

# Role for the Sphingosine kinase 1/Sphingosine-1-phosphate pathway in colon carcinogenesis

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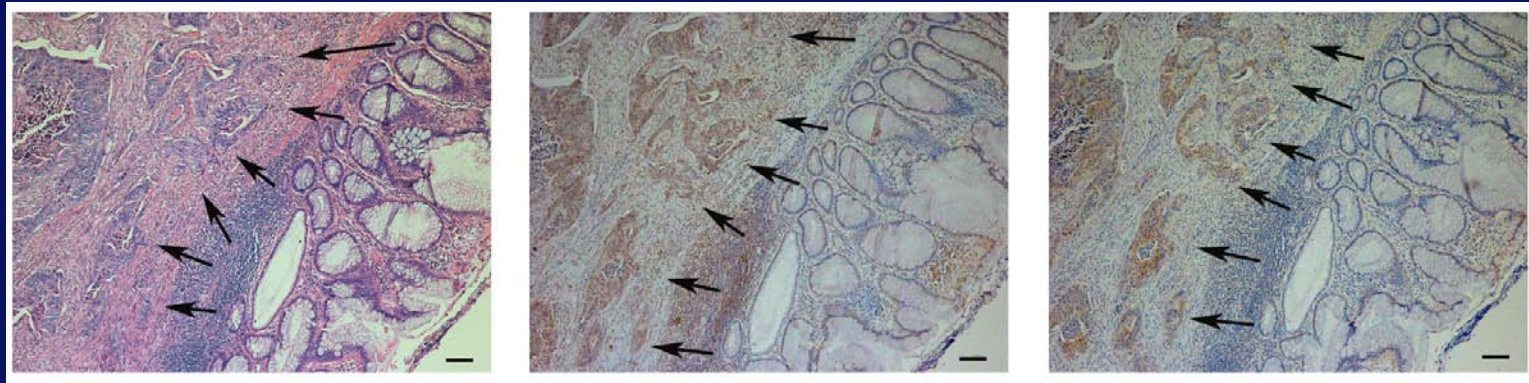
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# Introduction

- Colorectal cancer is the 2<sup>nd</sup> leading cause of cancer related deaths in the US.
- Accumulating evidence suggests that dietary factors, especially fat (lipids), are important for colon carcinogenesis.
- Our recent study suggests that the sphingosine kinase 1 (SphK1)/sphingosine-1-phosphate (S1P) may mediate COX-2 expression and PGE<sub>2</sub> production in colon cancer cells and SphK1 is overexpressed in rodent colon cancer tissues (*Faseb J 20: 386, 2006*).

We hypothesized that Sphk1/S1P pathway mediate colon carcinogenesis and constitute novel targets for colon cancer chemoprevention.

# Immunohistochemistry in human colon cancers



H&E

SphK1

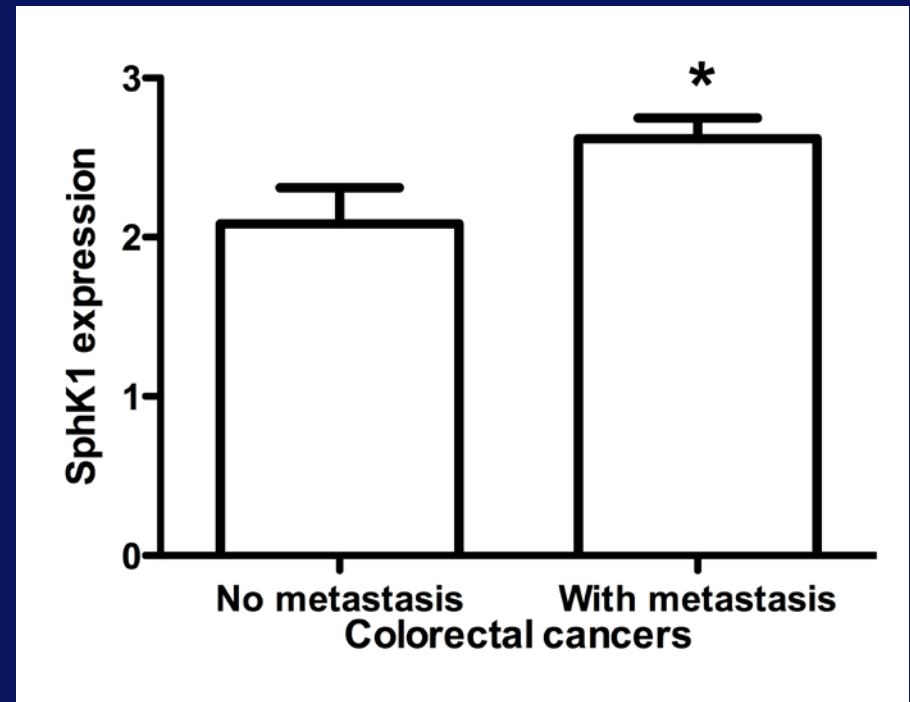
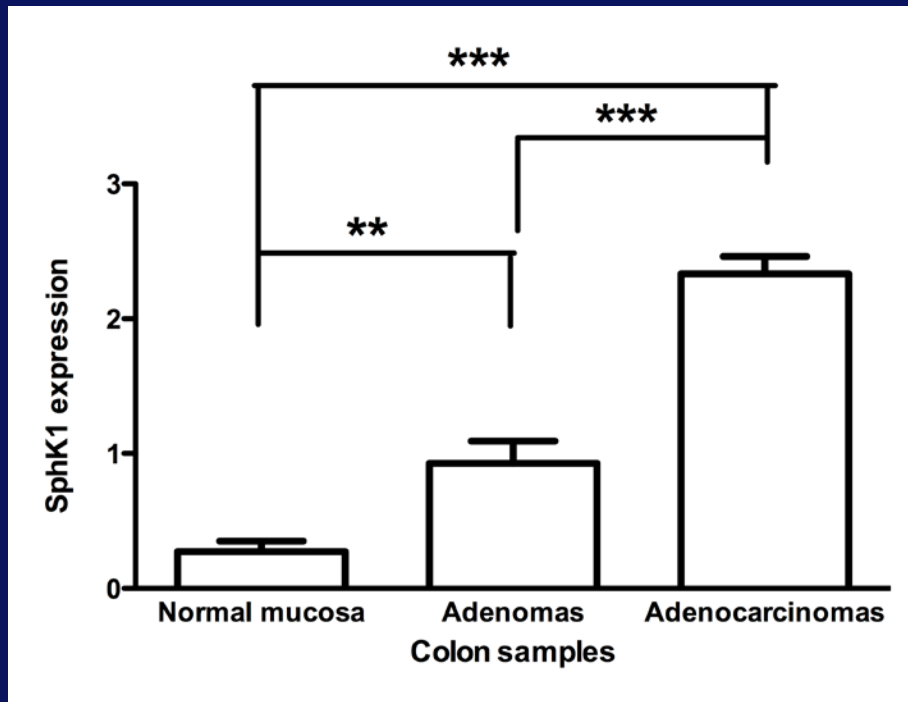
COX-2

