Deletion ≠ Inhibition: from DNA-PKcs to PARPs

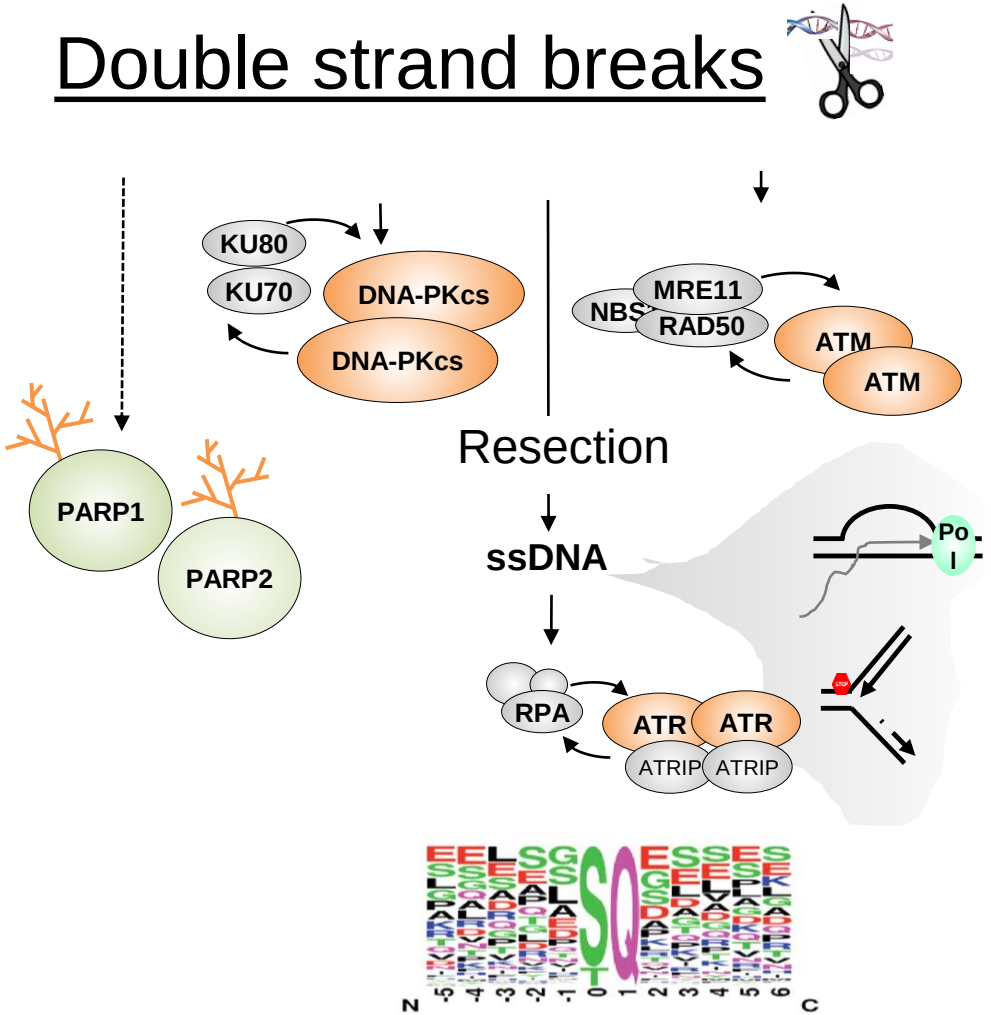
in DNA damage responses to cancer therapy

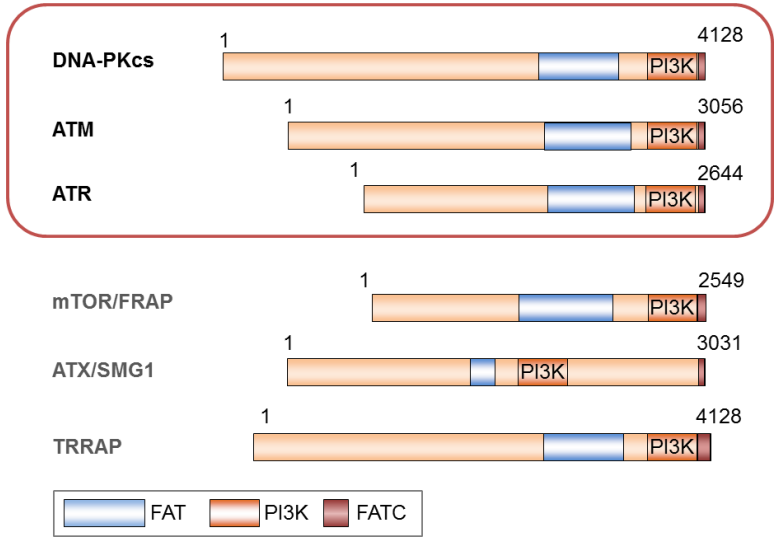
Shan Zha
Columbia University

Sept 21, 2021

DNA Damage Responses

Double strand breaks

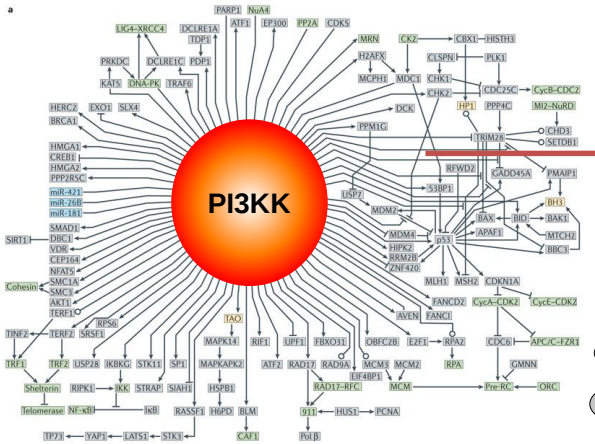


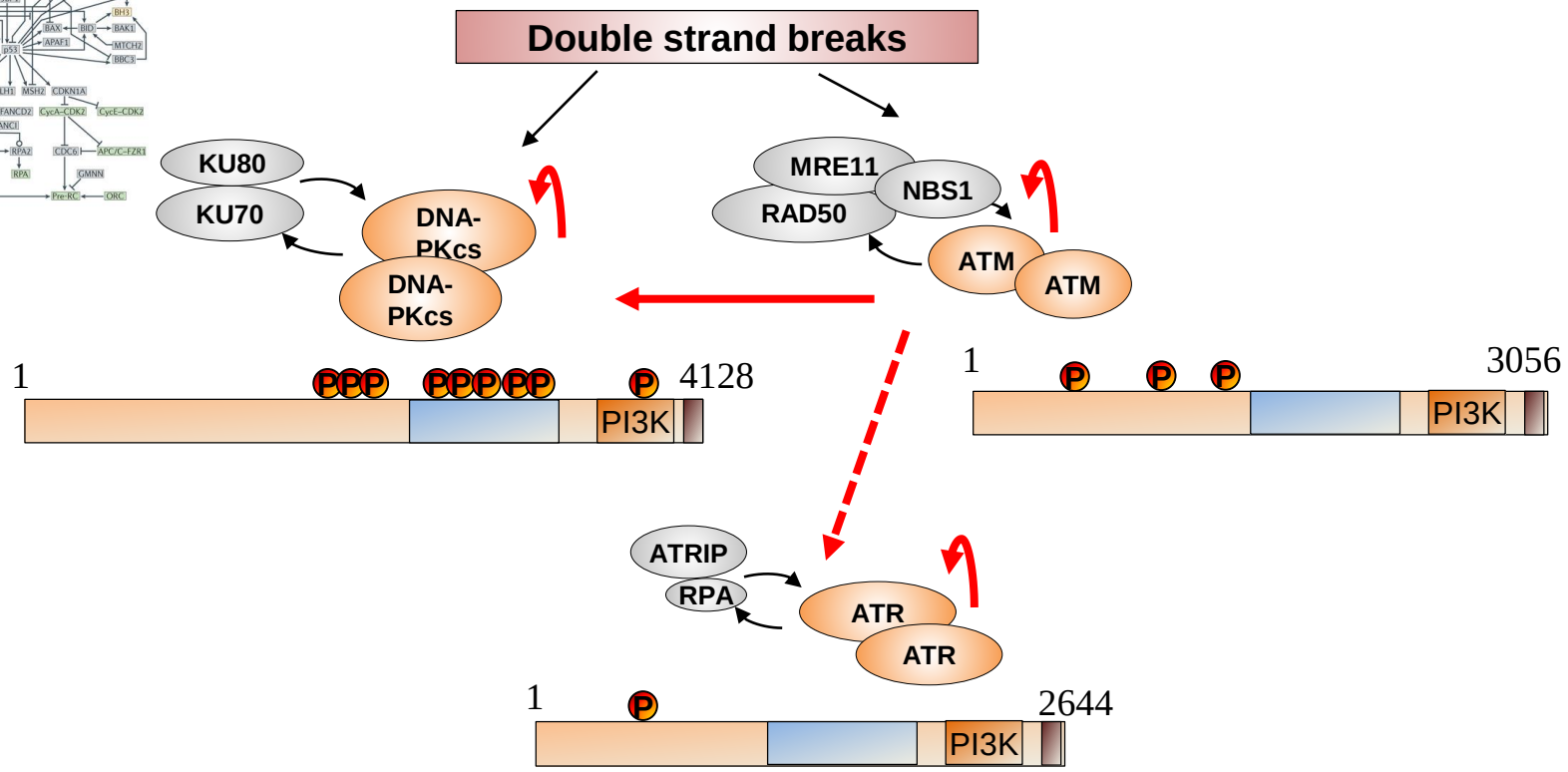


Inhibition = or ≠ deletion

Modified from Science 25 May 2007: Vol316 (1160-1166)
Peter J McKinnon EMBO Rep. 2004;5:772-776

Beyond Signaling ???

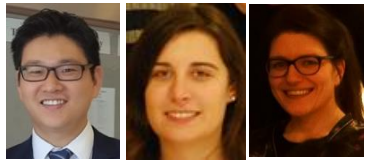




Nature Reviews Molecular Cell Biology 14, 197-210

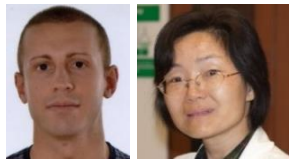
Catalytic Inactivation Models

1. **ATM Kinase Dead (2012, 2016, 2020, 2021).**

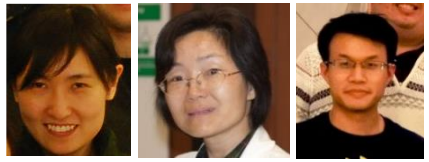


2. **DNA PKcs Kinase Dead (2015, 2018, 2020)**

3. **ATR Kinase Dead (2018)**



4. **Catalytically inactive PARP1 and PARP2**



Kinase	PI3KK Core	Mouse	Human
ATM	LGLGDRH	D2880	D2870
DNA-PKcs	LGIGDRH	D3922	D3889
ATR	LGLGDRH	D2466	D2475

Catalytic Inhibition ≠ Deletion ≠ phosphorylation

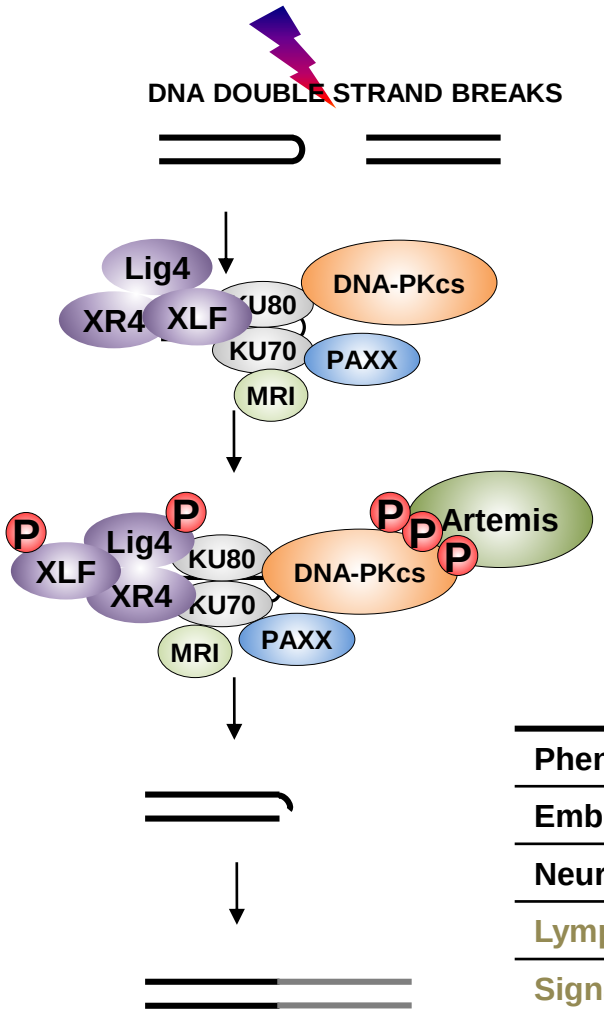
Gene	Deletion	Catalytic inactive	Rescued	Pho mut
ATM	Viable	Lethal E9.5	Mre11 deletion	No phenotype
ATR	+/- normal	Male infertility		N/A
DNA-PK	Viable	Lethal E15.5	Ku70/80 deletion	Moderate NHEJ defects,

ATM -| strand cleavage (Replication/HR),

ATR –| long ssDNA (*e.g.*, Cisplatin, meiosis),

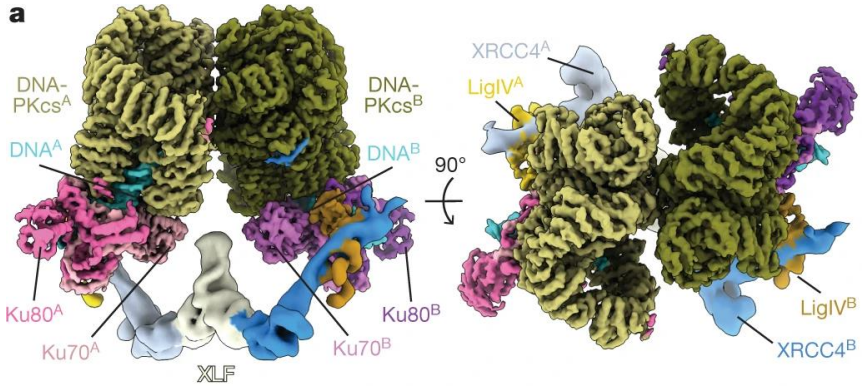
DNA-PKcs –| end-ligation (NHEJ), rRNA and rDNA

Non-Homologous End-Joining



End-ligation: Ku70/80, Lig4, XRCC4, XLF, PAXX and MRI (CYREN)

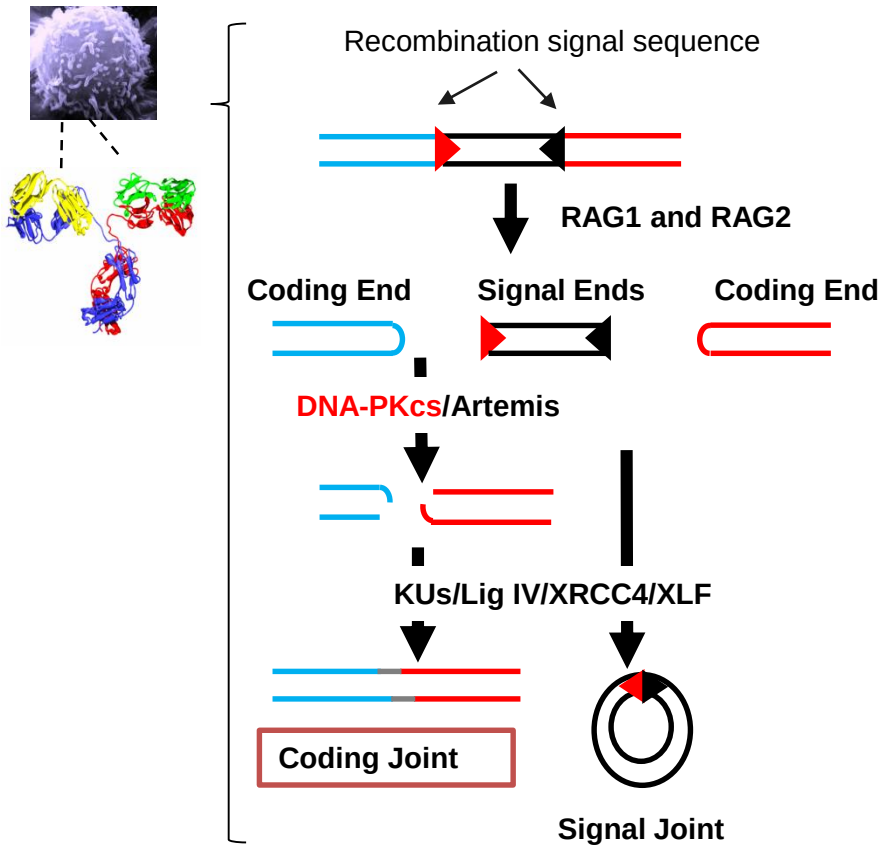
End-processing: DNA-PKcs, Artemis

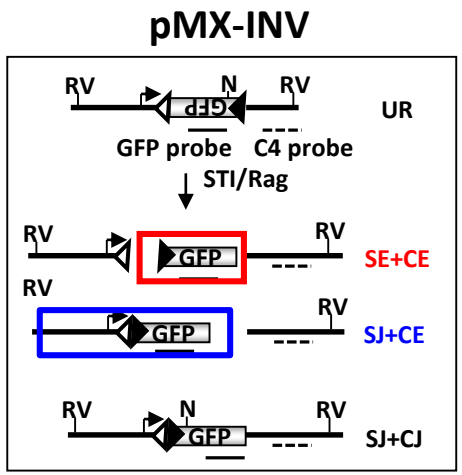


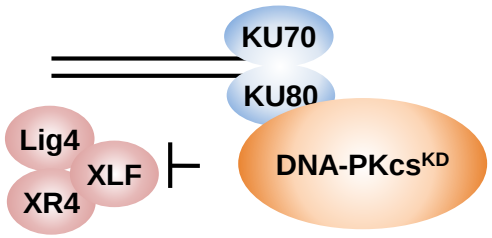
Phenotype	Lig4/XRCC4	DNA-PKcs/Artemis
Embryonic Dev.	E16.5 lethal	Normal size
Neuronal Apoptosis	Severe	Not observed
Lymphocyte	Blocked	Blocked
Signal Join	Blocked	**Normal**

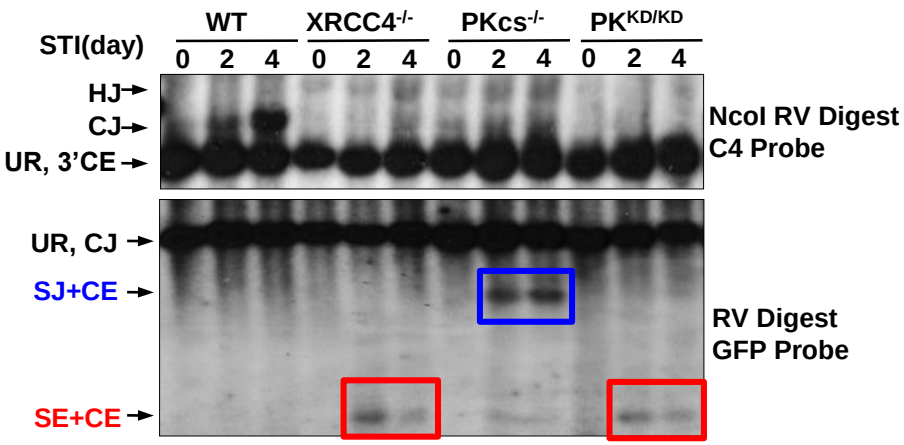
Chen et al. Nature 2021 May;593(7858):294-298
Chaplin & Blundell et al. Mol Cell. 2021 Aug 19;81(16):3400-3409

