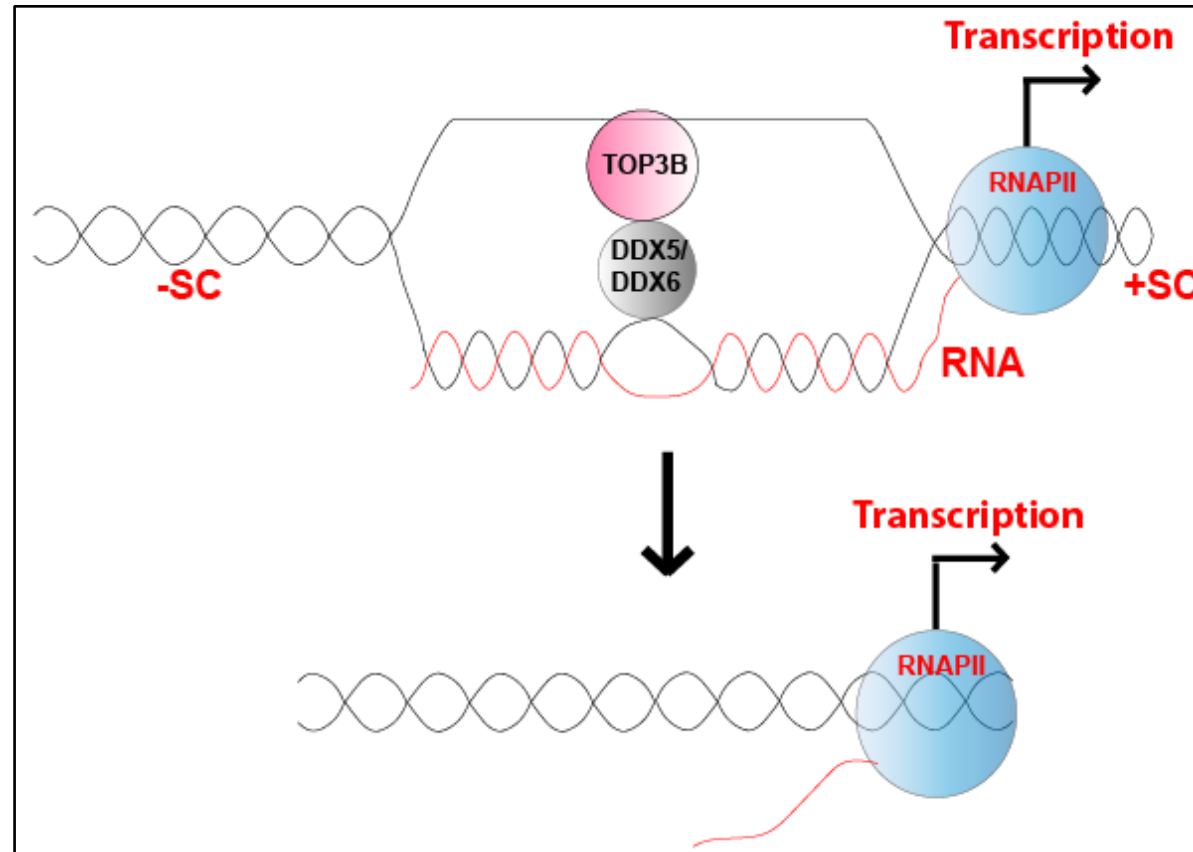# Human DNA/ RNA Topoisomerase TOP3-beta (TOP3B) is coordinated with the DEAD-box Helicases DDX5 and DDX6 to resolve R-Loops
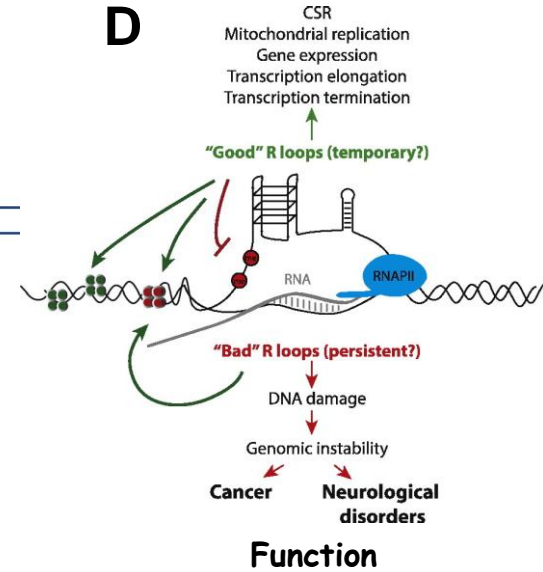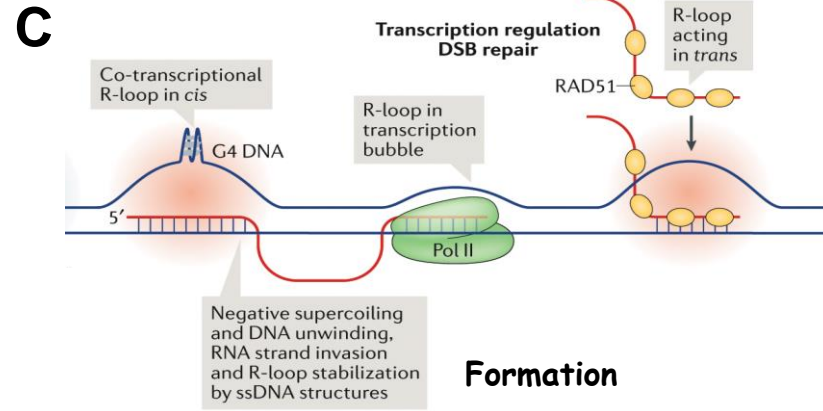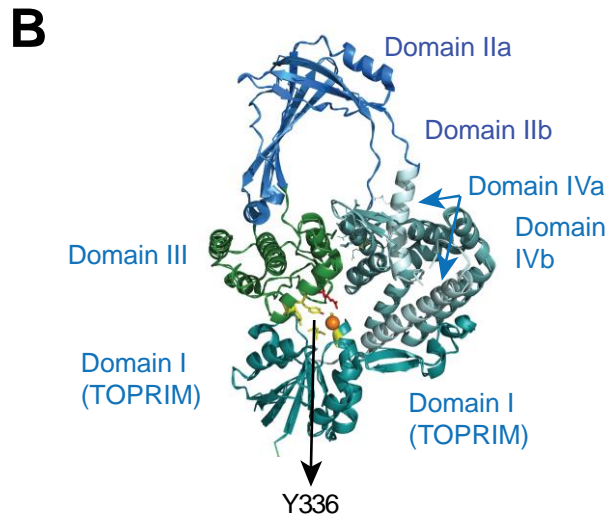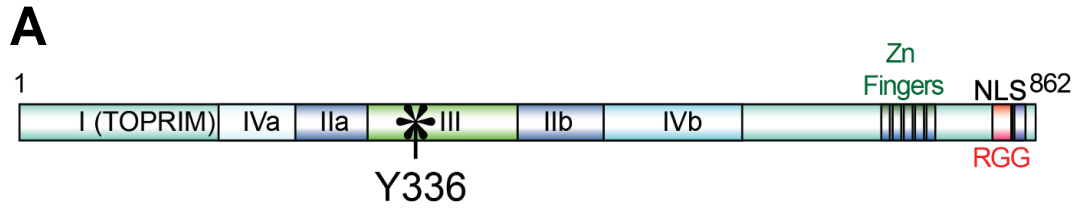
Sourav Saha[a] *and Yves Pommier[a] *

