

Mechanisms of Tumor Suppressor Gene Demethylation in Colon Cancer by Berries



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Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, 2010

Estimated New Cases*

			Males	Females			
Prostate	217,730	28%			Breast	207,090	28%
Lung & bronchus	116,750	15%			Lung & bronchus	105,770	14%
Colon & rectum	72,090	9%			Colon & rectum	70,480	10%
Urinary bladder	52,760	7%			Uterine corpus	43,470	6%
Melanoma of the skin	38,870	5%			Thyroid	33,930	5%
Non-Hodgkin lymphoma	35,380	4%			Non-Hodgkin lymphoma	30,160	4%
Kidney & renal pelvis	35,370	4%			Melanoma of the skin	29,260	4%
Oral cavity & pharynx	25,420	3%			Kidney & renal pelvis	22,870	3%
Leukemia	24,690	3%			Ovary	21,880	3%
Pancreas	21,370	3%			Pancreas	21,770	3%
All Sites	789,620	100%	All Sites	739,940	100%		

Estimated Deaths

			Males	Females			
Lung & bronchus	86,220	29%			Lung & bronchus	71,080	26%
Prostate	32,050	11%			Breast	39,840	15%
Colon & rectum	26,580	9%			Colon & rectum	24,790	9%
Pancreas	18,770	6%			Pancreas	18,030	7%
Liver & intrahepatic bile duct	12,720	4%			Ovary	13,850	5%
Leukemia	12,660	4%			Non-Hodgkin lymphoma	9,500	4%
Esophagus	11,650	4%			Leukemia	9,180	3%
Non-Hodgkin lymphoma	10,710	4%			Uterine Corpus	7,950	3%
Urinary bladder	10,410	3%			Liver & intrahepatic bile duct	6,190	2%
Kidney & renal pelvis	8,210	3%			Brain & other nervous system	5,720	2%
All Sites	299,200	100%	All Sites	270,290	100%		

- Colorectal cancers are third most common cancer and third major cause of cancer mortality in U.S.
- 5-year survival (1999-2005) = 66%

Black Raspberries (BRBs)

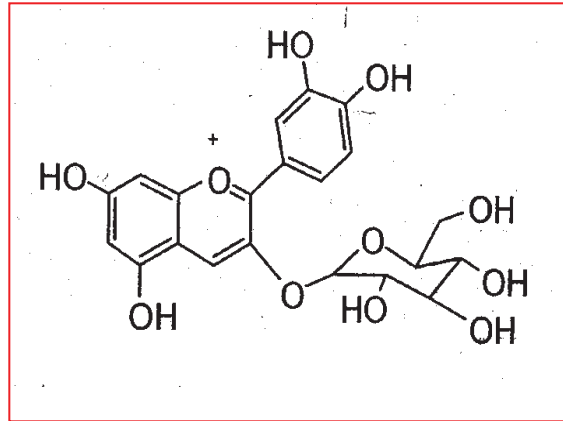
Freeze-dried powder



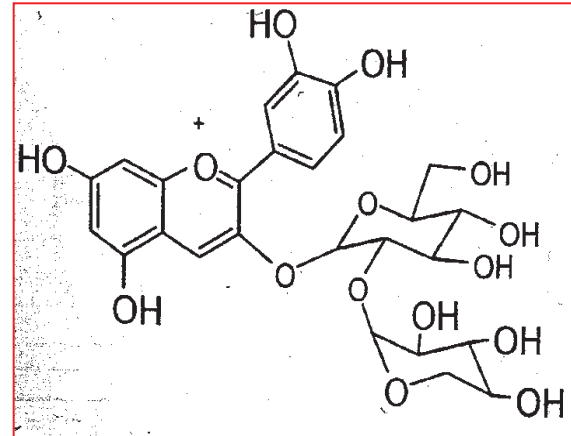
Some Chemopreventive Agents in Black Raspberries

- **Vitamins:** A, C, E, folic acid
- **Minerals:** calcium, selenium
- **Simple phenols:** ellagic acid, ferulic acid, coumaric acid, quercetin
- **Complex phenols:** anthocyanins
- **Phytosterols:** β -sitosterol, campesterol, stigmasterol

