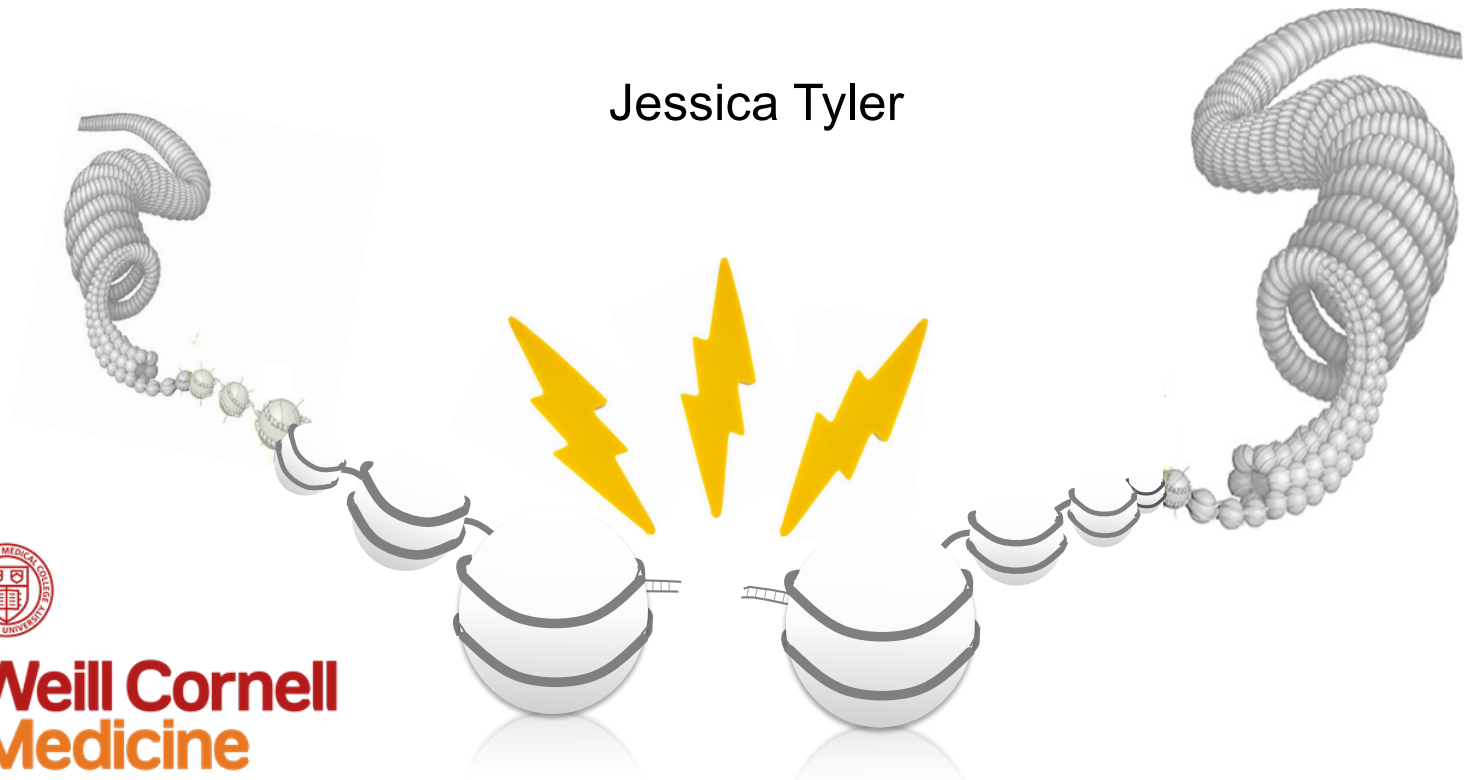




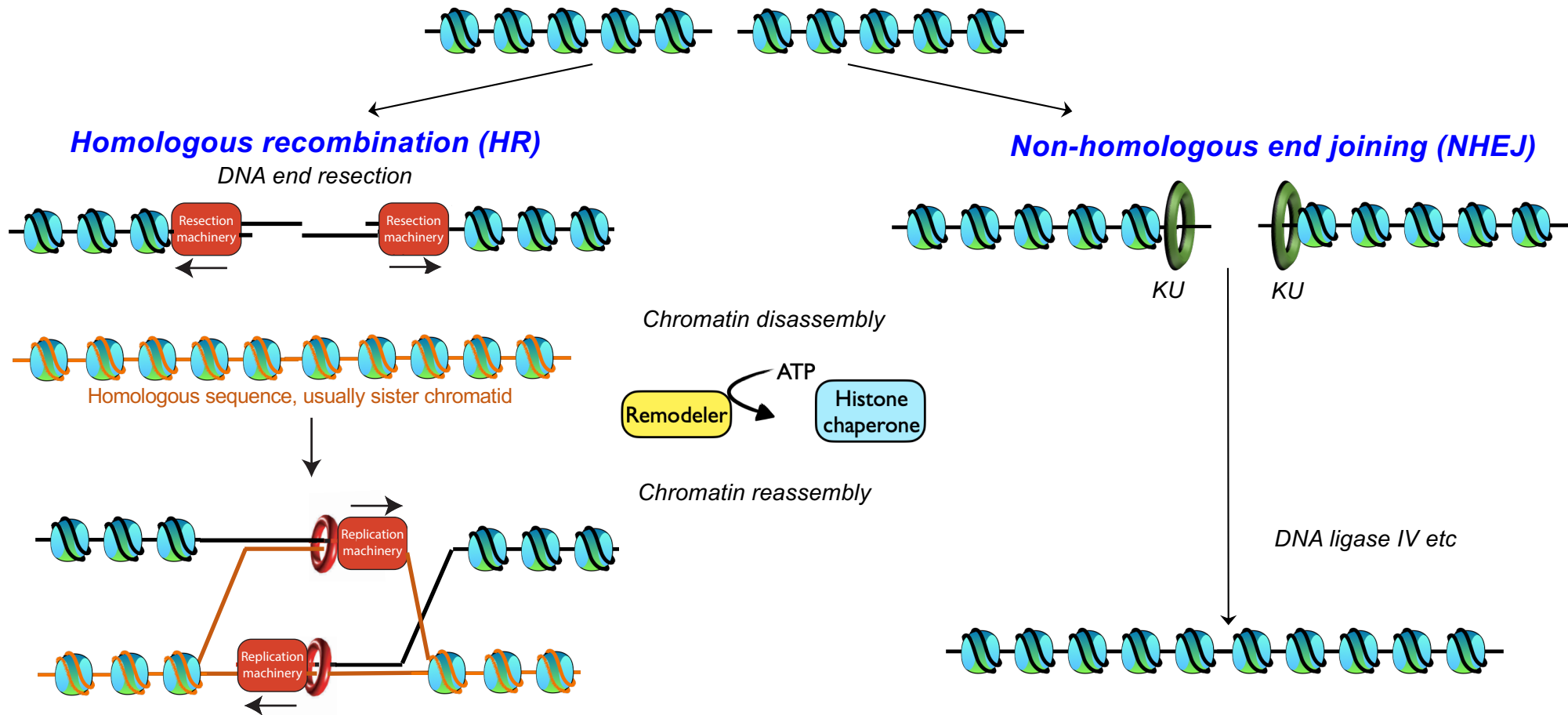
Novel mechanisms regulating DNA end resection at DNA double strand breaks

Jessica Tyler

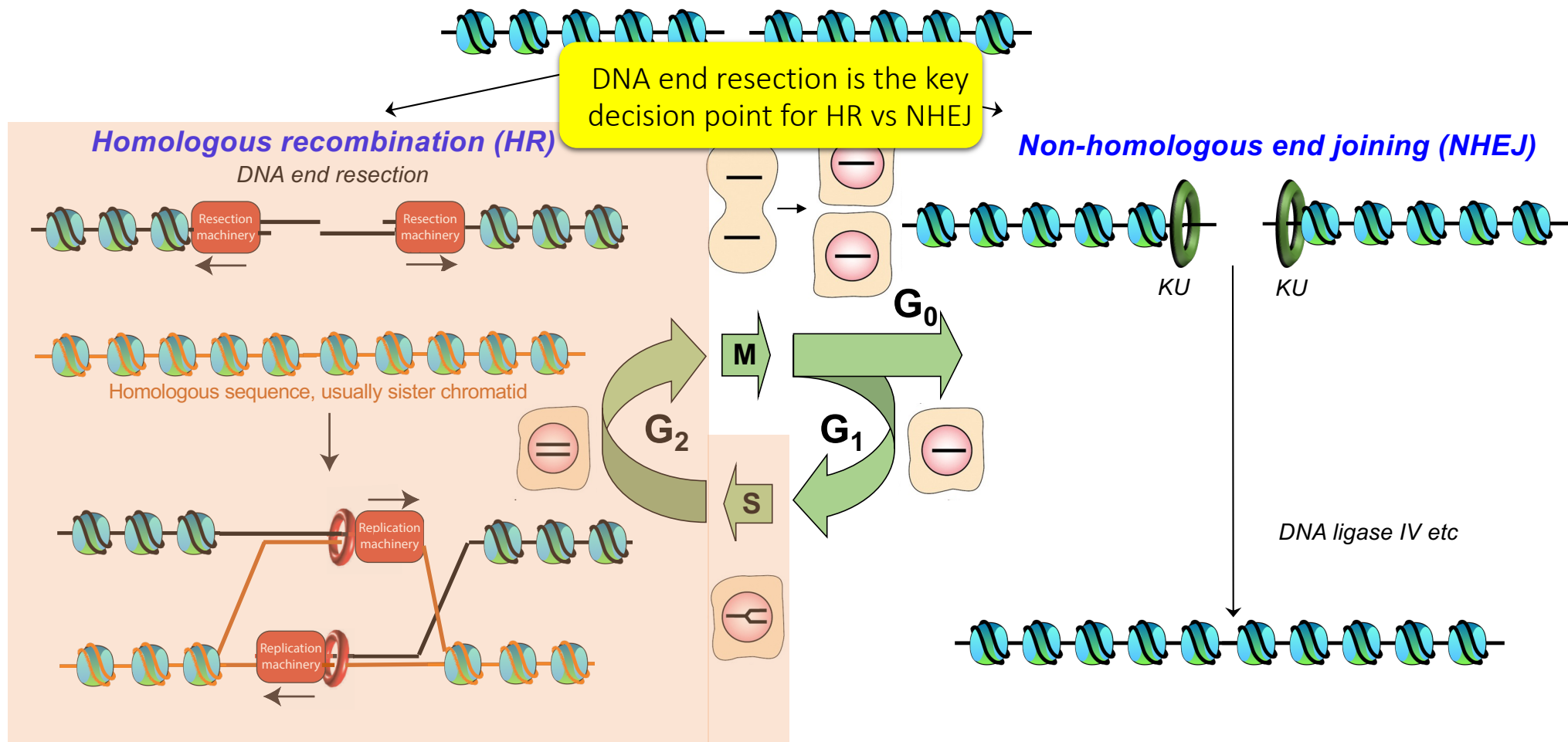


Weill Cornell
Medicine

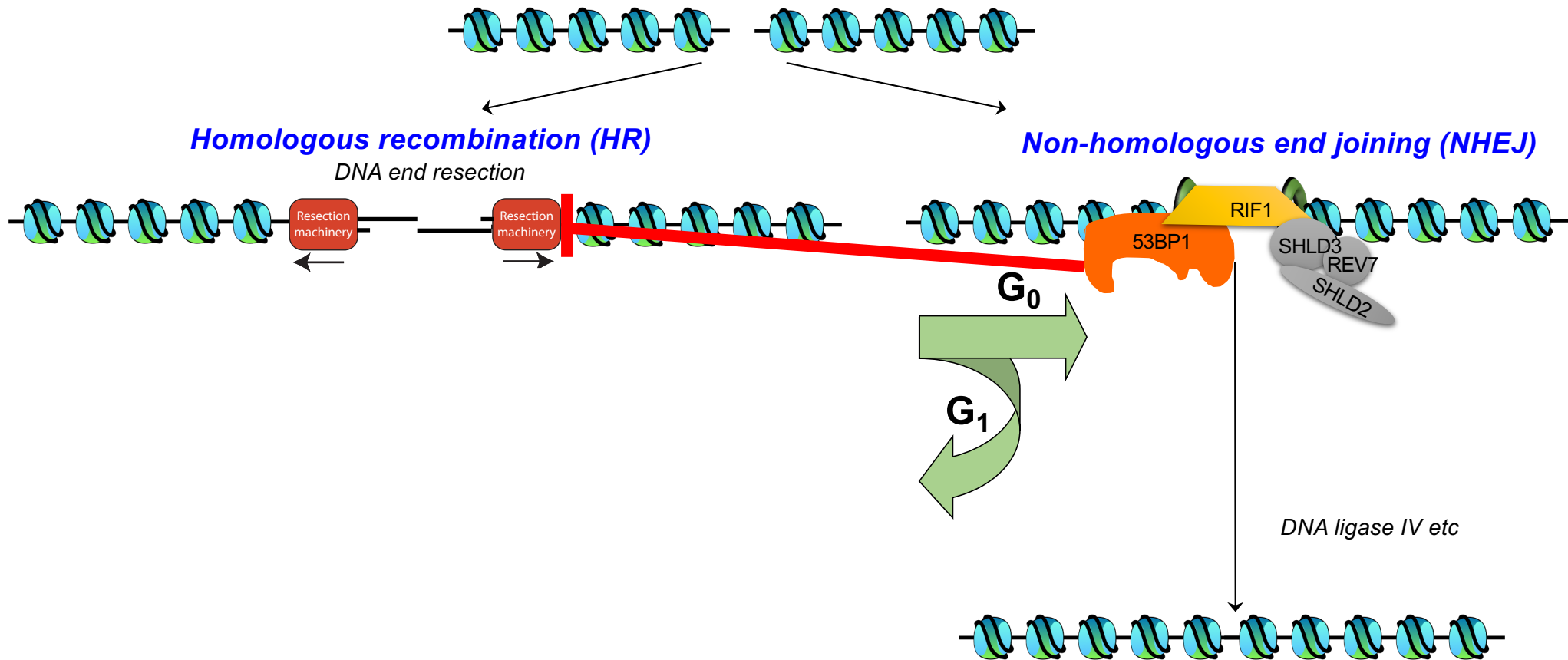
The two major pathways of DNA double strand repair



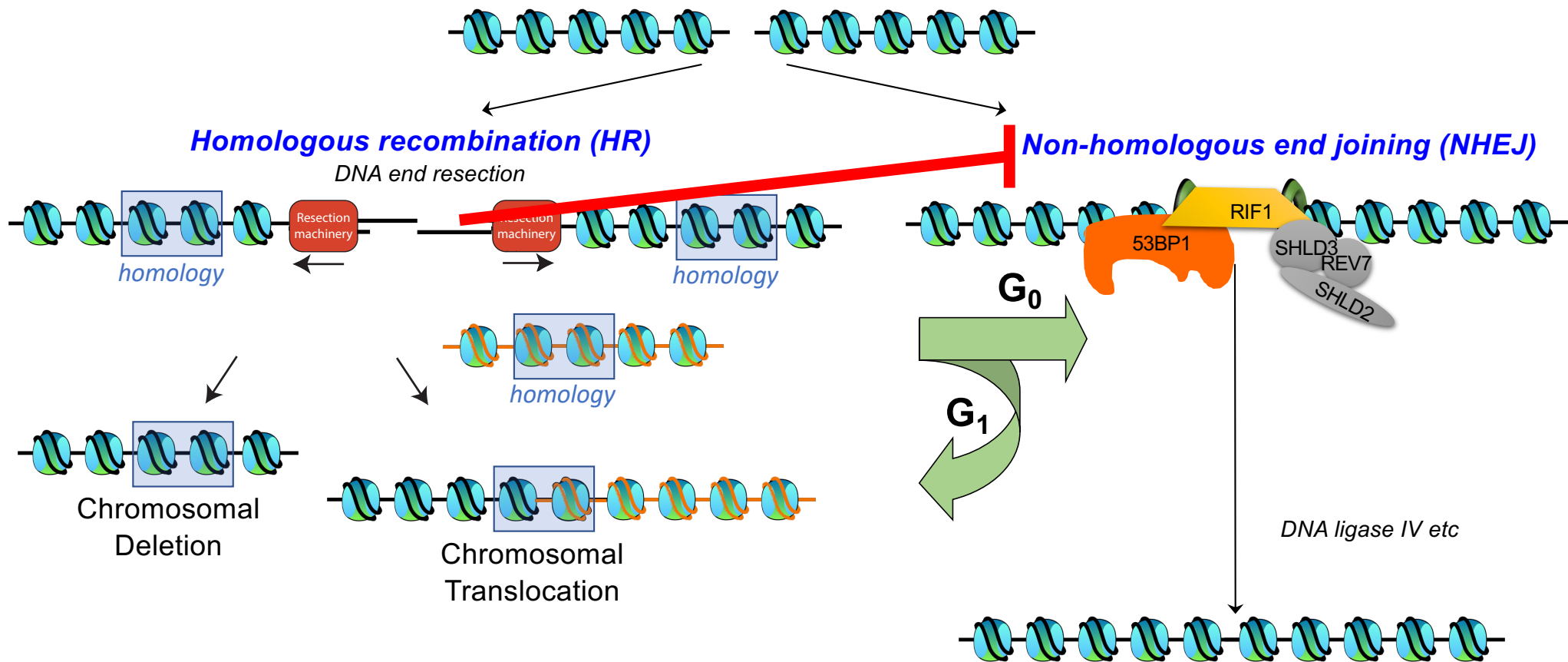
Cell cycle differences in DNA double strand repair



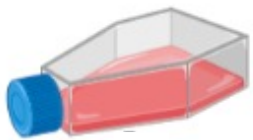
Extensive DNA end resection is blocked in G₁ phase cells



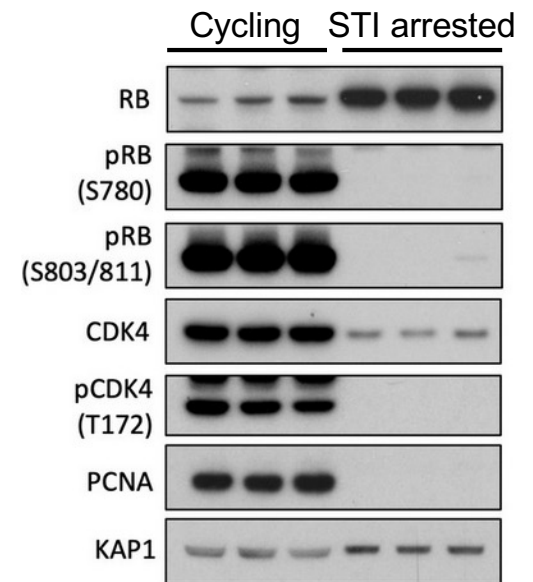
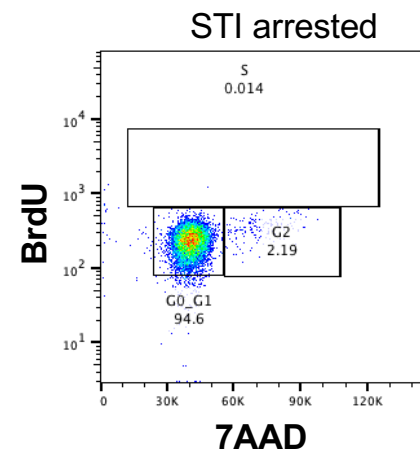
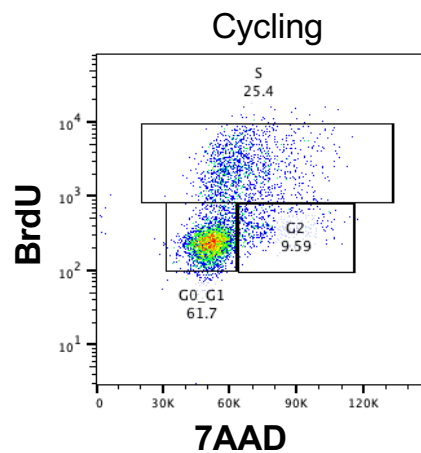
If aberrant end resection occurs in G_1 / G_0 cells.....



