DNA Damage Response to Naphthalene metabolites

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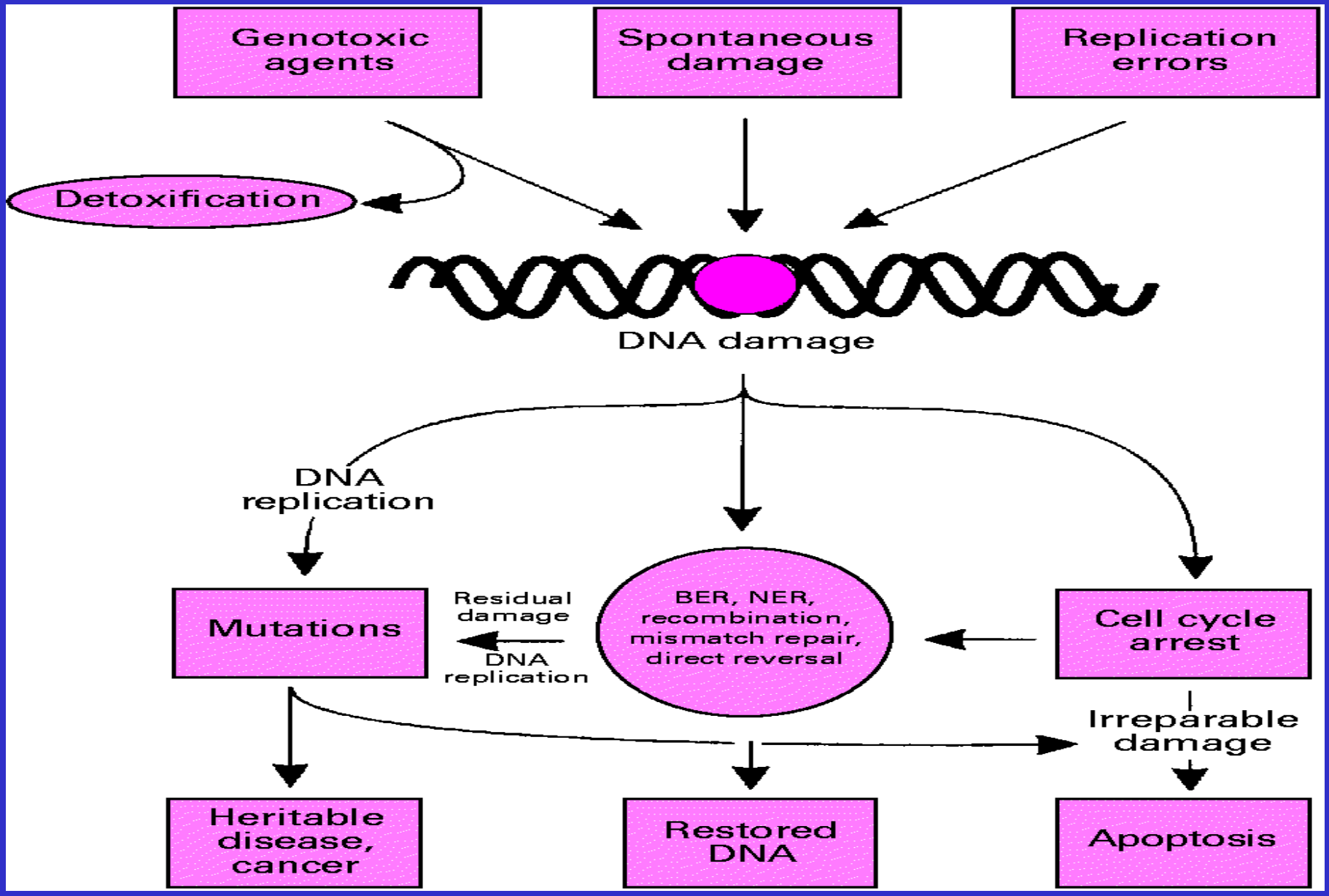
Establishment of novel assays

1] Abasic site assay (1998).

2] Real-time DNA SSB assay (2003).

3] DNA damage response analysis (2007).

What is the DNA damage response analysis?



[Krokan, et al., Biochem J, 1997]

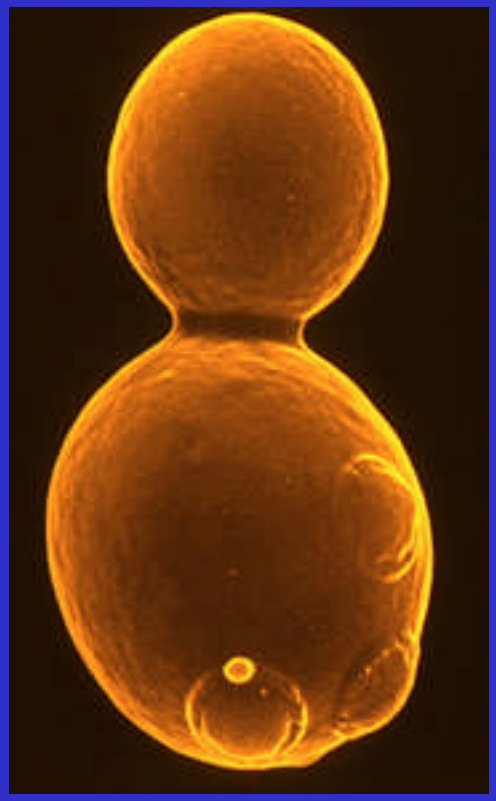
Global DNA damage responses

Specific DNA damage

DNA damage response project using genetic approach

1] Discover a new function of DNA repair gene.

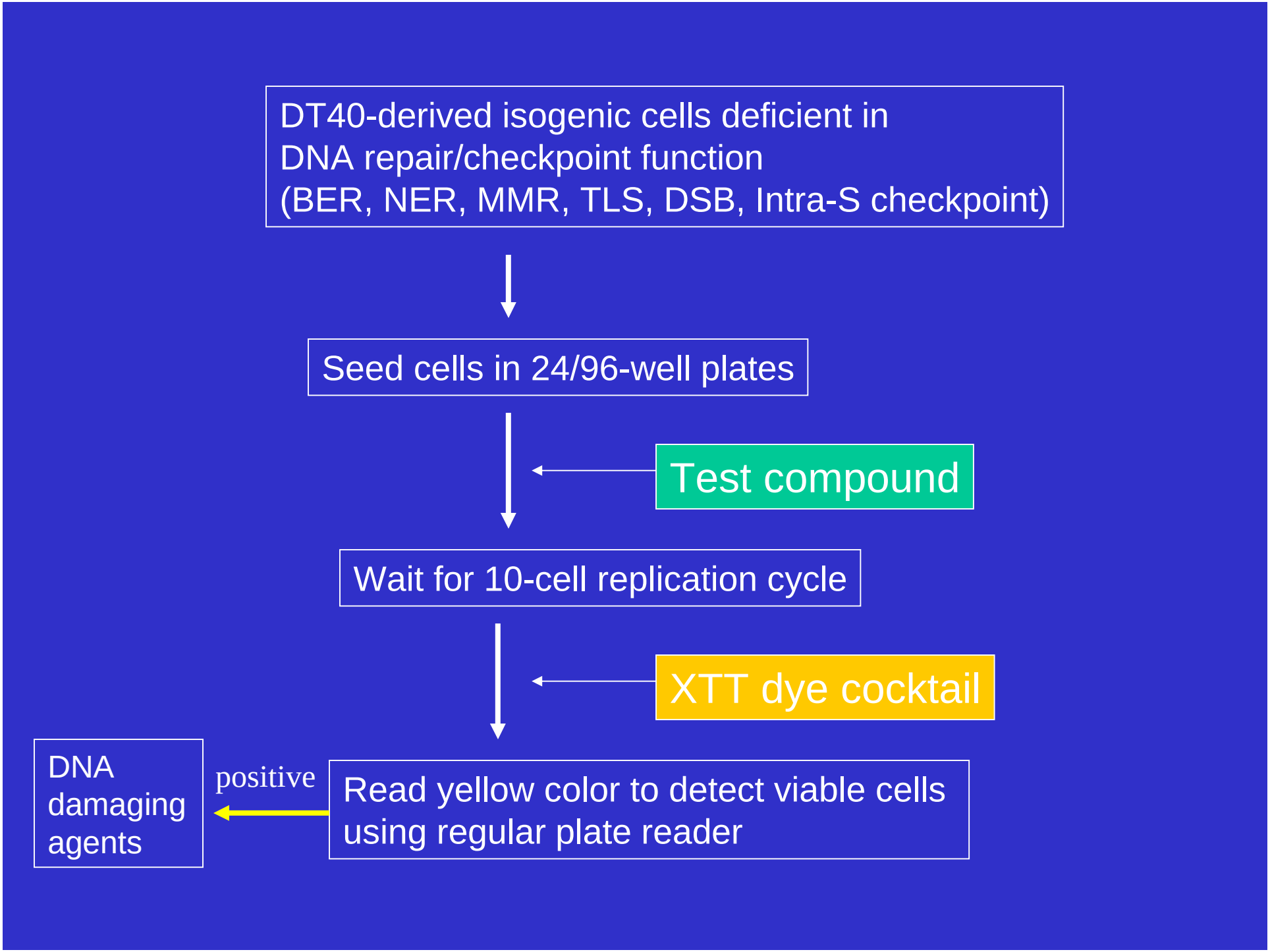
2] A novel screening method for detection of genotoxic compounds.



Chicken DT40 B-lymphocytes line

- **High gene targeting frequencies (10-90% among stable transfectants).**
- **Stable phenotype; ideal for analysis of DNA repair defects.**
- **DT40 cells express some proteins which yeast cells don't have (e.g., PARP1, Polβ, BRCA1, BRCA2, etc).**

Method of DNA damage response analysis


DT40-derived isogenic cells deficient in
DNA repair/checkpoint function
(BER, NER, MMR, TLS, DSB, Intra-S checkpoint)
Seed cells in 24/96-well plates
Test compound
Wait for 10-cell replication cycle
XTT dye cocktail
Read yellow color to detect viable cells
using regular plate reader
positive
DNA
damaging
agents

DNA damage response project using DT40 cells

1] Endogenous aldehydes:

formaldehyde, acrolein, crotonaldehyde, glyoxal, methylglyoxal

2] Naththale metabolites:

1,2-naphthoquinone, 1,2-naphthalenediol, 1,4-naphthoquinone, 1,4-naphthalenediol

3] Estrogen metabolites:

2-OHE2, 4-OHE2

Cells

Model system: DT40-derived B-lymphocytes

- **DT40 cells**
- **DT40-derived *pol*$\beta^{-/-}$ cells**
- **DT40-derived *fen1*$^{-/-}$ cells**

Exposure

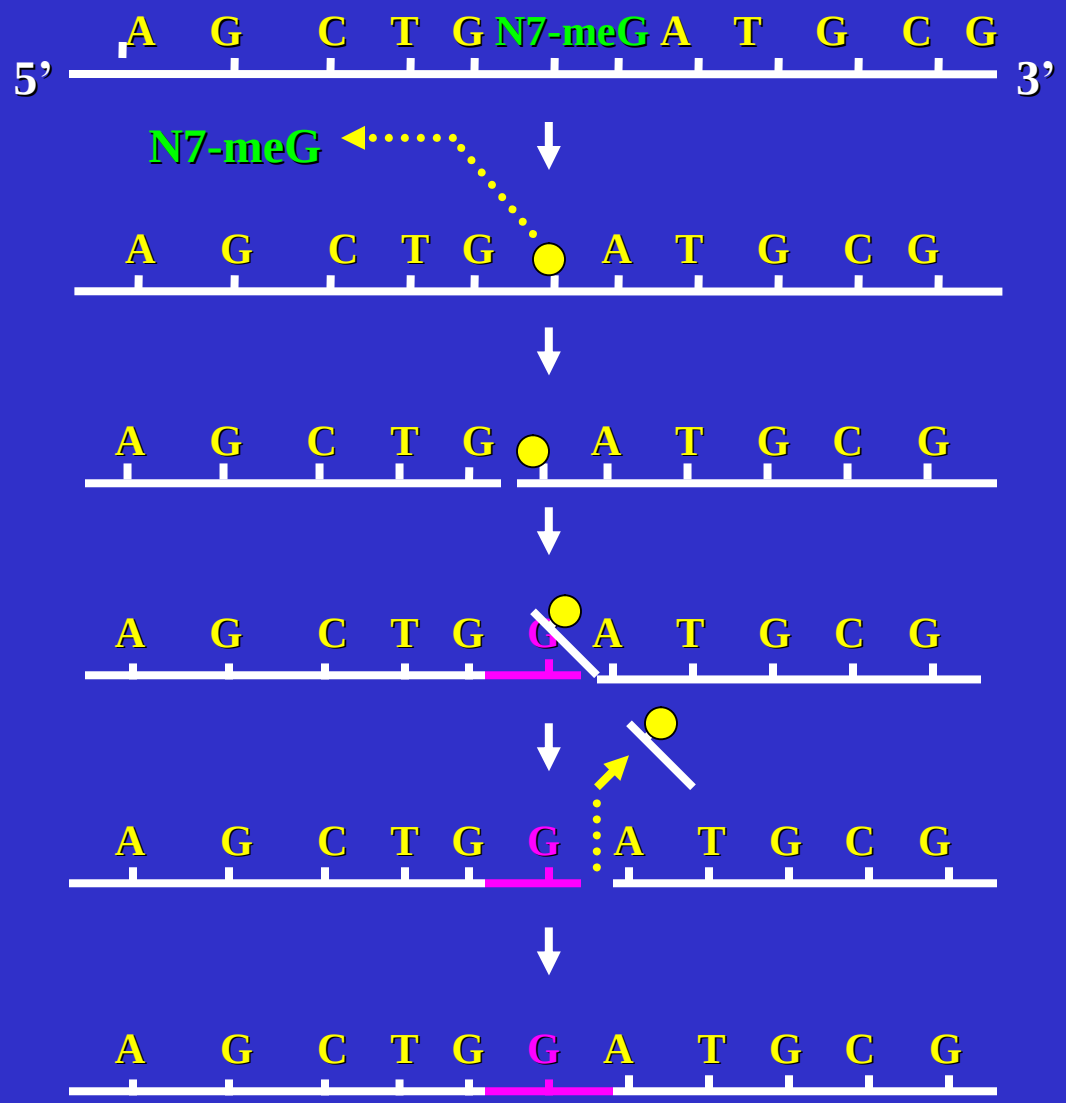
Methyl methanesulfonate (MMS)

