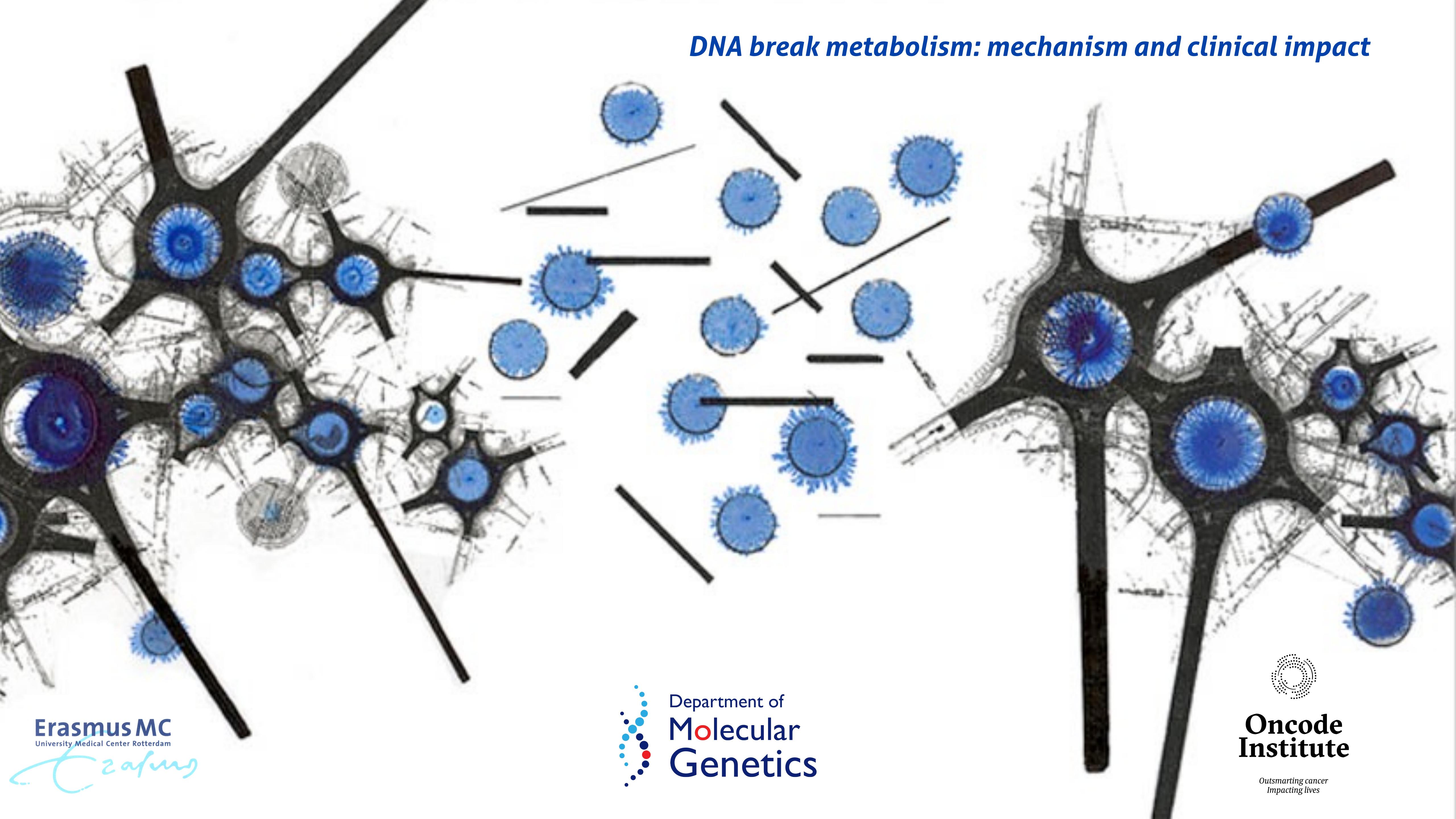
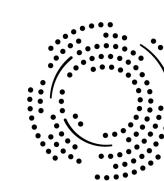


DNA break metabolism: mechanism and clinical impact



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**Molecular
Genetics**



**Oncode
Institute**
Outsmarting cancer
Impacting lives

DNA break metabolism: mechanism and clinical impact

Genome maintenance by DNA repair

Homologous recombination and BRCA2

Patient selection in breast cancer

Heterogenous disease

Different treatment options available

Patient selection in breast cancer

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Different treatment options available

