

第三届中国计算蛋白质组学研讨会

The 3rd China Workshop on Computational Proteomics **CNCP-2014**

November 12-13 ▶ Beijing

SIMM

# Technology Development for Membrane Protein Analysis and Its Applications

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**November 12, 2014**



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**Institute of Materia Medica**  
*Chinese Academy of Sciences*

**1. Technology Development for Membrane Protein Analysis**

**2. Applications in Drug-treated samples and Clinic Samples**

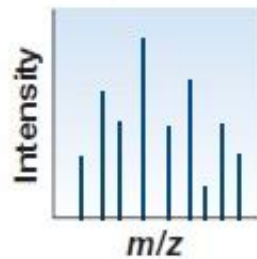
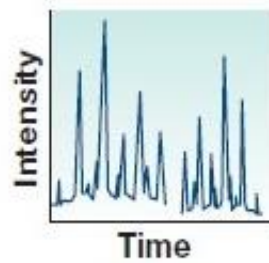


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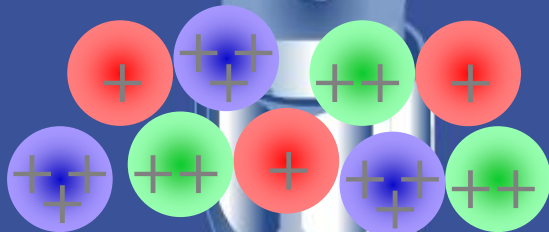
# Flowchart of Proteomic Analysis



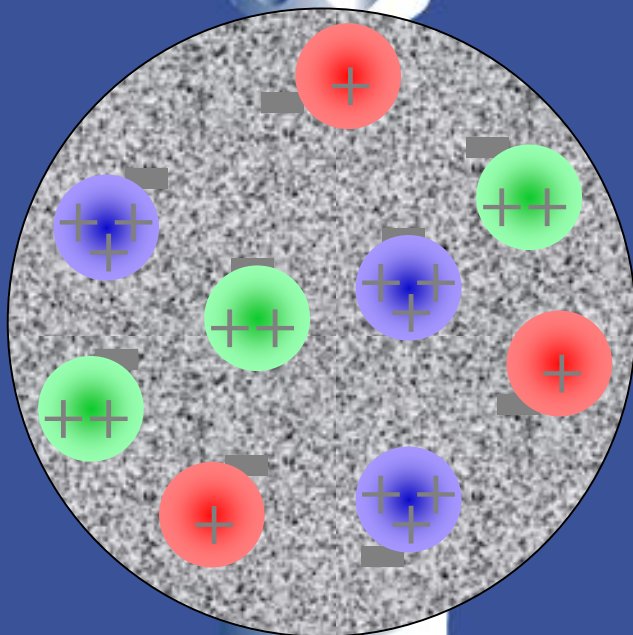


# Principle of Strong Cation Exchange

**Sample Loading**

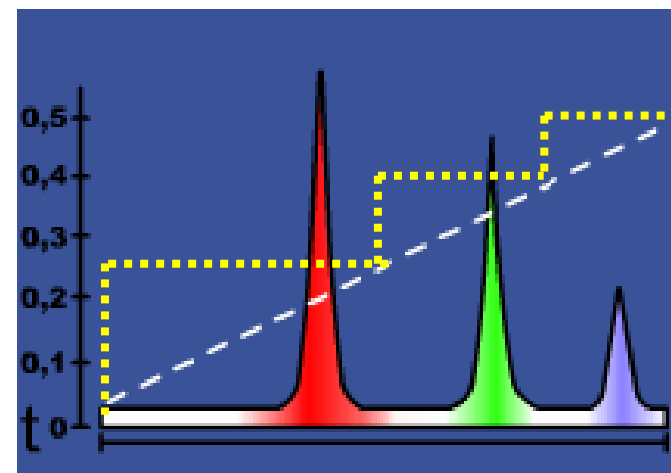


**Elution**

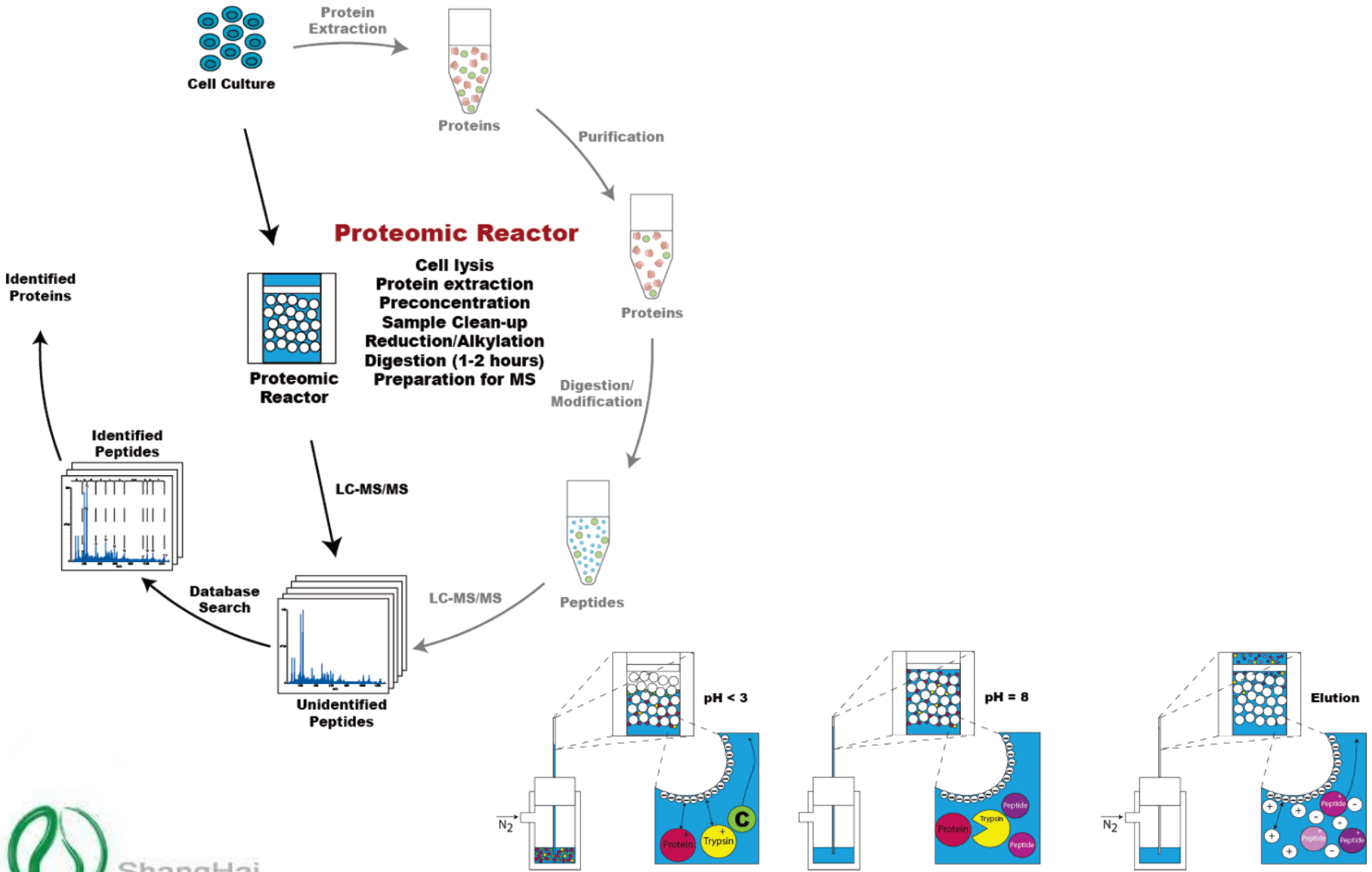


**Ion Strength**

**pH**



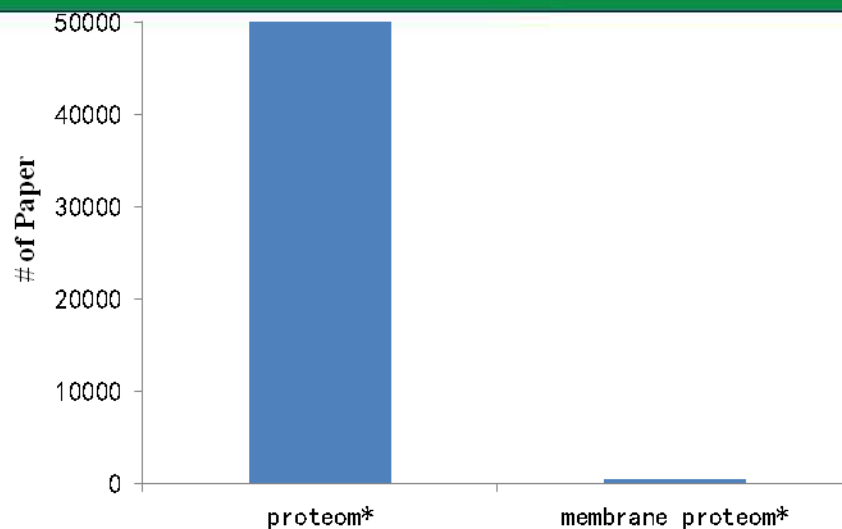
# Proteomic Reactor



# Membrane Protein Analysis is an Important and Challenging Topic in Proteomics

## Membrane Protein Analysis is Very Important in Proteomics

1. >20% human genes encode membrane proteins
2. the membrane associated proteins account for nearly 60% of pharmaceutical drug targets
3. >50% membrane proteins are glycosylated