Assays

survival assay

Base excision repair (BER) for alkylated purines

● : AP sites



Spontaneous depurination

APE1

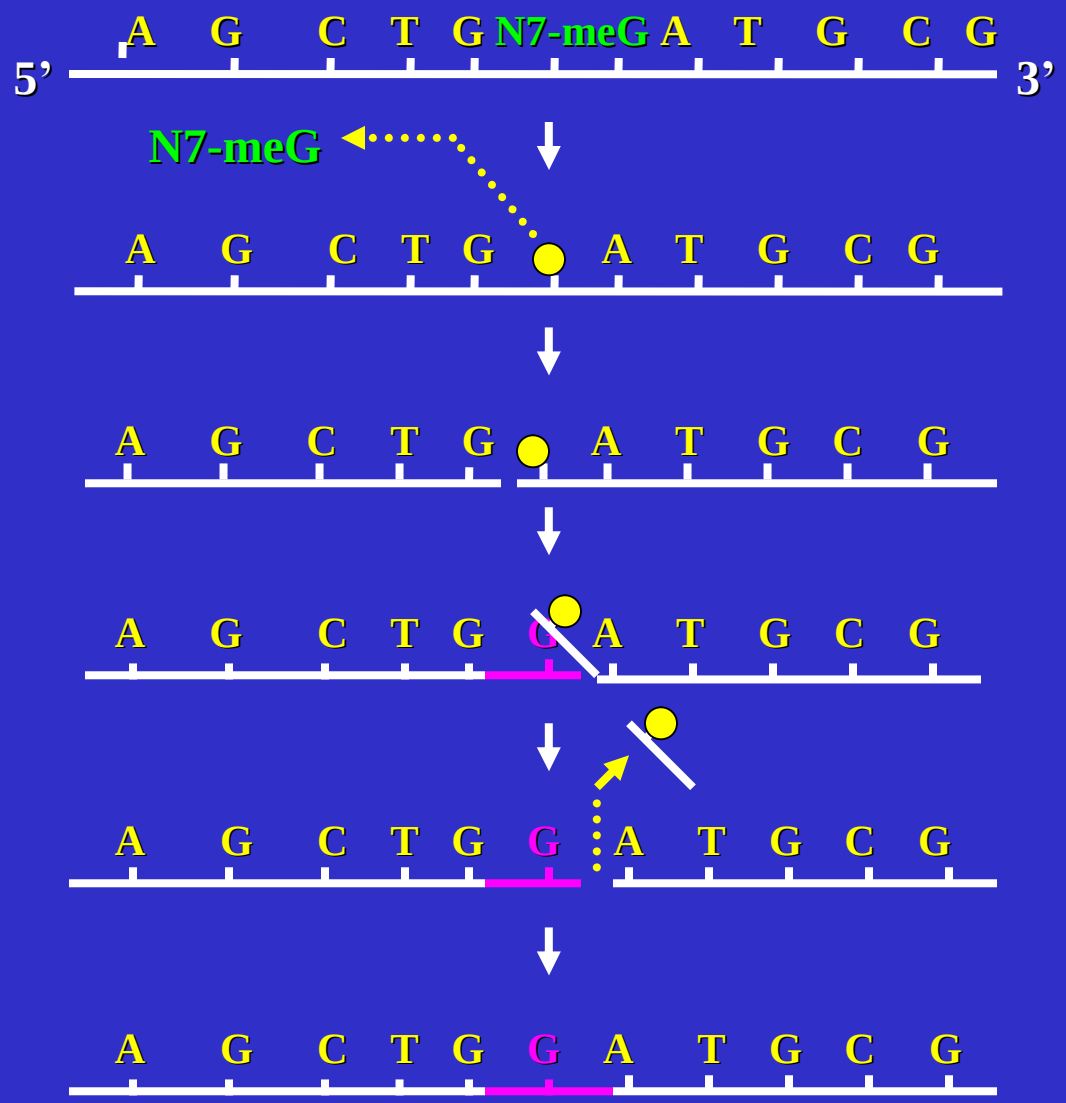
DNA polymerase β

DNA polymerase β

DNA Ligase III

Base excision repair (BER) for alkylated purines

● : AP sites



Spontaneous depurination

APE1

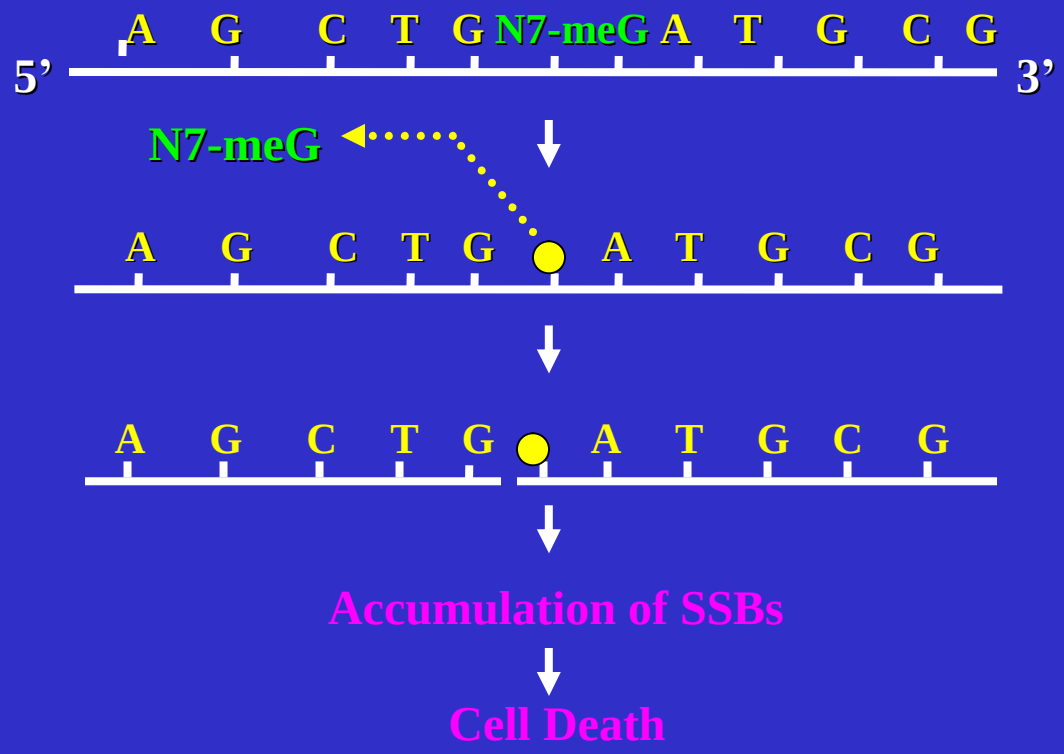
DNA polymerase β

DNA polymerase β

DNA Ligase III

Base excision repair (BER) for alkylated bases

● : AP sites



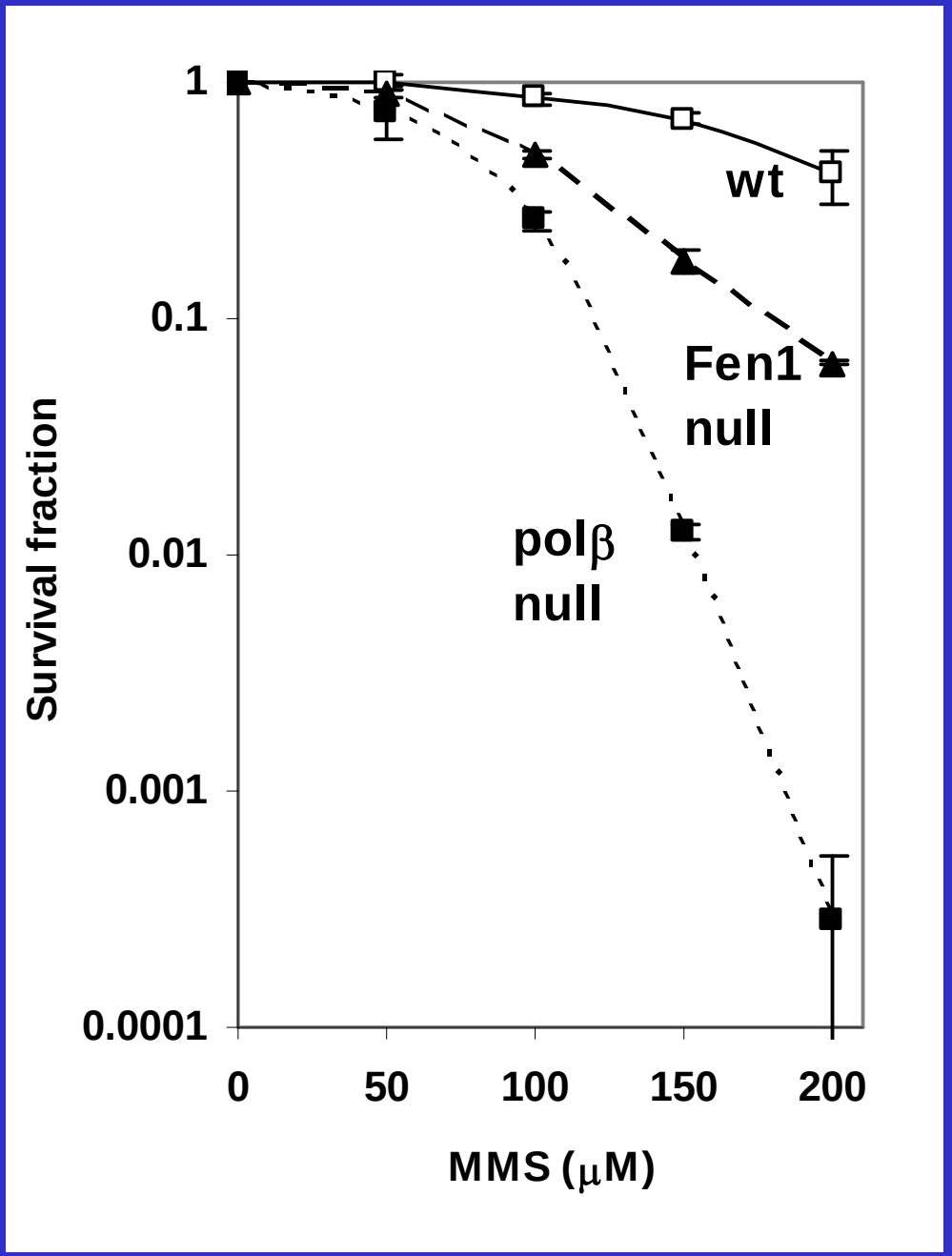
Spontaneous depurination

APE1

~~DNA polymerase β~~

~~DNA polymerase β~~

DNA Ligase III


1
0.1
0.01
0.001
0.0001
Survival fraction
0
50
100
150
200
MMS (μM)
wt
Fen1
null
polβ
null

Cells

Model system: DT40-derived B-lymphocytes

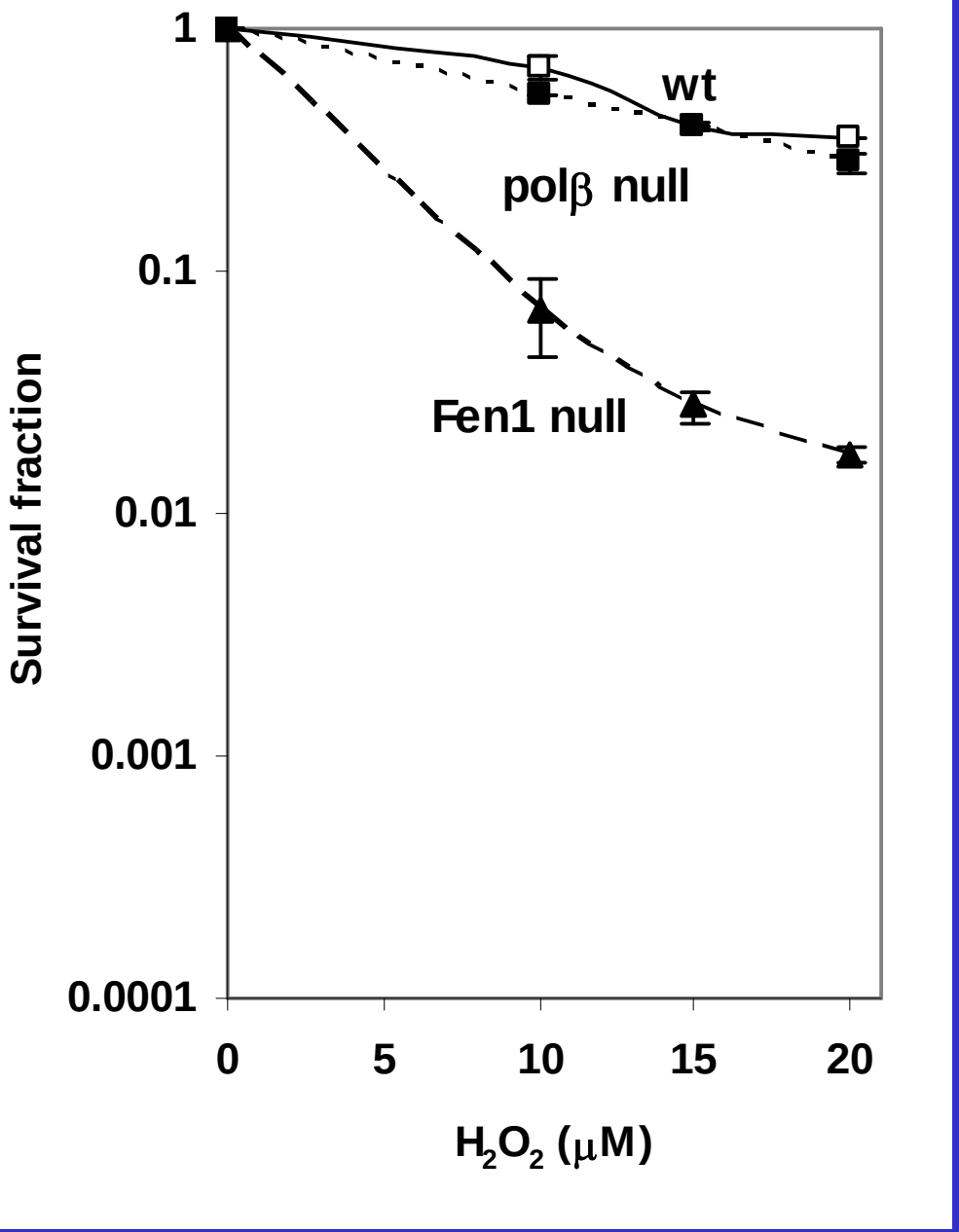
- **DT40 cells**
- **DT40-derived *pol*$\beta^{-/-}$ cells**
- **DT40-derived *fen1*$^{-/-}$ cells**

