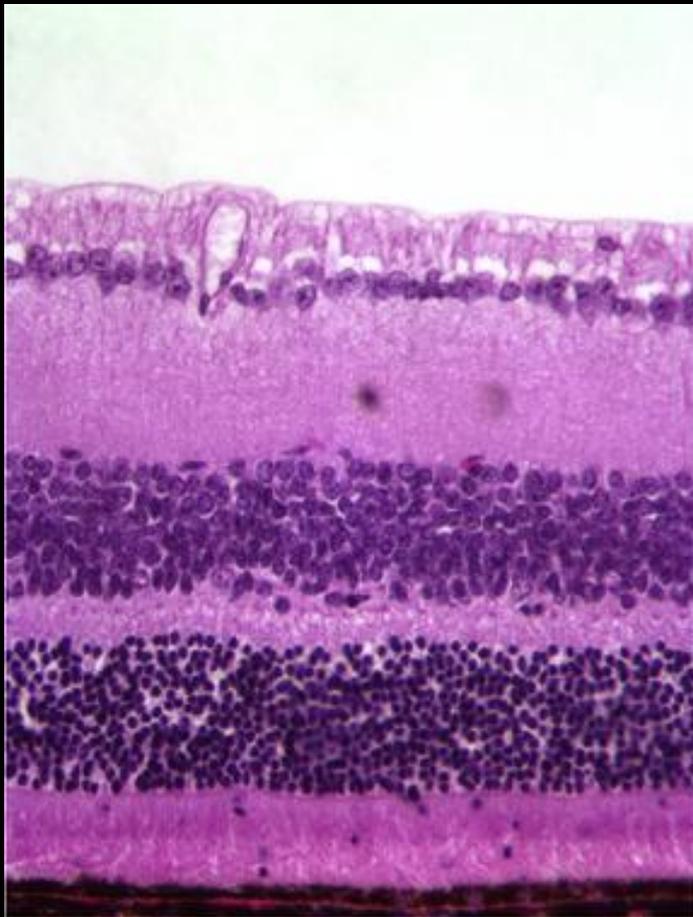
Retinal ChIP-chip

Gene expression

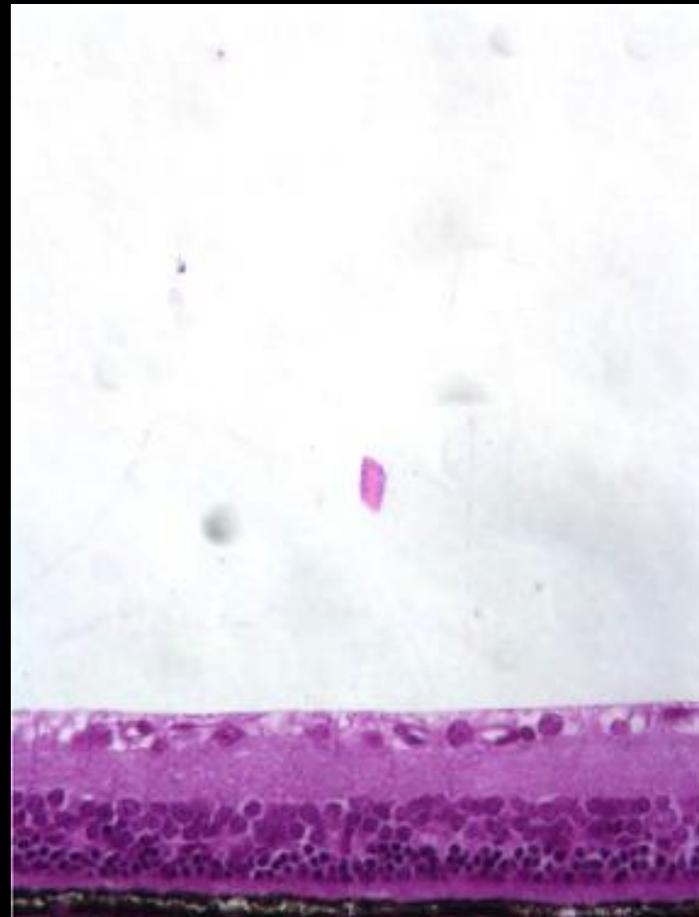


Cyclin D1^{-/-} retinal phenotype

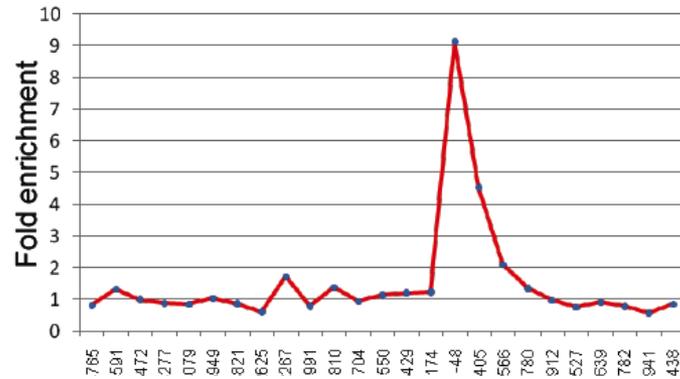
Wild-type retina



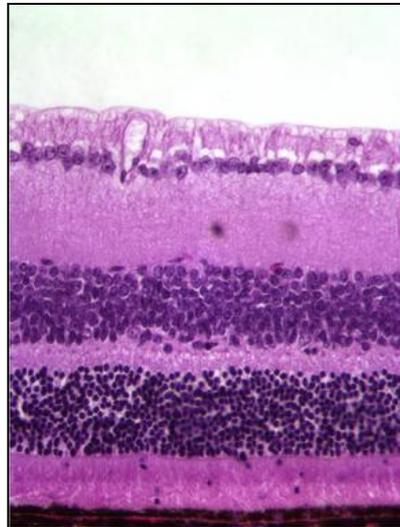
Cyclin D1^{-/-} retina



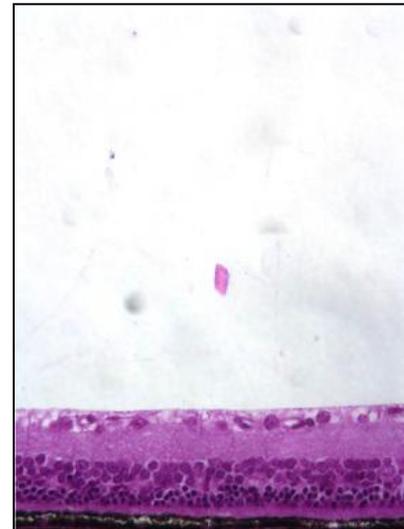
Interaction of cyclin D1 with the *Notch1* gene promoter



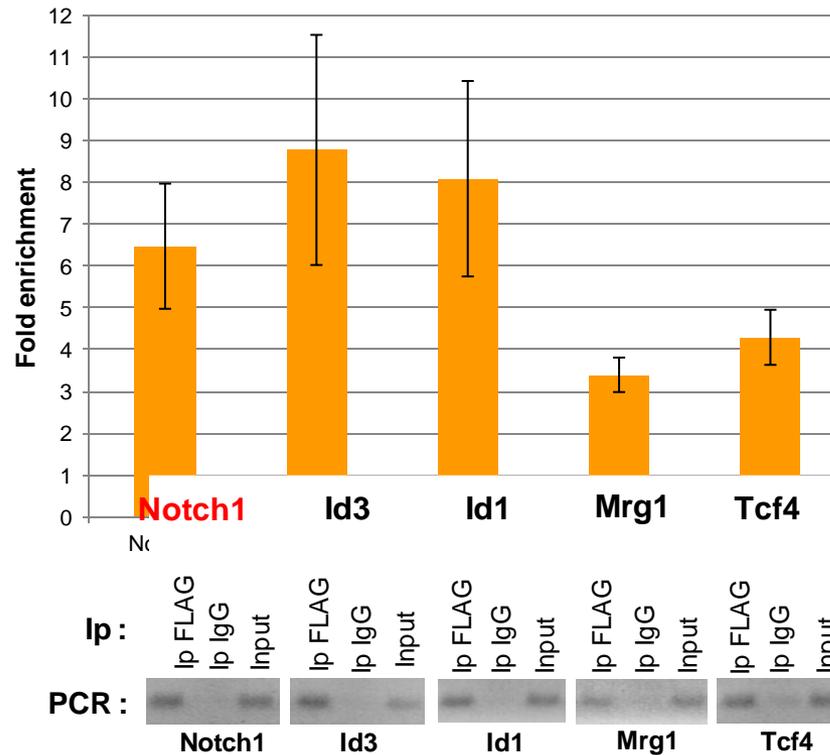
Wild-type retina

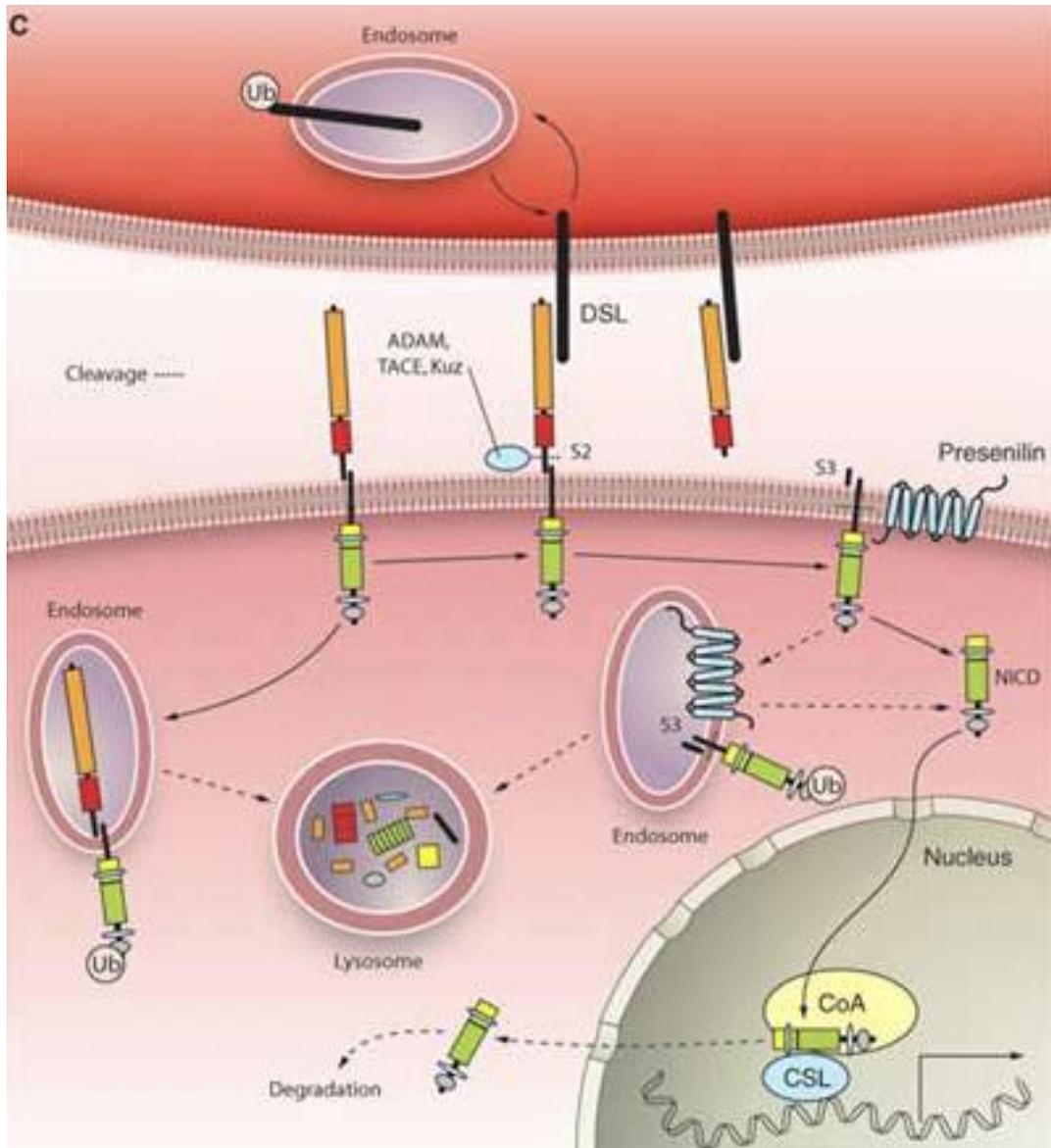


Cyclin D1^{-/-} retina

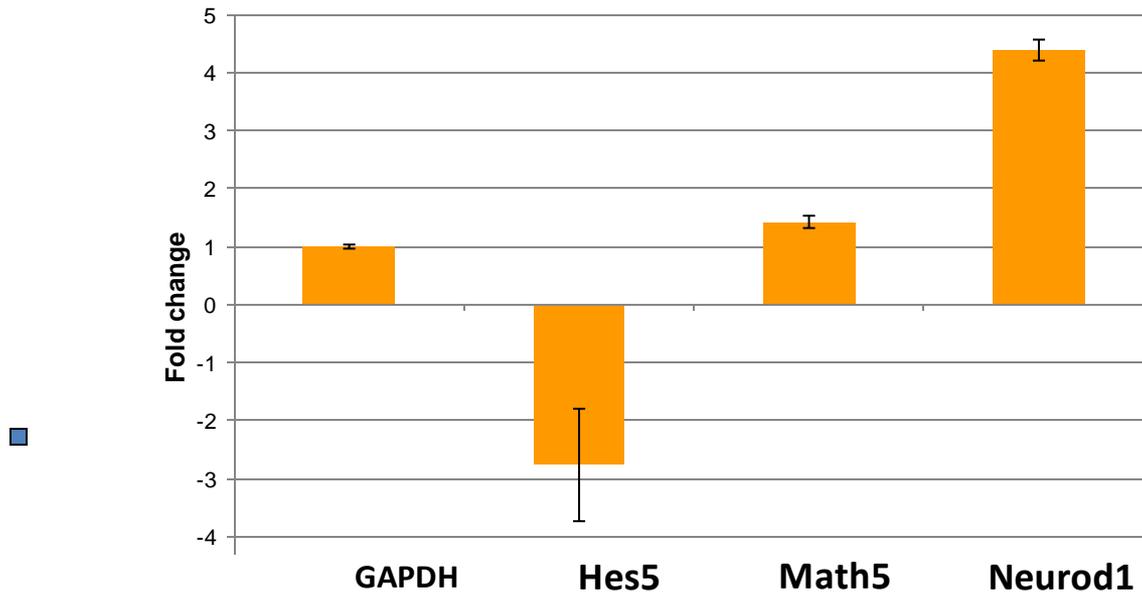
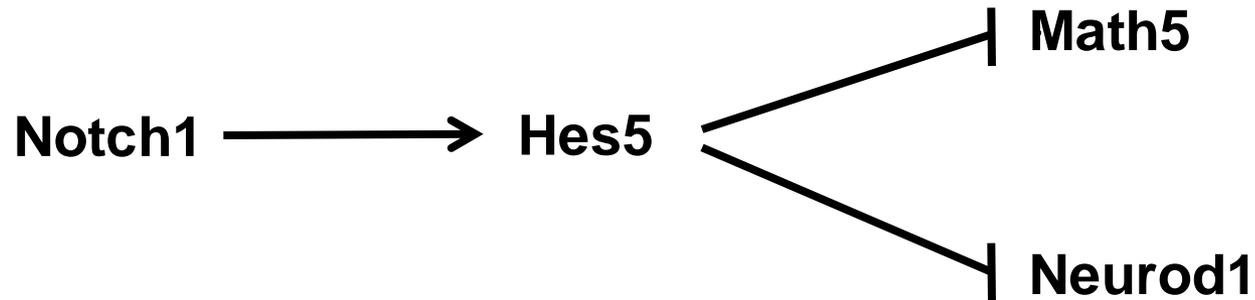