Abelson virus transformed murine pre-B cells can be induced to efficiently arrest in G₀



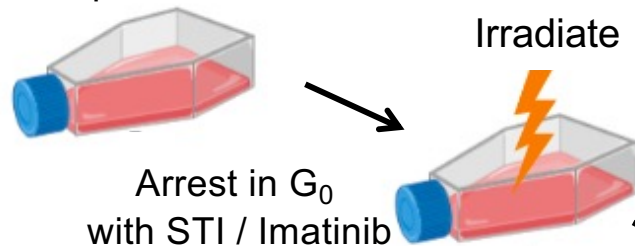
Arrest in G₀
with STI / Imatinib



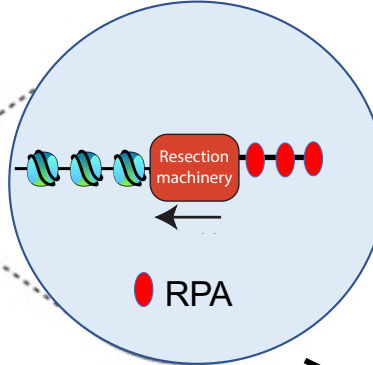
Bo-Ruei Chen

An assay for a proxy of end resection in G₀ cells

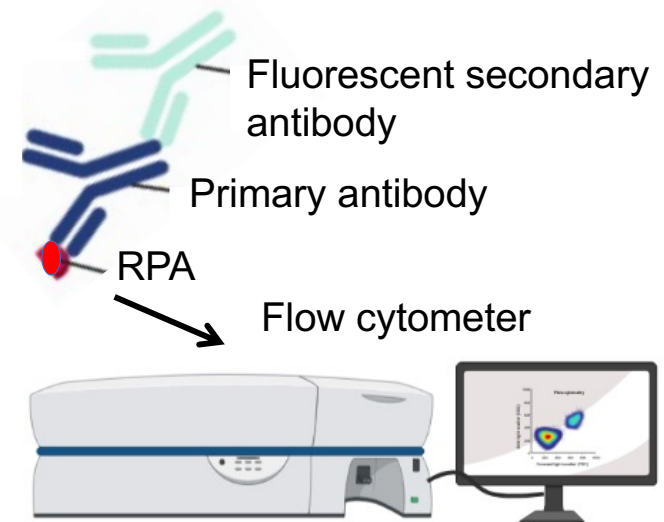
V-Abl transformed
murine pre-B cells



Triton-X extract to
removed soluble RPA

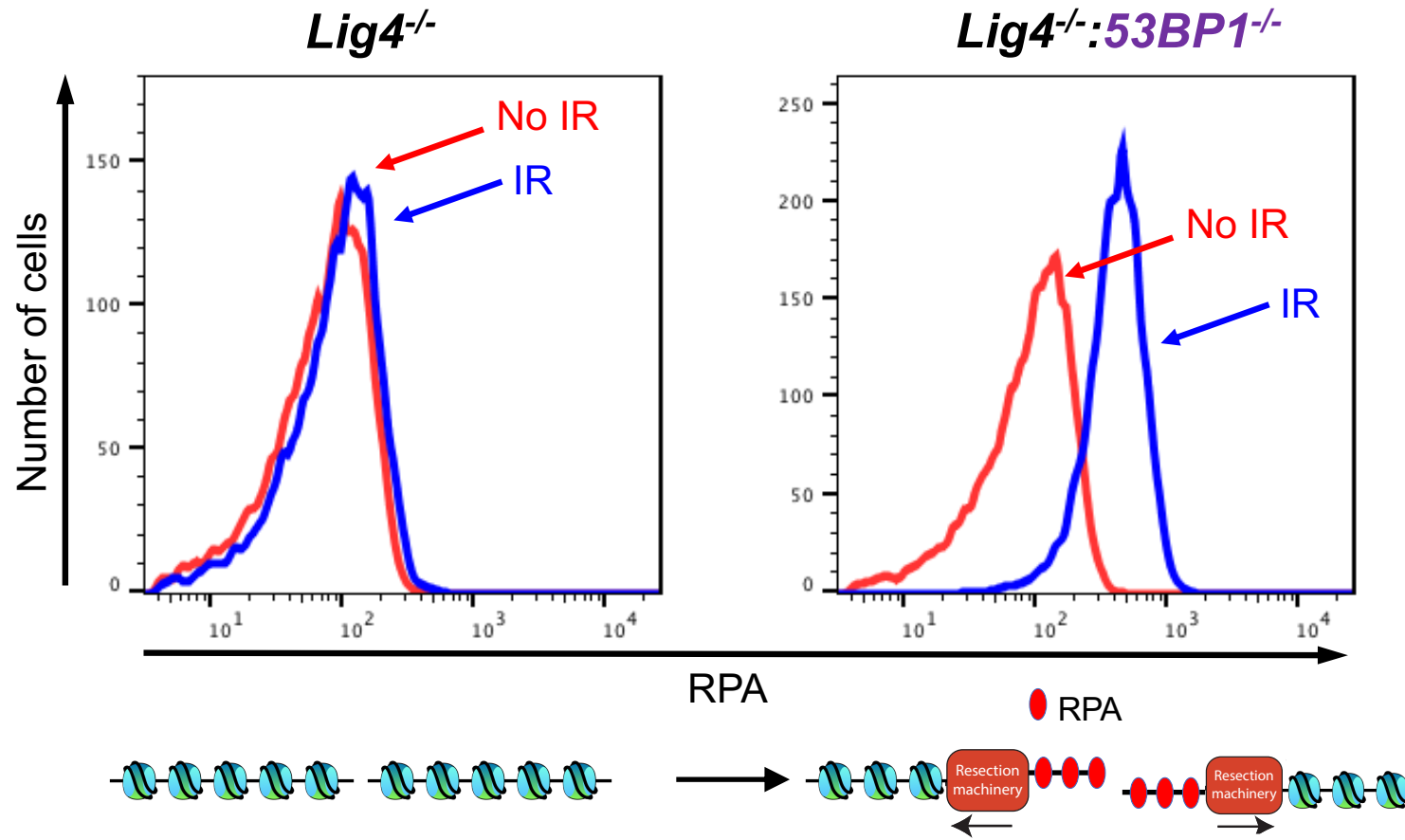


Forment & Jackson 2012

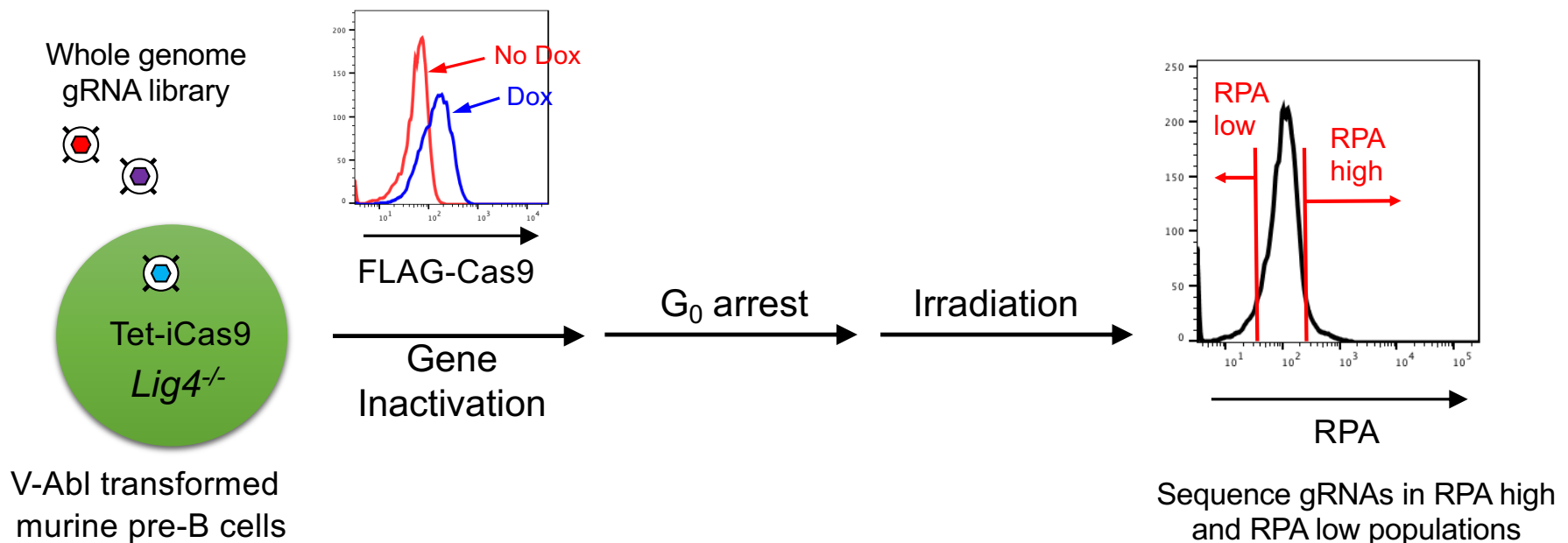


RPA binds single-stranded DNA at resected DNA ends

G₀ cells:



Whole genome gRNA screen for genes encoding proteins that prevent end resection in G_0 cells



Genes enriched in screen for proteins that prevent DNA end resection in G₀ cells

Gene	Fold enrichment in RPA high cells
53BP1	4
Rif1	4
Shld2	3
Rev7	3
Lin37	5
Lin52	4

53BP1/
Shieldin

MuvB / DREAM

RPA High Score:

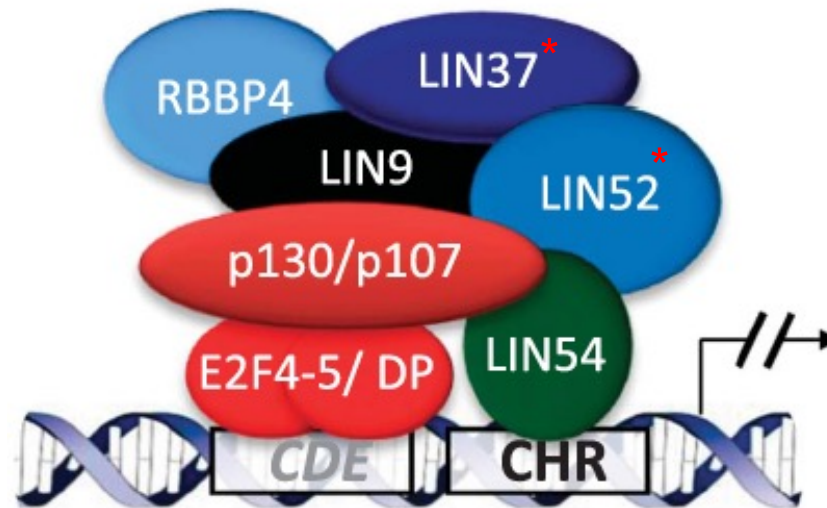
$$\frac{\text{Normalized reads (RPA high)}}{\text{Normalized reads (RPA low)}}$$

The DREAM complex

DREAM (*Drosophila* RBP, E2F, and Myb associated proteins)

DREAM and RB: cooperatively repress gene expression,
to maintain cells in G₁/G₀

DREAM complex

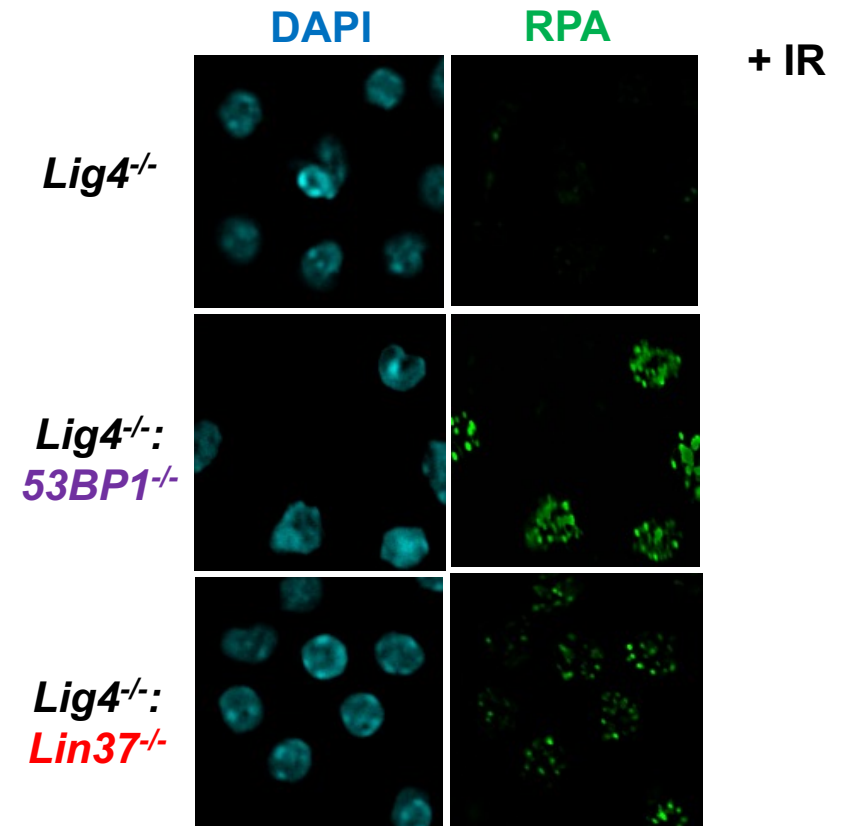
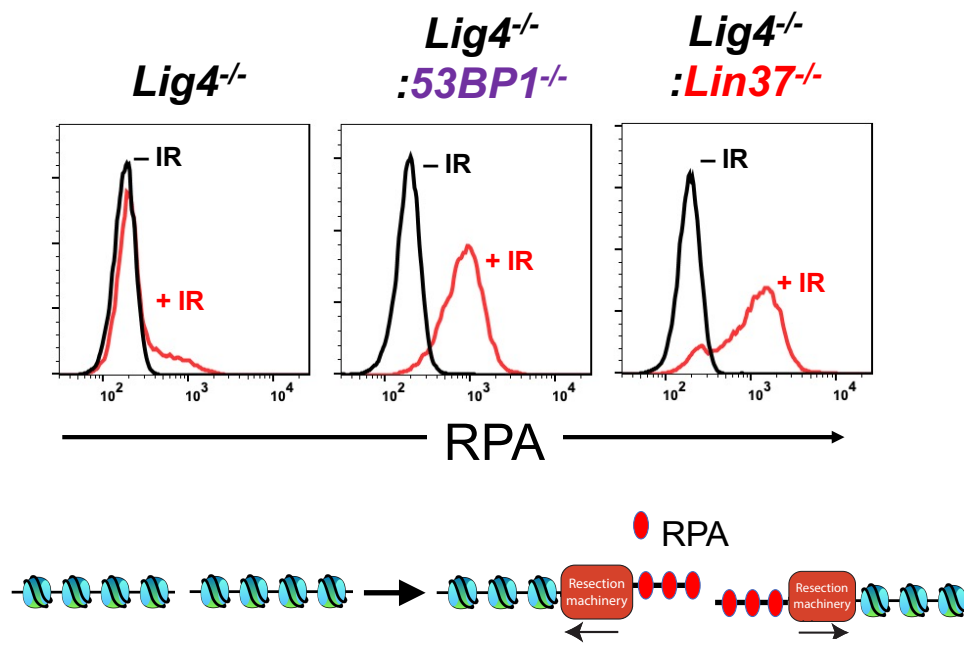


* Subunits identified
in our screen

Transcriptional repressor

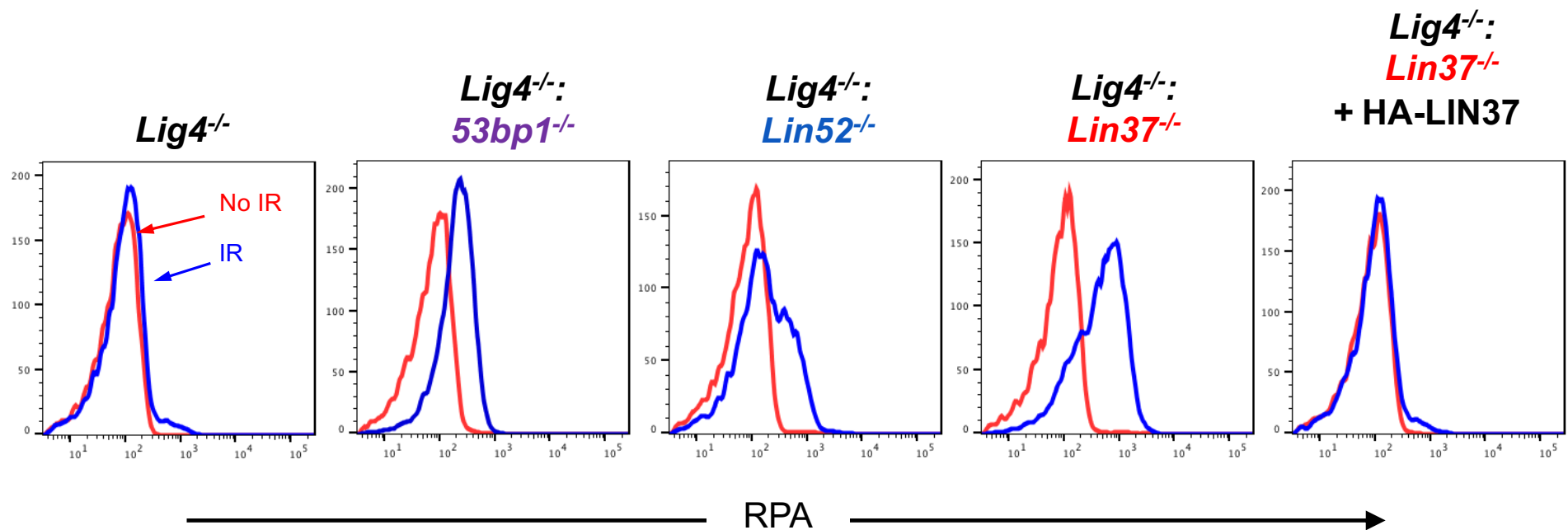
Loss of Lin37 leads to RPA accumulation at DSBs in G₀ cells

G₀ cells:



Loss of Lin52 or Lin37 leads to RPA accumulation at DSBs in G₀ cells

G₀ cells:

