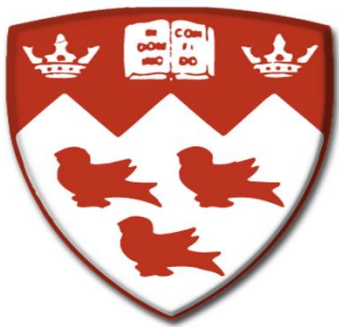




**Low tryptophan 2,3 dioxygenase (TDO) expression is associated with distinctive immuno-metabolism of Tryptophan and preserved Th17/Treg balance in HIV elite controllers**



**Jean-Pierre Routy M.D.**

**December 5 , 2013**



**6** HIV Persistence  
during Therapy  
Sixth International Workshop

Session VI: Immunology of HIV persistence

Miami, December 3-6, 2013



# Tryptophan (Trp) metabolism and HIV infection

- Immune activation in ART treated patients:
  - Linked with non-AIDS events
  - CTL and CD4 Help function
  - Reservoir size
- Trp catabolism in HIV infection
  - Th17/Treg differentiation <sup>1</sup>
  - Immune activation, dysbiosis and gut microbiota<sup>2</sup>
  - Immuno-metabolism pathway<sup>3</sup>



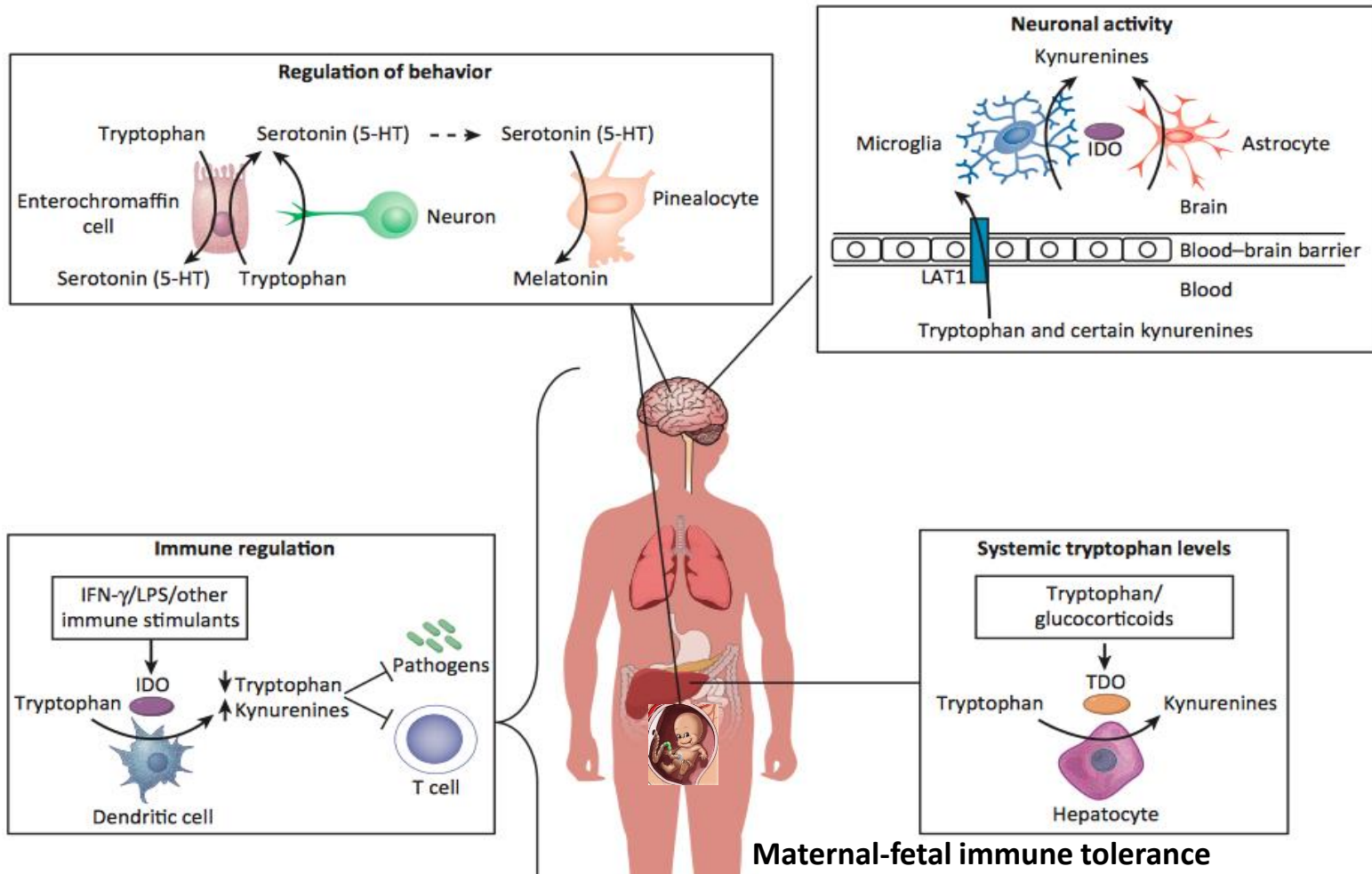
# Tryptophan



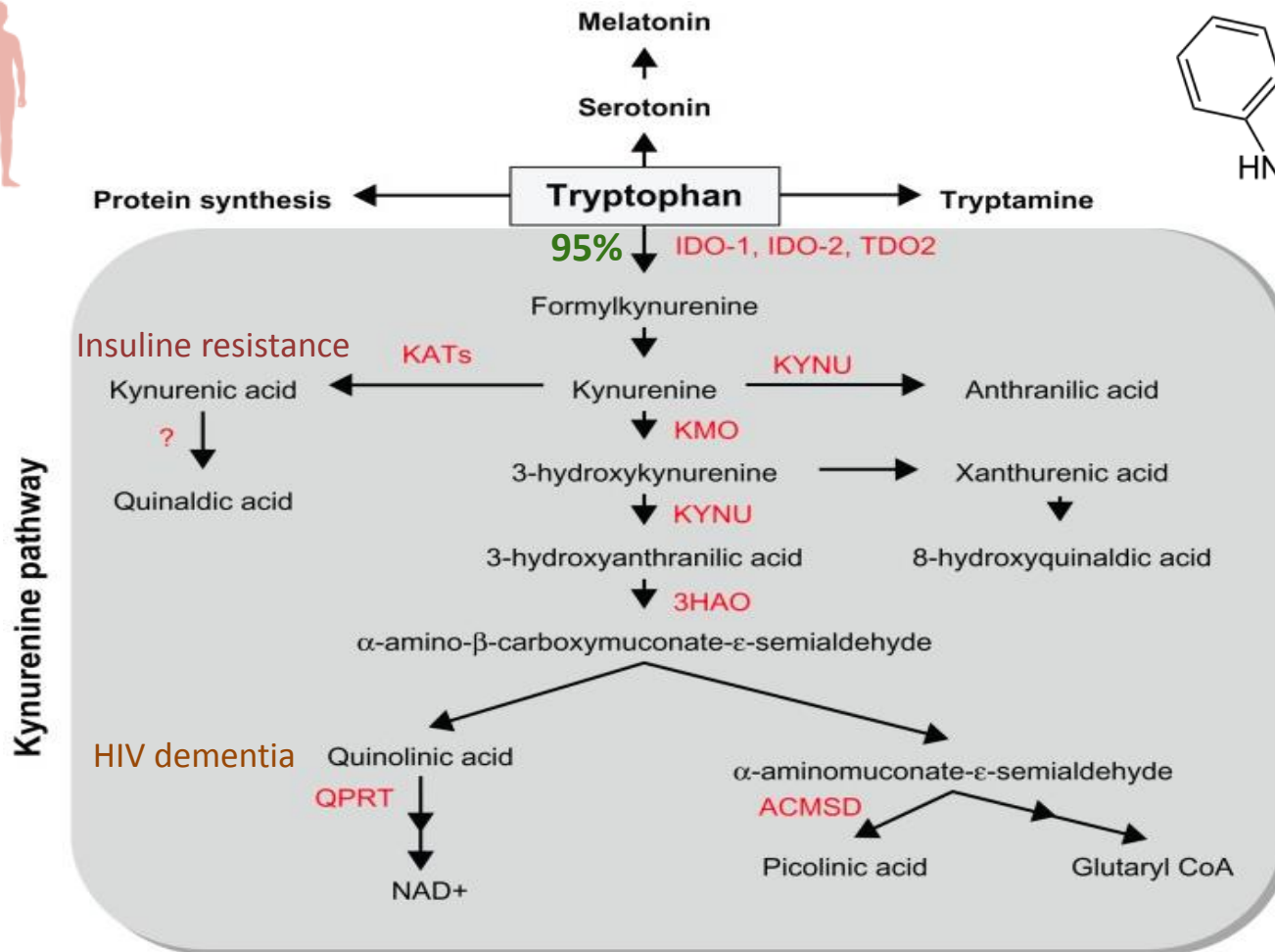
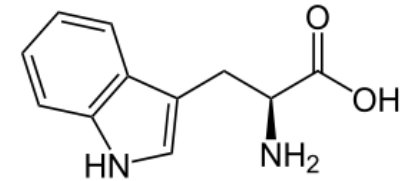
- 1 of the 9 essential AA only provided by:
  - diet and gut bacterial production<sup>1,2</sup>
- The only AA that bind to albumin (85-90%)
- BBB: Only free form via competitive L-type AA transporter
- Tryptophan levels determined by:
  - Food intake, type of bacterial production in the gut
  - Pathways that convert tryptophan: 95% through the kynurenine
- Role:
  - Protein synthesis
  - Production serotonin, melatonin, tryptamine
  - Tryptophan catabolism: **kynurenine pathway (95%)**
    - Synthesis of NAD<sup>+</sup> a coenzyme for energy metabolism
    - **Local immunosuppression and systemic tolerance via Treg**