Presence of cyclin D1 on retinal promoters



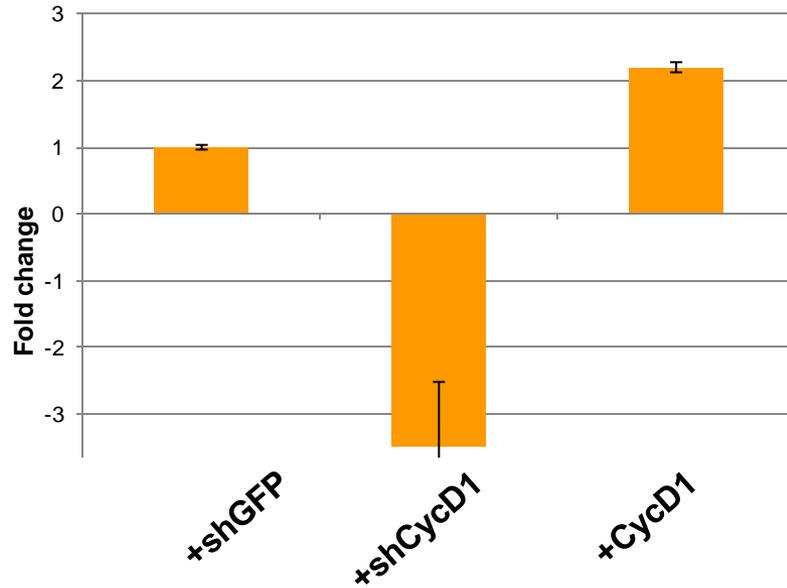


Notch1-signaling in cyclin D1^{-/-} retinas

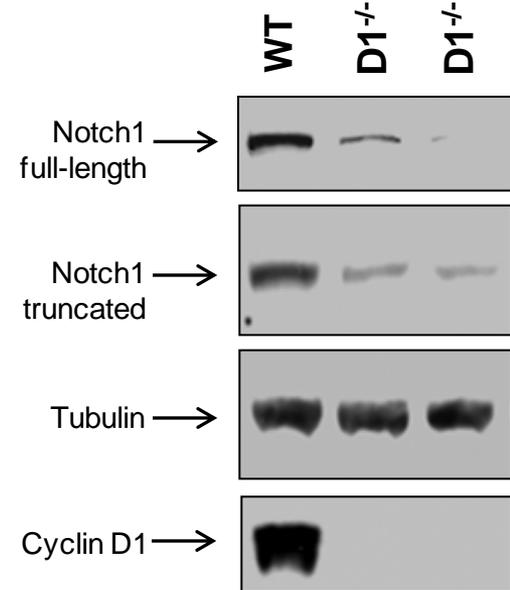


Notch1 levels are influenced by cyclin D1

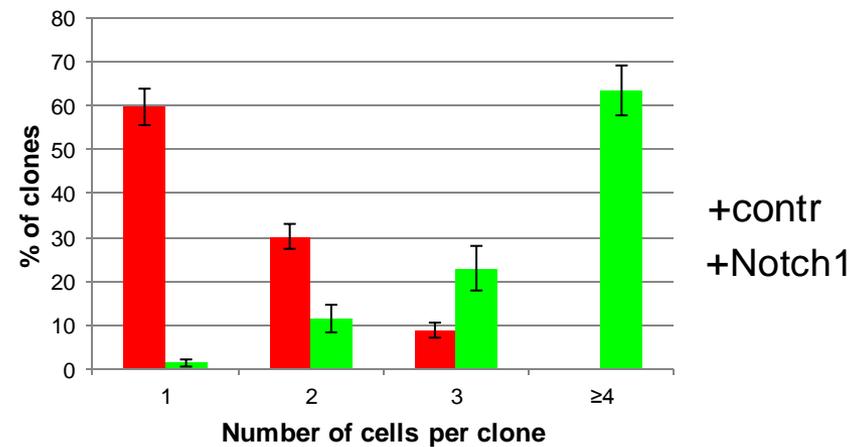
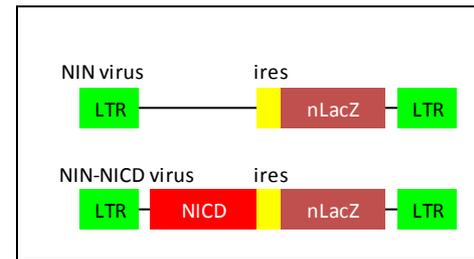
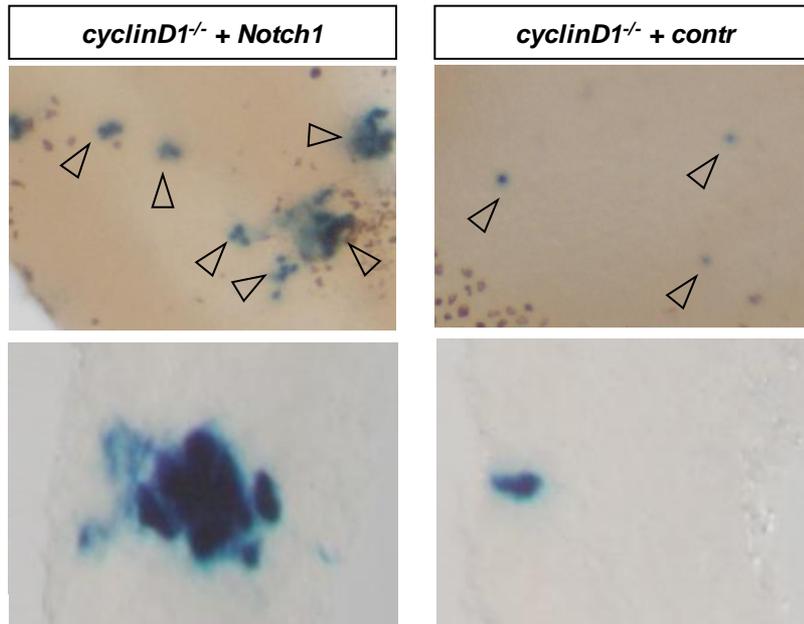
Notch1 levels in retinal precursor cells



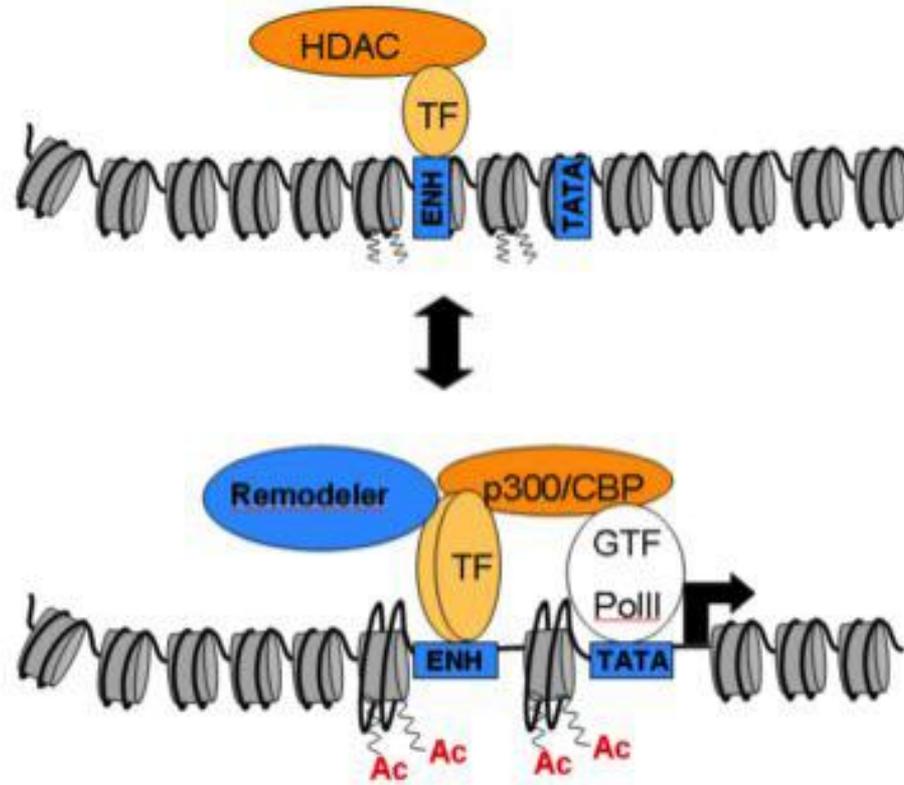
Retinas



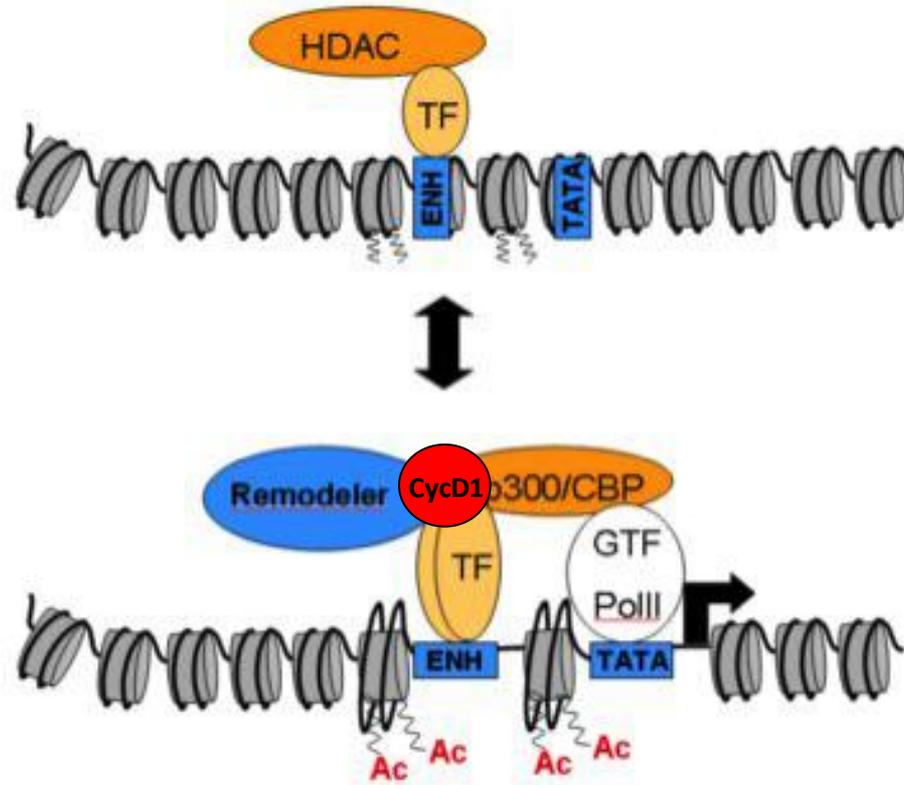
Transduction of cyclin D1^{-/-} retinas with Notch1