Pathological analysis

DNA sequencing

Patient selection in breast cancer

Heterogenous disease

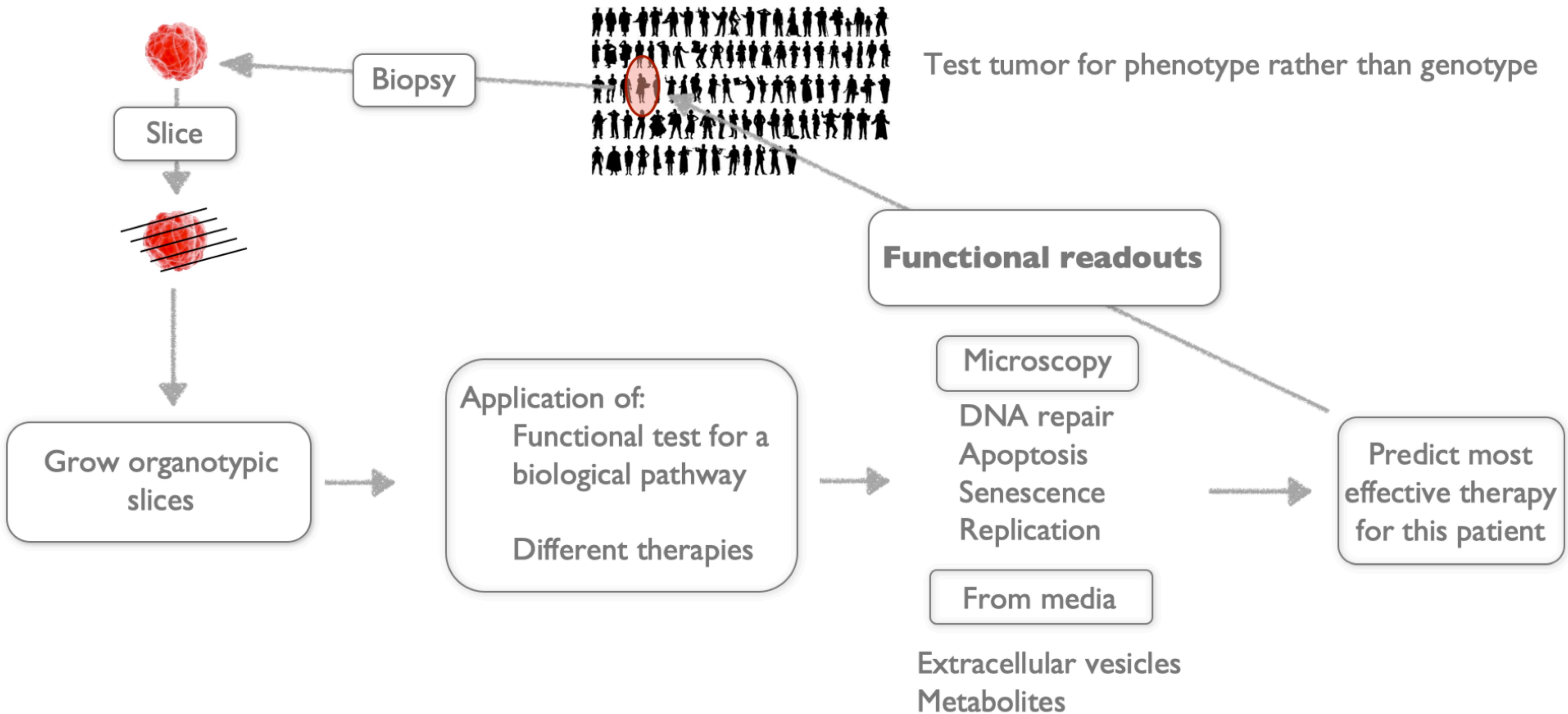
Different treatment options available

Pathological analysis

DNA sequencing

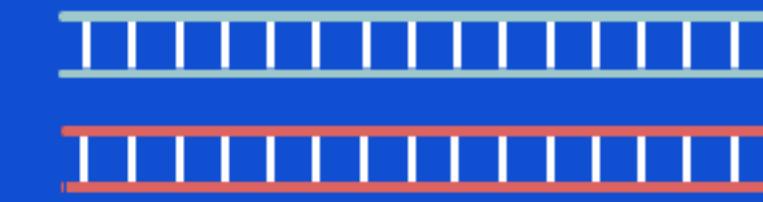
Functional pathology

Test viable patient tumor material for functionality in ex vivo tests

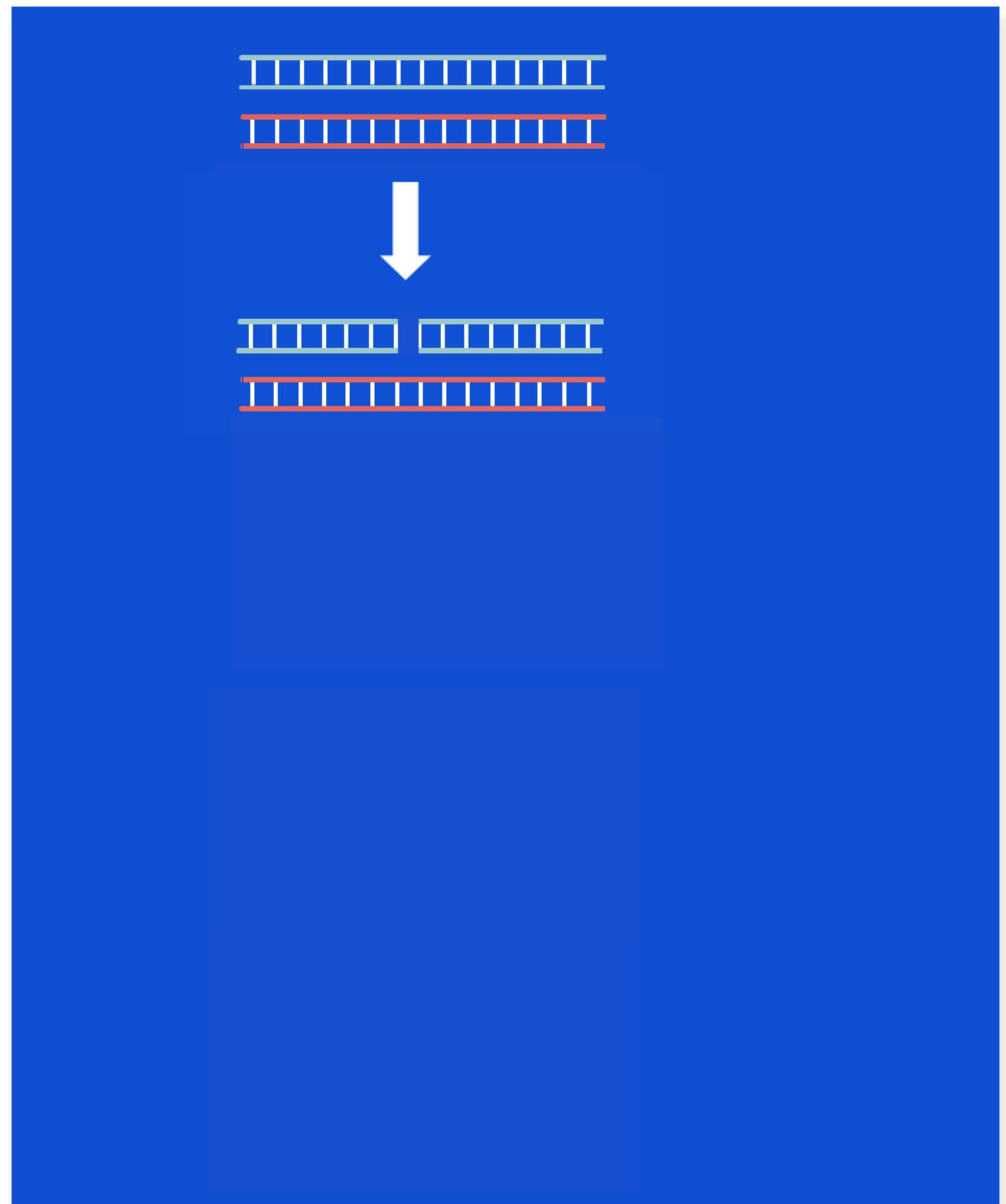


DNA repair by homologous recombination

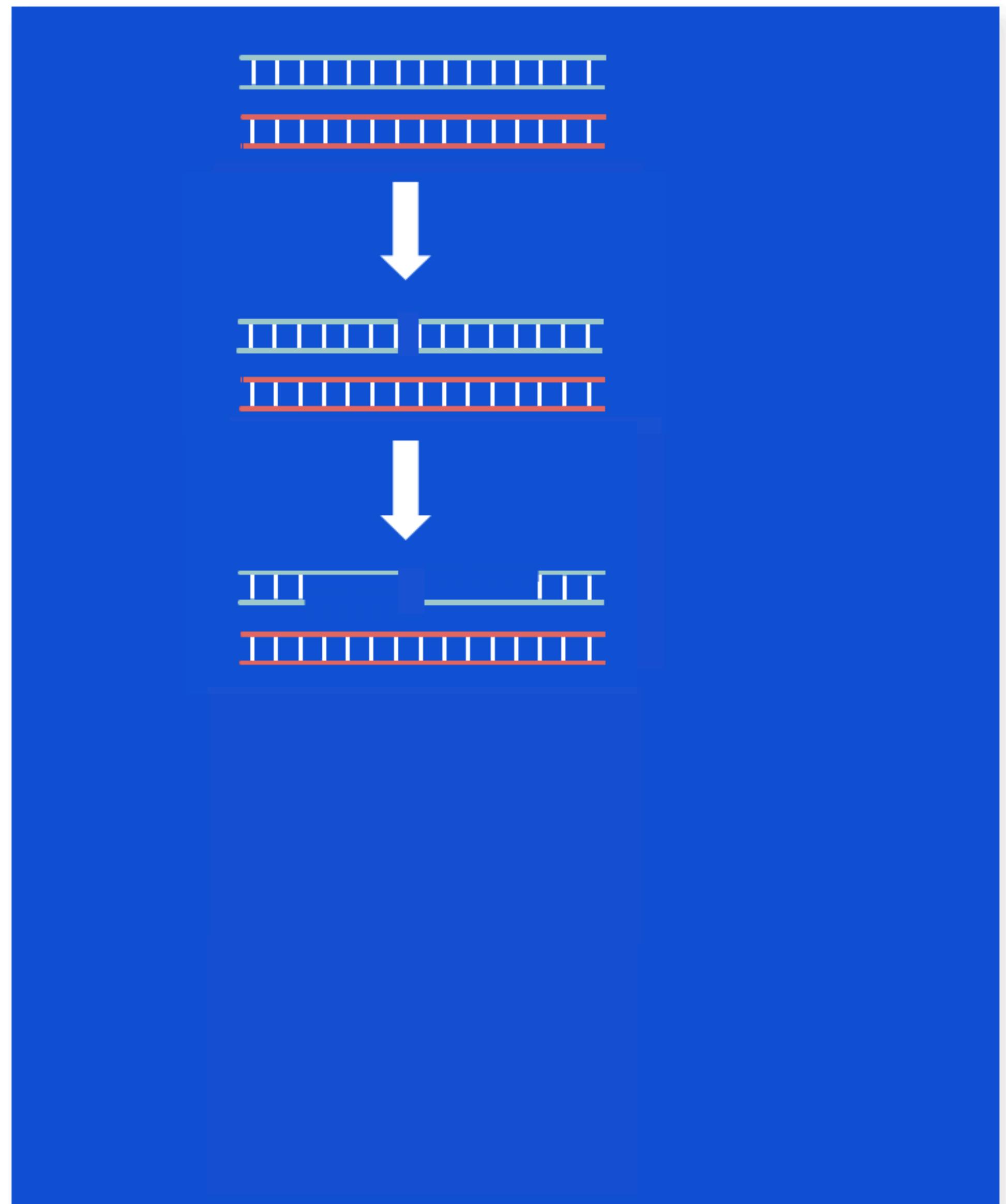
DNA repair by homologous recombination



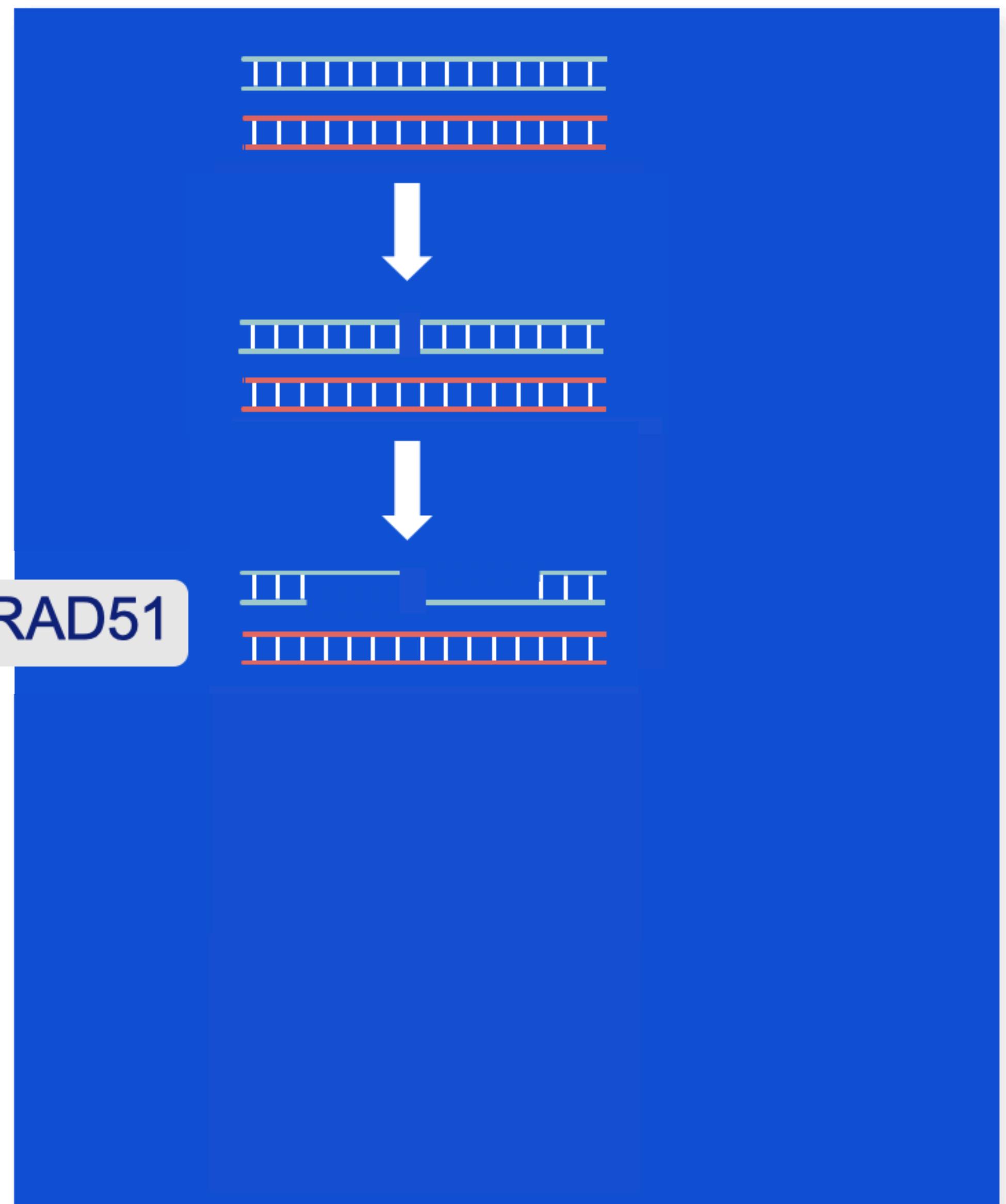
DNA repair by homologous recombination



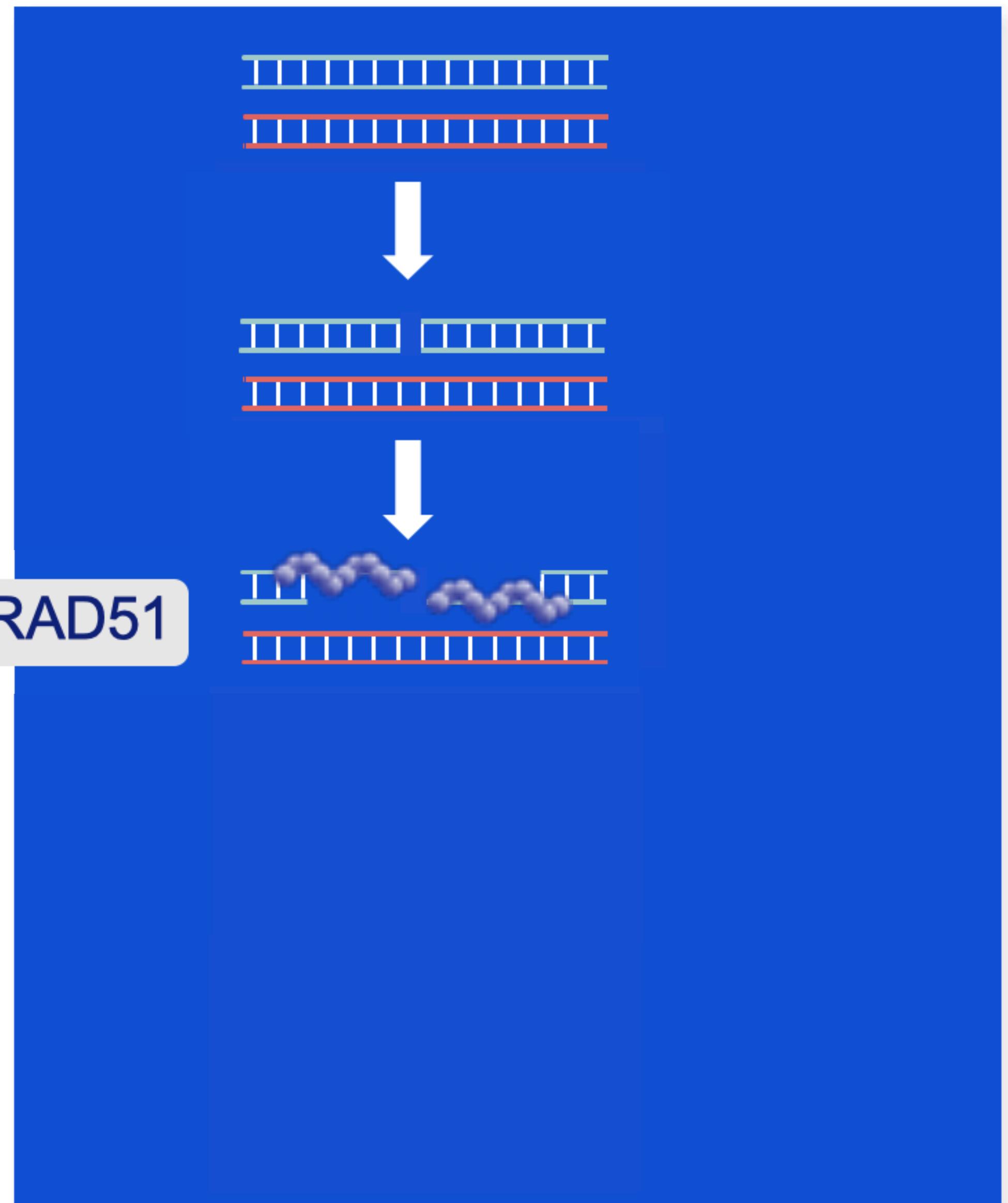
DNA repair by homologous recombination



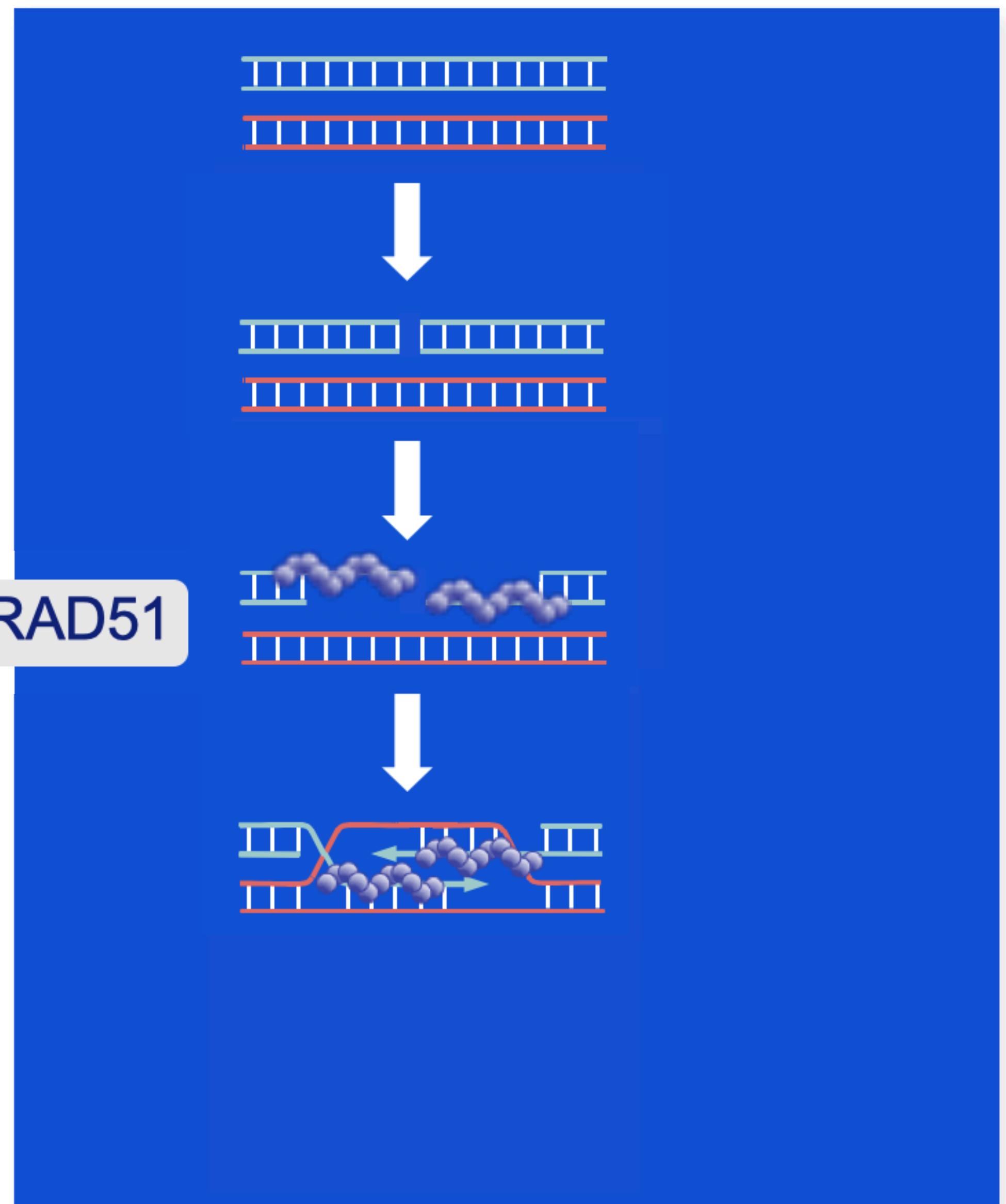
DNA repair by homologous recombination



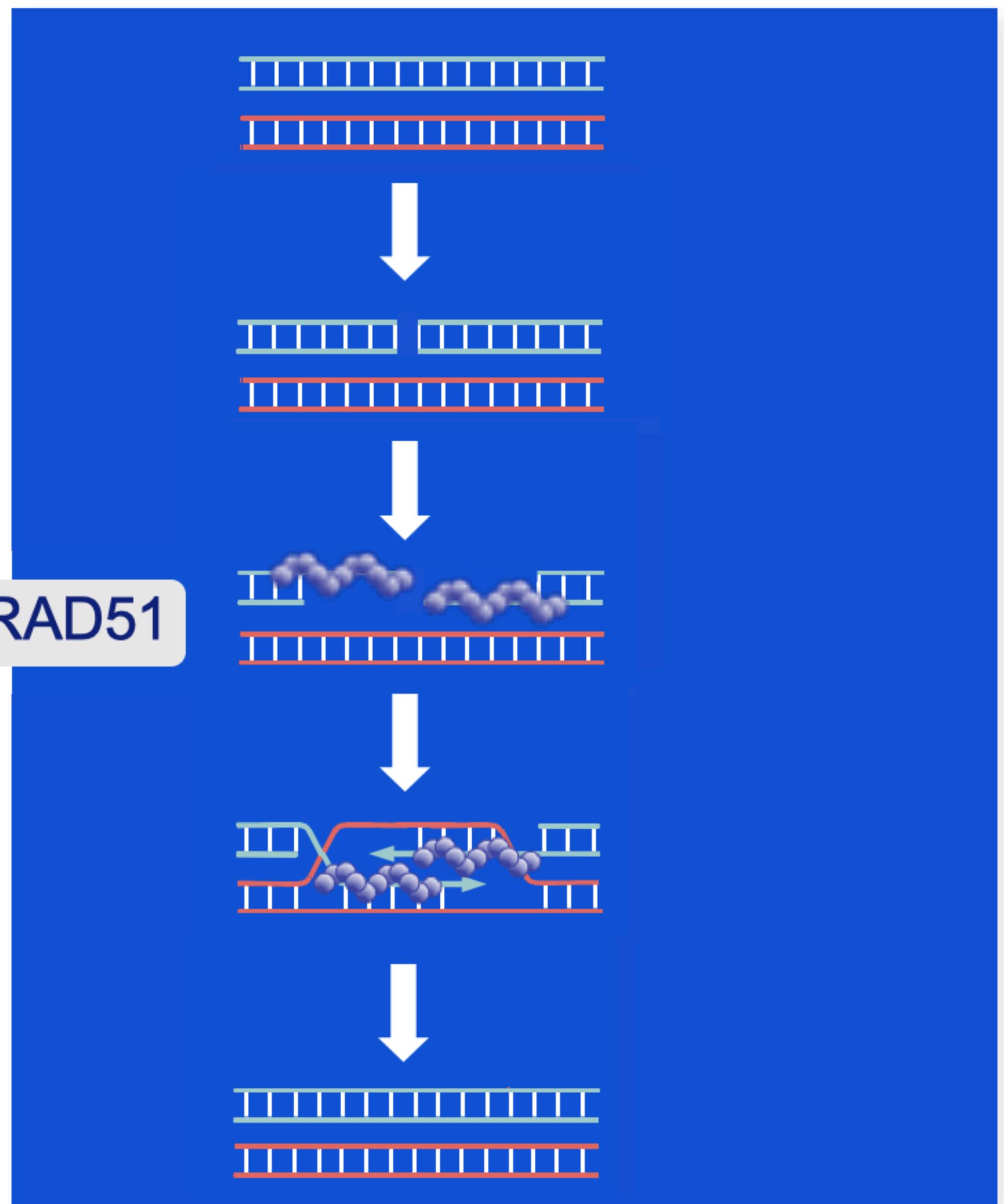
DNA repair by homologous recombination



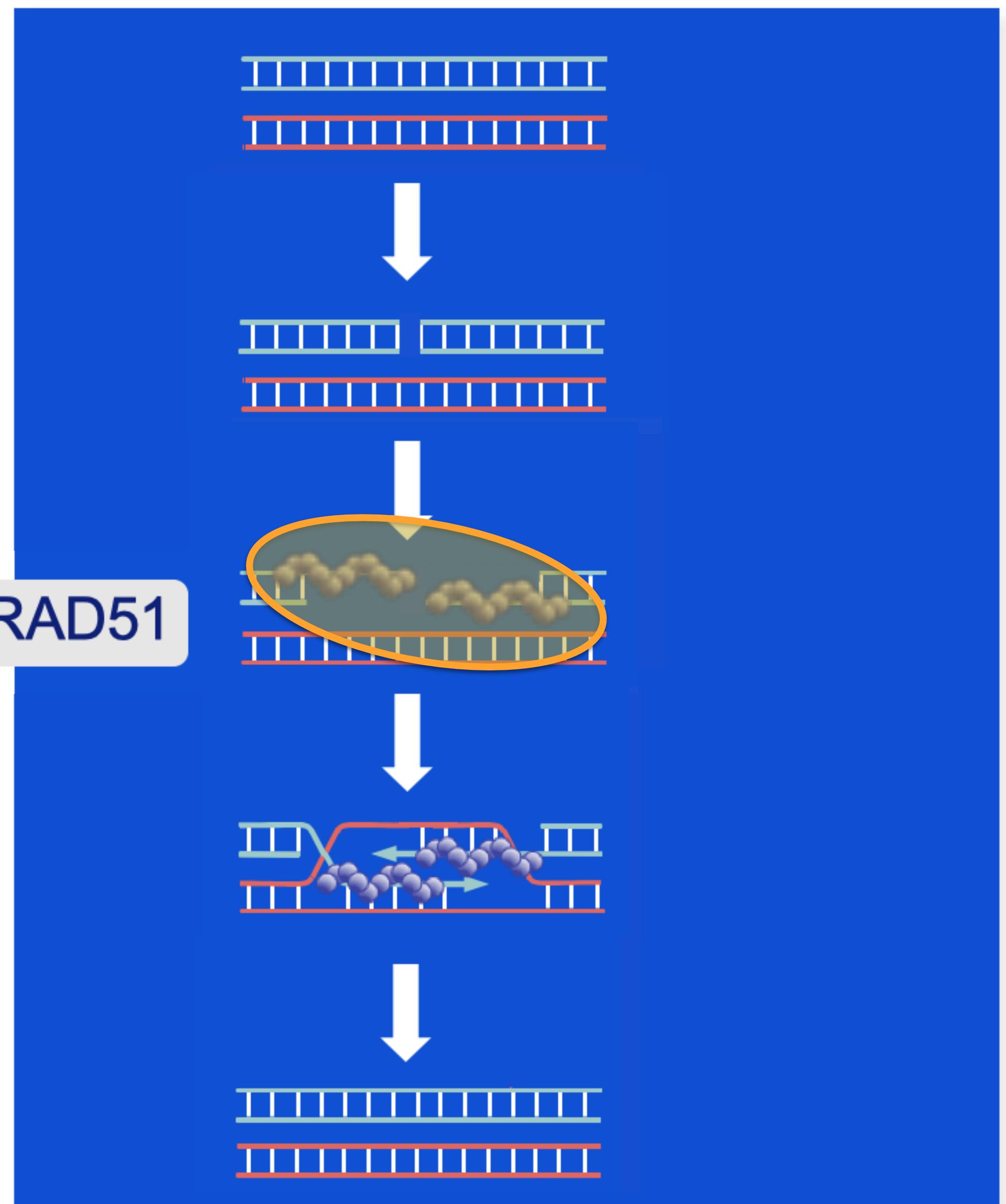
DNA repair by homologous recombination



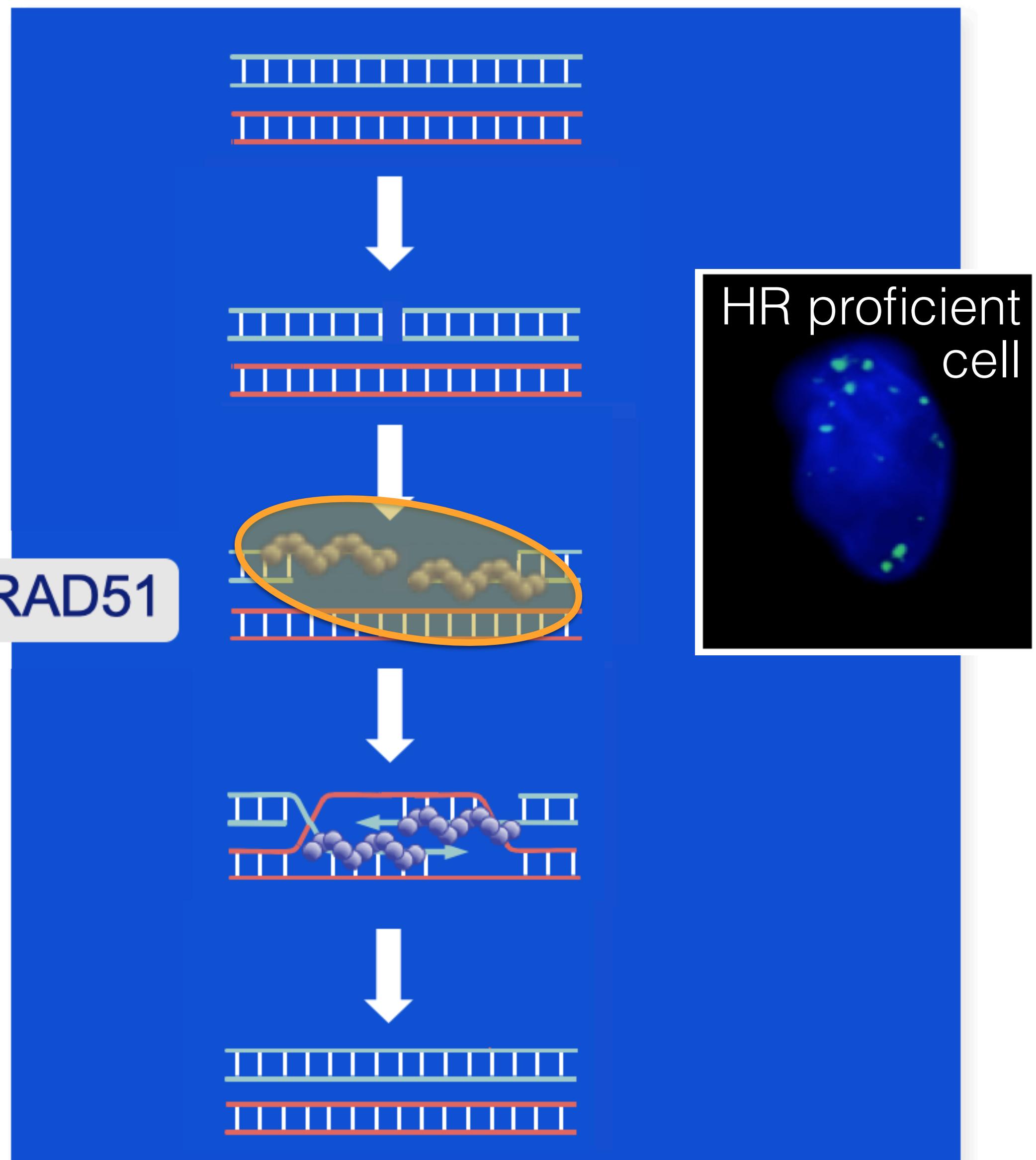
DNA repair by homologous recombination



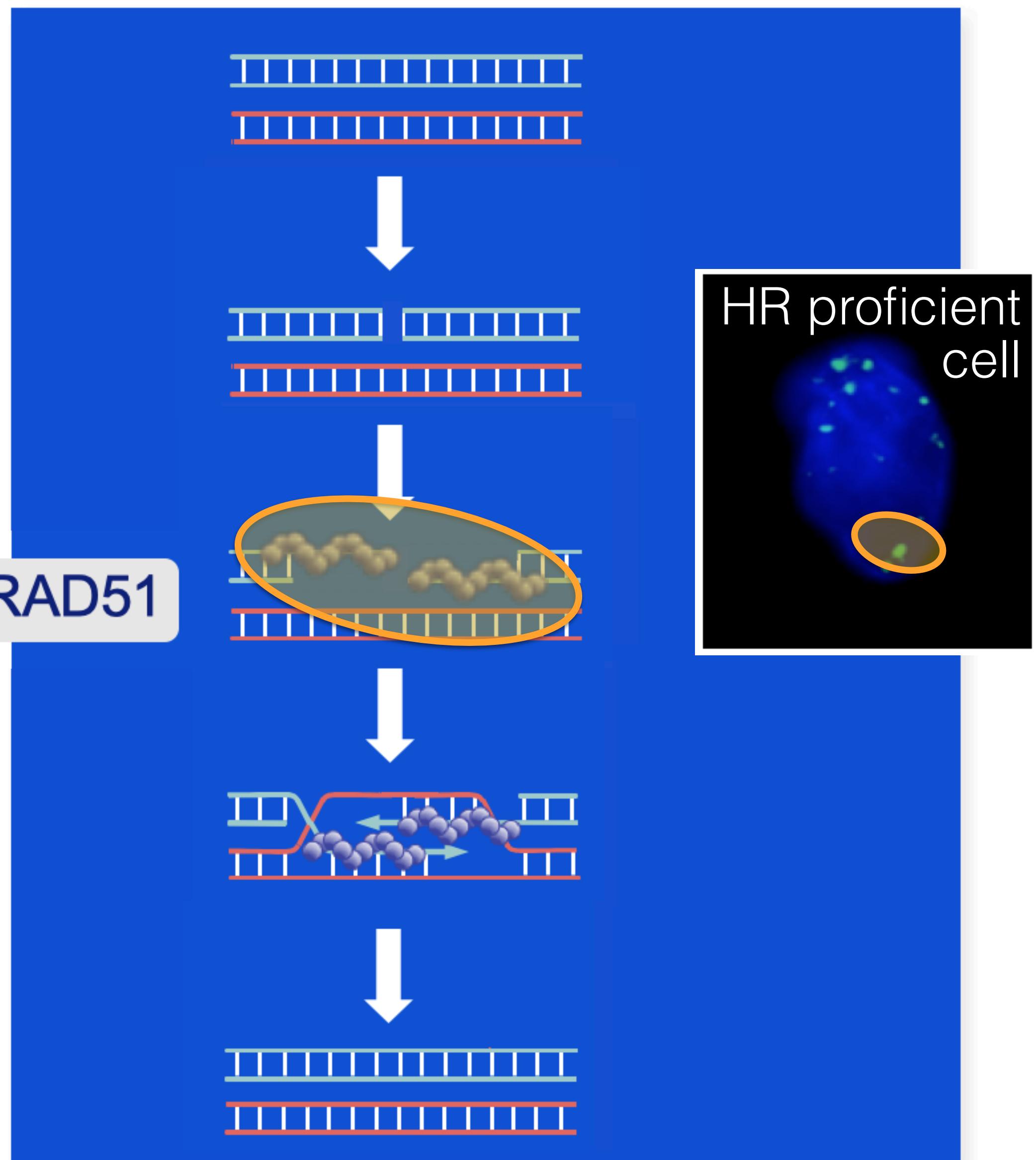
DNA repair by homologous recombination



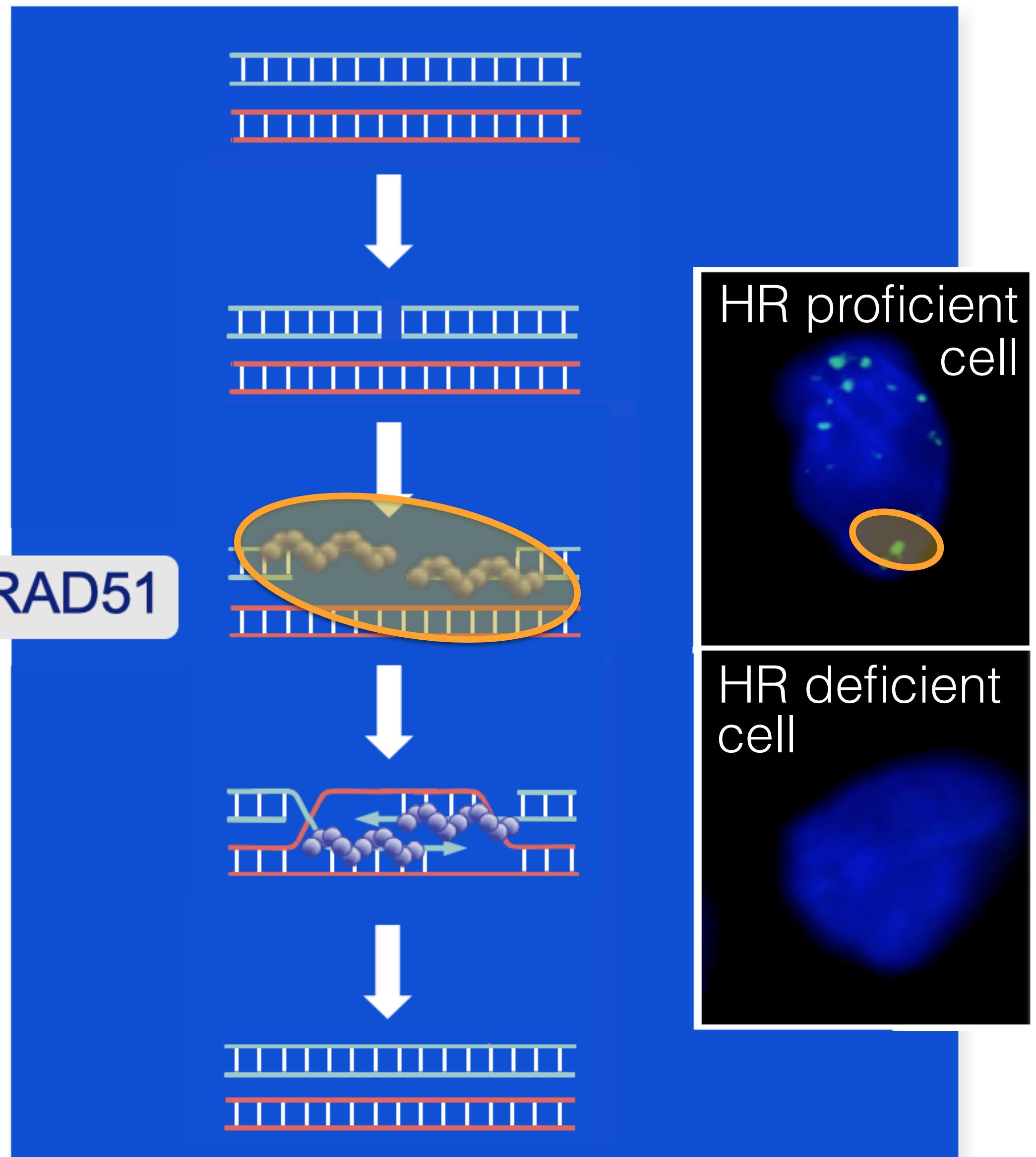
DNA repair by homologous recombination



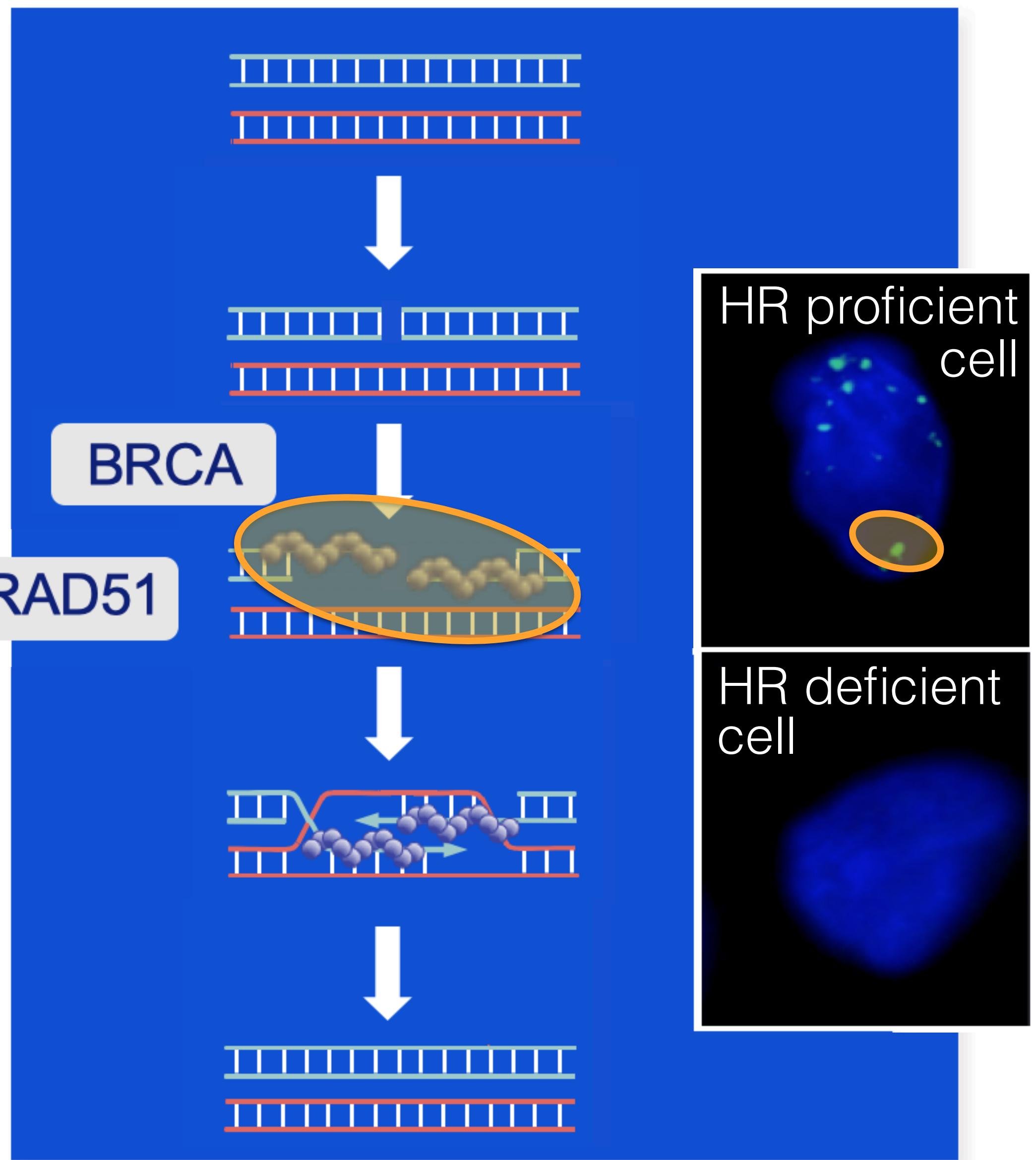
DNA repair by homologous recombination



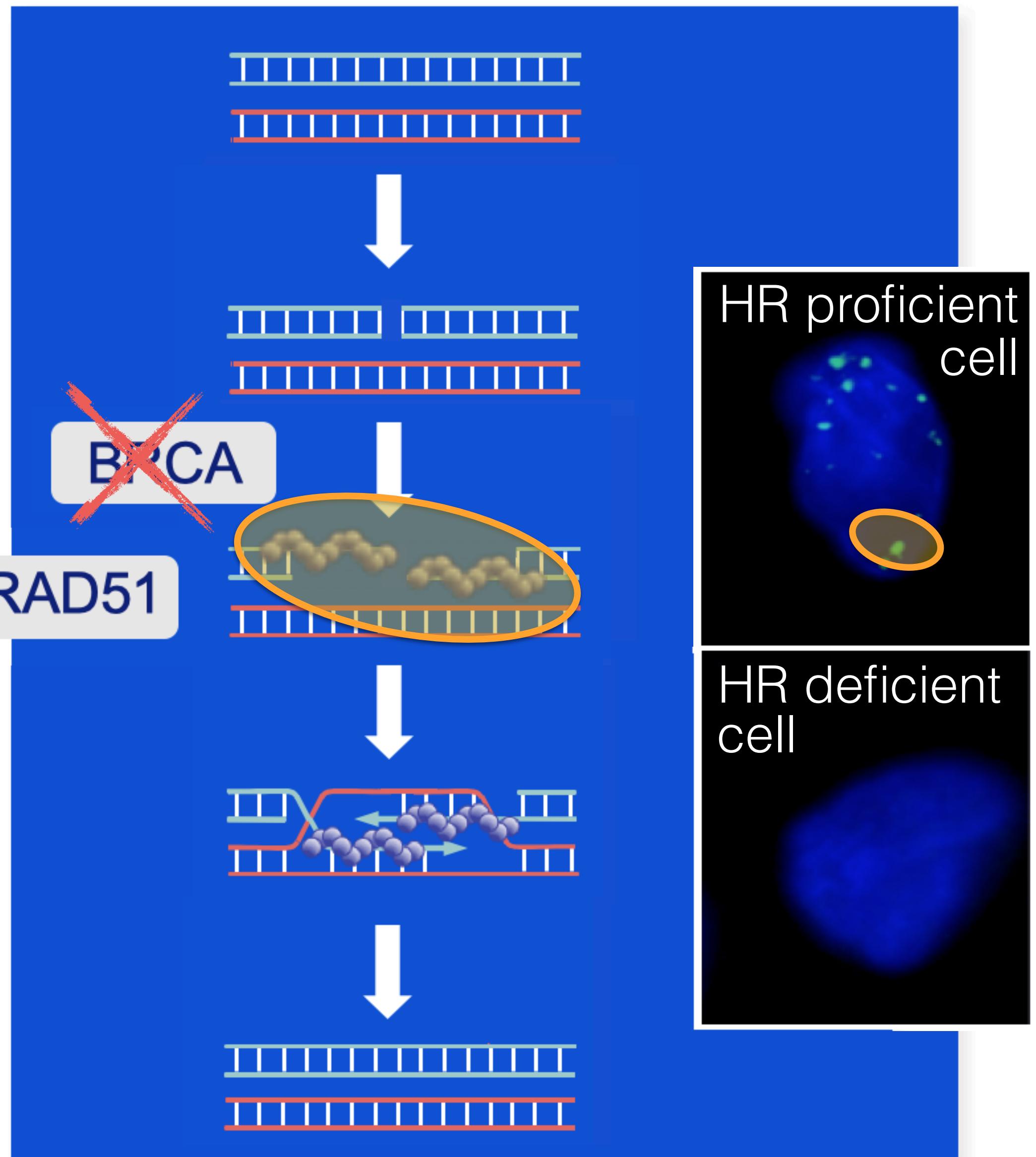
DNA repair by homologous recombination



DNA repair by homologous recombination



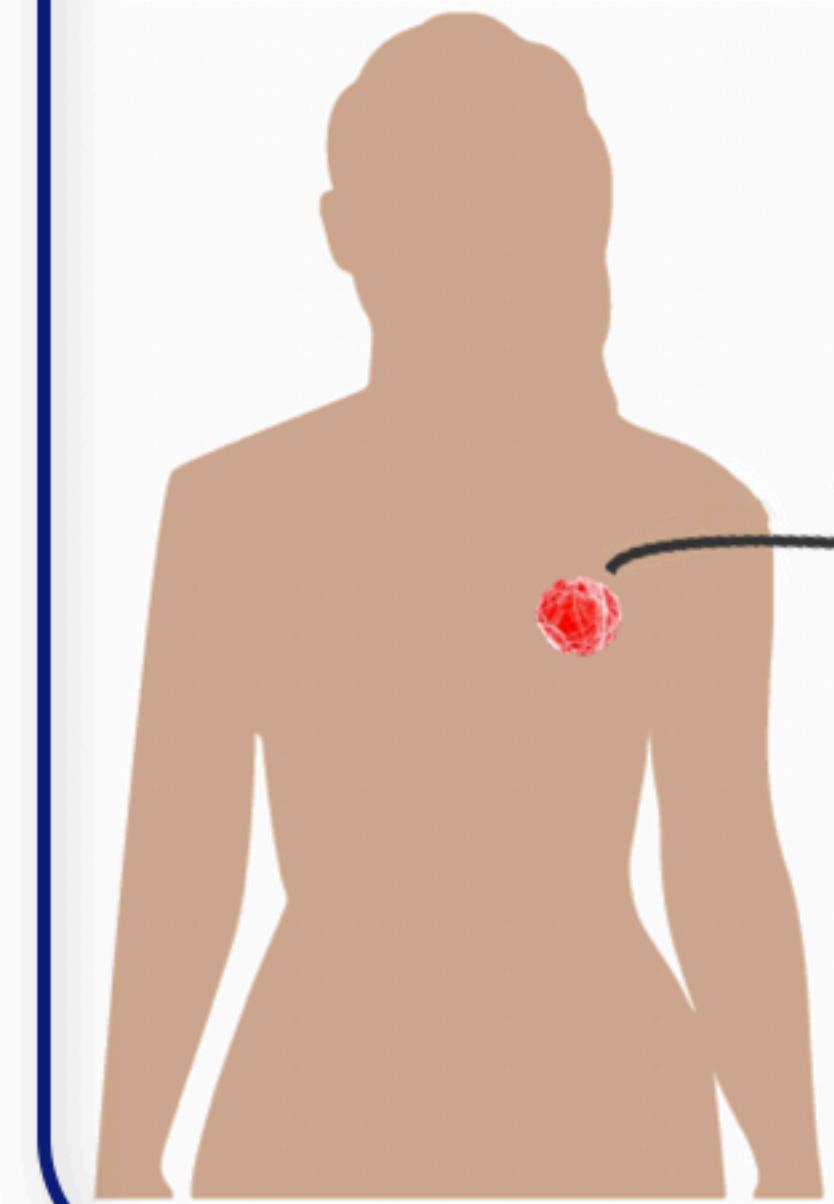
DNA repair by homologous recombination



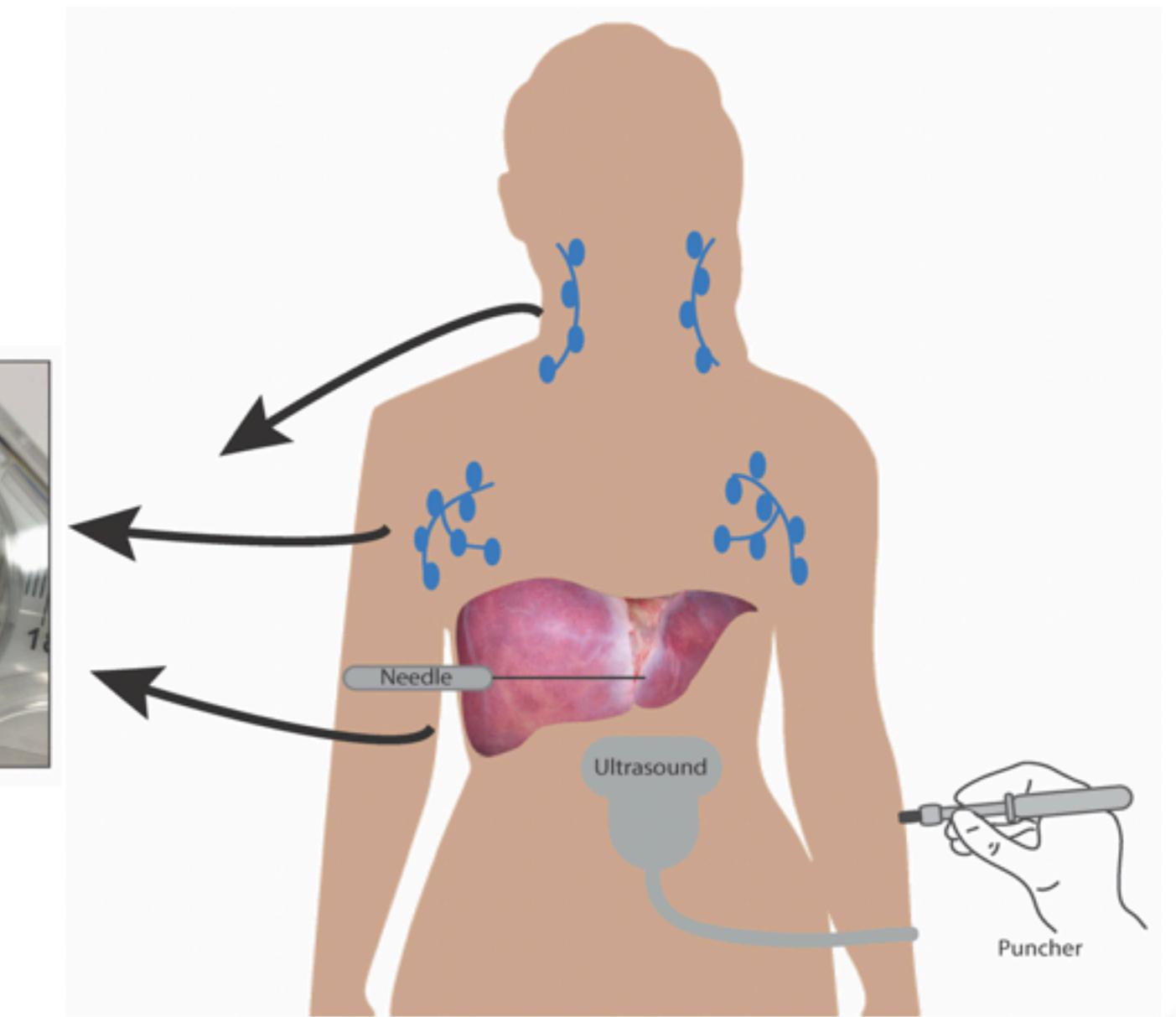
REpair CAPacity test

- Fresh tumor tissue

Primary tumor

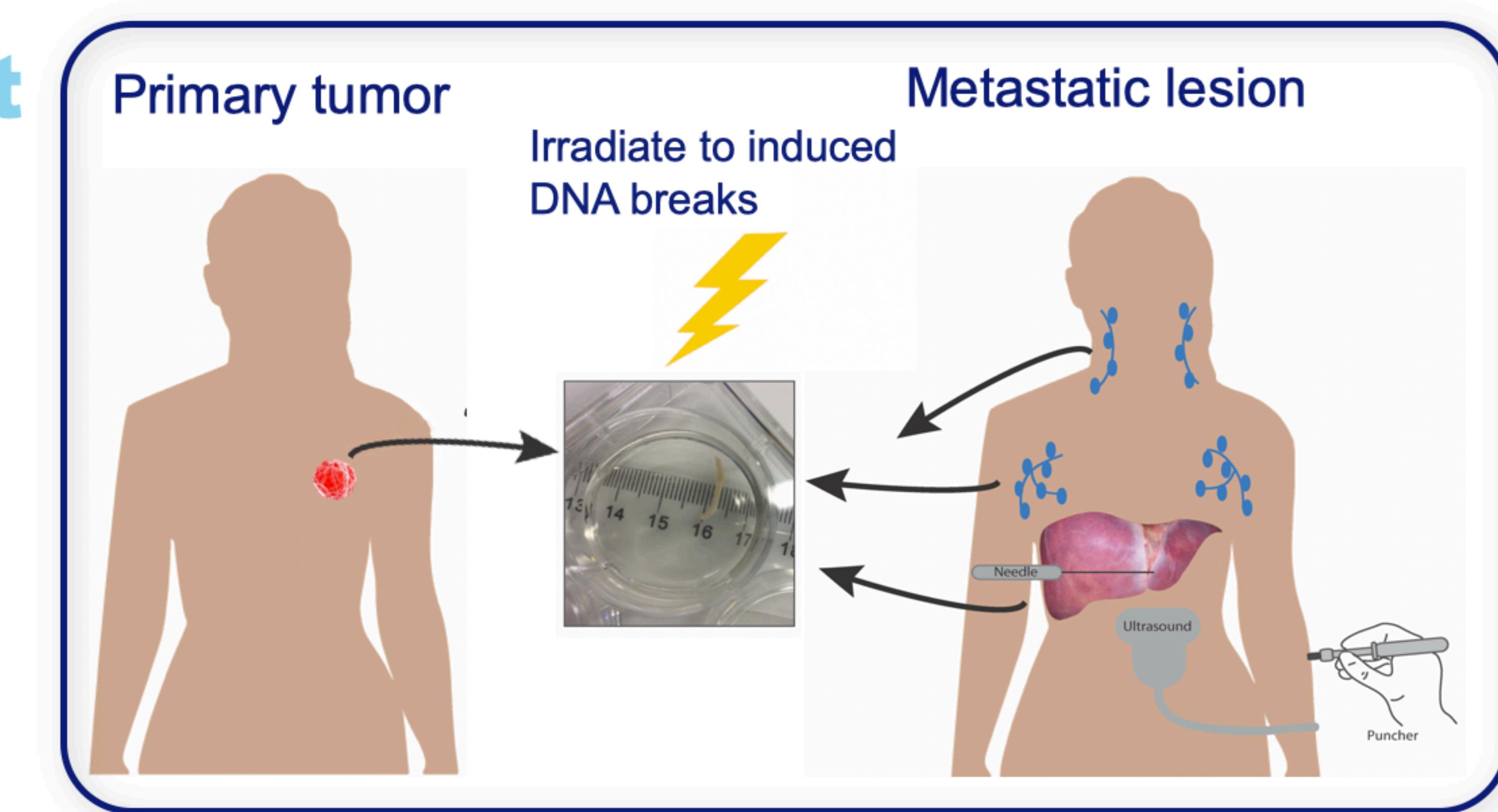


Metastatic lesion



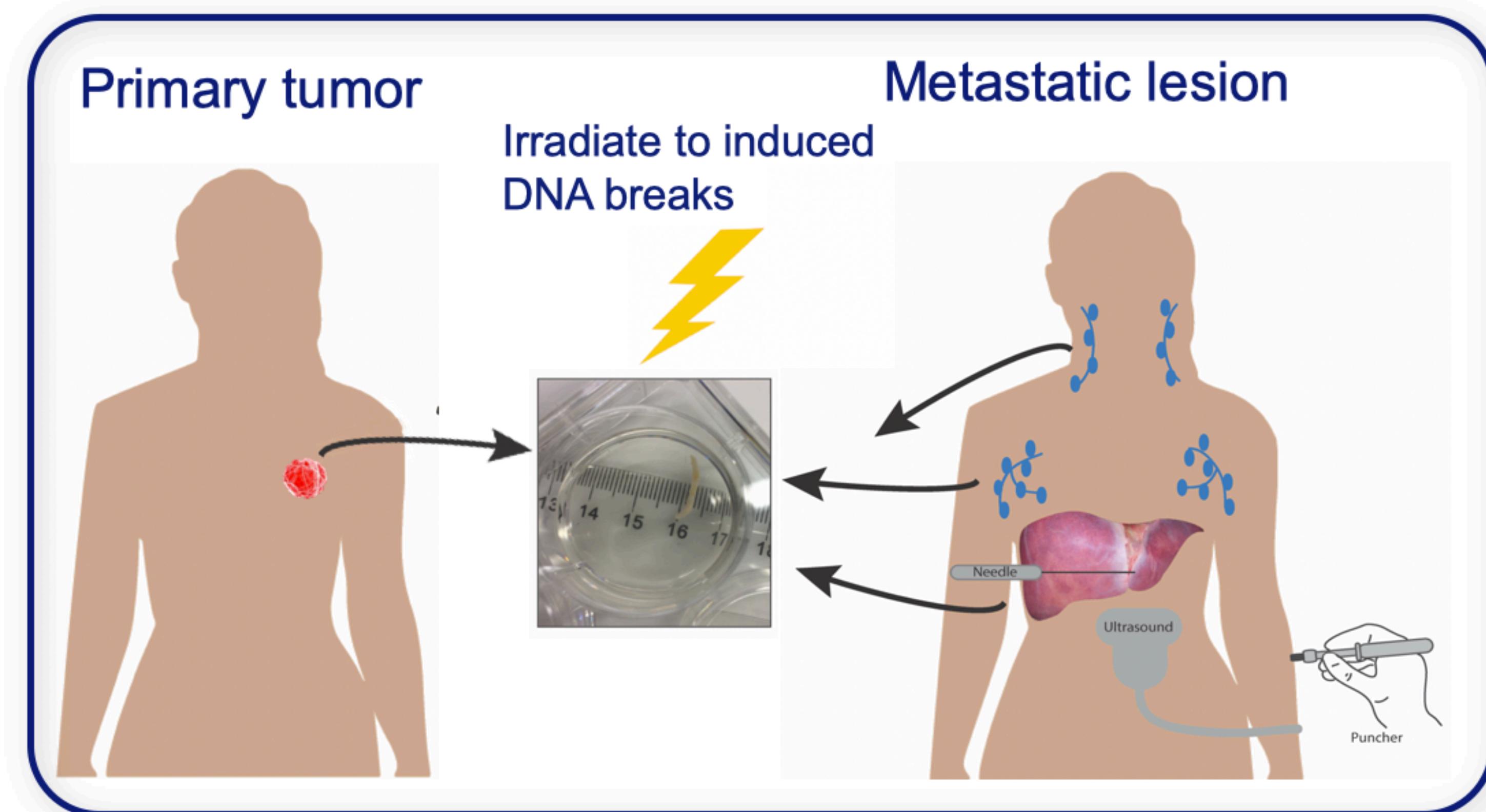
REpair CAPacity test

- Fresh tumor tissue
- Functional test for HRD

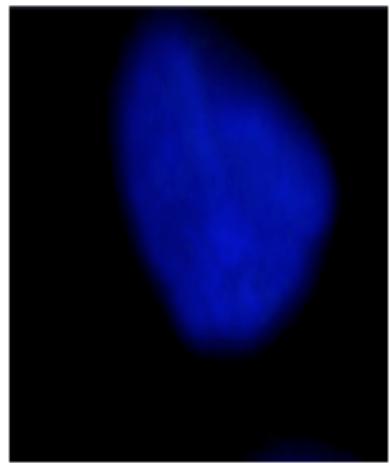
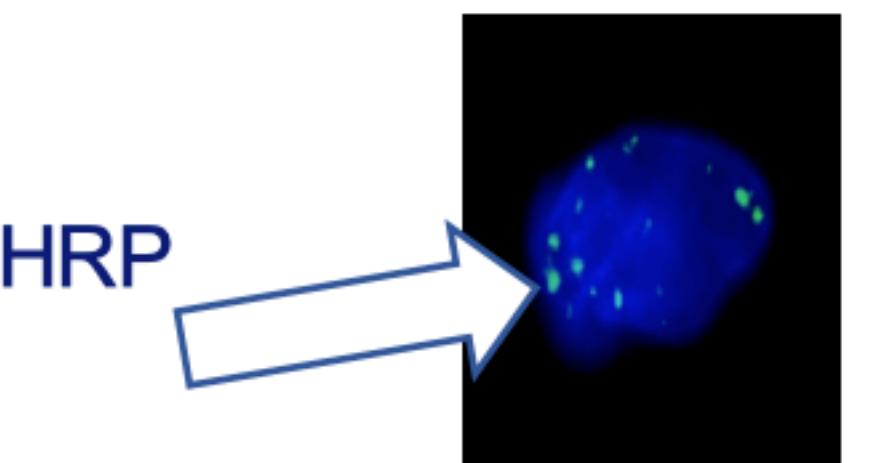


REpair CAPacity test

- Fresh tumor tissue
- Functional test for HRD



Functional test for homologous recombination efficacy

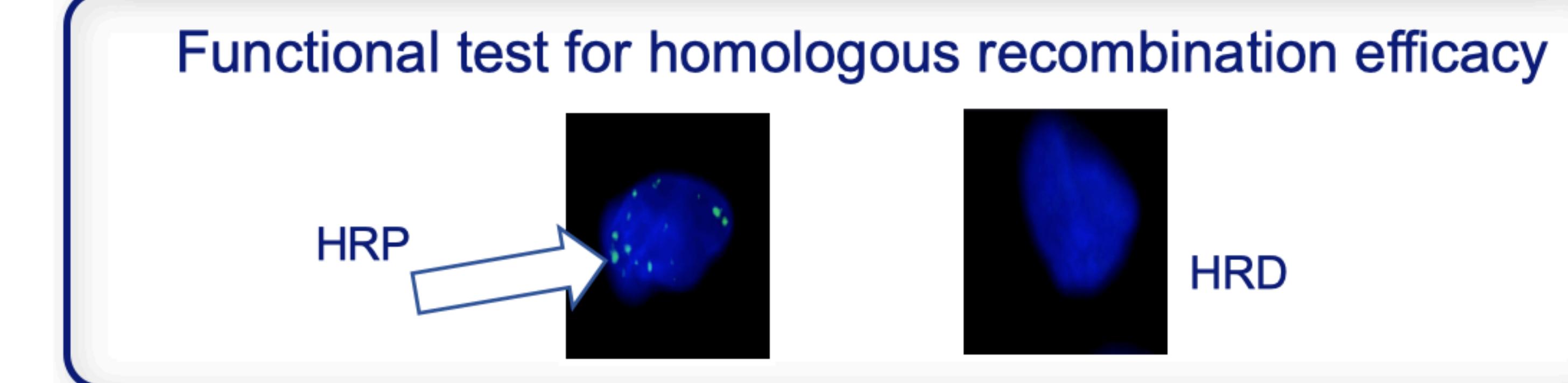
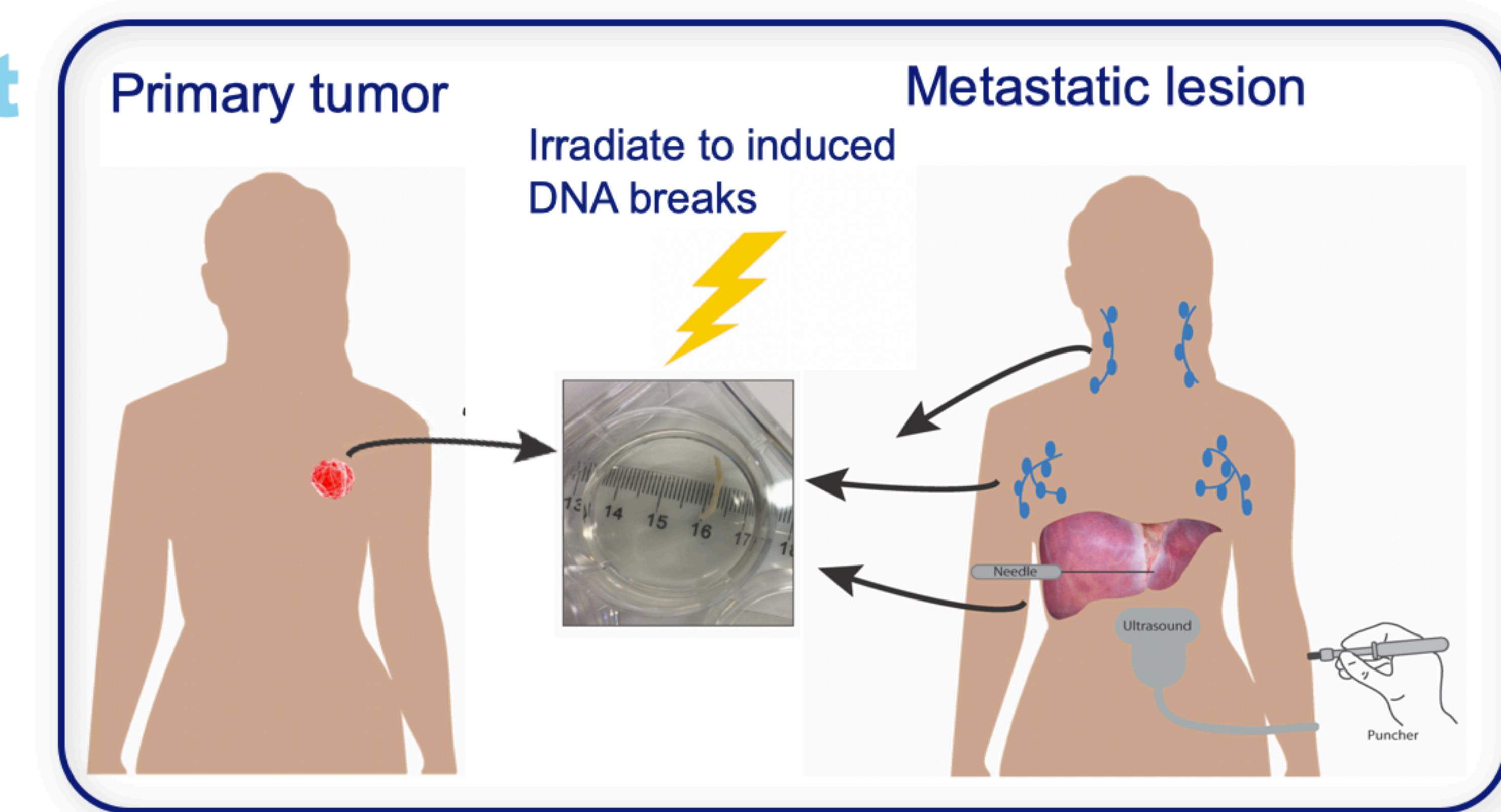


