

Functions of BARD1 and associated proteins and its application in cancer prognostics and treatment

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Tumor suppressor BARD1 patented:

1. Use as vaccine
2. Use for cell killing
3. Use as prognostic marker

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Clinical application of BARD1: background and principles

- The "normal" functions of BARD1
- BARD1 expression in tumors:
 - in breast and ovarian cancers
 - in lung cancer
- BARD1 expression the human cytotrophoblast:
 - a non-malignant cells with an invasive phenotype
- AND in amnion fluid-secreted by the cytotrophoblast?
- Detection of BARD1 in serum for cancer screening?
- Immunogenic BARD1 applied for tumor cell killing

Genes implicated in epithelial cancers: predisposition genes (tumor suppressor genes)

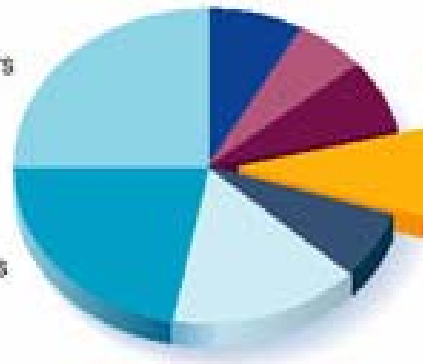
- p53 (lung, colon, breast, prostate)
- BRCA1 (breast, ovary, prostate)
- BRCA2 (breast, ovary)
- BARD1 (breast, ovary, uterus,
 - and other proliferative tissues)

Function: repair, apoptosis (cell death)

Old Age Cancers are derived from epithelial cells

Paediatric

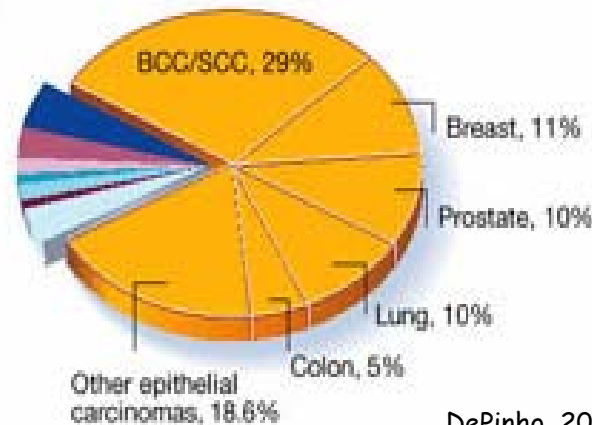
- Epithelial carcinomas, 9%
- Lymphomas
- Nervous system tumours
- Leukaemia
- Germ cell tumours
- Soft tissue sarcomas
- Malignant bone tumours
- Others/unspecified



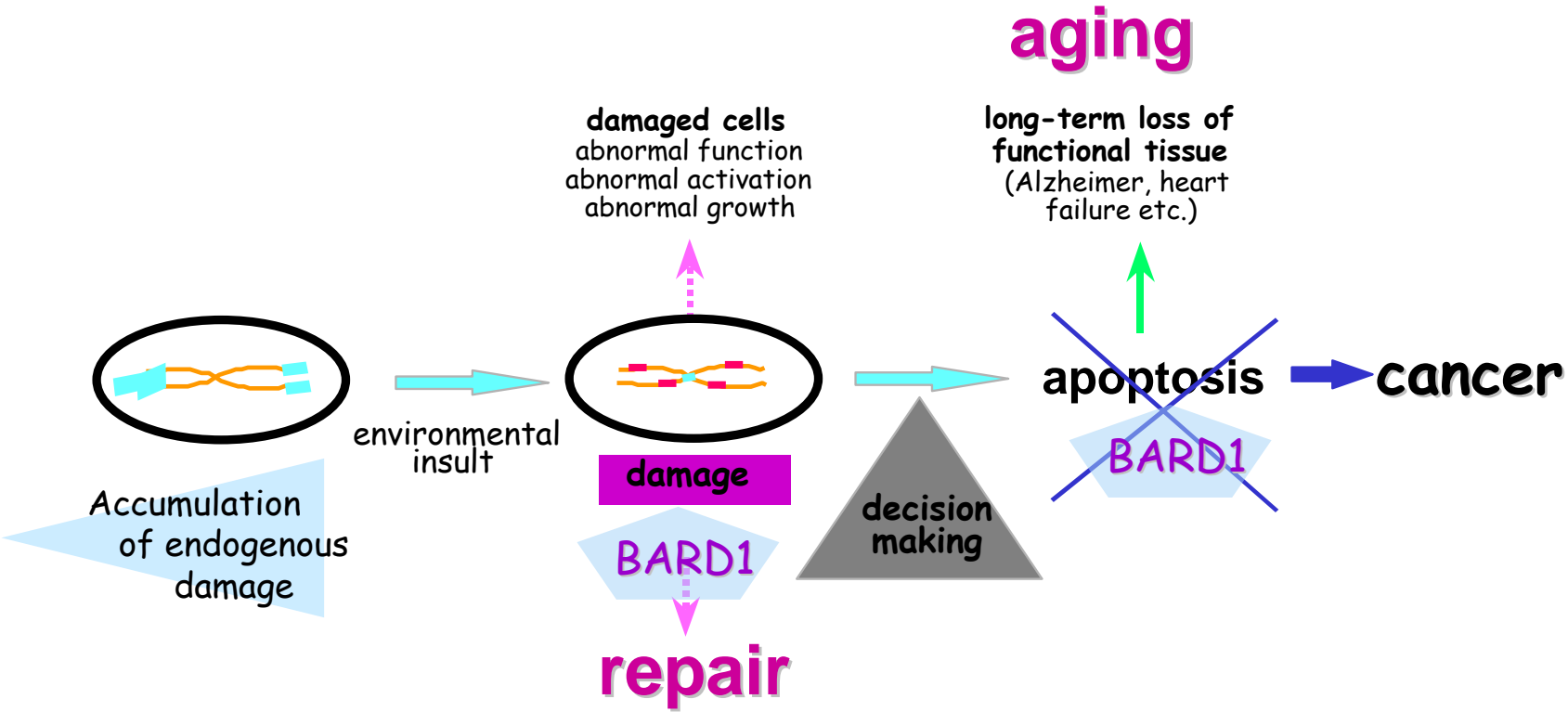
Adult

- Epithelial carcinomas, 83.6%
- Lymphoma
- Multiple myeloma
- Leukaemia
- Nervous system tumours
- Kidney
- Non-epithelial skin cancers
- Others/unspecified

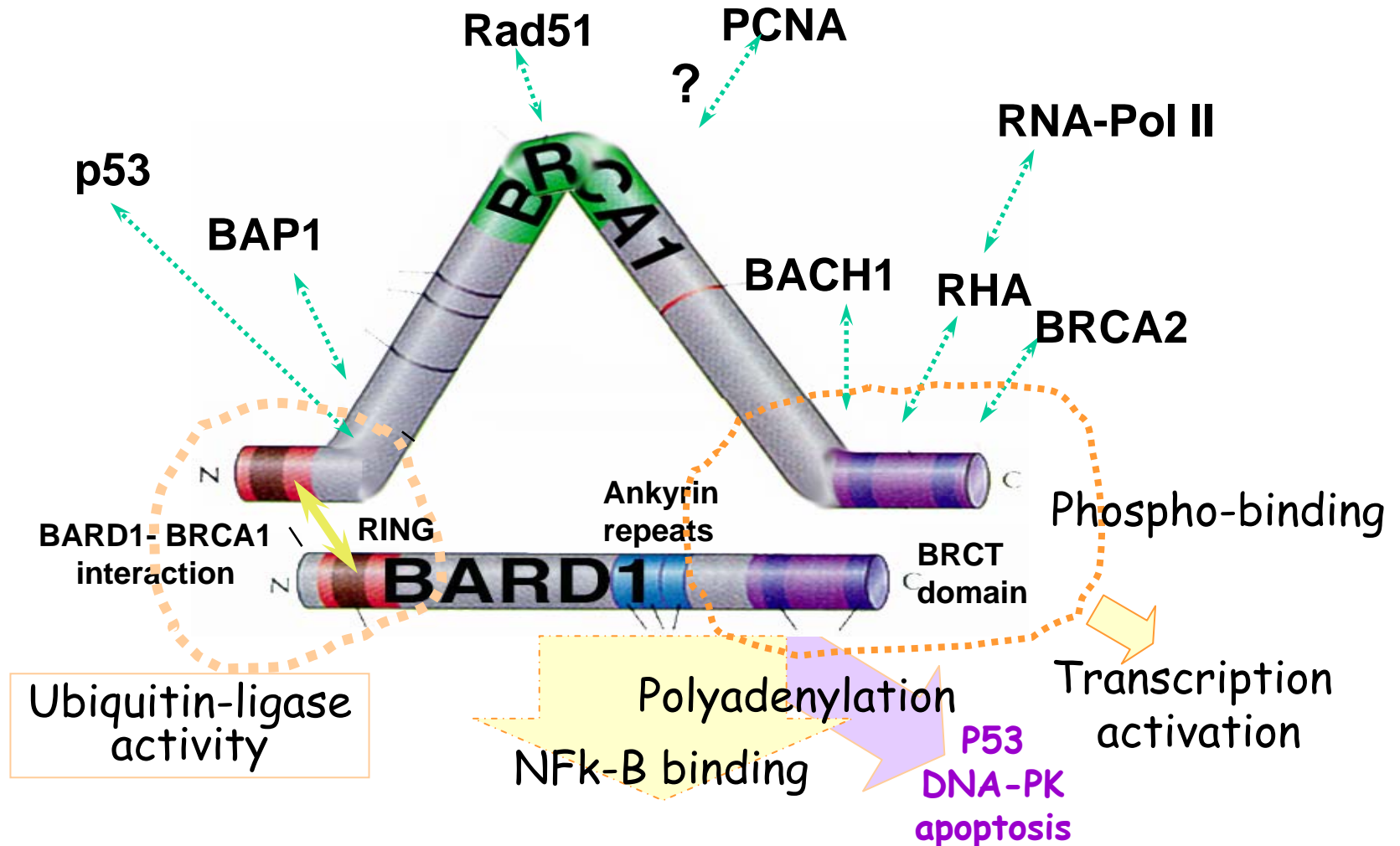
BCC Basal cell carcinoma
SCC Squamous cell carcinoma



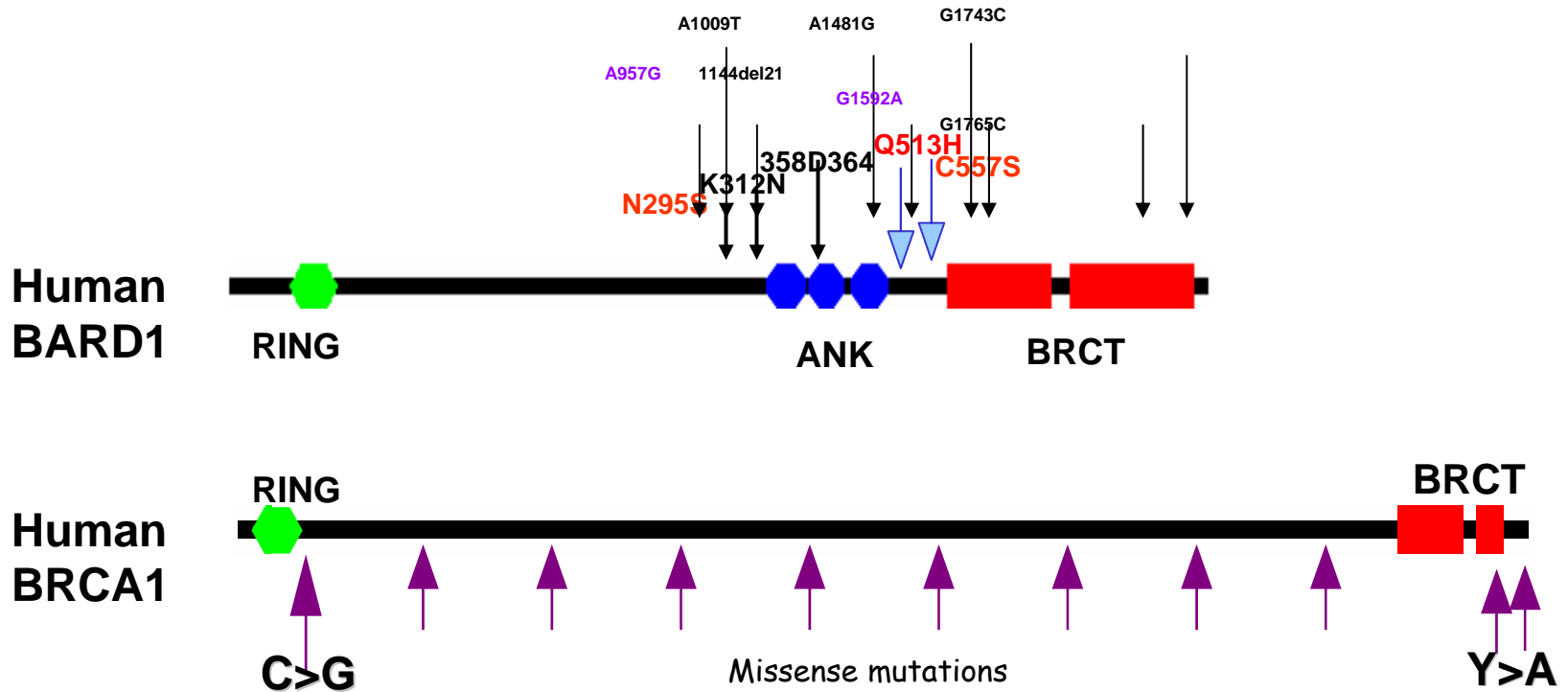
Accumulation of damage during aging



BARD1 and its interacting proteins: a converging vehicle

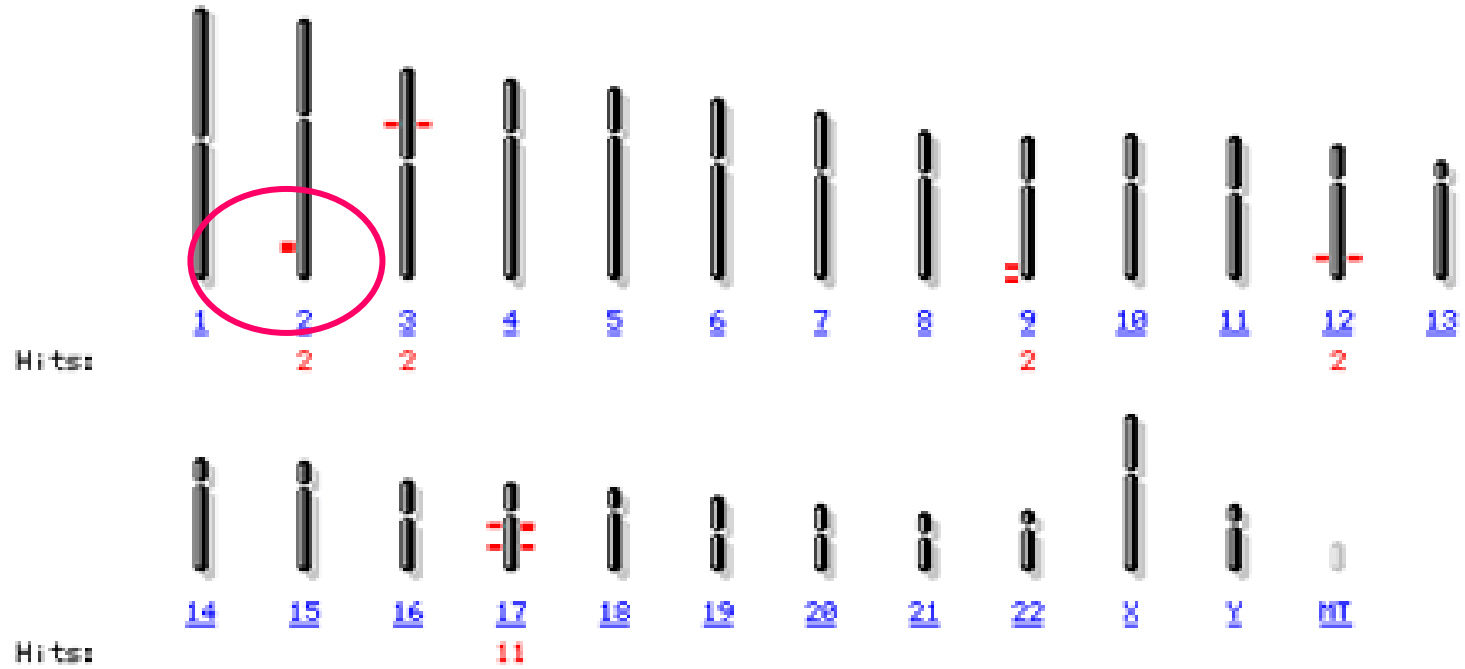


BARD1 and BRCA1 mutations predisposing to breast cancer

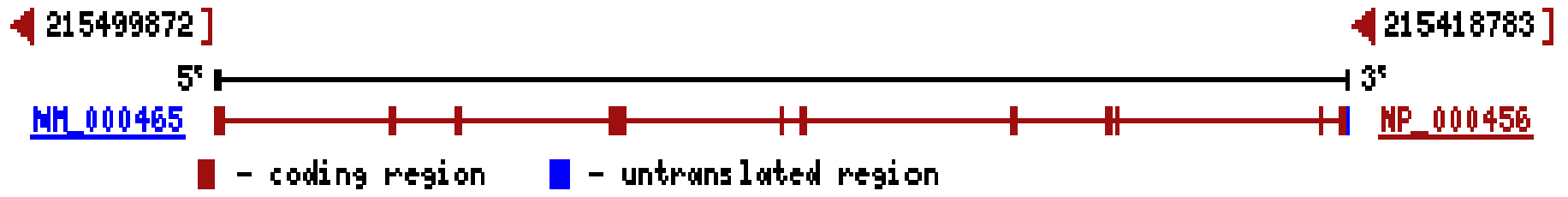


Homo sapiens genome view

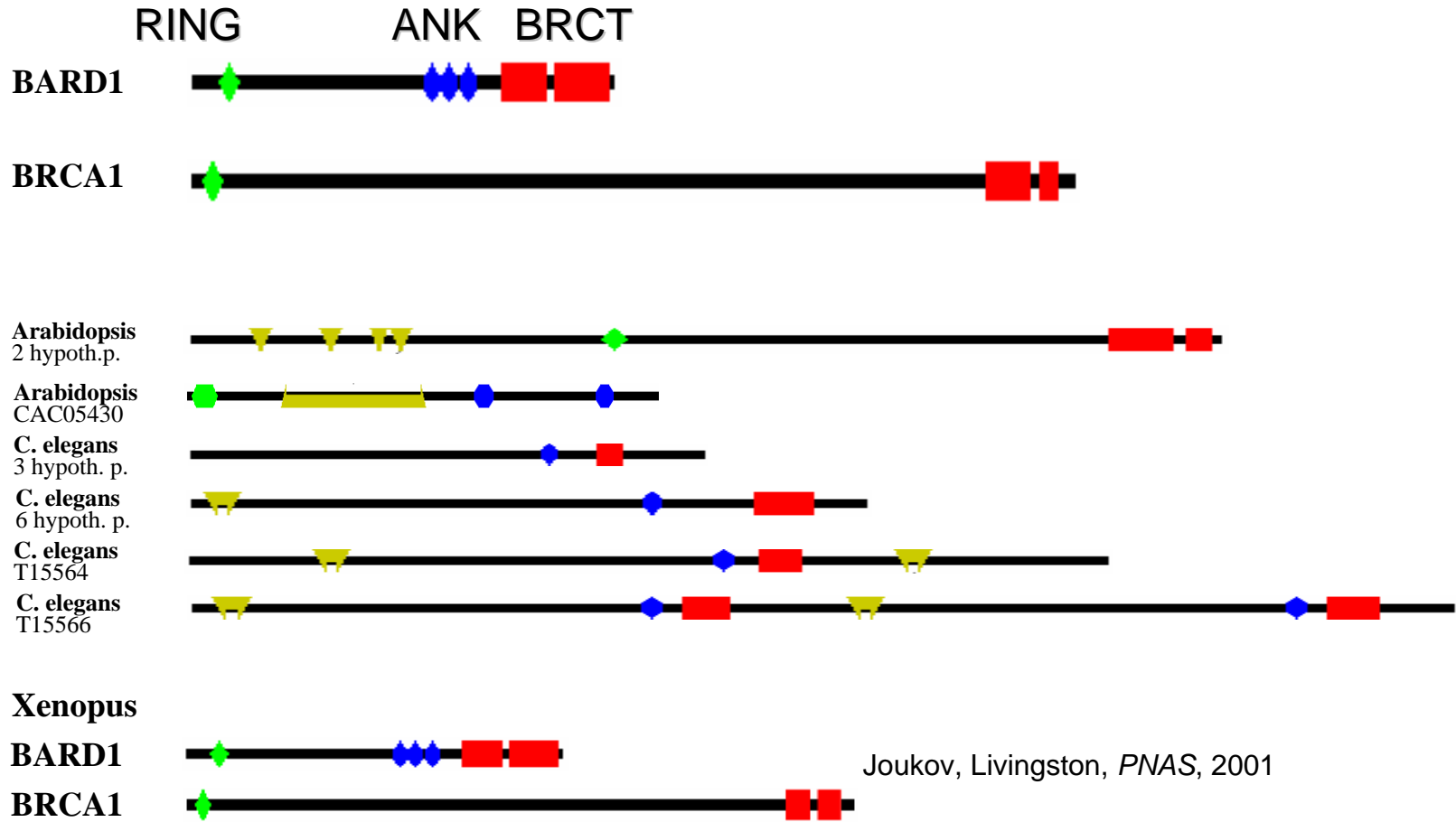
build 35 version 1 statistics



NC_000002



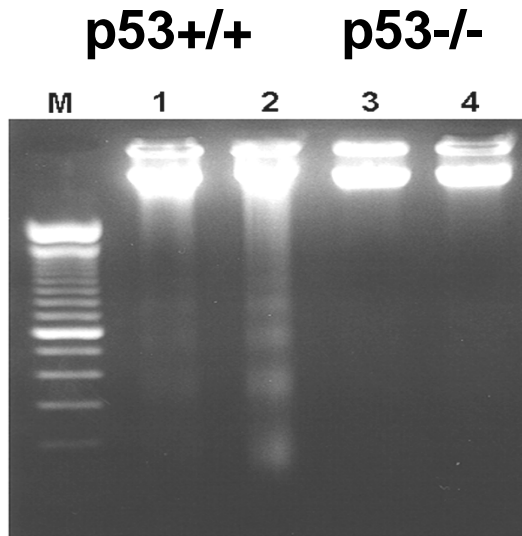
No one like BARD1



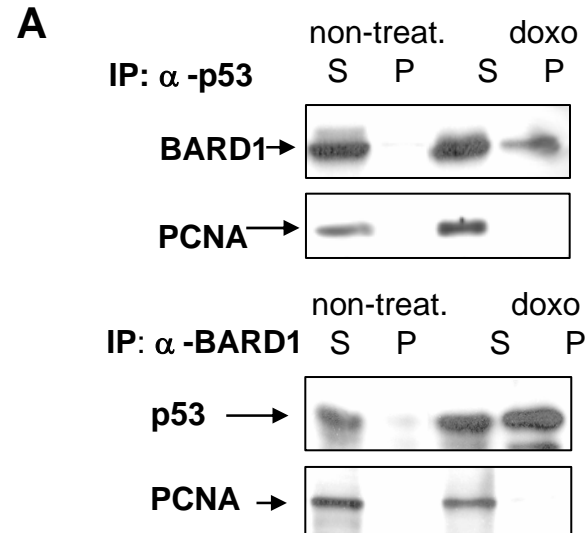
Joukov, Livingston, *PNAS*, 2001

BARD1 induces apoptosis in a p53-mediated pathway

transfection of BARD1



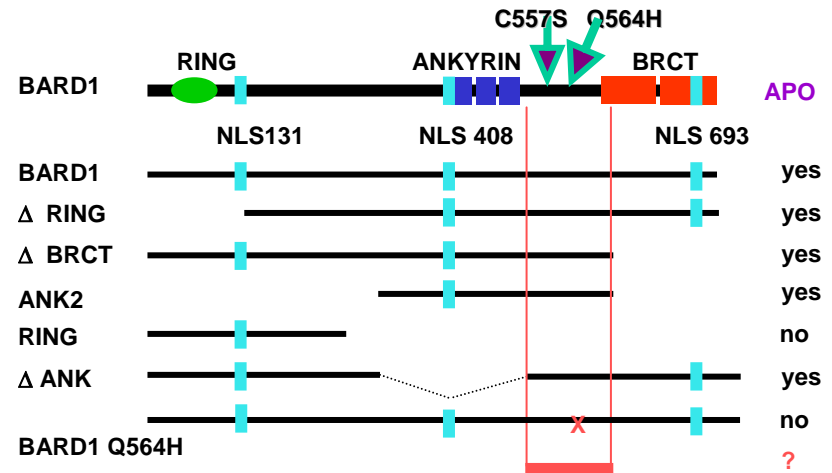
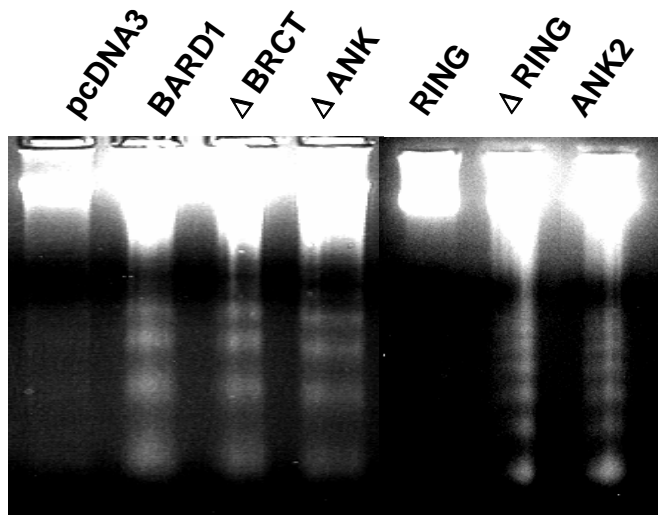
BARD1 binds to p53



BARD1 stabilizes p53



Mapping of the apoptotic region

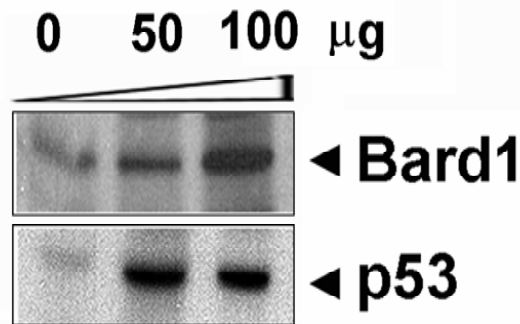


Deletion mutants lacking the BRCA1 interaction domain are efficient in apoptosis induction and have increased protein stability

