Glioma cancer stem cells mediate immune suppression that can be reversed with STAT3 blockade

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Immune suppression in Malignant Glioma Patients

Mechanisms

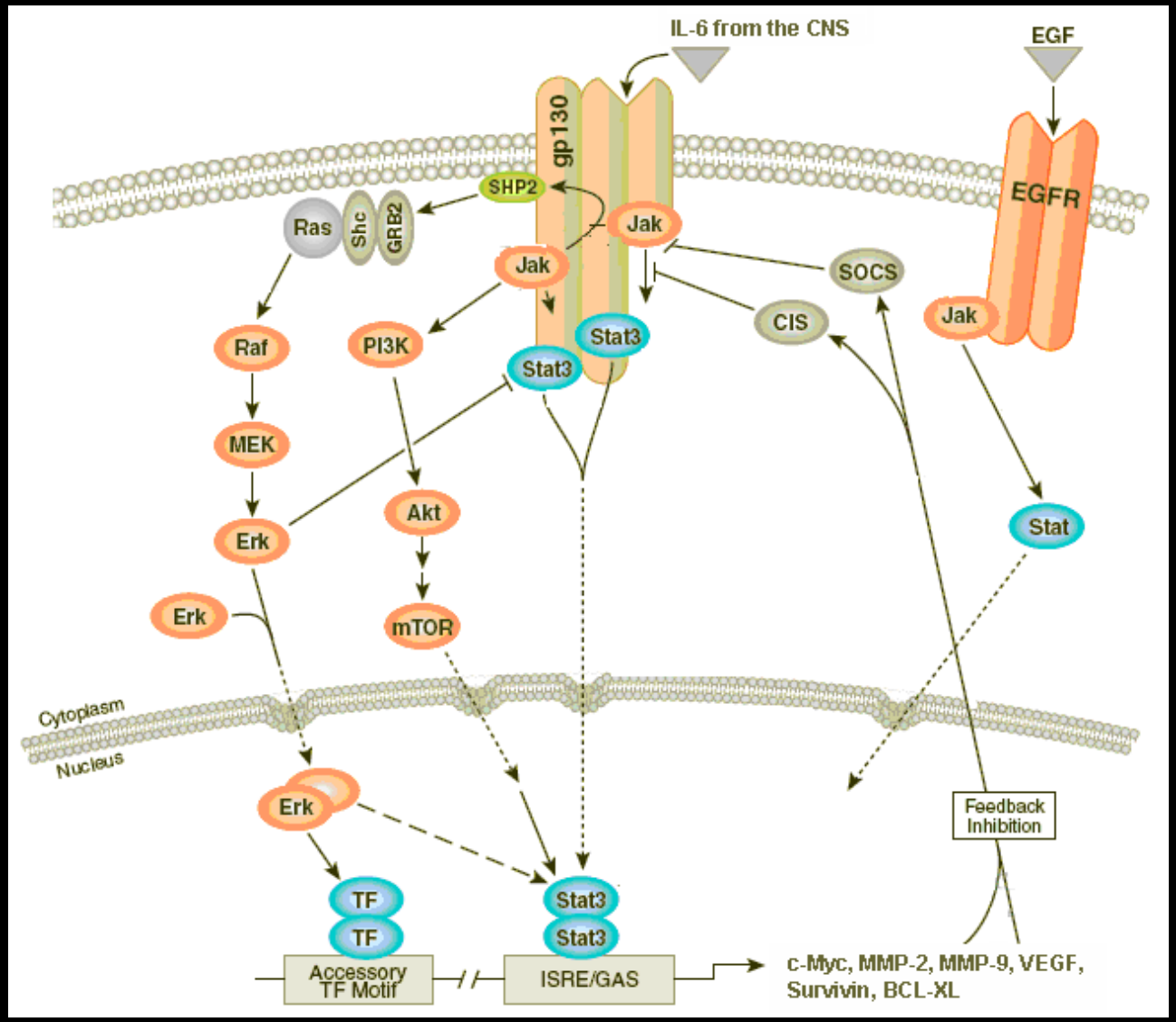
- Immune suppressive cytokines (TGF-β, IL-10, PGE2)
- Loss of antigen/MHC expression
- Lack of co-stimulation in the tumor microenvironment (induction of anergy)
- Induction of T cell apoptosis
- Expression of inhibitory co-stimulation (B7-H1)
- Enhanced Tregs
- Immune suppressive microglia/macrophages
- Cancer stem cells/cancer initiating cells?

Manifestations

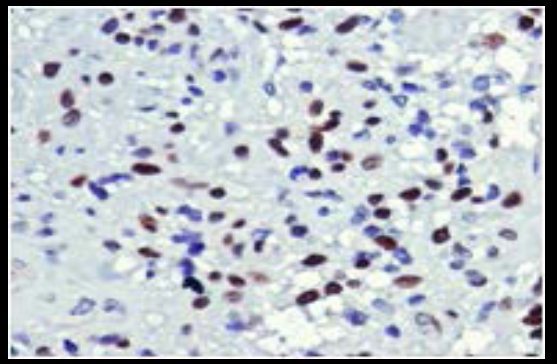
- Decreased delayed type hypersensitivity responses to recall antigens
- Diminished antibody responses
- Impaired T cell proliferation and responses to IL-2
- Impaired cytotoxic/effector T cell responses
- T cell anergy/unresponsiveness

Is there a common pathway?