[a] Laboratory of Molecular Pharmacology, Developmental Therapeutics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892
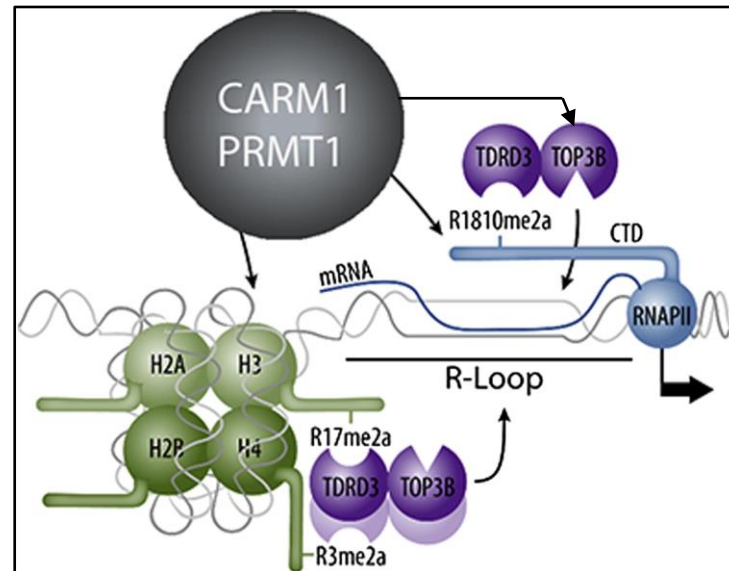
- sourav.saha@nih.gov and pommier@nih.gov
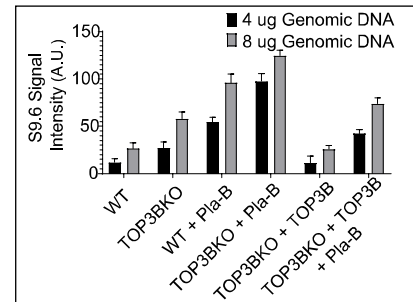
DNA/ RNA Topoisomerase 3-beta (TOP3B)

