SLFN11 Biology

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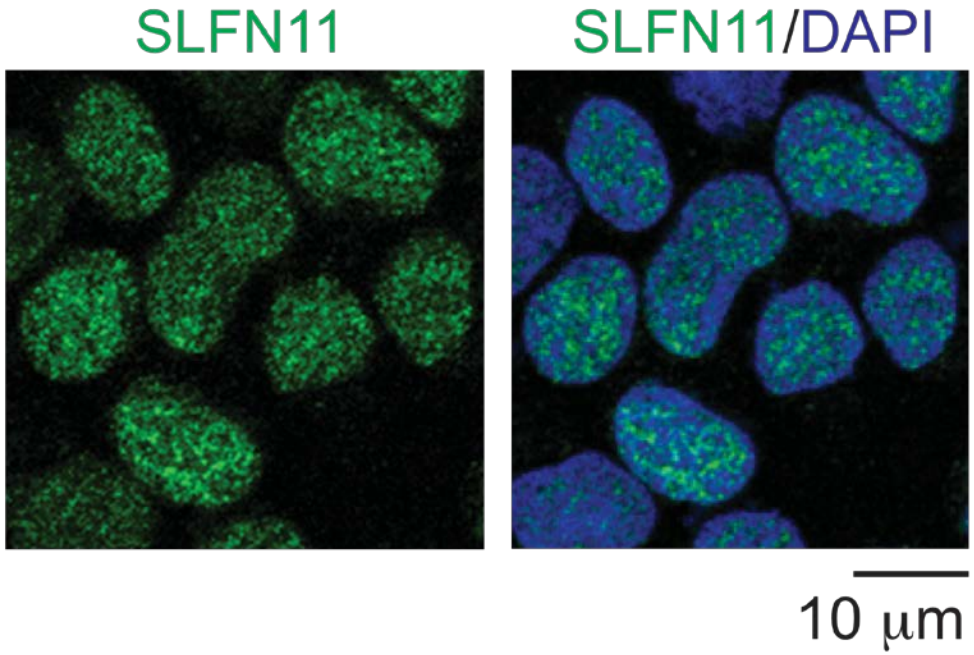
Schlafen 11: SLFN11

- A member of the SLFN (schlafen = sleep) family (innate immune response genes), found only in mammals
- Found by cancer cell line genomic analyses as determinant of response to a broad range of chemotherapeutics drugs
- A putative DNA/RNA helicase (Walker motif)
- Binds to chromatin, RPA at damage sites, tRNA…
- Located in nucleus (NLS, IHC and immunofluorescence)



Immunofluorescence microscopy

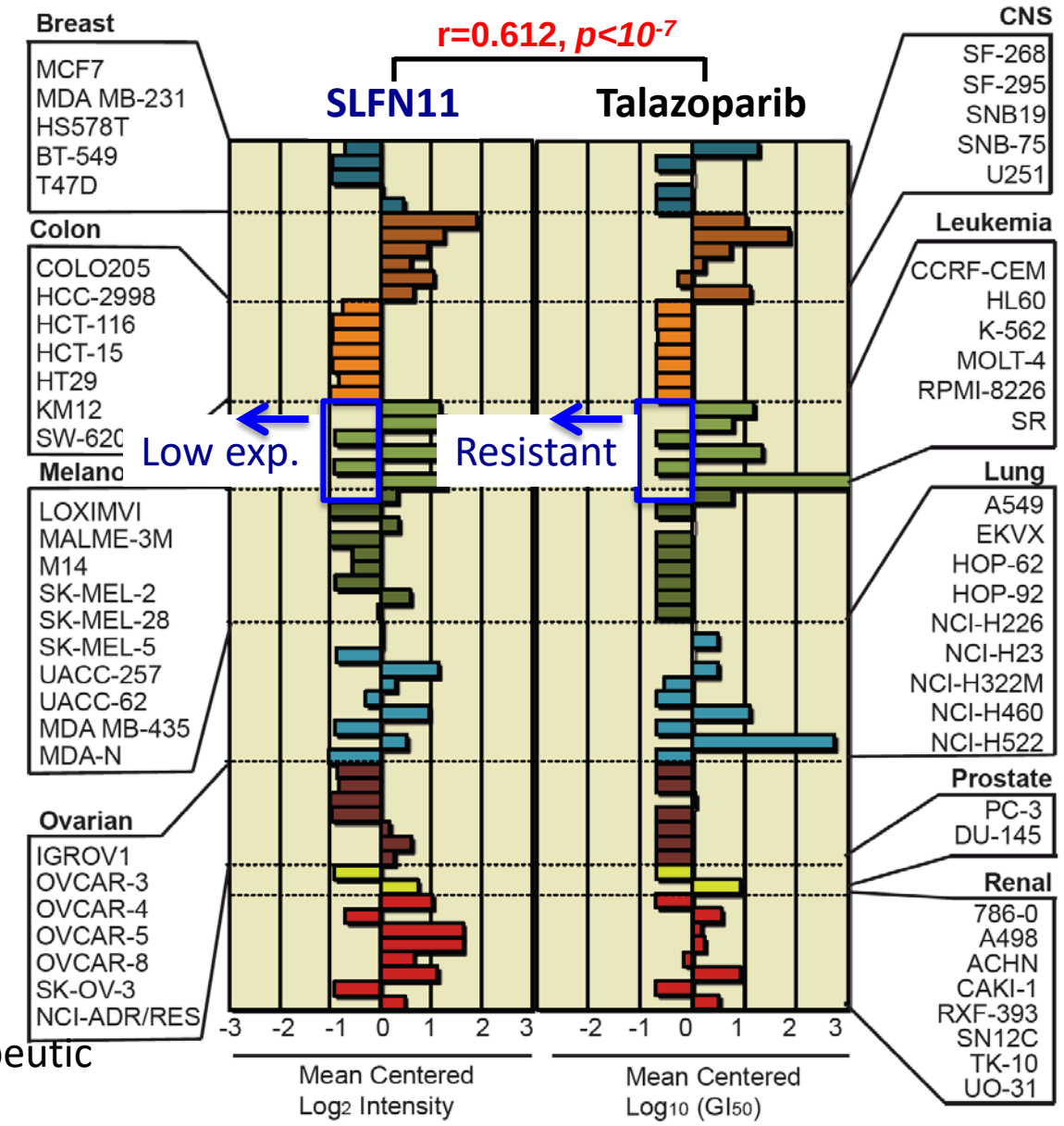
Outline

- Schlafen 11 (SLFN11) is a determinant of response to all drugs approved for SCLC treatment (cisplatin, etoposide, topotecan) and to PARP inhibitors beyond BRCA; i.e. replication inhibitors
- How does SLFN11 put cells with replicative damage to sleep?
- SLFN11 translation to the clinic?

Outline

- Schlafen 11 (SLFN11) is a determinant of response to all drugs approved for SCLC treatment (cisplatin, etoposide, topotecan) and to PARP inhibitors beyond BRCA; i.e. replication inhibitors

High correlation between expression of *Schlafen 11* (*SLFN11*) and cellular response to talazoparib in non-isogenic cell lines (NCI-60)



CellMiner
http://discover.nci.nih.gov/

NCI-60 Cancer Cells; Developmental Therapeutic Program

Murai, J. …Pommier, Y 2016

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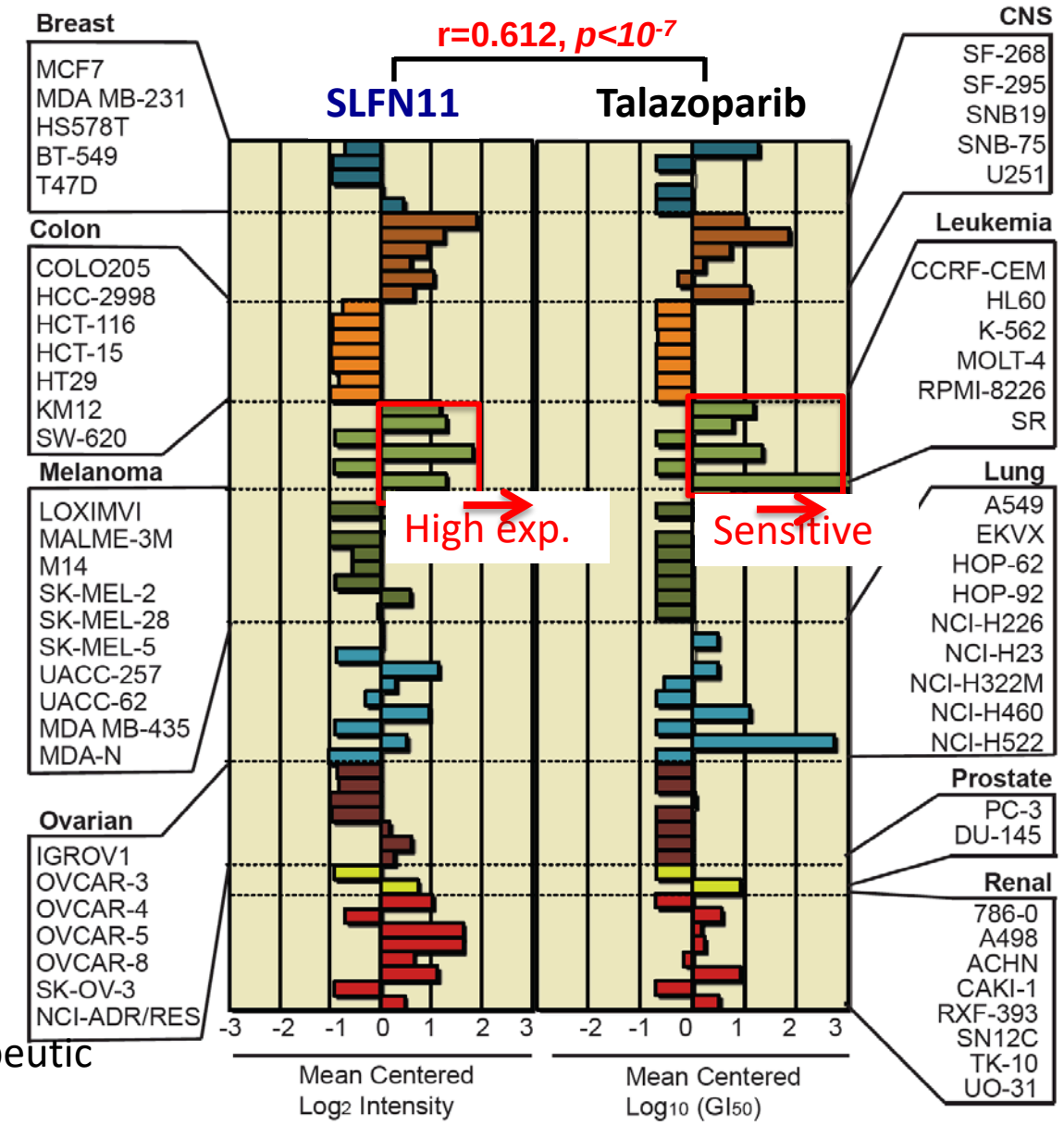

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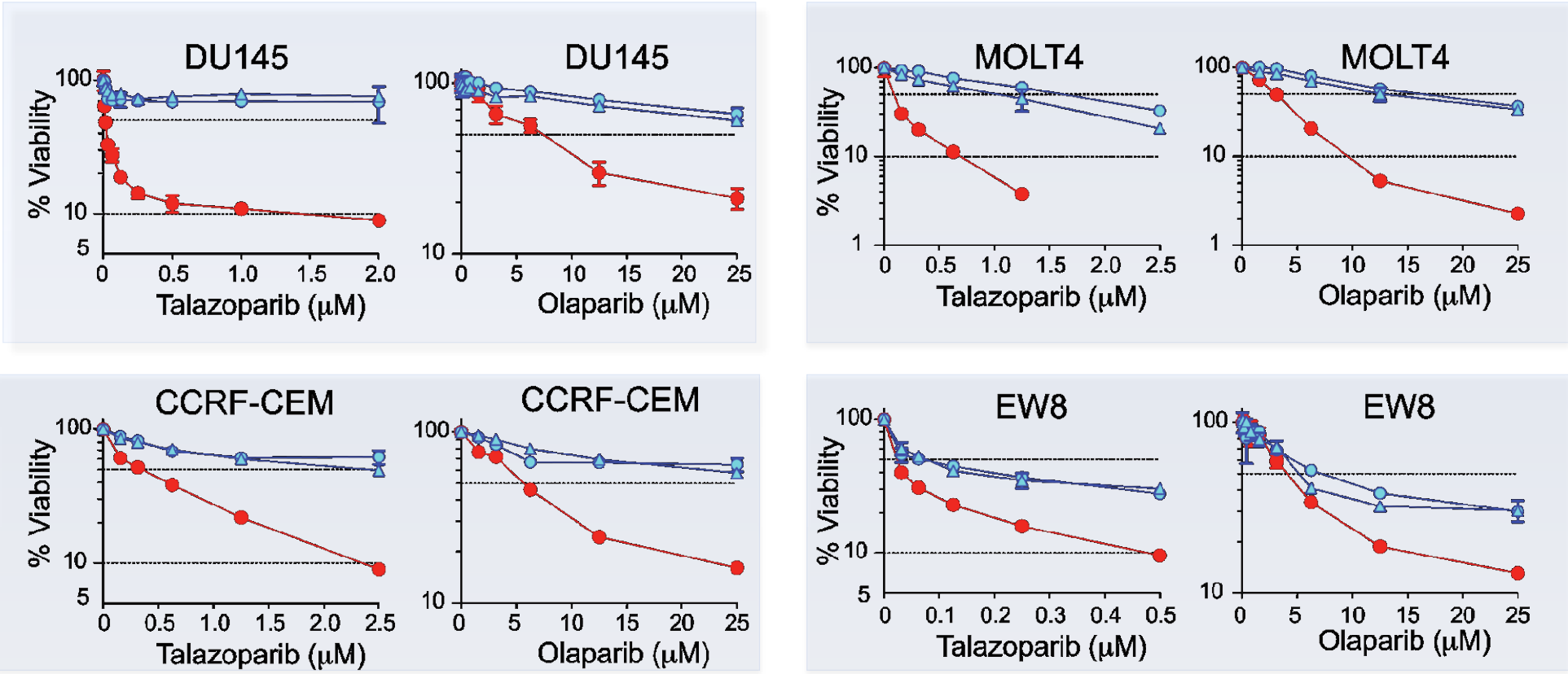
NCI-60 Cancer Cells;
Developmental Therapeutic Program

