

# Immune impact on cancer stemness and metastasis

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SITC workshop  
October 25, 2012, Bethesda, MD

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I have no financial relationships to disclose.

I will not discuss off label use and/or investigational use in my presentation.



SOCIETY FOR IMMUNOTHERAPY OF CANCER

October 24-28, 2012 • North Bethesda, MD

WORKSHOP • PRIMER • ANNUAL MEETING



# Think Big

1. Balance is the key to life:  
Immune imbalance in the tumor  
microenvironment.
  2. Oncogenesis model
- 

Use the simplest method and  
technology to address the most  
complex cross-functional issues

# Impact of immune imbalances in the human tumor microenvironment

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**Immune imbalances:**

**Inhibitory and stimulatory B7 family members**

**APC subsets**

**Effector and regulatory T cell subsets**

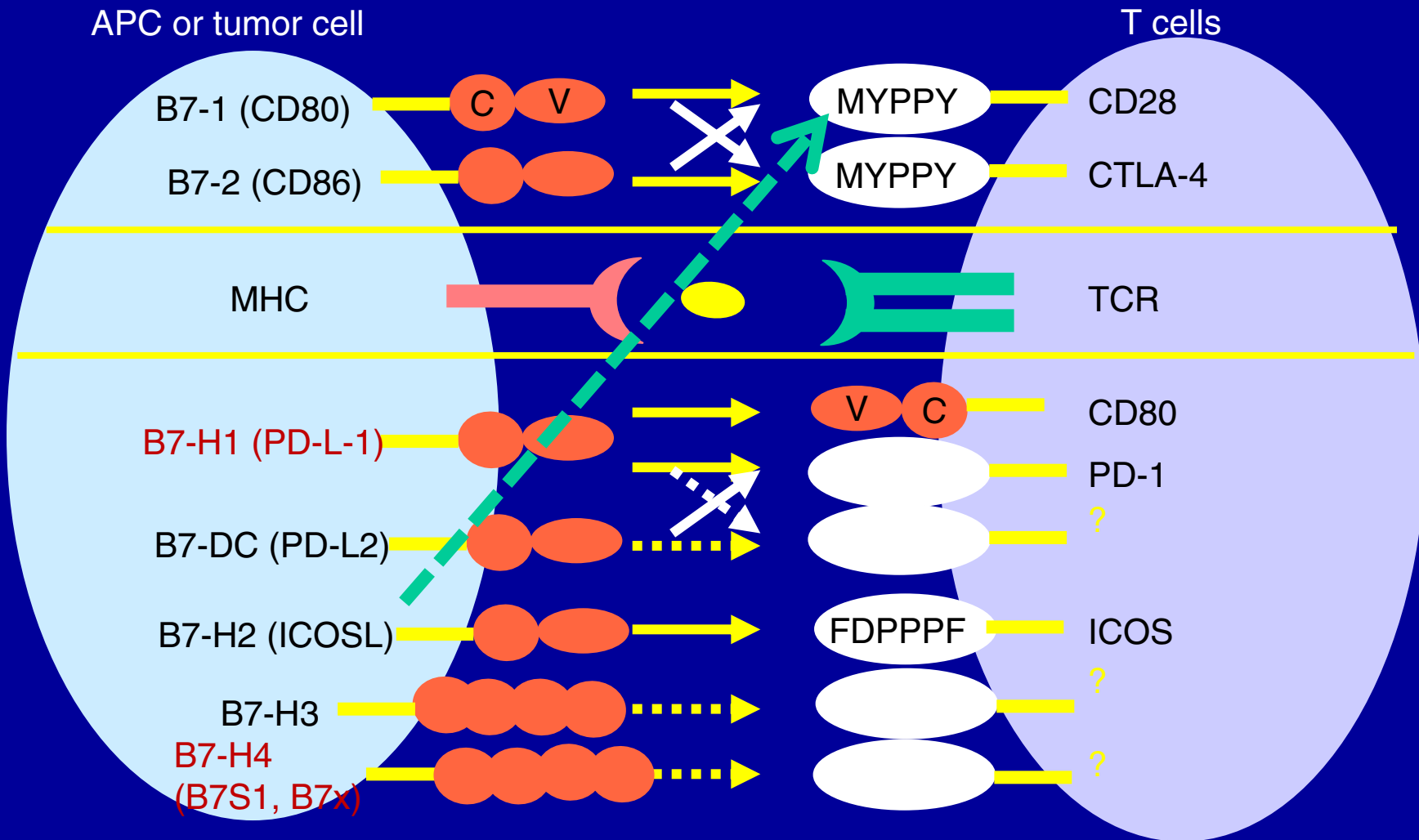
W Zou. Immunosuppressive networks in the tumor microenvironment and their therapeutic relevance. *Nature Review Cancer*, 5:263-274, 2005

W Zou. Regulatory T cells, tumor immunity and immunotherapy. *Nature Review Immunology*, 6:295-307, 2006

W Zou, L Chen. Inhibitory B7 family molecules in the tumor microenvironment. 8:467-477, *Nature Review Immunology*, 2008

W Zou and N Restifo. Th17 cells, tumor immunity and immune therapy. 10:248-256, *Nature Review Immunology*, 2010

# Inhibitory and stimulatory B7 imbalance



Nat Med, 2003, 2004; J Exp Med, 2006, Cancer Res, 2003-2011

# APC subset imbalance

MDC

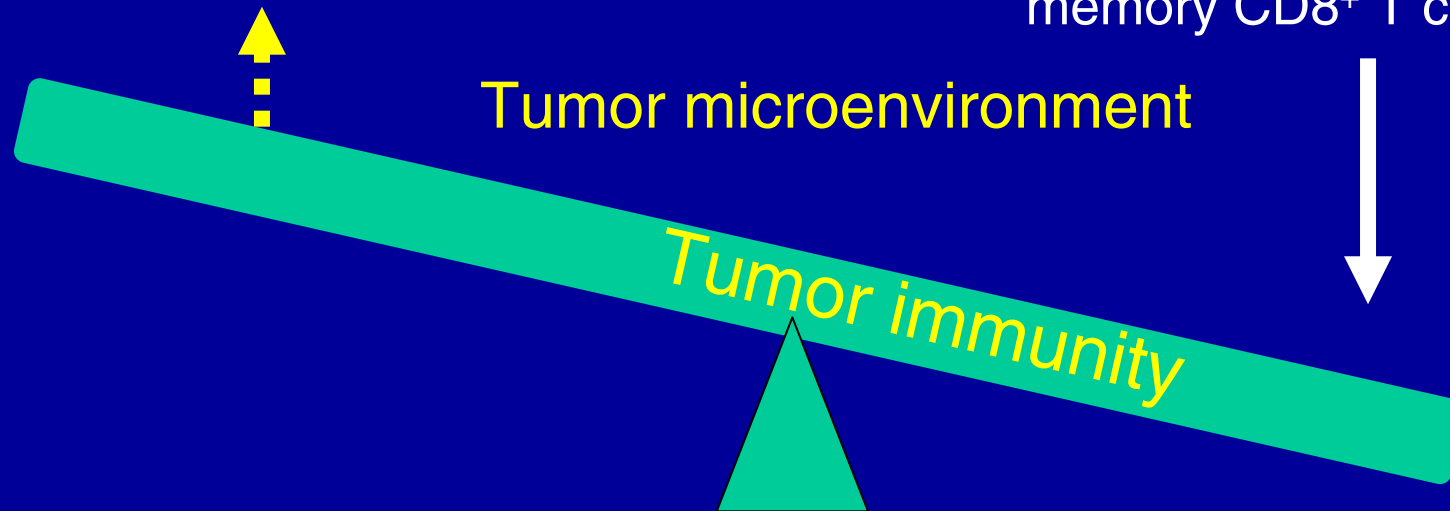


Potent IL-12, Th-1 polarization,  
TAA-specific effector memory CTL

PDC, MDSC, immature DC

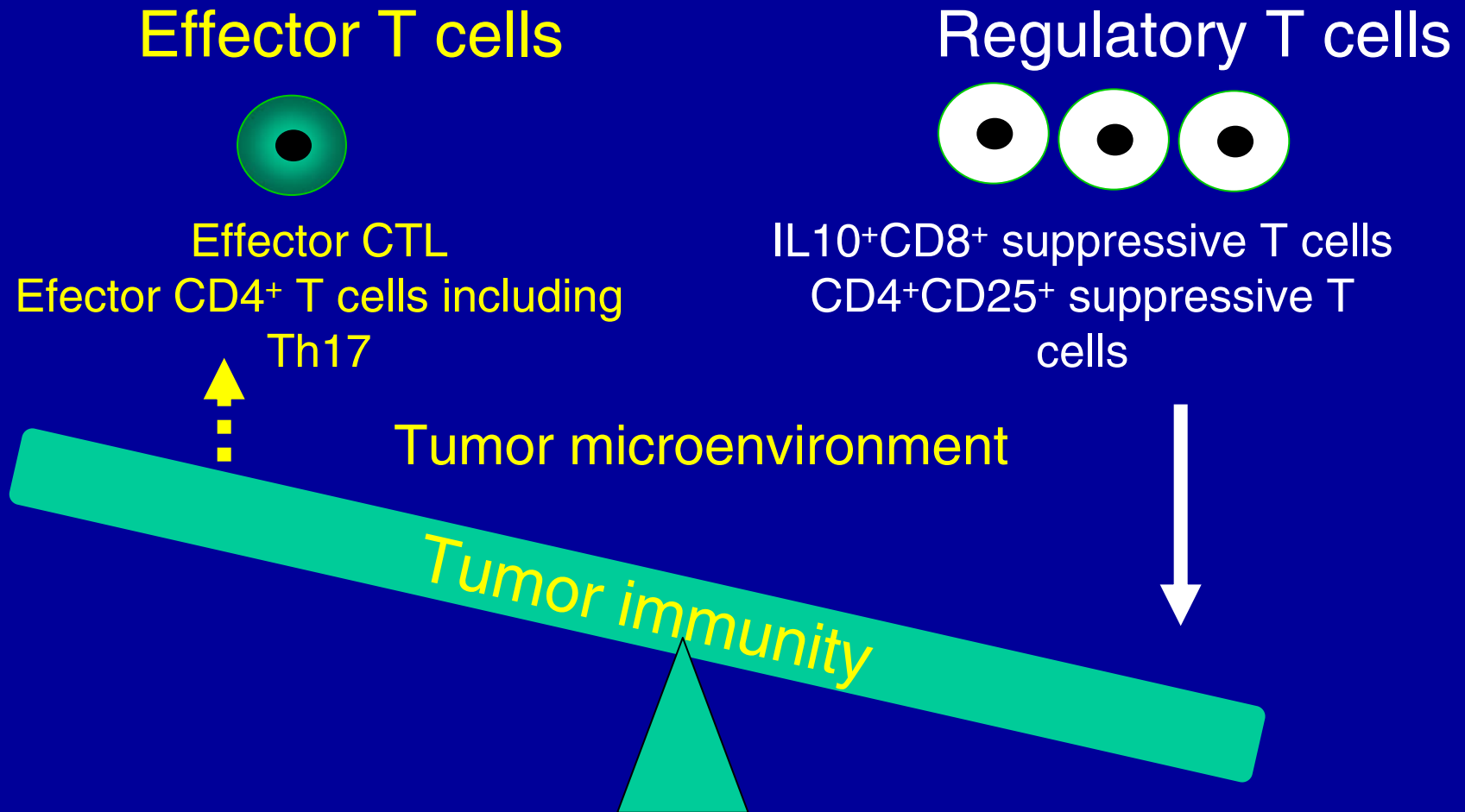


No IL-12, Th-2 polarization,  
TAA-specific IL10<sup>+</sup> central  
memory CD8<sup>+</sup> T cells?



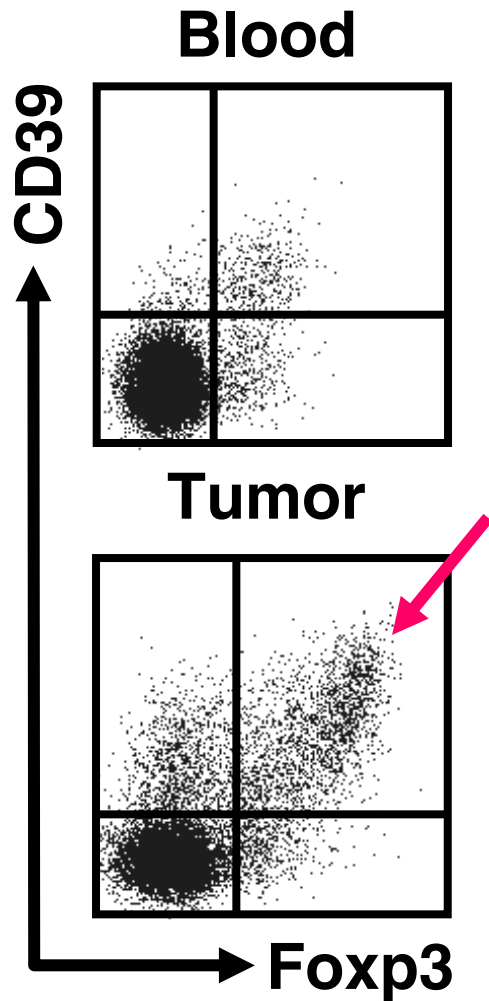
Nat Med, 2001, 2003, 2004; J Exp Med, 2006,  
J Immunol, 2002-2011; Cancer Res, 2003-2011

