TRANSCRIPTOMIC REGULATORY ANALYSIS OF THE DENDRITIC CELL RESPONSE TO POST-LUNG TRANSPLANT INVASIVE ASPERGILLOSIS

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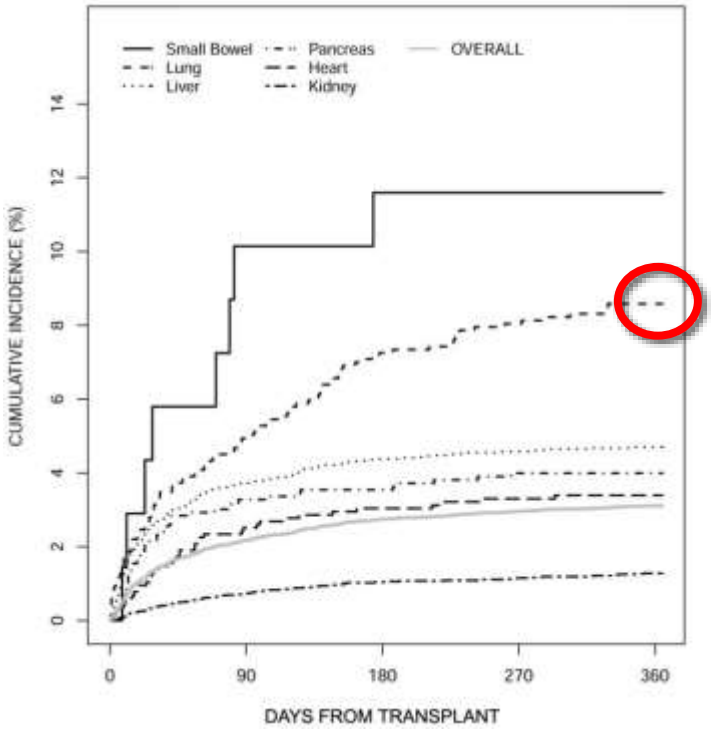
MRC Medical Research Council

NHS National Institute for Health Research

Disclosures

- Nil financial
- I’m not an infection doctor
- I’m a respiratory intensivist

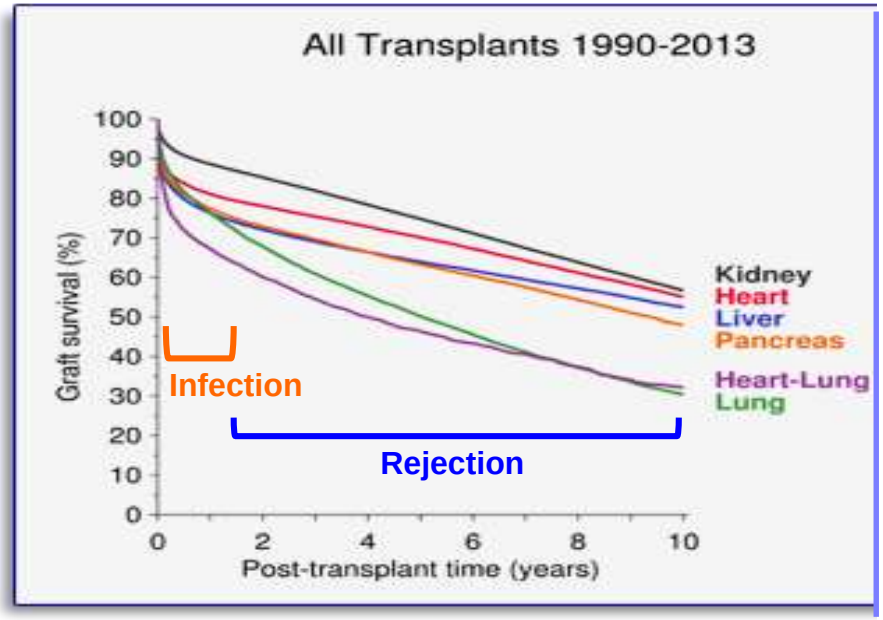
Lung transplantation



TRANSNET study

- risk of invasive fungal infection within 1yr of transplant ➔ highest with lung
- **~50%** of lung transplant IFI's were due to *Aspergillus fumigatus*

~3.8% of lung transplant recipients die within 1st year from *Aspergillus* infection, globally



5-year survival = 51%

Delsing et al. BMC Infectious Diseases 2014, 14:166
http://www.biomedcentral.com/1471-2334/14/166

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series

Corine E Delsing[1†], Mark S Gresnigt[1†], Jenneke Leentjens[1,2†], Frank Preijers[4], Florence Allantaz Frager[5], Matthijs Kox[2,3], Guillaume Monneret[5], Fabienne Venet[5], Chantal P Bleeker-Rovers[1], Frank L van de Veerdonk[1], Peter Pickkers[2], Alexandre Pachot[5], Bart Jan Kullberg[1] and Mihai G Netea[1,6*]

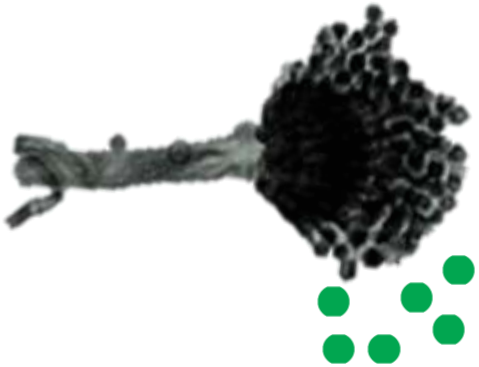
Exogenous Interferon-γ Immunotherapy for Invasive Fungal Infections in Kidney Transplant Patients

D. Armstrong-James[a,†], I. A. Teo[a,†], S. Shrivastava[b,d], M. A. Petrou[c], D. Taub A. Dorling[b,d,e] and S. Shaunak[a,*]

https://www.ishlt.org

Pappas P G et al. *Clin Infect Dis* 2010

Lung transplant



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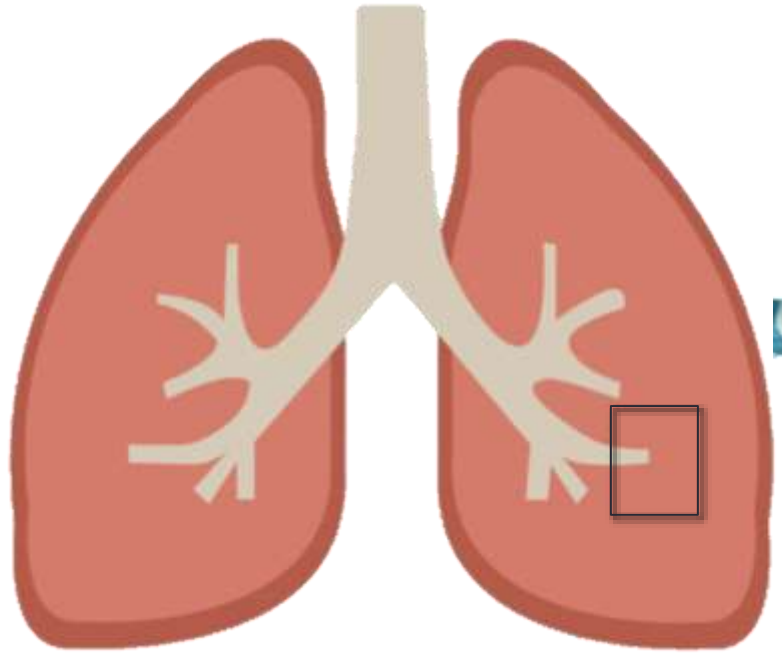

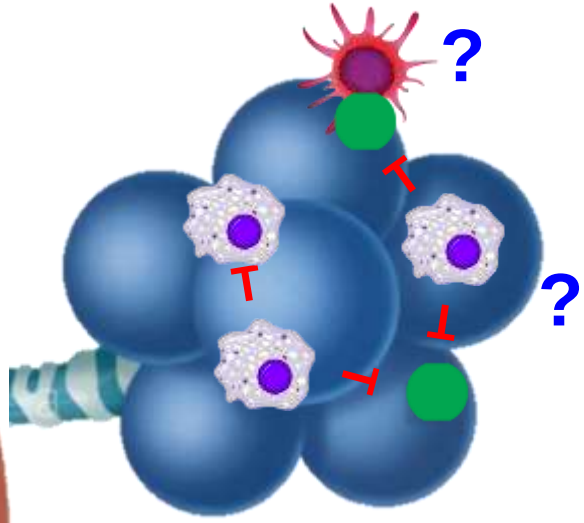

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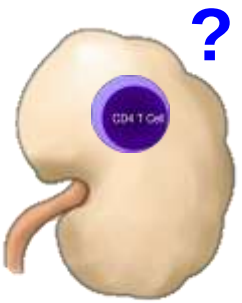

