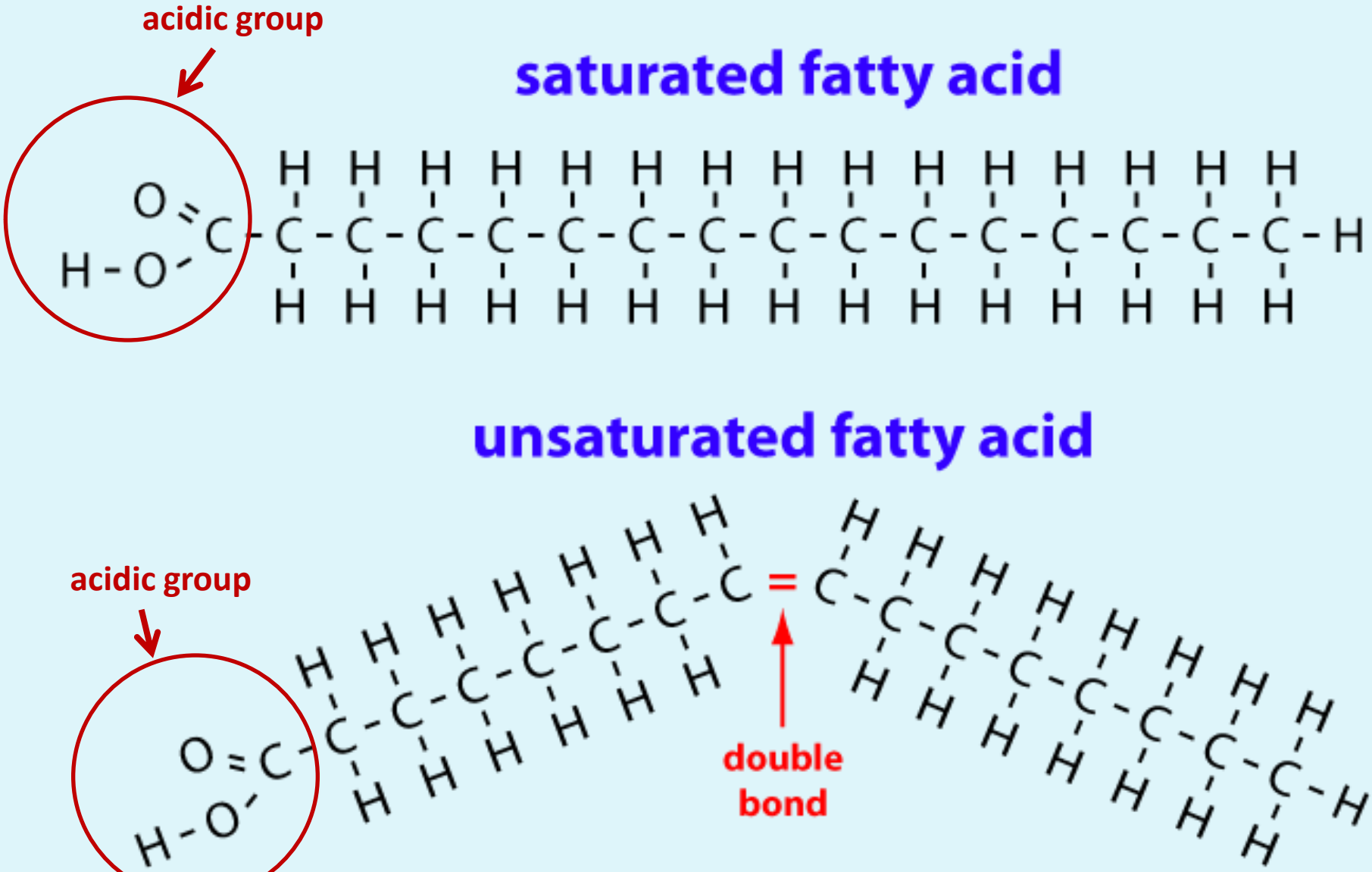
Long Chain Fatty Acids- a Key Component of Cellular Membranes

Biological Stain Commission and the New York State Histotechnological Society 2018

***William E. Grizzle, M.D., Ph.D.**
Dennis Otali, Ph.D.

***Trustee Emeritus of the Biological Stain Commission and Professor of Pathology University of Alabama at Birmingham**
Department of Pathology
Birmingham, AL. wgrizzle@uabmc.edu


acidic group
saturated fatty acid
unsaturated fatty acid
acidic group
double bond

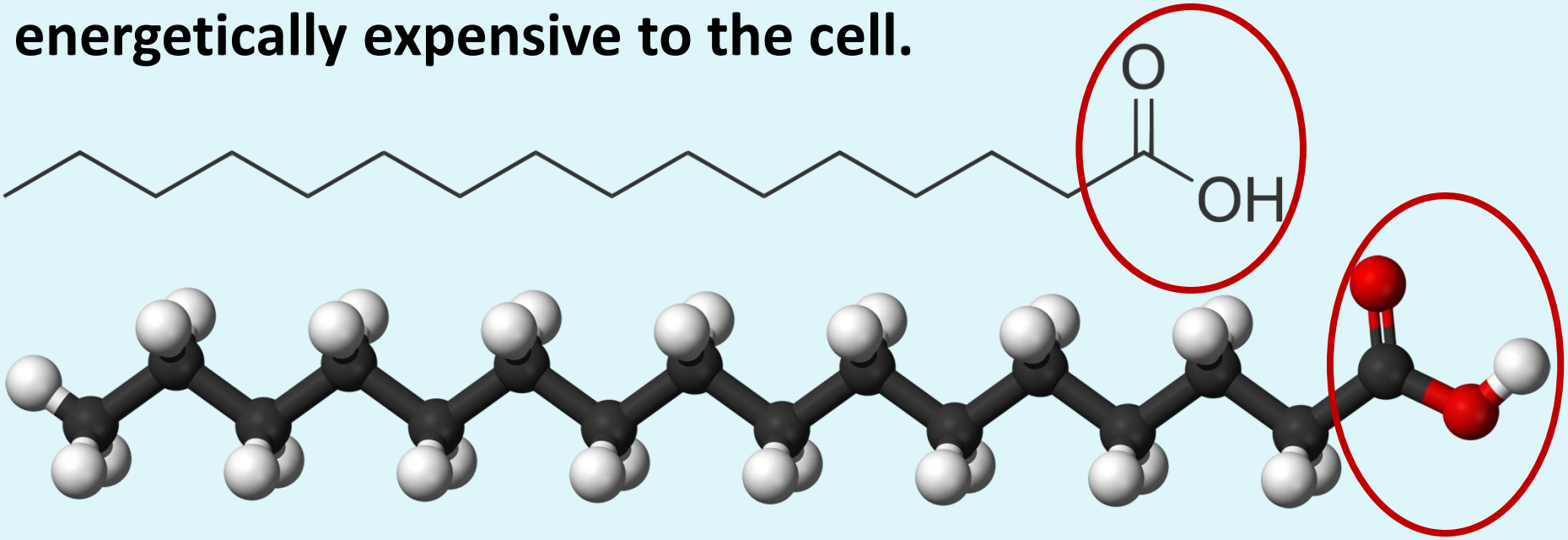
Fatty Acids

- The most common fatty acids.

	Carbon Atoms: Double Bonds	Common Name	Melting Point (°C)
	Saturated Fatty Acids		
Higher mp	12:0	Lauric acid	44
	14:0	Myristic acid	58
	16:0	Palmitic acid	63
	18:0	Stearic acid	70
	20:0	Arachidic acid	77
	Unsaturated Fatty Acids		
Lower mp	16:1	Palmitoleic acid	1
	18:1	Oleic acid	16
	18:2	Linoleic acid	-5
	18:3	Linolenic acid	-11
	20:4	Arachidonic acid	-49

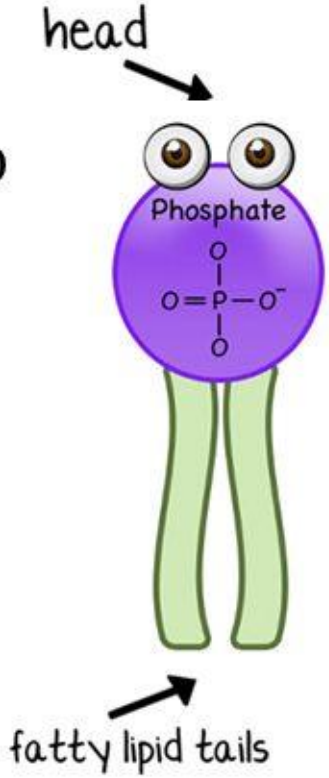
16 carbon atom fatty acid –palmitic acid

The acid group of the fatty acid (red circle) is the only active binding site. When a molecule binds to this group, the name changes, for example, from palmitic acid to palmitate. Also, during synthesis, which begins with the acid group, each pair of methyl groups (-CH_2- or –CH_3) added to the length of the fatty acid chain is very energetically expensive to the cell.