# T cell subset imbalance

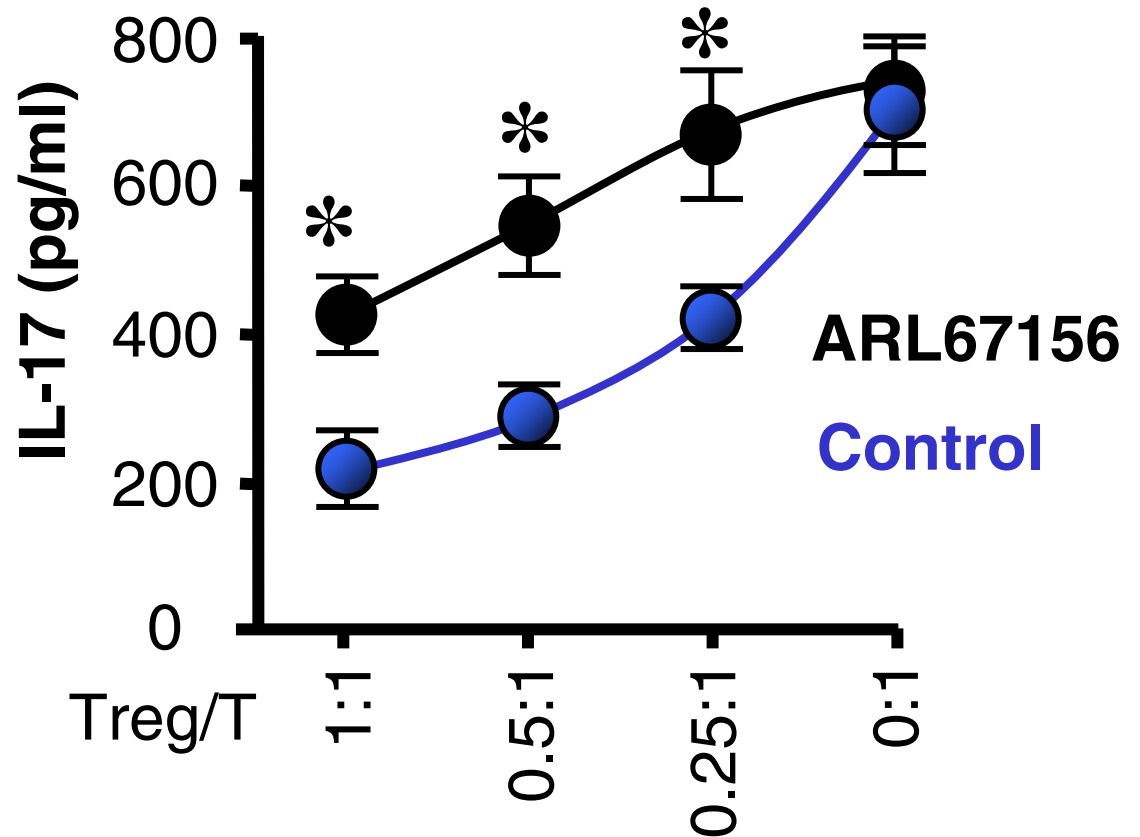


Nat Med, 2003, 2004, 2007, 2012; Science Translational Medicine, 2011; Blood, 2006, 2009, 2011; J Immunol, 2002-2011; Cancer Res, 2003-2012; Oncolimmune, 2012

# Imbalance between Treg and Th17: Treg suppressed Th17 via adenosinergic pathway



ARL67156 - a structural analogue of ATP and an ectonucleotidase inhibitor





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**I. Th17 stemness and cancer**

**II. MDSC, microRNA and  
cancer stemness**

# I. Th17 stemness and mechanisms

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a. Th17 cells: a minor population

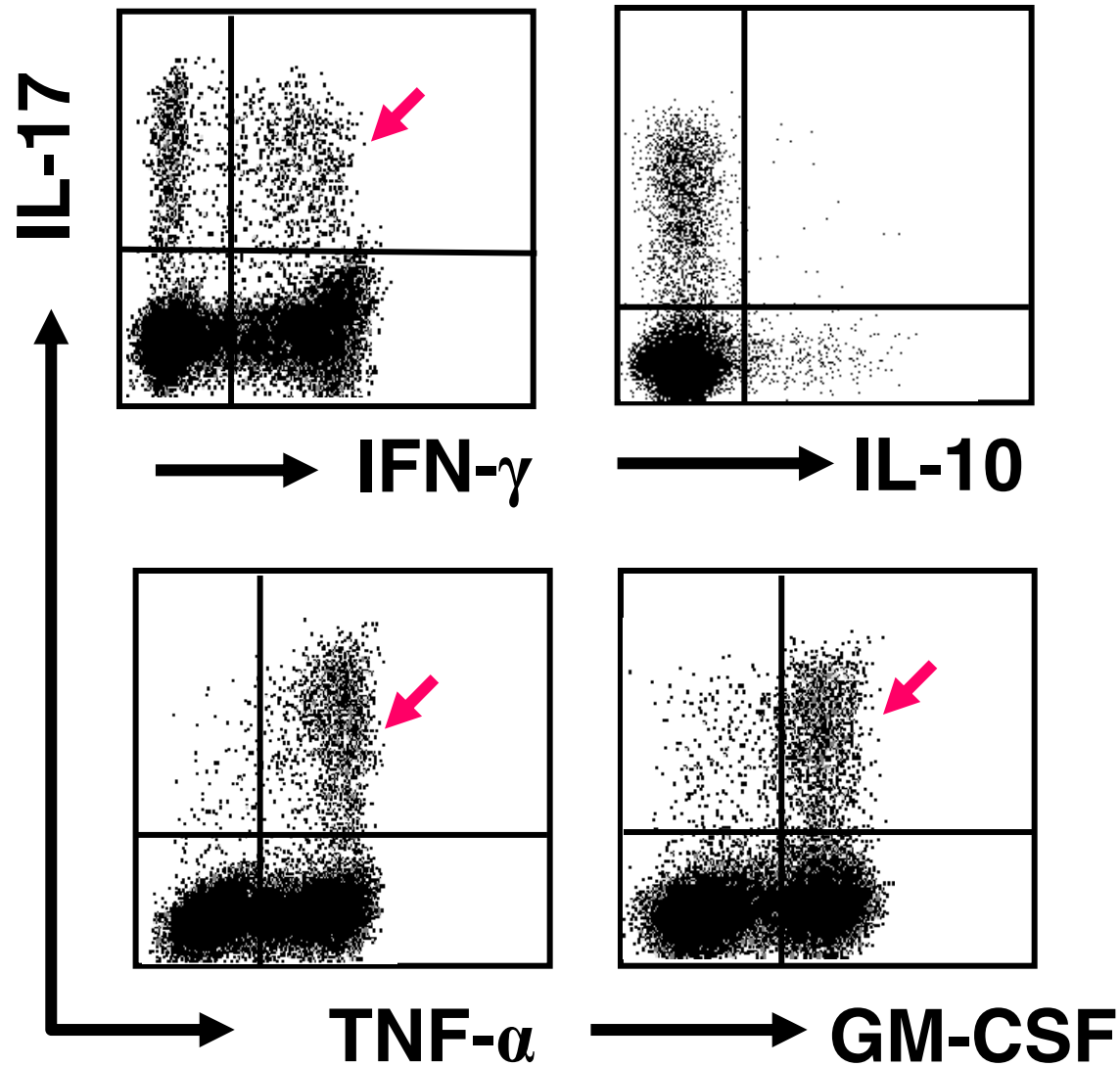
b. GVHD, autoimmunity, tumor immunity

## Forever Seventeen

**It is that quality possessed by some  
which draws all others with its  
magnetic force ---- Elinor Glyn**

# Polyfunctionality of Th17 cells in human cancer

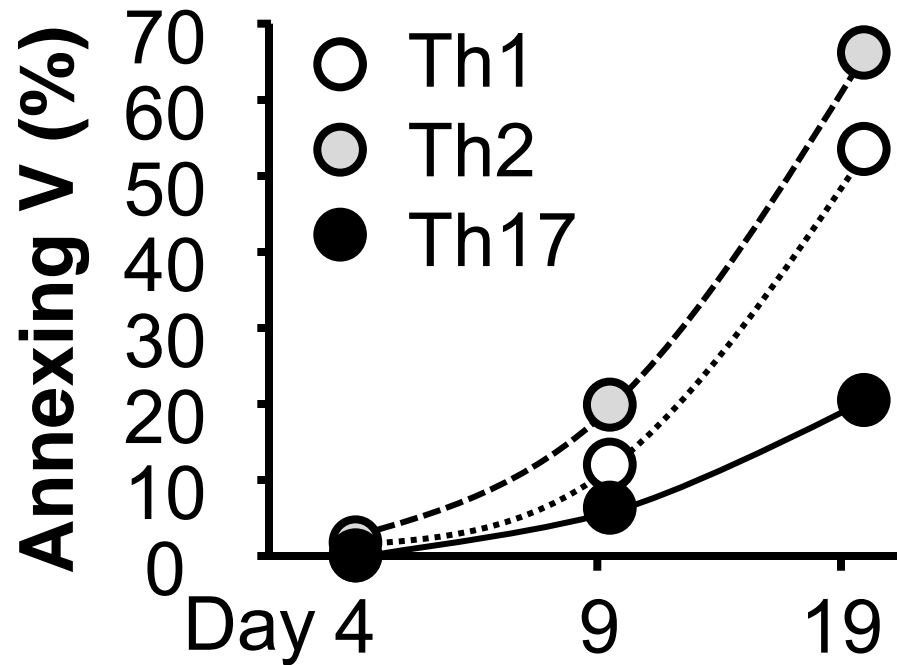
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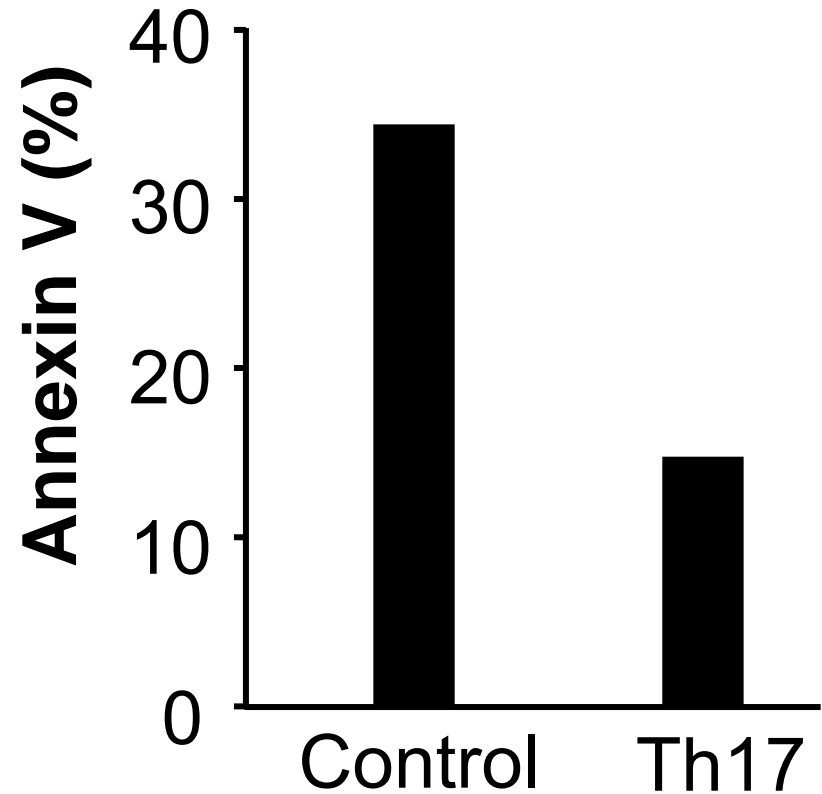
# High apoptotic resistance capacity

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## TCR engagement

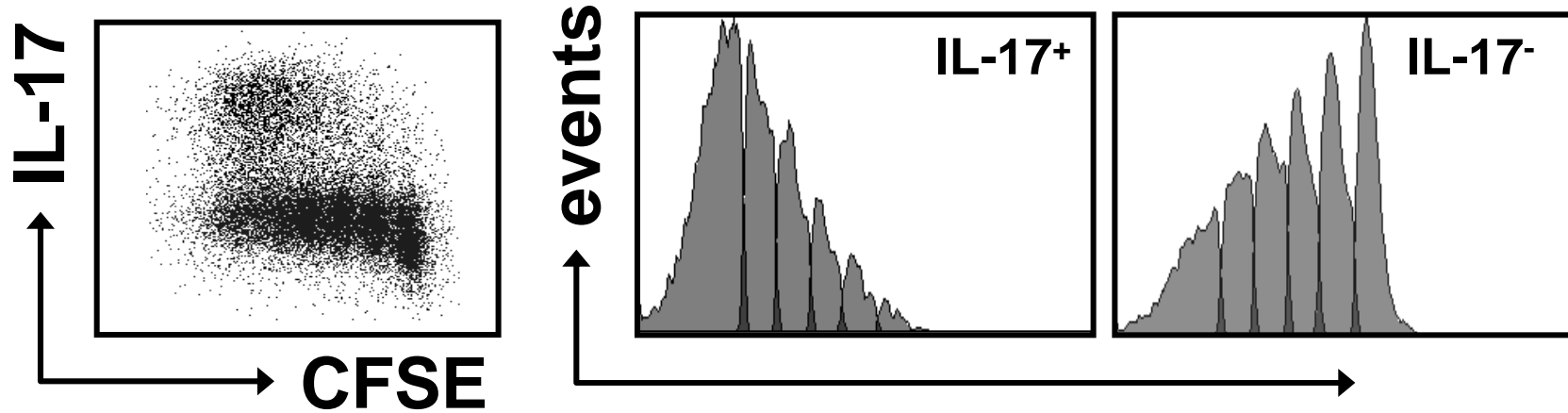


## Cisplatin treatment



# High proliferative renewal capacity

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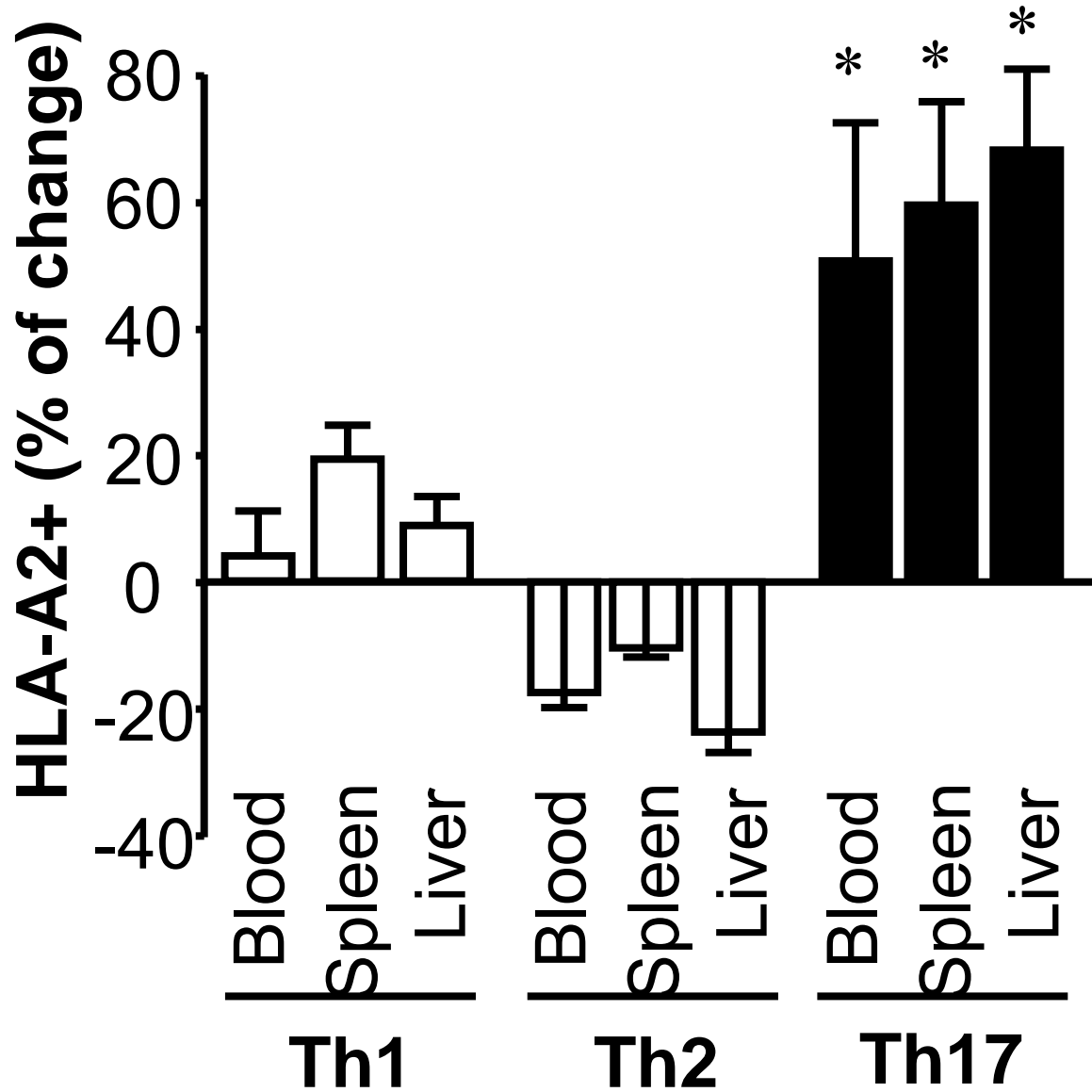


