

sTable I. Diet Ingredients

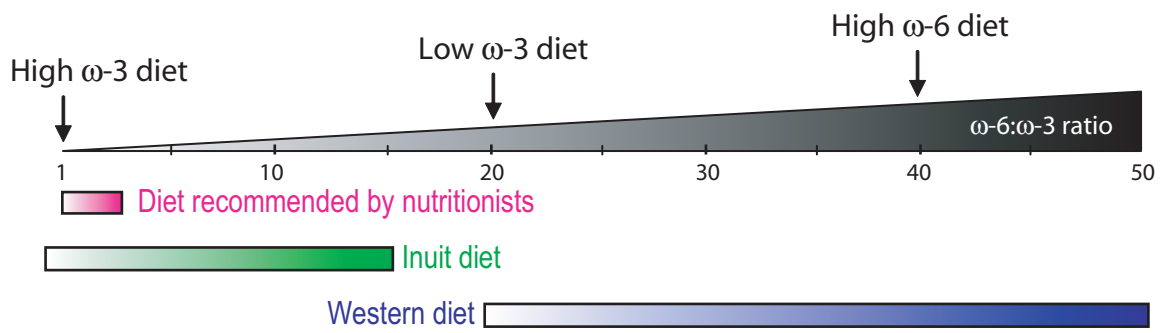
Ingredient	Diet Group (g/100 g Dry Weight)		
	High $\omega$ -3	Low $\omega$ -3	High $\omega$ -6
<b>Variable diet fat</b>			
Palm oil	8	10	6.5
Olive oil	3	0	0
Safflower oil	0	3	6.5
EPA <sup>1</sup>	1	0	0
DHA <sup>1</sup>	1	0	0
<b>Fixed diet ingredients</b>			
Wheat F1	30.0		
Sucrose	10.0		
Dextrin	10.0		
Casein	10.0		
Lactalbumin	10.0		
Alphacel	11.0		
$\beta$ -Sitosterol	0.015		
Vitamin mix <sup>2</sup>	1.0		
Mineral mix <sup>3</sup>	4.7		
Choline bitartrate	0.25		
$\alpha$ -tocopherol	0.002		
$\gamma$ -tocopherol	0.002		
Tenox 20A	0.002		

1. Incromege EPA; 500TG (50% EPA and 10% DHA) and Incromege DHA; 500TG (10% EPA and 50% DHA) from Bioriginal ([www.bioriginal.com](http://www.bioriginal.com)) were used. TG: triglyceride form.

2. AIN-93M; TD94049.

3. AIN-93; TD 94047.

Note: Diets contain 397 Kcal/100g and 30% of energy is from fat, 50% from carbohydrates, and 20% from proteins.