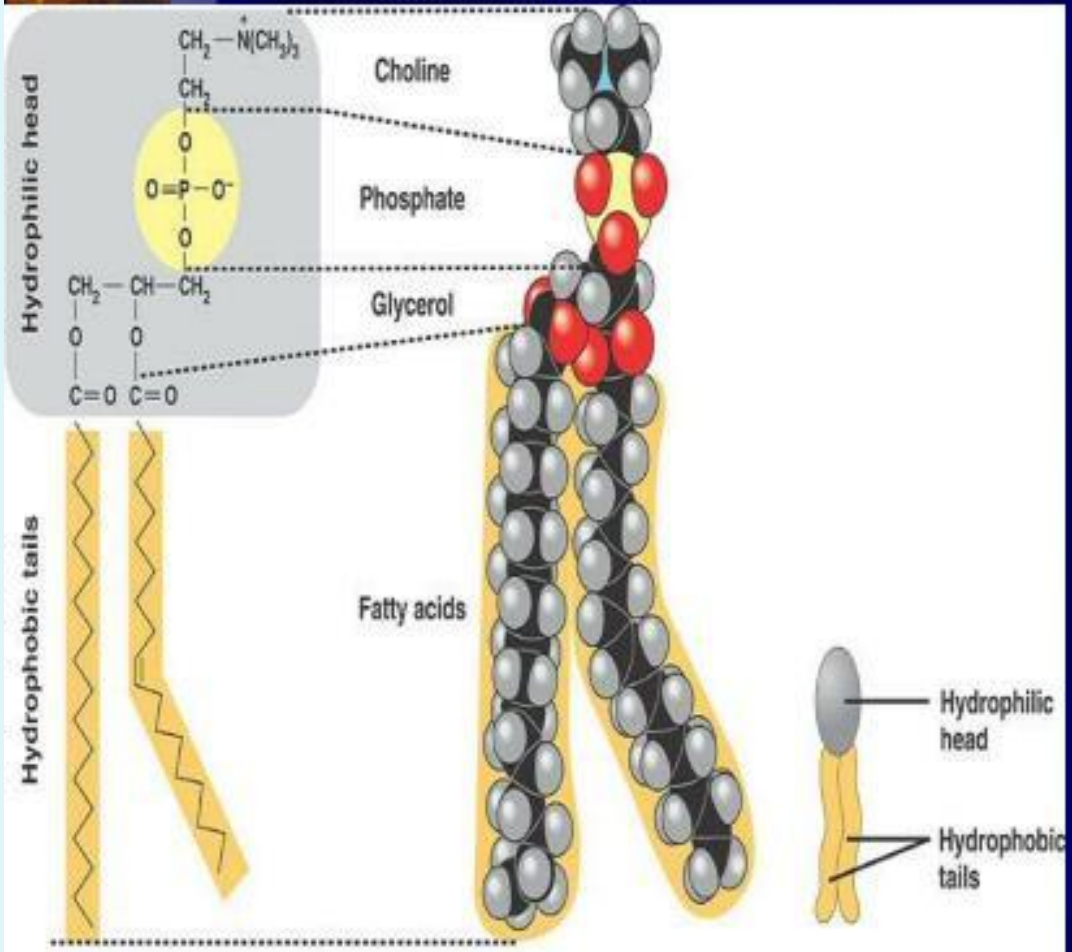
Phospholipid

- Cell membranes are made up of a lipid similar to triglycerides; a **phospholipid**
- Structure: Glycerol backbone attached to a phosphate group "**head**" and 2 fatty acid "**tails**"
  - The tails can be saturated or unsaturated
  - (*Animation*) **About 80% of the way down…**
- Function: Provide a selective **barrier** between the inside and outside of the cell and cellular compartments.



http://media1.shmoop.com/images/module_images/PhospholipidFlashcard_640x480.jpg

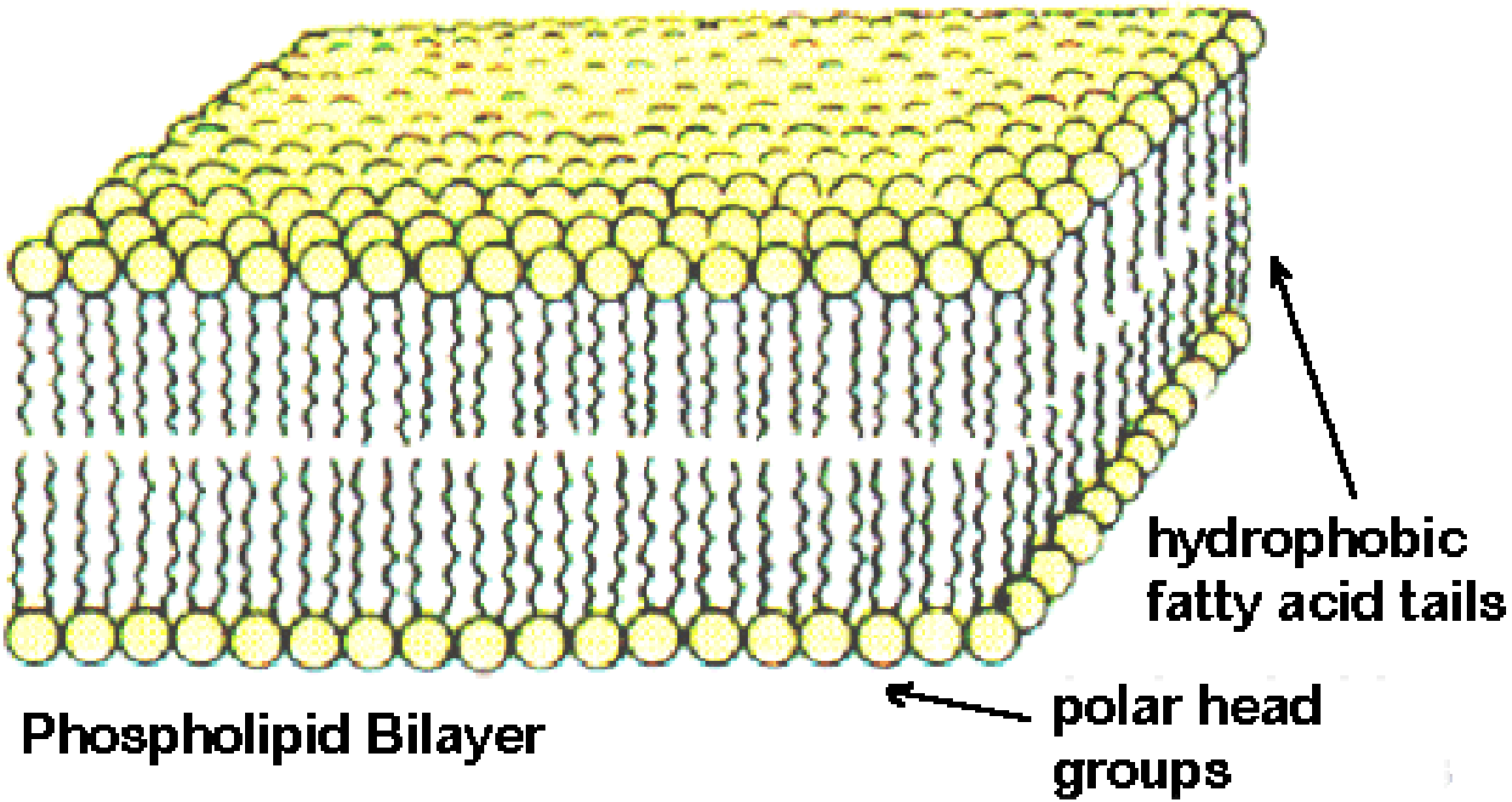
Phospholipids – cell membrane component



Phospholipids: fatty acids bound to glycerol, a phosphate group replaces one fatty acid.

Phosphate group is hydrophilic—"water loving head"

"Water fearing tails" are fatty acid chains—hydrophobic


hydrophobic
fatty acid tails
polar head
groups
Phospholipid Bilayer

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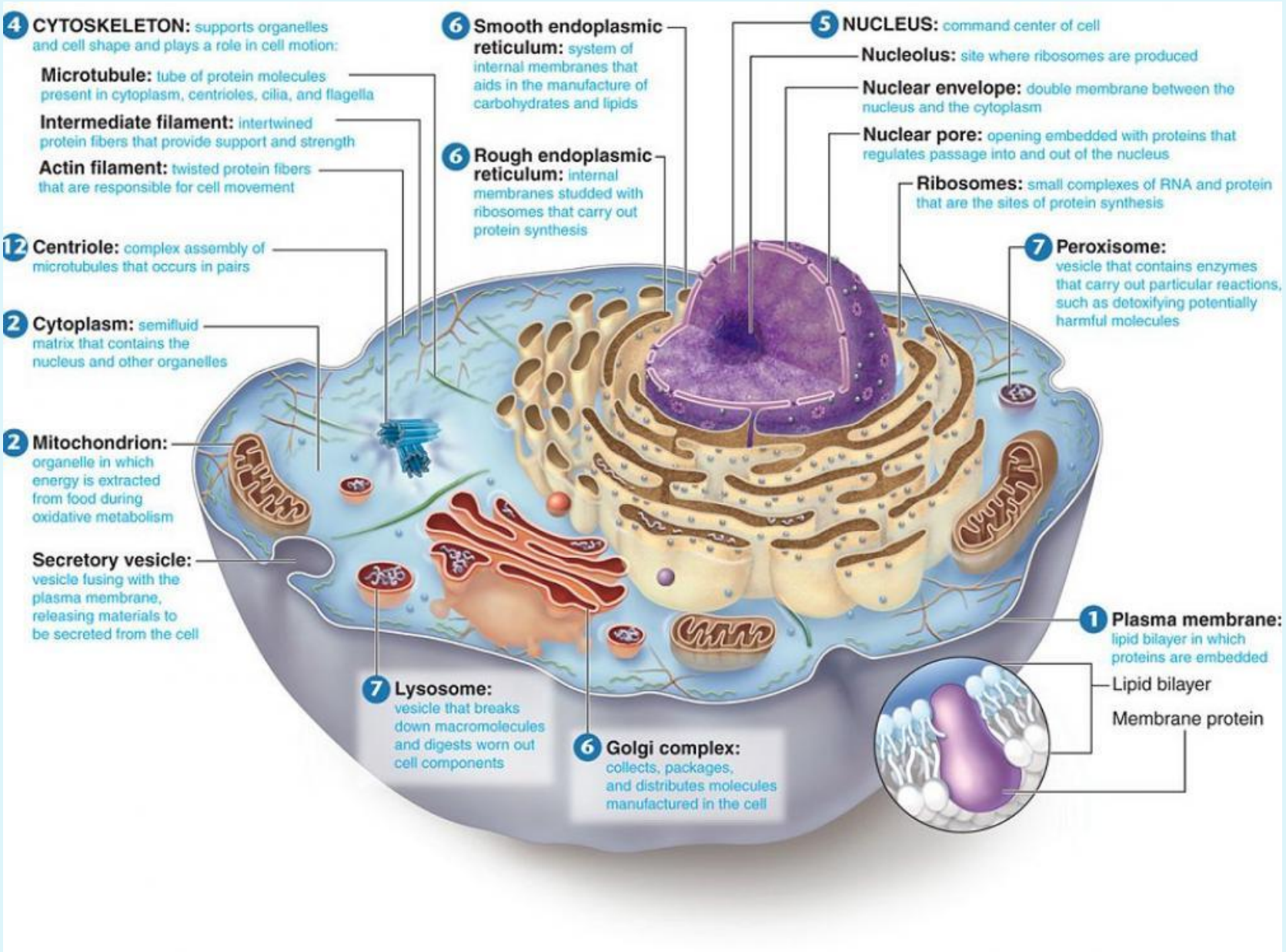
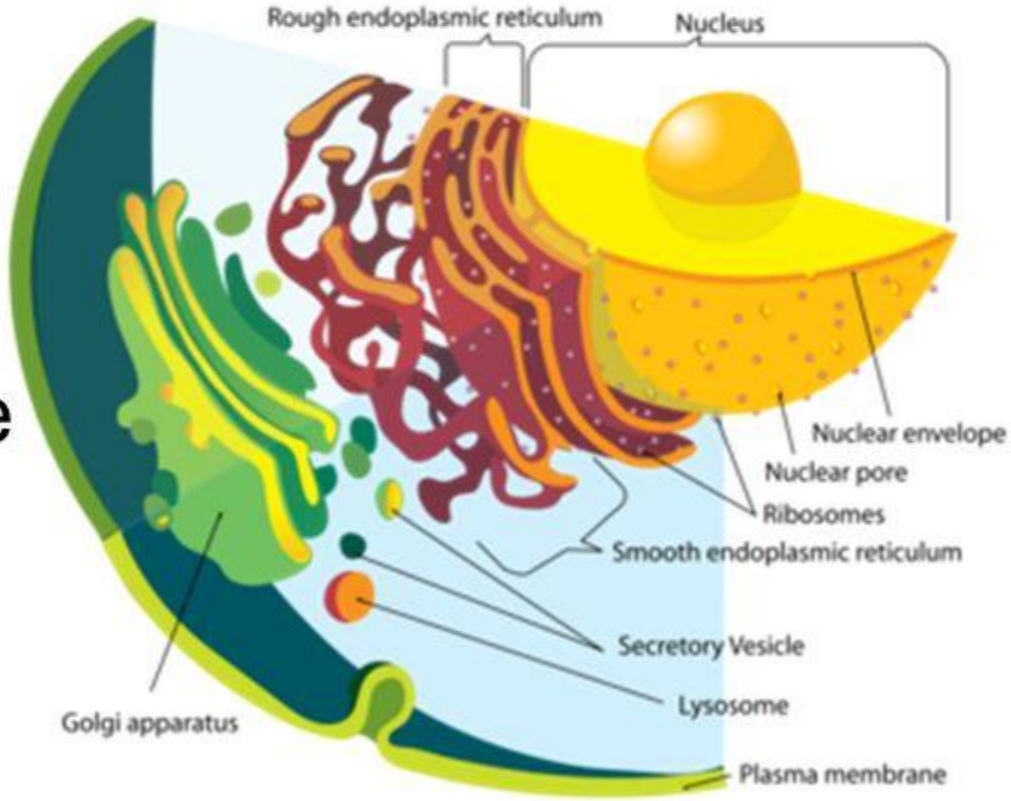