Exposure

H_2O_2

Assays

survival assay


1
0.1
0.01
0.001
0.0001
Survival fraction
wt
polβ null
Fen1 null
0
5
10
15
20
H2O2 (μM)

Cells

Model system: DT40-derived B-lymphocytes

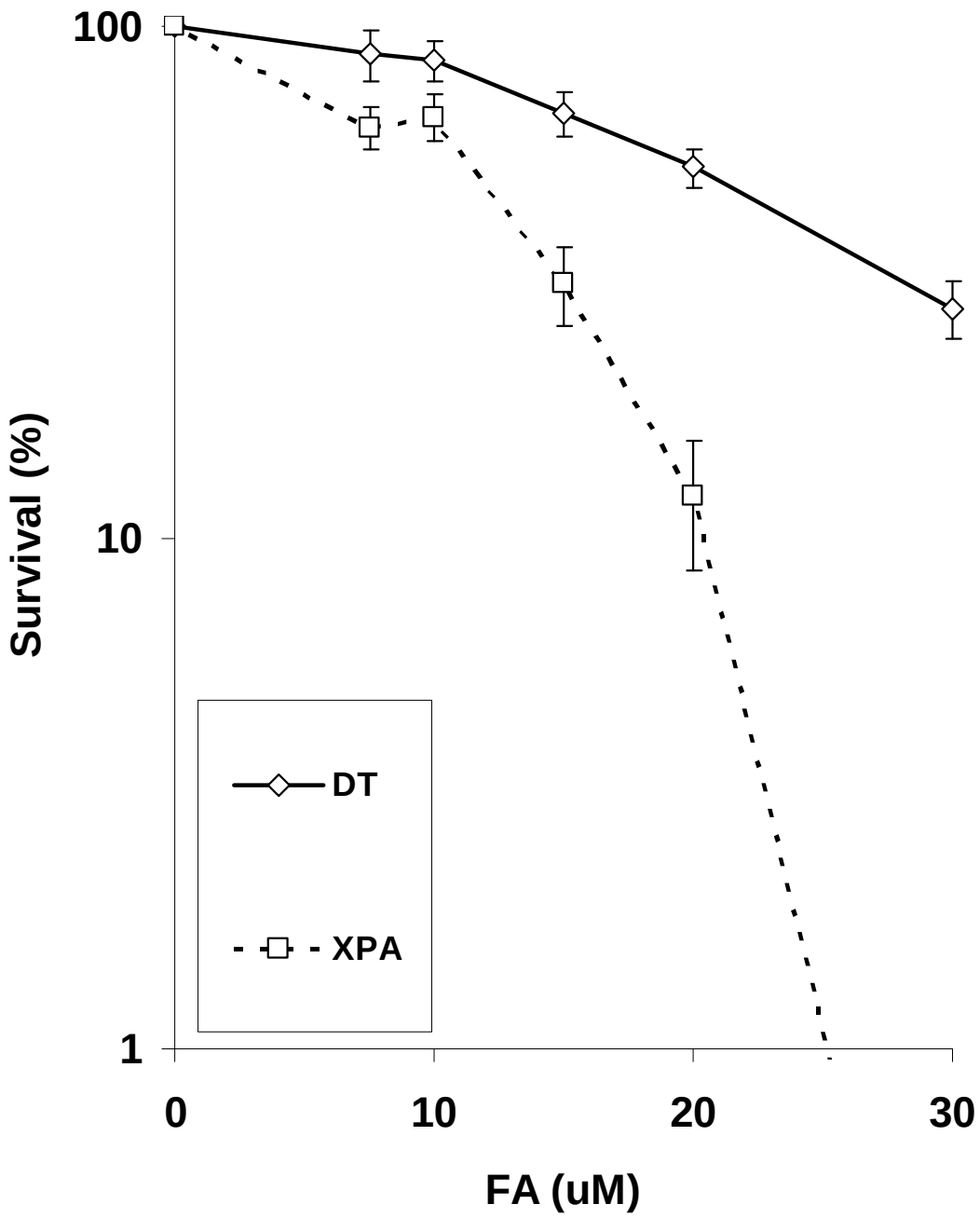
- **DT40 cells**
- **DT40-derived *xpa*$^{-/-}$ cells**

Exposure

formaldehyde

Assays

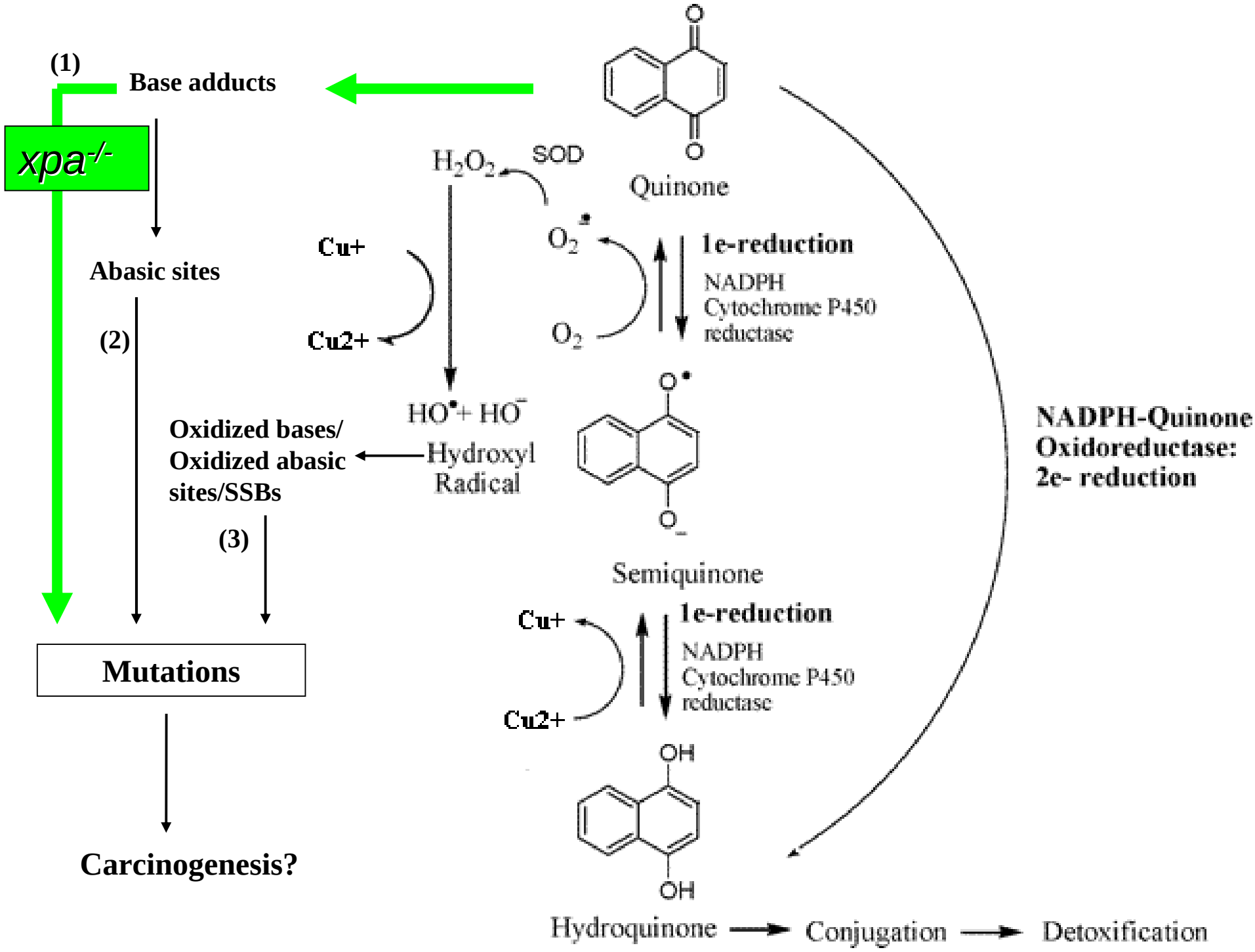
survival assay

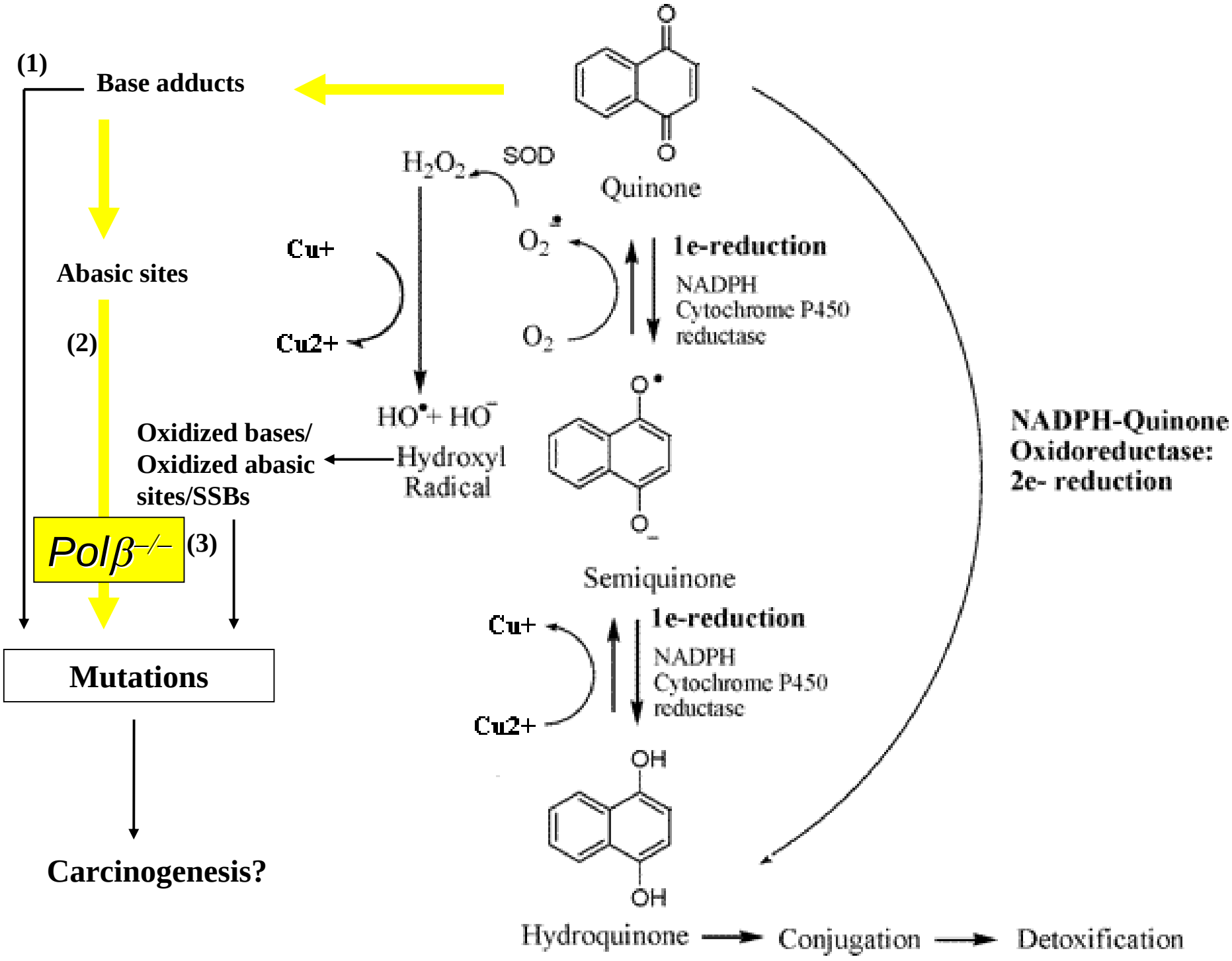

100
10
1
Survival (%)
0
10
20
30
FA (uM)
DT
XPA

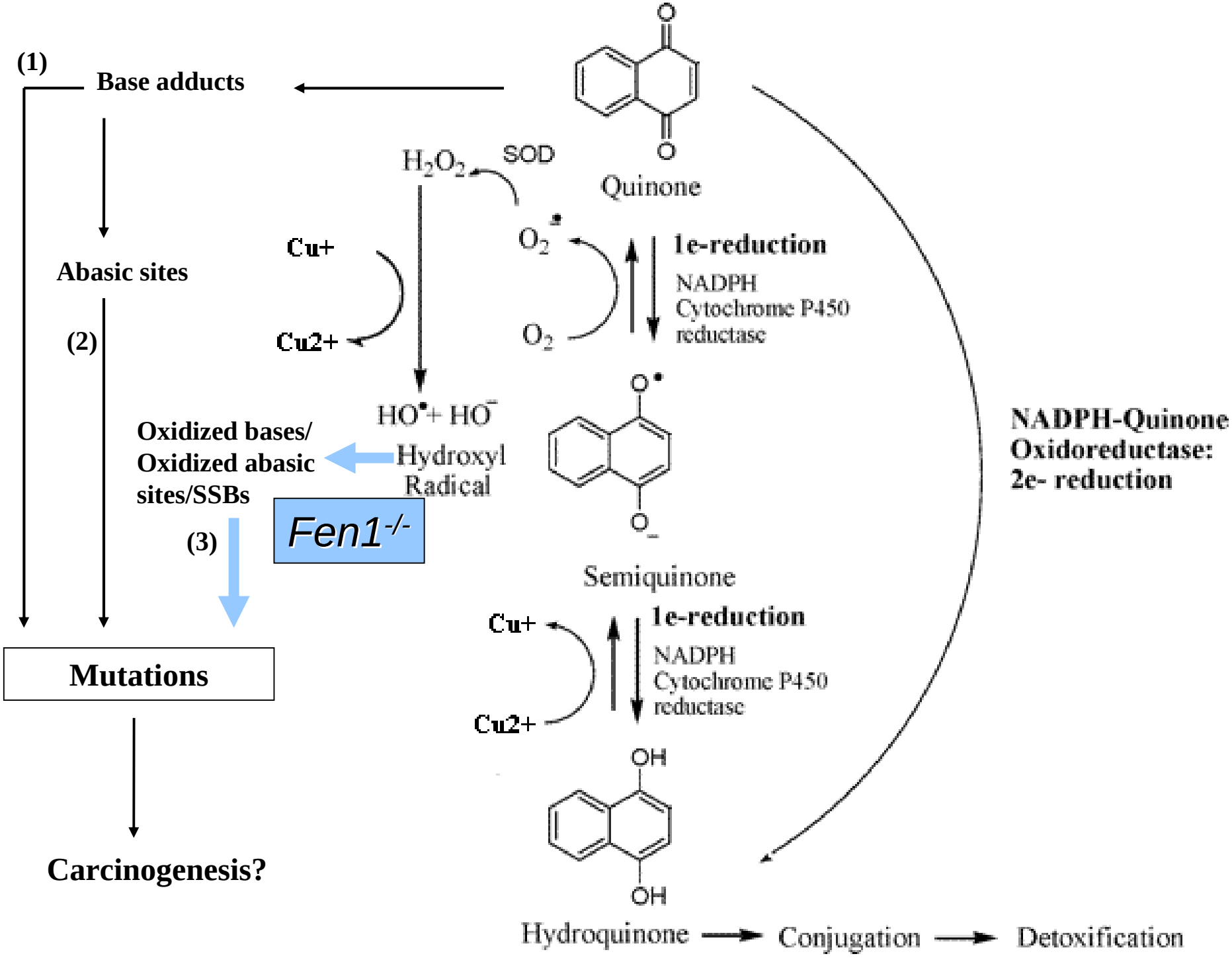
Question:

