Mammalian Translesion DNA Synthesis: Are Y-family Polymerases Essential?

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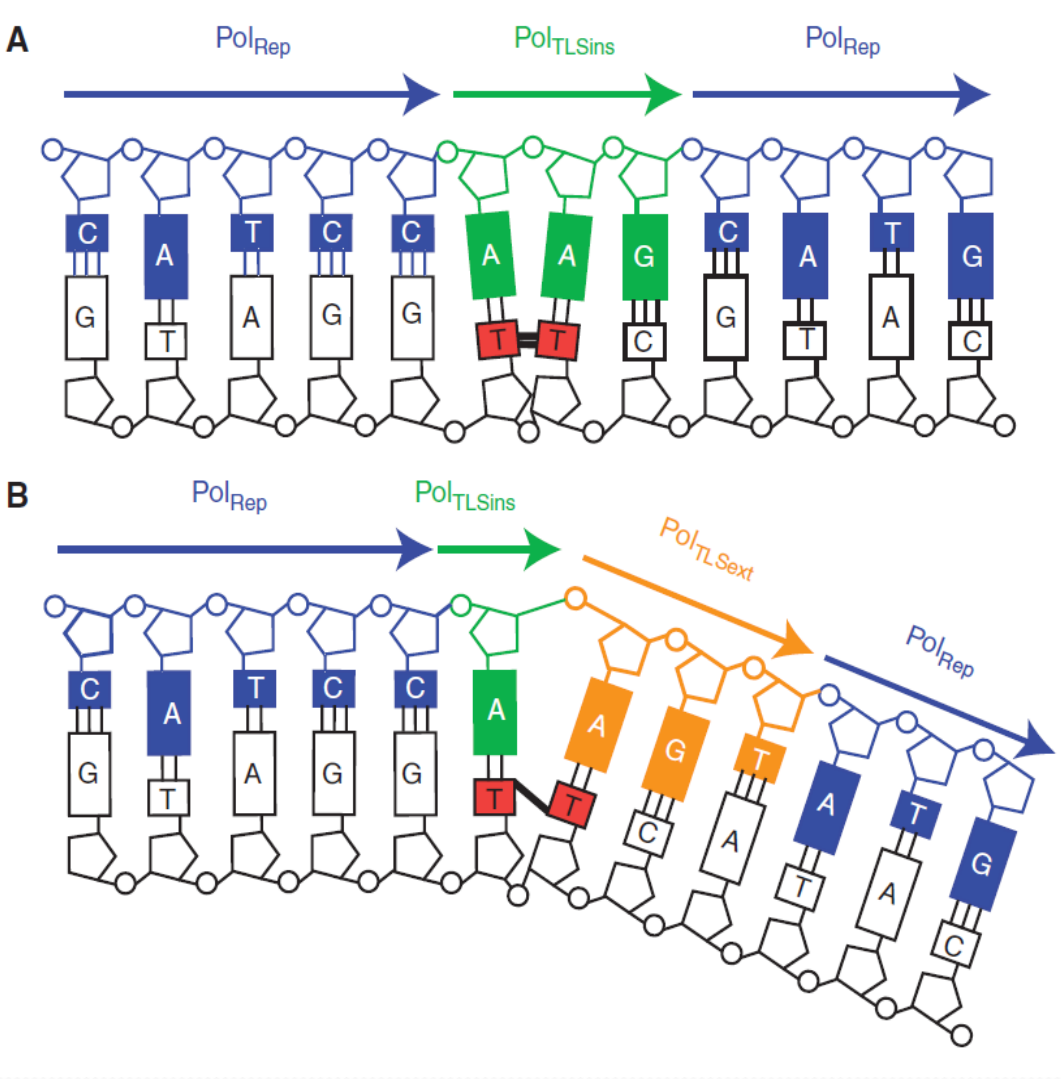
One-step versus Two-step TLS



One-step TLS

Inserter and Extender:

Pol η for CPD

Two-step TLS

Inserter:

Pol η, ι, κ, (Rev1)

Extender:

Rev1, pol ζ, (κ)