Murai, J. …Pommier, Y 2016

SLFN11 inactivation in 4 different isogenic cell lines confers high resistance to PARP inhibitors

=> SLFN11 inactivation is a causal and dominant mechanism of resistance to PARP inhibitors

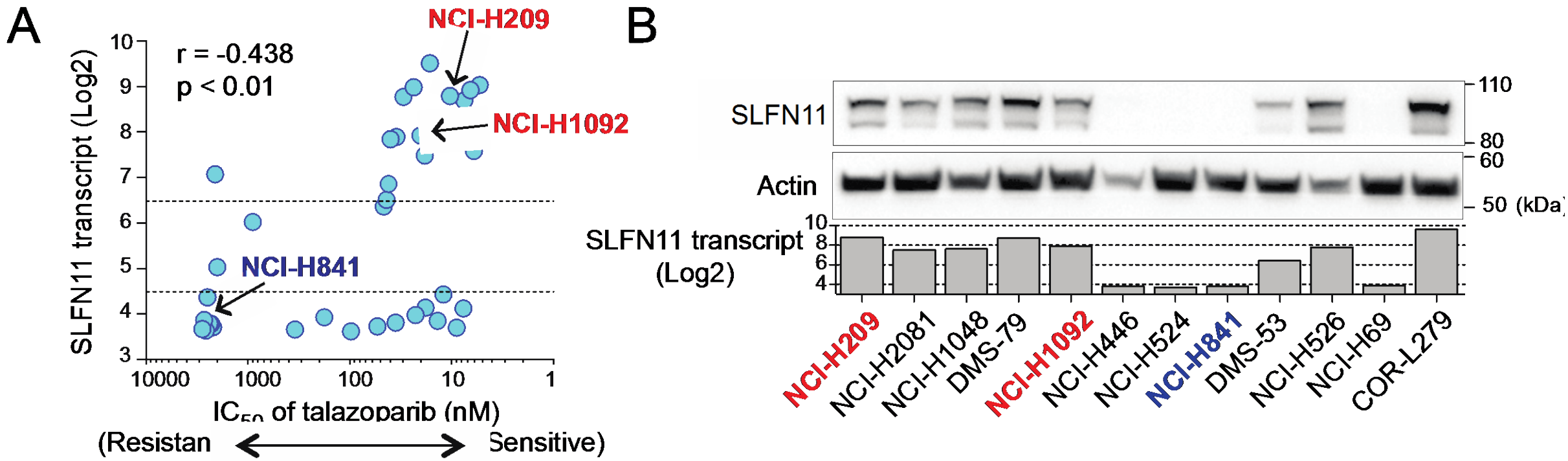


Murai, J.…Pommier, Y. 2016

DU145: Prostate cancer
MOLT4 and CCRF-CEM: Leukemia
EW8: Ewing's sarcoma
(CRISPR/Cas9)

SLFN11 determines response to a broad range of DNA-targeted agents:
TOP1, TOP2, PARP inhibitors, cisplatin, carboplatin, gemcitabine, hydroxyurea…

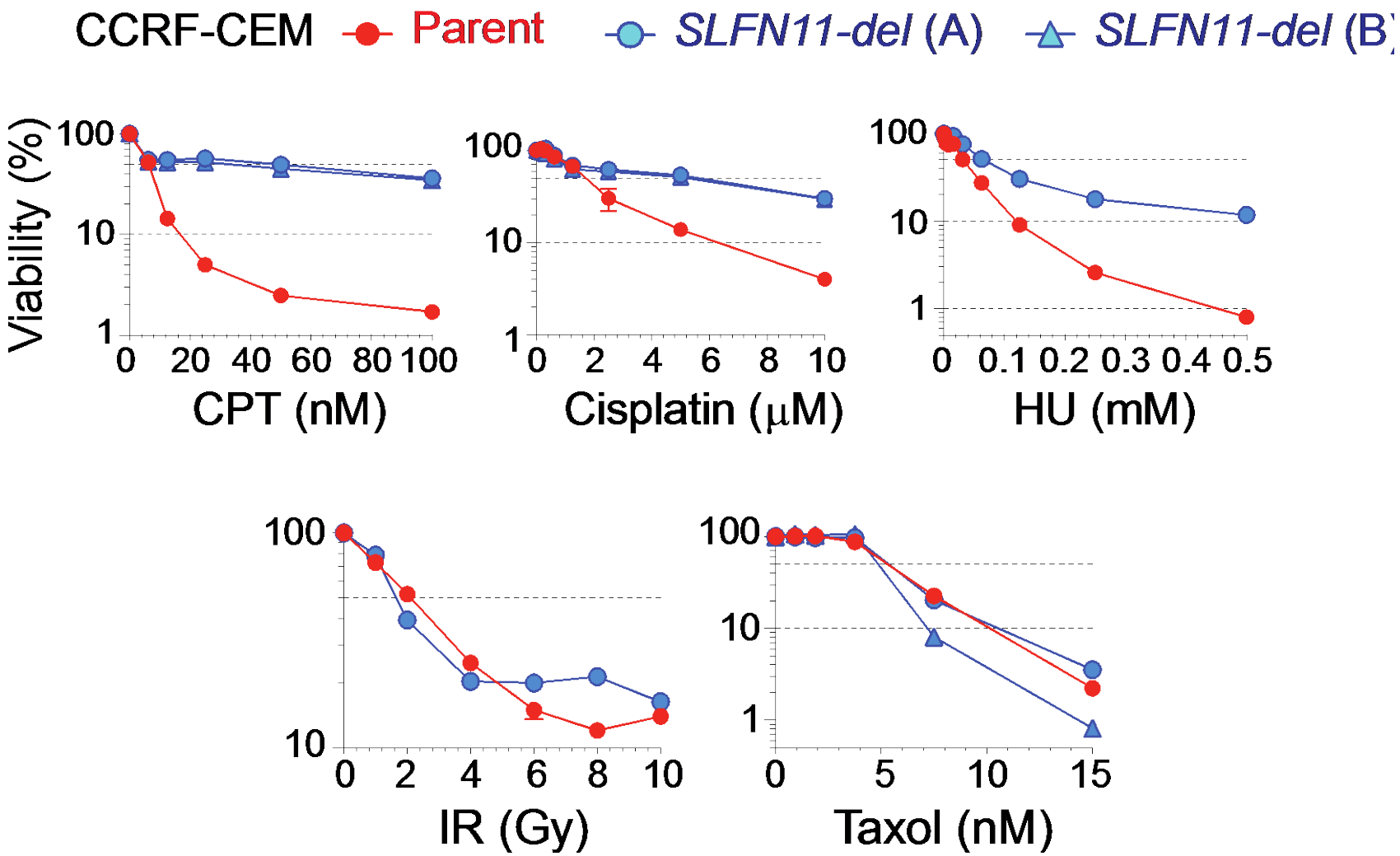
SLFN11 expression is correlated with sensitivity to talazoparib in small cell lung cancer (SCLC) cells



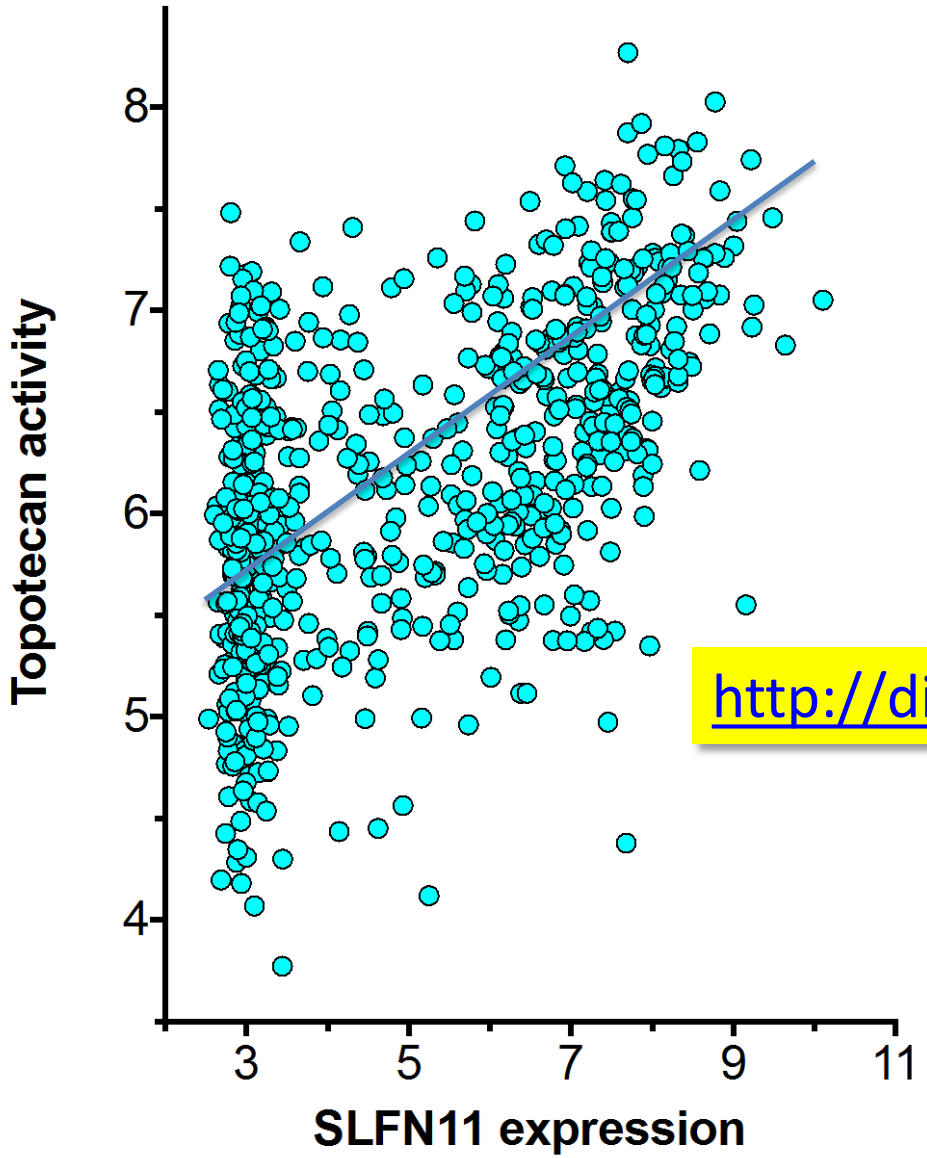
Note the high dynamic range of expression ("on" or "off") and correlation between transcript and protein levels

SLFN11 inactivation in isogenic cell lines confers high resistance to **Replication inhibitors** but not to IR or taxol or protein kinase inhibitors

=> SLFN11 inactivation is a causal mechanism of resistance



SLFN11 expression is highly correlated with sensitivity to topotecan in non-isogenic cell lines

