

# **Alkylation of cysteine 468 in Stat3 defines a novel site for therapeutic development**

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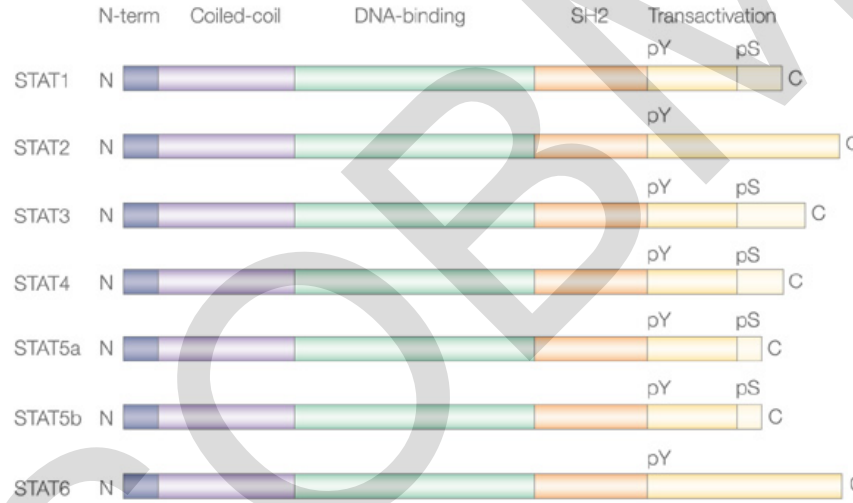
Beckman Research Institute

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Los Angeles, USA

# STAT family of transcription factors

(STAT = Signal Transducers and Activators of Transcription)

