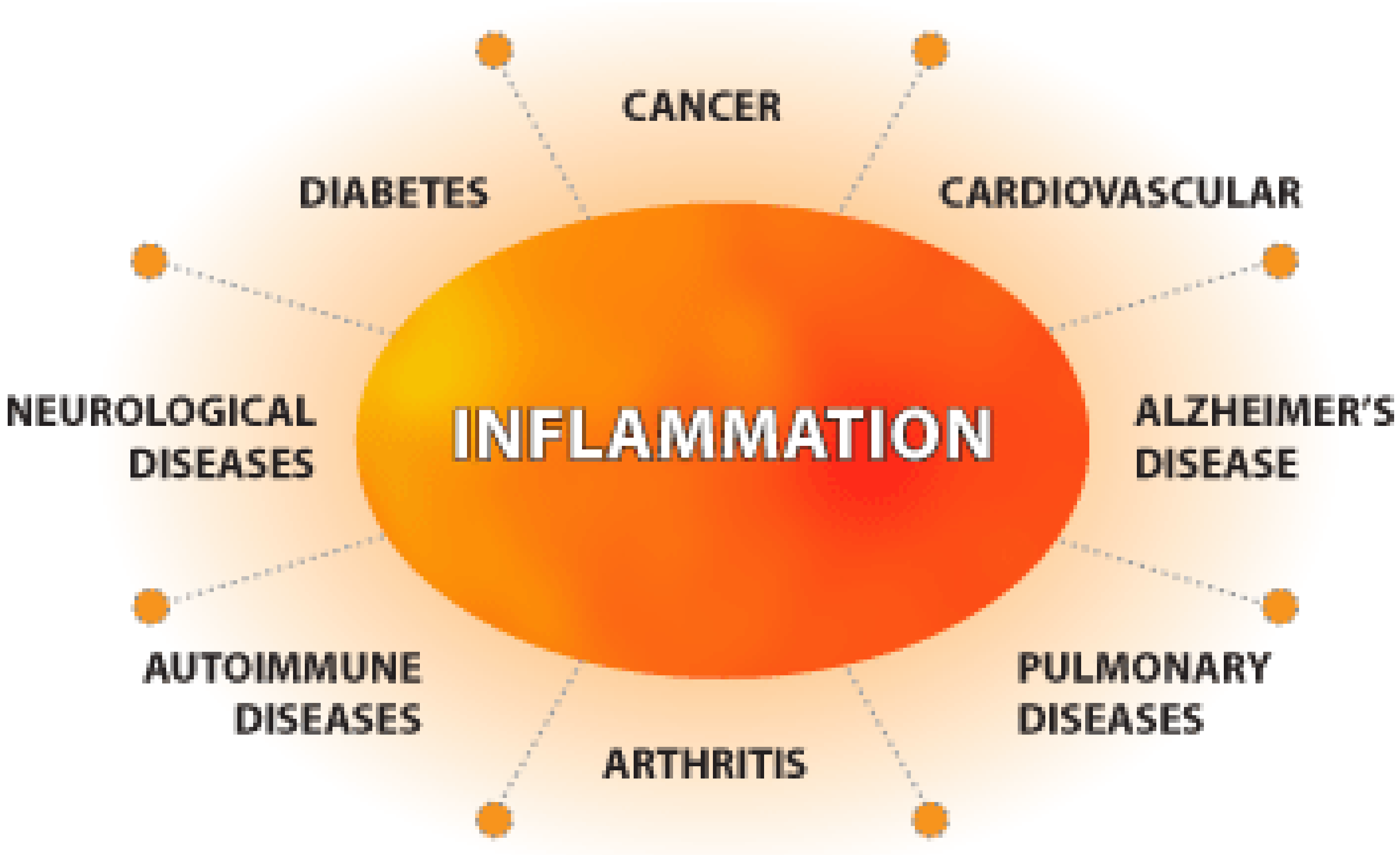
# Functional characterization of *de novo* designed peptide derived from CXCL8 in inflammation and breast cancer progression

生化學科 江信仲

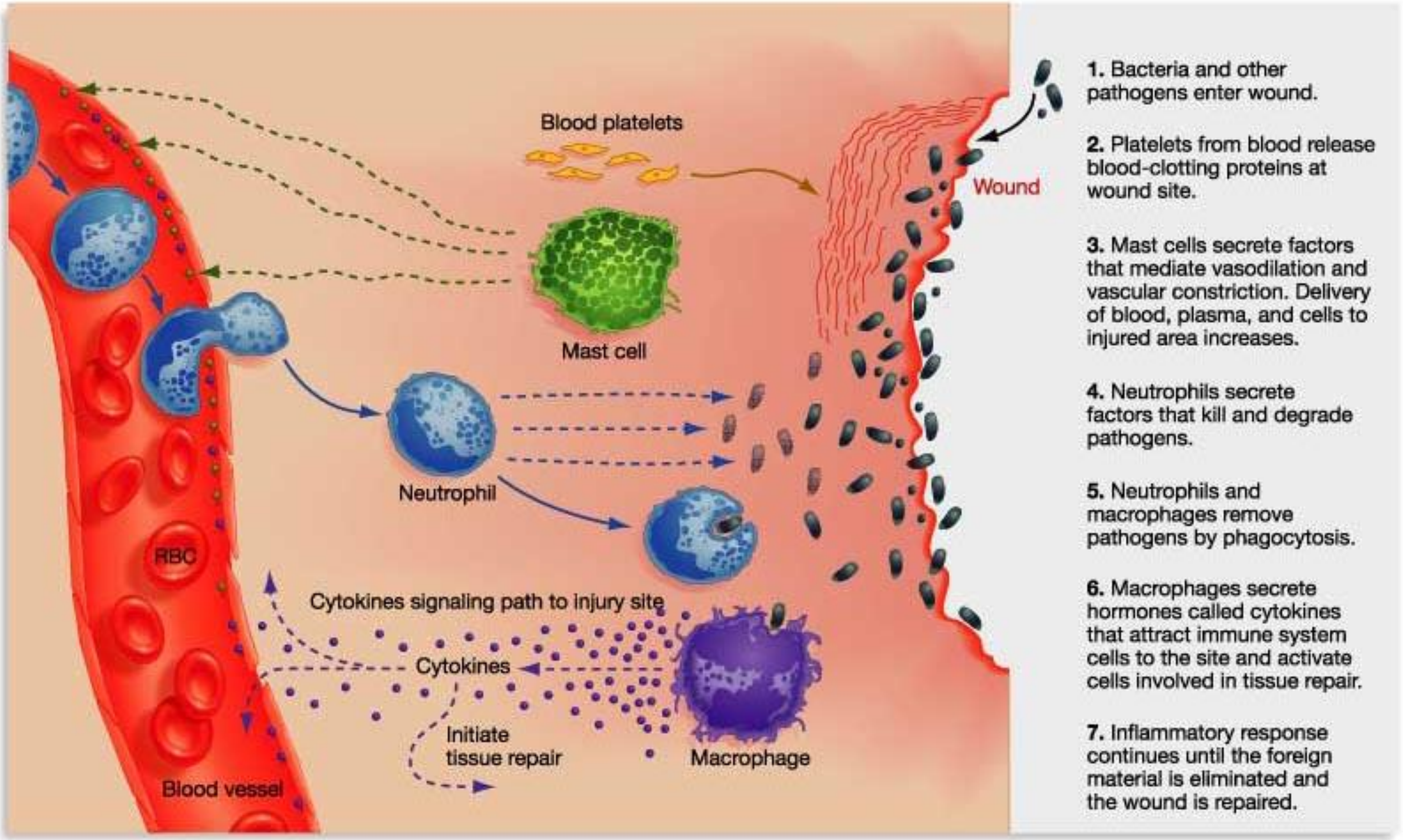
110/4/20

# Inflammation



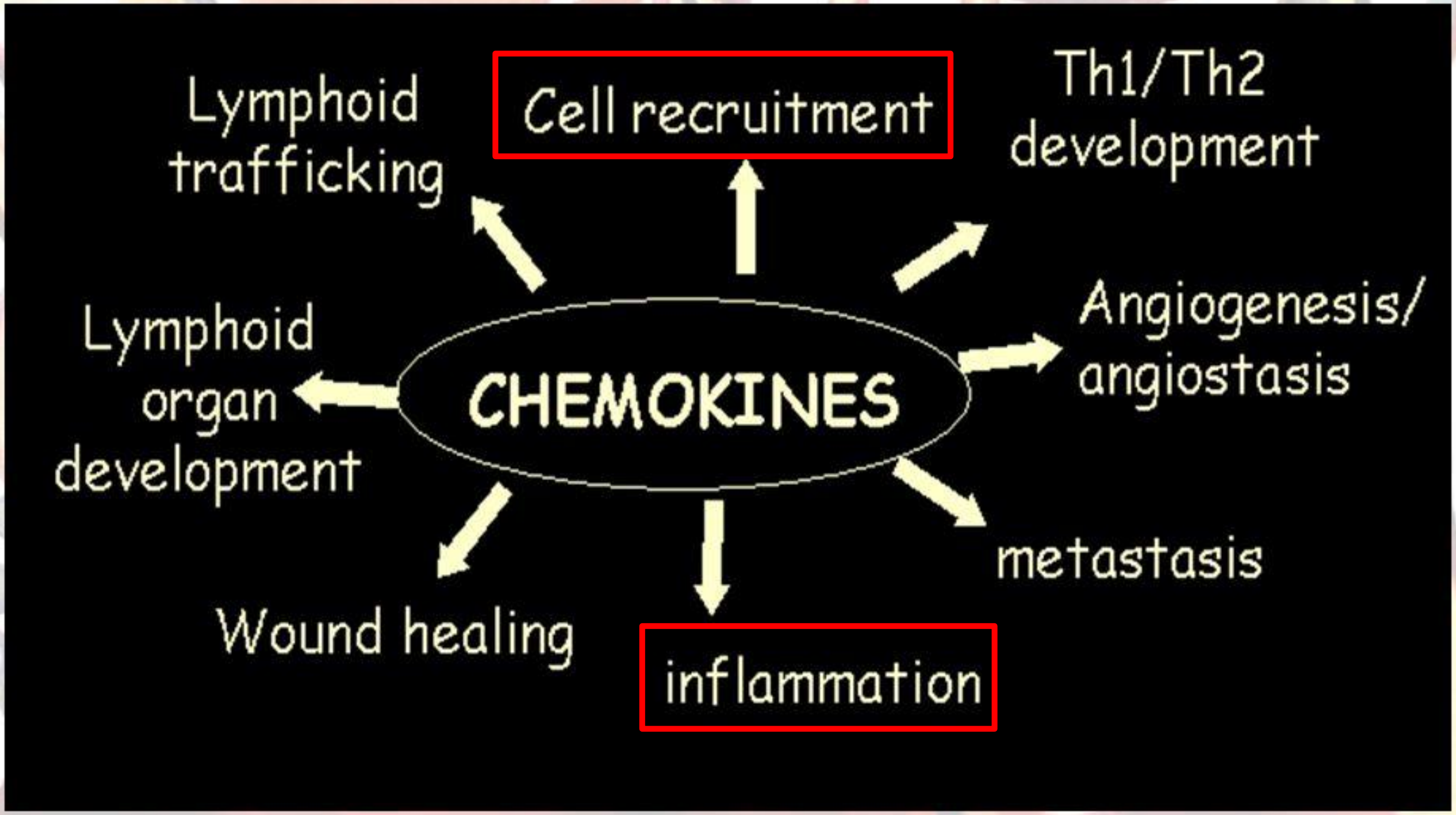

http://altered-states.net/barry/newsletter611/

# Inflammation



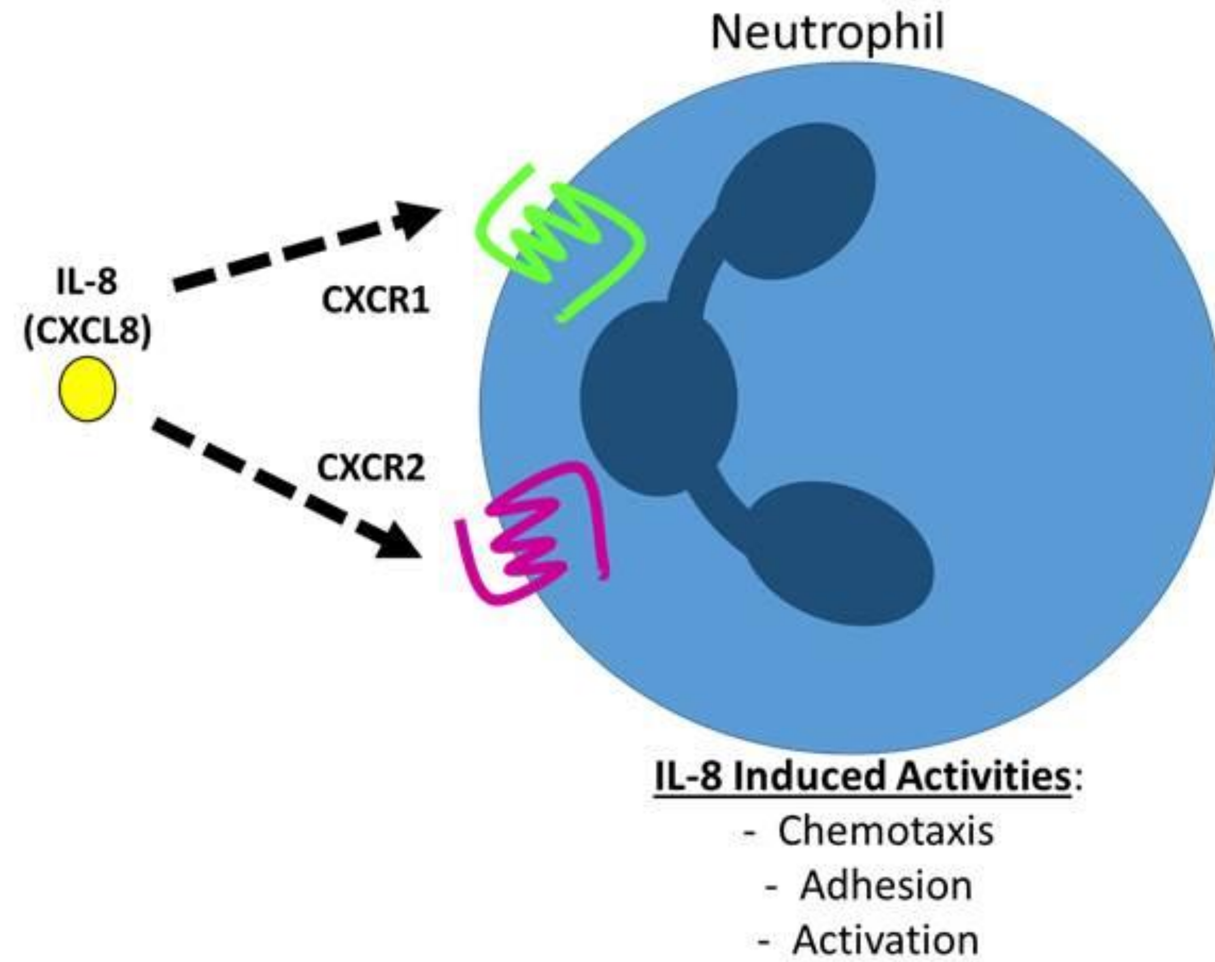

https://montereybayholistic.wordpress.com/2012/12/21/20-ways-to-fight-inflammation

