Base excision repair protein dysregulation as a driver of genomic instability and cellular transformation

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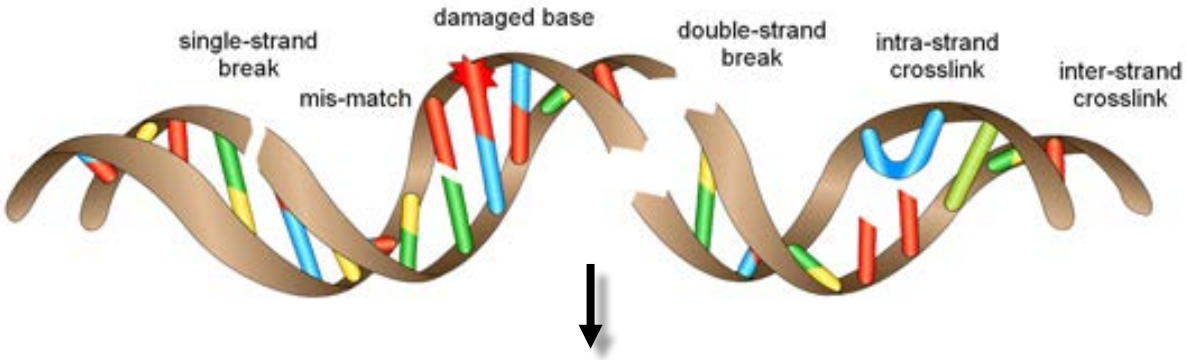
DNA Repair Interest Group

January 16, 2018

Multiple DNA repair pathways exist to handle various types of damage



DNA Repair

*Nucleotide Excision Repair (**NER**)*

*Non-homologous End Joining Pathways (**c-NHEJ**) and (**a-NHEJ**)*

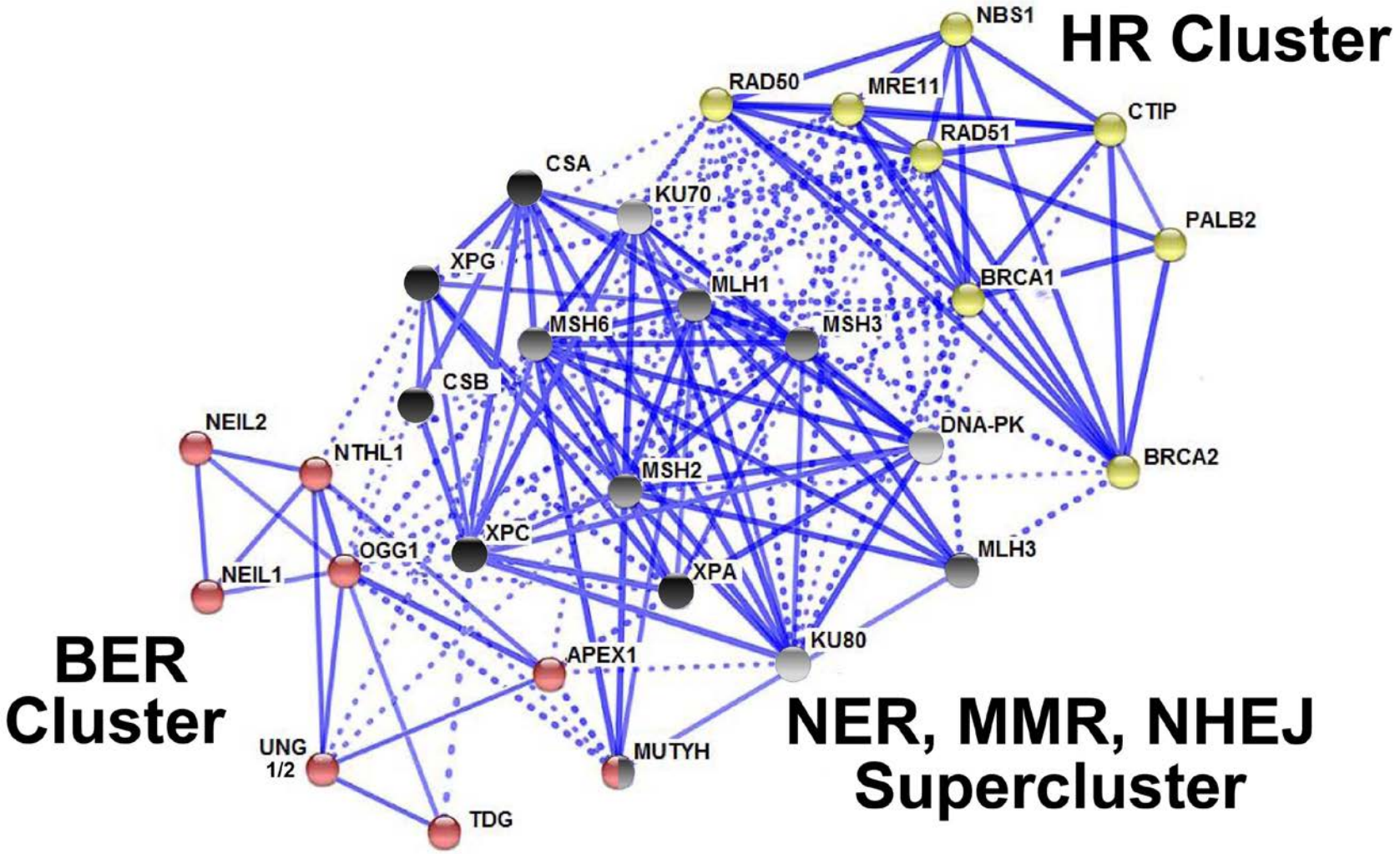
*Base Excision Repair (**BER**)*

*Homologous Recombination (**HR**)*

*Mismatch Repair (**MMR**)*

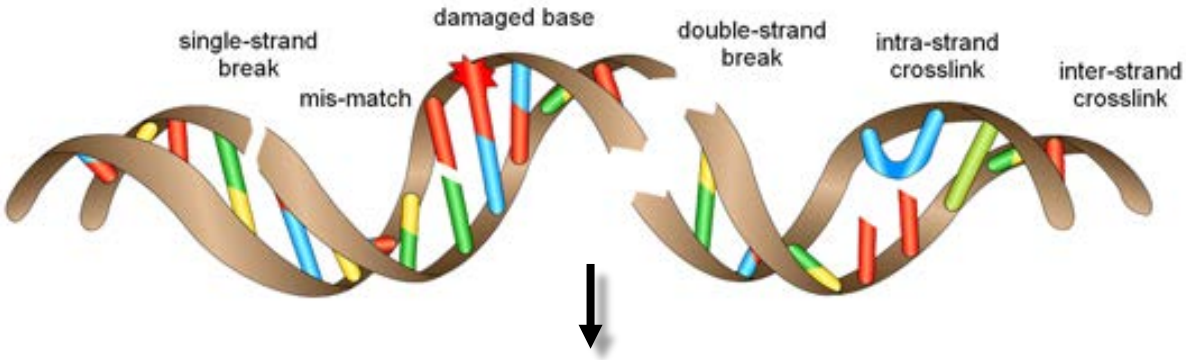
Direct Reversal

DNA repair pathway crosstalk
Regulation of DNA damage management?



Limpose, Corbett, and Doetsch. DNA Repair 2017

Multiple DNA repair pathways exist to handle various types of damage



DNA Repair

*Nucleotide Excision Repair (**NER**)*

Bulky base damage

*Non-homologous End Joining Pathways (**c-NHEJ**) and (**alt-NHEJ**)*

Double strand breaks DSBs

*Base Excision Repair (**BER**)*

Oxidation
Alkylation
Deamination
Uracil

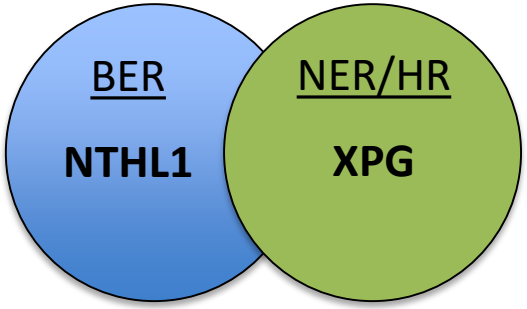
*Homologous Recombination (**HR**)*

Double strand breaks DSBs

BER protein pathway crosstalk examples

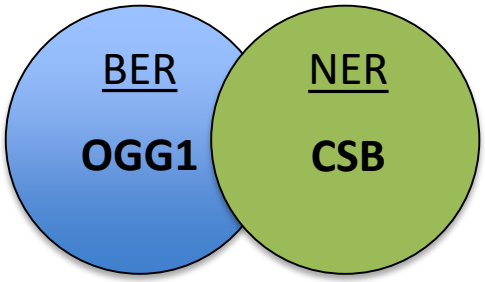
NTHL1 glycosylase

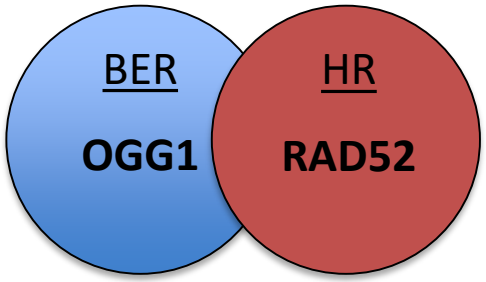
stimulates NTHL1 incision and turnover from DNA



OGG1 glycosylase

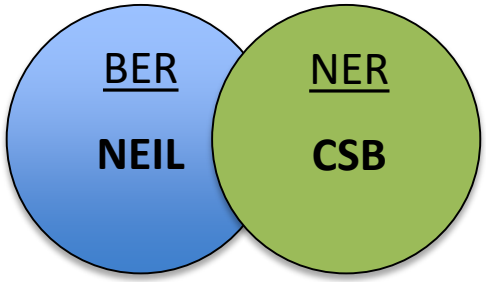
influences OGG1 binding and incision of substrate



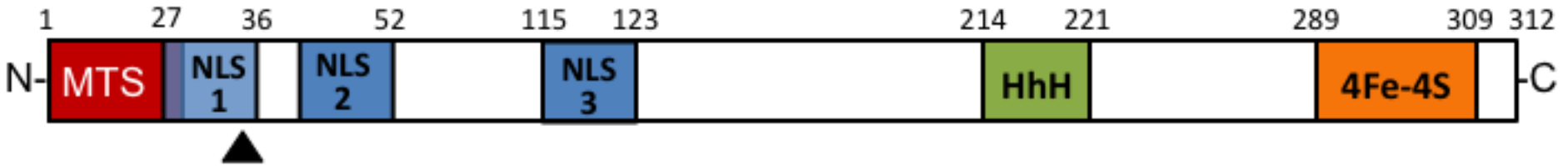


NEIL glycosylases

stimulates NEIL activity

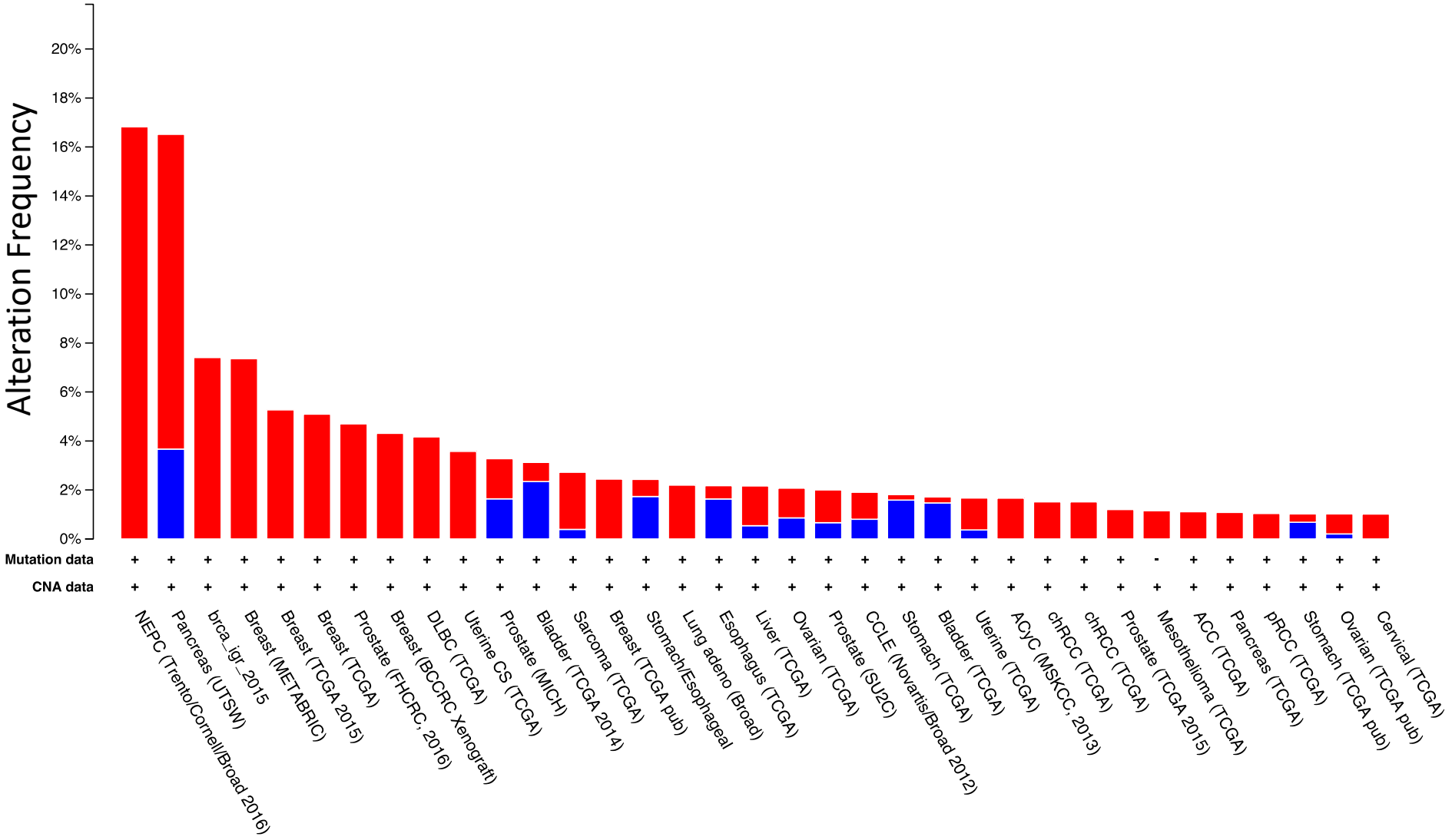


Endonuclease III-like glycosylase I (NTHL1)