TABLE 1 Summary of immunohistochemistry for SphK1 and COX-2 in human colorectal tissues

	N	SphK1 staining				COX-2 staining			
		—	±	+	2+	—	±	+	2+
Normal mucosa	33	24 (73)	9 (27)			27 (82)	6 (18)		
Adenoma	14	3 (21)	9 (64)	2 (14)		8 (57)	4 (29)	2 (14)	
Adenocarcinoma	33		5 (15)	12 (36)	16 (48)	3 (9)	13 (39)	13 (39)	4 (12)
Metastasis	14			6 (43)	8 (57)		6 (43)	5 (36)	3 (21)

Values in parentheses represent percentage, —, no staining; ±, weak staining in <50% of the cells; +, moderate staining in >50% of the cells; 2+, strong staining in >50% of the cells.

# SphK1 expression levels in human colorectal tumors



# ACF (aberrant crypt foci) formation assay in mice

AOM (10mg/kg) or saline ip injection



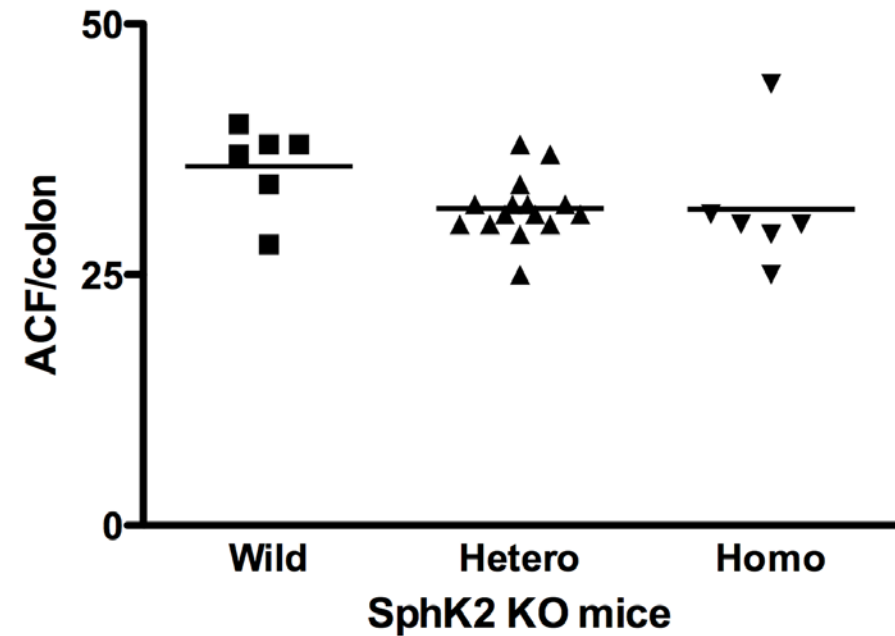
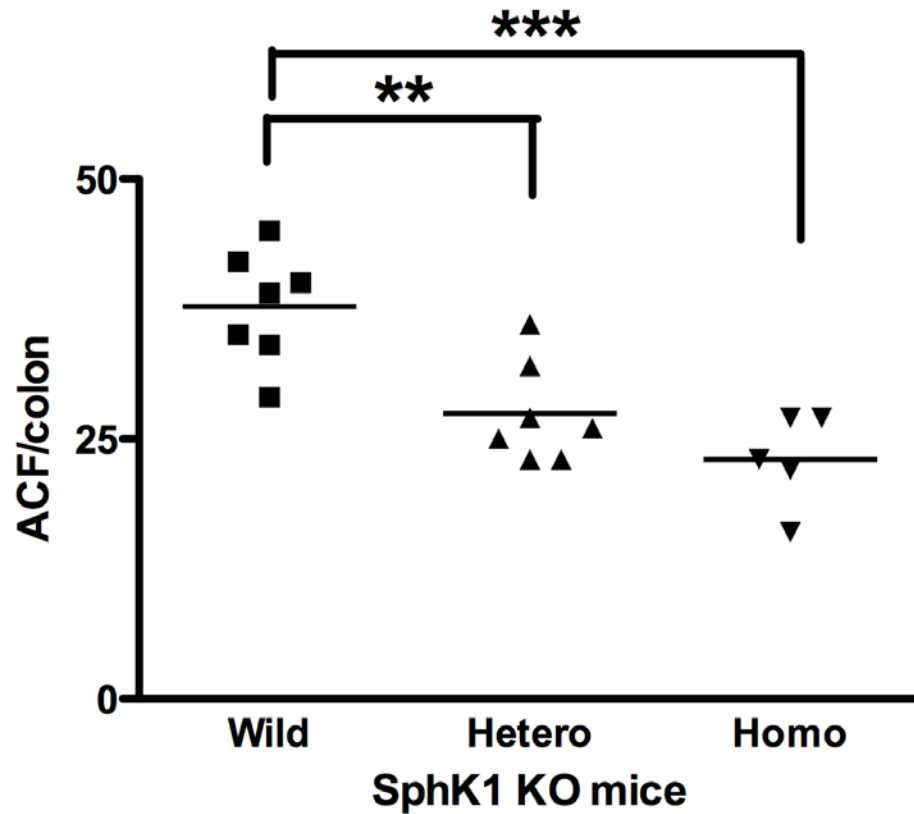
Wild type, SphK1 or SphK2 KO mice

ACF

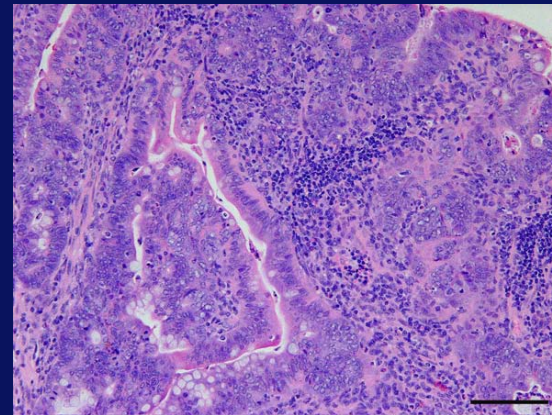
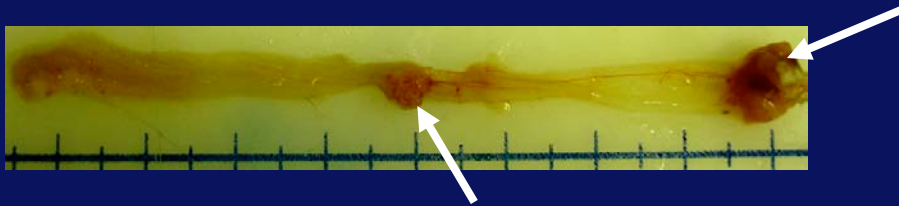
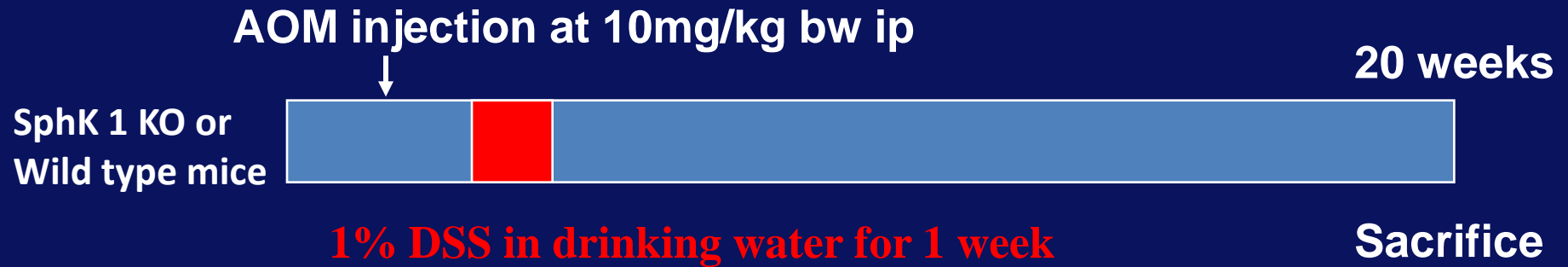


