Novel ‘elements’ of immune suppression within the tumor microenvironment

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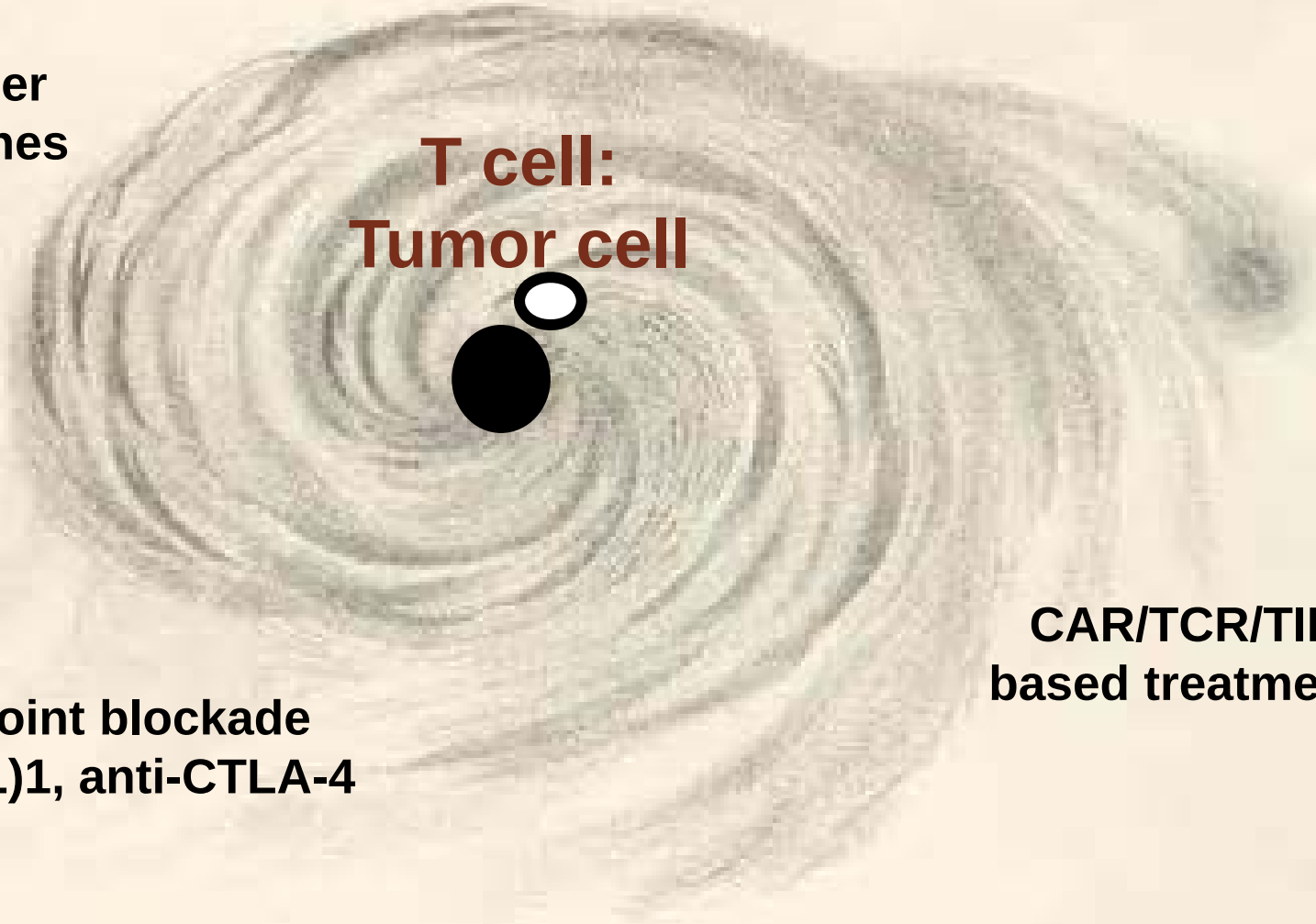
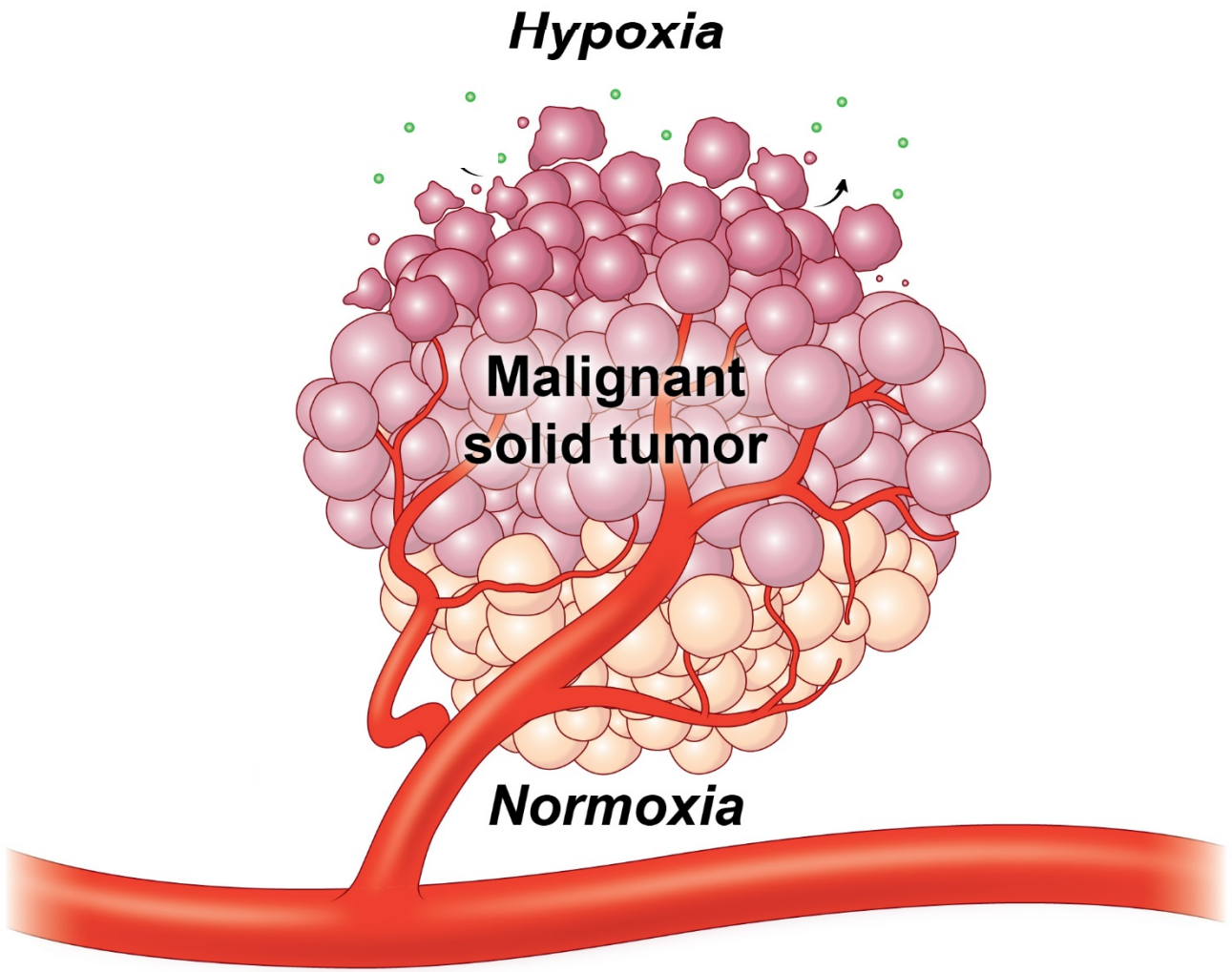
At the center of the galaxy of increasingly successful cancer immunotherapies



Understanding the tumor microenvironment during initiation and growth of tumor



Metastasis is the cause of >90% of all cancer deaths

- **Successful metastasis requires evasion of immunity at the secondary site**
- **The lung is a common site of metastasis for many cancers**
- **Vascular architecture has historically explained cancer's predisposition to disseminate to the lung**

Hypothesis

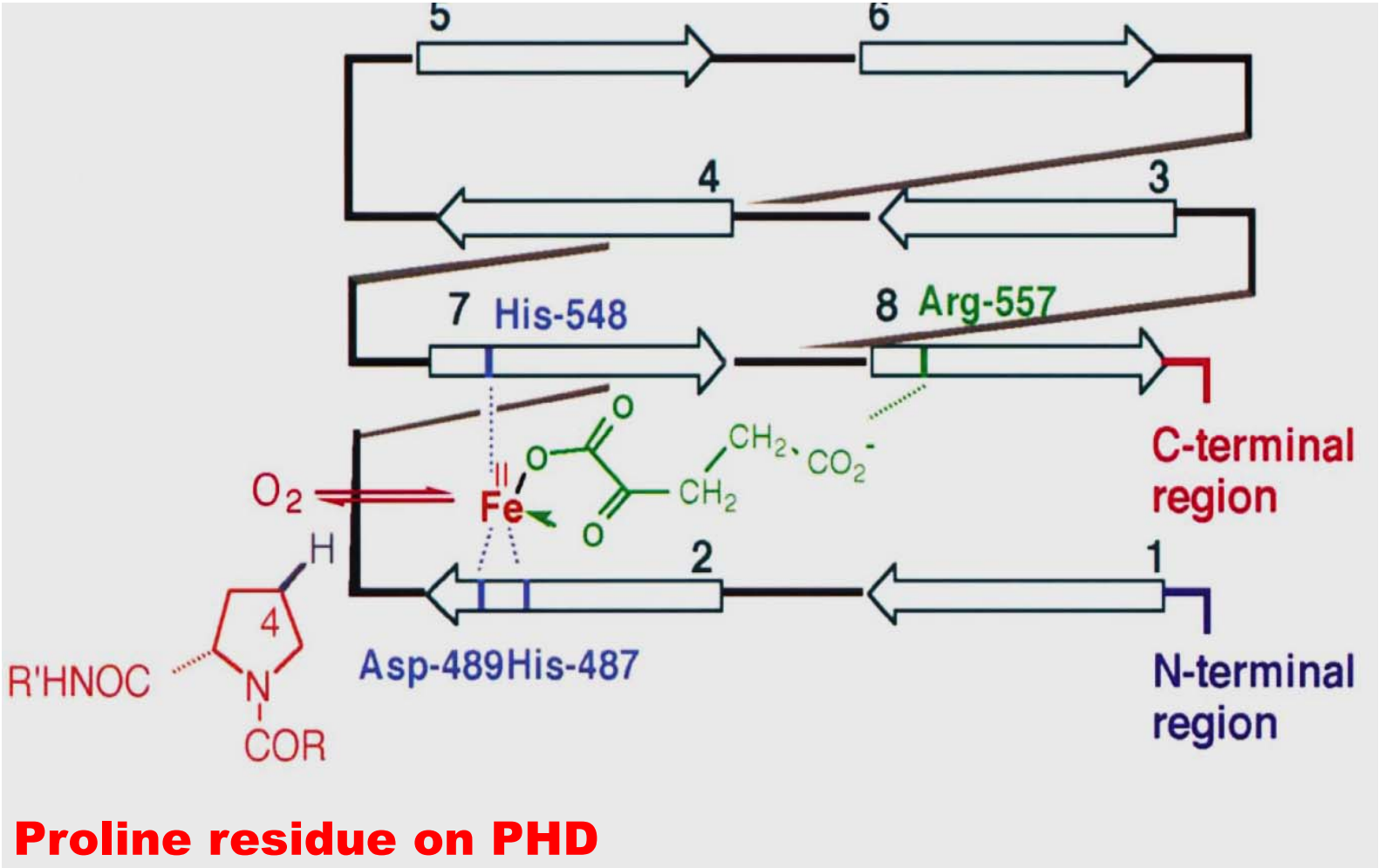
Site-specific environmental factors – such as Oxygen – help establish immunologically permissive sites for metastasis

How do anti-tumor T cells ‘sense’ Oxygen, and does this affect their function?

T cells use prolyl hydroxylase domain (PHD) containing proteins

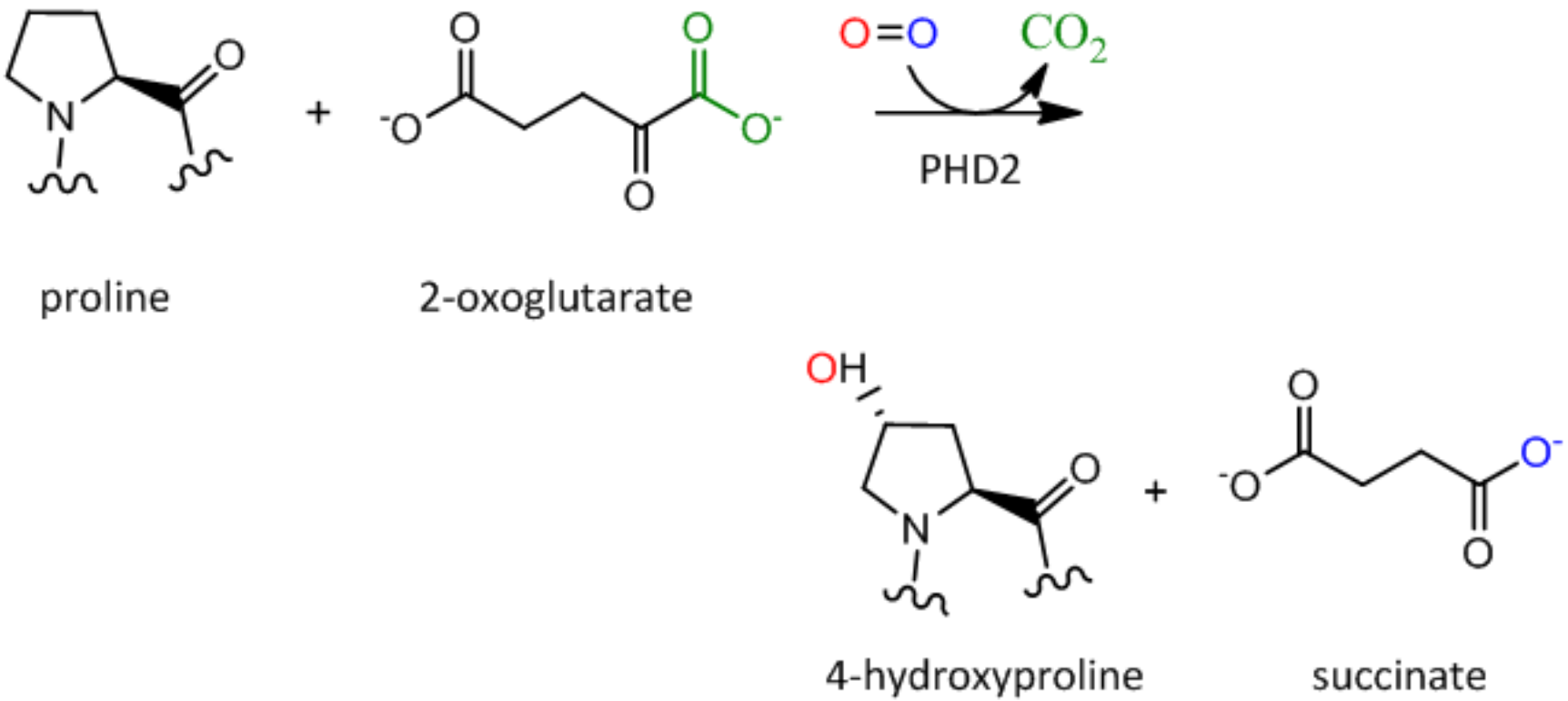
These dioxygenase (O_2) sensors containing non-heme–binding iron (Fe) that catalyzes the hydroxylation of proline residues

PHD proteins hydroxylate proline residues

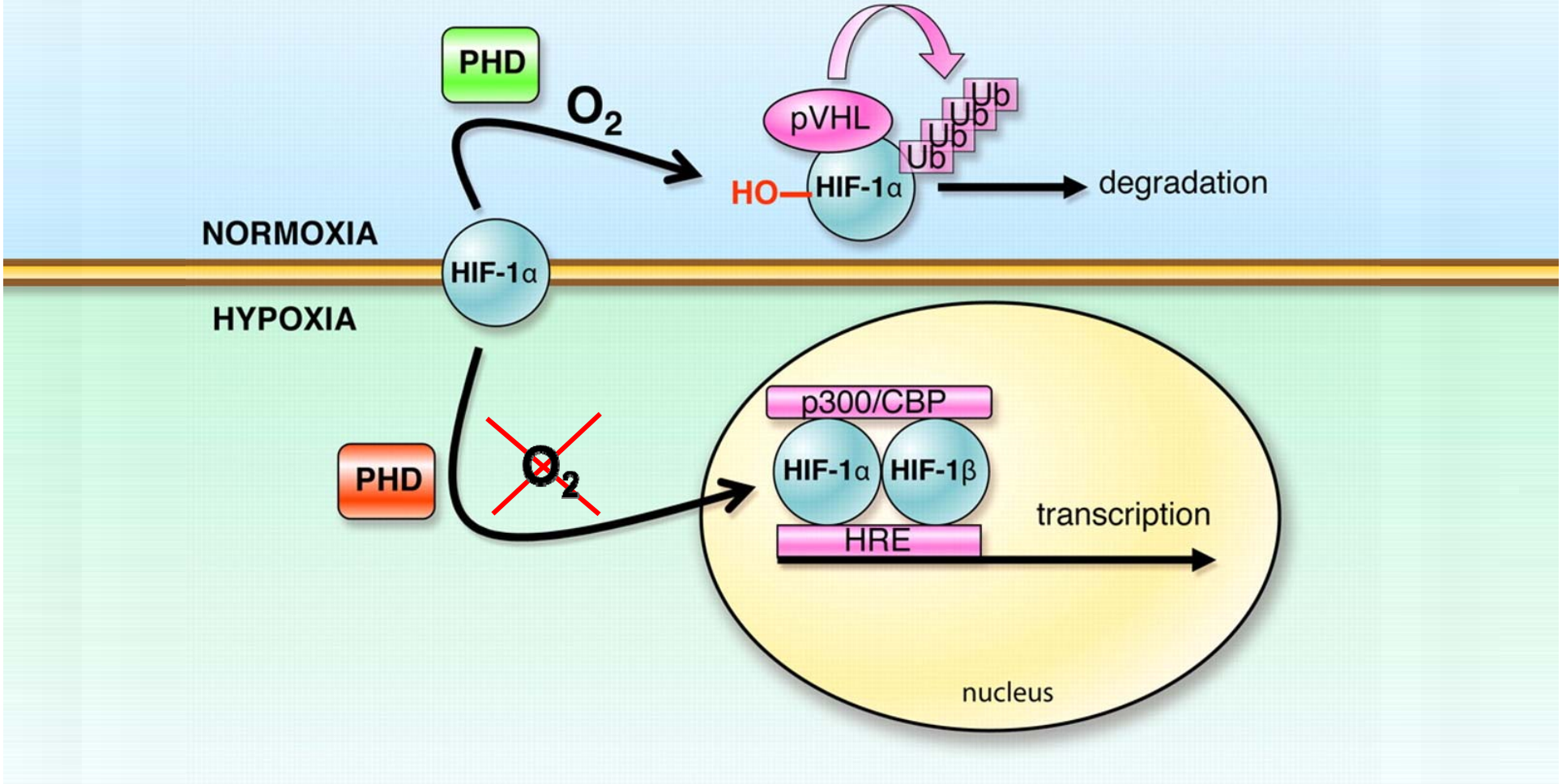


Proline residue on PHD target protein

The PHD enzyme splits dioxygen into hydroxylated proline and succinate

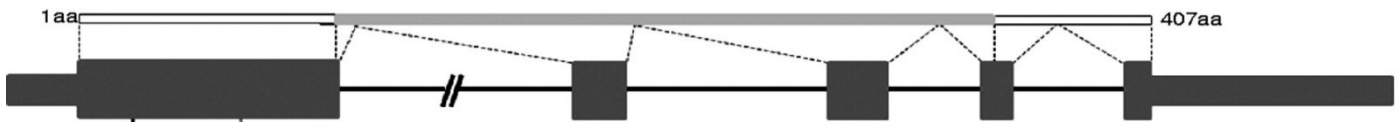


PHD enzymes degrade hypoxia inducible factor (HIF) – and possibly other proteins – in the presence of oxygen