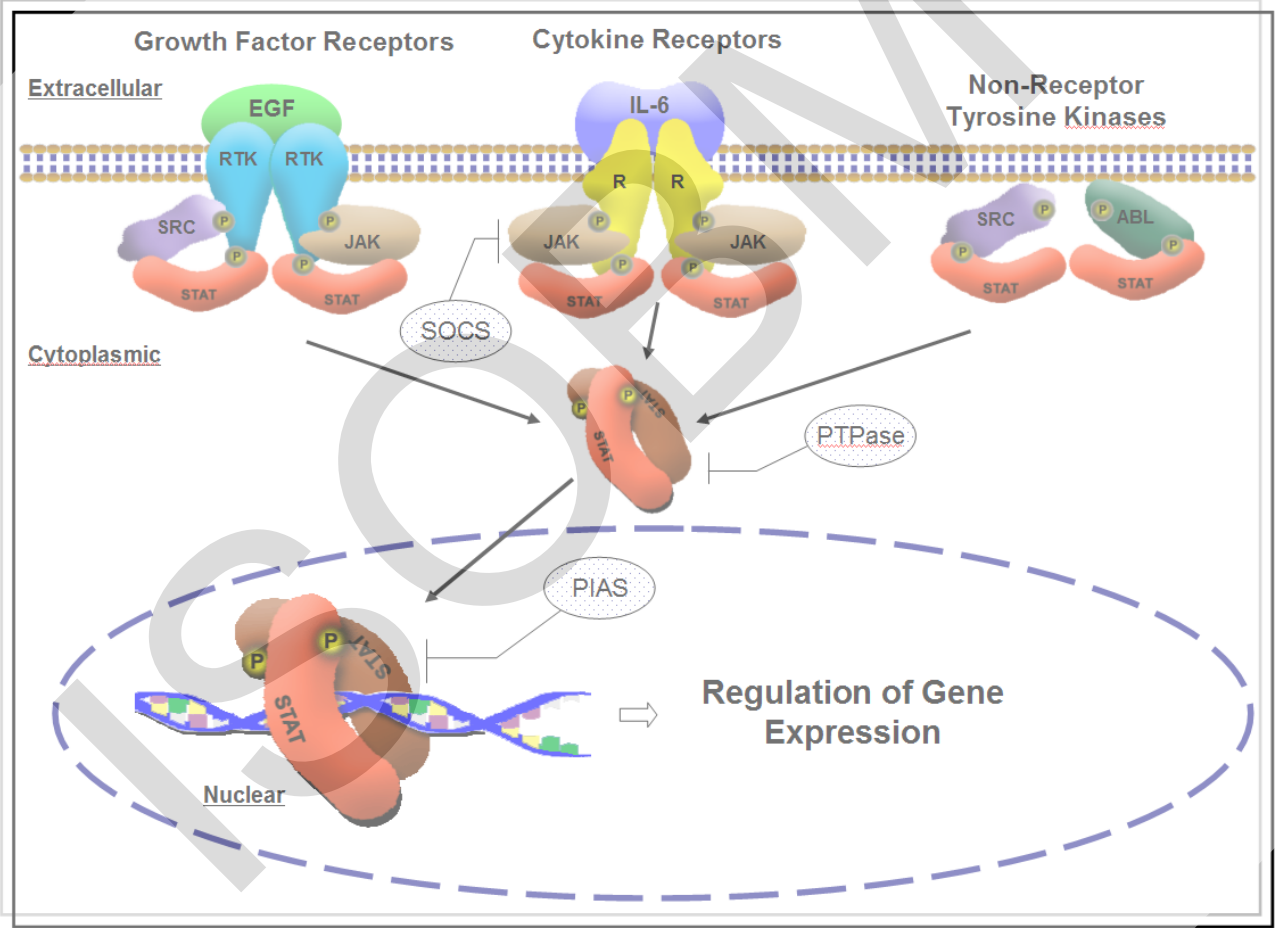


Yu and Jove. Nat Rev Cancer. 2004, 4:97-105.

STAT proteins participate in many physiological processes such as:

- Embryonic development
- Organ genesis and function
- Innate and adaptive immune function
- Regulation of cell differentiation, growth, and apoptosis

# Normal STAT signaling is transient and tightly regulated



Buettner et al. Clin Can Res. 2002, 8:945-954.

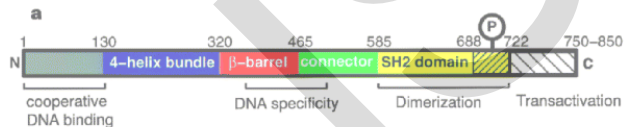
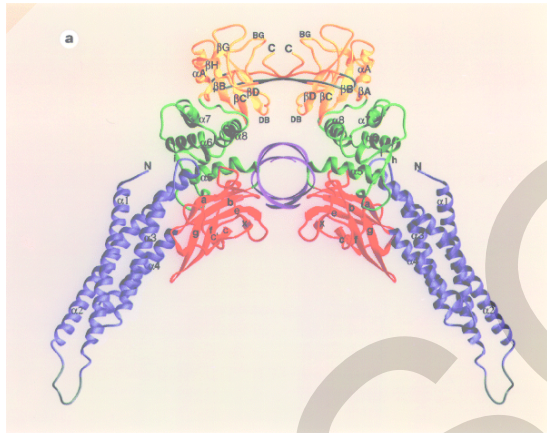
➔ Aberrant STAT signaling observed in many tumor cells



# Cells expressing Stat3C are tumorigenic

## 1998: Stat3 crystal structure

Becker et al. Nature. 1998, 394:145-51.



## 1999: Stat3 as an oncogene

Bromberg et al. Cell. 1999, 98:295-303.

Persistent active mutant form of Stat3 (Stat3C) generated by substitution of two cysteine residues with the SH2 domain:

- dimerizes spontaneously
- binds to DNA
- activates transcription
- causes cellular transformation in fibroblast cells
- causes tumor formation of transformed fibroblast cells (3Y1) in nude mice

# Persistent activation of STATs in human cancers

Table 1 | **Activation of STATs in human cancers**

Tumour type	Activated STAT
<b>Blood tumours</b>	
Multiple myeloma	STAT1, STAT3
Leukaemias:	
HTLV-1-dependent Erythroleukaemia	STAT3, STAT5
Acute myelogenous leukaemia (AML)	STAT1, STAT5
Chronic myelogenous leukaemia (CML)	STAT1, STAT3, STAT5
Large granular lymphocyte leukaemia (LGL)	STAT5
Lymphomas:	
EBV-related/Burkitt's	STAT3
Mycosis fungoides	STAT3
Cutaneous T-cell lymphoma	STAT3
Non-Hodgkins lymphoma (NHL)	STAT3
Anaplastic large-cell lymphoma (ALCL)	STAT3
<b>Solid tumours</b>	
Breast cancer	STAT1, STAT3, STAT5
Head and neck cancer	STAT1, STAT3, STAT5
Melanoma	STAT3
Ovarian cancer	STAT3
Lung cancer	STAT3
Pancreatic cancer	STAT3
Prostate cancer	STAT3

Based on references cited in REFS 12,17. EBV, Epstein-Barr virus; HTLV-1, human T-lymphotrophic virus-1.

