



EFFECT OF ESTRADIOL IN AN AOM/DSS-TREATED MOUSE MODEL OF COLORECTAL CANCER: IMPLICATION FOR SEX DIFFERENCE IN COLORECTAL CANCER DEVELOPMENT

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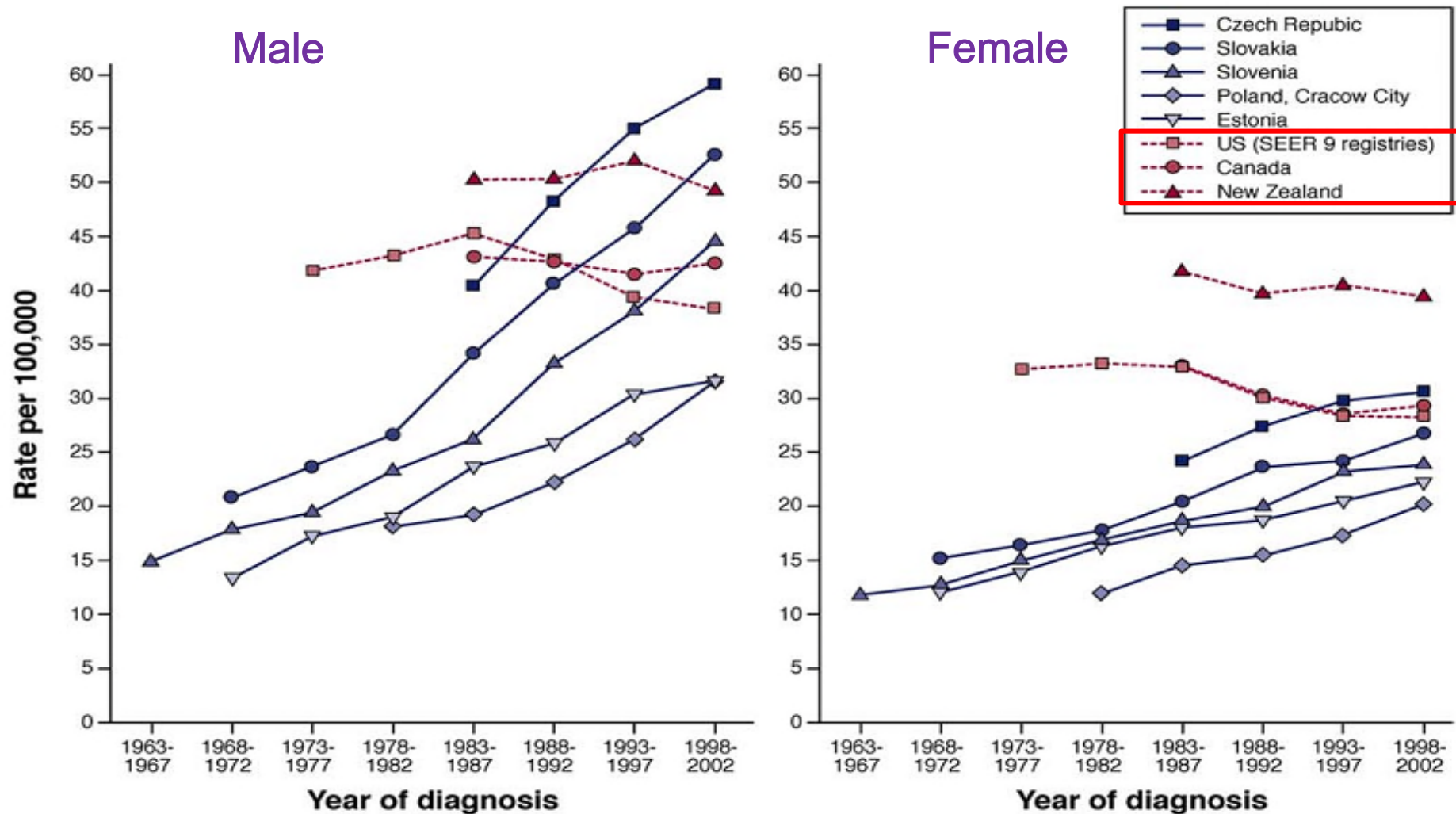
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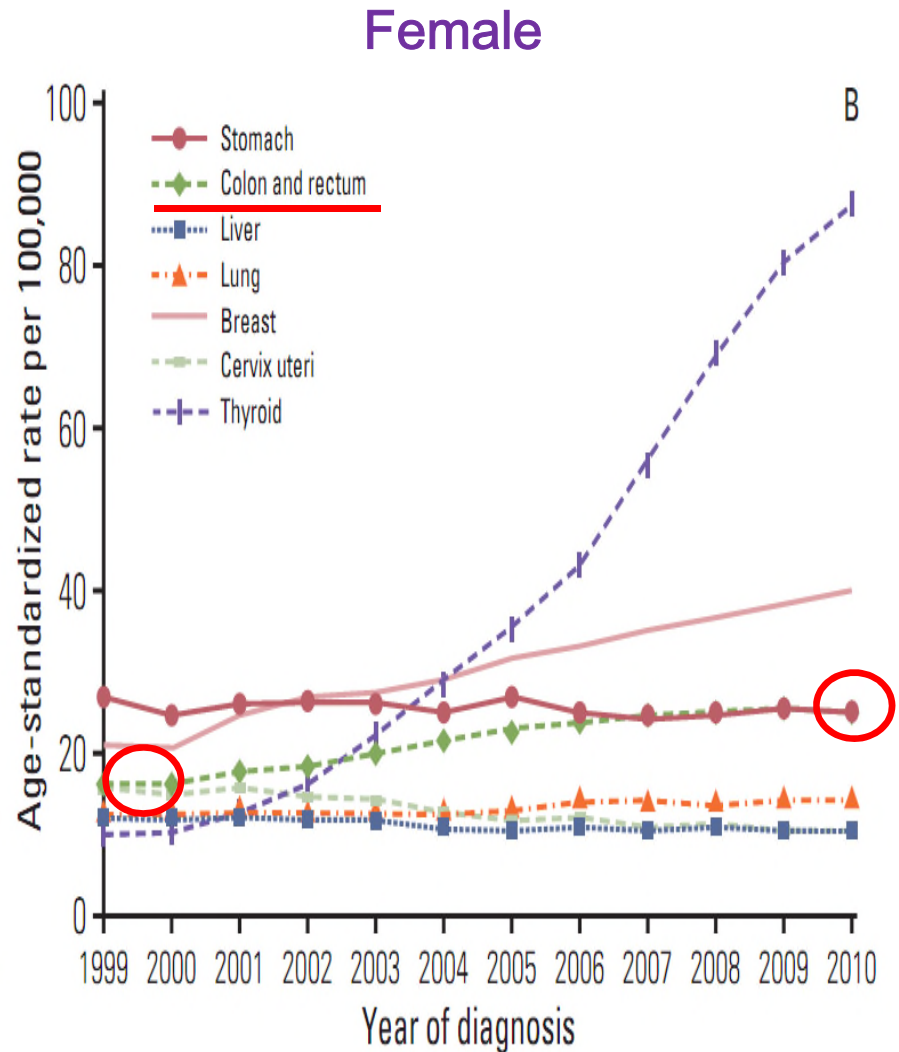
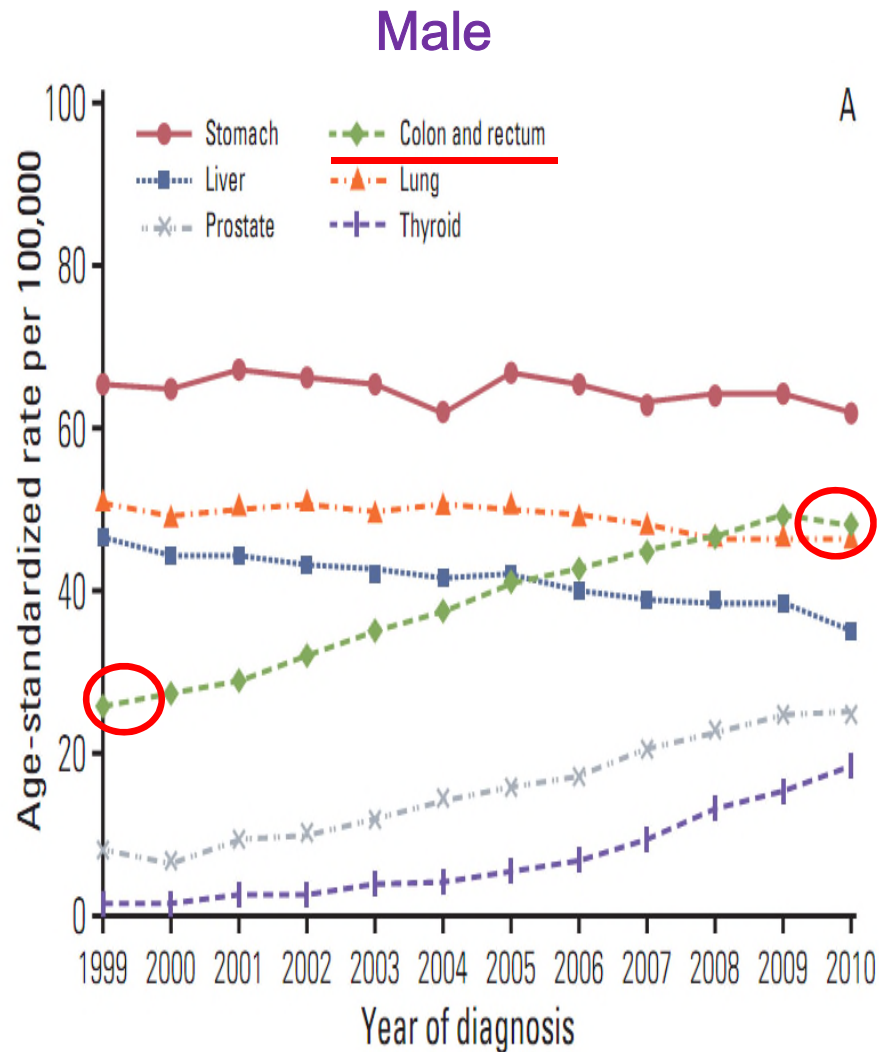
Seoul National University Bundang Hospital

