Viral pathogenesis in the era of COVID

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GEORGIA
RESEARCH
ALLIANCE

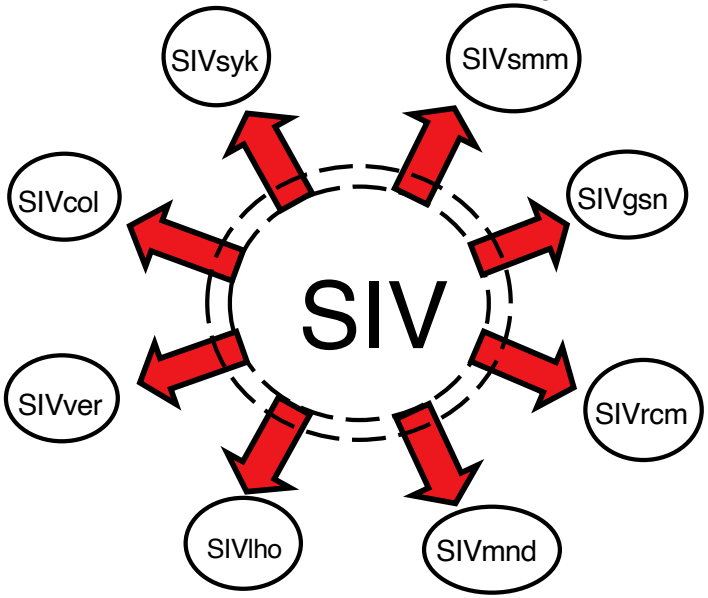
Origins of HIV

Mantled guereza

Vervet monkey

Sykes monkey

Sooty mangabey



L'Hoest's monkey

Mandrill

Greater spot-nosed monkey

Red-capped mangabey

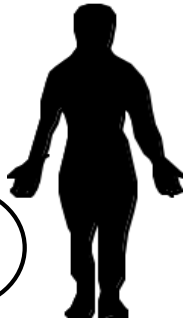
SIVmac

HIV-2

SIVcpz

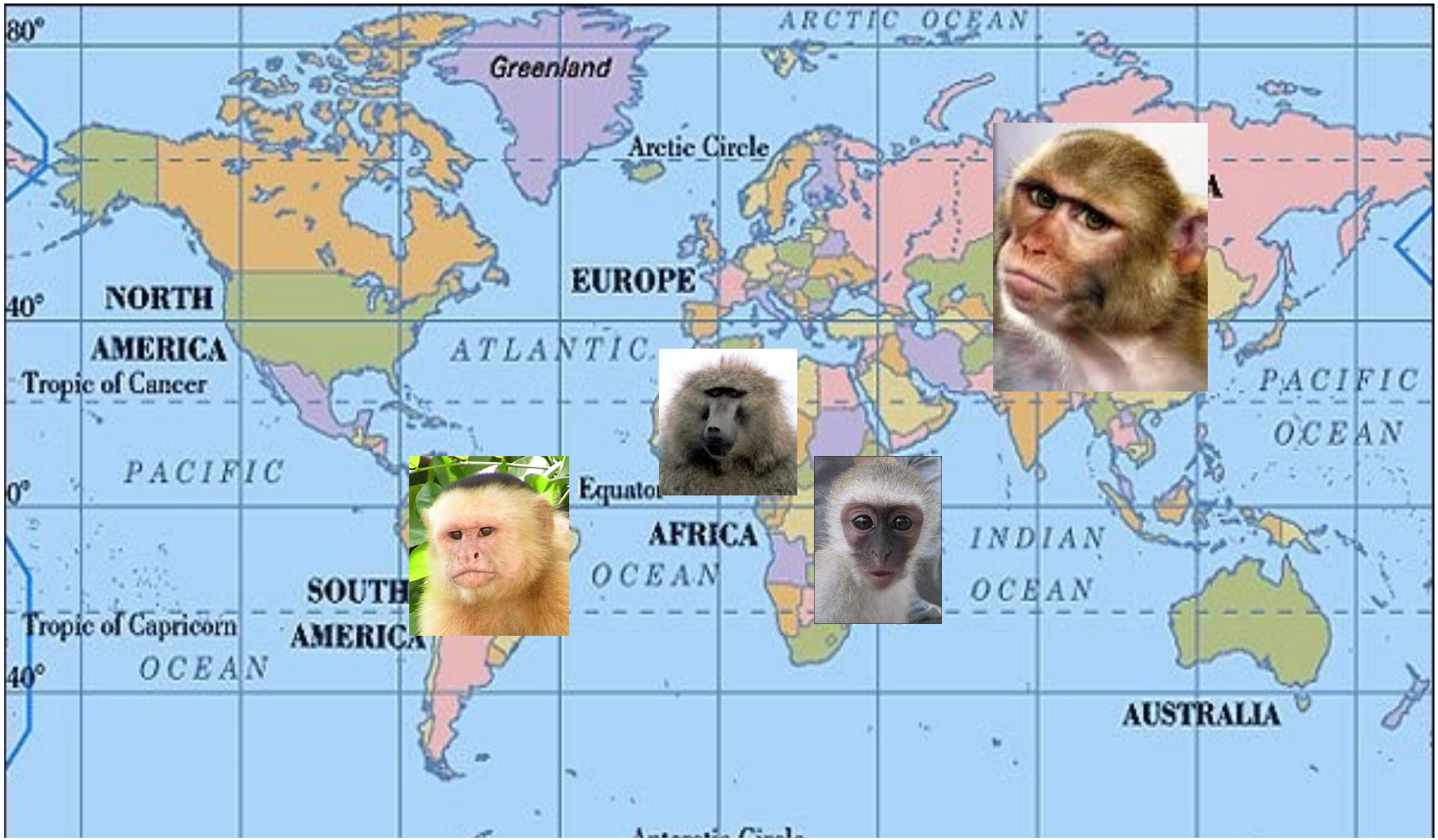
Chimpanzee

HIV-1

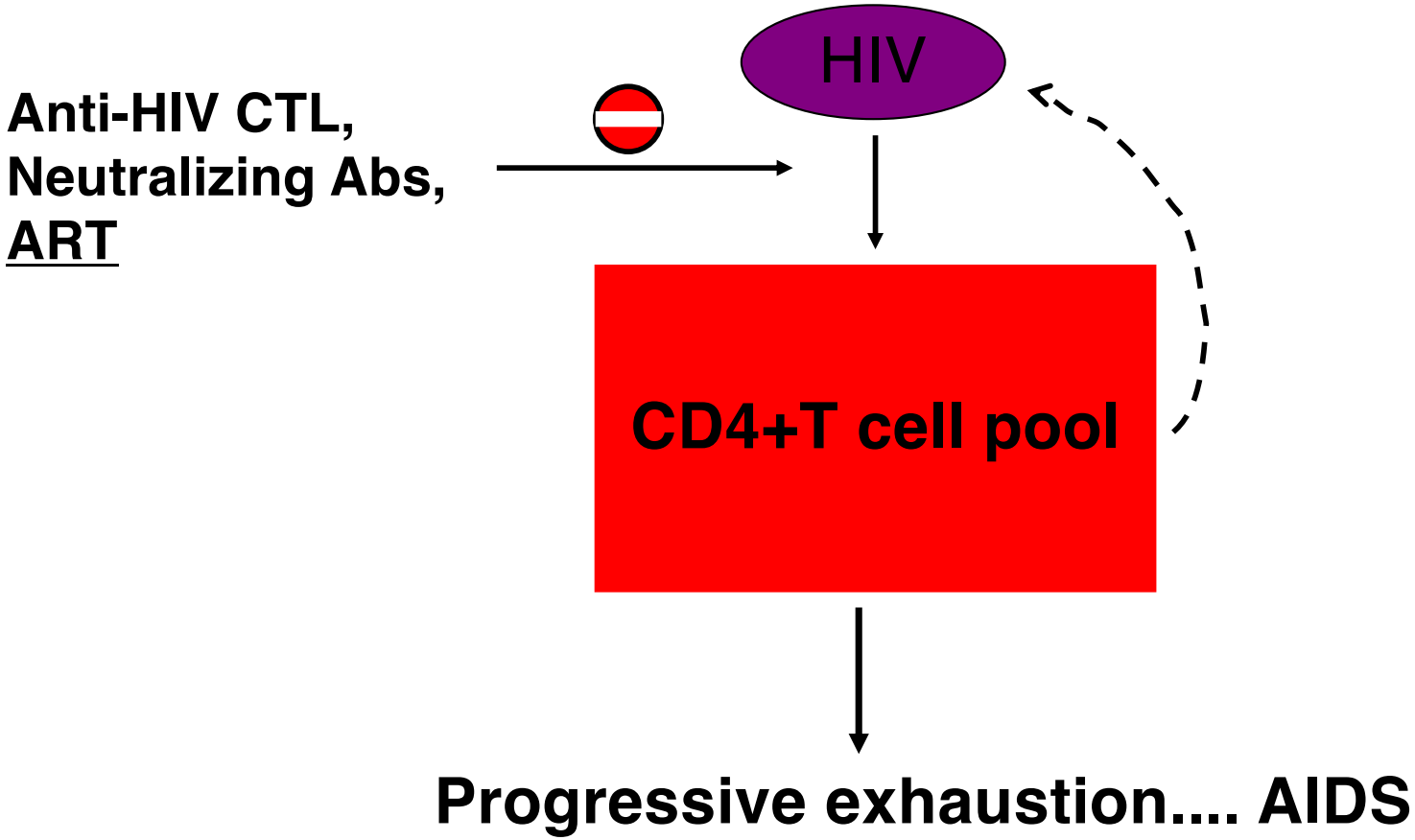


Slide courtesy of Beatrice Hahn

Monkey models:



The "Classical" Model of AIDS Pathogenesis



In support of the classical model:

- **HIV infects and kills CD4+ T cells.**
- **CD4+ T cells decline progressively during infection and the level of CD4+ T cells predicts the risk of developing AIDS.**
- **Suppression of HIV replication by antiretroviral therapy (ART) is followed by increase of CD4+ T cells.**

AIDS is more than an HIV-mediated loss of CD4+ T cells;

- Similar rate of cell death for CD4+ and CD8+ T cells
- Most of the CD4+ T cells that die during chronic HIV infection are NOT directly infected by the virus
- Chronic, generalized immune activation results in functional defects and increased apoptotic cell death of uninfected T cells (both CD4+ and CD8+)

Comparative AIDS Research:

SM
&
AGM

SIVmac RMs
SIVmac PTMs

SIVmac Chinese RMs
SIVsmE660 RMs
SHIVsf162p3

SIVcpz chimps

HIV-1 humans

SIVmac
RM EC

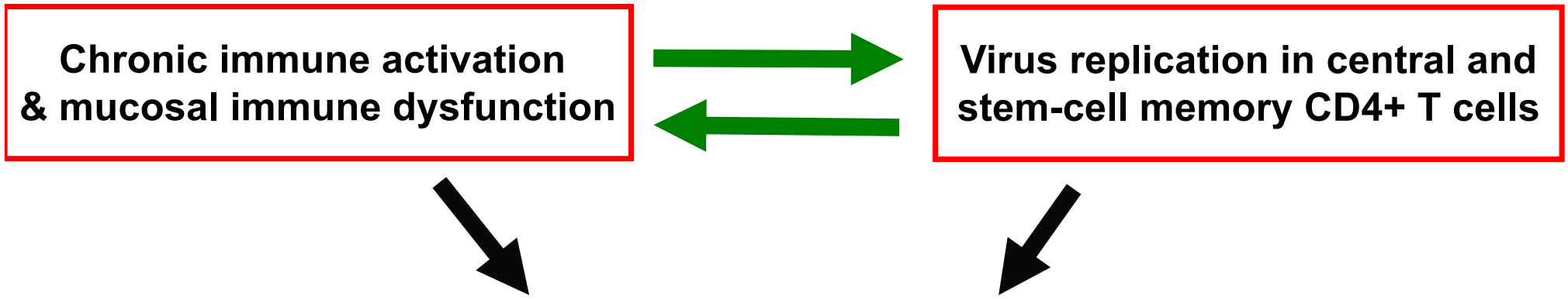
HIV-2 humans

HIV-1 humans
LTNP/EC

Virus replication

Pathogenicity

How HIV and SIV infection cause AIDS: Lessons from natural SIV infections



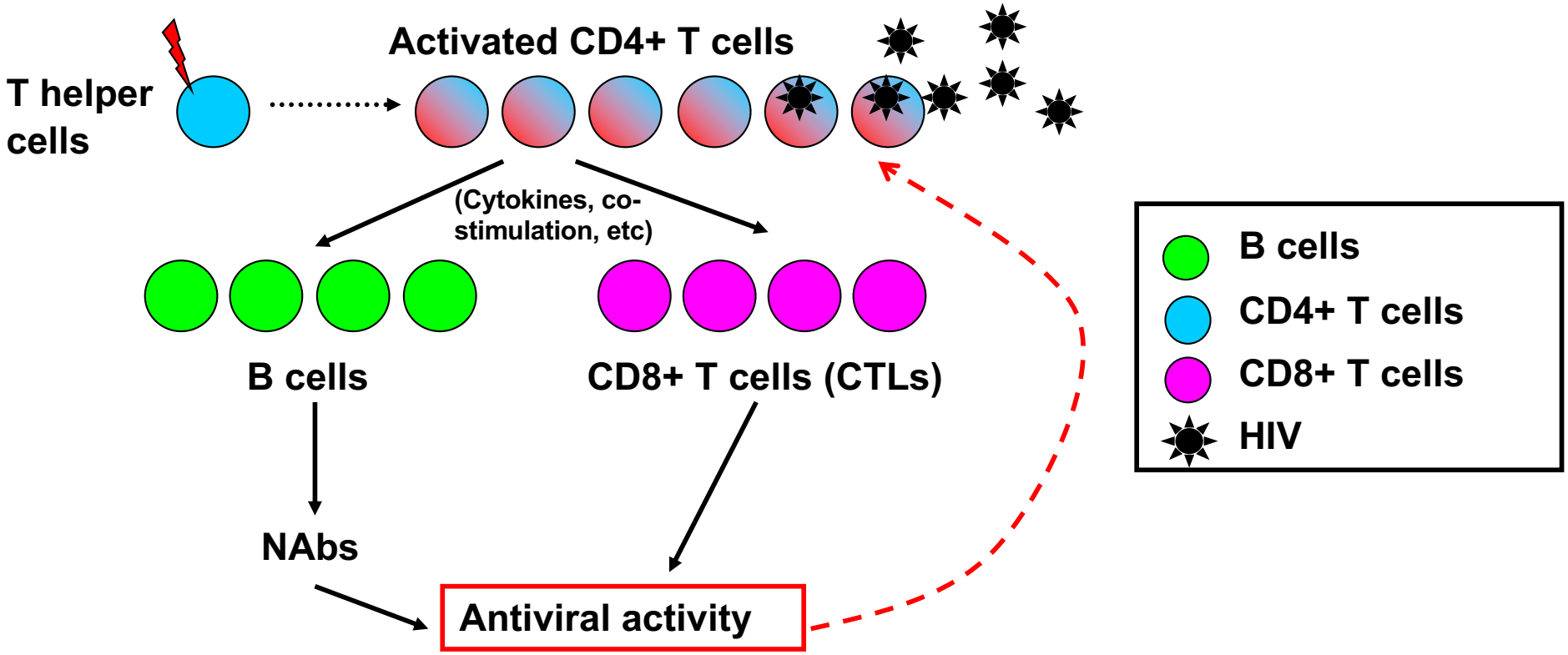
Silvestri et al. Immunity 2003
Dunham et al. Blood 2006
Brenchley et al. Nat Med 2006
Klatt et al. J Clin Invest 2008
Bosinger et al. J Clin Invest 2009
Brenchley et al., Immunity 2010
Reddick et al., PLoS Path 2011
Zhang et al., J Clin Invest 2011
Delmau et al., J Clin Invest 2011
Bosinger et al. PLoS Path 2013
Klatt et al. PLoS Path 2014
Mir et al. J Virol 2015
Palesch et al. Nature 2018
Joas et al. Cell Reports 2020

Garber et al. J Clin Invest 2004
Schindler et al. Cell 2006
Silvestri et al., J Clin Invest 2007
Sodora et al. Nat Med 2009
Keele et al. Nature 2009
Paiardini et al. Nat Med 2011
Ortiz et al. J Clin Invest 2011
Milush et al. J Clin Invest 2011
Chahroudi et al. Science 2012
Chahroudi et al. PLoS Path 2014
Cartwright et al., J Immunol 2015
Chahroudi et al., Science Transl Med 2016
Wetzel et al. PLoS Path 2019
Lee et al. PLoS Path 2021

MTCT in natural SIV hosts

- **MTCT is rare in all studied species of natural SIV hosts (Pandrea, J Virol 2008; Chahroudi, J Virol 2011).**
- **When infected, either naturally or experimentally, newborns of SMs and AGMs show low or undetectable viremia (Chahroudi, J Virol 2011; Brenchley, Blood 2012).**
- **Virus replication does not appear to be controlled by immune mechanisms, but rather by paucity of CD4+CCR5+ target cells (Chahroudi, PLoS Pathogens, 2014).**
- **Evolutionary pressure to protect newborns from SIV infection might have resulted in immunological adaptations that protect adults from pathogenesis (Chahroudi, Science 2013).**