The STAT3 pathway is active in many cancers including gliomas

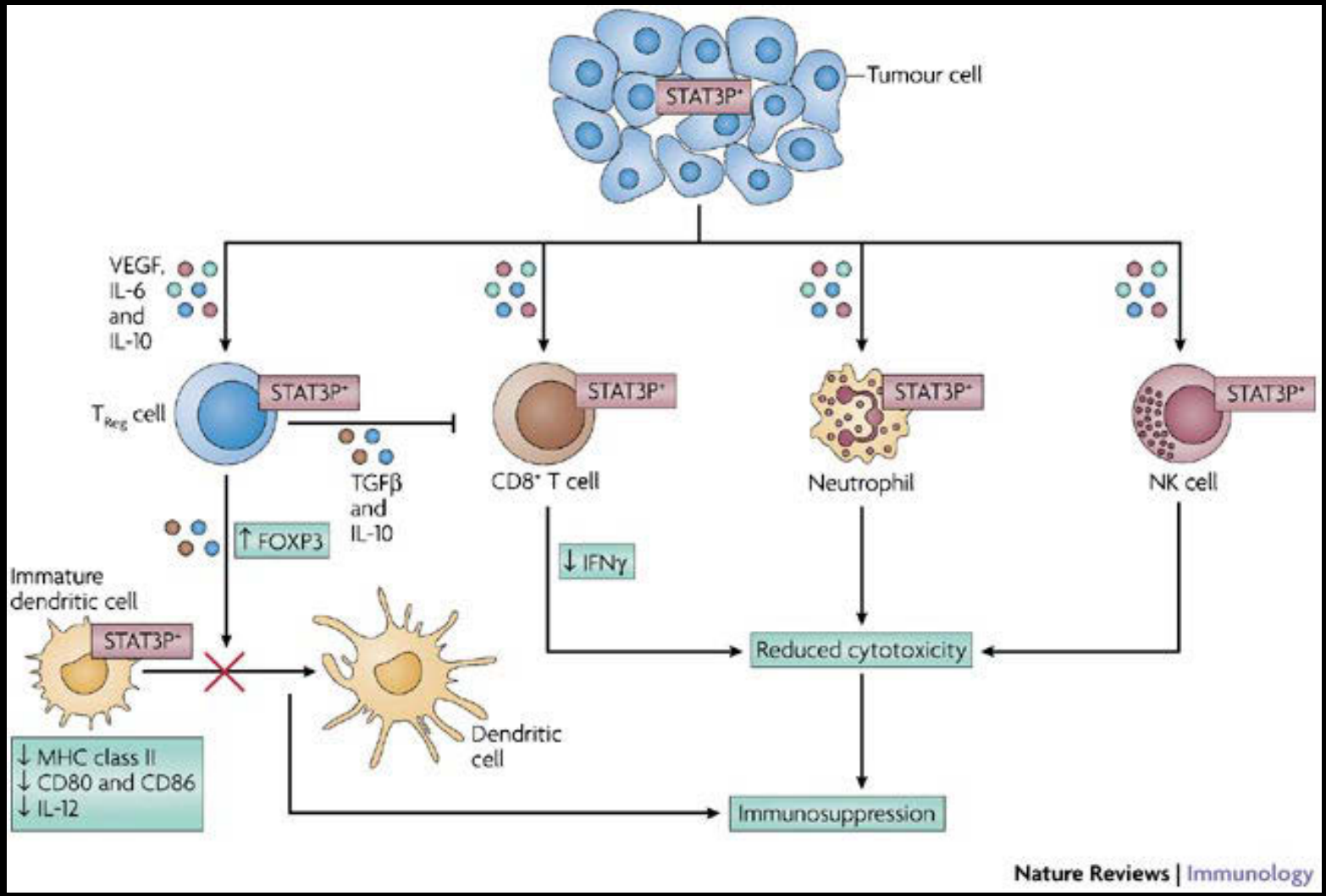


p-STAT3 Expression in GBM



Abou-Ghazal MK et al. Clinical Cancer Res. 2008, 14(24):8228-8235

The STAT3 pathway is a key regulatory pathway in immune suppression



Yu *et al*. *Nature Reviews Immunology* **7**, 41–51 (January 2007)

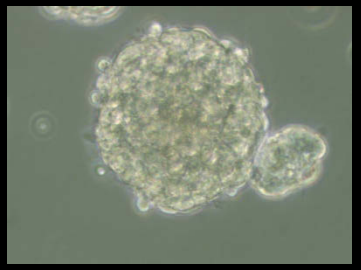
Glioma cancer stem cells (gCSCs)

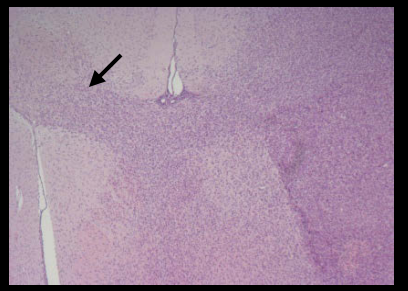
- gCSCs possesses marked capacity for proliferation, self-renewal and differentiation. (Singh S, et al. Cancer Res. 2003, 63: 5821-5828)
- gCSCs can establish tumors with all of the classical features of human GBM. (Galli R, et al. Cancer Res. 2004, 64: 7011-7021)
- gCSCs mediate resistance of radiotherapy and chemotherapy. (Bao S, et al. Nature 2006: 756-760; Murat A, et al. J Clin Oncol 2008: 3015-3024)

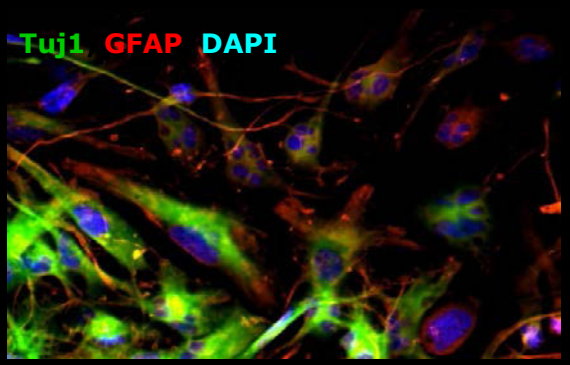
Hypothesis : gCSCs contribute to the immune suppression evident in glioma patients and STAT-3 pathway blockade can reverse this immunosuppression

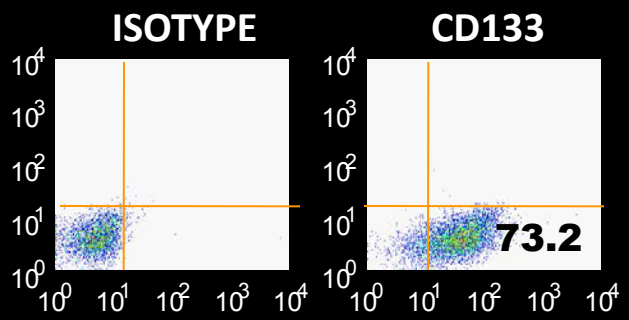
Properties of GBM Cancer Stem Cells (gCSC)/cancer initiating cells

- Form neurospheres
- Form infiltrating tumors
- Are induced to differentiate into various glia populations
- Express CD133