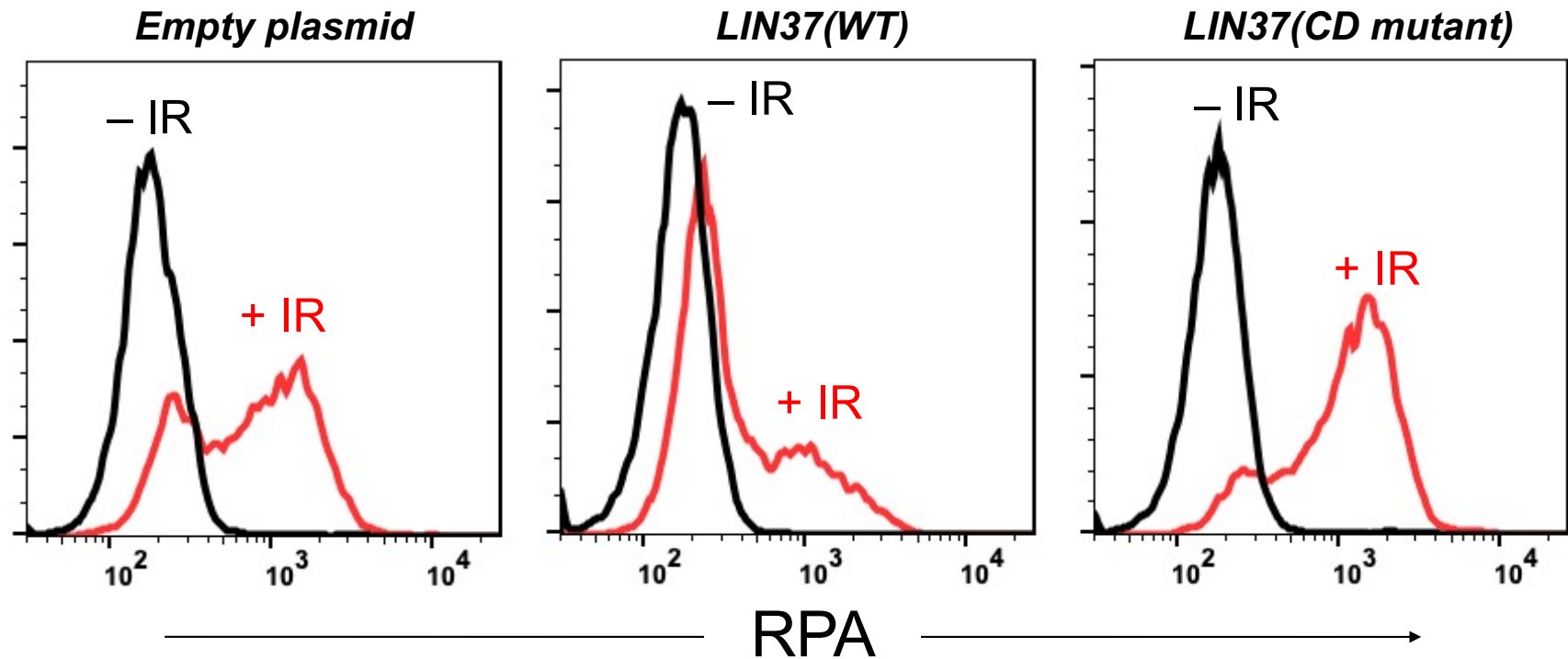


Lin37 function in end protection is within the DREAM complex

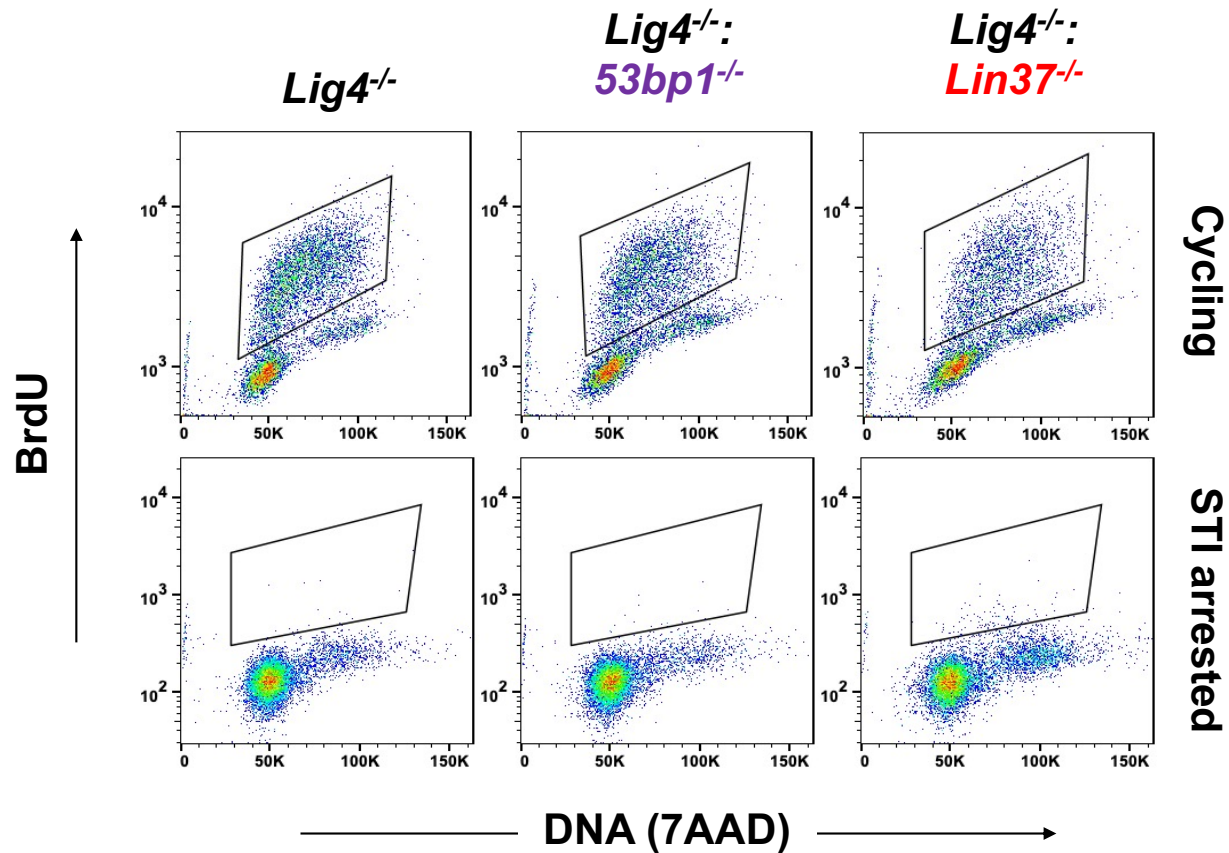
G₀ *Lig4*^{-/-}: *Lin37*^{-/-} cells

Rescue plasmid:

*Cannot assemble into
DREAM complex*

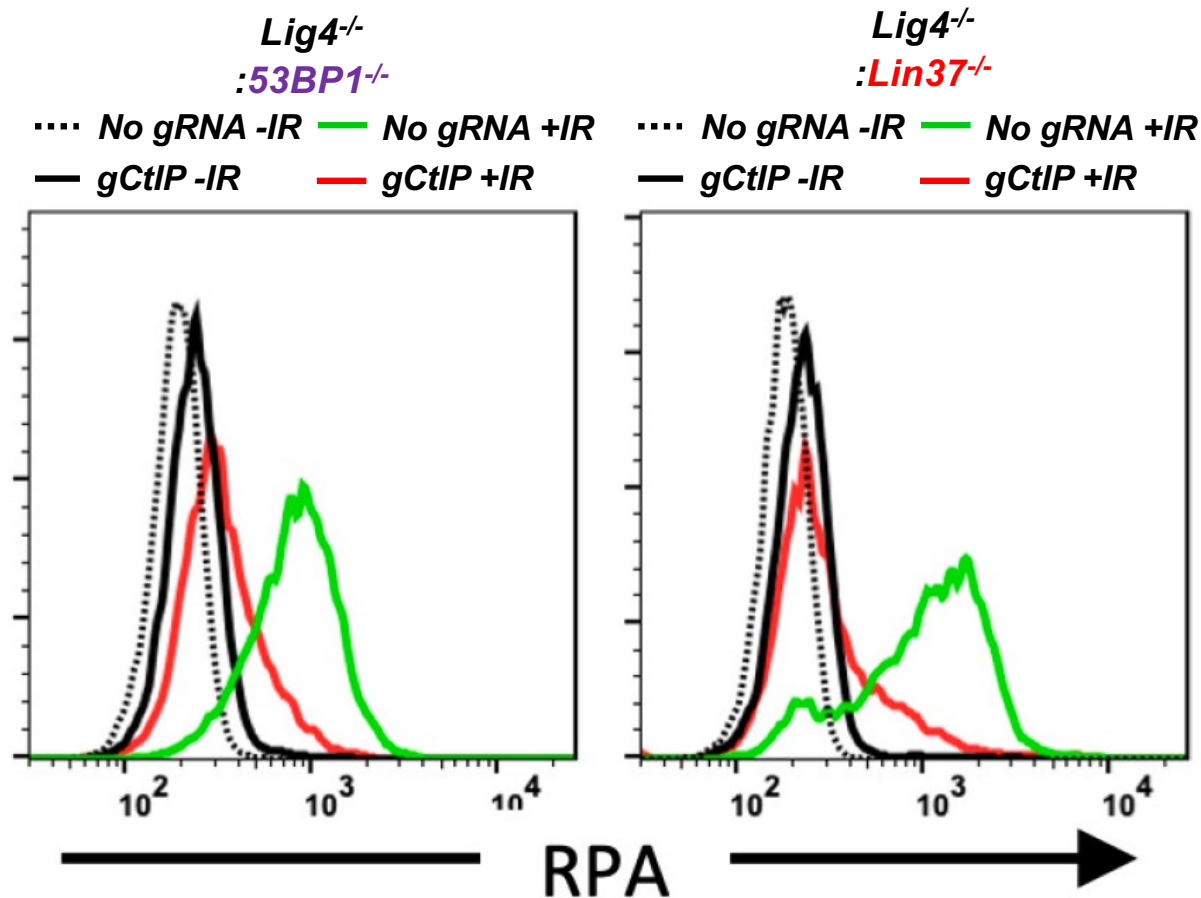


Loss of Lin37 does not cause entry into the cell cycle



CtIP-dependent DNA end resection in Lin37-deficient G_0 cells

G_0 cells:



Loss of Lin37 leads to DNA end resection in G₀ cells

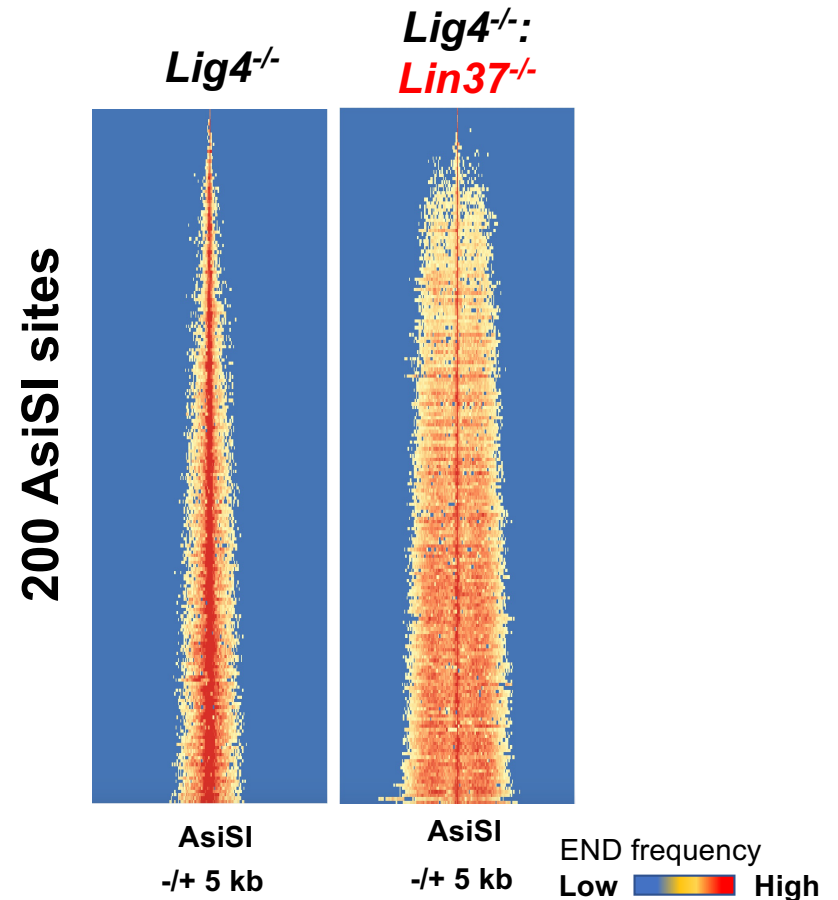
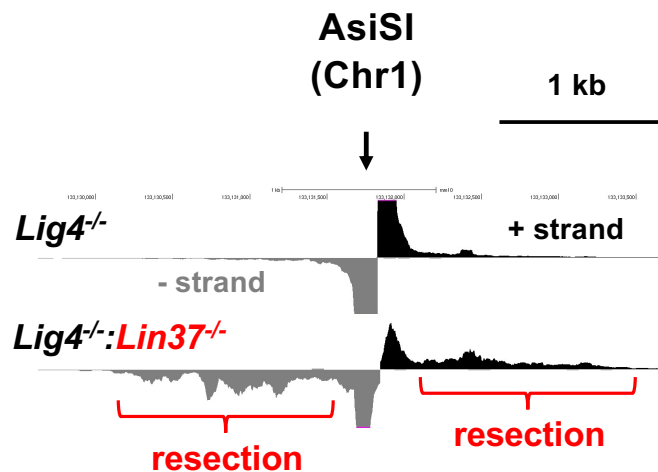
G₀ cells:

End-seq

Inducible AsiSI endonuclease



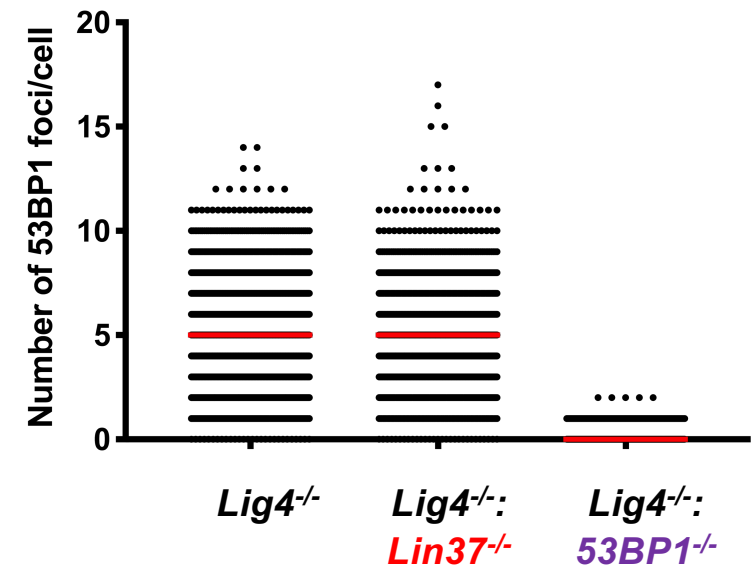
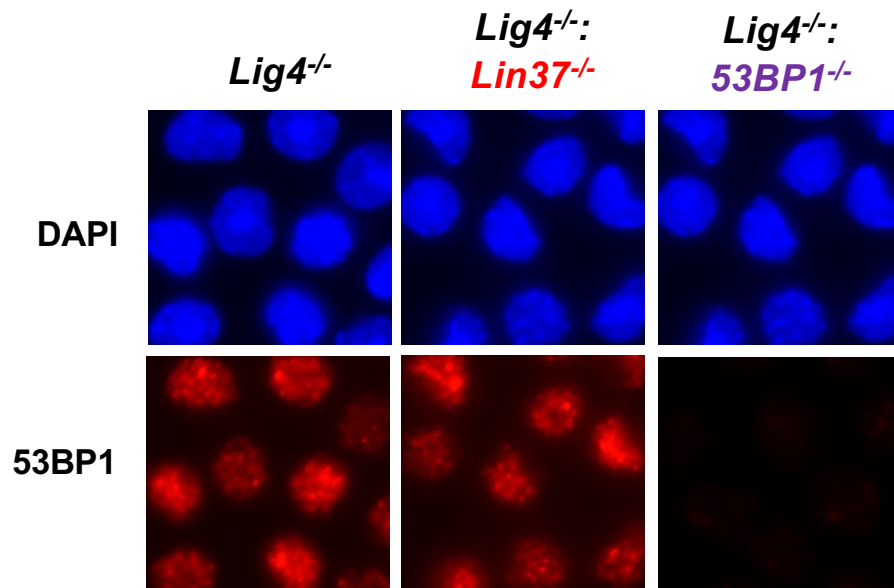
Canela and Nussenzweig 2016



Anthony Tubbs, Nick Zolnerowich, Wei Wu, Andre Nussenzweig

Loss of Lin37 does not impair 53BP1 recruitment to DSBs

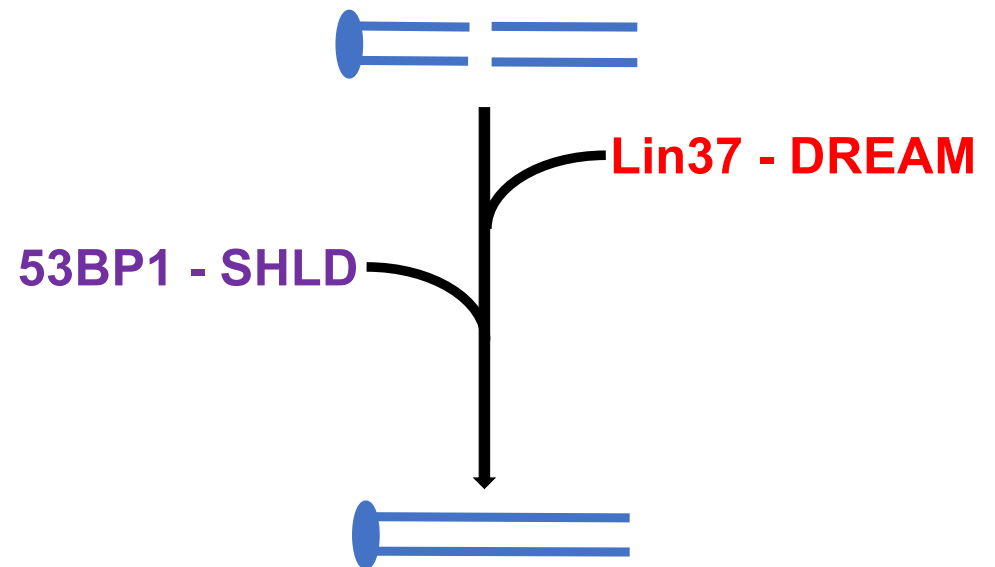
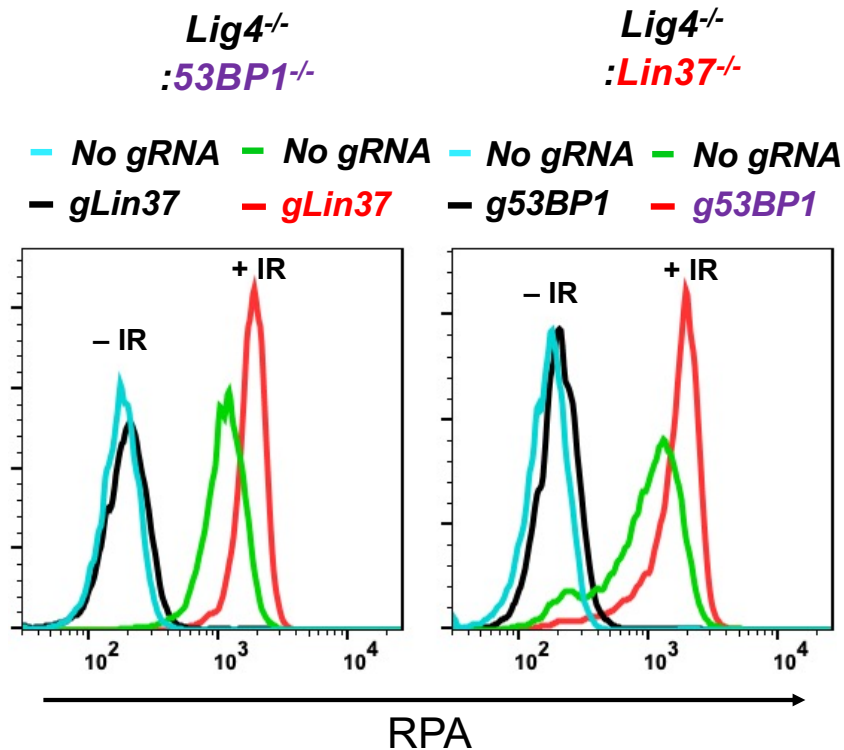
G₀ cells:
+ IR