Immune responses to HIV: a fine balancing act



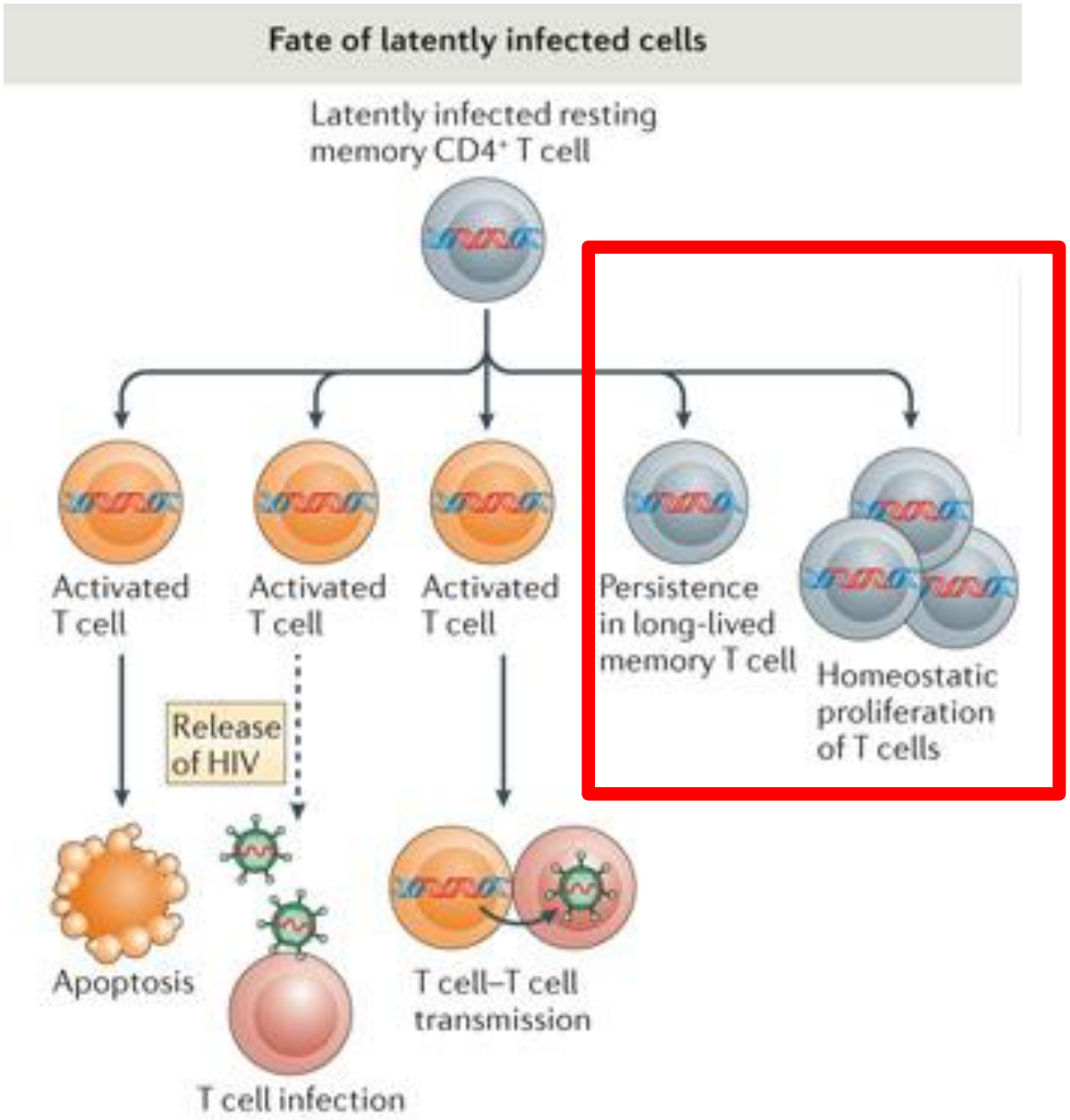
The key for an immunization strategy that successfully controls HIV transmission and/or early replication is to fully exploit the antiviral role of CD4+ T cells without the side effect of creating additional target cells for the virus.

Strategies to cure HIV/AIDS:

- **Full suppression of virus replication**
- **Identification of persistent reservoirs**
  -establishment
  -regulation/ractivation
  -persistence
- **Elimination of reservoirs**

Modified from Dr. Steve Deeks

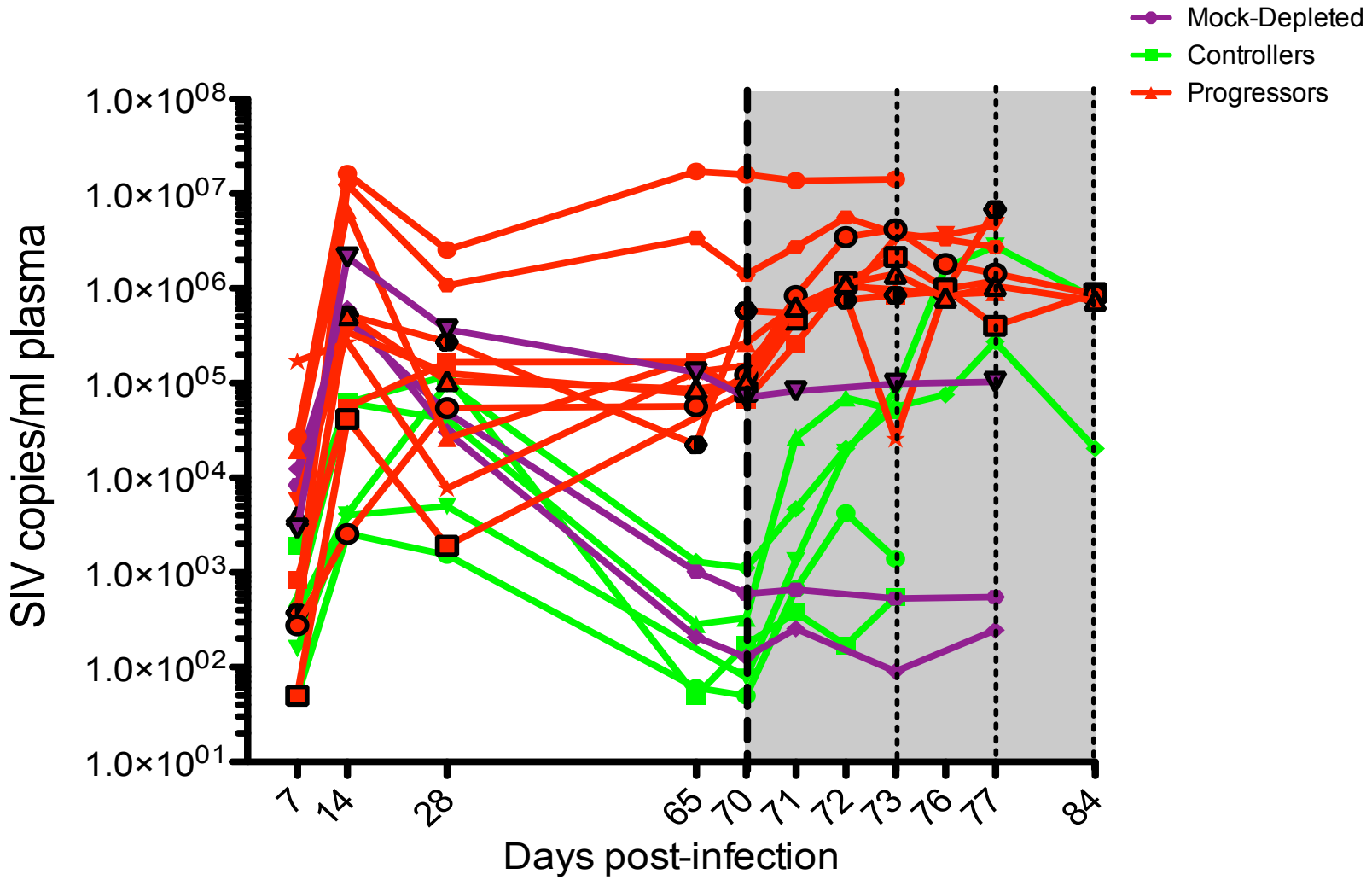
Mechanisms of HIV Persistence under ART



CD8+ T lymphocytes mediate viral control in HIV/SIV infection

- **Strong association between control of viremia and specific MHC class I alleles in both humans and macaques.**
- **Appearance of HIV/SIV-specific CTL responses is temporally coordinated with the postpeak decline in viral replication**
- **Escape mutations in CTL-targeted epitopes develop early during infection.**
- **LTNP/EC have more polyfunctional HIV-specific CD8+ T-cells compared to progressors.**
- **Antibody-mediated CD8+ lymphocytes depletion in SIV-infected macaques results in increased viremia and rapid disease progression.**

CD8 depletion is followed by dramatic increase of viremia in "controller" SIV-infected RMs



Chowdhury et al., J Virol 2015

KEY QUESTIONS:

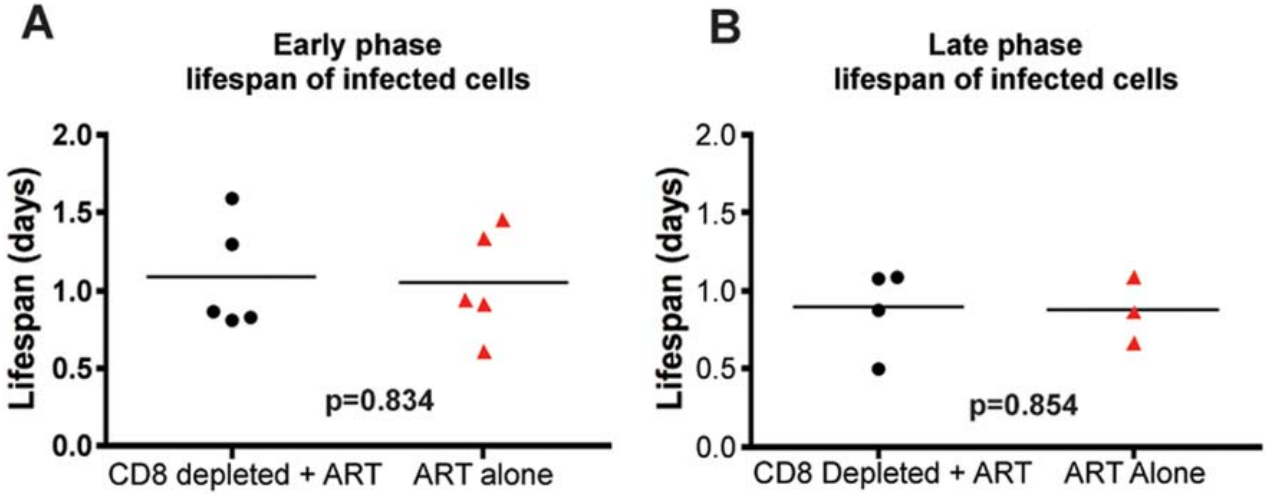
What is the role of CD8+ lymphocytes in controlling viremia on ART?

What is the role of CD8+ lymphocytes in the establishment and maintenance of the persistent reservoir of latently infected cells under ART?