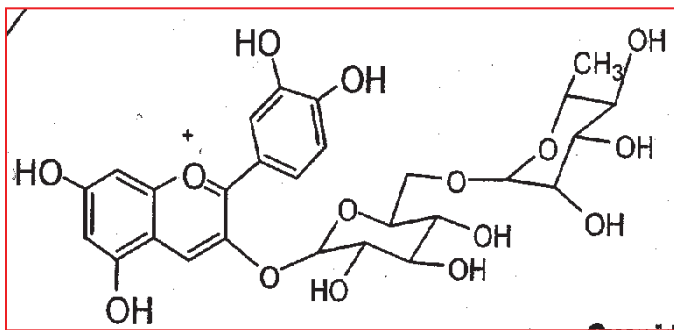
Anthocyanins in Black Raspberries



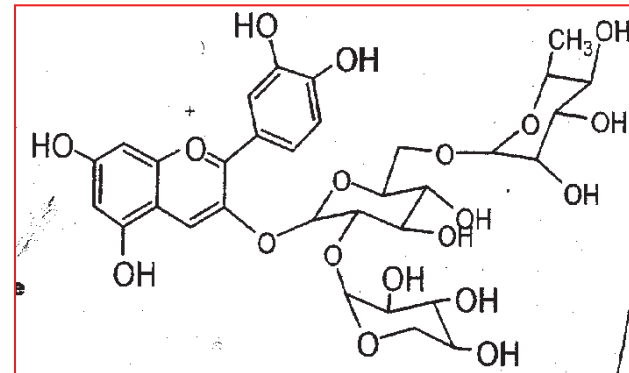
Cyanidin 3-O-glucoside



Cyanidin 3-O-sambubioside

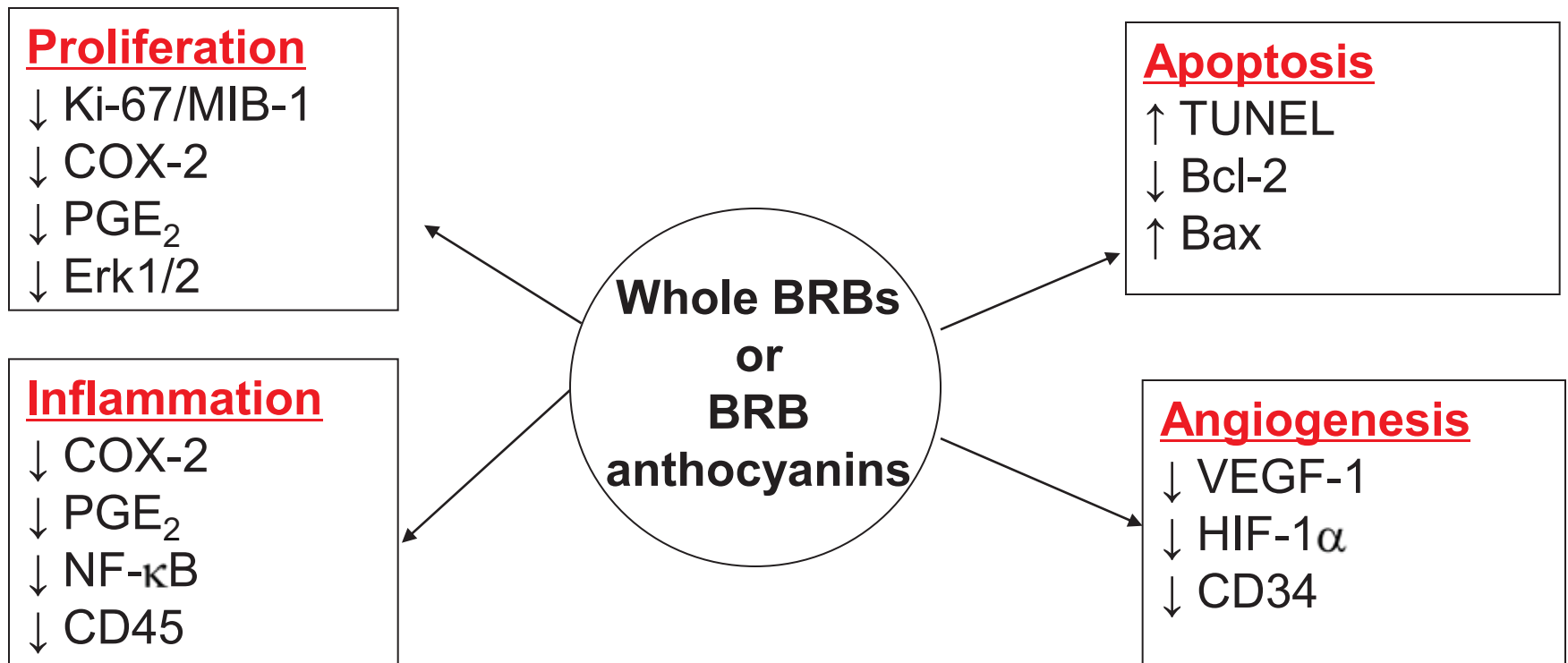


Cyanidin 3-O-rutinoside



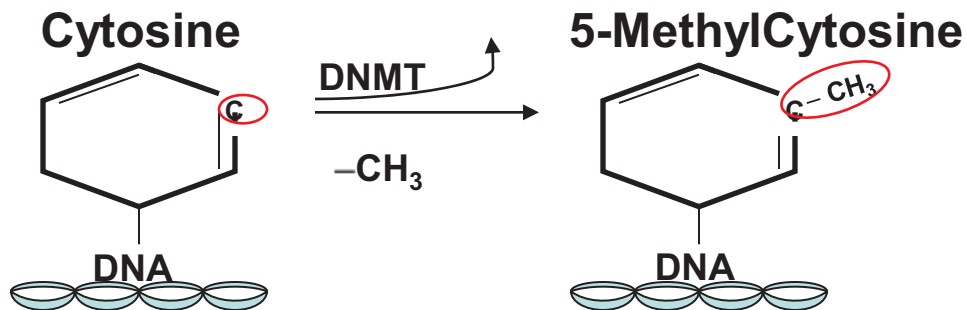
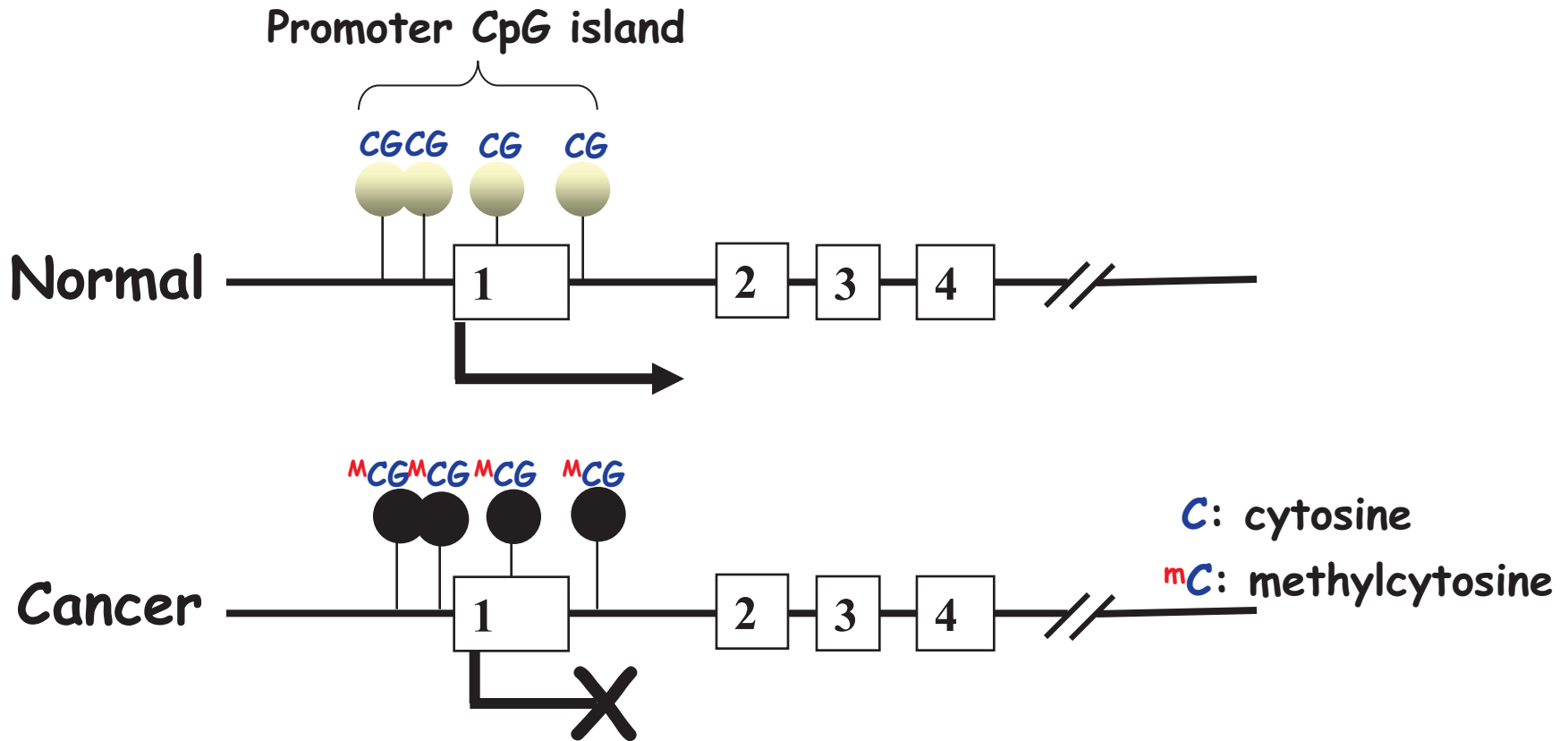
Cyanidin 3-O-(2^G-xylosylrutinoside)

Effects of Whole BRBs and BRB Anthocyanins on Cellular Events and Associated Genes in NMBA-Treated Rat Esophagus



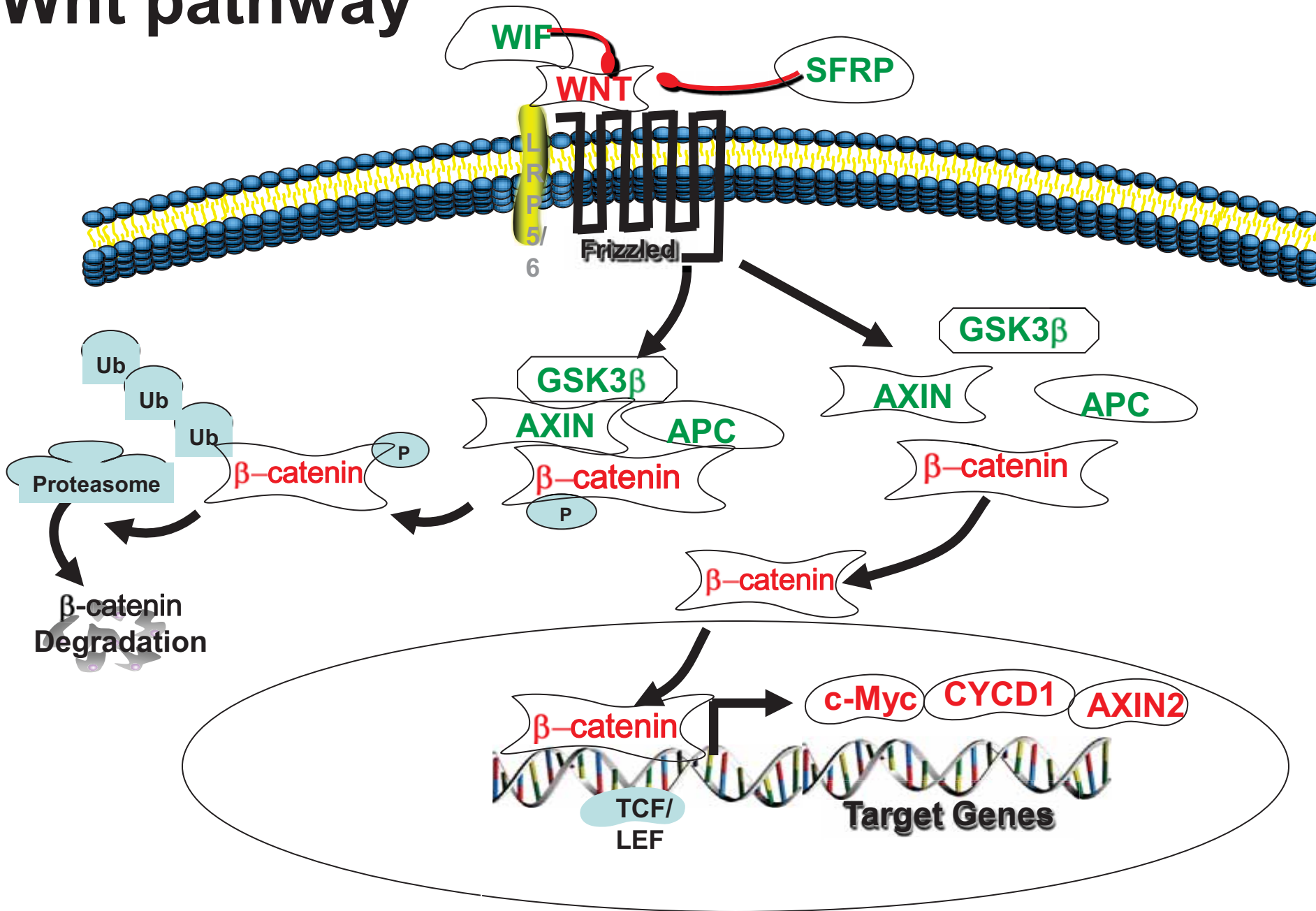
CD45 – leukocyte common antigen, CD34 – Microvessel marker