# Chemokine Function



# Interleukin 8 (CXCL8)

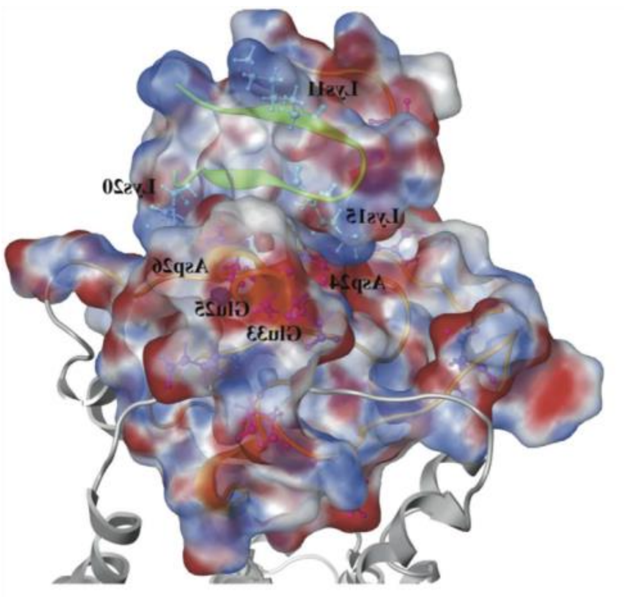
CXCL8



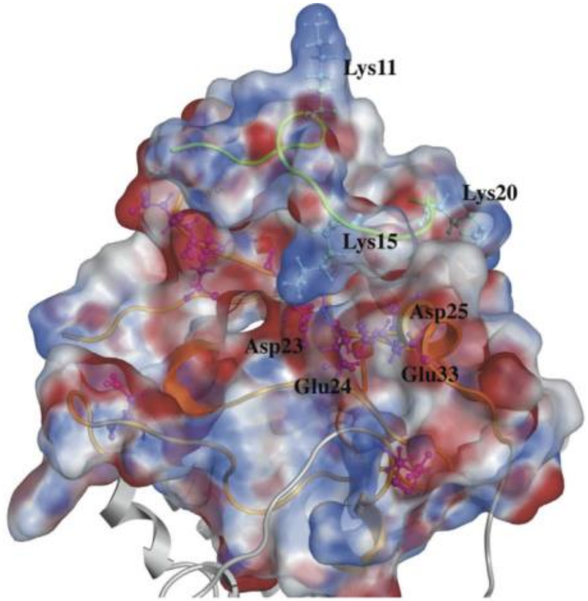

trophils

y factor

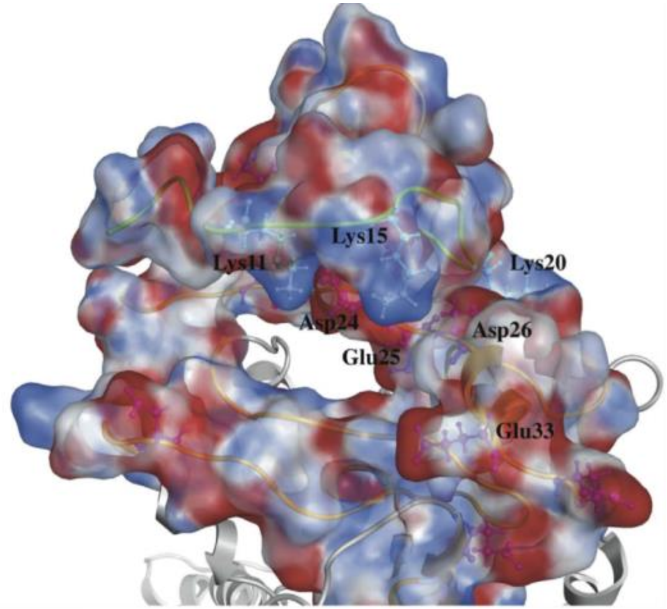
# Peptides design for blockage of CXCR1



p_wt14



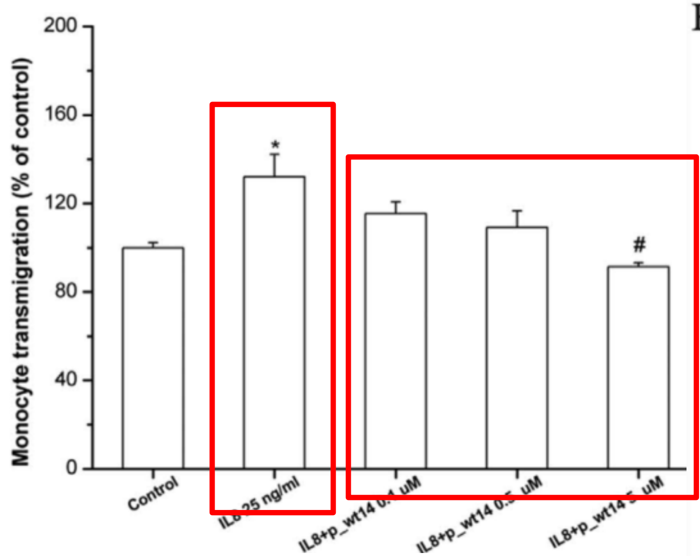

p_wt16




p_wt18

Shinn-Jong J, *et al* Scientific Reports. 2015

A

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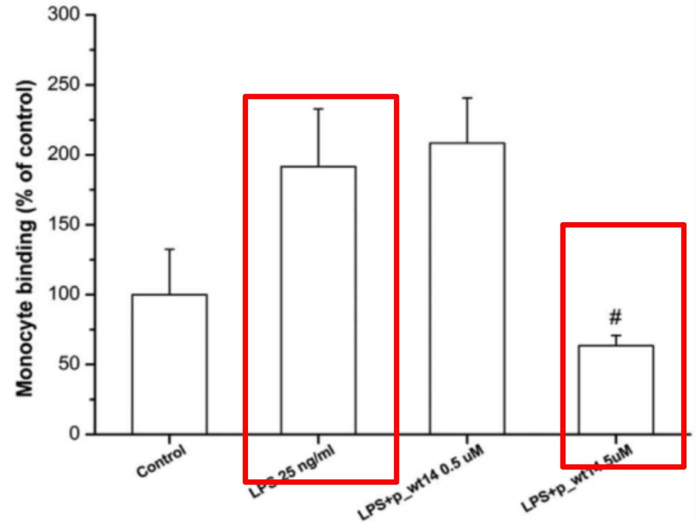
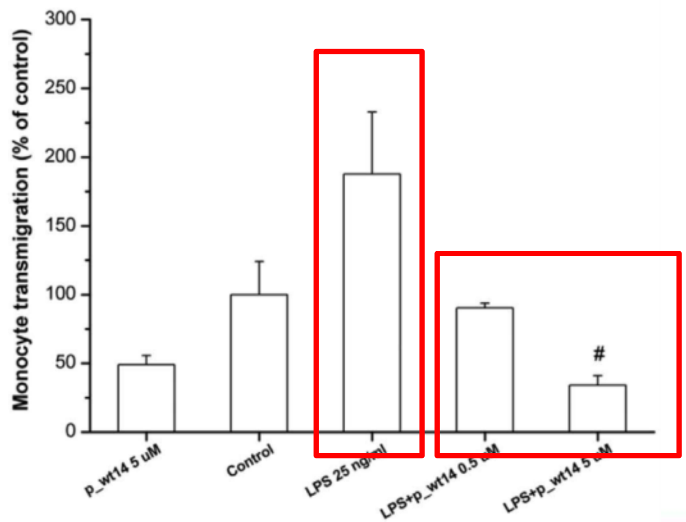
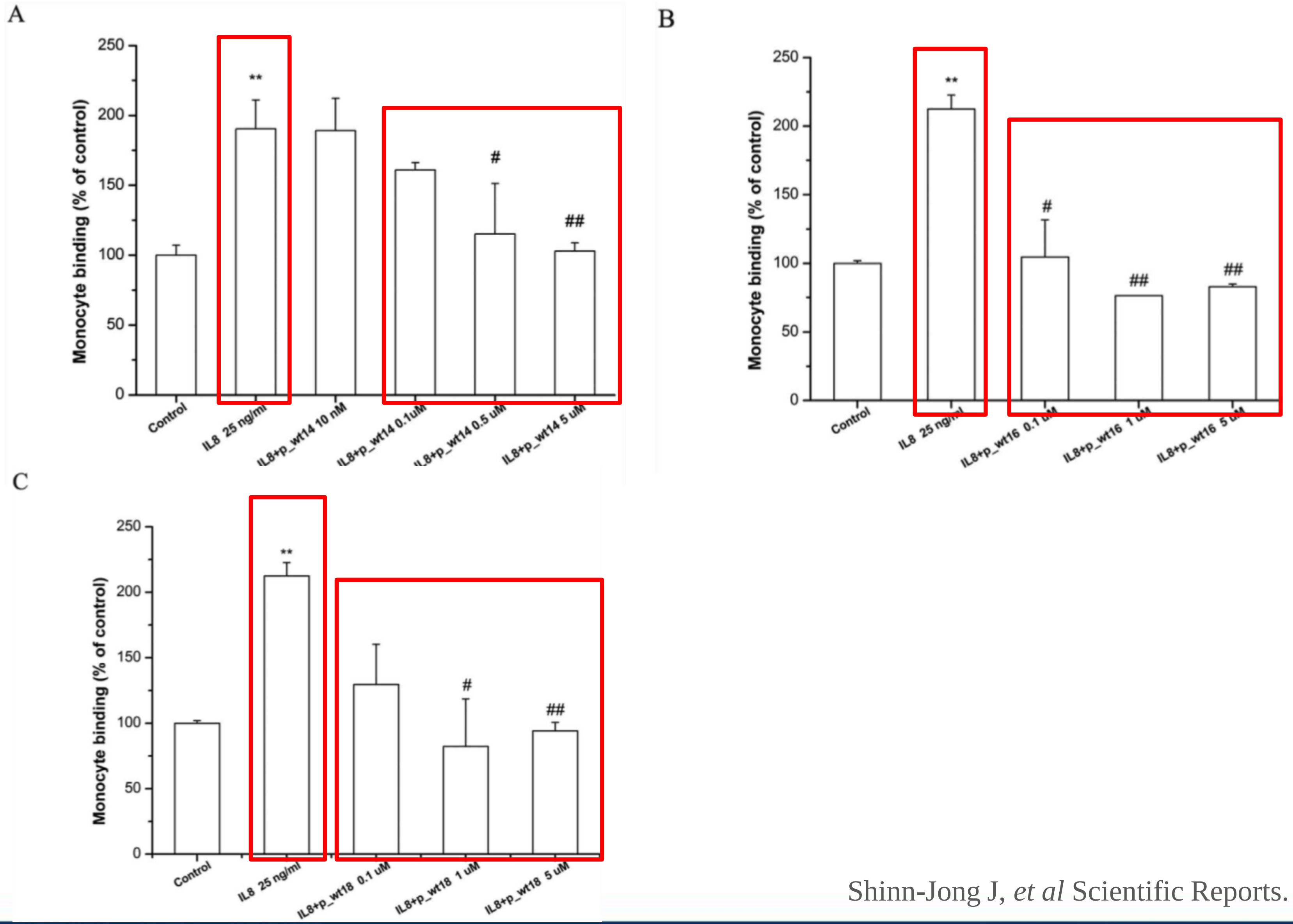



C




Shinn-Jong J, *et al* Scientific Reports. 2015




Shinn-Jong J, *et al* Scientific Reports. 2015

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

# Peptides derived from CXCL8 based on *in silico* analysis inhibit CXCL8 interactions with its receptor CXCR1

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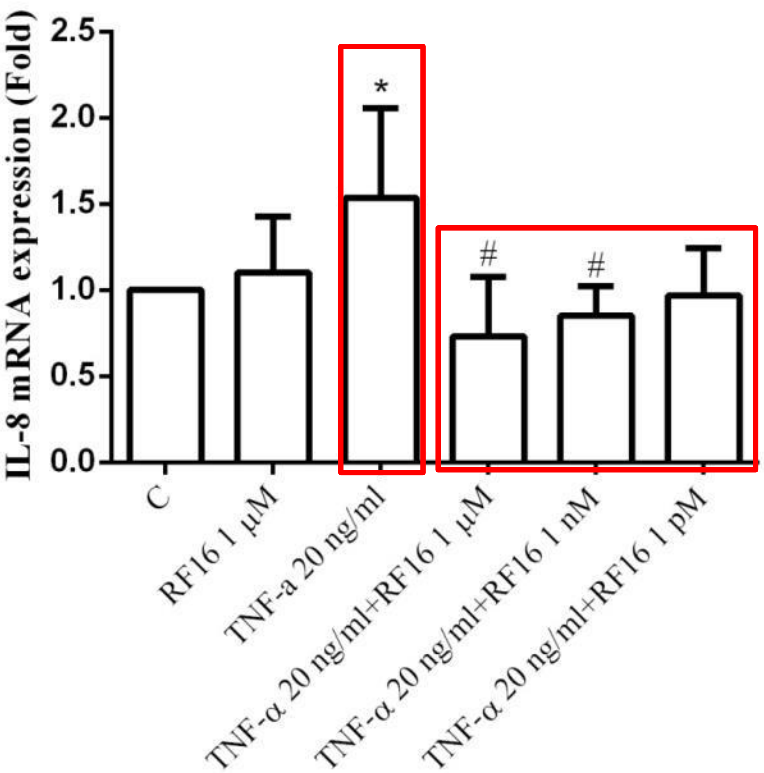
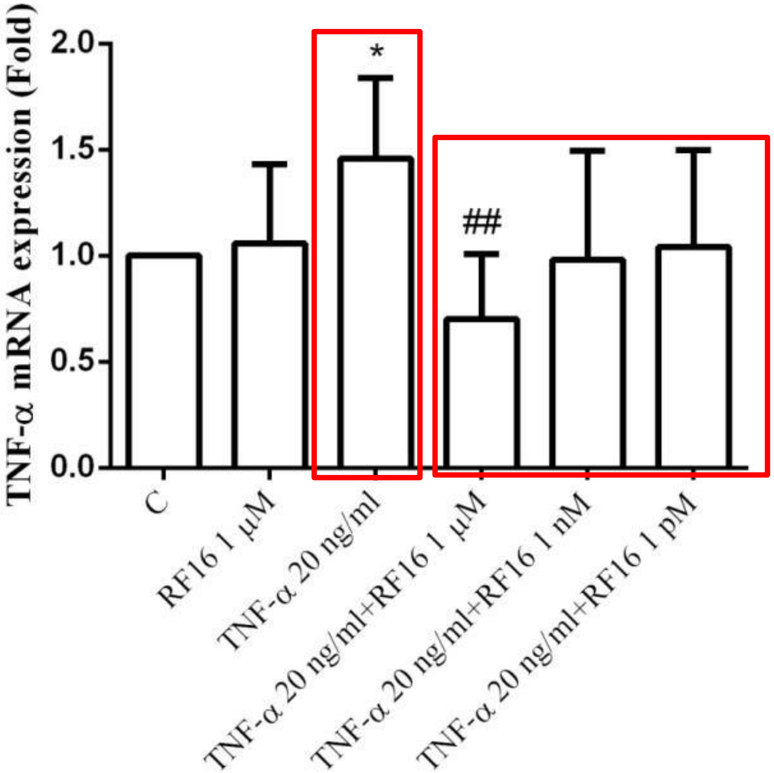
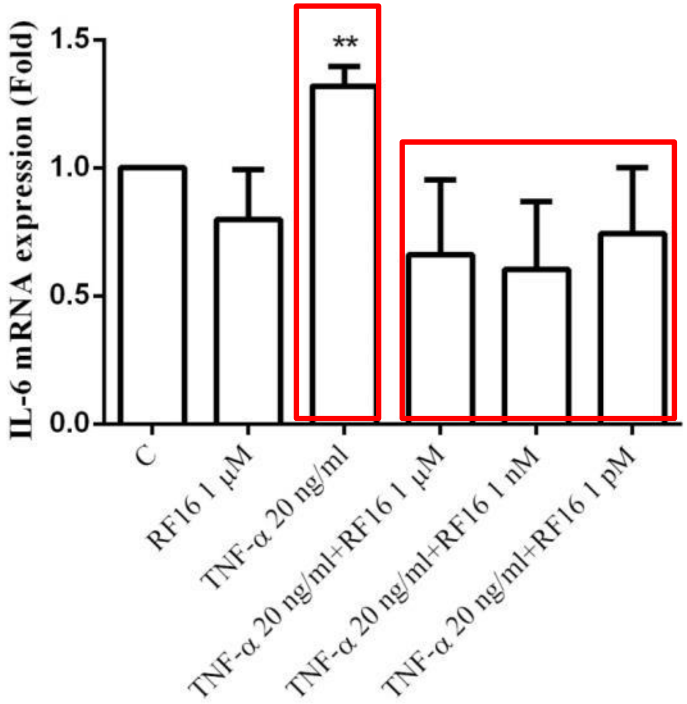
Shinn-Jong Jiang[1], Je-Wen Liou[1,2], Chun-Chun Chang[2,3], Yi Chung[4], Lee-Fong Lin[4] & Hao-Jen Hsu[4]