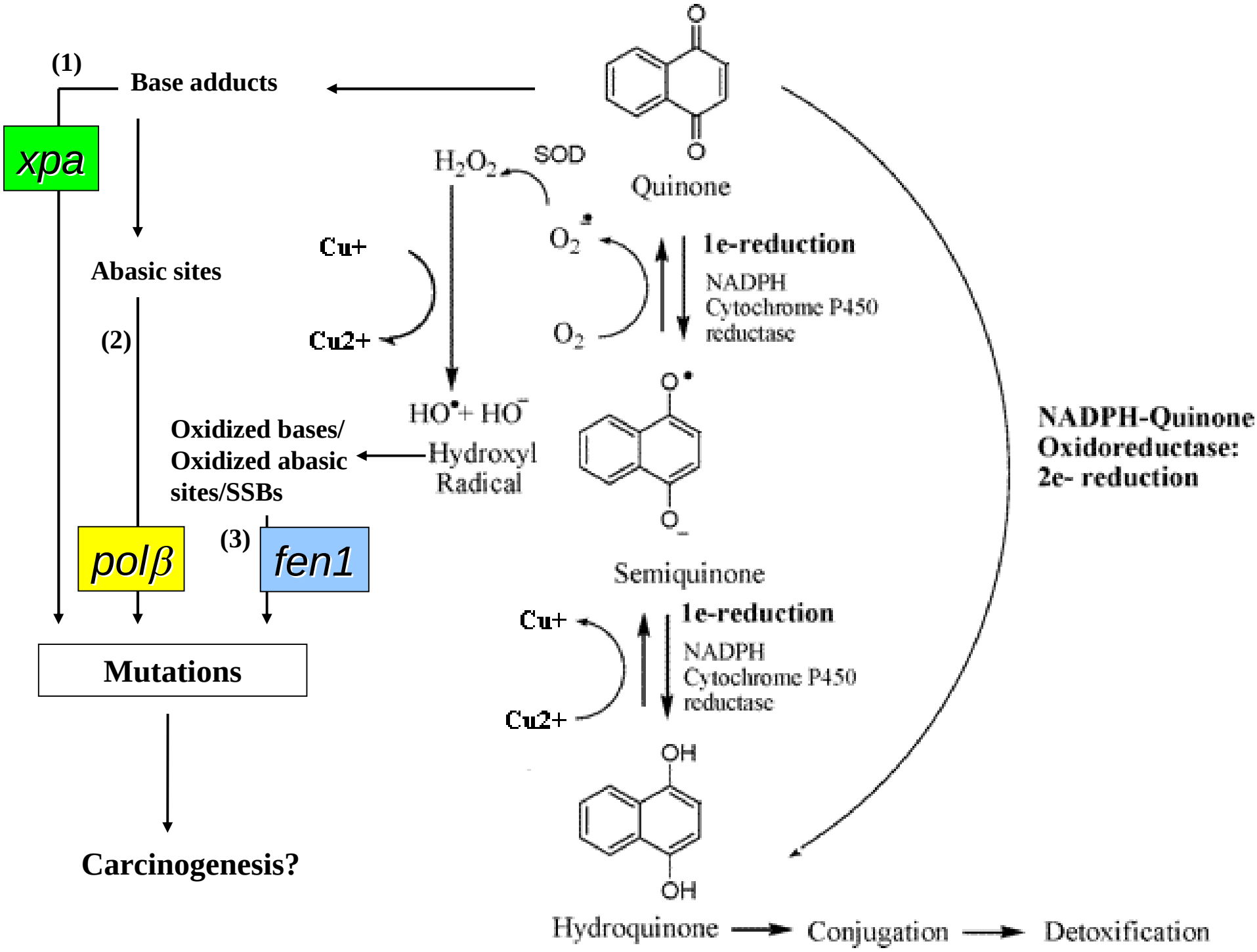
-Do naphthalene metabolites introduce DNA damage in cellular DNA?

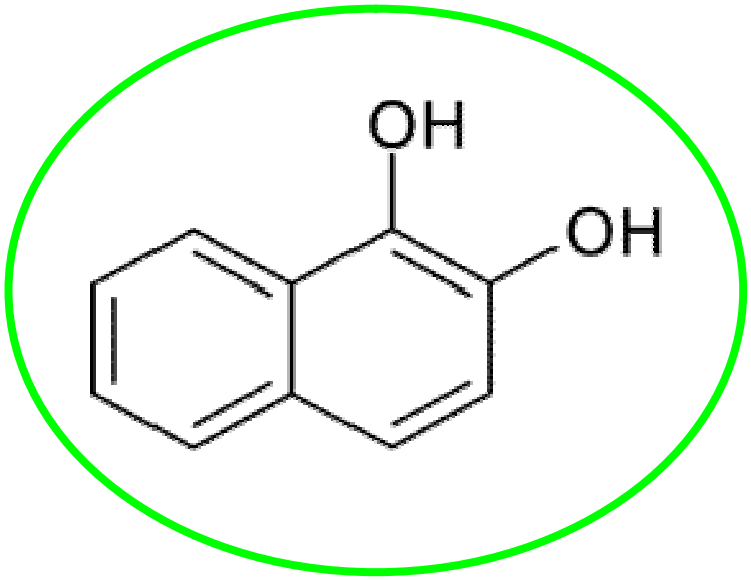
- What is a major impact of naphthalene metabolites on DNA damage response?


(1)
Base adducts
xpa-/-
Abasic sites
(2)
Oxidized bases/
Oxidized abasic
sites/SSBs
(3)
Mutations
Carcinogenesis?
H2O2
SOD
Quinone
Cu+
Cu2+
O2•
O2
1e-reduction
NADPH
Cytochrome P450
reductase
HO•+ HO−
Hydroxyl
Radical
Semiquinone
NADPH-Quinone
Oxidoreductase:
2e- reduction
OH
OH
Hydroquinone
Conjugation
Detoxification

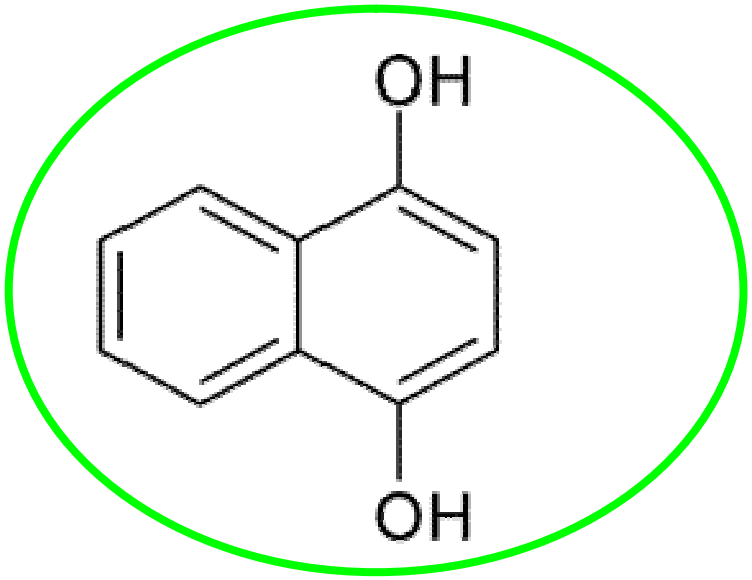

(1)
Base adducts
Abasic sites
(2)
Oxidized bases/
Oxidized abasic
sites/SSBs
Polβ−/−
(3)
Mutations
Carcinogenesis?
H2O2
SOD
Quinone
Cu+
Cu2+
O2•
O2
1e-reduction
NADPH
Cytochrome P450
reductase
HO• + HO−
Hydroxyl
Radical
Semiquinone
NADPH-Quinone
Oxidoreductase:
2e- reduction
OH
OH
Hydroquinone
Conjugation
Detoxification


(1)
Base adducts
Abasic sites
(2)
Mutations
Carcinogenesis?
Oxidized bases/
Oxidized abasic
sites/SSBs
(3)
Fen1-/-
H2O2
SOD
Quinone
Cu+
Cu2+
O2
O2
1e-reduction
NADPH
Cytochrome P450
reductase
HO• + HO−
Hydroxyl
Radical
Semiquinone
Cu+
Cu2+
1e-reduction
NADPH
Cytochrome P450
reductase
OH
OH
Hydroquinone
Conjugation
Detoxification
NADPH-Quinone
Oxidoreductase:
2e- reduction

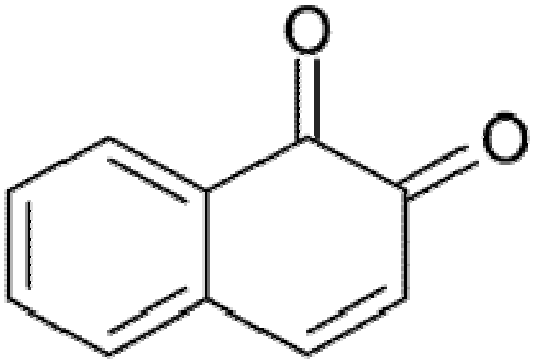

(1)
Base adducts
xpa
Abasic sites
(2)
polβ
(3)
fen1
Mutations
Carcinogenesis?
Oxidized bases/
Oxidized abasic
sites/SSBs
Cu+
Cu2+
H2O2
SOD
HO• + HO−
Hydroxyl
Radical
Quinone
O2•−
O2
1e-reduction
NADPH
Cytochrome P450
reductase
Semiquinone
Cu+
Cu2+
1e-reduction
NADPH
Cytochrome P450
reductase
OH
OH
Hydroquinone
Conjugation
Detoxification
NADPH-Quinone
Oxidoreductase:
2e- reduction