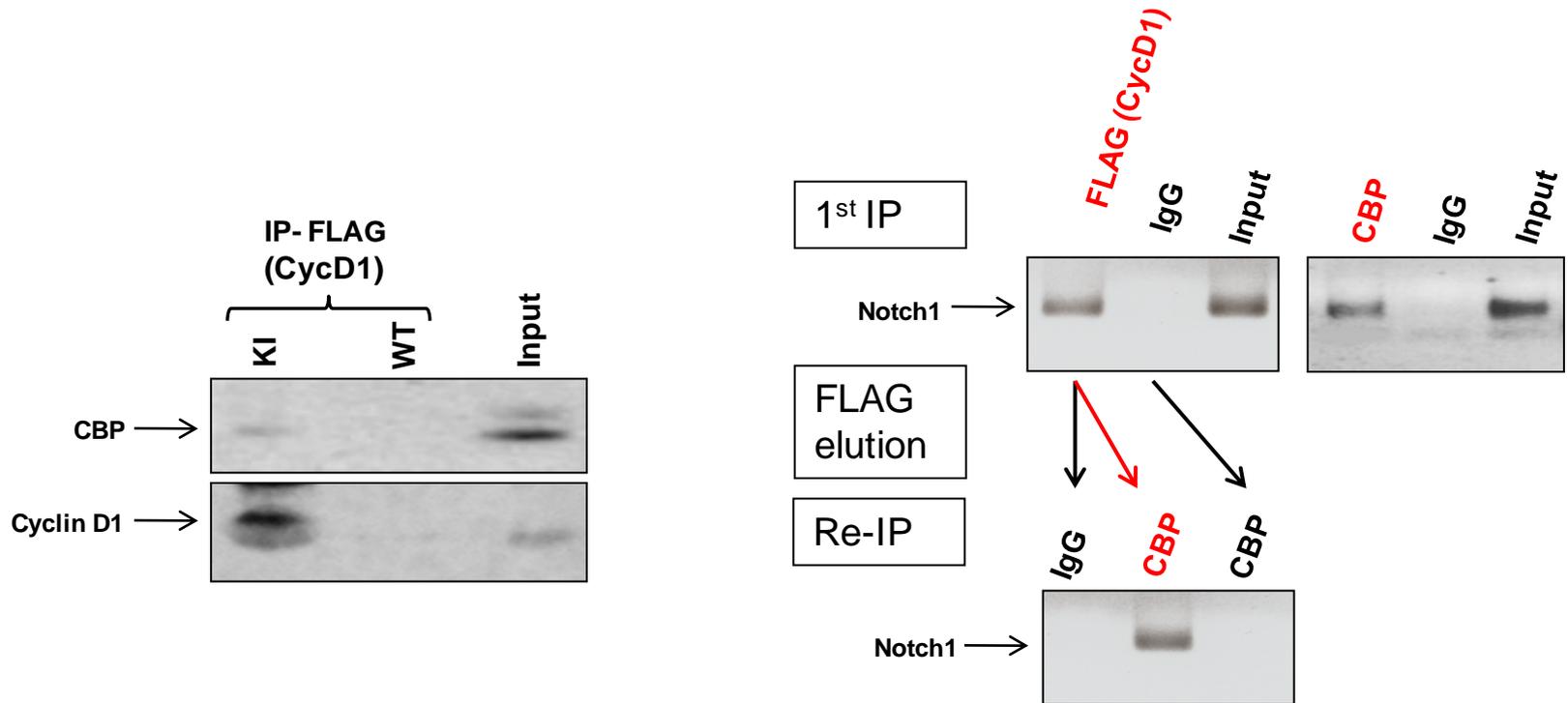
CBP histone acetyltransferase



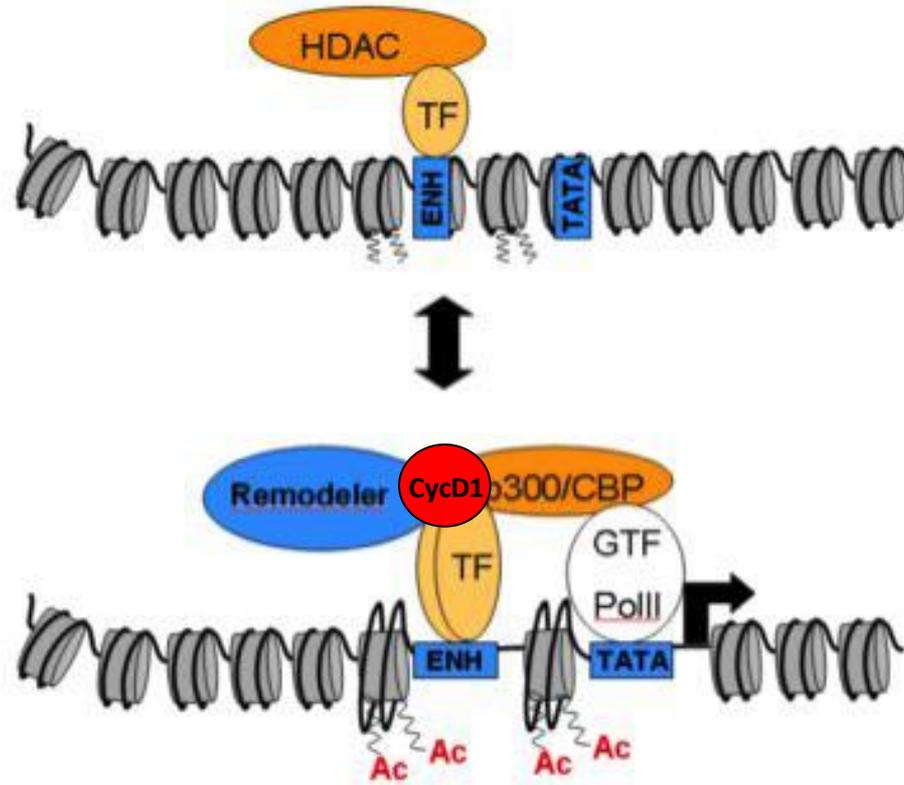
CBP histone acetyltransferase



Interaction of cyclin D1 with CBP in retinas

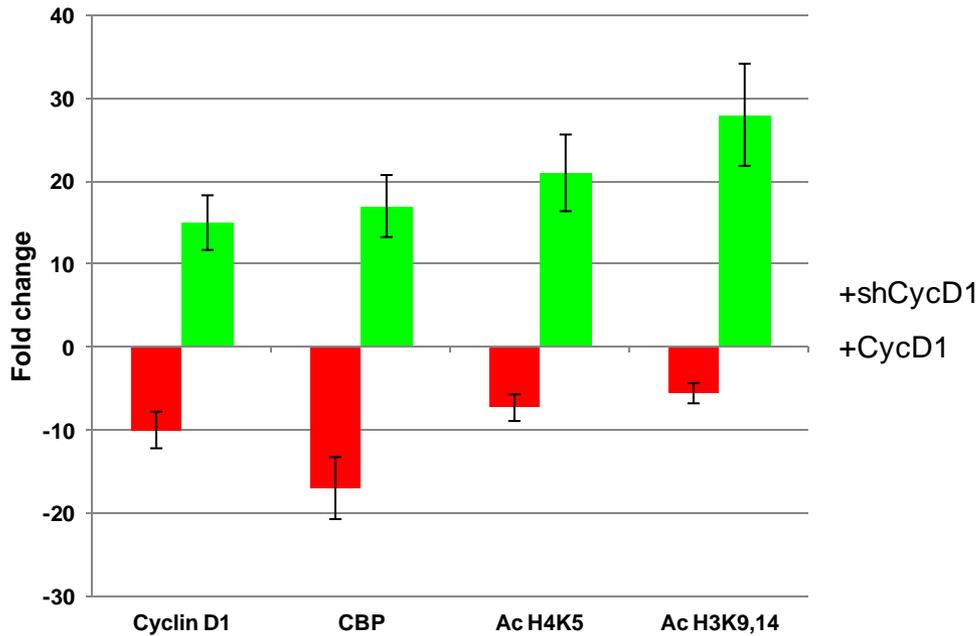


CBP histone acetyltransferase

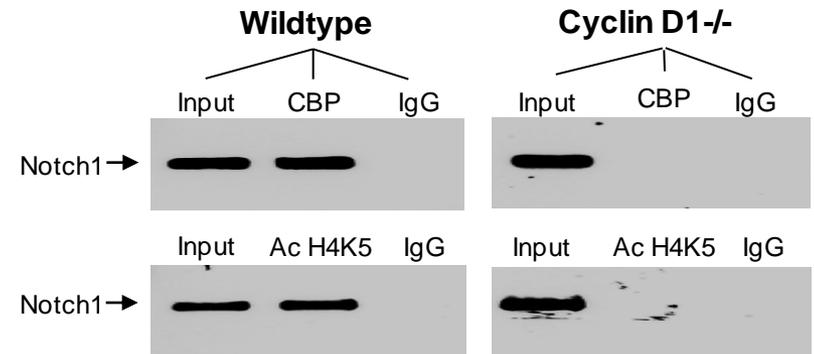


Interaction of cyclin D1 with CBP in retinas

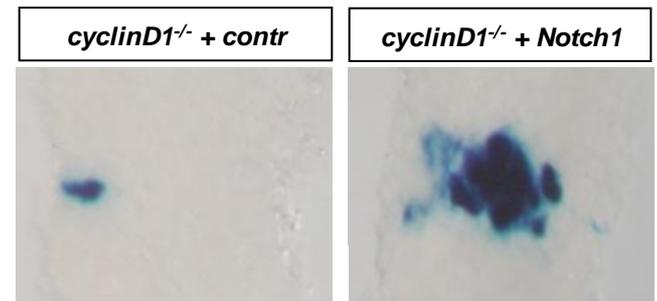
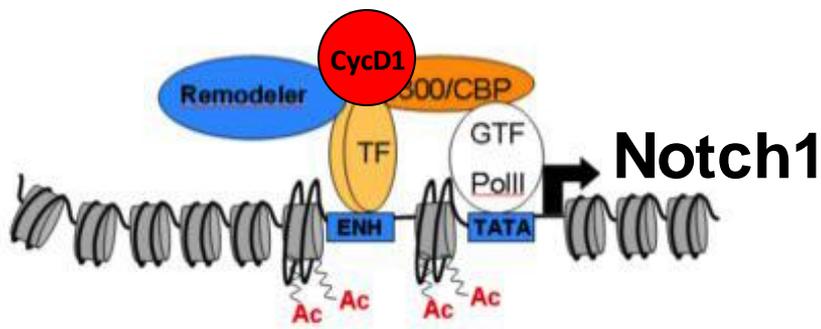
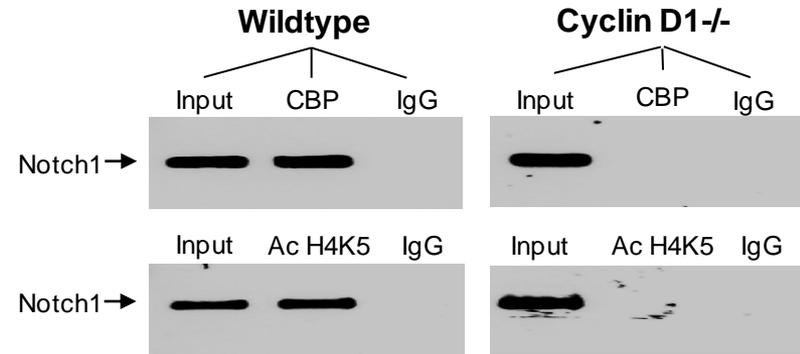
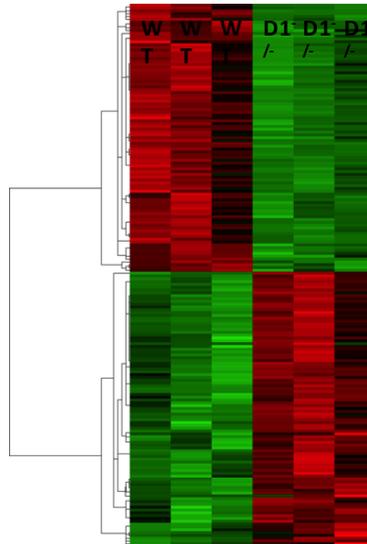
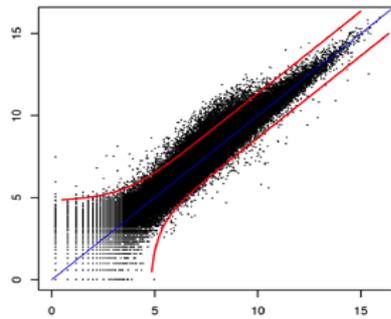
Retinal precursor cells



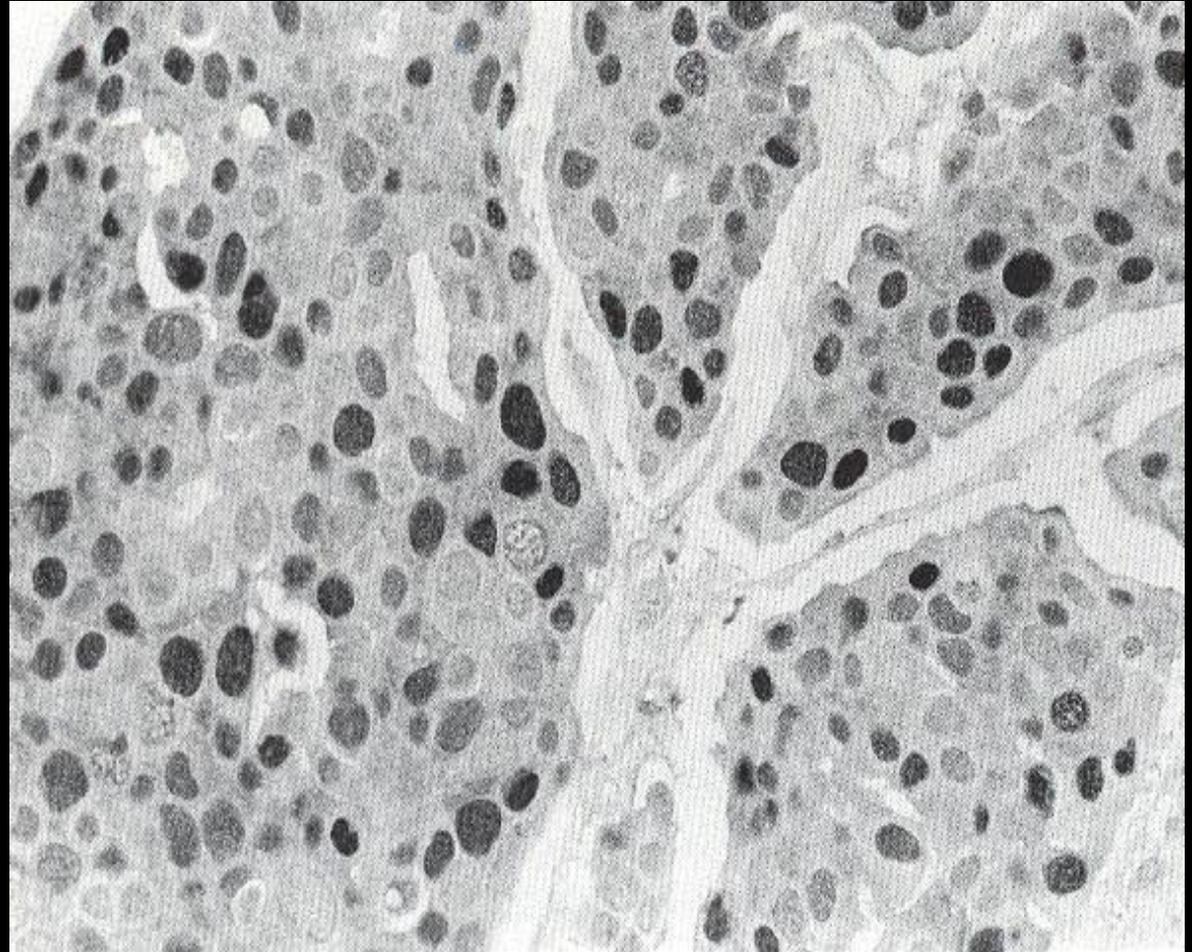
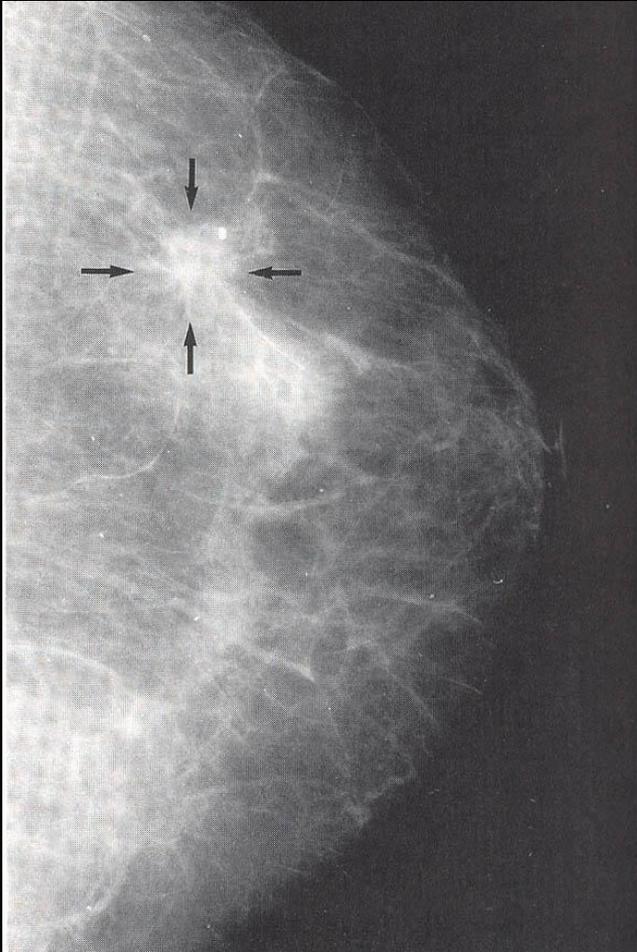
Retinas

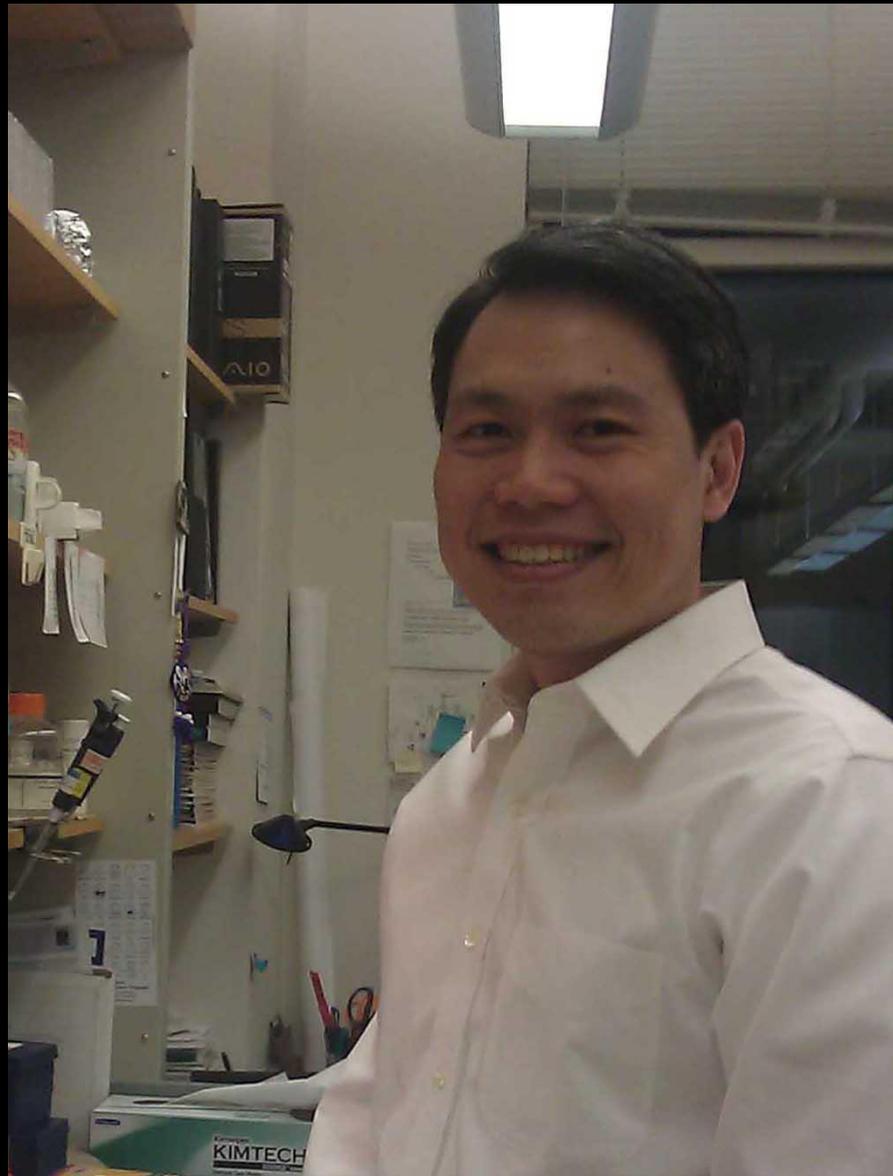


Summary I



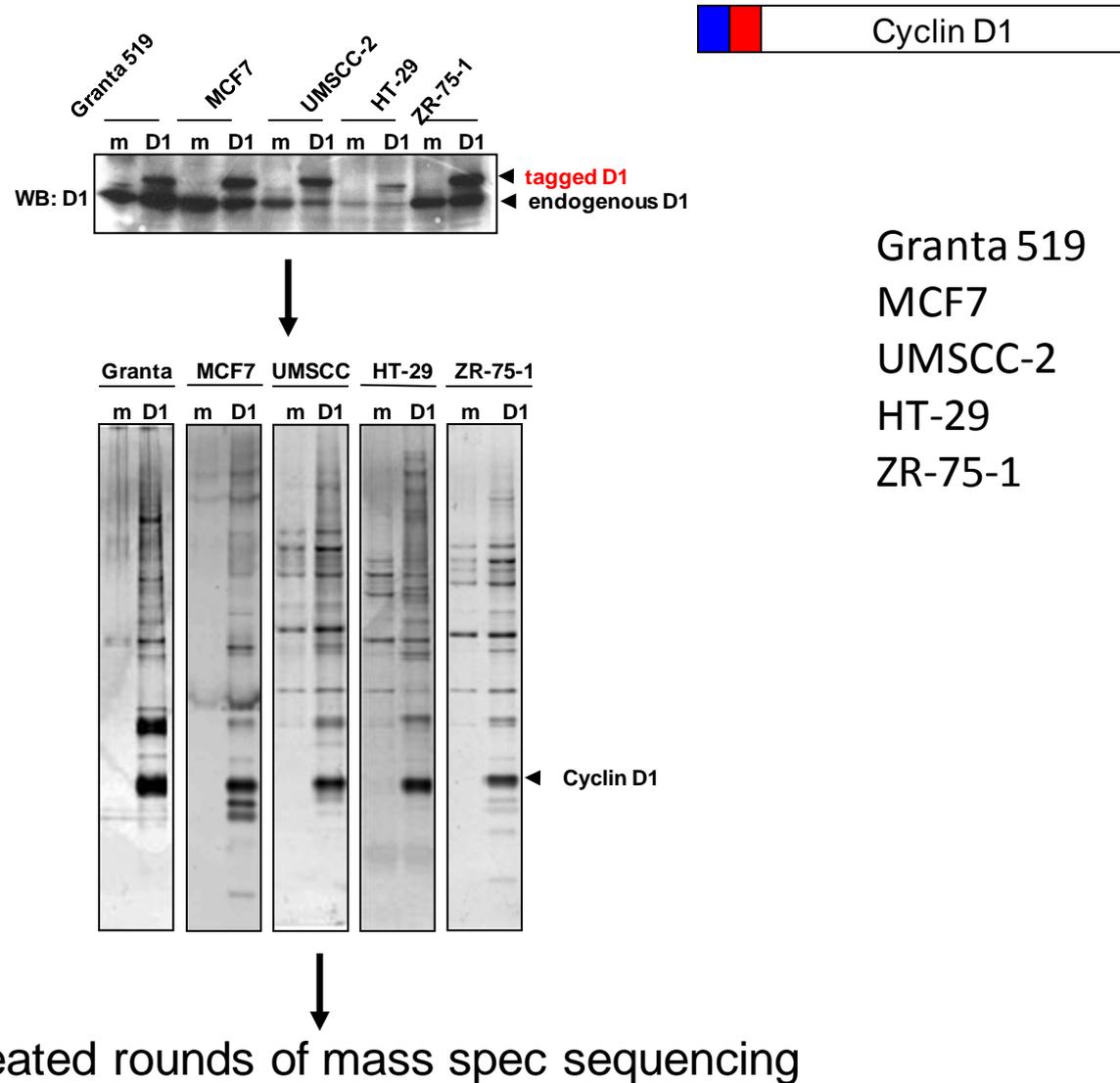
Cyclin D1 and cancer



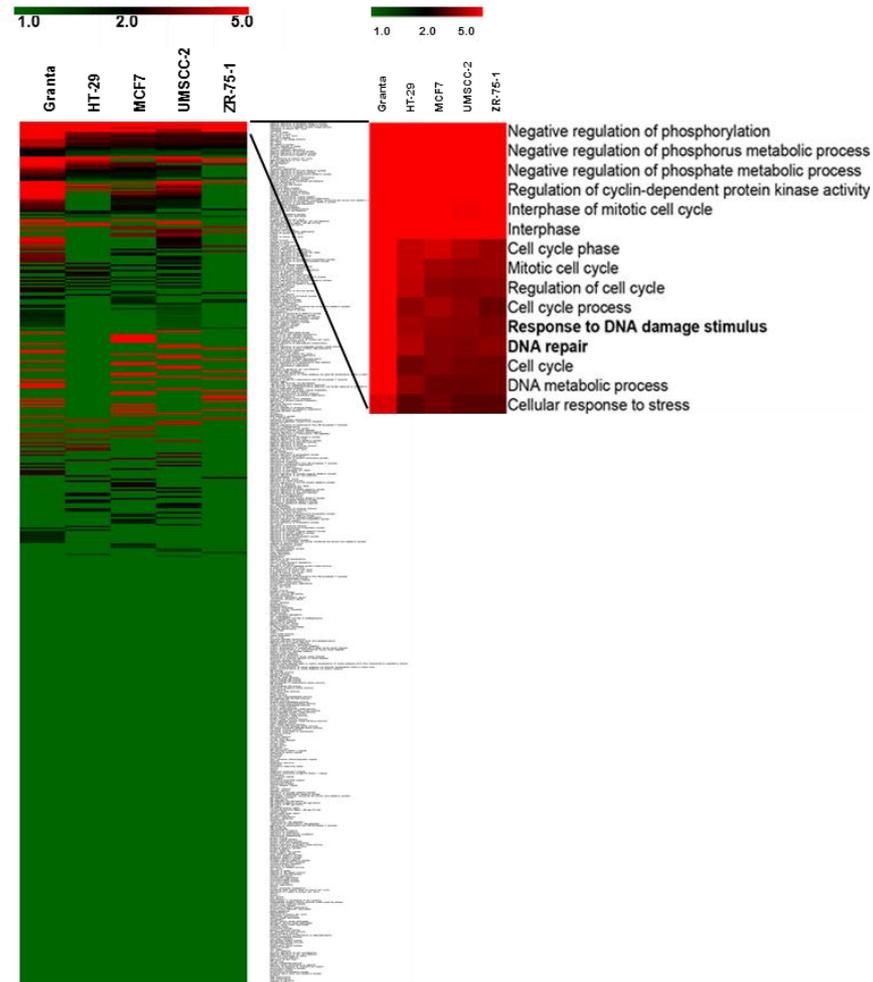


Siwanon Jirawatnotai

Purification of cyclin D1-associated proteins from human cancer cells

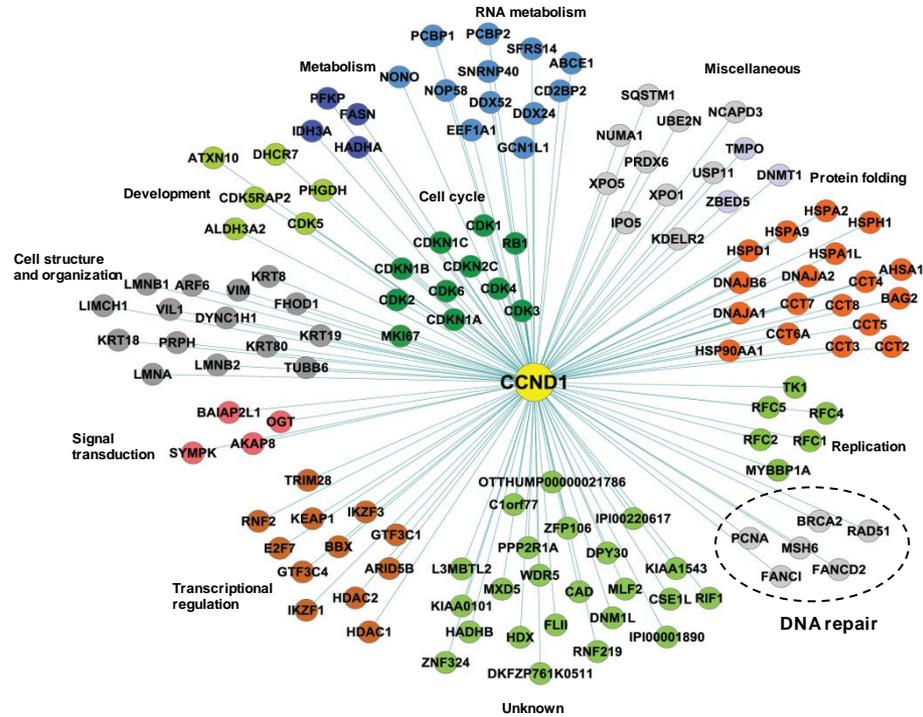


Bioinformatics analysis of cyclin D1 cancer “interactome”