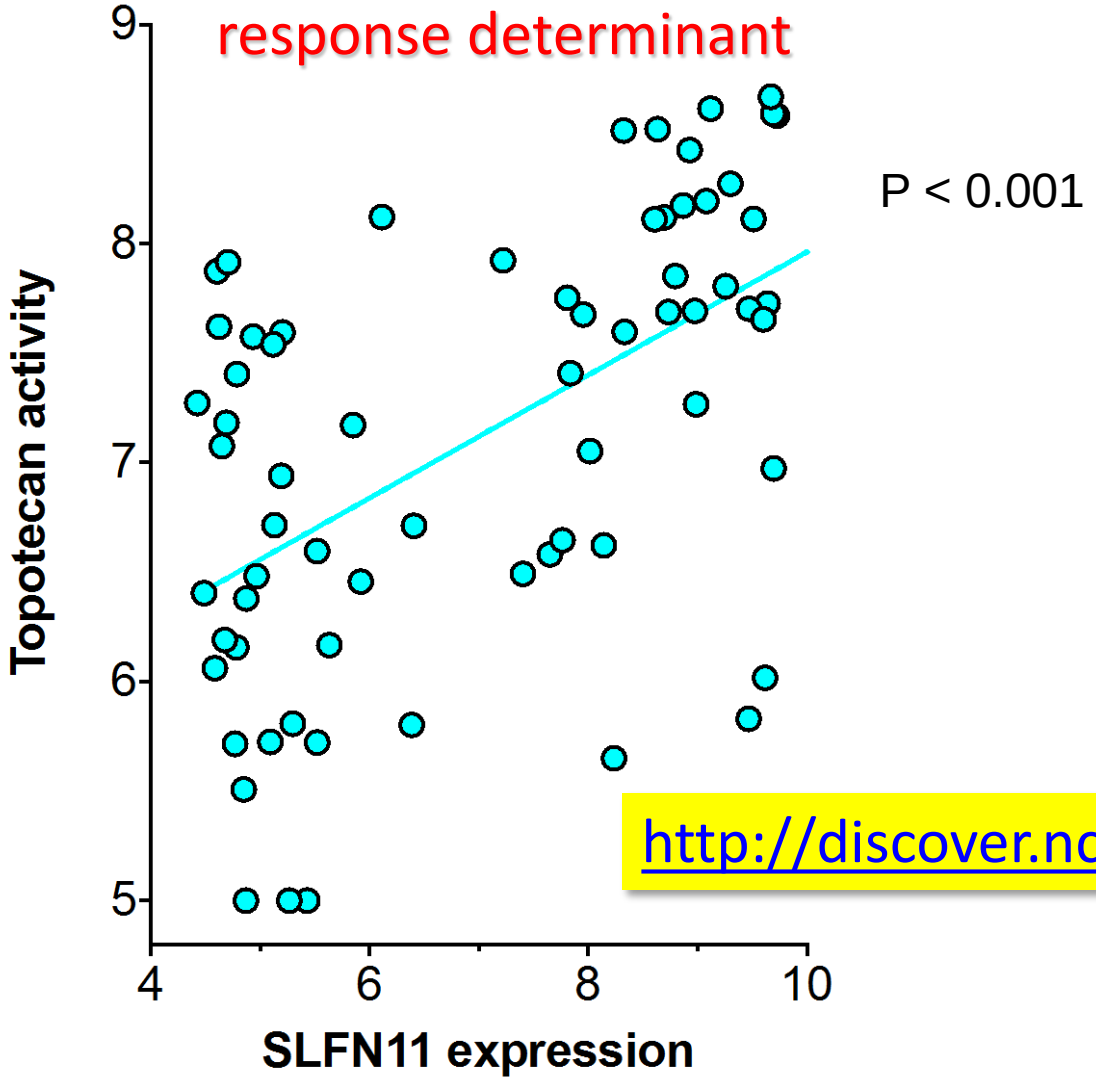


MGH-Sanger (GDSC) cancer cell line collection

$P < 0.001$

http://discover.nci.nih.gov/cellminercdb

SLFN11 expression is highly correlated with sensitivity to topotecan in non-isogenic SCLC cell lines ⇔ highly penetrant response determinant



http://discover.nci.nih.gov/cellminercdb

NCI-DTP SCLC cell line collection (Bev. Teicher)

SLFN11 expression is the highest genomic determinant correlated with sensitivity to topotecan, PARPi, etoposide, cisplatin ⇔ highly penetrant response determinant

CellMinerCDB | Univariate Analyses | Regression Models | Metadata | About

x-Axis Dataset
NCI/DTP SCLC

x-Axis Type
Drug Activity (-log10[IC50M])

ID: (e.g. 94600 or SLFN11)
Topotecan

y-Axis Dataset
NCI/DTP SCLC

y-Axis Type
Expression (log2)

ID: (e.g. 94600 or SLFN11)
SLFN11

☑ Show Color?

☐ Selected Tissues Only?

Color Specific Tissues?
all

Plot Data | Download Data | Search IDs | Compare Patterns

- cop: Copy Number
- mut: Mutation
- exp: Expression (Z-Score)
- xai: Expression (Avg. log2 Int.)
- pro: Protein (RPLA)
- mir: MicroRNA
- mda: Metadata
- swa: Protein (SWATH-MS)

http://discover.nci.nih.gov/cellminercdb

Pattern Comparison: Molecular Data

With Respect to: x-Axis Entry

Show 10 entries Search:

Data Type	Gene	Location	Correlation	P-Value	Annotation
All	All	All	All	All	All
exp	SLFN11	17q12	0.543	0.00000118	DNA Damage Response (DDR)
exp	KCNJ11	11p15.1	0.424	0.000258	potassium ion transport;negative regulation of insulin secretion
exp	PAK6	15q14	0.422	0.000276	Protein Kinases
exp	ASTN2	9q33.1	0.414	0.000367	

Outline

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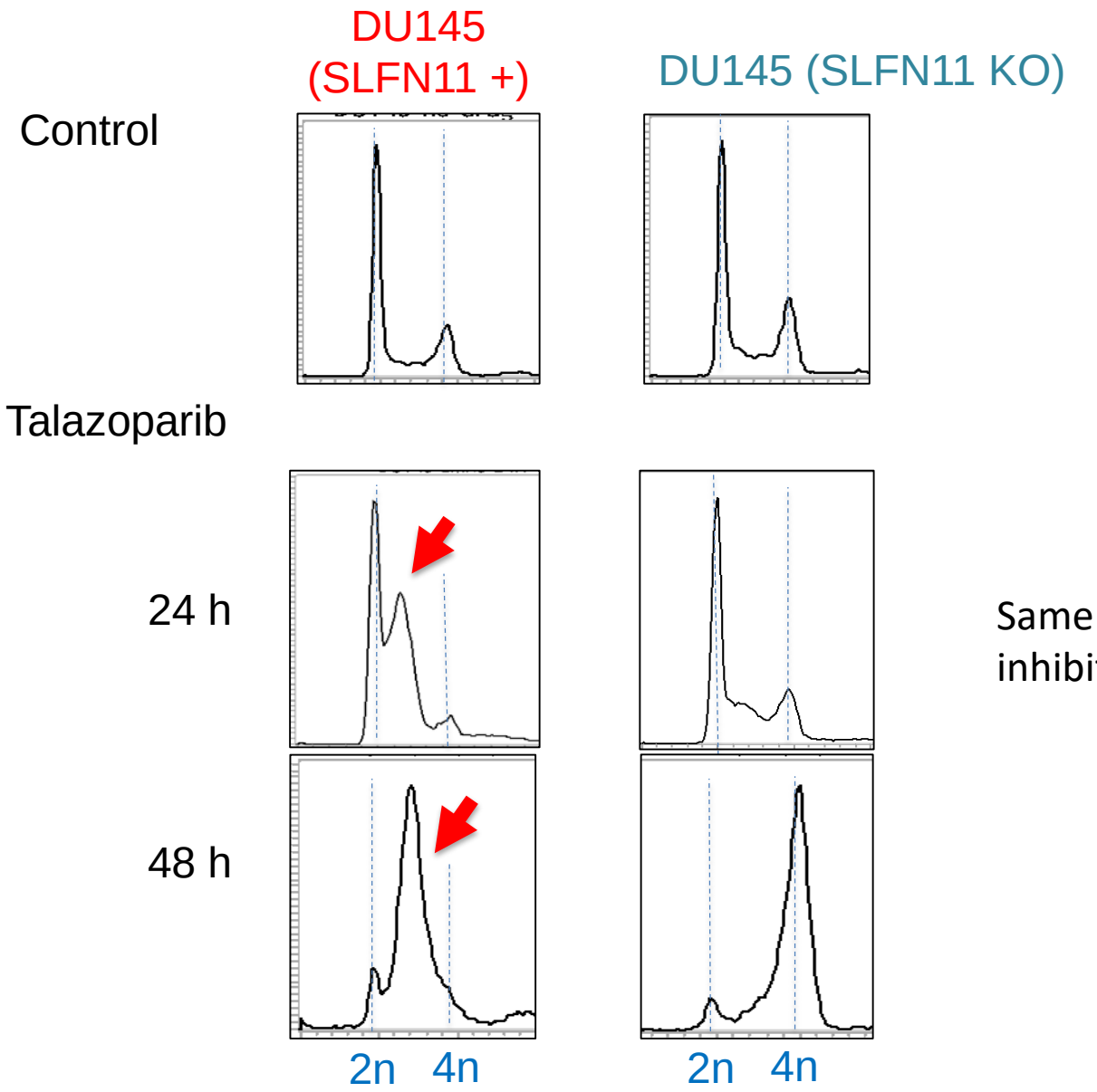
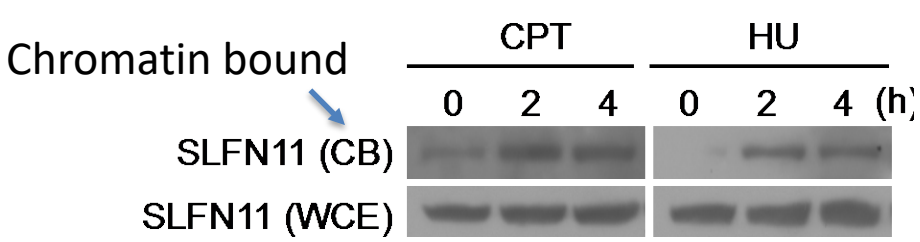
- How does SLFN11 put cells with replicative damage to sleep?

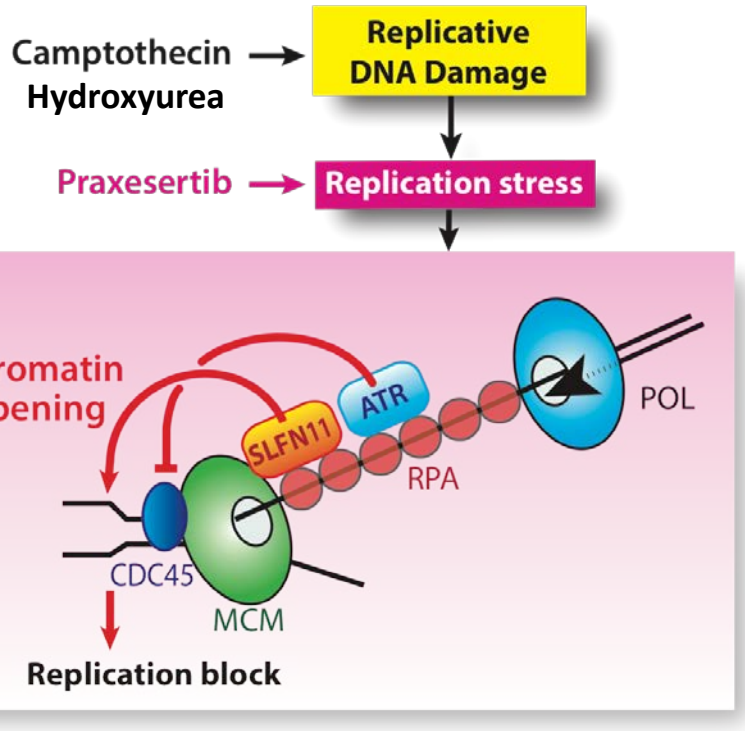
SLFN11 enforces irreversible cell cycle arrest in S-phase

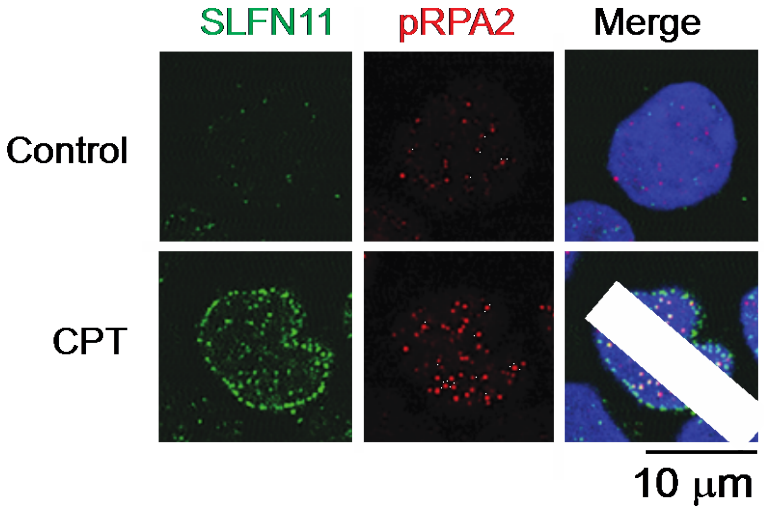


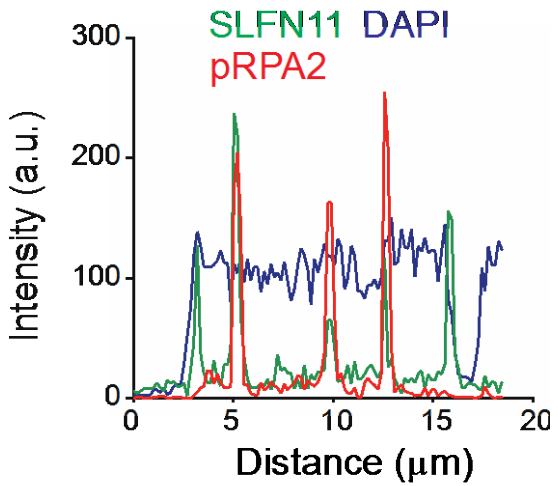
Same results with other replication inhibitors (camptothecin)

