Presenter disclosure information

- David H. Munn, MD

The following relationships exist related to this presentation:

NewLink Genetics, Inc. (Consultant, stock, SAB)

Immune Suppression by Stromal Cells

David H. Munn

Cancer Center

Georgia Health Sciences University

Potentially suppressive stromal cells

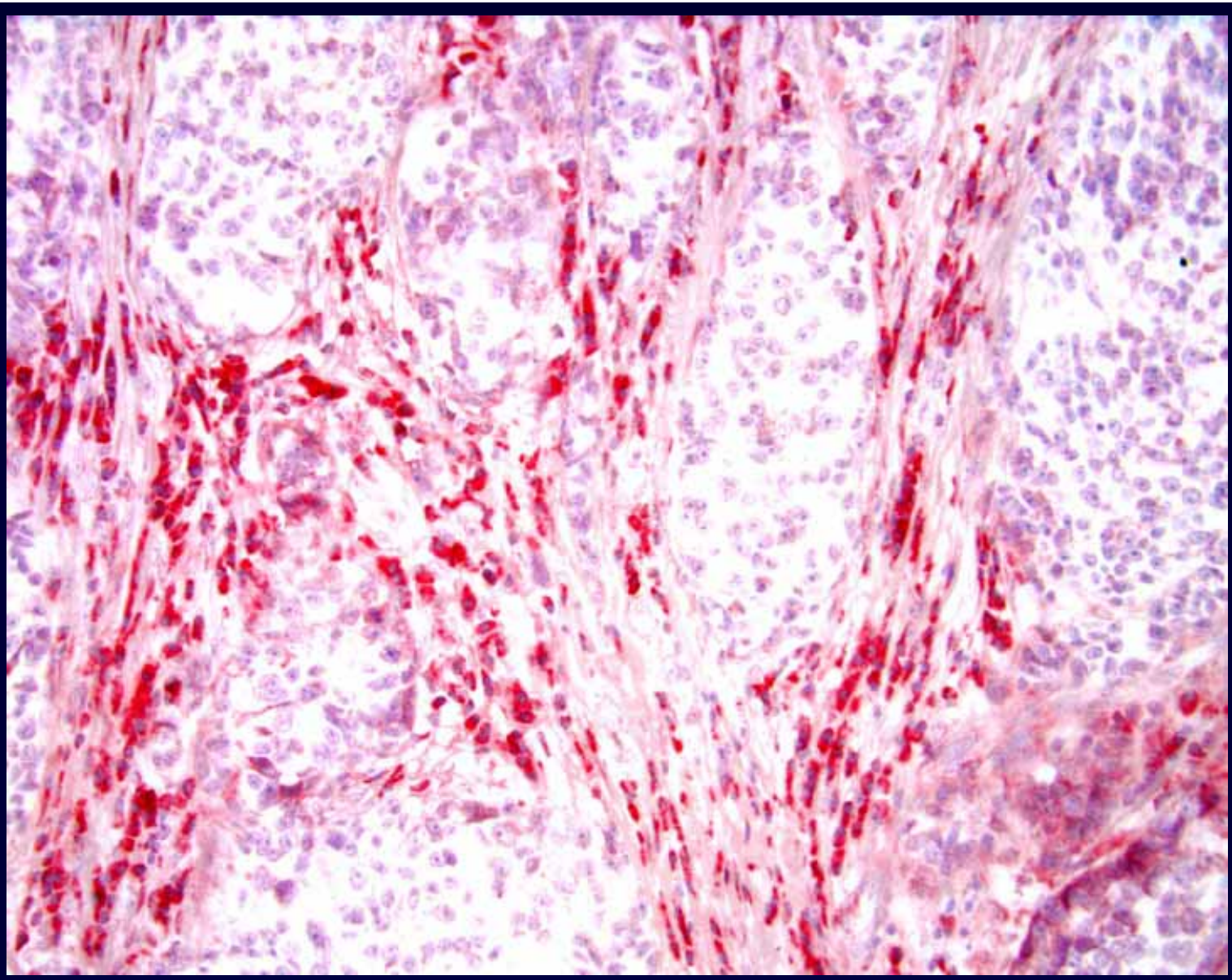
- tolerogenic DCs
- tumor-associated macrophages
- MDSCs
- endothelial cells

Potential molecular mechanisms

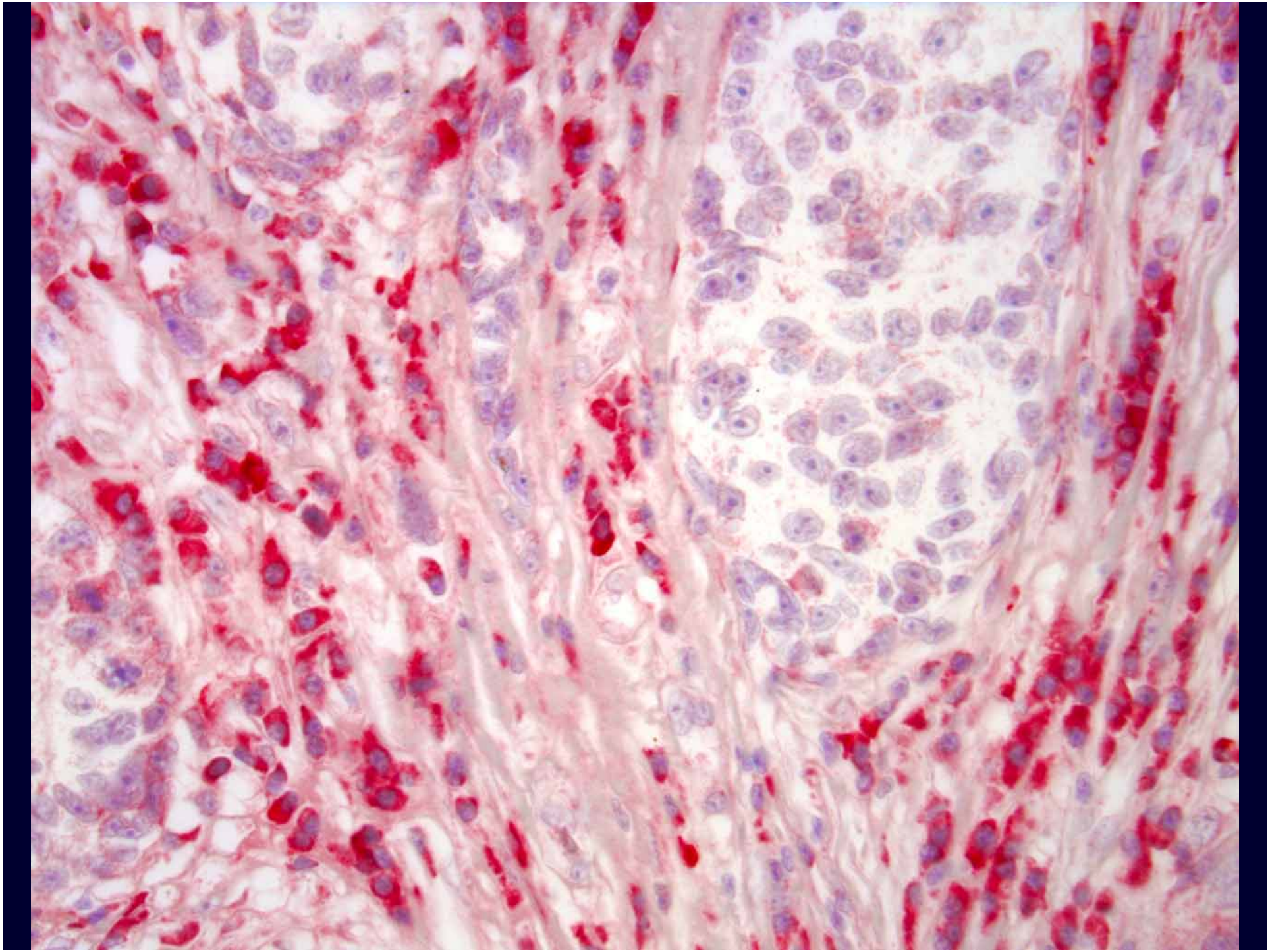
- indoleamine 2,3-dioxygenase
- arginase I
- Treg mechanisms (CTLA-4 & others)
- PD-ligands
- ROS, NO

Indoleamine 2,3-dioxygenase (IDO)