From J. Sale 2013

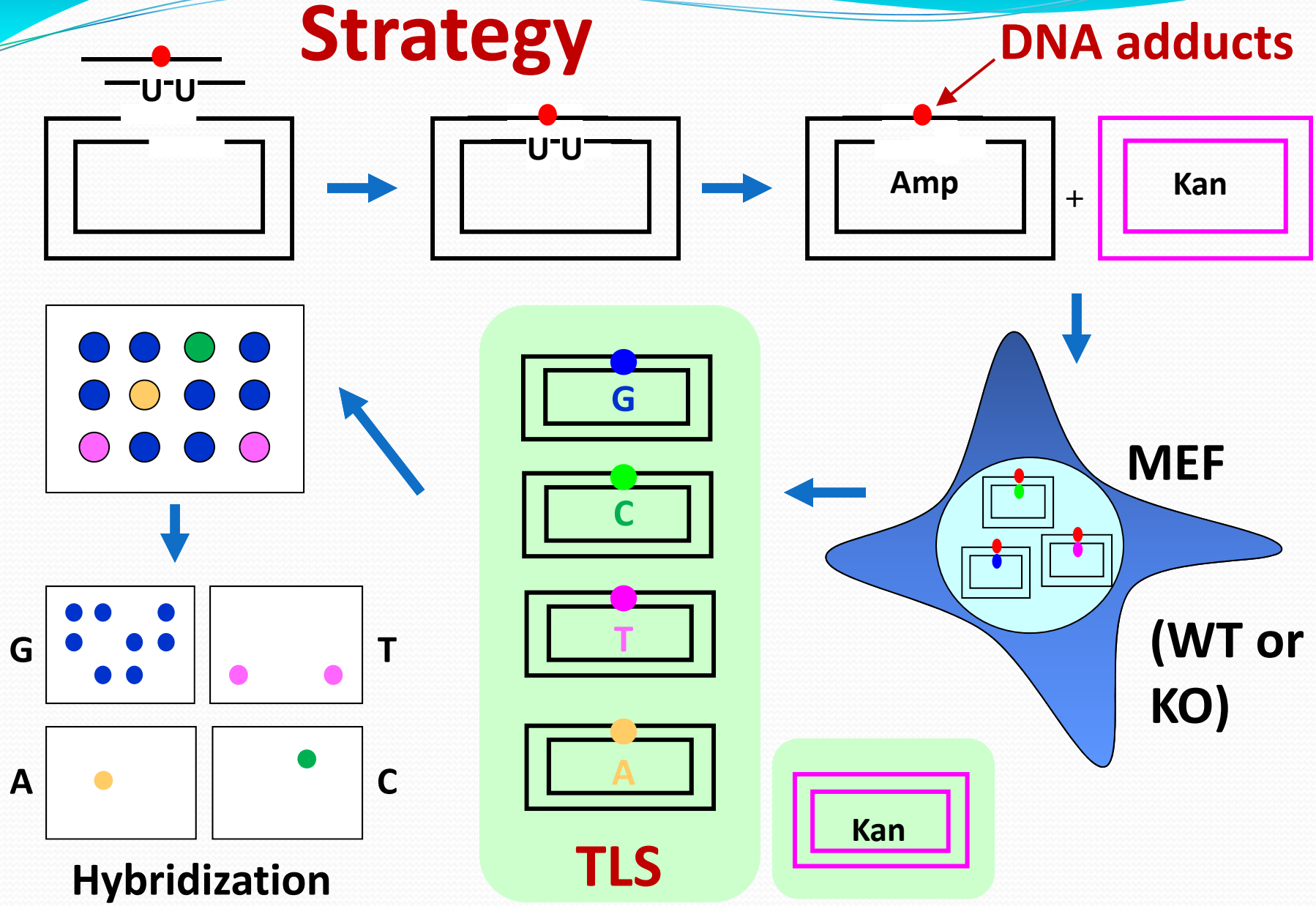
Examine two-step TLS

- Which pol(s) is the inserter?
- Which pol(s) is the extender?

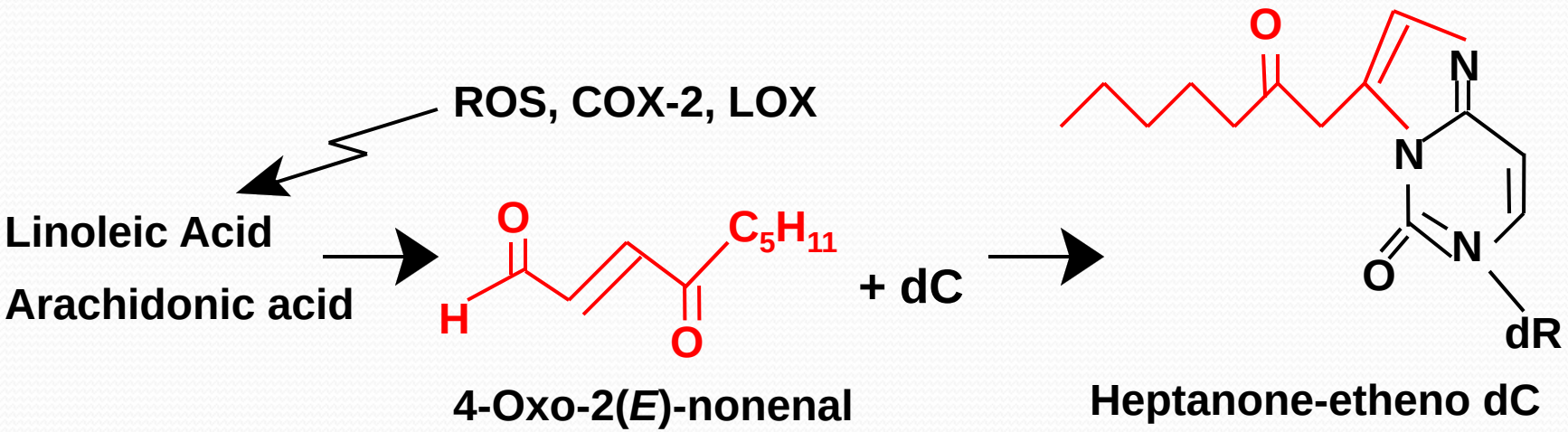
Three DNA adducts

- Heptanone-ε-dC (H-εdC)
- Bezo[*a*]pyrene-dG (BPDE-dG)
- Aristolactam-dA (dA-AL-I)

Strategy


U-U
U-U
DNA adducts
Amp
+
Kan
MEF
(WT or KO)
G
C
T
A
TLS
Kan
G
T
A
C
Hybridization

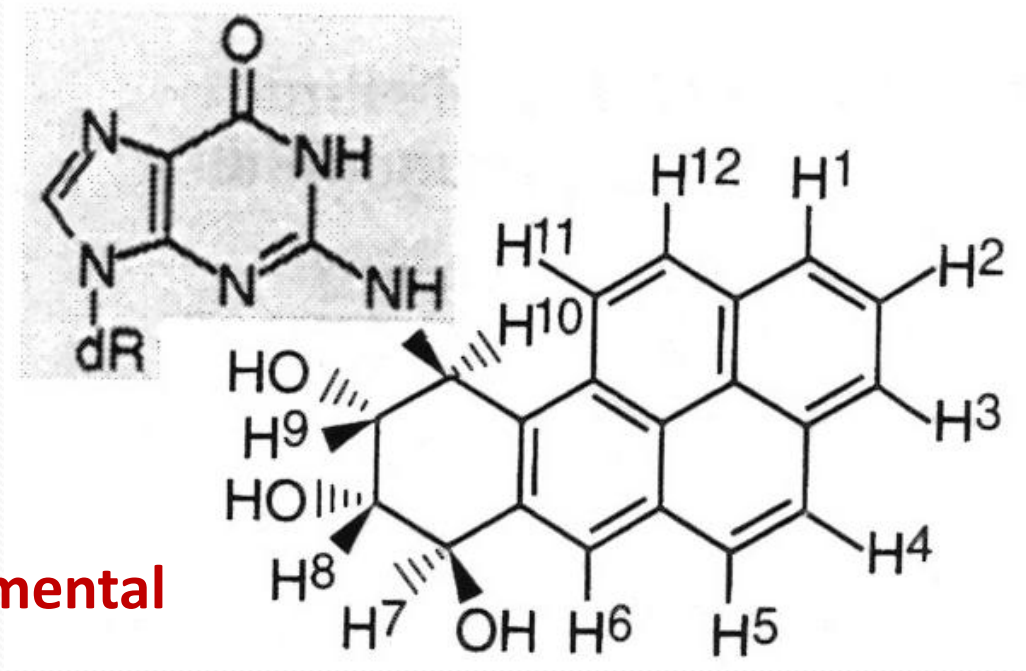
Heptanone-etheno-dC



- An **endogenous DNA adduct** and a biomarker of **lipid peroxidation**
- Detected in COX2-overexpressing tissues of a mouse colon tumor model
- **Detected in human autopsy tissues**
- **Extremely miscoding**

BPDE-dG

(bezo[*a*]pyrene-dG)