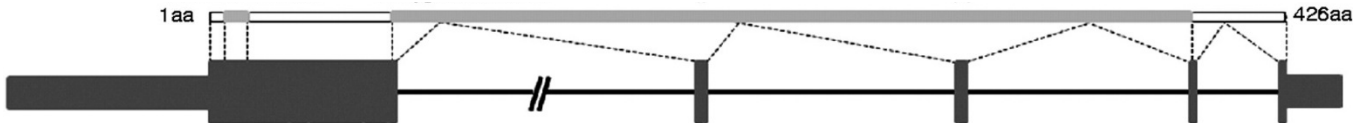


EGLN genes encoding PHD oxygen sensors are located at three different sites in human genome

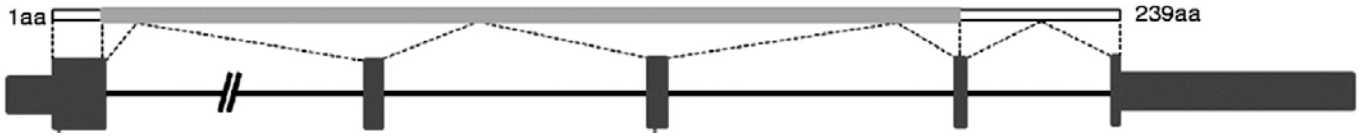
PHD1 (EGLN2): 19q13.2



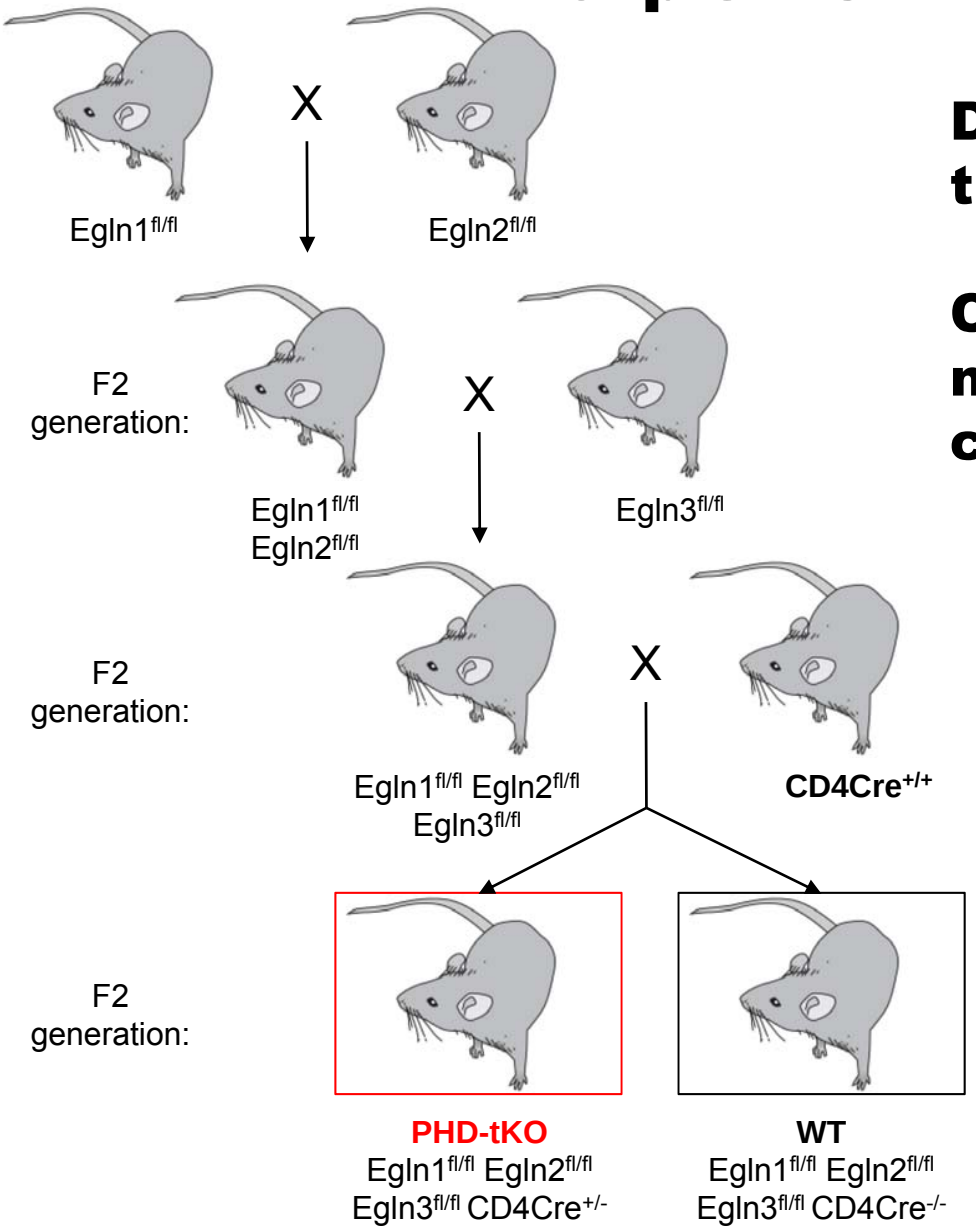
PHD2 (EGLN1): 1q42.1



PHD3 (EGLN3): 14q13.1



Studying T cell-intrinsic oxygen sensing required a triple KO mouse

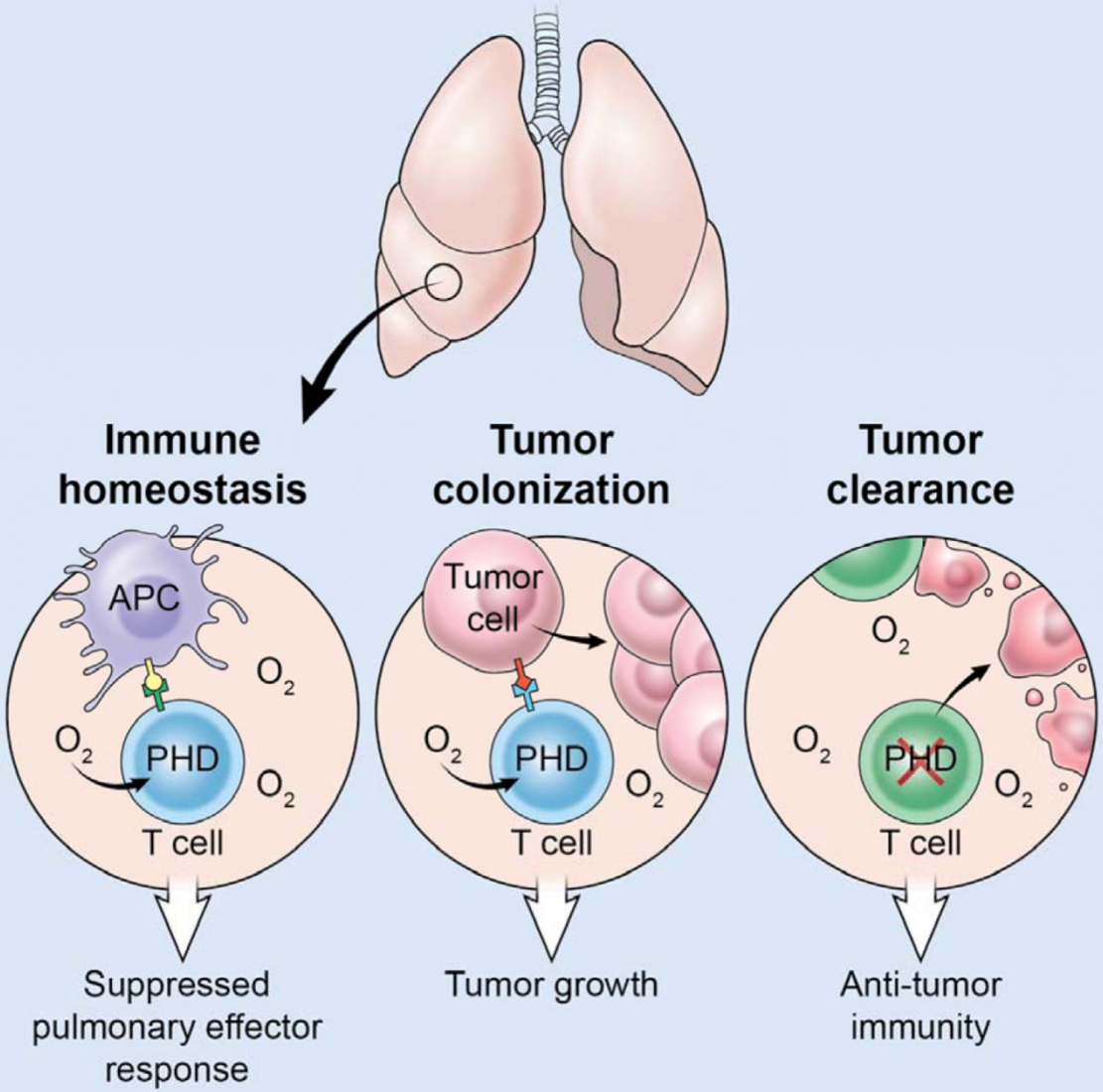


Does oxygen affect anti-tumor immunity?

Can oxygen sensing be manipulated to improve cancer immunotherapy?

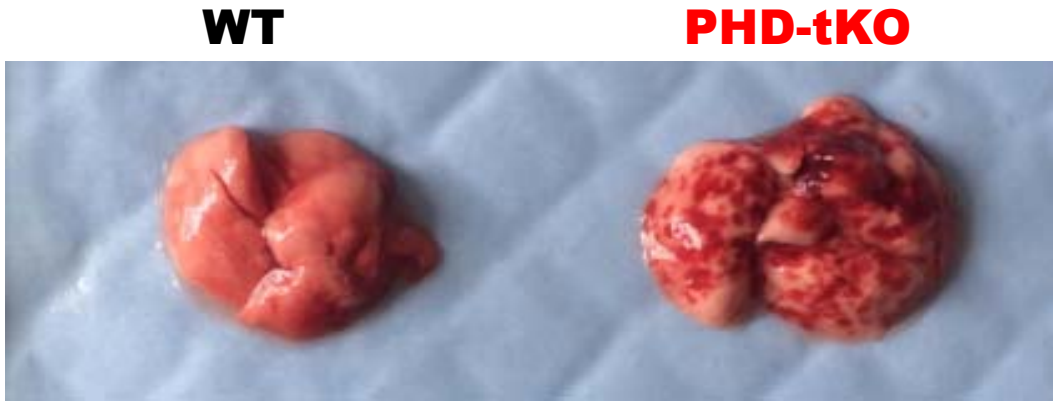
D Clever, Cell, August 25, 2016

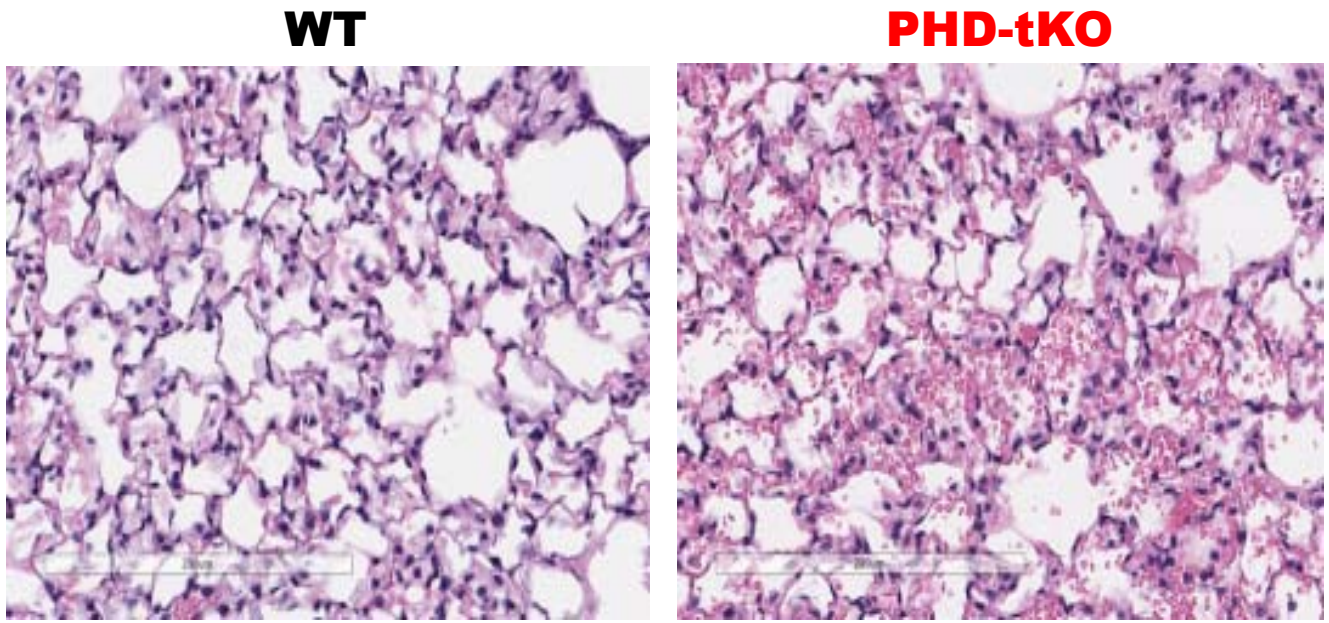
Oxygen Sensing by T Cells Establishes an Immunologically Tolerant Metastatic Niche



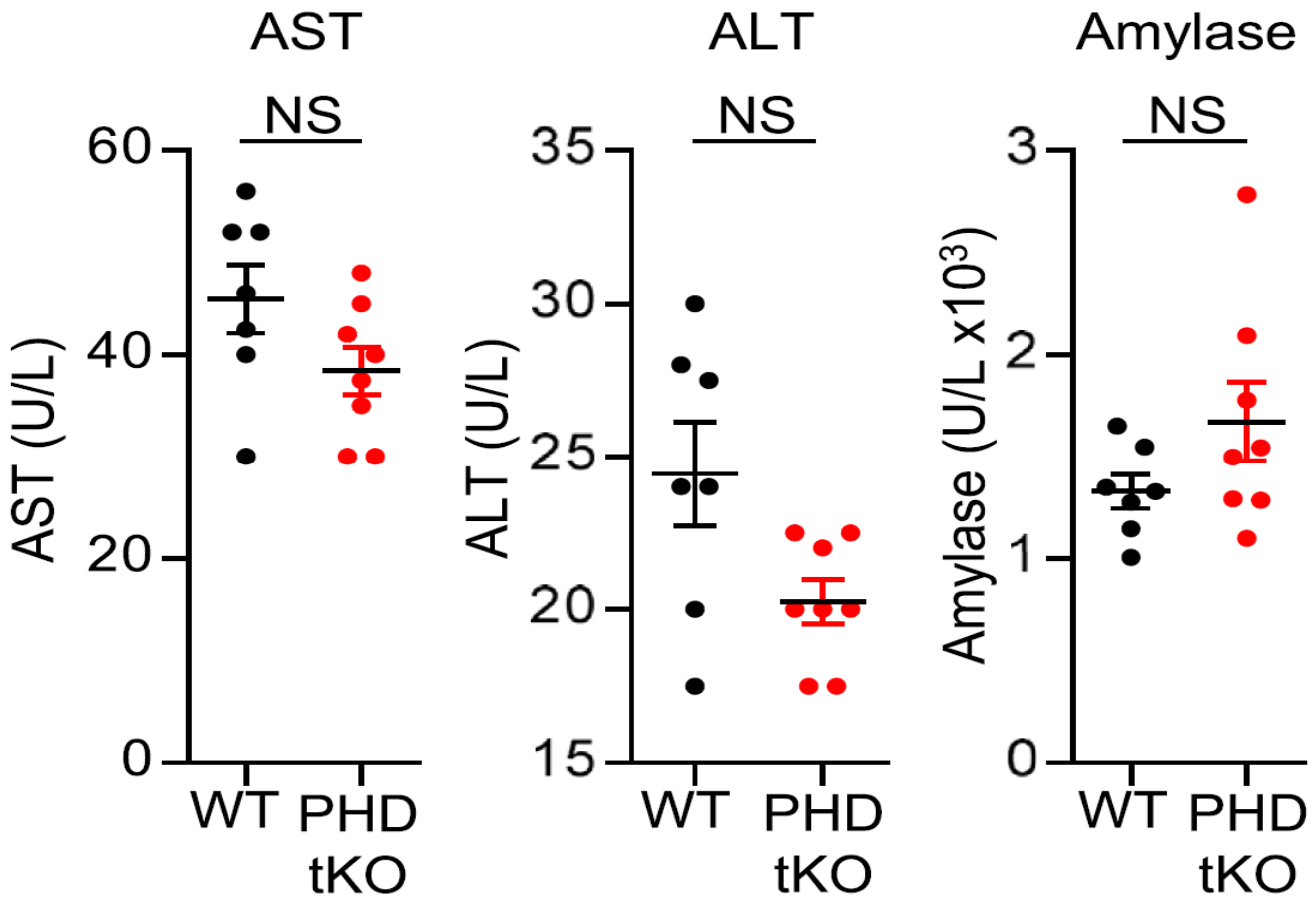
D Clever, R Roychoudhuri . . . A Goldrath, Y Belkaid and NP Restifo, Cell, August 25, 2016