<http://www.ncbi.nlm.nih.gov/pubmed>

## Improvements

Low Abundance

To Increase Identification Capability & Sequence Coverage

Strong Anion Exchange Beads and Multiple Enzymes

*Zhou H et al Anal Bioanal Chem. 2010 Aug;397(8):3421-30.*

Low Abundance

To Improve Low Abundant Protein Detectability

Proteomic Reactor with pH Fractionation

*Zhou H et al. Talanta. 2010 Feb; 80(4):1526-1531.*

Hydrophobicity

To Enhance Membrane Protein Solubility

Centrifugal Proteomic Reactor

*Zhou H et al. Mol Cell Proteomics. 2011 Oct;10(10):O111.008425*

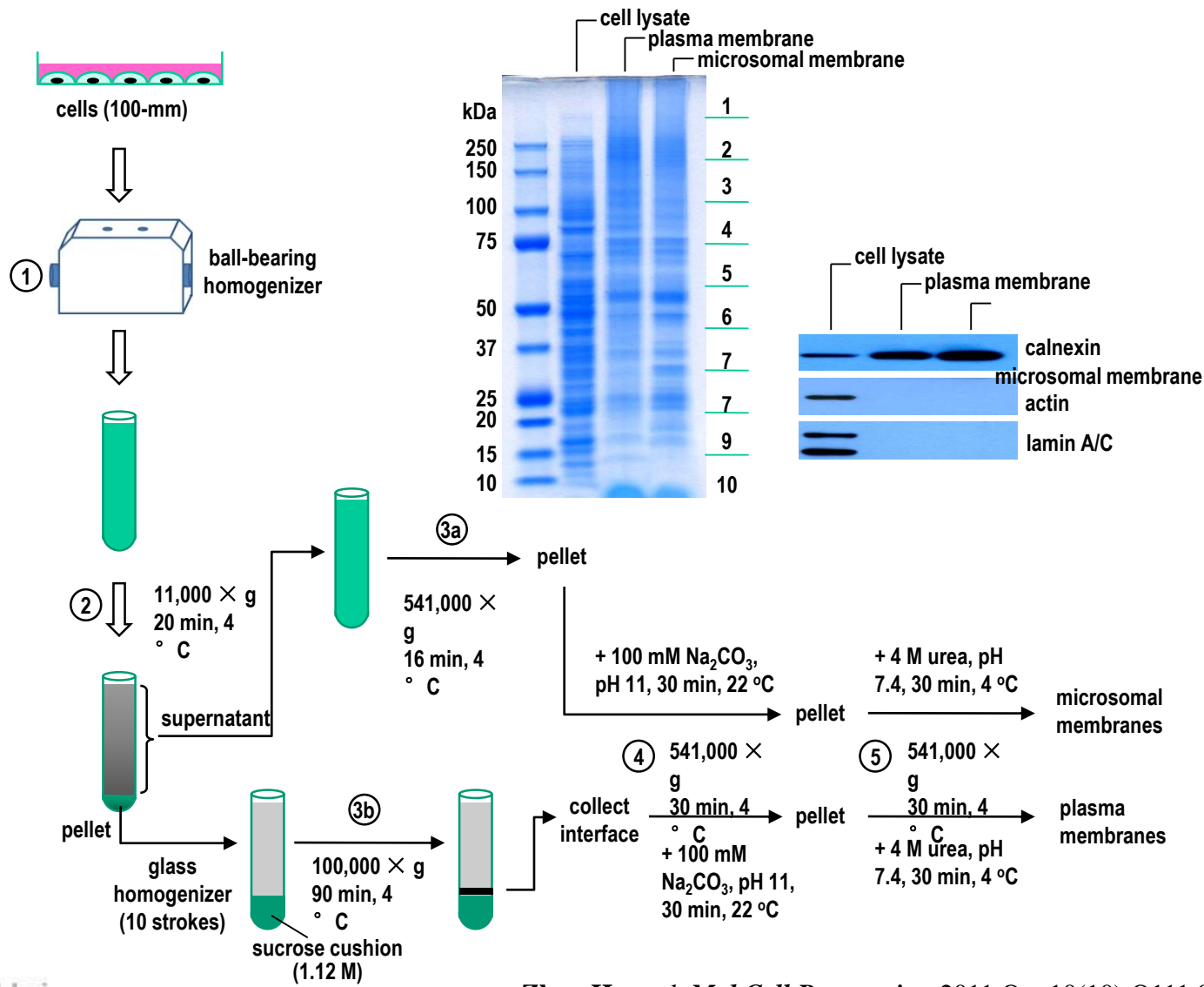
Glycosylation

To Identify Glycopeptides and Glycosylation Sites

Glycoproteomic Reactor

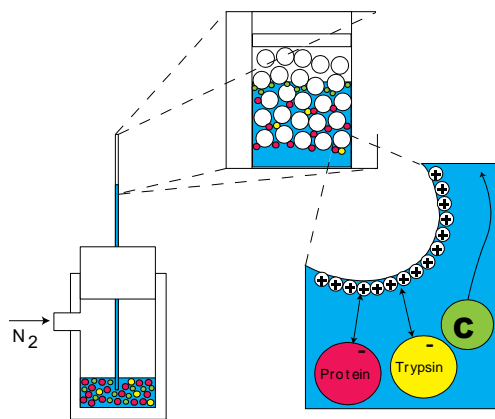
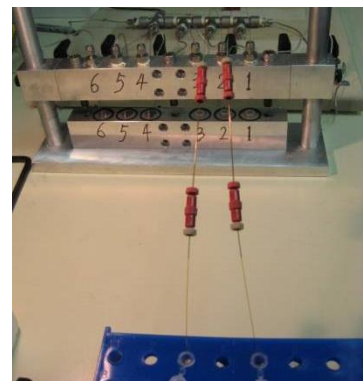
*Zhou H et al. J Proteome Res. 2009 Feb;8(2):556-66.*

# Subcellular Fractionation of Membrane Proteins





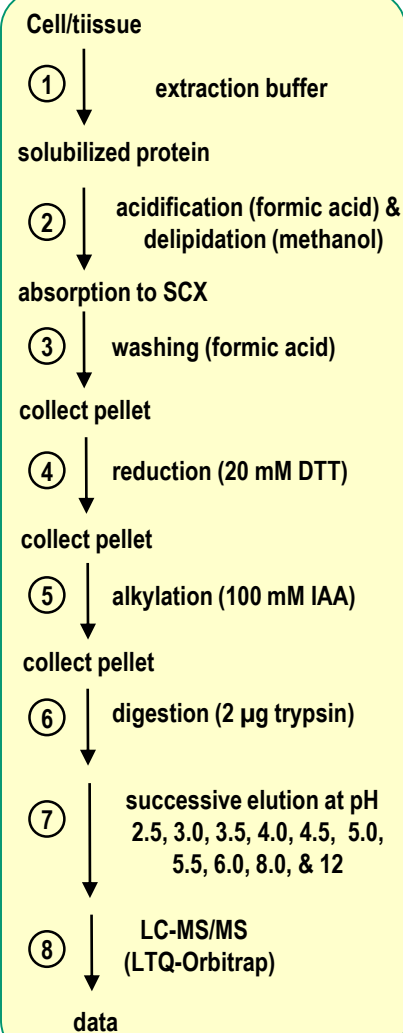
# Centrifugal Proteomic Reactor



SCX Column (in high pressurized vessel)



Bead in EP tube



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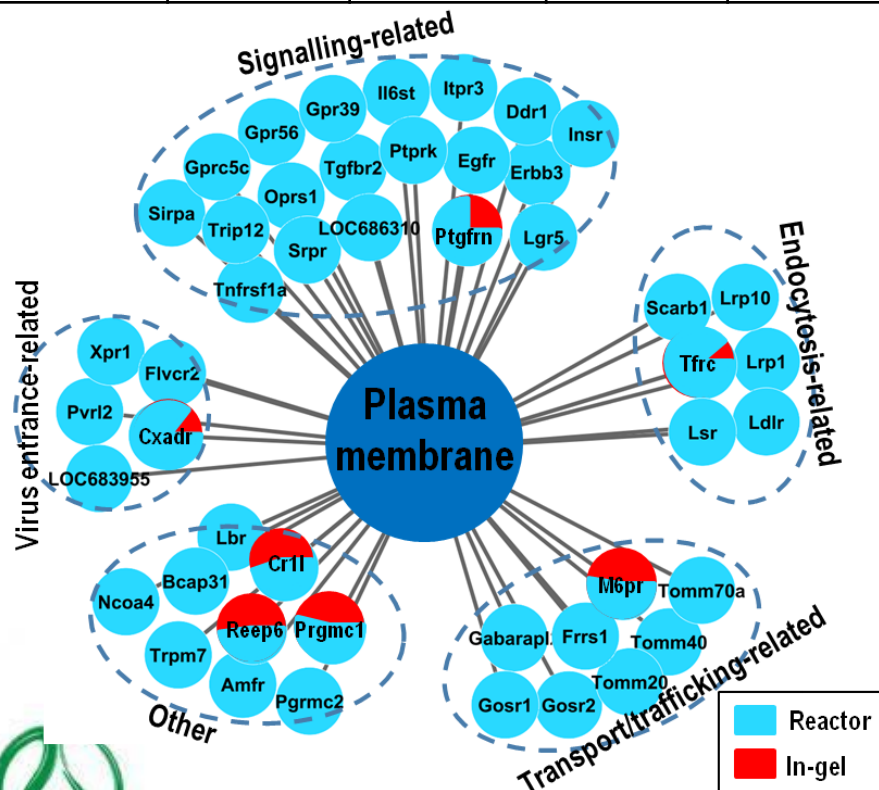
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Zhou H, et al. *Mol Cell Proteomics*. 2011 Oct;10(10):O111.008425.

# Centrifugal Proteomic Reactor

Method	CPR		In-gel Digestion	
Sample	Plasma membrane	Microsome Membrane	Plasma membrane	Microsome Membrane
# of proteins	<b>945</b>	<b>955</b>	<b>110</b>	<b>128</b>
# of membrane proteins	<b>591</b> (63%)	<b>447</b> (47%)	<b>72</b> (65%)	<b>70</b> (55%)



## Improvements

- User-friendly , only need a centrifuge
- Comparing to traditional in-gel digestion, # of proteins increase 7 fold, # of membrane proteins increase 6 fold
- The percentage of membrane proteins in plasma membrane fraction is >60%



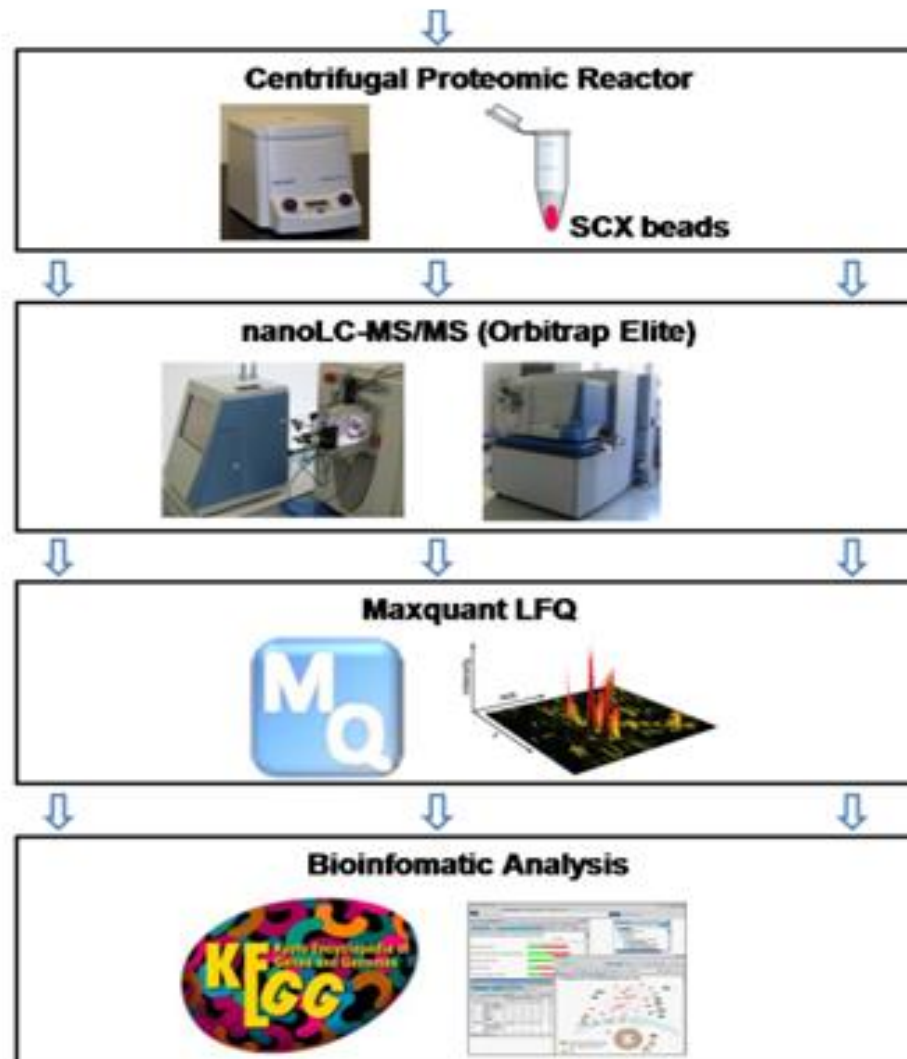
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## Paired Samples