sFigure 1

**A**

Percent distribution of total fatty acids

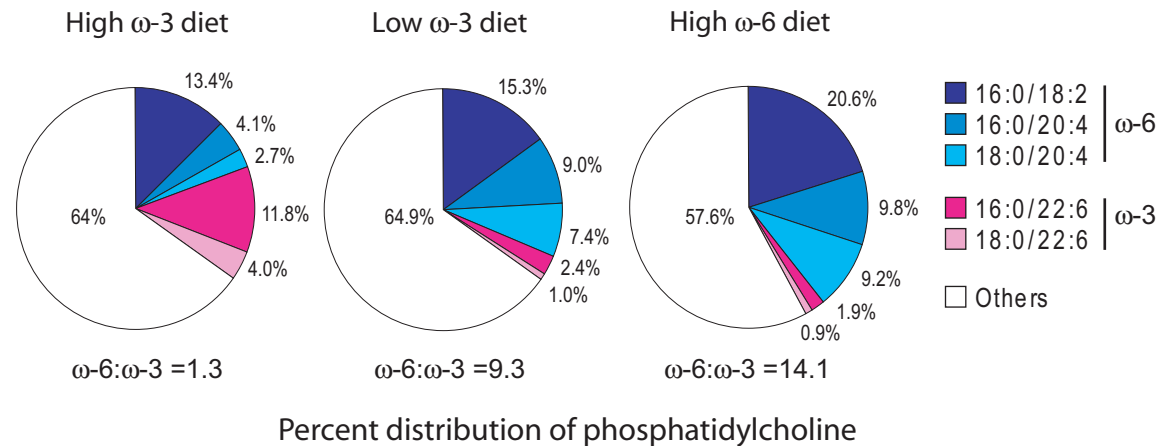
FA \ Diet	High $\omega$ -3	Low $\omega$ -3	High $\omega$ -6
14:0	0.44	0.36	0.30
14:1	0.00	0.00	0.00
16:0	22.74	19.60	19.72
16:1	0.93	1.76	0.86
18:0	8.74	8.07	10.03
18:1(Total)	13.38	15.66	8.87
18:1(vaccenate)	0.89	1.75	1.02
18:1(oleate)	12.49	13.91	7.85
18:2	12.33	15.23	24.01
18:3	0.00	0.00	0.00
20:0	0.27	0.14	0.18
20:1	0.98	0.46	0.51
20:2	0.19	1.28	0.94
20:3	0.00	0.00	0.00
20:4	7.72	20.27	24.44
20:5	10.06	0.09	0.04
22:0	0.00	0.03	0.04
22:6	8.84	1.39	1.18