Nussenzweig lab

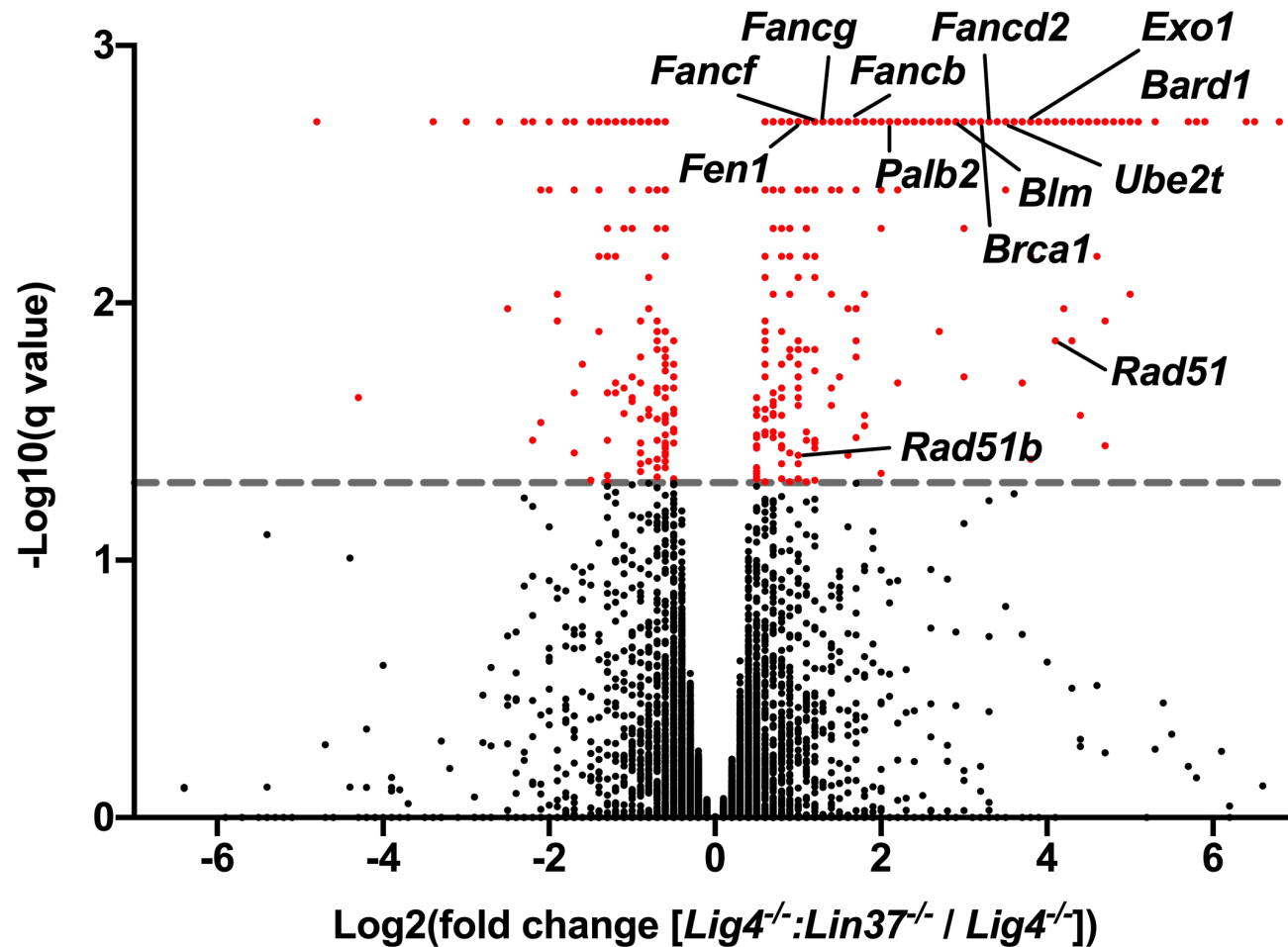
Lin37 and 53BP1 are in distinct pathways of DNA end protection

G₀ cells:

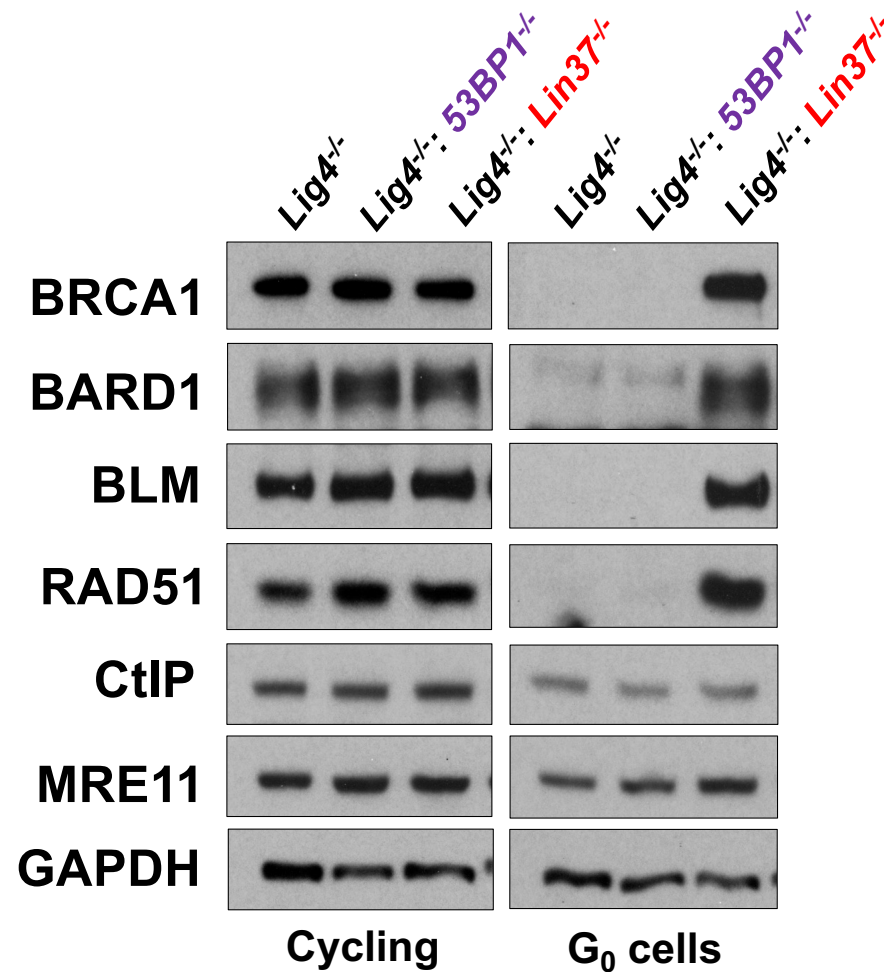


Gene expression changes in Lin37-deficient G_0 cells

G_0 cells:
RNA-seq

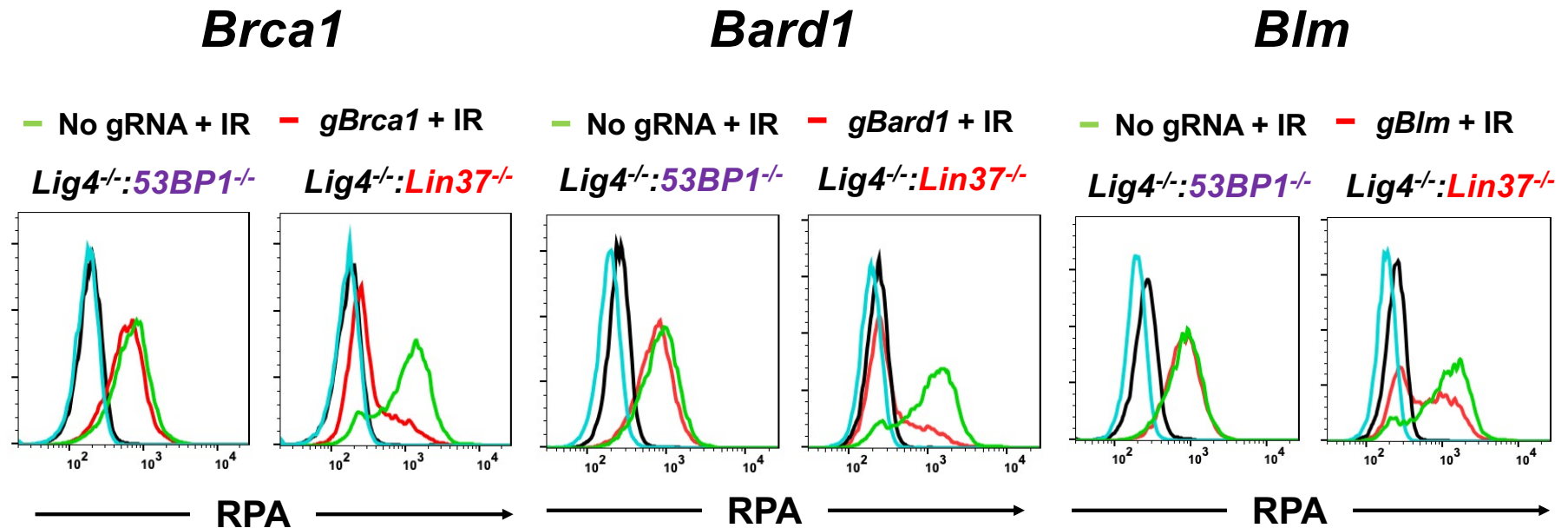


Expression of HR machinery in Lin37-deficient G₀ cells



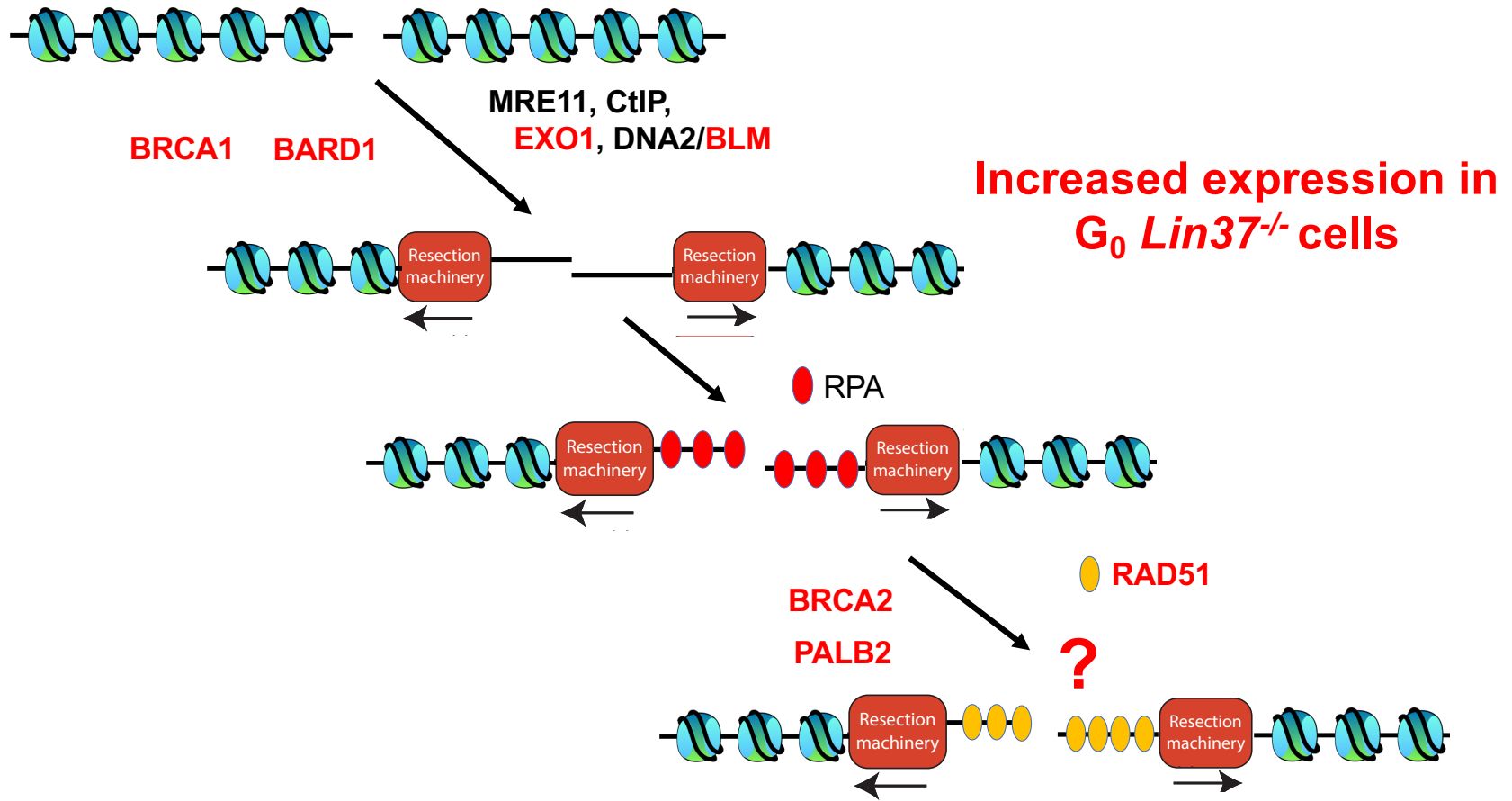
End resection in $\text{Lin37}^{-/-}$ G_0 cells depends on the HR machinery

G_0 cells:



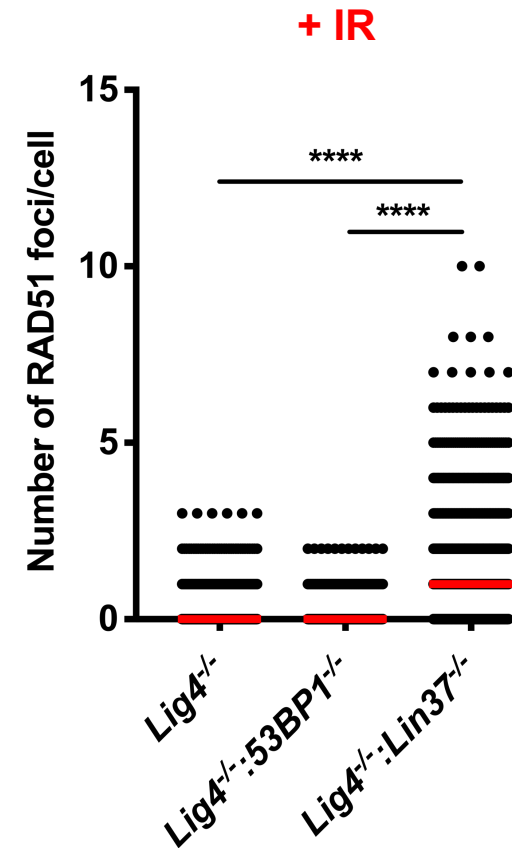
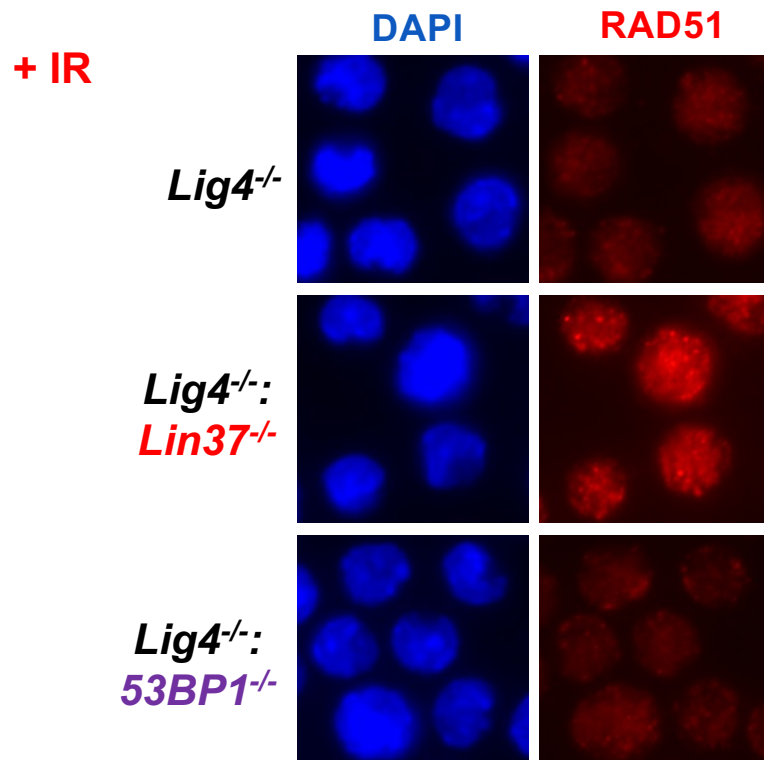
HR in Lin37-deficient G_0 cells?

