BARD1 functions in malignant transformation and in therapeutic application

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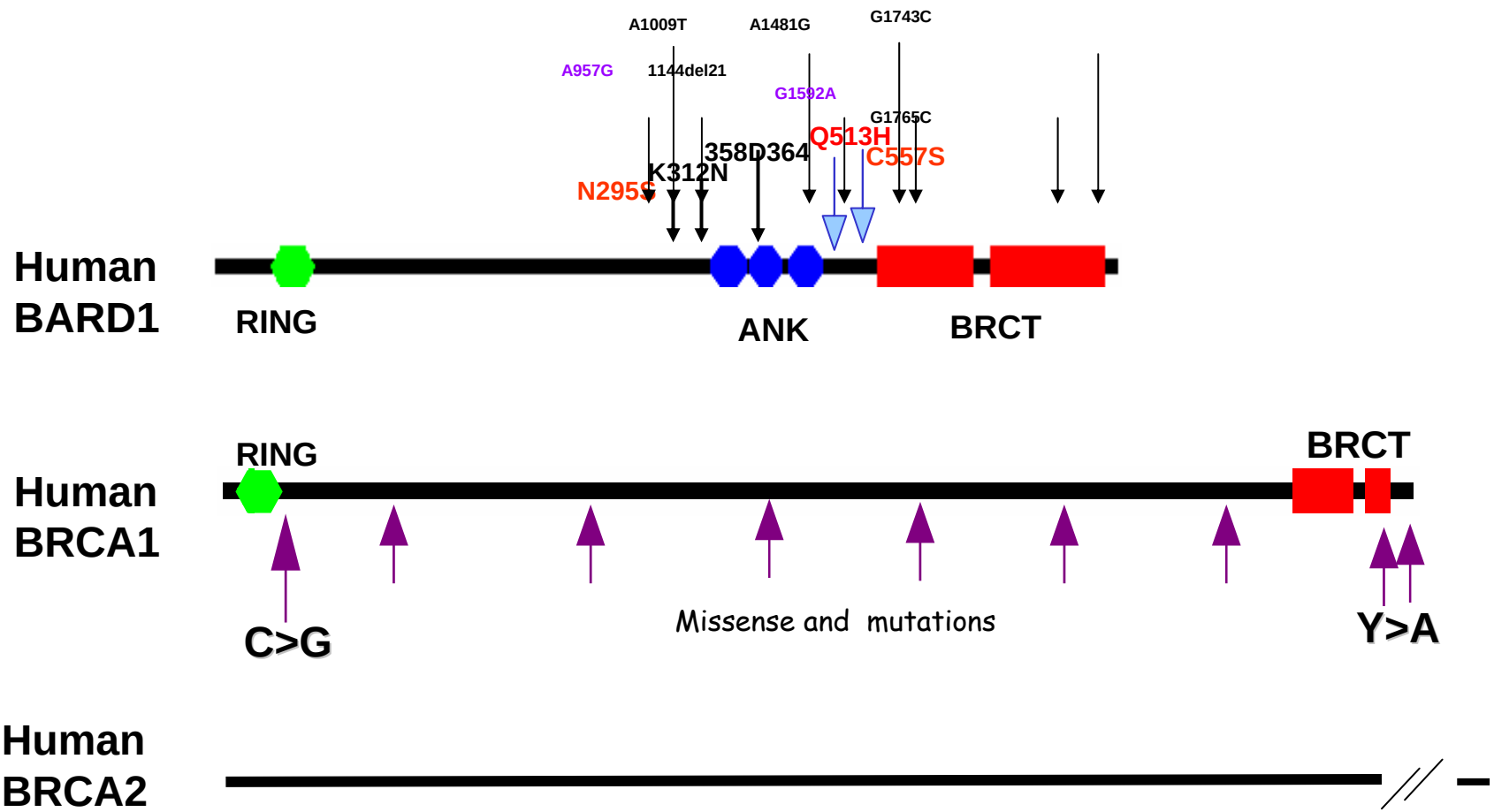
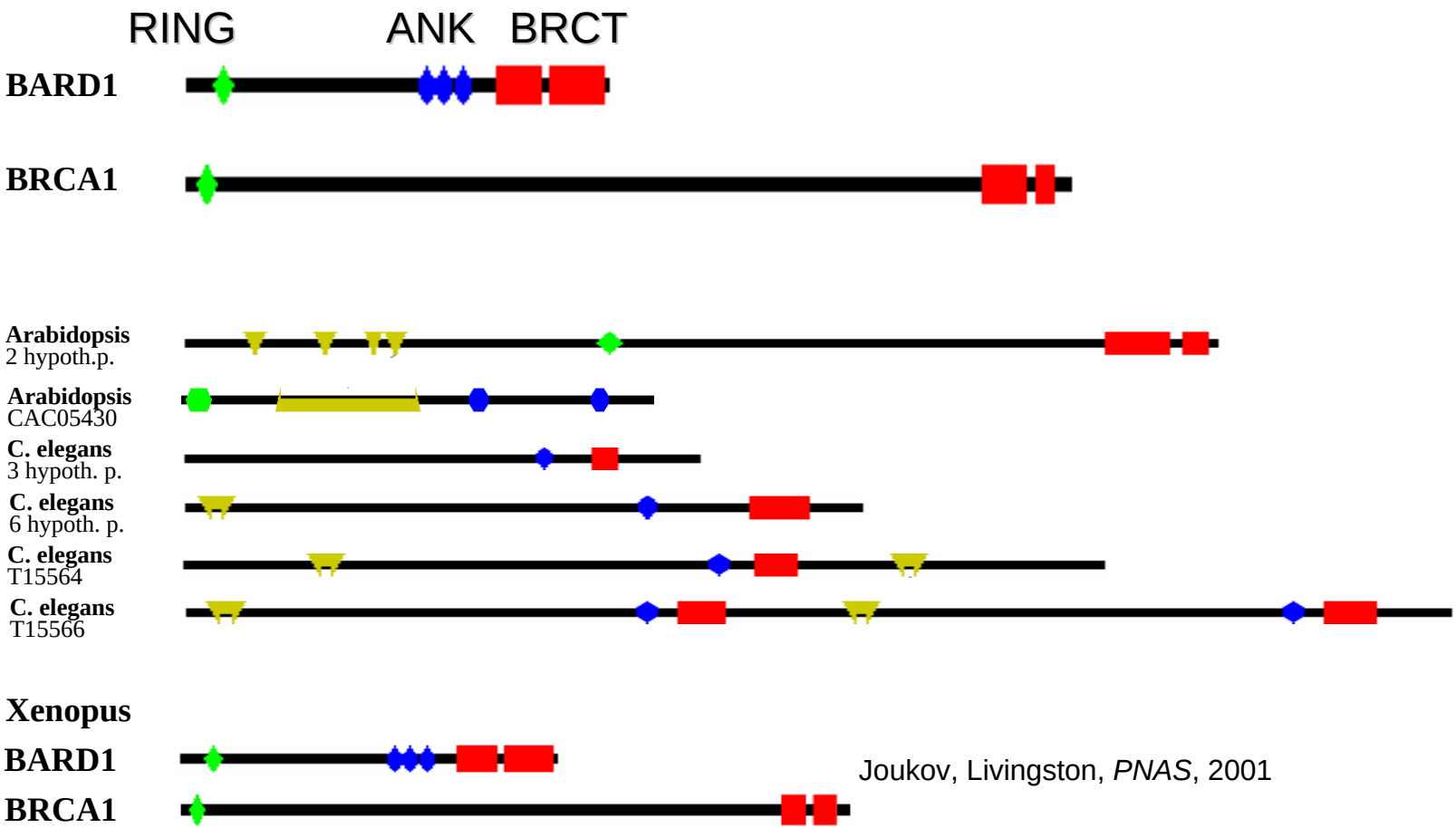
BARD1 and BRCA1 (and 2) mutations associated with breast and ovarian cancer



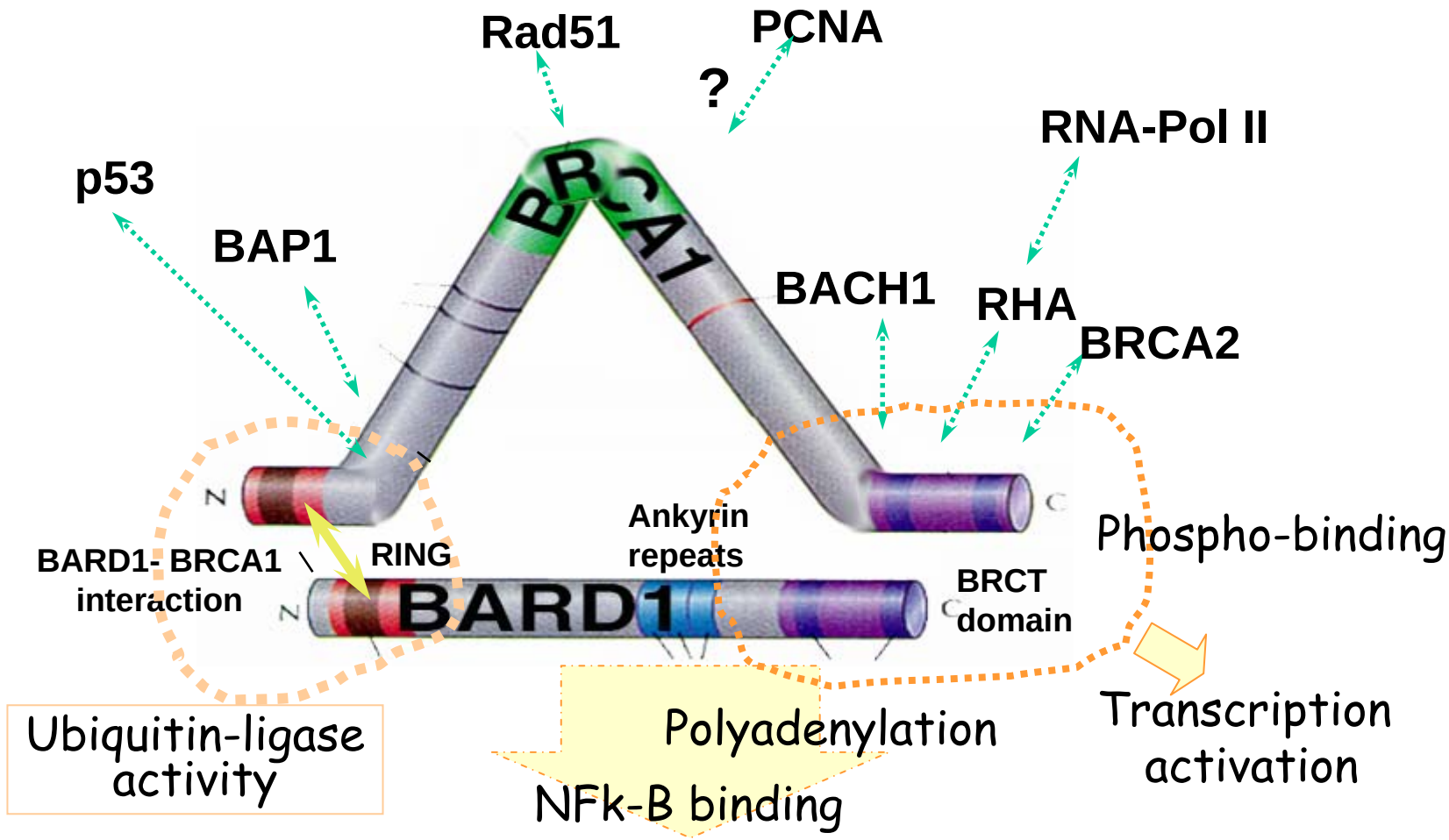
No one like BARD1



Joukov, Livingston, *PNAS*, 2001

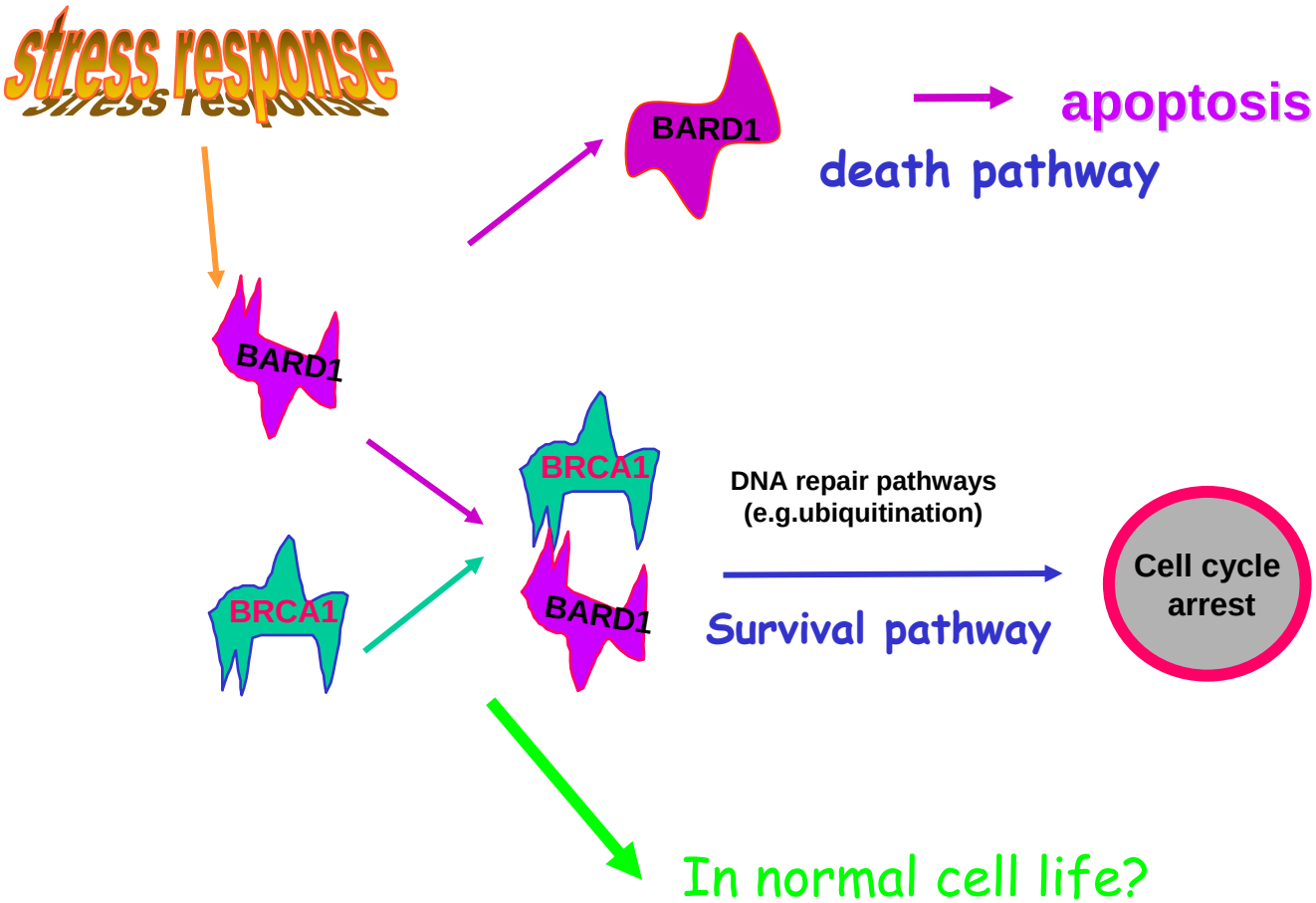
Irminger-Finger and Leung, IJBCB 2002

BARD1/BRCA1 heterodimer: a converging vehicle



Irminger-Finger et al., Bioch. 1999

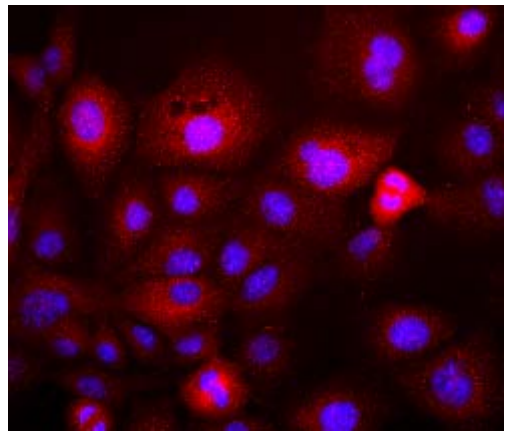
Presumed tumor suppressor pathways of BARD1

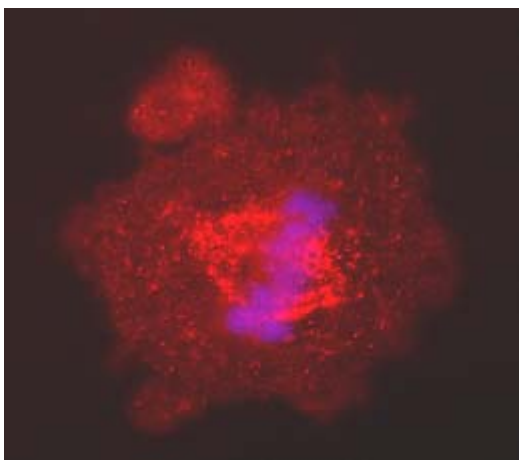


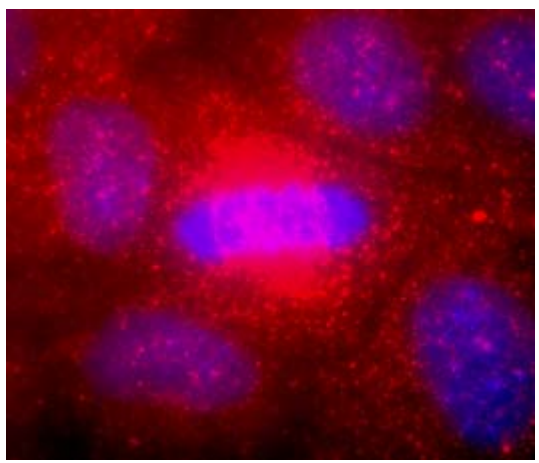
Role of BARD1, BRCA1, and BRCA2 in normal life

