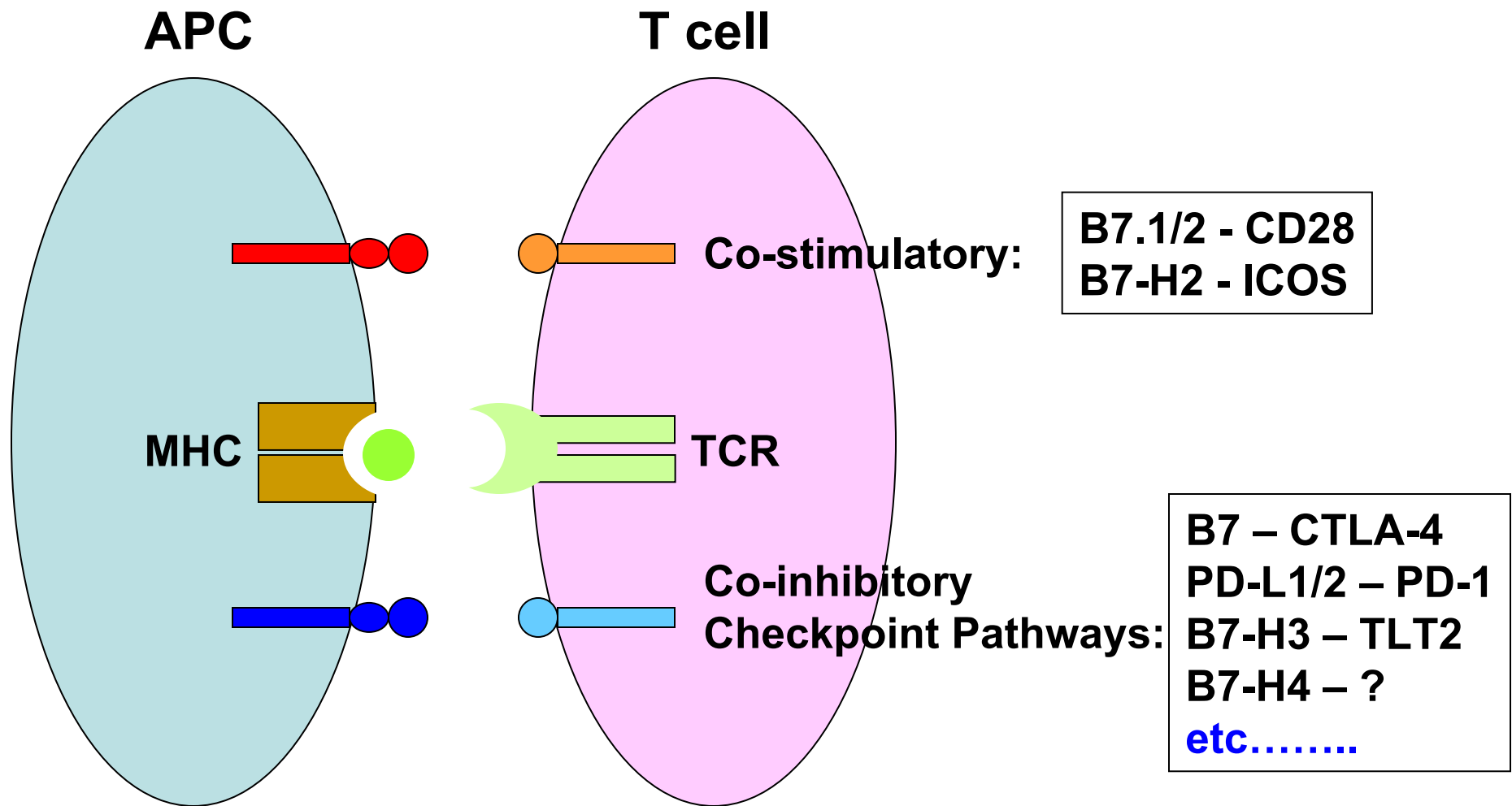


**VISTA, a novel immune checkpoint protein ligand
that suppresses anti-tumor T cell responses**

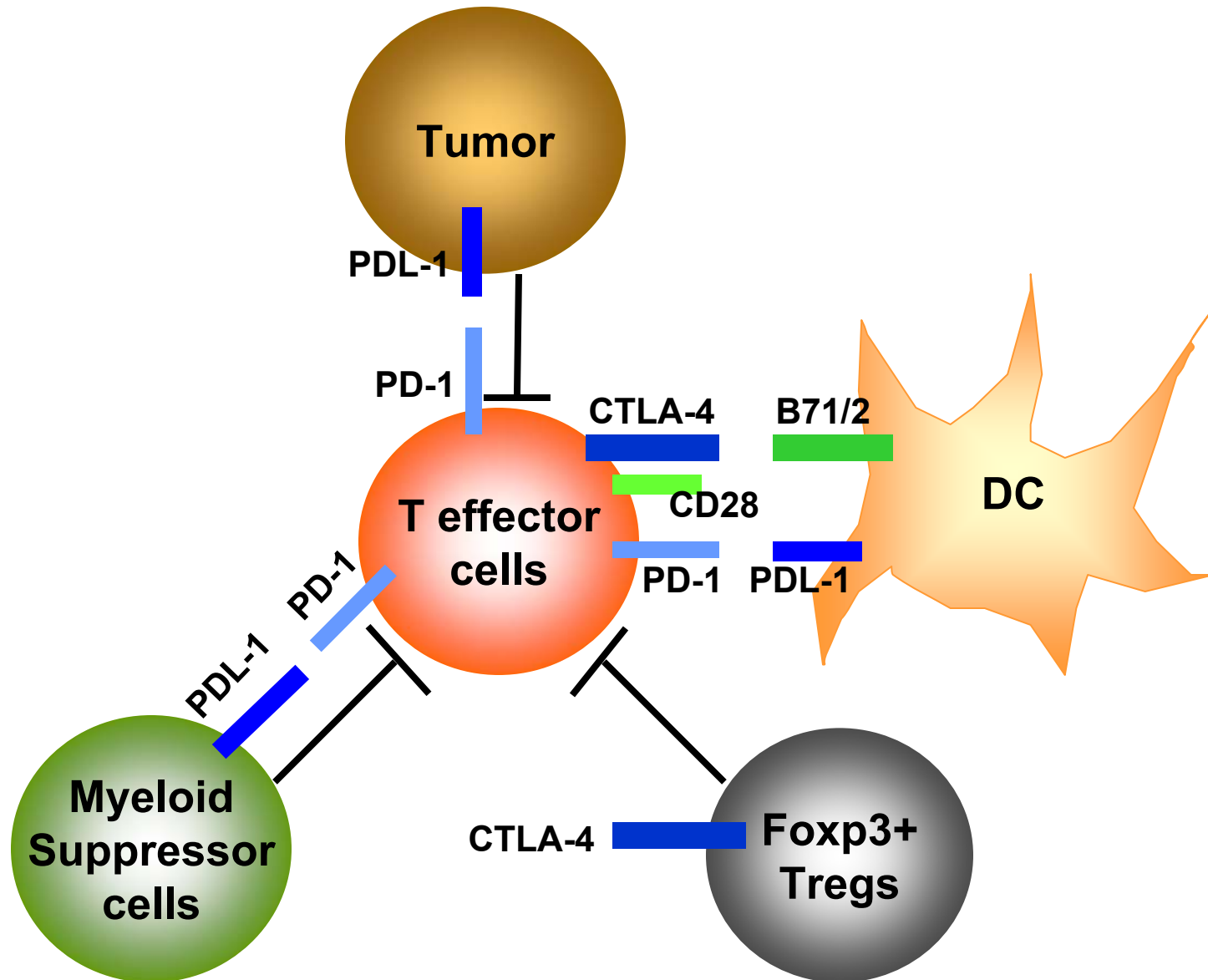
Li Wang

Dartmouth Medical School

The B7 Immunoglobulin Super-Family immune regulators



Immune checkpoint-mediated T cell suppression during tumorigenesis



Release the brake: Immune checkpoint blockade in cancer immunotherapy

- **Antibody-mediated CTLA-4 blockade in combination with a cellular vaccine (Gvax) induced regression of established poorly immunogenic B16 melanoma.**
 - **Elsas et.al. 1999, *J Exp Med* 190:355-366**

- **Ipilimumab, the human α CTLA-4 mab, was approved by the FDA in March 2011 to treat patients with late-stage melanoma.**

- **Ipilimumab has also undergone early phase trials for other cancers, such as lung cancer and prostate cancer.**
 - **Calabro et.al. 2010, *Semin Oncol* 37:460-467**