OPEN ACCESS Freely available online PLoS PATHOGENS

CD8+ Lymphocytes Control Viral Replication in SIVmac239-Infected Rhesus Macaques without Decreasing the Lifespan of Productively Infected Cells

Nichole R. Klatt[1,2], Emi Shudo[3], Alex M. Ortiz[1], Jessica C. Engram[1], Mirko Paiardini[1], Benton Lawson[2], Michael D. Miller[4], James Else[2], Ivona Pandrea[5], Jacob D. Estes[6], Cristian Apetrei[5], Joern E. Schmitz[7], Ruy M. Ribeiro[3], Alan S. Perelson[3], Guido Silvestri[1,2]*

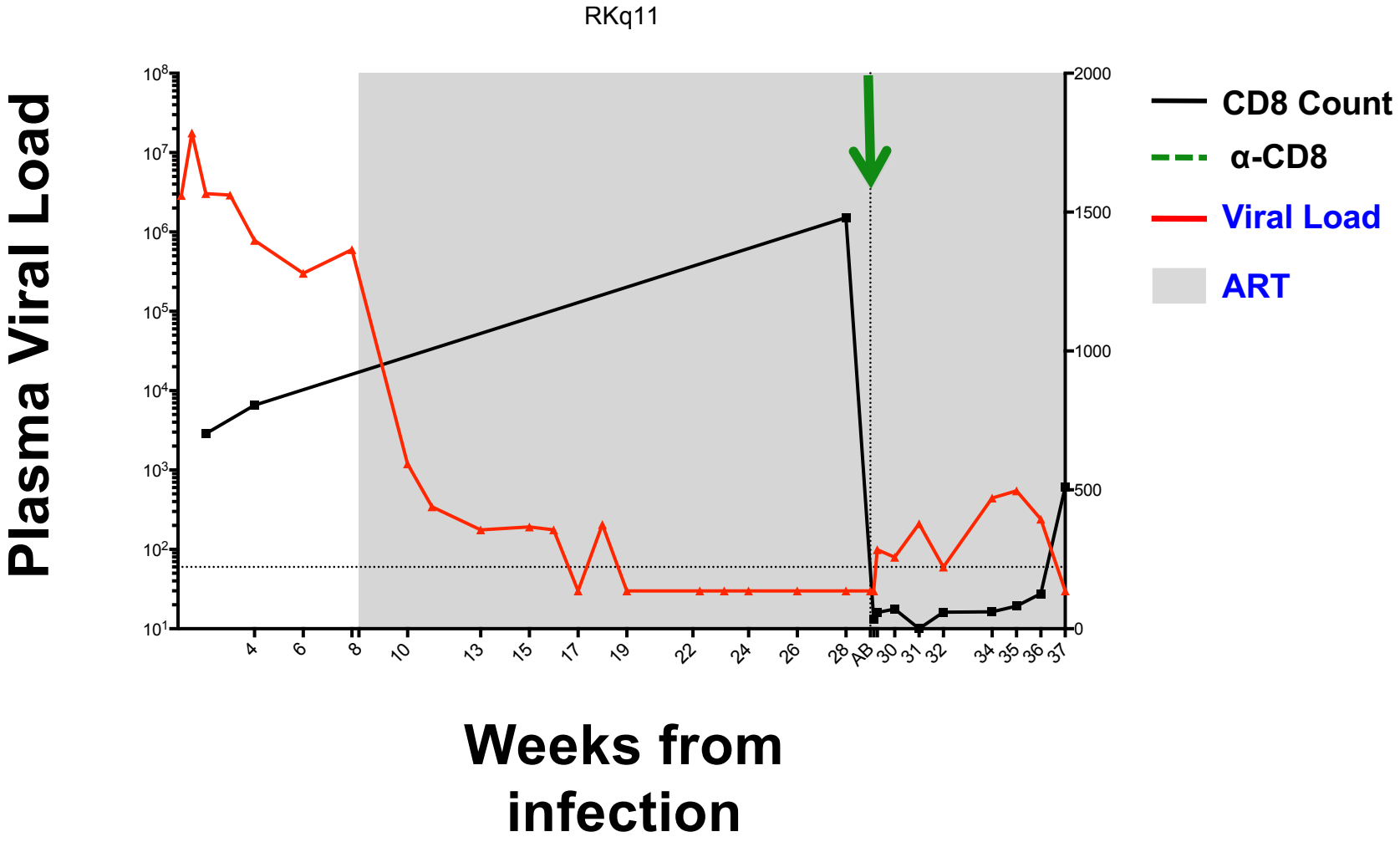


No difference in lifespan of productively infected cells in the presence or absence of CD8+ lymphocytes in both early or late chronic infection.

Similar result by Wong, Dandekar et al., PLoS Pathogens 2010.

We concluded that non-cytolytic mechanisms may contribute to CD8+ lymphocyte-mediated suppression of virus replication.

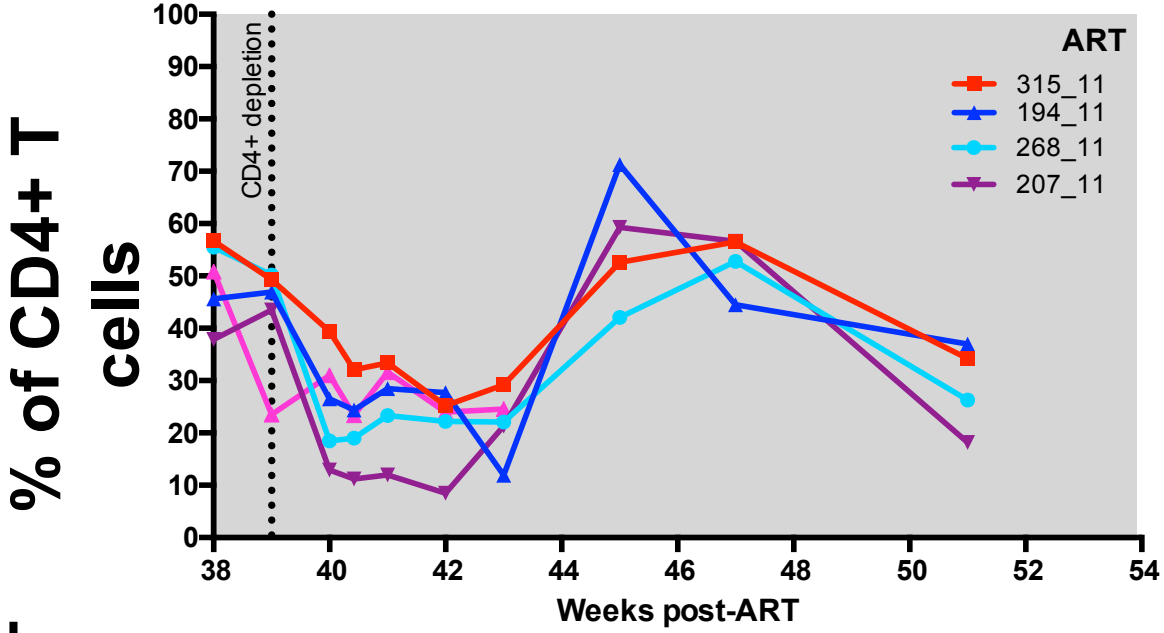
CD8 depletion is followed by increase in VL under highly suppressive ART

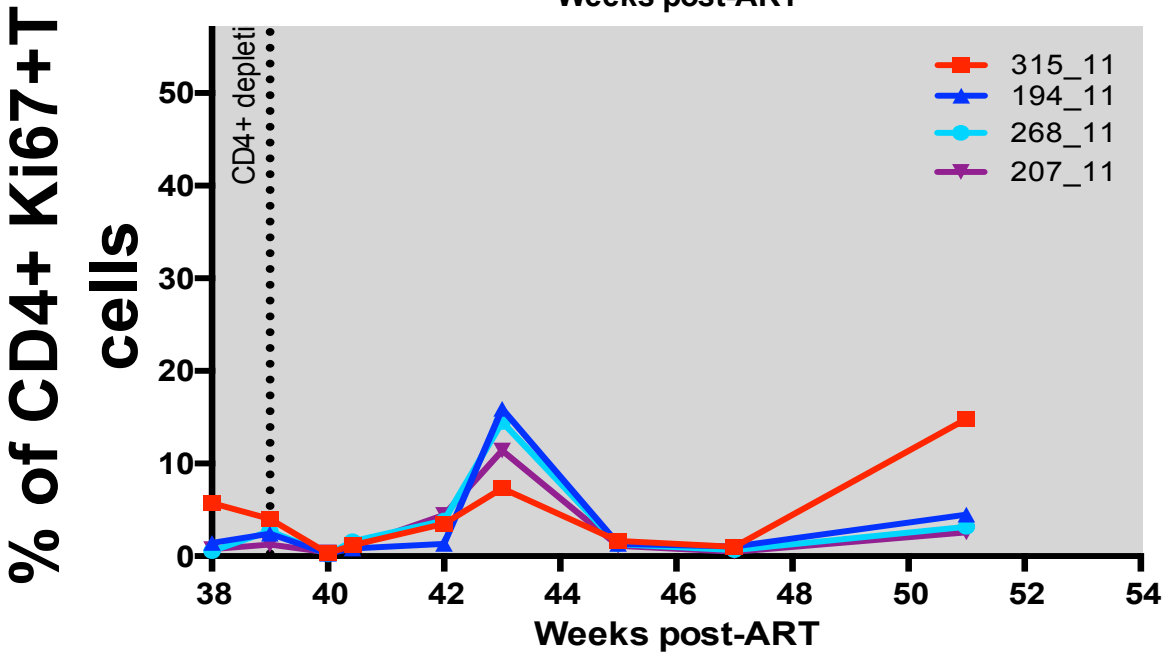


CD8+ lymphocytes are required to maintain undetectable viremia in ART-treated SIV-infected macaques (*Cartwright et al., Immunity, 2016*)

Is the virus production observed after CD8+ lymphocyte depletion in ART-treated SIV-infected macaques just due to increased levels of CD4+ T cell activation?

