CELLS WITH LEUKEMIA-ASSOCIATED ***DNMT3A*** MUTATIONS ARE MORE SENSITIVE TO CYTARABINE-INDUCED DNA DAMAGE

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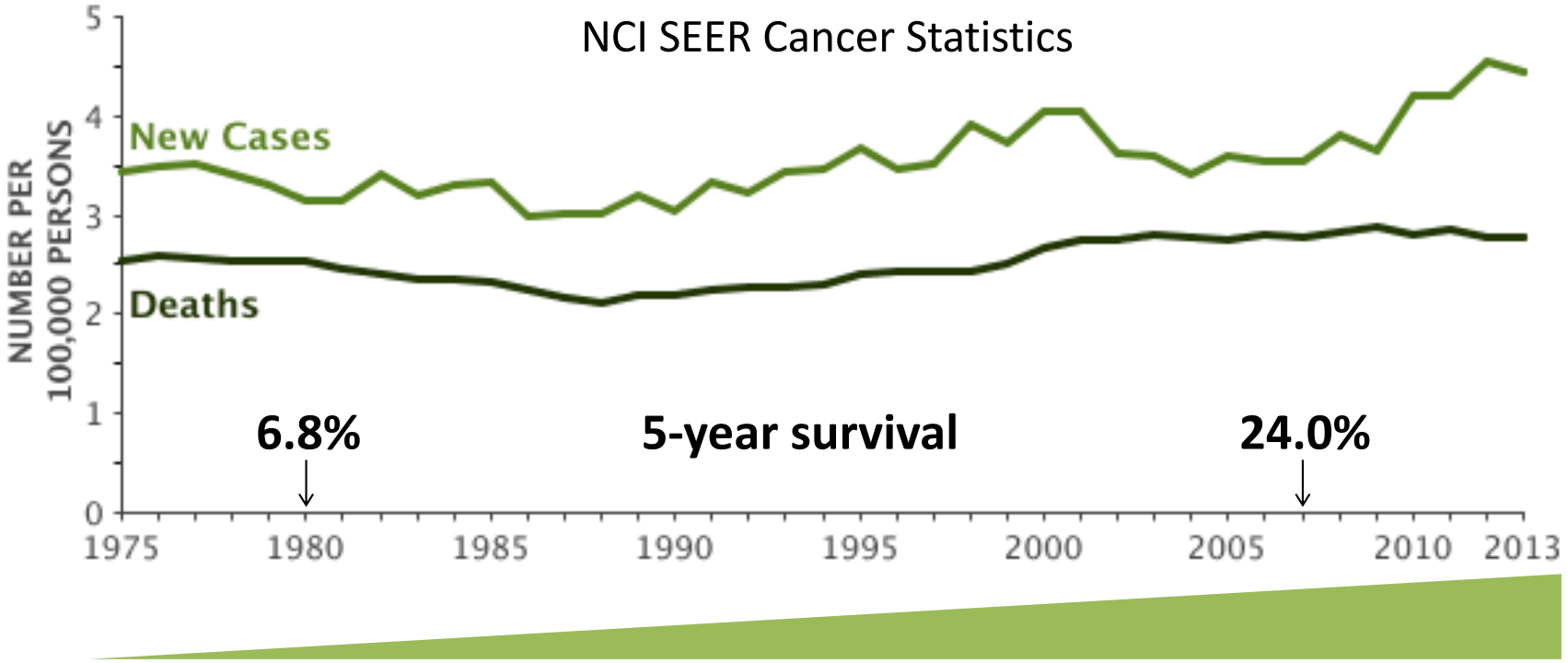
Acute myeloid leukemia (AML) is largely incurable



intensive chemo, **HSCT**, **better supportive care**

5-10 months

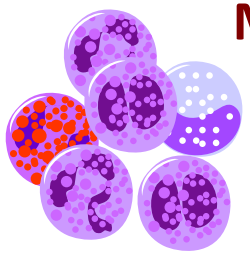
median survival

in patients 60+ years old unfit for high-dose chemo

Döhner, Weisdorf & Bloomfield, *NEJM* 2015

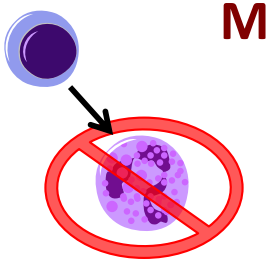
Two-hit model of AML pathogenesis

Class I proliferation (JAK2, FLT3)



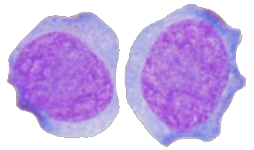
Myeloproliferative neoplasms (MPN)

Class II differentiation (RUNX1, CEBPA)



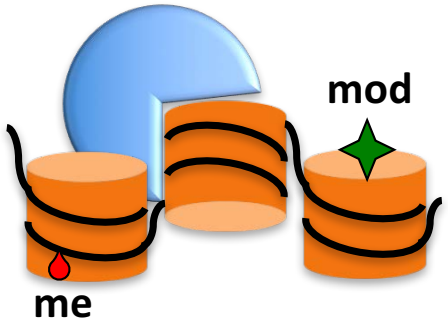
Myelodysplastic syndromes (MDS)

Acute myeloid leukemia



(AML)

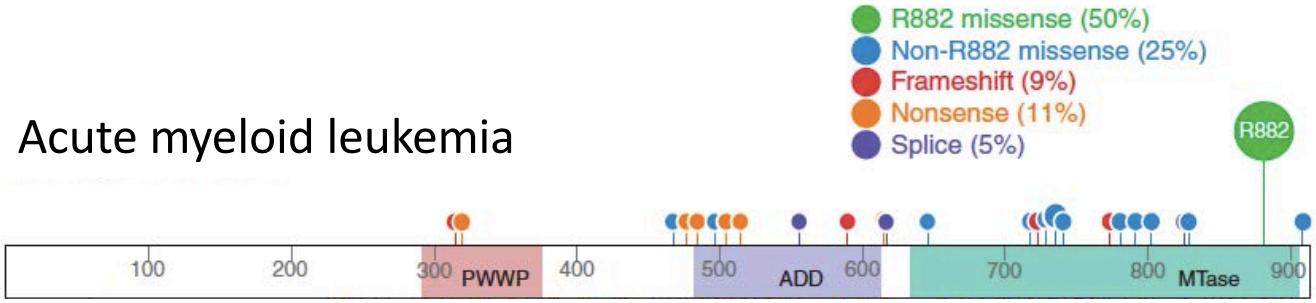
Other genes –
epigenetic and chromatin modifier genes
(*DNMT3A*, *TET2*, *IDH1/2*, *EZH2*, *ASXL1*),
etc.



Recurrent DNA methyltransferase 3A (***DNMT3A***) mutations in AML

Mutation — no./total no. (%)	
NPM1	54/200 (27)
FLT3	56/200 (28)
DNMT3A	51/200 (26)
IDH1 or *IDH2*	39/200 (20)
NRAS or *KRAS*	23/200 (12)
RUNX1	19/200 (10)
TET2	17/200 (8)
TP53	16/200 (8)
CEBPA	13/200 (6)
WT1	12/200 (6)
PTPN11	9/200 (4)
KIT	8/200 (4)