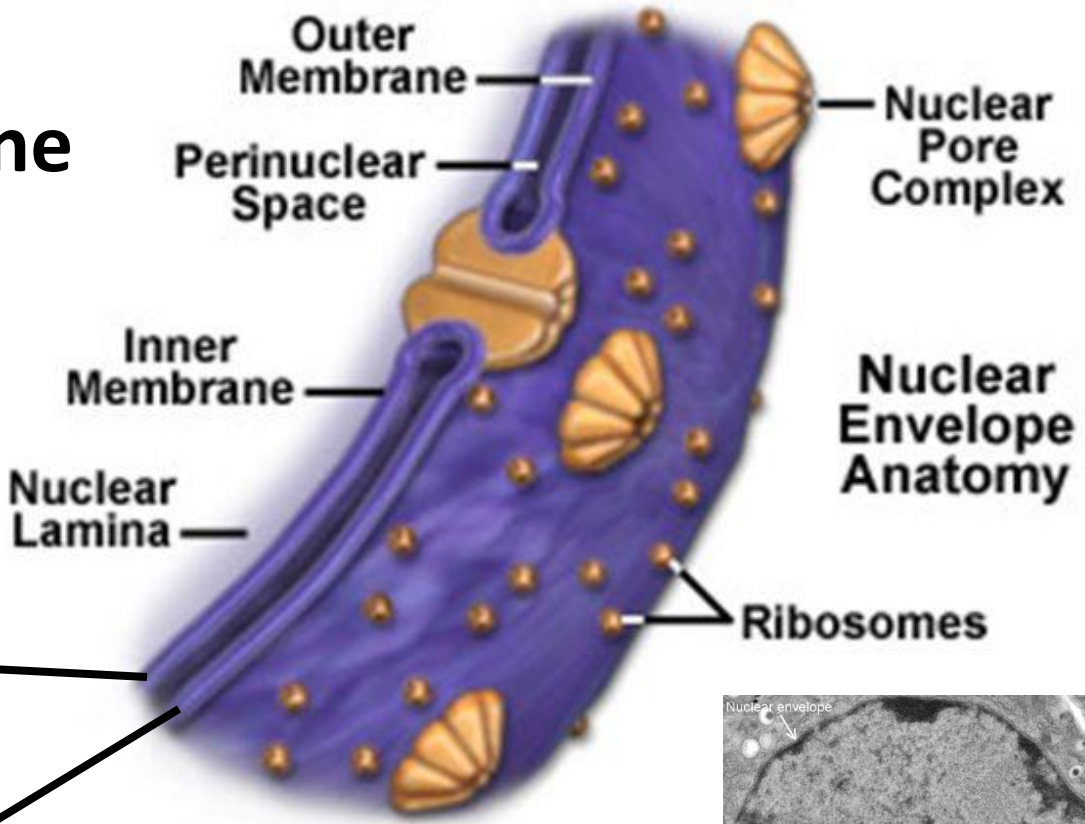
The ENDOPLASMIC SYSTEM

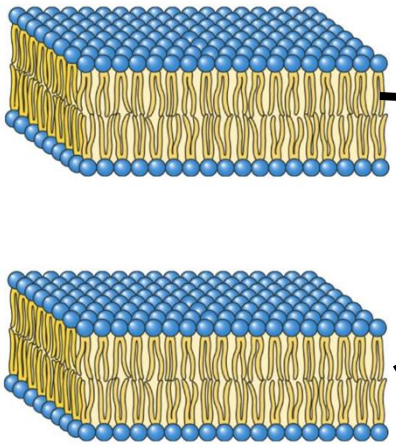
- The nuclear envelope
- Endoplasmic reticulum
- Golgi apparatus
- Lysosomes
- Vacuoles
- Plasma membrane