South Korea

Trends of Incidence of Colorectal cancer in the World

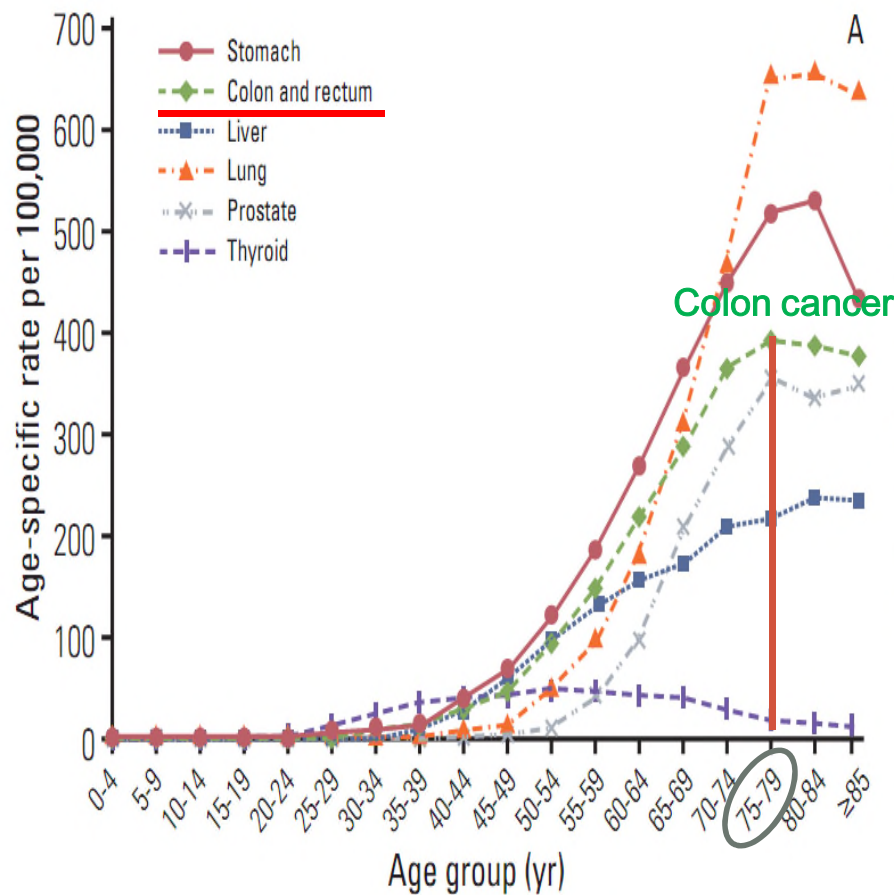


Trends of Incidence of Colorectal cancer in South Korea

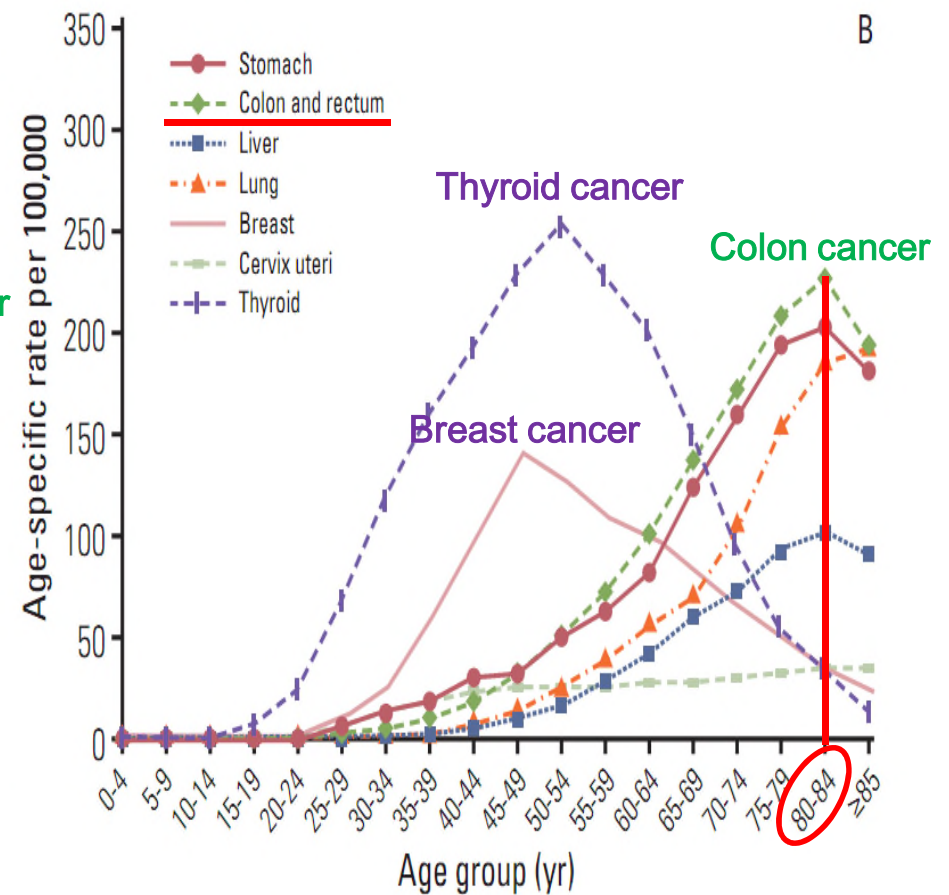


Sex disparities in CRC prevalence by age in Korea

Male



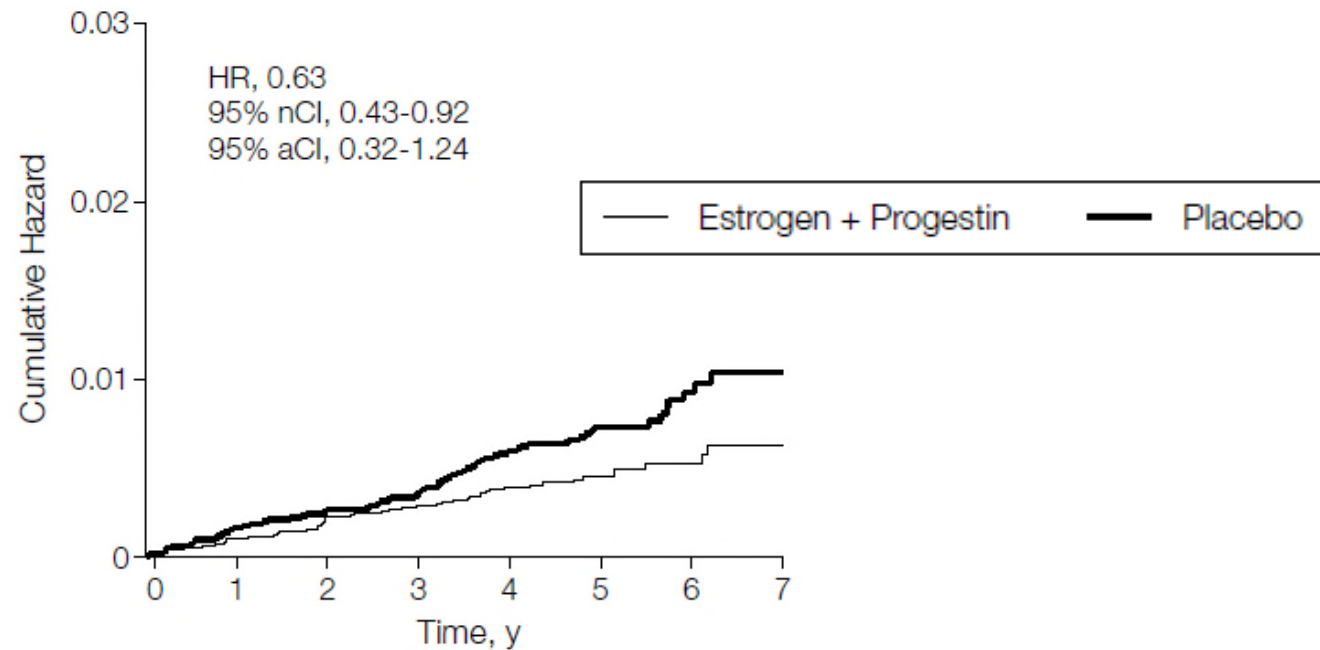
Female



Proximal and distal colon tumors depending on sex

Feature	Proximal (Right)	Distal (Left)
Age at diagnosis	Older	Younger
Gender	More females	More males
Mucinous tumours	Frequent	Infrequent
Familial cancer syndrome	HNPCC	FAP
5FU chemotherapy benefit	Good	Marginal or none
Ploidy	Mostly diploid	Mostly aneuploid
Loss of heterozygosity	Infrequent	Frequent
<i>TP53</i> mutation	20–30%	50–60%
MSI+	25%	2–3%
CIMP+	25–40%	3–10%

Effects of sex hormones in CRC: Epidemiologic study



	All PMH			E alone			EP		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
Never [†]	454	414	Reference	454	414	Reference	454	414	Reference
Ever	550	648	0.9 (0.7-1.1)	297	294	1.0 (0.8-1.3)	130	183	0.7 (0.5-1.0)
Current	357	457	0.8 (0.6-0.9)	199	226	0.9 (0.7-1.1)	98	147	0.6 (0.5-0.9)
<5 y	57	59	0.9 (0.6-1.4)	22	19	0.9 (0.4-1.9)	29	27	1.1 (0.6-2.0)
≥5 y	298	396	0.7 (0.6-0.9)	177	207	0.9 (0.7-1.2)	69	120	0.6 (0.4-0.8)
<i>P</i> trend			0.008			0.28			0.002
Former	193	191	1.1 (0.8-1.4)	94	68	1.5 (1.0-2.2)	31	36	0.9 (0.5-1.7)
<5 y	91	79	1.3 (0.9-2.0)	54	30	1.9 (1.1-3.2)	20	24	1.1 (0.5-2.2)
≥5 y	97	112	0.9 (0.6-1.3)	40	38	1.0 (0.6-1.8)	11	12	0.8 (0.3-2.1)
<i>P</i> trend			0.94			0.236			0.77

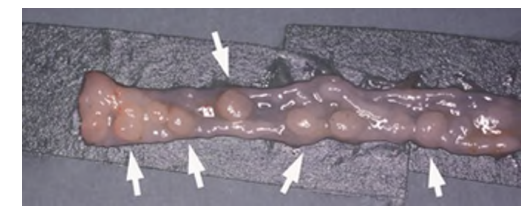
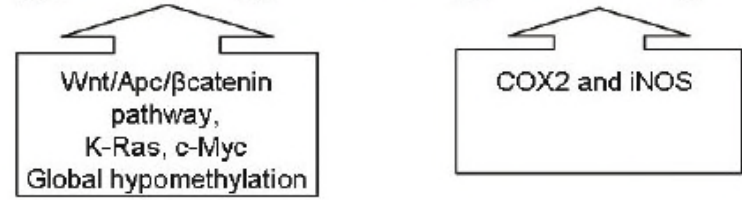
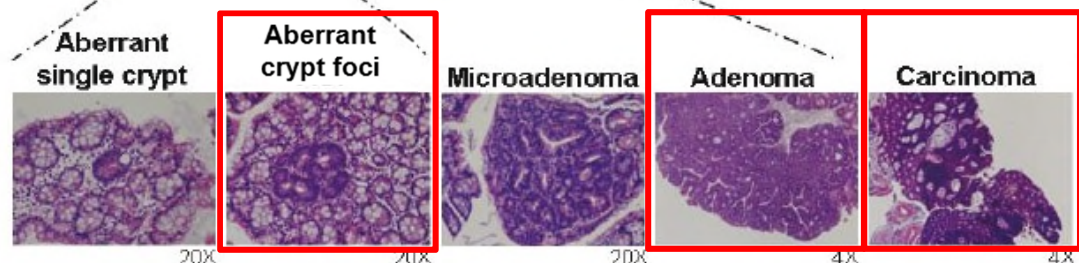
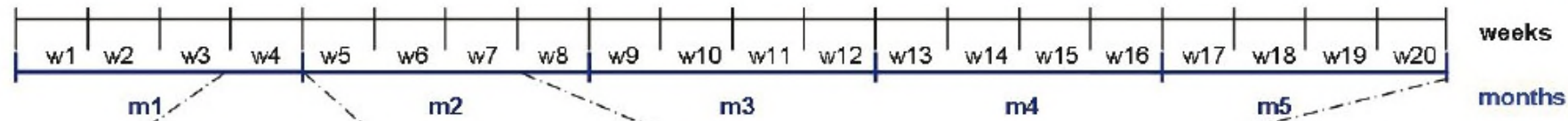
JAMA 2002;288:321-33.

Newcomb, Polly A., et al. *Cancer Res* 2007;67:7534-9.

AOM/DSS colorectal cancer mice model

Five-week-old ICR mice

AOM i.p. injection (10 mg/kg) **AOM: azoxymethane – carcinogen** → metabolite causes **DNA mutation**



Morphology of AOM/DSS-induced cancer in colon (Macroscopic assessment)

- A major chemically - induced CRC model
- Synergic actions of tumor-inducing and tumor-promoting effects of AOM and DSS
- Follows the aberrant crypt foci-adenoma-carcinoma sequences and shows similar molecular features as a human CRC

HYPOTHESIS 1

Estrogen supply in male AOM/DSS mice model would reduce colorectal tumorigenesis by modulating inflammation

HYPOTHESIS 2

Ovarectomy in female AOM/DSS mice model would increase colorectal tumorigenesis which will be reduced by supplementation of estrogen.