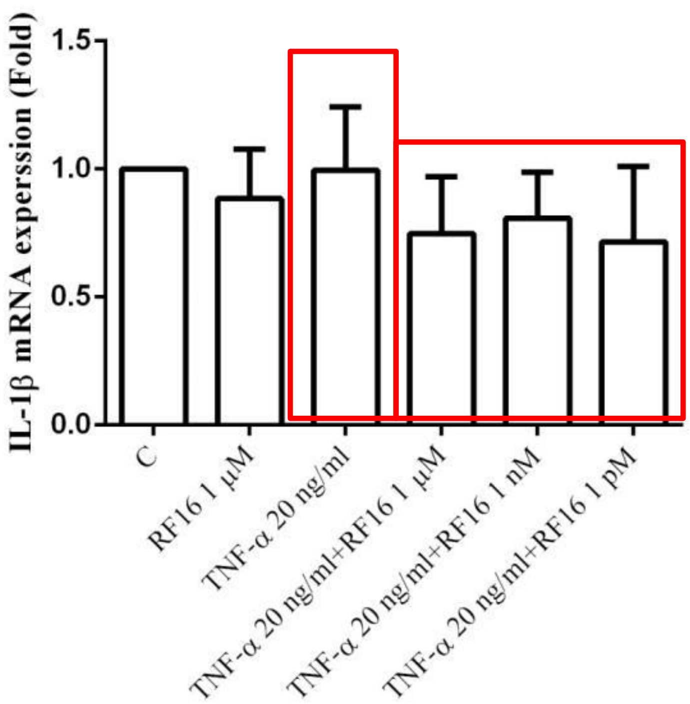
# Effect of RF16 peptide on TNF-α-induced pro-inflammatory cytokine mRNA expressions



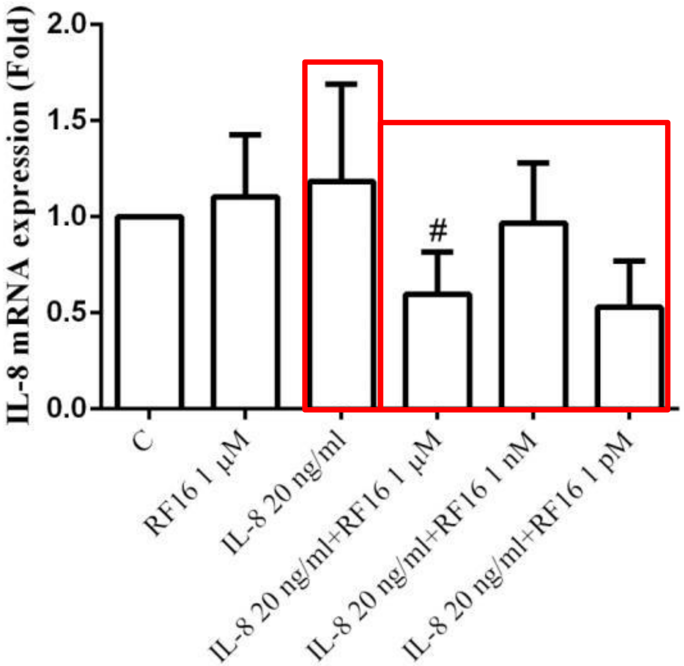



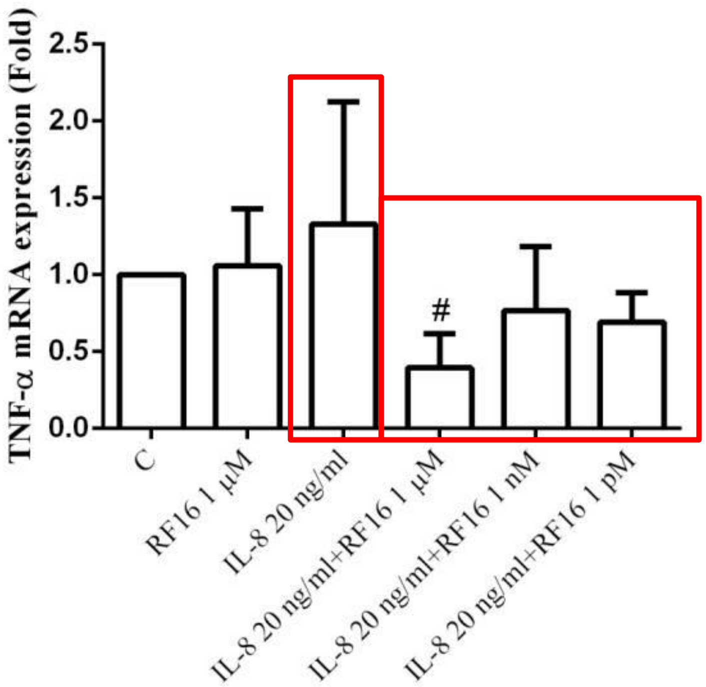
# Effect of RF16 peptide on TNF-α-induced pro-inflammatory cytokine mRNA expressions



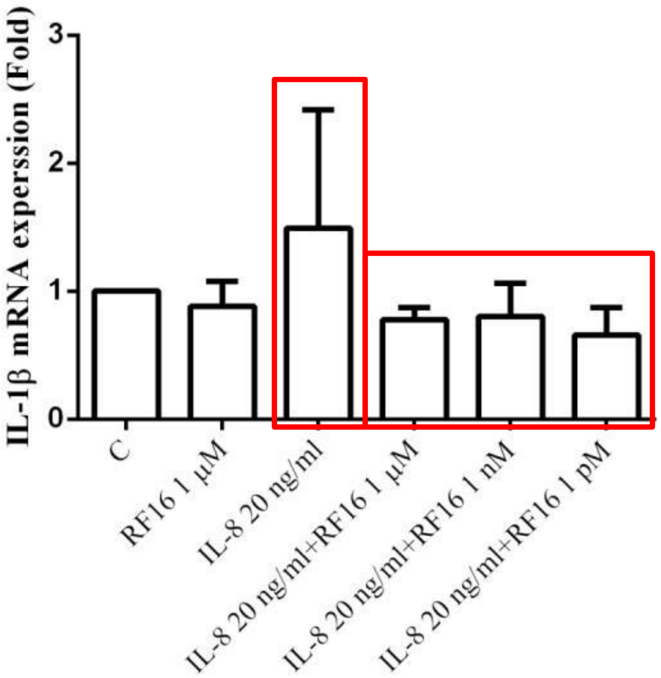



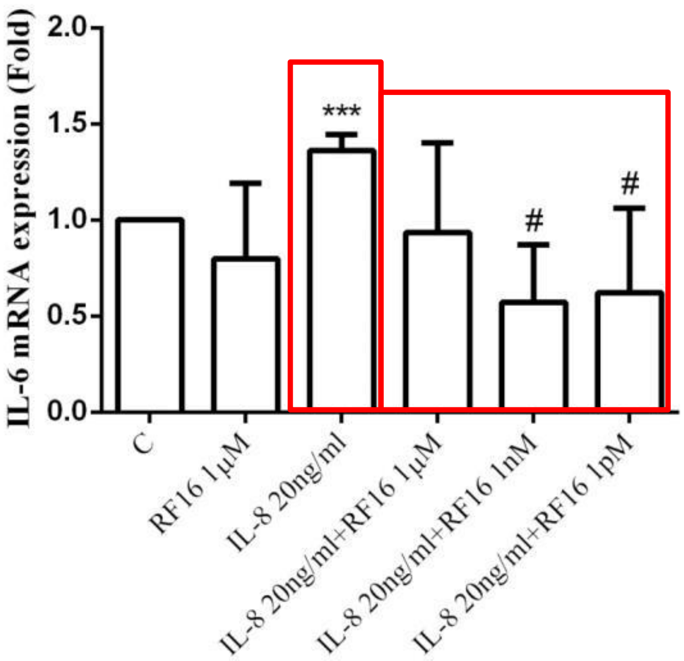
# Effect of RF16 peptide on IL-8 induced pro-inflammatory cytokine mRNA expressions



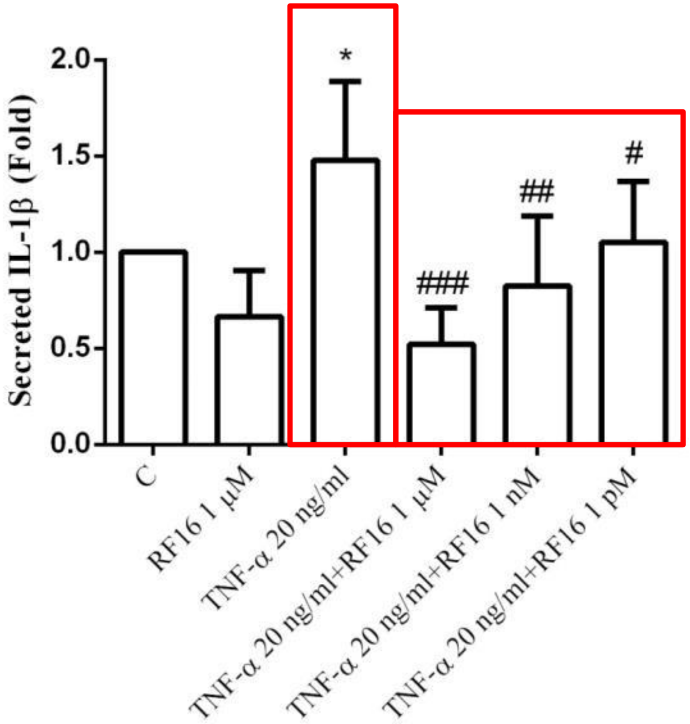



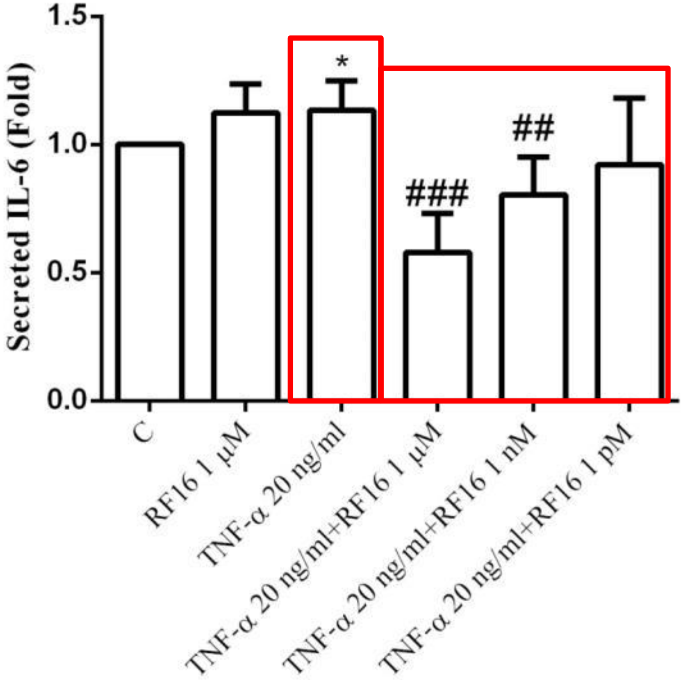
# Effect of RF16 peptide on IL-8 induced pro-inflammatory cytokine mRNA expression



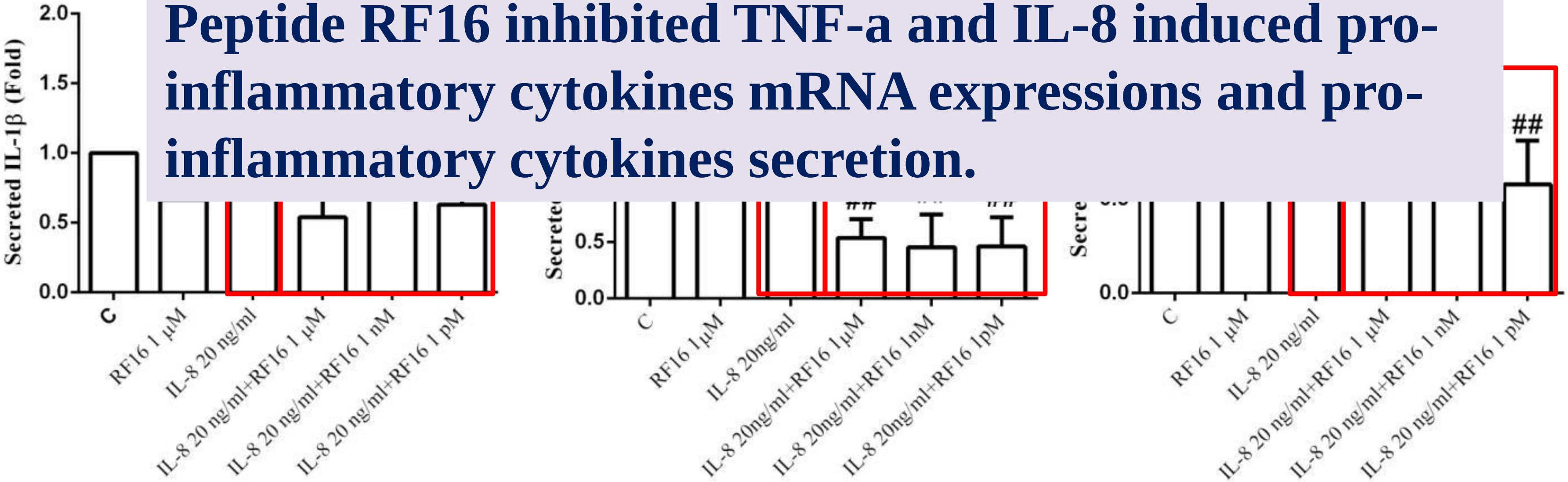
# Effect of RF16 peptide on TNF-α-induced pro-inflammatory cytokines secretion



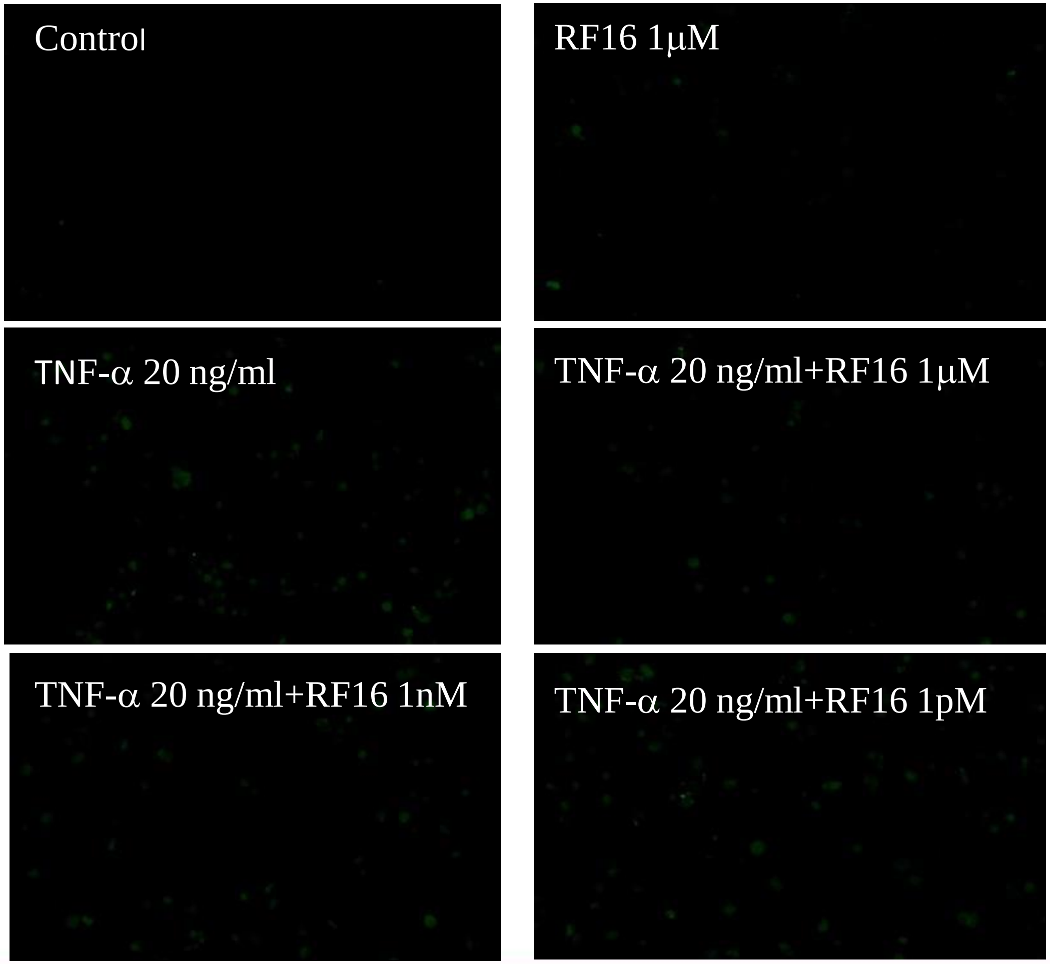



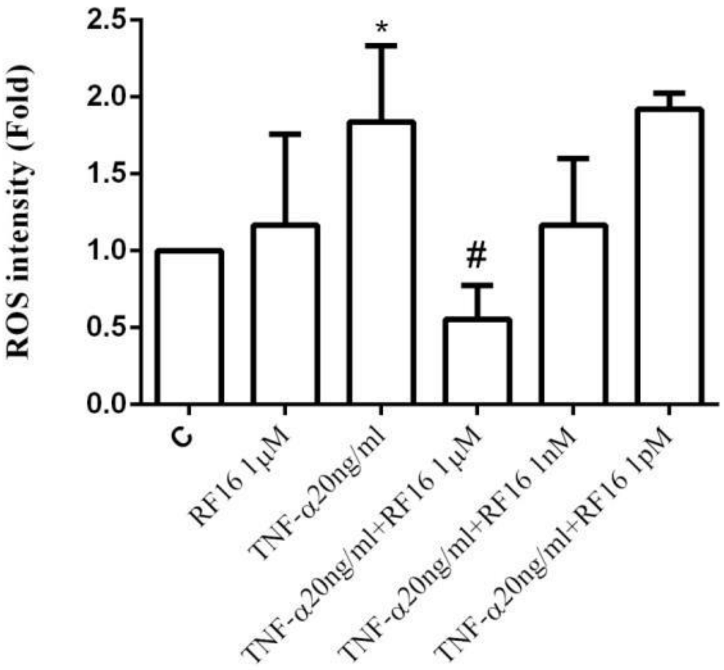
# Effect of RF16 peptide on IL-8 induced pro-inflammatory cytokines secretion