Sacrifice animals

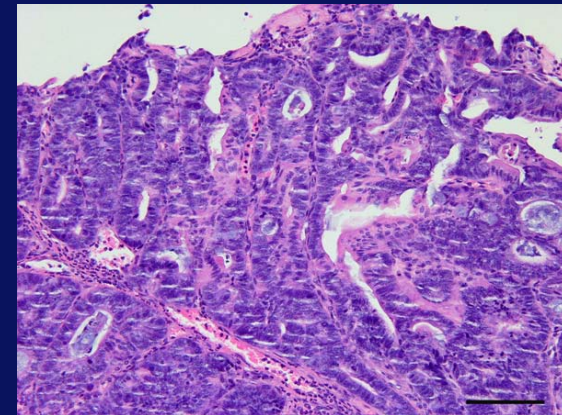
# SphK1, not SphK2, deficiency significantly inhibits ACF formation



# Inflammation-related colon cancer model

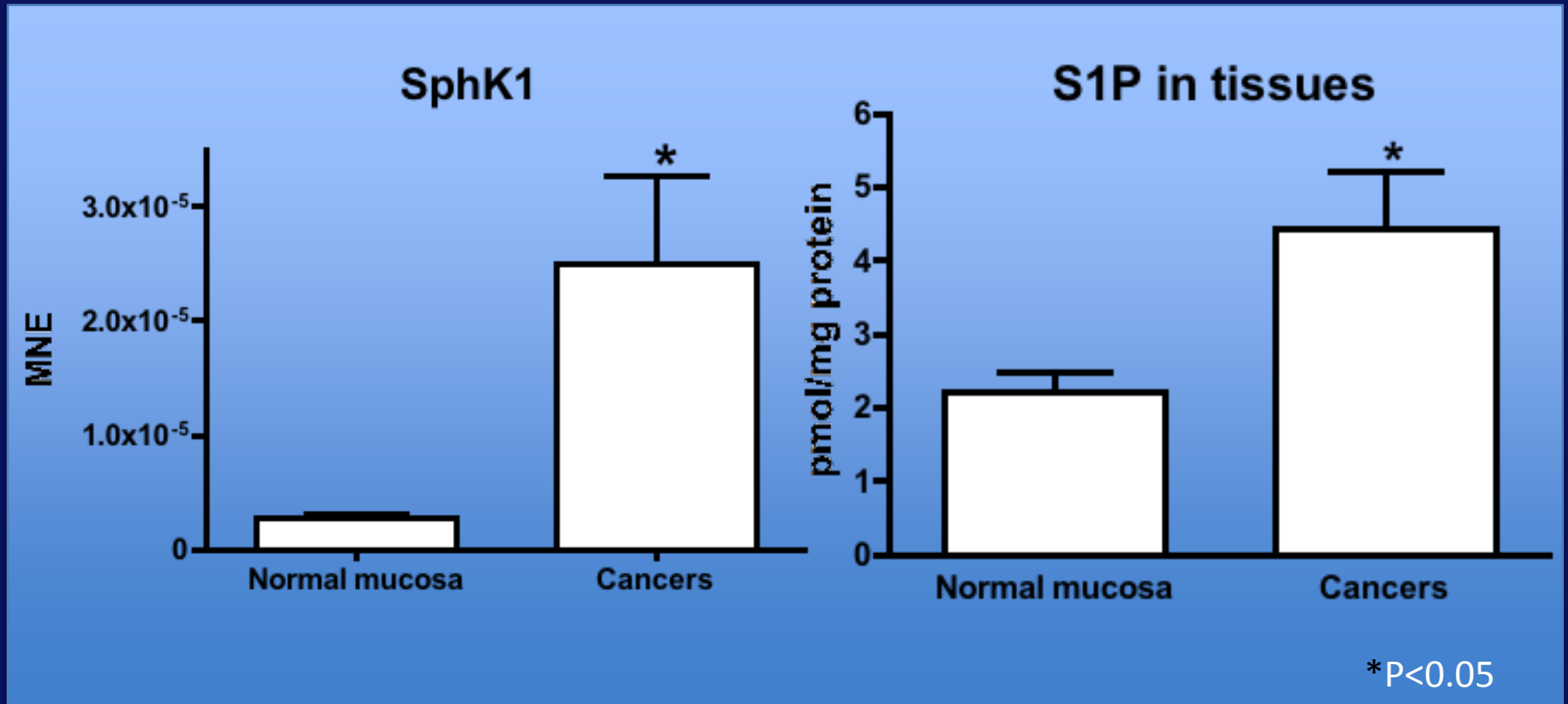


WT

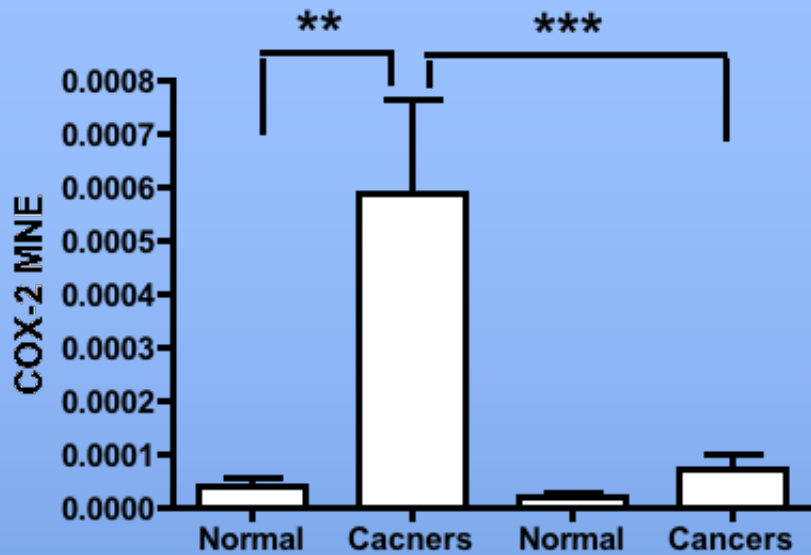


SphK1 KO

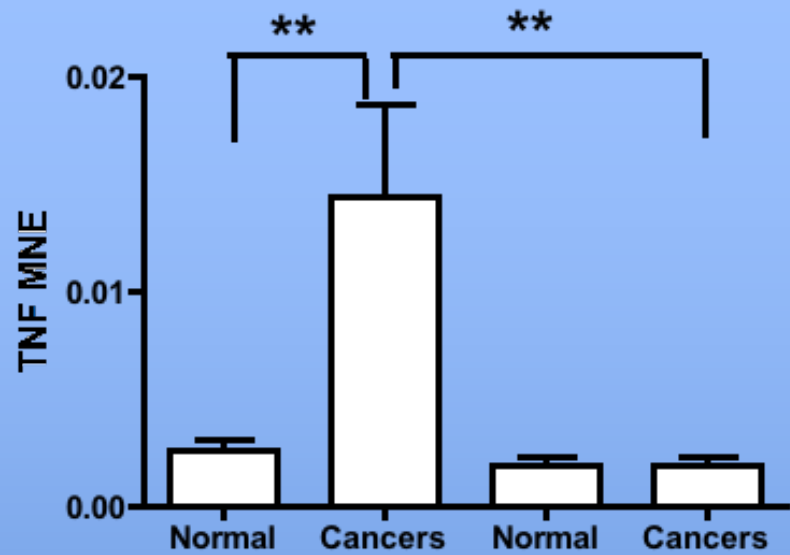
# SphK1 is up regulated in colon cancers in wild type mice