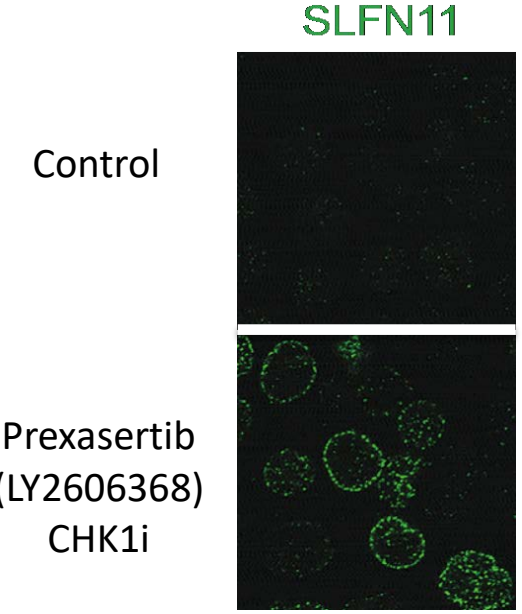
SLFN11 is recruited to chromatin in response to replication damage (CPT: camptothecin; HU: hydroxyurea; CHK1 inhibitor: Prexasertib)





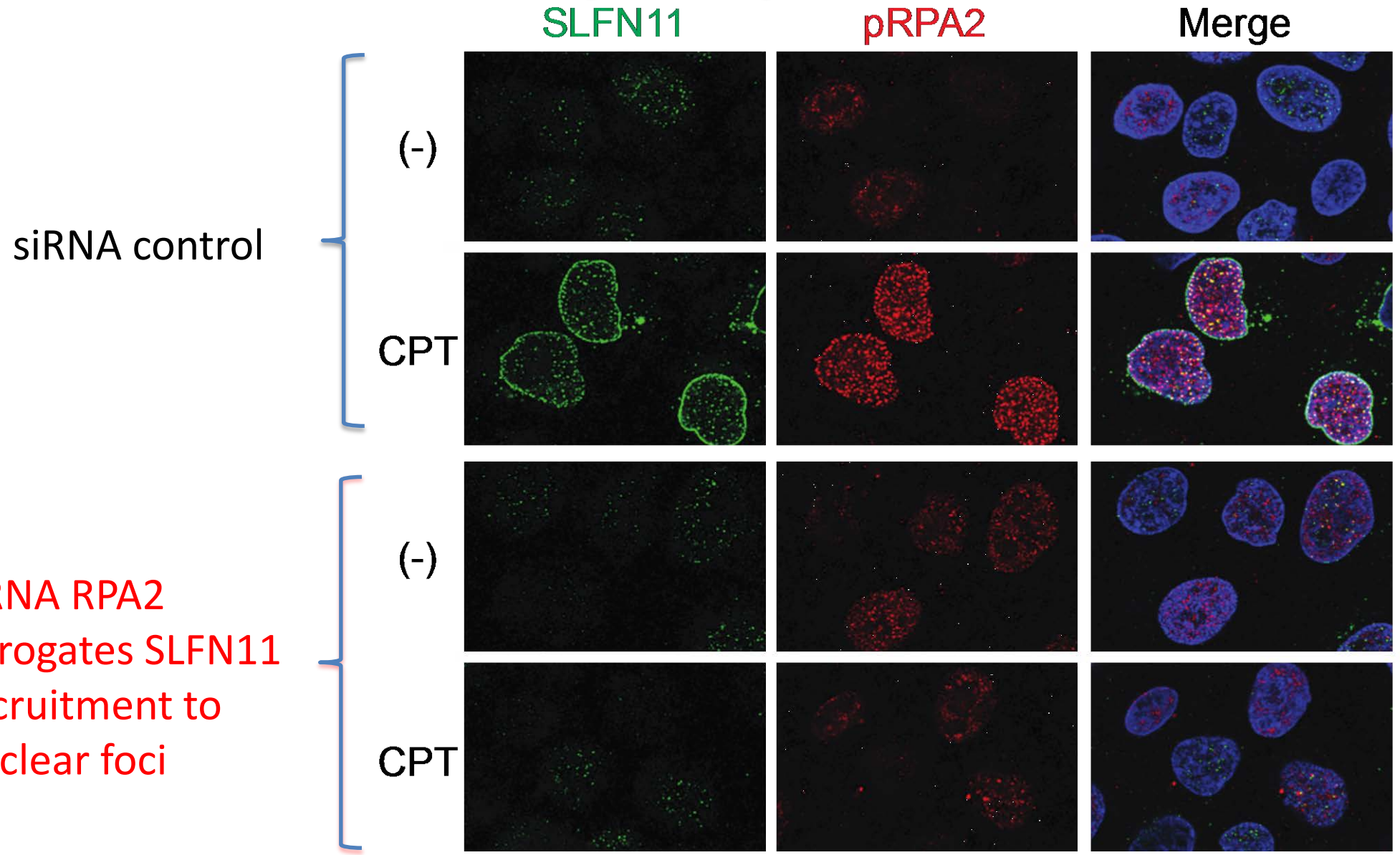




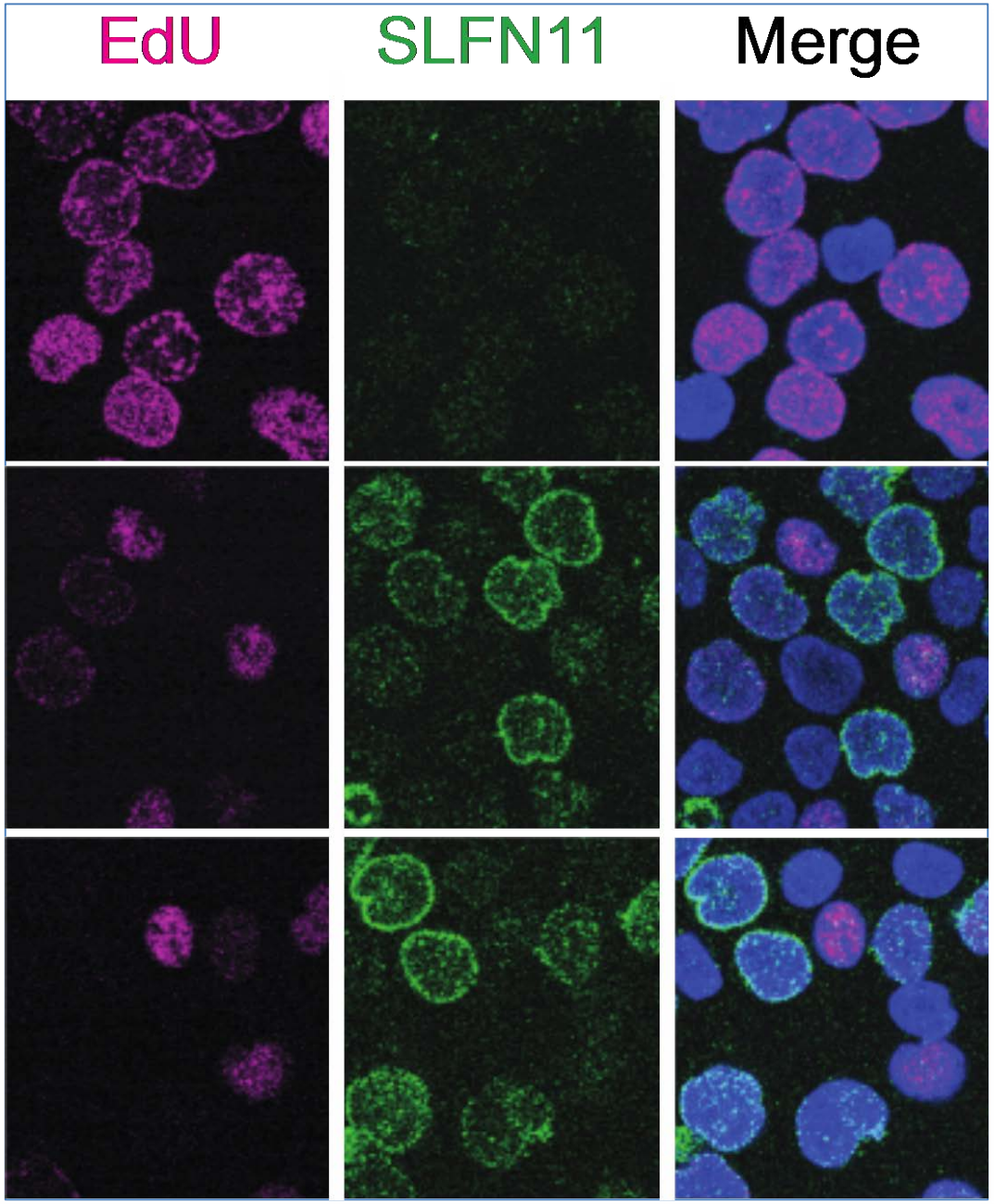


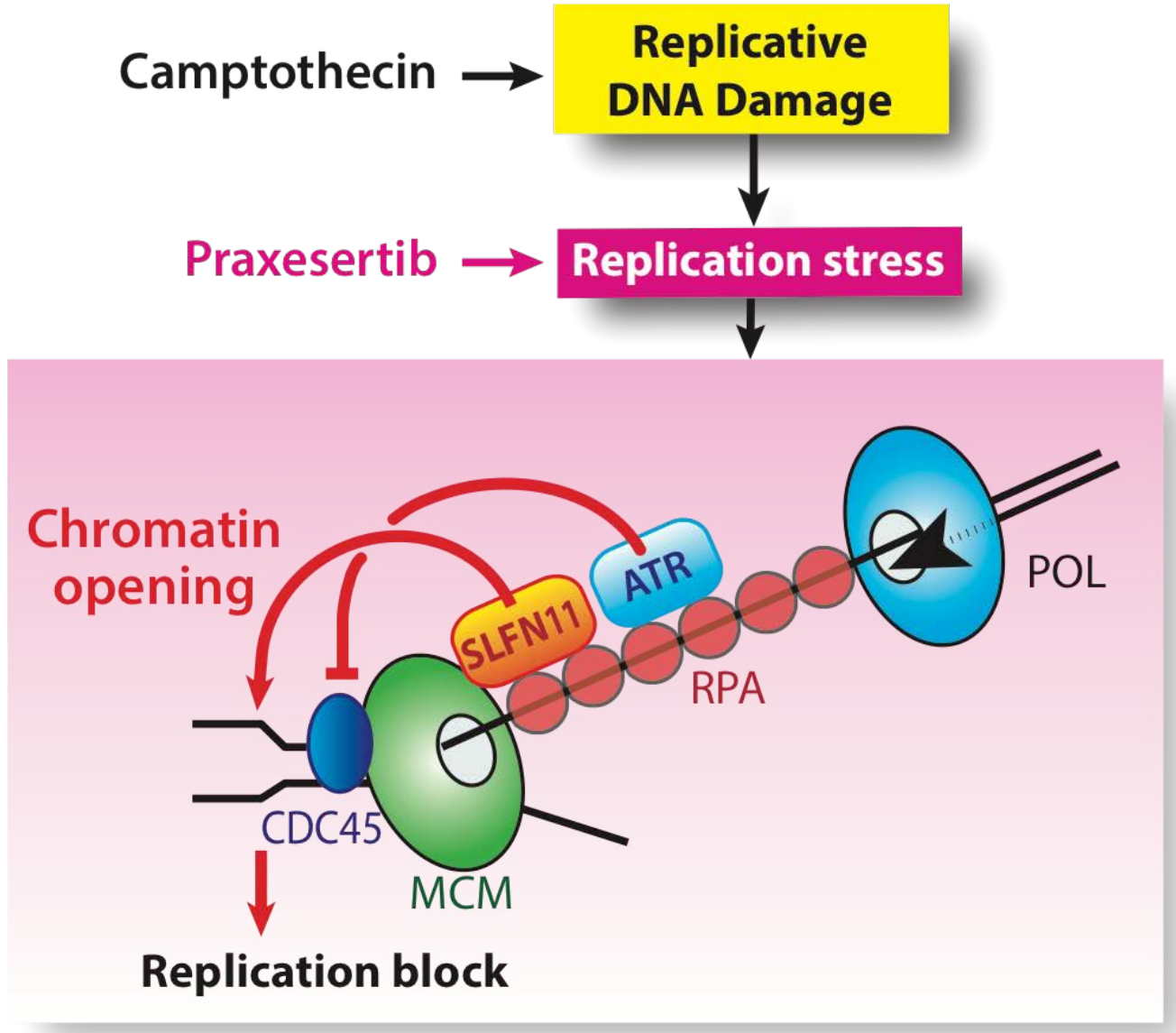
Immunofluorescence microscopy after non-ionic detergents to detect tight chromatin binding

SLFN11 is recruited to chromatin by the single-strand binding replication proteins RPA