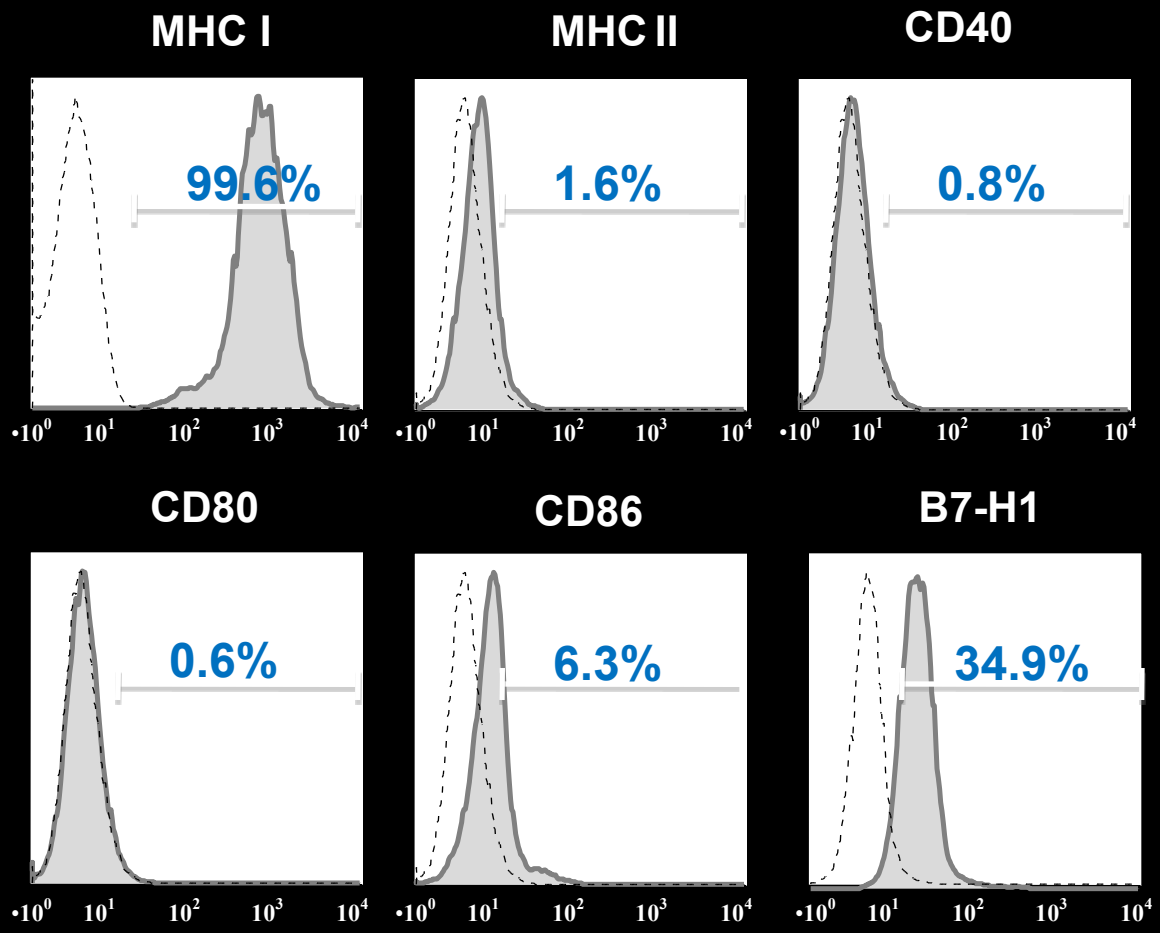




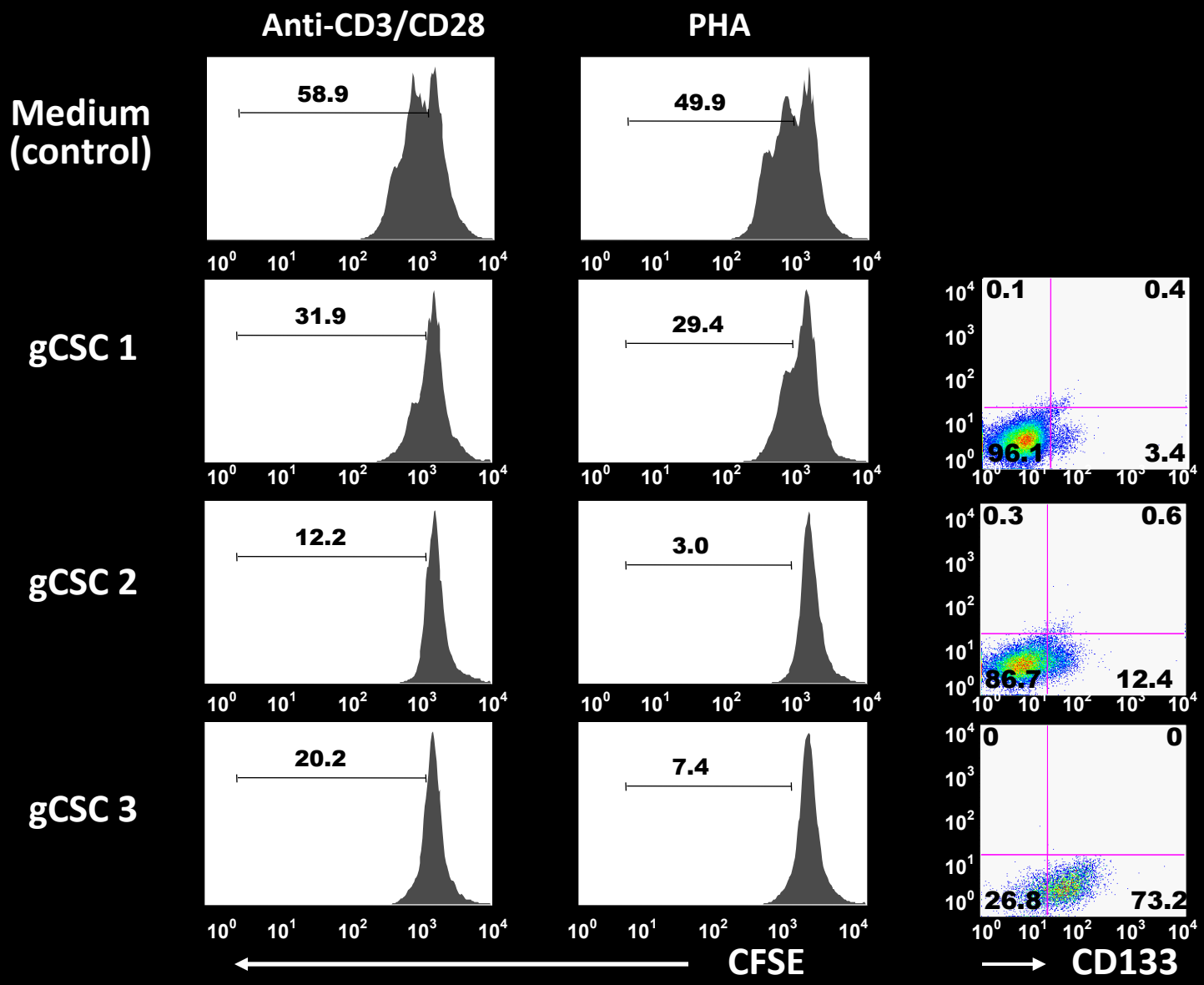


DMEM-F-12 supplemented with
EGF (20ng/mL), bFGF and B27 (1:50)