DNA Methylation and Tumor Suppressor Gene Silencing



DNMT: DNA methyltransferase
DNMT family: DNMT1, DNMT3A, DNMT3B, etc

Wnt pathway





**Clinical
Cancer
Research**

Highlights

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Selected Articles from This Issue

Gene Demethylation by Berries in Colorectal Cancer

Wang *et al.*

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New strategies are needed in the chemoprevention of colorectal cancer. Here, Wang and colleagues investigated whether short-term treatment with freeze-dried black raspberries (BRB) could modulate biomarkers of tumor development in a cohort of colorectal cancer patients. They found that BRBs protectively modulated biomarkers of cell proliferation, apoptosis, and angiogenesis in both tumor and adjacent normal specimens. Further, BRBs caused demethylation of the promoter regions of relevant tumor suppressor genes in adjacent normal tissues and colorectal tumors, and this effect was dependent on total dose. These results provide rationale for further evaluation of BRBs in colorectal cancer prevention.

Colorectal Cancer Study – Pre-surgical model

1. Pre-Treatment Colorectal Biopsies - Adjacent normal and tumor
2. Blood and Urine Samples

1. Post-Treatment Colorectal Biopsies - Adjacent normal and tumor
2. Blood and Urine Samples

20 Patients - Black Raspberry Slurry (20g/3x/day)

Treatment Time

(Range 1-9 wks, Average 3 wks)

Black Raspberry Slurry



Colorectal Cancer Patient Demographics

	No. of Patients (%)
Gender	
Female	3 (15)
Male	17 (85)
Age	
<45	2 (10)
45-60	8 (40)
>60	10 (50)
Average 59	
Tumor location	
Transverse, descending, and sigmoid colon	3 (15)
Cecum and ascending colon	3 (15)
Rectum	14 (70)
Metastatic disease	
Lymph node involved	2 (10)
No evidence	18 (90)

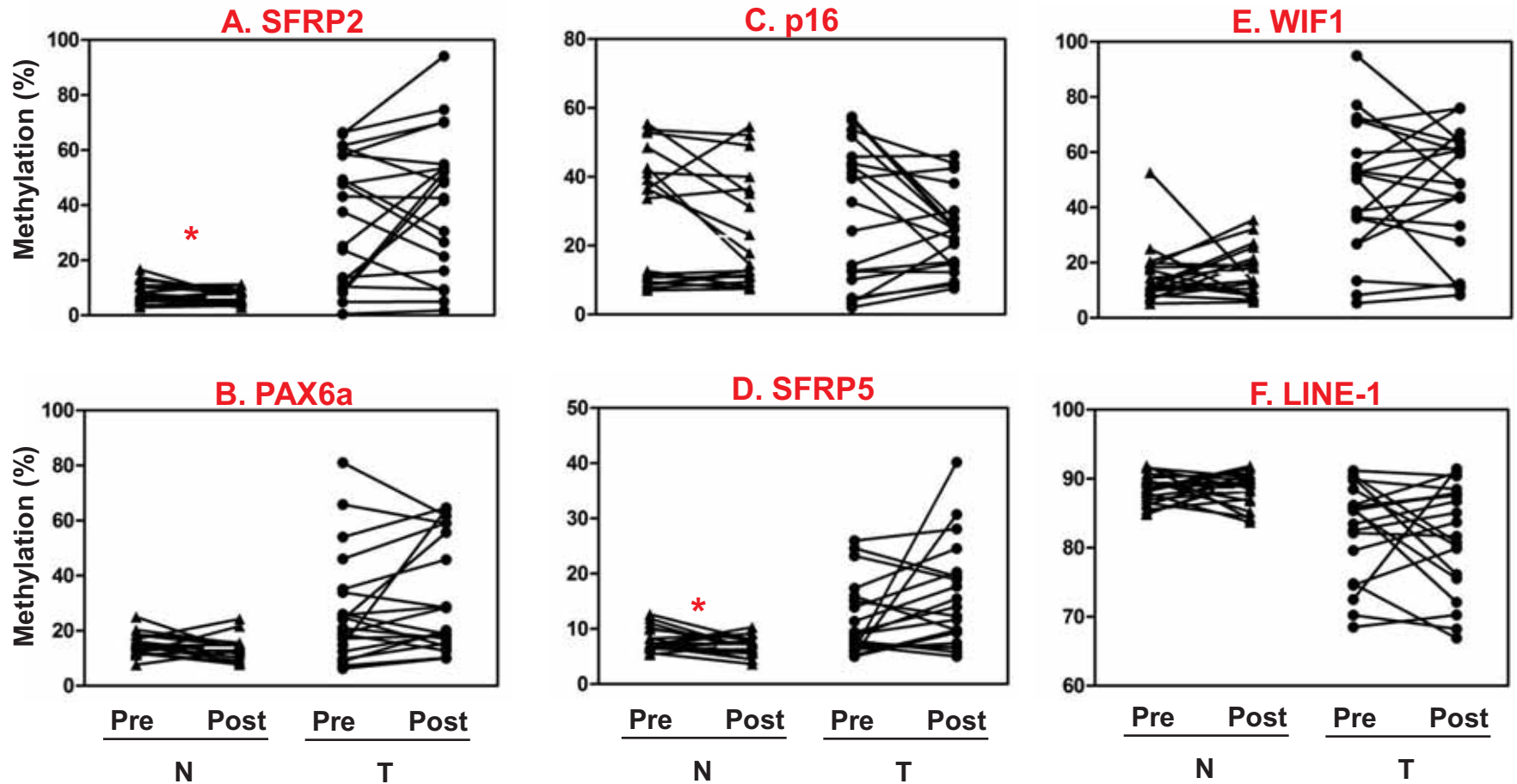
Colon Cancer Patient Demographics (cont'd)

	No. of Patients (%)		No. of Patients (%)
Berry treatment (wks)		Compliance (%)	
≤ 3	11 (55)	80-90	1 (5)
3-4	6 (30)	90-100	8 (40)
>4	3 (15)	100	11 (55)
Range 1-9, Average 3		Toxicities reported	
Berry doses (20g/dose, 3 doses/day)		Diarrhea	3 (15)
≤ 50	8 (40)	Constipation	4 (20)
50-100	10 (50)	None	13 (65)
>100	2 (10)	Improved bowel movements	15 (75)
Average 67			