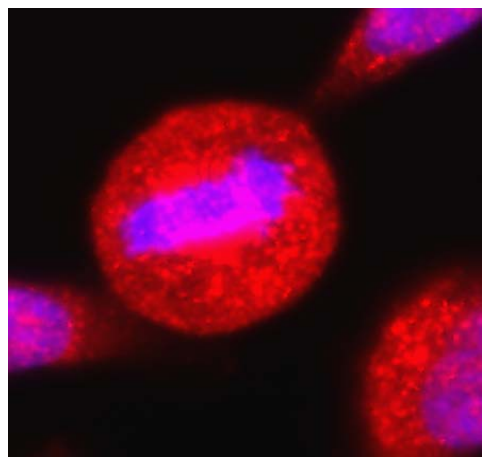
- BARD1, BRCA1 and BRCA2 ko are embroynic lethal
- BARD1 and BRCA1 ko cells are cellular lethal
- Depletion of BARD1, BRCA1 or BRCA2 cause genetic instability
  - Irminger-Finger et al., 1998
  - Jukov et al., 2002
  - McCarthy et al., 2003
- Genetic instability is the hallmark of tumors with mutations in BARD1, BRCA1 and BRCA2

BARD1 is upregulated in mitosis





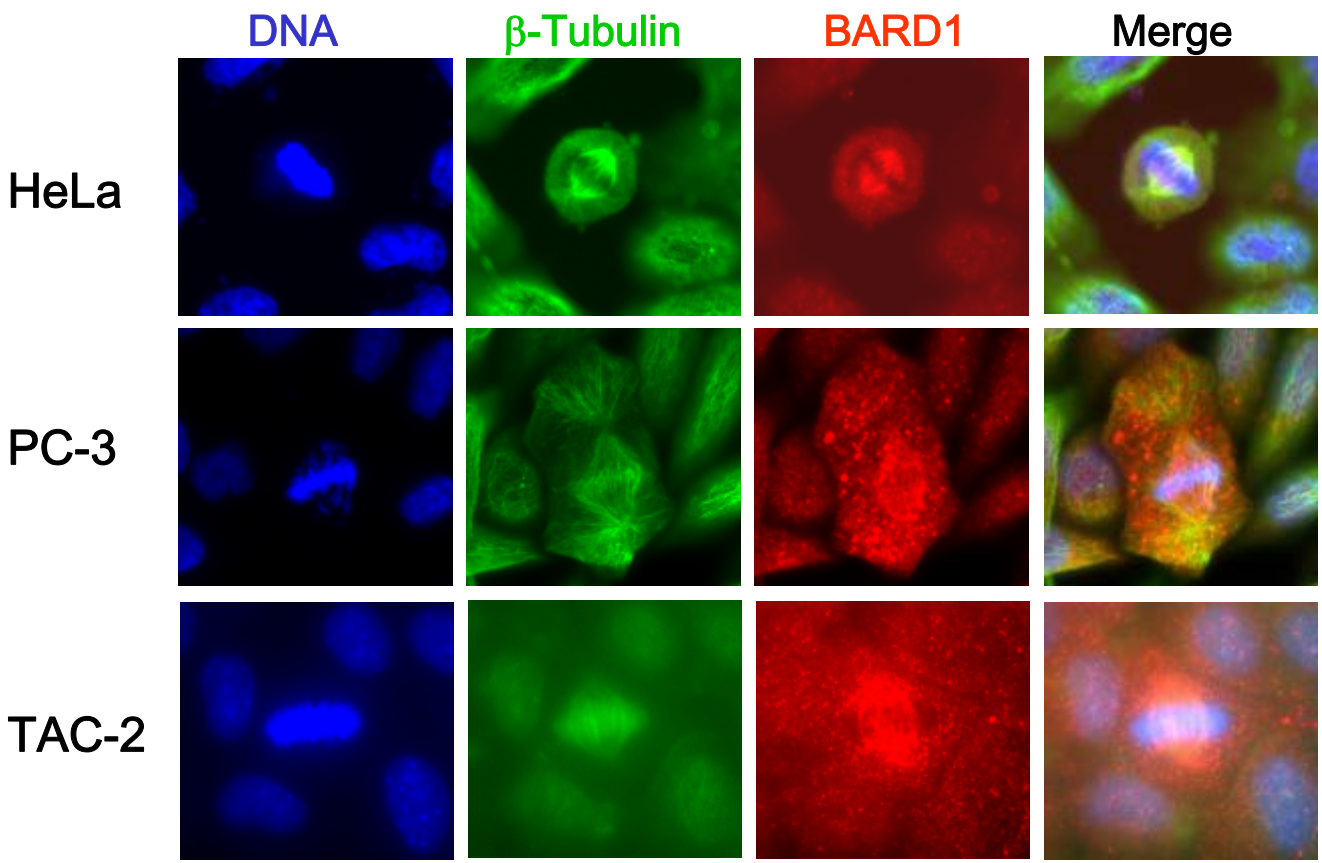


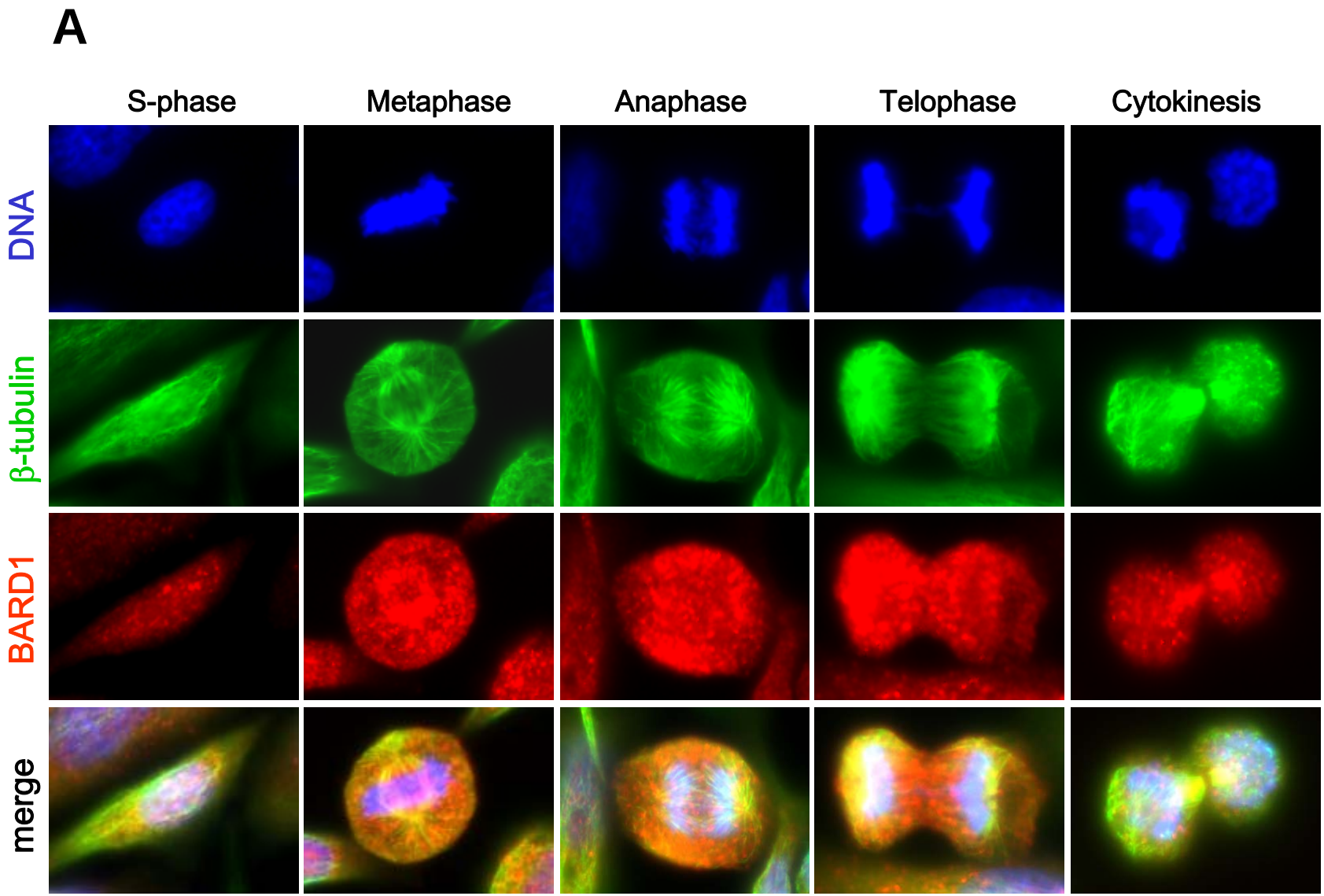


HeLa **TAC-2** **PC-3**

BARD1 (H-300) red Alexa555 DNA blue DAPI

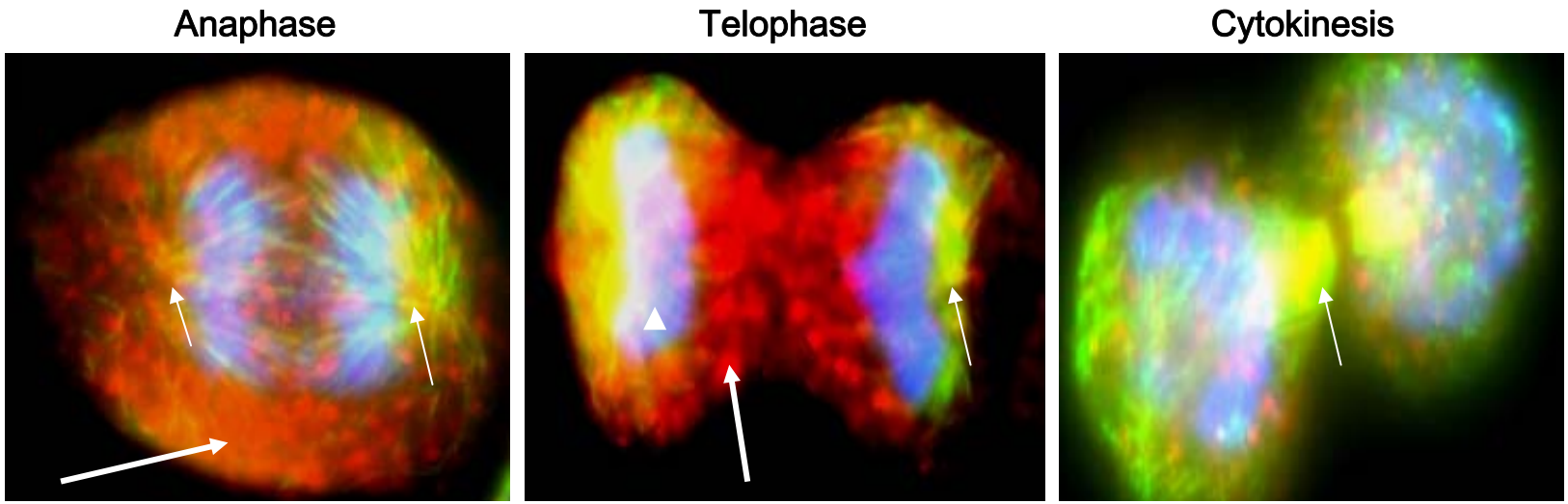
BARD1 localizes to spindle microtubles




A
S-phase
Metaphase
Anaphase
Telophase
Cytokinesis
DNA
β-tubulin
BARD1
merge

BARD1 a relay player in mitosis

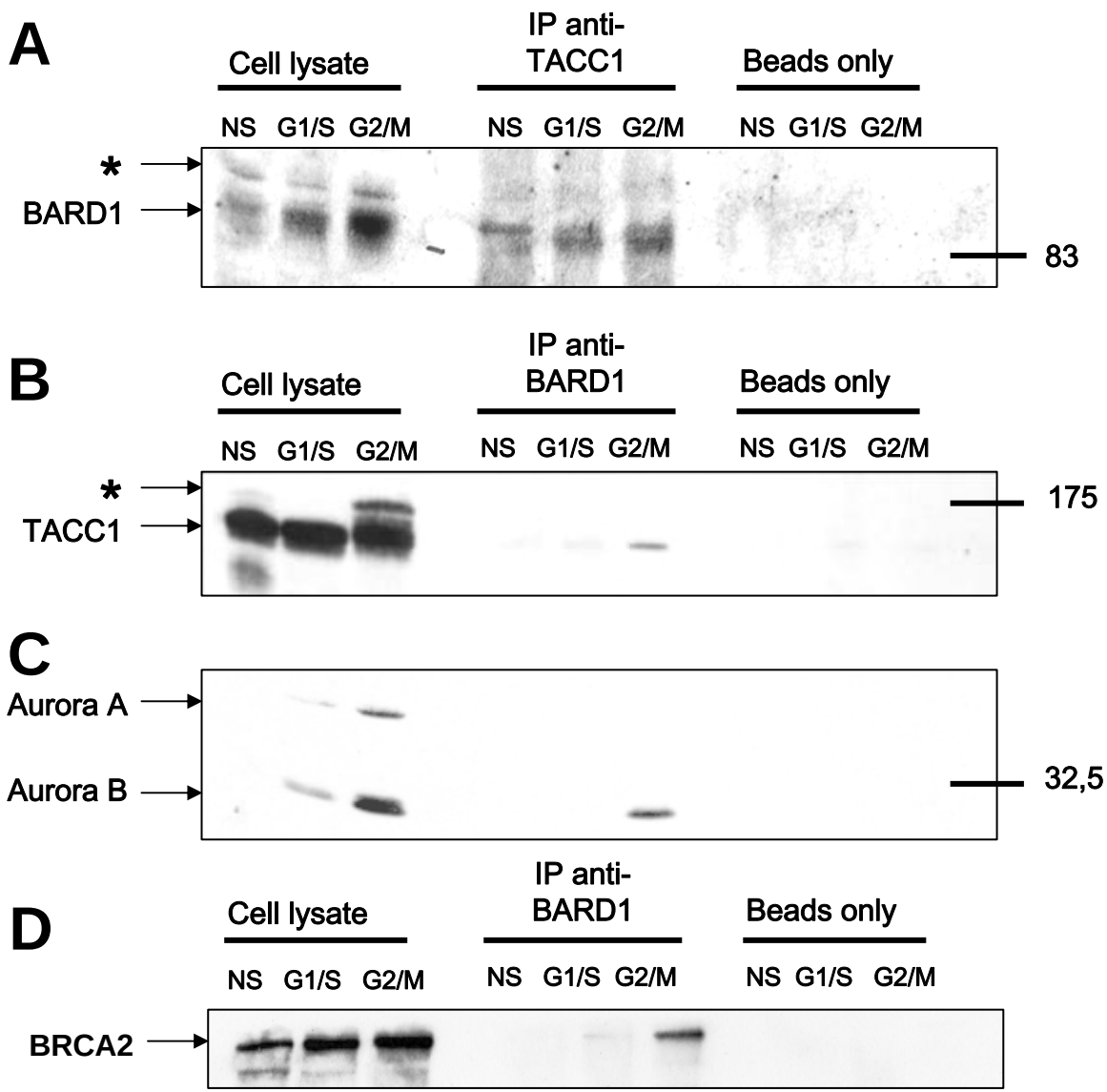
B



BARD1/BRCA1
Ubiquitin ligase
targeting
γtubulin

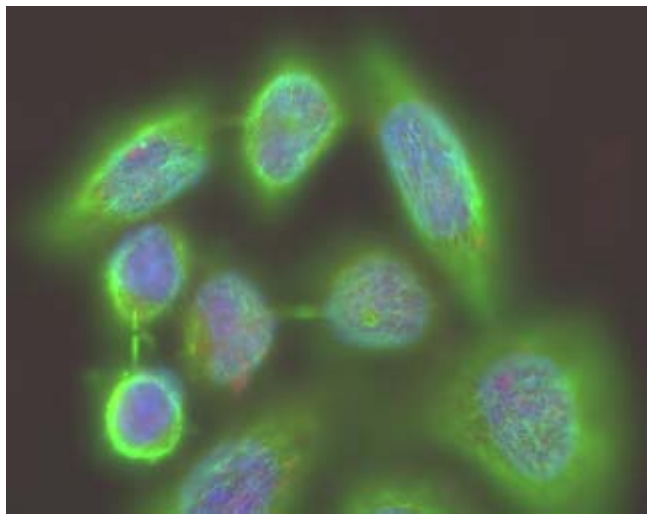
BARD1/BRCA2
control of
cytokinesis

Who are the partners?