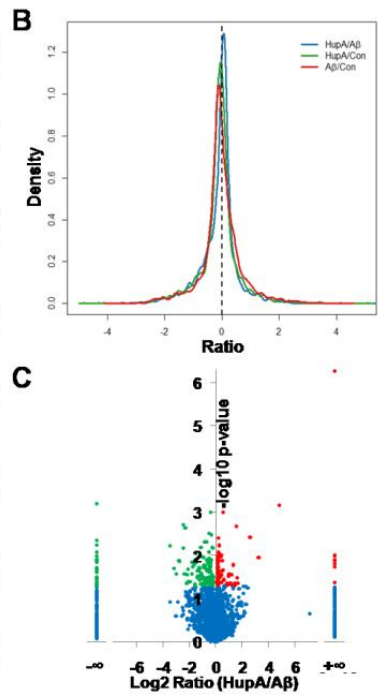
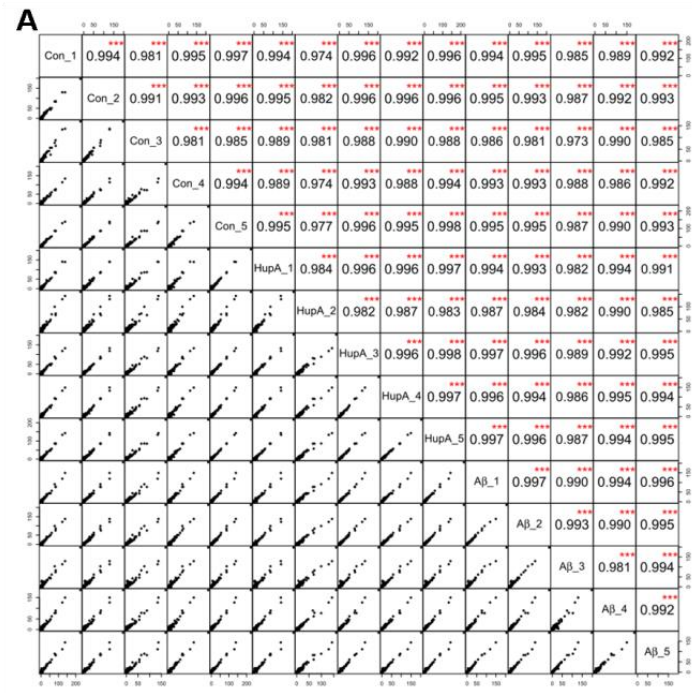
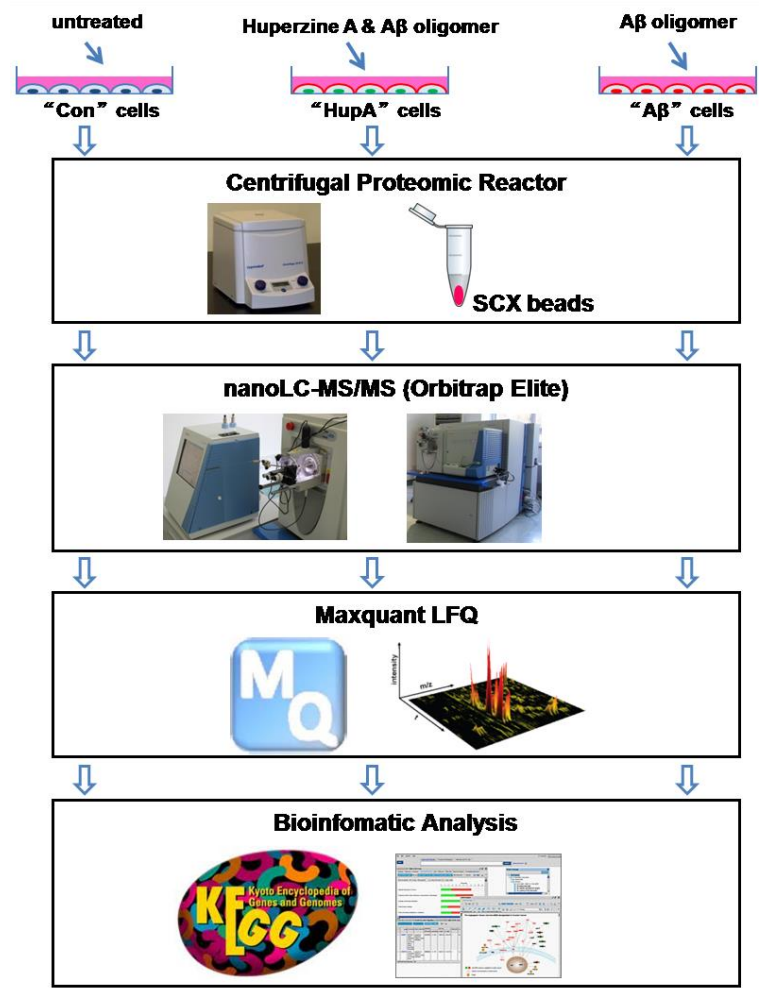


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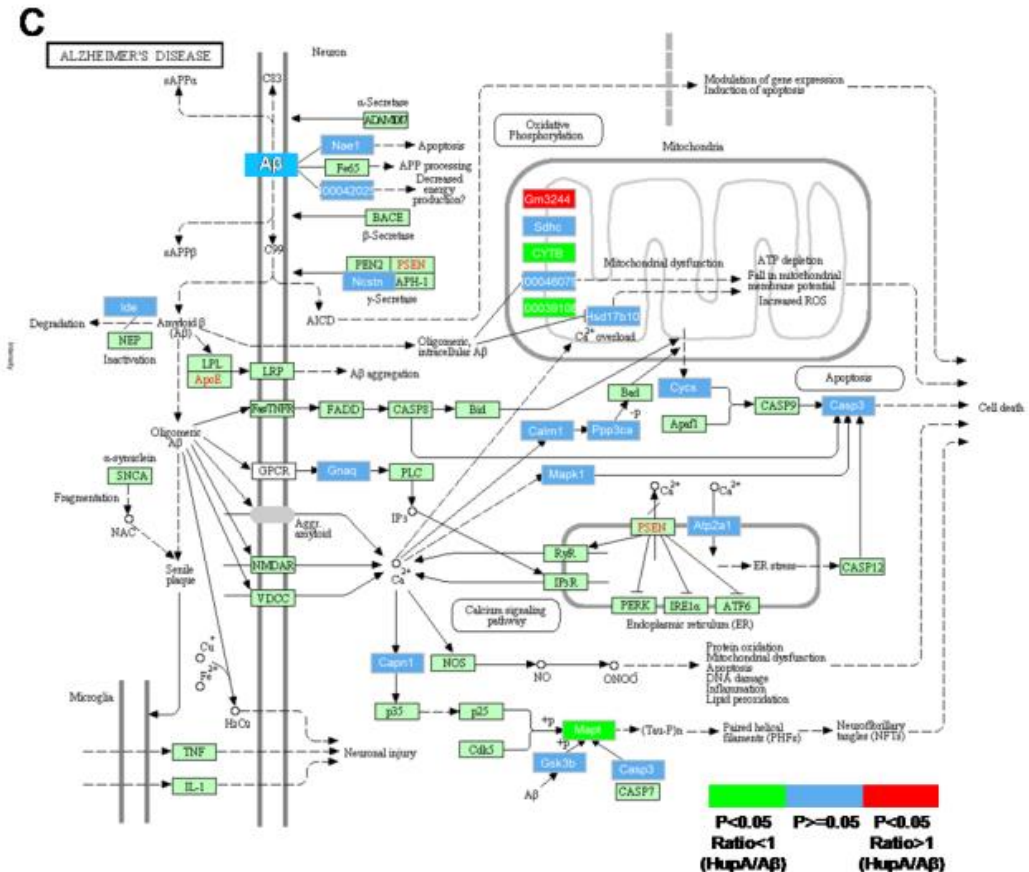
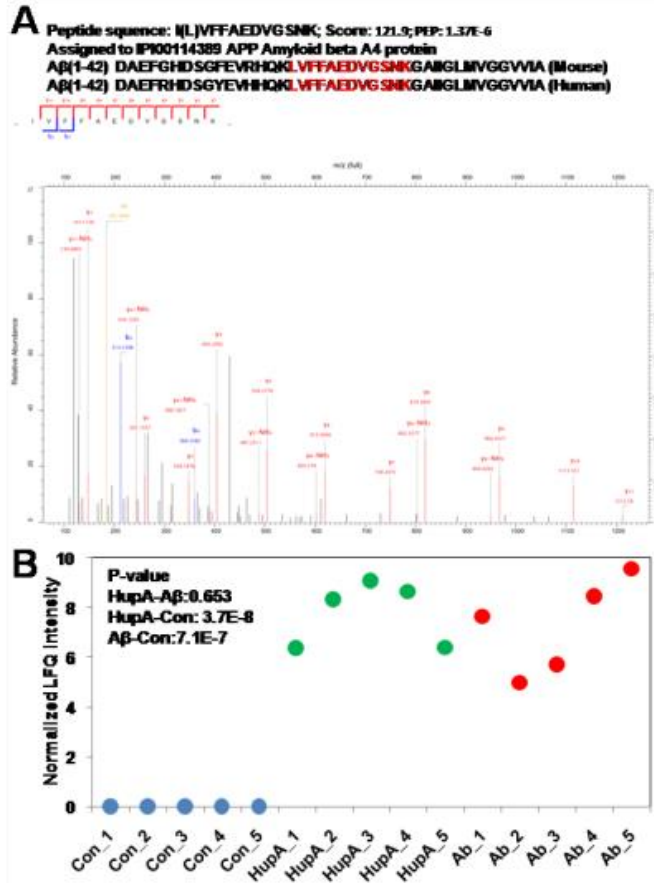
Chinese Academy of Sciences

# Quantitative proteomic analysis reveals the neuroprotective effects of huperzine A for amyloid beta treated neuroblastoma N2a cells