**Peptide RF16 inhibited TNF-a and IL-8 induced pro-inflammatory cytokines mRNA expressions and pro-inflammatory cytokines secretion.**



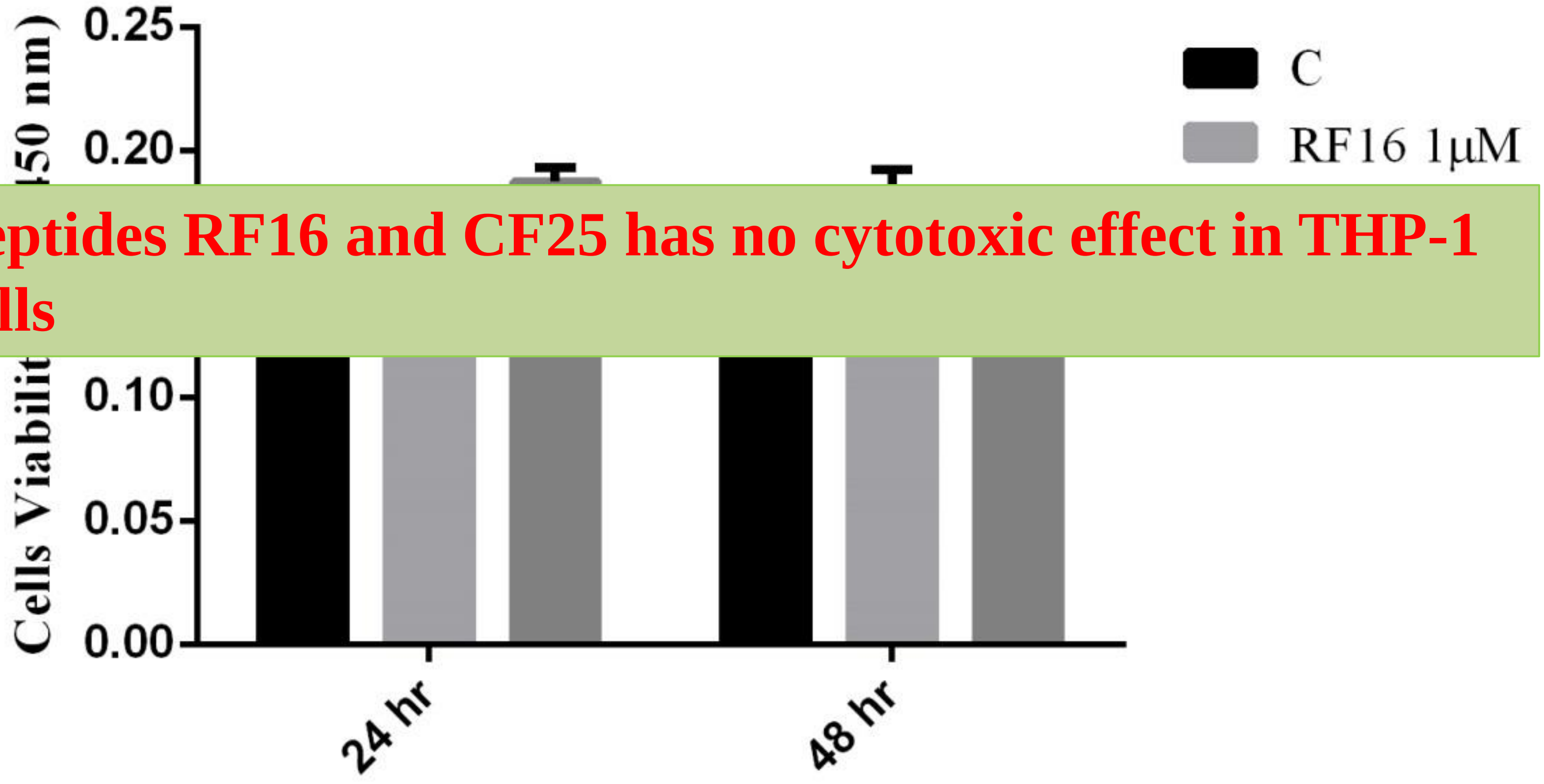
# Effect of RF16 peptide on TNF-α-induced Reactive Oxygen Species