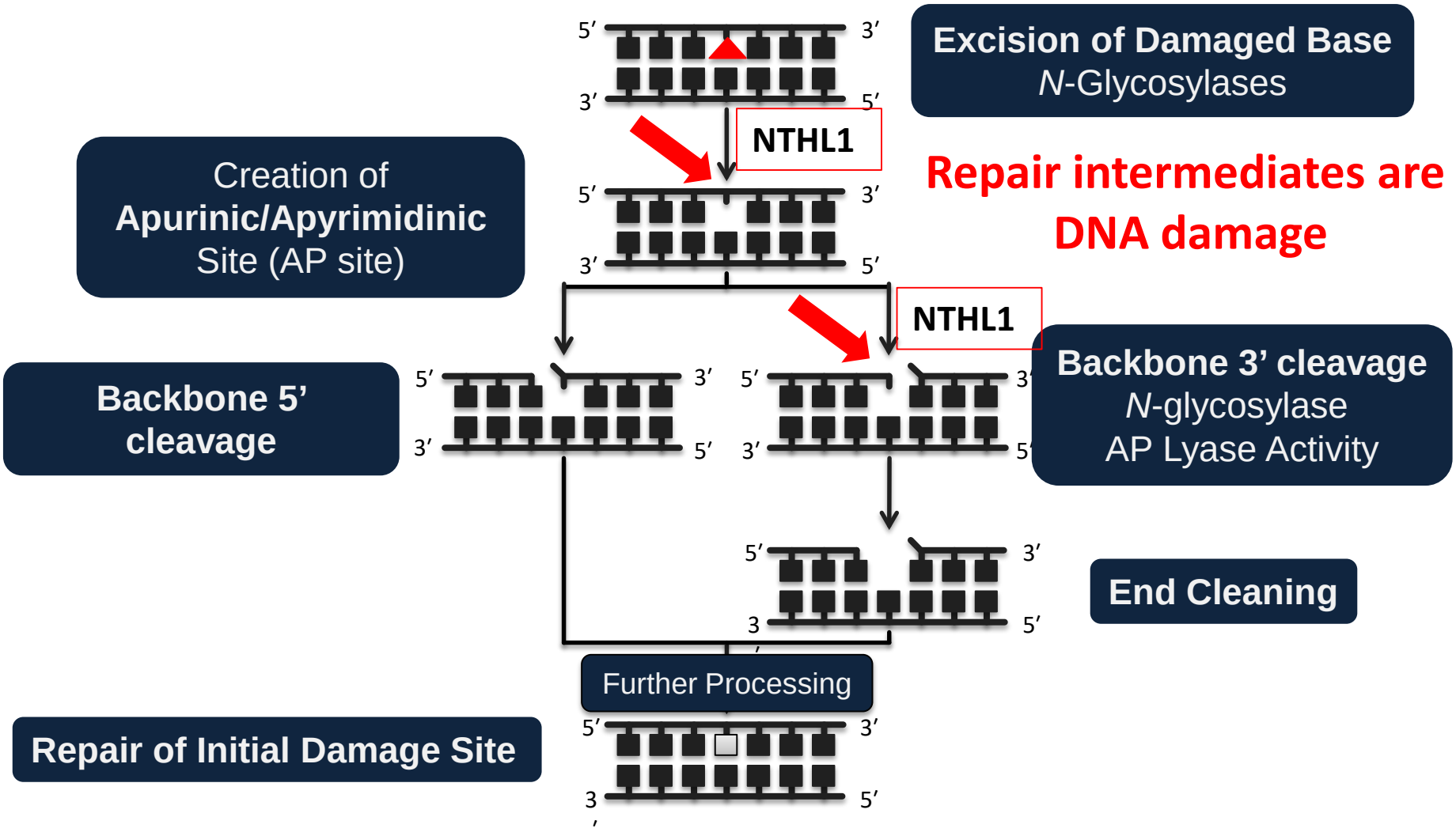


- Highly conserved from bacteria to humans
- Functions in both the nucleus and mitochondria
- Repairs a suite of oxidative DNA damage
  - Major substrate: Pyrimidine derivatives (cytosine and thymine)
  - Minor substrate: ring opened guanine
- Catalytic activity resides in the HhH motif at lysine 220
  - Lysine to glutamine (K220Q) mutagenesis abolishes glycosylase and AP lyase activity (Ikeda, Mitra et al. JBC, 1999)
- Germline *NTHL1* variant causes LOSS of NTHL1 protein
  - Patients are predisposed to colon cancer (Rivera et al. *NEJM*, 2015) (Weren et al. *Nat Gen* 2015)
  - loss of NTHL1 → accumulated DNA damage → mutations → cancer

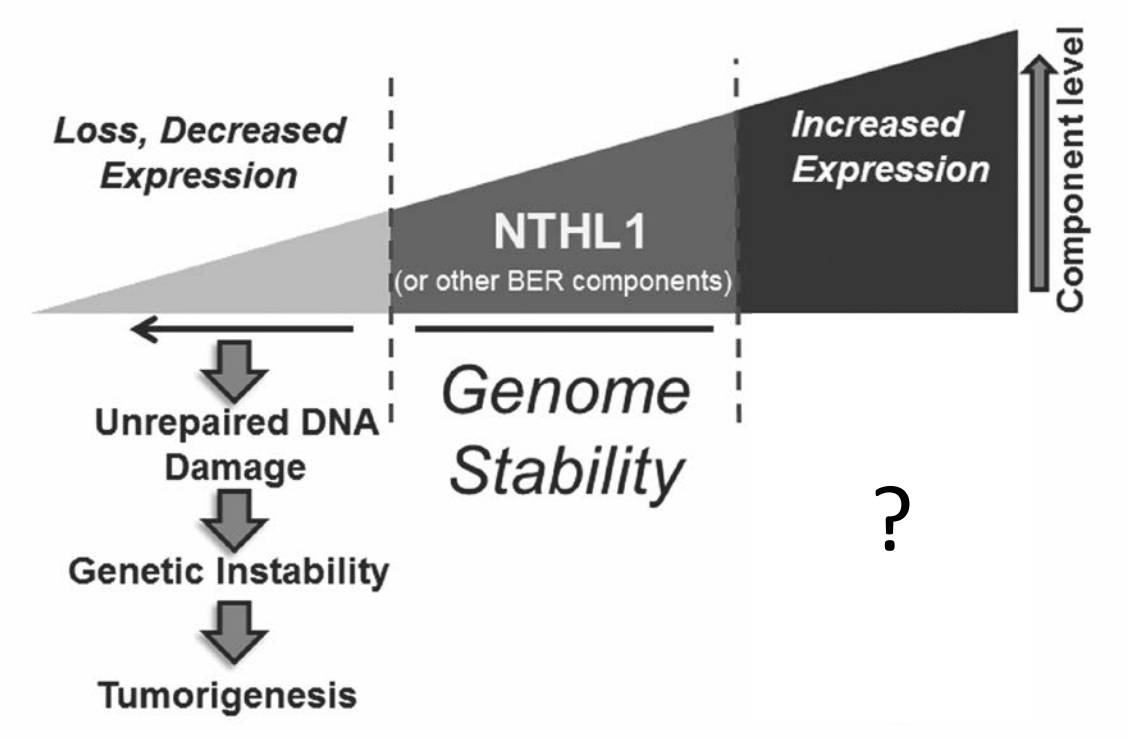
NTHL1 amplification (instead of deletion) is found in multiple cancer types



Base Excision Repair (BER)
Main system for repairing oxidative base damage



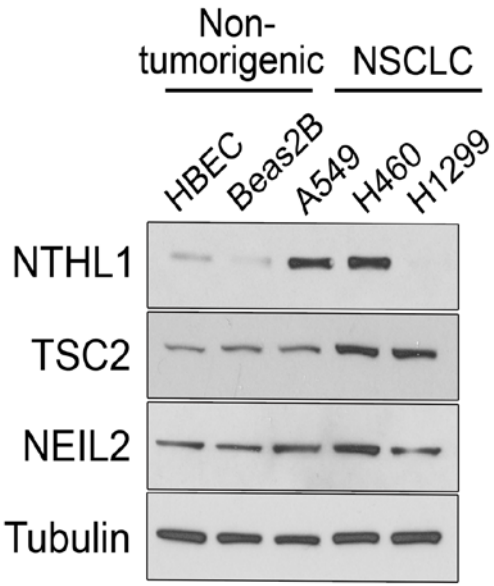
Hypothesis: *NTHL1 overexpression contributes an oncogenic advantage*

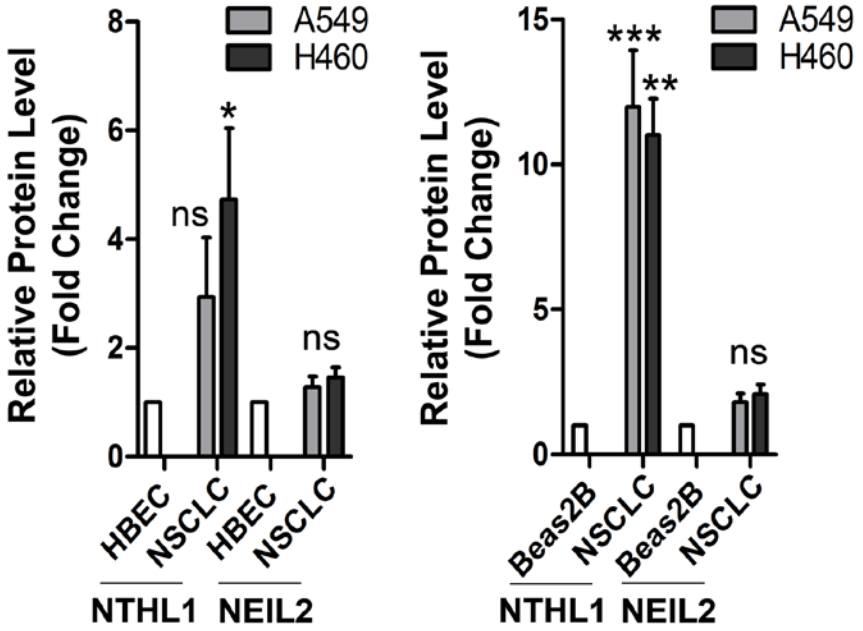


Kristin Limpose

NTHL1 protein levels are elevated in non-small cell lung cancer (NSCLC) cell lines

Non-Small Cell Lung Cancer (NSCLC)					
BER gene	% Amplified	% Loss	% Mutated	% mRNA upregulation	% mRNA downregulation
NTHL1	4.3	0	2.7	17	0
OGG1	1	1.5	2.7	11.6	1.2
NEIL1	0.9	0.5	3.3	10.2	0
NEIL2	1.6	22.3	2	20.9	9.1
NEIL3	0.4	11.3	13.7	26	0
APE1	7.8	0	1.3	33	0





Steady-state NTHL1 levels are increased in NSCLC cell lines

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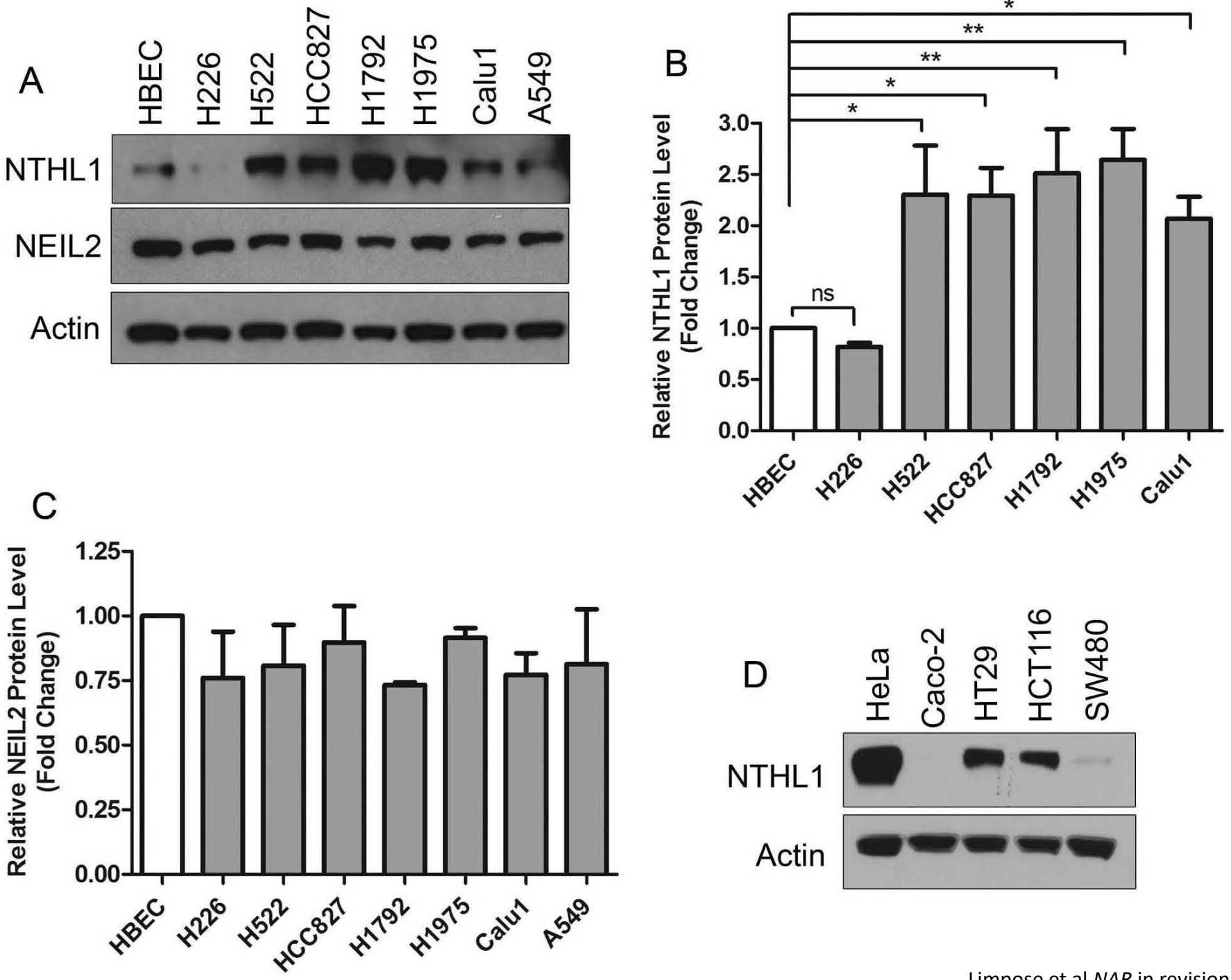
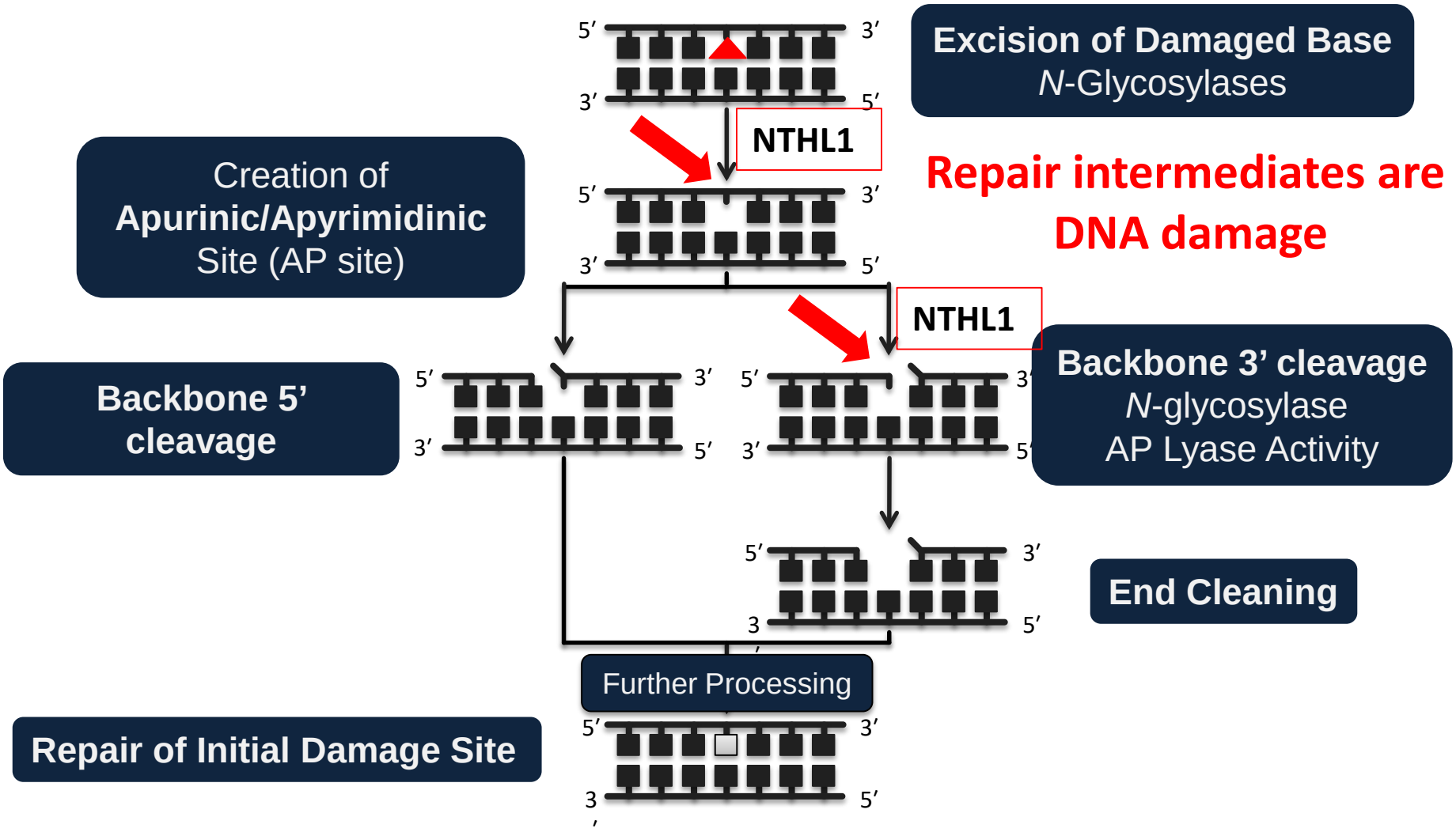


