Mechanistic Studies on the Anti-tumor Activities of Omega-3 Polyunsaturated Fatty Acids on Human Neuroblastoma Cells

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Contents

- Introduction Human Neuroblastoma (NB) and Omega-3 Polyunsaturated Fatty Acids (n-3 PUFA)
- Anti-tumor Effects of n-3 PUFA on Human Neuroblastoma Cells
- Mechanistic Studies and Molecular Action Mechanisms of n-3 PUFA
- Conclusions and Future Perspectives

What is Neuroblastoma?

- An extracranial tumor derived from primitive cells of the sympathetic nervous system
- The most common and deadly solid tumor of childhood
- Accounts for 8 - 10% of all childhood cancers and ~15% of all childhood cancer-related deaths

Symptoms：

Abdomen



Chest



Bones around the eyes or orbits



Etiology of Neuroblastoma

Risk factors:

- Smoking
- Alcohol consumption
- Use of hormones and anti-fertility drugs
- Maternal use of hair dye
- Use of medicinal drugs during pregnancy

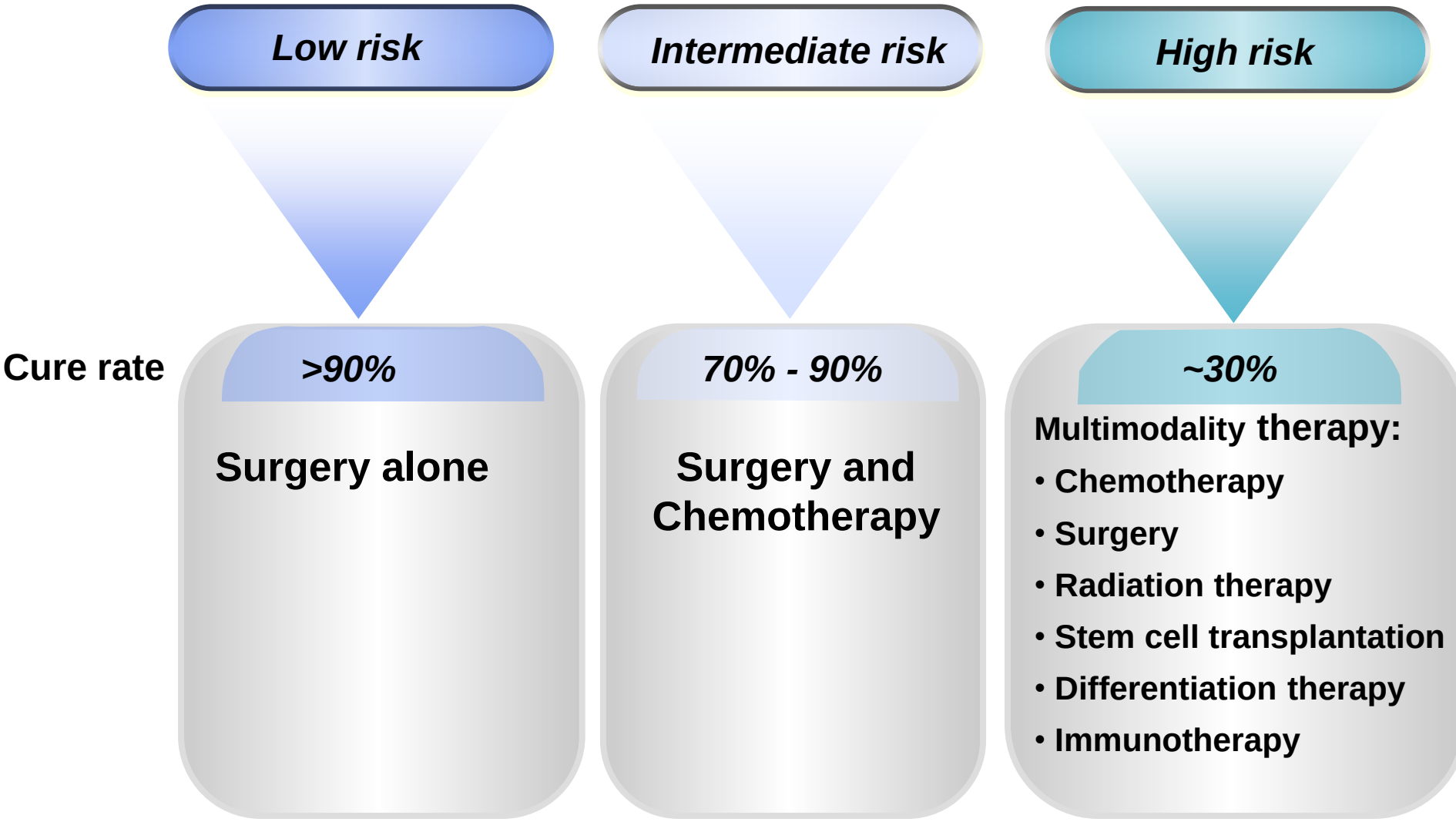
Protective factors:

- Folic acid supplements
- Vitamin supplements

Molecular Pathogenesis of Neuroblastoma

- ***N-myc*** **amplification state: a key factor in the genesis of human neuroblastoma**
- **Chromosomal abnormalities**
- **Mutations in the *PHOX2B* and *ALK* genes**
- **Disruption of cell division cycle, apoptosis and other signaling pathways**

Treatment of Neuroblastoma



Side Effects of Treatment

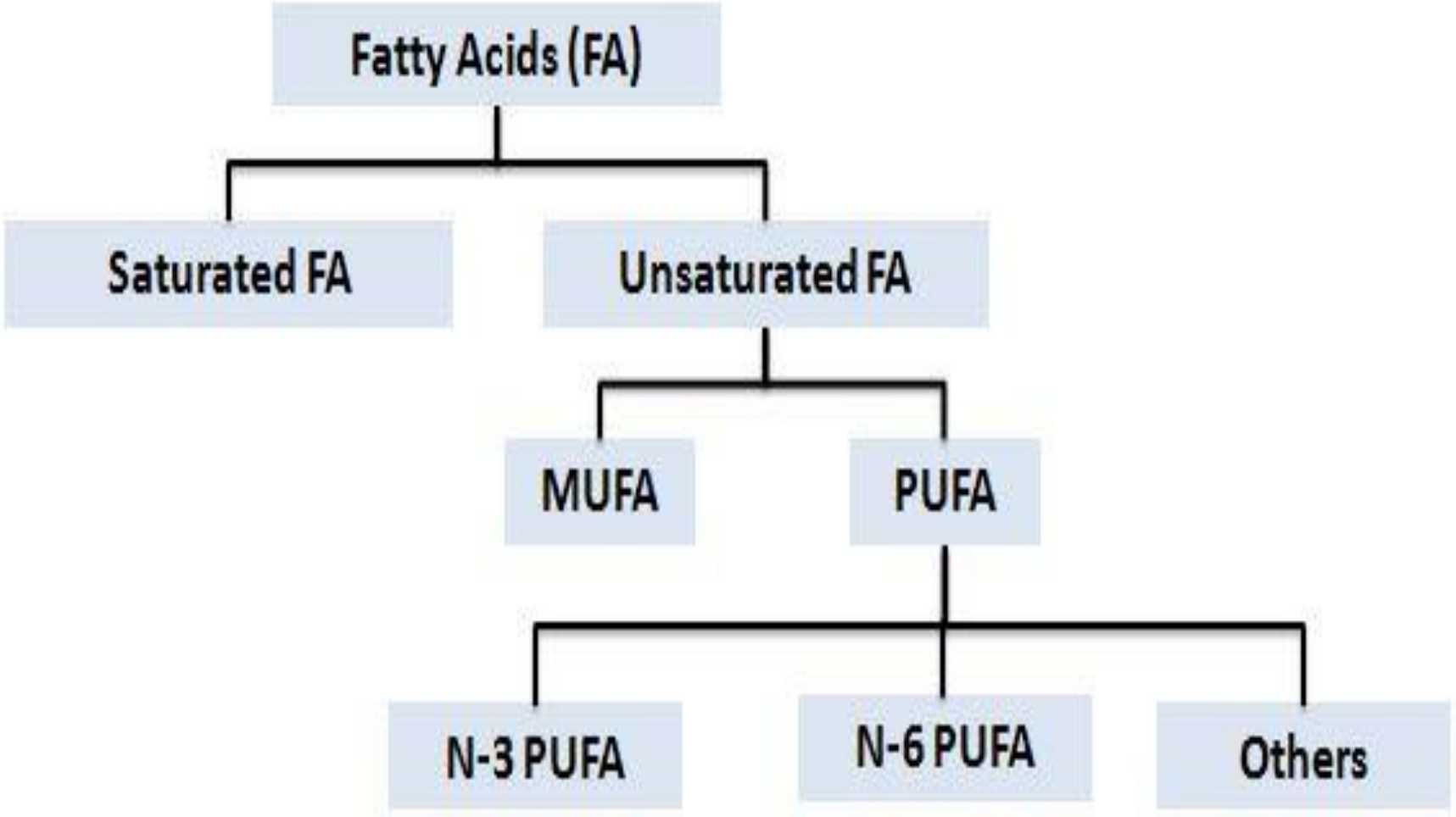
Early side effects:

- Nausea and vomiting
- Diarrhoea
- Hair loss
- Increased risk of infection
- Bruising and bleeding
- Tiredness

Late side effects:

- Affects heart and kidneys functions
- Hearing problems
- Fertility problems
- Growth reduction
- Increased risk of developing secondary cancer

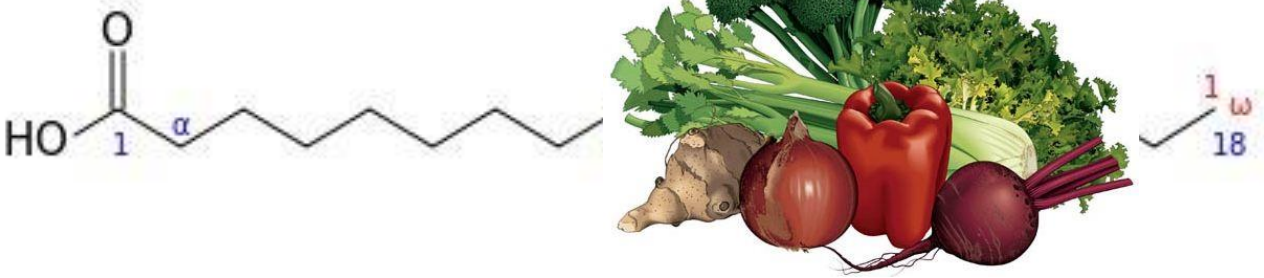
Dietary Fatty Acids as Naturally-occurring Anti-tumor Agents