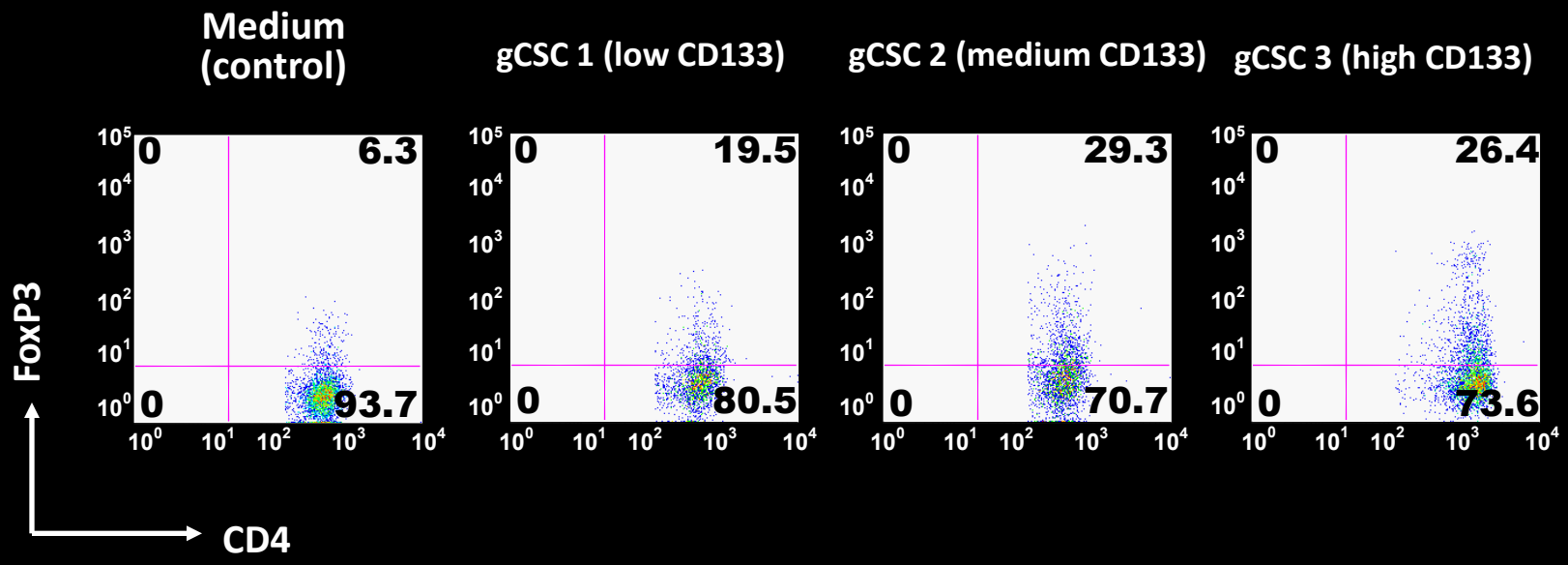
The immune phenotype of gCSCs are immune inhibitory