BARD1 is upregulated in response to cellular stress

On the protein level

TAC-2 cells
Doxorubicin

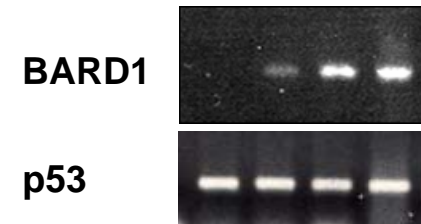


Western

On the mRNA level

ES cells

UV - 1 4 - min
doxo - - - 10 μ g



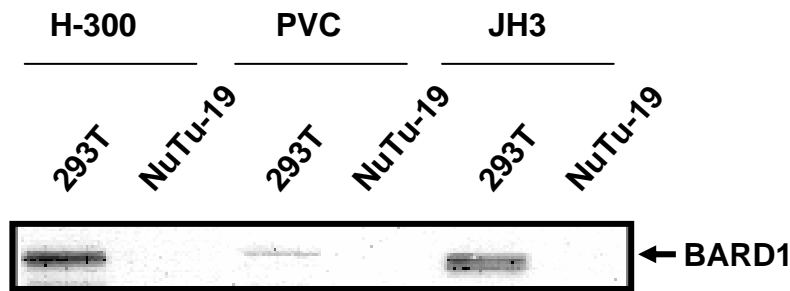
RT-PCR

Mechanism of apoptosis induction by BARD1

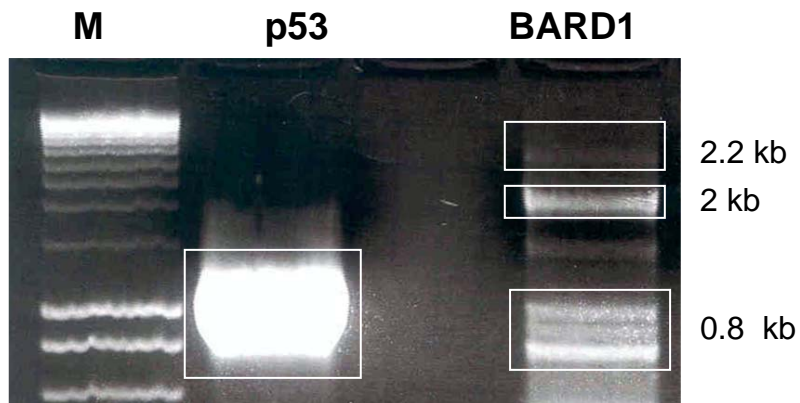
- Exogenous expression of BARD1 leads to apoptosis
- BARD1-induced apoptosis depends on functional p53
- Upregulation of BARD1 results in stabilization of p53 protein
- Repression/deletion of BARD1 results in apoptosis resistance

BARD1 deficient tumor cells: NuTu-19 resistant to apoptosis

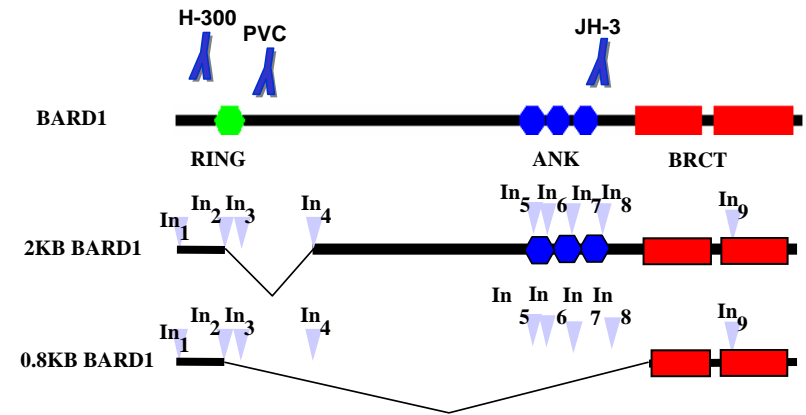
A



B

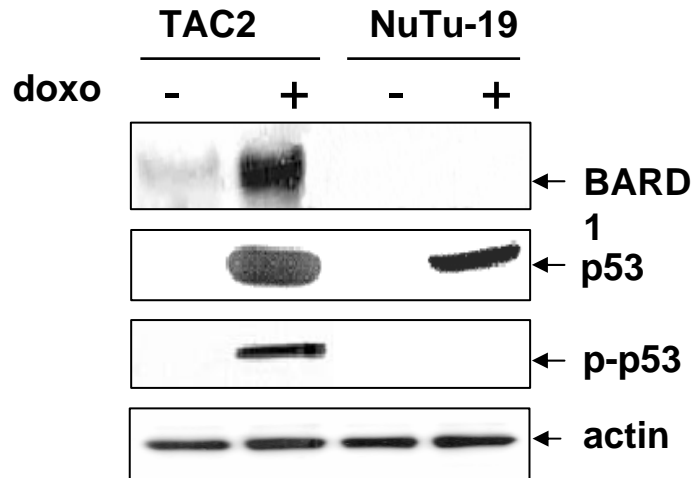


C

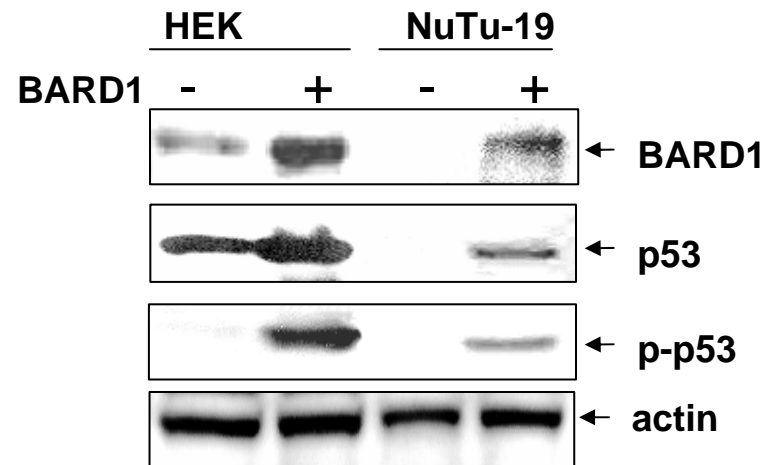


BARD1 required for p53 phosphorylation

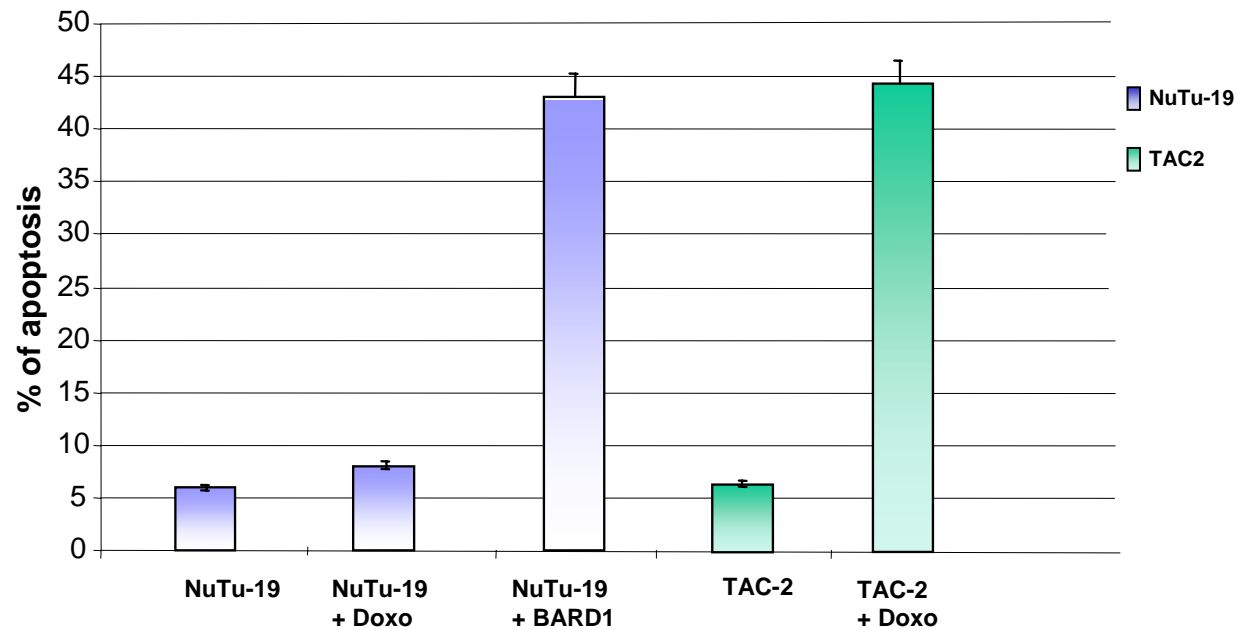
Apoptotic treatment



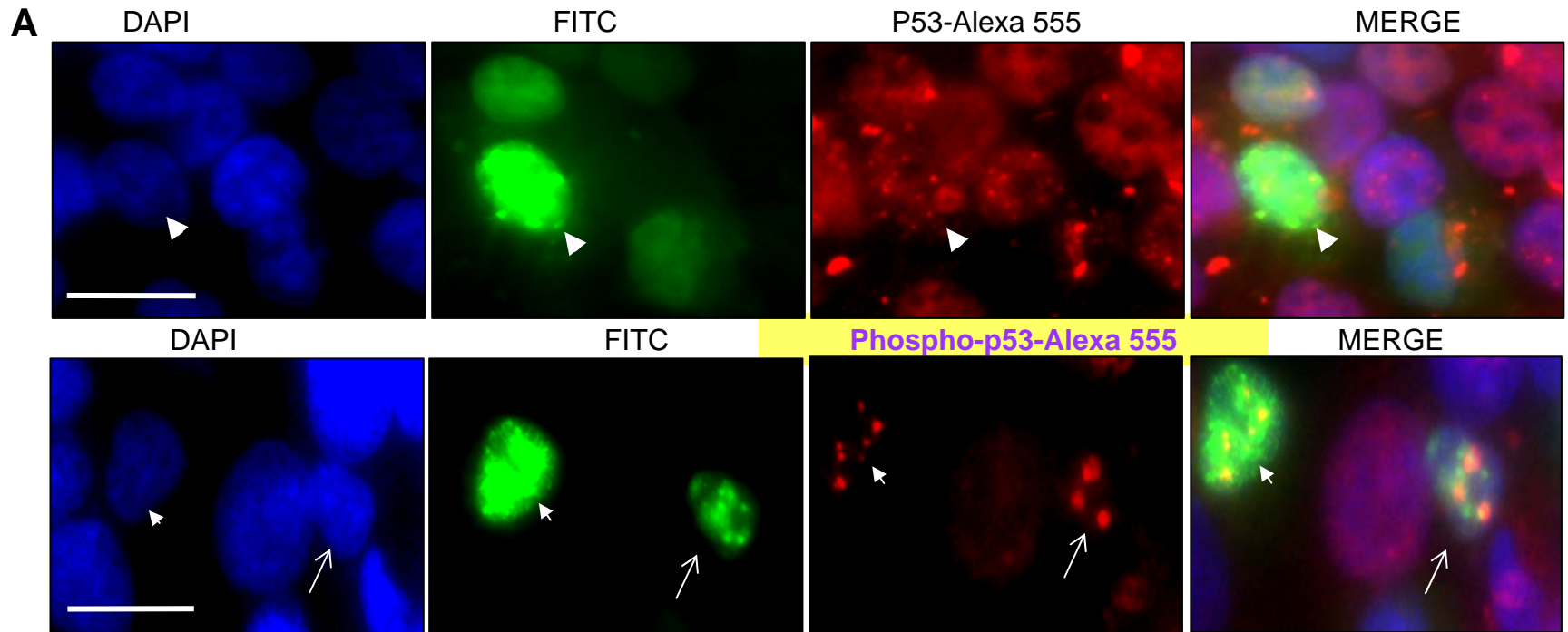
BARD1 transfection



Exogenous expression of BARD1 can induce apoptosis in NuTu-19 cells

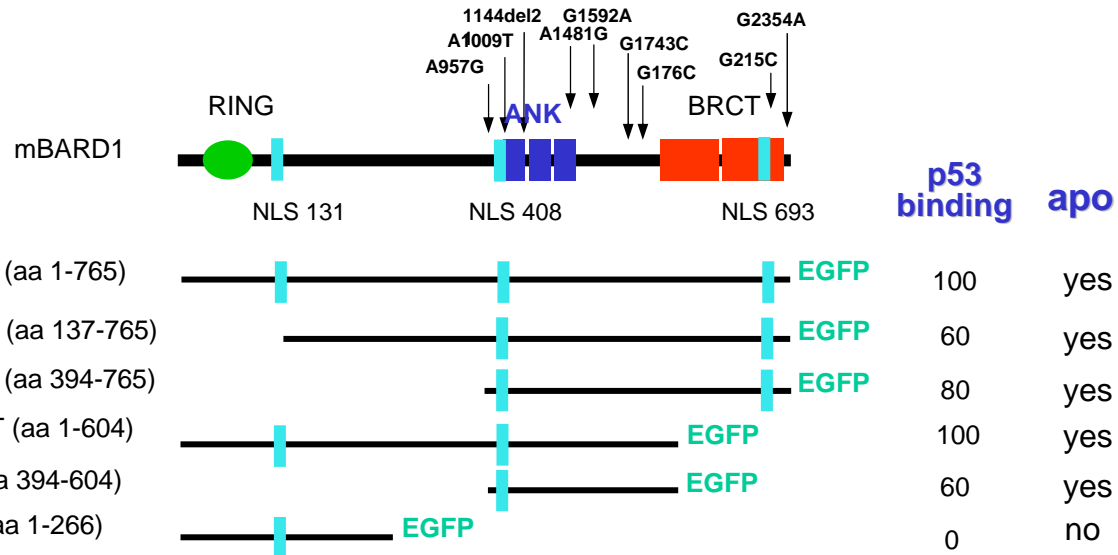
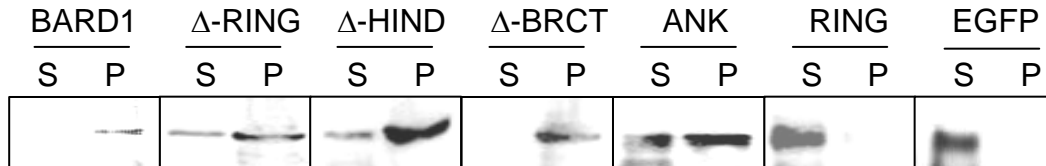


BARD1 colocalizes with phospho-p53



Mapping p53 binding region

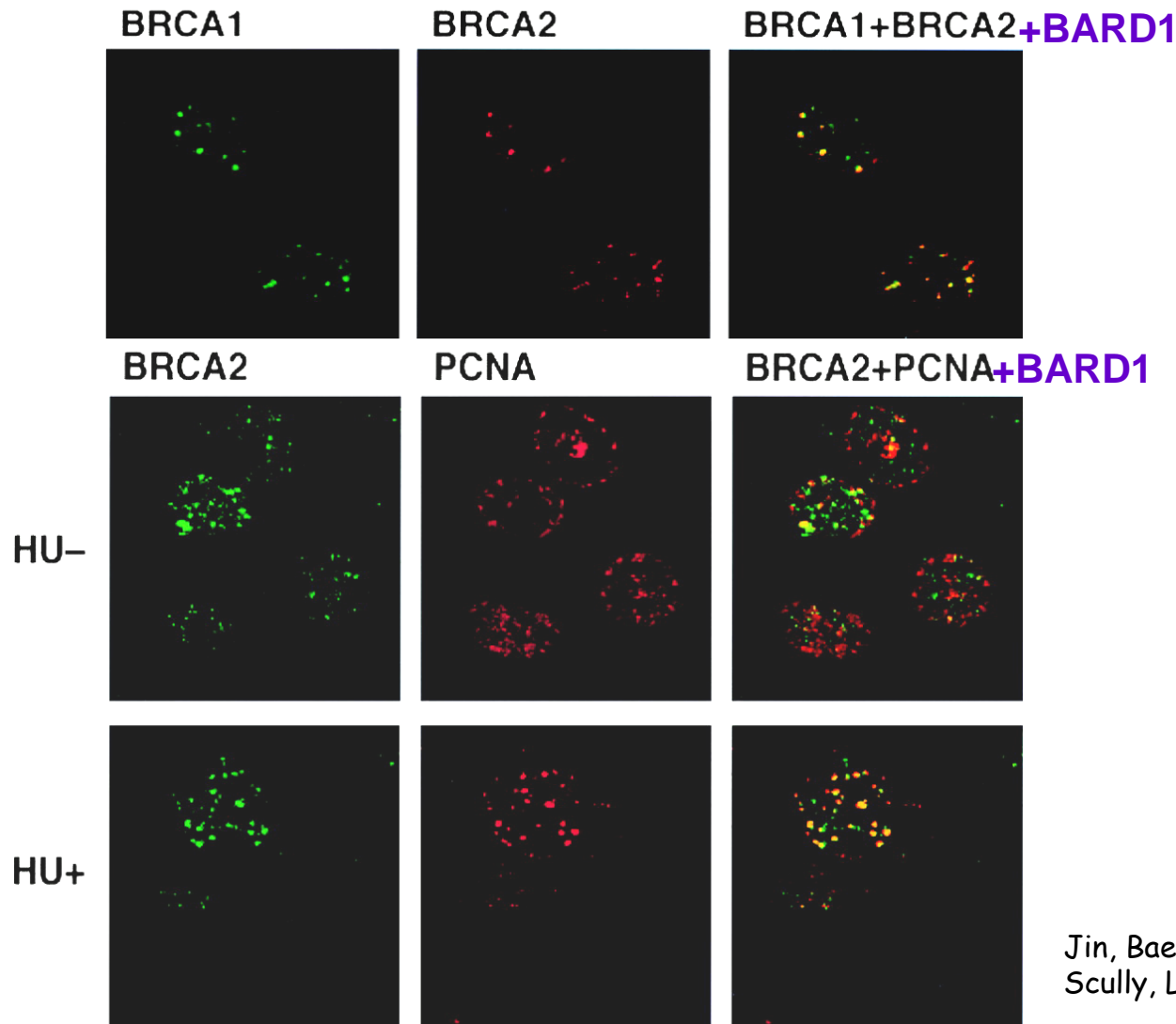
IP p53



BARD1 induces apoptosis by catalyzing phosphorylation of p53

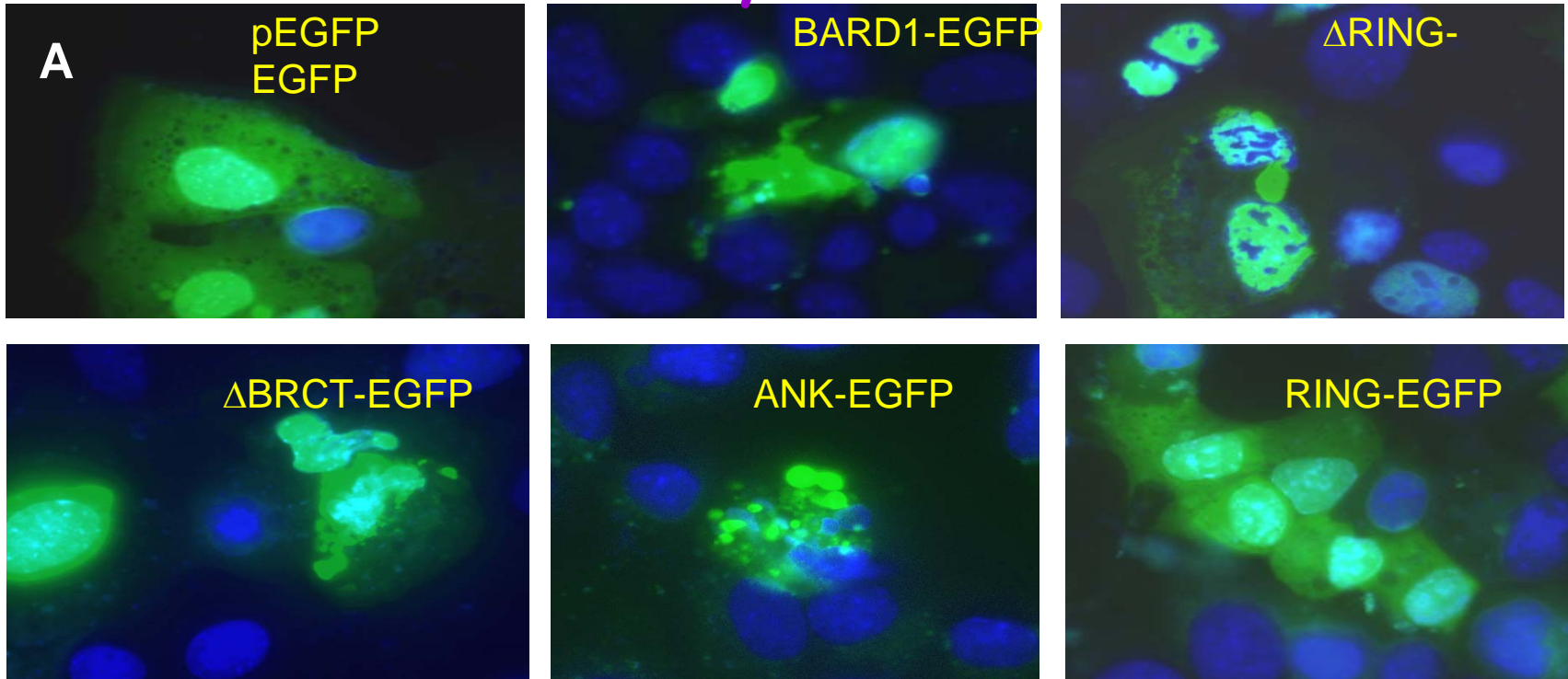
- BARD1 binds to p53 and to phosphorylated p53
- BARD1 binds to Ku-70, the catalytic subunit of DNA-PK but not ATM
- Exogenous expression of BARD1 in BARD1-deficient cells induces p53 phosphorylation
- Exogenous expression of BARD1 in BARD1 deficient cells restores apoptosis sensitivity

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

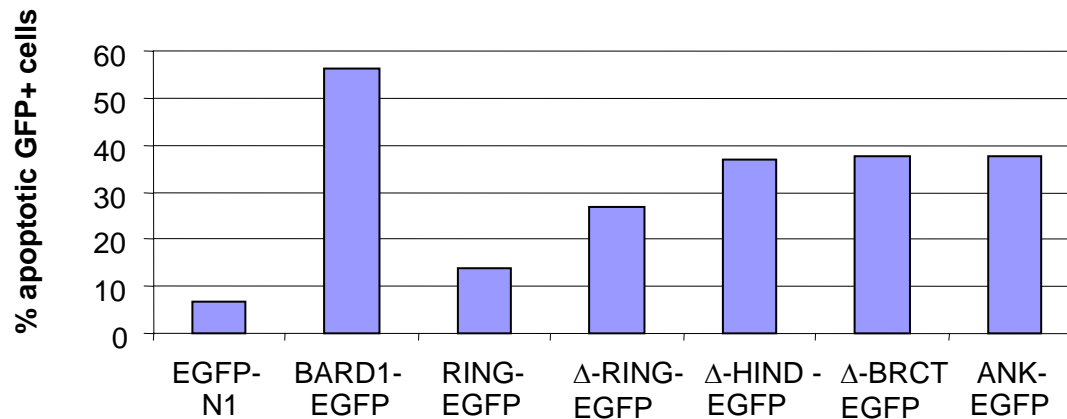


Jin, Baer, *JBC* 1997;
Scully, Livingston, *Cell* 1997

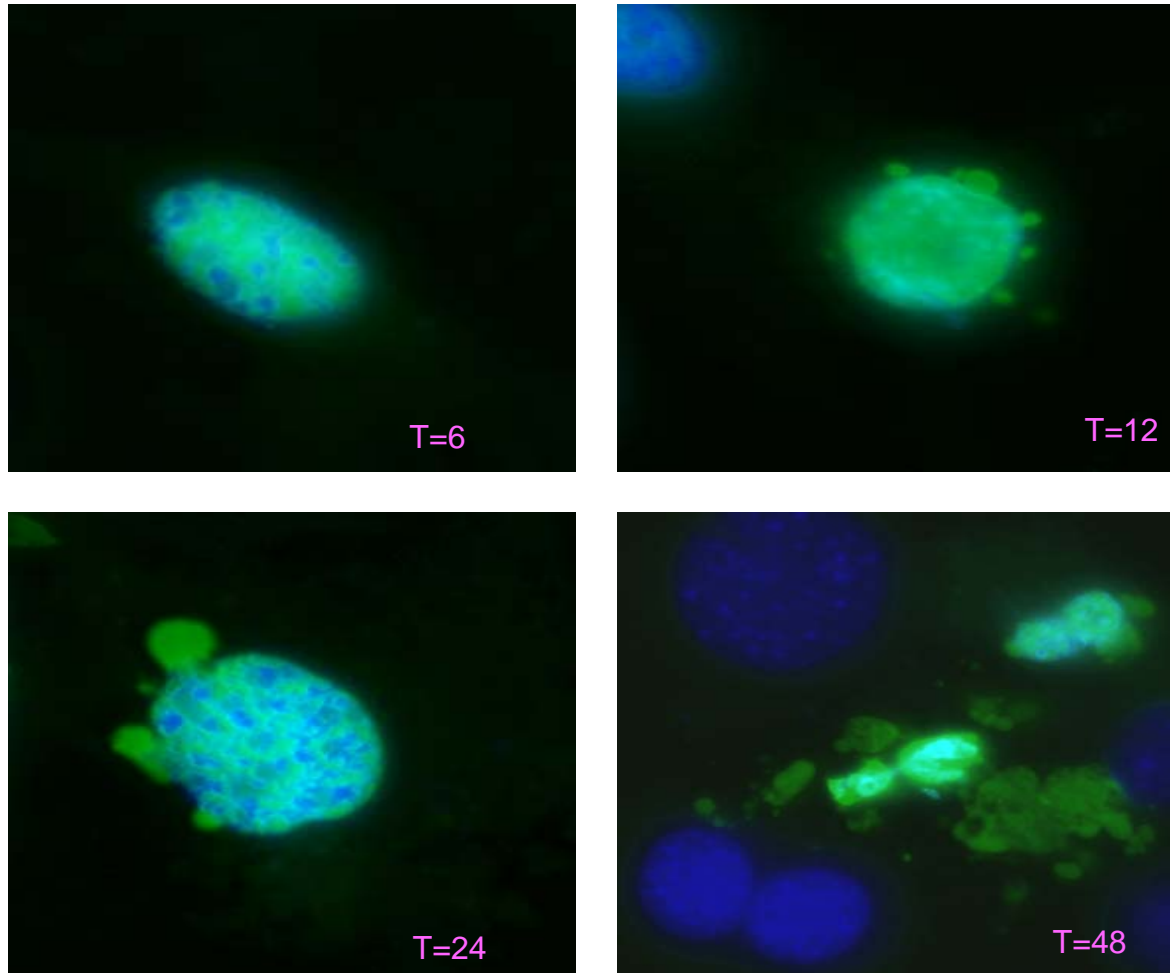
....but not only "nuclear dots"