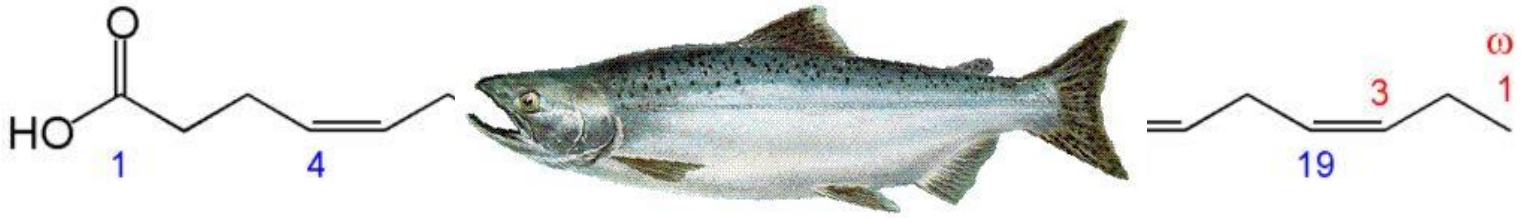
Omega-3 Polyunsaturated Fatty Acids

- Essential long chain fatty acids with more than one C=C double bond

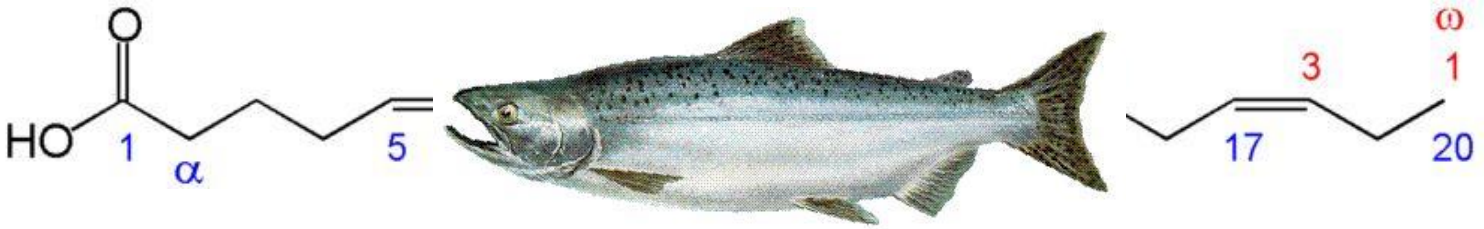
α-Linolenic acid (ALA) 18



Docosahexaenoic acid (DHA) C22:6



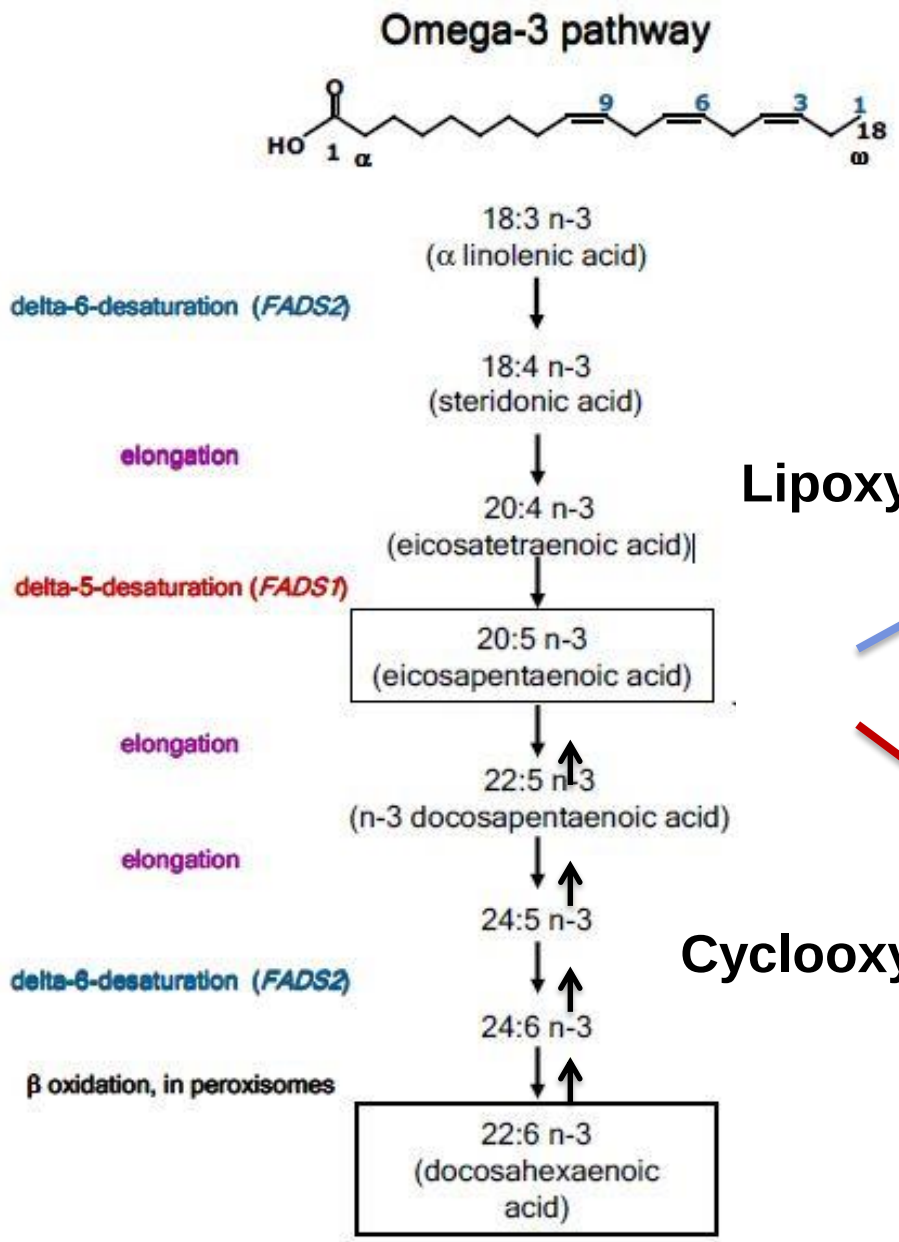
Eicosapentaenoic acid (EPA) C20:5



Major Sources of n-3 PUFA

Source	EPA (mg/100g)	DHA (mg/100g)	EPA + DHA (mg/100g)
Herring	909	1105	2014
Salmon	862	1429	1840
Swordfish	127	772	899
Tuna	233	629	862
Oyster	274	210	484
Clams	138	146	284
Lobster	117	78	195
Shrimp	50	52	102

Metabolism of n-3 PUFAs



Lipoxygenase

Leukotrienes,
Lipoxins etc.

Eicosanoids

Cyclooxygenase

Prostaglandins,
Thromboxanes etc.

N-3 PUFAs and Cancer

Chemopreventive

Epidemiological studies

↑ Fish intak

↓ GI cance

↓ Prostate cancer

↓ Breast cancer

Anti-tumor

…Arrest in Hepatocellular
…, 2010)

…s in Melanoma Cells

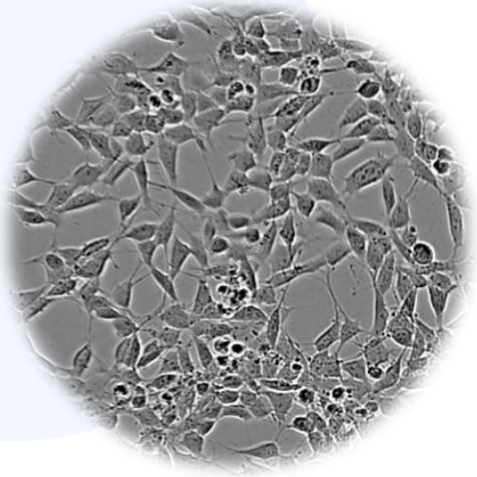
…R stress and Growth Arrest
… (Jakobsen *et al*., 2008)

How about neuroblastoma?

N-3 PUFAs show potential inhibitory effects on cancers

Human Neuroblastoma Cells Used in This Study

LA-N-1 Cells

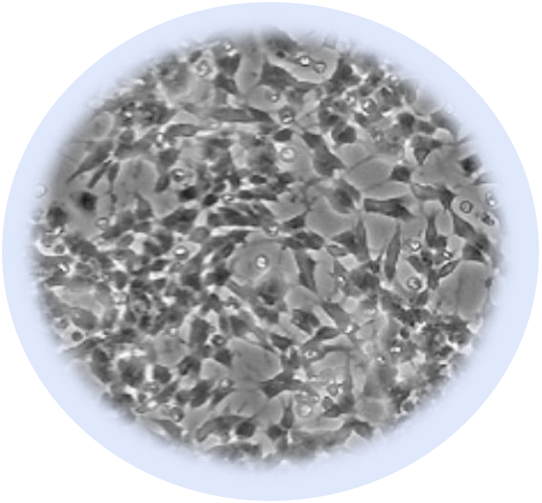


From bone marrow of a child

N-myc amplification (20-30 fold)

Highly invasive and tumorigenic in nude mice

SH-SY5Y Cells



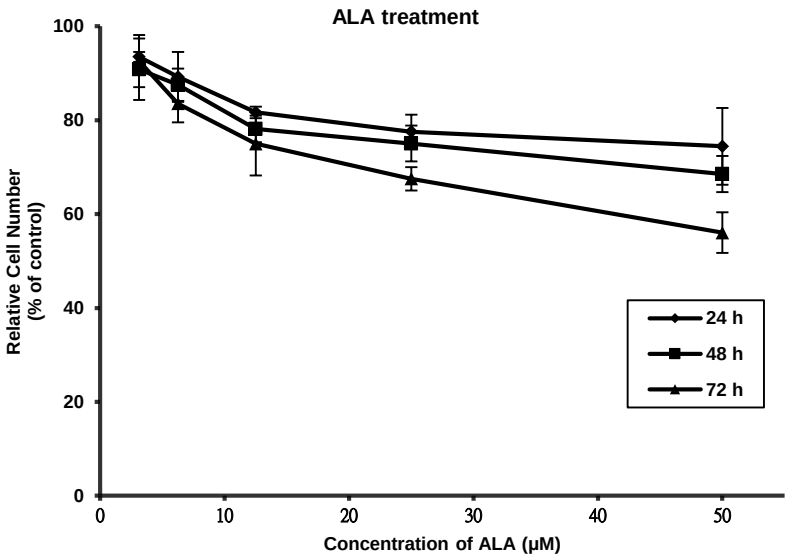
From bone marrow of a child

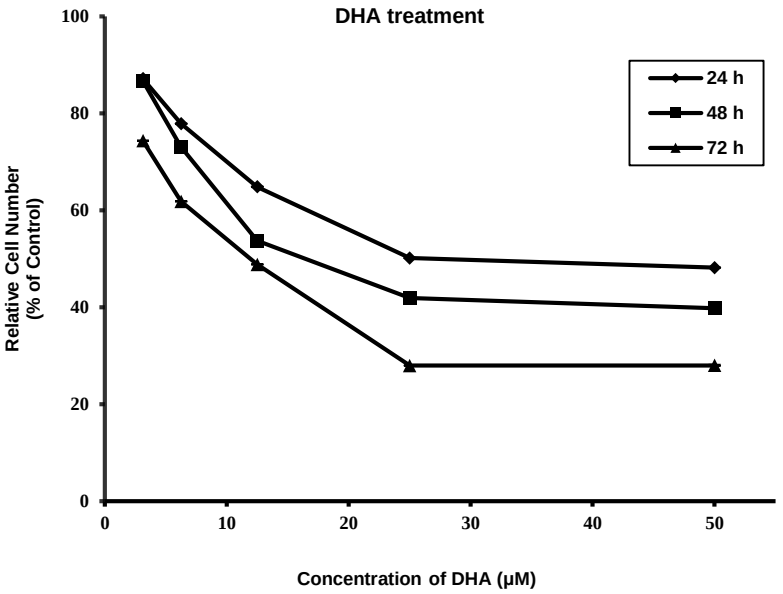
No *N-myc* amplification

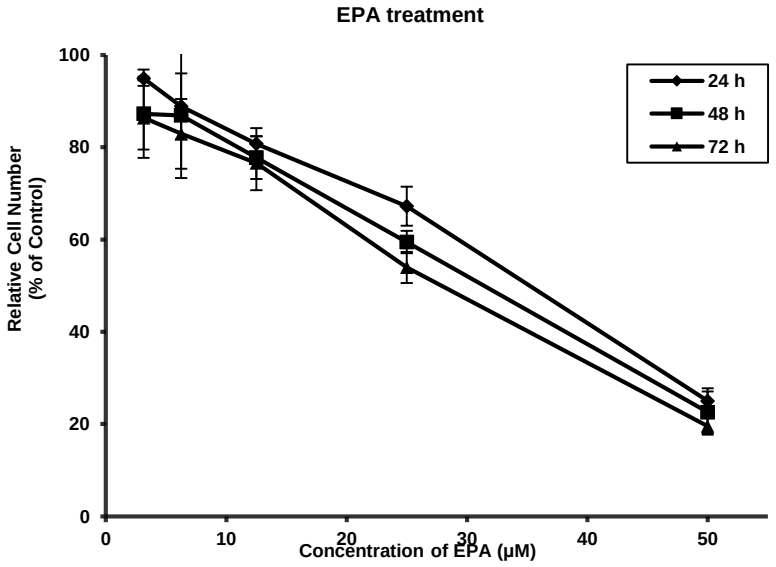
Less invasive and tumorigenic in nude mice

