Molecular Events Underlying Cell-Associated HIV Transmission

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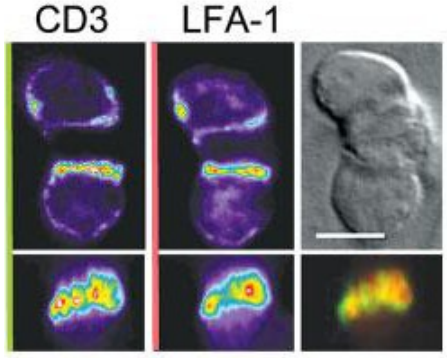
HIV – Dendritic Cell Interactions

- Robust virus replication in dendritic cell (DC) – T cell co-cultures (*Cameron et al, 1992*).
- Mature DCs can transfer HIV-1 particles to $CD4^+$ T cells at an enhanced efficiency

– primarily because of establishment of stable DC – T cell conjugates (*Macdonald et al, 2003*)

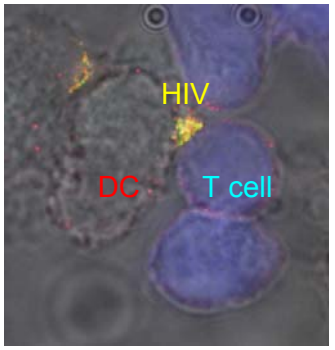
DC – T cell Immunological Synapse



EJI 2005 35:1741

MHC – TCR (CD3)
CD4 or CD8
ICAM1 – LFA1
CD80/86 – CD28

DC – T cell Virological Synapse



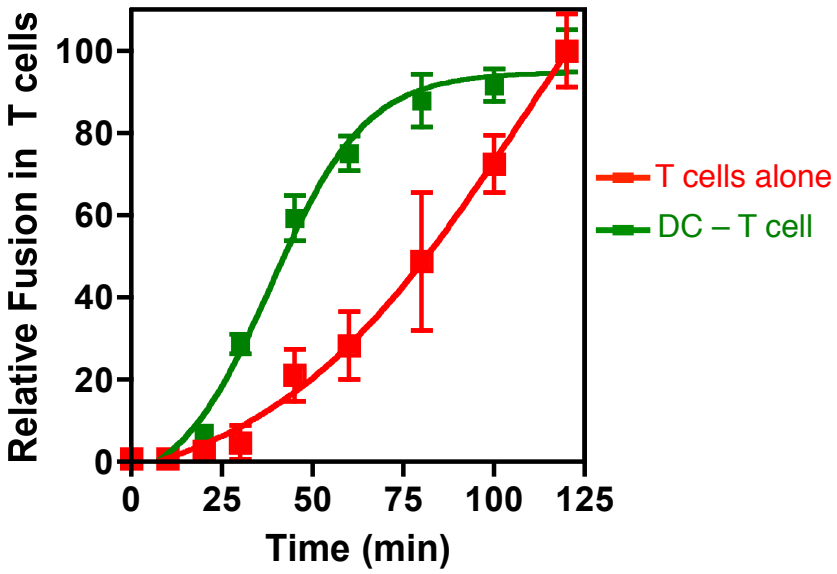
MHC – TCR (CD3)
CD4 or CD8
ICAM1 – LFA1
CD80/86 – CD28
CD81

– Putative evasion from neutralizing antibody responses (*Sagar et al, 2012*)

Dendritic Cells Facilitate HIV-1 Infection

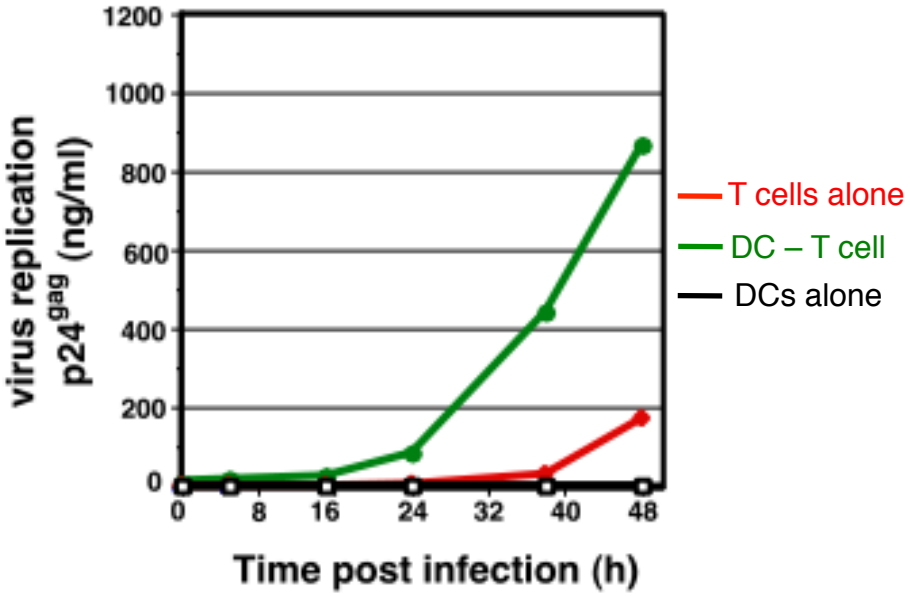
- DC – T cell co-cultures are sites of robust viral replication

Kinetics of Establishment of Productive Infection in CD4+ T cells is Enhanced in the Setting of DC – T cell Co-cultures



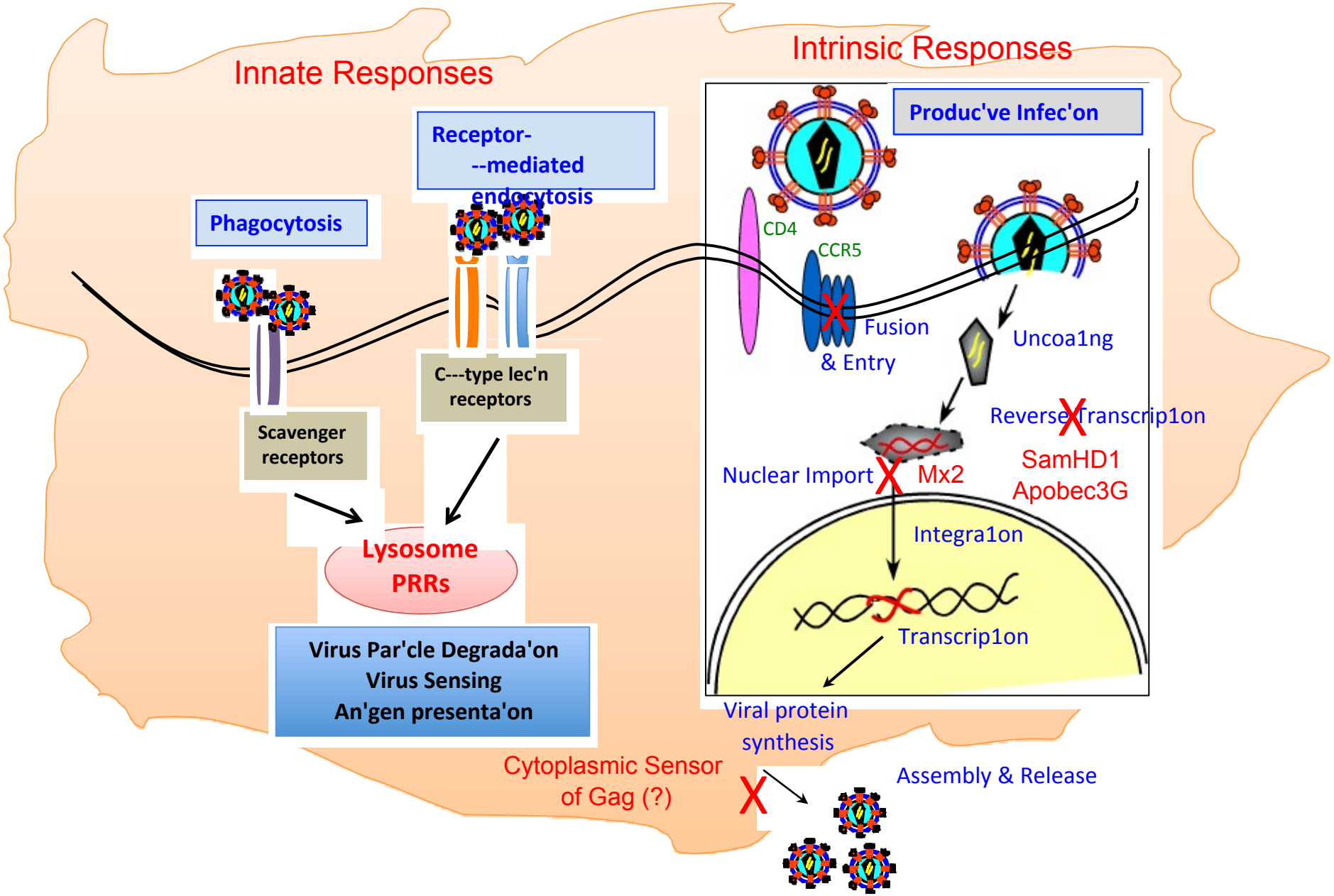
DC - T transfer (50% fusion) = 38.6 ± 1.75 min
T cells alone (50% fusion) = 84 ± 2.5 min

Magnitude of Virus Replication in CD4+ T cells is Enhanced in the Setting of DC – T cell Co-cultures

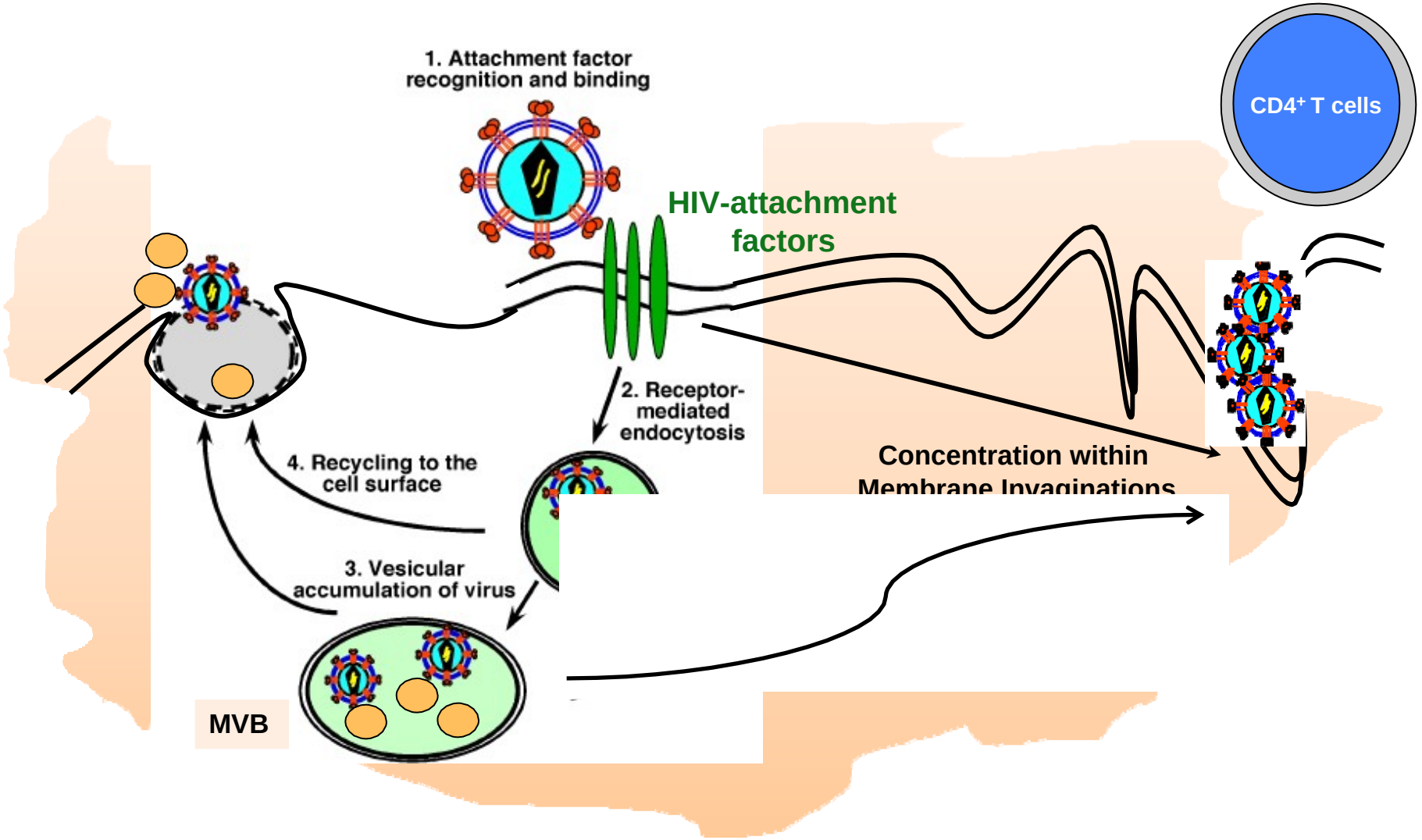


J. Virol. 2002 76: 10692 - 71

Dendritic Cells: Hostile Cellular Environment for HIV

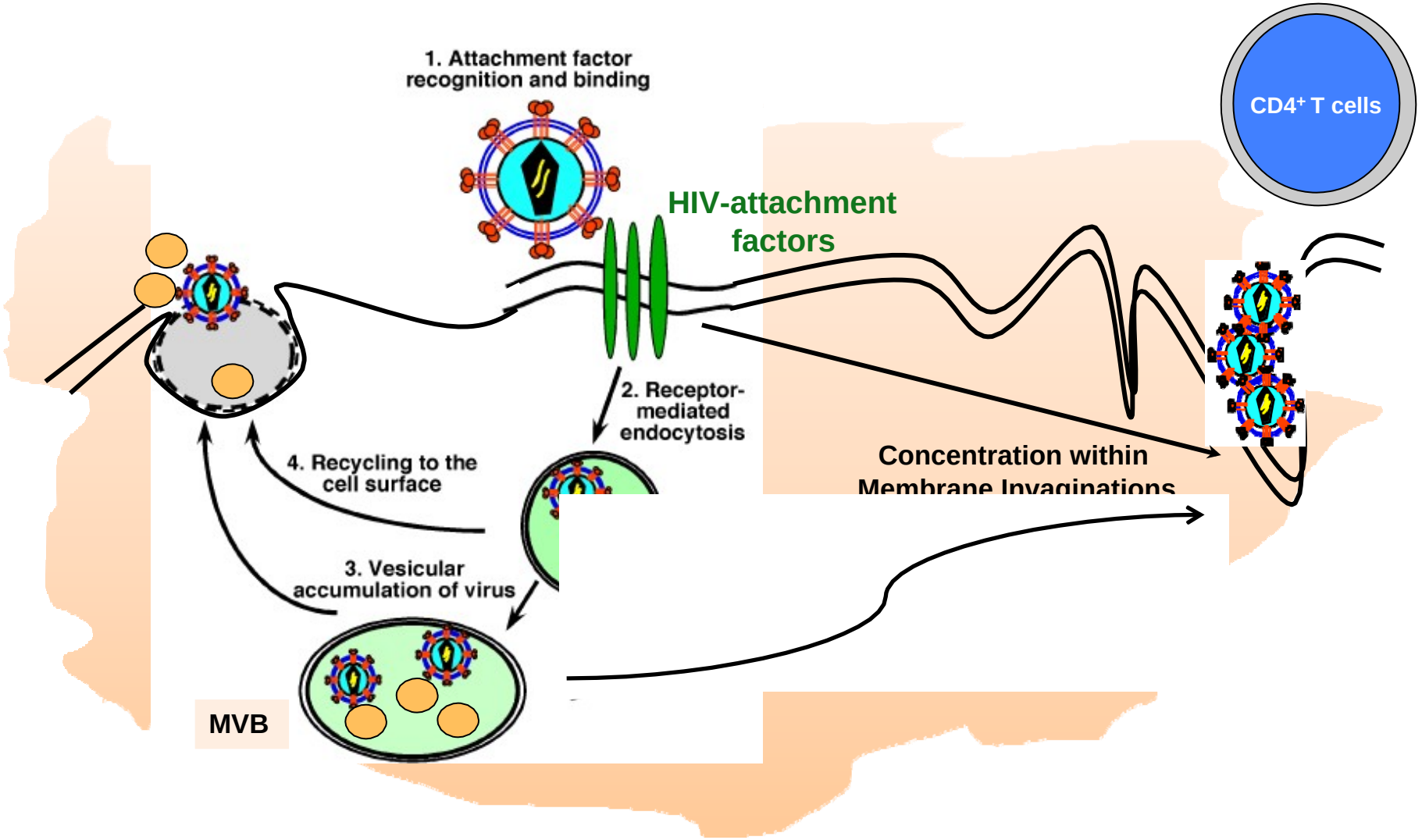


Dendritic Cell-Associated HIV-1 trans Infection



Captured HIV-1 Particles transferred to T cells
Without de novo virus replication in Dendritic Cells

