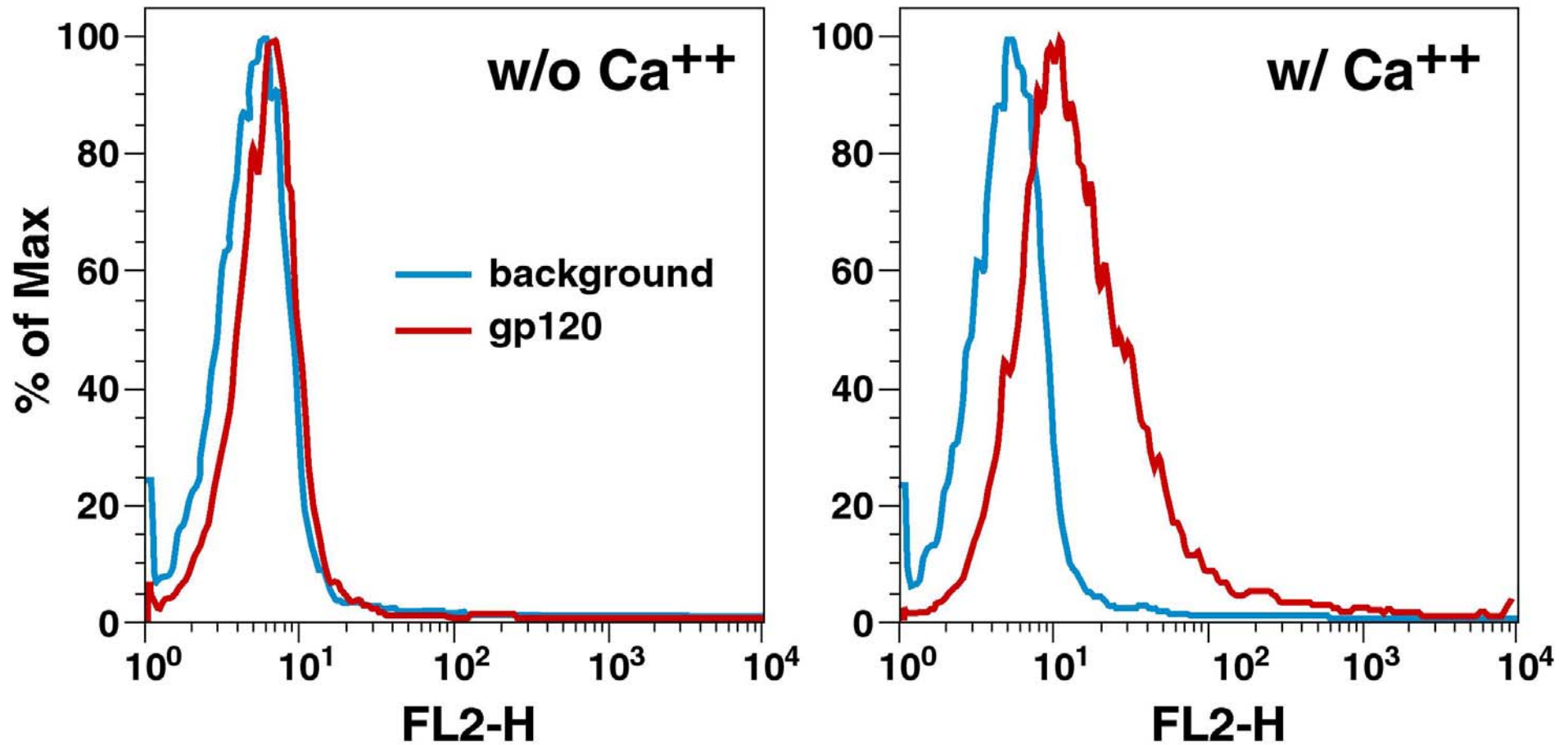


HIV gp120 Binds to Integrin $\alpha_4\beta_7$ by Mimicking its Natural Ligand: A Novel Role for the V2 Loop

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HIV-1 gp120 Binds to NK Cells but Requires Ca^{2+}



Identification of NK Cell gp120 Receptor

★ Strategy:

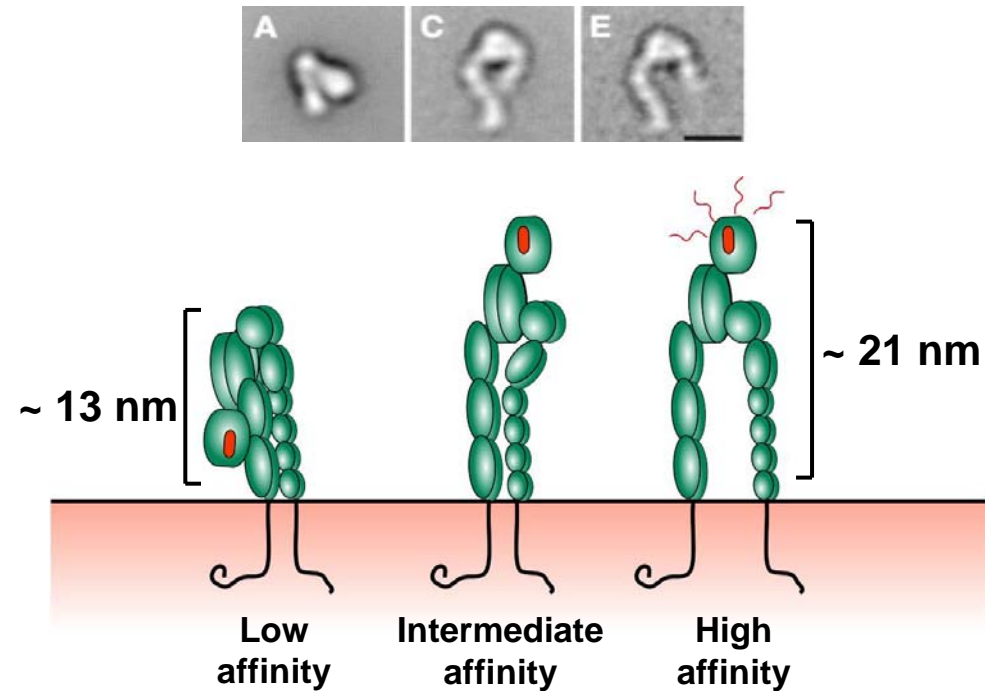
- Prepared NK cell solubilized membranes.
- Passed over a gp120 affinity column.
- Bound proteins eluted and identified by tandem-mass spectroscopy.

★ Results:

- No C-type Lectins were found.
- Integrin β_7 among the proteins identified.
- β_7 forms a heterodimer with α_4 to form the integrin $\alpha_4\beta_7$.

