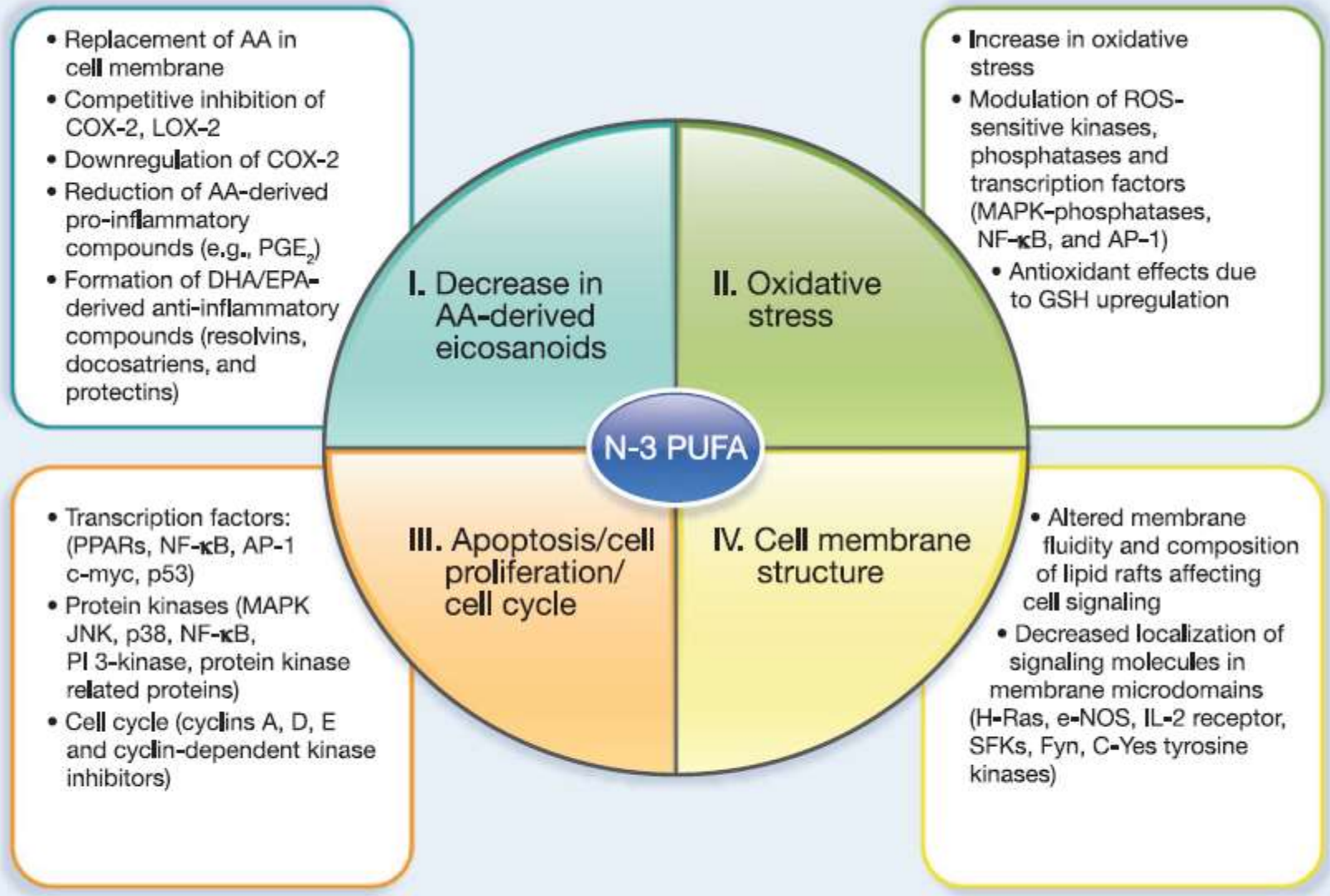
SUCCESS AND CHALLENGES IN BREAST CANCER PREVENTION

- Tamoxifen and Raloxifene are effective chemopreventive agents against ER positive breast cancer, but neither drug reduces the incidence of estrogen receptor negative tumors
- This deficiency is likely due to the fact that multiple cellular pathways in addition to ER contribute to breast cancer development. Therefore, a multi-targeted approach is needed employing interventions with complementary mechanisms of action
- We believe that the addition of omega-3FA to antiestrogens will increase the spectrum of preventable breast cancer subtypes by suppressing multiple oncogenic pathways which may cross-talk with ER signaling and may be responsible for de novo or acquired antiestrogen resistance



SUSAN G. KOMEN PROMISE GRANT
(A. Manni, K. El-Bayoumy)

- Experimental systems
  - MNU rat mammary tumor ***(Thompson)***
  - Polyoma middle T transgenic mice
- Human studies
  - Postmenopausal women with dense breast

END POINTS

-Rodent studies
- histopathology
- Tissue and circulating biomarkers (hormone responsiveness, oxidative stress, growth factor signaling, genomics, PPARγ expression and function)

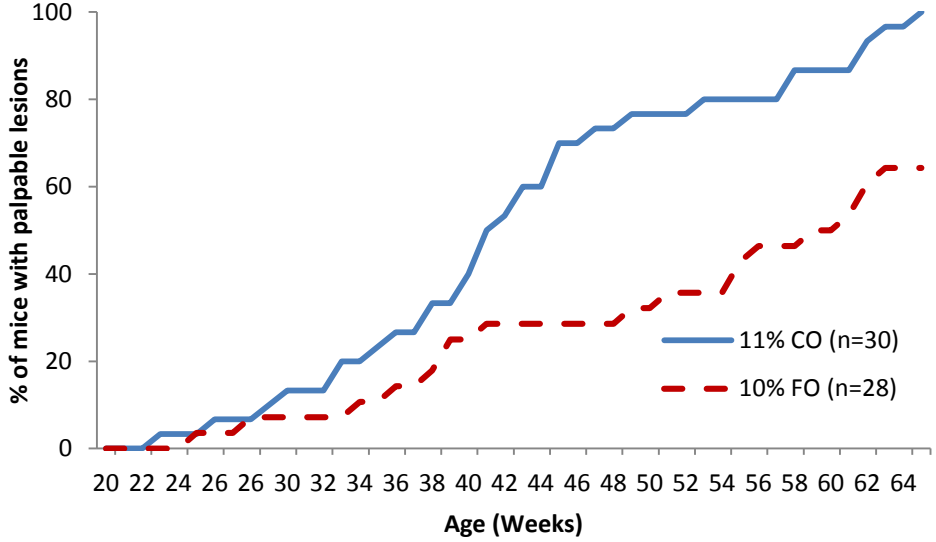
- Human studies
- breast density
- circulating and urinary biomarkers (oxidative stress, estrogen metabolism, inflammation, growth factor signaling)