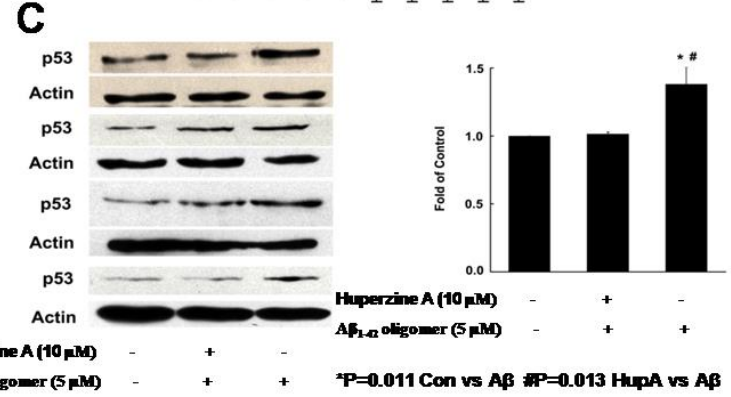
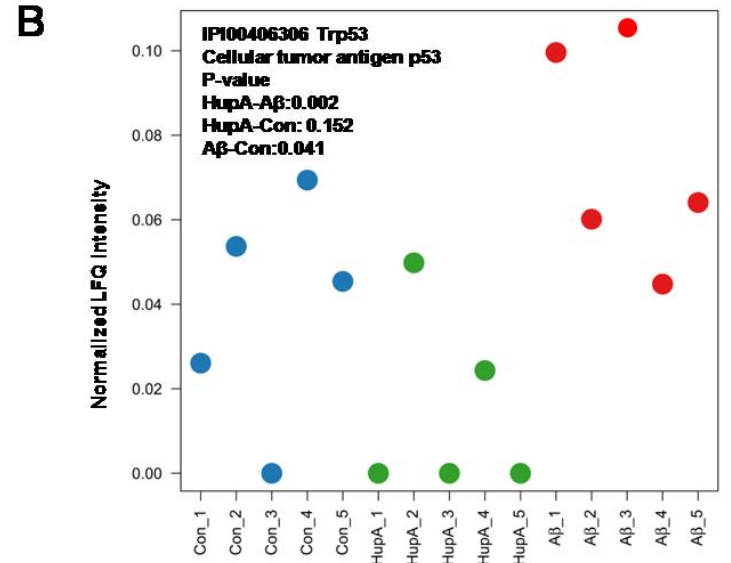
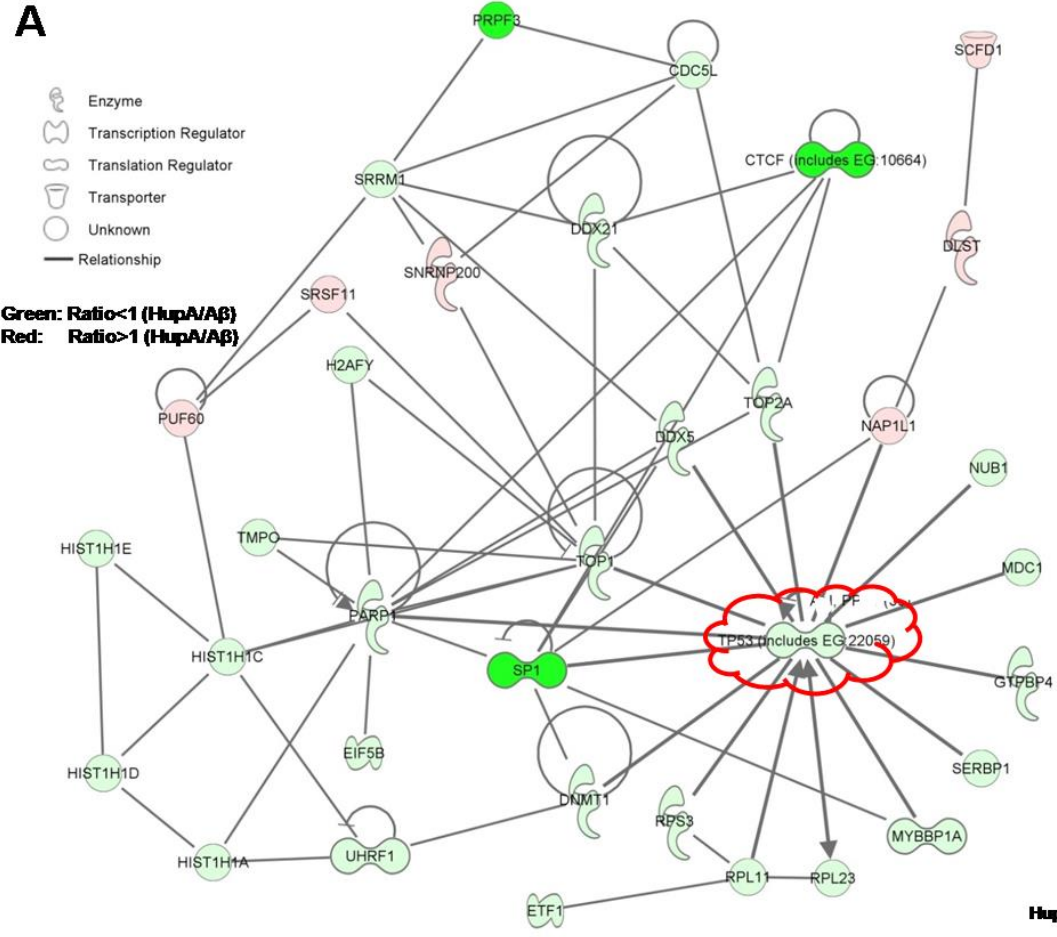
Proteomics. 2013 Apr;13(8):1314-24.

# Quantitative proteomic analysis reveals the neuroprotective effects of huperzine A for amyloid beta treated neuroblastoma N2a cells



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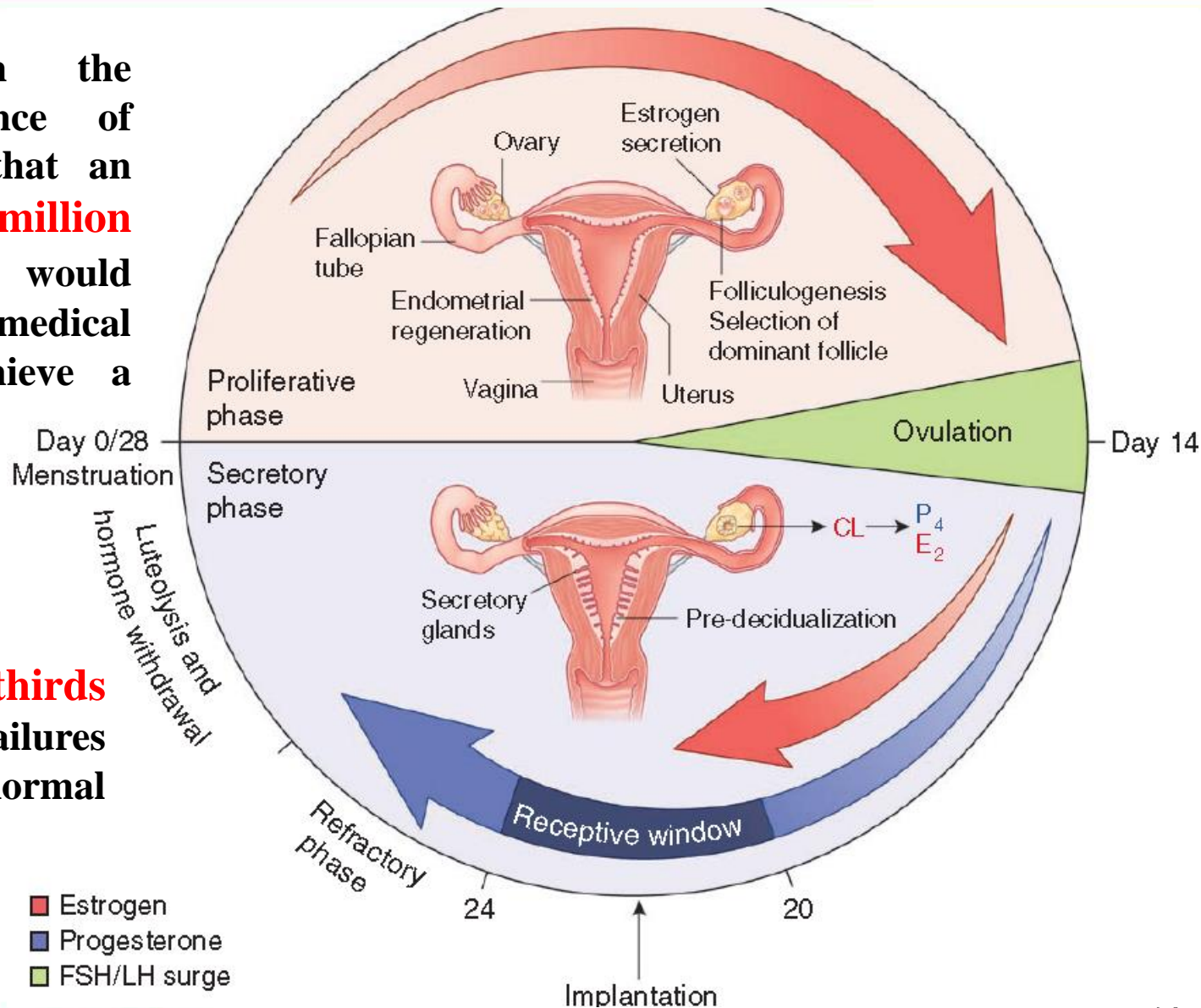
# Quantitative proteomic analysis reveals the neuroprotective effects of huperzine A for amyloid beta treated neuroblastoma N2a cells



# Quantitative Proteomic Analysis of Human Endometrial Tissues in Receptive and Proliferative Phases

Recent data on the worldwide prevalence of infertility indicate that an estimated **48.5 million couples** worldwide would need some form of medical intervention to achieve a pregnancy

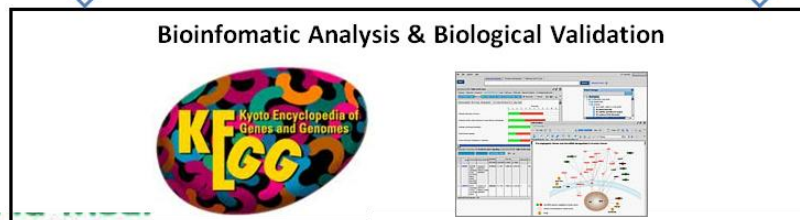
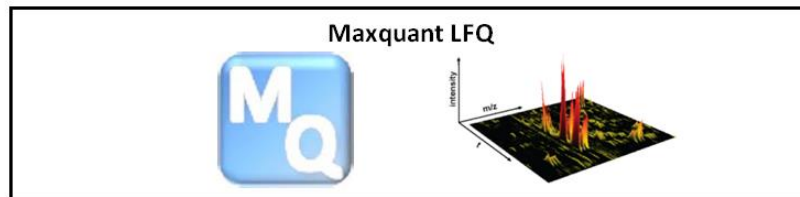
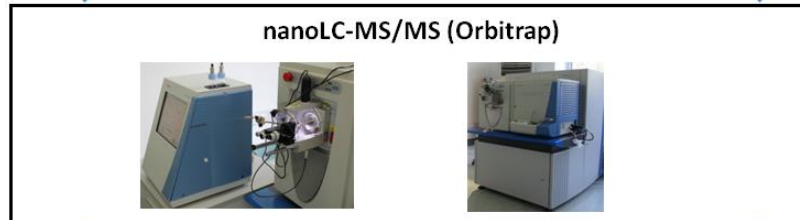
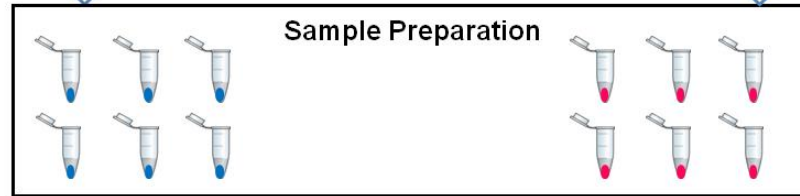
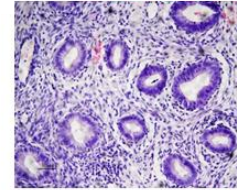
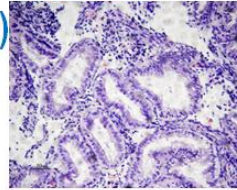
Approximately **two-thirds** of implantation failures result from abnormal uterine receptivity



