Regulating the Regulators for Cancer Immunotherapy: LAG-3 Finally Catches Up

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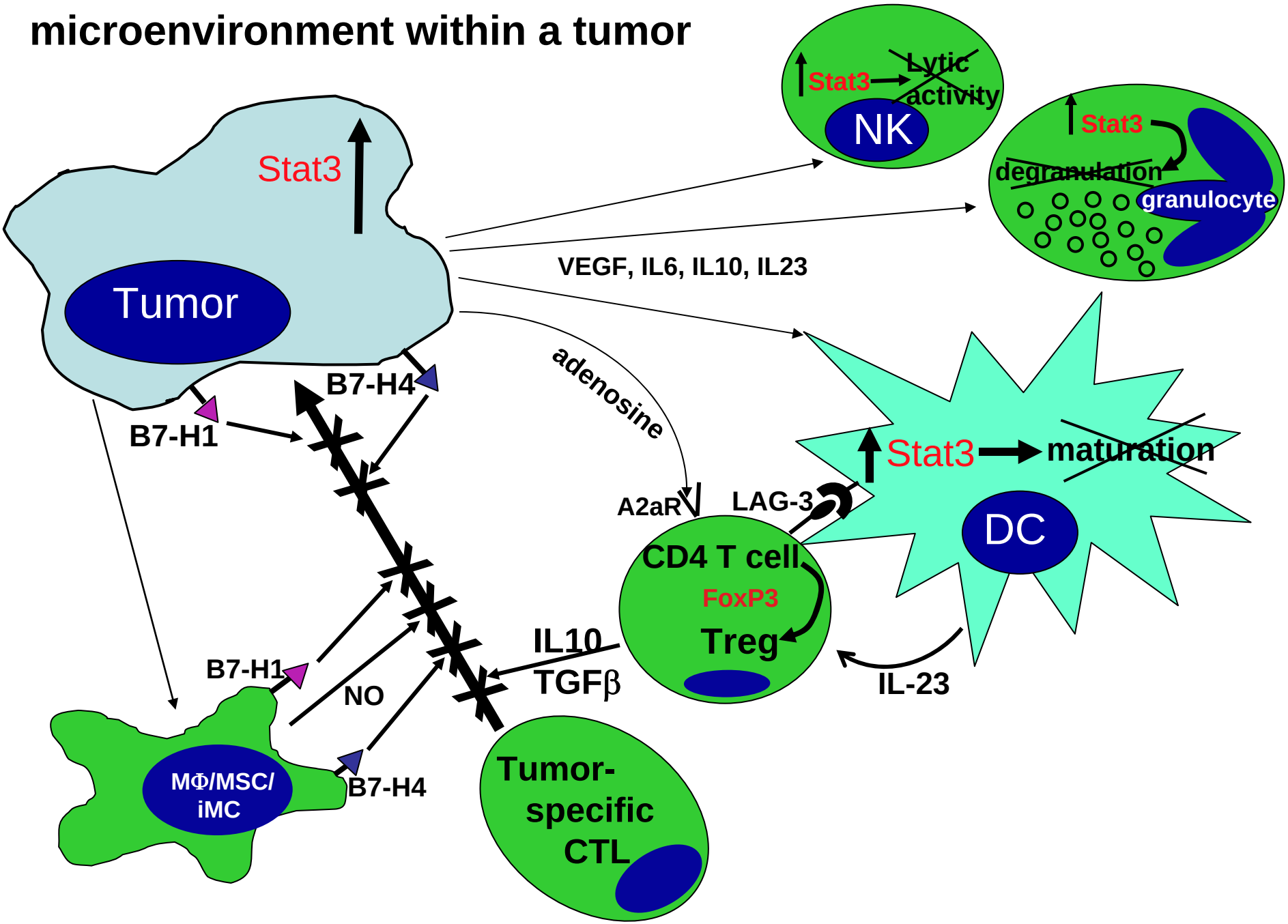
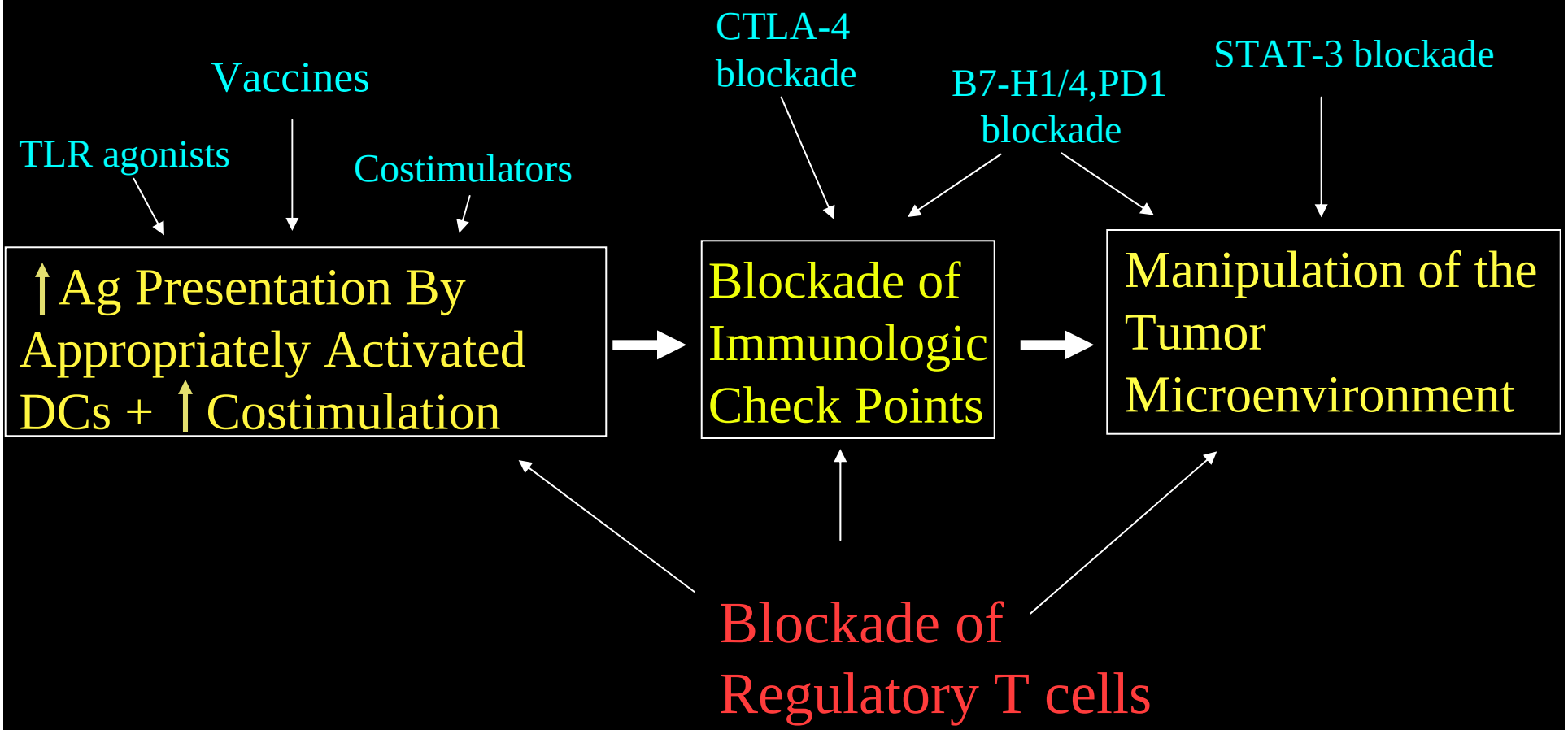
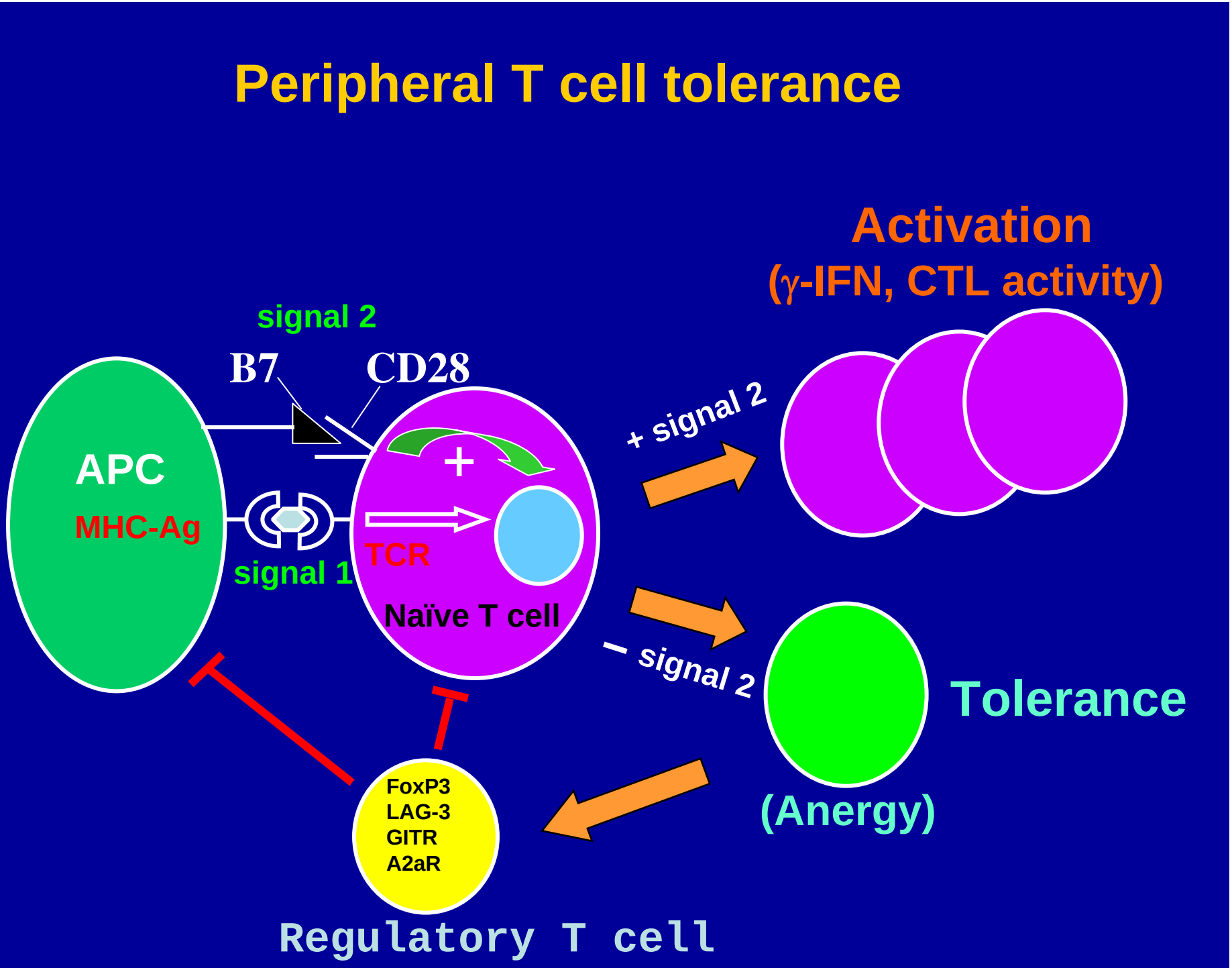
The hostile immune microenvironment within a tumor