REpair CAPacity test

- Fresh tumor tissue
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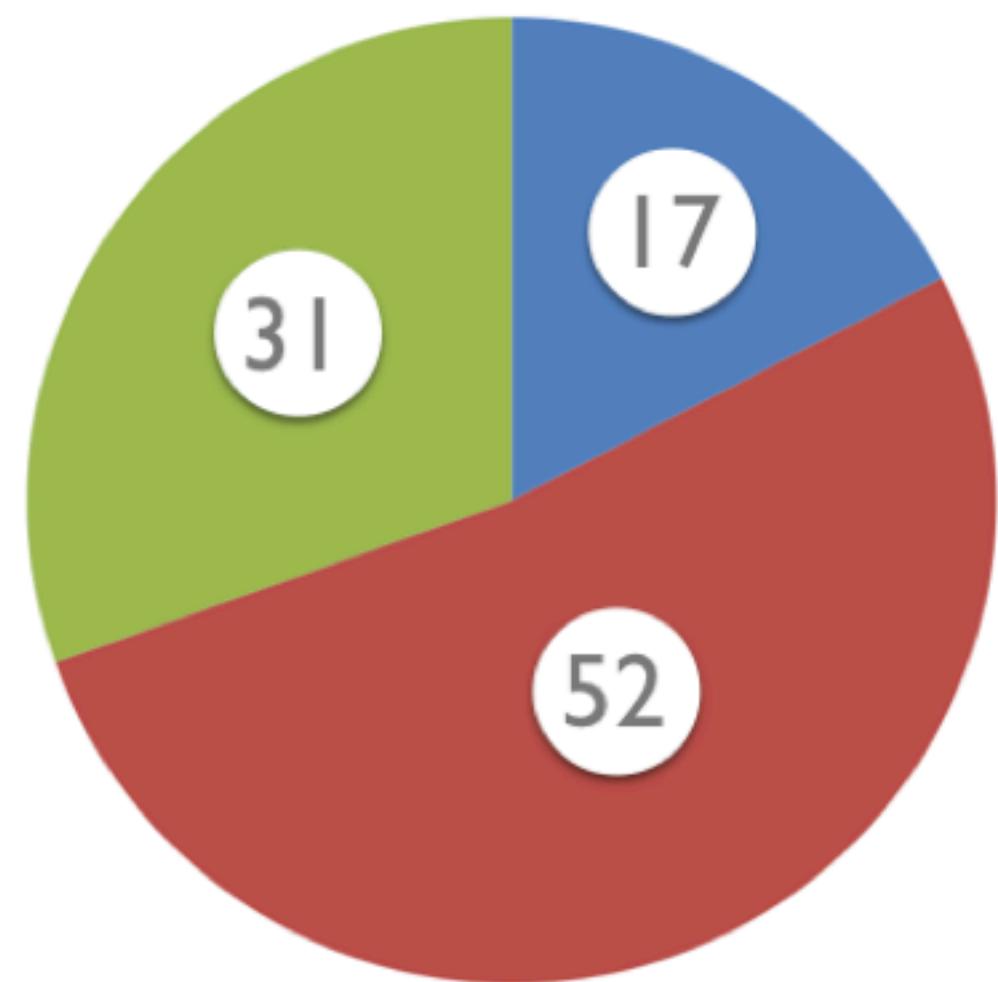
Primary tumors
~20% RECAP negative

Metastatic lesions
~30% RECAP negative



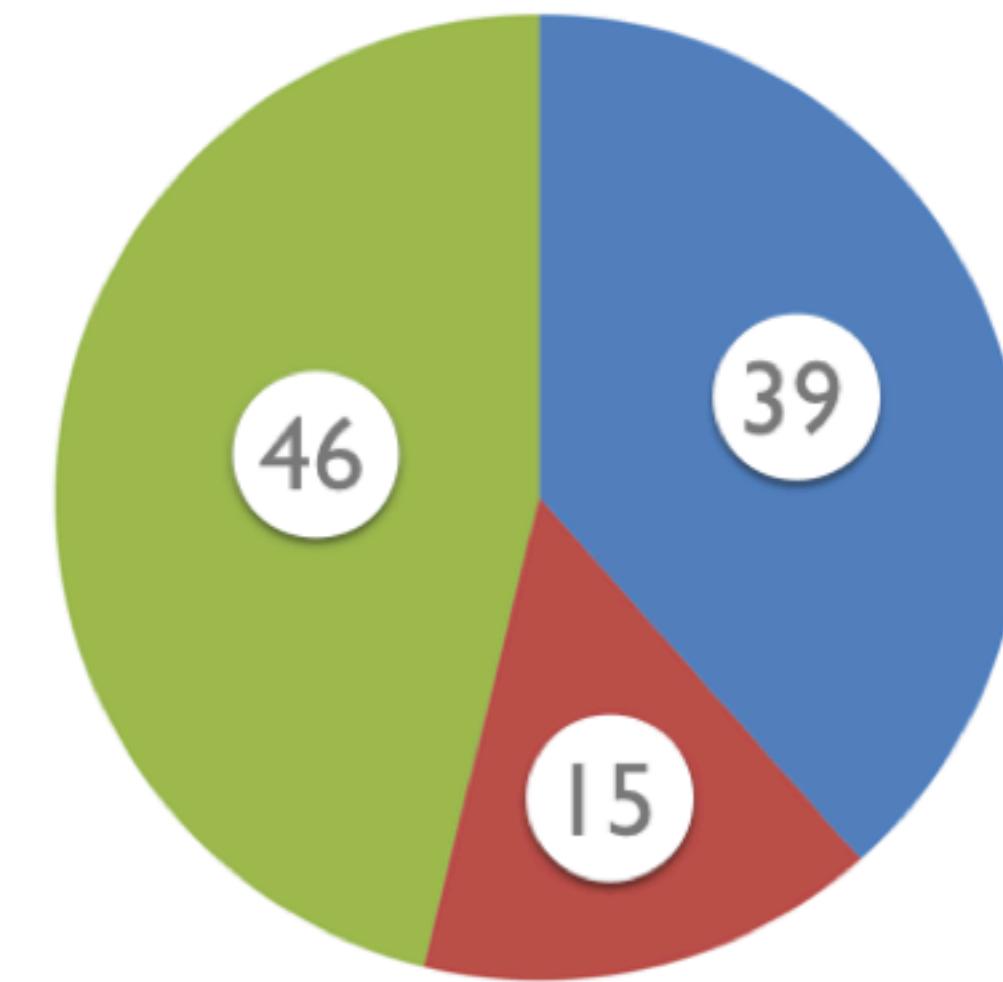
HRD tumors in breast cancer: not always BRCA

HRD primary tumors



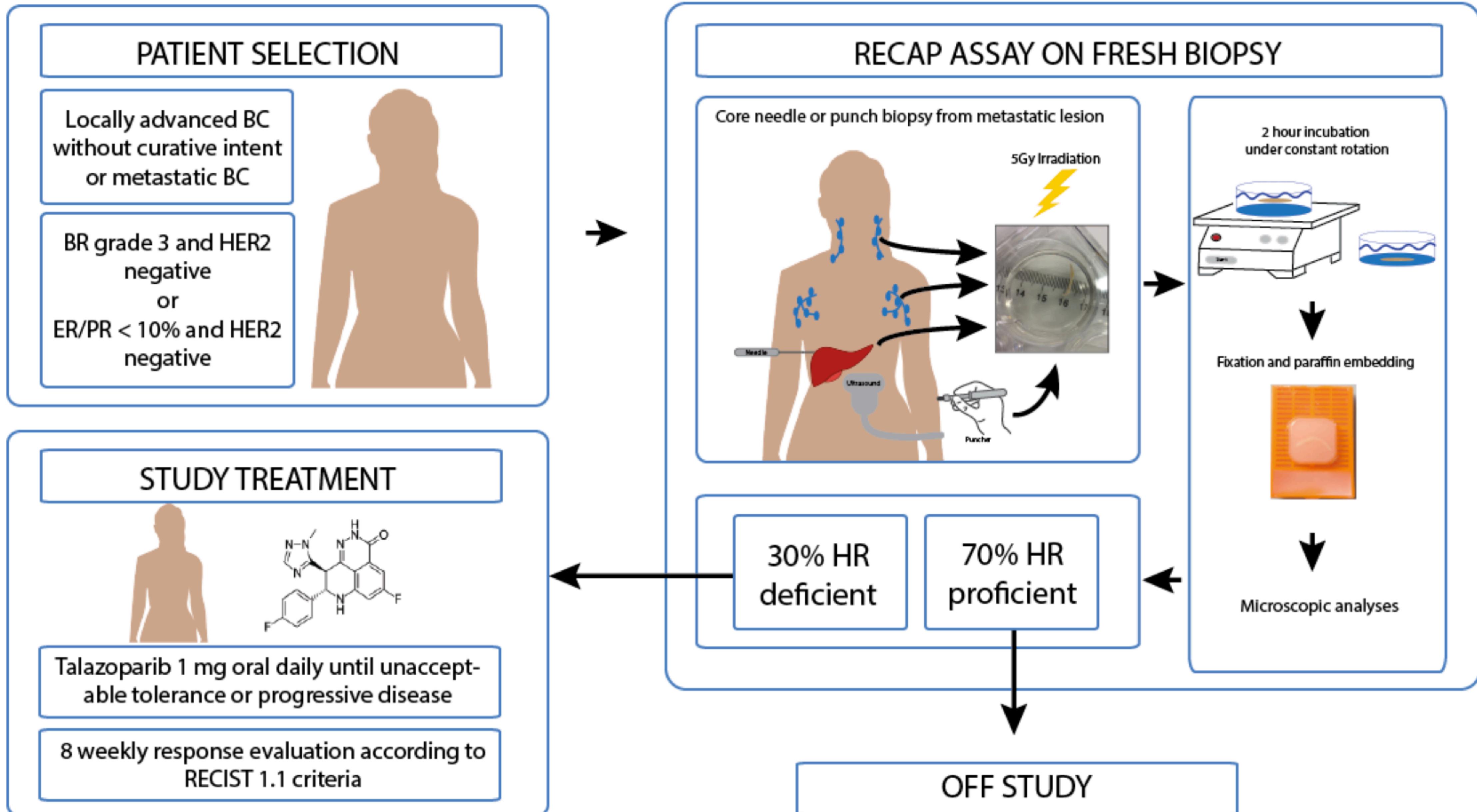
N = 24

HRD metastatic lesions



N = 13

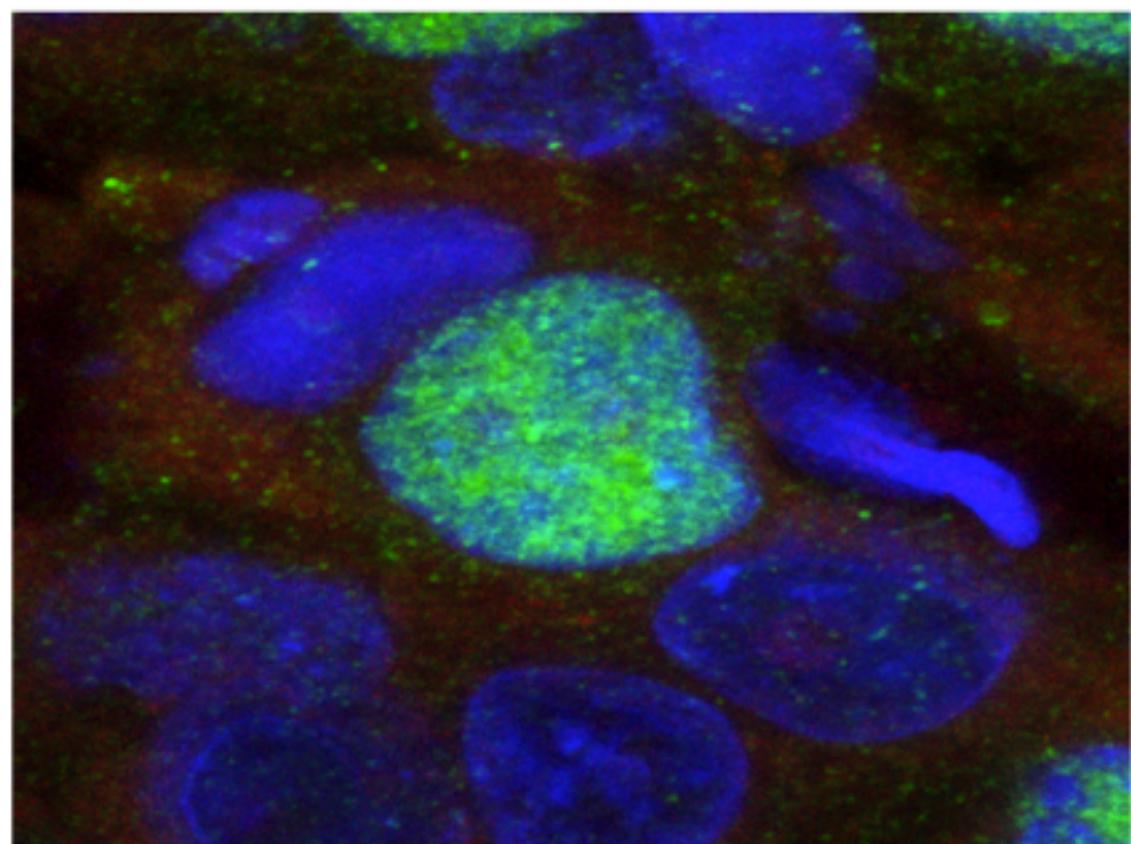
Clinical study: The FUTURE trial



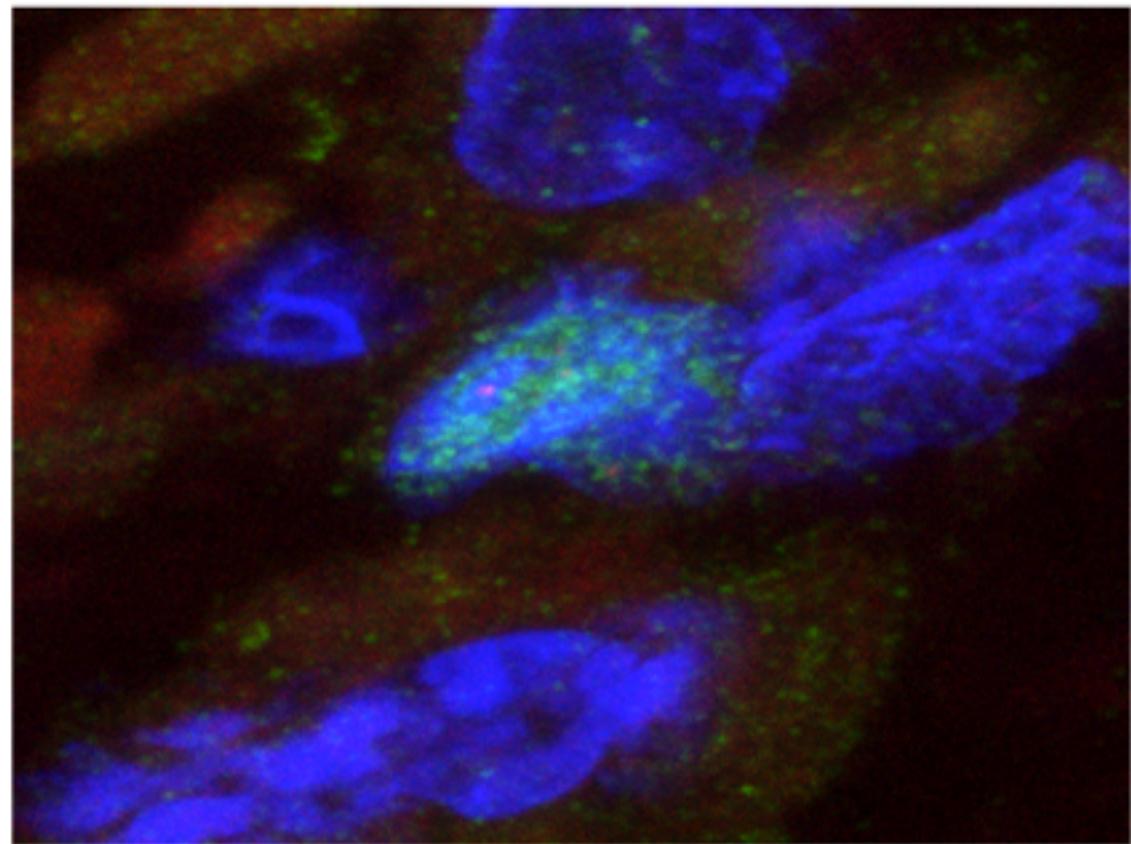
Real-time measure of HRD reversion

HRD phenotype

Pre-treatment



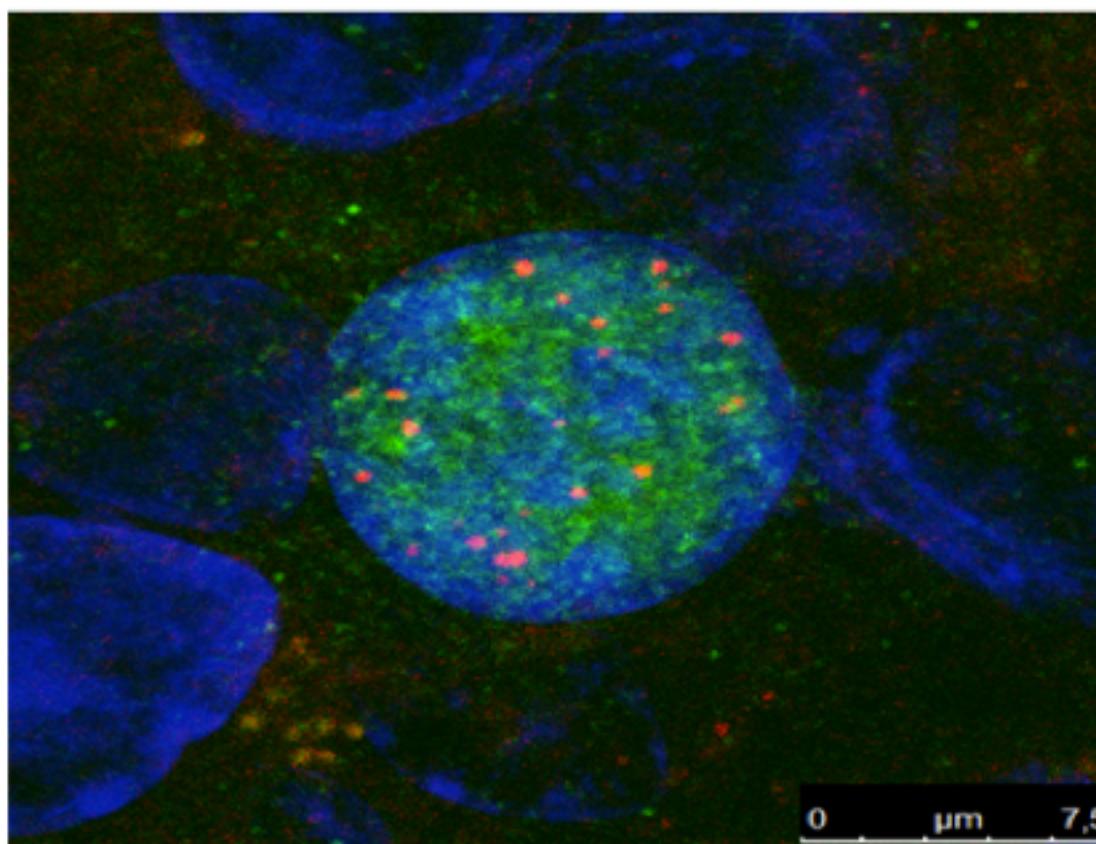
M242: Negative



RAD51+ stromal cell (internal control)

Reversion to HRP upon treatment with carboplatin and PARPi

Post-treatment

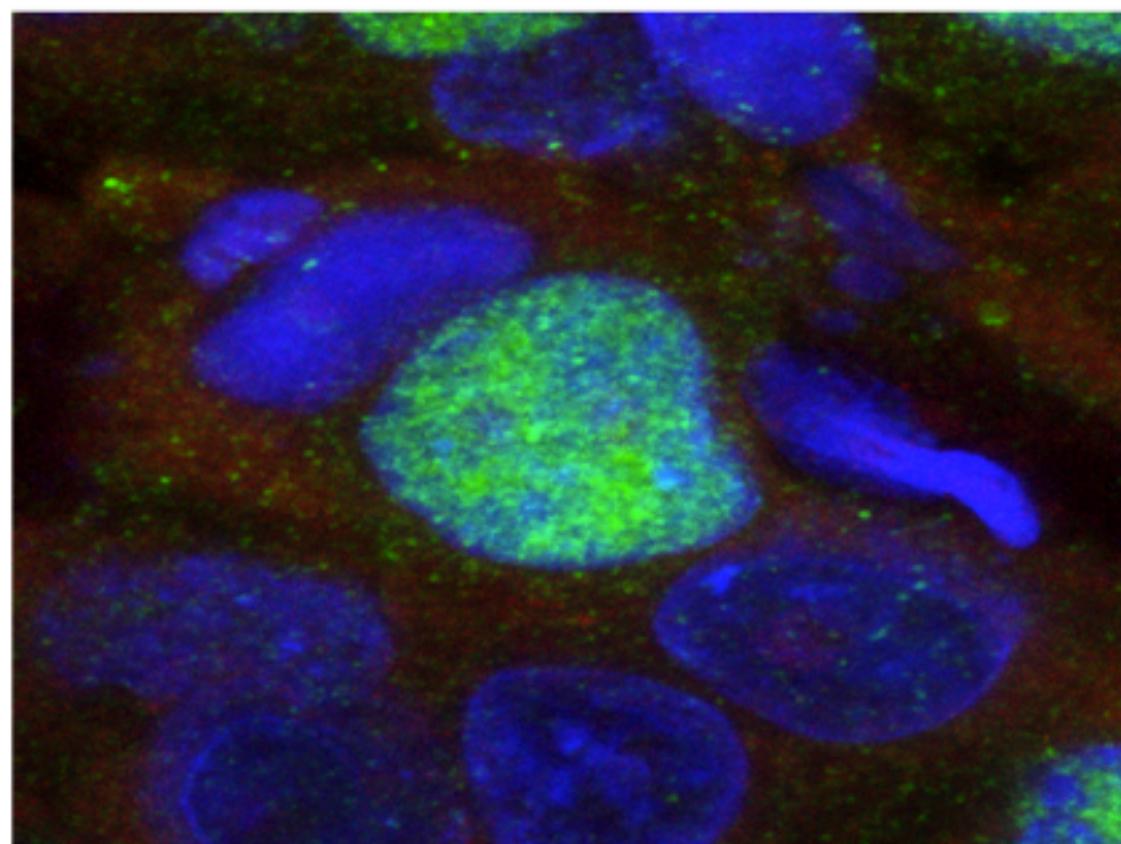


M303: Positive

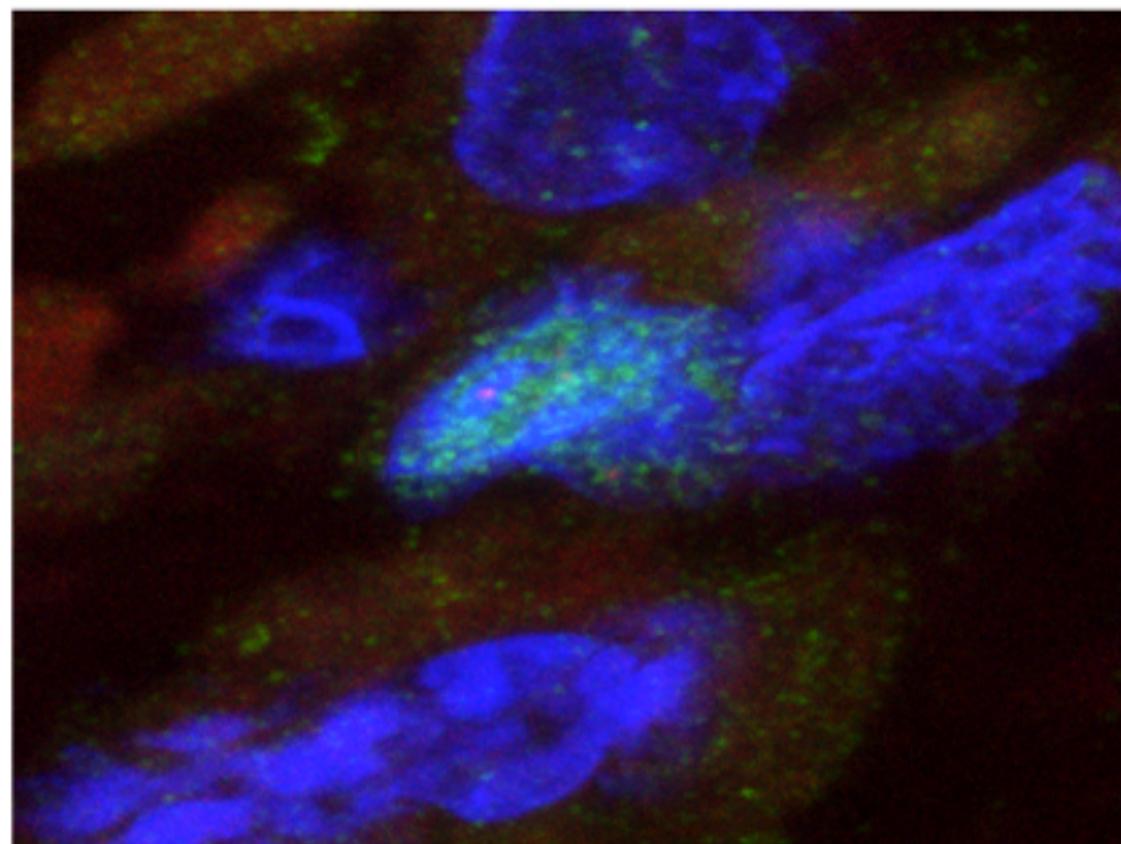
Real-time measure of HRD reversion

HRD phenotype

Pre-treatment



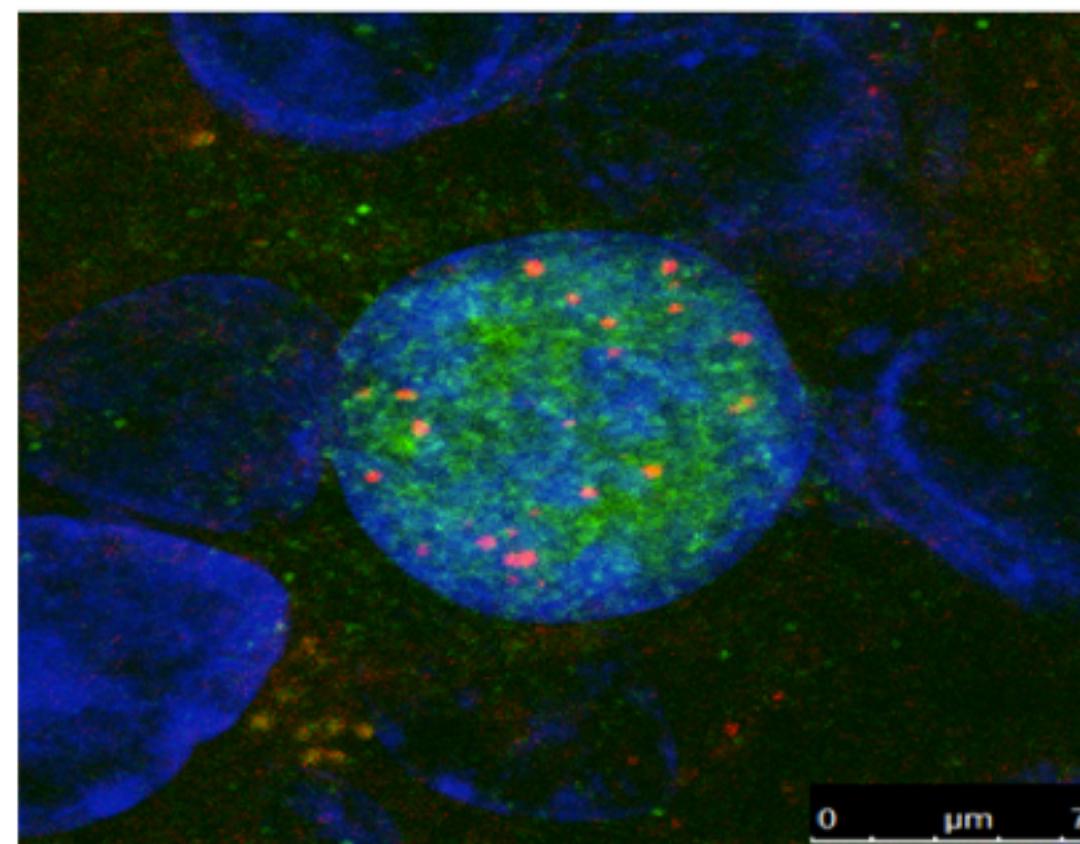
M242: Negative



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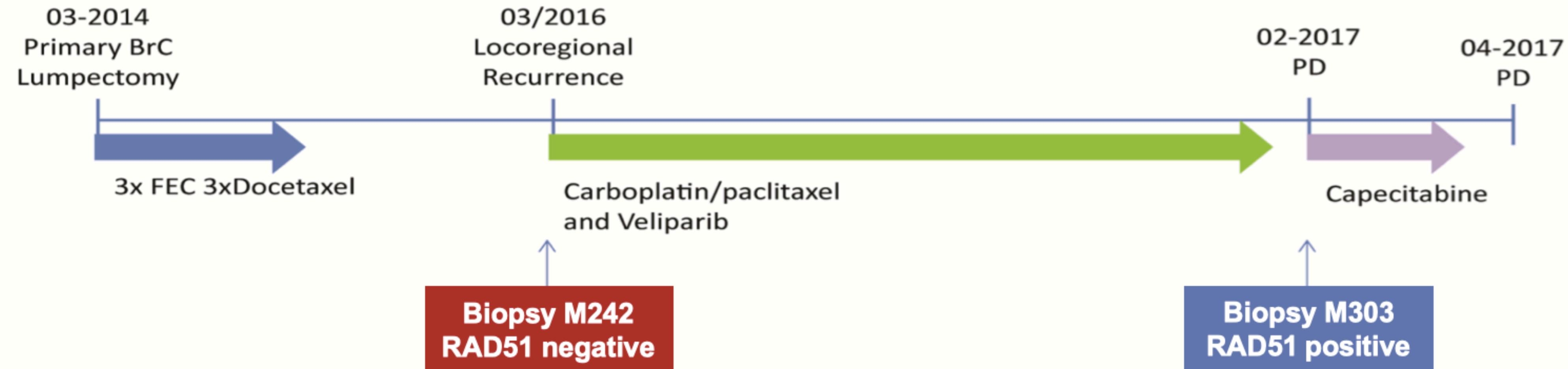


M303: Positive

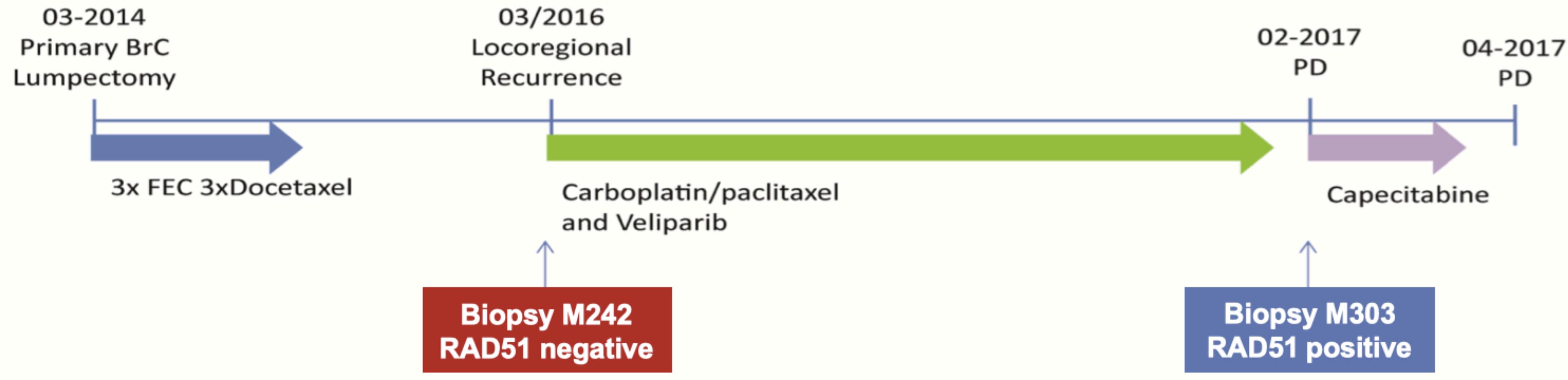
Three patients were assayed upon start of treatment and after disease progression while on DNA crosslinking agents and/or PARPi

All regained RAD51 foci formation capacity in RECAP

BRCA1 reversion mutation explains resistance



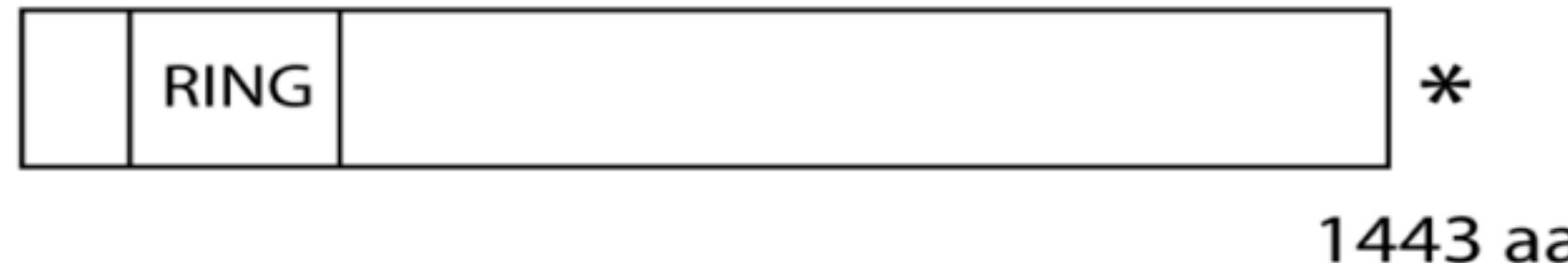
BRCA1 reversion mutation explains resistance



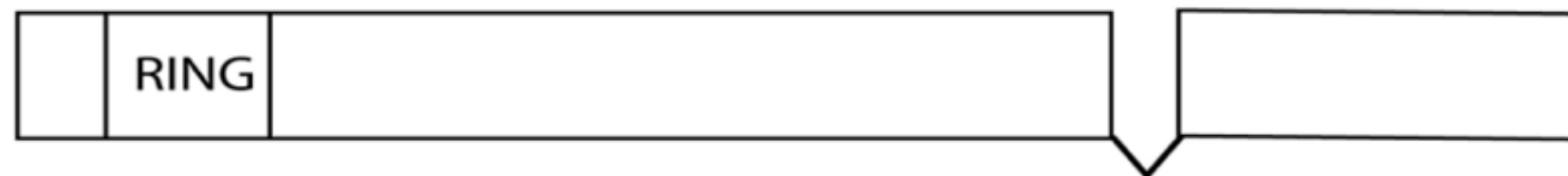
BRCA1 wild-type



BRCA1 primary mutation
c.4327C>T
p.Arg1443*



BRCA1 secondary mutation
c.4327-4329delinsTGG
p.Arg1443_delinsTrp



Second point mutation

Conclusion

- RAD51 foci are a read out for HR capacity
- ~20% of primary mammary tumors and ~30% of metastases is RECAP negative
- RECAP detect tumors that are HRD without BRCA mutations
- Reversion of HRD can also be monitored by RECAP
- Concordance with DNA-based HRD tests?