DNA-PKcs blocks end-ligation until activated





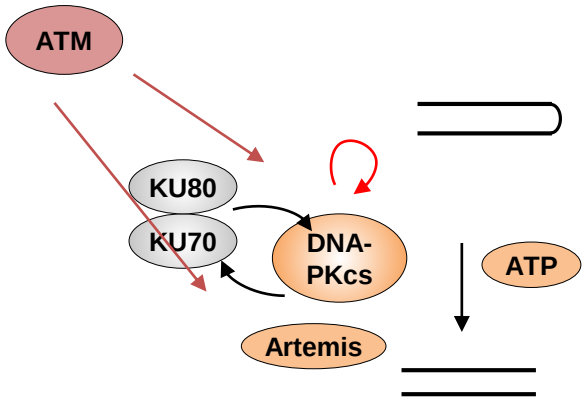


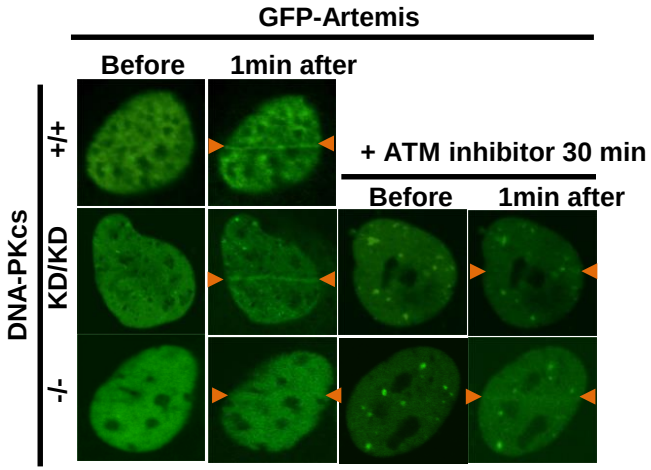


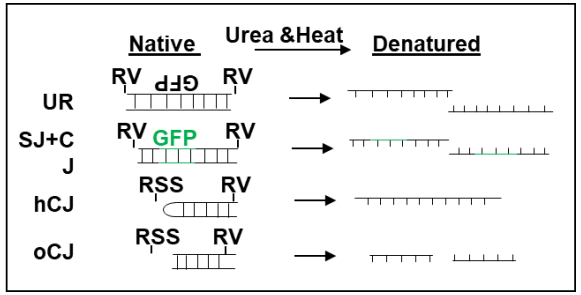
DNA-PKcs	+/+	+/KD	KD/KD	Total
Ku70+/-	20	42	0	62
Ku70-/-	7	20	8	35
Ku80+/-	9	16	0	25
Ku80-/-	6	12	5	23

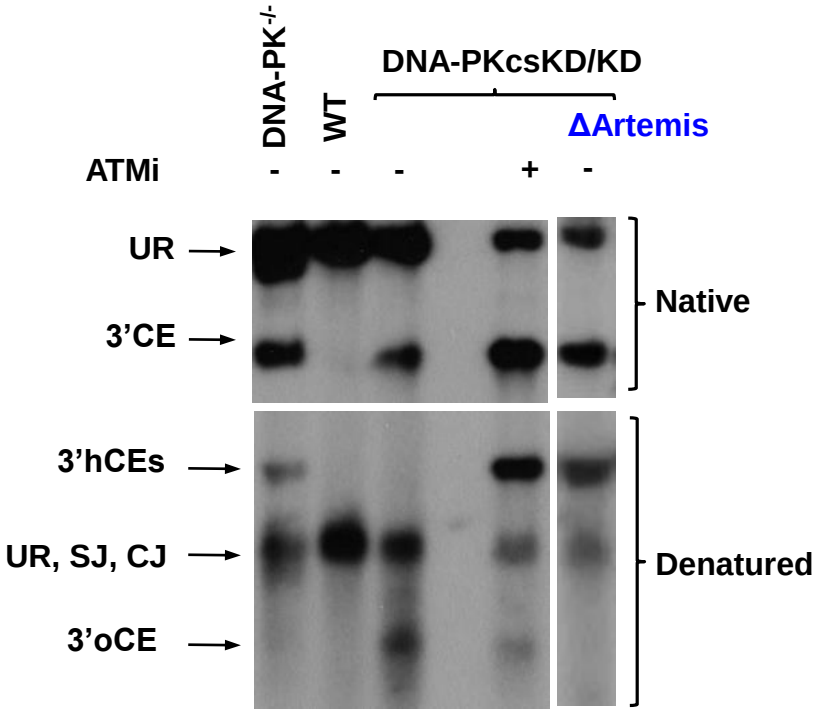
Jiang, Crowe and Liu et. al. Mol Cell 2015

DNA-PKcs$^{KD/KD}$ cells open hairpin in a ATM and Artemis dependent manner.



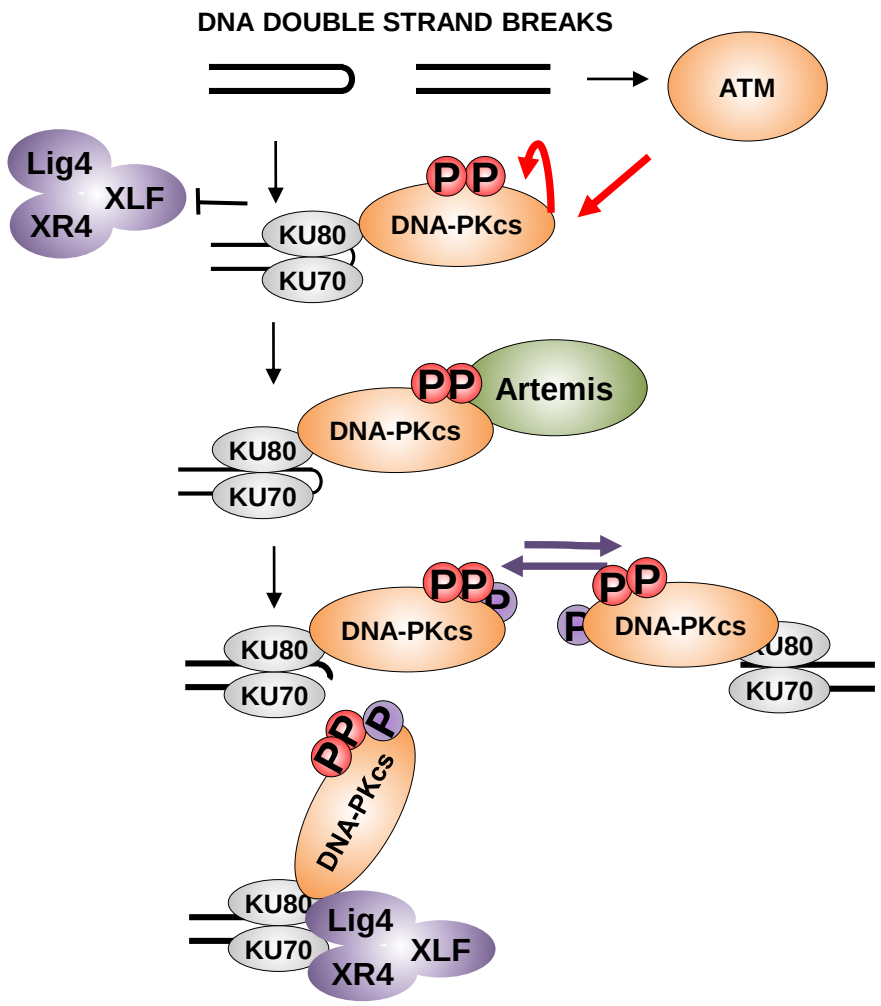


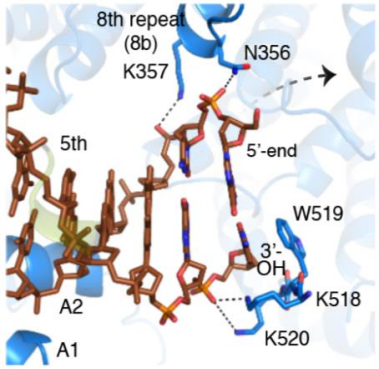




Jiang et. al. Mol Cell 2015

Working Model & Perspectives

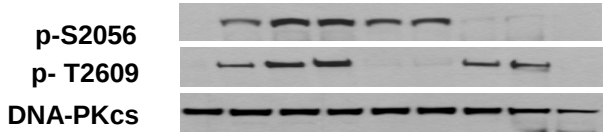


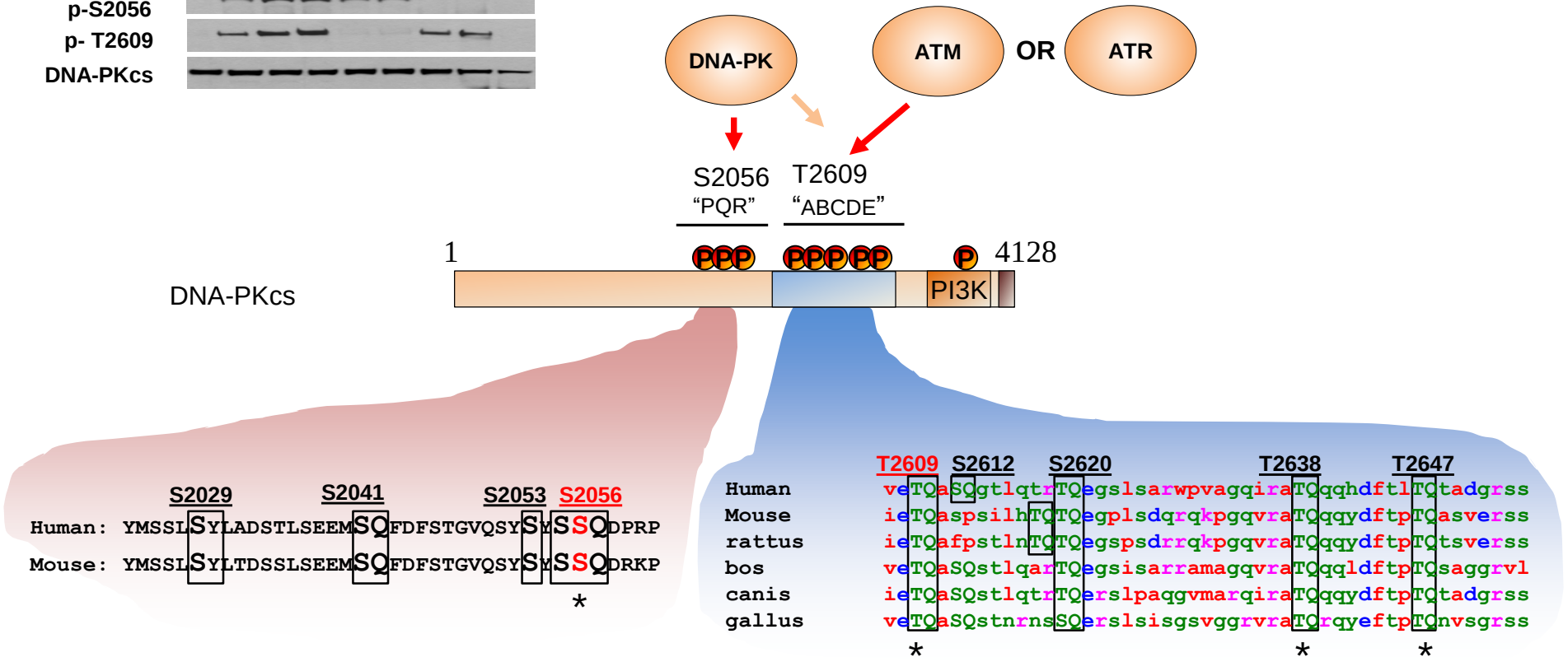


Chen & Yang et al. Mol Cell. 2021 Feb 18;81(4):801-810

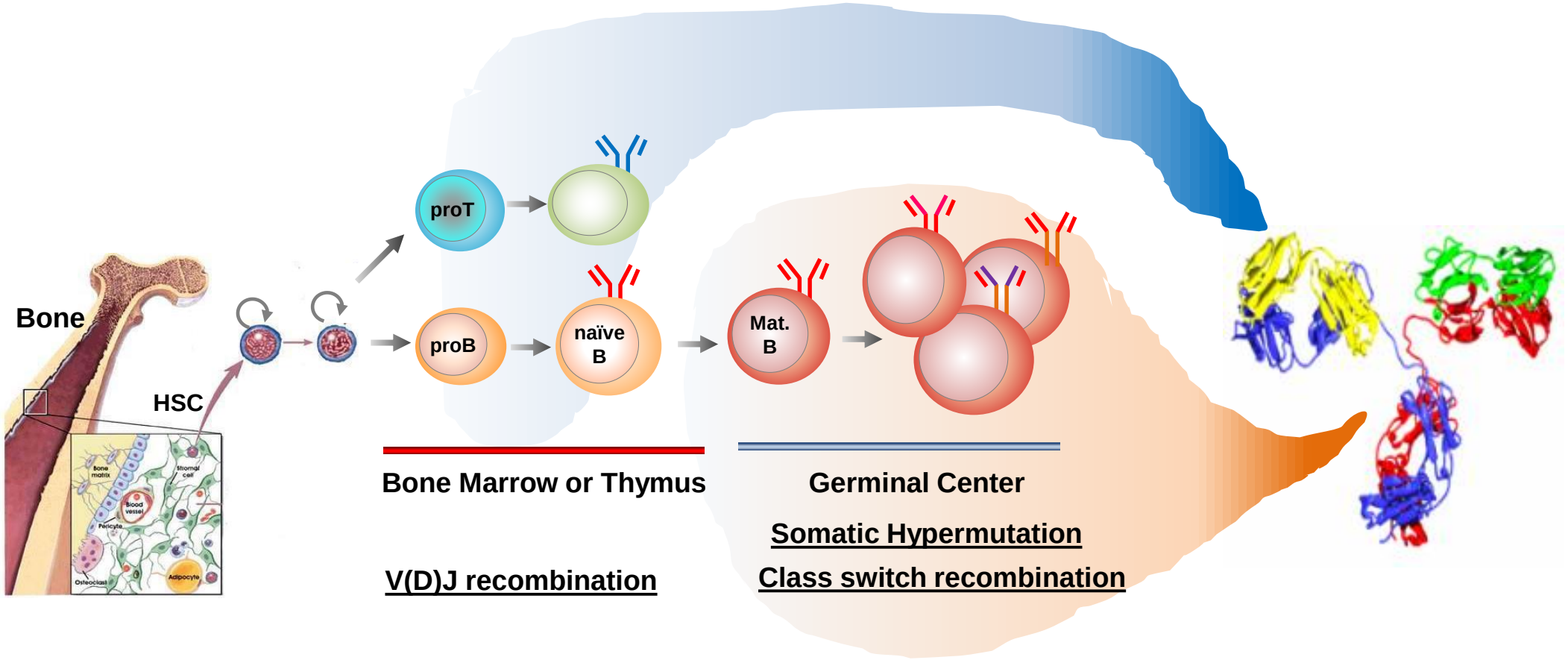
“Auto”-Phosphorylation Clusters

IR	-	30Gy							
Min after IR	-	10	30	60	30	60	30	60	60
ATM inh	-	-	-	-	+	+	-	-	+
DNA-PK inh	-	-	-	-	-	-	+	+	+

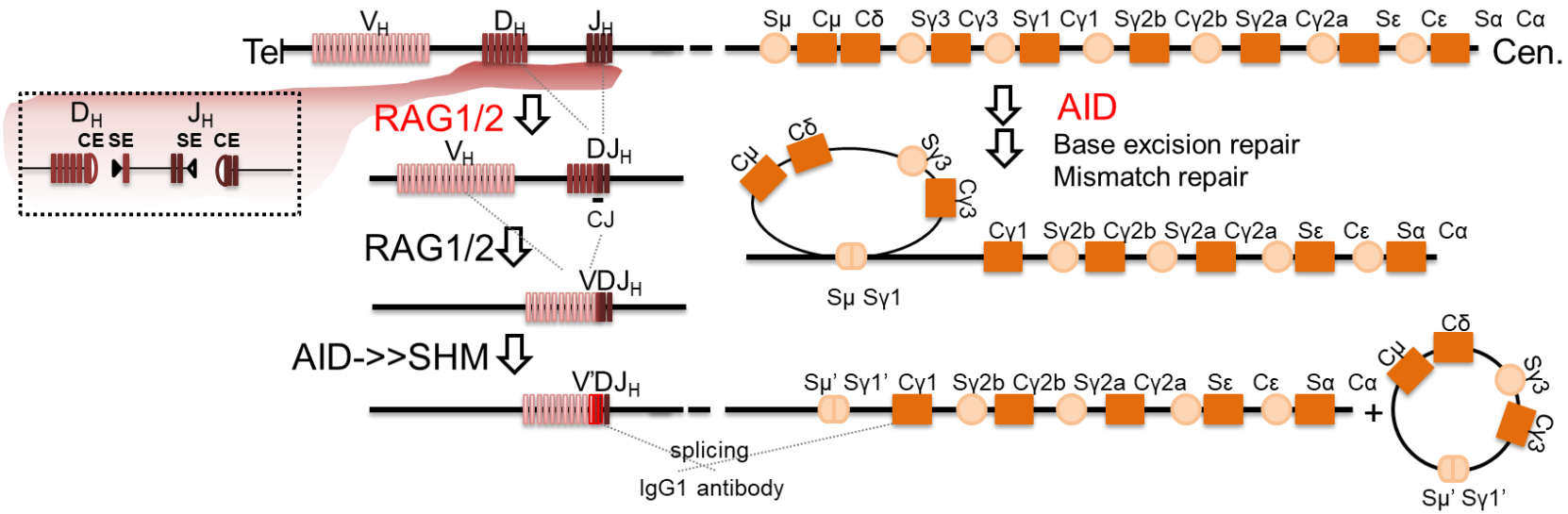




Lymphocyte Development



V(D)J recombination and Class switch recombination



<u>V(D)J recombination</u>
G1 (no replicating)

Hairpin/Blunt Ends

NHEJ

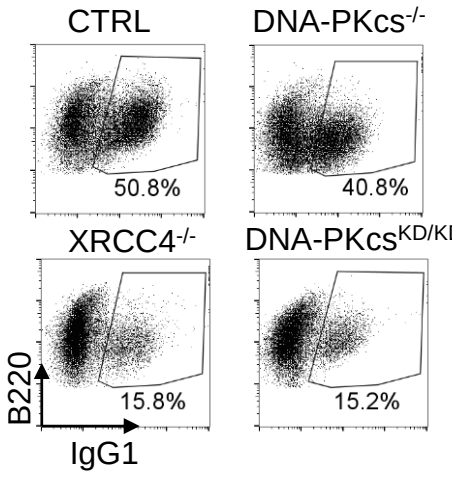
<u>Class switch recombination</u>
G1->S proliferating

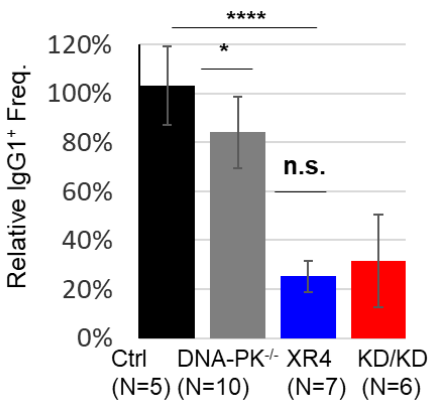
Staggered breaks, G/C rich repetitive region, resection and in the context of replication

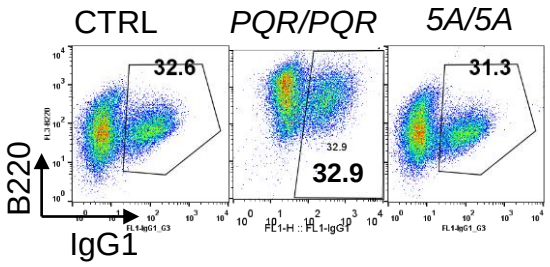
NHEJ and Alternative-End Joining

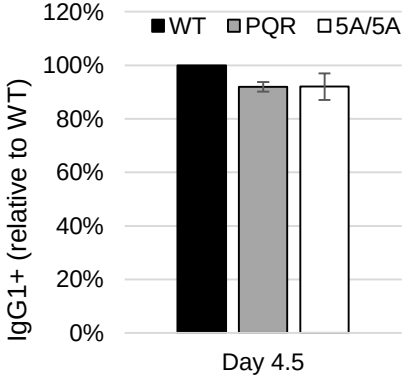
DNA Repair (Amst). 2020 Oct;94:102874

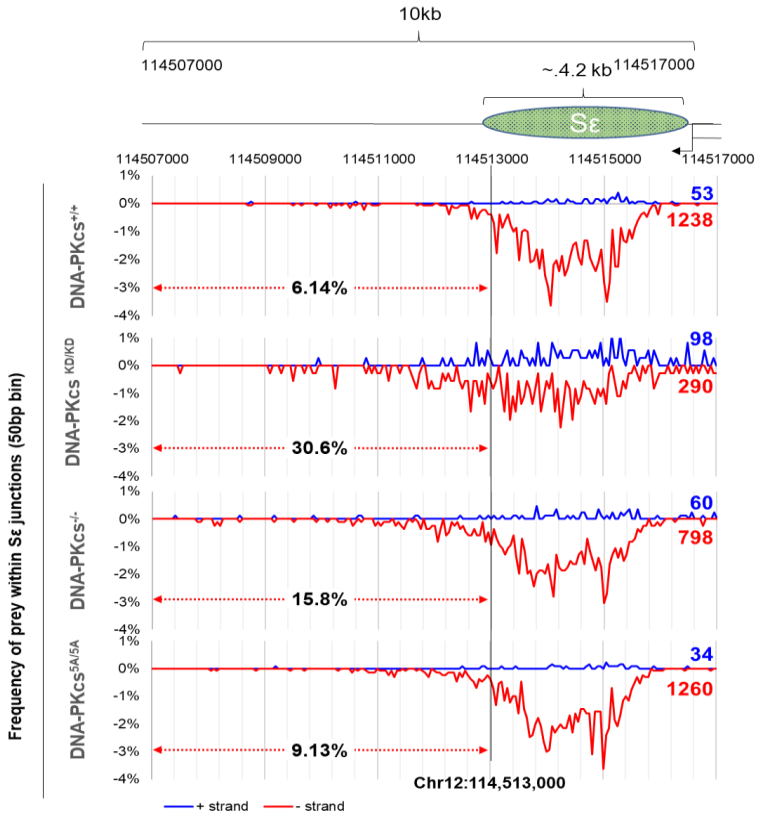
Kinase dead ≠ auto-phosphorylation

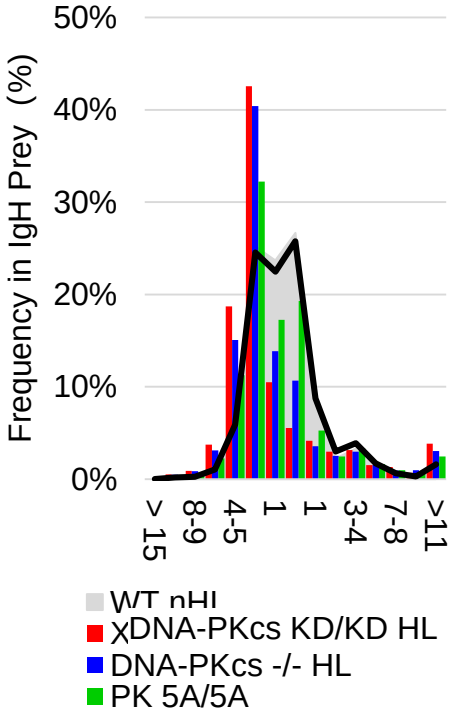












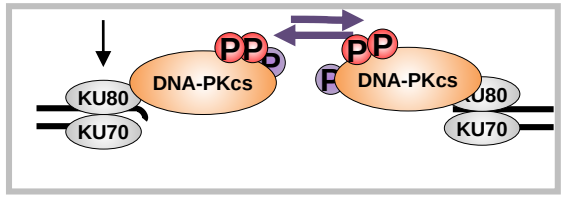
Crowe J et. al 2018 PNAS
Jiang W et.al 2019 J Immunology
Crowe J & Wang X et. al 2020 PNAS