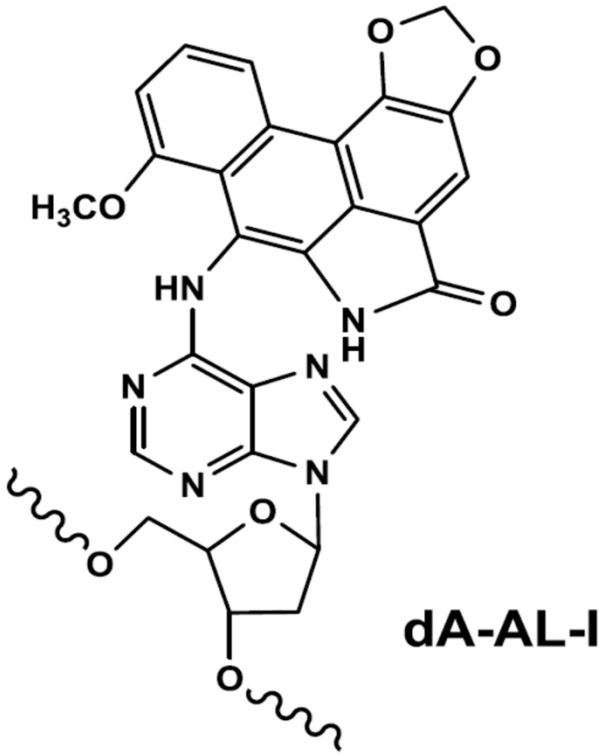


From E. Friedberg et al 1995

- Benzo[*a*]pyrene is
  - ✓ a well known **environmental carcinogen**
  - ✓ present in the smokes of **tobacco** and **fossil fuel** combustion
- Its metabolite, **benzo[*a*]pyrene dihydrodiol epoxide (BPDE)**, forms bulky DNA adducts mainly on dG
- **Highly mutagenic**

dA-AL-I

7-(deoxyadenosin-N6-yl)-aristolactam I



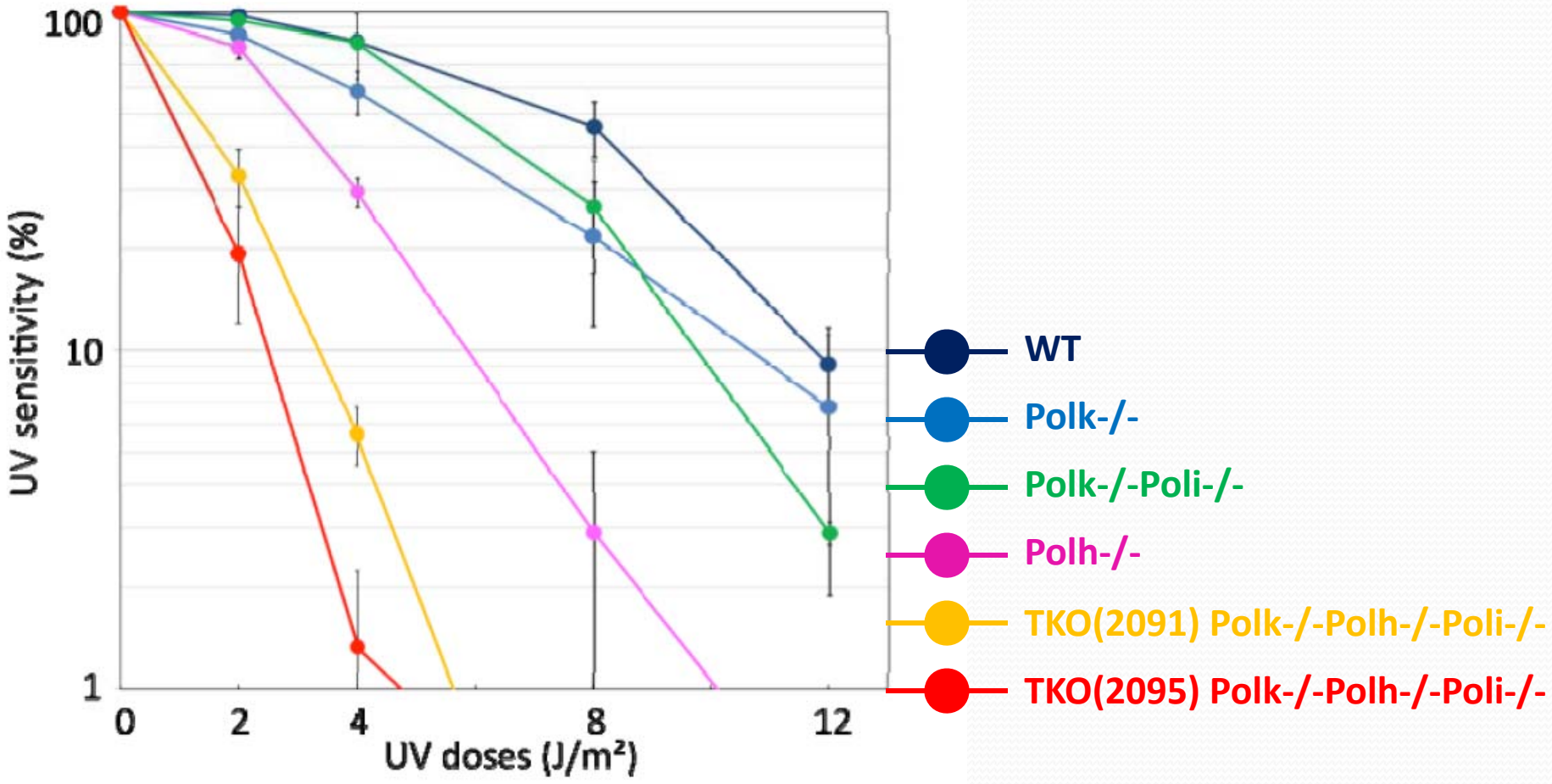
dA-AL-I

- **Aristolochic acid** is
  - ✓ **nephrotoxin** & **human carcinogen**
  - ✓ observed in all ***Aristolochia*** **plants**
  - ✓ associated with **chronic kidney disease** and **urothelial carcinomas of upper urinary tract**
- **AL-DNA adducts** cause **signature mutations**:
  - ✓ **A to T transversions** (73% of single-base substitutions)
  - ✓ located predominantly **on the non-transcribed strand**