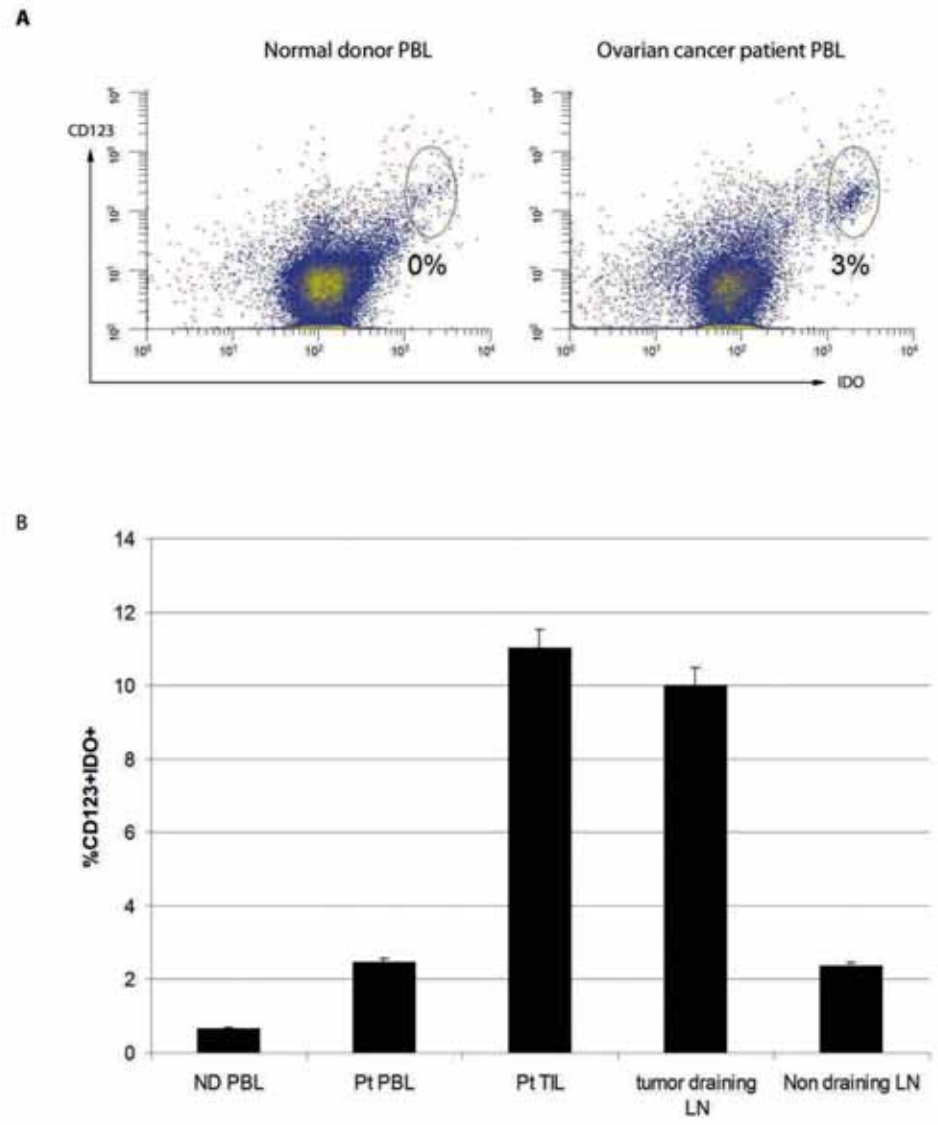
- IDO is a natural endogenous molecular mechanism of immune suppression
- IDO can create acquired peripheral tolerance *de novo*
- IDO is counter-regulatory (i.e., induced by inflammation but suppressive for immune responses)
- IDO regulates both innate and adaptive responses
  - control of local inflammation, IL-6, etc
  - suppresses effector T cells, activates Tregs



Ovarian carcinoma (sample courtesy of Dr. Kunle Odunse)



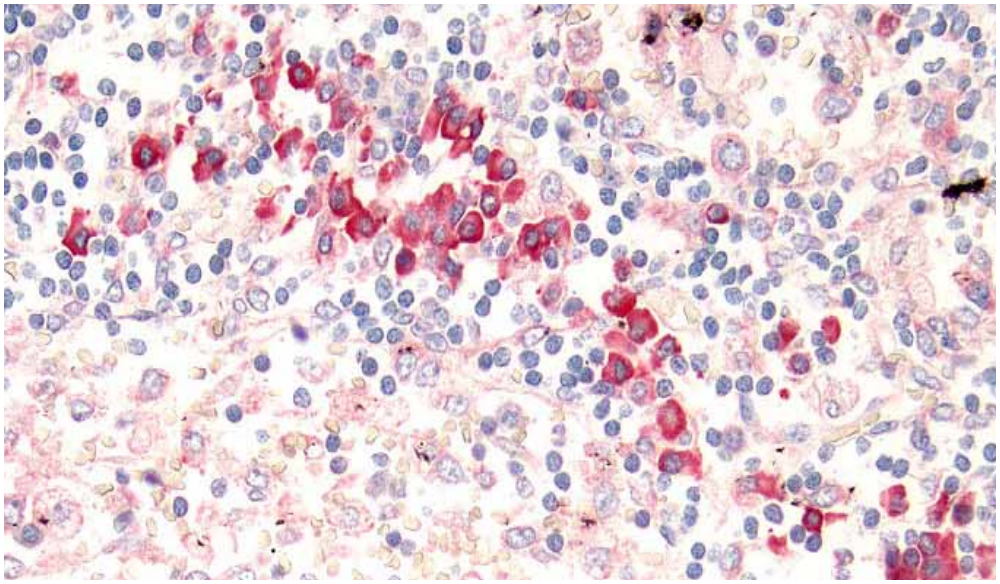
QIAN et al. Supplement figure S5



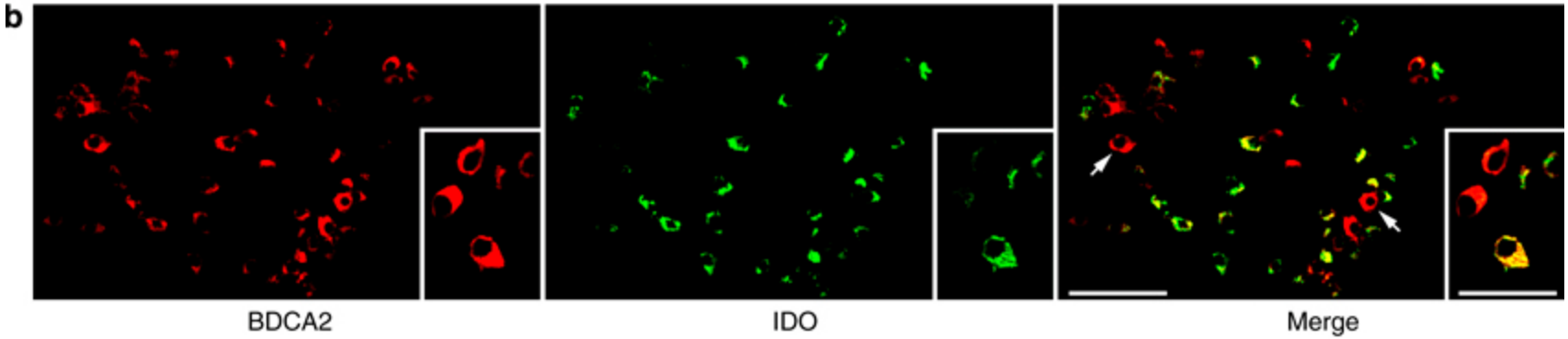
[From Qian, Odunsi and colleagues, Cancer Res 2009;69:5498–504]

IDO in melanoma sentinel LNs is expressed by plasmacytoid DCs

IDO staining
(From Munn et al, Science 2002)



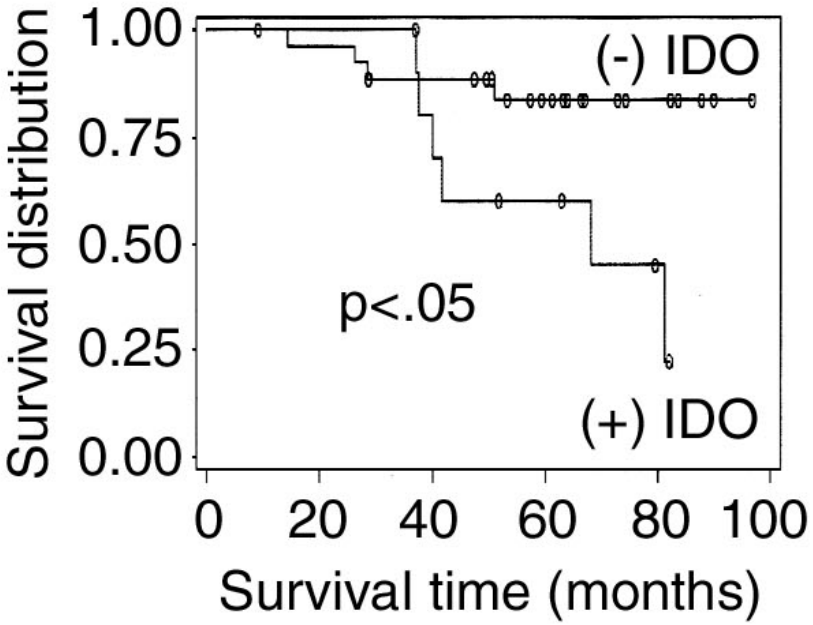
BDCA2 vs IDO
(From Gerlini et al, *J Invest Derm* 130:898, 2010)



Predictive value of abnormal IDO expression in human tumor-draining lymph nodes

- 40 patients with cutaneous malignant melanoma, no metastases
- sentinel lymph node obtained at time of initial diagnosis