<sup>1</sup>Vujkovic-Cvijin et al. Sci transl Med, 2013 <sup>2</sup>Zelante Immunity 2013 Munn Tren in Immunol 2013

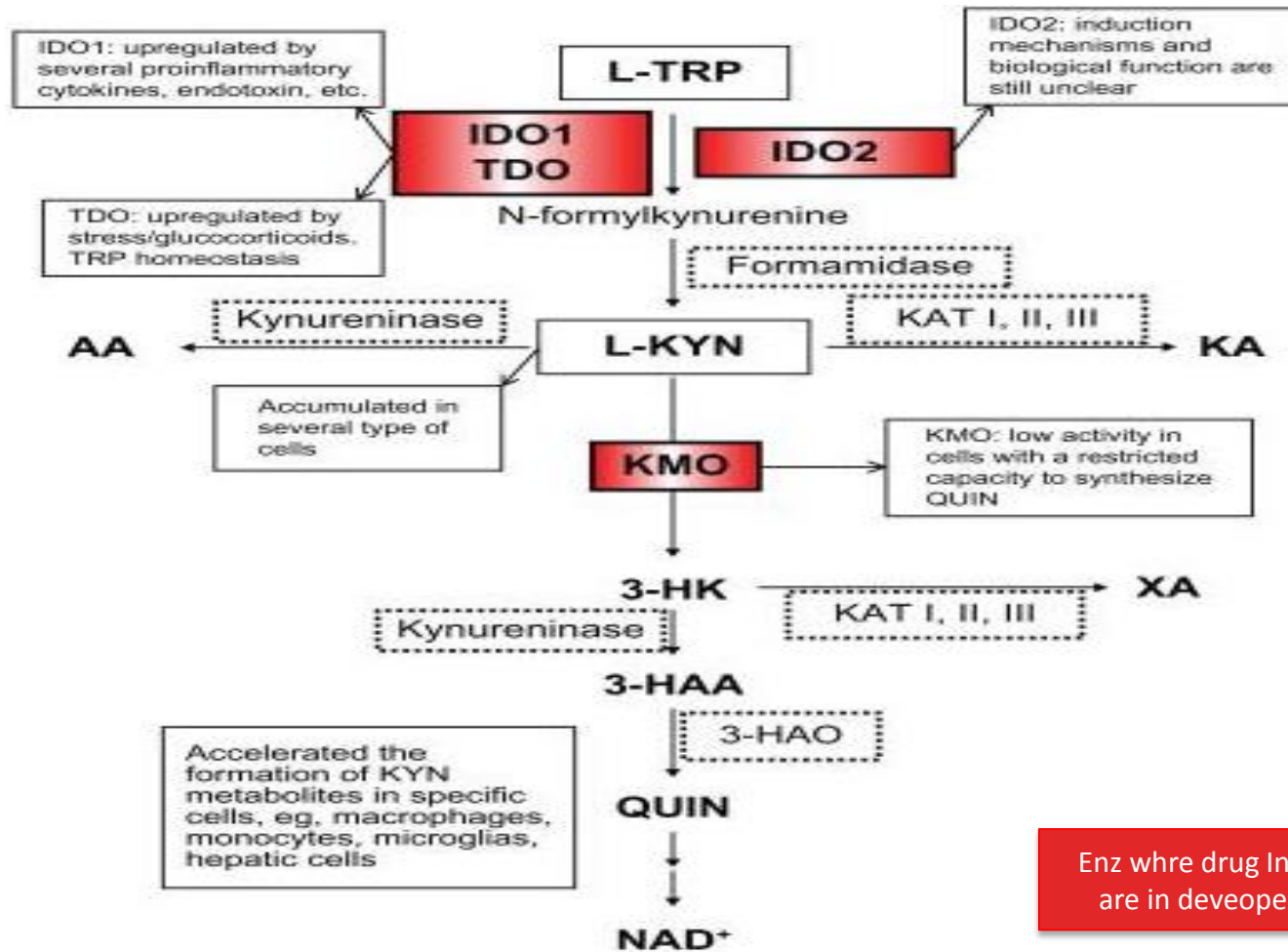
# Tryptophan metabolism in health and disease



# Tryptophan catabolism by IDO or TDO and downstream enzymes



# Trp pathway: enzymes and catabolites



# IDO



- Isolated in rabbit intestine 1967<sup>1</sup>
- Expression in **certain tissues and cancer cells**
  - Epithelial cells: Gut
  - APC: DCs, macrophages, B cells
  - Microglia, neurons, astrocytes
- Regulated by inflammatory signals:
  - INF- $\gamma$ , LPS, TNF $\alpha$ , amyloid peptides, TAT protein
- IDO-mediated Trp catabolism: **Treg, tolerogenic APC**
  - Prevents fetus rejection during pregnancy<sup>2</sup>
  - Induces peripheral immune tolerance in transplants<sup>3</sup>
  - Suppress immunity in cancers and chronic infections<sup>4,5</sup>

<sup>1</sup>Yamamoto et al. J Bio Chemistry 1967 <sup>2</sup>Munn et al 1998 <sup>3</sup>Gillemin GJ neuroreport 2003

<sup>4</sup>Friberg et al. 2002; Uyttenhove et al. 2003. <sup>5</sup>Planes R Plos One 2013

# TDO

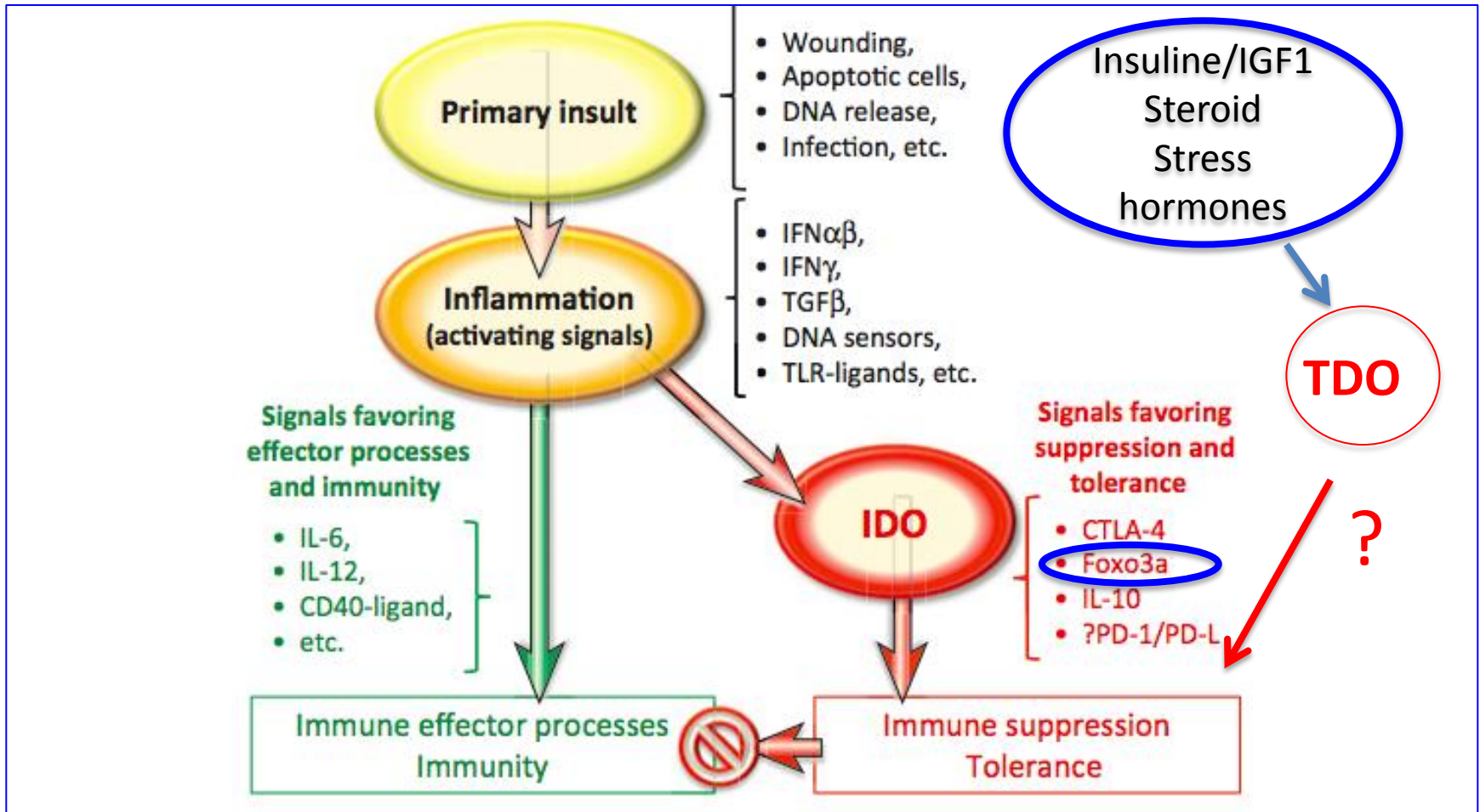


- First Trp catabolite enzyme discovered in 1949
- Isolated in liver<sup>1</sup>
- Rate-limiting reaction<sup>1</sup>
- Predominant expression in:
  - Liver, astrocytes and some neurons<sup>2</sup>
  - Cancer cells
- Regulated by endocrine signals:
  - Induced by cAMP (insulin), cortisol<sup>3</sup>
  - Glucocorticoid receptor (CGR)
- When inhibited or depleted:
  - Reversal of tumoral immune resistance<sup>4</sup>
  - Delay aging in *C.Elegans*<sup>5</sup>