**EFFECT OF ESTRADIOL IN AN
AOM/DSS-TREATED MOUSE MODEL OF
COLORECTAL CANCER: IMPLICATION
FOR SEX DIFFERENCE IN CRC
DEVELOPMENT**

Background

Many epidemiologic data suggest [protective role of estrogen](#) in CRC development.

Caiazza F, et al. Front Oncol 2015;5:19

There was [relationship between estrogen and Nrf2](#) (nuclear factor erythroid 2-related factor 2, a transcriptional factor) in breast cell line.

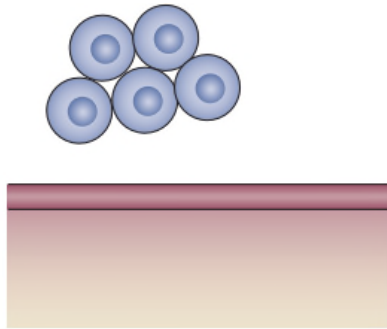
Exp Cell Res. 2014;328:351-60

[PKC \$\delta\$](#) (protein kinase C δ) activates [Nrf2](#) in the $G\alpha_{13}$ signaling pathway.

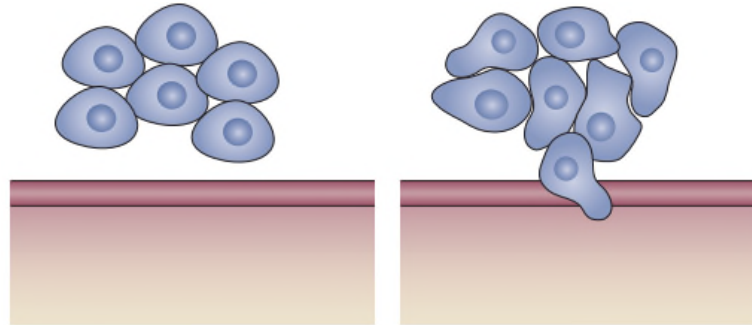
Li T, et al. Am J Physiol Heart and Circ Physiol. 2014;306:H1105-15

Dual role of NRF2 activation depending on tumor stage

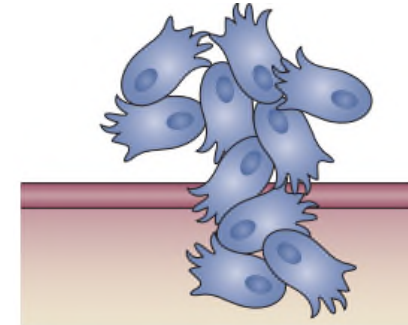
Normal cells



Premalignant or early malignancy



Malignant cells



Status of NRF2 signalling:

Activated by drugs



Biological outcome:

Induction of anti-inflammatory and anti-oxidative genes



Cytoprotection and detoxification:
• suppression of tumour promotion and progression
• chemoprevention of cancer

Pro- or anti-tumorigenic role of NRF2 unclear

Constitutively active by mutation in some tumours

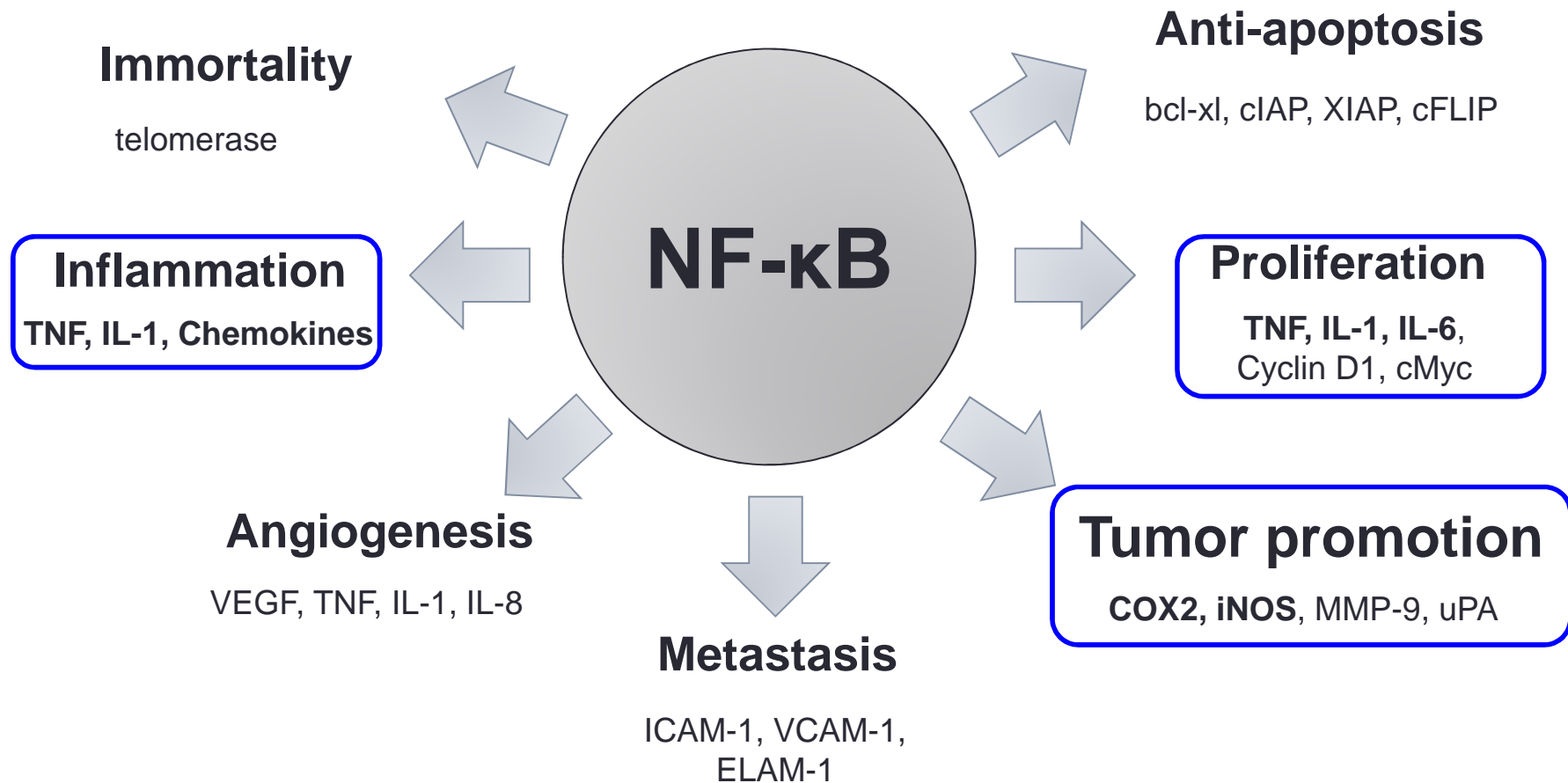


Protection against high endogenous levels of ROS



Increased resistance to chemotherapy and increased survival of tumour cells:
poor outcome in patients

Role of NF- κ B in development of cancer

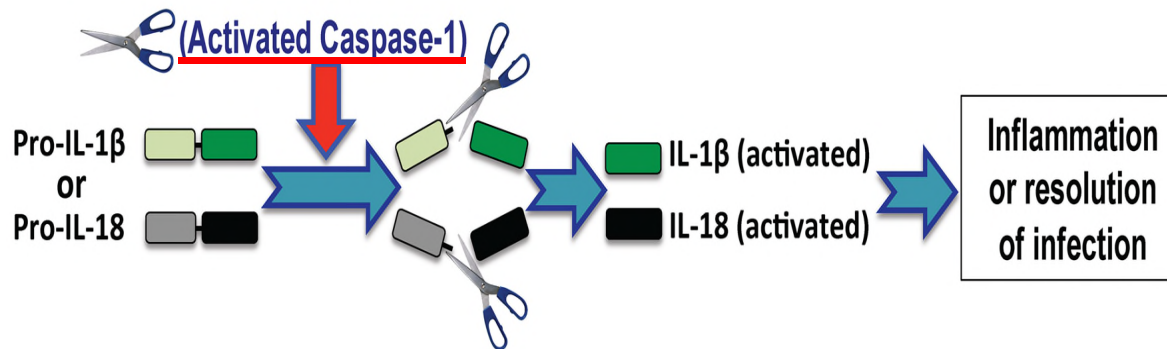


Cross-talk between Nrf2 and NF- κ B downregulate pro-inflammatory signaling by suppressing NF- κ B directly.

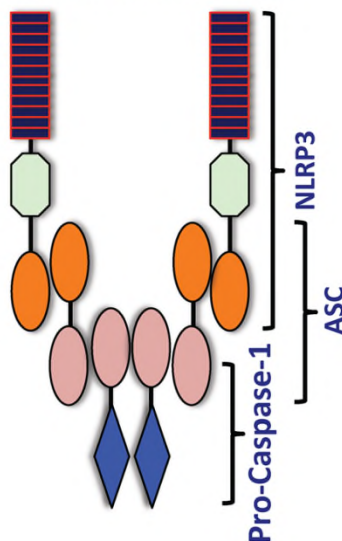
IL-1 β , caspase-1 and Inflammasome

IL-1 β is expressed as an inactive pro-IL-1 β moiety, and cleavage by caspase-1 activates IL-1 β .

C Activation of Pro-IL-1 β /pro-IL-18



NLRP3 inflammasome



Activation of caspase-1 is achieved by cytosolic multiprotein complexes called inflammasomes, formed by a sensor protein, an adaptor protein, and an inflammatory caspase.

NLRs (nucleotide-binding domain leucine-rich repeat proteins or NOD-like receptors)

ASC (apoptosis-associated speck like protein containing a caspase recruitment domain— CARD)

Inflammasome, Nrf2, estrogen and CRC

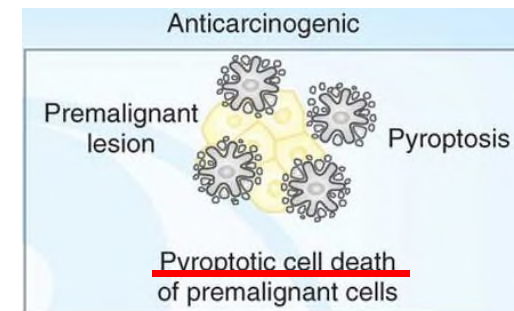
Estrogen activates the inflammasome, and the relationship of Nrf2 with the activating mechanism of the NLRP3 inflammasome was reported.

Zhao C, et al. J Biol Chem. 2014;289:17020-9

Caspase-1 activated by NLRP3 inflammasome triggers pyroptosis which might elicit an anti-cancer immune reaction.

Miao EA, et al. Immunol Rev. 2011;243:206-14

Kepp O, et al. Eur J Immunol. 2010;40:627-30



However, NLR gene such as NLRP3 could be a biomarker of CRC and cancer progression.

Liu R, et al. Oncotarget, 2015;6,;33456

Thus, a double-edge sword behavior of the NLRP3 inflammasome, with anti- and pro-cancer activities is possible.