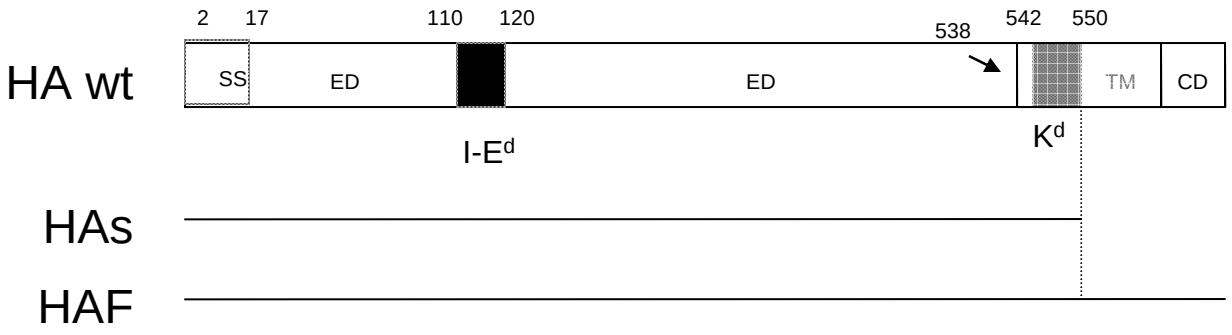
Combinatorial immune therapies to hit distinct steps in the evolution of antitumor immunity