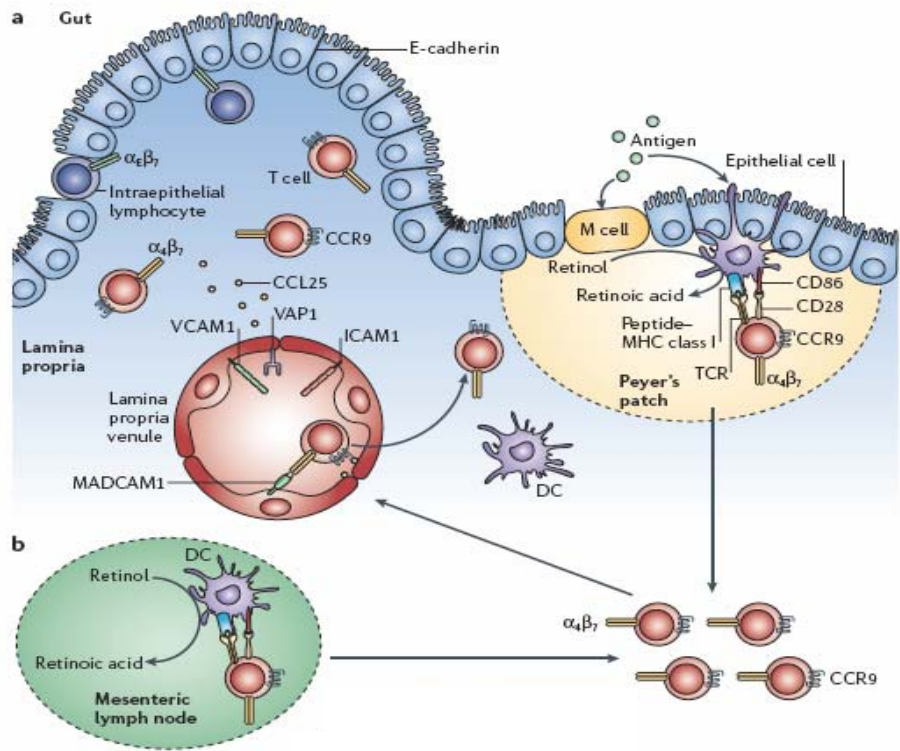
Integrin $\alpha_4\beta_7$

- ★ $\alpha_4\beta_7$ mediates homing to, and retention of leukocytes to GALT.
- ★ $\alpha_4\beta_7$ also provides costimulatory functions to T cells.
- ★ Three natural ligands: MadCAM, VCAM-1 and Fibronectin.
- ★ $\alpha_4\beta_7$ is structurally dynamic and exist in three distinct states of activation.
- ★ $\alpha_4\beta_7$ activity is strictly dependent on divalent cations ($\text{Ca}^{++} < \text{Mg}^{++} < \text{Mn}^{++}$).

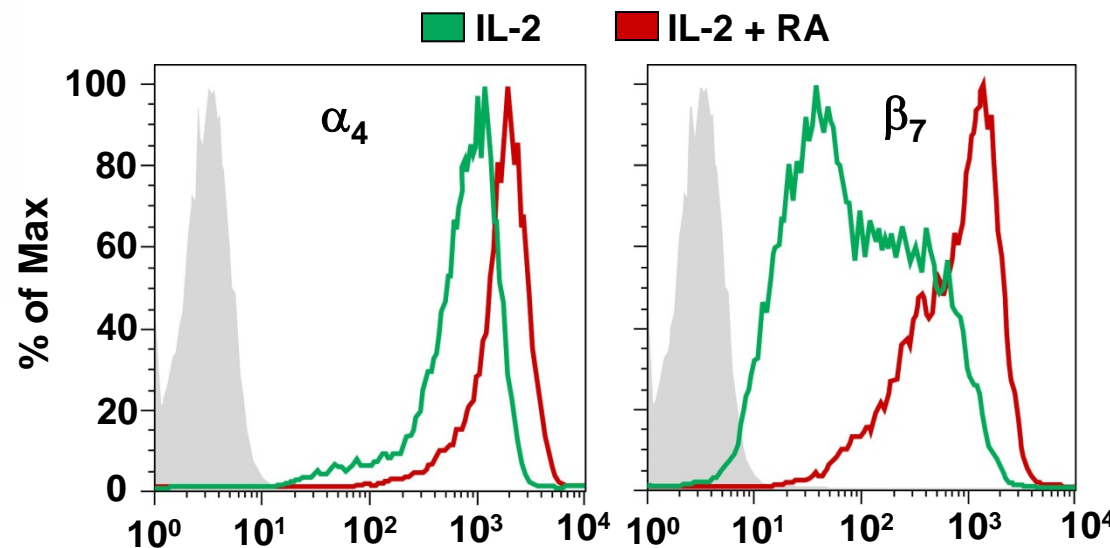


Adapted from Kinashi Nature Reviews Immunology 5 (July 2005)