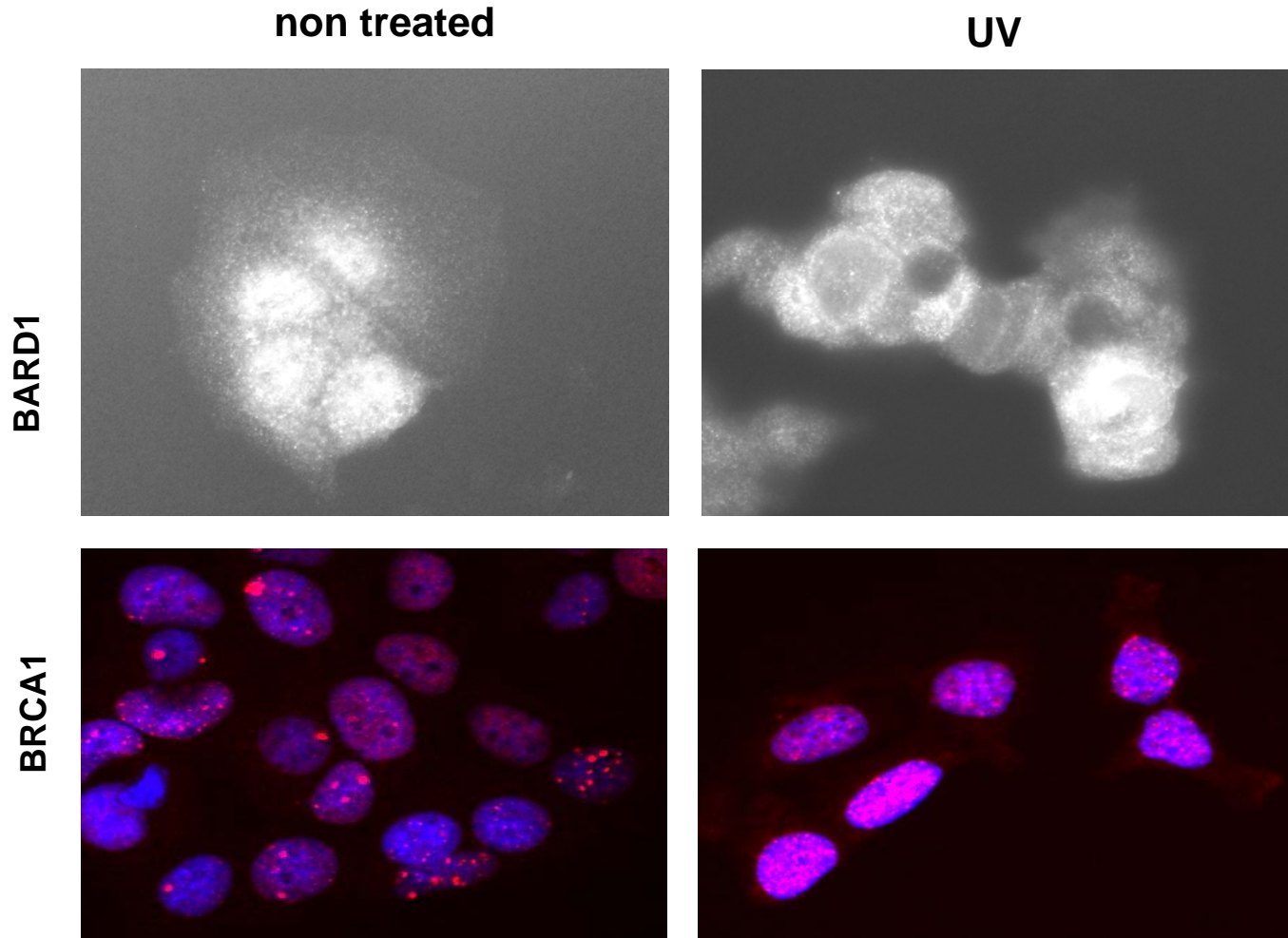
B



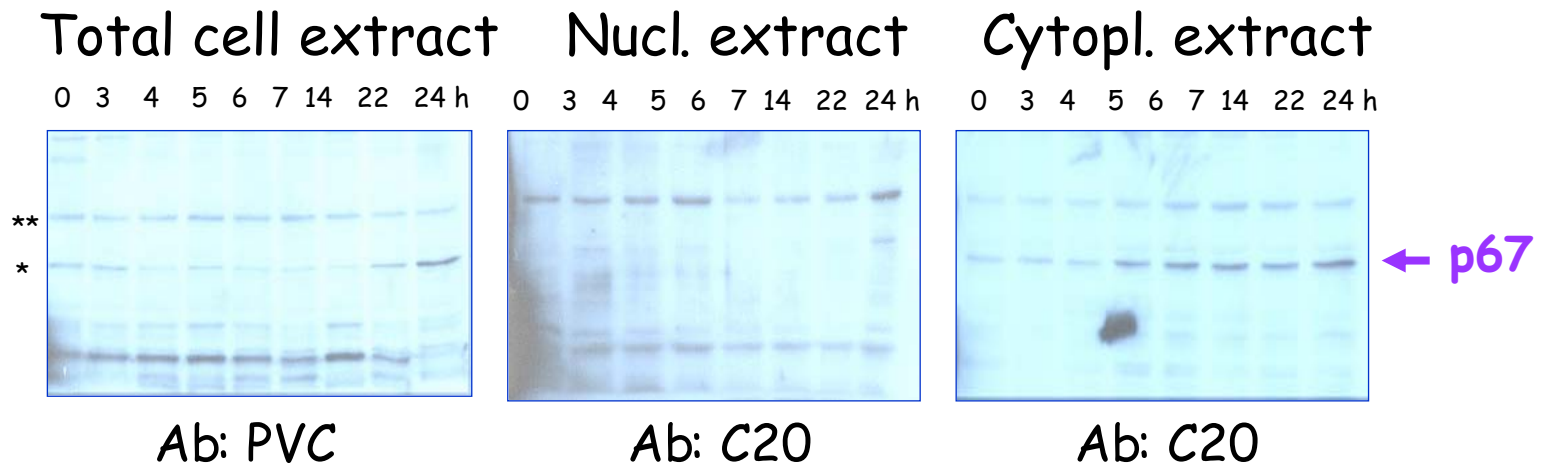
BARD1 translocates to the cytoplasm during apoptosis



Intracellular localization of BARD1 upon apoptosis induction



BARD1 is translocated to the cytoplasm during apoptosis



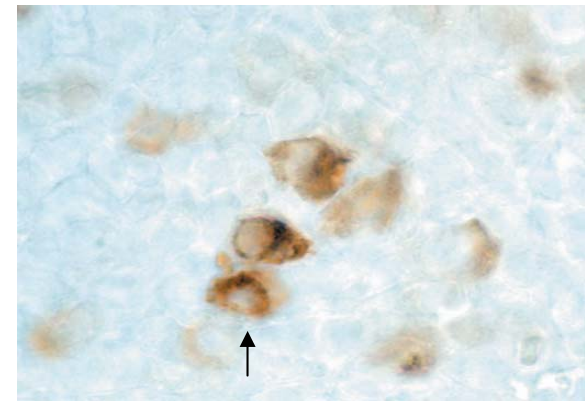
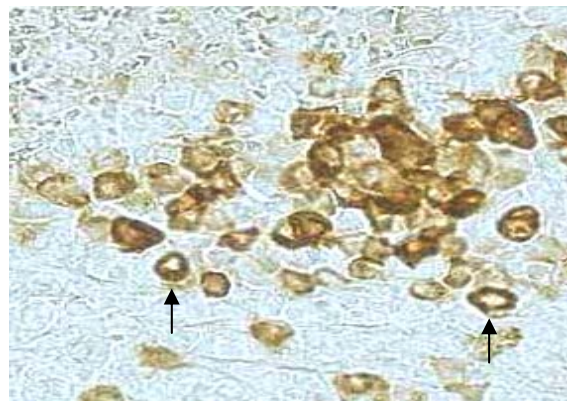
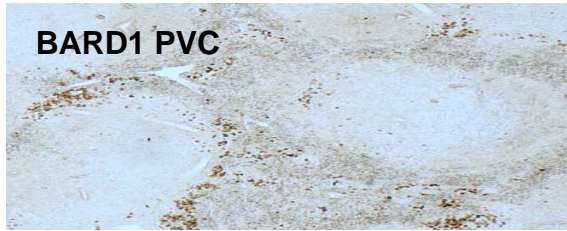
The C-terminal p67 is translocated to the cytoplasm and is immunogenic and anti-tumorigenic in an animal model of colon cancer (Gautier, Irminger-Finger et al., Cancer Res 2000).

Apoptotic function of BARD1 in vivo

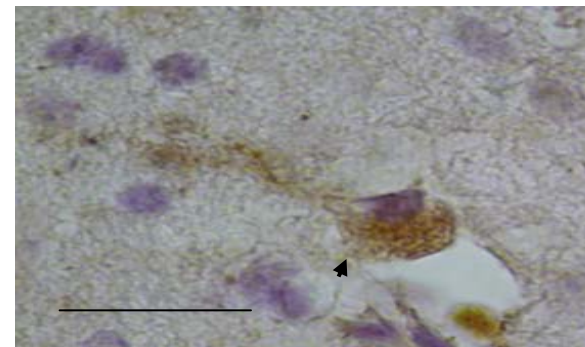
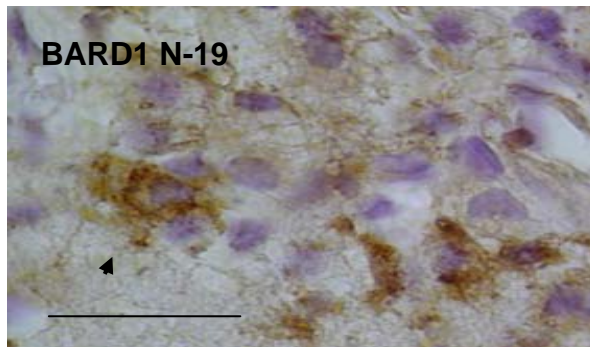
- In morphogenesis
spermatogenesis
- In homeostasis
Spleen, bone
- In tumor suppression

Cytoplasmic localization of BARD1 in vivo

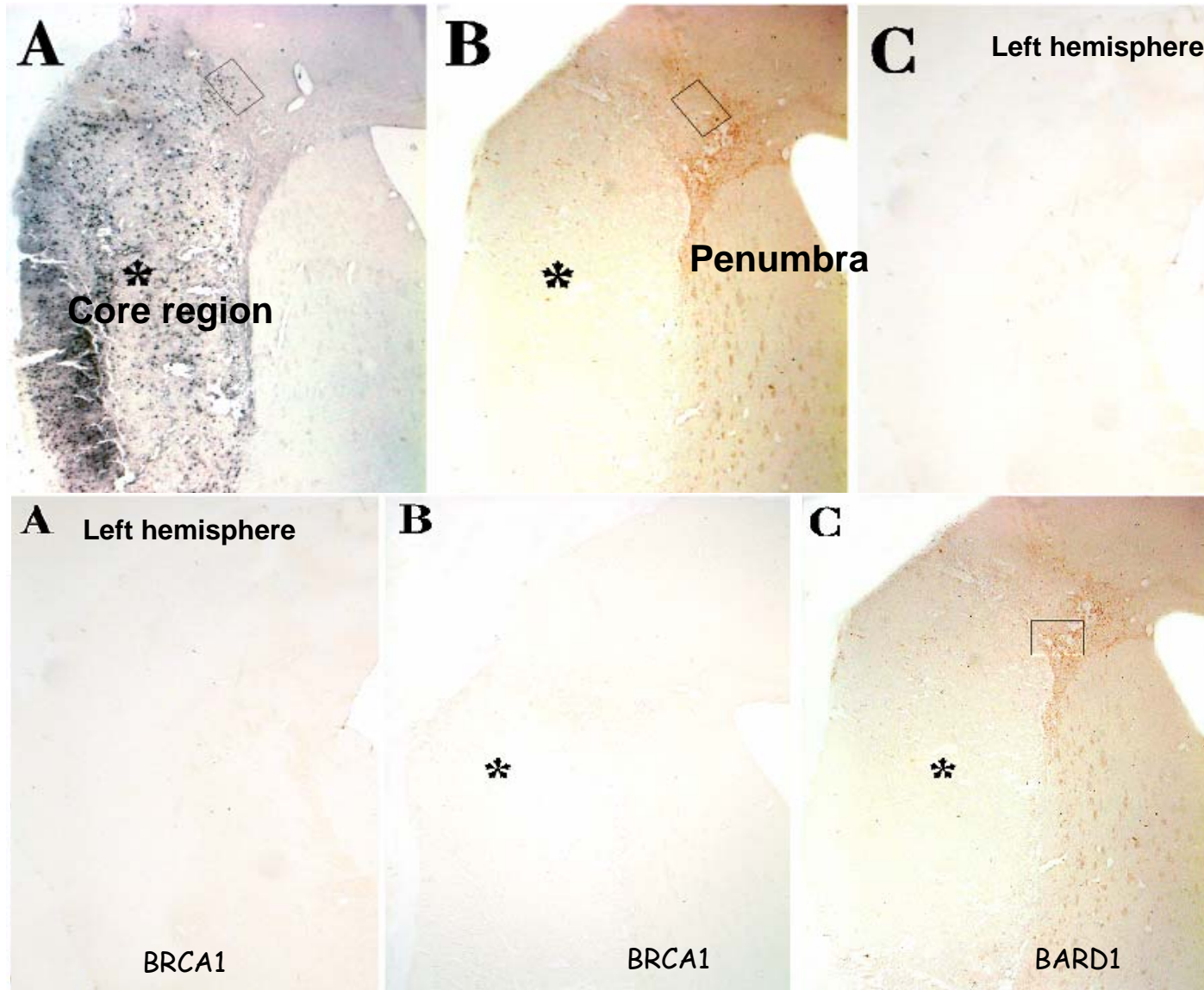
A



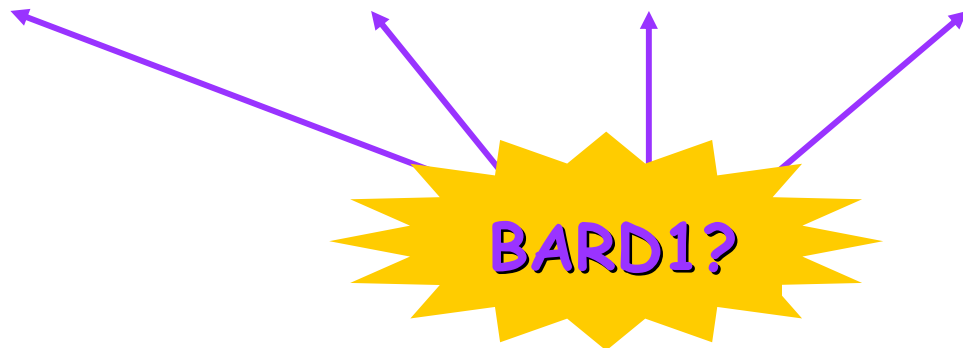
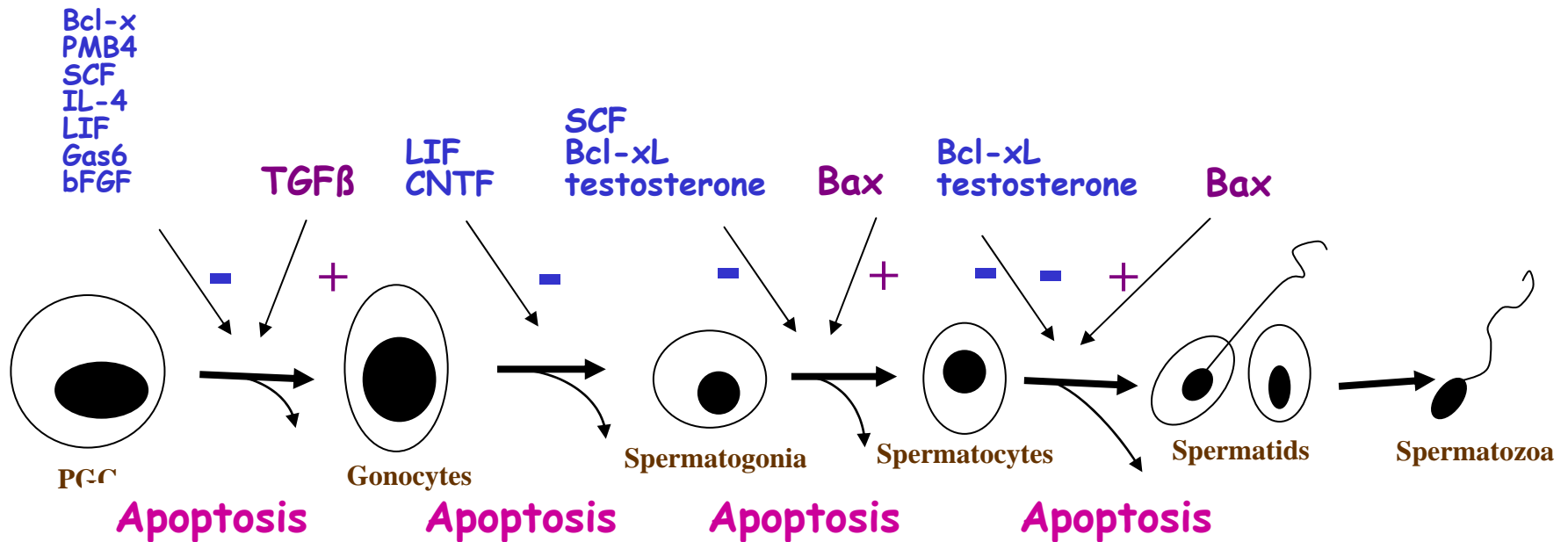
B



BARD1 upregulation in vivo by ischemia

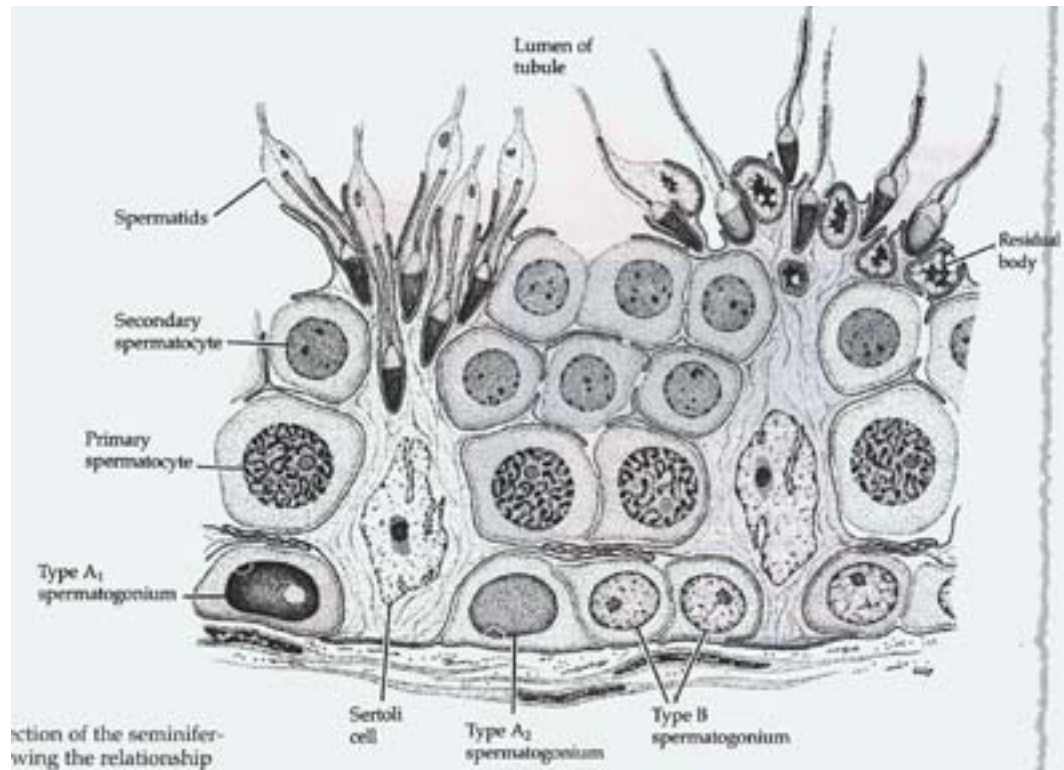


Apoptosis and (meiotic) repair during spermatogenesis



Modified from:
Print, *Bioassays*, 2000

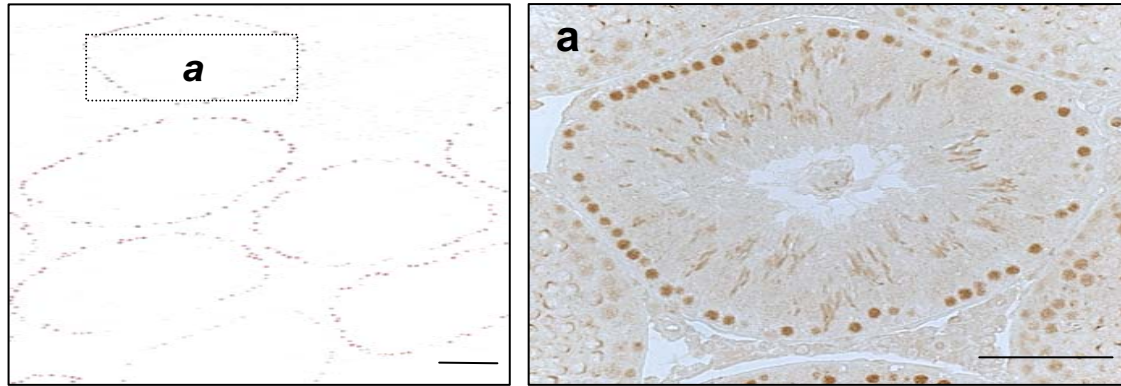
Schematic diagram of seminiferous tube



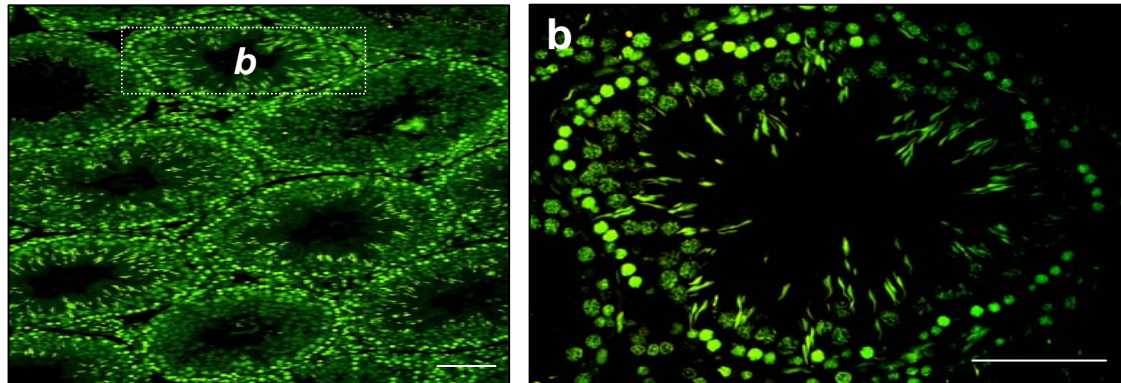
BARD1 expression is correlated with apoptosis markers

A

anti-BARD1



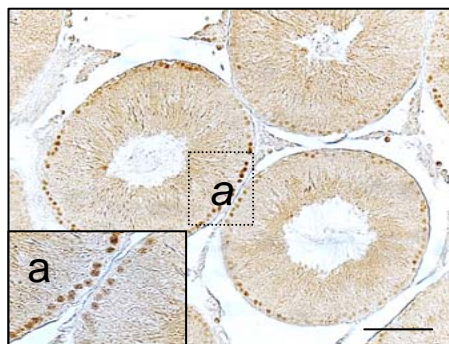
TUNEL



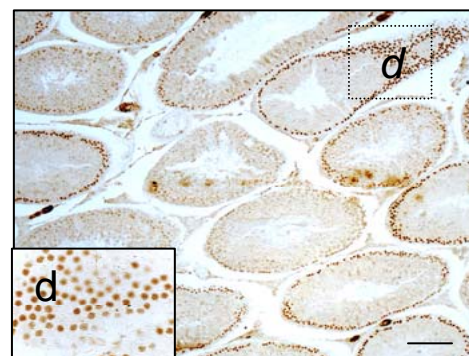
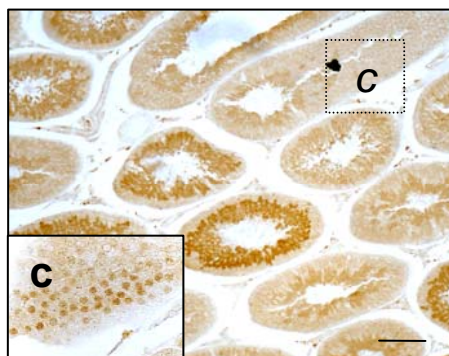
B

activated caspase 3

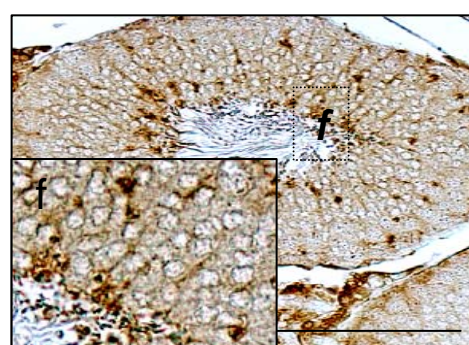
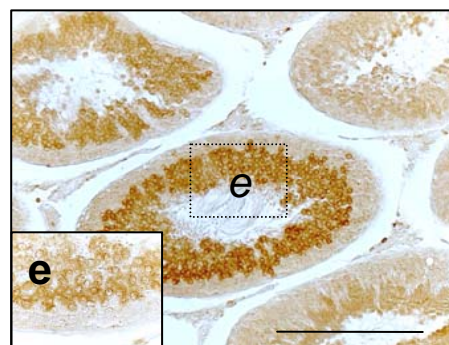
anti-BARD1