Peripheral T cell tolerance

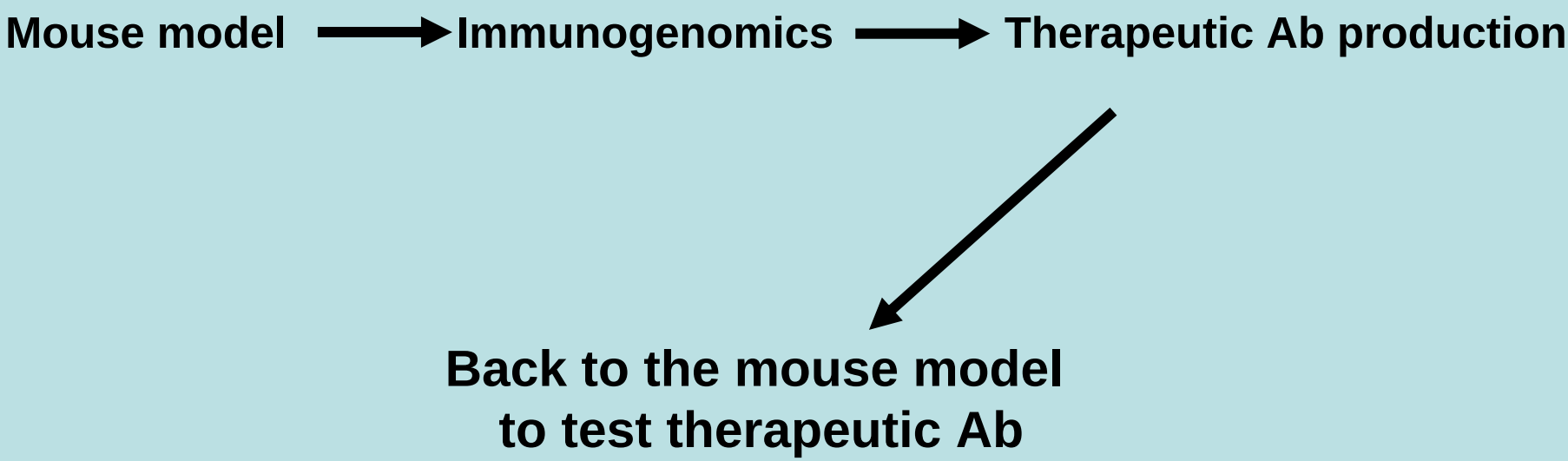


The Probasin – Hemagluttinin (ProHA) Transgenic Mouse

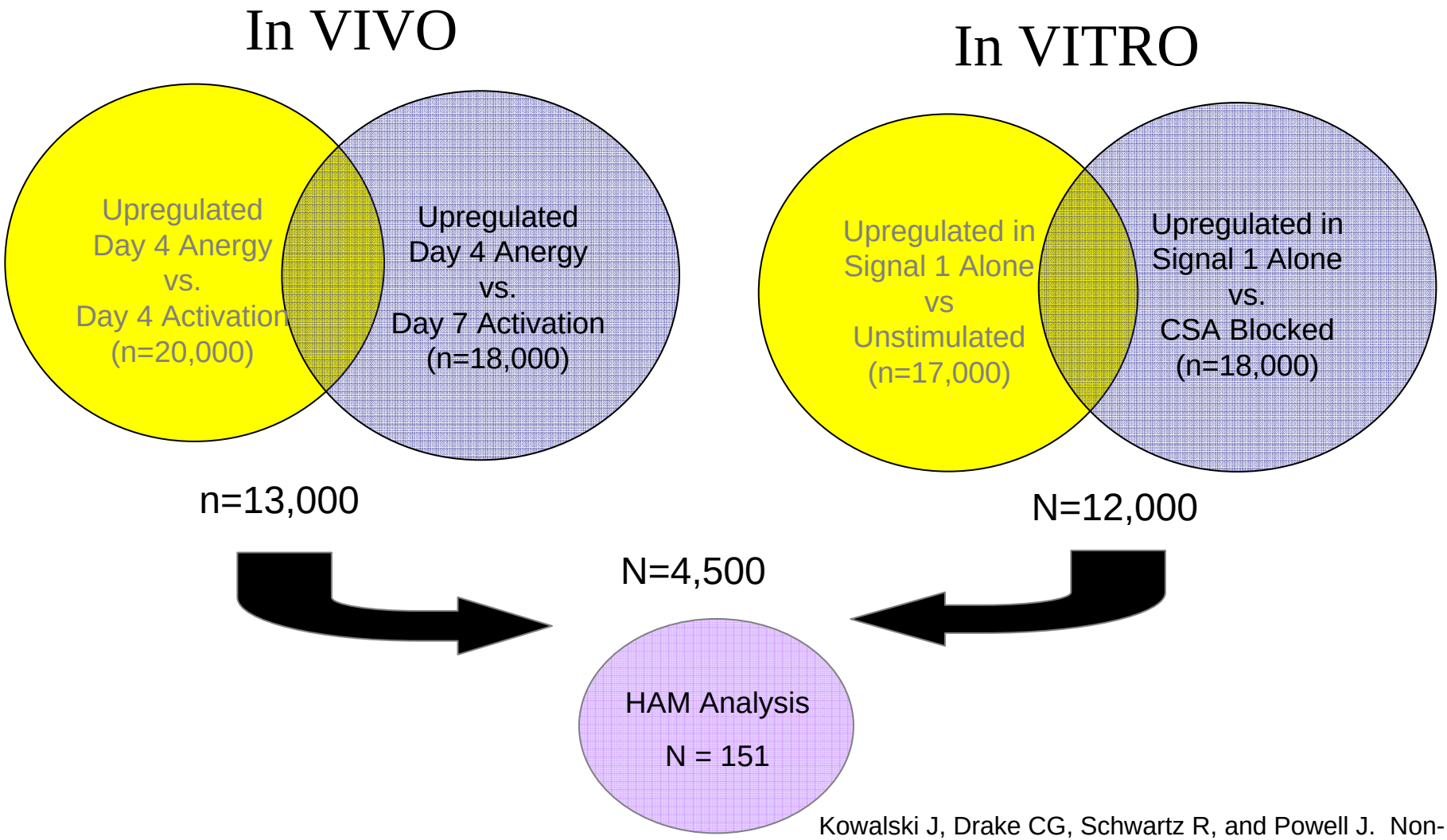


The ProHA x TRAMP Mouse(ProTRAMP) A tumor tolerance model

- ProSV40 – Oncogenic
- ProHA – A Tumor / and Tissue Specific Antigen
- Disease grossly identical to TRAMP
- 12’th Generation Intercross onto B10.D2
- HA-specific CD4 and CD8 TCR transgenic T cells transferred into ProTRAMP are TOLERIZED. HA-specific CD4s become Treg

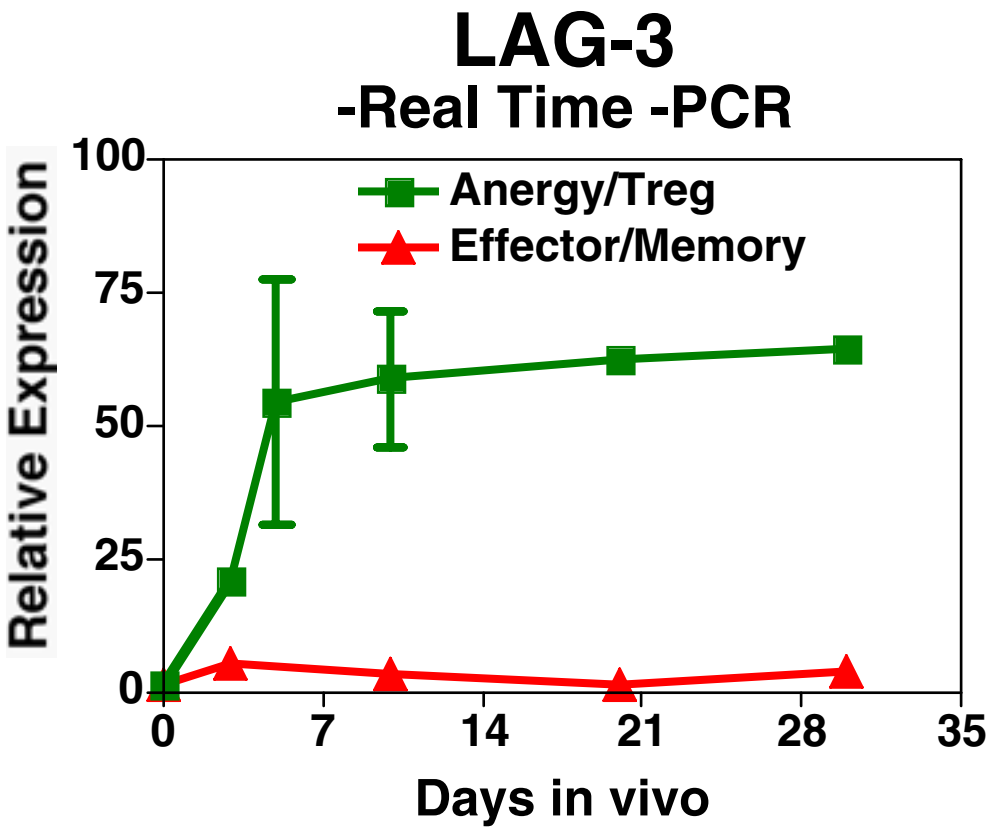

Mouse model
Immunogenomics
Therapeutic Ab production
Back to the mouse model
to test therapeutic Ab