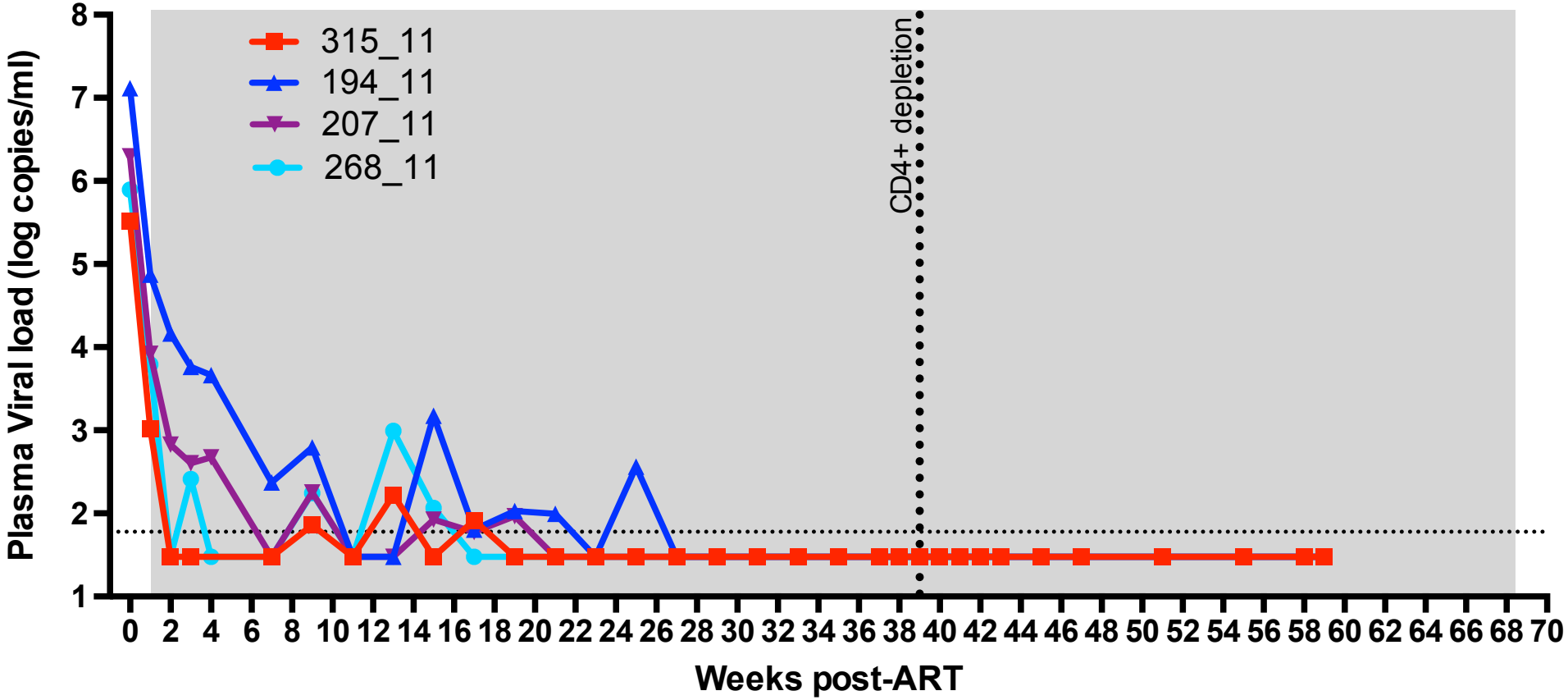
CD4+ T cell depletion is followed by a significant increase of proliferating, Ki67+ CD4+ T cells





Kumar et al., J Virol, 2018

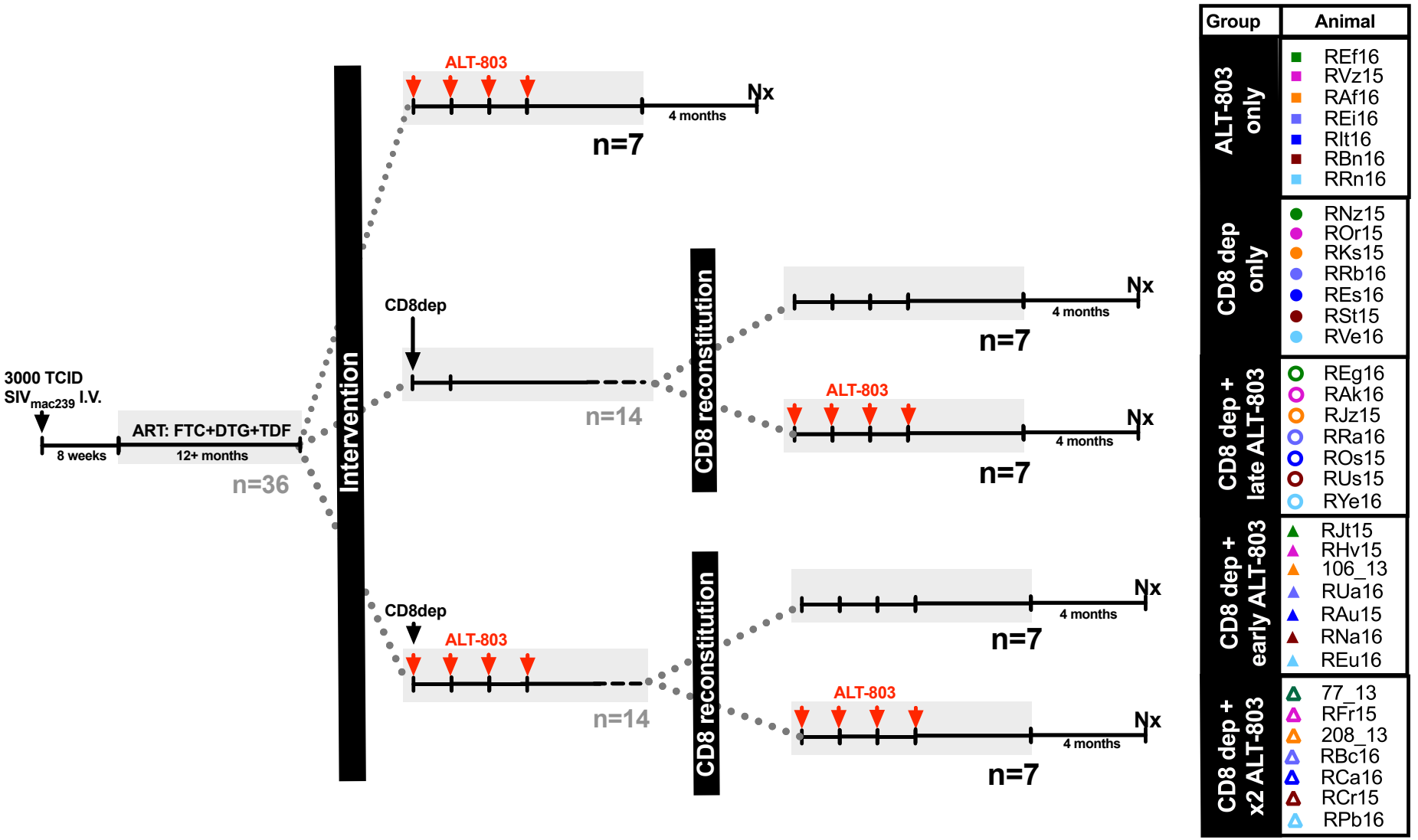
… but is NOT associated with increase in viremia



