

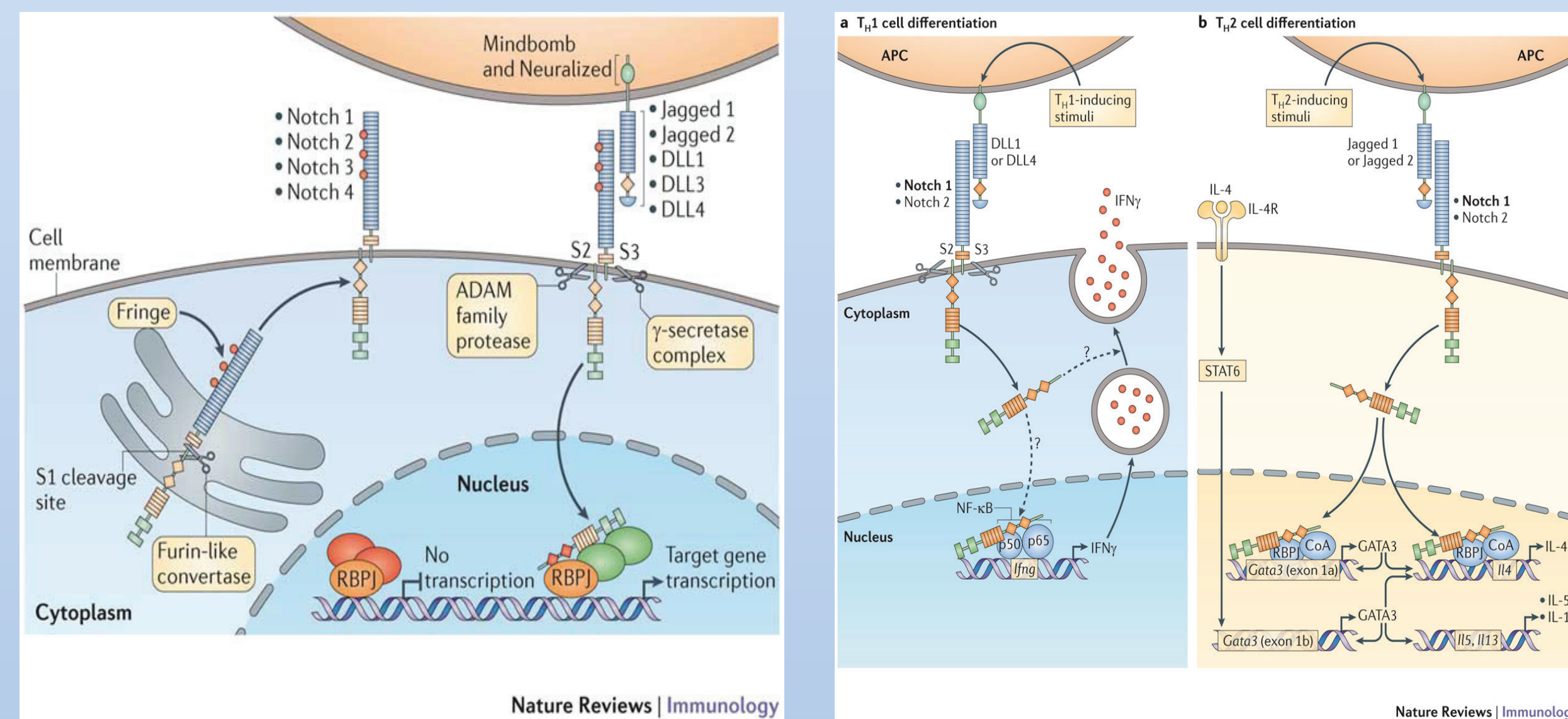


Abstract

Myeloid-derived suppressor cells (MDSC) are a group of immature precursors that play a key role in the inhibition of immune responses in patients and animal models with cancer and other diseases. MDSC utilize various mechanisms for T cell suppression, but not all are well-understood. Some of these include the production of peroxynitrite and reactive oxygen species, the expression of arginase 1, and nitric oxide synthase 2, and the depletion of arginine and cysteine. However, there are no current therapies to specifically and globally block MDSC. Our long term goal is to characterize new therapies to block MDSC function in tumor-bearing hosts.