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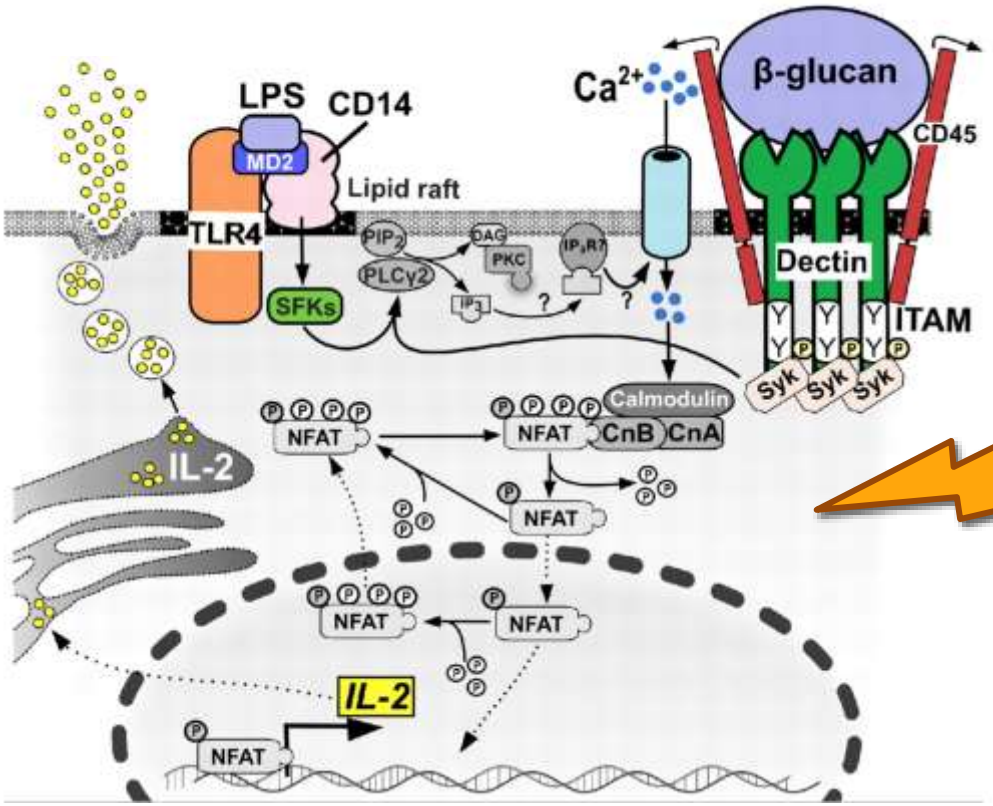
FK506