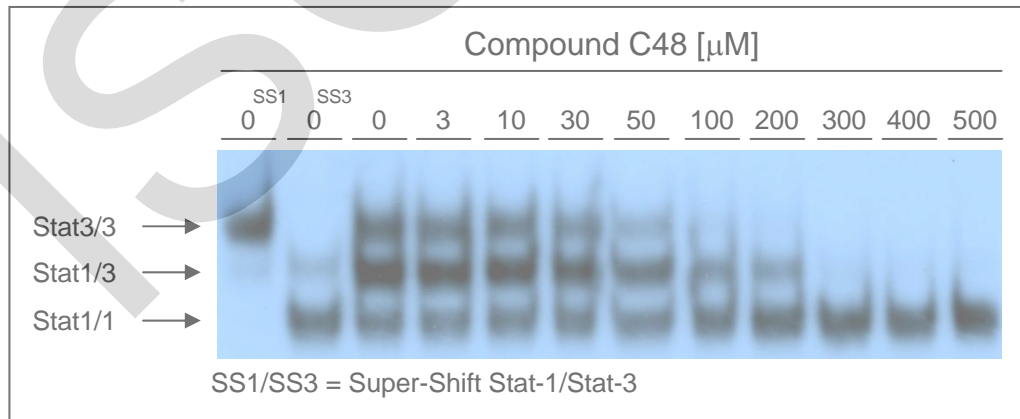
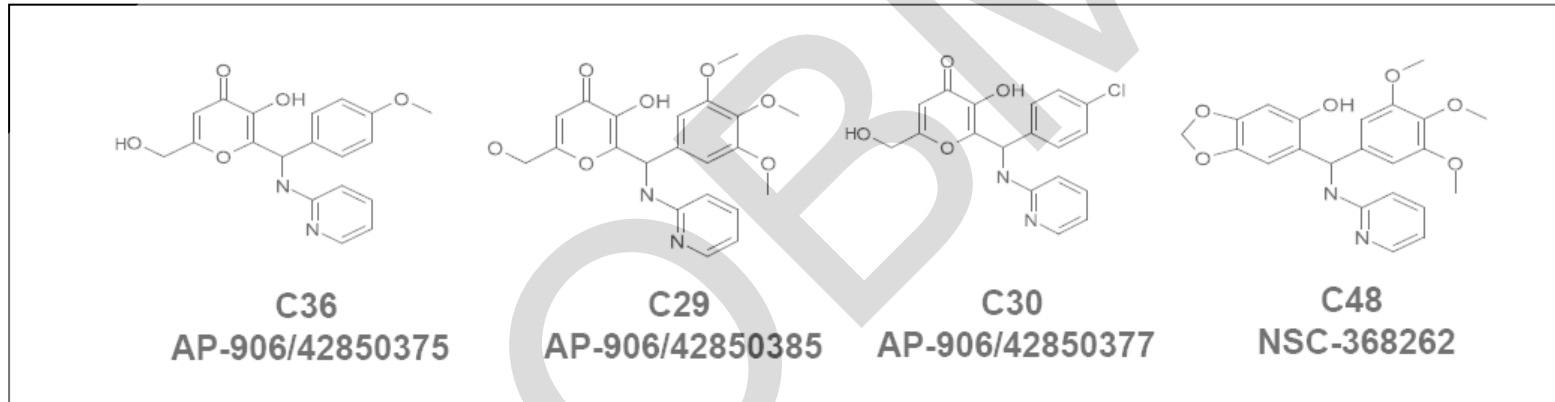
Yu and Jove. Nat Rev Cancer. 2004, 4:97-105.

