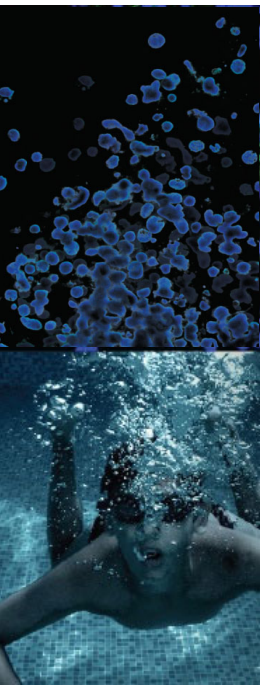
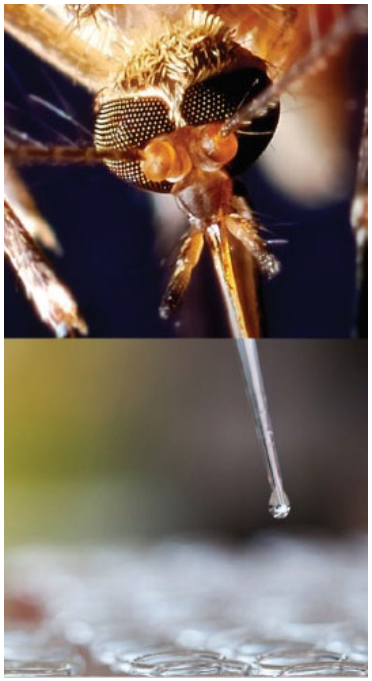
Extracellular Vesicles, HIV-1, and Neurospheres: Touching a Nerve



Heather Branscome, Ph.D.
Supervisor, Laboratory Operations, ATCC

Fatah Kashanchi, Ph.D.
Professor, George Mason University

Credible Leads to Incredible™



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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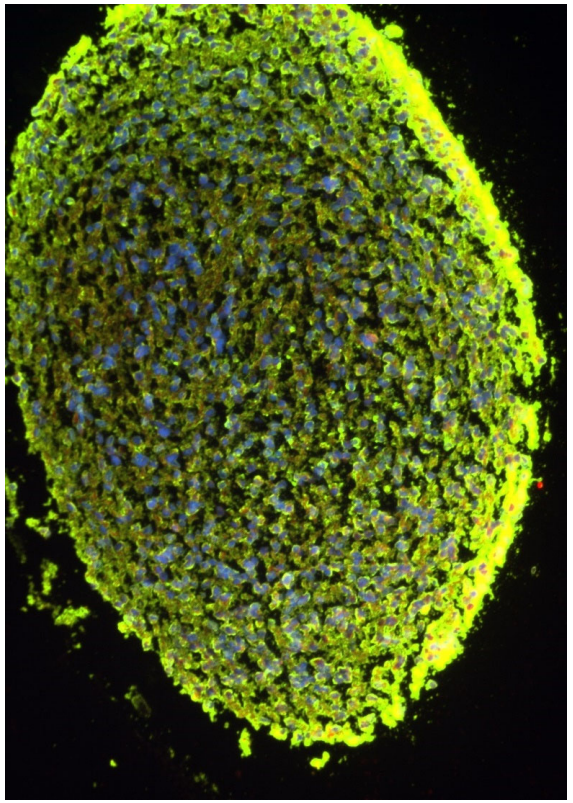
Overview

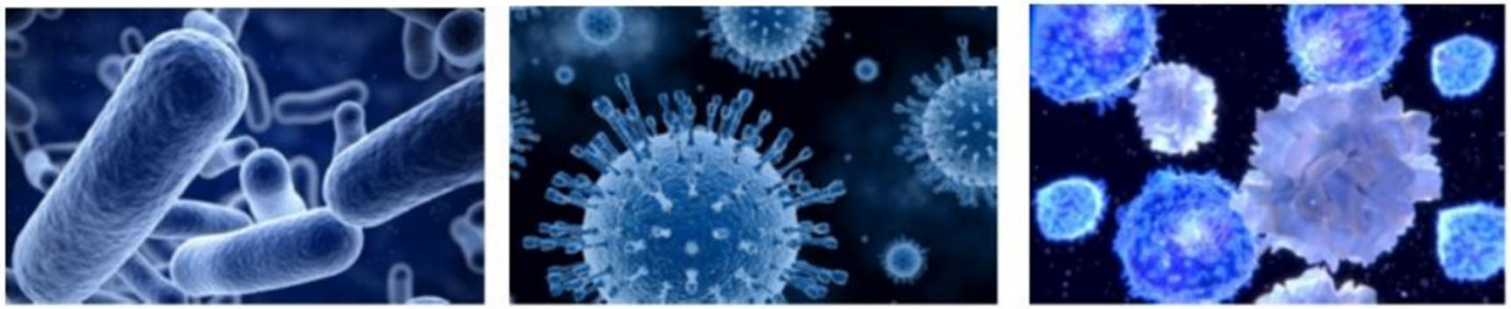
- Update on the most recent literature surrounding EVs from virally infected cells (“Damaging EVs”)
- Generation and infection of iPSC-derived neurospheres
- Effect of stem cell EVs (“Reparative EVs”) on HIV-1 infected neurospheres
- Summary



Neural progenitor cell-derived neurosphere

Meeting of the American Society for Intercellular Communication

Mason Marketplace



It gives us great pleasure to annouce the 1st meeting of the **American Society for Intercellular Communication (ASIC)**

The meeting will be held on **October 21st – October 23rd 2021.**

The meeting is organized by a group of investigators seeking to create a platform for informal exchange of ideas on emerging questions and cutting-edge developments in the field of Extracellular Vesicles (EVs), Extracellular Particles (EPs), and particulate carriers of extracellular RNA (exRNA) as biological mediators, regulators and diagnostic analytes. Our program of relatively short talks (15 and 30 minutes) encourage discussions that can take place after each scientific talk, where the audience and attendees can debate the concepts presented to foster a dynamic scientific exchange.

We invite you to join us for a 3-day meeting (Thursday afternoon, all day Friday and Saturday until noon; October 21st – October 23rd) at the Bolger Center in Potomac, Maryland. The program will include the functions of EVs, EPs, exRNA in cancer, CNS diseases, infections (both bacterial and viral), the mechanistic basis for their biogenesis and activities, and technological and diagnostic advances in the field. *In the interest of everyone's health and safety, vaccines are required to attend this meeting in person. We will also be following CDC guidelines for this meeting.*

Late Breaking Abstracts are due by September 30, 2021

Late Breaking Registration: September 30, 2021

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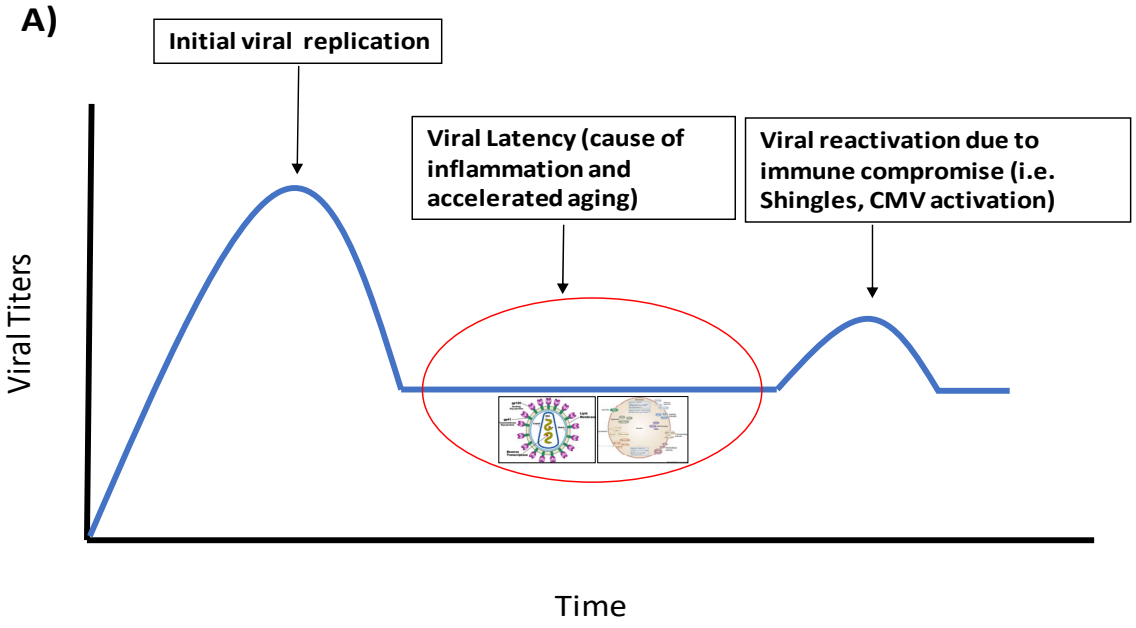
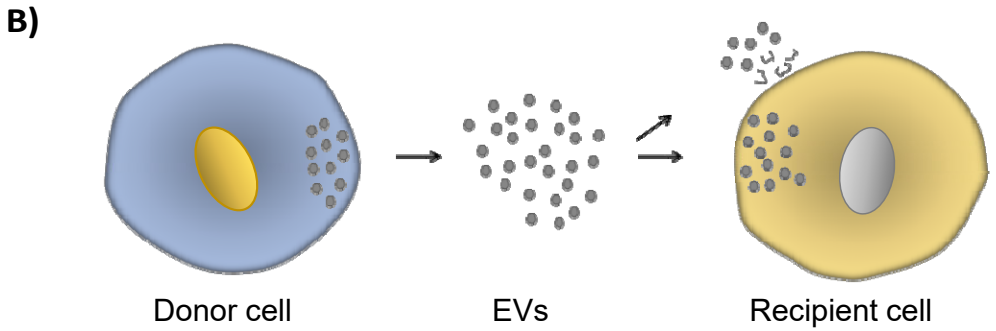
EVs from latent cells





Epidemiology

A)

Table 1. Baseline Characteristics Among 190 Study Subjects

Characteristic	Value
Age, years	51 (44–57)
Male sex, % of patients	92
$CD4^+$ T-cell count, cells/mm^3	523 (249–728)
T cells expressing CD4, %	23 (16–32)
$CD8^+$ T-cell count, cells/mm^3	844 (606–1185)
T cells expressing CD8, %	48 (37–57)
Nadir $CD4^+$ T-cell count, cells/mm^3	113 (29–227)
Duration of HAART suppression, months	31 (14–66)
Agent included in HAART, no. of patients	
Protease inhibitor	124
NNRTI	73
Raltegravir	14
Maraviroc	1
Enfuvirtide	3

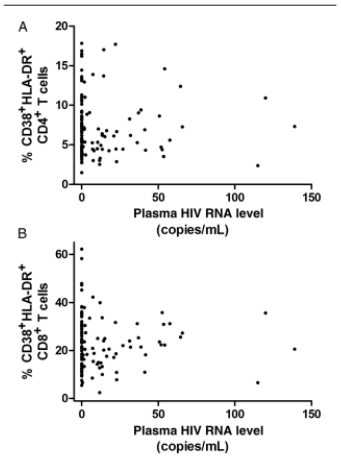


Figure 1. No associations between ultrasensitive plasma human immunodeficiency virus (HIV) RNA levels and T-cell activation. Ultrasensitive plasma HIV RNA levels were measured using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0. All *P* values are >.40.

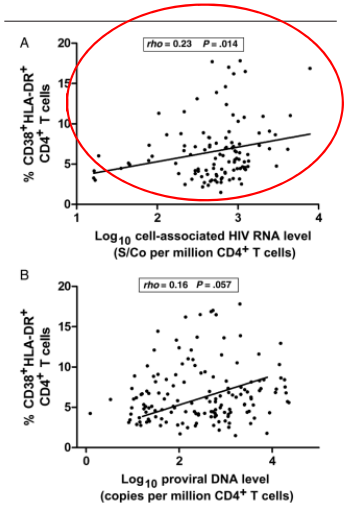


Figure 2. Cell-based measures of viral persistence are modestly associated with immune activation.

B)

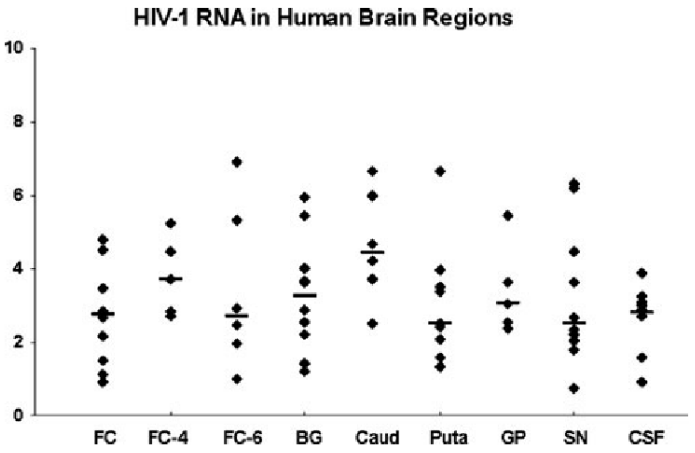
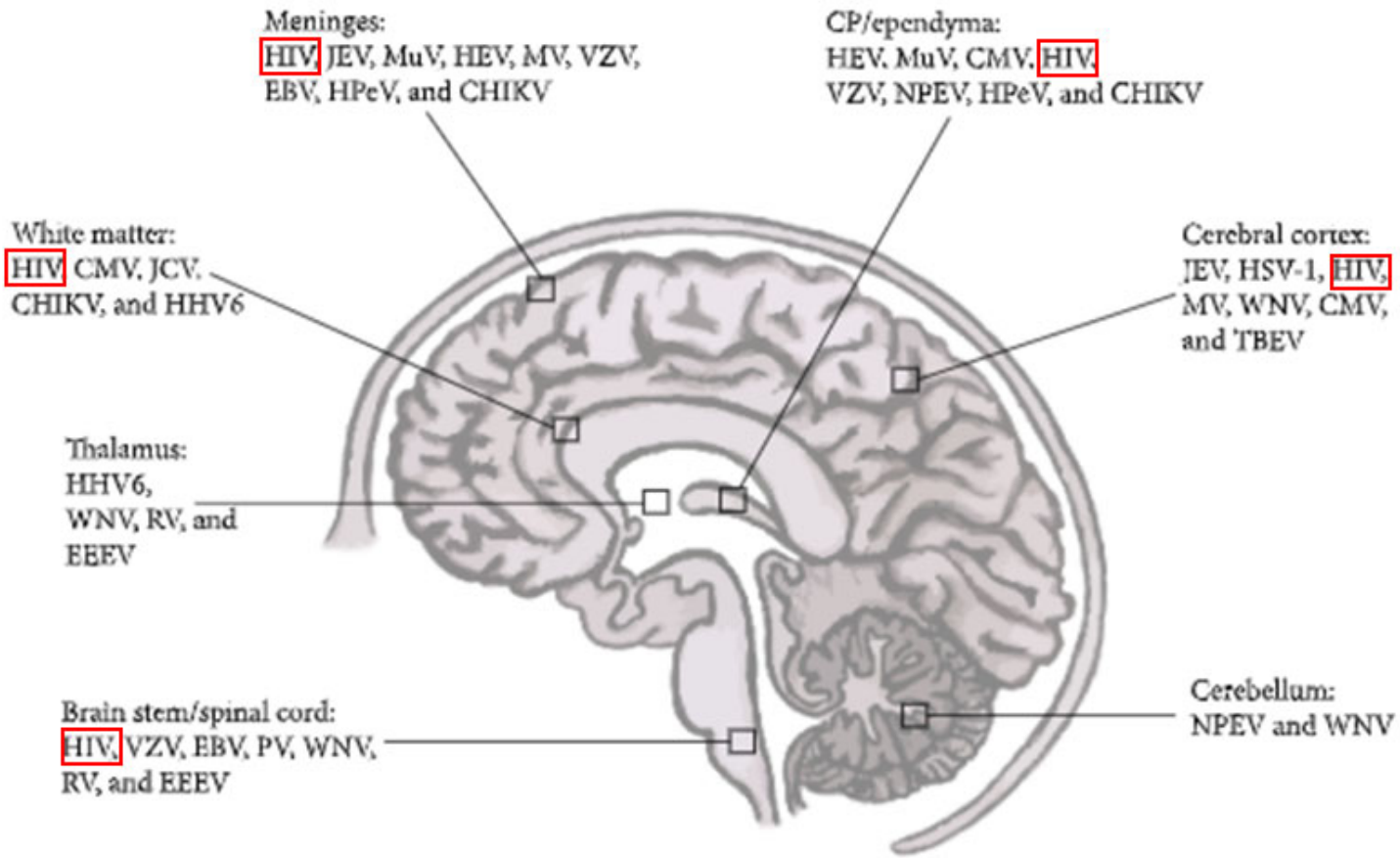


Figure 1 HIV-1 RNA load (log_{10} copies/g tissue) in each region of HIV-1+ individuals is presented in a scatter plot. The horizontal bars represent the median value in each region. Viral load values were higher in caudate nucleus compared to that in the other regions (FC-4, FC-6, BG, putamen, globus pallidus, SN), and the lowest values were found in FC and CSF.

A) Hatano/Deeks et. al., JID. 2013
B) Kumar et. al., J Neurovirol. 2007

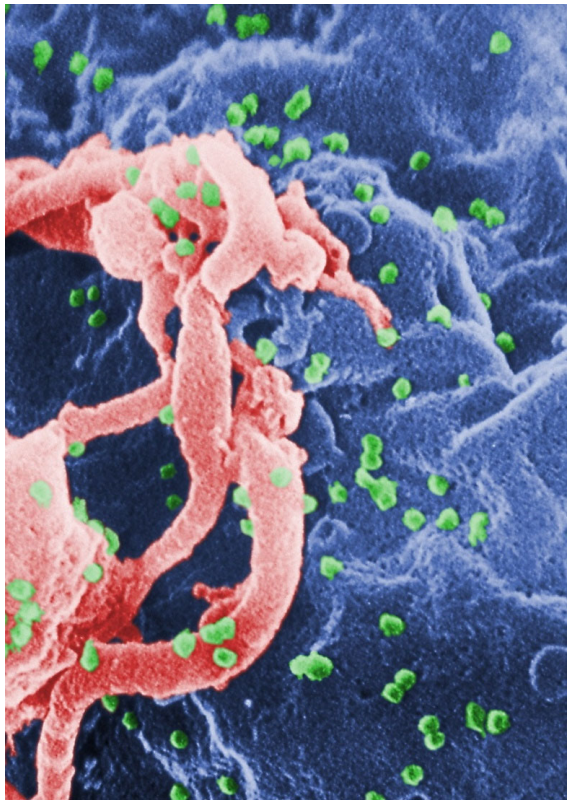
CNS Viral Infections



Dahm et al. Mediators Inflamm. 2016.

HIV-1 and HAND

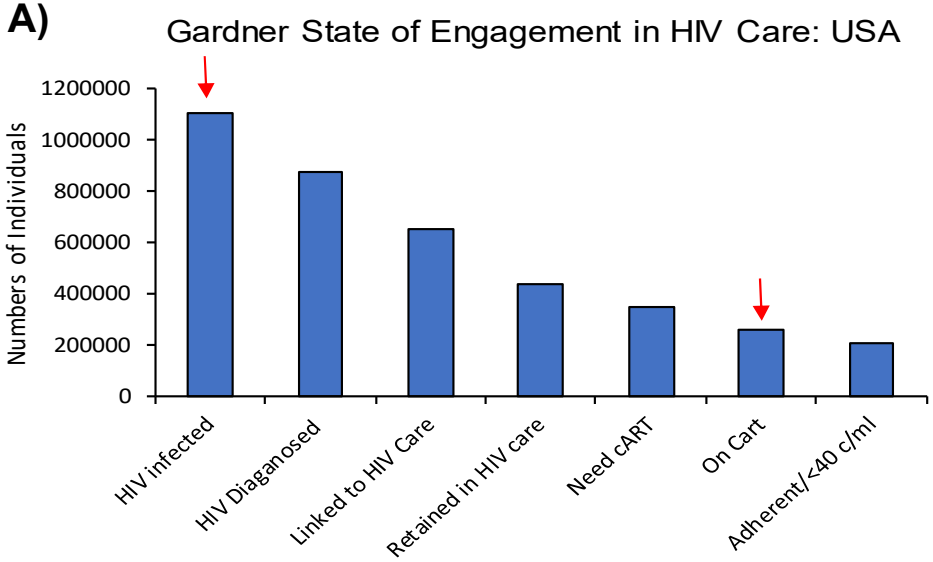
- HIV-associated neurocognitive disorders (HAND) are comprised of a range of neurological dysfunctions which are commonly associated with HIV-1 infection.
- Despite cART, HAND still persists in HIV-1 patients (>50%).
- Exosomes secreted from HIV-1 infected cells under cART can damage CNS cells (damaging exosomes).
  - Damaging exosomes contain viral non-coding RNA (TAR) and other viral proteins (Tat, Nef, Env) that cause neuroinflammation.
- Damaging exosomes have also been isolated from 6 other CNS tropic viral infections including HTLV-1, Zika, RVFV, Ebola, HSV-1, and HHV-6A.



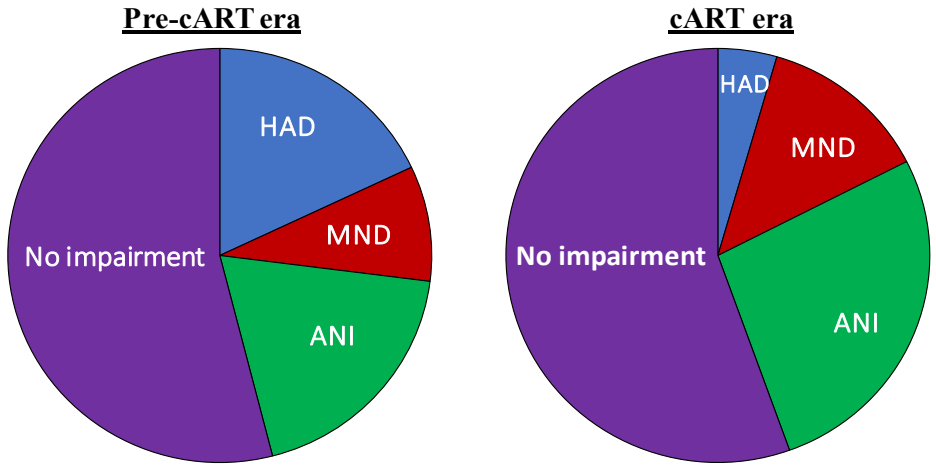
HIV-1 budding (in green) from cultured lymphocyte. Credit: C Goldsmith, P Feorino, EL Palmer, WR McManus, Centers for Disease Control and Prevention (CDC)

HIV-1 and HAND

A)



B)



HIV-associated dementia (HAD), asymptomatic neurocognitive impairment (ANI), and mild neurocognitive disorder (ANI); from Justin McArthur at Hopkins