OMEGA-3FA AND MAMMARY CARCINOGENESIS

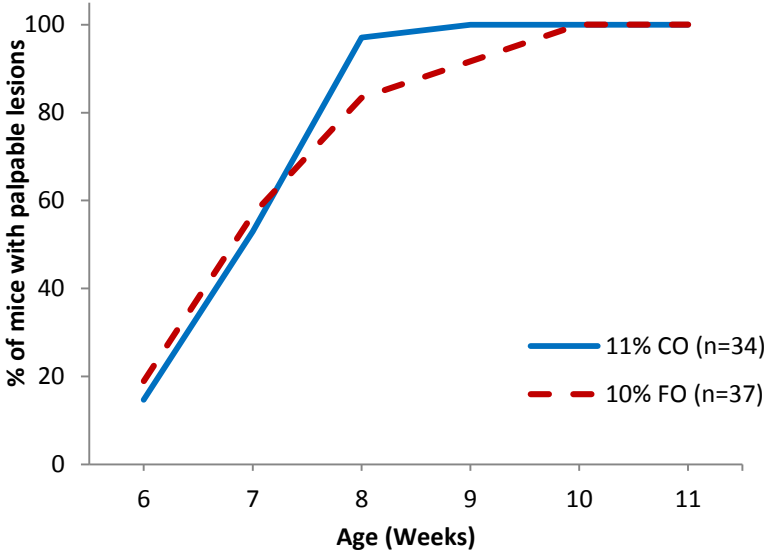
- Despite the perception that n-3FA protect against breast cancer, epidemiological studies have yielded inconsistent results (Signori C, *et al., Cancer Research* 71:6091, 2011)
- A recent meta-analysis of 21 independent prospective cohort studies revealed that dietary intake of marine n-3FA was associated with a 14% reduction in breast cancer risk (Zheng JS, *et al., BMJ* 346:3706, 2013)
- Preclinical studies have been in general more supportive a protective role of n-3FA against mammary carcinogenesis, although inconsistencies still remain as reviewed by us (Signori C, *et al., Cancer Research* 71:6091, 2011)

EFFECT OF n-3 FA IN TRANSGENIC MODELS OF MAMMARY CARCINOGENESIS

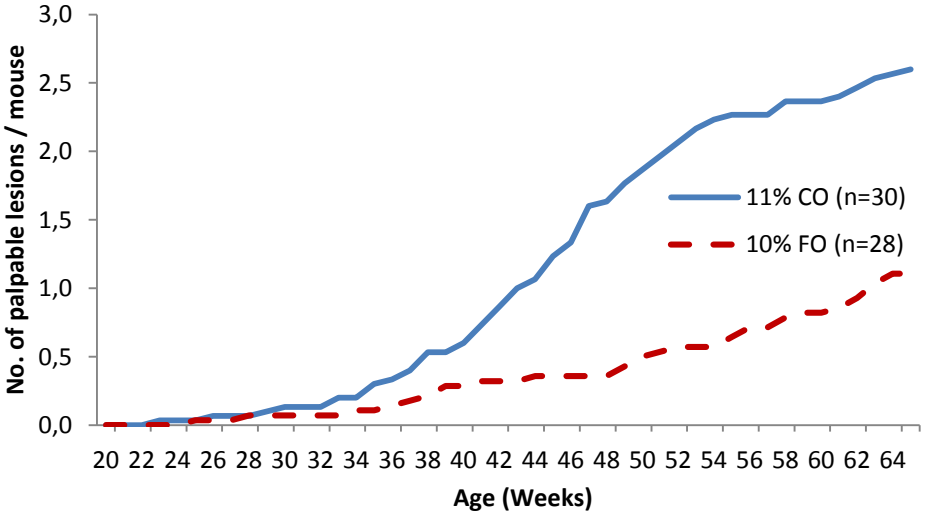
Tumor Incidence (Her2/neu Mice)



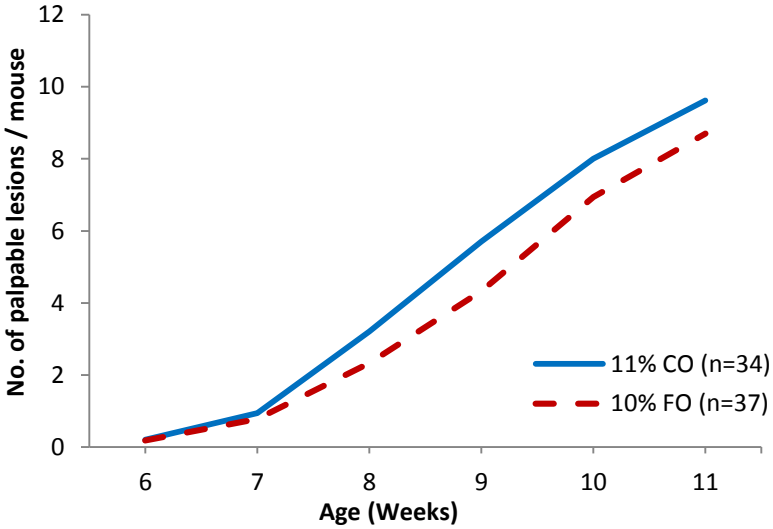
Tumor Incidence (PyMT Mice)



Tumor Multiplicity (Her2/neu Mice)



Tumor Multiplicity (PyMT Mice)



Effect of dietary omega-3 to omega-6 ratio on carcinogenic response in the mammary gland[a] (Jiang W, *et al, Cancer Research,* 72(15):3795, 2012)

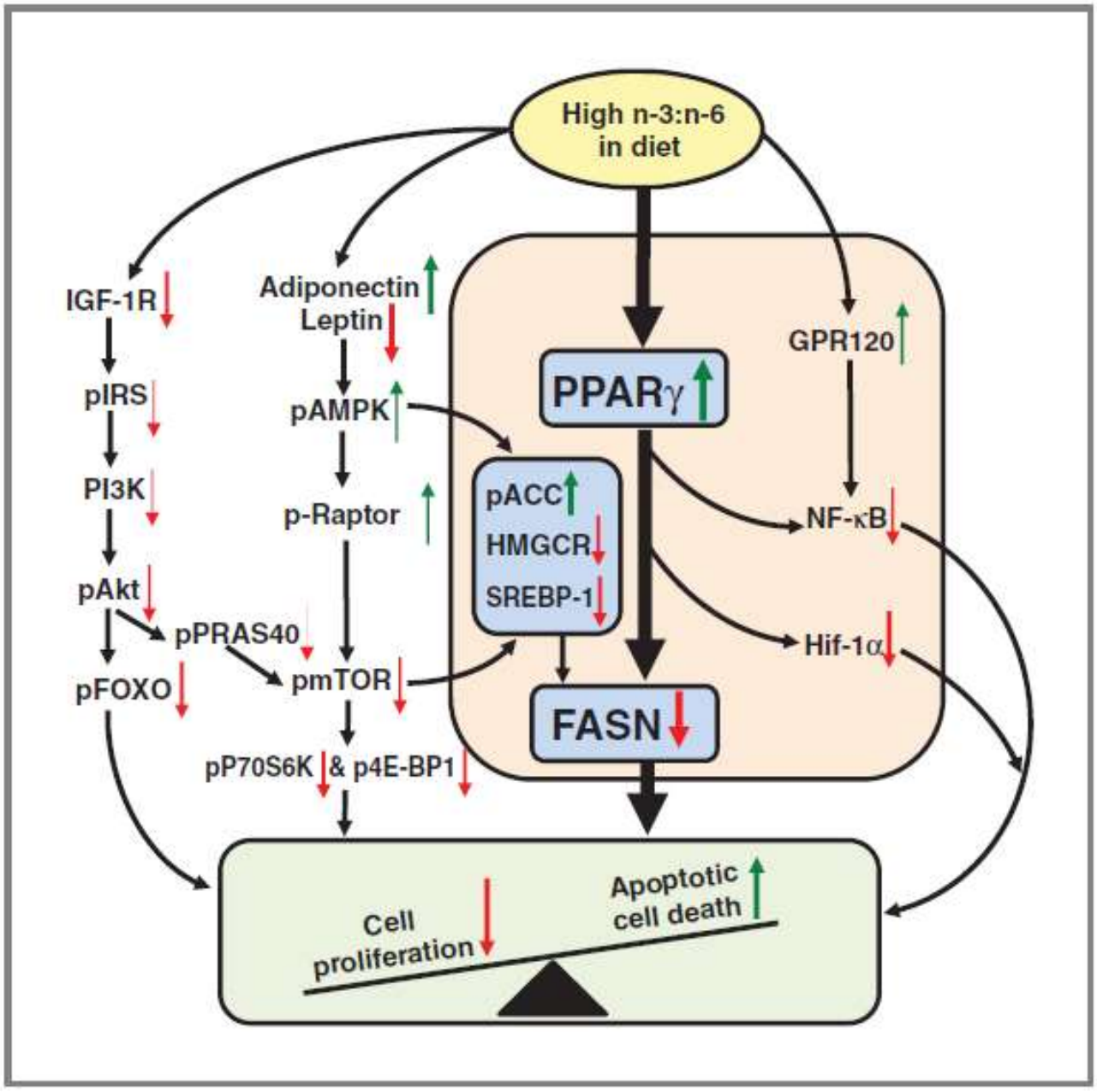
Dietary n-3:n6 ratio	Low (0.6)	High (14.6)	P
Cancer Incidence (%)	96.7	76.7	<0.0001
Cancer Multiplicity (No. carcinomas/rat)	3.0 ± 0.4	2.1 ± 0.3	<0.0001
Cancer Burden (Cancer mass/rat (g))	1.44 ± 0.39	0.29 ± 0.09	<0.0001
Cancer Latency (day)	38 ± 2	42 ± 2	<0.0001