<sup>1</sup>Heidelberger J Biol Chem 1949, <sup>2</sup>Pocivavsek A, *Eur J Neurosci*. 2012 <sup>3</sup>Ren S, et al *Arch Biochem Biophys*. 2000 <sup>4</sup>Pilote et al. *Proc Natl Acad Sci* 2012 van der Goot PNAS 2012



# IDO is activated by inflammation and induces immune suppression and tolerance



# Study objectives

- As Trp metabolism is involved in immune suppression in HIV infection (Favre Sci Transl Med. 2010)
- To assess in circulating blood:
  - Trp metabolite levels & Trp enzyme expression
- Study population: **Elite controllers (EC)**
  - Higher CD14<sup>++</sup>CD16<sup>+</sup> mono. compared to controls<sup>1,2</sup>
  - Transcription factor phosphorylation<sup>3</sup>
    - Longer CM T cell survival linked to inactivation of the transcription factor **FOXO3a**
    - Good for CD8 memory<sup>4</sup>, stem cells<sup>5</sup> and for aging too<sup>6</sup> !!

<sup>1</sup>Van Grevenynghe & Sekaly Nat Med 2008 <sup>2</sup>Hatano H Plos Path 2013 <sup>3</sup>Krishnan S JID 2013

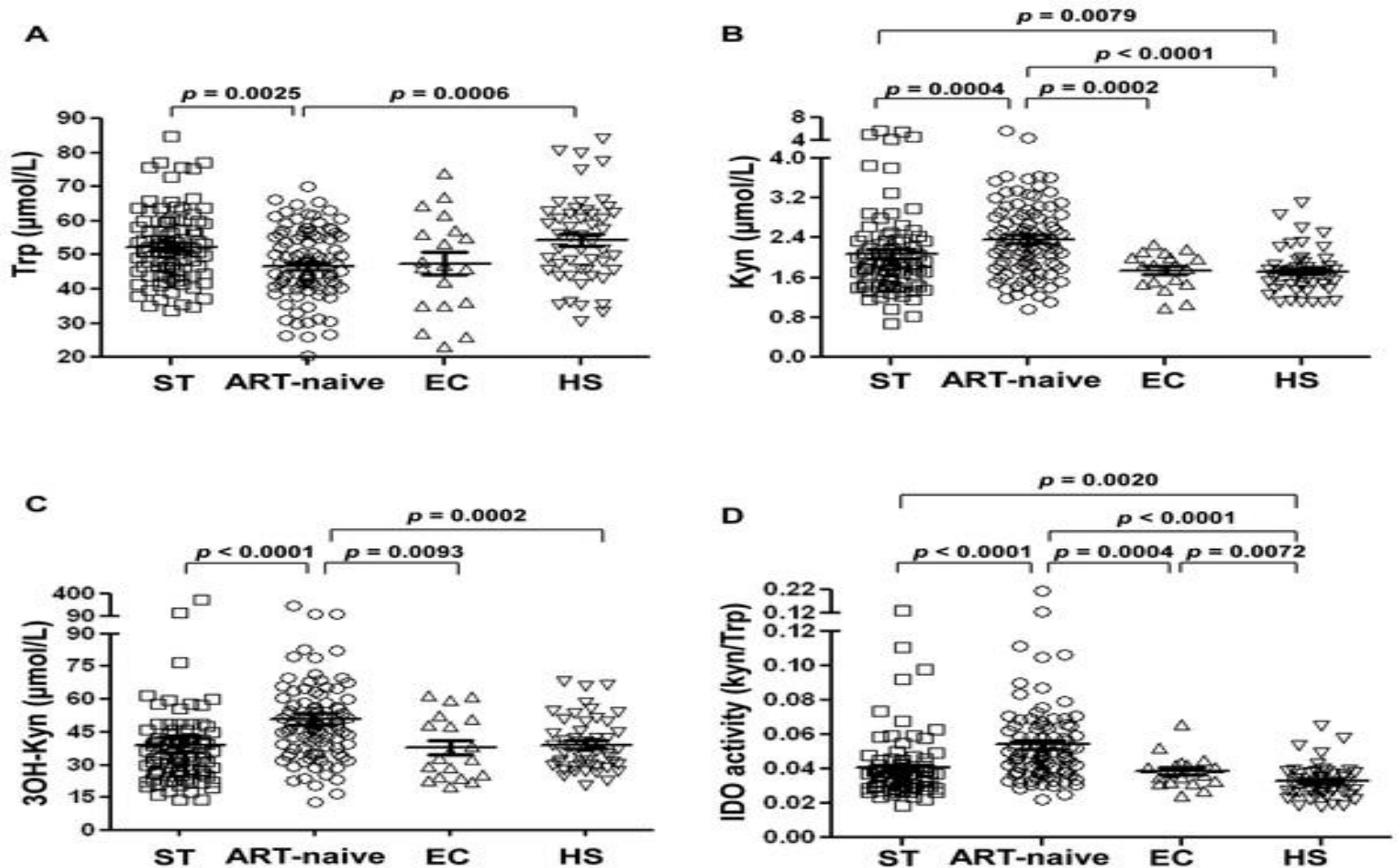
<sup>4</sup>Tzelepis J Immunol 2013 <sup>5</sup>Warr MR Nature 2013, <sup>6</sup>Brooks-Wilson Hum Genet 2013