BRB Anthocyanins Were Detected in Urine and Tissue from Colorectal Cancer Patients After Average 3 Wks of BRB Treatment

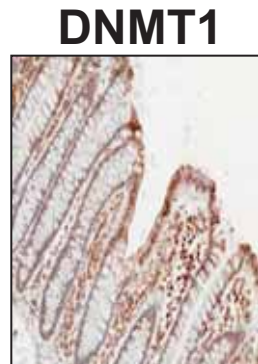
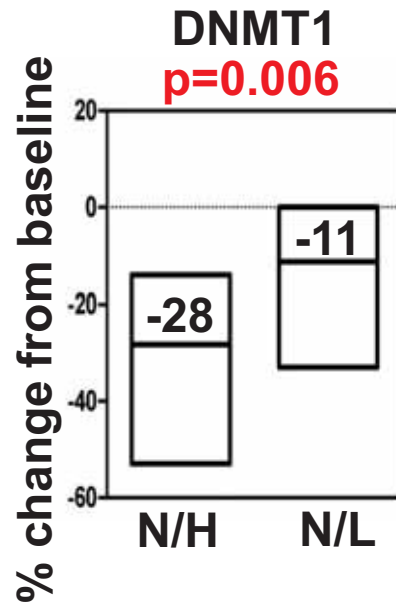
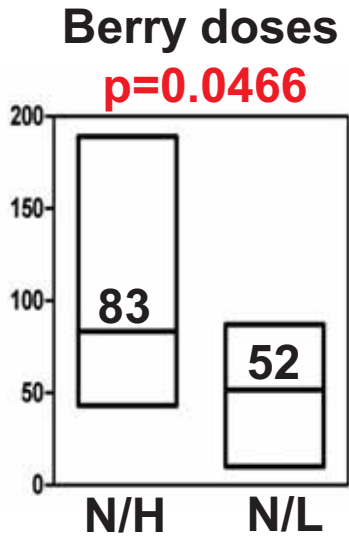
	Pre-treatment		Post-treatment		
	Mean	S.D.	Mean	S.D.	
	(Range)		(Range)		
Urine (pmol/mL)	0.0	0.0	423.9	429.3	
	(0.0-0.0)		(56.2-1822.1)		
Adjacent normal (fmol/mg)	0.0	0.0	299.9	754.9	
	(0.0-0.0)		(1.7-2011.5)		
Adenocarcinoma (fmol/mg)	0.0	0.0	55.4	60.8	p=0.21
	(0.0-0.0)		(2.2-109.2)		

Effects of BRBs on promoter methylation and on LINE-1 repetitive element in adjacent normal tissues and colorectal adenocarcinomas from 20 patients before and after 1-to-9 wks of berry treatment

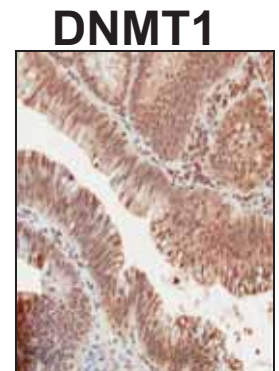
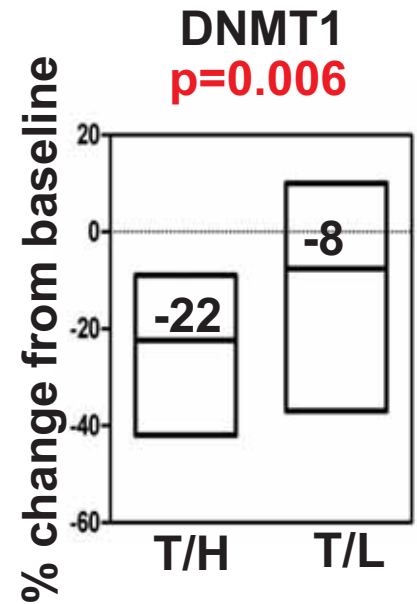
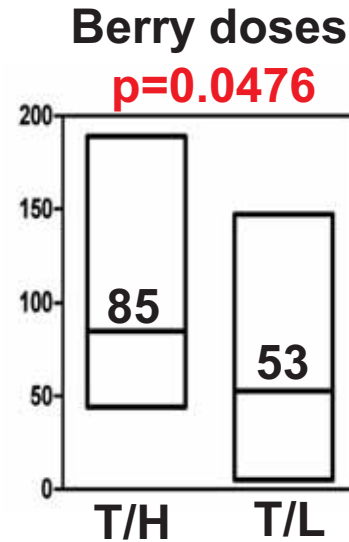


Percent Change from Baseline in DNMT1 Protein Expression in Adjacent Normal and Tumor Tissues in Colorectal Cancer Patients Who Were Treated with Berries – High vs Low Doses