[a]Values are means ± SEM (n=30) except incidence. Cancer incidence was evaluated by Fisher's exact test, cancer multiplicity by ANOVA following square root transformation of cancers counts per rat, cancer burden by the Kruskall Wallis rank test, and cancer latency by survival analysis

Effect of dietary n-3:n-6 ratio on cellular processes regulating cell proliferation and apoptosis

Dietary n-3:n-6 ratio	Low 0.6	High 14.6	P
Cell proliferation			
Ki67 index (%)	34.9 ± 1.6	14.0 ± 0.9	<0.0001
Rb^{Ser780} ratio	0.41 ± 0.03	0.26 ± 0.02	<0.0001
Cyclin-D1	1,302 ± 29	967 ± 29	<0.0001
P21	465 ± 31	664 ± 40	<0.001
P27	326 ± 11	393 ± 10	<0.0001
Apoptosis			
Apoptotic index (%)	1.71 ± 0.05	3.92 ± 0.13	<0.0001
Bax	188 ± 8	242 ± 9	<0.0001
Bcl-2	589 ± 28	527 ± 26	0.117
Bax/Bcl-2	0.32 ± 0.01	0.47 ± 0.02	<0.0001
Apaf-1	392 ± 9	457 ± 17	0.005
PARP89	795 ± 26	577 ± 53	0.002
PARP116	547 ± 15	292 ± 28	<0.0001
PARP89/116 ratio	1.45 ± 0.02	1.99 ± 0.02	<0.0001

Jiang W, *et al., Cancer Research* 72(15):3795, 2012



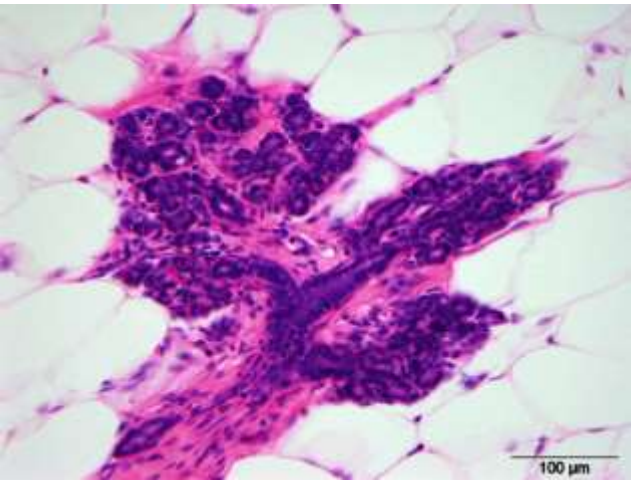
Jiang W, *et al., Cancer Research* 72(15):3795, 2012

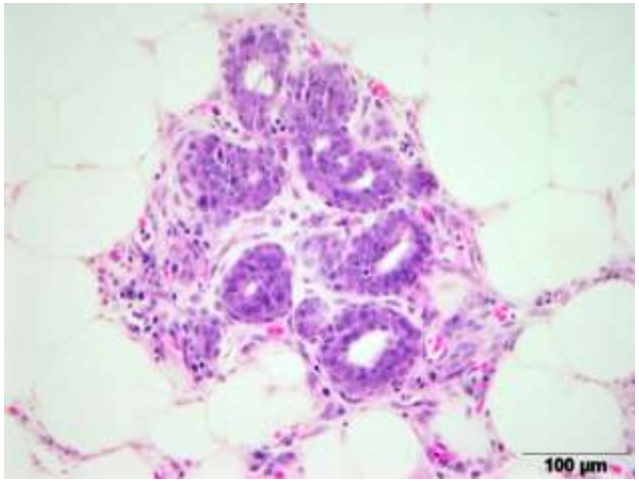
Individual and Combined Effects of the FO rich diet and Tam on MNU mammary tumor incidence, multiplicity and volume (Manni A, *et al., Int J Cancer* 134:1549, 2014)

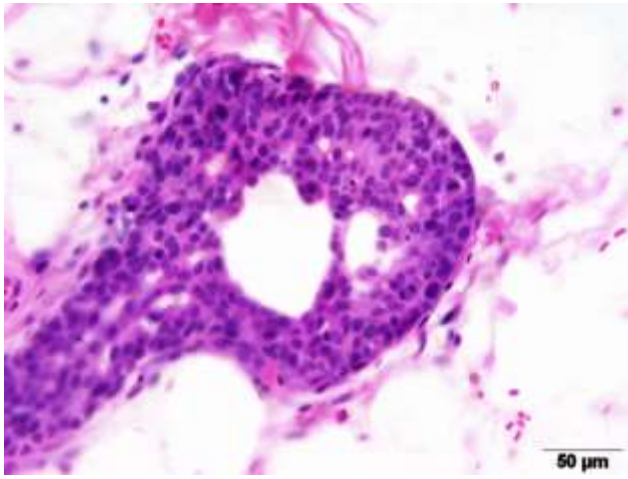
Experimental groups	No. of rats	No. of rats with tumors	Incidence (%)	No. of Tumors per rat (mean ± SE)	Tumor volume (mm^3) mean ± SE
1. 20% CO	18	16	88.9	2.78 ± 0.53	1666.63 ± 443.69
1. 20% CO + Tam 0.6 ppm	17	9	52.9	0.76 ± 0.22[c]	1437.44 ± 592.80
1. 10% FO + 10% CO	18	13	72.2	2.11 ± 0.45	1302.87 ± 358.07
1. 10% FO + 10% CO + Tam 0.6 ppm	18	5	27.8[b]	0.33 ± 0.14 [c]	184.31 ± 63.51