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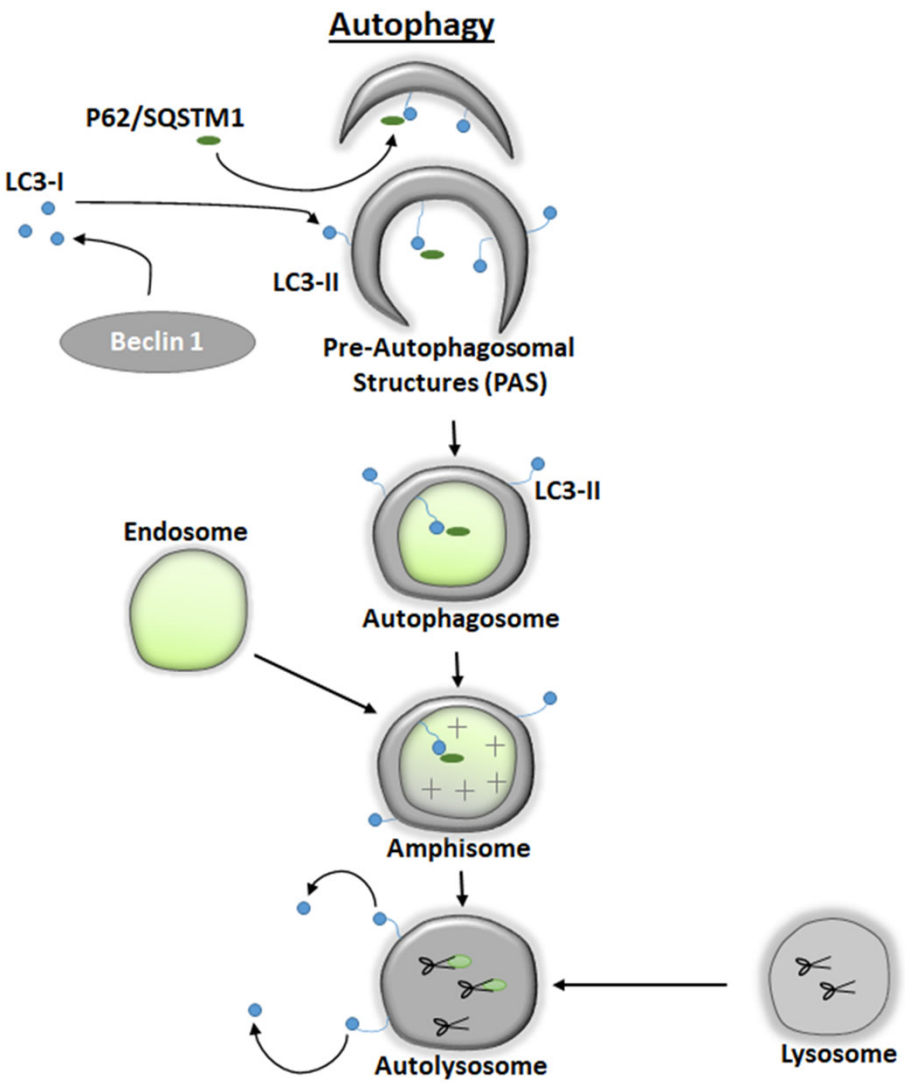
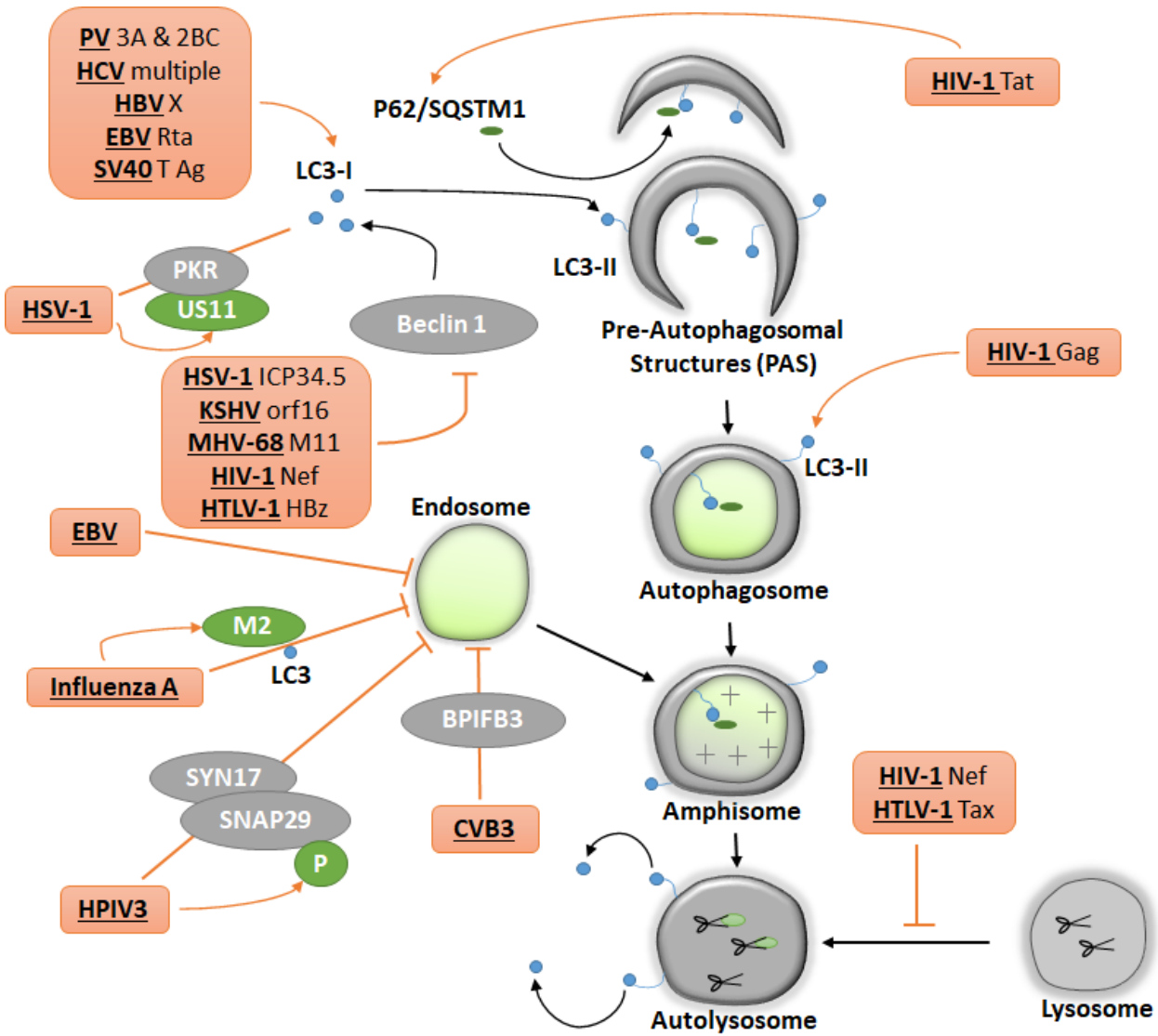
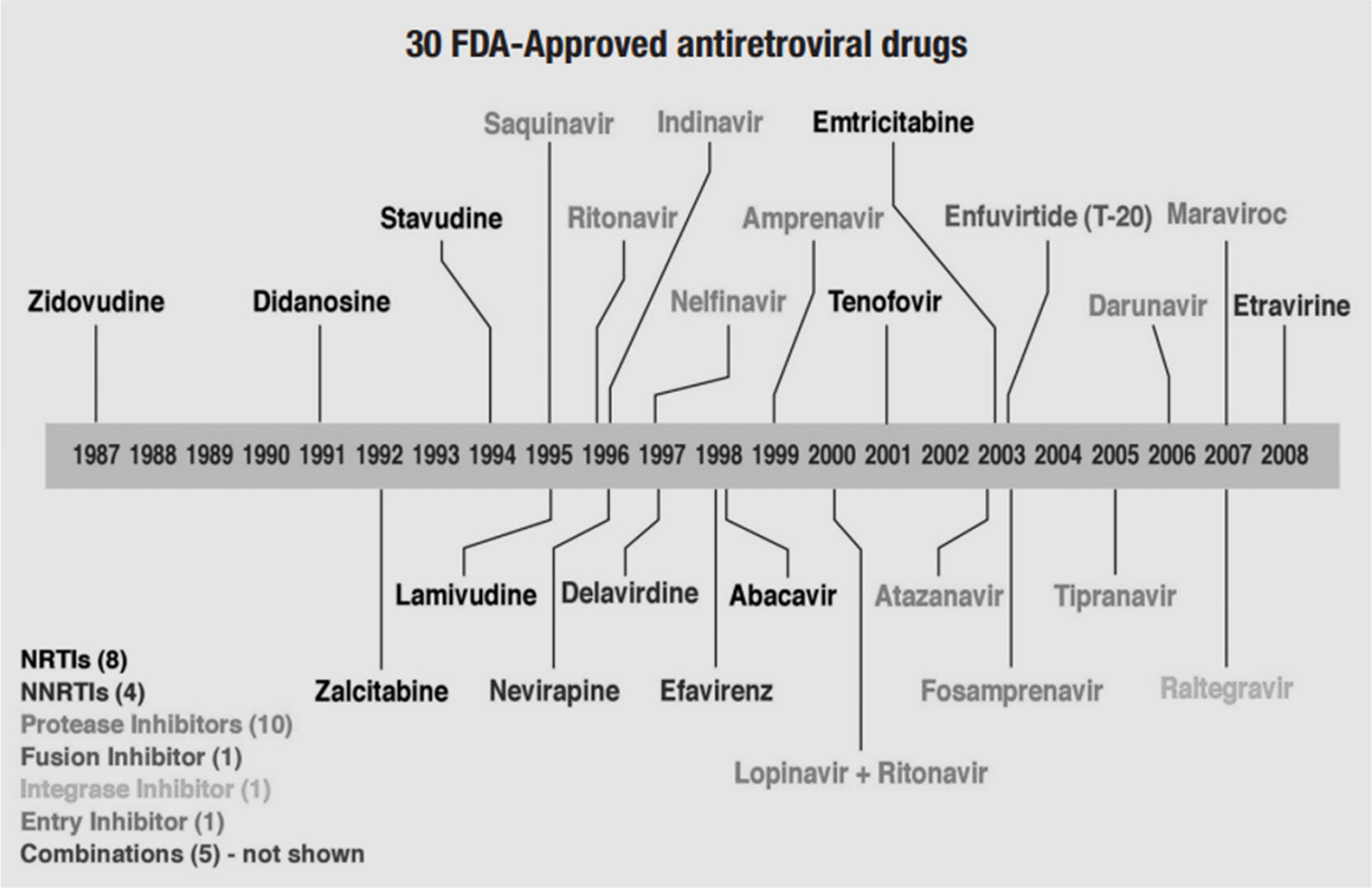

Autophagy
P62/SQSTM1
LC3-I
LC3-II
Beclin 1
Pre-Autophagosomal
Structures (PAS)
LC3-II
Endosome
Autophagosome
Amphisome
Autolysosome
Lysosome

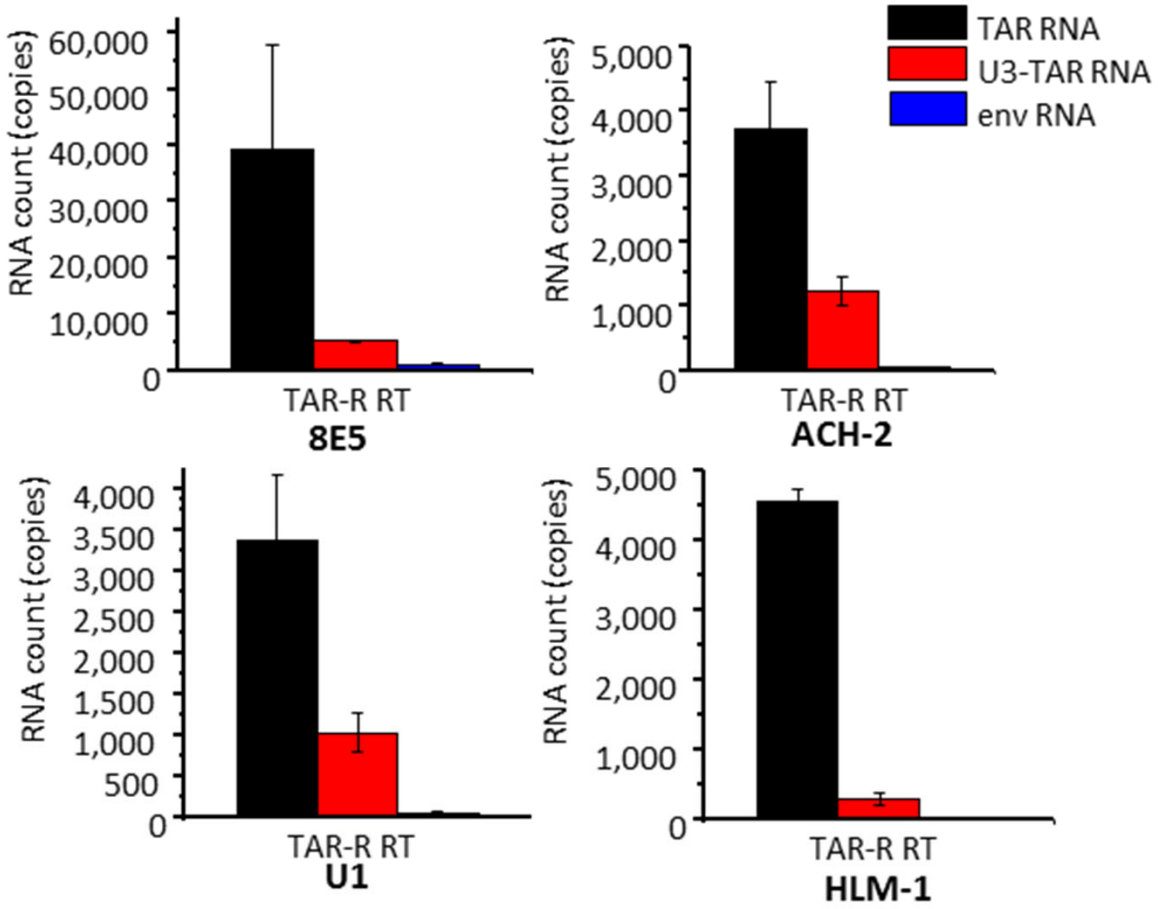

PV 3A & 2BC
HCV multiple
HBV X
EBV Rta
SV40 T Ag
P62/SQSTM1
HIV-1 Tat
LC3-I
LC3-II
PKR
HSV-1
US11
Beclin 1
Pre-Autophagosomal Structures (PAS)
HIV-1 Gag
HSV-1 ICP34.5
KSHV orf16
MHV-68 M11
HIV-1 Nef
HTLV-1 HBz
LC3-II
Endosome
EBV
Autophagosome
M2
LC3
Influenza A
BPIFB3
Amphisome
SYN17
SNAP29
P
HIV-1 Nef
HTLV-1 Tax
CVB3
HPIV3
Autolysosome
Lysosome

No FDA-approved transcription inhibitors

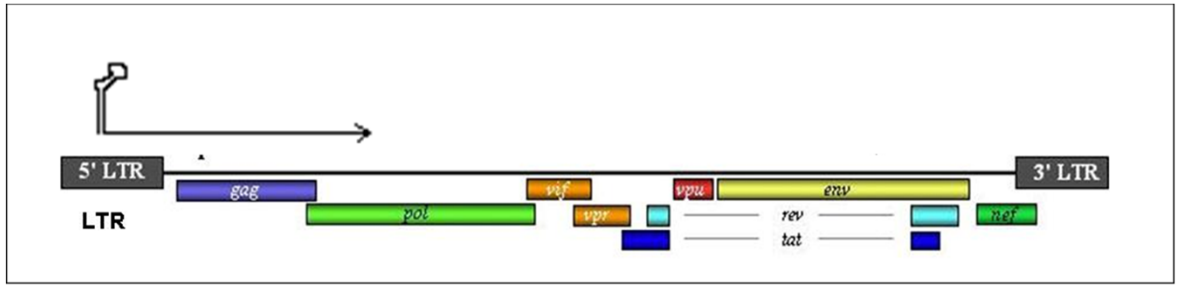


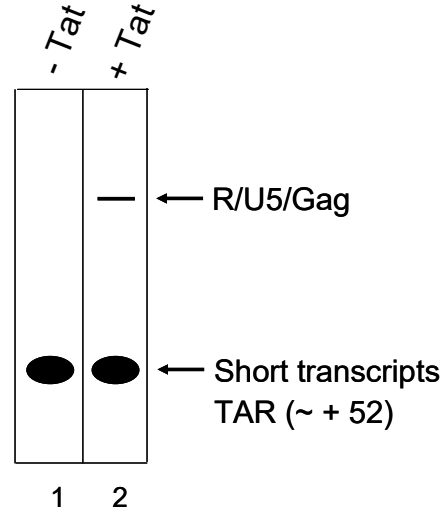
Palmisano L and Vella S, Ann Ist Super Sanita. 2011

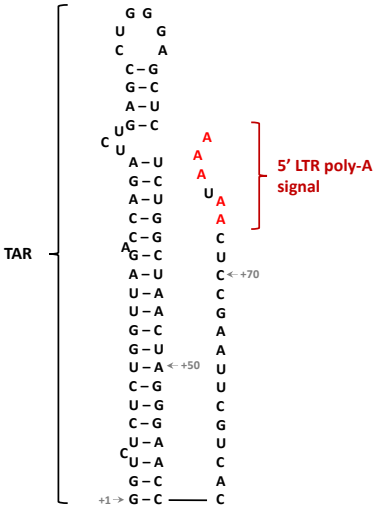
TAR RNA is present in culture supernatants of HIV-1 infected cells



TAR RNA (short transcripts)







- More than 70 papers from late 80s to present
- Detected in vitro, in vivo and latent patient cells

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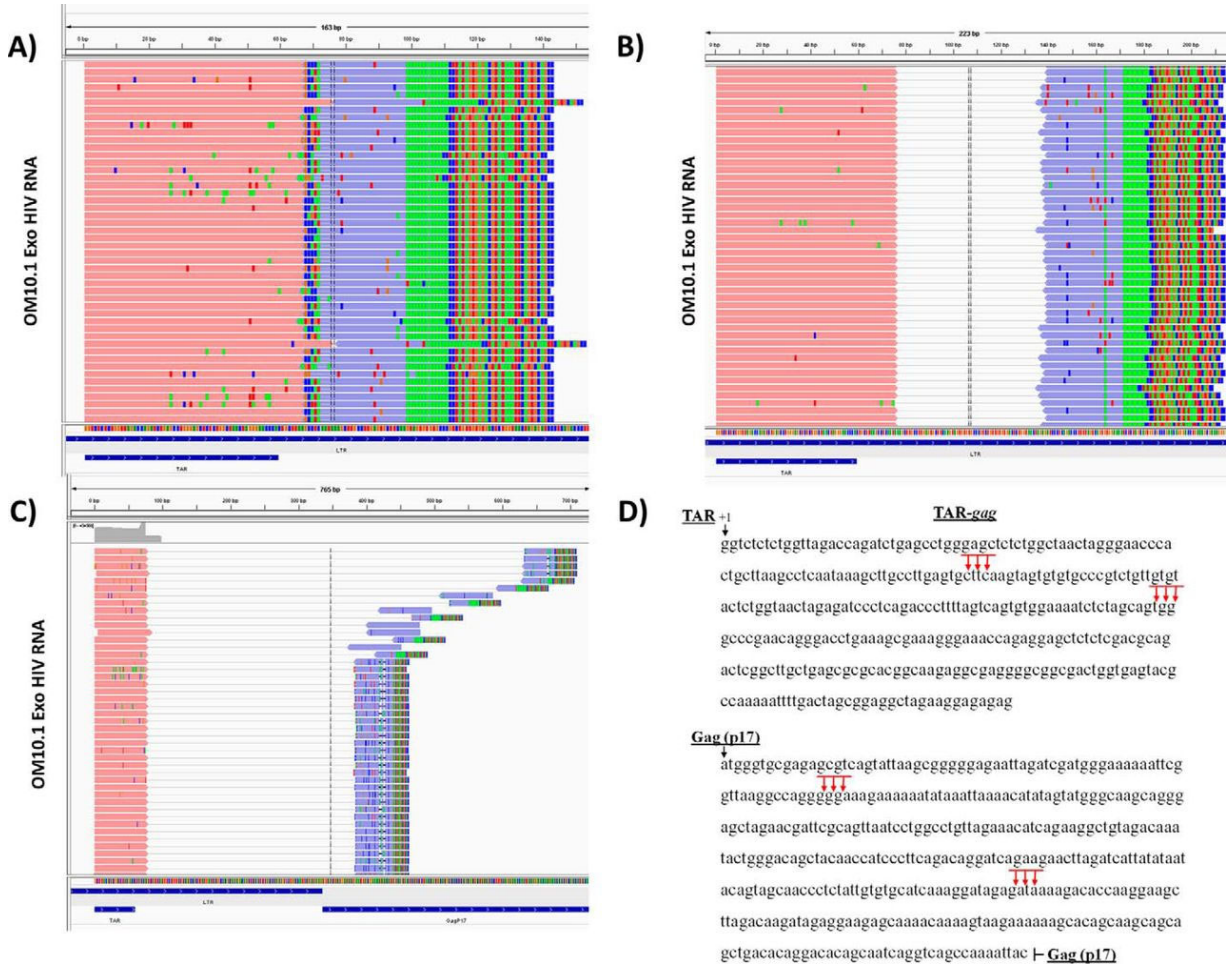
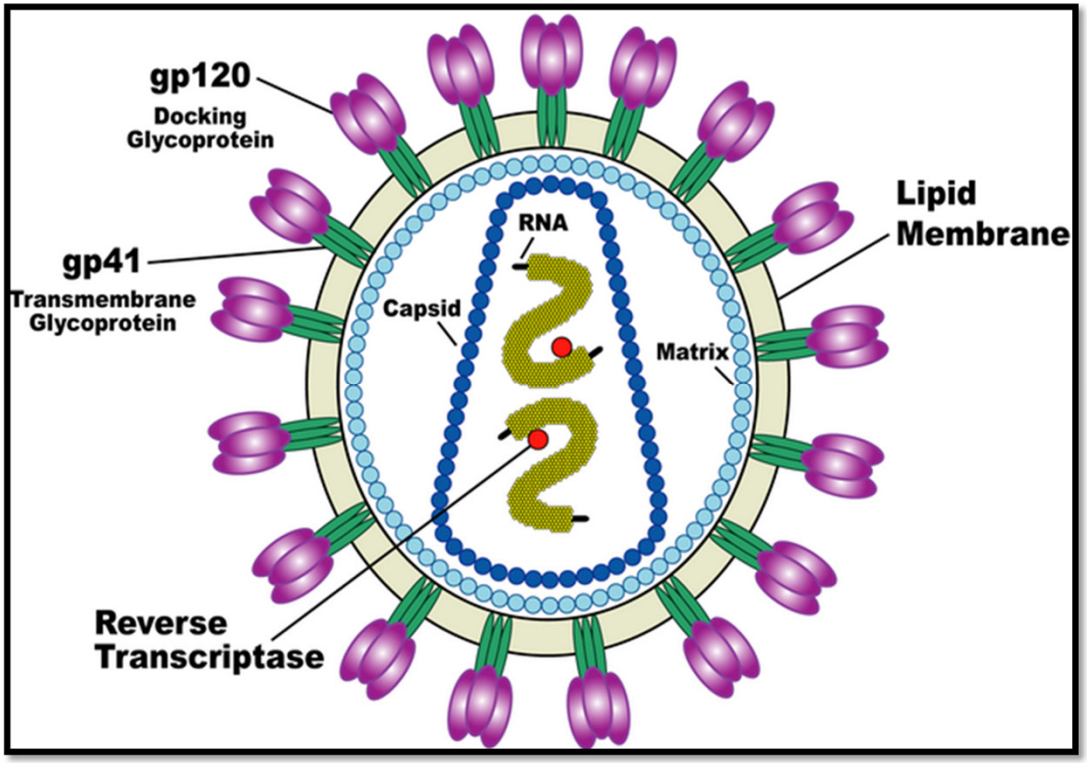
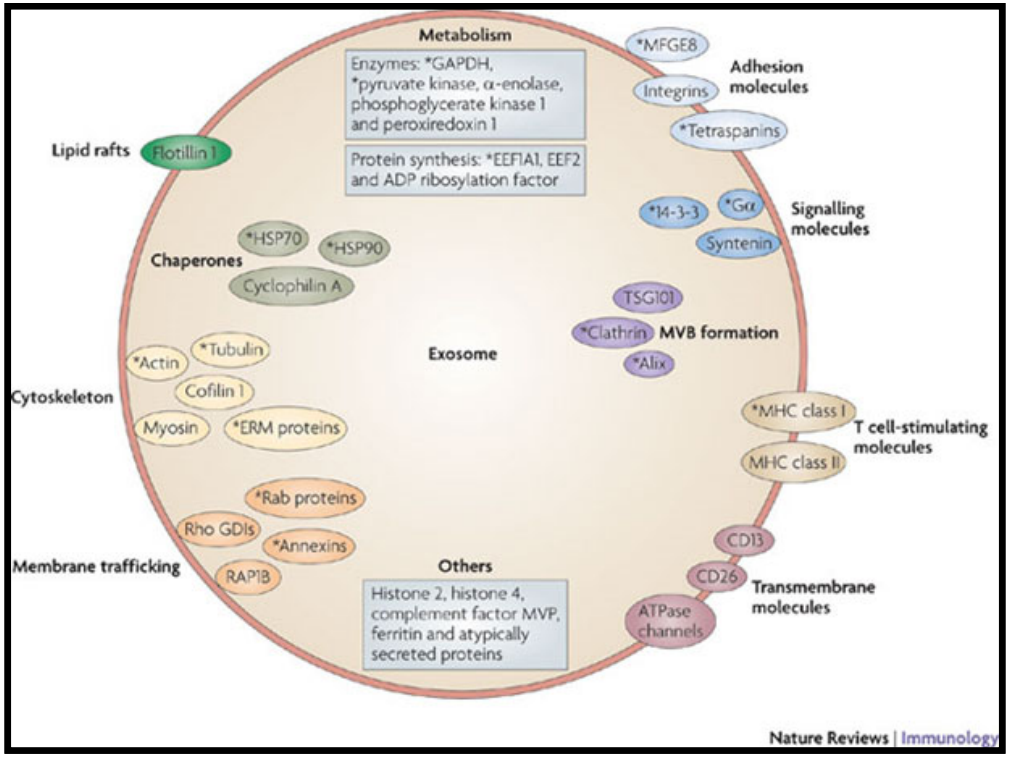
Sequence alignment of exosomal RNA using Integrated Genomics Viewer



Barclay et al., J Biol Chem. 2017.

Membrane vesicles as conveyors of immune responses





Thery et al., Nat Rev Immunol. 2009.

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SCIENTIFIC REPORTS

Correction: Publisher Correction

OPEN

Antiretroviral Drugs Alter the Content of Extracellular Vesicles from HIV-1-Infected Cells

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Accepted: 1 May 2018
Published online: 16 May 2018

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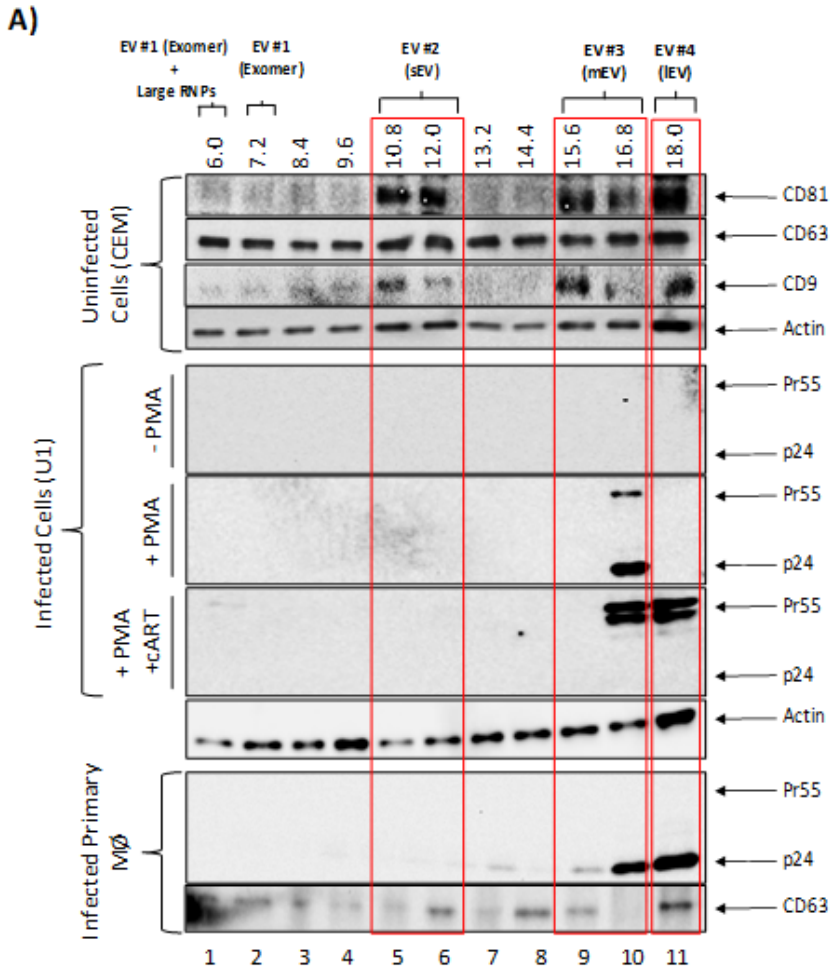
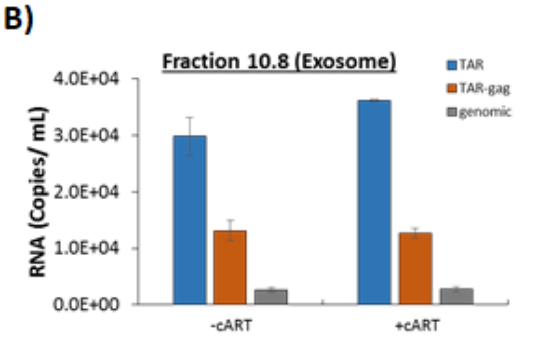
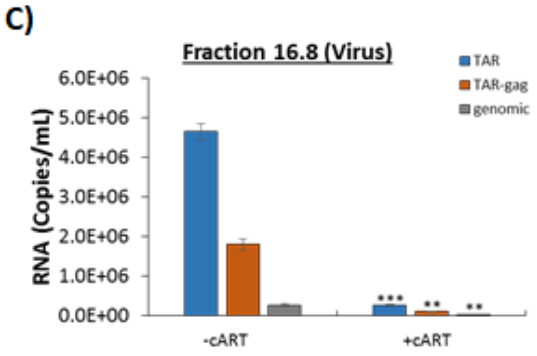
To date, the most effective treatment of HIV-1 is a combination antiretroviral therapy (cART), which reduces viral replication and reverses pathology. We investigated the effect of cART (RT and protease inhibitors) on the content of extracellular vesicles (EVs) released from HIV-1-infected cells. We have previously shown that EVs contain non-coding HIV-1 RNA, which can elicit responses in recipient cells. In this manuscript, we show that TAR RNA levels demonstrate little change with the addition of cART treatment in cell lines, primary macrophages, and patient biofluids. We determined possible mechanisms involved in the selective packaging of HIV-1 RNA into EVs, specifically an increase in EV-associated hnRNP A2/B1. More recent experiments have shown that several other FDA-approved drugs have the ability to alter the content of exosomes released from HIV-1-infected cells. These findings on cART-altered EV content can also be applied to general viral inhibitors (interferons) which are used to treat other chronic infections. Additionally, we describe unique mechanisms of ESCRT pathway manipulation by antivirals, specifically the targeting of VPS4. Collectively, these data imply that, despite antiretroviral therapy, EVs containing viral products are continually released and may cause neurocognitive and immunological dysfunction.