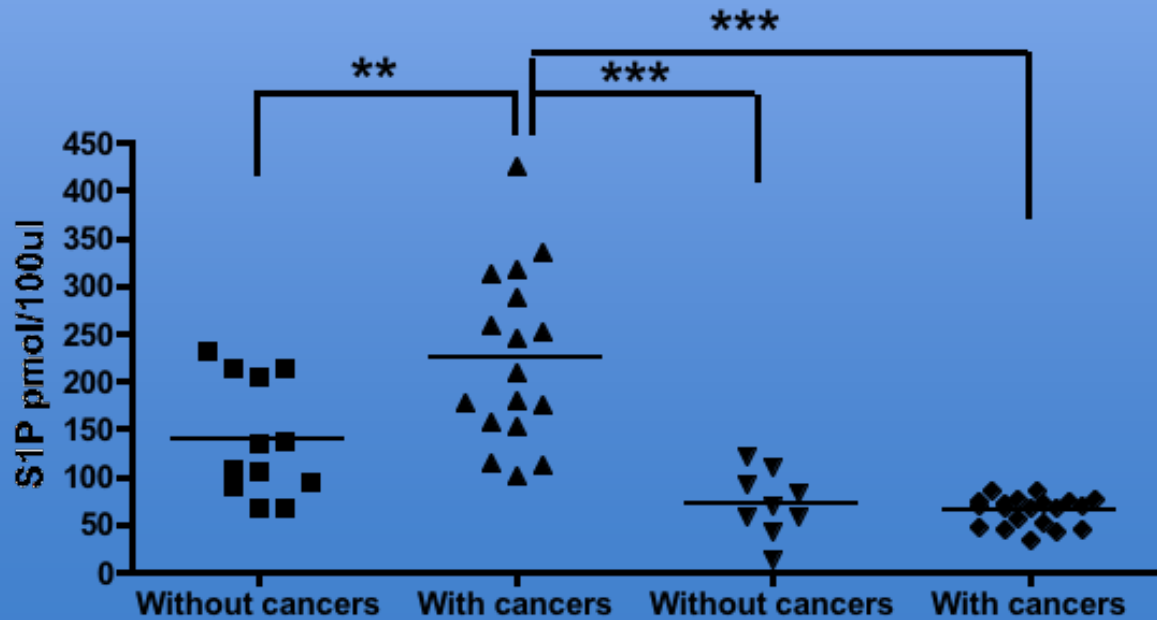
# COX-2



# TNF



# S1P in blood



\*\*P<0.01,  
\*\*\*P<0.001

WT

SphK1 KO

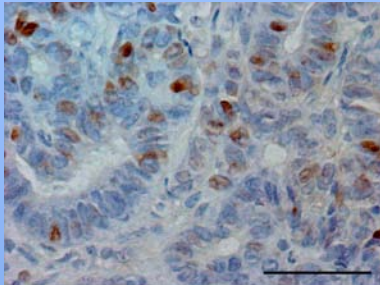
TABLE 2 Results of AOM/DSS-induced colon carcinogenesis in SK1 KO mice

Mouse	Incidence (% mice with colon tumors)			Multiplicity (no. tumors/mouse)			Cancer
	Total	Adenoma	ADC	Total	Adenoma	ADC	Volume (mm <sup>3</sup> )
Wild type	73	23	60	1.17±0.20	0.23±0.08	0.97±0.21	17.4±4.41
SK1 KO	38 (48)**	10 (57)	31 (48)**	0.41±0.11 <sup>#</sup>	0.10±0.06	0.31±0.09 <sup>##</sup>	4.77±1.86

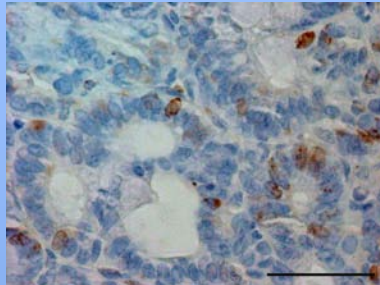
Totals include adenomas and adenocarcinomas (ADC). Values in parentheses represent percentage inhibition from wild type mice. Multiplicity and volume values are mean ± SE. \*\* $P < 0.01$  vs. wild type;  $\chi^2$  test. <sup>#</sup> $P < 0.005$ , <sup>##</sup> $P < 0.01$  vs. wild type; Student's  $t$  test.