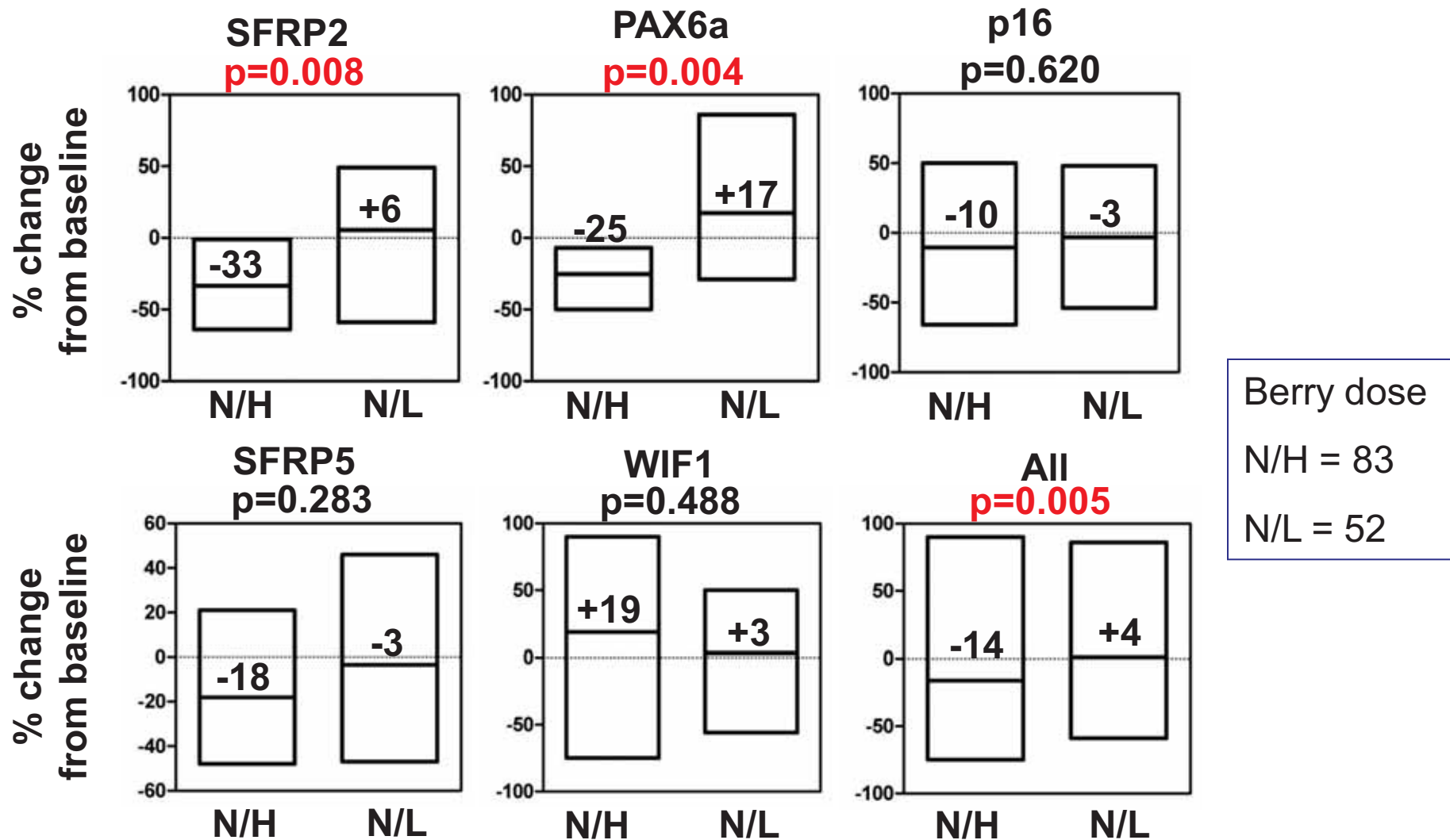
Adjacent normal



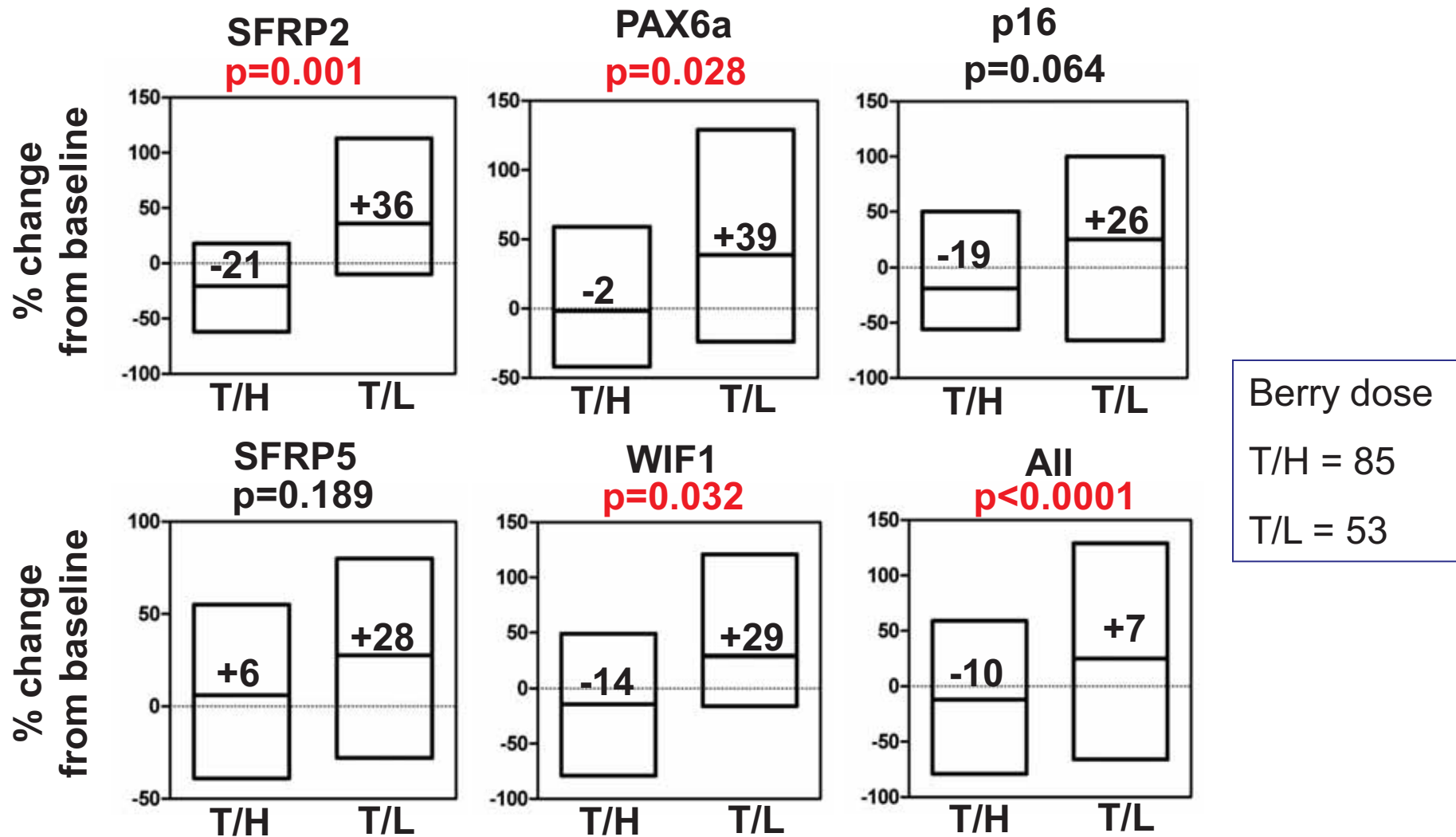
Adenocarcinoma



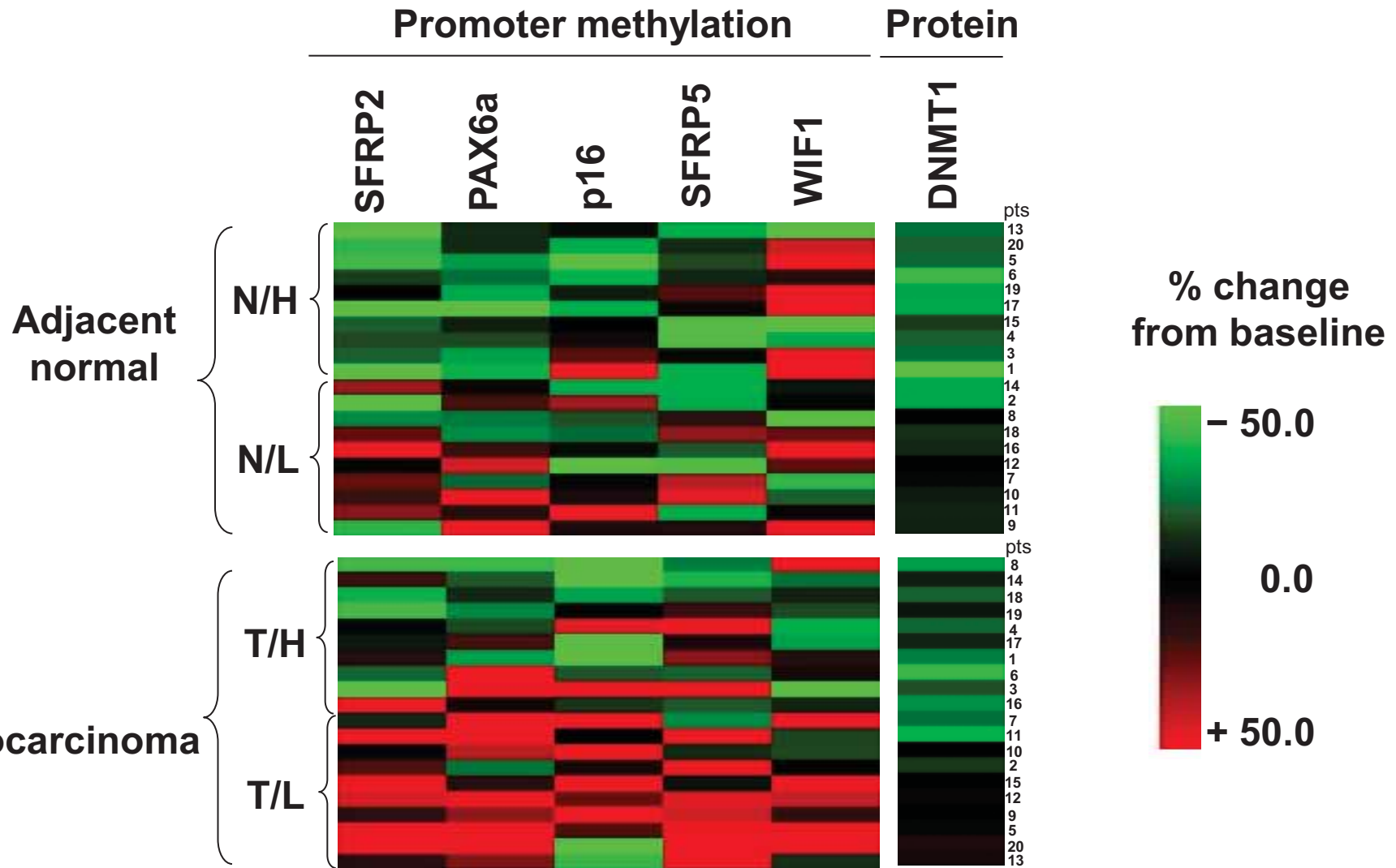
Percent Change from Baseline in Promoter Methylation in Adjacent Normal Tissues in Colorectal Cancer Patients Who Were Treated with Berries – High vs Low Doses