[b]$p<0.01$ vs Group 1

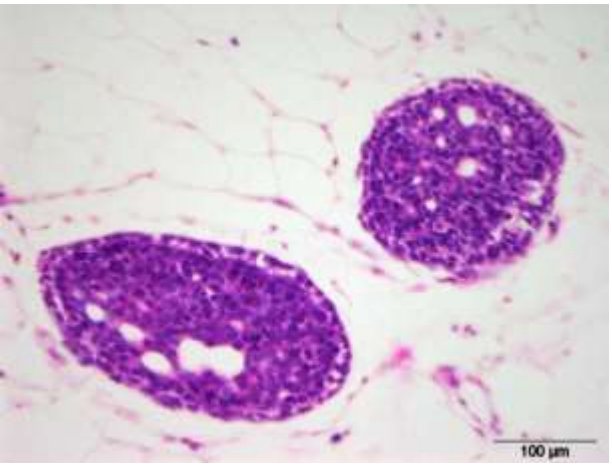
[c]$p<0.001$ vs Group 1

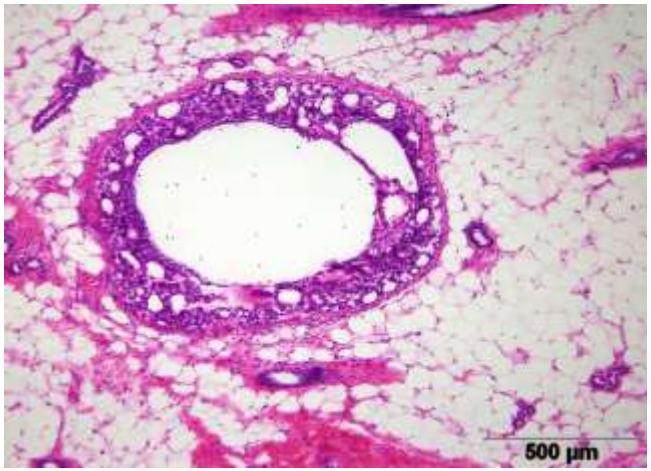


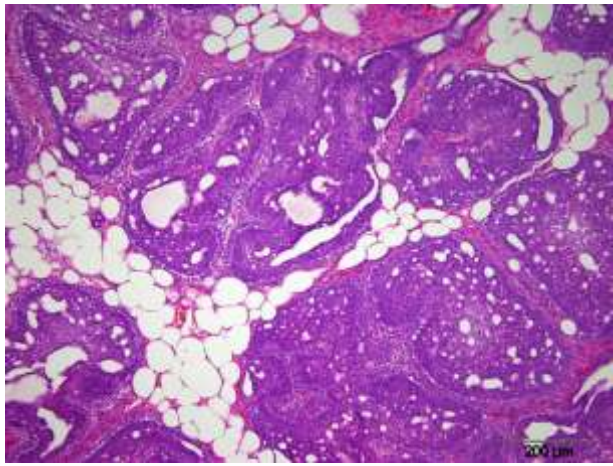




NORMAL → MILD IDP → MODERATE IDP →







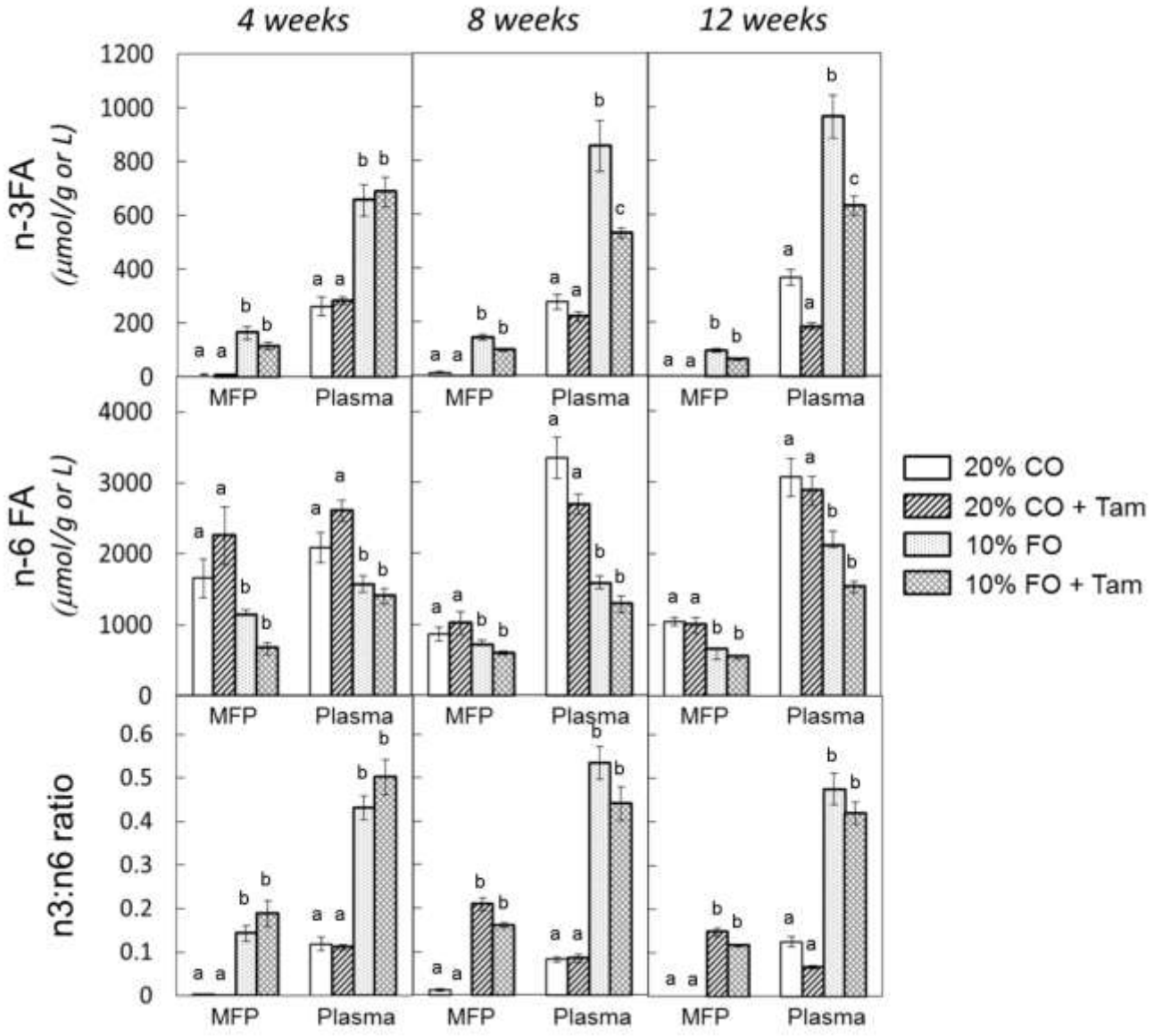
FLORID IDP → DCIS → INVASIVE CANCER

FLORID IDP → INVASIVE CANCER

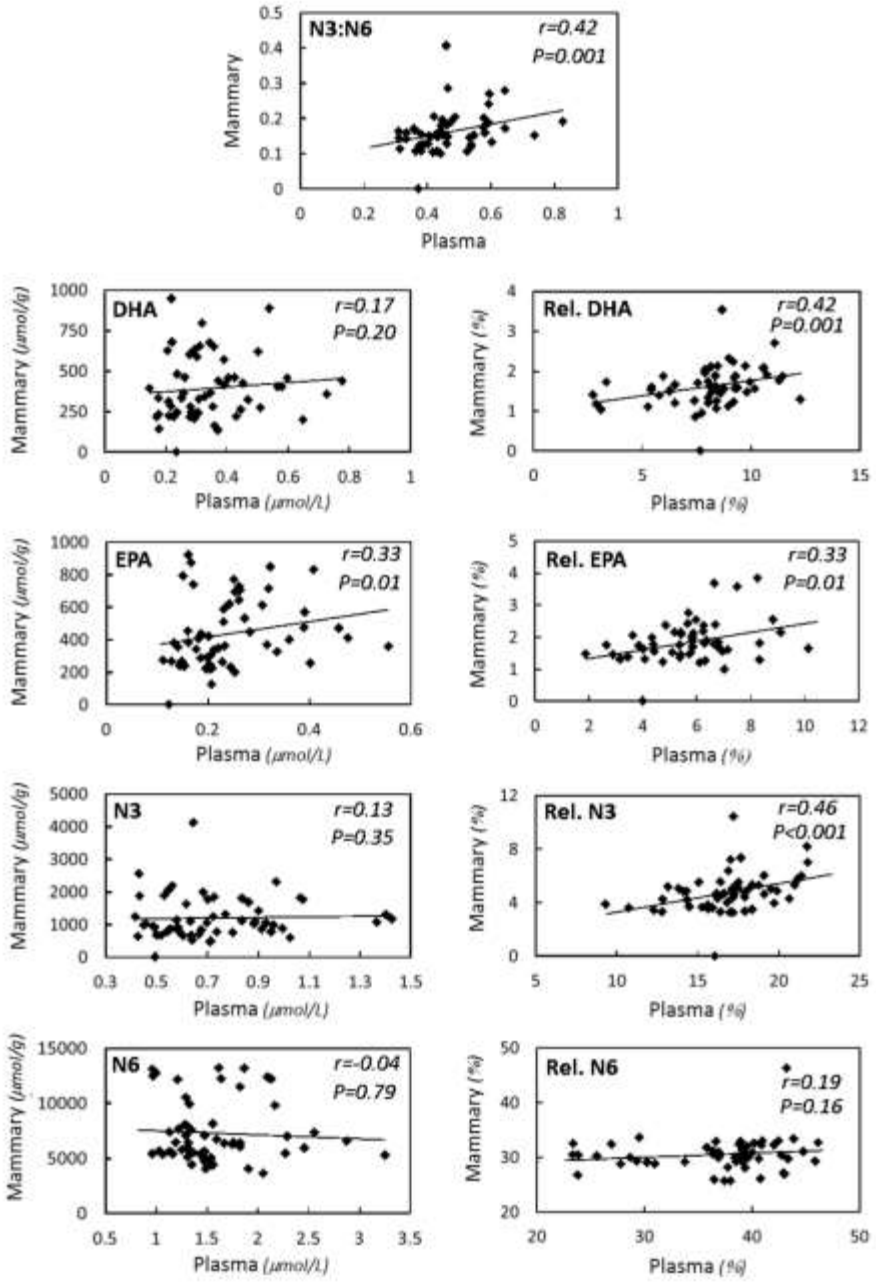
Individual and combined time dependent effects of FO and Tam on histologic parameters of MNU-induced mammary carcinogenesis

	Experimental Groups			
	20% CO	Tam 0.6 ppm	10% FO	Tam 0.6 ppm + 10% FO
Lesion Multiplicity (mean±SE)				