Acute myeloid leukemia

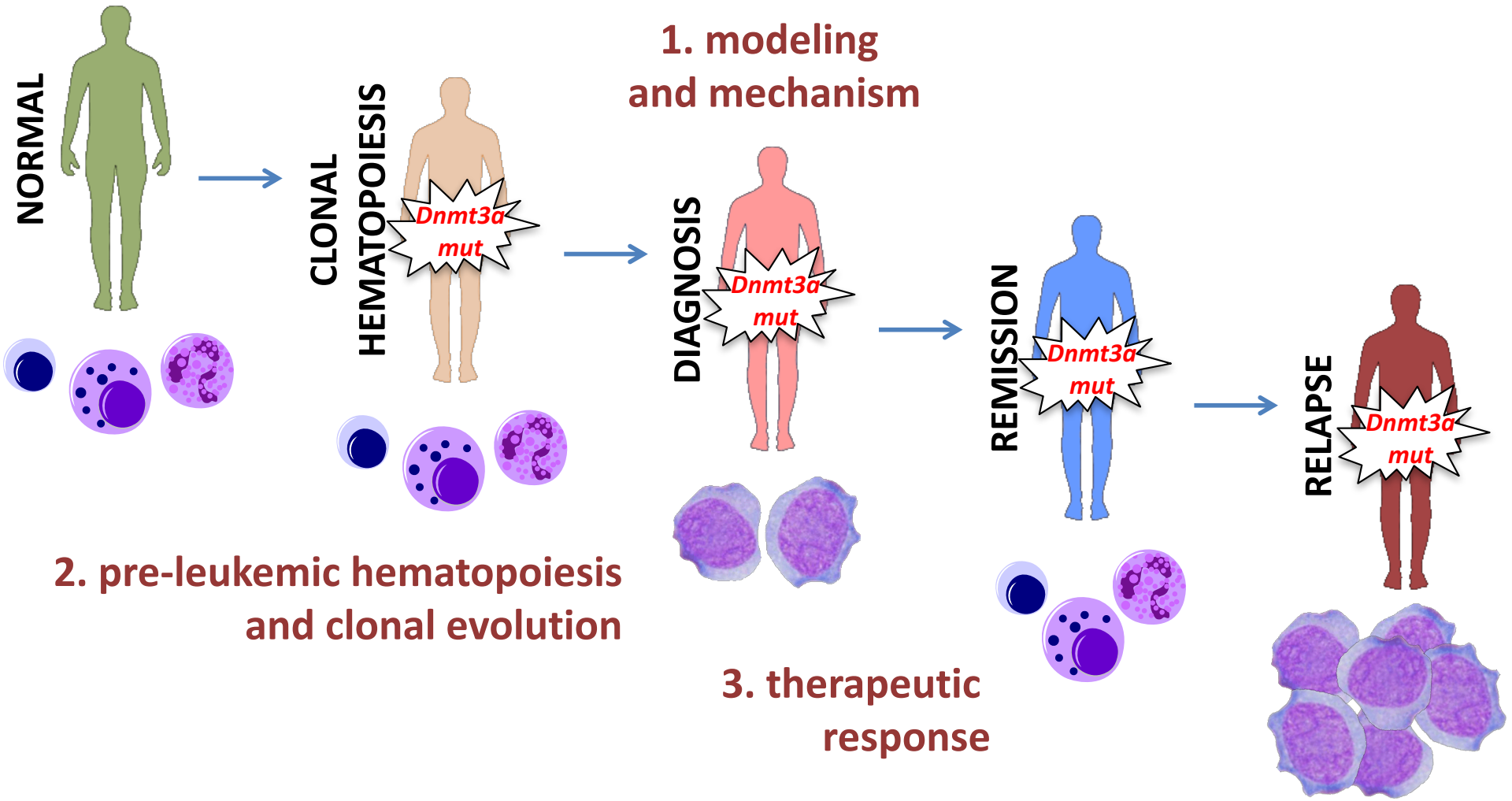


modest DNA hypomethylation
not associated with gene dysregulation

TCGA, 2013

Brunetti *et al*. 2017

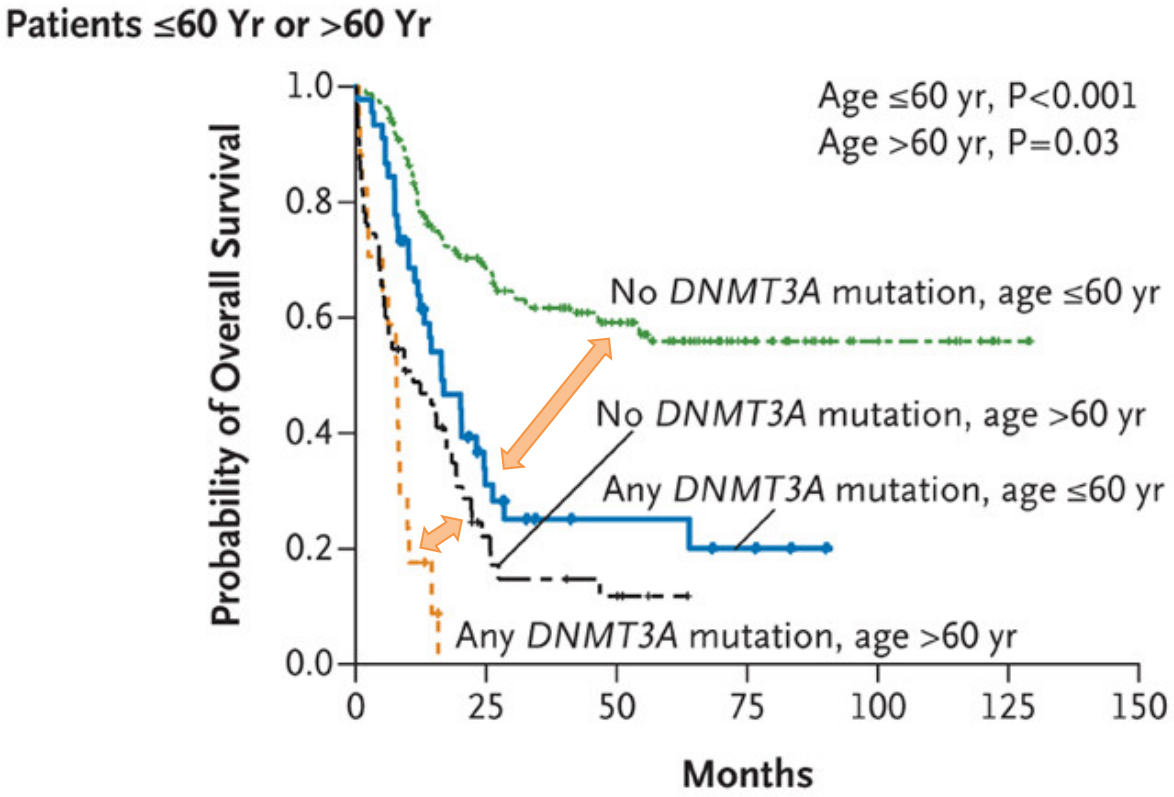
OG lab research interests



DNMT3A mutations are associated with advanced age and unfavorable outcome in AML

	***DNMT3A* WT**	***DNMT3A* MUT**
Age at diagnosis *	60 yrs	67 yrs

* $p < 0.001$



Balasubramanian *et al*. 2018

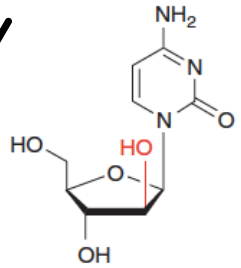
Ley *et al*. 2010, Yan *et al.* 2011

Low-dose cytarabine – a treatment option for elderly AML patients

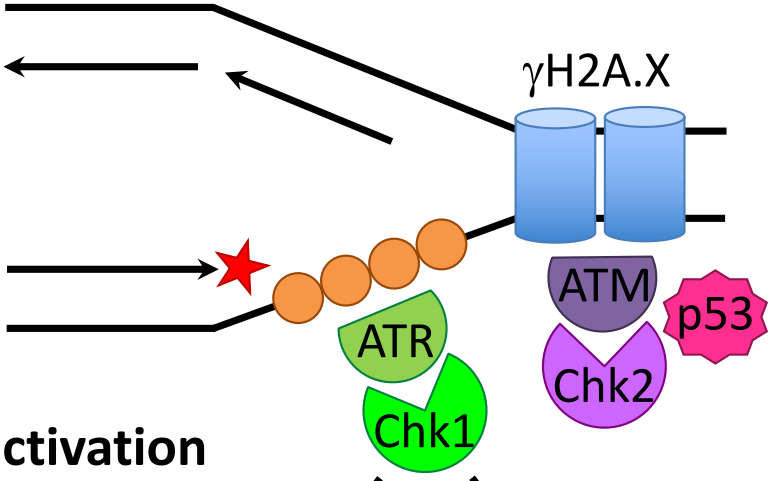
60+ years old, poor performance status

supportive care

low-dose cytarabine



investigational therapy (clinical trial)



Chain termination

Fork collapse

Checkpoint activation

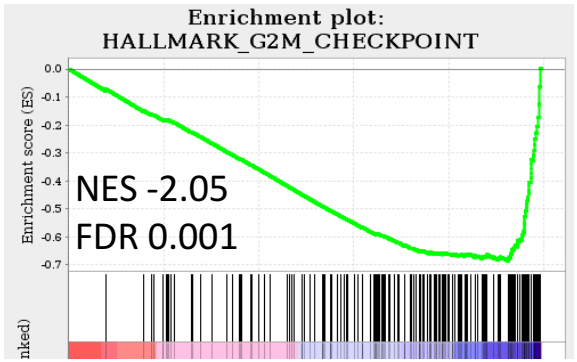
Apoptosis

G2→M checkpoint

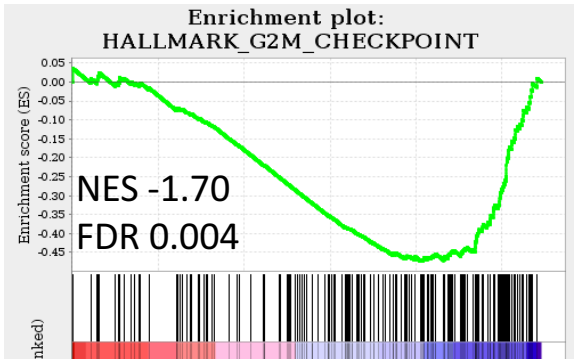
origin firing

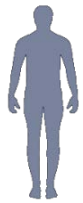
Negative enrichment of the cell cycle-related signatures in cells expressing **DNMT3A^{R882}**

***+/m* vs *WT* stem&prog cells**



***DNMT3A* R882 vs WT (TCGA)**



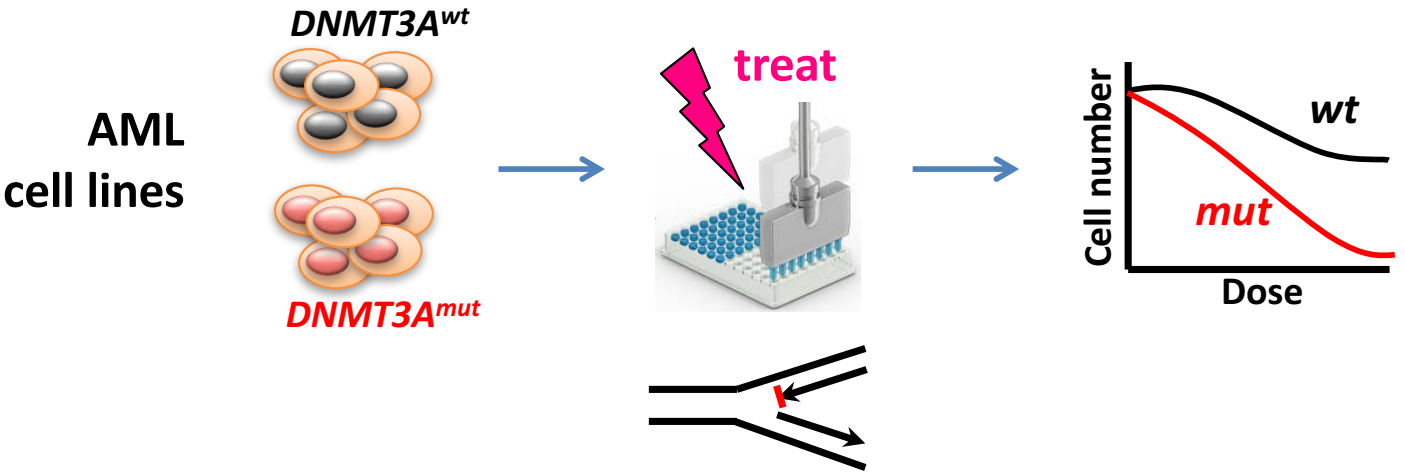


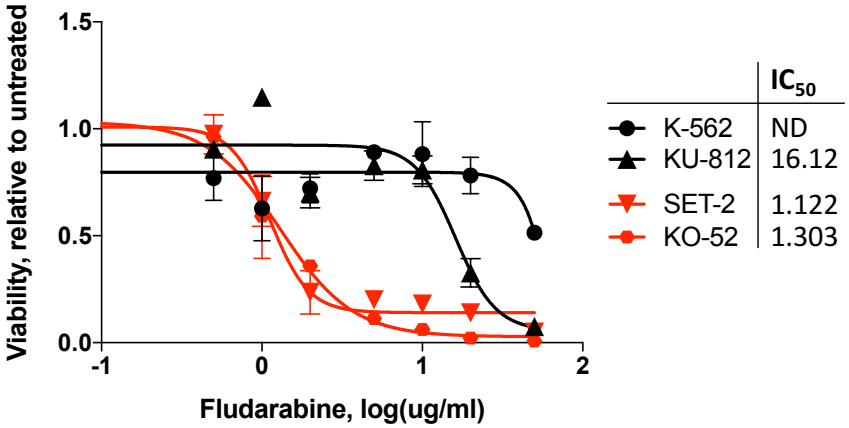
Guryanova *et al*. (2016) *Nat Med*

TCGA (2013) *NEJM*
Glass *et al.* (2017) *Cancer Discov*

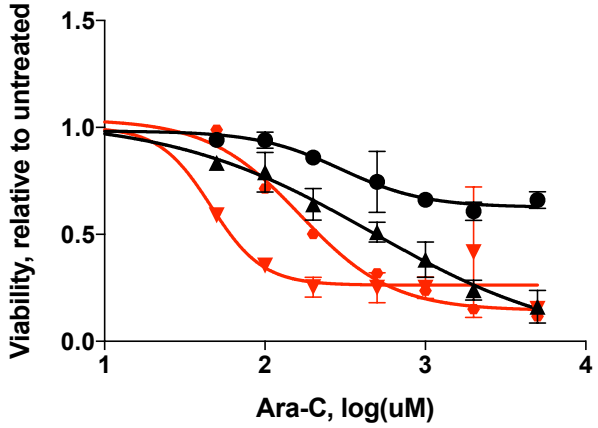
DNMT3A directs therapeutic response to replication stress-inducing agents