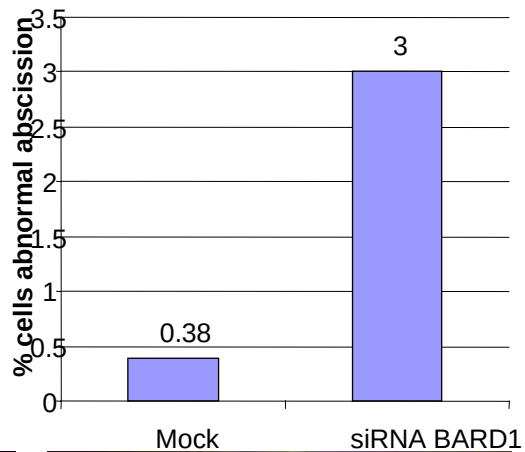


siRNA depletion of BARD1

A



B



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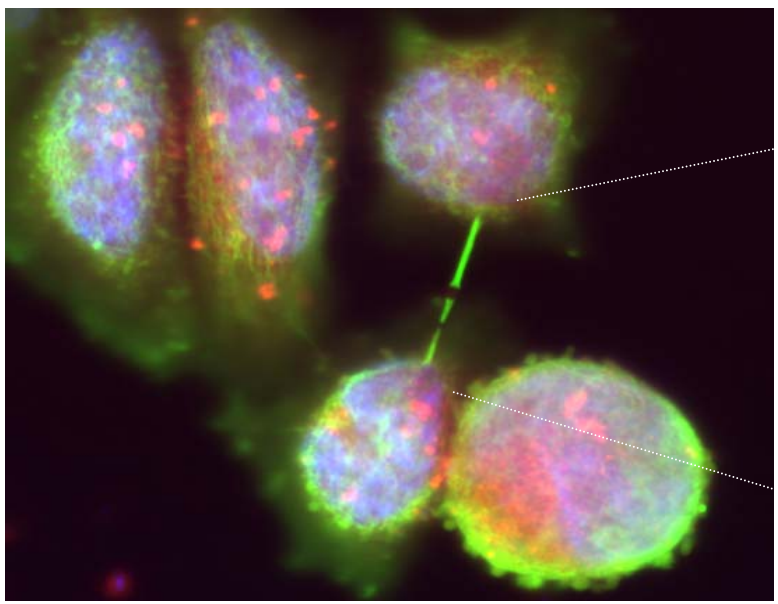
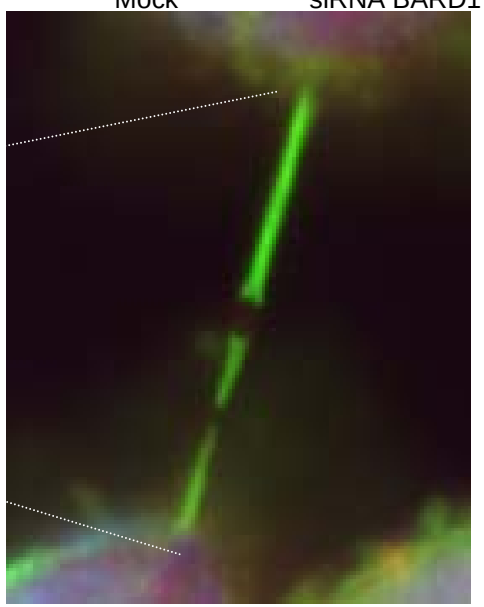
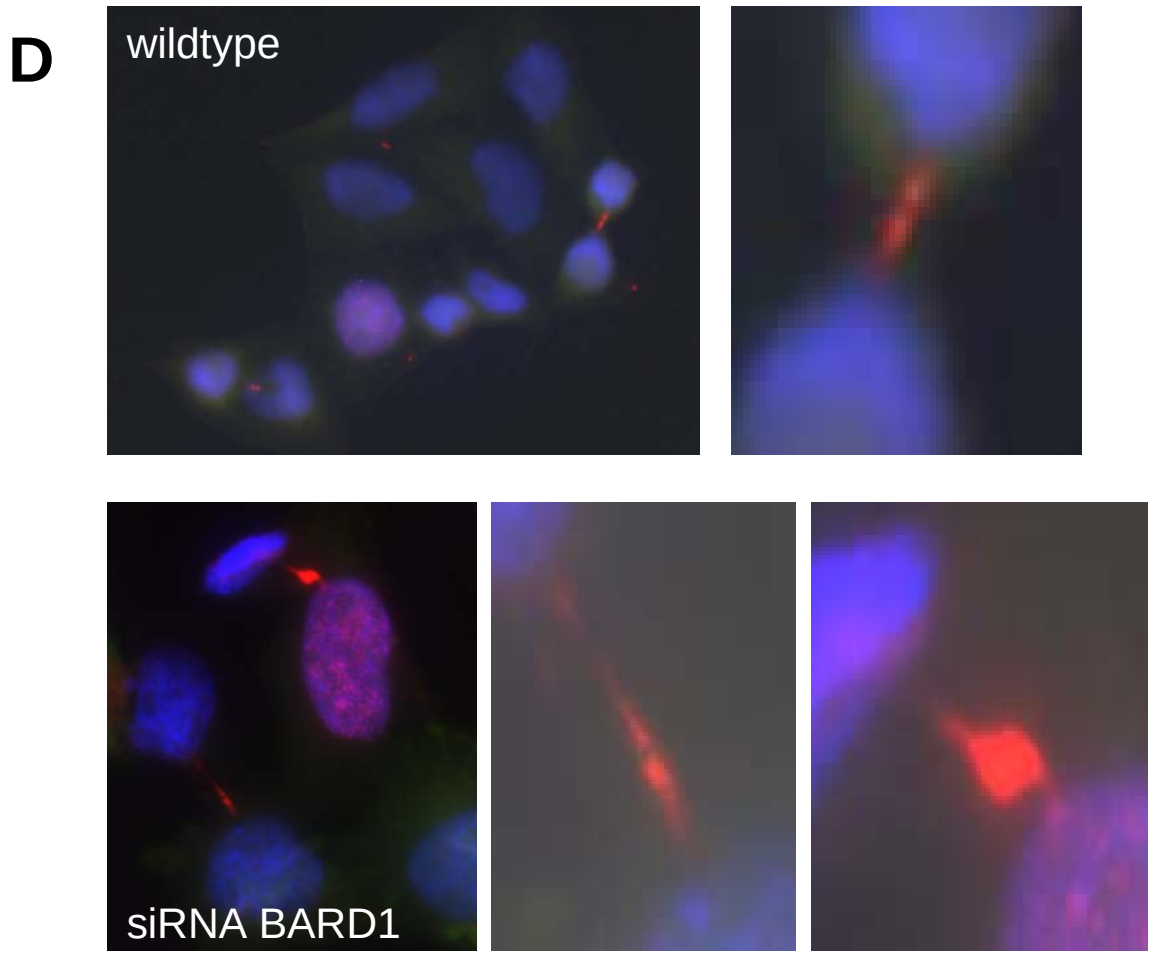
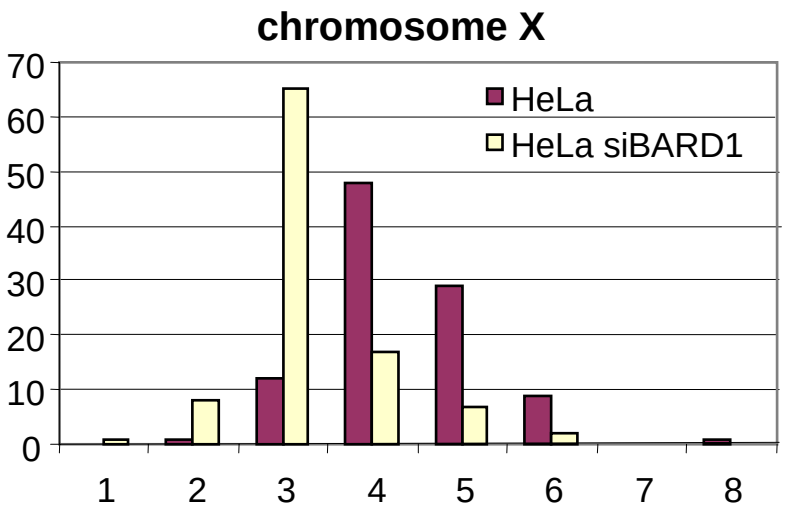


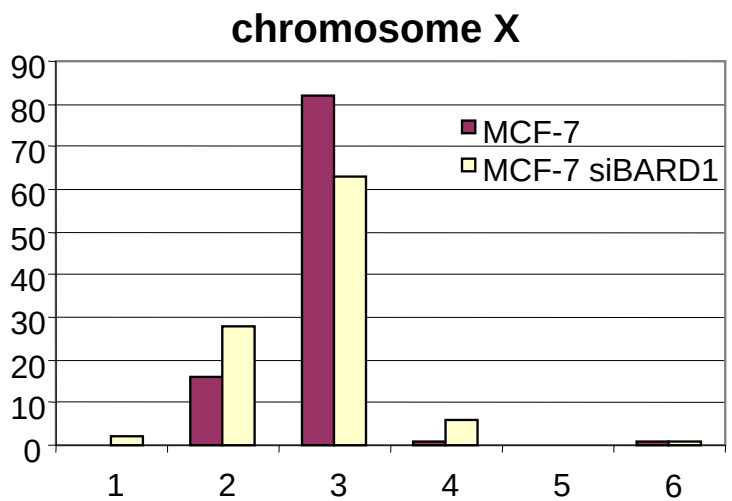


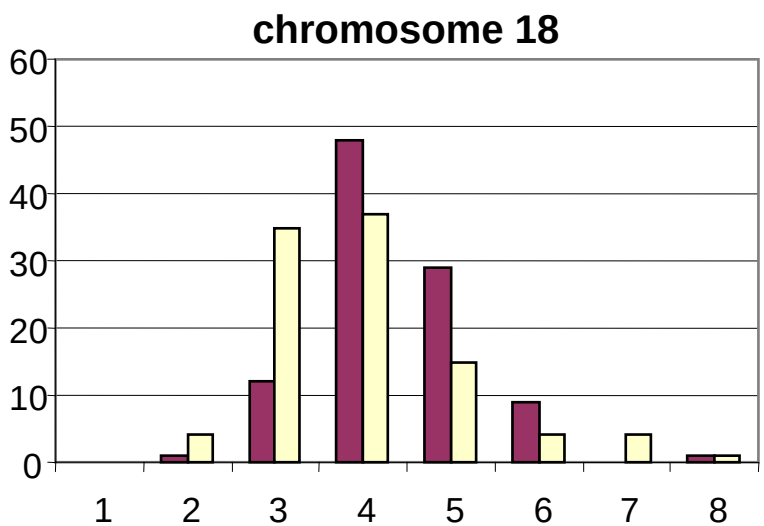
Aurora B remains at the midbody

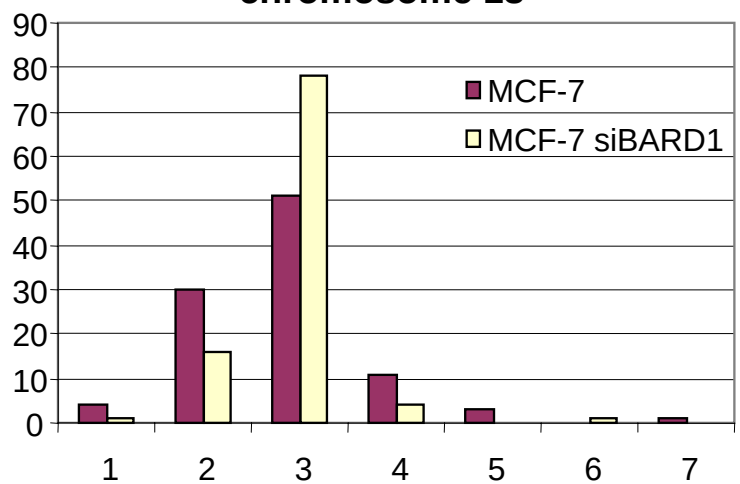


Numerical chromosomal instability in BARD1 depleted cells





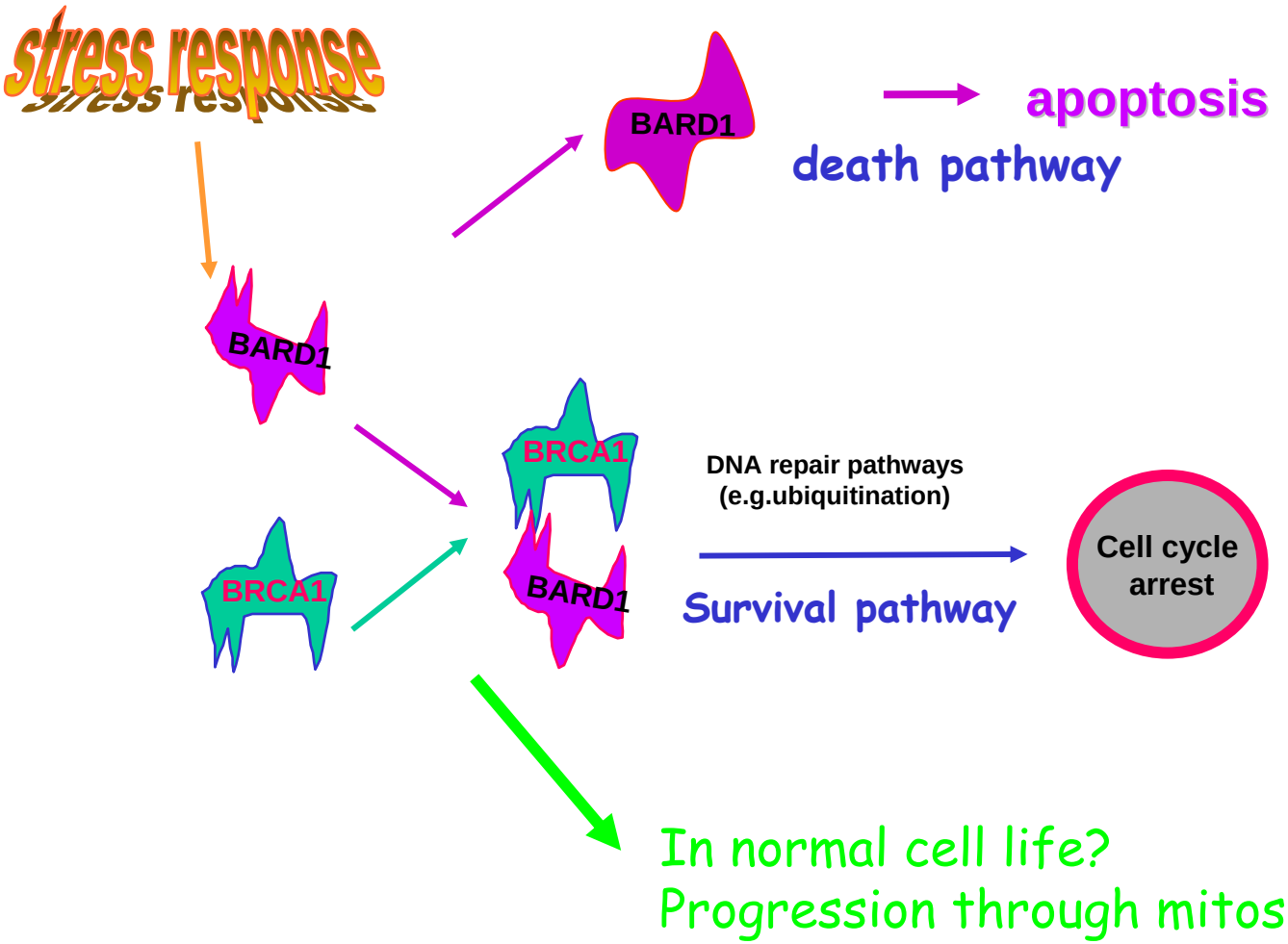




Role of BARD1 in normal life

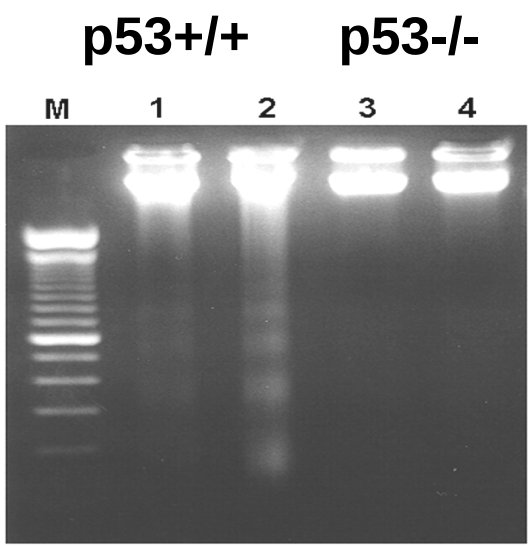
- BARD1 increased during mitosis
- Localizes to mitotic spindle via TACC1
- Controls centromer duplications via BARD1/BRCA1 ubiquitin ligase activity
- Controls cytokinesis via interaction with Aurora B and BRCA2
- Failure of doing all these jobs leads to genetic instability