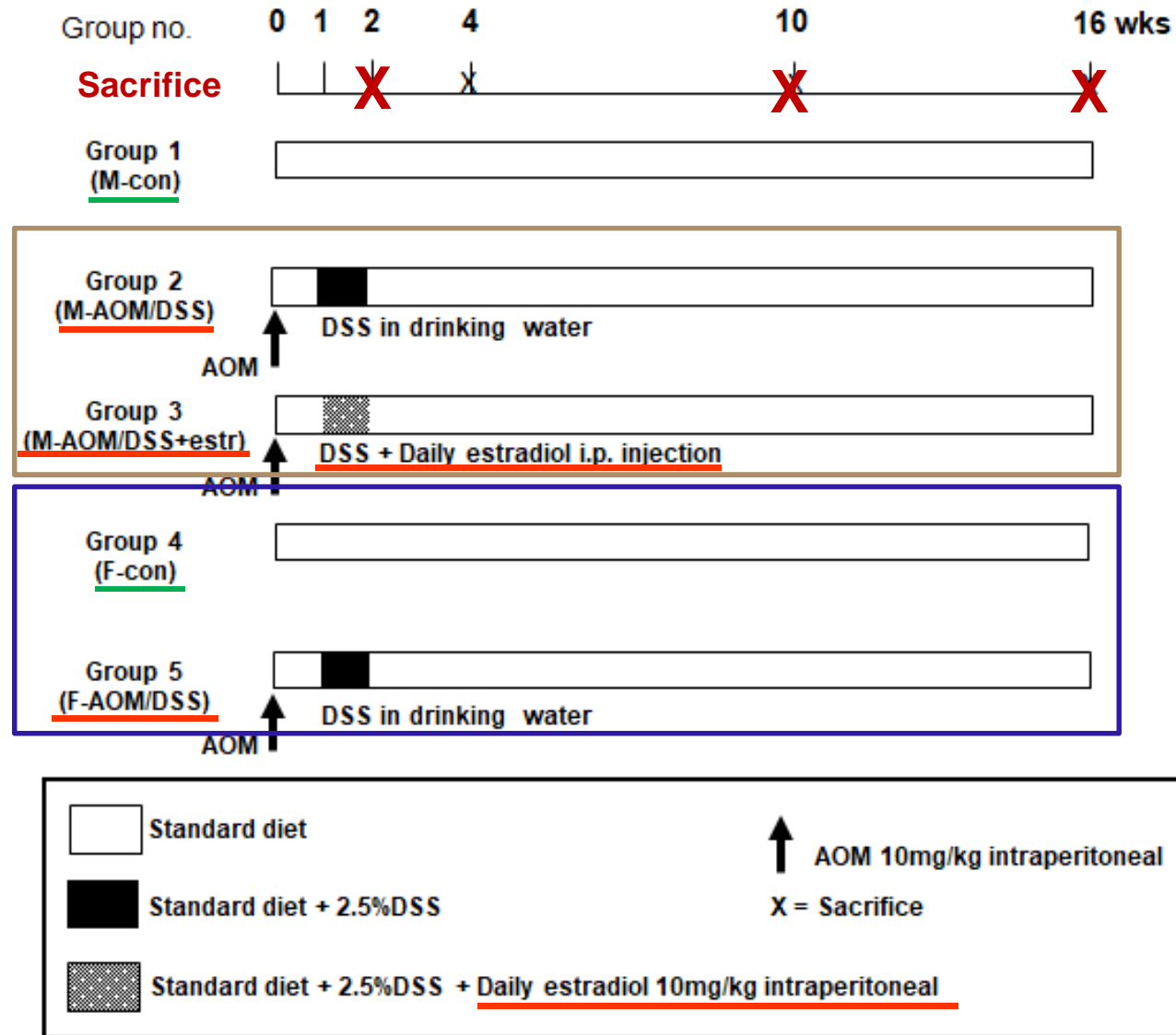
Kolb R, et al. Protein Cell. 2014;5:12-20

Aim

To investigate the effects of estrogens in male mice on inflammation and tumorigenesis by evaluation of Nrf2, NF-kB, and inflammasome pathway

Animal experimental design

5-week-old at the point of AOM injection



Experimental protocols (I)

Disease Activity Index (DAI)

- Body weight loss
- Stool consistency
- Hematochezia

DAI scoring

Score	Weight loss	Stool consistency	Hematochezia
0	None	Normal	Absence
1	0-10%		
2	10-15%	Loose	Blood tinged
3	15-20%		
4	>20%	Diarrhea	Presence

Gross measurement

Colon length (2, 10,16 weeks)

Counting tumor lesions (10,16 weeks)

- Histopathology

- Colonic epithelial damage (2 week)
- Depth of infiltration with inflammatory cells (2 weeks)
- Scoring of microscopic adenoma/cancer (10,16 weeks)

Experimental protocols (II)

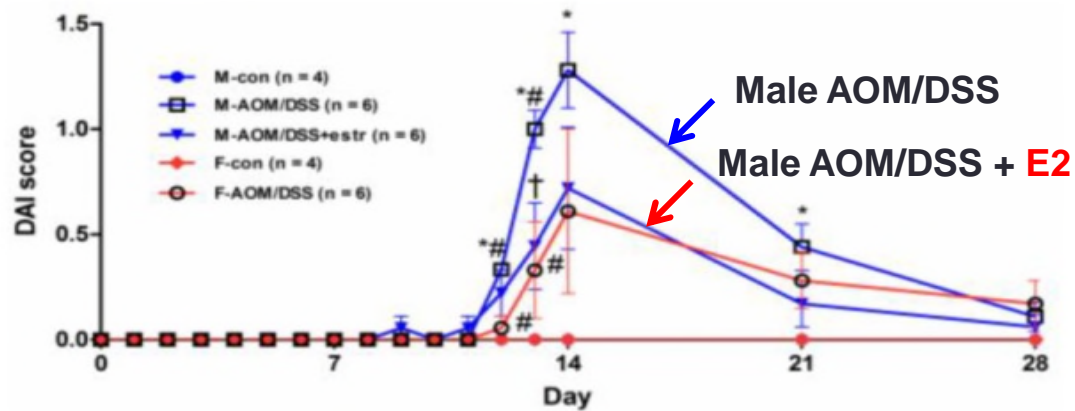
- **Measurement of inflammatory cytokines** such as MPO, COX2 and IL-6 (ELISA) NFkB (Western blot)
- . **Underlying cancer mechanism**
 - Anti-oxidation:** Nrf2, Heme oxygenase-1 (HO-1), GCLC, GCLM, NQO1
 - Inflammasome :** NLRP3, IL1 β , caspase 3 (Western blot, RT PCR)



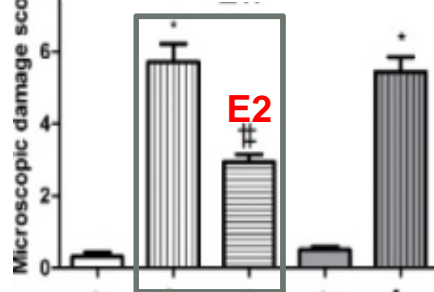
Results

Estradiol prevents inflammation in AOM/DSS-induced colitis (Week 2)

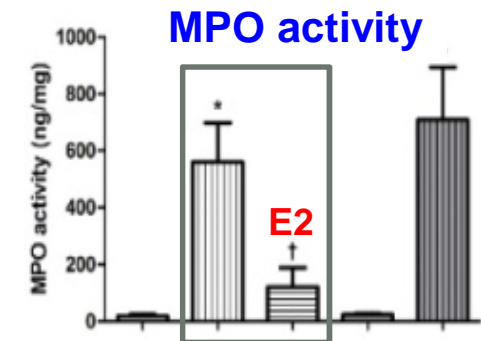
Disease activity index (DAI)



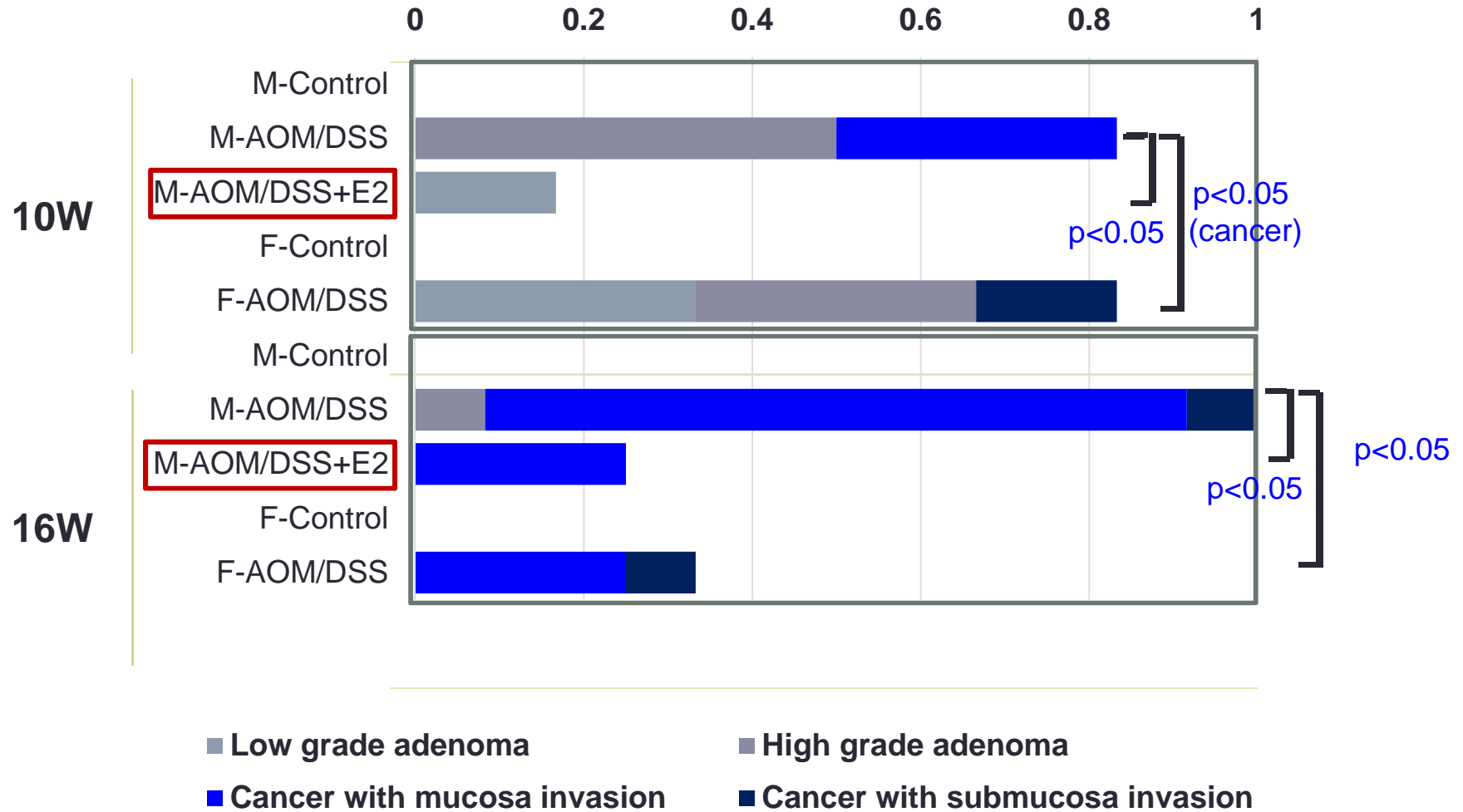
Microscopic damage



Histopathologic findings of the colonic mucosa

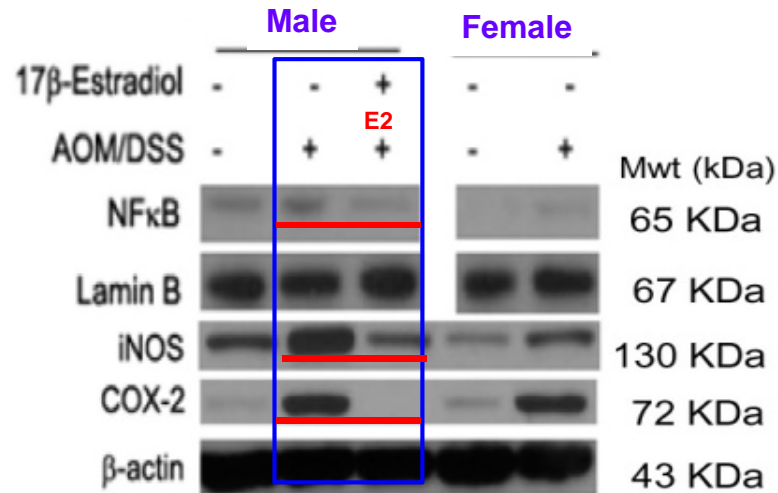


AOM/DSS-induced tumorigenesis are reduced by estradiol (Week 10 and 16)