Leukemia cell lines with ***DNMT3A*** mutations are more sensitive to replication stress inducers



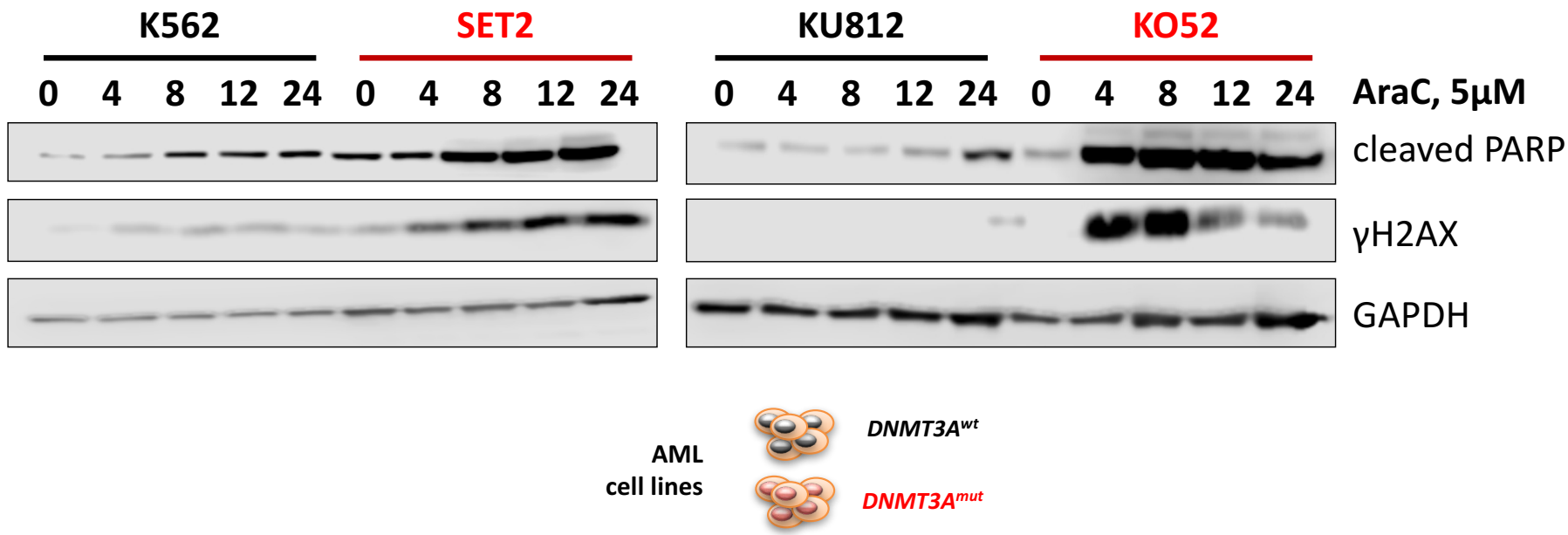


	IC_{50}
K-562	ND
KU-812	16.12
SET-2	1.122
KO-52	1.303

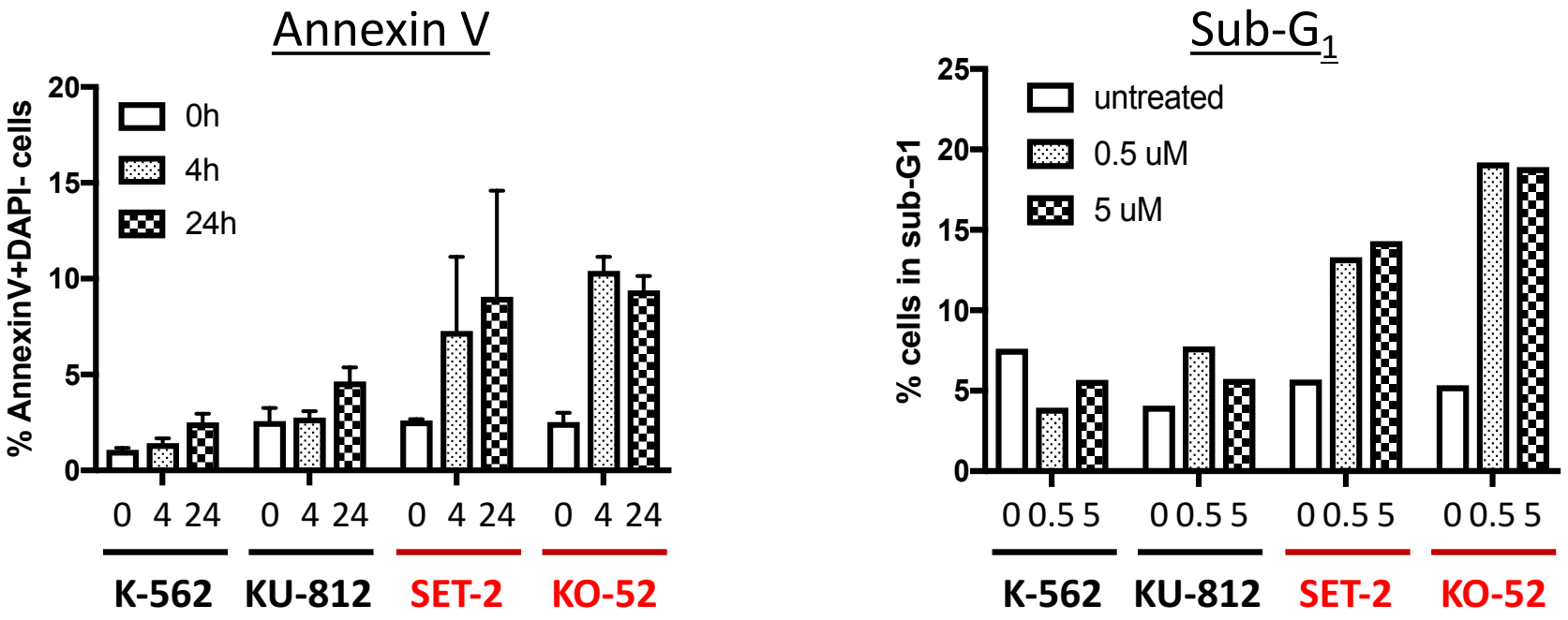


	IC_{50}
K-562	297.6
KU-812	465.2
SET-2	46.1
KO-52	165.8

DNMT3A-mutant cell lines treated with Ara-C accumulate markers of DNA damage and apoptosis