Genes Involved in Expression of the Tolerant Phenotype in T Cells

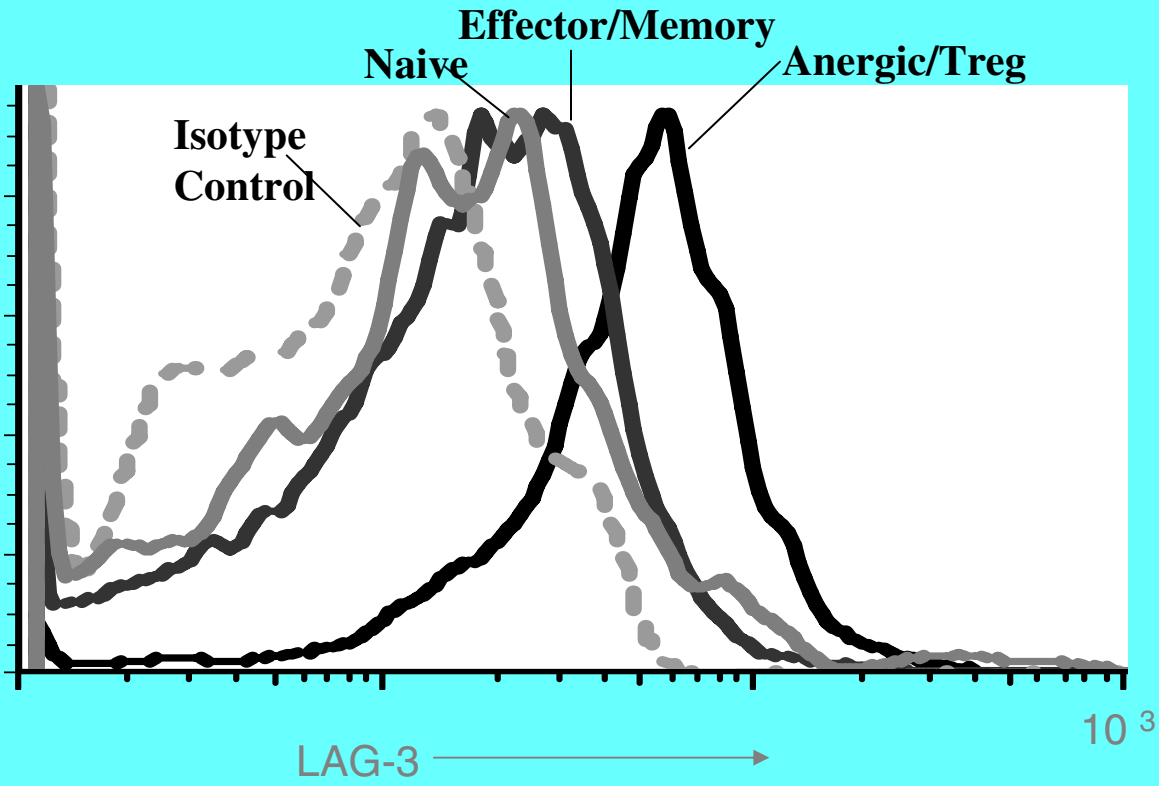


Kowalski J, Drake CG, Schwartz R, and Powell J. Non-Parametric, hypothesis-based analysis of microarrays for comparison of several phenotypes. *Bioinformatics* 20: 364-373, 2004

Symbol	IN VIVO Ratio Anergy / Memory	IN VITRO Ratio Anergy / Resting	NAME - FROM GO Ontogeny Search
Lag3	3.6	8.7	Lymphocyte-activation gene 3
Bcl3	3.5	14.6	B-cell leukemia/lymphoma 3
Tnfrsf4	2.9	5.7	tumor necrosis factor receptor superfamily member 4
Nfatc1	2.7	14.3	NF-ATc isoform a (NF-ATca)
Il5	2.7	10.7	Interleukin 5
Bcat1	2.6	34.0	branched chain amino acid transferase 1, cytosolic
Ptprs	2.5	4.5	Protein tyrosine phosphatase, receptor type, S
Mapkapk2	2.4	10.5	MAP kinase-activated protein kinase 2
Tubb5	1.8	4.5	beta-tubulin (isotype Mbeta 5)
Bcap37	1.8	4.1	B-cell receptor-associated protein 37
Fhl2	1.7	8.3	Four and a half LIM domains
Il1r2	1.5	3.9	Interleukin 1 receptor, type II
Cish	1.4	5.9	Cytokine inducible SH2-containing protein
Ndrg1	1.4	8.0	N-myc downstream regulated gene 1
Etf1	1.4	4.6	Eucaryotic tranlation termination factor 1
Prkch	1.4	6.0	Protein kinase C, eta
Cnil	1.3	24.7	cornichon-like protein
Tnfsf11	1.2	26.0	Tumor necrosis factor (ligand) superfamily, member 11
Il13	1.2	95.7	Interleukin 13
Kcnn4	1.2	5.2	calcium -activated potassium channel, small conductance
Ccl1	1.1	159.9	chemokine (C-C) motif ligand 1
Egr2	1.1	75.9	Early growth response 2
Ier3	1.1	8.7	immediate early response 3
Gch	1.1	30.5	GTP cyclohydrolase 1
Rgs16	1.1	13.4	regulator of G protein signalling 16
Csf1	1.1	3.7	colony-stimulating factor 1 (macrophage)
Fkbp8	1.0	8.2	FK506 binding protein 8
Nr4a1	1.0	46.9	nuclear receptor subfamily 4, group A member 1
Lcp2	1.0	4.4	lymphocyte cytosolic protein 2
Dnajc5	1.0	16.1	DnaJ (Hsp40) homolog


LAG-3
-Real Time -PCR
Relative Expression
100
75
50
25
0
Anergy/Treg
Effector/Memory
0
7
14
21
28
35
Days in vivo

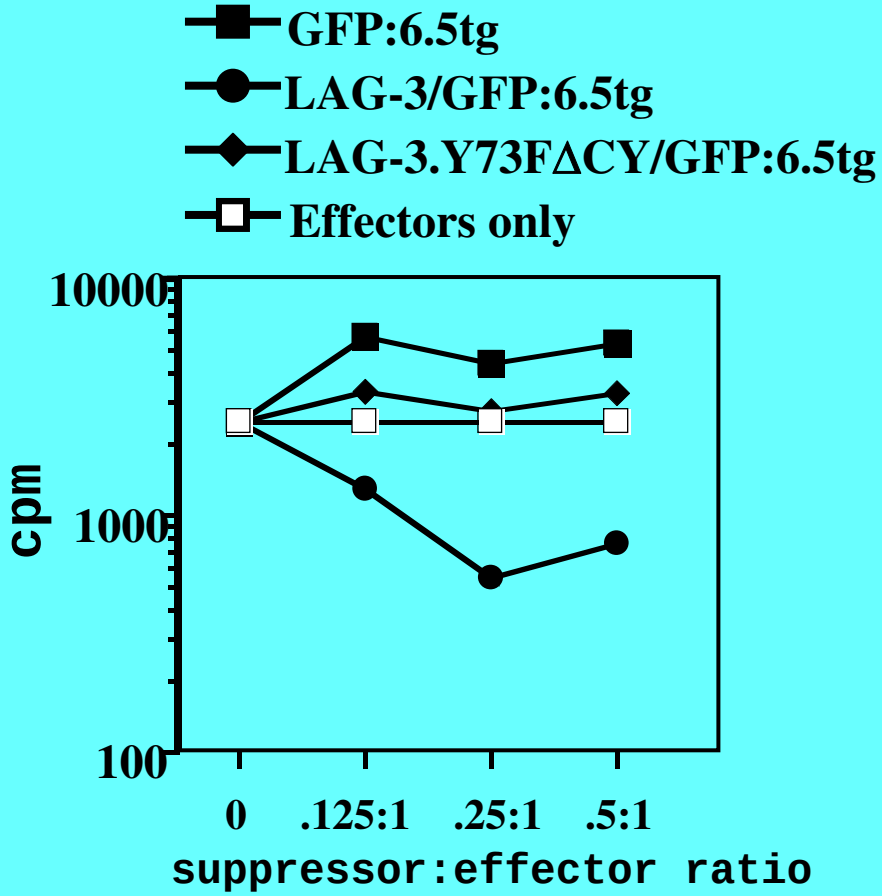
LAG-3 is highly expressed on the surface of induced Treg



LAG-3

- Cloned in early 1990s
- CD4 homologue
- Does not substitute for CD4 in T cell development or helper T cell function
- Binds MHC II with higher affinity than CD4 but at a distinct site from CD4
- Function unclear - reported to play a role in modulating NK function, T cell function, APC function but no clear conclusions
- Cytoplasmic tail completely different from CD4. Signaling pathways unclear

Transduction of CD4+CD25- T cells with wild type LAG-3 confers regulatory capacity



Can LAG-3 blockade alter endogenous T cell function?

