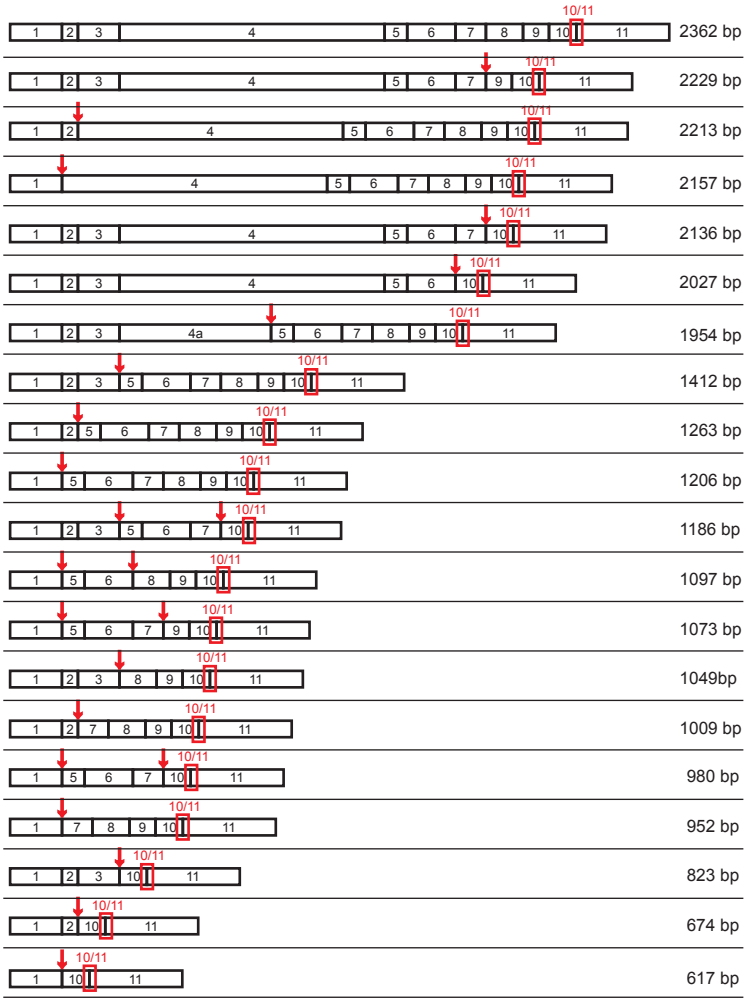
A



B

FL	**ORF**	**N19**	**PVC**	**WFS**	**C20**
FL del 8	1-7	N19	PVC	WFS	
Kappa	**4-11**			**WFS**	**C20**
Beta	**ORF**			**WFS**	**C20**
FL del 8-9	1-7	N19	PVC	WFS	
FL del 7-9	1-6	N19	PVC	WFS	
Pi	**ORF**	**N19**	**PVC**	**WFS**	**C20**
Gamma	**1-3 stable***	**N19**	**PVC**		
Gamma del 3	1-2	N19			
Gamma del 2-3	1	N19			
Gamma del 8	1-3	N19	PVC		
Gamma del 2-3, 7	1	N19			
Gamma del 2-3, 8	1	N19			
Gamma del 5-7	1	N19			
Phi	**ORF**	**N19**			**C20**
Gamma del 2-3, 8 -9	1	N19			
Delta	**ORF**	**N19**			**C20**
Epsilon	**ORF**	**N19**	**PVC**		**C20**
Epsilon del 3	1-2	N19			
Eta	**ORF**				**C20**

BARD1 isoforms in colon cancer.

A) BARD1 mRNA isoforms in colon cancer as reported by Sporn et al., 2011 (antibody used: E11 monoclonal undefined regions aa 1-330).

B) BARD1 splice variants identified in (**A**) are identical with already known isoforms (Li et al., 2007; Zhang et al., 2011); or forms with or without additional deletions, which results in premature termination of the respective ORFs and presumably unstable proteins. *Expression confirmed by si-RNA repression.