# Study population

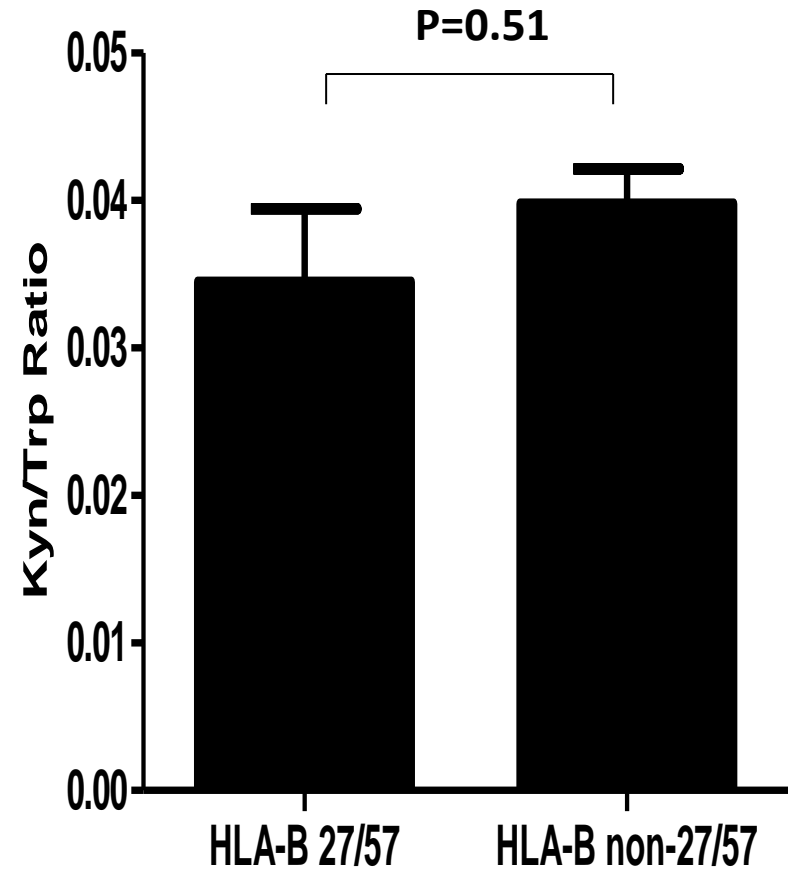
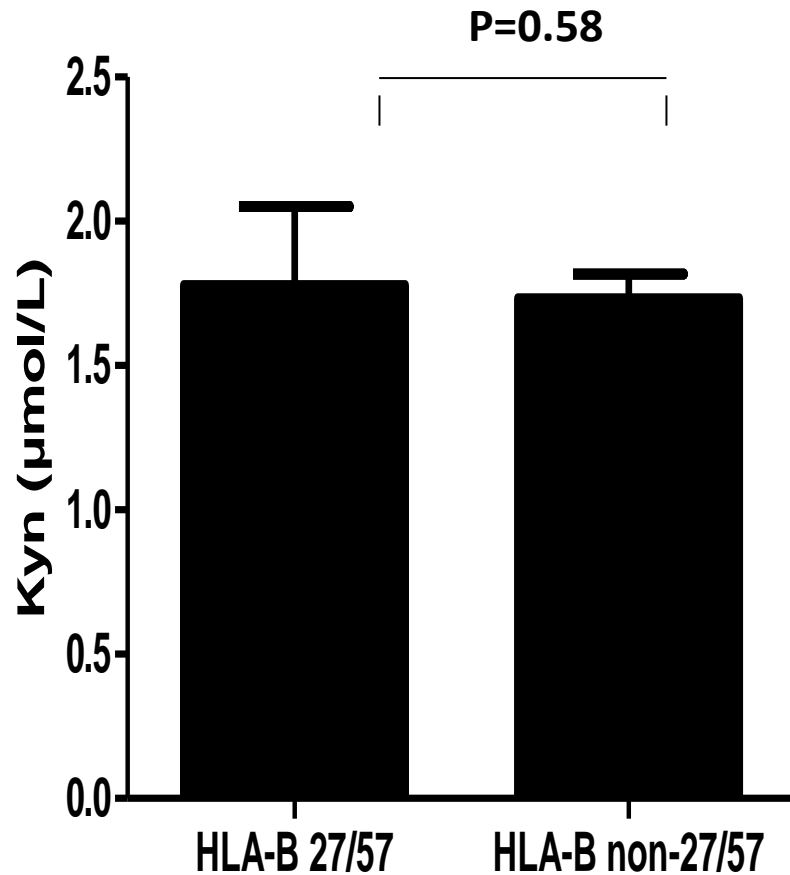
Characteristics	Healthy subjects	Study population N=253		Successfully treated
	HS (n=50)	Elite (n=19)	Naïve (n=96)	ST (n=88)
Age (years) [Mean ± SD (range)]	48.5 ± 7.2 (40-62)	49.4 ± 7.8(41-62)	40.5 ± 7.4 (23-53)	47.7 ± 7.6 (29-63)
Male [n (%)]	34 (76%)	10 (50%)	76 (79%)	12 (71%)
Risk factors:				
MSM [n (%)]	NA	7 (35%)	57 (59%)	10 (59%)
Heterosexual [n (%)]		10 (50%)	23 (24%)	4 (24%)
Time since HIV-1 diagnosis (years) [Mean ± SD (range)]	NA	12.8± 7.0 (7-20)	6.8 ± 7.8 (0-22)	8.9± 4.3 (3-18)
CD4 T cell count (cells/μl) [Mean ± SD (range)]	788.3.3 ± 275.6 (281-1224)	622.1 ± 204.7 (417-1341)	391.9 ± 168.3 (30-814)	556 ± 173 (400-1116)
CD8 T cell count (cells/μl) [Mean ± SD (range)]	358.7 ± 172 95-732)	569.4 ± 301.9 (162-1193)	743.1 ± 324.6 (293-1759)	758 ± 227 (452-1151)
CD4:CD8 ratio [Mean ±SD (range)]	2.53 ± 0.95 (0.38-4.34)	1.32 ± 0.94 (0.35-2.26)	0.60 ± 0.34 (0.06-2.09)	0.78 ± 0.29 (0.39-1.51)
Viral load (log10copies/mL) [Mean ±SD (range)]	NA	< 1.6	4.11 ± 0.92 (1.73-6.35)	< 1.6
Time since Viral load < 50 copies/ml (years) [Mean ± SD (range)]	NA	12.8± 7.0 (7-20)	NA	5.2±1.9 (2-8)
Time since start of ART (years) [Mean ± SD (range)]	NA	NA	NA	7.1±3.04(2-12)

Controlled for BMI, Albumin, glucose, cholesterol, depression, anti-depressive drugs

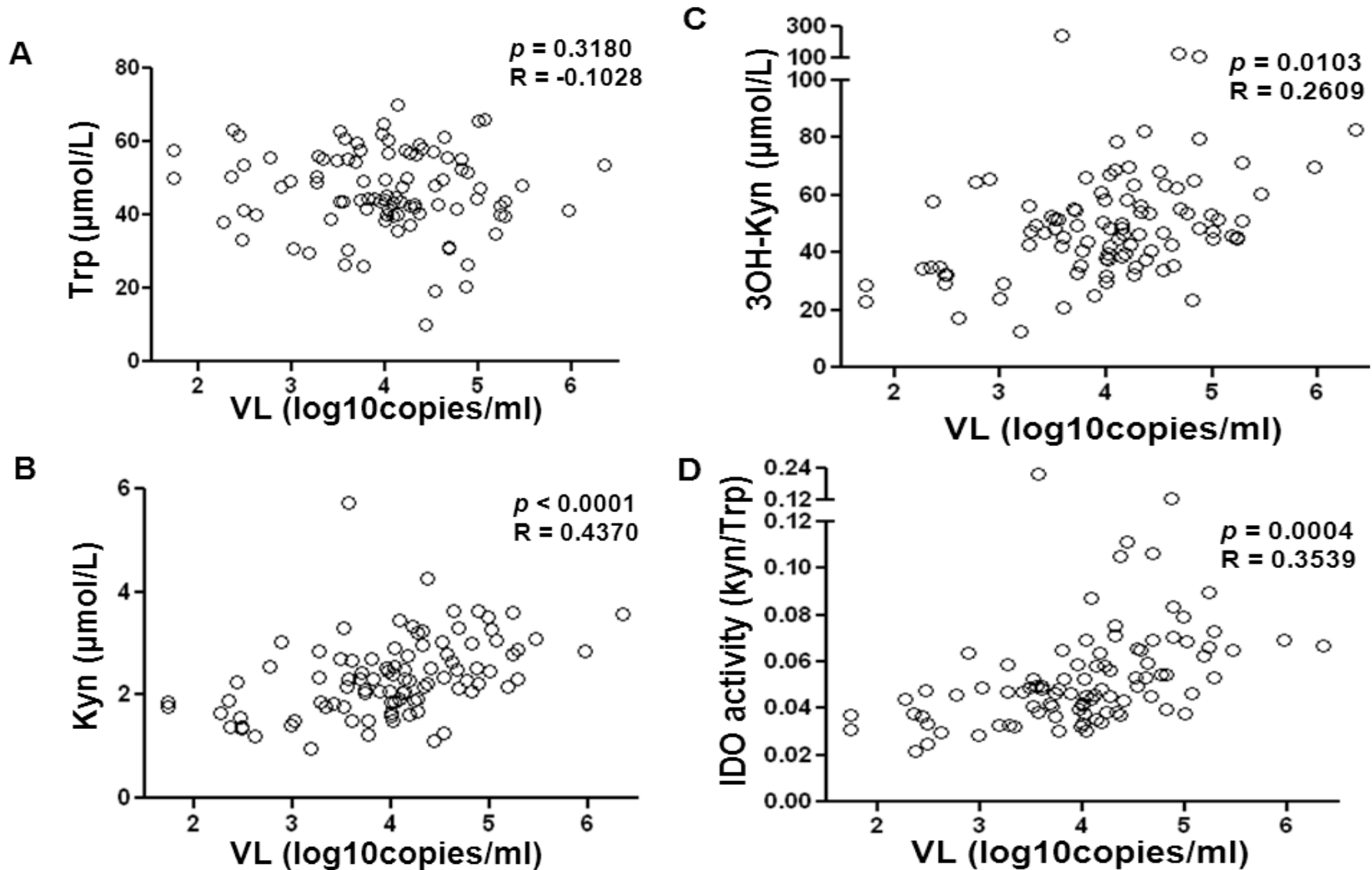
# Trp catabolites and enzymes: In different study groups