## Colon cancer

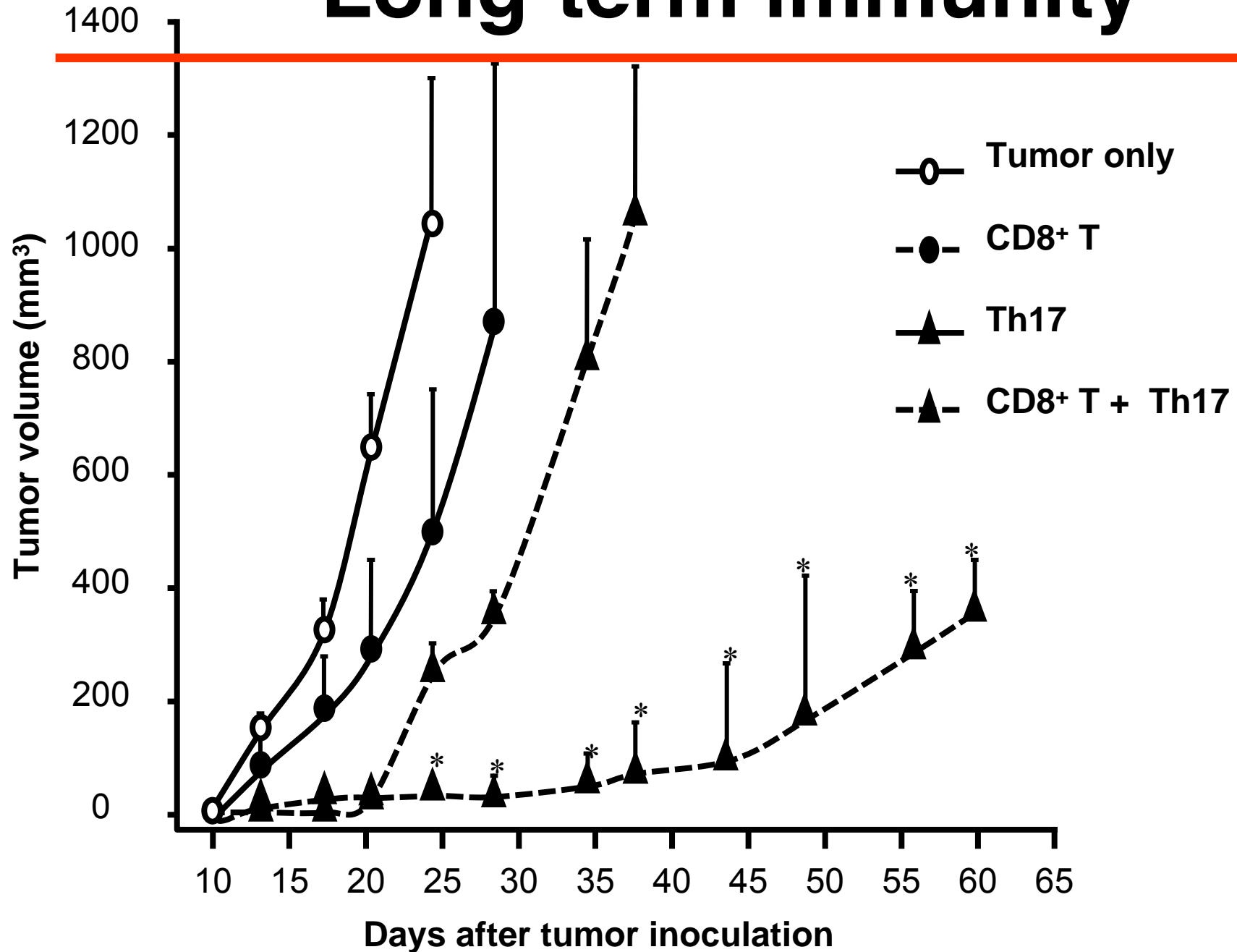


# Lasting persistence

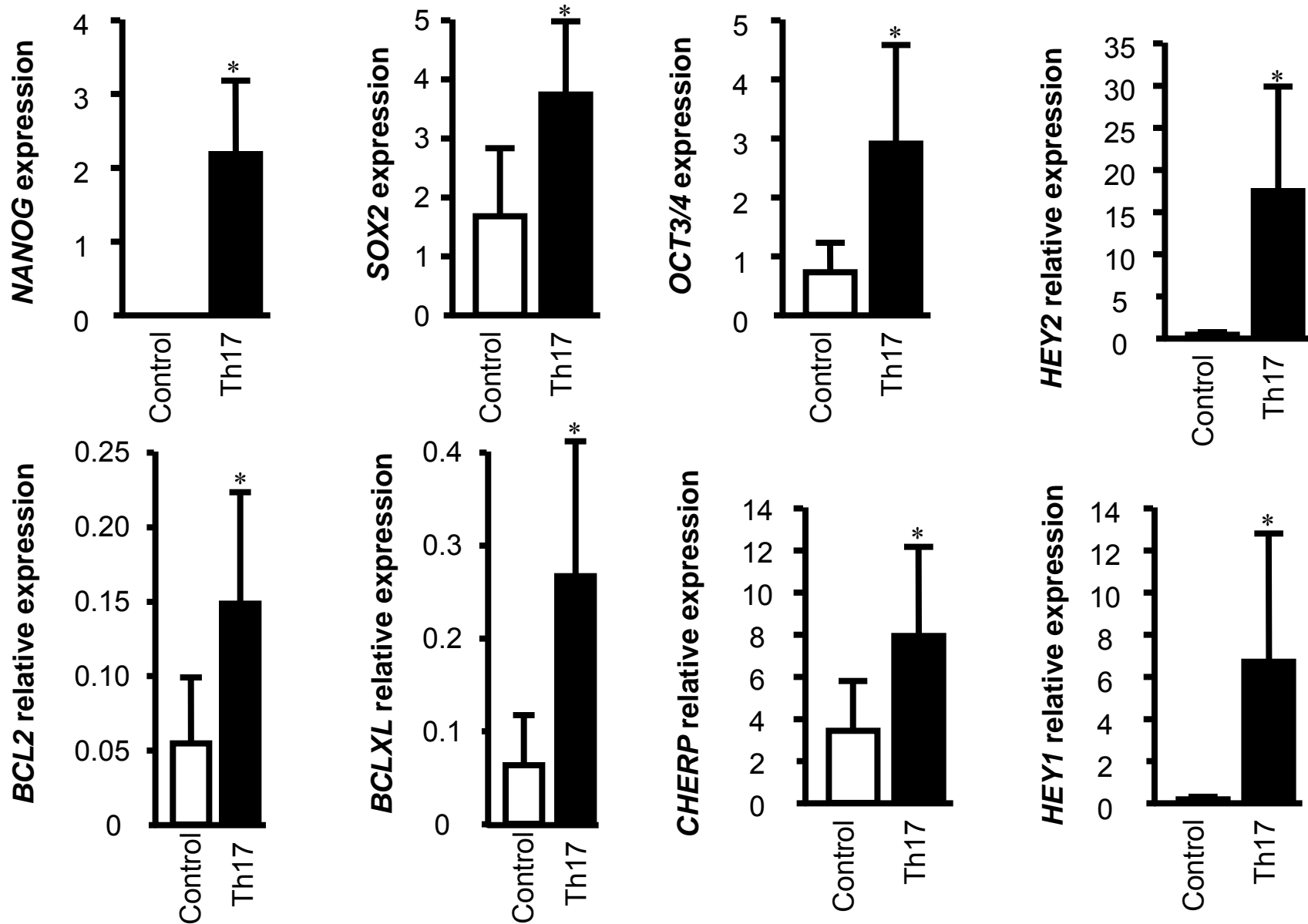
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# Long-term immunity



# Stem cell, anti-apoptosis, and Notch genes





# Th17 cells may have stem cell character

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**Proliferative self-renewal**

**Apoptosis resistance**

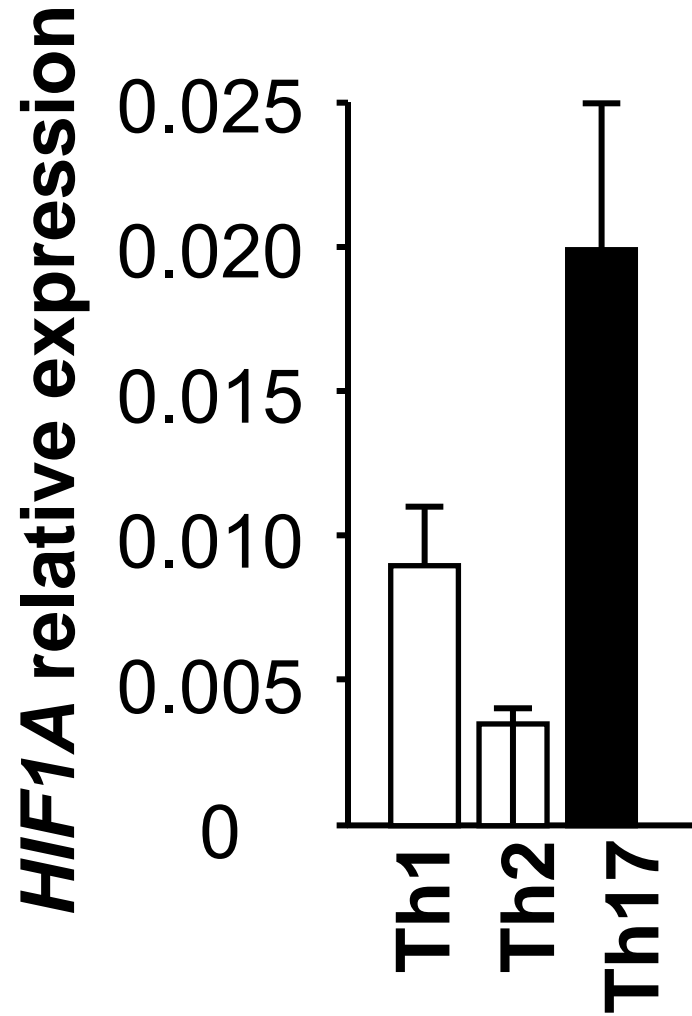
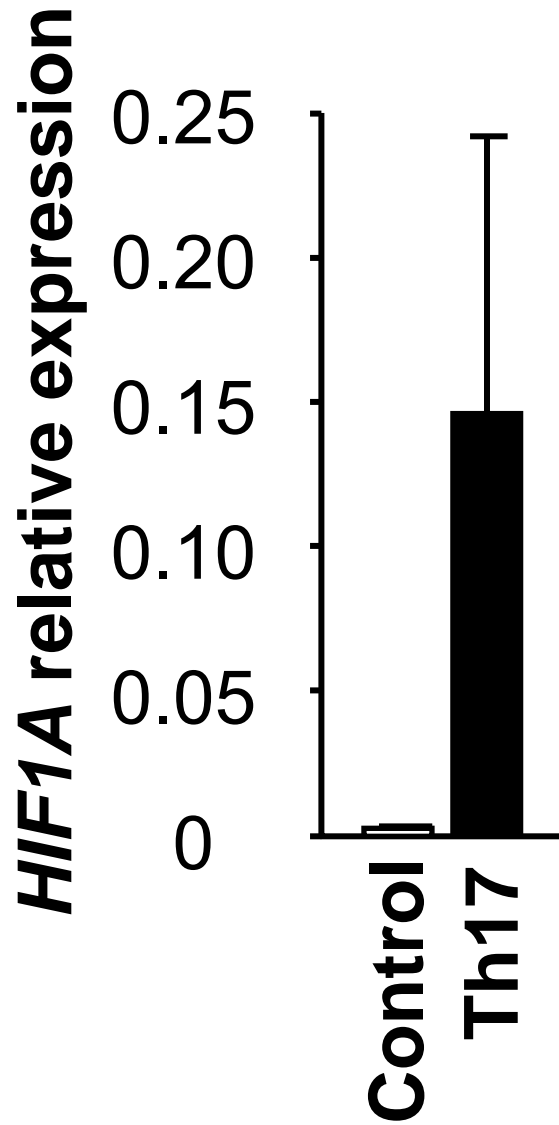
**Long-lived and lasting persistence**