TLS in TKO (triple knock-out) MEFs

- Deficient in Y-family inserter pols, η, ι, and κ
- Extremely sensitive to UV

TKO cells are extremely sensitive to UV



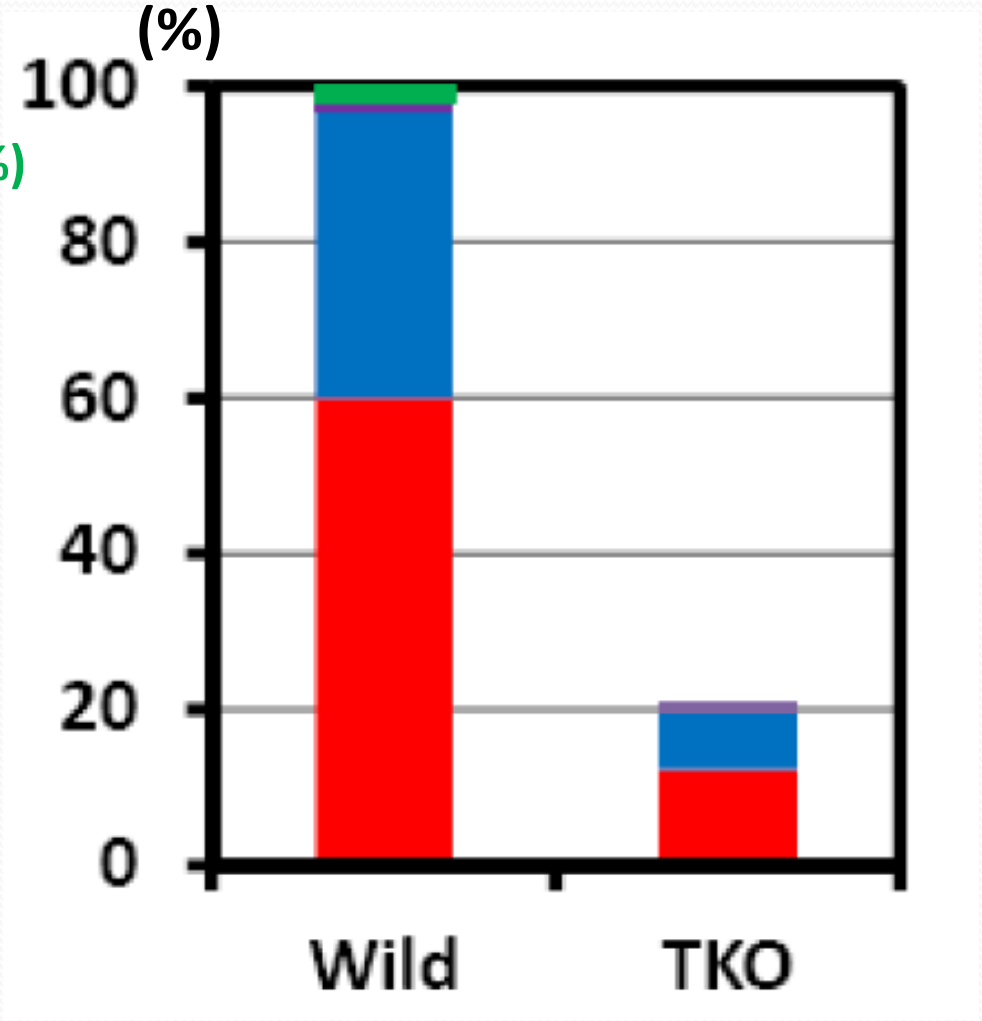
TLS across Heptanone-ε-dC is reduced in TKO Cells

H (Heptanone-ε-dC) → G (2%)

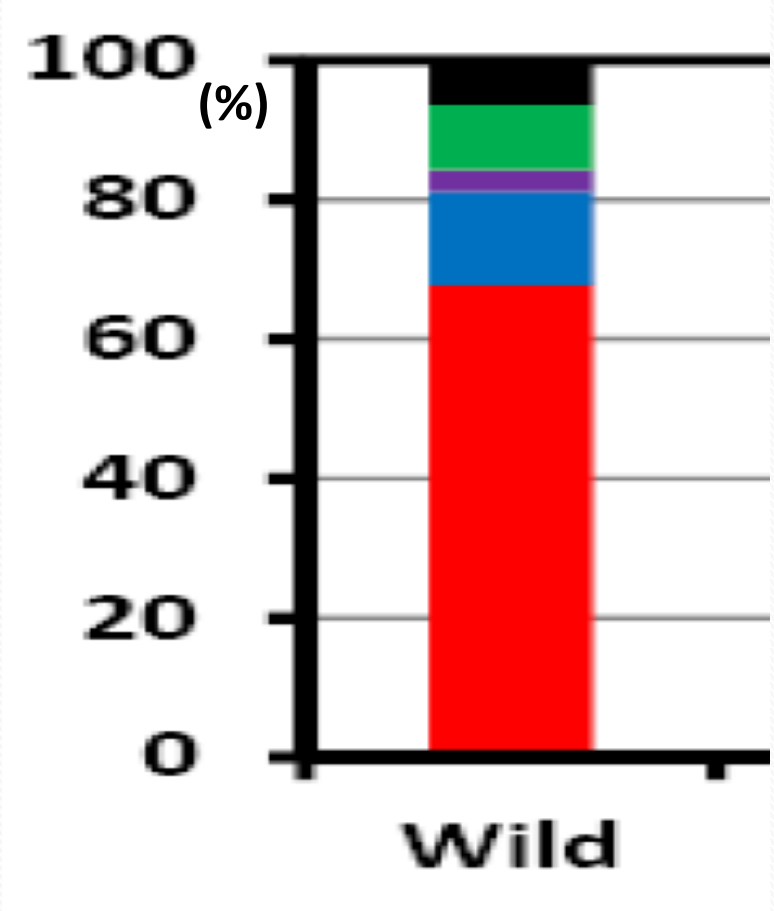
H → C (correct TLS is 1%)

H → A (37%)

H → T (60%)



TLS across BPDE-dG is highly mutagenic (>90%)



B (BPDE-dG) → others (5%)