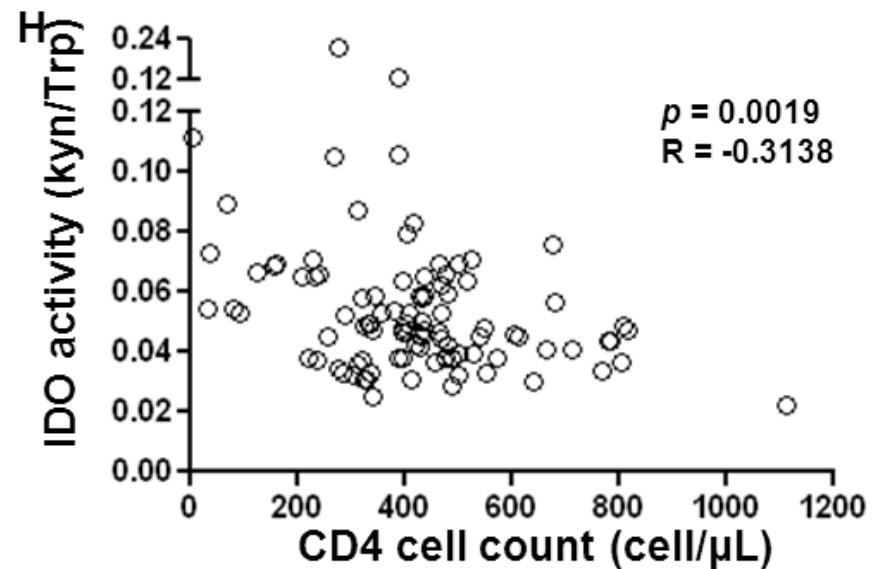
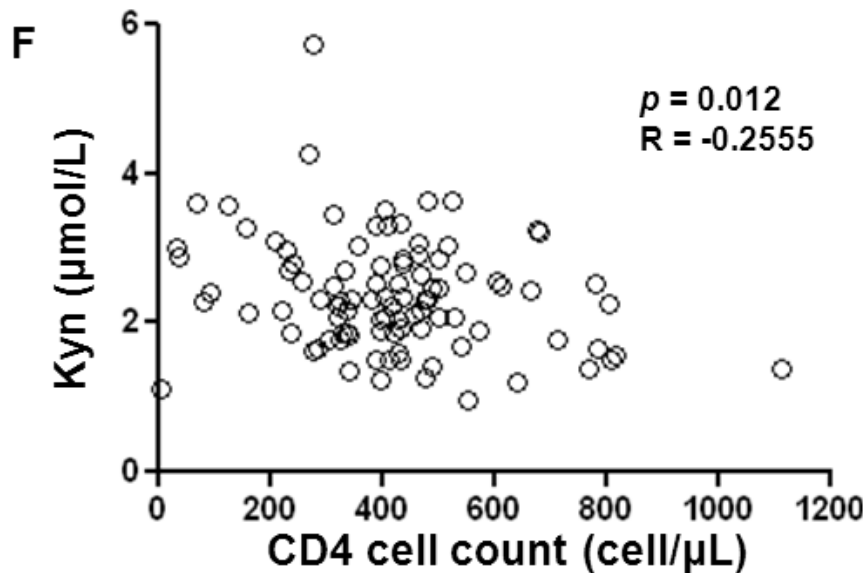
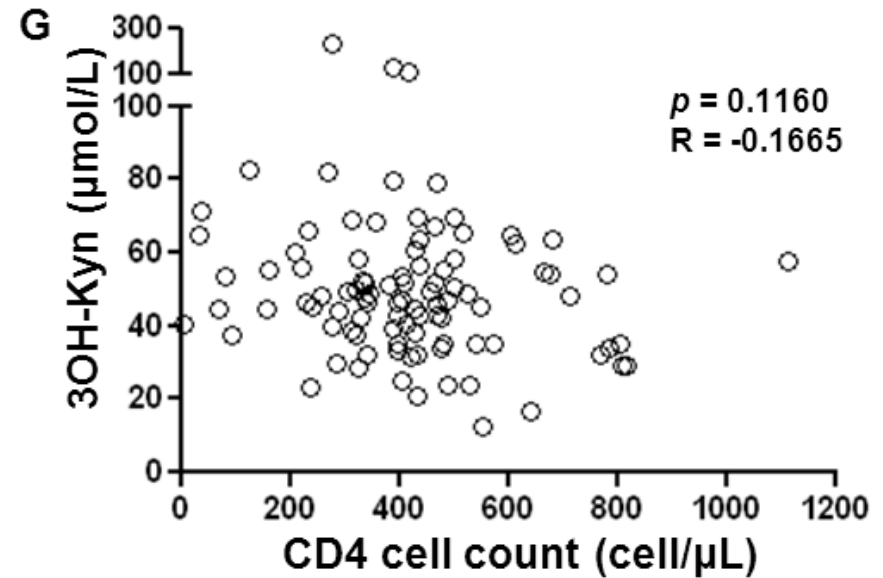
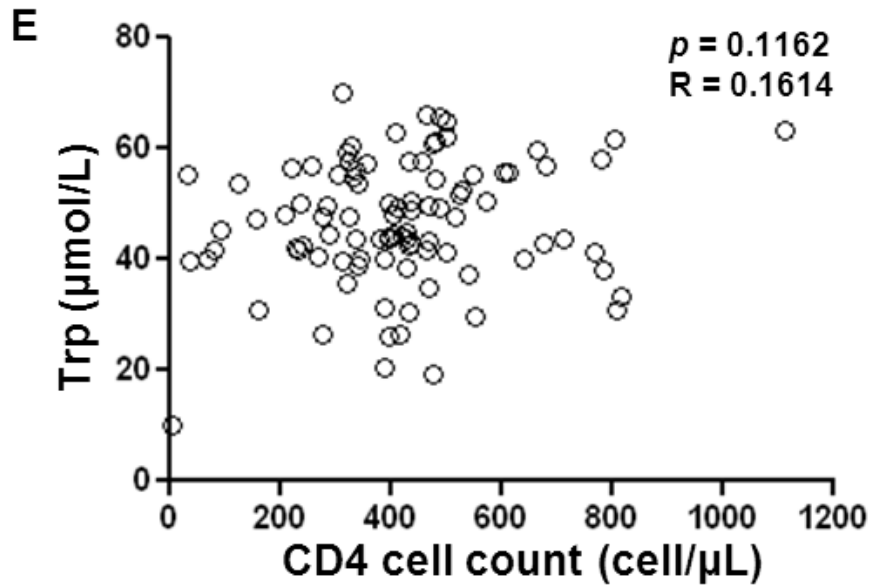
# Trp metabolism in EC is not associated with HLA \*B57 or \*27 haplotypes