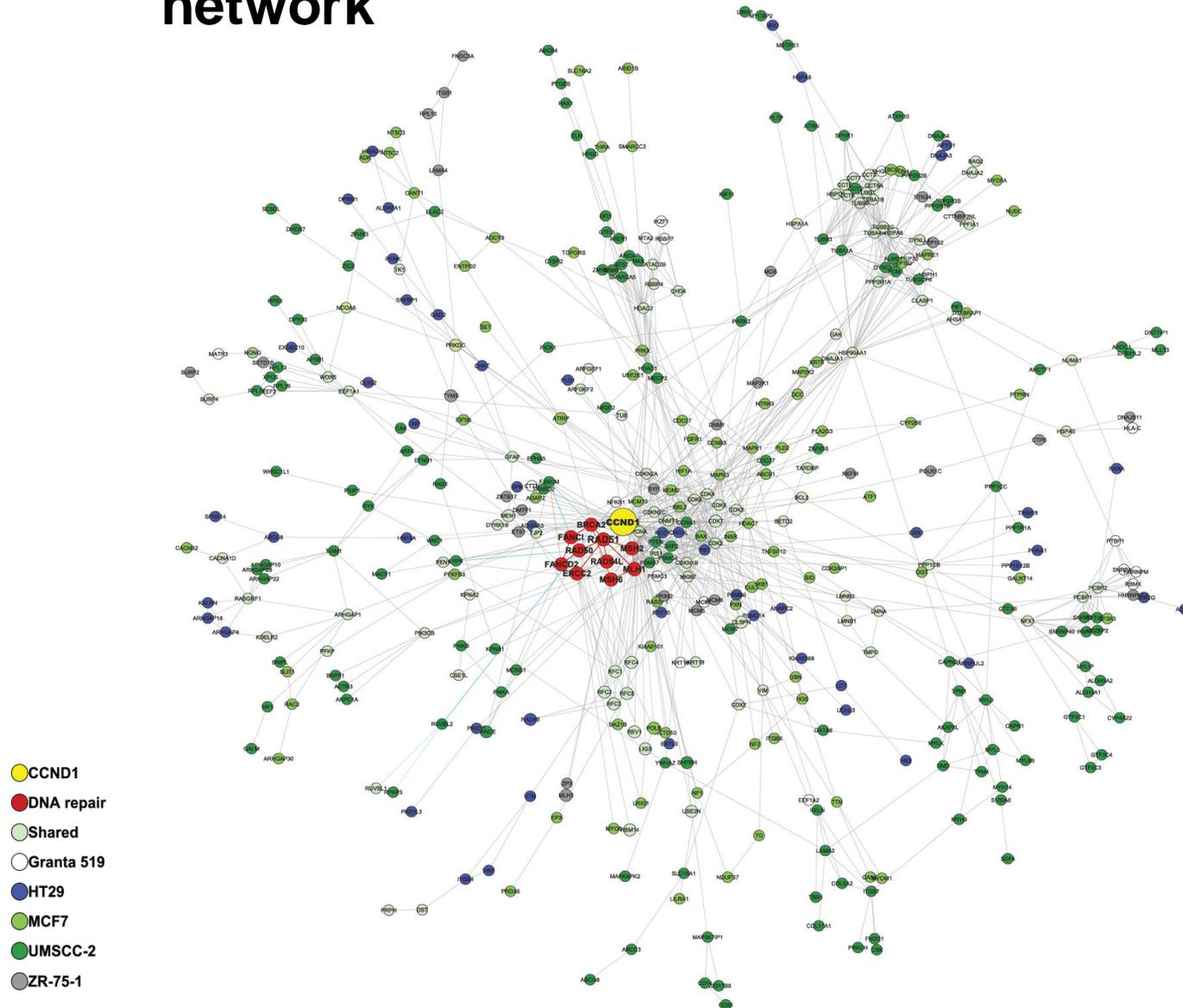


Yaoyu Wang
Mick Correll
John Quackenbush

Cyclin D1 cancer “interactome”

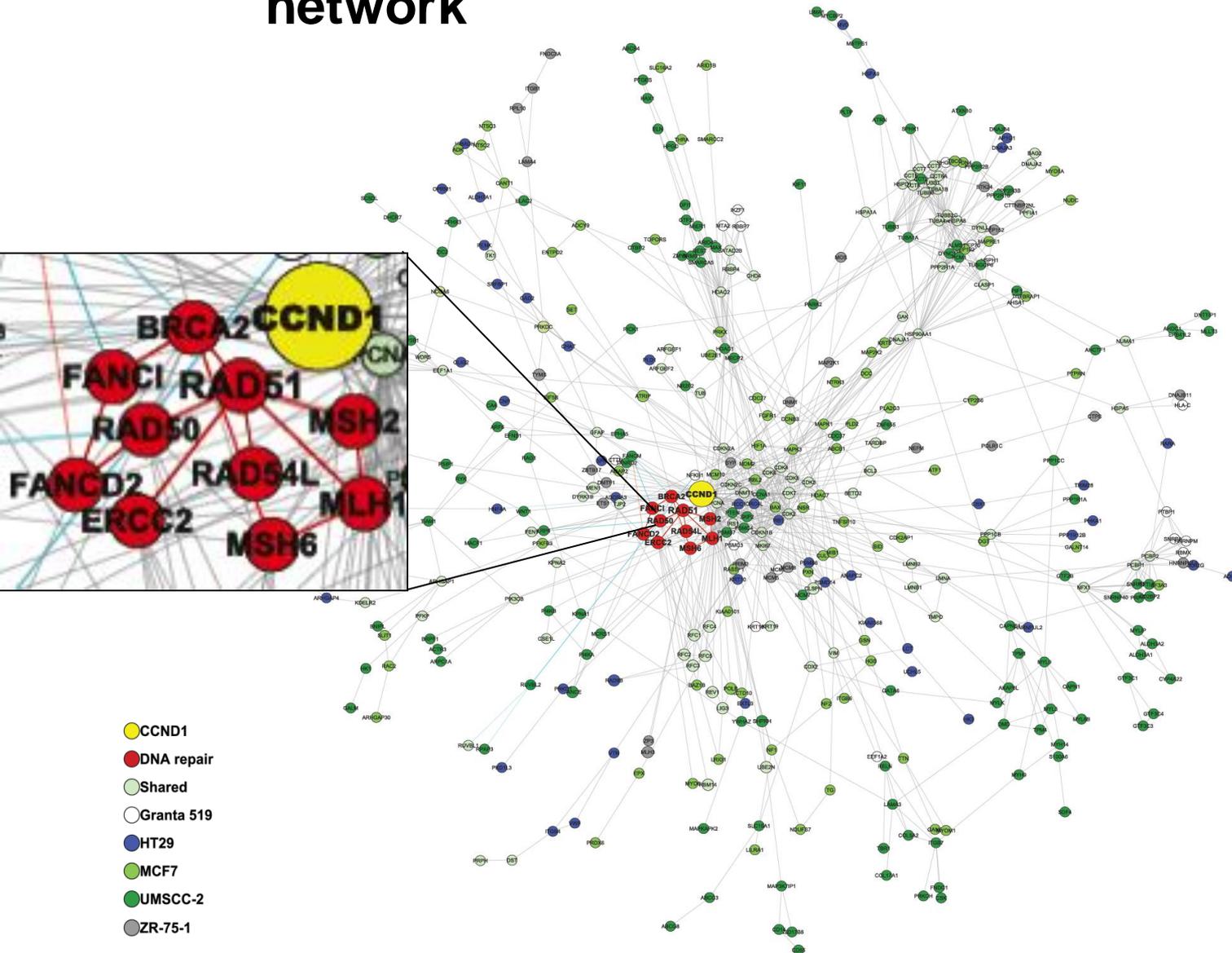


Cyclin D1 cancer “interactome” network



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Mick Correll
John Quackenbush

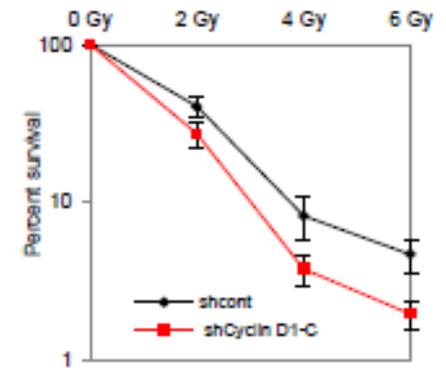
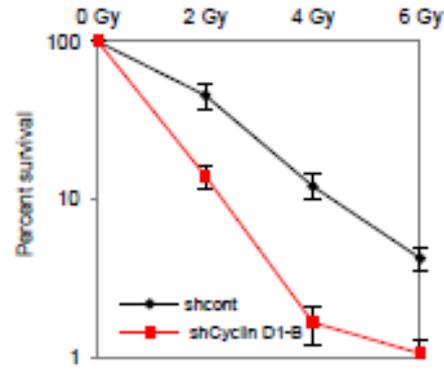
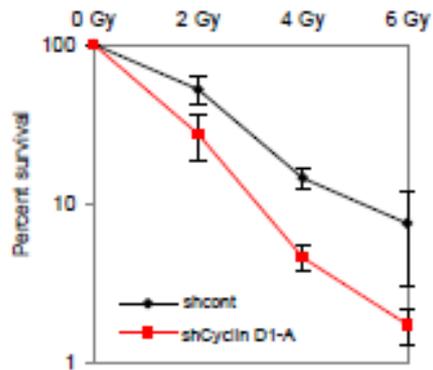
Cyclin D1 cancer “interactome” network



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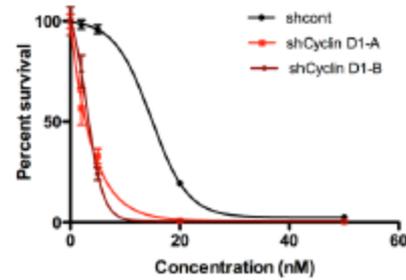
Analyses of radiation sensitivity

HeLa

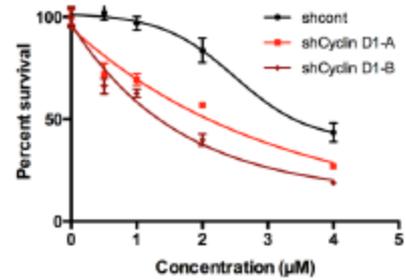


Sensitivity to DNA damaging agents

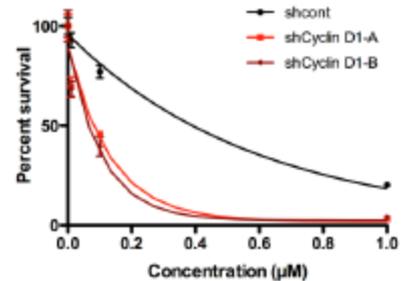
Camptothecin



Etoposide

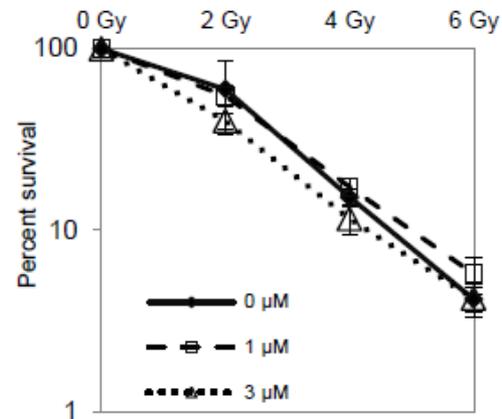
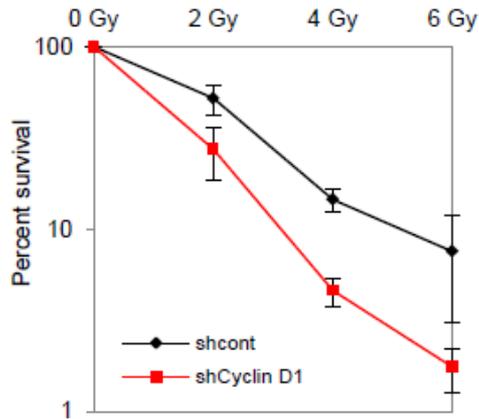


Olaparib



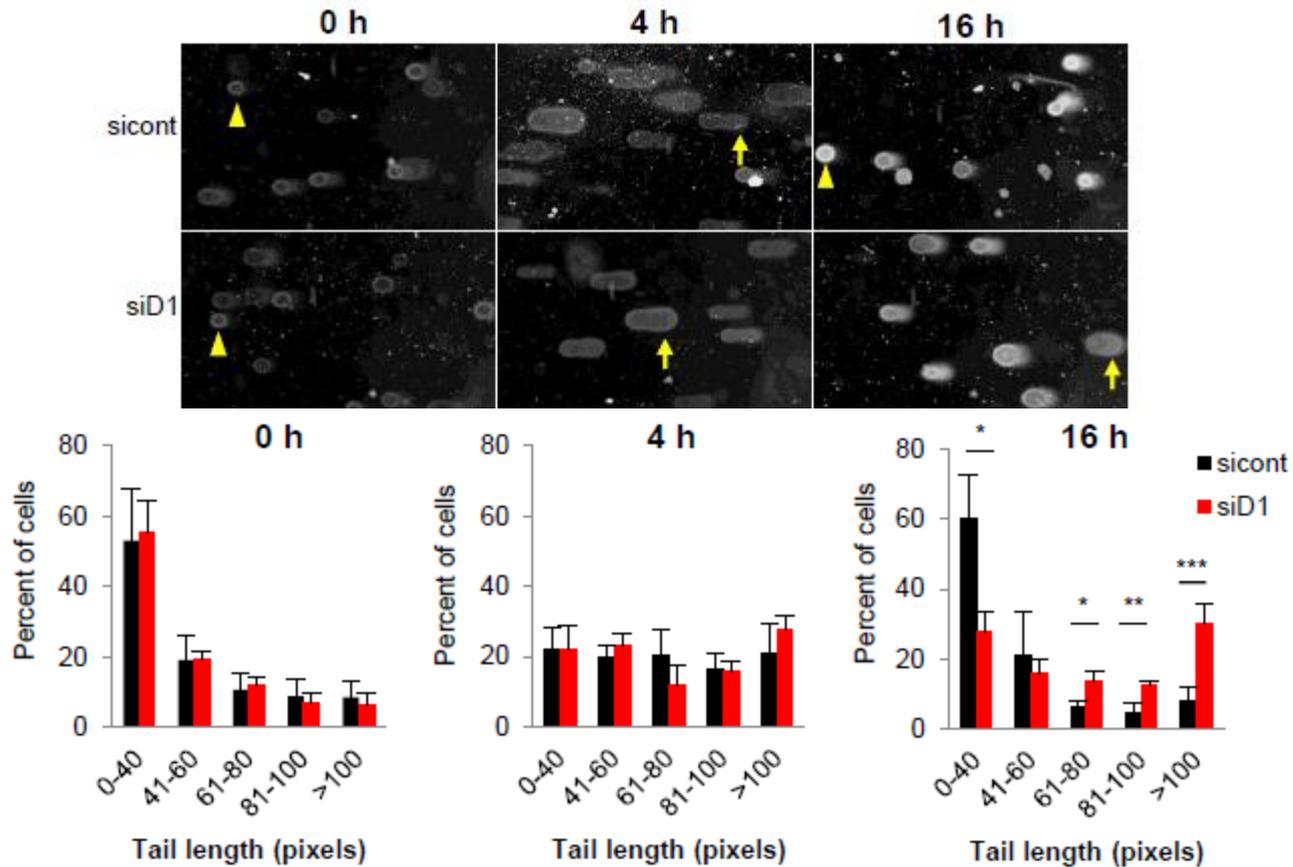
CDK kinase-independent function in DNA damage response