A

Y336

R-loop

C

Formation

D

CSR
Mitochondrial replication
Gene expression
Transcription elongation
Transcription termination

"Good" R loops (temporary?)

"Bad" R loops (persistent?)

DNA damage

Genomic instability

Cancer   Neurological disorders

Function

B

TOP3B and R-loop Connection

(B) Goto-Ito et. al., Sci. Rep. 2017;
(C) Niehrs and Luke, Nature Reviews Molecular Cell Biology, 2020;
(D) Richard and Manley, J Mol Biol., 2017;
(E) Yang et. Al., Mol. Cell, 2014

Increased R-loops in TOP3BKO cells and induction of cellular TOP3B cleavage complexes during R-loop formation
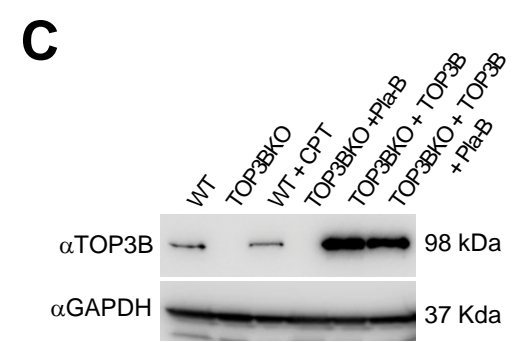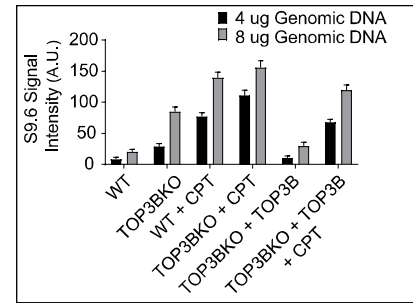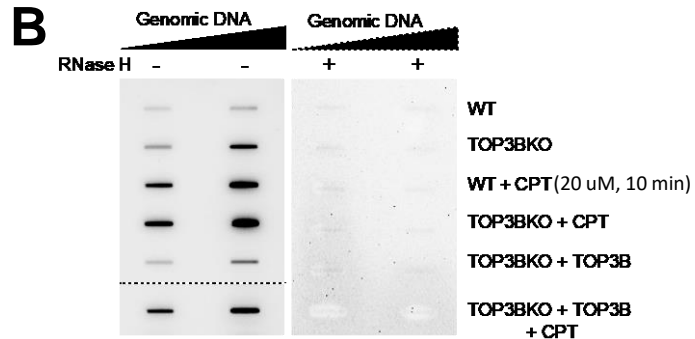
R-loop detection by DOT-BLOT with s9.6 Ab