**Stem cell associated genes**

**Mechanism?**

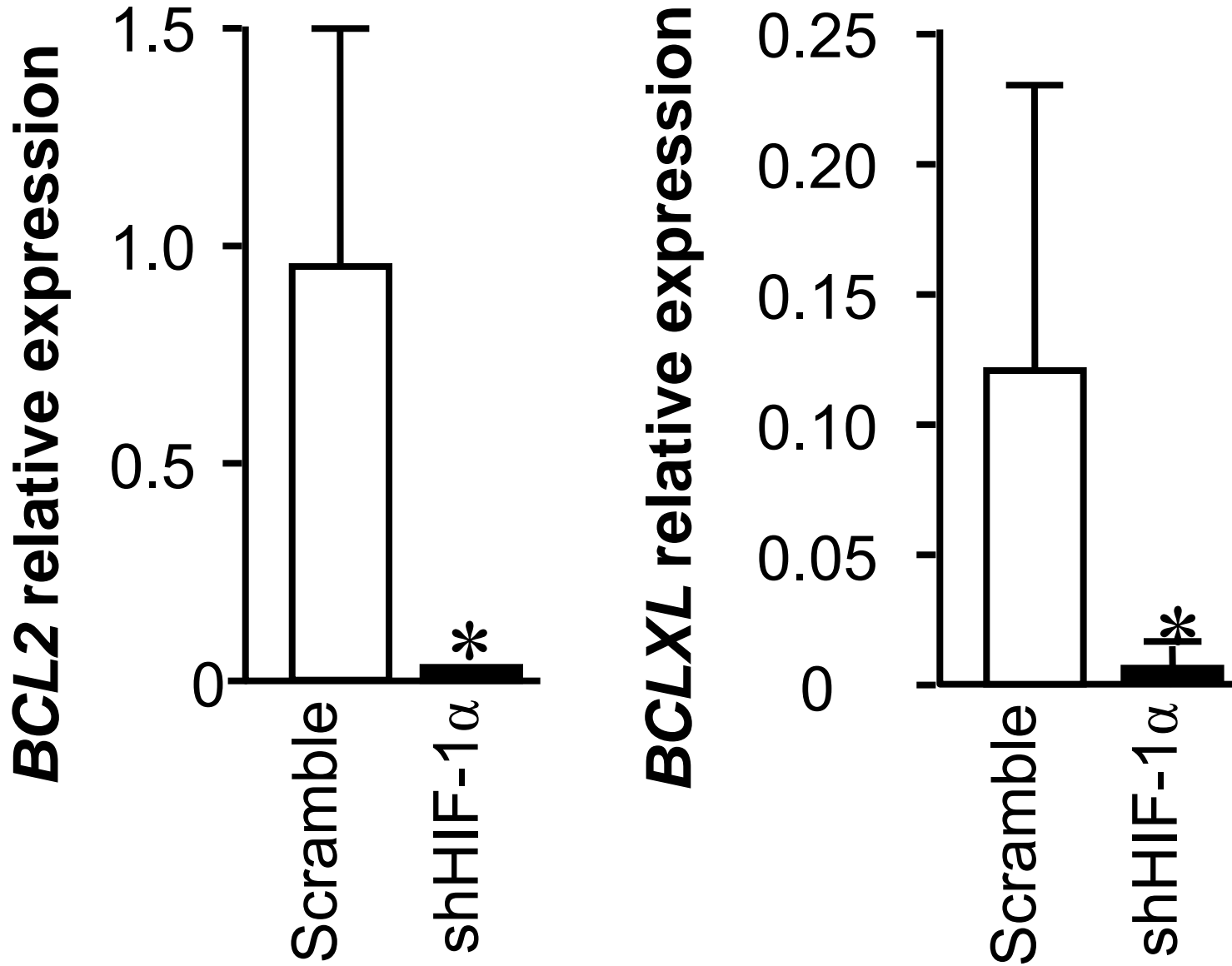
# Th17 cells express HIF-1 $\alpha$

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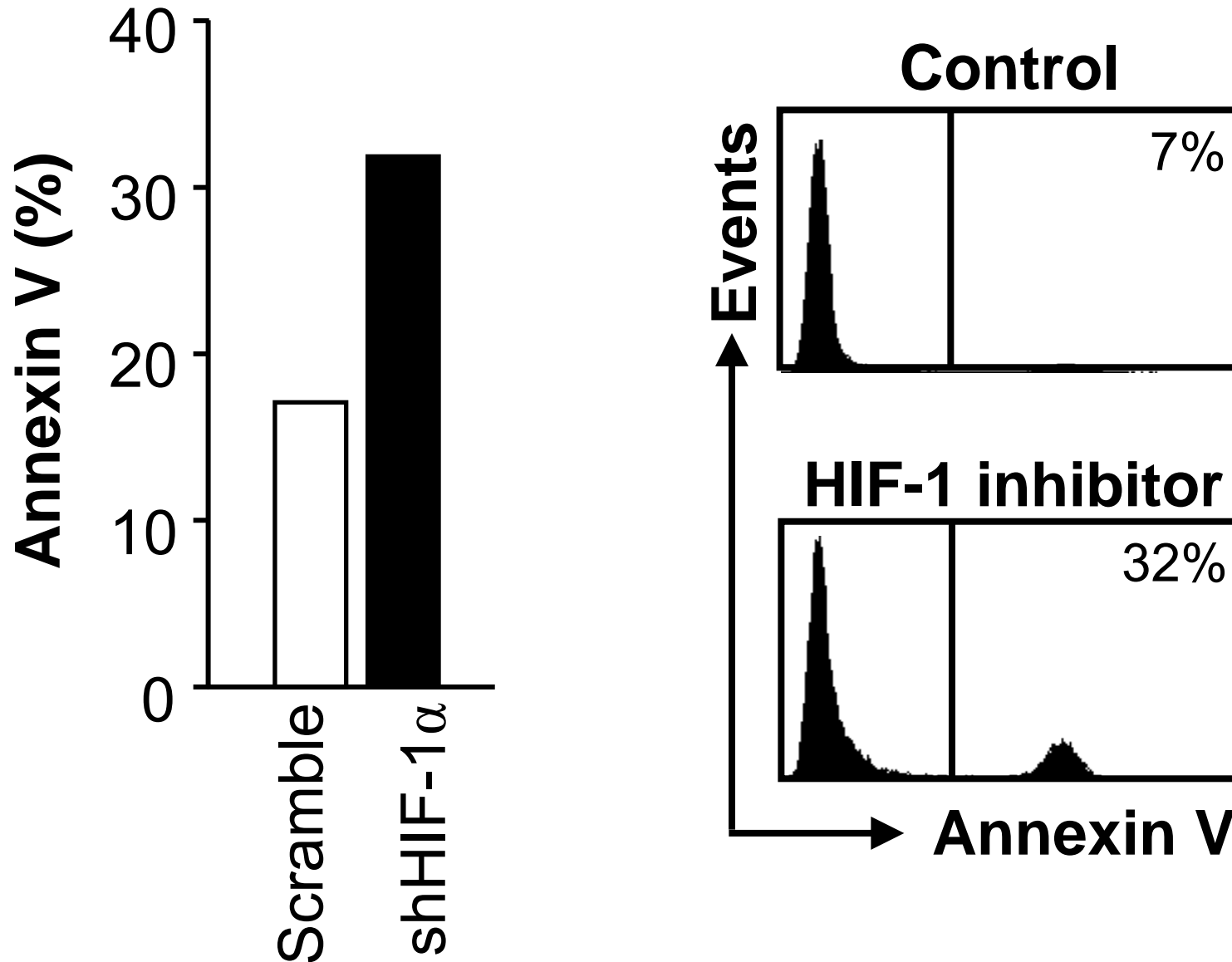


# HIF-1 $\alpha$ controls Bcl expression in Th17 cells

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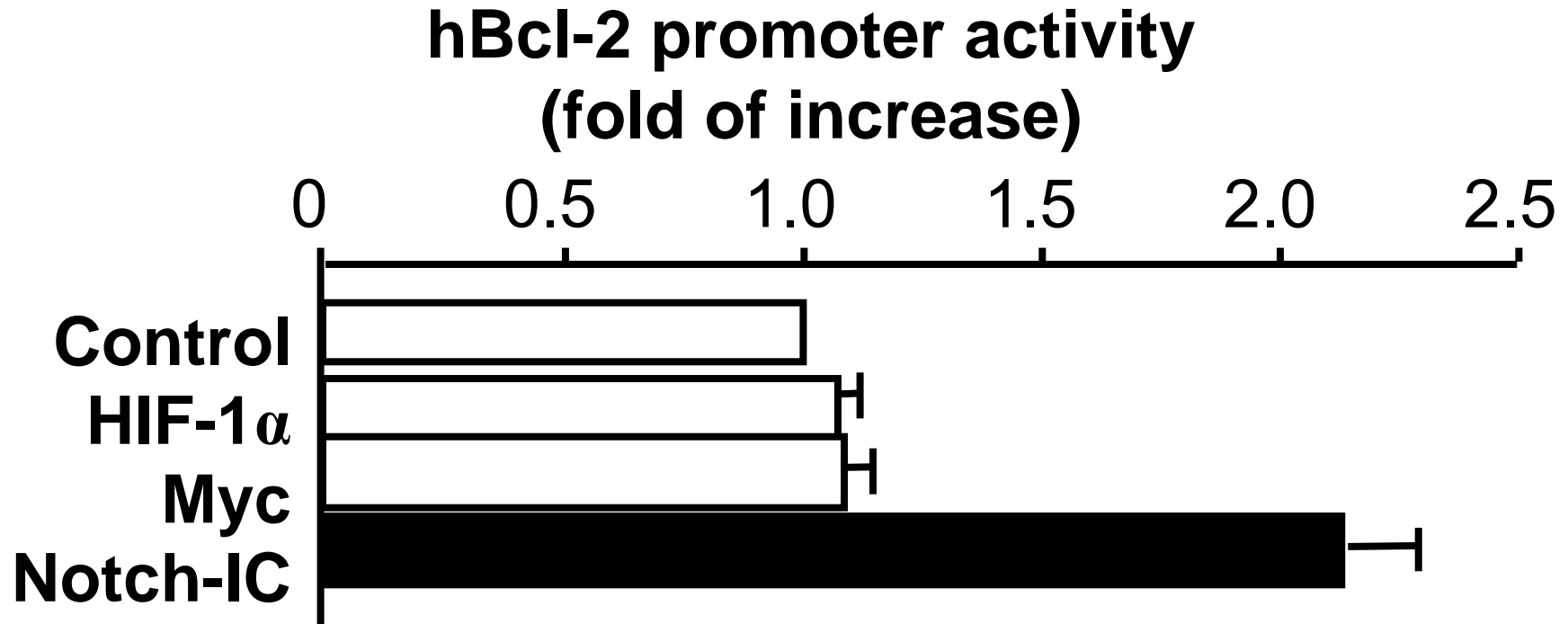
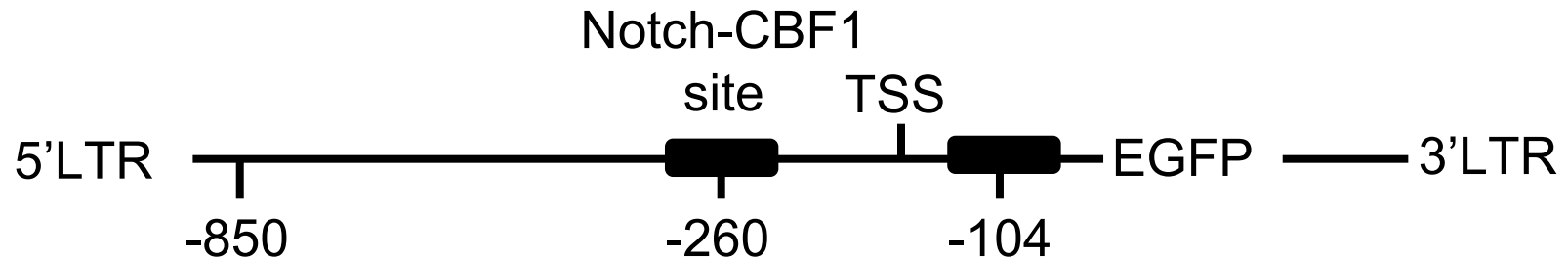


# HIF-1 $\alpha$ controls Th17 cell survival



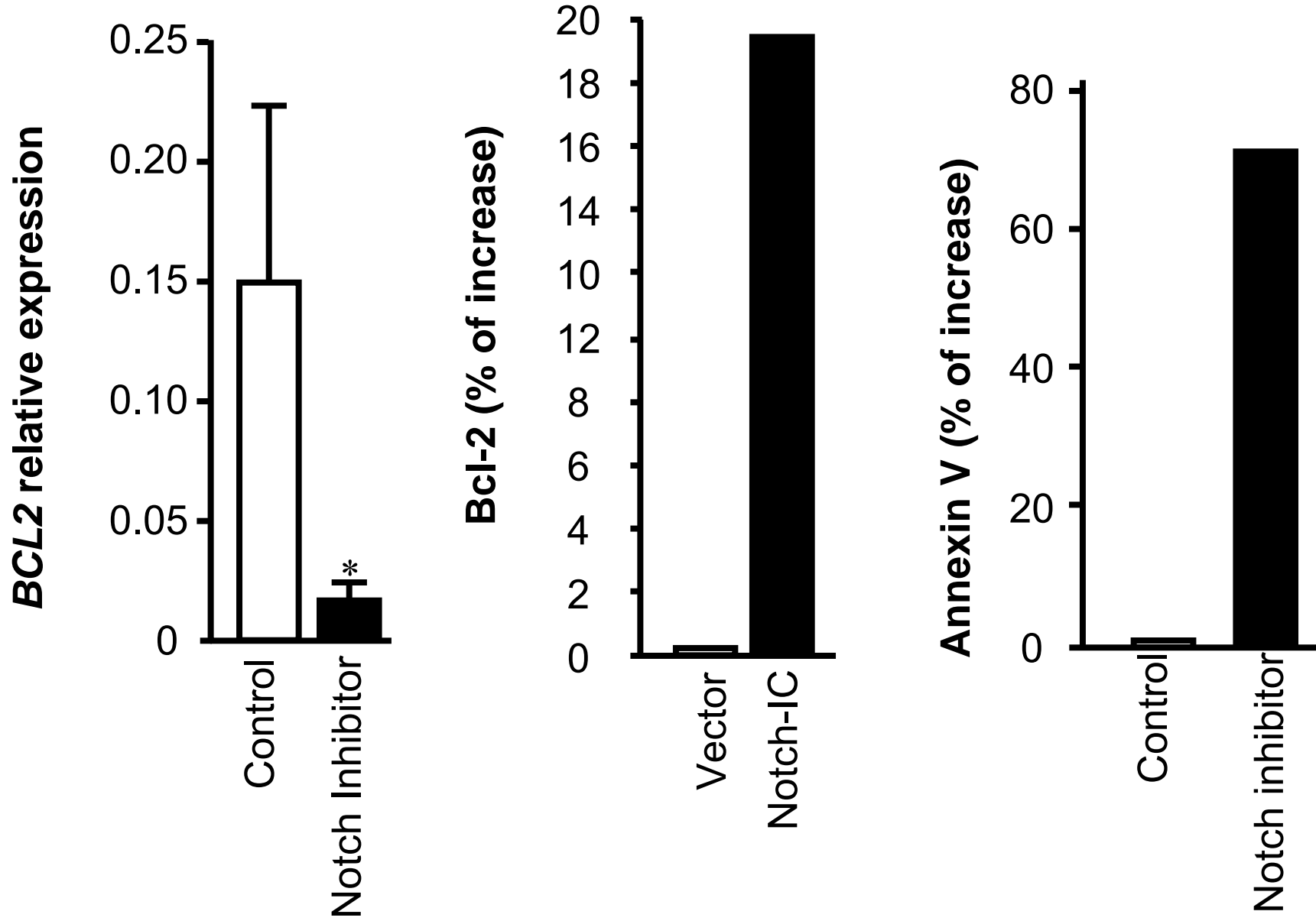
# Notch, not HIF directly regulates Bcl expression in Th17 cells

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# Notch directly regulates Bcl and Th17 survival