- in collaboration with Scott Antonia at Moffitt Cancer Center

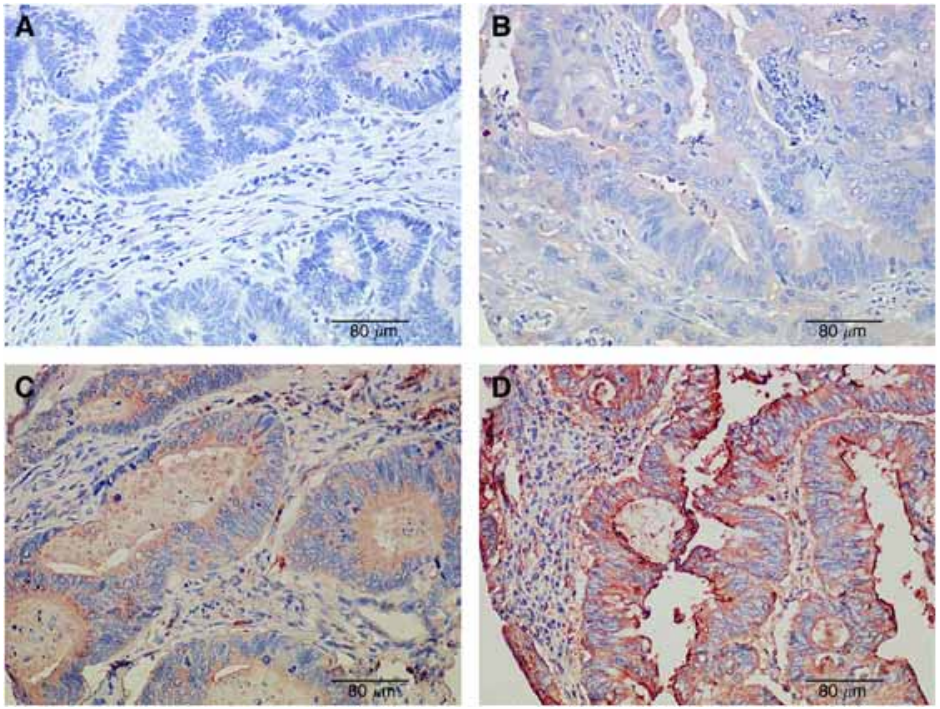


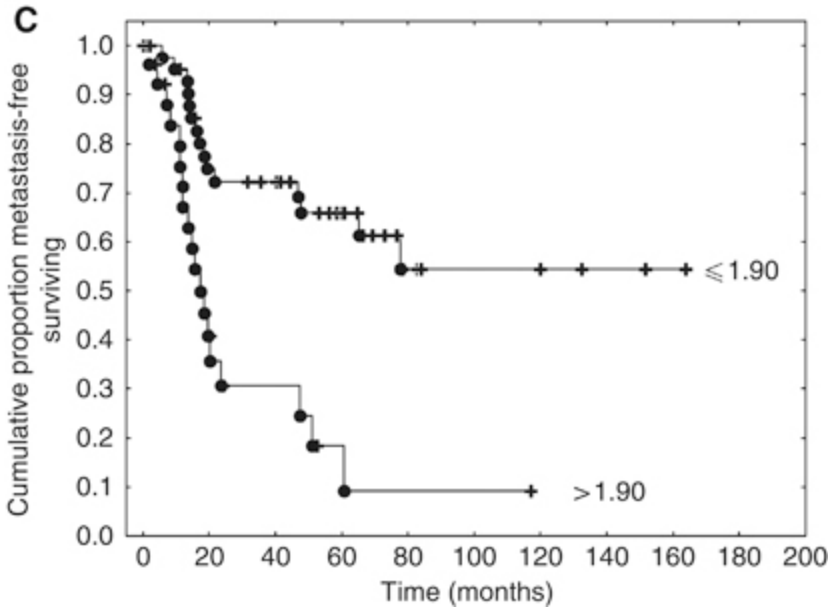
normal pattern
n=29

abnormal IDO+
n=11

from Munn et al, *J. Clin. Invest.*, 2004

Prognostic significance of IDO in colorectal carcinoma

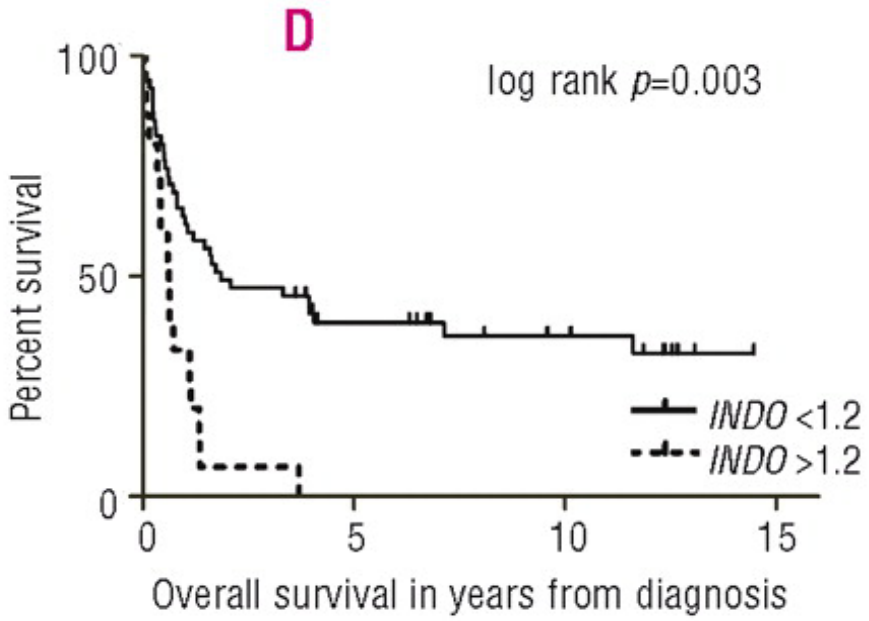




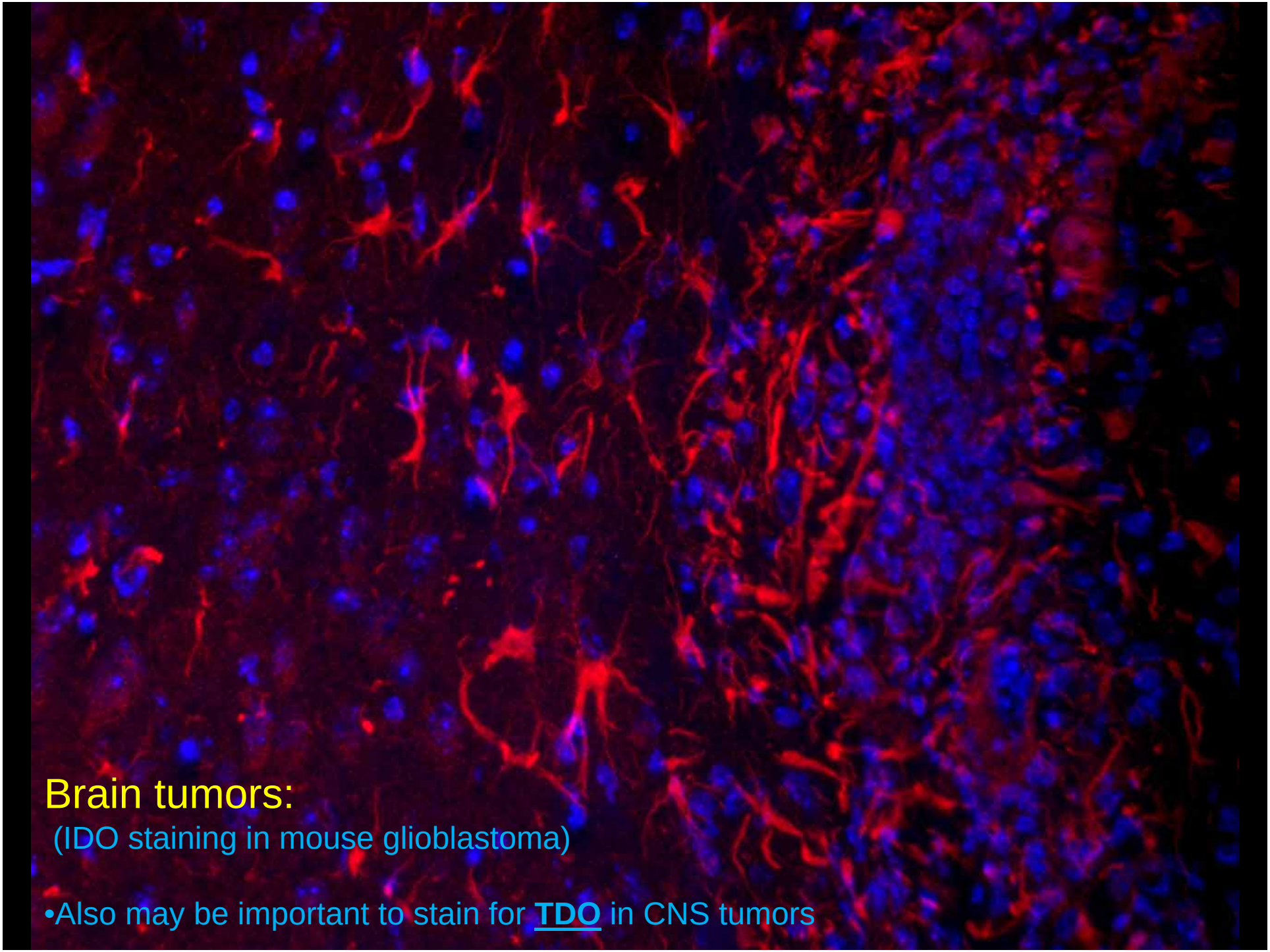
From Ferdinande et al **Clinicopathological significance of indoleamine 2,3-dioxygenase 1 expression in colorectal cancer**

British Journal of Cancer (2012) **106**, 141–147

INDO expression by microarray and by qPCR correlated to clinical outcome in patients with adult AML