4 week	1.30±0.26	1.70±0.30	1.40±0.34	1.60±0.3
8 week	1.70±0.45	1.00±0.30	3.00±0.58	1.40±0.22
12 week	3.60±0.62	1.35±0.27[c]	3.22±0.46	0.61±0.18[c]
Composite lesion score (mean±SE)				
4 week	1.90±0.38	2.50±0.45	2.30±0.50	2.10±0.38
8 week	6.80±2.32	1.70±0.65	9.90±2.69	2.50±0.81
12 week	15.6±2.81	4.94±1.11[d]	12.6±2.29	2.00±0.71[c]

From Manni A, *et al., Int J Cancer* 134:1549, 2014



From Manni A, *et al., Int J Cancer* 134:1549, 2014



From Manni A, *et al., Int J Cancer* 134:1549, 2014

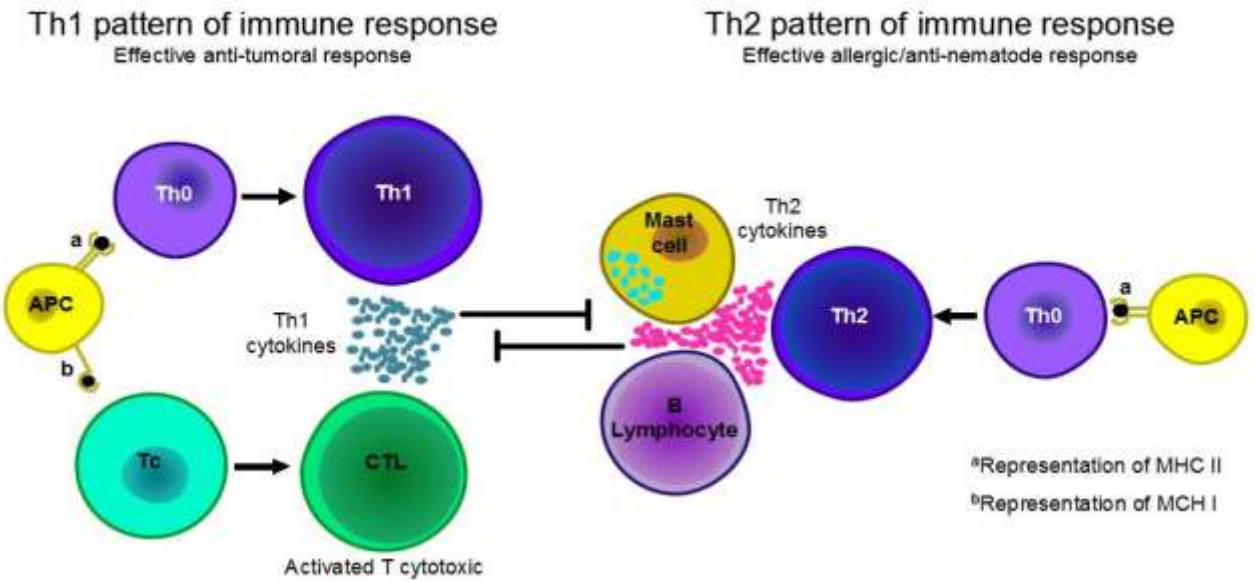
Correlation between FA levels and biomarker expression by mammary gland lesions [a]