T-cell intrinsic PHD proteins suppress spontaneous pulmonary inflammation

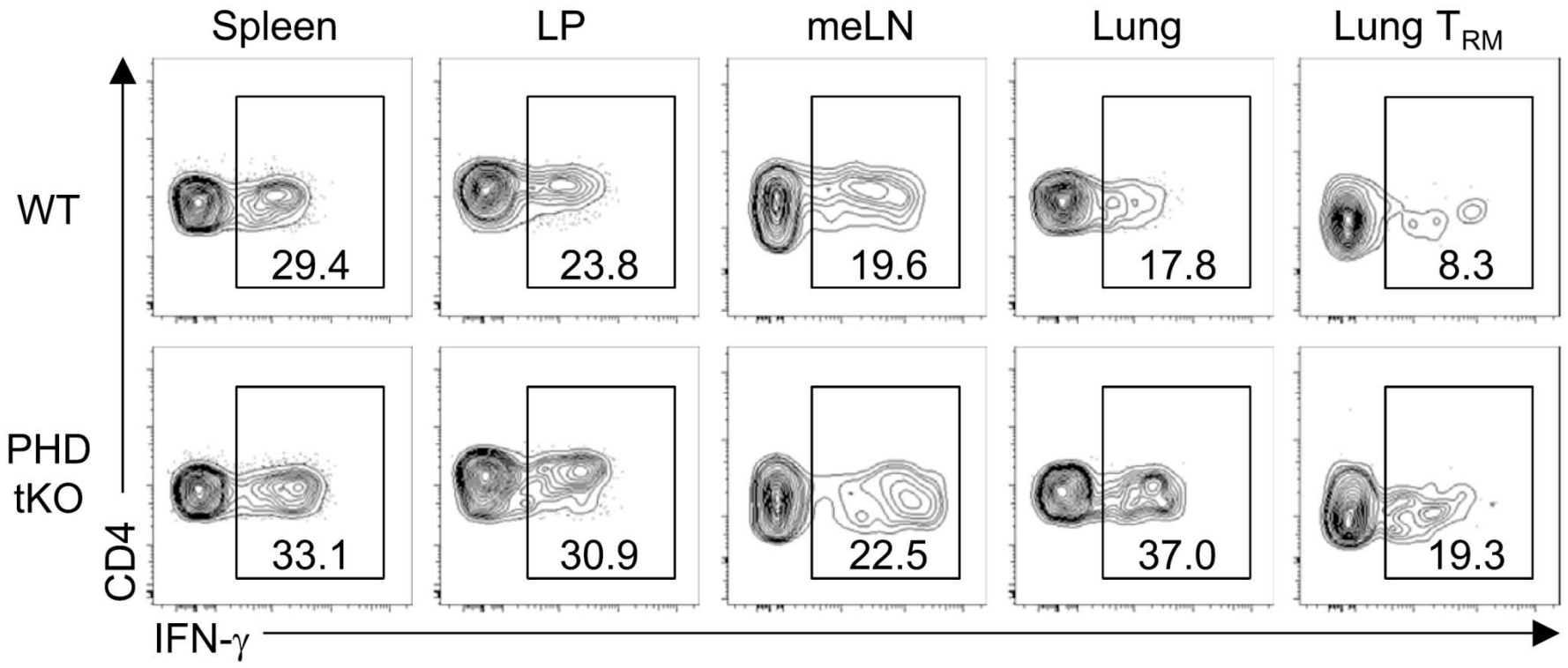




T-cell intrinsic PHD proteins do not trigger spontaneous inflammation in the gut

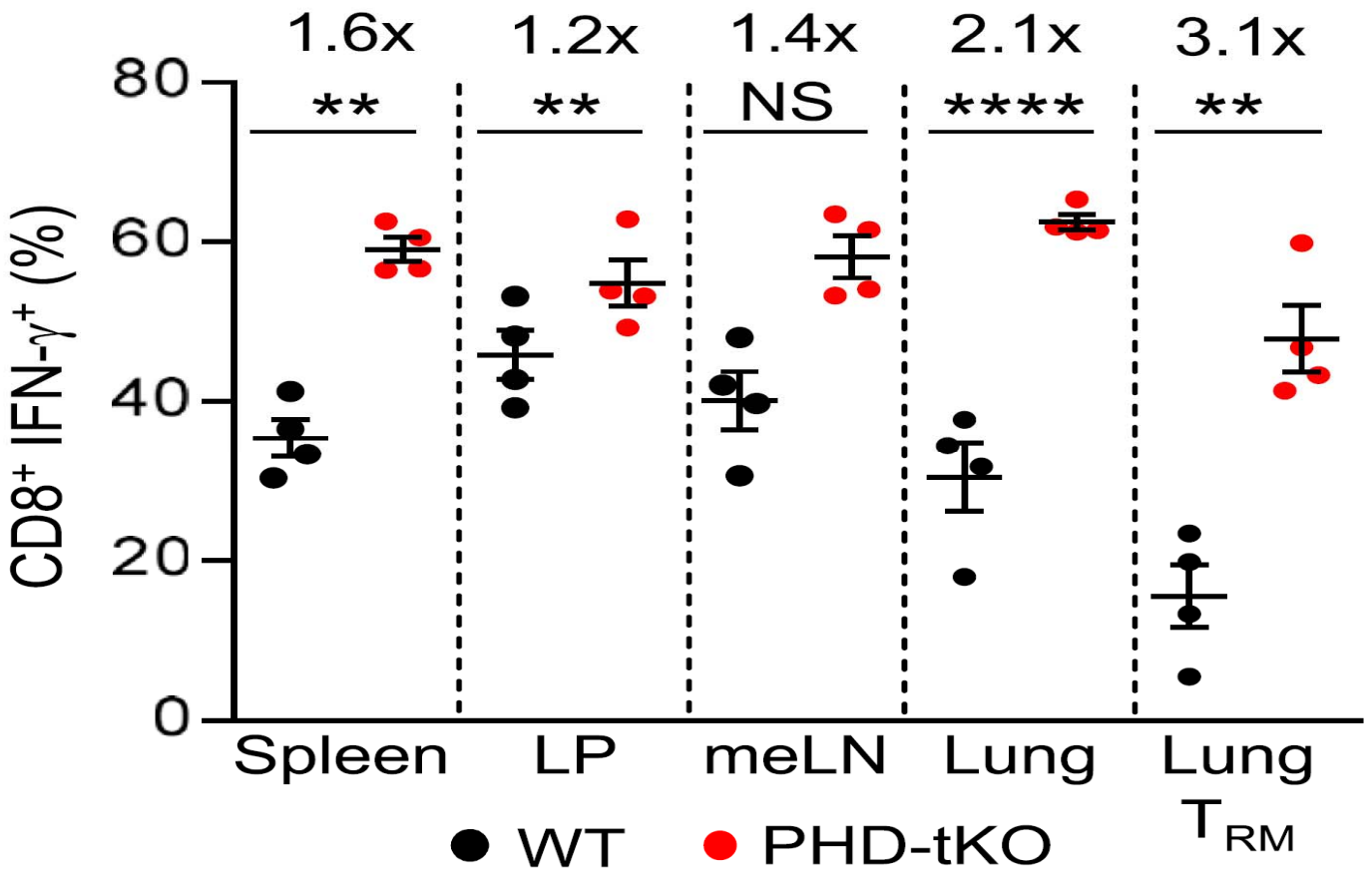


CD4+ T cells lacking PHD proteins are prone to produce IFN-γ after stimulation

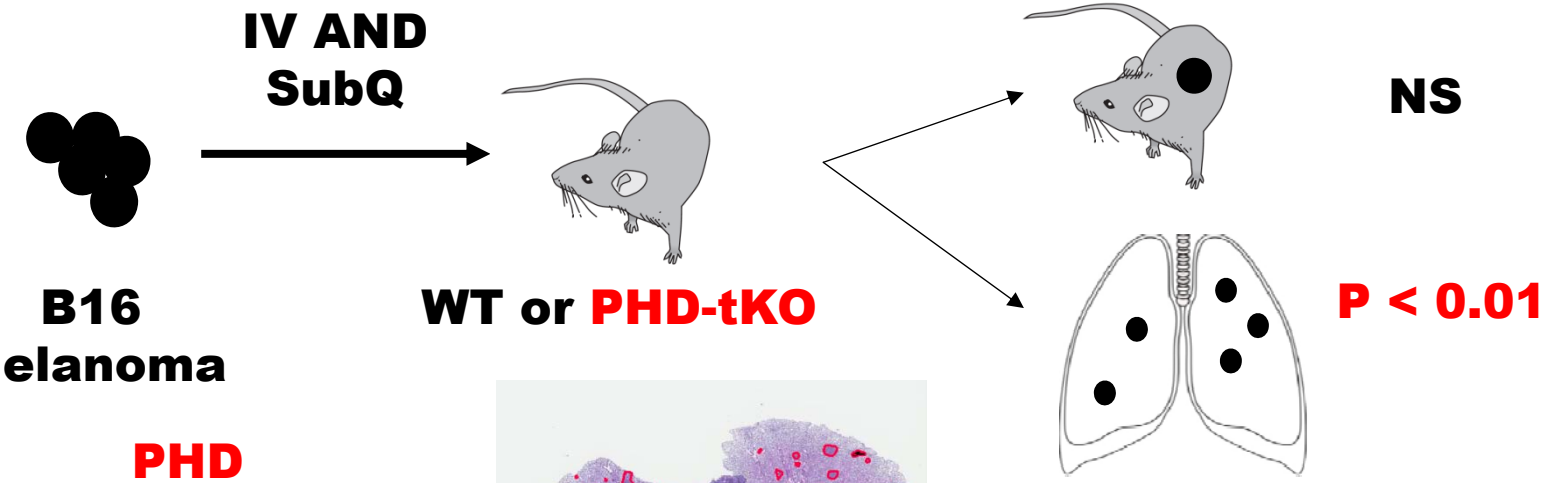


D Clever, Cell, August 25, 2016

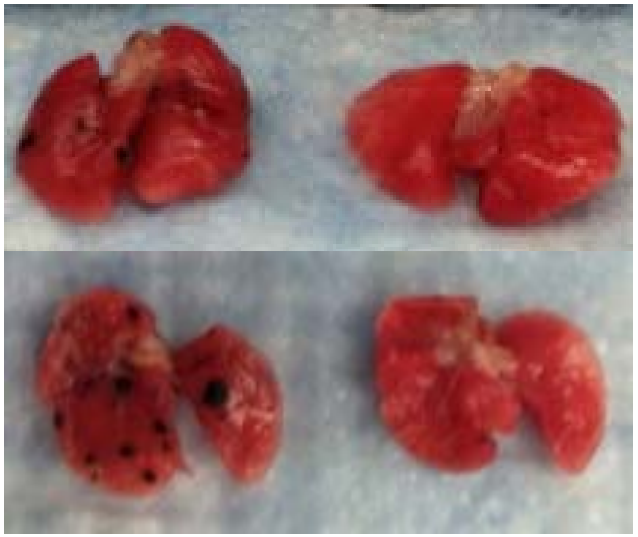
CD8⁺ T cells lacking PHD proteins are prone to produce IFN-γ after stimulation

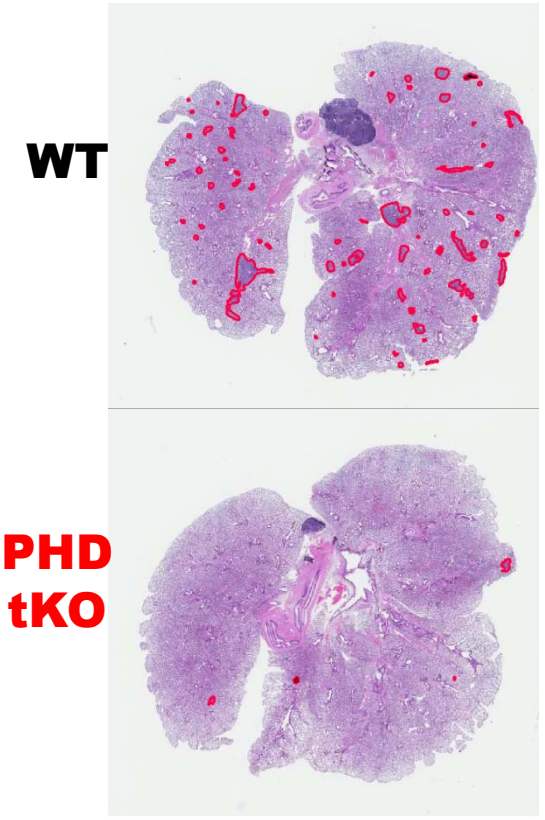


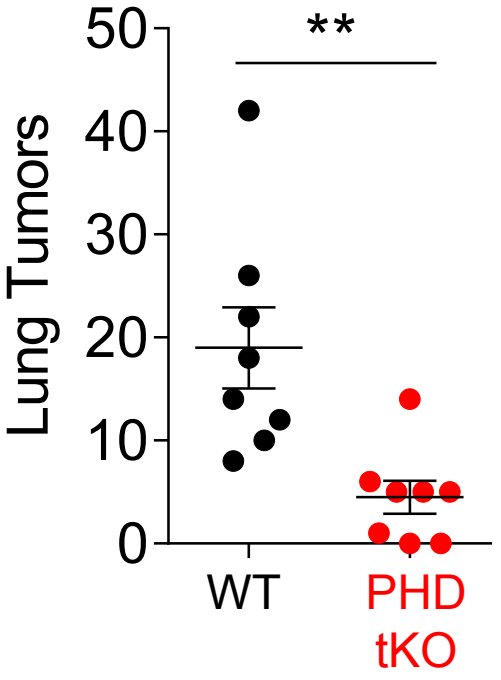
T cell-intrinsic expression of PHD proteins licenses tumor colonization in the lung but not SQ tissue



WT PHD tKO







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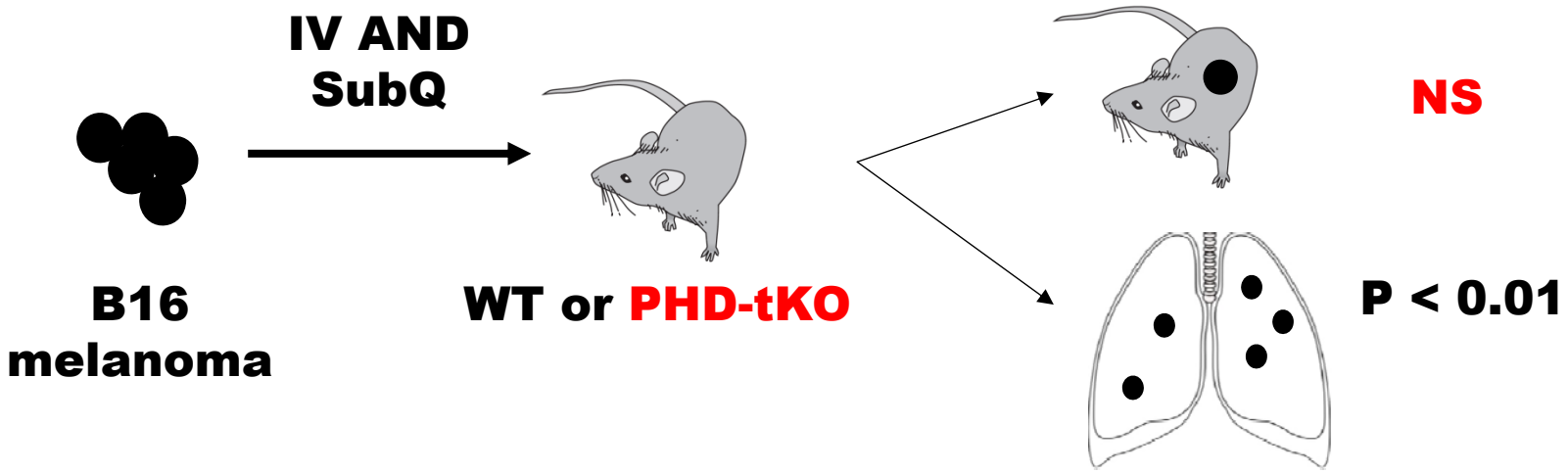
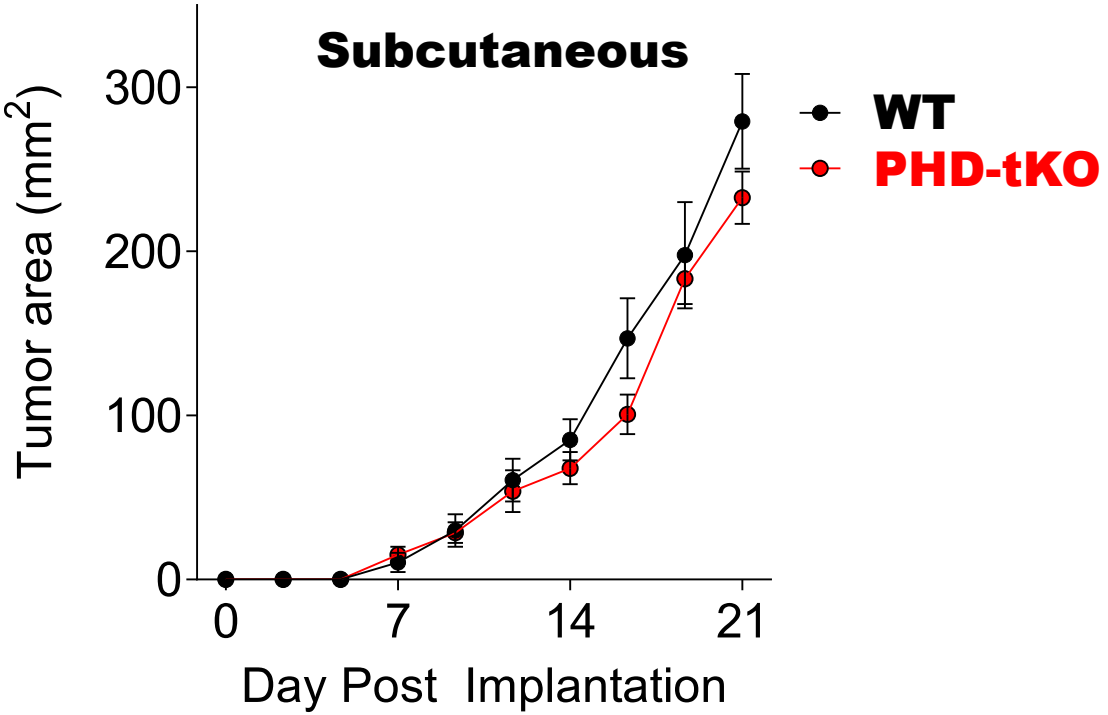
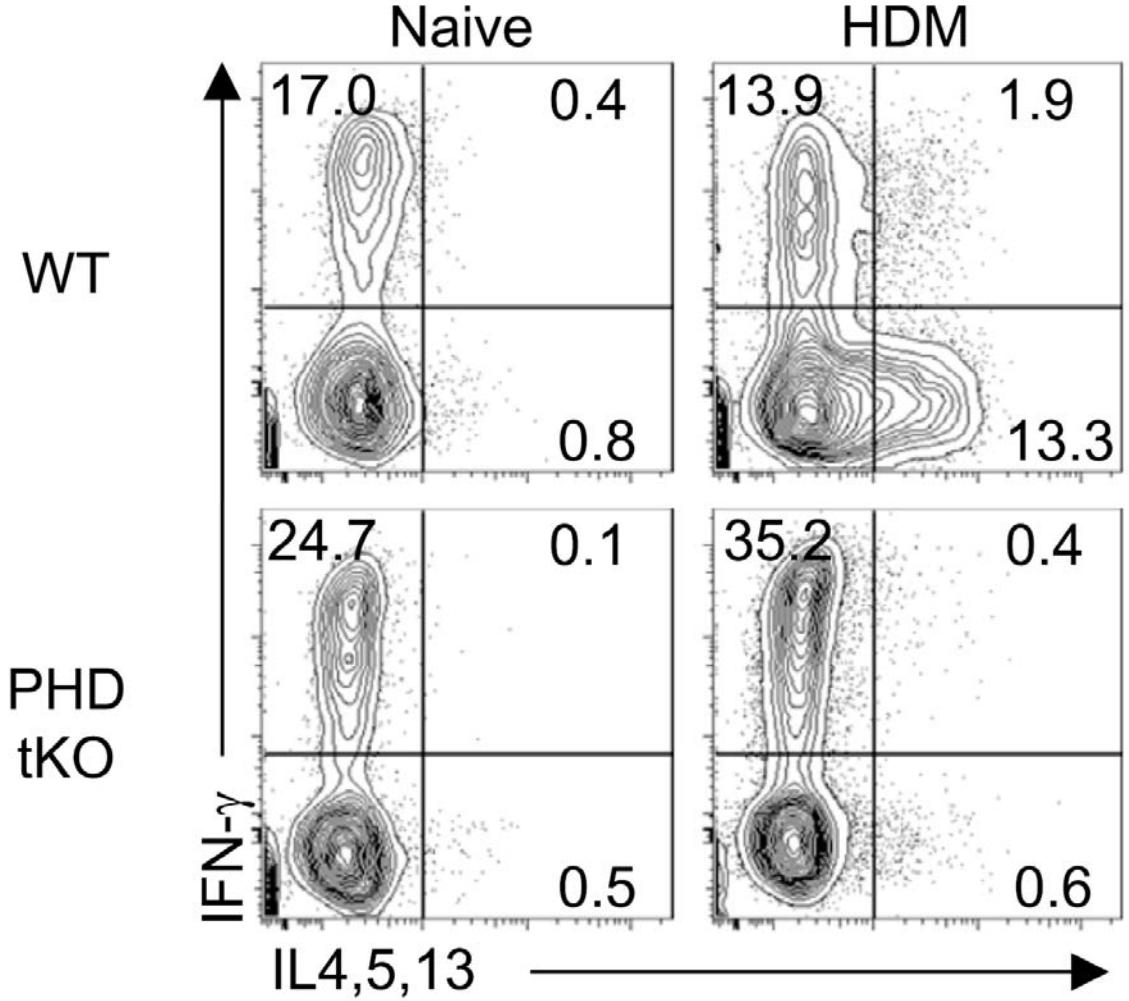
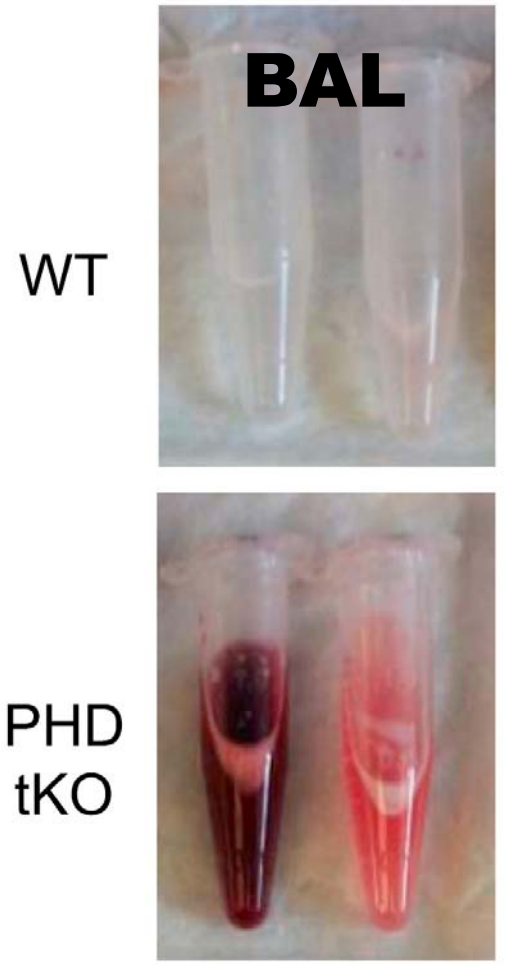