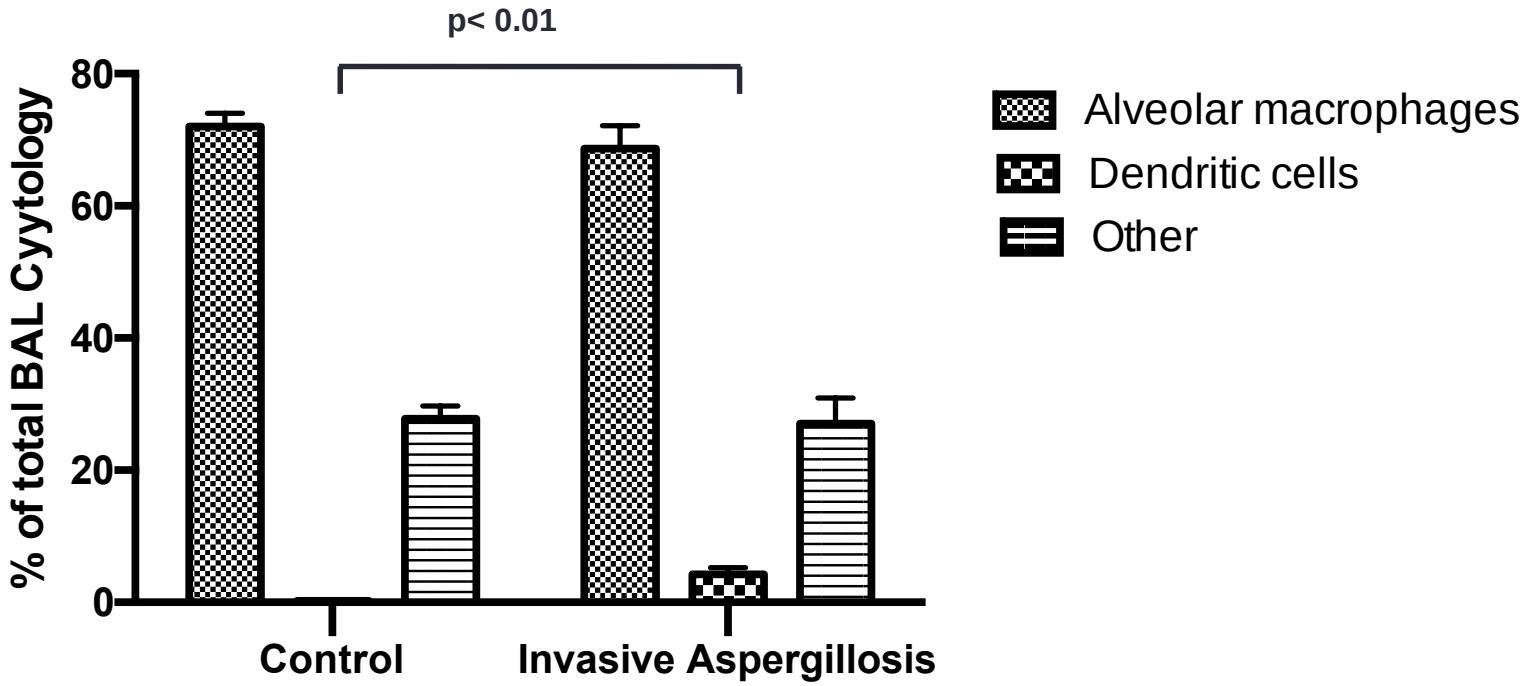
Herbst et el. *EMBO Mol Med* 2015

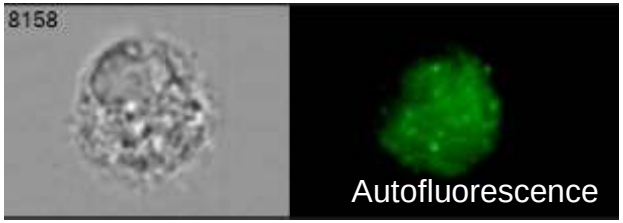
Calcineurin-NFAT axis in myeloid cells

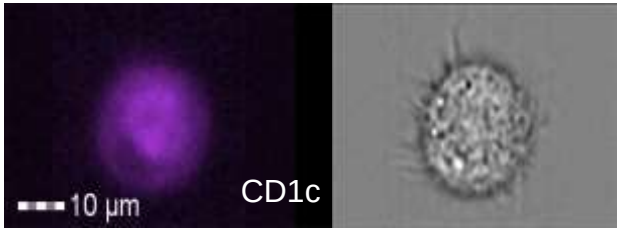


Fric et el. *Blood.* 2012

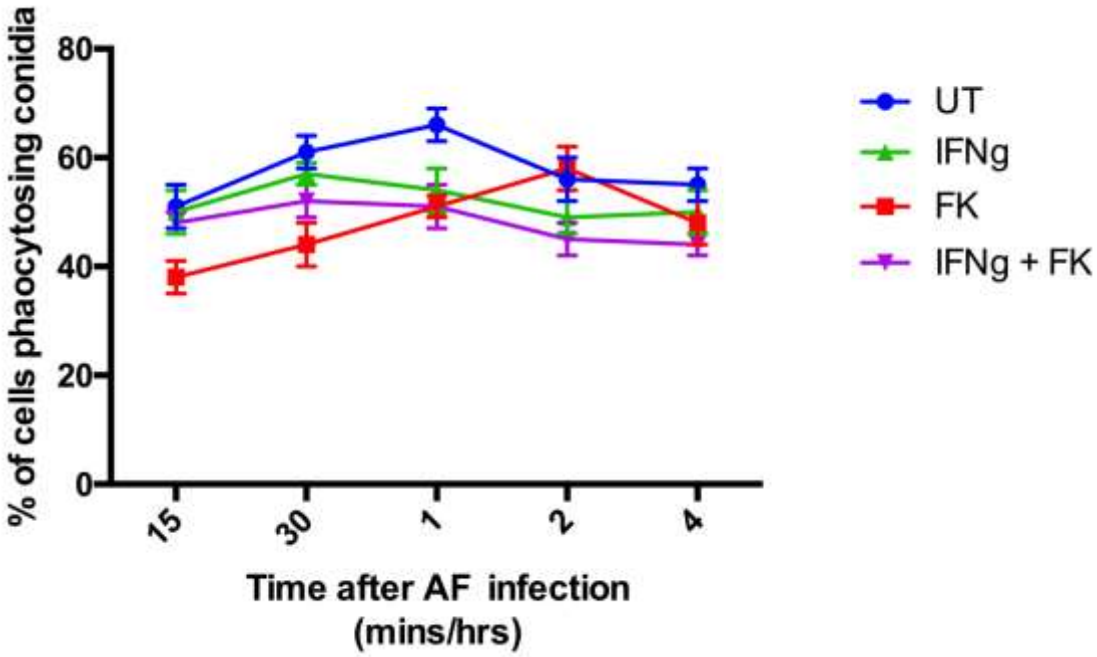
Monocyte-derived dendritic cells migrate to the alveoli in lung transplant recipients with invasive aspergillosis

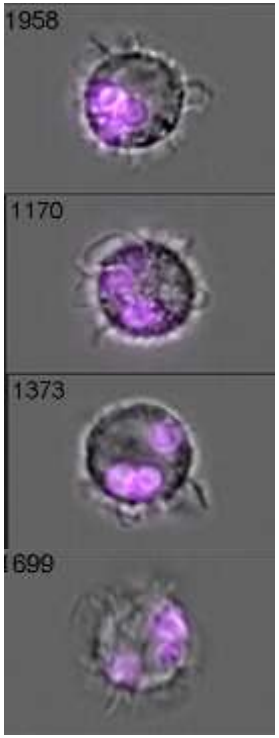






FK506 and IFN-γ alter phagocytosis of *A.fumigatus* swollen conidia by DC's



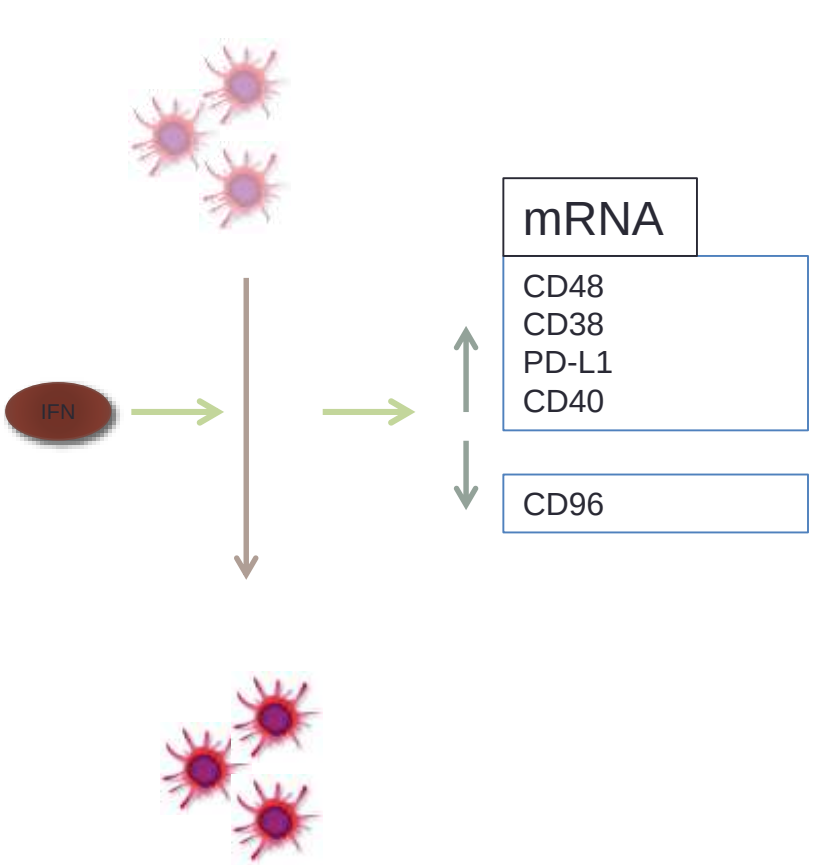


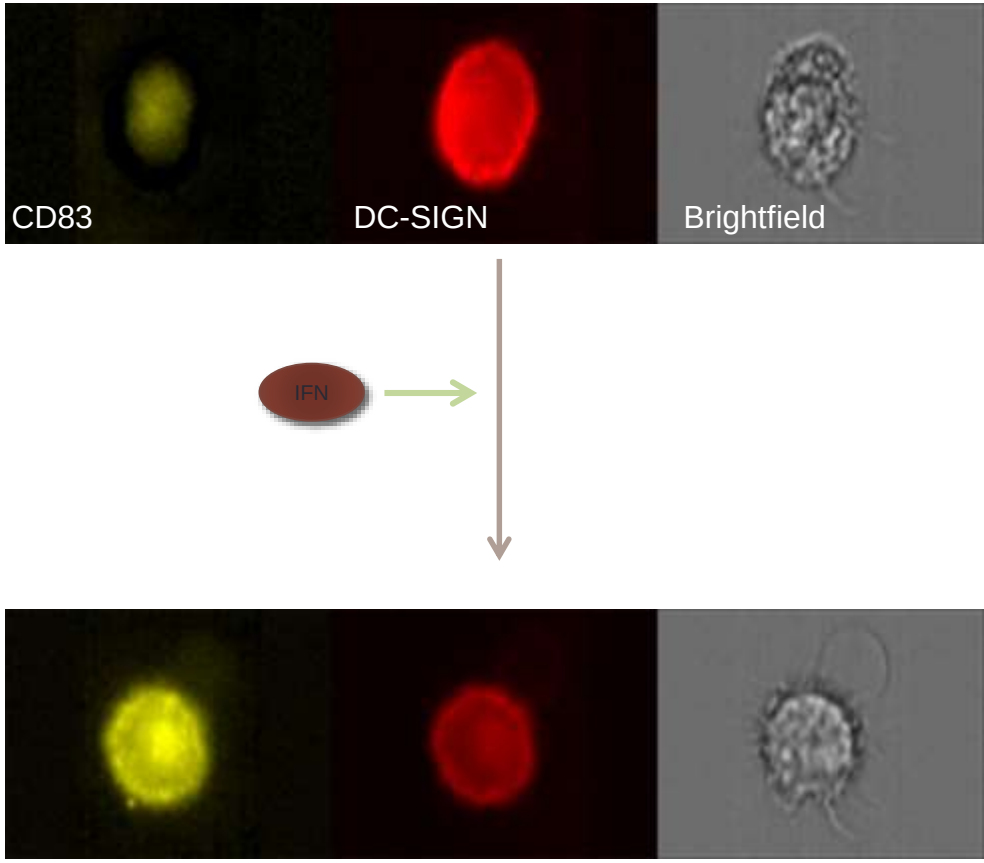
Phagocytosis is delayed by FK506 (p<0.001)

Peak phagocytosis is reduced by IFN-γ (p<0.01)