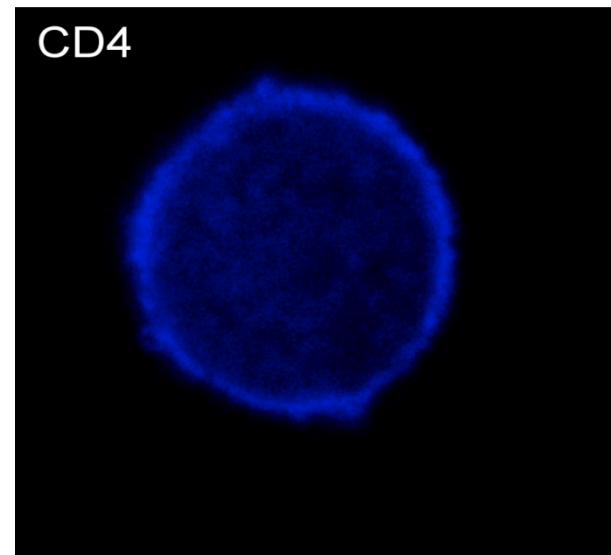
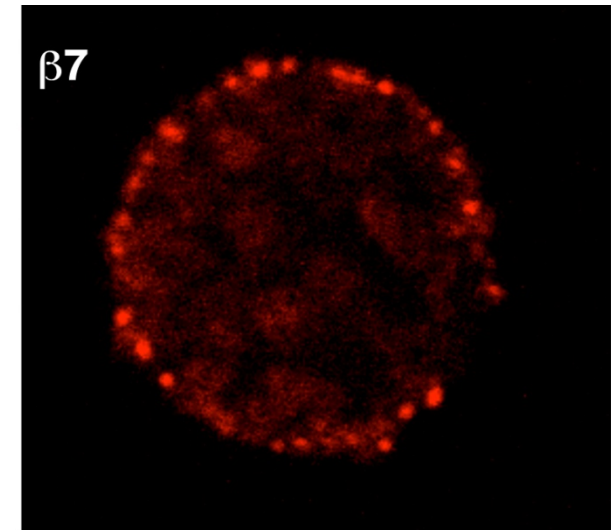
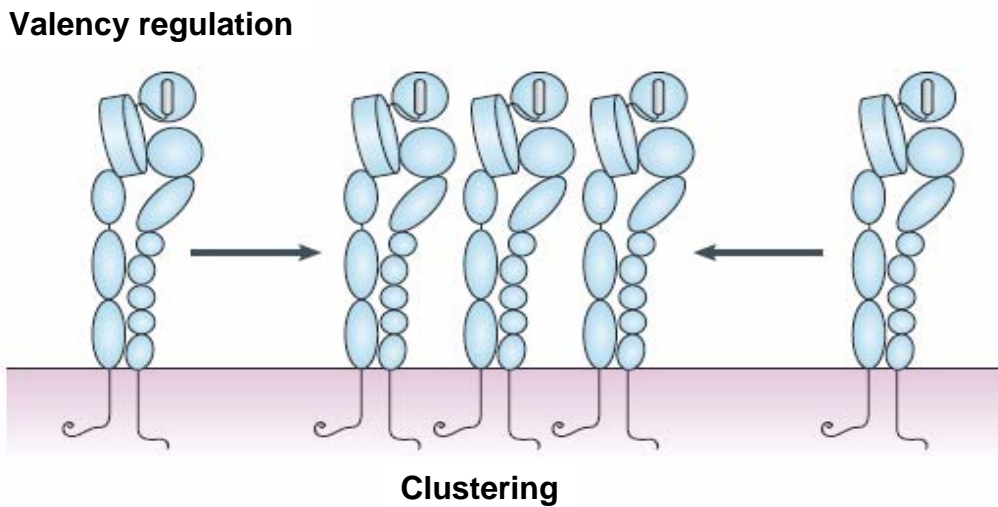
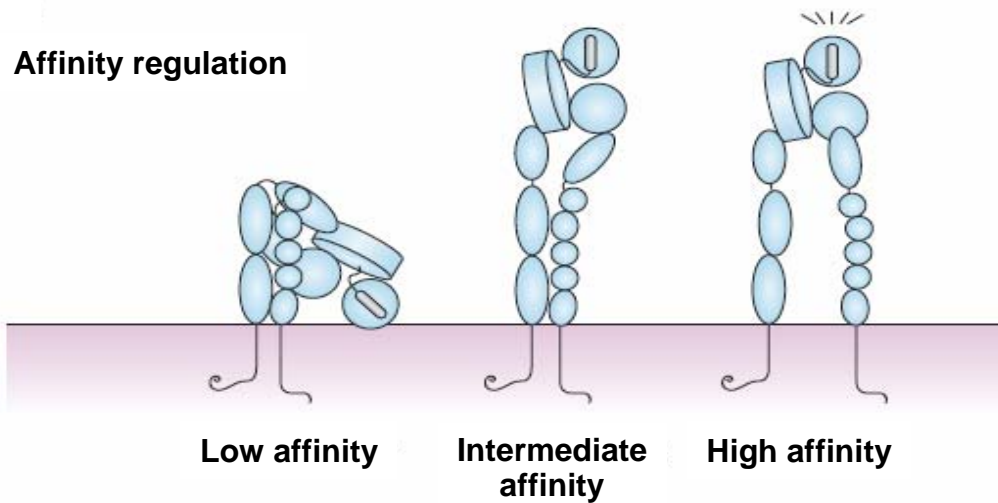
$\alpha_4\beta_7$ Mediates Homing and Retention of Lymphocytes to the GALT



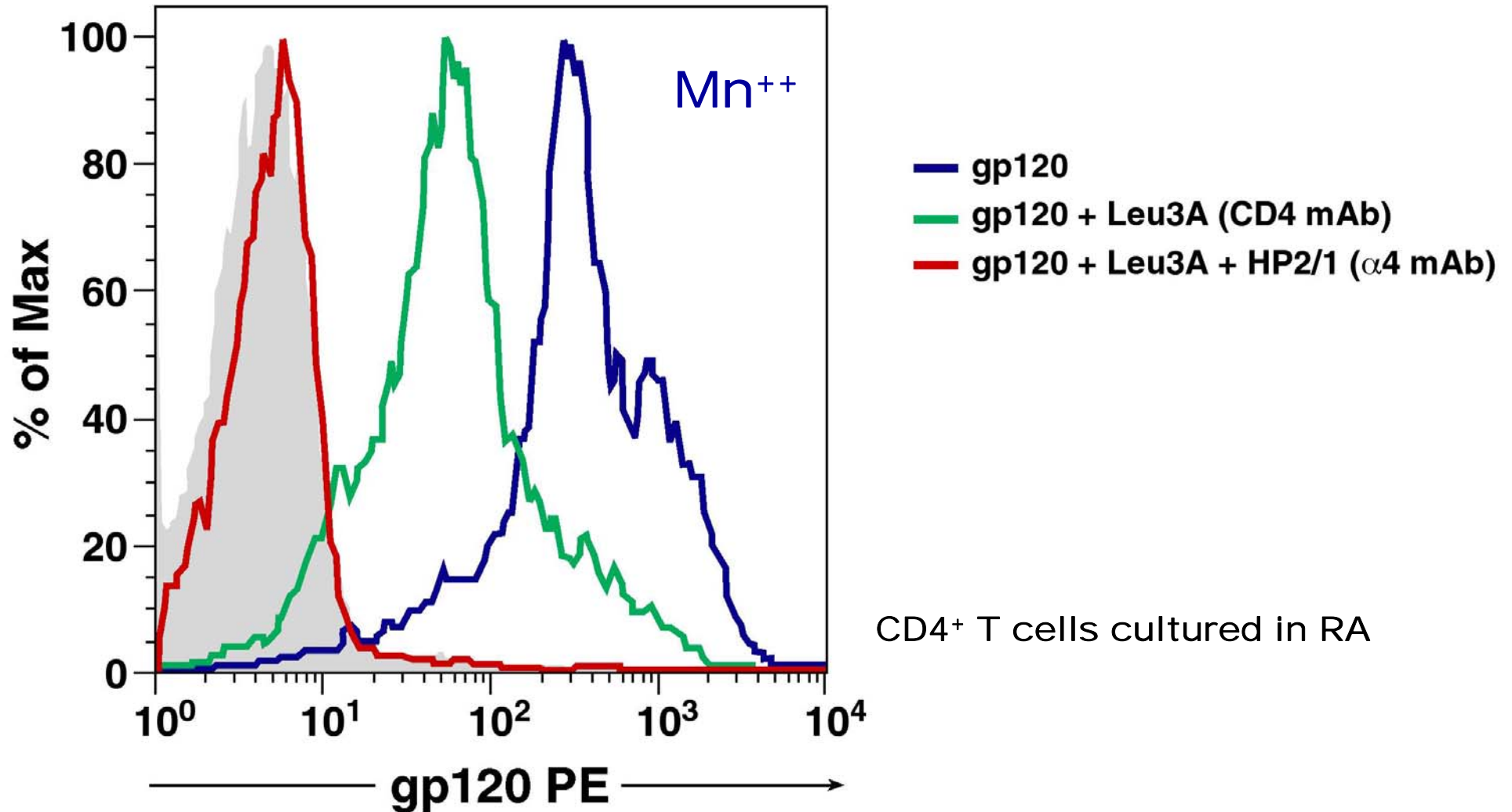
- ★ Gut DCs secrete retinoic acid (RA) which acts to increase the expression/activation state of $\alpha_4\beta_7$.
- ★ A subset of peripheral T cells are positive for both α_4 and β_7 , but little (< 2%) of the $\alpha_4\beta_7$ is in an active conformation.
- ★ Culturing peripheral T cells in RA increases the expression/activation state of $\alpha_4\beta_7$.