Notch receptors (1-4) control multiple functions of immune cells through binding to ligands DLL1 (Delta like ligand), DLL3, DLL4, JAG1, and JAG2. Binding of Notch receptors to DLL1 and 4 increase proliferation and IFN- γ production in T cells, while binding to JAG1 and 2 promote IL-4 production. In this study, we aimed to determine the role of the decreased Notch signaling in T cells in the suppression induced by tumor-MDSC. In addition, we aimed to characterize the differential expression of Notch ligands in tumor vs. splenic MDSC. Our results suggest the major importance of the decreased T cell-Notch signaling in the suppression of T cell responses in tumors. Also, we show the specific modulation of Notch ligands in MDSC by tumors which could potentially play a key role in their regulatory function. Continuation of this research is expected to enable therapies to overcome tumor induced immune energy, enhancing the effect of immunotherapy.

Notch receptor-ligand reaction



Radtke F et al. *Nature Reviews Immunology*. (2013)

Specific Aims

- Determine the effects of MDSC on T cell Notch expression and understand the mechanisms leading to this effect.
- Test whether rescuing of Notch signaling in T cells renders them resistant to MDSC suppression *in vivo*.
- Determine the expression of Jag and DLL ligands in tumor and spleen MDSC. Test the role of tumors in the modulation of Notch Ligands.

Methods

- Some of the methods worth noting are the following:
- Tumor model: C57BL/6 mice (4 to 6-wk-old female) were obtained from Harlan (Indianapolis, IN). 3LL Lewis lung carcinoma cells (1×10^6 cells) were injected s.c. into the mice.
 - Cell sorting: Anti-Gr1 EasySep[®] positive selection from Stemcell technologies was used for the magnetic sorting of MDSC from the tumor extract.
 - Real-time PCR: mRNA expression was established by real-time PCR using the cost-efficient SYBR Green dye.
 - ELISpot: We measure the frequencies of antigen-specific T cells producing IFN- γ using a commercially available kit.

