

# **Abrogation of collagen-induced arthritis by a peptidyl arginine deiminase inhibitor is associated with modulation of T cell-mediated immune responses**

Joanna Kawalkowska<sup>1\*</sup>, Anne-Marie Quirke<sup>1\*</sup>, Fatemeh Ghari<sup>2</sup>, Simon Davis<sup>4</sup>, Venkataraman Subramanian<sup>3</sup>, Paul R. Thompson<sup>3</sup>, Richard O. Williams<sup>1</sup>, Roman Fischer<sup>4</sup>, Nicholas B. La Thangue<sup>2</sup> and Patrick J. Venables<sup>1</sup>

<sup>1</sup>Kennedy Institute, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Roosevelt Drive, Oxford OX3 7FY, UK

<sup>2</sup>Laboratory of Cancer Biology, Department of Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, UK

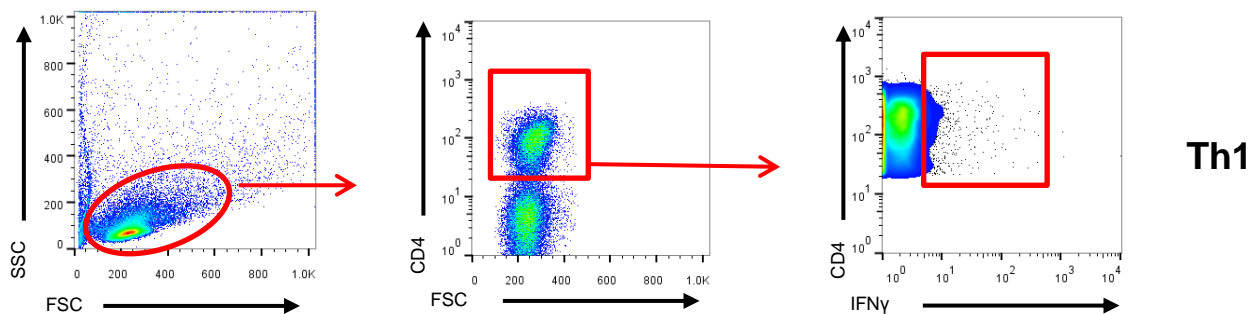
<sup>3</sup>Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, LRB 826, 364 Plantation Street, Worcester, MA, 01605, USA

<sup>4</sup>Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7FZ, UK

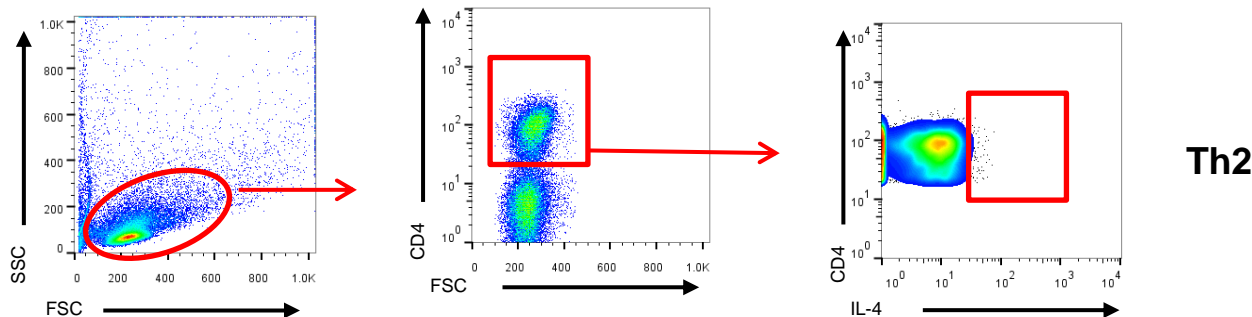
\* Contributed equally to the study as joint first authors