PHD proteins suppress type I responses against innocuous house dust mite (HDM) Ag





Summary

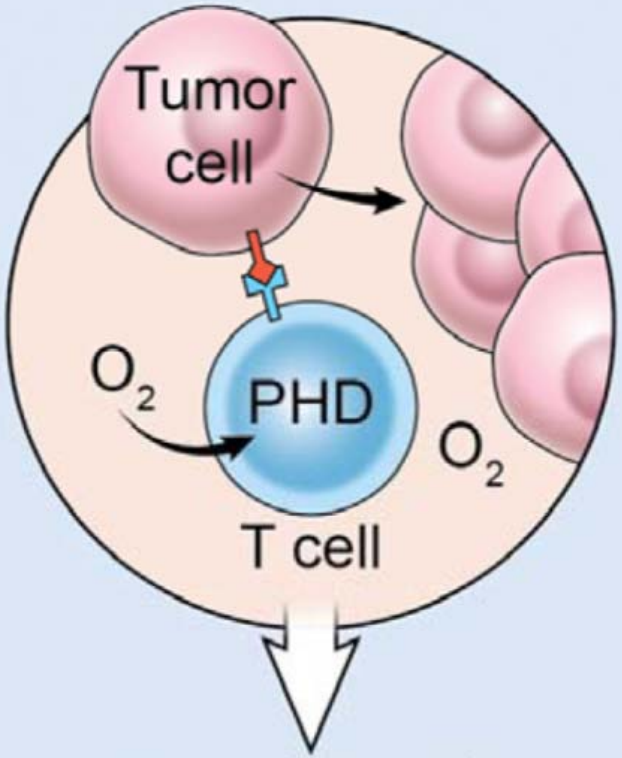
1. T-cell intrinsic PHD proteins suppress spontaneous pulmonary inflammation

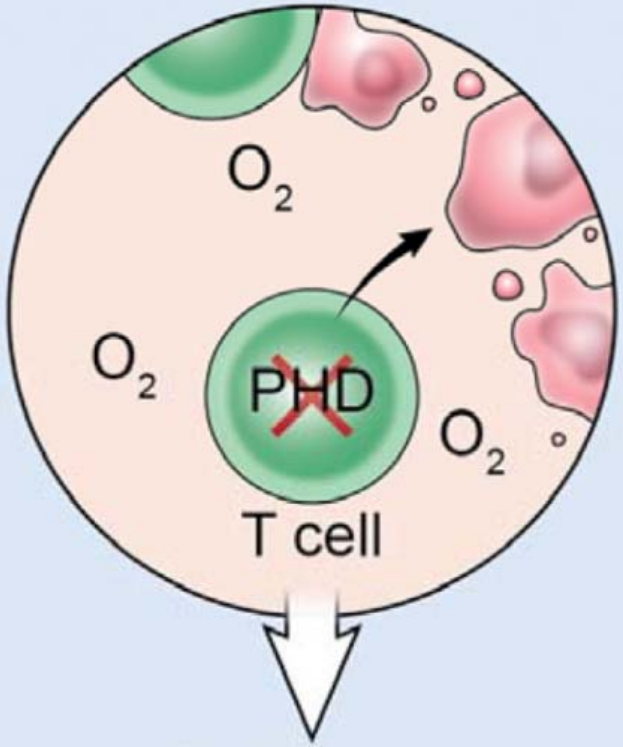
2. $CD8^+$ and $CD4^+$ T cells lacking PHD proteins are prone to produce IFN-γ after stimulation

3. T cell-intrinsic expression of PHD proteins licenses tumor colonization in the lung but not SQ tissue

4. PHD proteins suppress type I responses against innocuous house dust mite (HDM) Ag

The Problem





Normal homeostasis

Tumor colonization

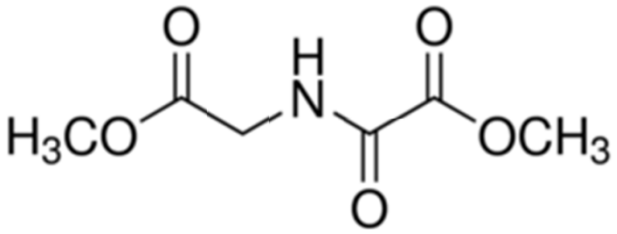
Hyper-responsiveness to innocuous Ag

Tumor clearance

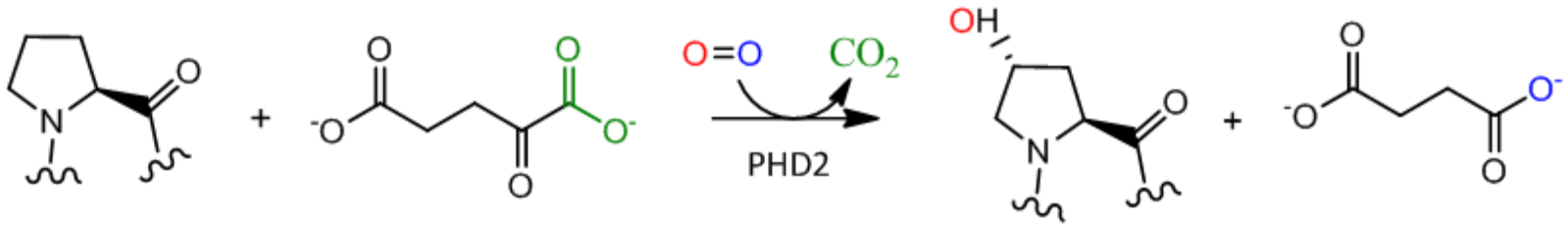
A Solution

Knockout or drug PHD proteins only in T cells specific for tumor antigens while leaving all other T cells intact

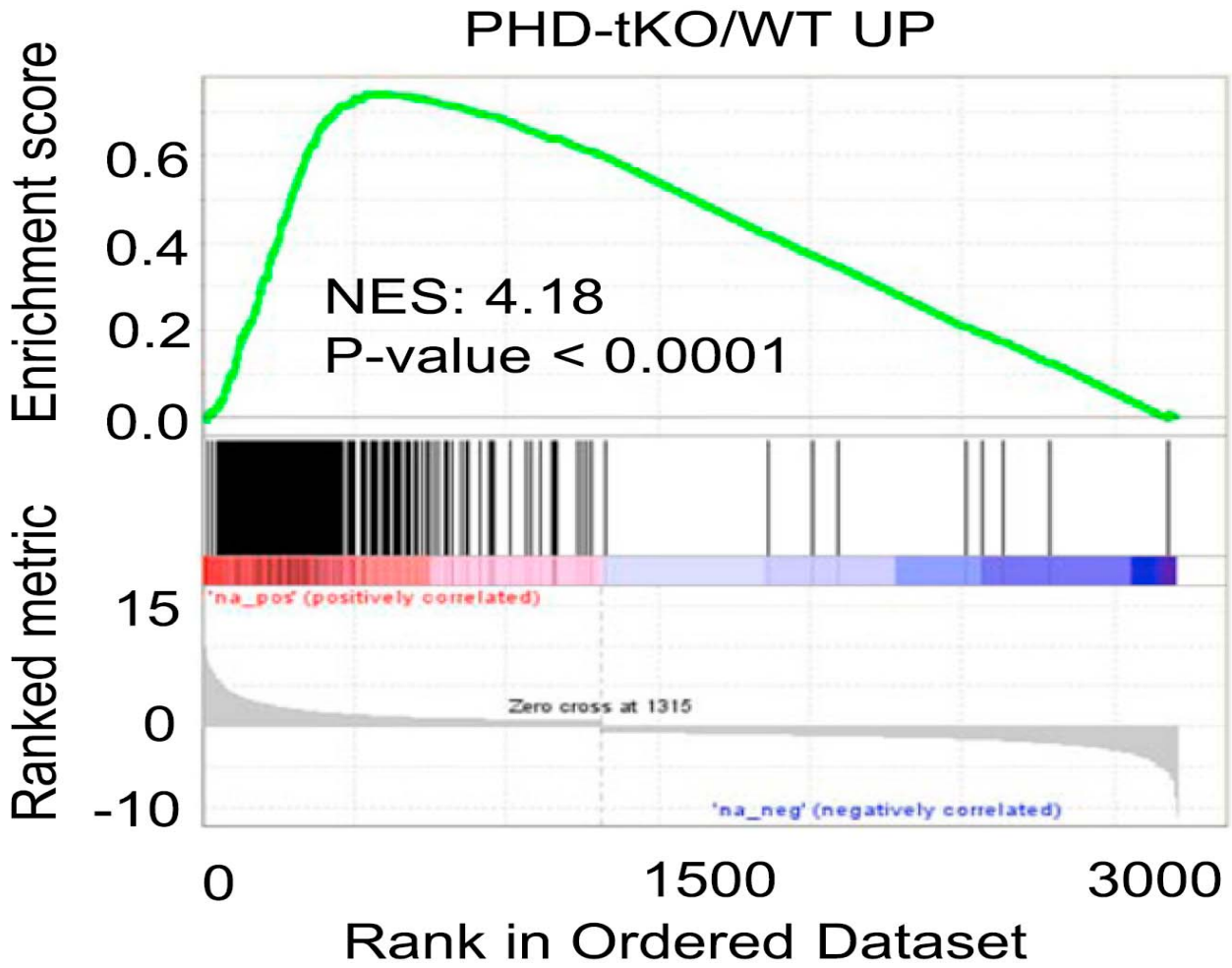
DMOG blocks the oxygen sensing PHD proteins



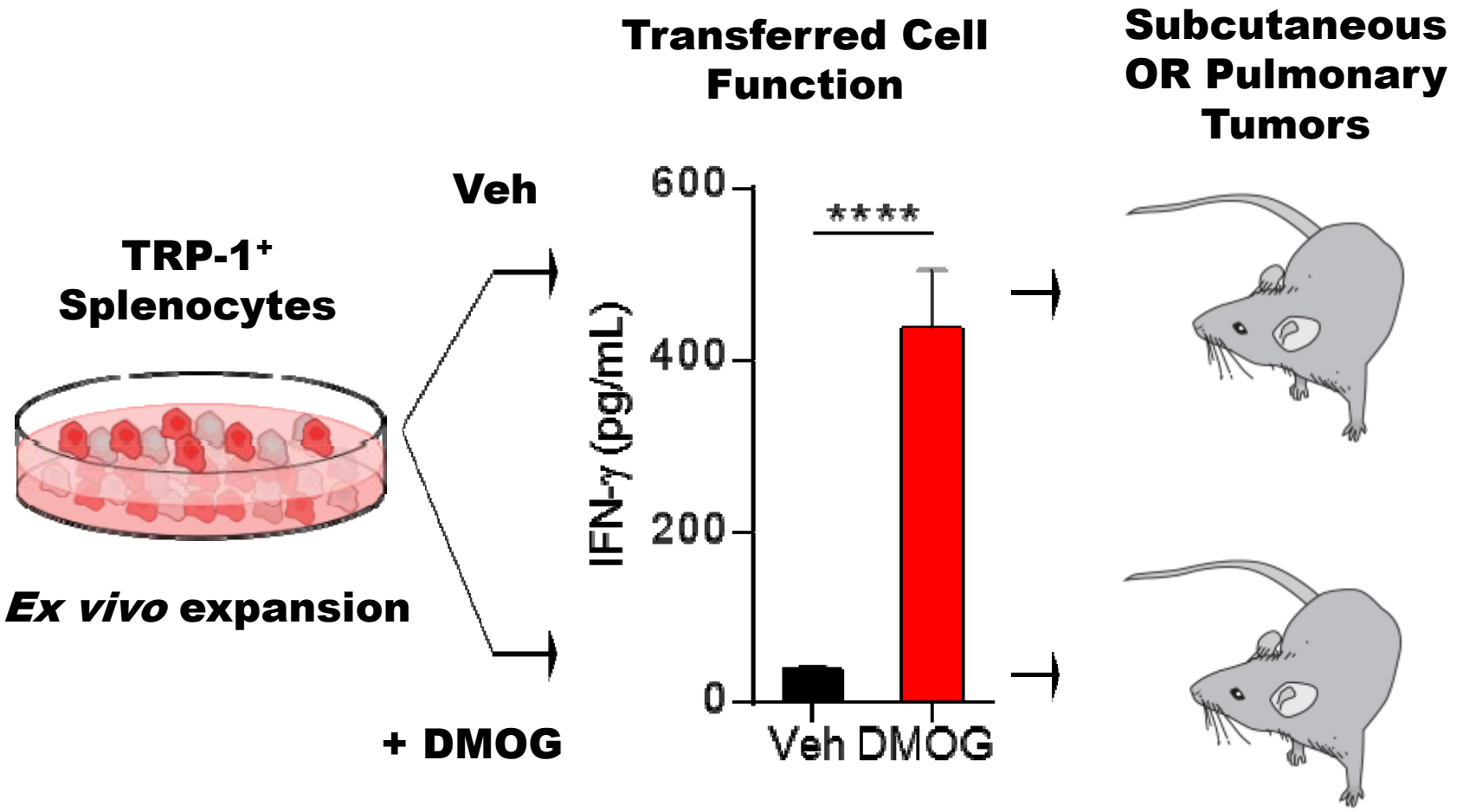
Dimethyloxalylglycine (DMOG)



Gene set enrichment analysis (GSEA) shows that DMOG/vehicle induces similar gene expression changes as PHD-tKO/WT