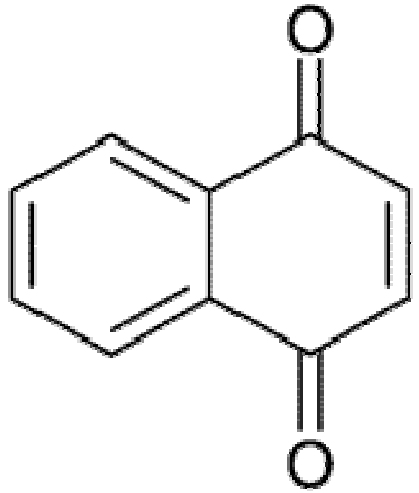
NCAT



NHQ



1,2-NQ



1,4-NQ

Cells

Model system: DT40-derived B-lymphocytes

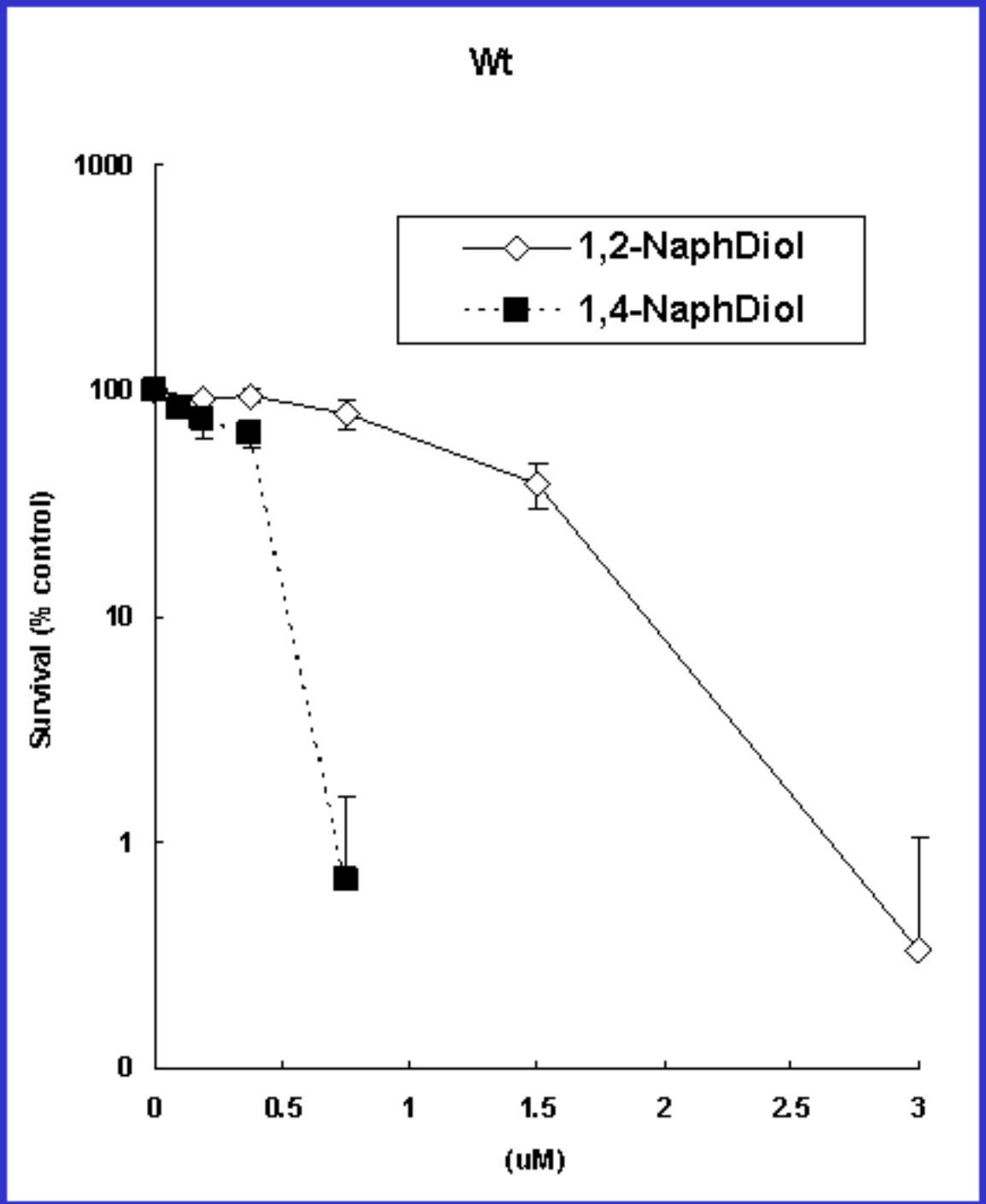
- wt DT40 cells

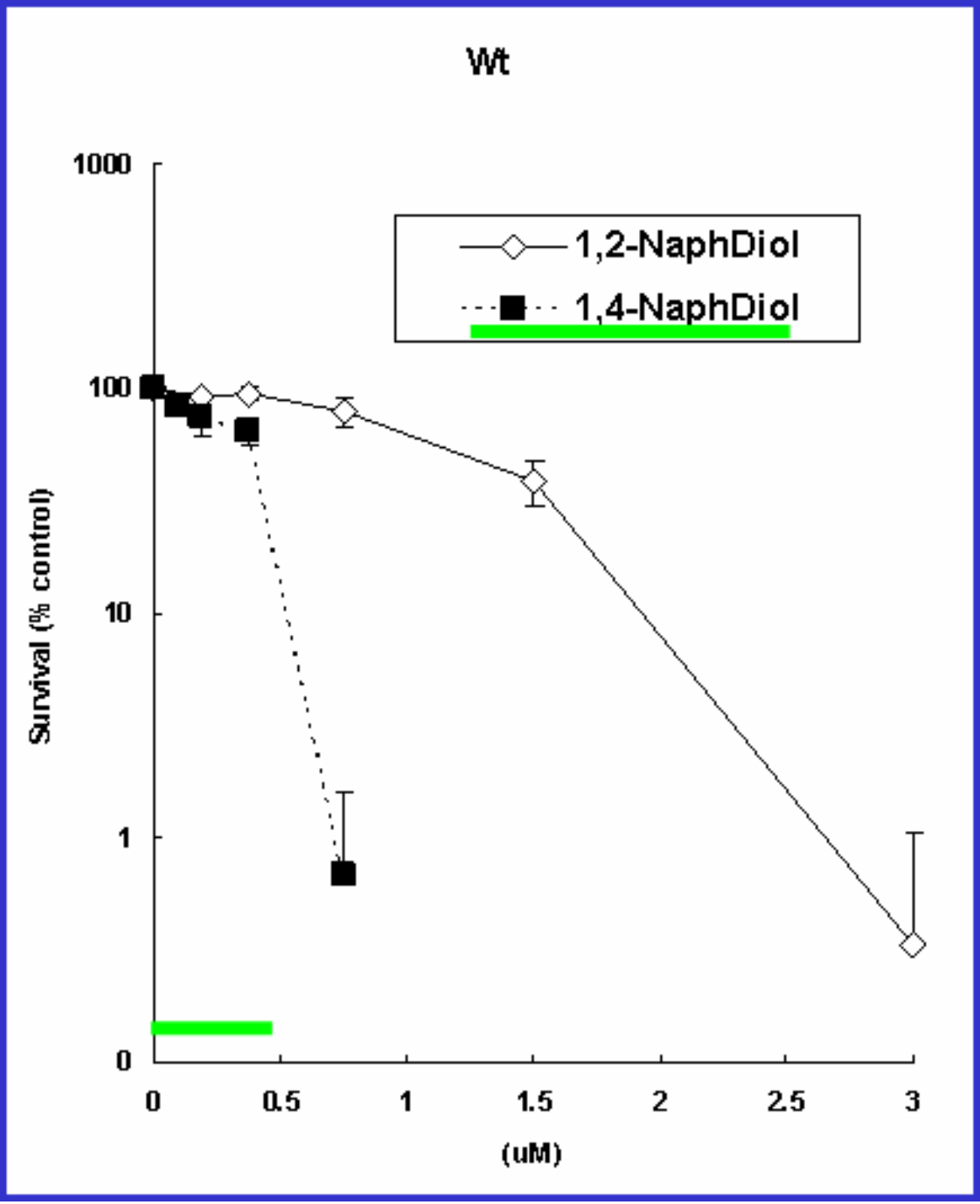
Exposure

1,2-NaphDiol vs 1,4-NaphDiol

Assays

survival assay


Wt
1000
100
10
1
0
Survival (% control)
1,2-NaphDiol
1,4-NaphDiol
0
0.5
1
1.5
2
2.5
3
(uM)


Wt
1000
100
10
1
0
Survival (% control)
1,2-NaphDiol
1,4-NaphDiol
0
0.5
1
1.5
2
2.5
3
(uM)

Cells

Model system: DT40-derived B-lymphocytes

- wt DT40 cells

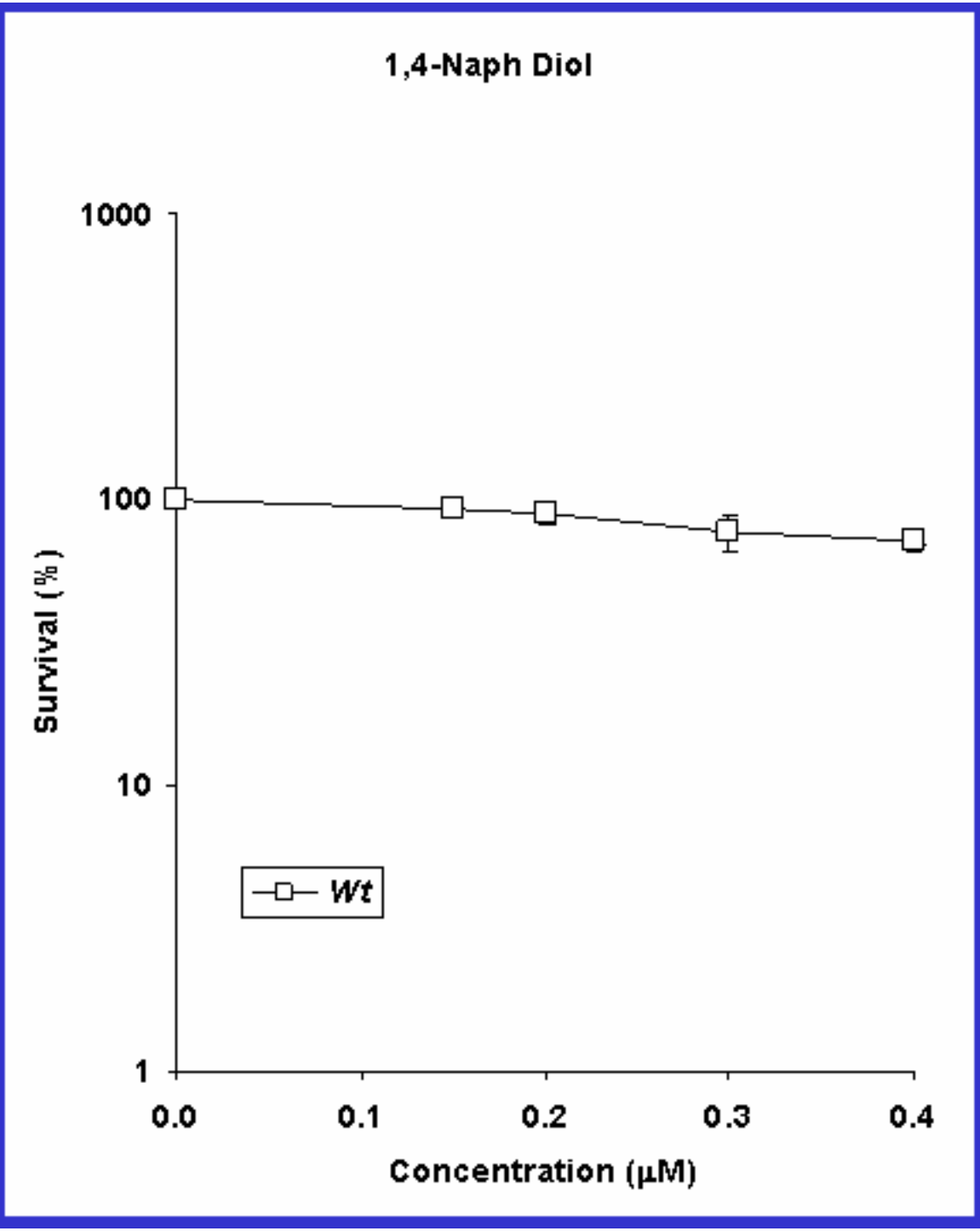
- DT40 cells deficient in DNA damage response