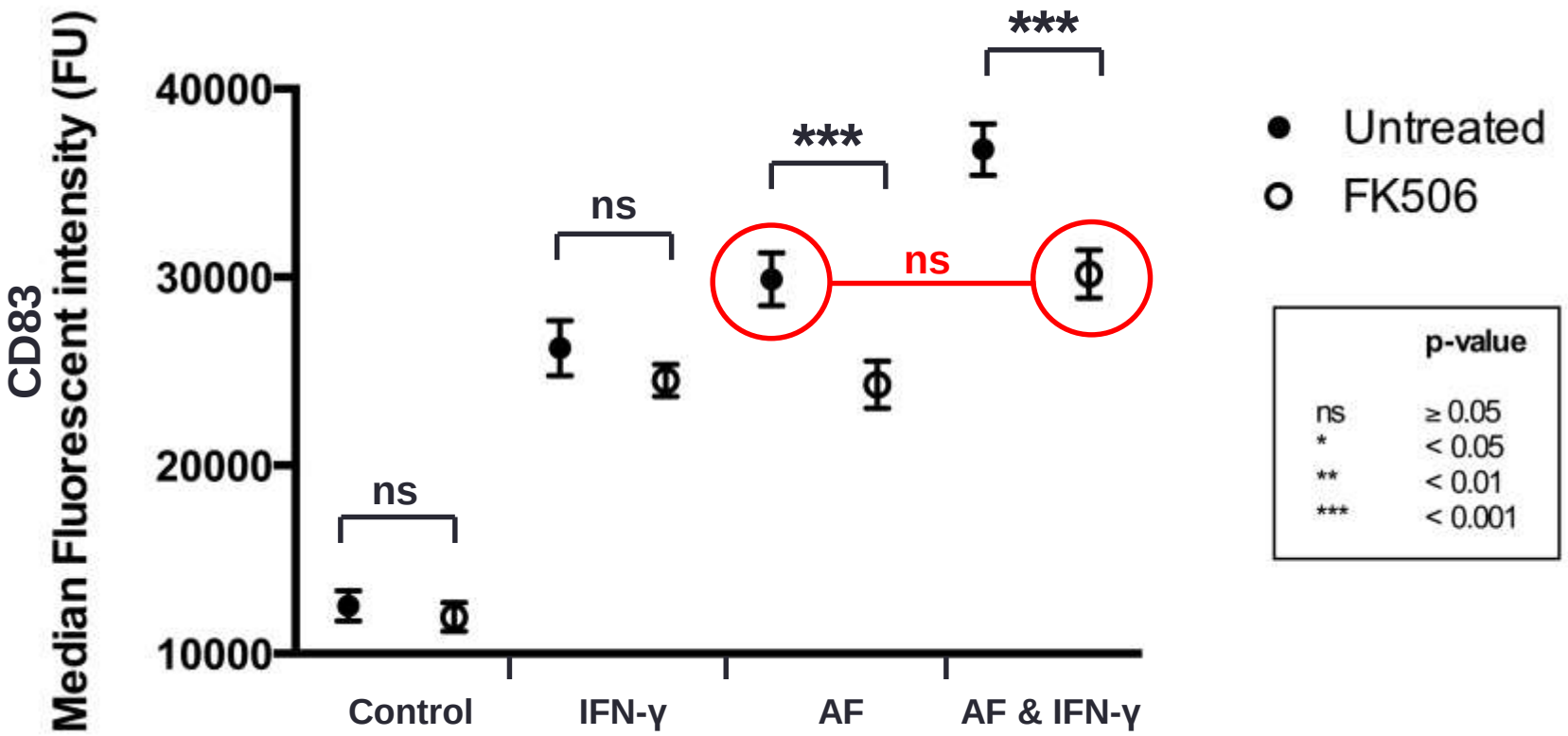
IFN-γ treatment of an FK506-treated DC permits earlier phagocytosis, but reduces peak phagocytosis (p<0.001, p<0.01)