Inhibition of PHD proteins with DMOG before adoptive cell transfer immunotherapy



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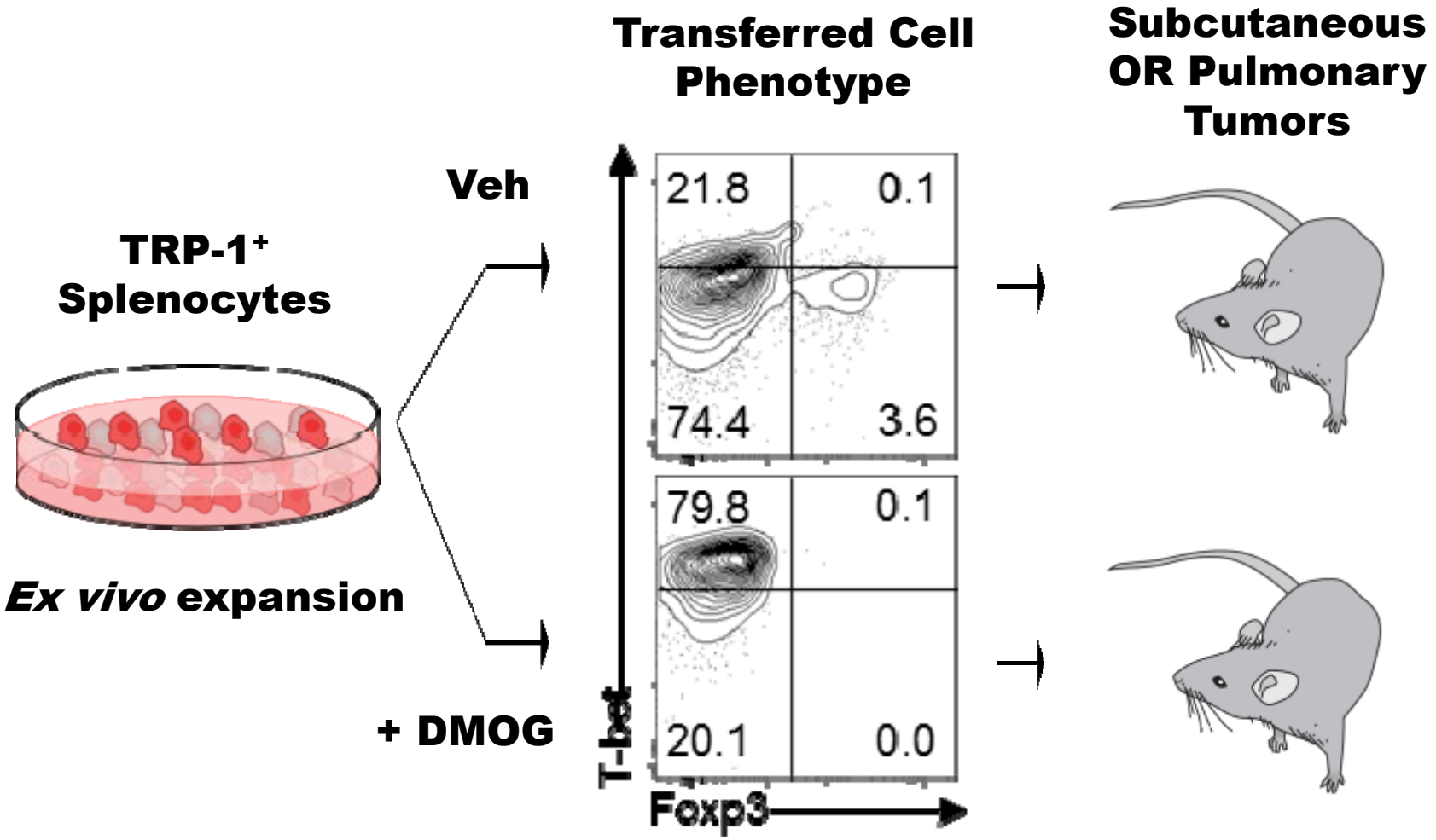
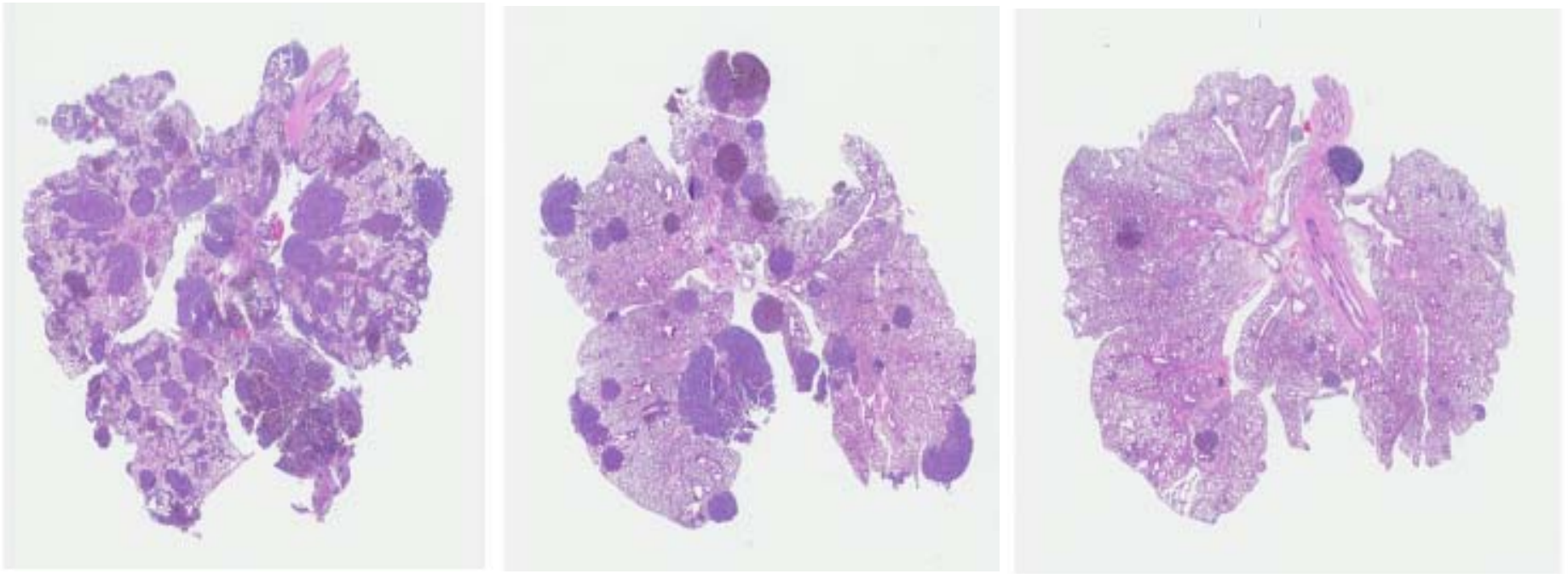


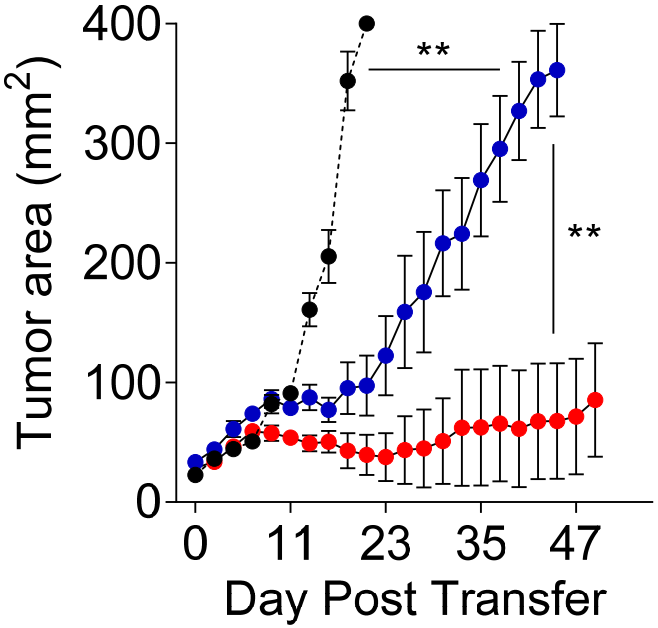
Inhibition of PHD proteins with DMOG improves adoptive cell transfer immunotherapy

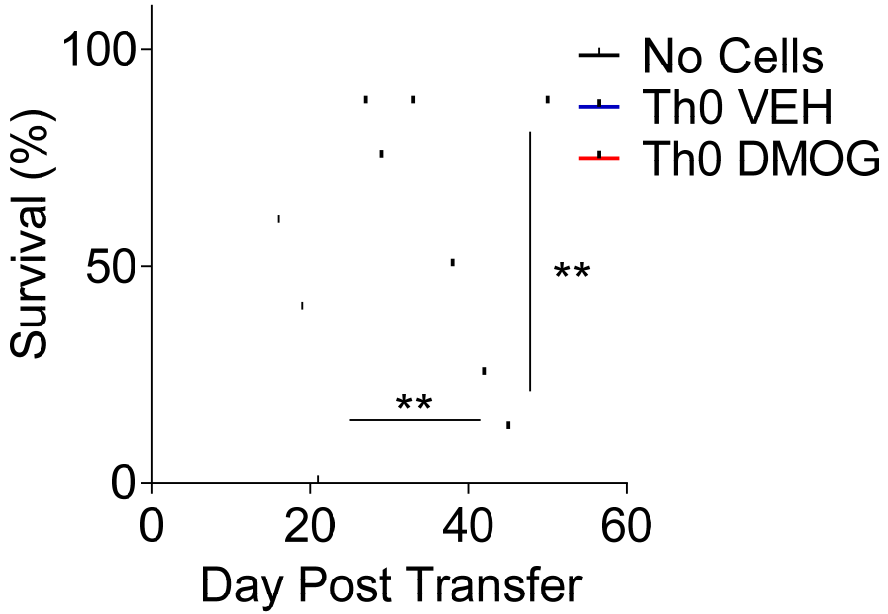
No Cells | **Trp-1 VEH** | **Trp-1 DMOG**



D Clever, Cell, 2016

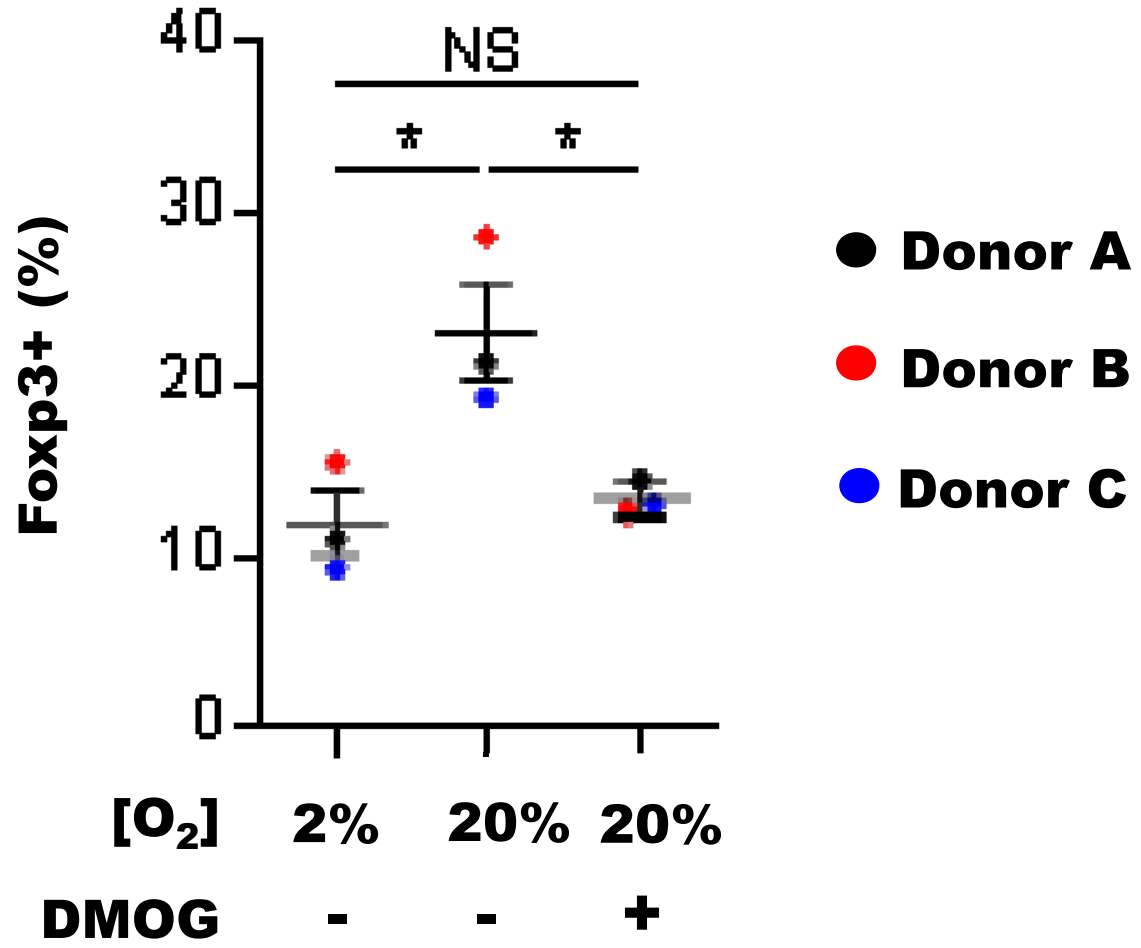
Improved efficacy of DMOG-cultured cells for established subcutaneous tumors





D Clever, Cell, 2016

Foxp3$^+$ iTreg fate specification of human CD4$^+$ T cells cultured with DMOG



Summary

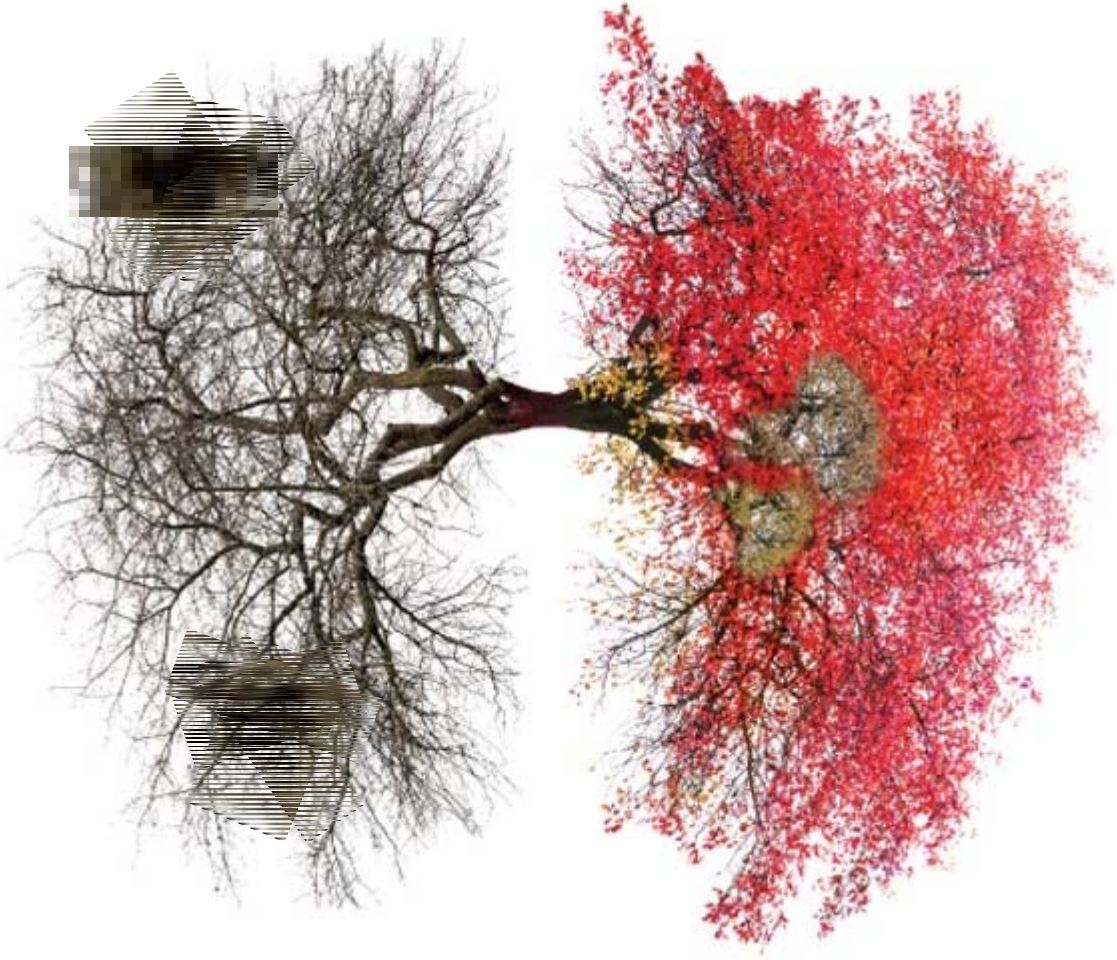
1. DMOG blocks the oxygen sensing PHD proteins as evidenced by RNA seq and gene set enrichment analysis (GSEA)

2. Inhibition of PHD proteins with DMOG changes the function and phenotype of T cells . . .

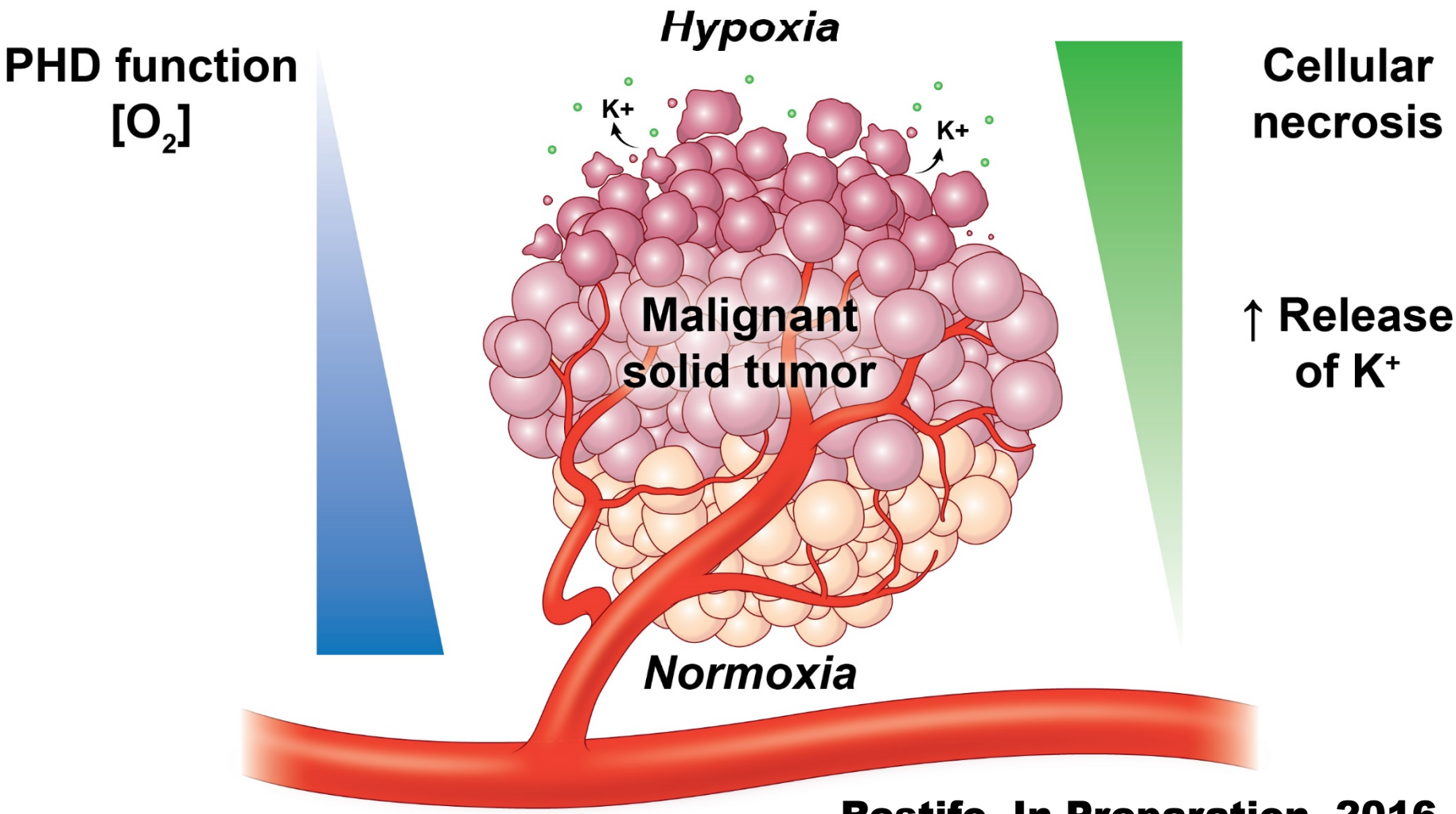
3. . . . and improves adoptive cell transfer immunotherapy

4. Finally, similar maneuvers can be done with human $CD4^+$ T cells

How do tumor immune suppressive mechanisms change with progressive growth?