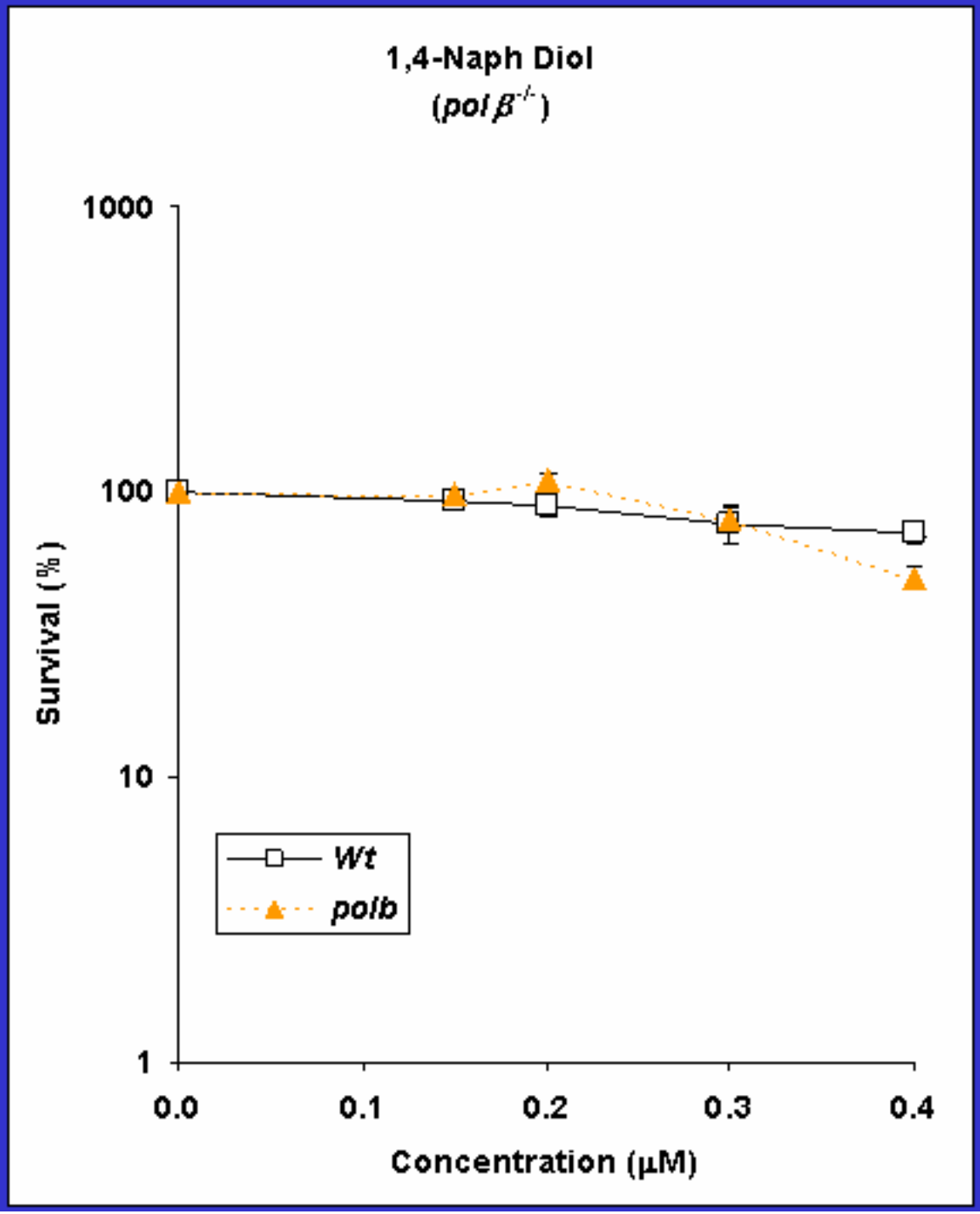
Exposure

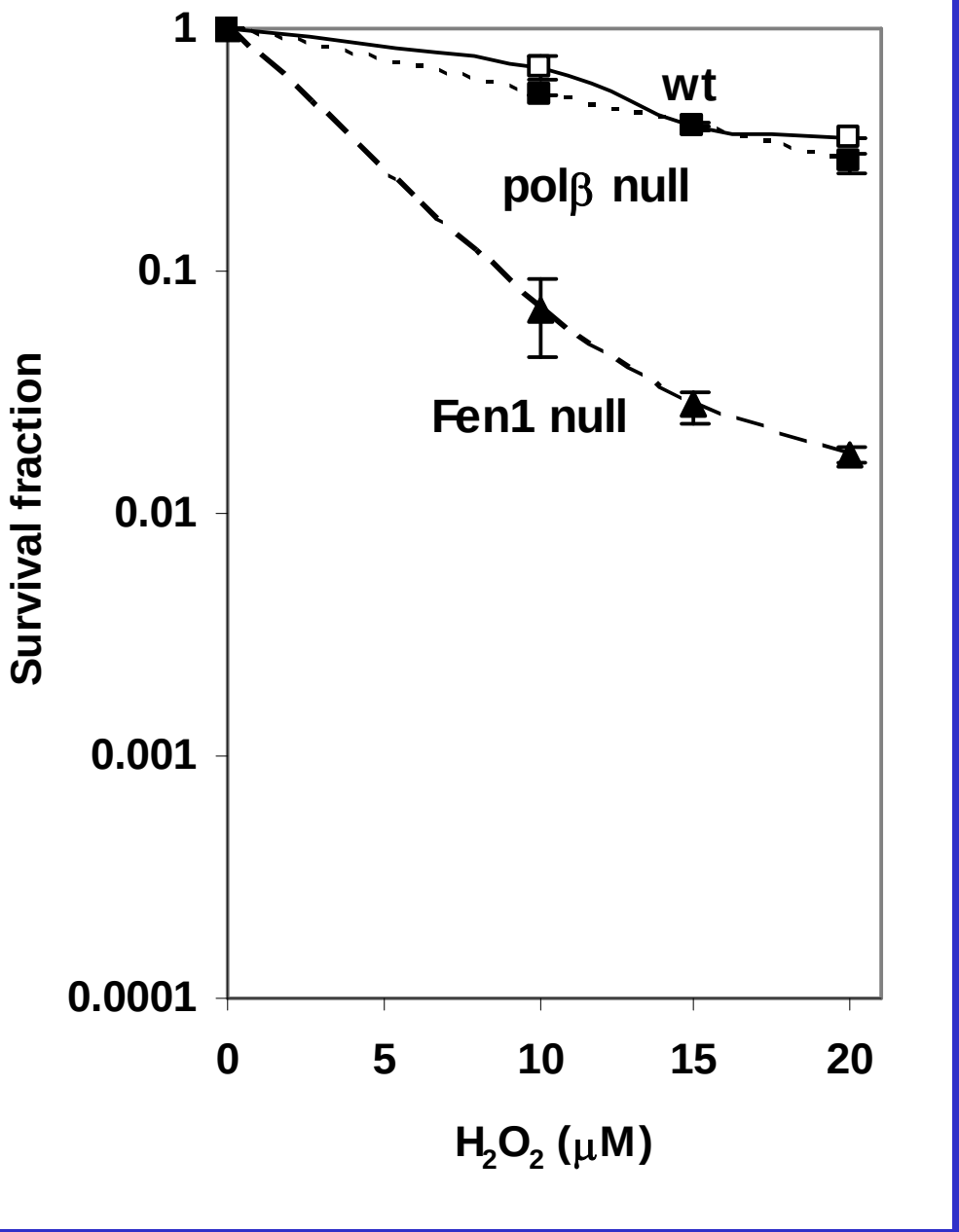
1,4-NaphDiol

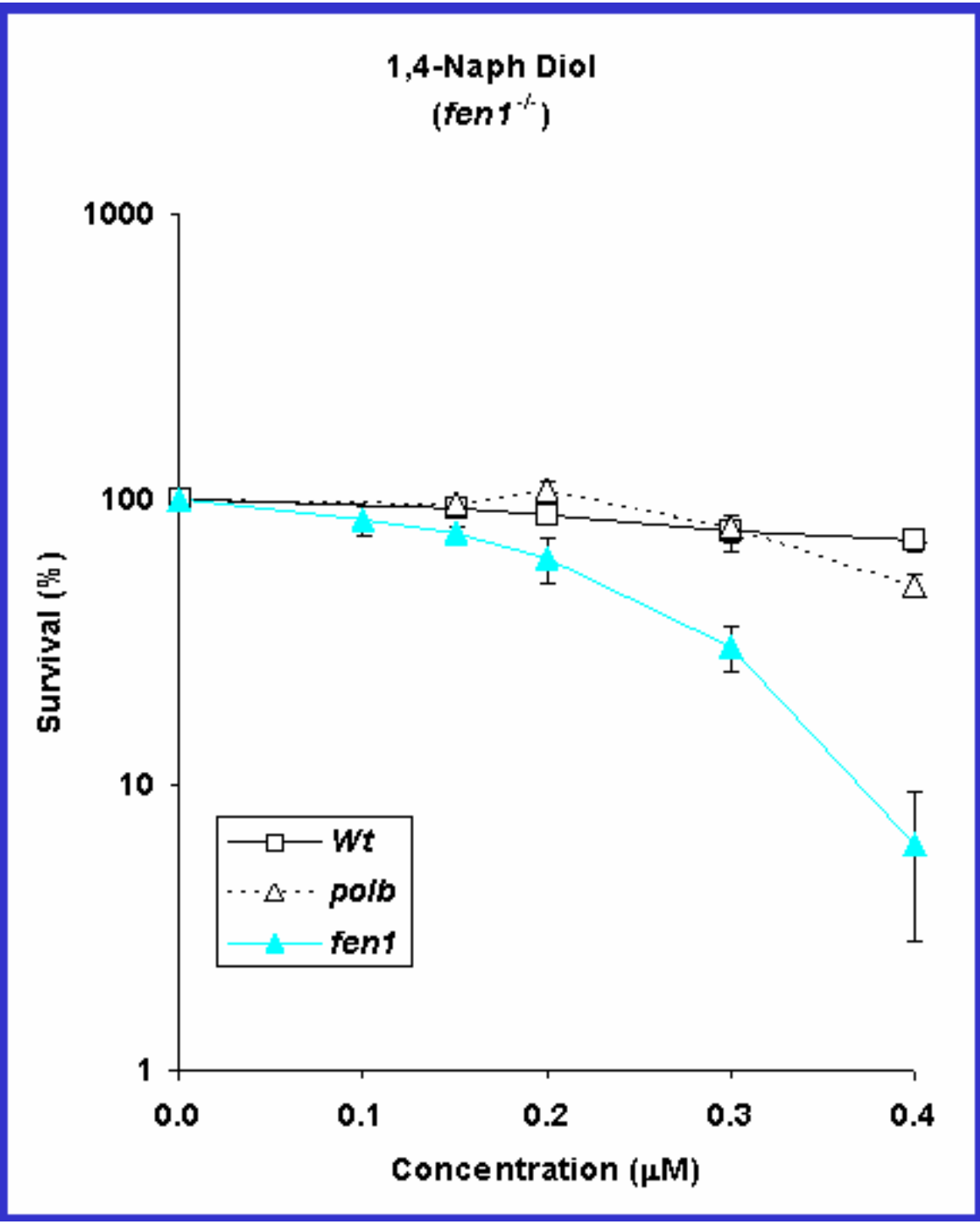
Assays

survival assay


1,4-Naph Diol
1000
100
10
1
Survival (%)
Wt
0.0
0.1
0.2
0.3
0.4
Concentration (µM)


1,4-Naph Diol
(pol β-/-)
1000
100
10
1
Survival (%)
Wt
polb
0.0
0.1
0.2
0.3
0.4
Concentration (µM)


1
0.1
0.01
0.001
0.0001
Survival fraction
wt
polβ null
Fen1 null
0
5
10
15
20
H2O2 (μM)


1,4-Naph Diol
(fen1 -/-)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
fen1

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Vertebrate POLQ and POLβ Cooperate in Base Excision Repair of Oxidative DNA Damage

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Kyoto 606-8507

[3] Research Reactor Institute
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Osaka 590-0494
Japan

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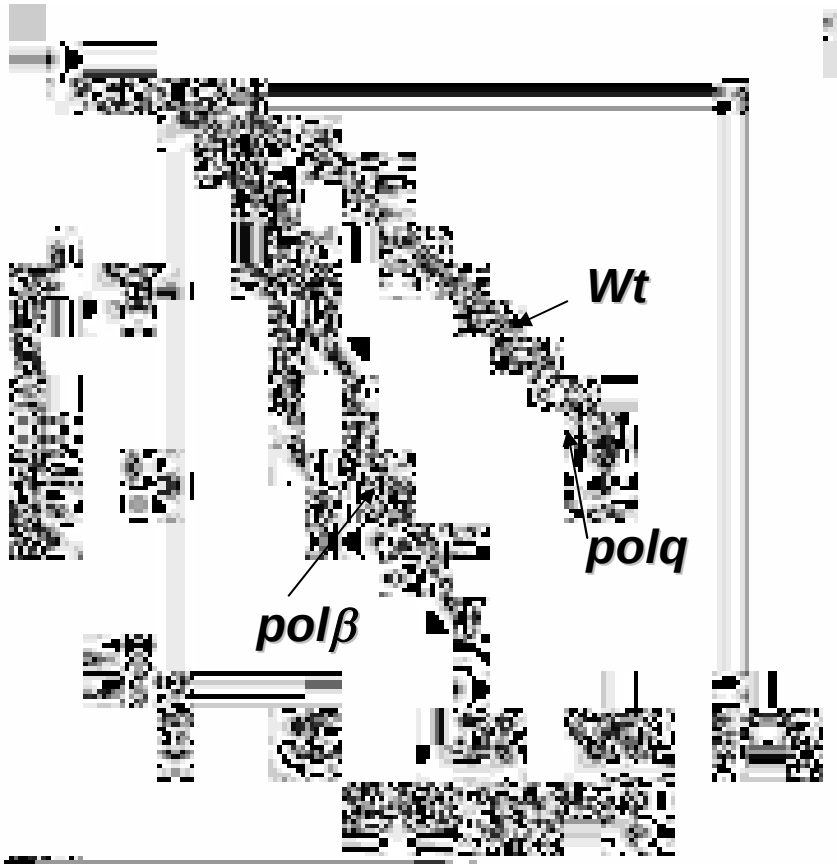
Chapel Hill, North Carolina 27599

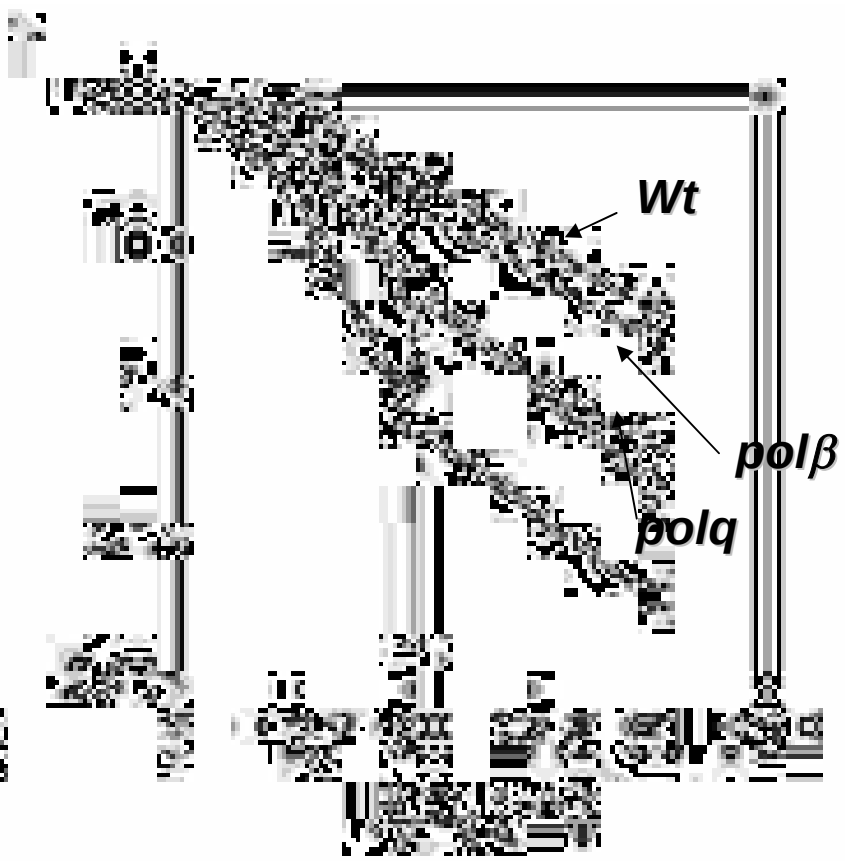
Summary

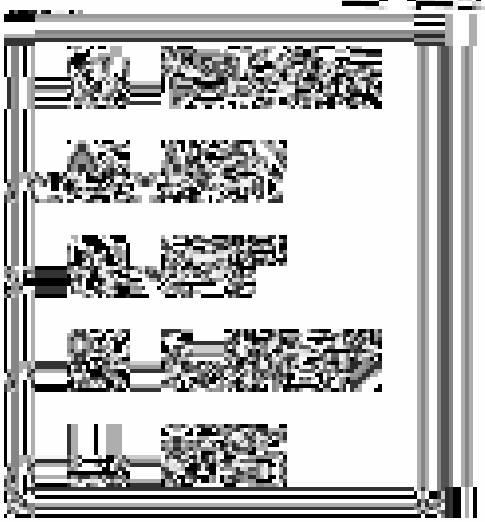
Base excision repair (BER) plays an essential role in protecting cells from mutagenic base damage caused by oxidative stress, hydrolysis, and environmental factors. POLQ is a DNA polymerase, which appears to be involved in translesion DNA synthesis (TLS) past base damage. We disrupted POLQ, and its homologs HEL308 and POLN in chicken DT40 cells, and also created *polq/hel308* and *polq/poln* double mutants. We found that *POLQ*-deficient mutants exhibit hypersensitivity to oxidative base damage induced by H_2O_2, but not to UV or cisplatin. Surprisingly, this phenotype was synergistically increased by concomitant deletion of the major BER polymerase, POLβ. Moreover, extracts from a *polq* null mutant cell line show reduced BER activity, and POLQ, like POLβ, accumulated rapidly at sites of base damage. Accordingly, POLQ and POLβ share an overlapping function in the repair of oxidative base damage. Taken together, these results suggest a role for vertebrate POLQ in BER.