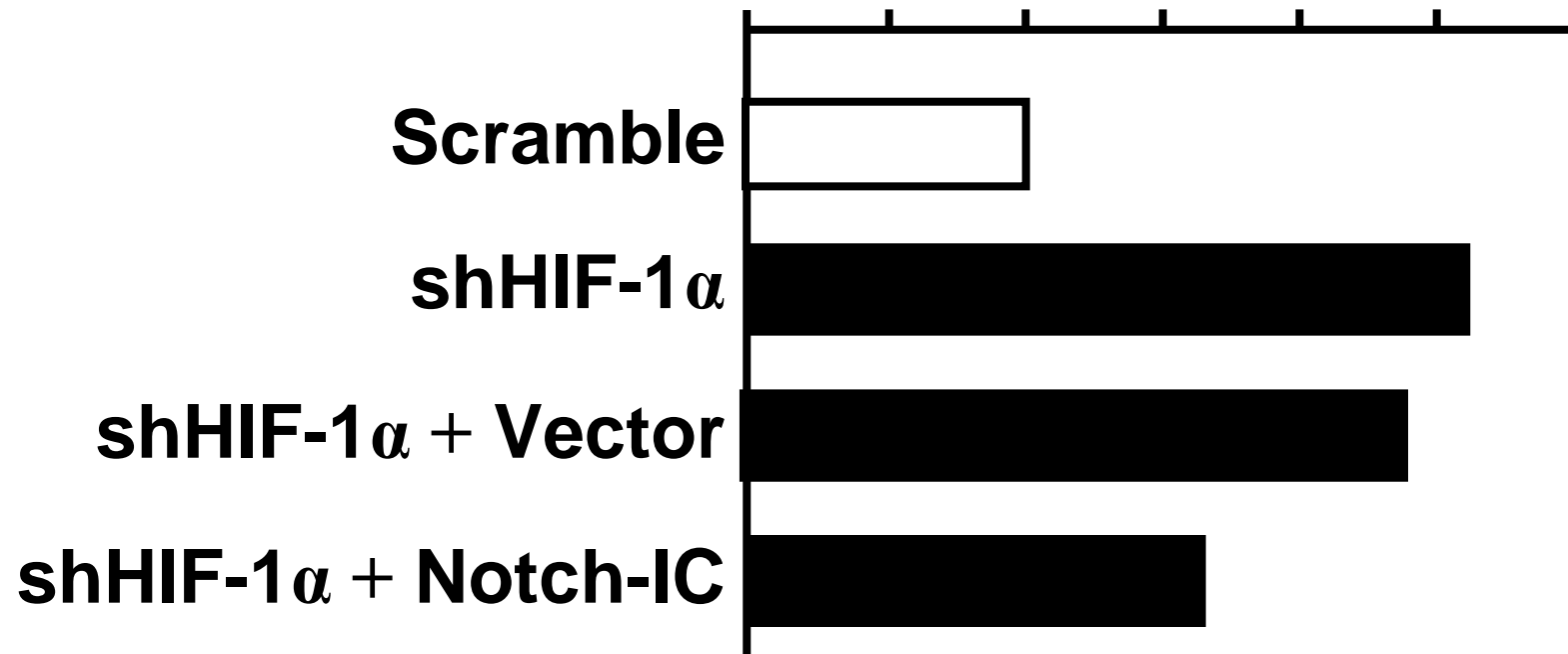
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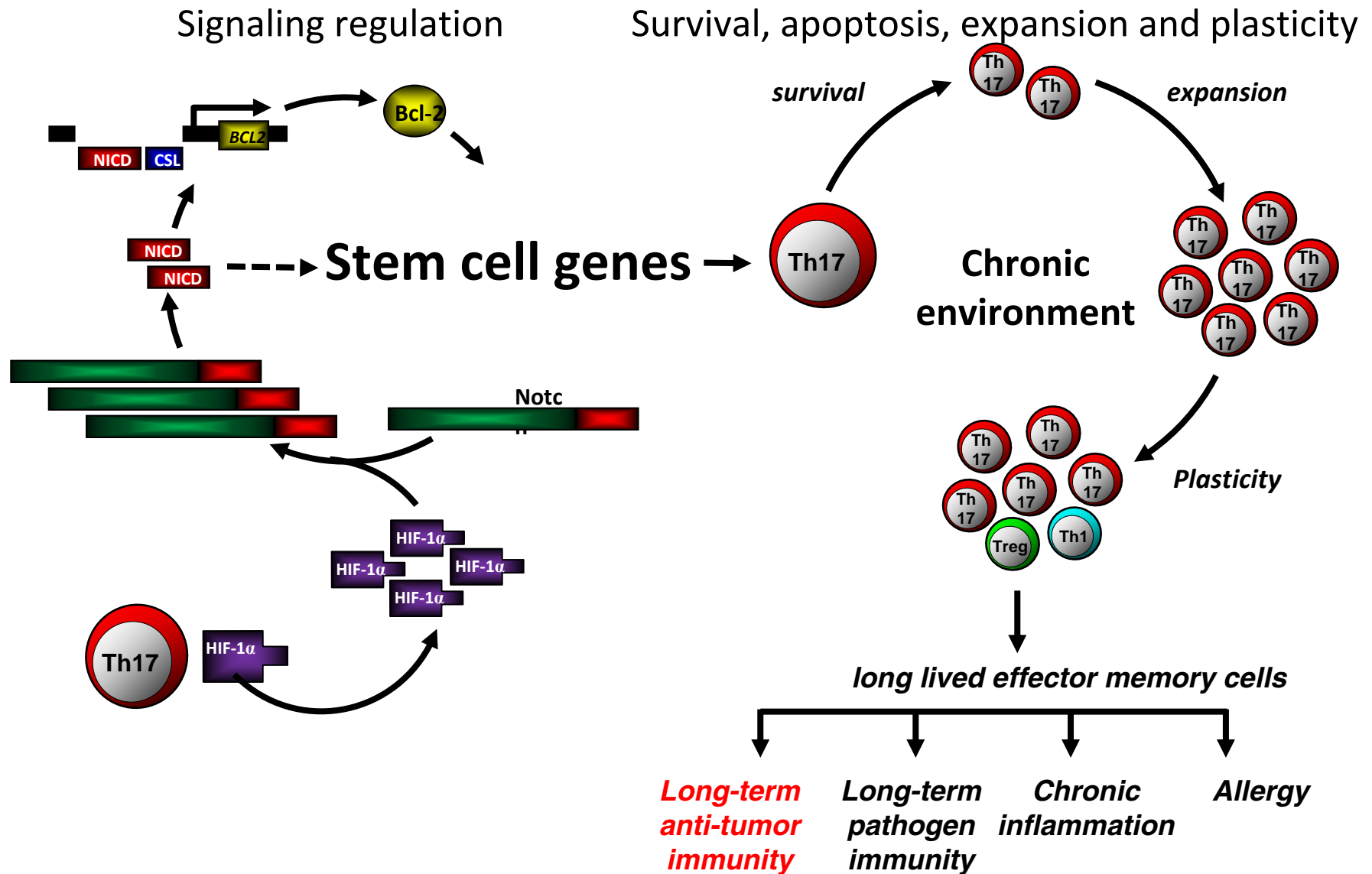
# Notch directly regulates Bcl and Th17 survival

Annexin V<sup>+</sup> cells (fold of increase)

0 0.5 1.0 1.5 2.0 2.5 3.0



# Th17 cell stemness and HIF/Notch/Bcl-2





# Th17 cell stemness and HIF/Notch/Bcl-2

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1. Muranski P, Borman ZA, Kerkar SP, Klebanoff CA, Ji Y, Sanchez-Perez L, Sukumar M, Reger RN, Yu Z, Kern SJ, Roychoudhuri R, Ferreyra GA, Shen W, Durum SK, Feigenbaum L, Palmer DC, Antony PA, Chan CC, Laurence A, Danner RL, Gattinoni L, Restifo NP. **Th17 Cells Are Long Lived and Retain a Stem Cell-like Molecular Signature. *Immunity* 2011;35:972-85 (December)**
2. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM, Pan F. **Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 2011;146:772-84 (September).**
3. Kryczek I, Zhao E, Liu Y, Wang Y, Vatan L, Szeliga W, Moyer J, Klimczak A, Lange A, Zou W. **Human TH17 Cells Are Long-Lived Effector Memory Cells. *Science Translational Medicine* 2011;3:104ra100 (October).**

# Th17 in human tumor immunity

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## 1. Th17 dynamics and tumor immunity

Th17 cells are polyfunctional, and mediate protective tumor immunity

Th17 and Th1 cells collaboratively impact tumor immunity

## 2. Th17 stemness and mechanisms

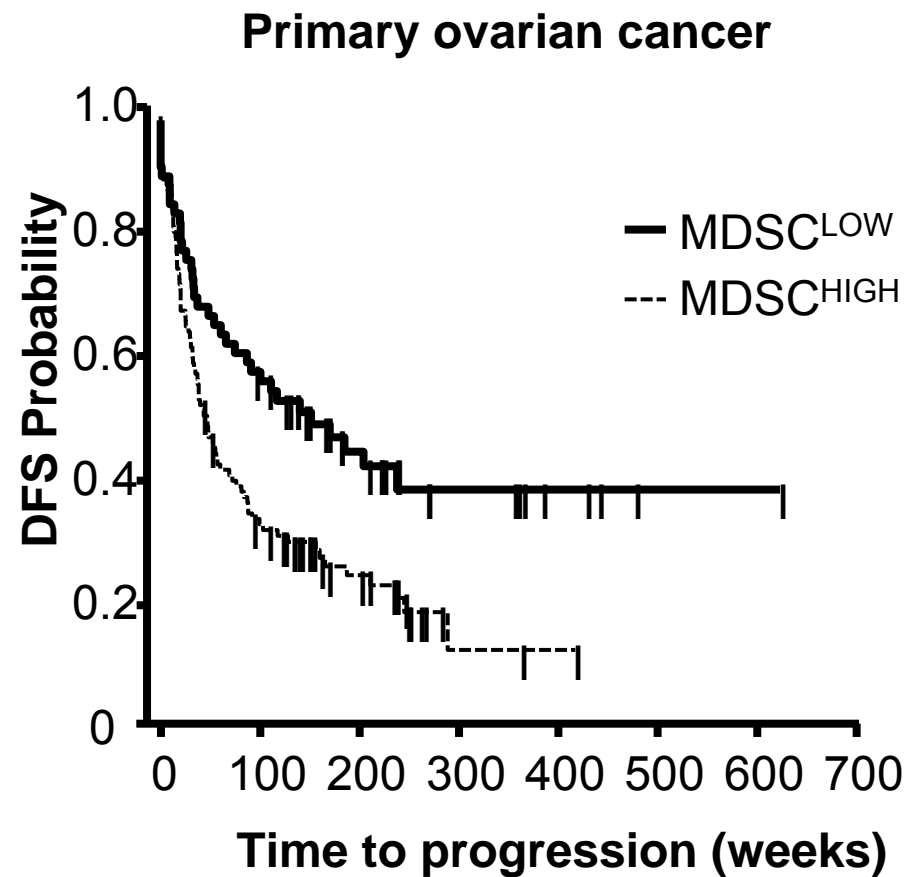
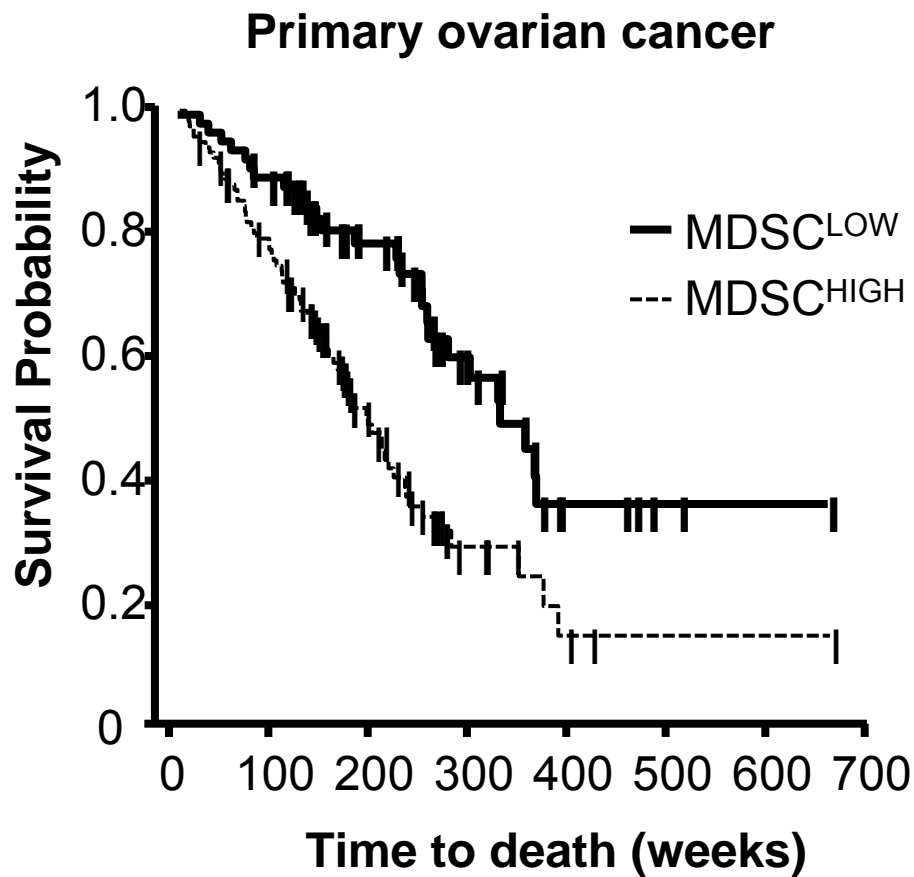
Human Th17 cells have stem cell properties