Dik van Gent and Agnes Jager

Titia Meijer

Marjolijn Ladan

Nicole Verkaik

John Martens

Carolien van Deurzen

Jos Jonkers (NKI)





De Rotterdam

HSF2BP, a BRCA2 interactome component

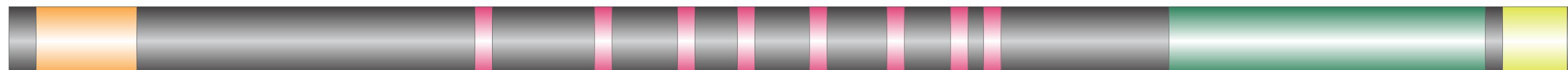
Department of
**Molecular
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Erasmus MC
University Medical Center Rotterdam

**Oncode
Institute**

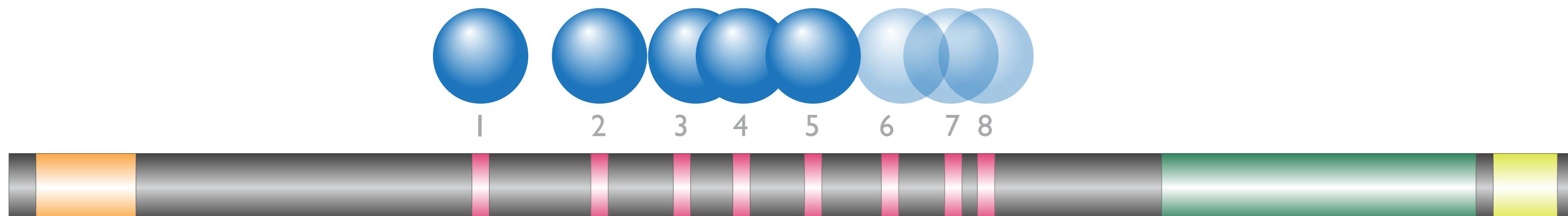
Outsmarting cancer
Impacting lives

BRCA2



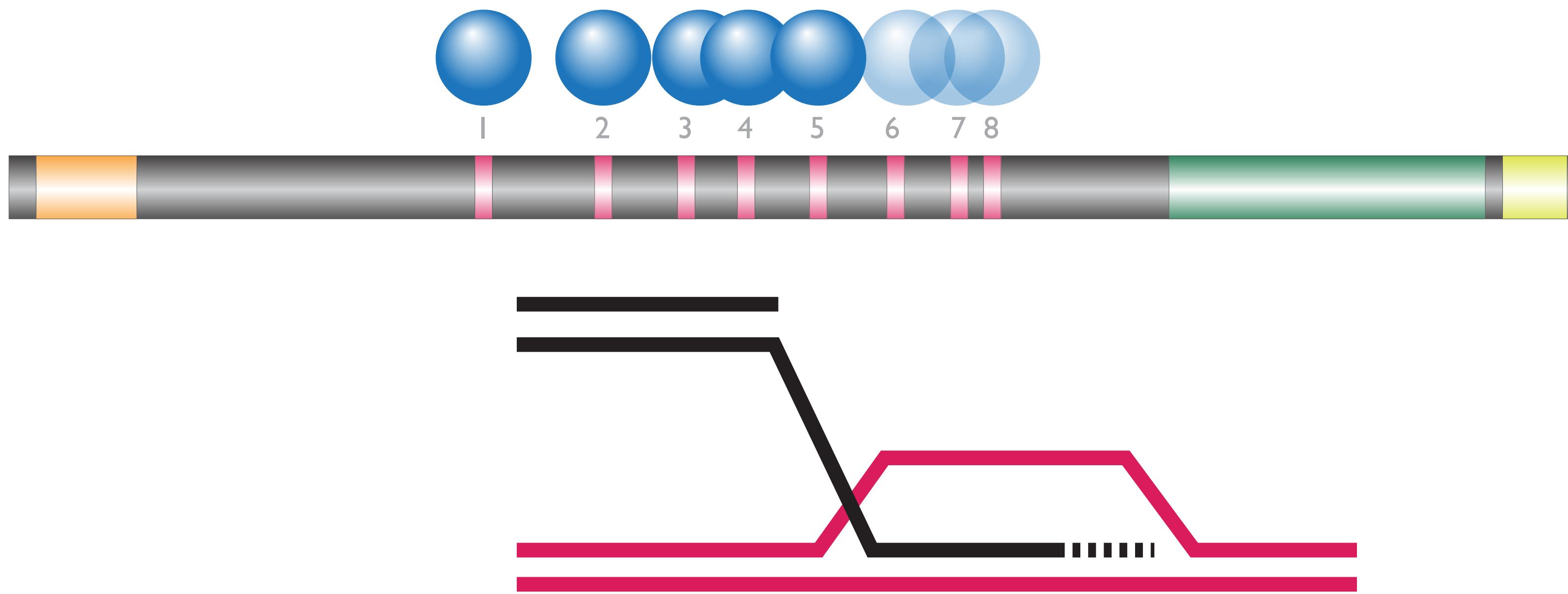
BRCA2, 370 kDa

BRCA2



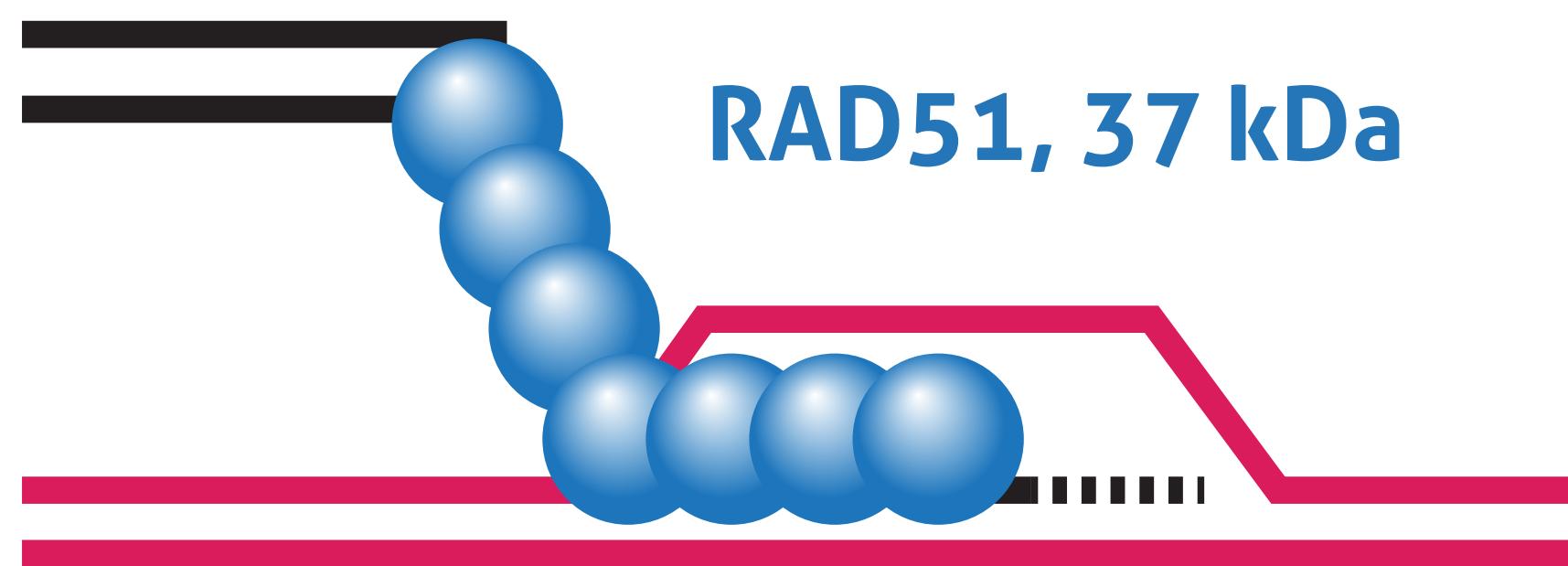
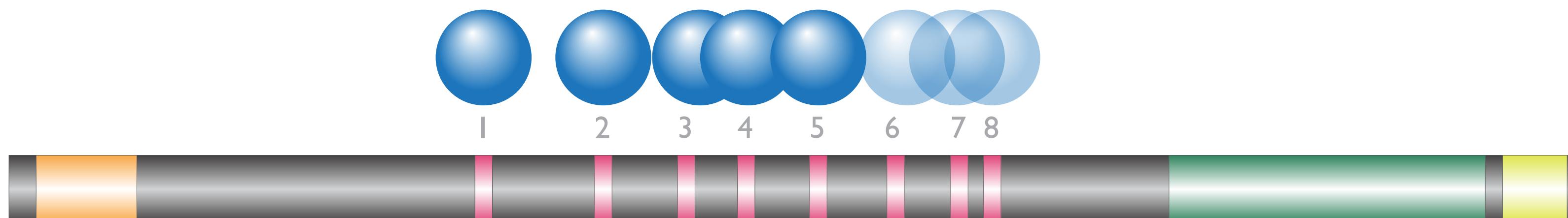
BRCA2, 370 kDa

BRCA2

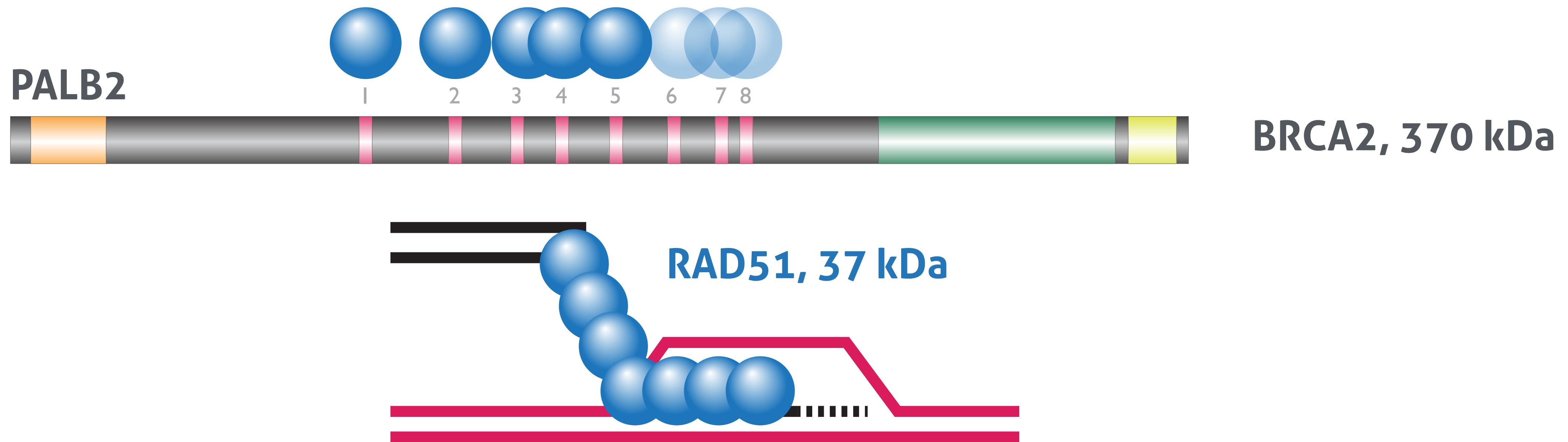


BRCA2, 370 kDa

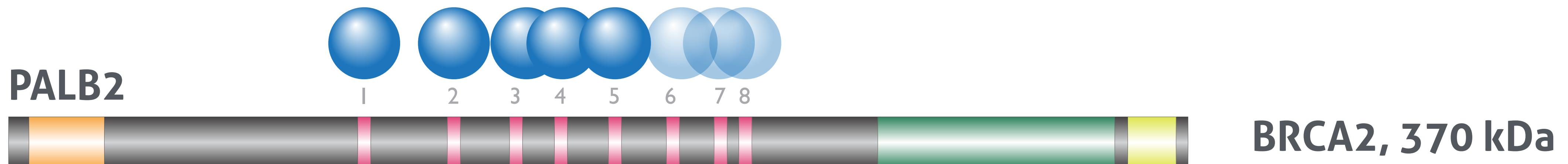
BRCA2



BRCA2

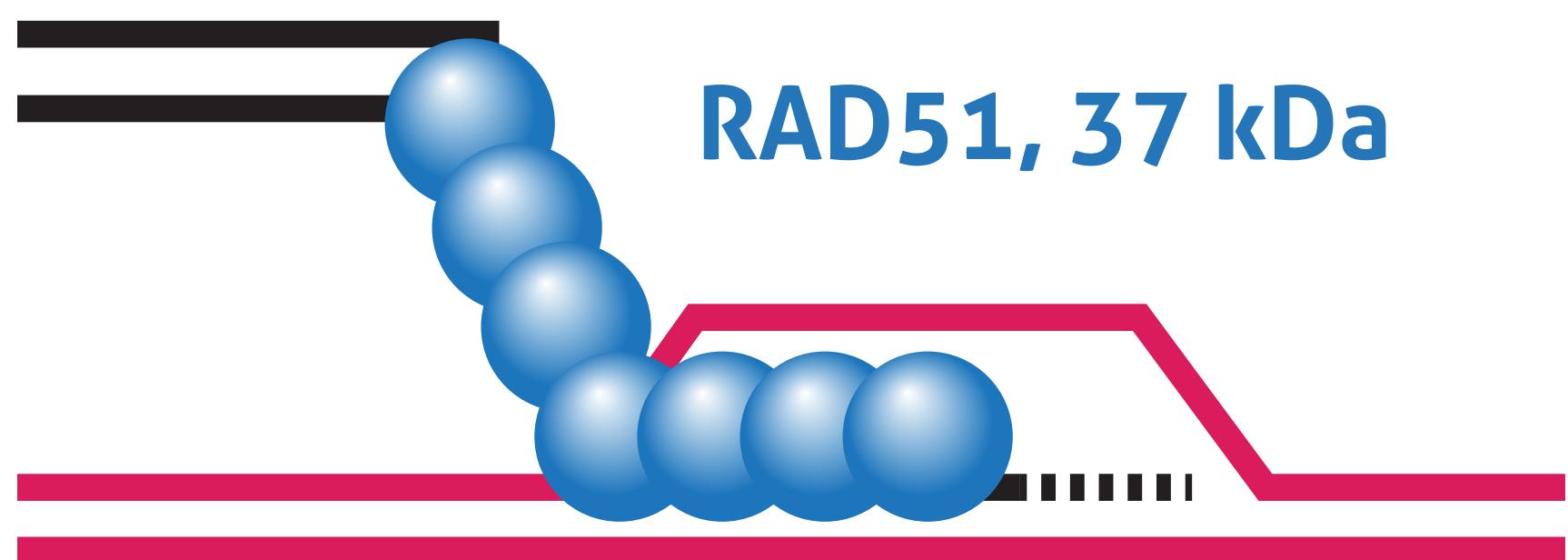


BRCA2

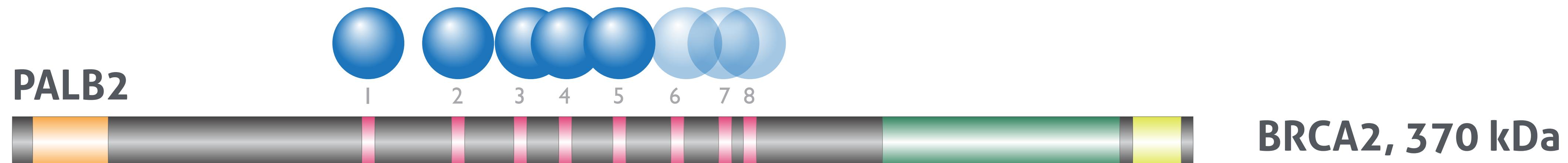


Homologous Recombination

DSB Repair

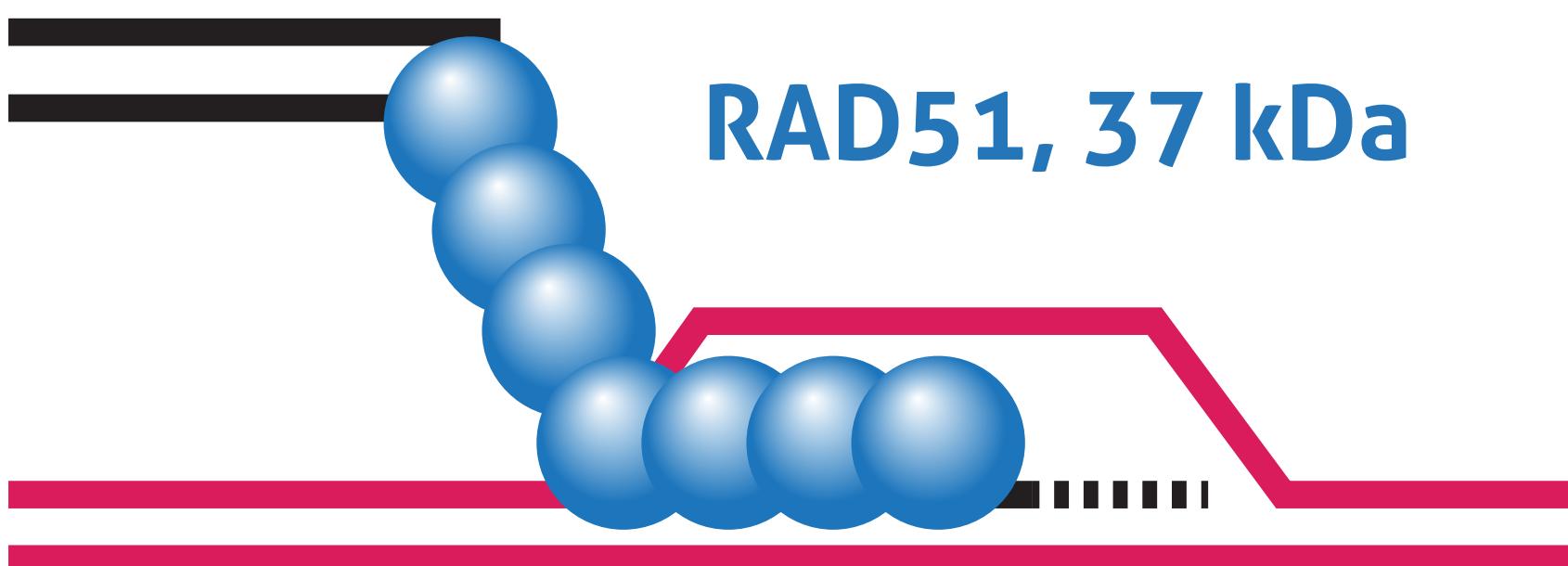


BRCA2



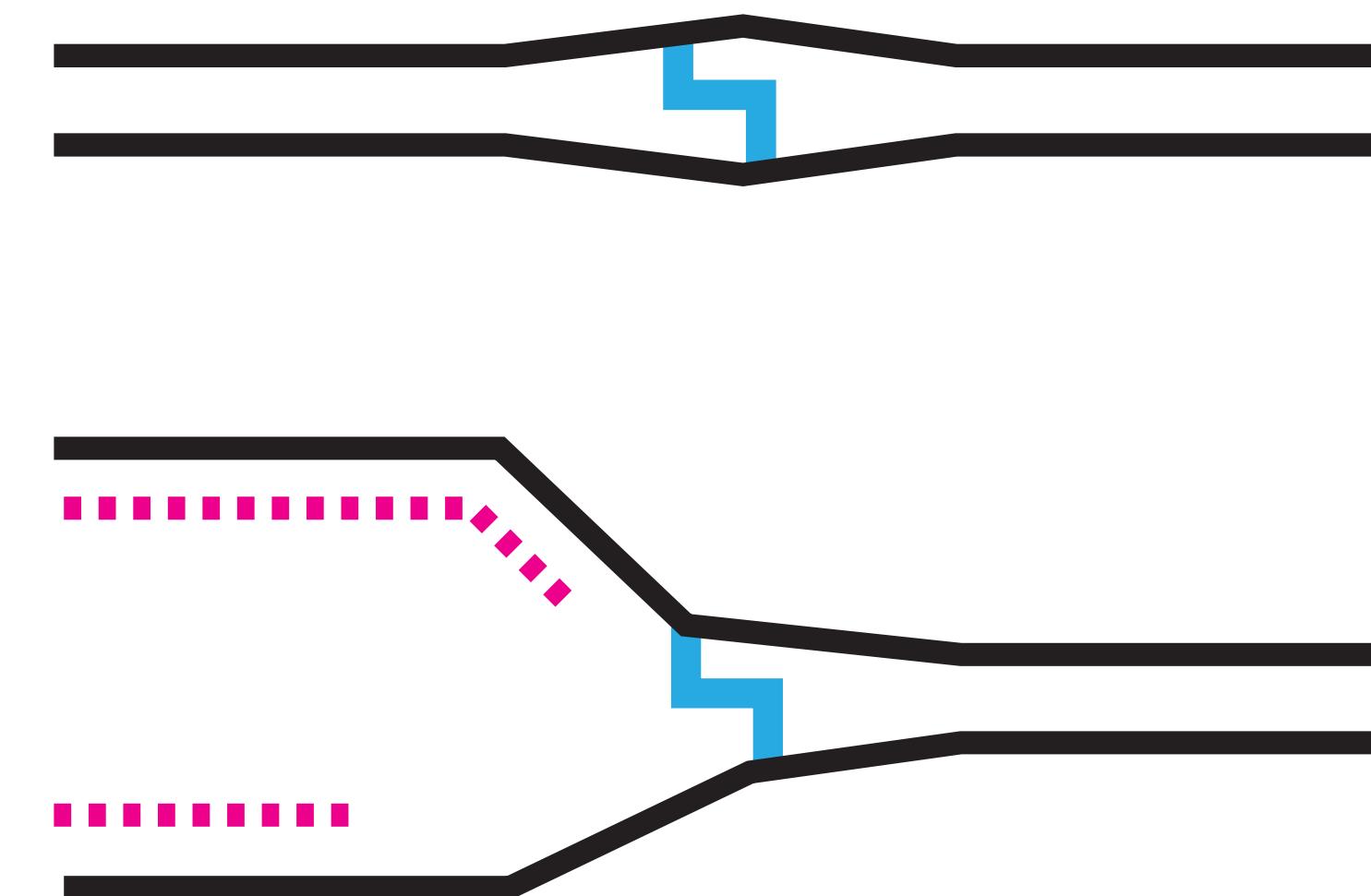
Homologous Recombination

DSB Repair

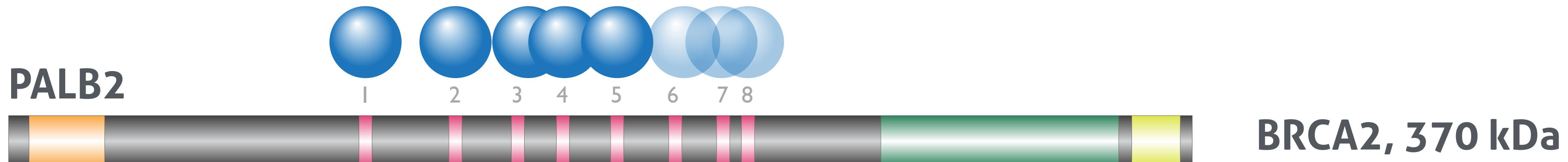


Interstrand Crosslink (ICL)

Fanconi anemia pathway

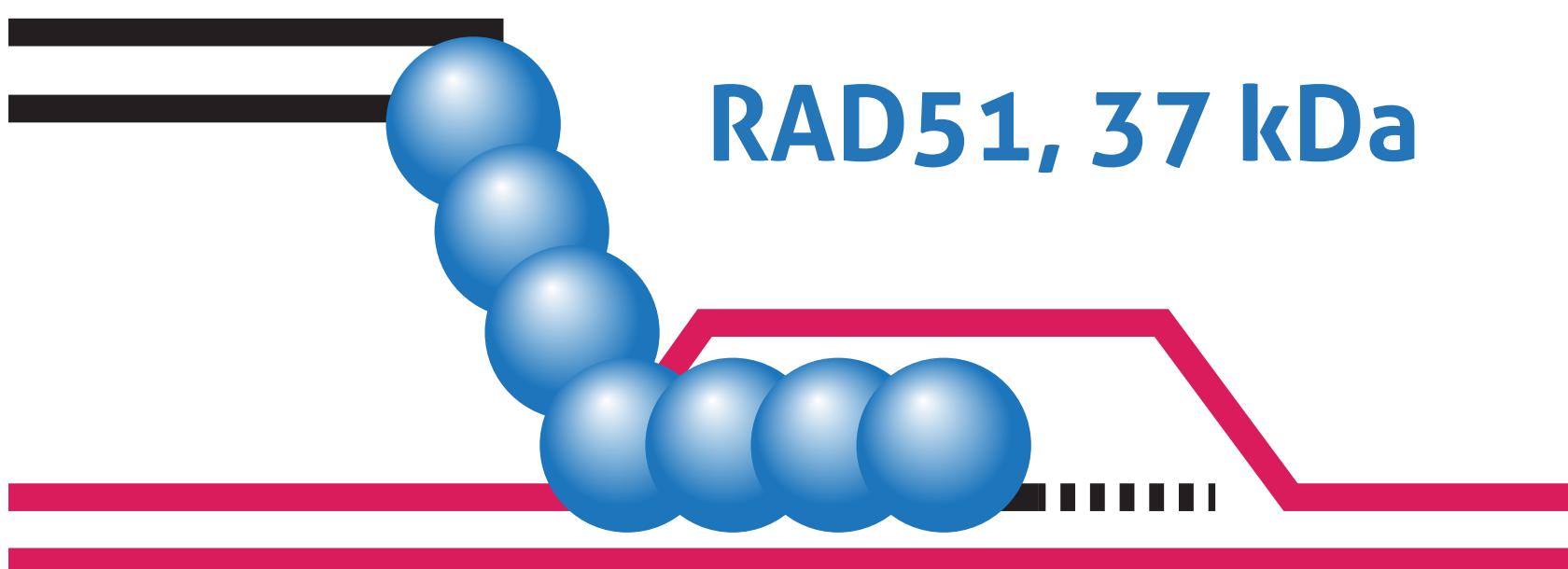


BRCA2



Homologous Recombination

DSB Repair



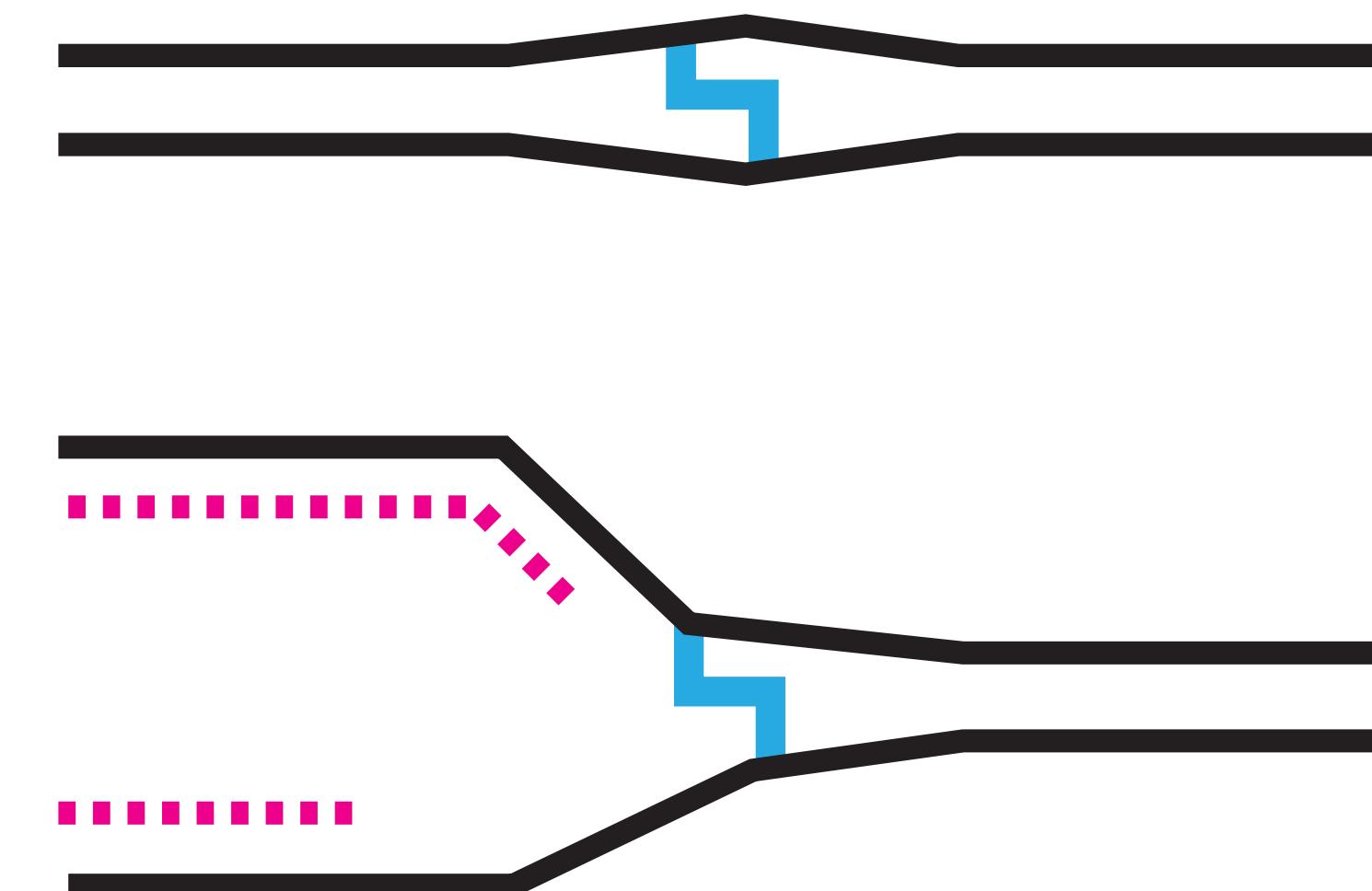
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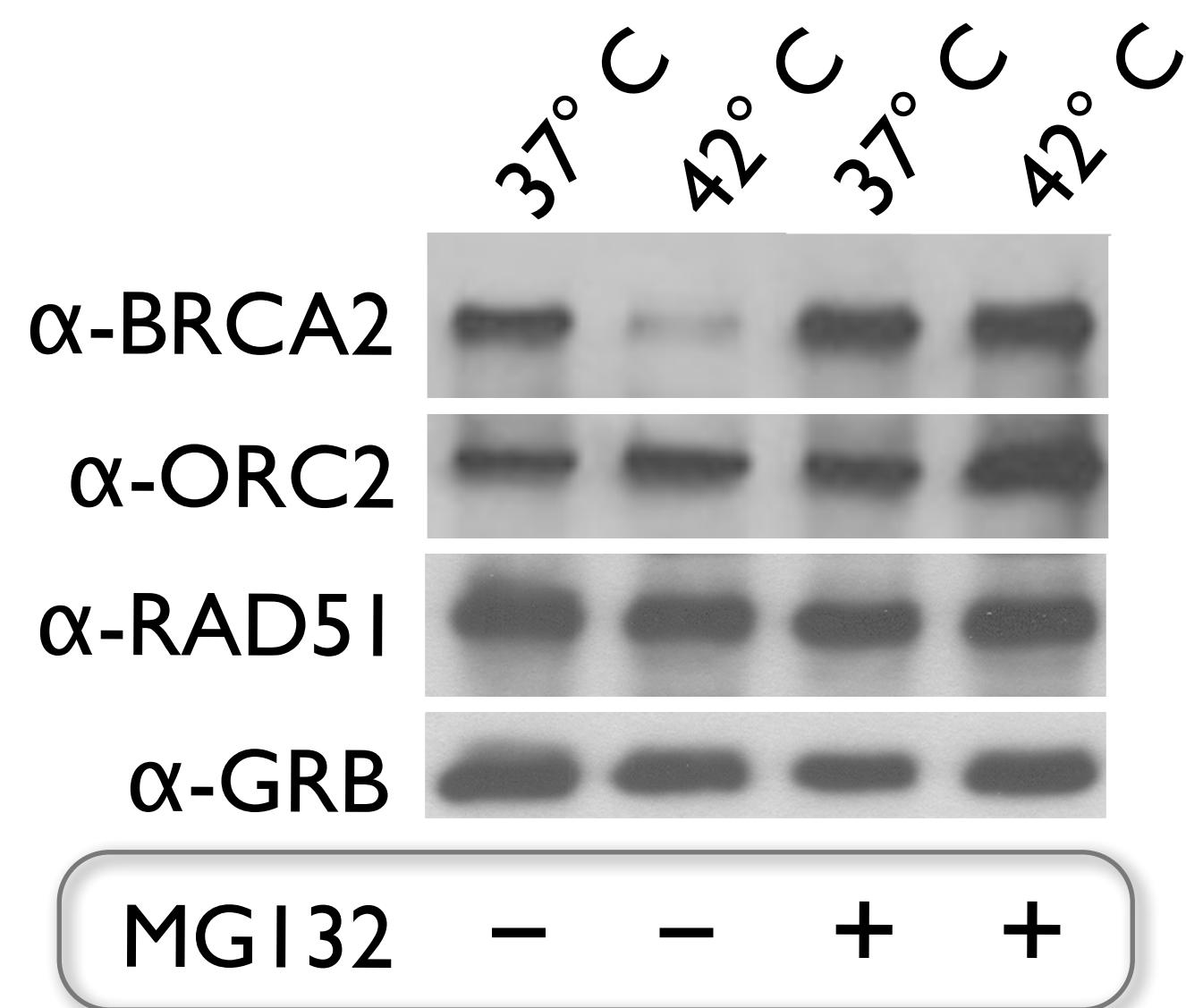
BRCA2 = FANCD1

PALB2 = FANCN

RAD51 = FANCR

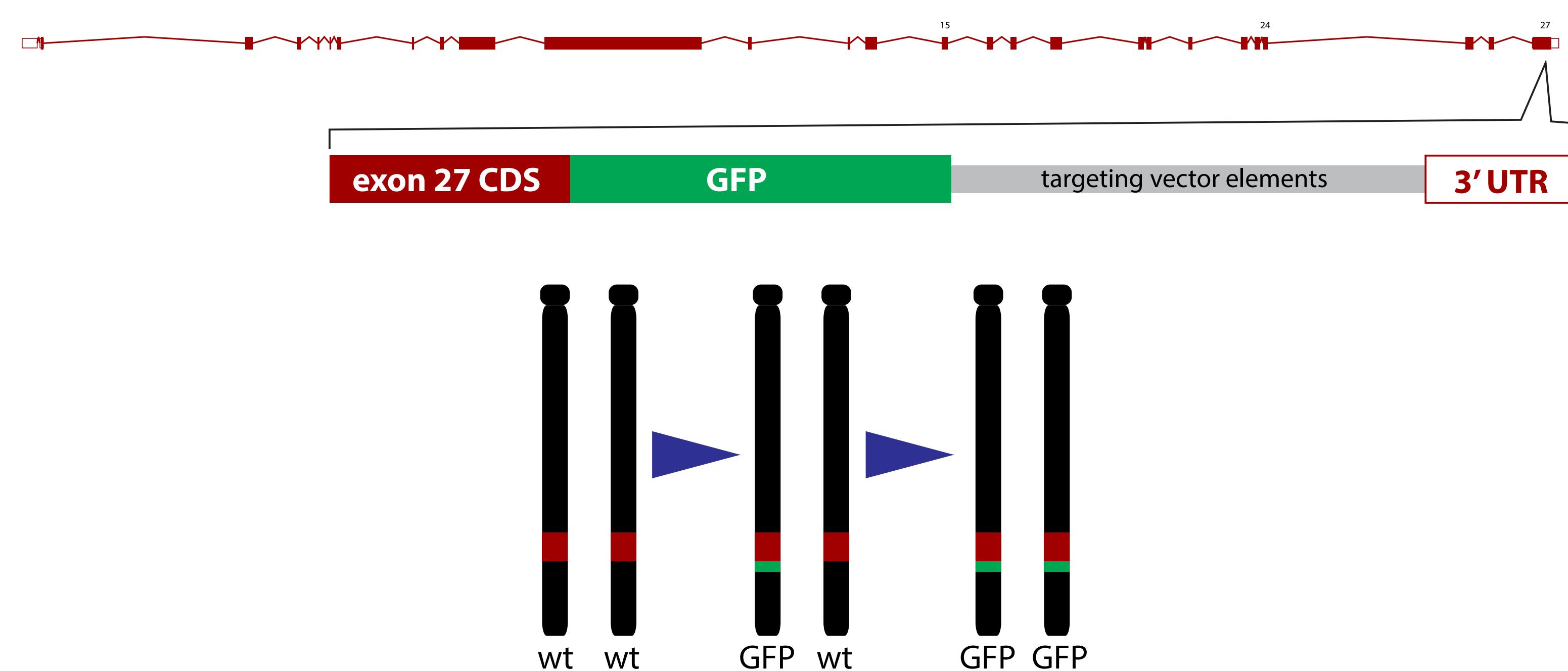
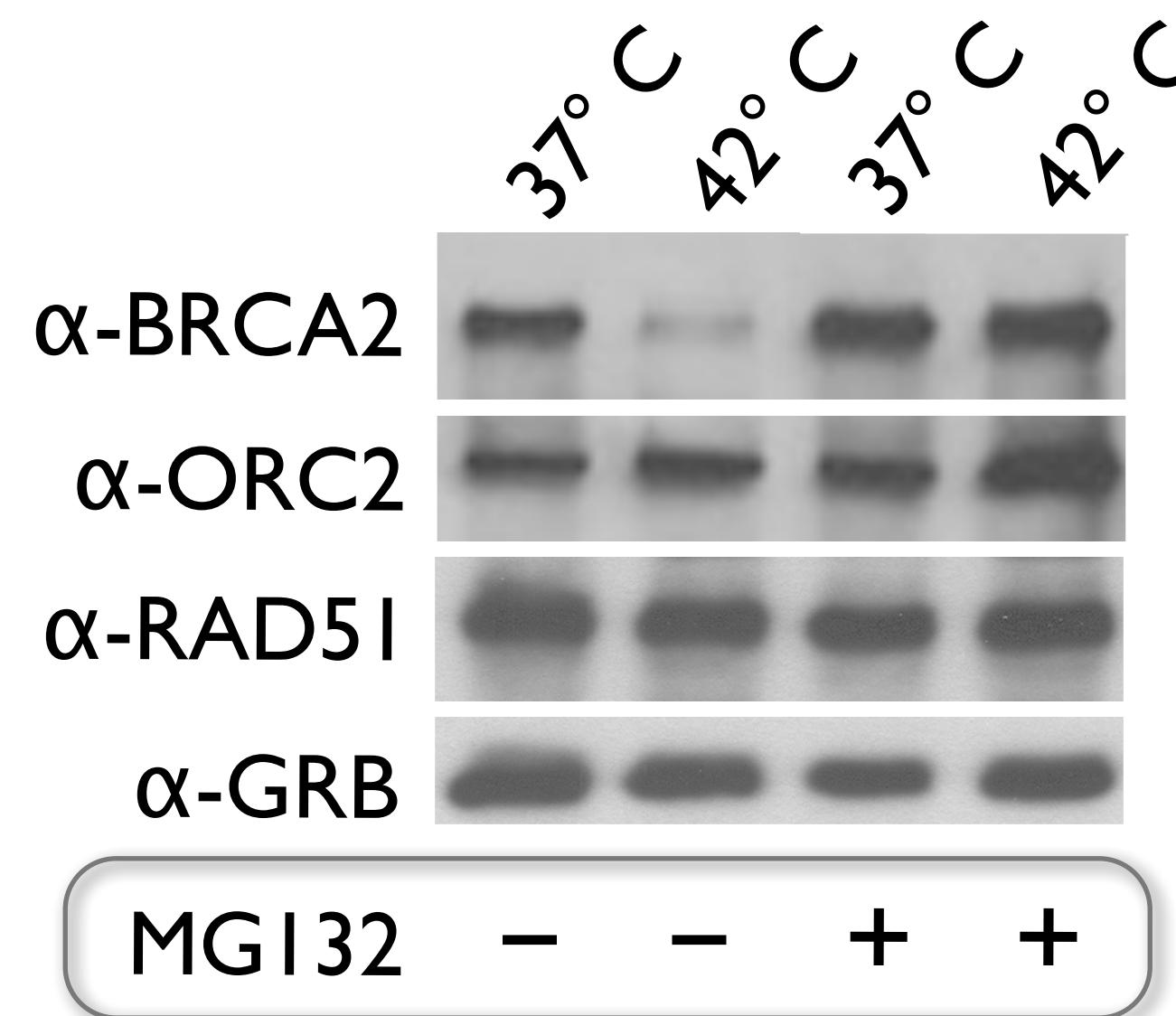


MILD HYPERHERMIA TRIGGERS DEGRADATION OF BRCA2



MILD HYPERHERMIA TRIGGERS DEGRADATION OF BRCA2

Brca2^{GFP/GPF} ES cells



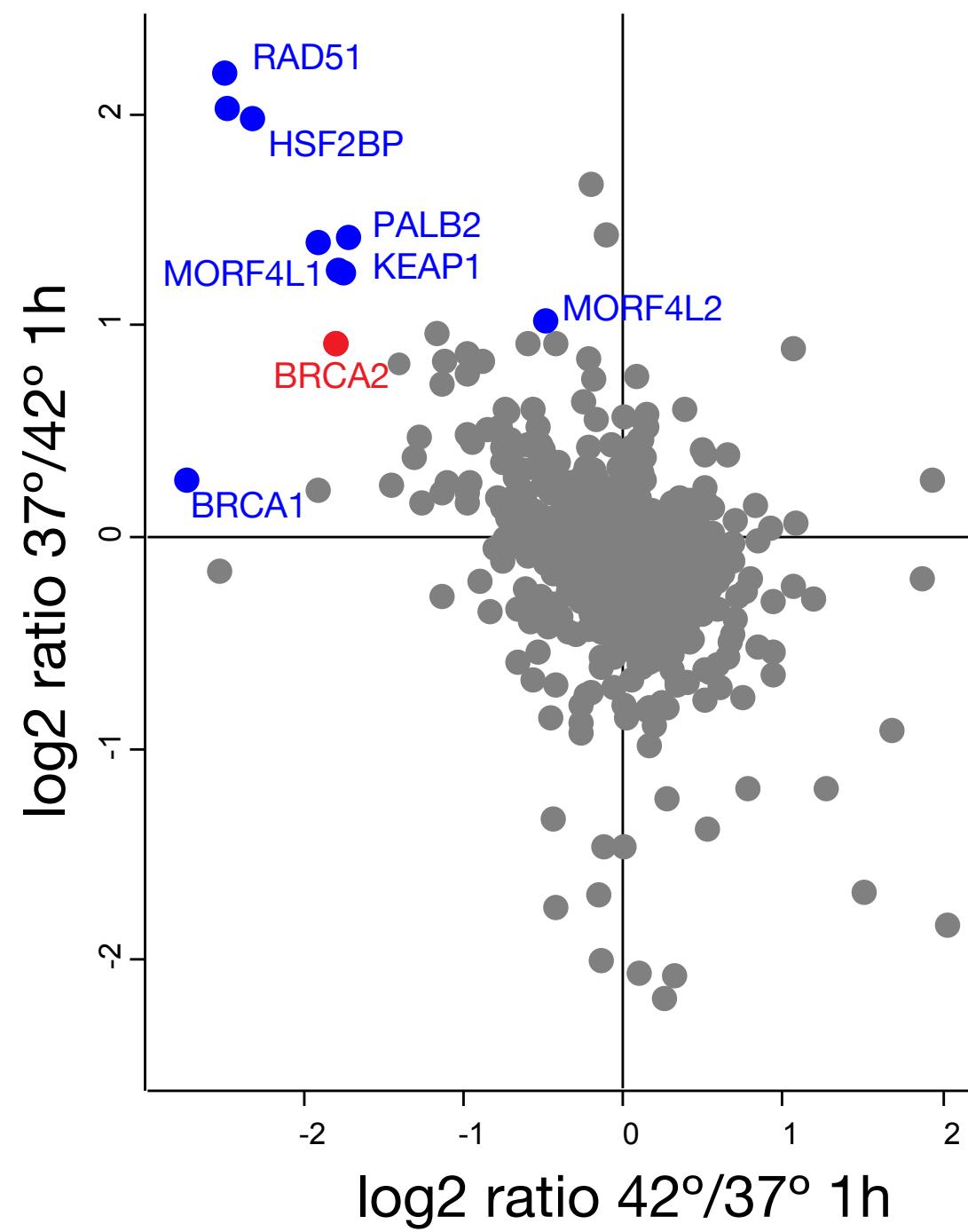
GFP IP label swap SILAC mass spectrometry experiments