Results

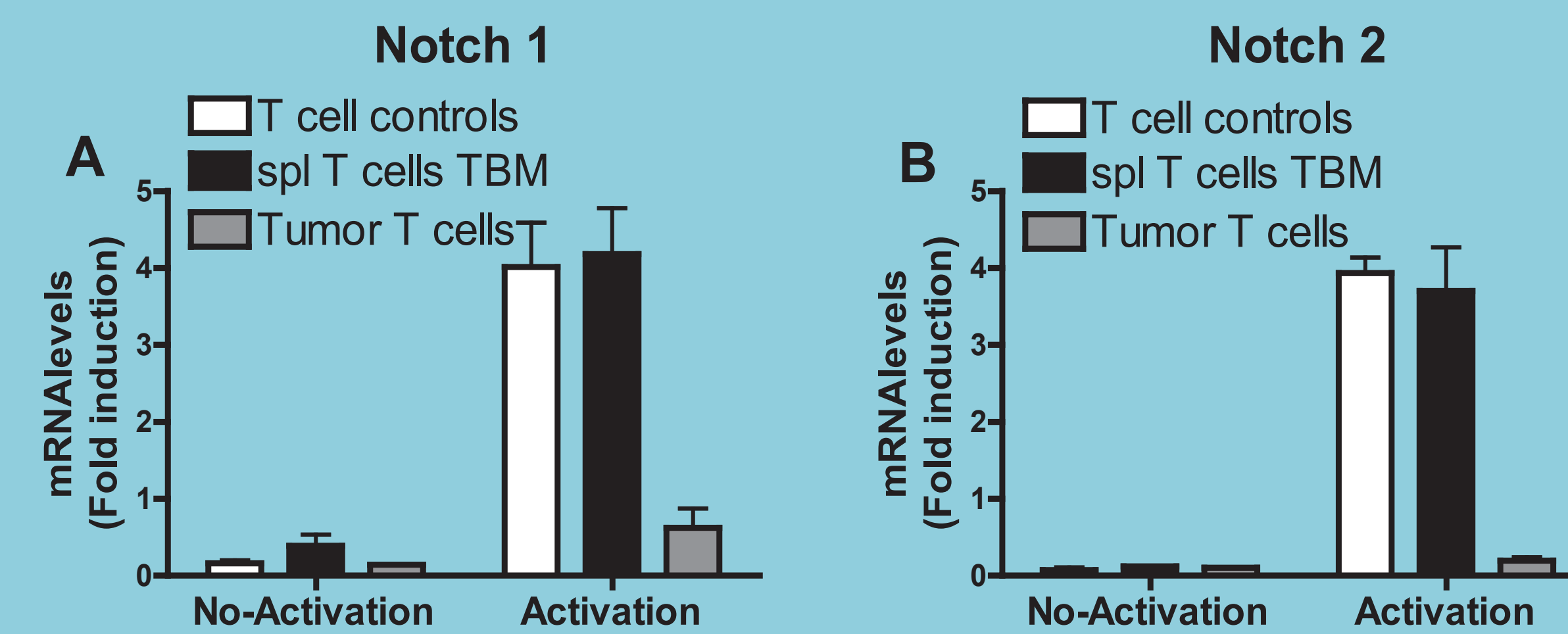


Figure 1. Tumor-infiltrating T cells had a decreased expression of Notch-1 and -2. T lymphocytes were isolated from tumors and spleen of mice bearing s.c. 3LL tumors for 17 days or spleens from mice without tumors. Then, T cells were activated with anti-CD3/CD28 for 24 hours and tested for Notch-1 and -2 mRNA by real-time PCR. Results represent mean \pm SD from 4 different animals and tested triplicates.