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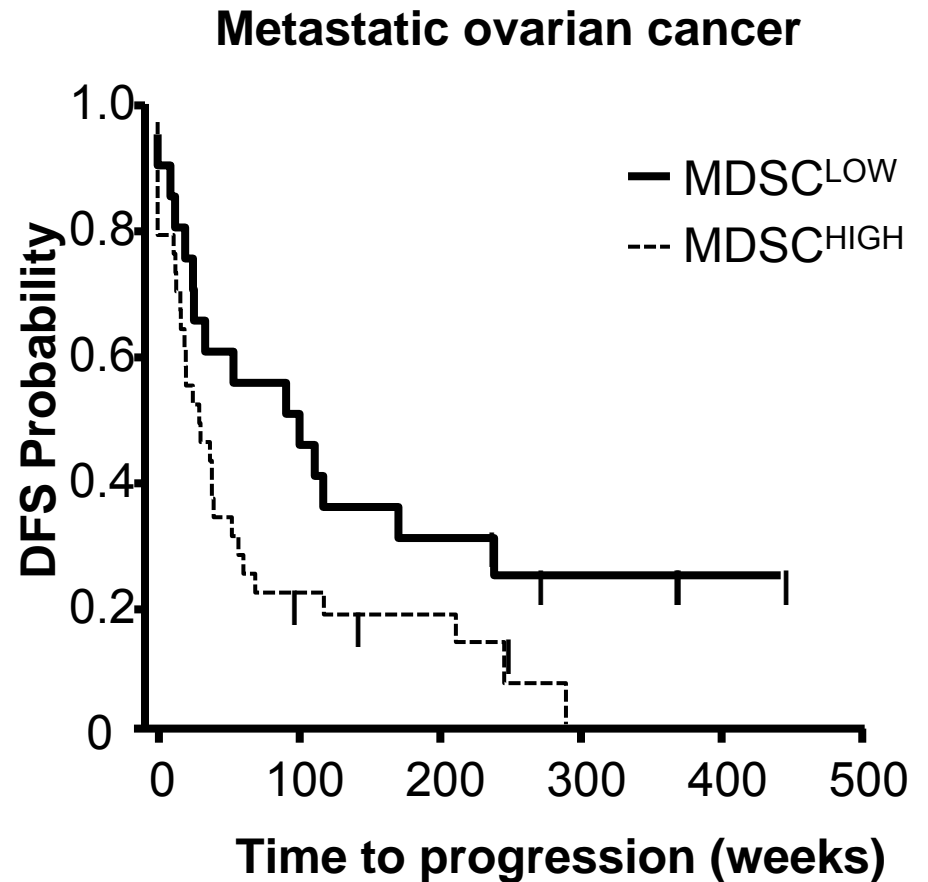
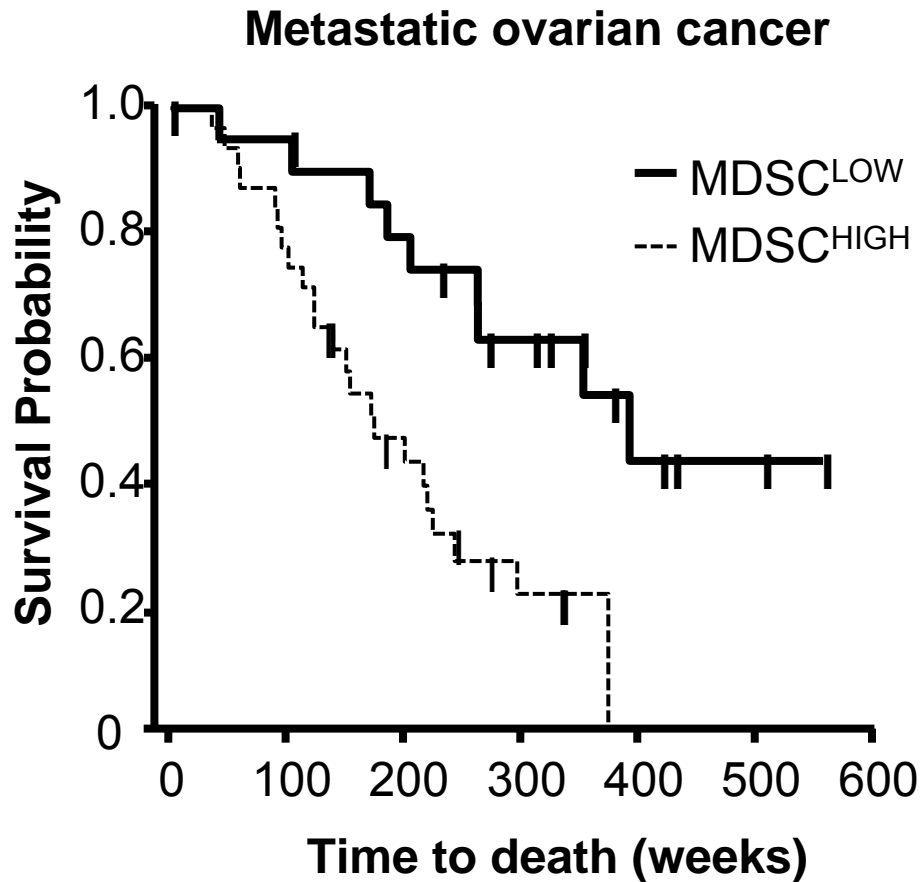
## **II. Myeloid derived suppressor cells (MDSCs), microRNA and cancer stemness**

# MDSCs and primary ovarian cancer progression

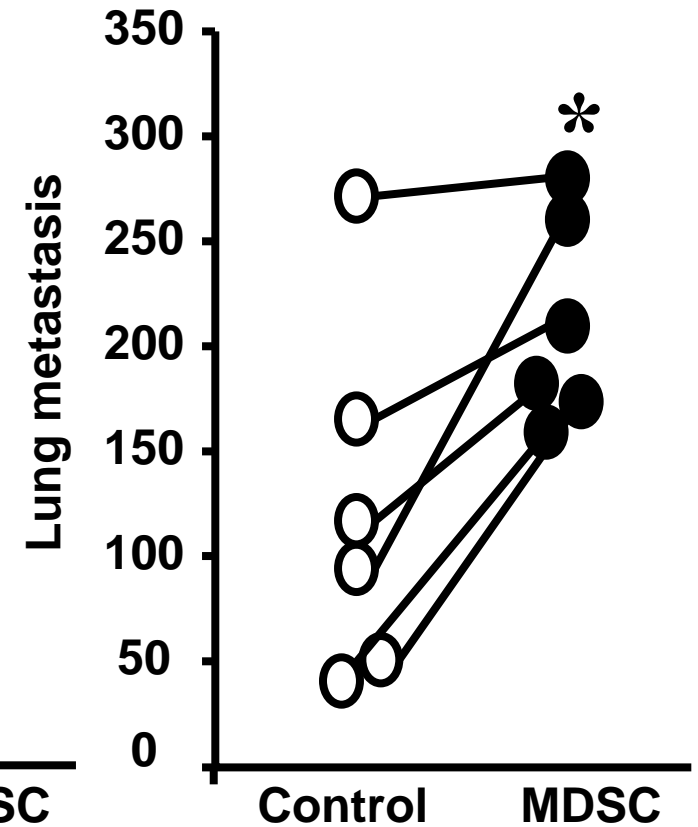
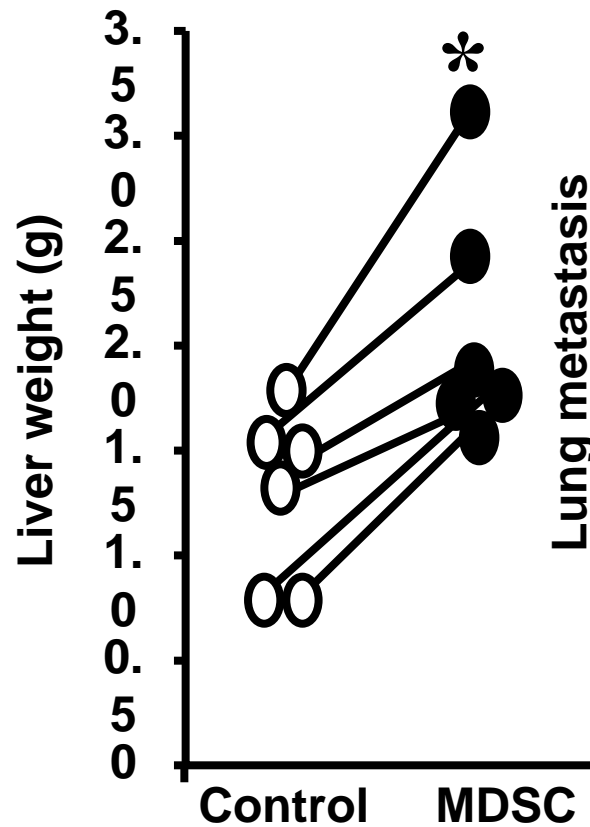
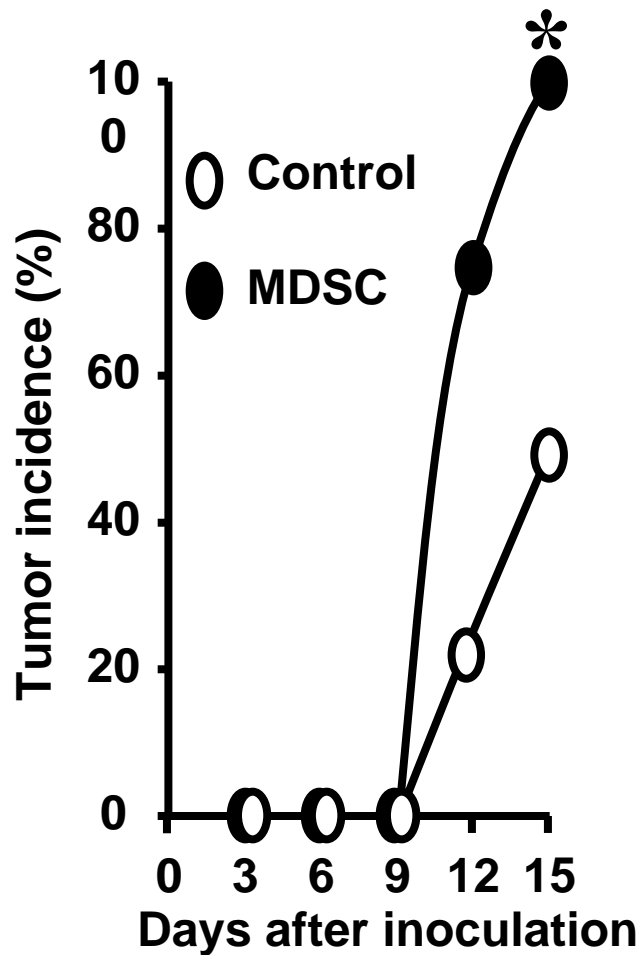
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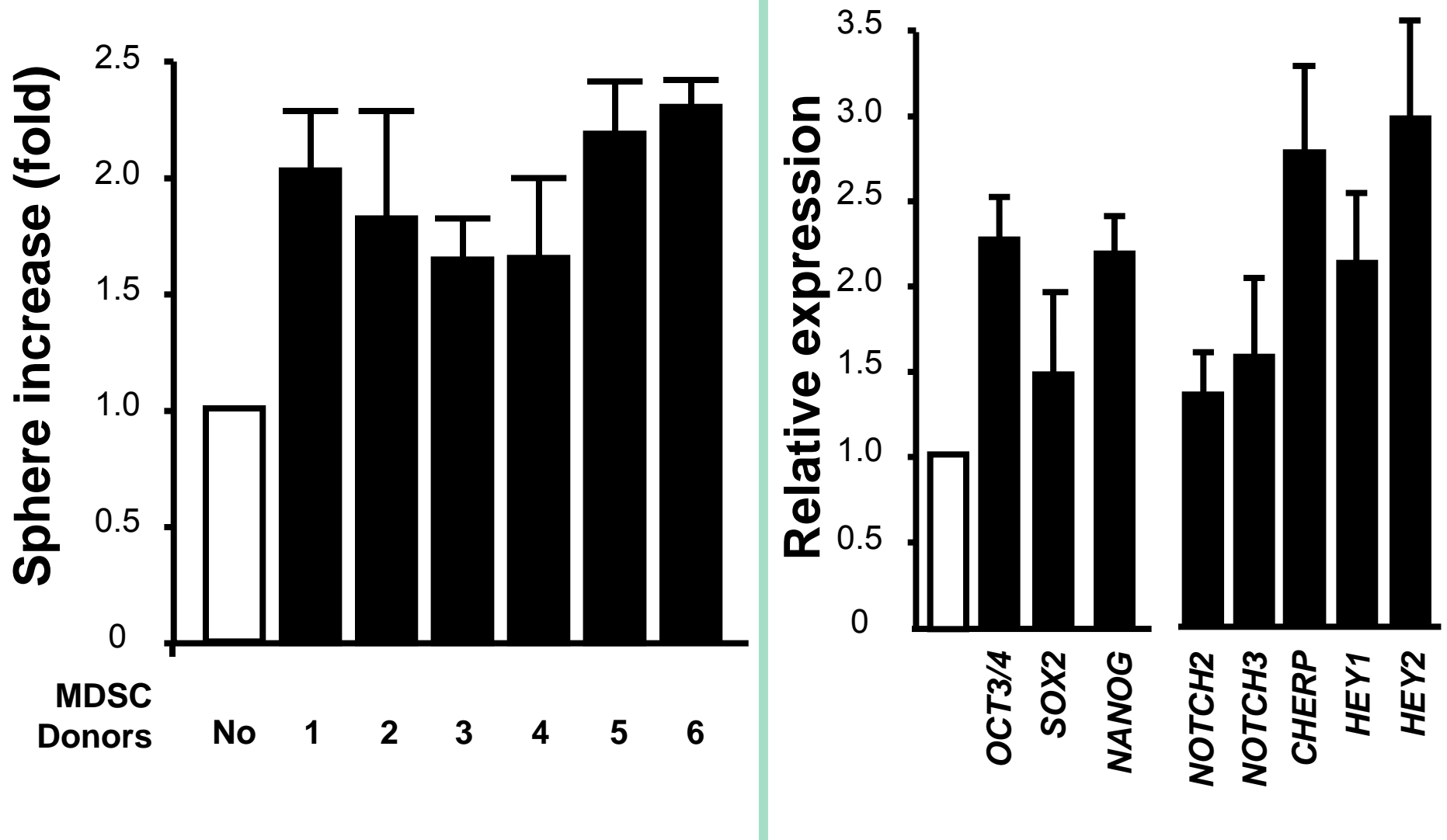
# MDSCs and ovarian cancer metastasis



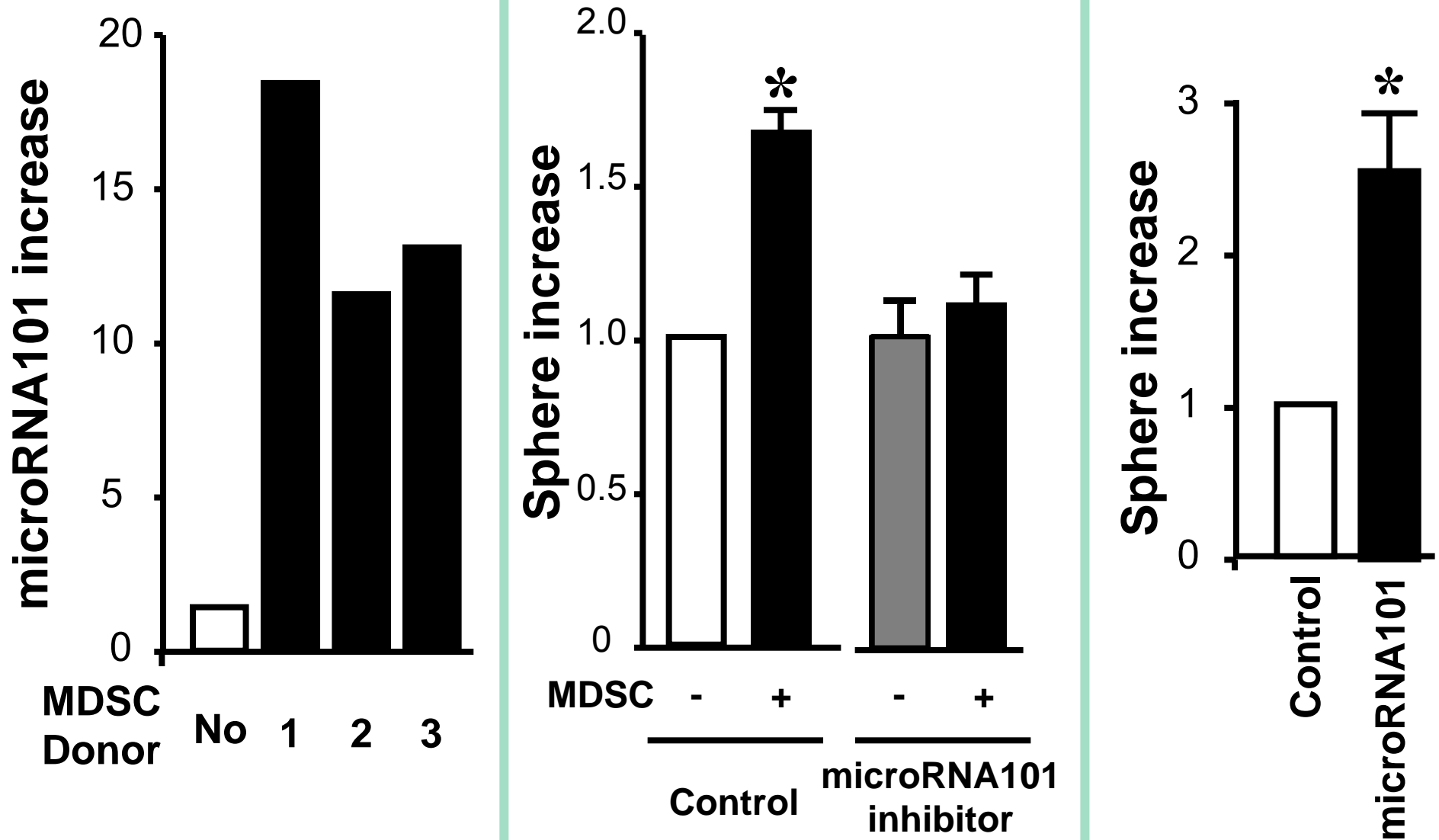
# MDSCs promote ovarian cancer metastasis