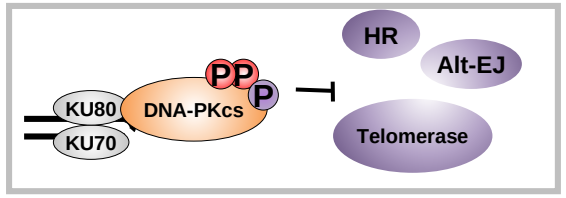
DNA-PKcs-phosph is not required for chromosomal V(D)J recombination and Hairpin Opening

	V(D)J recombination		Class switch recombination		
	Hairpin	End-ligation	Efficiency (IgG1)	Resection	Blunt
WT	Open	Fine	100%	+	40%
PQR	Open	Fine	100%	+ +/-	40%
5A	Open	Fine/↓fidelity (80%)	100%	++	25%
Null	Closed	Fine/↓fidelity (85%)	90%	+++	20%
Kinase Dead	Open	Blocked	50%	+++++	15%

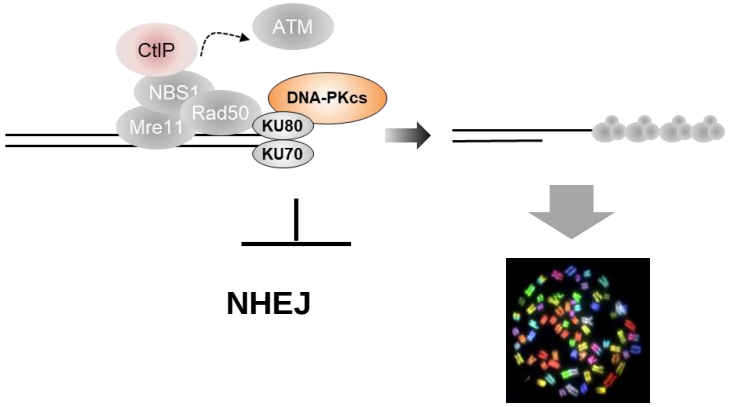
Crowe J et. al 2018 PNAS
Jiang W et.al 2019 J Immunology
Crowe J & Wang X et. al 2020 PNAS

Structural function of DNA-PKcs





Impact on other repair pathway -end resection? - NO

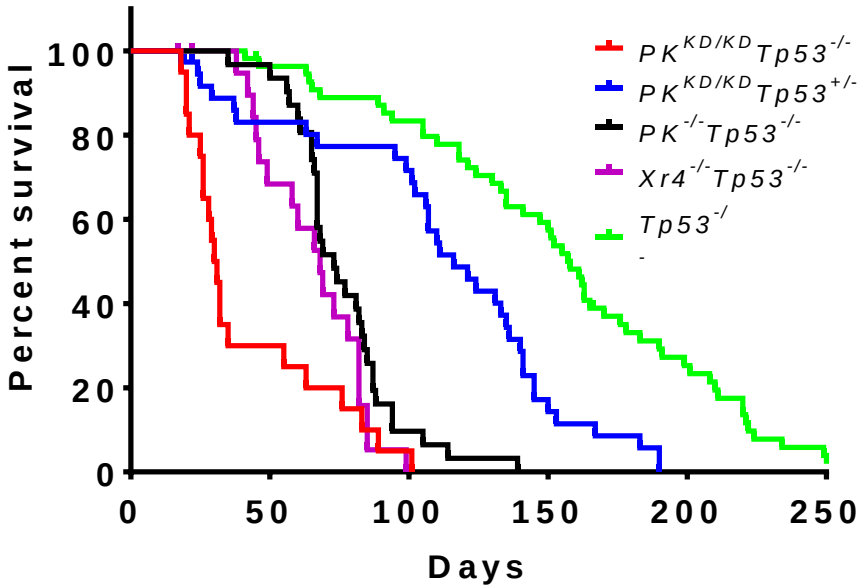


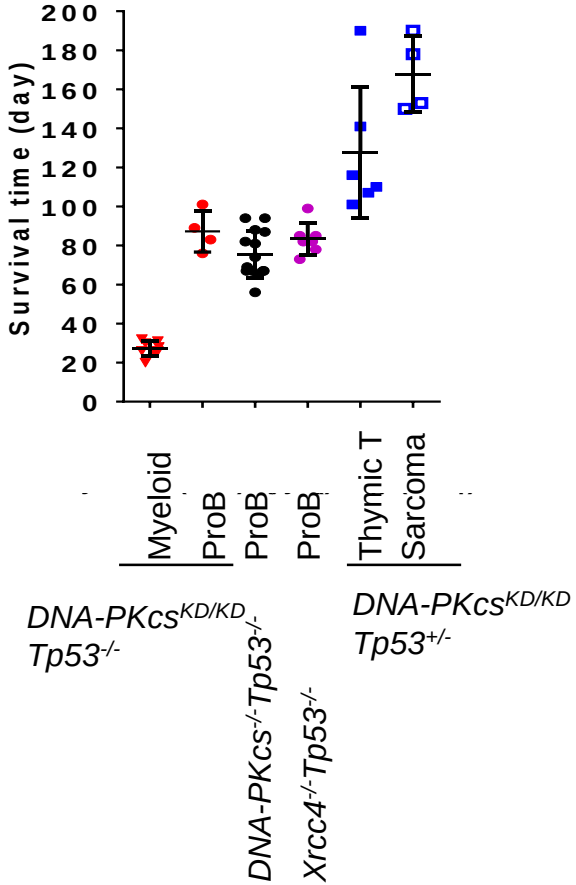
Translocation

~~TP53~~

<u>Lymphoma and Leukemia</u>

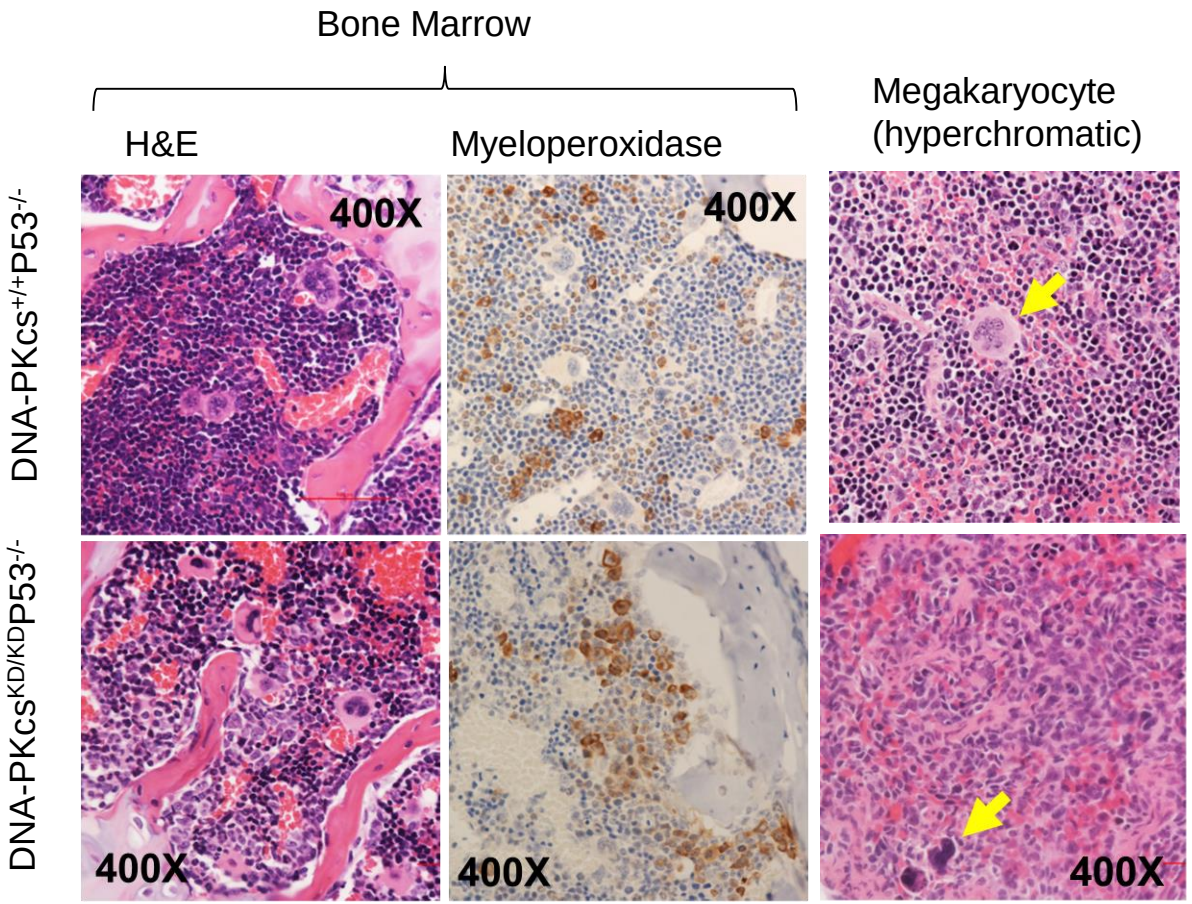
DNA-PKcs$^{KD/KD}$p53$^{-/-}$ mice died lymphomas free

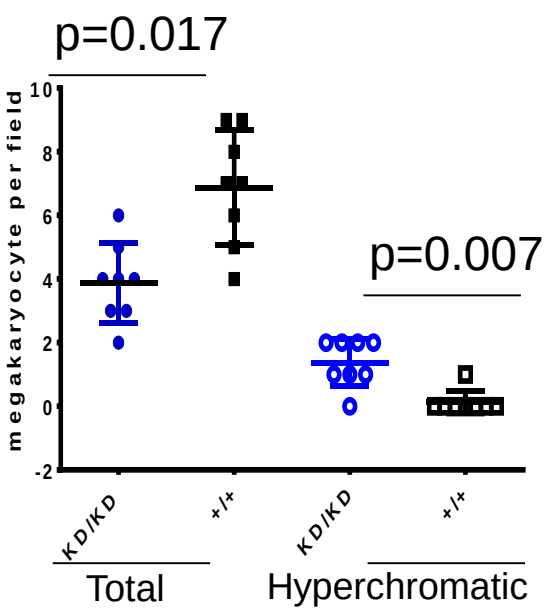




Jennie Crown

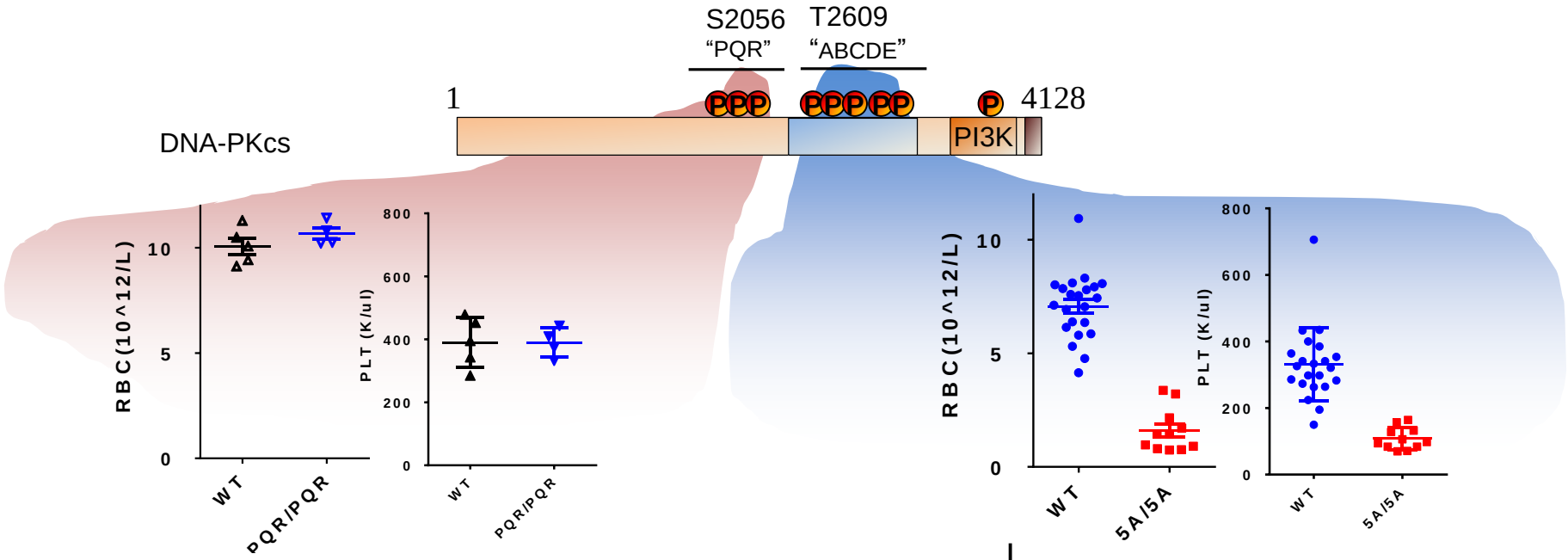
DNA-PKcs$^{KD/KD}$p53$^{-/-}$ mice developed Myelodysplasia Like Disease