Dendritic Cell-Associated HIV-1 trans Infection



Captured HIV-1 Particles transferred to T cells
Without de novo virus replication in Dendritic Cells

HIV-1 Attachment factors on Dendritic Cells

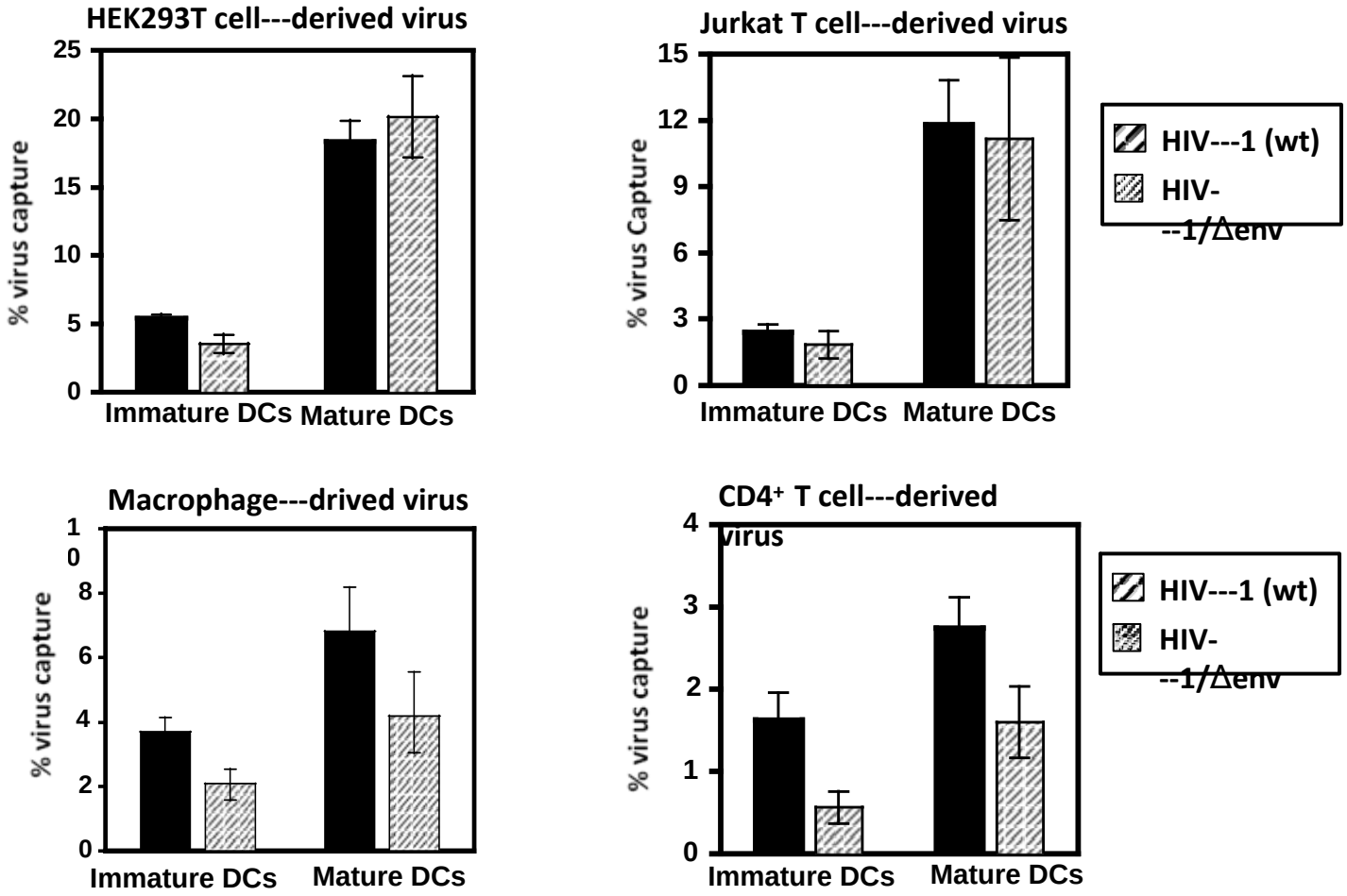
Envelope Glycoprotein gp120 - Dependent Binding

- C-type lectin receptors
  - Recognition of the glycosylated gp120
  - DC-SIGN, Langerin, DCIR

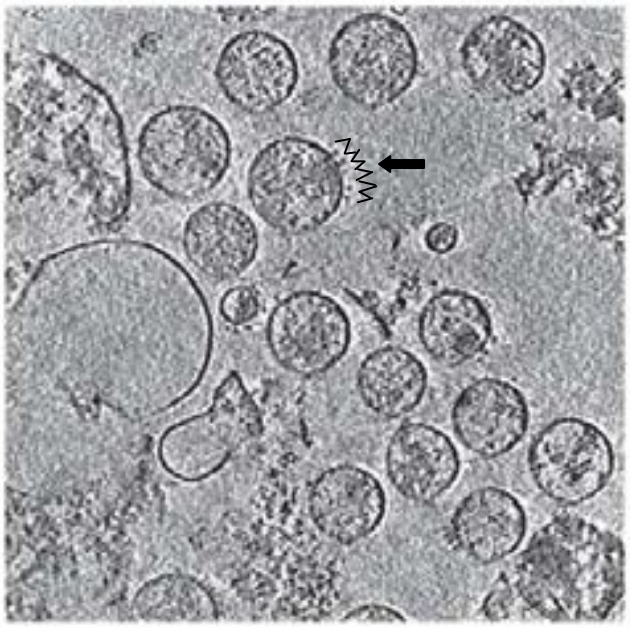
- Heparan sulfate proteoglycans (Syndecans)
  - Polyanionic surface
    - Charge-based interactions with gp120

Geijtenbeek et al, Cell 2000; Turville et al, Nat. Immunol 2002; de Witte et al, PNAS 2007; Lambert et al, Blood 2008;

Envelope Glycoprotein-Independent Capture of HIV-1 Particles by Dendritic Cells

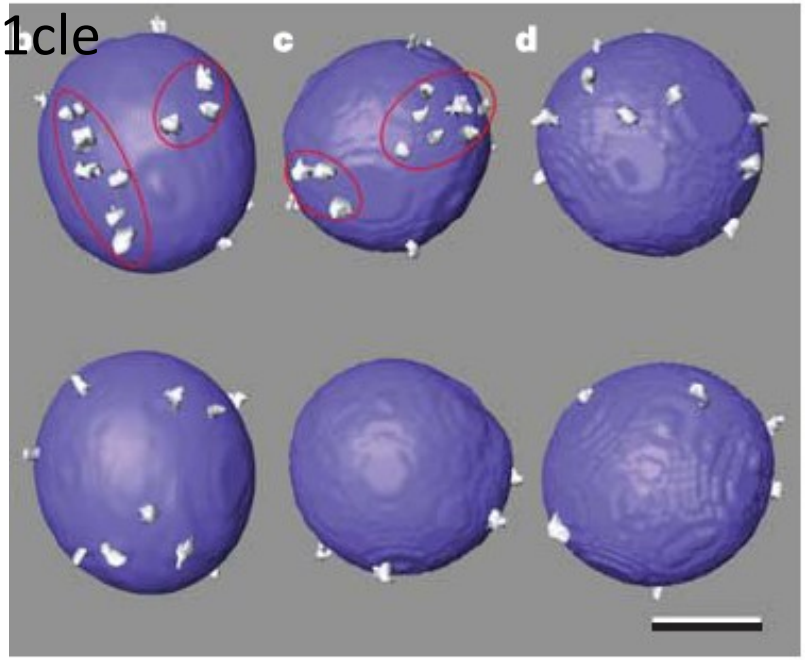


Env---deficient virus par'cles derived from divergent cellular sources remain competent for capture by dendri'c cells (*J. Virol. 2009 83: 3496---3506*)



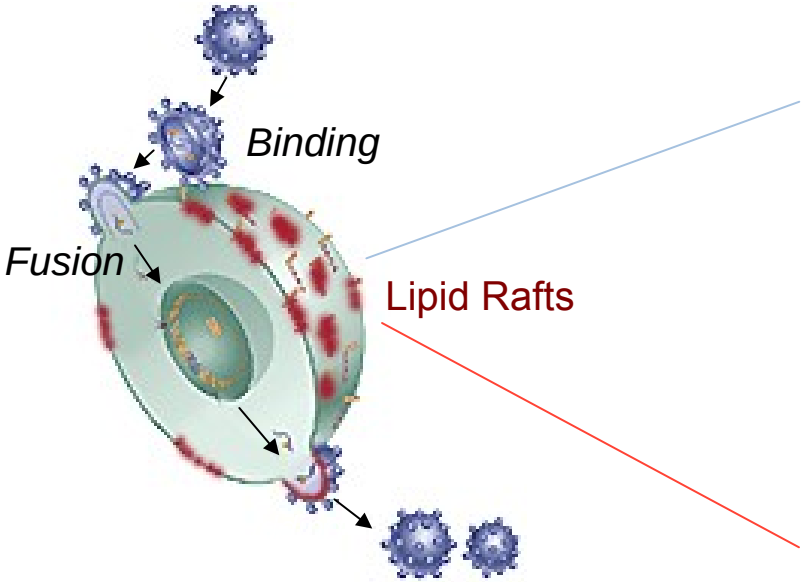
Zhu et al., Nature 2006
Sougrat et al., PLoS Pathogens 2007

~ 7 --- 14 Env trimers per par1cle

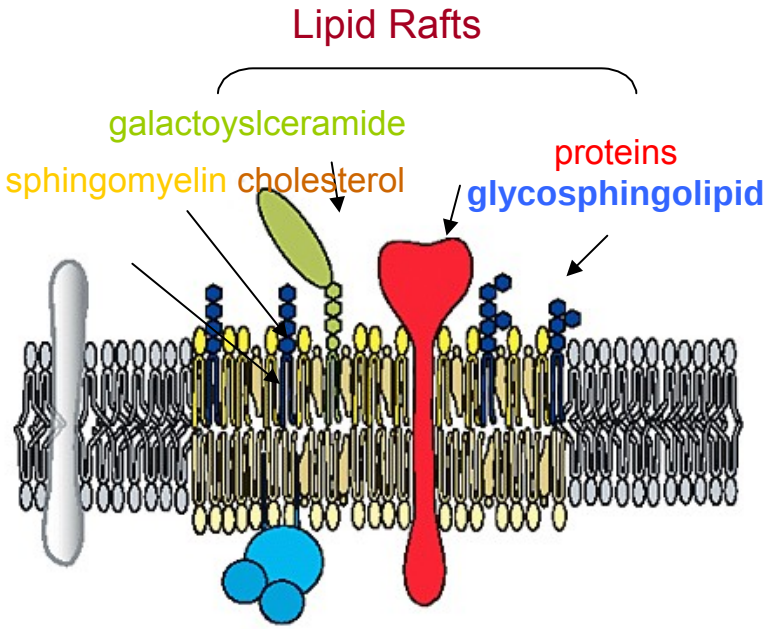


Host cellular determinants incorporated in virus particle membrane might play a role in HIV capture by dendritic cells.

HIV Incorporates Molecules from Host Membrane



Modified from progenics.com/hiv.cfm



Modified from Higashi, Glycoworld

•Lipid and protein composition of the virus particle membrane is determined by the site of virus assembly and exit.

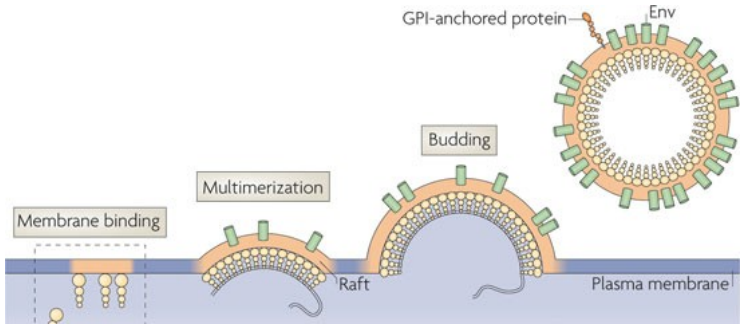
•Virus particles enriched in cholesterol, sphingolipids, glycosphingolipids, and lipid raft associated cellular proteins such as CD59 and tetraspanin proteins.

•Strategy to identify HIV determinant necessary for capture by DCs
- Selective depletion of virus particle membrane constituents

Virus Particle Associated Glycosphingolipids a re Essential for HIV-1 Capture by DCs re?