TOP3Bcc detection by RADAR Assay
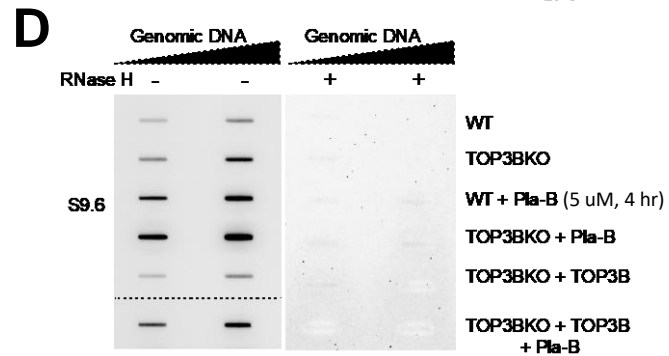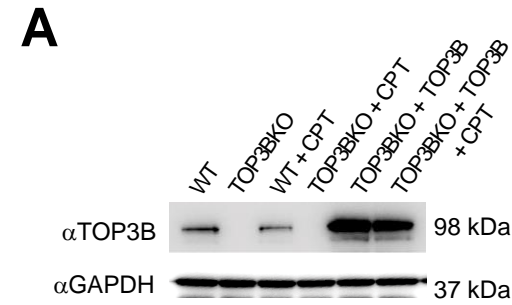
F **RADAR assay:** (rapid approach to DNA/RNA adduct recovery): purification of DNA/RNA by chaotropic agents such as guanidinium isothiocyanate. (Kiianitsa and Maizels, 2013)
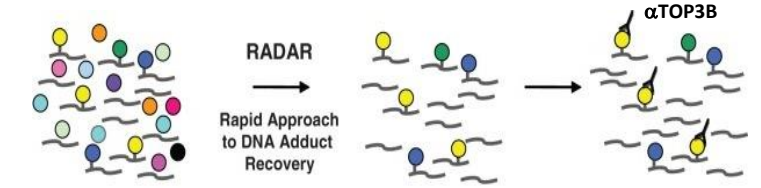
Kiianitsa and Maizels, Nucl. Acids Res., 2013
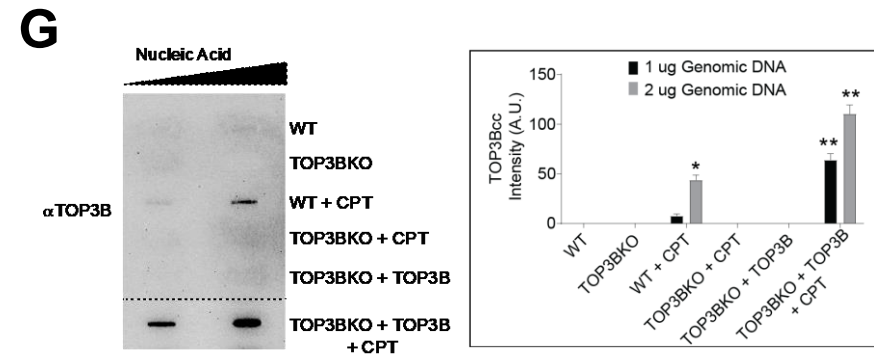
R-loop Confocal Microscopy

Green = R-loop (S9.6 Ab)