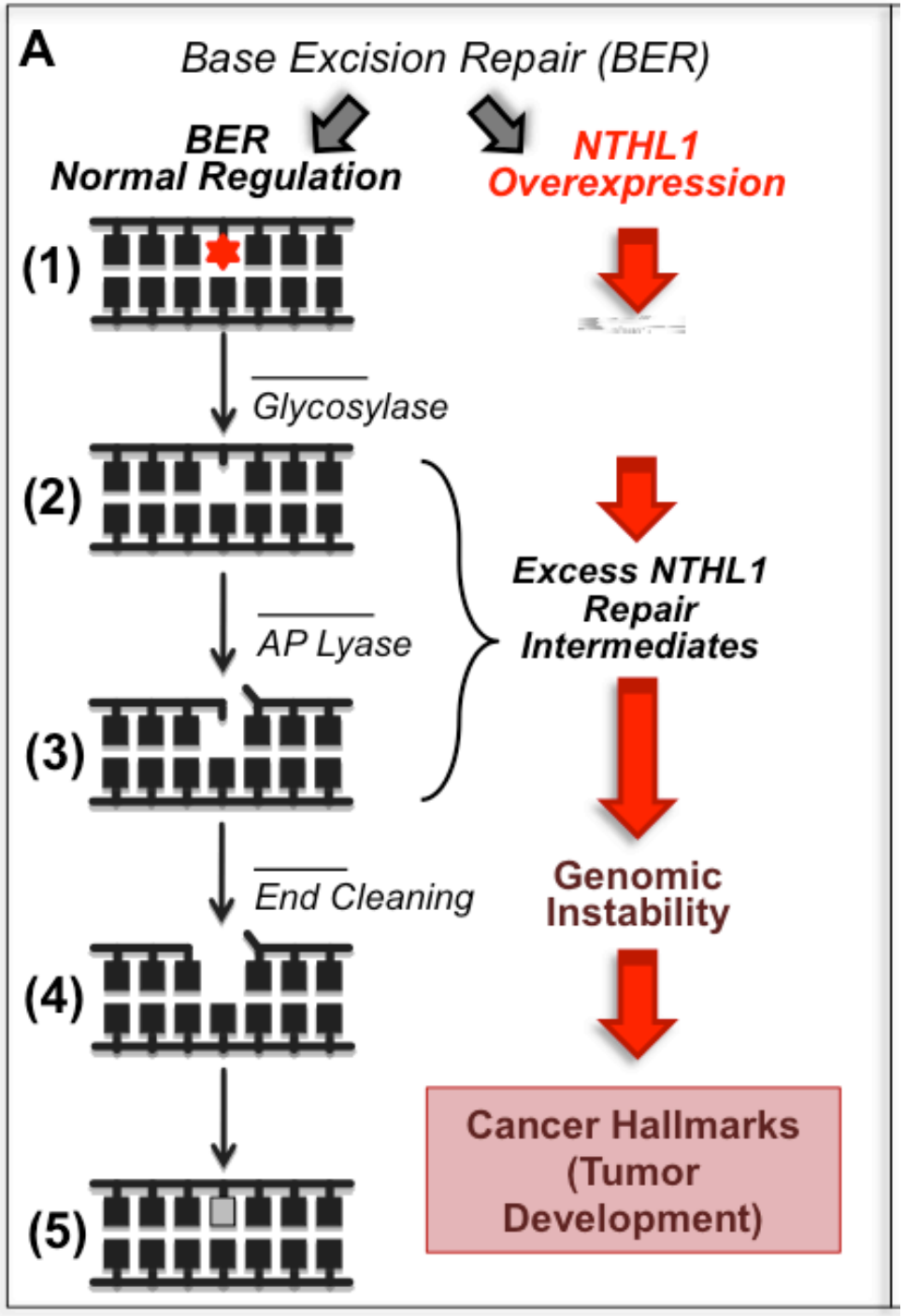
Limpose et al *NAR* in revision

Base Excision Repair (BER)
Main system for repairing oxidative base damage

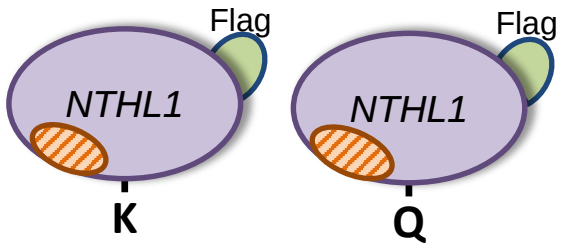


Initial predicted consequence of NTHL1 overexpression:

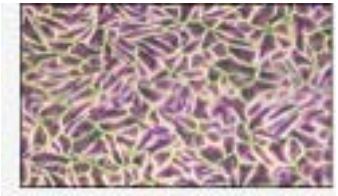
Mutagenic BER repair intermediates overwhelm the downstream BER repair processes resulting in genomic instability



An initial glimpse into the biology of BER protein overexpression: two versions of NTHL1 in non-cancerous human bronchial epithelial cells (HBEC-3KT)



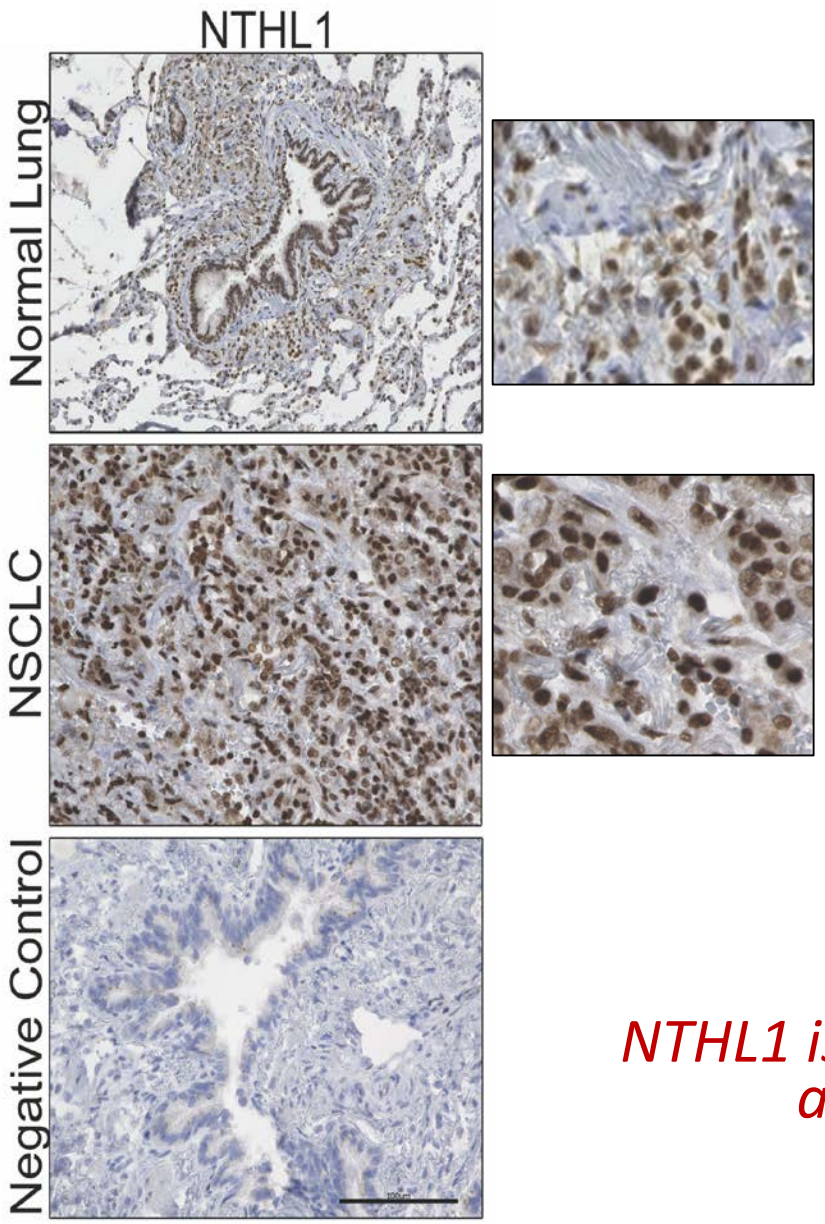
	WT	Cat_{mut}
Protein Impact	Wild Type	**No** catalytic activity
Functional Impact	?	?

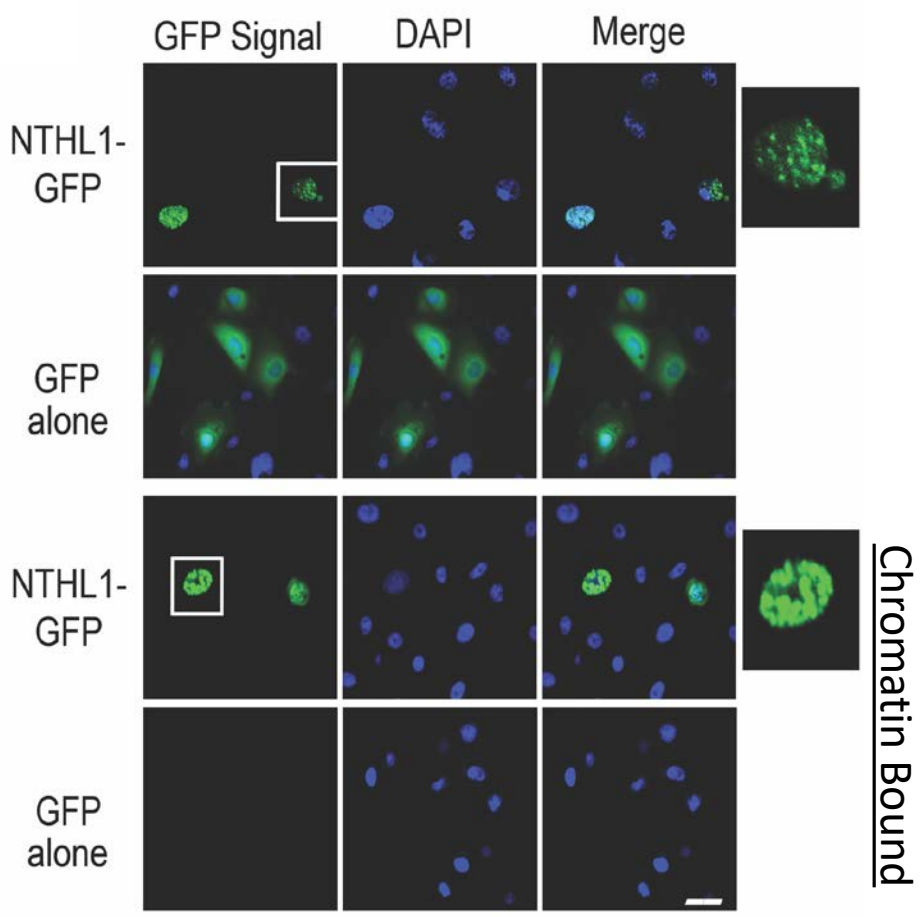


HBEC-3KT

Continuously replicating cell line via expression of *hTERT* and *Cdk4* (no colonies in soft agar or tumors In nude mice)

NTHL1 is primarily localized to the nucleus in both NSCLC and overexpressing HBEC cells





NTHL1 is not mislocalized upon overexpression and is associated with chromatin

IHC staining: Emory University Pathology Core

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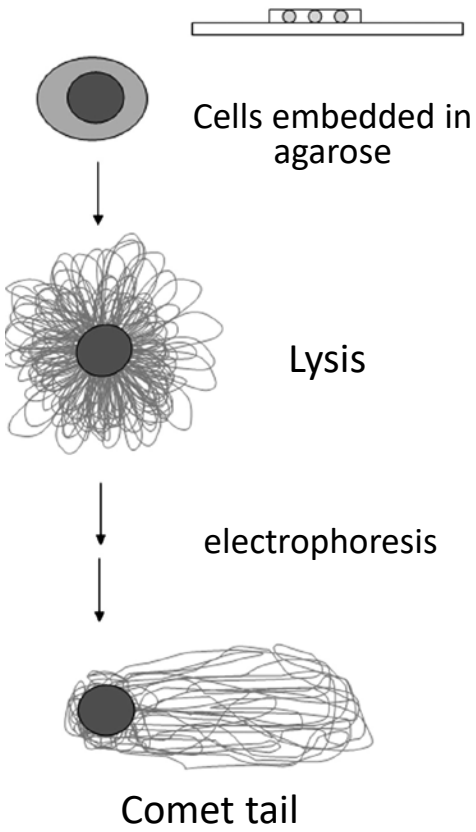
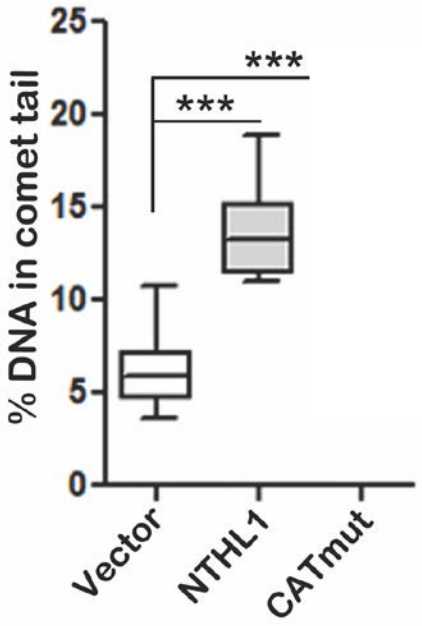
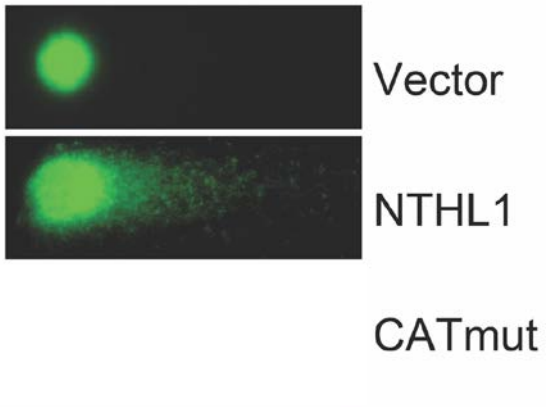
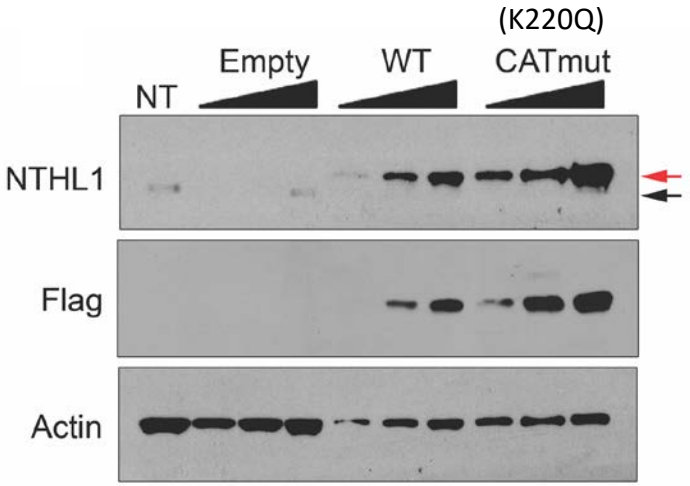
DNA damage accumulates upon overexpression of NTHL1 and CATmut