N-3 PUFAs Inhibited Growth of LA-N-1 Cells

Cell line: LA-N-1

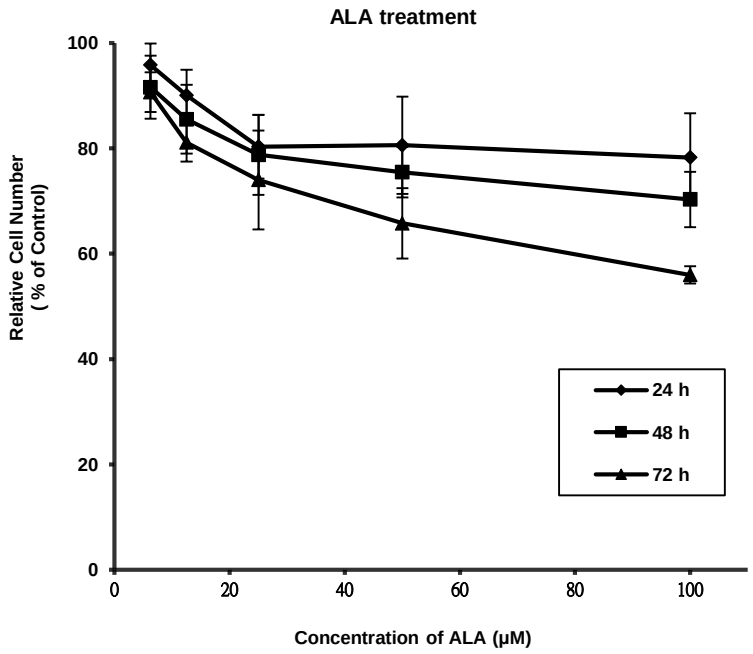


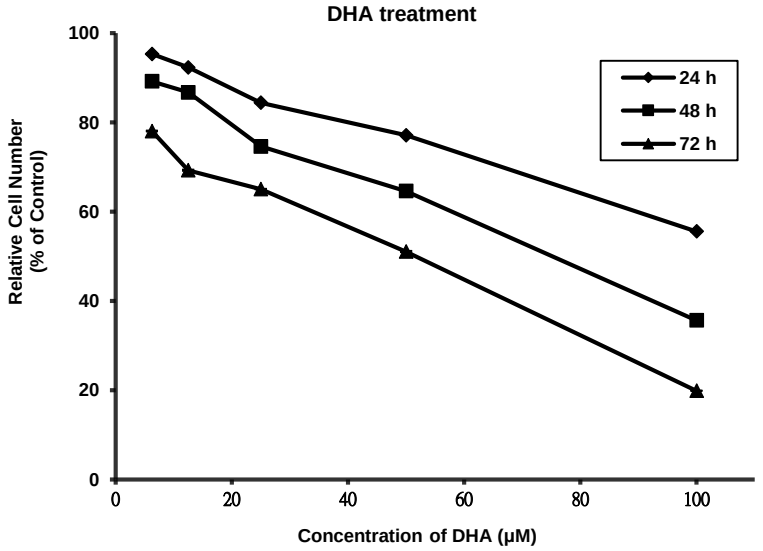


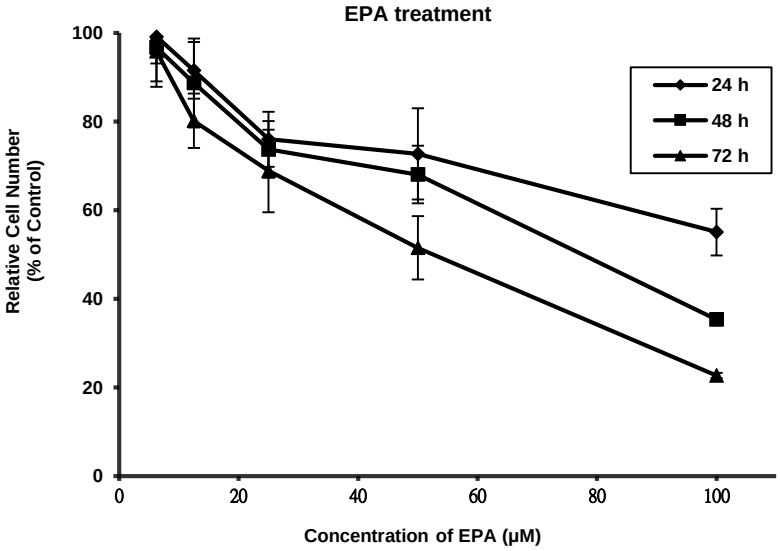


N-3 PUFAs Inhibited Growth of SH-SY5Y Cells

Cell line: SH-SY5Y





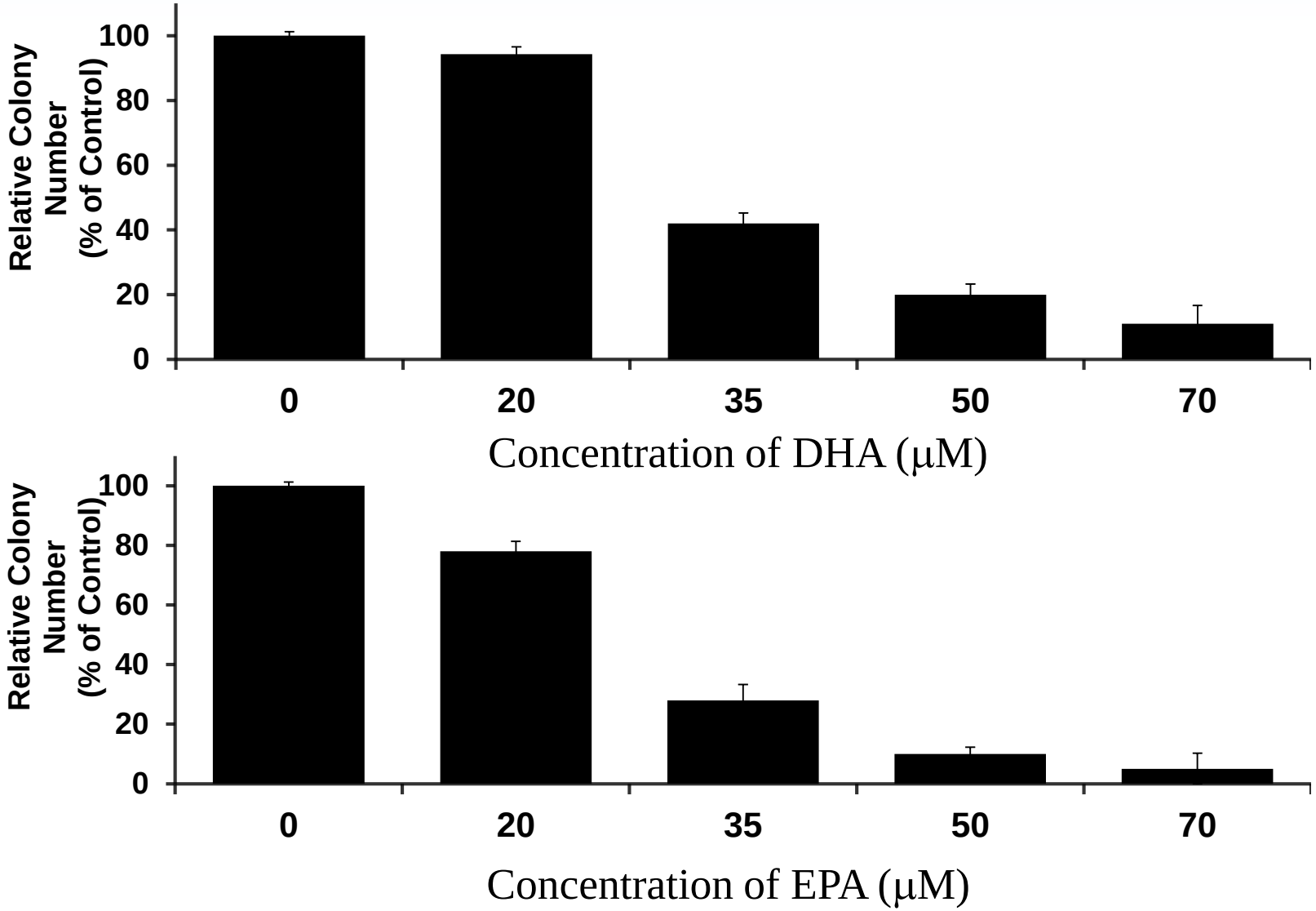


Approximate IC_{50} Values of N-3 PUFAs on Human Neuroblastoma Cells at 48 h of Treatment

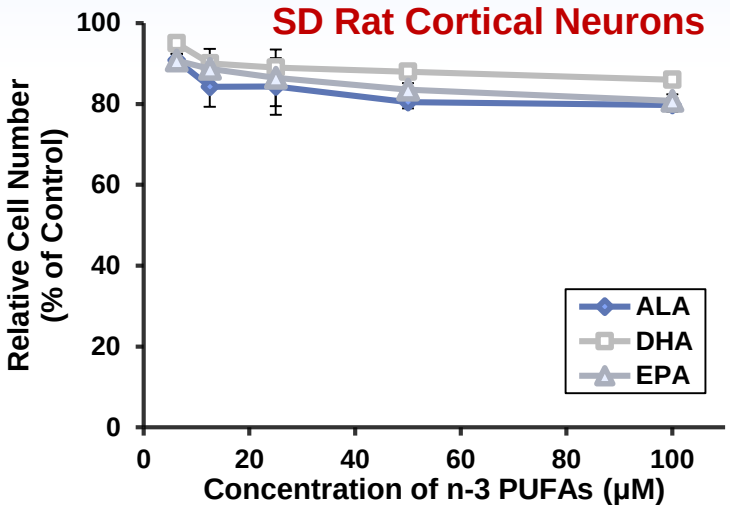
Cell line: LA-N-1	
ALA	>50 μM
DHA	18 ± 1 μM
EPA	35 ± 2 μM