Presumed tumor suppressor pathways of BARD1

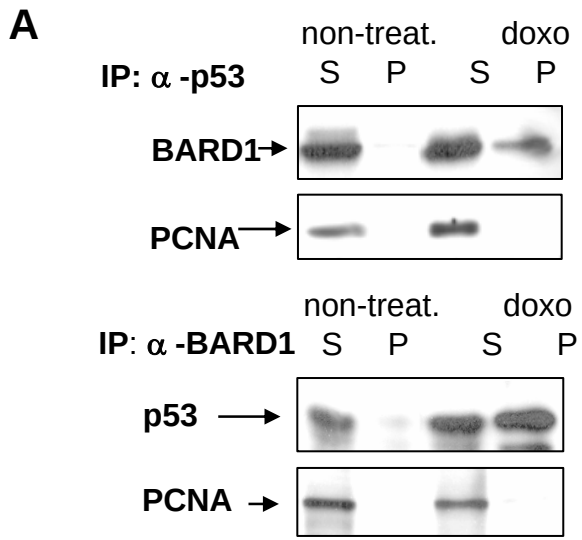


BARD1 as tumor suppressor: induction of p53-mediated apoptosis

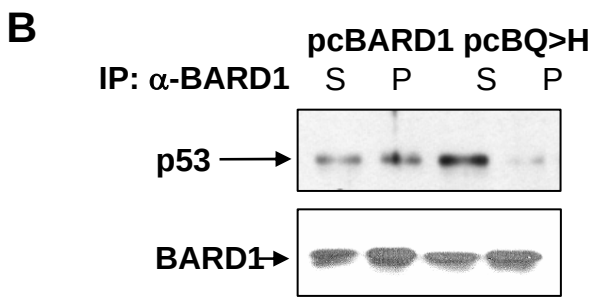
transfection of BARD1



BARD1 binds to p53

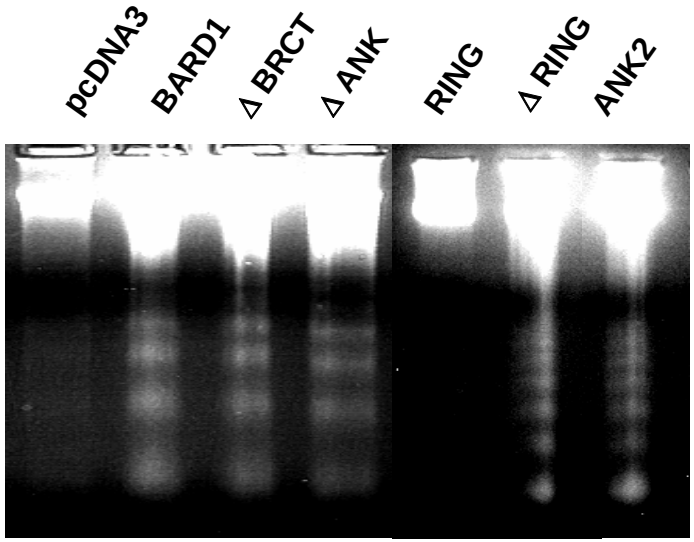


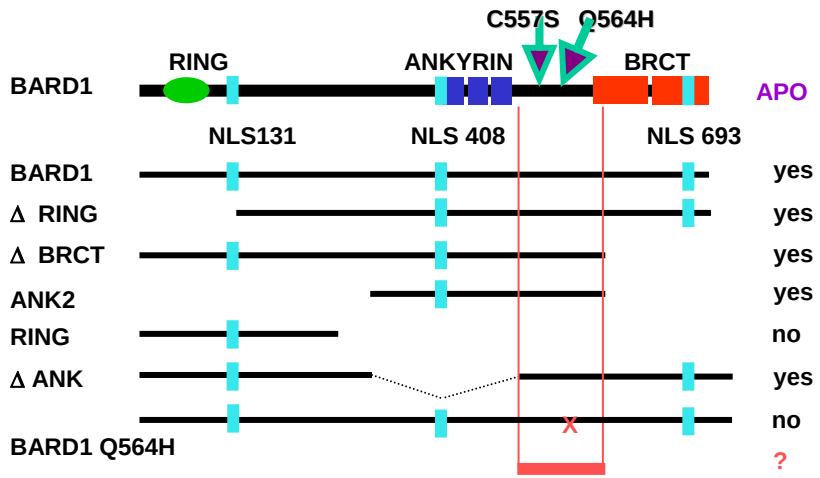
BARD1 stabilizes p53



Irminger-Finger et al., Mol Cell 2001

Mapping of the apoptotic region



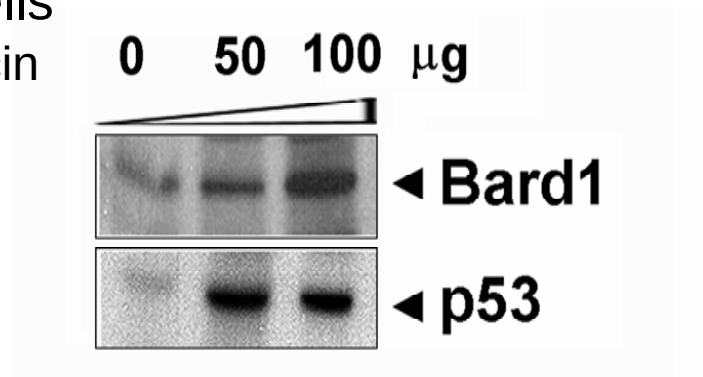


Deletion mutants lacking the BRCA1 interaction domain are efficient in apoptosis induction and have increased protein stability.
BRCA1 competes apoptosis by BARD1 in co-transfection assays.

BARD1 is upregulated in response to cellular stress

On the protein level

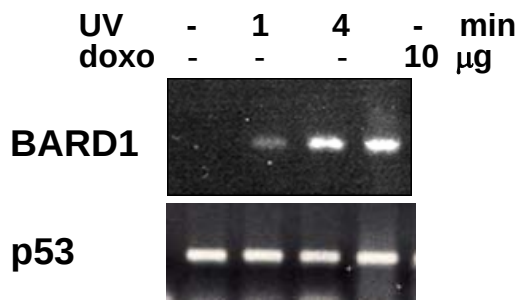
TAC-2 cells
Doxorubicin



Western

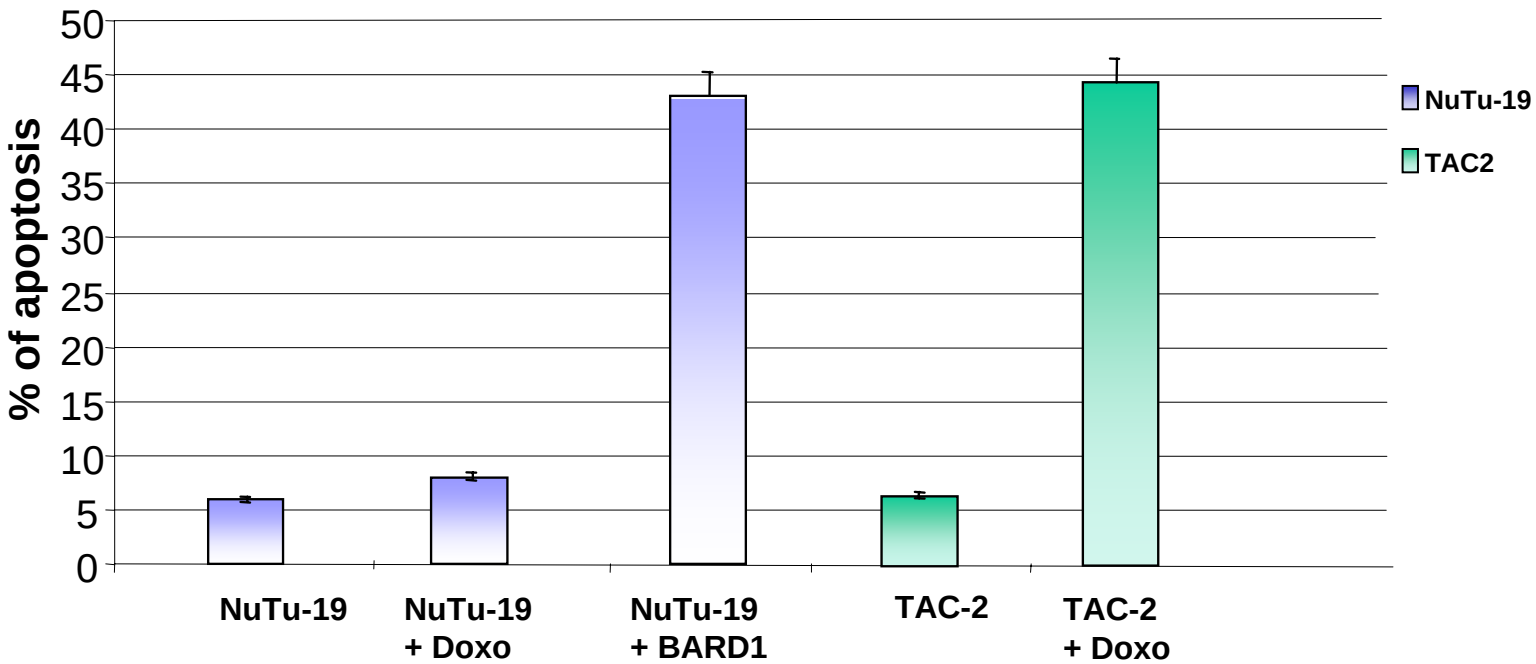
On the mRNA level

ES cells



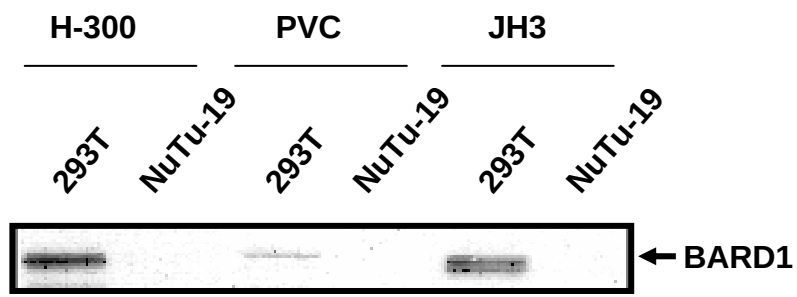
RT-PCR

Exogenous expression of BARD1 is sufficiant for apoptosis induction in apoptosis-resistant NuTu-19 cells



Apoptosis resistant NuTu-19 cells lack functional BARD1

A



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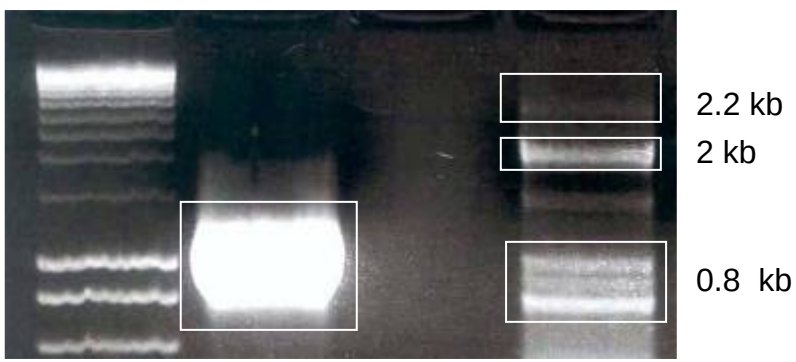

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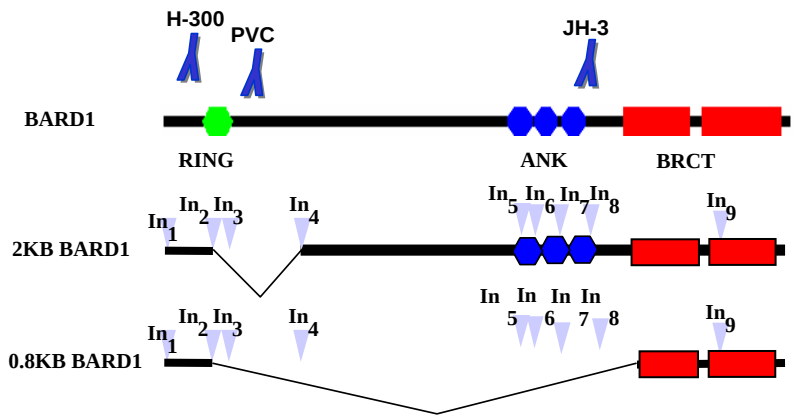
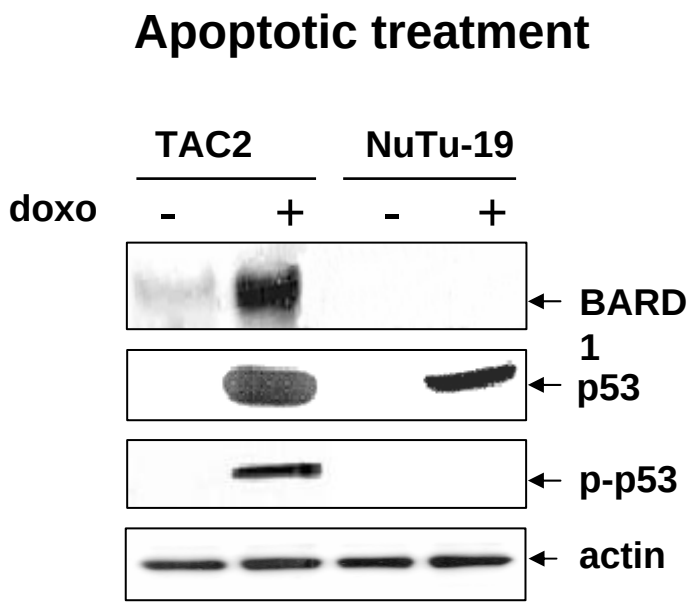
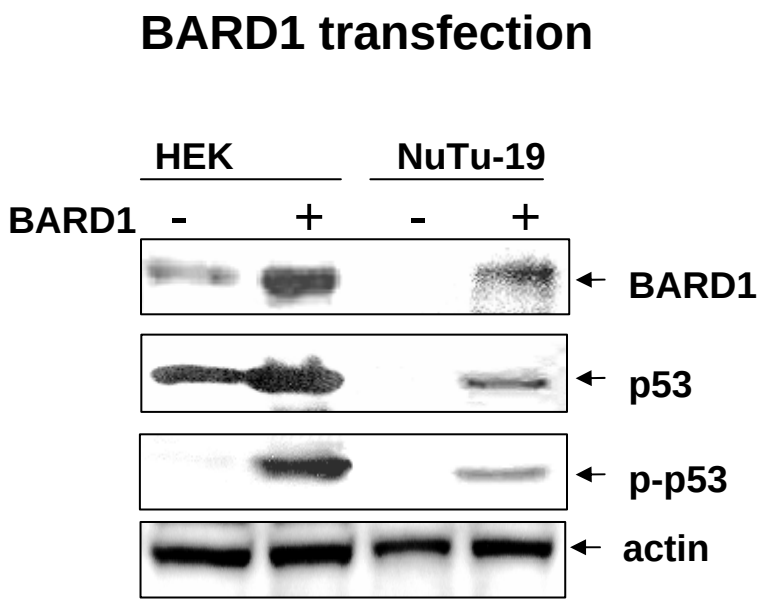


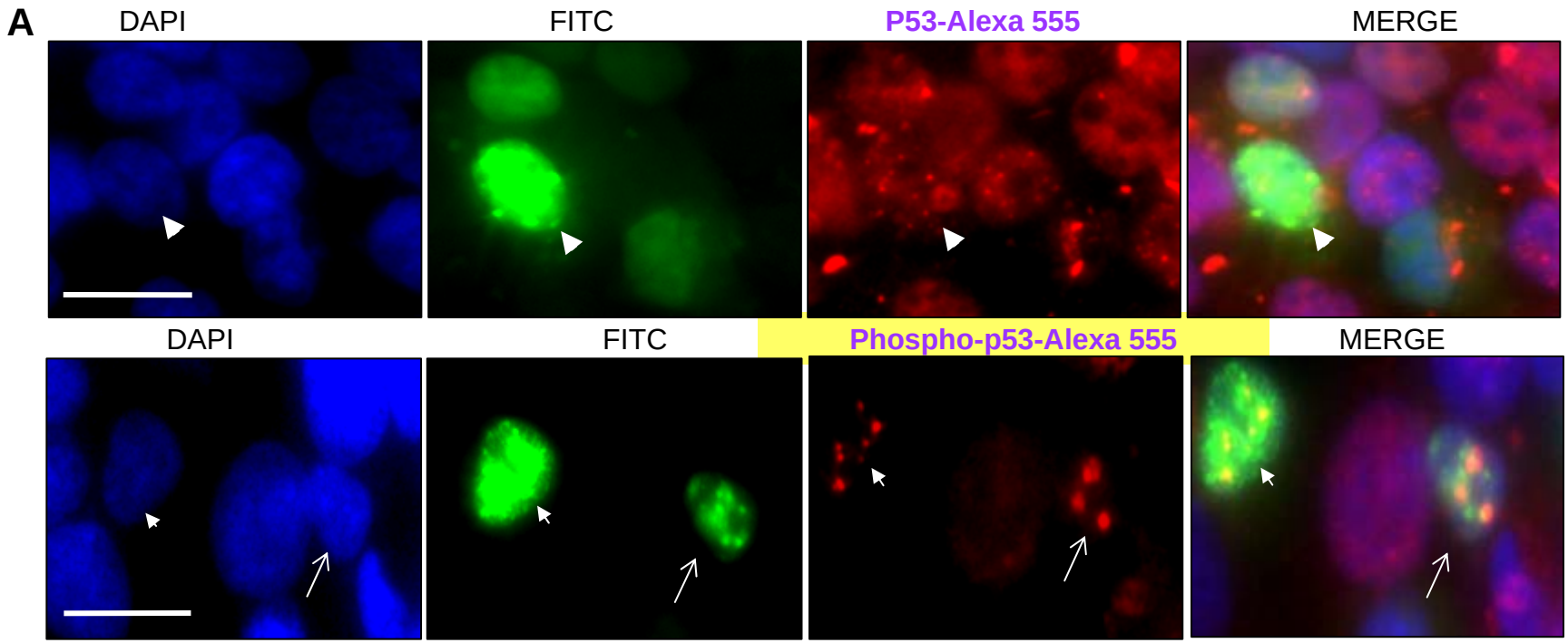
Feki et al., & Irminger-Finger, Oncogene 2005