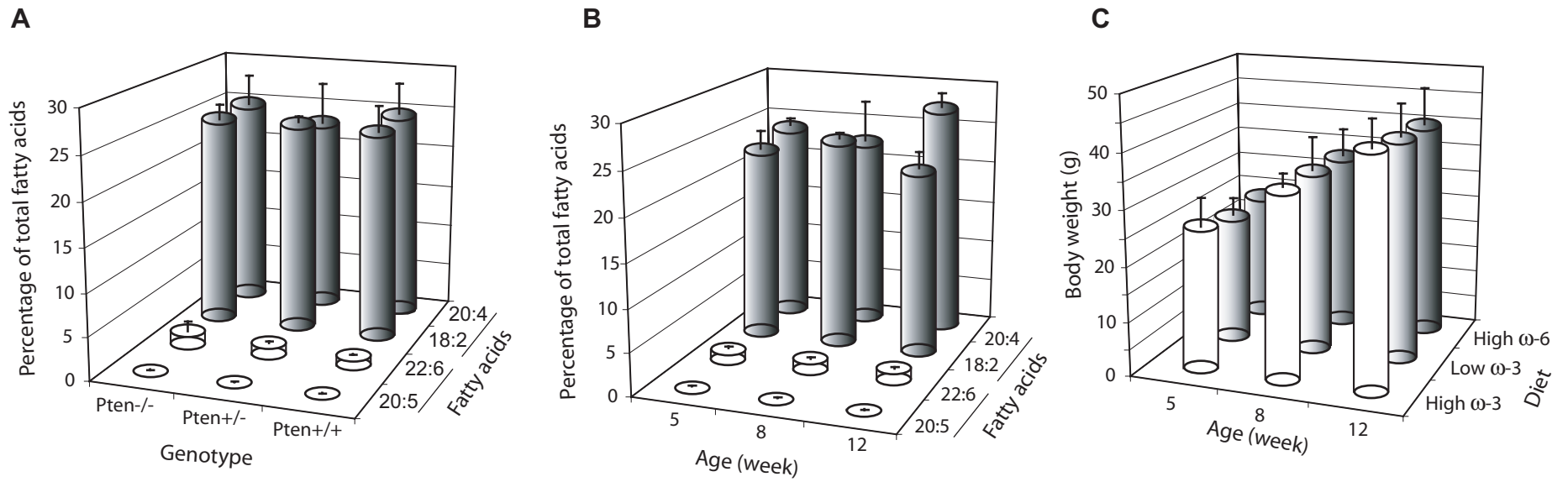
**B**

$\omega$ -6 to  $\omega$ -3 PUFA ratios

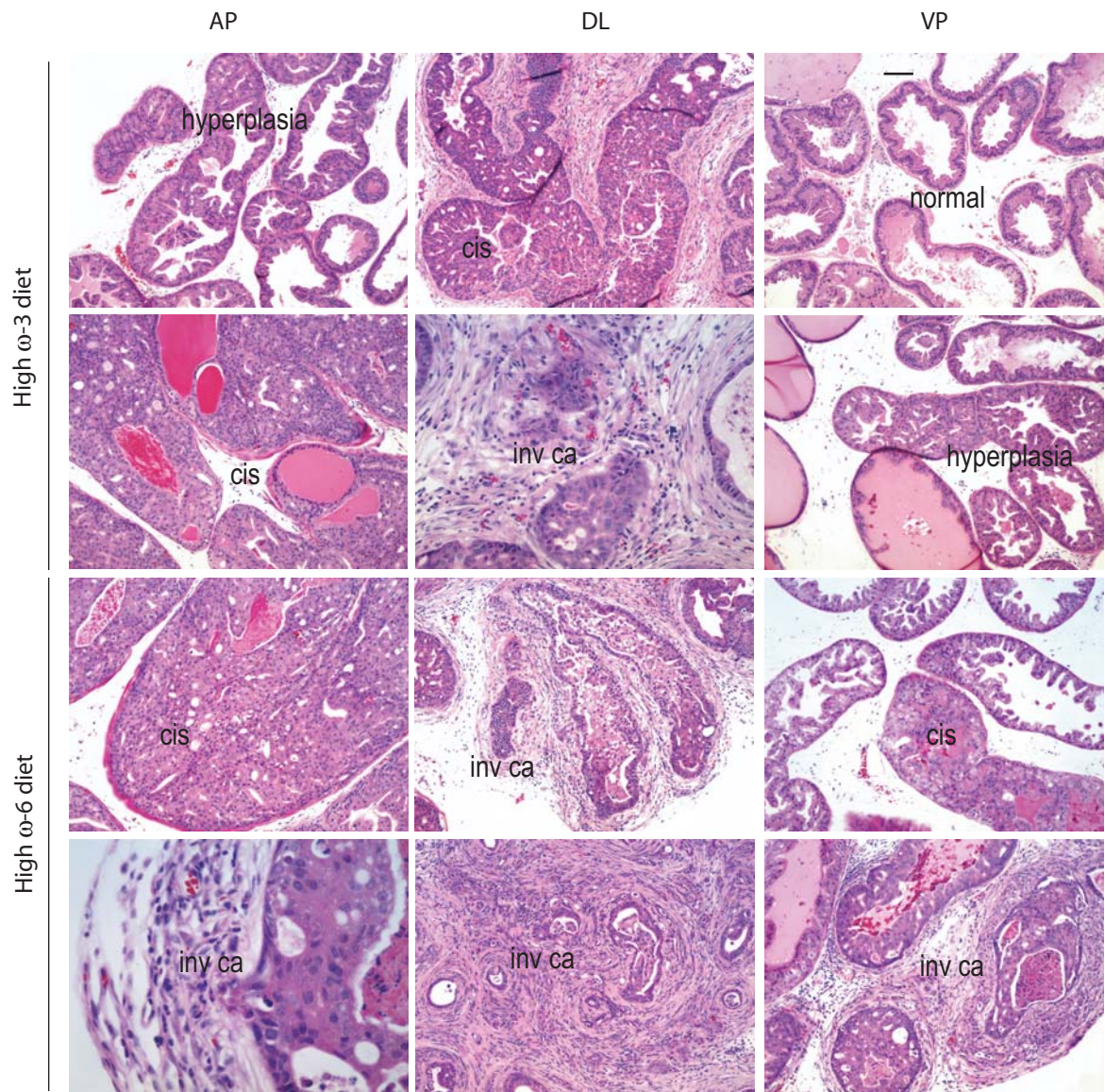
	High $\omega$ -3 diet			Low $\omega$ -3 diet			High $\omega$ -6 diet		
	$\omega$ -3	$\omega$ -6	$\omega$ -6: $\omega$ -3	$\omega$ -3	$\omega$ -6	$\omega$ -6: $\omega$ -3	$\omega$ -3	$\omega$ -6	$\omega$ -6: $\omega$ -3
Food	9.9	9.8	1.0	0.5	10.5	21.0	0.5	21.7	43.4
Blood	18.9	20.0	1.1	1.5	35.5	23.7	1.2	48.5	40.4
Prostate	9.1	8.3	0.9	0.7	12.9	18.4	0.4	21.3	48.4