# MDSCs promote ovarian cancer stemness



# MDSCs stimulate microRNA101 and promote ovarian cancer stemness





# MicroRNA101 targets CtBP2 and promote ovarian cancer stemness

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## WT 3'UTR-CtBP2

5' ... AGUGUGAGUUACCGU U

3' AAGUCAAUAGUC

has-microRNA101

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## Mutant 3'UTR-CtBP2

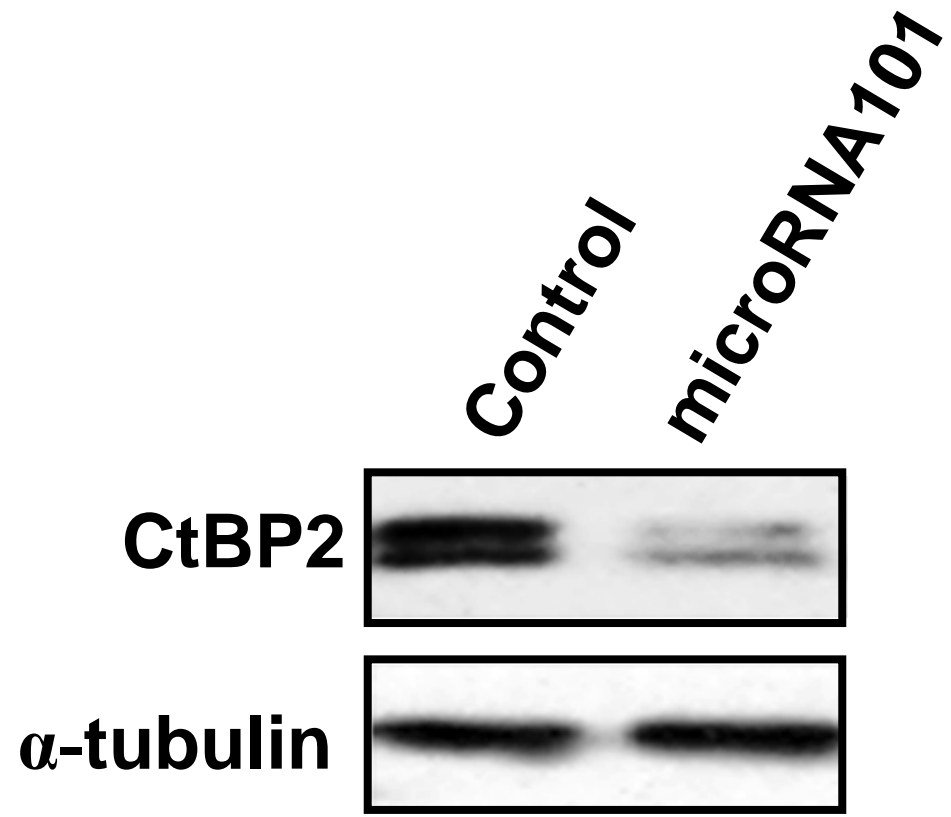
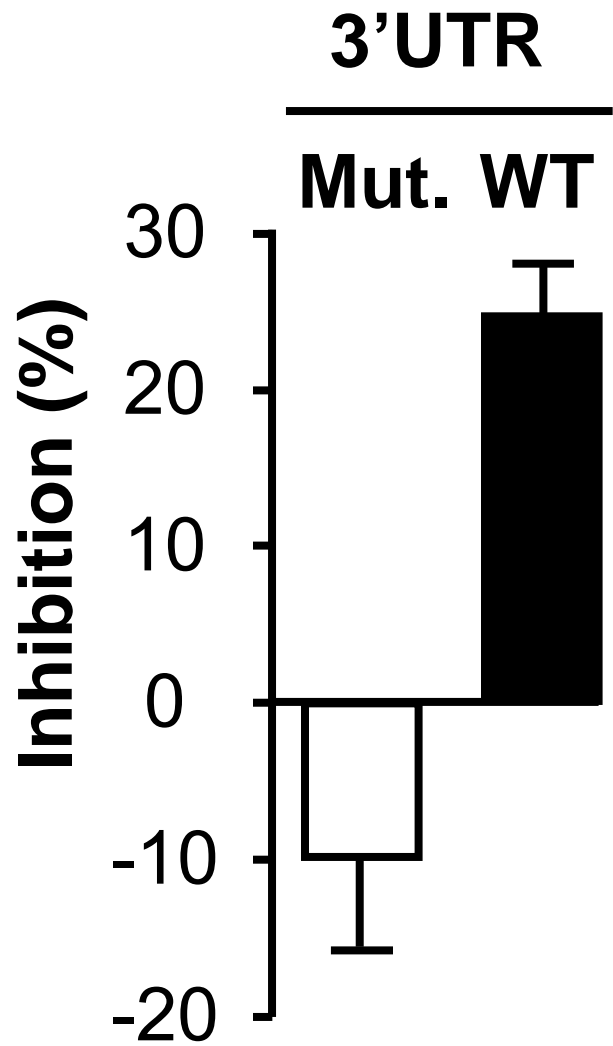
5' ... AGUGUGAGUUACCGU U

3' AAGUCAAUAGUC

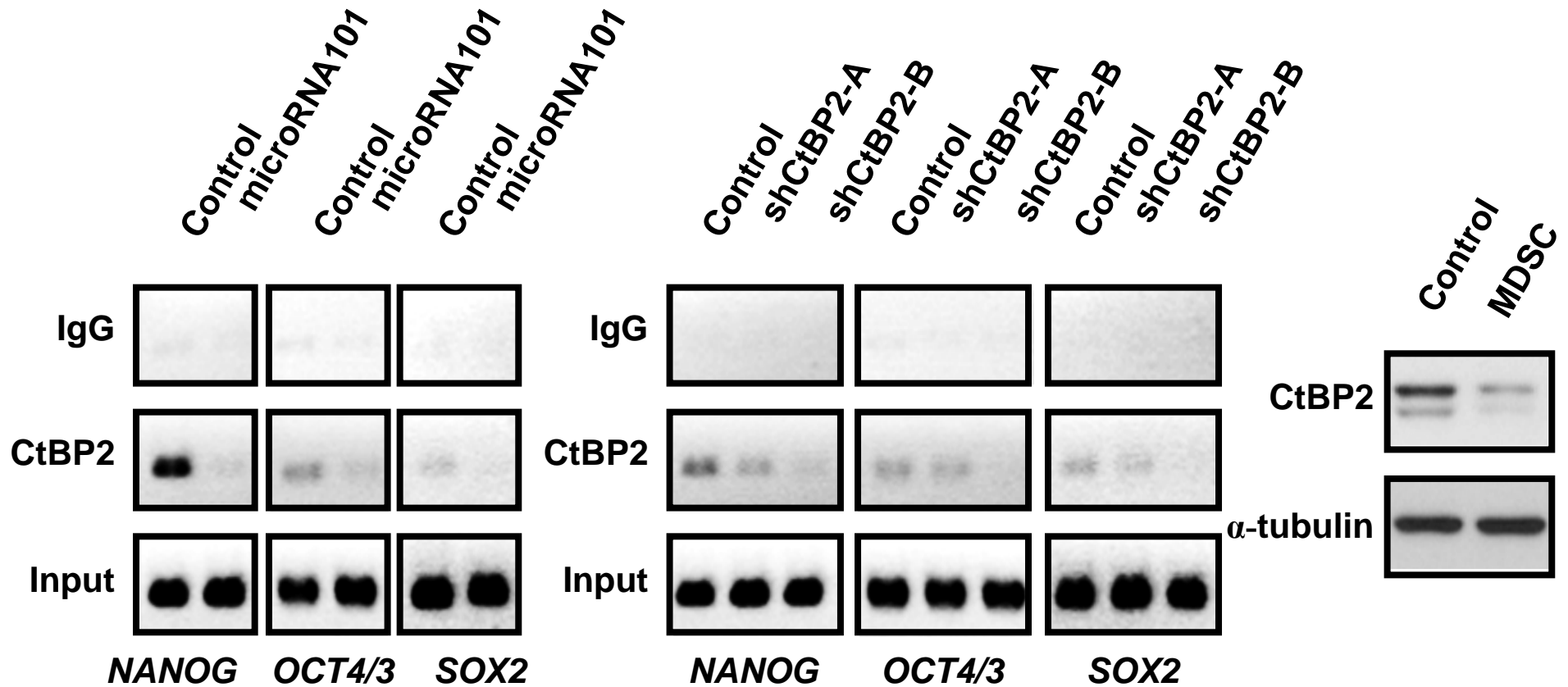
has-microRNA101

# MicroRNA101 targets CtBP2 and promote ovarian cancer stemness

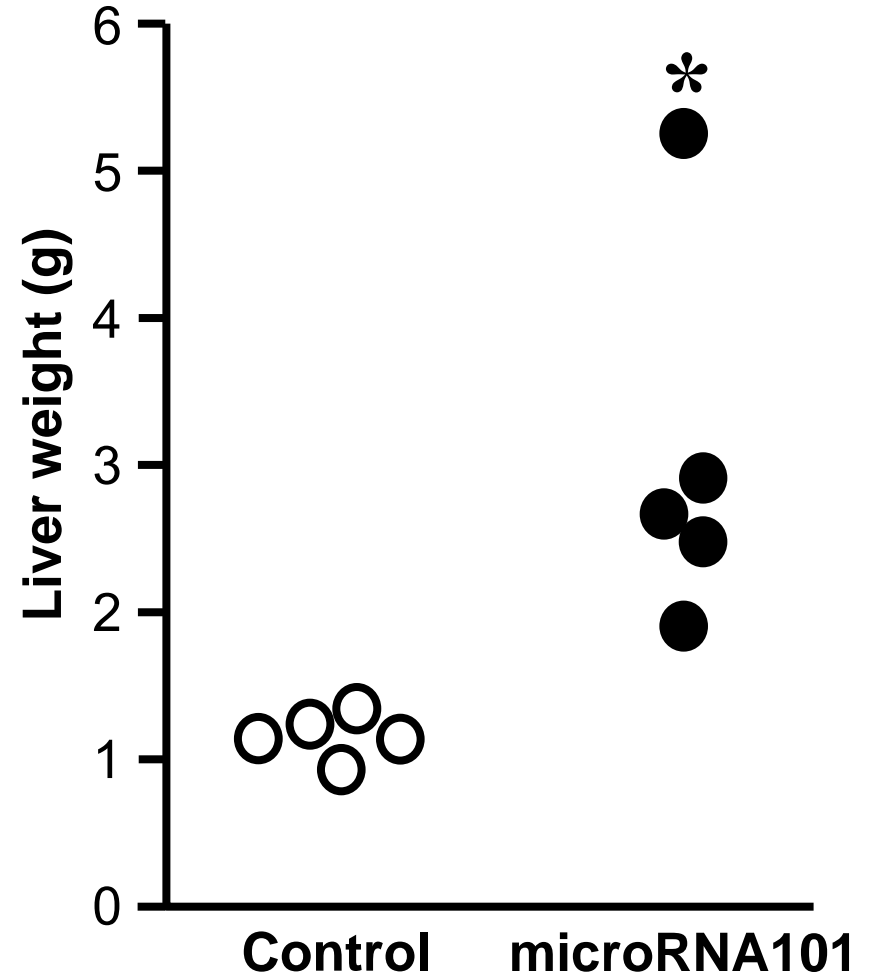
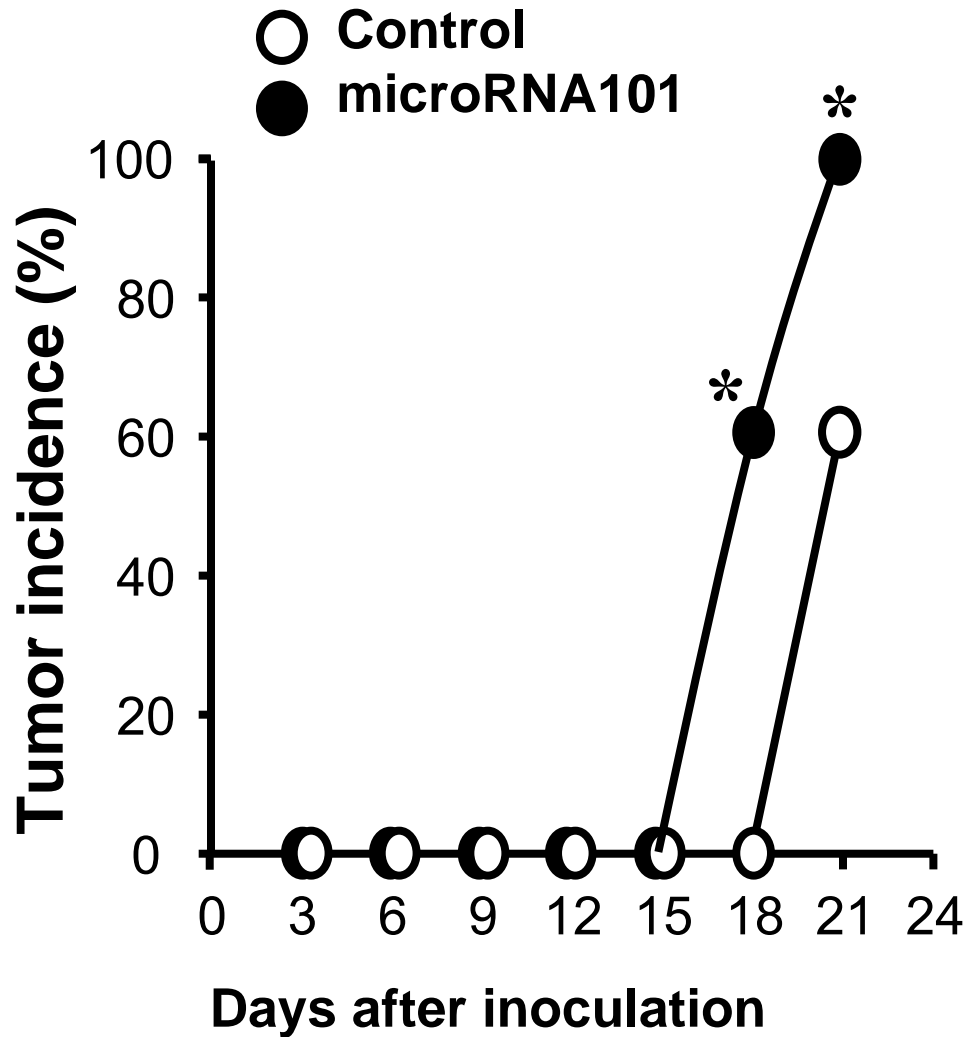
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# MicroRNA101 represses CtBP2 and promote ovarian cancer stemness