Release the brake: Immune checkpoint blockade in tumor immunotherapy

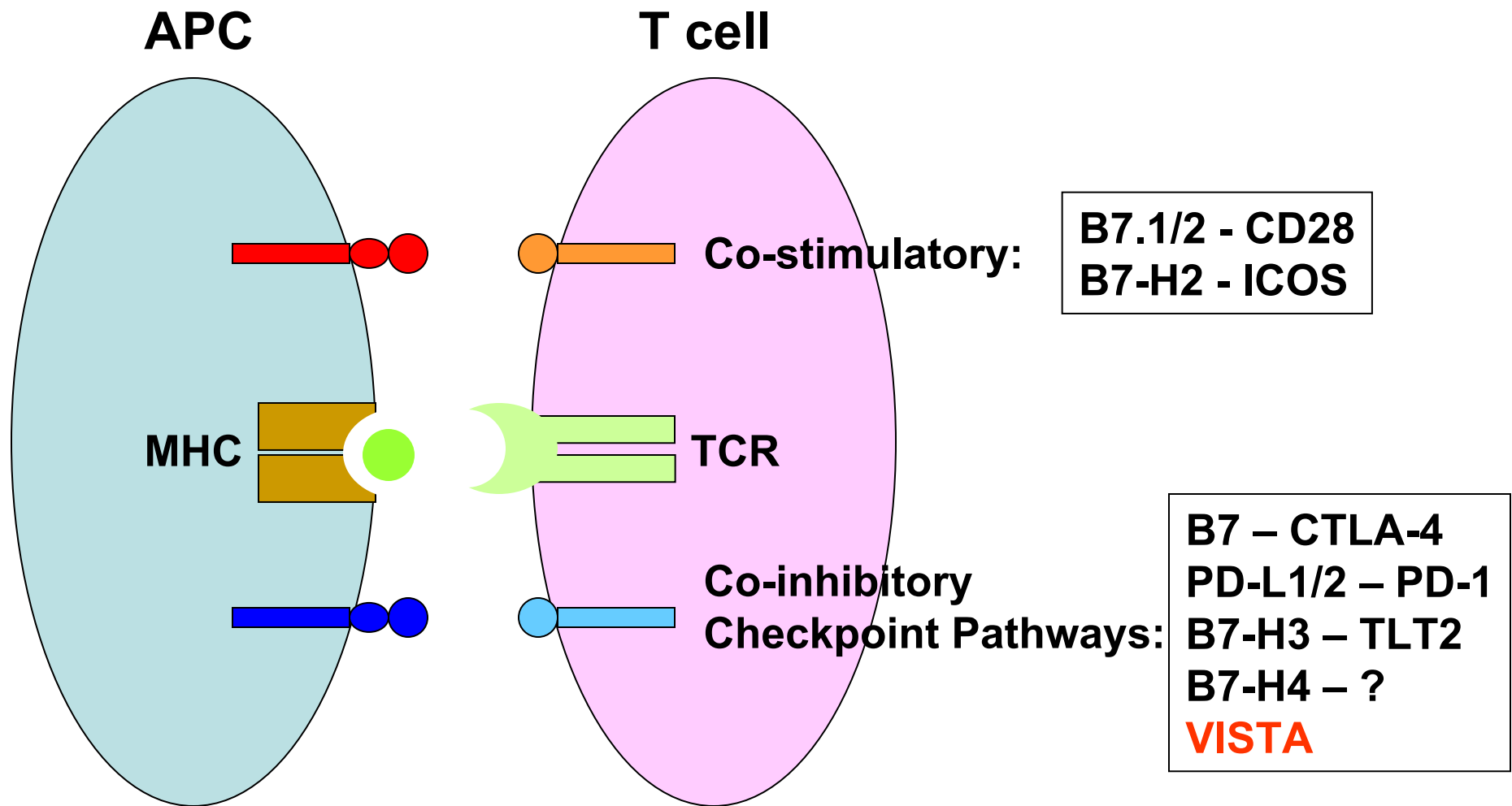
➤ **Blocking the PD-L1:PD-1 pathway, in conjunction with other immune therapies, inhibits tumor progression.**

- **Blank et.al. 2005, *Cancer Immunol Immunother* 54:307-314**
- **Hirano et.al. 2005, *Cancer Res* 65:1089-1096**
- **Geng et.al. 2006, *Int J Cancer* 118:2657-2664**
- **Li et.al. 2009, *Clin Cancer Res.* 15: 1632-4**
- **Pilon-Thomas et.al. 2010 *J Immunol.* 184: 3442-9**
- **Weber J, 2010, *Semin Oncol* 37(5):430-9**

➤ **MDX-1106, the human anti-PD-1 mab has entered clinical trials.**

- **Brahmer JR et.al. 2010, *J Clin Oncol.* 28(19):3167-75**
Phase I study of anti-programmed death-1 (MDX-1106) as single-agent in refractory solid tumors is well tolerated and demonstrates clinical anti-tumor activity.

The B7 Immunoglobulin Super-Family immune regulators



VISTA: a new checkpoint protein, and a V-domain Immunoglobulin Suppressor of T cell Activation

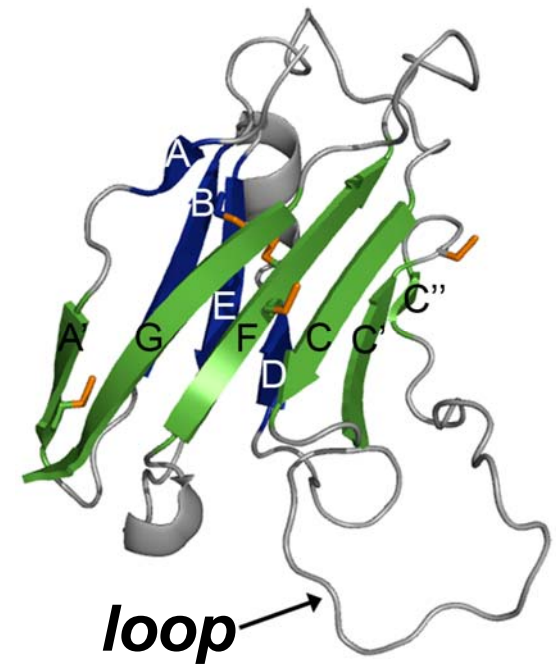
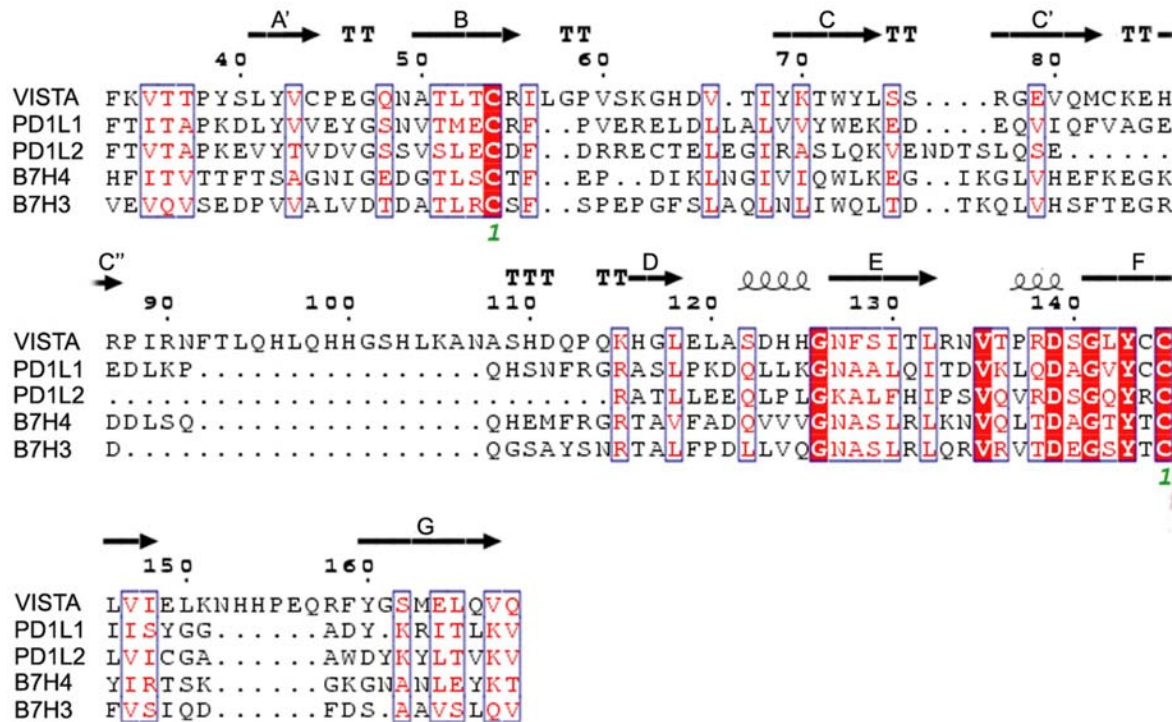
Ig-v domain