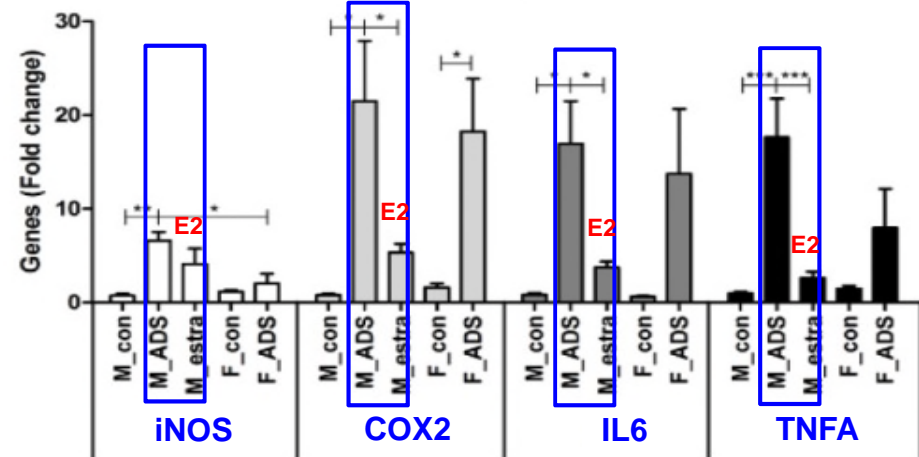
Effect of estradiol during colitis and cancer progression in terms of NF-κB

NF-κB-regulated proteins

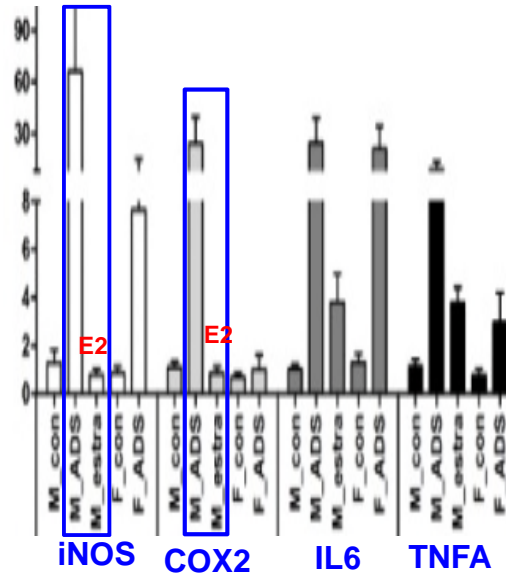
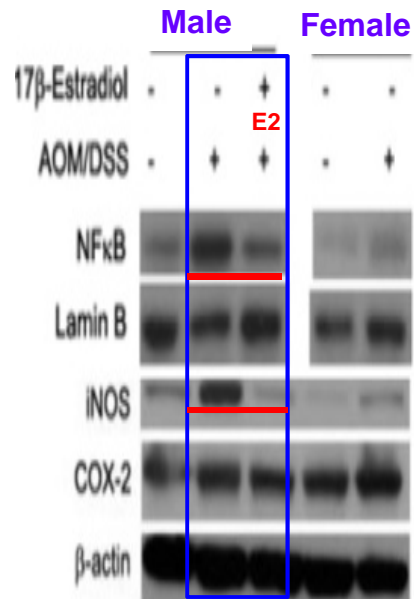


Week 2

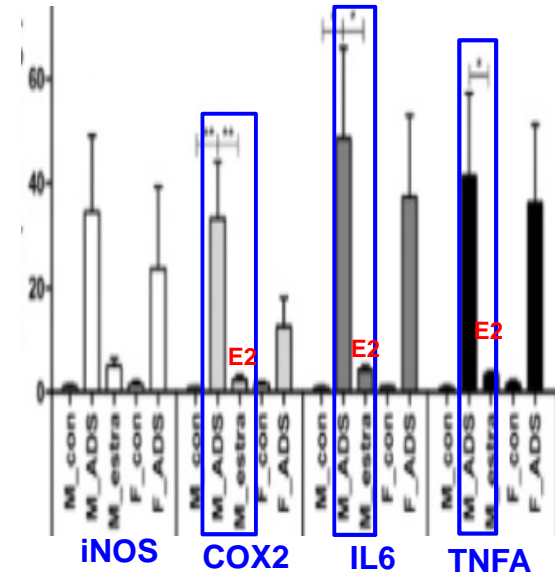
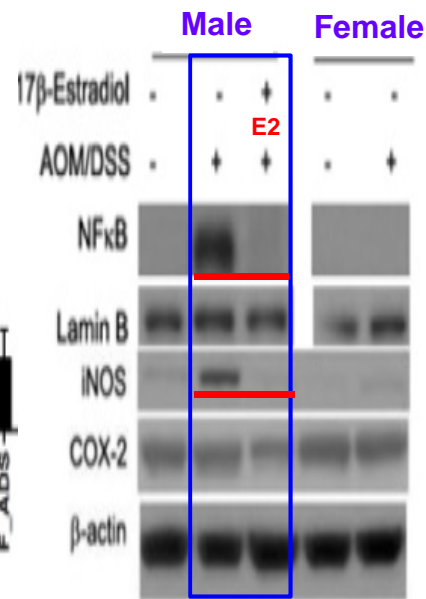
NF-κB-regulated genes



Week 10

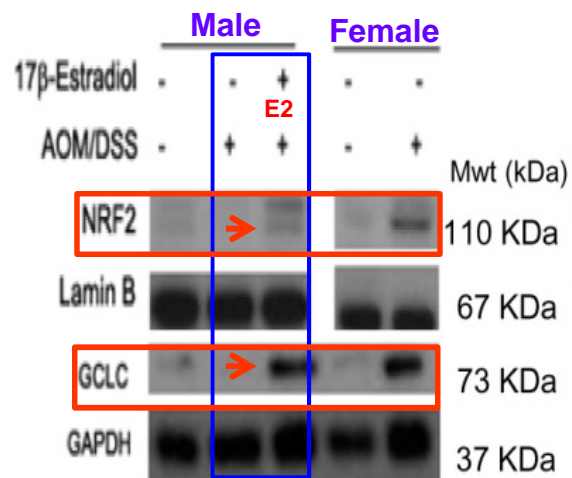


Week 16



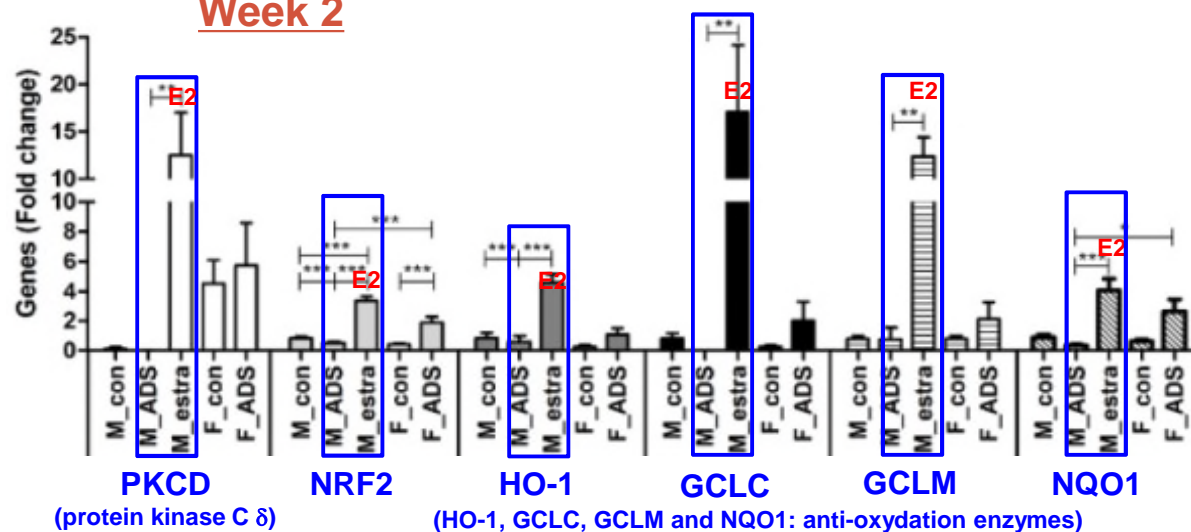
Effect of estradiol during colitis and cancer progression in terms of Nrf2