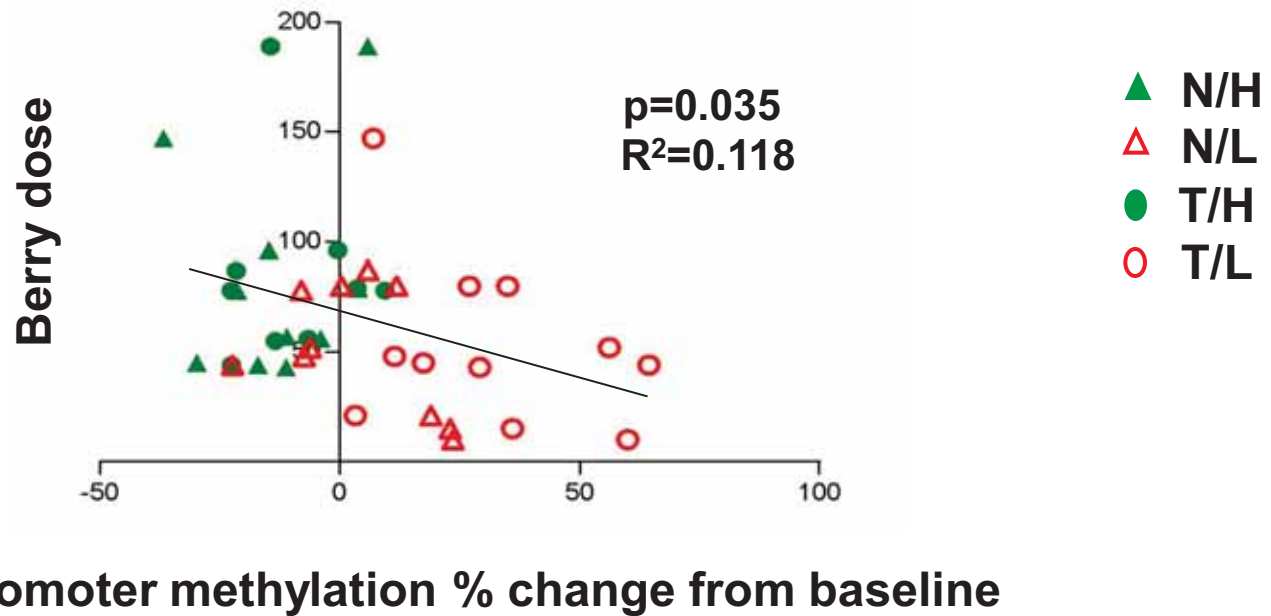
Percent Change from Baseline in Promoter Methylation in Adenocarcinoma in Colorectal Cancer Patients Who Were Treated with Berries – High vs Low Doses



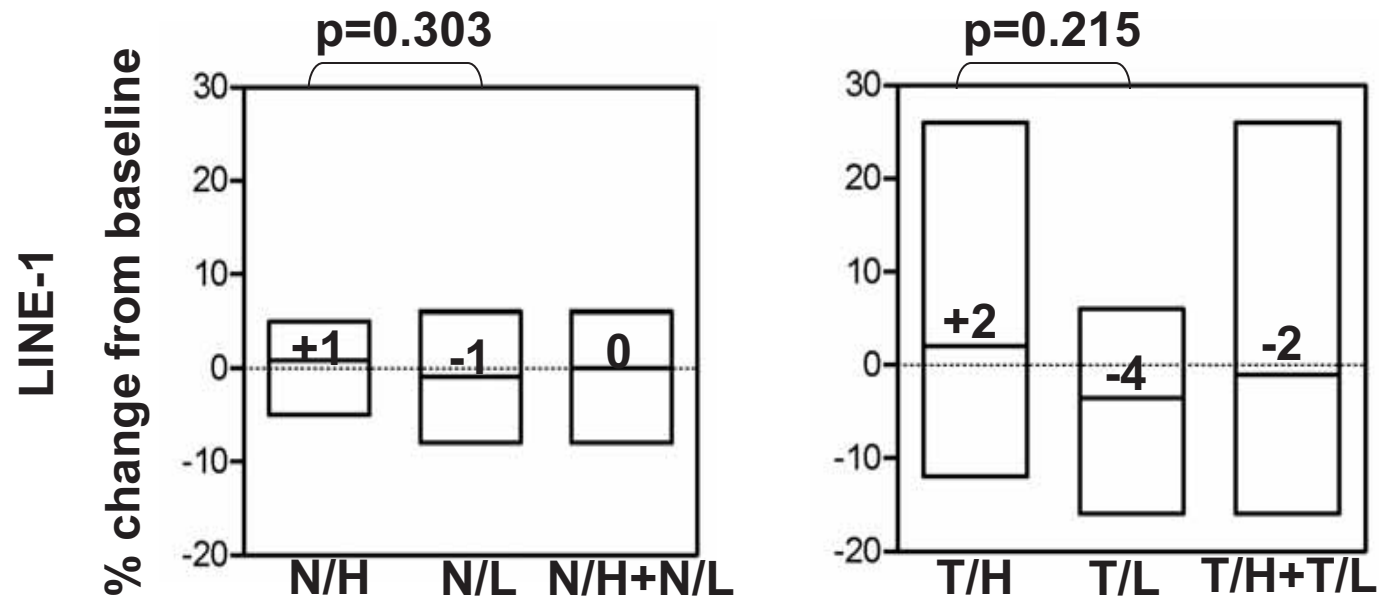
Heat-maps of Promoter Methylation Changes from Baseline of *SFRP2*, *PAX6a*, *p16*, *SFRP5* and *WIF1*, and Protein Expression Change of DNMT1



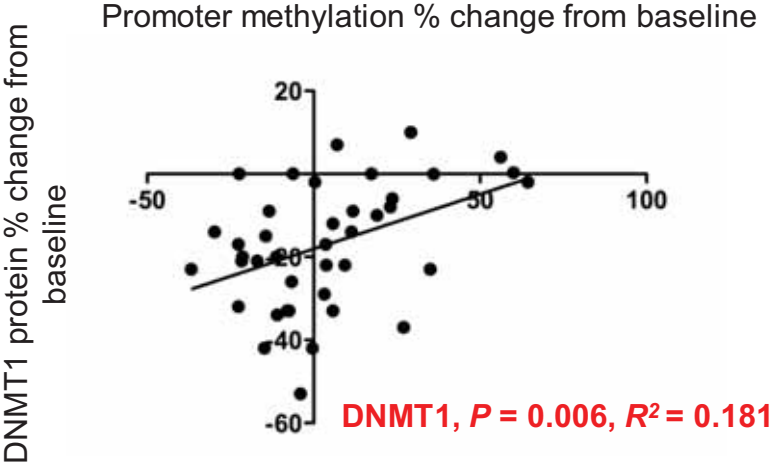
Correlation of Methylation Change with Total Berry Dose