Chamuleau, M. E.D. et al. Haematologica 2008;93:1894-1898

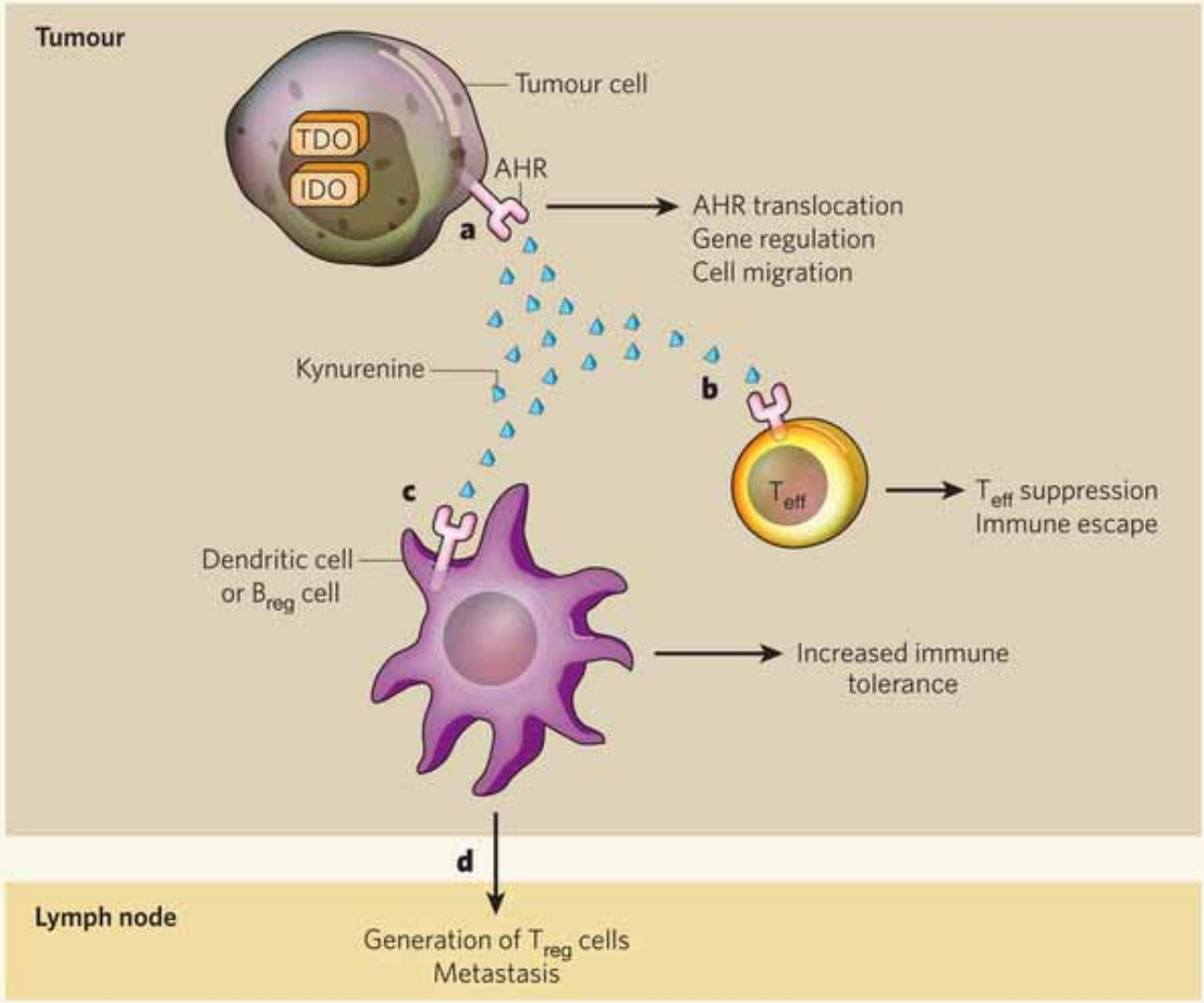


Brain tumors:

(IDO staining in mouse glioblastoma)

•Also may be important to stain for **TDO** in CNS tumors

IDO and TDO in glioblastoma

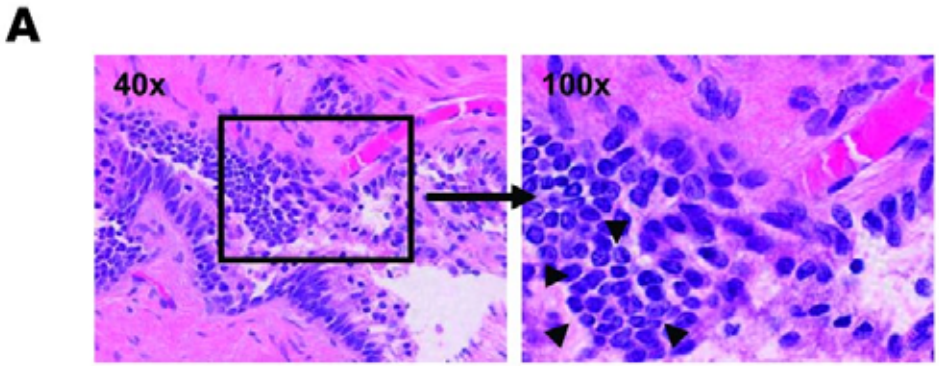


from Prendergast, Nature N&V on
Opitz, Platten and colleagues, Nature 478, 197–203 (2011)

How does IDO get turned on?

DCs in prostate tumors express FOXO3, which induces IDO expression and a suppressive DC phenotype

From Watkins et al, *J Clin Invest.* 2011;121(4):1361–1372 (Andy Hurwitz lab).



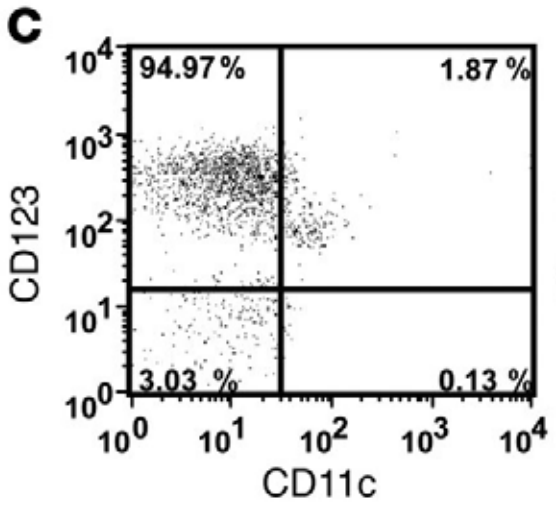


Table 2

Human TADCs have elevated expression of genes associated with tolerance

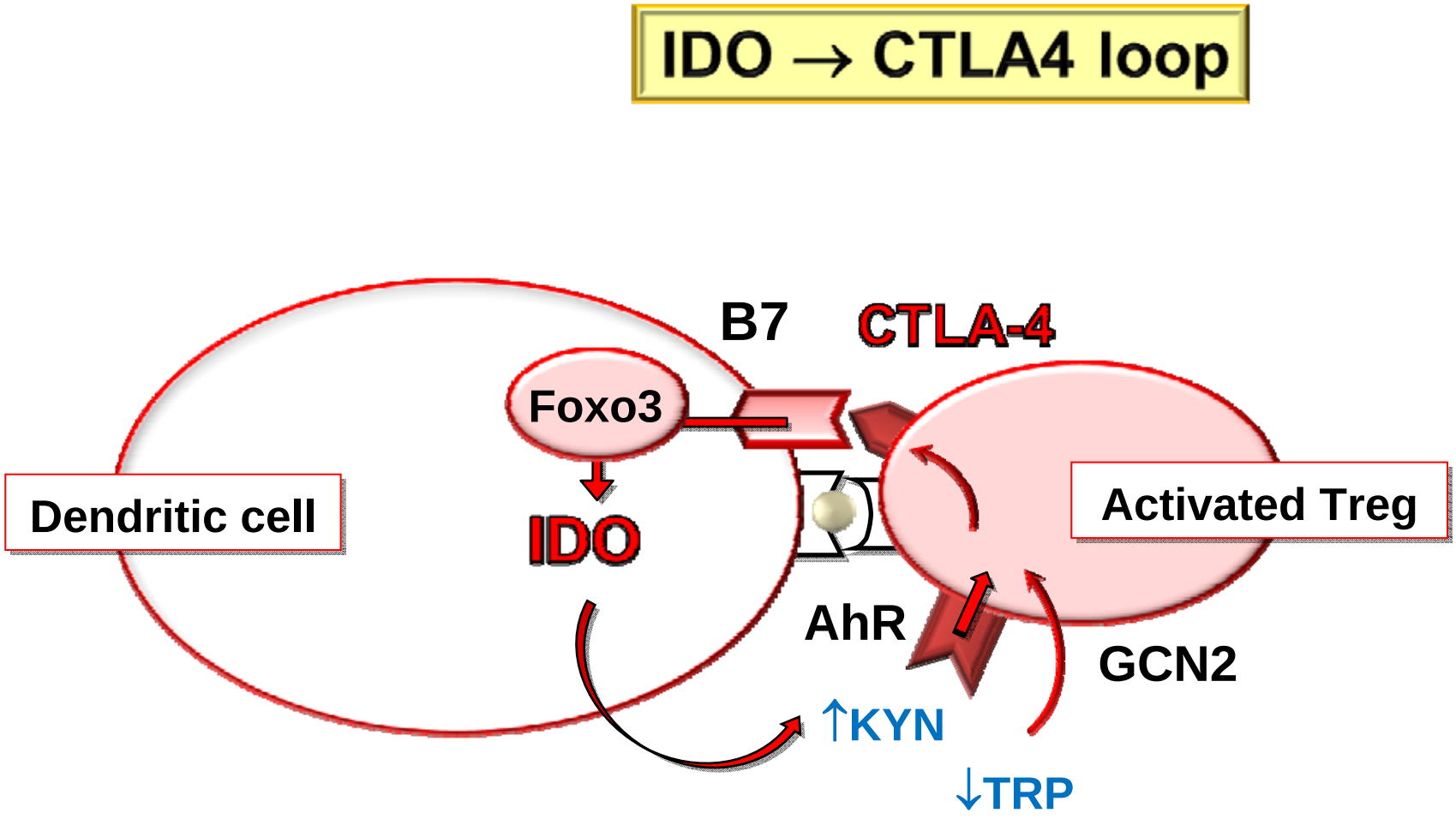
Gene	Fold change tumor/non-tumor
FASLG	5.2
IDO1	7.3
CD274	3.1
STAT3	5.1
FOXO3	6.9

RNA was isolated and hybridized to Affymetrix Human Gene 1.0 ST arrays. Fold change values have corresponding *P* values of less than 0.00001 (ANOVA). Data are representative of 5 independent microarrays for tumor and non-tumor biopsies.