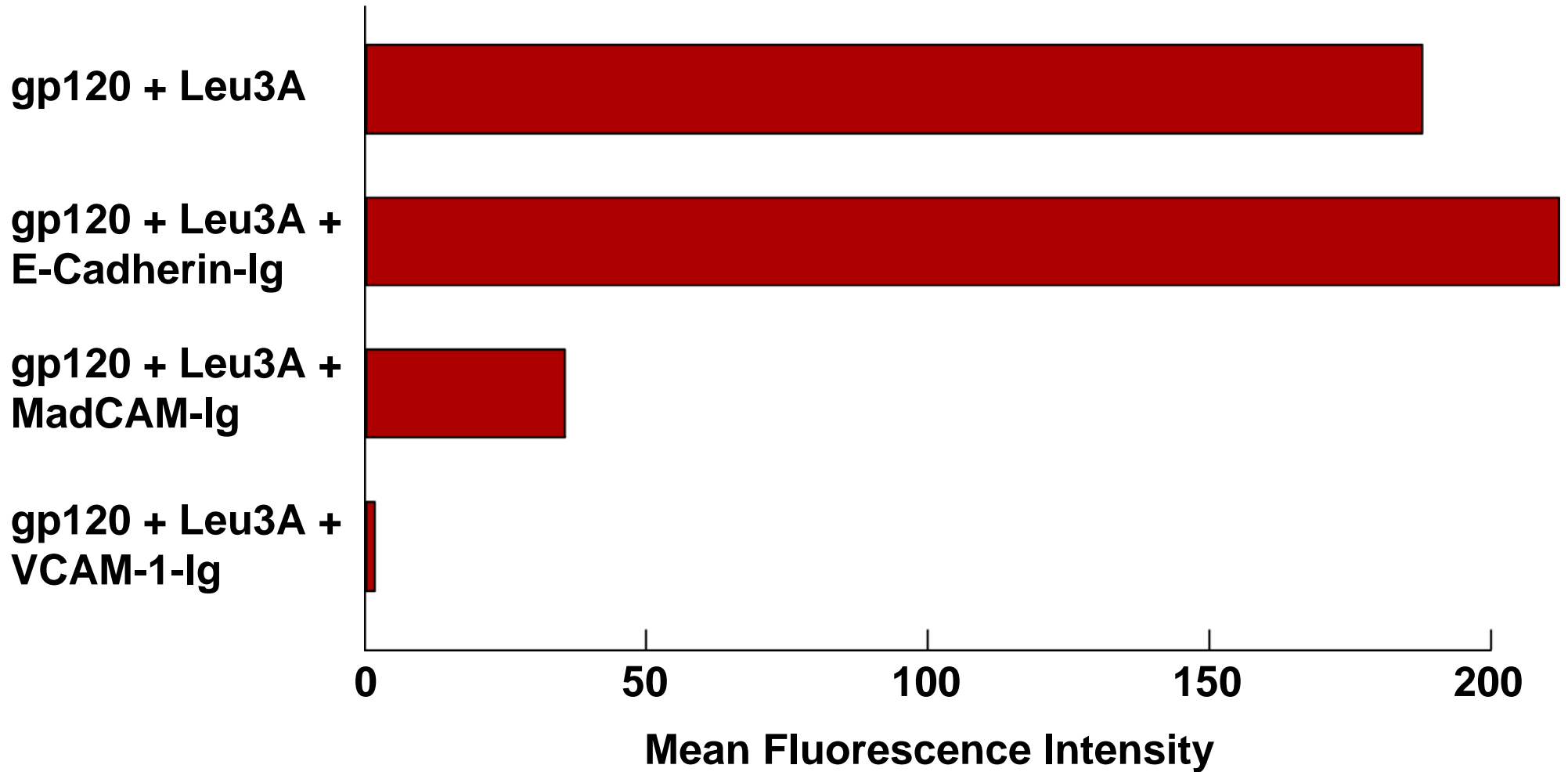
Integrins Regulate Affinity and Activity by Clustering on the Cell Surface



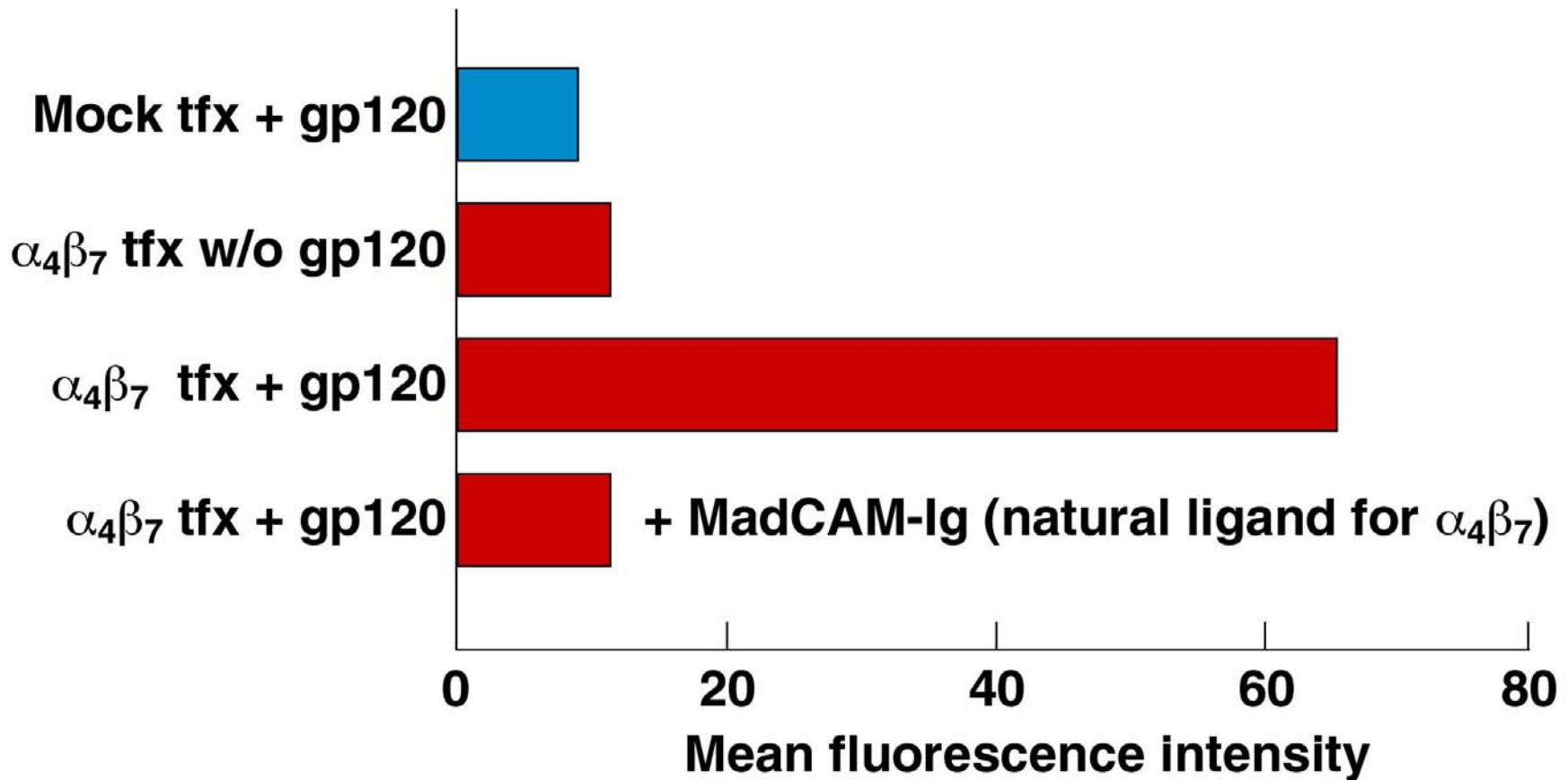
gp120 Binds to CD4⁺ T Cells in a CD4 Independent Manner