Increased apoptosis in ***DNMT3A***-mutant cell lines after Ara-C treatment

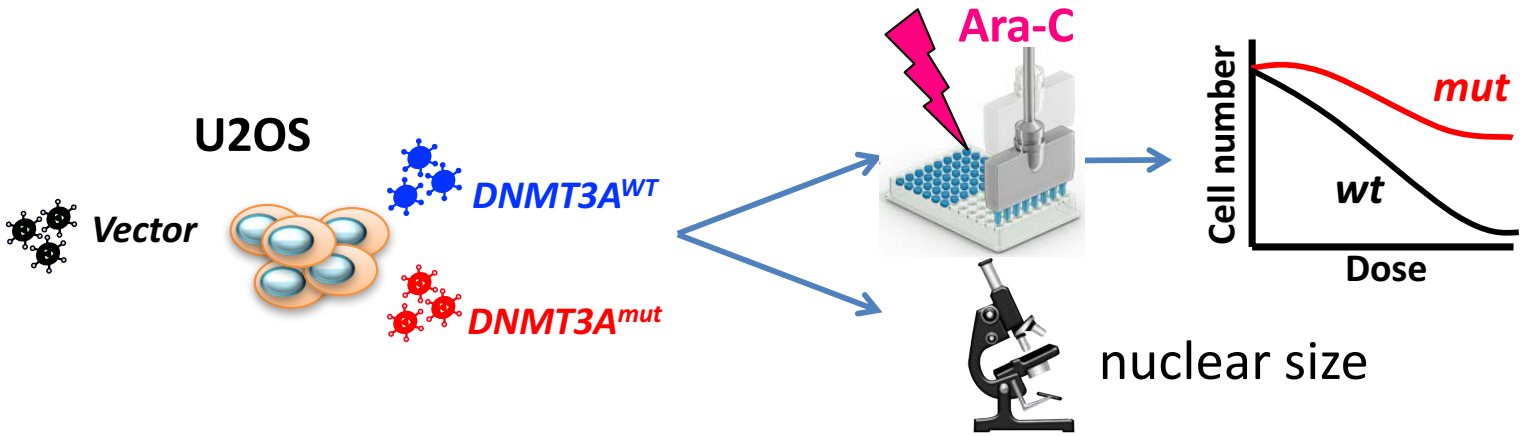


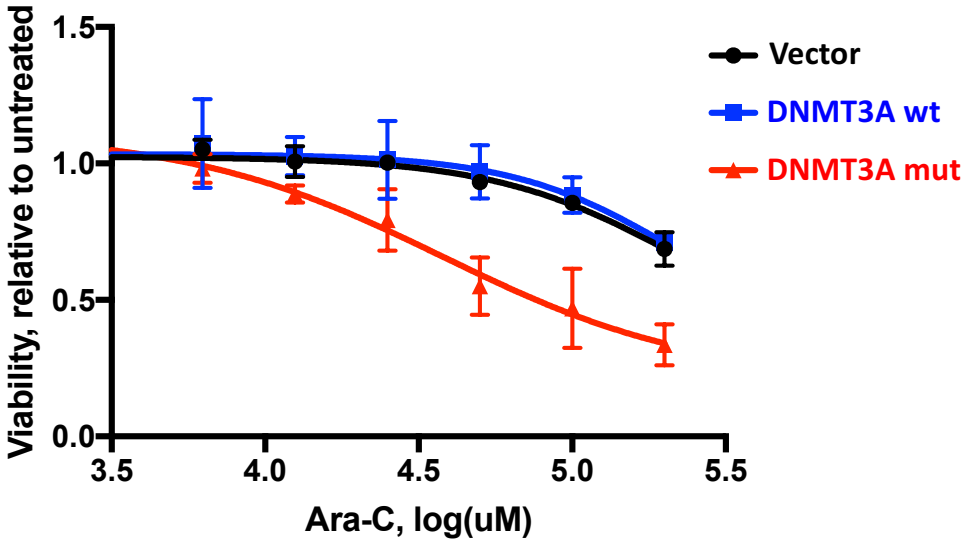
AML cell lines

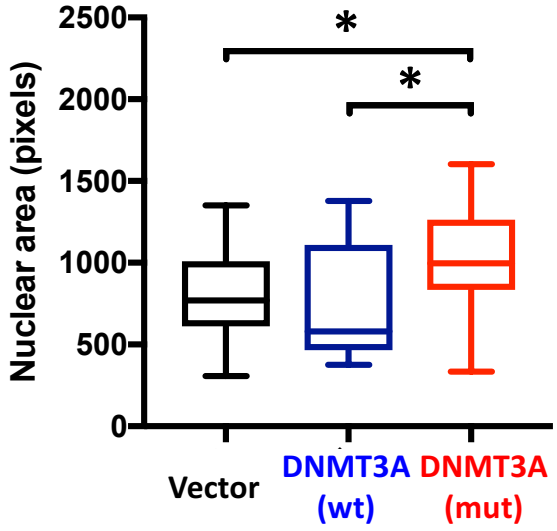
$DNMT3A^{wt}$

$DNMT3A^{mut}$

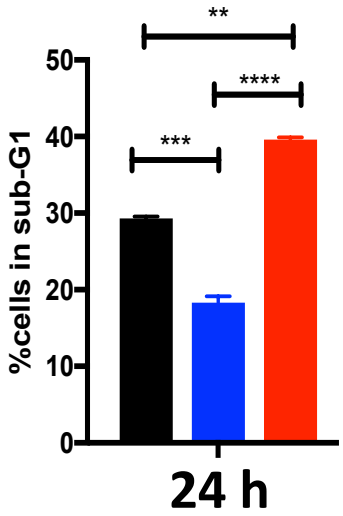
Ara-C sensitivity correlates with nuclear structure changes in cells expressing **DNMT3A** variants

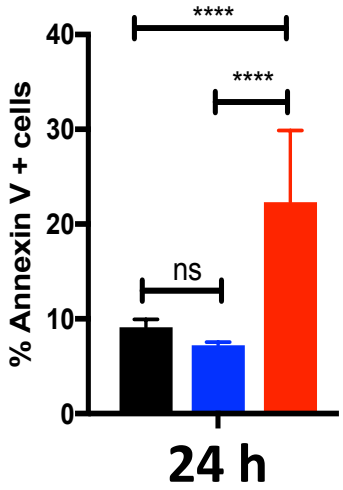


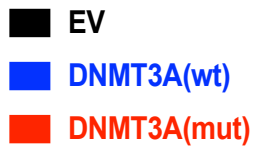




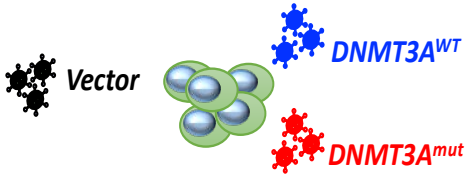
Increased apoptosis after Ara-C exposure in cells expressing **DNMT3A** mutants



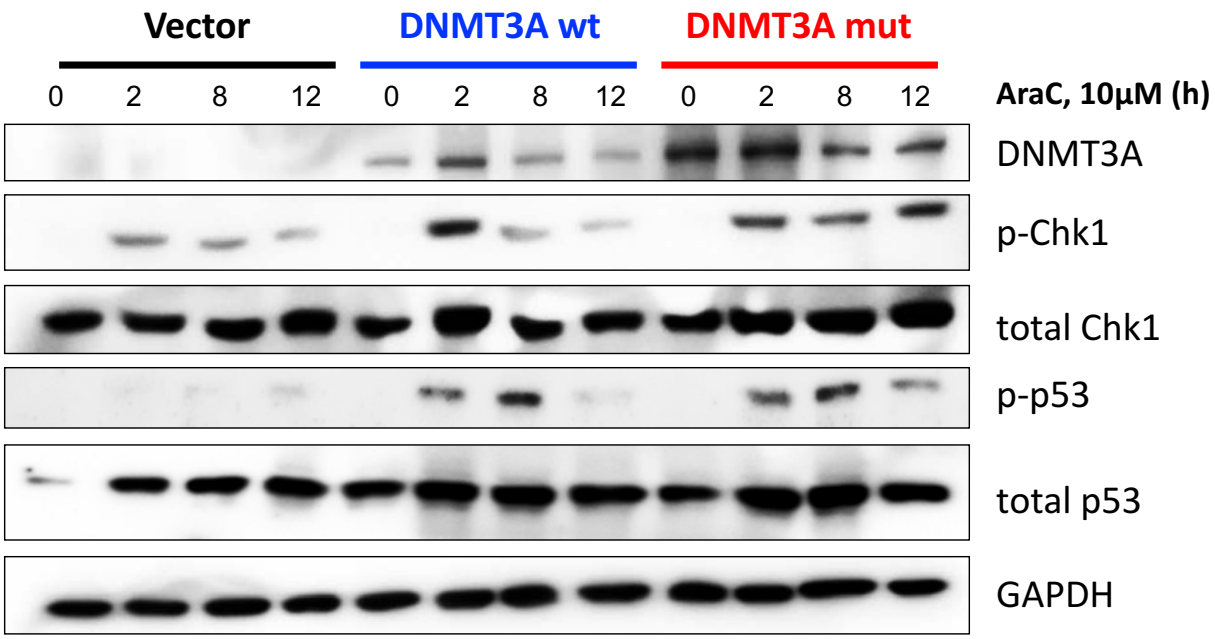


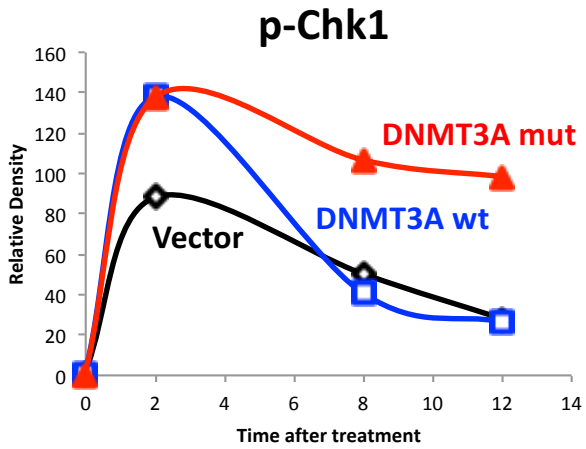


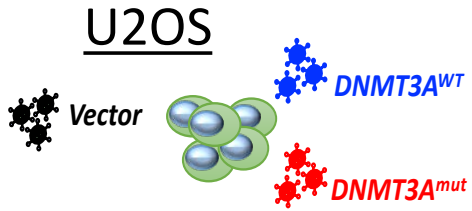
AraC, 50µM



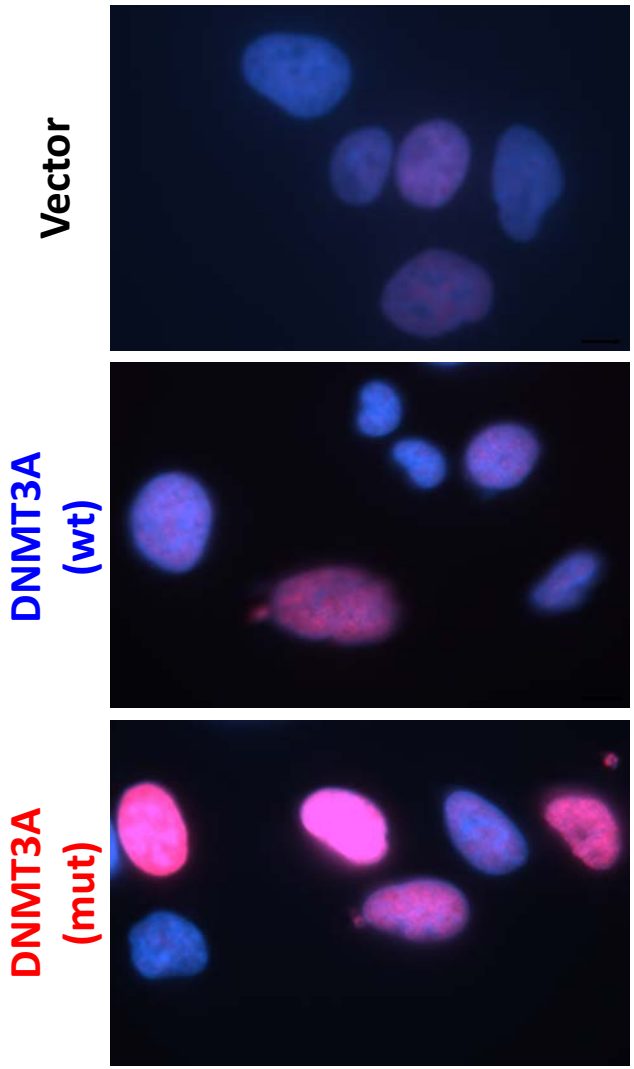
Cells expressing mutant **DNMT3A** show persistent DNA damage signaling after Ara-C



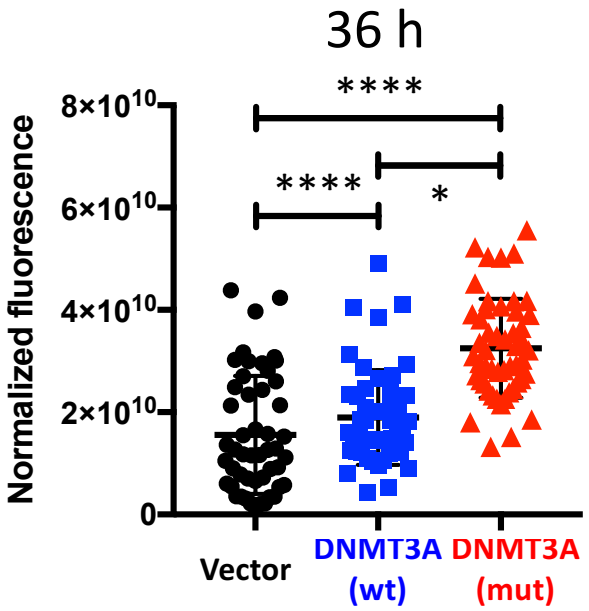


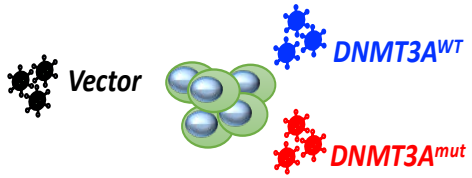


Accumulation of DNA damage marker γH2A.X after Ara-C exposure in cells with **DNMT3A** mutations

