

## HLDA10 VALIDATION FILE FOR CD370\_DNGR Clec9A

### More than two antibodies that have same expression pattern on primary cells Binding to transfectants

MOLECULE NAME: Clec9A

ALTERNATIVE NAMES: DNGR

GENE FAMILY: Group V C-type Lectin Family

PROTEIN: Type II transmembrane glycoprotein

FUNCTION: Functions as an endocytic receptor on a small subset of myeloid cells specialized for the uptake and processing of material from dead cells. Recognizes filamentous form of actin in association with particular actin-binding domains of cytoskeletal proteins, including spectrin, exposed when cell membranes are damaged, and mediate the cross-presentation of dead-cell associated antigens in a Syk-dependent manner.

EXPRESSION: CD141 DC Subset (1)

#### ANTIBODY INFORMATION

##### 10-09

Antibody Name: 9A11

Specificity: Human CLEC9A/DNGR1

Antibody Species: Mouse

Ig Isotype: IgG1

Immunogen: Fc:Clec9A

Epitope Recognised:

Submitter: Gordon Brown

#### INFORMATION PROVIDED BY SUBMITTER

Publications: (2, 3)

Reactivity: Flow cytometry  
Western blotting, under nonreducing conditions but not under reduced conditions  
Does not work in paraffin-fixed tissues

##### 10-02, 10-65

Antibody Name: 8F9

Specificity:

Antibody Species: Mouse

Ig Isotype: IgG2a, κ

Immunogen: RBL-2H3 cells expressing hDNGR-HA

Epitope Recognised:

Submitter: Caetano Reis e Sousa

INFORMATION PROVIDED BY SUBMITTER

Publications: (4)

Licensed to: BioLegend (Submitted the PE labelled conjugate)

**10-45**

Antibody Name: Clone 683409, FAB6049P  
 Specificity: Tested for binding to HEK293/eGFP transfectants, Binds to approx 50% of CD3-CD141+ HLA-DR+ and to CD16 monocytes  
 Antibody Species: Mouse  
 Ig Isotype: IgG1  
 Immunogen: NS0-derived Recombinant human Clec9A (aa 57-241)  
 Epitope Recognised  
 Submitter: R&D Systems

References

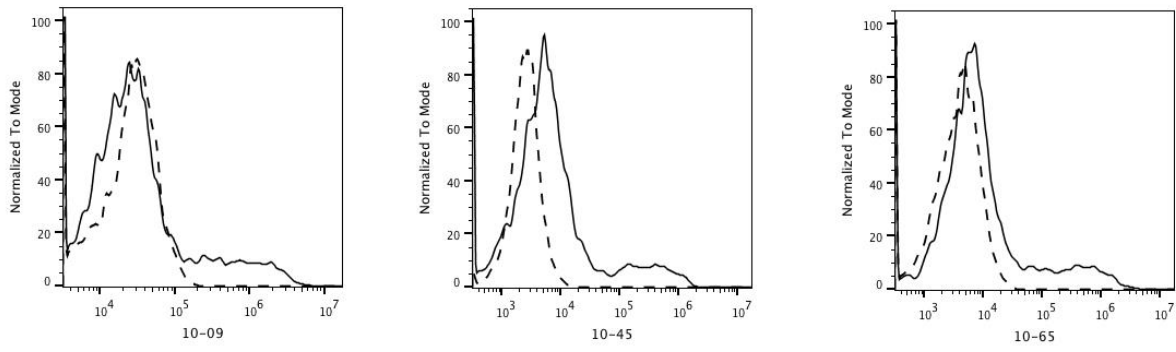
SPECIFICITY

	10-02	10-09	10-45	10-65
Expression on transfected cells Figure 1.	Yes (n=2)	Yes	Yes	Yes
Expression on cell line	Yes	Yes	Yes	Yes
Expression on normal primary cell	CD141+ DC	CD141+ DC	Approx 50% of CD3-CD141+ HLA-DR+	CD141+ DC
Thymic DC	CD141+ DC	CD141+ DC	CD141+ DC	CD141+ DC
In vitro derived cells (MoDC, CD34 derived LC, Macrophage)	Neg	Neg	Neg	Neg
Paraffin Staining	Neg	Neg	Neg	Neg

CELL LINE EXPRESSION

	Cell lines	10-02	10-09	10-45	10-65
Burkitt lymphoma B cell lines	Raji	+/-	+/-	-	-
T cell leukemia	Jurkat	-			-
Myeloid Leukemia	HEL	-	-	+/-	+
	HL-60	+	-	+/-	+/-
	NB4	+		-	-
	THP-1	+	-	+/-	-
	U-937	+	+/-	+/-	+
Hodgkins derived line	KM-H2	-	-	-	-

Figure 1. CHO-K1 transfected with hClec9A cDNA stained with each CLEC9A antibody submitted .