HIV Proteome

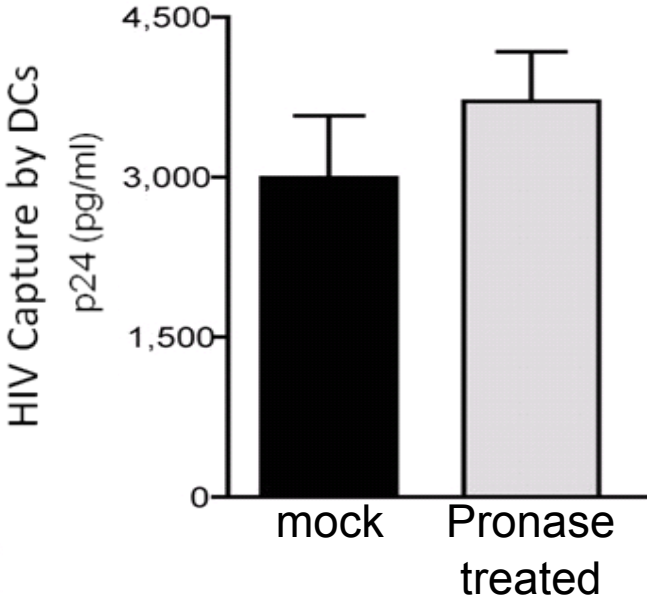
Tetraspanins
GPI-linked proteins
Class II MHC



HIV Lipidome

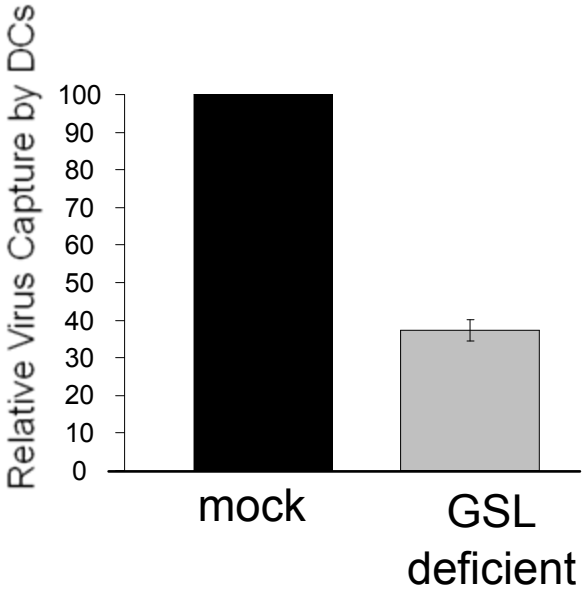
Cholesterol
Sphingomyelin
Glycosphingolipids

Removal of Protein from the virion



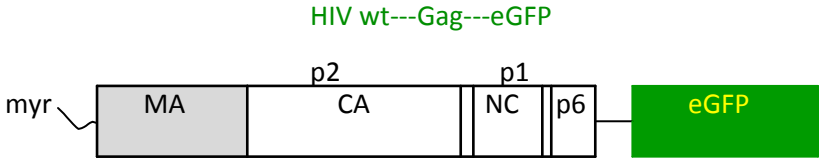
Izquierdo-Useros, Blood 2009

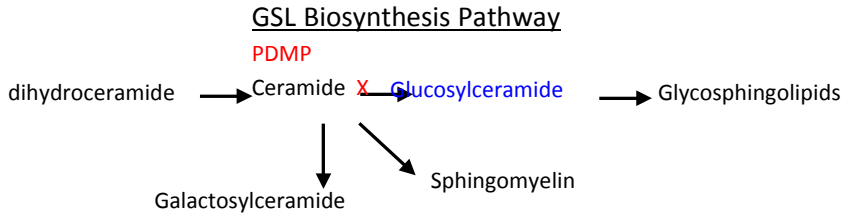
Removal of GSLs from the virion



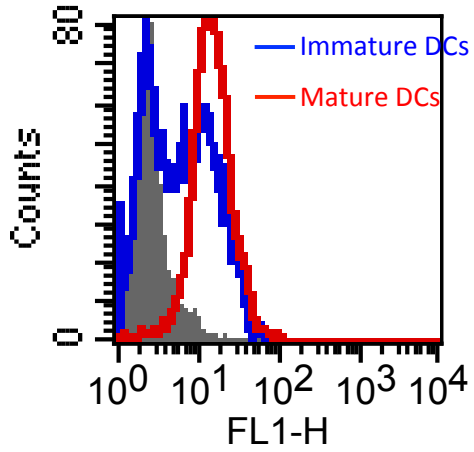
Hatch, J.Virol 2009

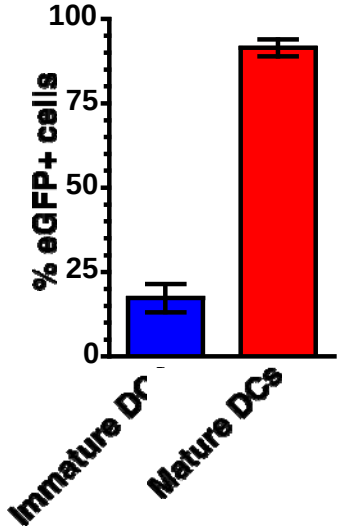
Maturation of DCs Enhances Env-Independent Glycosphingolipid Mechanism of HIV-1 Capture

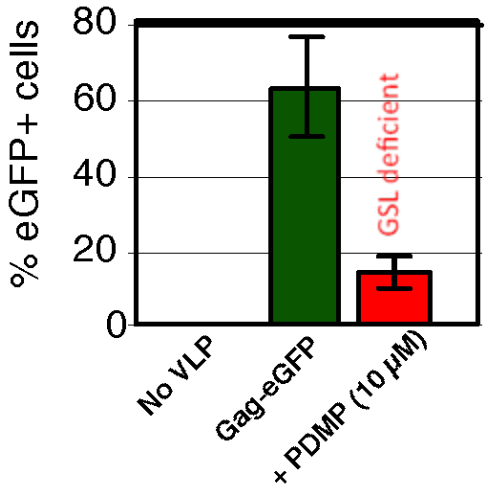


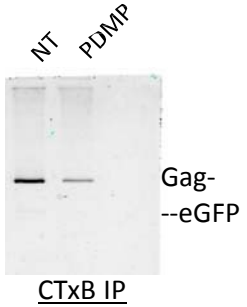


Gag---eGFP VLP Capture





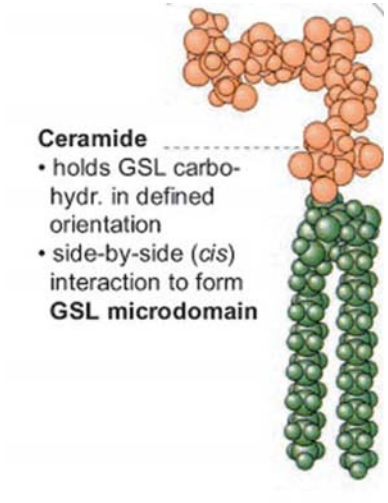




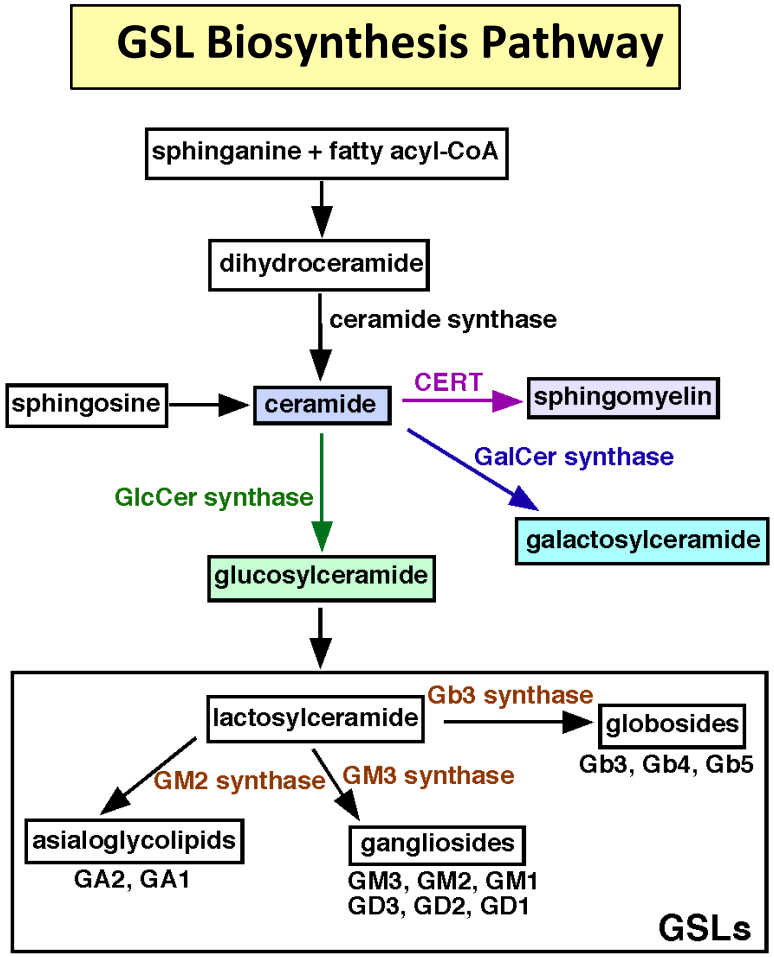
HIV VLPs produced in the presence of PDMP are impaired for capture by mature DCs

Hatch et al, J. Virol. 2009 83: 3496---3506; Izquierdo--Useros et al, Blood 2009 113:2732---2741

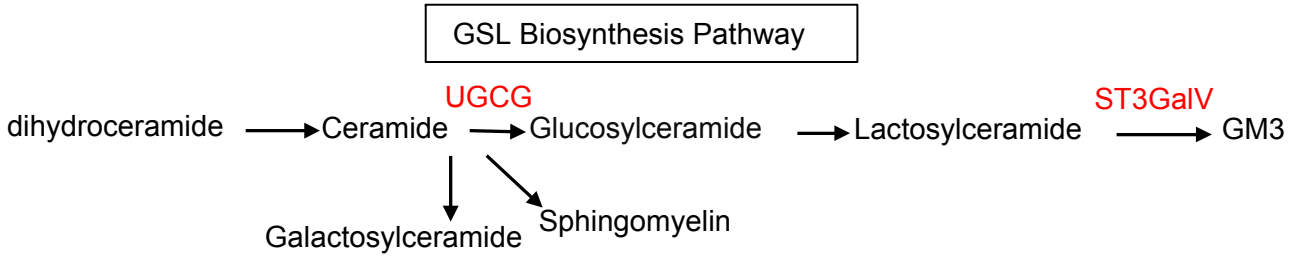
Glycosphingolipids (GSLs): Raft-Resident Lipids

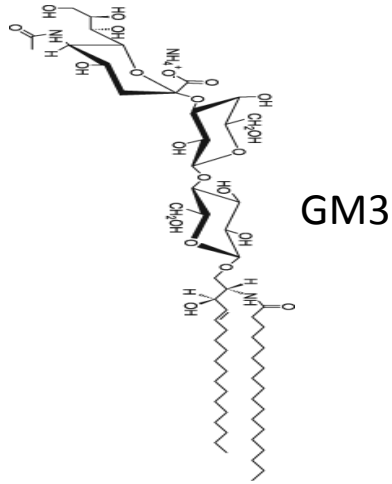


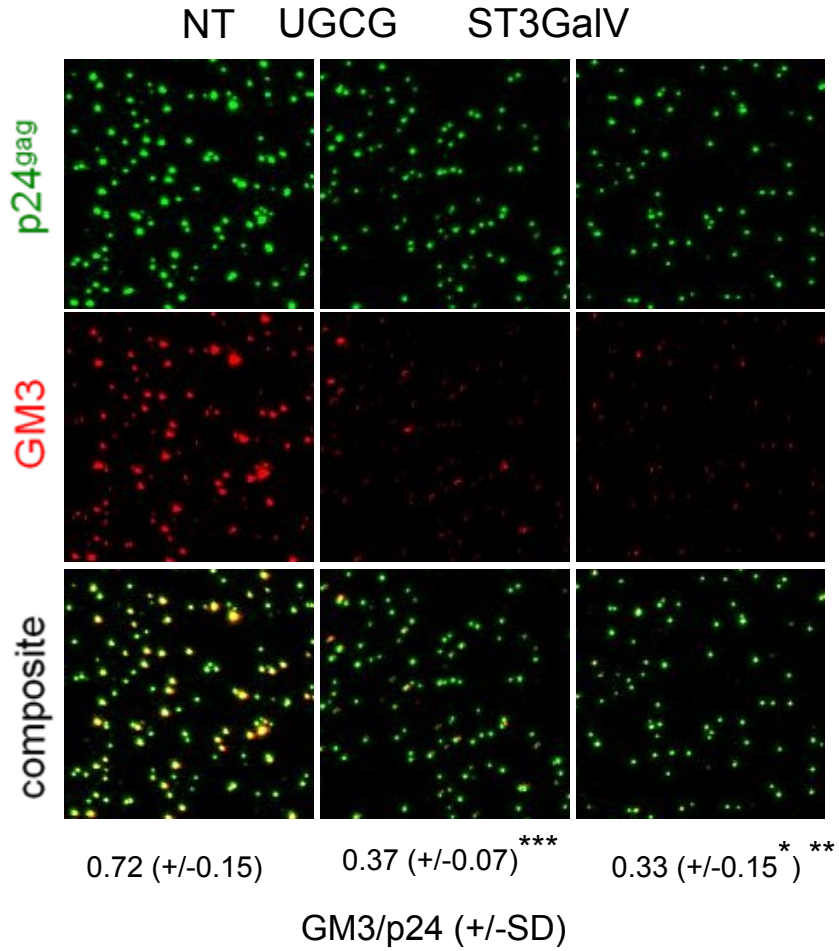
•Lipid por'on of GSL, ceramide, is embedded in the outer leaflet of the plasma membrane, and their glycan moie'es project into the extracellular space.