- Stat3 and Stat5 oncogenic
- Stat1 associated with inhib. of growth → tumor suppressor

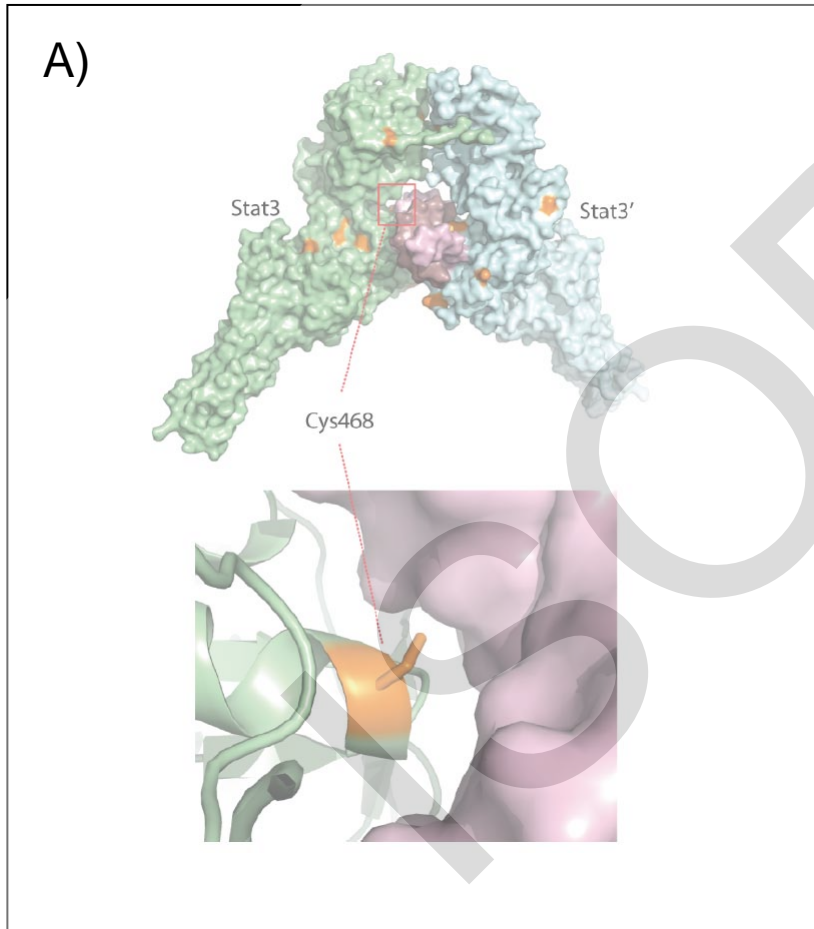
# Development of small molecule inhibitors of Stat3

- Many publications have confirmed persistent activation of Stat3 as a valid target for therapeutic intervention
- No selective, clinically relevant small molecule inhibitor of Stat3 available
- → We applied molecular dynamics (MD) modeling and virtual ligand screening (VLS) to identify selective, small molecular inhibitors of Stat3