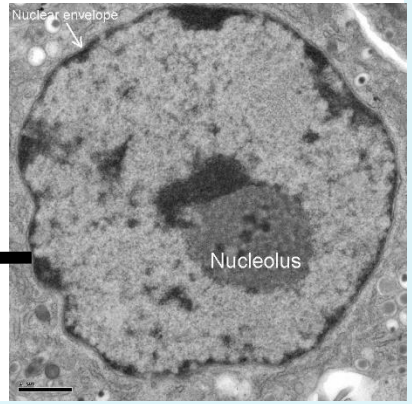


Nuclear Envelope

- Also called the nuclear **membrane**
- Double membrane
- Porous

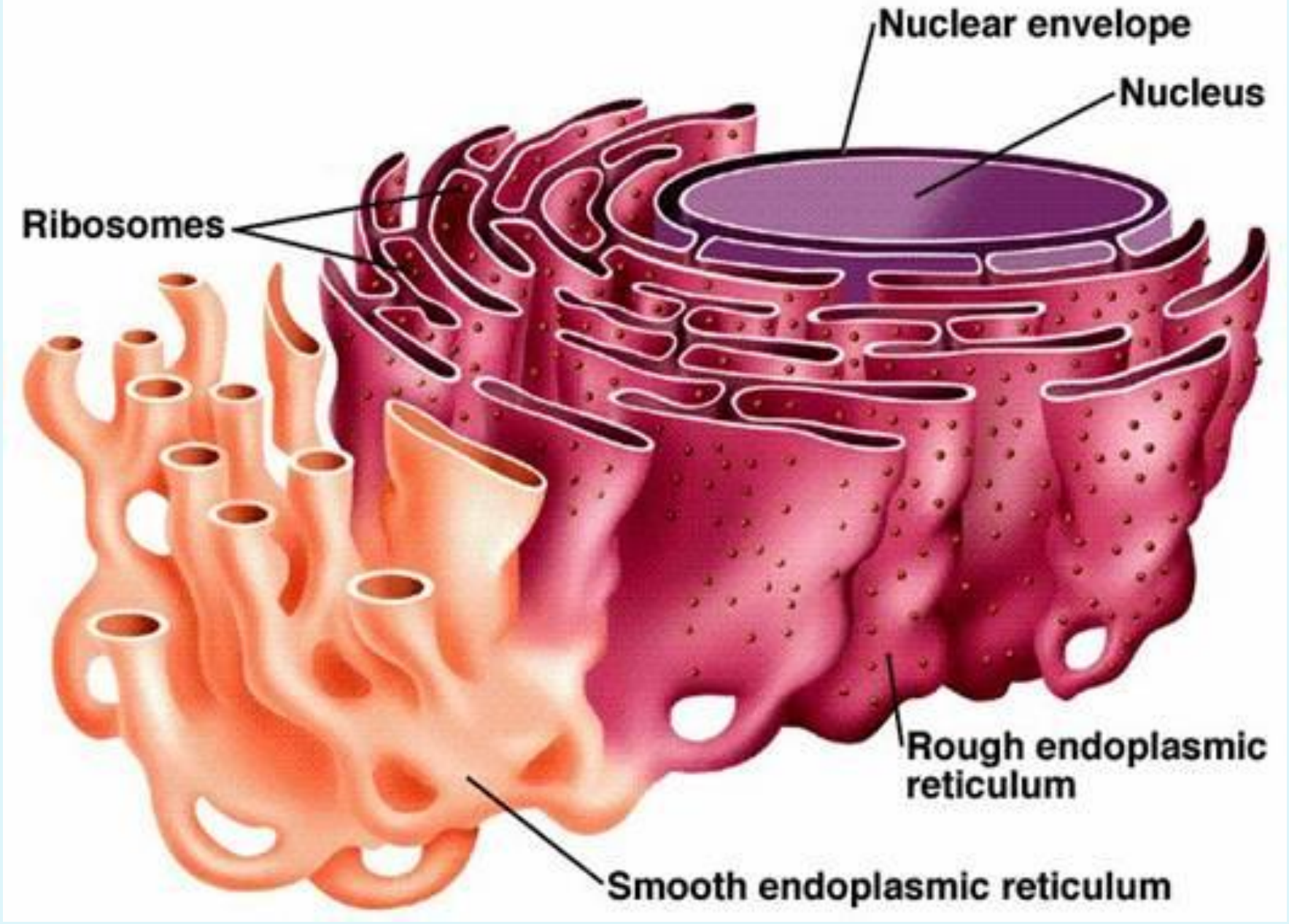






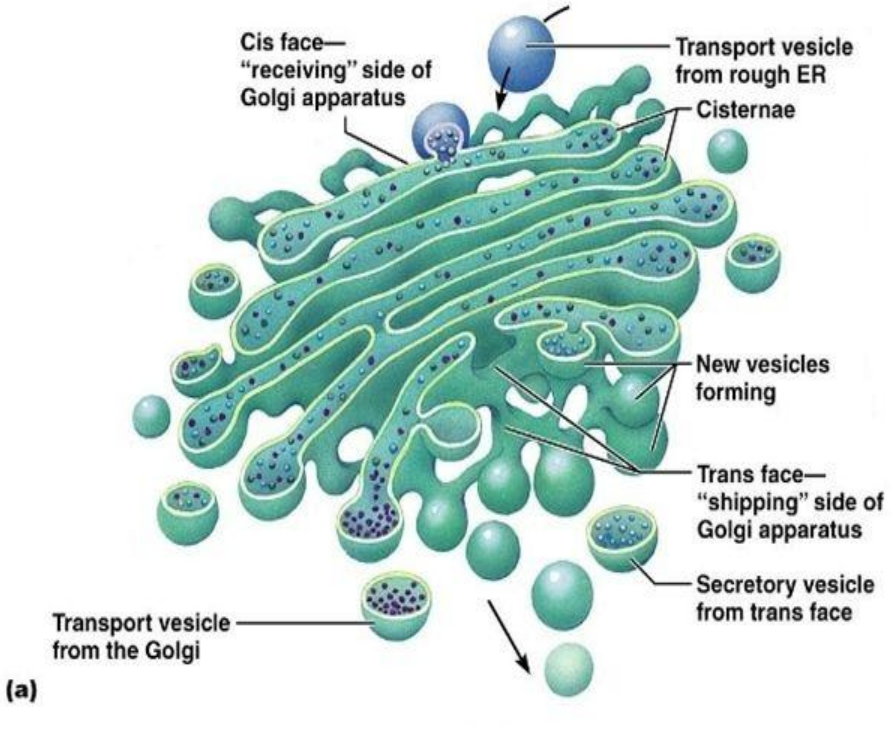
Nuclear Envelope

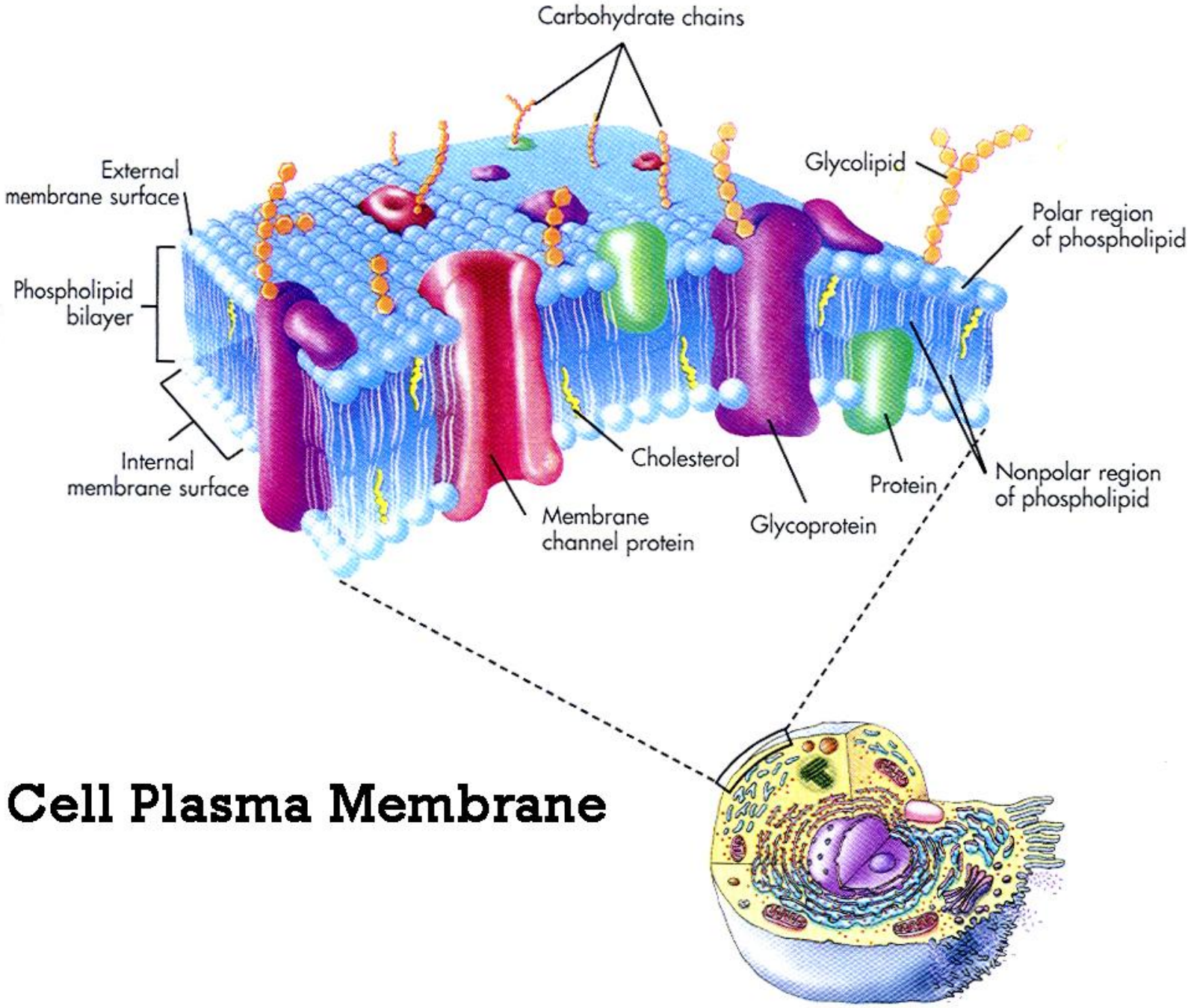
SOME MEMBRANE SYSTEMS OF ANIMAL CELLS



Golgi Apparatus

- Modifies proteins by adding signaling sugars onto surface of protein.
- Unmodified protein arrives at Golgi inside a transport vesicle.
- Fuses with Golgi and is modified as it travels through Golgi
- Golgi membrane pinches off with modified protein inside.





Cell Plasma Membrane

- **Palmitate is one of the major fatty acid components of the human body; it is especially high in adipocytes and in cell membranes.**

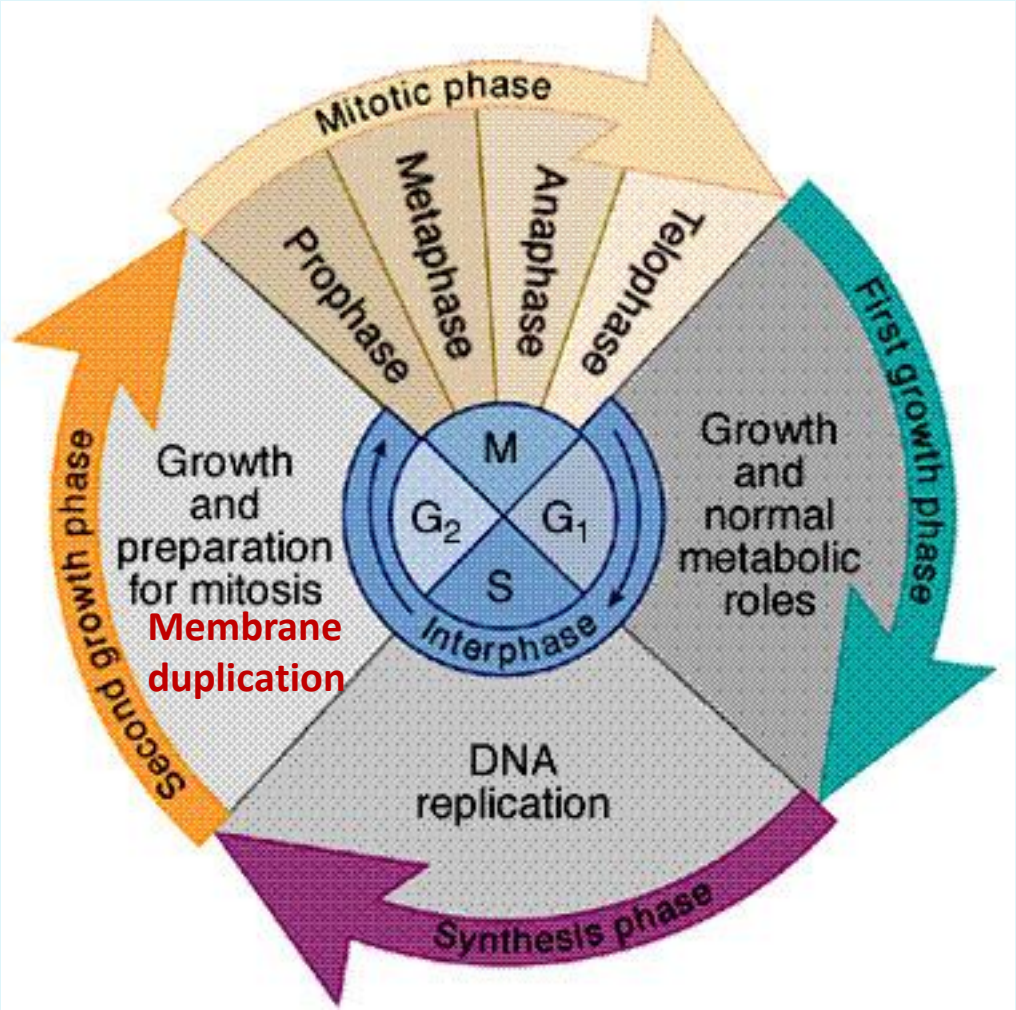
- **The fatty acid composition of cell membranes varies widely with diet and other factors. Specifically, the composition of the membranes of cells can change during culture when different fatty acid salts are provided in the media.**

- **Fatty acids and their components can be absorbed from the diet or can be synthesized endogenously.**

- **Long chain fatty acids are synthesized in cells by fatty acid synthase (FASN). This synthesis is very energy-expensive for each carbon addition to the growing fatty acid chain.**

- **Palmitic acid (16 – carbon atoms) is the main fatty acid synthesized by FASN.**

- **Because fatty acids are required for construction of all cell membranes, fatty acids are required by all cells, especially rapidly proliferating cells.**



Our studies of the involvement of fatty acids in cancer utilizes prostate cancer as a model.