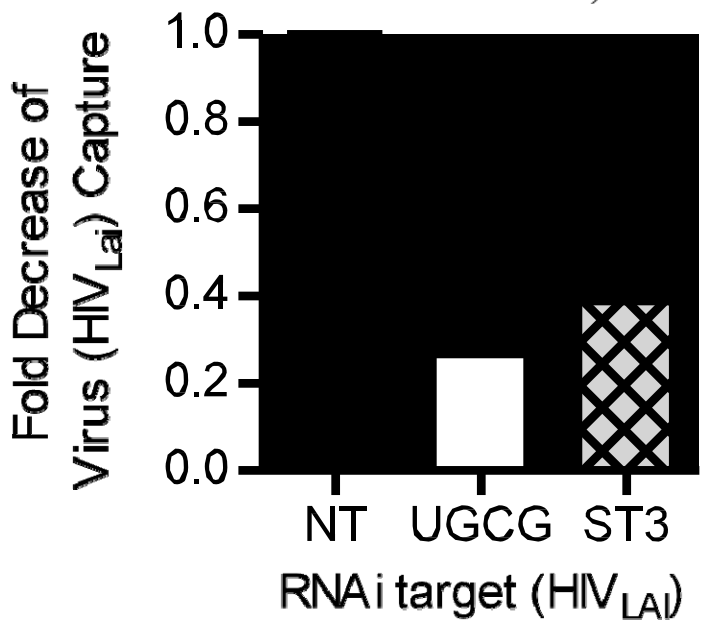


GM3-Depleted Virions are Deficient for Capture by Mature DCs



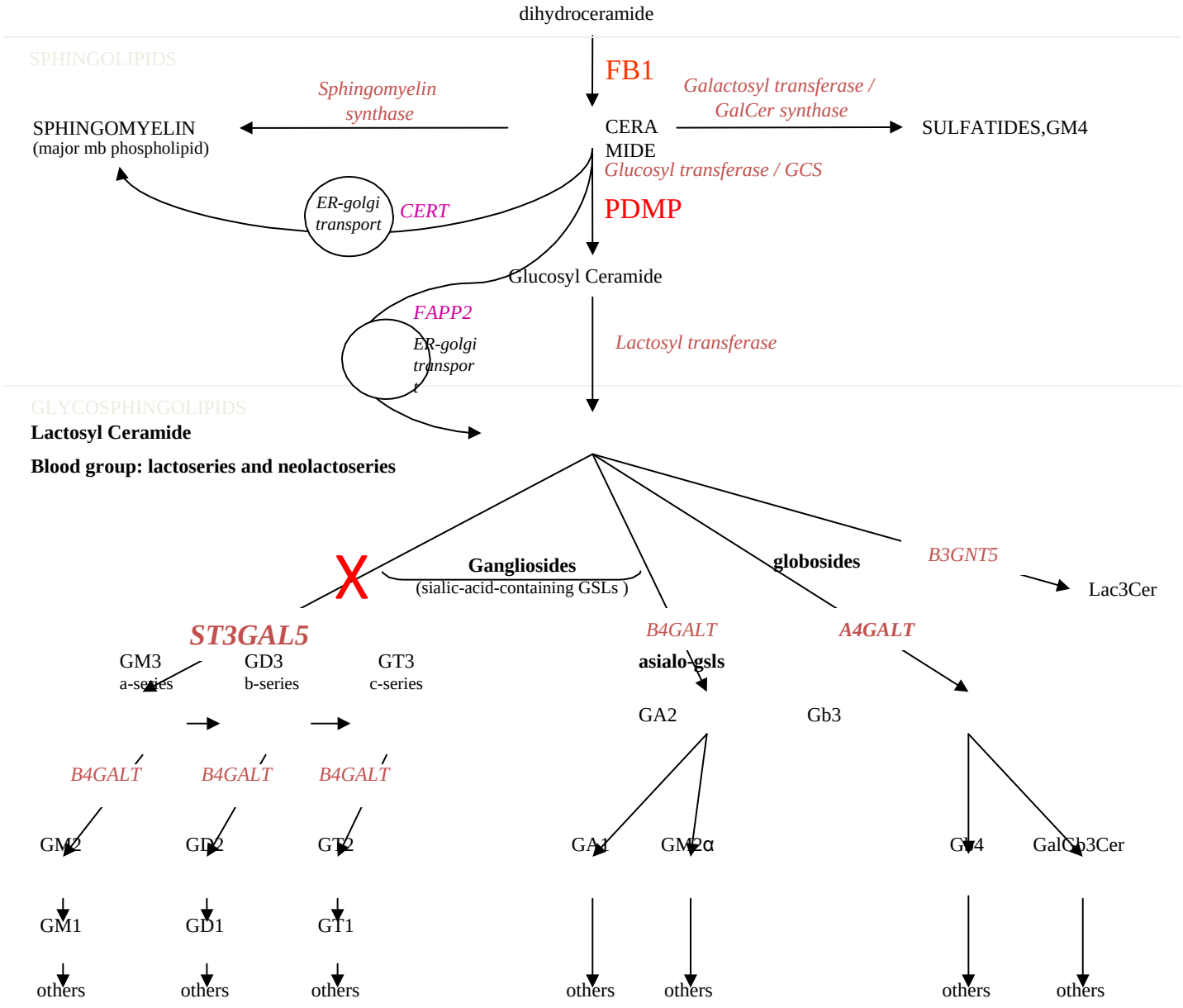






Puryear et al, PNAS 2012 109: 7475 – 7480

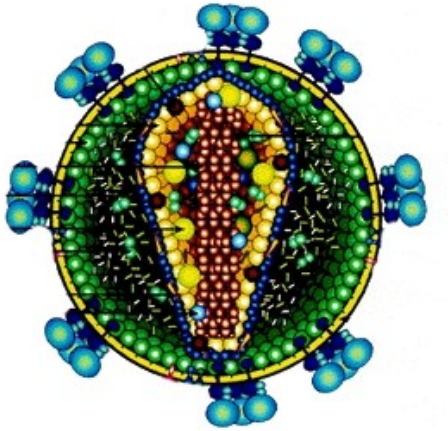
GSL Biosynthesis Pathway



Liposome system for analyzing HIV Capture by Mature DCs

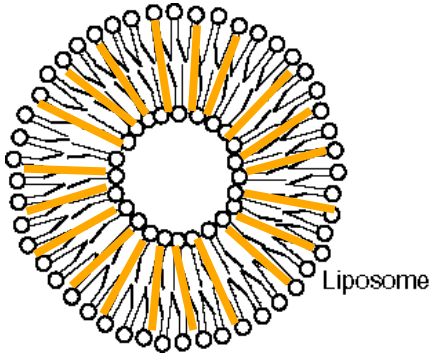
--- 150nm (~ virus sized) par1cles
--- cholesterol coupled to TopFluor dye

HIV



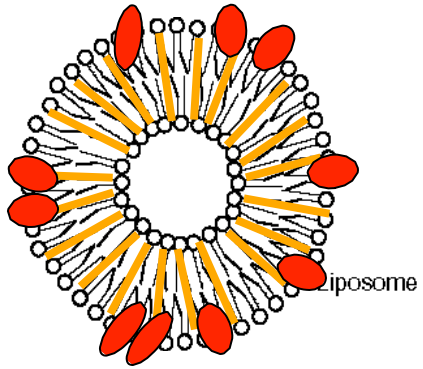
Composi1on
~ 45% cholesterol
>30% phospholipids
~10% PS
0.5---3.5% GM3

Mock (PS)



Composi1on 45%
cholesterol* 54%
DPPC
1% PS

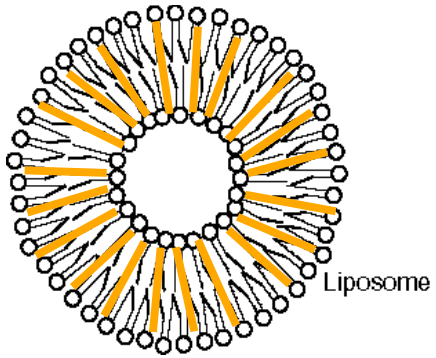
+Lipid



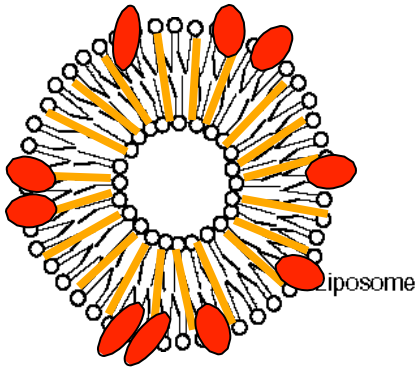
Composi1on 45%
cholesterol* 53%
DPPC
1% PS
1% Lipid

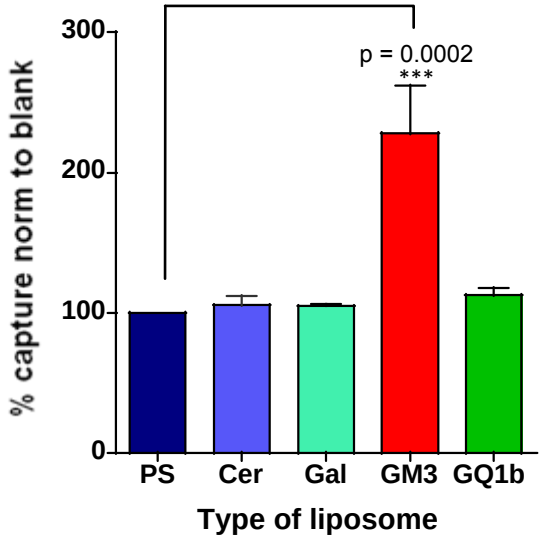
Inclusion of GM3 in Virus-sized Liposomes Results in Enhanced Mature DC capture

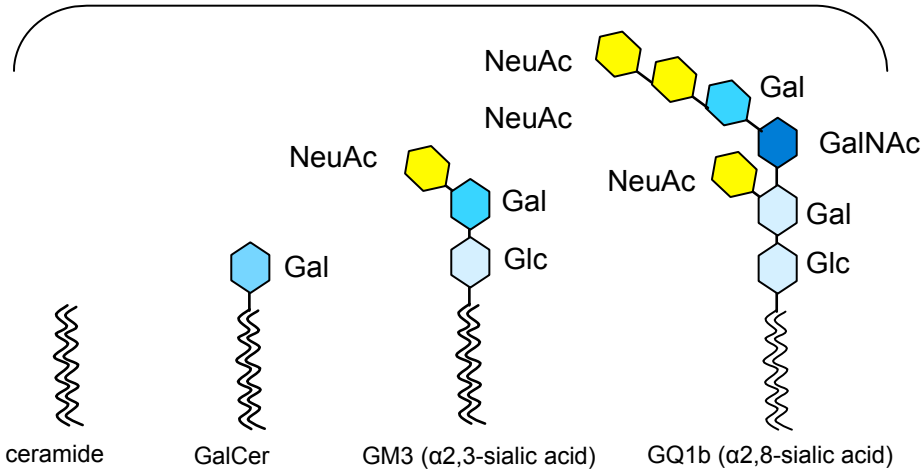
Mock (PS)



+Lipid

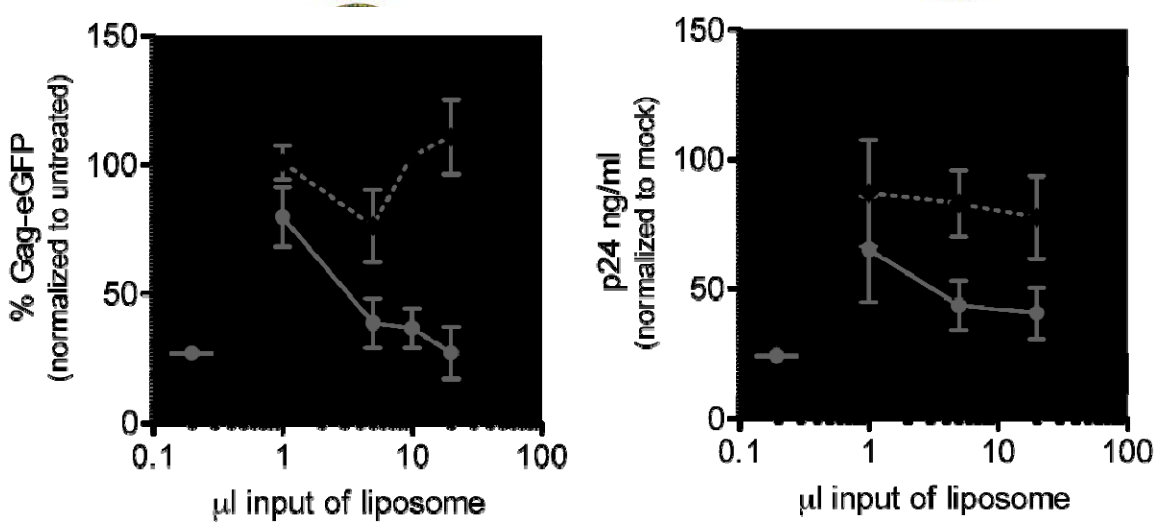




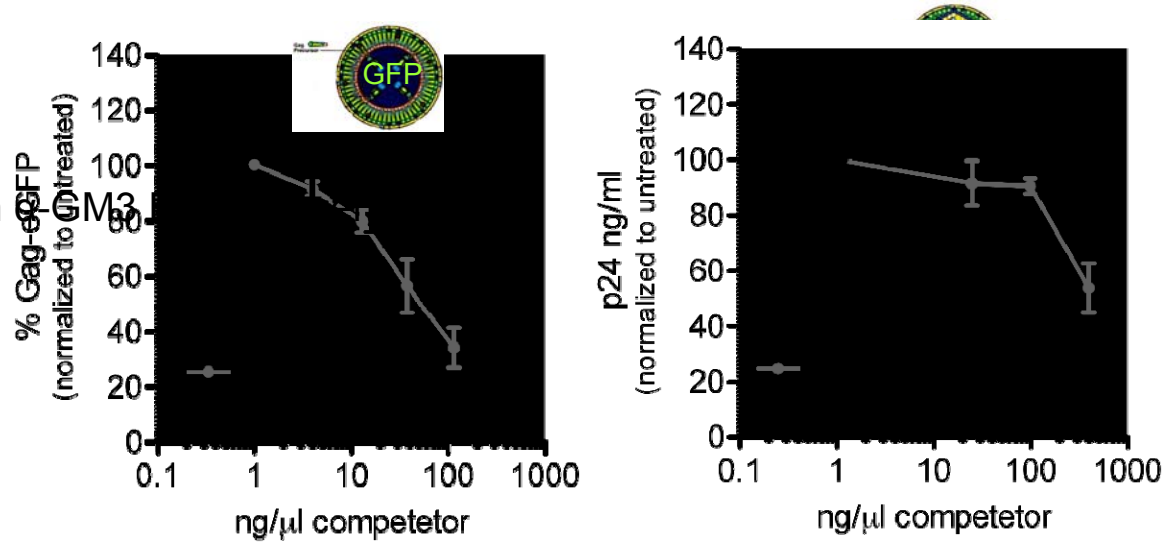


Blocking GM3 Results in Attenuated Capture of HIV by Mature Dendritic Cells

Competition With Liposomes



Competition With GM3



Immune Activation and GM3 Enrichment in HIV-1 Particles

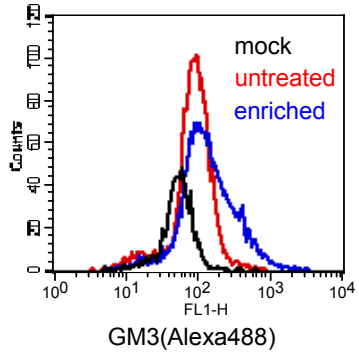
- GM3 is the major ganglioside in the plasma membrane of lymphocytes and monocytes

- GM3 synthase activity is upregulated
  - upon immune activation, and
  - upon initiation of monocyte to macrophage differentiation

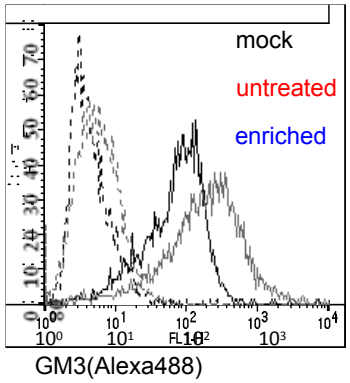
GM3 enrichment in Virus Producer Cells Produces HIV that is Captured by DCs with Enhanced Efficiency