N-19

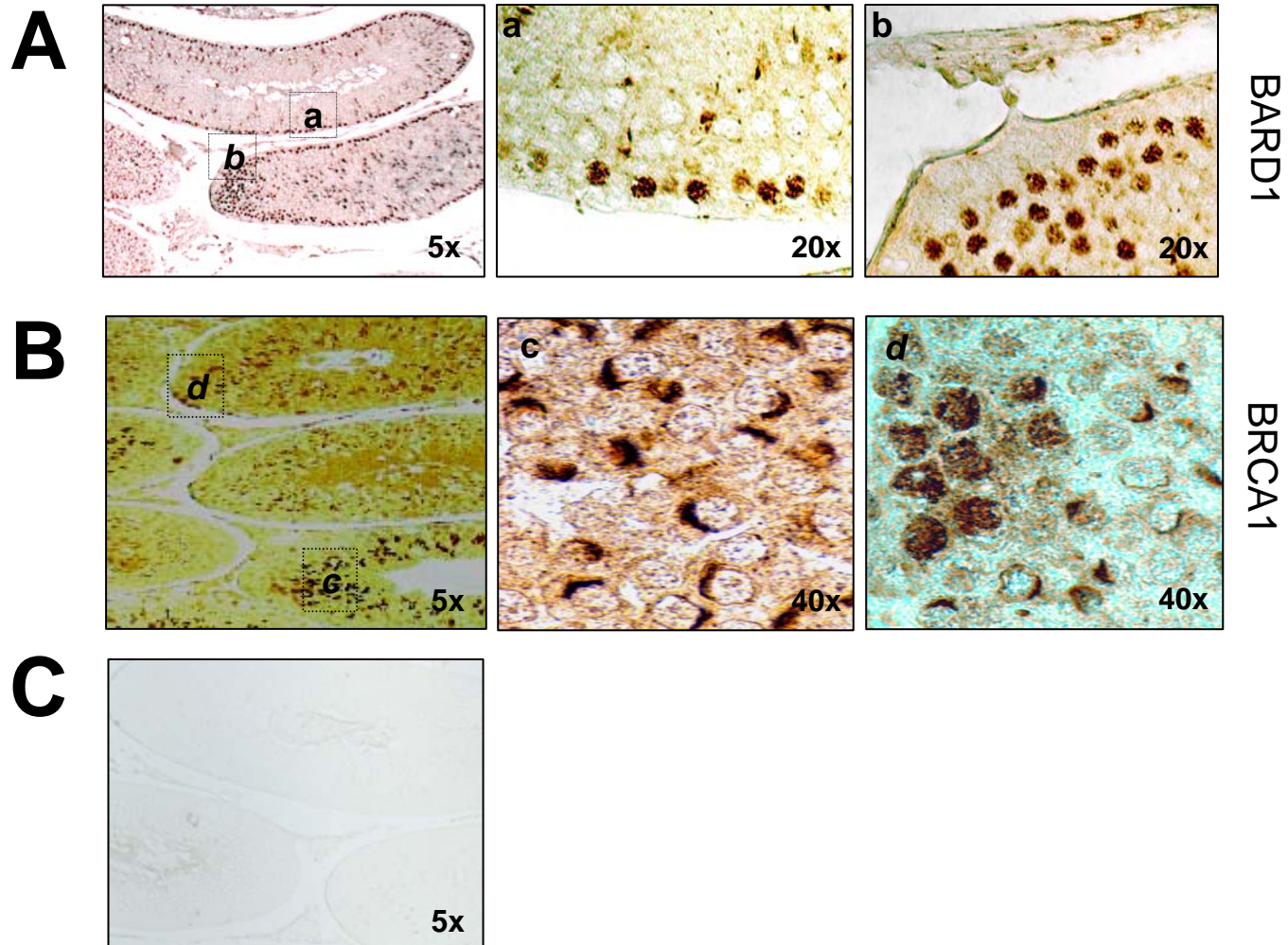


N-19

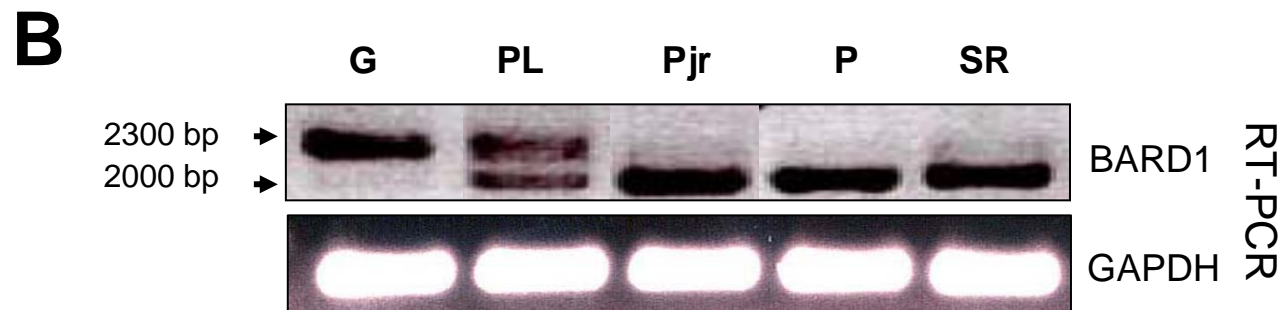
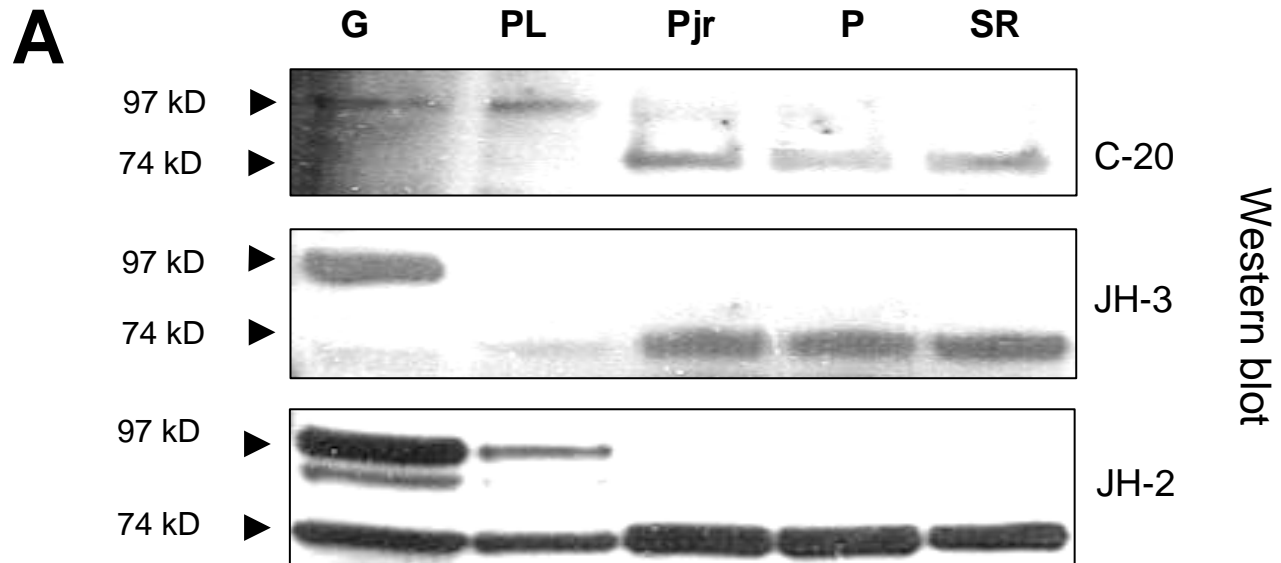


JH3

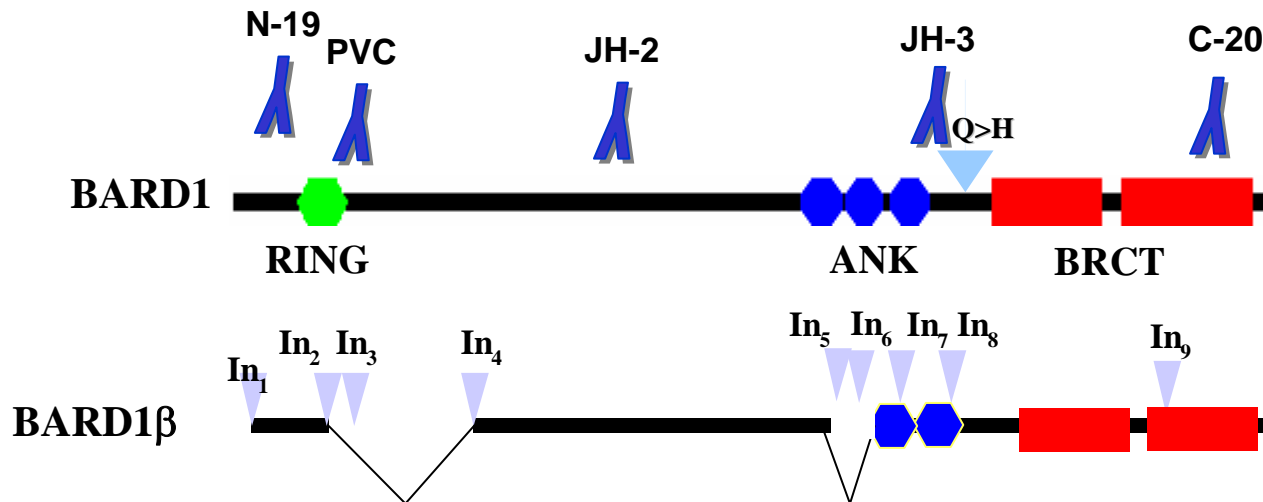
BARD1 and BRCA1 expression overlap partially



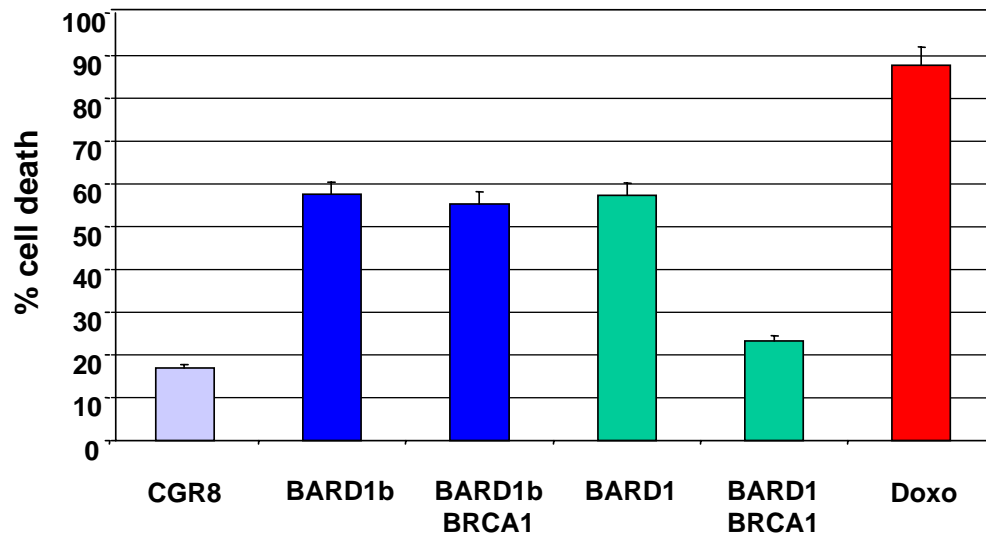
Different BARD1 isoforms are expressed at different stages



BARD1 β lacks the RING finger



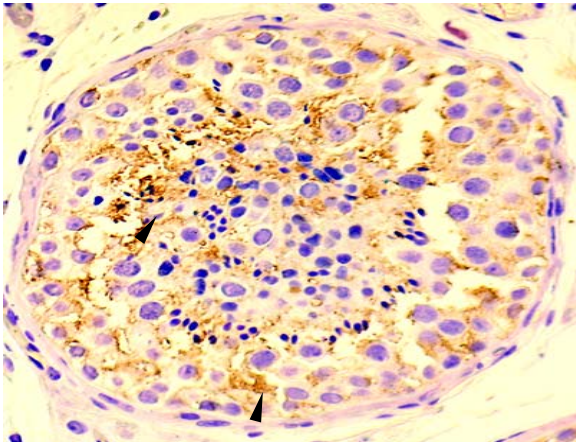
BARD1 β is an efficient apoptosis inducer



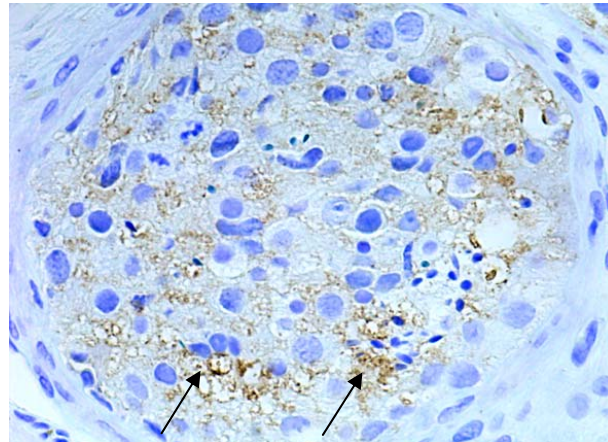
BARD1 expression associated with abortive spermatogenesis

A

Obstructive Azoospermia

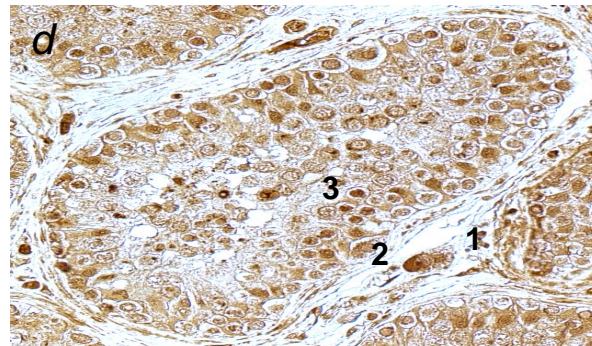
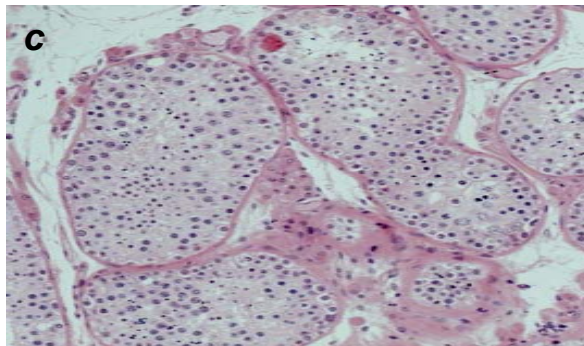


Sertoli cell Syndrome

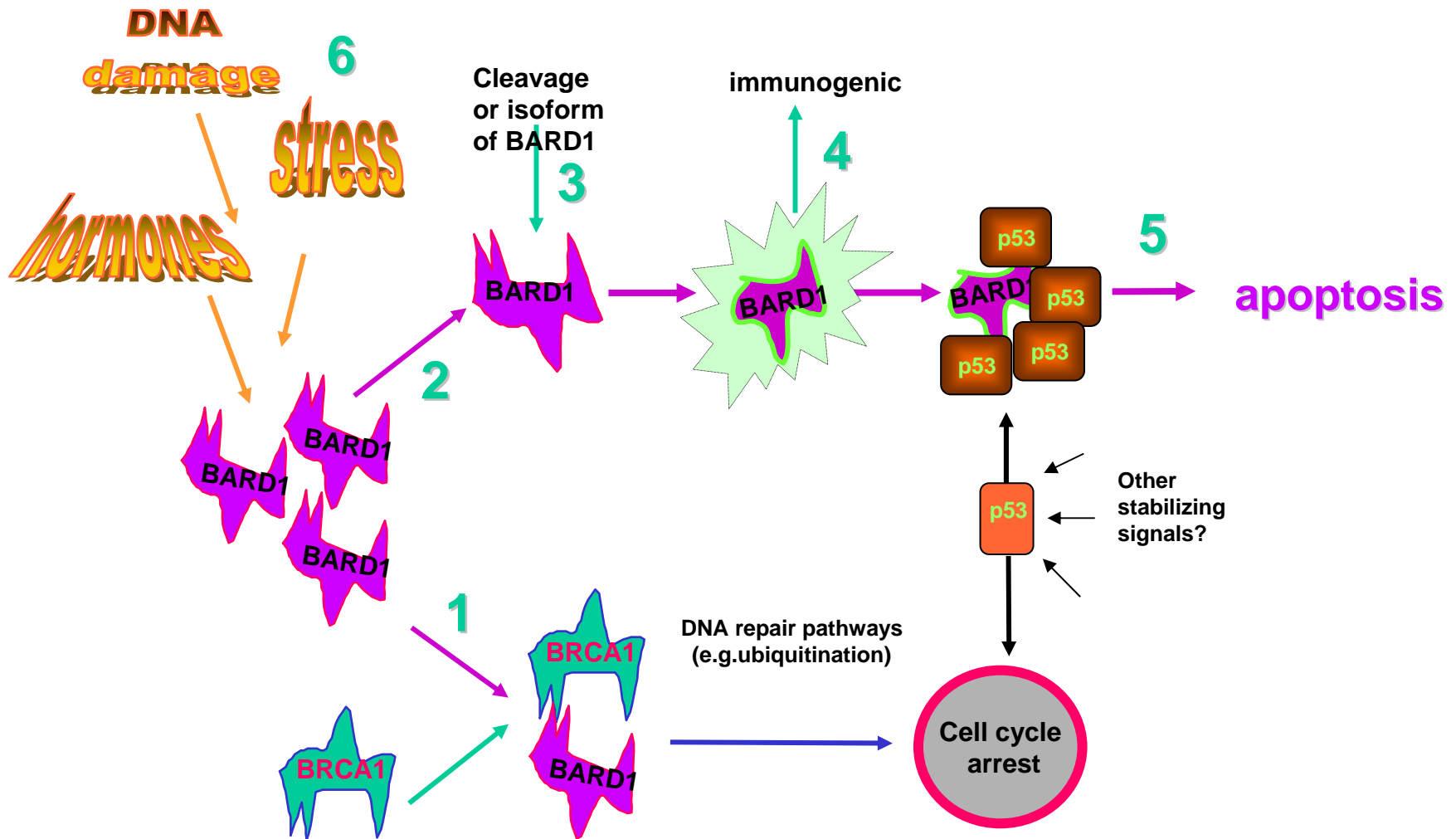


B

cryptorchidy

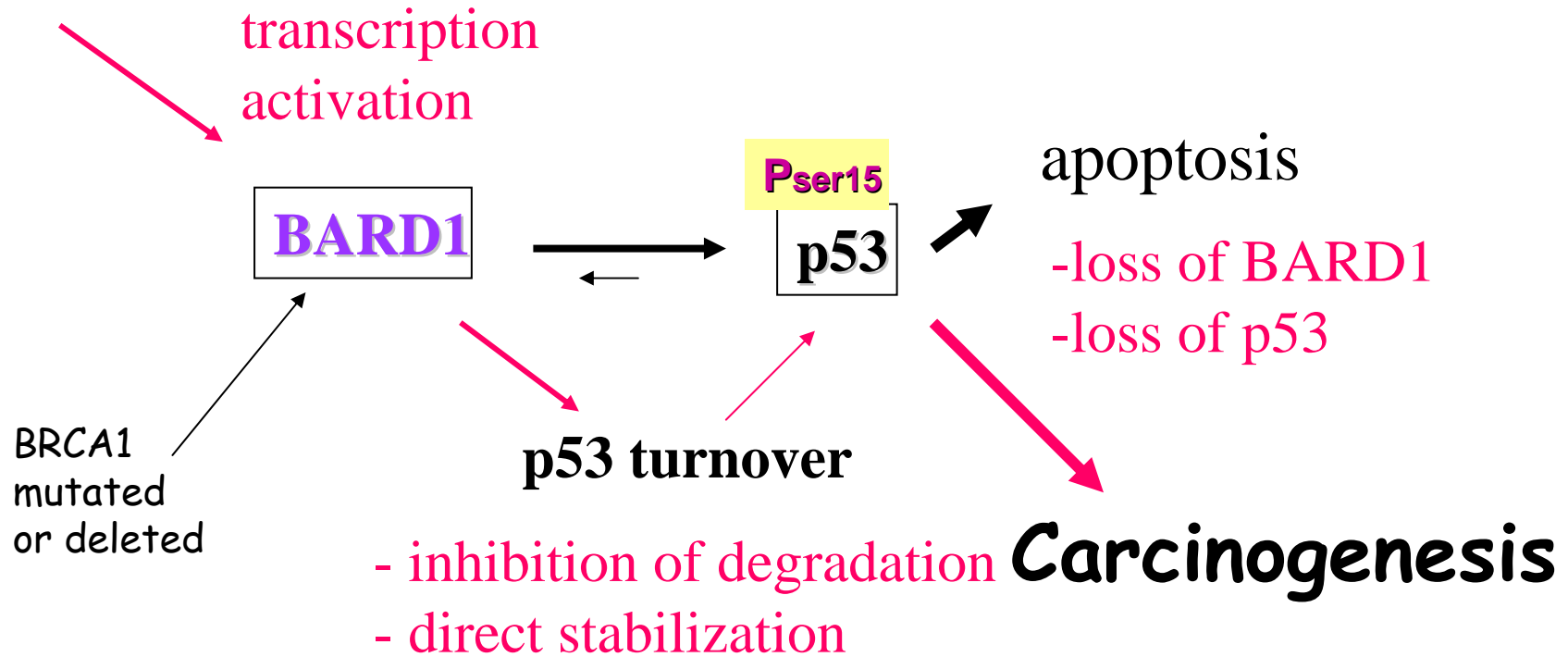


Presumed tumor suppressor pathways of BARD1

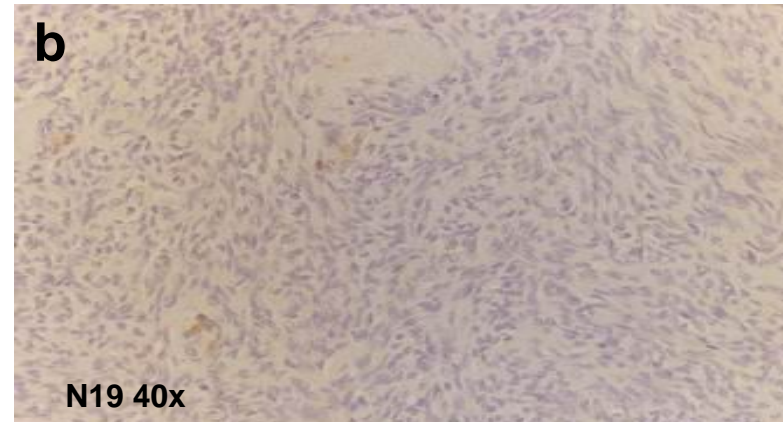
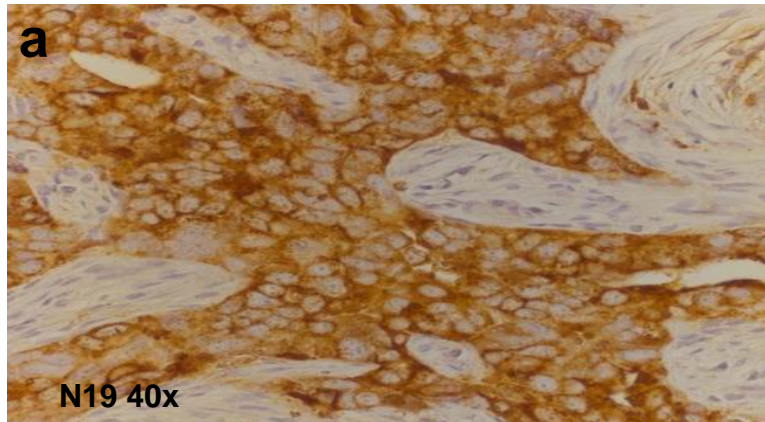
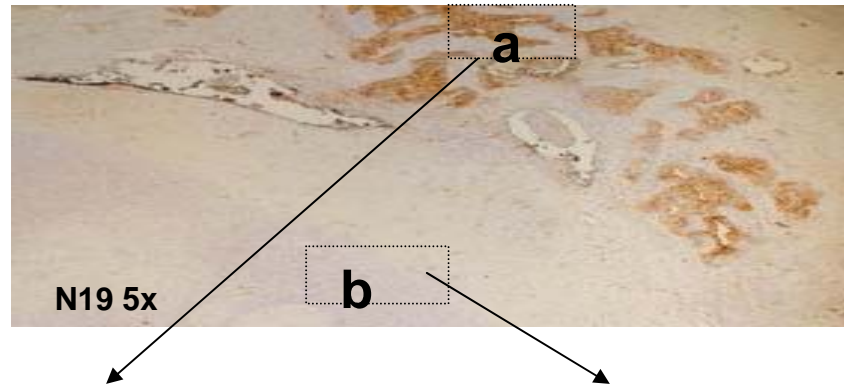


Expected loss of BARD1 in tumorigenesis

Carcinogenic stress

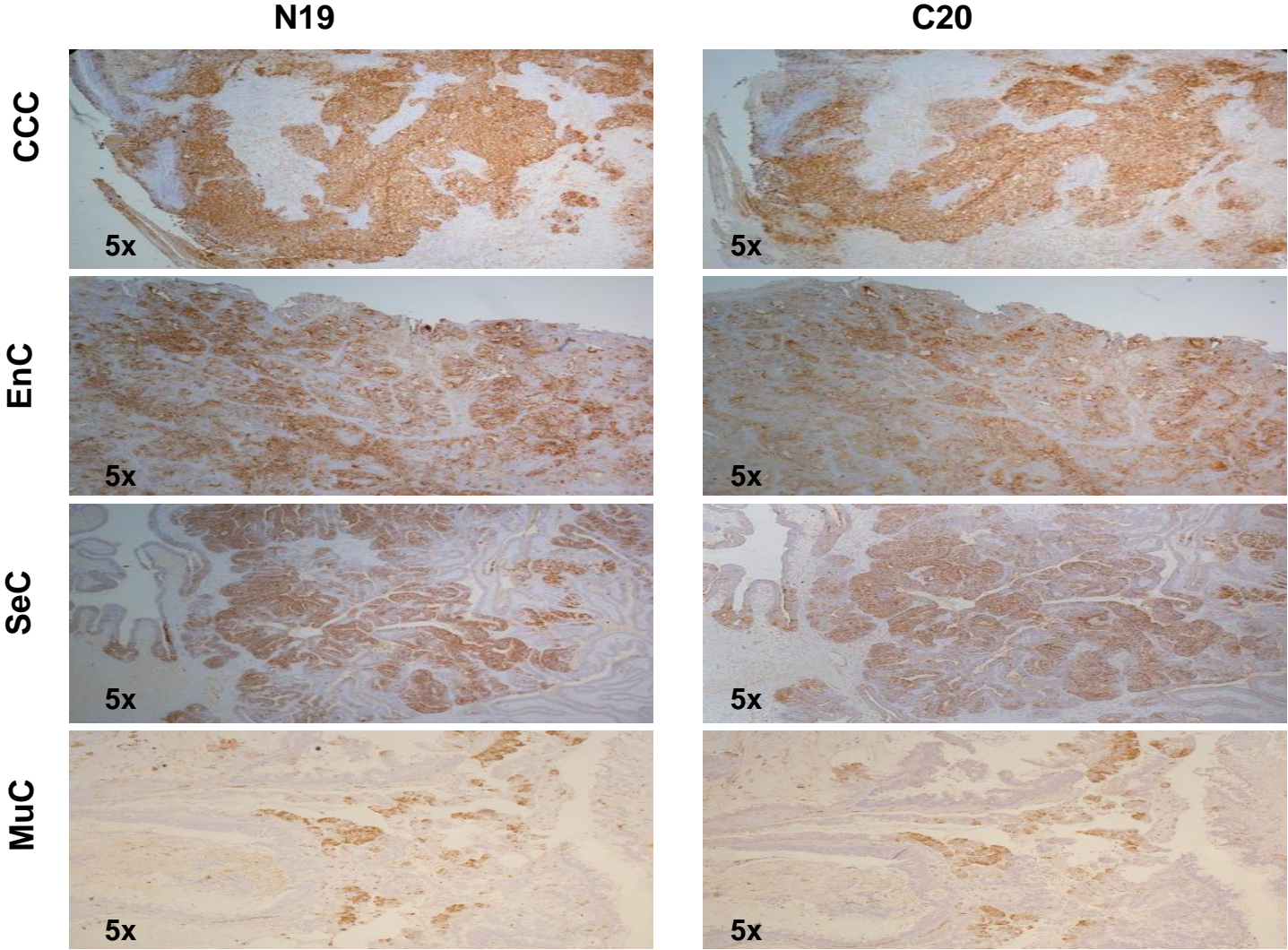


However, BARD1 is upregulated in tumors

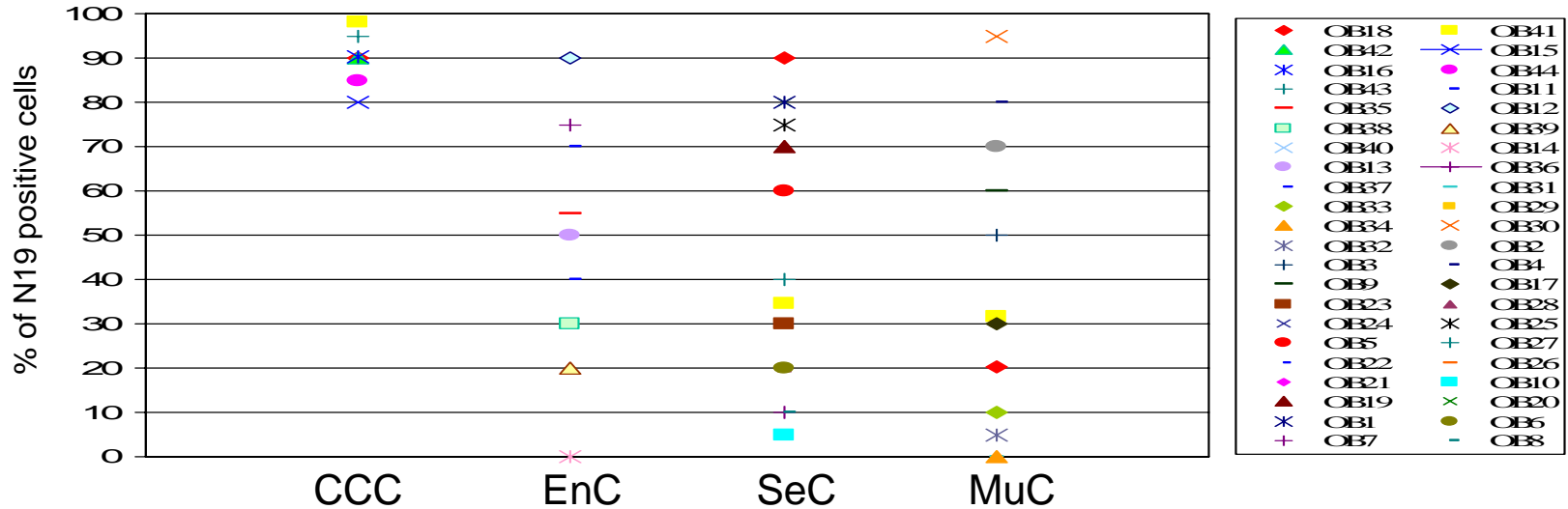
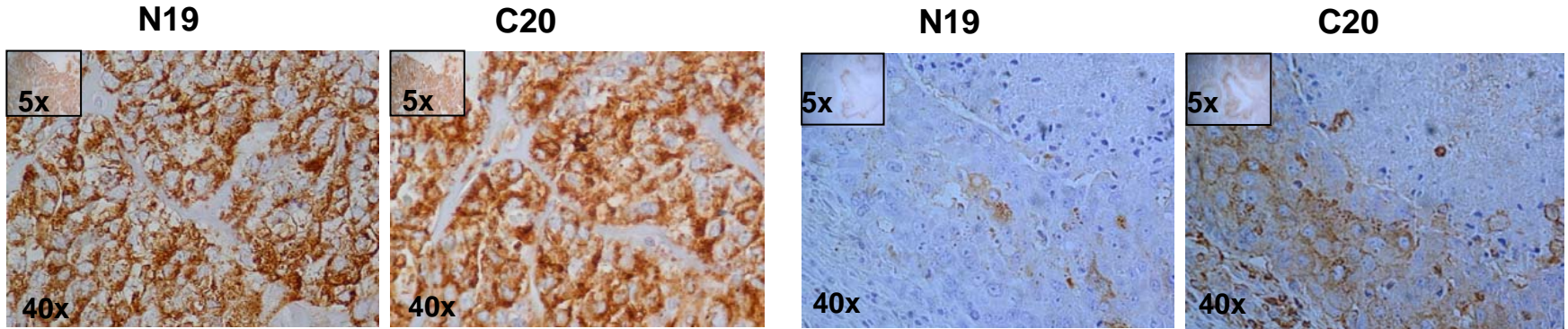