Comet Assay: Erica Werner

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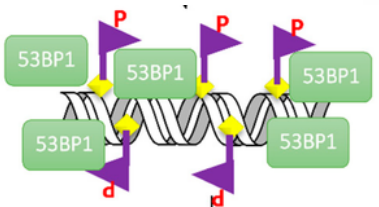
DSBs accumulate upon overexpression of NTHL1 and CATmut



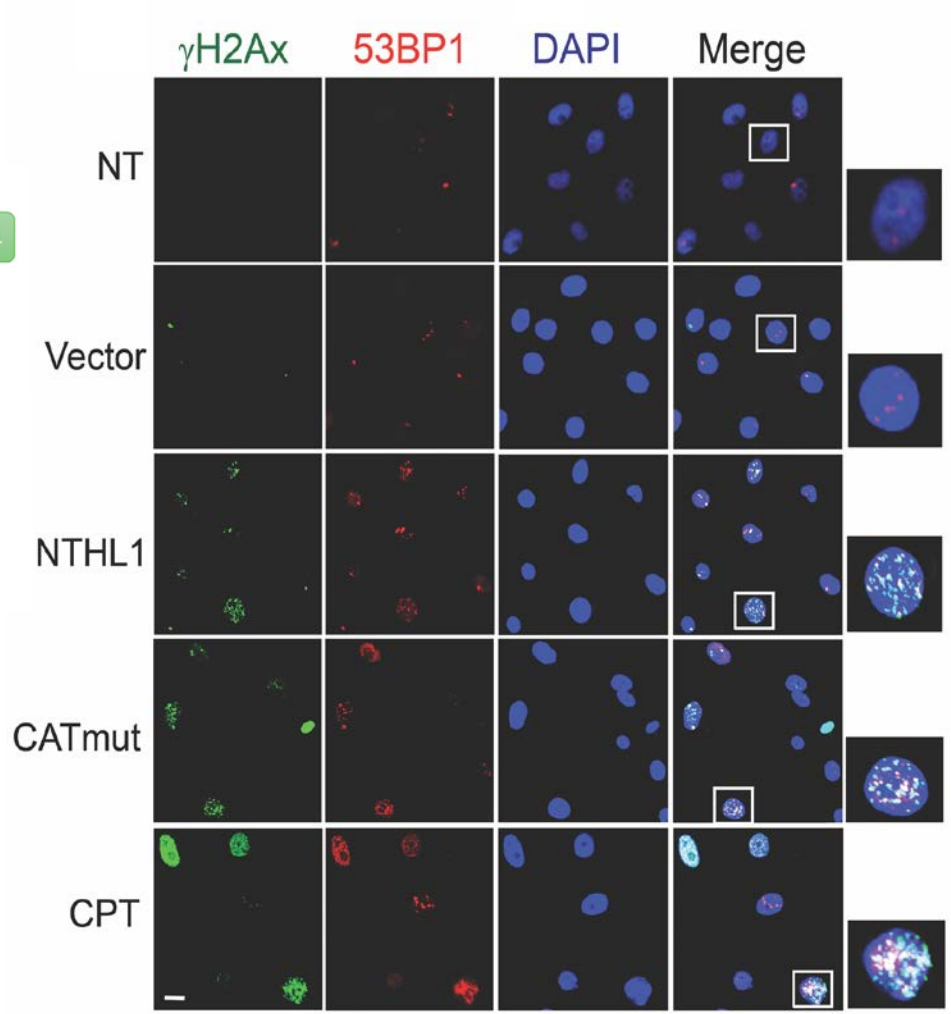
Repair

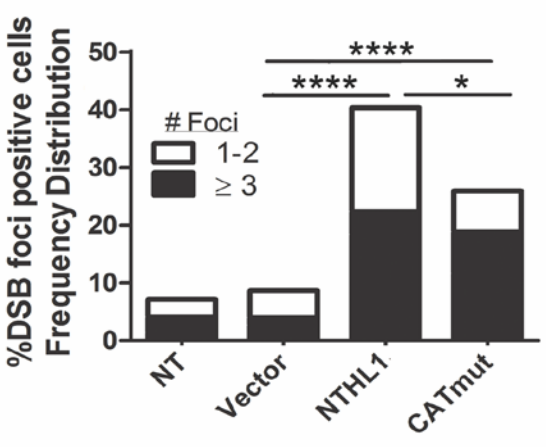
Cell survival

DNA damage site

H2AX marks

phosphorylated H2AX





Elevated NTHL1 levels increase the cellular load of DNA damage in a manner that does not depend on NTHL1 enzymatic activity (greater effect seen with enzymatically active NTHL1)

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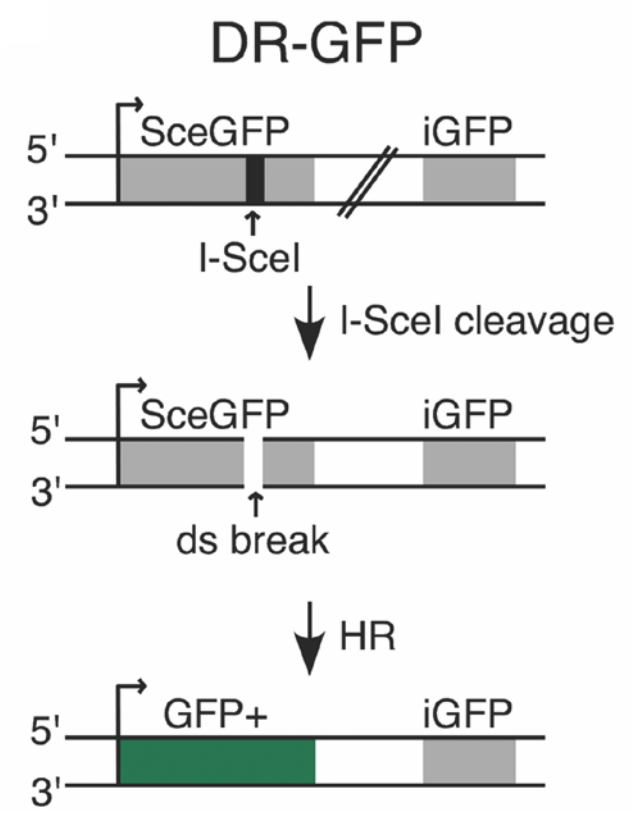
DSBs accumulate upon overexpression of NTHL1 and CATmut

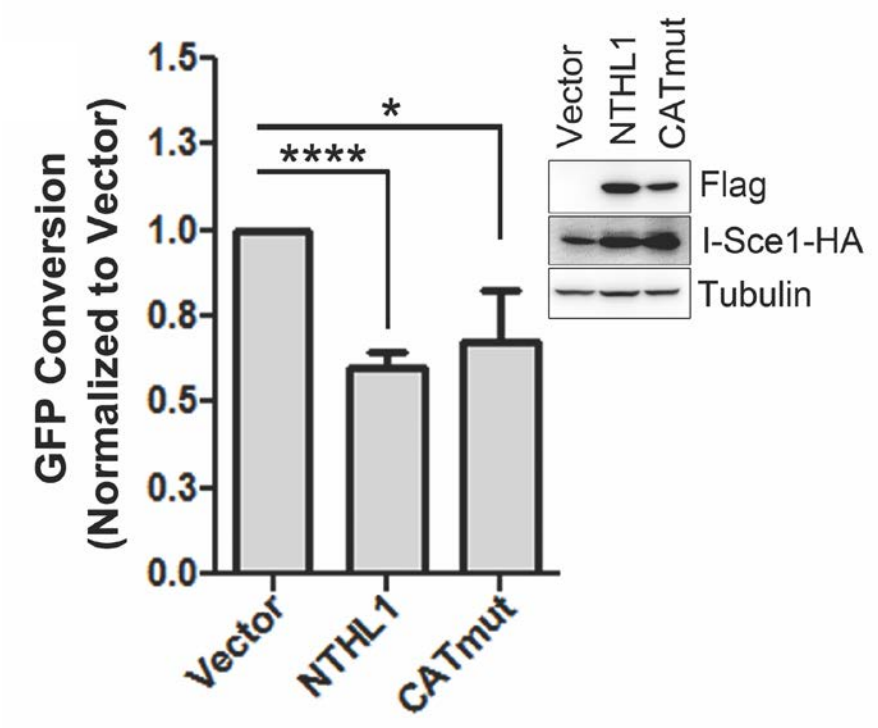
Is this caused by direct induction of DSBs or inhibition of DSB repair (HR or NHEJ)?

<u>HR measurement</u>: Gene conversion in direct repeat GFP (DR-GFP) reporter construct integrated into the DR-U2OS cell line (Pierce et al Genes Dev. (1999)) – Interrogates HR after I-SceI-induced DSB in GFP – Cleaved GFP repaired by second, transcriptionally inactive GFP – Results in recovery of GFP fluorescence.

DSB repair (HR) is compromised by overexpression of NTHL1 and CATmut

DSB Repair Assay





Increased NTHL1 or CATmut protein inhibits HR repair of DSBs in a manner that does not depend on NTHL1 enzymatic activity (~50% decrease in HR)

DR-GFP assay: Kelly Trego
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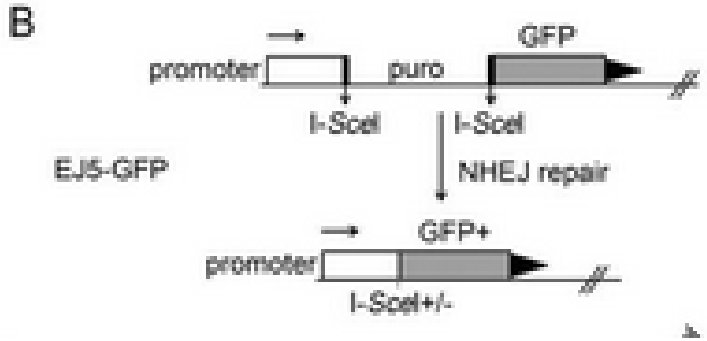
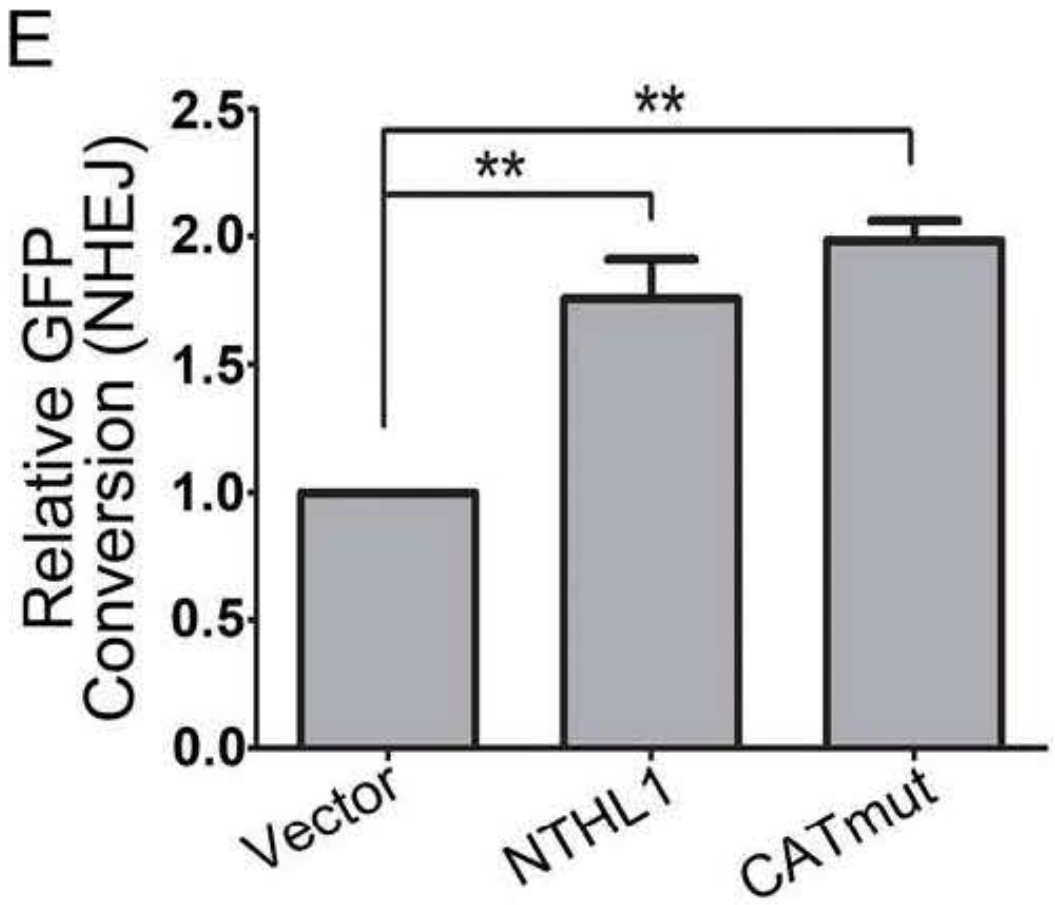
<u>NHEJ measurement</u>: End joining employing reporter in DsR-7F4 cells (Li et al. Radiat. Res (2013)) – Generate DSB flanking puromycin cassette – separates transcriptional promoter and promoterless GFP gene – I-SceI-induced DSB yields proximal and distal ends – when re-ligated via EJ restores transcriptionally active GFP gene.