DC maturation signal with IFNγ treatment

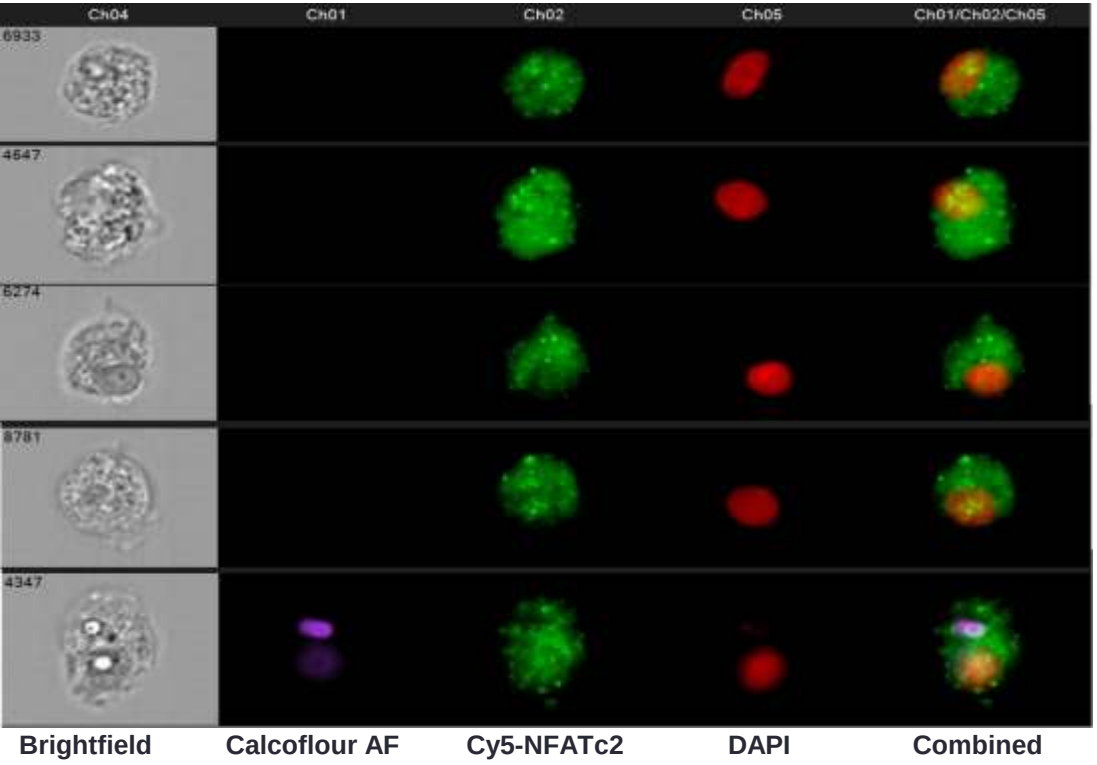


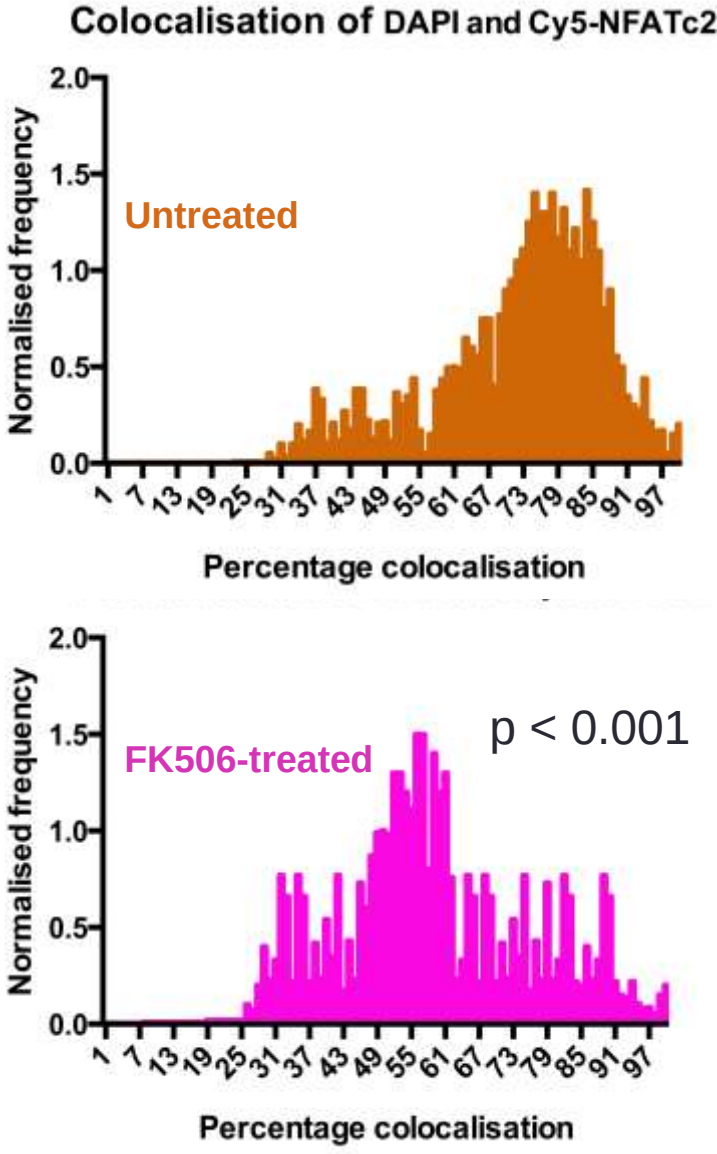


FK506-impaired maturation of DC's is restored by IFNγ

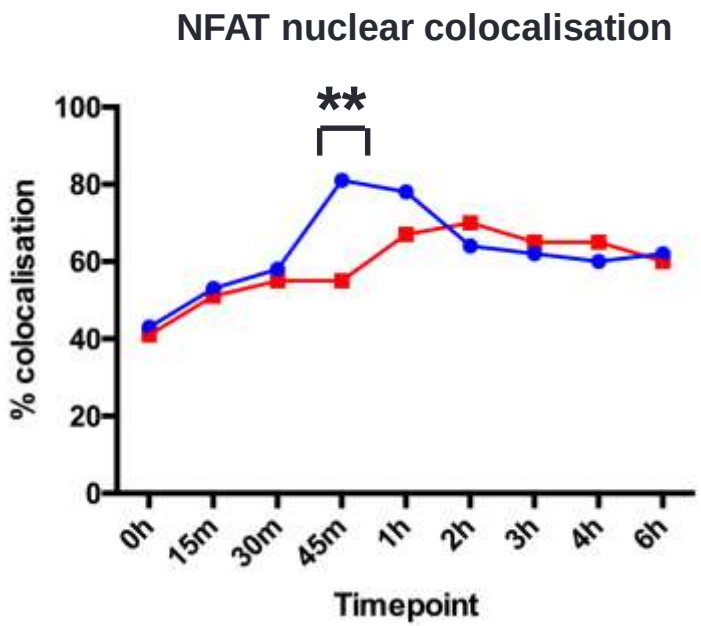


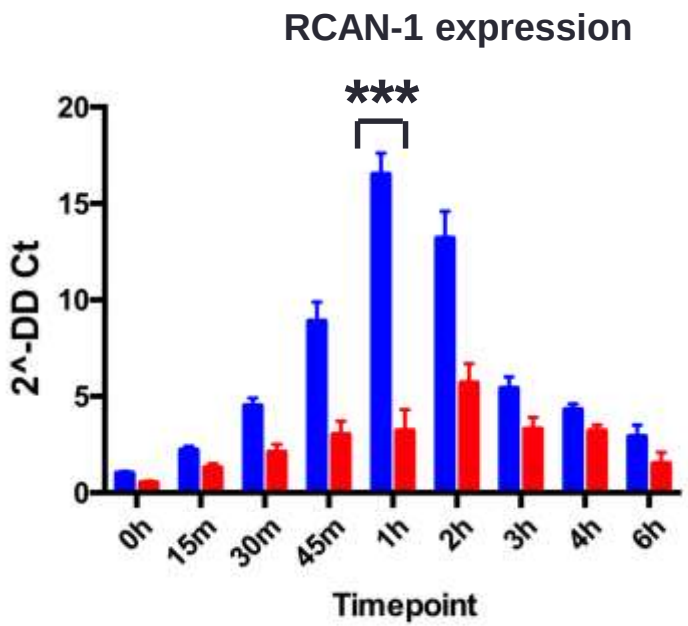
FK506 impairs translocation of NFAT in *A fumigatus* infected DC's





FK506 diminishes and delays NFAT nuclear translocation and target gene expression