Total n = 8 animals

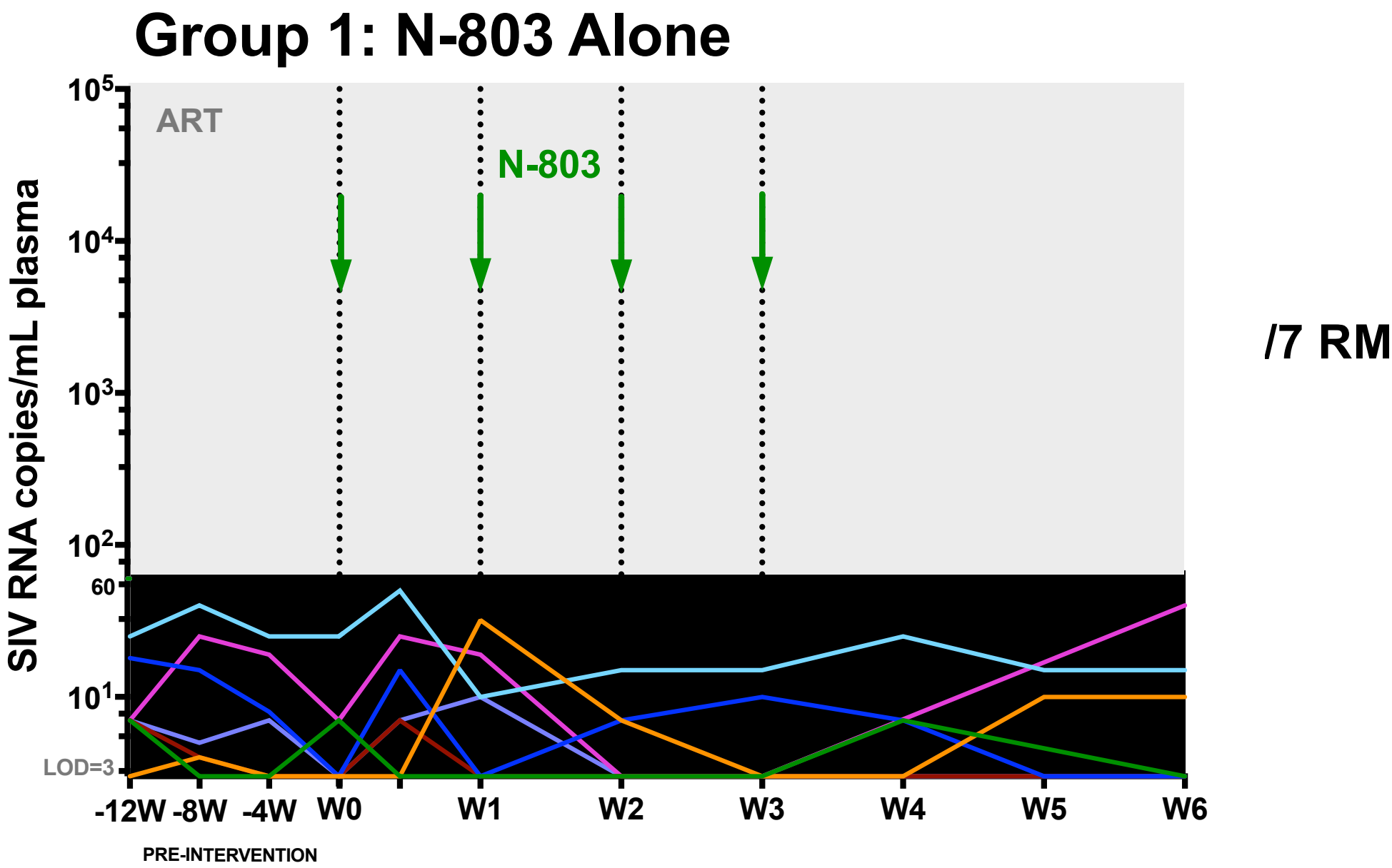
Kumar et al., J Virol, 2018

Study Design



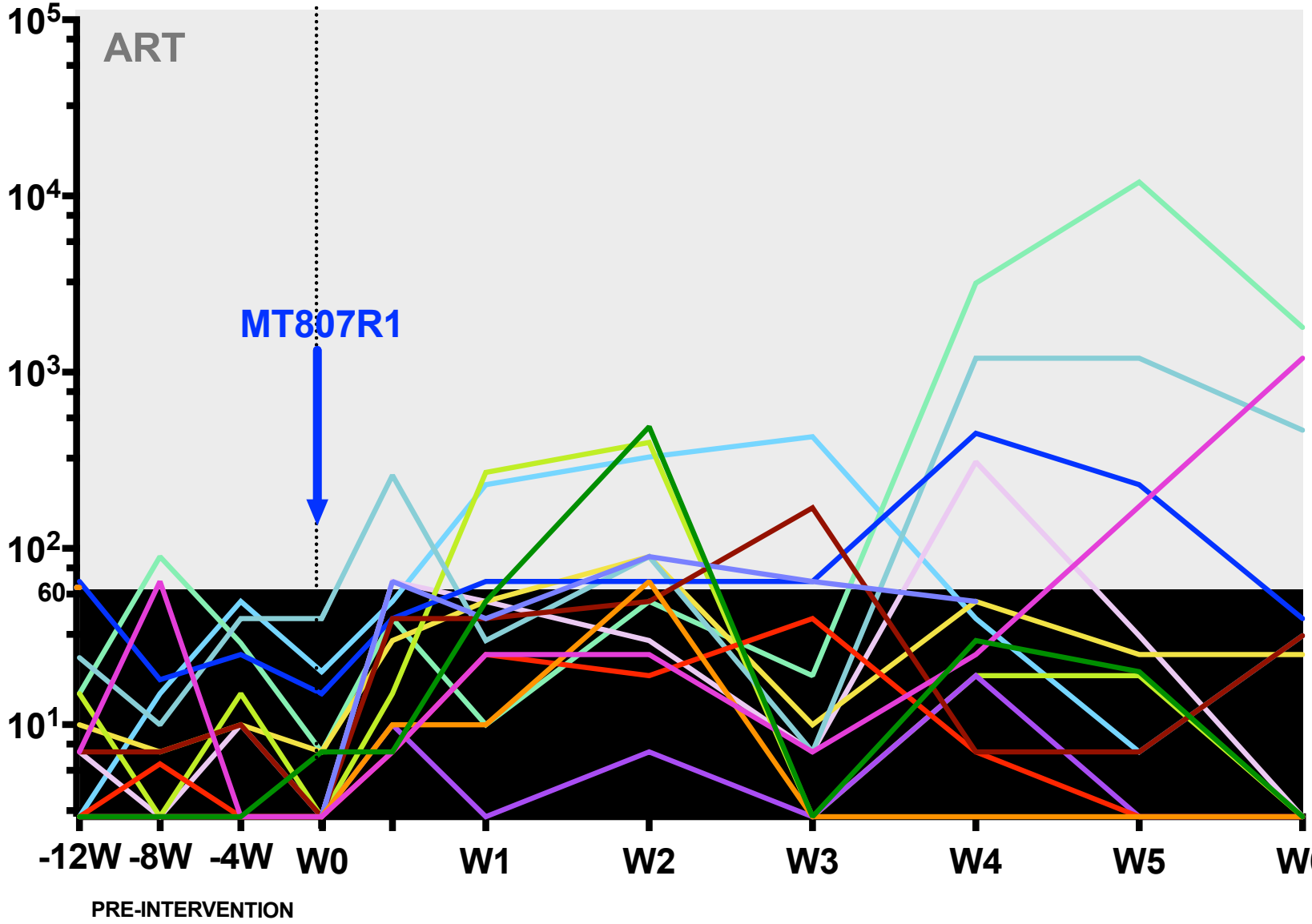
McBrien et al., Nature 2020

N-803 does not induce virus reactivation in ART-treated SIV-infected macaques



CD8 depletion is still associated with increased virus production even after 12 months of ART

Group 2: CD8 Depletion Alone



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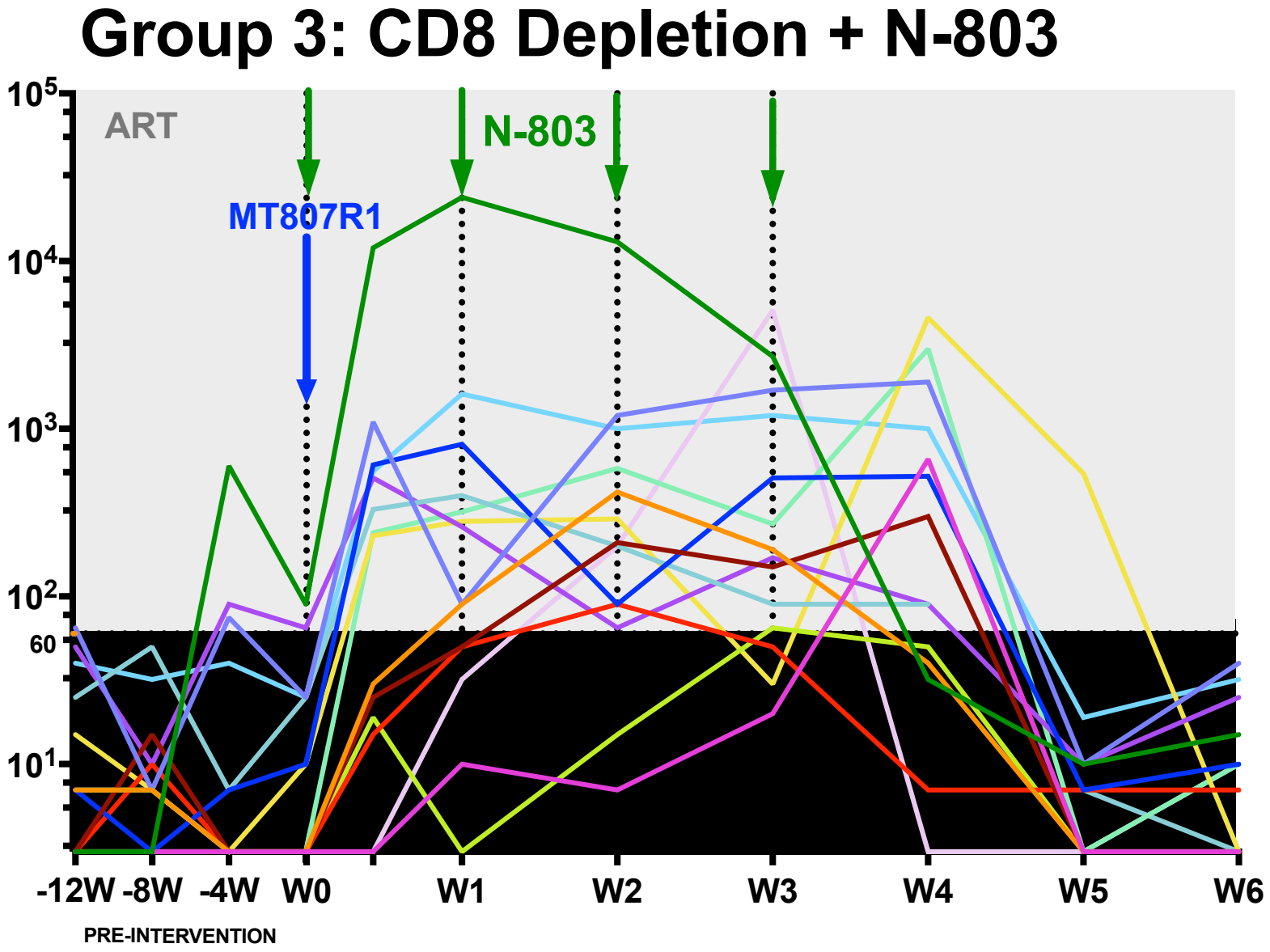
N-803 induce strong and persistent virus reactivation in ART-treated CD8-depleted macaques