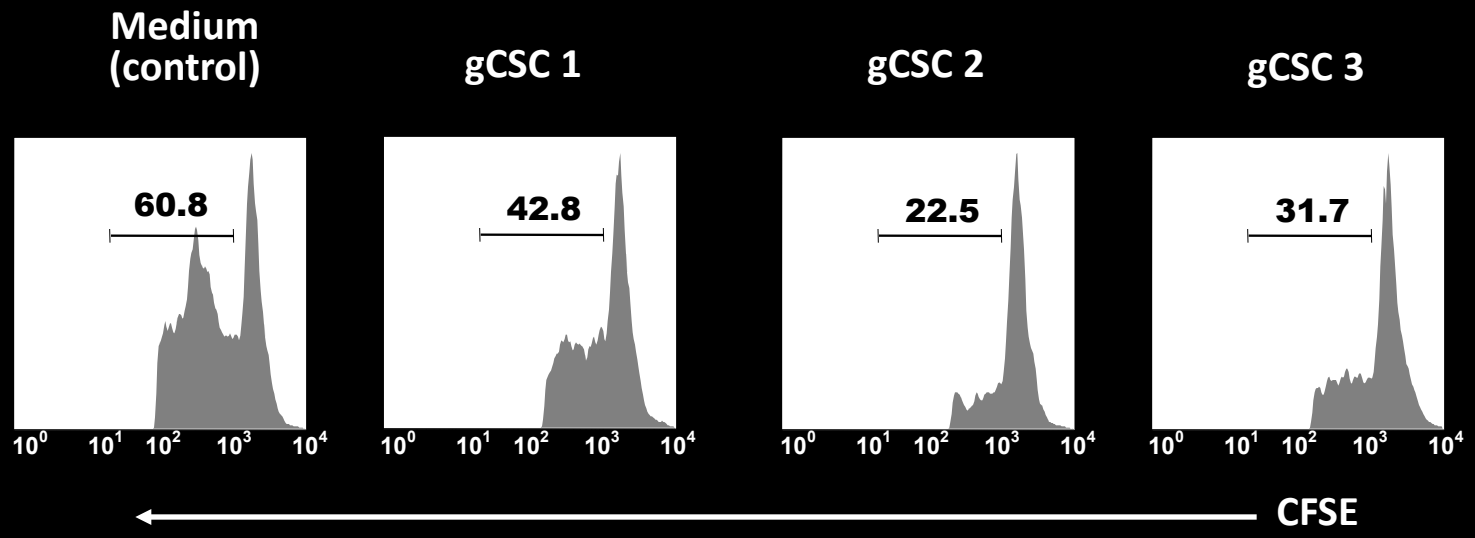


gCSCs inhibit T cell proliferation

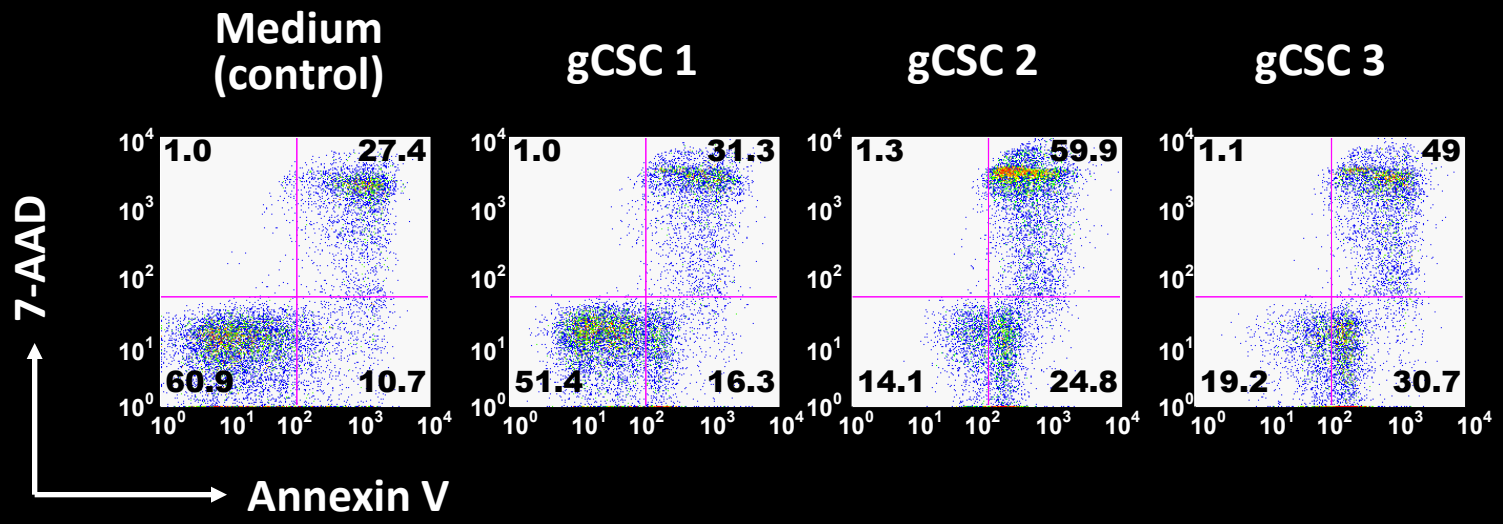


gCSCs induce FoxP3+ Tregs

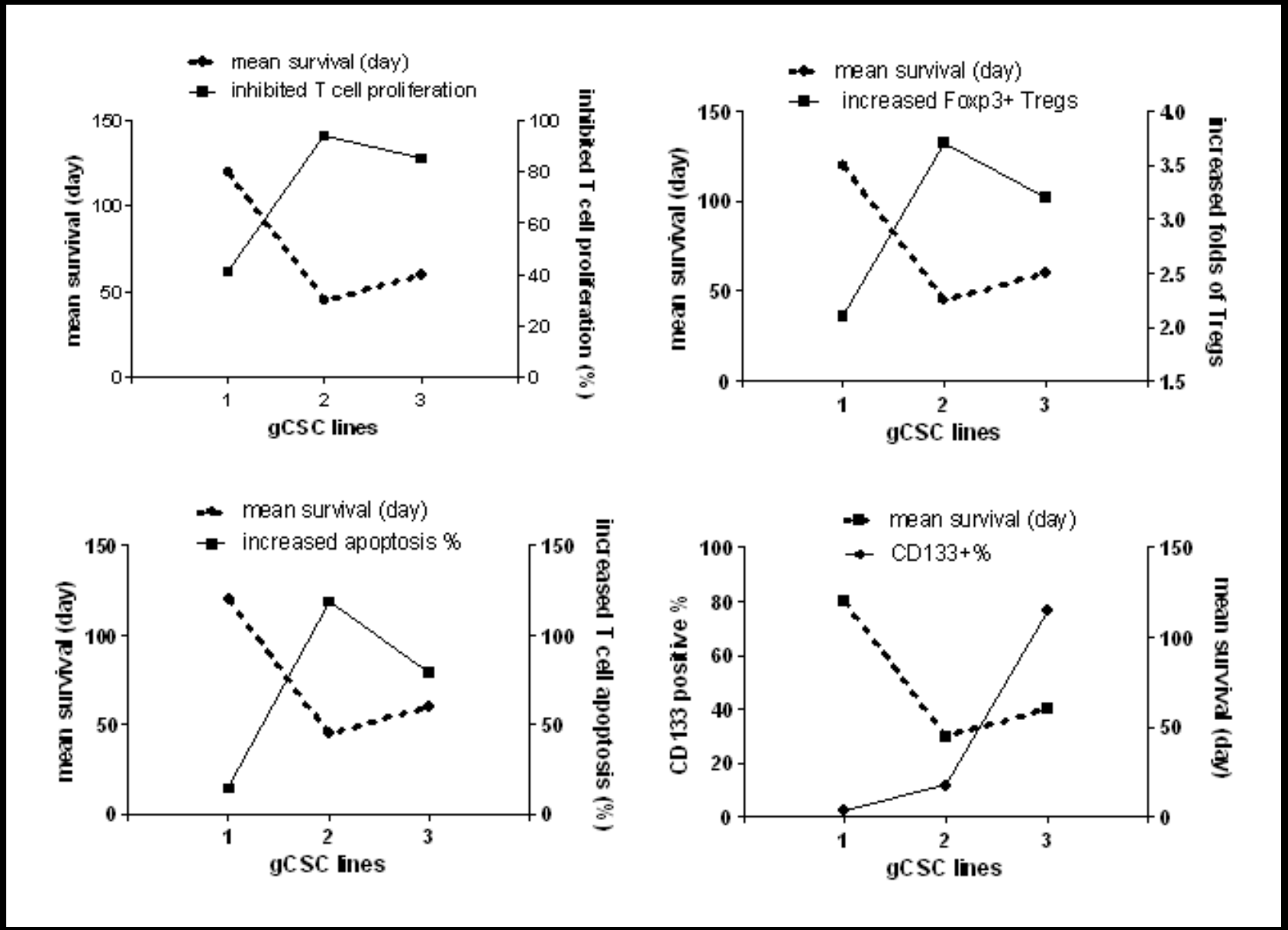




gCSCs induce T cell apoptosis



The degree of gCSC-mediated immune suppression correlates with *in vivo* tumorigenicity