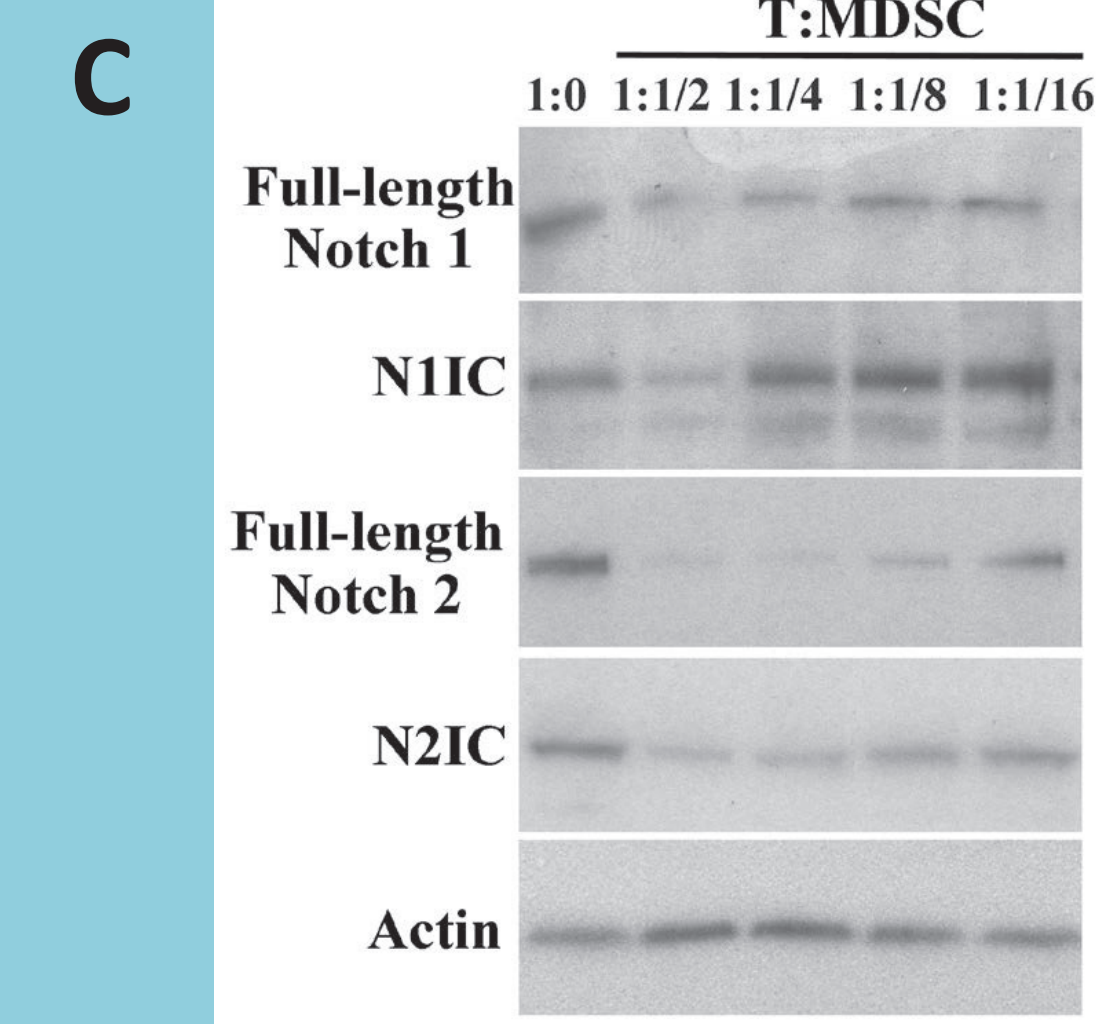


Figure 2. MDSC block Notch 1 and 2 upregulation in T cells. Activated CD3⁺ T cells were co-cultured at different ratios with tumor-infiltrating MDSC for 48 hours. Then, T cells were negatively isolated using anti-CD11b beads and whole protein extracts harvested and tested for the expression of Notch-1 and -2 by western blot. A representative plot of 3 replicate experiments is shown.