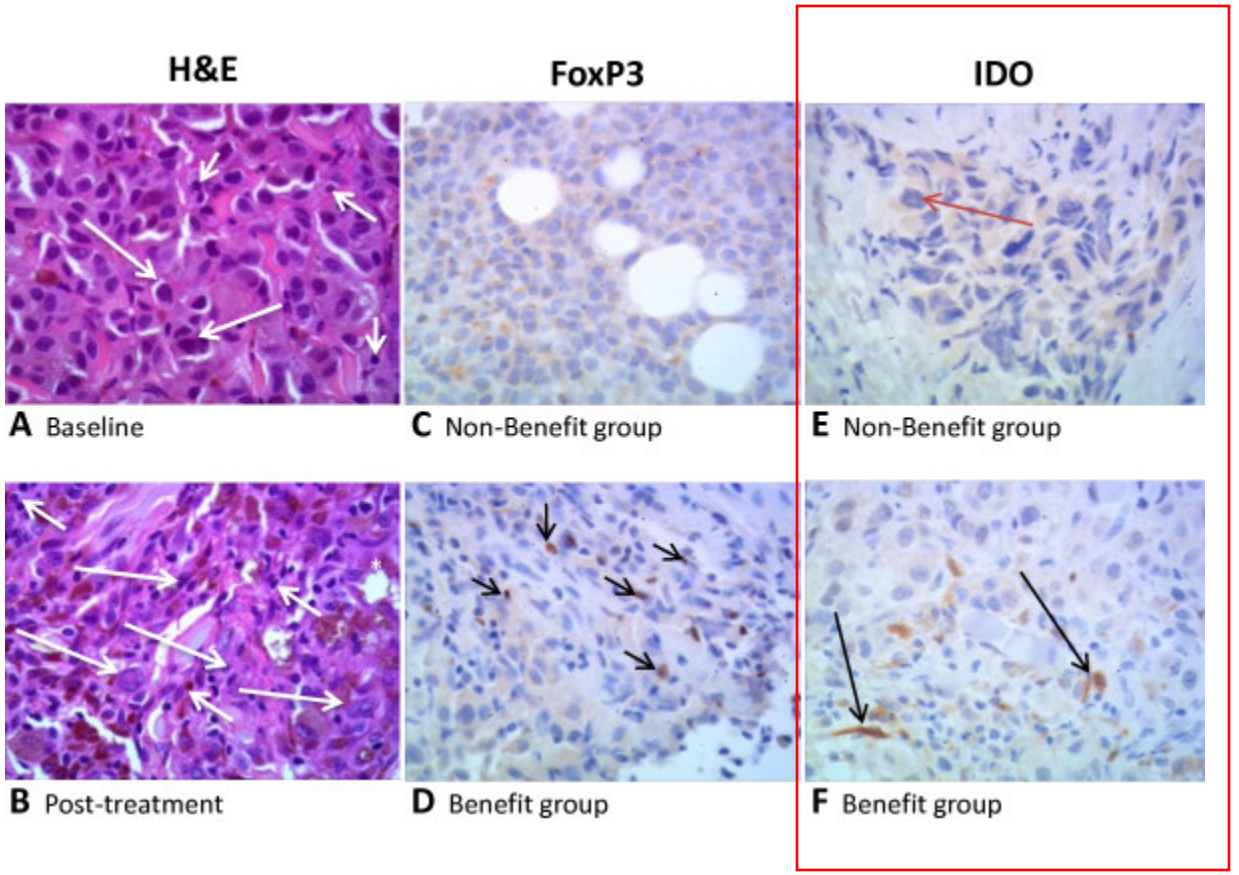
Technical caveats in IDO staining

- different IDO antibodies may give different staining patterns (validated antibodies are crucial)
- tumor IDO may stain differently than host IDO (mutation, alternant splicing, etc)
- antibodies may cross-react with IDO1 and IDO2
- some epitopes are labile in room air (need to stain fresh-cut sections)
- most IDO antibodies need antigen retrieval for paraffin

Links between IDO, CTLA-4 and Tregs



High expression of IDO and Foxp3 at baseline may be a predictor of clinical response to ipilimumab



from Hamid *et al. Journal of Translational Medicine* 2011 **9**:204

Why does the presence of tumor automatically create a tolerogenic microenvironment?

•Virchow said that a tumor was a “wound that never heals” …

- constant tissue remodeling
- new blood-vessel formation
- chronic, macrophage-driven inflammation

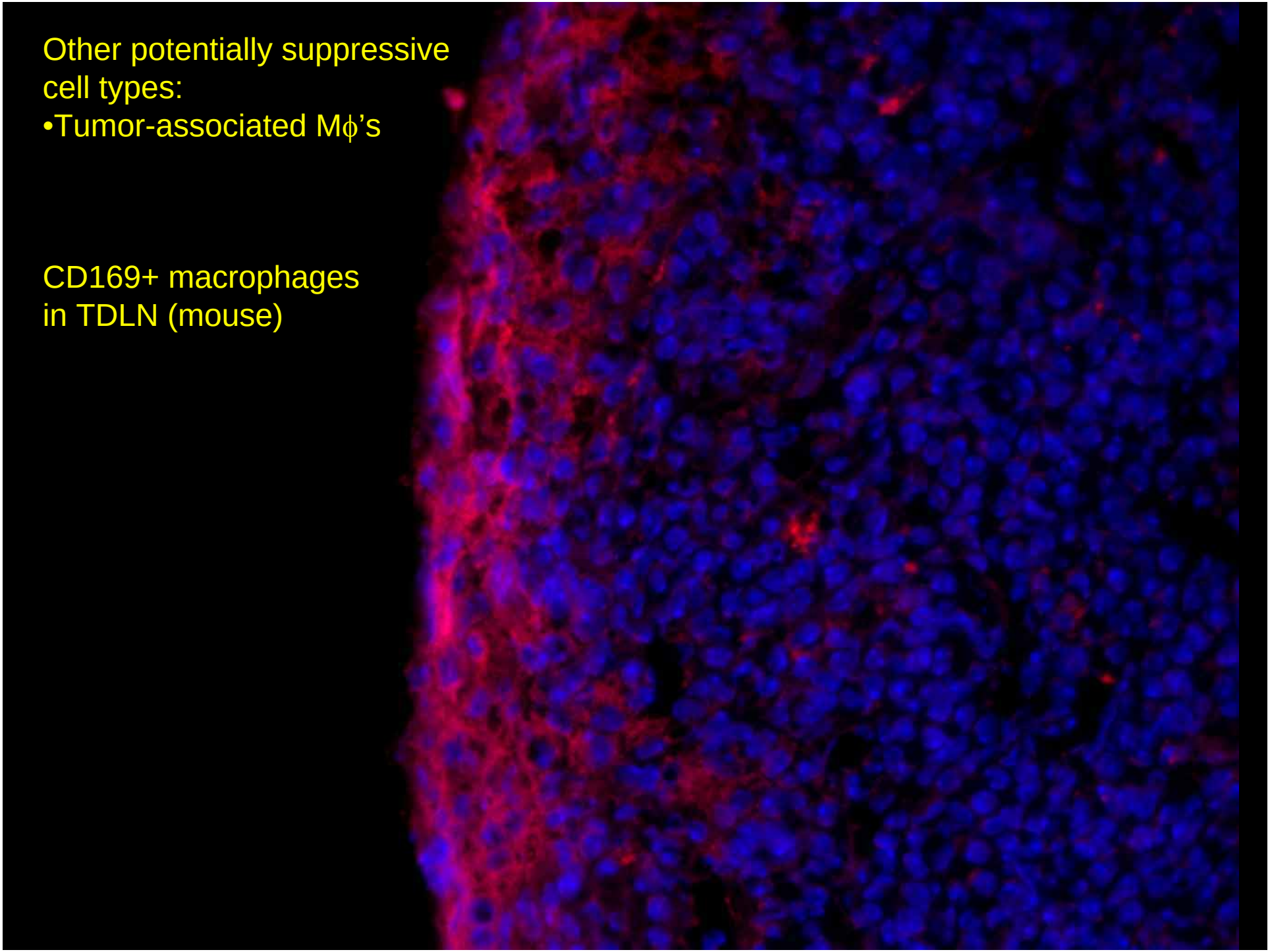
•all healing wounds (if sterile) may be an inherently tolerogenic milieu for T cells