BARD1 expression is specific for tumor type

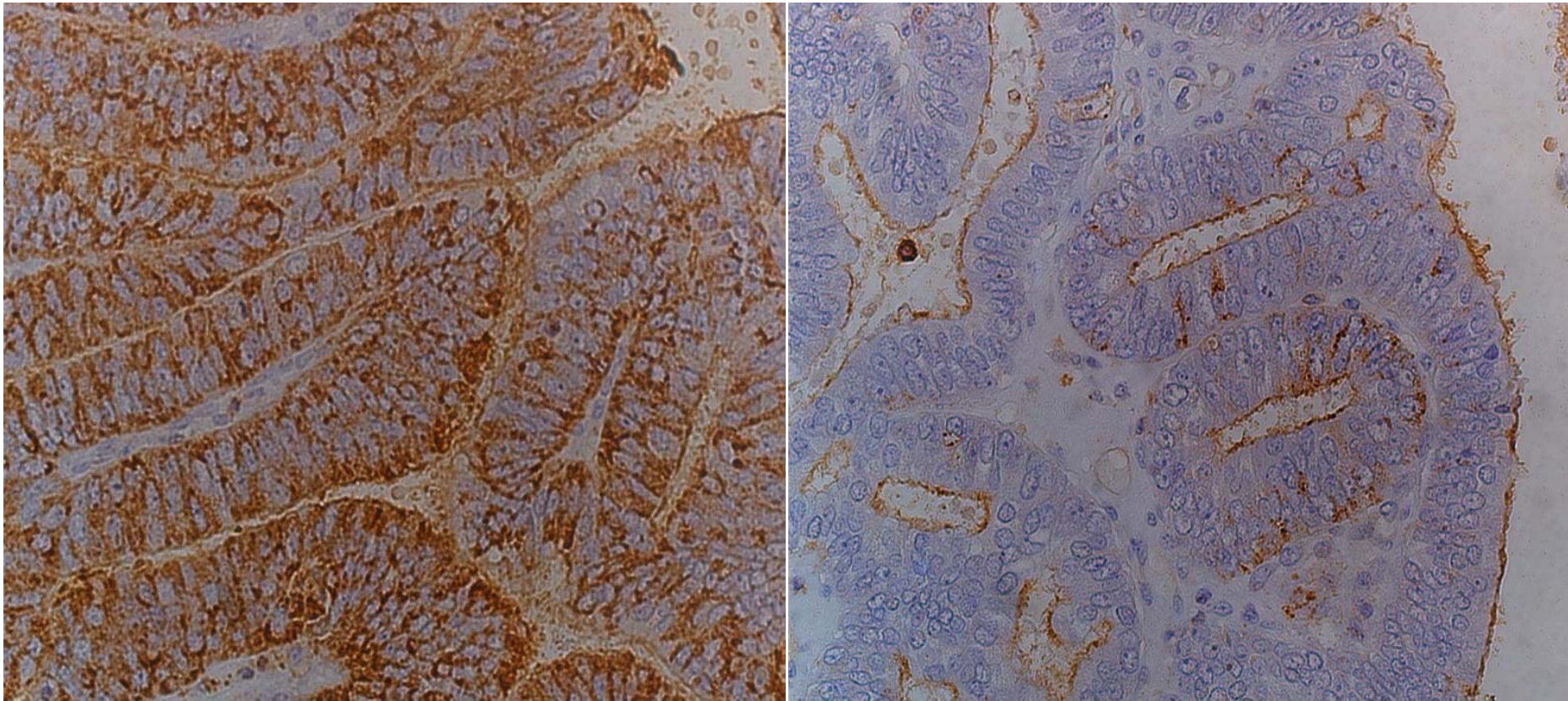
A

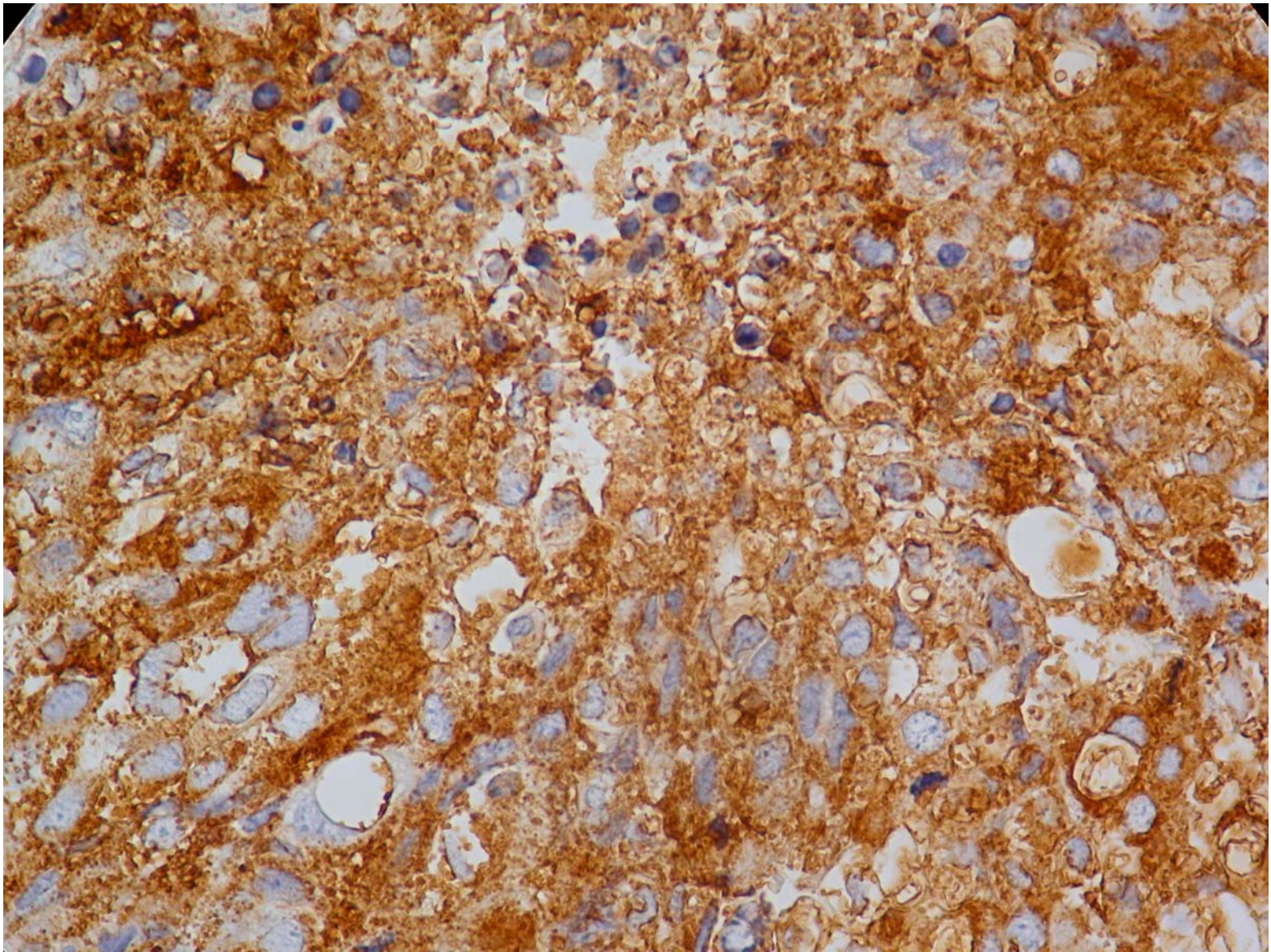


Expression of N- and C-terminal epitopes



Correlation of BARD1 expression with tumor grade



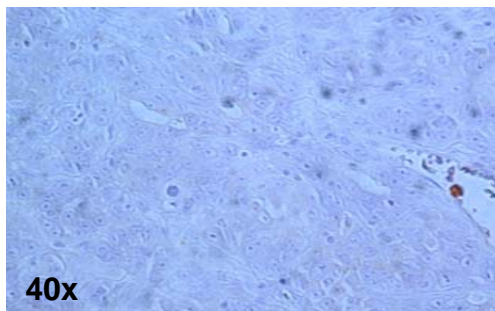
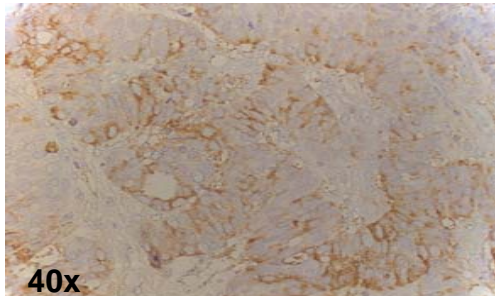


OB16 A1 Clair cell carcinoma C20 x40

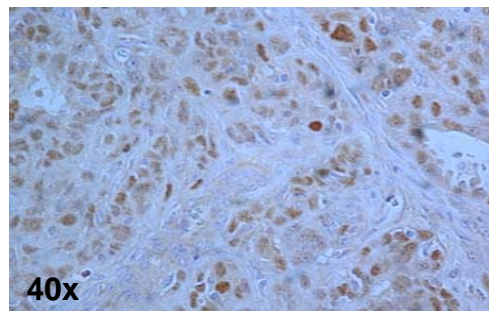
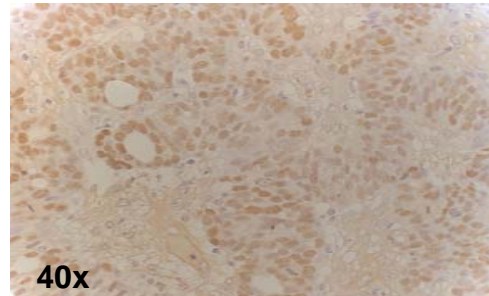
BARD1 but not p53 is correlated with tumor type

A

N19

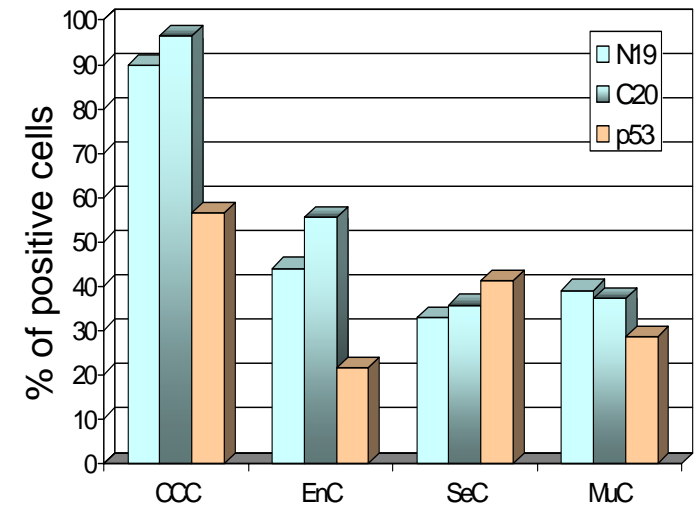


p53



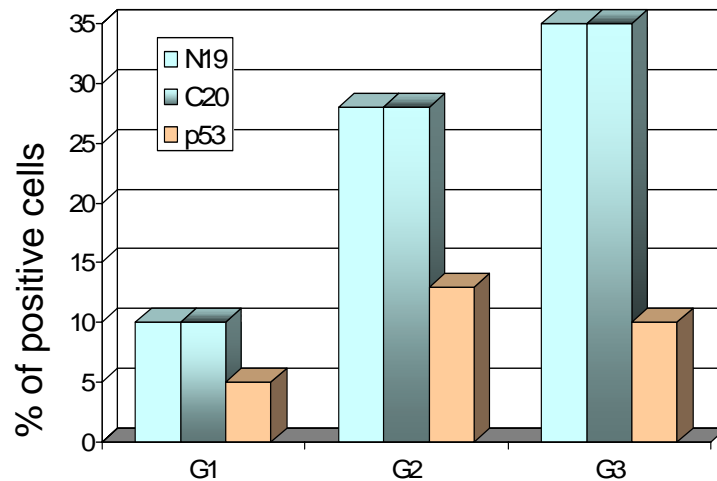
B

Correlation of BARD1 and p53 with histological type of ovarian carcinoma

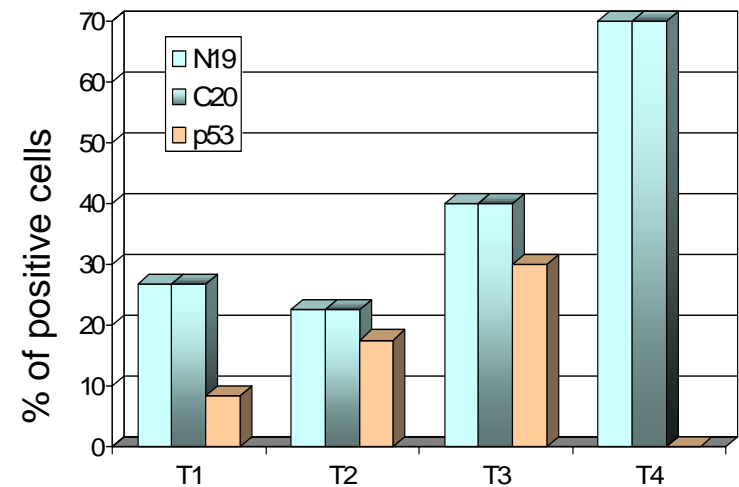


Correlation of BARD1 expression with tumor grade and stage

Correlation of BARD1 and p53 with differentiation in sporadic breast cancer

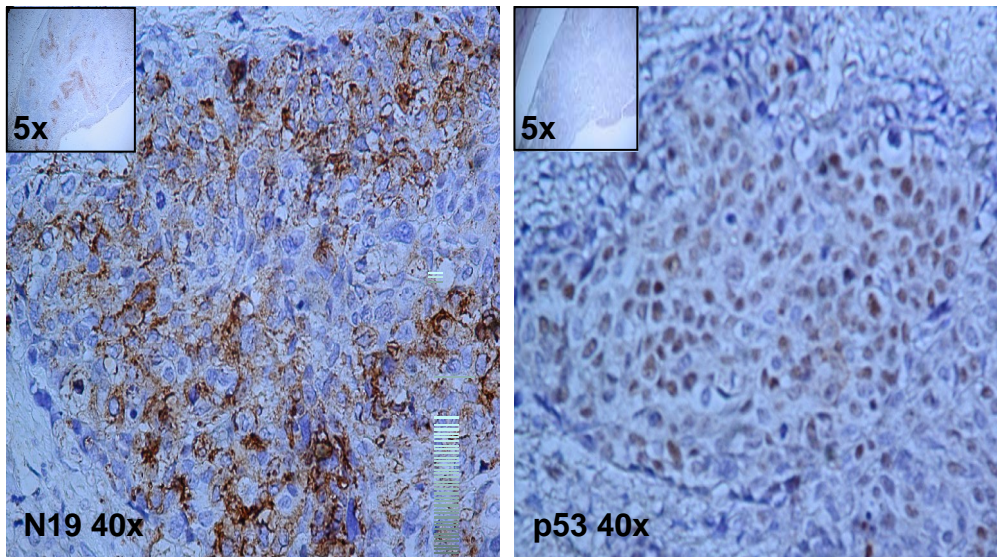


Correlation of BARD1 and p53 with tumor size in sporadic breast cancer



No correlation of BARD1 and p53 expression in NSCLC

A



B

Distribution of N19 expression in different pathological types

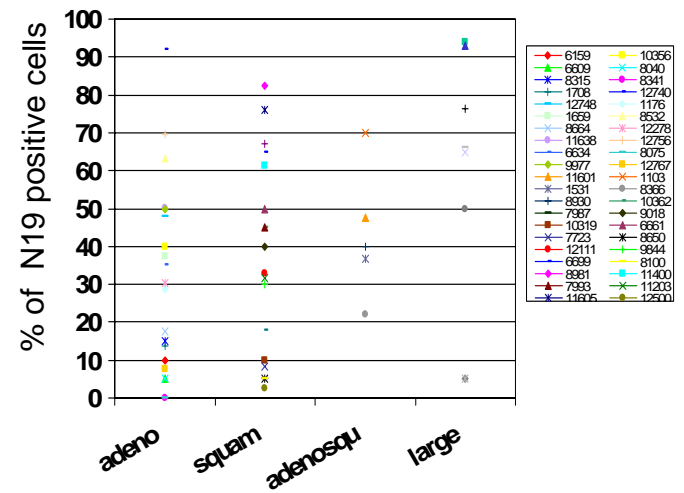
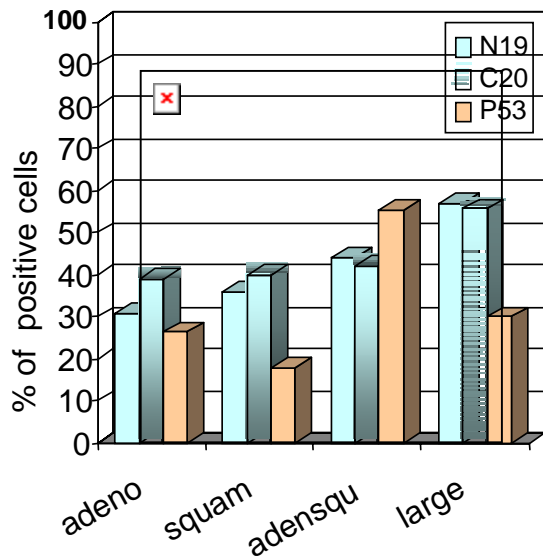


Figure 5

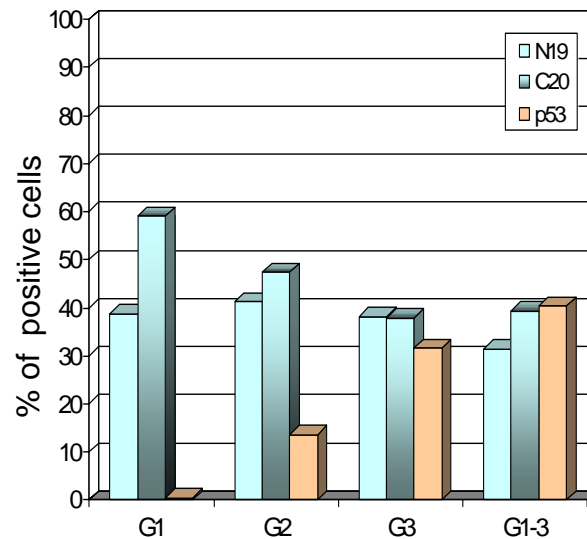
No correlation of BARD1 expression with tumor grade and stage in NSCLC

C

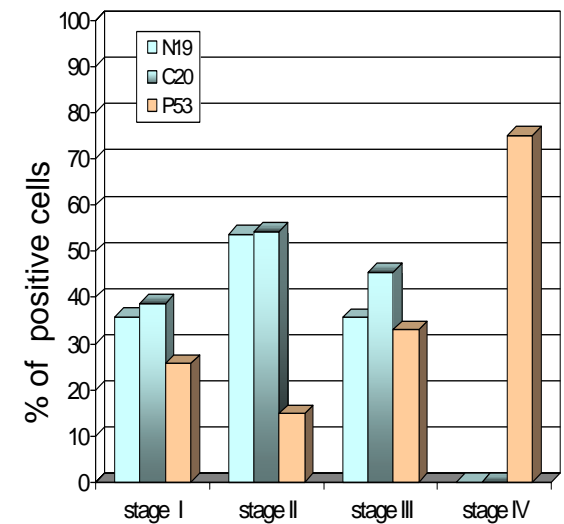
BARD1(N19/C20) and p53 in different pathological type



BARD1(N19/C20) and p53 in different pathological grade



BARD1(N19/C20) and p53 in different stages



Tumors express an aberrant form of BARD1

A

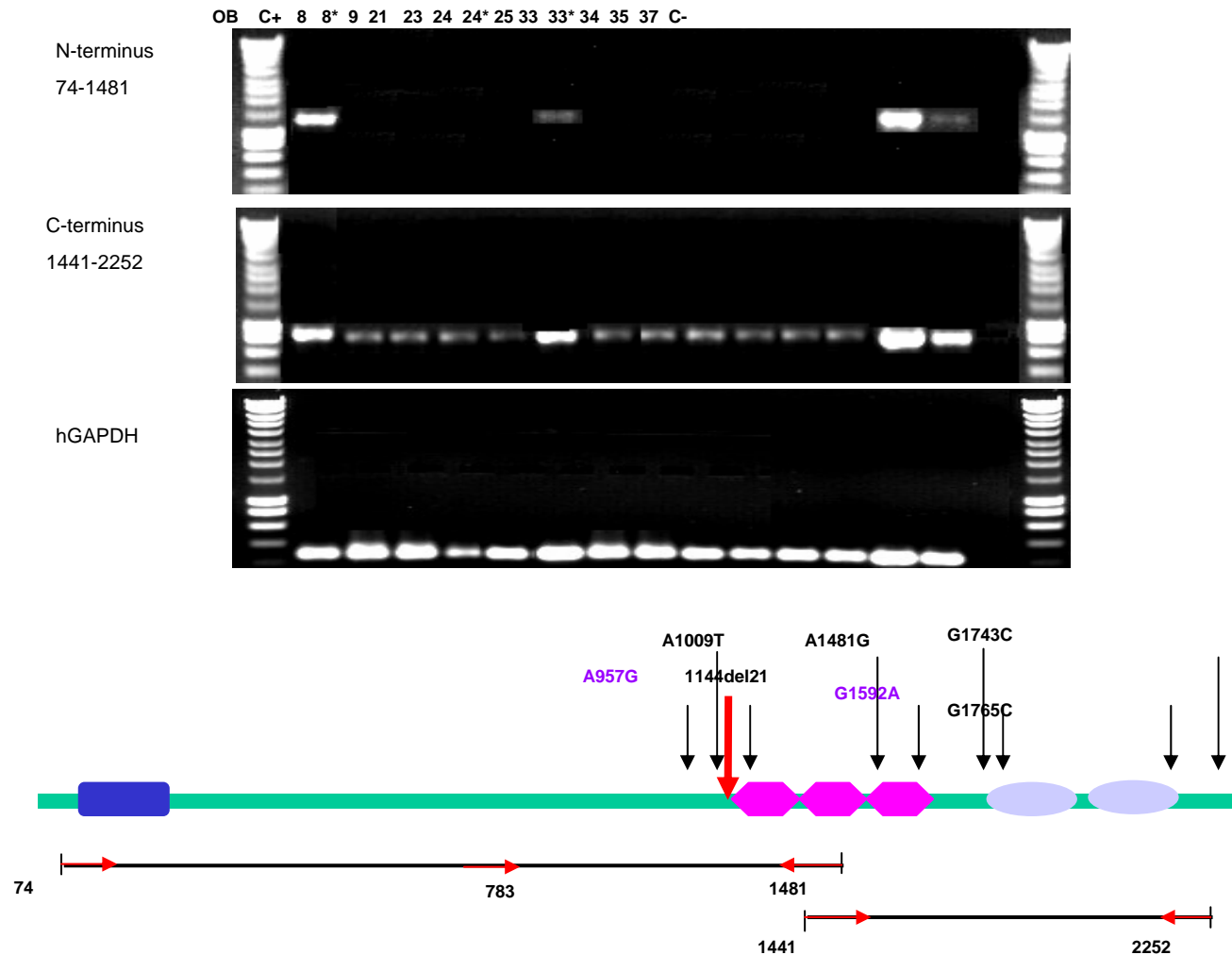


Figure 6

BARD1 is transcriptionally altered in rat ovarian cancer cells and in human cancers?