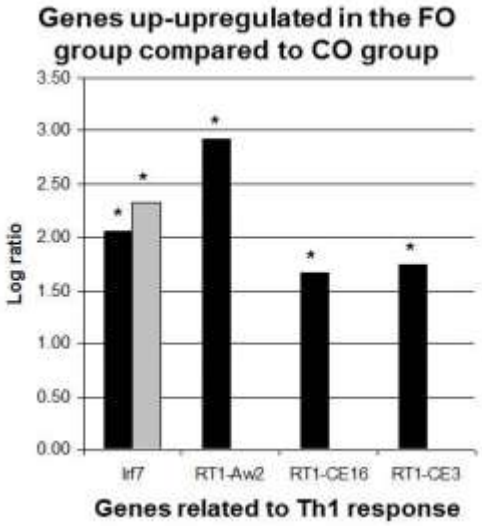
	Fatty Acids [b]															
	Absolute amount									Relative concentrations						
Biomarkers	ALA	AA	EPA	DPA	DHA	N3	N6	N3:N6	Total	ALA	AA	EPA	DPA	DHA	N3	N6
p-ERK	**-0.47**	-0.39	-0.12	-0.21	-0.32	-0.27	-0.09	-0.28	-0.22	**-0.57**	**-0.47**	-0.12	-0.26	-0.30	-0.20	0.16
p-AKT	-0.22	0.05	-0.11	-0.32	**-0.46**	-0.32	0.15	**-0.46**	-0.07	-0.36	0.05	-0.08	-0.29	**-0.45**	-0.31	**0.57**
p-mTOR	0.07	0.32	0.13	0.08	0.19	0.16	0.11	0.04	0.15	-0.15	0.32	0.01	-0.05	0.12	0.00	-0.15
p-S6	-0.27	-0.15	-0.05	-0.20	-0.28	-0.20	0.14	-0.36	-0.10	**-0.50**	-0.26	-0.07	-0.33	-0.35	-0.18	**0.50**
p-nuclear NFkB	-0.29	-0.32	**-0.52**	**-0.55**	**-0.50**	**-0.50**	**-0.57**	-0.27	**-0.61**	-0.09	-0.04	-0.25	-0.30	-0.23	-0.24	0.13
Ki-67	**-0.44**	-0.38	-0.27	-0.37	**-0.40**	-0.38	-0.04	**-0.42**	-0.13	**-0.55**	-0.34	-0.21	-0.34	**-0.44**	-0.35	-0.36
CC3	-0.33	-0.17	-0.28	-0.36	-0.33	-0.33	0005	**-0.43**	-0.15	-0.34	-0.32	-0.25	-0.25	-0.33	-0.27	**0.46**

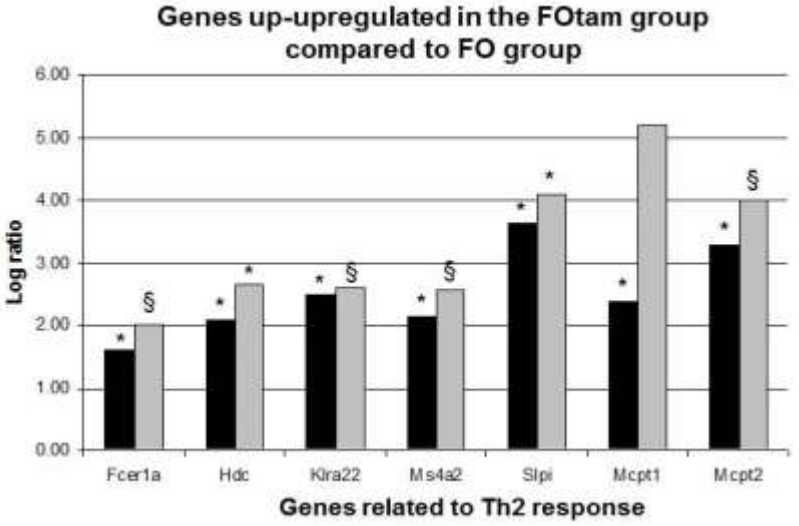
[a] Tissue levels (absolute and relative amounts) of the indicated FA was correlated with the expressions of the indicated biomarkers in the contralateral mammary gland of the same rat. Since n3-FA were undetectable in the MFPs of CO fed rats, only the groups of rats fed the FO diet (with and without Tam) are included. For this analysis, the data from the groups sacrificed at 8 and 12 weeks are combined. The MFPs from rats sacrificed at 4 weeks are excluded since they mostly comprised normal mammary glands were biomarkers could not be analyzed by IHC due to the low cellularity of the specimen which prevented reliable scoring.

[b] Values are correlation coefficients (r) n=26-33; values in **bold** are statistically significant ($p<0.05$)

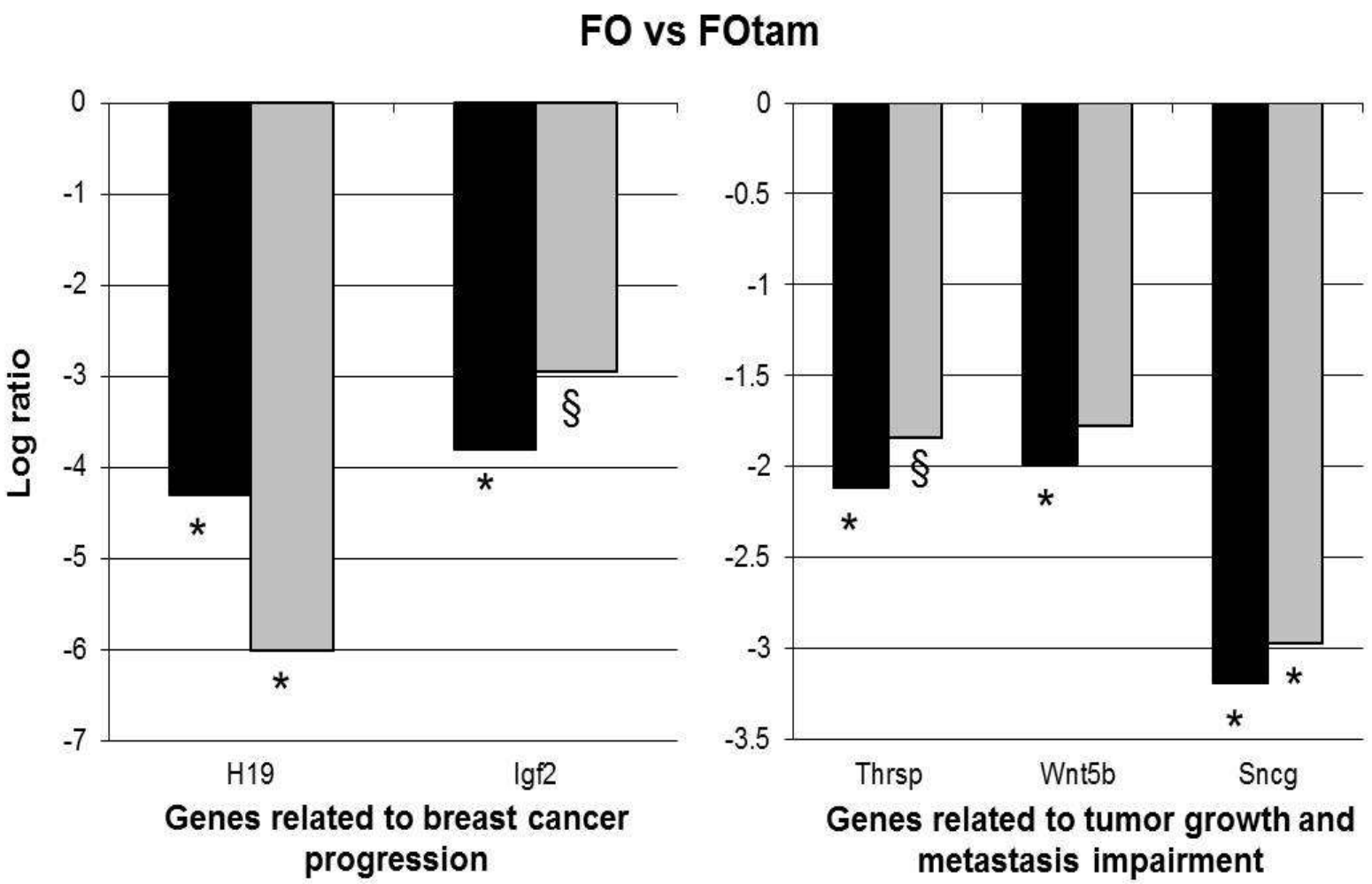
From Manni A, *et al., Int J Cancer* 134:1549, 2014