TM

cytoplasmic

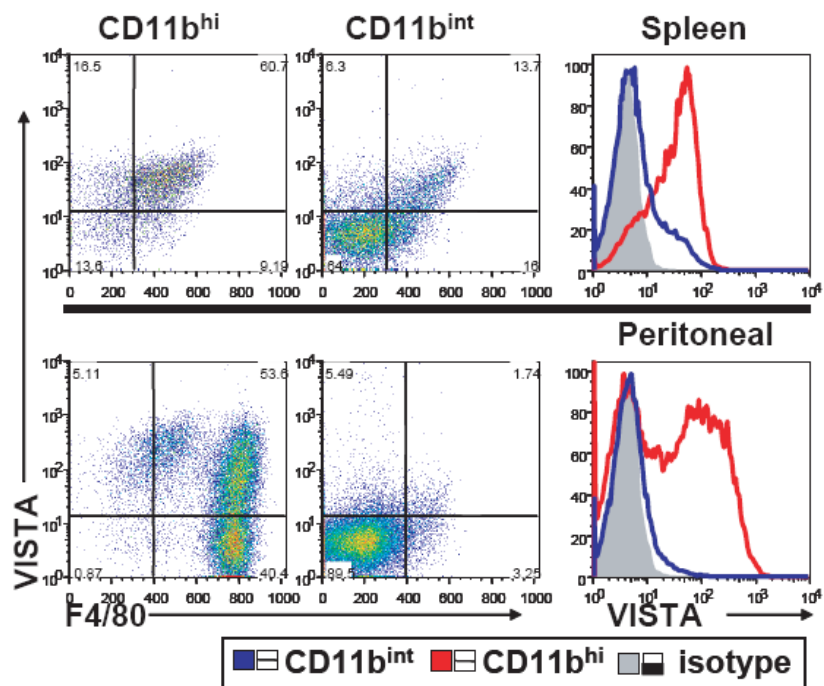
Ig-v domain structural
model of VISTA, using
PD-L1 as template

Sequence of the Ig-v domain

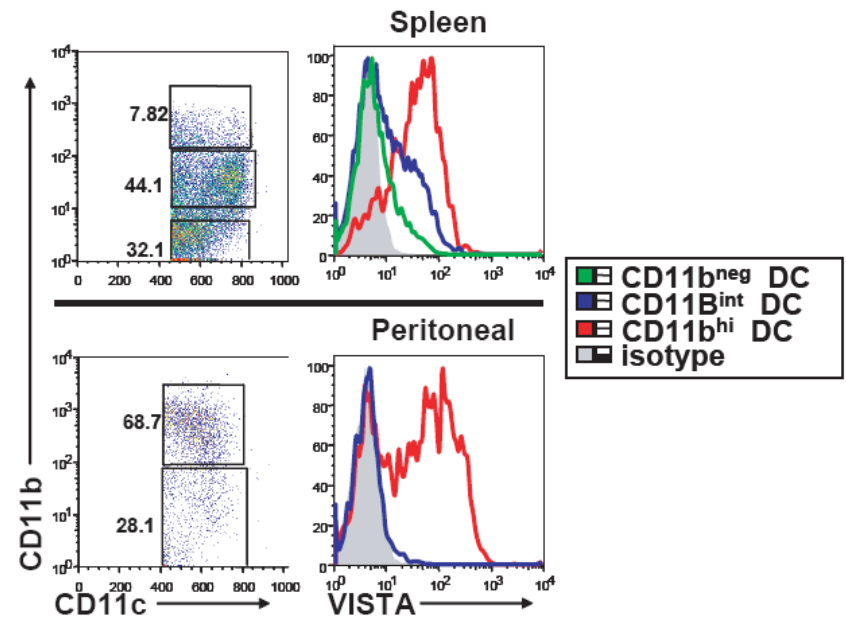


VISTA is highly expressed on CD11b^{hi} myeloid cells

Monocytes/macrophages



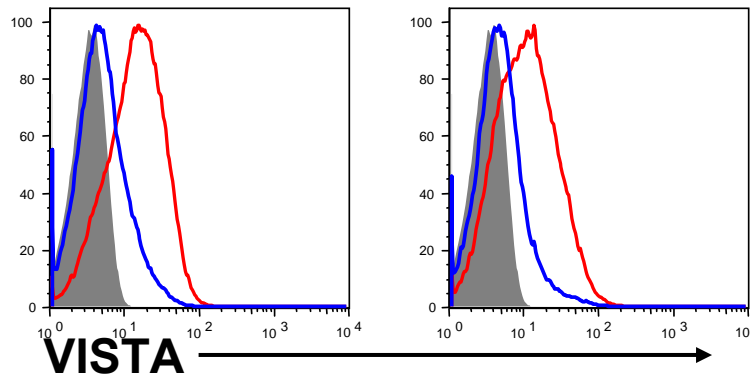
DCs



VISTA is expressed on T cells

Peripheral LN

Spleen

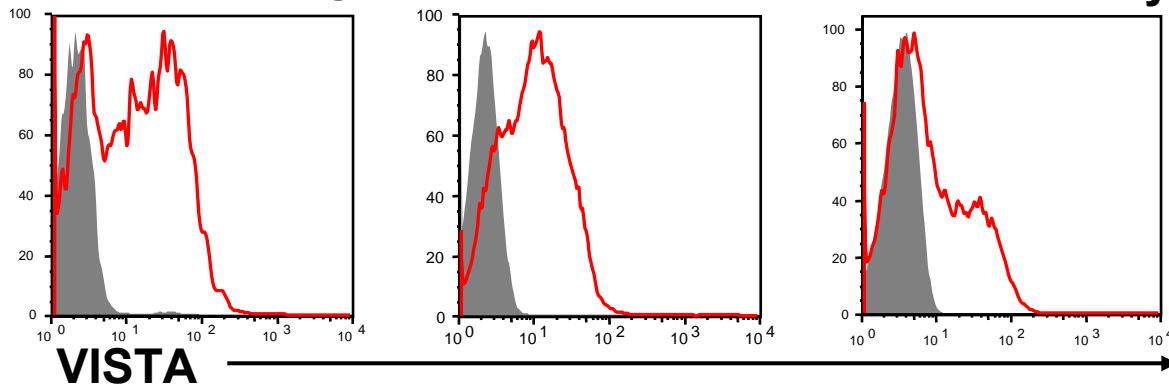


■ CD8+ cells ■ CD4+ cells ■ isotype

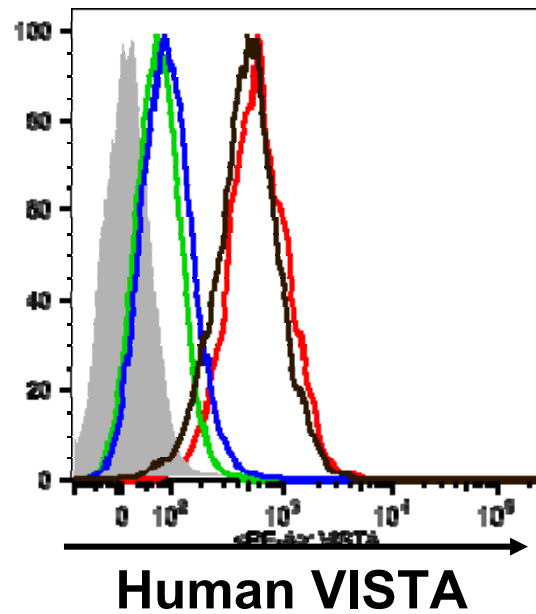
Foxp3⁺ nTregs

CD4⁺ naive

CD4⁺ memory



VISTA expression on human PBMC cells



- CD11c⁺
- CD8⁺
- CD4⁺
- Isotype
- CD11b⁺

Data contributed by Janet L. Lines

Immobilized VISTA-Ig fusion protein inhibits T cell activation

T cell proliferation

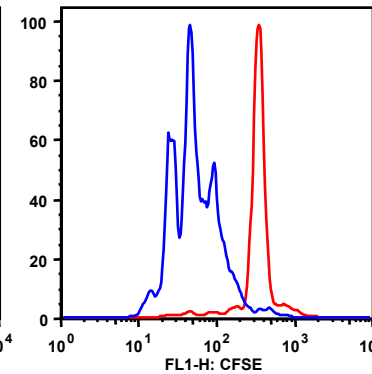
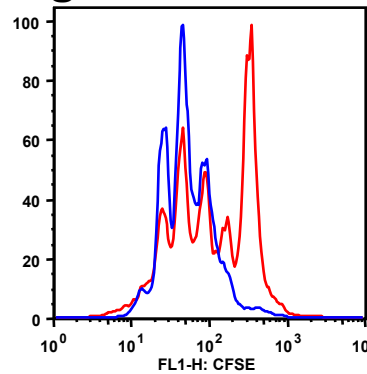
Plate-bound α CD3 + VISTA-Ig or control Ig