NHEJ is moderately elevated by overexpression of NTHL1 and CATmut



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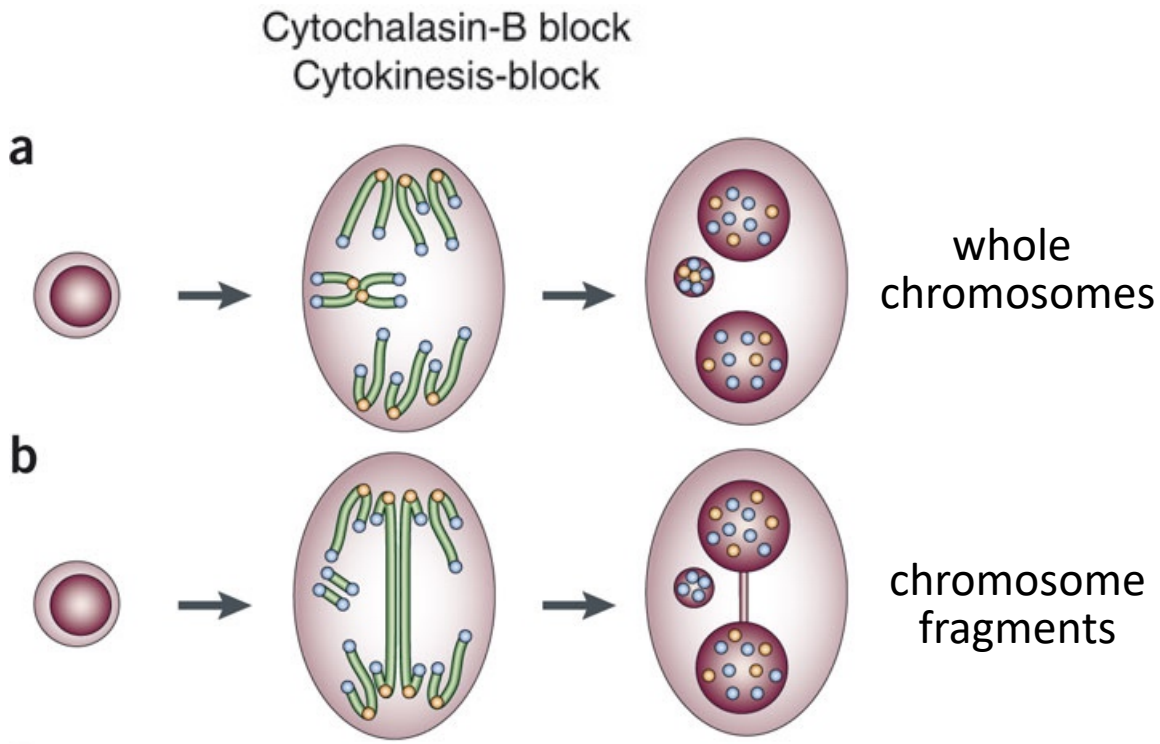
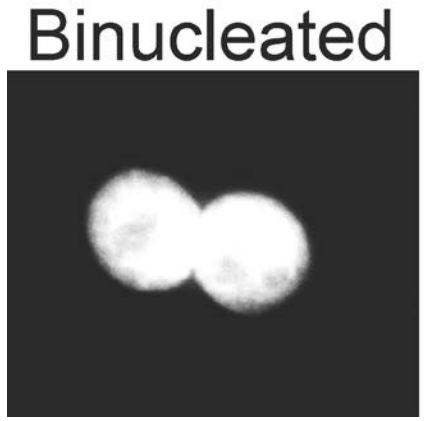
Results suggest : Increased NTHL1 or CATmut protein inhibits HR-mediated repair of DSBs

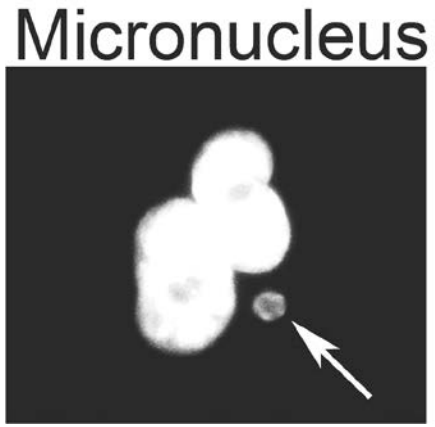
Micronucleus assay: A gold standard for measuring genomic instability



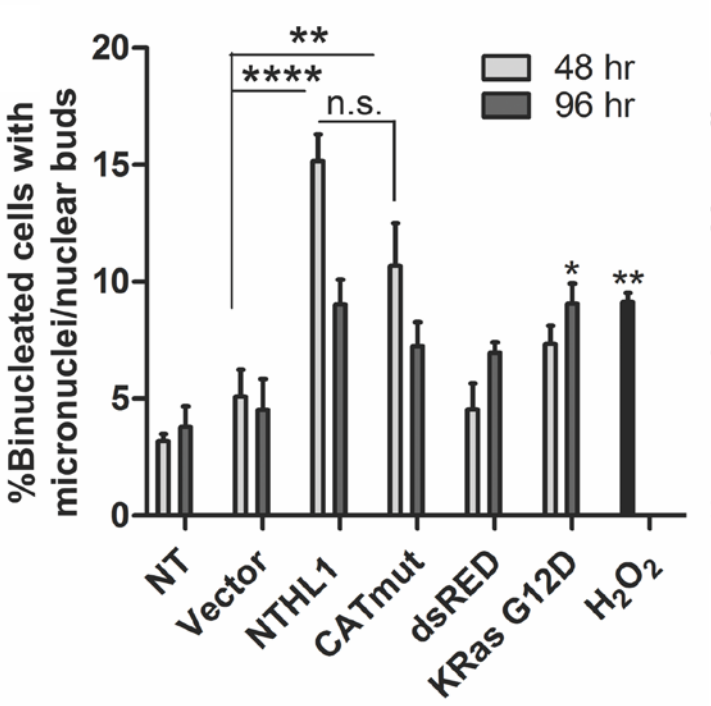
whole chromosomes

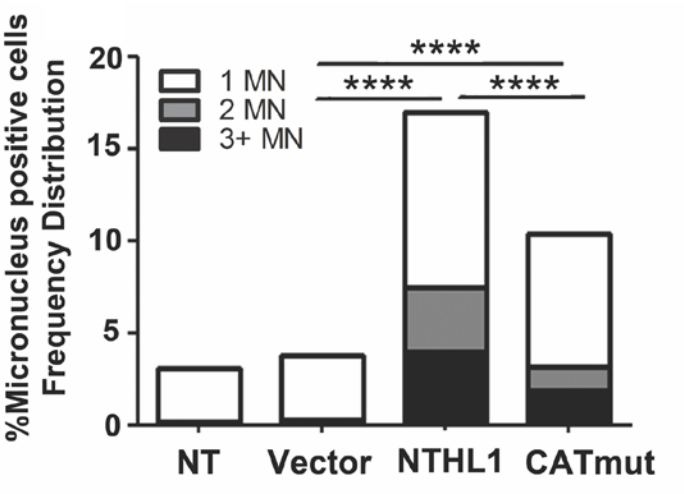
chromosome fragments

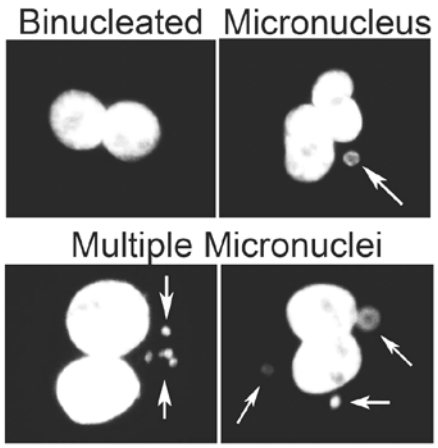




Genomic instability is induced by transient NTHL1 and CATmut overexpression







Genomic instability is present at 48 hours following overexpression

NTHL1 and CATmut overexpression results in multiple micronuclei

Occurs independent of NTHL1 enzymatic activity (greater effect seen with enzymatically active NTHL1)

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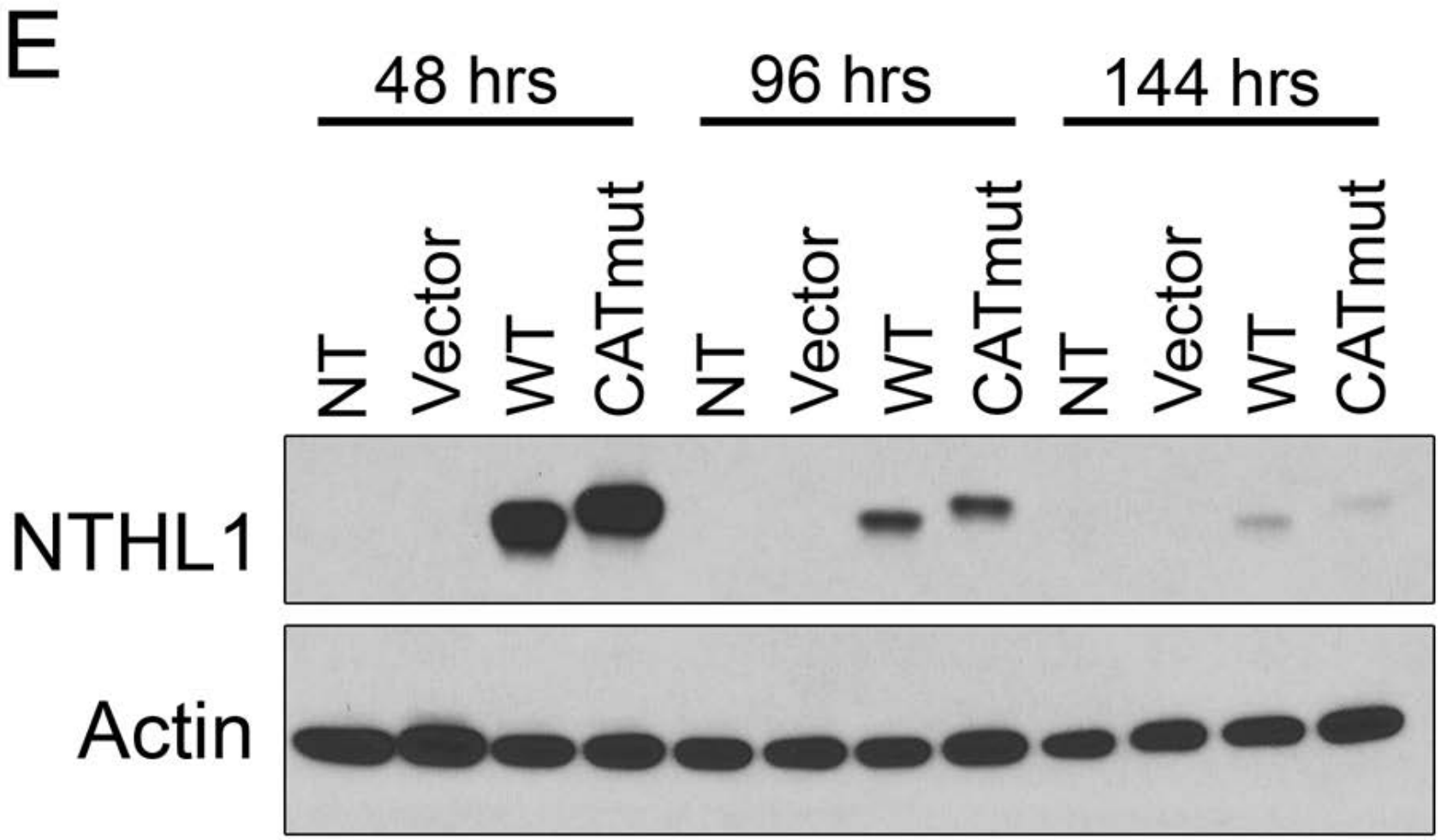
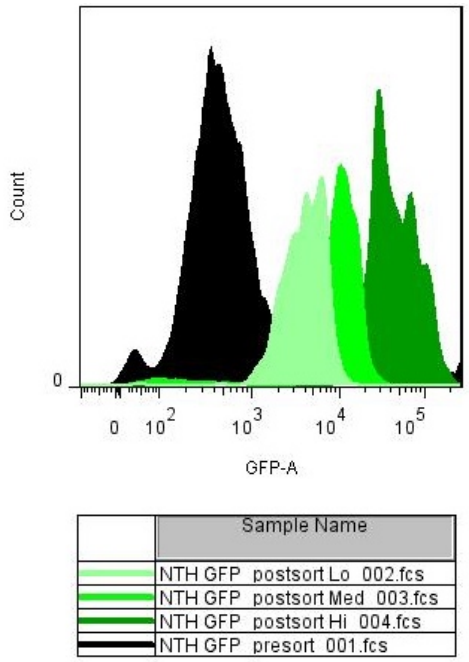
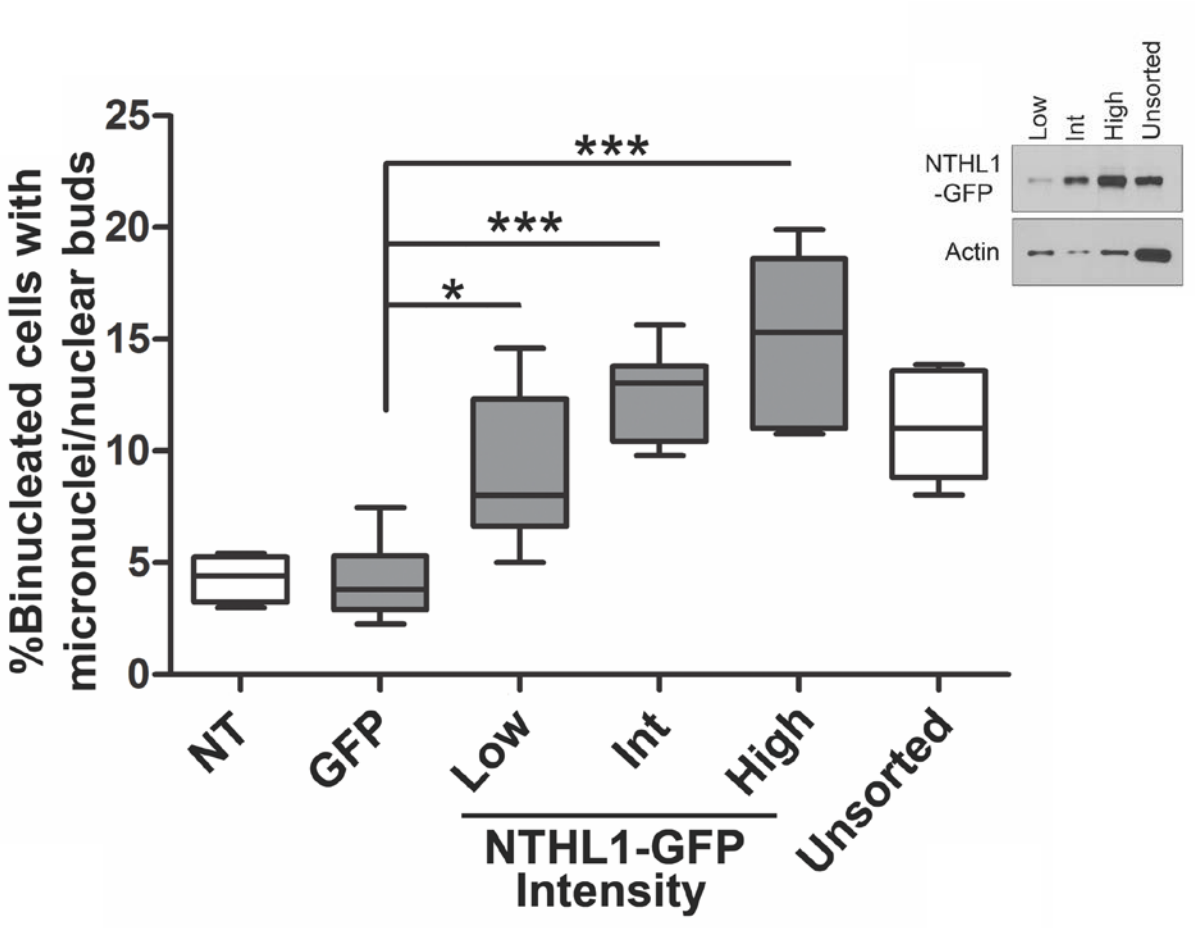

E
48 hrs
96 hrs
144 hrs
NT
Vector
WT
CATmut
NT
Vector
WT
CATmut
NT
Vector
WT
CATmut
NTHL1
Actin

Genomic instability is proportional to the level of NTHL1 overexpression





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Does replication stress contribute to the observed cellular phenotypes?