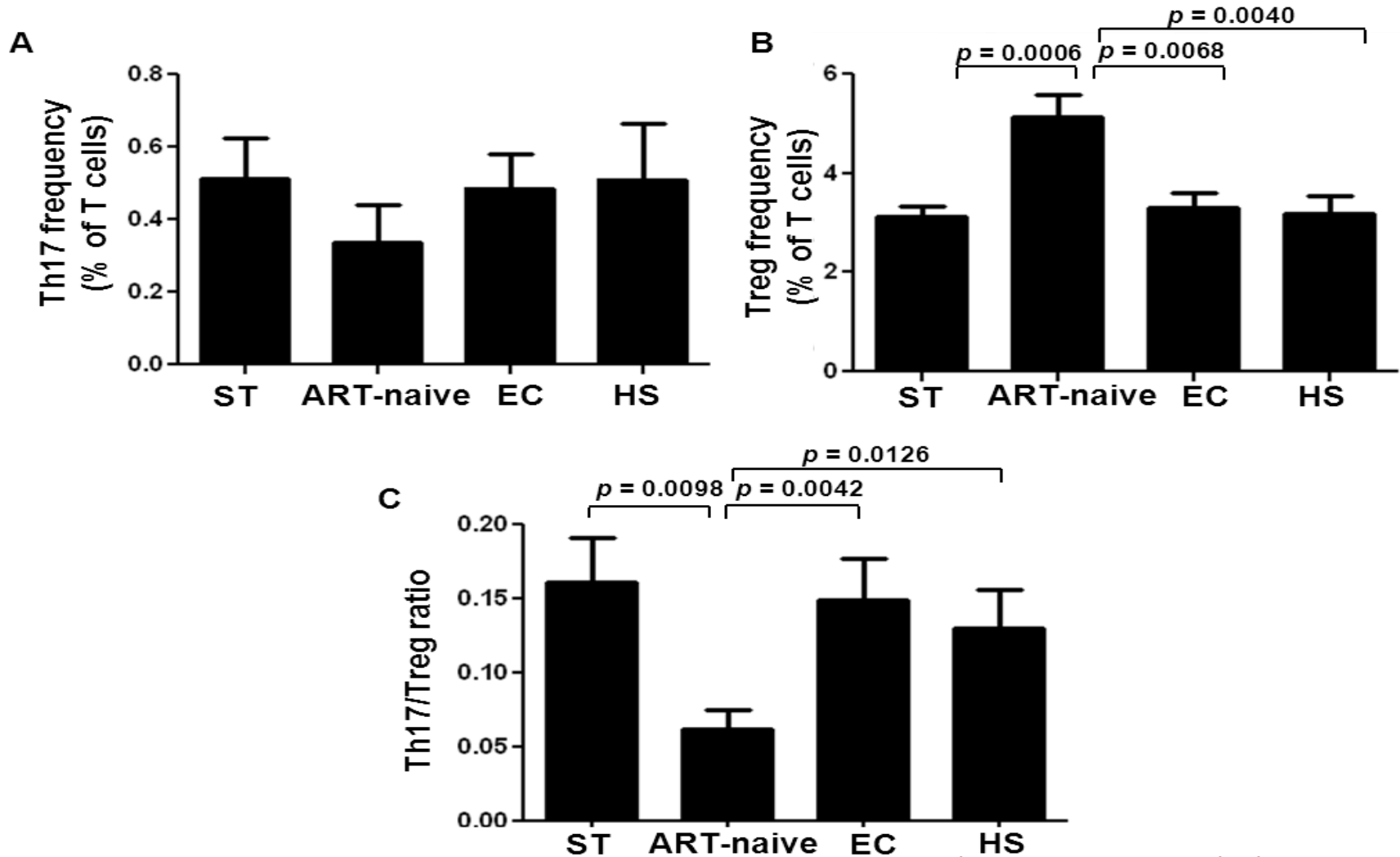
# Trp catabolites and Viral load



# Trp catabolites and CD4 T cells

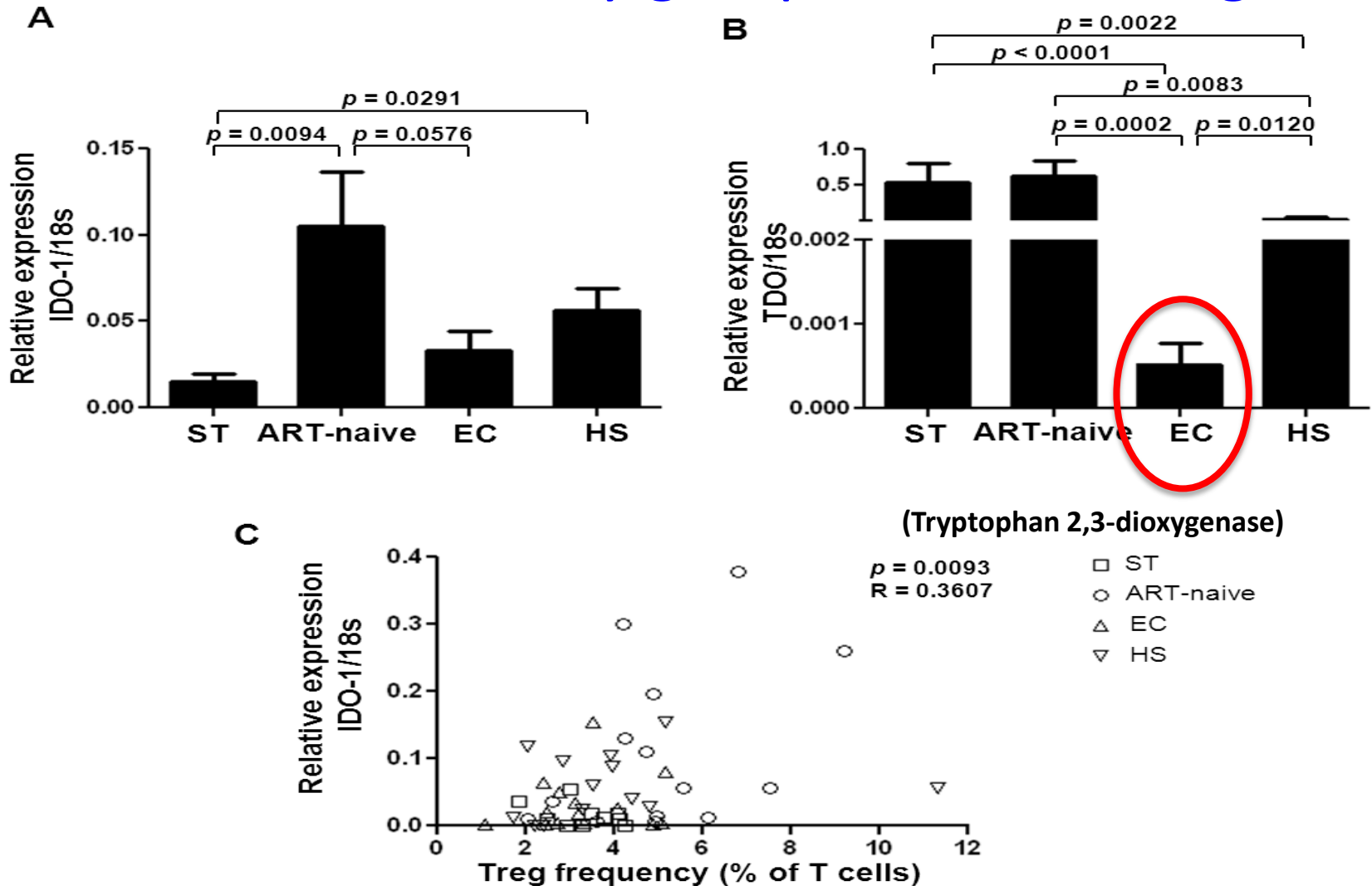


# Th17 and Treg frequency in blood: In different study groups

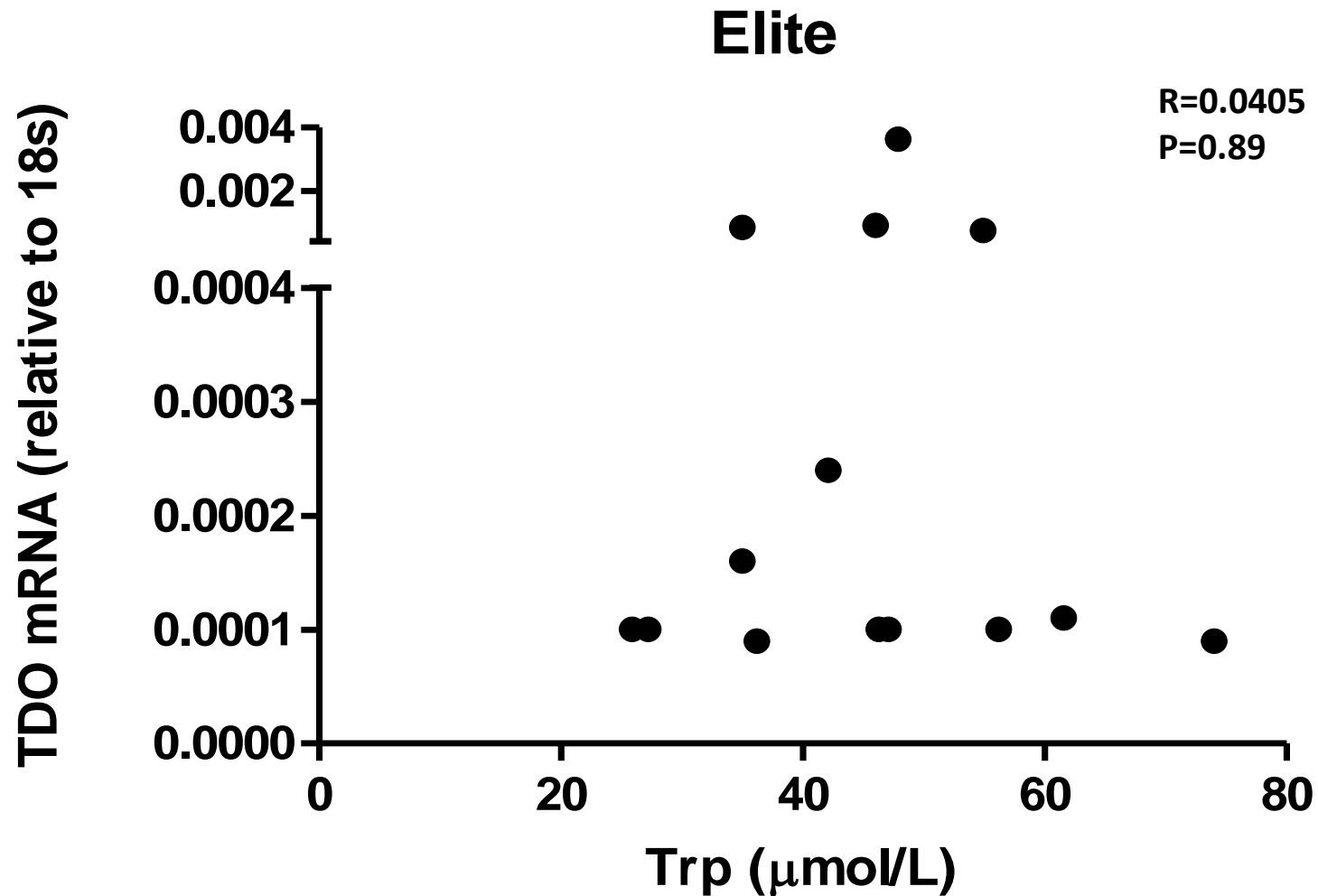




# mRNA expression of IDO and TDO: in different study groups and with Treg



# No correlation between Trp levels and TDO expression



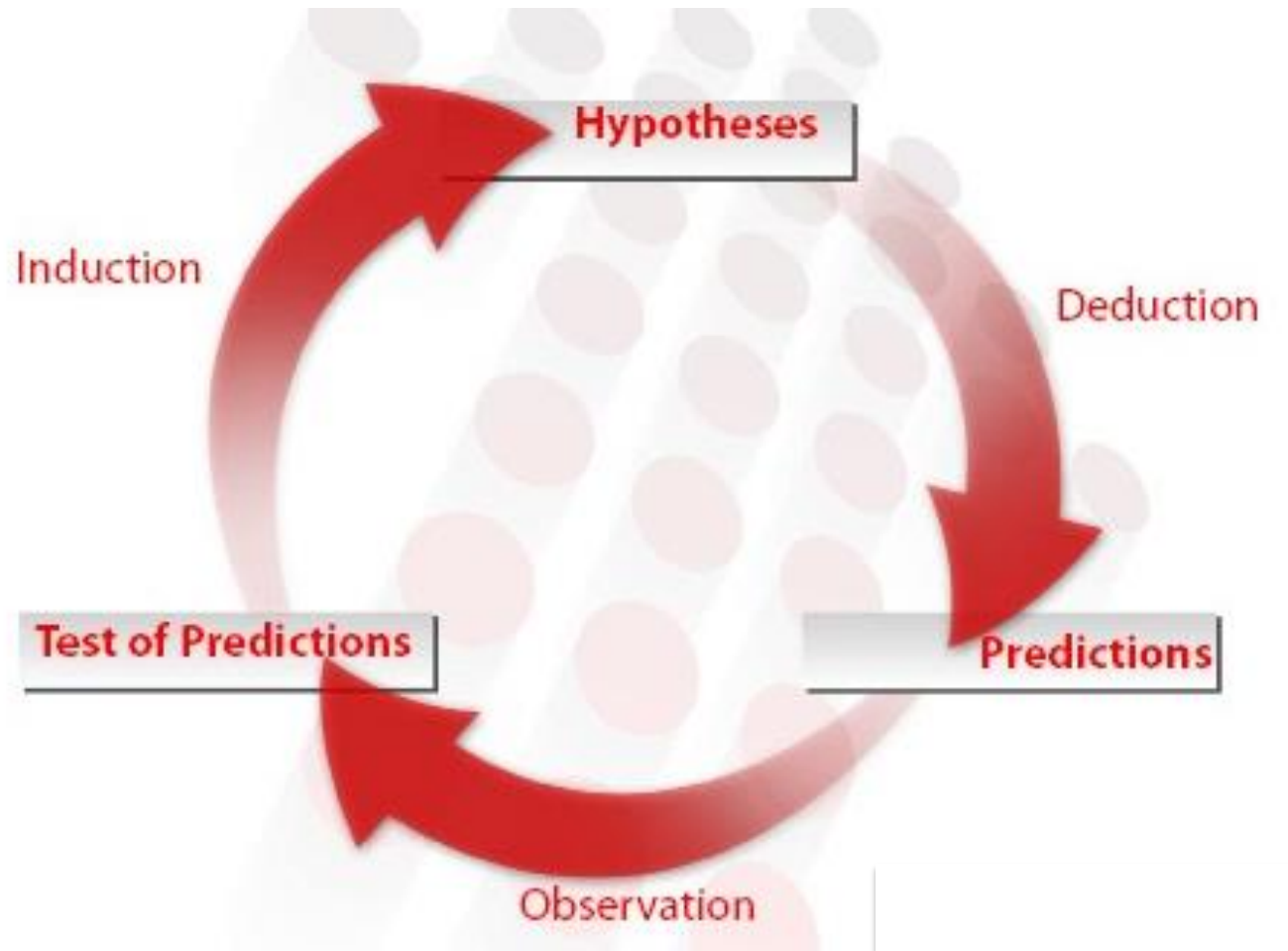