Alex Zelensky

The BRCA2 interactome

BRCA2 interactors

Identified HSF2BP

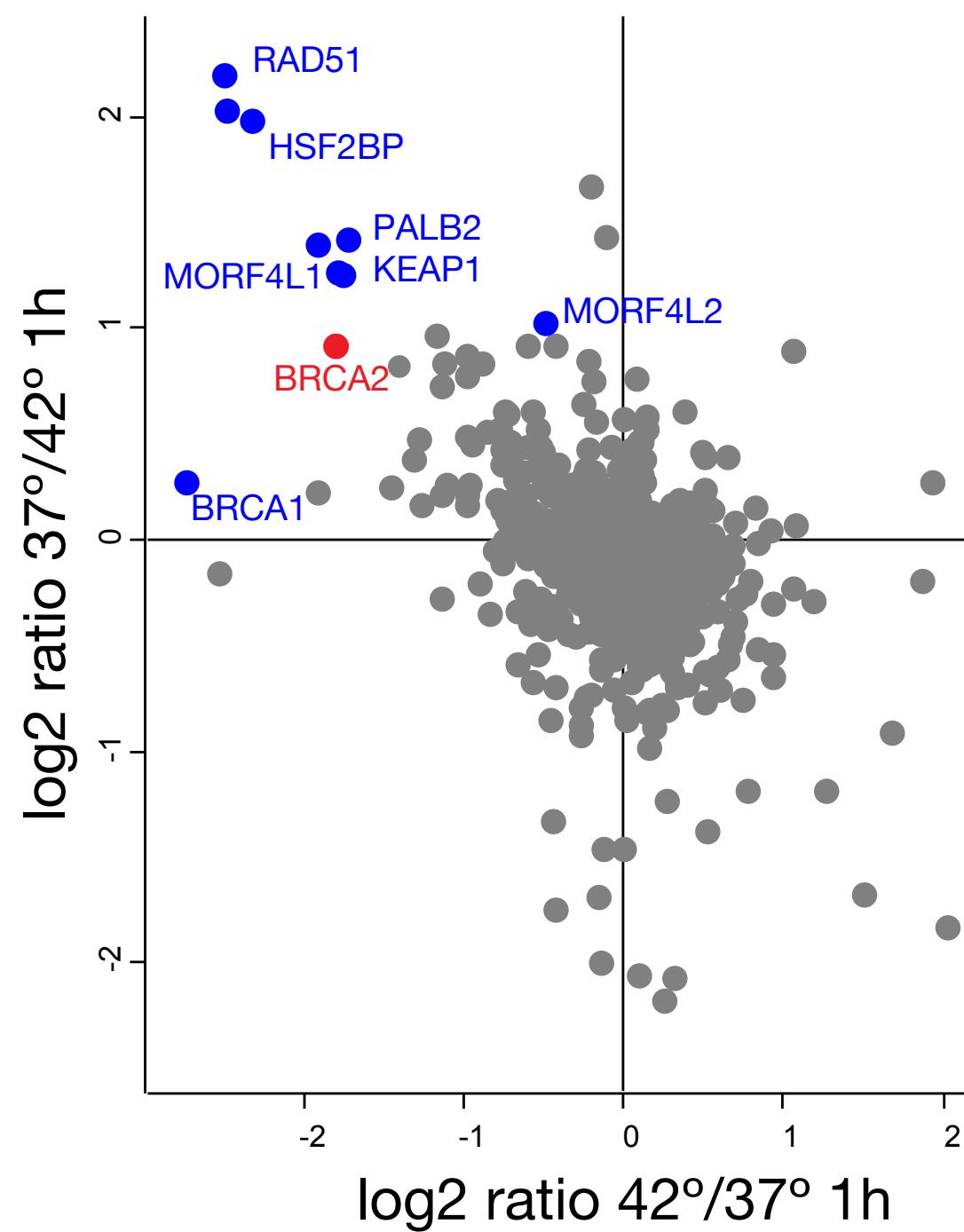


Label swap SILAC mass spectrometry experiments

The BRCA2 interactome

BRCA2 interactors

Identified HSF2BP



Generate: $Hsf2bp^{GFP/wt}$ ES cells

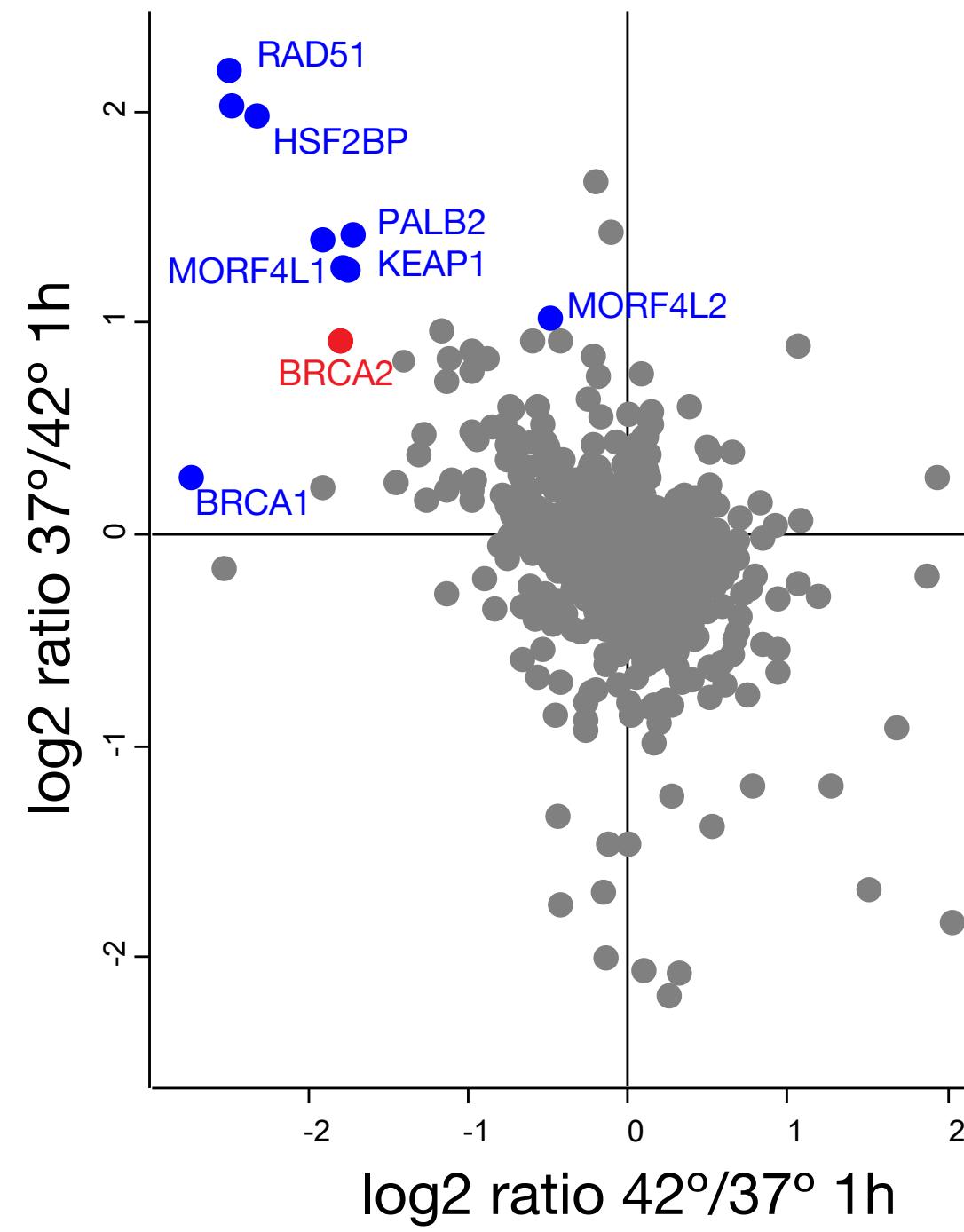
$Palb2^{GFP/GFP}$ ES cells

$Rad51ap1^{GFP/GFP}$ ES cells

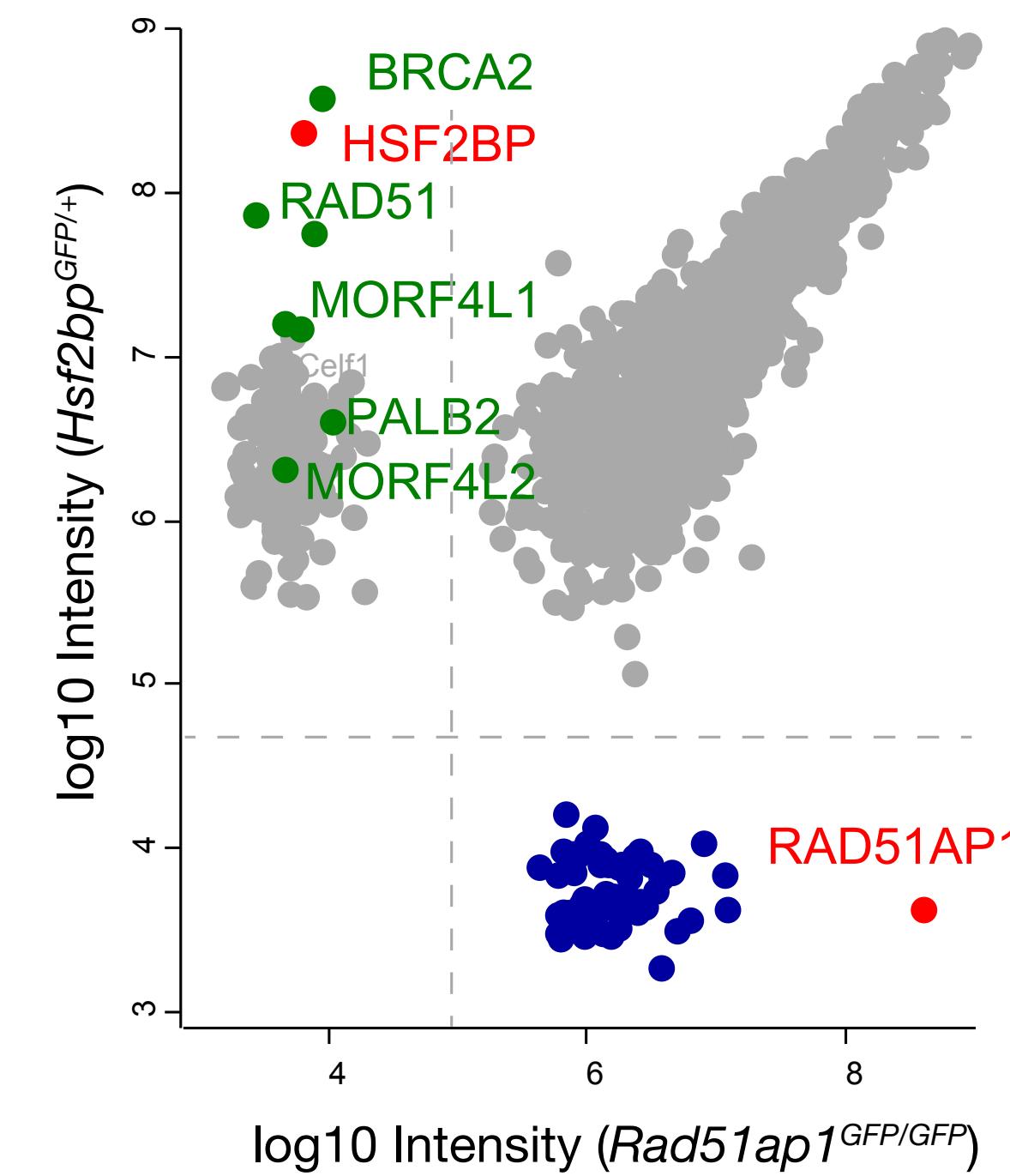
Label swap SILAC mass spectrometry experiments

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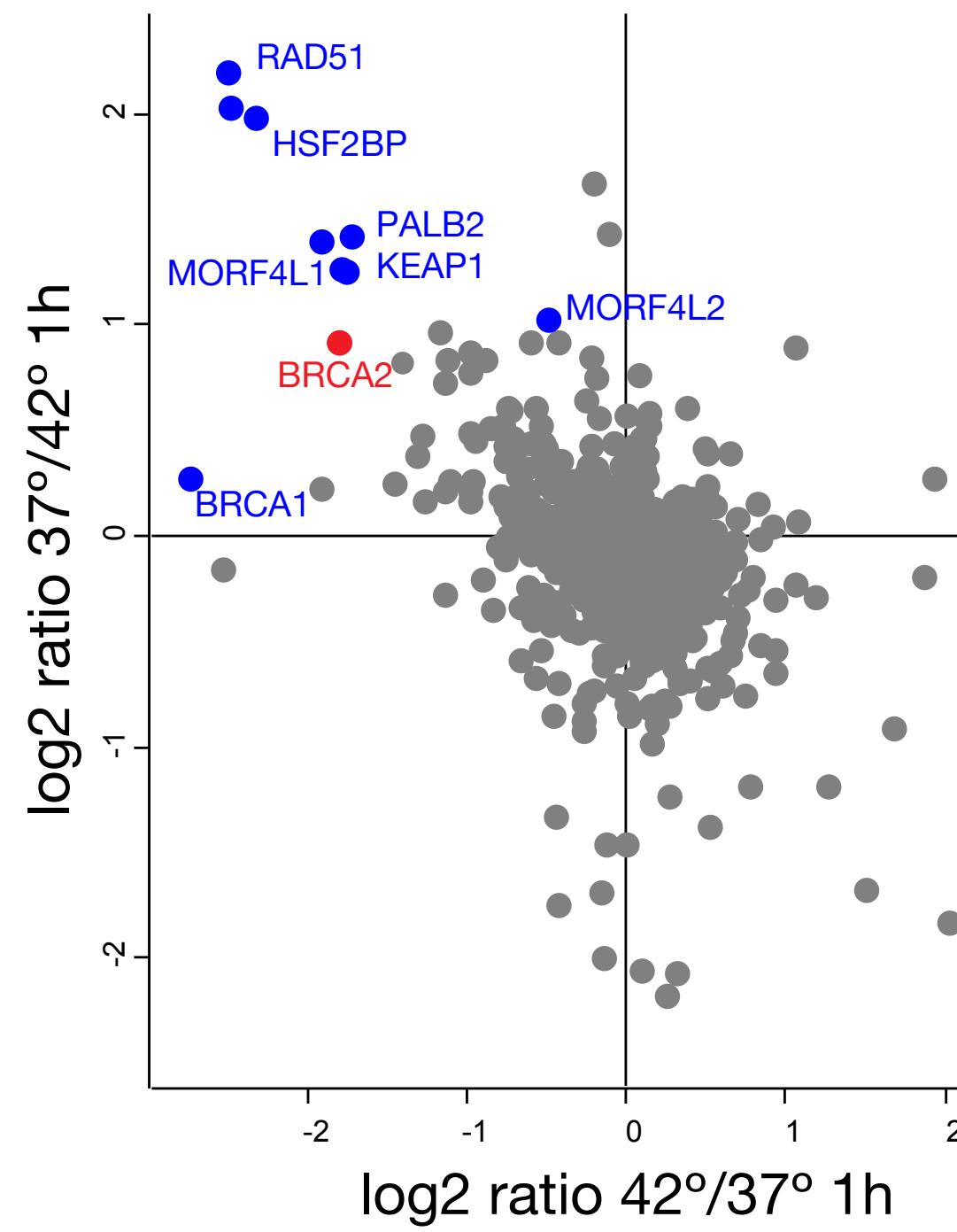
Reciprocal interaction
via HSF2BP IP



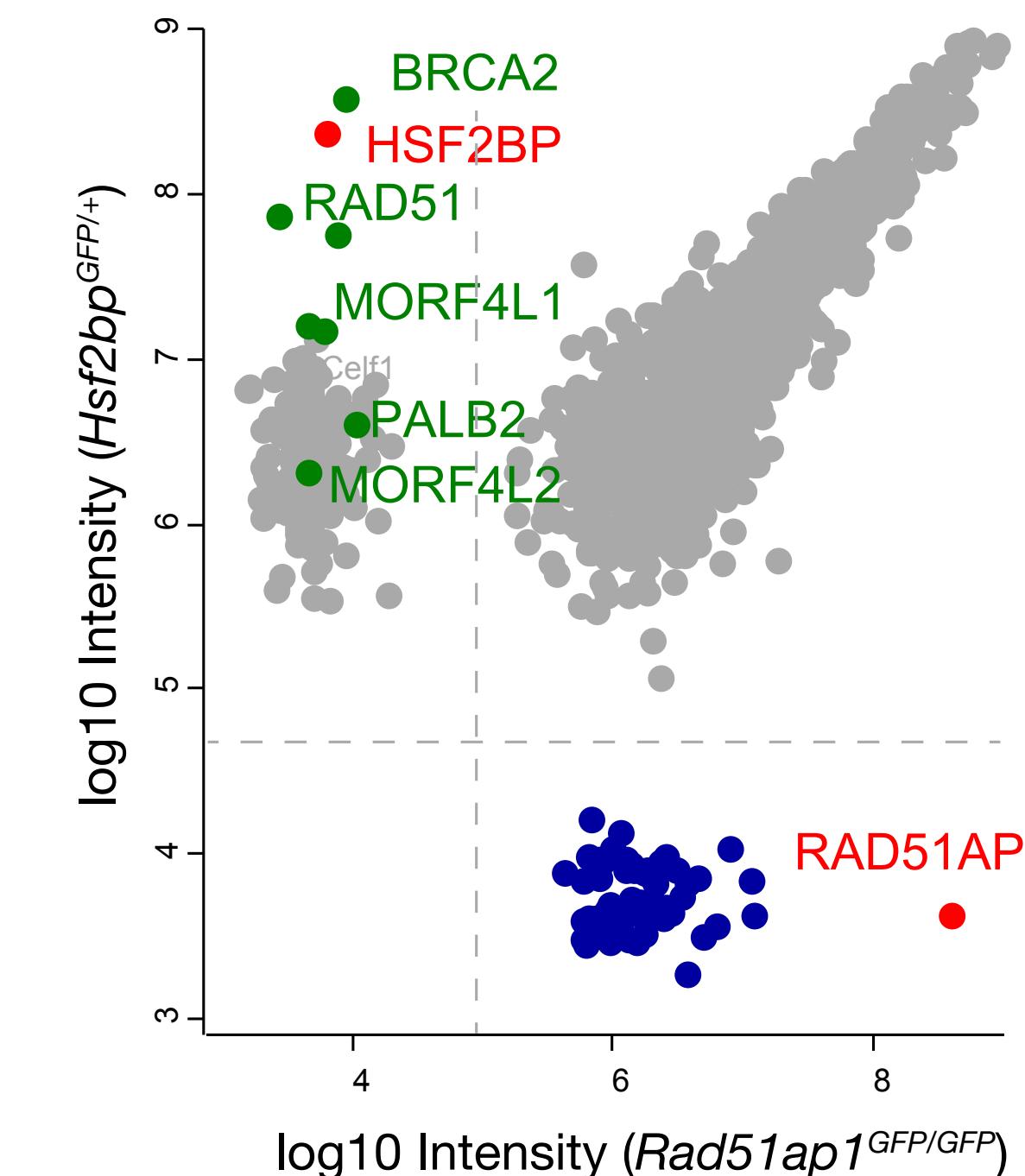
Label swap SILAC mass spectrometry experiments

The BRCA2 interactome

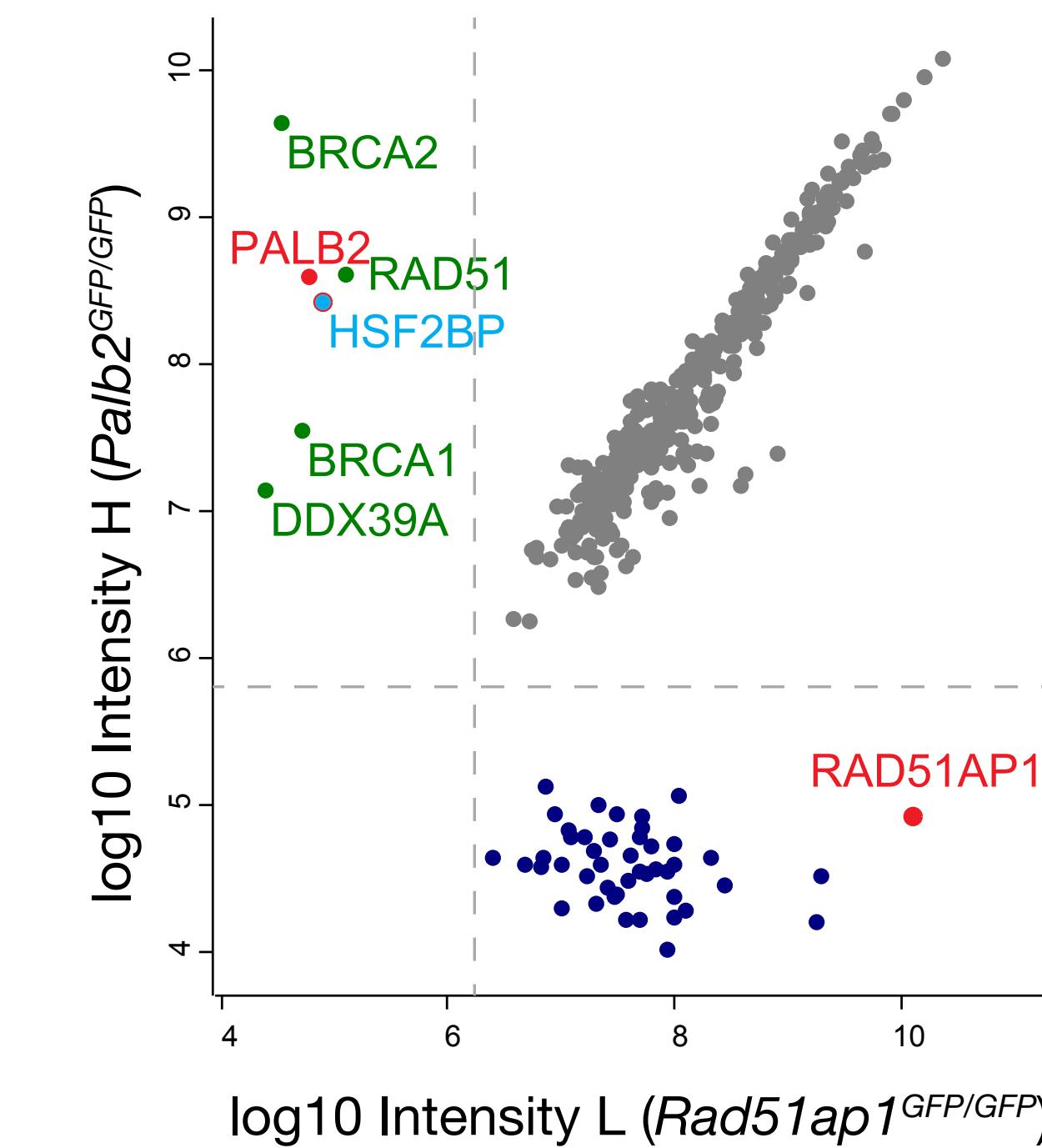
BRCA2 interactors
Identified HSF2BP



Reciprocal interaction
via HSF2BP IP



Also via PALB2 component



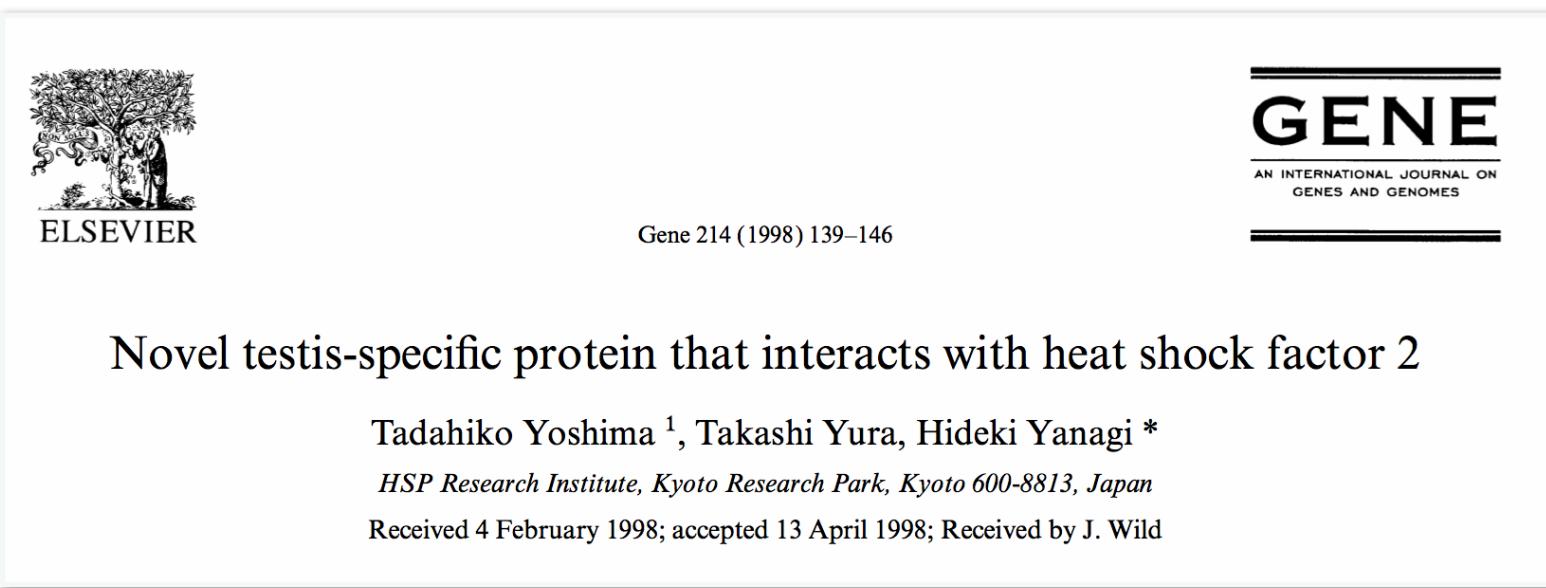
Label swap SILAC mass spectrometry experiments

HSF2BP

HSF2BP is highly conserved from fish to mammals (450 million years)

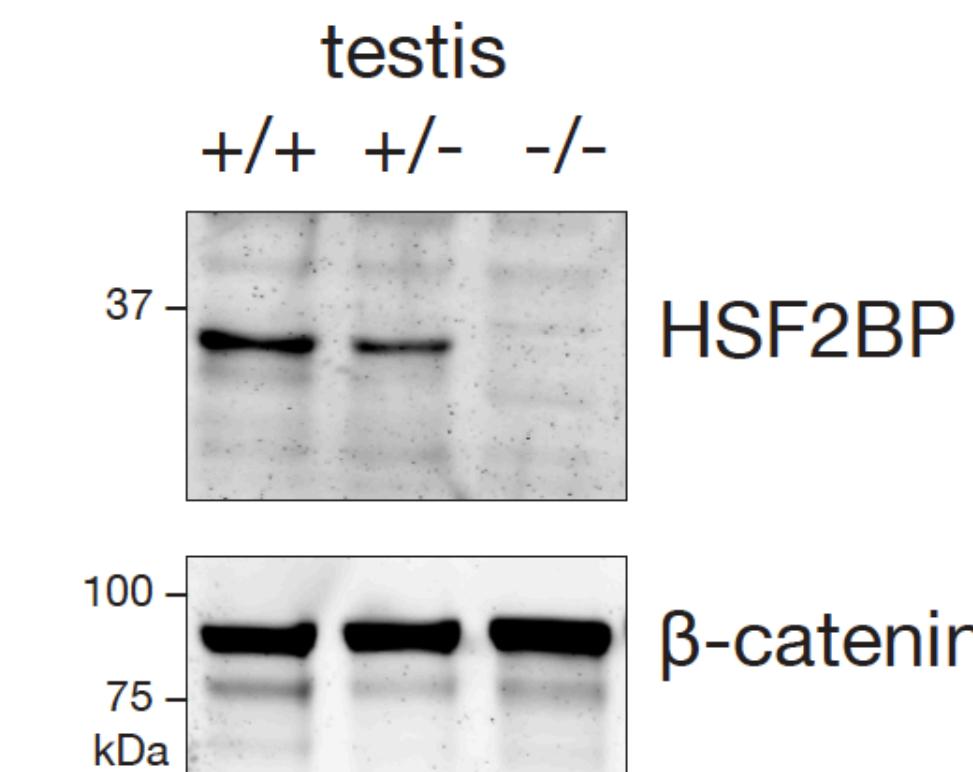
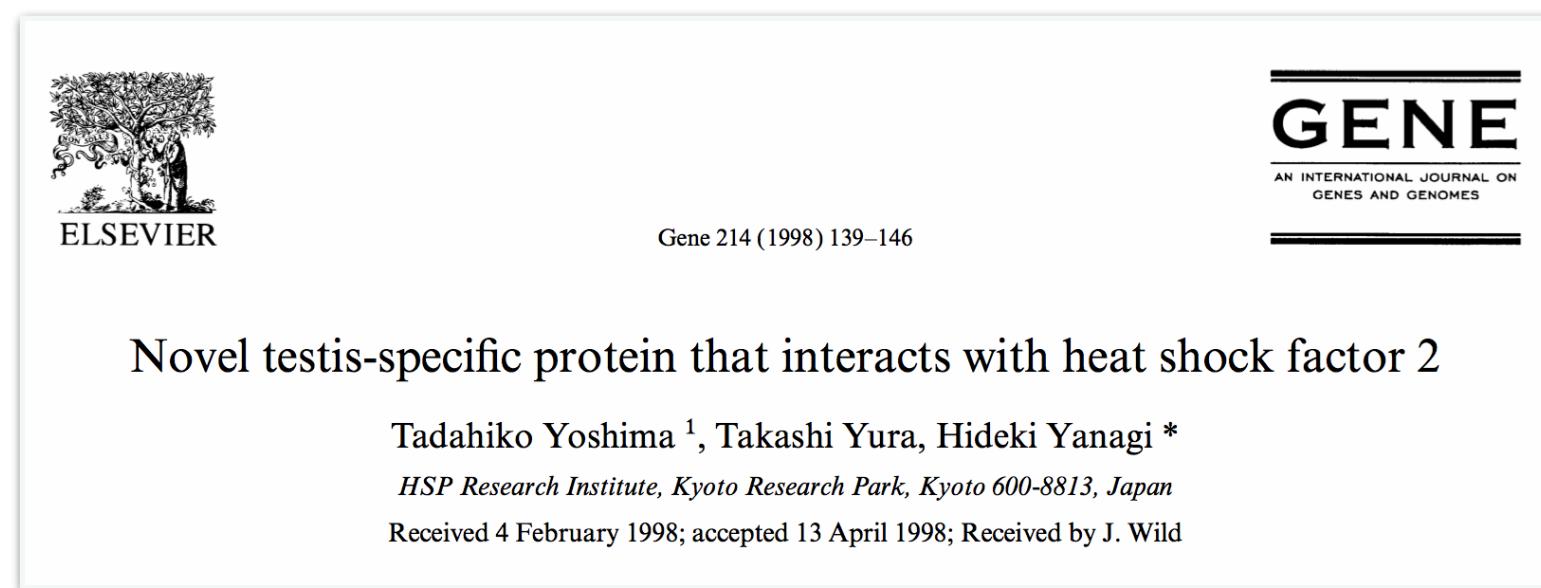
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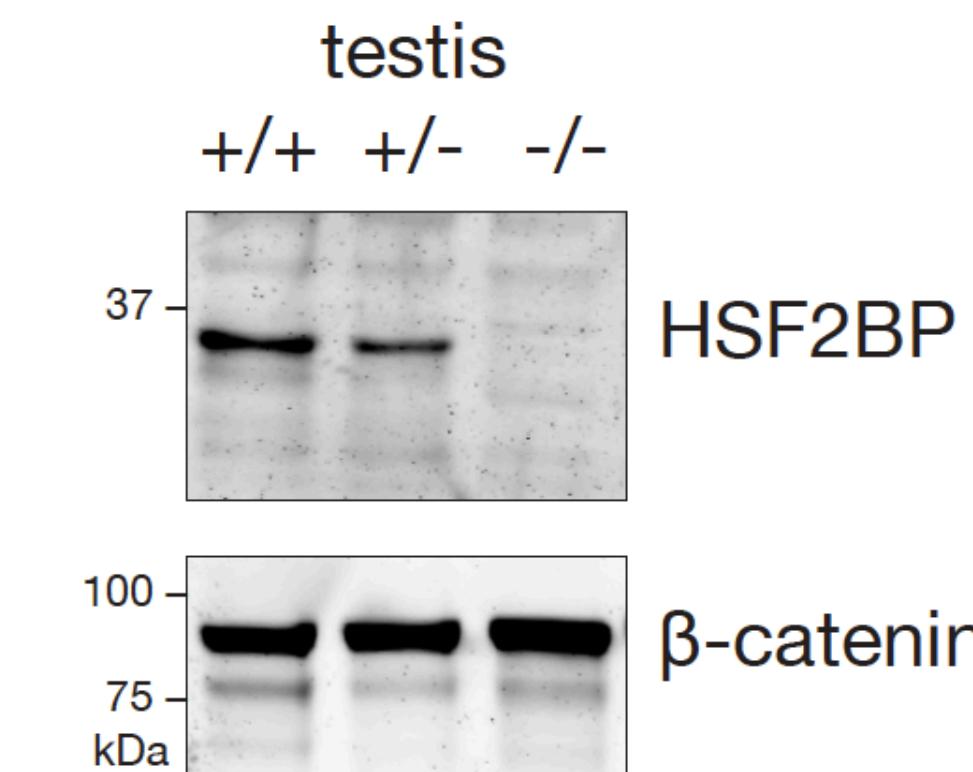
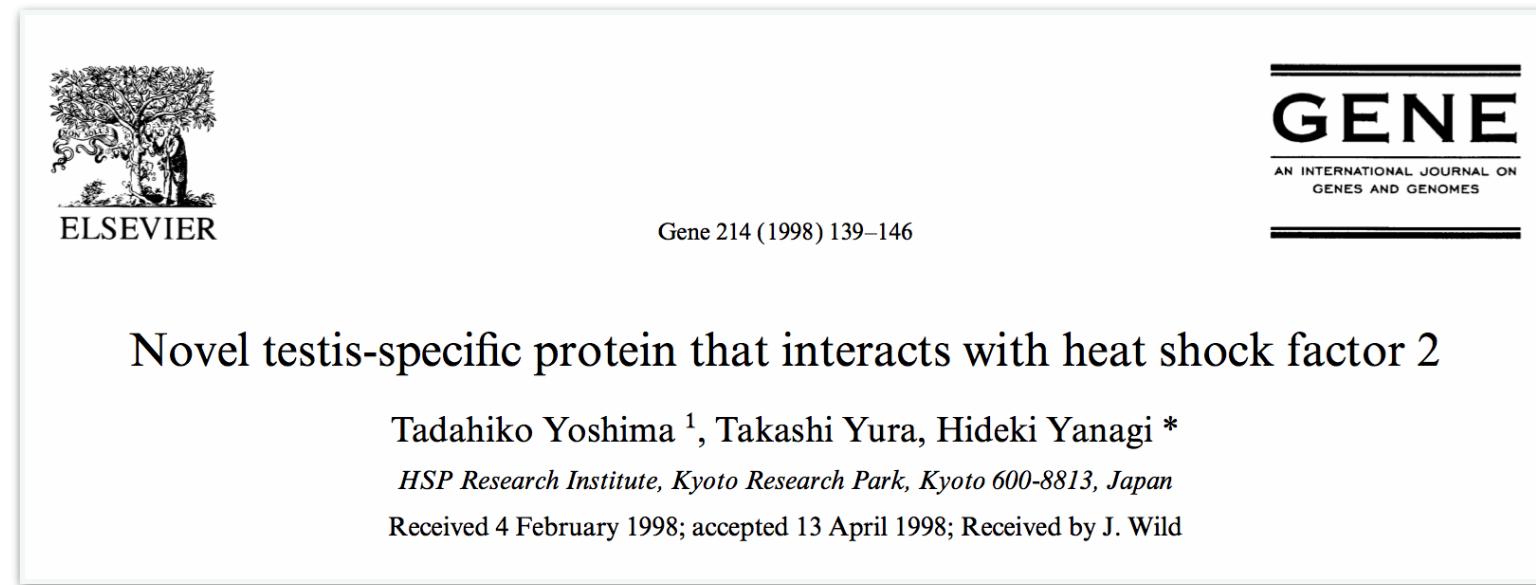
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HSF2BP