BARD1 required for p53 phosphorylation

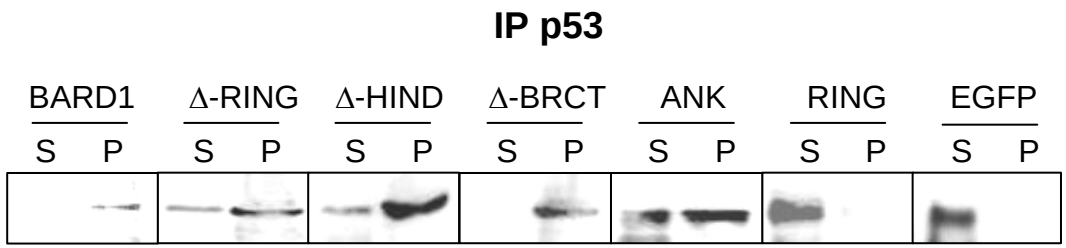


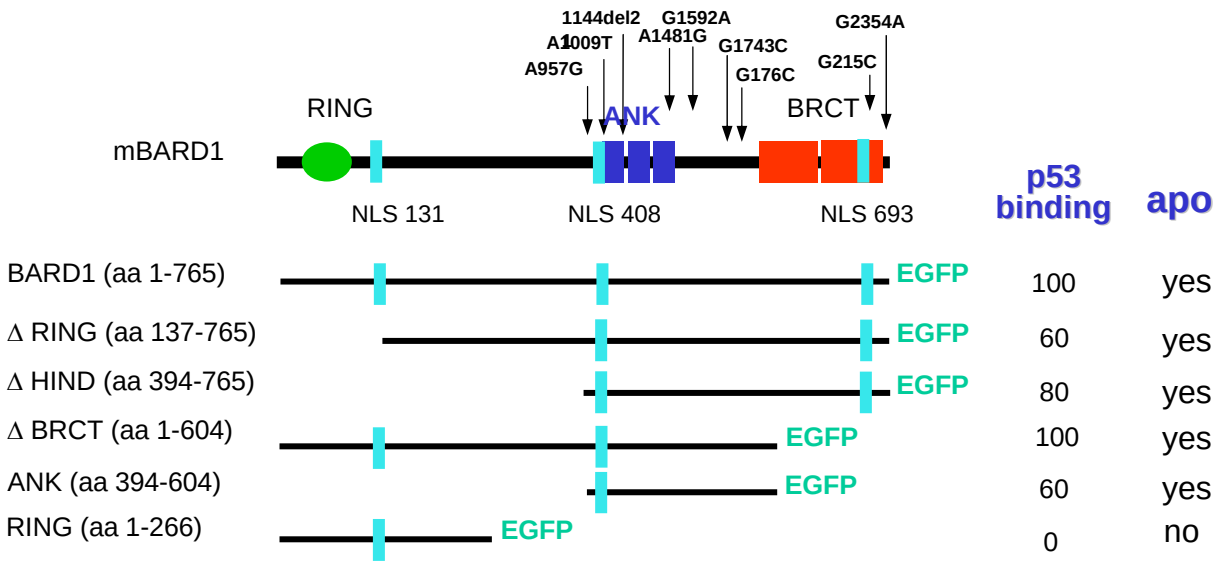


BARD1 colocalizes with phospho-p53



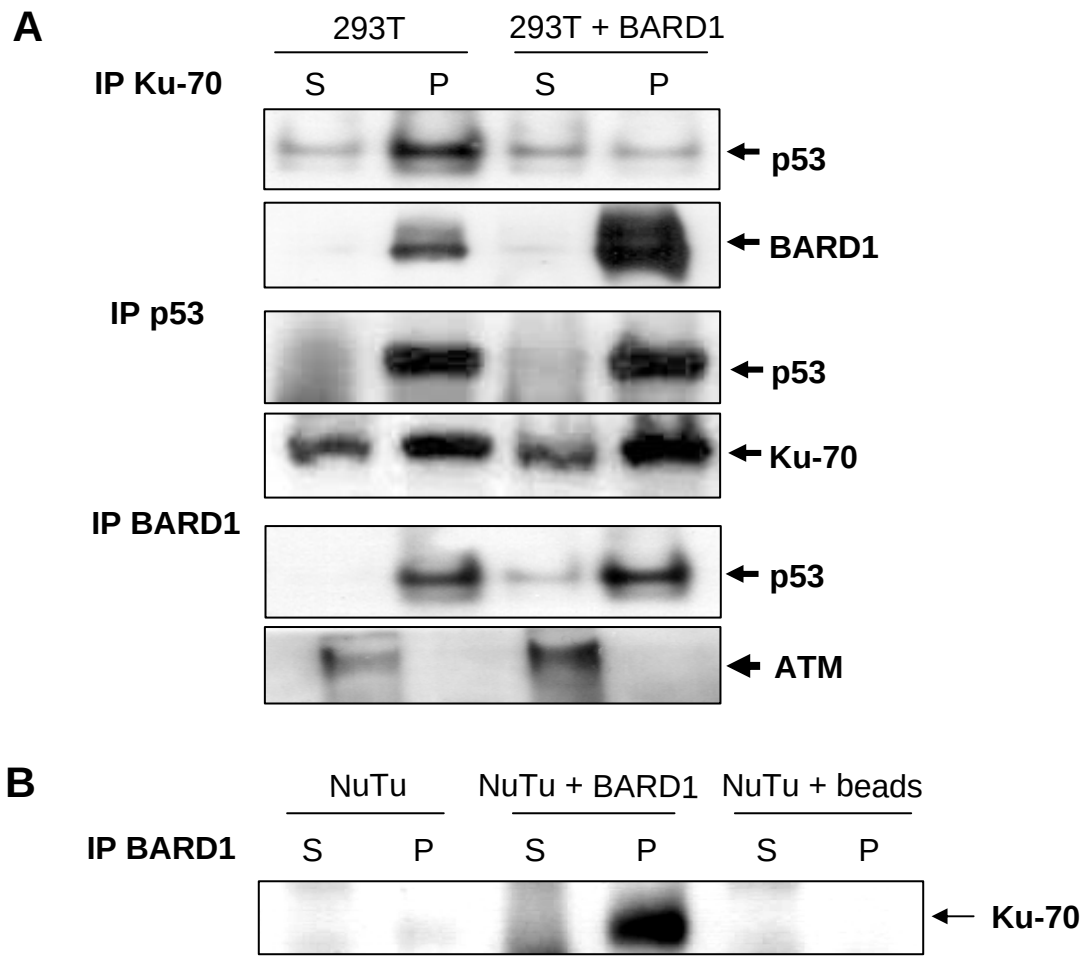
Mapping the p53 binding region



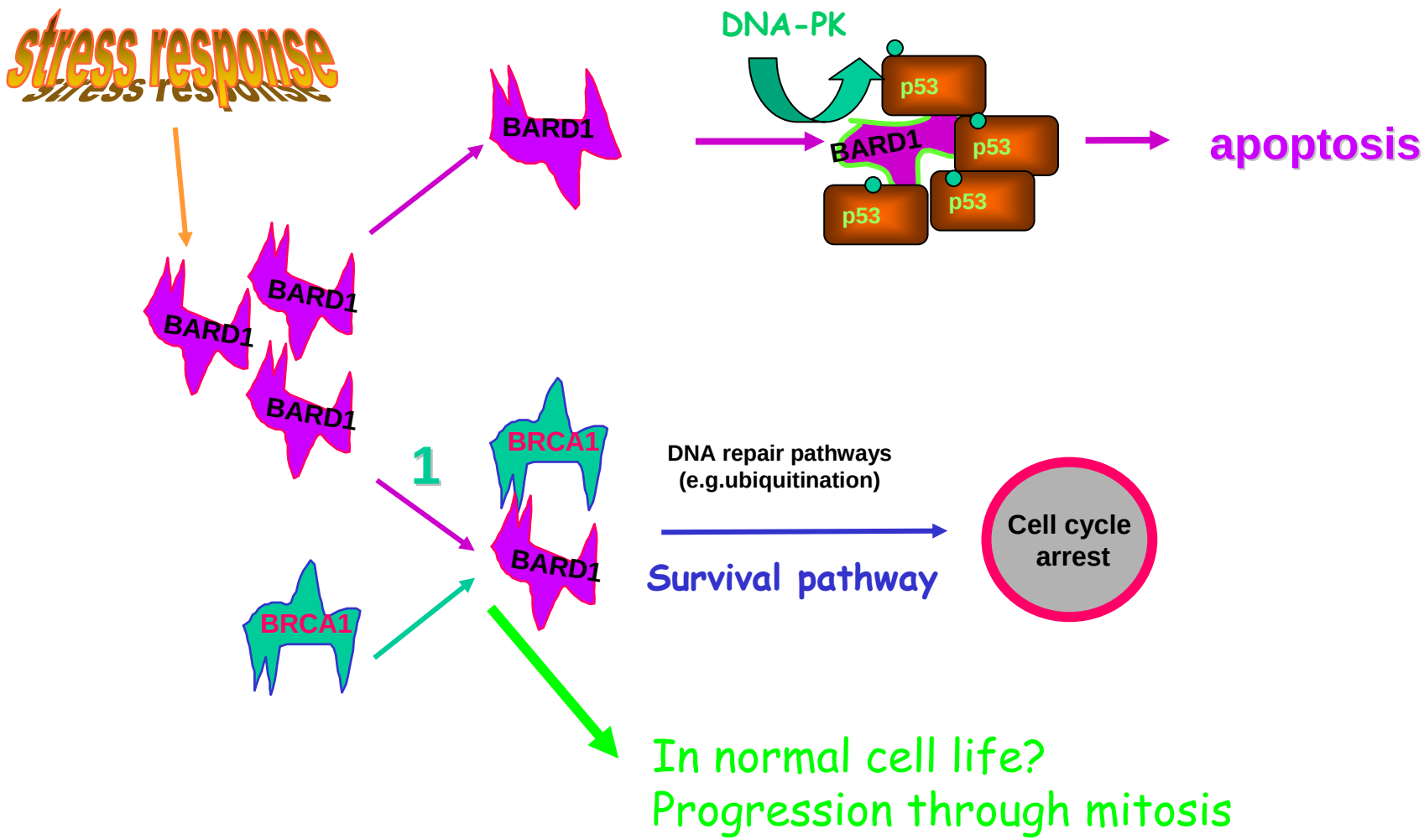


Feki et al., Oncogene 2005

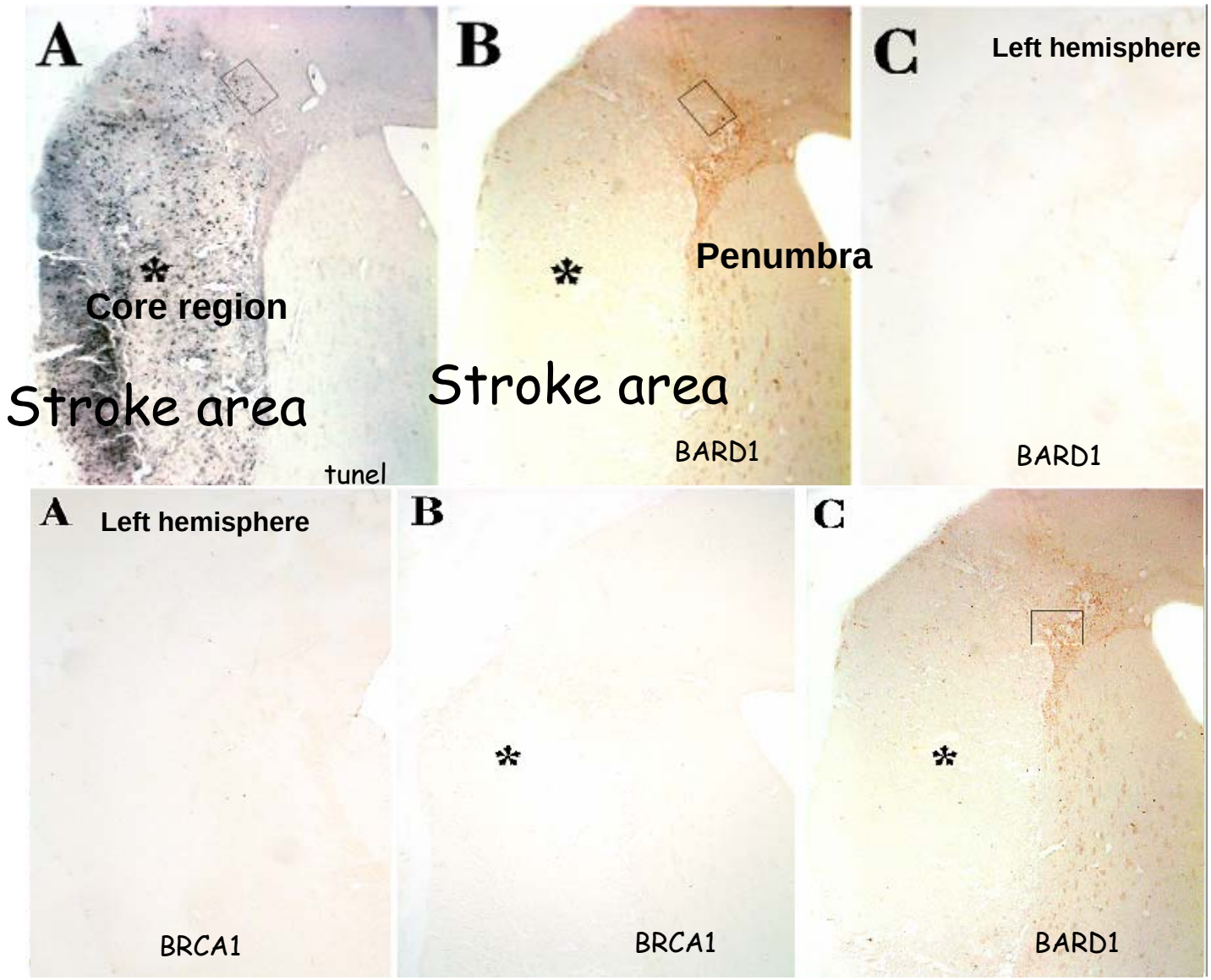
BARD1 brings the kinase to its target



Presumed tumor suppressor pathways of BARD1

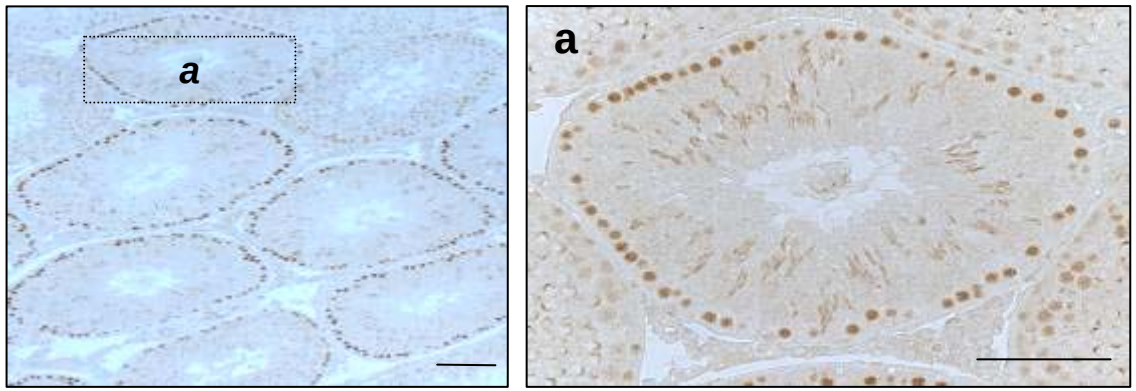


BARD1 upregulation in vivo by ischemia

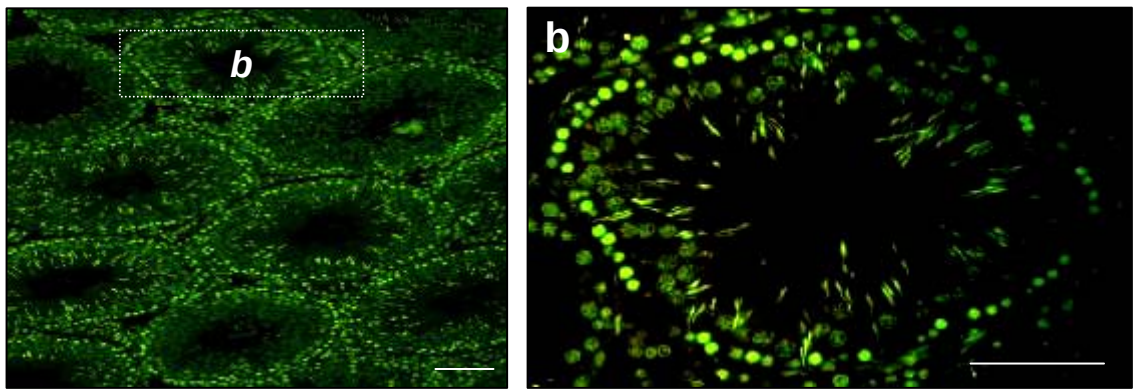


BARD1 expression is correlated with apoptosis in spermatogenesis

anti-BARD1



TUNEL

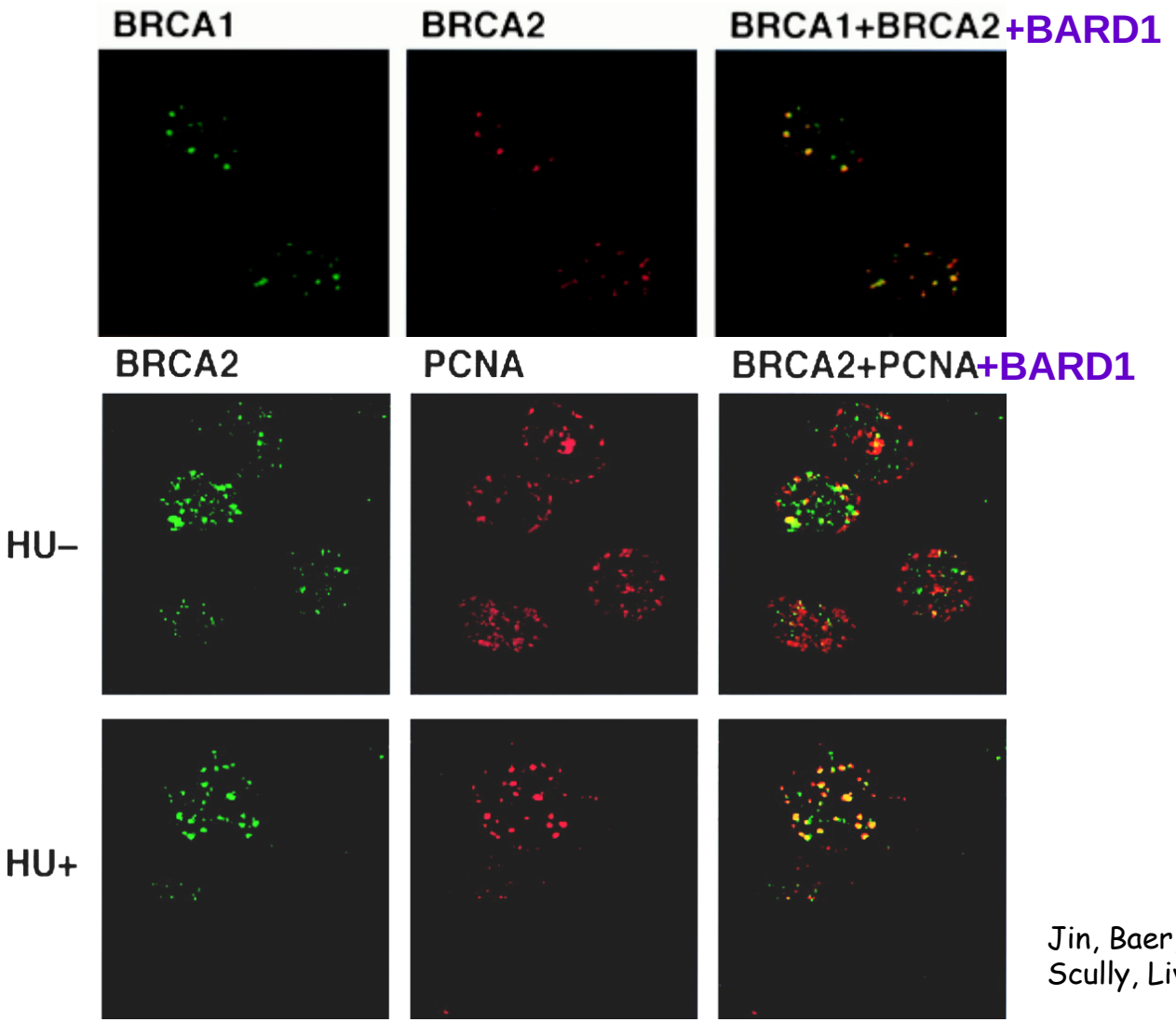


Feki et al., & Irminger-Finger, BOR 2004

Mechanism of apoptosis induction by BARD1

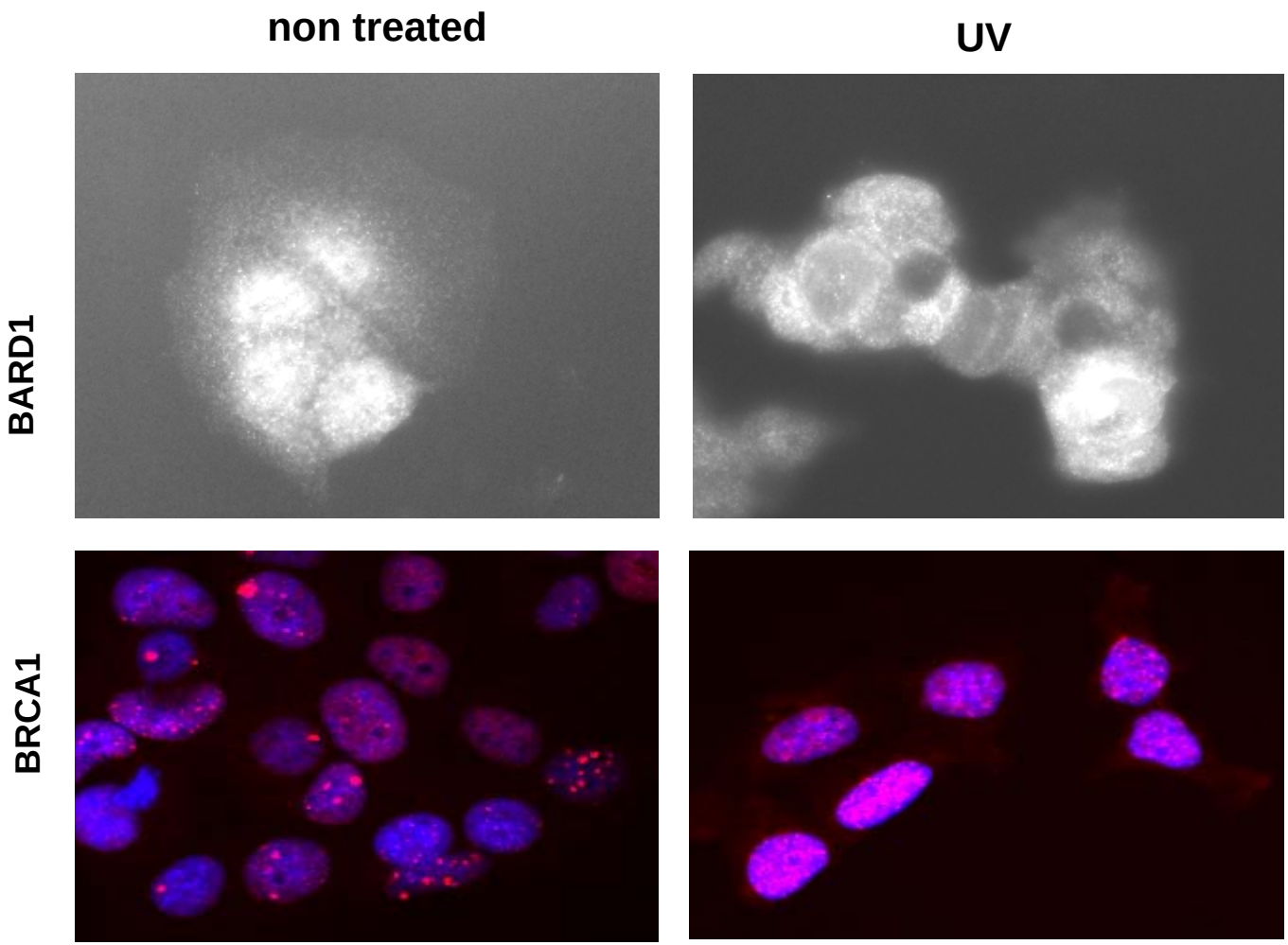