Nrf2-regulated proteins

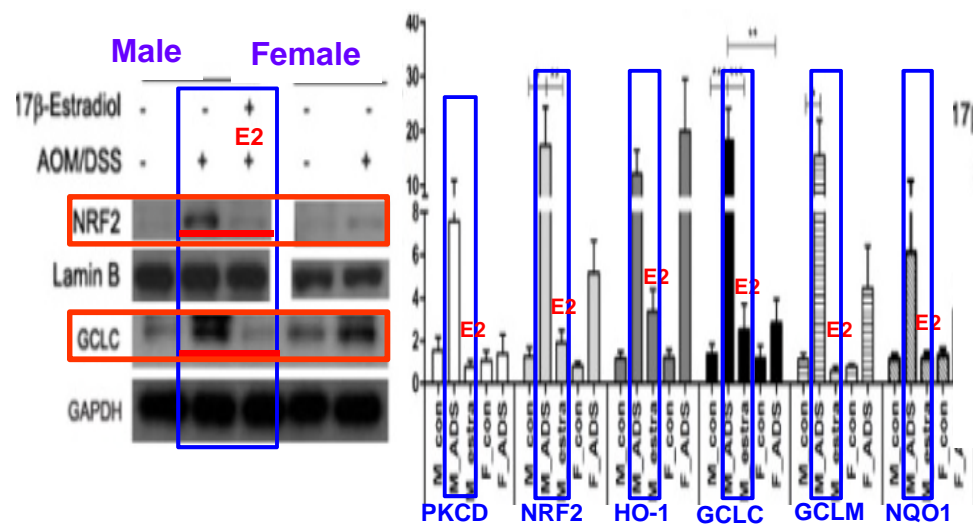


Nrf2-regulated genes

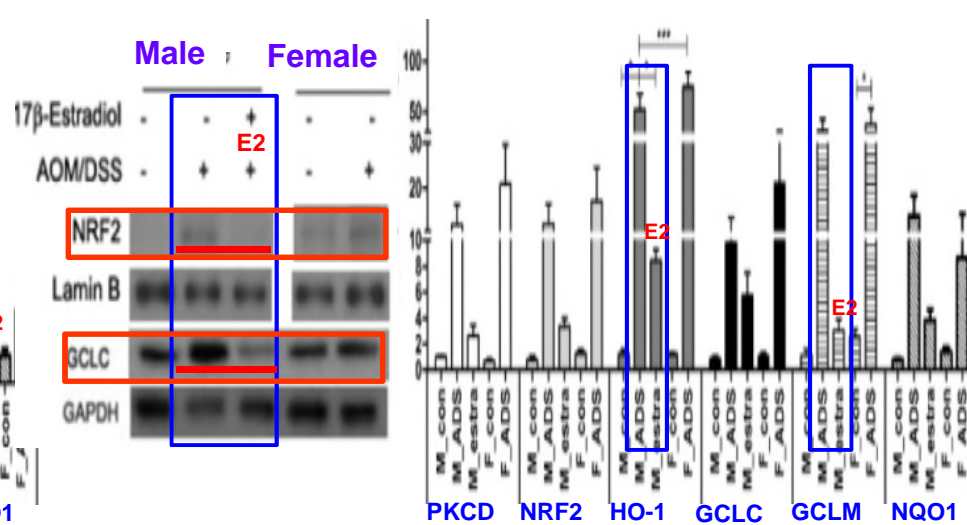
Week 2



Week 10

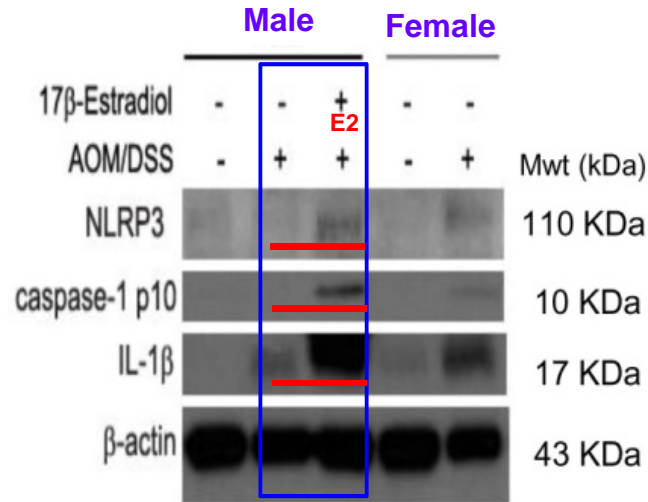


Week 16



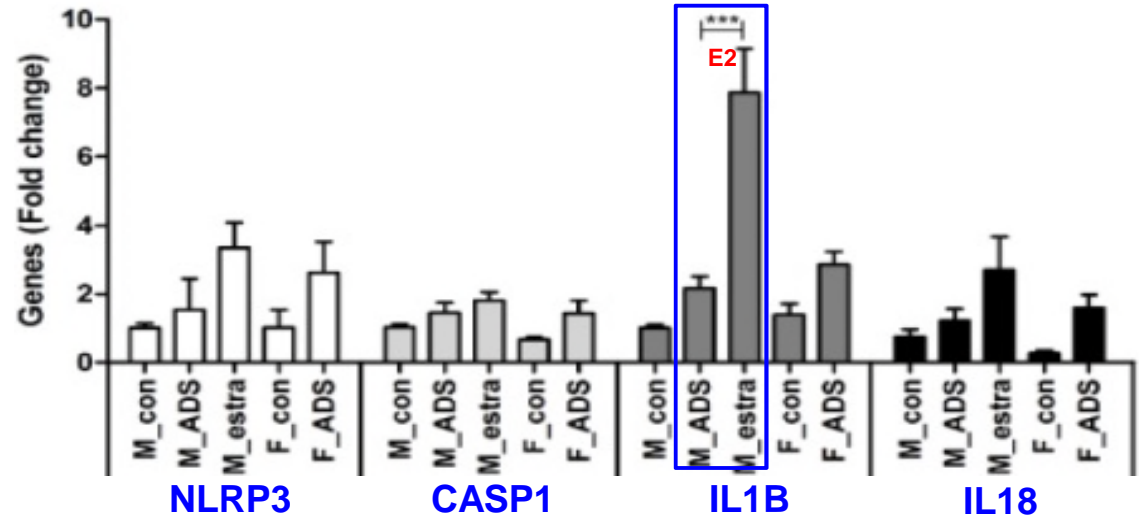
Effect of estradiol during colitis and cancer progression in terms of NLRP3

NLRP3 inflammasome -regulated proteins



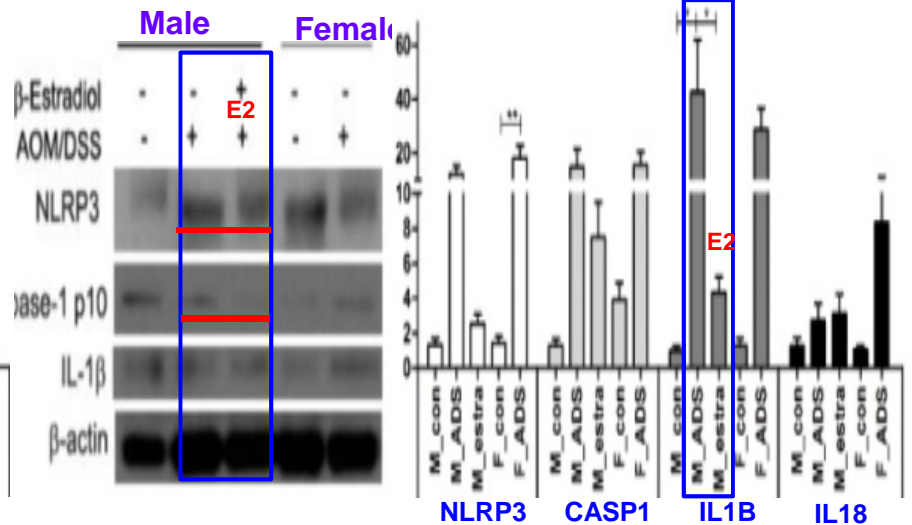
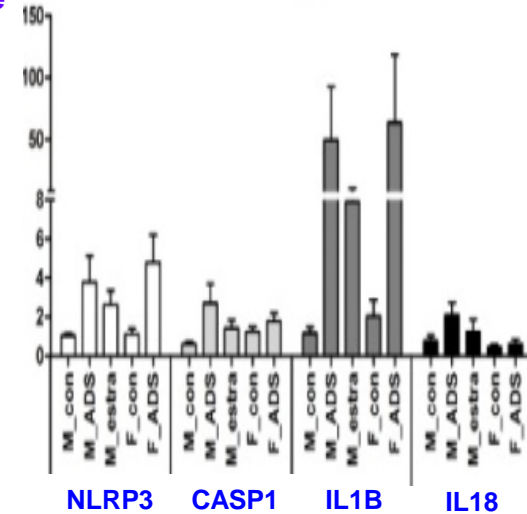
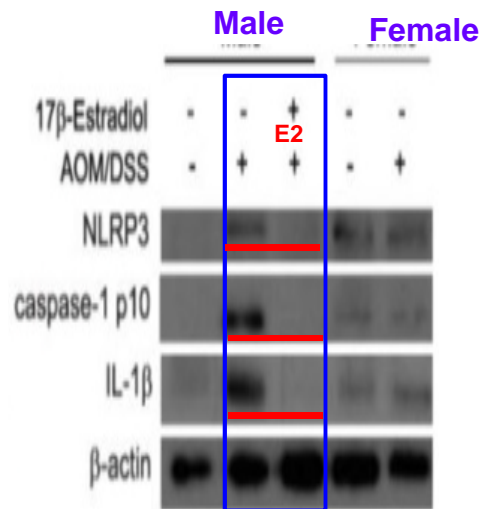
Week 2

NLRP3 inflammasome -regulated genes



Week 10

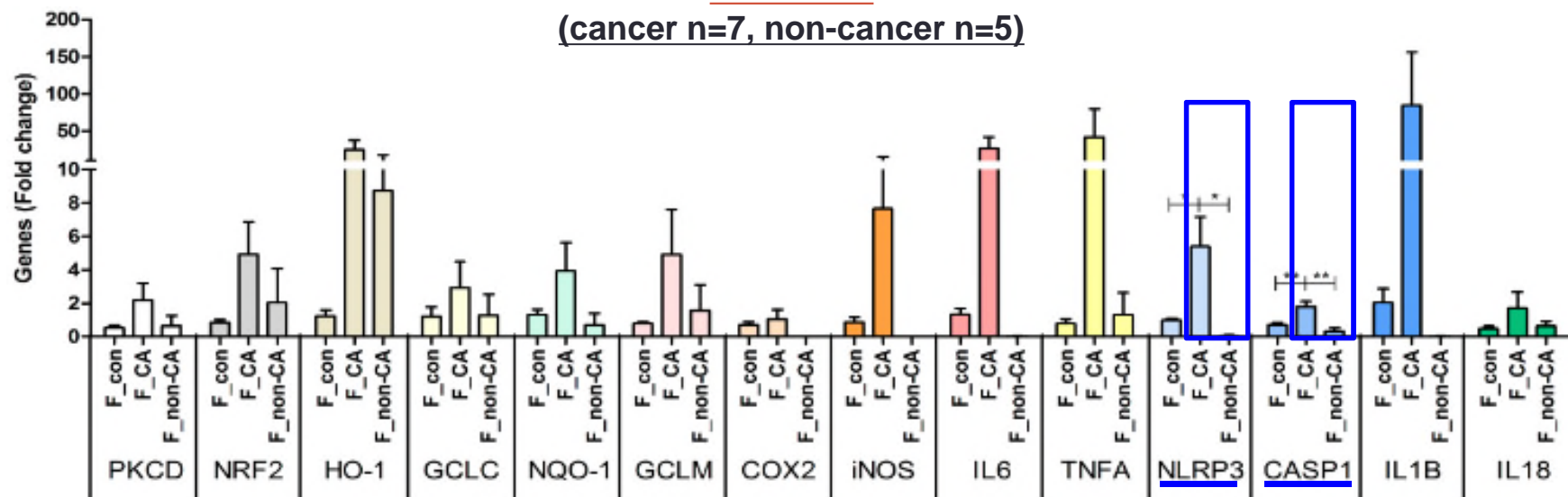
Week 16



Difference between cancer and non-cancer group in F-AOM/DSS group

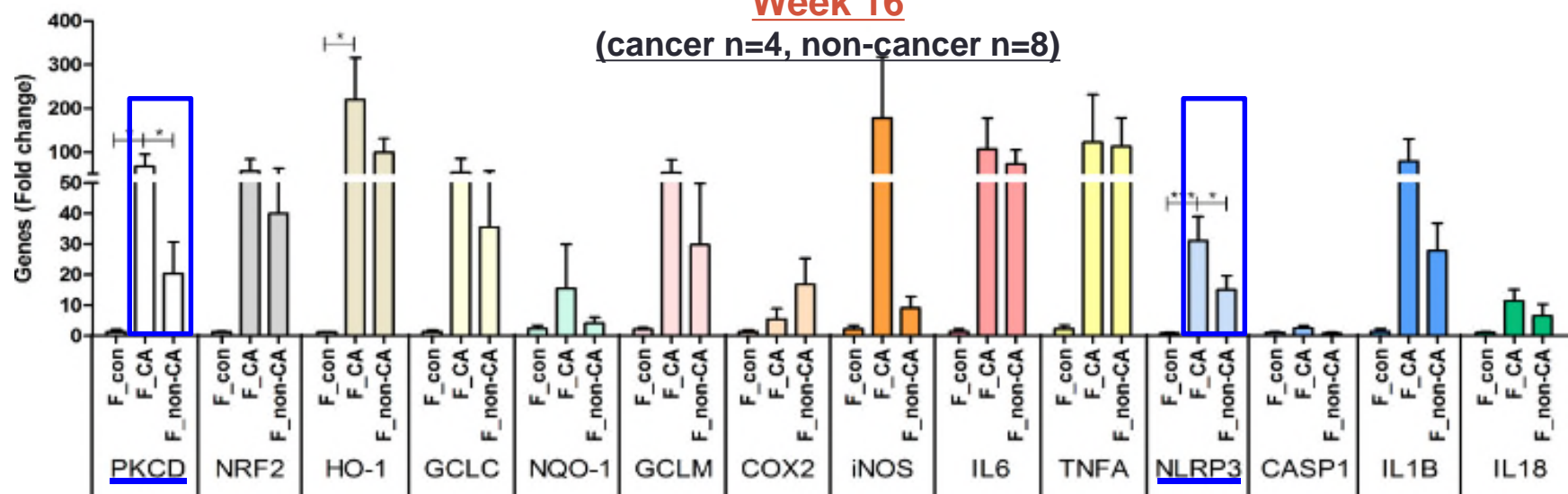
Week 10

(cancer n=7, non-cancer n=5)



Week 16

(cancer n=4, non-cancer n=8)



Summary of 1st Experiment

The effect of estradiol administration into M-AOM/DSS

	Week 2 (inflammation)	Week 10 and 16 (tumorigenesis)
DAI and histologic severity of colitis	decrease	
Tumor incidence		decrease
NF-kB	decrease	decrease
Nrf2	increase	decrease
Inflammasome	increase	decrease

Inflammasome in F-AOM/DSS depending on cancer

	Cancer	Non-cancer
NLRP3, caspase 1	high	low

Conclusion of 1st Experiment

The data indicate that **estrogen inhibits the initiation of colorectal cancer** by up-regulating **Nrf2 or inflammasome-related pathways**.