Day 0: α-LAG-3 (0.2mg)

Day 1: VAC-HA

Day 3: α-LAG-3 (0.2mg)

Day 6: Adoptive transfer targets

ProTramp

Mix 1:1; inject i.v.

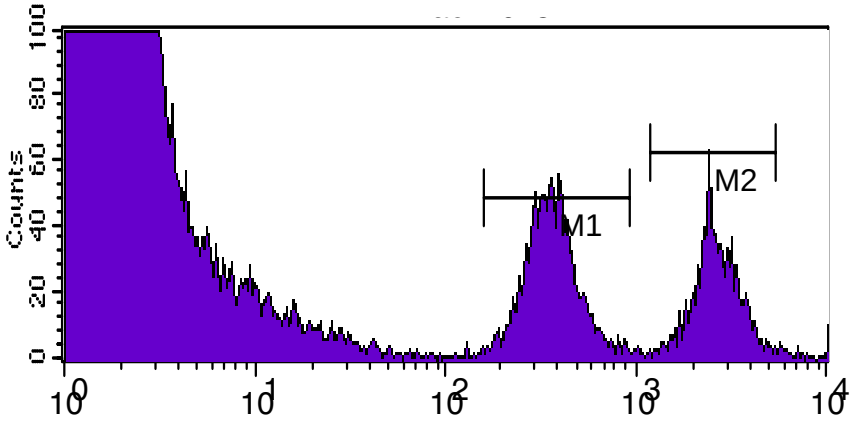
Unstimulated — CFSE^{lo}

HA peptide-pulsed — CFSE^{hi}

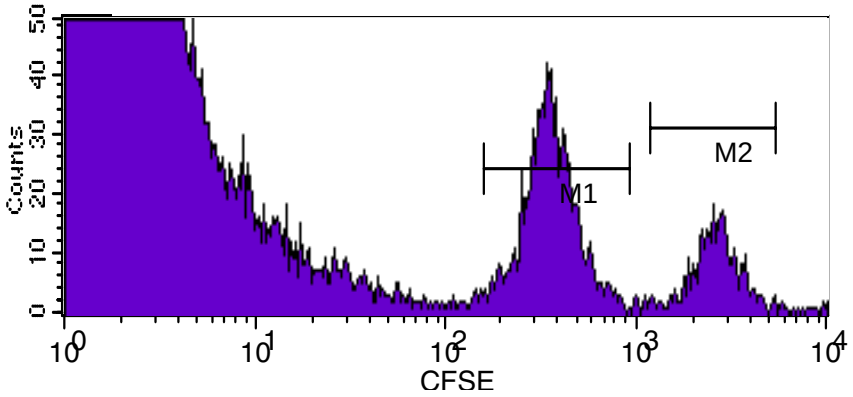
B10.D2 splenocytes

Day 7: Harvest spleens; Flow

ProTRAMP+vaccine

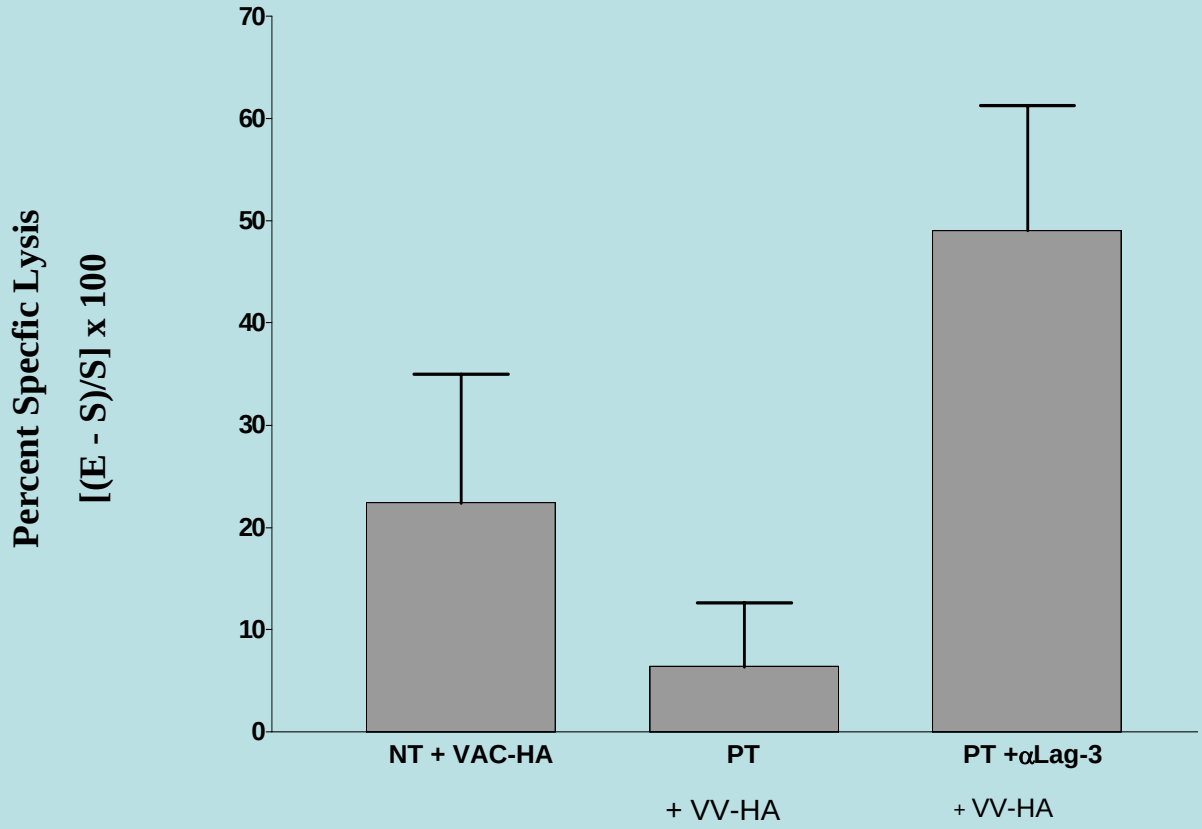


ProTRAMP+vaccine+anti-LAG3



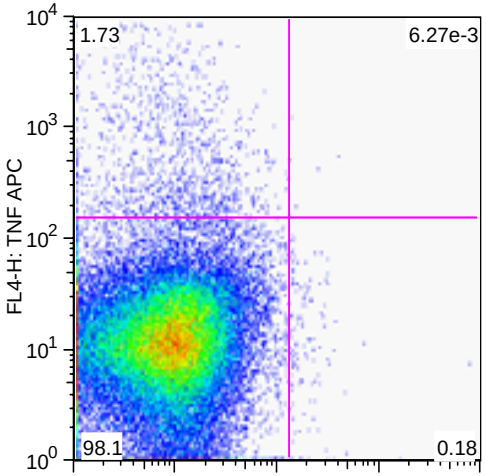
Tumor-tolerized endogenous CTL regain effector function in vivo after LAG-3 blockade

ProTramp (14-16 wk old)