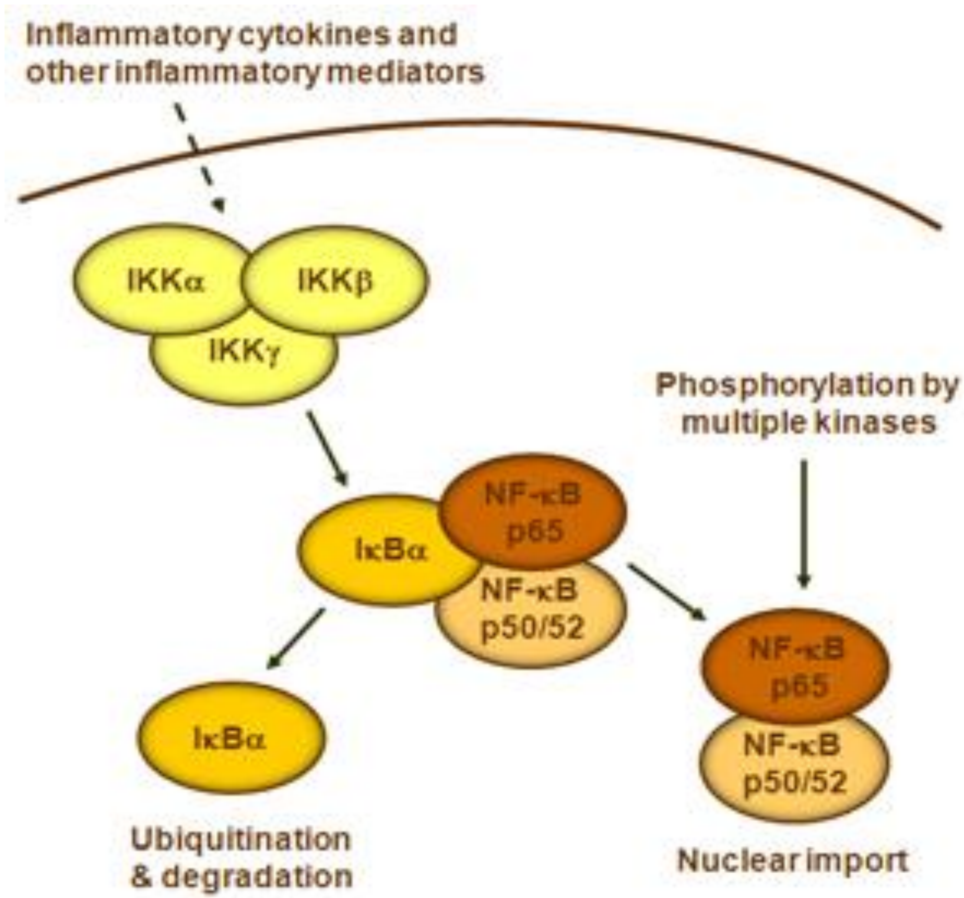

H2DCFDA

# Detection of RF16 and CF25 peptides induced cytotoxicity in THP-1 cells




**Peptides RF16 and CF25 has no cytotoxic effect in THP-1 cells**

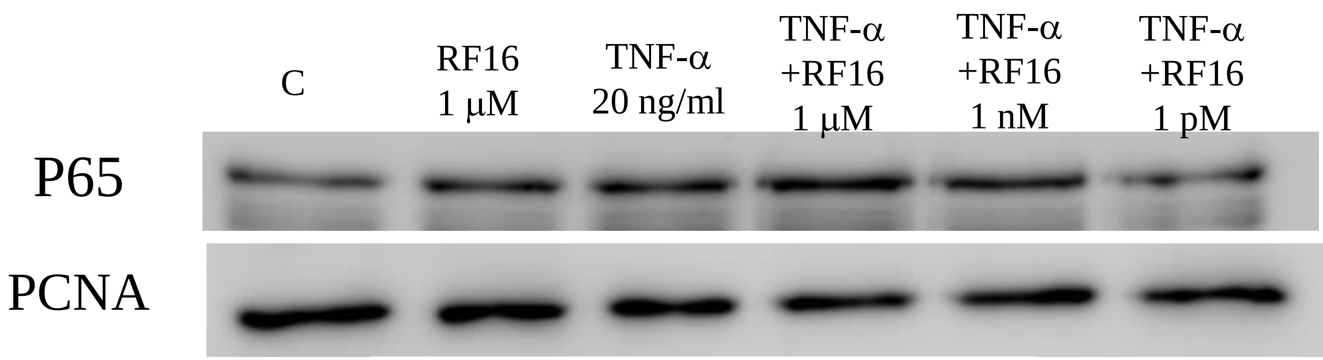
# NF-κB pathway

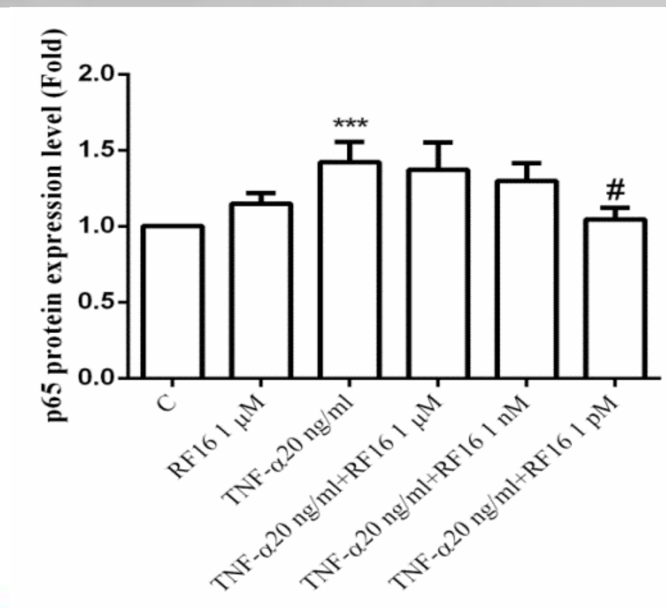


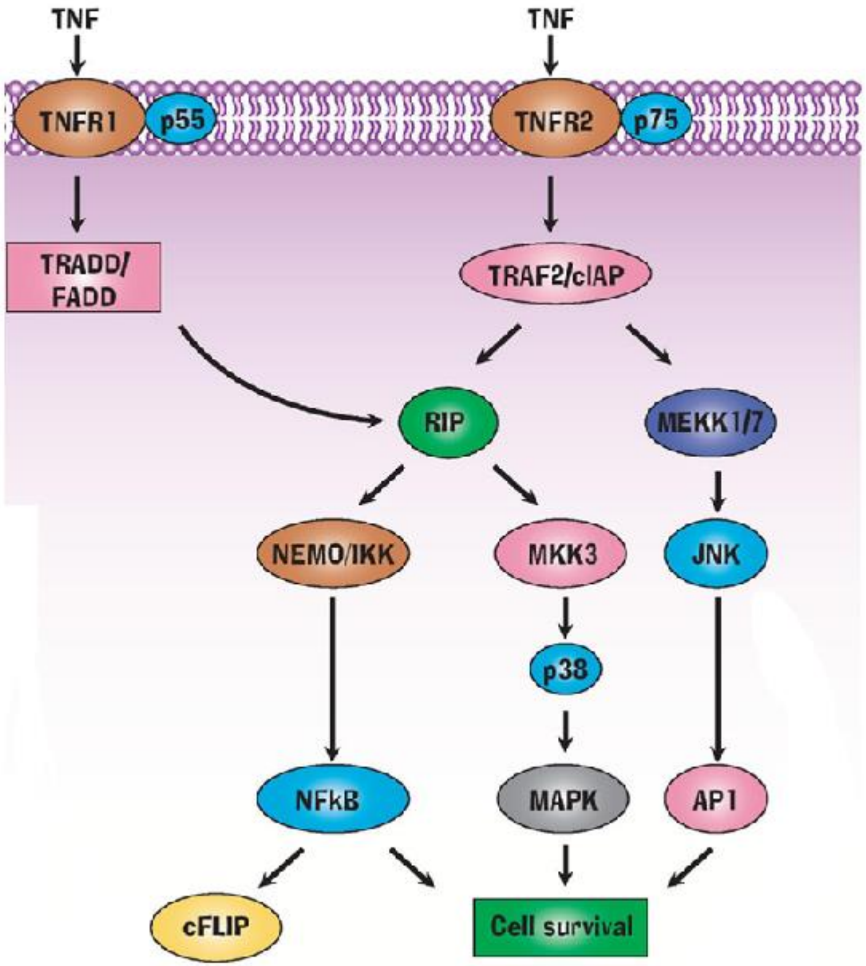



http://tgrbio.com/alphascreen-surefire/surefire-nf-kb-signaling/

# Effect of peptide RF16 on TNF-α-activated NF-κB pathway

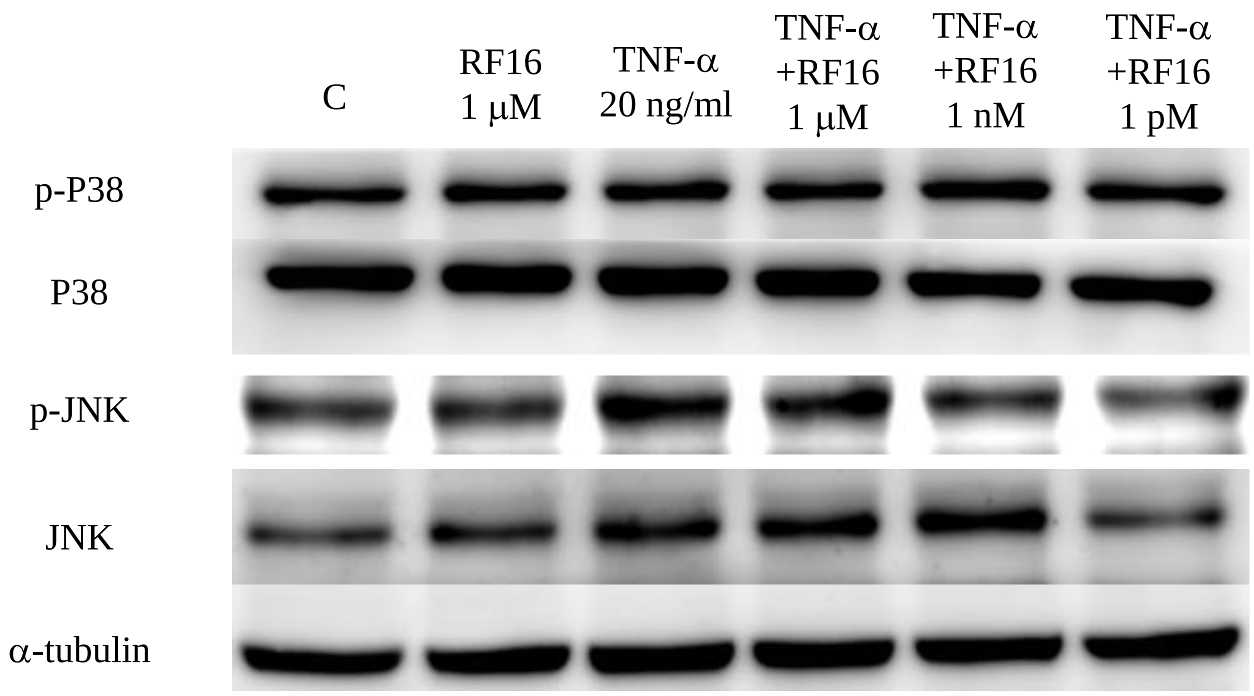


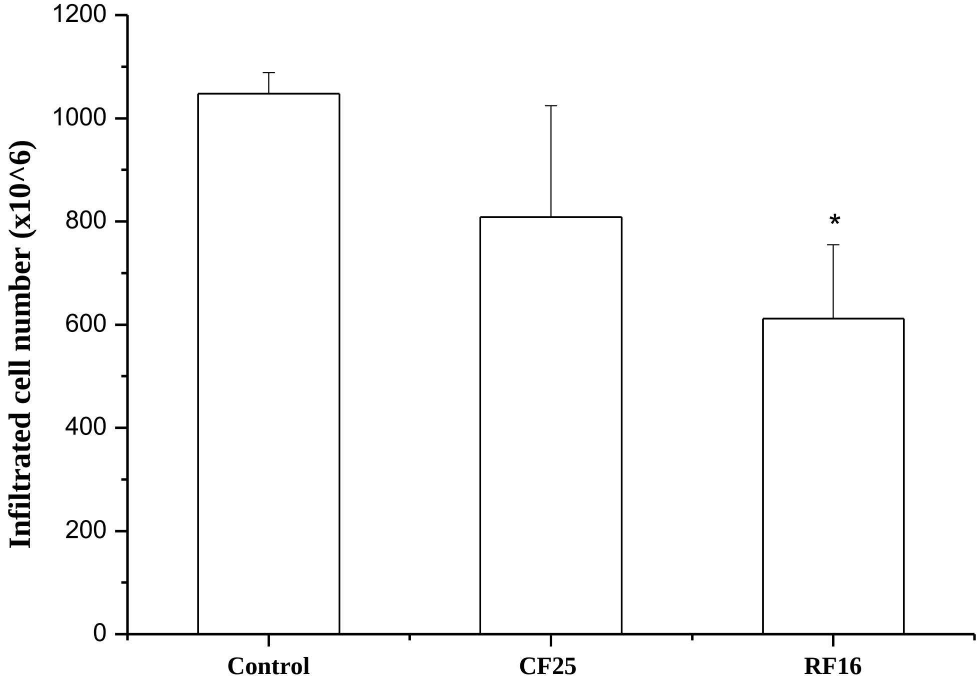



Denise L. Faustman, *et al Front. Immunol.*, 2013

# Effect of RF16 peptide on TNF-α-activated P38/JNK signal pathway



# Effect of RF16 peptide on thioglycolate-induced peritonotis model

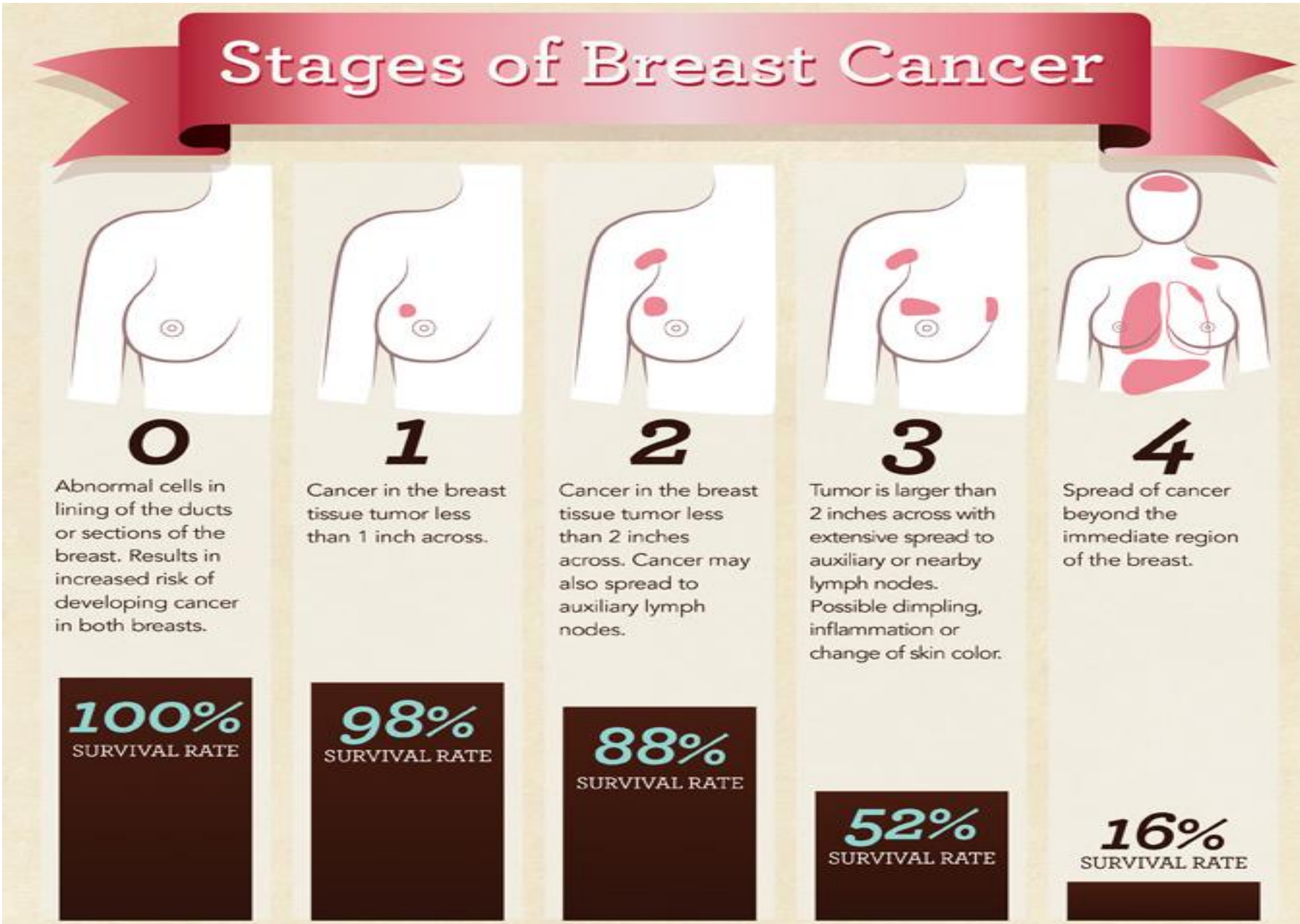


Research article

# CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts

Christophe Ginestier,[1,2] Suling Liu,[1] Mark E. Diebel,[1] Hasan Korkaya,[1] Ming Luo,[3] Marty Brown,[1] Julien Wicinski,[2] Olivier Cabaud,[2] Emmanuelle Charafe-Jauffret,[2] Daniel Birnbaum,[2] Jun-Lin Guan,[3] Gabriela Dontu,[1] and Max S. Wicha[1]

2010 Feb;120(2):485-97. doi: 10.1172/JCI39397.


Stages of Breast Cancer
0
Abnormal cells in lining of the ducts or sections of the breast. Results in increased risk of developing cancer in both breasts.
100% SURVIVAL RATE
1
Cancer in the breast tissue tumor less than 1 inch across.
98% SURVIVAL RATE
2
Cancer in the breast tissue tumor less than 2 inches across. Cancer may also spread to auxiliary lymph nodes.
88% SURVIVAL RATE
3
Tumor is larger than 2 inches across with extensive spread to auxiliary or nearby lymph nodes. Possible dimpling, inflammation or change of skin color.
52% SURVIVAL RATE
4
Spread of cancer beyond the immediate region of the breast.
16% SURVIVAL RATE

# Breast Cancer Subtypes

| Breast cancer subtype | Estrogen receptor and/or Progesterone receptor | HER2 |
|---|---|---|
| **ER-positive** (Hormone receptor positive) | + | - |
| **HER2-positive** | + or - | +++ |
| **Triple-negative** | - | - |

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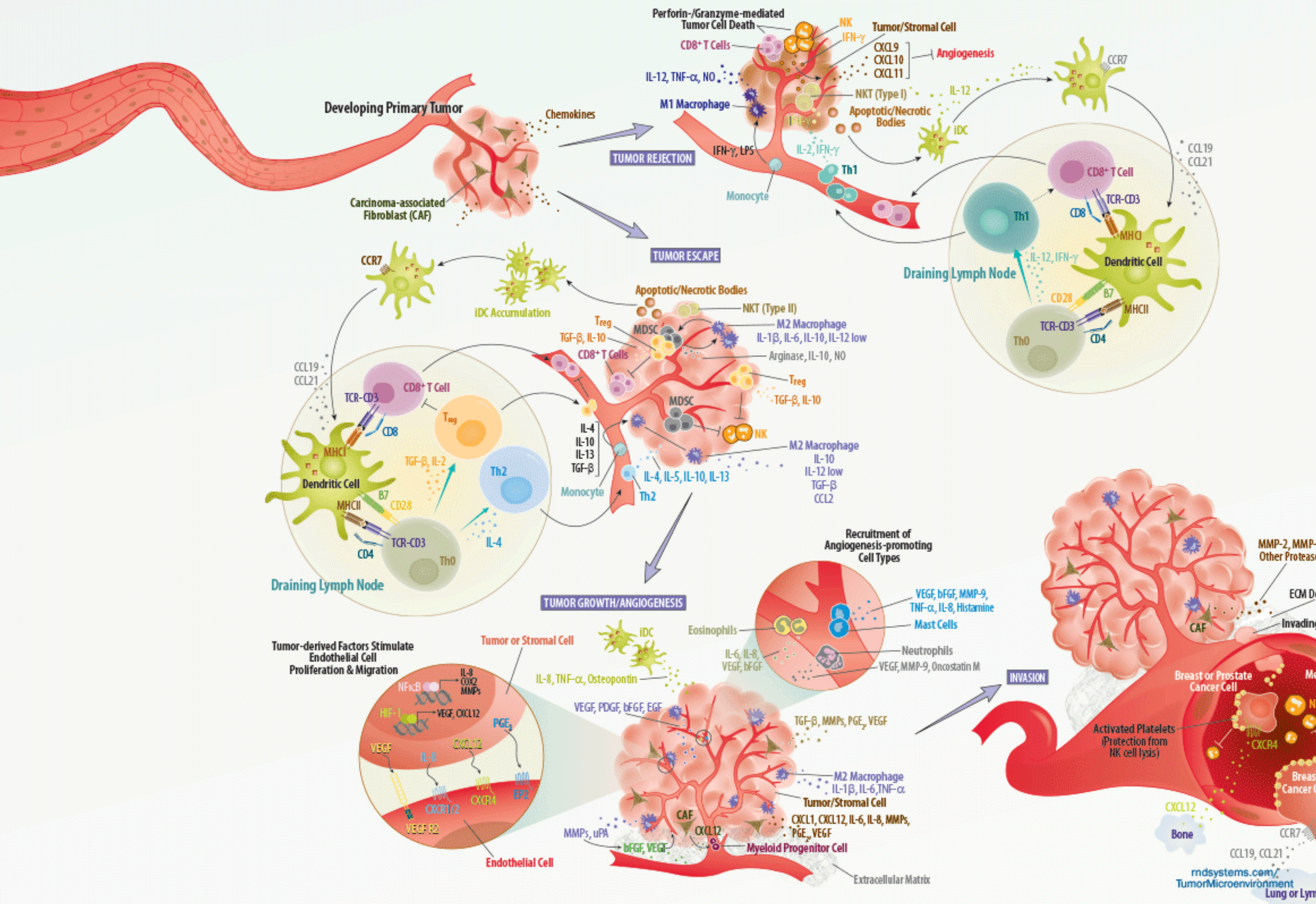
# Microenvironmental Regulation of Tumor Growth & Metastasis