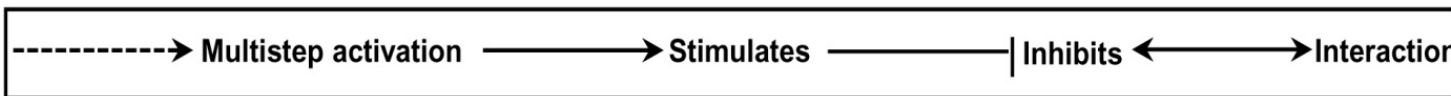
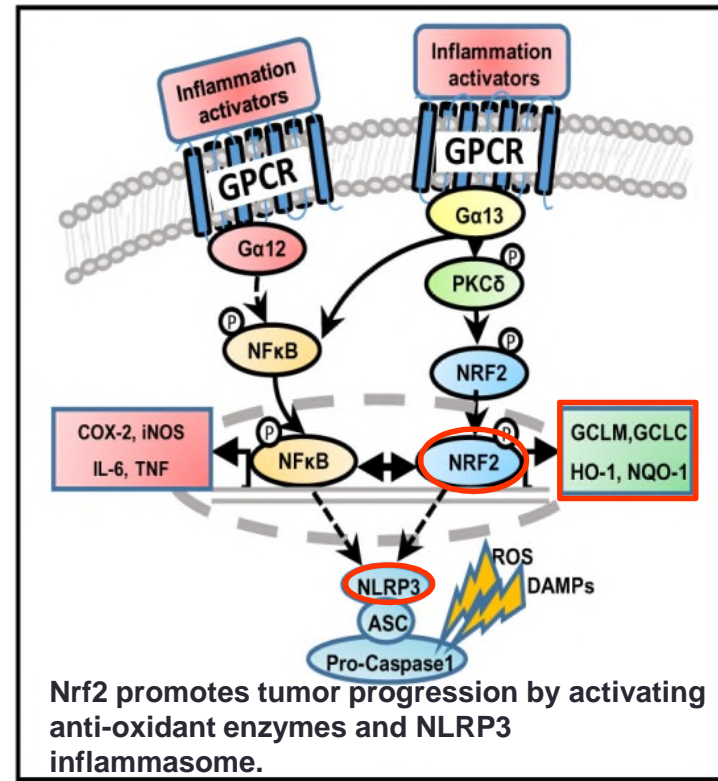
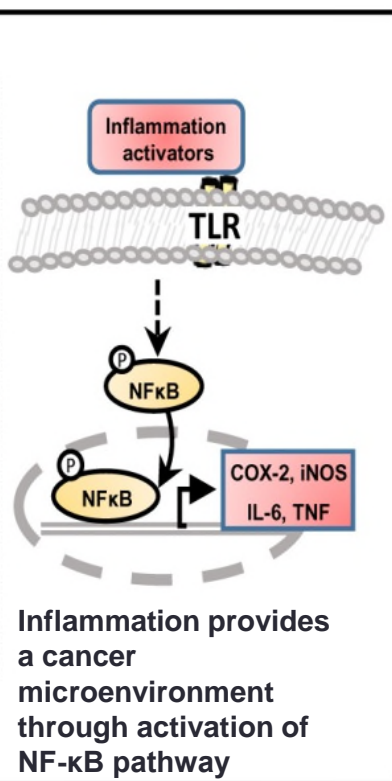
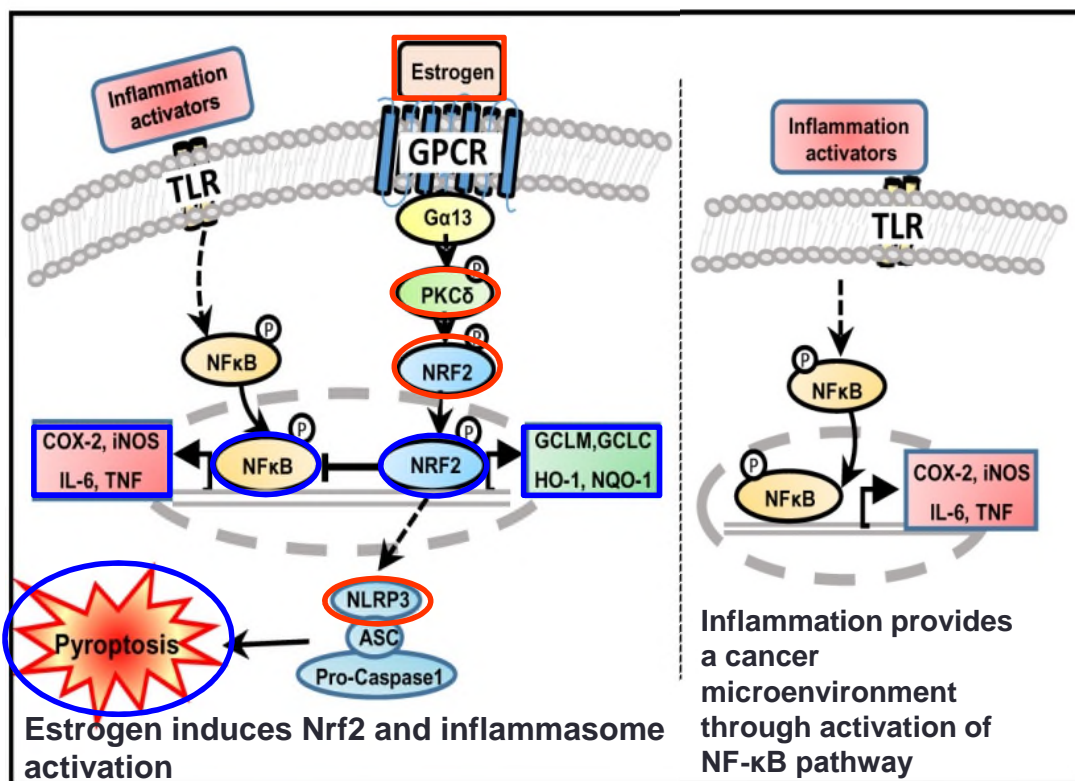
However, on premalignant or malignant stage (week 10 and 16) **estrogen prevents CRC by inhibition of Nrf2 and NLRP3 inflammasome**.

These data suggest dual role of **Nrf2 / inflammasome in the tumorigenesis of CRC**.