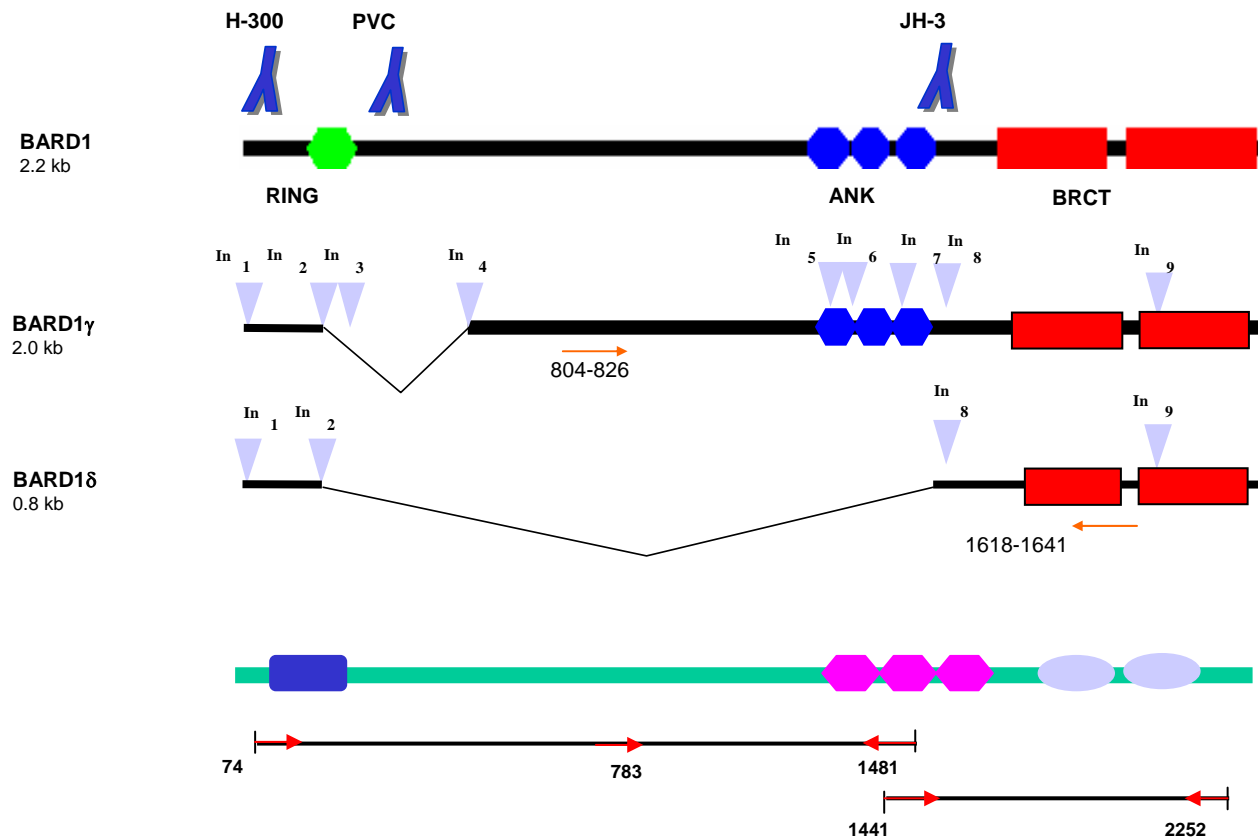
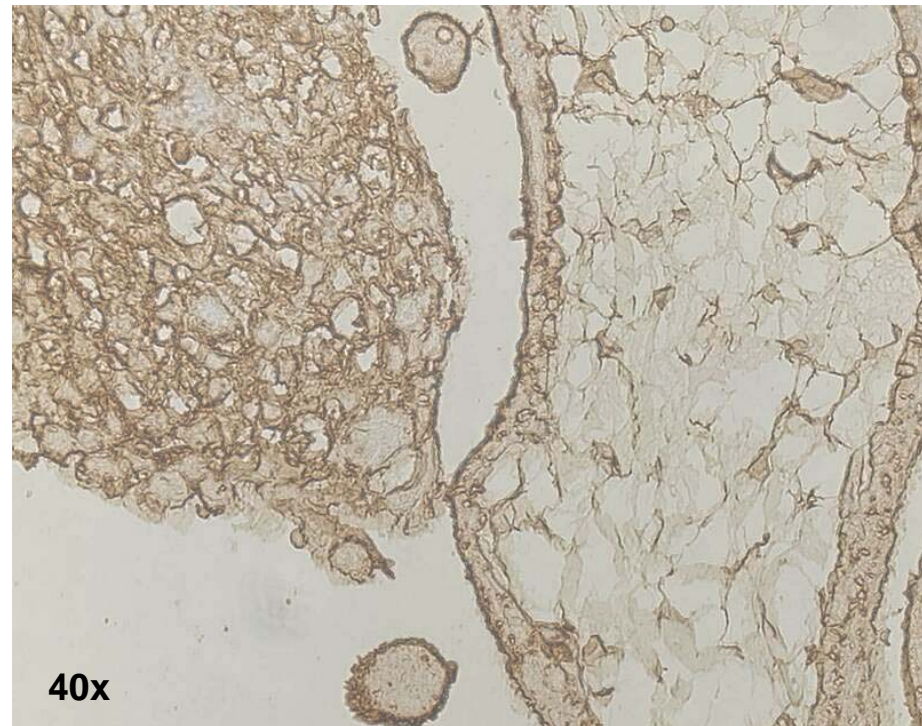
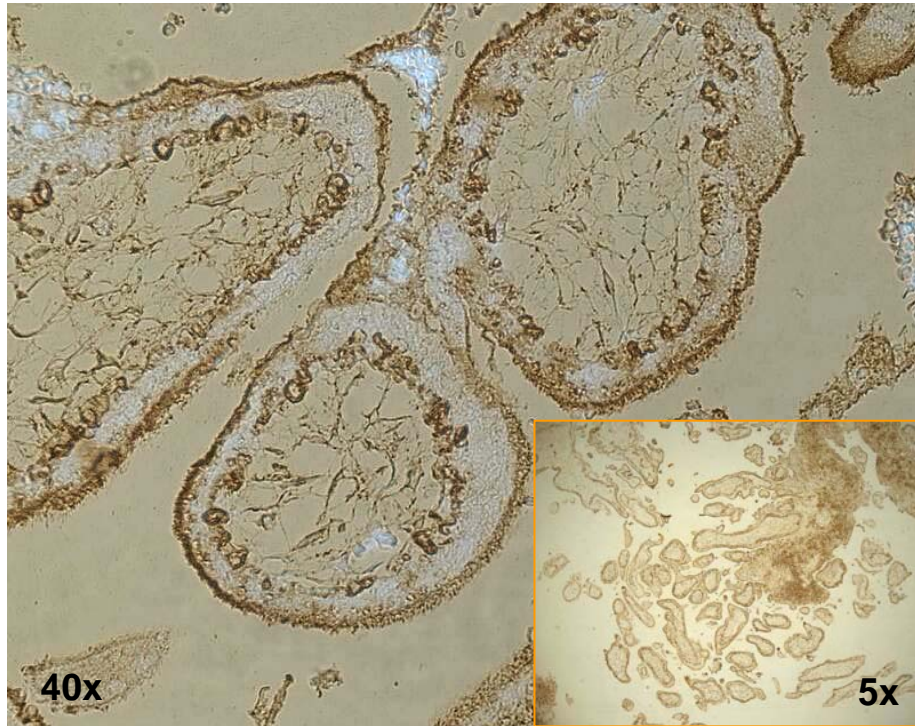


Figure 5A-C

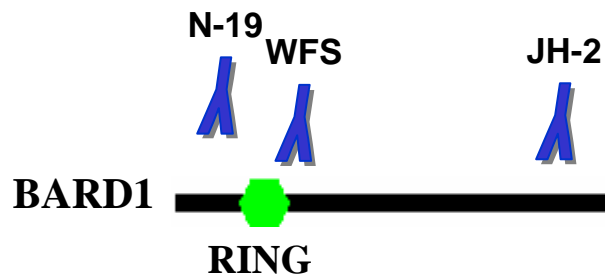
BARD1 expression in human cancers

- Elevated expression of BARD1 in cancer
- Cytoplasmic localization of BARD1 in tumors
- Loss of 5' region
- Expression is correlated with tumor type, grade and stage in breast and ovarian cancer
- No correlation in NSCLC
- No correlation of BARD1 and p53 expression

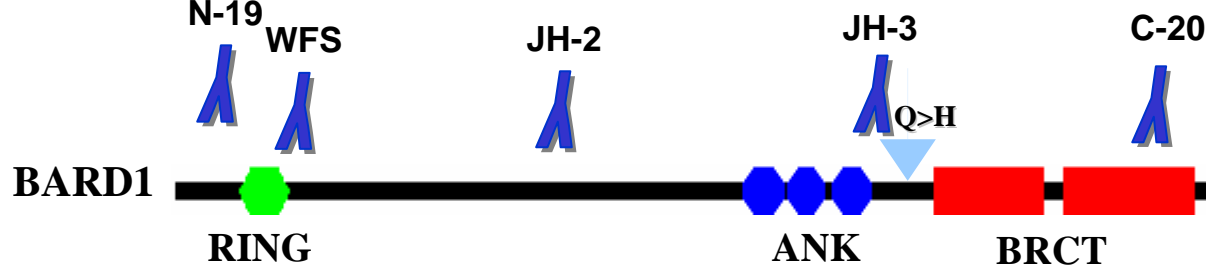
BARD1 expression in human cytotrophoblast



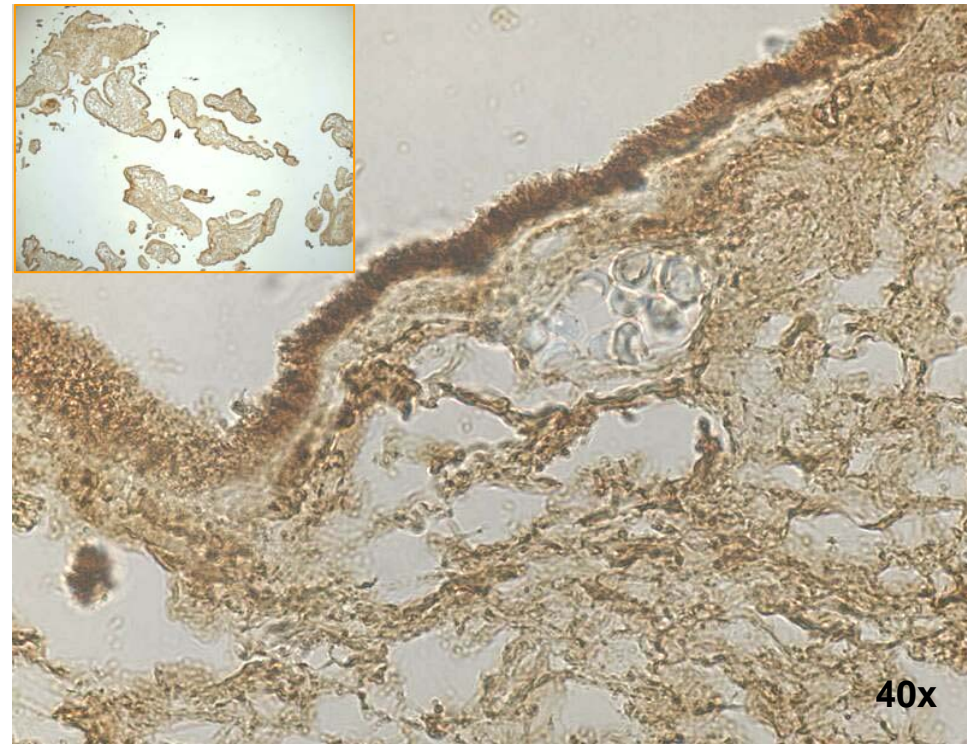
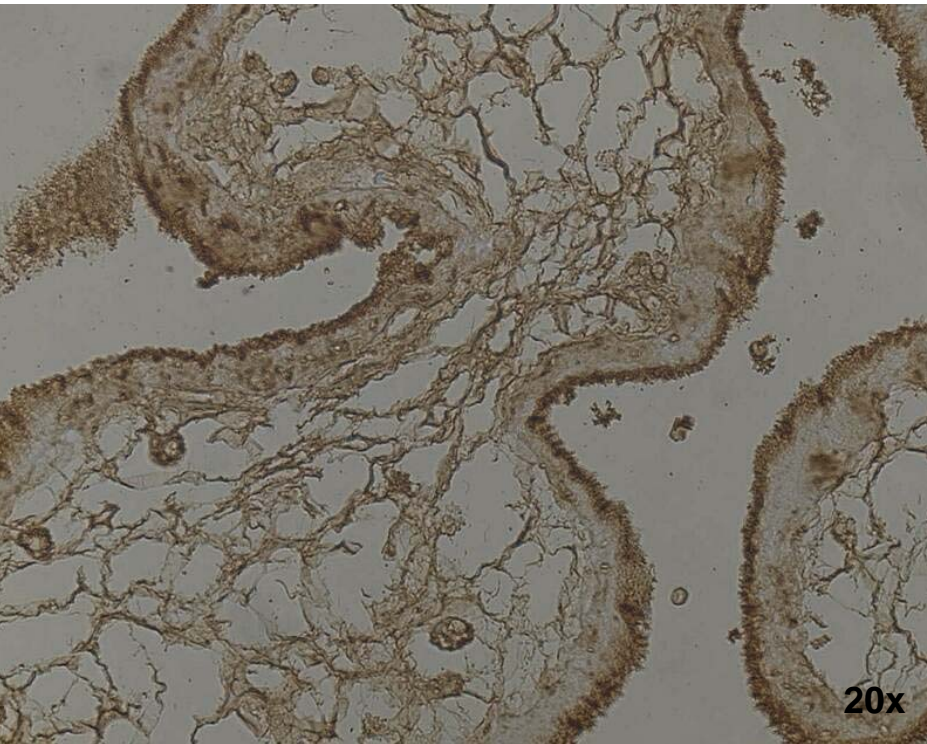
BARD1 N-19



BARD1 WFS



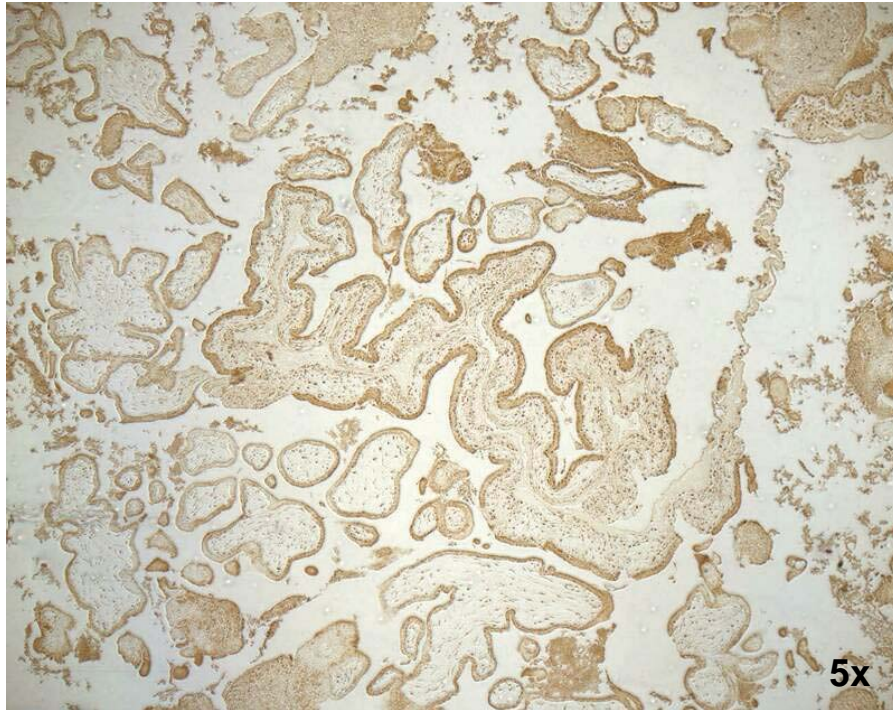
BARD1 expression in placenta at 12 weeks of pregnancy



BARD1 N-19

Immunohistochemistry of human placenta at 12th weeks of pregnancy. BARD1 N-19 antibody was used on sections of human placentas of the first trimester of pregnancy. Representative images at 12 weeks of pregnancy demonstrate BARD1 redistribution to the brush border membrane. This subcellular localization suggests that BARD1 might be secreted.

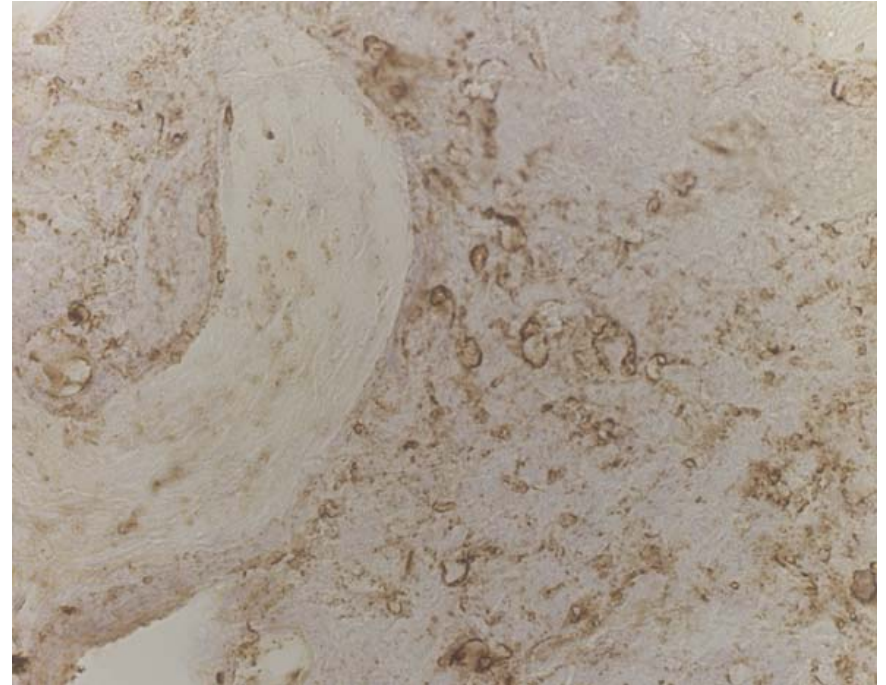
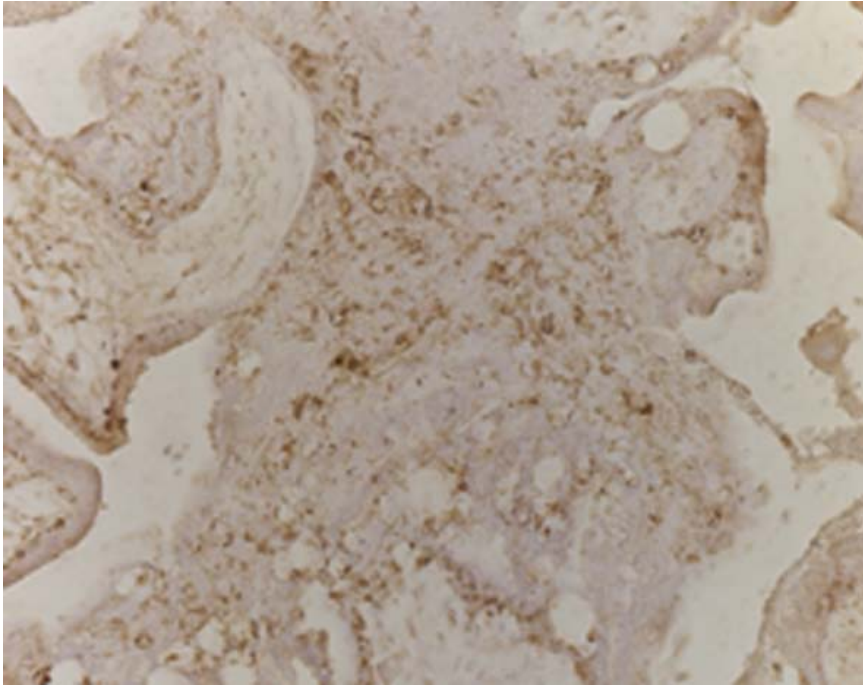
BARD1 expression is correlated with apoptotic markers



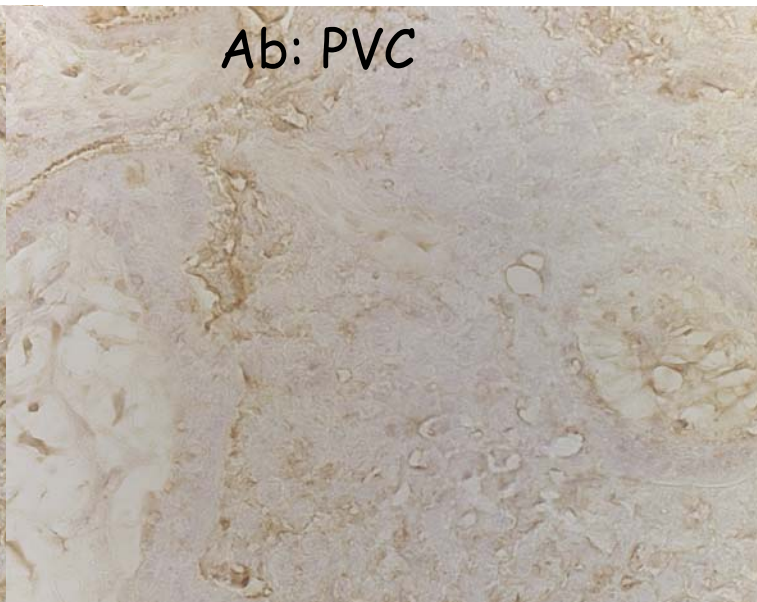
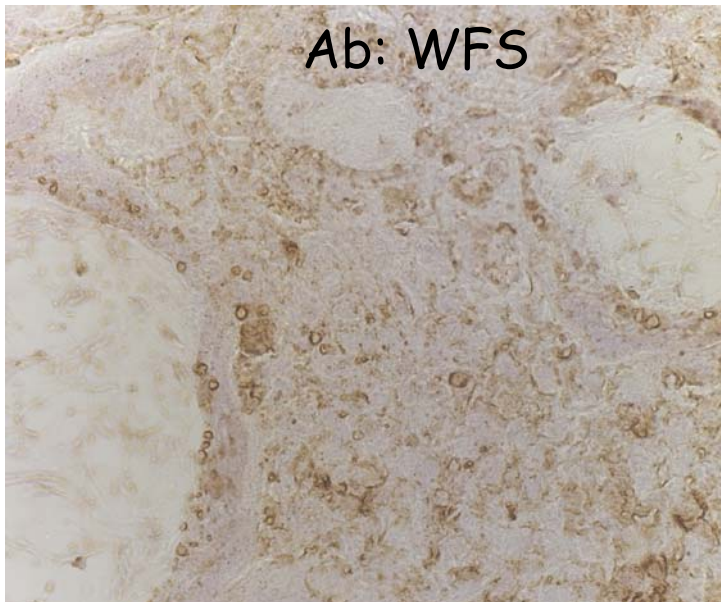
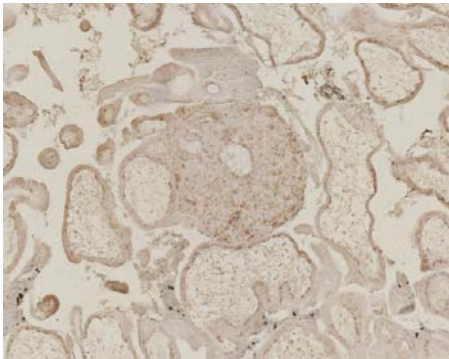
Activated Caspase 3

Identification of potential apoptotic cells. Since BARD1 expression can induce apoptosis in vitro and in vivo, activating a p53-caspase pathway, we stained placenta sections with antibodies against activated caspase 3. A complete correlation of BARD1 expression and activated caspase 3 expression can be observed. However, whether the BARD1 expressing cells actually undergo apoptosis can not be determined by these experiments.

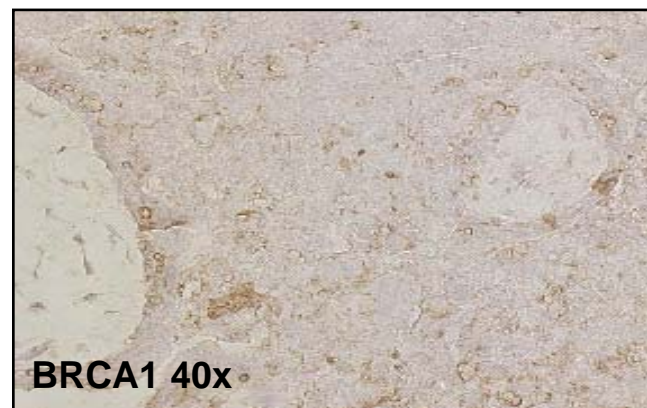
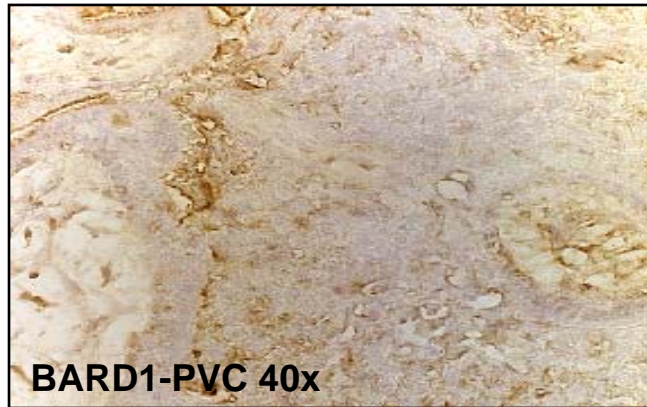
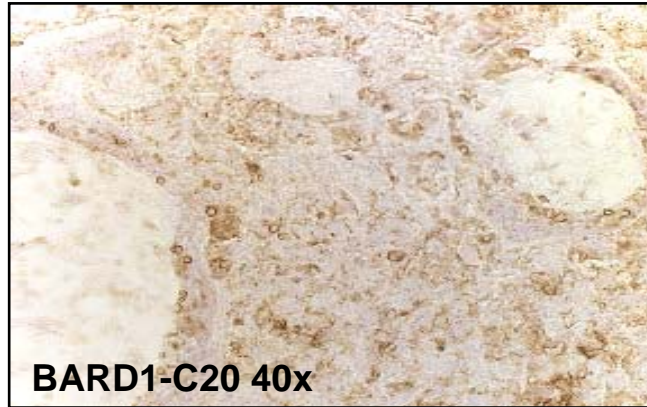
BARD1 expression in invasive cells



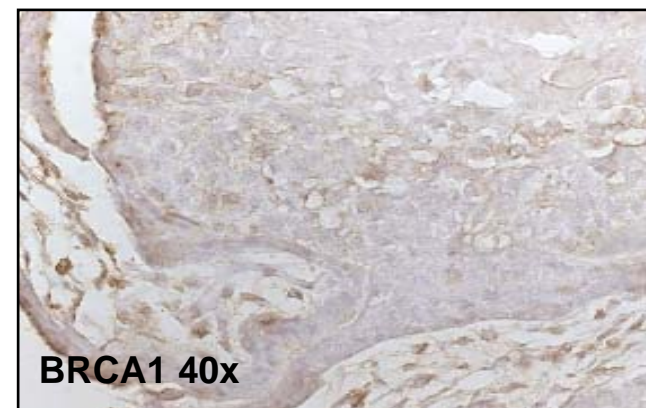
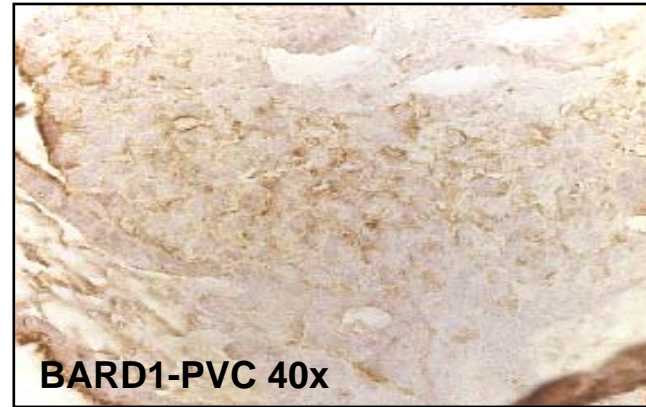
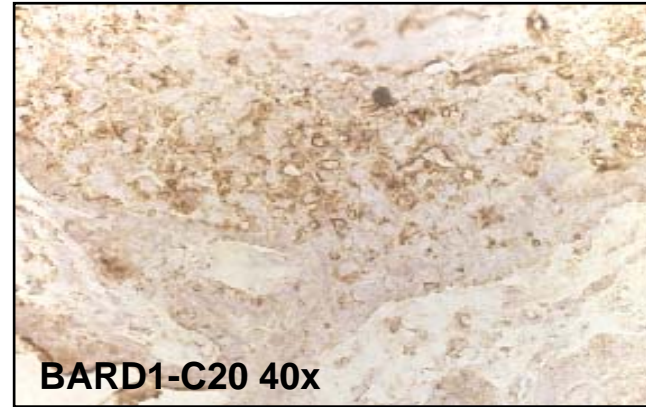
Only BARD1 γ expressed in invasive cells