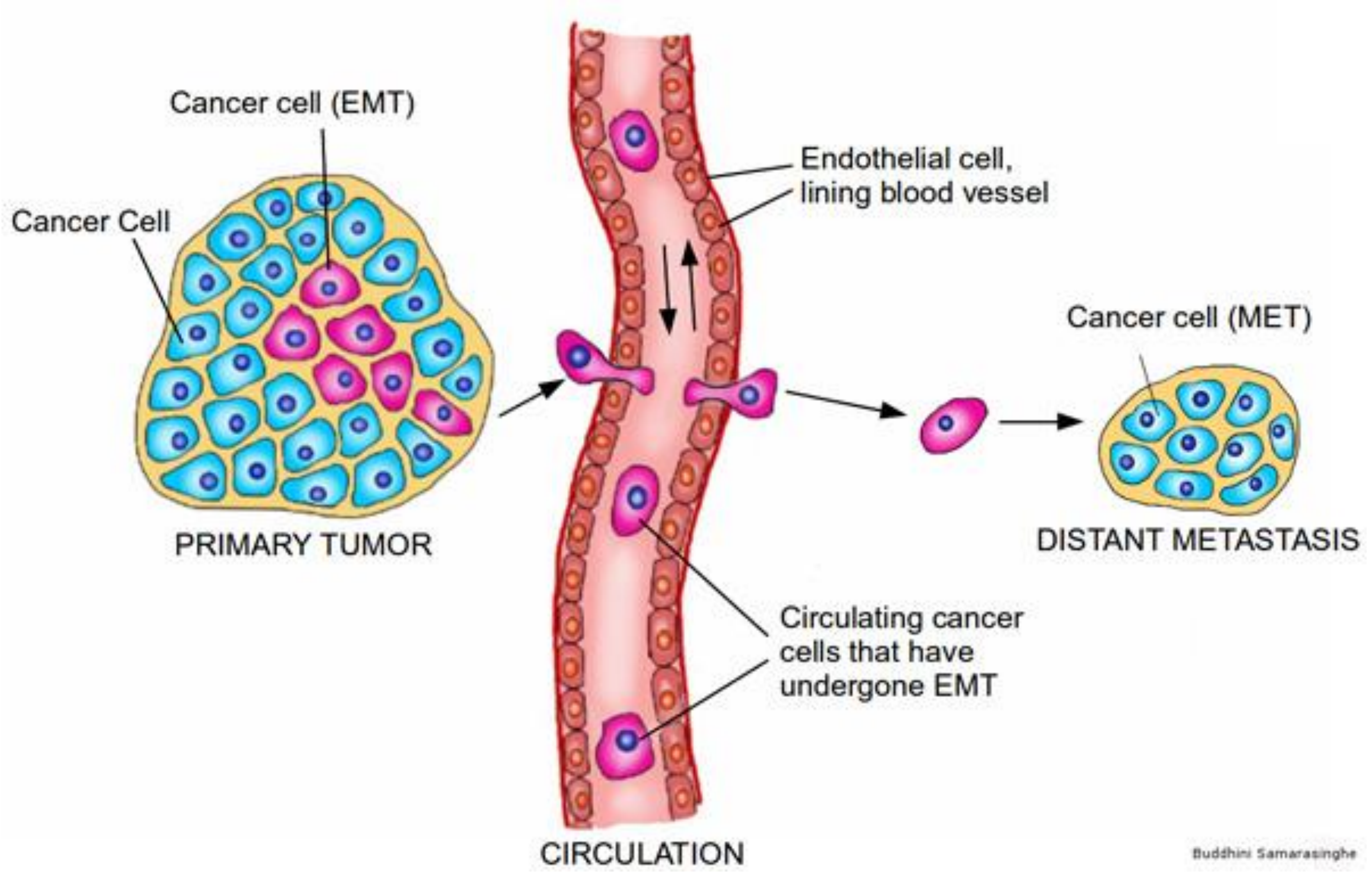



**TUMOR-DERIVED CHEMOKINES/CYTOKINES RECRUIT IMMUNE EFFECTOR CELLS THAT MAY PROMOTE TUMOR GROWTH**

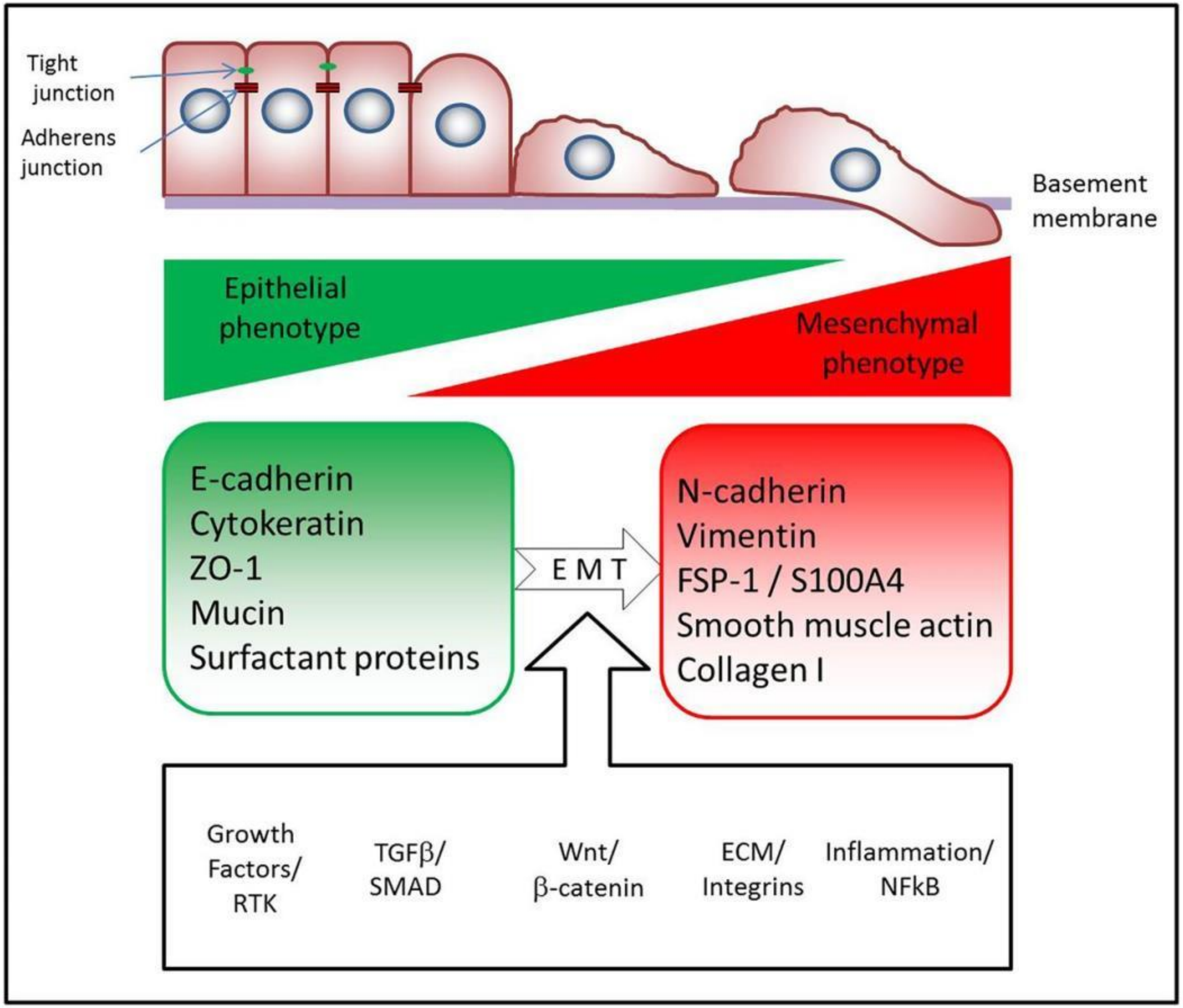
| Cell Type – Tumor-promoting Effects | Receptors | Tumor-derived Chemokines/Cytokines |
|---|---|---|
| **Monocytes/Macrophages (Type 2) – Immunosuppression, proliferation & angiogenesis** | | |
| | CCR1, CCR5 | CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL8/MCP-2 |
| | CCR2 | CCL2/MCP-1 |
| | M-CSF R | M-CSF |
| **Dendritic Cells – Immunosuppression, tumor antigen tolerance, angiogenesis** | | |
| | CCR1, CCR5 | CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES |
| | CCR6 | CCL20/MIP-3α, β-Defensin |
| | CXCR1, CXCR2 | CXCL8/IL-8 |
| | CXCR4 | CXCL12/SDF-1 |
| **Regulatory T Cells ($T_{reg}$) & Th2 Cells – Immunosuppression** | | |
| | CCR4 | CCL17/TARC, CCL22/MDC |
| **Myeloid-derived Suppressor Cells – Immunosuppression, proliferation & angiogenesis** | | |
| | CCR2 | CCL2/MCP-1 |
| | CXCR2 | CXCL5 |
| | CXCR4 | CXCL12/SDF-1 |
| **Mast Cells – Immunosuppression, proliferation & angiogenesis** | | |
| | CCR3 | CCL11/Eotaxin |
| | CCR5 | CCL5/RANTES |
| | CXCR4 | CXCL12/SDF-1 |
| | SCF R/c-kit | SCF/c-kit Ligand |
| **Neutrophils – Proliferation & angiogenesis** | | |
| | CXCR1 | CXCL6/GCP-2, CXCL8/IL-8 |
| | CXCR2 | CXCL1/GROα, CXCL2/GROβ, CXCL3/GROγ, CXCL5, CXCL8/IL-8 |
| **Eosinophils – Angiogenesis** | | |
| | CCR3 | CCL11/Eotaxin |

NOTE: This poster conveys a general overview and should be considered neither comprehensive nor definitive. The details of this information are understood to be subject to interpretation. 

# Metastasis

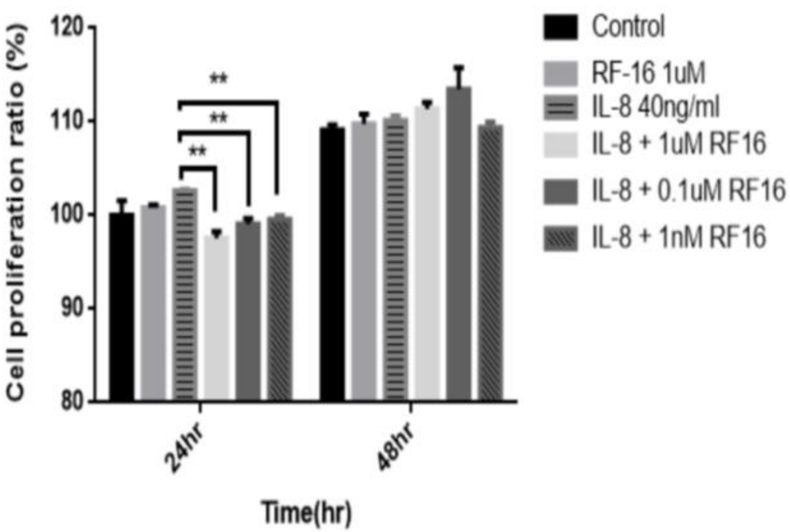

Cancer cell (EMT)
Cancer Cell
Endothelial cell, lining blood vessel
Cancer cell (MET)
PRIMARY TUMOR
DISTANT METASTASIS
Circulating cancer cells that have undergone EMT
CIRCULATION
Buddhini Samarasinghe

# EMT (epithelial-mesenchymal transition)

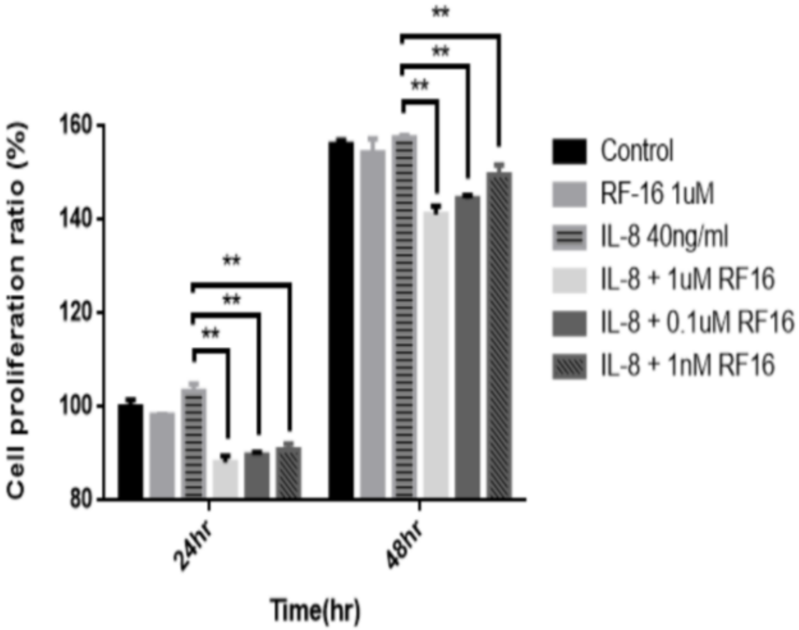


# Effect of RF16 peptide on IL-8-induced proliferation

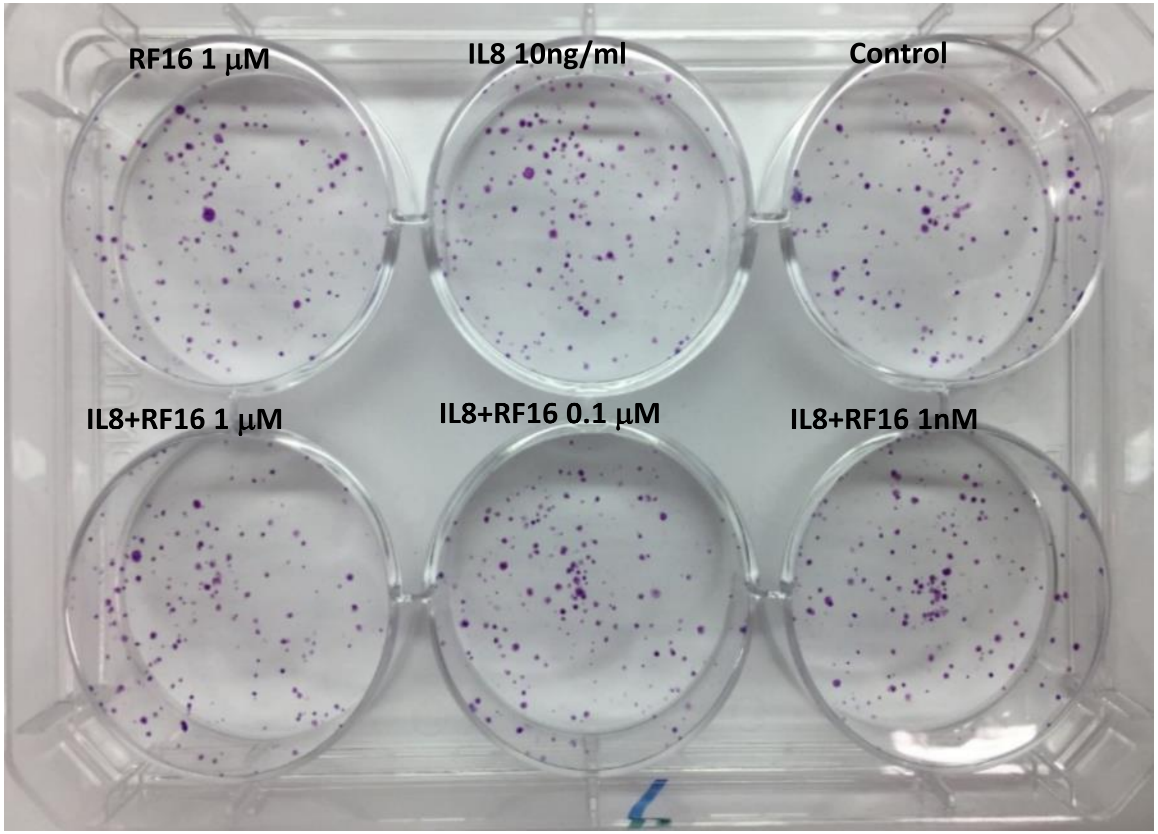
**MCF-7**



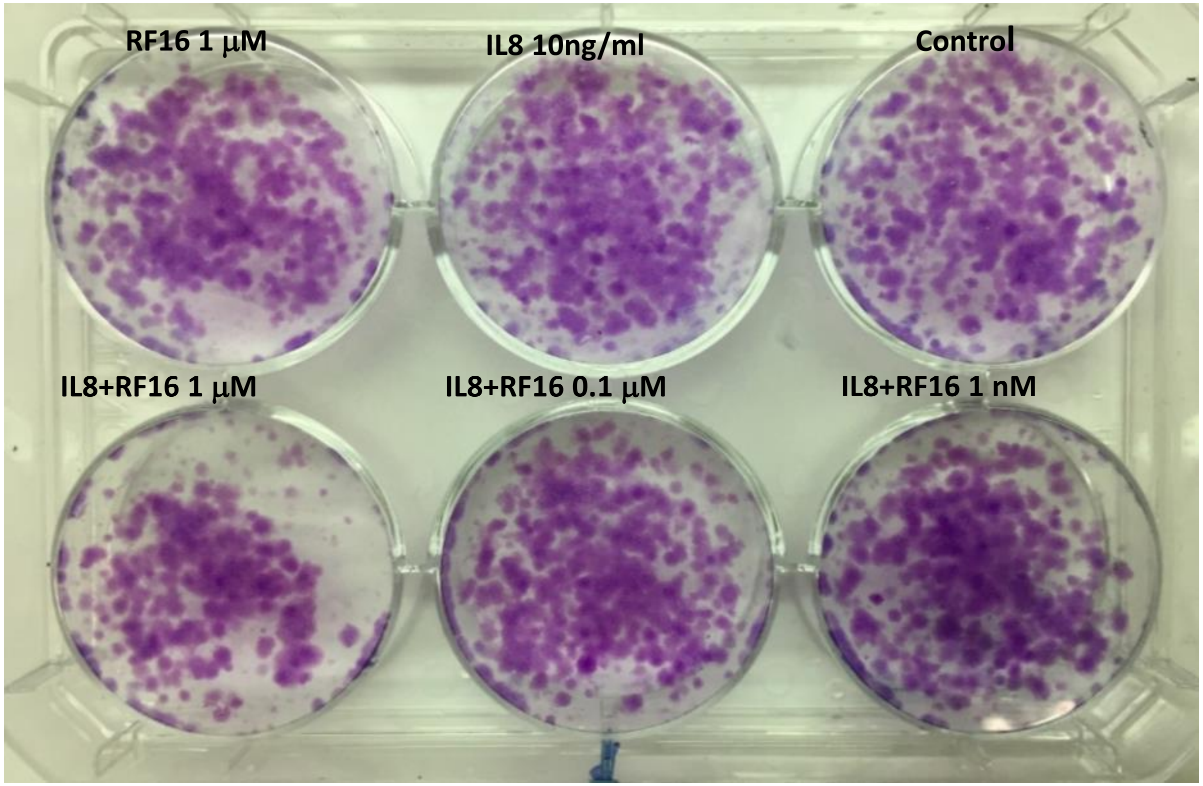

**MDA-MB-231**



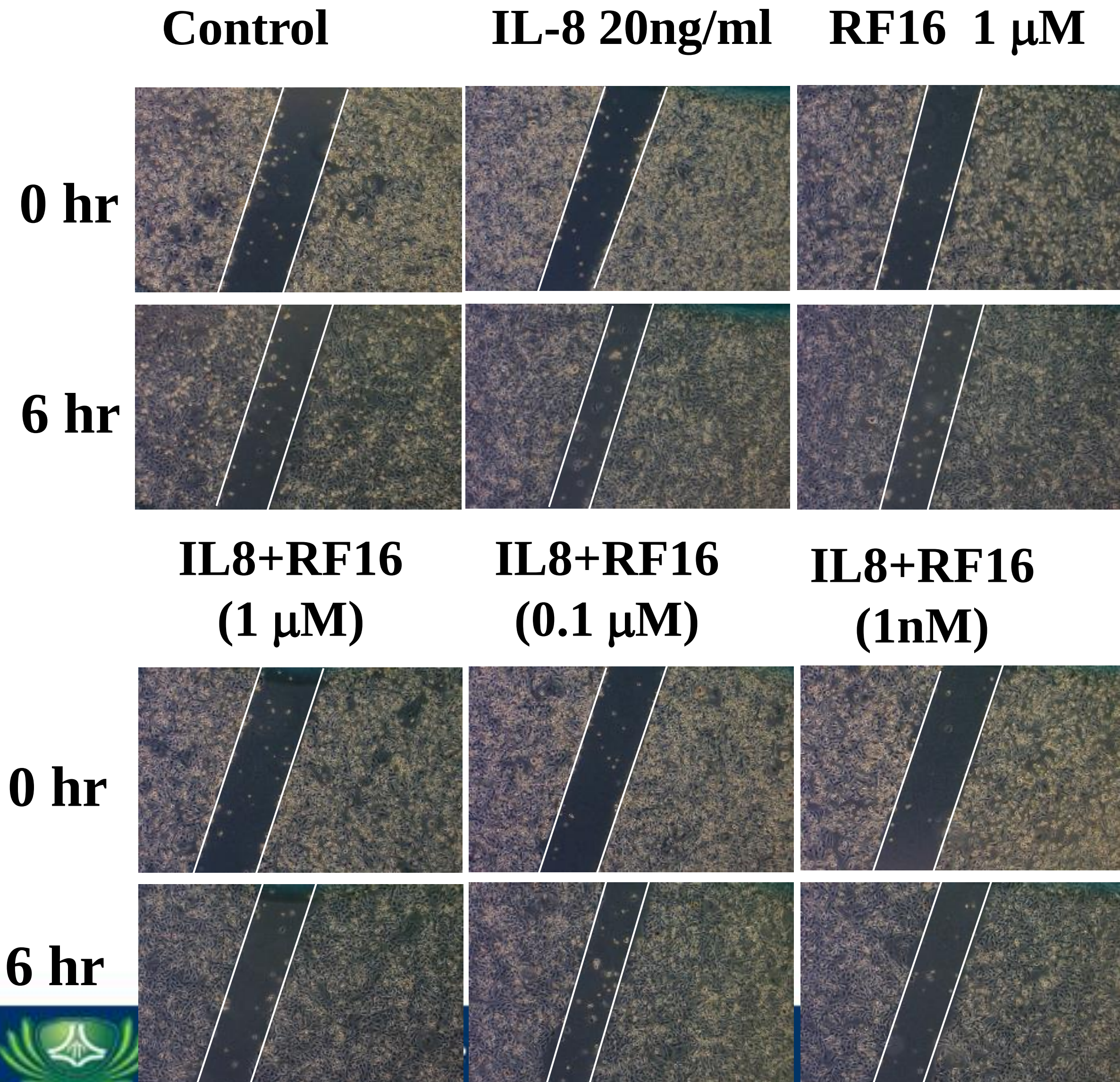
# Colony Formation (MCF-7)