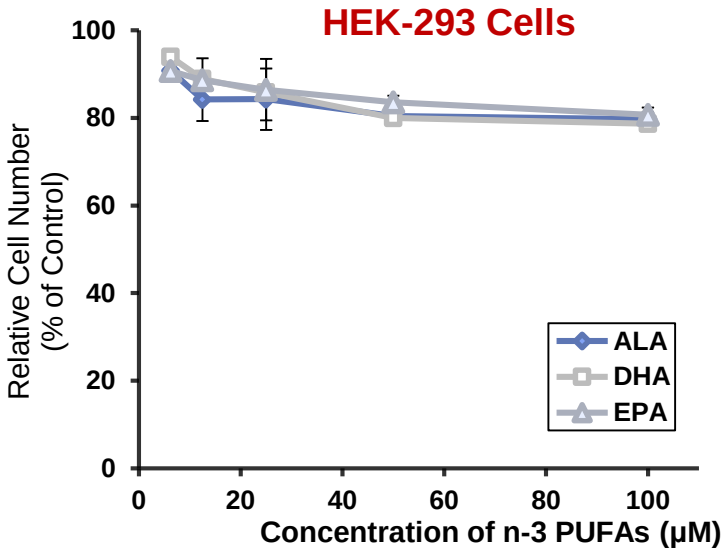
Cell line: SH-SY5Y	
ALA	>100 μM
DHA	100 ± 8 μM
EPA	95 ± 7 μM

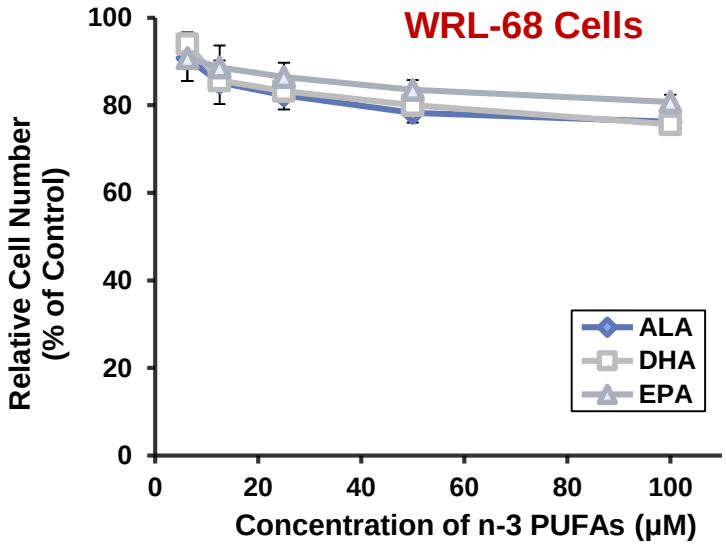
N-3 PUFAs Suppressed the Colony-forming Ability of Human Neuroblastoma LA-N-1 Cells



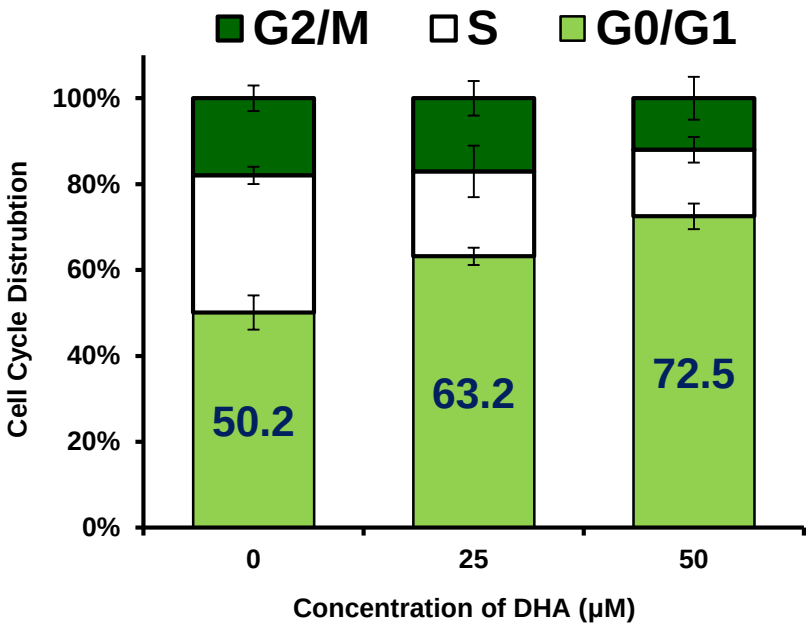
Effect of N-3 PUFAs on the Growth of Normal or Non-tumorigenic Cells

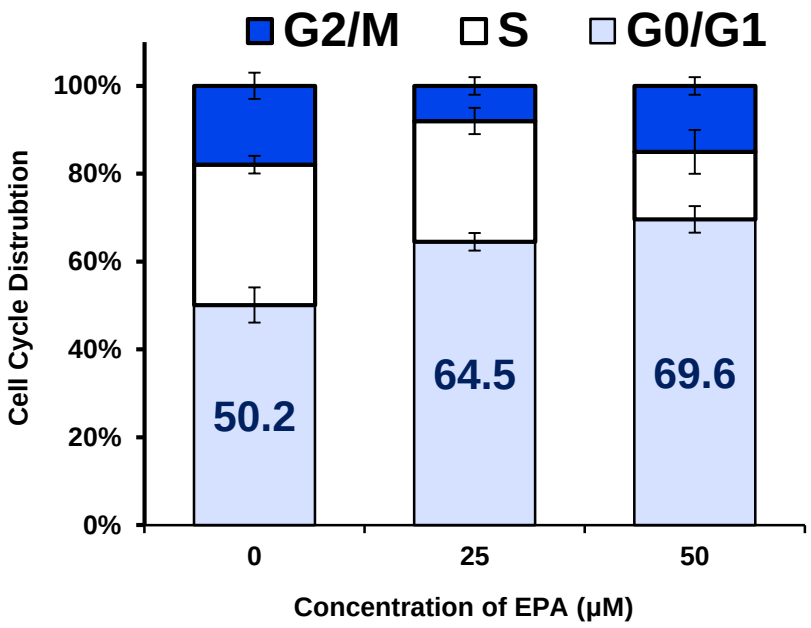






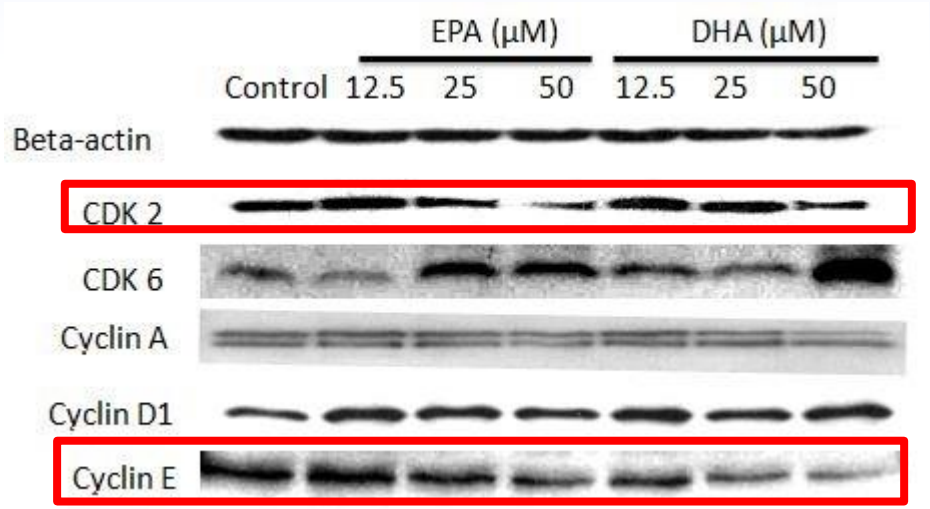
N-3 PUFAs Induced Cell Cycle Arrest in Human Neuroblastoma LA-N-1 Cells

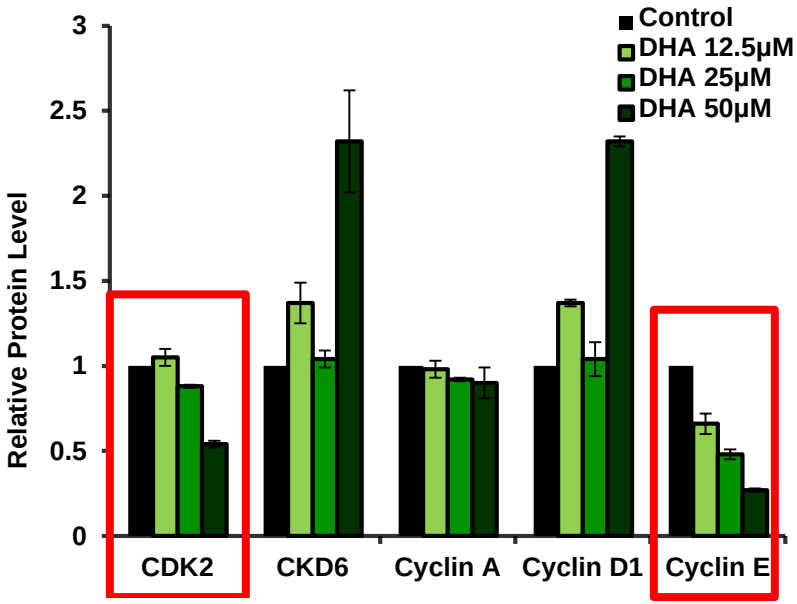


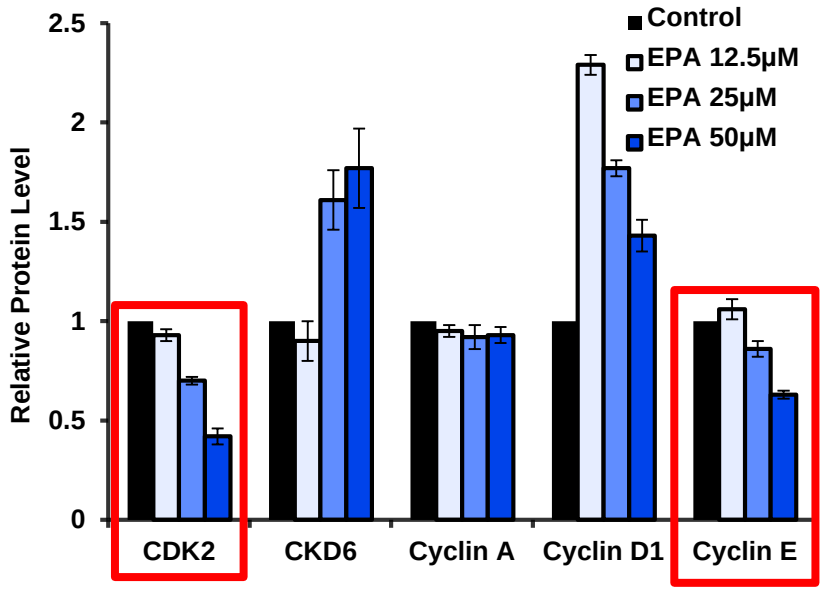


N-3 PUFAs induced cell cycle arrest of neuroblastoma LA-N-1 cells at G_0/G_1 phase