G_0 cells:



Rad51 filaments form in Lin37-deficient G₀ cells

G₀ cells:

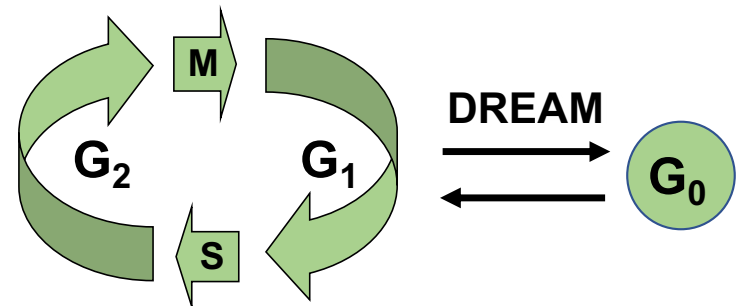
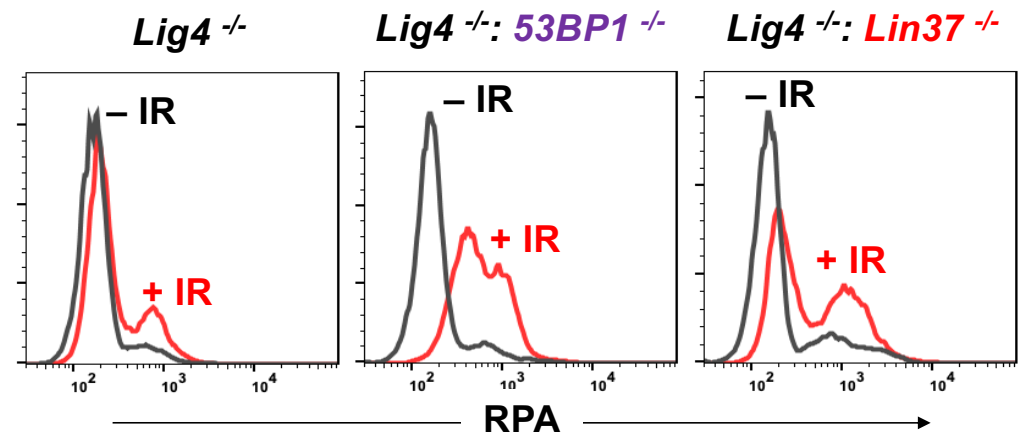
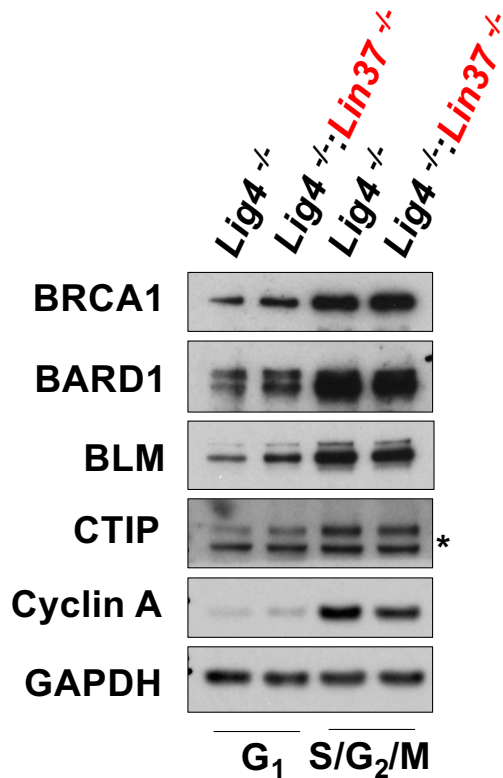


Lin37 only has a minor role in DNA end protection in G₁ phase

Same result in G₂ cells

Cycling cells:

G₁ phase cells isolated from cycling population:



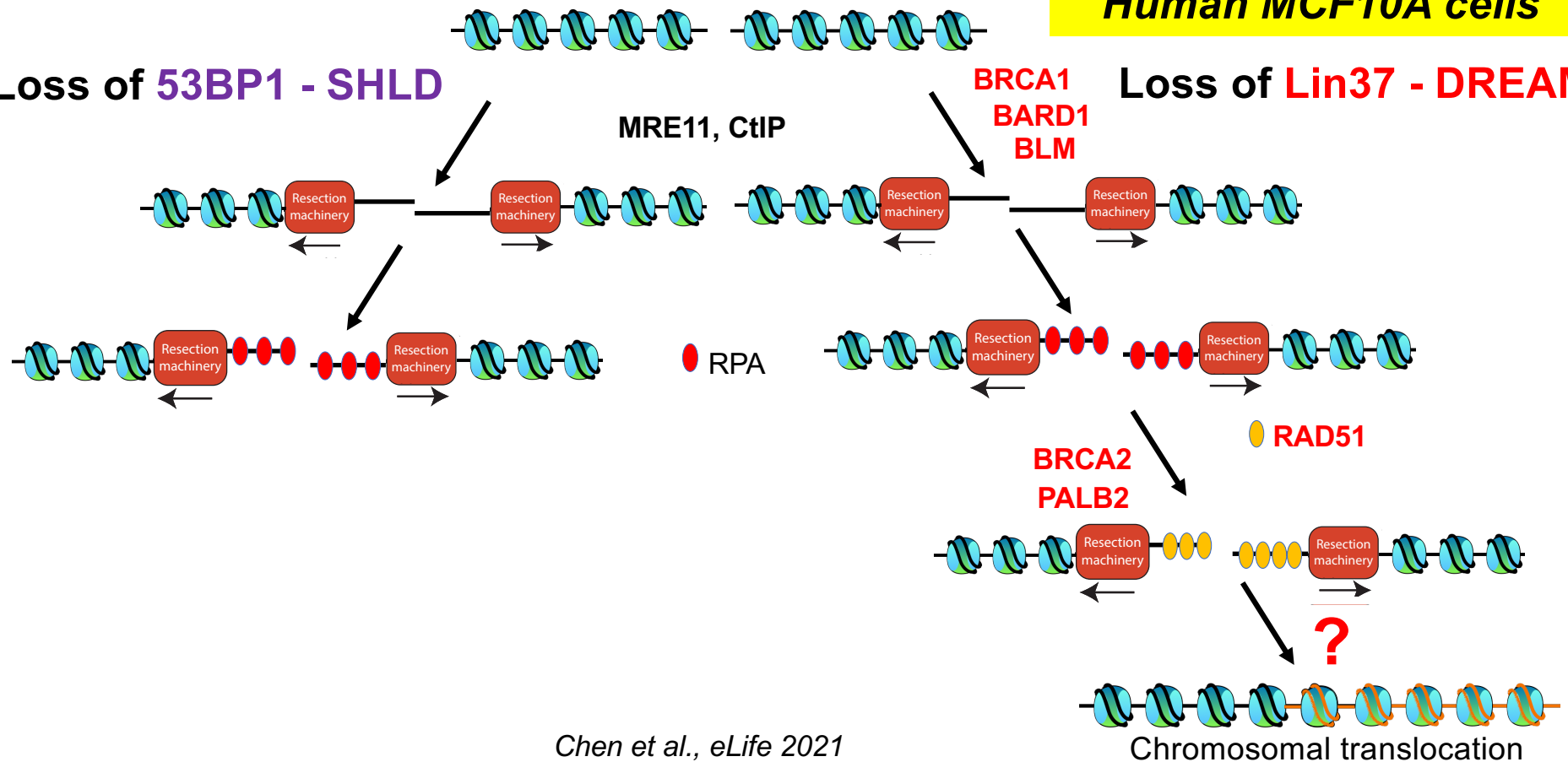
DNA end protection in G₀ cells

G₀ cells:

**See same thing in
Human MCF10A cells**

Loss of 53BP1 - SHLD

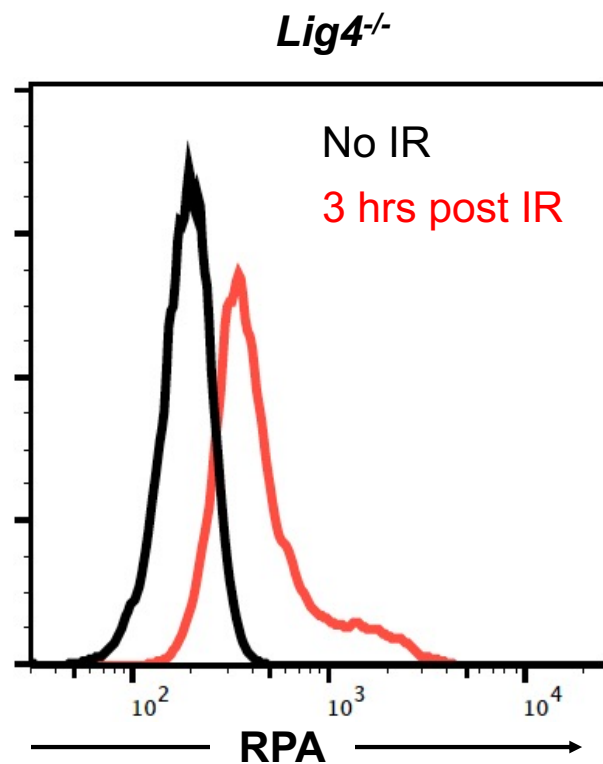
Loss of Lin37 - DREAM



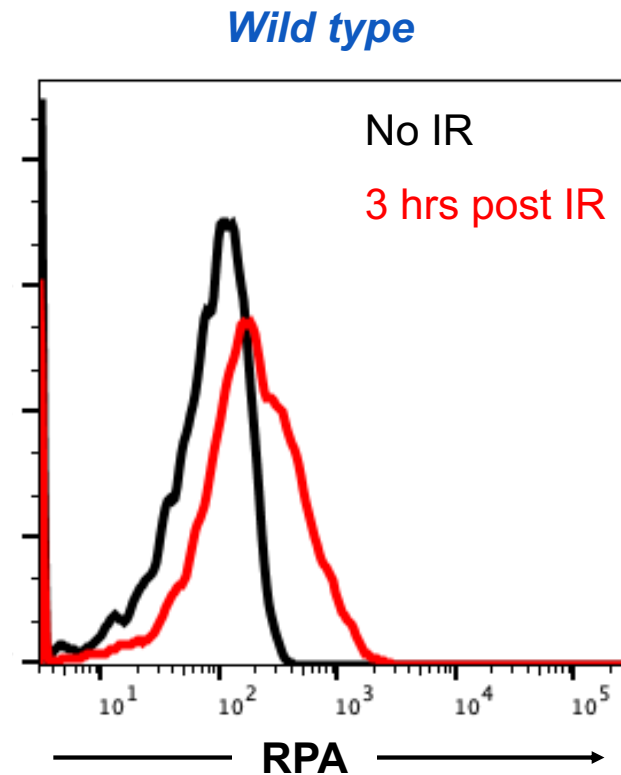
Chen et al., eLife 2021

Potential DNA end resection in G₀ cells 3hrs after IR

G₀ cells:



Faith Fowler

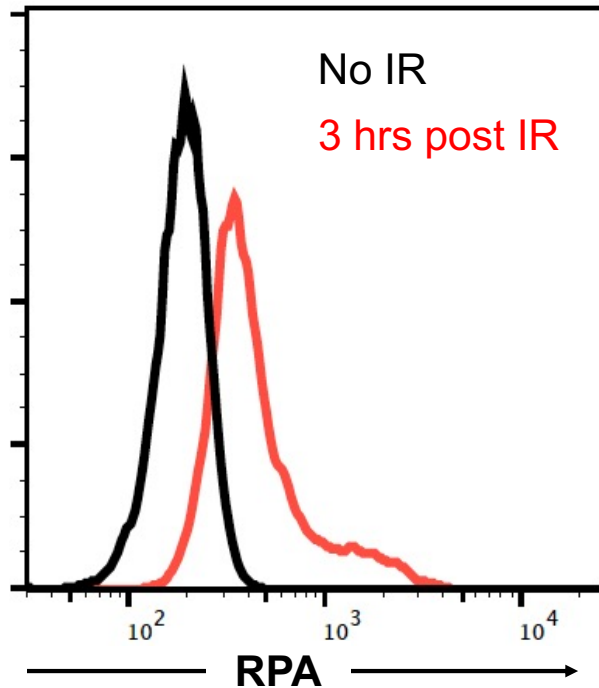


Potential DNA end resection in G_0 cells also occurs in human cells

G_0 cells:

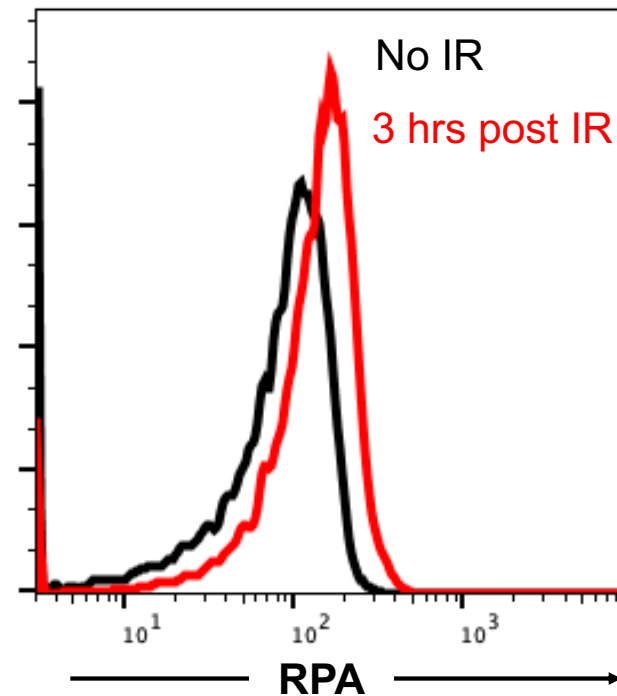
Murine pre-B cells

Lig4^{-/-}



MCF10A cells

Wild type



RPA localizes to DNA damage foci in G₀ cells

MCF10A cells 3 hrs post IR

G₀ cells:

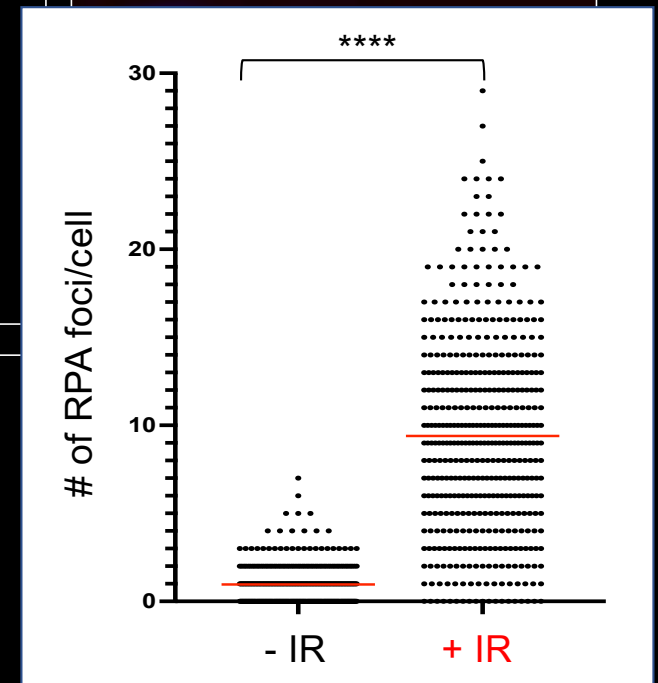
DAPI

RPA

Merge

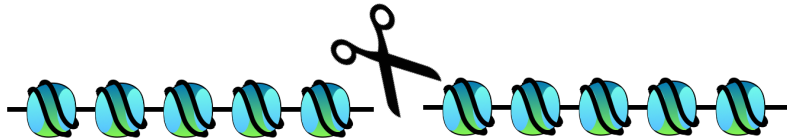
- IR

+ IR

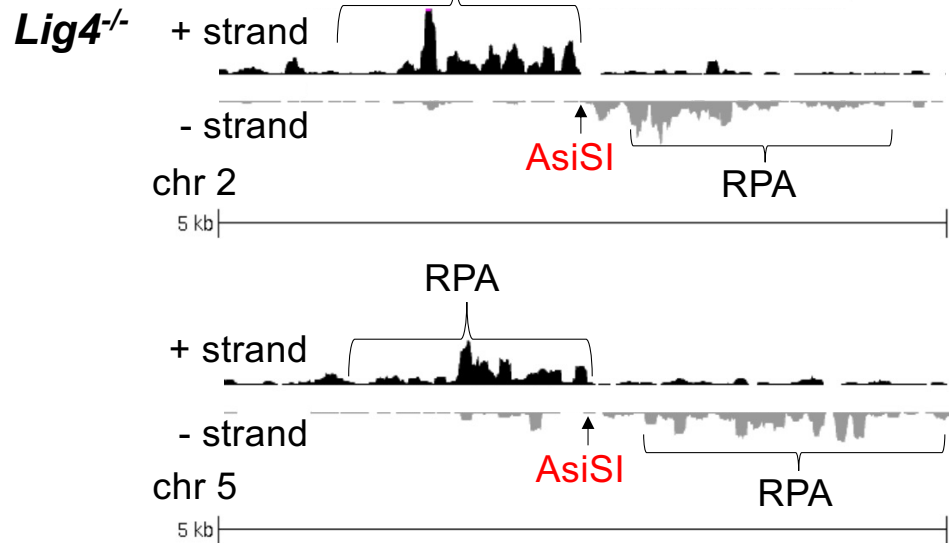


RPA localizes to sites of DNA damage in G₀ cells

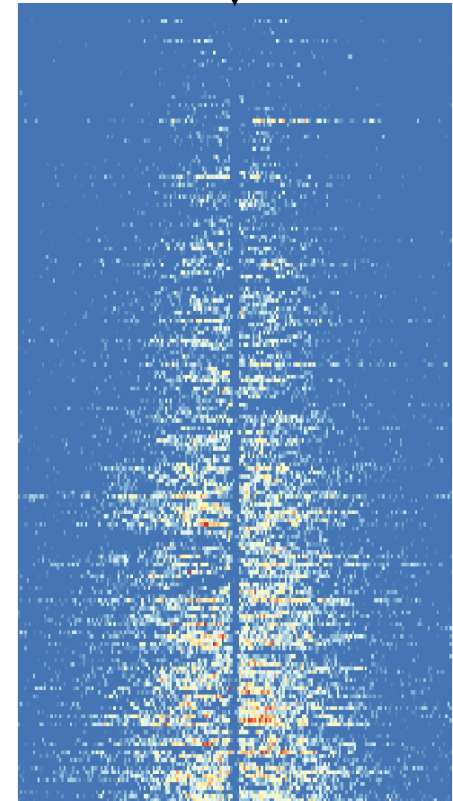
G₀ cells:
Inducible AsiSI endonuclease



RPA-ChIPseq



-5 (kb) AsiSI +5

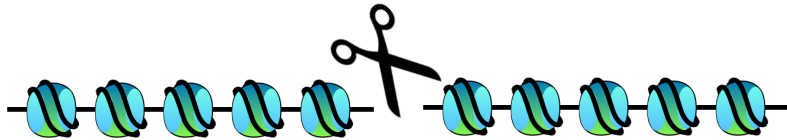


Low High

Raphael Pavani, Wei Wu, Andre Nussenzweig

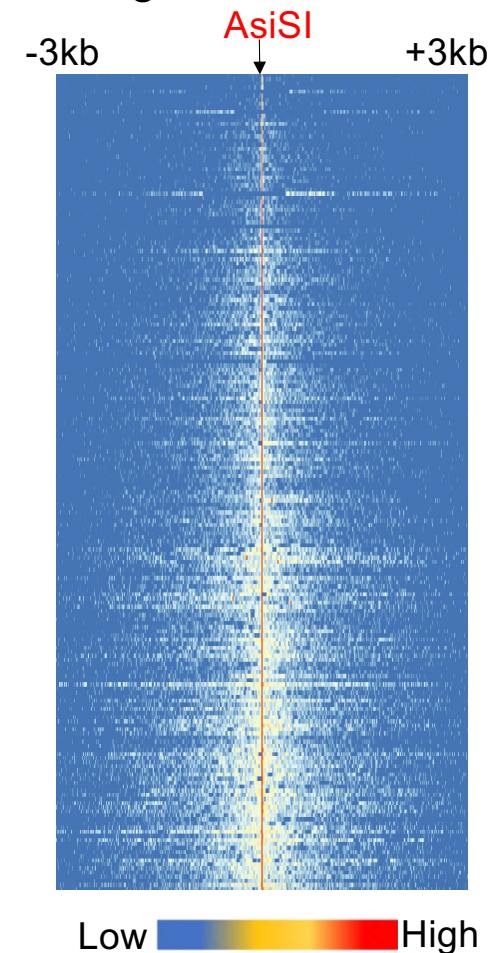
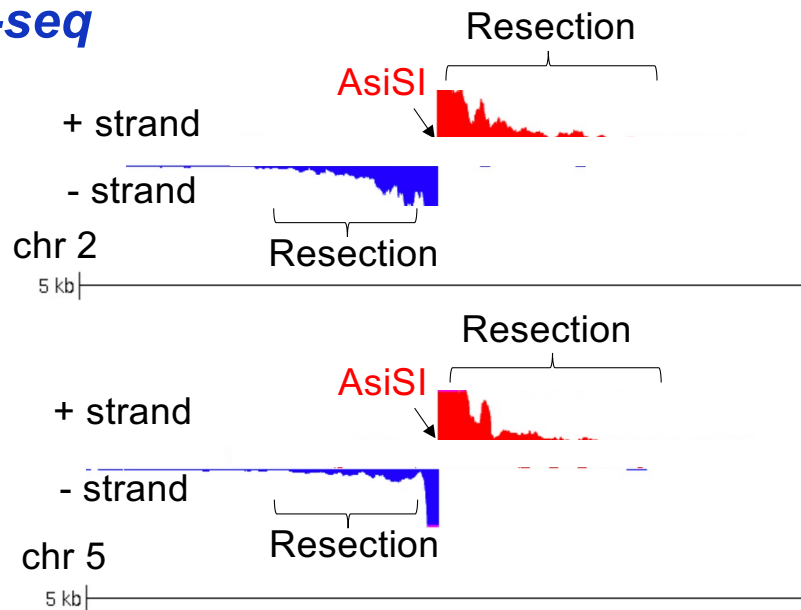
There is DNA end resection in G₀ cells

G₀ cells:
Inducible AsiSI endonuclease



End-seq

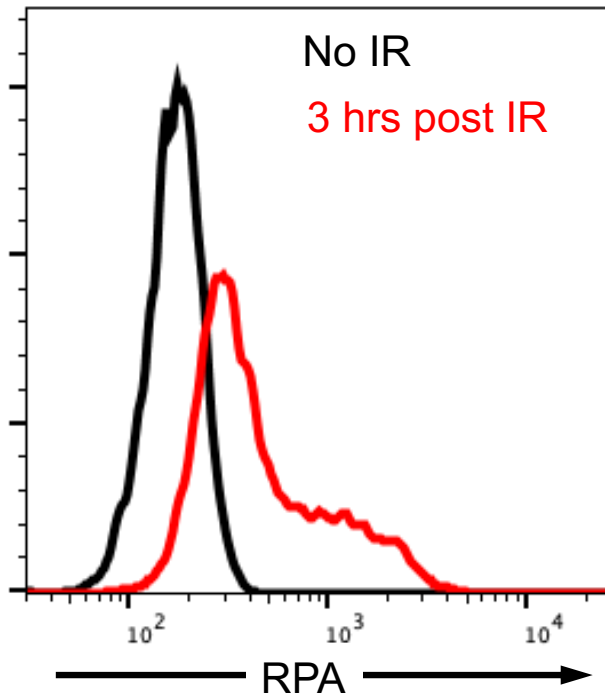
Lig4^{-/-}



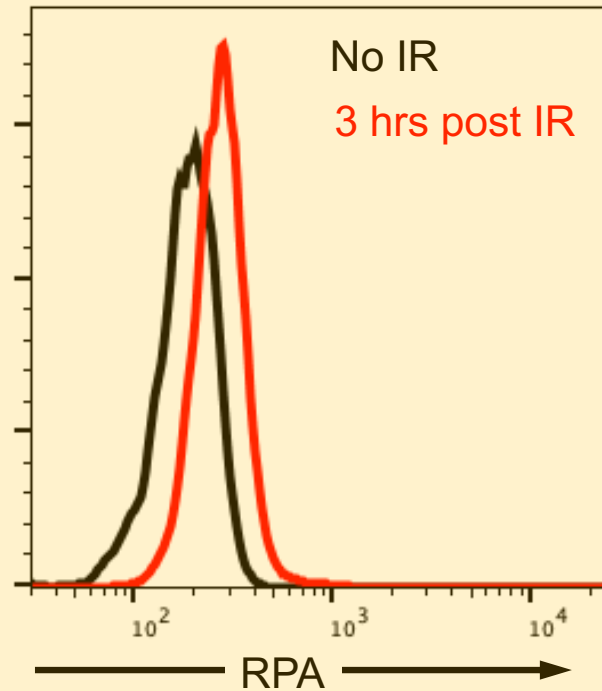
Nick Zolnerowich, Jacob Paiano, Wei Wu, Andre Nussenzweig

The DNA end resection machinery is involved....

