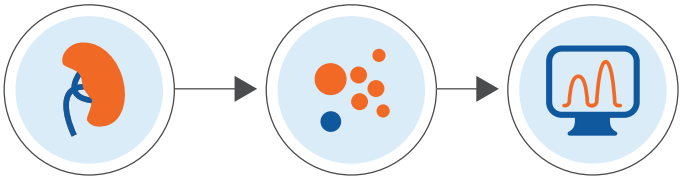
Delineation of Functional Treg Subsets Using Cell Surface Marker Expression

Featured clones:

- Clone V46-1954 is a highly specific anti-mouse CD304 (Nrp-1) antibody that does not cross-react with Nrp-2.
- Clone Y23-1185 is a novel anti-mouse CD39 (NTPDase 1) antibody.



Background

Regulatory T cells (T_{regs}) comprise a heterogenous group of CD4 T cells that modulate immune responses and maintain immune system homeostasis. Naturally occurring T_{regs} were first characterized within the $CD4^+CD25^+$ T cell subpopulation. These cells express the transcription factor Foxp3, which is essential for T_{regs} stability and immunosuppressive functions. A growing number of cell surface markers support the identification of specific T_{regs} subsets including functional subsets. Neuropilin-1 (Nrp-1 or CD304) is one such marker used to distinguish natural T_{regs} from peripheral and induced T_{regs} in murine models. In both humans and mice, ectonucleoside triphosphate diphospho-hydrolase 1 (NTDase 1 or CD39) regulates adenosine-triggered immunosupressive effects in T_{regs} and has become a novel potential immune checkpoint inhibitor target in cancer immunotherapies.

Cell Preparation and Analysis

C57BL/6 mouse spleen was mechanically dissociated and single-cell suspensions were sequentially incubated with Mouse BD Fc Block™ and a cocktail of antibodies. The cells were acquired using a BD FACSymphony™ A5 Cell Analyzer and analyzed in FlowJo™ v10.7.1 Software. Uniform Manifold Approximation and Projection (UMAP) was used for visualization of immune cell populations. Cell populations were annotated based on the expression of lineage-specific markers (Figure 1A). Additionally, CD304 (Nrp-1) expression was shown in a heatmap (Figure 1B). Flow cytometric analysis of the correlated expression of CD304 versus CD25 was derived from $CD45^+CD3^+CD4^+$ T cells (Figure 2A). Then, CD39 expression was determined within $CD304^-CD25^-$, $CD304^+CD25^-$ and $CD304^+CD25^+$ subsets (Figure 2B).

Laser	Fluorochrome	Specificity	Clone
UV	BUV395	CD8α	53-6.7
	BUV496	CD45	30-F11
	BUV563	F4/80	T45-2342
	BUV615	CD314	CX5
	BUV661	CD21	7G6
	BUV737	Ly-6C	AL-21
	BUV805	B220	RA3-6B2
Violet	BV421	CD304	V46-1954
	BV510	I-A/I-E	M5/114.15.2
	BV570	CD4	GK1.5
	BV605	TCRgδ	GL3
	BV650	CD23	B3B4
	BV711	CD27	LG.3A10
	BV750	CD192	475301
	BV786	IgM	R6-60.2
Blue	BB515	CD62L	MEL-14
	BB630	CD11c	HL-3
	BB660	CD357	DTA-1
	PerCP-Cy5.5	IgD	11-26c.2a
	BB755	Ly-6G	1A8
	BB790	NK1.1	PK136
Yellow-Green	PE	CD39	Y23-1185
	PE-CF594	CD93	AA4.1
	PE-Cy5	NKG2A/E/C	20D5
	PE-Cy5.5	CD25	PC61.5
	PE-Cy7	CD43	S7
Red	APC	CD3	145-2C11
	APC-R700	CD19	1D3
	APC-Cy7	CD11b	M1/70

Table 1. Deeper T reg insights from a single tube with broad 29-color immunophenotyping of mouse splenocytes.
BB: BD Horizon Brilliant™ Blue; BUV: BD Horizon Brilliant™ Ultraviolet; BV: BD Horizon Brilliant Violet™

Cat. No	Description	Clone
567112	BD Horizon Brilliant Violet™ 421 Rat Anti-Mouse CD304	V46-1954
567104	BD Pharmingen™ PE Rat Anti-Mouse CD39	Y23-1185