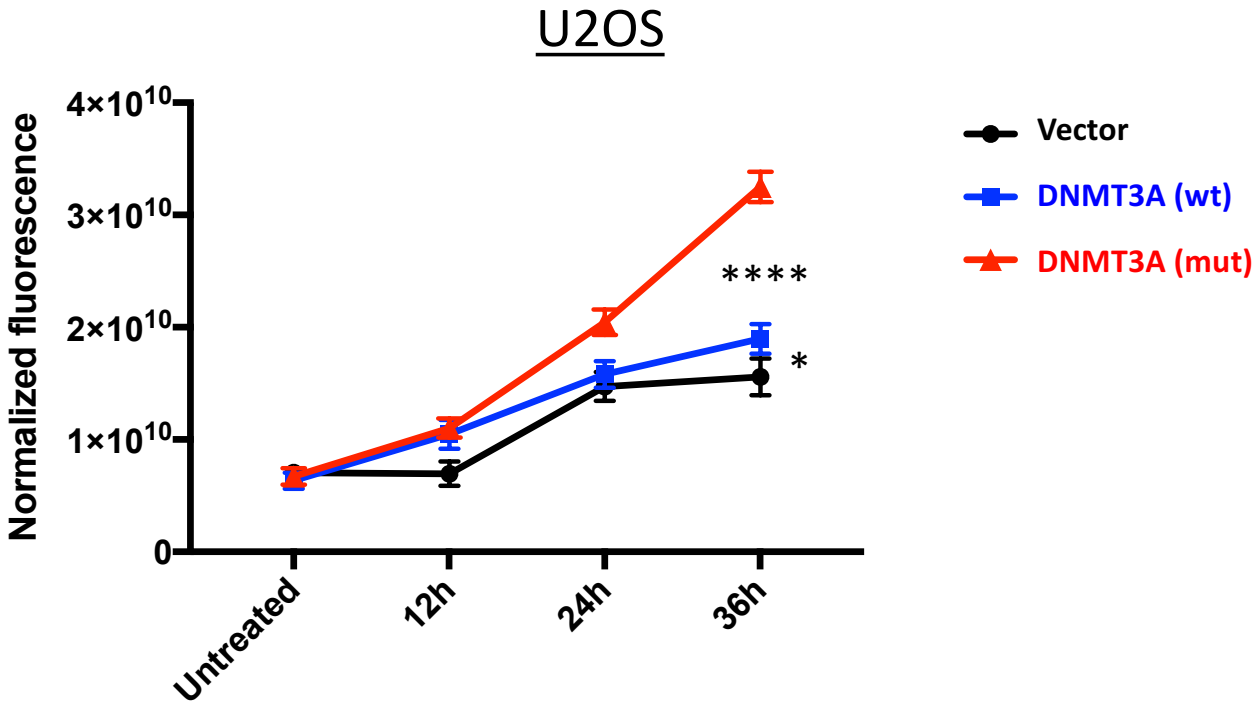


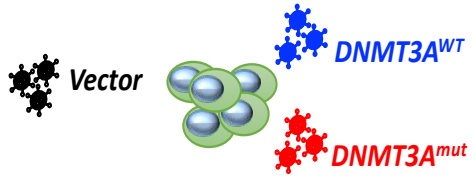
U2OS





Accumulation of DNA damage marker γH2A.X after Ara-C exposure in cells with **DNMT3A** mutations





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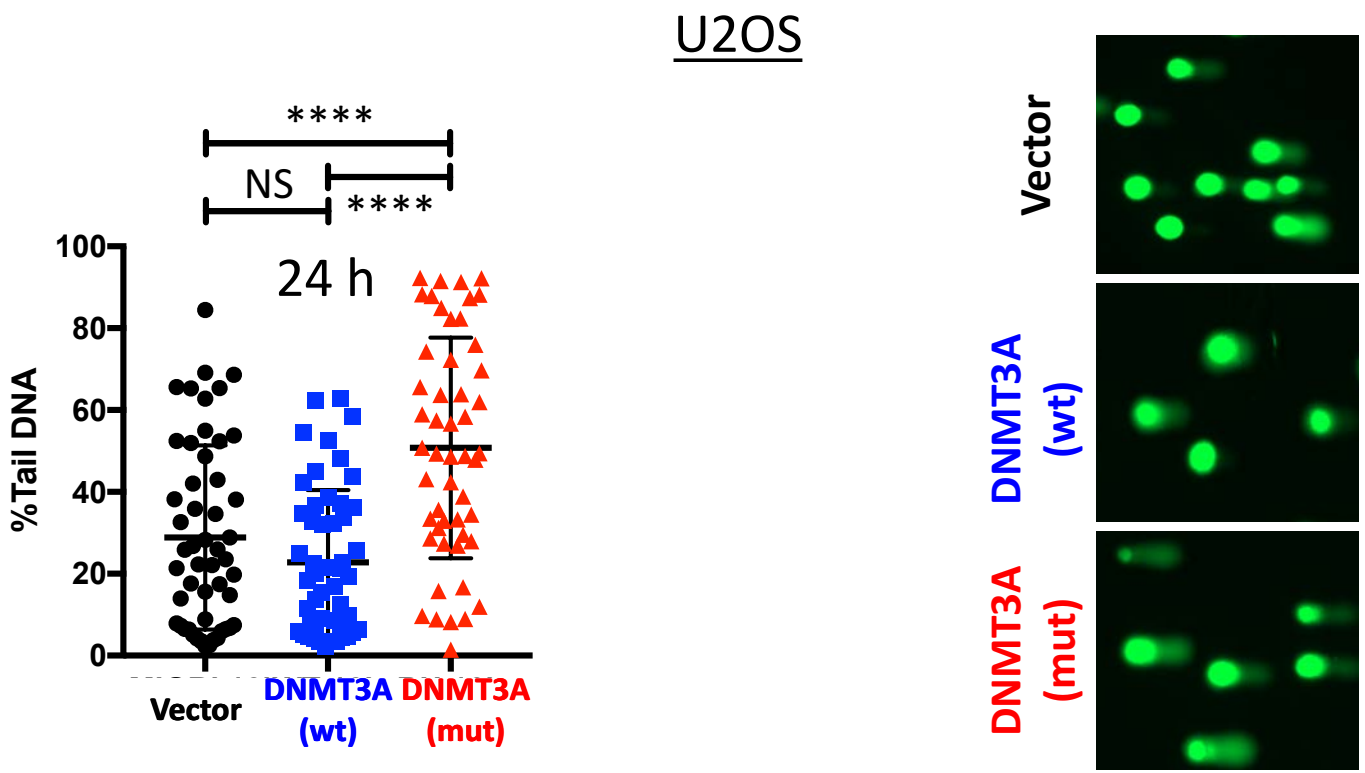
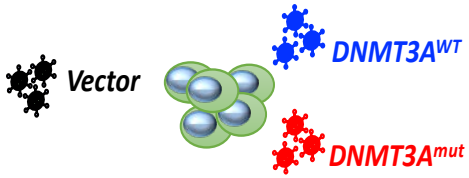
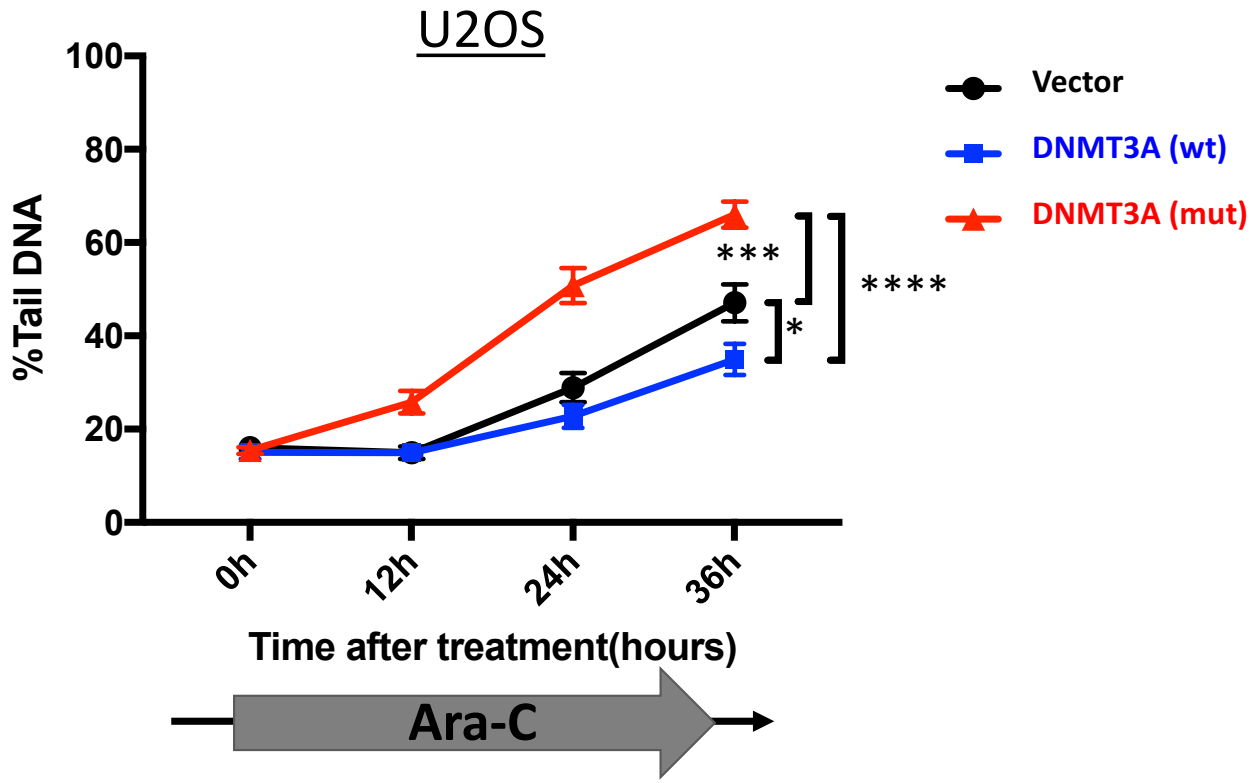
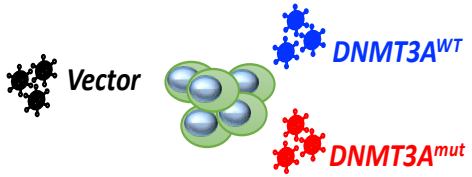