GM3 Expression on Virus Producer Cells

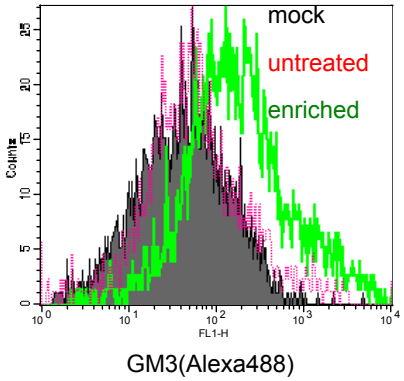
HeLa



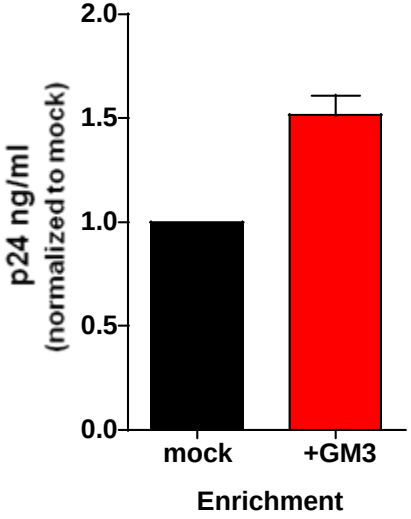
Monocyte → Macs

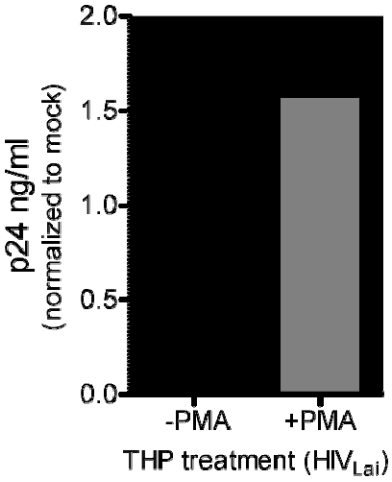


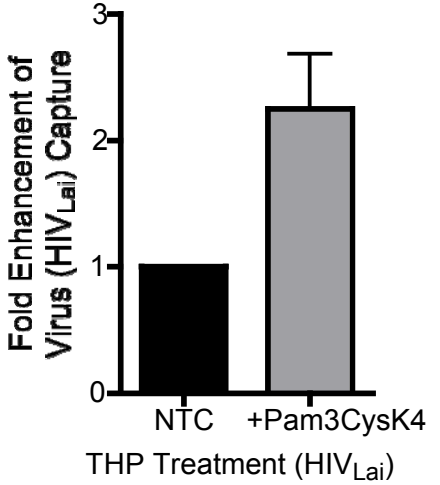
Monocyte/Pam$_3$CysK$_4$



HIV-1 Capture by Dendritic Cells

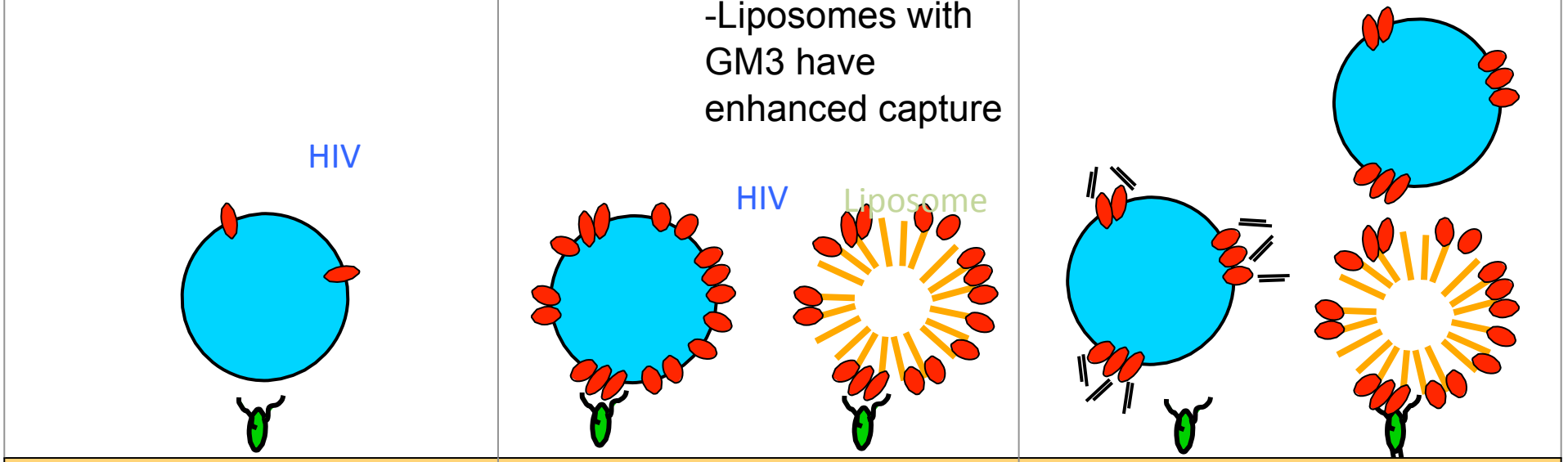






Summary:
GM3 mediates HIV capture by DCs

Loss of Function	Gain of Function	Competition Assays
- Knockdown of GM3 synthase results in decreased capture	-Enrichment with GM3 (immune activation or lipid addition) results in enhanced capture -Liposomes with GM3 have enhanced capture	- GM3 liposomes compete for capture - α-GM3 Fab blocks capture

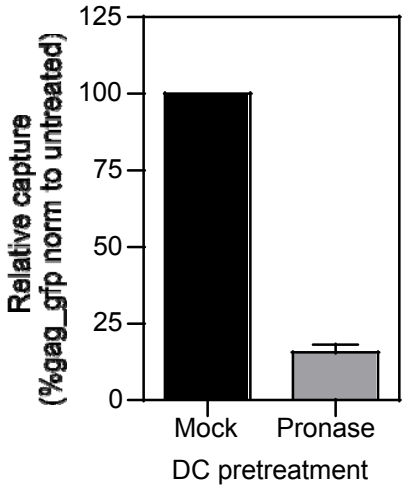


Puryear et al, PNAS 2012 109: 7475 – 7480

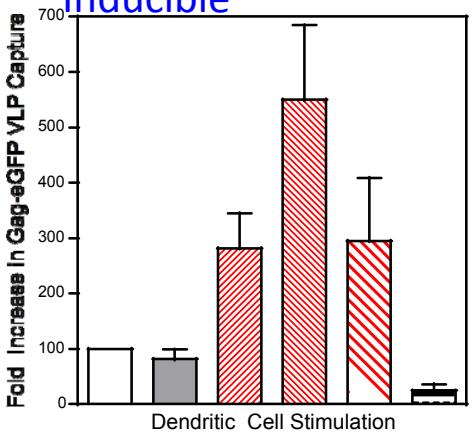
What is the DC---receptor responsible for GM3---dependent HIV---1 capture?

Characteristics of the GM3-Dependent HIV Capture Mechanism on Dendritic Cells

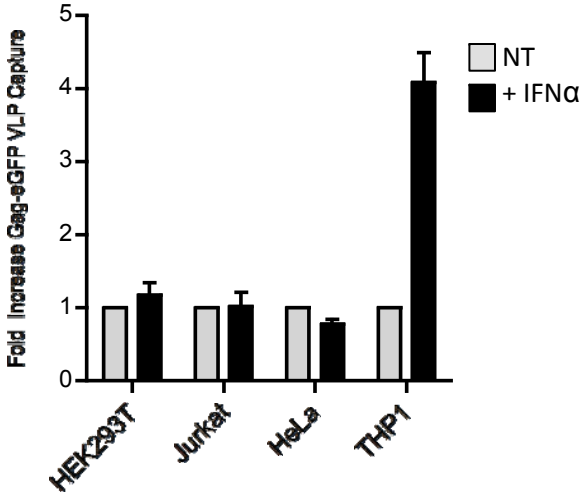
1. Pronase Sensi1ve



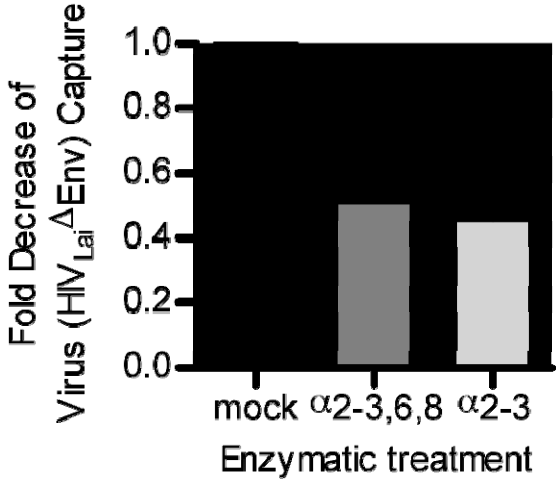
2. IFN---α Inducible



3. Myeloid Cell Specific



4. α2,3---Sialic Acid Requirement

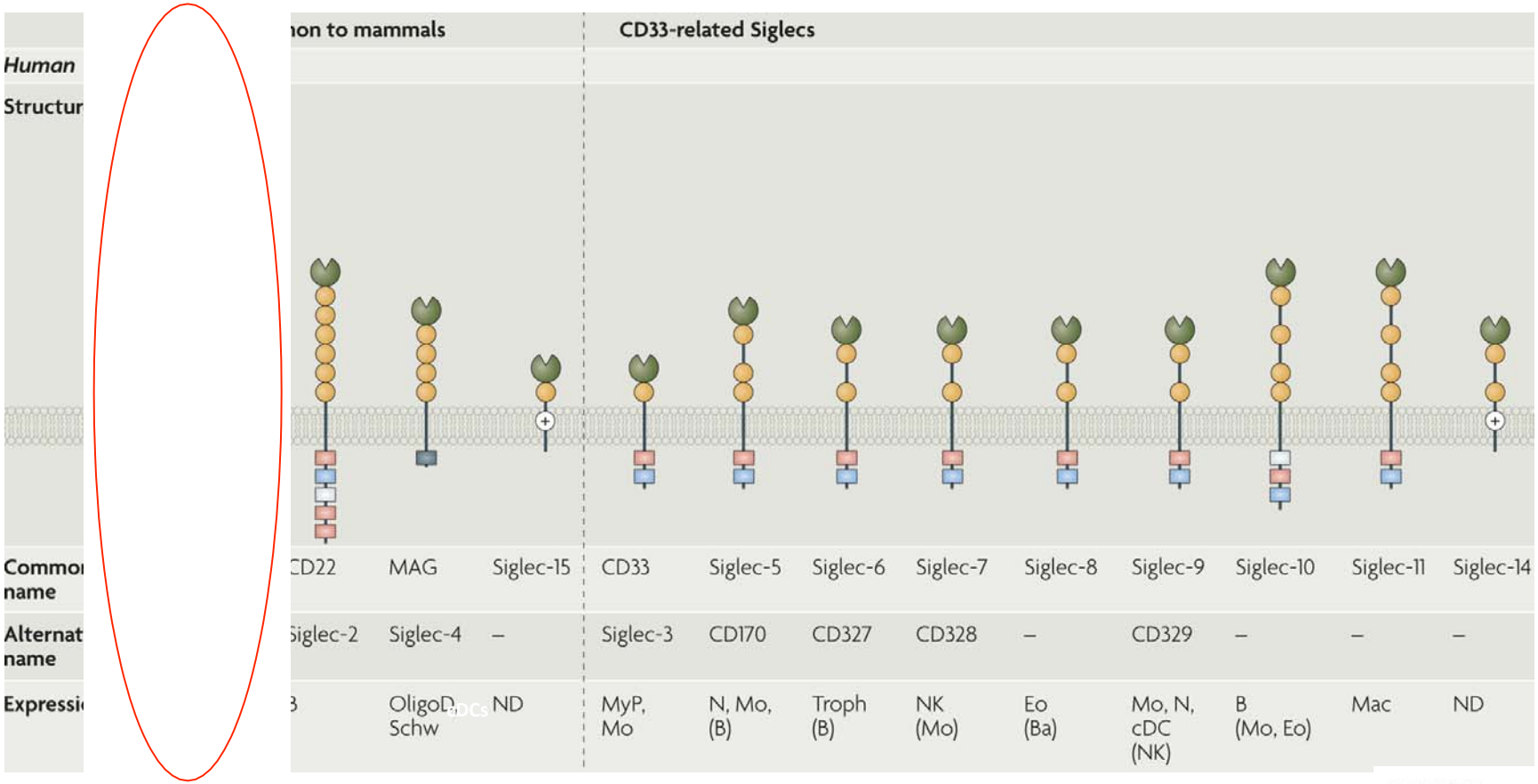


Neuraminidase Treatment of HIV

SIGLEC Family of Lectins

- Sialic acid-recognizing Ig-superfamily lectins
- Major homologous family of I-type lectins divided into 2 sub-groups
  - Evolutionary conserved sub-group (Siglecs-1, -2 and -4)
  - Rapidly evolving CD33 and Siglec-3-related sub-group (Siglecs-3 and -5-13 in primates)
- Broadly expressed in cells of the immune system

SIALIC-ACID-BINDING IMMUNOGLOBULIN-LIKE LECTINs



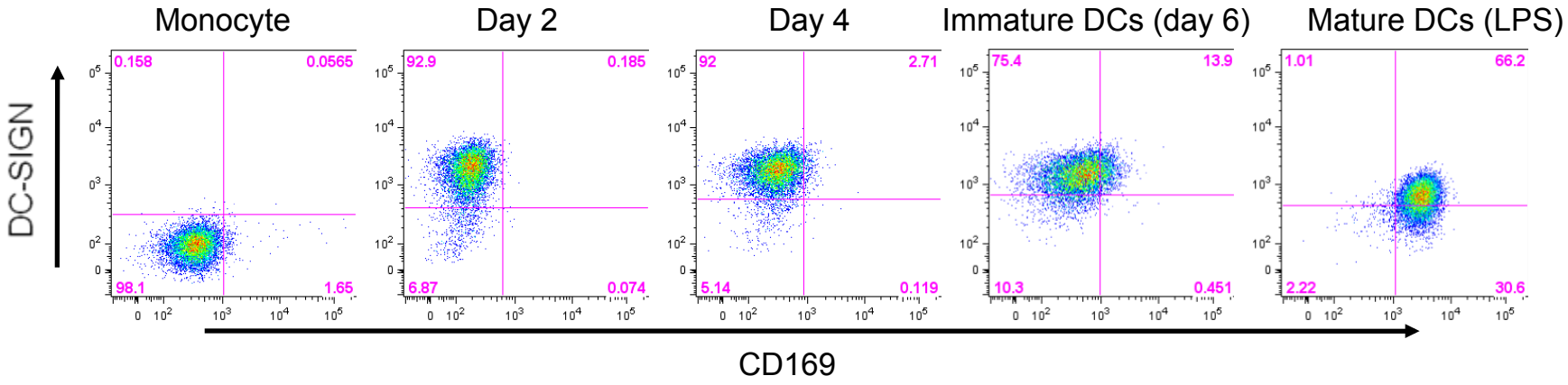
	...on to mammals			CD33-related Siglecs								
Human												
Structur												
Commo... name	...CD22	MAG	Siglec-15	CD33	Siglec-5	Siglec-6	Siglec-7	Siglec-8	Siglec-9	Siglec-10	Siglec-11	Siglec-14
Alternat... name	...iglec-2	Siglec-4	–	Siglec-3	CD170	CD327	CD328	–	CD329	–	–	–
Expressi...	...3	OligoD... Schw	ND	MyP, Mo	N, Mo, (B)	Troph (B)	NK (Mo)	Eo (Ba)	Mo, N, cDC (NK)	B (Mo, Eo)	Mac	ND