Isolation of EVs away from virus

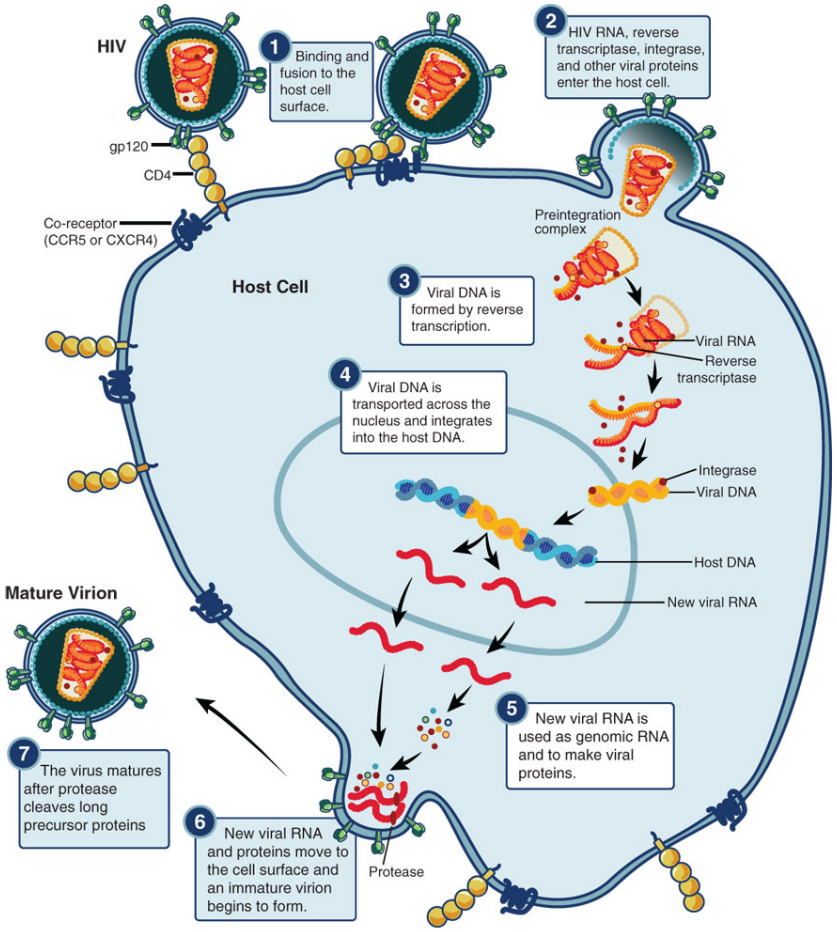


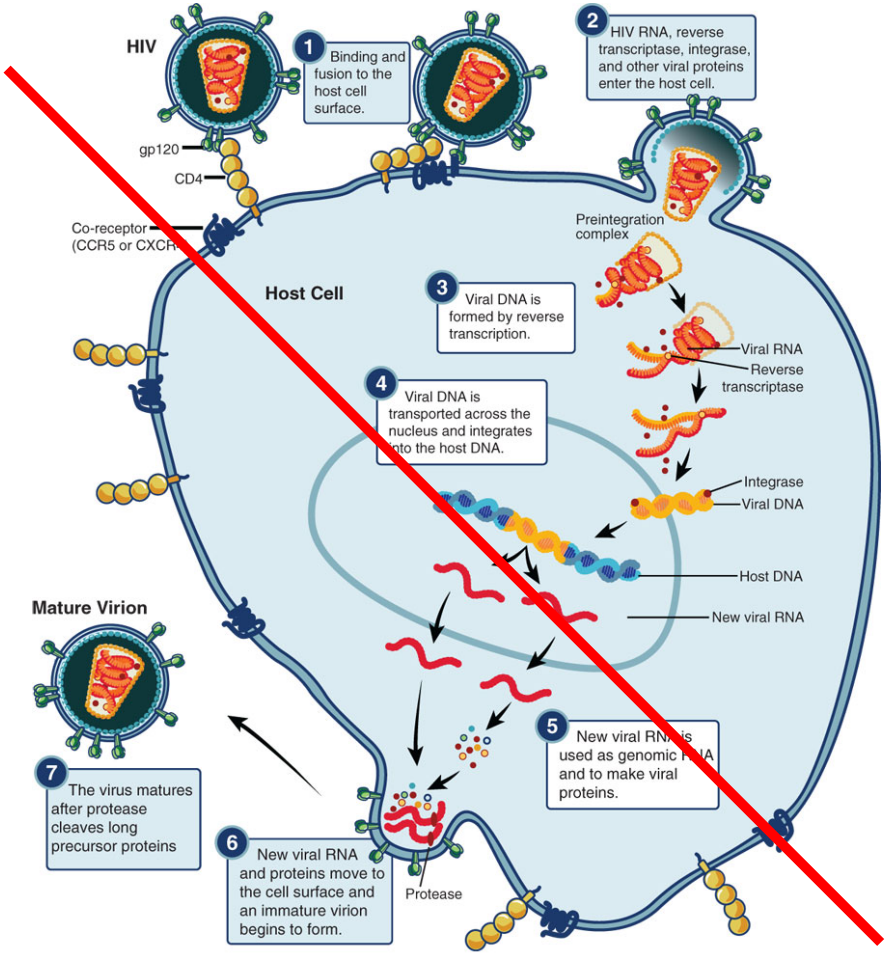




DeMarino et al., Sci Rep. 2018

HIV life cycle





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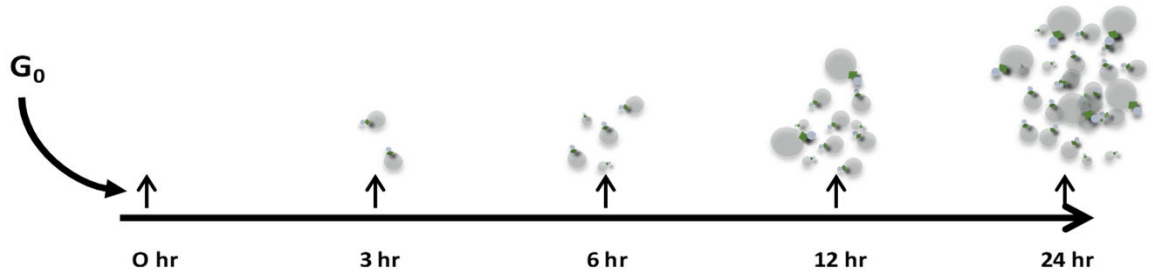
Question: Which particles are released first from infected cells- EVs or Viruses ?



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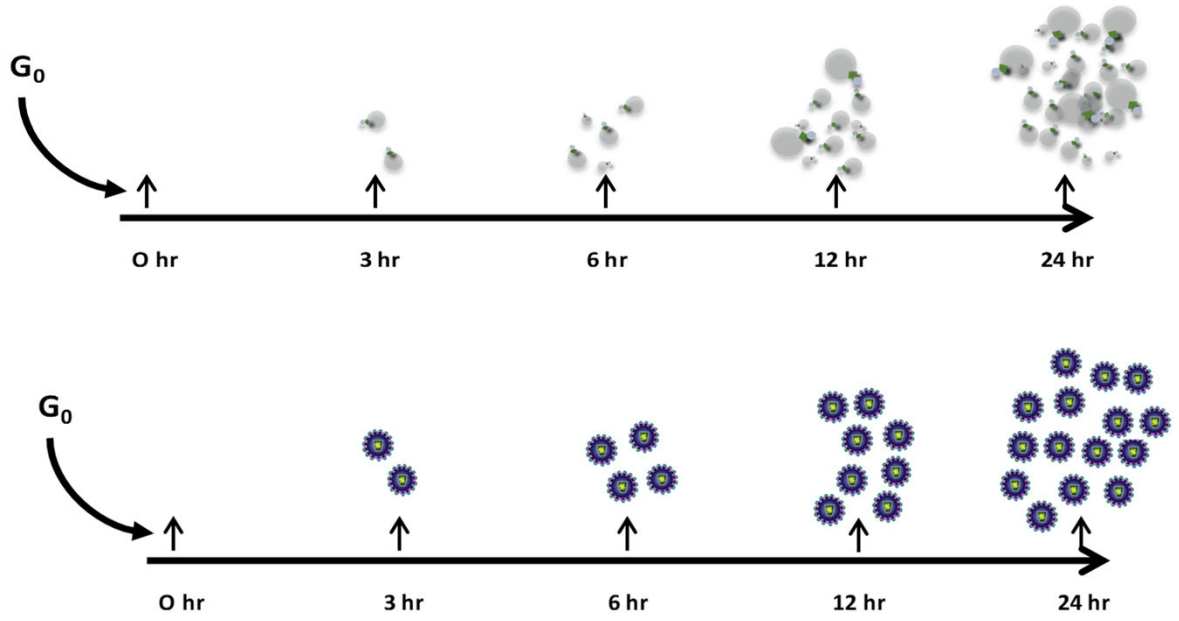

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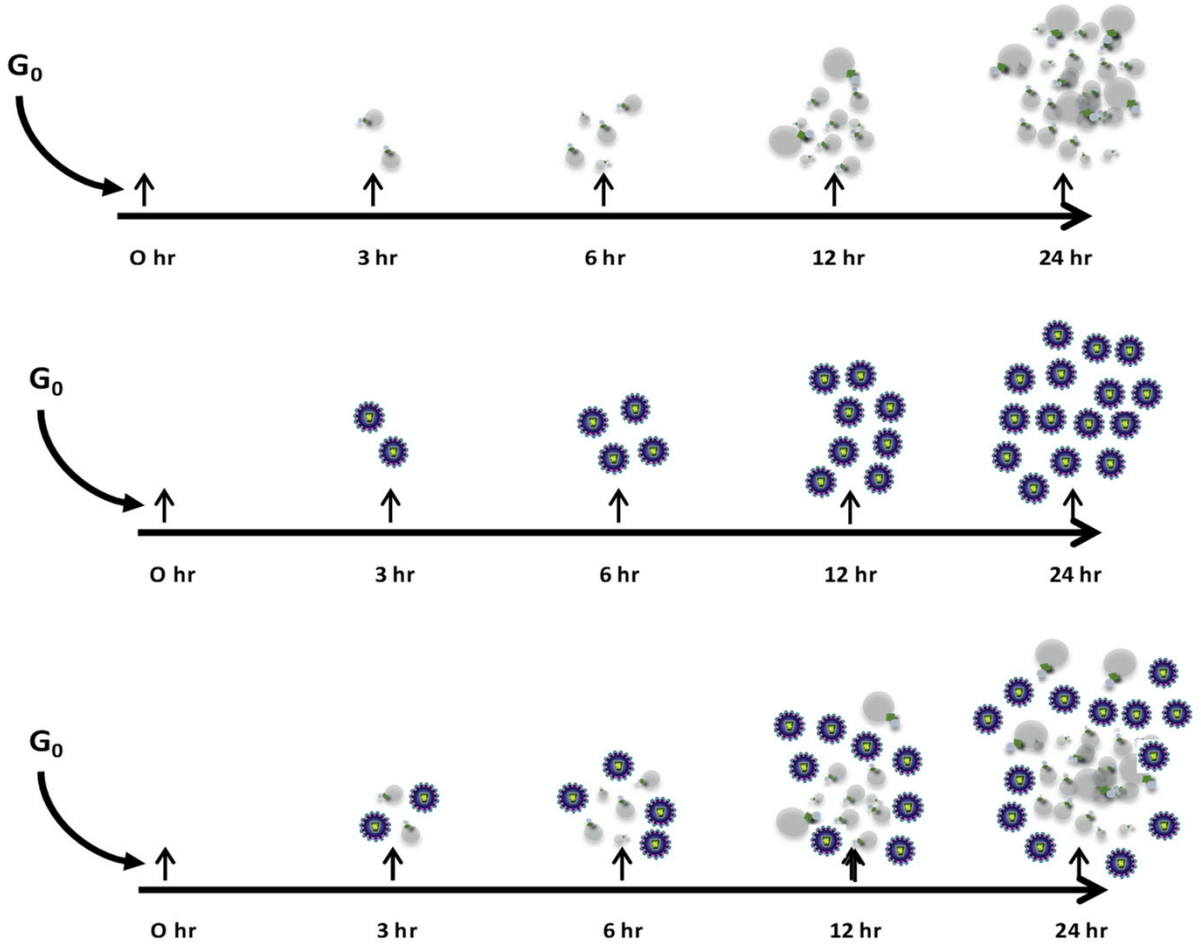
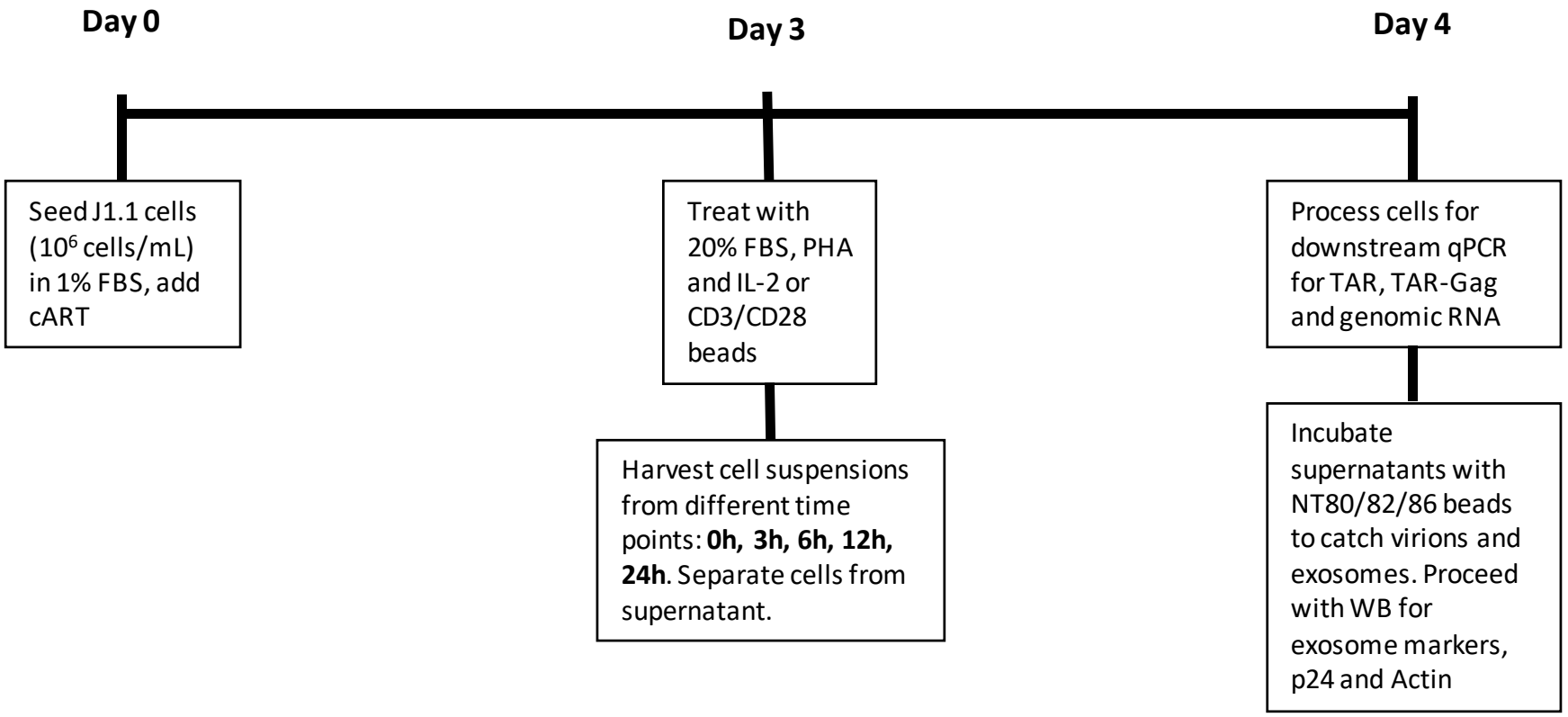
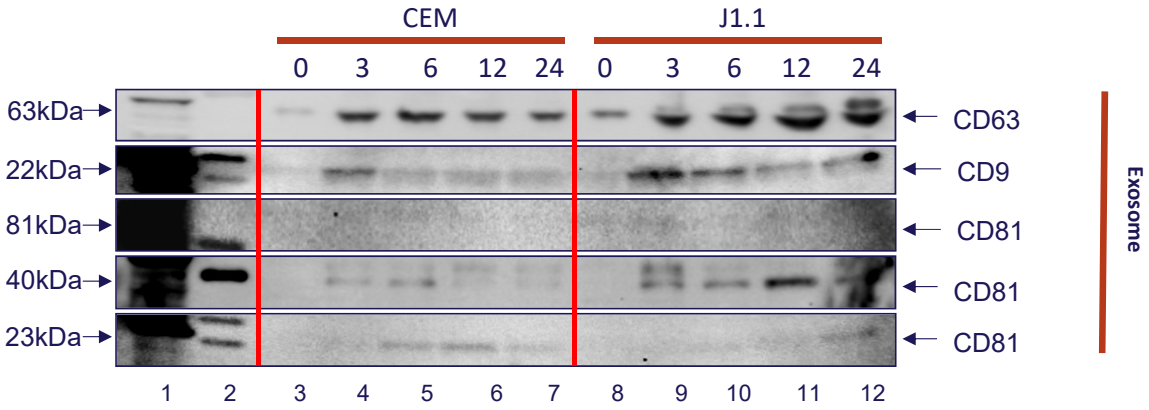