α CD3 : VISTA or control Ig

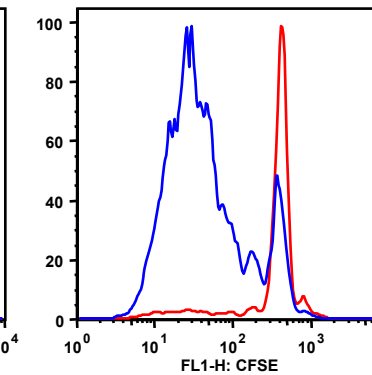
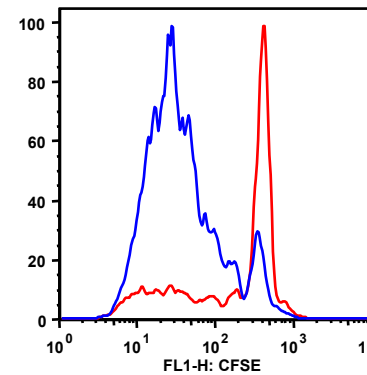
1:1

1:4

CD4⁺
T cells



CD8⁺
T cells



Control Ig
VISTA-Ig

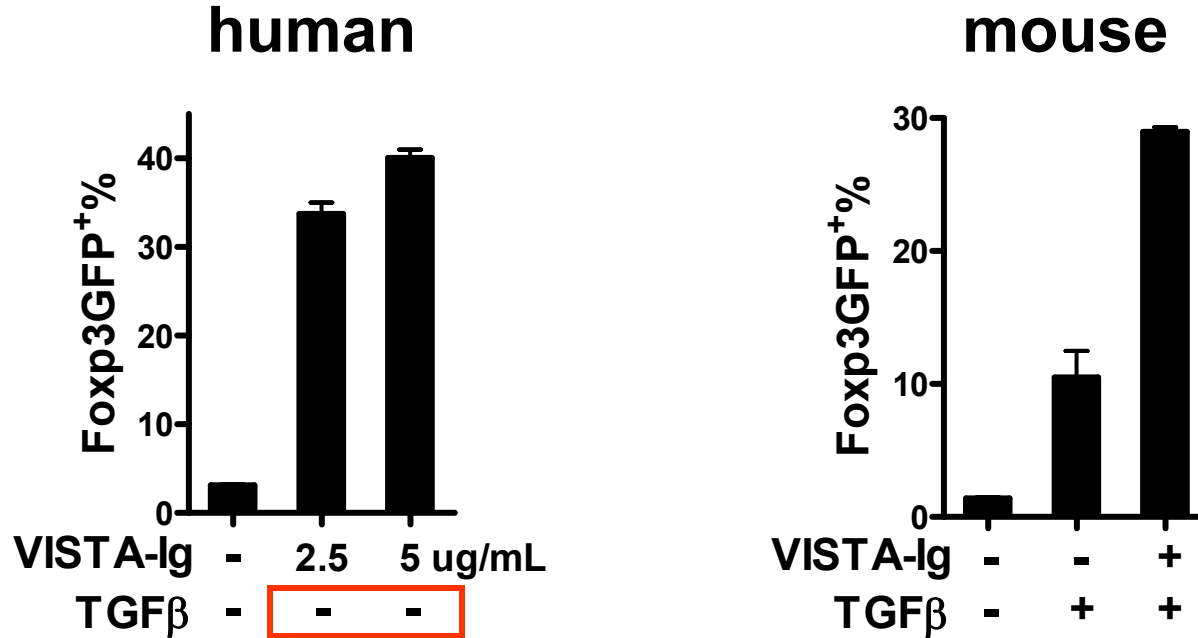
Immobilized VISTA-Ig fusion protein inhibits T cell activation

Plate-bound α CD3 + VISTA-Ig or control Ig

- ▶ Inhibit proliferation, but do not enhance apoptosis.
- ▶ Inhibit activation markers: CD69, CD44, CD62L.
- ▶ Inhibit T cell cytokine production (IL2, IFN γ etc).
- ▶ Suppression can be partially rescued by exogenous IL-2.
- ▶ PD-1 KO T cells are also inhibited.

VISTA promotes the induction of adaptive Tregs

Plate-bound α CD3 + VISTA-Ig or control-Ig +/- TGF β

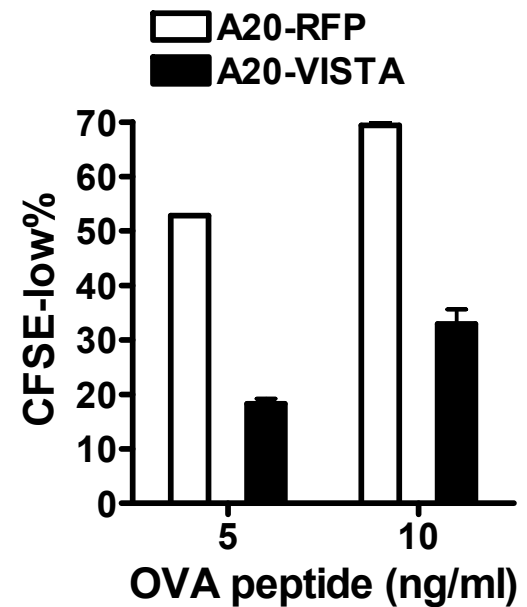
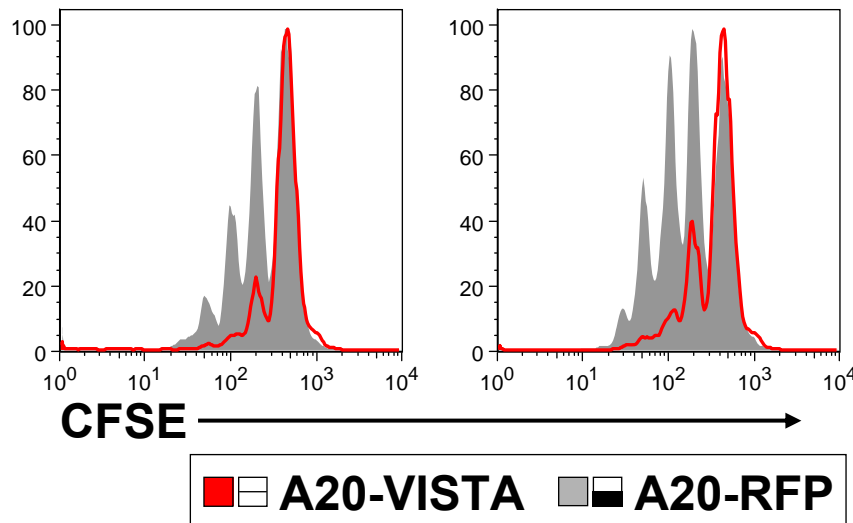


VISTA expression on APC suppress T cell proliferation

APC (A20 cells) + T cells (OTII) + peptide

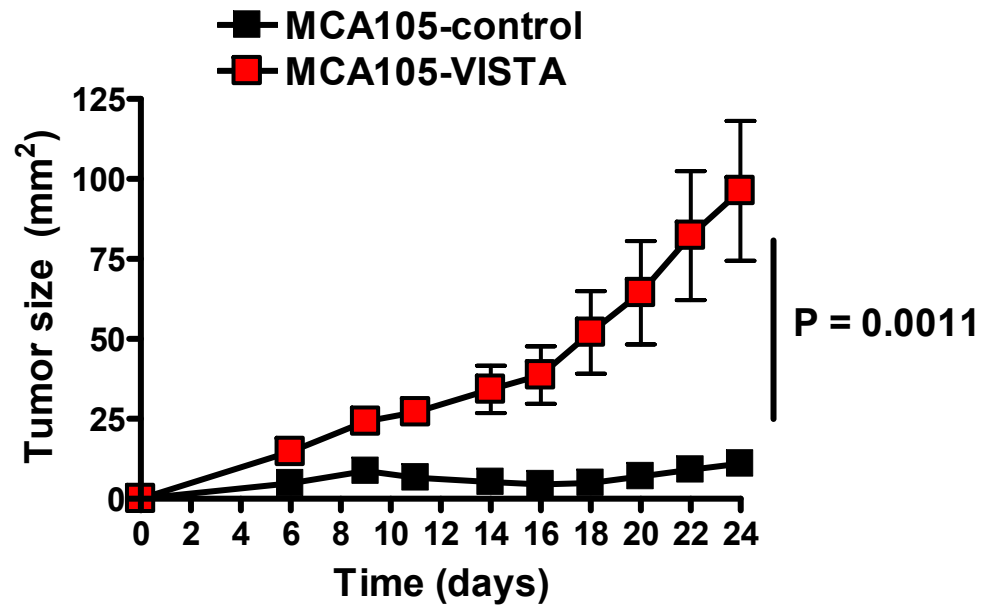
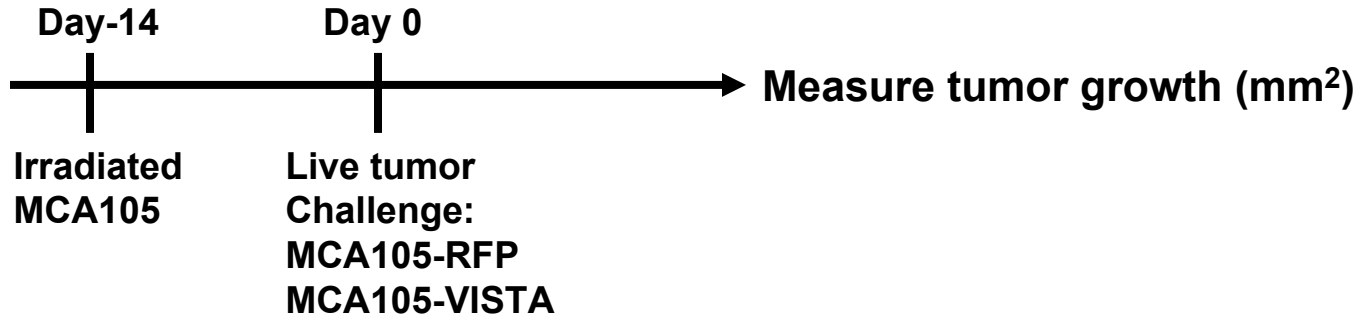
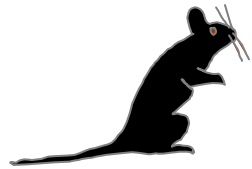
OVA peptide: 5 ng/mL