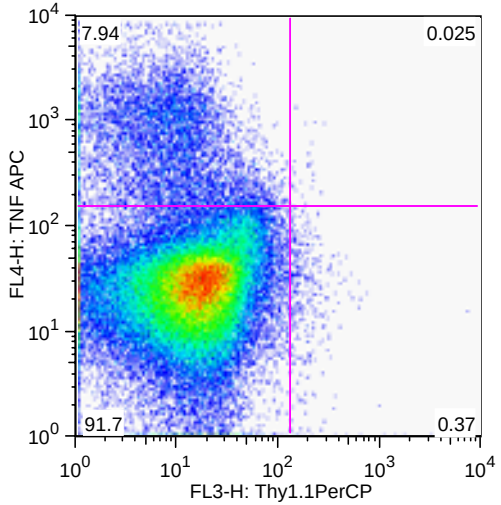


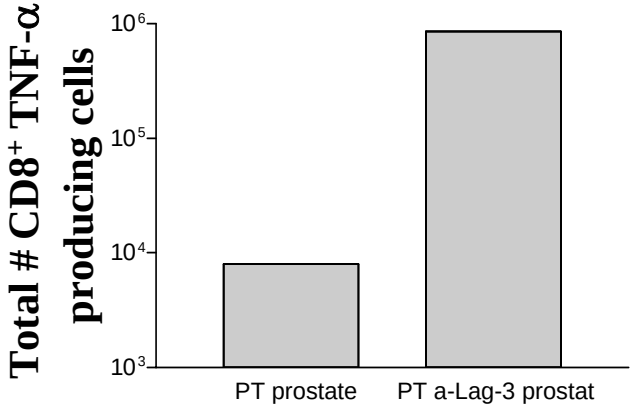
α-LAG-3 leads to endogenous CD8 migration and TNF-α production within prostates of ProTRAMP mice

ProTramp



ProTramp + α-LAG-3

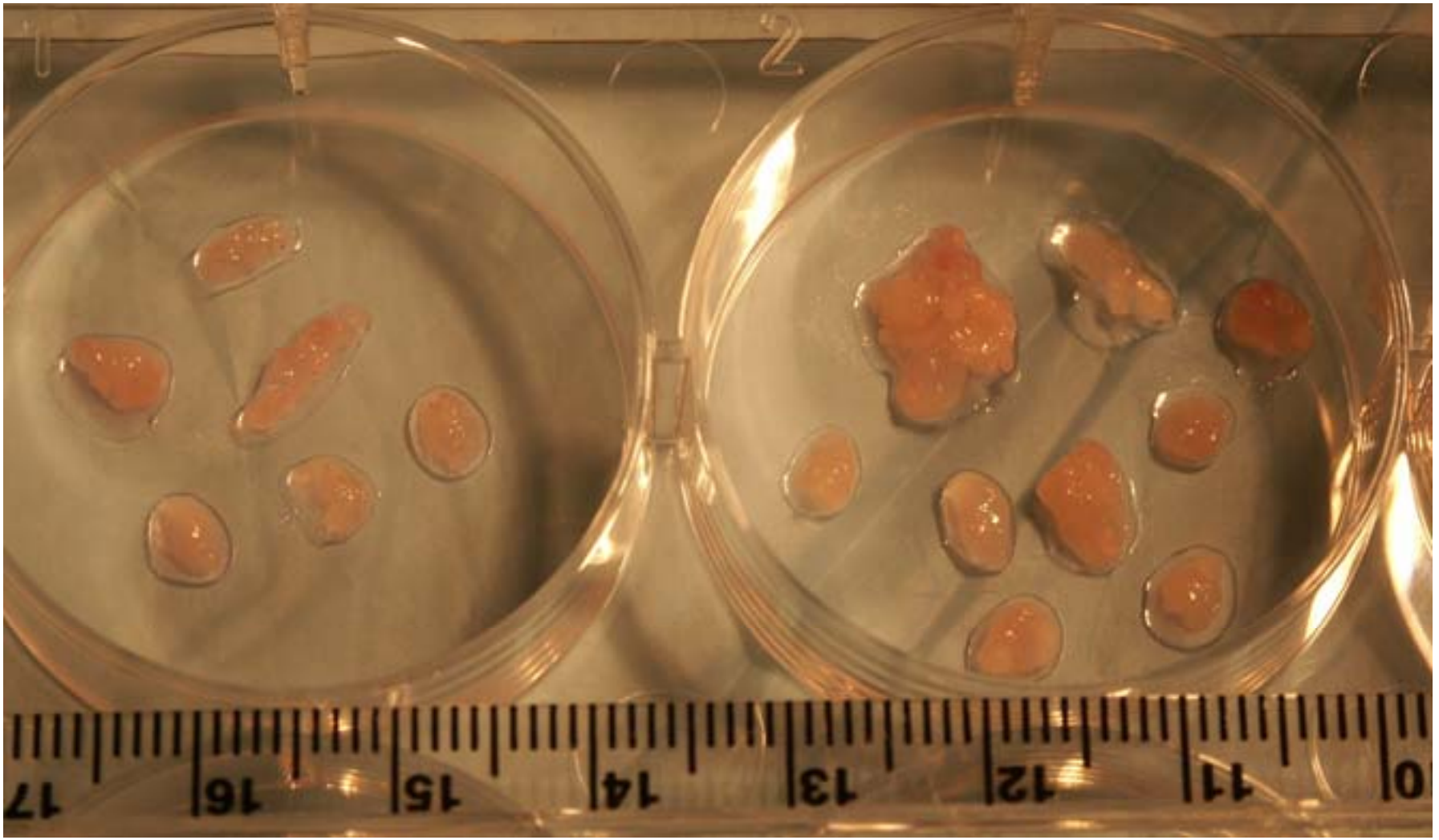




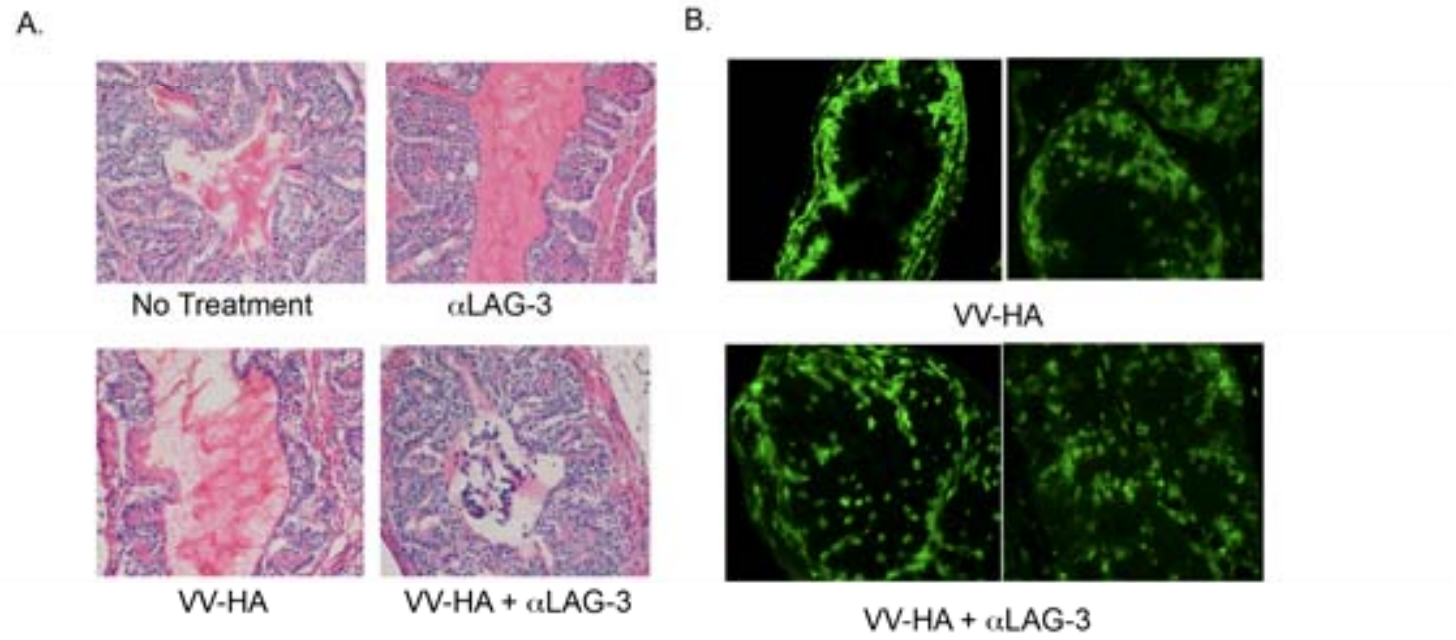
Prostate tissue from Pro-Tramp mice 7 days post-AT