Cytokines elaboration by gCSC screened by RayBio Cytokine Antibody Array and verified by ELISA

TGF-β 76 (39-118) pg/mL

PGE_2 169 (79-277) pg/mL

CCL-2 210 (0-391) pg/mL

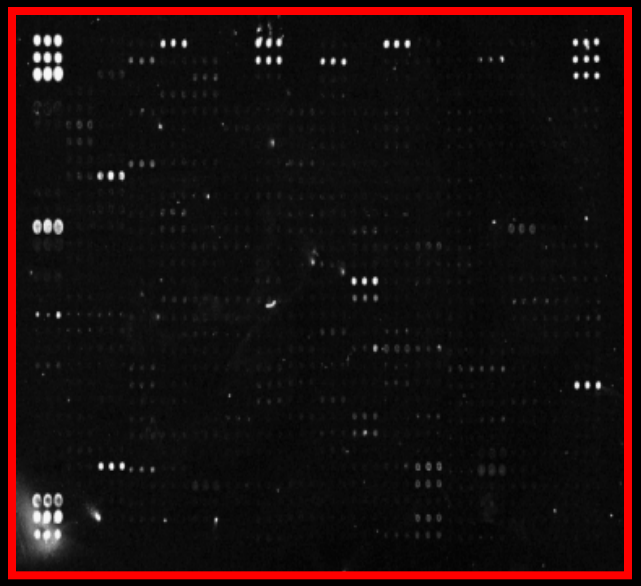
Galectin-3 2,600 (500-4,500) pg/mL

IL-10 0 pg/mL