HeLa

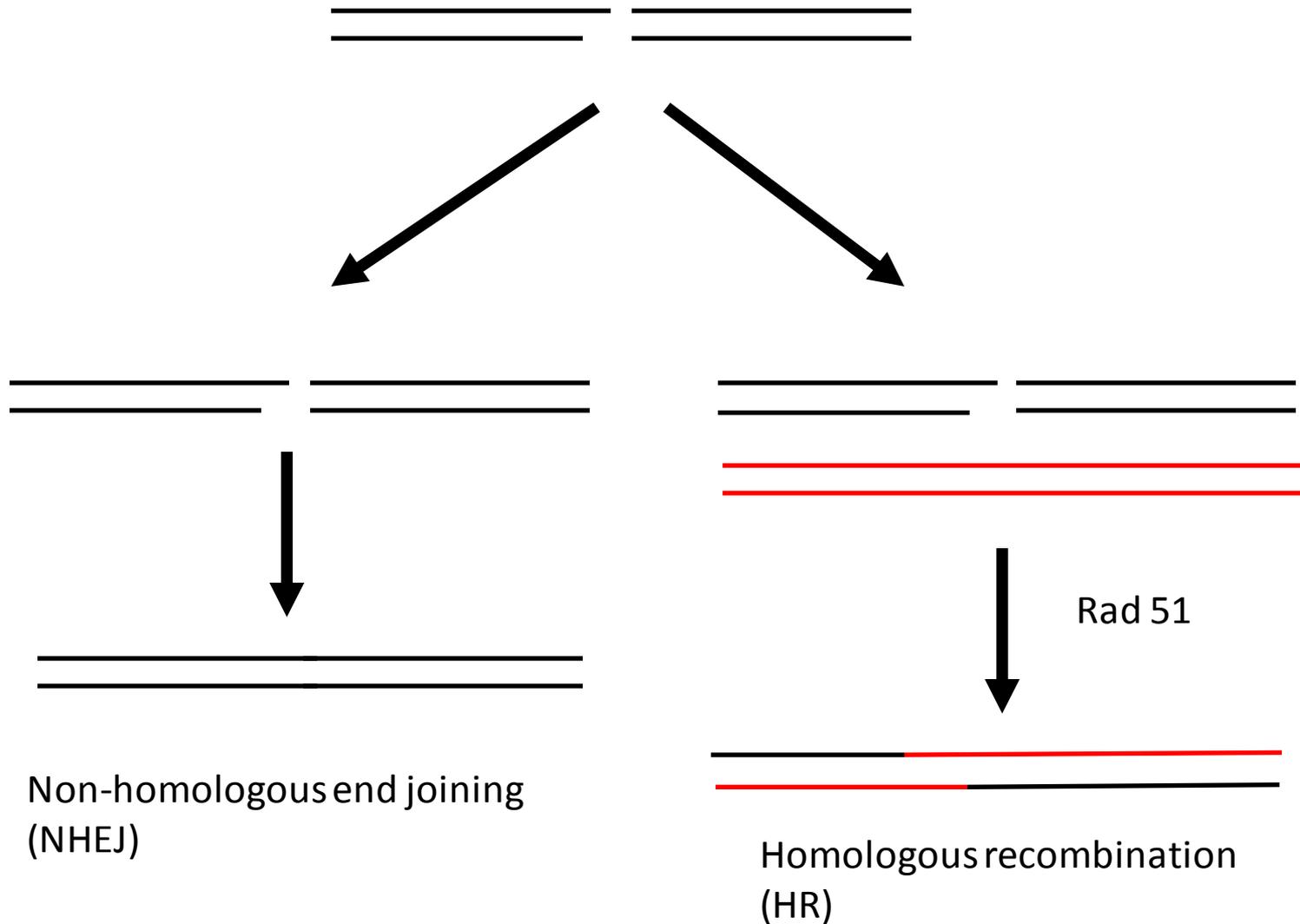


PD 0332991

Comet assays to measure broken DNA



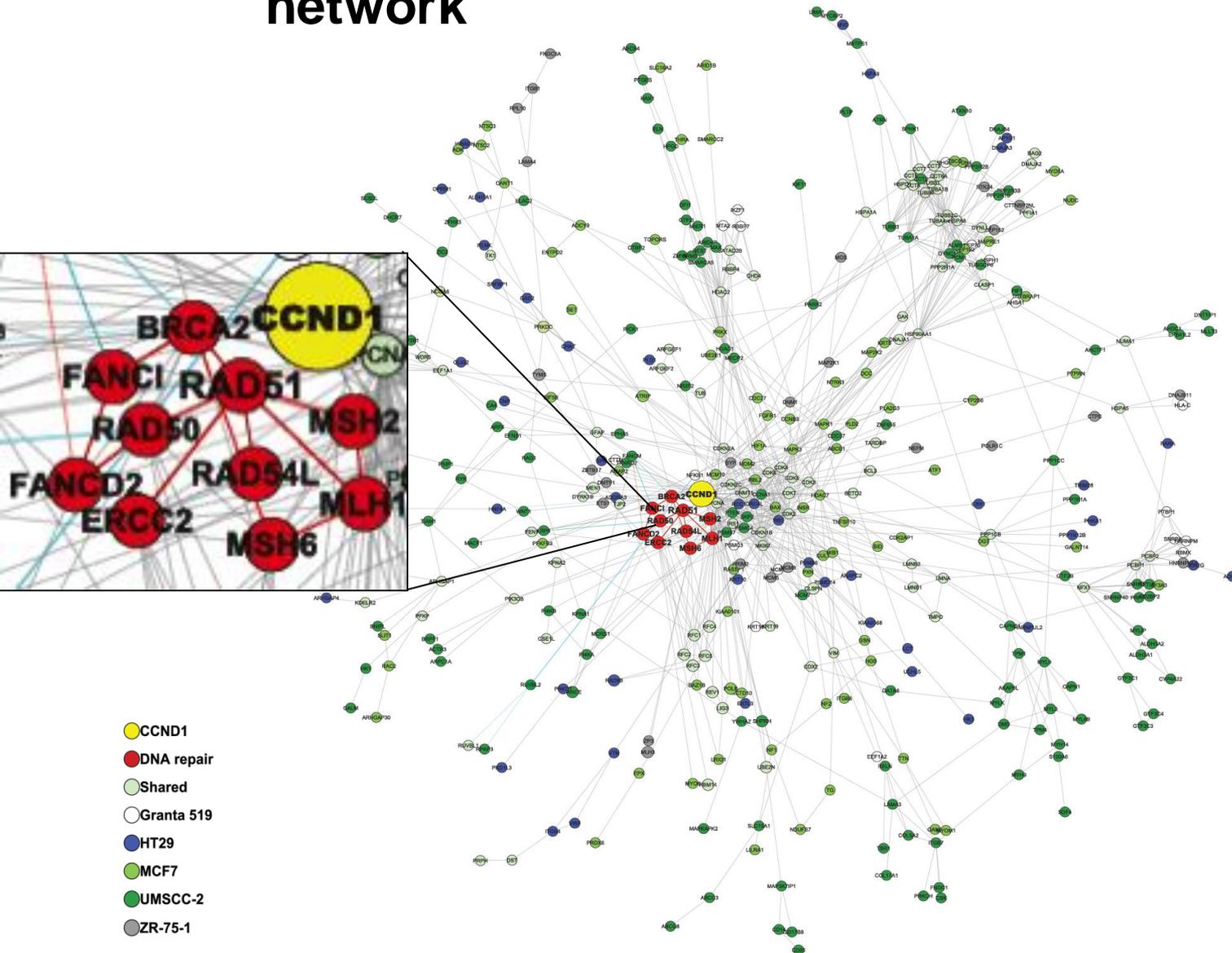
DNA repair



Non-homologous end joining
(NHEJ)

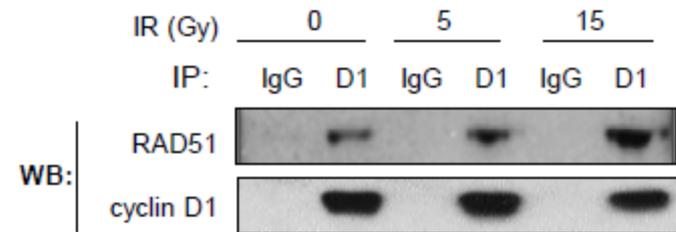
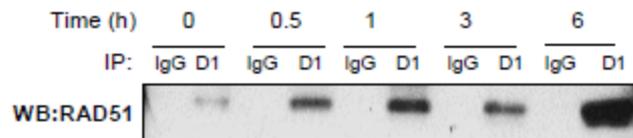
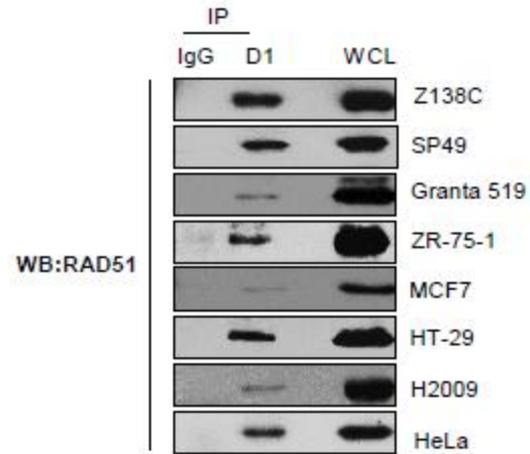
Homologous recombination
(HR)

Cyclin D1 cancer “interactome” network

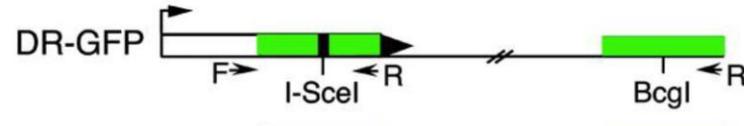


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John Quackenbush

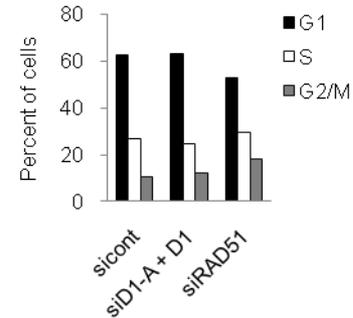
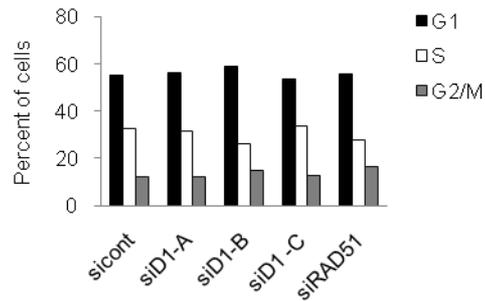
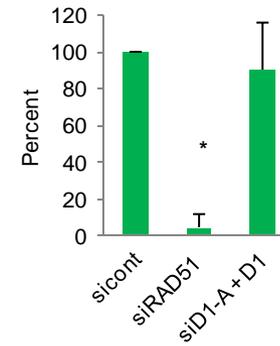
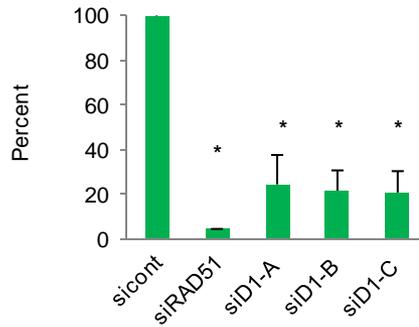
Analyses of cyclin D1 - Rad51 interaction *in vivo*



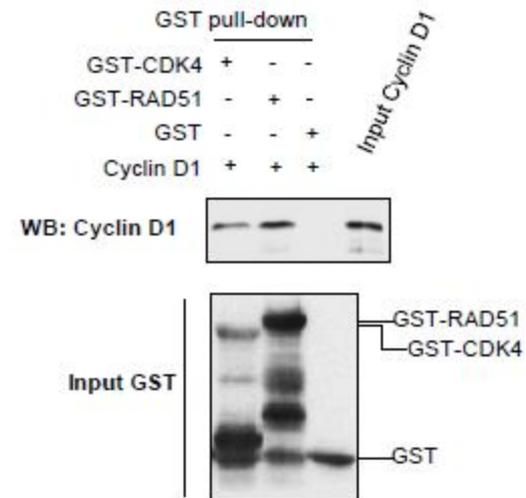
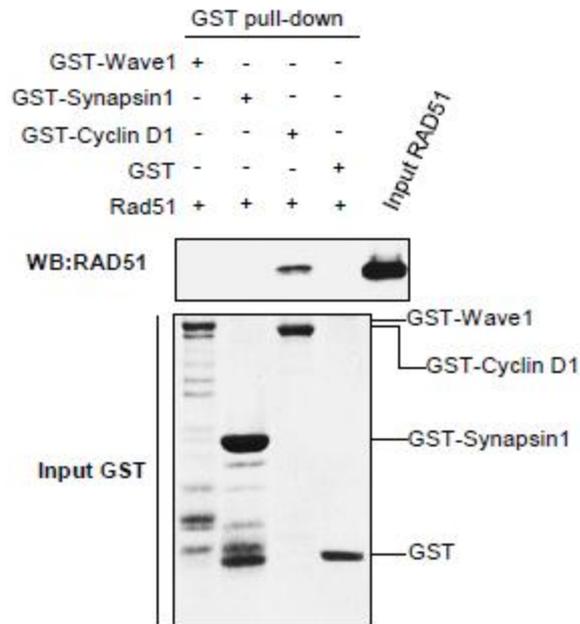
Homologous recombination assays



Pierce A., et al. 1999

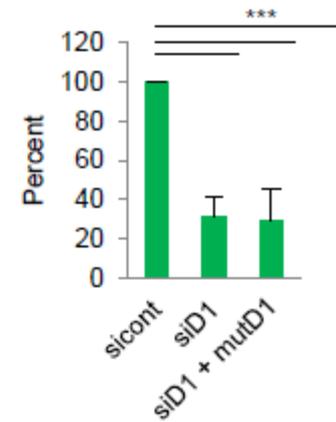
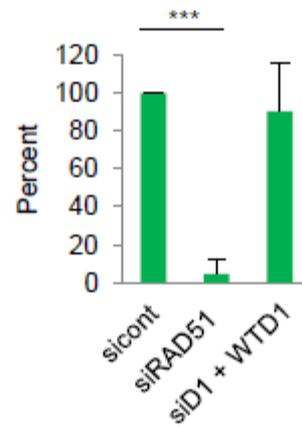
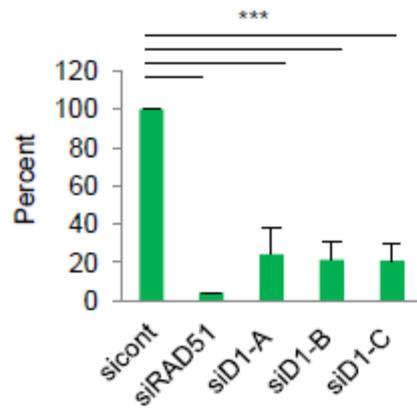
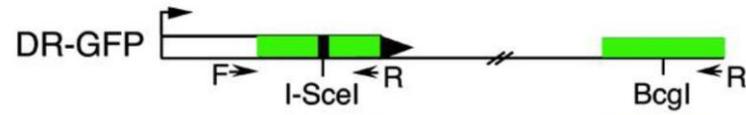


Analyses of cyclin D1 - Rad51 interaction *in vitro*

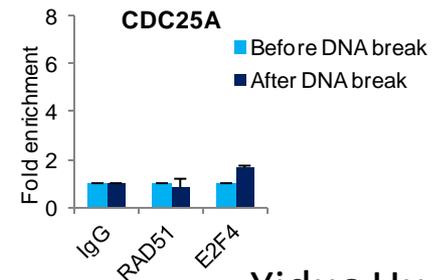
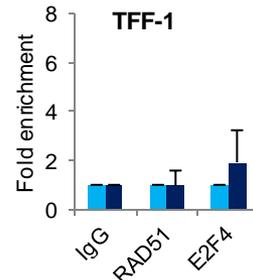
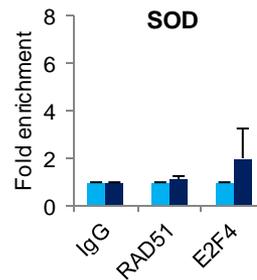
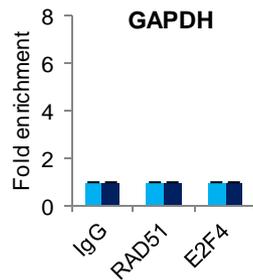
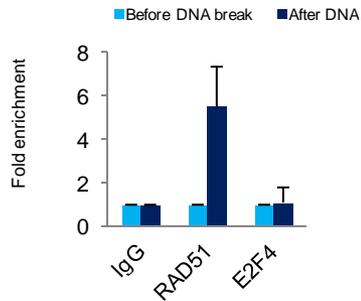
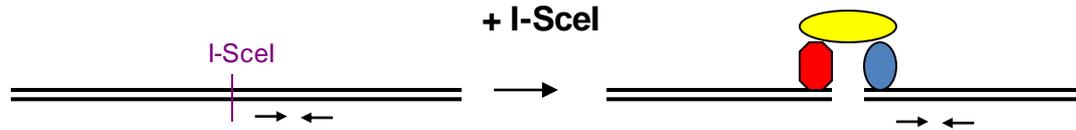