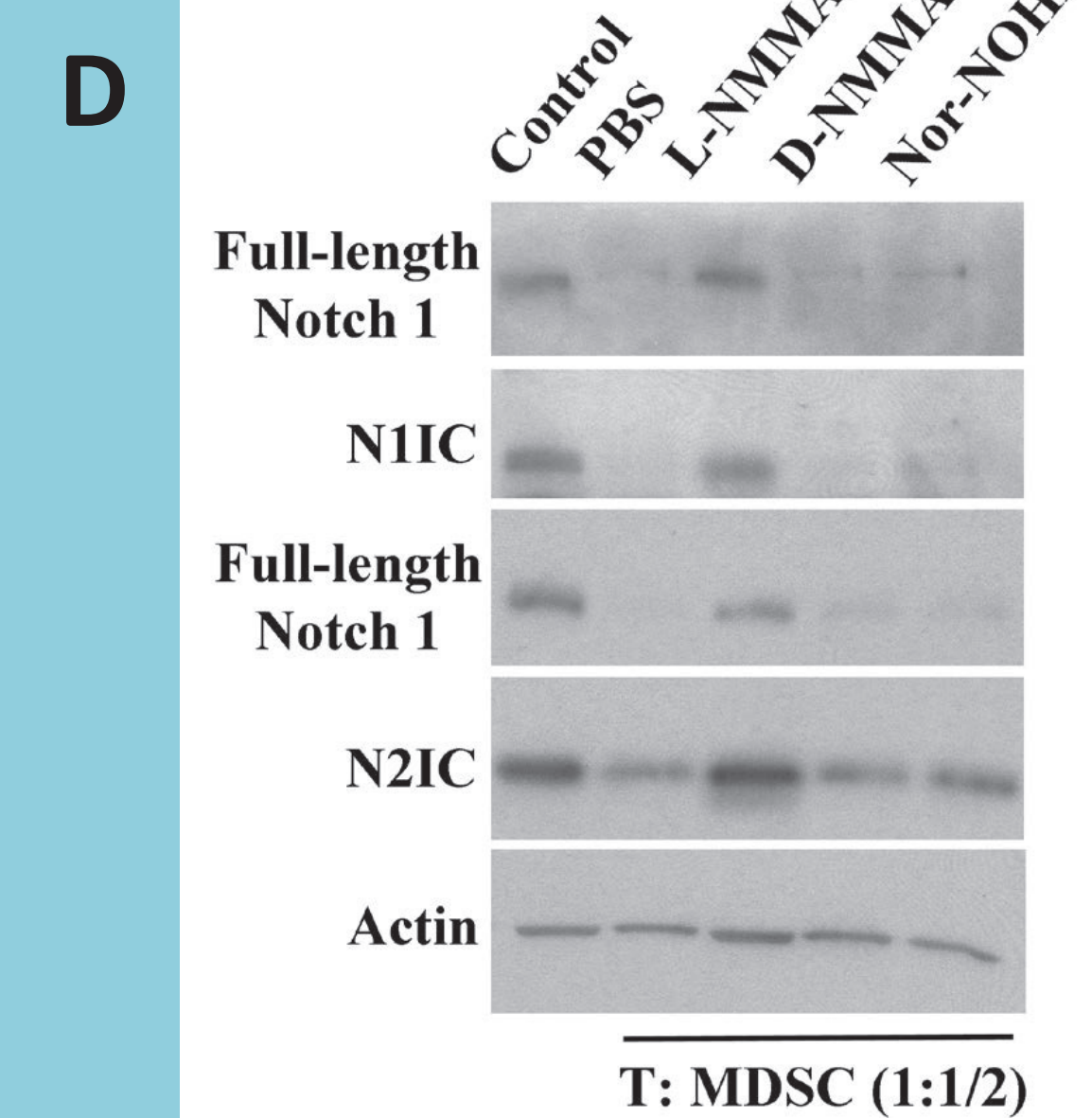


Figure 3. Inhibition of T cell Notch by MDSC is mediated by Nitric oxide synthase. Activated T cells co-cultured with MDSC at a 1:1/2 ratio were treated with L-NMMA (500 μ M), D-NMMA (500 μ M), and NN (200 μ M) for 48 hours. Then, extracts were isolated and used as in (C). Representative results are from 3 similar experiments.