Approach: immunoblot for known replication stress signaling proteins.

ATR phosphorylation (pATR T19898)

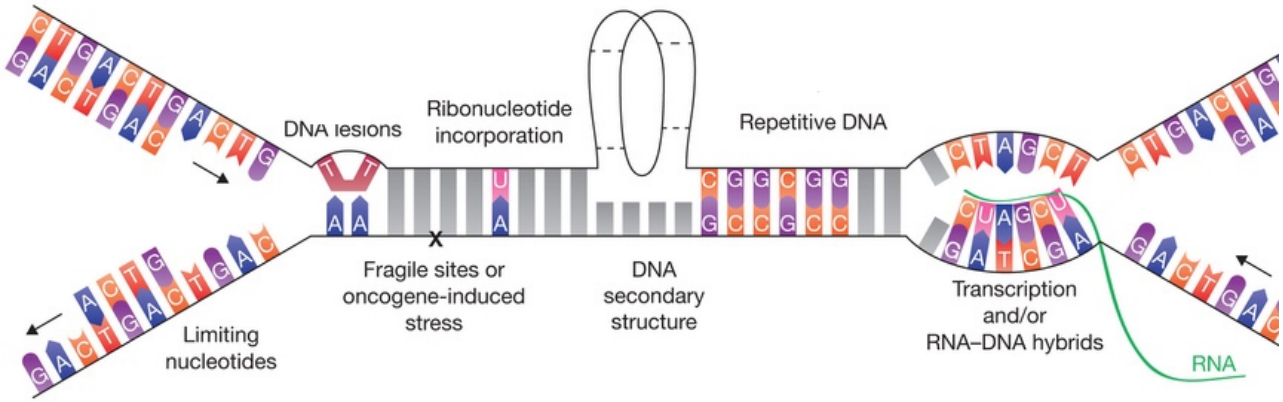
Chk1 phosphorylation – intra-S phase checkpt (S317)

Replication fork collapse -> DSBs -> ATM activation

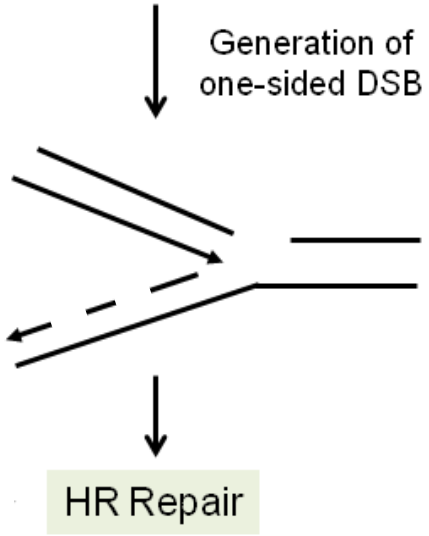
-> phosphorylation of downstream effectors -> Chk2 (pChk2 T68)

-> phosphorylation of RPA (pRPA S4/S8)

DSBs can occur during each replication cycle



Fork collapse and double strand break (DSB) formation



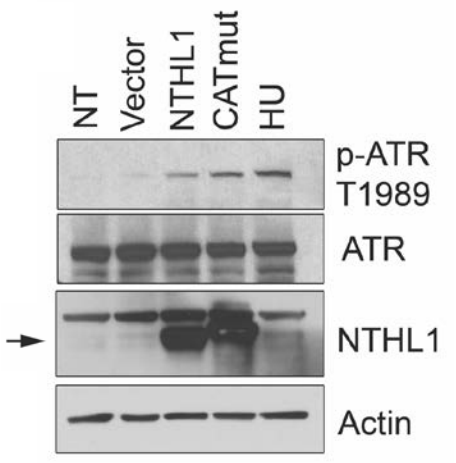
Markers

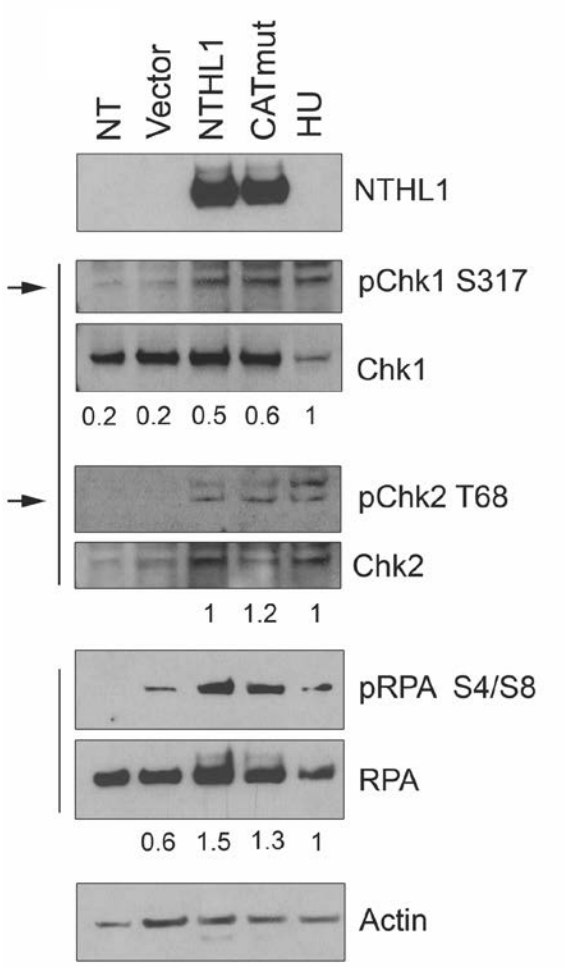
Replication stress:
pATR
pChk1

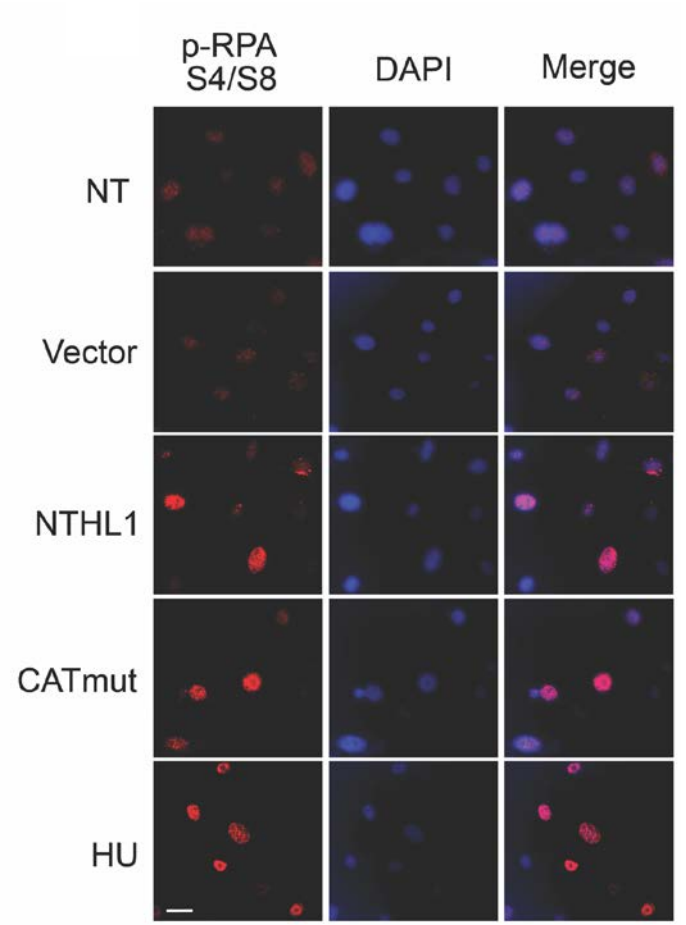
DSB signaling:
yH2Ax
53BP1
pChk2

HR signaling:
pRPA S4/S8

Replication stress signaling assessment following NTHL1 and CATmut overexpression







Replication stress:
pATR
pChk1

DSB signaling:
yH2Ax
53BP1
pChk2

HR signaling:
pRPA S4/S8

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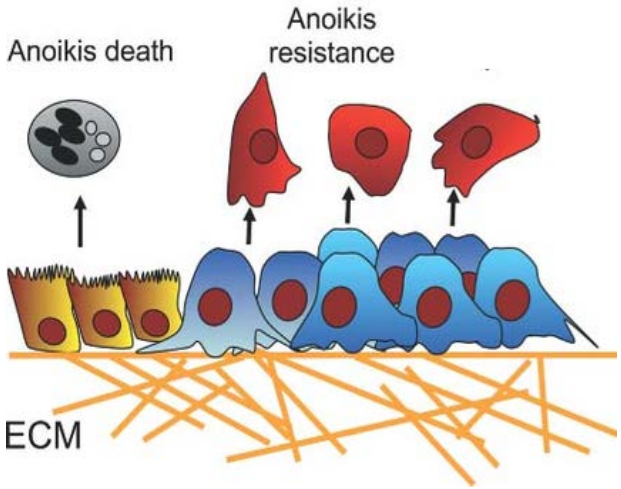
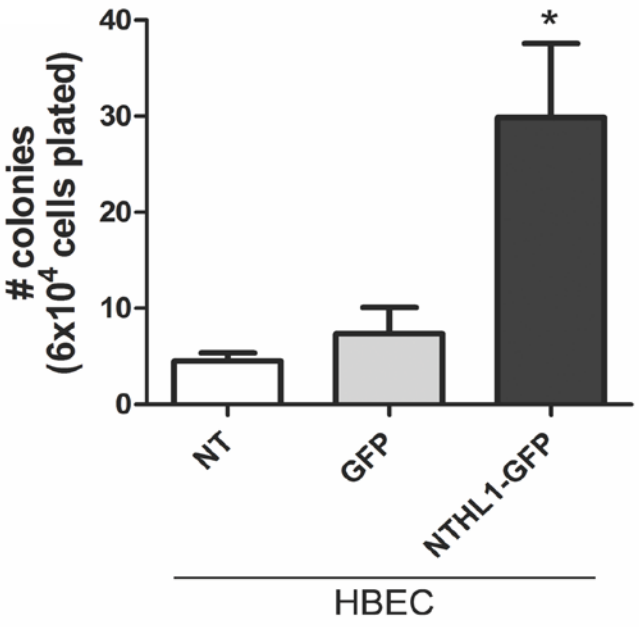
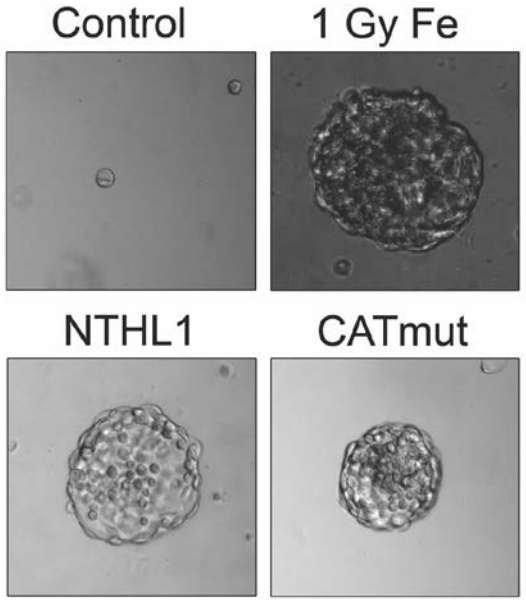
Conclusion:

NTHL1 and CATmut overexpression induce replication stress signaling

Early cancer hallmarks are conferred by NTHL1 and CATmut overexpression







HBEC cells overexpressing NTHL1 and CATmut form colonies in soft agar

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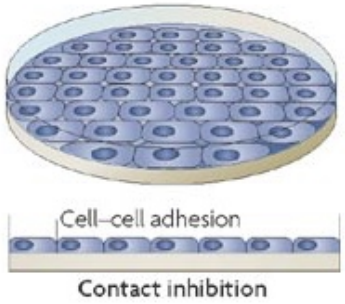
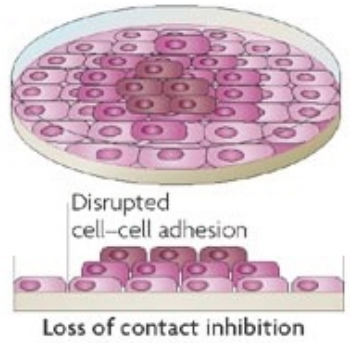
Early markers of cellular transformation are conferred by NTHL1 and CATmut overexpression

Normal cells



Transformed cells



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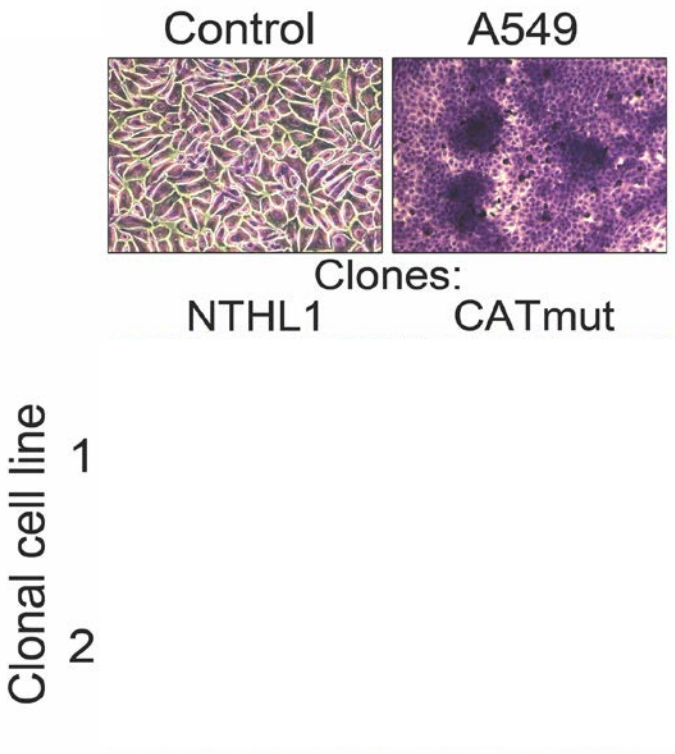