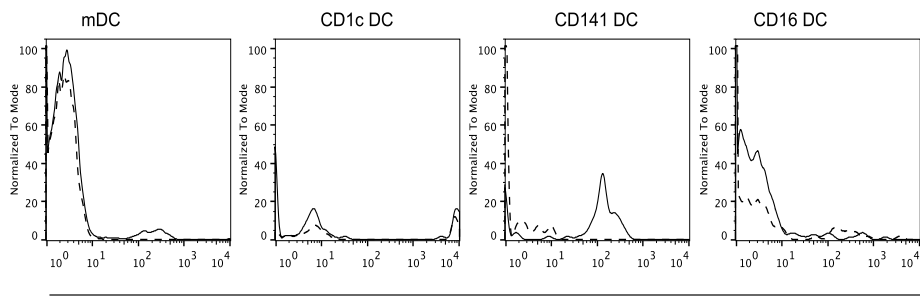
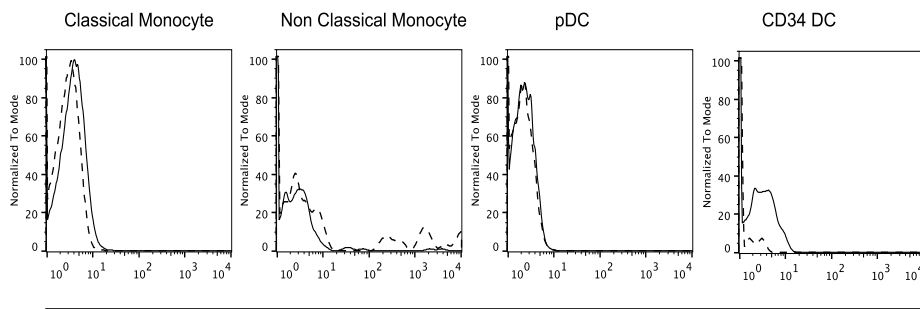
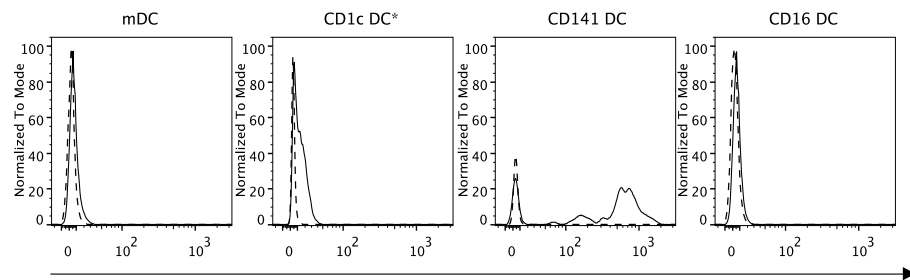
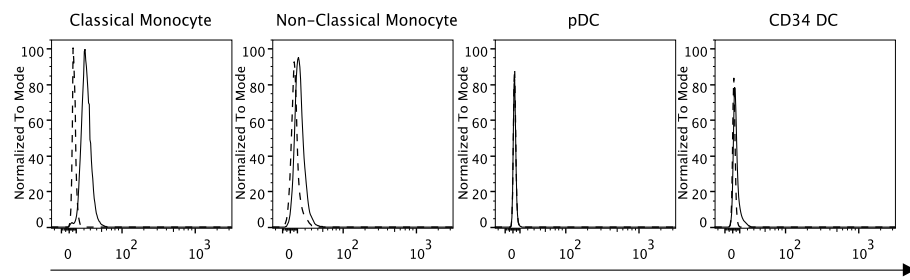
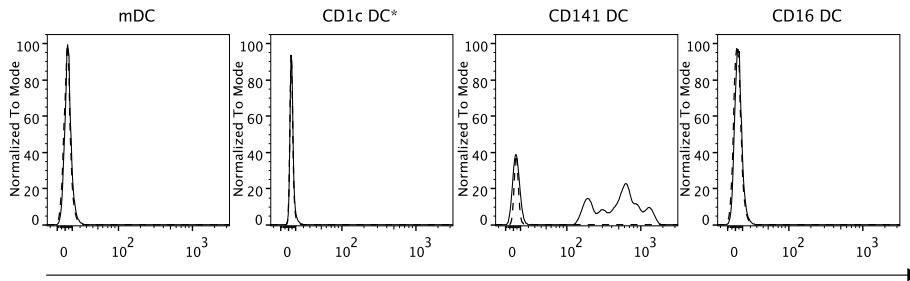
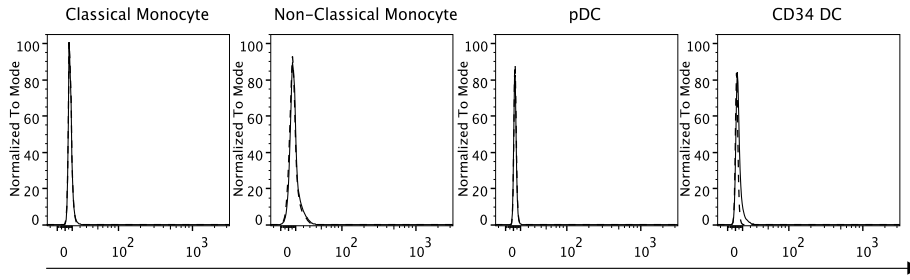
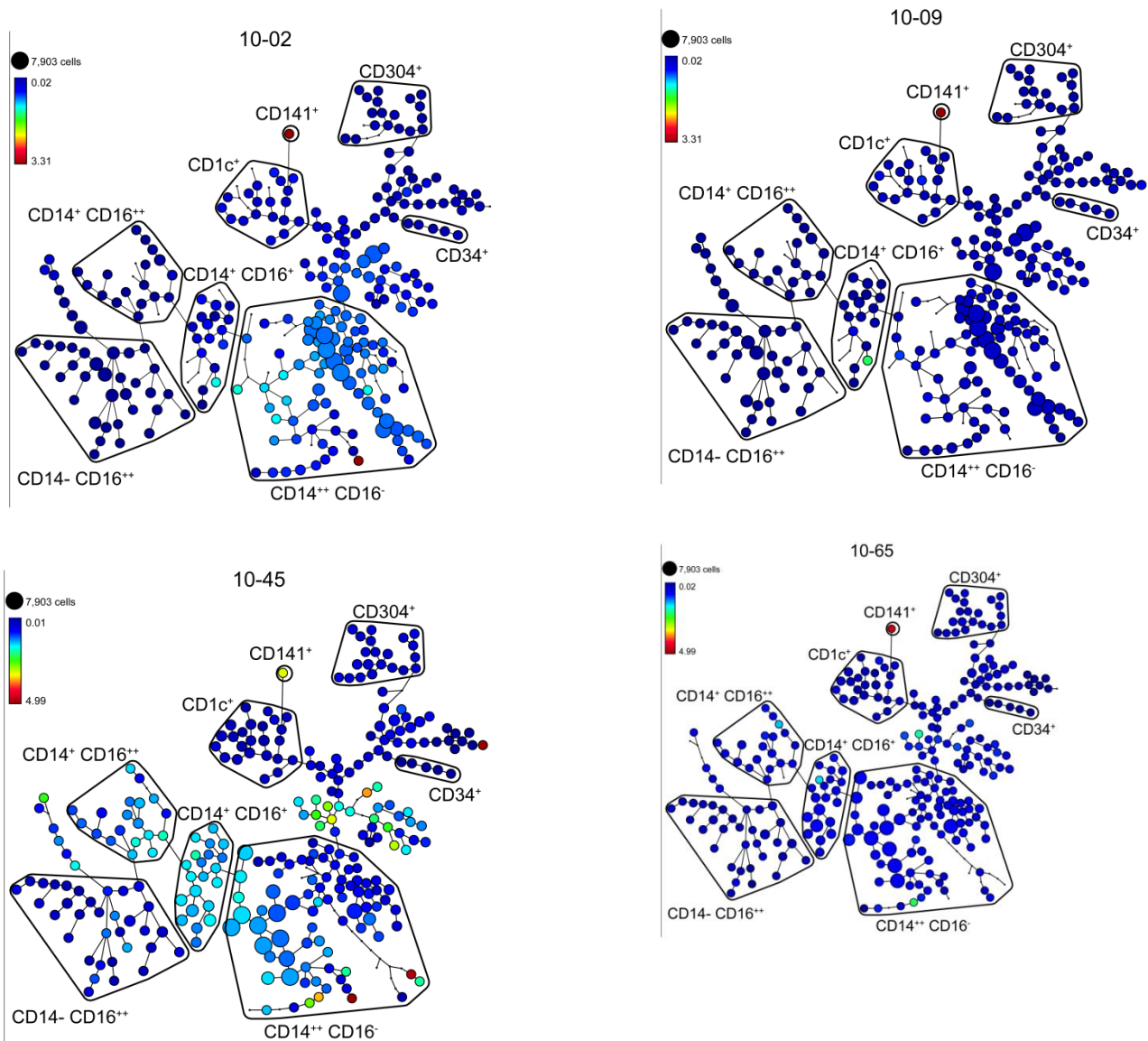


Figure 2: Binding of each antibody with fresh blood DC. Solid line = test antibody, dashed line = isotype control.

Figure 3. Clustering analysis using SPADE to demonstrate expression of Clec9A antibodies on fresh blood DC.



## PUBLICATIONS USING ANTIBODIES

(5)

1. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *The Journal of experimental medicine*. 2010;207(6):1247-60. Epub 2010/05/19.
2. Huysamen C, Willment JA, Dennehy KM, Brown GD. CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. *The Journal of biological chemistry*. 2008;283(24):16693-701. Epub 2008/04/15.
3. Schreiber G, Klinkenberg LJ, Cruz LJ, Tacke PJ, Tel J, Kreutz M, et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells. *Blood*. 2012;119(10):2284-92. Epub 2012/01/12.
4. Sancho D, Mourao-Sa D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, et al. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *The Journal of clinical investigation*. 2008;118(6):2098-110. Epub 2008/05/24.

5. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *The Journal of experimental medicine*. 2010;207(6):1261-71. Epub 2010/05/19.