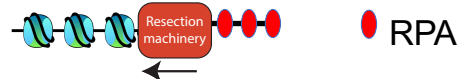
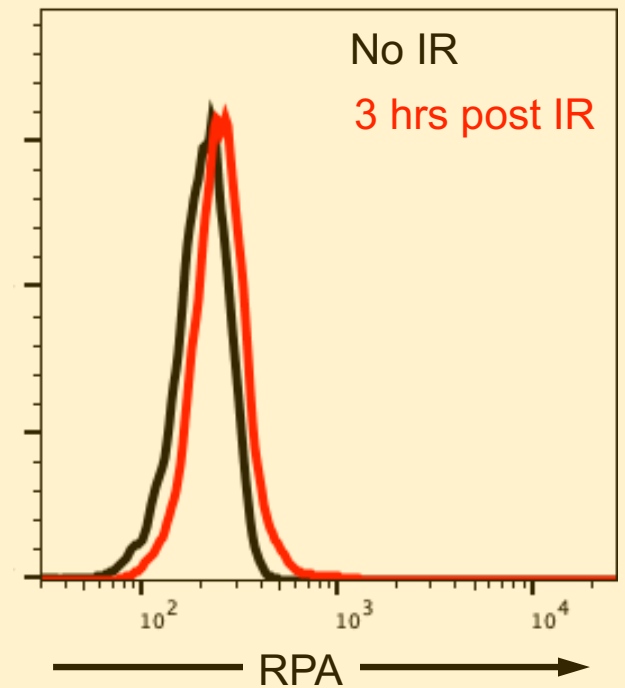
G₀ cells: *Lig4*^{-/-} - gRNA



Lig4^{-/-} + gMre11

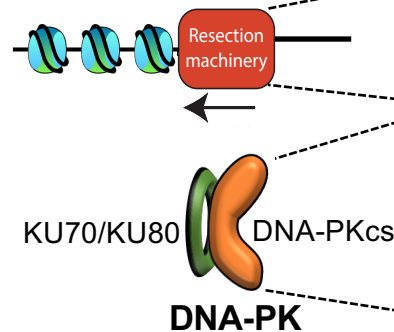
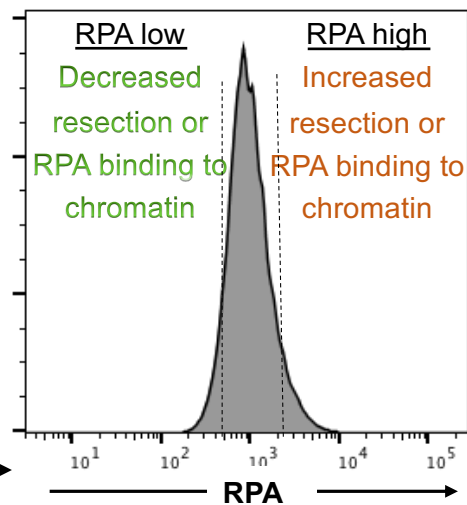
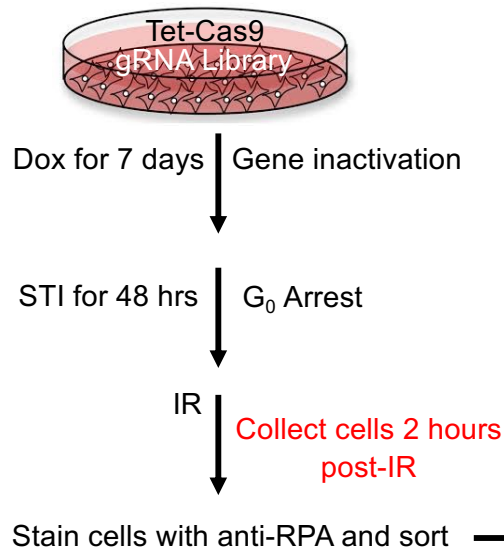


Lig4^{-/-} + gCtIP



CRISPR/Cas9 screen for regulators of end resection

Lig4^{-/-} Abelson Cells



Gene	RPA <u>LOW</u> Fold Enrichment	Gene	RPA <u>HIGH</u> Fold Enrichment
<i>CtIP</i>	7,3,2,2,1	<i>Rnf168</i>	5,4,4,3,2
<i>Nbn</i>	5,5,4,3,2	<i>53bp1</i>	11,10,8,4,4
<i>Prkdc</i>	20,14,12,7,6	<i>Shld1</i>	6,5,4,4,3
<i>Ku70</i>	33,14,9,7,5	<i>Rif1</i>	5,4,2,2
<i>Ku80</i>	16,16,11,9,6	<i>Fbxl12</i>	64,33,26,22,20

DNA-PKcs-dependent RPA loading in G₀ cells

3 hrs post IR

G₀ cells:

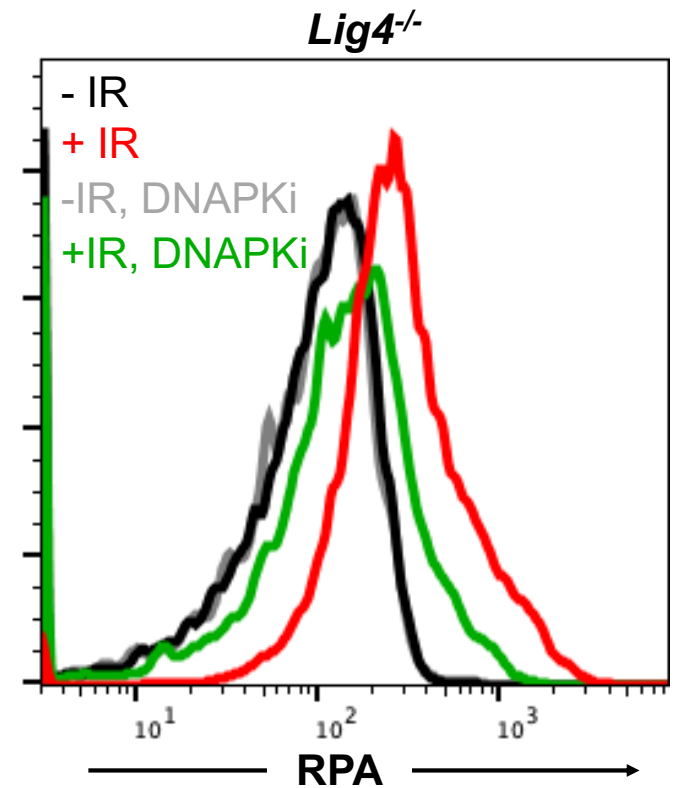
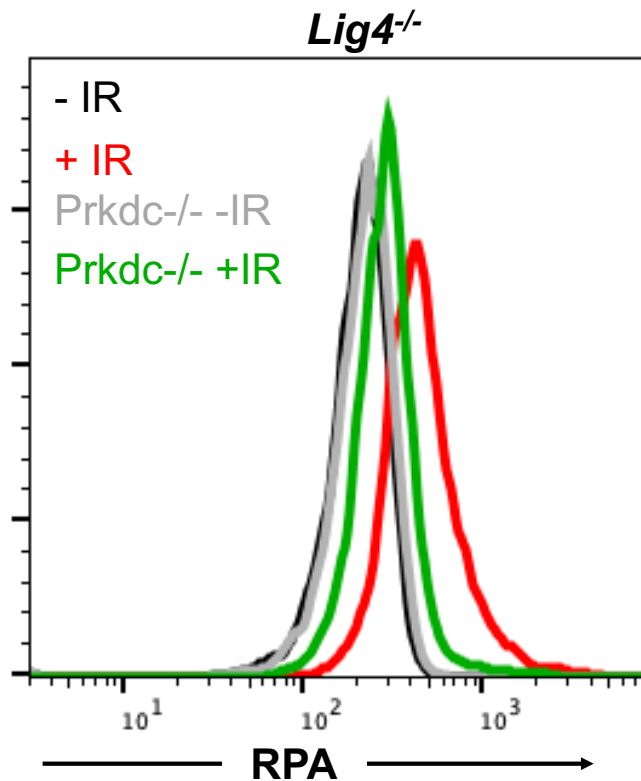
Deletion of DNA-PKcs encoding gene:

DNA-PK

KU70/KU80

DNA-PKcs

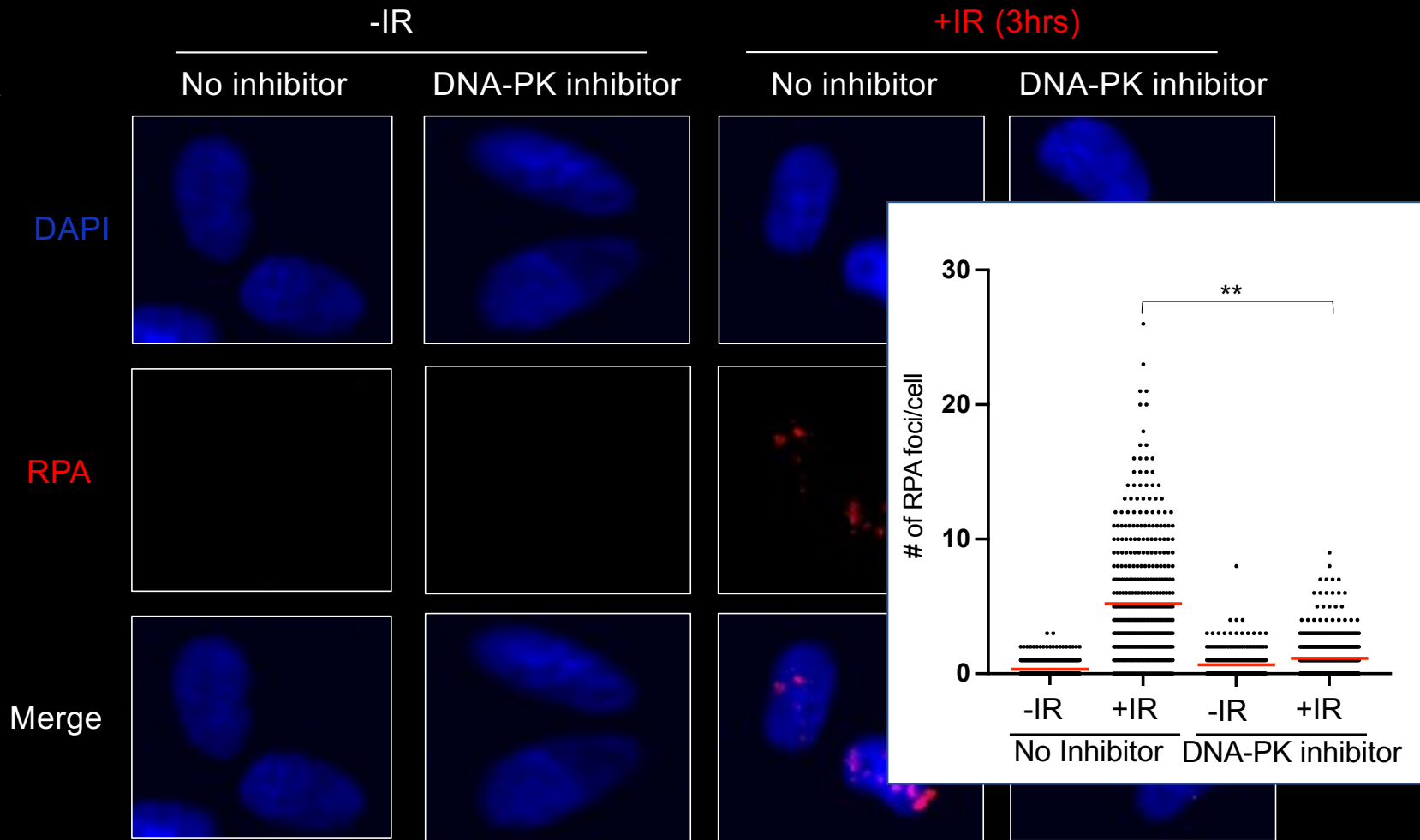
Inhibition of DNA-PKcs:



DNA-PK-dependent RPA localization to damage foci

***G₀* arrested
MCF10A cells**

3 hrs post IR

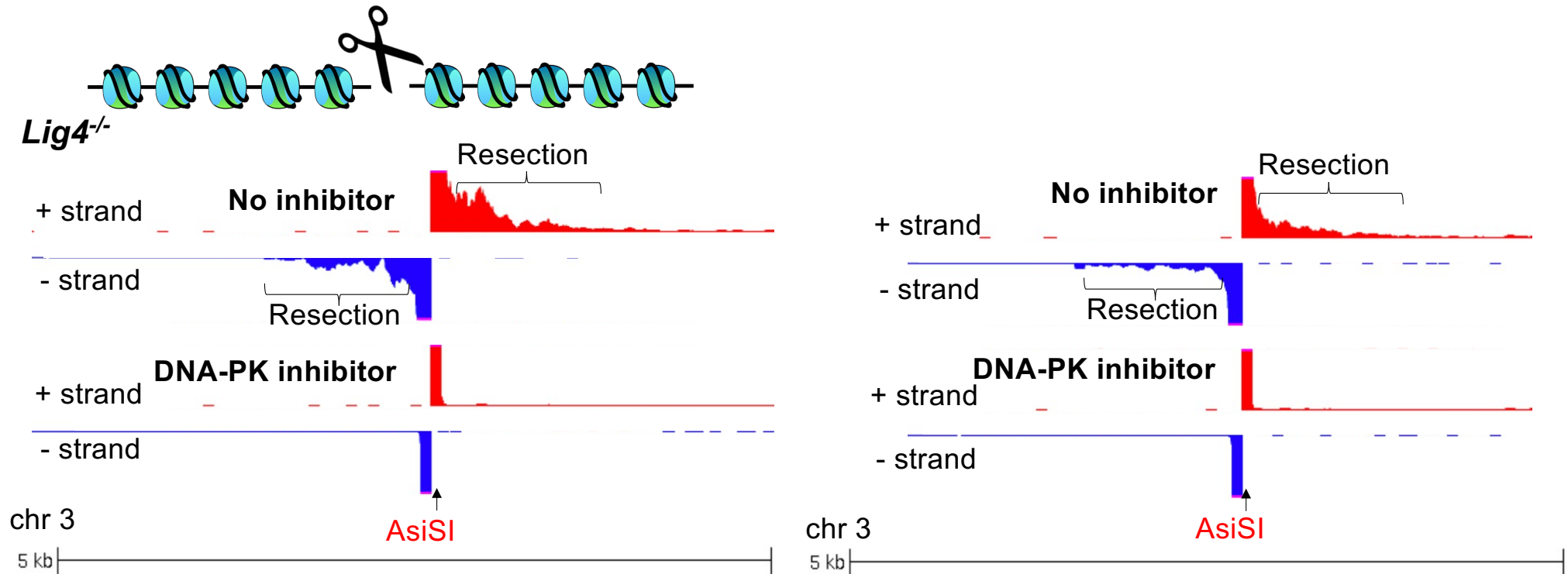


DNA-PKcs-dependent DNA end resection in G₀ cells

G₀ cells:

End-seq

Inducible AsiSI endonuclease



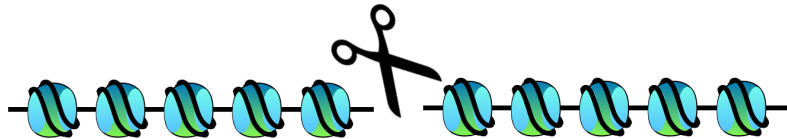
Nick Zolnerowich, Jacob Paiano, Wei Wu, Andre Nussenzweig

DNA-PKcs-dependent DNA end resection in G₀ cells

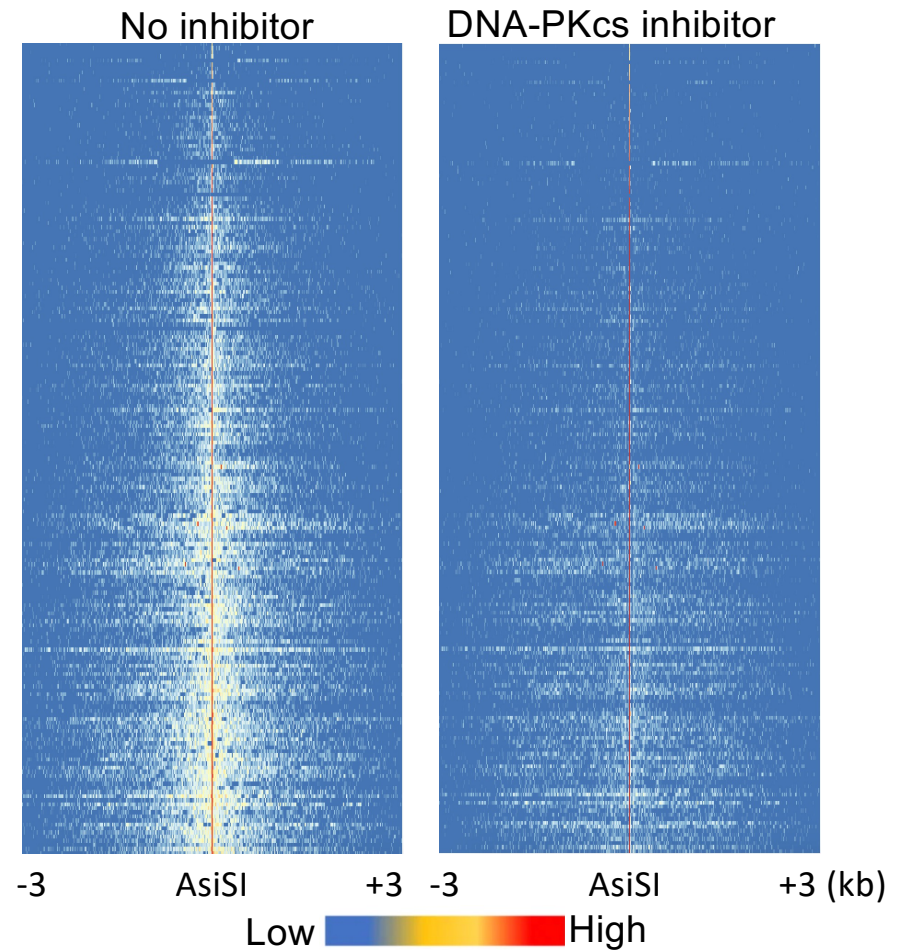
G₀ cells:

End-seq

Inducible AsiSI endonuclease



Lig4^{-/-}

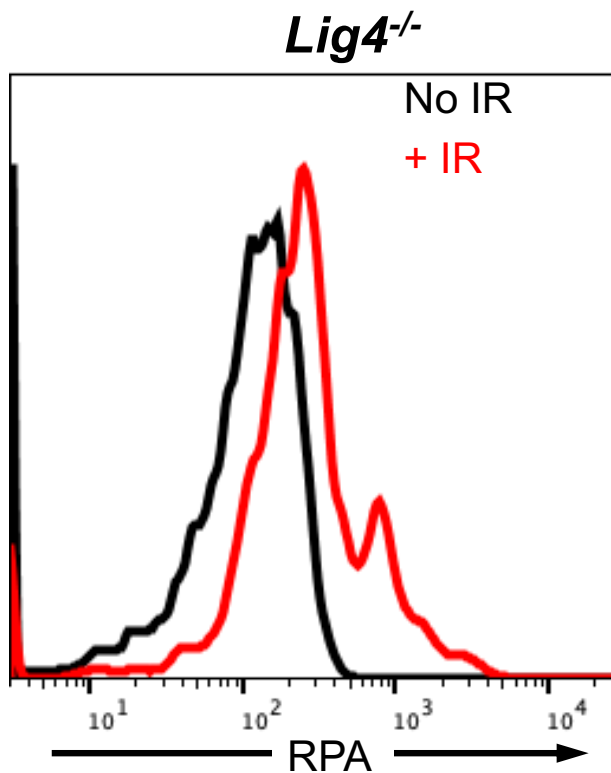


Nick Zolnerowich, Jacob Paiano, Wei Wu, Andre Nussenzweig

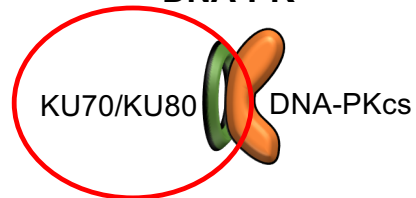
KU70-dependent RPA loading in G₀ cells

3 hrs post IR

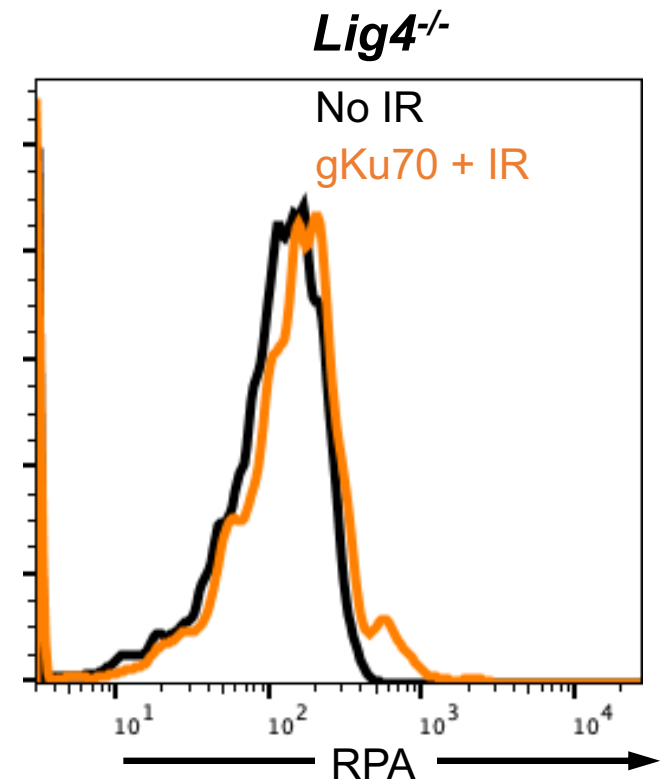
G₀ cells:



DNA-PK

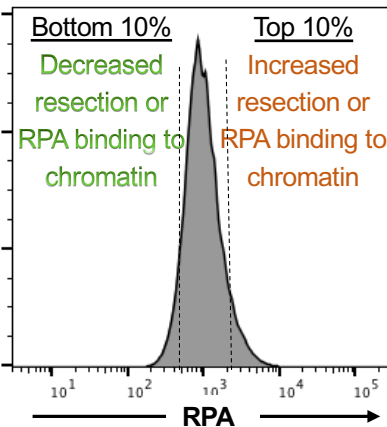
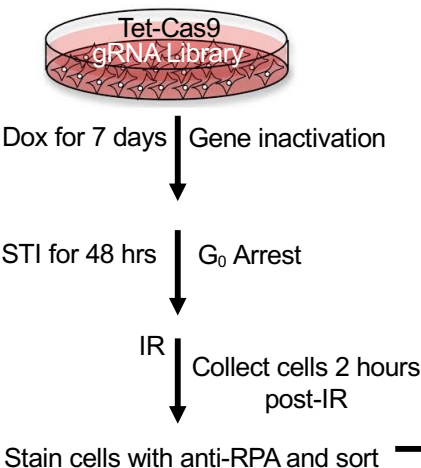


Deletion of KU70 encoding gene:

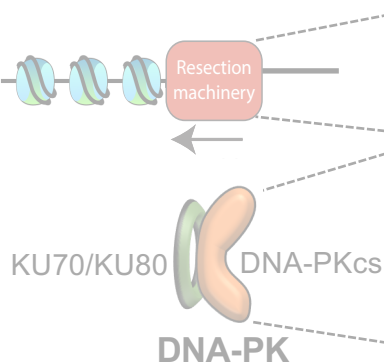


CRISPR/Cas9 screen for regulators of end resection

Lig4^{-/-} Abelson Cells

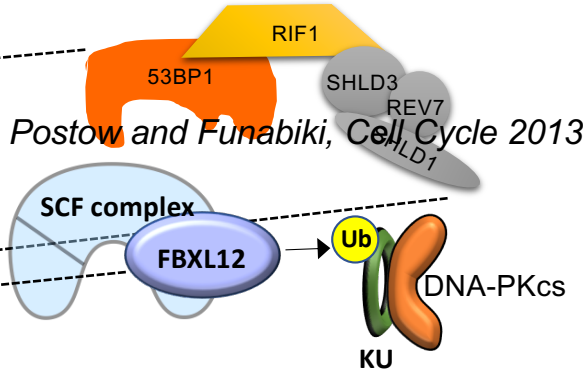


Pro DNA end resection



Gene	RPA LOW Fold Enrichment	Gene	RPA HIGH Fold Enrichment
<i>CtIP</i>	7,3,2,2,1	<i>Rnf168</i>	5,4,4,3,2
<i>Nbn</i>	5,5,4,3,2	<i>53bp1</i>	11,10,8,4,4
<i>Prkdc</i>	20,14,12,7,6	<i>Shld1</i>	6,5,4,4,3
<i>Ku70</i>	33,14,9,7,5	<i>Rif1</i>	5,4,2,2
<i>Ku80</i>	16,16,11,9,6	<i>Fbx12</i>	64,33,26,22,20

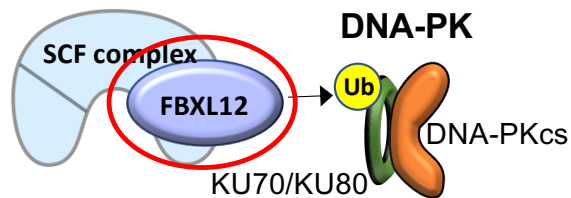
Pro DNA end protection



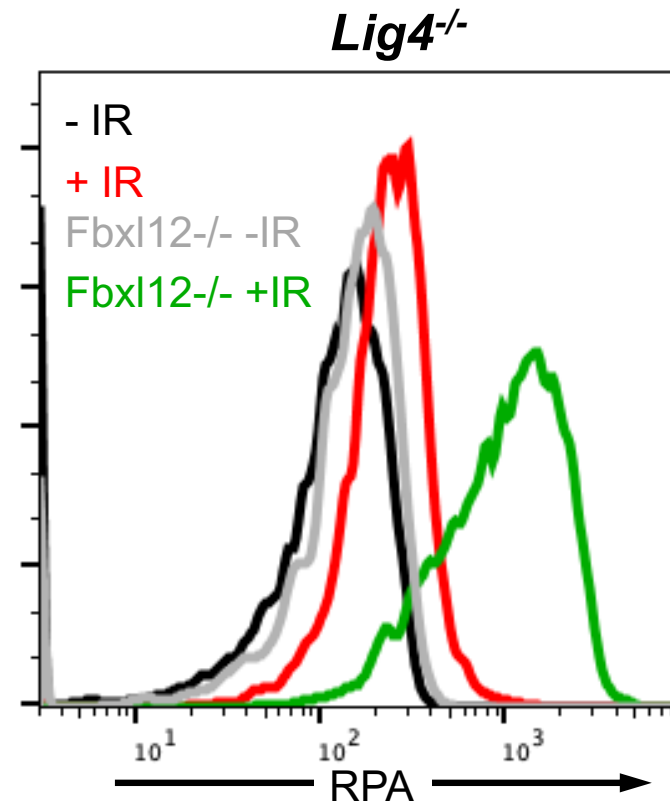
FBXL12 prevents overloading of RPA in G₀ cells

3 hrs post IR

G₀ cells:



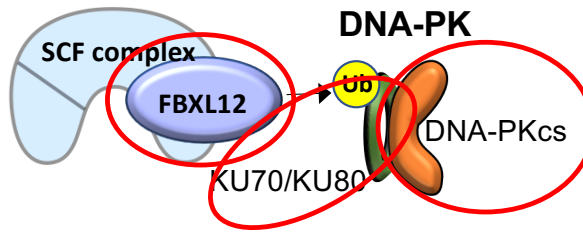
Deletion of Fbxl12 encoding gene:



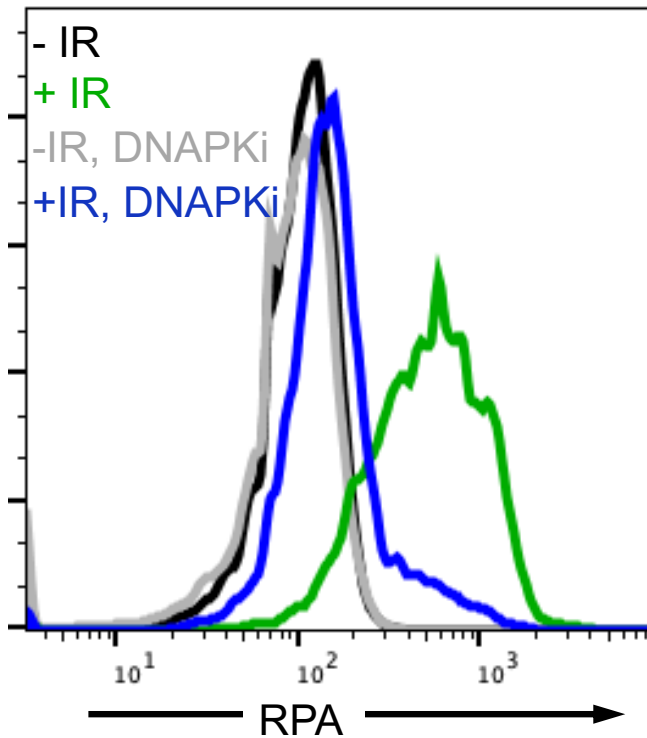
FBXL12 prevents overloading of RPA in G₀ via DNA-PK

3 hrs post IR

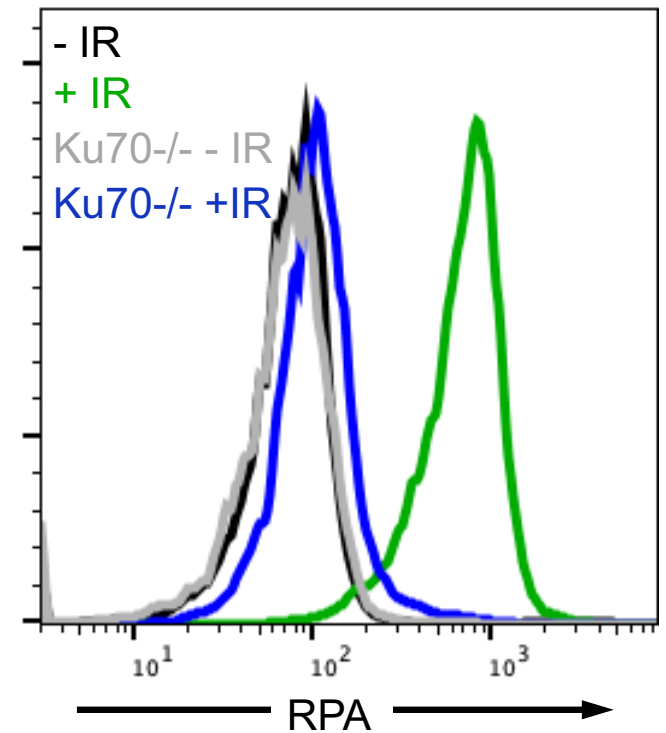
G₀ cells:



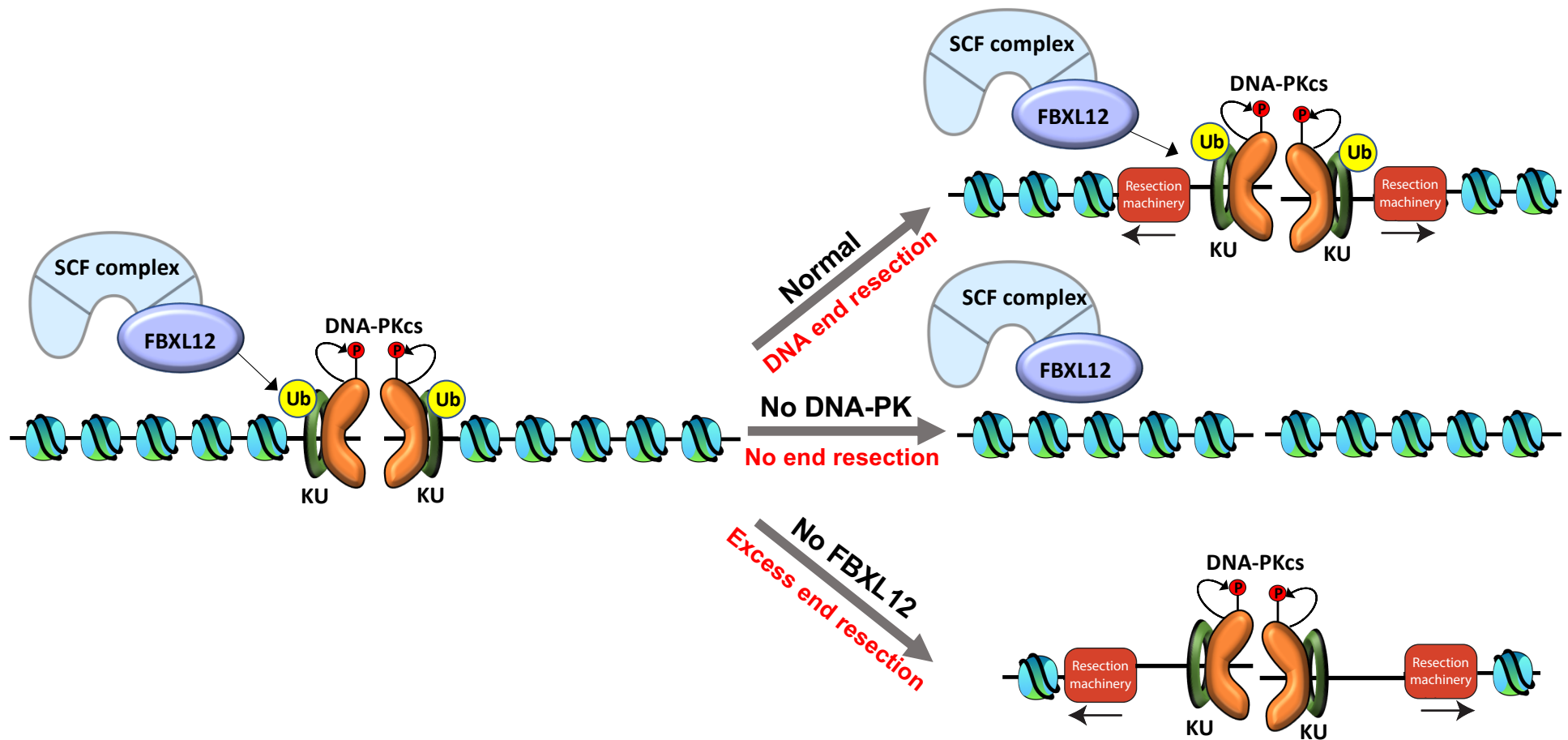
Lig4^{-/-}:Fbx12^{-/-}



Lig4^{-/-}:Fbx12^{-/-}



Summary of data so far in G_0 cells at earlier times after DSB induction



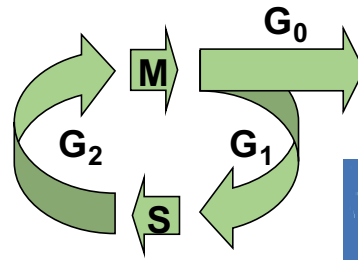
There is no DNA-PK-dependent resection in G₂ phase