## Cell proliferation

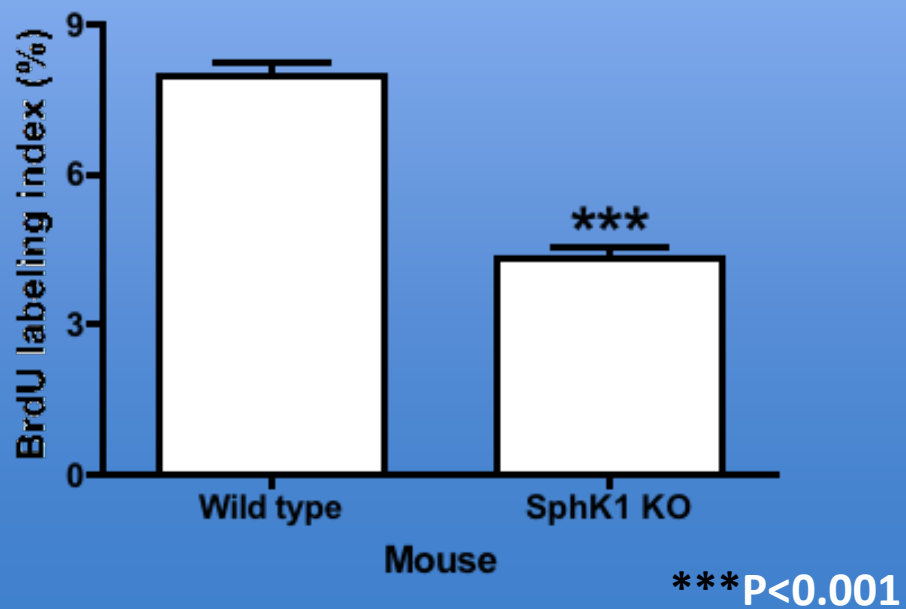
A



B

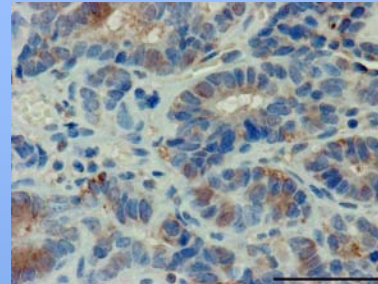


C

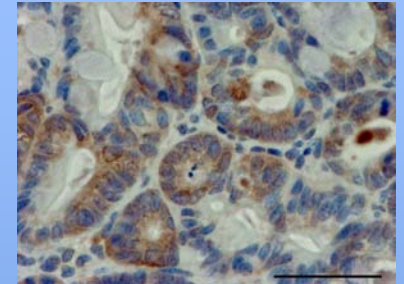


## Apoptosis

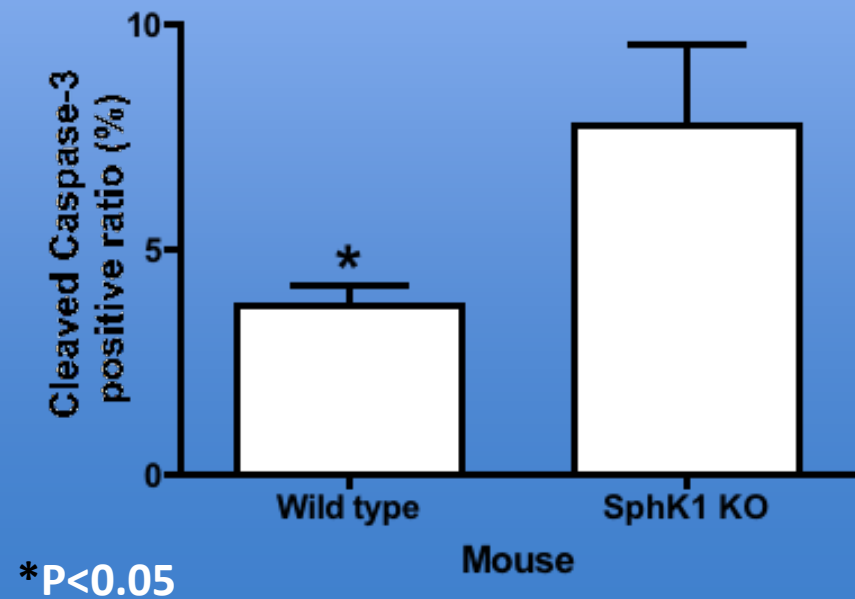
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E