Experimental procedure

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J1.1
0 3 6 12 24 0 3 6 12 24
63kDa
22kDa
81kDa
40kDa
23kDa
CD63
CD9
CD81
CD81
CD81
Exosome
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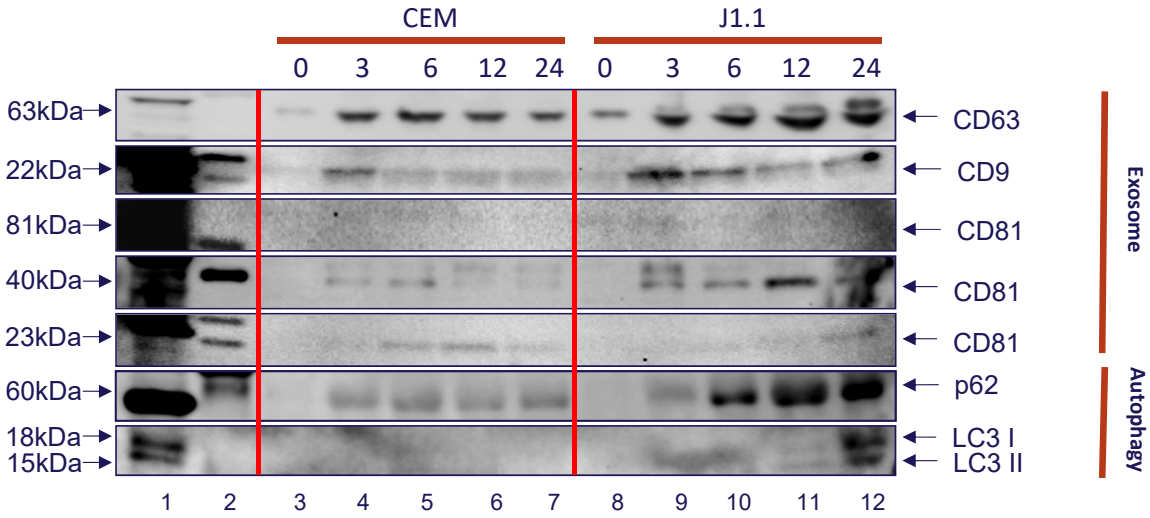
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40kDa
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18kDa
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CD9
CD81
CD81
CD81
p62
LC3 I
LC3 II
Exosome
Autophagy
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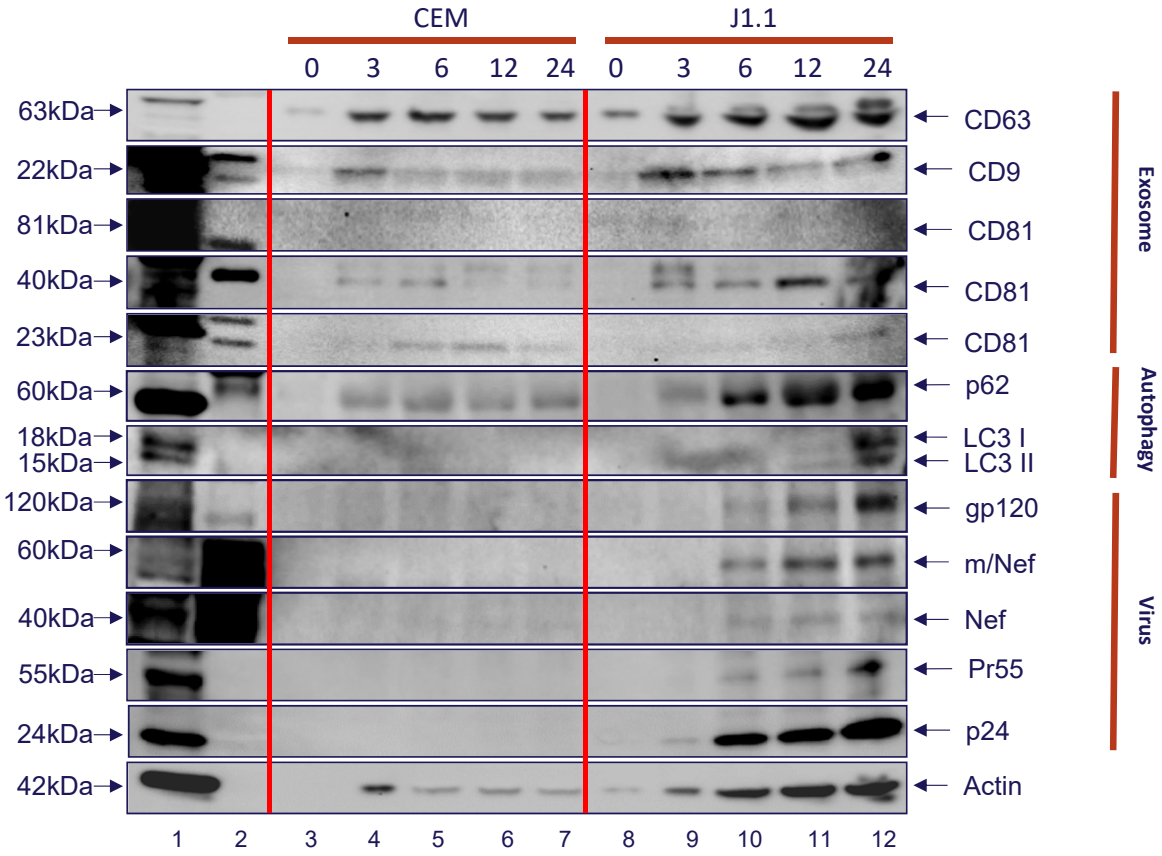
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55kDa
24kDa
42kDa
CD63
CD9
CD81
CD81
CD81
p62
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p24
Actin
Exosome
Autophagy
Virus
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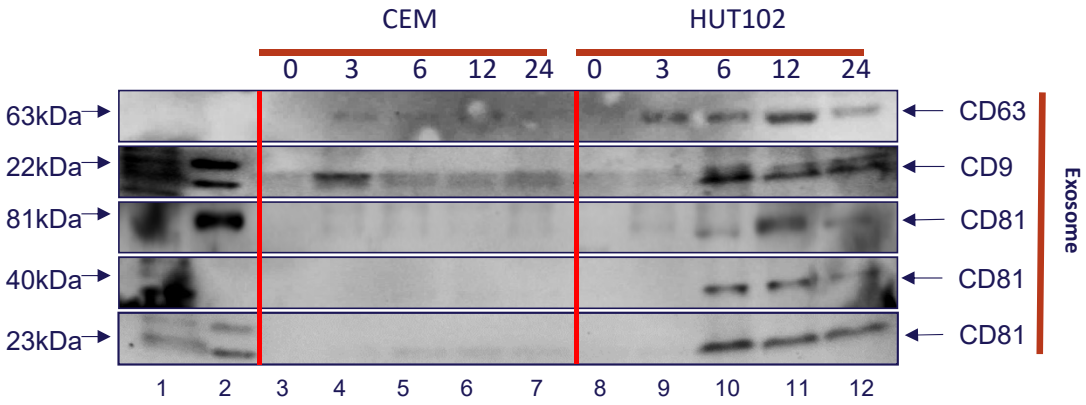
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CD9
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Exosome
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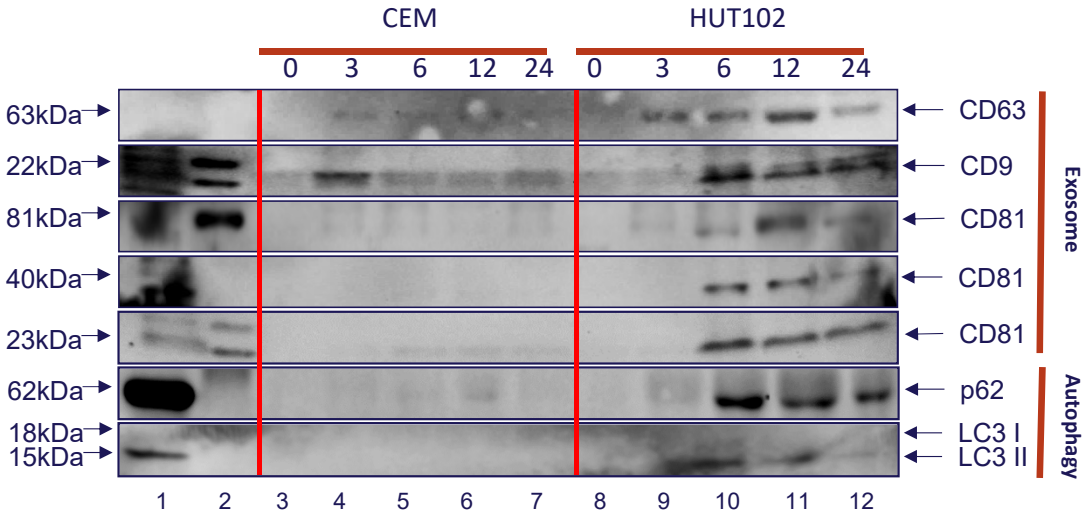
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CD9
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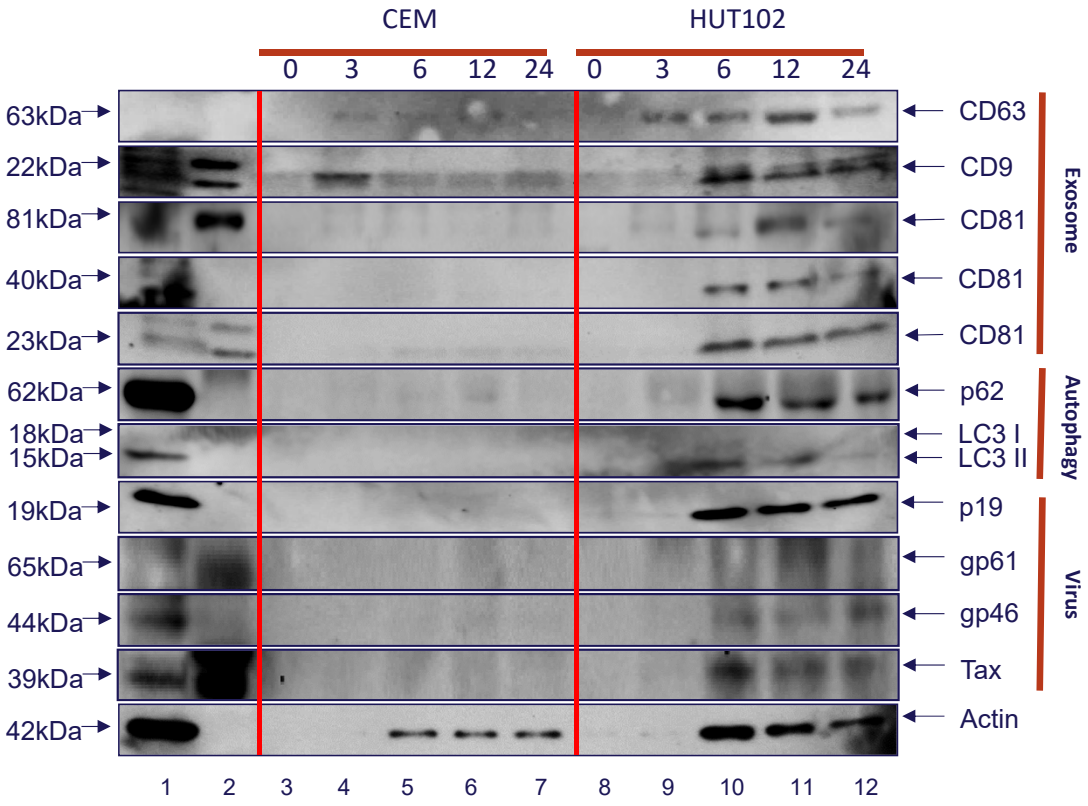
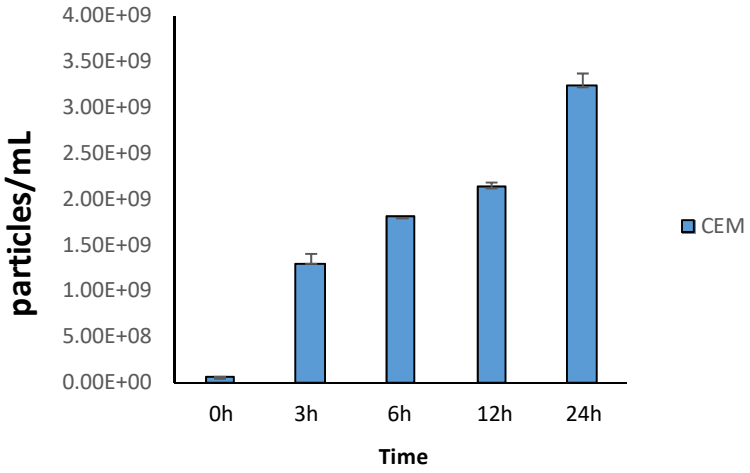
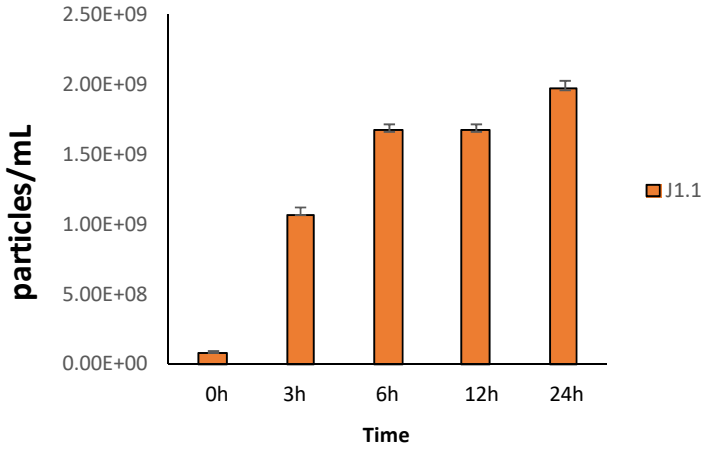
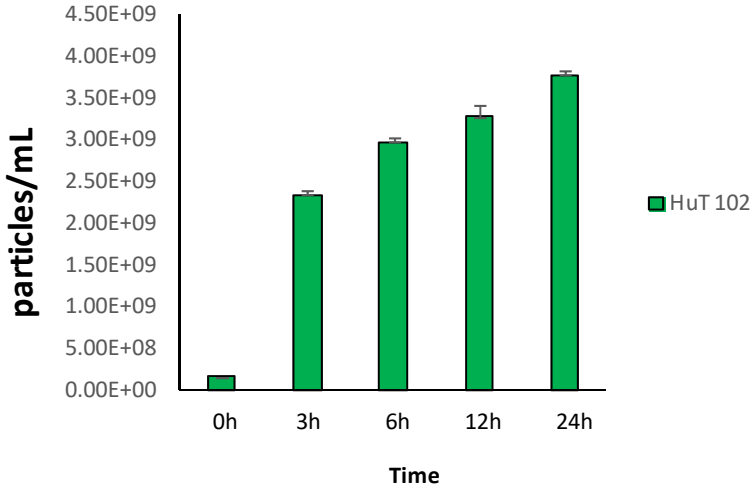
CD81
CD81
p62
LC3 I
LC3 II
Exosome
Autophagy
1 2 3 4 5 6 7 8 9 10 11 12


CEM
HUT102
0 3 6 12 24
0 3 6 12 24
63kDa
22kDa
81kDa
40kDa
23kDa
62kDa
18kDa
15kDa
19kDa
65kDa
44kDa
39kDa
42kDa
CD63
CD9
CD81
CD81
CD81
p62
LC3 I
LC3 II
p19
gp61
gp46
Tax
Actin
Exosome
Autophagy
Virus
1 2 3 4 5 6 7 8 9 10 11 12

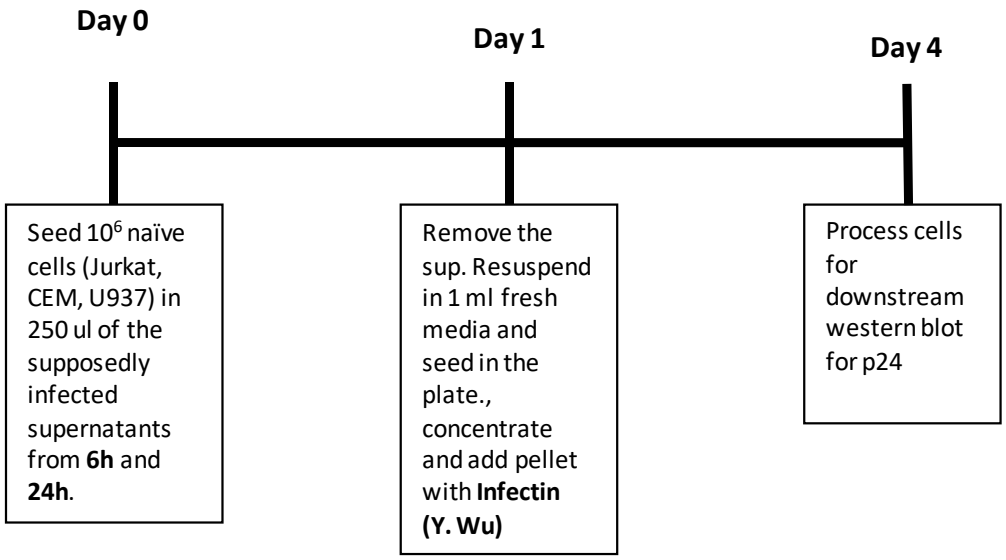
Particle release over time

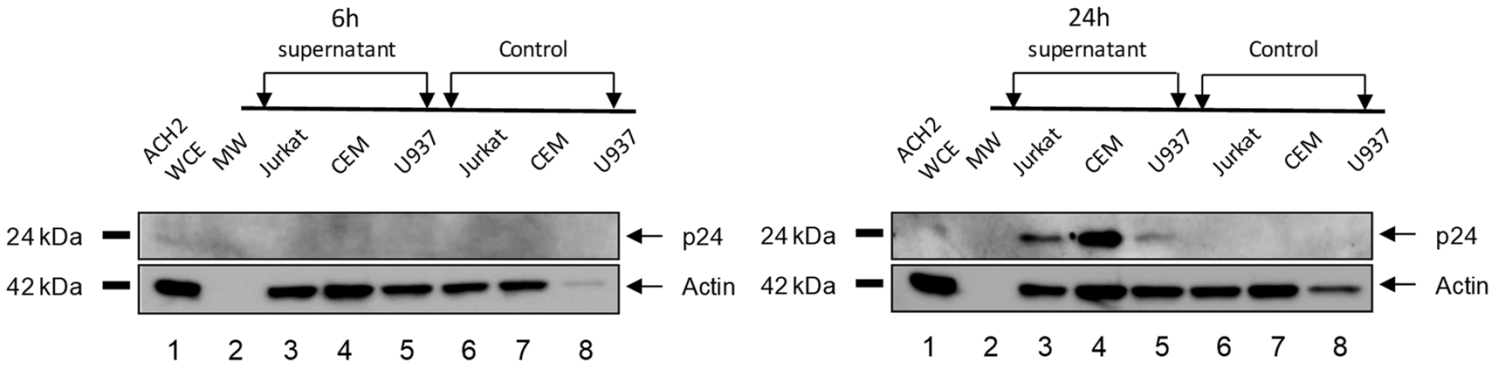


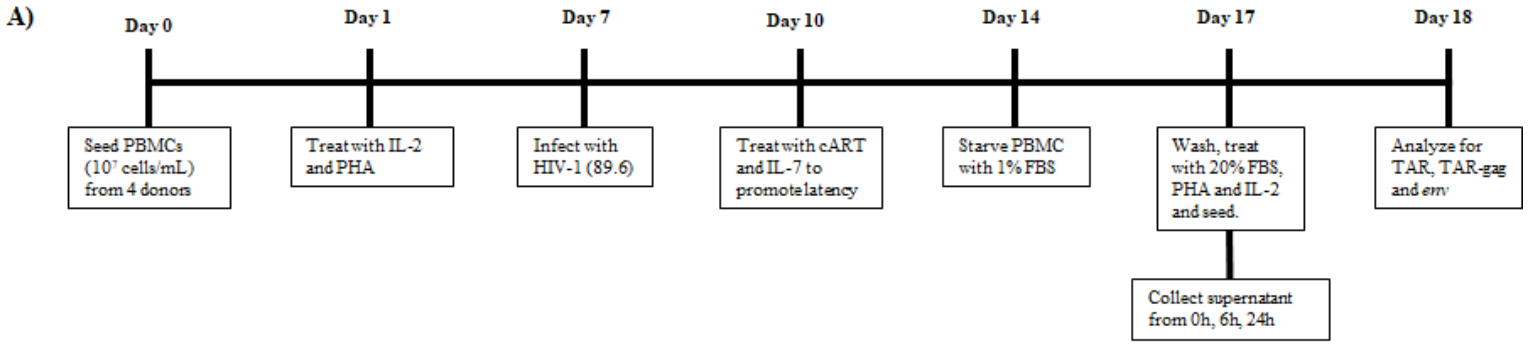




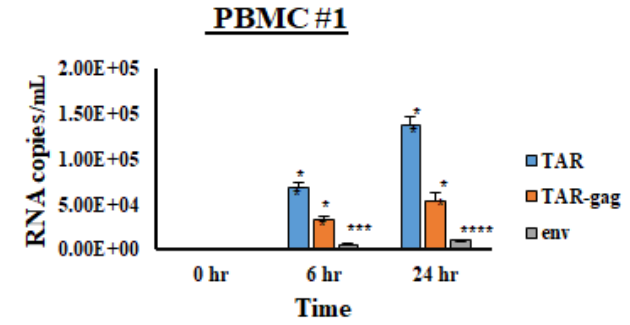
Virus rescue assay

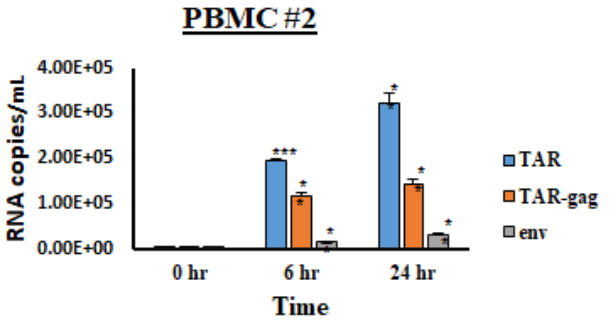


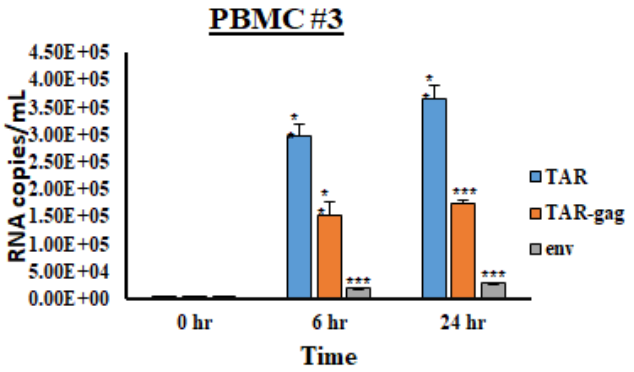


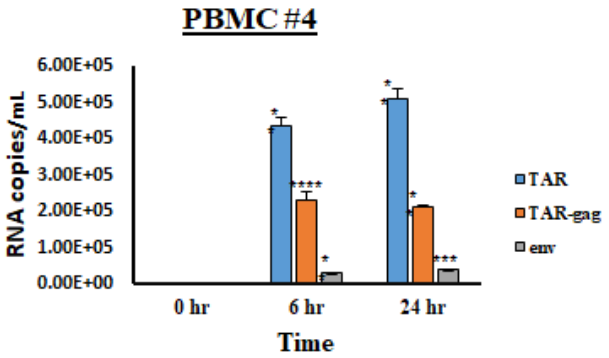

A)
Day 0
Day 1
Day 7
Day 10
Day 14
Day 17
Day 18
Seed PBMCs (10⁷ cells/mL) from 4 donors
Treat with IL-2 and PHA
Infect with HIV-1 (89.6)
Treat with cART and IL-7 to promote latency
Starve PBMC with 1% FBS
Wash, treat with 20% FBS, PHA and IL-2 and seed.
Collect supernatant from 0h, 6h, 24h
Analyze for TAR, TAR-gag and env

B)

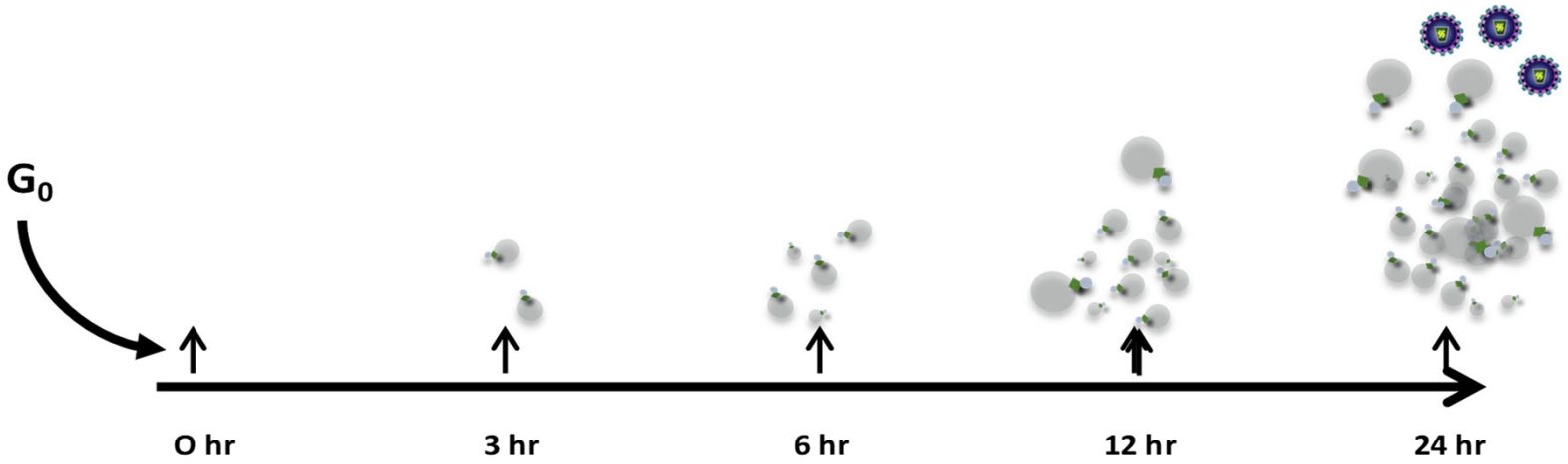

PBMC #1
RNA copies/mL
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
0 hr
6 hr
24 hr
Time
TAR
TAR-gag
env

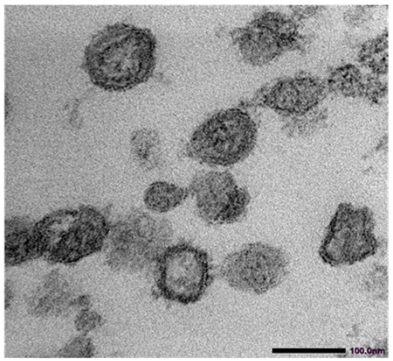

PBMC #2
RNA copies/mL
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
0 hr
6 hr
24 hr
Time
TAR
TAR-gag
env

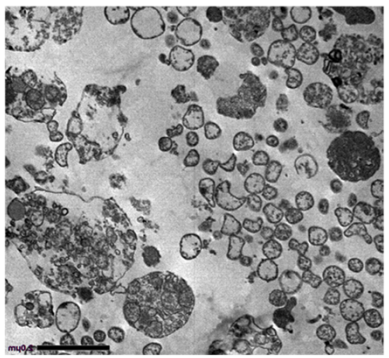

PBMC #3
RNA copies/mL
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
0 hr
6 hr
24 hr
Time
TAR
TAR-gag
env


PBMC #4
RNA copies/mL
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
0 hr
6 hr
24 hr
Time
TAR
TAR-gag
env

Answer: EVs come out of infected cells first and then a mixture of both EVs and viruses at a later time



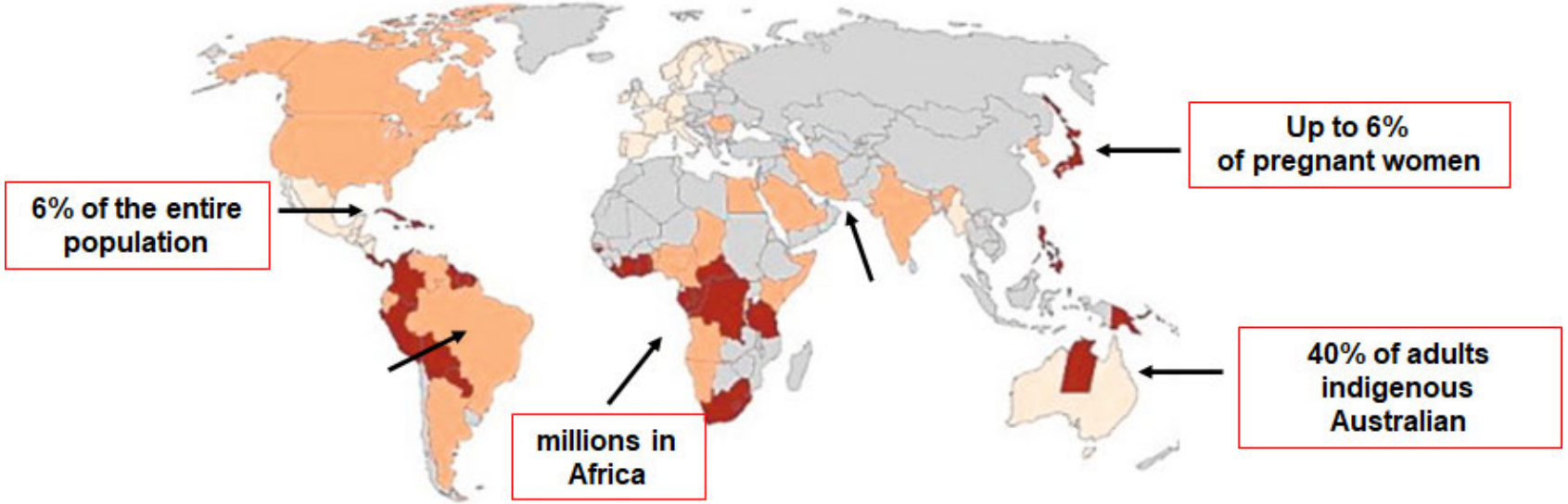




Human T-lymphotropic virus type 1 (HTLV-1)

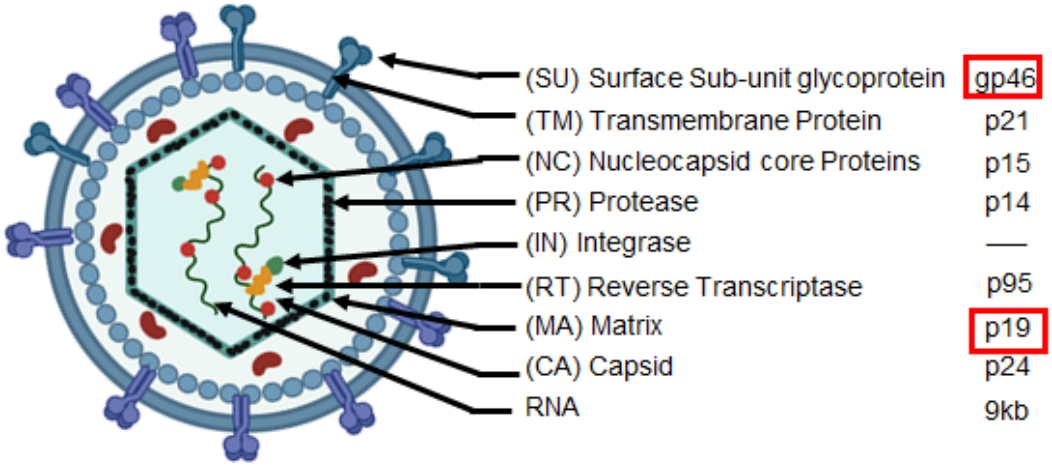
Epidemiology

- Approximately **5-10 million** patients worldwide infected with HTLV-1.
- The true figure is greater as many cases are unreported from highly populated regions, such as **China, India, Northwest Africa (i.e., the Arab Maghreb), and East Africa**.
- It is estimated that the actual global prevalence may be as **high** as **20 million** worldwide.