- Excess BARD1 over BRCA1 leads to apoptosis
- BARD1-induced apoptosis depends on functional p53
- Upregulation of BARD1 results in stabilization of p53 protein
- Repression/deletion of BARD1 results in apoptosis resistence

Co-localization of BRCA1 and BARD1 with repair proteins in "*nuclear dots*"

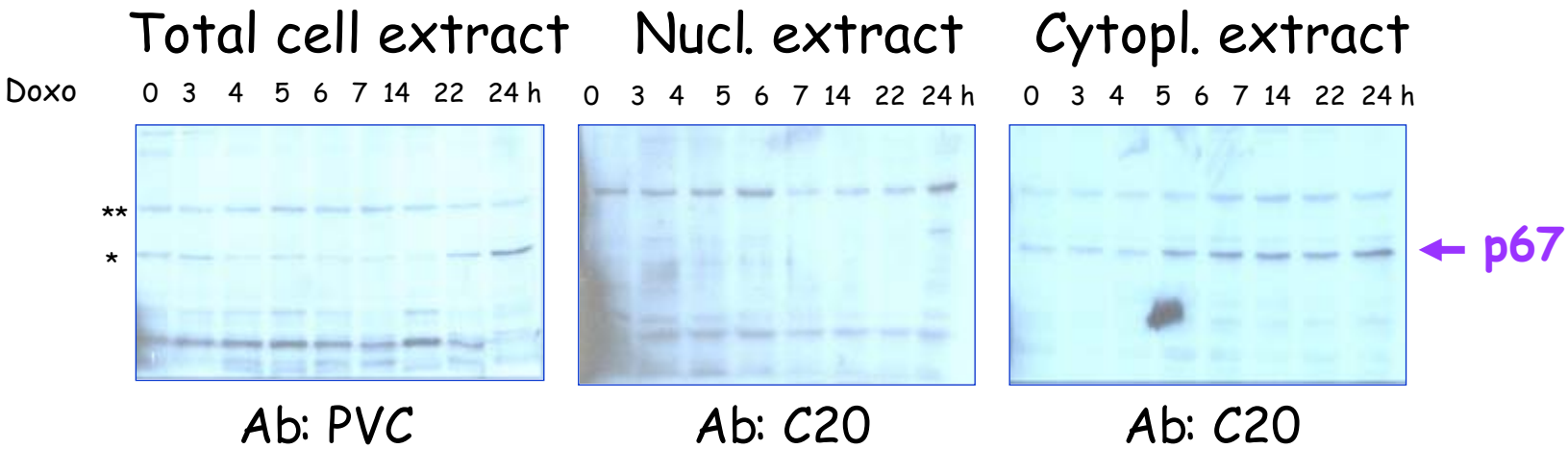


Jin, Baer, *JBC* 1997;
Scully, Livingston, *Cell* 1997

BARD1 translocates to the cytoplasm upon apoptosis induction

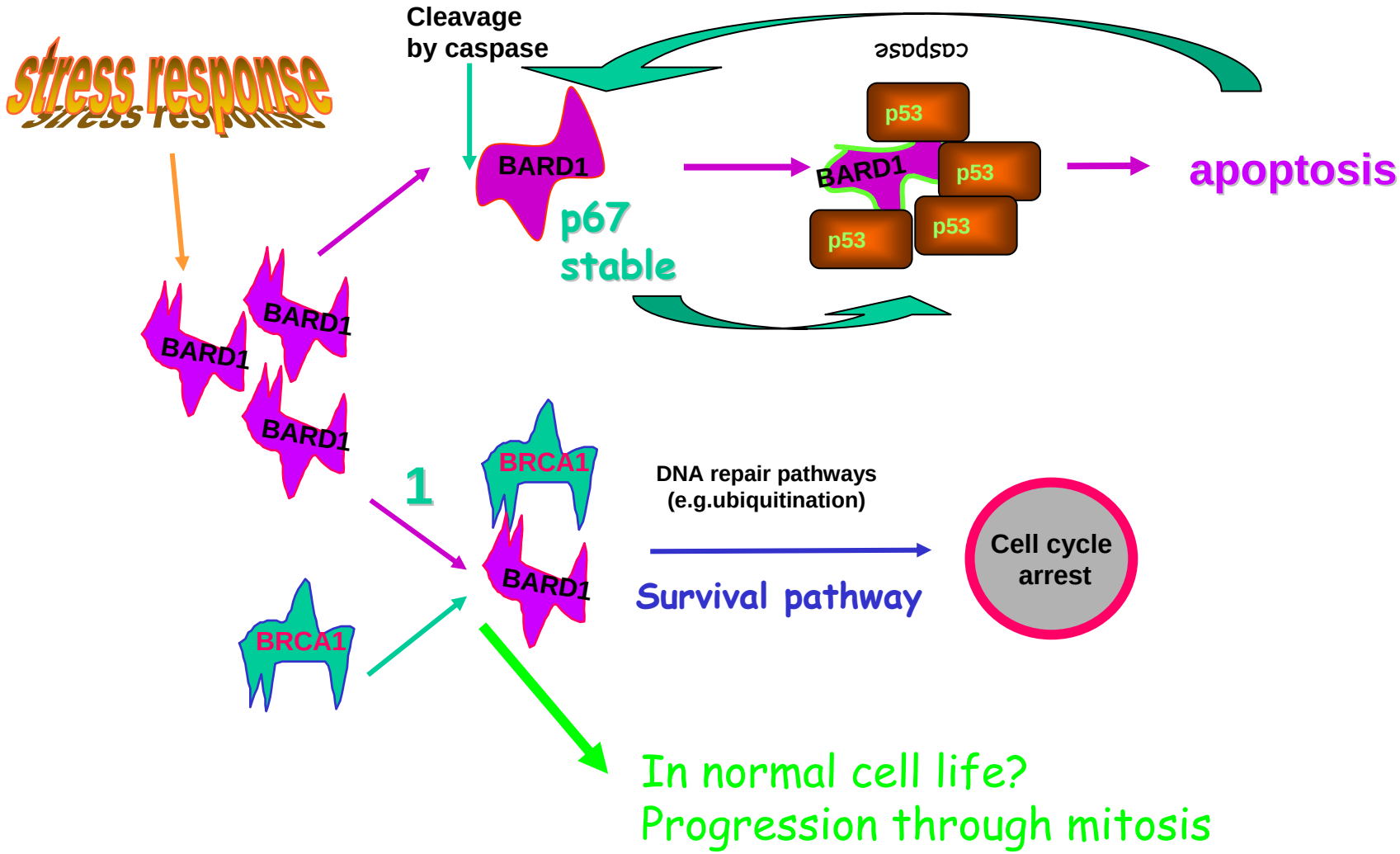


BARD1 is translocated to the cytoplasm during apoptosis

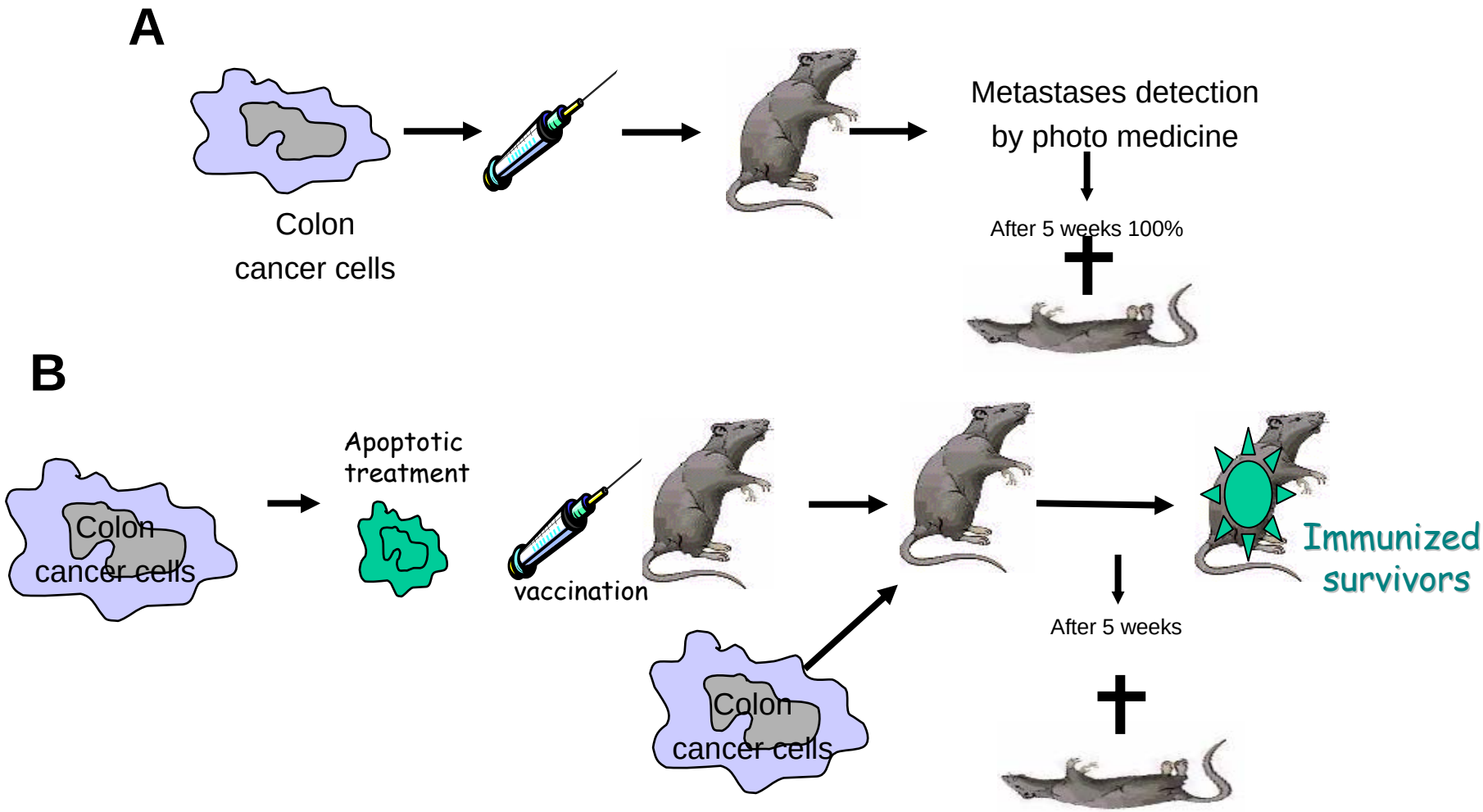


The C-terminal p67 appears in the cytoplasm. p67 is immunogenic and anti-tumorigenec in an animal model of colon cancer (Gautier, Irminger-Finger et al., Cancer Res 2000).