- **For a cell to proliferate, all cell membranes must be duplicated prior to mitosis. This requires high levels of fatty acids.**

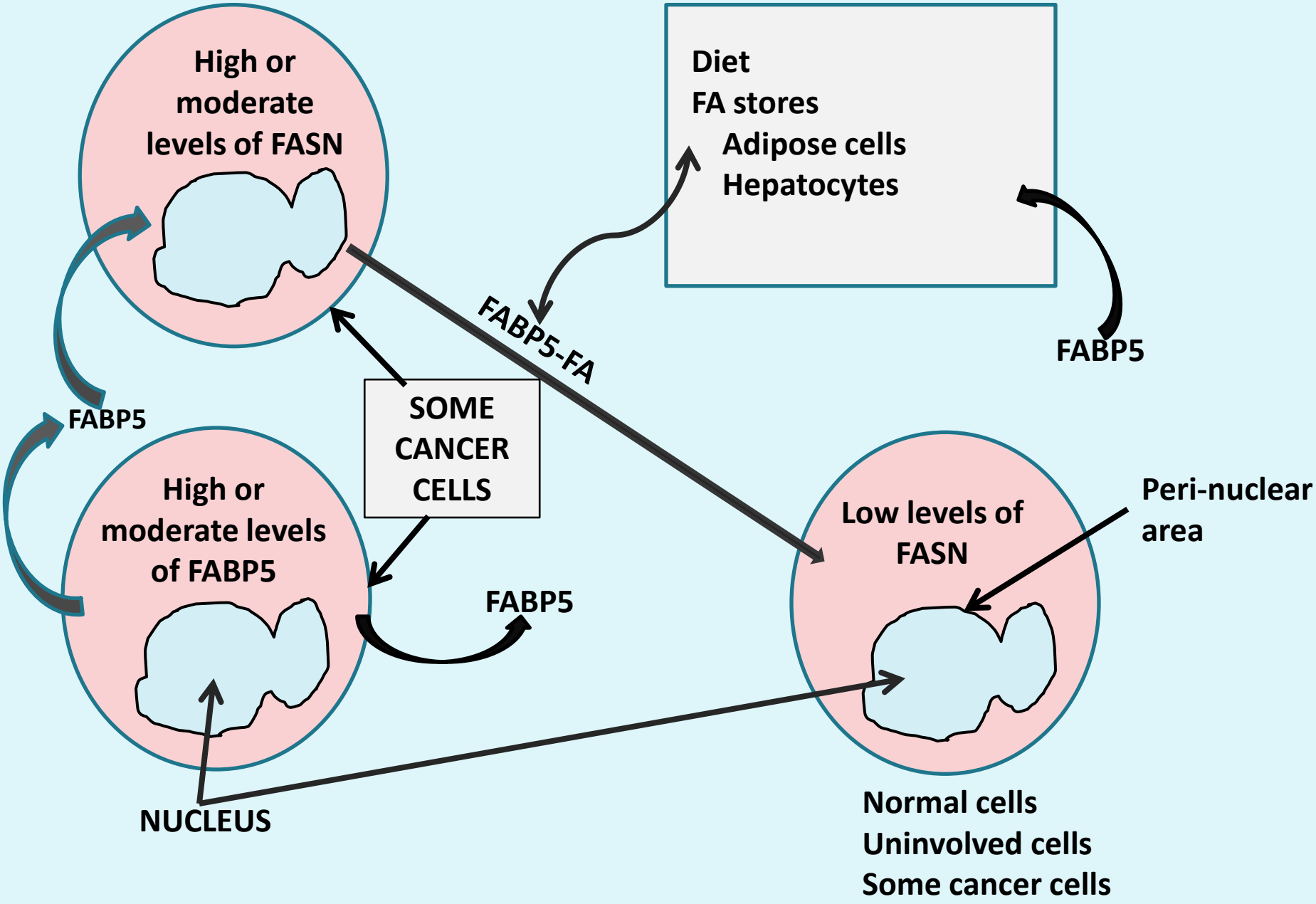
- **Thus, cancers require an increased amount of fatty acids to grow rapidly; this has been confirmed by many studies which have found that high levels of FASN are correlated with tumor aggressiveness.**

- **Less proliferative cells (e.g., normal cells) require less fatty acids for which adequate levels may be provided by diet.**

- **Fatty acids (FA) have limited solubility and must be transported to, into, and out of cells by a carrier protein.**

- **The carrier proteins for fatty acids vary**
- **with cell type.**

- **Our laboratory has been focused on the carrier protein, epidermal fatty acid binding protein (e-FABP) or fatty acid binding protein 5 (FABP5) which has variable expression in prostate cancer.**

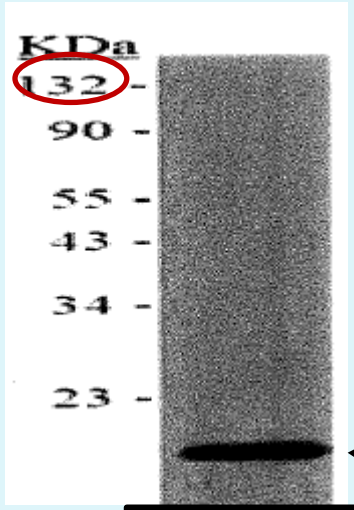

High or moderate levels of FASN
Diet
FA stores
Adipose cells
Hepatocytes
FABP5-FA
FABP5
FABP5
SOME CANCER CELLS
High or moderate levels of FABP5
FABP5
Low levels of FASN
Peri-nuclear area
NUCLEUS
Normal cells
Uninvolved cells
Some cancer cells

As part of our effort to study ad understand fatty acids in prostate cancer we need to study FABP5 and FASN.

Problems in Immunohistochemistry

A. Tissues
B. Primary antibodies
C. Secondary detection system
D. Performance of assay (testand standard operating procedures
E. Controls
F. Evaluating immunohistochemistry
G. Problem solving

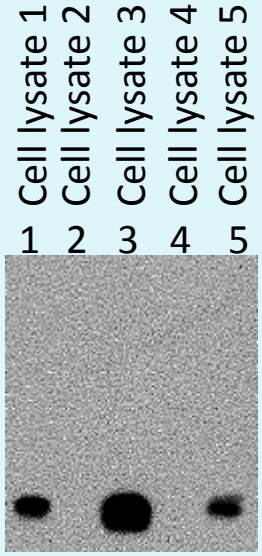
Western blot analysis of FABP5 MW= 15KDa