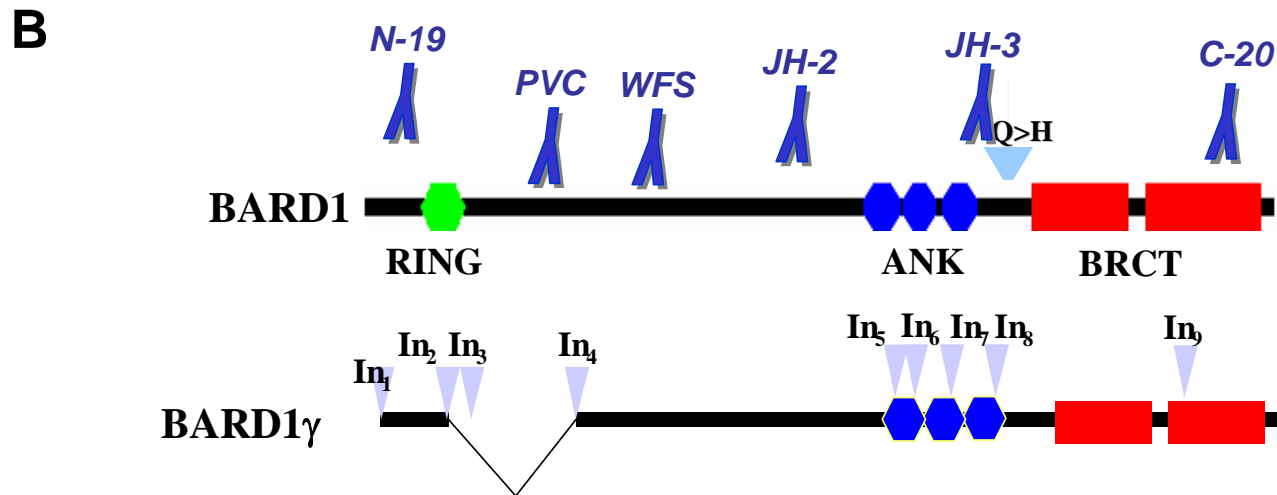
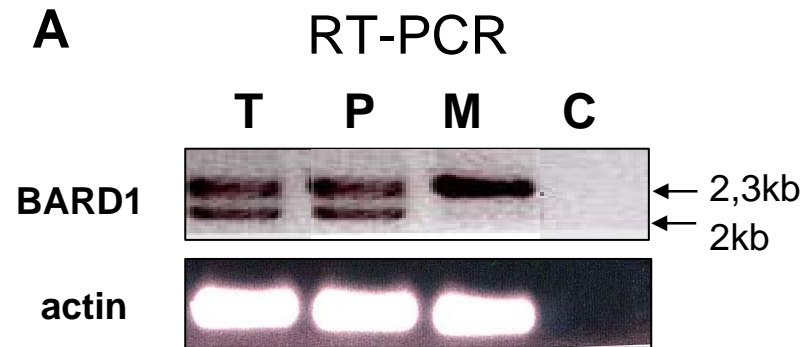
5 weeks



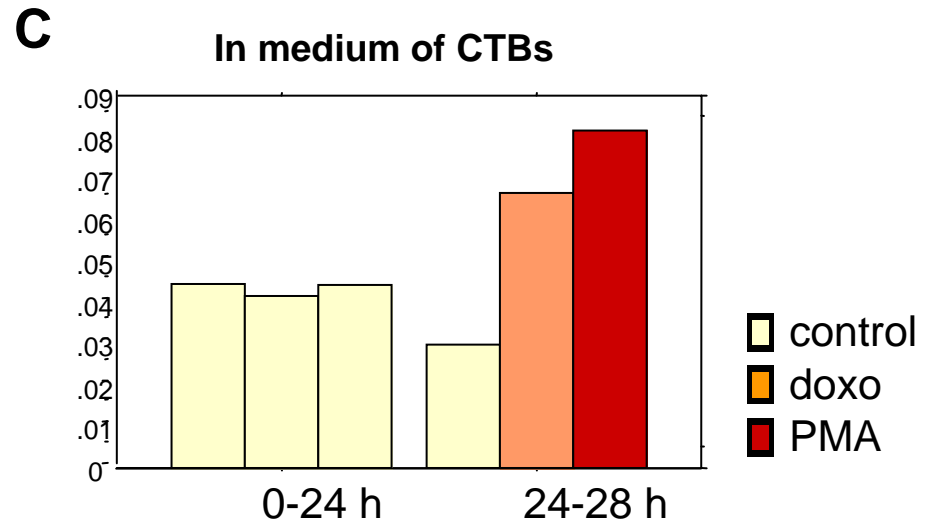
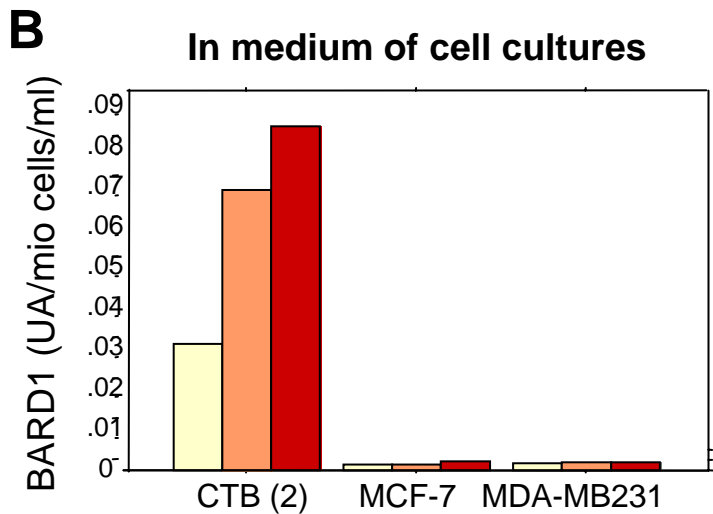
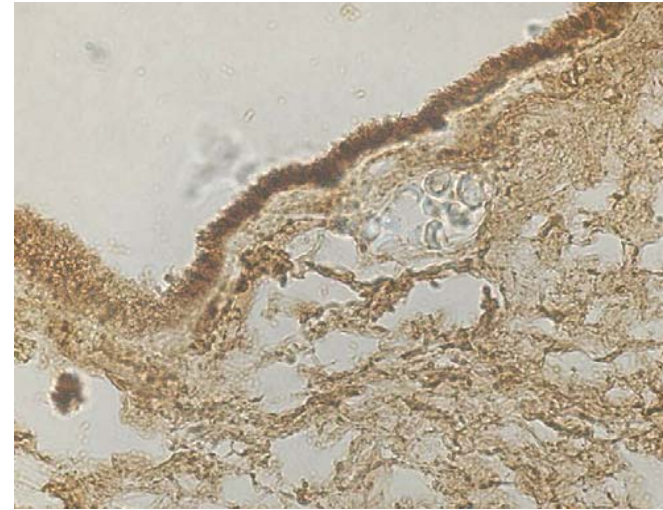
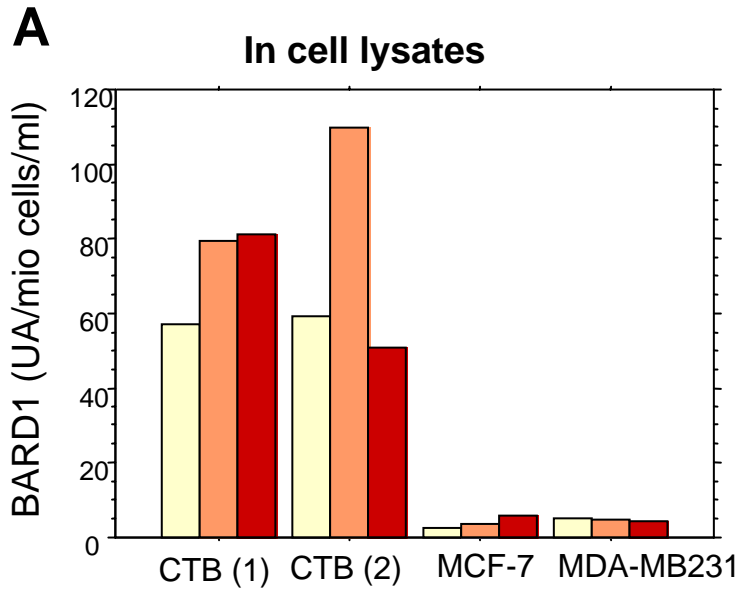
12 weeks



Differentially spliced isoform of BARD1 in cytotrophoblast and placenta lack RING finger

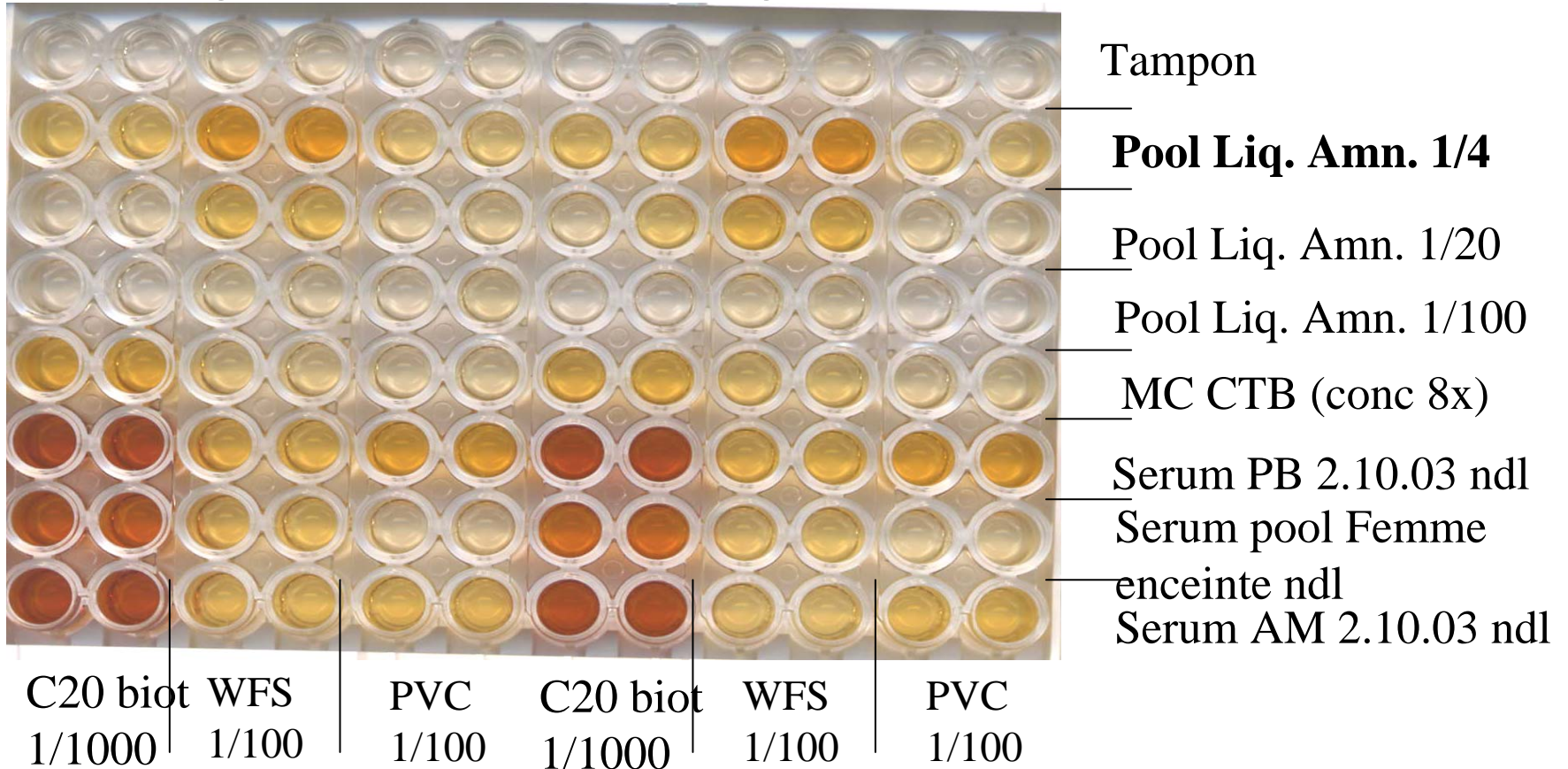


BARD1 is secreted from CTBs in culture



Quantification of BARD1 by ELISA

Coating C 20 (1/200) Coating N19 (1/200)

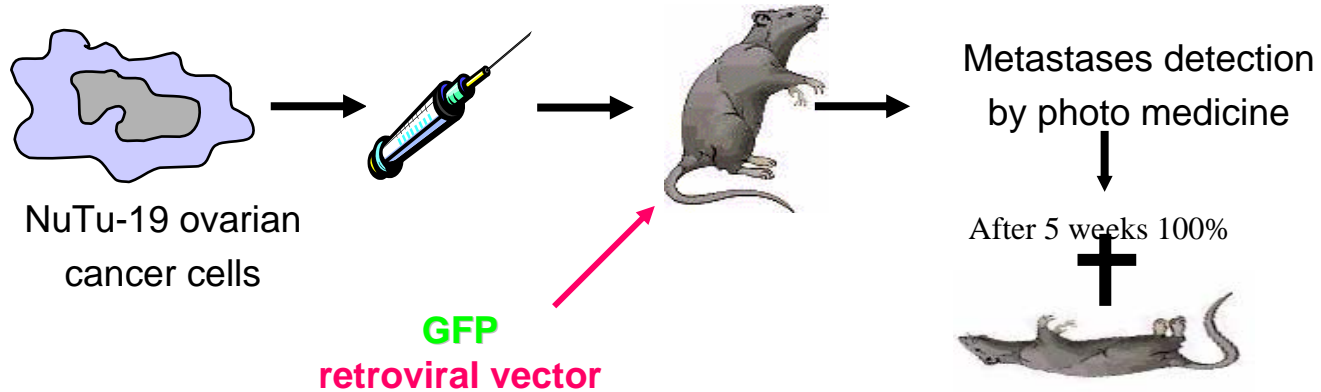


Conclusions

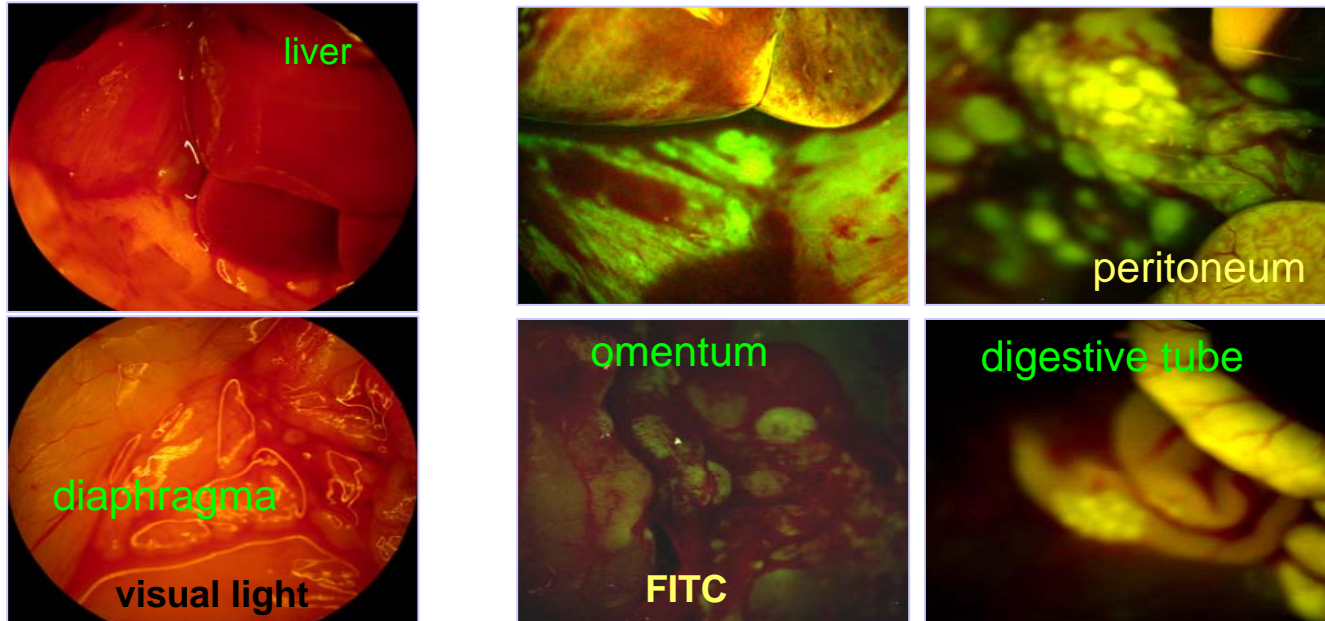
- BARD1 has a novel cellular function in CTBs and placenta
 - Regulated at different times of pregnancy
 - Regulated expression of different epitopes
- Cytotrophoblast is expressing full length and splice variant γ of BARD1
- BARD1 is secreted from CTBs
- A function of BARD1
 - in invasion?
 - in angiogenesis?

Animal cancer model for tumor cell killing

A

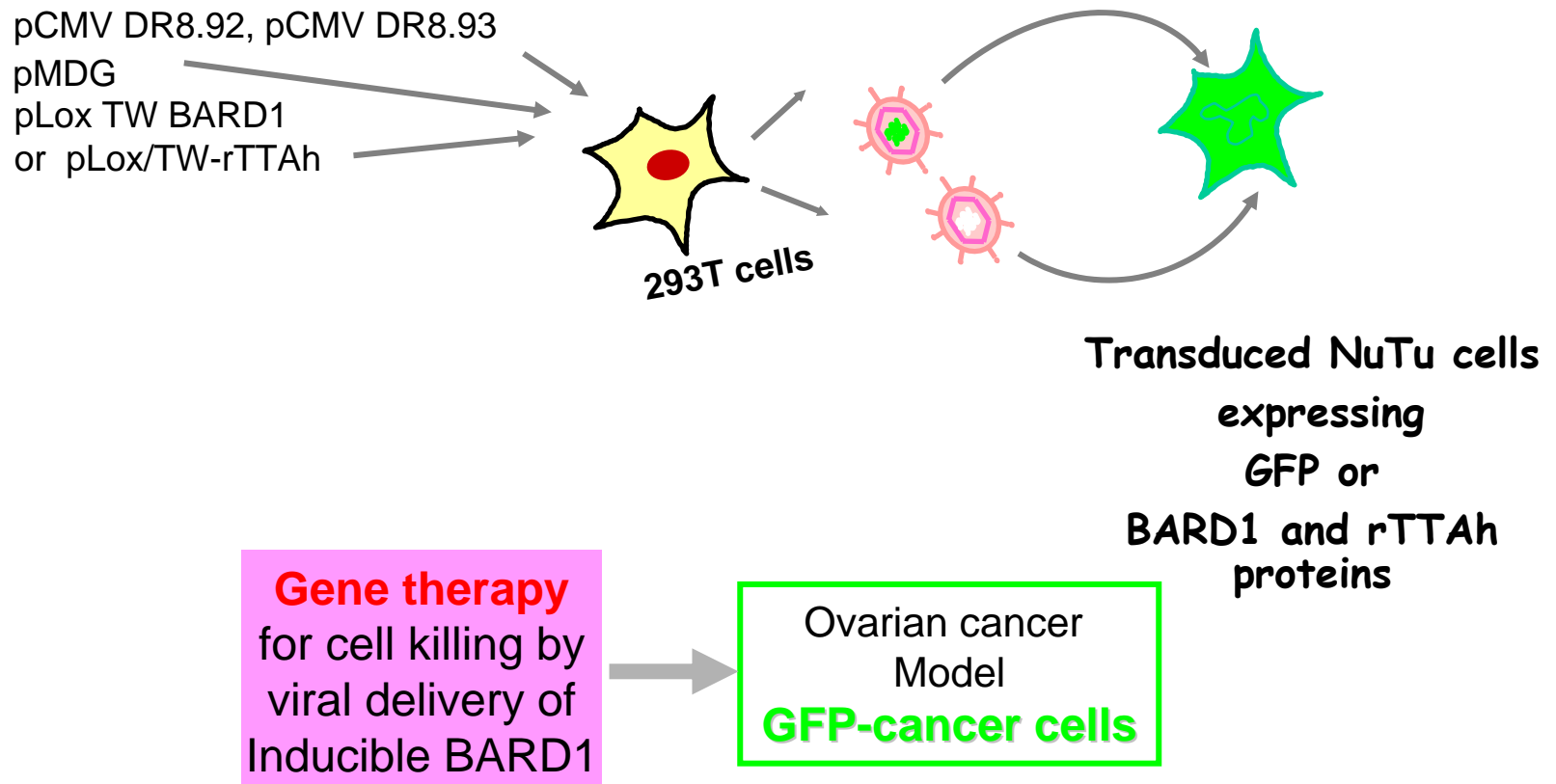


B



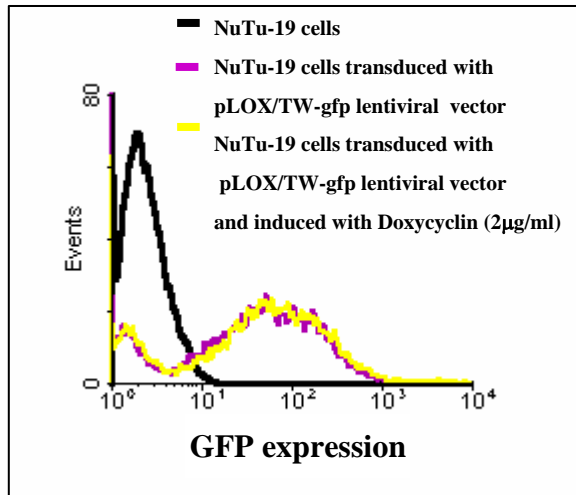
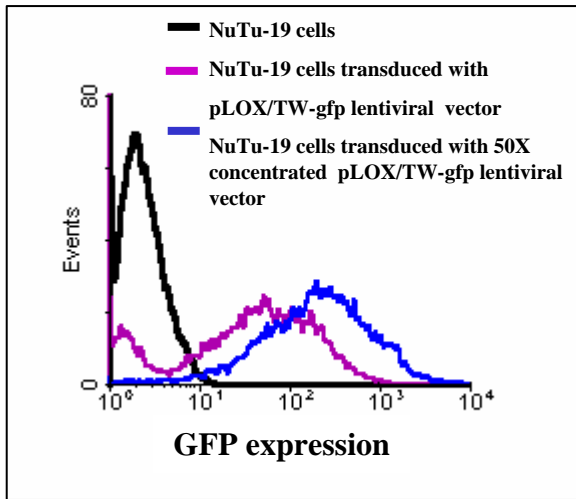
Transduction of NuTu-19 cells with lentiviral vectors expressing BARD1 or GFP