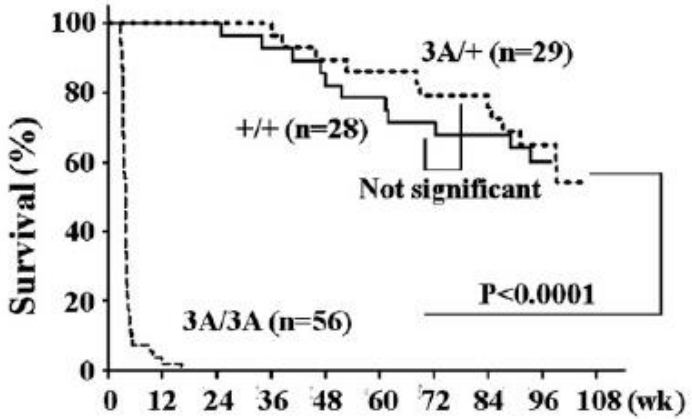


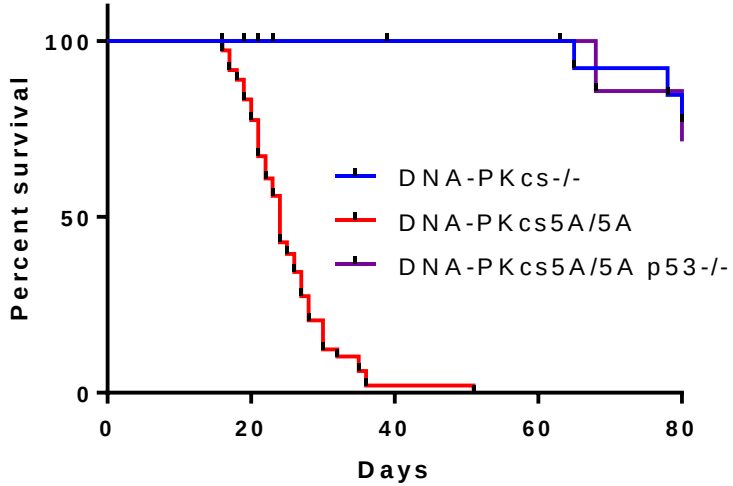


Helped by Dr. Govind Bhagat

T2609A, but not S2056A leads to bone marrow failure

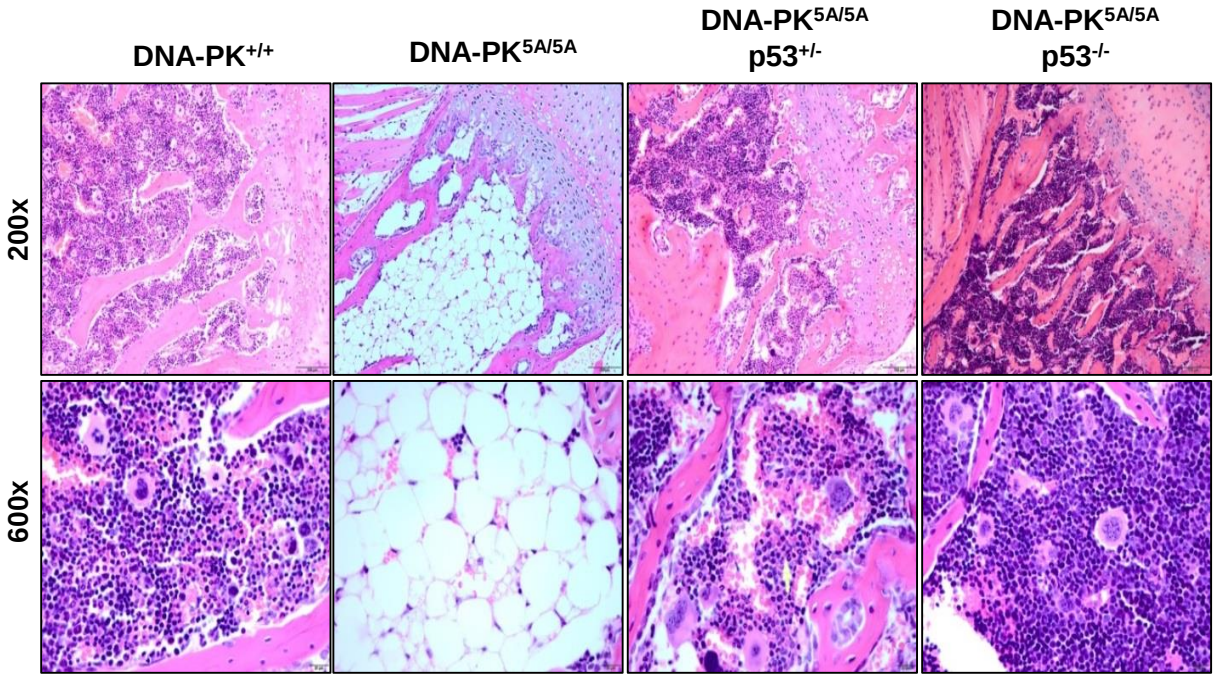


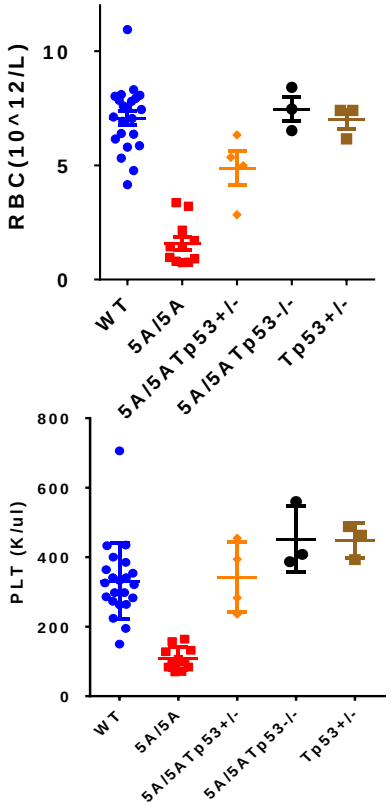




Jennie Crowe
Zhang et al JCB 2011

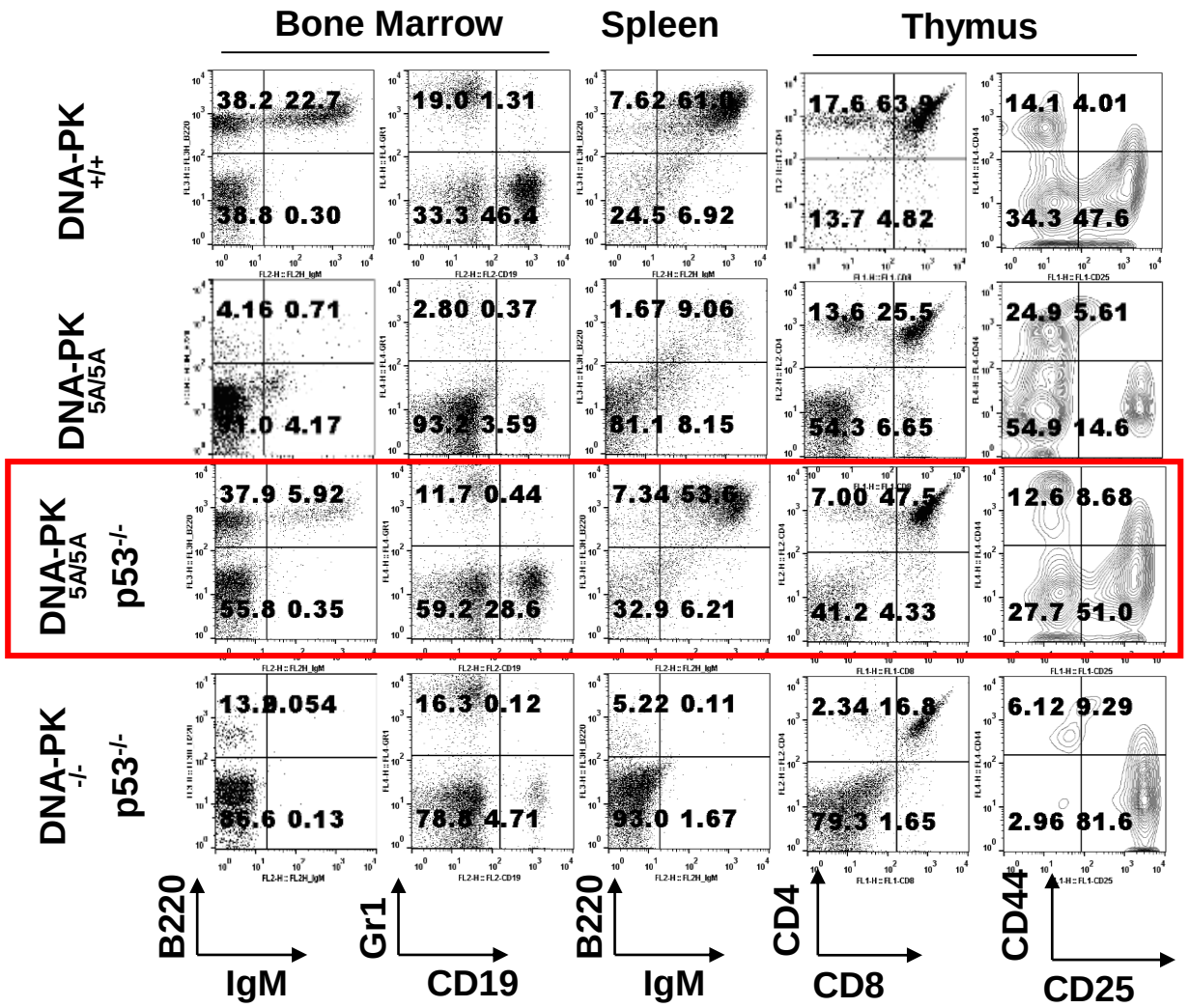
P53 dependent Bone Marrow Failure

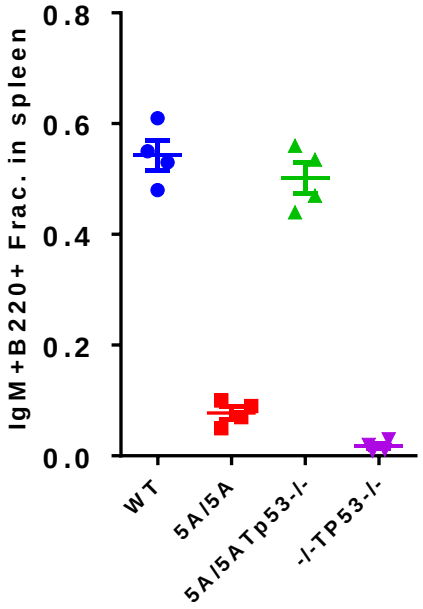




Jennie Crowe

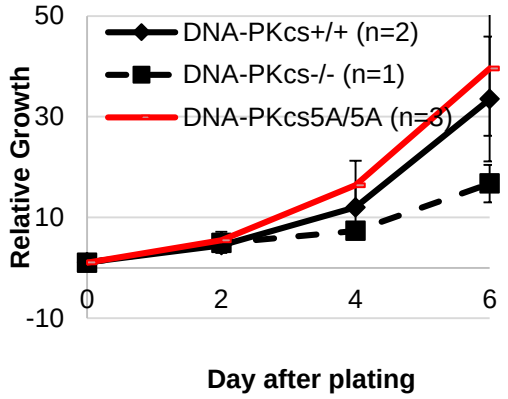
T2609A does not affect early lymphocyte development

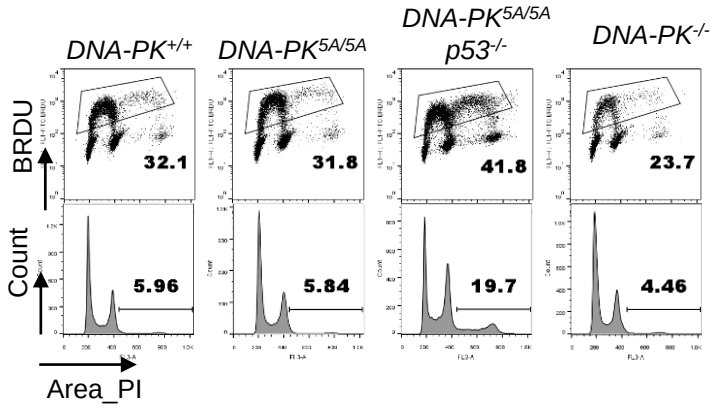


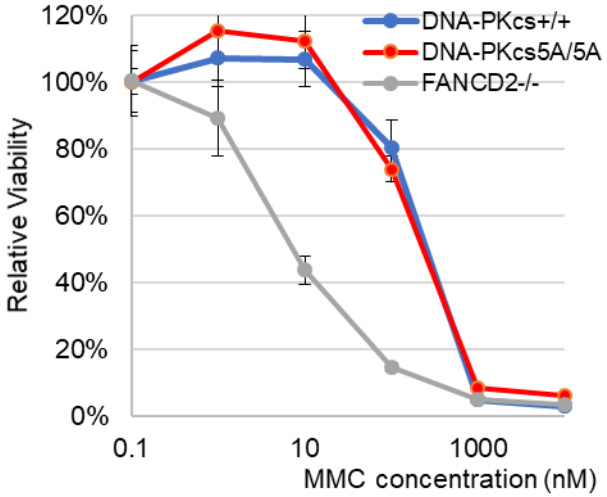


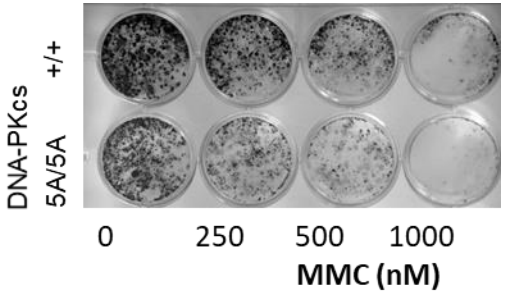
Jennie Crowe

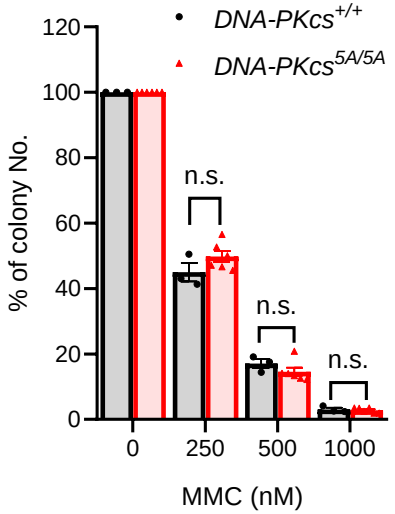
T2609A did not cause severe crosslink defects



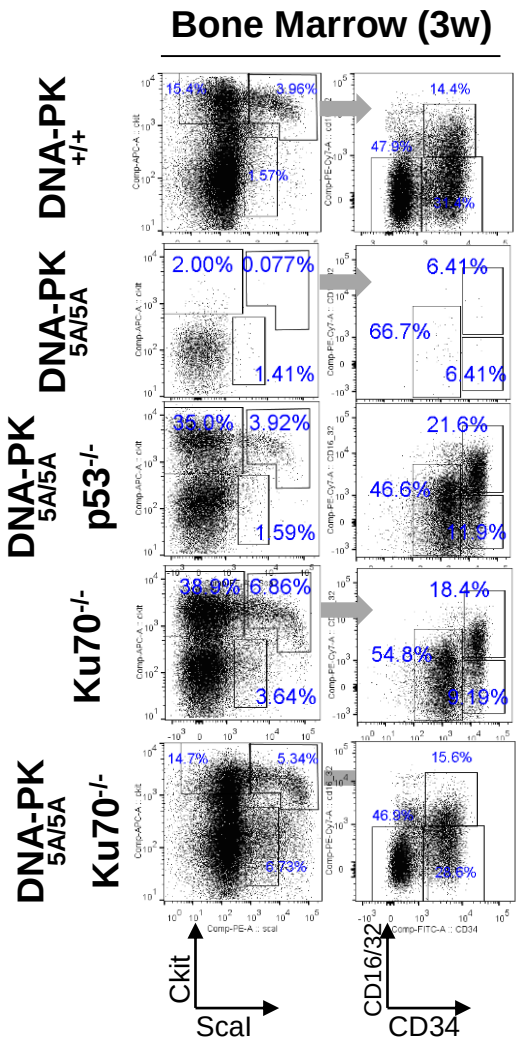


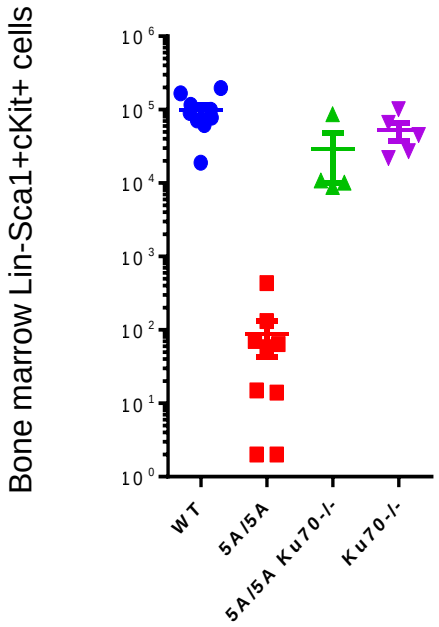


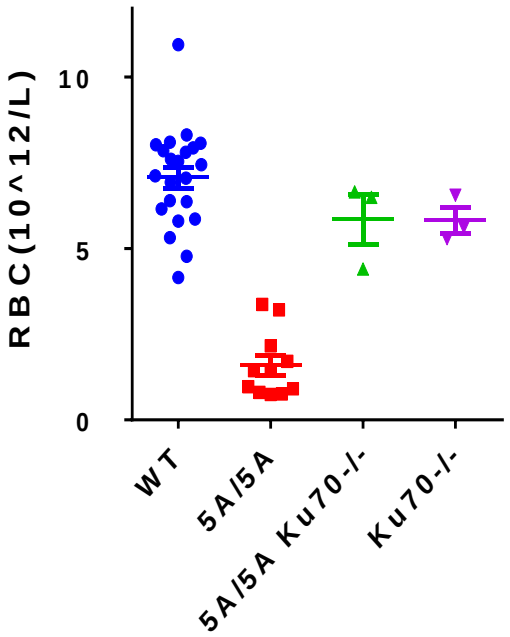




T2609A and KD cause Ku dependent HSC defects



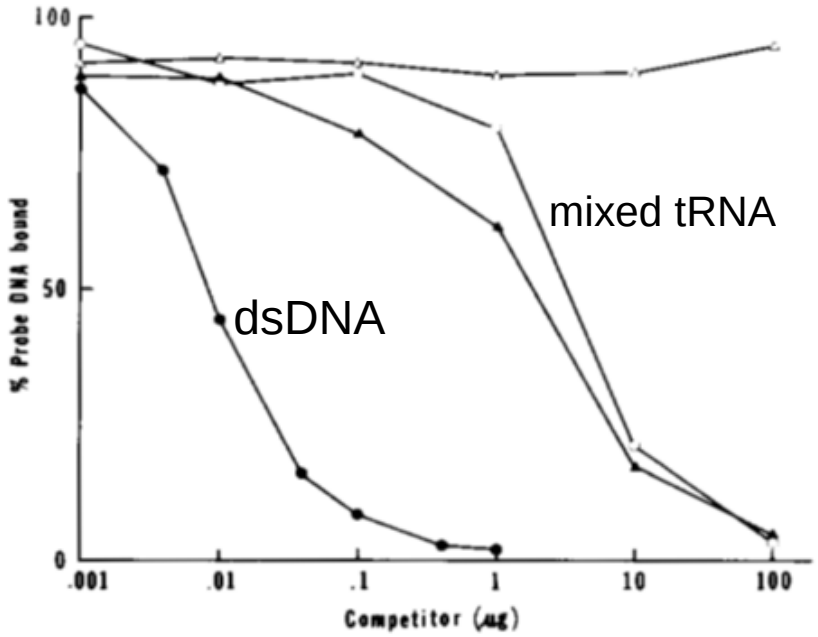




Jennie Crowe, Zhengping Shao

Why?

Mimori T and Hardin JA JBC 1996



Cell

Mutually Exclusive Binding of Telomerase RNA and DNA by Ku Alters Telomerase Recruitment Model

Jennifer S. Pfingsten,[1,3] Karen J. Goodrich,[1] Cornelius Taabazuing,[1,4] Faissal Ouenzar,[2] Pascal Chartrand,[2] and Thomas R. Cech[1,*]

[1]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado Biofrontiers Institute, Boulder, CO, 80309-0215, USA

[2]Department of Biochemistry, Université de Montréal, Montréal, Quebec H3C 3J7, Canada

[3]Present address: SomaLogic, Inc., Boulder, CO 80301, USA

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RNA recognition by the DNA end-binding Ku heterodimer

ANDREW B. DALBY, KAREN J. GOODRICH, JENNIFER S. PFINGSTEN,[1] and THOMAS R. CECH[2]

Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado BioFrontiers Institute, Boulder, Colorado 80309-0596, USA

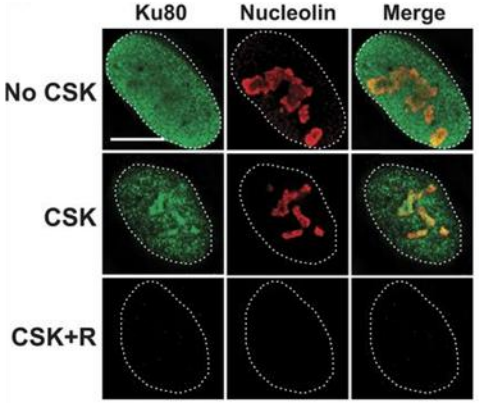
Table 1. Summary of Binding and Competition Data

	3xmutTLC1-KBS RNA	TLC1-KBS RNA	Blunt End
Apparent K_D [nM]	353 ± 49	10.5 ± 0.7	0.18 ± 0.06
k_{off} [min^{-1}]		2.6 ± 1.2	0.007 ± 0.005
$t_{1/2}$ [min]		0.27	99
k_{on} [$M^{-1}s^{-1}$]		3.4×10^6	6.3×10^5

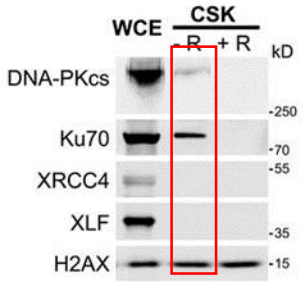
Ku and DNA-PKcs bind to nucleoli

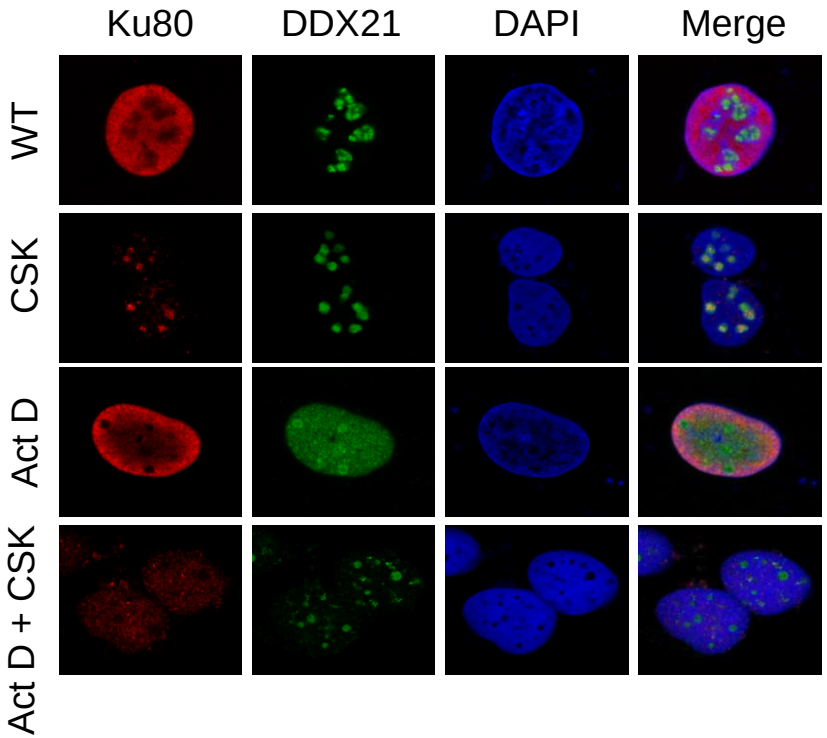
A new method for high-resolution imaging of Ku foci to decipher mechanisms of DNA double-strand break repair

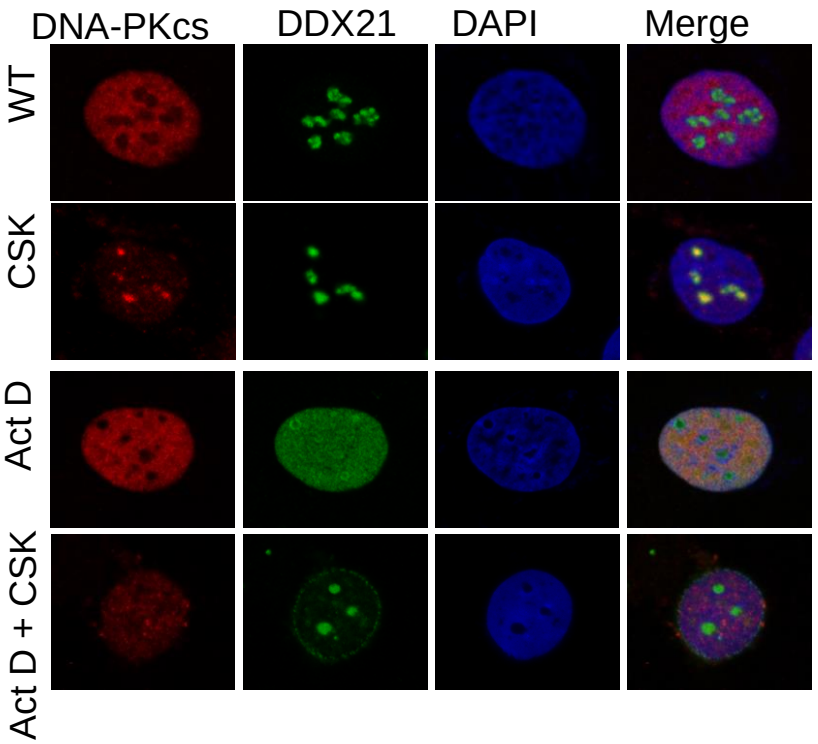
Sébastien Britton,[1] Julia Coates,[1] and Stephen P. Jackson[1,2]



R=Rnase+







Yimeng Zhu

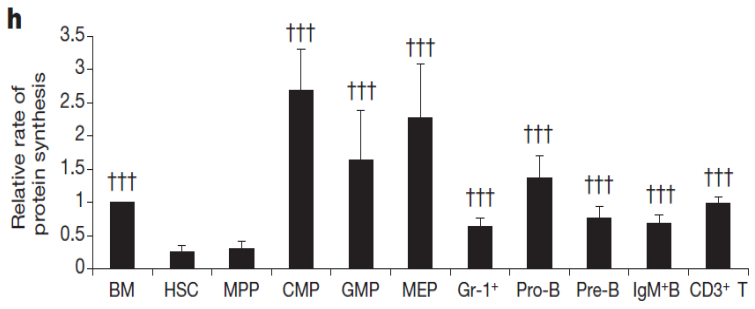
CMP/MEP requires high translation.