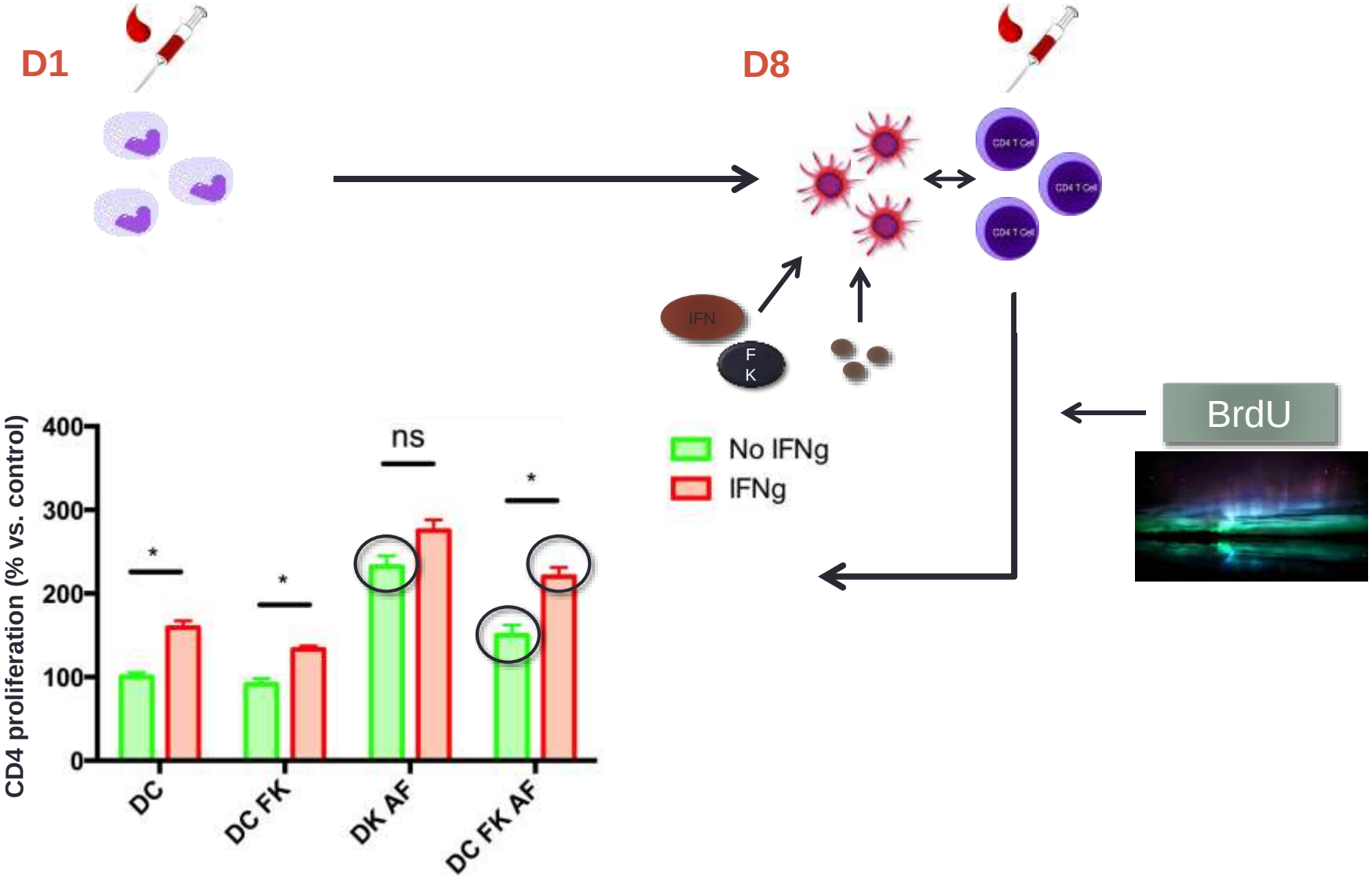




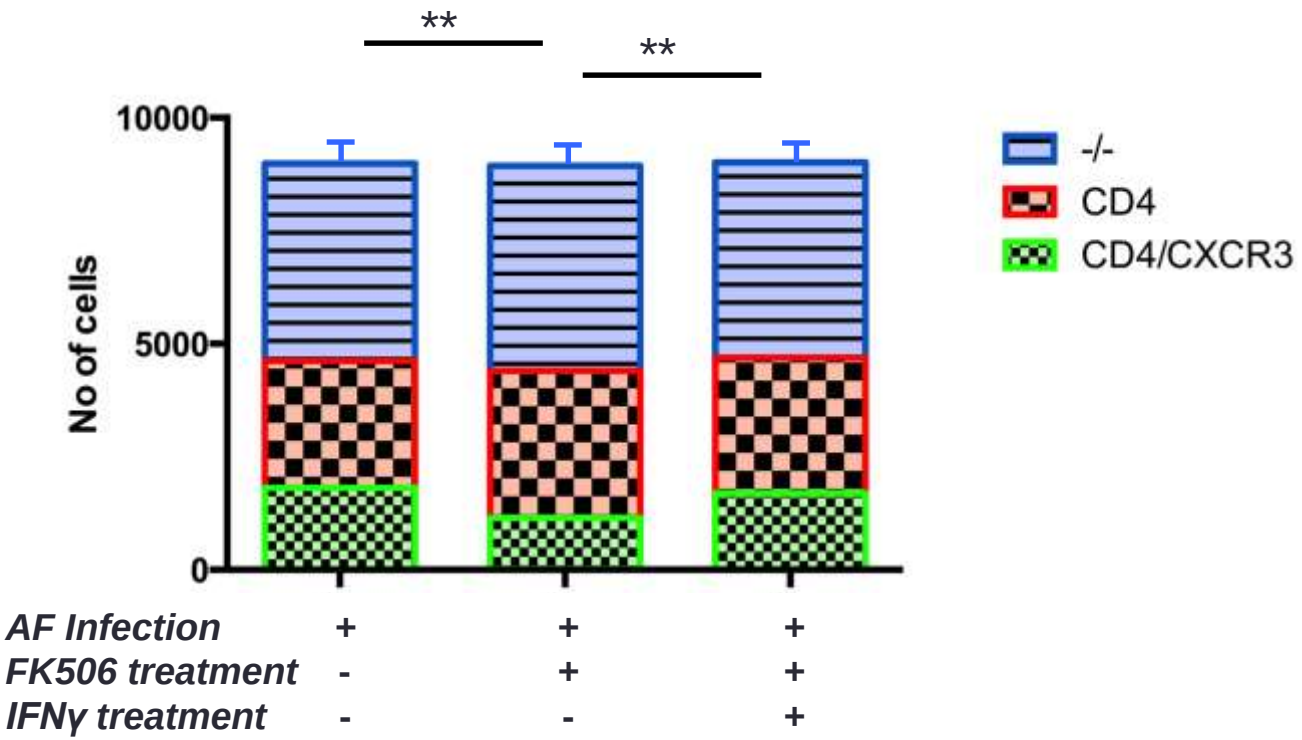
ChIP-Seq

RNA-Seq

Interferon-γ reverses the inhibitory effect of FK506 on CD4 T cell proliferation by DC's in co-culture

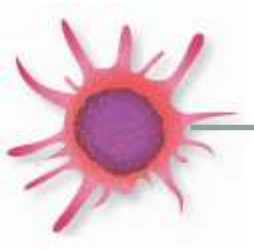


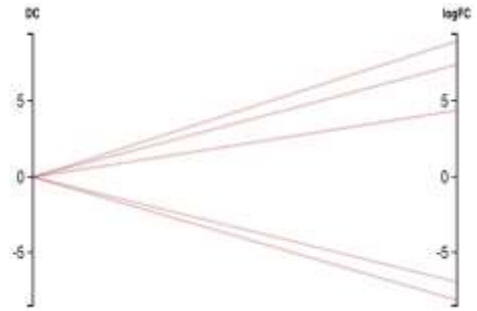
Interferon-γ repolarizes DC-co-cultured CD4 T-cells towards a Th1 phenotype



** = P < 0.01

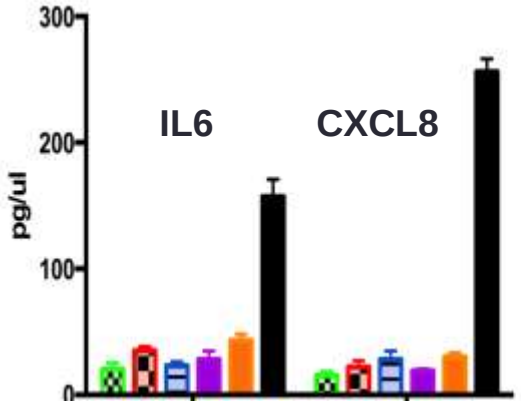
IFNγ treatment of DC's/CD4 T-cells restores fungal killing capacity of macrophages



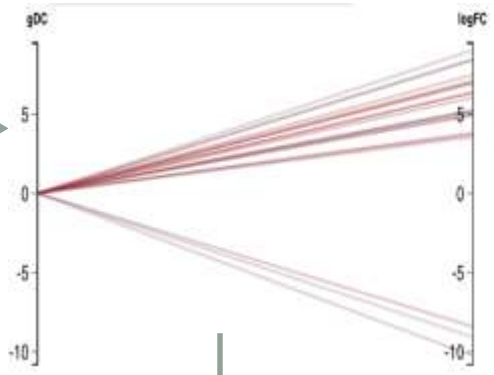


HSPA1B
MDC1

AATF
AZU1
NAP1L4
ITGB1P1



NR4A2 — T-cell differentiation
EGR3 — Acute stress response (downstream of NFAT)



DUSP1
EDN1
IL6
CXCL2
BTG2
CXCL3
CXCL8
FOSB
NFKBIZ
CCL20

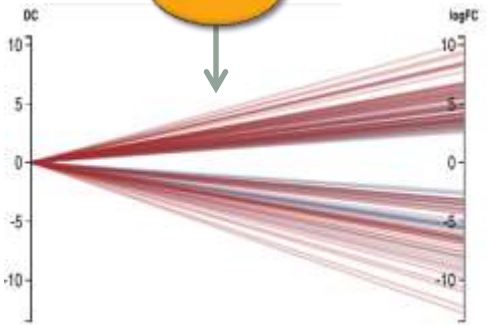
NTAN1

required for Jak-STAT microbial killing
neutrophil chemotaxis

controls myeloid cell migration
neutrophil chemotaxis, phagocytosis
part of AP-1 complex, co-transcriptor for NFkB

lymphocyte (CD4) chemotaxis

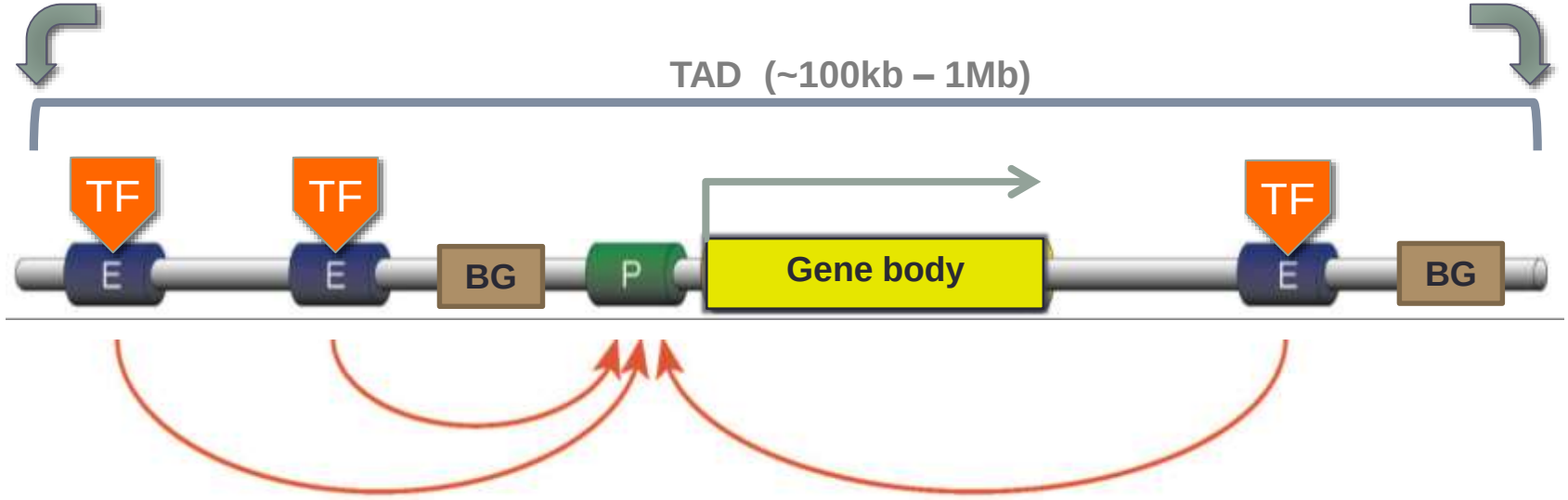
FK

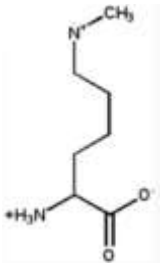


CD40L — Required for T-cell activation
CD38 — DC maturation marker
CCL4 — Chemoattracts macrophages and NK cells