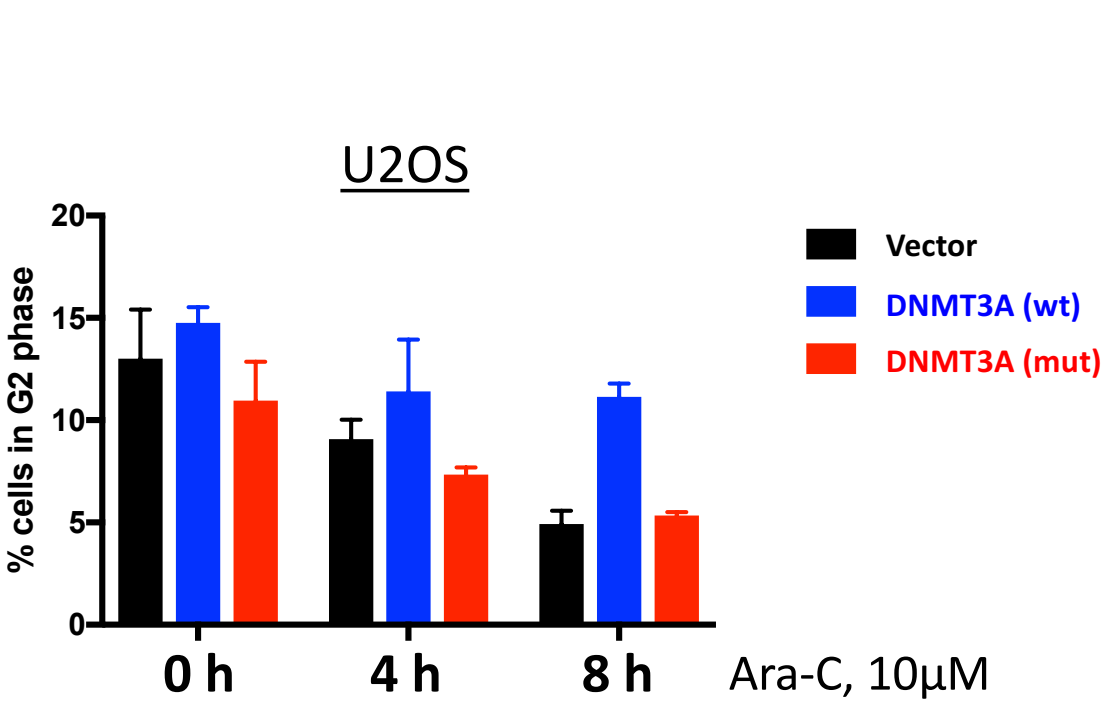


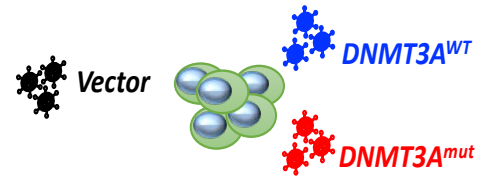
Accumulation of DNA damage in cells with ***DNMT3A*** mutations after continuous Ara-C exposure

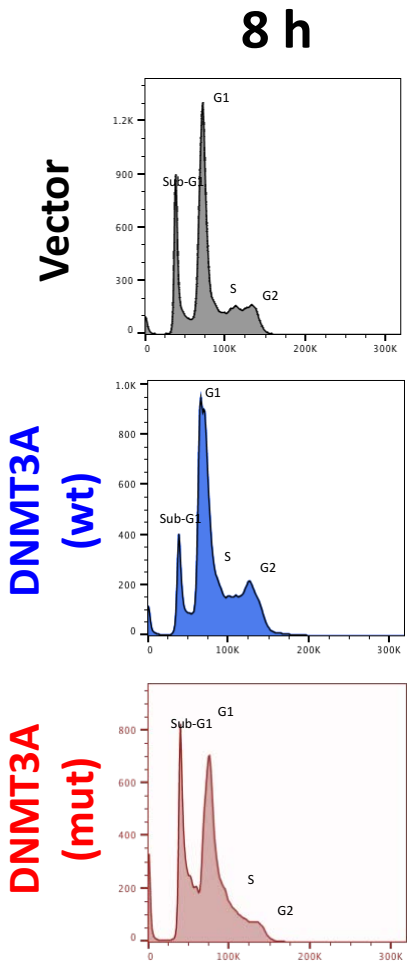




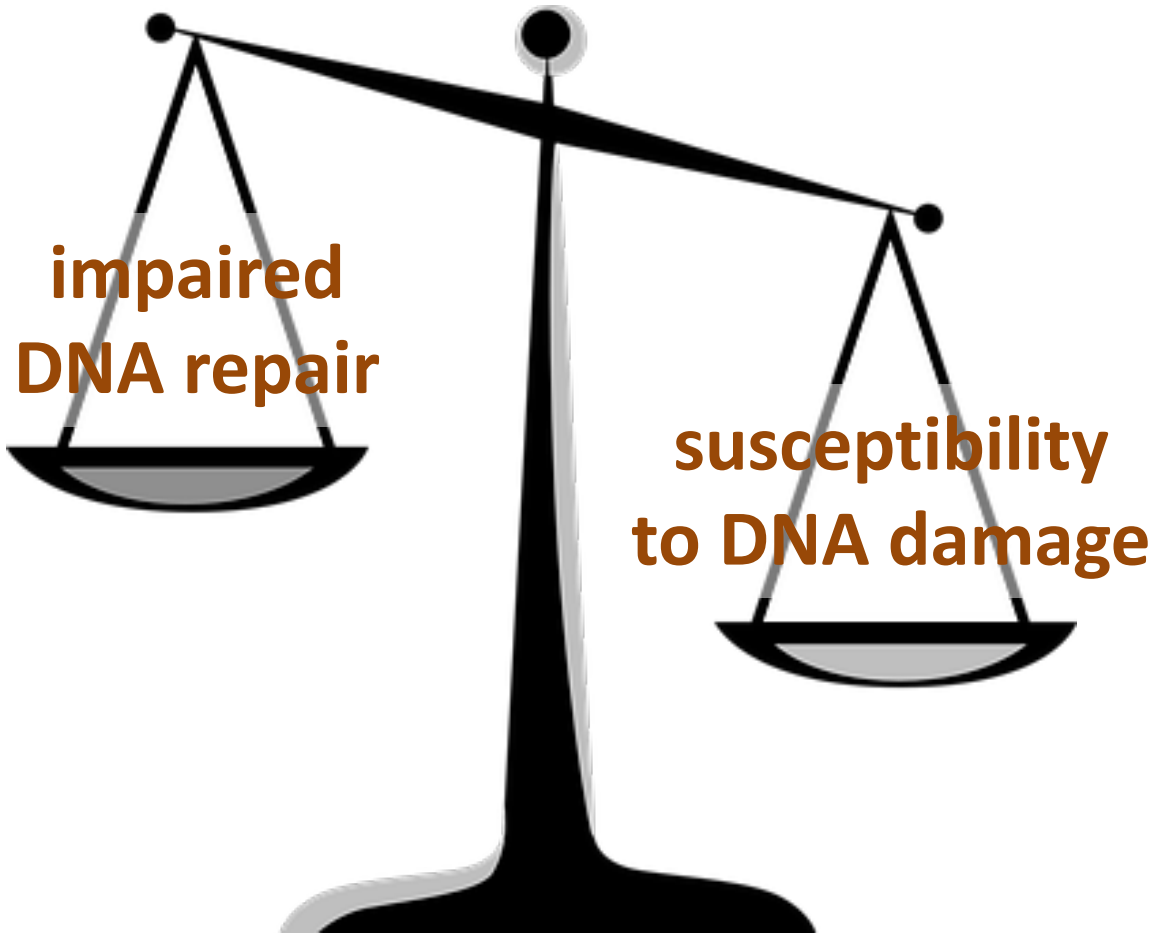
Altered cell cycle profile after Ara-C treatment in cells with ***DNMT3A*** mutations