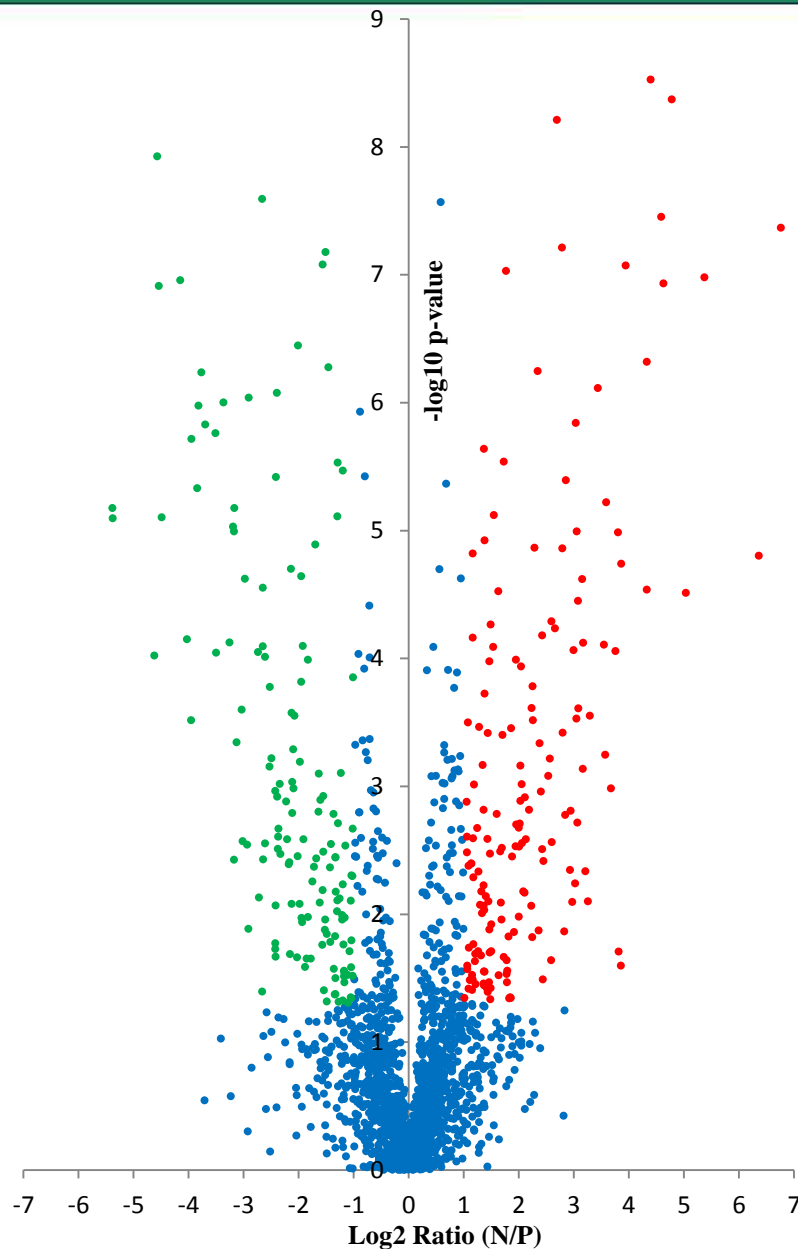
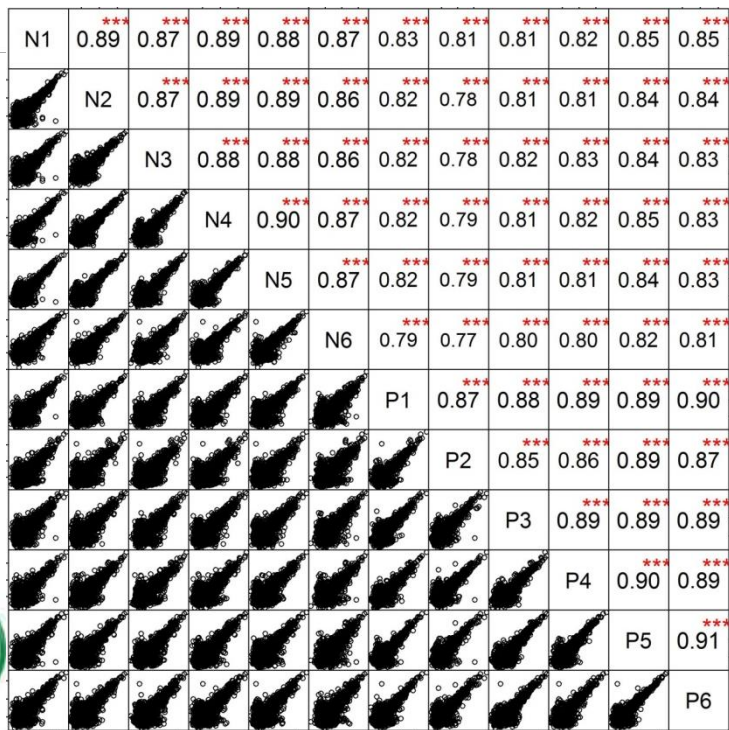
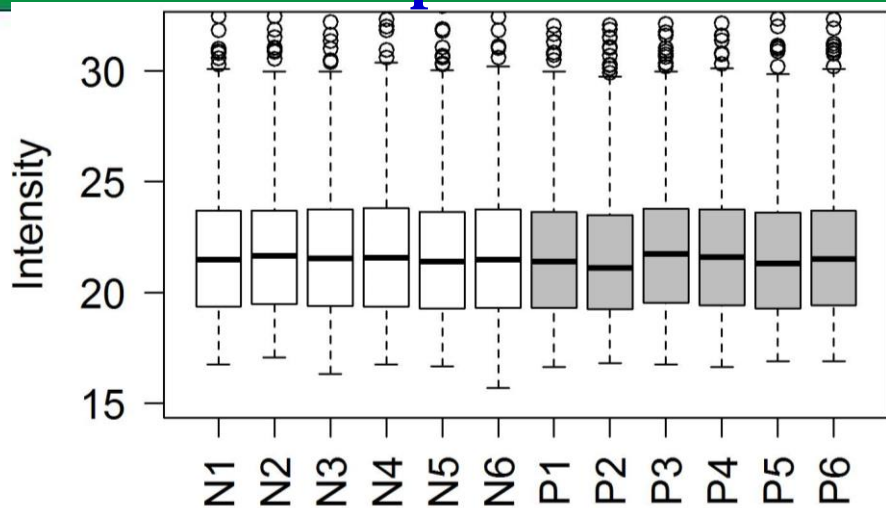


# Quantitative Proteomic Analysis of Human Endometrial Tissues in Receptive and Proliferative Phases

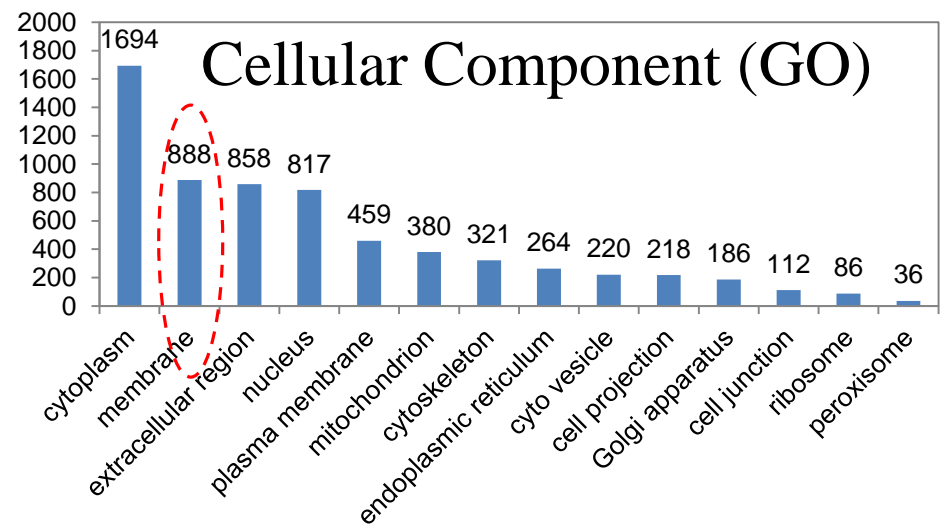
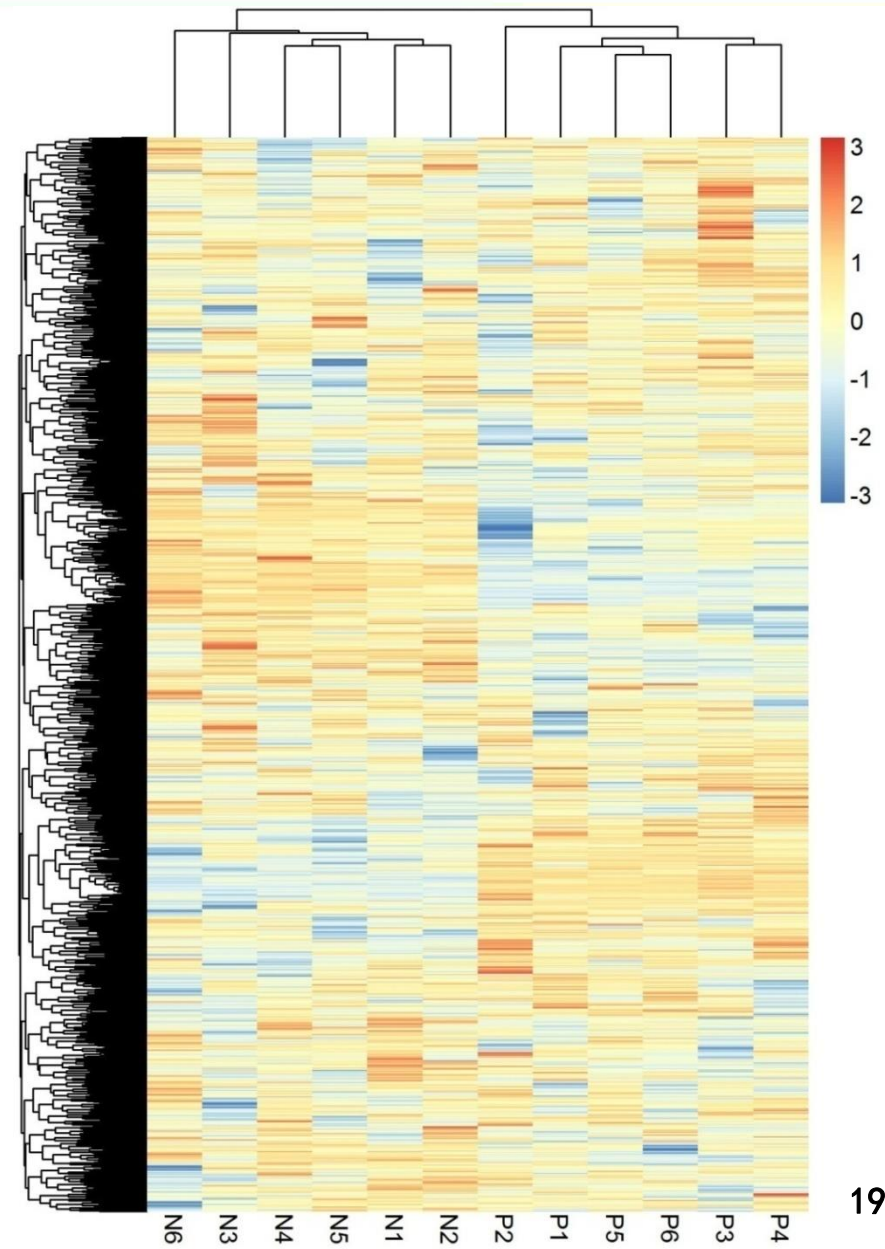
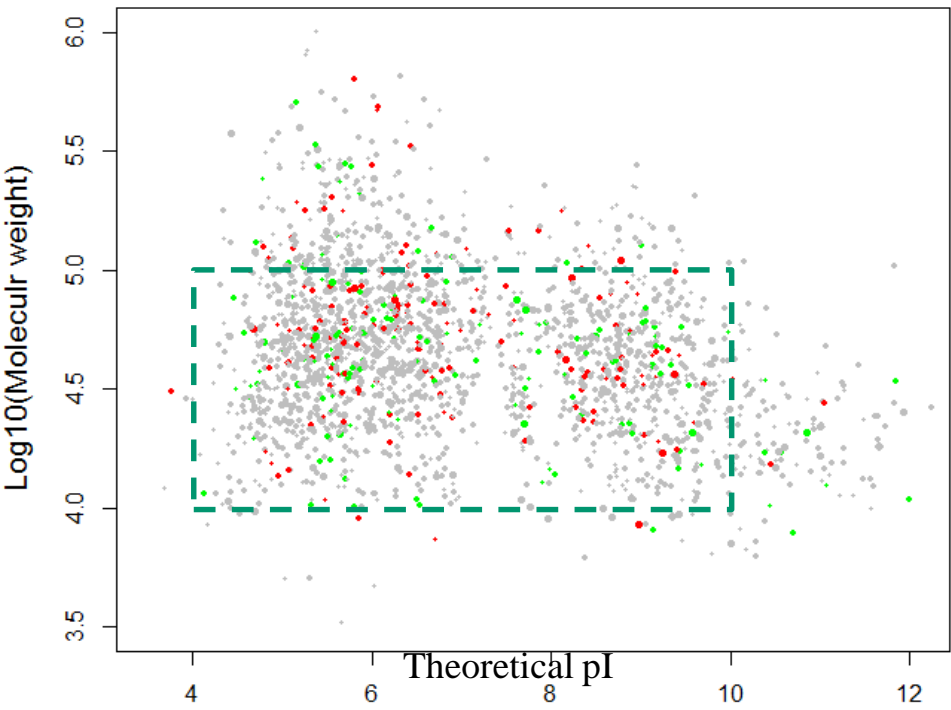
Receptive phase endometrial tissue (N1..N6)      Proliferative phase (P1...P6)