**C**

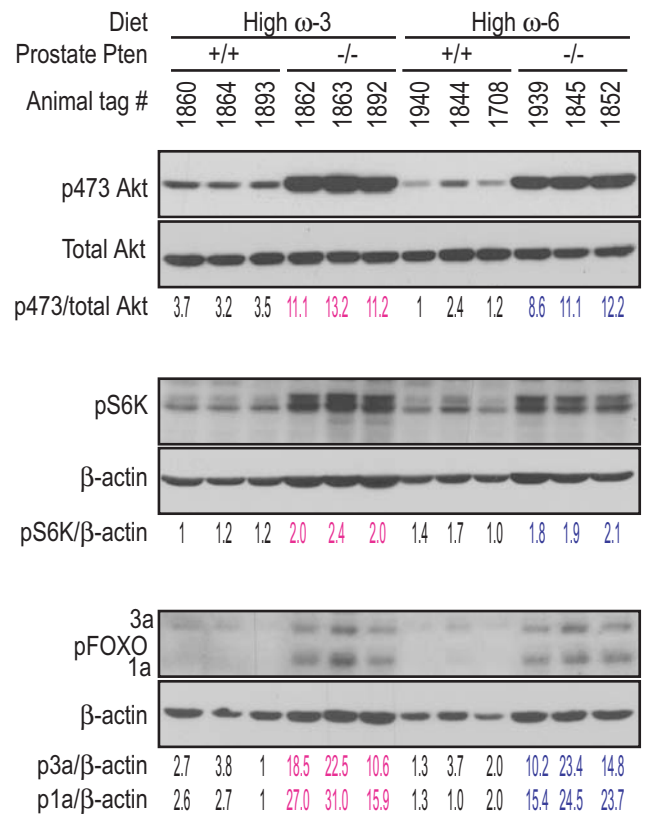




sFigure 3



sFigure 4



sFigure 5

## Supplemental figures

### sTable I. Diet Ingredients.

**sFig. 1. Omega-6 to 3 PUFA ratio in diets.** Omega-6:3 ratio in the high omega-3 diet is 1, which is recommended for human consumption by a panel nutritionists and found in some populations such Inuit. Omega-6:3 ratio is 20 in the low omega-3 diet and 40 in the high omega-6 diet, which are within the range of omega-6 to 3 ratios found in typical American diets.

### sFig. 2. Fatty acid distribution and incorporation of omega-3 and omega-6 PUFA in phospholipids.

**A.** Total fatty acids were measured in the food, blood and prostate tissues of *Pten*<sup>P+/+</sup>, *Pten*<sup>P+/-</sup> and *Pten*<sup>P-/-</sup> mice fed with the experimental diets. Percent distribution of total fatty acids in blood samples is shown (averaged from 5 mice per group). By convention, fatty acids are named after the number of carbon atoms and the number of double-bonds. As expected, mice fed with the three diets showed major differences in omega-6 PUFA [linoleic acid (18:2) and arachidonic acid (20:4)], as well as omega-3 PUFA [eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6)]. Omega-3 PUFA linolenic acid (18:3) was low in food and prostate tissues and not detectable in the blood.