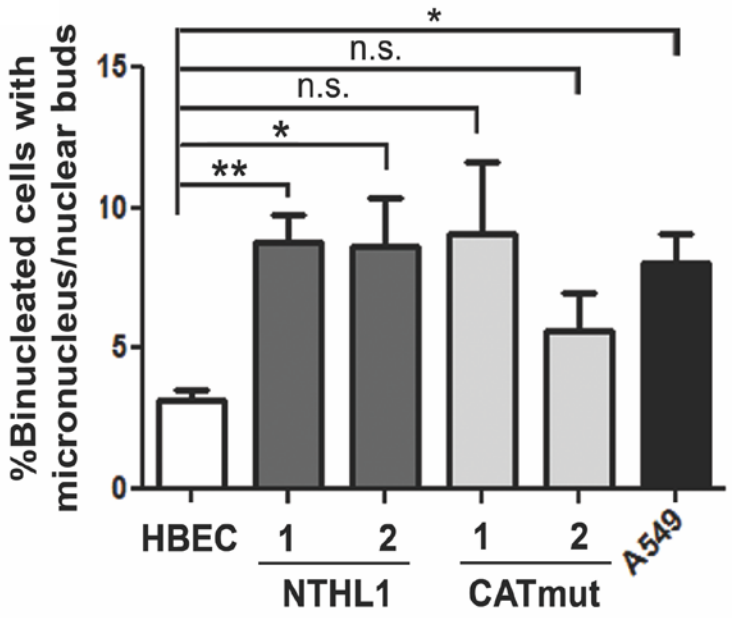

Loss of contact inhibition develops in cells that acquired the capability to grow in soft agar

Persistent genomic instability is a permanent characteristic of these transformed cells

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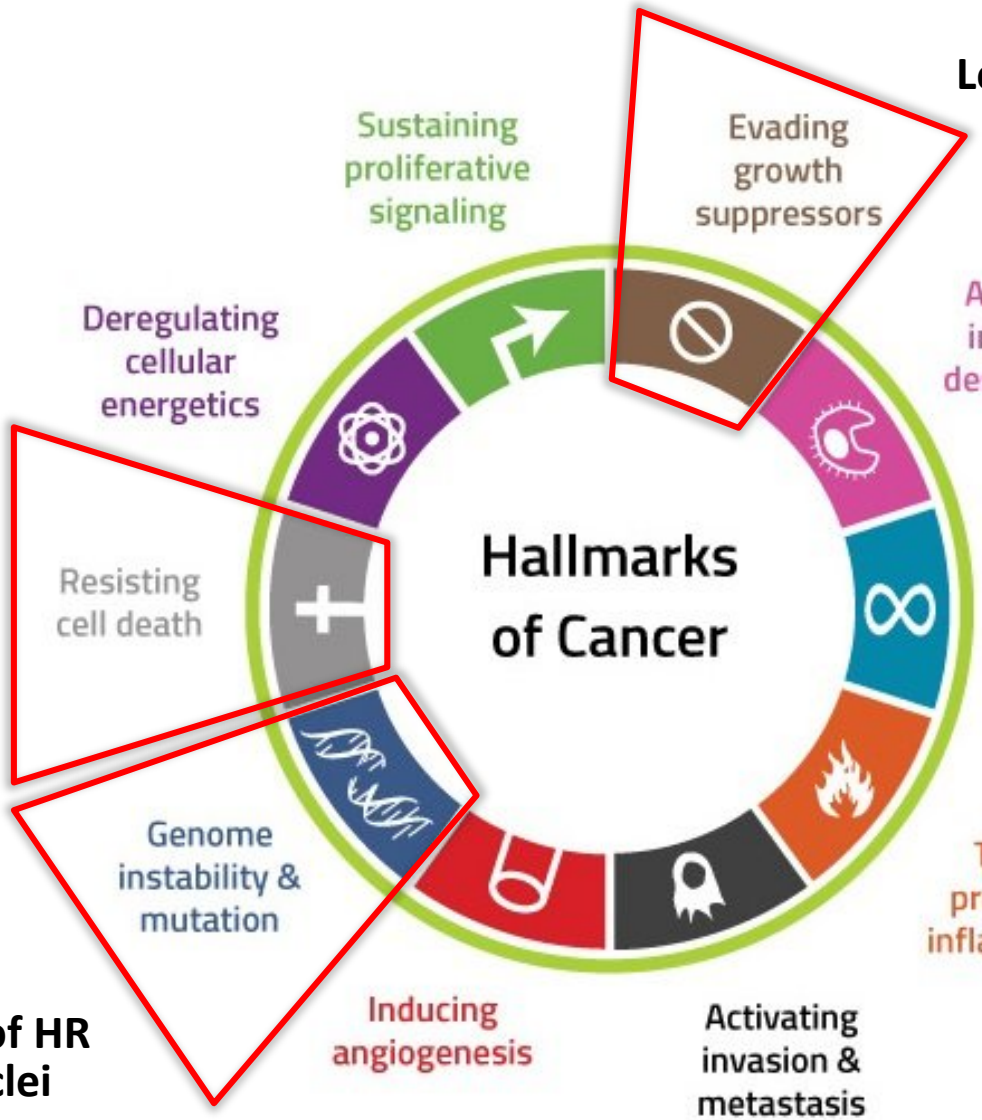
Acquired markers of cellular transformation



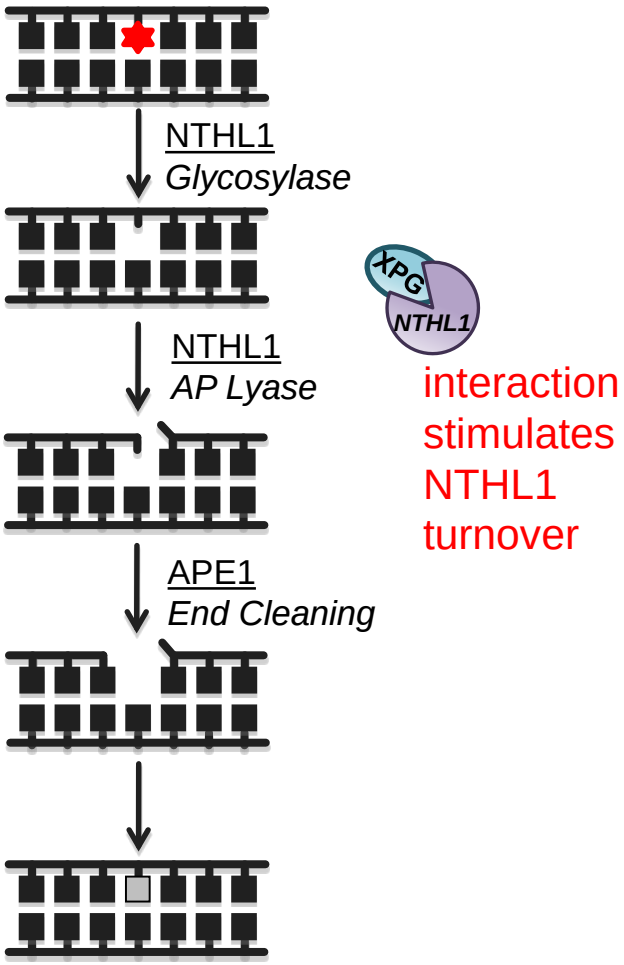
Loss of contact inhibition

Anoikis
Anchorage independent growth

DSBs
Inhibition of HR
Micronuclei

The Hallmarks of Cancer. Originally published in Cell, 144, Hanahan D & Weinberg RA, Hallmarks of Cancer: The Next Generation, 2011.

In addition to DNA damage in the form of BER intermediates caused by catalytic activity of NTHL1 what could the other effects be?



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Base Excision Repair of Oxidative DNA Damage Activated by XPG Protein

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Summary

Oxidized pyrimidines in DNA are removed by a distinct base excision repair pathway initiated by the DNA glycosylase—AP lyase hNth1 in human cells. We have reconstituted this single-residue replacement pathway with recombinant proteins, including the AP endonuclease HAP1/APE, DNA polymerase β, and DNA ligase III-XRCC1 heterodimer. With these proteins, the nucleotide excision repair enzyme XPG serves as a cofactor for the efficient function of hNth1. XPG protein promotes binding of hNth1 to damaged DNA. The stimulation of hNth1 activity is retained in XPG catalytic site mutants inactive in nucleotide excision repair. The data support the model that development of Cockayne syndrome in XP-G patients is related to inefficient excision of endogenous oxidative DNA damage.

Introduction

Damaged base residues in DNA can be removed by one of two separate excision repair processes. Lesions generated endogenously by hydrolysis or exposure to active oxygen are corrected by base excision repair (BER), with release of the altered base in free form by a DNA glycosylase and formation of an abasic site (apurinic/apyrimidinic site, AP site) as a key intermediate. On the other hand, dipyrimidine adducts generated by exposure to ultraviolet light and other types of base damage that cause major helix distortion are processed by nucleotide excision repair (NER), in which a DNA chain is incised on each side of the lesion to release an oligonucleotide containing the damaged residue. These two pathways are completely distinct and their major forms in mammalian cells share no enzymes or other protein factors (Wood, 1996; Lindahl et al., 1997). Defects in key activities of the BER process, such as AP endonuclease, DNA polymerase β, or the XRCC1-DNA ligase III heterodimer, lead to embryonic lethal phenotypes in the mouse, indicating that repair of endogenous DNA lesions is essential during development (Wilson and Thompson, 1997). In contrast, NER defects generally are nonlethal, and mutations in any of the 7 key genes *XPA* to *XPG* can be the cause of the inherited cancer-prone disease xeroderma pigmentosum in man.

Deamination of cytosine to uracil in DNA is counteracted by BER; the repair process involves DNA polymerase β–catalyzed substitution of a single dCMP residue in DNA to replace the excised uracil and deoxyribose phosphate moieties and has been reconstituted with purified human factors (Kubota et al., 1996; Nicholl et al., 1997; Srivastava et al., 1998). Oxidized DNA bases such as thymine glycol (Tg) and 8-oxoguanine are believed to be repaired in a similar way, although the initial step is carried out by bifunctional enzymes, which can both release a damaged base by DNA glycosylase activity and cleave the DNA chain at the abasic site by AP lyase activity. Excision of various ring-saturated and ring-fragmented oxidized derivatives of thymine and cytosine is due to a Tg-DNA glycosylase-AP lyase activity, the human counterpart of *E. coli* endonuclease III or Nth. The three-dimensional structure of the bacterial enzyme has been established (Kuo et al., 1992); the homologous human enzyme hNth1 retains relevant key features and has been expressed in active form from a cloned cDNA (Aspinwall et al., 1997; Hilbert et al., 1997). Characteristic structural properties include a conserved helix–hairpin–helix region that accounts for binding of the damaged pyrimidine and also contains an active site Lys residue required for DNA glycosylase activity, a C-terminal 4Fe-4S cluster involved in the interaction with the DNA sugar phosphate backbone, and a highly conserved Asp residue required for AP lyase activity (Kuo et al., 1992; Thayer et al., 1995; Doherty et al., 1996; Aspinwall et al., 1997).

Recently, there have been unexpected indications for overlapping functions of some components of the BER and NER pathways, in particular with regard to repair of oxidative DNA damage. XPG protein is a structure-specific endonuclease, which accounts for DNA strand cleavage on the 3′ side of a lesion during NER (O'Donovan et al., 1994; Cloud et al., 1995; Evans et al., 1997a). XP-G patients that only produce a truncated version of the protein also suffer from the clinically distinct severe disease Cockayne syndrome (CS) (Vermeulen et al., 1993; Nouspikel et al., 1997). In addition to the NER

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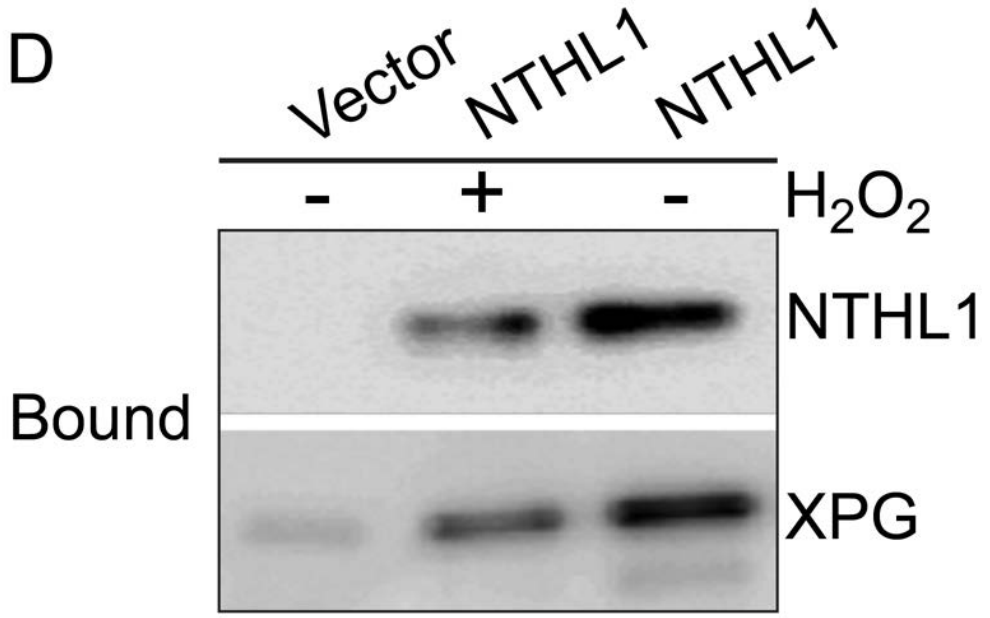
NTHL1 interacts with XPG (Co-IP)



