

HLDA10 VALIDATION FILE FOR CD366 TIM-3

MOLECULE NAME	TIM-3
ALTERNATIVE NAMES	Hepatitis A virus cellular receptor 2, HAVCR-2, T-cell immunoglobulin and mucin domain-containing protein 3, TIMD-3, T-cell immunoglobulin mucin receptor 3, TIM-3, T-cell membrane protein 3
GENE FAMILY	
PROTEIN	Single pass type I membrane protein
FUNCTION	Regulates macrophage activation. Inhibits T-helper type 1 lymphocyte (Th1)-mediated auto- and alloimmune responses and promotes immunological tolerance. May be also involved in T-cell homing. Receptor for LGALS9
EXPRESSION	T-helper type 1 lymphocyte (Th1)-specific
	On 90% monocytes, NK cells
	Mainly mouse studies

10-24

Antibody Name	Clone 344823, FAB2365P
Specificity	Binds monocytes
Antibody Species	Rat
Ig Isotype	IgG2a
Immunogen	
Epitope Recognised	
Submitter	R&D
References	

10-75

Antibody Name	F38-2E2
Specificity	
Antibody Species	Mouse
Ig Isotype	IgG1. K
Immunogen	Human Tim-3 fusion protein
Epitope Recognised	
Submitter	BioLegend
References	

INFORMATION FOR CONFIRMATION OF SPECIFICITY

	10-24	10-75
Expression on transfected CHO	Yes	Not confirmed
Expression on cell line	Weak pos on Raji and Daudi	

Expression on normal primary cell	Yes pos on monocytes and brighter on CD1c DC that CD16 population Bright on pDC in one study.	Yes pos on monocytes and brighter on CD1c DC that CD16 population
Thymic DC	Pos	Pos
AML	Pos on AML blasts – 3 samples	Pos on AML blasts – 3 samples
In vitro derived cells	Bifid peak pos on GC-csf derived macrophages, downregulated by LPS/IFN and IL-4, pos on m-csf macrophage which is increased by LPS/IFN, IL4 or IL10, pos on MoDC but slightly downreg by LPS	Bifid peak pos on GC-csf derived macrophages, downregulated by LPS/IFN and IL-4, pos on m-csf macrophage which is increased by LPS/IFN, IL4 or IL10, pos on MoDC but slightly downreg by LPS

CELL LINE EXPRESSION

	Cell lines	10-24	10-75
Burkitt lymphoma B cell lines	Raji	-	-
	Daudi		
T cell leukemia	Jurkat		
Myeloid Leukemia	HEL	-	-
	HL-60	-	-
	NB4	+	+
	THP-1	+/-	+/-
	U-937	+/-	+/-
Hodgkins derived lines	KM-H2		