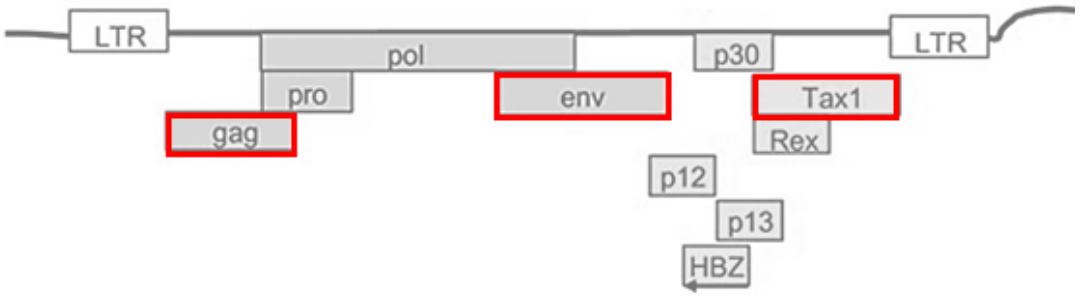


Robert R. McKendall. Handbook of Clinical Neurology. 2014.

A) VIRION

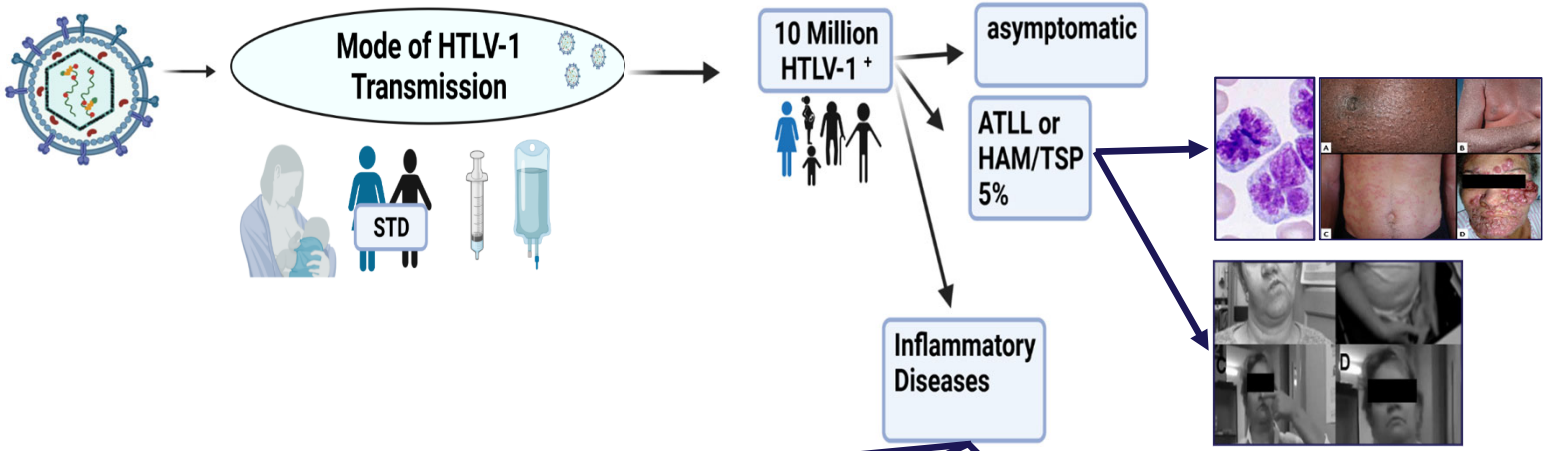


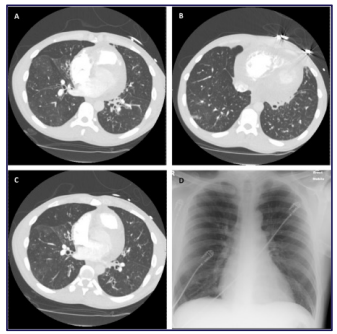
B) HTLV-1 Genome Structure

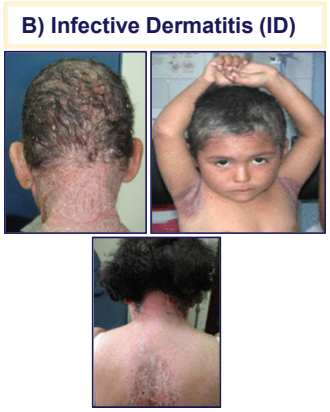


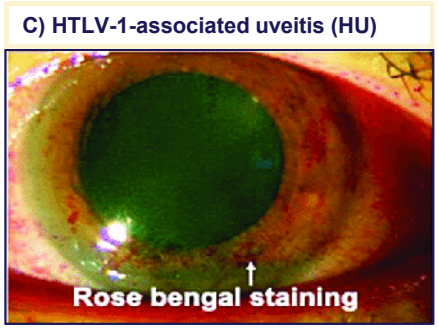
Higuchi and Fujii, Retrovirology. 2009.

HTLV-1 overview



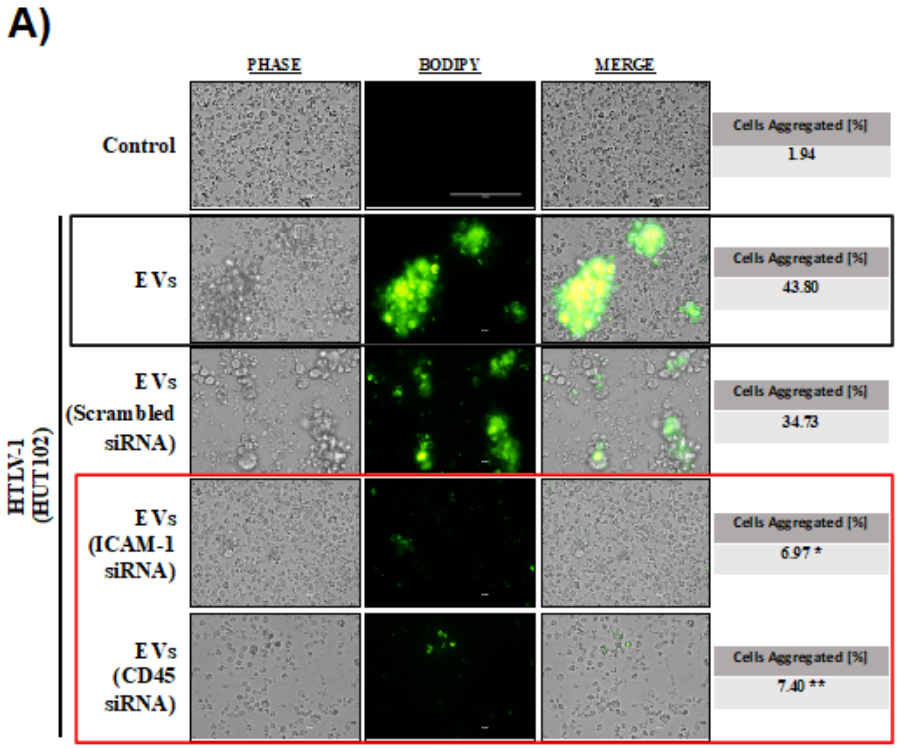


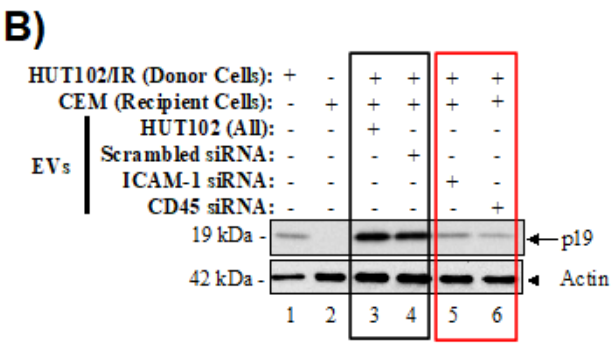


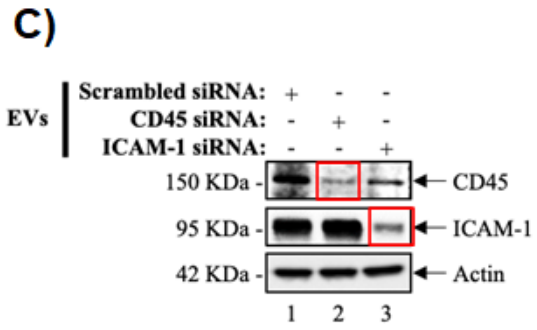


Oliveira PD et al., Rev Assoc Médica Bras. 2016.
Kamoi K., Frontiers in Microbiology. 2020.
Bravo FG., Sem Diagnostic Pathol. 2020.
Chiong et al., IDCases, 19, e00714.

Inhibition of ICAM-1 and CD45 via small interfering RNA prevents cell-to-cell contact

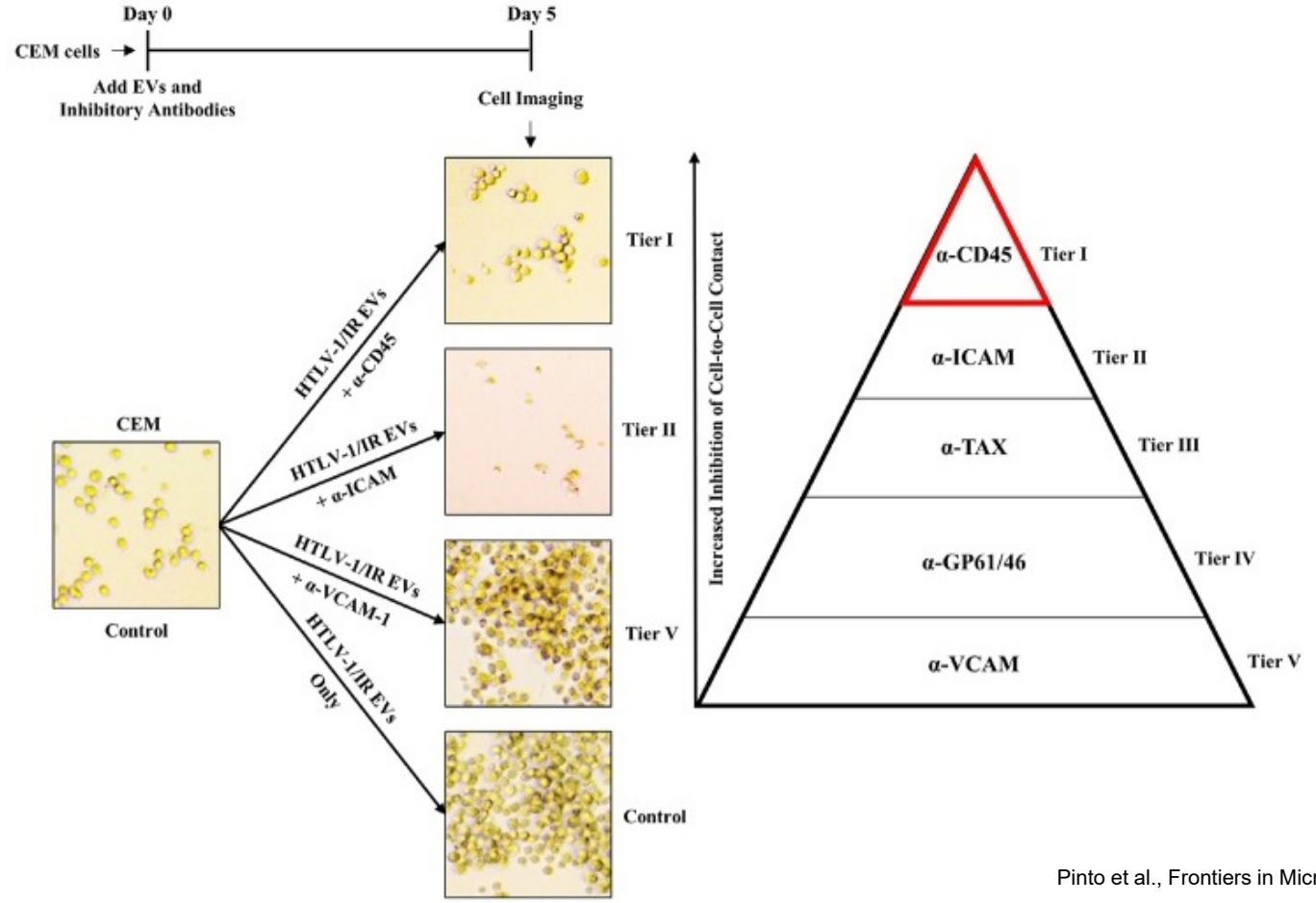






Pinto et al., Retrovirology. 2021.

Antibodies against specific cellular surface receptors inhibit cell-to-cell contact



Pinto et al., Frontiers in Microbiology. 2019.

Proteomic analysis of the 2k, 10k, and 100k showed that HTLV-1 EVs carry viral/host proteins

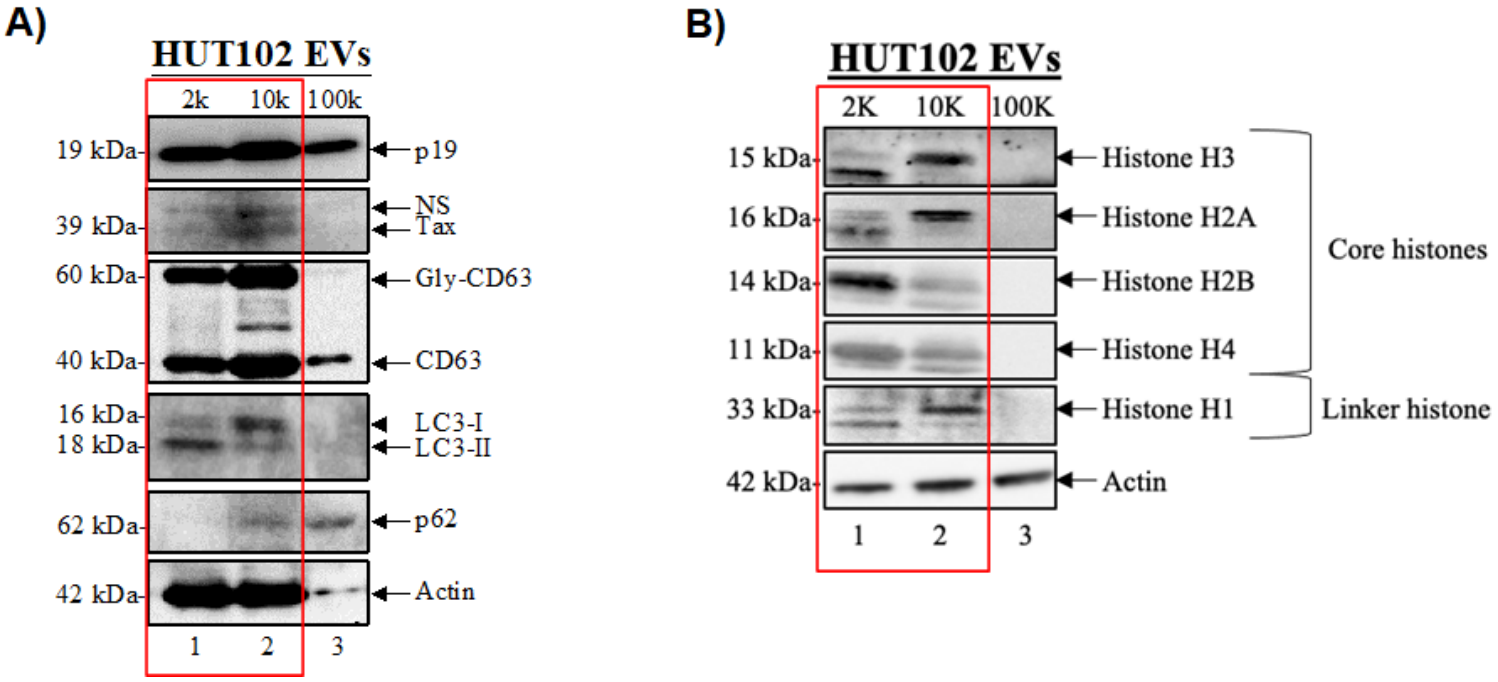
A)

	Viral Proteins		
EVs	**Reference**	**Hits**	**Sequence (Most Abundant Peptide)**
2k	Polyprotein (Gag-Pro-Pol)	57	KPPPNQPFR
	Gag (p19)	15	IALETPVWIPINYSLLASLLPK
	Envelope	11	PYLGQSWTPYTGAVSSPYWK
	Tax	4	VIGSALQFLIPR
10k	Polyprotein (Gag-Pro-Pol)	28	KPPPNQPFR
	Gag (p19)	16	IALETPVWIPINYSLLASLLPK
	Gag-Pol	9	DQIYLNPSQVQSLVQLR
	Envelope	5	NGGGYYSASYSDPSLK
	Protease	5	NTSVLGAGGQTQDHFK
100k	Polyprotein (Gag-Pro-Pol)	30	DPSWASILQGLEEPYHAFVER
	Gag (p19)	12	IALETPVWIPINYSLLASLLPK; VQANNPQQQGLR
	Envelope	2	NGGGYYSASYSDPSLK

B)

Pinto et al., Retrovirology. 2021.

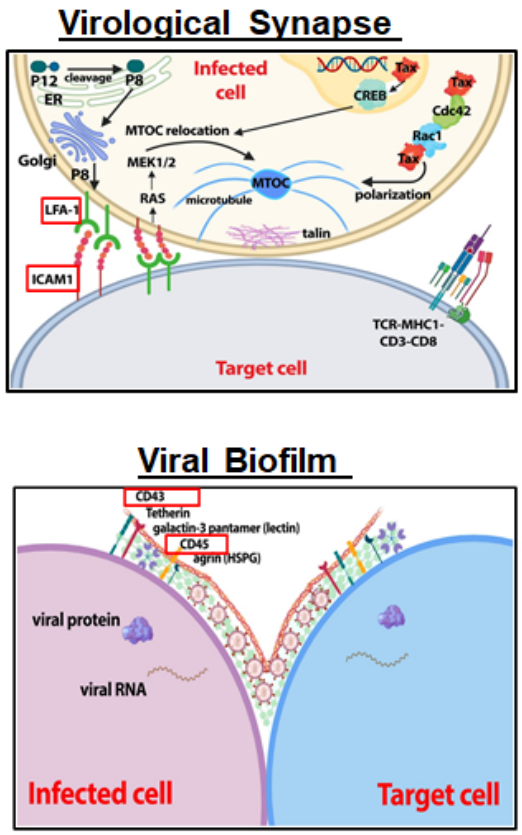
2k & 10k HTLV-1 EVs contain highest amount of viral/host proteins



Pinto et al., Retrovirology. 2021.

HTLV-1 EVs contain CD45, ICAM-1 and LFA-1 may contribute to enhance cell-to-cell contact

A)



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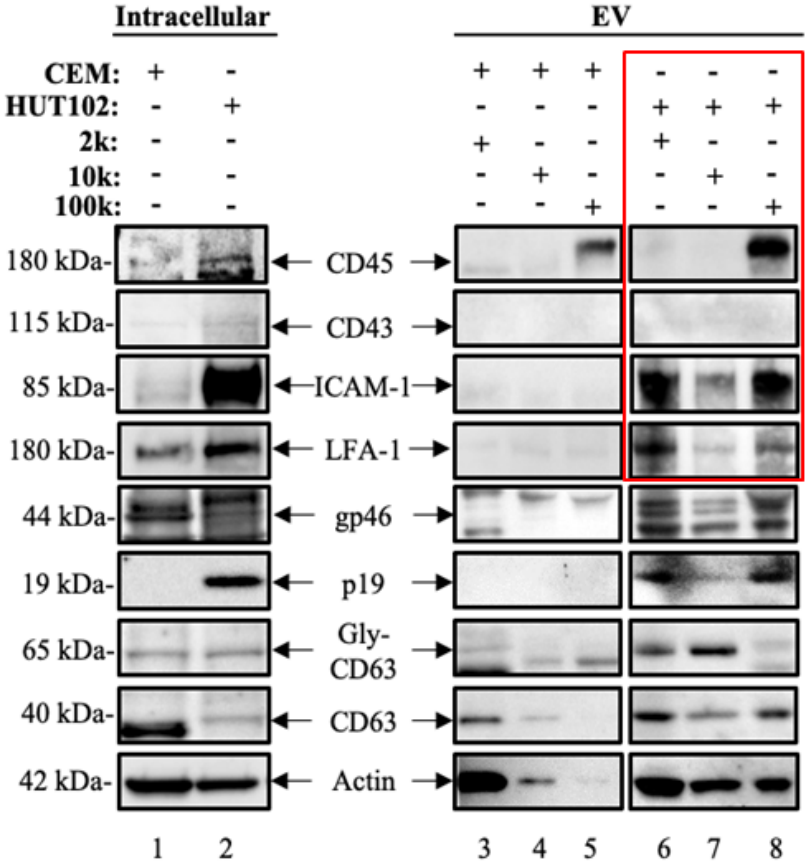
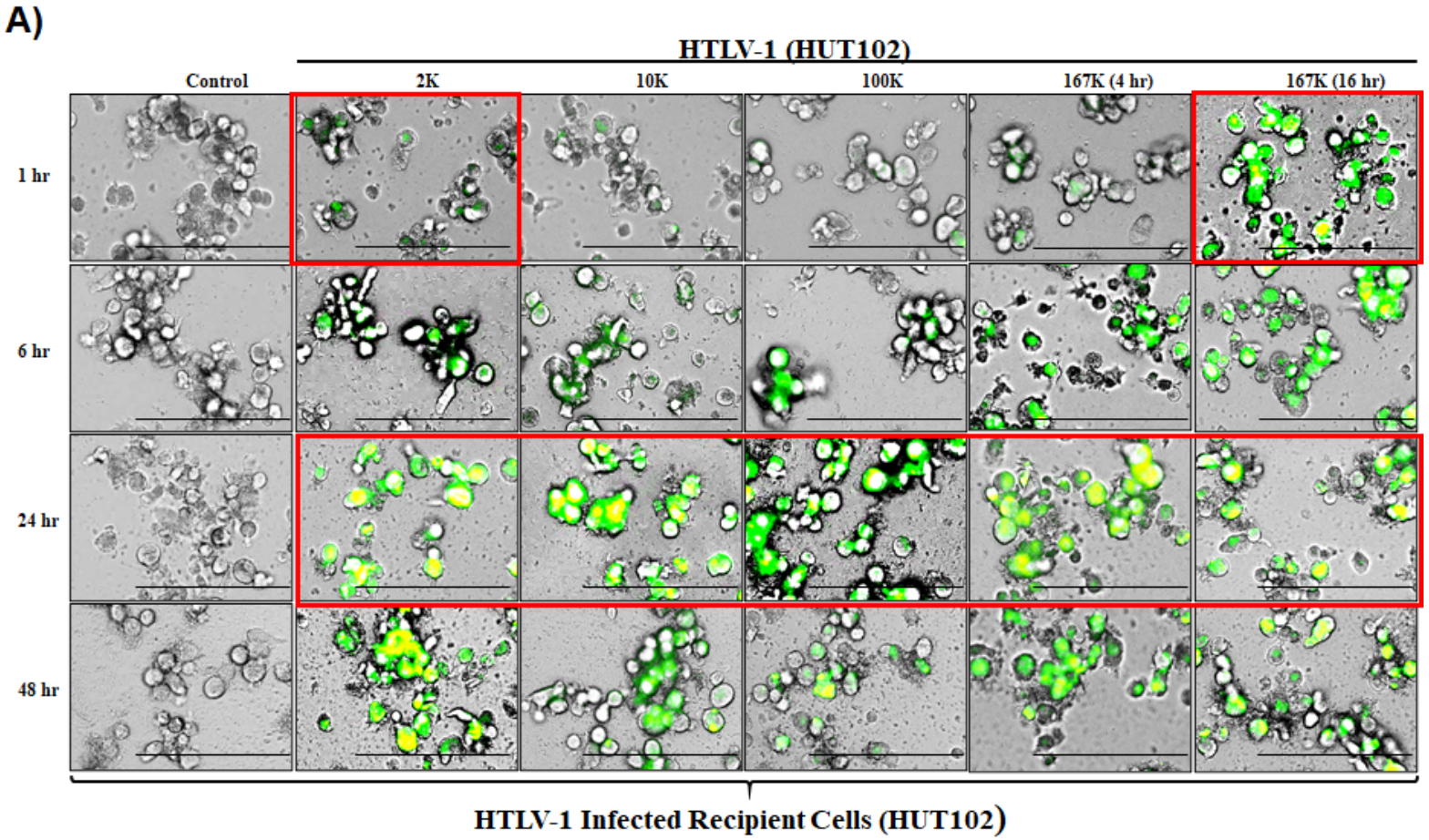
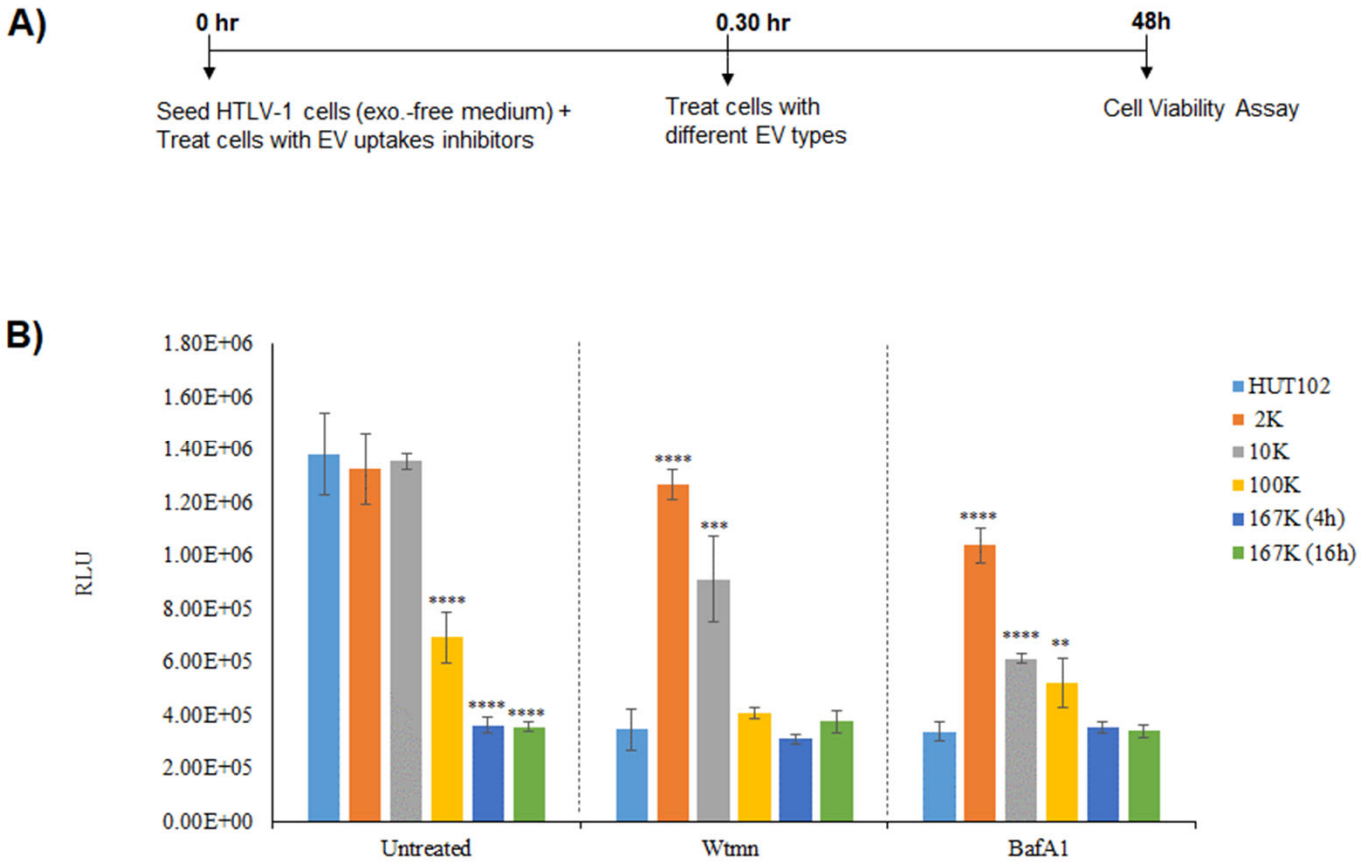


Pinto et al., Retrovirology. 2021.