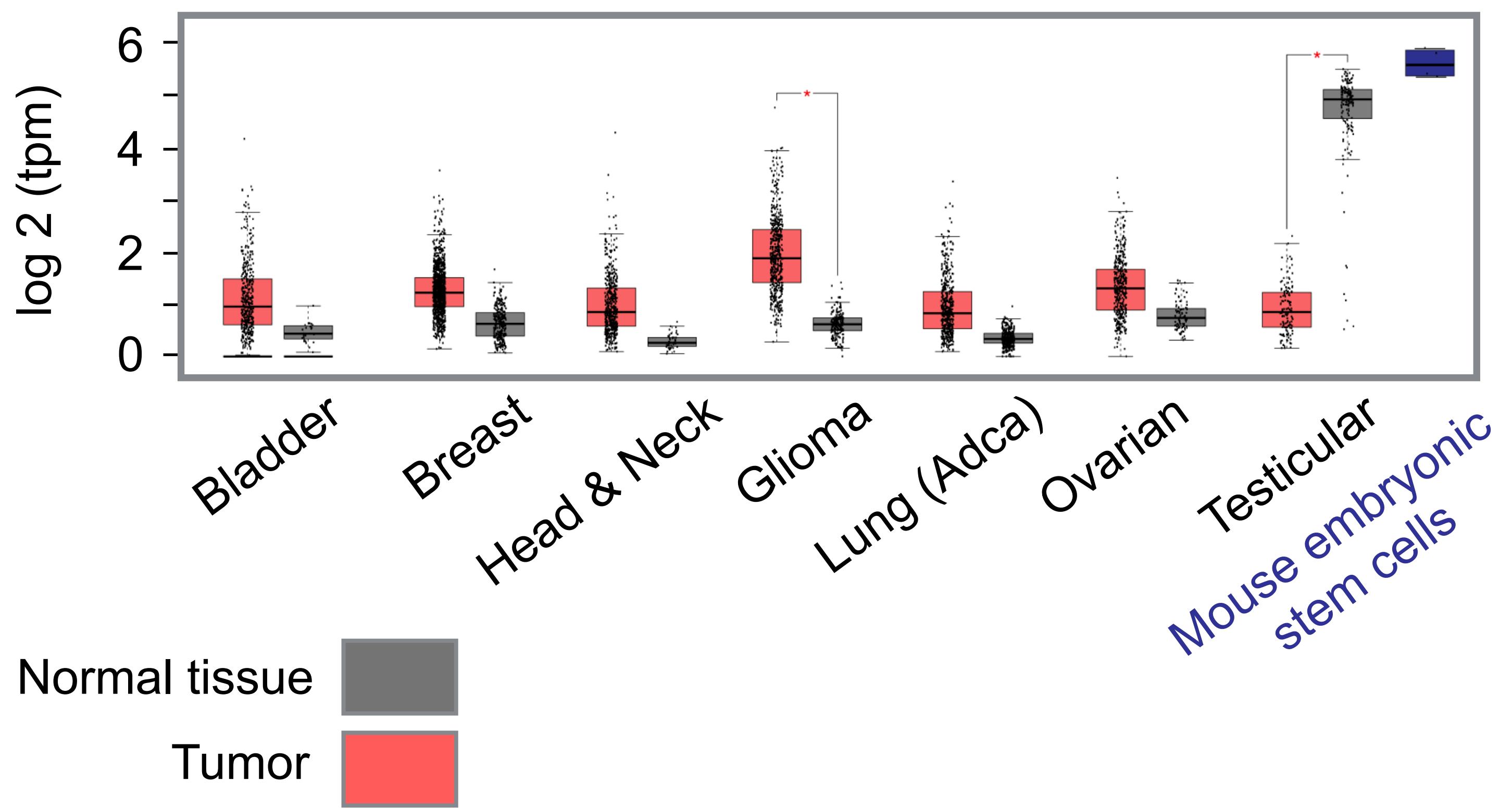
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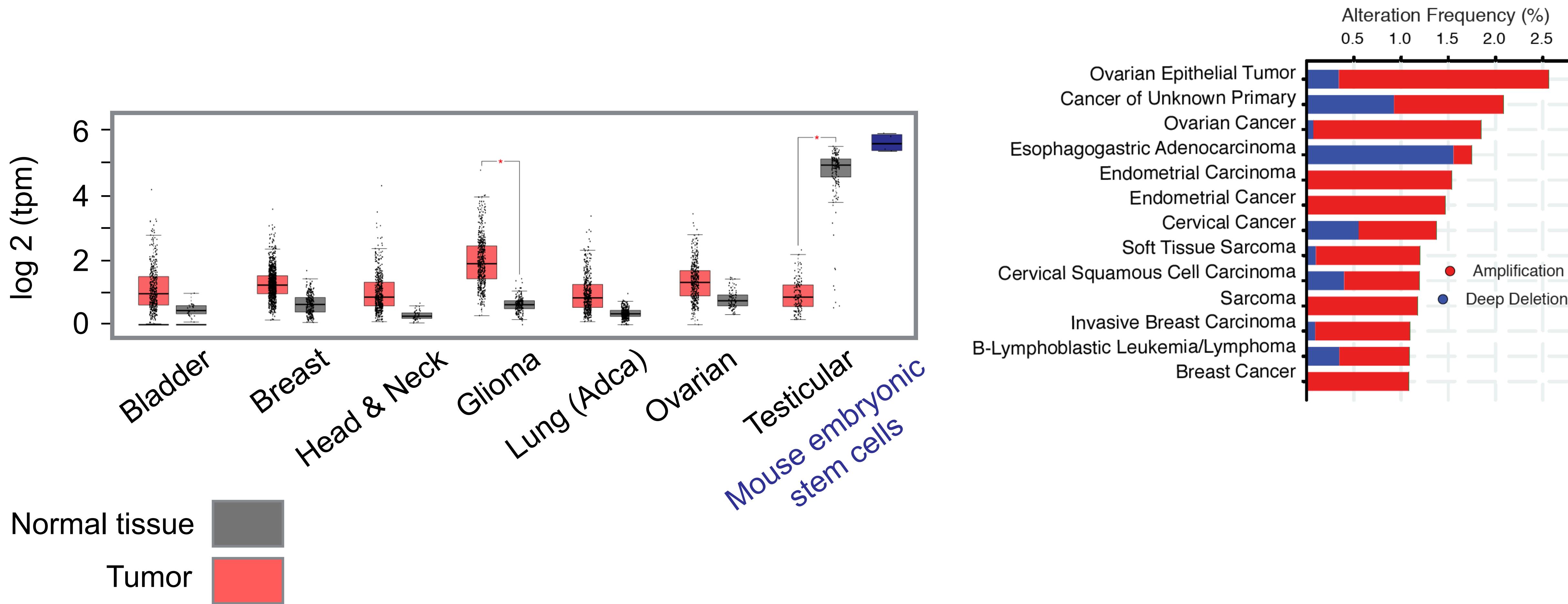
**HSF2BP affects meiosis: mislocalization RAD51 and DMC1
defective chromosome synapsis**

HSF2BP ectopic expression and amplification in tumors

HSF2BP ectopic expression and amplification in tumors

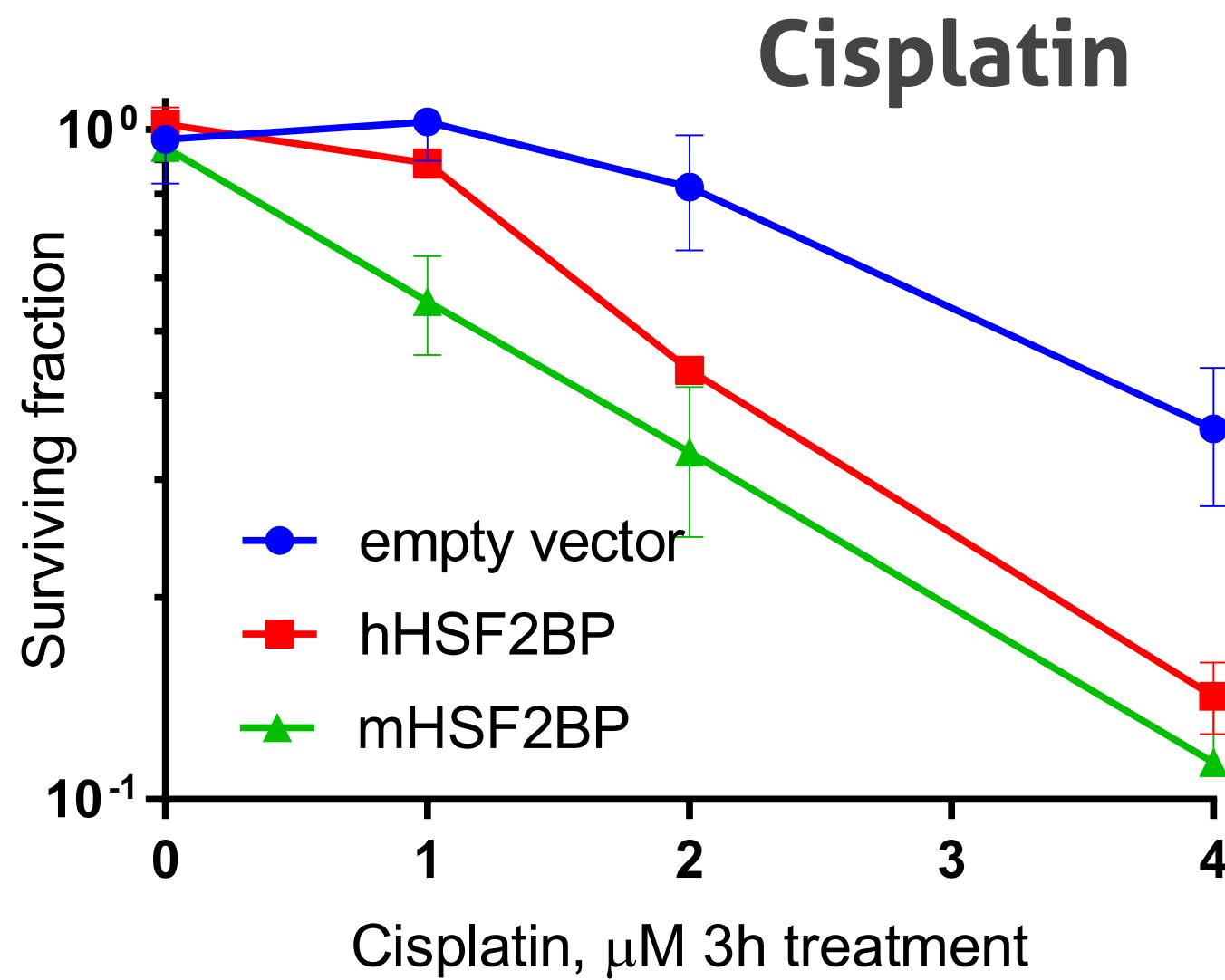


HSF2BP ectopic expression and amplification in tumors



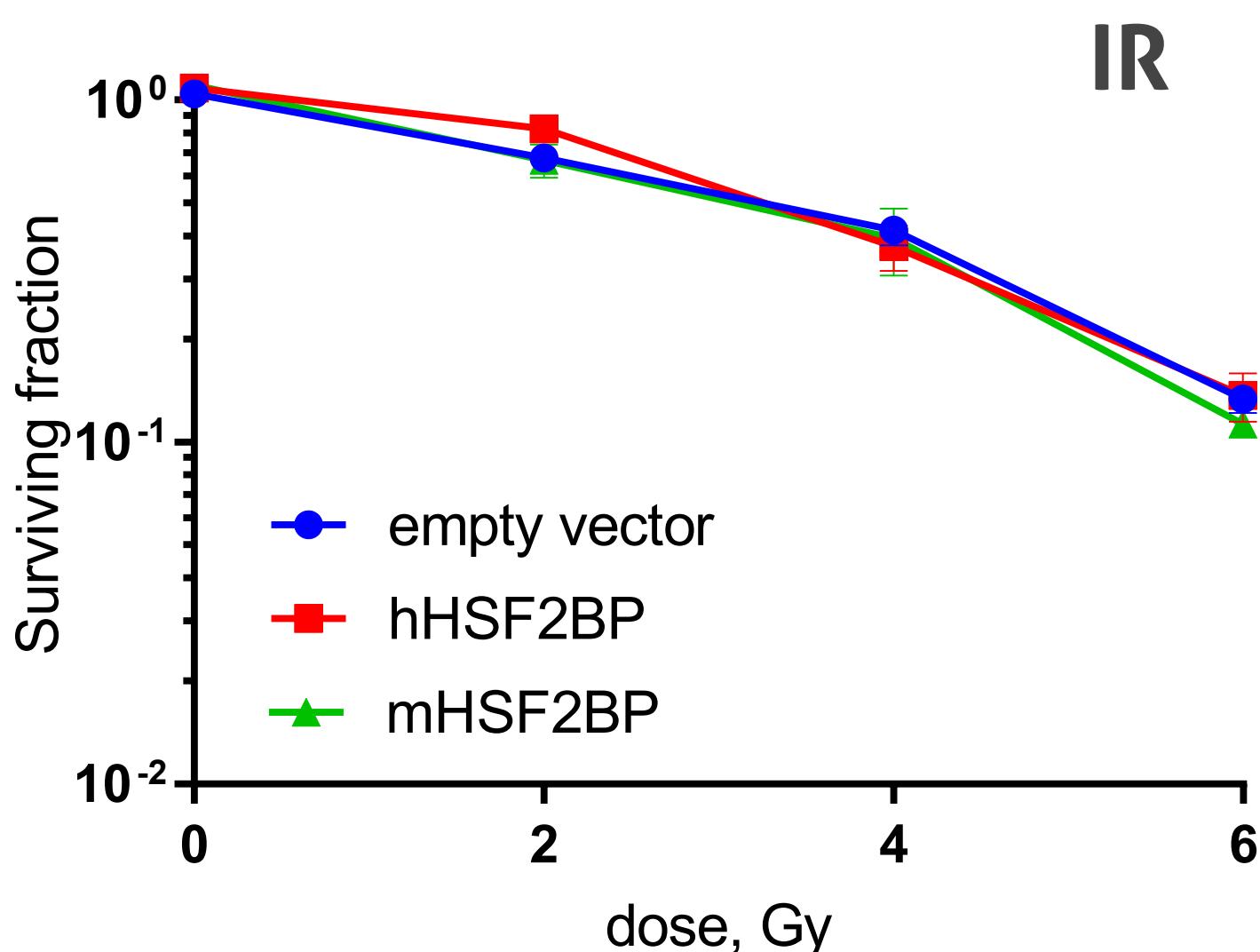
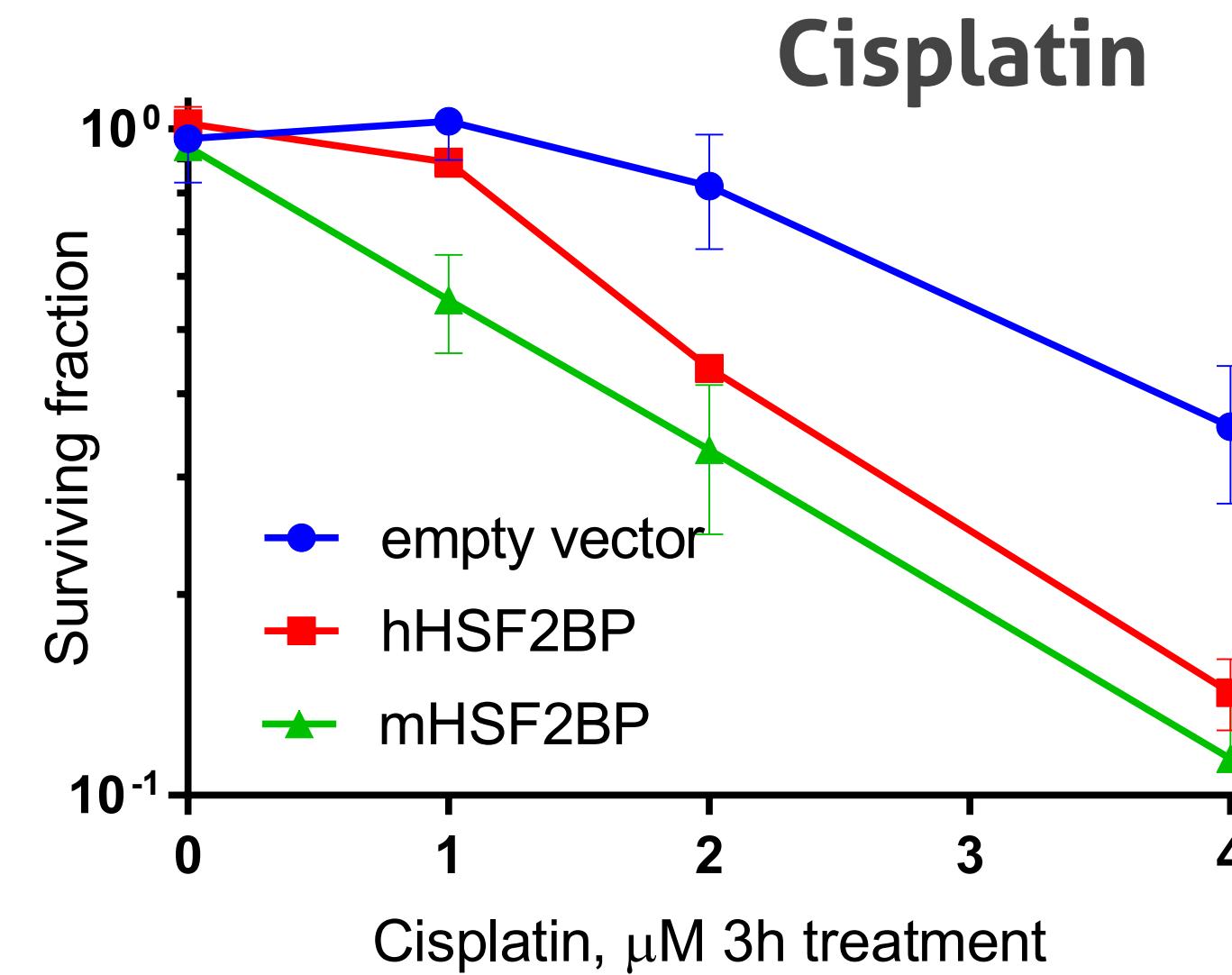
HSF2BP over expression sensitizes to ICLs

HeLa cells
with untagged
mouse HSF2BP
or human HSF2BP



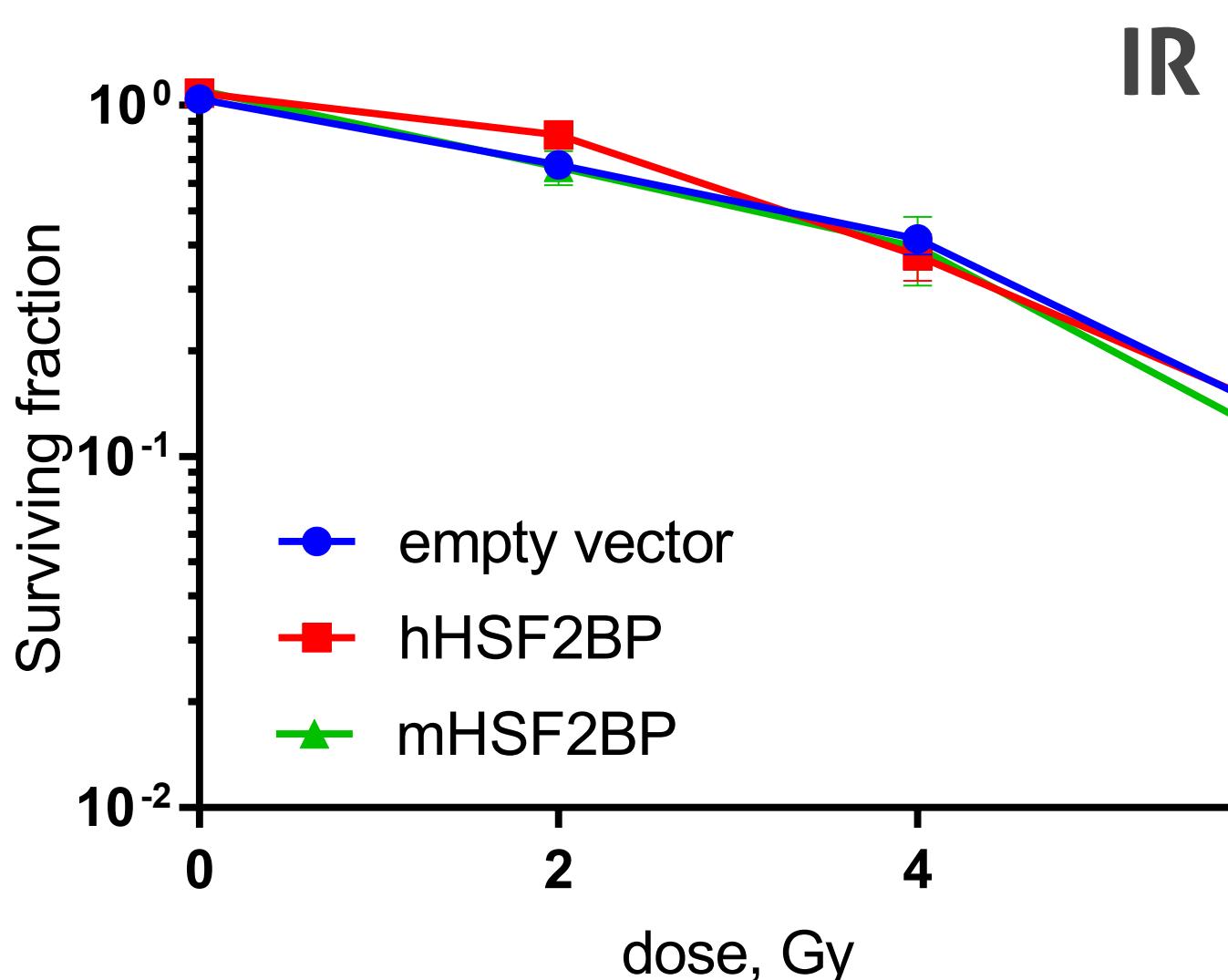
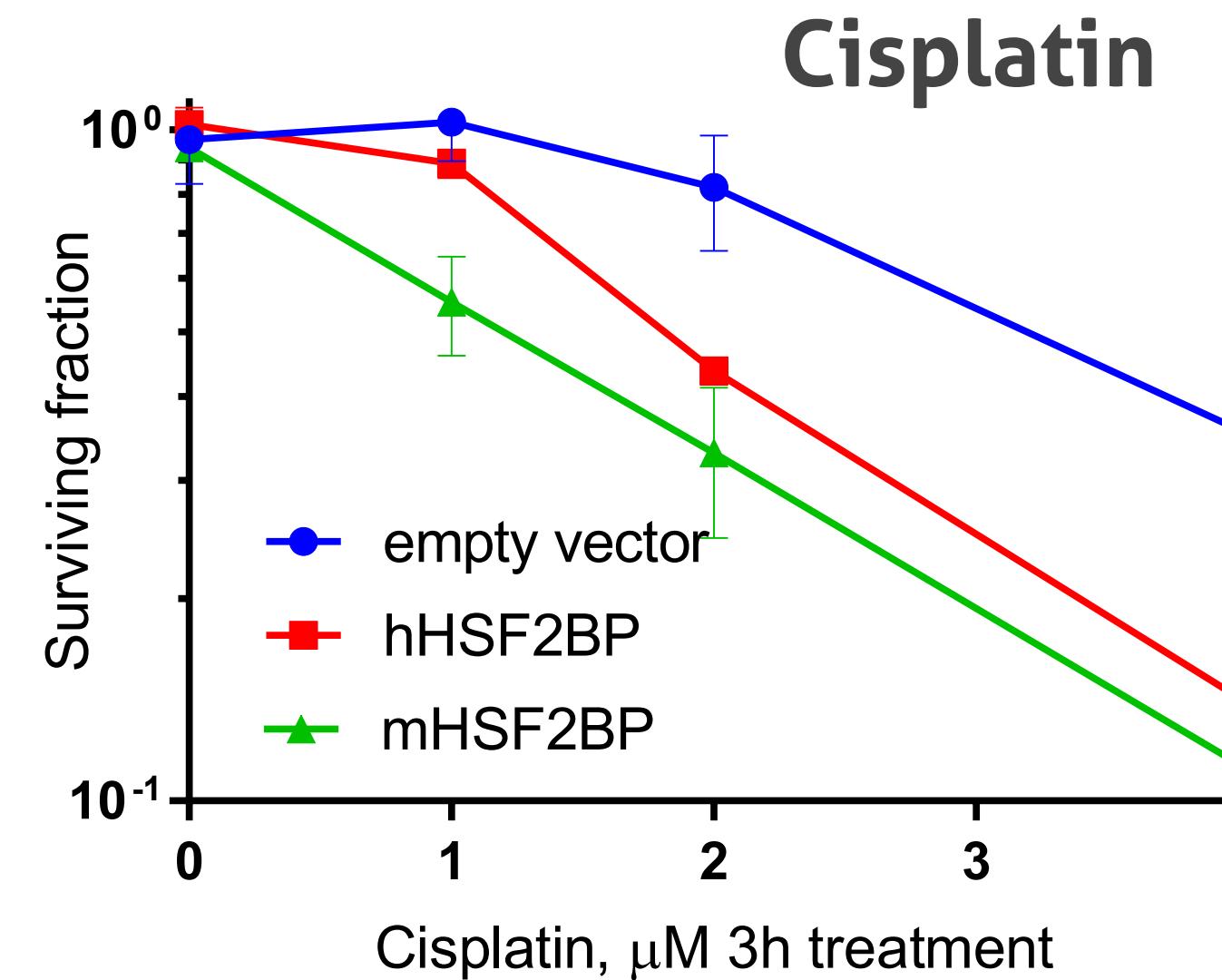
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or human HSF2BP

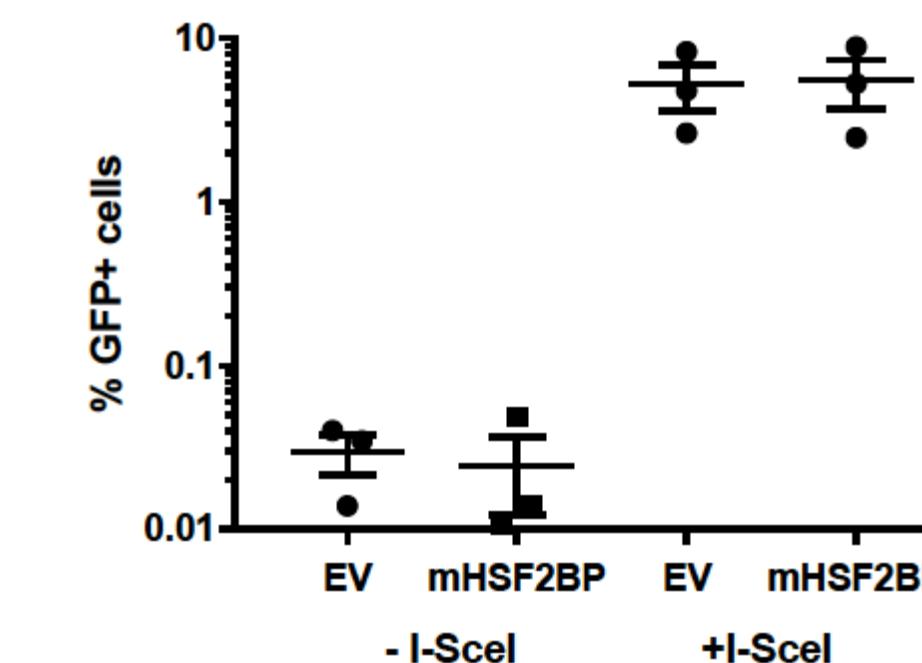


HSF2BP over expression sensitizes to ICLs

HeLa cells
with untagged
mouse HSF2BP
or human HSF2BP

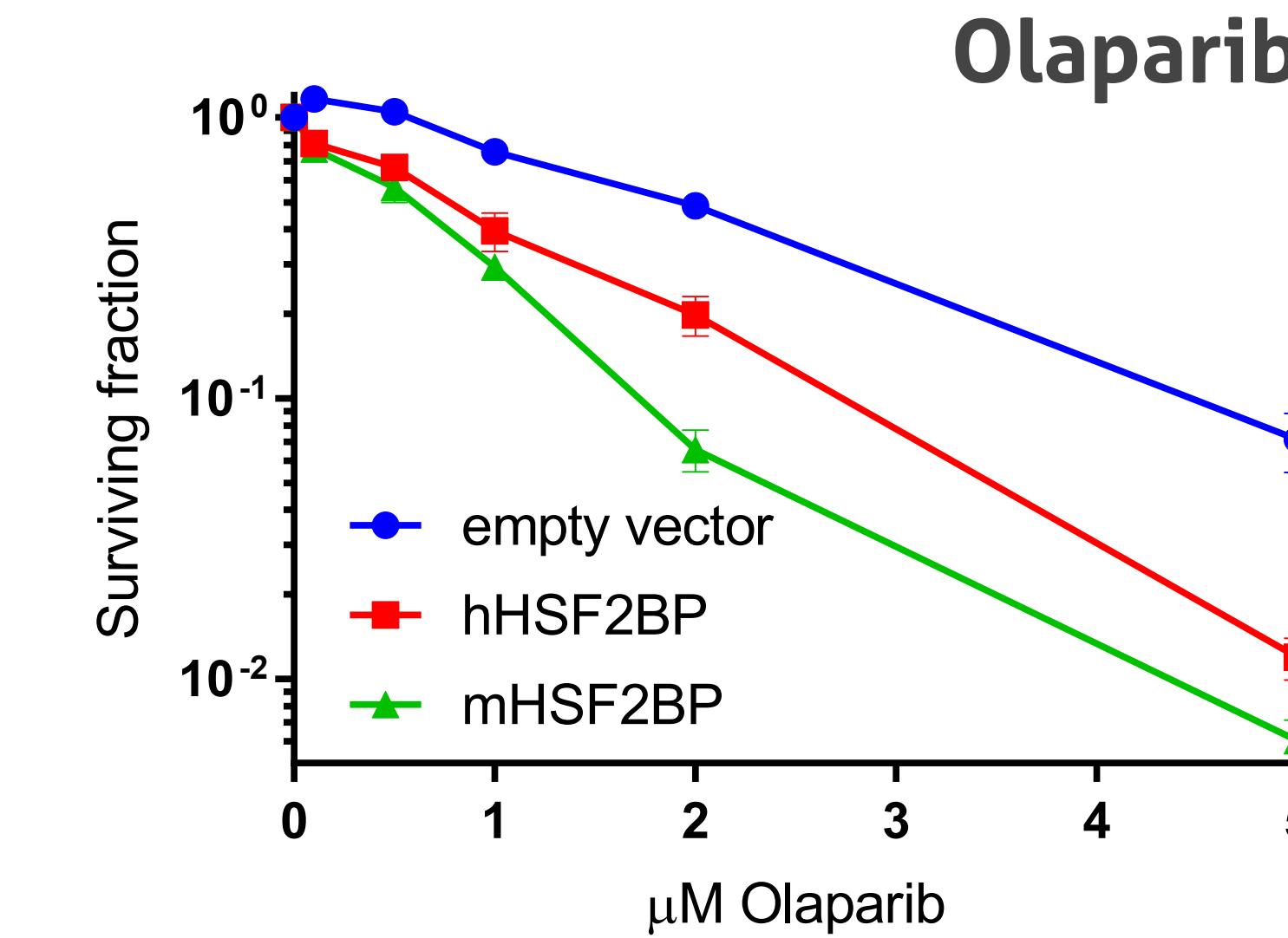
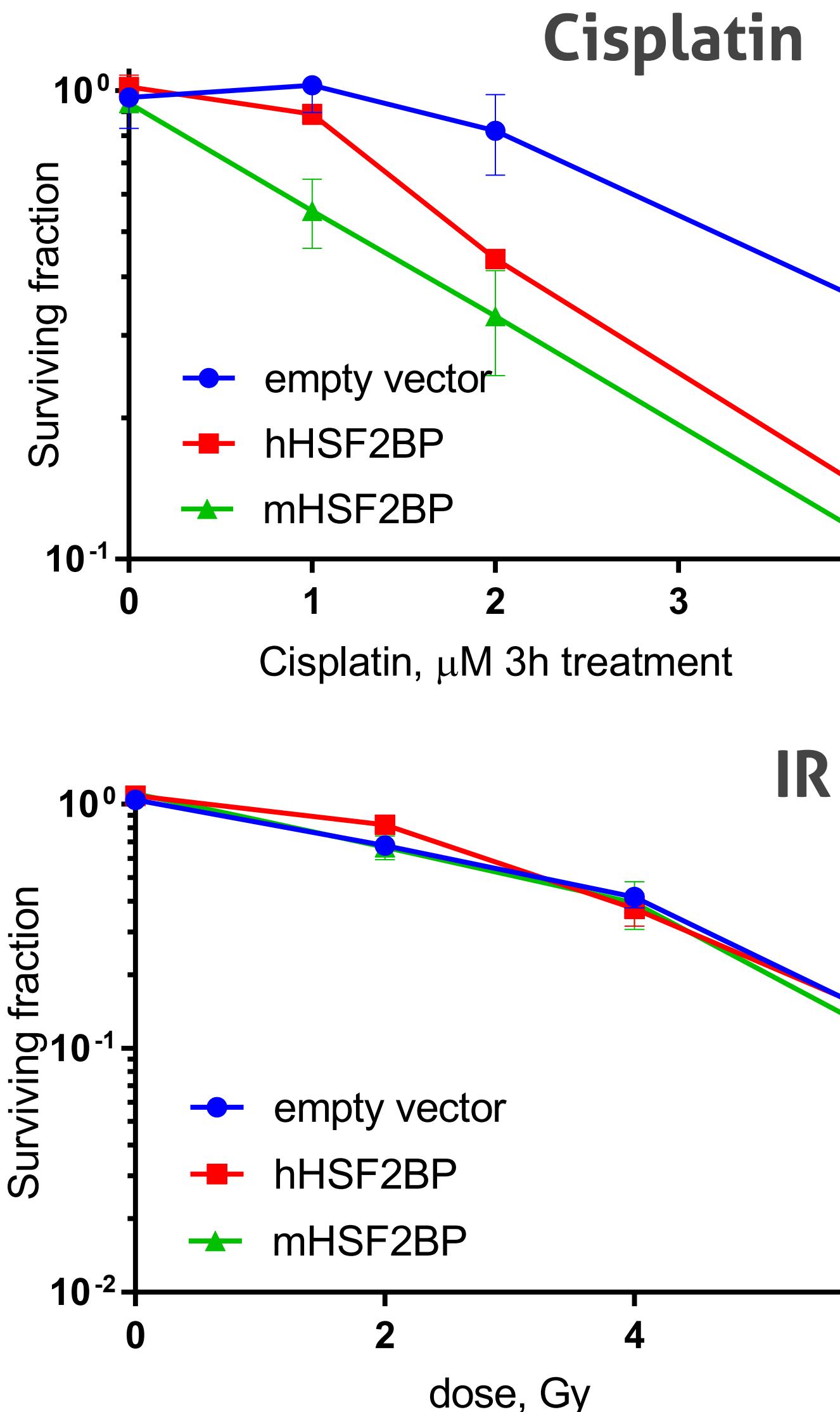


DR GFP gene conversion

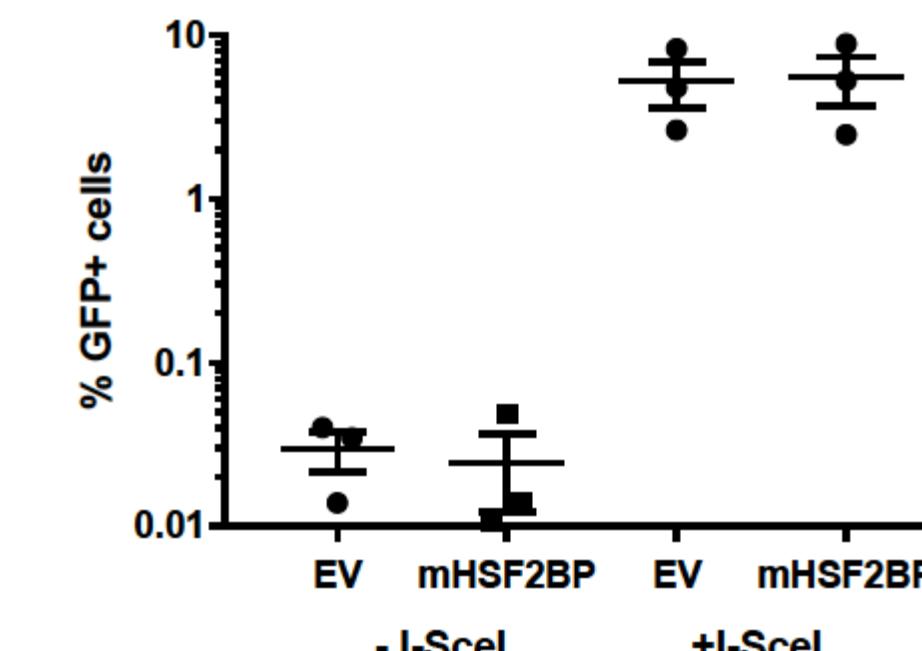


HSF2BP over expression sensitizes to ICLs

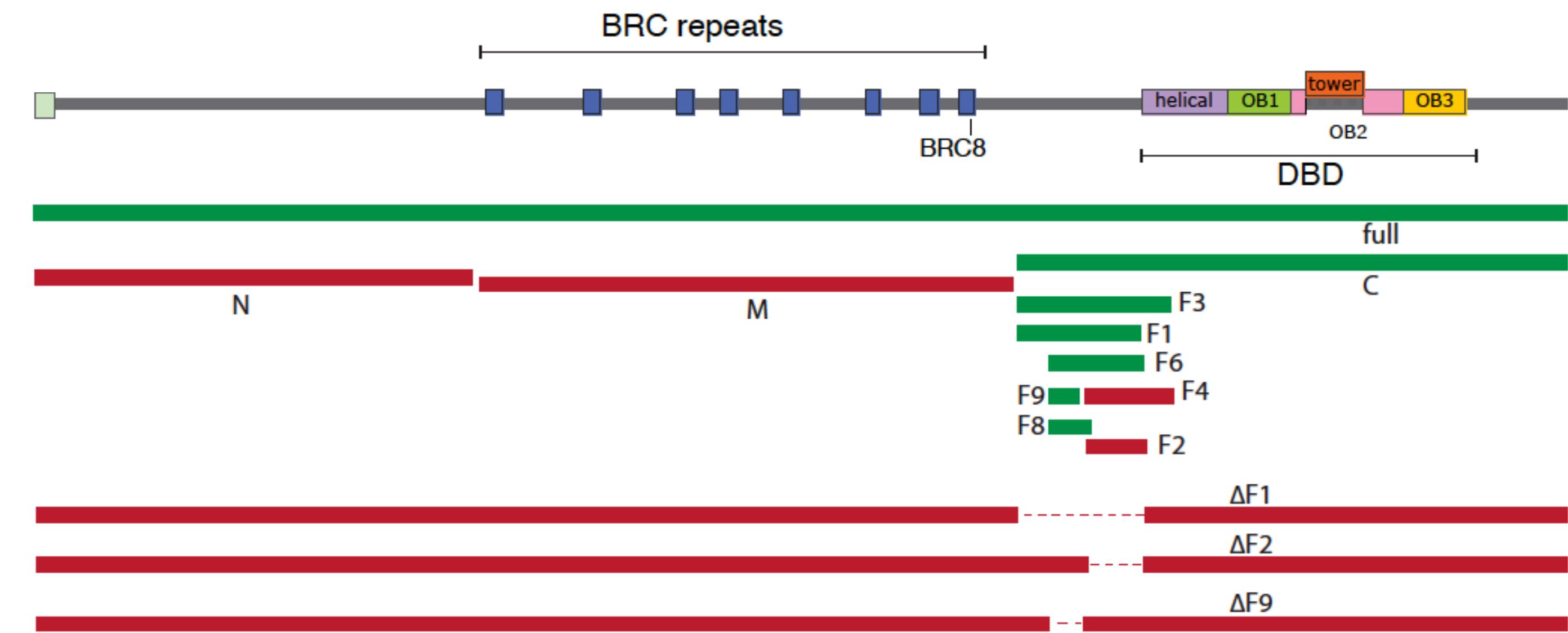
HeLa cells
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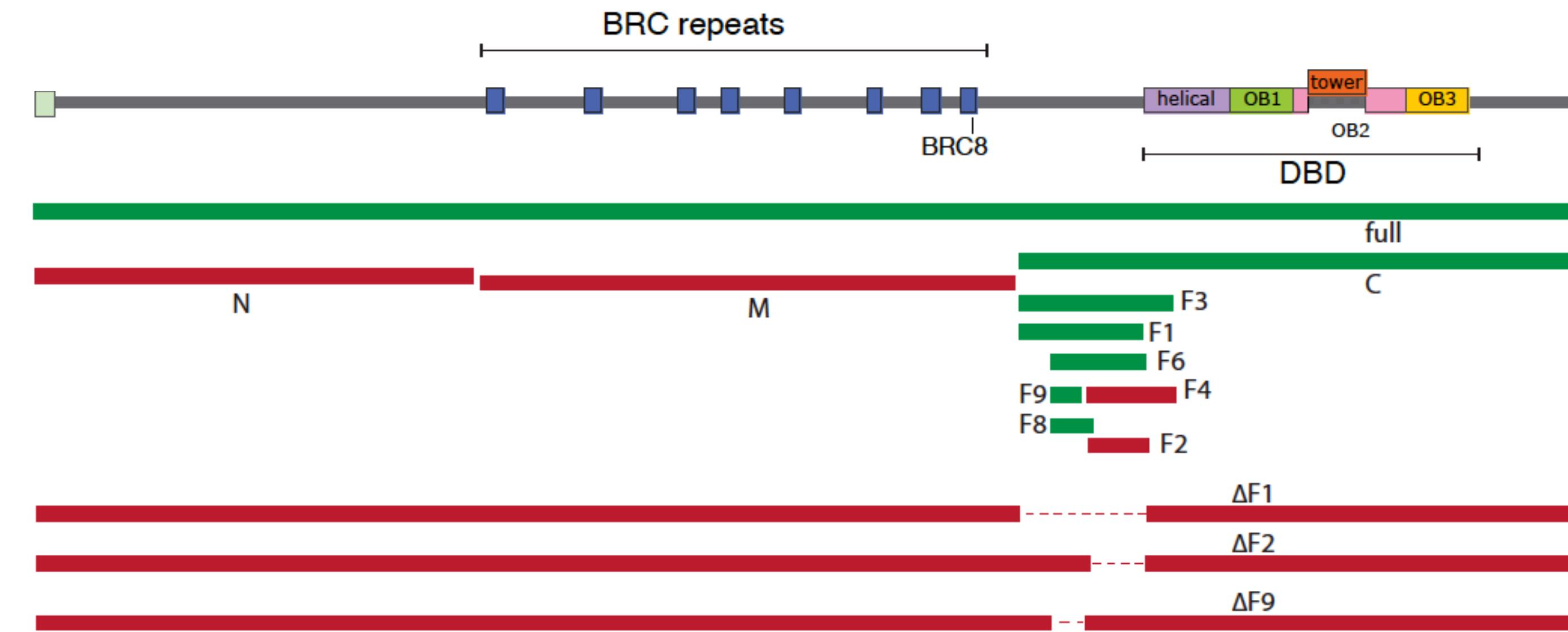
DR GFP gene conversion