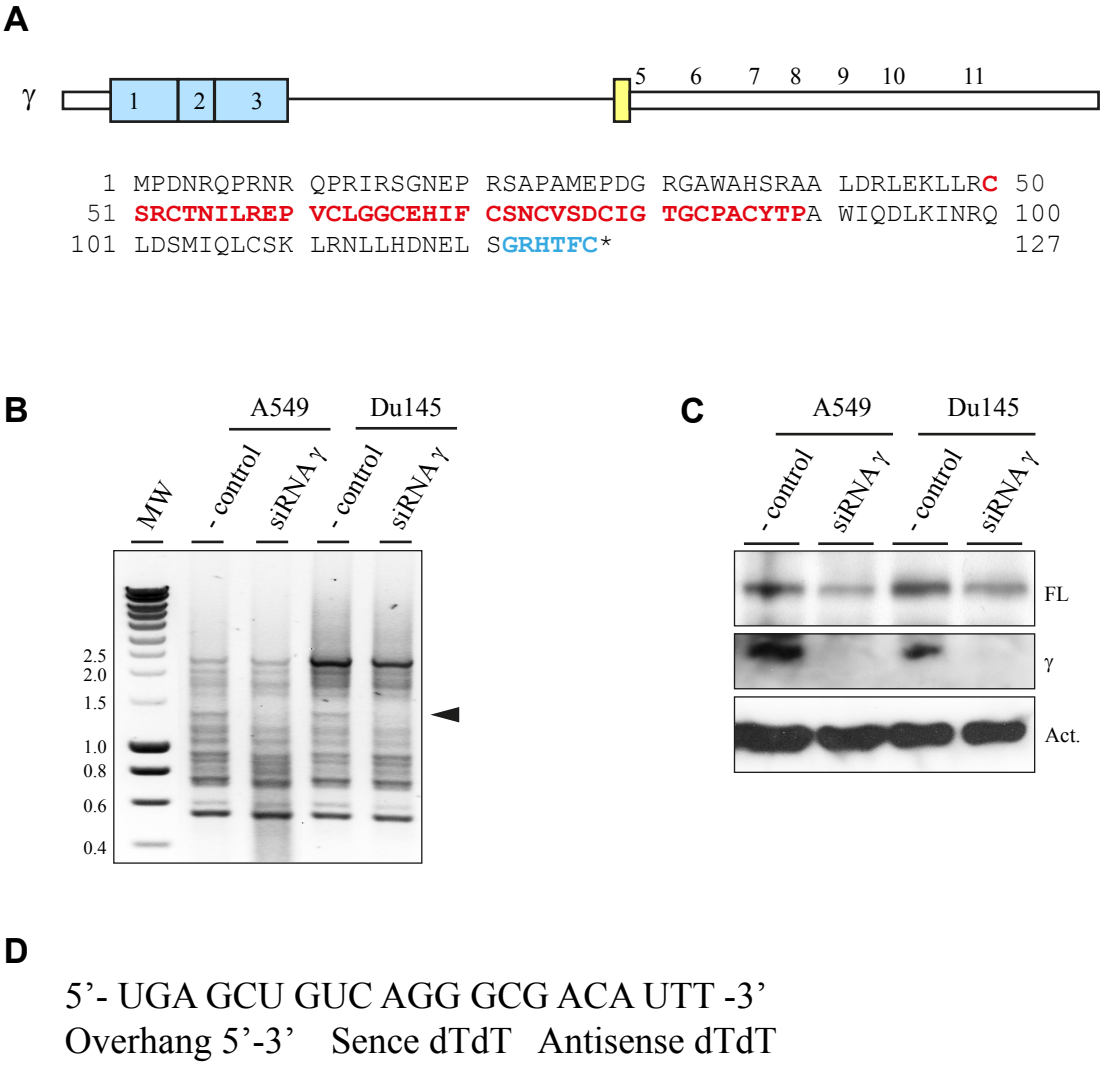
Names of known isoforms are indicated on the left. Known isoforms have an open reading frame (ORF) and are most likely translated. Isoforms with additional deletions are listed as name of known isoform plus indication of deleted exons (del x). These isoforms have shorter ORFs over fewer exons that are indicated. Presence of epitopes recognized by antibodies used in this study (N19, PVC, WFS, C20) is indicated for each isoform.

References:

Li, L., Ryser, S., Dizin, E., Pils, D., Krainer, M., Jefford, C.E., Bertoni, F., Zeillinger, R., and Irminger-Finger, I. (2007) Oncogenic BARD1 isoforms expressed in gynecological cancers. Cancer Res. 67: 11876–11885, doi:10.1158/0008-5472.CAN-07-2370.