RNA/DNA Hybrid IP-Western blotting using S9.6 antibody

Cleavage of R-loop mimicking substrate by TOP3B

A

B

C

Nguyen et. al., Mol. Cell, 2017

D

E

F
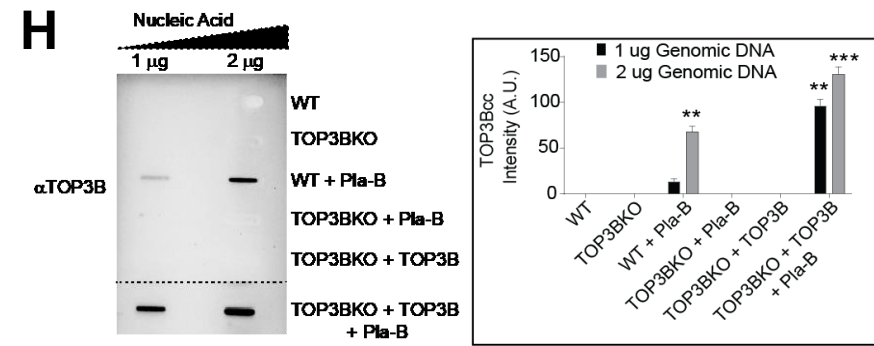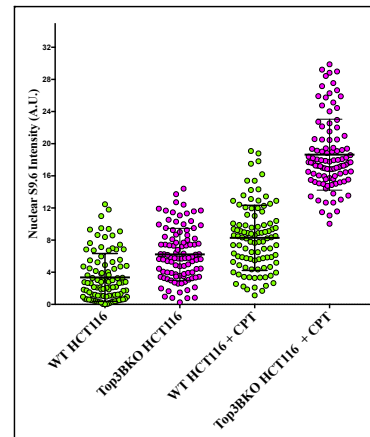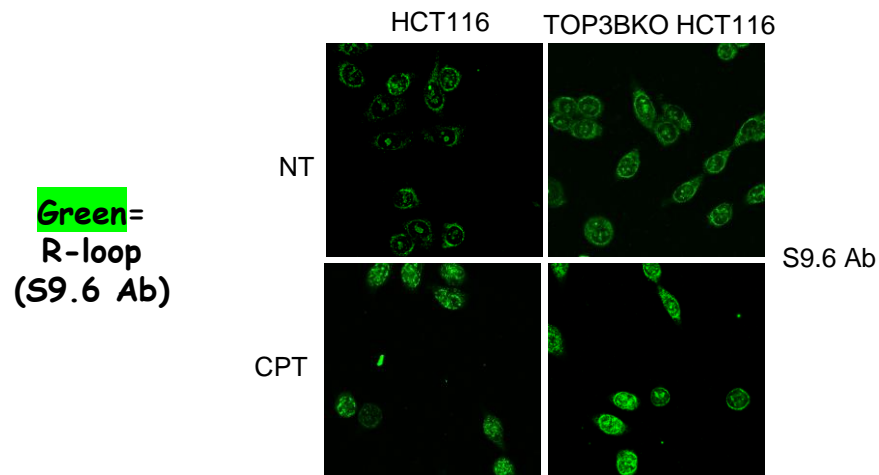
TOP3B-helicase coupling resolves R-loops??

A

How does TOP3B activity on single stranded DNA in R-loops promote R-loop resolution?

TOP3B can only cleave single strand DNA (ssDNA) in R-loop structures

↓

A helicase coupled with TOP3B can unwind DNA-RNA hybrid of R-loop to generate ssDNA

↓

TOP3B-mediated strand passage through ssDNA Resolves R-loop

B

IP-Mass Spec Experiments

TOP3BKO HCT116
↓
Transfection with FLAG tagged TOP3B

No Treatment   Pla-B Treatment
↓   ↓
Cell Lysis
↓
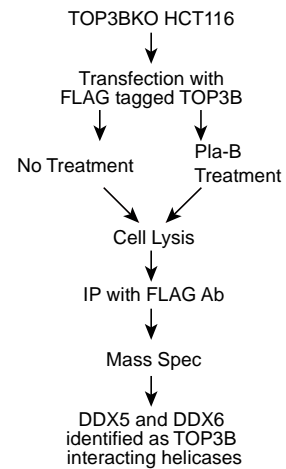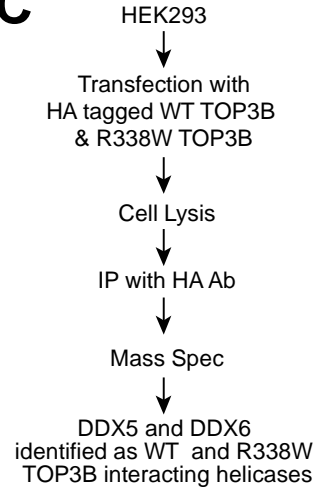IP with FLAG Ab
↓
Mass Spec
↓
DDX5 and DDX6 identified as TOP3B interacting helicases

Helicases	# PSM TOP3BKO + TOP3B	# PSM TOP3BKO + TOP3B+ Pla-B	Remark
DDX5	58	57	Interacts before and after R-loop induction
DDX6	6	9	Interacts before and after R-loop induction