Effects of N-3 PUFA on the Expression of Cell Cycle Regulatory Proteins



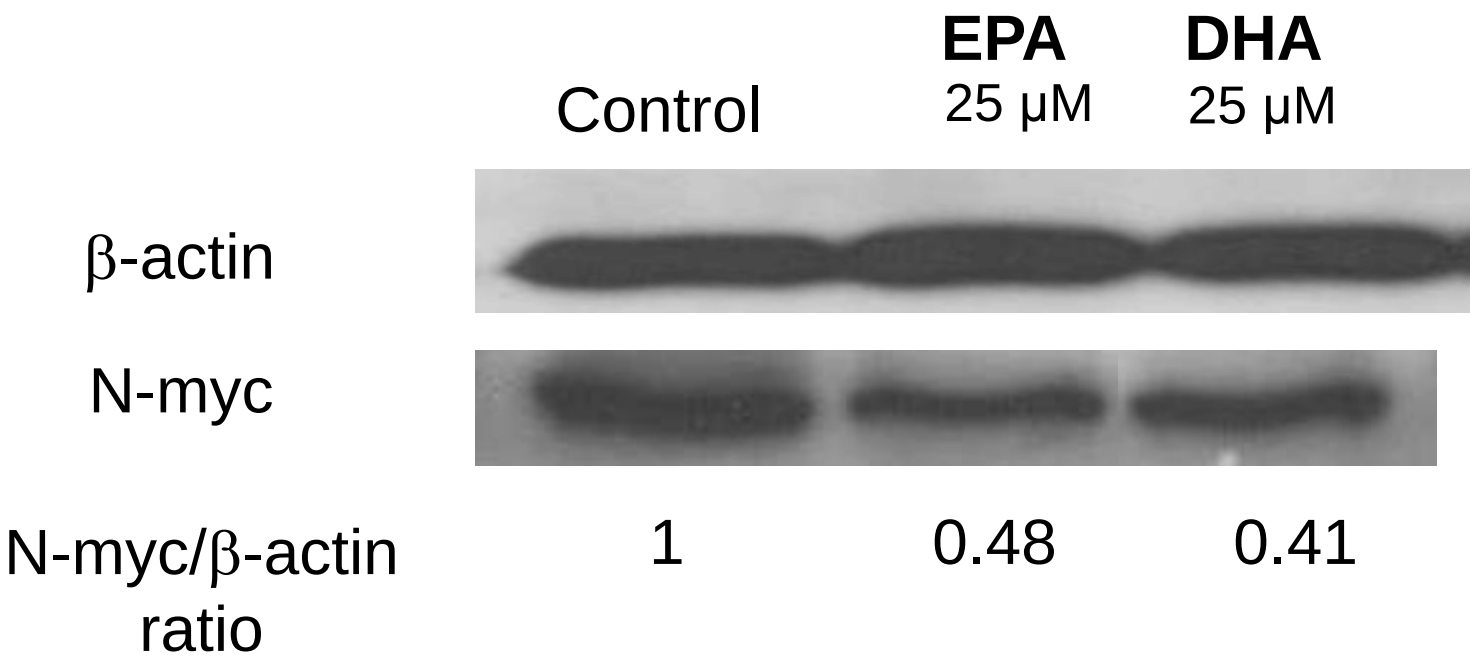




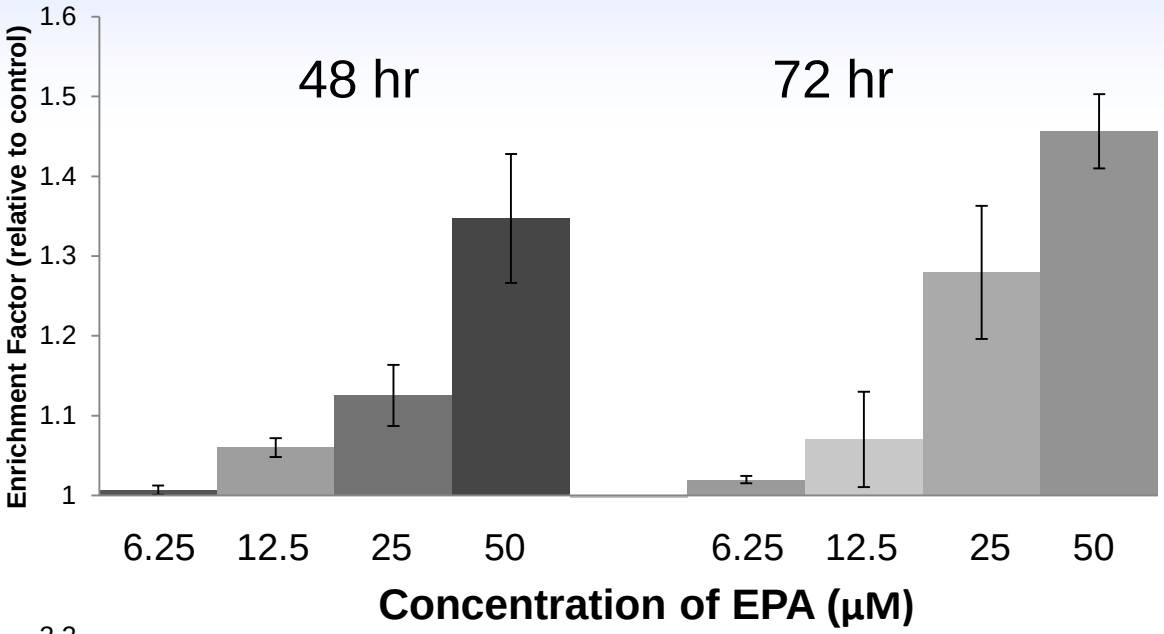
N-3 PUFAs Reduced N-myc Expression in LA-N-1 Cells

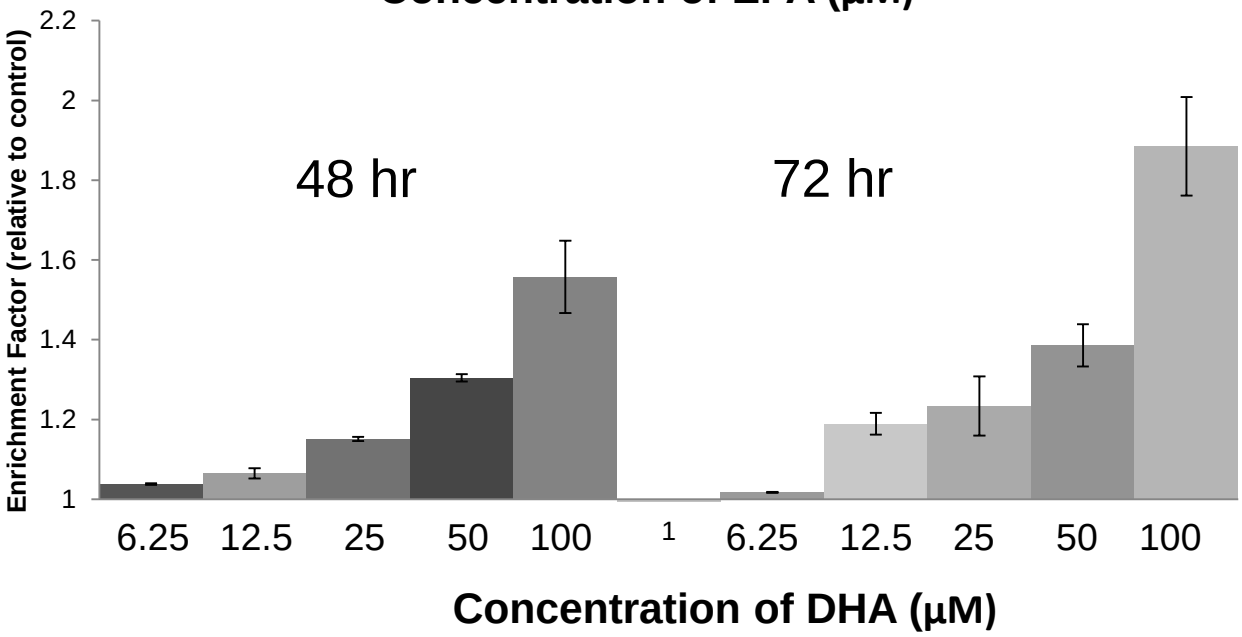
N-myc

- Proto-oncogene protein belonging to Myc family
- A transcription factor that controls expression of many target genes including proliferation, cell growth, apoptosis and differentiation
- Usually amplified in high stage neuroblastoma



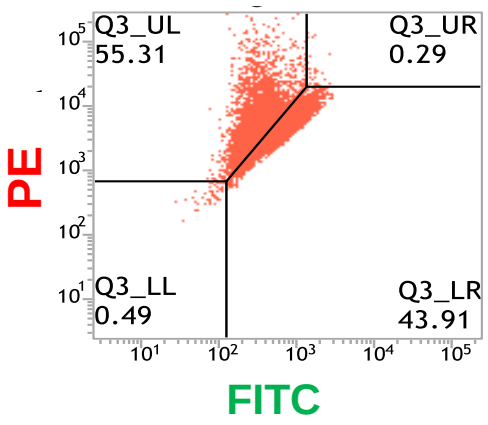
DHA and EPA Induced Apoptosis in LA-N-1 Cells