Proposed regulatory mechanism of estrogen in colitis-associated CRC

Week 2

Week 10 or 16

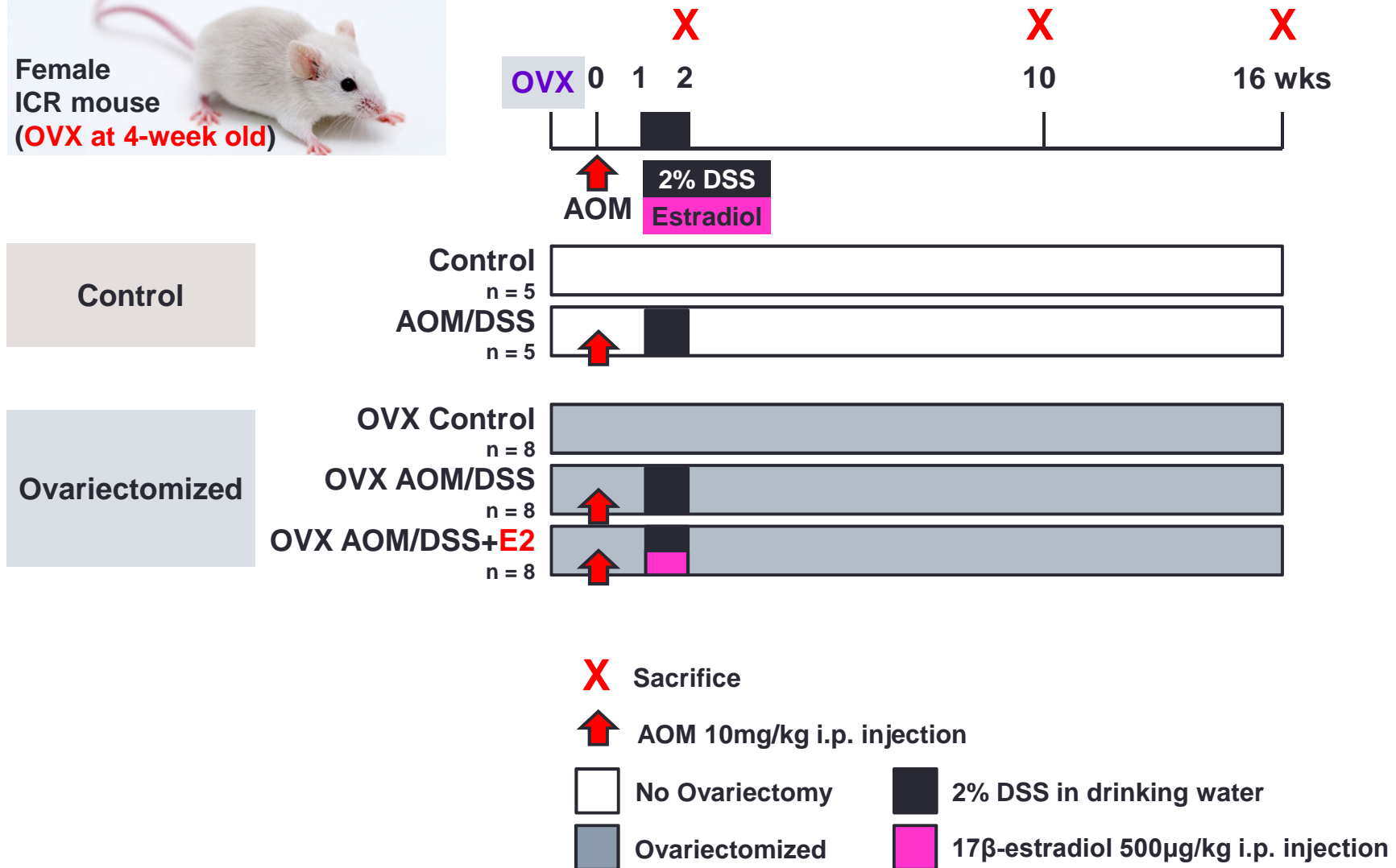


**EFFECT OF OVARECTOMY IN AN AOM/DSS-
TREATED MOUSE MODEL OF COLORECTAL
CANCER**

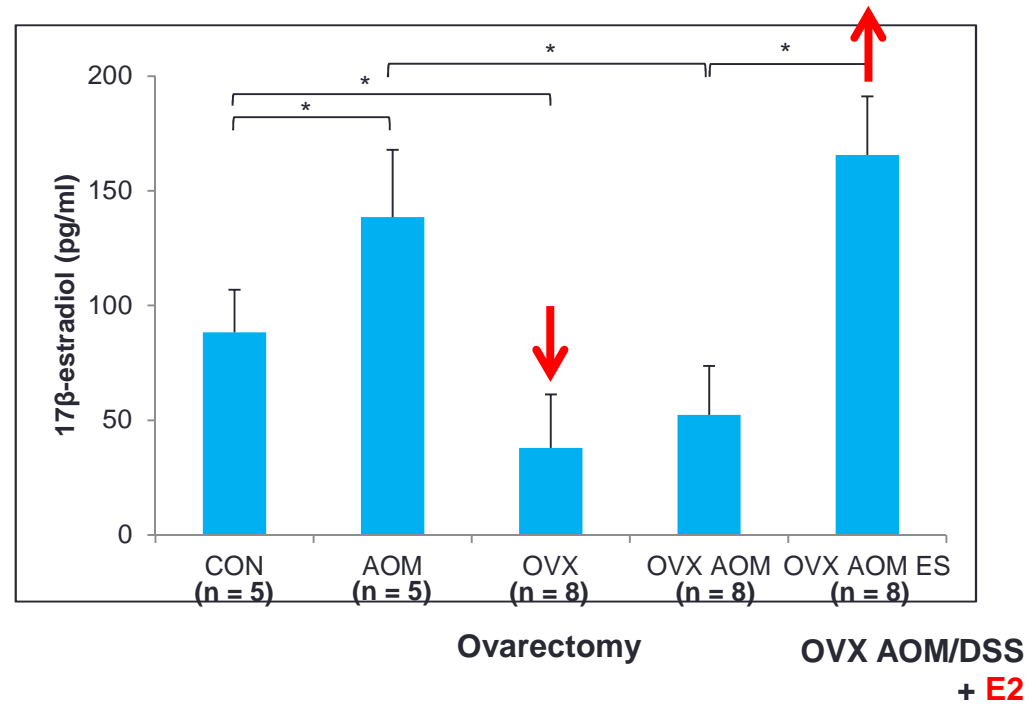
Aim of 2nd Experiment

To investigate whether ovariectomy in female AOM/DSS mice model increases colorectal tumorigenesis, and whether tumorigenesis is reduced by supplementation of estrogen after ovariectomy.

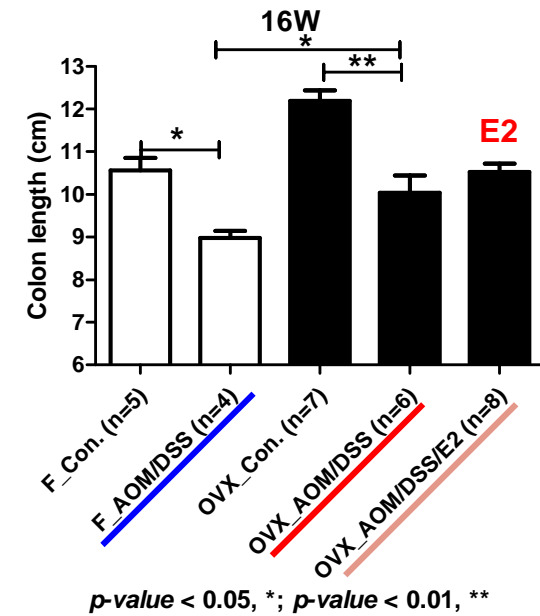
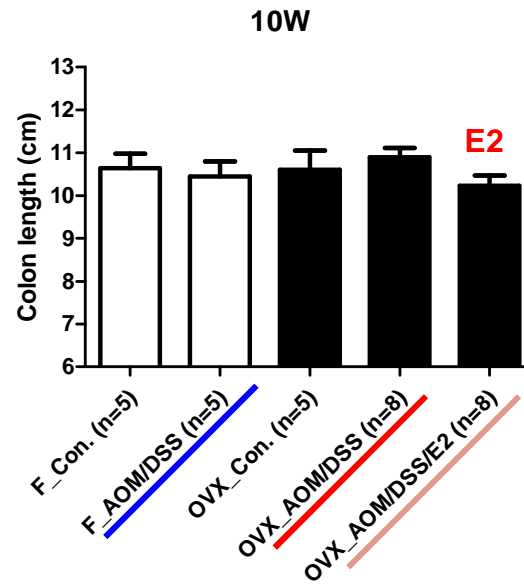
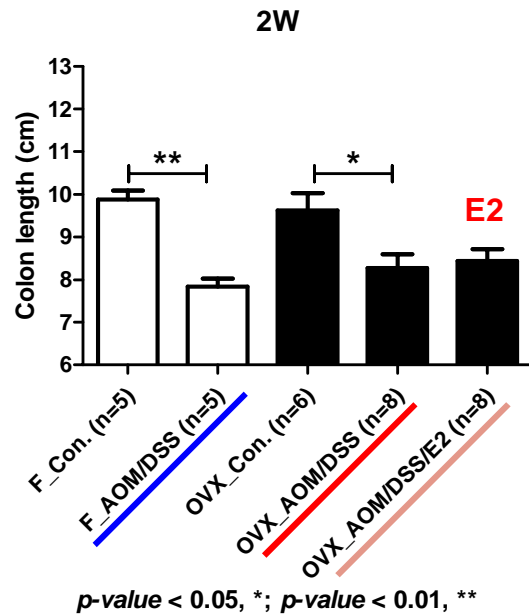
Ovarectomy and AOM/DSS and estradiol injection



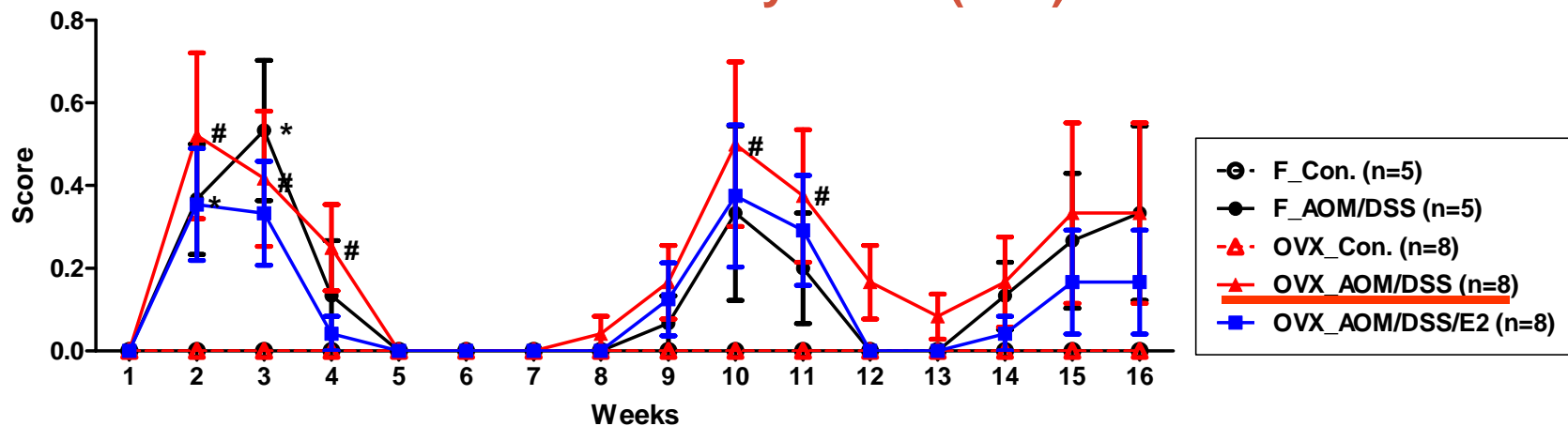
17 β -estradiol ELISA (blood) in female mice on week 2



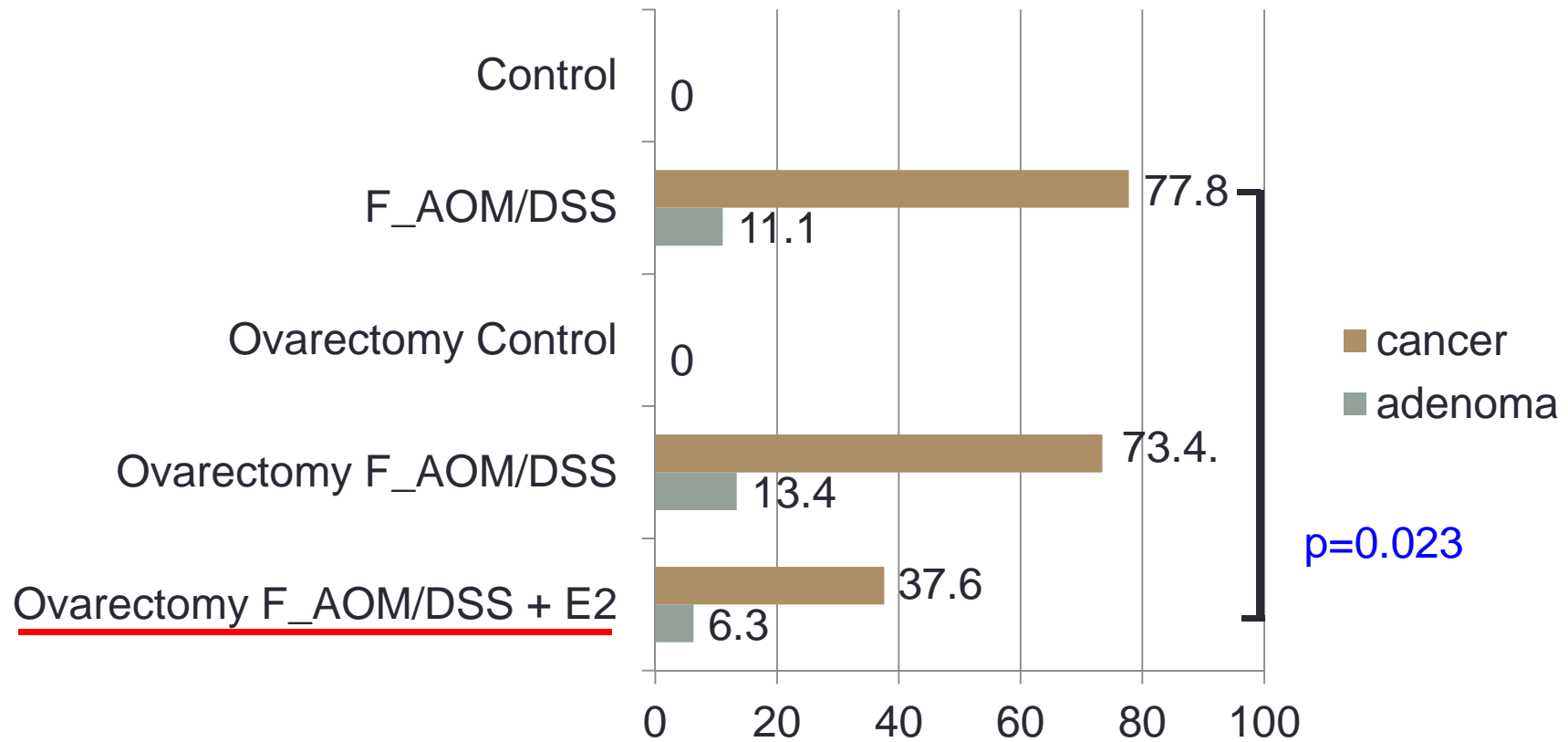
Colon length



Disease activity index (DAI)



**Ovarectomy did not increase tumor but E2 prevented tumor
in AOM/DSS-induced colitis (Week 10 + 16)**



Summary of 2nd experiment

Ovarectomy did not aggravate inflammation in female AOM/DSS model.

Ovarectomy did not increase tumor but E2 prevented tumor in AOM/DSS-induced colitis (Week 10 + 16).

Take home message

- ◆ Estrogen treatment prevented CRC in male mice.
- ◆ Increase of Nrf2 and inflammasome by estradiol in the inflammation stage contributes to the prevention of CRC in male mice. However, in the tumorigenesis period the role could be opposite.
- ◆ Ovariectomy in female mice did not affect inflammation or tumorigenesis, so far.
- ◆ Investigation regarding the effect of orchiectomy is necessary in the future.



Thank you for your attention