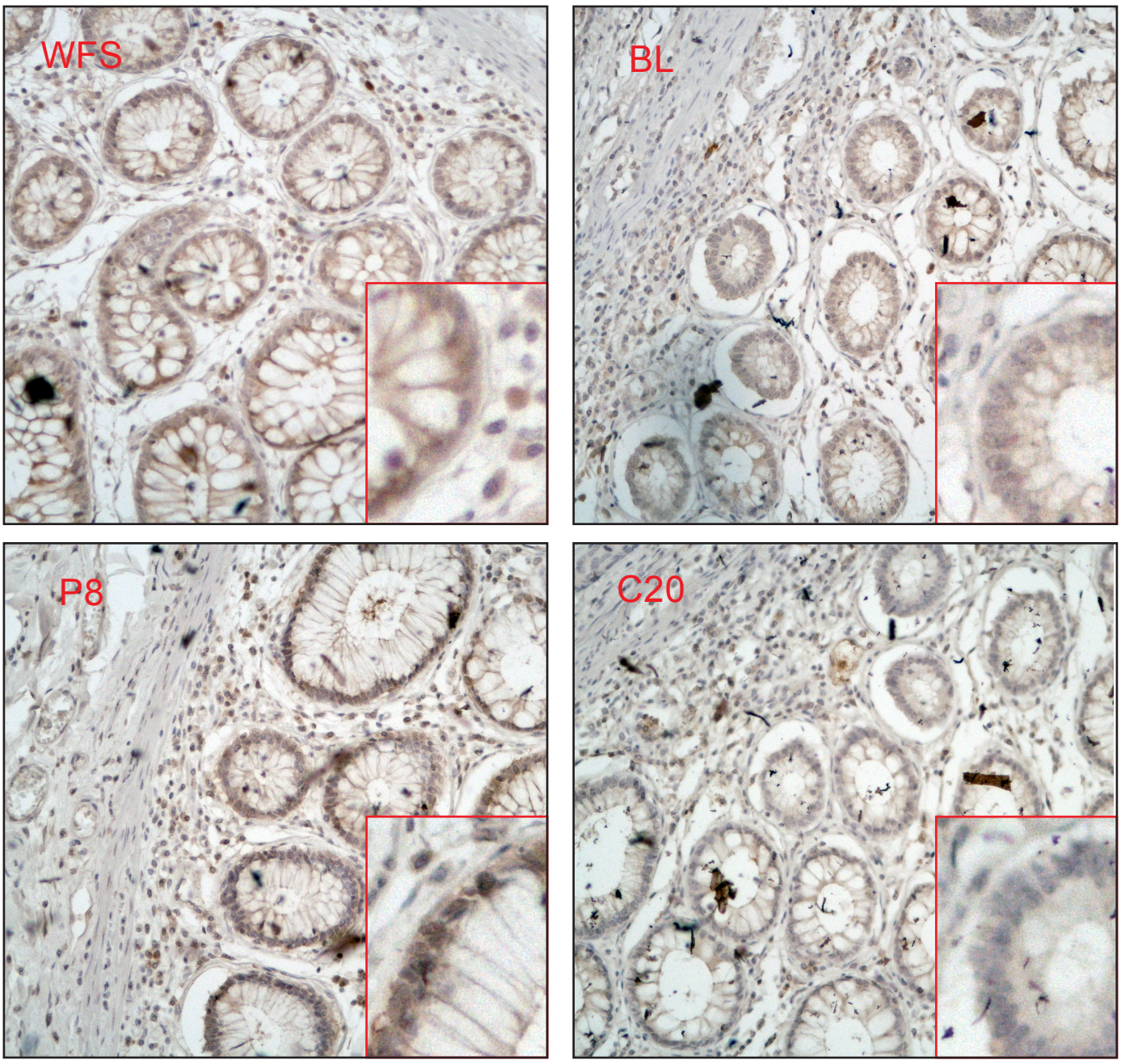
Sporn, J.C., Hothorn, T., and Jung, B.H. (2011) BARD1 expression predicts outcome in colon cancer. Clin Cancer Res. , doi:10.1158/1078-0432.CCR-11-0263.

Zhang, Y.-Q., Bianco, A., Malkinson, A.M., Leoni, V.P., Frau, G., Rosa, N.D., André, P.-A., Versace, R., Boulvain, M., Laurent, G.J., Atzuri, L., and Irminger-Finger, I. (2011) BARD1: An independent predictor of survival in non-small cell lung cancer. International Journal of Cancer. Journal International Du Cancer. [Epub ahead of print], doi:10.1002/ijc.26346.



BARD1γ encodes a RING-finger protein and regulates the level of FL BARD1.

A. BARD1 gamma mRNA scheme. Non-coding sequences are represented with white bars; alternatively translated sequences are shown as yellow bars. BARD1γ mRNA may encode 14.5kDa polypeptide containing RING-finger domain (shown in red). **B.** Specific BARD1γ siRNA knockdowns BARD1γ mRNA (corresponding RT-PCR fragment is marked with an arrowhead). Non-treated control cells show no change of BARD1γ mRNA level. **C.** The protein corresponding to the expected BARD1γ product is recognized by N-terminus specific N19 antibody and dramatically reduced following BARD1γ siRNA depletion (middle panel). Beta-actin was used as loading control (lower panel). Full length BARD1 was reduced in the cells treated with BARD1γ siRNA (upper panel). This reduction correlates with the reduction of BARD1γ protein level (lower panel). **D.** The sequence of siRNA specific for BARD1 isoform Gamma.



Comparative staining of colon cancer tumor tissue by antibodies used in the research.

To support the hypothesis that the C20 epitope is present, but not accessible in most isoforms, we have used an antibody against a different sequence in exon 11, as used in a study of lung cancer (Zhang et al., Int J Cancer 2011), for immunohistochemistry of colon cancer samples and compared it to the C20 staining pattern. Similarly, we have performed immunohistochemistry with a commercial antibody BL (exon 4) and compared its staining pattern to that of WFS (exon 4) on a selected number of colon cancer tissue samples.

The staining demonstrates that all four (WFS, BL , P8 and C20) antibodies demonstrate the identical distribution of the signal indicating their specificity. At the same time, C20 staining is weaker that support our hypothesis that C20 epitope is present, but not accessible in most isoforms.