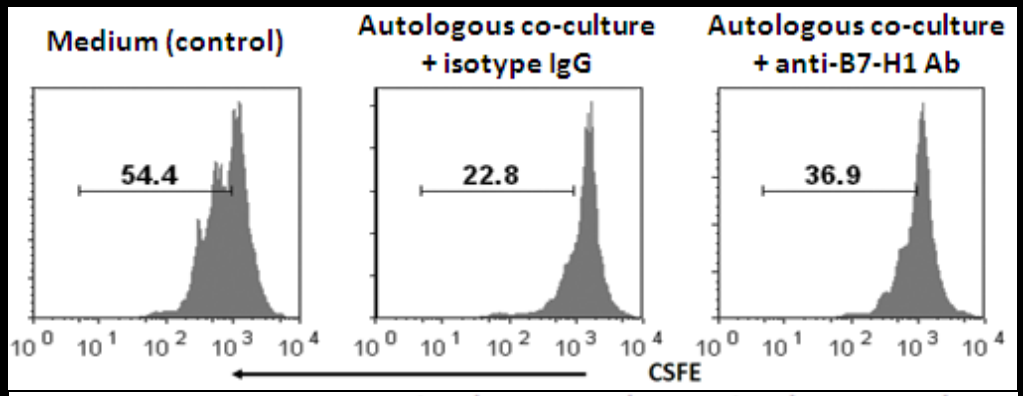
Soluble Fas 0 pg/mL

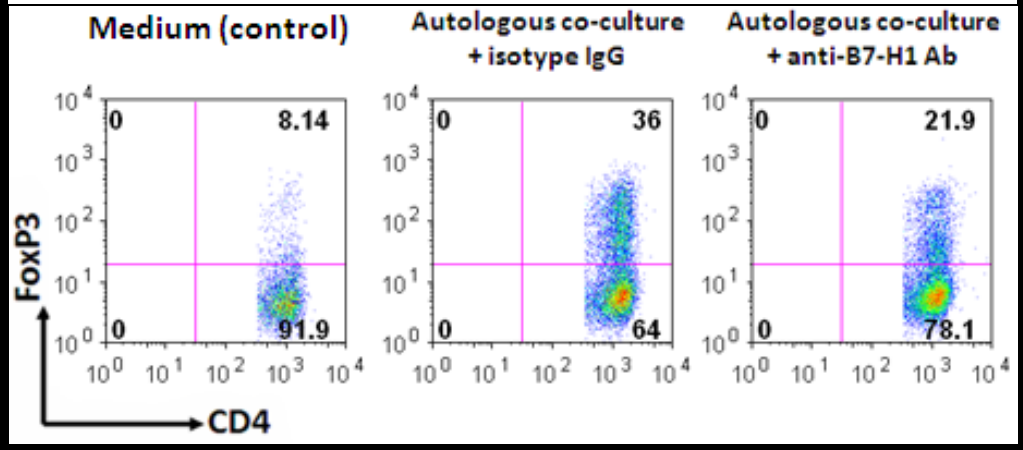
IL-6 0 pg/mL

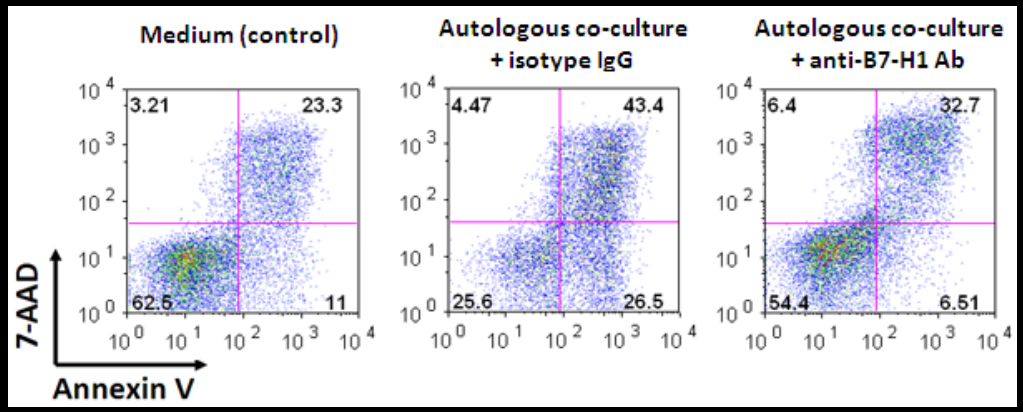
NO 0 pg/mL



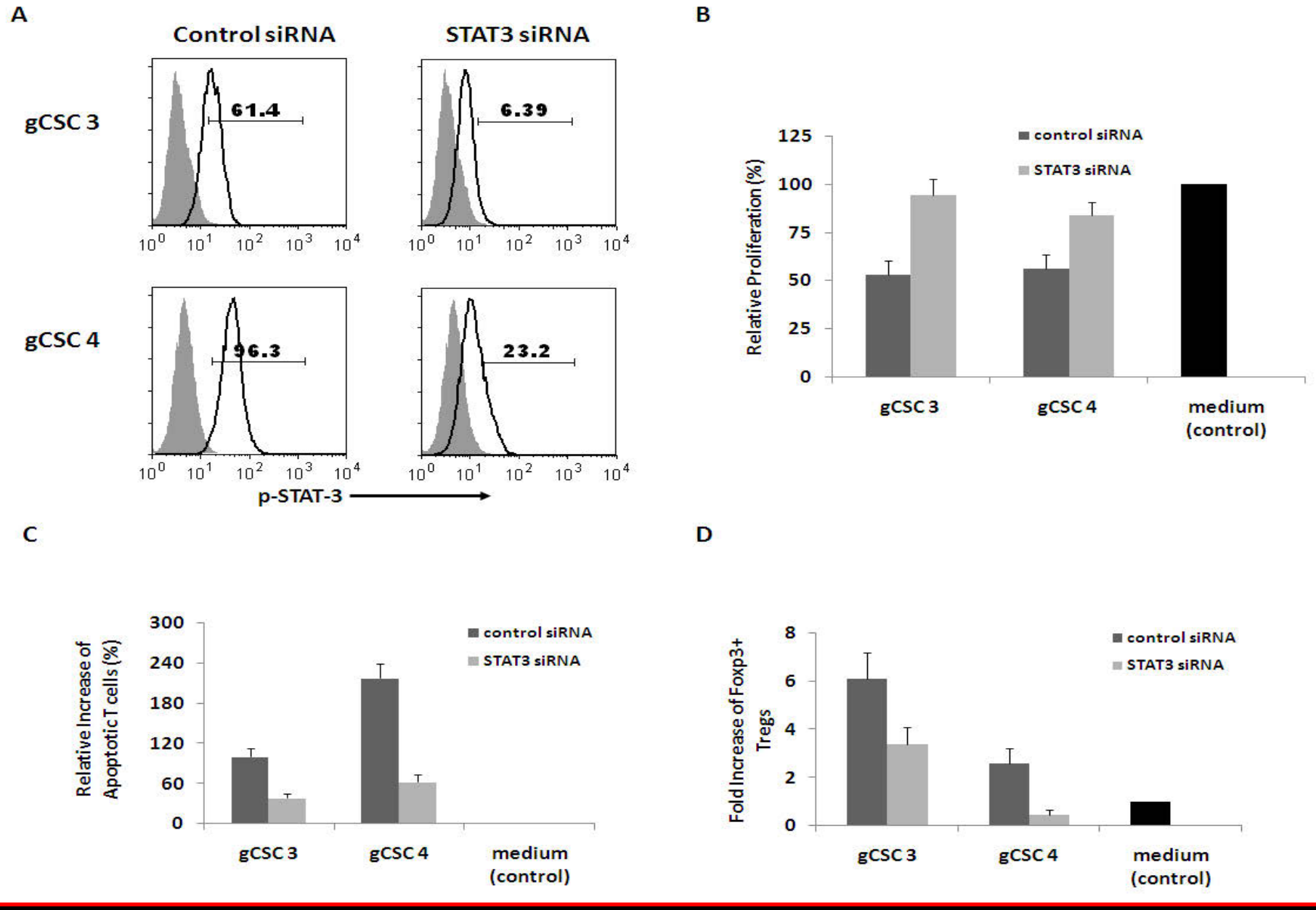
B7-H1 contributes to cell-contact dependent immune suppression