Adapted from Bidinotto LT, *et al., Nutrition and Cancer 64:991, 2012*

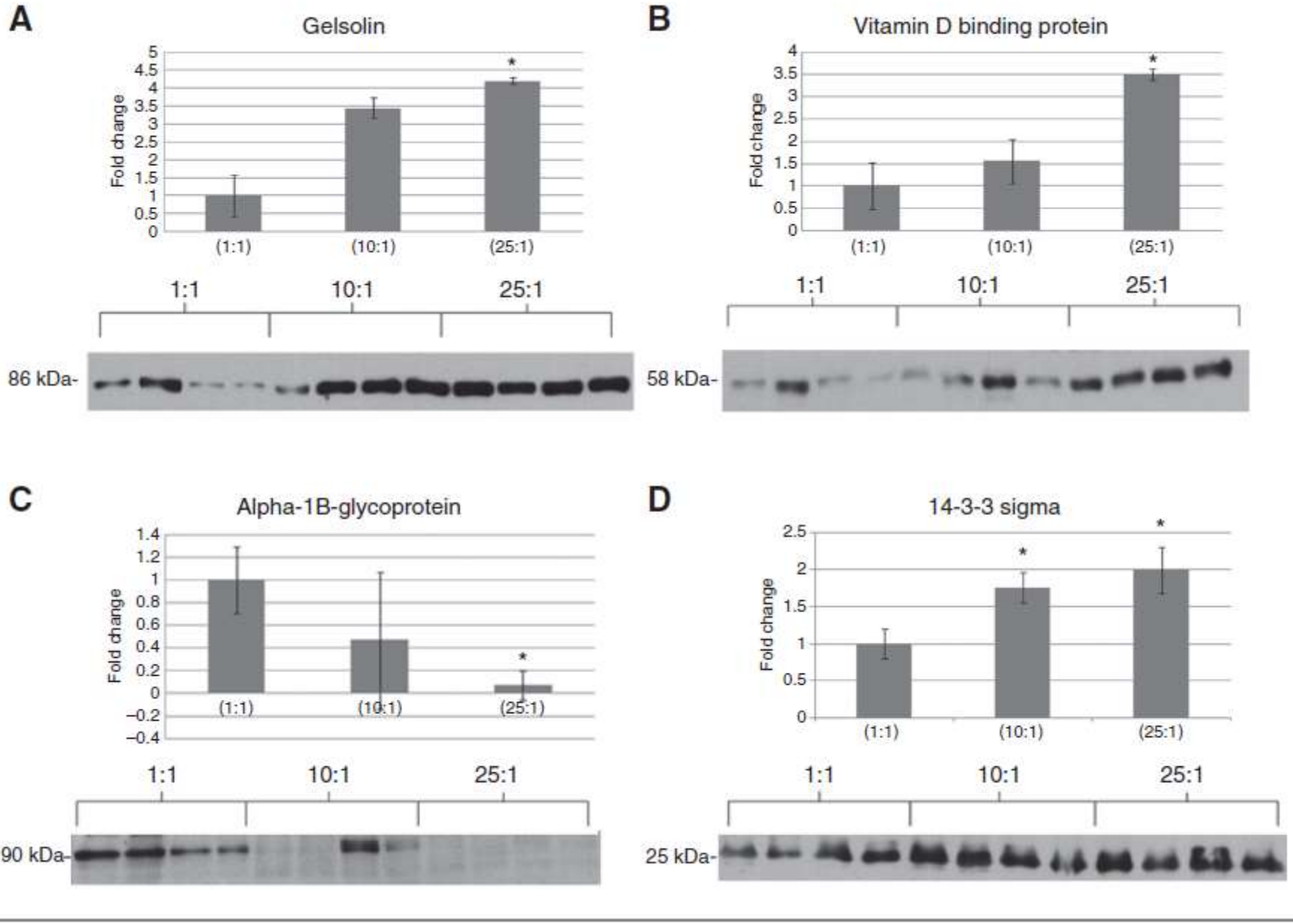


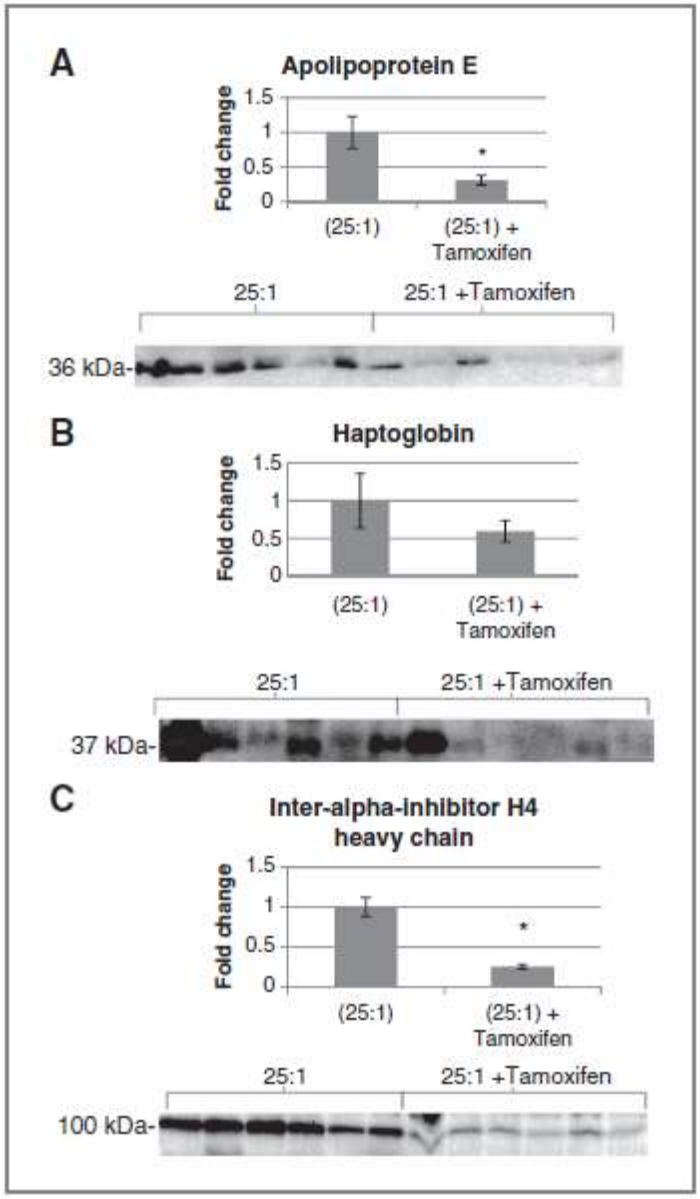
Adapted from Bidinotto LT, *et al., Nutrition and Cancer 64:991, 2012*