gp120 Binding to $\alpha_4\beta_7$ is Inhibited by $\alpha_4\beta_7$ Ligands MadCAM and VCAM-1



Transient Transfection (tx) of $\alpha_4\beta_7$ in 293T-cells Promotes gp120 Binding



$\alpha_4\beta_7$ Binds to its Three Ligands via Tripeptide Motifs

	<u>Motif</u>
MadCAM	LDT
VCAM-1	IDS
Fibronectin	LDV

Removal of the core Aspartic Acid residue abrogates $\alpha_4\beta_7$ binding

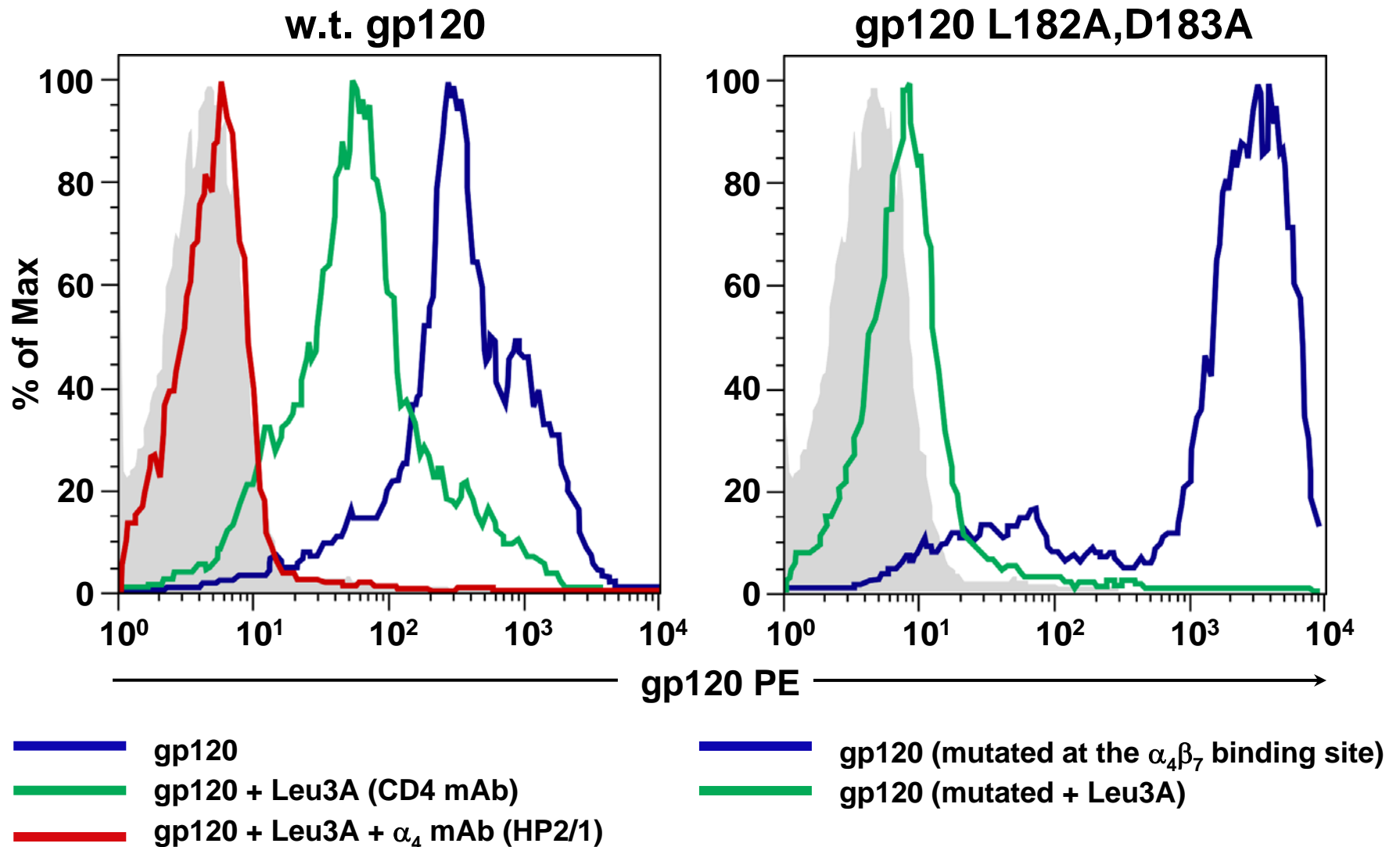
Sequence Conservation in the V2 Loop of HIV-1: Molecular Mimicry of the $\alpha_4\beta_7$ - Binding Motif