# SUPPLEMENTARY FIGURE 1

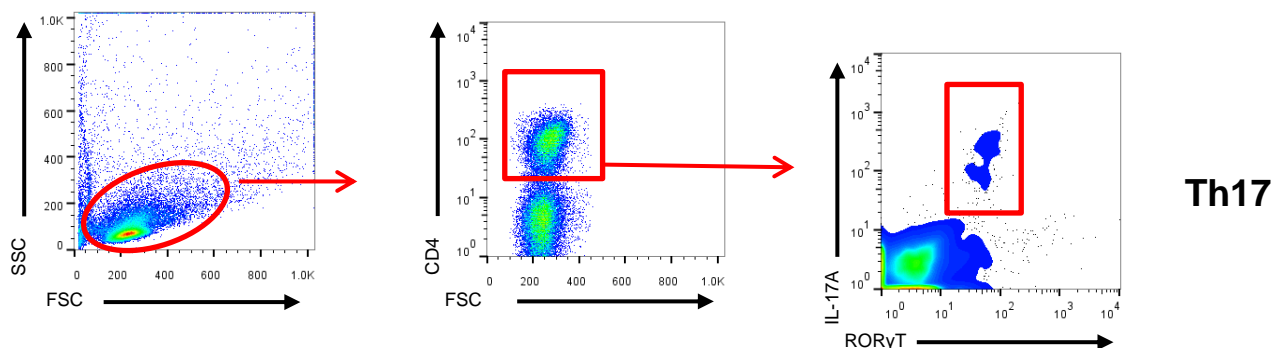
**a**



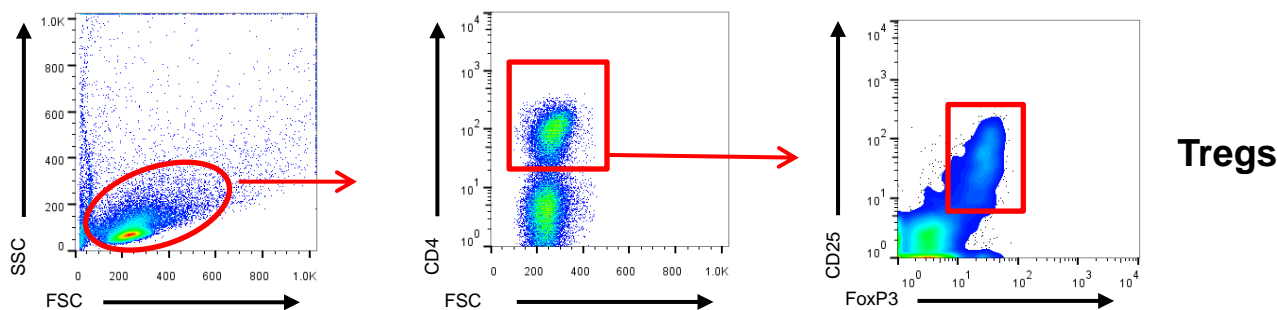
**b**



**c**



**d**



The gating strategies used in flow cytometry to identify CD4<sup>+</sup> T cell subpopulations in the lymph nodes of arthritic mice

(a-c) T helper subsets were identified with antibodies targeted against CD4 and IFN- $\gamma$  (Th1), IL-4 (Th2) or IL-17A (Th17). (d) Tregs were designated as CD4<sup>+</sup> CD25<sup>+</sup> FoxP3<sup>+</sup>.

**SUPPLEMENTARY TABLE 1**

Mass spectrometry analysis of citrulline residues in proteins isolated from the lymph nodes of mice with collagen-induced arthritis. The number of mice (out of 5-6 mice per group) which had citrulline detected in that protein is shown.

<b>Protein</b>	<b>Accession</b>	<b>Site</b>	<b>Vehicle</b>	<b>1 mg BB-Cl</b>	<b>10 mg BB-Cl</b>	<b>Naive</b>
Prothrombin	H7BX99	R490	3	2		
Actin, , cytoplasmic 1	P60710	R290	2	1		
Elongation factor 1-alpha	P10126	R266	2	1		
RNA demethylase ALKB5	Q3TSG4	R287	2			
Histone H4	P62806	R93	1	1	2	
Histone H3.2	P84228	R117	1	1		
YLP motif containing protein	F6YTL8	R579	1	2		
Actin-related protein 2	P61161	R319	1			
14-3-3 protein epsilon	P62259	R19	1			
Gelsolin	P13020	R449	1			
Probable ATP-dependent RNA helicase	Q80Y44	R478	1			
Keratin type 1 cytoskeletal17	Q9OWL7	R410	1			
Myosin-9	Q8VDD5	R143	1			
Beta-globin	A8DUK4	R410	1			
Probable ATP-dependent RNA helicase	Q61656	R2523	1			
Spondin-1	Q8VCC9	R93	1			
Acyl-CoA dehydrogenase 10	Q8K370	R456	1			
Collagen alpha-1(VII)	Q63870	R2523		1	1	
Tropomyosin alpha 3 chain	D3Z6I8	R124		1		
Trifunctional enzyme subunit alpha	Q8BMS1	R610		1		
Fat1	F2Z4A3	R3775		1		
Fatty acid synthase	P19096	R2019		1		
Histone deacetylase 1	O09106	R229		1		
RNA demethylase ALKBH5	Q3TSG4	R287		1		
Elongation factor 1-alpha	P10126	R134		1		
Inter-alpha-trypsin inhibitor heavy ITIH2	P19823	R341		1		
Inter-alpha-trypsin inhibitor heavy ITIH3	Q06033	R70		1		
Heterogeneous nuclear ribonuclearprotein A3	Q8BG05	R68		1		
Keratin, type2 cytoskeletal 1b	Q6IFZ6	R342		1		
Keratin, type2 cytoskeletal 75	Q8BGZ7	R239		1		
Beta-2-glycoprotein 1	Q01339	R139		1		
Treacle protein	O08784	R1068				1
Serin/arginine-rich splicing factor 5	O35326	R48				1

## SUPPLEMENTARY TABLE 2

The antibodies used in flow cytometry to identify CD4<sup>+</sup> T cell subpopulations in the lymph nodes of arthritic mice and in T cell cultures

<b>Antibody</b>	<b>Clone</b>	<b>Manufacturer</b>
CD4	GK1.5	eBioscience
CD25	PC61.5	eBioscience
IL-17A	eBio117B7	eBioscience
ROR $\gamma$ T	Q31-378	BD Biosciences
IFN- $\gamma$	XMG1.2	eBioscience
IL-4	11B11	eBioscience
FoxP3	FJK-16s	eBioscience
GATA3	TWAJ	eBioscience