C

HEK293
↓
Transfection with HA tagged WT TOP3B & R338W TOP3B
↓
Cell Lysis
↓
IP with HA Ab
↓
Mass Spec
↓
DDX5 and DDX6 identified as WT and R338W TOP3B interacting helicases

Helicases	# PSM Empty Vector	# PSM WT TOP3B	# PSM R338W TOP3B
DDX5	6	62	48
DDX6	0	7	6

D

HEK293
↓
Cell Lysis
↓
IP with TOP3B Ab
↓
Mass Spec
↓
DDX5 and DDX6 identified as WT and R338W TOP3B interacting helicases

Helicases	# PSM HCT116	# PSM TOP3BKO
TOP3B	95	0
DDX5	143	47

E

IP-Western blotting Experiments

FLAG IP

| Pla-B (5 uM, 4 hr) | — | — | + |
| FLAG TOP3B | — | + | + | kDa |

DDX6	54
DDX5	68
TDRD3	83
TOP3B	98

Whole Cell Extract

DDX6	54
DDX5	68
TDRD3	83
TOP3B	98
GAPDH	37

DDX5 and DDX6 are candidate helicases working with TOP3B......

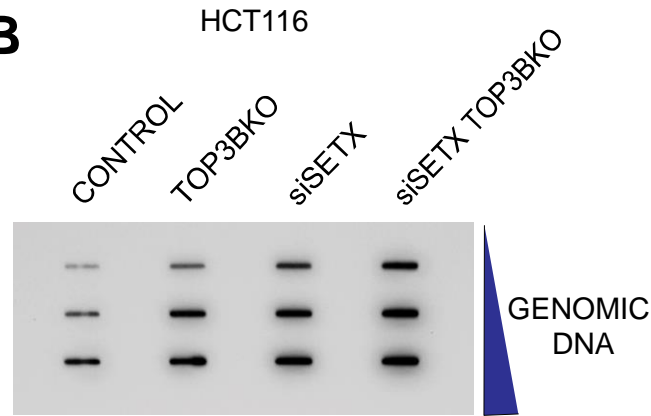# DDX5/ DDX6 but not senetaxin (SETX) are epistatic with TOP3B to resolve R-loops
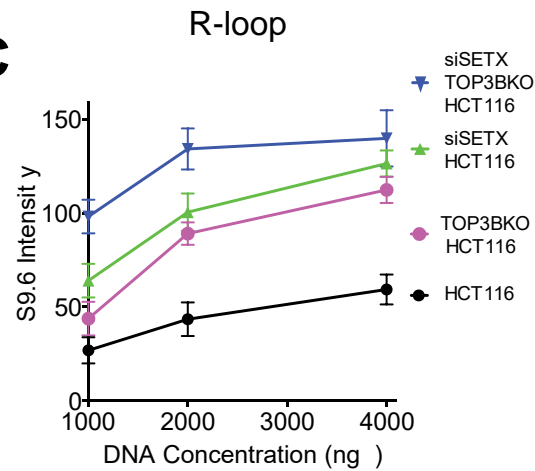
A

Senataxin (SETX)
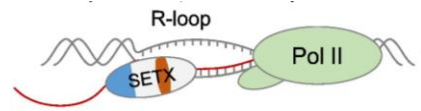
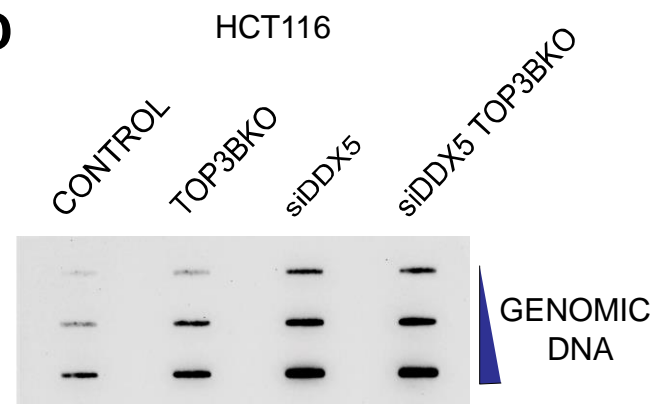Known R-loop resolving Helicase (Groh et. al., JMB, 2017)