MAPPING THE BRCA2 - HSF2BP interaction

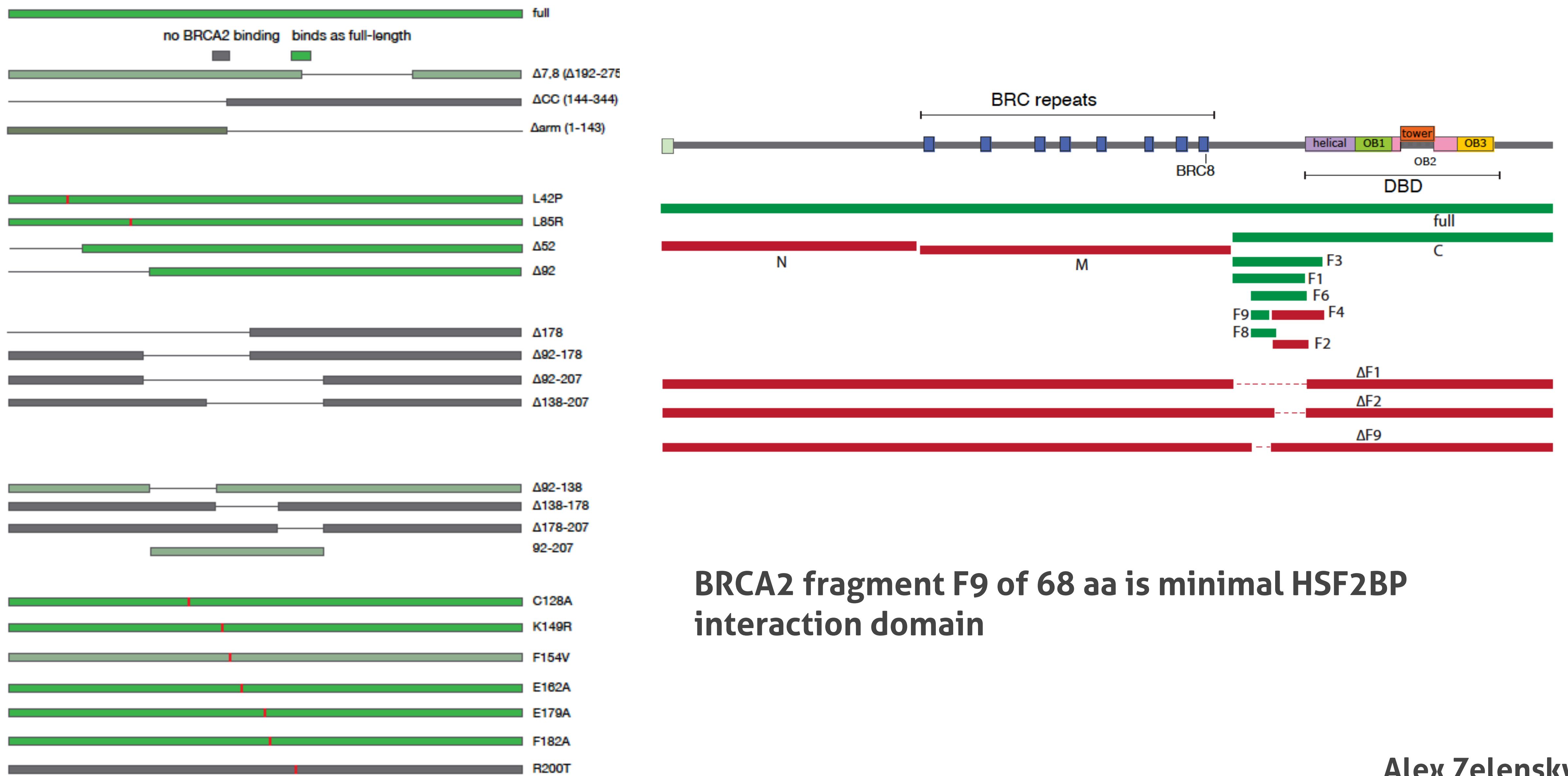


MAPPING THE BRCA2 - HSF2BP interaction

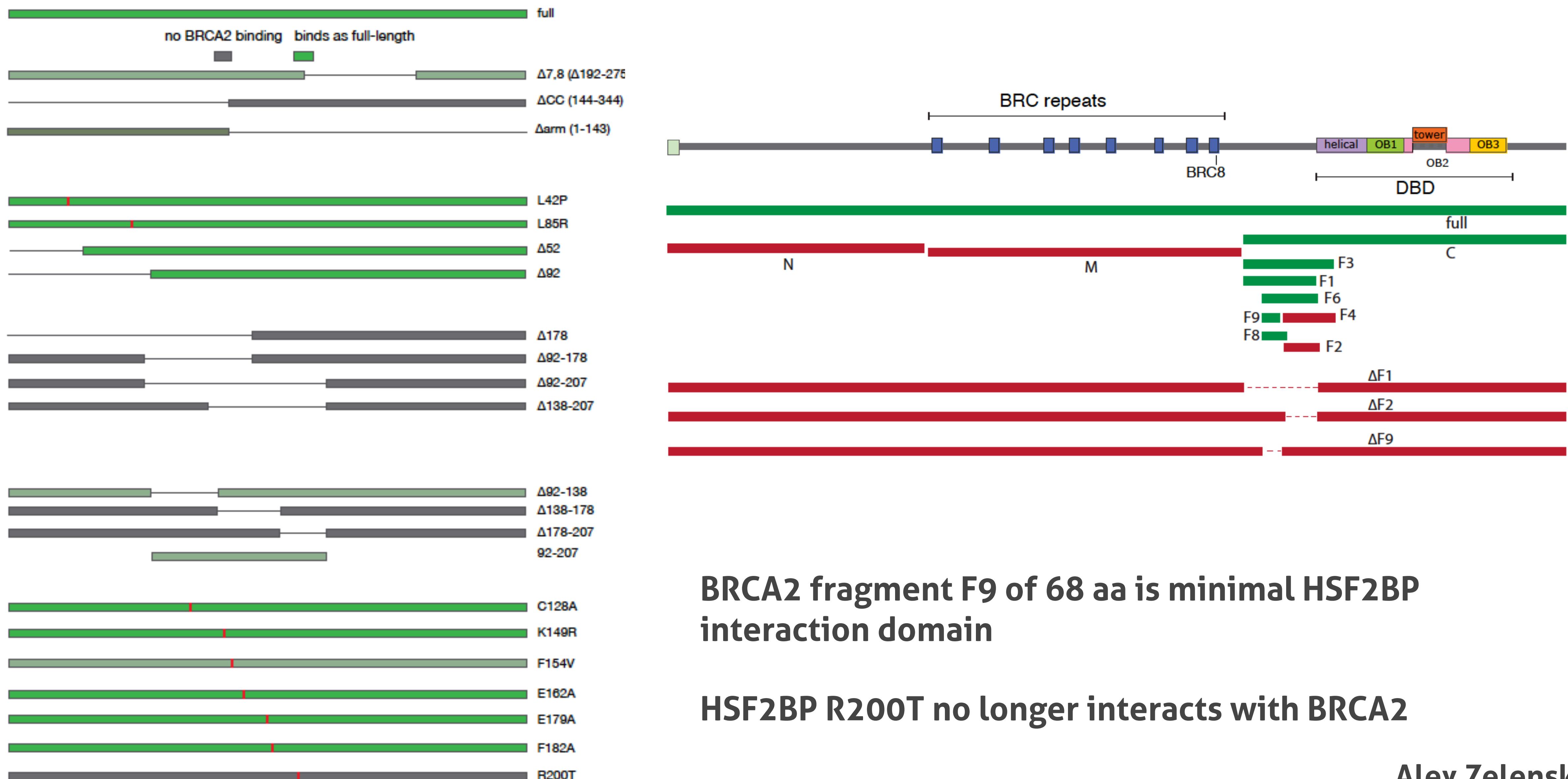


BRCA2 fragment F9 of 68 aa is minimal HSF2BP interaction domain

MAPPING THE BRCA2 - HSF2BP interaction

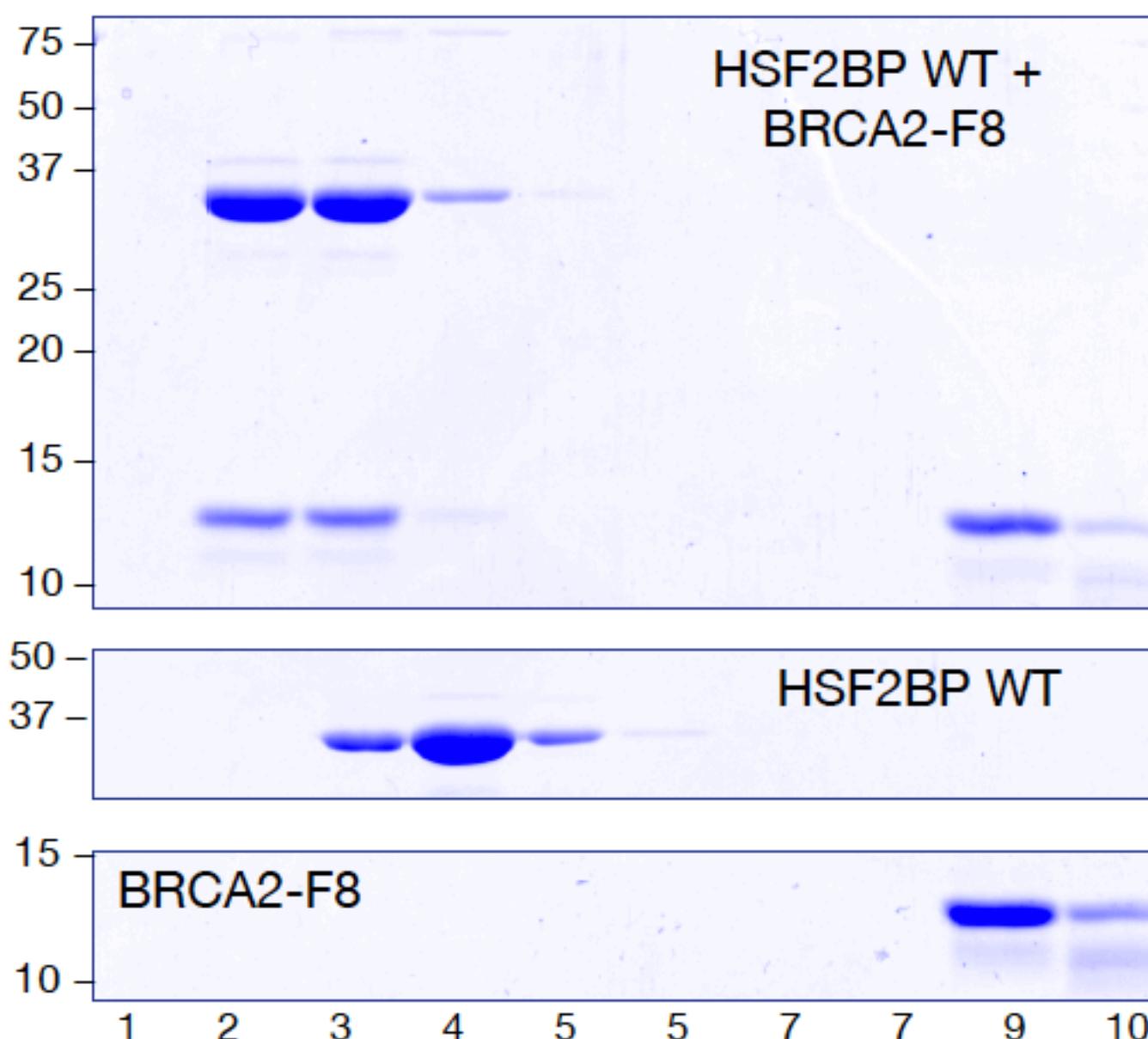
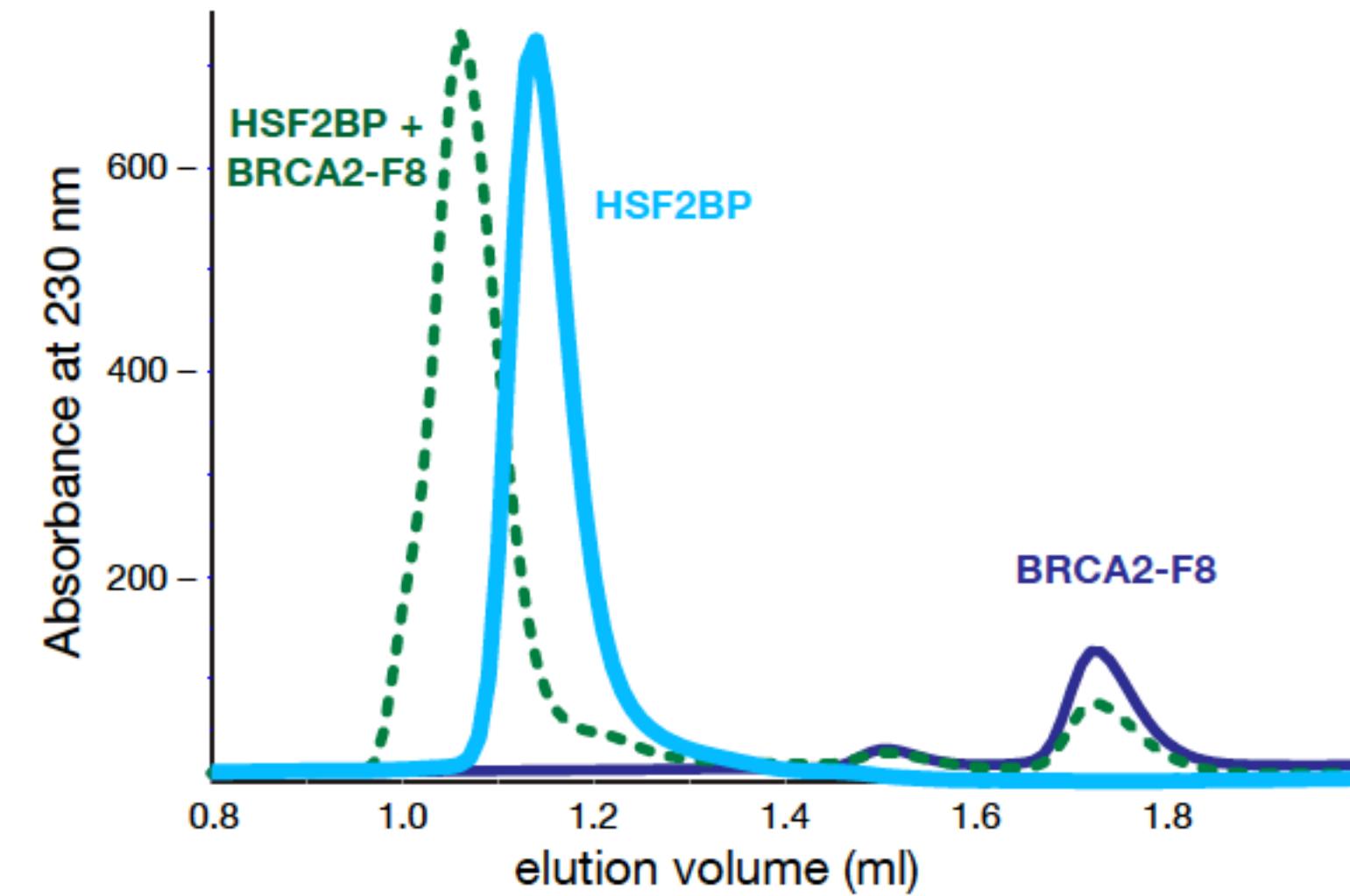


MAPPING THE BRCA2 - HSF2BP interaction



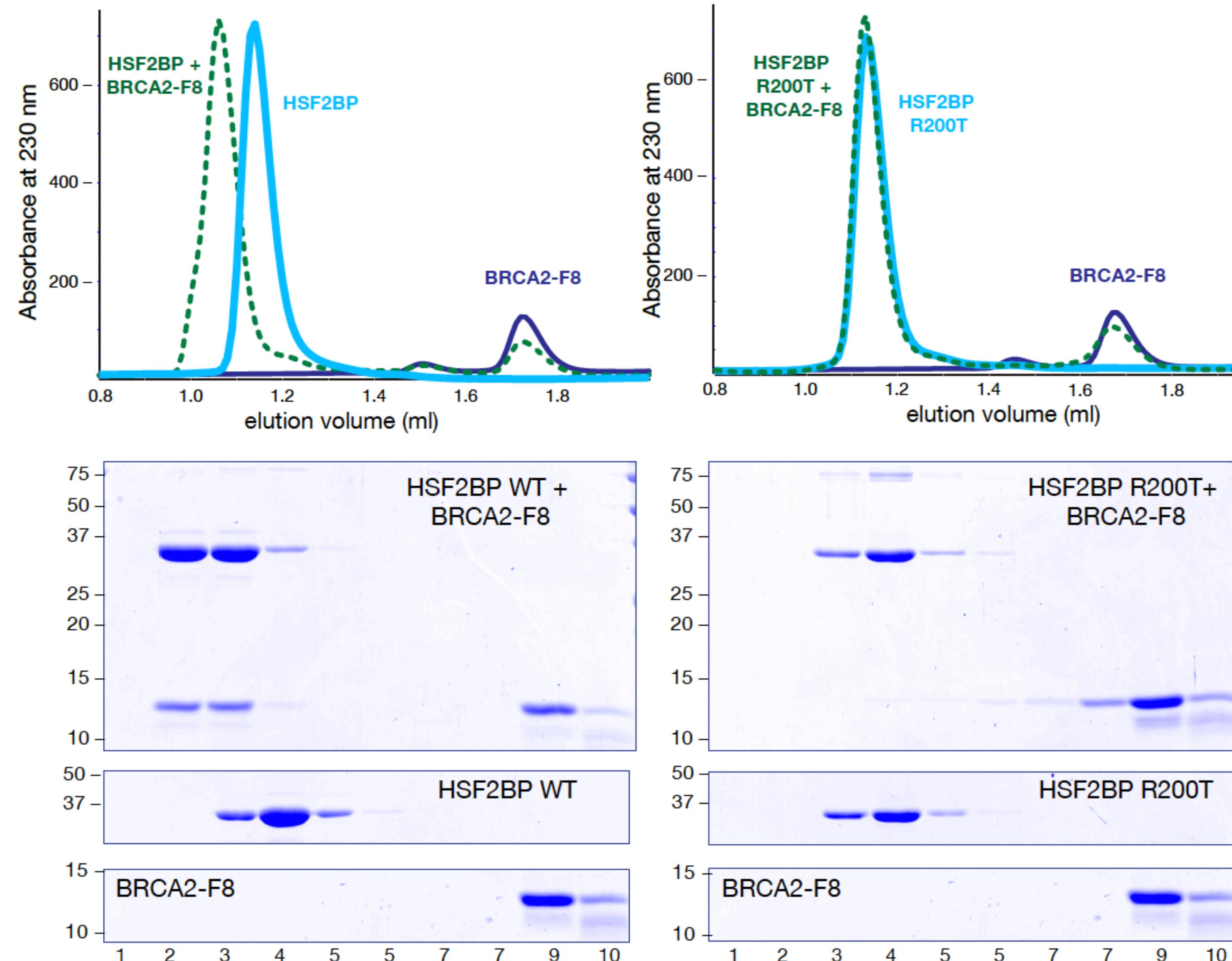
Alex Zelensky

MAPPING THE BRCA2 - HSF2BP interaction



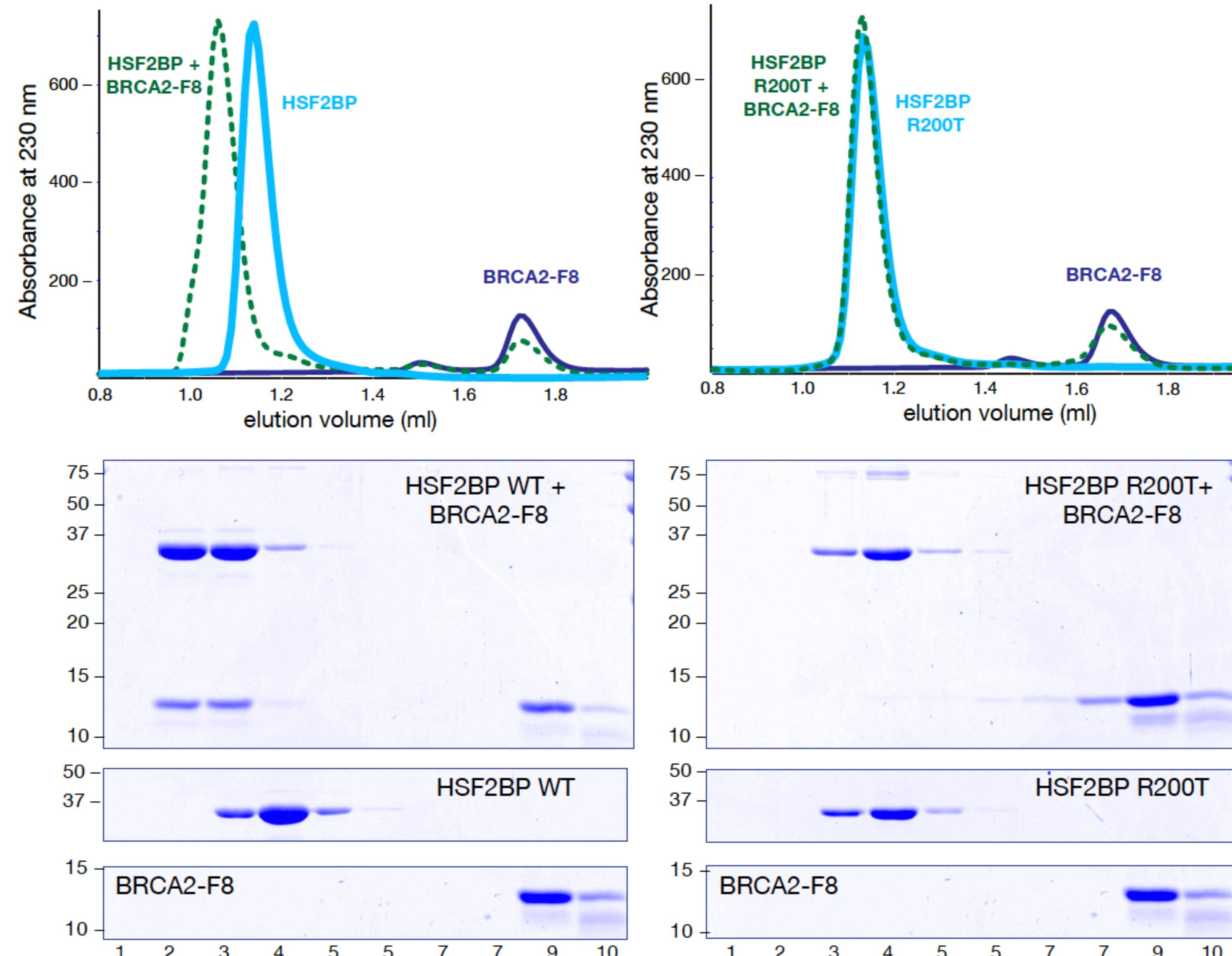
Gelfiltration of purified untagged HSF2BP (WT and R200T) with BRCA2 F8 fragment

MAPPING THE BRCA2 - HSF2BP interaction



Gelfiltration of purified untagged HSF2BP (WT and R200T) with BRCA2 F8 fragment

MAPPING THE BRCA2 - HSF2BP INTERACTION

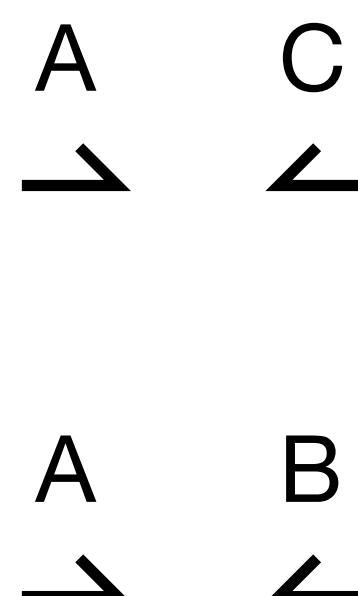
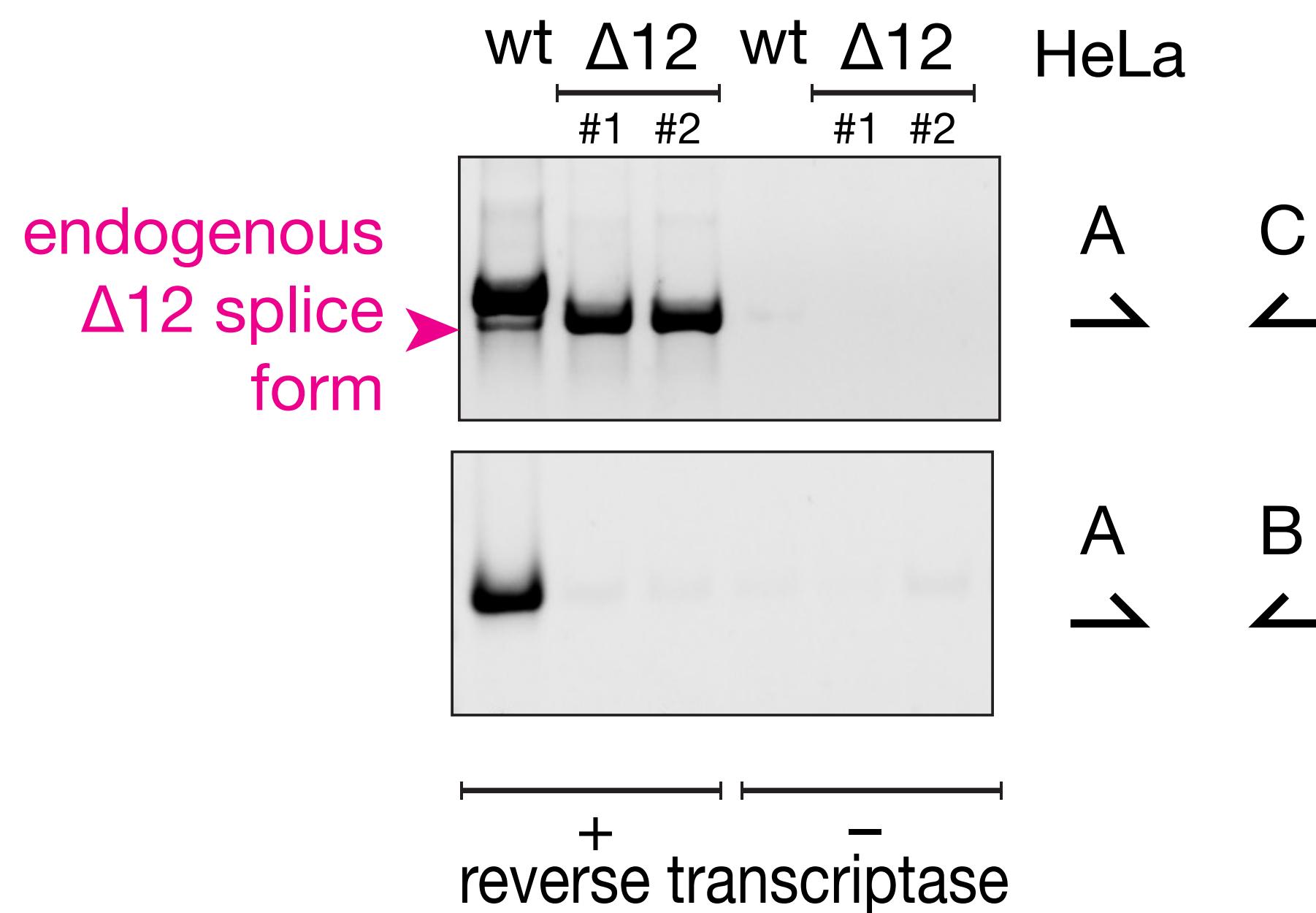
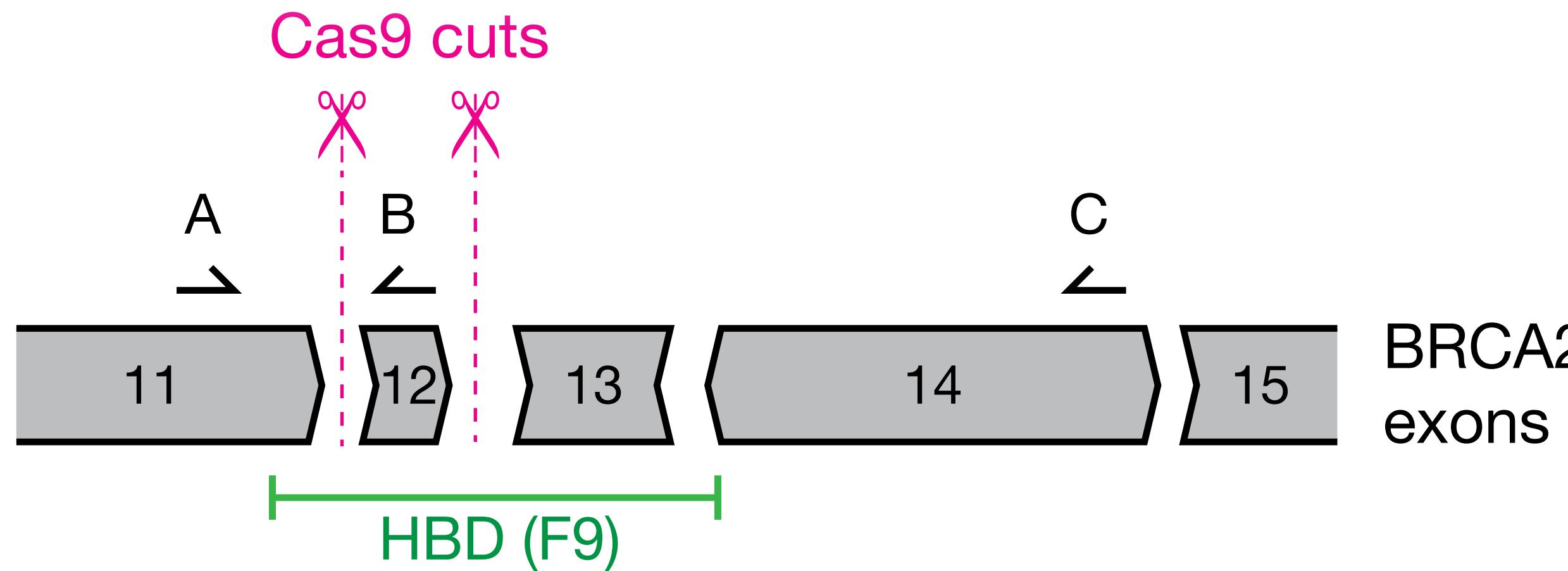