Wojciech Michowski

Homologous recombination assays

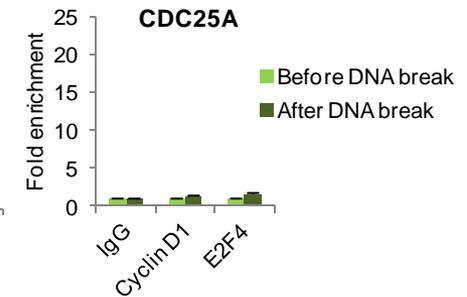
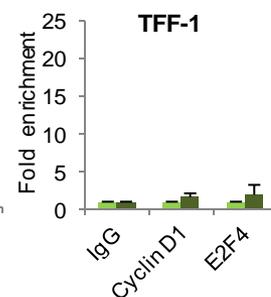
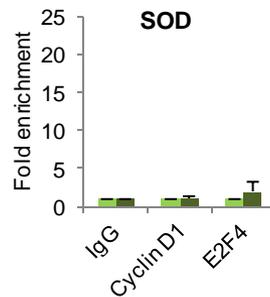
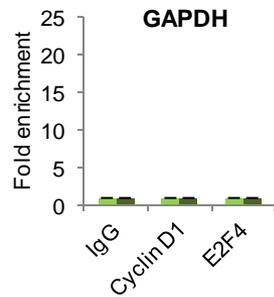
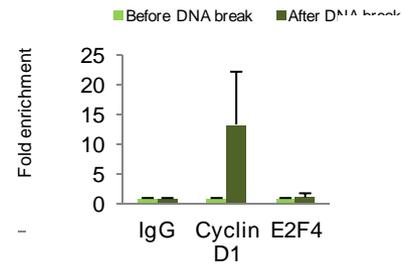
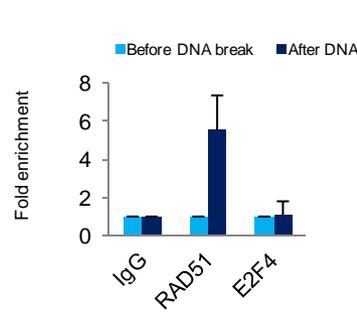
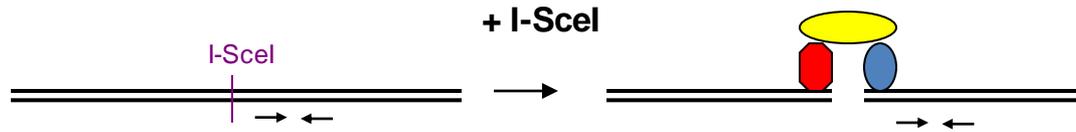


Localization of Rad51 to ds DNA breaks



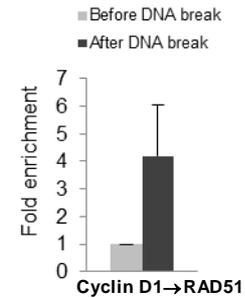
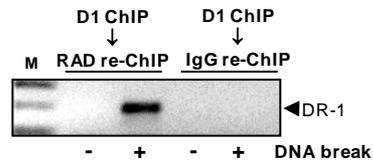
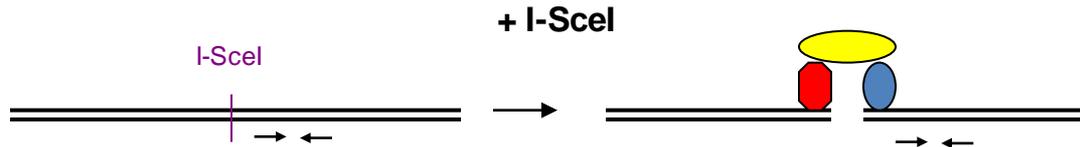
Yiduo Hu
David Livingston

Localization of cyclin D1 to ds DNA breaks



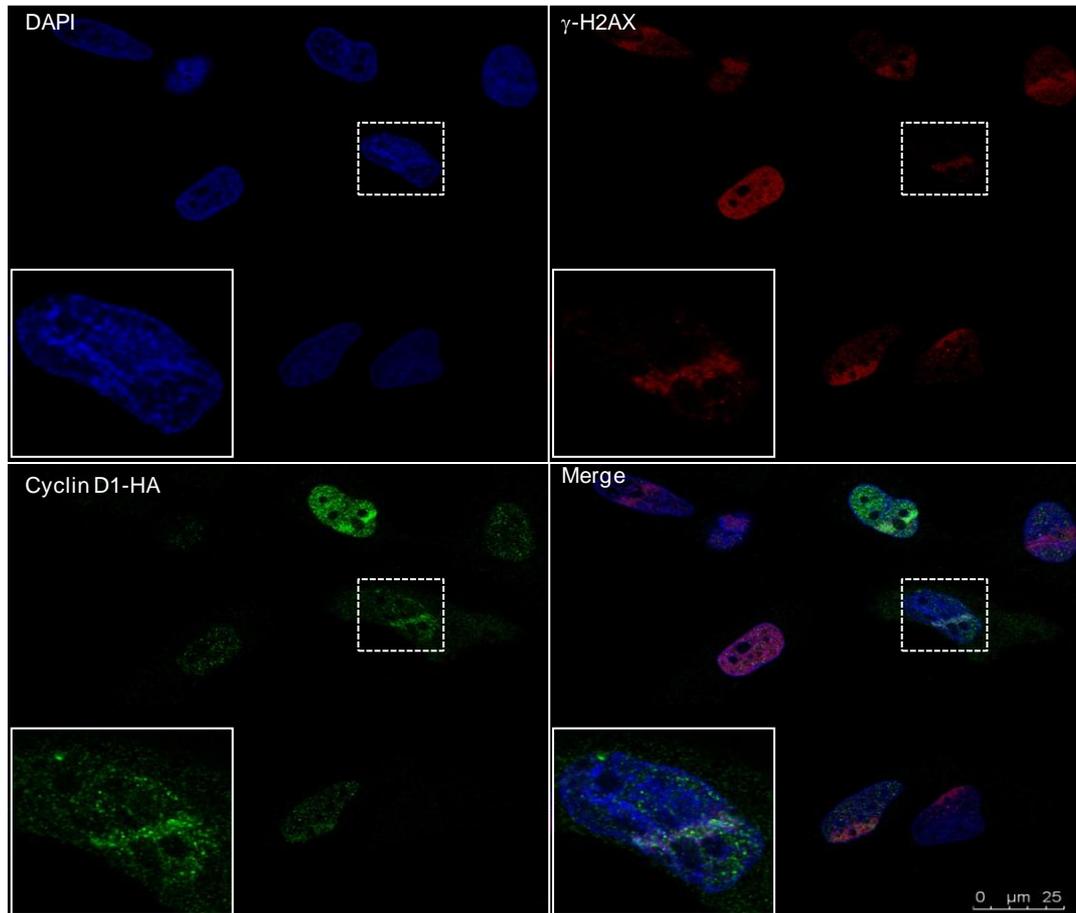
Yiduo Hu
David Livingston

Co-localization of cyclin D1 and Rad51 on ds DNA breaks



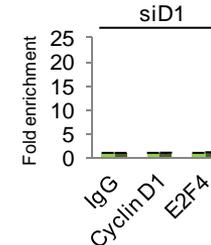
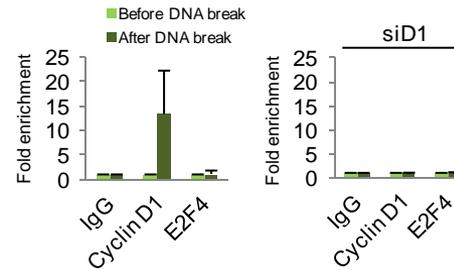
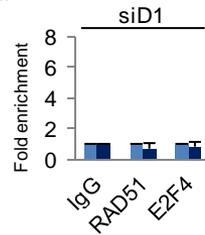
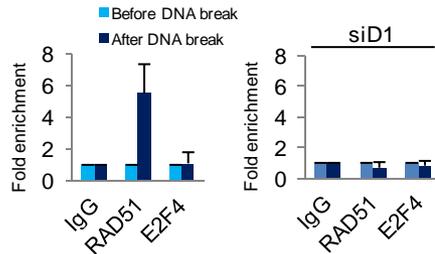
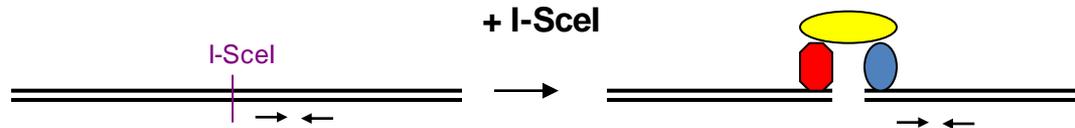
Yiduo Hu
David Livingston

Localization of cyclin D1 on DNA damage stripes

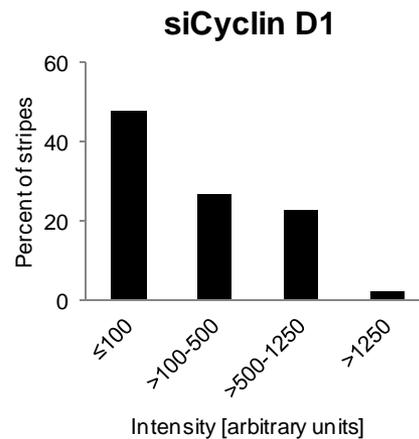
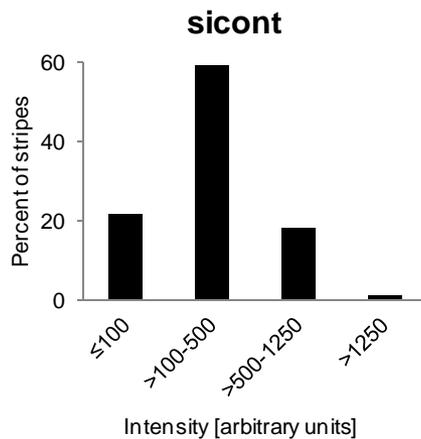
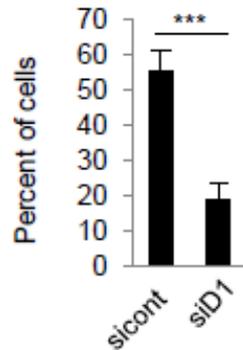