- tissue remodeling releases many self antigens – may need active tolerance induction
- the local milieu is rich in TGFβ, VEGF (tolerogenic cytokines)
- macrophage-mediated inflammation may suppress rather than activate T cells

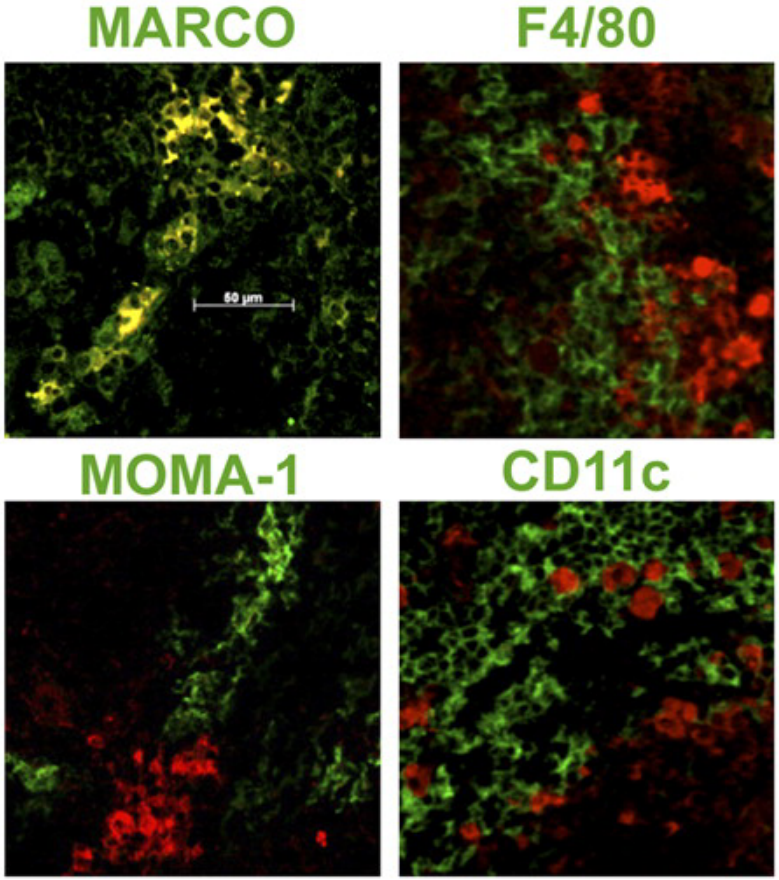
Other potentially suppressive cell types:

- Tumor-associated Mϕ's

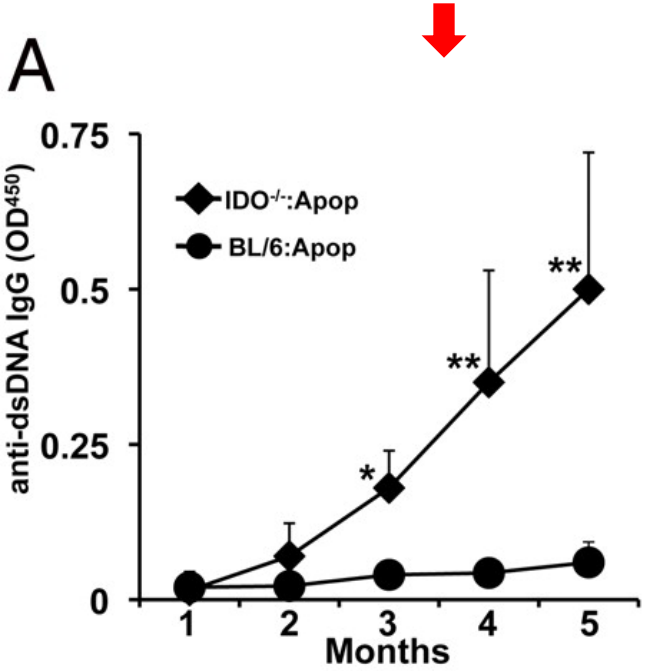
CD169+ macrophages in TDLN (mouse)