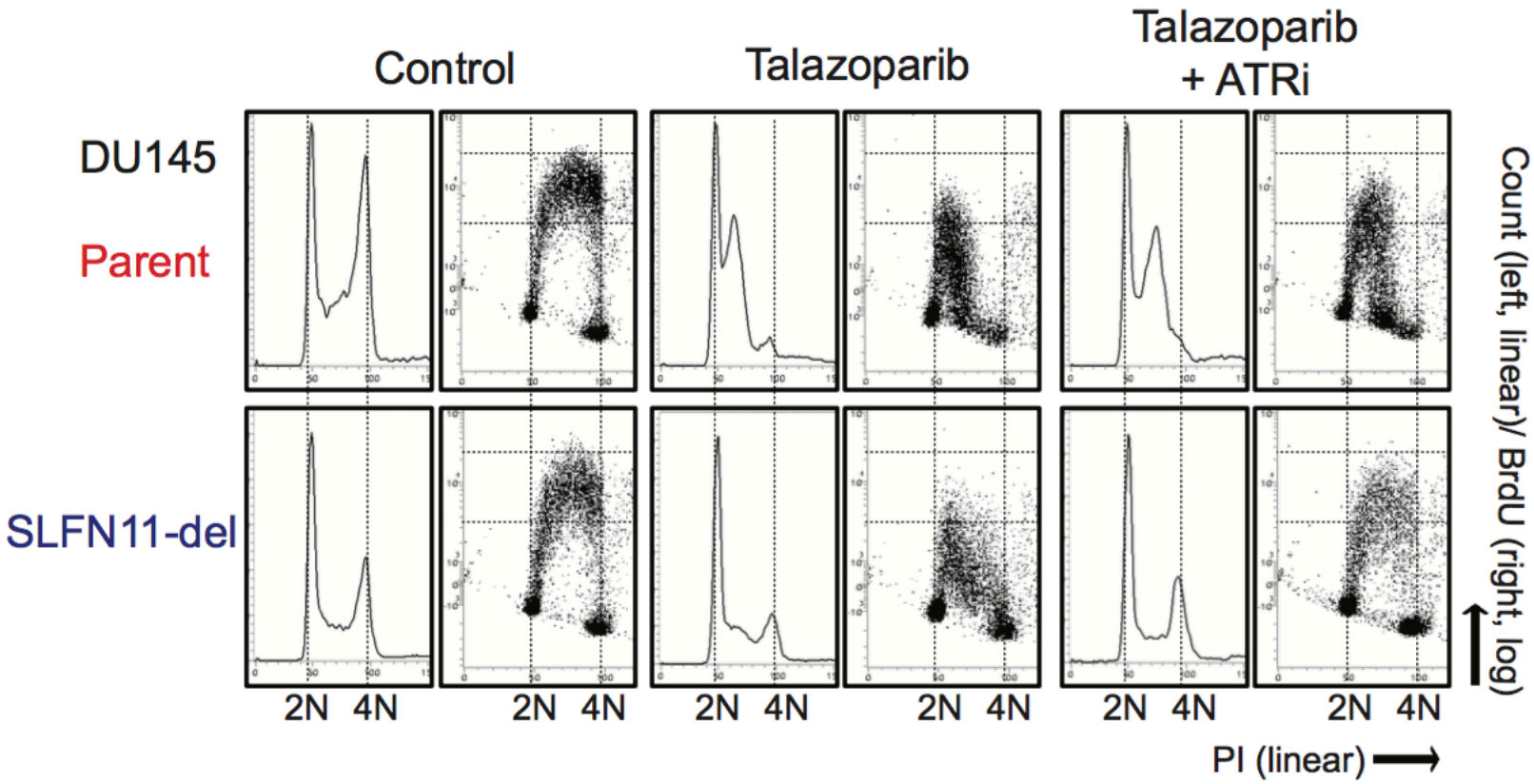
SLFN11 is recruited to chromatin by RPA independently of ATR



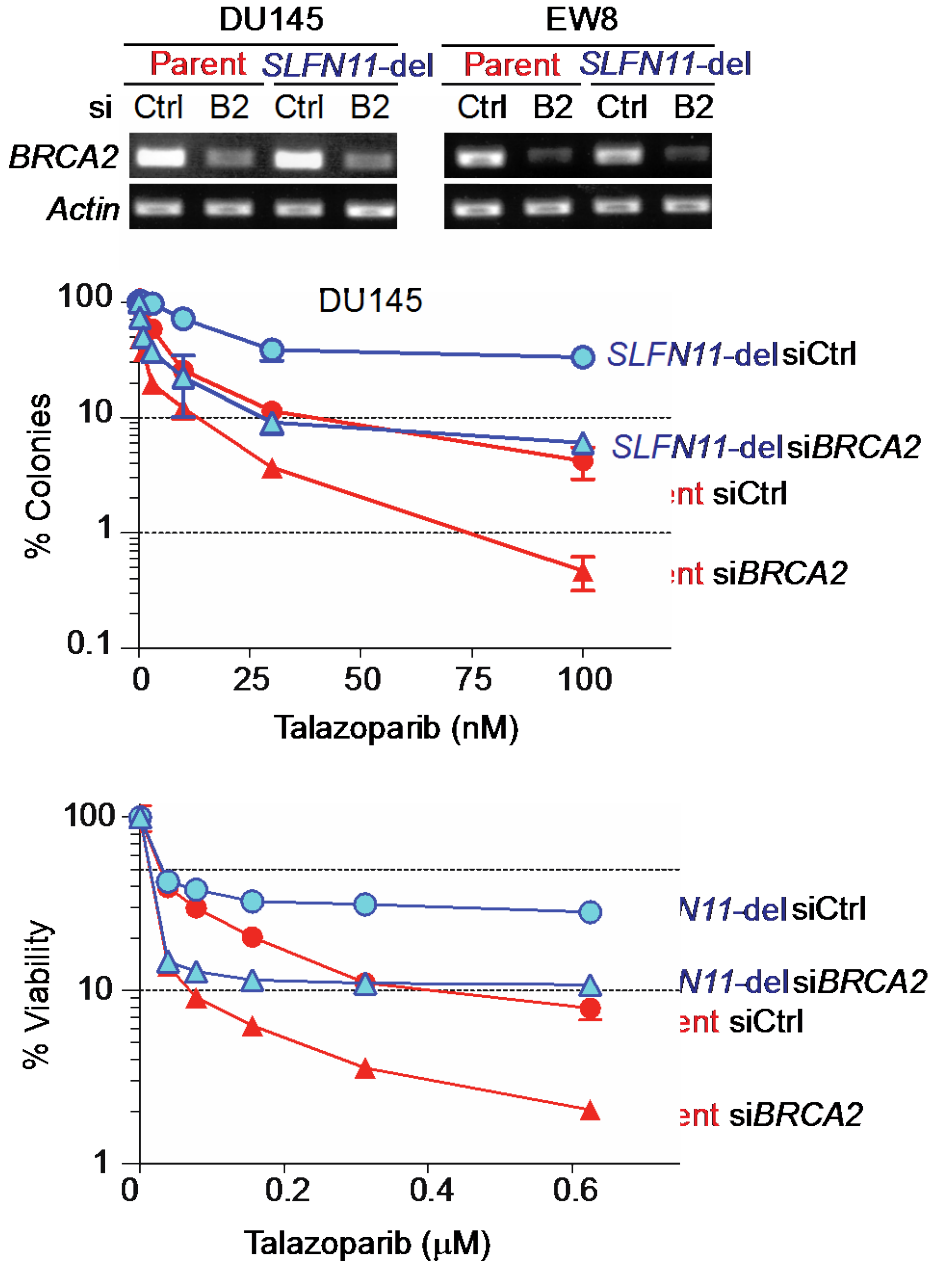


Murai, J.…Pommier, Y. 2018 Mol Cell

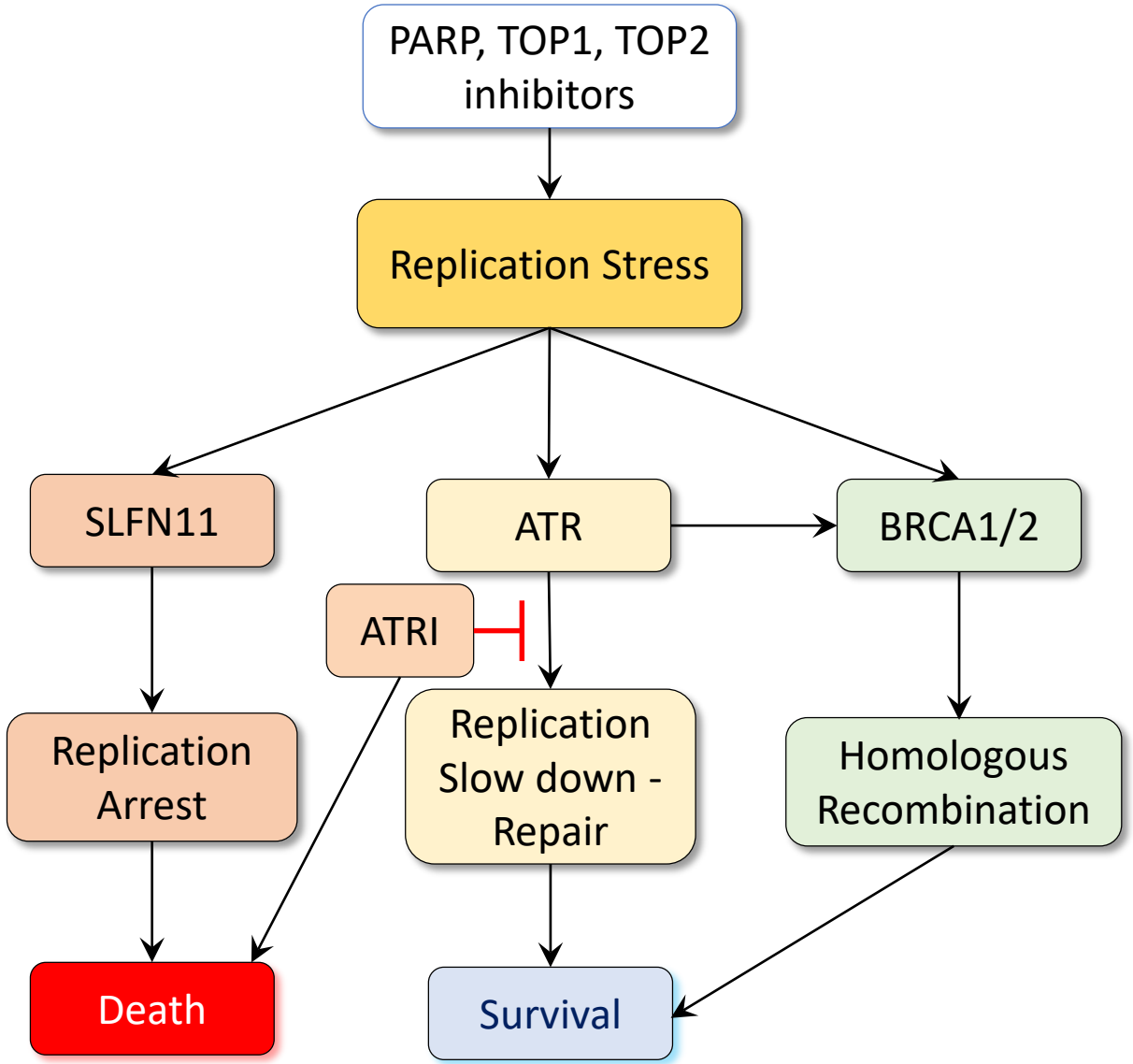
SLFN11 induces replication arrest independently of ATR



SLFN11 acts independently of BRCA2 and HR



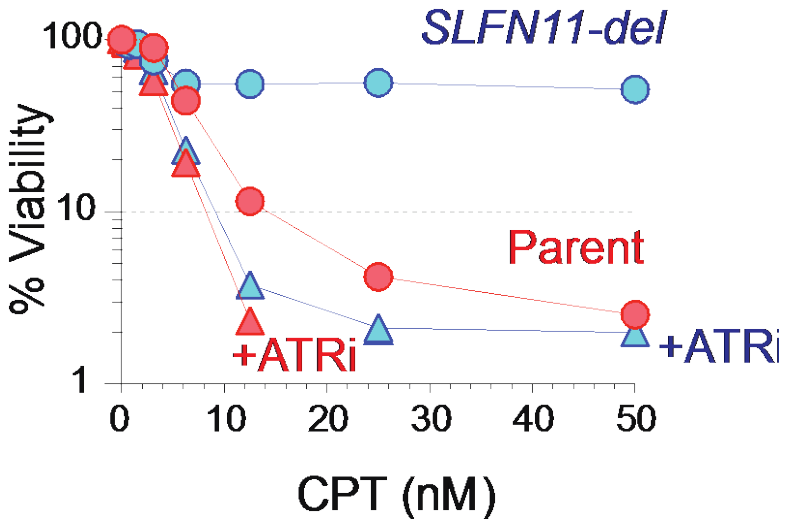
SLFN11 induces lethal replication arrest independently of ATR and BRCA1/2