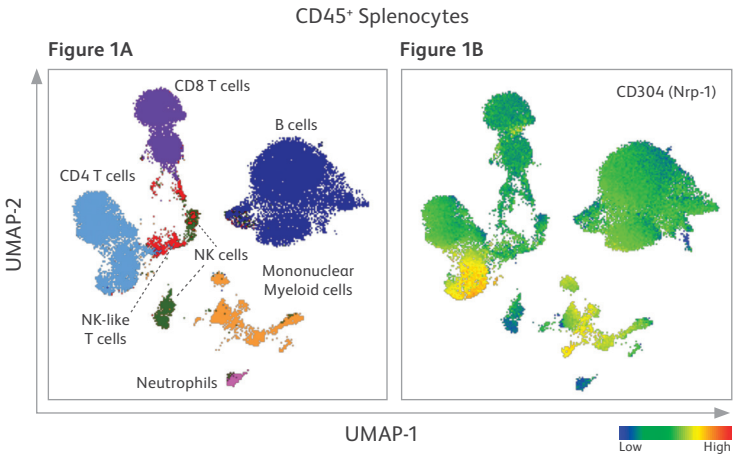


Figure 1. CD304 (Nrp-1) expression most correlated with subset of CD4 T cells and mononuclear myeloid cells.
A. Cell population annotation. **B.** Heatmap showing CD304 (Nrp-1) expression.

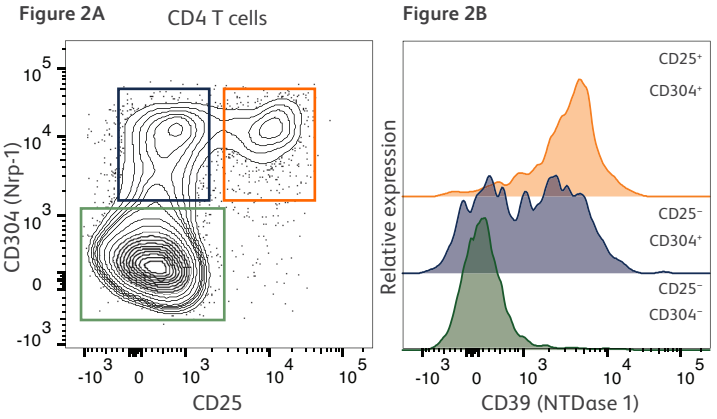


Figure 2. Clean separation of CD4 T cell subsets based on CD304 (Nrp-1) expression and subsequent differential expression of CD39 (NTDase 1).
A. Bivariate contour plots showing CD304 versus CD25 expression on CD45⁺CD3⁺CD4⁺ T cells. **B.** Histogram overlays showing CD39 (NTDase 1) expression in CD4 T cell subsets.

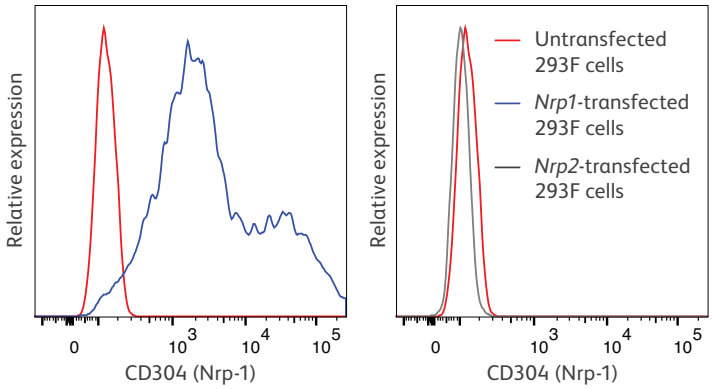


Figure 3. Clone V46-1954 specifically binds CD304 (Nrp-1) and not closely related Nrp-2.
Flow cytometric analysis of CD304 (Nrp-1) expression on transfected cells. 293F cells were left untransfected (red histogram) or transfected with either *Nrp1* (blue histogram) or *Nrp2* (grey histogram). Cells were stained with anti-CD304 (Nrp-1) antibody (clone V46-1954). The histograms show clone V46-1954 is specific to Nrp-1 but not Nrp-2.

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