# Compound C48 preferentially inhibits Stat3 DNA-binding activity in EMSA



# Surface cysteines in Stat3

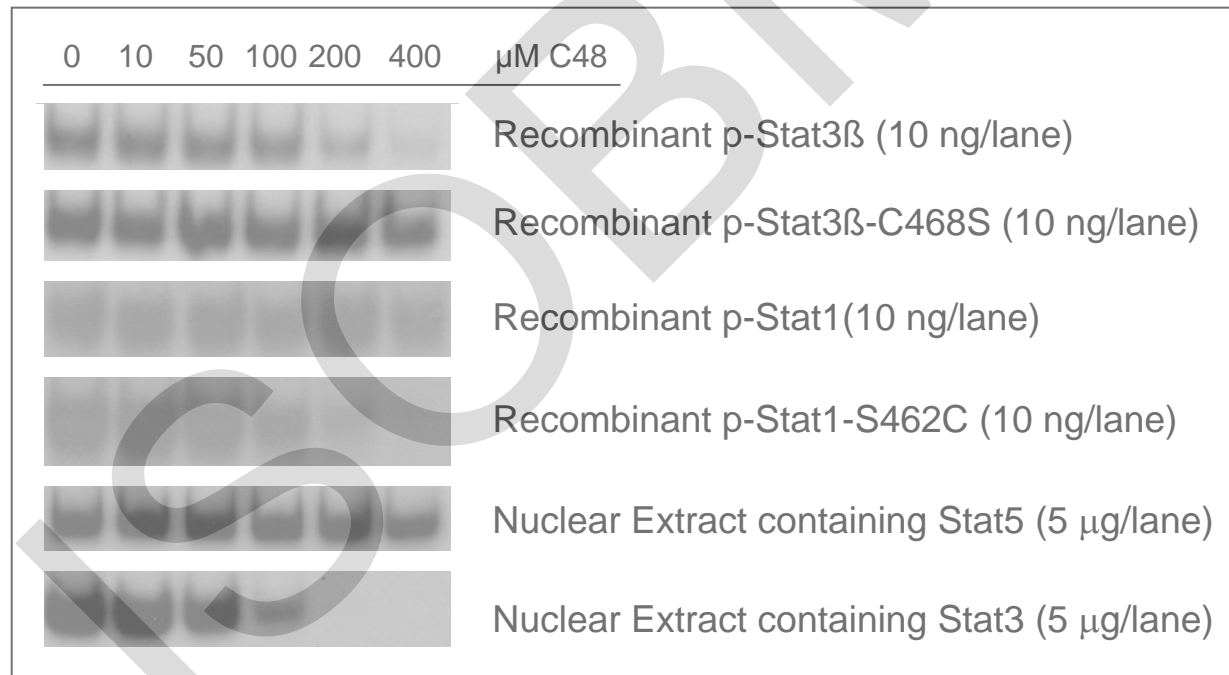


- 9 cysteines on Stat3 surface
- Cys468 at protein/DNA interface
- Cys468 not present in other STATs  
→ suggesting a potential mechanism for isoform specificity

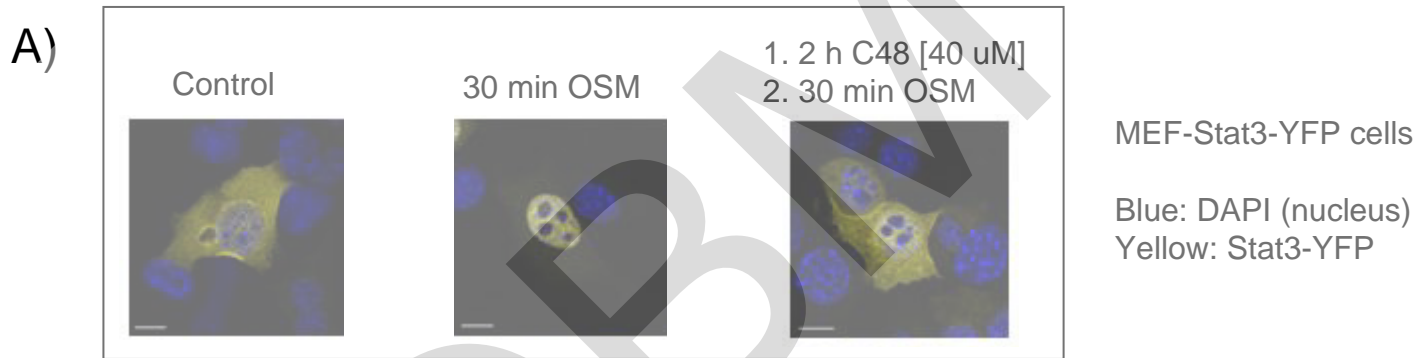
B)

STAT1	$\alpha/\beta$	455	VVISNVSQ LP SGWAS
STAT2		453	VIISNMNQLSIAWAS
STAT3	$\alpha/\beta$	461	VVISNICQ MP NAWAS
STAT4		451	VMISNVSQ LP NAWAS
STAT5	A/B	466	VVIVHGSQ DH NATAT
STAT6		410	VVIVHGNQ DN NAKAT
			*:* : * . *:

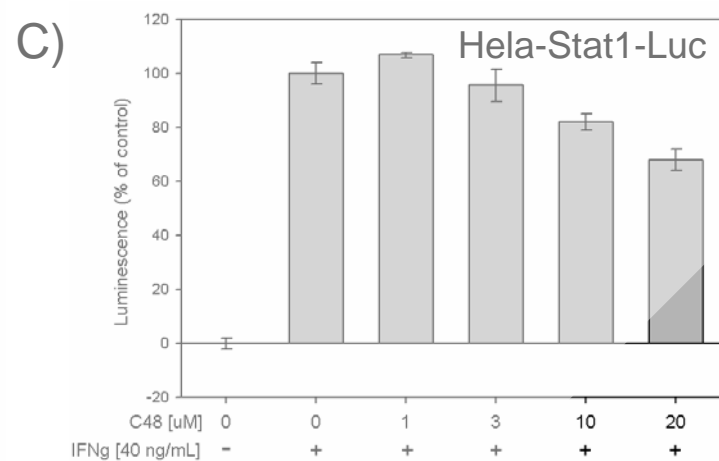
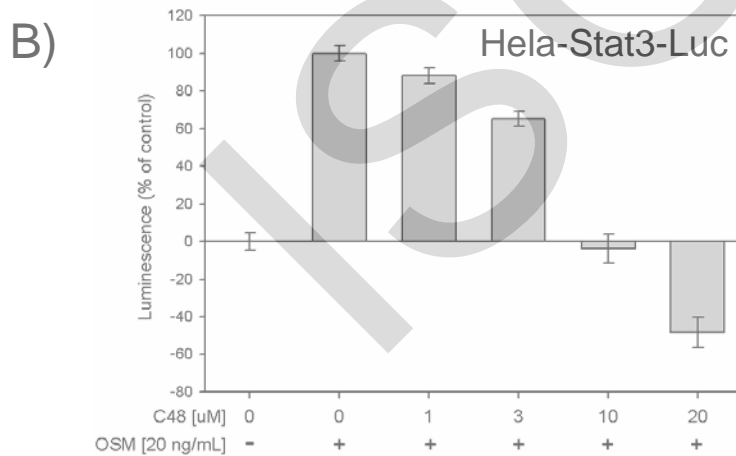
# Cys468 to Ser468 mutation desensitizes Stat3 to inhibition by compound C48



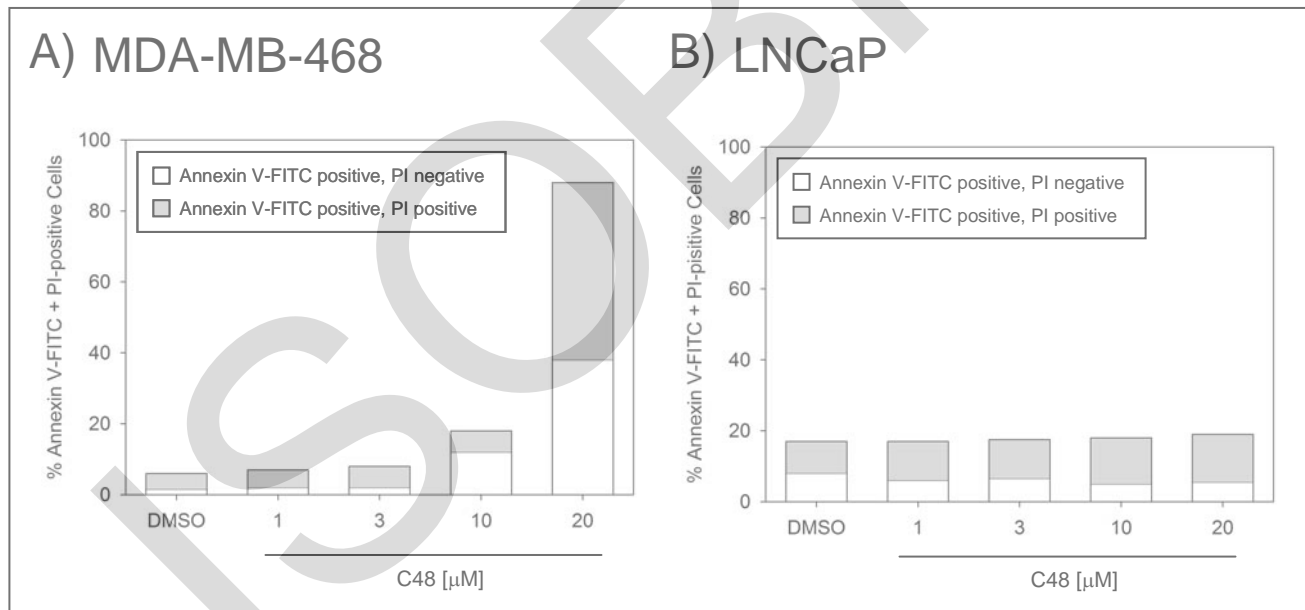
# Compound C48 inhibits Stat3 nuclear translocation