Rcan1 — Negative regulator of NFAT

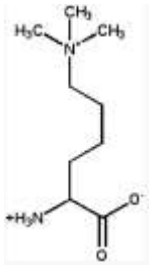
Gene expression is regulated by transcription factor binding events at distant regulatory elements within topologically associated domains (TAD's)





E

P



E Enhancer

P Promoter

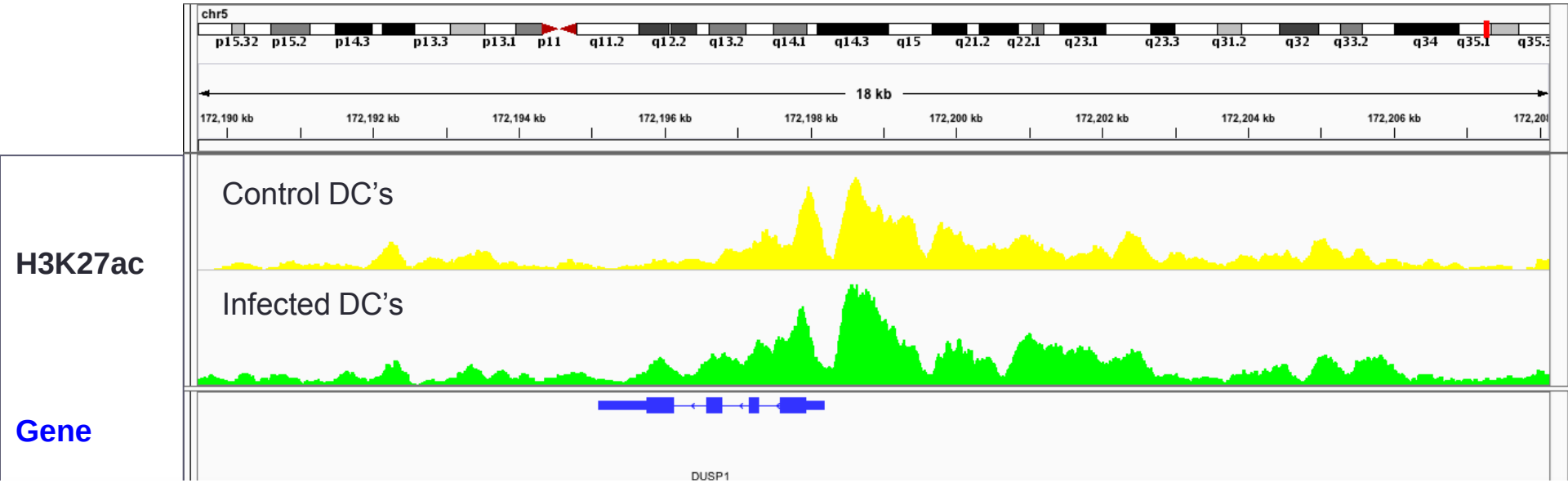
BG Background gene

TF Transcription factor

		H3K4me1	H3K4me3
H3K27ac	+ve	Active enhancer	Active promoter
	-ve	Poised enhancer	Inactive promoter

Whalen et el, *Nature Genetics,* 2016
Pope et al. *Nature* 2014
Harmston et al, *bioRxiv* 2016
Dixon et al. *Nature* 2015
Rao et al, *Cell* 2014
Creyghton el, *PNAS,* 2010
Shlyueva, *Nature Genteics,* 2014

Identification of differentially activated enhancer regions within TAD's around genes of interest



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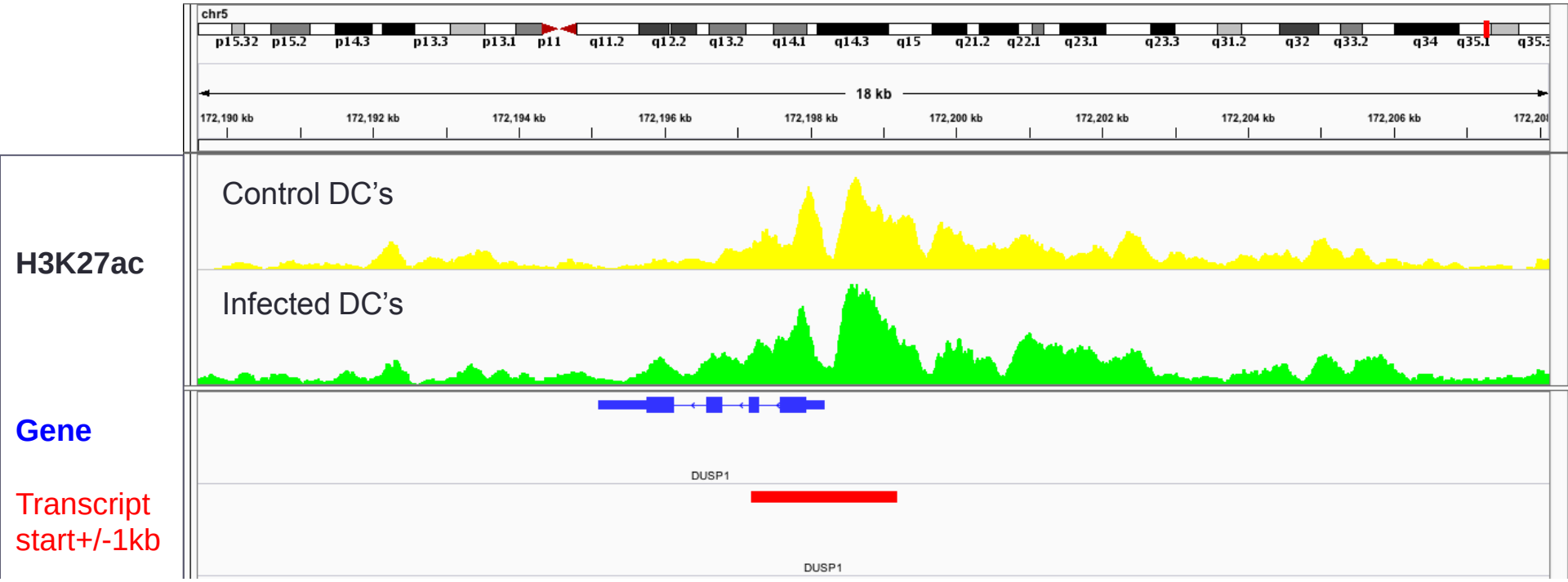