V2 Loop

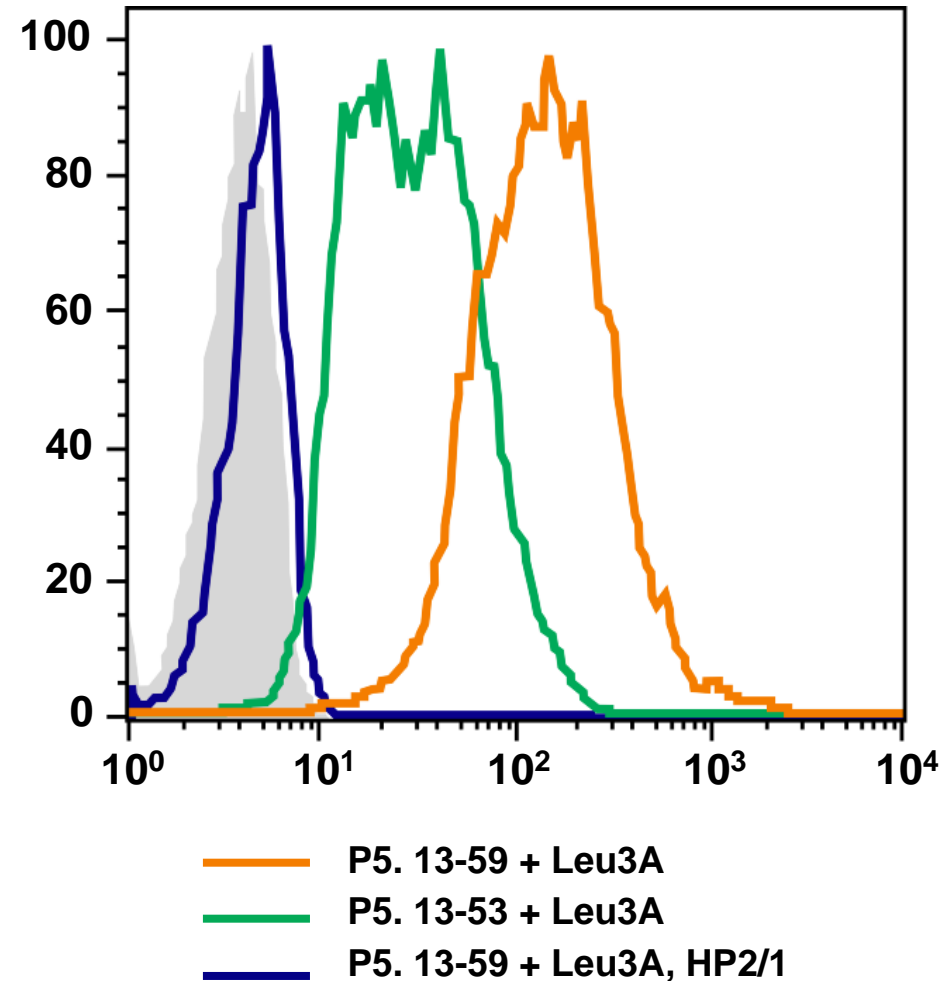
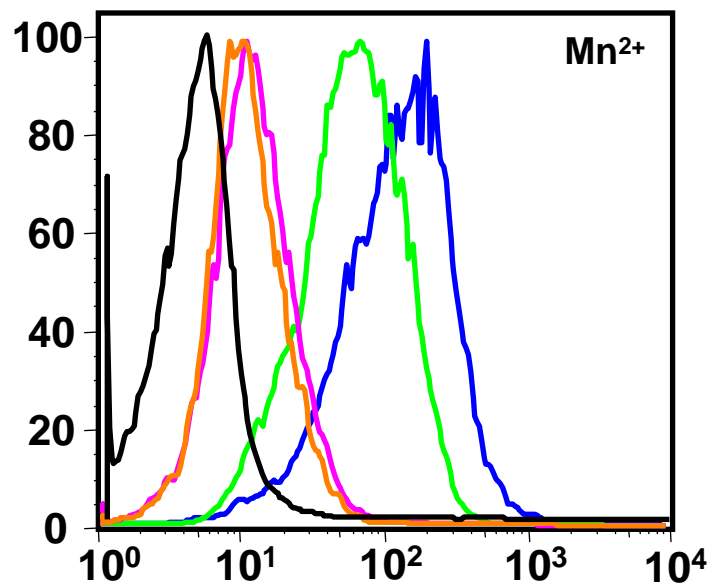
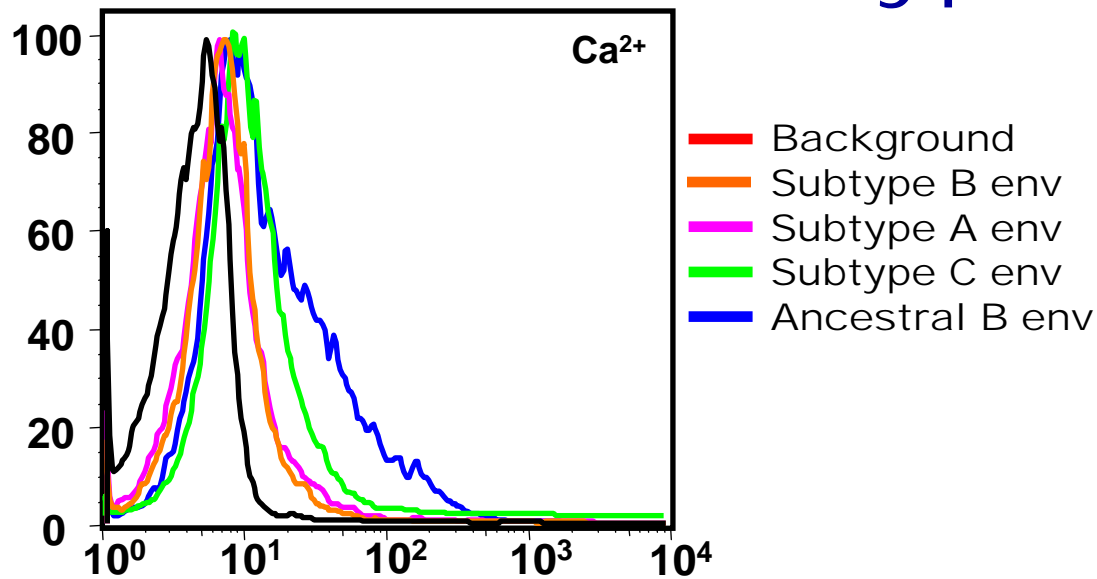
HXB2:	156	CSFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYSLTSC	199
CONSENSUS:		CSFNITTEIRDK K K K VYALFYK LDV VPID-DNNSYRLINC	
CONSENSUS A1:		LDV	
CONSENSUS A2:		LDV	
CONSEBSUS B		LDV	
CONSENSUS C		LDI	
CONSENSUS D		LDV	
ANCESTRAL A1		LDV	
ANCESTRAL B		LDV	
ANCESTRAL C		LDI	
CONSENSUS 0		DLV/M	
CONSENSUS SIVsmm		DLV	
CONSENSUS HIV-2		KDV	