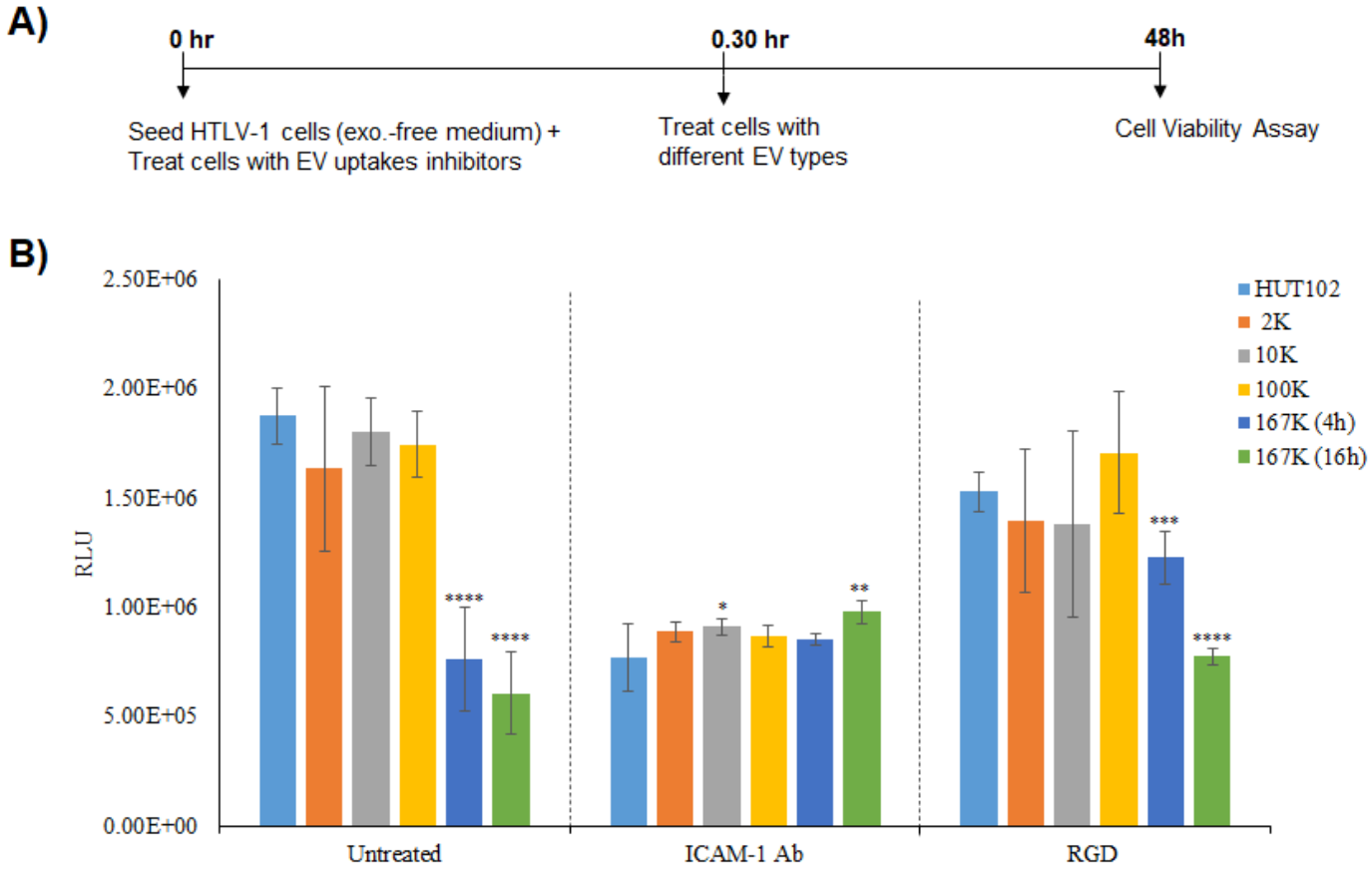
2K & 167K (16 hr) HTLV-1 EV uptake occurs before other EV subpopulations by the same cells



Inhibition of micropinocytosis attenuates 10K and 100K EV internalization and decrease cell viability in HUT102 cells

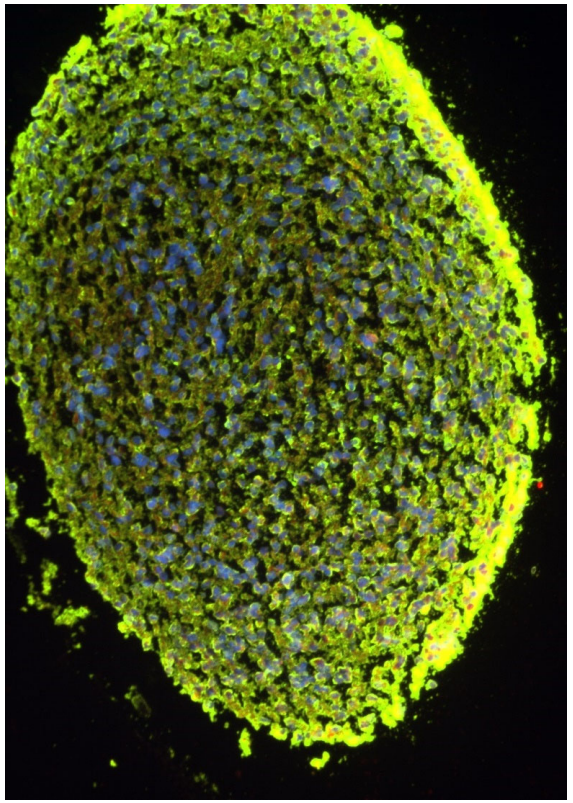


Inhibition of receptor-mediated endocytosis attenuates EV internalization and decrease cell viability in HUT102 cells



Overview

- Update on the most recent literature surrounding EVs from virally infected cells (“Damaging EVs”)
- Generation and infection of iPSC-derived neurospheres
- Effect of stem cell EVs (“Reparative EVs”) on HIV-1 infected neurospheres
- Summary



ATCC

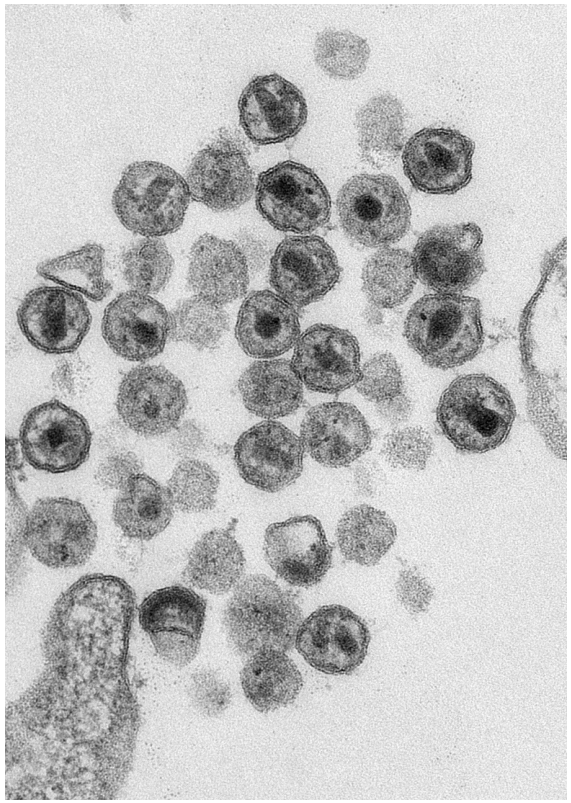
Background

Epidemiology

- HIV-associated neurocognitive disorders (HAND) are commonly associated with HIV-1 infection
- Despite combination antiretroviral therapy (cART) HAND persists in HIV-1 patients (>50%)

Significance

- There is an unmet need to develop novel platforms for CNS disease modeling and therapeutic intervention
- EVs from stem cells have demonstrated reparative properties in a wide range of pathologies, including those related to the CNS



HIV particles; Photo credit: M Metcalfe, T Hodge, CDC

REPORT

Zika virus impairs growth in human neurospheres and brain organoids

Patricia P. Garcez[2,1,*], Erick Correia Loiola[1,†], Rodrigo Madeiro da Costa[1,†], Luiza M. Higa[3,†], Pablo Trindade[1,†], Rodrigo Delvecchio[3], Juliana Minardi Nascimento[1,4], Rodrigo Brindeiro[3], Amilcar Tanuri[3], Stevens K. Rehen[1,2,*]

SCIENTIFIC REPORTS

OPEN

Zika virus disrupts molecular fingerprinting of human neurospheres

Received: 01 August 2016
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Published: 23 January 2017

Patricia P. Garcez[1,2], Juliana Minardi Nascimento[1,3], Janaina Mota de Vasconcelos[4], Rodrigo Madeiro da Costa[3], Rodrigo Delvecchio[3], Pablo Trindade[3], Erick Correia Loiola[1], Luiza M. Higa[3], Juliana S. Cassoli[3], Gabriela Vitória[1], Patricia C. Sequeira[5], Jaroslaw Sochacki[1], Renato S. Aguiar[5], Hellen Thais Fuzii[7], Ana M. Bispo de Filippis[6], João Lídio da Silva Gonçalves Vianez Júnior[6], Amilcar Tanuri[3], Daniel Martins-de-Souza[3] & Stevens K. Rehen[1,2]

D'Aiuto et al. Stem Cell Research & Therapy (2018) 9:134
https://doi.org/10.1186/s13287-018-0881-6

Stem Cell Research & Therapy

RESEARCH Open Access

Generation of three-dimensional human neuronal cultures: application to modeling CNS viral infections

Leonardo D'Aiuto[1*], Jennifer Naciri[1], Nicholas Radio[2], Sesha Tekur[2], Dennis Clayton[3], Gerard Apodaca[3], Roberto Di Maio[4], Yun Zhi[5], Peter Dimitrion[7], Paolo Piazza[6], Matthew Demers[1], Joel Wood[1], Charleen Chu[4,9,12], Jason Callio[12], Lora McClain[13], Robert Yolken[7], James McNulty[8], Paul Kinchington[9], David Bloom[10] and Vishwajit Nimgaonkar[1,11]

Neuroinvasive potential of SARS-CoV-2 revealed in a human brain organoid model

Eric Song, Ce Zhang, Benjamin Israelow, Peiwen Lu, Orr-El Weizman, Feimei Liu, Yile Dai, Klara Szigeti-Buck, Yuki Yasumoto, Guilin Wang, Christopher Castaldi, Jaime Heltke, Evelyn Ng, John Wheeler, Mia Madel Alfajaro, Benjamin Fontes, Neal G. Ravindra, David Van Dijk, Shrikant Mane, Murat Gunel, Aaron Ring, Craig B Wilen, Tamas L. Horvath, Angeliki Louvi, Shelli F. Farhadian, Kaya Bilguvar, Akiko Iwasaki

doi: https://doi.org/10.1101/2020.06.25.169946

ACS Chemical Neuroscience

pubs.acs.org/chemneuro Viewpoint

iPSCs-Derived Platform: A Feasible Tool for Probing the Neurotropism of SARS-CoV-2

Xiao-Yuan Mao* and Wei-Lin Jin*

THE EMBO JOURNAL

SARS-CoV-2 targets cortical neurons of 3D human brain organoids and shows neurodegeneration-like effects

Anand Ramani, Lisa Müller, Philipp Niklas Ostermann, Elke Gabriel, Pranty Abida-Islam, Andreas Müller-Schiffmann, Aruljothi Mariappan, Olivier Goureau, Henning Gruell, Andreas Walker, Marcel Andrée, Sandra Hauka, Torsten Houwaart, Alexander Dilthey, Kai Wohlgemuth, Heymut Omran, Florian Klein, Dagmar Wieczorek, Ortwin Adams, Jörg Timm, Carsten Korth, Heiner Schaal, Jay Gopalakrishnan

Modeling HIV-1 neuropathogenesis using three-dimensional human brain organoids (hBORGs) with HIV-1 infected microglia

Roberta S. dos Reis[1], Shilpa Sant[2,3✉], Hannah Keeney[3], Marc C. E. Wagner[1] & Velpandi Ayyavoo[1✉]

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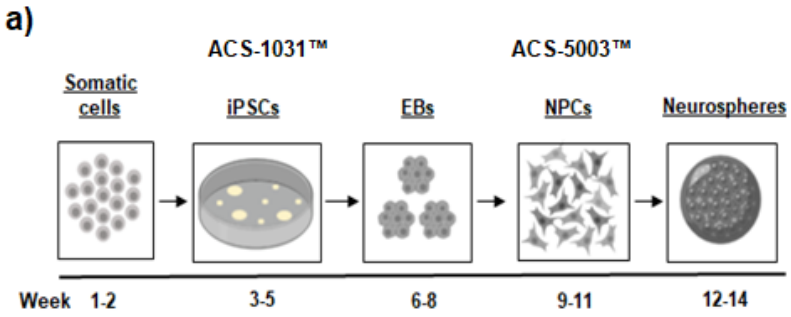
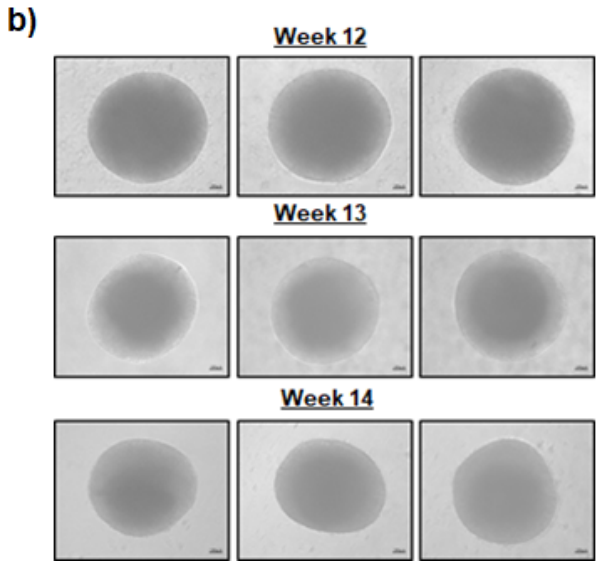
Retroviral Infection of Human Neurospheres and Use of Stem Cell EVs to Repair Cellular Damage

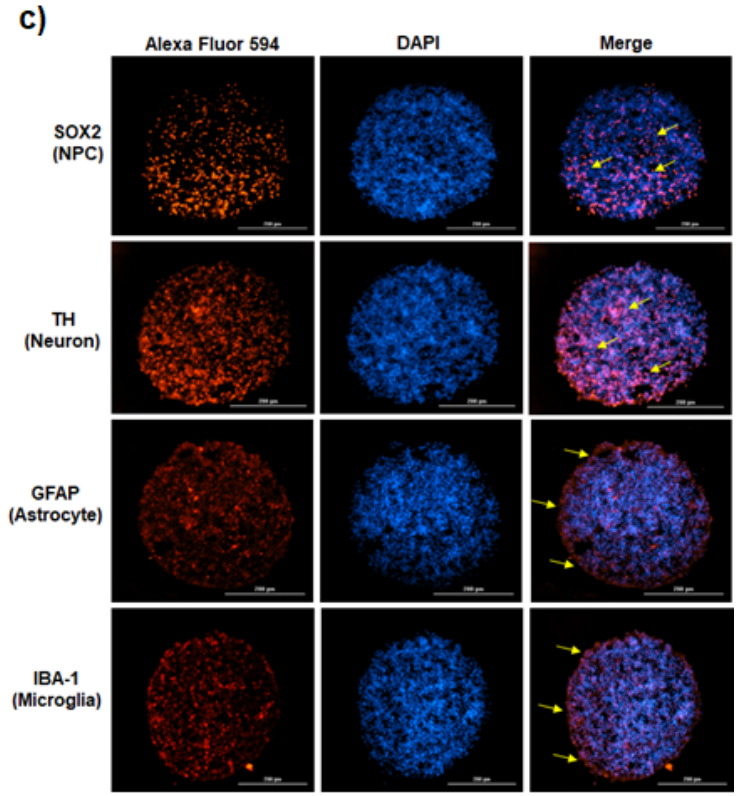
Heather Branscome, Dezhong Yin, Sheela Jacob, Maria Cowen, Yuriy Kim, Pooja Khatkar, James Erickson, Nazira El-Hage, Lance A. Liotta, Fatah Kashanchi