Presumed tumor suppressor pathways of BARD1



Colon cancer model: immunotherapy



Gautier, Irminger-Finger et al., Cancer Research 2000

Apoptotic BARD1 isoform p67 is immunogenic

Immunized survivors

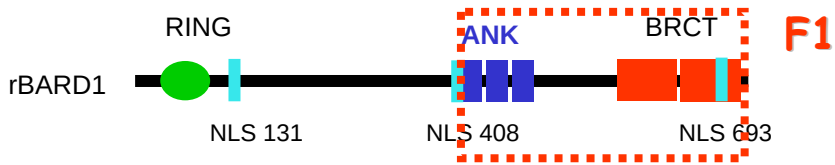
Western blot

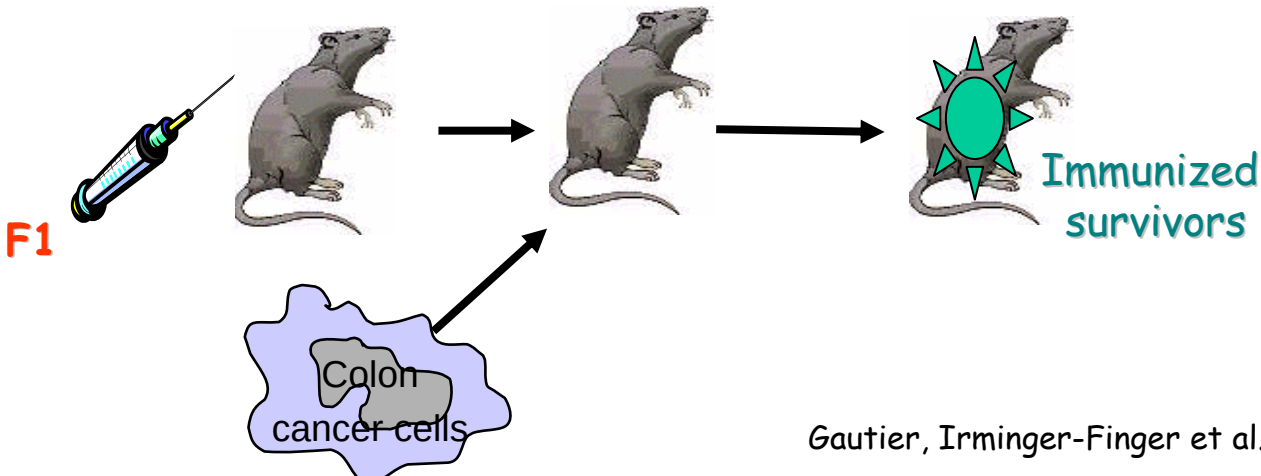
p67

Screening of Expression library

Identification of Epitopes:

BARD1 partial sequence

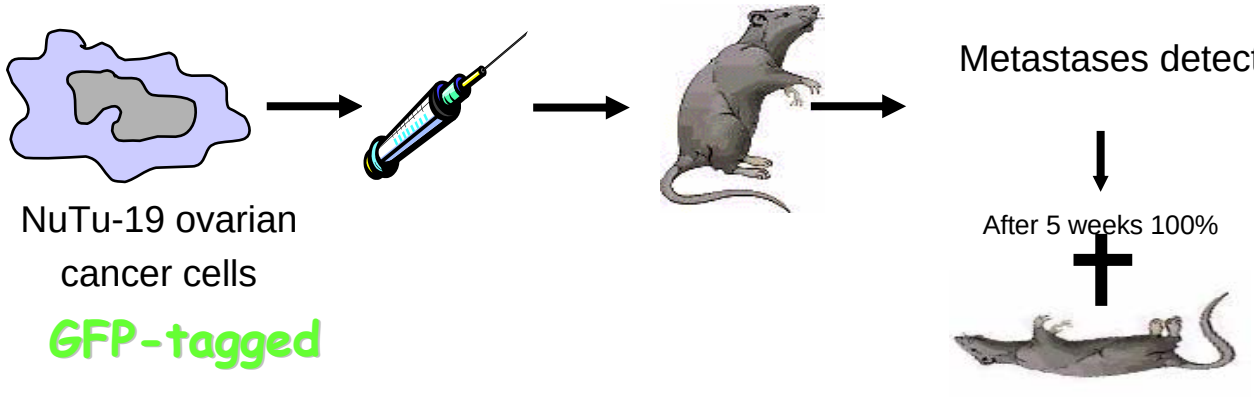




Gautier, Irminger-Finger et al., Cancer Research 2000

Animal cancer model for tumor cell killing

A



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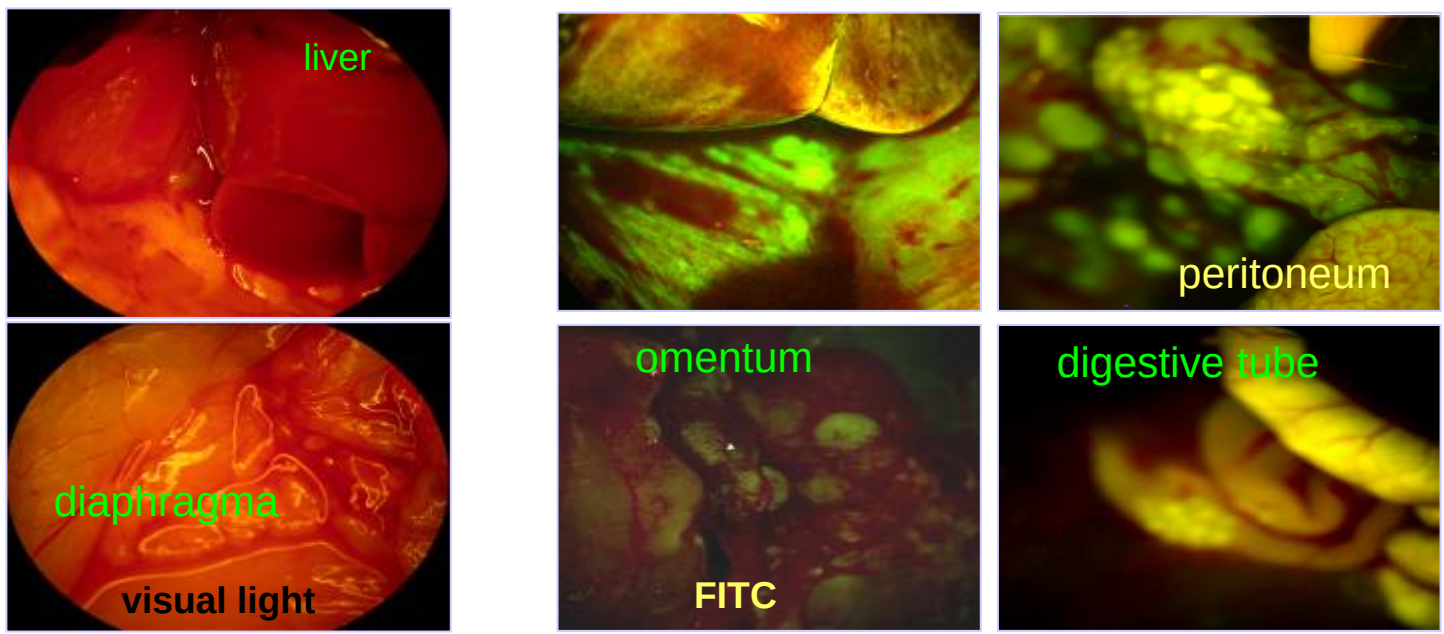
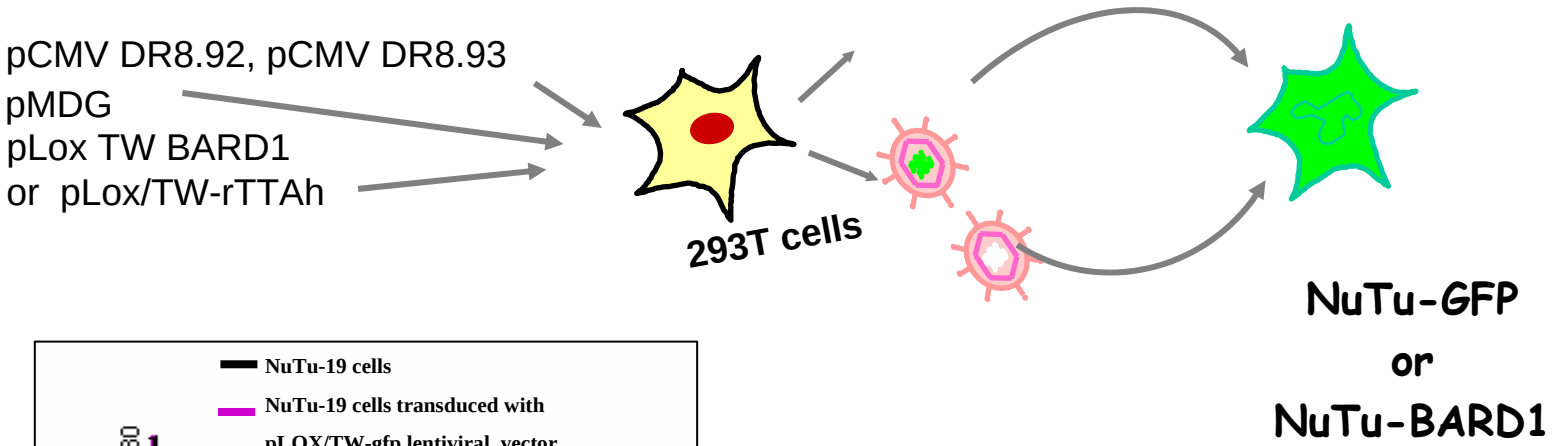
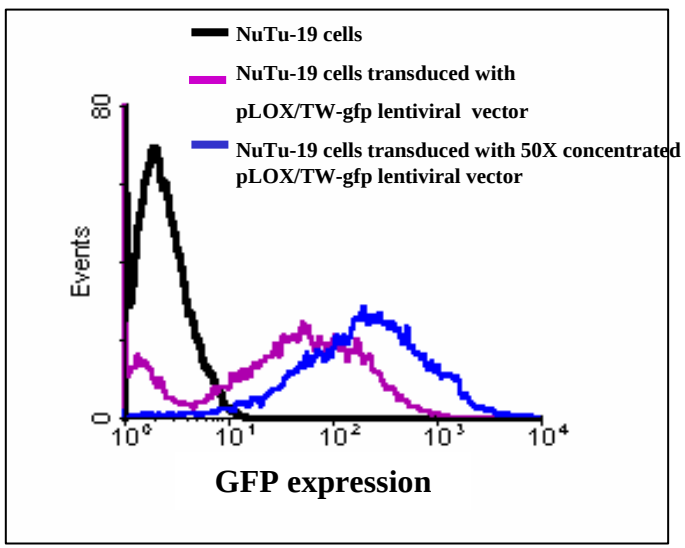


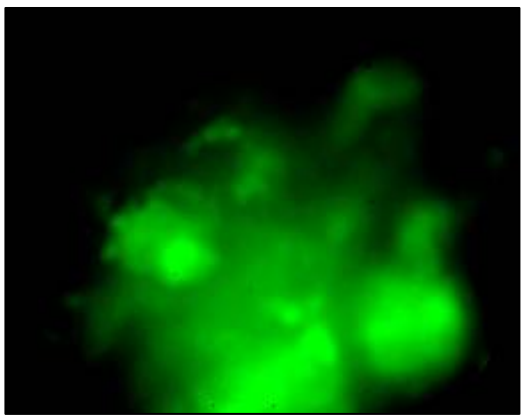
Transduction of NuTu-19 cells with lentiviral vectors expressing BARD1 or GFP

Packaging plasmids: pCMV DR8.92, pCMV DR8.93 (core and enzymatic components of HIV-1)
Envelop plasmid: pMDG (VSV-G protein)
Transducing vectors: pLOX/TW-BARD1 or pLOX/TW-rTTAh