10 ng/mL

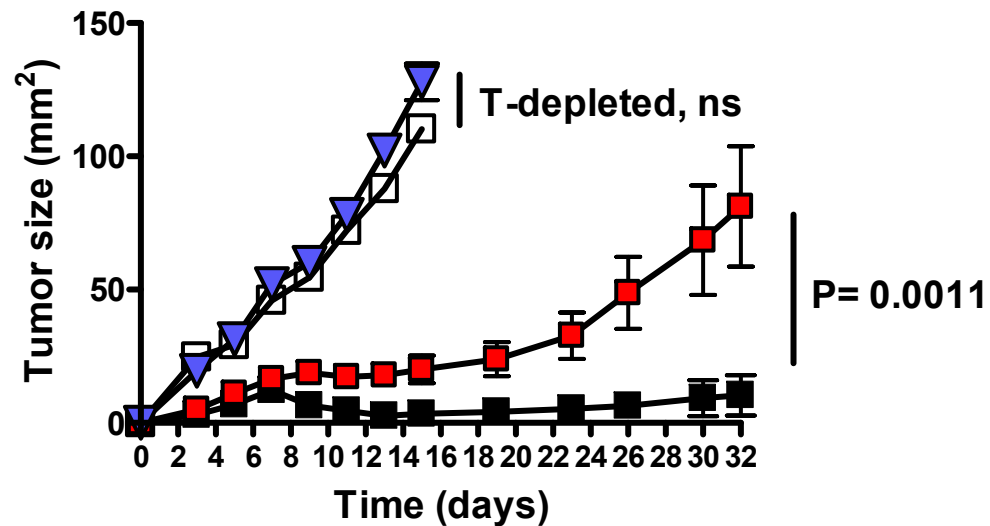
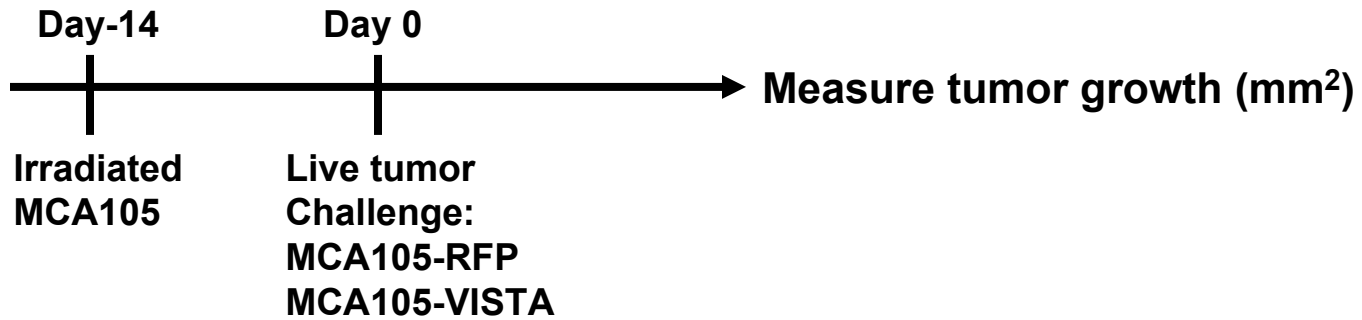
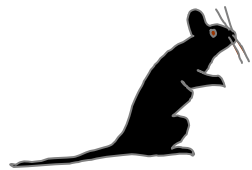


- Similar results are obtained using BM-derived DCs that are transduced with VISTA-expressing retrovirus

VISTA expression on tumors impairs protective α tumor immunity

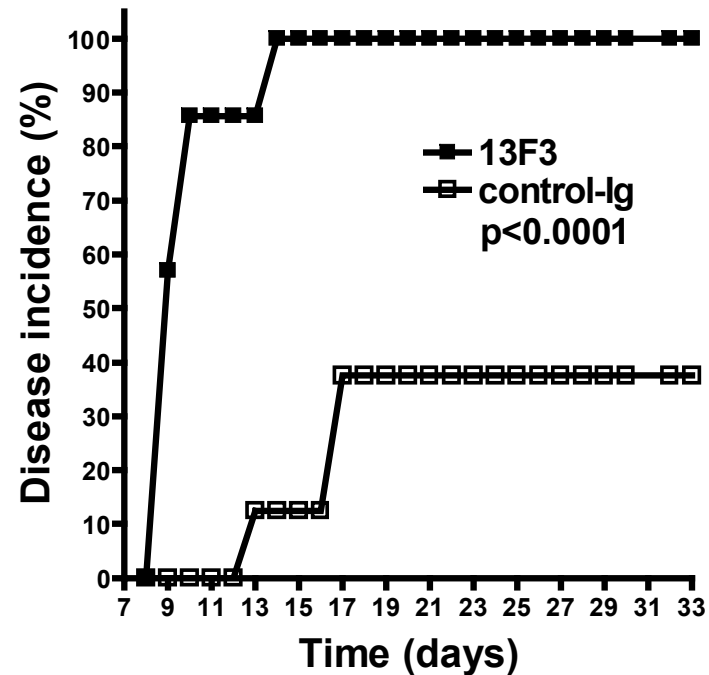
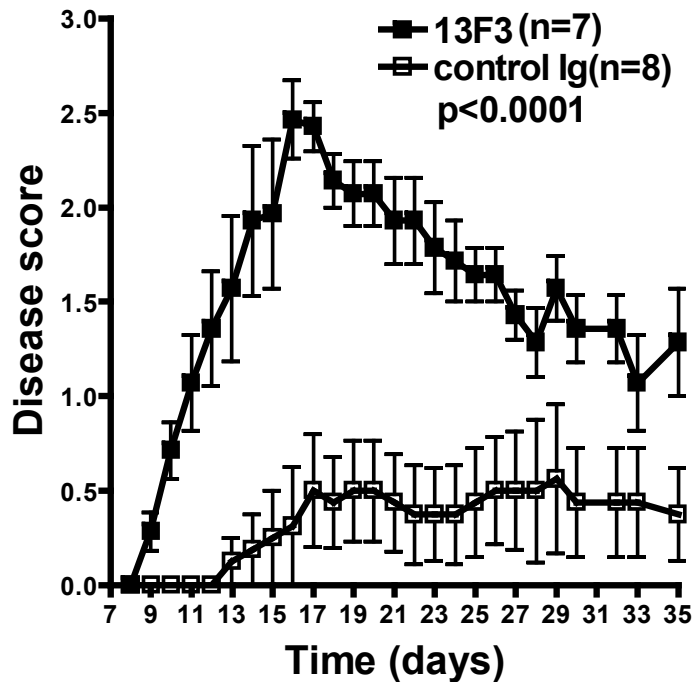
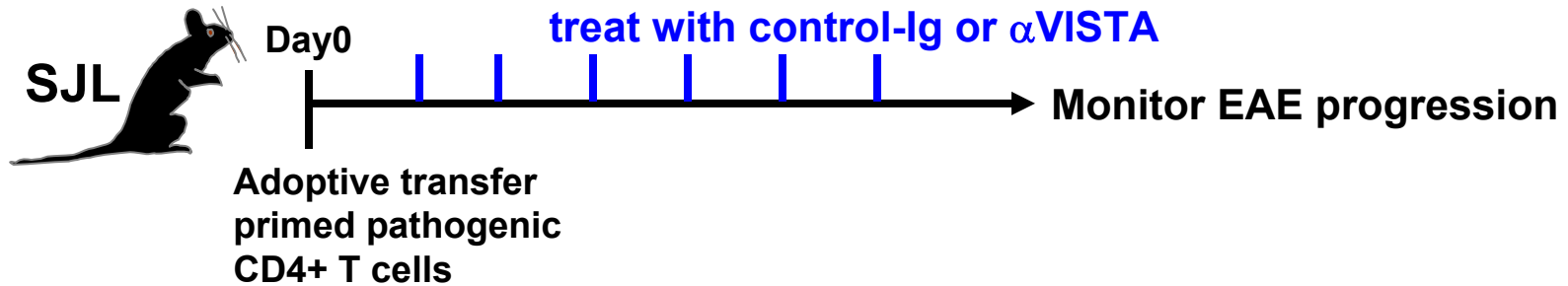