# Study findings and limitations

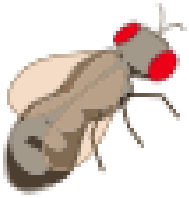
- EC low and homogenous TDO expression:
  - Measured in total PBMCs
  - Not measured in APC: Monocytes, DC, B cells
- TDO expression is very tissue specific but:
  - Can be induced:
    - During pregnancy in the uterus
    - By cancer cells
- TDO and immune regulation



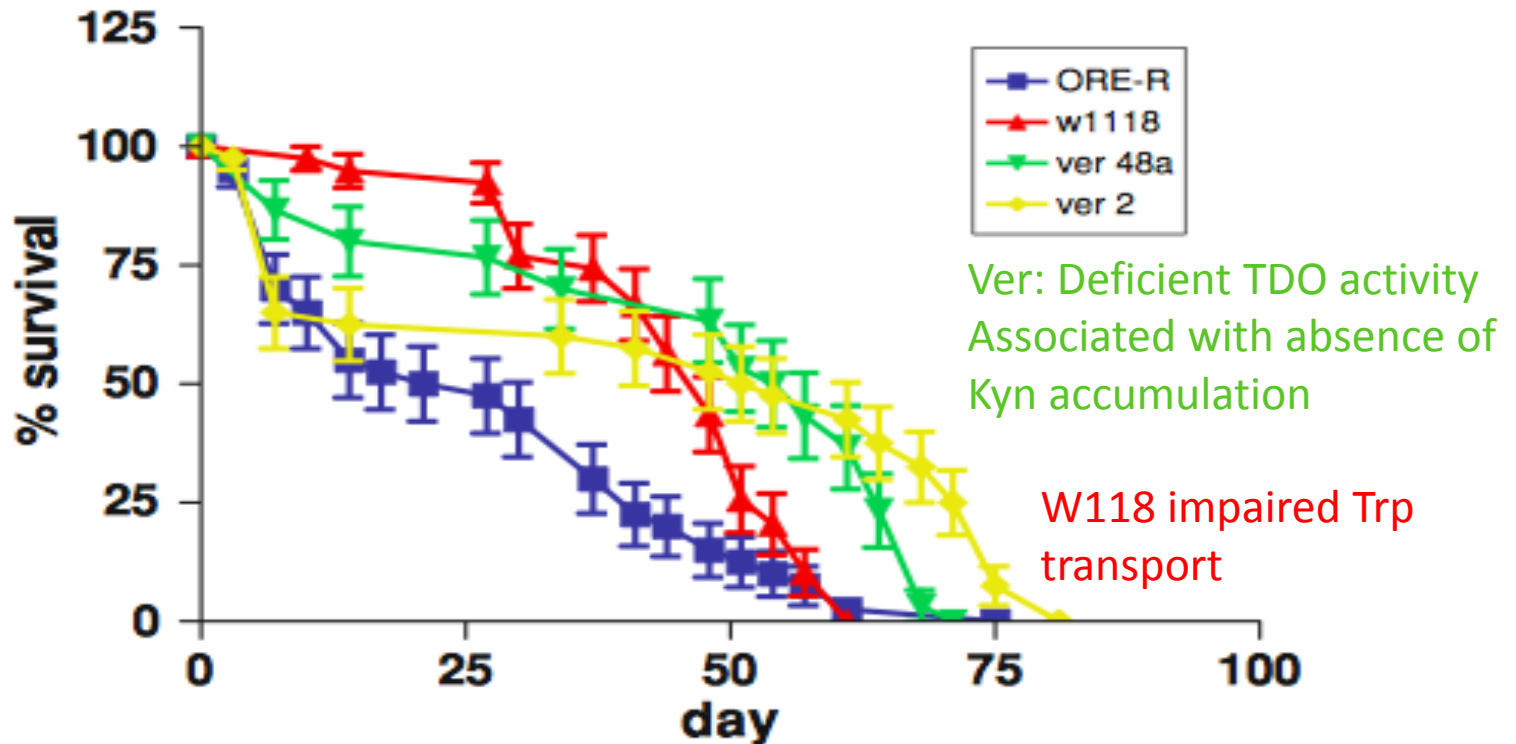
Crystal structure of the tryptophan 2,3-dioxygenase