ARTICLE

doi:10.1038/nature13035

Haematopoietic stem cells require a highly regulated protein synthesis rate

Robert A. J. Signer[1], Jeffrey A. Magee[1], Adrian Salic[2] & Sean J. Morrison[1]



LETTERS

nature medicine

Altered translation of GATA1 in Diamond-Blackfan anemia

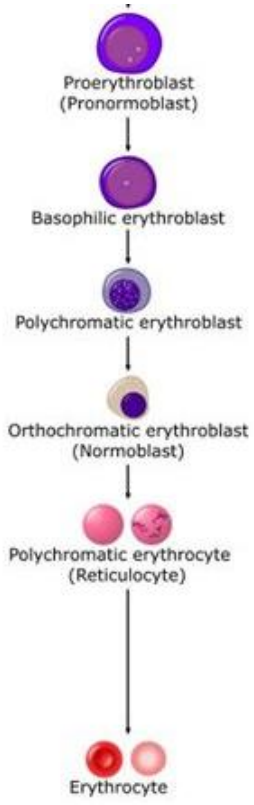
Leif S Ludwig[1–6], Hanna T Gazda[4,7,8], Jennifer C Eng[3,4], Stephen W Eichhorn[3,9], Prathapan Thiru[3], Roxanne Ghazvinian[7], Tracy I George[10], Jason R Gotlib[11], Alan H Beggs[7,8], Colin A Sieff[1,2,8], Harvey F Lodish[3,4,9], Eric S Lander[4,9,12] & Vijay G Sankaran[1–4,8]

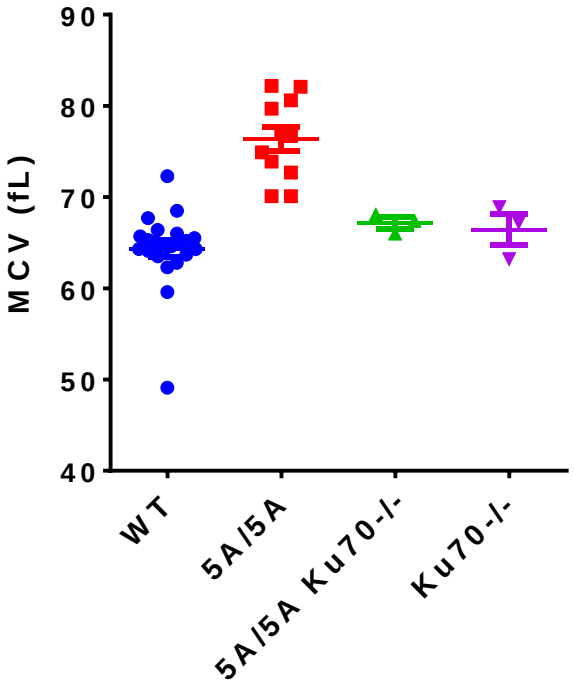
Nucleoli Stresses Disorders

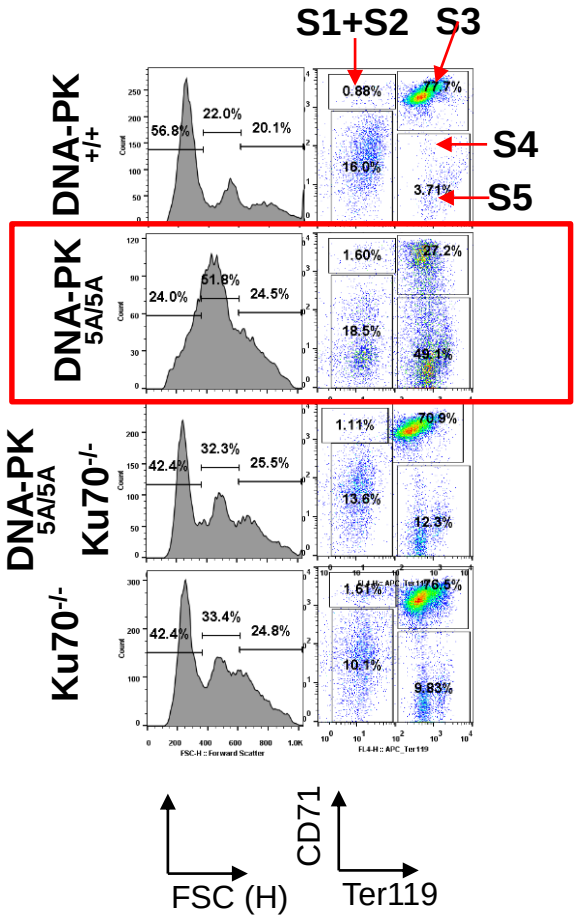
- Blackfan-Diamond Anemia are often caused by mutations in one of 18 ribosomal protein (RP) genes
- Hyperpigmentation
- Hyper activation of p53 without DNA damage
- Macrocytic Anemia and bone marrow filature
- Increased risk for MDS and AML

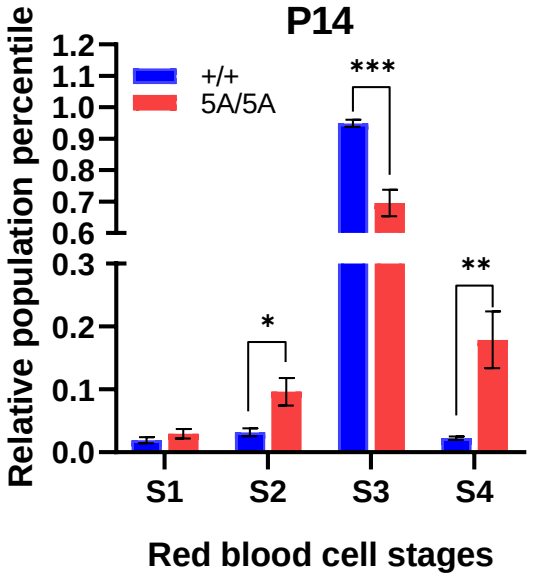
Harding et al., 2015, Cell Reports 13, 251–259
Carkins et al., 2013 Nucleic Acids Res. 41(15): 7378–7386.
Dejmek et al.2009 Mol Cancer Res. 7(4):581-91

DNA-PKcs mutant mice has "*Macro*"cytic Anemia

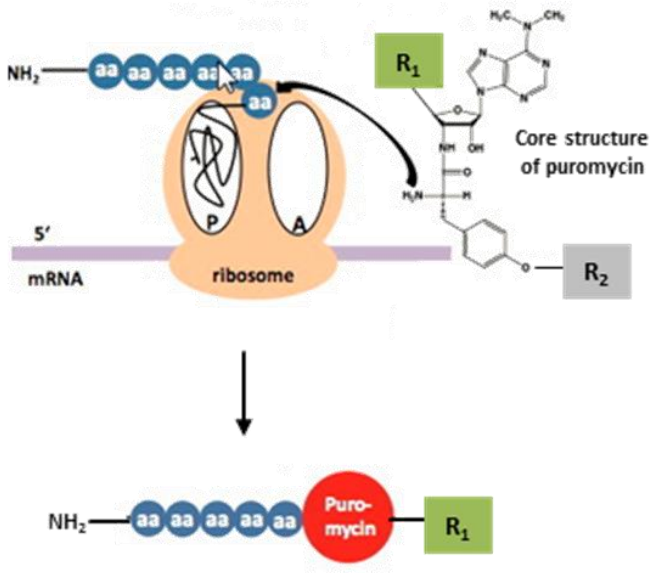








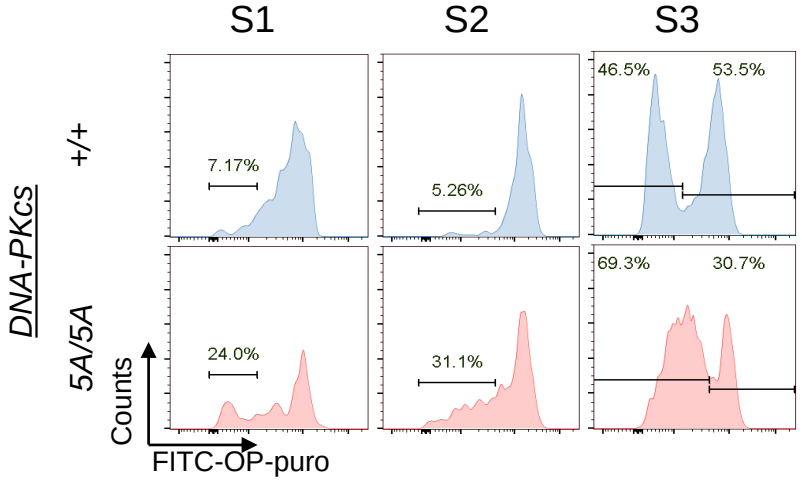
Translation defects in erythroid progenitors

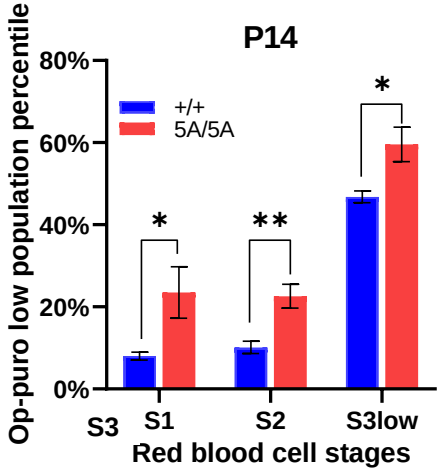


Puromycin conjugates participate in the peptide bond formation with the nascent polypetpide chain on the ribosome resulting in C-terminal labeled proteins .

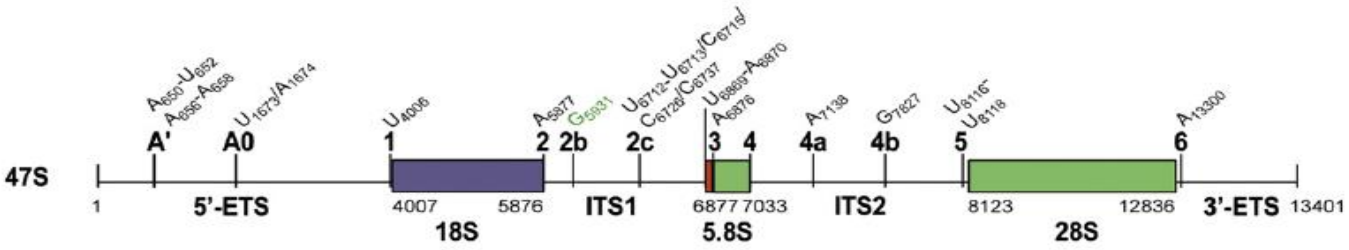
Labeled dC-puromycin conjugates:
R_1: Fluorescein/Biotin-labeled dC
R_2: CH_3

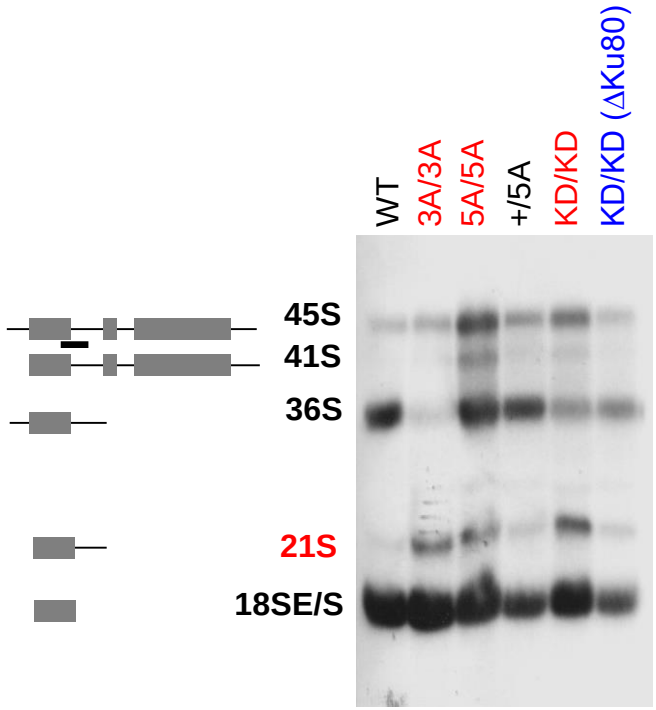
O-Propargyl-puromycin:
R_1: OH
R_2: CH_2-C-CH (Alkyne group)

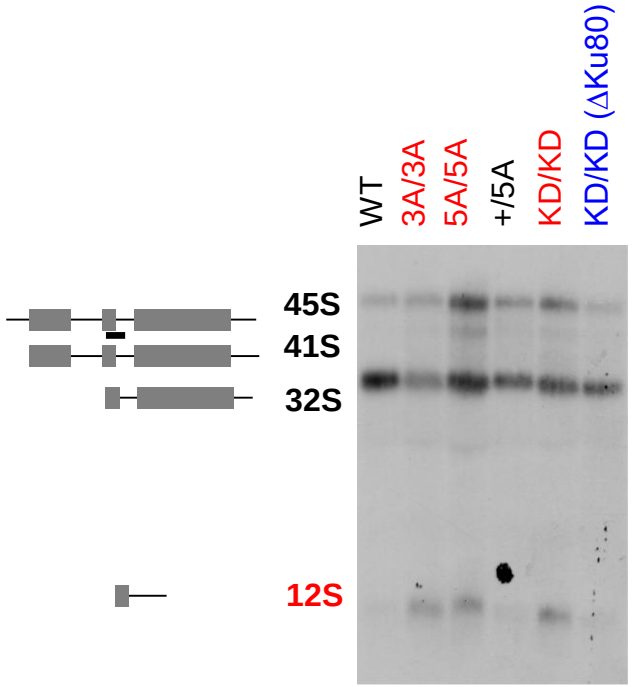




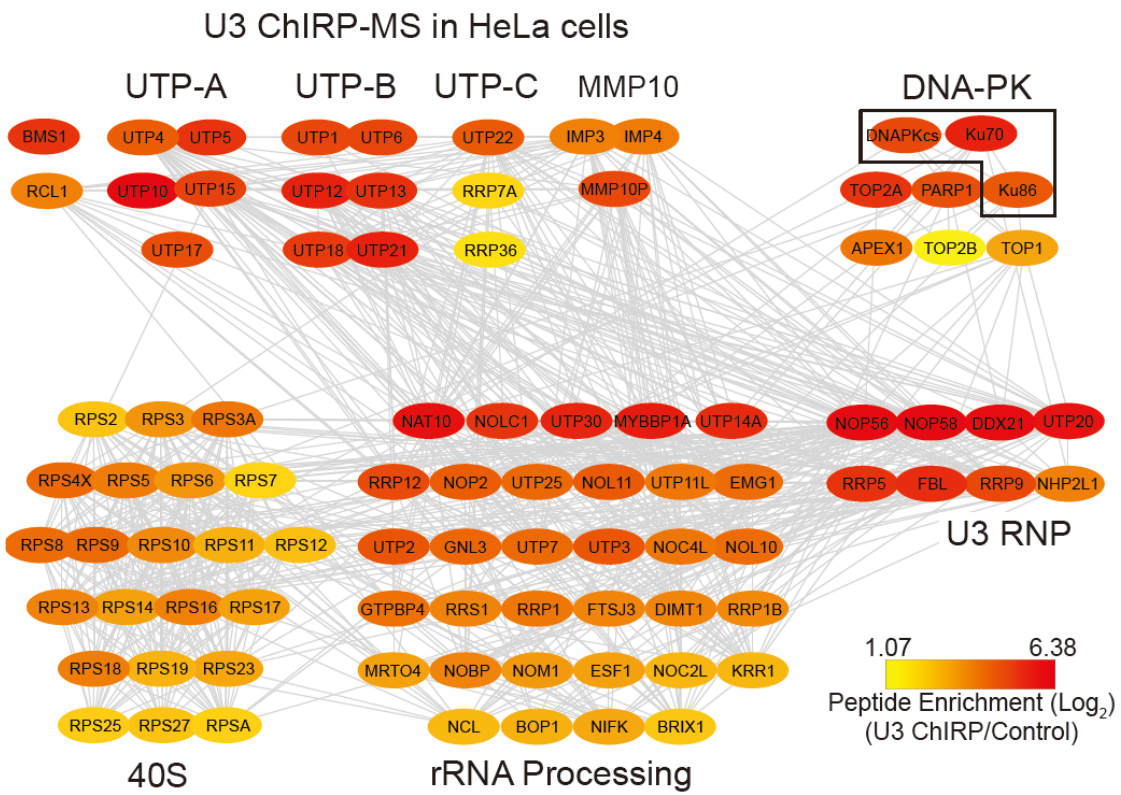
rRNA processing defects

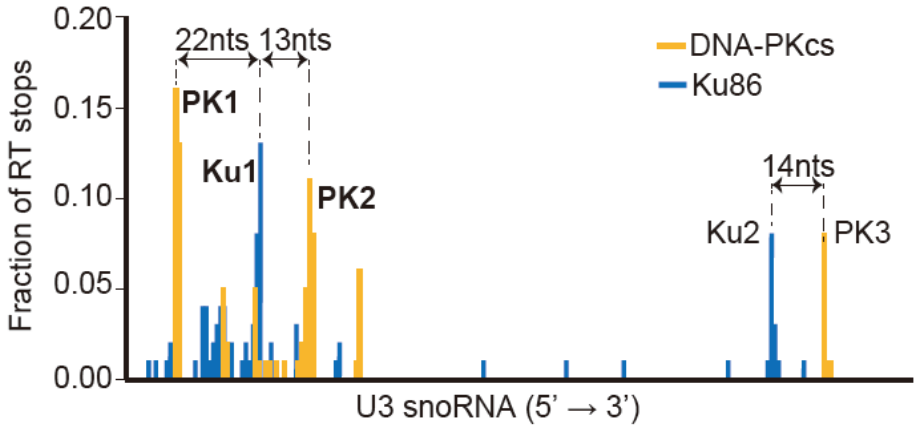


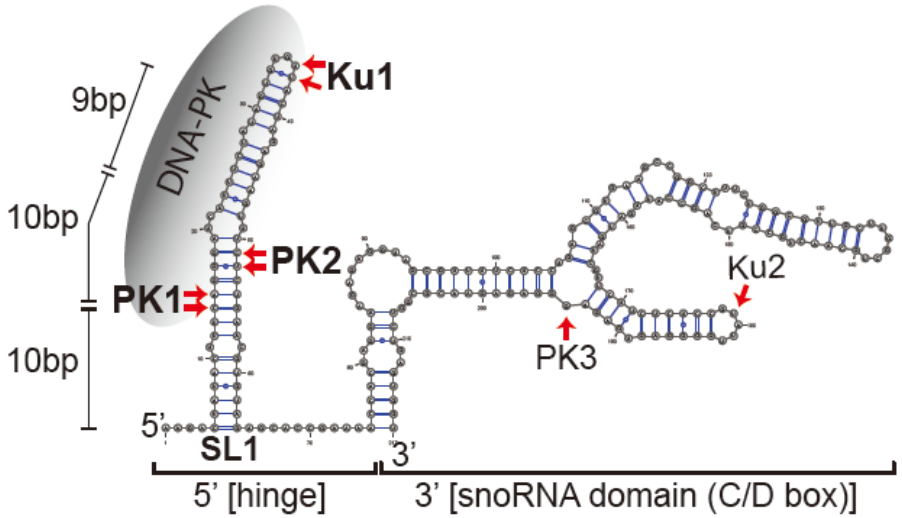




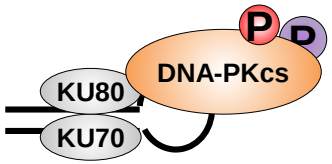
rRNA processing factor binding by KU



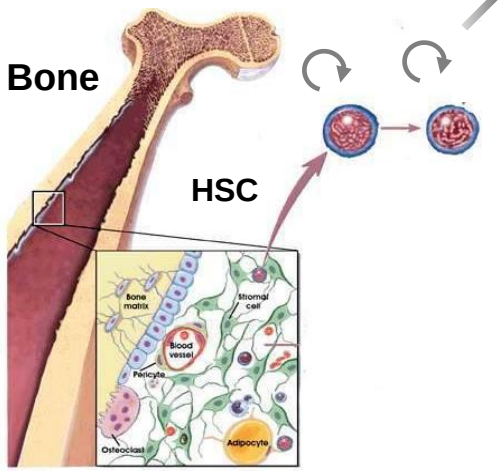


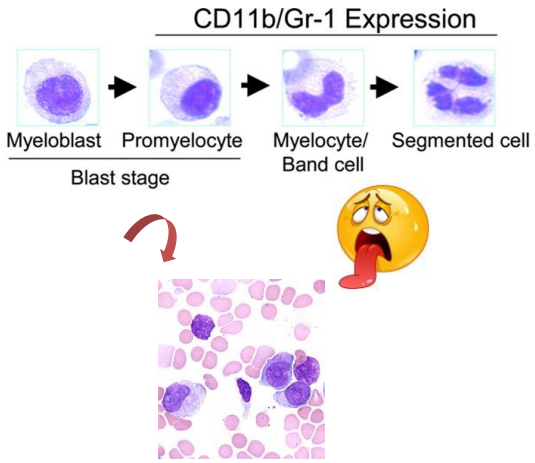


Working Model



rRNA intermediates





DNA-PKcs T2609 or KD mutations

⊥

rRNA processing
rDNA integrity

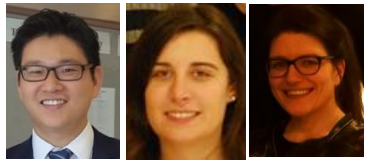
Protein Translation → Myeloid/MEP differentiation