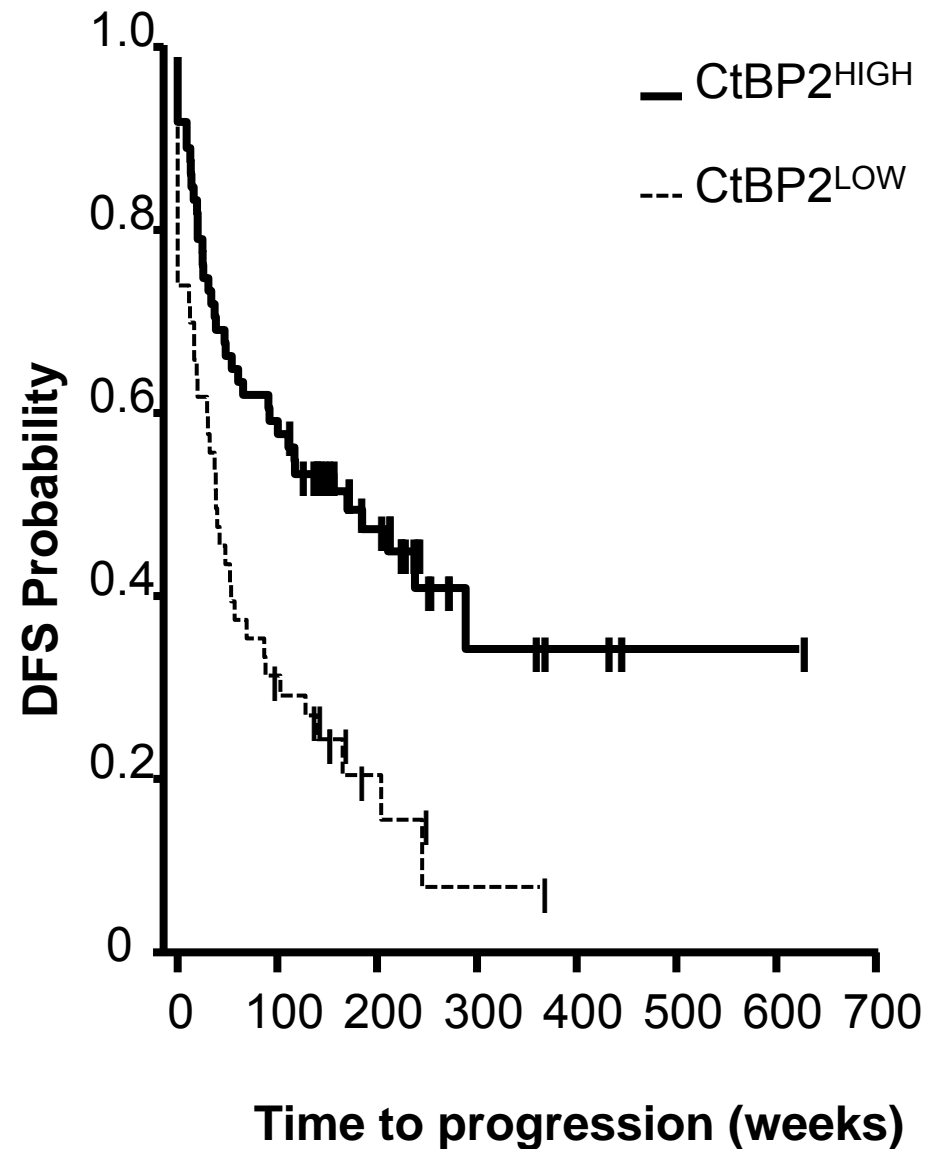
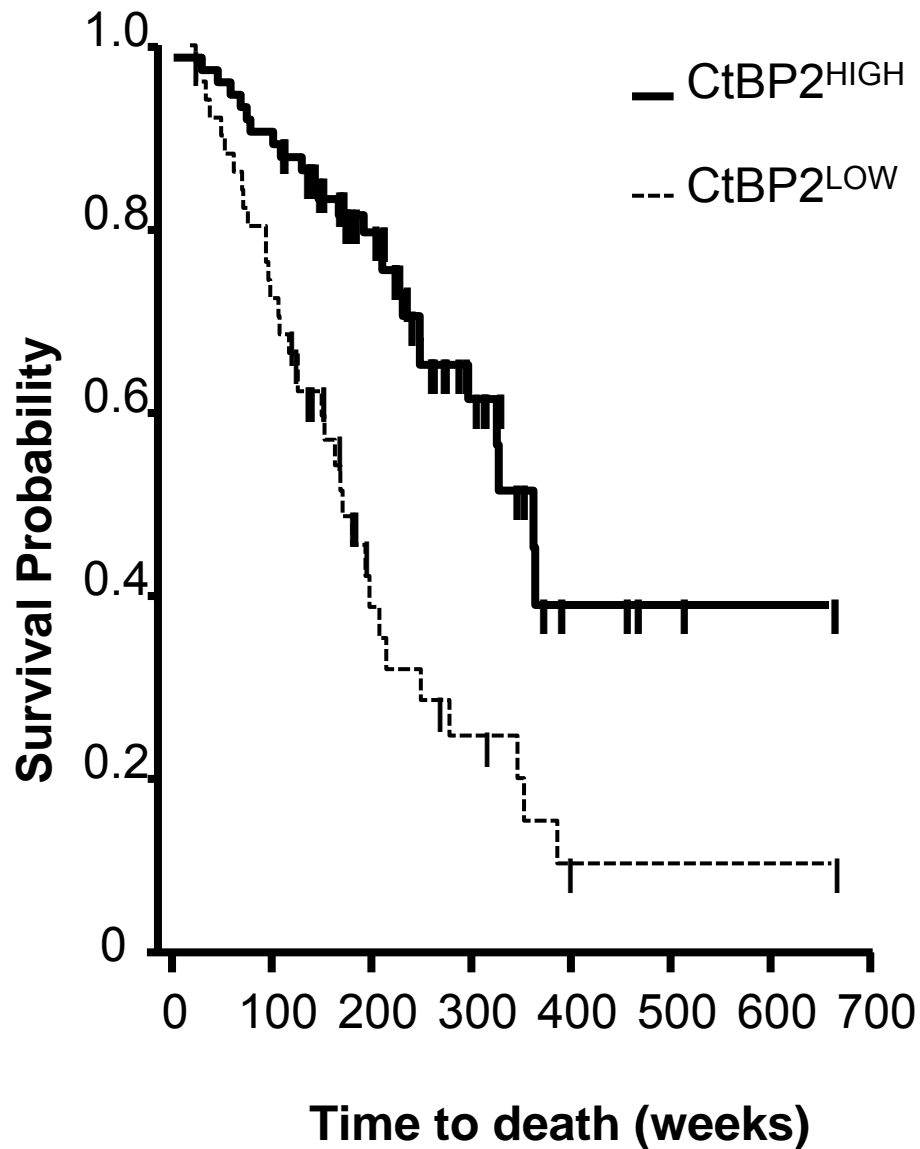


# MicroRNA101 promote ovarian cancer incidence and metastasis

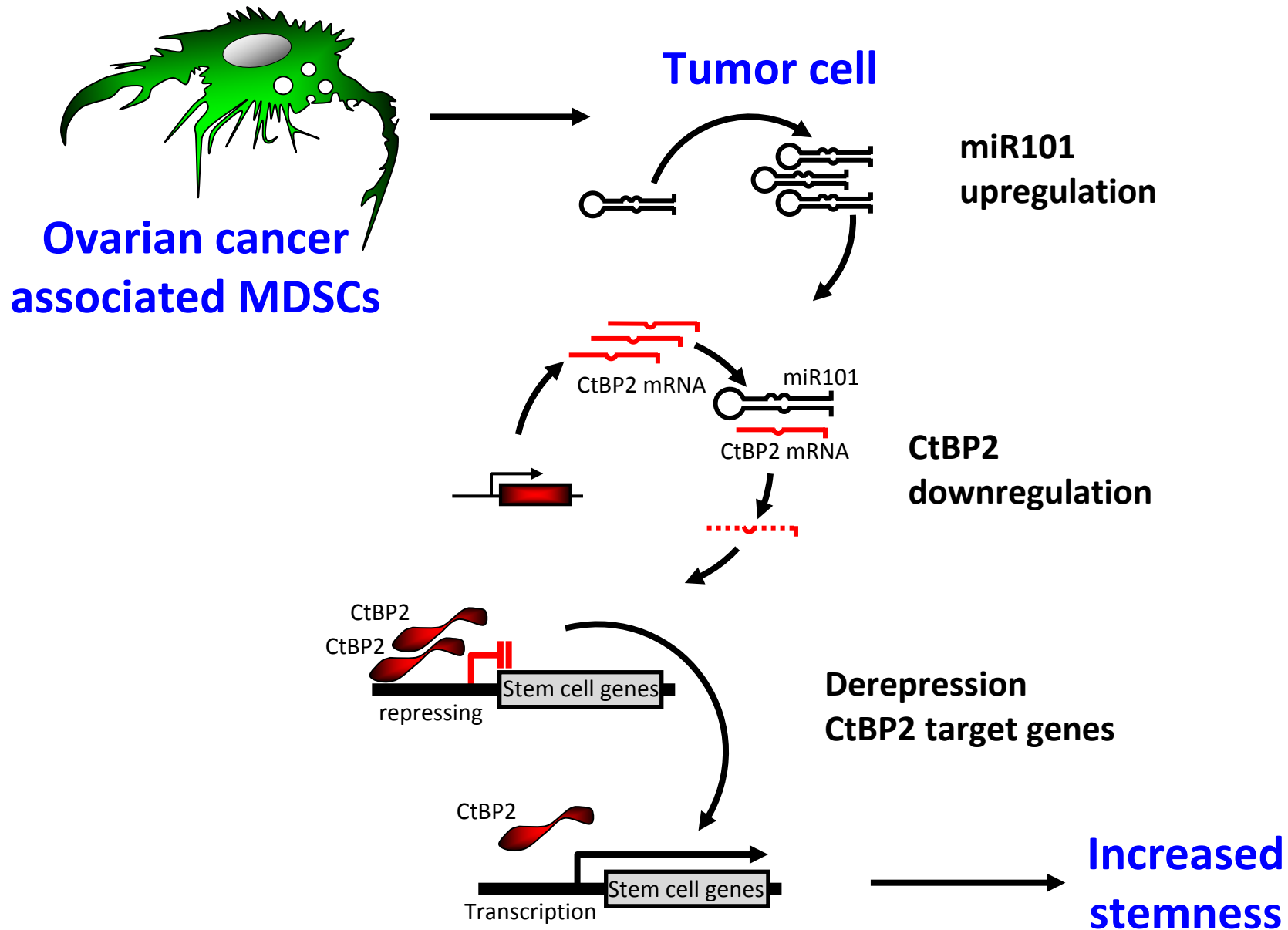


# Low CtBP2 is associated with poor survival

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# MDSCs cancer stemness via microRNA101/CtBP2



# MDSCs, microRNA and cancer stemness

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## 1. MDSCs: Immune evasion

Creating and maintaining immune suppressive environment

## 2. MDSCs: Stem niche

Promoting and sustaining cancer stem cell pool

# Oncogenesis model

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Tumor initiation: Genetic mutations and instability

Knudson hypothesis: Genetic signal, signal 1:

Intraclonal genetic alternation:  $10^{-8} \times 10^{-8} = 10^{-16}$

Interclonal genetic alternation:  $10^{-8} + 10^{-8} = 2 \times 10^{-8}$

Extrinsic stemness signal (MDSCs, macrophages, fibroblasts), signal 2:

Environmental stem cell niche (Signals for stemness maintenance).

Mutation + extrinsic signals  $> 10^{-8}$

Immune suppressive signal (MDSCs, Tregs), signal 3:

Mutation + environmental niche + suppressed immunity  $\gg 10^{-8}$

## Three signal oncogenesis model



Ilona Kryczek  
Wojciech Szeliga  
Saleh Altuwaijri  
Cailin Wilke  
Linda Vatan  
Ke Wu  
Takashi Tanikawa  
Joel Crespo  
Nisha Nagarsheth  
Tracy Cui  
Dongjun Peng  
Yanwei Lin  
Allen Bruce  
Johann Gudjonsson  
Andrew Johnston  
Abhishek Aphale  
James Elder  
Theodore Welling  
Yin Wang  
Yan Liu  
Shuang Wei  
Rebecca Liu  
Emily Finlayson  
Emina Huang  
Diane Simeone  
Alfred Chang  
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Yale University

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Alan Gordon  
Arizona Gynecologic Oncology, Phoenix

Robert Edwards  
University of Pittsburg

CA092652, CA099985, CA100227, CA123088,  
CA133620, DOD OC020173  
Concern Foundation  
Ovarian Cancer Research Foundation  
Rivkin Ovarian Cancer Research Center

# Controversial?

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## Inequalities in evaluating Th17 and tumor

Th17  $\neq$  IL-17<sup>+</sup> cell

Th17  $\neq$  IL-17

Th17  $\neq$  IL-23

Exogenous IL-17  $\neq$  Endogenous IL-17

ROR gene expression  $\neq$  Th17

Mouse  $\neq$  Human

Immunodeficient  $\neq$  Immune Competent

Early Cancer Stage  $\neq$  Advanced Cancer Stage

Chemical Carcinogen-induced Cancer  $\neq$  Chronic Infection-

Associated Cancer  $\neq$  Spontaneous Cancer



**Th17 cells in human spontaneous epithelial carcinoma**