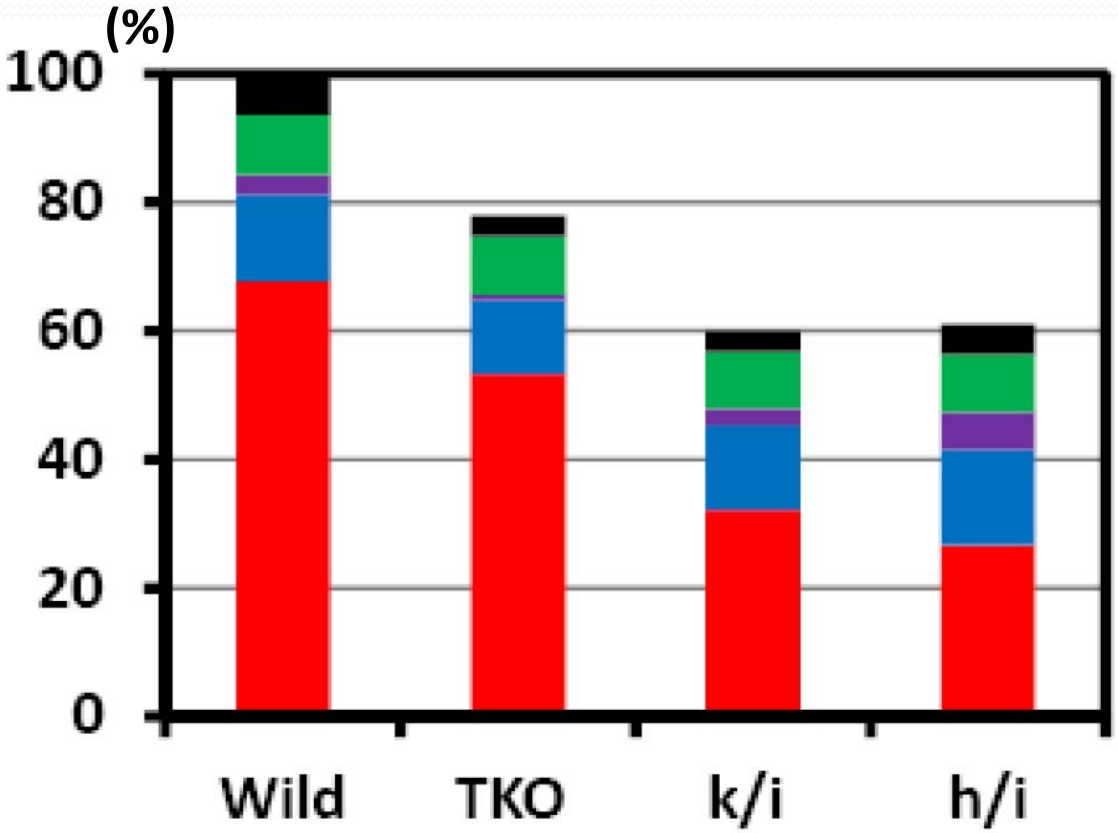
B → G (correct TLS is 9%)

B → C (1%)

B → A (12%)

B → T (73%)

TLS across BPDE-dG is Relatively Efficient in TKO Cells



B (BPDE-dG) → others

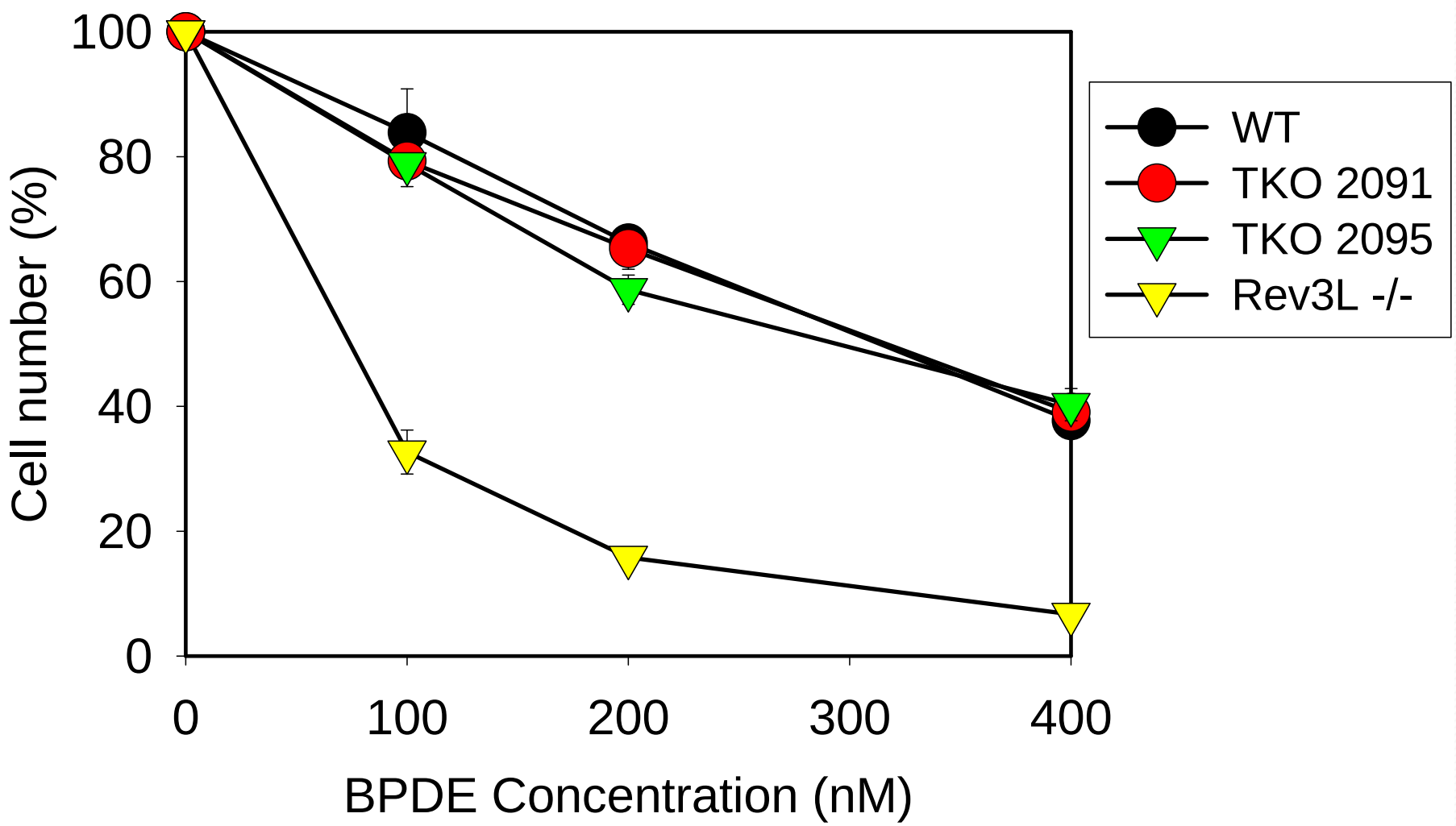
B → G (correct TLS)