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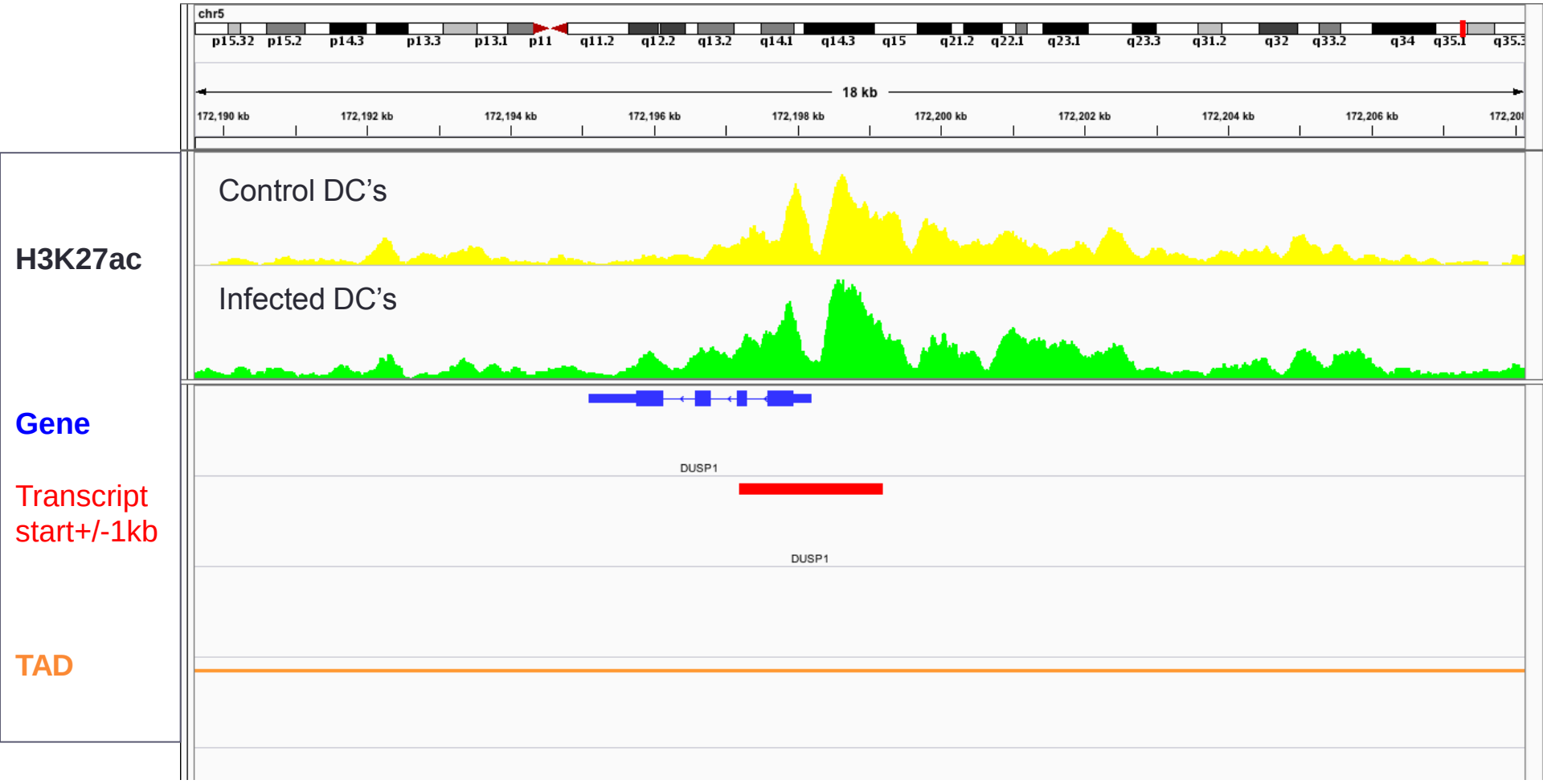

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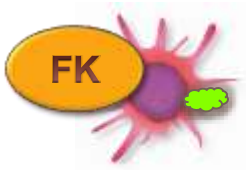
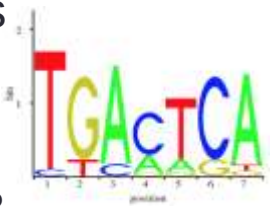
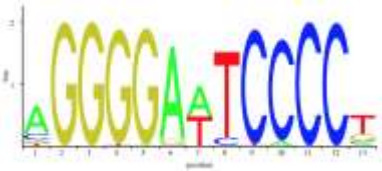


Condition	*Key TF*	*logFC*	*p-val (FDR)*
Infection	c-Fos	2.23	2.8e-08
	NFkB	3.05	6.0e-36
	STAT3	2.20	3.0e-25
	(NFATc2 motif)	1.34	2.8e-08
FK506 Rx	NFATc2 motif	- 2.01	3.1e-03
IFNg Rx	STAT1	7.32	1.4e-35
	IRF1	7.16	1.5e-24
	NFATC2 motif	1.64	5.9e-13
	c-Fos	- 2.43	3.3e-47
	STAT3	- 2.30	1.9e-31

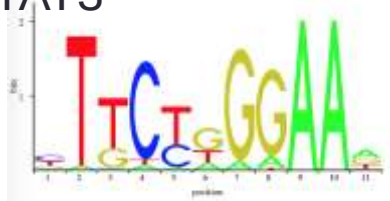
c-Fos



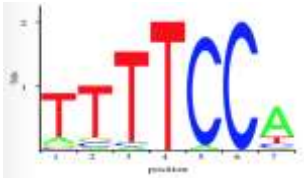
NFkB



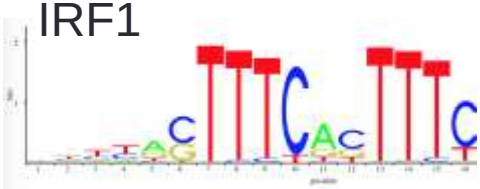
STAT3



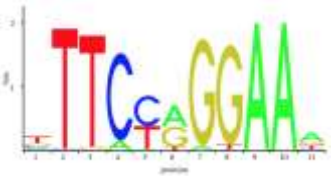
NFATc2



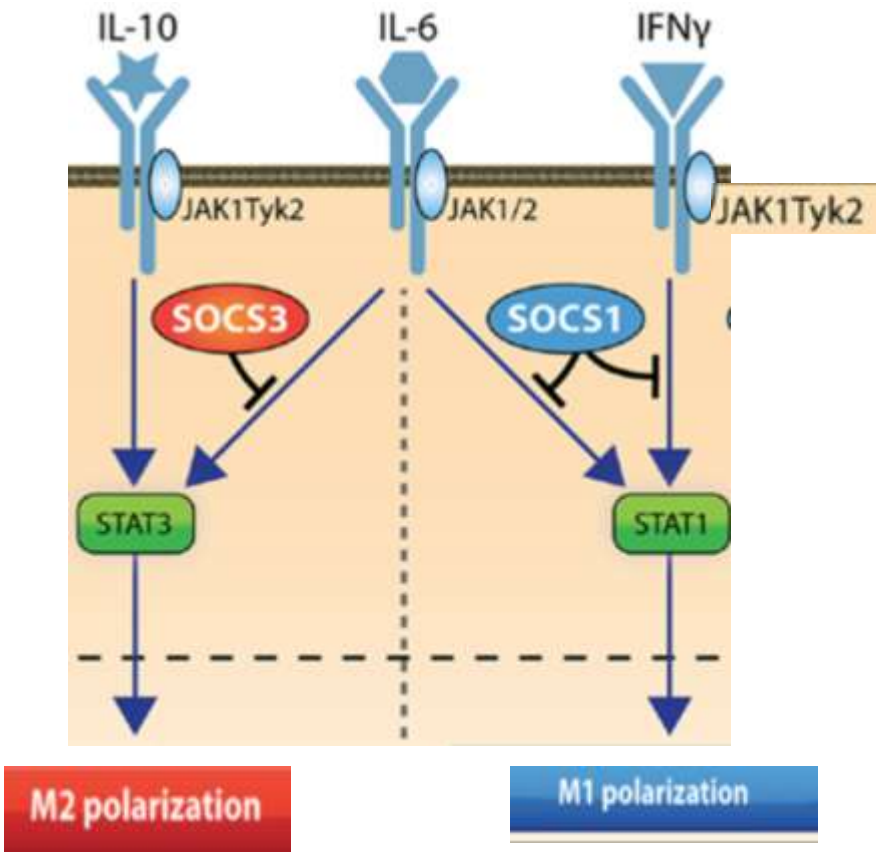
IRF1



STAT1



JAK-STAT pathway in macrophages



Wilson et al. *Frontiers Imm* 2014

Summary

Interferon gamma treatment of DC's in an FK506-suppresed model of *A. fumigatus* infection -

- Promotes:-
  - Phagocytosis
  - DC maturation
  - CD4 T cell proliferation + Th1 polarisation
  - CD4-mediated fungal killing by macrophages
- Leads to a gene regulatory switch from STAT3 to STAT1

JAK-STAT signalling may have a previously-undefined regulatory role in:-

- the CNI-mediated impaired DC response to *A. fumigatus* infection
- the enhancement of the innate response by Interferon gamma in this context

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- Harefield Hospital

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- Elizabeth Ing-Simmons
- Malcolm Perry
- Ge Tan

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- Anand Shah

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