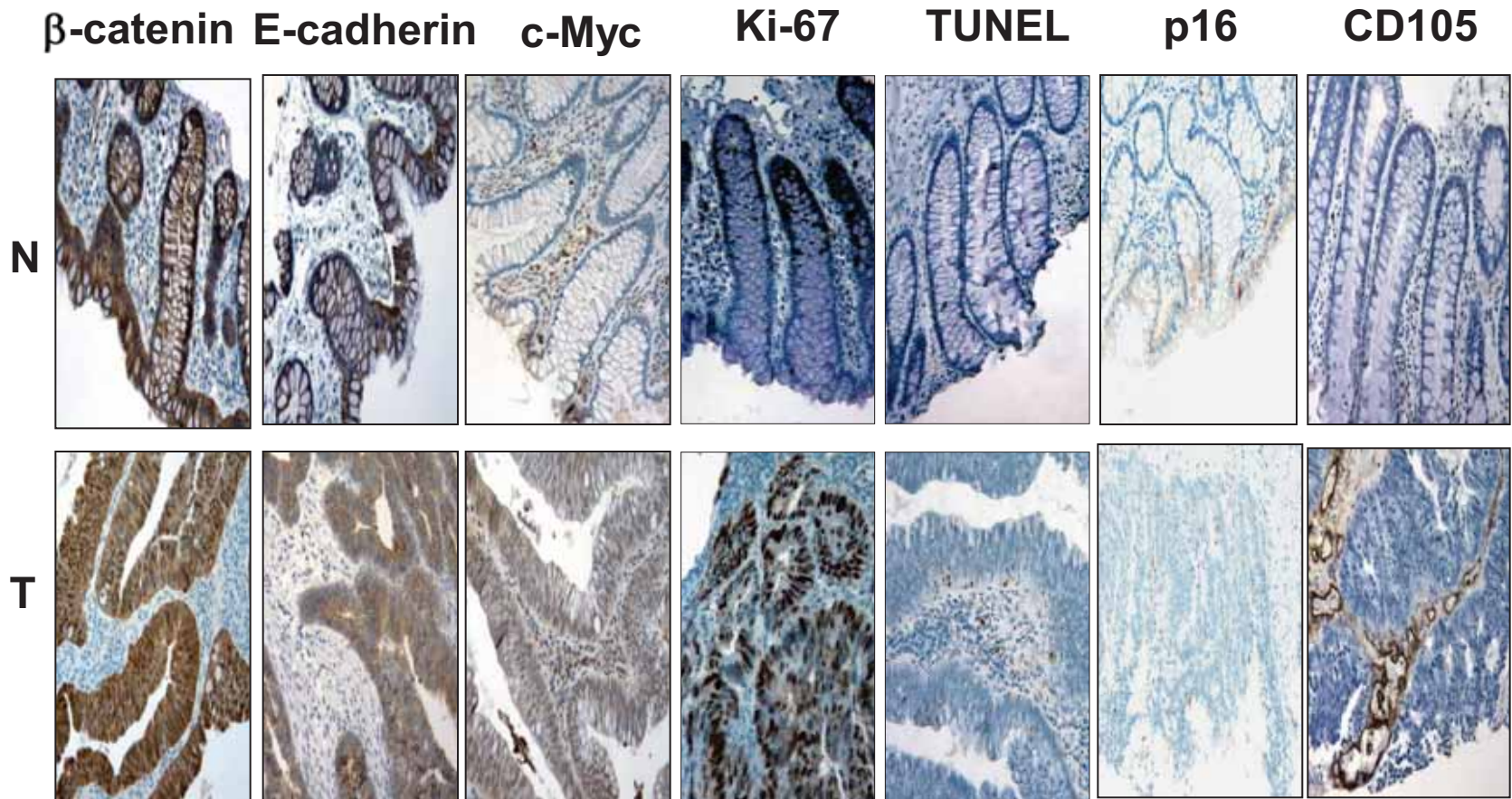
BRB Treatment Did Not Affect Global Methylation



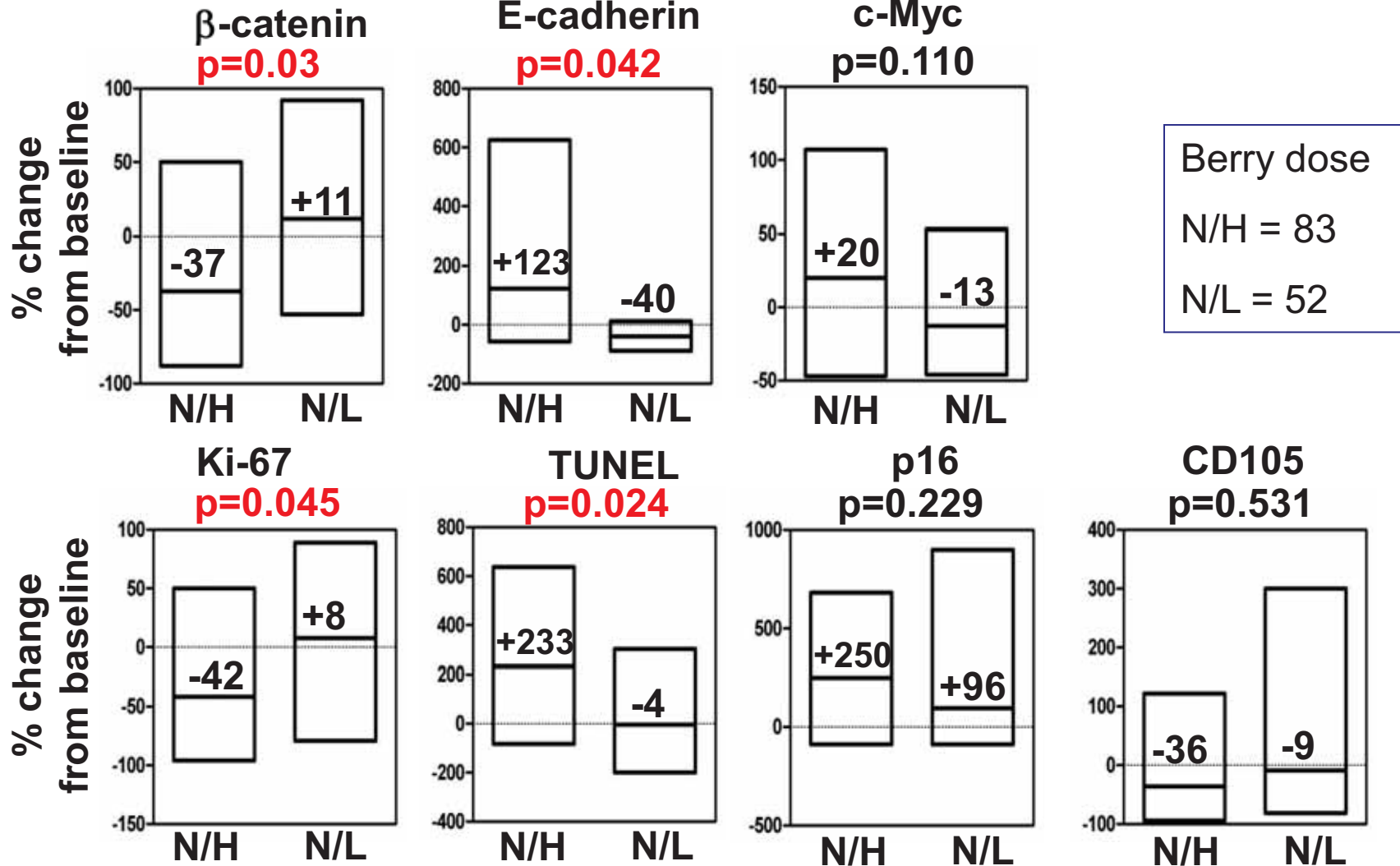
Correlation of Methylation Change with DNMT1 Changes