B → C

B → A

B → T

TKO cells are NOT sensitive to BPDE



TLS across dA-AL-I is NOT affected in TKO Cells

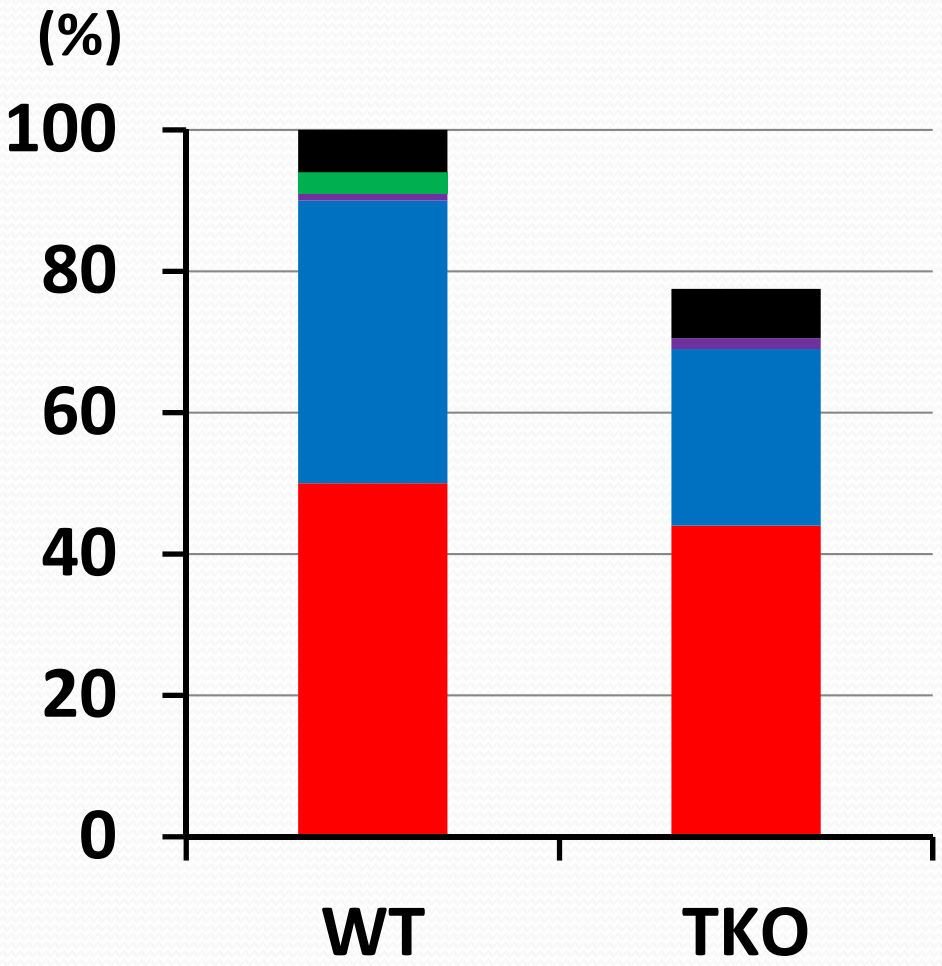
AL → Others (6%)

AL → Δ (3%)

AL → C (1%)

AL → A (correct 40%)

AL → T (50%)

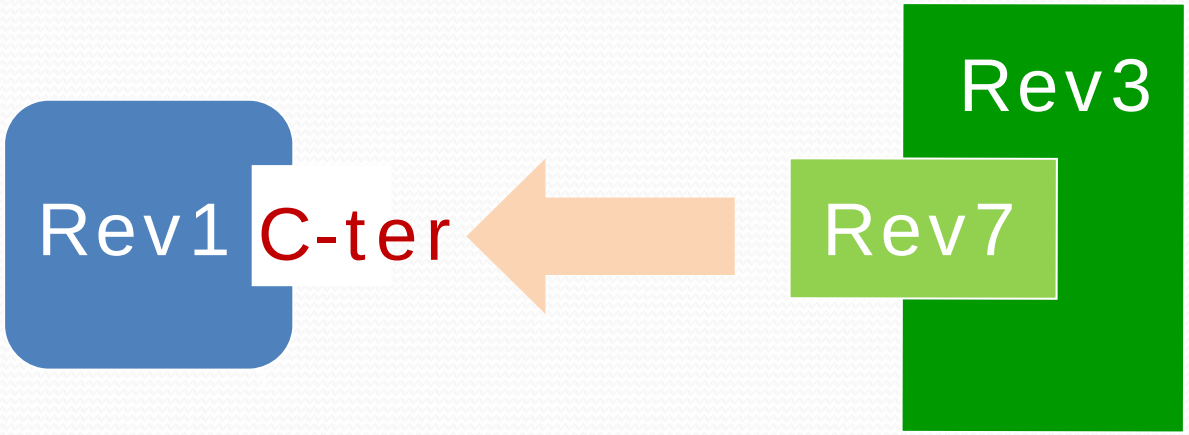


Requirement of Pol η, ι, or κ

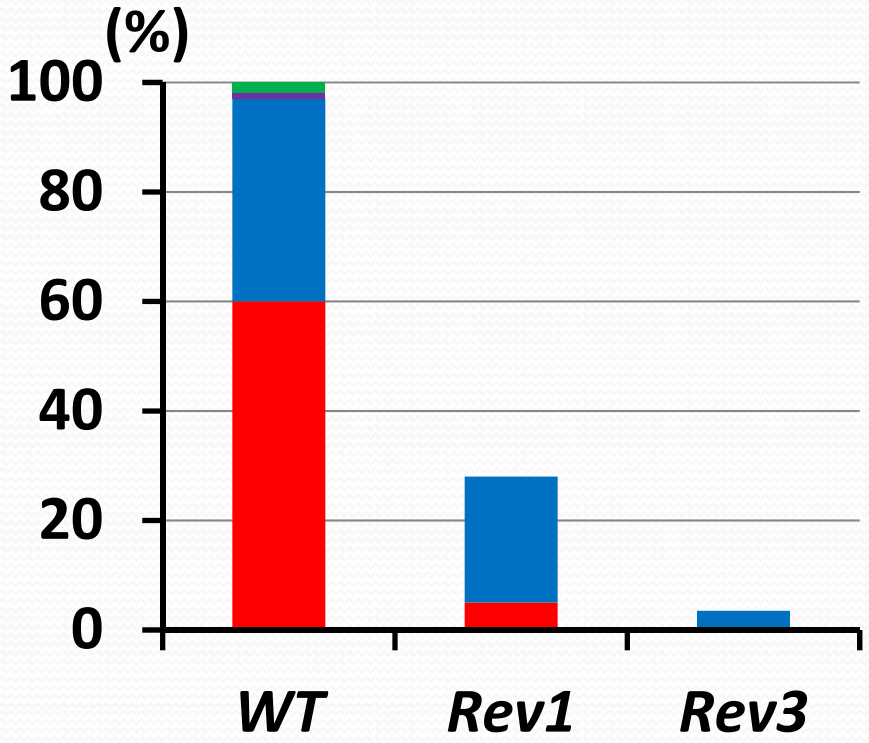
Heptanone-ε-dC	Yes
BPDE-dG	No
AL-I-dA	No

TLS in Rev1 KO MEFs and Rev3 KO MEFs

- **Rev1** C-terminal region recruits pol ζ (Rev3 + Rev7) through the interaction with Rev7
- **Pol ζ catalyze extension step**