doi: https://doi.org/10.1101/2020.12.31.424849

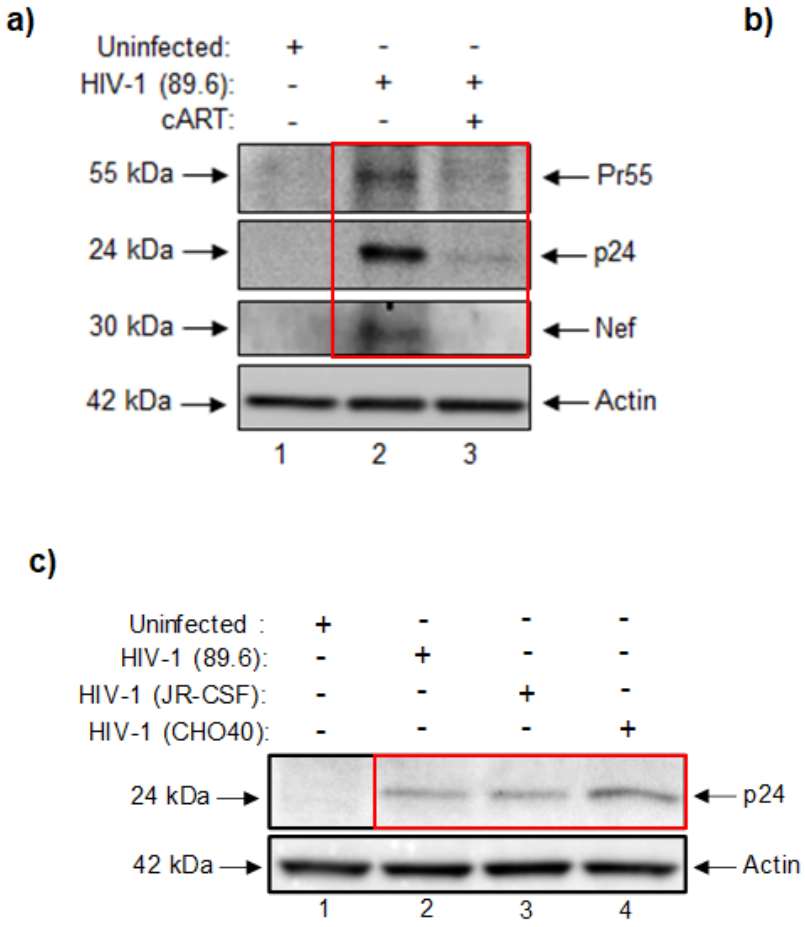
Generation of neurospheres



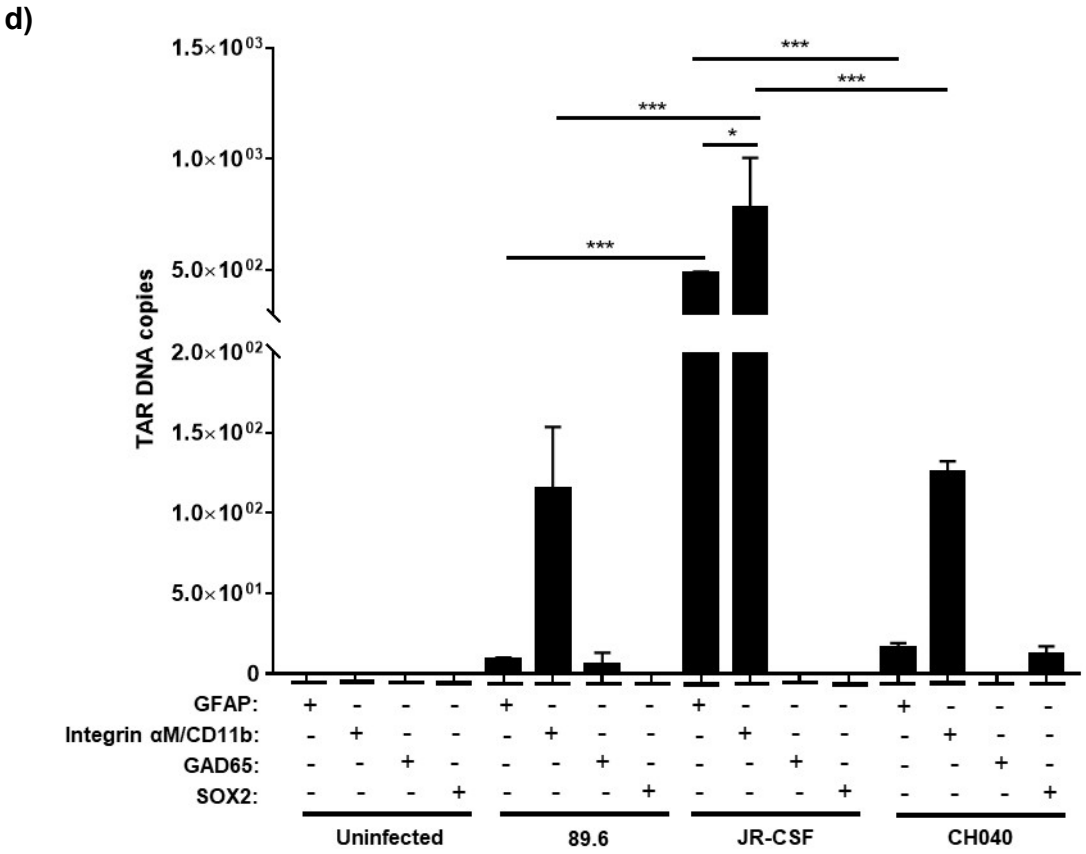




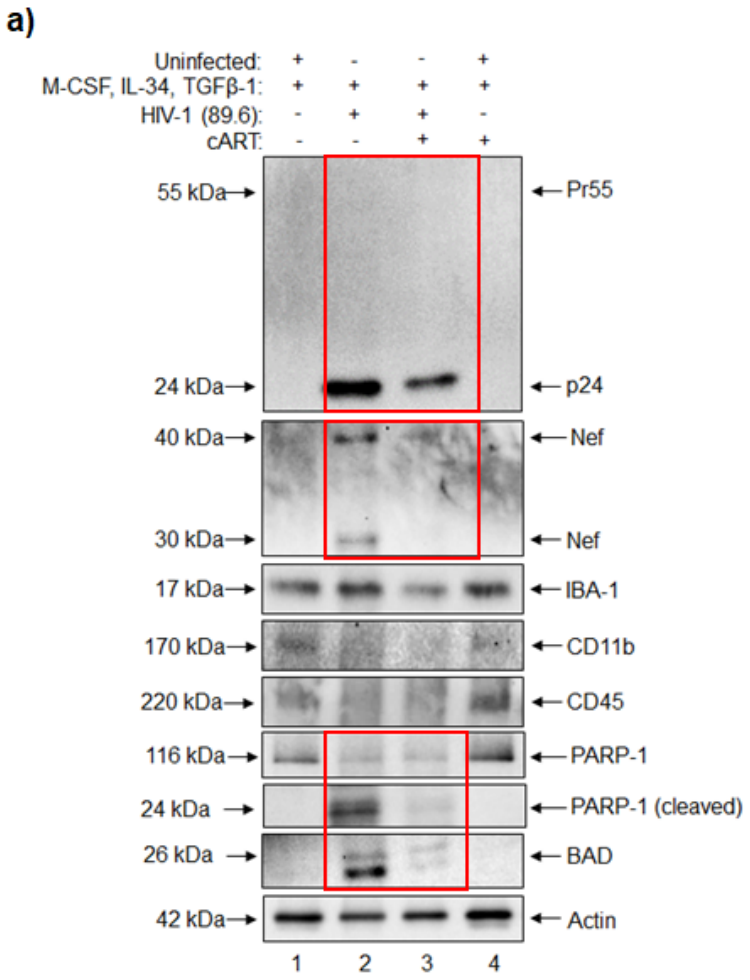
Infection of neurospheres

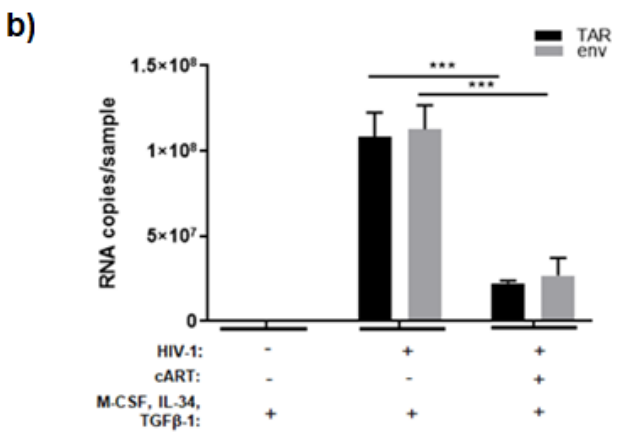


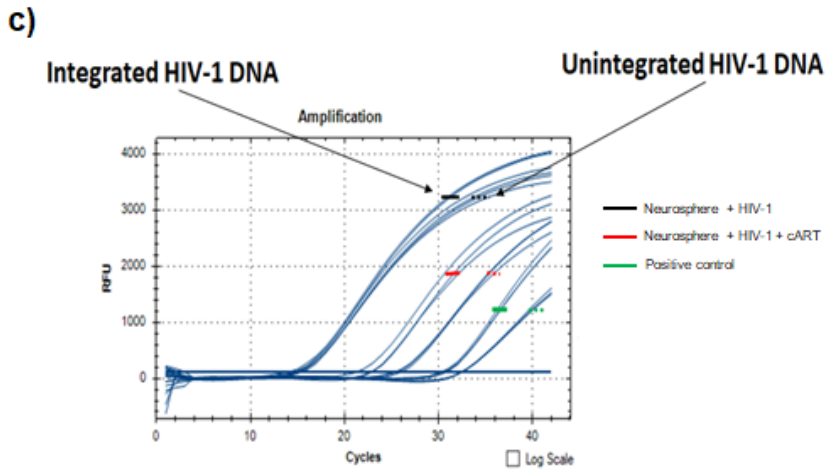
HIV-1 tropism



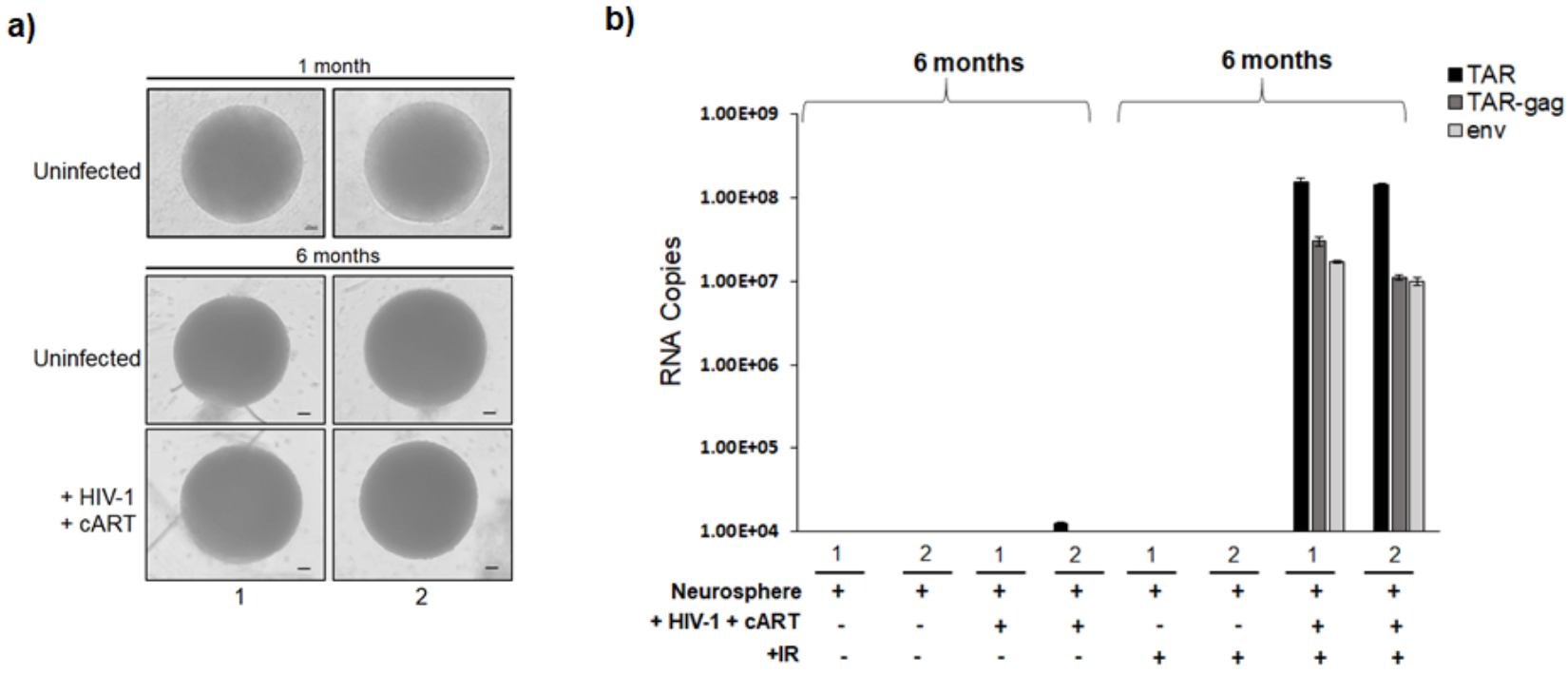
Infection of neurospheres (+ cytokines)



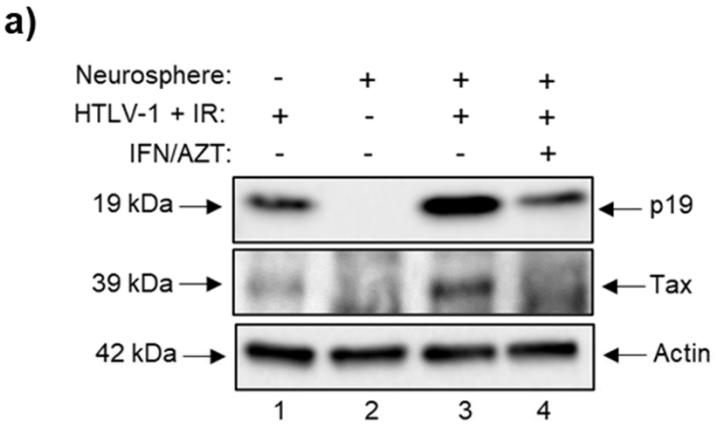


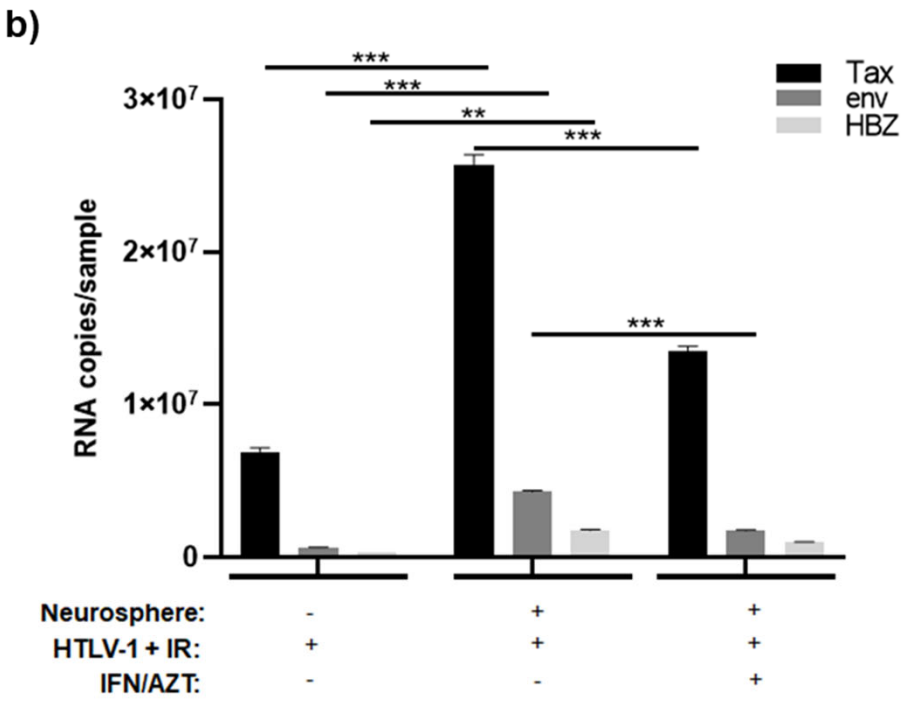


Infection of neurospheres (long-term)



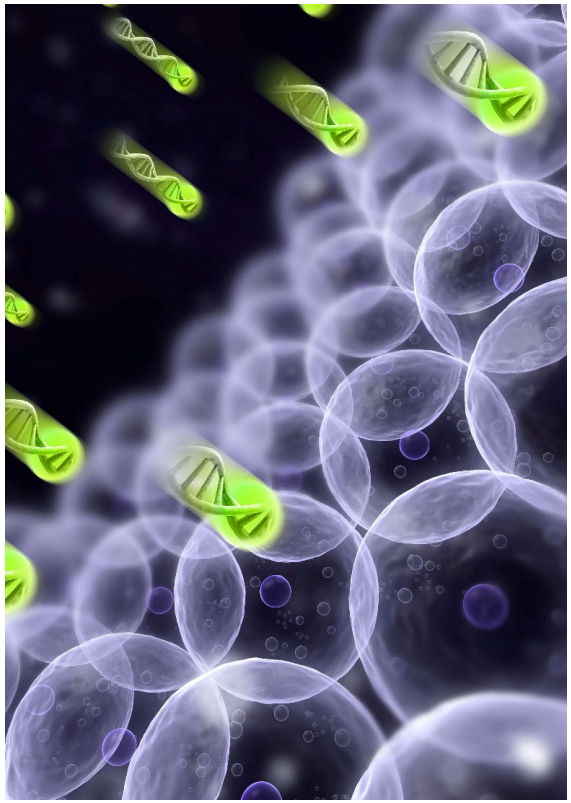
Infection of neurospheres (HTLV-1)





Stem cell EVs

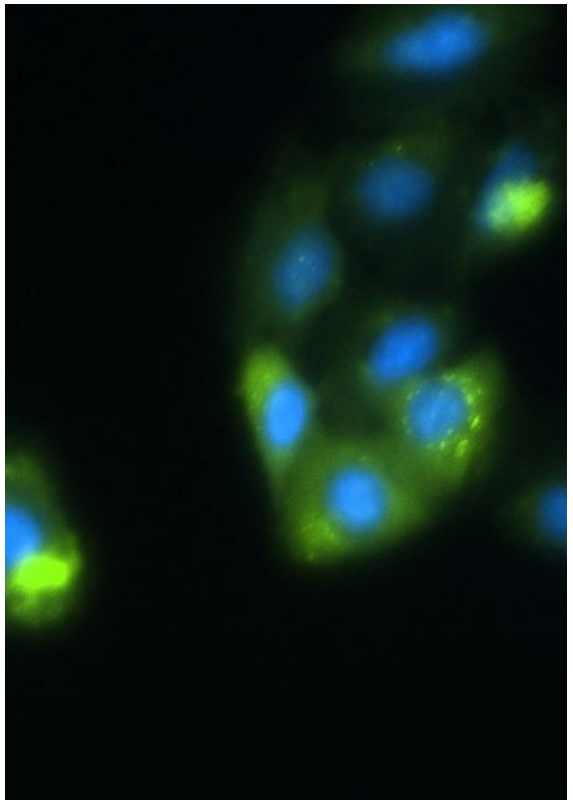
- Contain various biological cargo (miRNAs, lncRNAs, proteins, cytokines) that can be transferred to recipient cells
- **Proposed to play a role in homeostasis through tissue repair, regeneration, and immunomodulation**
- Potential alternative to stem cell therapy due to higher potency, increased stability/shelf life, and lower immunogenicity
- Widely studied for reparative purposes (e.g., skin/wound healing, cardiac repair, **CNS-related pathologies**)



ATCC

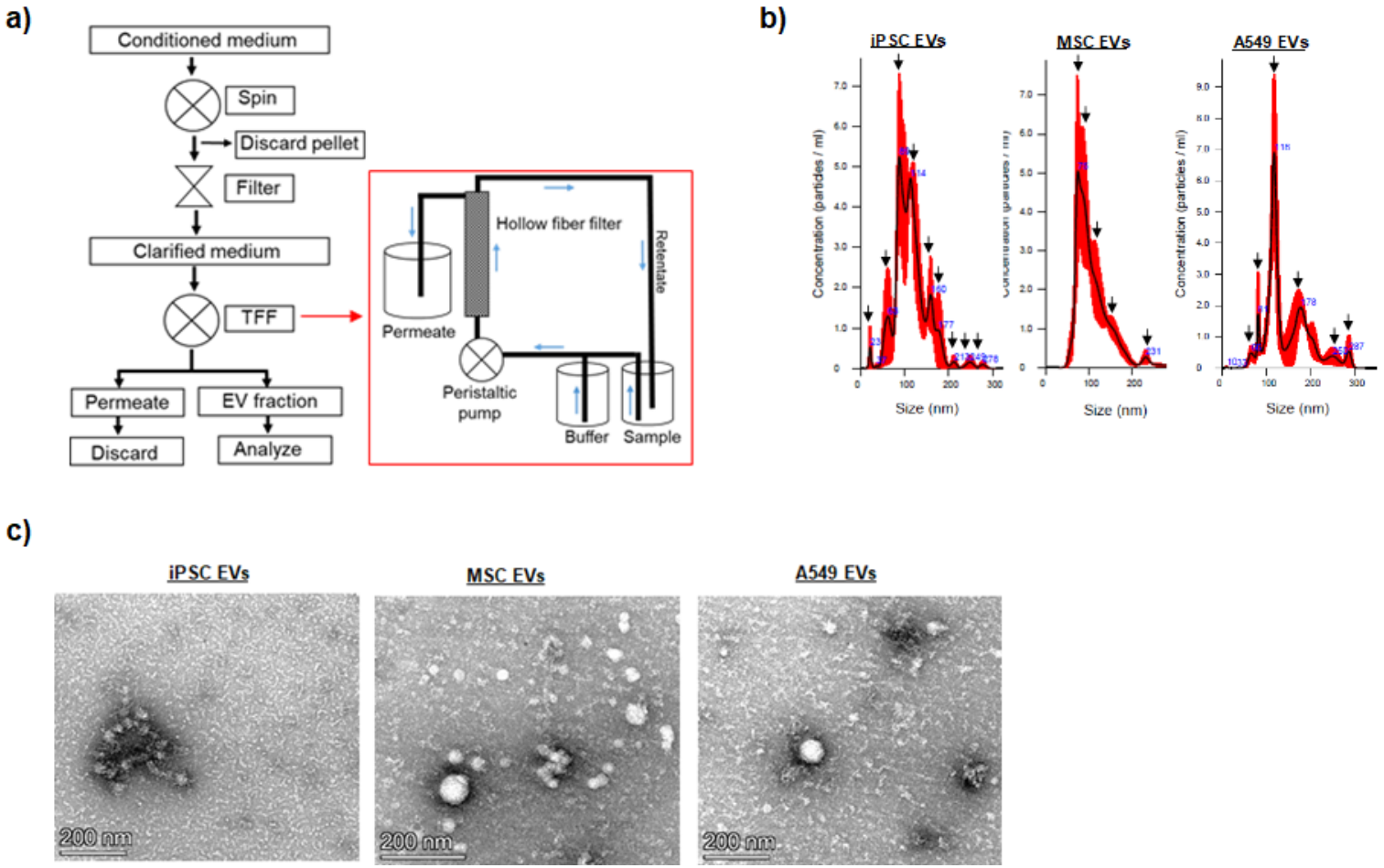
EVs and donor cells

- Mesenchymal Stem Cells (MSCs; ATCC® PCS-500-012™)
  - Human, normal
  - Bone-marrow derived
  - Authenticated for characteristic surface marker expression (CD90, CD73, CD105 positive; CD14, CD34, CD45 negative)
  - Multi-lineage differentiation potential (adipocyte, chondrocyte, osteocyte)
  - Induced Pluripotent Stem Cells (iPSCs; ATCC® ACS-1019™)
- Human, normal
  - Foreskin fibroblast-derived
  - Sendai virus reprogrammed
  - Authenticated for expression of stem cell markers (TRA-1-60, SSEA-4 positive; SSEA-1 negative)
  - Evaluated for pluripotency
- A549 Lung Carcinoma (ATCC® CCL-185™)
  - Control used for large-scale manufacturing and isolation
  - Equivalent to CCL-185-EXM™ exosomes (also: ATCC® SCRC-4000-EXM™, CRL-1435-EXM™, CCL-247-EXM™)