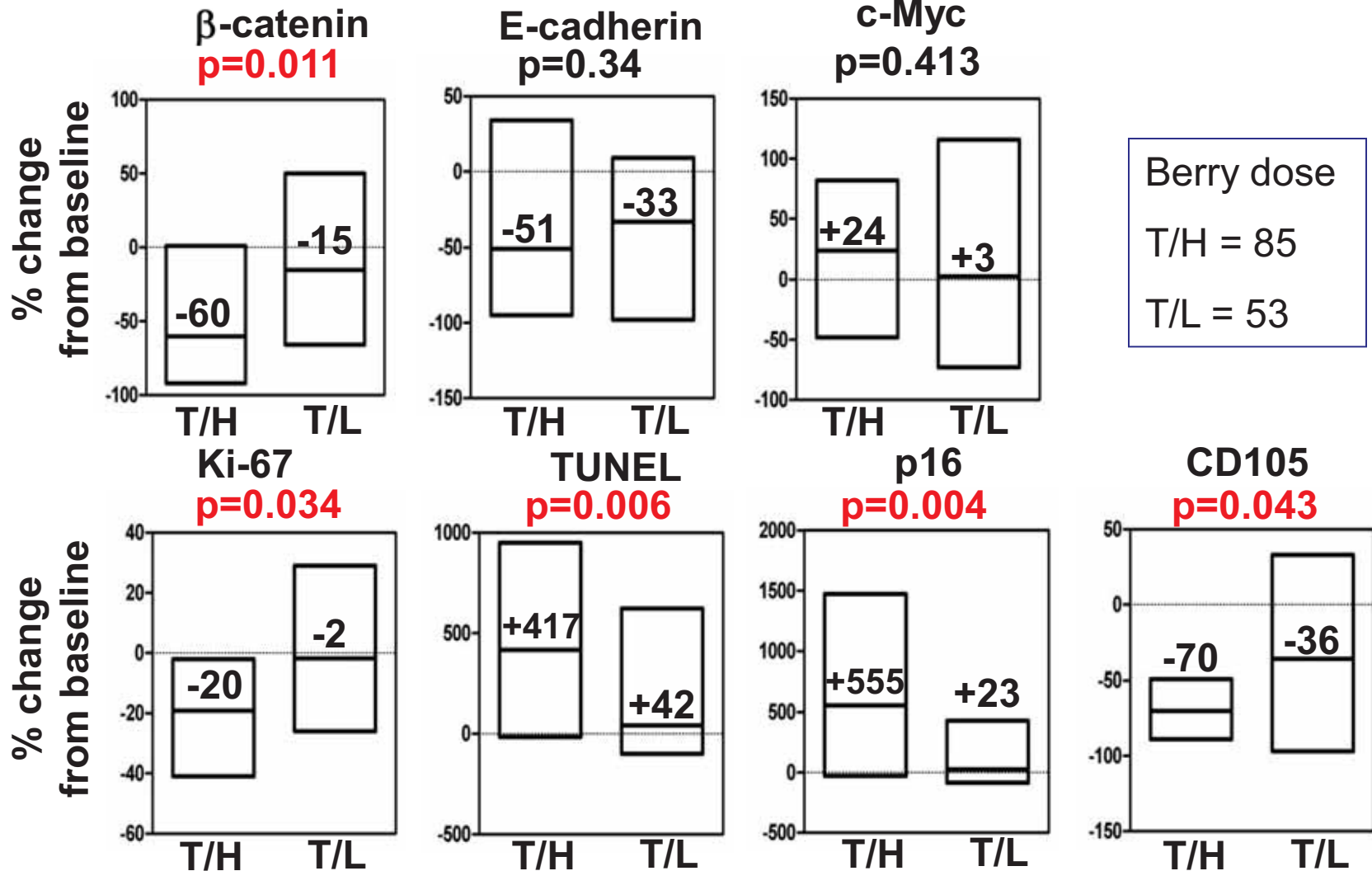
Representative Immunohistochemical Staining in Adjacent Normal and Colorectal Tumors



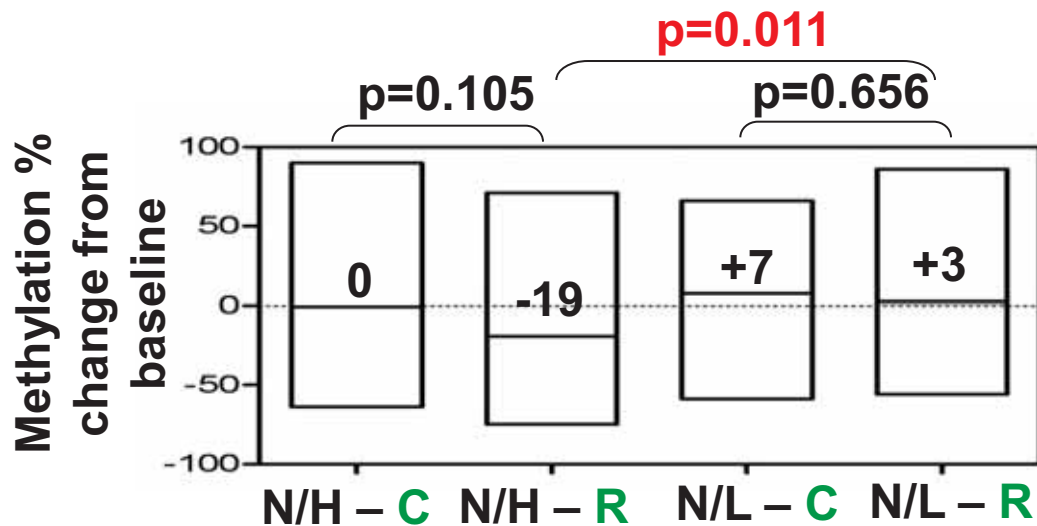
Percent Change from Baseline in Protein Expression in Adjacent Normal Tissues in Colorectal Cancer Patients Who Were Treated with Berries – High vs Low Doses



Percent Change from Baseline in Protein Expression in Adenocarcinoma in Colorectal Cancer Patients Who Were Treated with Berries – High vs Low Doses



Differential Responses of Adenocarcinoma from the Colon and Rectum to Berry Treatment



N/H – normal / high dose

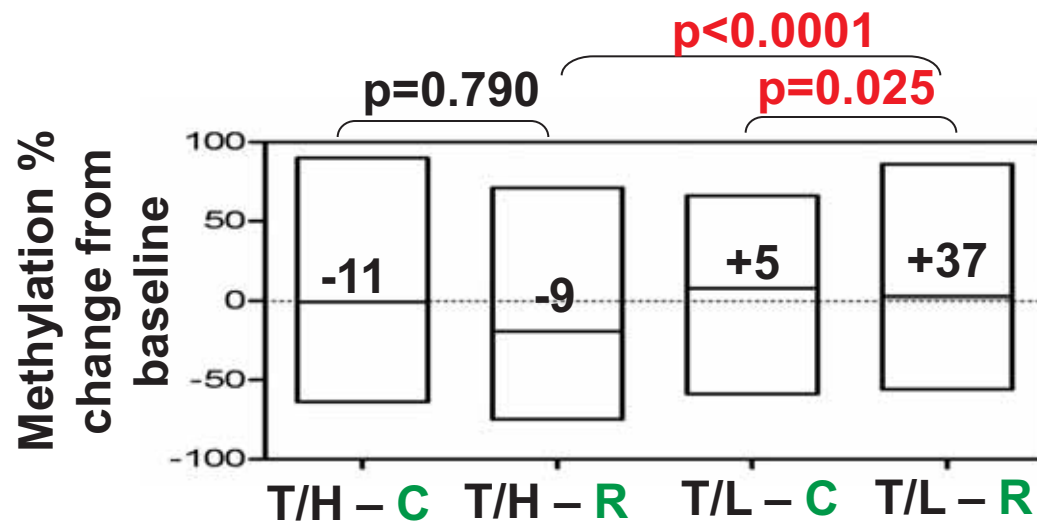
N/L – normal / low dose

T/H – Tumor / high dose

T/L – Tumor / low dose

C, Colon

R, Rectum



Summary

- The methylation of three Wnt inhibitors, *SFRP2*, *SFRP5* and *WIF1*, upstream genes in the Wnt pathway, and *PAX6a*, a developmental regulator, was modulated in a protective direction by BRBs in adjacent normal colon and in colorectal tumors. This was associated with decreased expression of DNMT1.
- BRBs also modulated the expression of genes downstream of the Wnt pathway as well as cell proliferation, apoptosis and angiogenesis in a protective direction.
- Rectal adjacent normal and adenocarcinomas were less responsive to short-term (~2 wks) berry treatment.

Conclusions

Pilot study in humans indicate that black raspberries (BRBs):

- **Are well tolerated.**
- **Protectively modulated DNA methylation markers. This was associated with decreased expression of DNMT1 and protective modulation of cellular functions e.g. cell proliferation and apoptosis.**
- **Could be chemopreventive in human colorectal cancer.**

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