Adapted from
Crocker, Nat Rev 2007

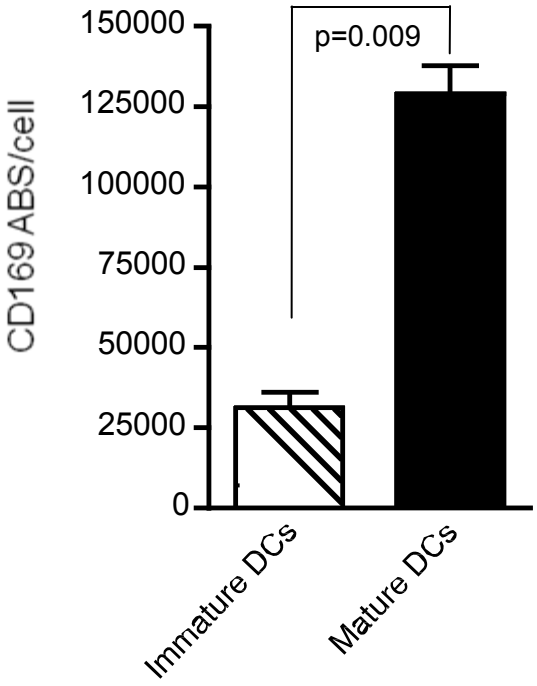
Siglec1 (CD169)

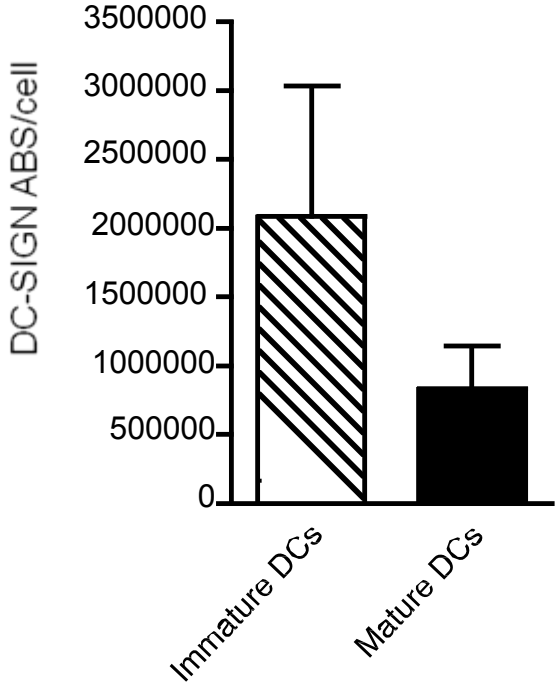
- expressed exclusively on myeloid cells
- expression induced by type I IFN
- binds to terminal α2,3-linked sialic acid residues

Maturation of Dendritic Cells Enhances CD169 Expression

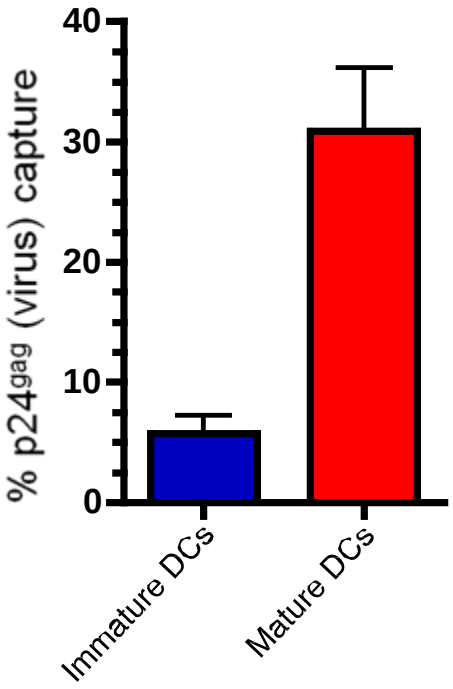


Quantitative FACS



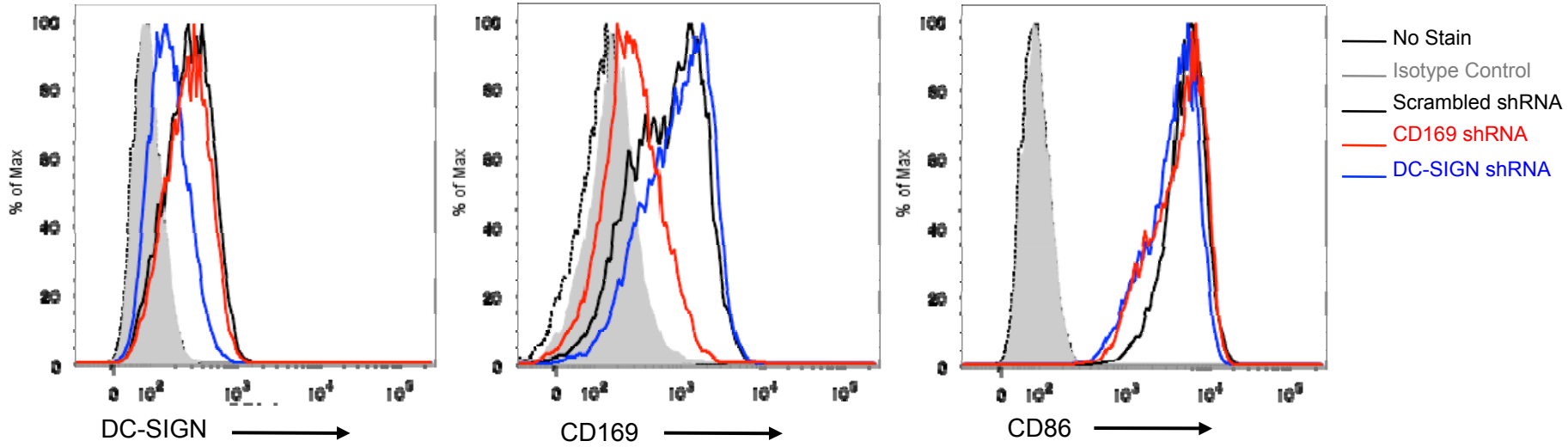


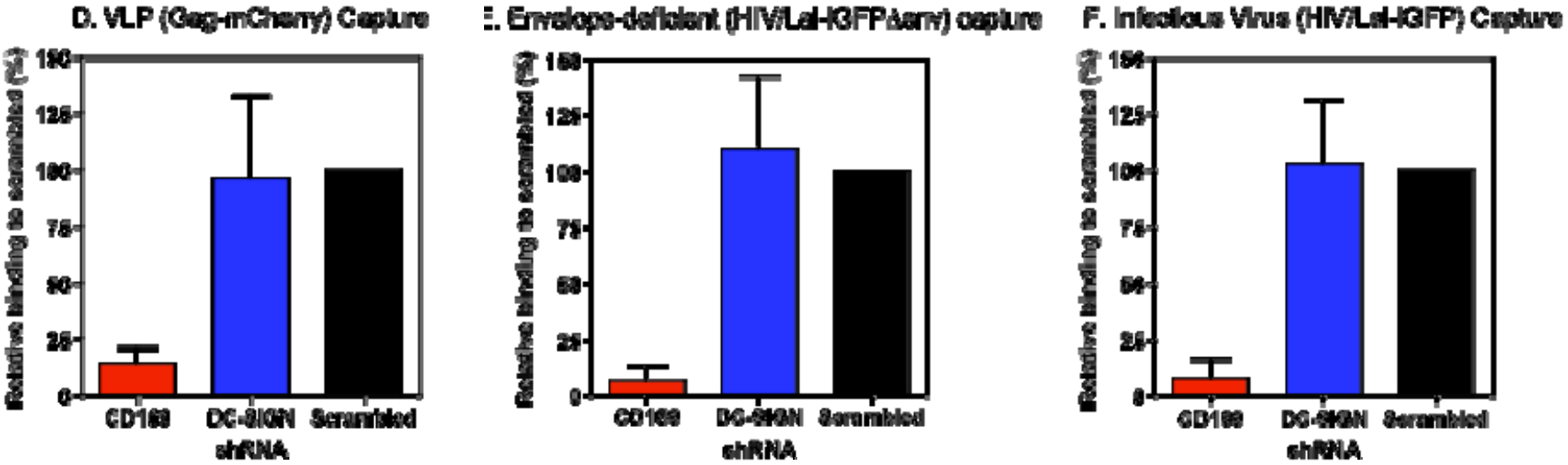
HIV-1 Capture



Selective Depletion of CD169 in Mature Dendritic Cells Abrogates HIV-1 Capture

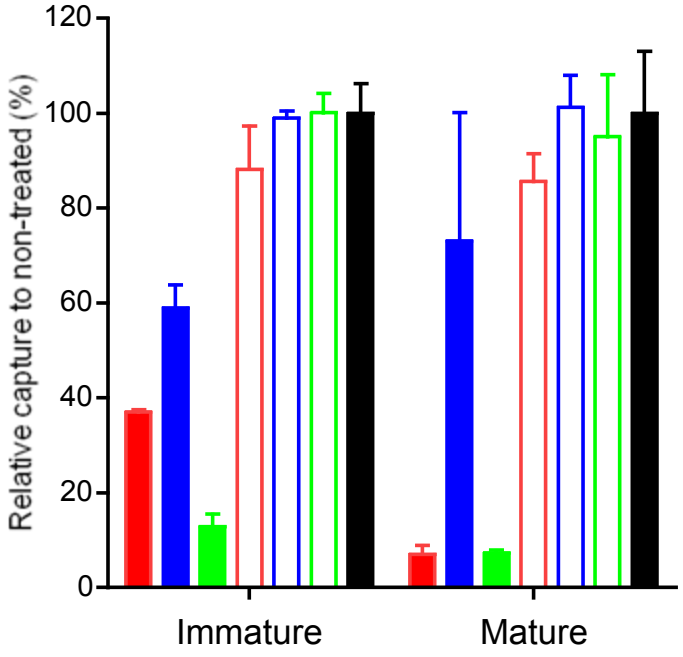
- Lentivector/shRNA-mediated knockdown of CD169 or DC-SIGN in mature DCs



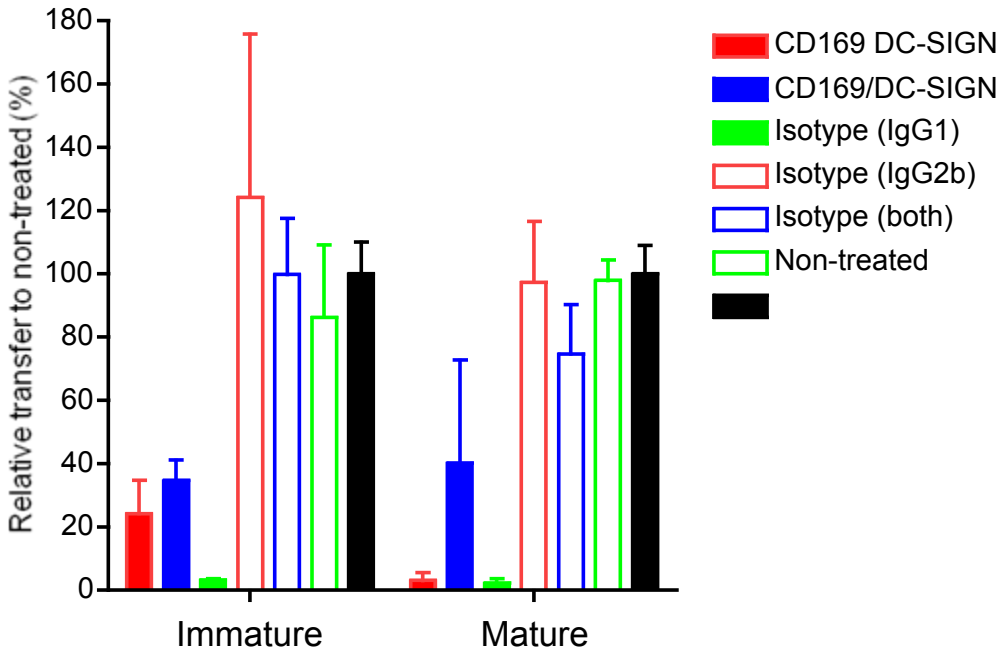


Neutralizing Antibodies Against CD169 block DC Mediated HIV-1 Capture & Trans Infection

Virus Binding

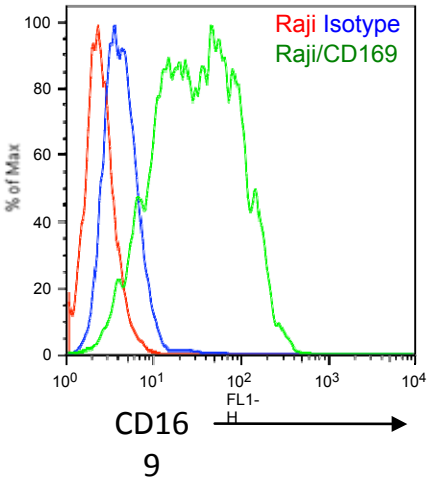


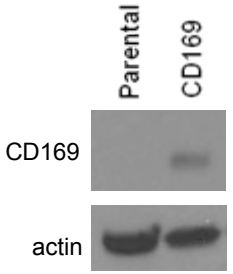
HIV-1 trans Infection

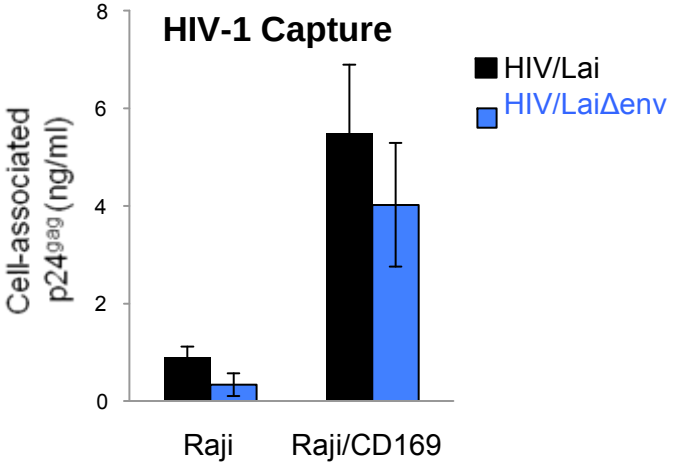


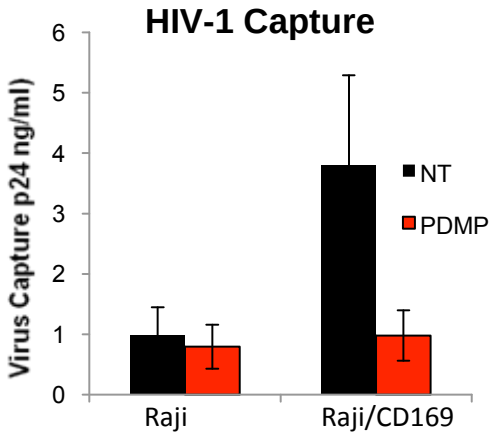
Exogenous Expression of CD169 in Receptor-Naïve Cells Rescues GSL-Dependent HIV-1 Capture & Trans Infection