↓

P53 Activation → Progenitor apoptosis

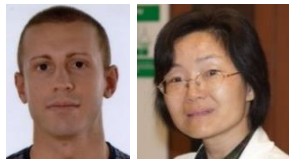
Catalytic Inactivation Models

1. **ATM Kinase Dead (2012, 2016, 2020, 2021).**



2. **DNA PKcs Kinase Dead (2015, 2018, 2020)**

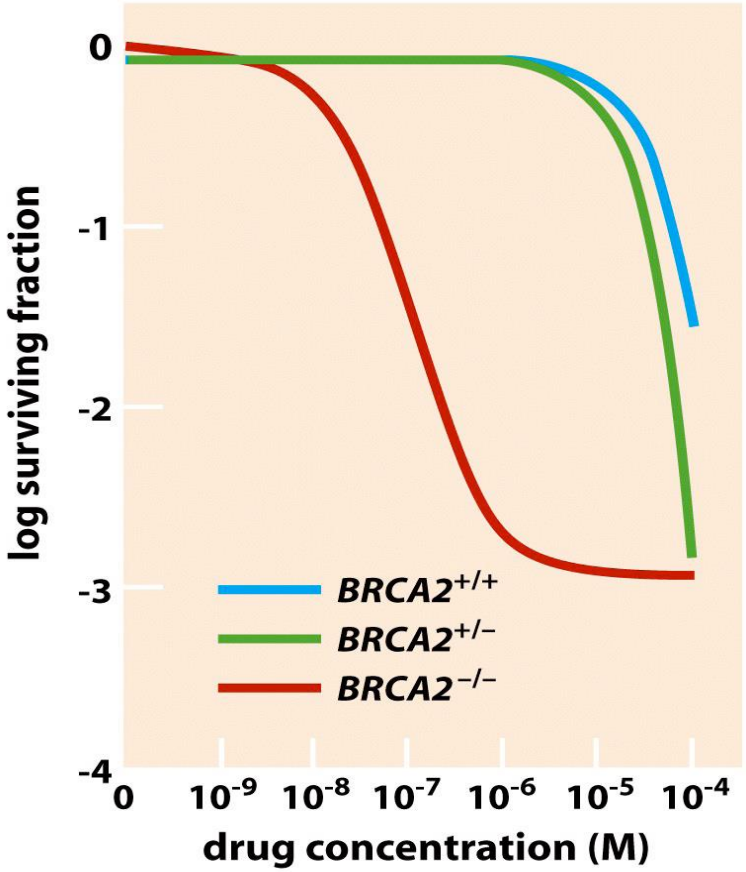
3. **ATR Kinase Dead (2018)**



4. **Catalytically inactive PARP1 and PARP2**

Kinase	PI3KK Core	Mouse	Human
ATM	LGLGDRH	D2880	D2870
DNA-PKcs	LGIGDRH	D3922	D3889
ATR	LGLGDRH	D2466	D2475

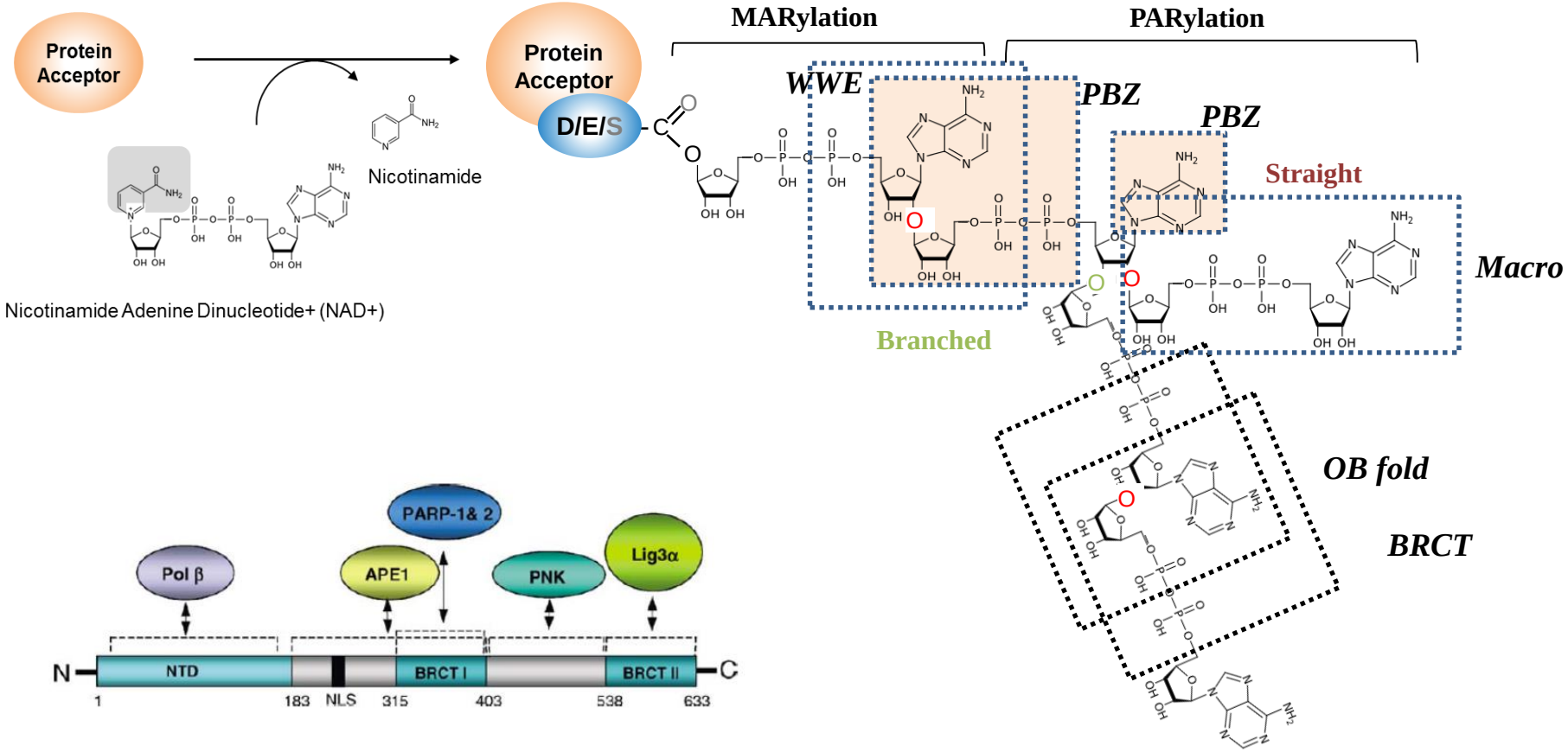
Synergistic lethality with BRCA mutants

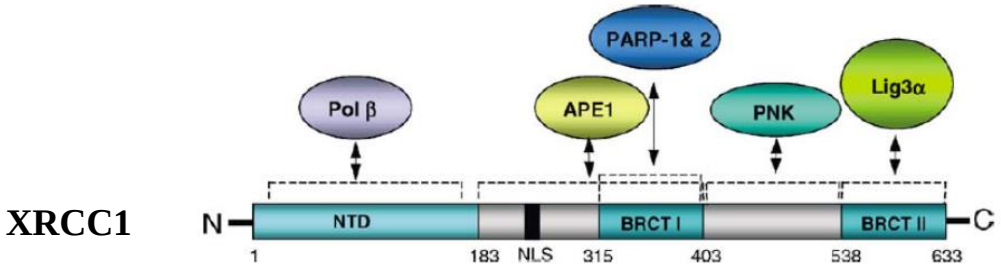


Agent	Tumor types	Company
Olaparib (Lynparza)	Breast, endometrial, gastric, glioblastoma, head and neck, lung, ovarian, pancreatic, prostate, sarcomas	AstraZeneca
Rucaparib	Breast, ovarian, pancreatic	Clovis Oncology
Niraparib (MK4827)	Breast, Ewing sacroma, ovarian	Tesaro
Veliparib (ABT-888)	Breast, cervical, colorectal, glio-blastoma, head and neck, lung, leukemias, multiple myeloma, non-Hodgkin lymphoma, ovarian, pancreatic, prostate	AbbVie
Talazoparib (BMN-673)	Breast, endometrial, leukemias, ovarian, solid tumors	BioMarin Pharmaceutical

Farmer H ….Ashworth A. Nature. 2005 Apr 14;434(7035):917-21.
Bryant HE….Helleday T. Nature. 2005 Apr 14;434(7035):913-7.

The activity of PARP1&2

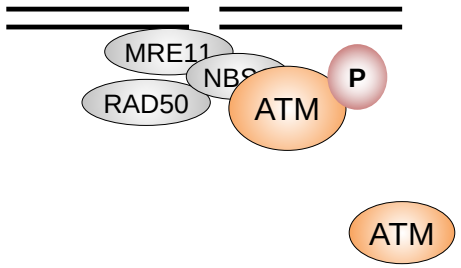


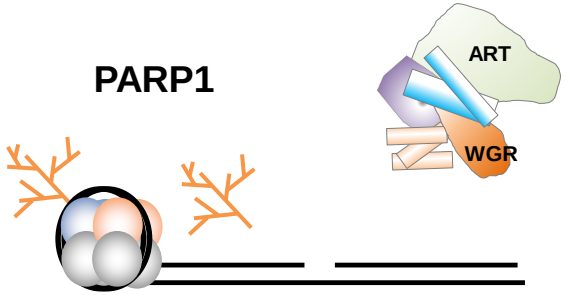


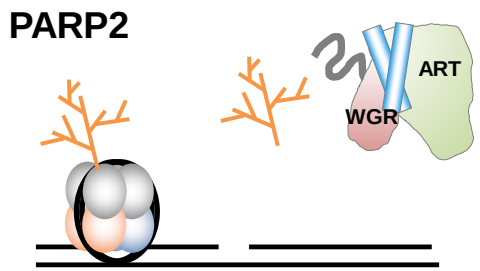
Nucleic Acids Research 2015, 43 (14) 6934–6944

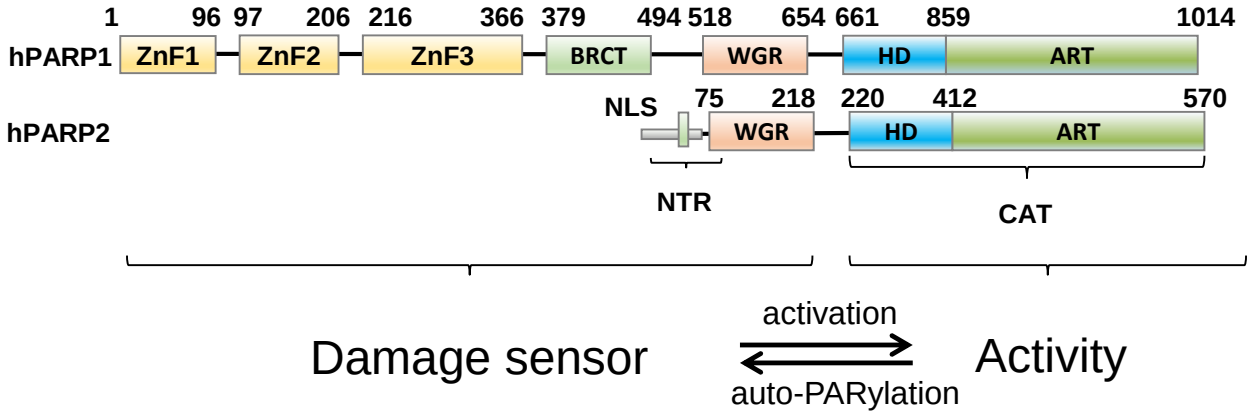
Reader of PAR and MAR: WWE, PIN, BRCT, OB fold, Macrodomain, RRM, RGG, FHA, RS/KR

DNA damage induced activation



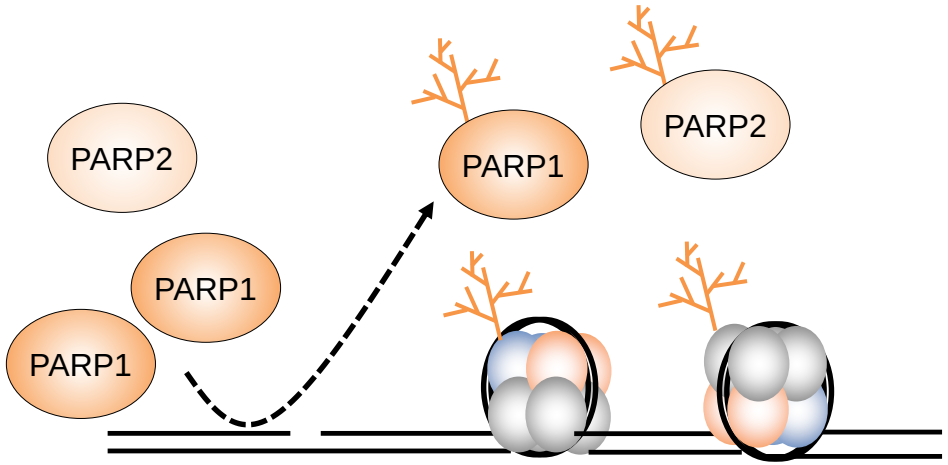


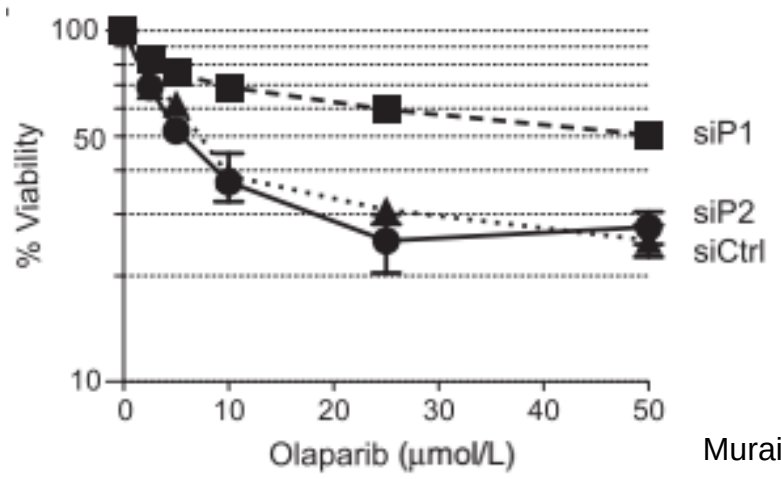




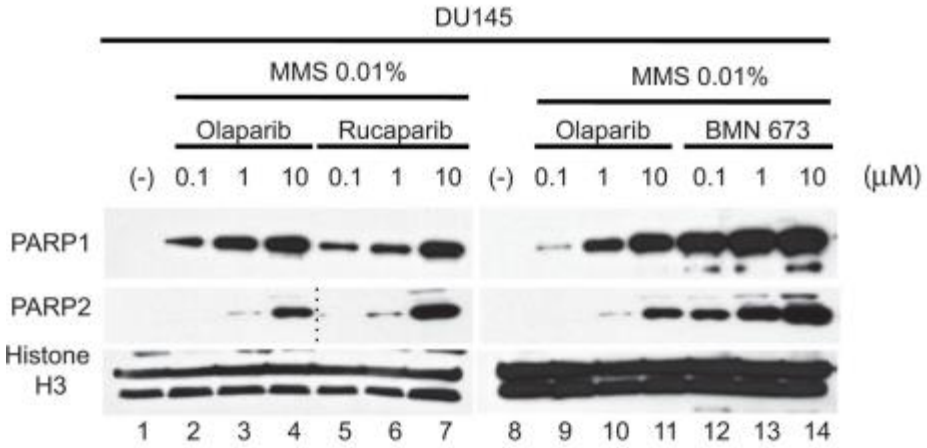
- Damage sensor
- Recruitment
- Allosteric activation at site
- Auto-modification

PARP1 Trapping



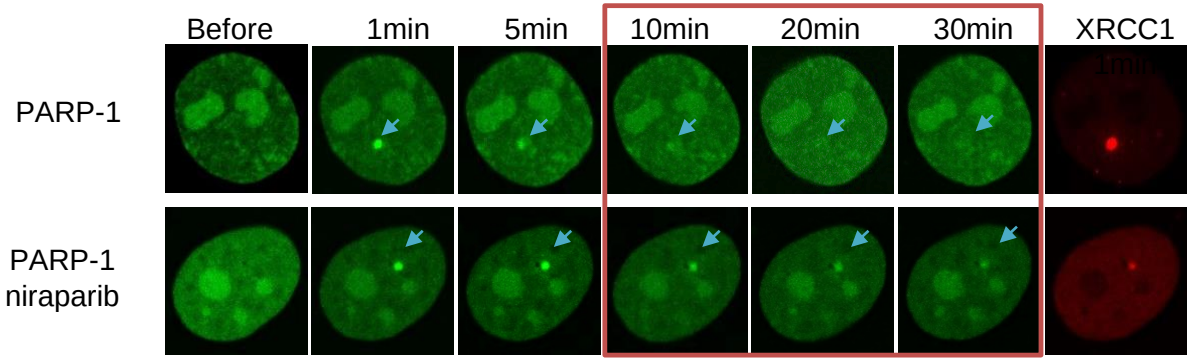


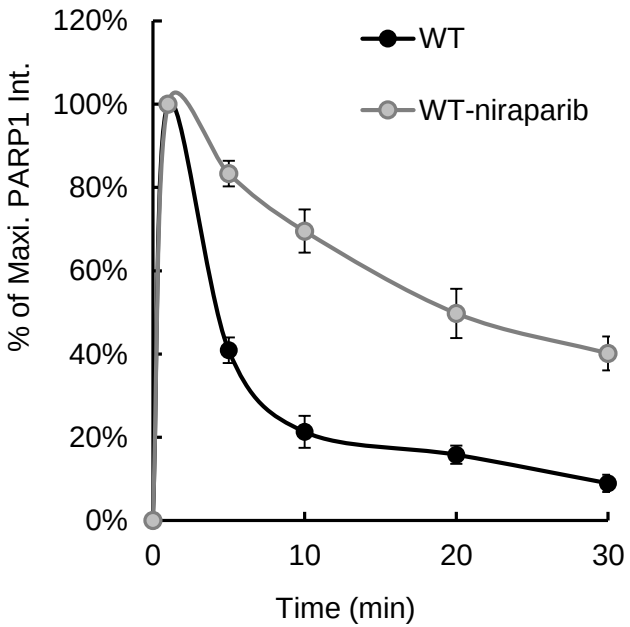
Murai J *et al Cancer Res,* 2012



Murai J *et al. Mol Cancer Ther*, 2014

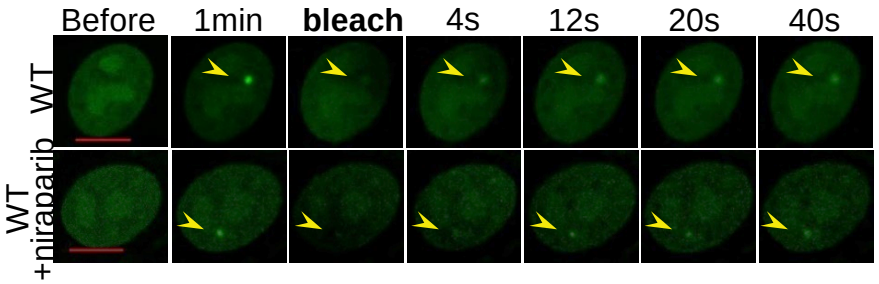
Persistent Foci can be seen in live cell imaging