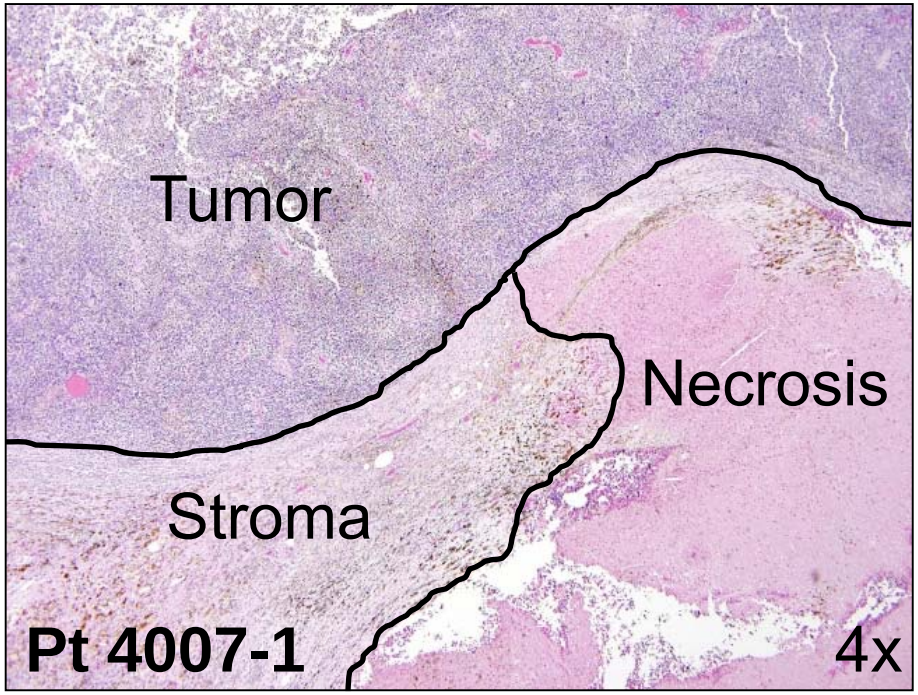


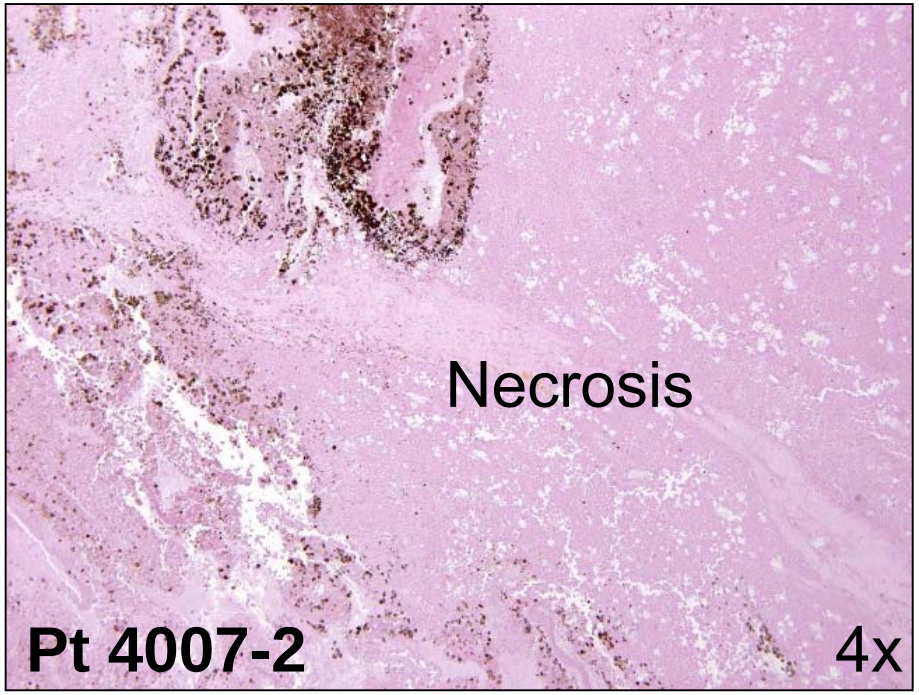
Increased hypoxia accompanies progressive tumor growth



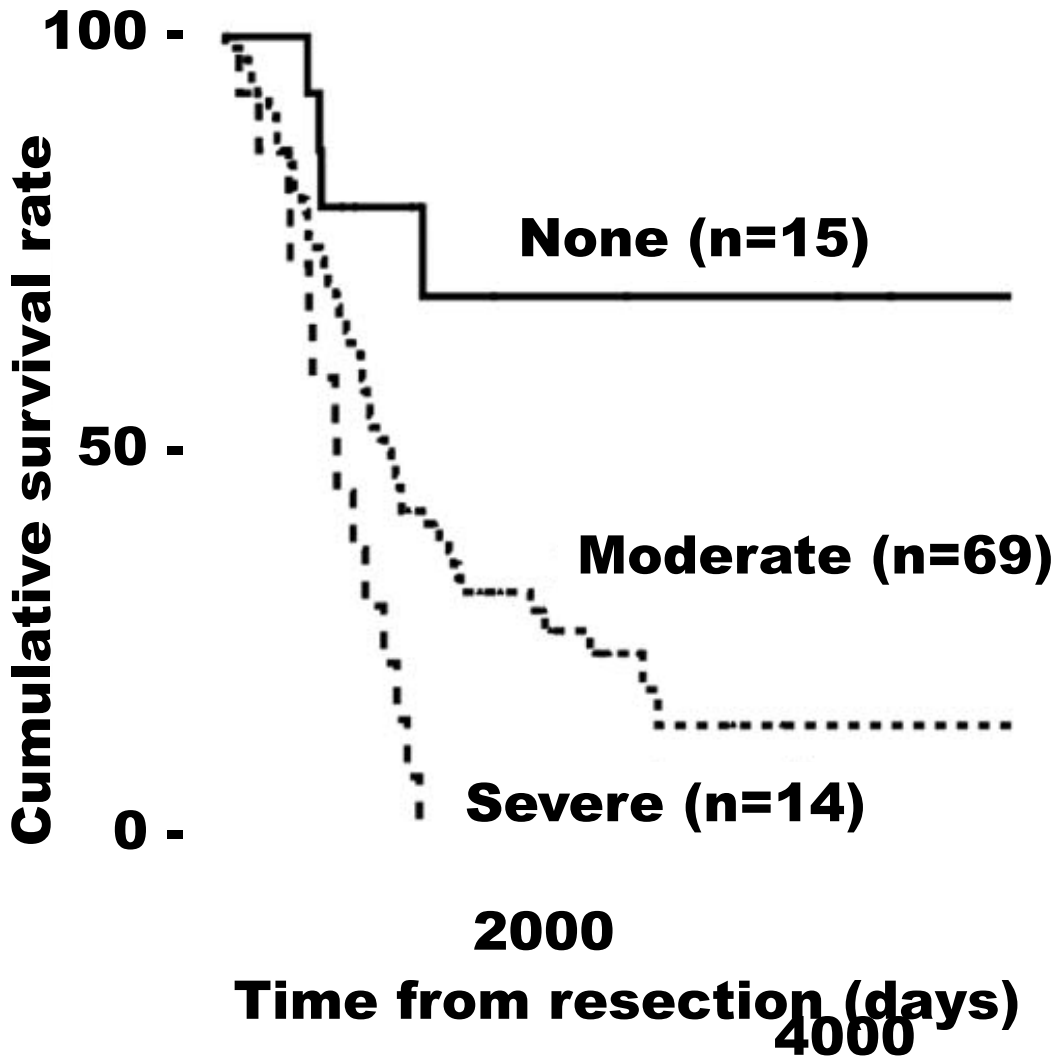
Restifo, In Preparation, 2016

The tumor microenvironment is characterized by a high tissue density of necrosis



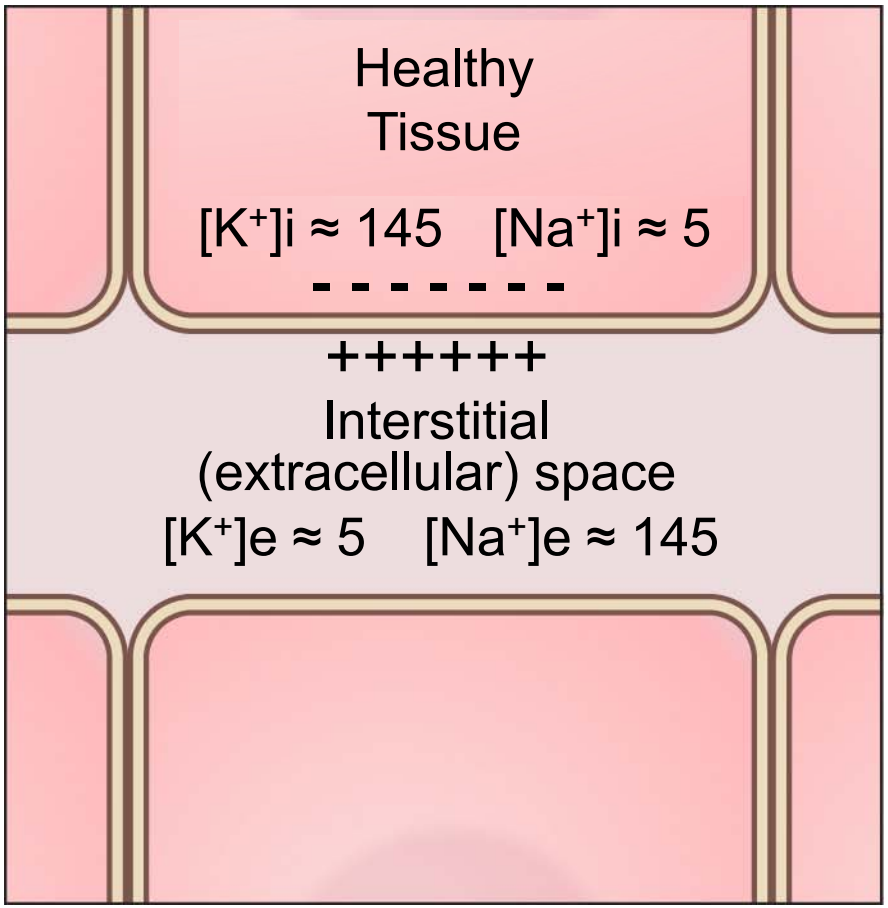


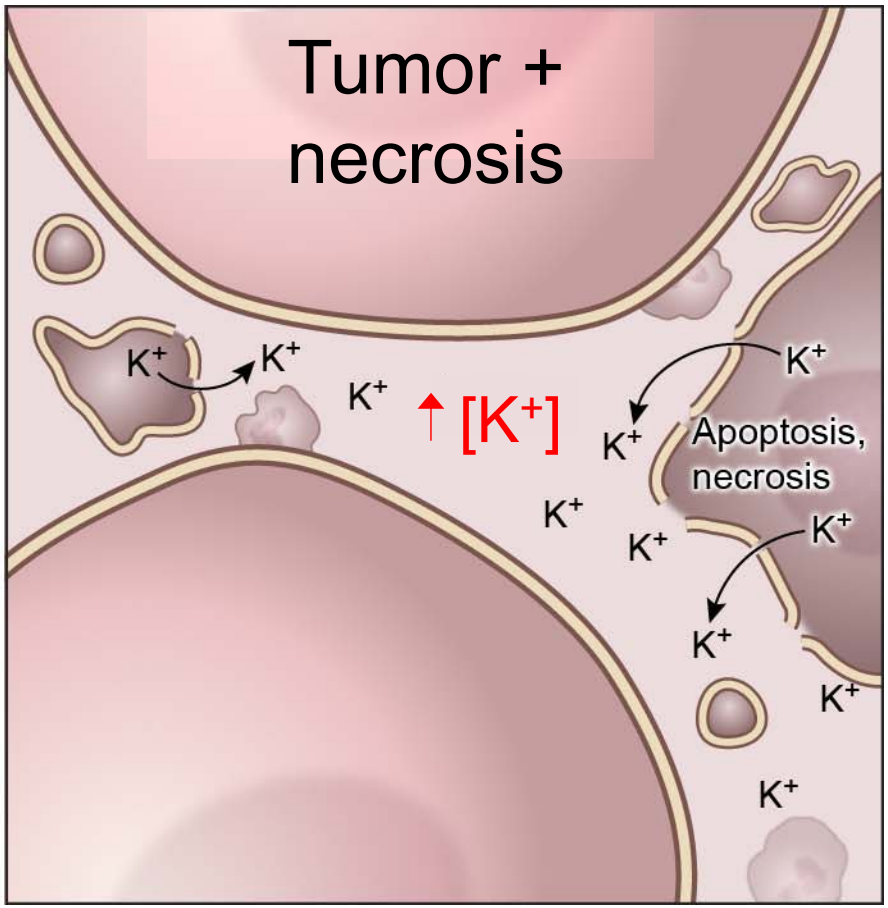
Severe tumor necrosis is associated with a poor prognosis



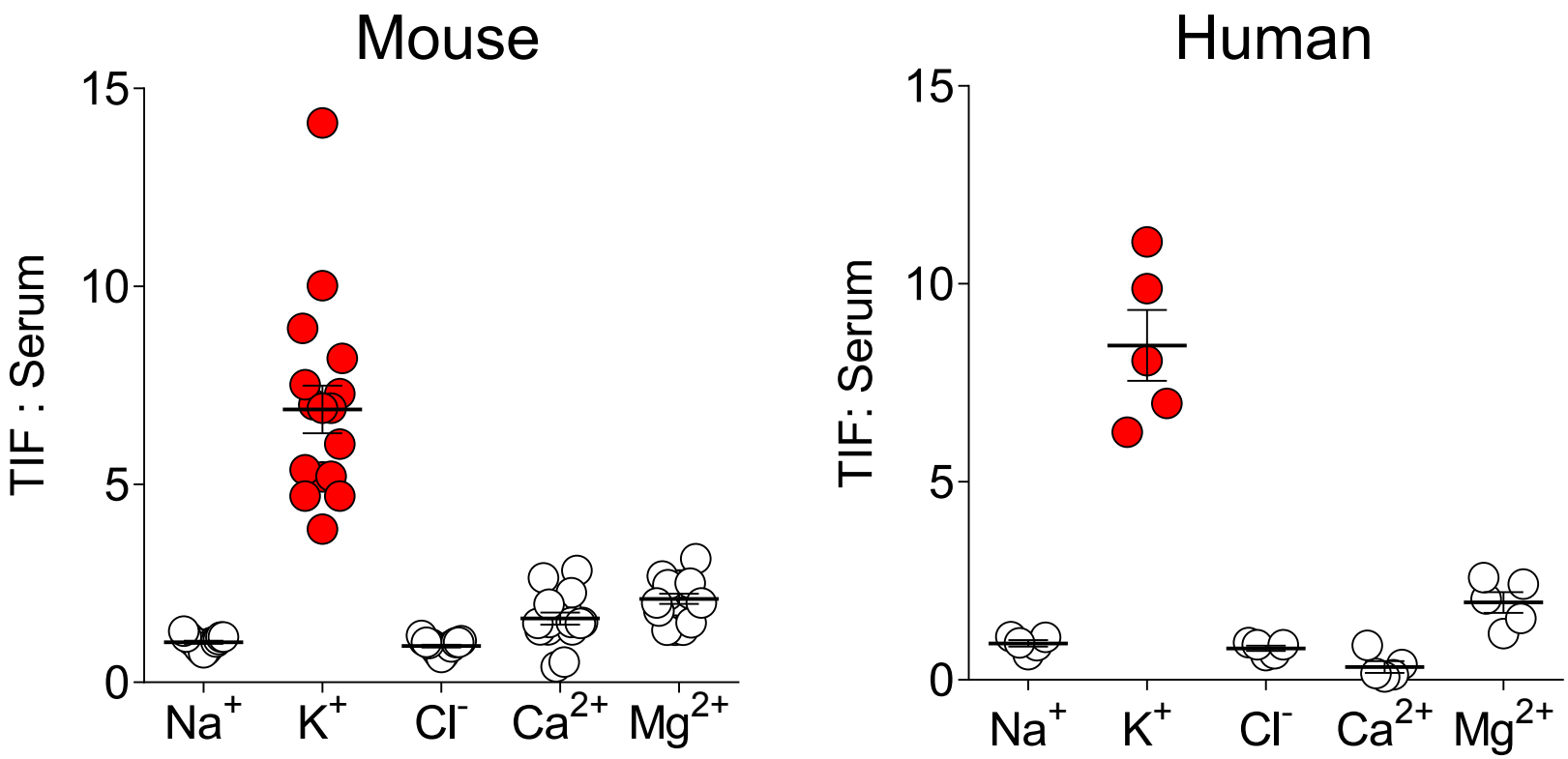
Komori et al. *Anticancer Res.* 2013

Necrosis releases intracellular ions into the extracellular space

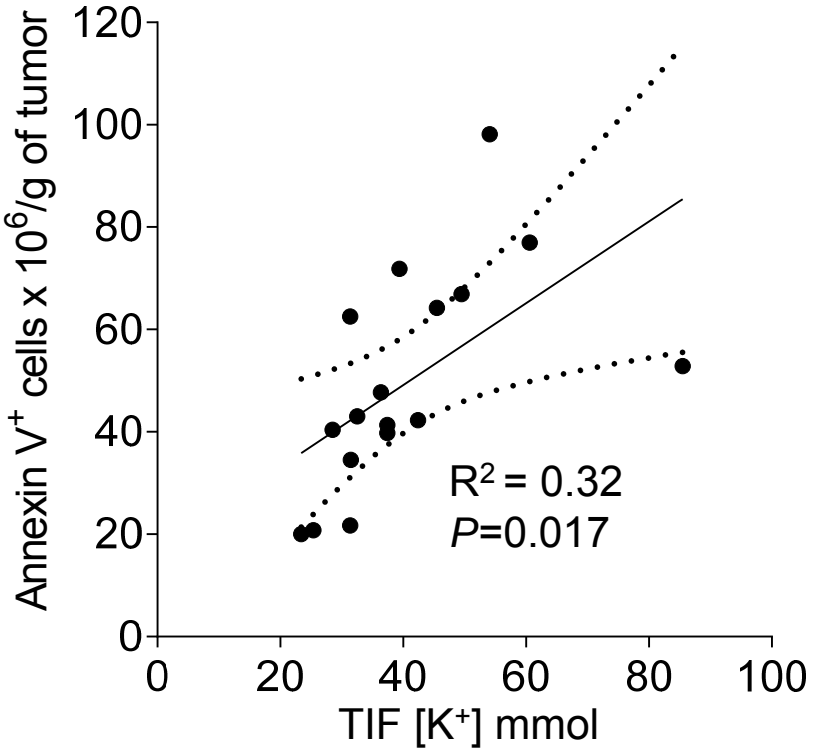




Tumor interstitial fluid (TIF) has an elevated concentration of extracellular potassium ([K^+])