Proteomic changes induced by effective chemopreventive ratios of n-3:n-6 fatty acids and Tamoxifen against MNU-induced mammary cancer in the rat

Christine G. Skibinski, Henry J. Thompson, Arunangshu Das, Andrea Manni, James D. Bortner, Anne Stanley, Bruce A. Stanley, and Karam El-Bayoumy (Cancer Prevention Research 6(9):1-10, 2013)

- We used the isobaric tags for relative and absolute quantitation method (iTRAQ) to analyze the proteomic profile of plasma samples of rats fed the following diets with varied n-3:n-6 ratios
  - Group 1 = 1:1
  - Group 2 = 10:1
  - Group 3 = 25:1
  - Group 4 = 25:1 + Tam (1 mg/kg diet)
- Select differentially expressed proteins were further validated by Western analysis


A
Gelsolin
Fold change
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
(1:1)
(10:1)
(25:1)
1:1
10:1
25:1
86 kDa-
B
Vitamin D binding protein
Fold change
4
3.5
3
2.5
2
1.5
1
0.5
0
*
(1:1)
(10:1)
(25:1)
1:1
10:1
25:1
58 kDa-
C
Alpha-1B-glycoprotein
Fold change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
*
(1:1)
(10:1)
(25:1)
1:1
10:1
25:1
90 kDa-
D
14-3-3 sigma
Fold change
2.5
2
1.5
1
0.5
0
*
*
(1:1)
(10:1)
(25:1)
1:1
10:1
25:1
25 kDa-



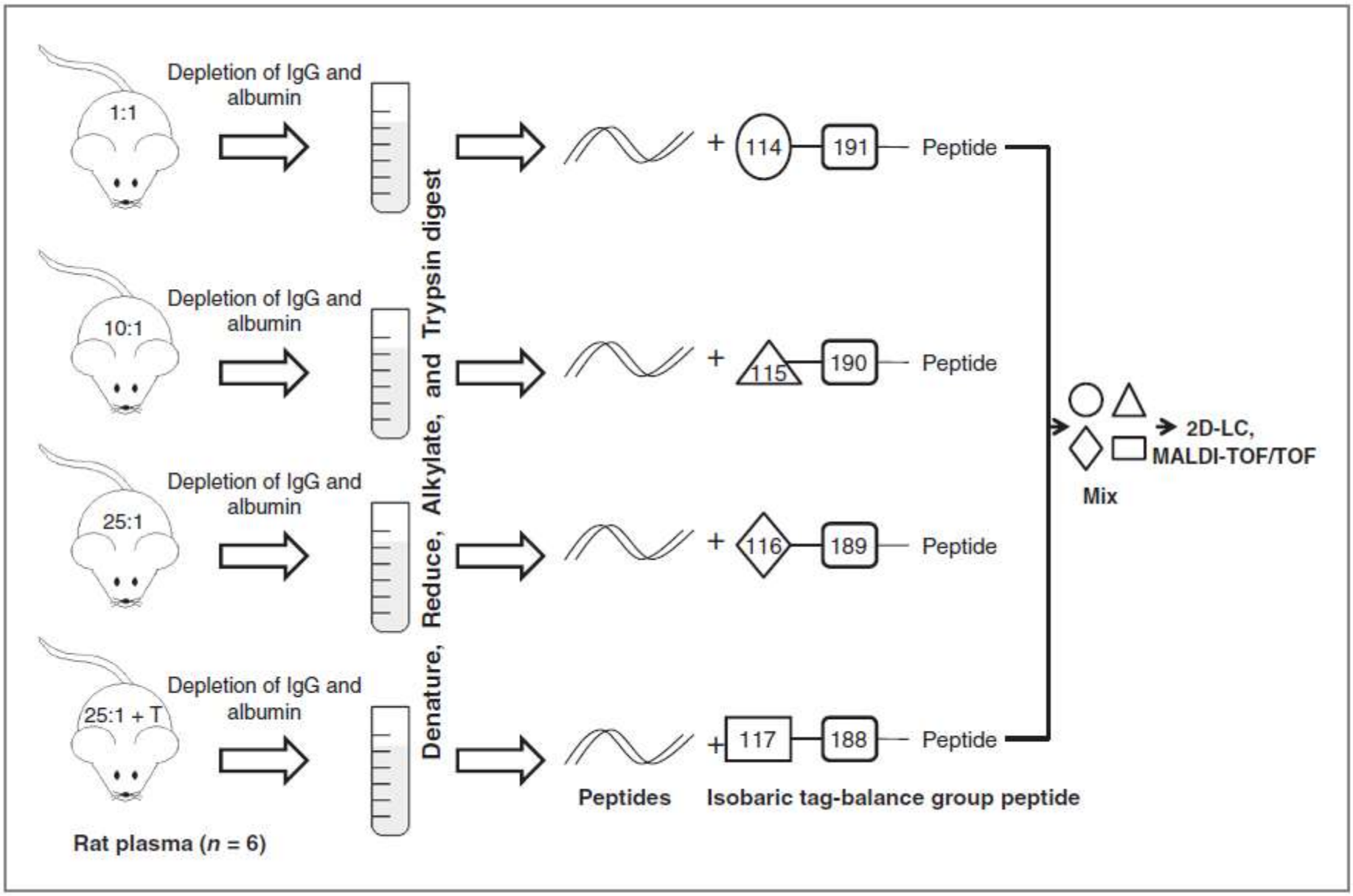
Adapted from Skibinski CG, *et al., Cancer Prev Res* 6:979, 2013

CONCLUSIONS
N-3FA ALONE

- Diets containing clinically achievable ratios of n-3FA:n-6FA (up to 2.3) had only a marginal tumor suppressive effect when used alone in the MNU-mammary tumor model.
- Calculated n-3FA:n-6FA ratios in excess of 10 (translationally irrelevant) were necessary to significantly suppress chemically-induced mammary carcinogenesis
- These results suggest that a component of n-3FA (e.g., DHA) and/or its metabolites may account for the chemoprevention provided by such high ratio
- Our results in transgenic models of mammary carcinogenesis suggest that diet-gene interactions play an important role in mediating the tumor suppressive action of n-3FA

CONCLUSIONS
n-3FA COMBINED WITH ANTIESTROGENS

- Our data suggest that the combination of translationally relevant amounts of n-3FA and Tamoxifen is superior to the individual interventions in suppressing chemically induced mammary carcinogenesis in rats
- Our signaling, genomic, and proteomic studies suggest complementarity in the mechanism of antitumor action of Tamoxifen and n-3FA
- Such complementarity, if confirmed in humans, will allow the use of lower and less toxic doses of antiestrogens in combination with n-3FA without losing chemopreventive efficacy


1:1
Depletion of IgG and albumin
10:1
Depletion of IgG and albumin
25:1
Depletion of IgG and albumin
25:1 + T
Depletion of IgG and albumin
Denature, Reduce, Alkylate, and Trypsin digest
+ 114 191 Peptide
+ 115 190 Peptide
+ 116 189 Peptide
+ 117 188 Peptide
Mix
2D-LC, MALDI-TOF/TOF
Rat plasma (n = 6)
Peptides
Isobaric tag-balance group peptide