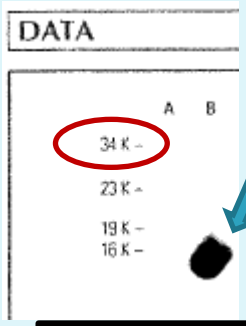


Company 1

Polyclonal antibody

15 KDa





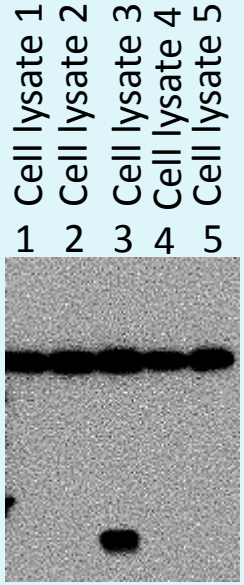
Company 2

Cell line A transfected with FABP5 as in B

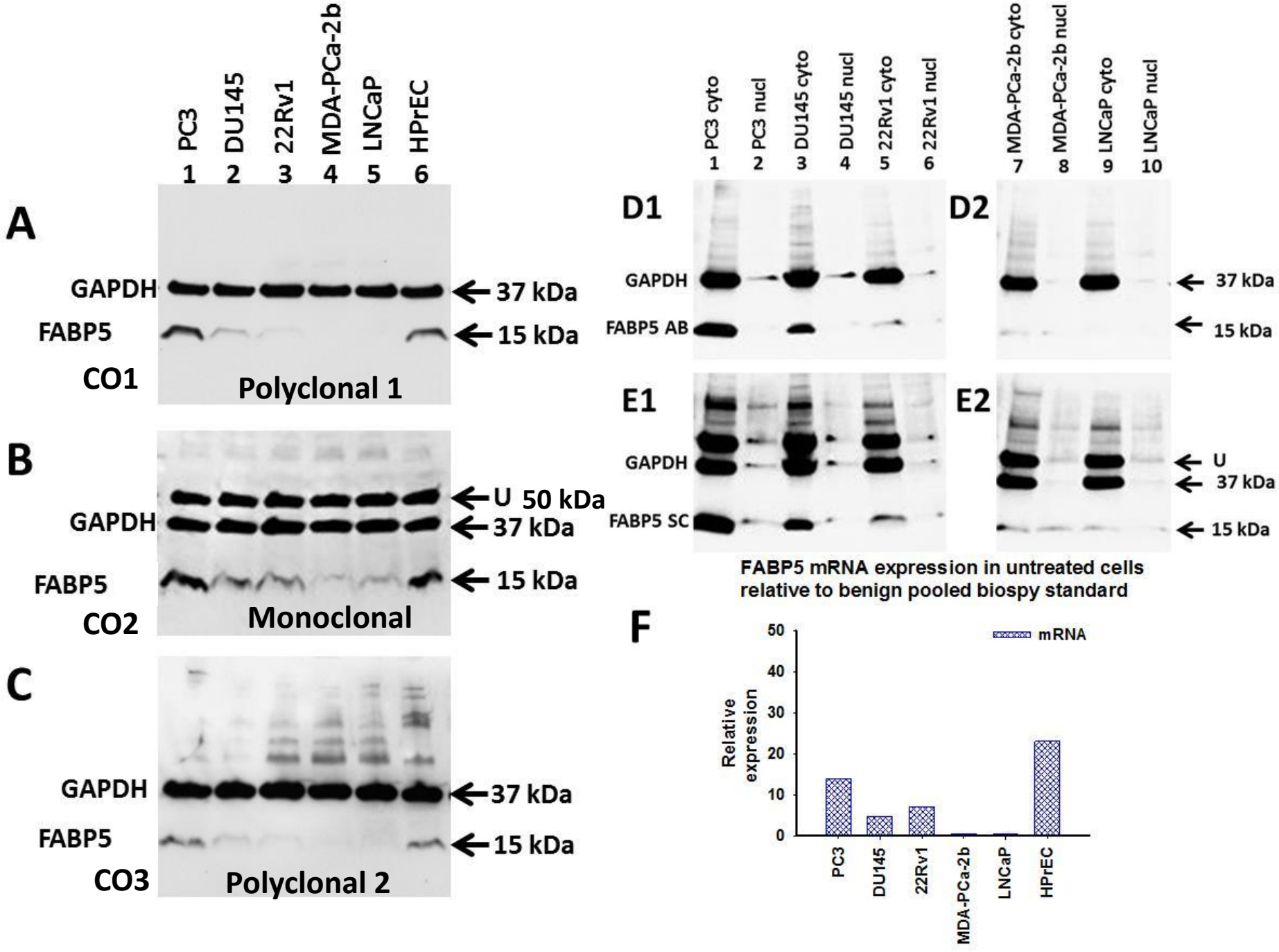
50 KDa

Monoclonal antibody

15 KDa



Will this band affect specificity in IHC


PC3
DU145
22Rv1
MDA-PCa-2b
LNCaP
HPrEC
1
2
3
4
5
6
A
GAPDH
FABP5
CO1
Polyclonal 1
37 kDa
15 kDa
B
GAPDH
FABP5
CO2
Monoclonal
U 50 kDa
37 kDa
15 kDa
C
GAPDH
FABP5
CO3
Polyclonal 2
37 kDa
15 kDa
PC3 cyto
PC3 nucl
DU145 cyto
DU145 nucl
22Rv1 cyto
22Rv1 nucl
MDA-PCa-2b cyto
MDA-PCa-2b nucl
LNCaP cyto
LNCaP nucl
1
2
3
4
5
6
7
8
9
10
D1
GAPDH
FABP5 AB
D2
37 kDa
15 kDa
E1
GAPDH
FABP5 SC
E2
U
37 kDa
15 kDa
FABP5 mRNA expression in untreated cells
relative to benign pooled biospy standard
F
mRNA
Relative expression
50
40
30
20
10
0
PC3
DU145
22Rv1
MDA-PCa-2b
LNCaP
HPrEC