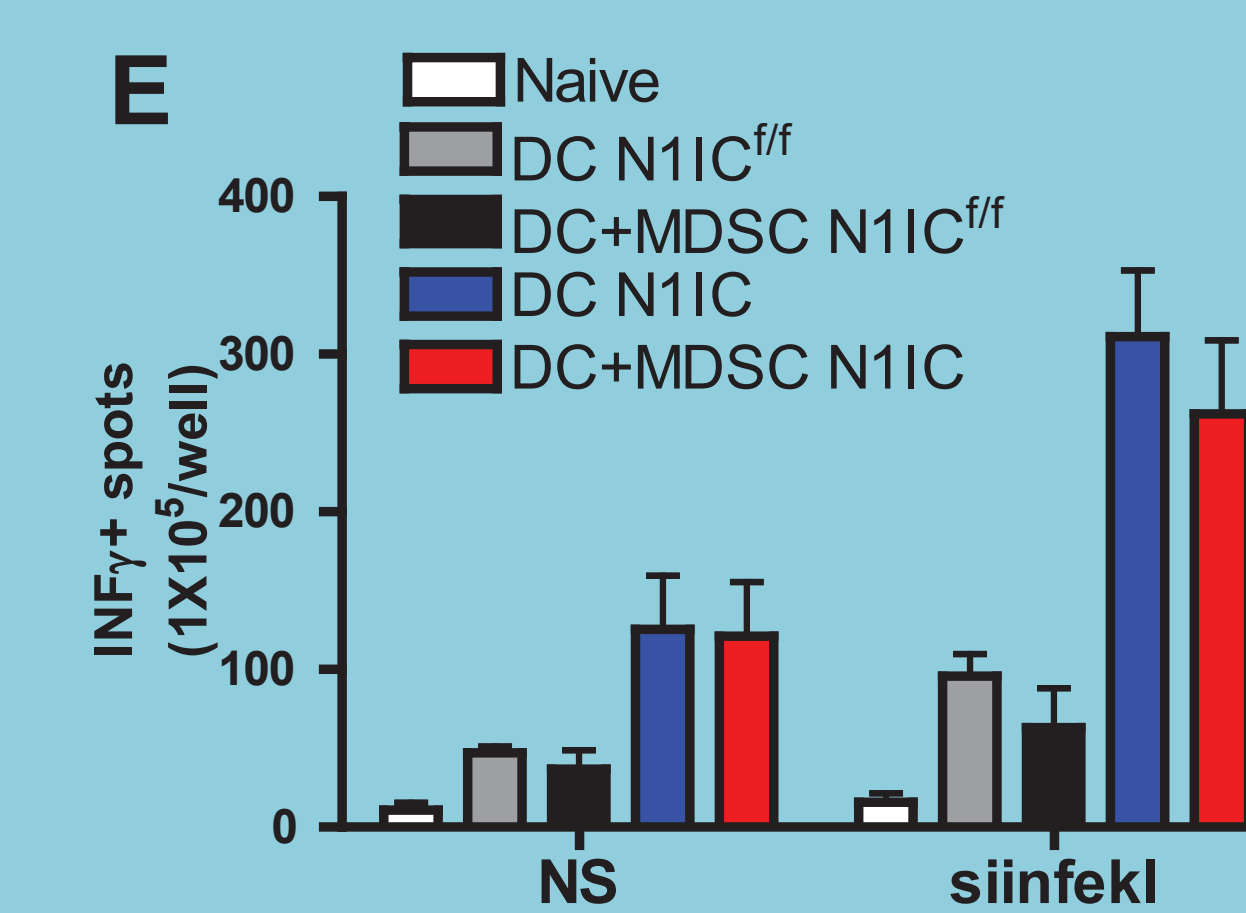


Figure 4. N1IC expression in T cells overcomes tolerogenic effect induced by MDSC *in vivo*. CD8⁺ T cells from CD45.2⁺ N1IC or N1IC^{fl/fl} mice were transferred i.v. into CD45.1⁺ congenic recipients. Following transfer, mice were vaccinated with a mix of dendritic cells (DC) and/or MDSC pulsed with siinfekl and the draining lymph nodes harvested, activated with siinfekl, and tested for production of IFN γ using ELISpot. Data represent mean \pm SD from 2 similar independent experiments.