IDO helps maintain tolerance to self antigens derived from apoptotic cells

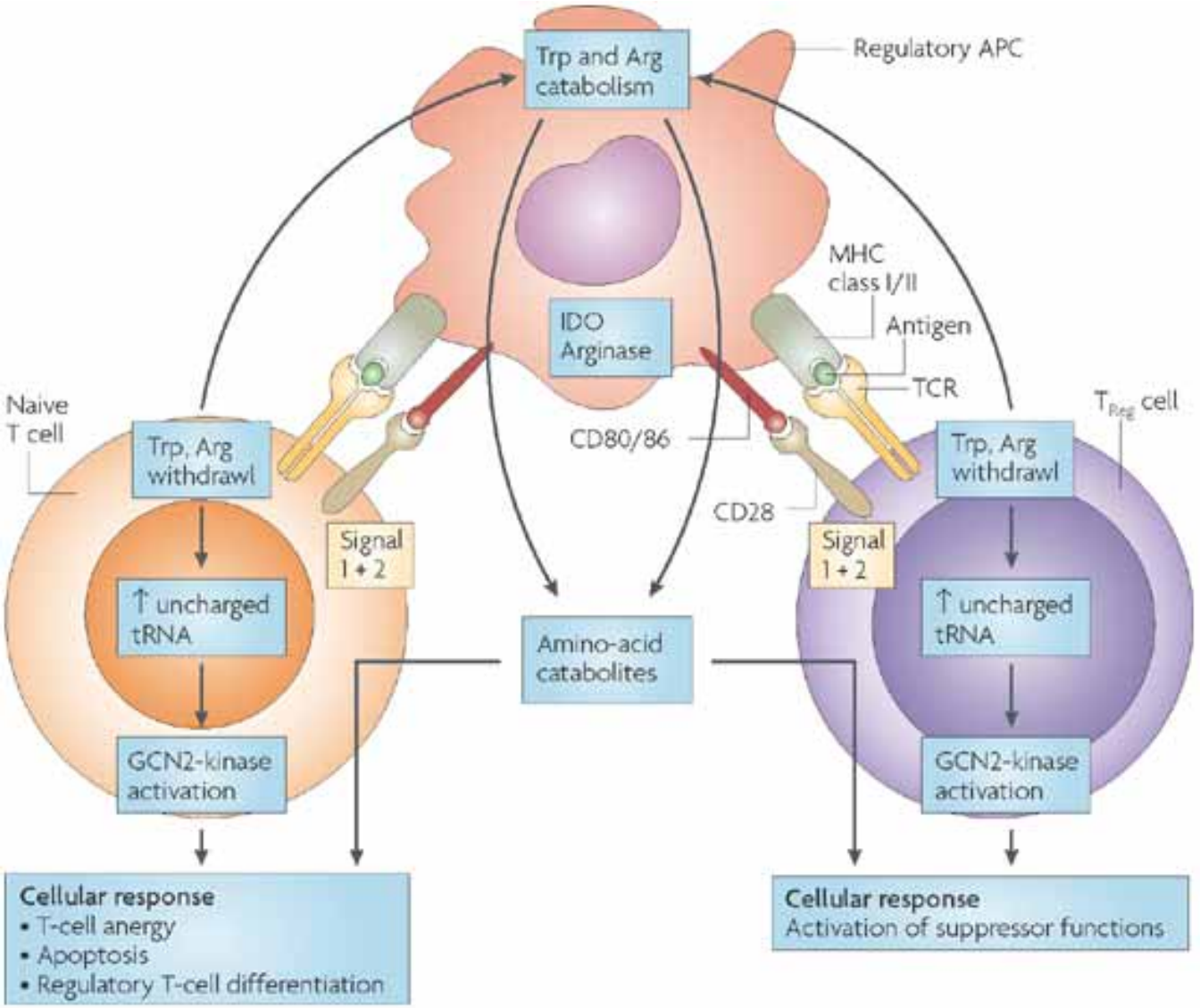


IDO-KO mice develop lupus when challenged with apoptotic cells



From Tracy McGaha lab
Ravishankar B et al. PNAS 2012

IDO can regulate adaptive T cell immune responses

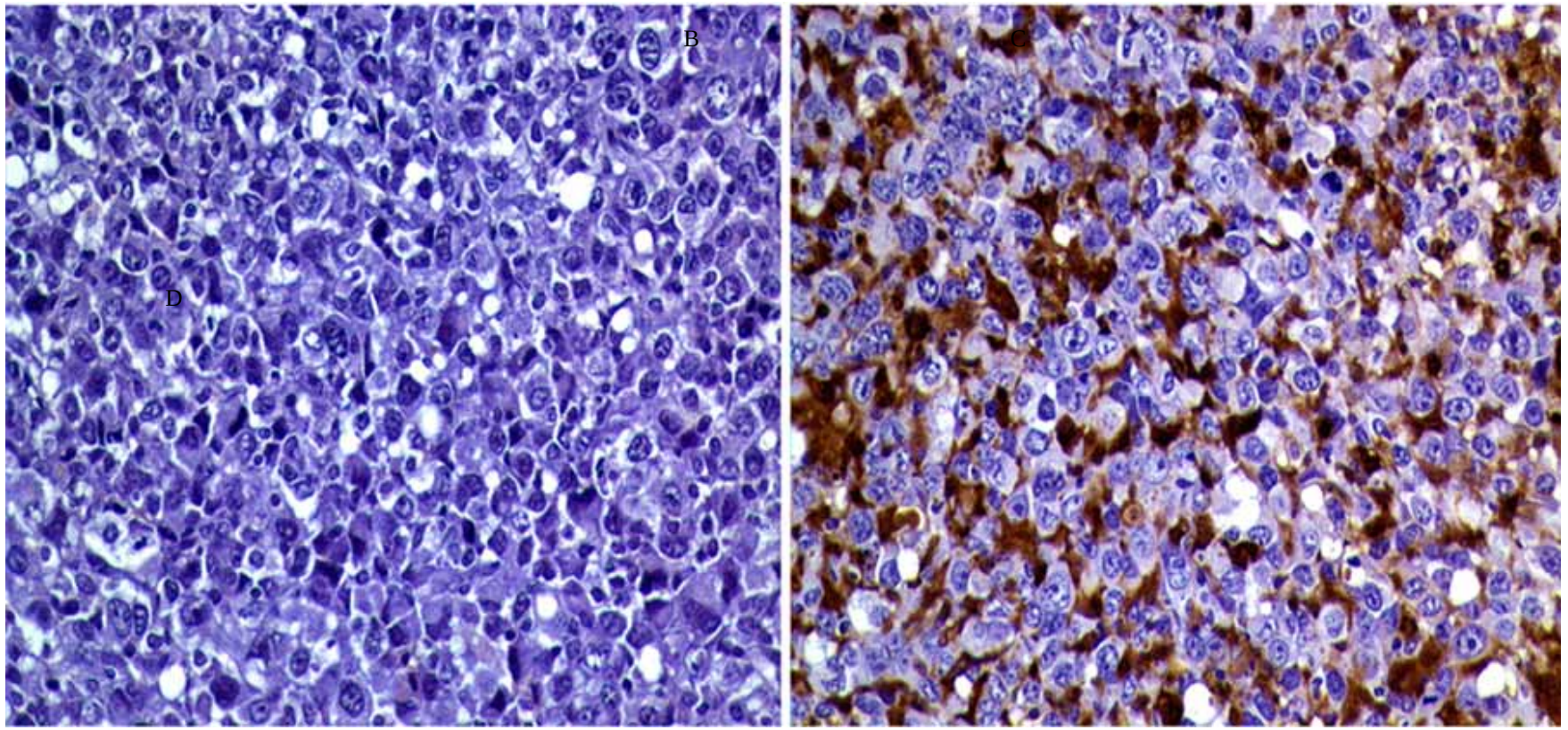


from Mellor & Munn, **Nature Reviews** | Immunology, 2008

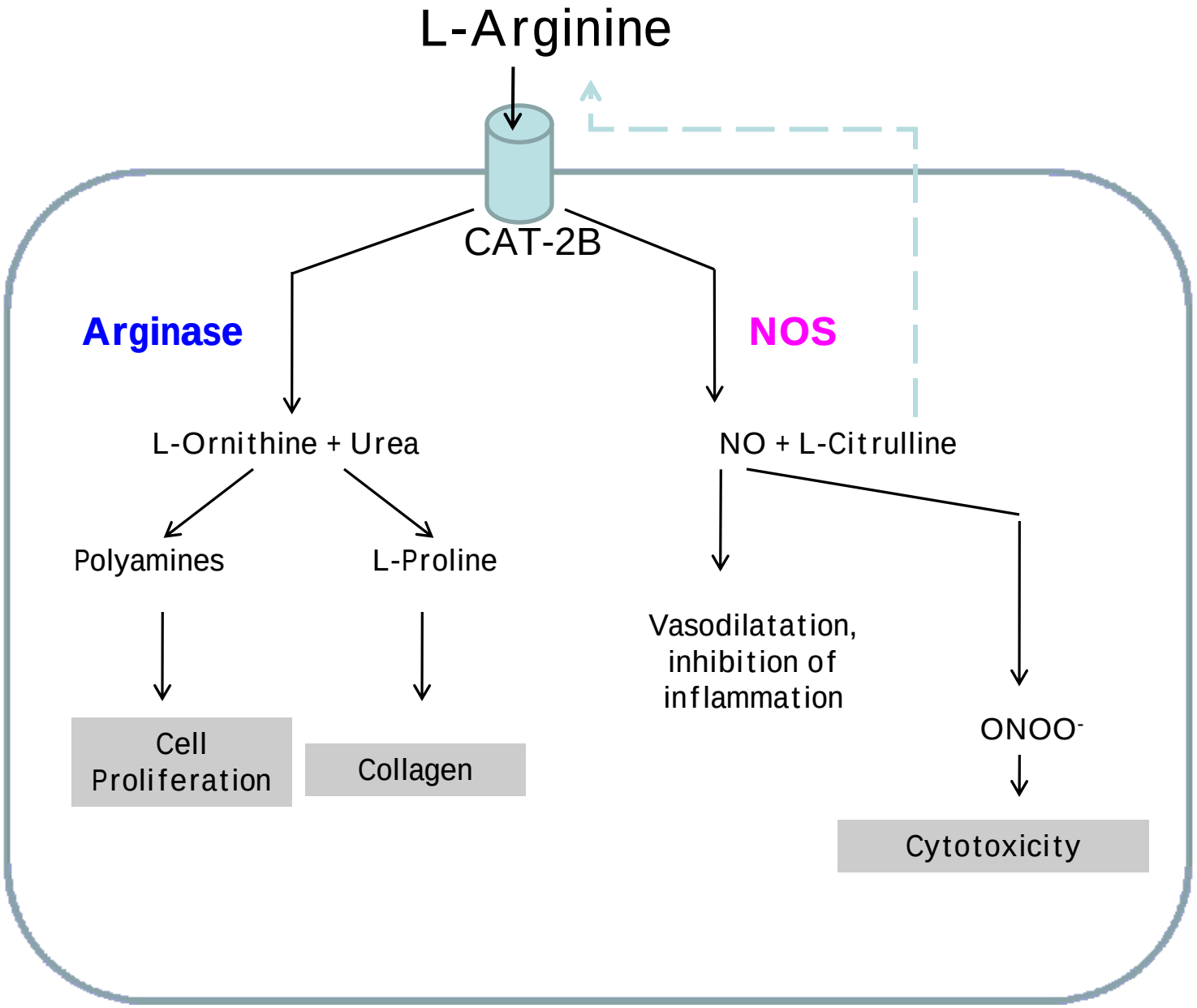
Arginase I Expression in 3LL Tumor

Isotype

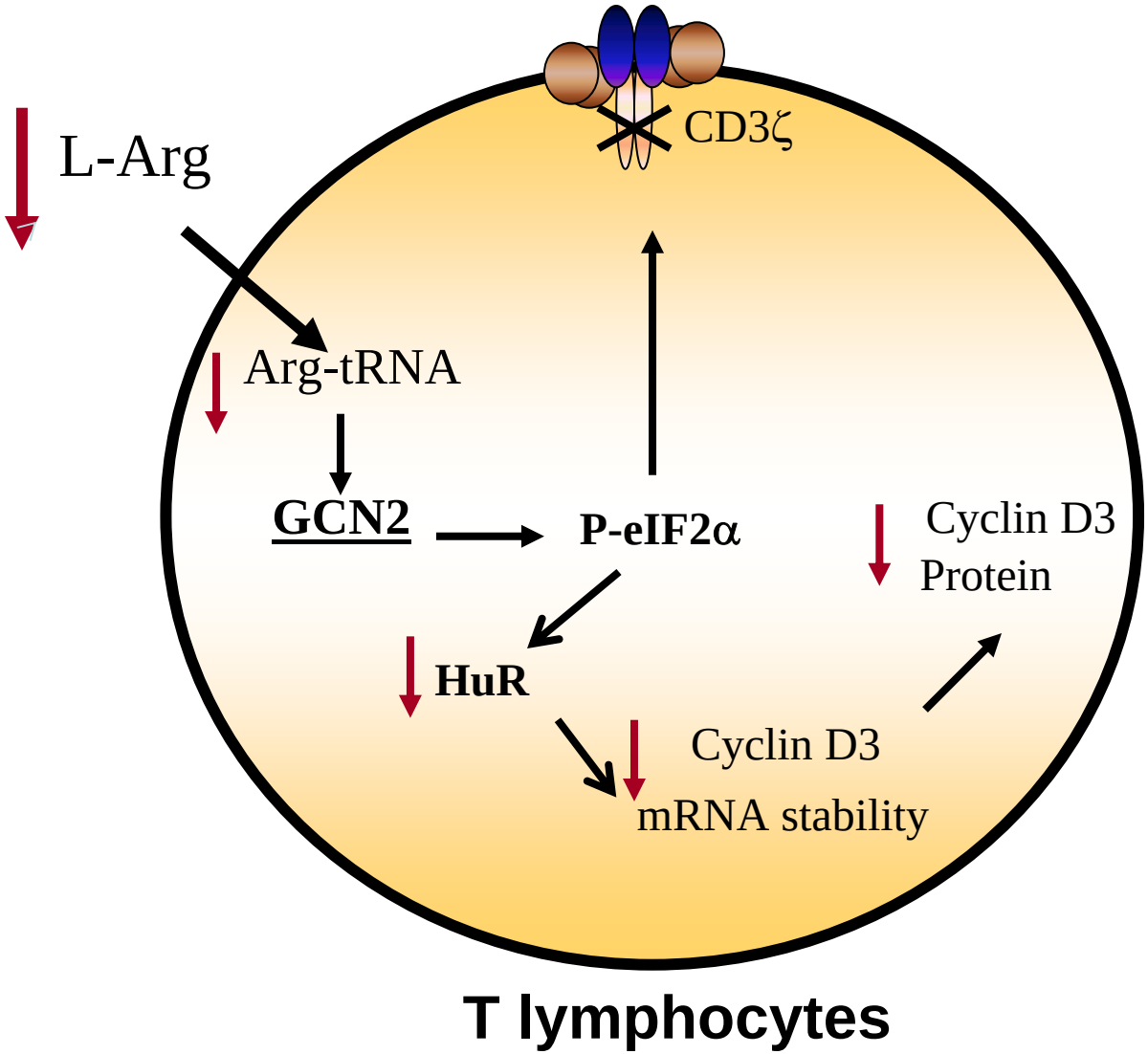
Arginase I



Courtesy of Dr. Augusto Ochoa's lab