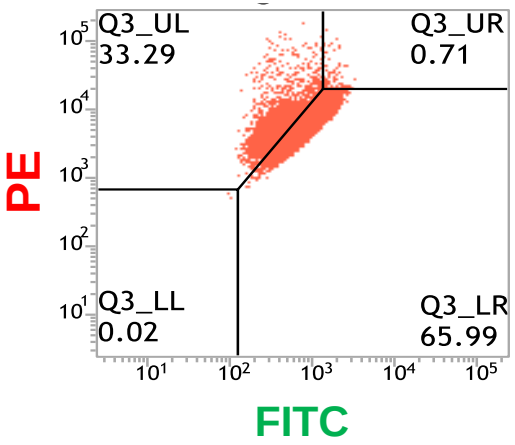


N-3 PUFAs Triggered Mitochondrial Membrane Depolarization in LA-N-1 cells

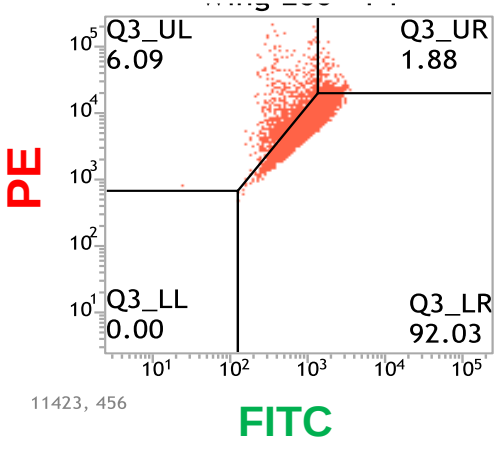
Control



EPA 30 µM

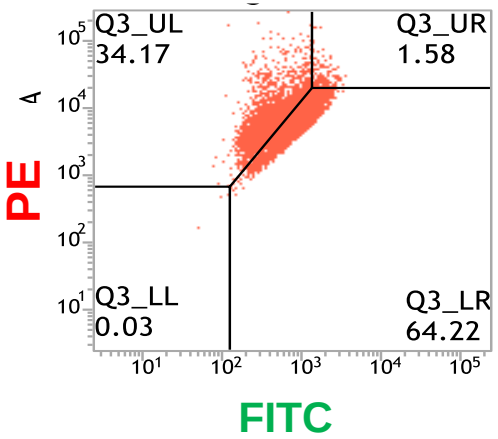


EPA 50 µM

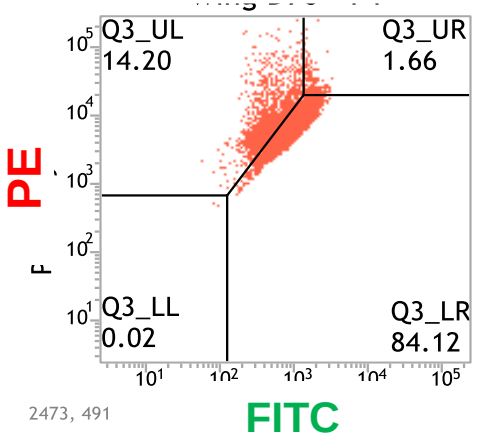


Red to Green shift indicates decrease in membrane potential

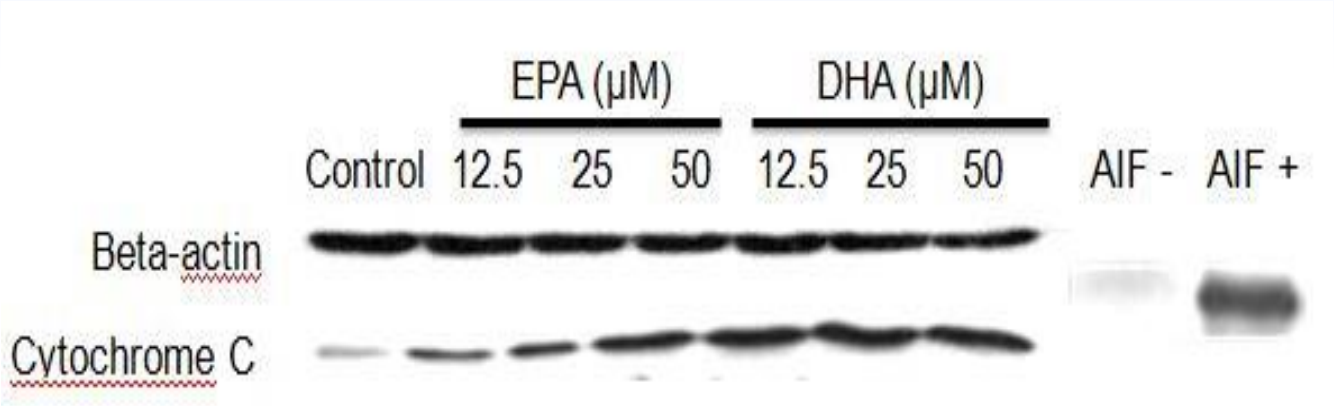
DHA 30 µM

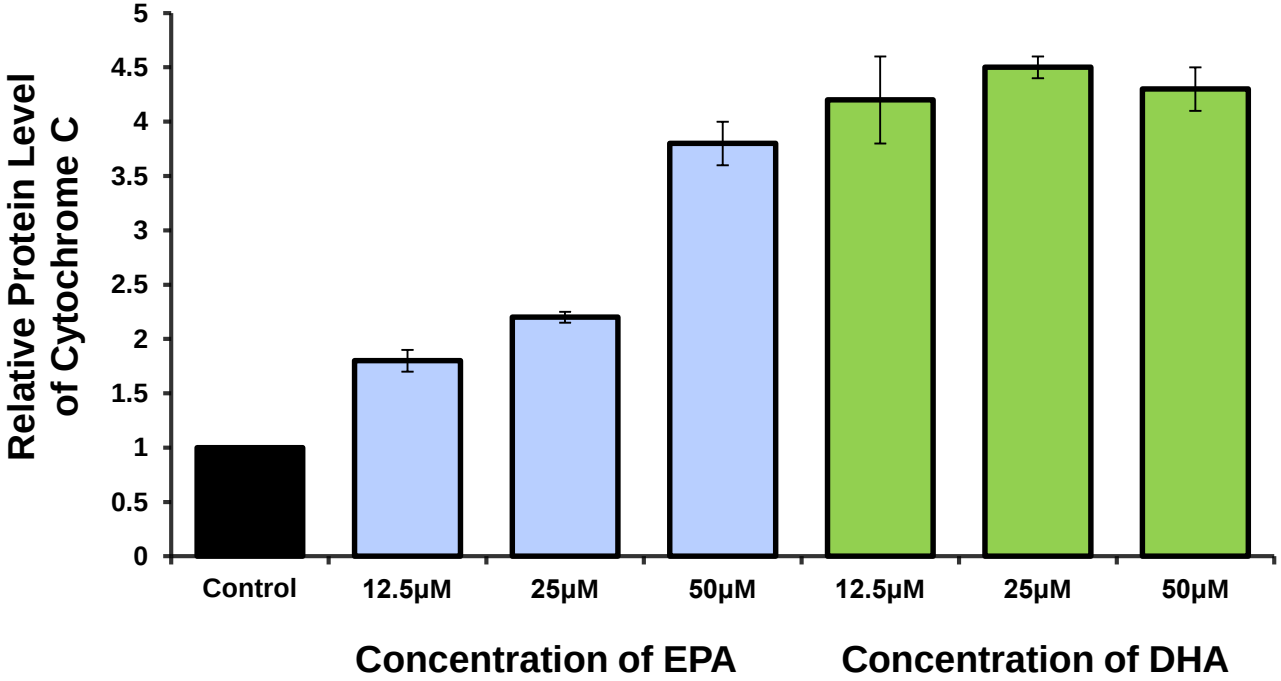


DHA 50 µM

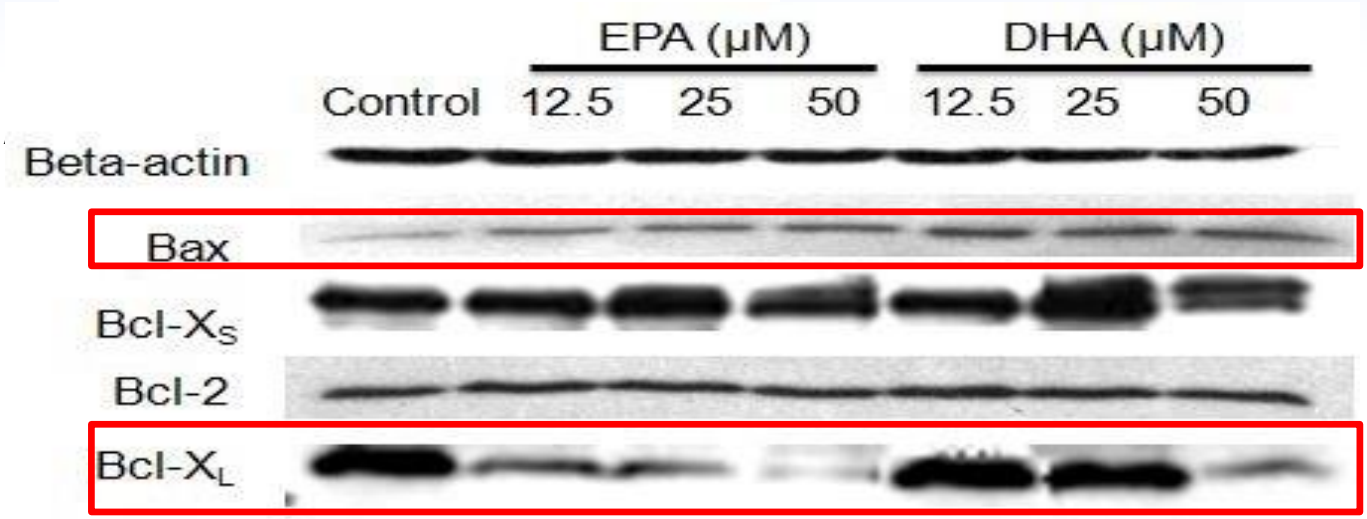


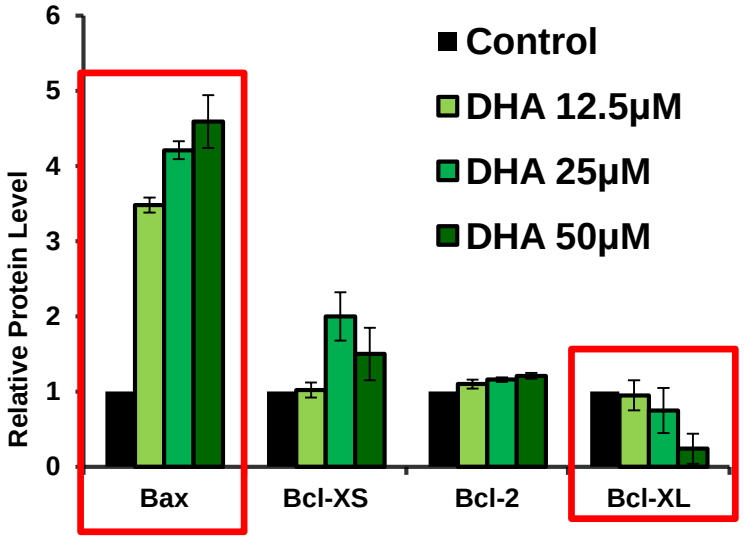
N-3 PUFAs Induced Cytochrome C Release from Mitochondria in LA-N-1 Cells

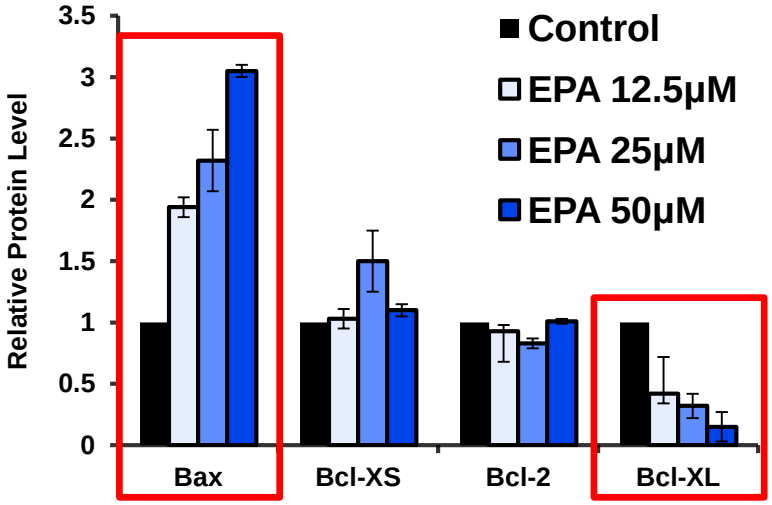




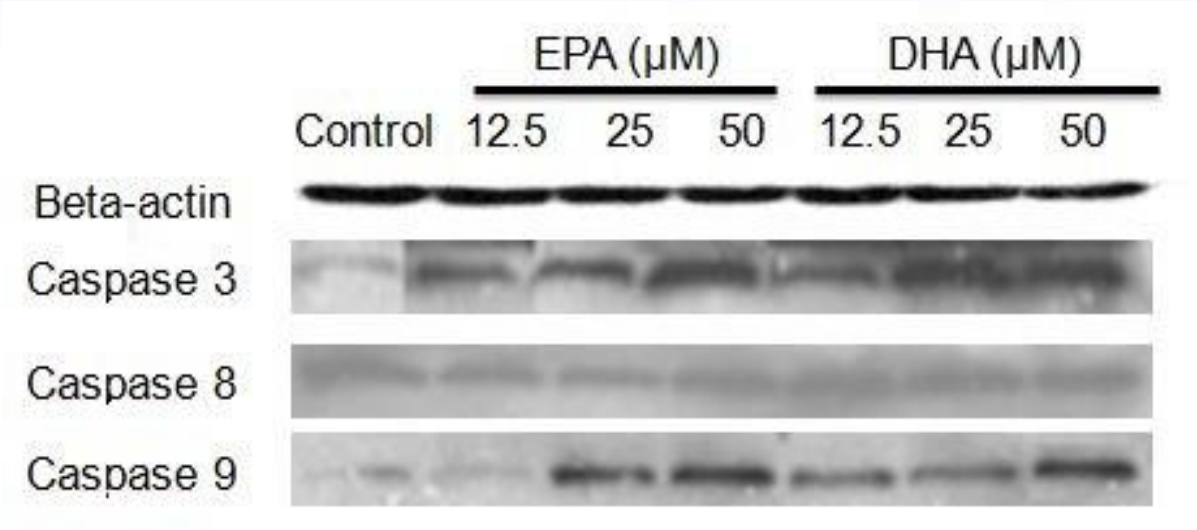
Expression of Apoptosis-related Proteins in N-3 PUFA-treated LA-N-1 Cells