LNCAP PROSTATE CANCER CELLS STAINED BY A RABBIT POLYCLONAL ANTIBODY TO FABP5

Rabbit polyclonal dilution 1/400

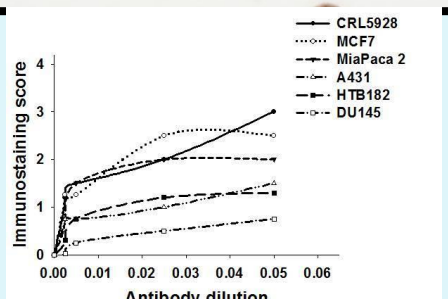
Rabbit polyclonal dilution 1/300

Rabbit polyclonal dilution 1/200

Rabbit polyclonal dilution 1/100

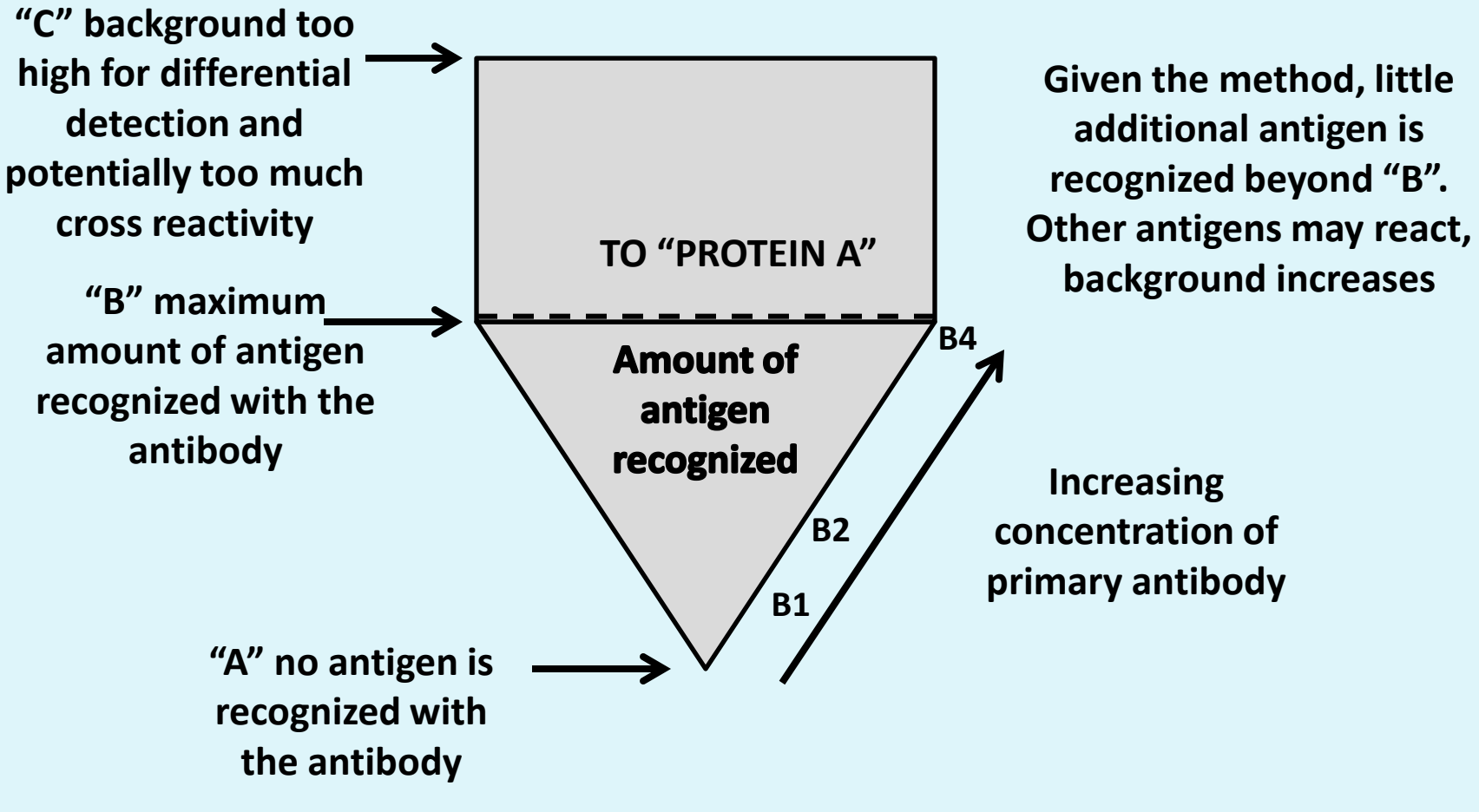
Rabbit polyclonal dilution 1/50

Rabbit polyclonal dilution 1/10

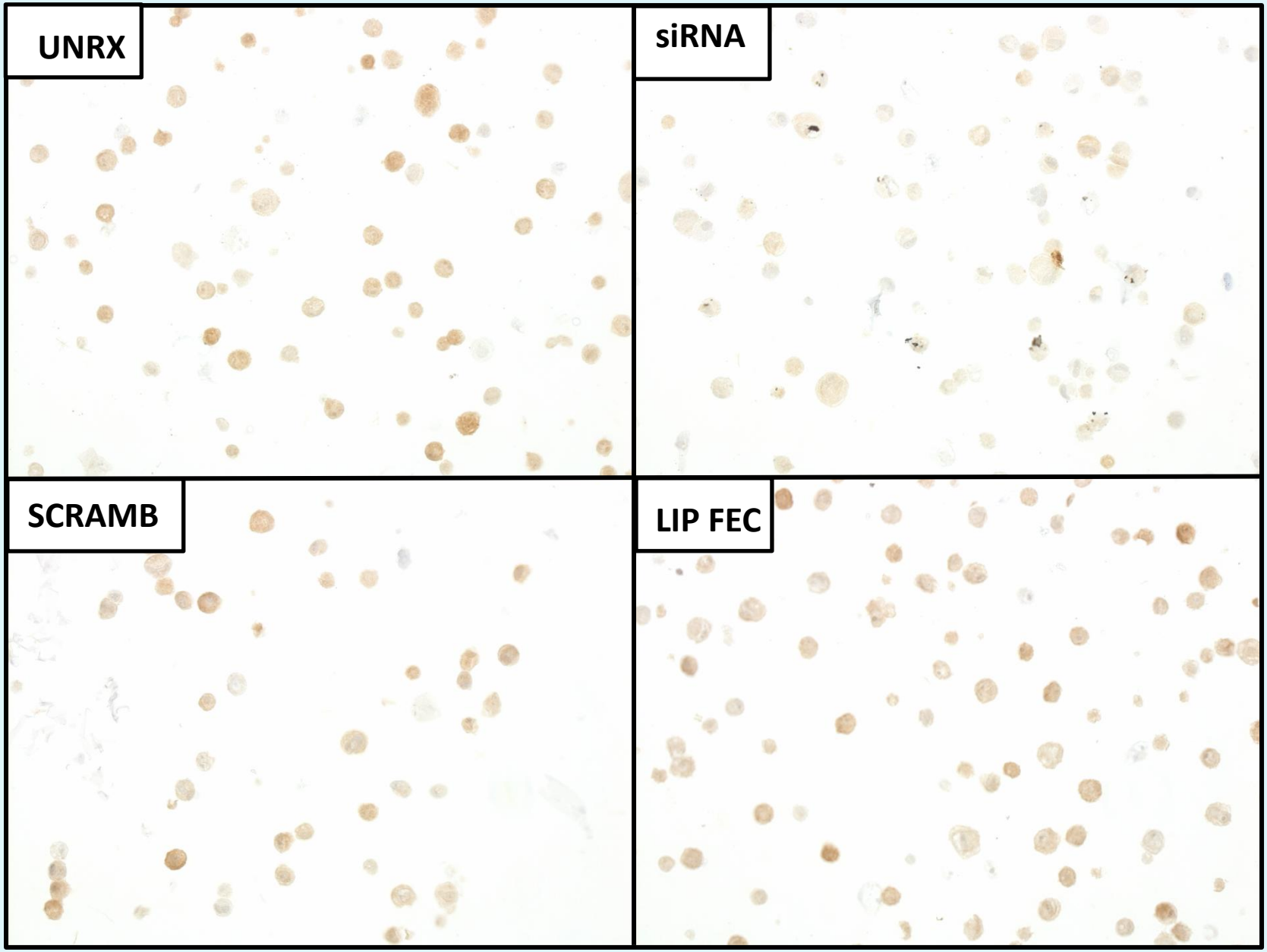


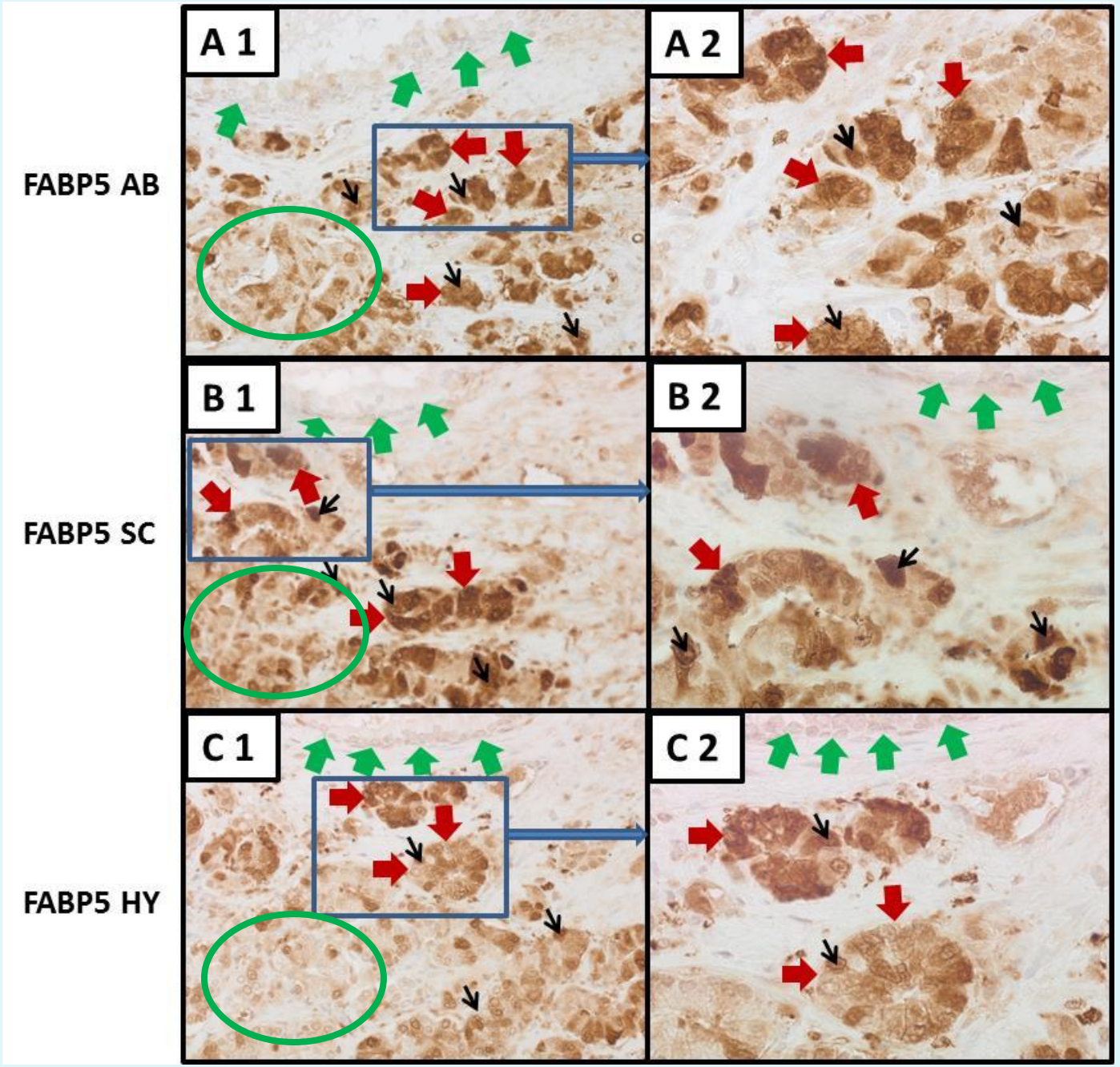
Problems in Immunohistochemistry

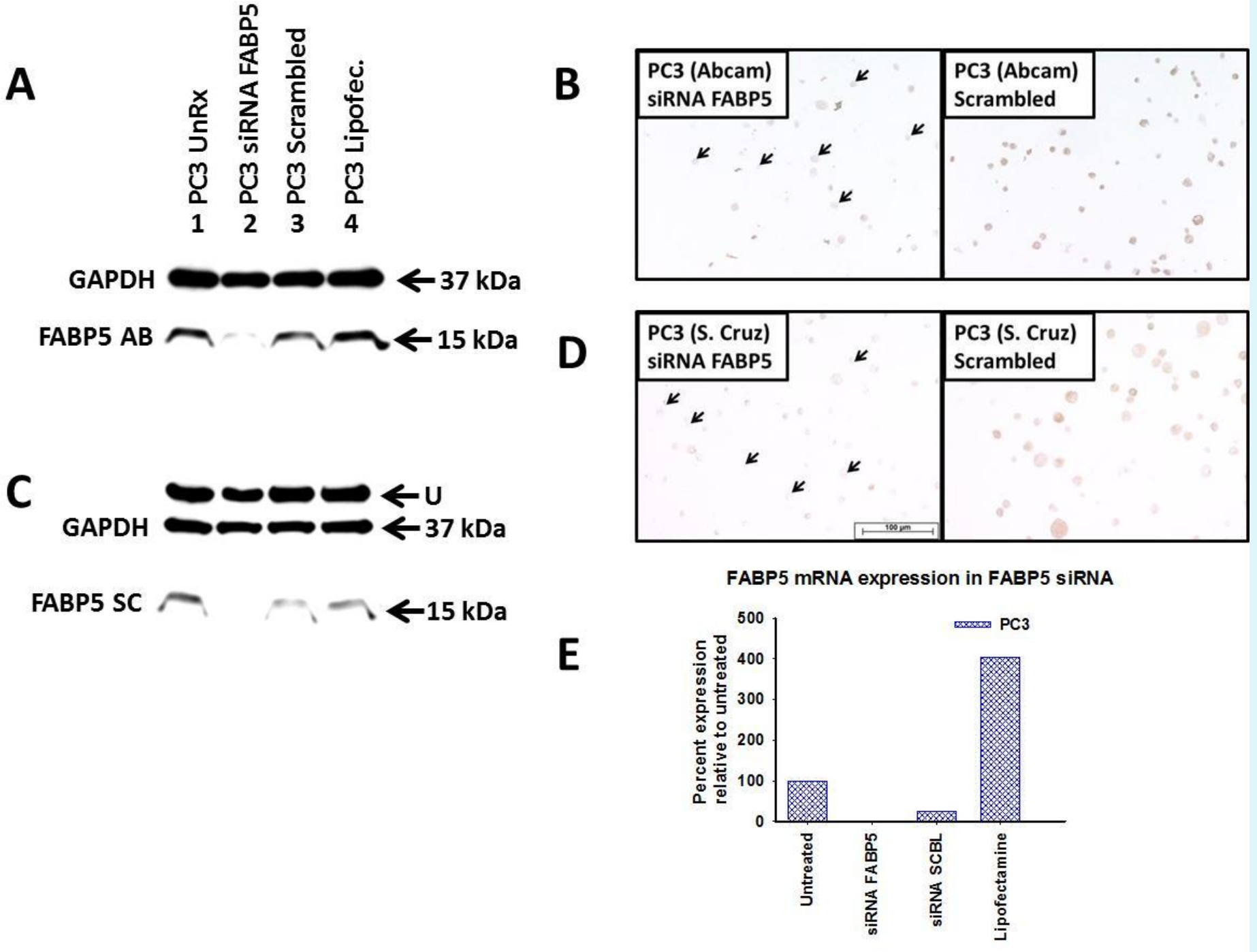
Primary Antibody – Sensitivity

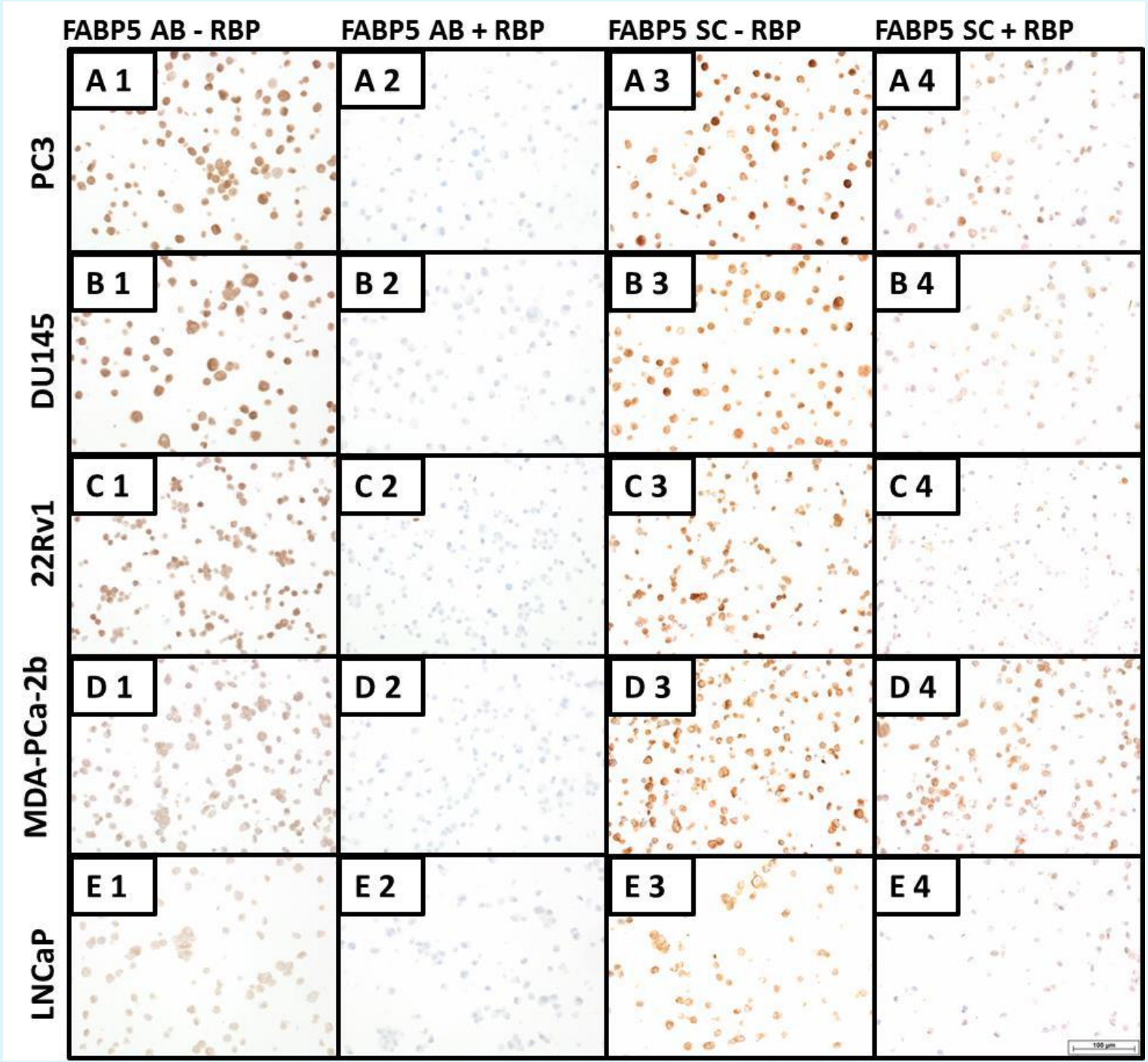


FABP5 Downregulated by siRNA in PC3 Cells and Stained by Antibody from Company 2

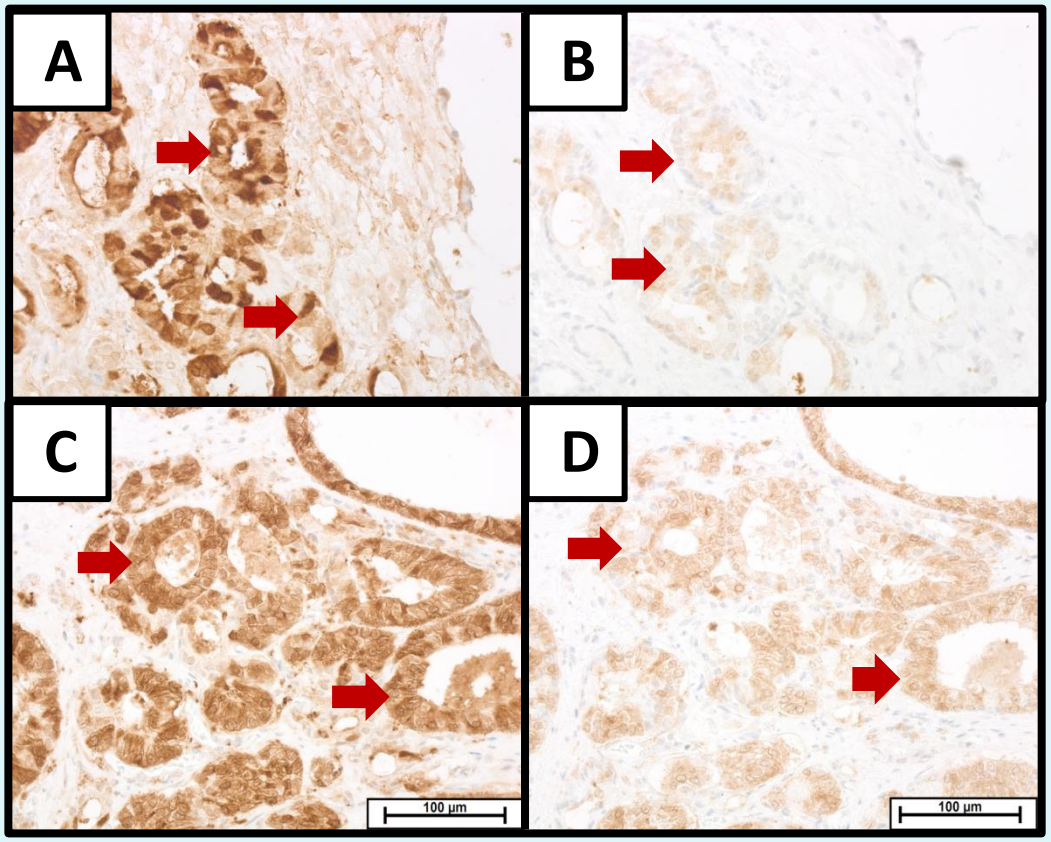



FABP5 AB
FABP5 SC
FABP5 HY
A 1
A 2
B 1
B 2
C 1
C 2