HSF2BP - BRCA2 interaction is in 1.2 nM range

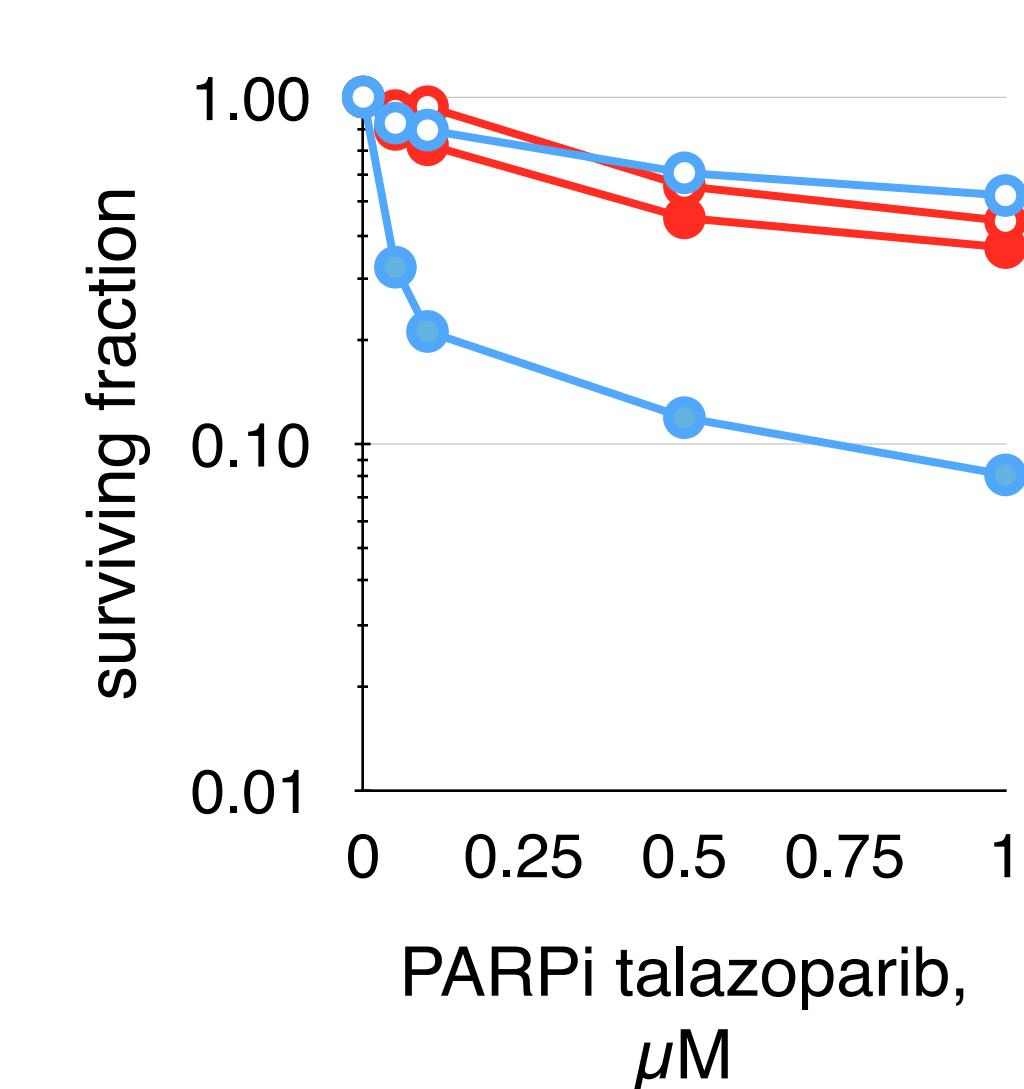
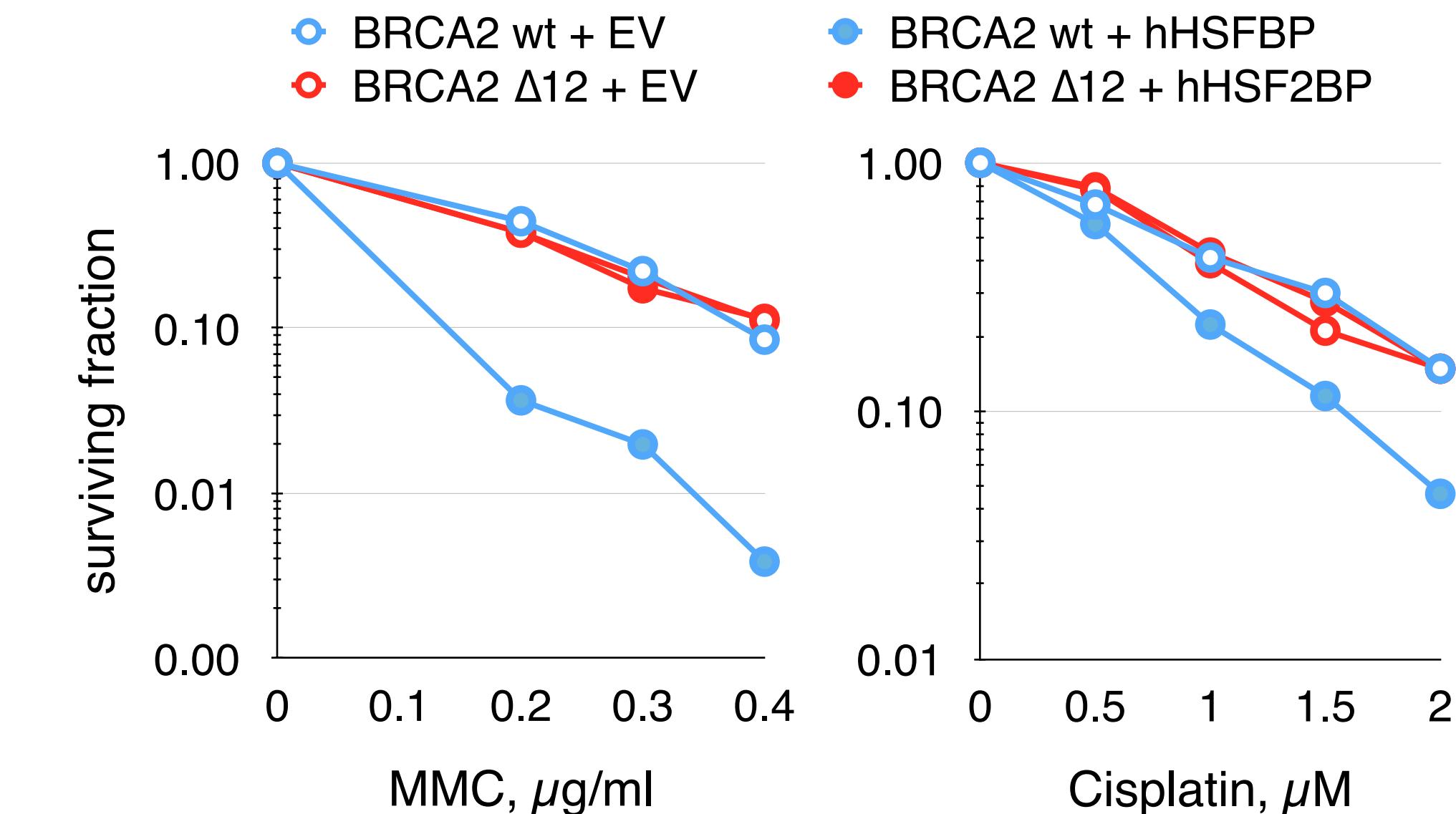
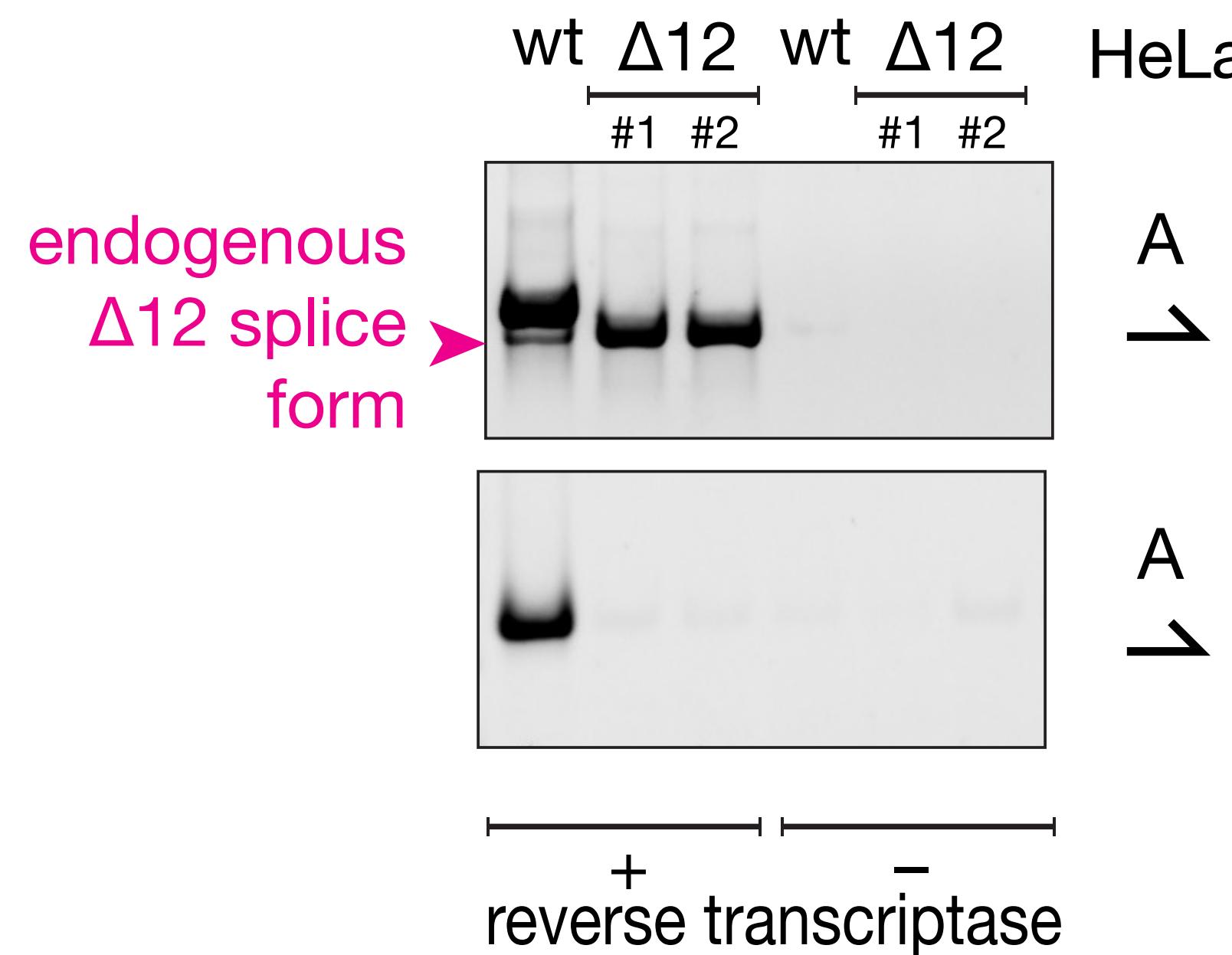
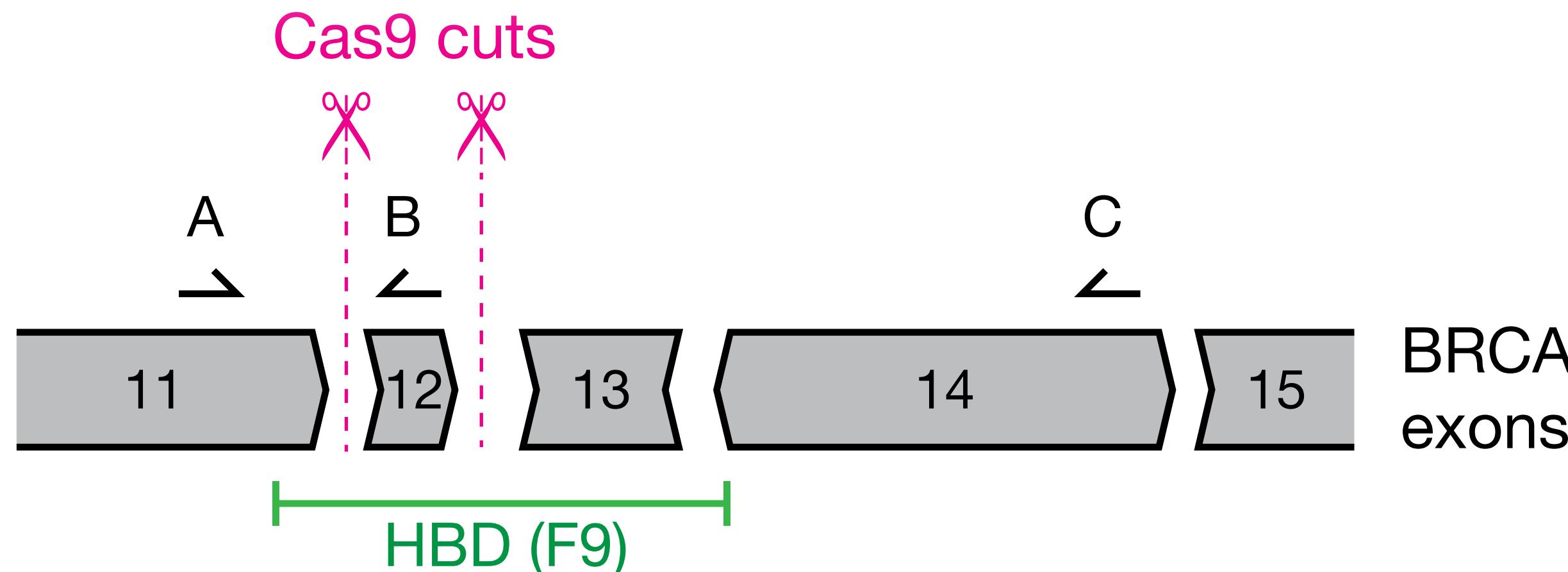
HSF2BP is a tetramer interacting with 2 BRCAs

Gelfiltration of purified untagged HSF2BP (WT and R200T) with BRCA2 F8 fragment

HSF2BP-BRCA2 interaction is necessary for sensitization



HSF2BP-BRCA2 interaction is necessary for sensitization



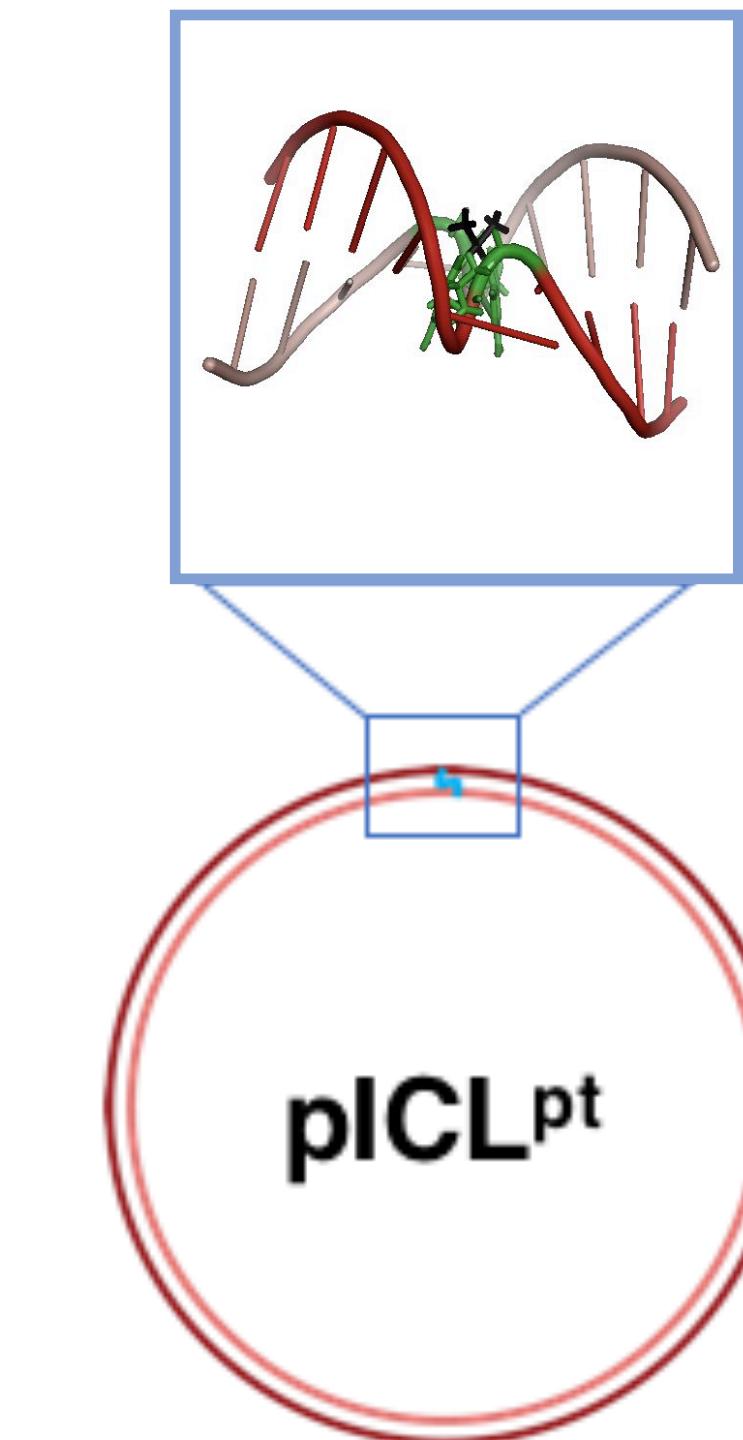
ICL REPAIR IN *XENOPUS* EGG EXTRACT

X. laeves eggs



egg extract

+



cisplatin ICL

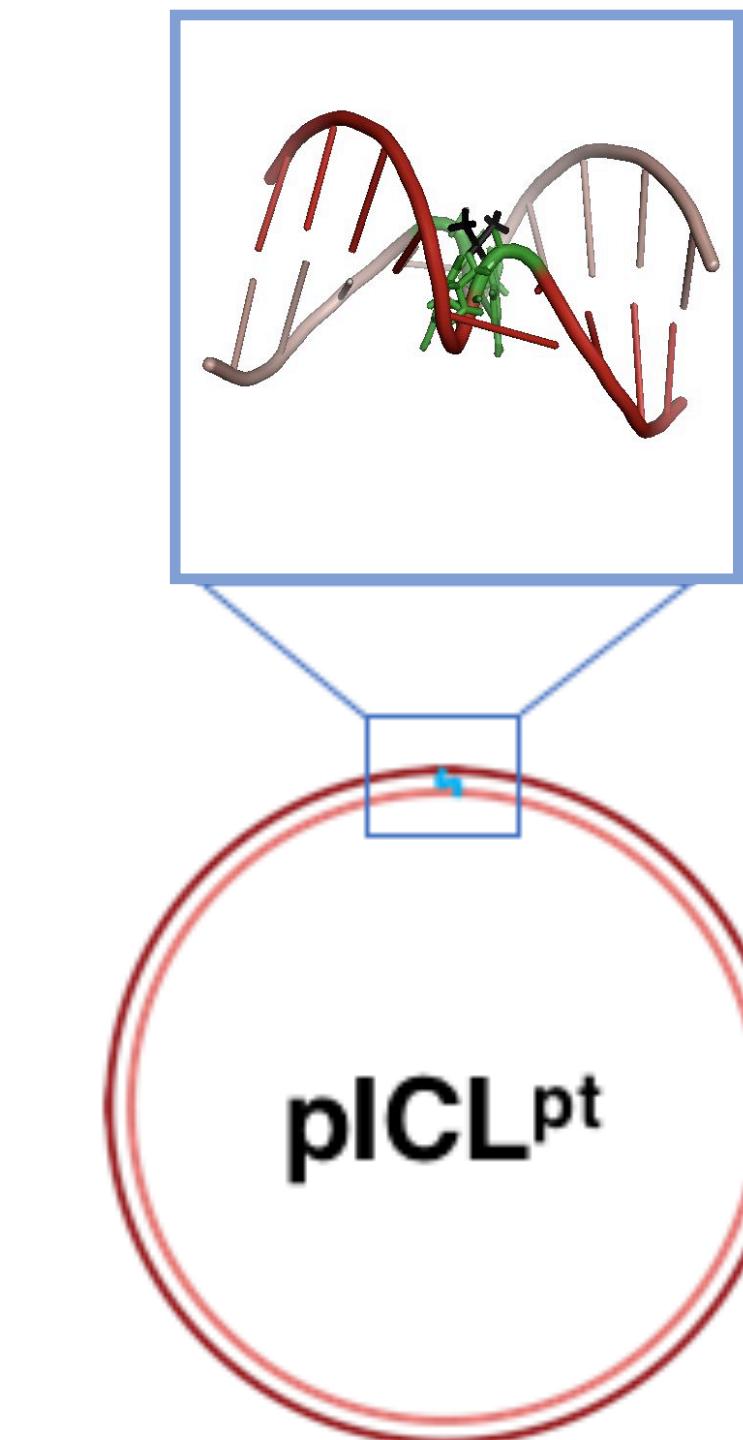
ICL REPAIR IN *XENOPUS* EGG EXTRACT

X. laeves eggs

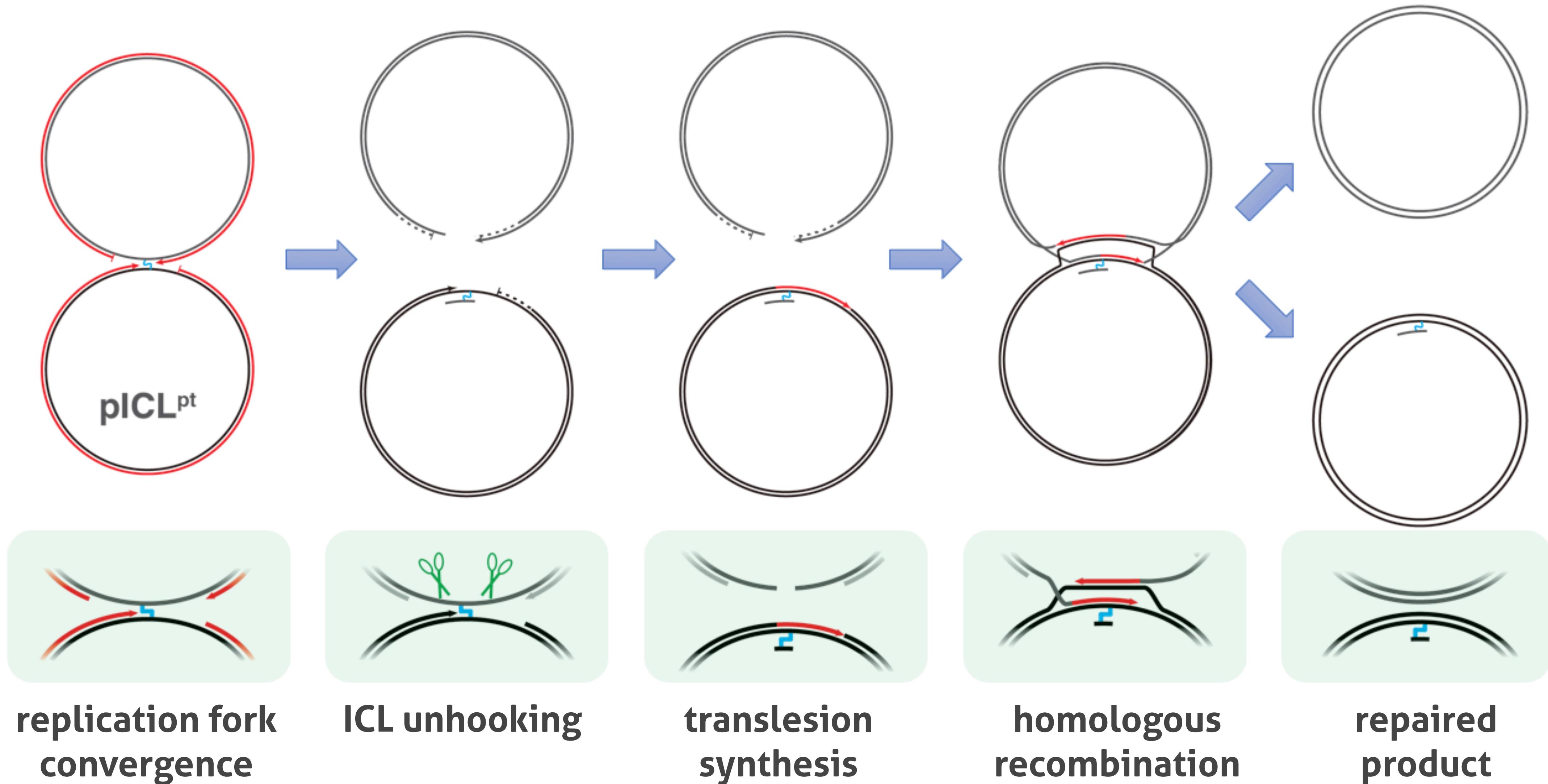


egg extract

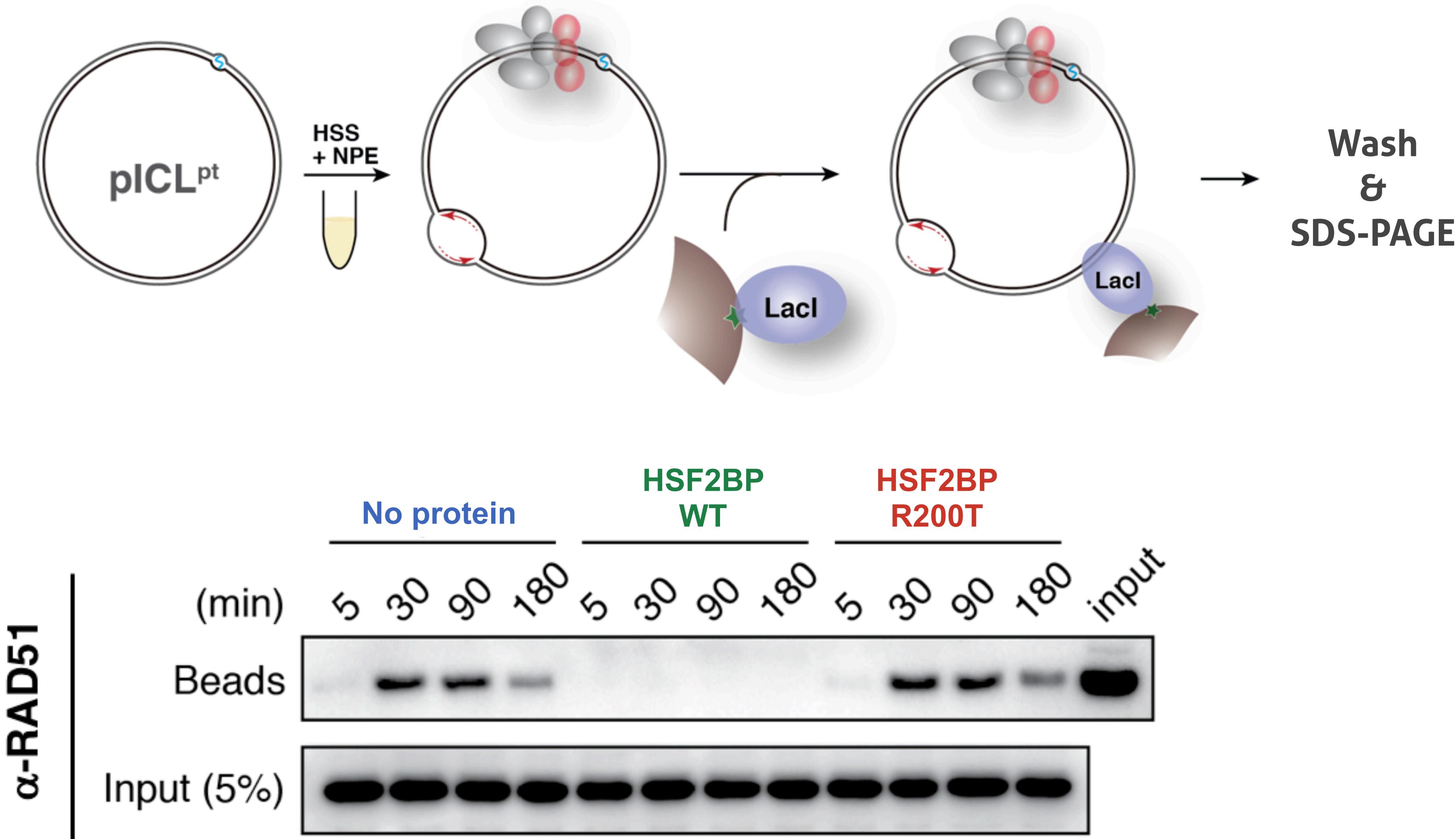
+



ICL REPAIR IN XENOPUS EGG EXTRACT

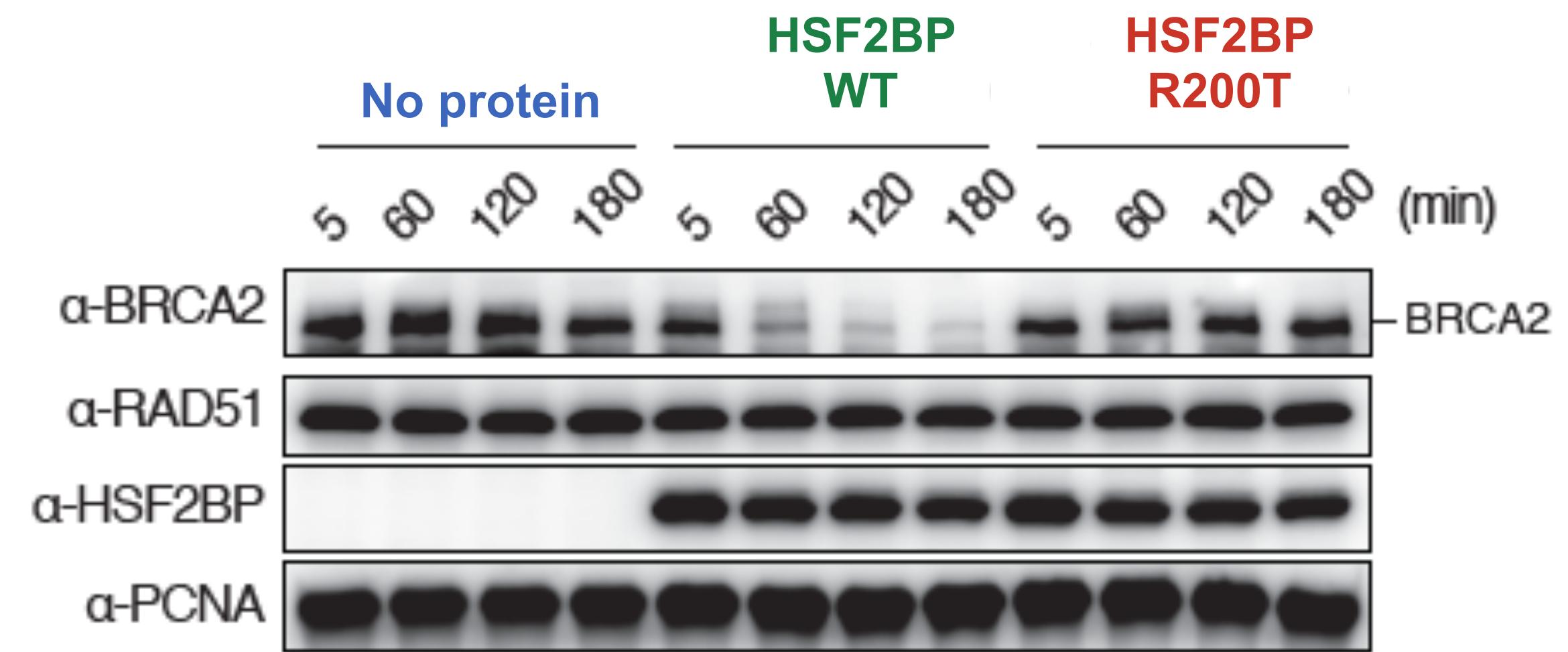


HSF2BP abrogated RAD51 loading



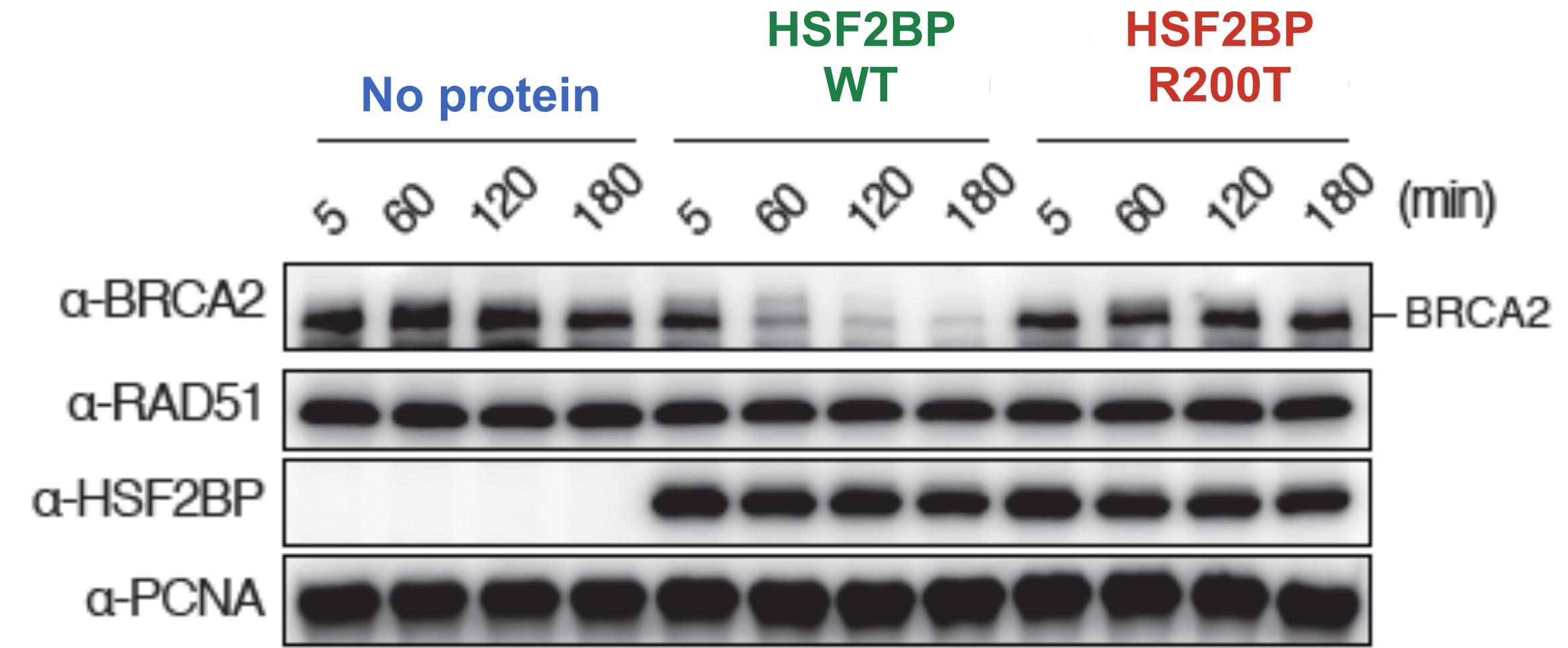
BRCA2 INACTIVATION BY HSF2BP

BRCA2 is progressively degraded during repair



BRCA2 INACTIVATION BY HSF2BP

BRCA2 is progressively degraded during repair



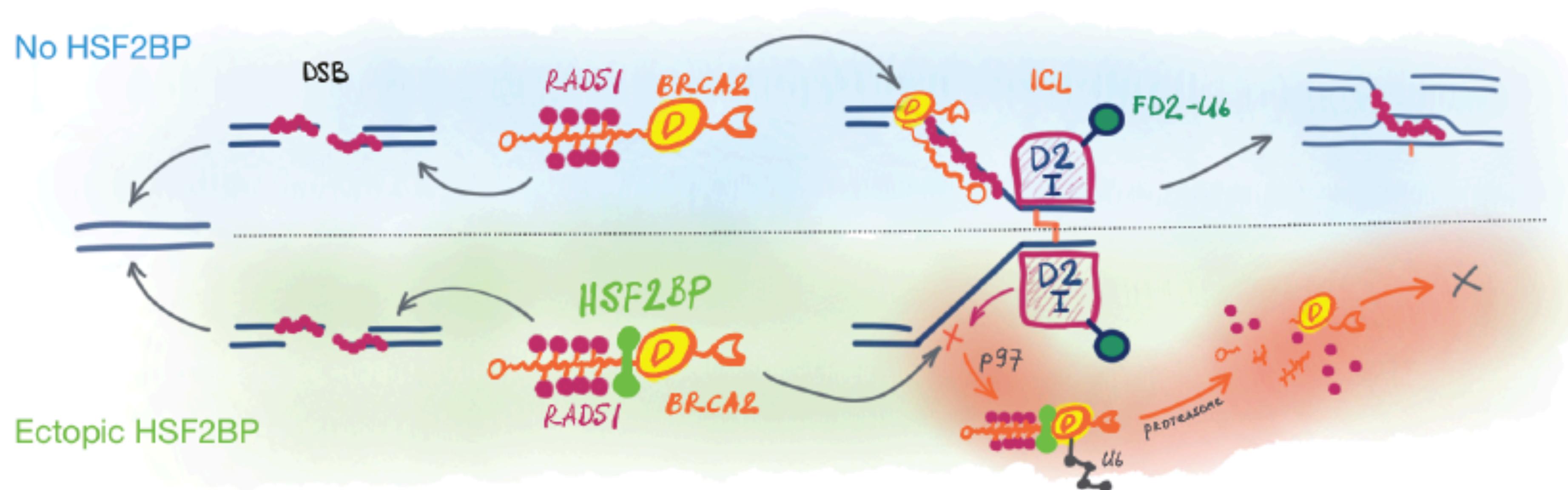
BRCA2 degradation is proteome mediated

BRCA2 degradation is ICL dependent

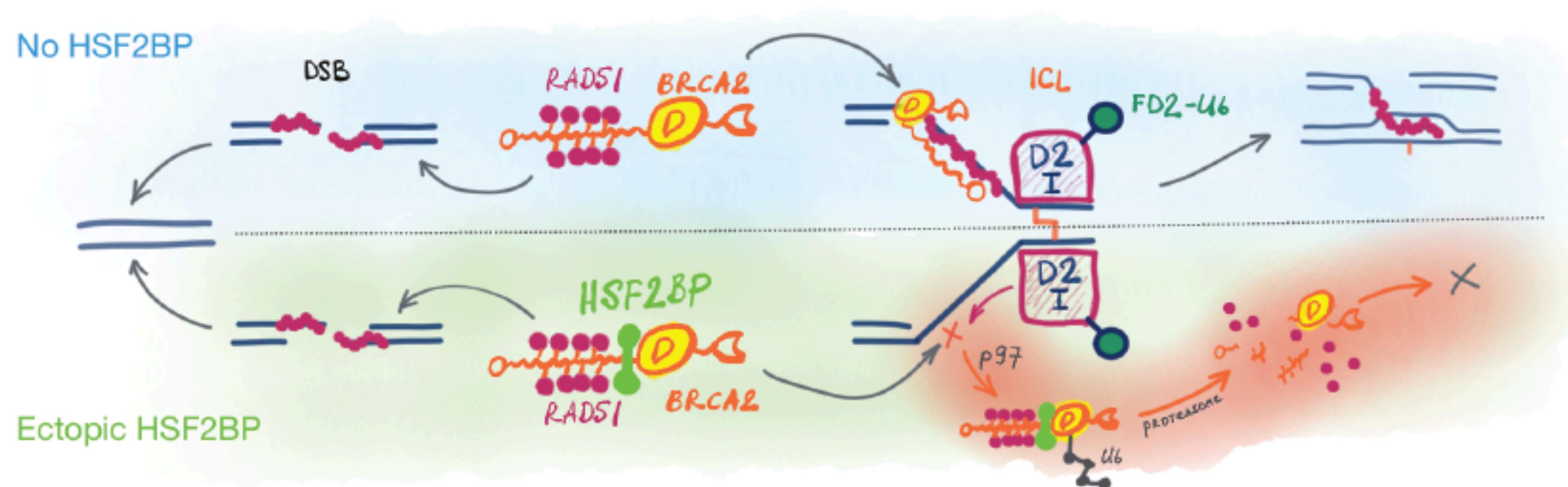
Proteosome inhibition still results in loss of BRCA2 from the ICL site

Inhibition of p97 segregase prevents HSF2BP-induced BRCA2 degradation

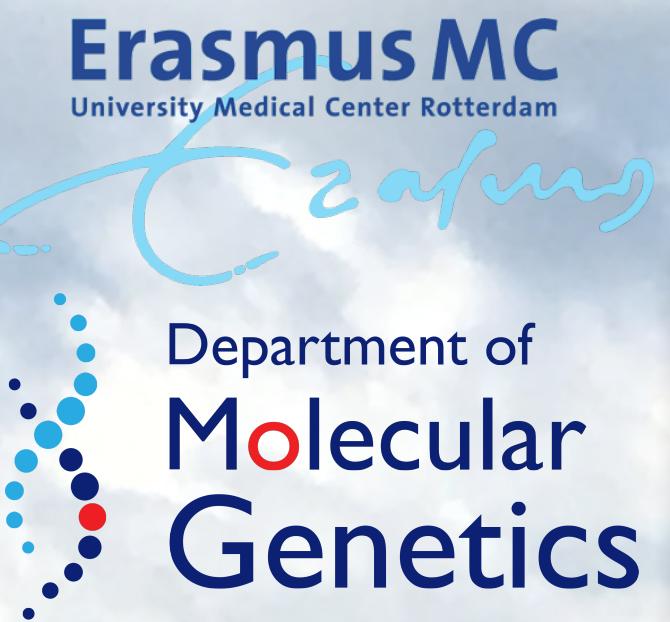
BRCA2 INACTIVATION BY HSF2BP



BRCA2 INACTIVATION BY HSF2BP



Detecting HSF2BP levels in tumor may suggest therapeutic options for patients

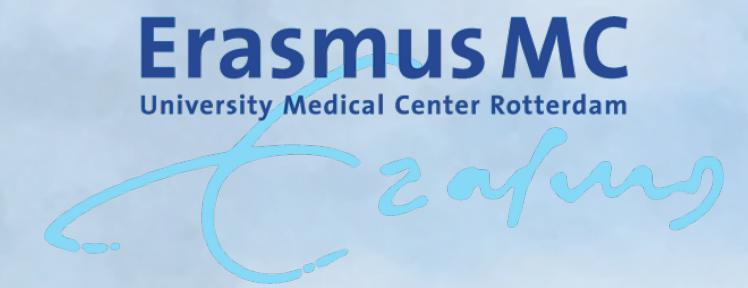


Alex Zelensky

Inger Brandsma	Nicole Verkaik
Sari van Rossum	Nicole van Vliet
Marcel Reuter	Hanny Odijk
Joyce Lebbink	Dik van Gent
Claire Wyman	Jeroen Essers



Koichi Sato
Puck Knipscheer



Dept. Developmental Biology
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