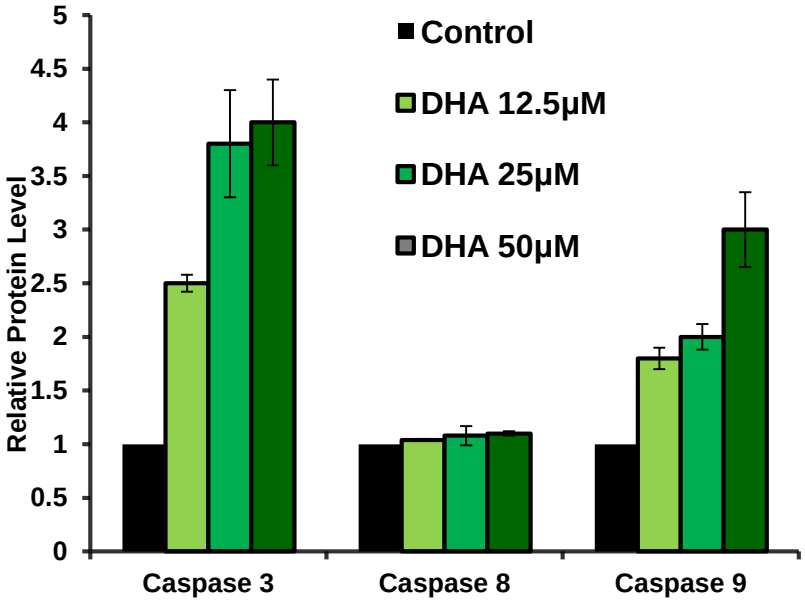


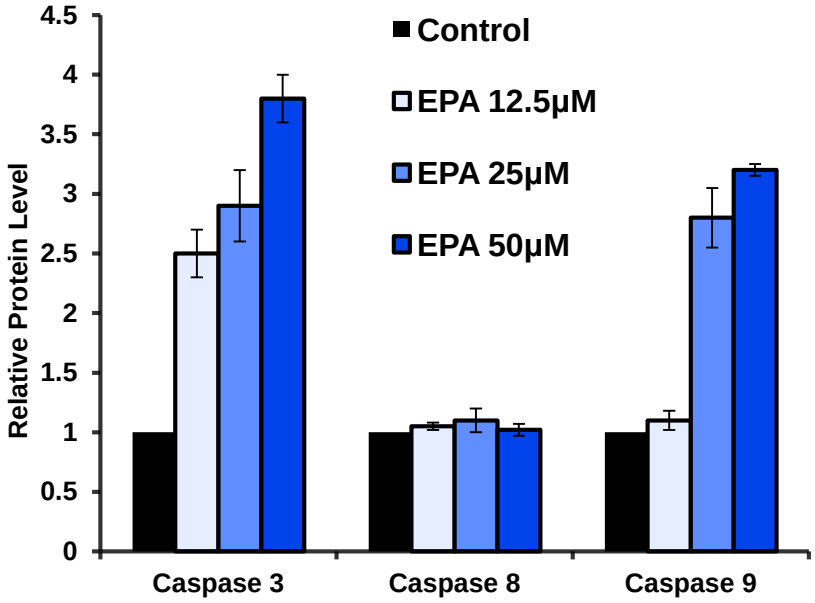




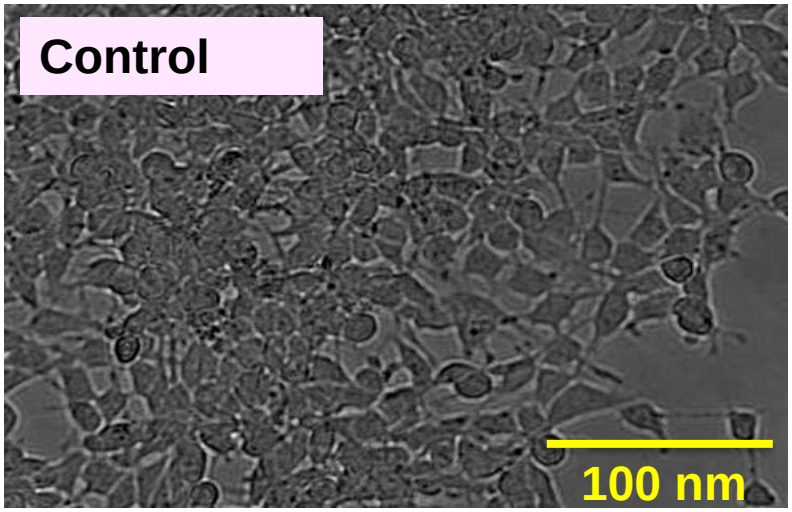
N-3 PUFAs Increased Cleavage of Caspase 3 and 9 in LA-N-1 Cells