B HCT116

CONTROL TOP3BKO siSETX siSETX TOP3BKO

GENOMIC DNA

R-loop

C

siSETX TOP3BKO HCT116
siSETX HCT116
TOP3BKO HCT116
HCT116

S9.6 Intensity vs DNA Concentration (ng)

D HCT116

CONTROL TOP3BKO siDDX5 siDDX5 TOP3BKO

GENOMIC DNA

R-loop

E

siDDX5 TOP3BKO HCT116
siDDX5 HCT116
TOP3BKO HCT116
HCT116

S9.6 Intensity vs DNA Concentration (ng)

F HCT116

CONTROL TOP3BKO siDDX6 siDDX6 TOP3BKO

GENOMIC DNA

R-loop

G

siDDX6 TOP3BKO HCT116
siDDX6 HCT116
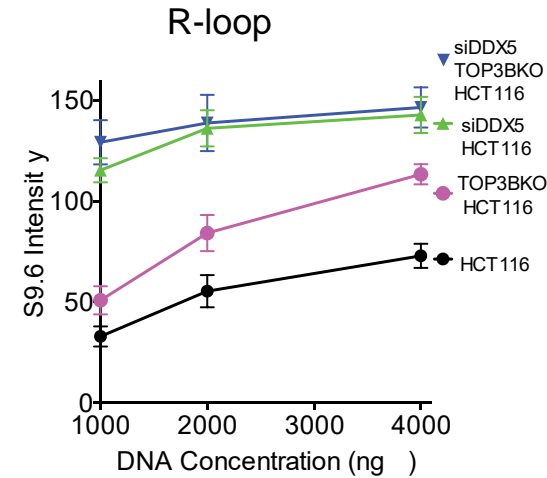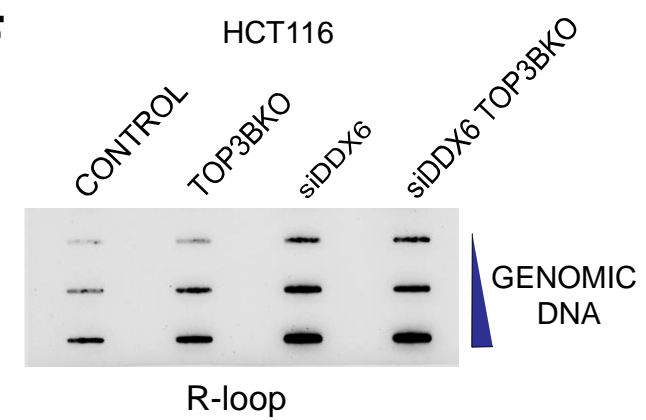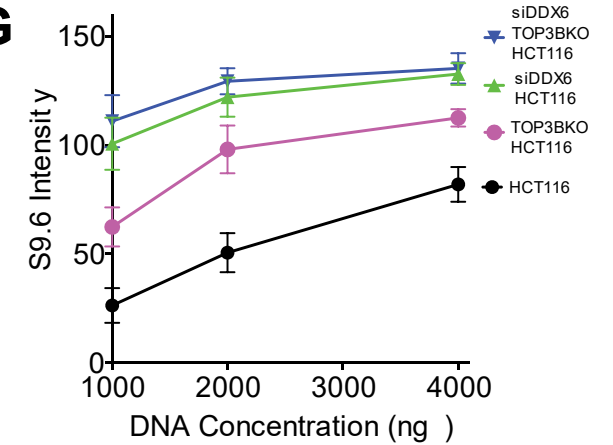TOP3BKO HCT116
HCT116

S9.6 Intensity vs DNA Concentration (ng)

Conclusions

❖ TOP3BKO cells have increased R-loops and ectopic expression of TOP3B resolves R-loops
❖ TOP3B is recruited to R-loops
❖ TOP3B cleaves the single-stranded DNA segment of R-loops by forming TOP3Bcc
❖ We propose thatTOP3B resolves R-loops in coordination with the DEAD-box helicases DDX5 and DDX6