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David Livingston

Impaired recruitment of Rad51 to ds DNA breaks in cyclin D1-depleted cells

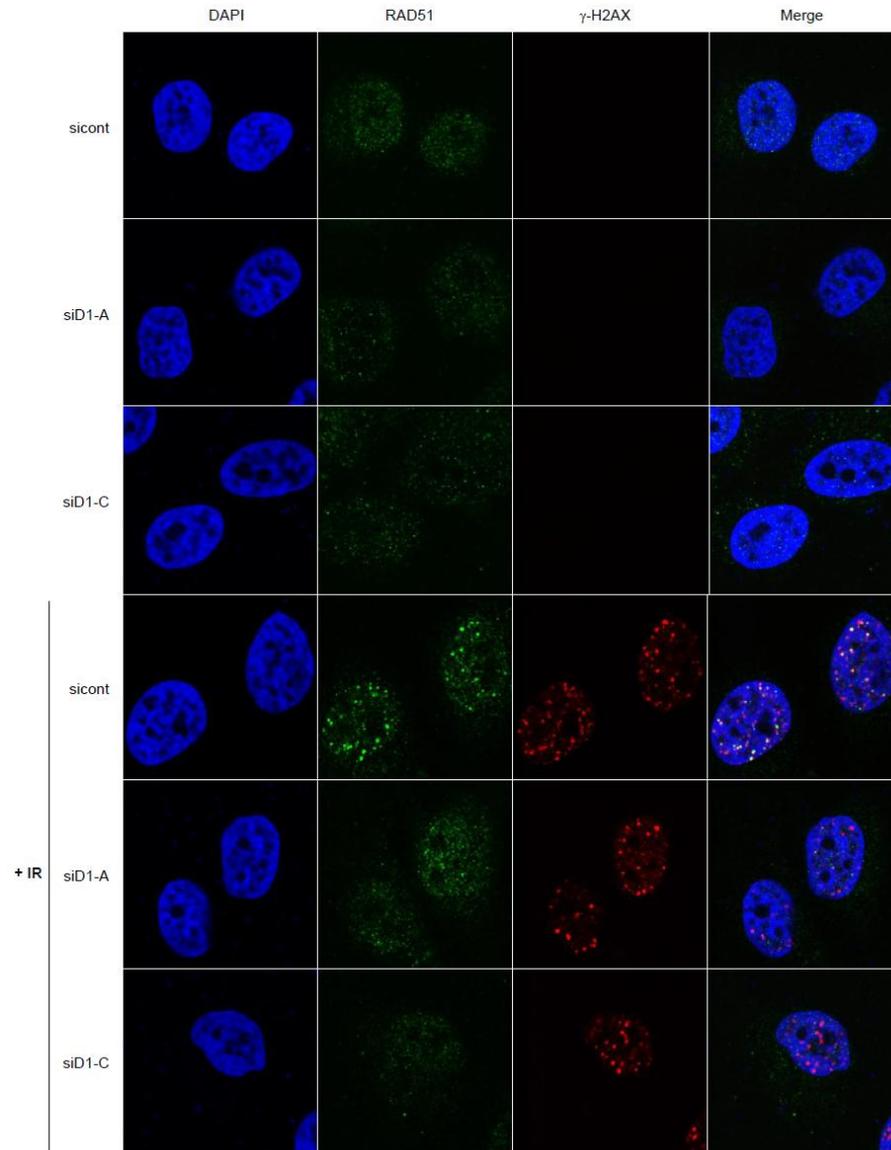


Impaired recruitment of Rad51 to DNA damage stripes in cyclin D1-depleted cells



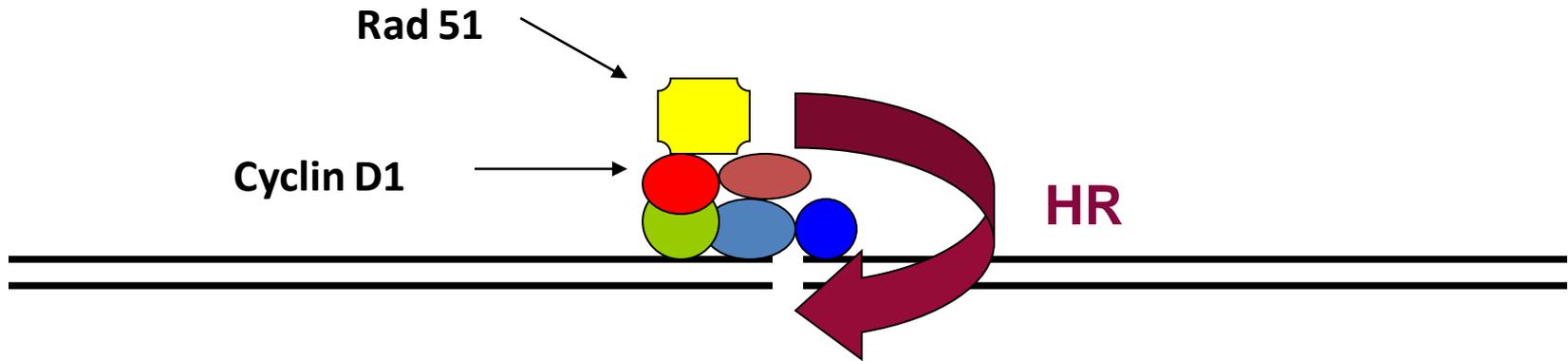
Yiduo Hu
David Livingston

Impaired recruitment of Rad51 to DNA damage foci in cyclin D1-depleted cells



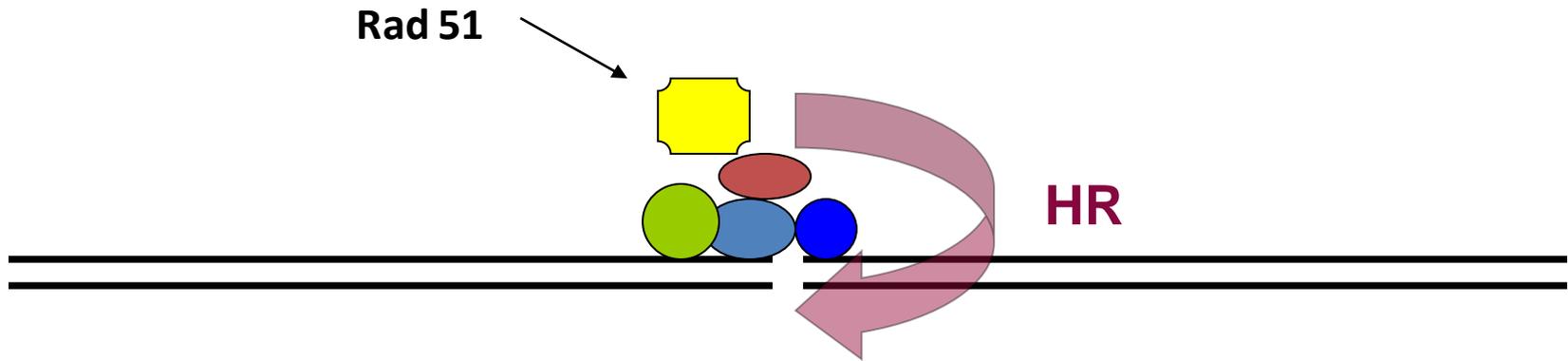
MODEL

Cyclin D1 localizes to DNA breaks and helps to recruit RAD51



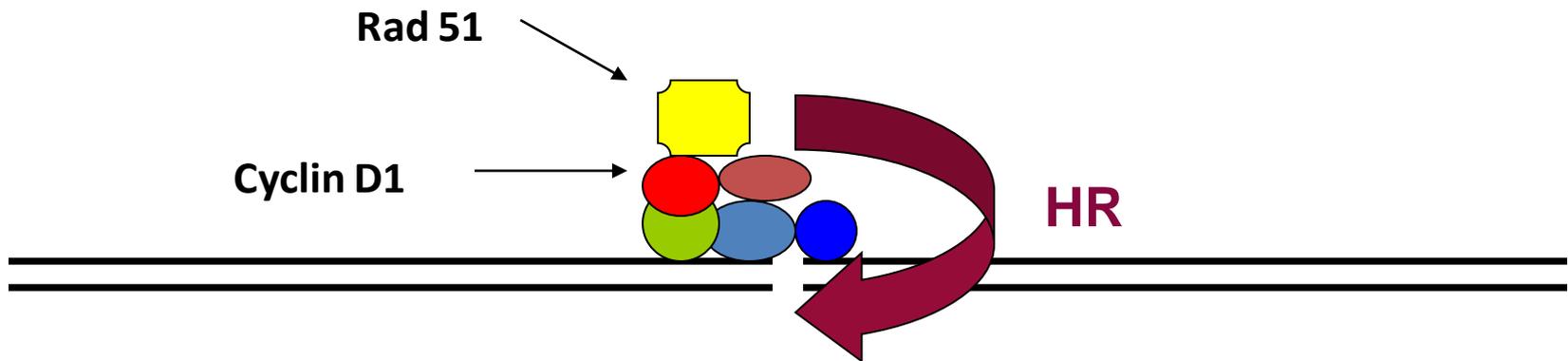
MODEL

Cyclin D1 localizes to DNA breaks and helps to recruit RAD51



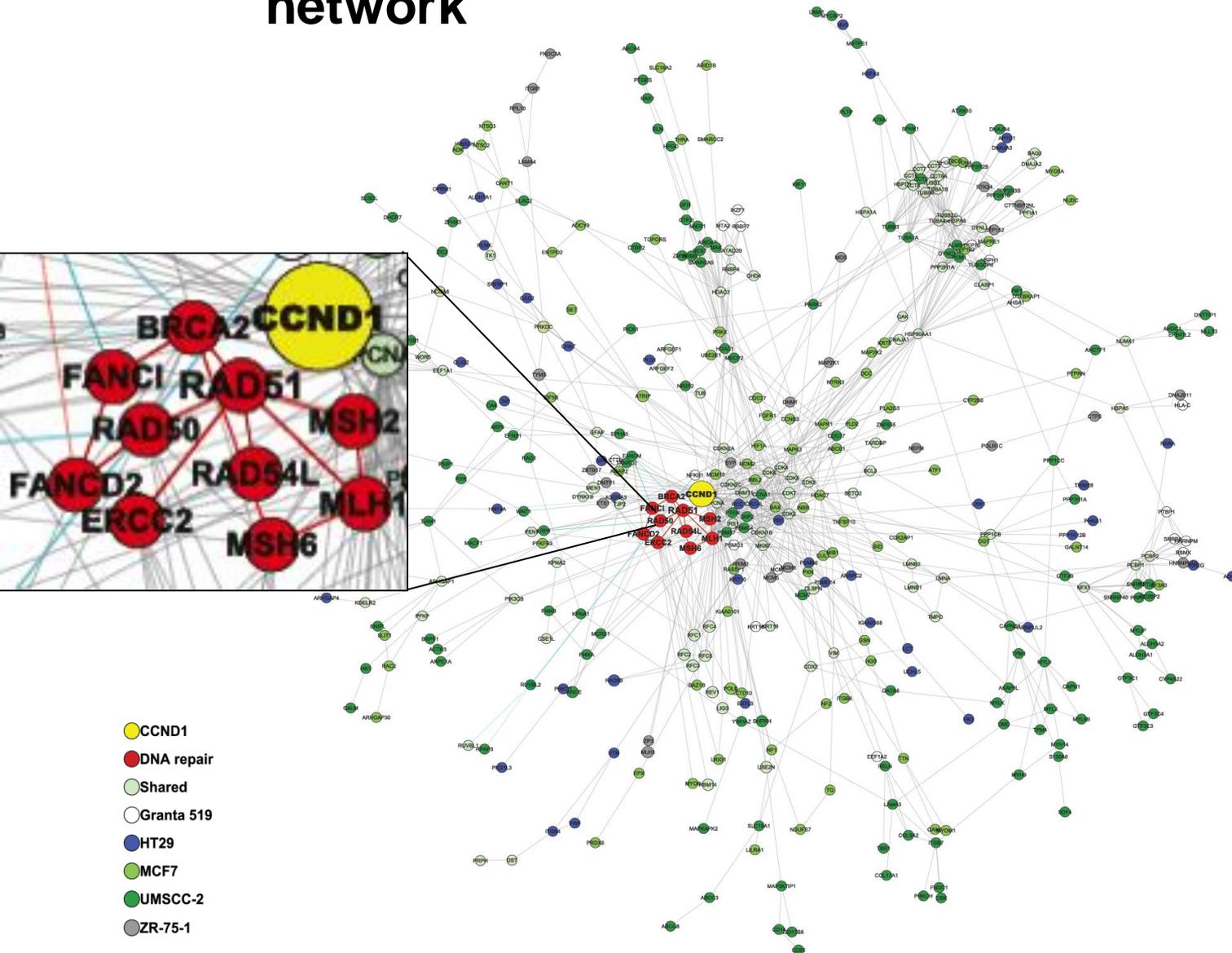
MODEL

Cyclin D1 localizes to DNA breaks and helps to recruit RAD51



How is cyclin D1 recruited to DNA breaks?

Cyclin D1 cancer “interactome” network



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John Quackenbush

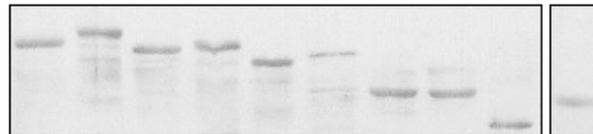
Interaction of cyclin D1 with BRCA2

GST-BRCA2-1 aa1-454	+	-	-	-	-	-	-	-	-	-
GST-BRCA2-2 aa420-906	-	+	-	-	-	-	-	-	-	-
GST-BRCA2-3 aa885-1362	-	-	+	-	-	-	-	-	-	-
GST-BRCA2-4 aa1338-1781	-	-	-	+	-	-	-	-	-	-
GST-BRCA2-5 aa1744-2115	-	-	-	-	+	-	-	-	-	-
GST-BRCA2-6 aa2106-2472	-	-	-	-	-	+	-	-	-	-
GST-BRCA2-7 aa2438-2824	-	-	-	-	-	-	+	-	-	-
GST-BRCA2-8 aa2780-3197	-	-	-	-	-	-	-	+	-	-
GST-BRCA2-9 aa3189-3418	-	-	-	-	-	-	-	-	+	-
GST	-	-	-	-	-	-	-	-	-	+
cyclin D1	+	+	+	+	+	+	+	+	+	+

WB: cyclin D1



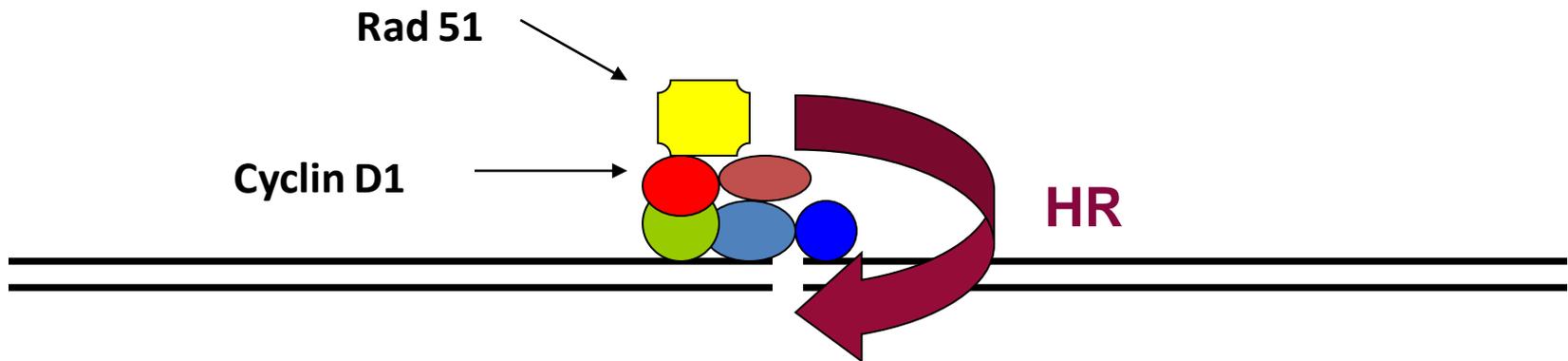
Input GST



Input cyclin D1

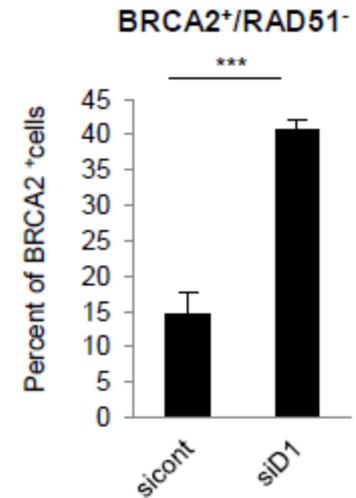
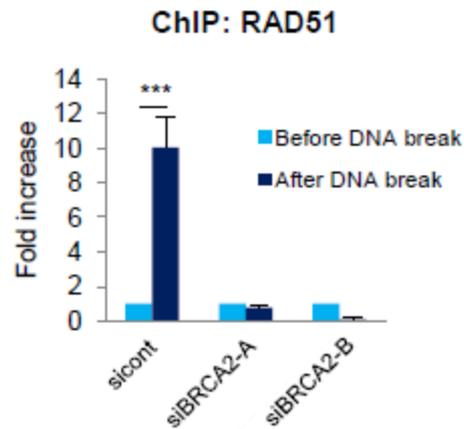
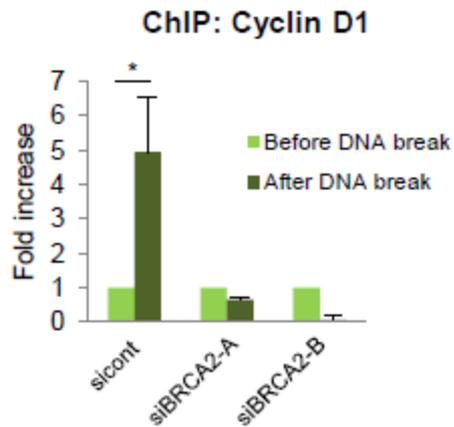
MODEL

Cyclin D1 localizes to DNA breaks and helps to recruit RAD51



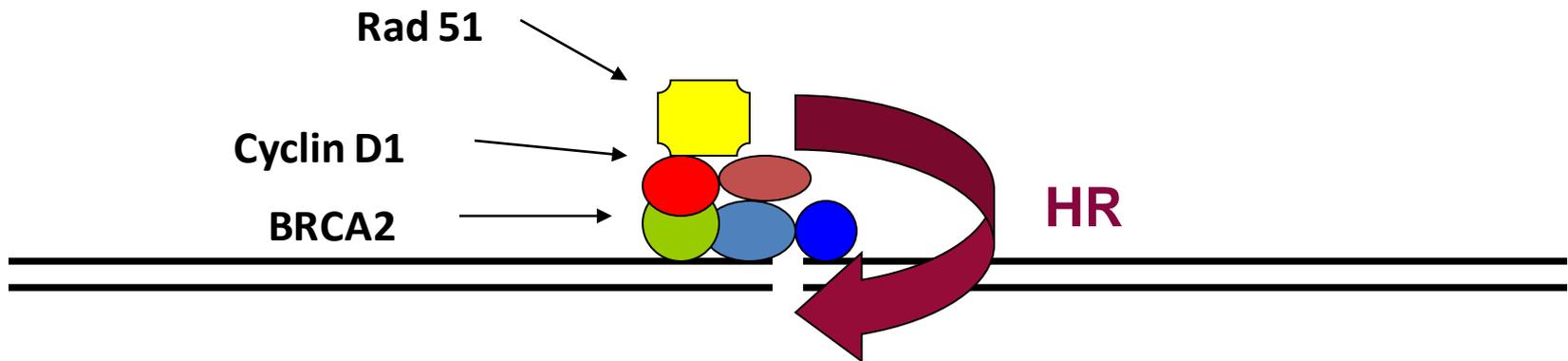
How is cyclin D1 recruited to DNA breaks?

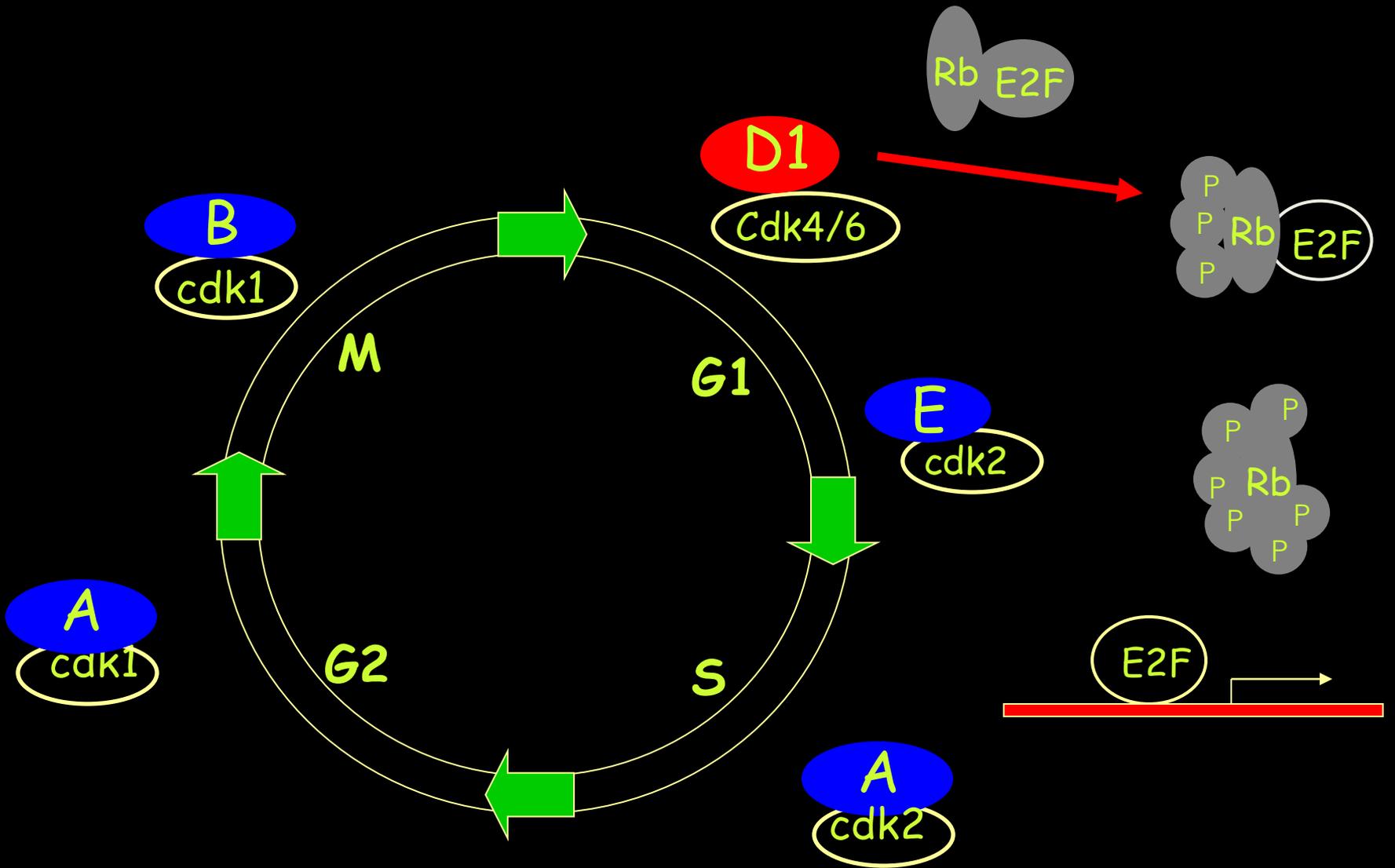
Impaired recruitment of cyclin D1 to ds DNA breaks in BRCA2-depleted cells