# Compound C48 blocks Stat3 transcriptional activity

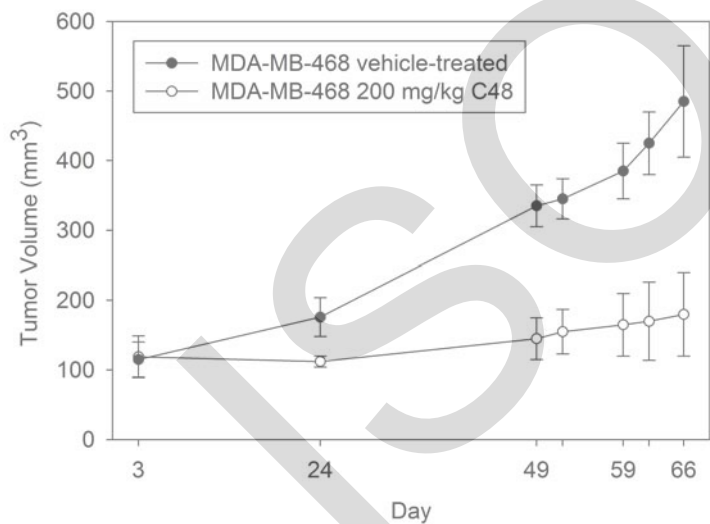


# Compound C48 induces apoptosis in Stat3-dependent tumor cells

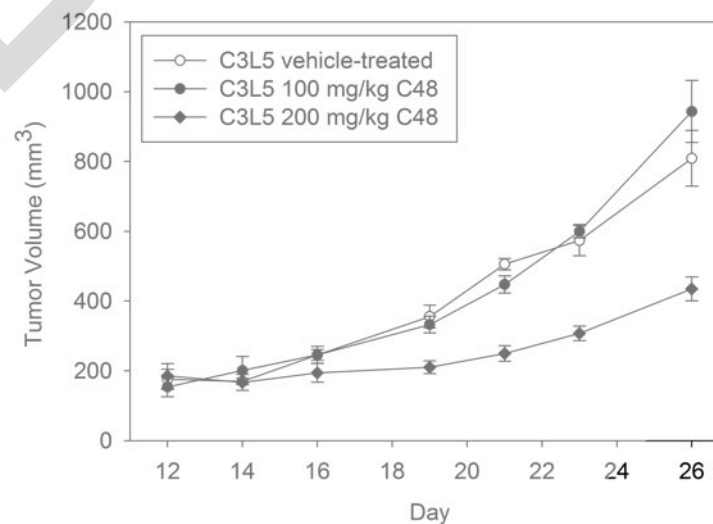


# Compound C48 inhibits *in vivo* growth of breast cancers

A) Xenograft nude mouse model

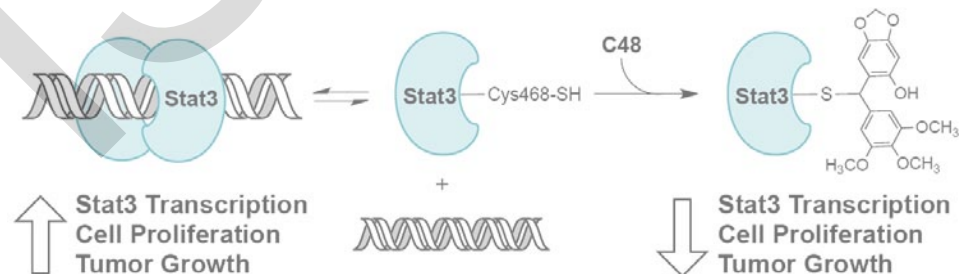


B) Syngeneic mouse model



# Summary

- **Compound C48** alkylates Stat3, induces apoptosis in tumor cell lines that depend on Stat3, and possesses *in vivo* anti-tumor activity.
- Selective alkylation of cysteine 468 in Stat3 defines a novel site for further therapeutic development of selective Stat3 inhibitors for cancer treatment.



# Conclusion

→ Our findings further validate Stat3 as a promising molecular target for cancer therapy and identify a potential new lead compound for drug development.

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