**B.** Percent of omega-3 (LNA 18:3, EPA 20:5 and DHA 22:6 in pink text) and omega-6 (LA 18:2 and AA 20:4 in blue text) as well as the omega-6 to 3 ratios are shown for food, blood and prostate tissue of mice on each diet. Omega-6:omega-3 ratios in the blood and prostate reflected those of the corresponding diet.

**C.** Phosphatidylcholine profiles were determined in blood and prostate samples. Data is shown are percentages of the major PUFA-containing PC species in blood samples from animal on each diet. Ratios of omega-6 to omega-3 PUFA-containing PCs are indicated below each chart. PC species are identified by the fatty acids at the *sn-1* and *sn-2* positions (shown as *sn-1/sn-2*): 16:0/18:2, 1-palmitoyl-2-linoleoyl-

sn-glycero-3-phosphocholine; 16:0/20:4, 1-palmitoyl-2-arachonoyl-sn-glycero-3-phosphocholine; 18:0/20:4, 1-stearoyl-2-arachonoyl-sn-glycero-3-phosphocholine; 16:0/22:6, 1-palmitoyl-2-docosahexaenoic-sn-glycero-3-phosphocholine; 18:0/22:6, 1-stearoyl-2-docosahexaenoic-sn-glycero-3-phosphocholine. “Others” refers to PC species with saturated and monounsaturated fatty acids at the *sn*-2 position. A higher percentage of PC molecules contained omega-3 PUFA at the *sn*-2 position (22:6) in mice fed the high omega-3 diet compared to the other diets. PC molecules were enriched in omega-6 PUFA at the *sn*-2 position (18:2 and 20:4) in mice fed the high omega-6 diet. Omega-3 PUFA containing PC increased by a factor of 4.6 between the high and low omega-3 diets, and by a factor of 5.2 between the high omega-3 and high omega-6 diets. Phospholipid profiling of PE showed major differences in 18:0/**20:4**, 16:0/**22:6** and 16:0/**20:5** species, and omega-3 containing PE were also increased by a factor of 5.2 in the prostate of animals on the high omega-3 compared to the high omega-6 diet (data not shown). Major PS differences were in 18:0/**20:4**, 20:0/**20:4**, 16:0/**22:6** and 18:0/**22:6** species, with a 2.9-fold increase in omega-3-containing PS in animals on the high omega-3 compared to the high omega-6 diet (data not shown).

**sFig. 3. Fatty acid distribution and body weight gained over time.** **A:** percent of total fatty acids for 20:5, 22:6, 18:2 and 20:4 are shown for *Pten*<sup>P+/+</sup>, *Pten*<sup>P+/-</sup> and *Pten*<sup>P-/-</sup> mice fed with the high omega-6 diet. The levels of omega-3 and omega-6 PUFA remained constant regardless of genotype. Fatty acid distribution was similarly conserved among genotypes in the high omega-3 and low omega-3 diet. **B:** percent of total fatty acids for 20:5, 22:6, 18:2 and 20:4 are shown for mice fed the high omega-6 diet at 5, 8 and 12 weeks of age. The level of omega-3 and omega-6 PUFA remained constant regardless of age. **C:** Similar body weight gain over 5 to 12 weeks in mice fed the high omega-3, low omega-3 and high omega-6 diet.



**sFig. 4. Histology of *Pten*<sup>P-/-</sup> prostates.** Histology of prostates from 8 week-old mice fed the high omega-3, low omega-3 or high omega-6 diet. Cis: carcinoma *in situ*, inv ca: invasive carcinoma, AP: anterior prostate, DL: dorsolateral prostate, VP: ventral prostate. Bar, 100  $\mu$ m.

**sFig. 5. Phosphorylation of S6K and FOXO.** Prostate tissues were obtained from 8-week-old *Pten*<sup>P+/+</sup> and *Pten*<sup>P-/-</sup> mice fed the high omega-3 and high omega-6 diets. Protein lysates were used for Western blotting of Akt, Akt pS473, S6K pT389, Foxo and  $\beta$ -actin. Ratios are indicated at the bottom of corresponding panel.