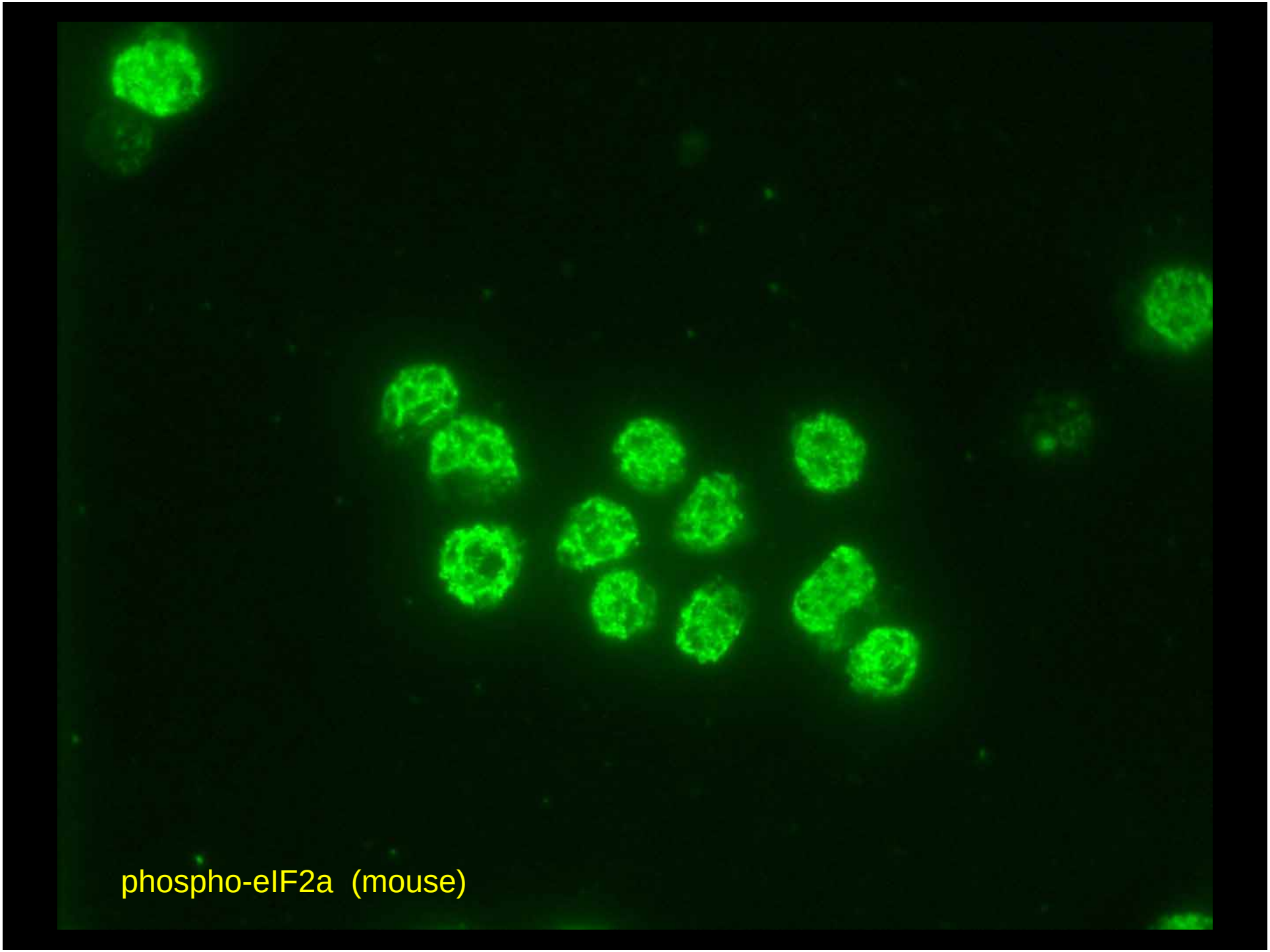


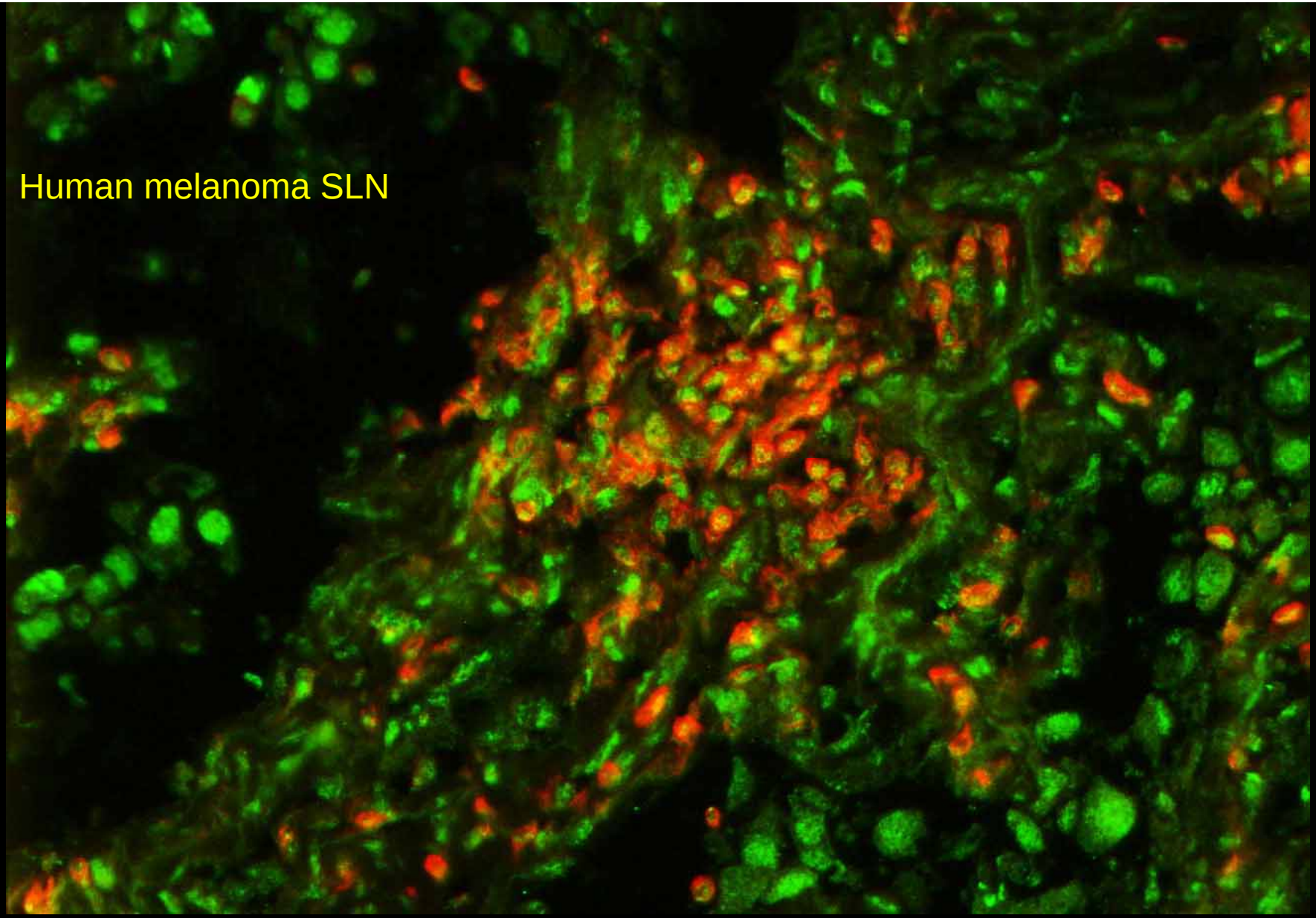
Courtesy of Dr. Augusto Ochoa's lab



Courtesy of Dr. Augusto Ochoa's lab

Rodriguez et al. *Immunol Rev*. 2008. **222**:180


phospho-eIF2a (mouse)


Human melanoma SLN
CD3
Gadd153 (CHOP)

Conclusions and future directions

- IDO and Arginase are two potential stroma-derived suppressor mechanisms in tumors and TDLNs
- These mechanisms activate a shared pathway of amino-acid depletion (eIF2a > GCN2 > CHOP)
- They may link mechanistically to other pathways (e.g., CTLA-4, Tregs, mTOR)


GHSU Cancer Research Center