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-rTTA_h

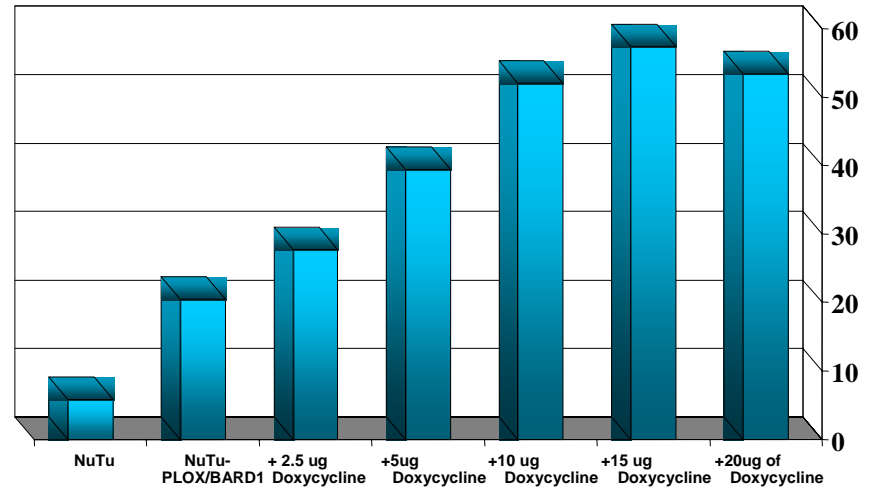


Apoptosis induction by inducible BARD1

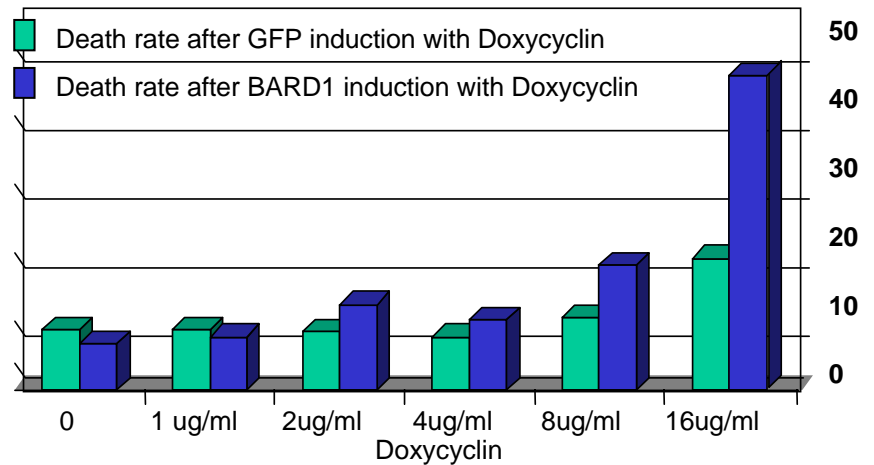
A



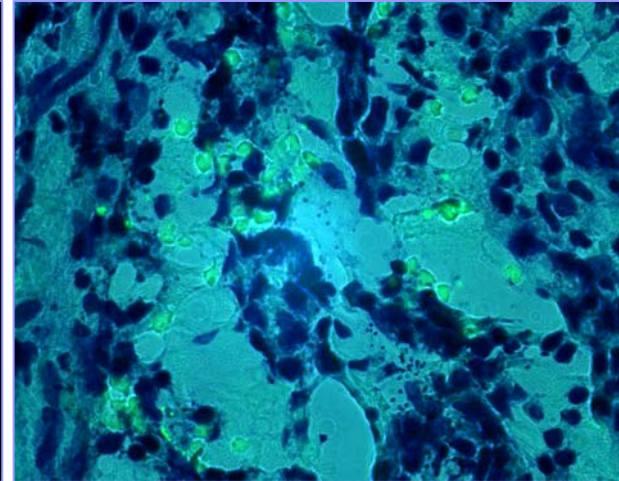
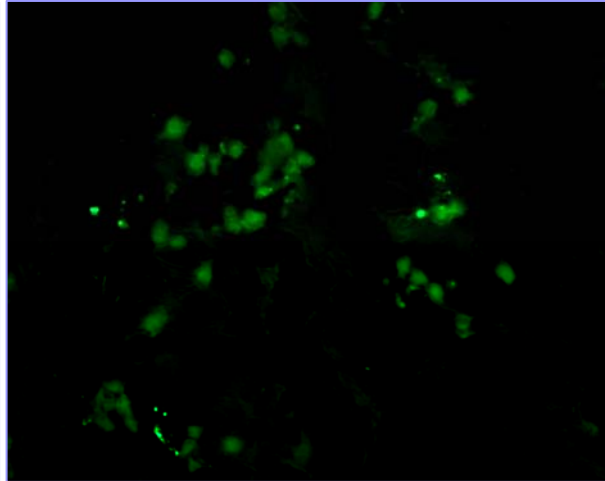
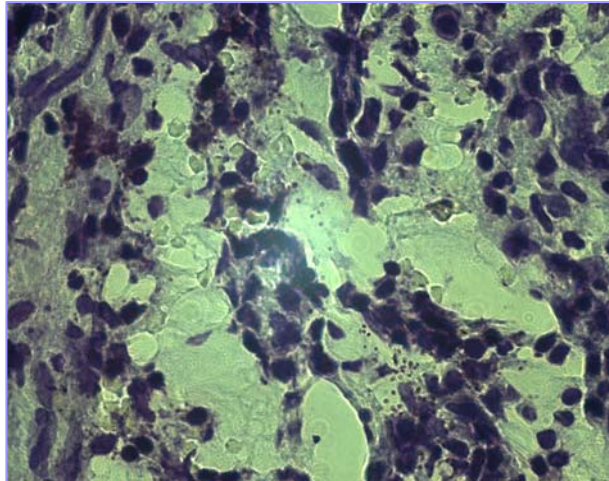
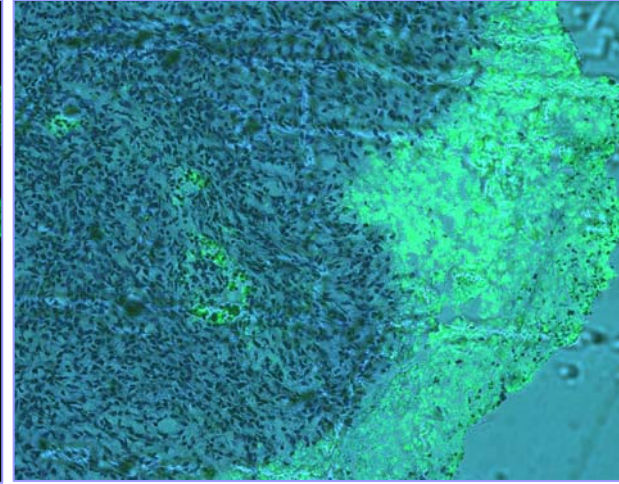
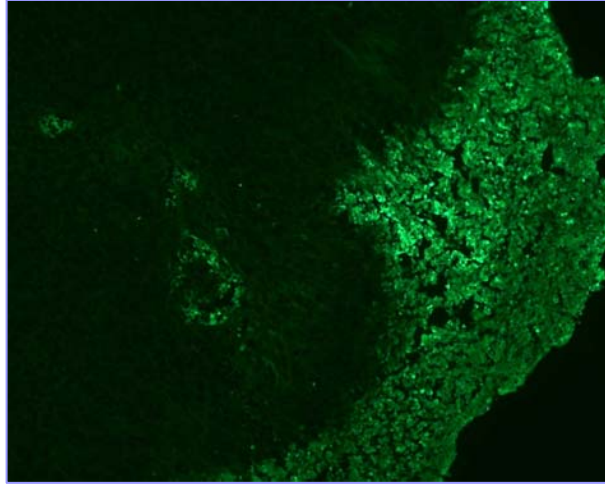
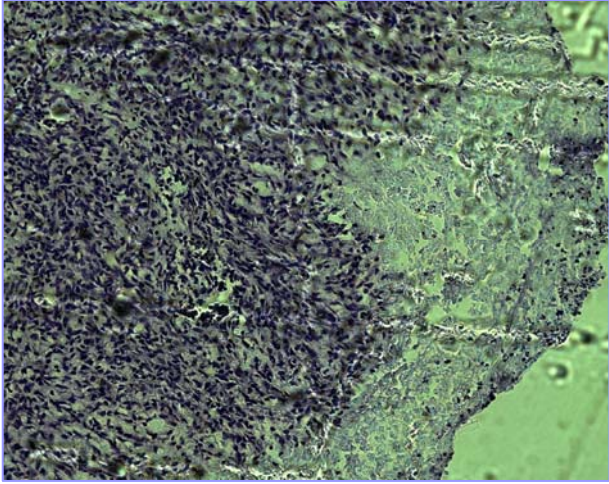
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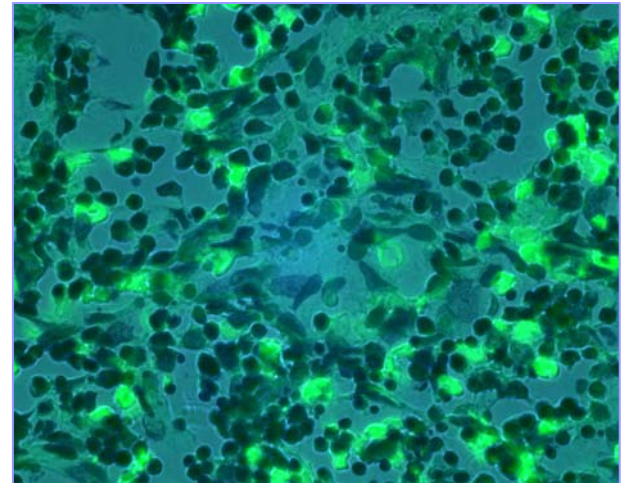
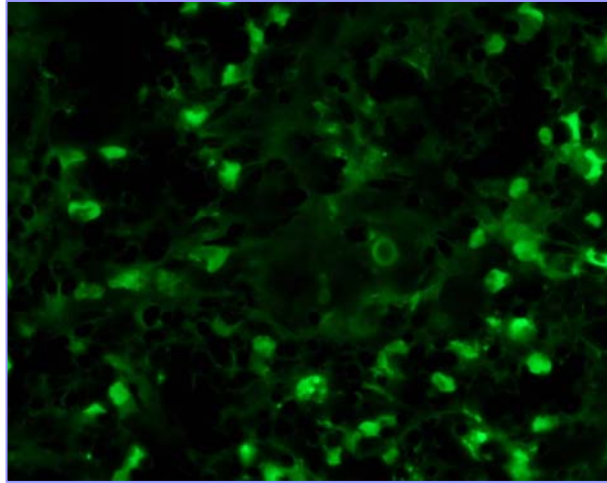
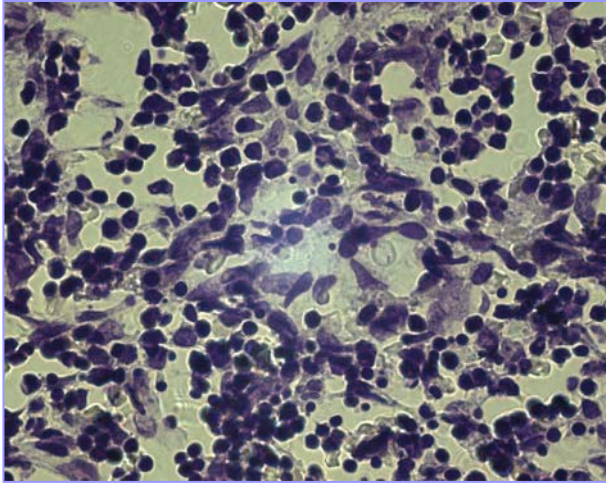
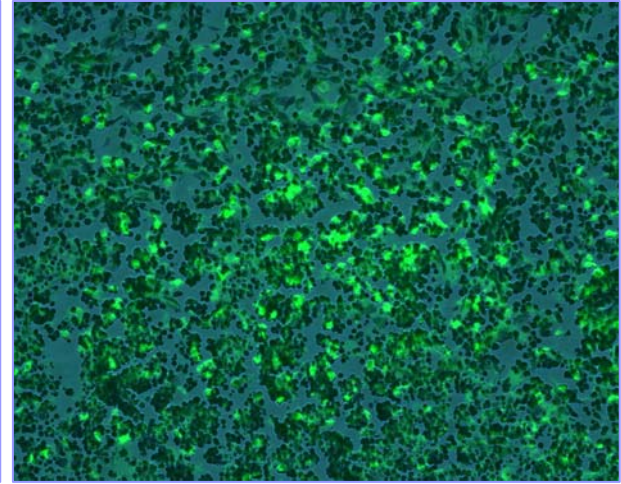
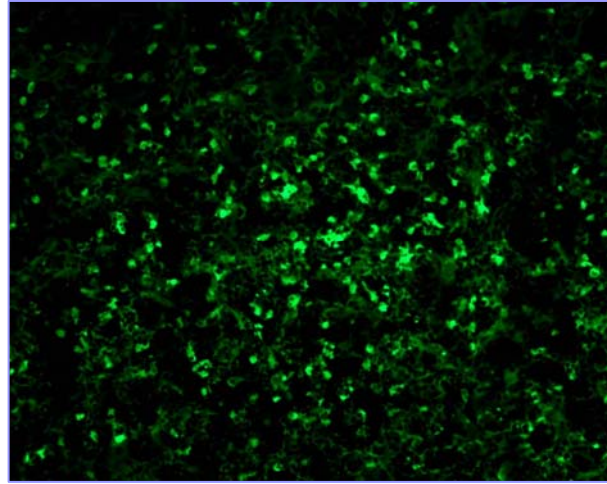
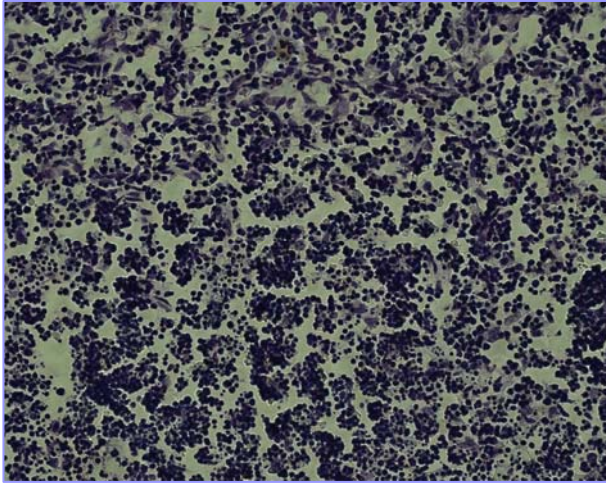
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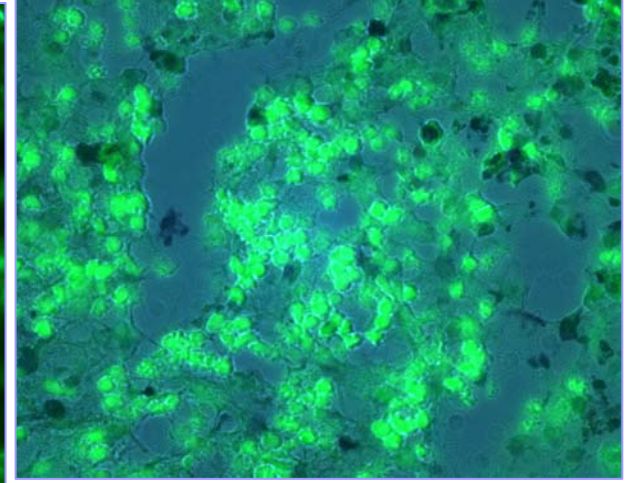
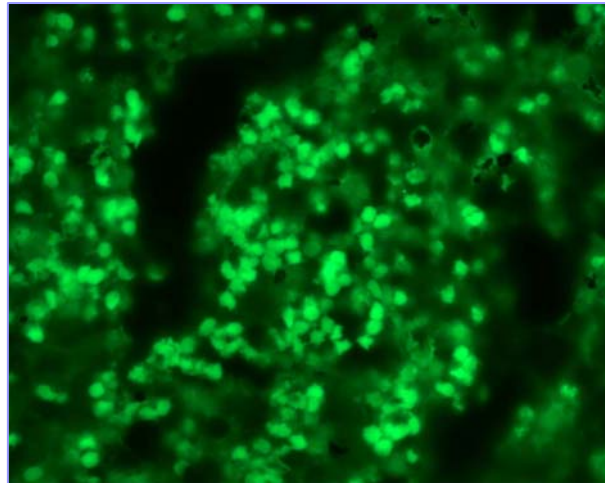
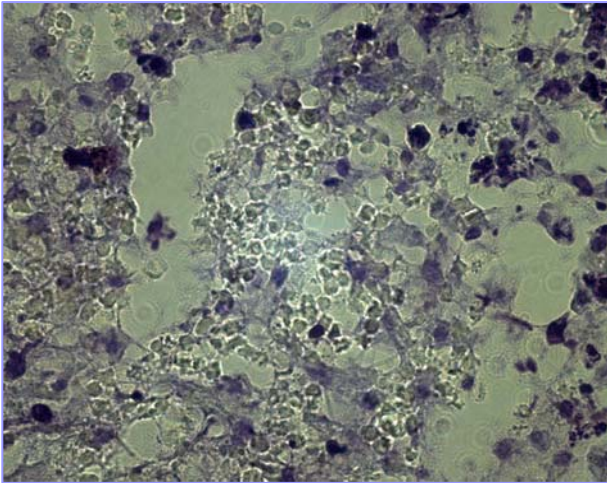
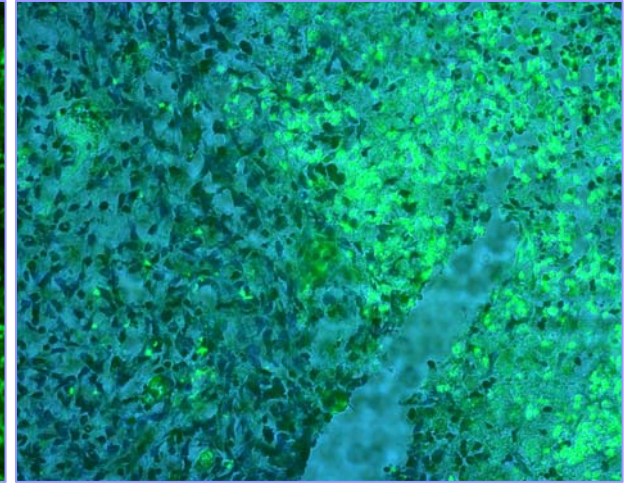
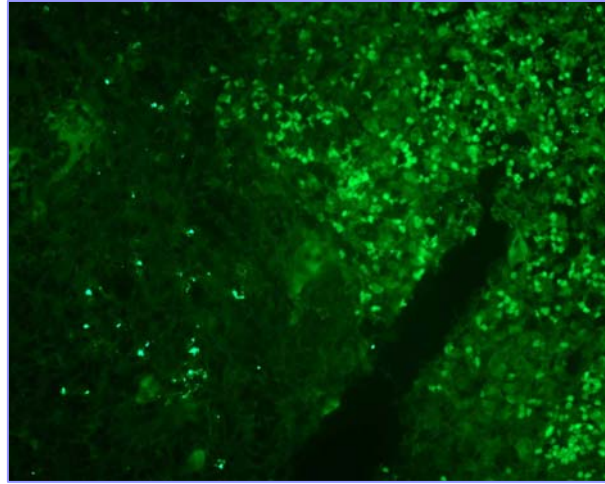
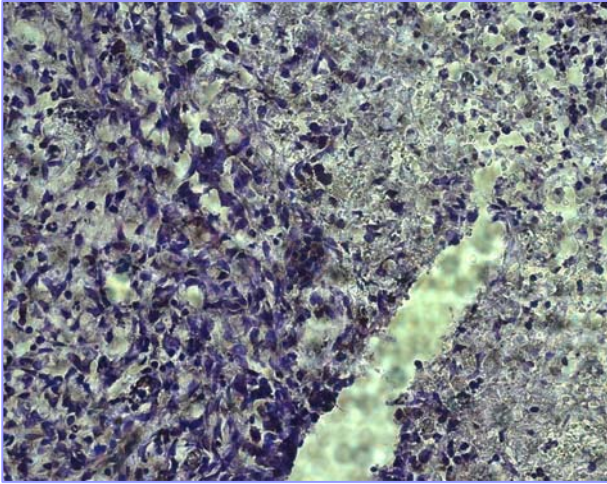
Diaphragm



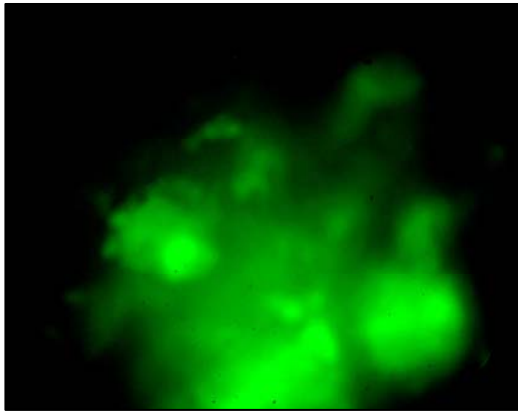
Thymus



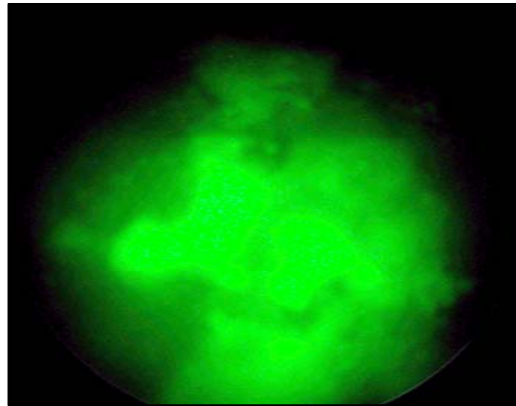
Omentum



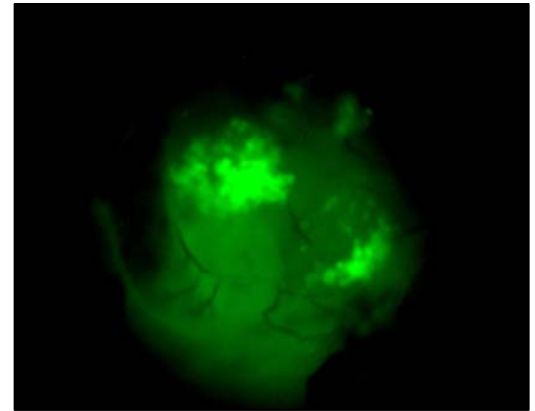
Nutu-19-EGFP tumors in Fisher rat



Diaphragm

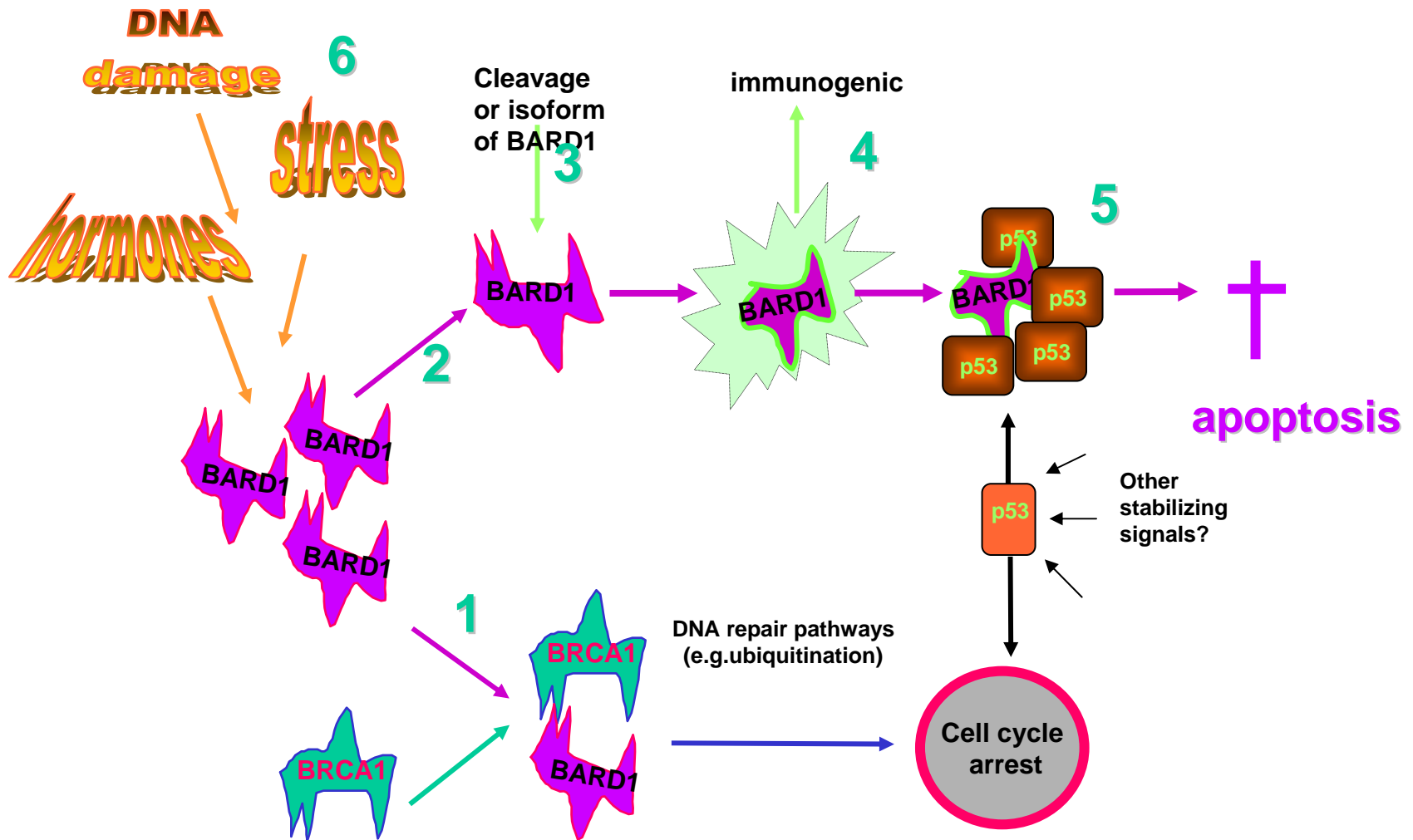


Omentum

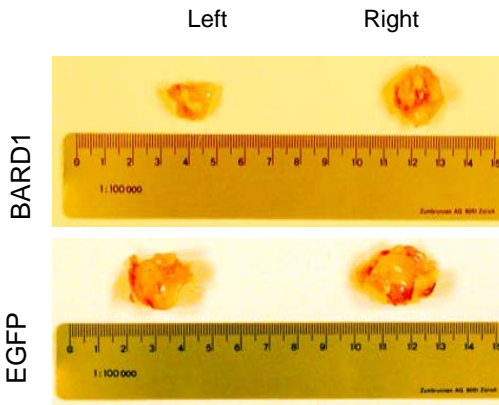
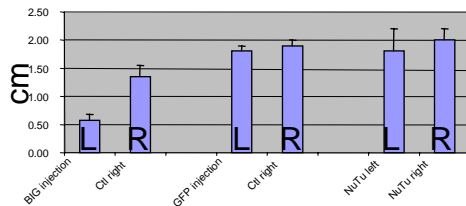
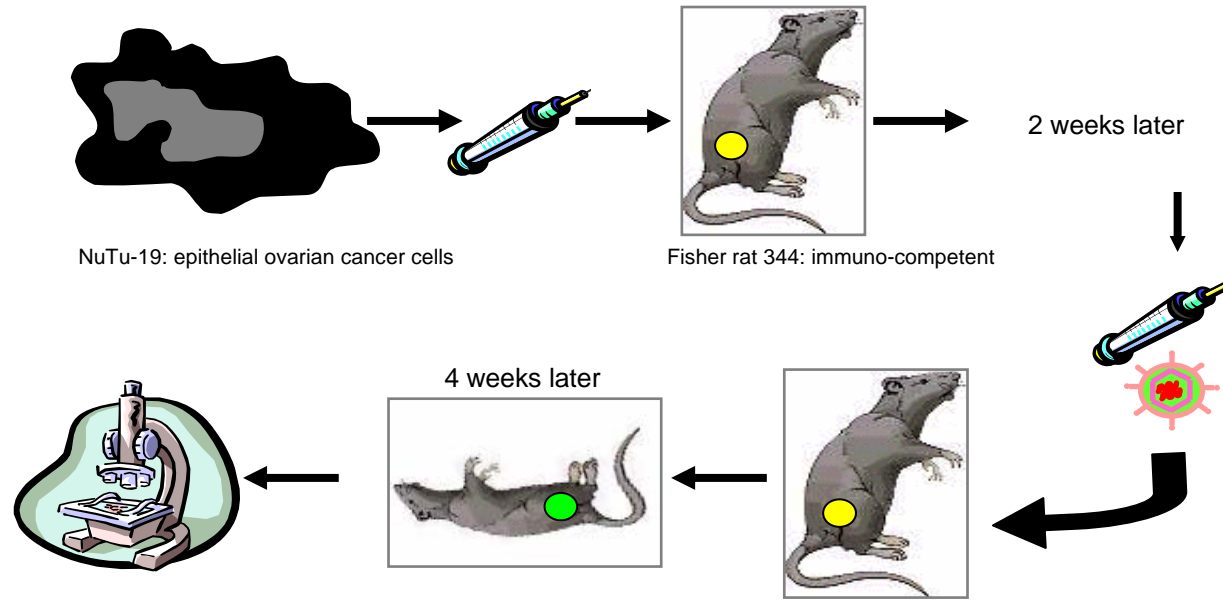


Thymus

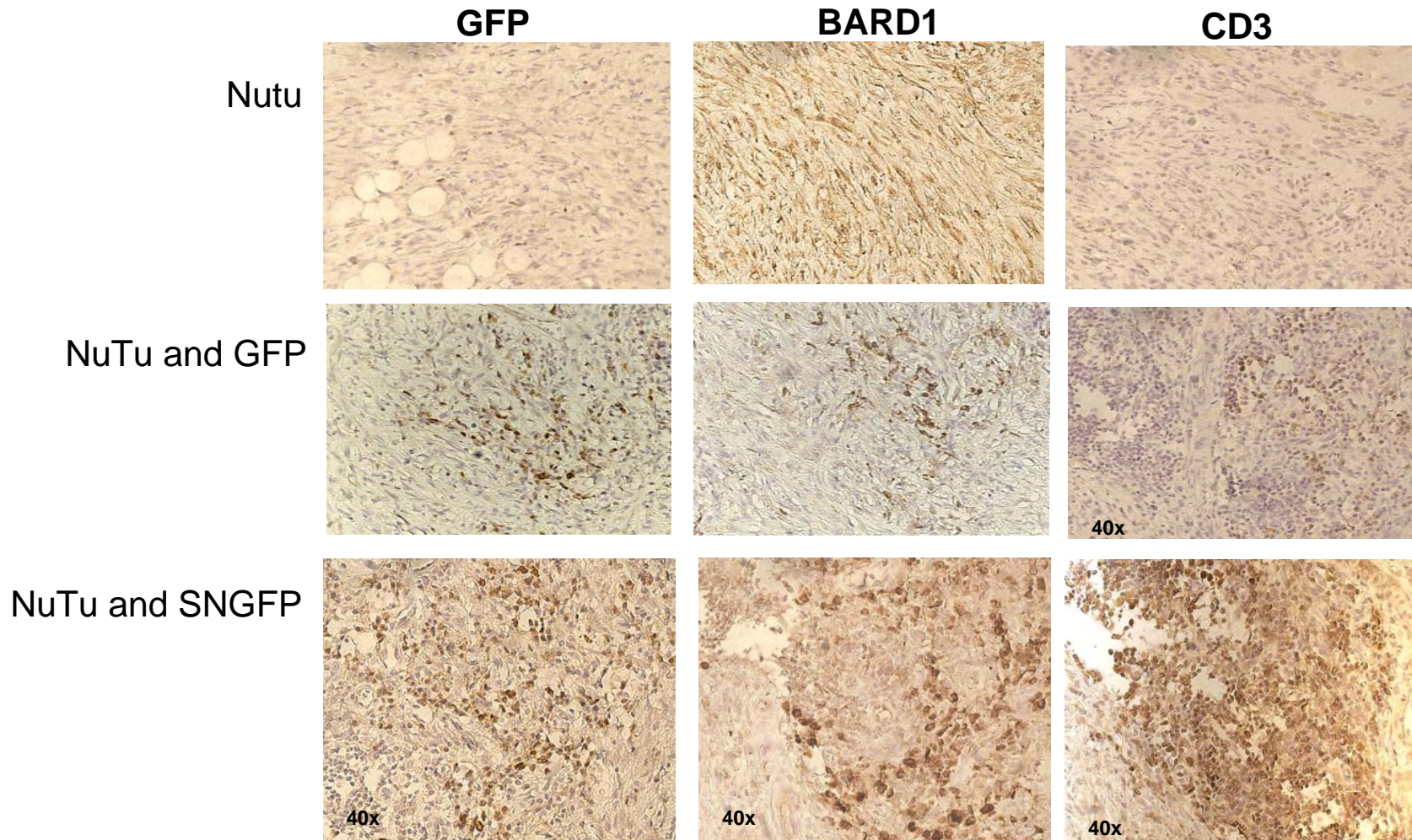
Presumed tumor suppressor pathways of BARD1



Viral BARD1 injection causes tumor shrinking



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
- Use BARD1 in anti-tumor vaccination



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