# Colony Formation (MDA-MB-231)

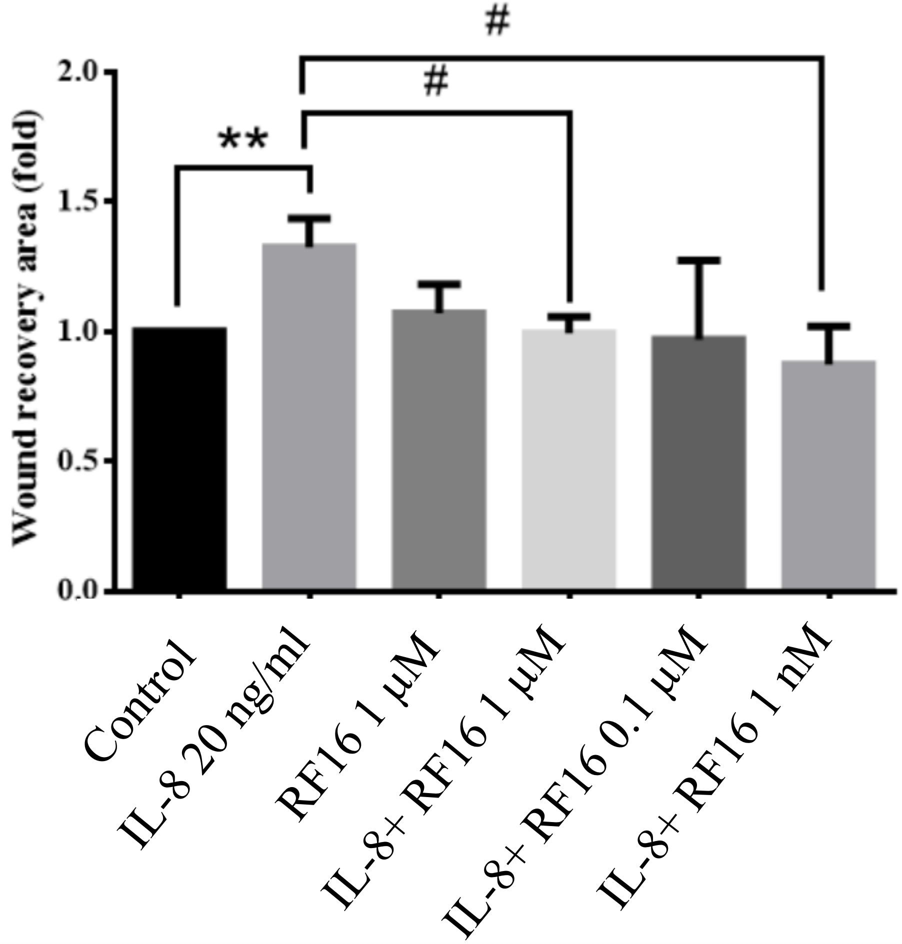


# Effect of RF16 peptide on IL-8-induced migration




MDA-MB-231

# Effect of RF16 peptide on IL-8-induced migration



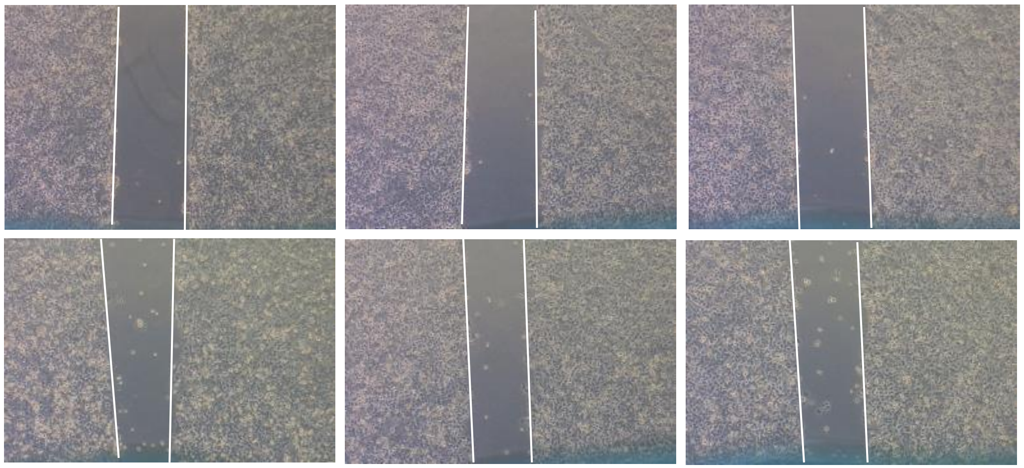
# Effect of RF16 peptide on IL-8-induced migration

MCF-7

**Control** | **IL-8 40ng/ml** | **RF16 1 μM**

**0 hr**

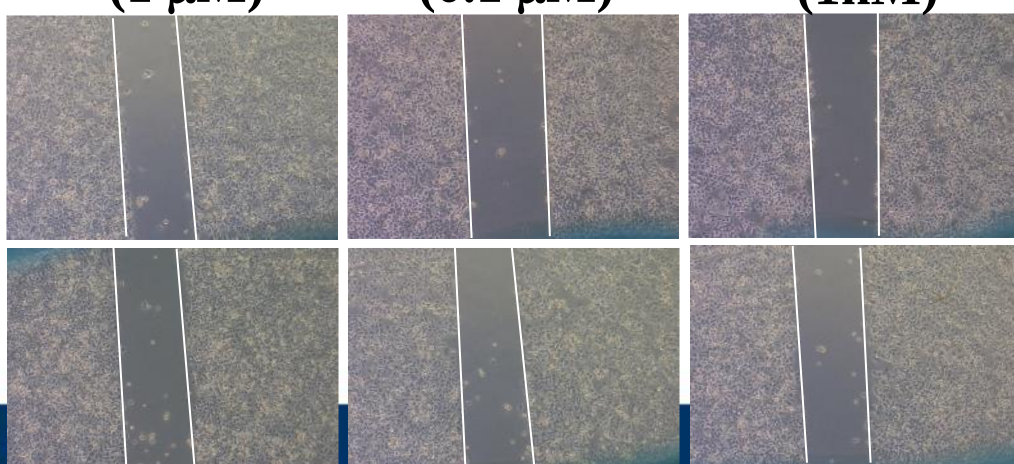
**24 hr**



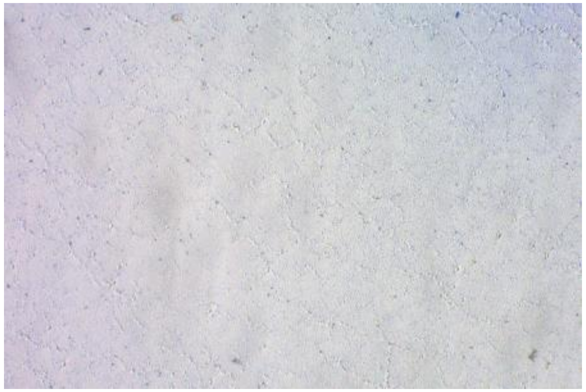
**IL8+RF16 (1 μM)** | **IL8+RF16 (0.1 μM)** | **IL8+RF16 (1nM)**

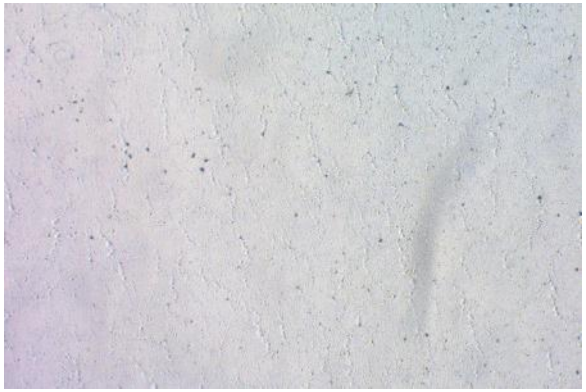
**0 hr**

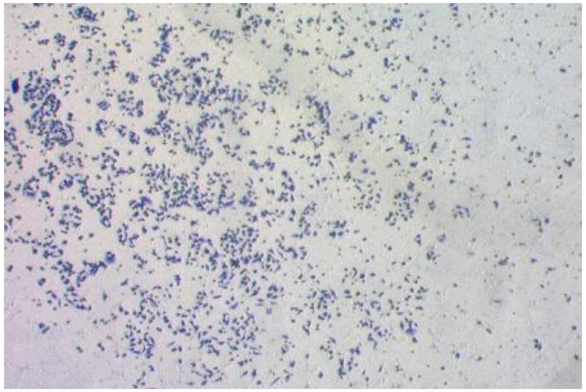
**24 hr**

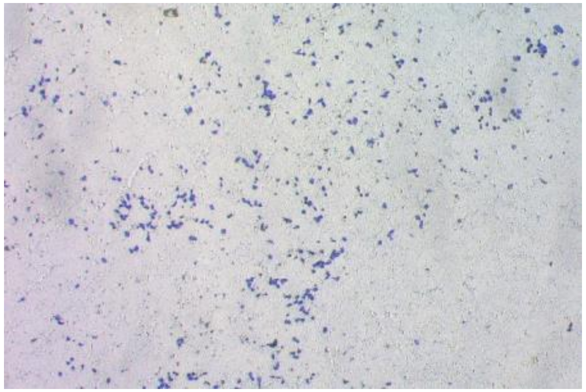


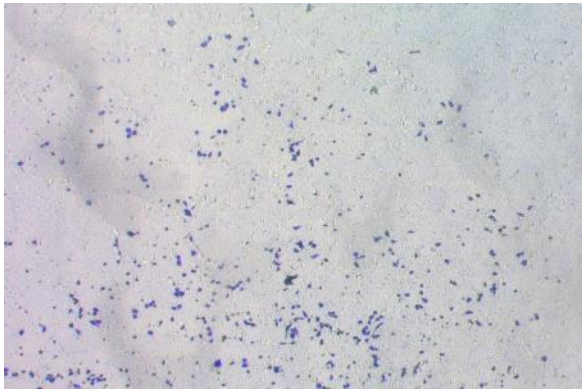
# Effect of RF16 peptide on IL-8-induced invasion

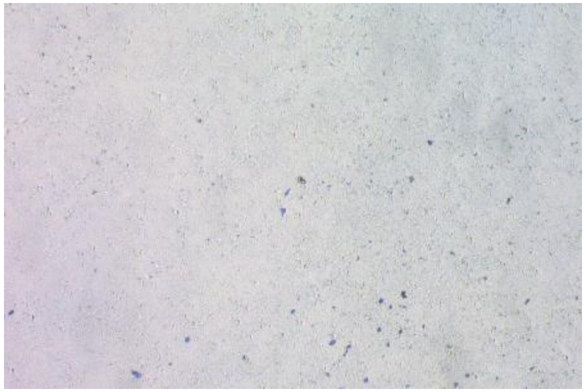

Control


RF16 1uM

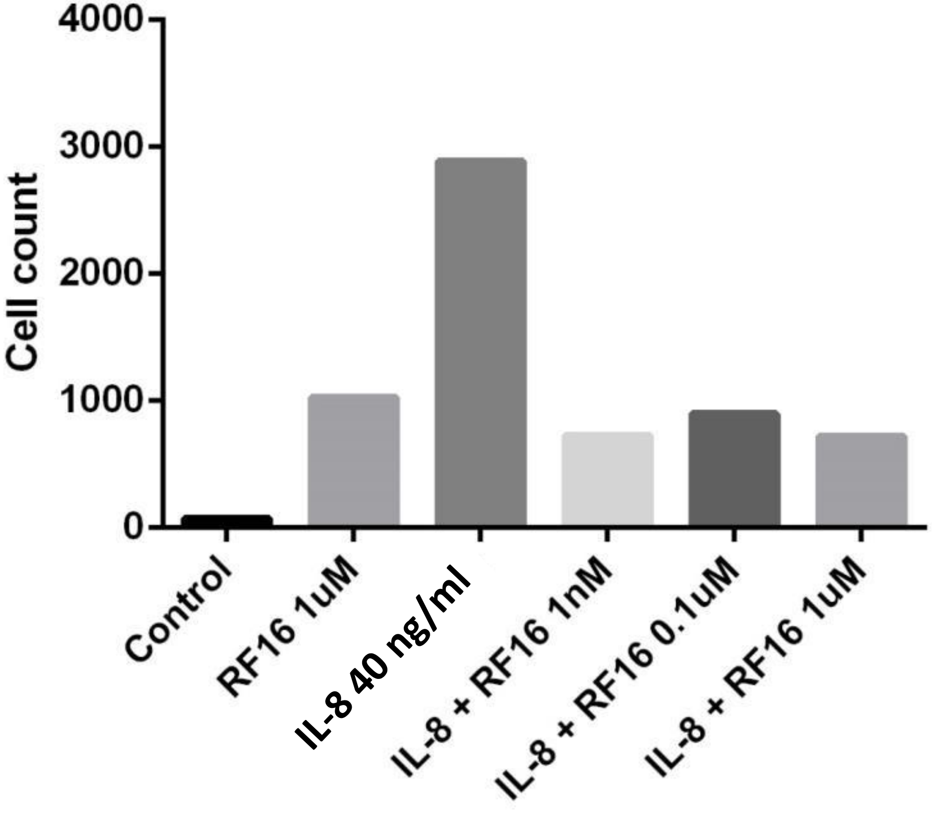

IL-8 40 ng/ml


IL-8 + RF16 1nM


IL-8 + RF16 0.1uM


IL-8 + RF16 1uM

# Effect of RF16 peptide on IL-8-induced invasion



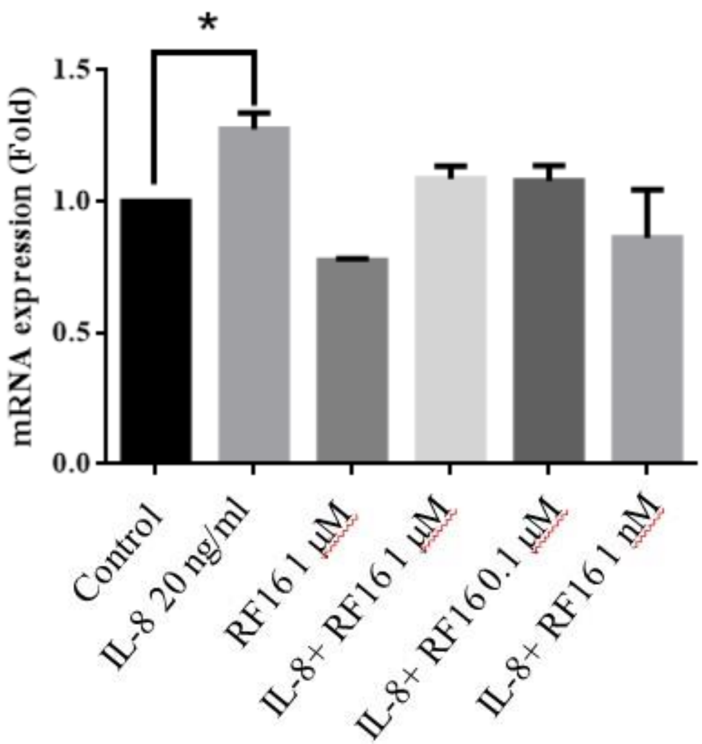
# Effect of RF16 peptide on IL-8-induced MMP activity expressions