98% of HIV-1 gp120s in sequence database contain Aspartic Acid at position 183

A Conserved LDV Epitope in the V2-Loop Mediates gp120 Binding to $\alpha_4\beta_7$



gp120 Isolated from Multiple HIV-1 Subtypes Bind to $\alpha_4\beta_7$



Evidence Suggesting Molecular Mimicry

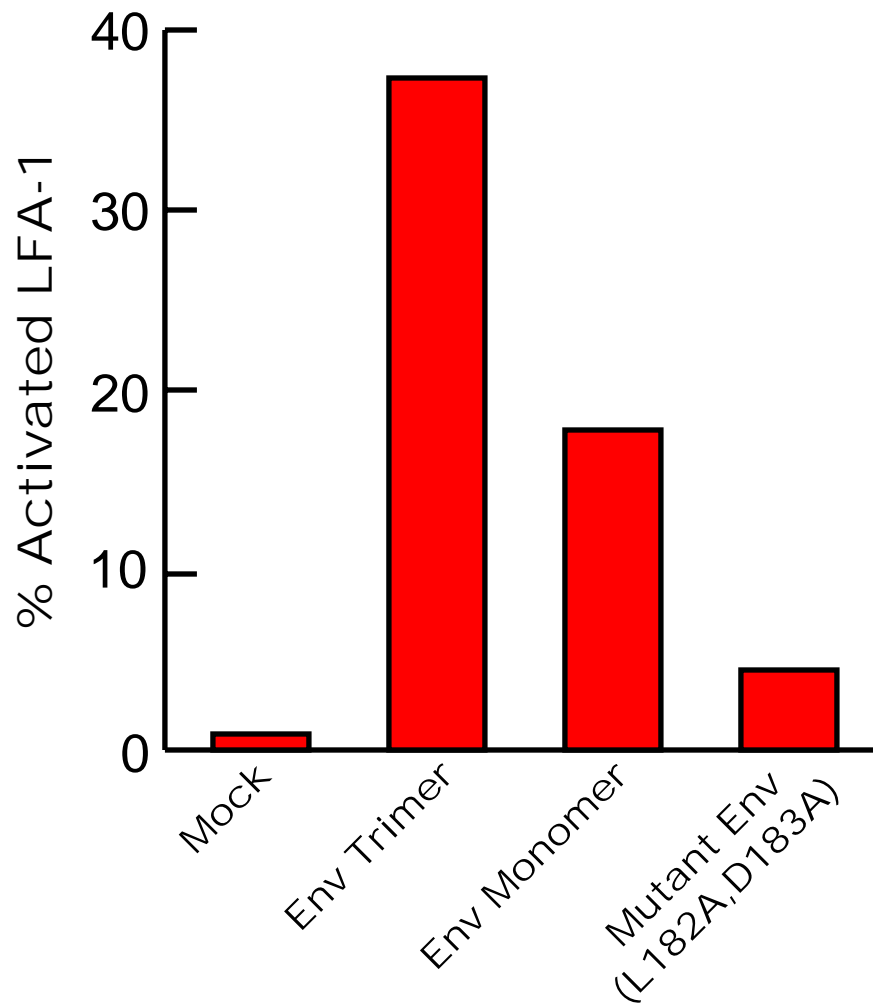
- ✦ gp120 competes with the natural ligands of $\alpha_4\beta_7$
- ✦ Binding is cation dependent and greatly enhanced in the presence of Mn^{++}
- ✦ gp120 utilizes a tripeptide motif that is structurally related to tripeptide motifs present in all three natural ligands

What is the selective
advantage to HIV of
gp120: $\alpha_4\beta_7$ interaction?

Integrins Used by Different Viruses

Virus Family	Virus Type	Integrins Used	Role in Infection
Adenovirus	Adenovirus 2/5	$\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha M\beta 2$, $\alpha v\beta 5$	Cell entry, signaling, endosome escape
Herpesvirus	HCMV	$\beta 1$, $\alpha v\beta 3$	Cell entry, signaling
	HHV-8	$\alpha 3\beta 1$, $\alpha 2\beta 1$	Cell entry, signaling
Hantavirus	SNV	$\alpha IIb\beta 3$, $\alpha v\beta 3$	Attachment, entry (?)
	PHV	$\beta 1$	Attachment, entry (?)
Picomavirus	EV1	$\alpha 2\beta 1$	Attachment, signaling, entry
	FMDV	$\alpha 5\beta 1$, $\alpha v\beta 3$	Attachment, entry
	CA9	$\alpha v\beta 3$, $\alpha v\beta 6$	Attachment, entry
	EV9	$\alpha v\beta 3$	Attachment, entry
Reoviridae	Rotavirus	$\alpha x\beta 2$, $\alpha 2\beta 1$, $\alpha v\beta 1$, $\alpha 4\beta 7$, $\alpha v\beta 3$	Attachment, entry
	Reovirus (Lang)	$\beta 1$	Cell entry

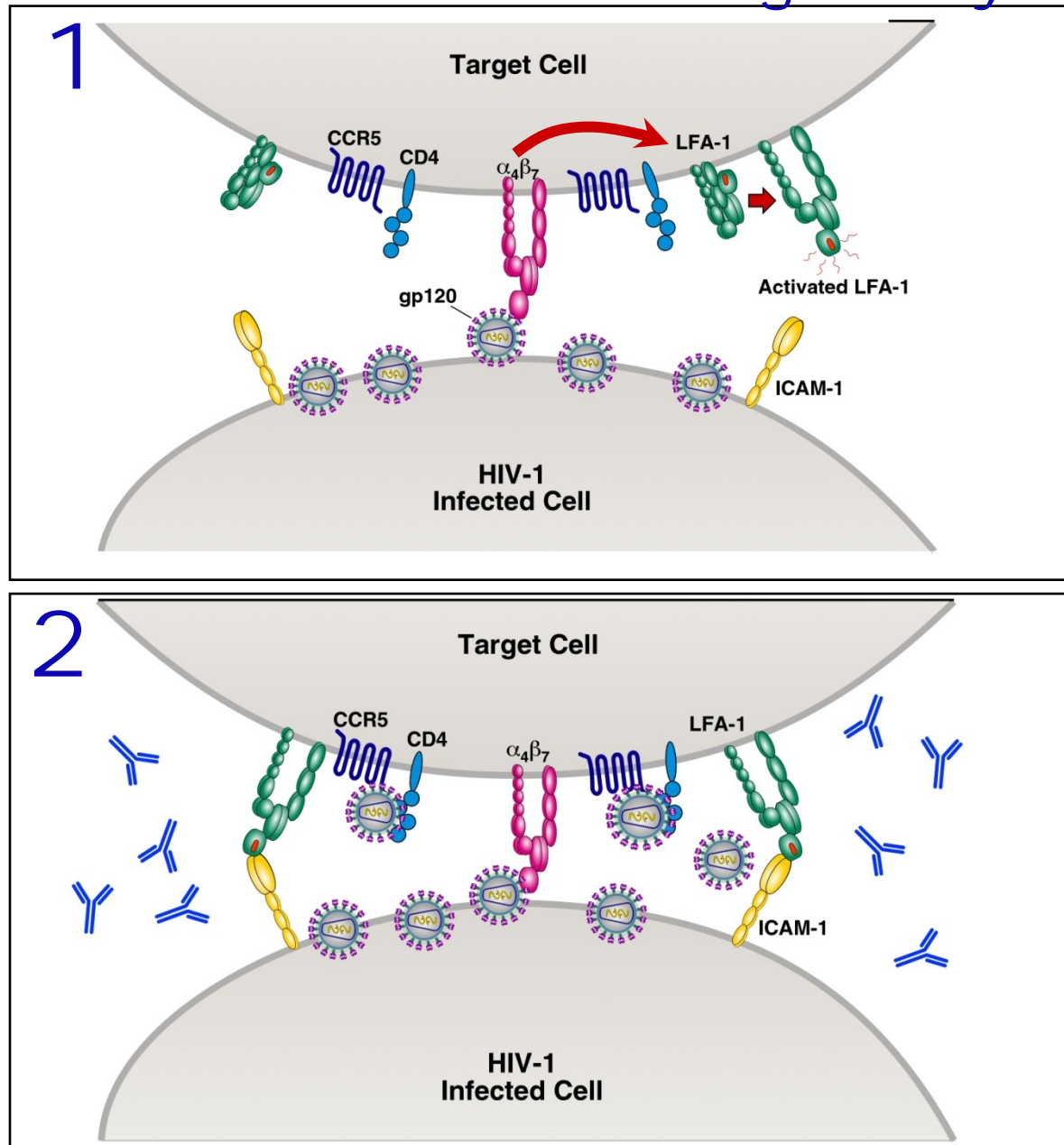
gp120 Rapidly Activates LFA-1 ($\alpha_L\beta_2$) on CD4⁺ T Cells in an $\alpha_4\beta_7$ Dependent Manner