Cell death correlates with levels of K^+ in the extracellular space



R Eil, Nature (In Press), Fall, 2016

Background and Experimental Question

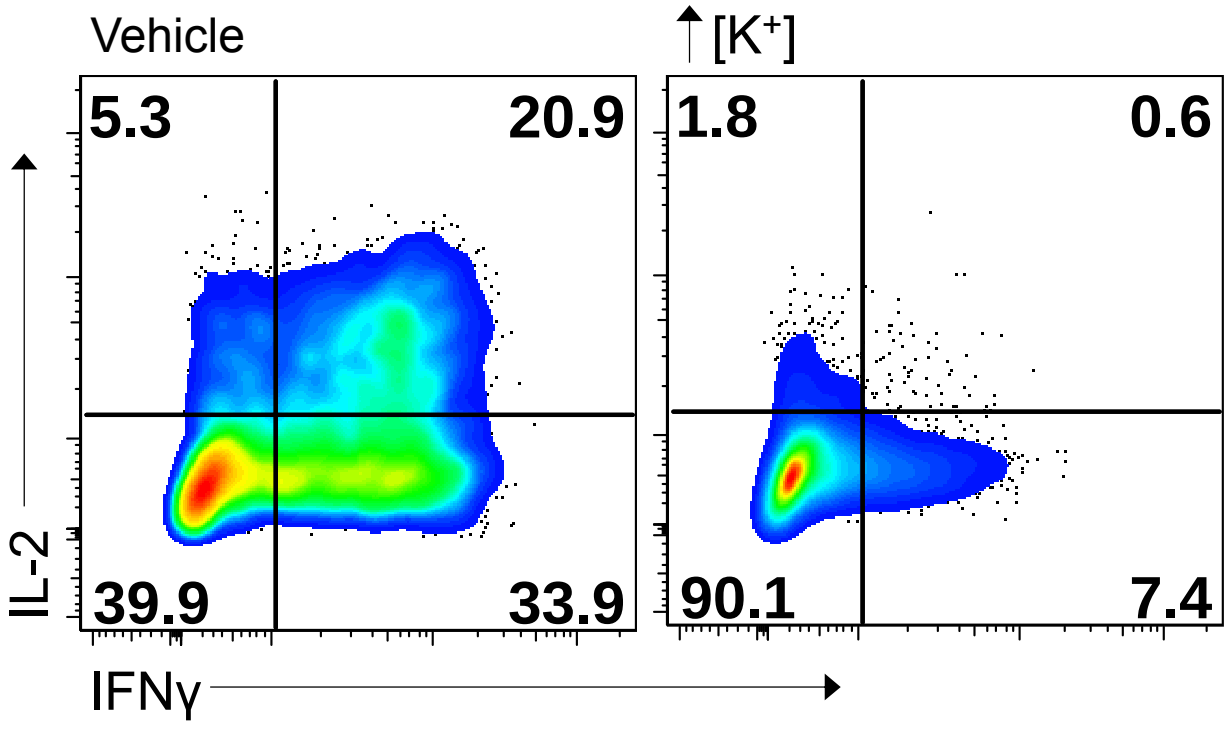
1. Human tumors persist and progress despite infiltration by tumor-specific effector T cells

2. Mouse and human tumors contain dense areas of cell necrosis

3. Cell necrosis leads to the release of an intracellular ion, potassium, into the extracellular space

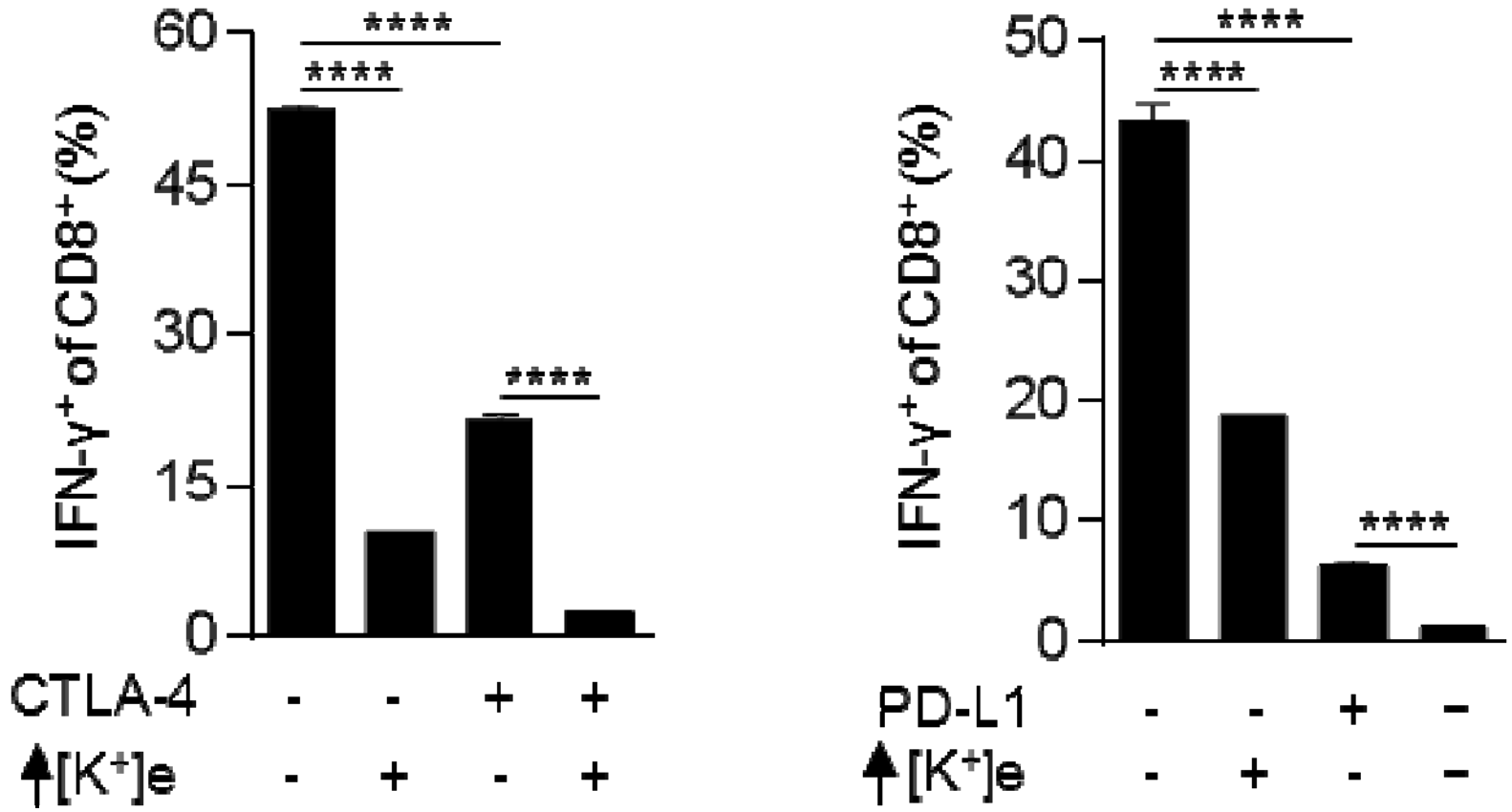
4. Do elevated concentrations of extracellular potassium ($[K^+]$) have any effect on T cell function?

Elevated [K^+] acutely inhibits T cell effector function



R Eil, Nature (In Press), Fall, 2016

Hyperkalemia augments checkpoint inhibition of T cells that may already be in place



R Eil, Nature (In Press), Fall, 2016

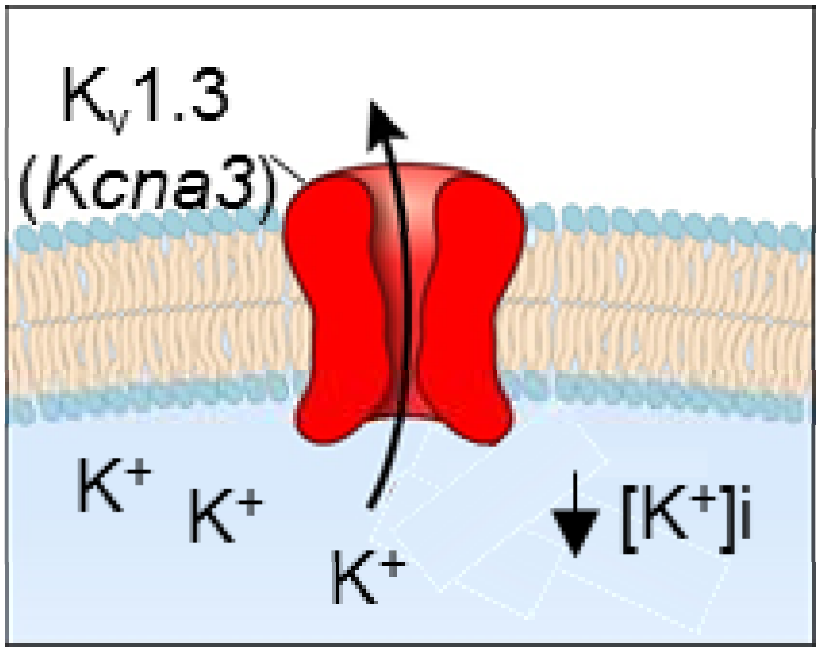
Tumor Interstitial Fluid (TIF) contains ~ 40 mm of K^+

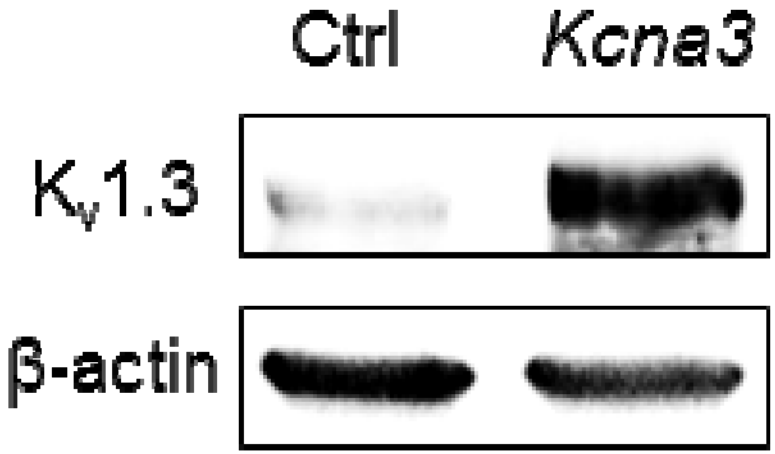
1. Elevated [K^+] produces profound suppression of human and mouse T cell TCR induced effector function

2. Hyperkalemia produces profound suppression of T cell receptor-induced transcripts including IL-2 and IFN-γ

3. Tumor associated hyperkalemia augments checkpoint inhibition of T cells that may already be in place

Naturally-occurring T cells express low levels of the potassium ion channel *Kcna3* encoding Kv1.3





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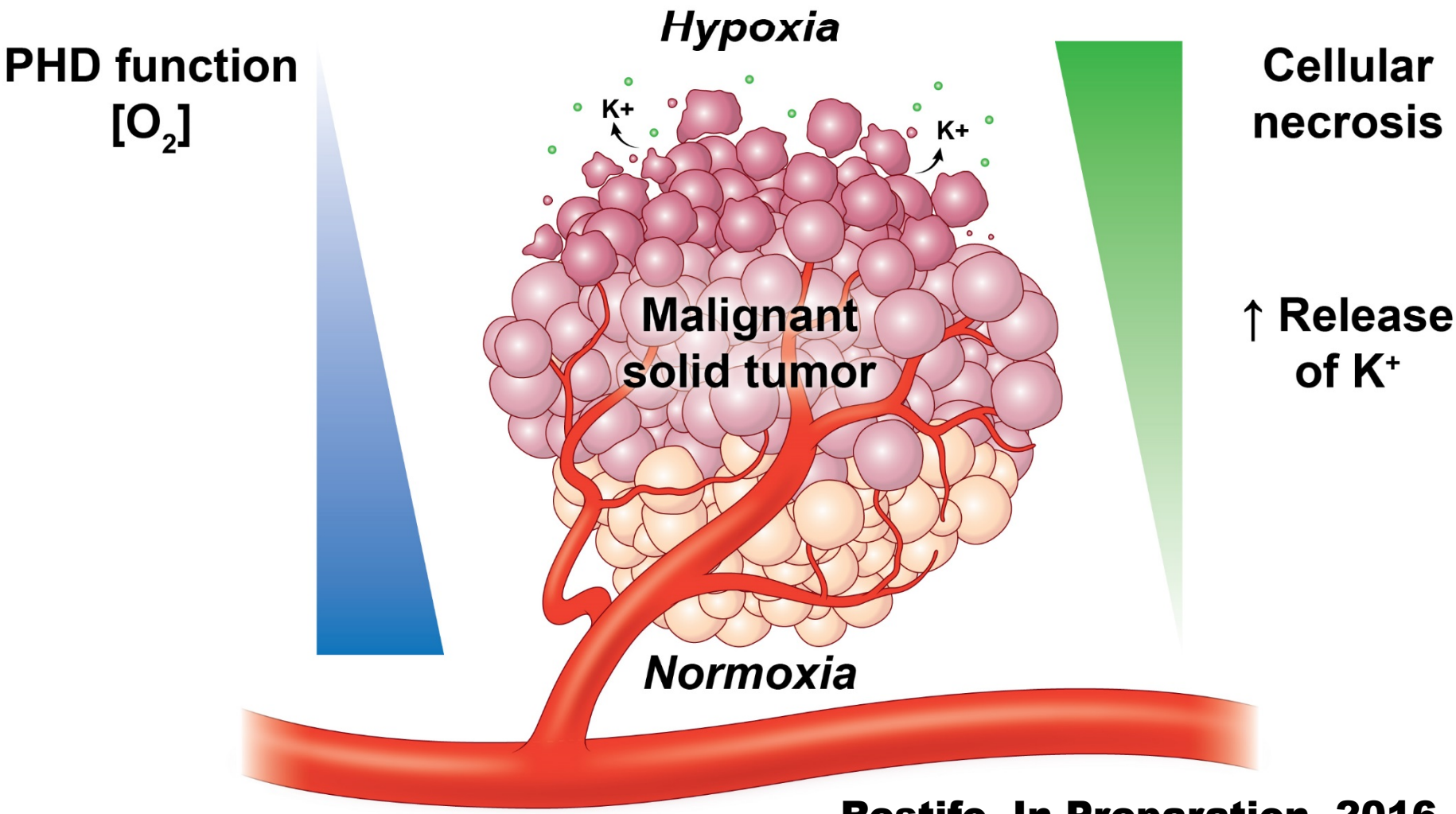
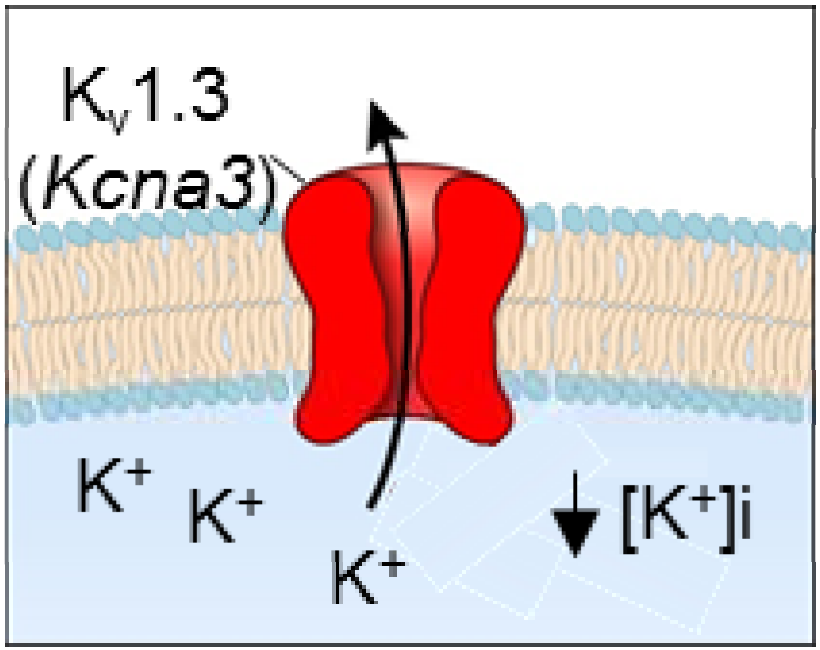
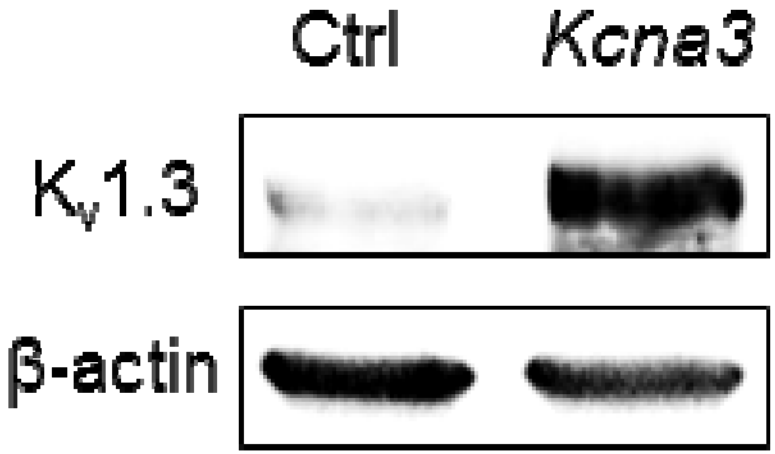


Restifo, In Preparation, 2016

Genetically engineering anti-tumor T cells to over-express the potassium ion channel *Kcna3*