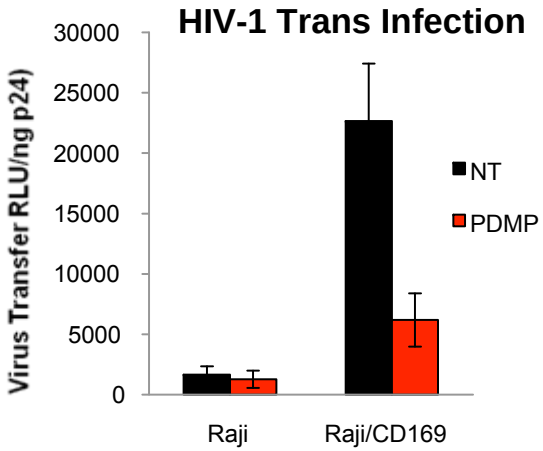
- Express human CD169 in Raji B cell line (non-permissive for HIV capture)
- Incubate with WT virus (NT), virus deficient in GSL (PDMP), or Env-deficient virus
- Co-culture with T cells





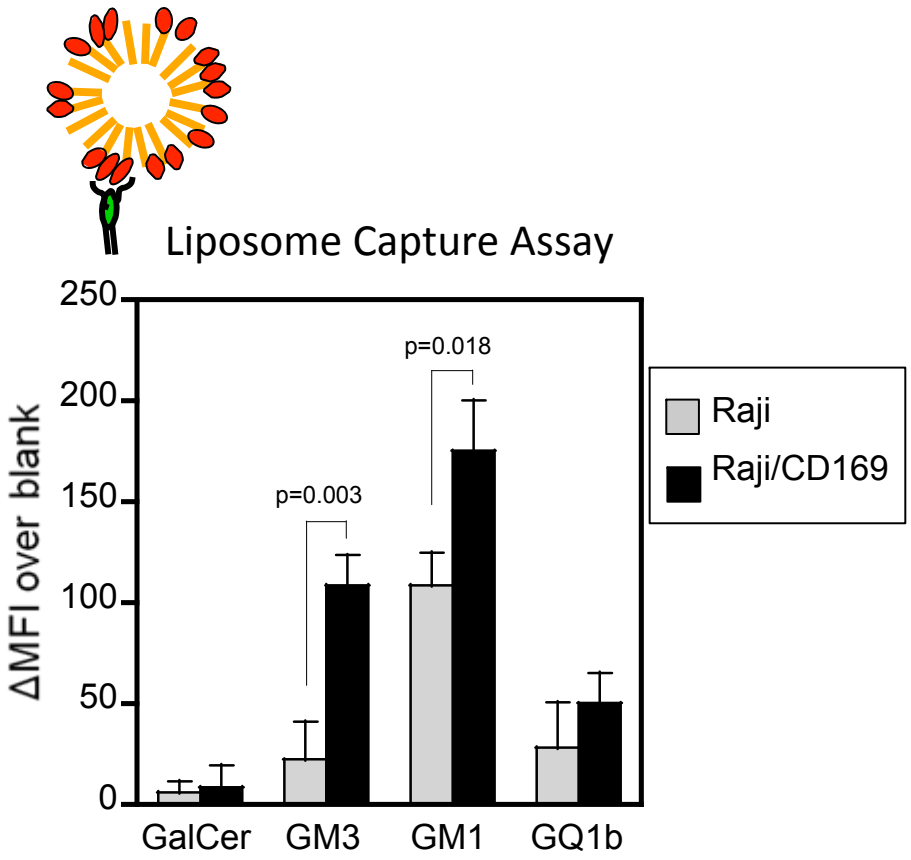


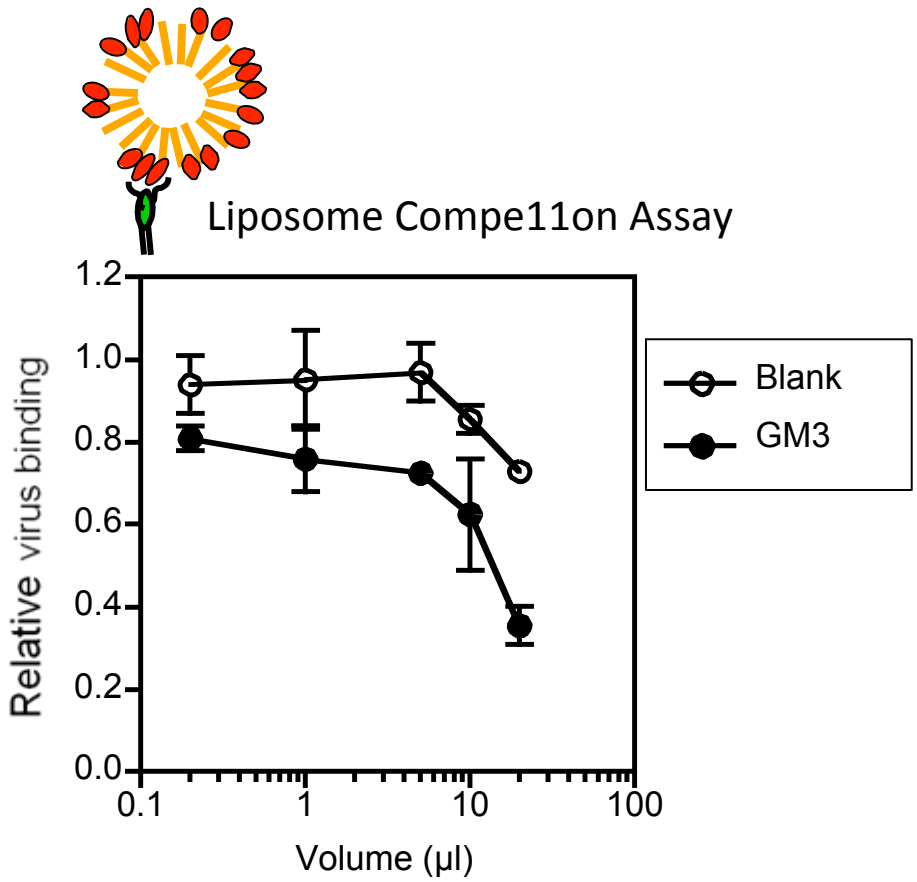




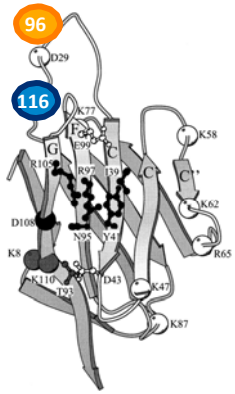
Capture and transfer of GSL-deficient HIV-1 particles by Raji/CD169 cells is attenuated

GM3 Recognition by CD169 is Essential for HIV-1 Capture





Lack of HIV-1 Capture by Cells Expressing CD169 Mutant Deficient for Sialic Acid Recognition

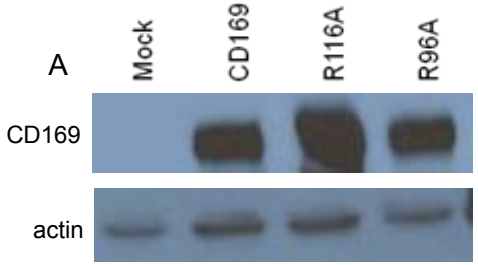


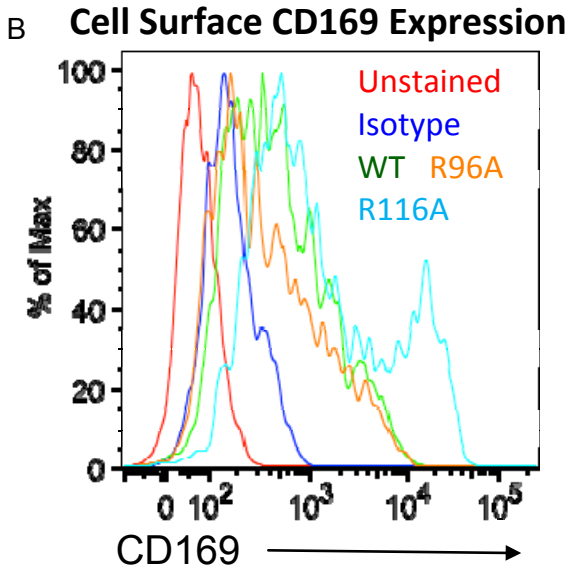
WT = human CD169

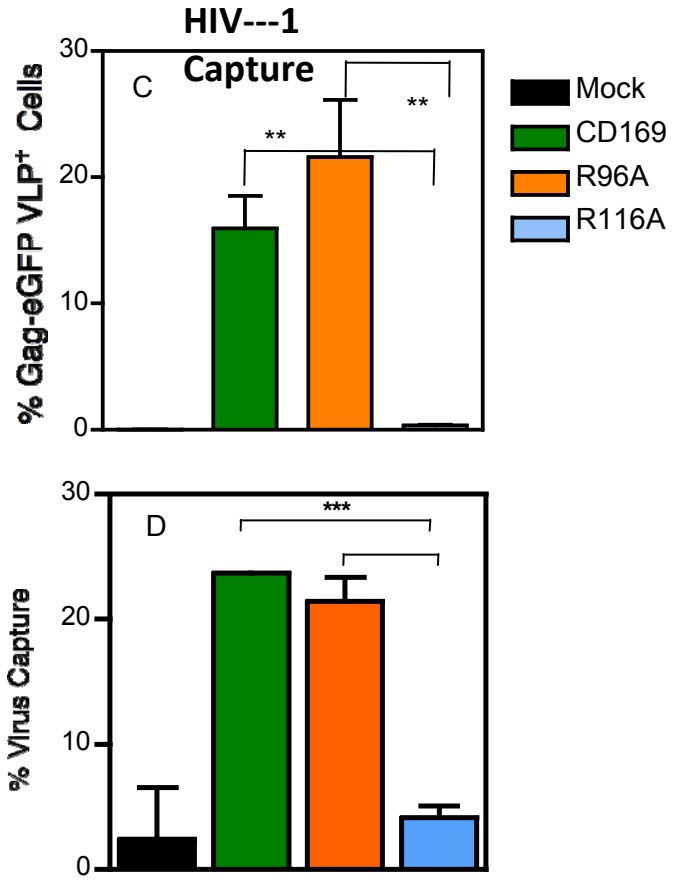
R96A = no affect on sialic acid binding

R116A = abrogates sialic acid binding

Vinson M et al, JBC 1996

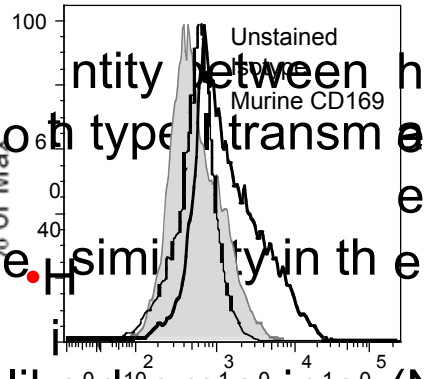


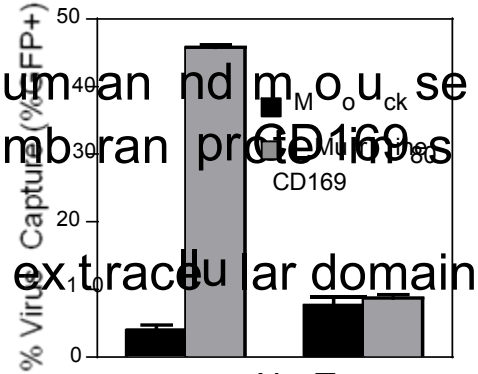


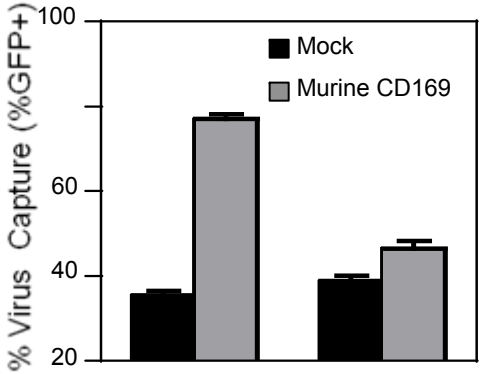


Murine CD169 can Mediate Retroviral Capture and Trans Infection

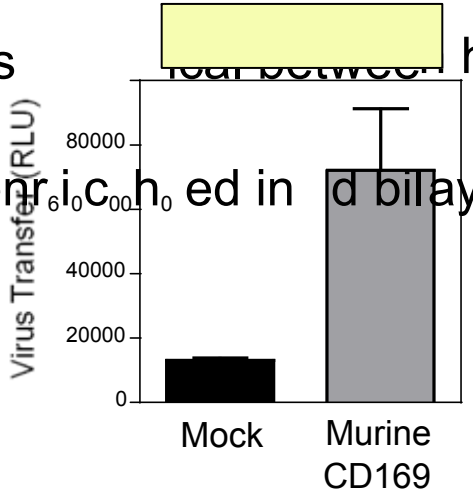
- Muri Murine CD169 Expression n als HIV-1 Capture yeloid cell MLV Capture