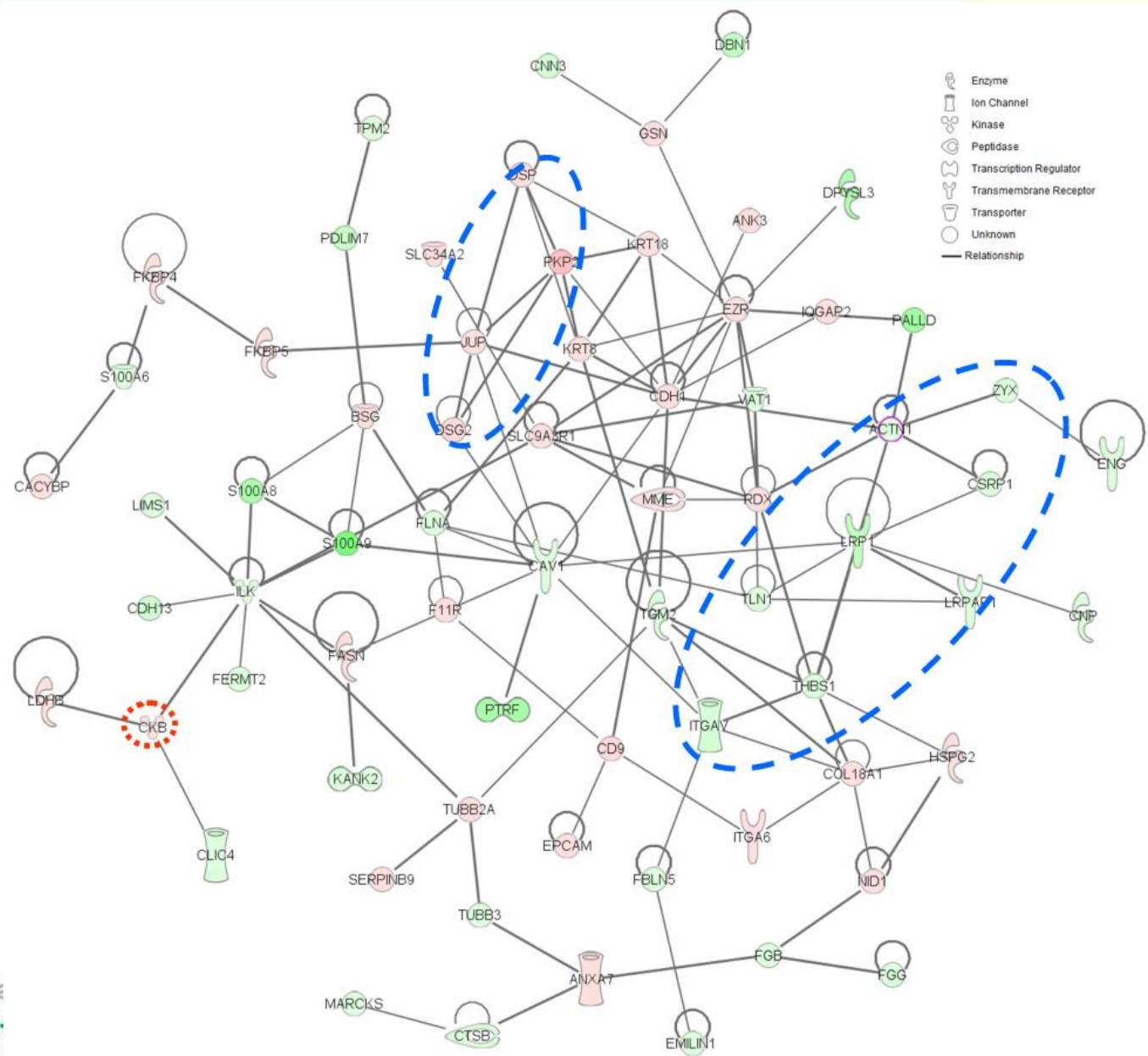
# Quantitative Proteomic Analysis of Human Endometrial Tissues in Receptive and Proliferative Phases



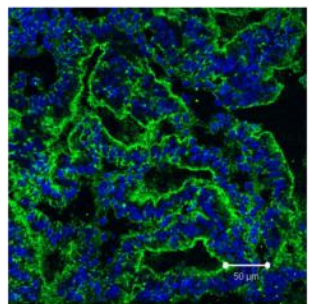
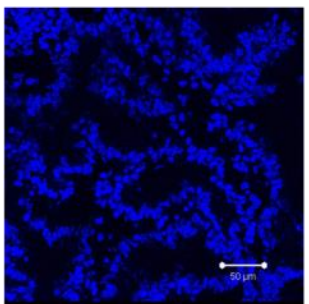
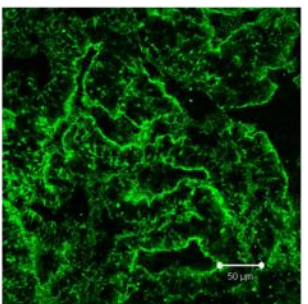
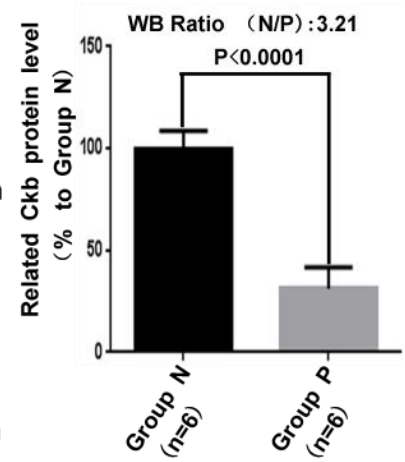
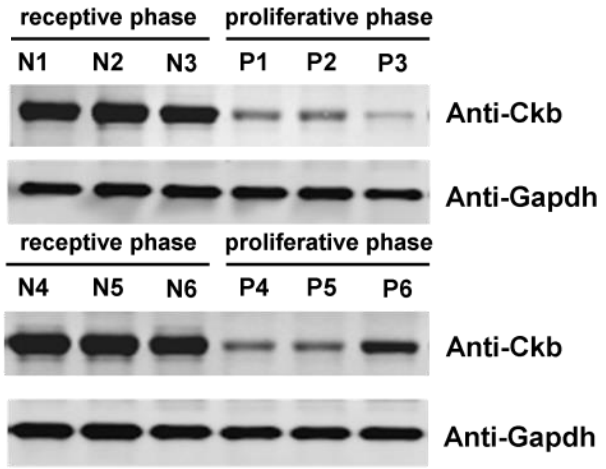
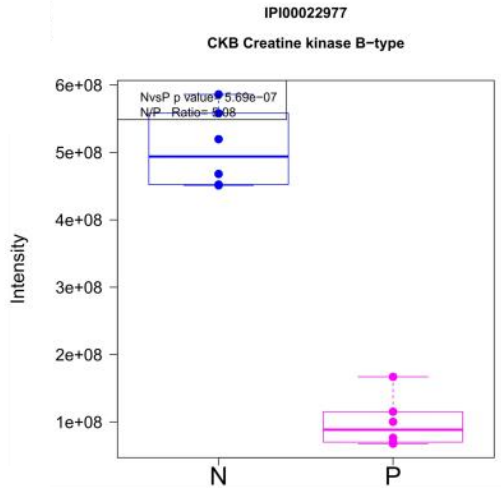
# Quantitative Proteomic Analysis of Human Endometrial Tissues in Receptive and Proliferative Phases



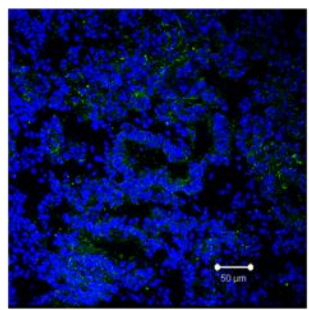
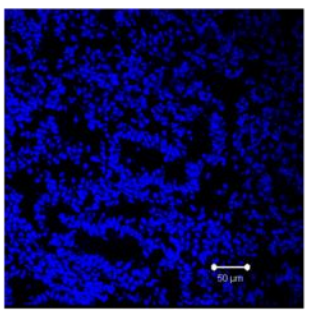
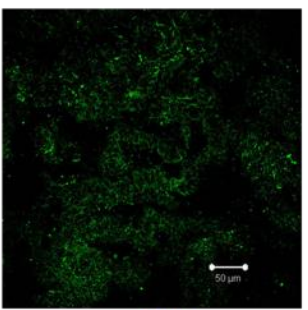
# Direct Protein-Protein Interaction Network Analysis



# Ckb Protein is Up-regulated in Receptive Phase



Endometrial tissue in receptive phase (group N)

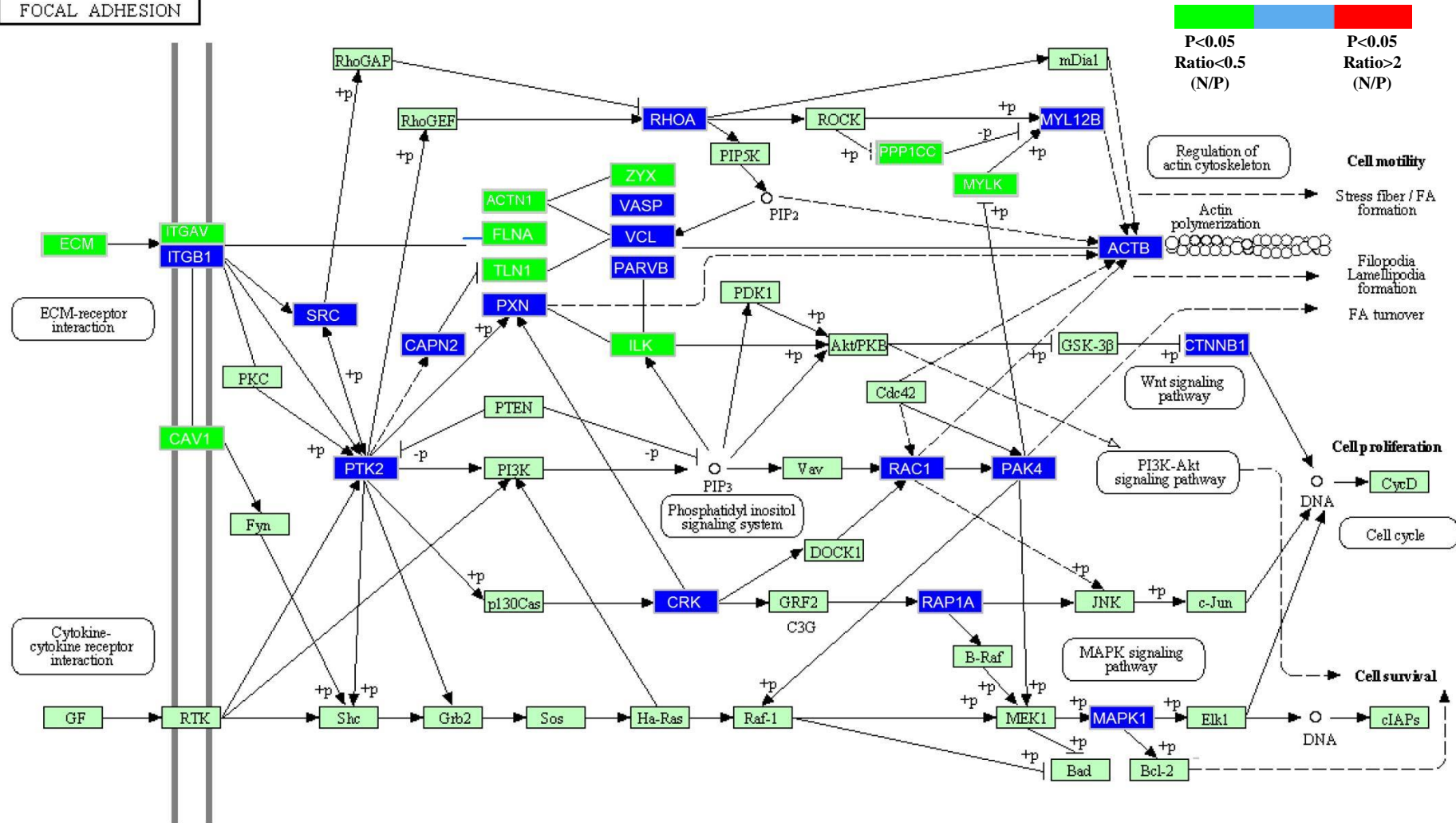


Endometrial tissue in proliferative phase (group P)