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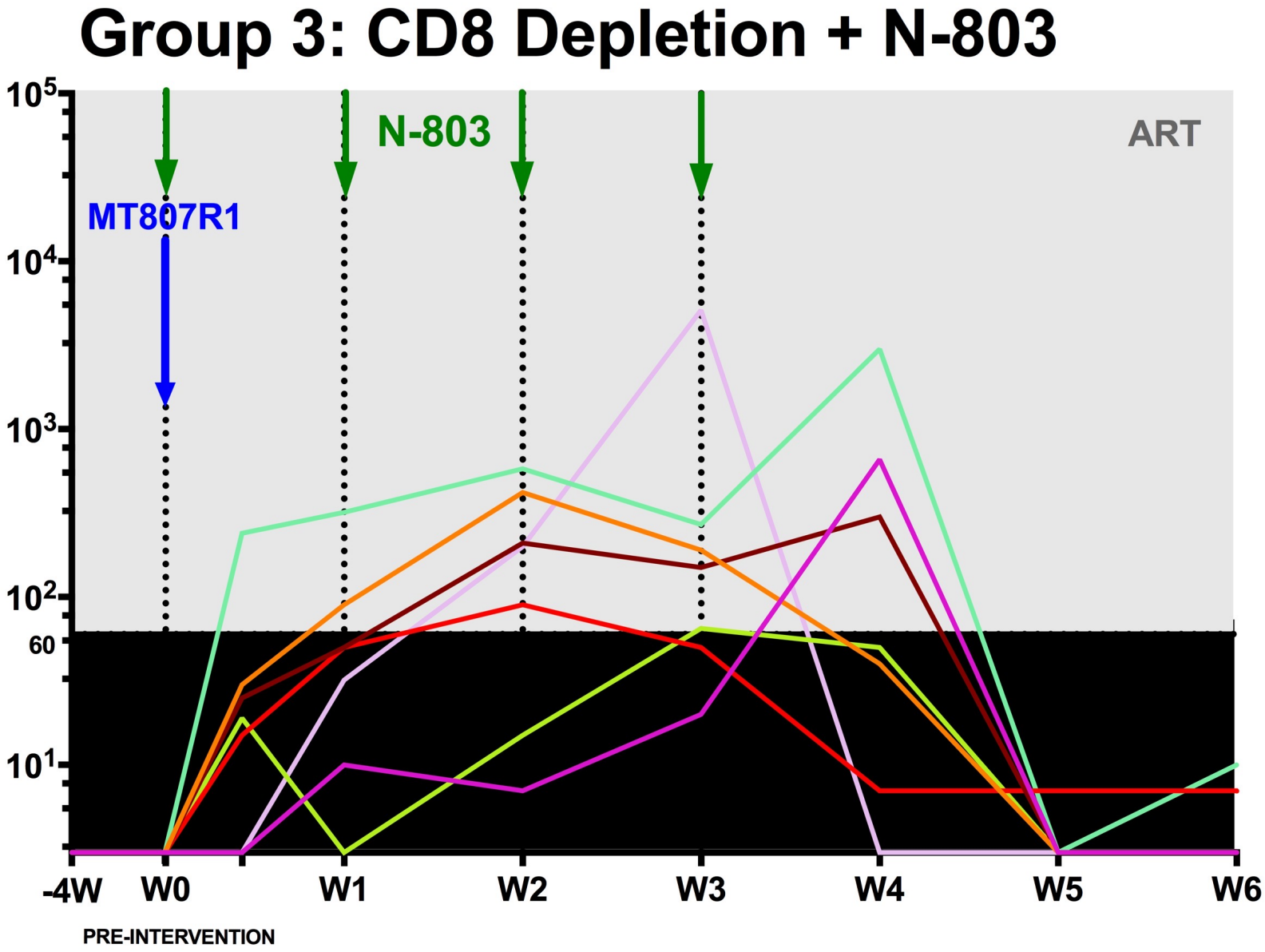
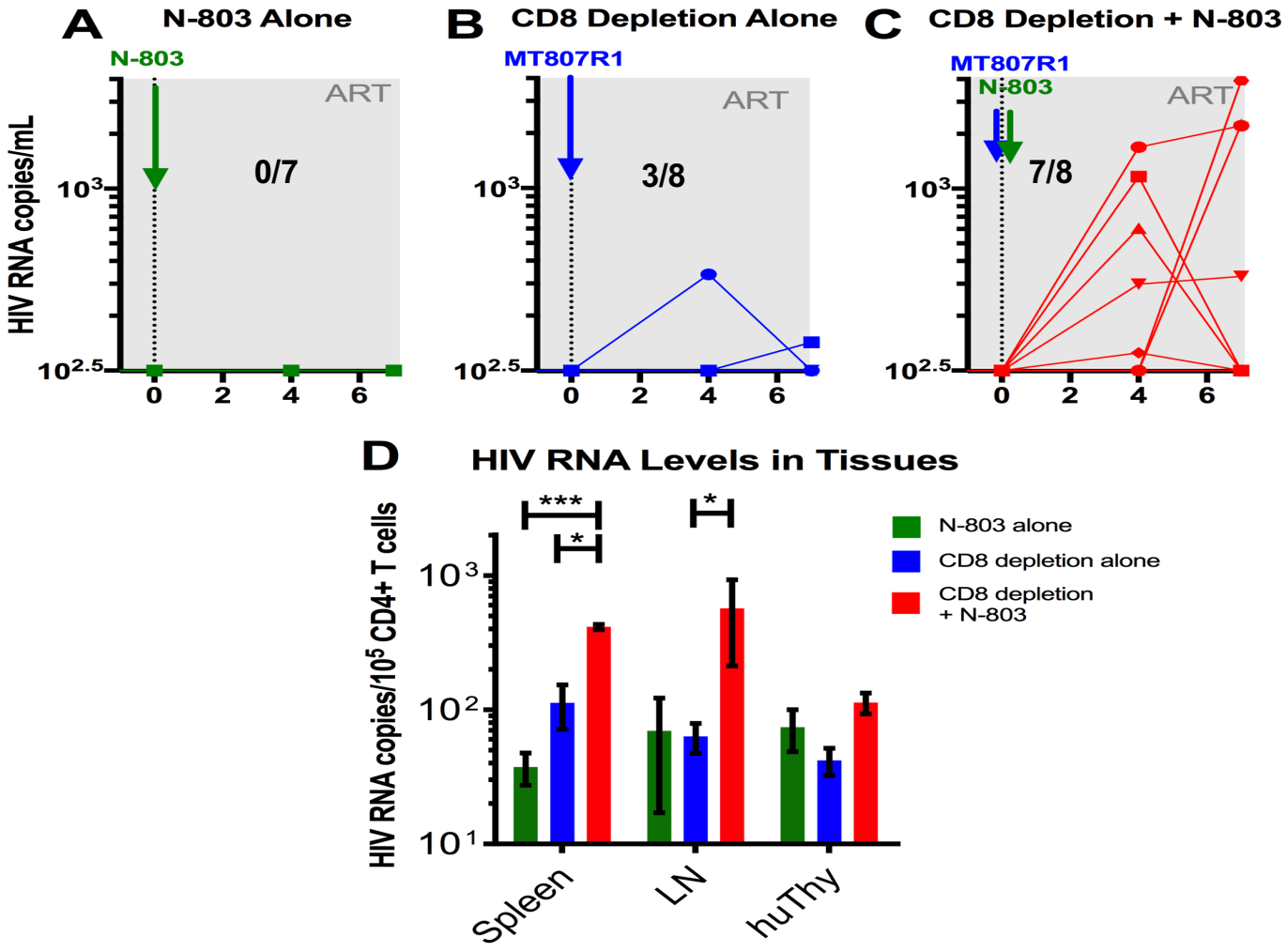
N-803 induce strong and persistent virus reactivation in ART-treated CD8-depleted macaques