TLS across Heptanone-ε-dC is greatly affected in Rev1 or Rev3 knockout cells



H (Heptanone-ε-dC) → G

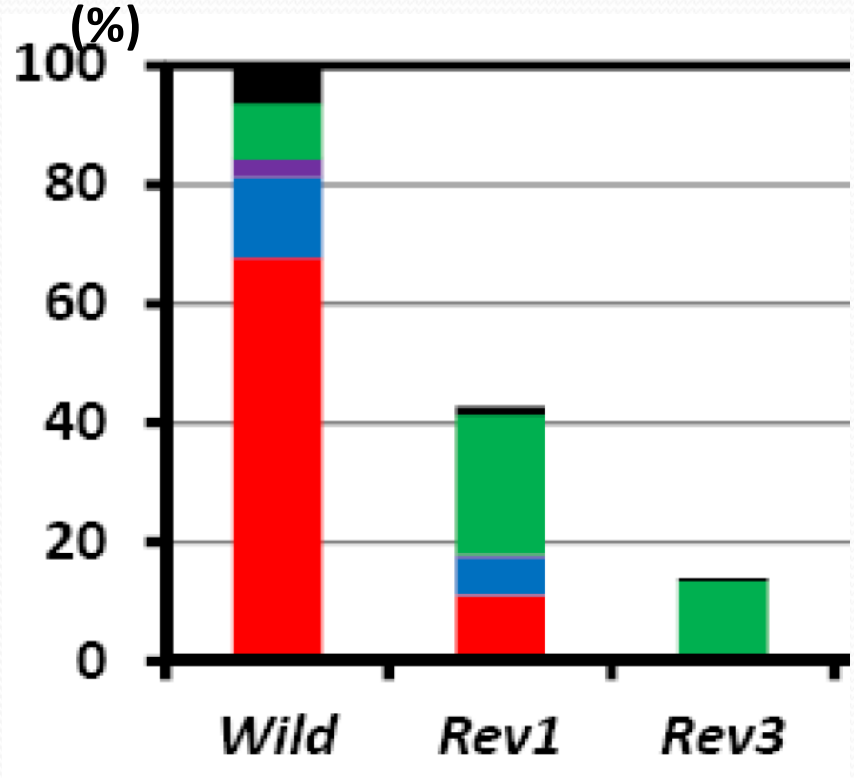
H → C

H → A

H → T

H→T is more dependent on Rev1 and Polζ than H→A

TLS across BPDE-dG is greatly affected in Rev1 or Rev3 knockout cells



B (BPDE-dG) → others

B → G (correct TLS)