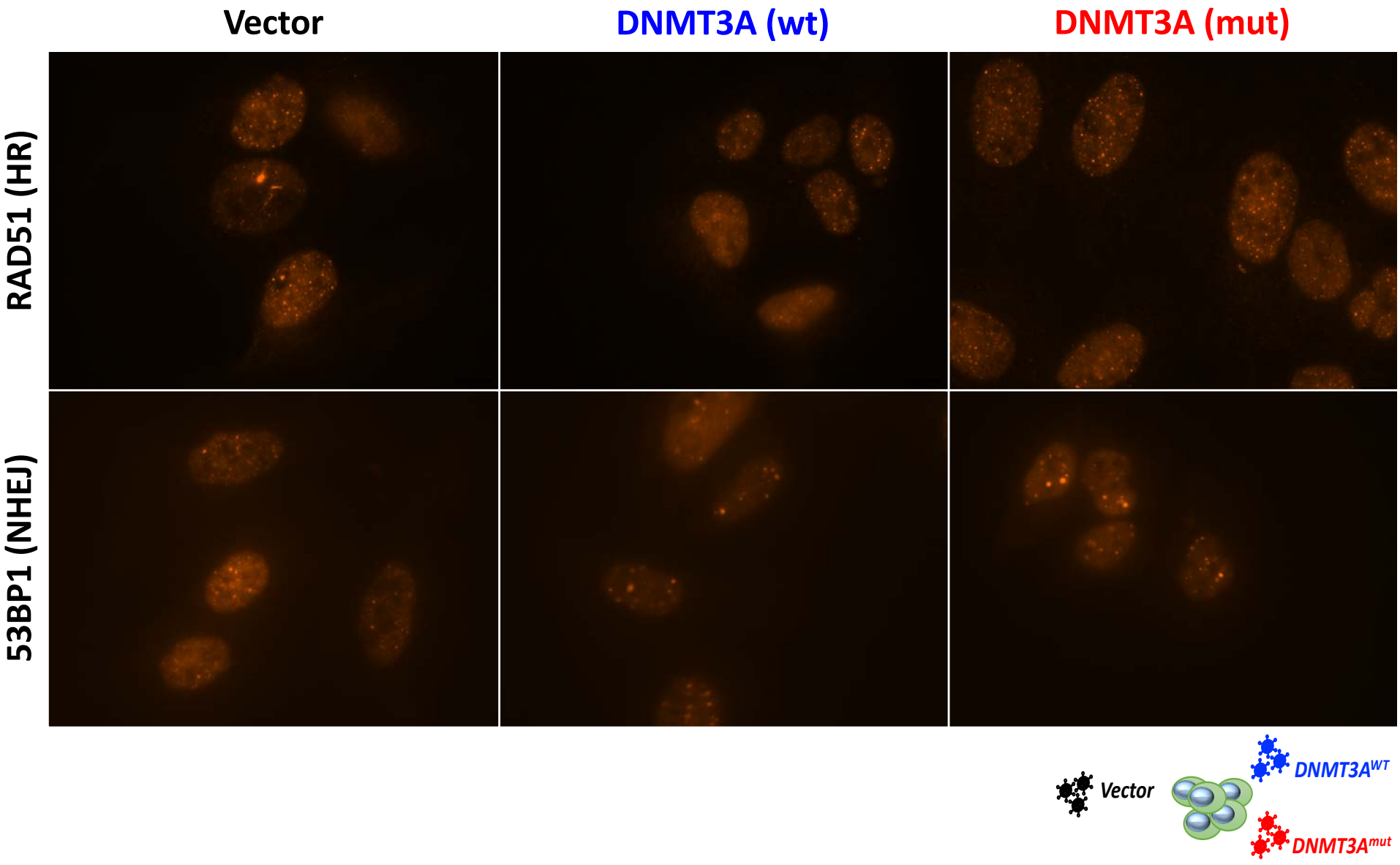




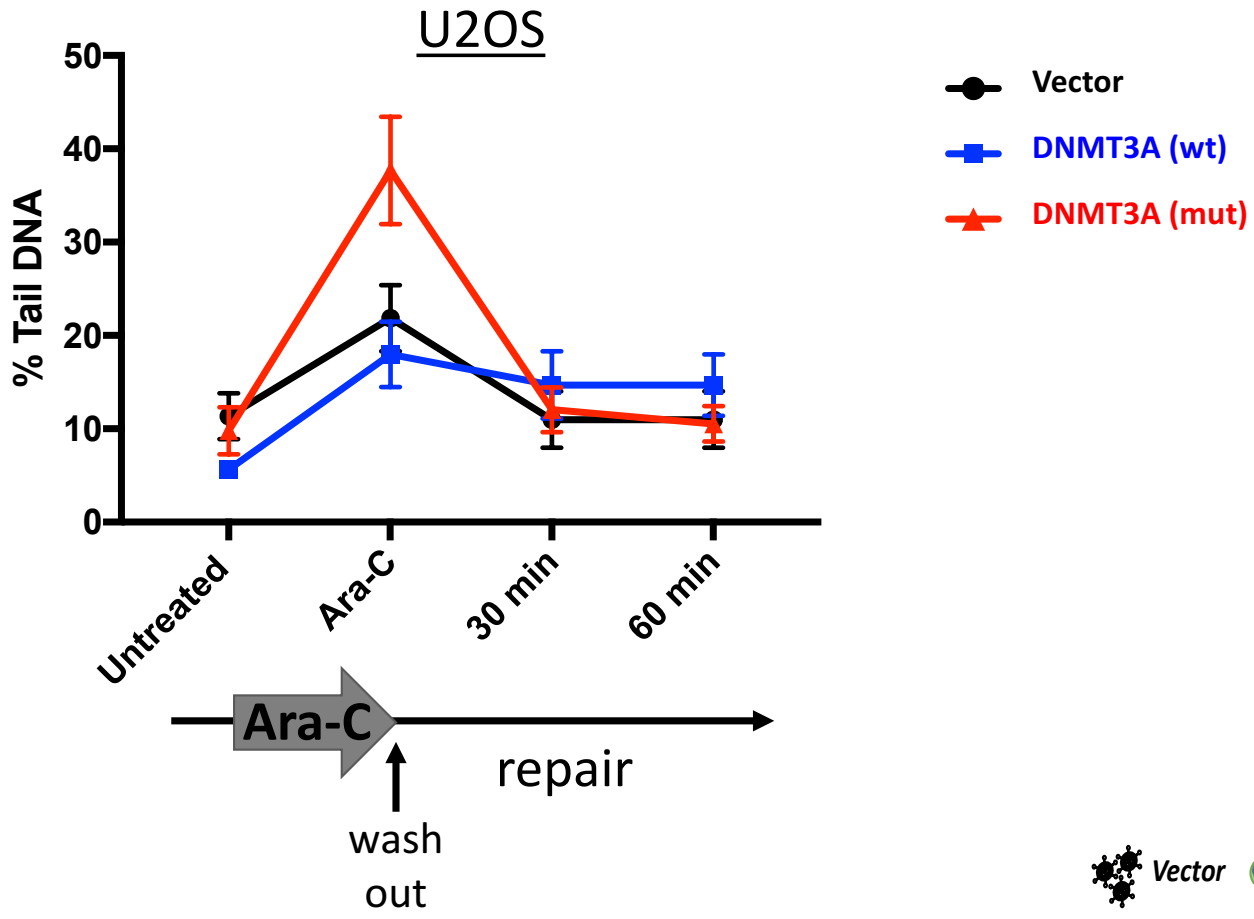
Why do cells with ***DNMT3A*** mutations accumulate DNA damage after Ara-C?

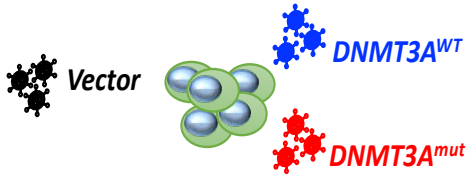


No defect in HR or NHEJ in cells with **DNMT3A** mutations treated with Ara-C

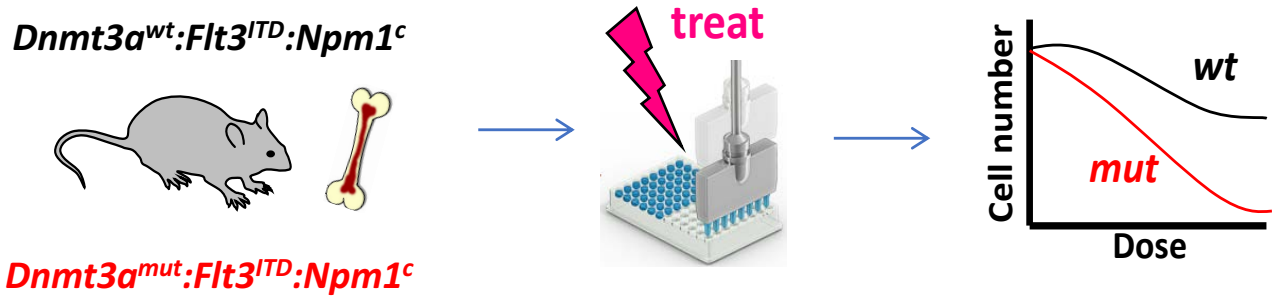


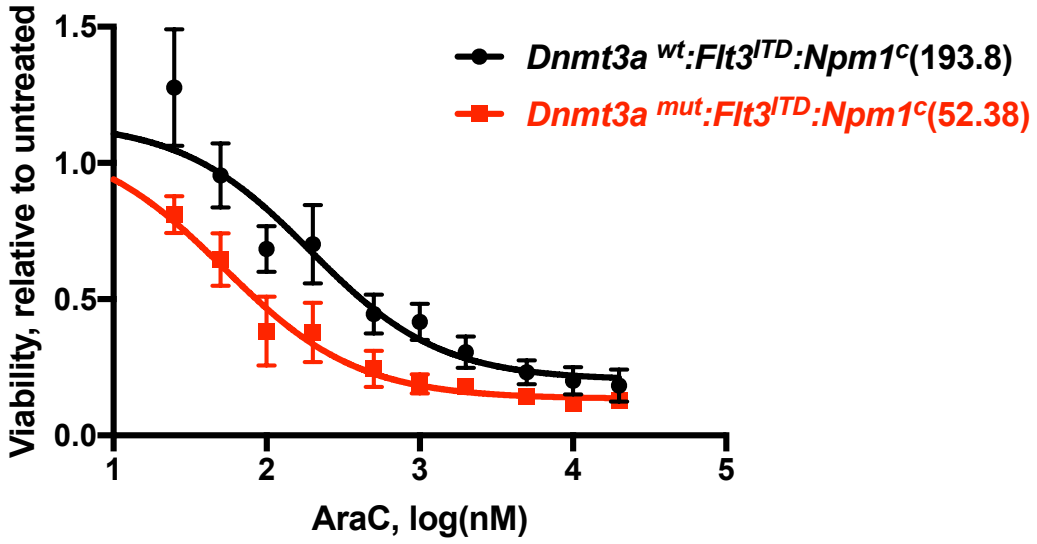
DNMT3A mutant cells efficiently resolve Ara-C induced DNA damage



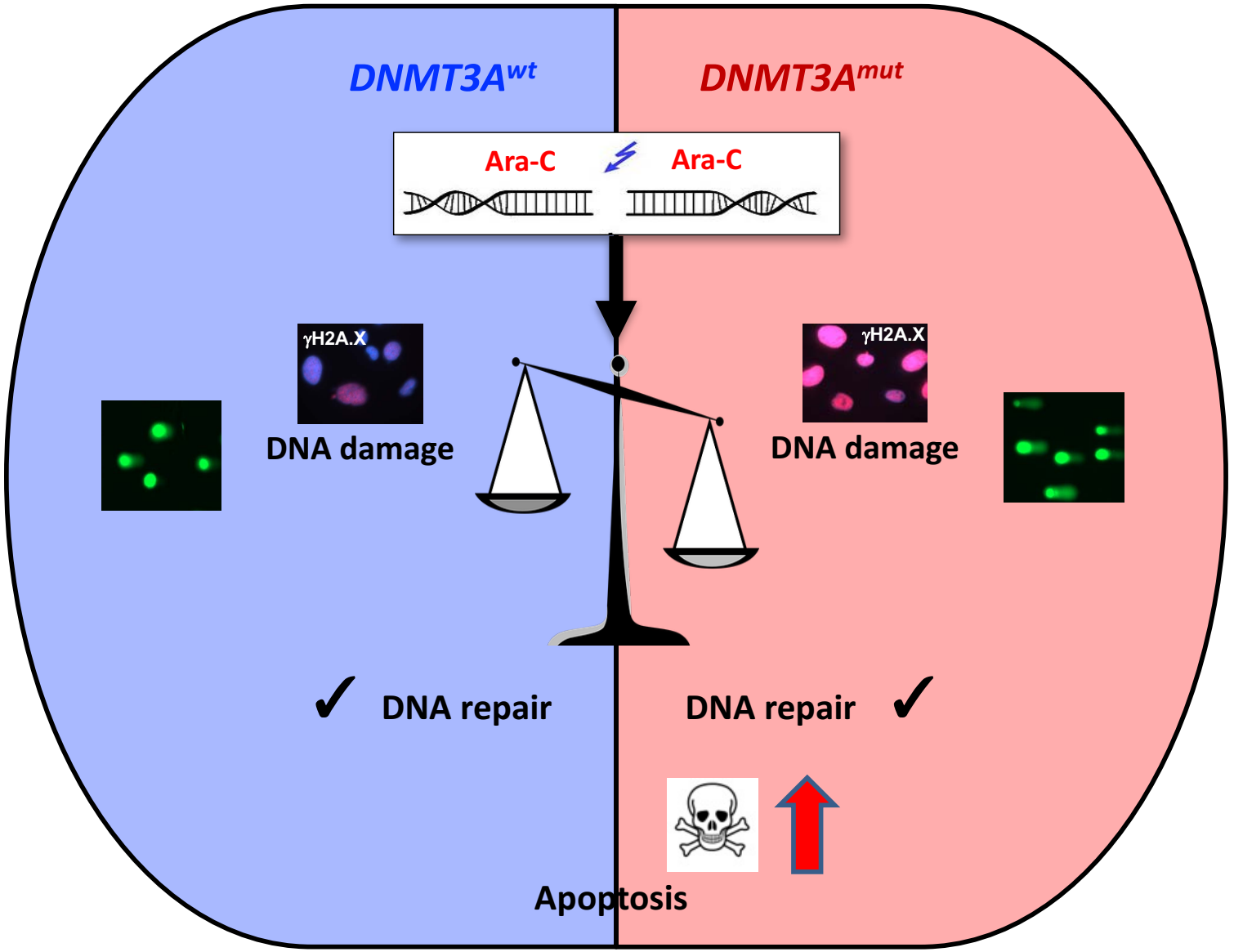


Mouse leukemia with a ***Dnmt3a*** mutation is more sensitive to cytarabine *ex vivo*