Clone 4 Adoptive Transfer

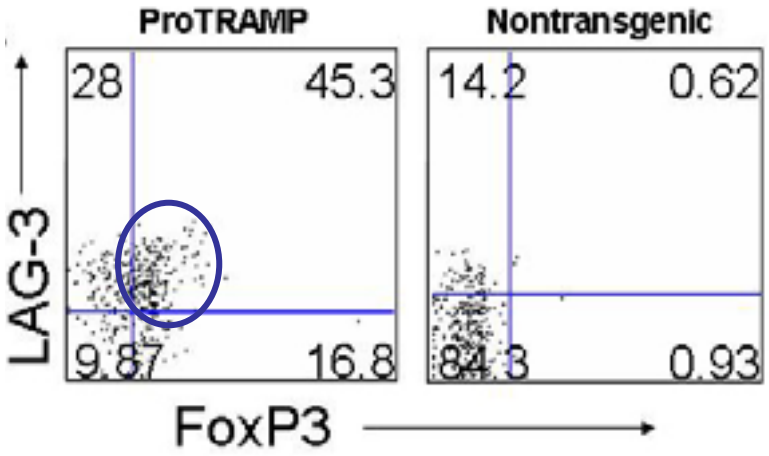
Clone 4 Adoptive Transfer + anti-LAG-3



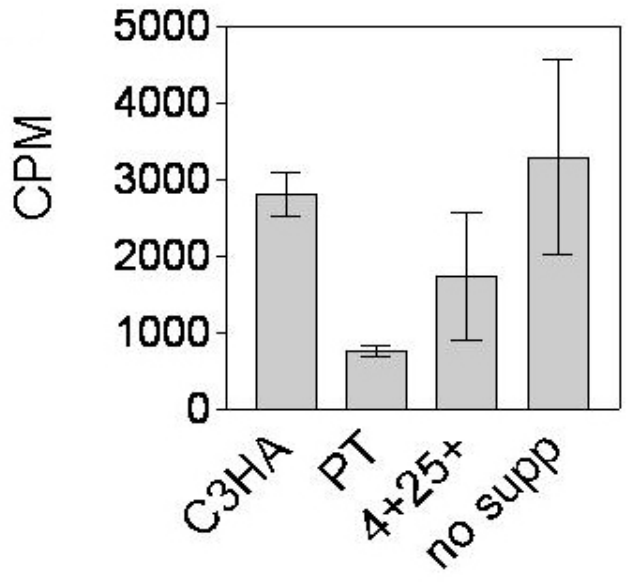
Histology of prostate cancers treated with vaccine + anti-LAG-3 antibodies



LAG-3 and FoxP3 expression are concordant on antigen-specific CD4 T cells only when antigen is present as self or tumor



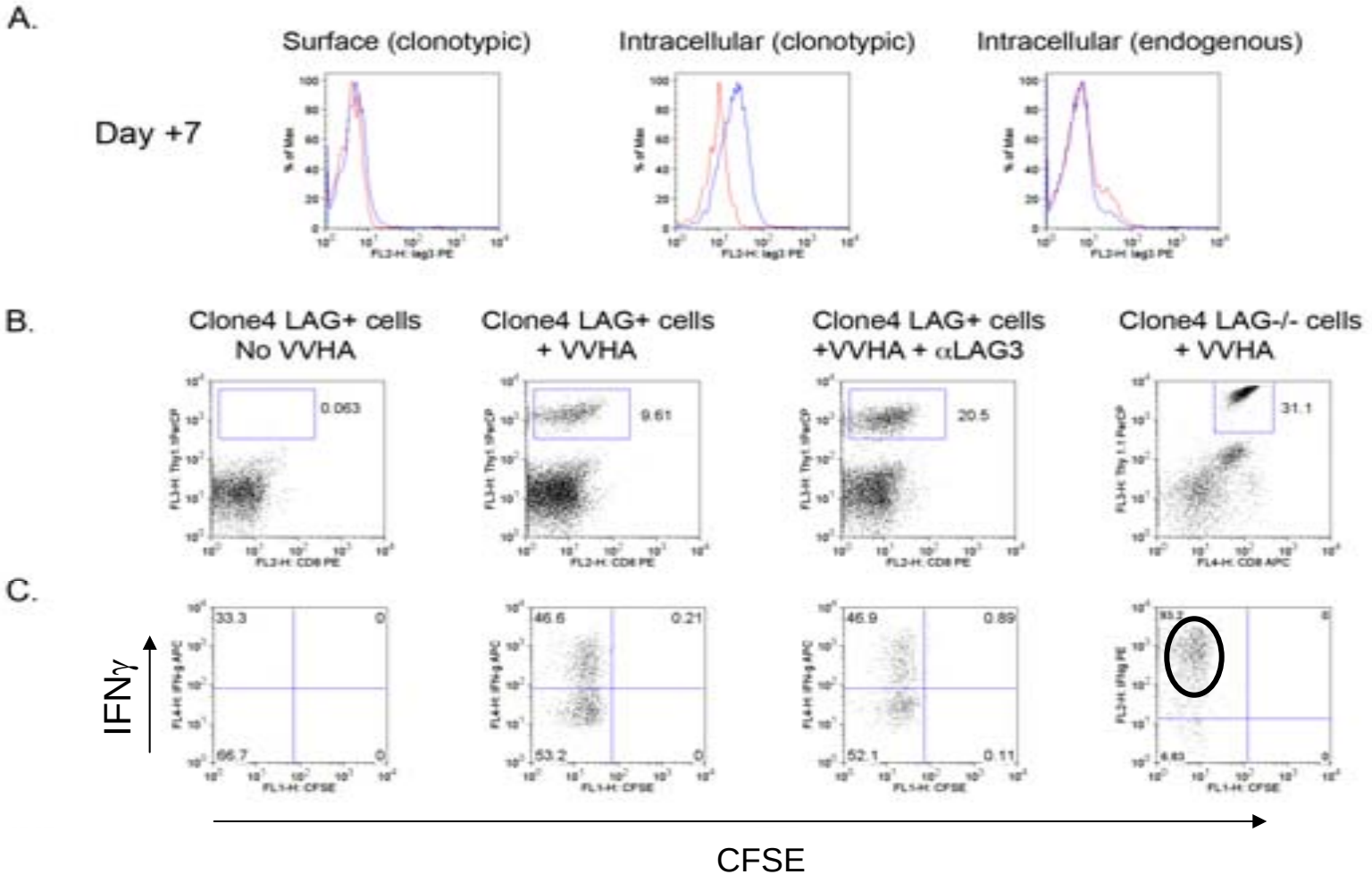
CD4+25+ Treg from tumor bearing mice are highly suppressive in *in vitro* assays