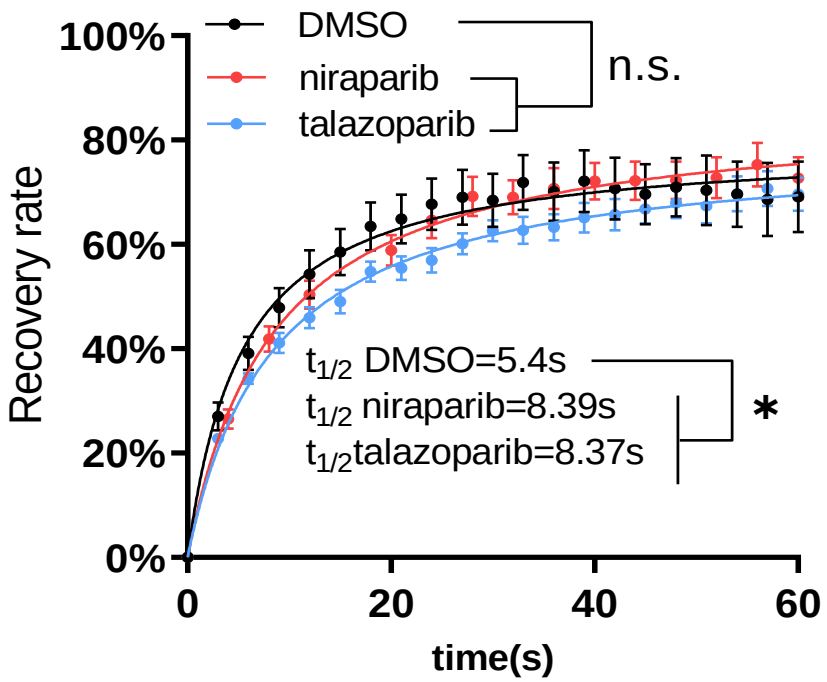




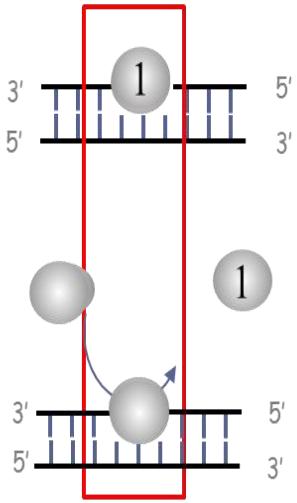
Shao et al. NAR 2020

PARP trapping ≠ Physical Retention





"Persistent" Foci

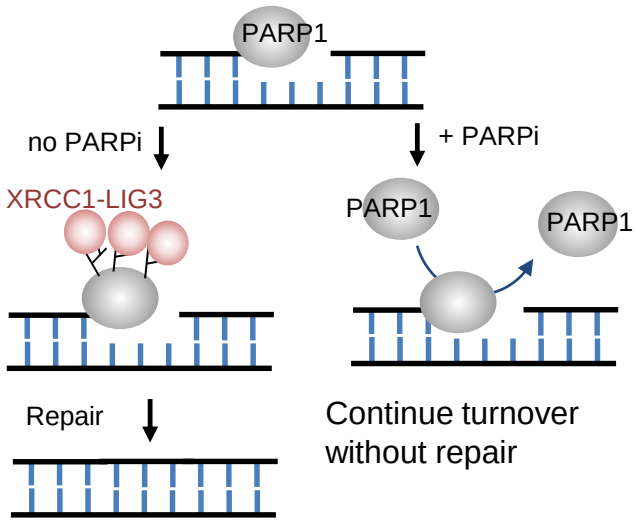


Stalling

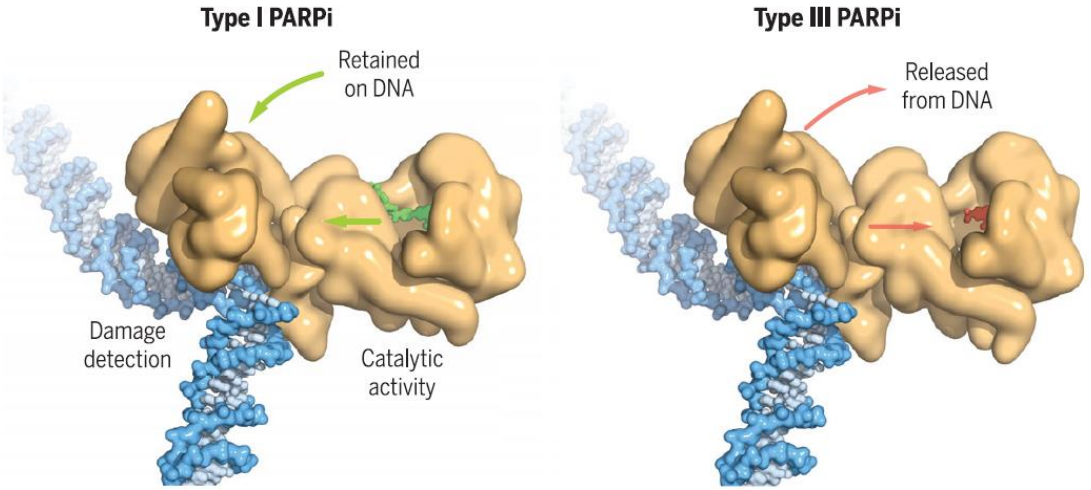
Go through

Shao et al. NAR 2020

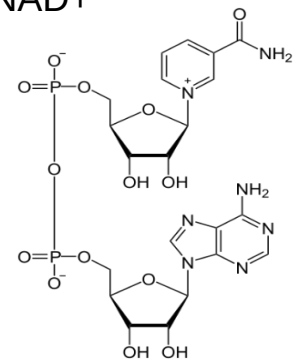
The nature of PARP1 trapping

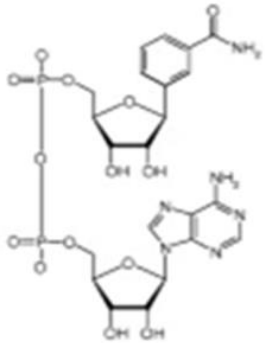


Shao et al. NAR 2020



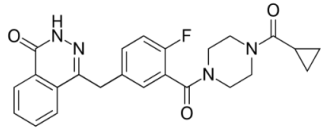
NAD+



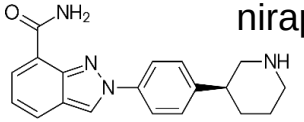


Benzamide adenine
dinucleotide (BAD)

olaparib

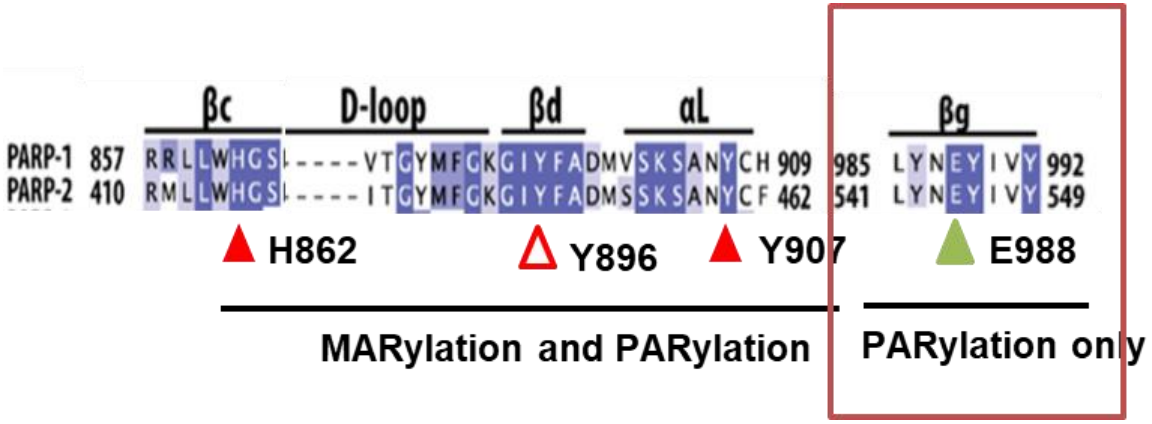


niraparib



Langelier et al. Nature Communications 2018
Zandarashvili et al. Science 2020

Physiological consequences?

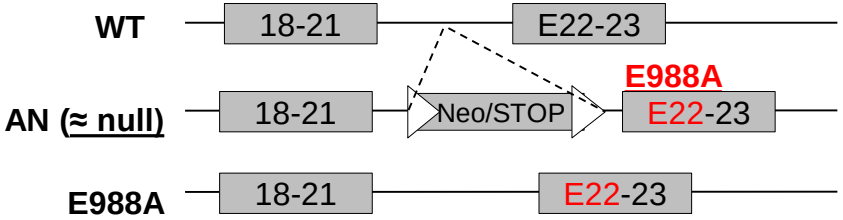


Gene	Deletion	PARylation deficient
PARP1	Viable	Embryonic lethal <E9.5
PARP2	Viable	Embryonic lethal at E14.5

Wang ZQ, et al.,Genes Dev. 1995 Mar 1;9(5):509-20
de Murcia JM, et al., PNAS. 1997 Jul 8;94(14):7303-7
Murcia JM, et al., EMBO J. 2003 May 1;22(9):2255-63

Parp1$^{+/E988A}$ are under representative

Inducible E988A Allele

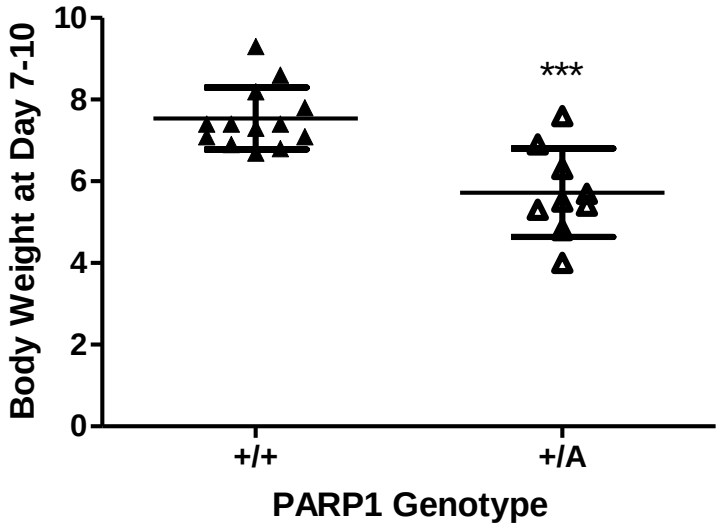


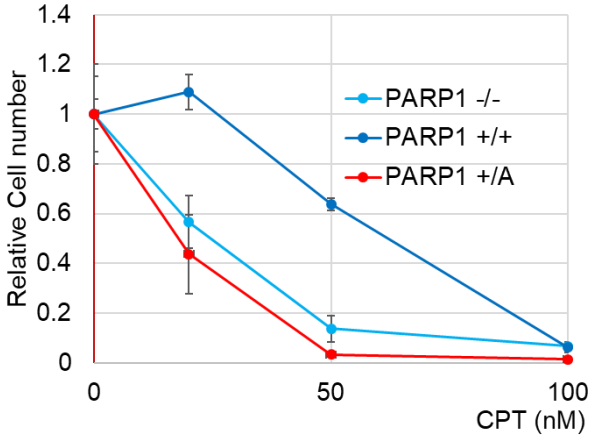
Parp1 +/AN x Rosa Flip/Flip

↓ Flipase express on/before E3.5

Rosa +/Flip Parp1 +/+ or **Rosa +/Flip Parp1 +/A**

Genotype	+/+	+/A	P
E3.5	53	11	P<0.01
Birth	165	35	6.3E-12





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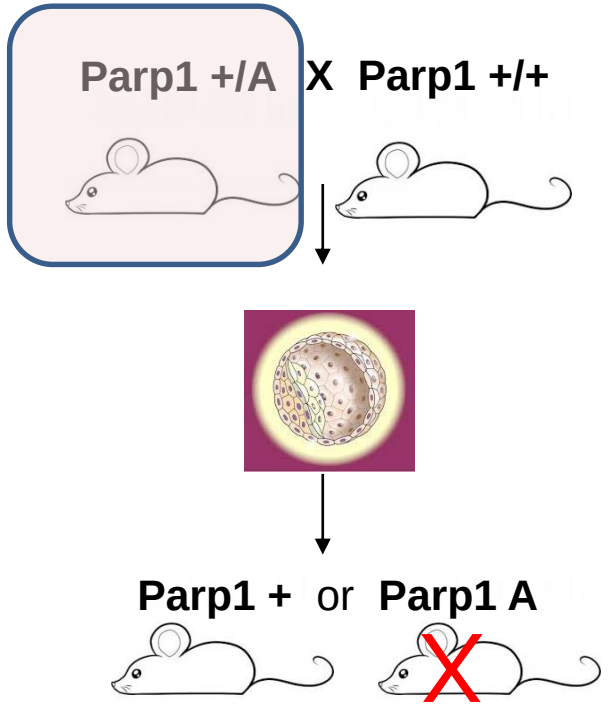
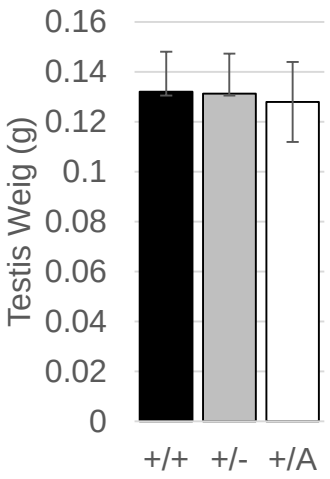
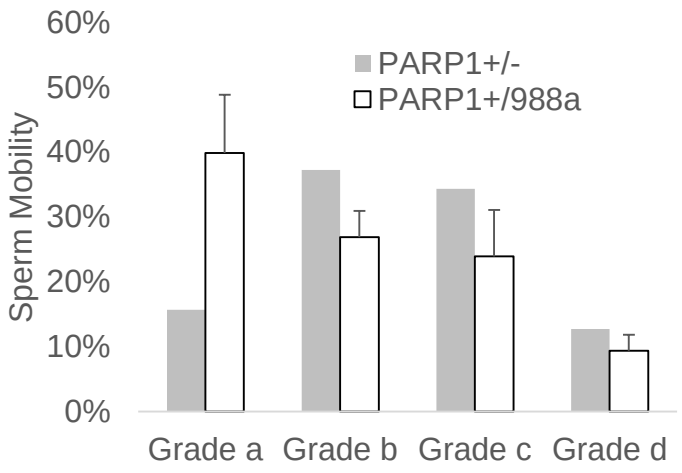
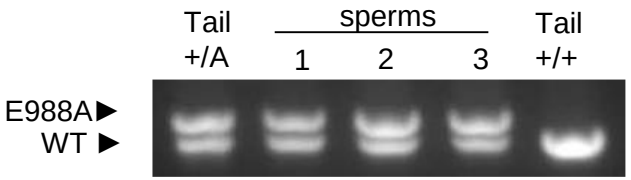
Chen Li, Zhengping Shao

PARP1$^{+/E988A}$ mice is fertile, but……..