End-seq

Lig4^{-/-} No inhibitor

G₂

DNA-PKcs inhibitor

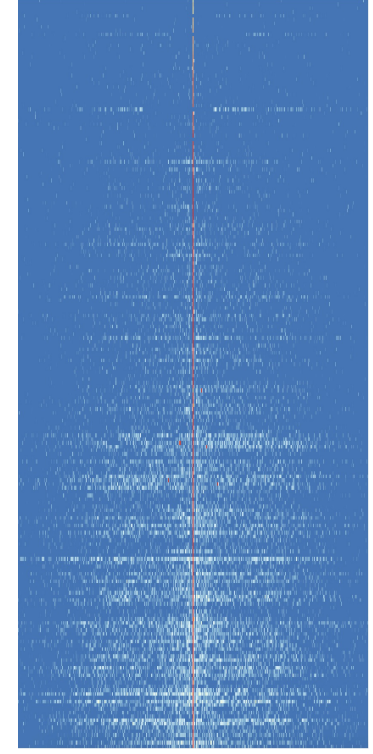
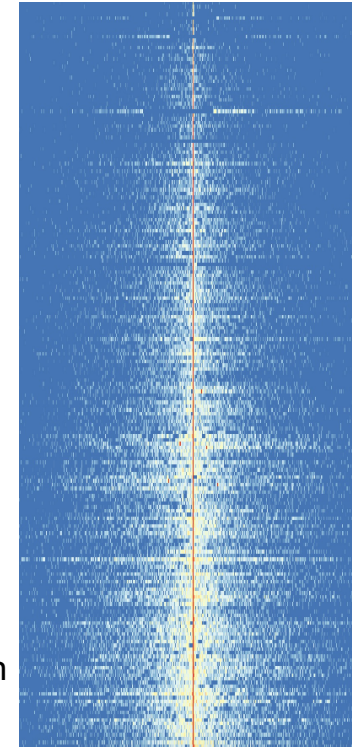
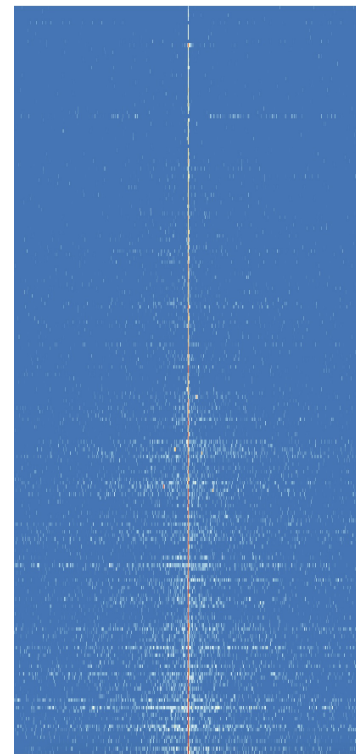
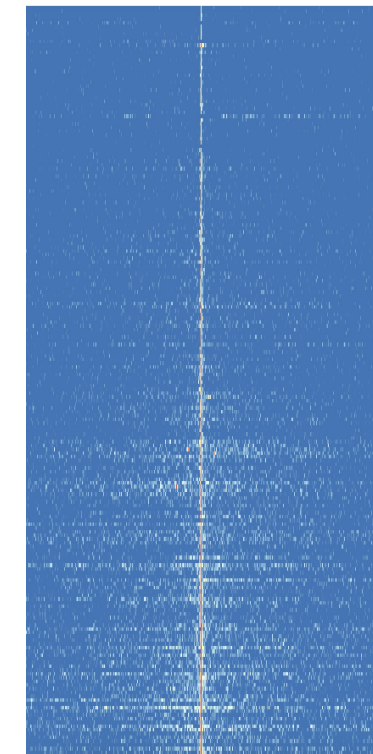


No inhibitor

G₀

DNA-PKcs inhibitor

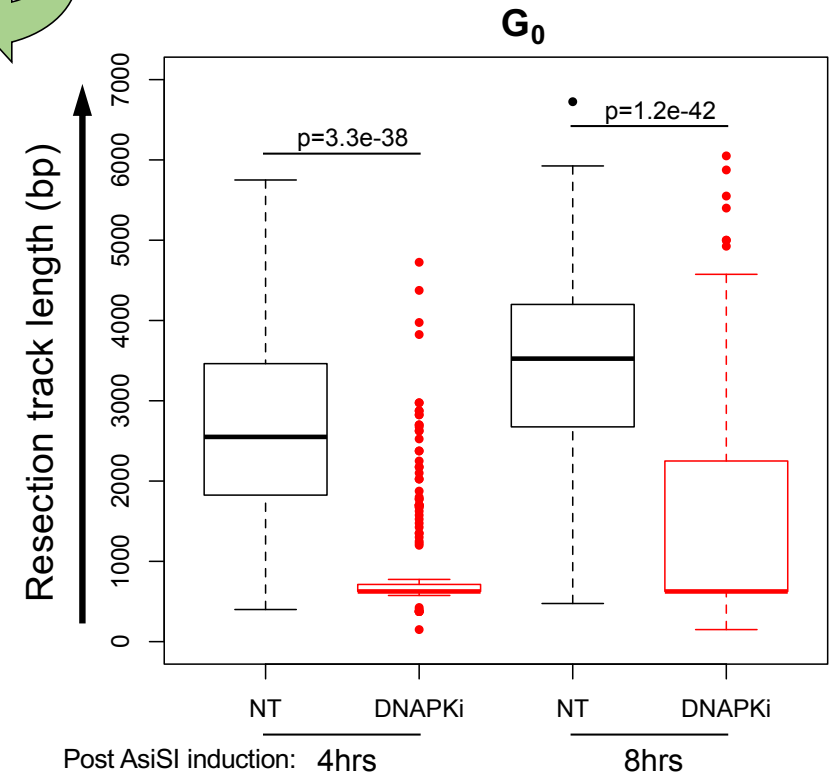
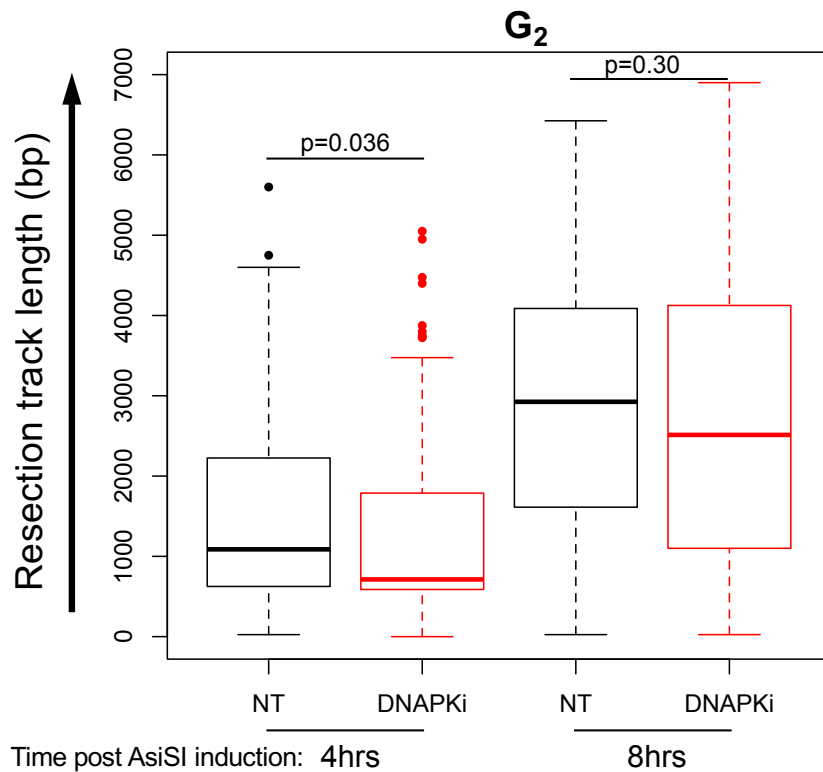
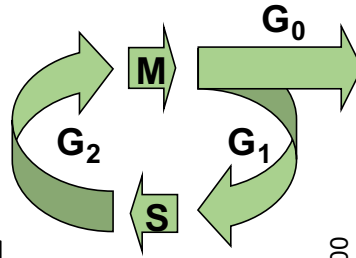
Low  High



Nick Zolnerowich, Jacob Paiano, Wei Wu, Andre Nussenzweig

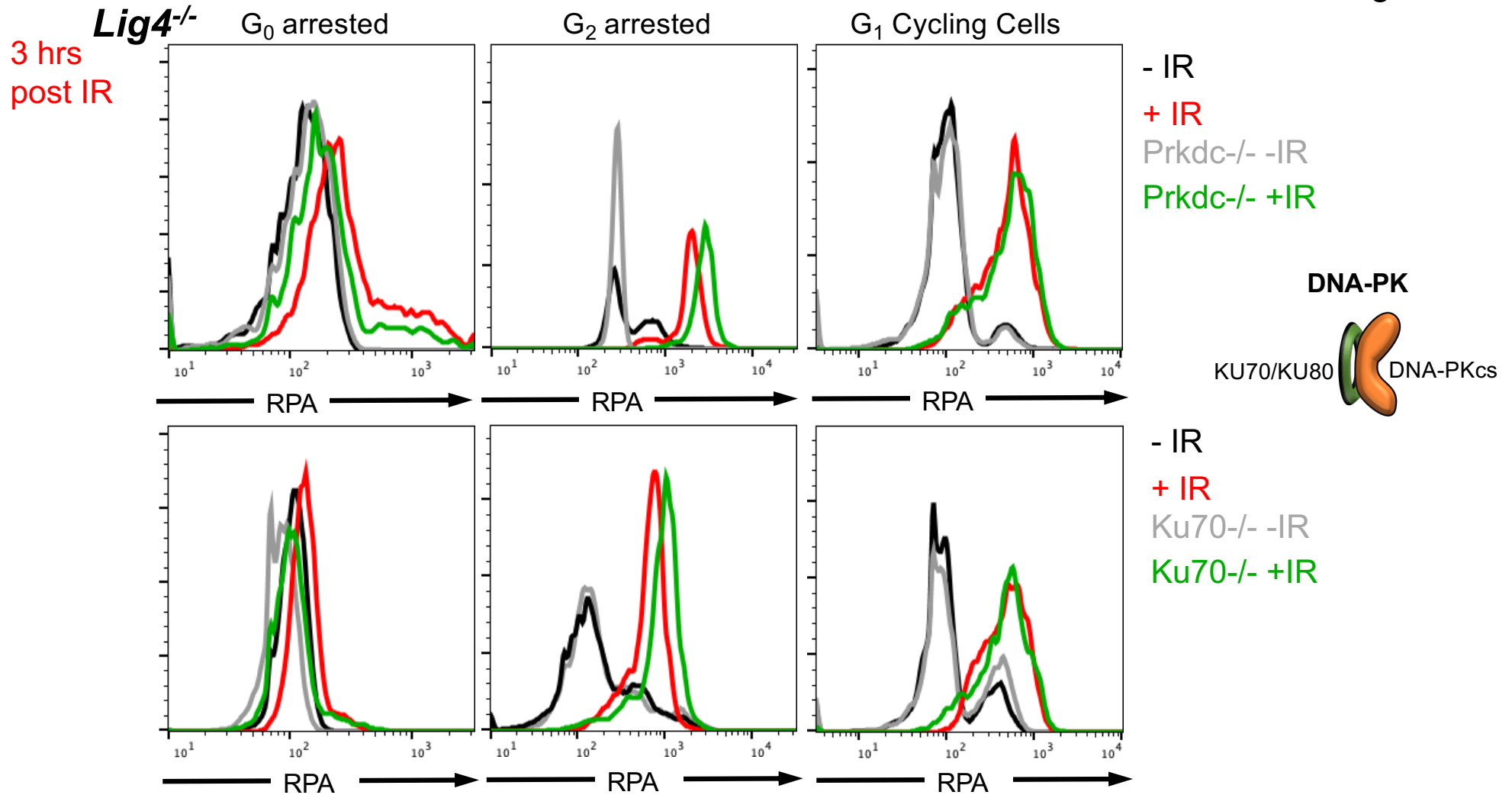
Quantitation of resection tract lengths

Lig4^{-/-}

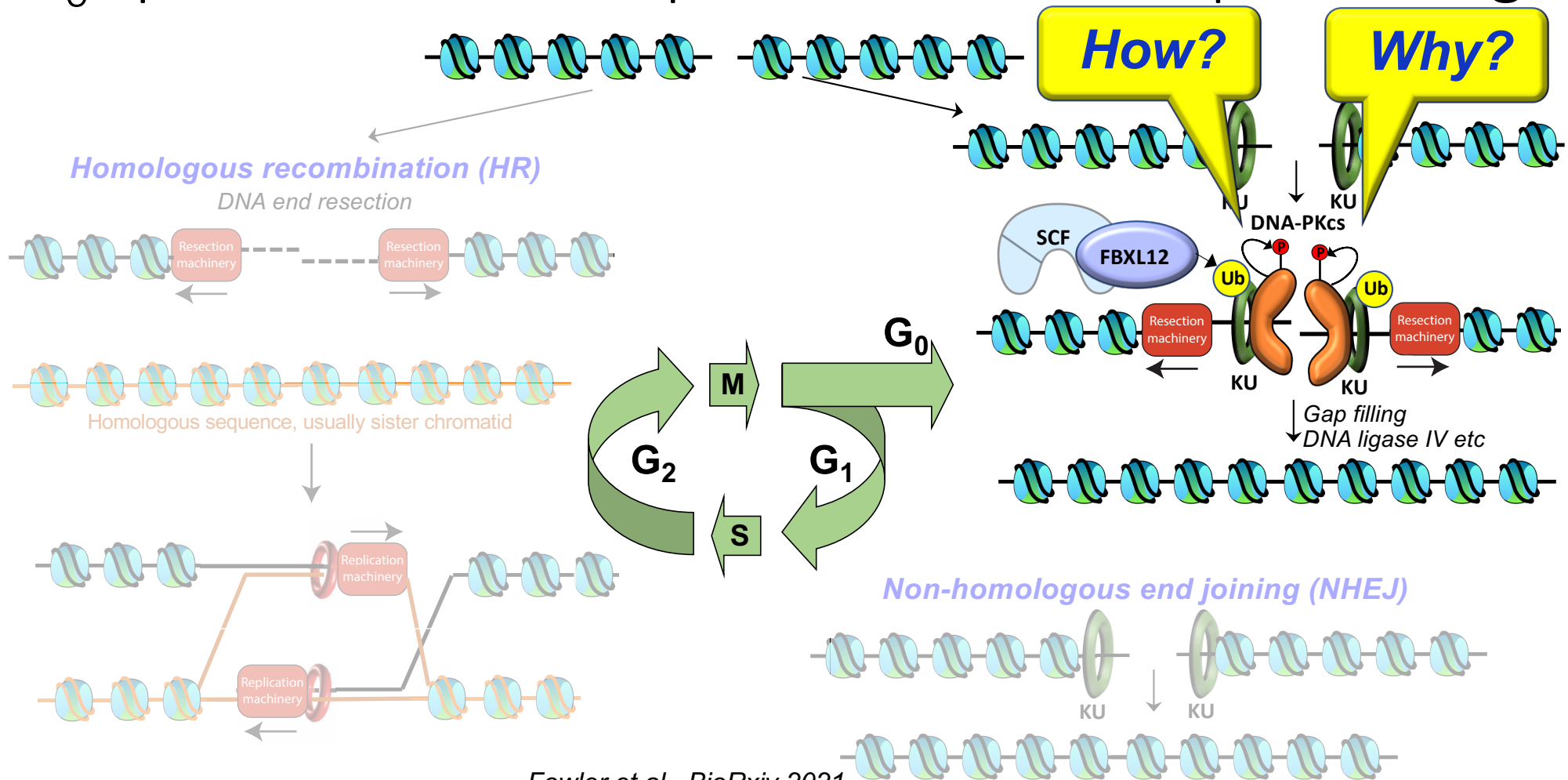


Nick Zolnerowich, Jacob Paiano, Wei Wu, Andre Nussenzweig

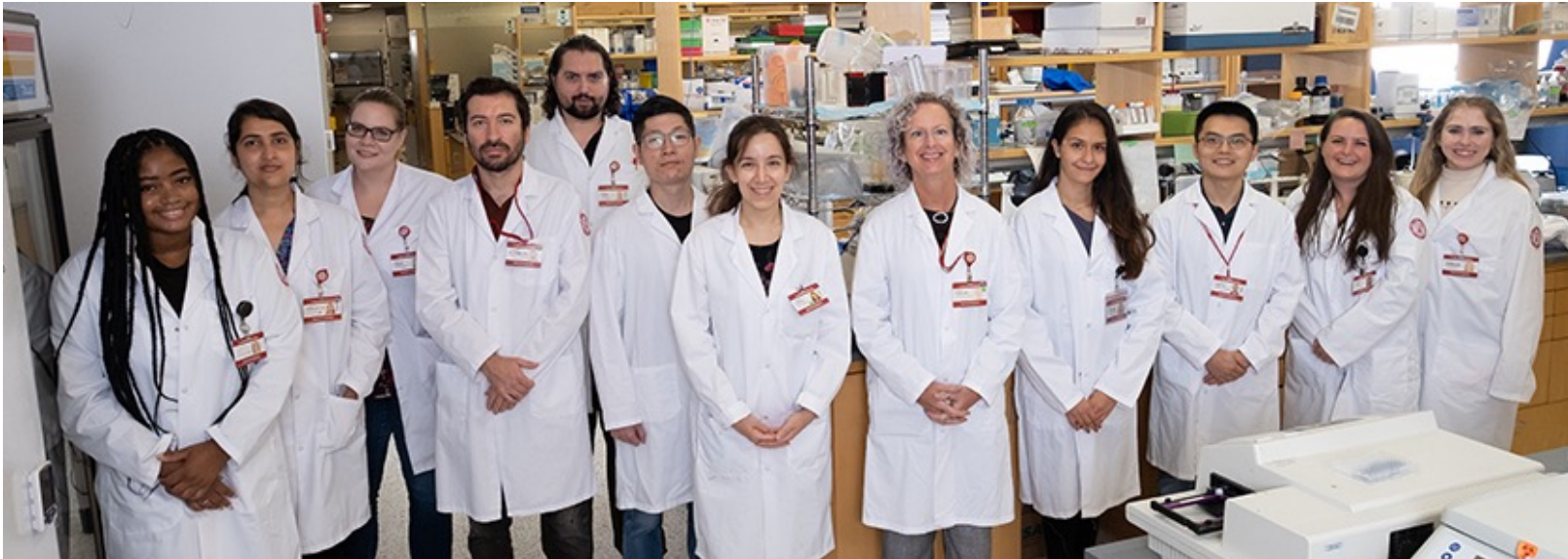
DNA-PK dependent end resection is unique to G₀ cells



G_0 specific DNA-PK dependent DNA end processing



Acknowledgements



Tyler lab (WCM)

Faith Fowler
Chitra Mohan
Kaylah Birmingham
Zulong Chen
Nina Arslanovik
Rhiannon Aguilar

Ujani Chakraborty
Helen Hoxie
Chelsea Peart
Ignacio Gutierrez
Spike Postnikoff



Sleckman lab (UAB)

Bo-Rui Chen
Lance Reynolds
Kim Manalang
Zih-Jie Shen



Andre Nussenzweig lab (NCI)

Jacob Paiano
Raphael Pavani
Anthony Tubbs
Wei Wu
Nicholas Zolnerowich
Dali Zong

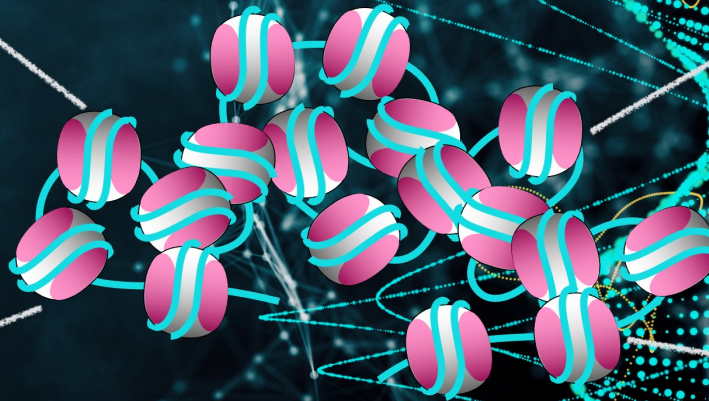


The interplay between epigenetic regulation and genome stability

Sept 28th-October 2nd, 2022
Sheraton Grand Seattle, Seattle

Save
the date

Most talks will be
chosen from
submitted
abstracts



Organizers: Jessica Tyler, Zhiguo Zhang, and Guohong Li

Keynote speakers



Geneviève Almouzni



John Diffley

Speakers

Irene Chiolo, University of Southern California
Daniel Durocher, University of Toronto
Susan Gasser, Friedrich Miescher Institute for Biomedical Research
Steven Henikoff, Fred Hutchinson Cancer Research Center
Qing Li, Peking University
Dirk Remus, Sloan Kettering Institute

Evi Soutoglou, University of Sussex
Toshio Tsukiyama, Fred Hutchinson Cancer Research Center
Haico van Attikum, Leiden University
Ruiming Xu, Institute of Biophysics, Chinese Academy of Sciences
Hongtao Yu, Westlake University