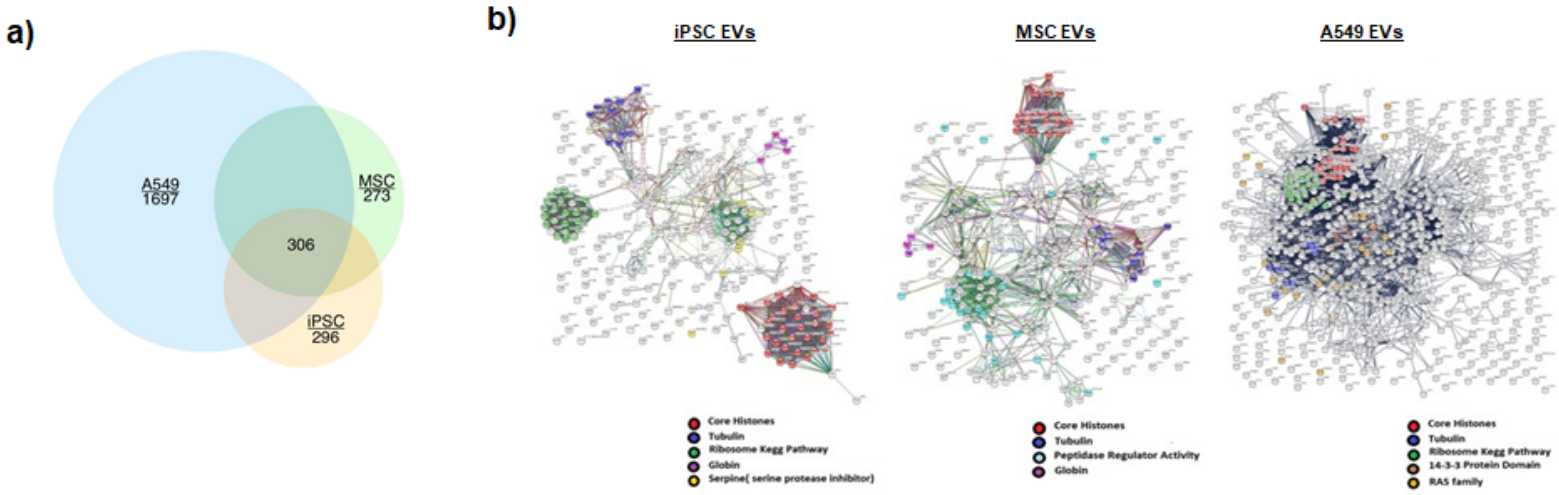


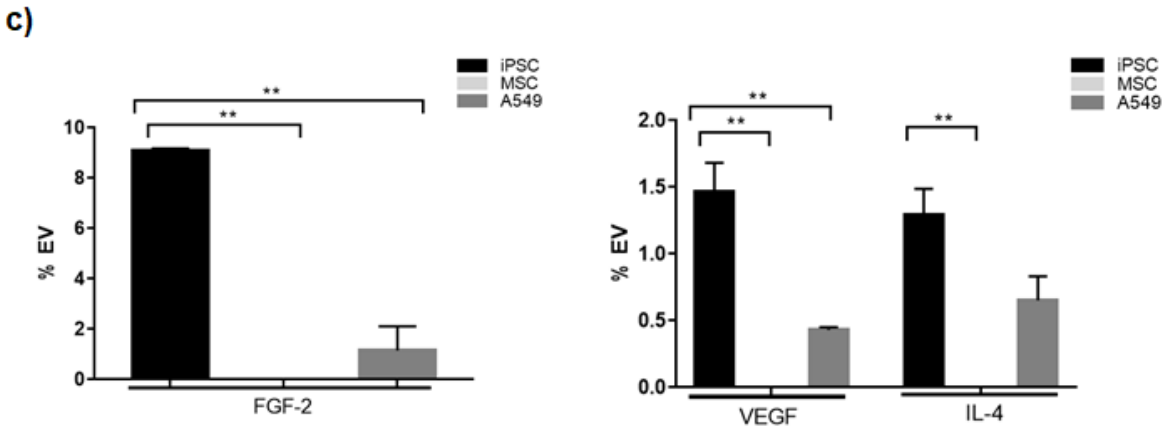
EVs synthesized in A549 cells

EV isolation and characterization

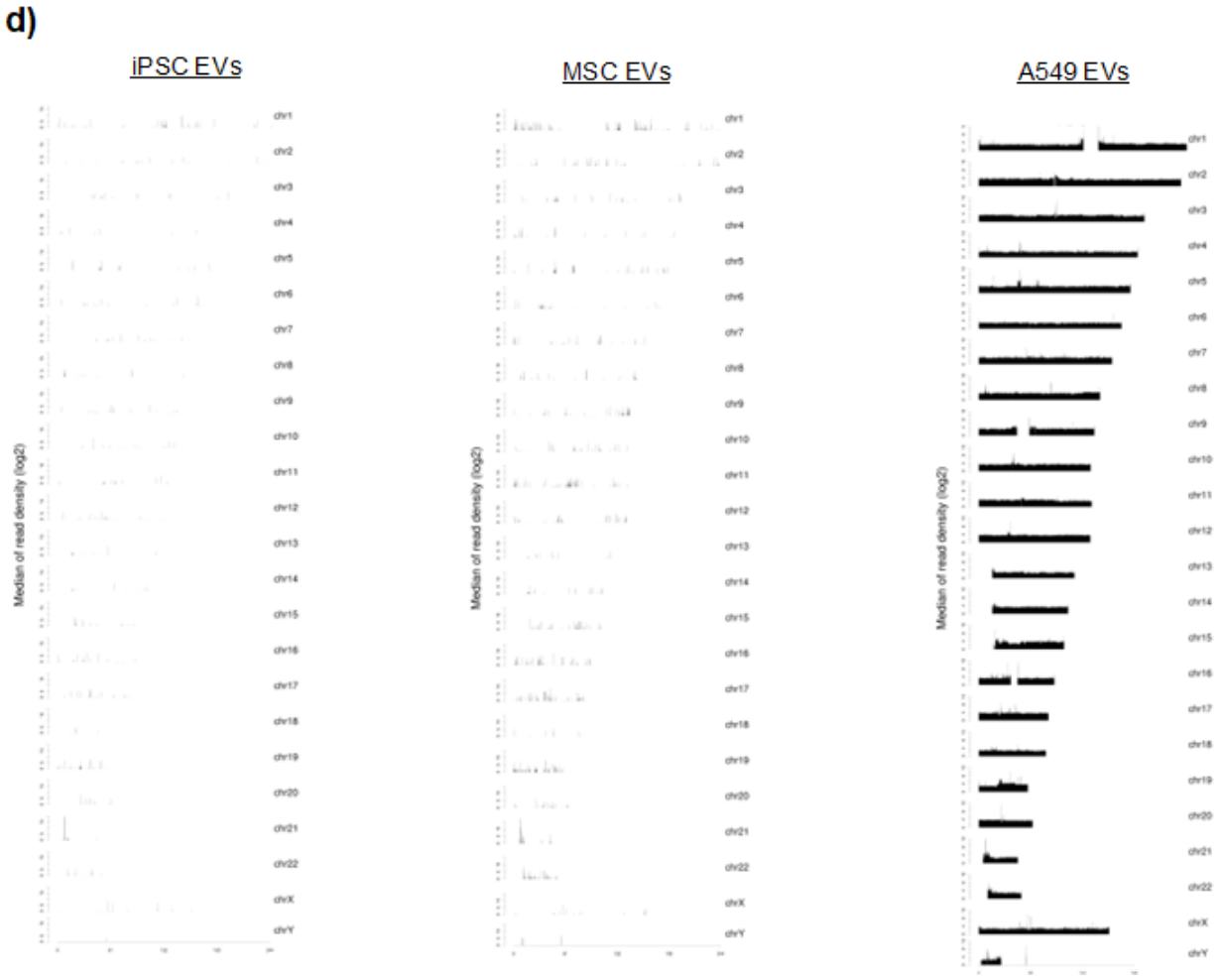


EV characterization

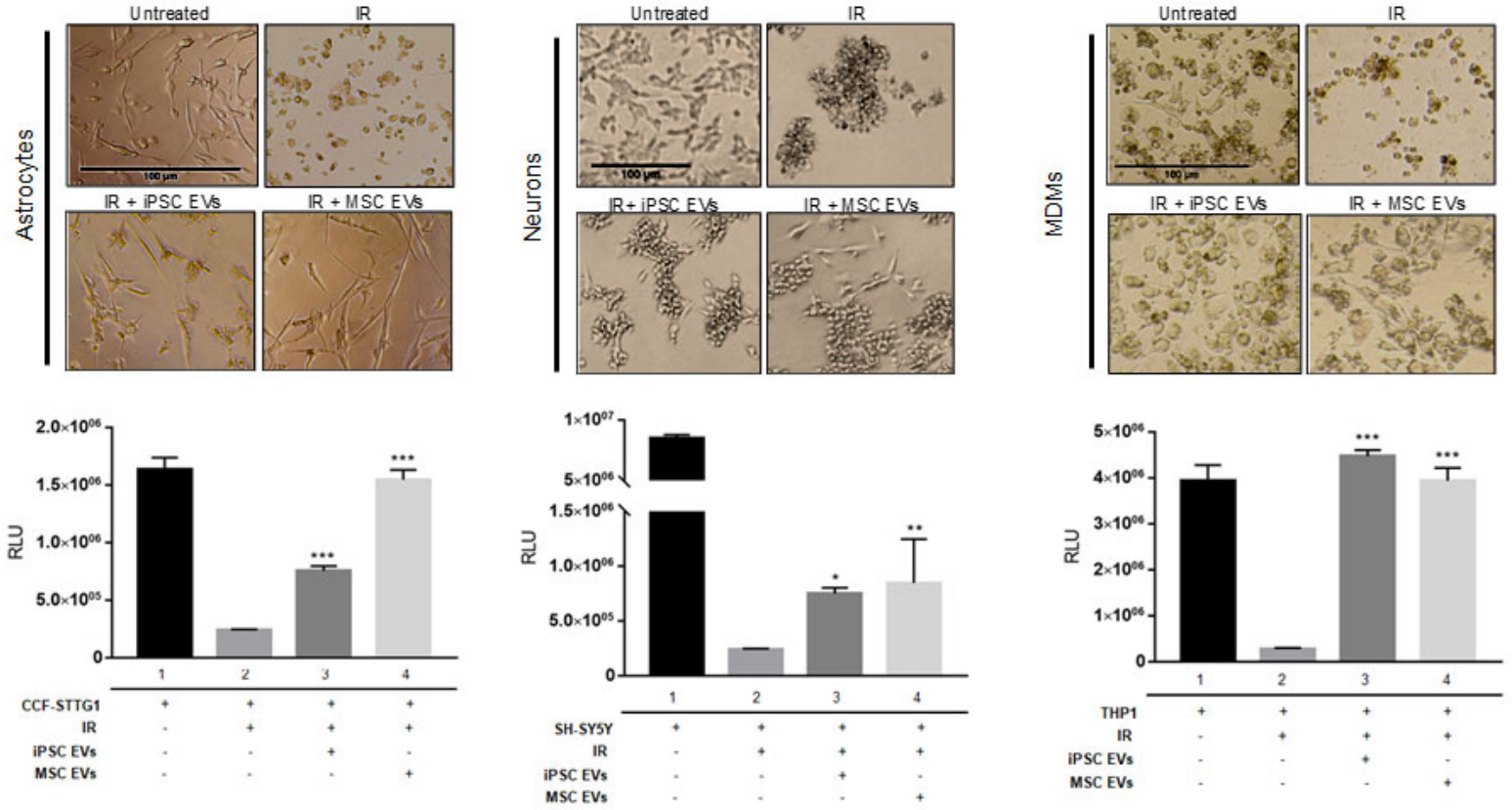




EV characterization



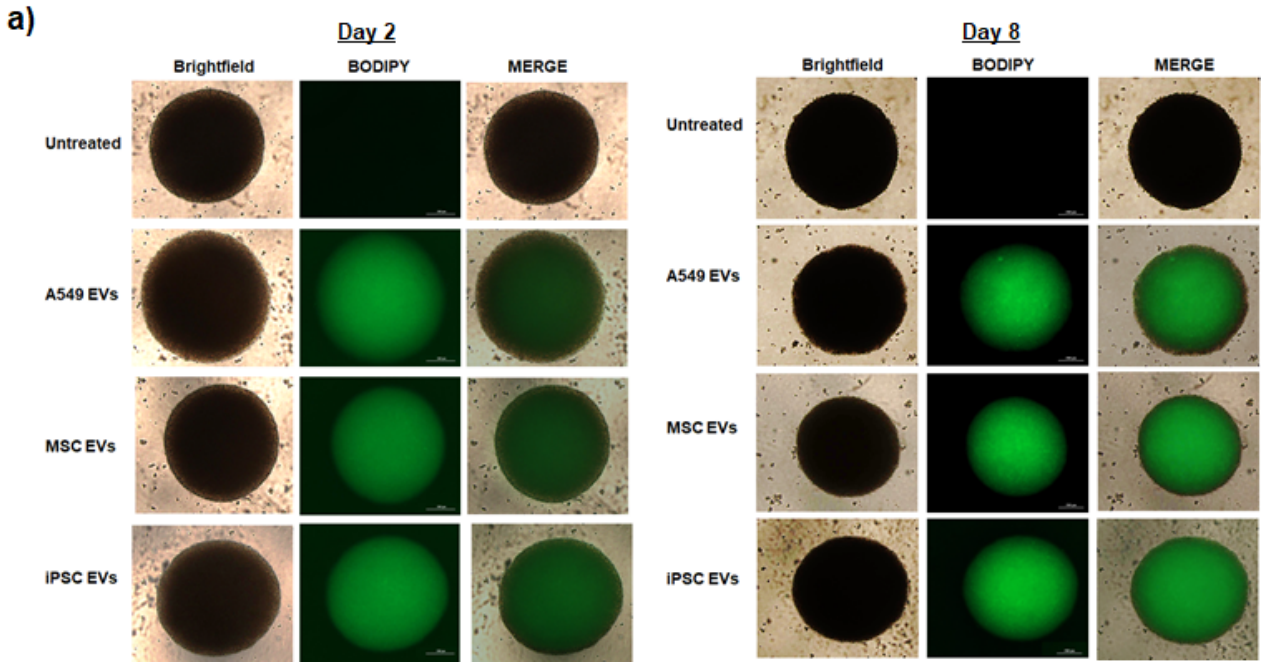
EV function

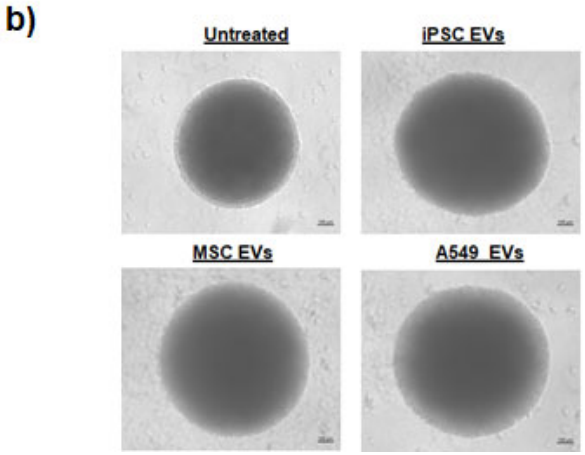


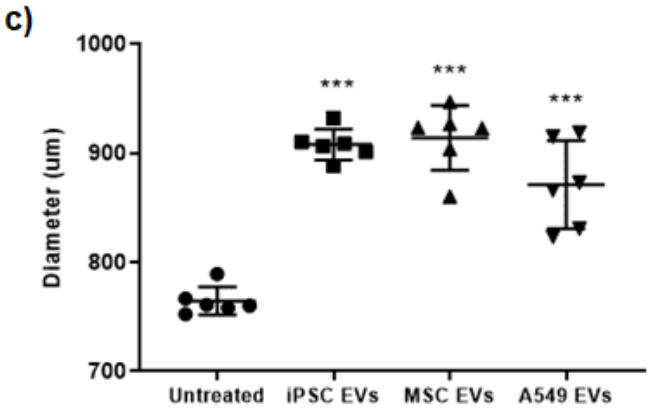
Stem Cell Extracellular Vesicles and Their Potential to Contribute to the Repair of Damaged CNS Cells

Heather Branscome[1,2], Siddhartha Paul[1], Pooja Khatkar[1], Yurly Kim[1], Robert A. Barclay[1], Daniel O. Pinto[1], Dezhong Yin[3], Weidong Zhou[4], Lance A. Liotta[4], Nazira El-Hage[5], Fatah Kashanchi[1,*]

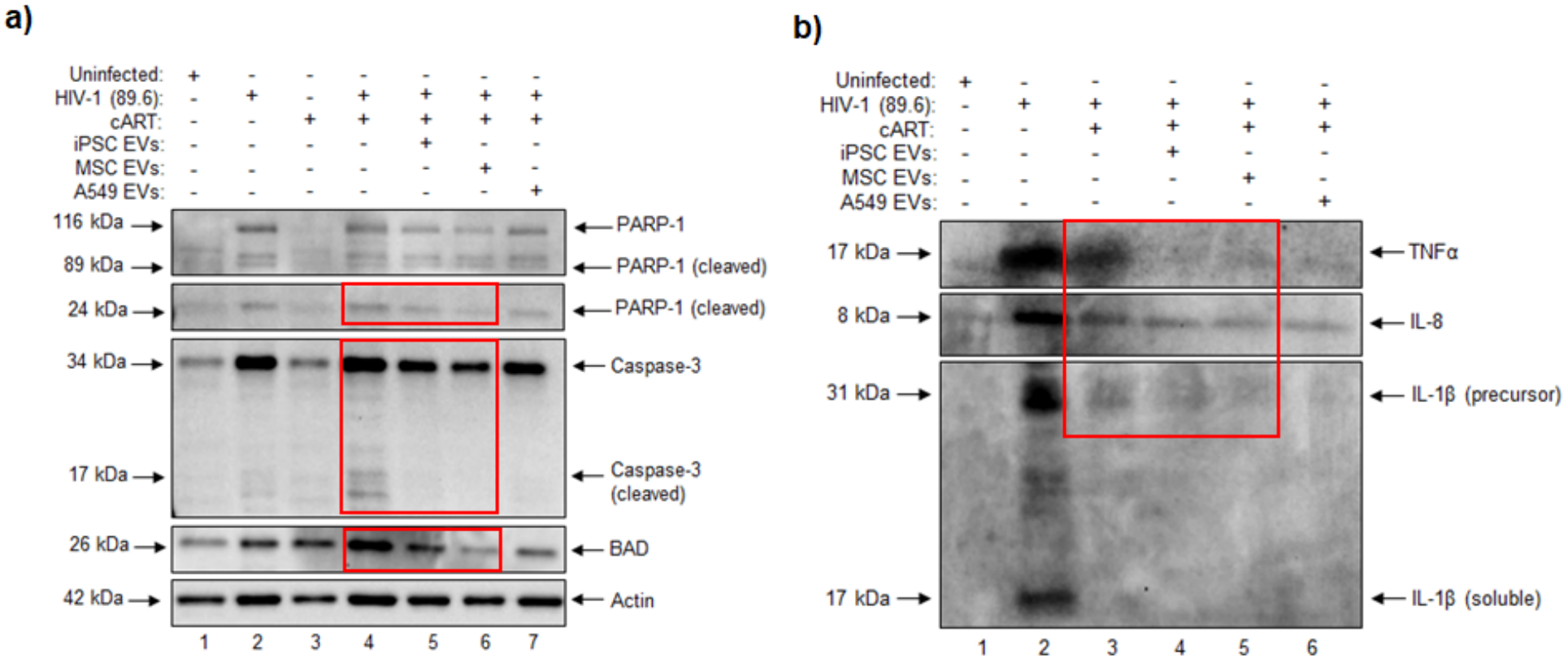
EV function


a)
Day 2
Brightfield
BODIPY
MERGE
Untreated
A549 EVs
MSC EVs
iPSC EVs
Day 8
Brightfield
BODIPY
MERGE
Untreated
A549 EVs
MSC EVs
iPSC EVs

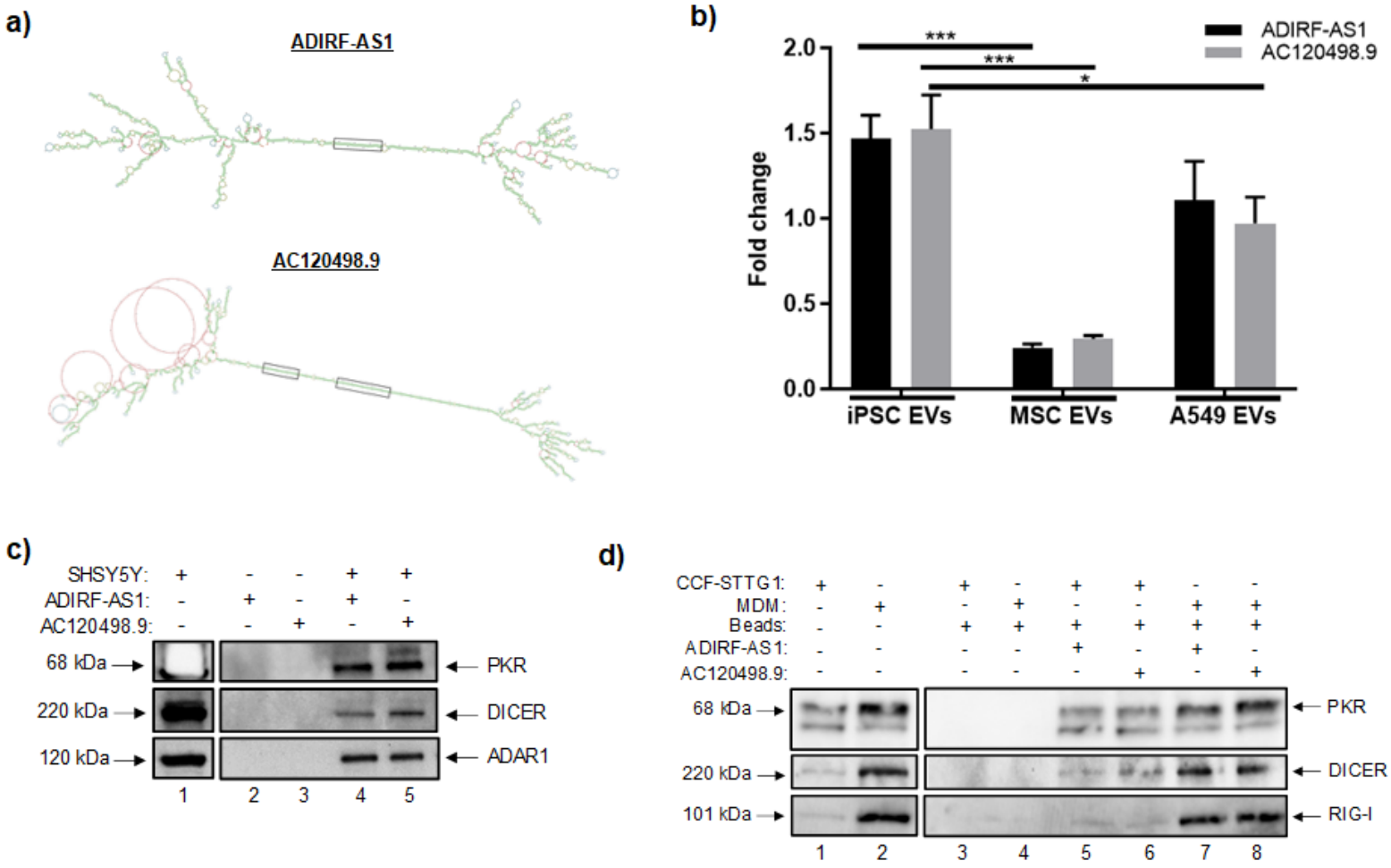

b)
Untreated
iPSC EVs
MSC EVs
A549 EVs


c)
Diameter (um)
1000
900
800
700
***
***
***
Untreated
iPSC EVs
MSC EVs
A549 EVs

EV function


a)
Uninfected:
HIV-1 (89.6):
cART:
iPSC EVs:
MSC EVs:
A549 EVs:
116 kDa
89 kDa
24 kDa
34 kDa
17 kDa
26 kDa
42 kDa
PARP-1
PARP-1 (cleaved)
PARP-1 (cleaved)
Caspase-3
Caspase-3 (cleaved)
BAD
Actin
1 2 3 4 5 6 7
b)
Uninfected:
HIV-1 (89.6):
cART:
iPSC EVs:
MSC EVs:
A549 EVs:
17 kDa
8 kDa
31 kDa
17 kDa
TNFα
IL-8
IL-1β (precursor)
IL-1β (soluble)
1 2 3 4 5 6

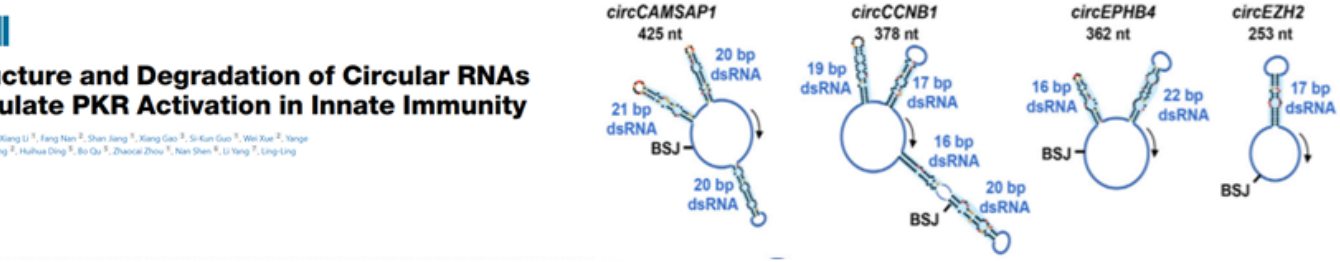
EV function


a)
ADIRF-AS1
AC120498.9
b)
ADIRF-AS1
AC120498.9
***
***
*
Fold change
2.0
1.5
1.0
0.5
0.0
iPSC EVs
MSC EVs
A549 EVs
c)
SHSY5Y:
ADIRF-AS1:
AC120498.9:
68 kDa
220 kDa
120 kDa
PKR
DICER
ADAR1
1 2 3 4 5
d)
CCF-STTG1:
MDM:
Beads:
ADIRF-AS1:
AC120498.9:
68 kDa
220 kDa
101 kDa
PKR
DICER
RIG-I
1 2 3 4 5 6 7 8

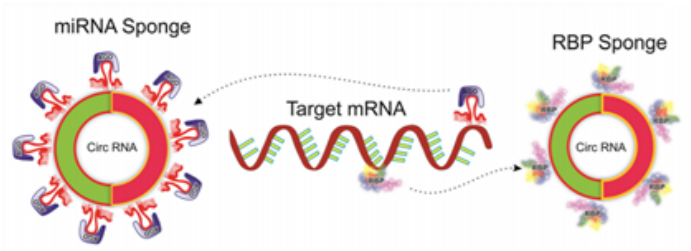
Future directions

- Further assessment of potential functional effects of EV-associated lncRNAs

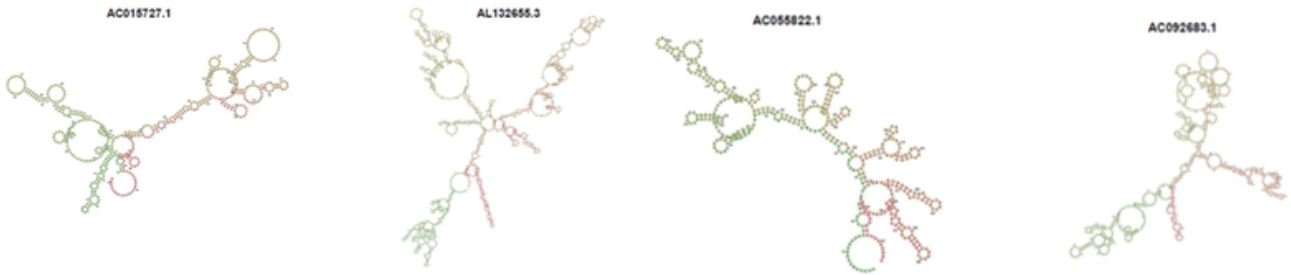
A)



B)

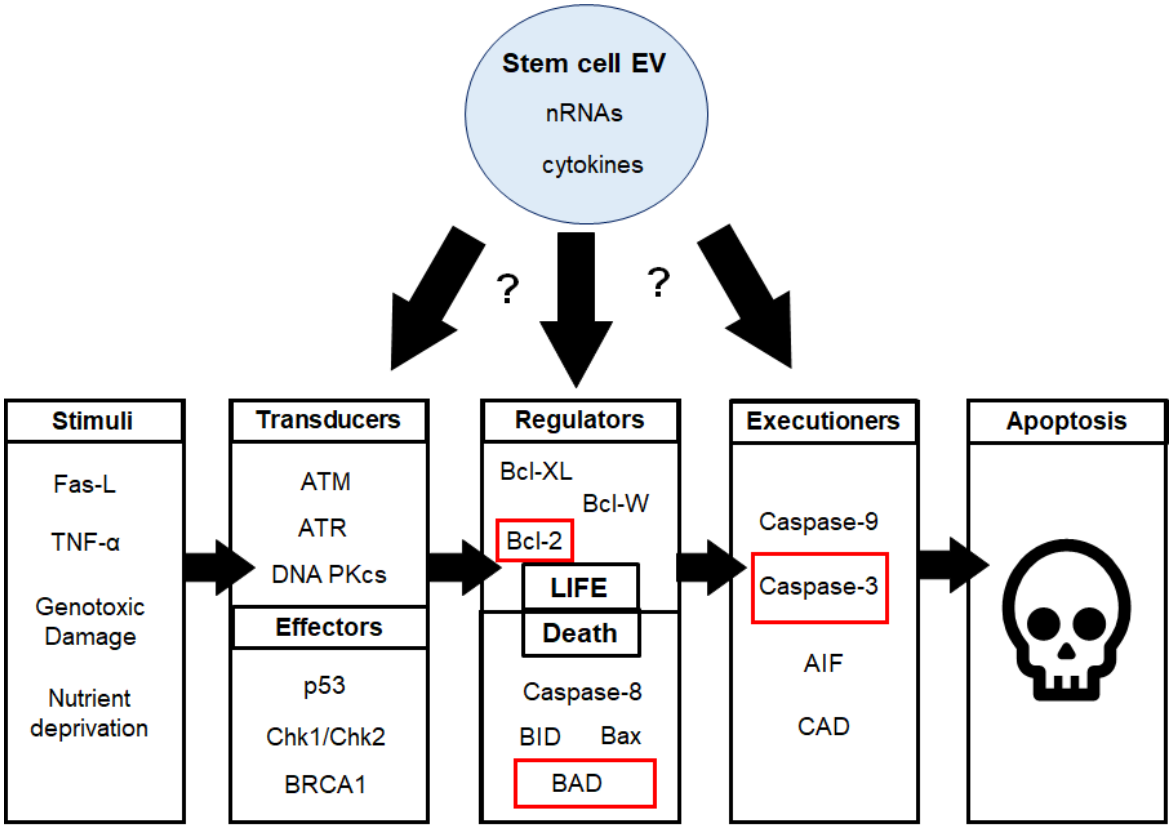


C)



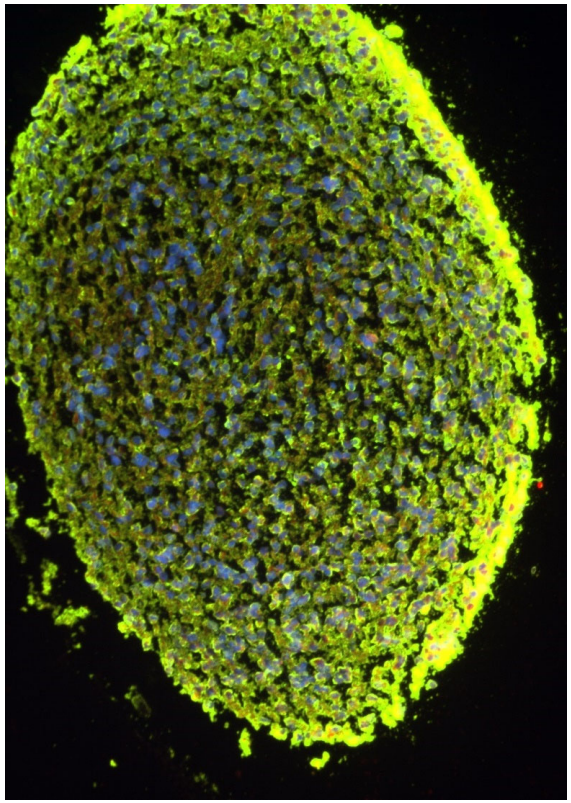
Future directions

- Better definitions of death and mechanisms of repair



Overview

- Update on the most recent literature surrounding EVs from virally infected cells (“Damaging EVs”)
- Generation and infection of iPSC-derived neurospheres
- Effect of stem cell EVs (“Reparative EVs”) on HIV-1 infected neurospheres
- Summary

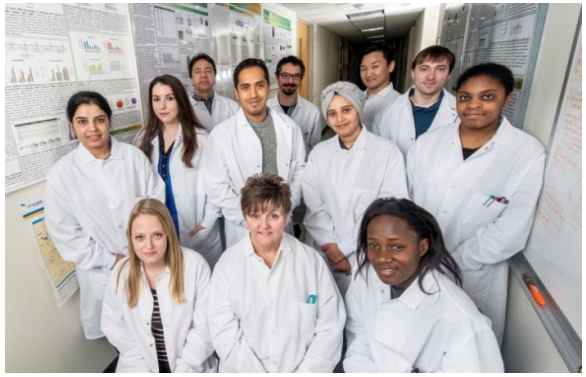


Summary

- HIV-1 TAR as non-coding RNA in EVs can be found in cell lines and patient samples.
- EVs are released first prior to viral release to potentially prime the neighboring uninfected cells.
- Early EV samples (i.e., 6 hours) contain p24, Nef, gp120 proteins, non-coding RNA, or coding RNAs; however, they are not infectious virions.
- The majority of the particles released (i.e., 24 hours) from infected activated cells are not infectious virions.
- NPC-derived 3-D neurospheres can be generated in a reproducible manner and can differentiate mature CNS cell types.
- 3-D neurospheres are permissive to retroviral infection.
- High yields of stem cell EVs can be recovered using advanced filtration methods.
- Stem cell EVs can exert functional effects in CNS-related cells in both 2-D and 3-D cultures.

ATCC

Acknowledgements



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- V. Planelles
- Lena Al-Harthi
- Kevin Morris

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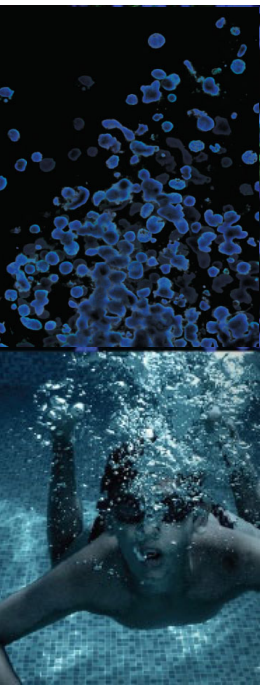
THANK YOU



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