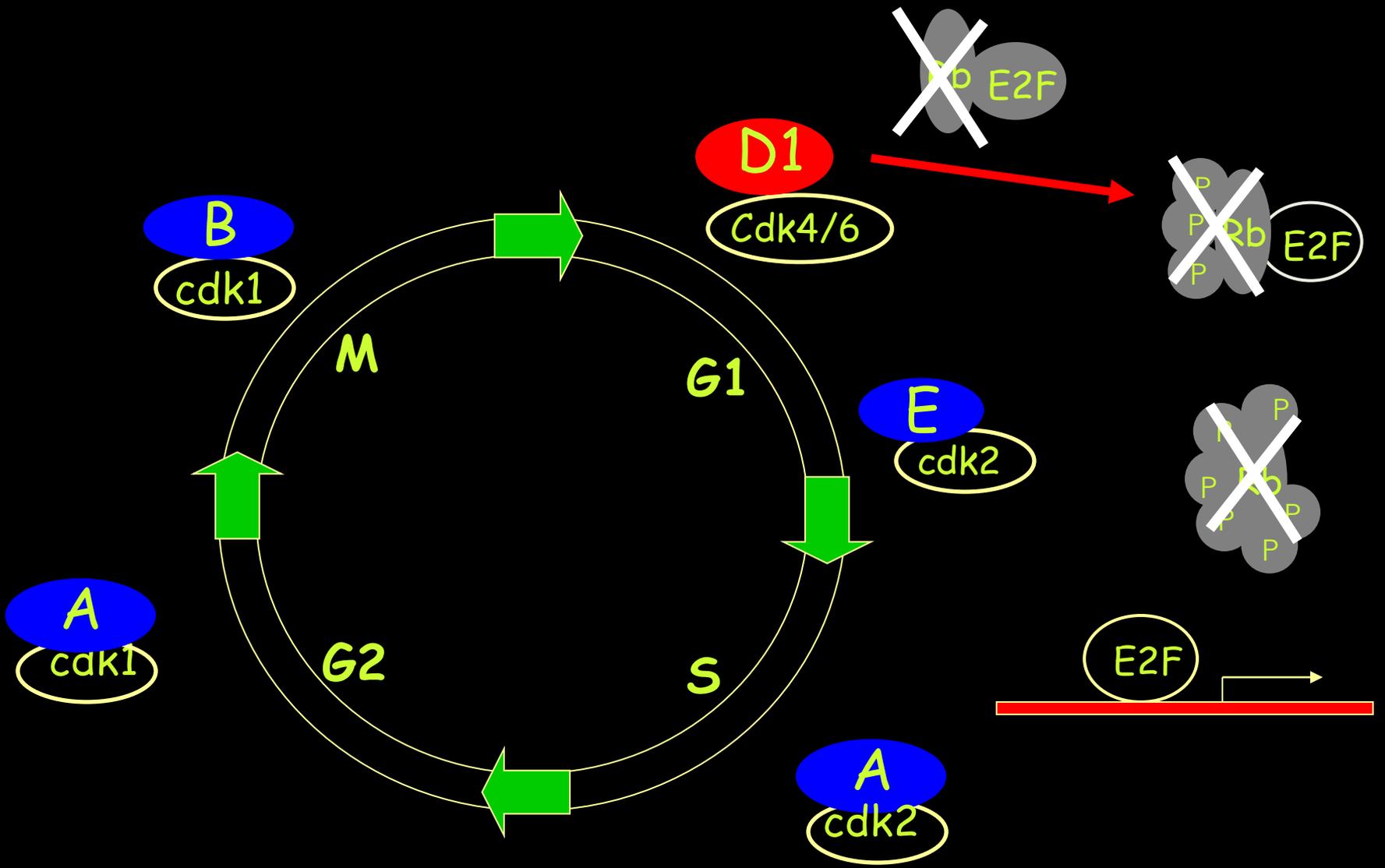


MODEL

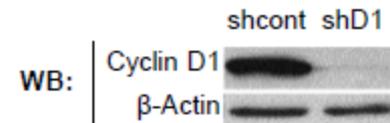
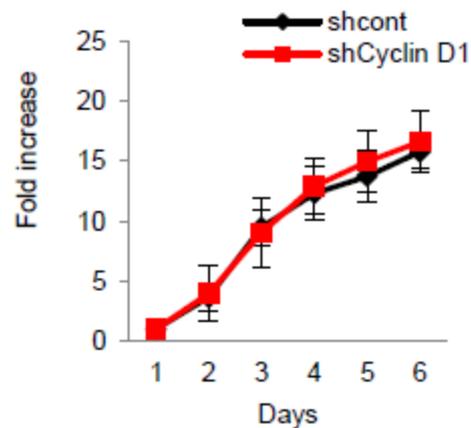
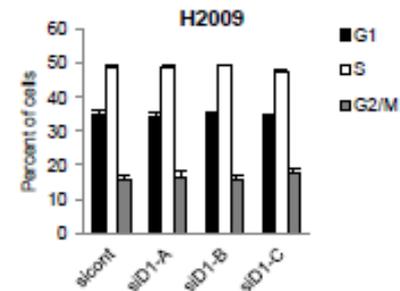
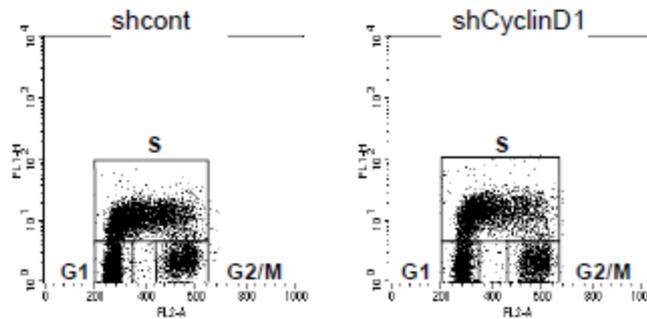
**BRCA2 re-recruits cyclin D1
cyclin D1 helps to recruit RAD51**



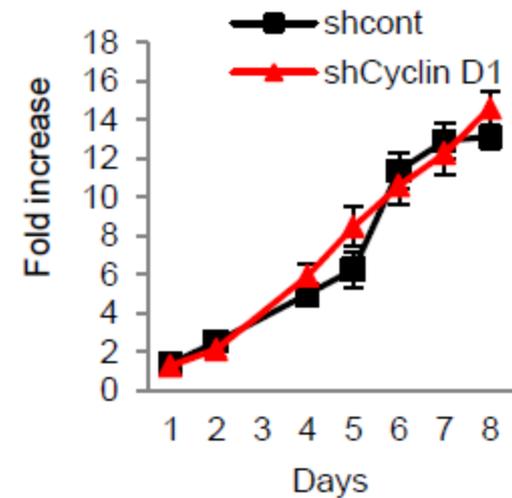
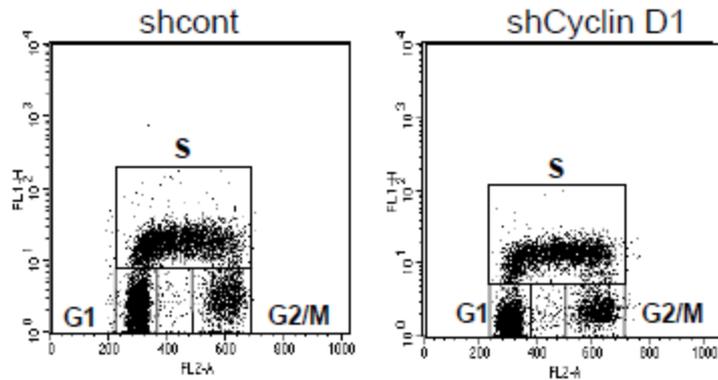




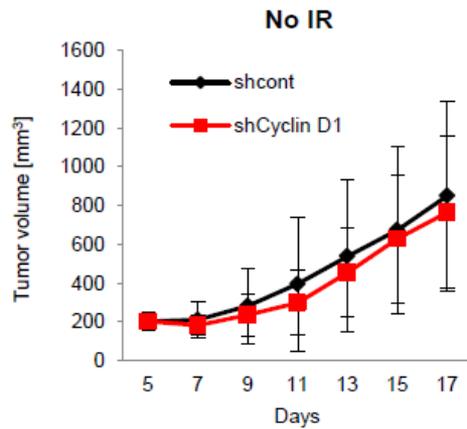
Retinoblastoma-negative lung cancer cells do not require cyclin D1 for proliferation



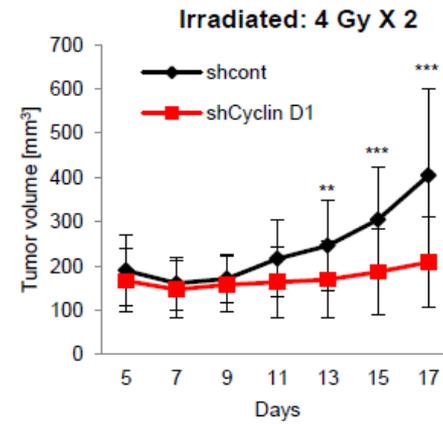
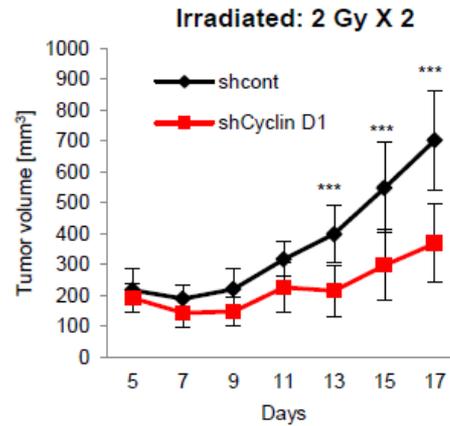
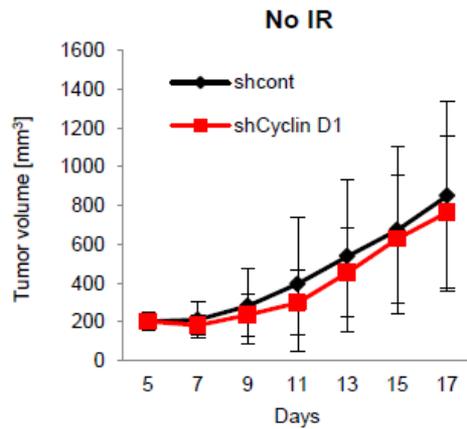
Retinoblastoma-negative HeLa cancer cells do not require cyclin D1 for proliferation



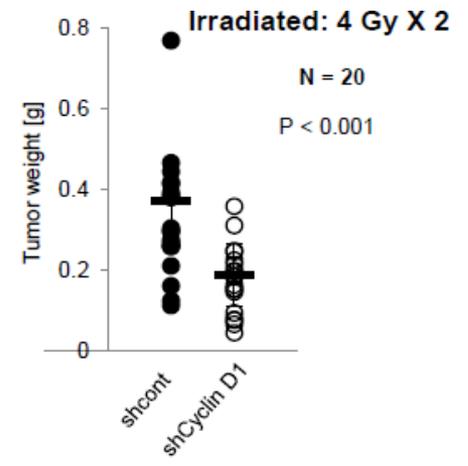
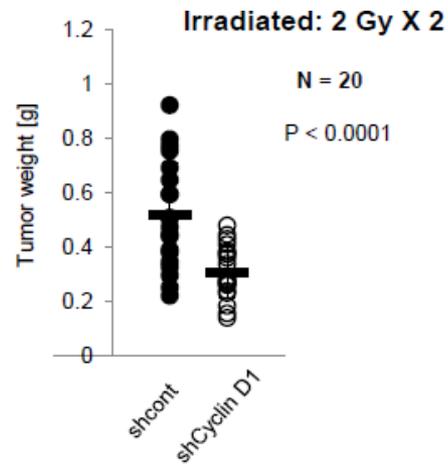
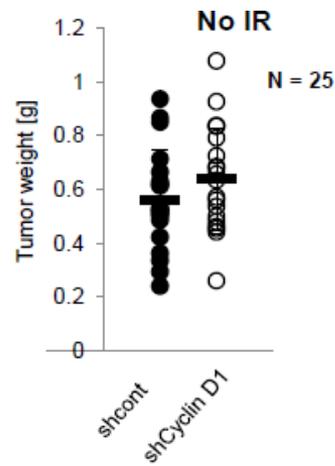
In vivo growth of H2009 lung cancer cells



In vivo growth of H2009 lung cancer cells

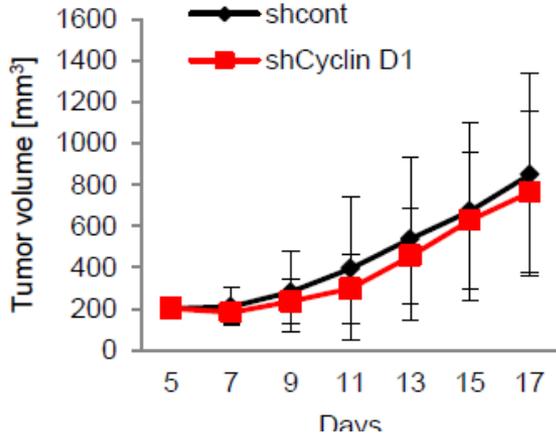


In vivo growth of H2009 lung cancer cells

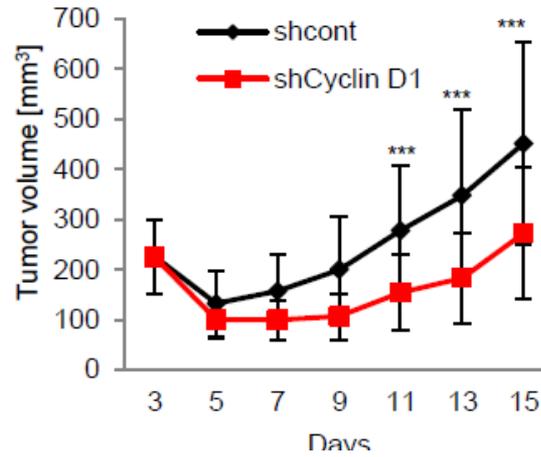


In vivo growth of H2009 lung cancer cells

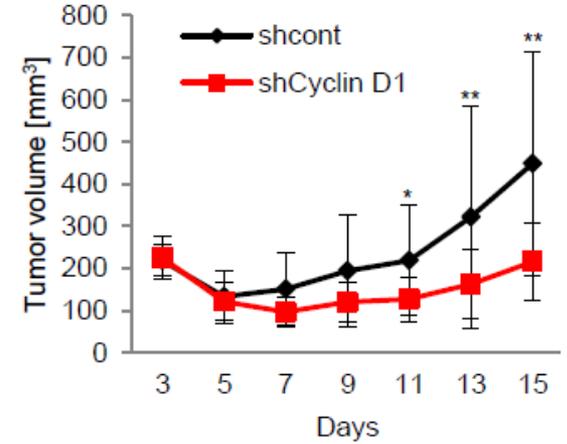
No IR



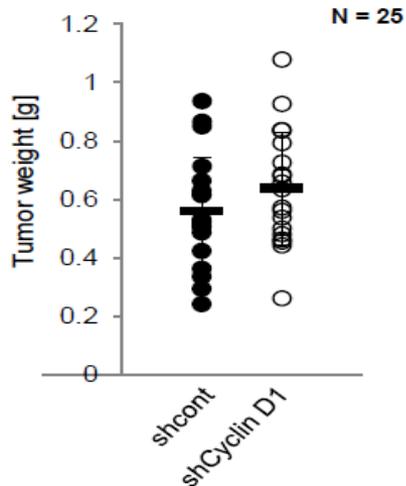
Irradiated: 4 Gy



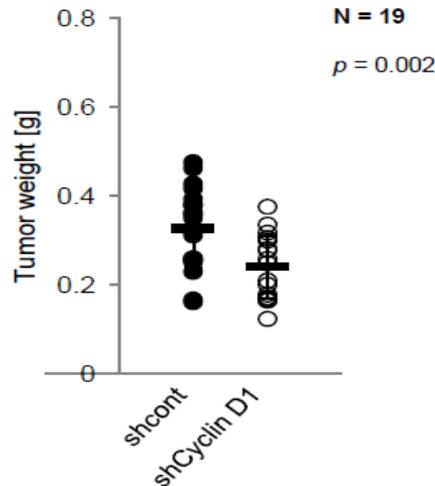
Irradiated: 8 Gy



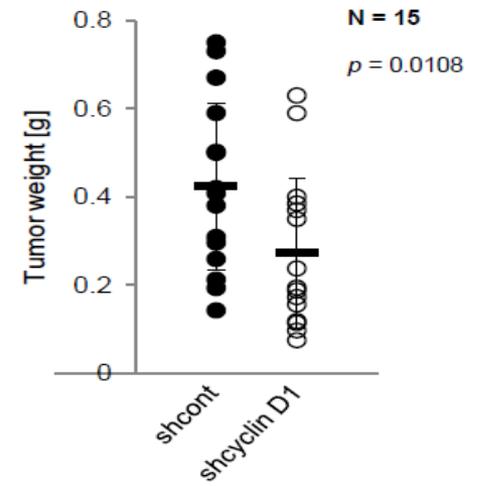
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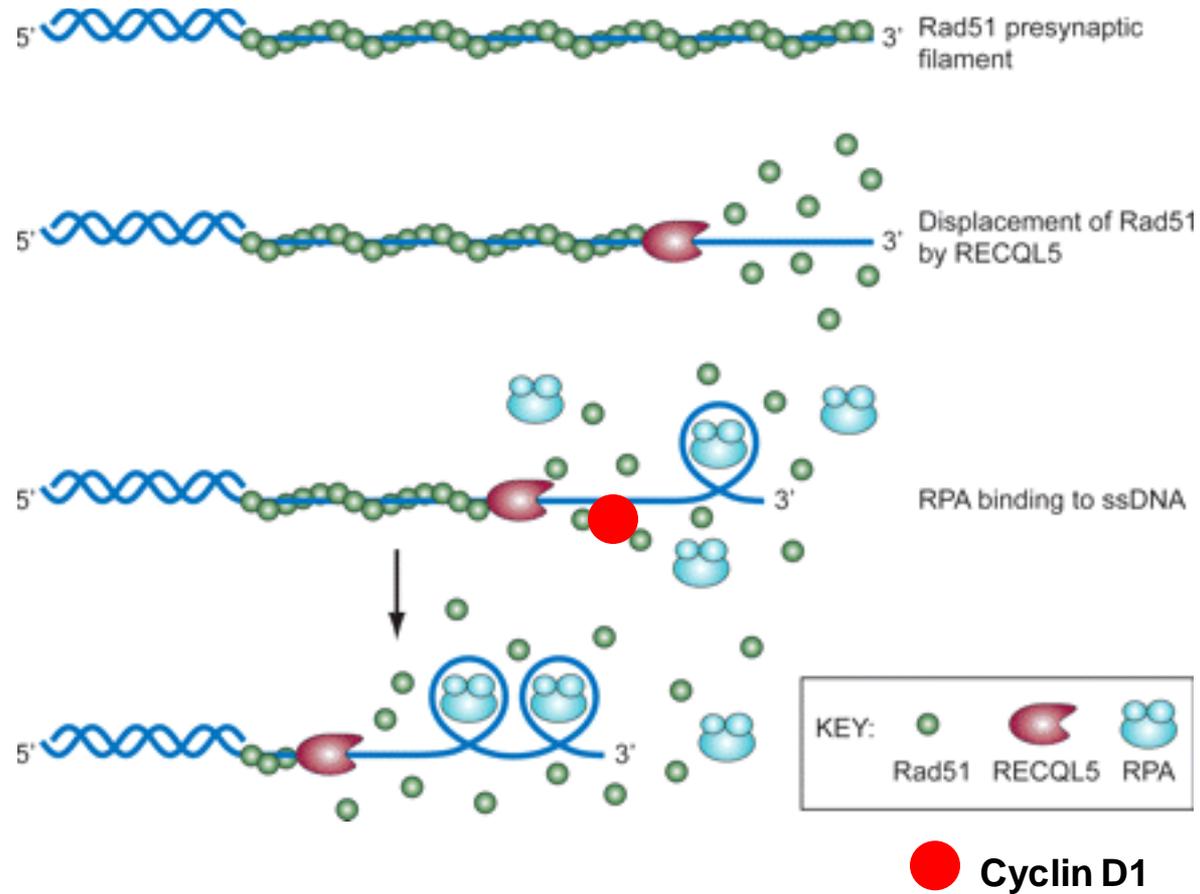
Irradiated: 4 Gy



Irradiated: 8 Gy



Cyclin D1 in DNA repair



Cyclin D1 may be a therapeutic target also in pRB-negative tumors

Siwanon Jirawatnotai

Frederic Bienvenu

Lisa Becks
Agnieszka Zagozdzon
Dana-Farber Cancer Institute

Joshua Elias
Tapasree Goswami
Steven Gygi
Department of Cell Biology
Harvard Medical School

Yiduo Hu
David Livingston
Dana-Farber Cancer Institute

Alexander Marson
Garrett Frampton
Megan Cole
Richard Young
Whitehead Institute

Yaoyu Wang
Mick Correll
John Quackenbush
Dana-Farber Cancer Institute