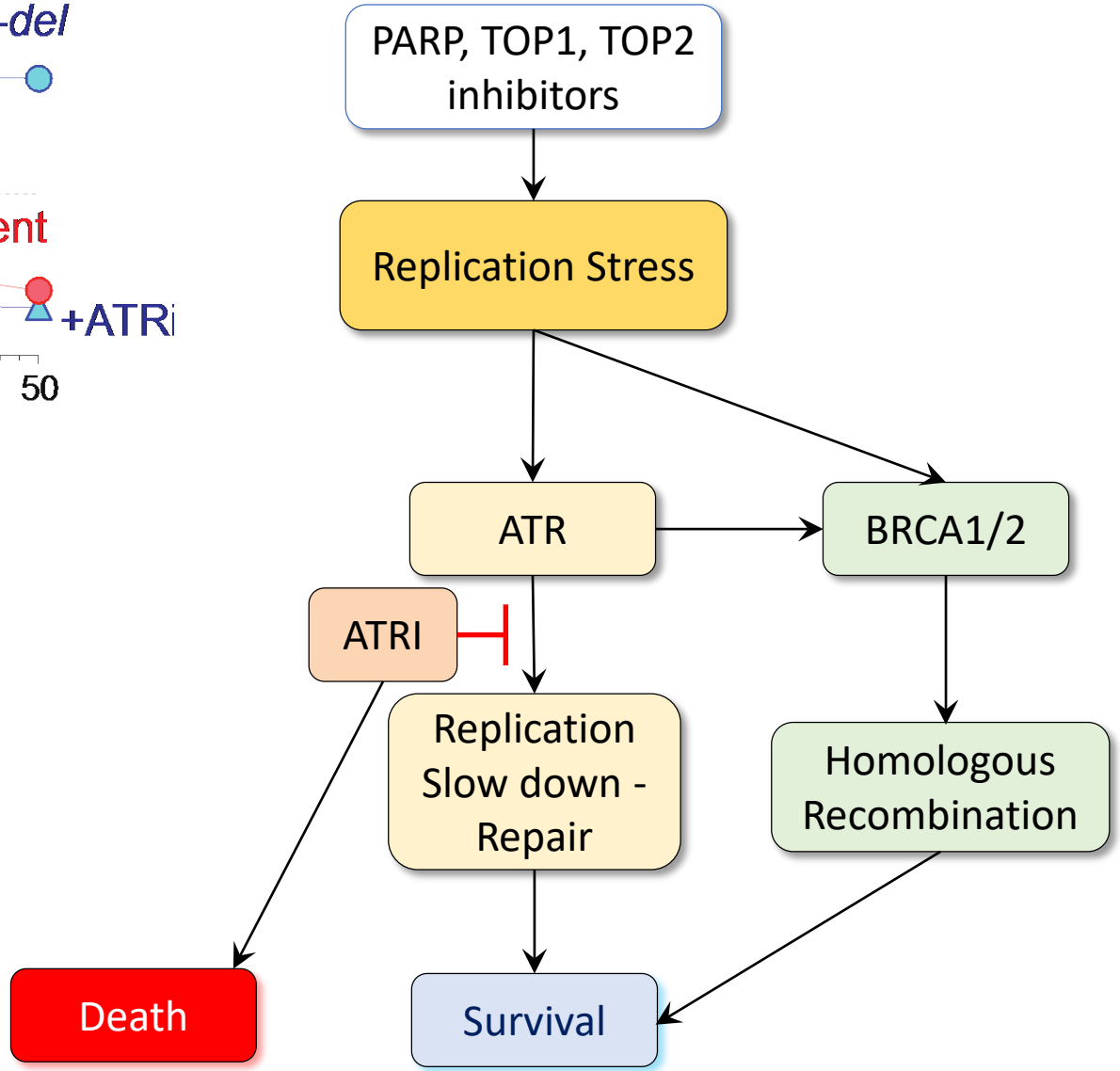


Murai…Pommier Oncotarget 2016 and ongoing studies

SLFN11 deficient cancer cells

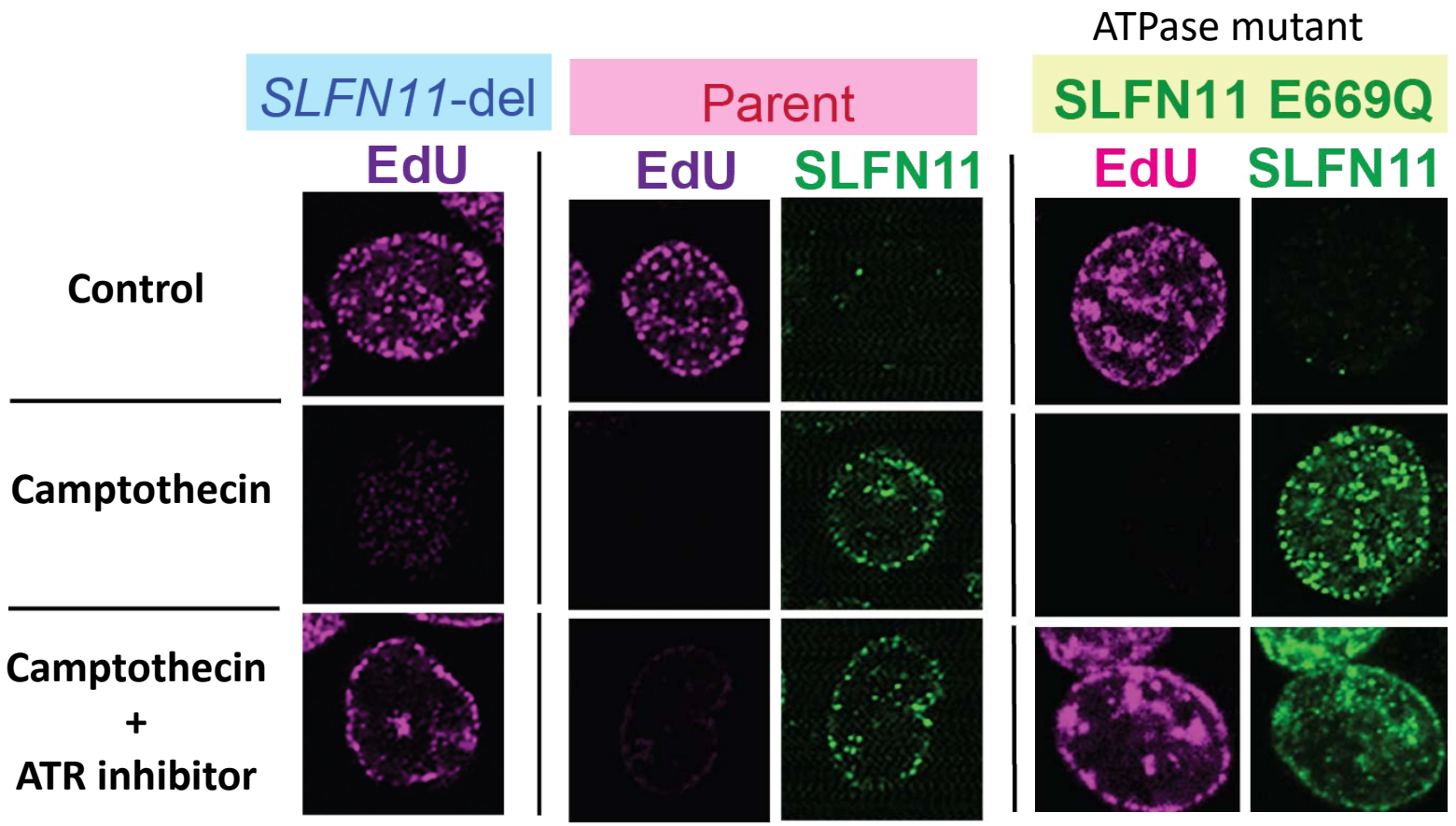
are hypersensitive to ATR inhibitors combined with DNA-replication-targeted therapies





Murai…Pommier Oncotarget 2016 and Mol Cell 2018

How does SLFN11 irreversibly arrest replication?



4 hour treatment with
100 nM CPT
± 1 µM AZD6738 (ATRi)

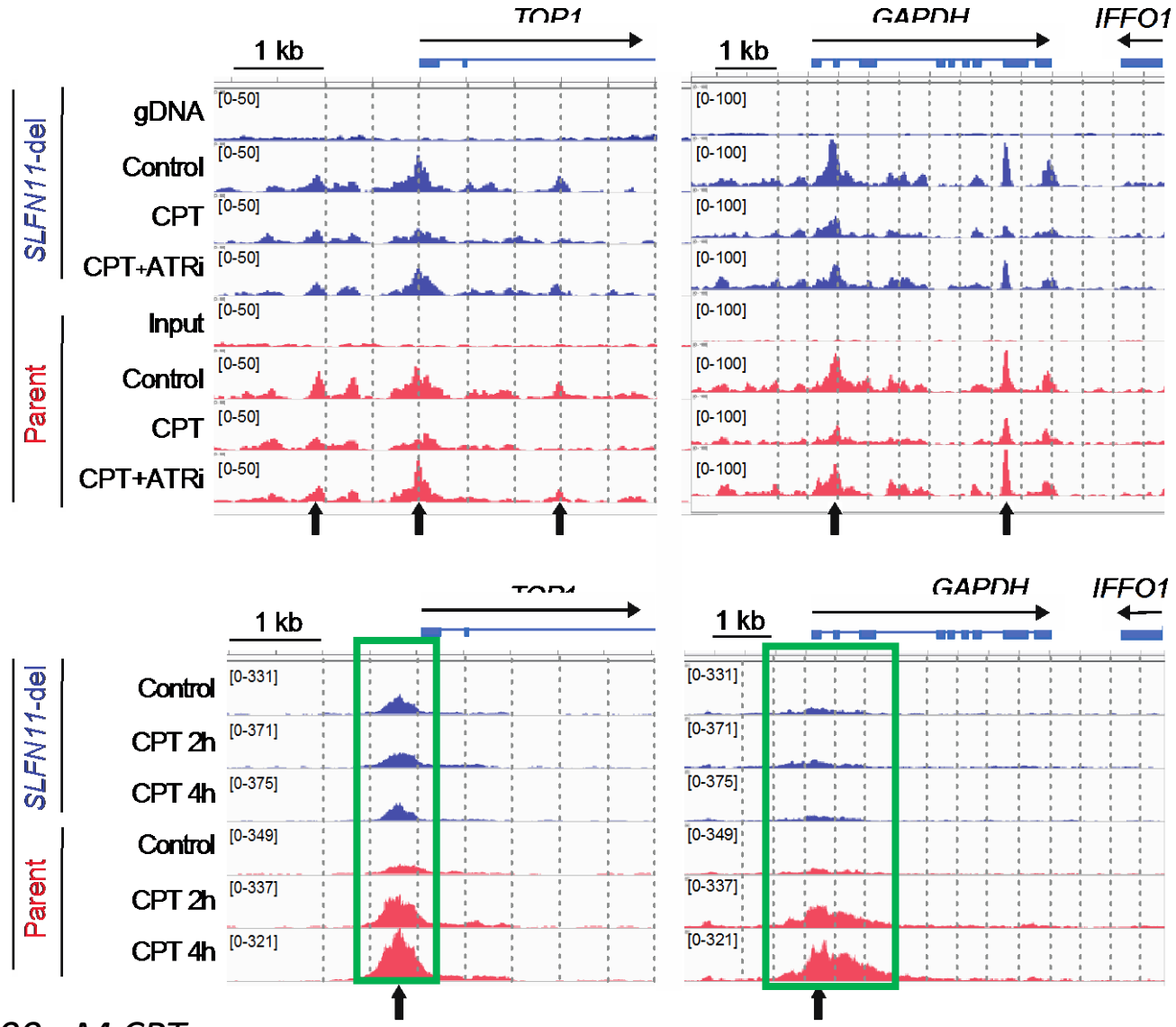
- Binding of SLFN11 to replication factories
- ATR-independent replication block due to ATPase activity (SLFN11 E669Q mutant)

Murai, J….Pommier, Y. 2018 Mol Cell

SLFN11 irreversibly arrest replication by opening chromatin

Nascent strand DNA sequencing:
Replication origins fire independently of SLFN11

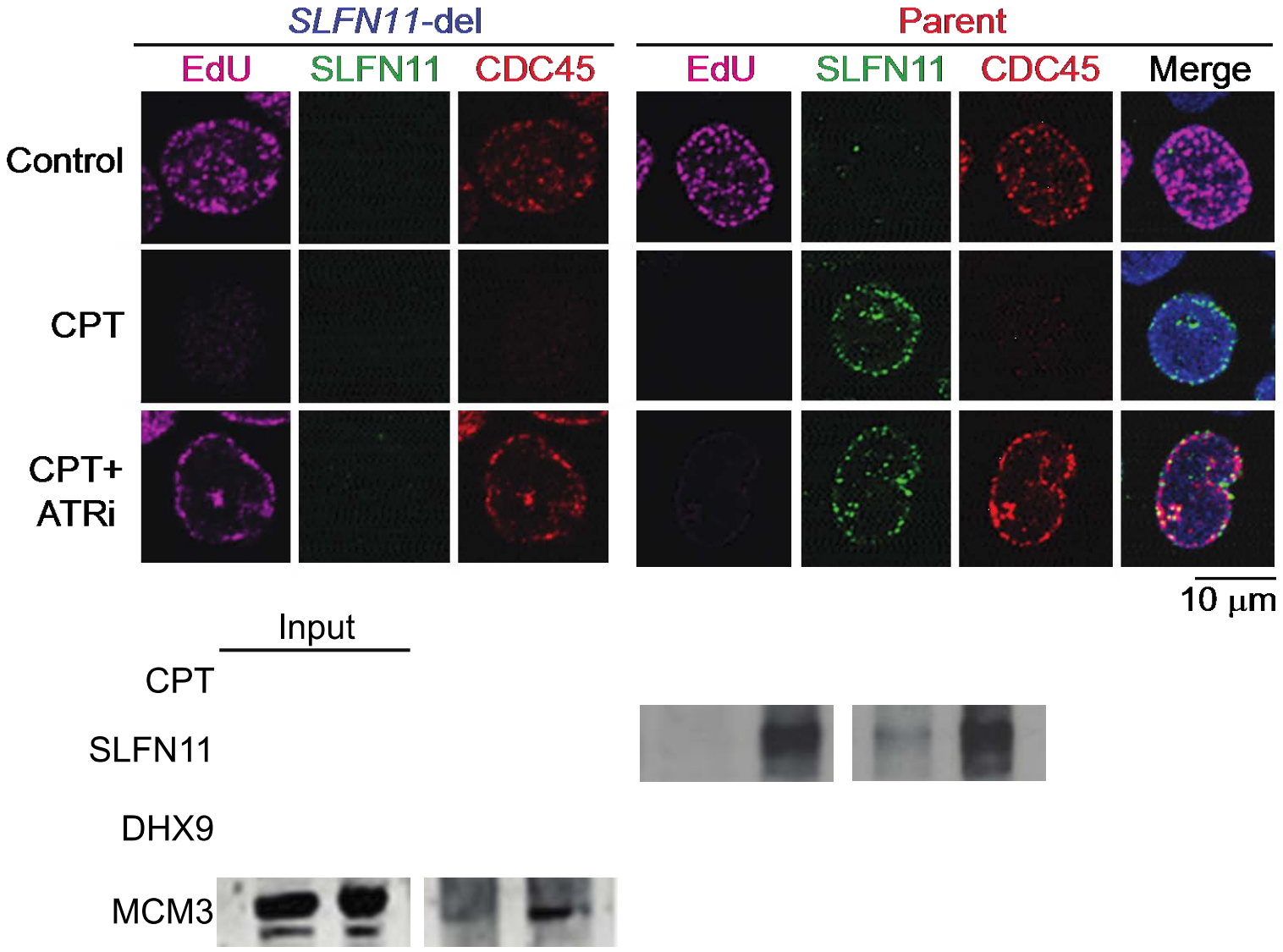
ATAC-seq:
Chromatin opening by SLFN11 in response to CPT