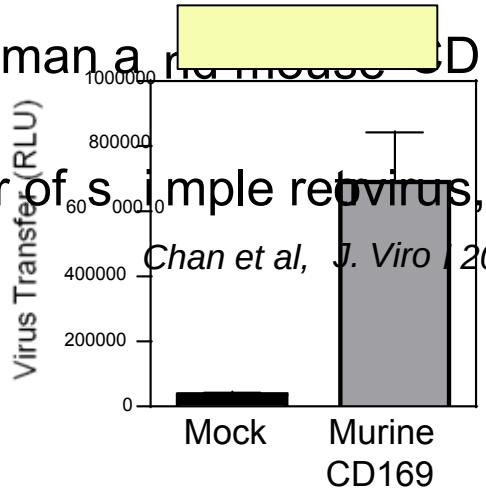






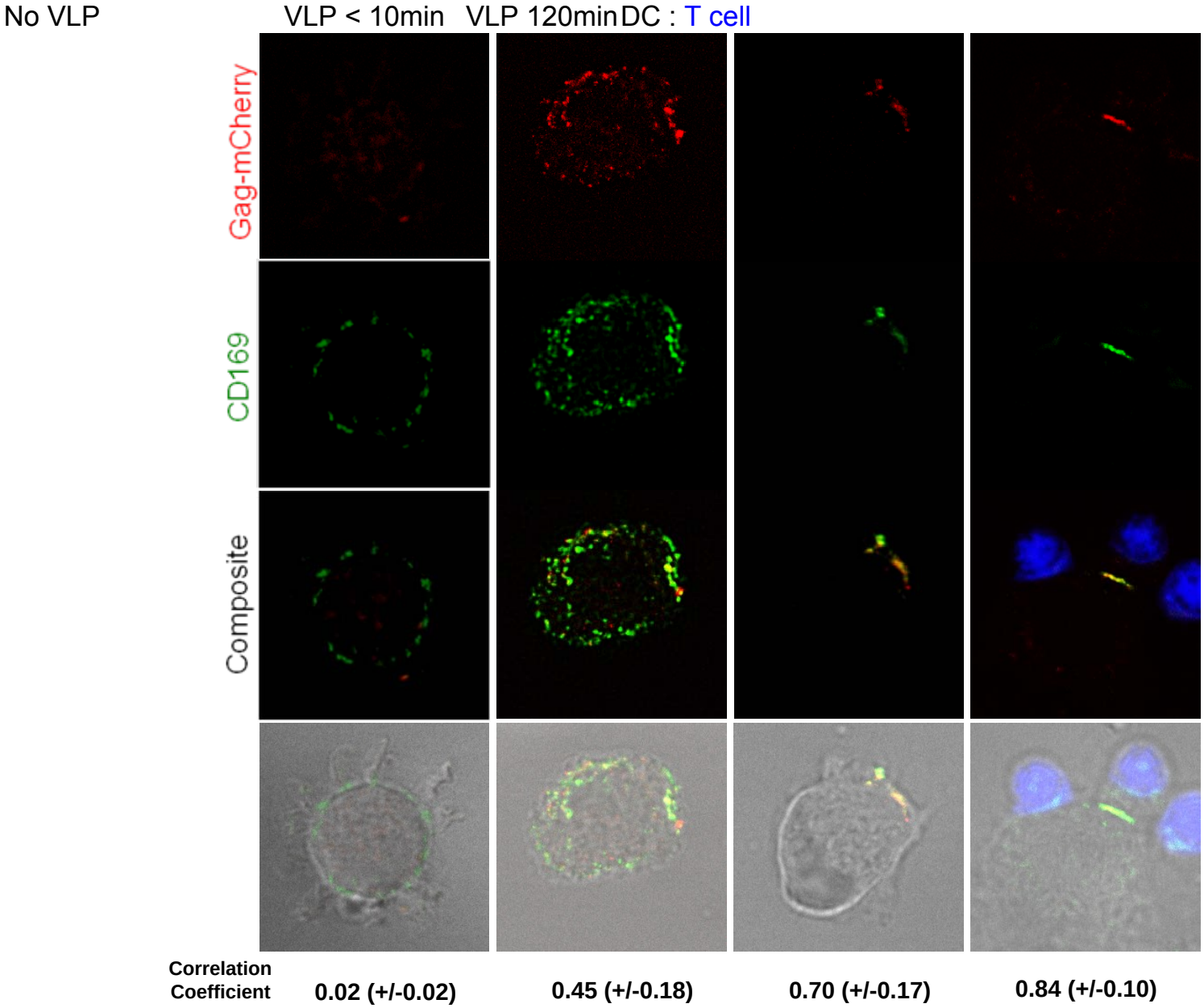
- 72% ntity etween hum an nd m o u ck se id e- bo th type II transm emb ran prote in s e
- H he simi ty in th e ex t race u lar domain i
- 17 lg like d om a in s (N-terminal V T-set d o m ain and 16 C- T set do m a ins)
- Sialic acid binding site is human a d mouse CD169 i d e nt
- GM3 gangliosides are enr i c h ed in d bilayer of s imple retrovirus, MLV lipi



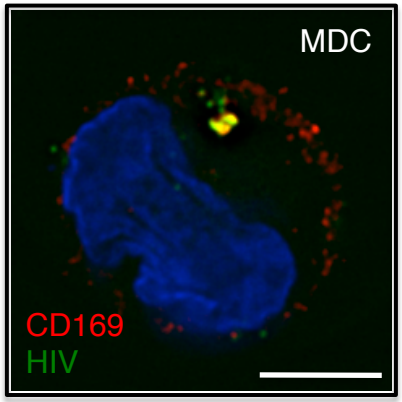


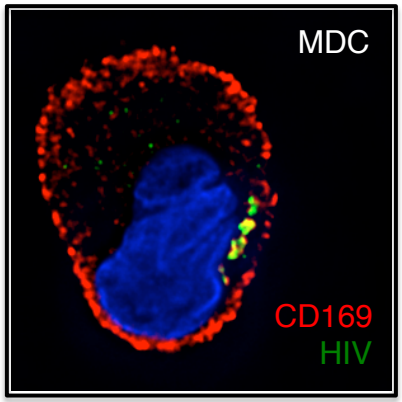
Chan et al, J. Virol 2008

HIV Gag-mCherry VLPs Co-localize with CD169 on Mature DC Surface and at the DC – T cell Virological Synapse

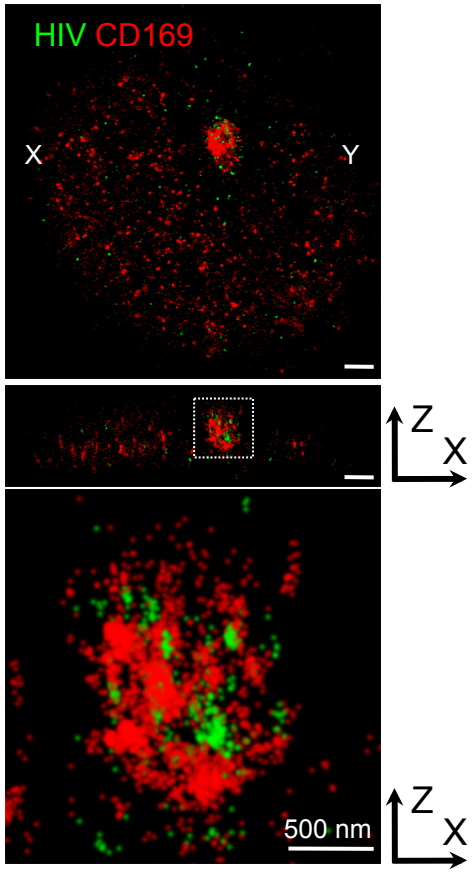


Localization of HIV-1 Particles in CD169^{+} Plasma Membrane Invaginations of Myeloid DCs

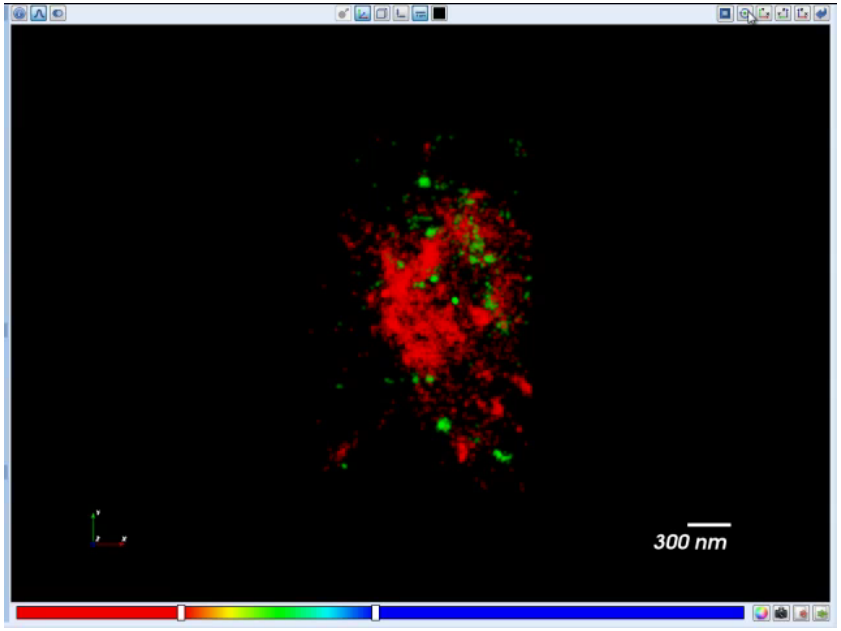




Deconvolution Microscopy

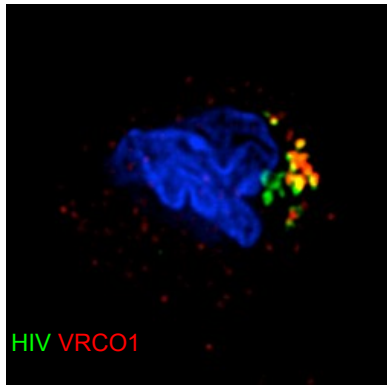


Super-resolution Microscopy

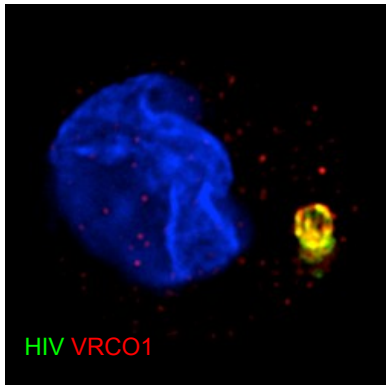


HIV-1 Particles Captured by CD169 are Trafficked to Non-Lysosomal Compartments

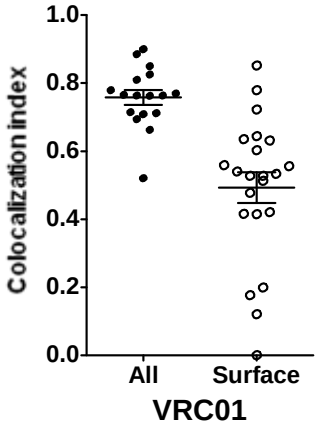
HIV-1 Evasion of Adaptive Immune Responses

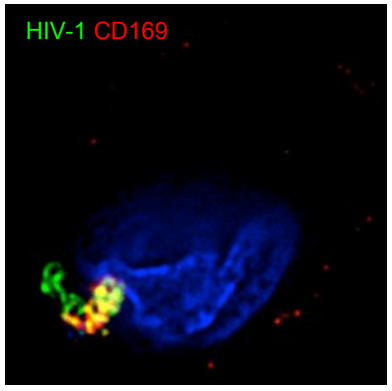


Surface



Permeabilized





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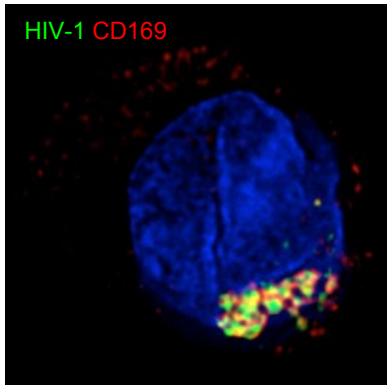

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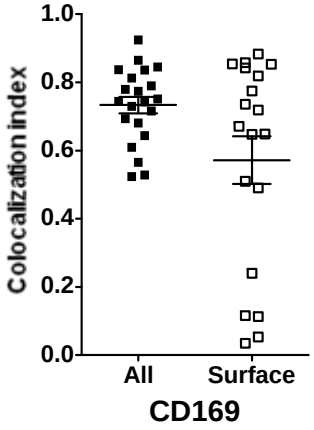


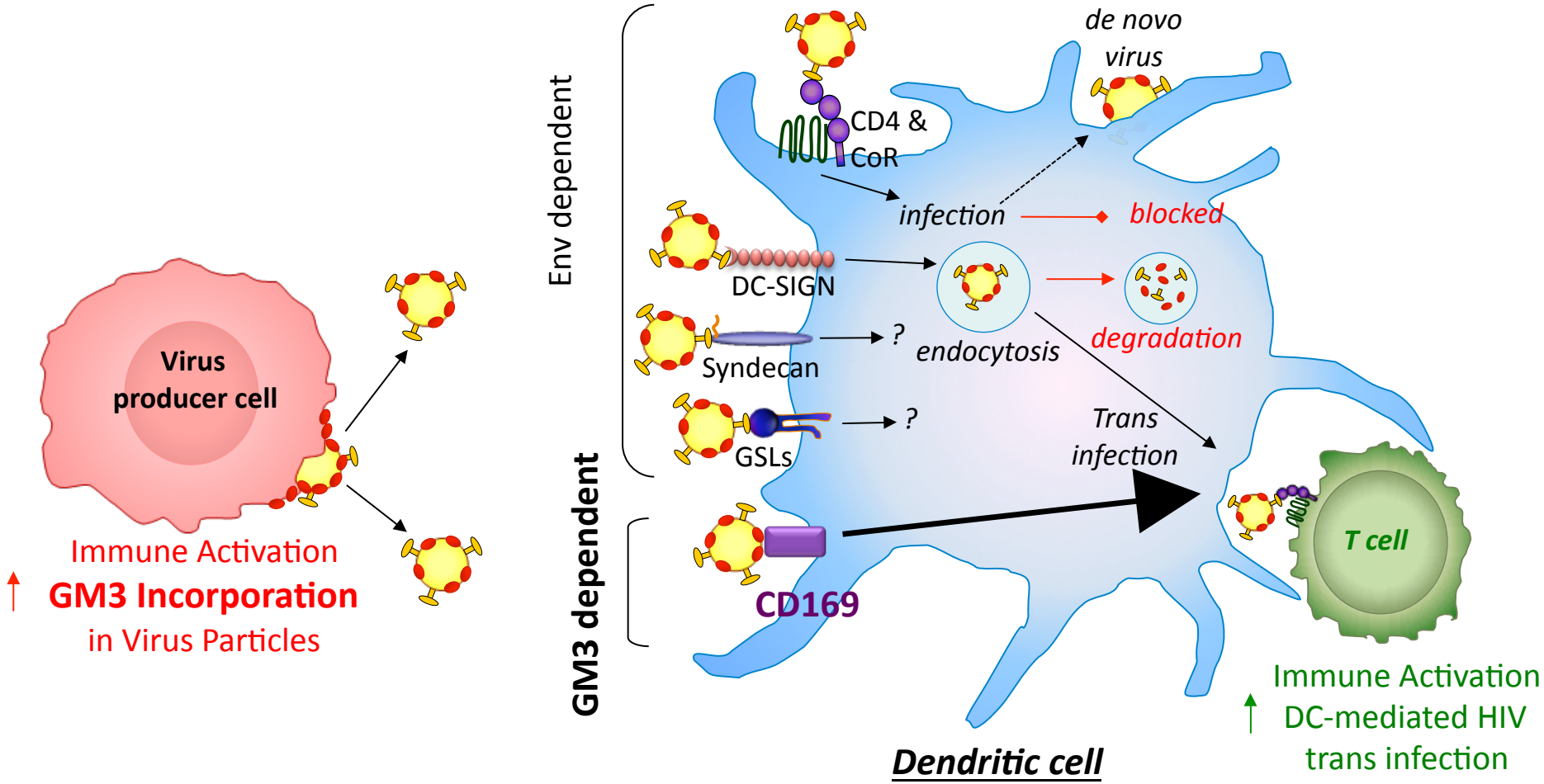
Conclusions

- Expression of CD169 on monocyte-derived DCs, inflammatory DCs and blood myeloid DCs is required for GM3-dependent HIV-1 capture.
- Blocking CD169 with neutralizing antibody or depletion via shRNA results in loss of DC-dependent HIV-1 capture and trans infection.
- Induced expression of CD169 in a non-permissive cell confers the ability to capture and transfer virus.
  - Mutation that eliminates sialic acid binding (R116A) results in attenuation of virus capture by CD169.
- HIV-bound CD169 re-localizes to a polarized region of DC and is co-localized with HIV-1 particles at the DC – T cell infectious synapse.
- HIV-1 particles captured by CD169 are trafficked to plasma membrane invaginations
  - Invariably accessed by surface-applied anti-gp120 monoclonal antibodies

Puryear & Akiyama et al, PLoS Path 2013 Izquierdo-Useros et al, PLoS Biology 2012

CD169+ DC Mediated HIV-1 Trans Infection

- Dependent on a host cell-derived ligand (GM3) and receptor (CD169)
- Parasitization of cell-to-cell recognition mechanism by HIV for virus dissemination
- Protection from innate and adaptive immune responses



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