VISTA expression on tumors impairs protective α tumor immunity



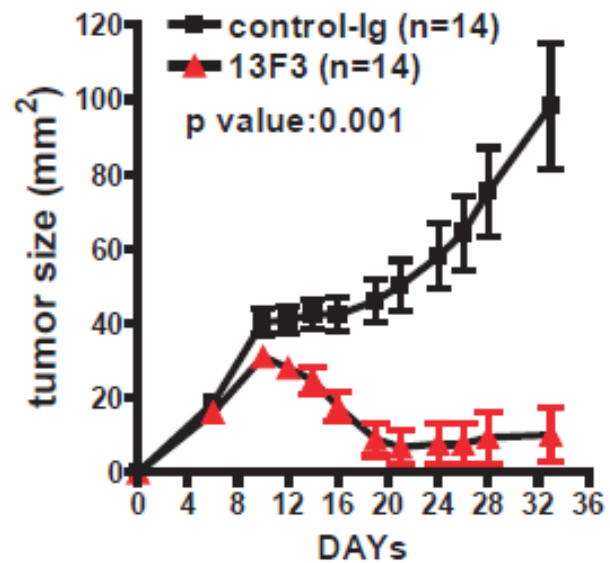
- MCA105-control
- MCA105-VISTA
- MCA105-control T-depletion
- ▼ MCA105-VISTA T-depletion

VISTA monoclonal antibody treatment enhances inflammatory disease

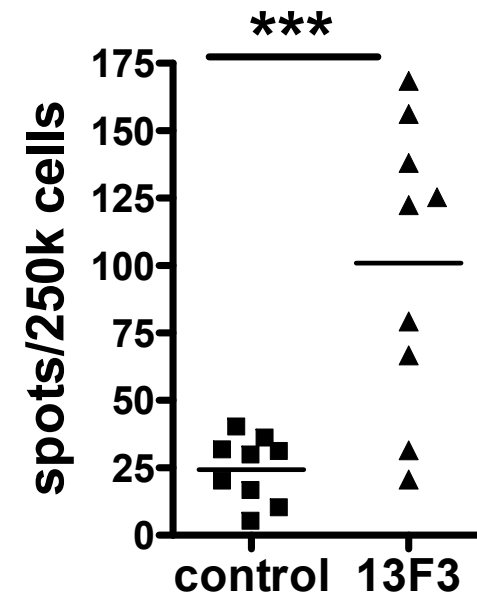


VISTA-specific antibody controls tumor growth

Bladder tumor MB49 (s.c)

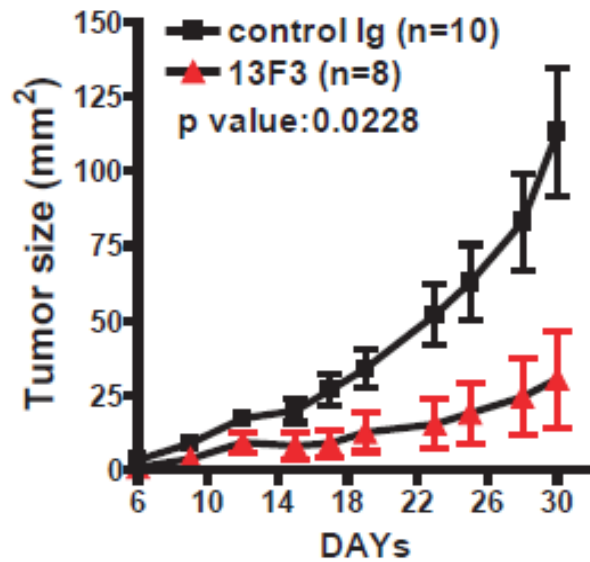


IFN γ ELISPOT

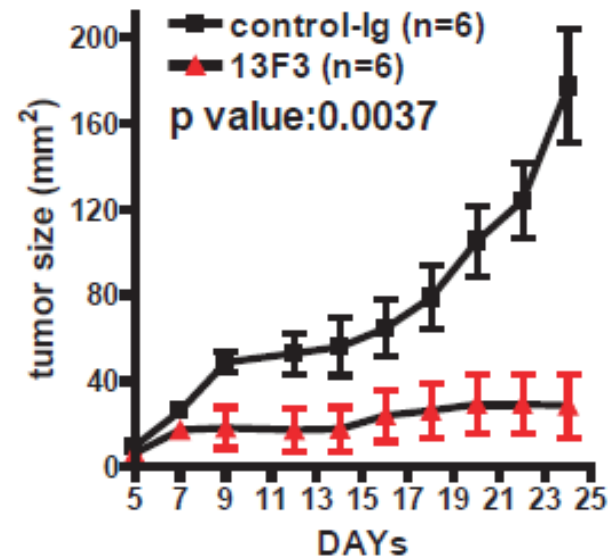


VISTA-specific antibody controls tumor growth

Fibrosarcoma MCA105 (s.c)

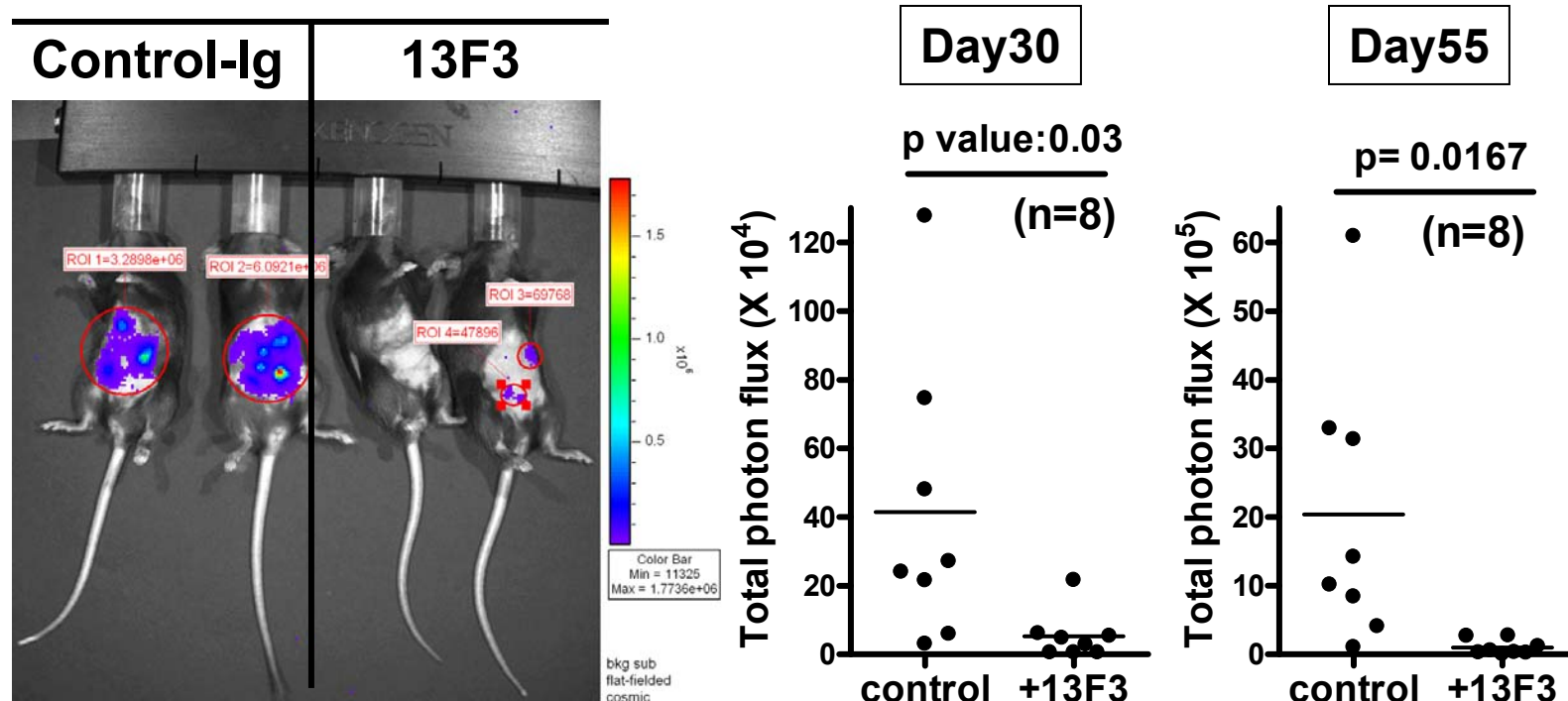


Thymoma EG7 (s.c)