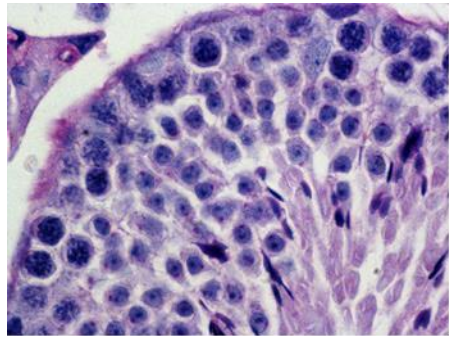




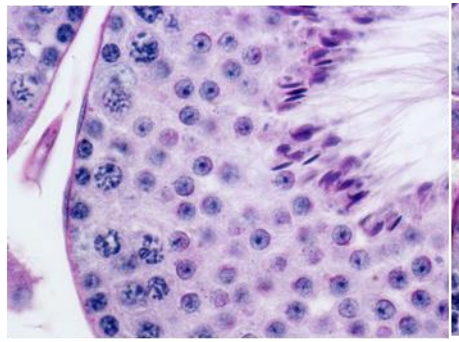


Genotype	+/+	+/A
E3.5	64	0
Birth	72	0

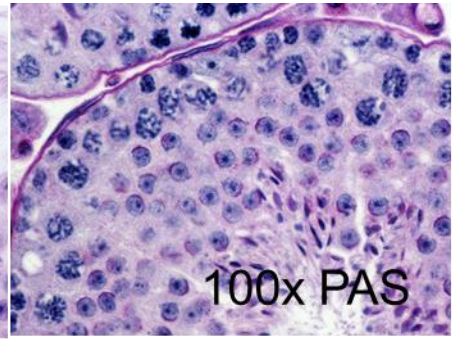
Parp1 +/+



Parp1 +/-

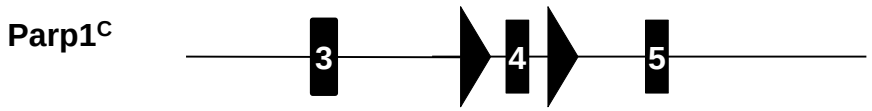


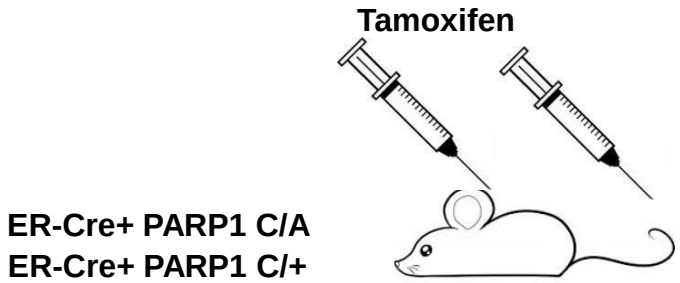
Parp1 +/A

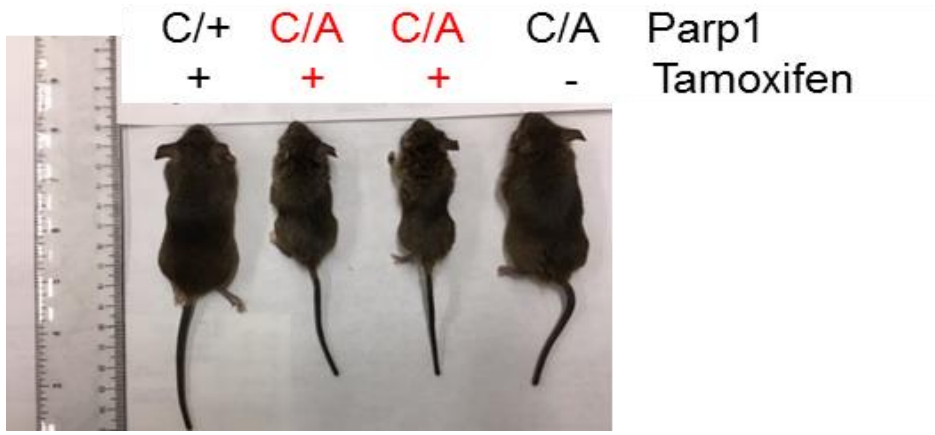


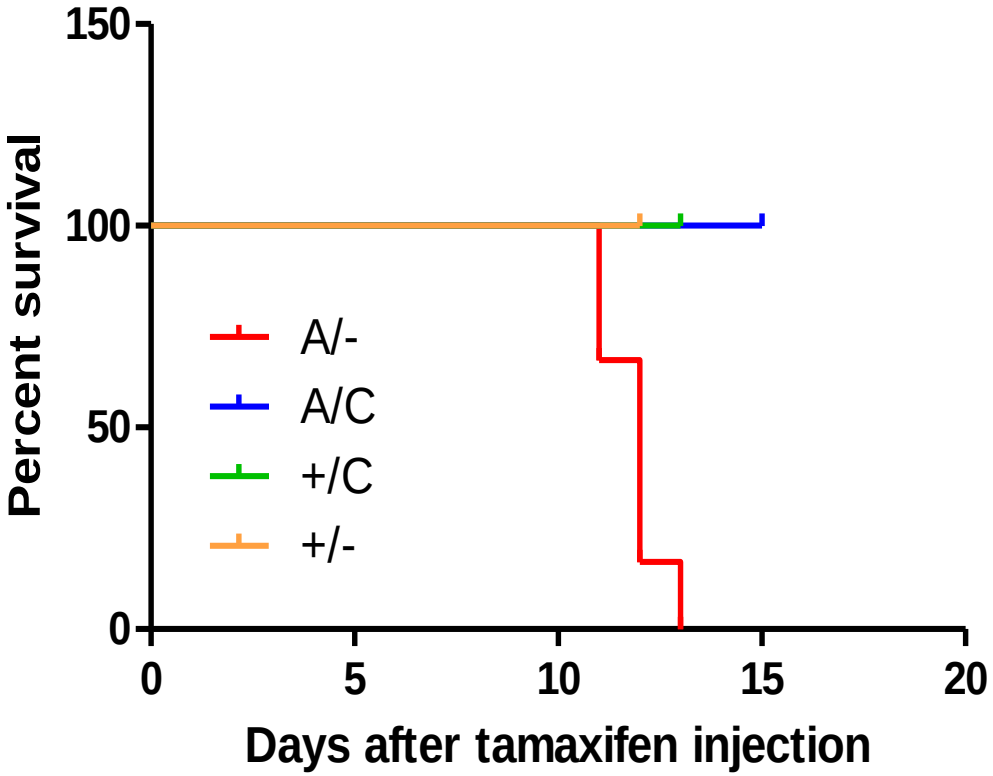
Zhengping Shao

Somatic Expression of the E988A alone causes lethal bone marrow failure



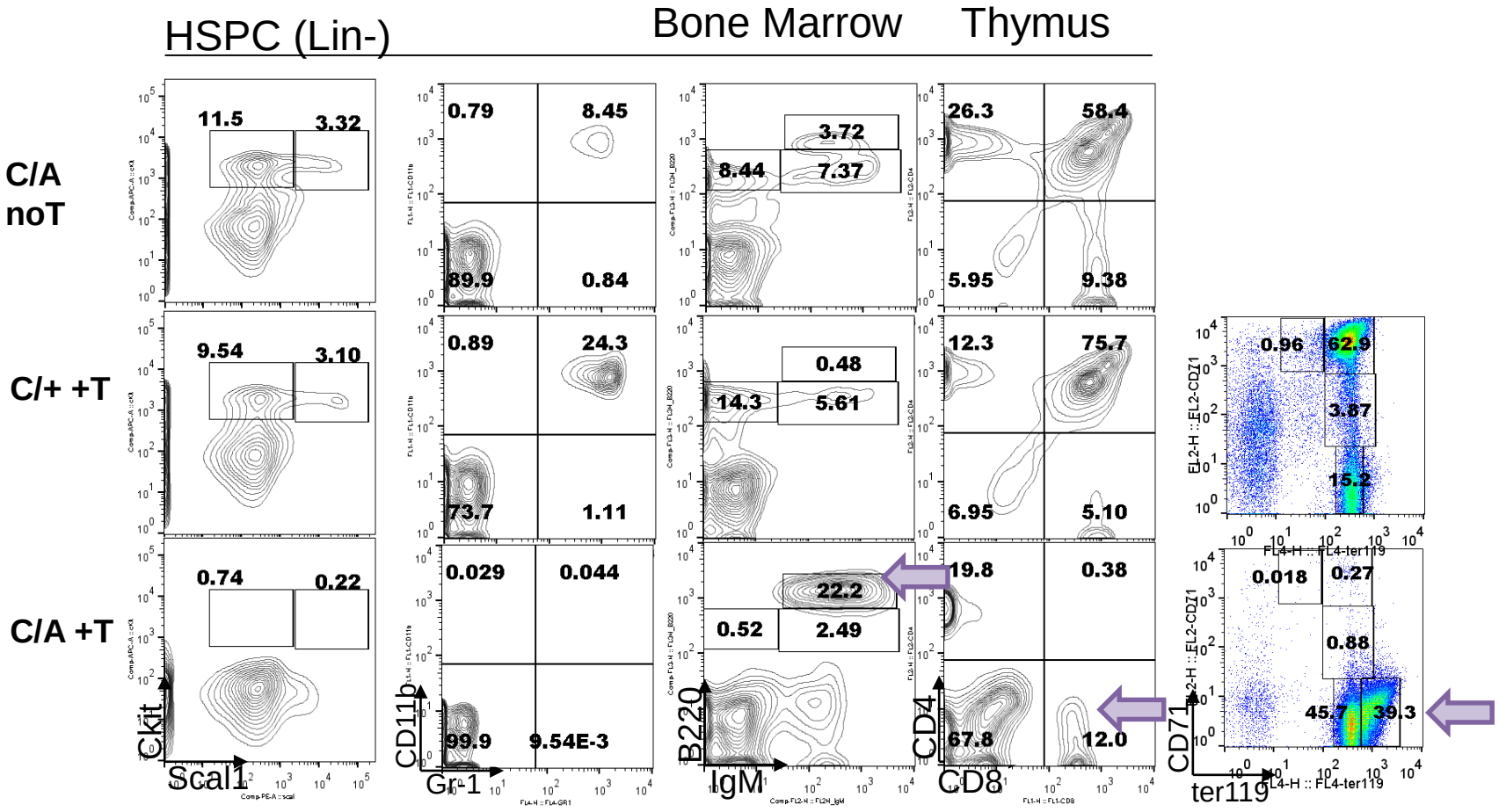






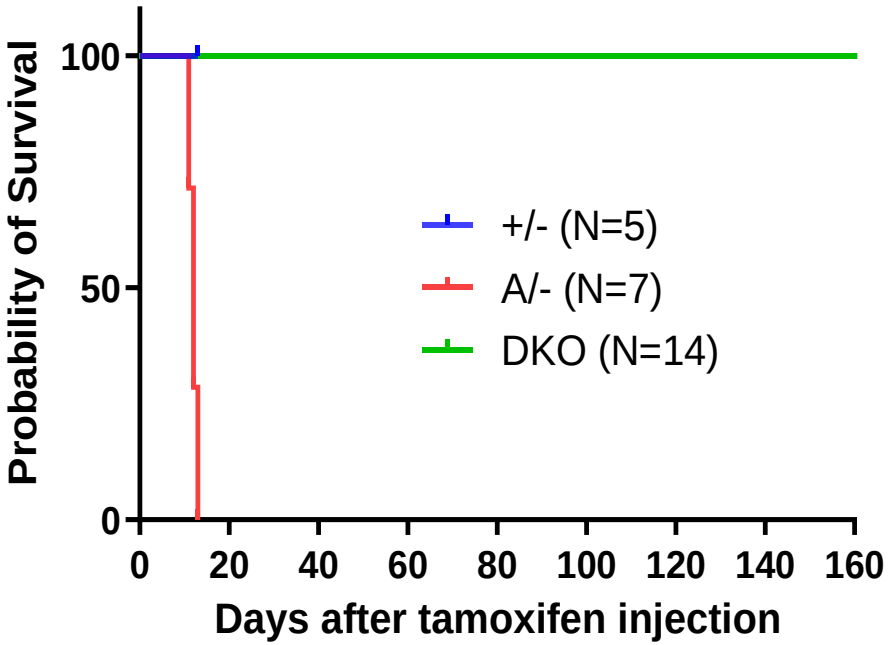
Zhengping Shao, Wenxia Jiang

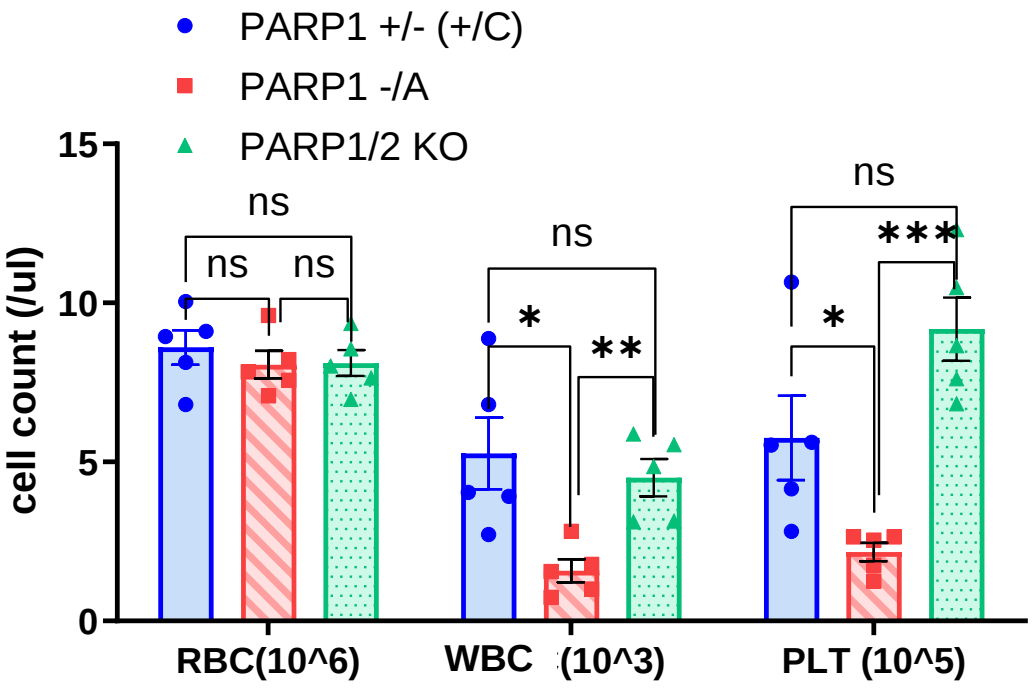
Somatic activation of A/- depletes hematopoietic stem and progenitor cells



Zhengping Shao

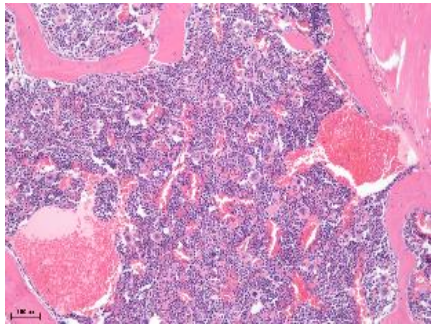
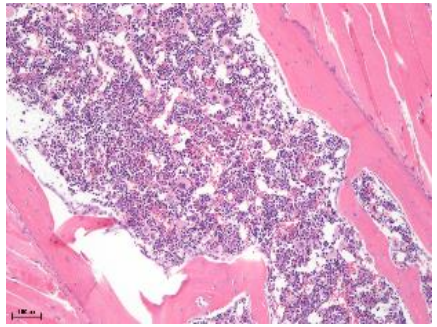
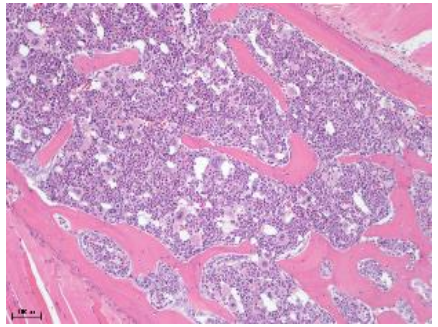
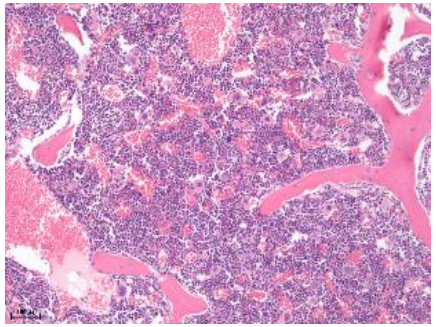
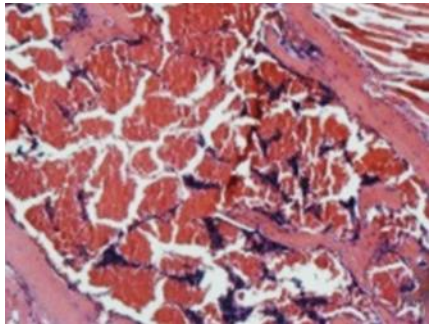
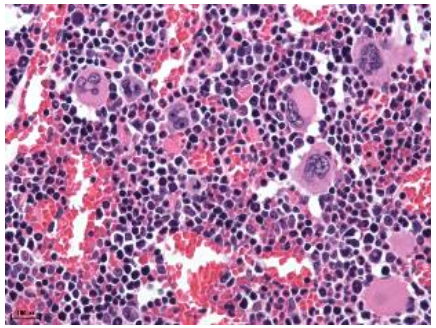
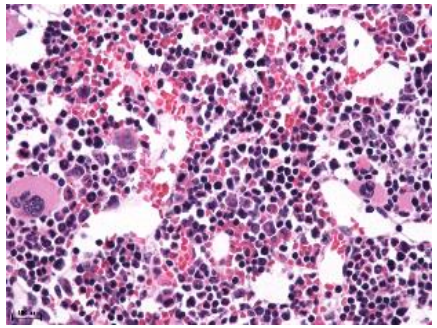
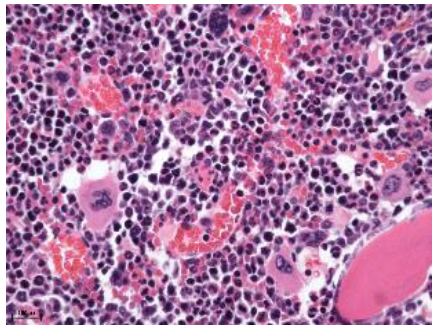
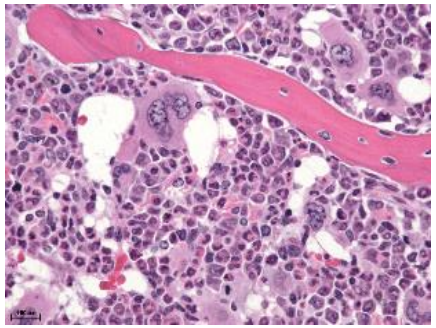
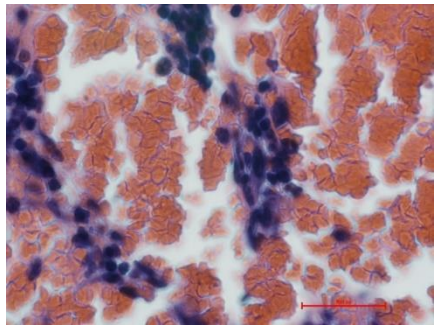
The lethality of Parp1 EA/- can not be explained by loss of both PARP1 and PARP2





Parp1-E988A causes pan bone marrow failure

Bone marrow *ROSA$^{+/ERcre}$ + TAM*

	Parp1$^{+/+}$Parp2$^{+/+}$	*Parp1$^{C/C}$Parp2$^{+/+}$*	*Parp1$^{+/+}$Parp2$^{C/C}$*	*Parp1$^{C/C}$Parp2$^{C/C}$*	*Parp1$^{C/A}$Parp2$^{+/+}$*
100X					
400X					

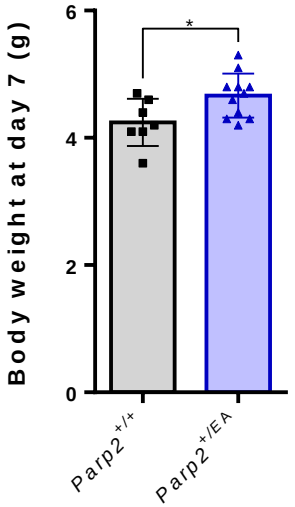
How about PARP2 ?

Dr. Xiaohui Lin

Parp2$^{E543A/E543A}$ mice are embryonic lethal with severe anemia

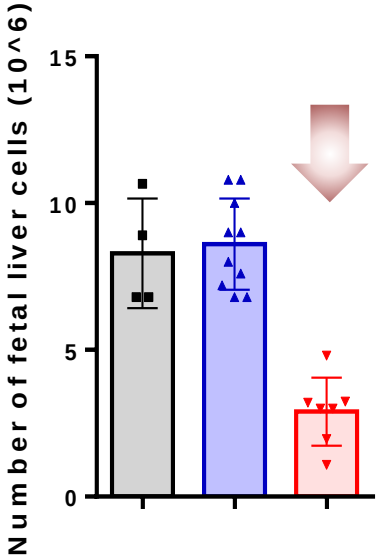
Parp2 +/EA X **Parp2 +/EA**

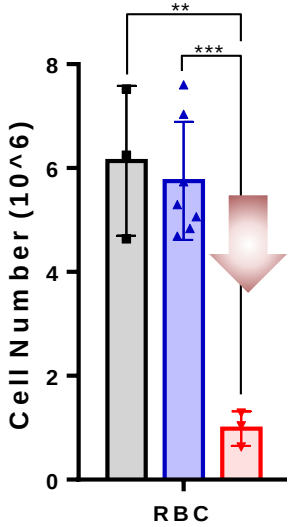
	PARP2$^{+/EA}$ x PARP2$^{+/EA}$				
	+/+	+/EA	**EA/EA**	total	p=
Birth	14	18	0	32	0.007



EA/EA +/EA **EA/EA** **EA/EA** +/EA +/EA

Fetal liver



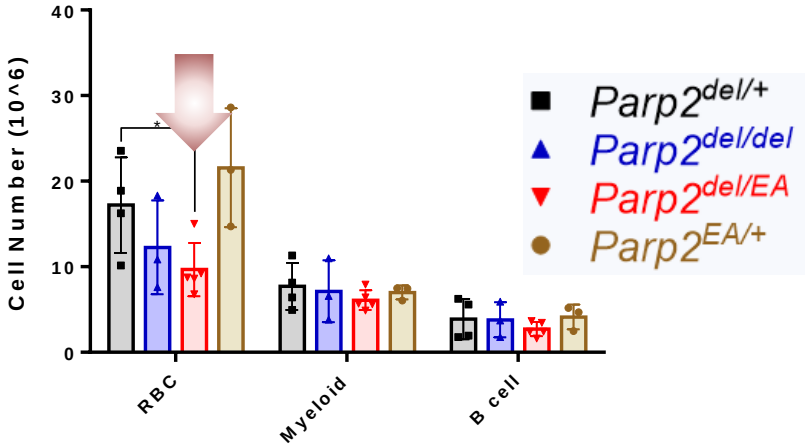


PARP2$^{del/E543A}$ mice are viable with isolated RBC defects

Parp2 +/EA X **Parp2 +/-**

PARP2$^{+/del}$ *x PARP2*$^{+/EA}$						
	+/+	+/del	+/EA	del/EA	total	p=
Birth	19	12	17	12	60	0.74

PARP2$^{+/EA}$ x PARP2$^{+/EA}$					
	+/+	+/EA	**EA/EA**	total	p=
Birth	14	18	0	32	0.007



The phenotype depends on the presence and the dose of PARP2- EA protein.

Catalytic Inhibition ≠ Deletion ≠ PTM

Gene	Deletion	Catalytic inactive	Rescued
ATM	Viable	Lethal E9.5	Mre11 deletion
ATR	+/- normal	Male infertility	
DNA-PK	Viable	Lethal E15.5	Ku70/80 deletion
PARP1	Normal	Lethal	
PARP2	Normal	Lethal E16.5	

ATM -| strand cleavage (Replication/HR),

ATR –| long ssDNA (*e.g.*, Cisplatin, meiosis),

DNA-PKcs –| end-ligation (NHEJ), rRNA and rDNA

PARP1 -| BER, Fork?

PARP2-| ???

Mouse genetics, Live cell Imaging, Metabolism, Genomic tools to analyze rDNA and chromosomal translocations

Thank you

We are recruiting… email sz2296@cumc.columbia.edu

Current member
Demis Menolfi
Zhengping Shao
Summer Wang
Yimeng Zhu
Xiaohui Lin
Brian Lee
Olivia Copu

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Xiangyu(Louis) Liu
Lisa Sprinzen
Denis Loredan
Chen Li
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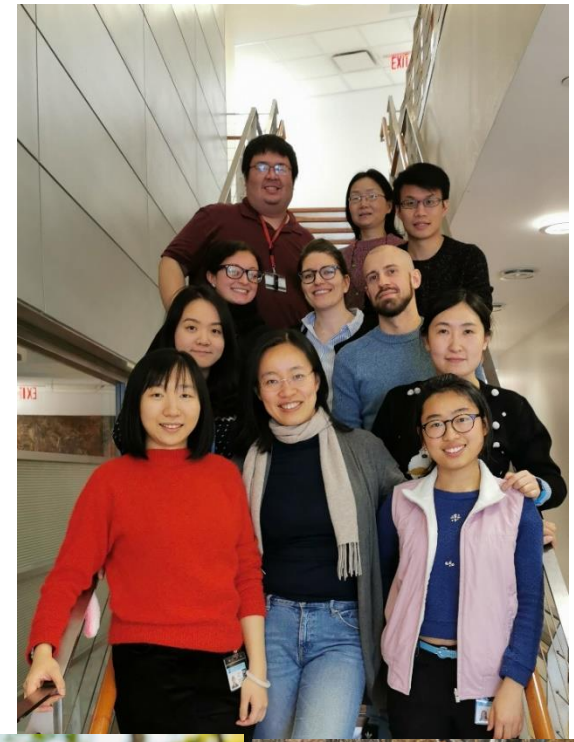
Serine Avagyan
Kerice Pinkney
Tina Xiang
Yu Tao
Verna Este

Columbia Colleagues
Wei Gu
Luke Berchowitz

Lorraine Symington
Richard Baer
Jean Gautier



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The Scripps Institute Laura J. Niedernhofer
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