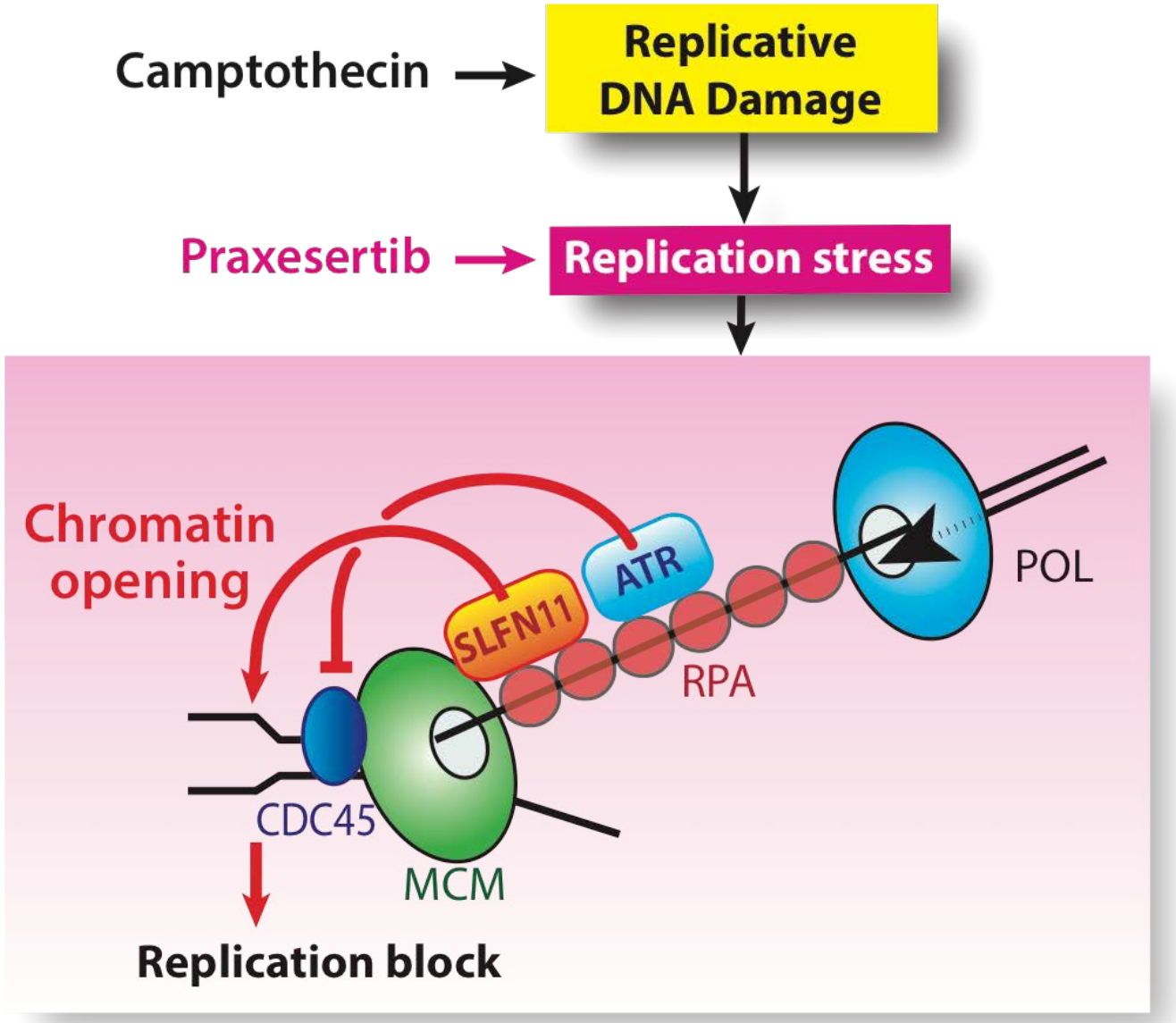


4 hour treatment with 100 nM CPT
± 2 µM VE-821 (ATRi)

Murai, J.…Pommier, Y. 2018 Mol Cell

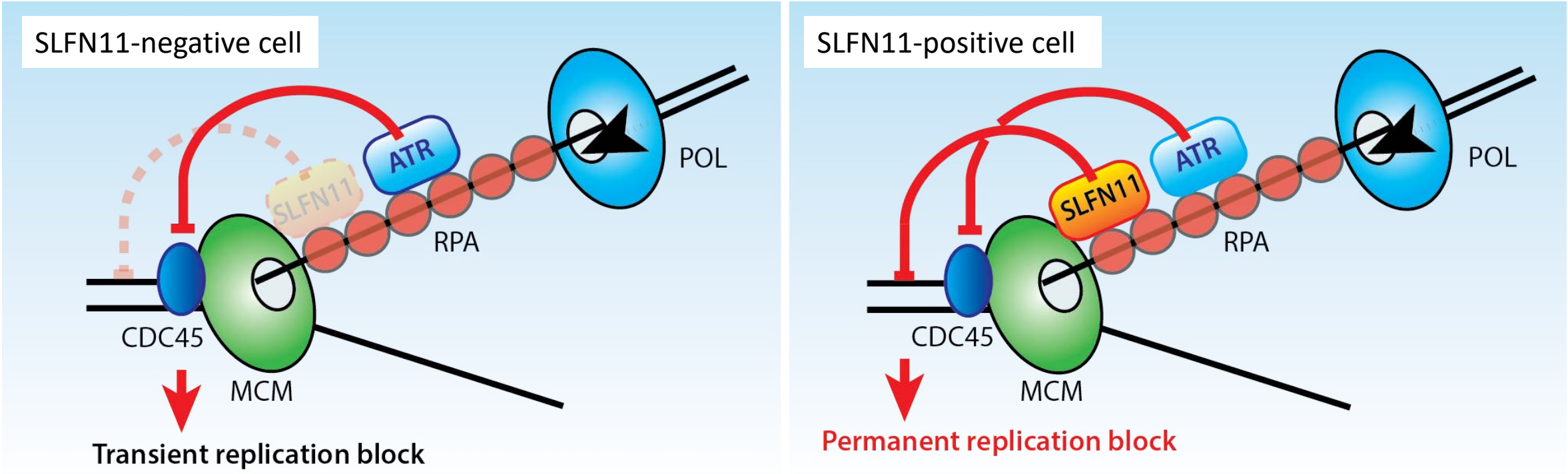
SLFN11 binds the CMG (CDC45, MCM2-7, GINS) replication helicase and DHX9 RNA helicase A





Murai, J.…Pommier, Y. 2018 Mol Cell

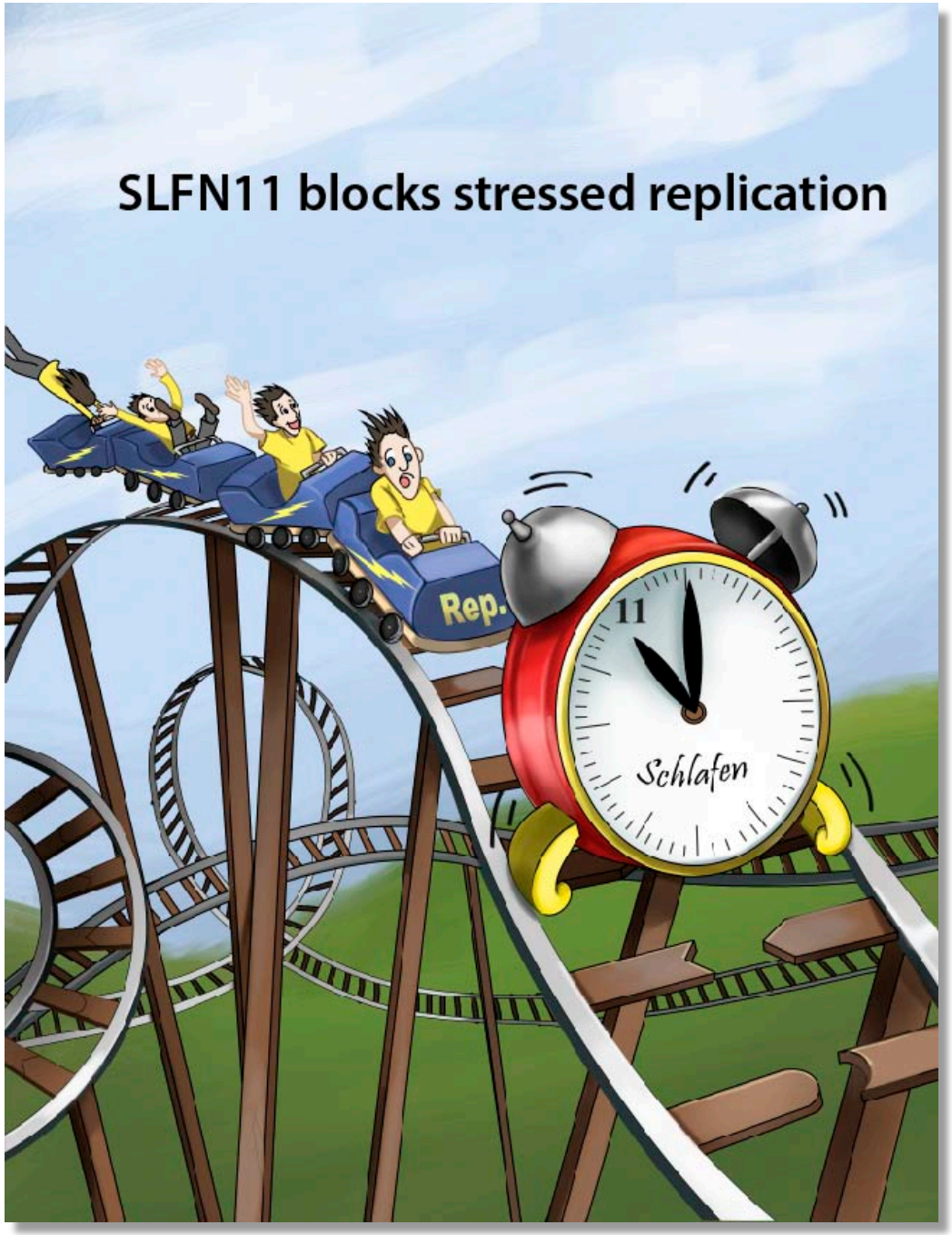
Working model for replication block by SLFN11



In the absence of SLFN11
(≃ 50% cancer cell lines: HeLa, U2OS, HCT116, RKO, MCF7, MDA-MB231…), ATR-CHK1 transiently arrests replication to allow DNA repair

SLFN11 binds to stressed replication forks through RPA, and arrests replication by blocking the replicative helicase complex