VISTA-specific antibody controls tumor growth

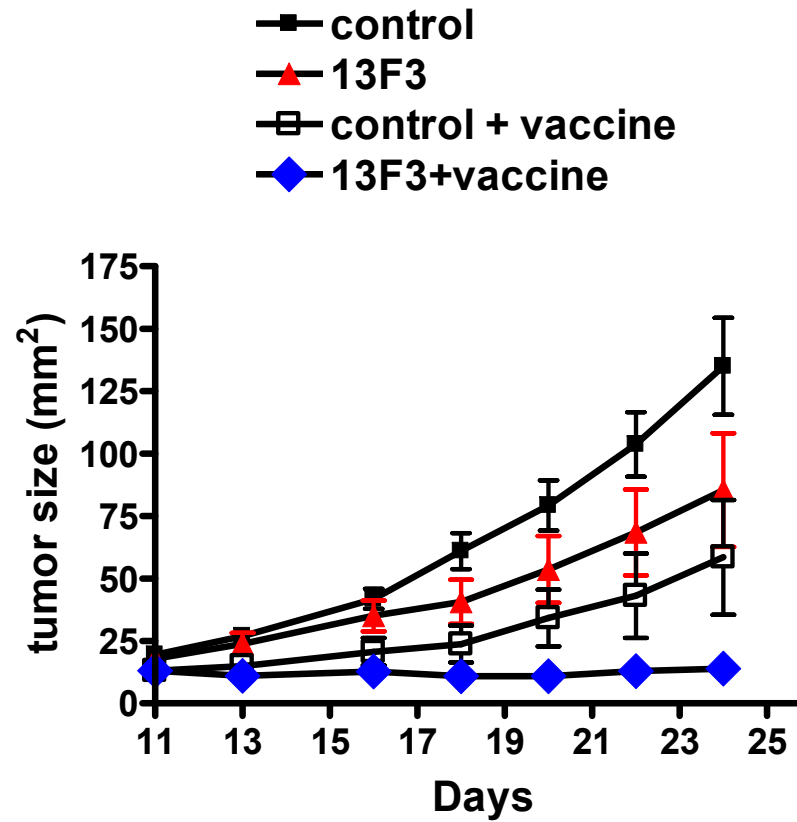
Ovarian tumor ID8-luciferase (peritoneal)



VISTA-specific antibody controls tumor growth

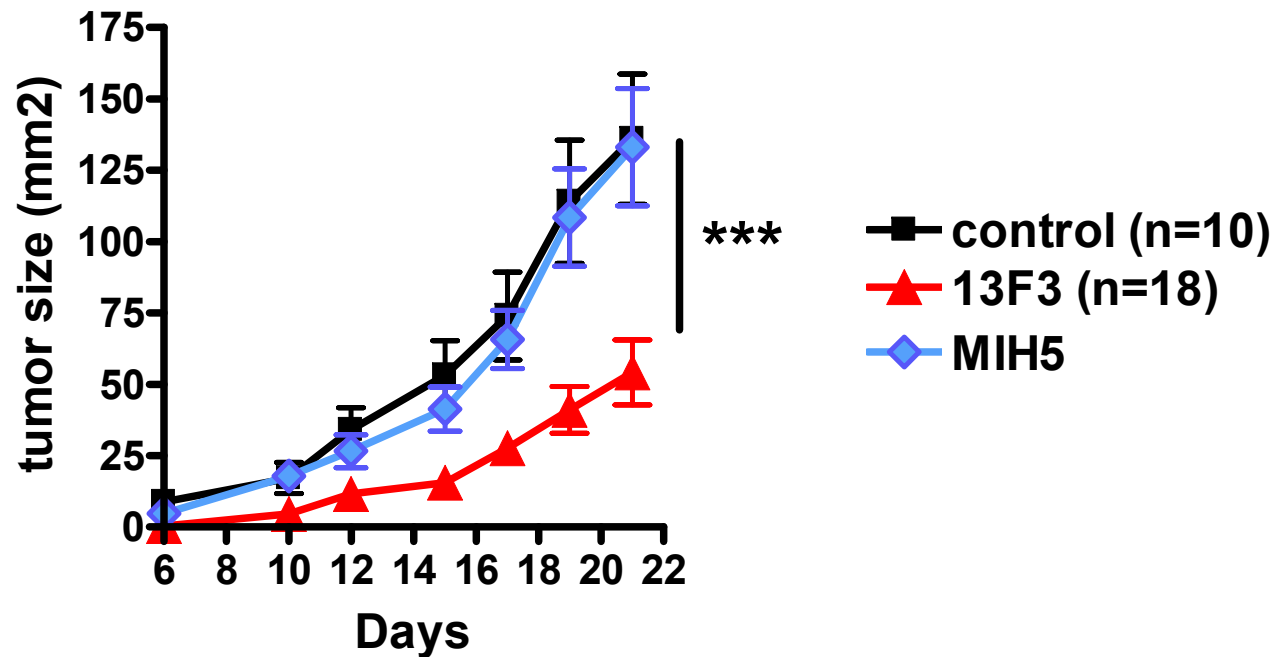
Skin tumor B16F10-OVA

Vaccine: CD40 agonist + LPS + OVA



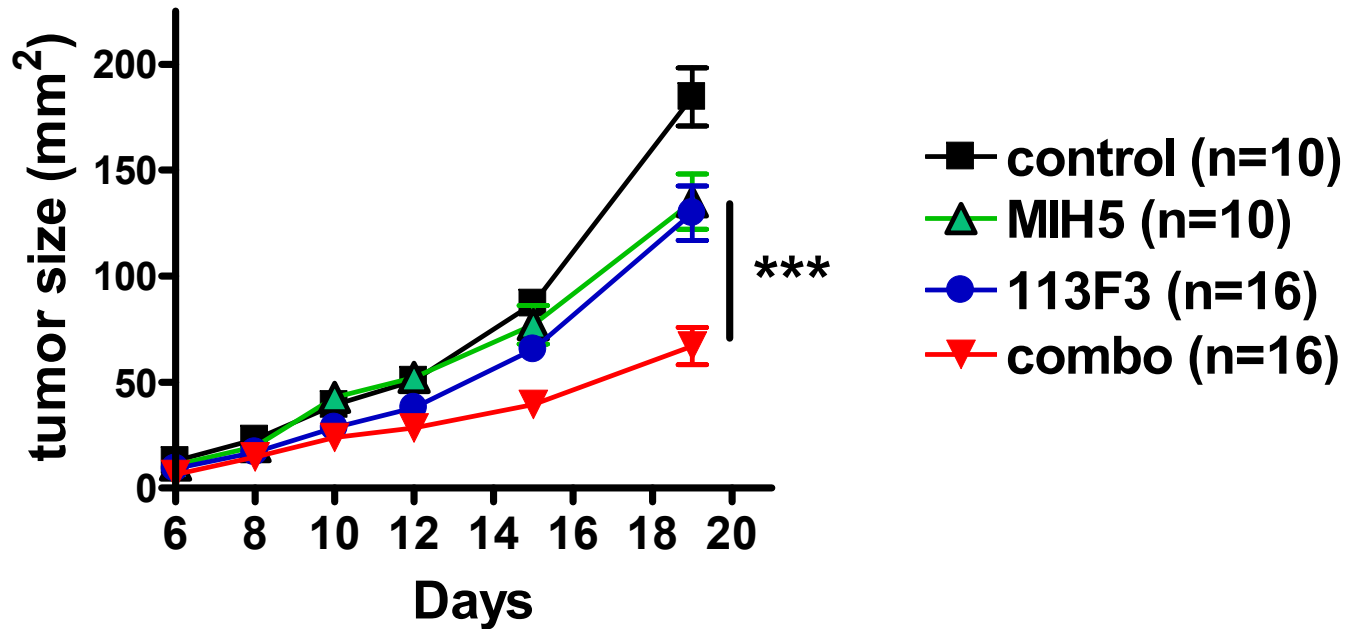
VISTA-specific antibody controls tumor growth

B16F10 (day-2 prophylactic treatment)