**IL-8 50 ng/ml**

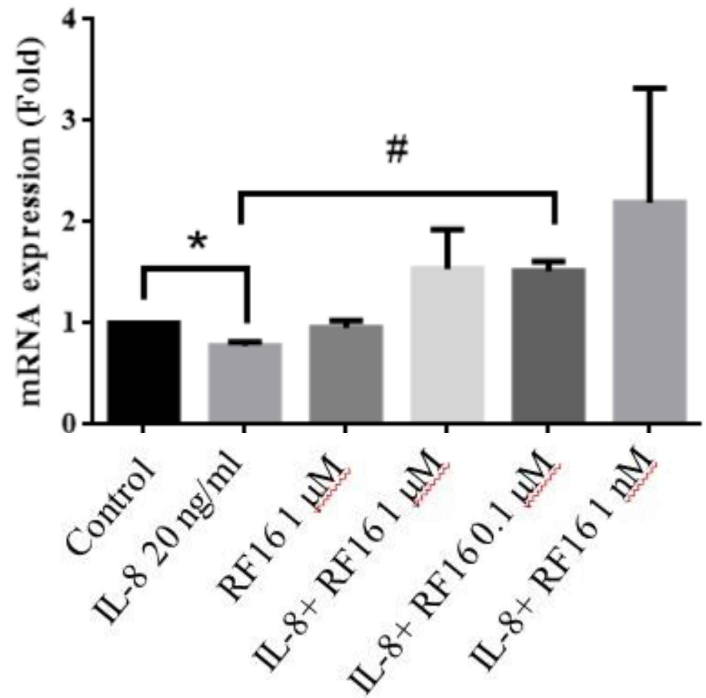
| Control | - | RF16 1 nM | RF16 0.1 μM | RF16 1 μM | RF16 1 nM | RF16 0.1 μM | RF16 1 μM |
|---|---|---|---|---|---|---|---|

# Effect of RF16 peptide on IL-8-modulated E-cadherin and Fibronectin mRNA expressions

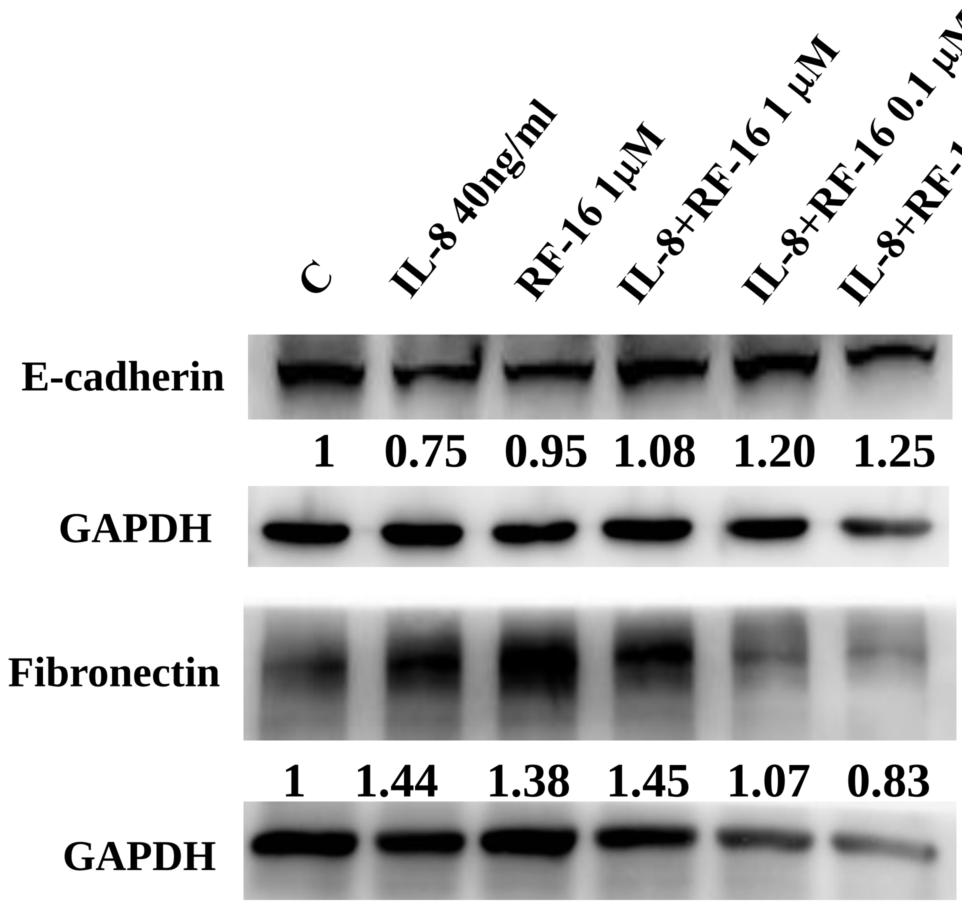
Fibronectin



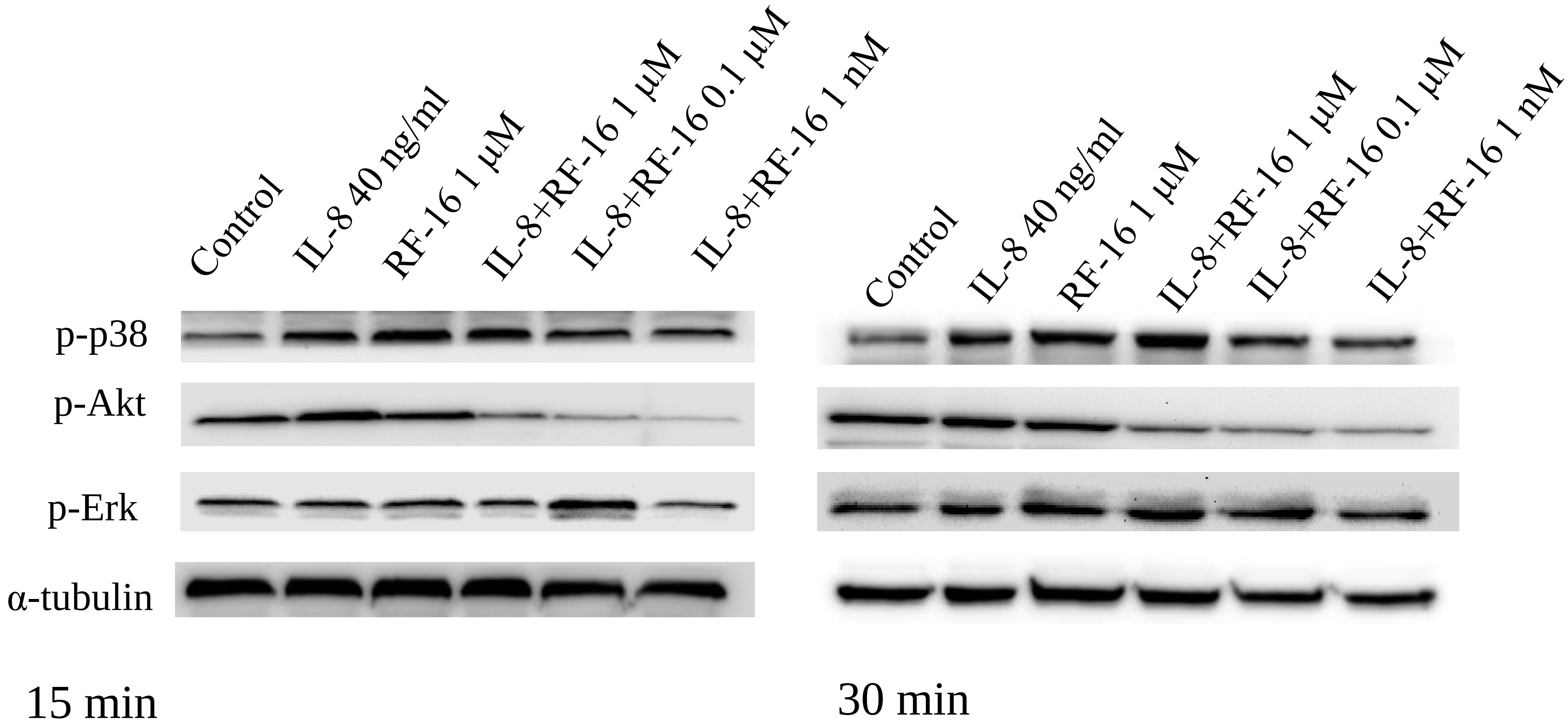

E-cadherin



# Effect of RF16 peptide on IL-8-modulated E-cadherin and Fibronectin expressions



# Effect of RF16 peptide on IL-8-induced p38 and PI3K activations



# Xenograft breast tumor model

(MDA-MB-231 cell
$1 \times 10^{6}$ cells /mouse)

30-35days

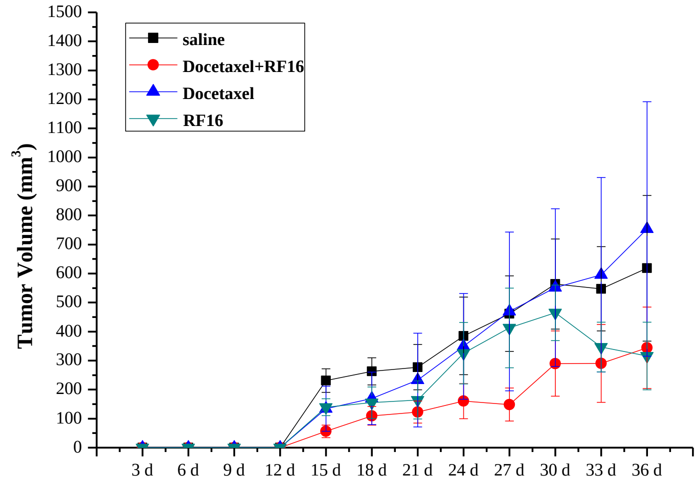
Control
Prptide RF-16 only (5 mg /kg)
Docetaxel (Doce) only (10 mg/kg)
Doce +RF-16

(1 time/week)

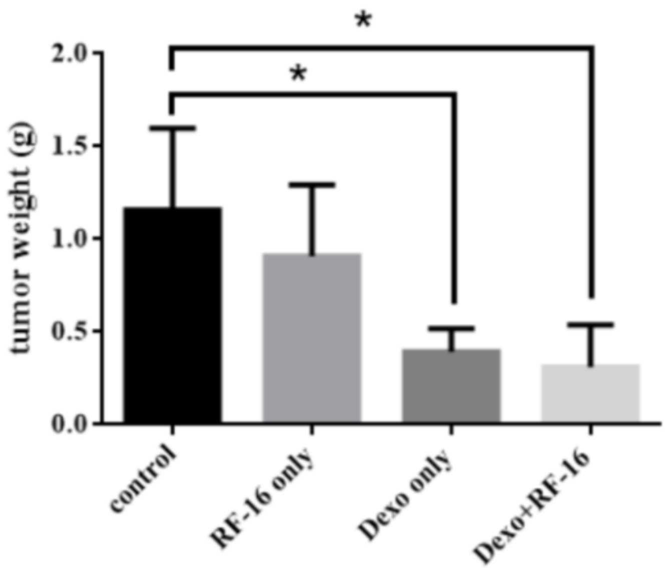
36 days

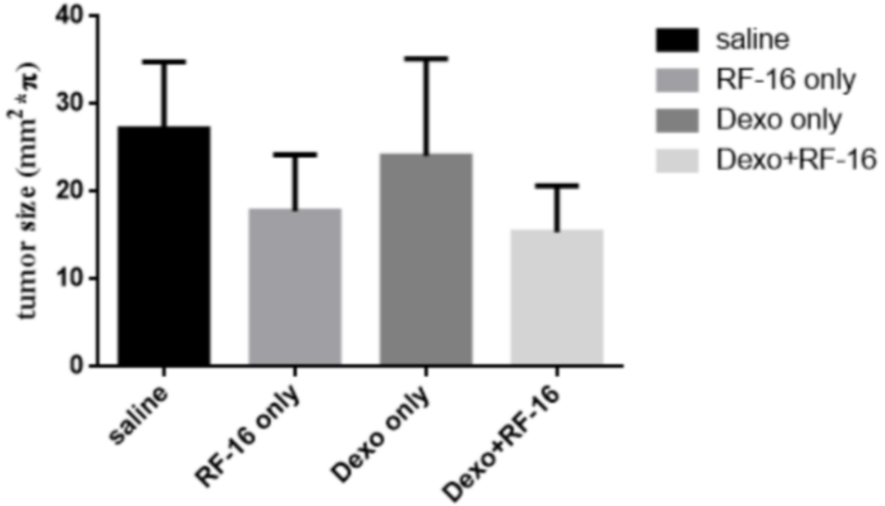
Sacrifice

# Xenograft breast tumor model

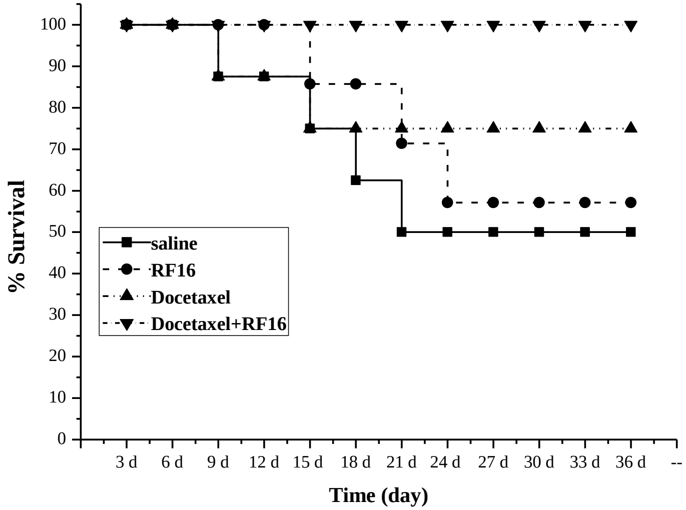



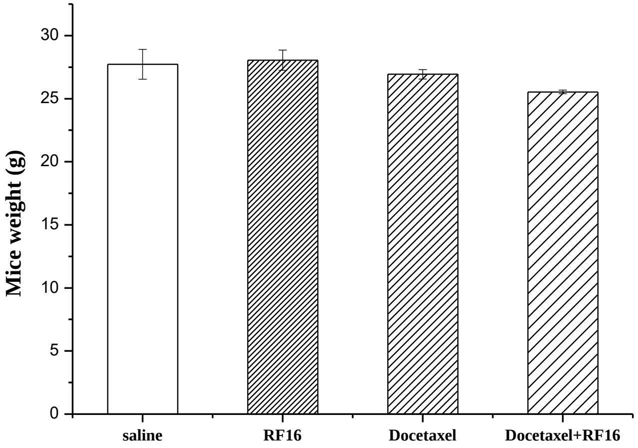
# Xenograft breast tumor model



# Xenograft breast tumor model



# Conclusion

***De novo* designed peptide derived from CXCL8 can decline TNF-α-induced inflammation on macrophages and obtains the synergistic effect on docetaxel promoted breast tumor suppression**

# 誌謝


生命科學系　　許豪仁老師

花蓮慈院
一般外科　　張群銘醫師
檢驗醫學科　　張淳淳主任

學生
杜宣諭
賴宜萱

助理
孫毓婷

# 敬請指導