Figure 1. Binding of mAb to CHO-K1 transfected with TIM-3 cDNA

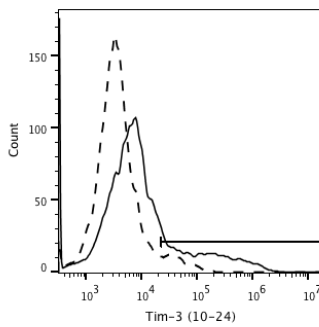


Figure 2. Binding of mAb to fresh blood DC

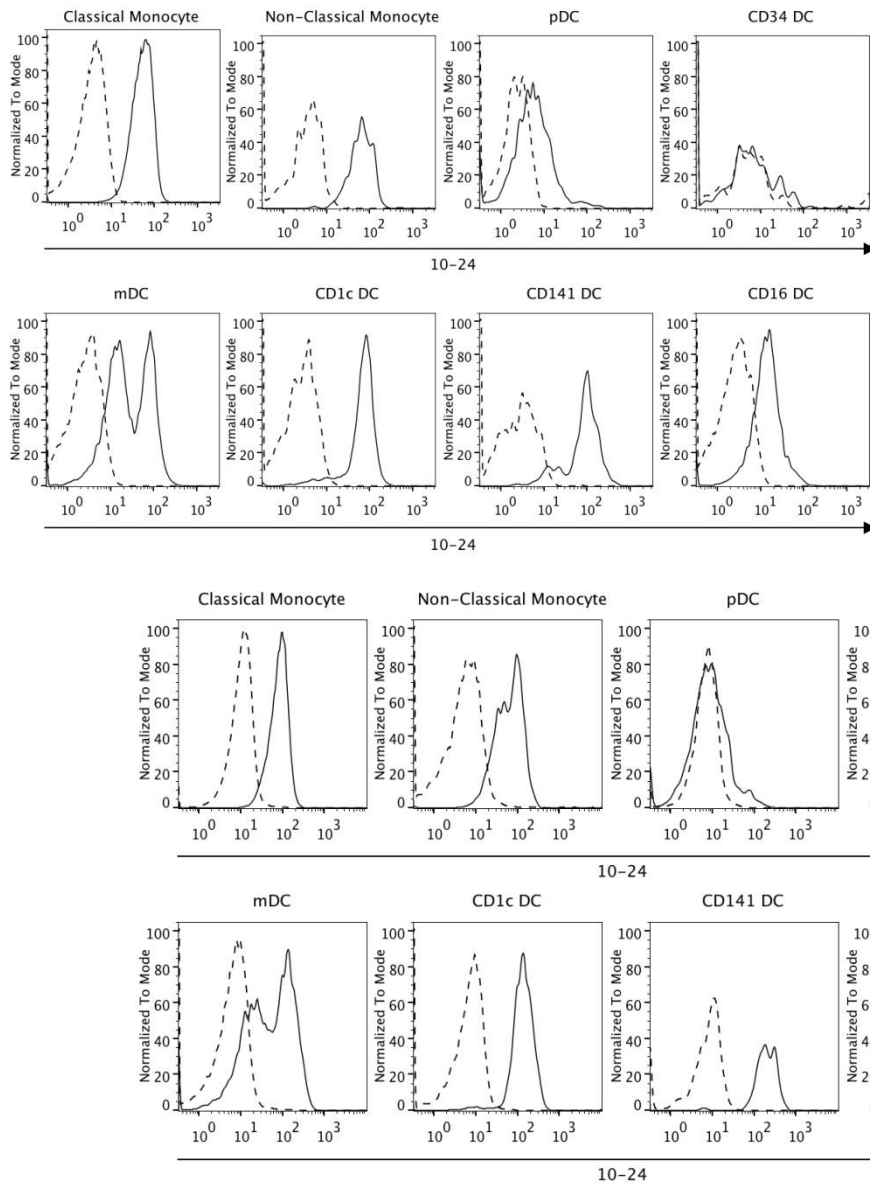
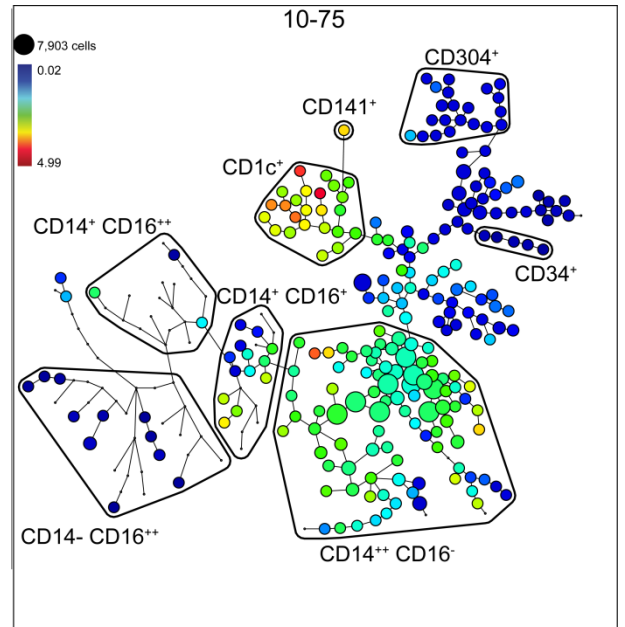
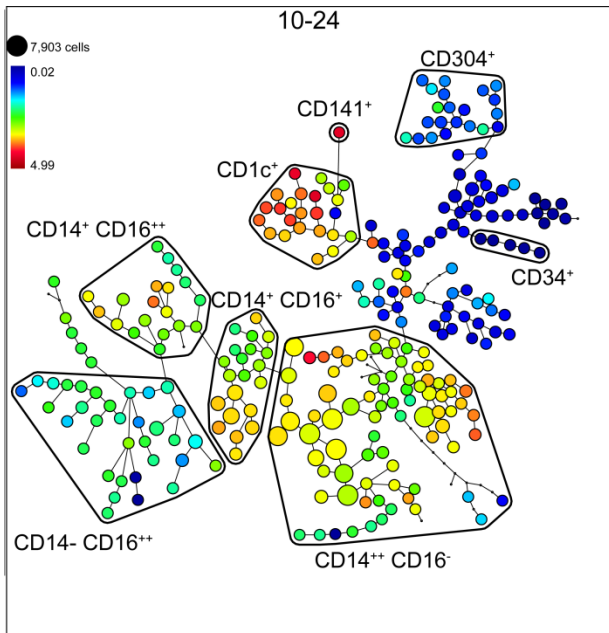


Figure 3. Clustering analysis using SPADE to demonstrate expression of TIM3 antibodies on fresh blood DC



PUBLICATIONS USING ANTIBODIES

(1)

1. Wei F, Zhong S, Ma Z, Kong H, Medvec A, Ahmed R, et al. Strength of PD-1 signaling differentially affects T-cell effector functions. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(27):E2480-9. Epub 2013/04/24.