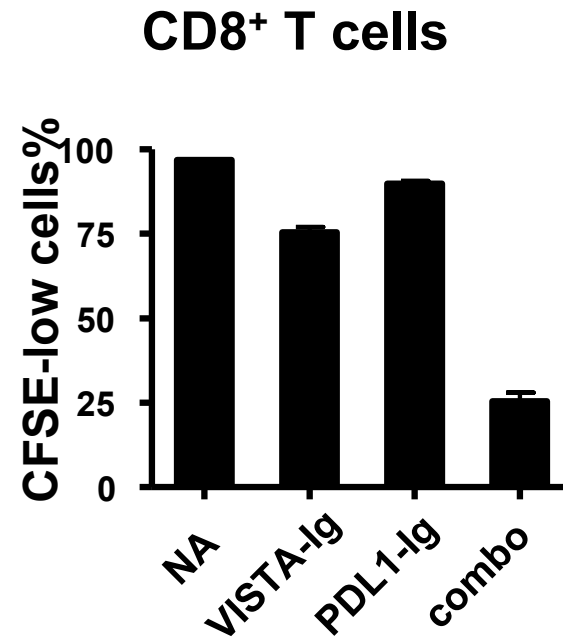
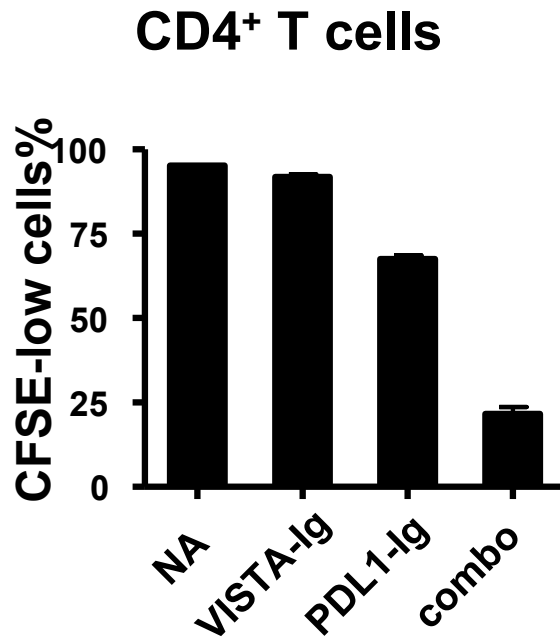
Combinatorial blockade of VISTA and PD-L1/PD-1 results in better tumor control

B16F10 (day+4 therapeutic treatment)



VISTA and PD-L1/PD-1 synergize to suppress T cell proliferation

VISTA-Ig fusion protein + PD-L1-Ig fusion protein + α CD3/CD28



The inducible melanoma model

Tyr::Cre/ERT2:

Tyrosinase promoter driven expression of Cre-ERT2, permitting tamoxifen-inducible, melanocyte-specific cre expression.

Braf^{CA}:

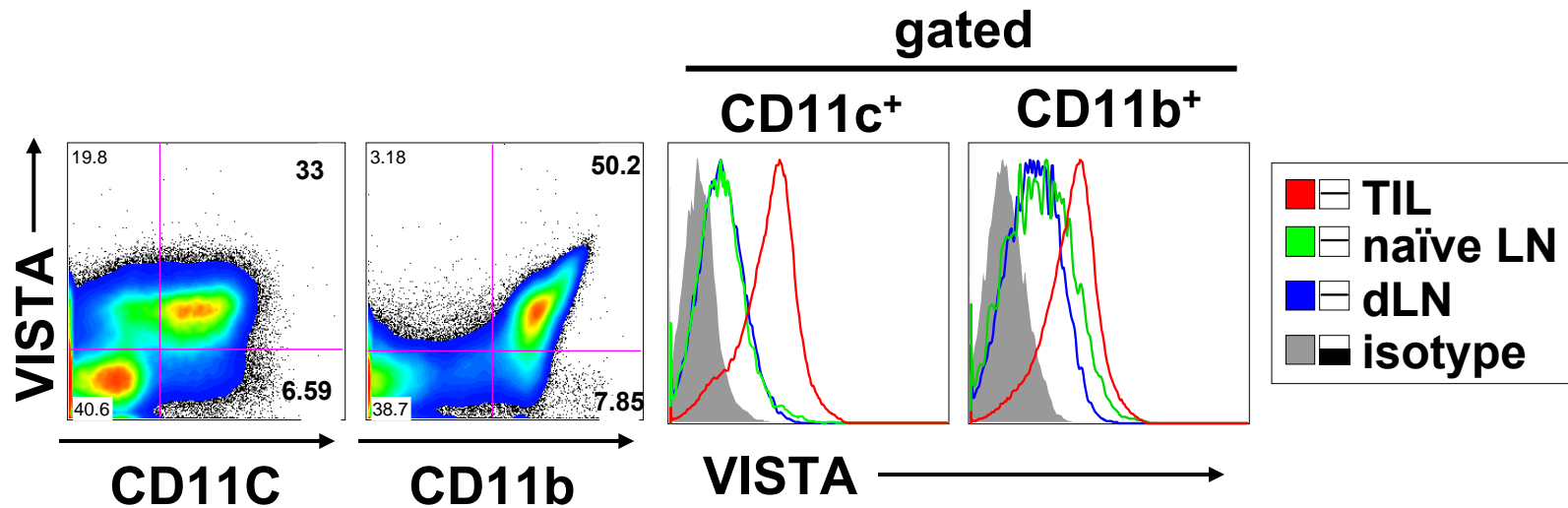
carrying a conditional Braf^{V600E} allele, permitting cre-mediated expression of Braf^{V600E}

Pten^{1ox5}:

carrying a conditional allele of Pten, permitting cre-mediated deletion of exon 5

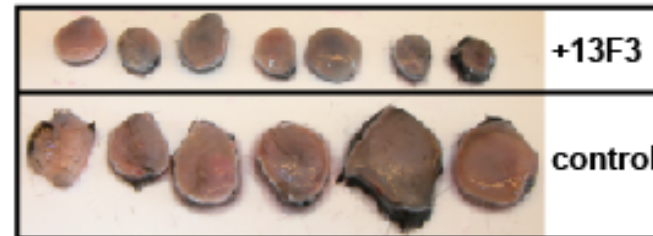
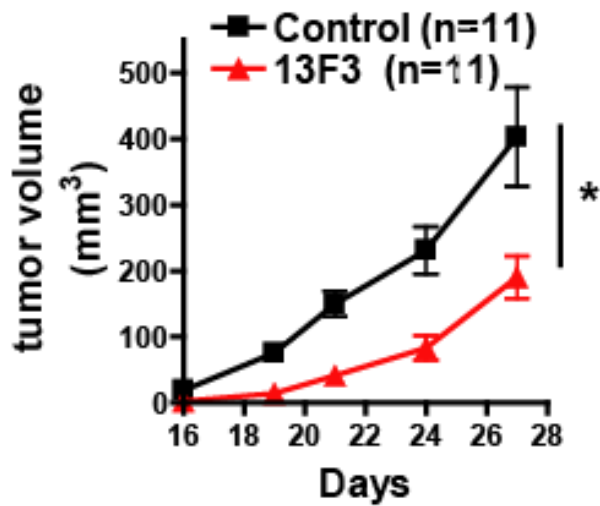
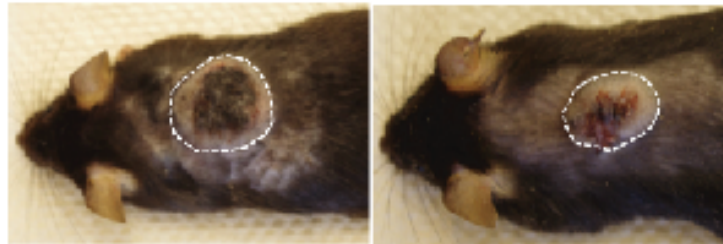
Dankort et al 2009 Nature Immunology

High VISTA expression within the tumor microenvironment



α VISTA blockade inhibited the growth of the inducible melanoma

induced melanoma



Conclusions

VISTA functions as a novel immune checkpoint protein ligand:

- ★ **controls inflammation and autoimmunity.**
- ★ **impairs the generation of anti-tumor immunity.**
- ★ **VISTA antibody-mediated blockade either alone, or in combination with other checkpoint blockade might provide a novel therapeutic strategy for cancer immunotherapy.**

J Exp Med, 2011, 208(3):577-92

ACKNOWLEDGEMENTS

Noelle Lab

(Dartmouth Medical School, NH)
(King's college in London, UK)

Randy Noelle

Janet Louise Lines

Cory Ahonen

Petra Sergent



Almo Lab

(Albert Einstein College of Medicine)

Steve Almo

Rotem Rubinsteine

Andras Fiser

(Albert Einstein College of Medicine)