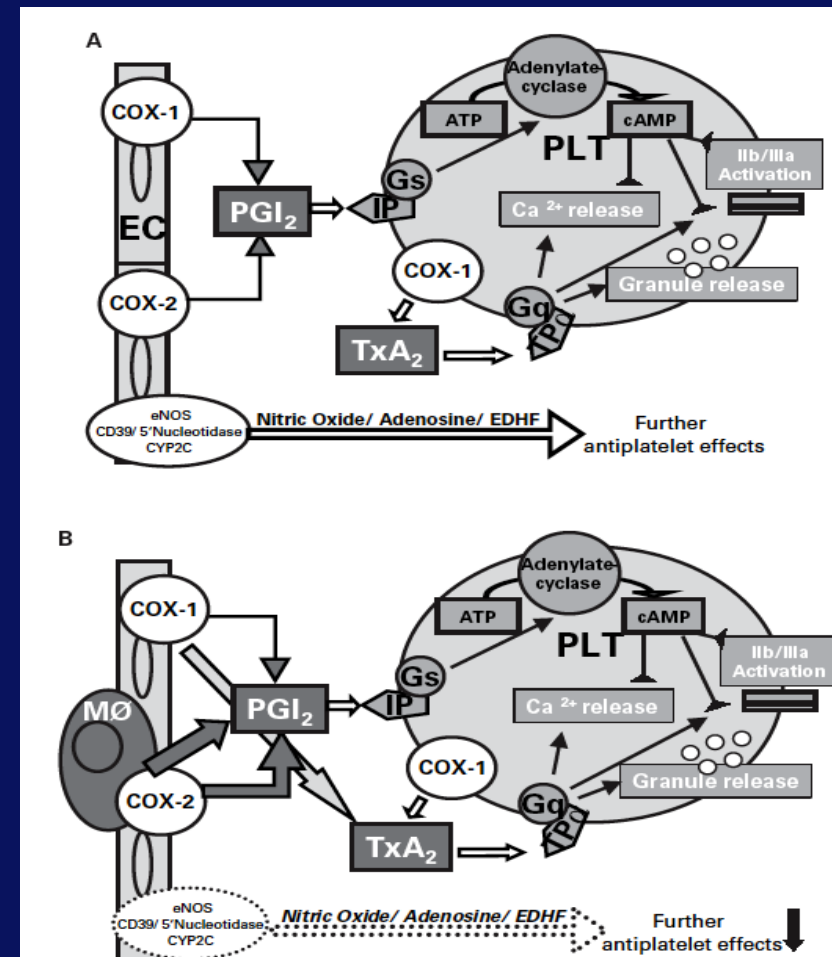
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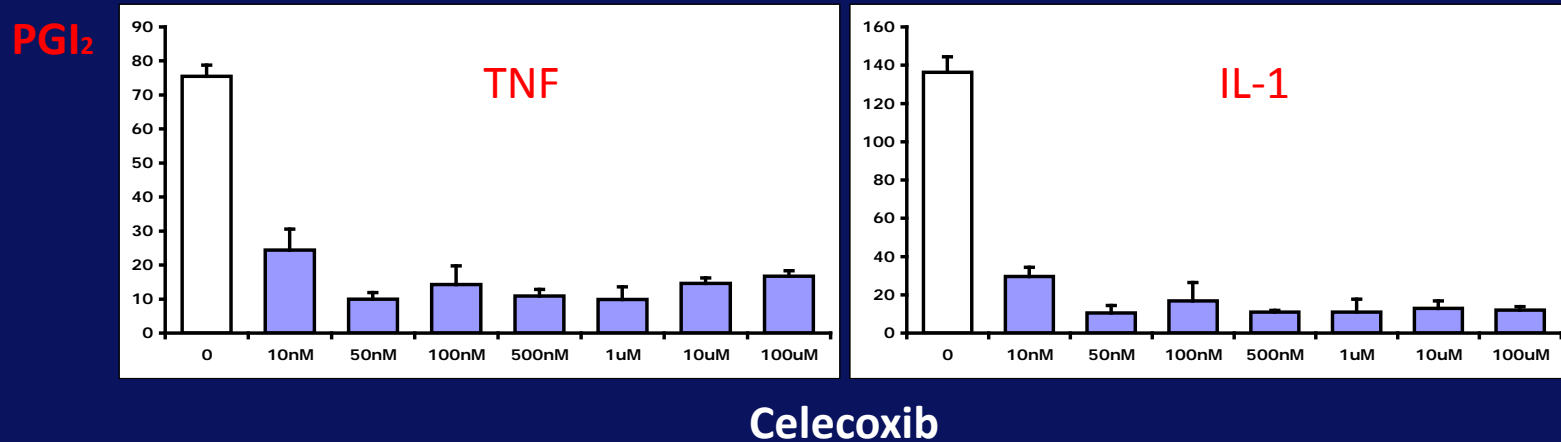
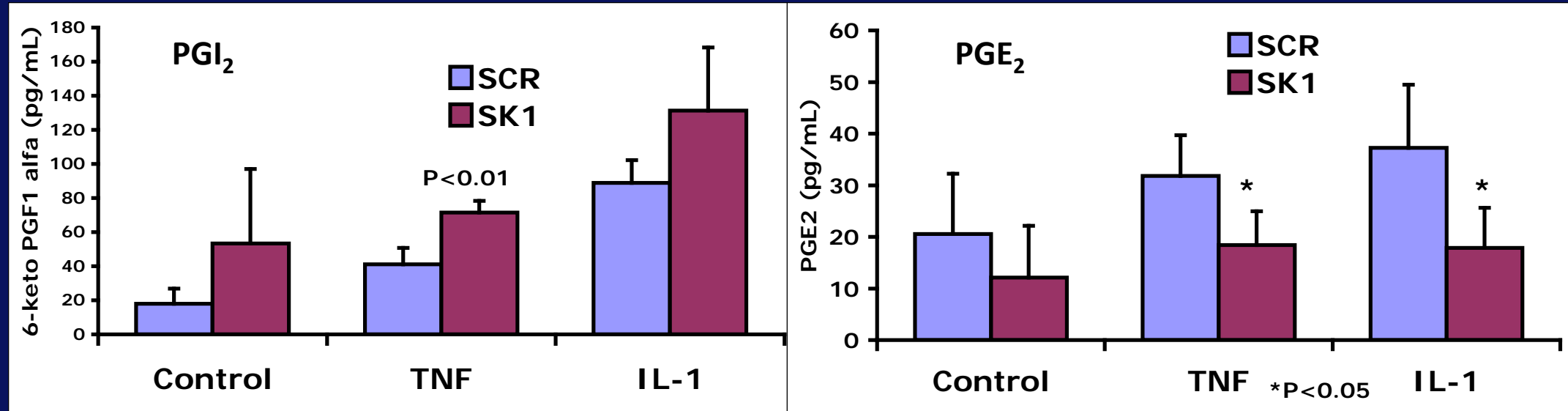
# Problems with COX-2 inhibitors

Selective COX-2 inhibitors show unwanted side effects such as increased cardiovascular risks and have been withdrawn from clinical chemoprevention and Alzheimer's disease trials.

One of the reasons may be due to a depression of PGI<sub>2</sub> formation by selective COX-2 inhibitors in endothelial cells which may elevate blood pressure, accelerate atherogenesis, and predispose patients receiving COX-2 inhibitors to an exaggerated thrombotic response to the rupture of an atherosclerotic plaque.



# SphK1 down-regulation does not inhibit PGI<sub>2</sub> production in HUVECs



# Summary

- SphK1 is overexpressed in human colon tumors including adenomas and adenocarcinomas.
- SphK1 expression is higher in primary colon cancers with metastasis than those without metastasis.
- SphK1 mRNA and S1P are up regulated in colon cancer tissues in mice.
- Levels of S1P in blood from mice with colon cancer are higher than those without colon cancer.
- SphK1, not SphK2, deficiency significantly reduced AOM-induced ACF formation, a preneoplastic lesion.
- SphK1 deficiency significantly inhibited colon cancer development with reduction of cell proliferation and induction of apoptosis.
- SphK1 down regulation does not reduce PGI<sub>2</sub> formation in HUVECs.

**The results provide evidence of a role for the SphK1/S1P pathway in colon carcinogenesis and chemoprevention.**

# Acknowledgments

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