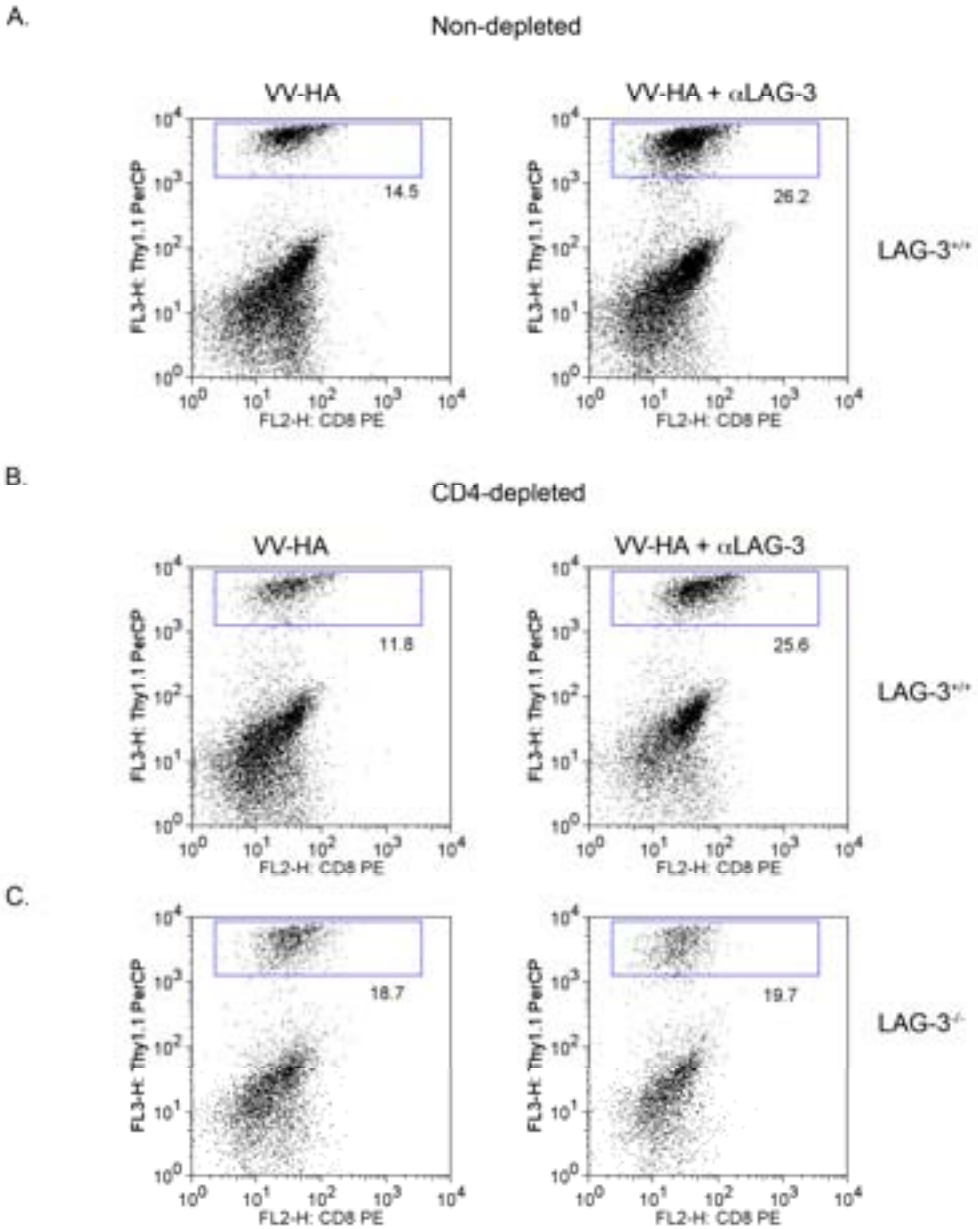
Membrane protein encoding genes upregulated among CD4+ cells infiltrating human prostate cancer

Probe ID	Fold Increase	Gene Definition	Gene Symbol	Cellular Component
TNFSF9	122	Tumor necrosis receptor superfamily, member 9 (41BB)	TNFRSF9	Membrane
234895_at	95	cytotoxic T-lymphocyte-associated protein 4	CTLA4	Membrane
206486_at	86	lymphocyte-activation gene 3	LAG3	Membrane
211269_s_at	58	interleukin 2 receptor, alpha (CD25)	IL2RA	Membrane
223851_s_at	31	tumor necrosis factor receptor superfamily, member 18 (GITR)	TNFRSF18	Membrane
224211_at	17	forkhead box P3	FOXP3	Nucleus

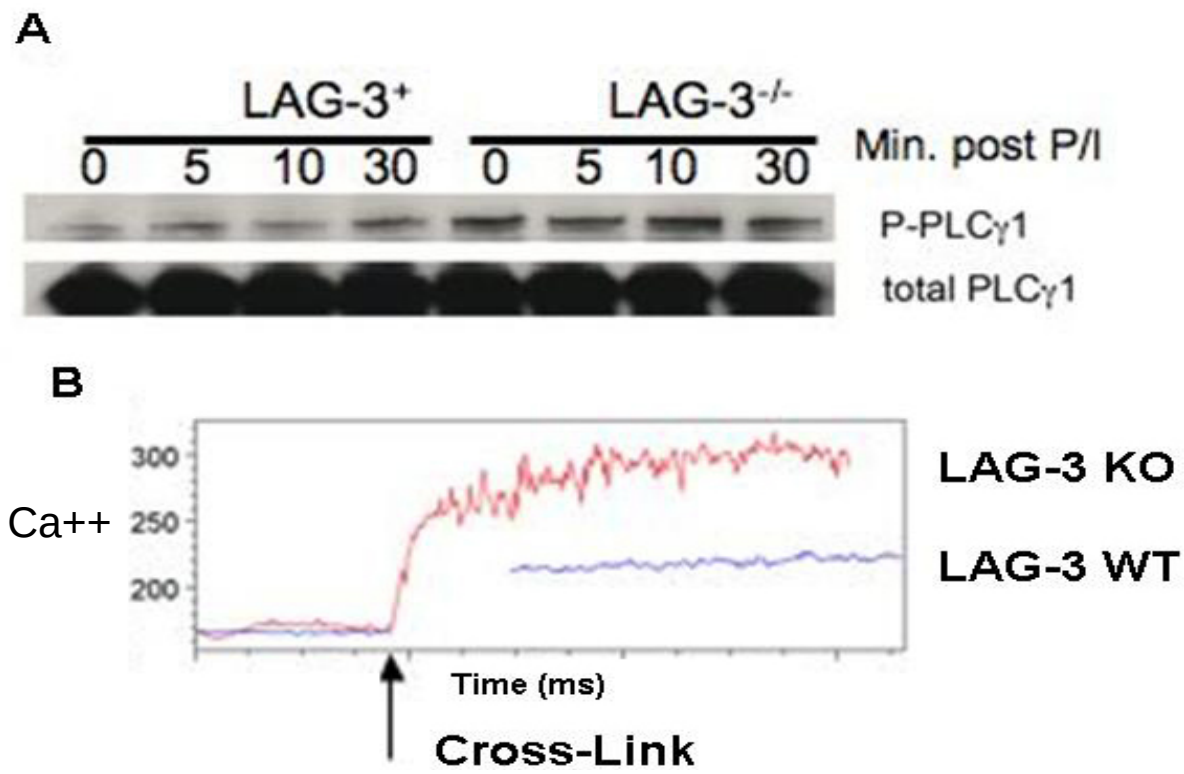
LAG3 expression on tumor specific CD8 T cells restrains their accumulation and function of in prostate cancers



CD4-independent role of LAG-3 for tumor-specific CD8 T cells



LAG-3 downregulates TCR dependent signaling



ProTRAMP model

Charles Drake

Adam Adler

LAG-3

Charles Drake

Ching-Tai Huang

Joe Grosso

Tulia Bruno

Ed Hipkiss

Christin Kelleher

Dario Vignale

Craig Workman