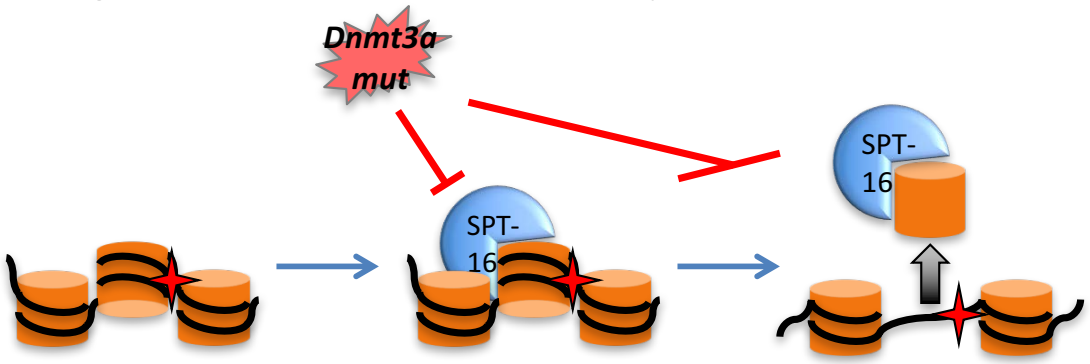
Summary



Continuous exposure = continuous intravenous infusion

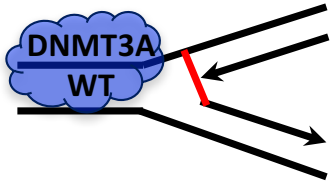
Ara-C sensitivity in *DNMT3A^{mut}* setting: Next steps

DNMT3A promotes recruitment of SPT-16 to DNA after torsional stress, through direct interaction; attenuated by DNMT3A mutations



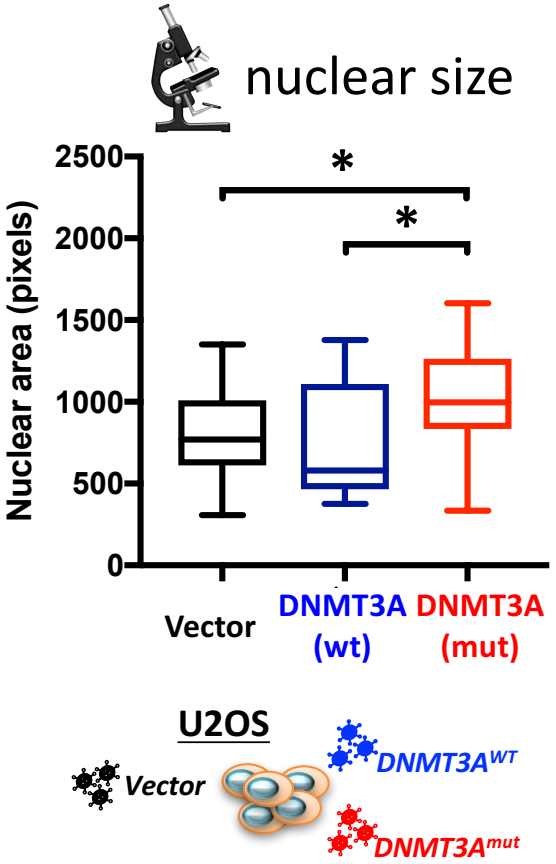
Guryanova *et al*. (2016) *Nat Med*

DNMT3A detected at stalled replication forks 30' after HU



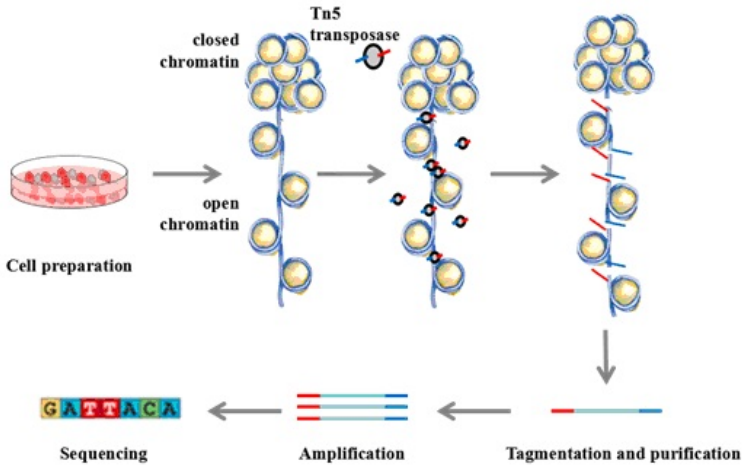
293T (HU)

Dungrawala *et al*. (2015) *Mol Cell*

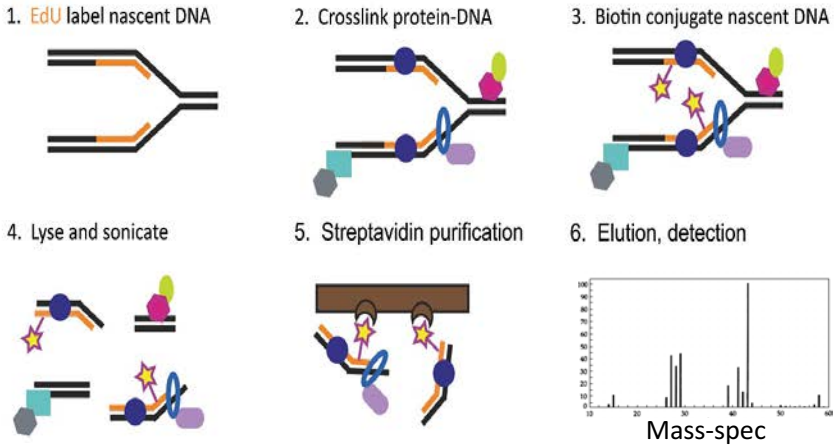


Ara-C sensitivity in ***DNMT3A^{mut}*** setting: Next steps

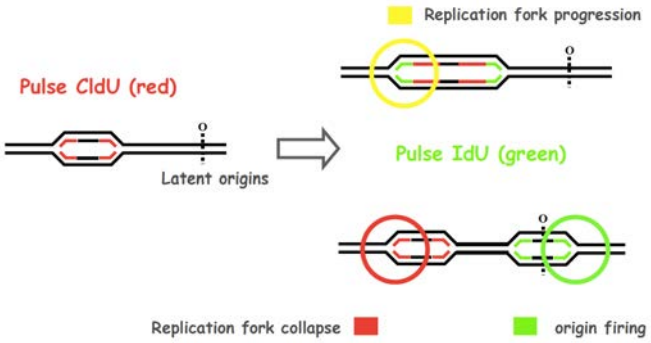
Chromatin accessibility – ATAC-seq



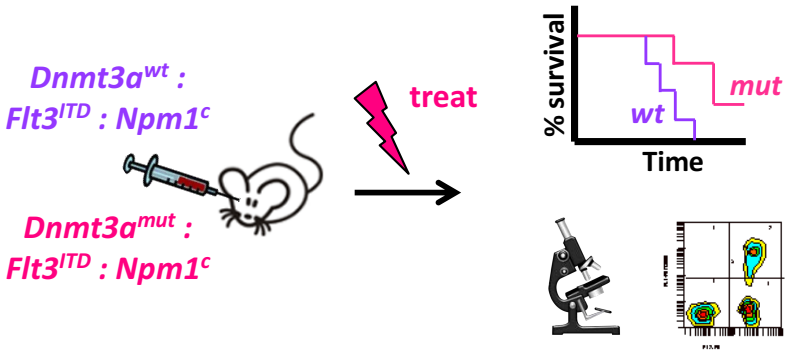
Proteome at replication forks – iPOND
(isolation of proteins on nascent DNA)



Replication fork dynamics – CldU/IdU labeling



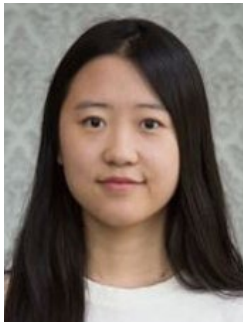
Differential sensitivity to cytarabine *in vivo*



Thank you!

Kartika Venugopal

Daniil Shabashvili



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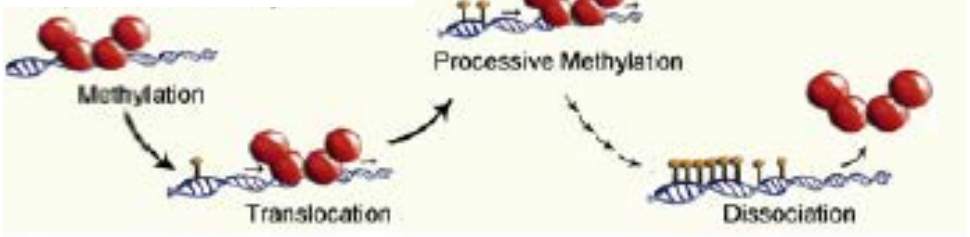
NIH National Institutes of Health
Turning Discovery Into Health

NIH/NCI K99/R00 Pathway to Independence Career Development Award

DNMT3A R882 mutations disrupt tetramerization and attenuate cooperative DNA binding

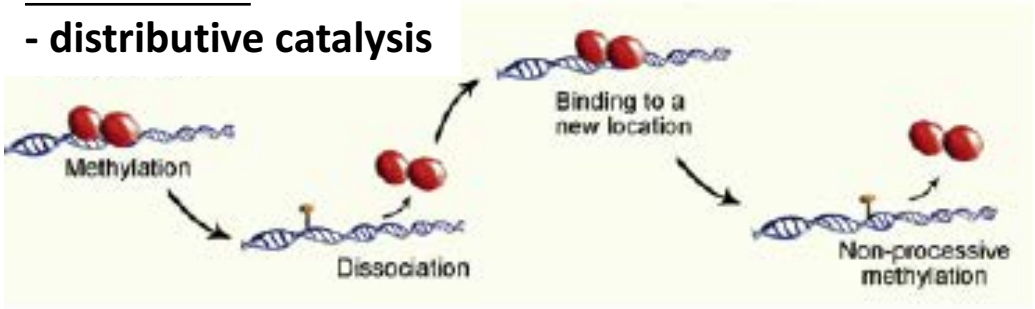
de novo DNA methyltransferase

Homotetramer
- processive catalysis



DNMT3A wt

Homodimer
- distributive catalysis



DNMT3A R882mut
↓processivity

Holz-Schietinger *et al*. 2012