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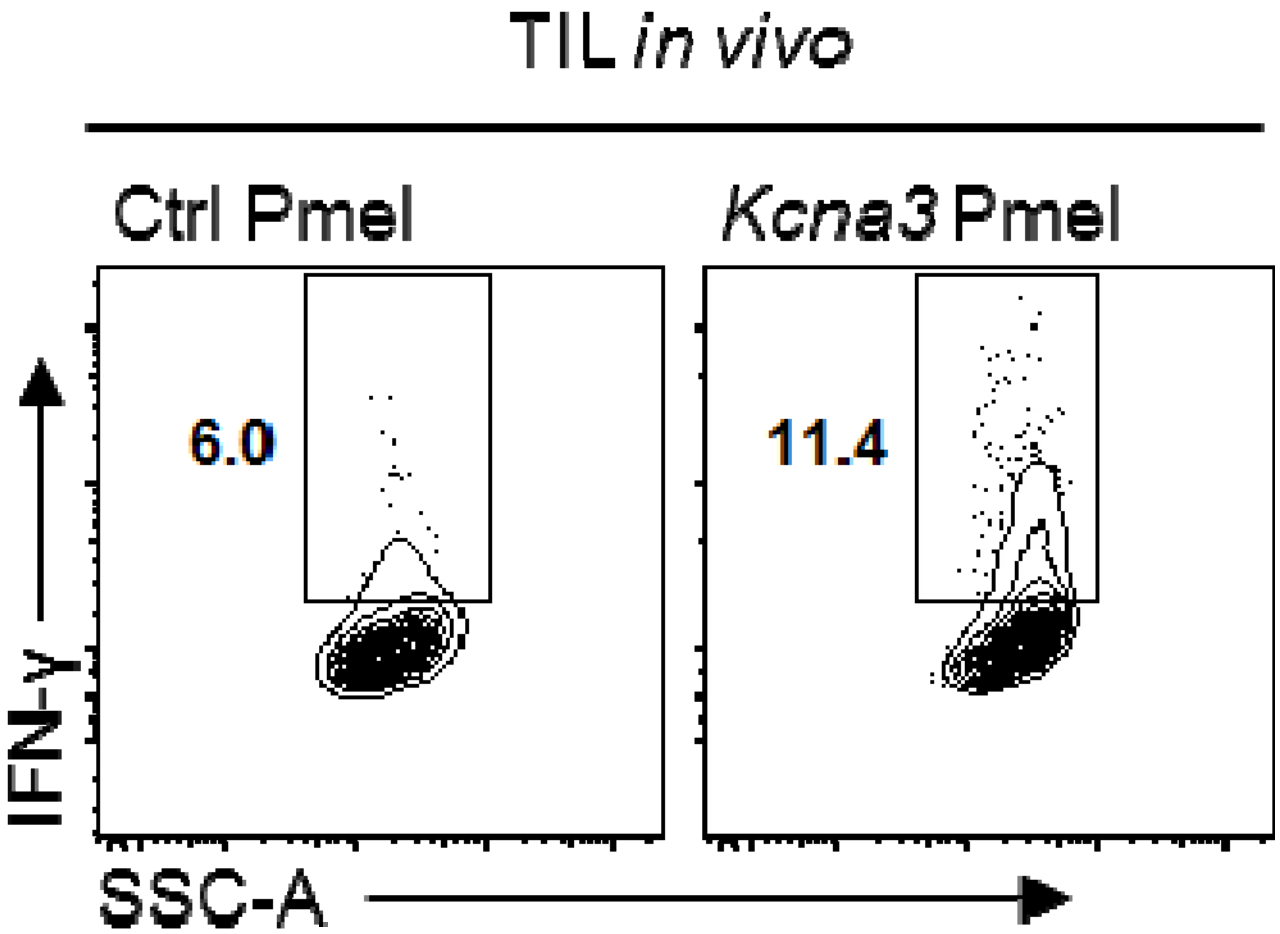
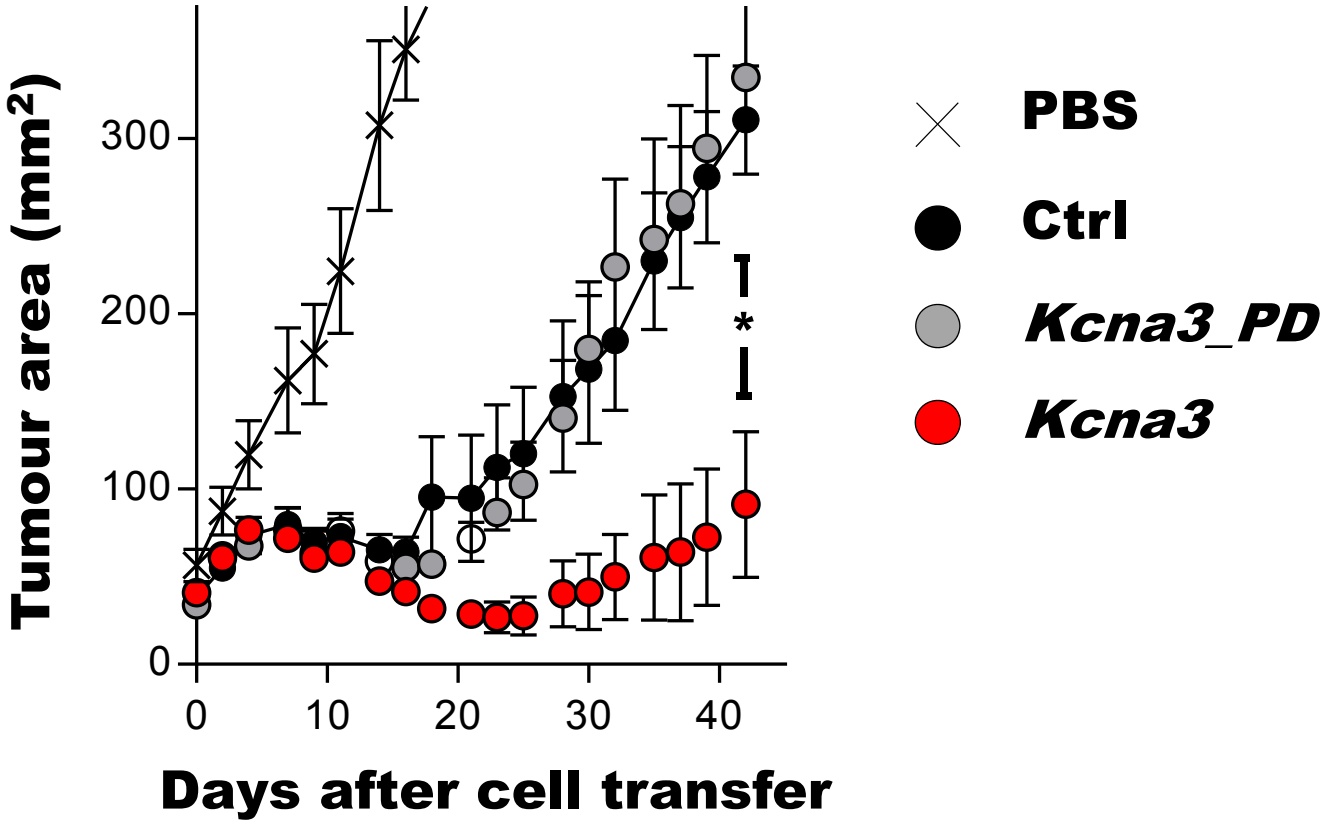
Kcna3 gene-engineered T cells make more IFN-γ *in vivo*



Anti-tumor T cells over-expressing *Kcna3* have enhanced therapeutic efficacy



R Eil, Nature (In Press), Fall, 2016

Overall summary

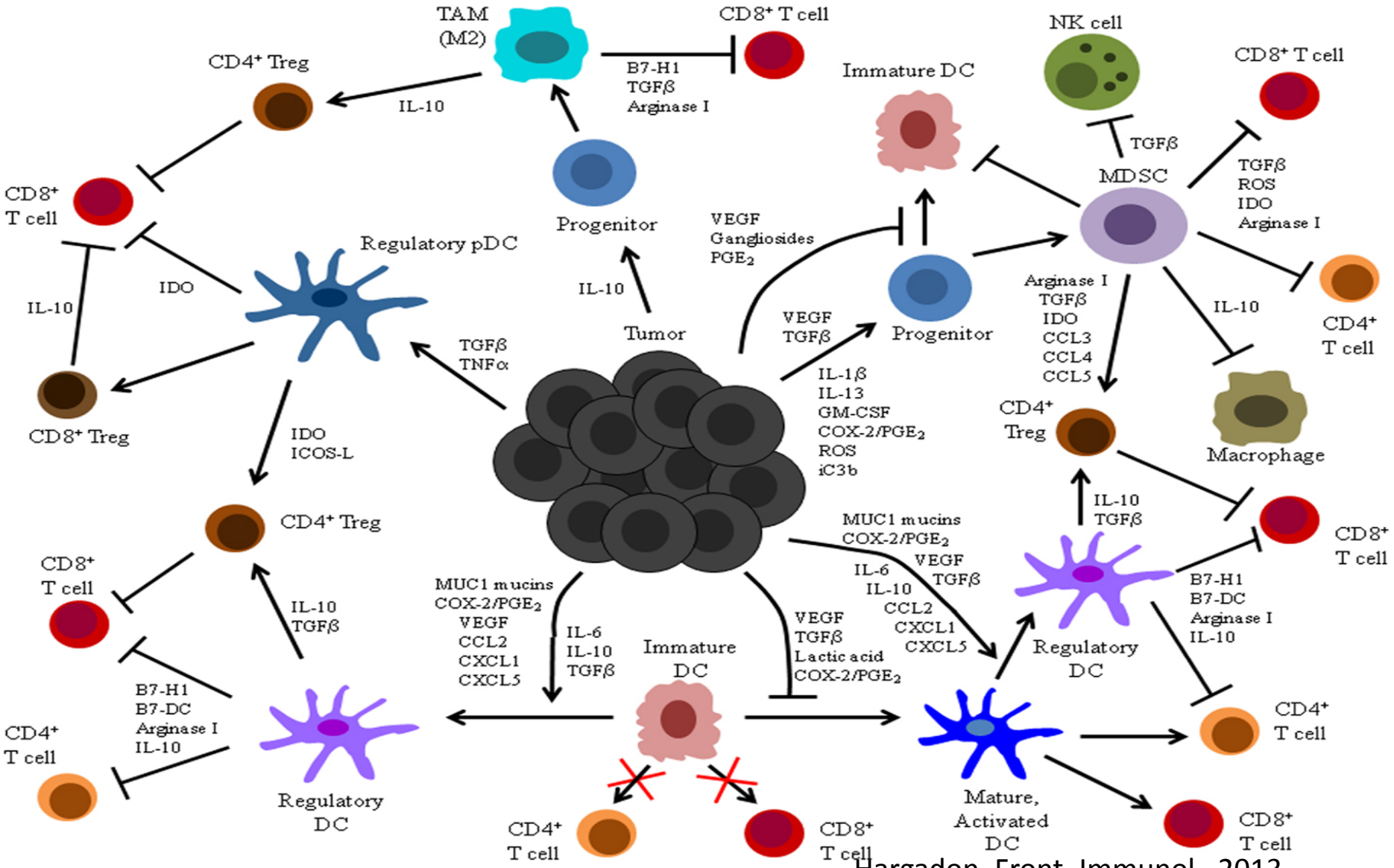
1. Tumor cell death creates elevated [K^+] in the tumor microenvironment.

2. This local hyperkalemia produces profound suppression of human and mouse T cells

3. T cells can be gene-engineered for resistance to hyperkalemia by over-expressing the [K+] ion transporter Kcna3

4. Anti-tumor T cells over-expressing Kcna3 have enhanced therapeutic efficacy

Tumor-induced immunosuppression is complicated



Hargadon, Front. Immunol., 2013

Composition of a human being

Element	Symbol	Percentage in Body
Oxygen	O	65.0
Carbon	C	18.5
Hydrogen	H	9.5
Nitrogen	N	3.2
Calcium	Ca	1.5
Phosphorus	P	1.0
Potassium	K	0.4
Sulfur	S	0.3
Sodium	Na	0.2
Chlorine	Cl	0.2
Magnesium	Mg	0.1
Trace elements include boron (B), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn).		less than 1.0

What is the immunology of the elements and how can it be used to destroy cancer?

8
O
Oxygen

19
K
Potassium

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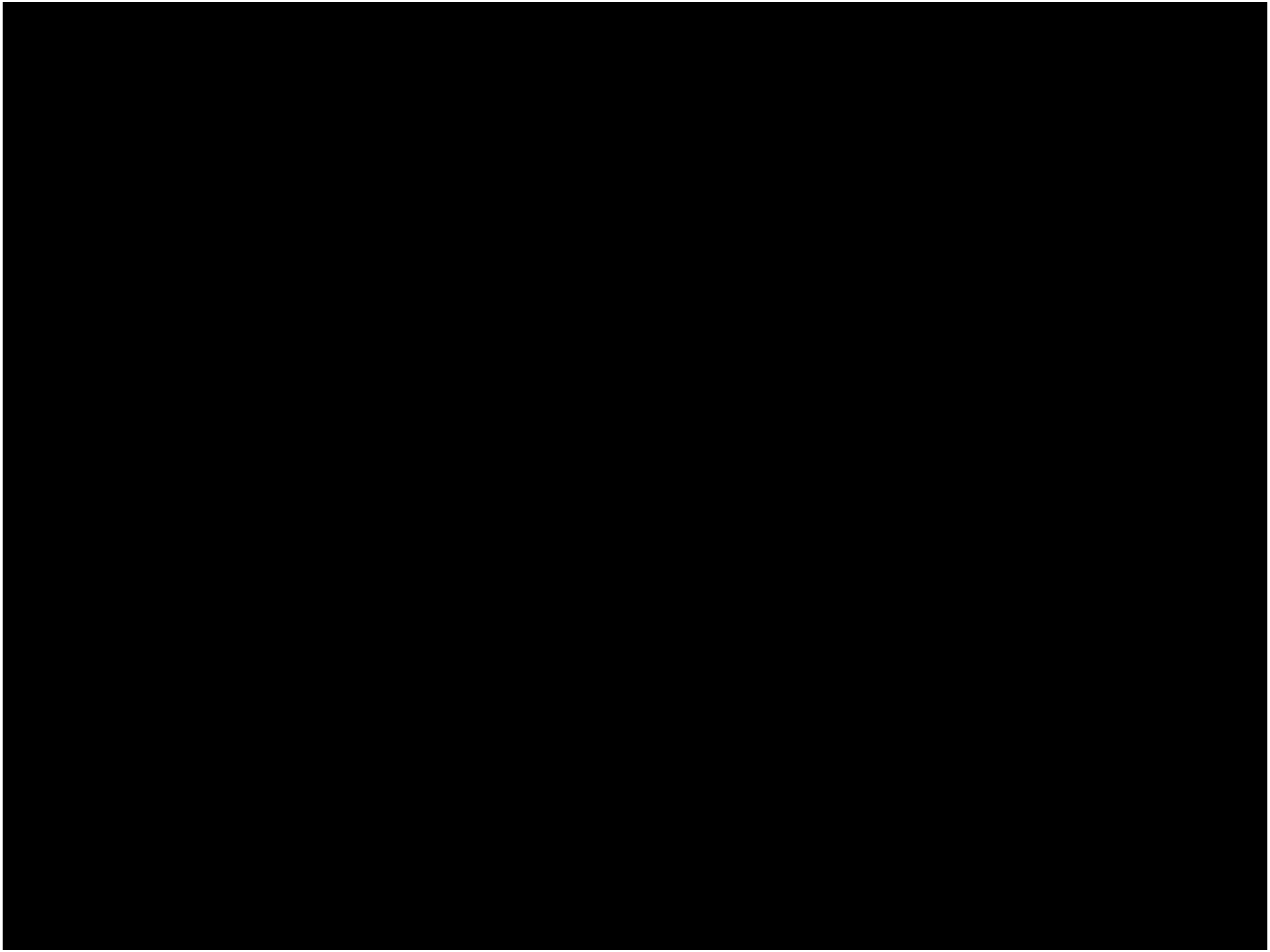
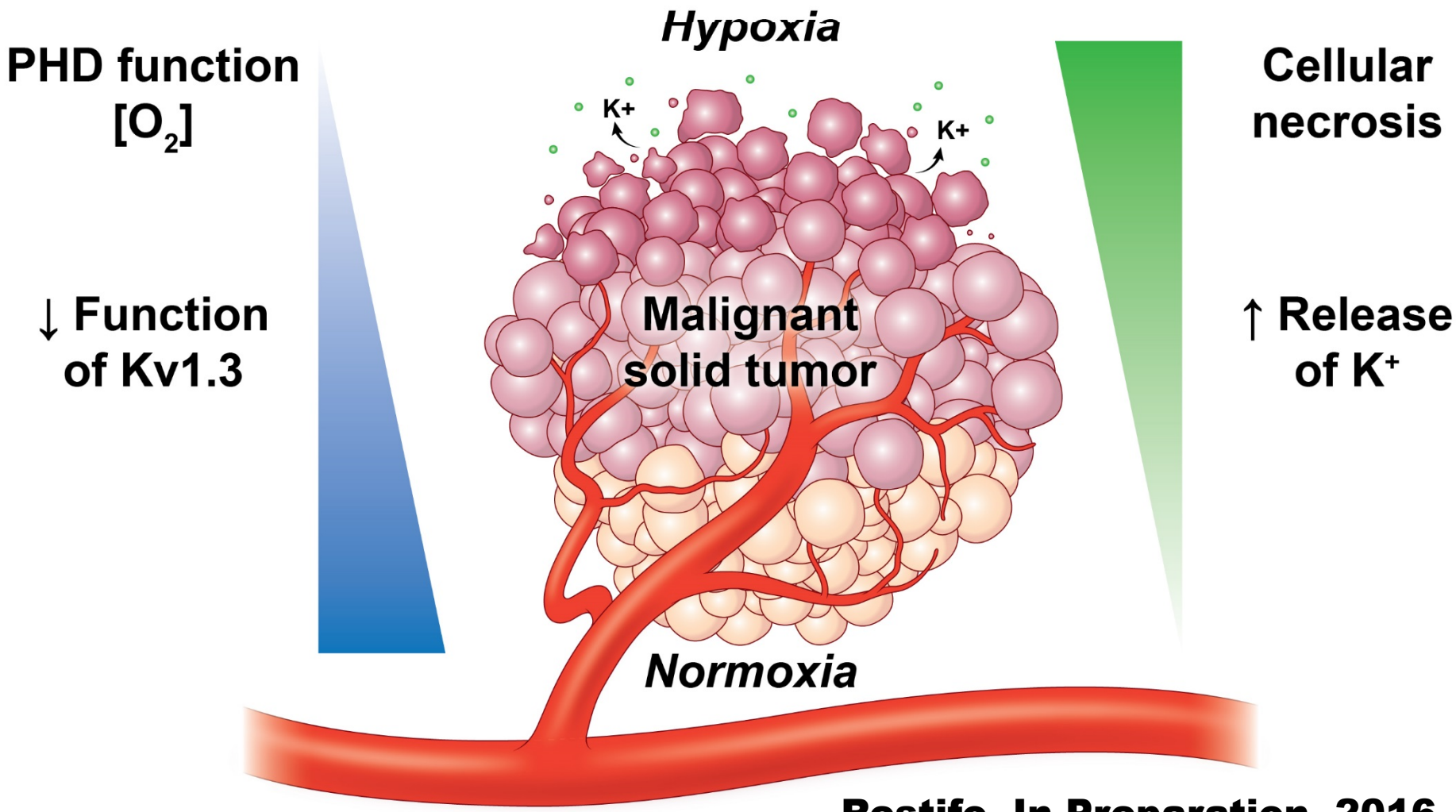


Increased hypoxia accompanies progressive tumor growth



Restifo, In Preparation, 2016