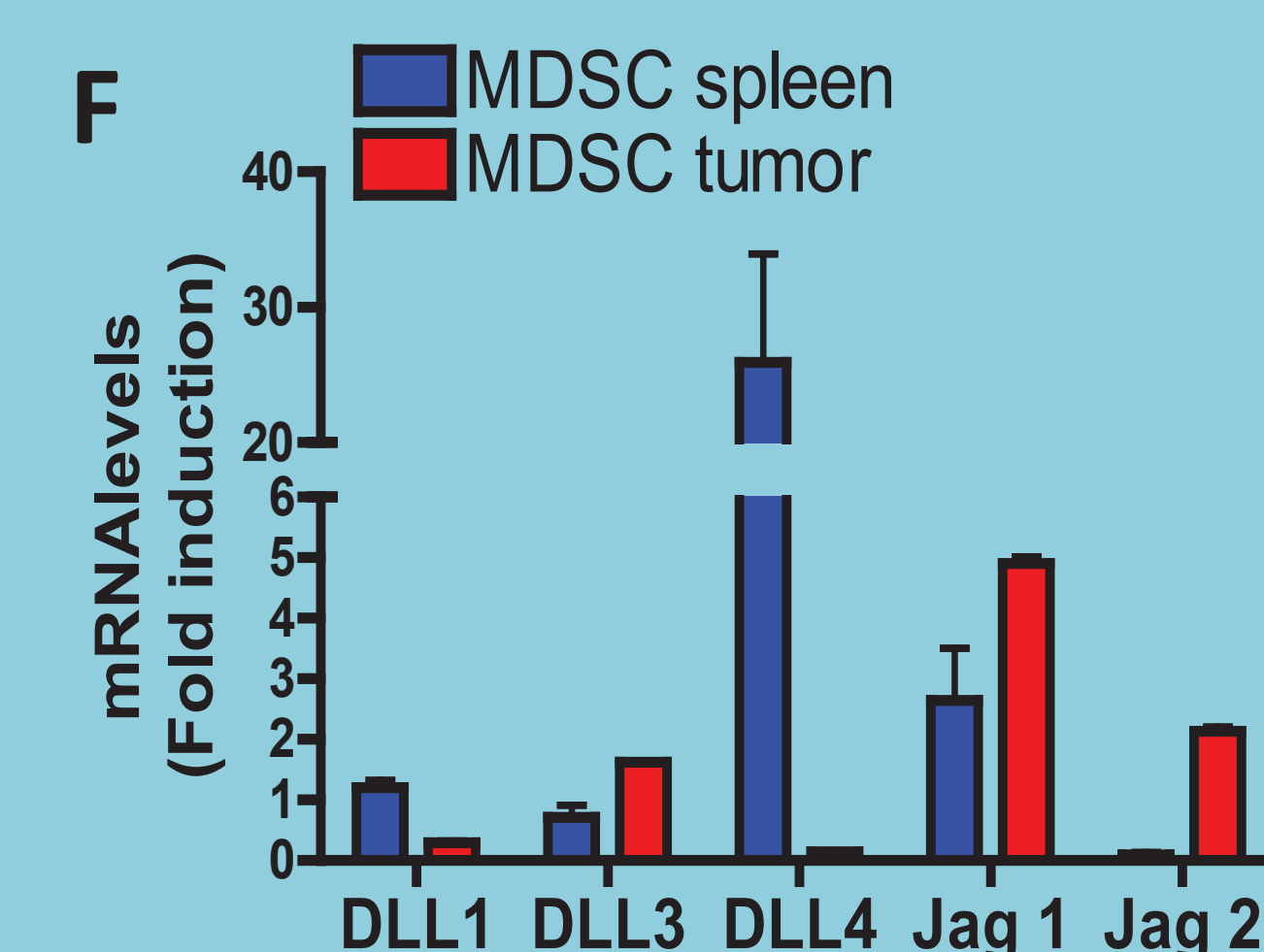


Figure 5. Differential expression of Notch ligands in splenic and tumor-MDSC. MDSC were isolated from tumors and spleens of mice bearing s.c. 3LL cells for 17 days using anti-Gr1 positive selection. Then, total RNA was isolated and tested for Notch ligands by real time PCR. Results represent mean \pm SD from 4 independent animals and tested in triplicate.