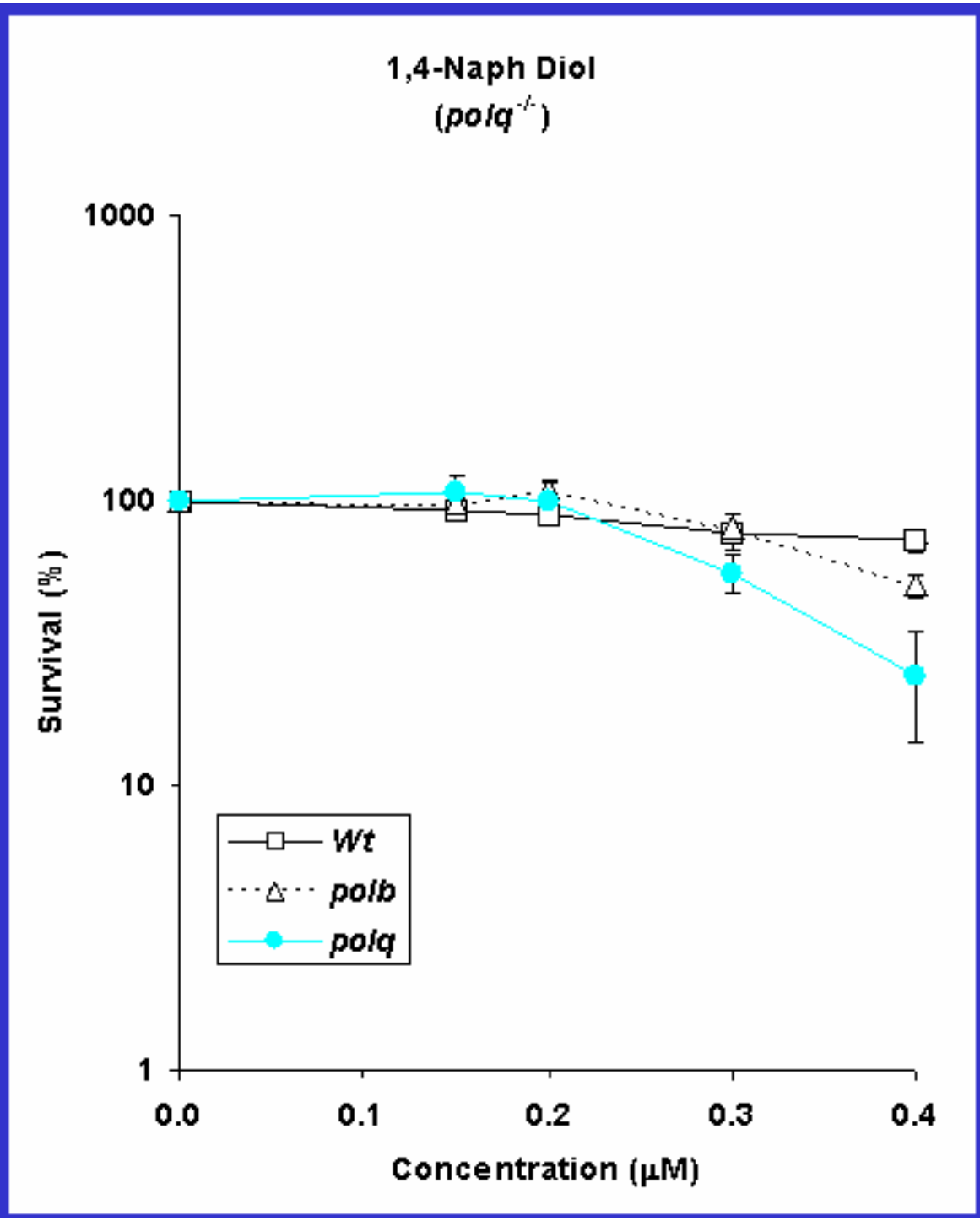
Introduction

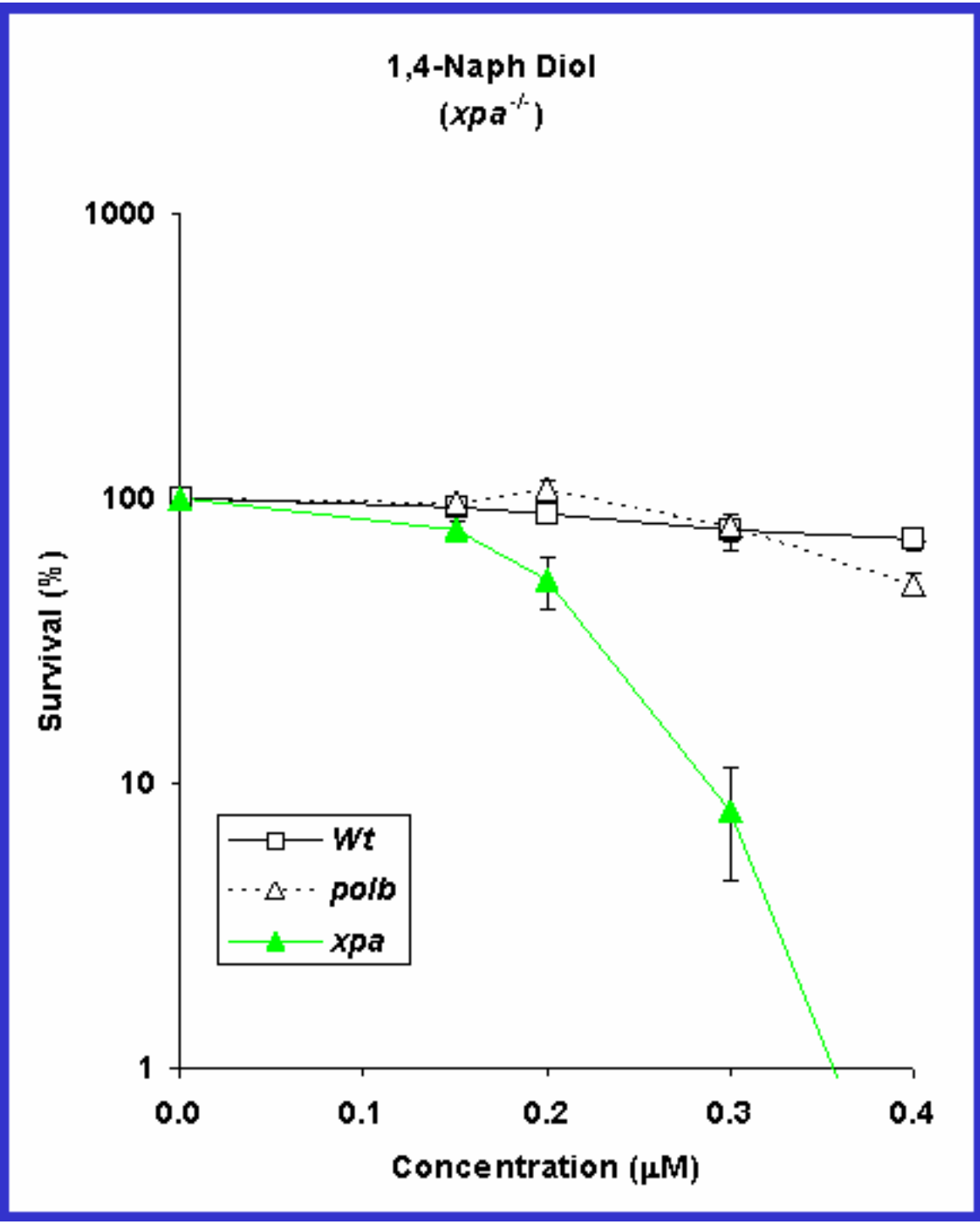
Base excision repair (BER) is a crucial process that eliminates many types of base damage that arise endogenously (Lindahl, 1993). Typically, BER begins with a lesion-specific DNA glycosylase that eliminates the damaged base from the deoxyribose of DNA strands (Barnes and Lindahl, 2004). Strand incision is carried