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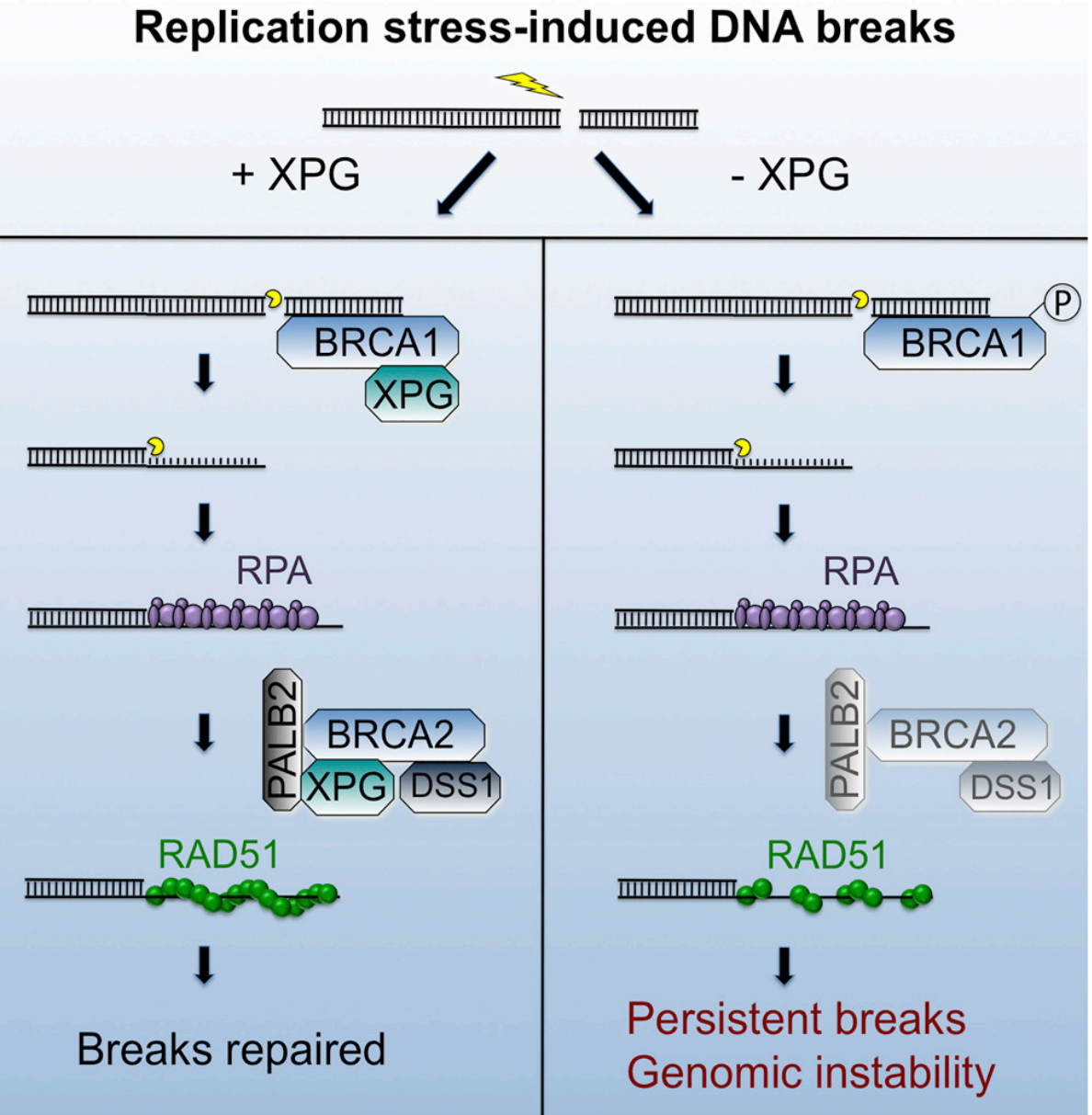
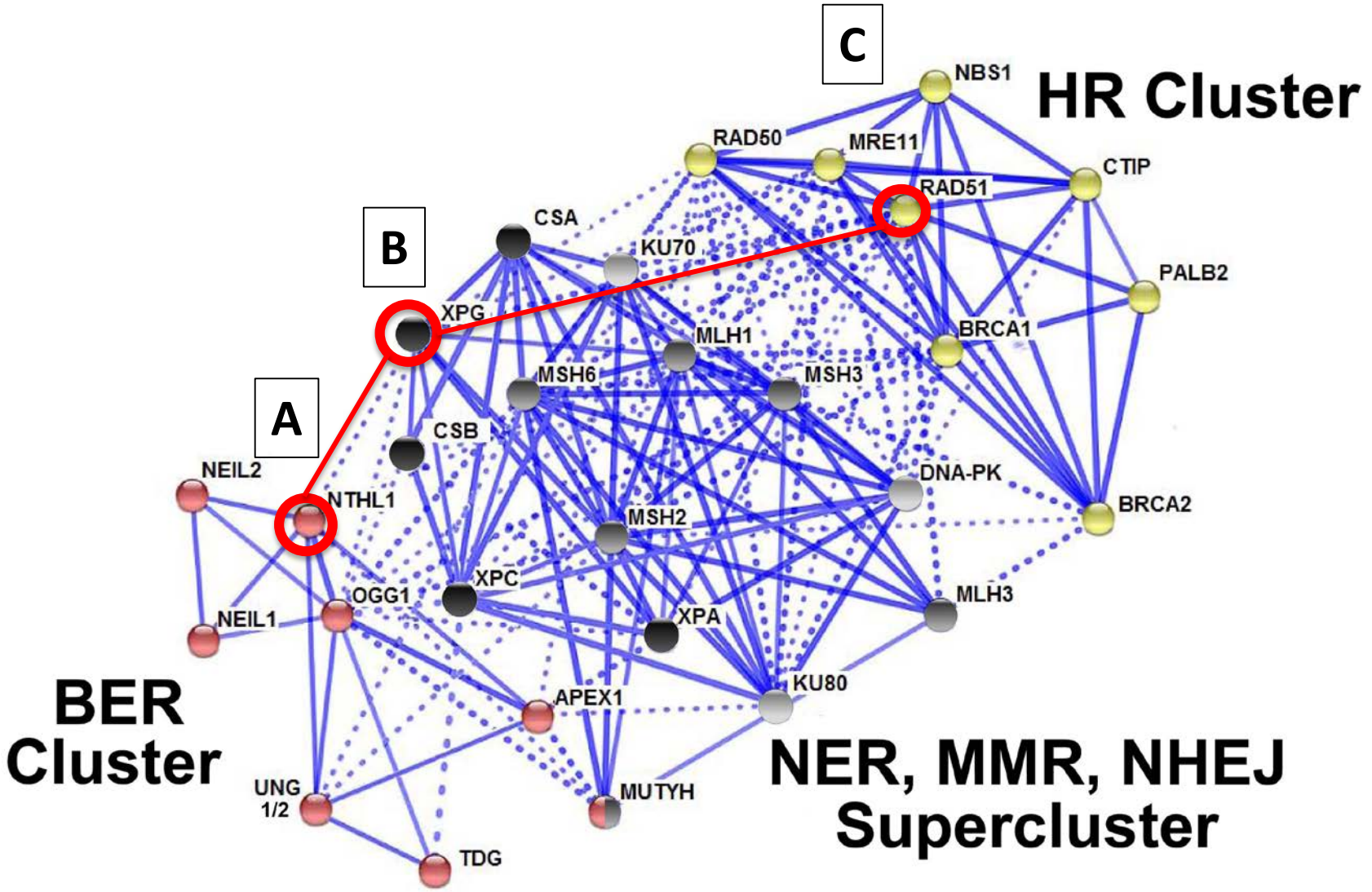
XPG is essential for HR repair of DSB resulting from collapsed replication forks (Trego et al. *Mol. Cell* 2016)



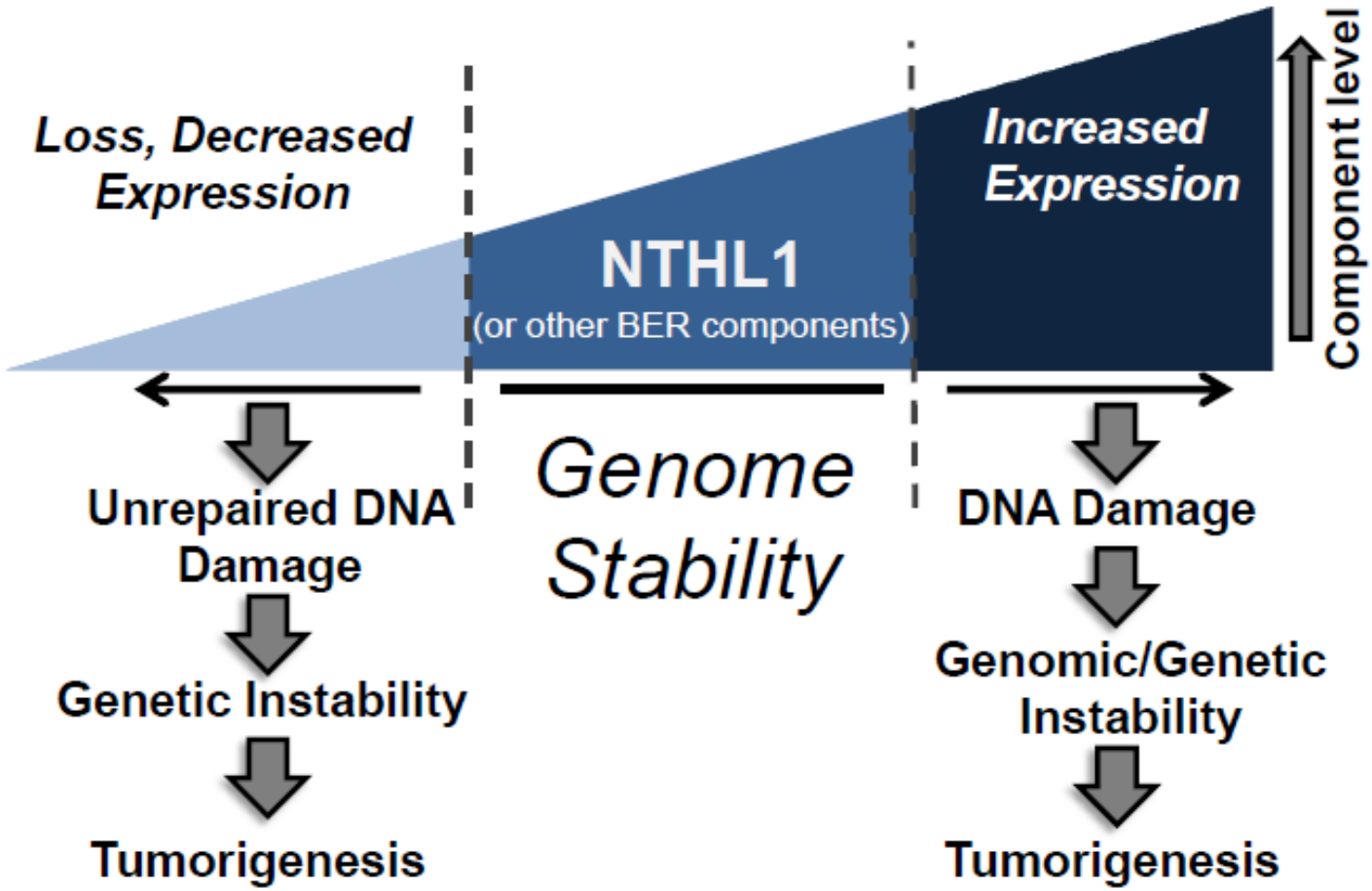
- HR function of XPG is not dependent on catalytic function

DNA repair pathway crosstalk regulates/impacts DNA damage management



Limpose, Corbett, and Doetsch. DNA Repair 2017

Current Model



Model

Replication stress

DSB Generation

Initiation of HR Repair

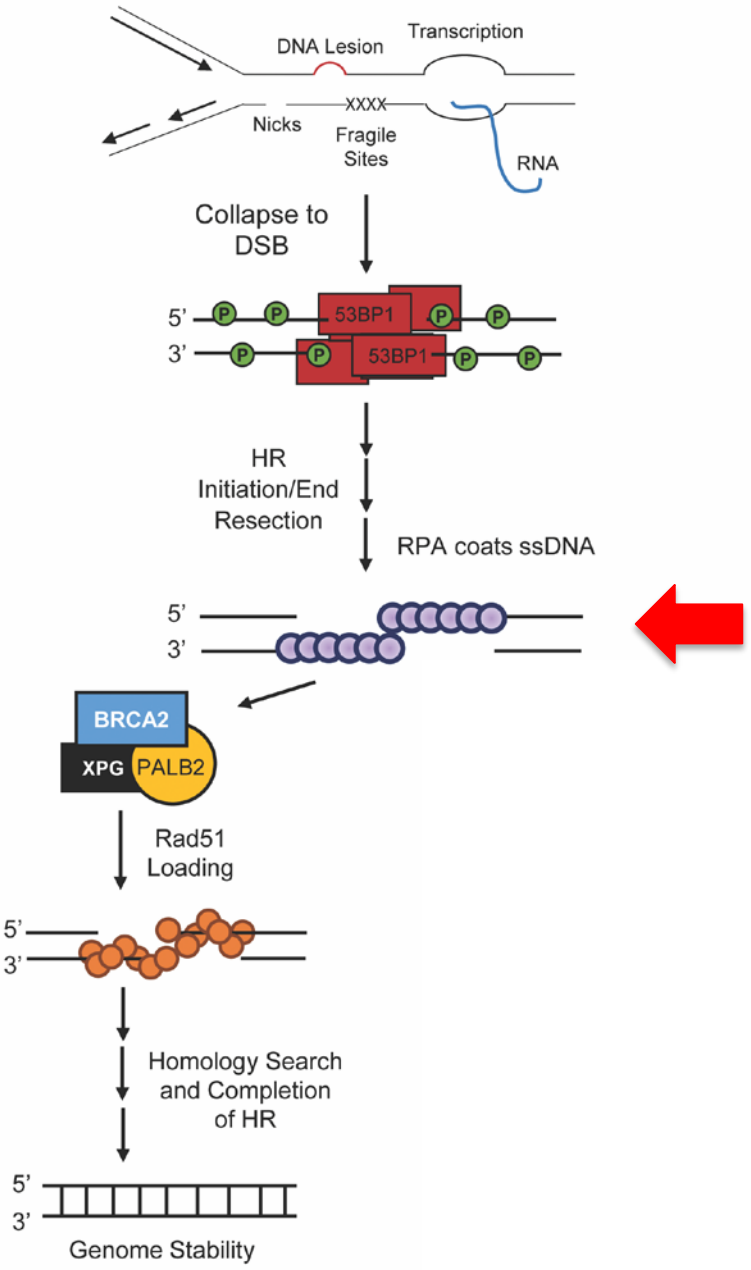
pRPA coats resected DNA

NTHL1 Sequestration of XPG

Inefficient loading of Rad51

Alt-NHEJ Initiated?

Genomic Instability



Supporting Result(s)

p-ATR T1989
p-Chk1 S317

γH2Ax/53BP1 foci
Comet Assay
p-Chk2 T68

p-RPA2 S4/S8

Decrease in DR-GFP
Comet assay

Micronucleus Formation

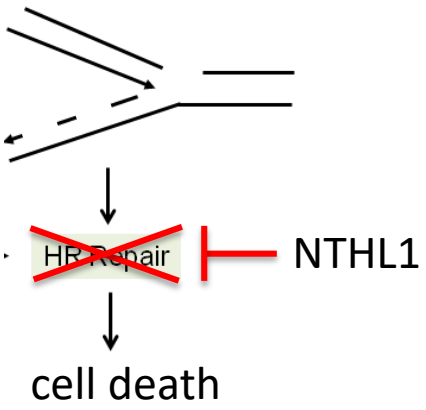
Conclusions

- Overexpression of a BER glycosylase causes DSB accumulation
- Overexpression of NTHL1 impairs DSB repair
  - independent of NTHL1 enzymatic activity
- Outcome of impaired DSB repair is genomic instability
- DSBs generated during replication likely exacerbate genomic instability
- Acquisition of multiple cellular transformation markers appear when NTHL1 is overexpressed

Clinical Implications

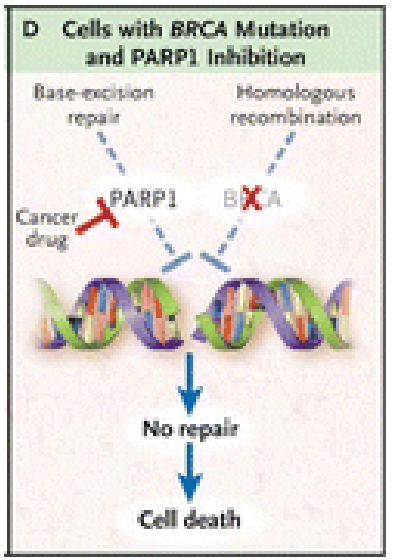
Prediction: tumor cells that overexpress NTHL1 could be sensitized to agents that induce DSBs

- Ionizing radiation (IR)
- Topoisomerase inhibitors



Novel combination strategies

- Sensitization to PARP inhibitors
- Exploiting the defect in HR



Biomarker potential

- Use to predict patient outcomes

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Biostatistics and Bioinformatics
Emory Integrated Genomics

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