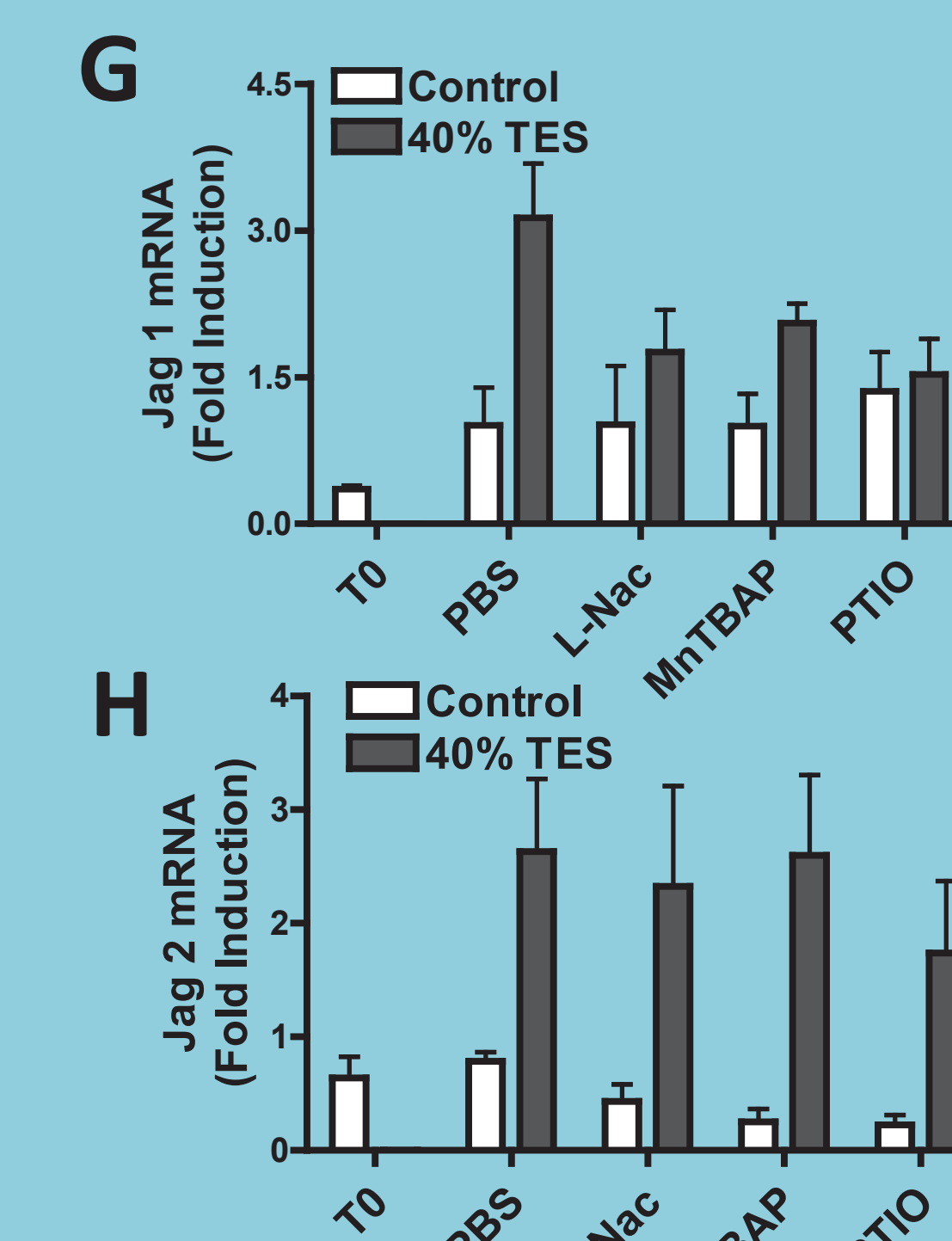


Figure 6. Tumors promote JAG expression in MDSC. G. Bone marrow-generated MDSC were cultured with 40% tumor explants supernatants (TES) for 48 hours, after which the expression of JAG 1 and 2 was tested by Quantitative PCR. H. Experiment G was repeated in the presence of anti-oxidant L-NAC or peroxynitrite (MnTBAP) or Nitric Oxide (PTIO) scavengers.

Conclusions

- Through our research, we have concluded the following:
- MDSC inhibit T cell function, in part through inhibition of Notch-1 and -2 expression. Rescuing of Notch signaling in T cells overcomes MDSC suppression.
 - A preferential induction of JAG1 and 2 occurs in MDSC infiltrating tumors. Therefore, potential inhibition of these ligands could have an impact on MDSC T cell interactions. Continuation of this study could lead to therapies to overcome immune suppression in cancer.

As for immediate future goals:

- Identify factors promoting JAG in MDSC.
- Determine the role of tumor-derived ROS/PNT in the induction of Notch ligands in MDSC.
- Test the effect of blocking JAG in tumor-MDSC.
- Test the effect of anti-JAG “probodyes” in the function of MDSC and the induction of T cell anergy in tumors.

Test the therapeutic effect of DLL 1-4 expression in MDSC

- Another viable way to overcome suppression by Jagged ligands could be the over-expression of Delta like ligands with positive T cell responses (DLL1 and DLL4) through the use of soluble clustered ligands.

References

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Acknowledgements

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