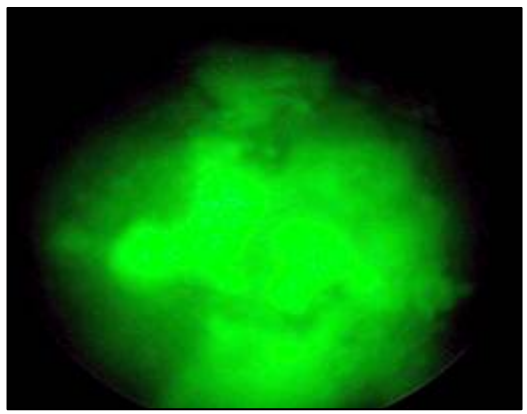




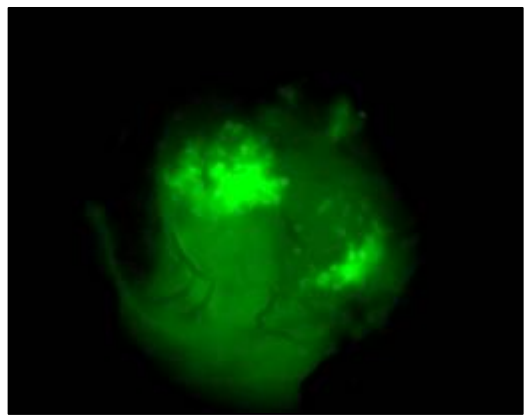
Nutu-19-EGFP tumors in Fisher rat



Diaphragm

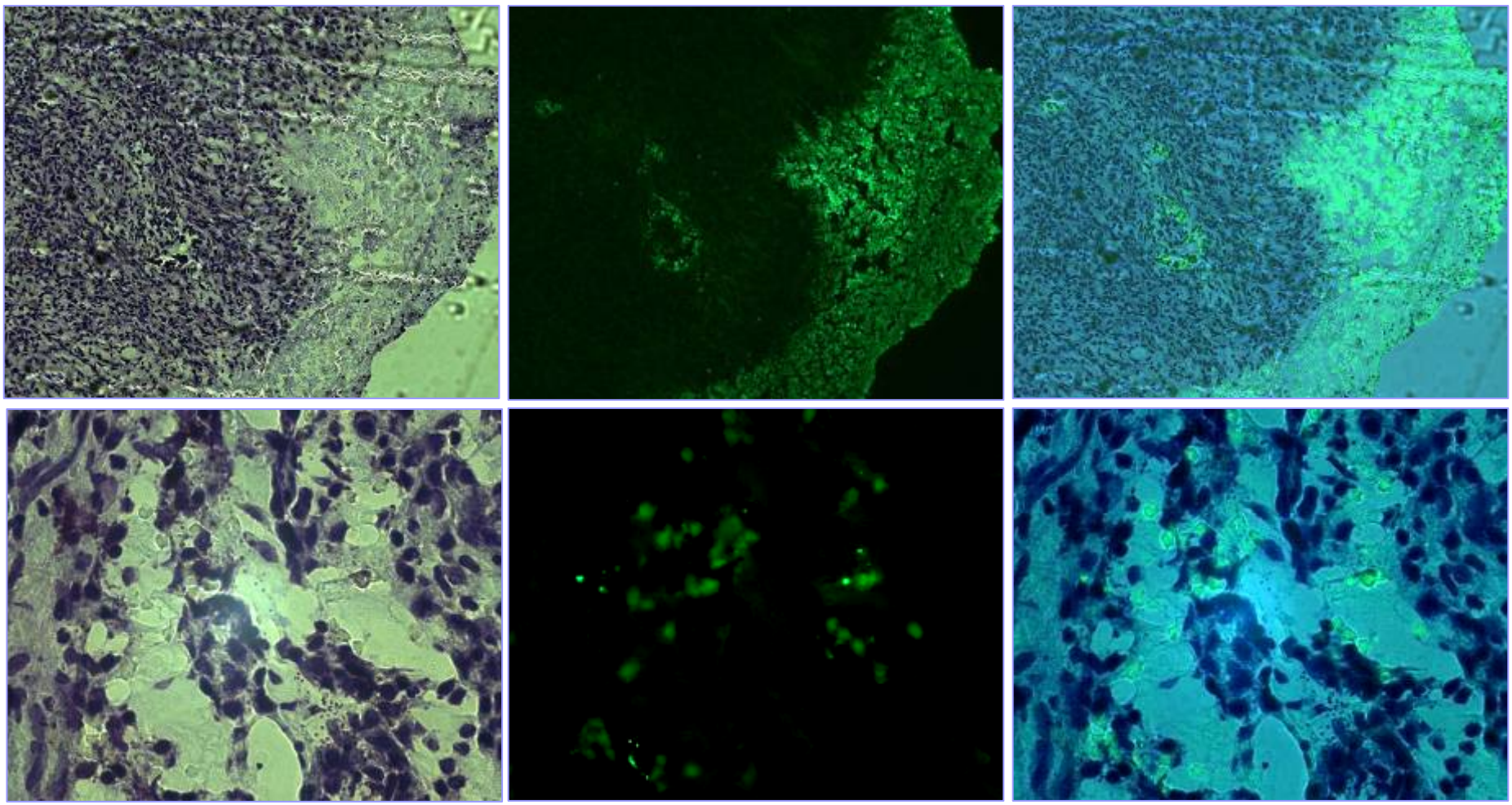


Omentum



Thymus

Diaphragm



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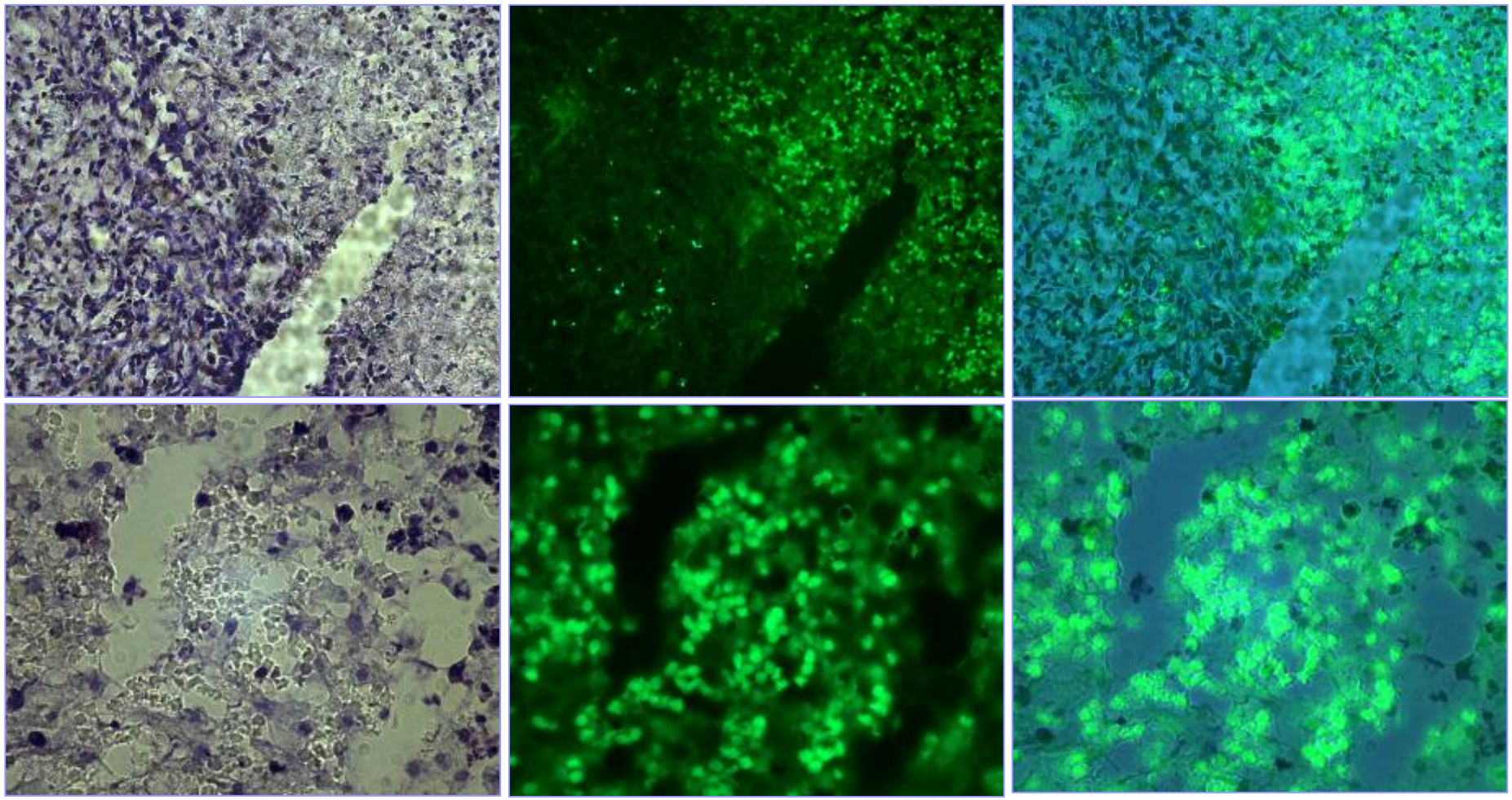
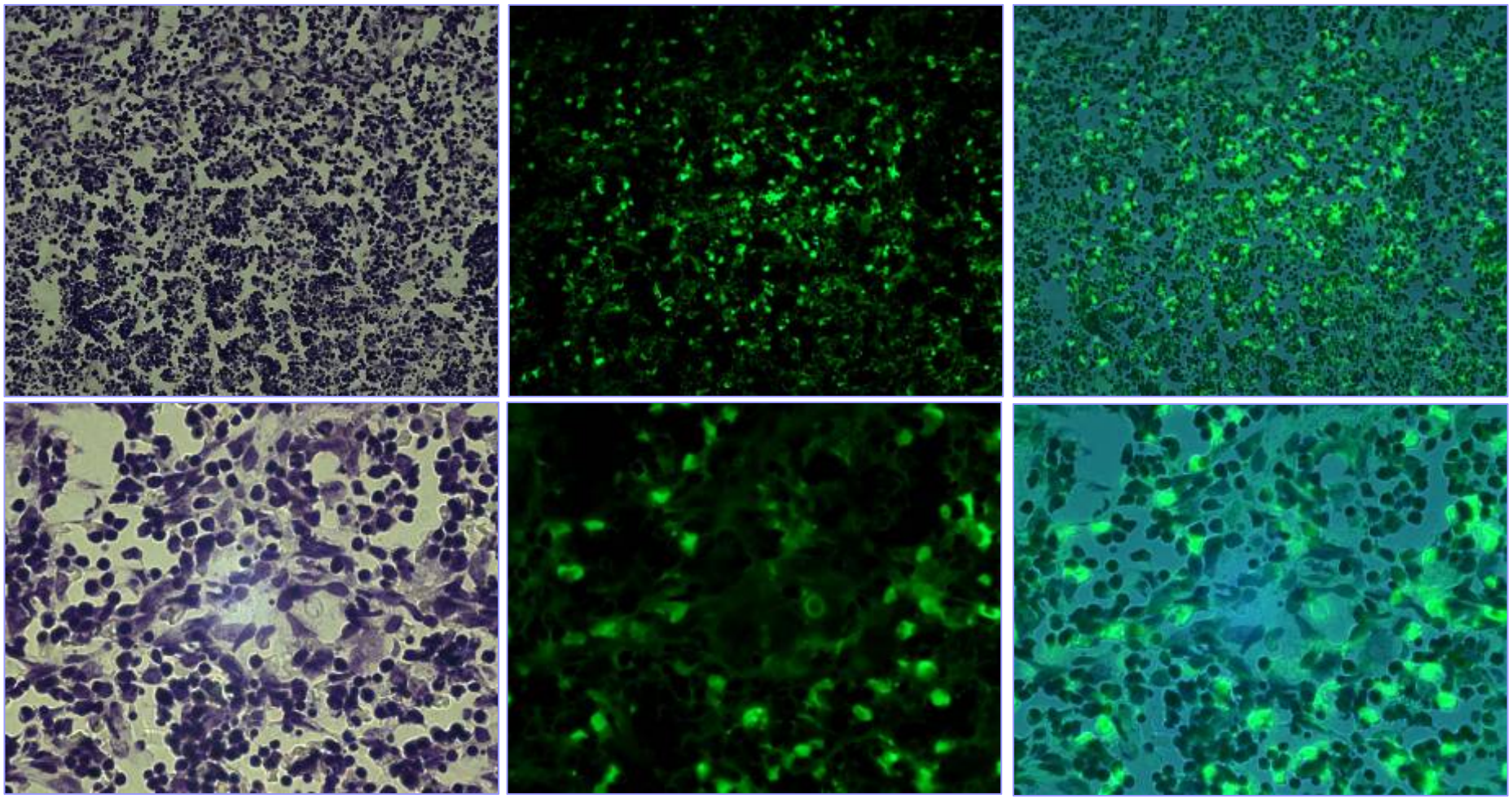
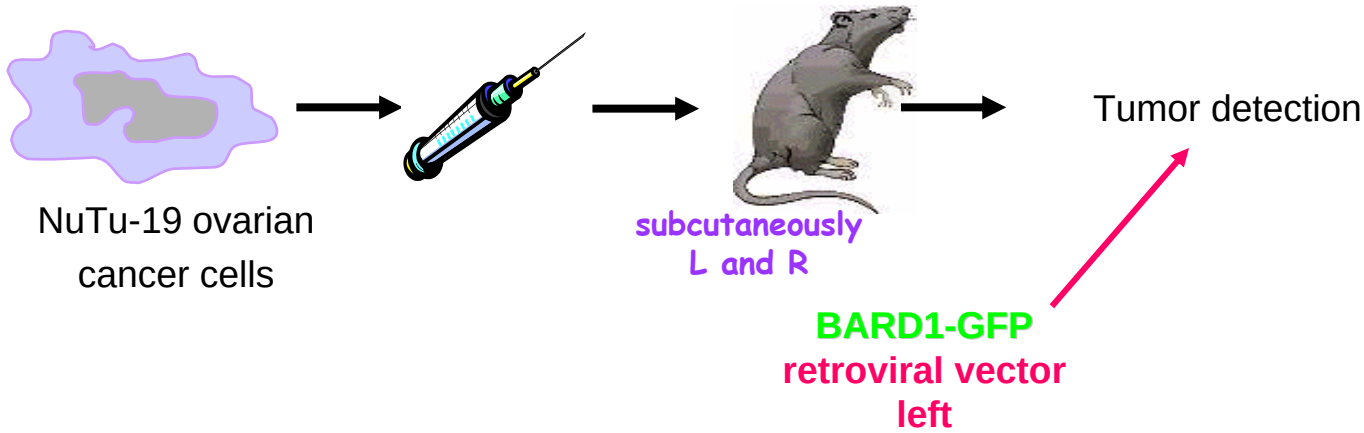


Thymus

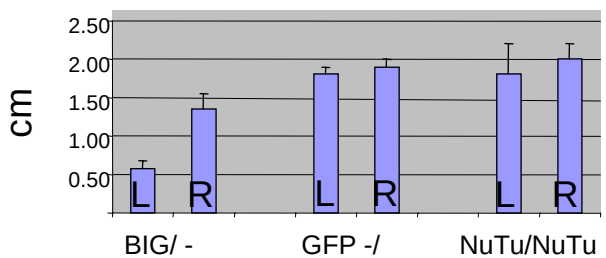


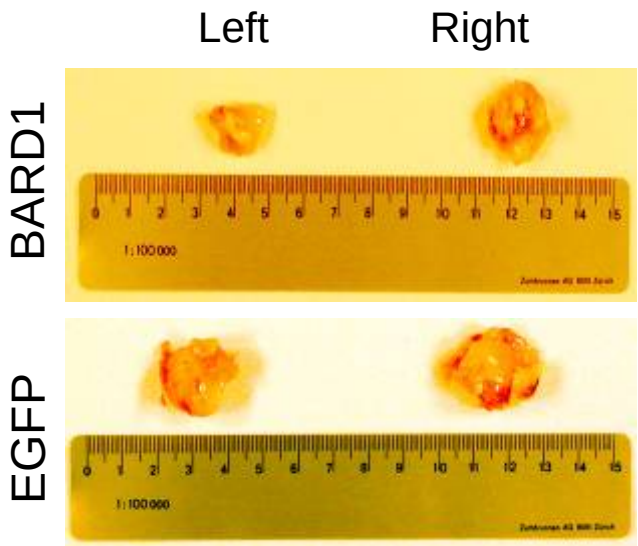
Viral delivered BARD1 causes tumor shrinking

A

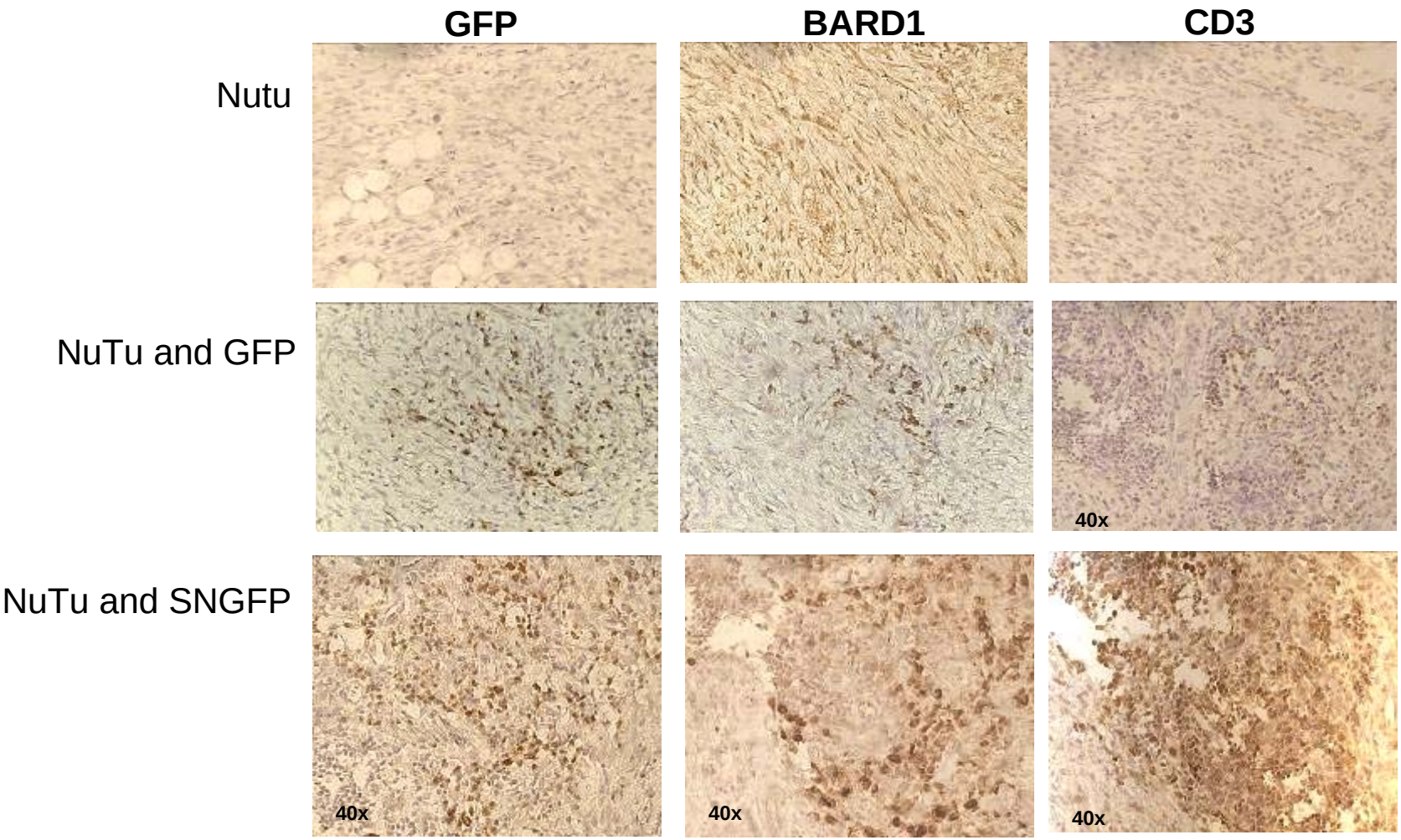


B





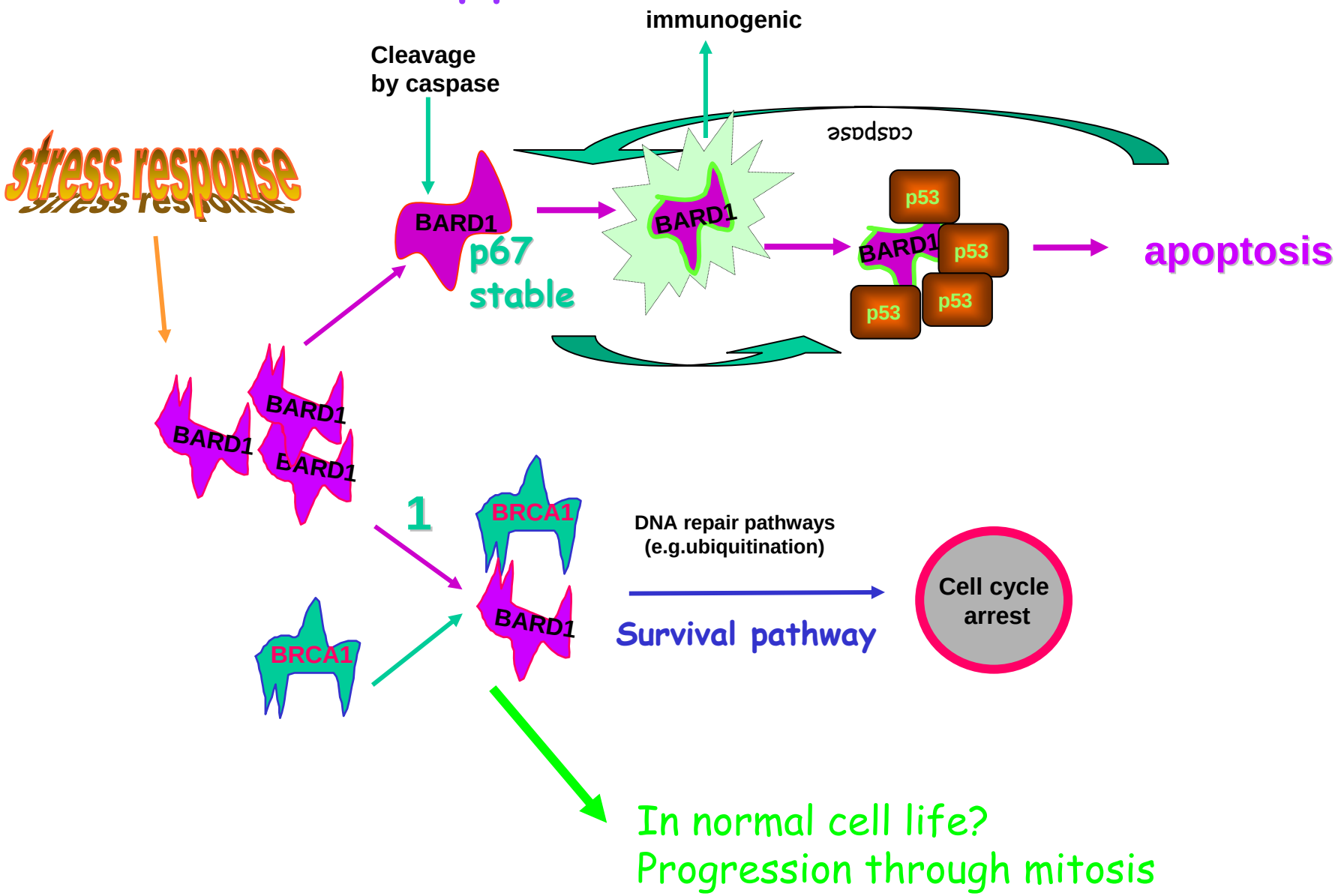
BARD1 expression in tumors causes immune response



BARD1 for tumor cell killing

- Ovarian cancer model
- BARD1 deficient tumor cells
- Viral delivery of functional BARD1
- Replace viral BARD1 by small molecules
- Anti-tumor vaccination

Several tumor suppressor functions of BARD1

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