# TDO and immune control



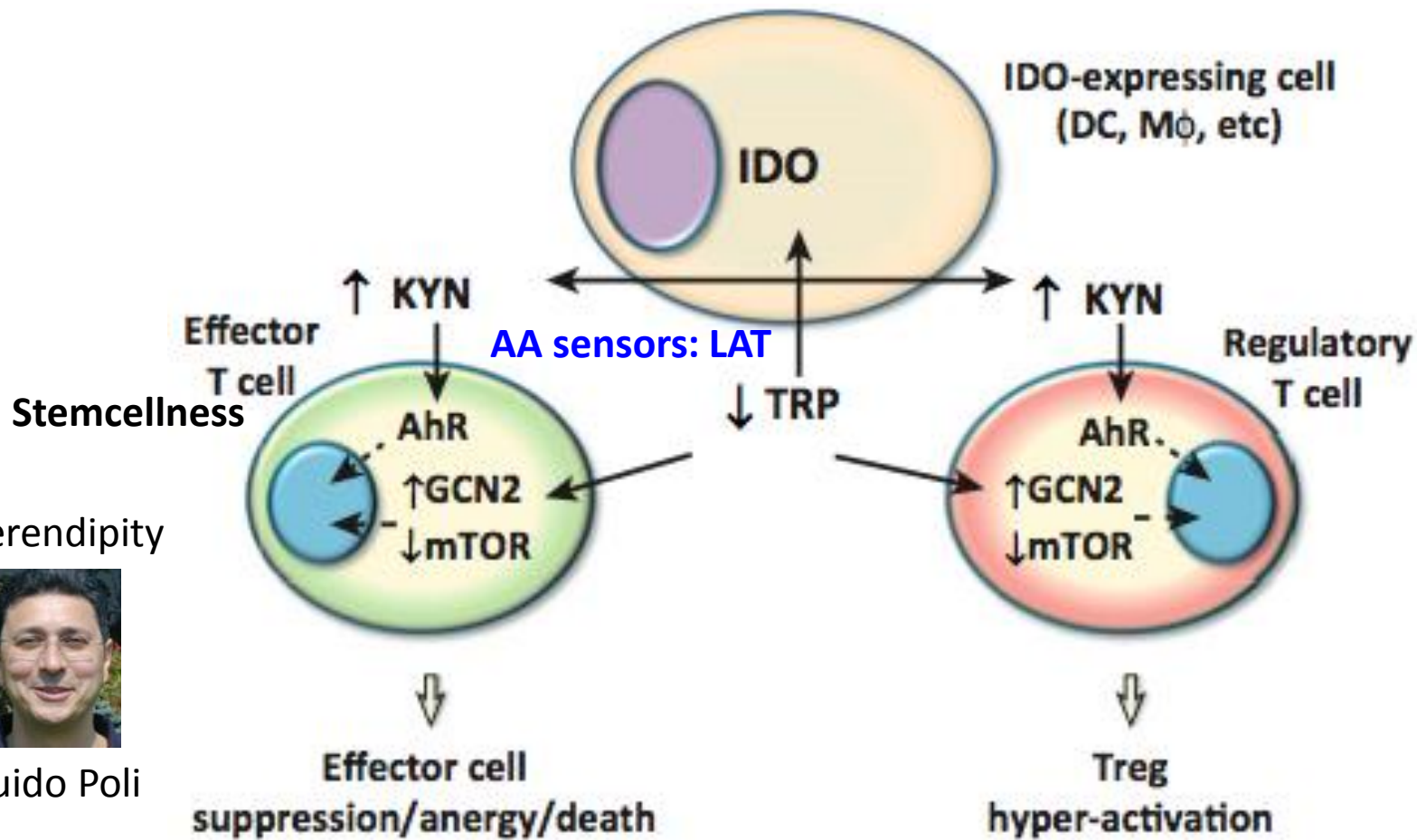


# TDO deficiency associated with increased survival in drosophila



**Fig. 1** Survival time of *Drosophila melanogaster* mutants with impaired formation of kynurenine. *ORE-R* Oregon-R, *w1118* white, *ver 48a* vermilion, *ver2* vermilion hypomorph

# Metabolic control of T cell and Treg responses via Trp pathway (IDO)



Serendipity



Guido Poli

**mTOR: AA sensor**

**GCN2 kinase: Molecular stress-response pathway**

# Tryptophan catabolism and immuno-metabolism control

AEGEAN CONFERENCES

Linking the international scientific community  
Bringing the humanity scholars together

2<sup>nd</sup>

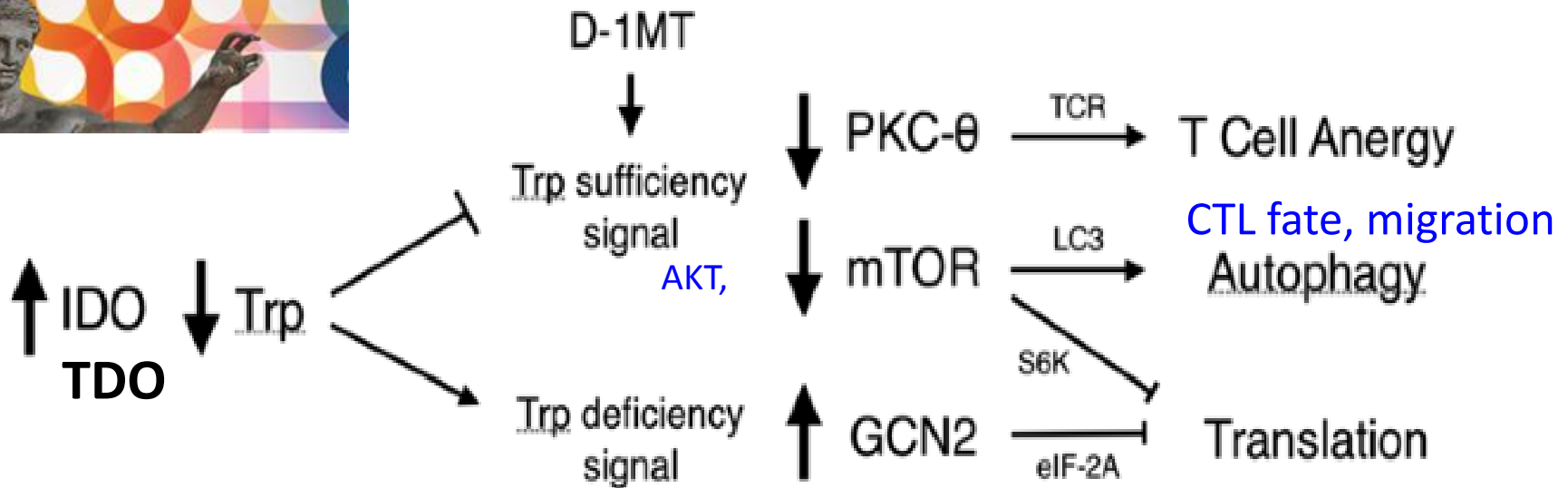
International Conference on ImmuneMetabolism:

Molecular and Cellular  
Immunology of Metabolism



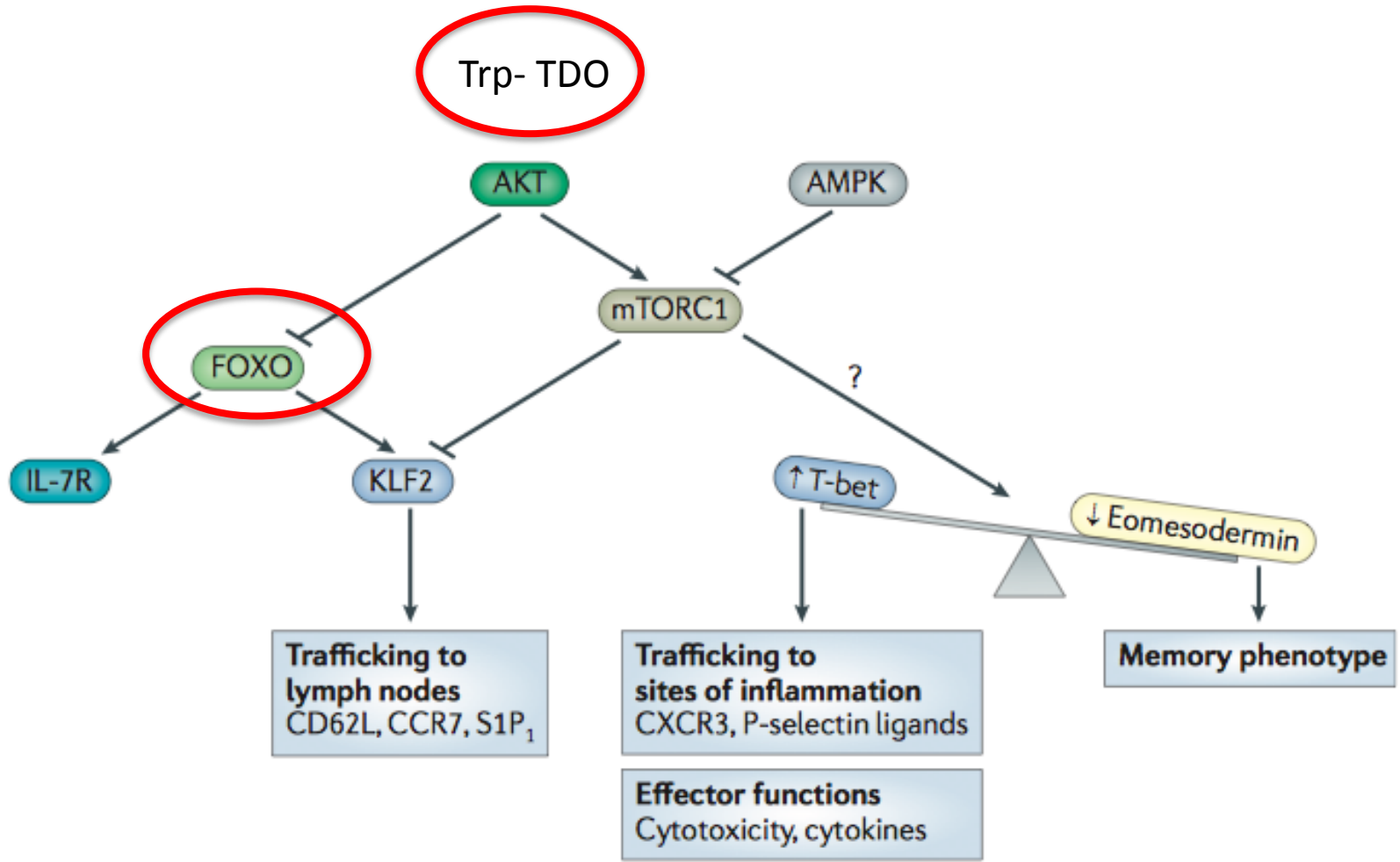
*"To lengthen thy life, lessen thy meals."*

Benjamin Franklin, Poor Richards Almanac (1737).



Metz et al. Oncoimmunology 2012

# Trp catabolism and CD8 fate in EC





# Conclusion

- EC have a distinctive Trp catabolism:
  - Low tryptophan like in viremic patients
  - Without accumulating the immunosuppressive Kyn
  - No Th17/Treg imbalance
- EC have low expression of TDO in PBMCs:
  - Very homogenous expression, not HLA B\*57,\*27 related
  - TDO deficiency in drosophila = increased survival
  - Cause or consequence of immune control ?
  - Importance for HIV reservoir size
- EC have 2 immune-metabolic signatures:
  - **FOXO3a and TDO:**
    - Handling well metabolic stress and aging !!



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