Murai, J.…Pommier, Y. 2018 Mol Cell



Chromatin opening
ATPase

Murai, J….Pommier, Y. 2018 Mol Cell

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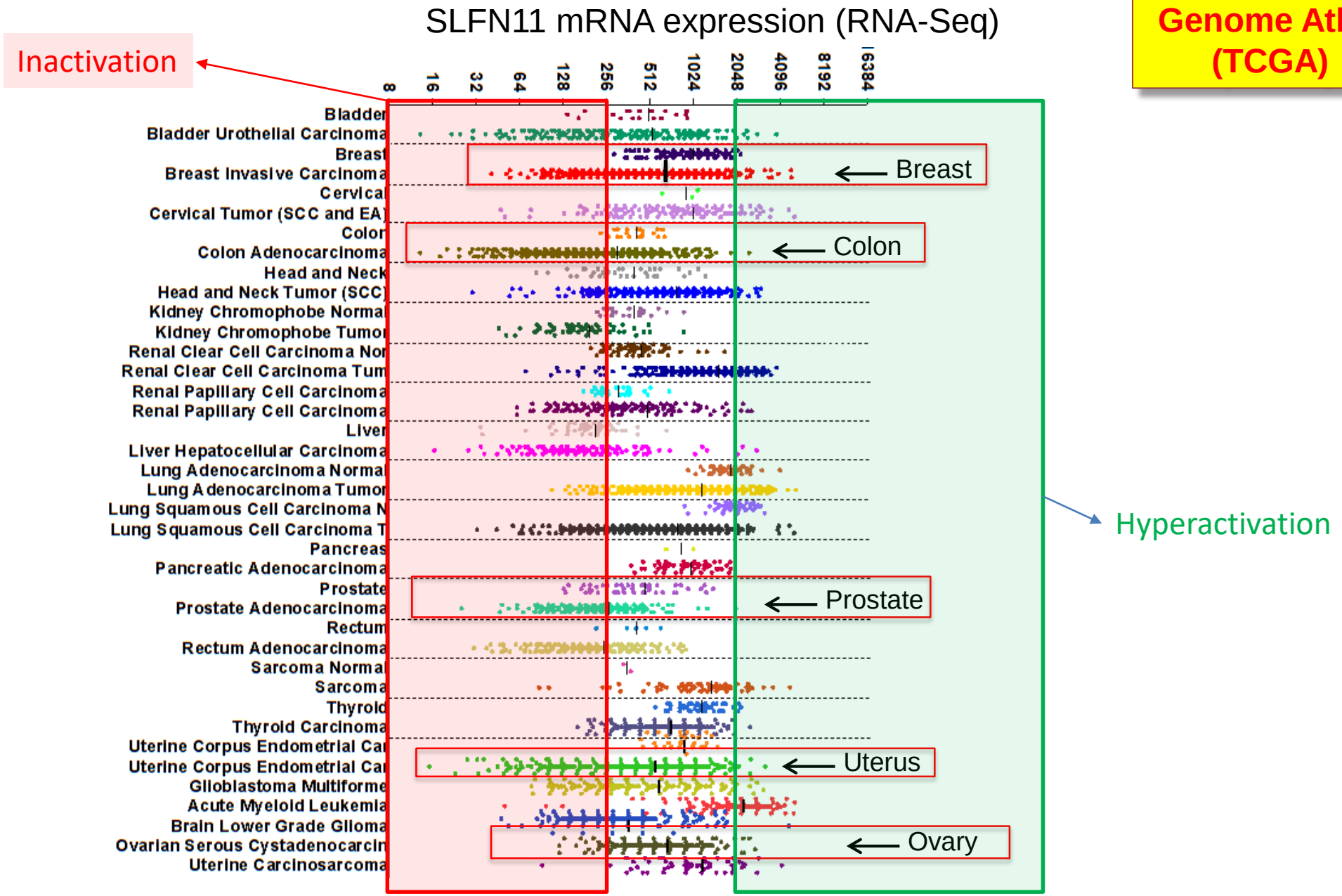
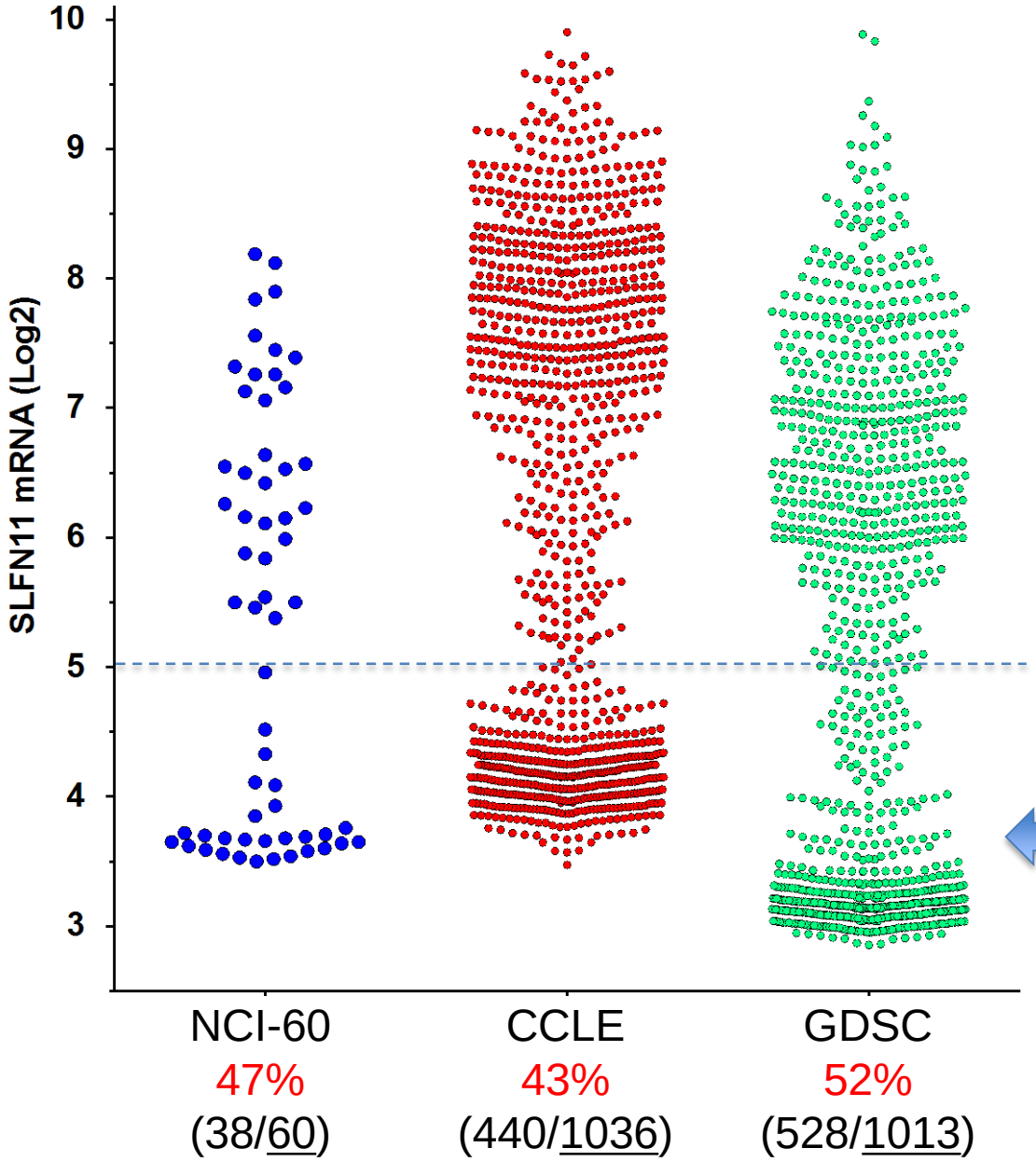
- Schlafen 11 (SLFN11) is a determinant of response to all drugs approved for SCLC treatment (cisplatin, etoposide, topotecan) and to PARP inhibitors beyond BRCA; i.e. replication inhibitors
- How is SLFN11 putting cells with replicative damage to sleep?
- SLFN11 translation to the clinic?

Broad range of SLFN11 expression in tumor tissue

The Cancer Genome Atlas (TCGA)



SLFN11 is inactivated in ≈ 45% of cancer cell lines



Especially in the cancer cell lines that are commonly used for screening resistance to PARP inhibitors

HeLa
U2OS
HCT116
RKO
MCF7
MDA-MB231
…

Translating SLFN11 to the clinic

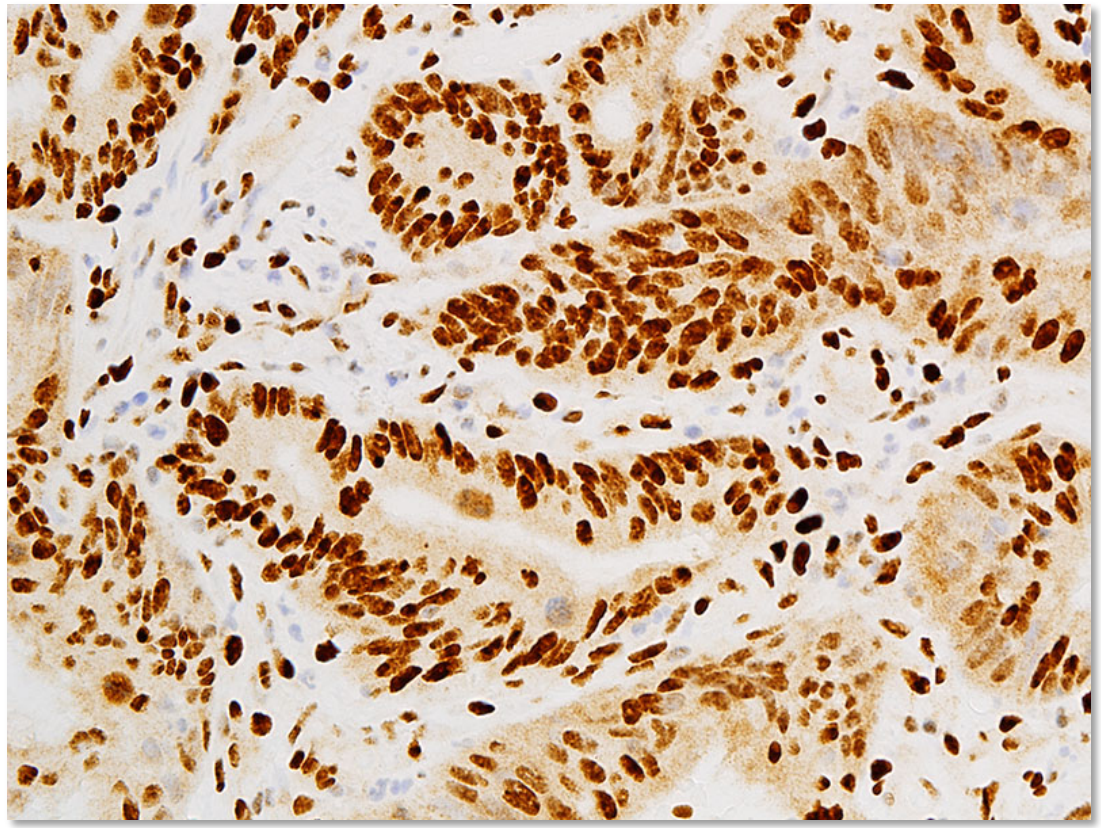
- **Transcript levels reflect protein expression – high dynamic range**
- **DNA methylation, histone deacetylation and EZH2 repress SLFN11 transcription**
- **IHC is readily feasible**

Slfn11 Antibody (D-2): sc-515071, mouse monoclonal, Santa Cruz

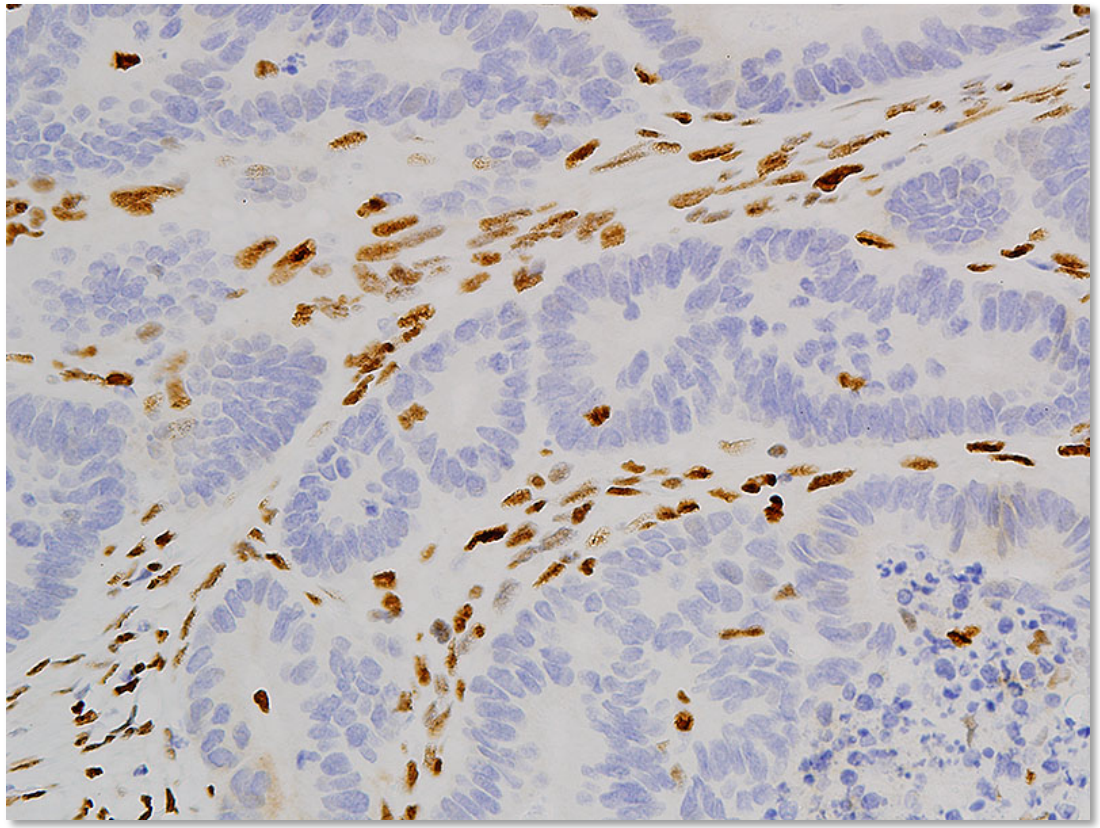
Selected by Junko Murai

Data from Drs. Anish Thomas and Markku Miettinen

Colon cancer (SLFN11-positive case)



Colon cancer (SLFN11-negative case)



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- Schlafen 11 (SLFN11) is a determinant of response to all drugs approved for SCLC treatment (cisplatin, etoposide, topotecan) and to PARP inhibitors beyond BRCA; i.e. replication inhibitors
- SLFN11 puts cells with replicative damage to sleep by opening chromatin with its ATPase activity and blocking stressed replicons with long single-stranded RPA filaments. SLFN11 may act as “guardian of normal replication”
- SLFN11 translation to the clinic?