Anti- β_2 MAb MEM-148:
Recognizes "activated"
form of LFA-1 ($\alpha_L\beta_2$)

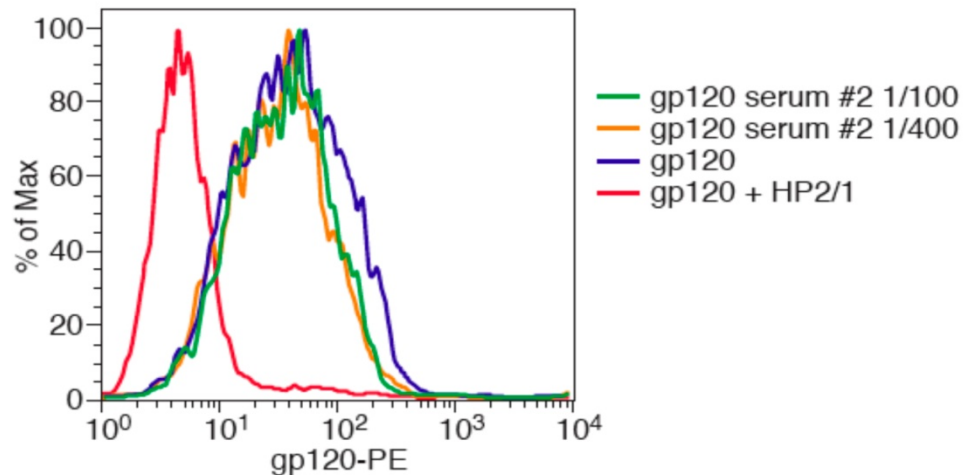
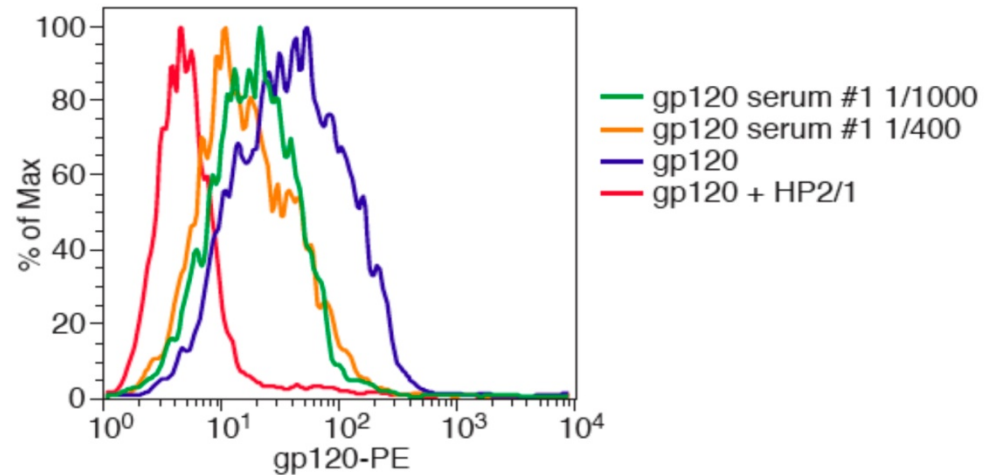
Claudia Cicala et.al.
Poster #P11-16

Hypothesis: gp120 Binding to $\alpha_4\beta_7$ Contributes to the formation of a Virological Synapse



Does HIV Immune Sera Inhibit gp120 Binding to $\alpha_4\beta_7$?

- Sera from two patients chronically infected with HIV-1 subtype B
- Inhibition of subtype B gp120 binding to $\alpha_4\beta_7$



Summary

- ★ HIV-1 gp120 binds to $\alpha_4\beta_7$ on NK Cells, CD4⁺ T Cells and CD8⁺ T Cells in a divalent cation dependent manner.
- ★ HIV-1 gp120 from all major subtypes binds to and signals through an **activated conformation** of integrin $\alpha_4\beta_7$ although binding efficiency among HIV isolates varies widely.
- ★ Culturing CD4⁺ T Cells in **Retinoic Acid** promotes gp120: $\alpha_4\beta_7$ binding.
- ★ We have identified a function for the V2 loop: by ligating $\alpha_4\beta_7$ it mediates the activation of LFA-1.
 - LFA-1 activation contributes to the formation of virological synapses, providing a mechanism for more efficient infection.
- ★ A highly conserved tripeptide motif (LDV/I) in the V2 loop mediates $\alpha_4\beta_7$ binding.

Acknowledgements

Laboratory of Immunoregulation
(NIAID)

James Arthos

Claudia Cicala

Diana Goode

Ravindra Gopaul

Shyam Kottlil

Katie Macleod

Elena Martinelli

Jonathan McNally

Massimiliano Pascuccio

Danlan Wei

Anthony Fauci

LPAT (NCI/SAIC)

Tom Conrads

Tim Veenstra

Zhen Xiao

LIB (NCI/SAIC)

Richard Lempicki

University of
Washington

Sherry McLaughlin

James I. Mullins