A
1 PC3 UnRx
2 PC3 siRNA FABP5
3 PC3 Scrambled
4 PC3 Lipofec.
GAPDH
37 kDa
FABP5 AB
15 kDa
C
U
GAPDH
37 kDa
FABP5 SC
15 kDa
B
PC3 (Abcam)
siRNA FABP5
PC3 (Abcam)
Scrambled
D
PC3 (S. Cruz)
siRNA FABP5
PC3 (S. Cruz)
Scrambled
100 µm
E
FABP5 mRNA expression in FABP5 siRNA
Percent expression relative to untreated
500
400
300
200
100
0
PC3
Untreated
siRNA FABP5
siRNA SCBL
Lipofectamine


FABP5 AB - RBP
FABP5 AB + RBP
FABP5 SC - RBP
FABP5 SC + RBP
PC3
DU145
22Rv1
MDA-PCa-2b
LNCaP
A 1
A 2
A 3
A 4
B 1
B 2
B 3
B 4
C 1
C 2
C 3
C 4
D 1
D 2
D 3
D 4
E 1
E 2
E 3
E 4

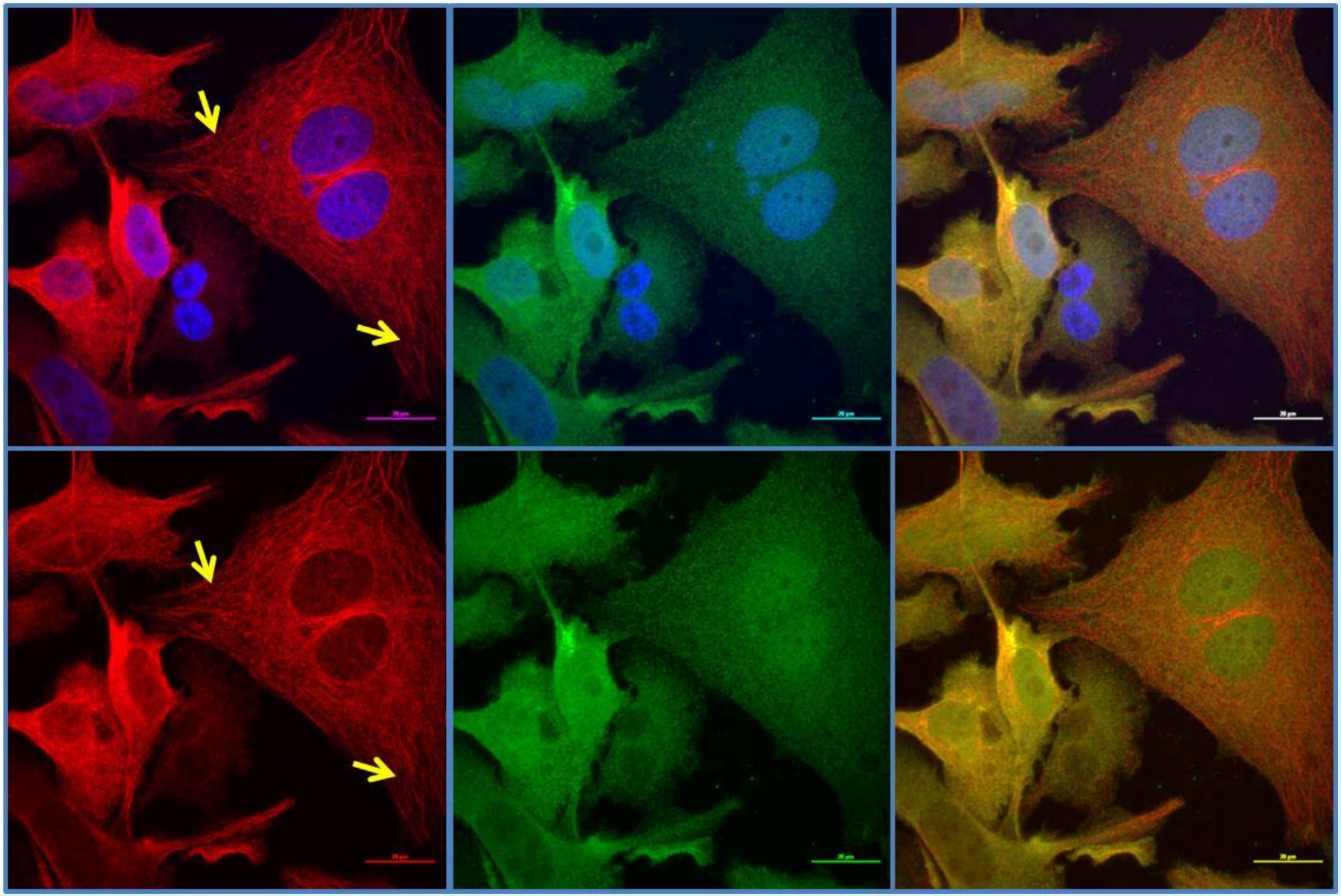
RECOMBINANT PEPTIDE BLOCKING OF ANTIBODY TO FABP5



FABP5 AB

FABP5 SC

PC3 SC (594) /AB (488) confocal microscopy



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