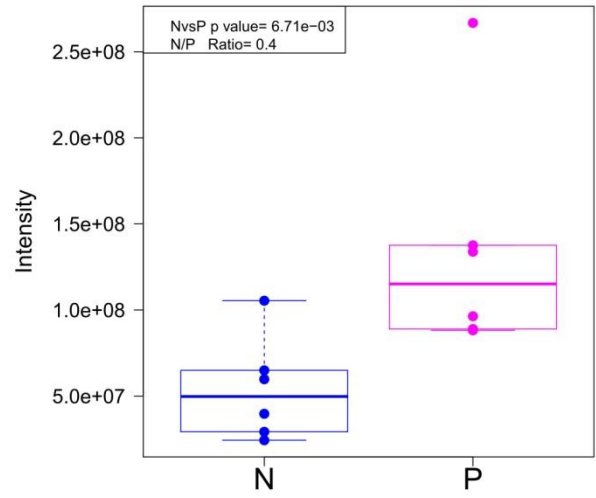
# KEGG Pathway Analysis

FOCAL ADHESION

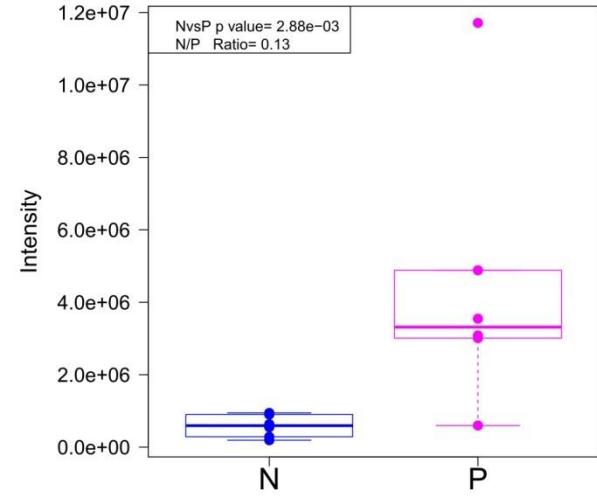


# KEGG Pathway Analysis

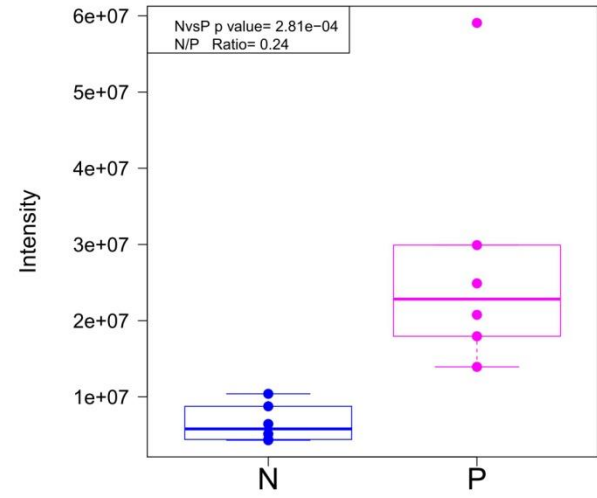
IPI00297646  
COL1A1 Collagen alpha-1(I) chain



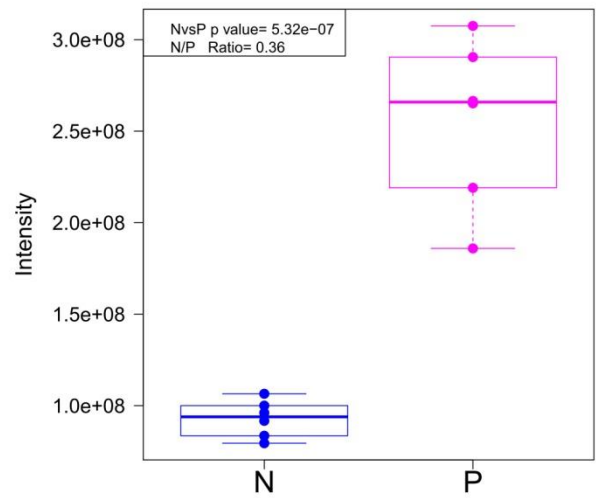
IPI00027505  
ITGAV Isoform 1 of Integrin alpha-V



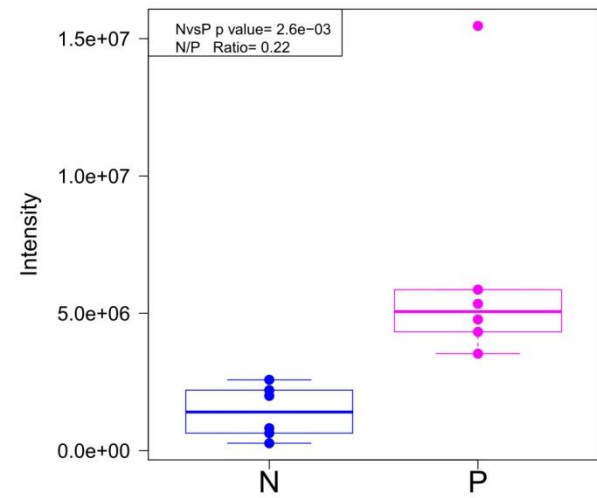
IPI00009236  
CAV1 Isoform Alpha of Caveolin-1



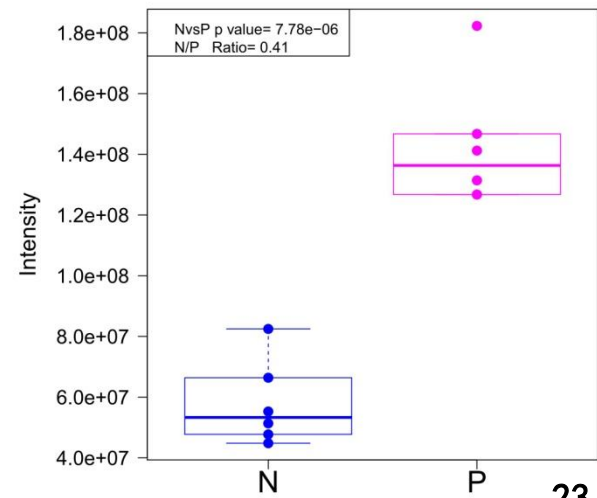
IPI00298994  
TLN1 Talin-1



IPI00926625  
ZYZX Zyxin



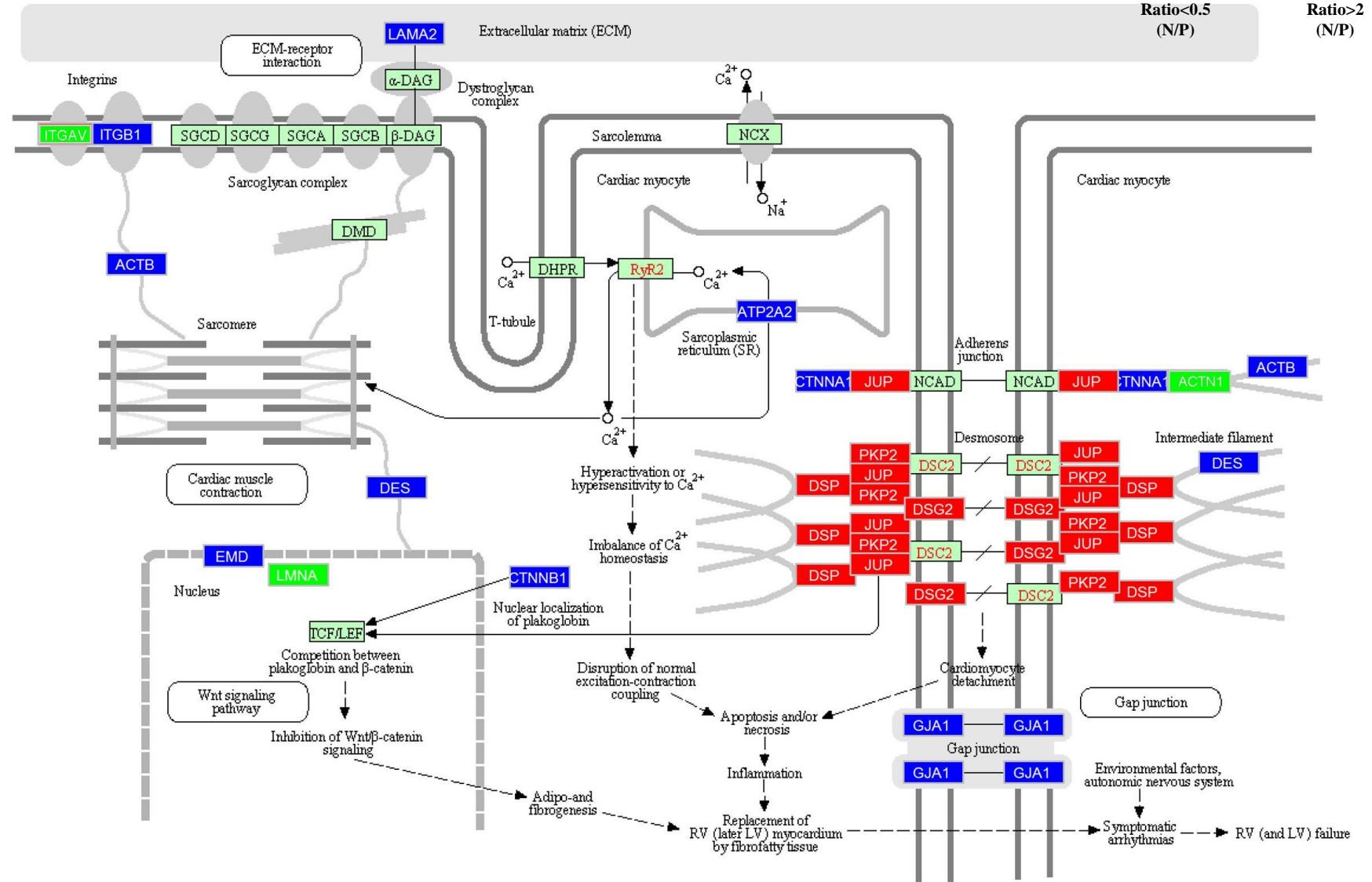
IPI00013508  
ACTN1 Isoform 1 of Alpha-actinin-1



# KEGG Pathway Analysis

## ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC)

P<0.05
Ratio<0.5
P<0.05  
Ratio<0.5
Ratio>2  
(N/P)
(N/P)