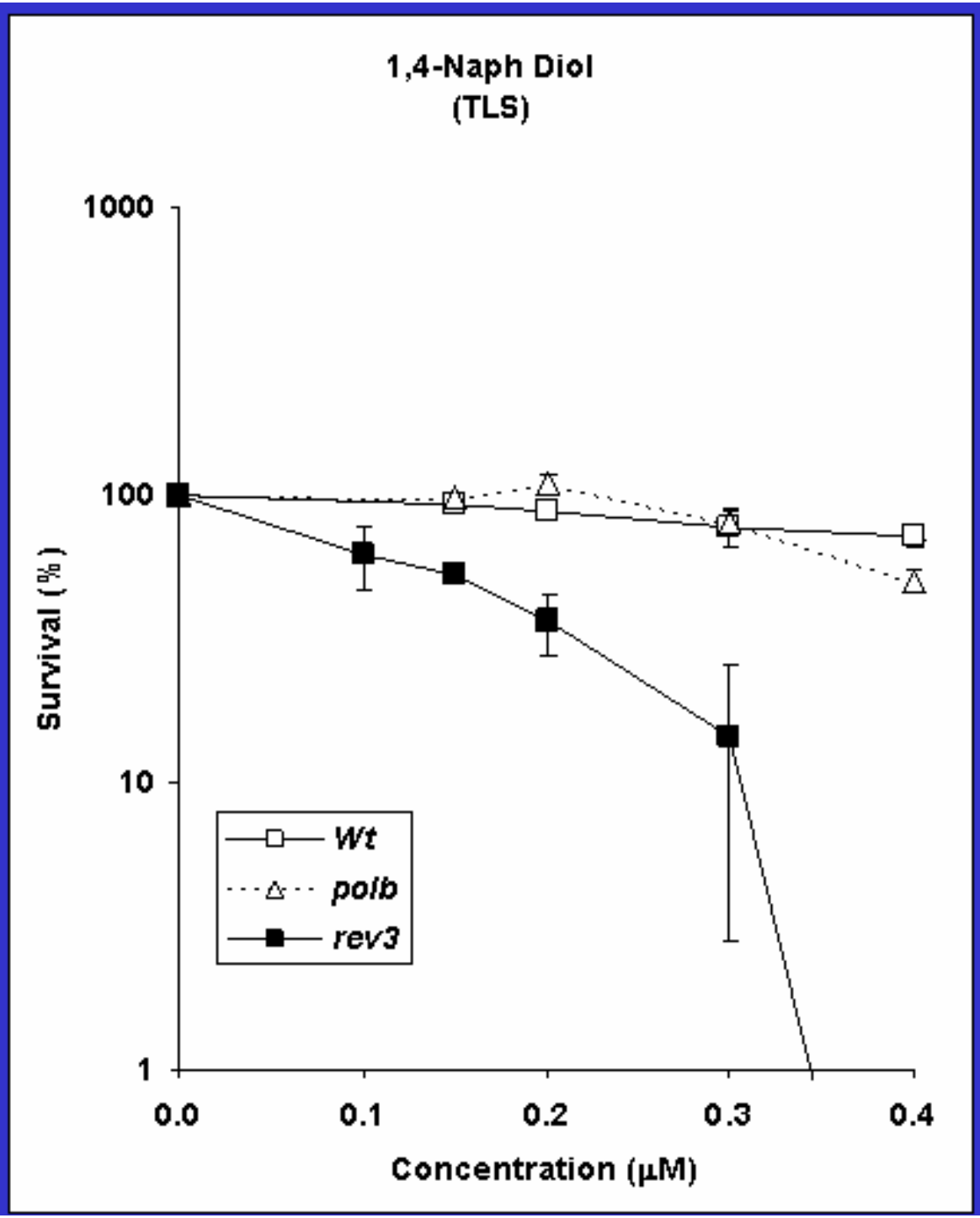

Wt
polq
polβ

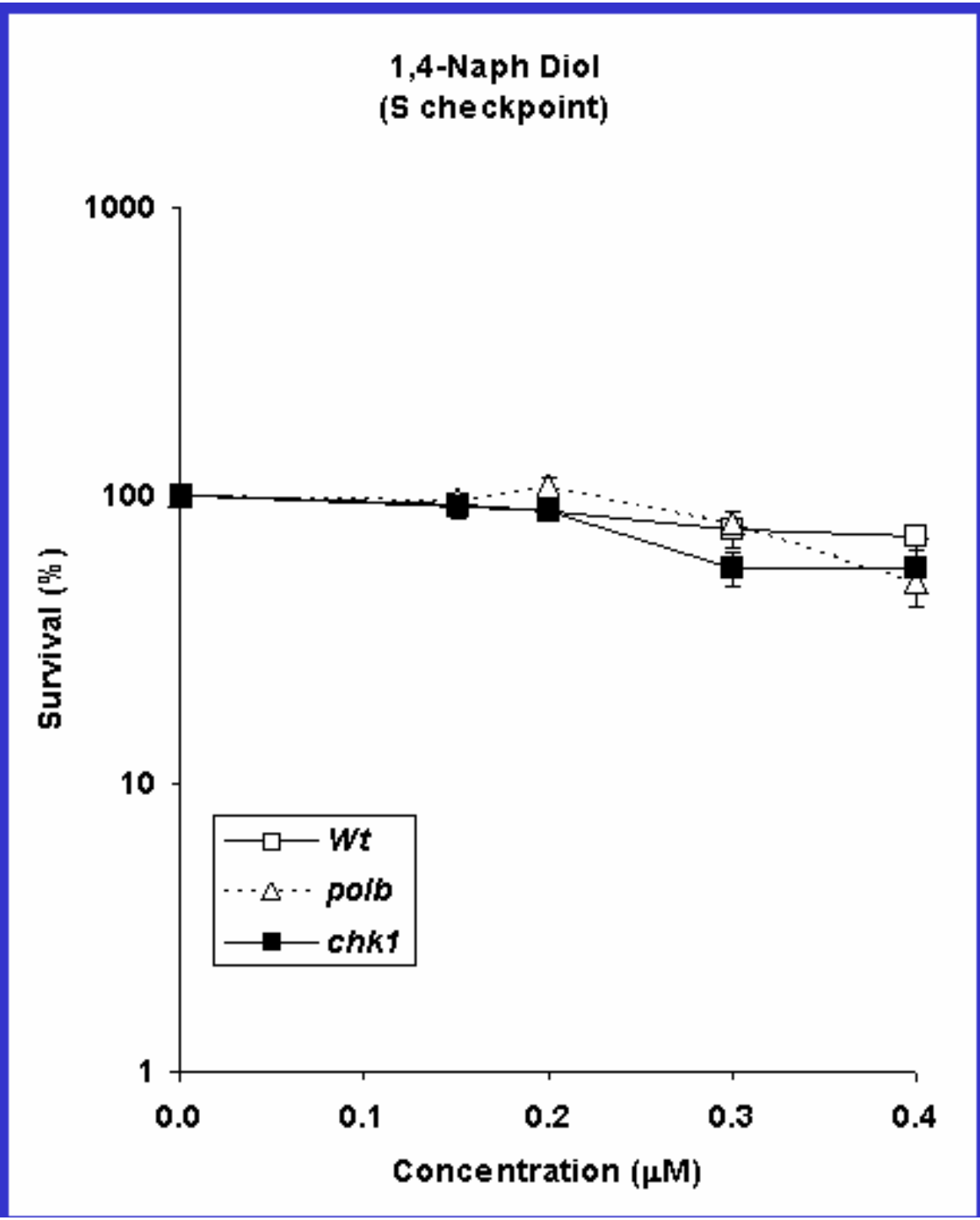

Wt
polβ
polq

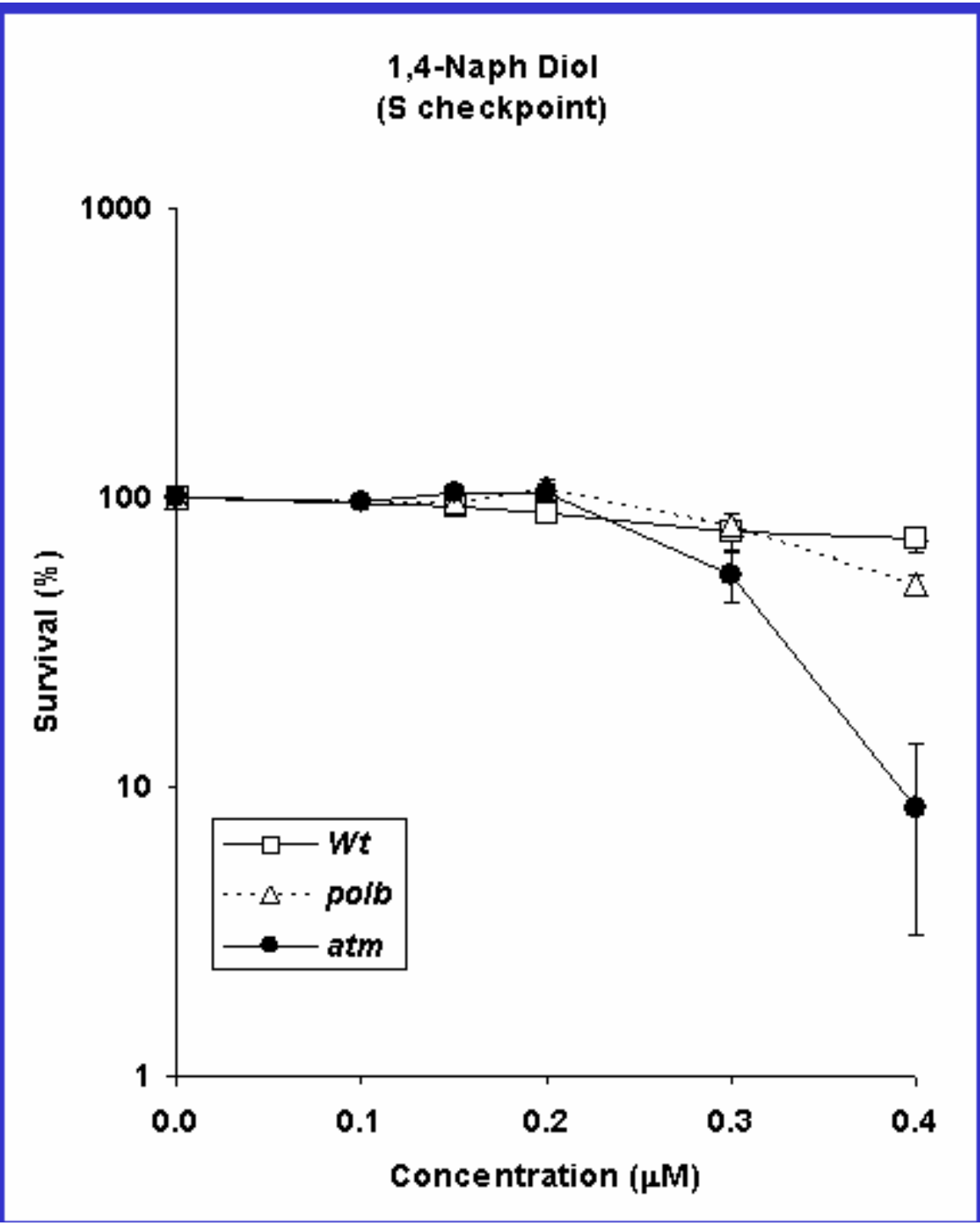


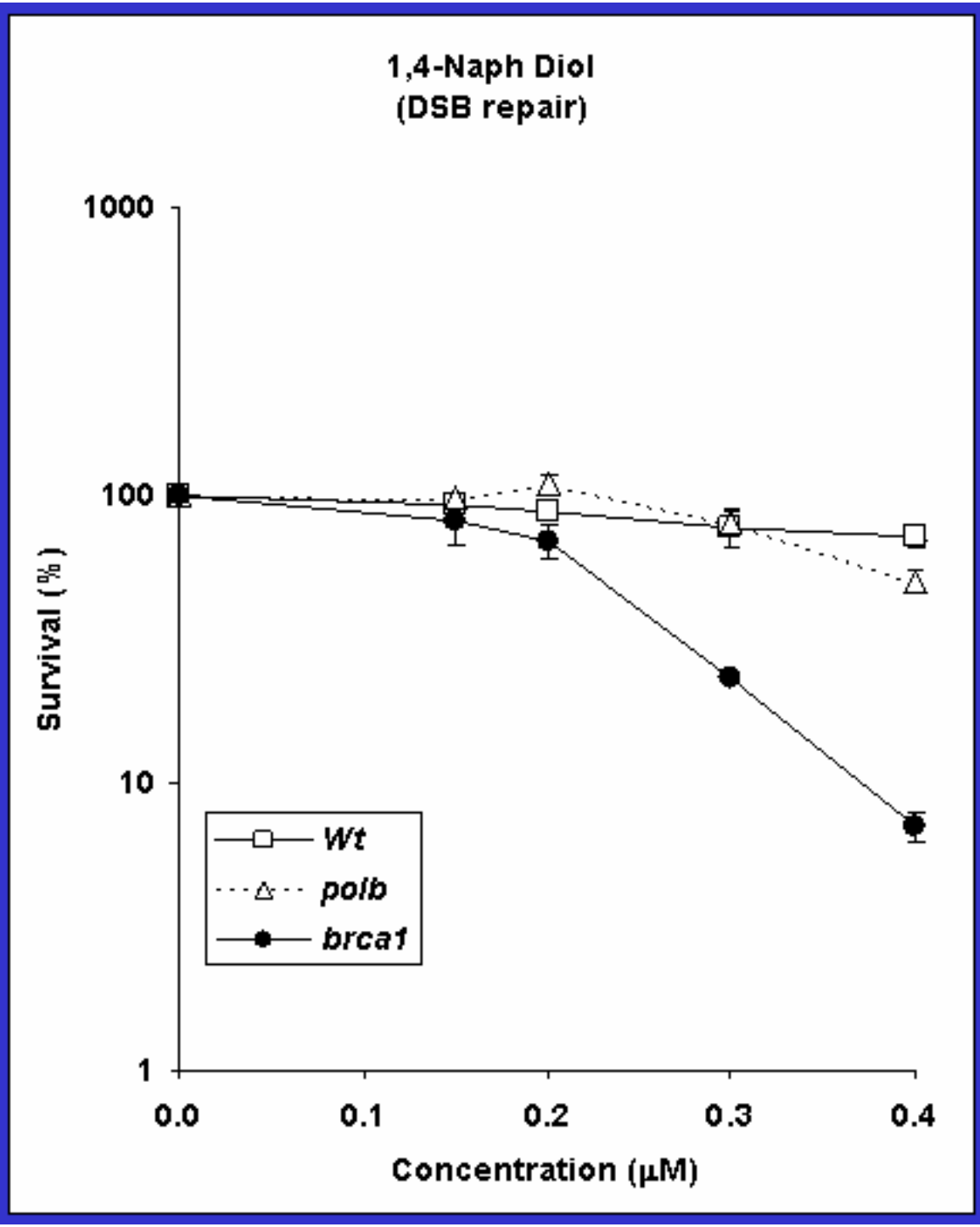

1,4-Naph Diol
(polq-/-)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
polq

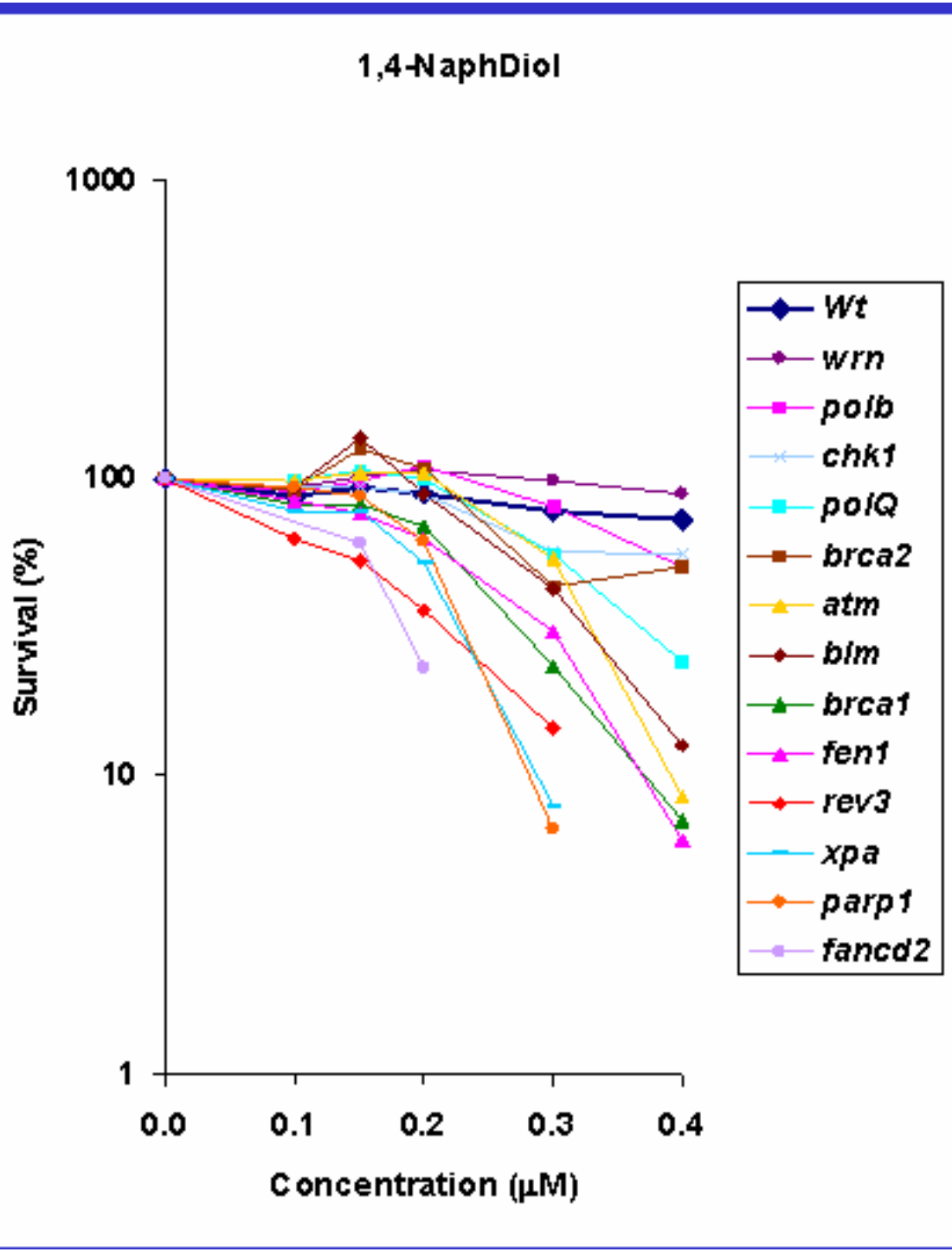

1,4-Naph Diol
(xpa-/-)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
xpa


1,4-Naph Diol
(TLS)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
rev3


1,4-Naph Diol
(S checkpoint)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
chk1


1,4-Naph Diol
(S checkpoint)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
atm


1,4-Naph Diol
(DSB repair)
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
polb
brca1


1,4-NaphDiol
1000
100
10
1
Survival (%)
0.0
0.1
0.2
0.3
0.4
Concentration (µM)
Wt
wrn
polb
chk1
polQ
brca2
atm
blm
brca1
fen1
rev3
xpa
parp1
fancd2

Conclusions

- DNA damage response analyses using isogenic DT40 cell model system are a novel and powerful experimental procedure to identify genotoxicity and DNA damage response for new chemicals .
- Abasic sites unlikely play a significant impact on DNA damage response in DT40 cells exposed to naphthalene metabolites.
- Oxidized DNA lesions and bulky adducts may cause cell death in DT40 cells deficient in various DNA damage response pathways.
- 1,4-NaphDiol introduces a serious cell death in some of DT40 cells deficient in DNA repair pathways at 300 nM, which cause little cytotoxic effects in wild-type DT40, indicating that naphthalene metabolites have a significant genotxicity.

Lab member and collaborators

- April Luke
- Brian F. Pachkowski
- John R. Ridpath
- Jennifer Dalrymple

- James A. Swenberg
- Shunichi Takeda (Kyoto University)
- Keizo Tano (Kyoto University)

Collaborations

- Need protocols
- Test your compounds
- Need new assays
- Other collaborations

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