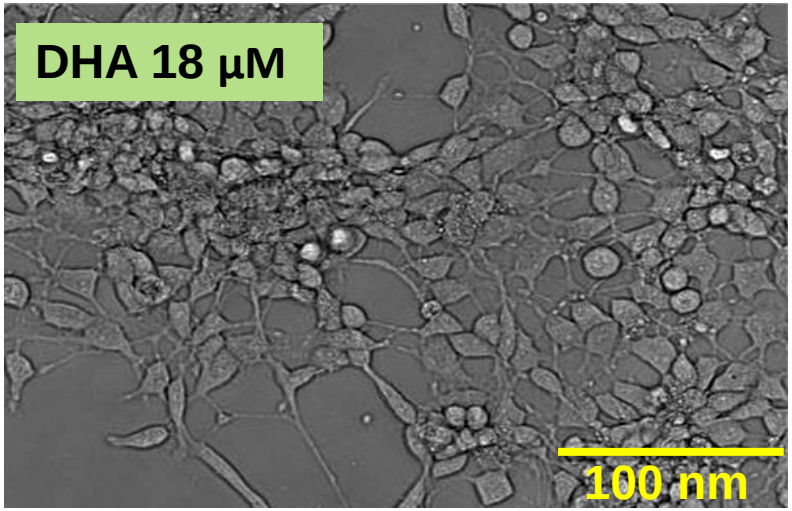


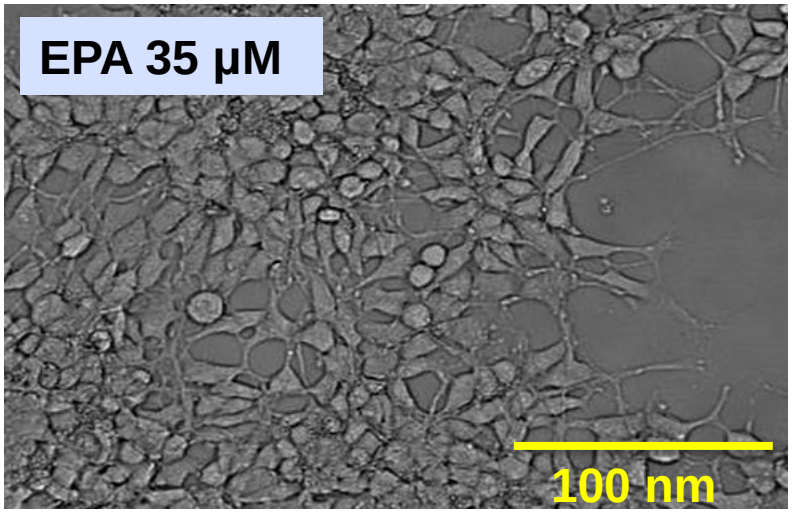


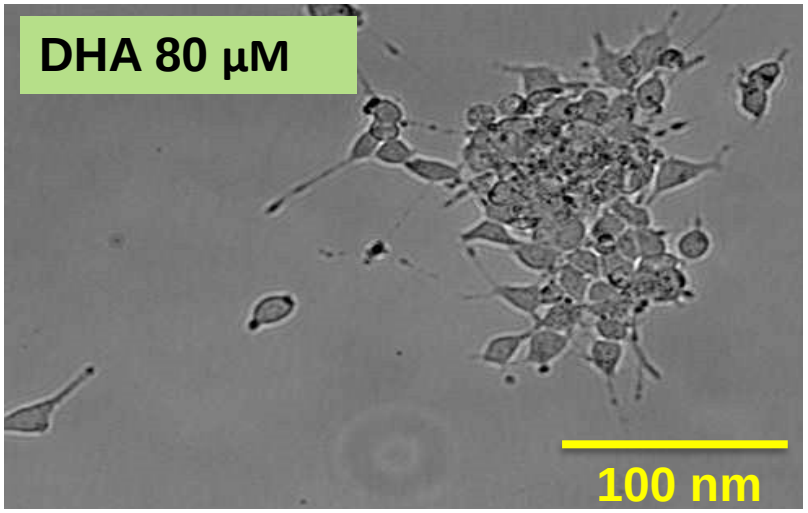


N-3 PUFA Failed to Induce Neuronal Differentiation of LA-N-1 Cells

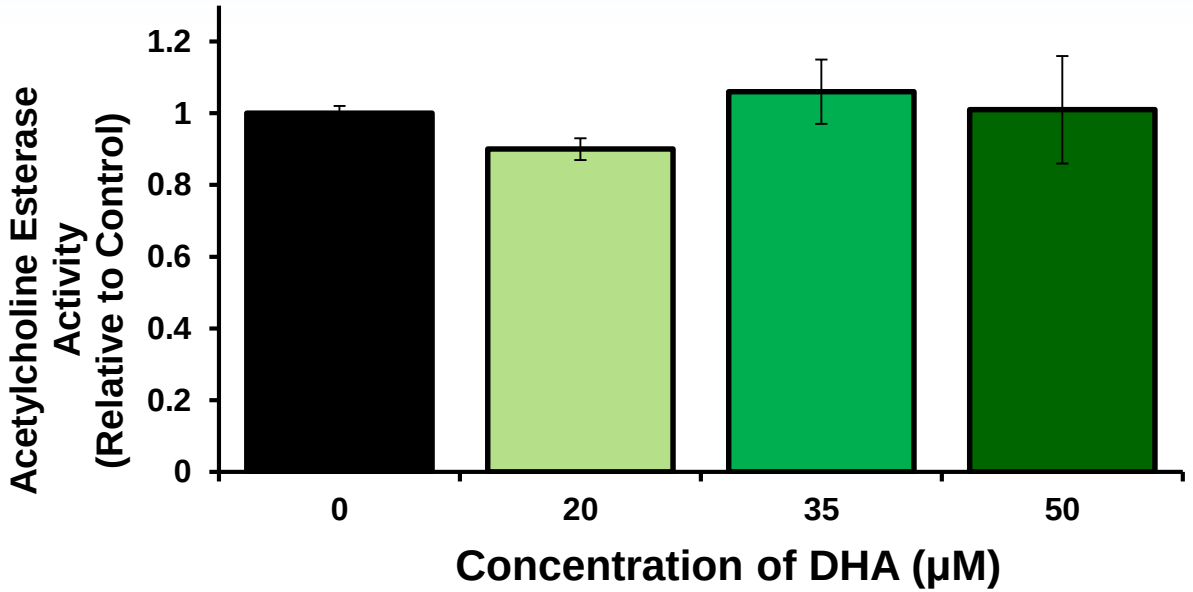


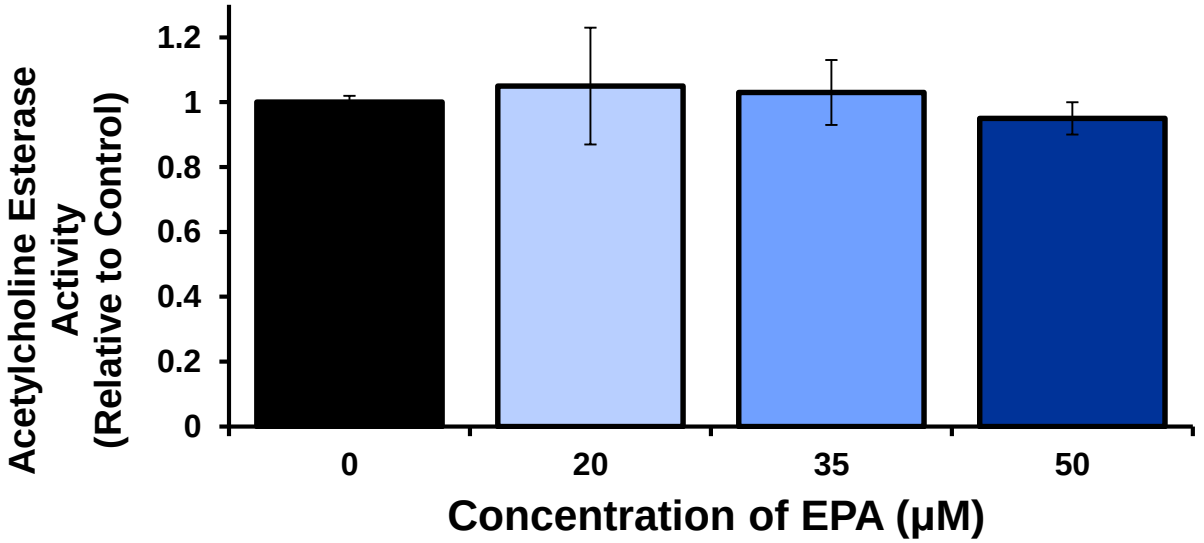




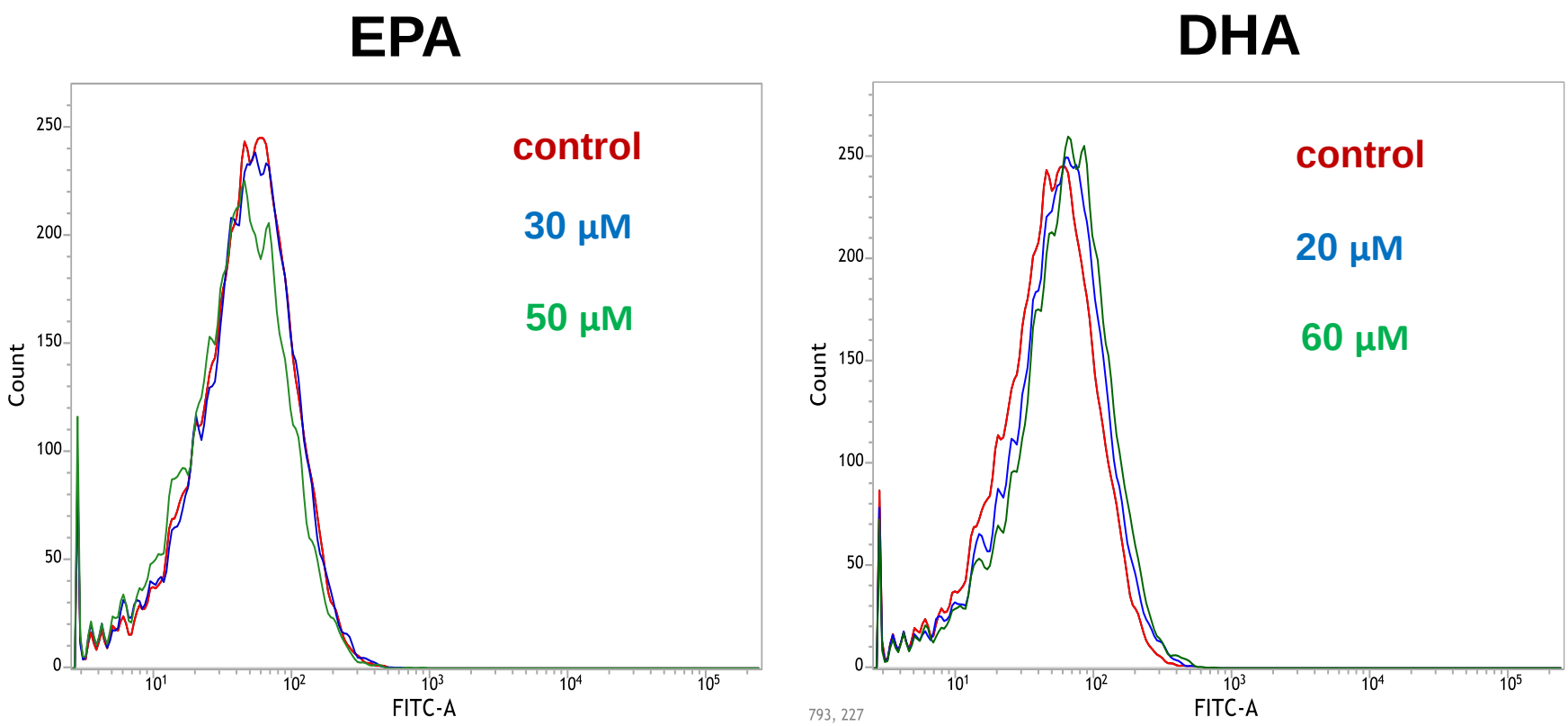


N-3 PUFA Did Not Induce Acetylcholine Esterase Activity in LA-N-1 Cells



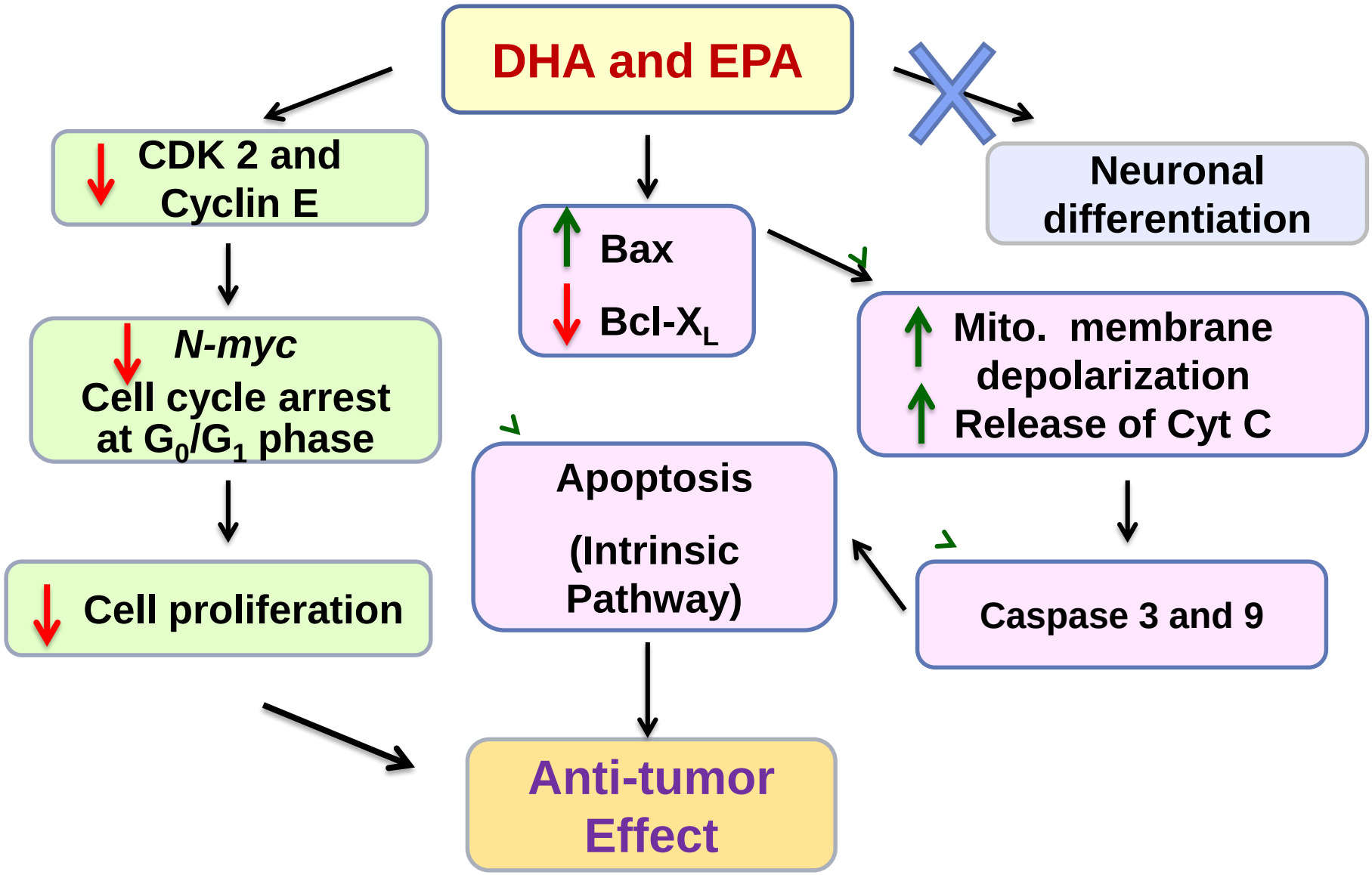


DHA and EPA Did Not Enhance the Generation of ROS in LA-N-1 Cells



Using DCFDA dye for staining

Summary


DHA and EPA
CDK 2 and Cyclin E
Neuronal differentiation
Bax
Bcl-X$_L$
N-myc
Cell cycle arrest at G$_0$/G$_1$ phase
Mito. membrane depolarization
Release of Cyt C
Apoptosis (Intrinsic Pathway)
Cell proliferation
Caspase 3 and 9
Anti-tumor Effect

Conclusions and Future Perspectives

- N-3 PUFA (DHA and EPA) are potential therapeutic agents for high risk neuroblastoma with *N-myc* amplification
- Do n-3 PUFA possess anti-angiogenic activities, both *in vitro* and *in vivo?*
- *In vivo* studies of n-3 PUFA in animal models (anti-tumor efficacy and acute and chronic toxicities)
- Any synergistic effects when used in combinations with other chemotherapeutic drugs or natural products?

Acknowledgements



Mr. SO Wai-wing (CUHK)
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Ms. Ada KONG Lai-ping (CUHK)
Prof. N.K. MAK (HKBU)
Prof. Z.Y. Chen (CUHK)

Thank you for your attention!