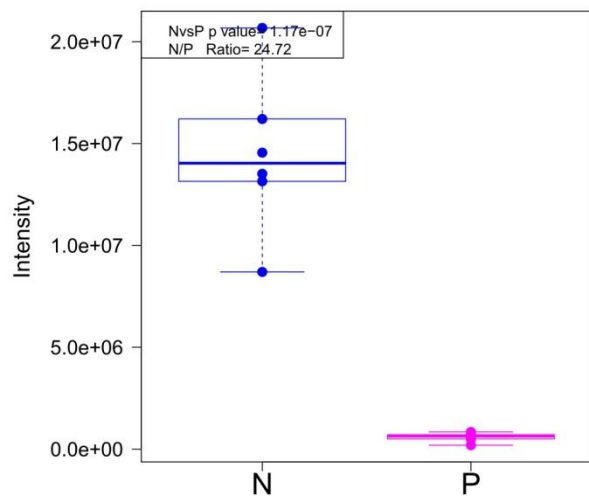




## KEGG Pathway Analysis

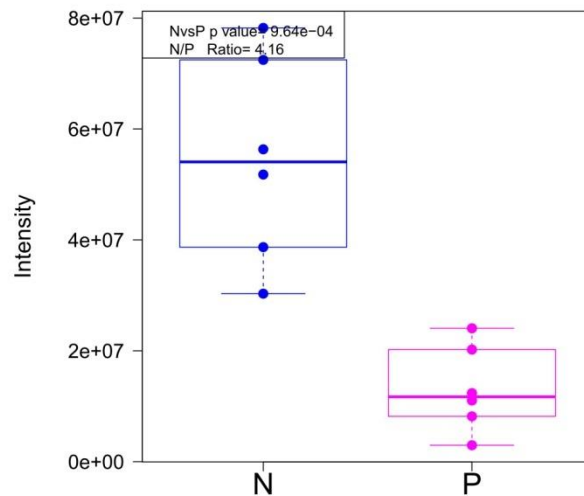
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PKP2 Isoform 2 of Plakophilin-2



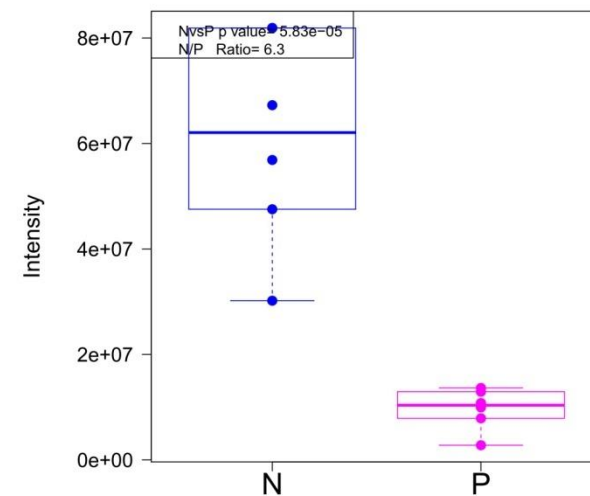
IPI00554711

JUP Junction plakoglobin



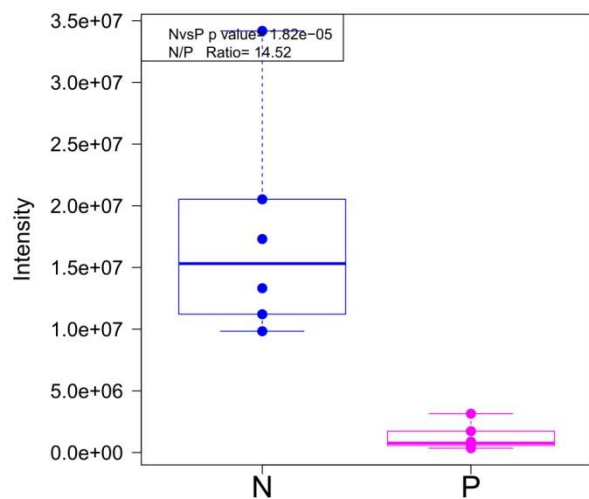
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DSP Isoform DPI of Desmoplakin



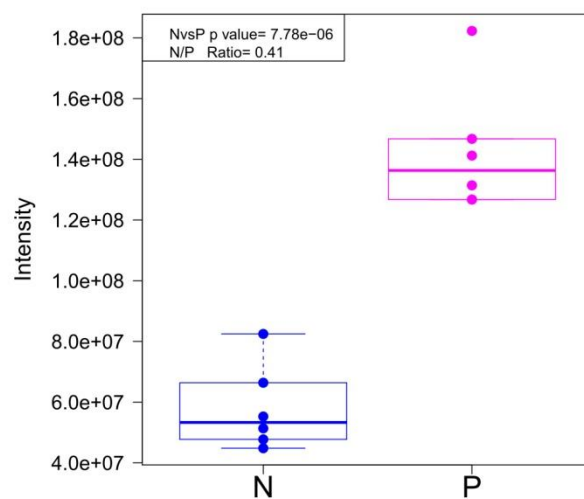
IPI00028931

DSG2 Desmoglein-2

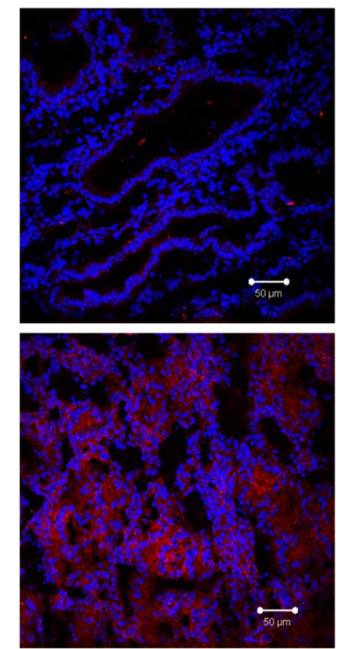
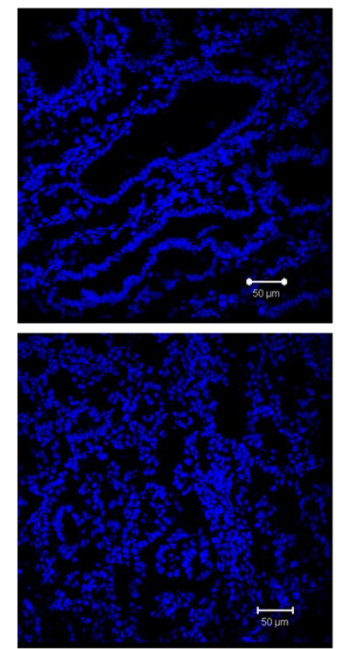
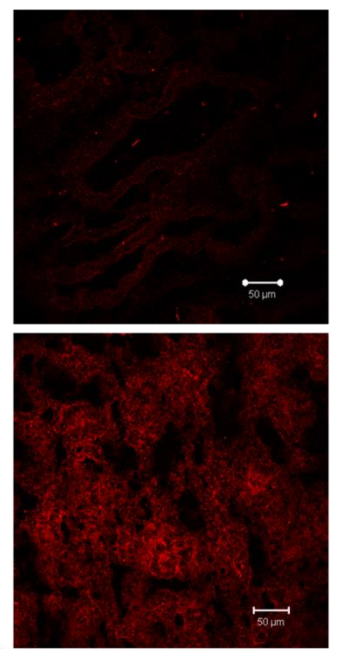
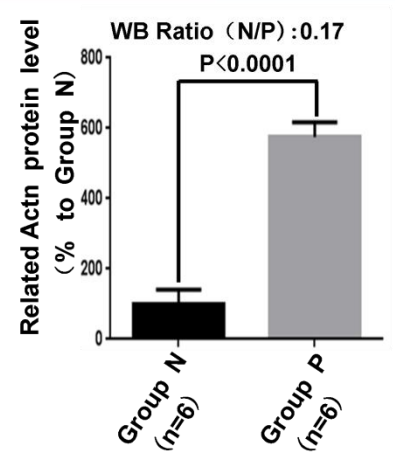
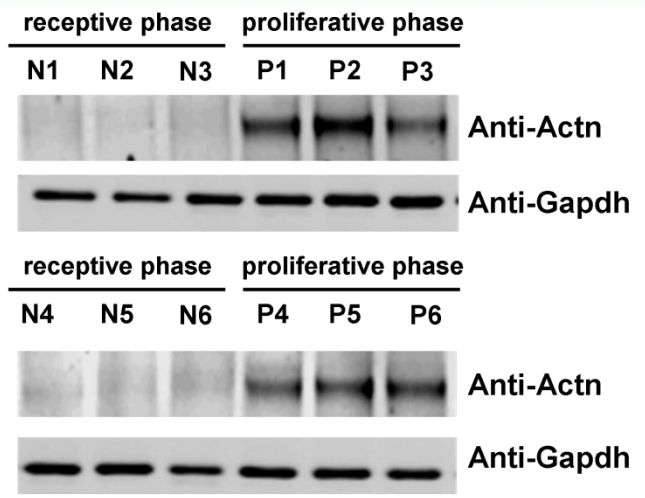
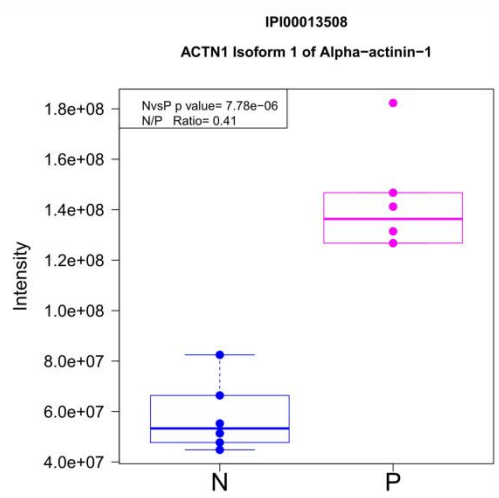


IPI00013508

ACTN1 Isoform 1 of Alpha-actinin-1



# Alpha-actinin is Down-regulated in Receptive Phase



Endometrial tissue in receptive phase (group N)

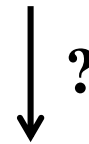
Endometrial tissue in proliferative phase (group P)

## Human Endometrial Tissue

Proliferative phase

Receptive phase

Ovarian hormones



Proteins involved in cell-cell focal adhesion



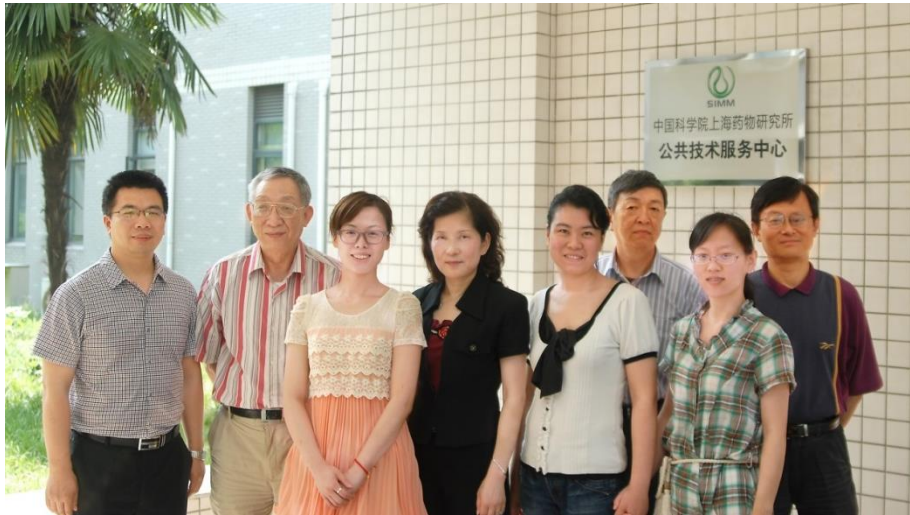
Proteins involved in desmosome



# Acknowledgements

**Dr. Daniel Figeys**

**The lab members in Dr. Figeys' lab, University of Ottawa**



**Collaborators:**

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**Dr. Huaiyu Yang (SIMM)**

**Dr. Hualiang Jiang (SIMM)**

**Dr. Chen Xu (Ruijin Hospital)**

**One Hundred Talent, CAS**

**Key Laboratory for Receptor Research, CAS**

**National Natural Science Foundation of China, No. 21375138**

# Thank you !



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