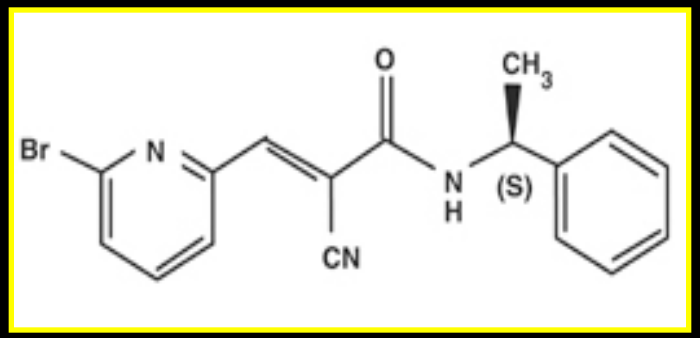


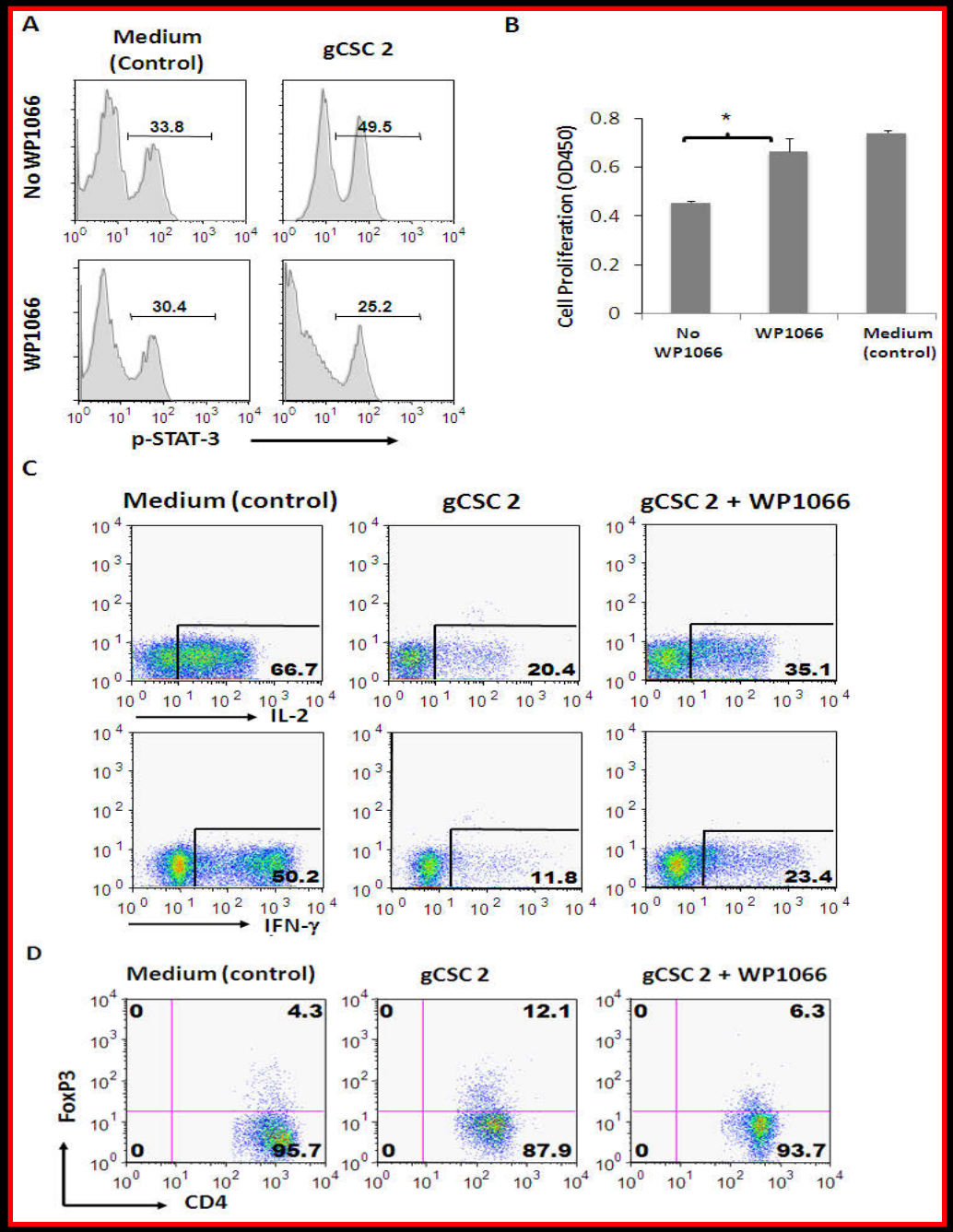


Inhibition of p-STAT3 reverses immune suppression



WP1066 restores immunological responses





Conclusions

gCSC contribute to malignant glioma patient immune suppression by:

- Inhibition of T cell proliferation, activation and effector function
- Induction of T cell apoptosis (via Galectin-3, B7-H1)
- Stimulation of Tregs (via TGF-β, B7-H1)

CD133 expression does not correlate with the degree of immune suppression

The degree of gCSC mediated immune suppression inversely correlates with *in vivo* survival

The immune suppressive properties of gCSCs on T cell effector function and proliferation can be reversed with inhibitors of the p-STAT3 pathway

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Conclusions

1. GBM cancer stem-like cells (gCSCs) possess intrinsic immunosuppressive properties;

2. Immunosuppressive properties of the gCSCs are reduced upon differentiation;

3. p-STAT-3 inhibitor, WP1066, can partially reverse immunosuppressive properties of the gCSCs.

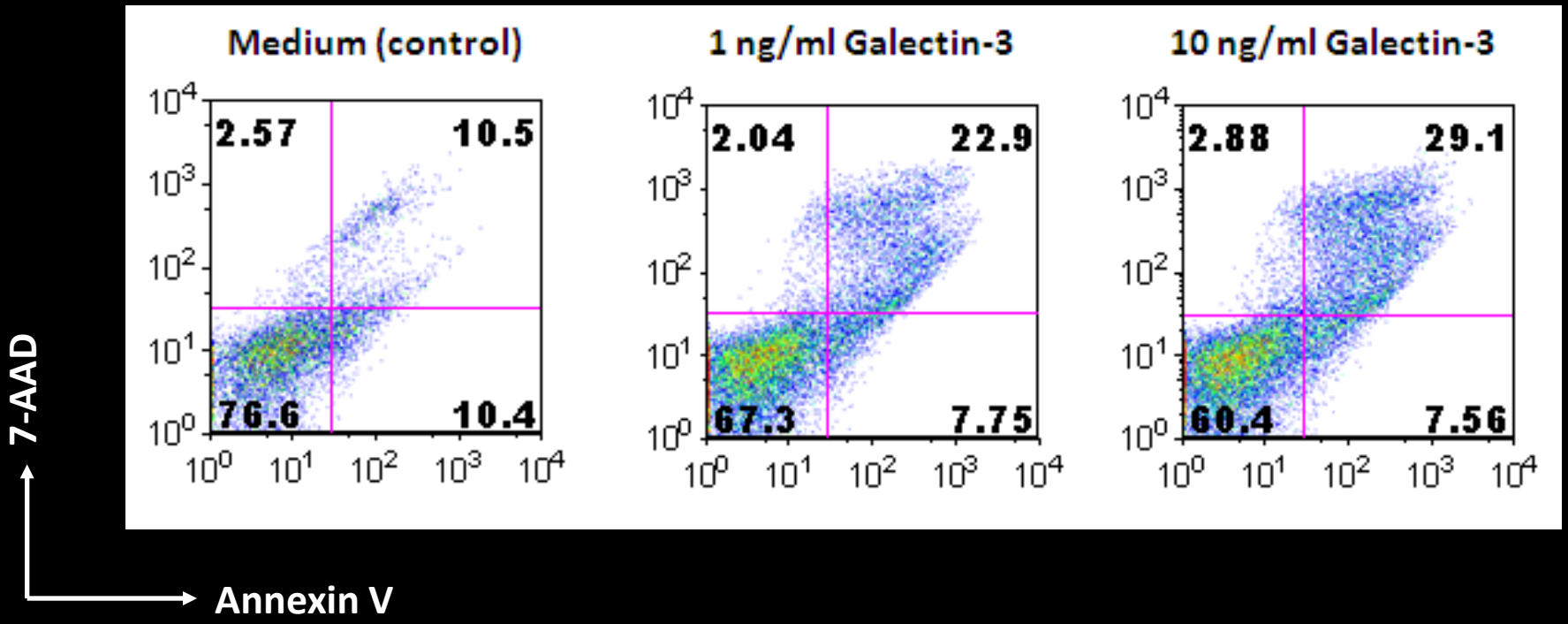
Future Direction

1. Identify the secreted product(s) from cancer stem cells mediating their immune suppressive properties;

2. Clarify mechanisms by which gCSCs induce regulatory T cells;

3. Ascertain whether immunosuppressive properties of gCSCs mainly depend on STAT-3 pathway.

Galectin-3 can induce T cell apoptosis



The degree of gCSC mediated immune suppression does not correlate with CD133 expression