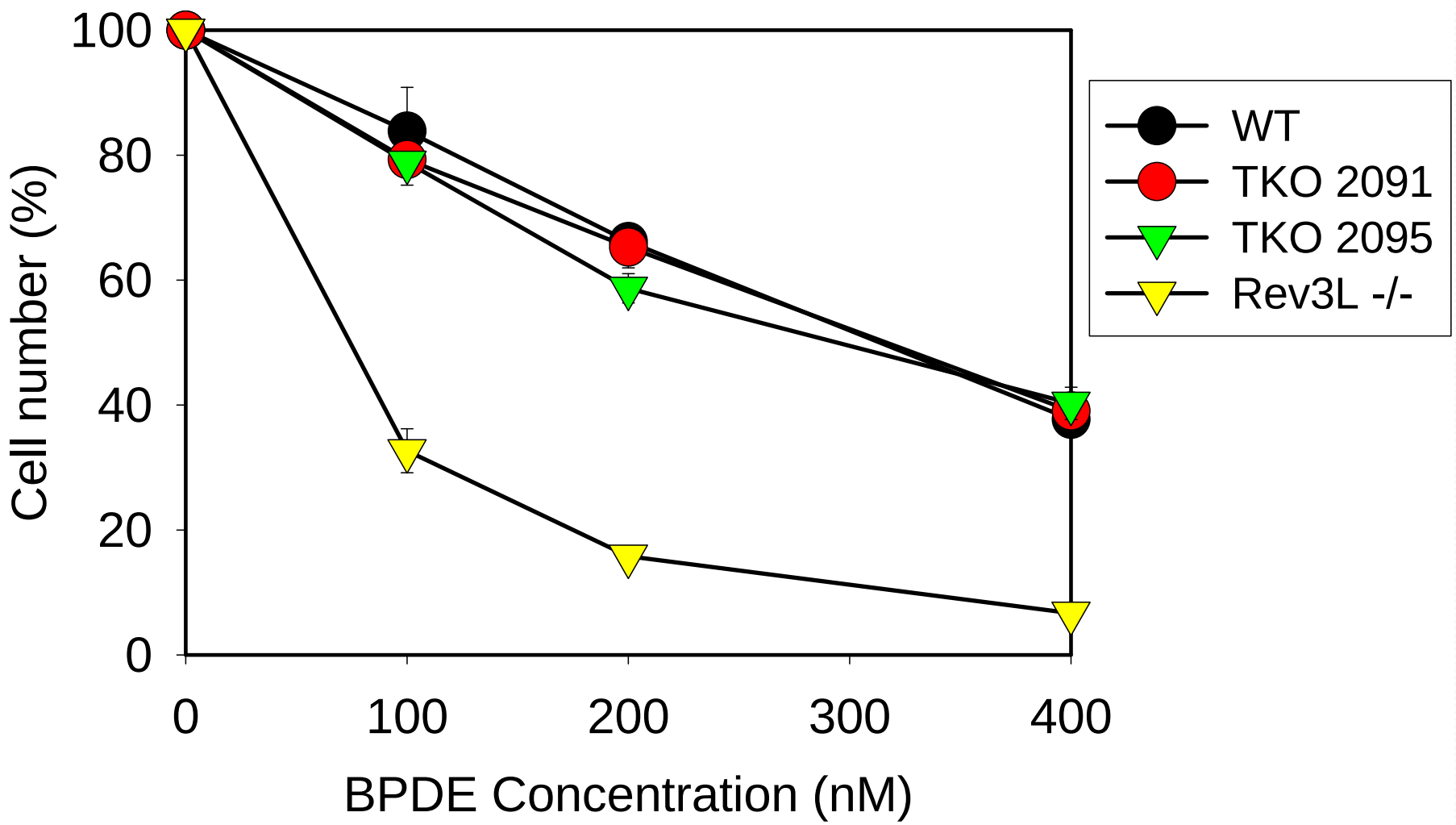
B → C

B → A

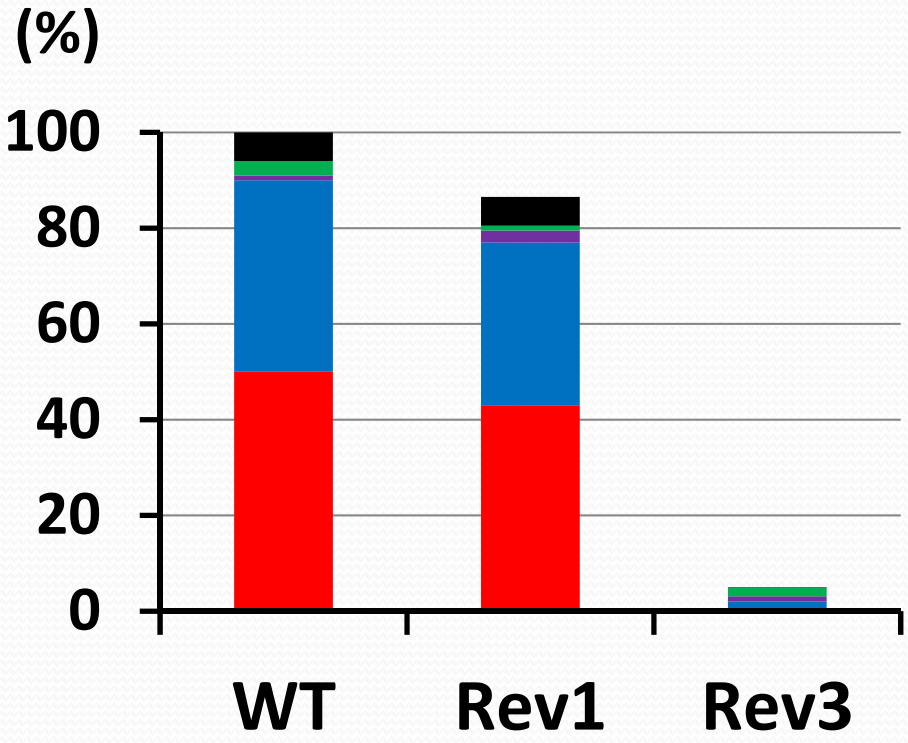
B → T

Mutagenic TLS is greatly dependent on Rev1 and Polζ

Rev3L-/- cells are sensitive to BPDE



TLS across dA-AL-I is NOT affected in Rev1, but greatly affected in Rev3 knockout cells



No AL → T in Rev3 KO cells

Rev1 and pol ζ are not epistatic

Requirement of Rev1 and Pol ζ

	Rev1	Pol ζ
Heptanone-ε-dC	Yes	Yes
BPDE-dG	Yes	Yes
AL-I-dA	No	Yes

Are Y-family pols essential?

	η, ι, κ	Rev1	Pol ζ
Heptanone-ε-dC	Yes	Yes	Yes
BPDE-dG	No	Yes	Yes
AL-I-dA	No	No	Yes

Which pol(s) inserts opposite adducts? When η, ι, and κ are Absent

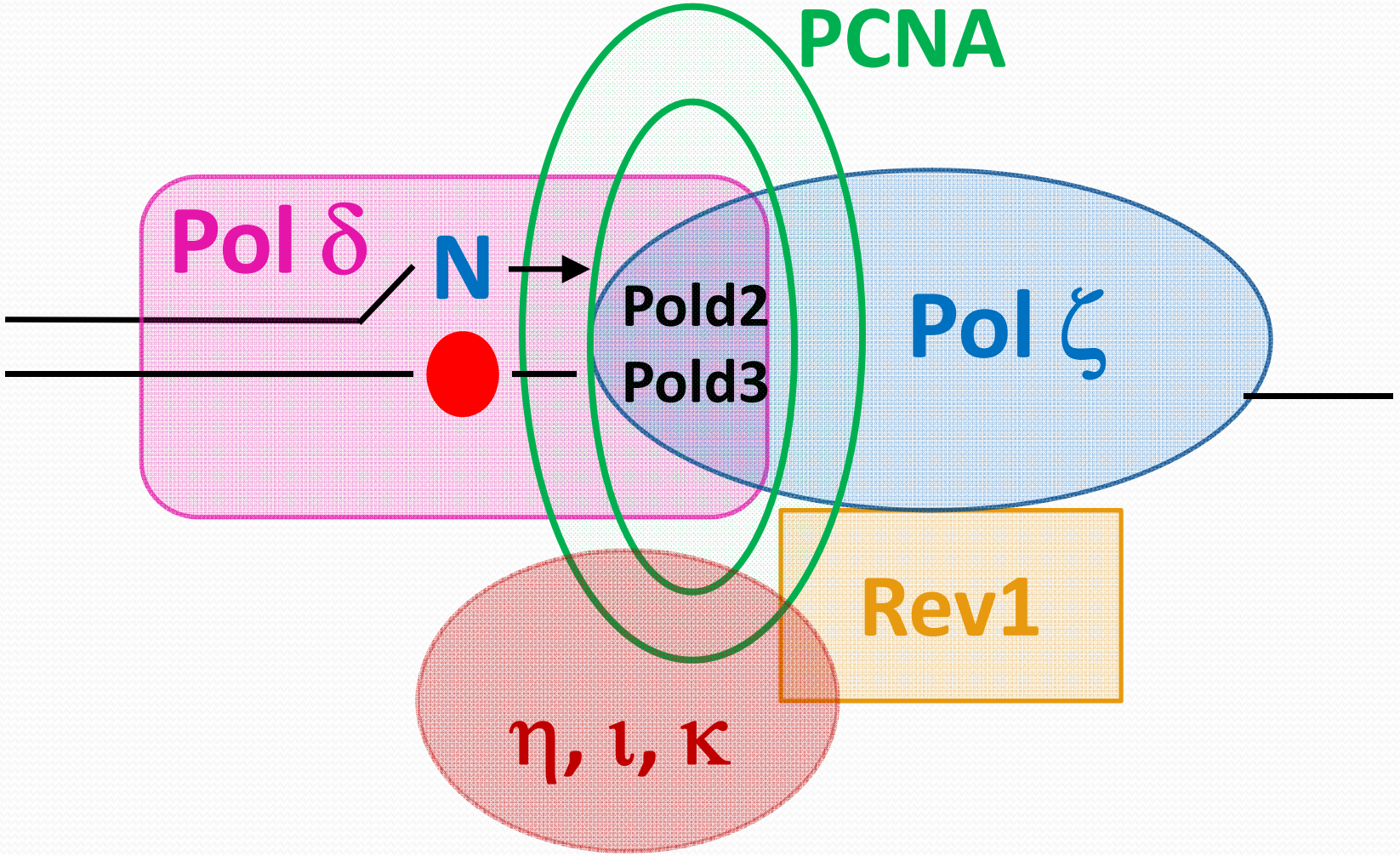
- Replicative **pol δ** inserts

  → Pol-switching with **Pol ζ** through shared subunits, **Pold2** and **Pold3**

- **Pol ζ** itself inserts

- **PrimPol** or other pols

How is Pol ζ recruited?



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