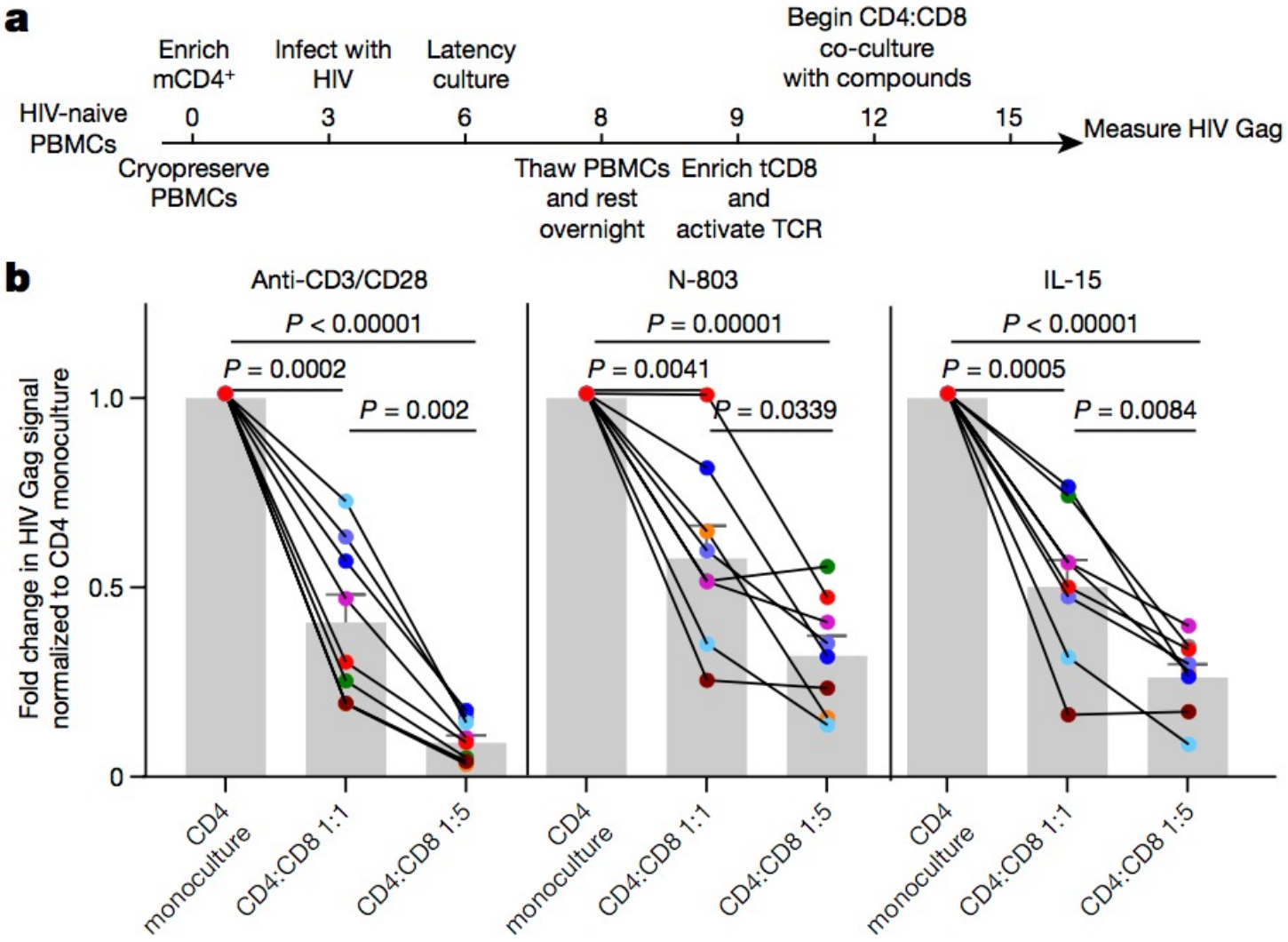


N-803 + CD8 depletion induces robust virus reactivation in HIV-infected ART-treated BLT humanized mice



McBrien et al., Nature 2020

Co-culture of latently infected CD4+ T cells with autologous unprimed CD8+ T cells results in decreased expression of HIV Gag after LRA treatment.

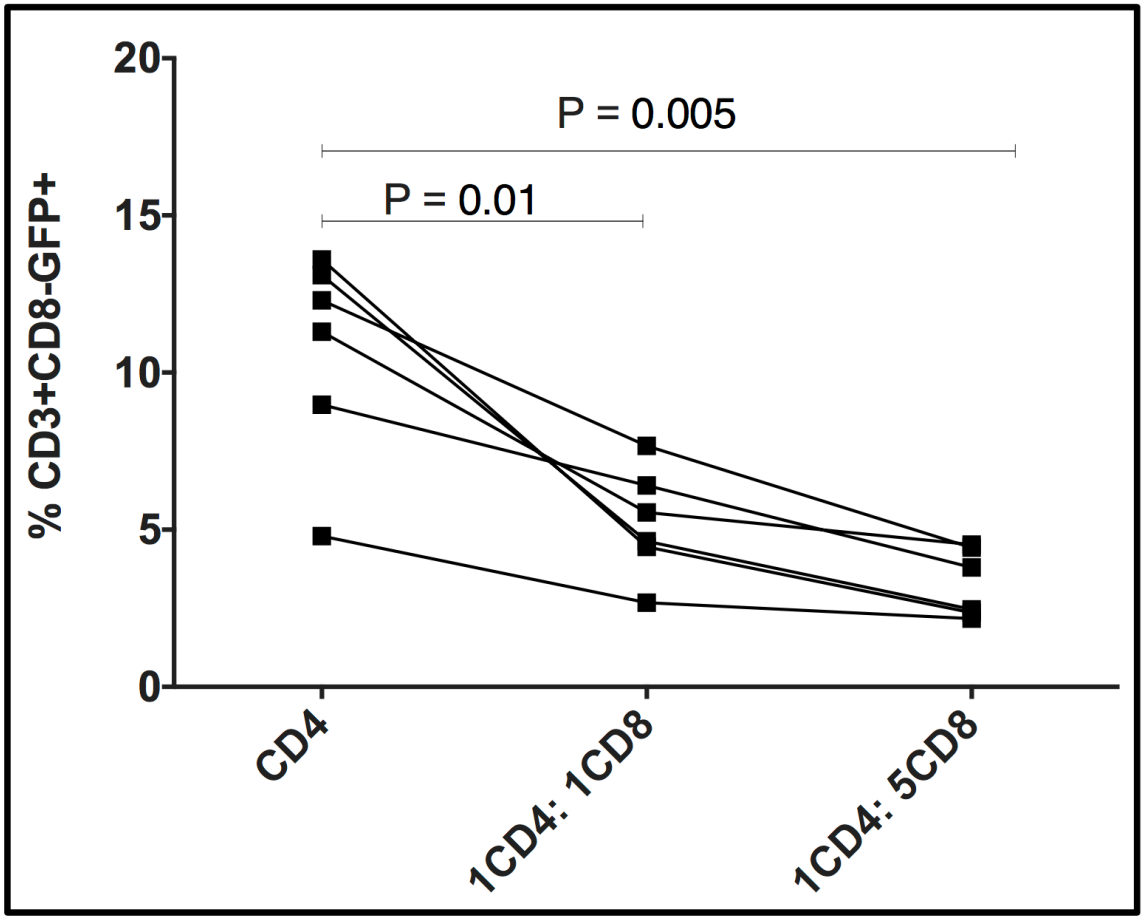


Mc Brien et al., Nature, 2020

Unprimed CD8+ T cells suppress HIV replication in a dose-dependent fashion

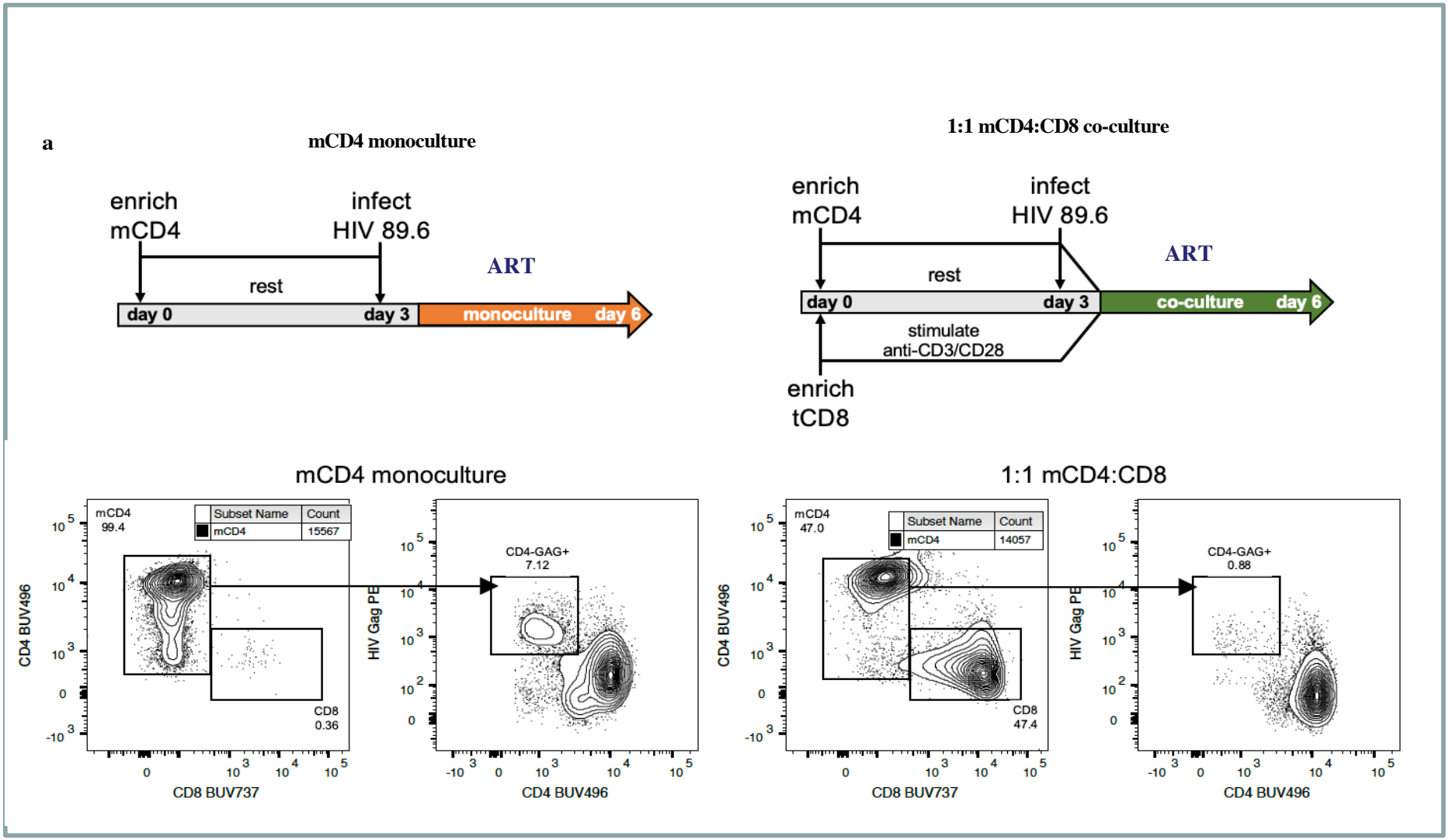
CD8+/CD4+ co-culture assay:

- ✓ Positive control wells with infected $CD4^+$ cells alone
- ✓ Co-culture wells with Infected CD4+ plus CD8+ at 1:1 and 5:1 effector-to-target cell (E:T) ratios
- ✓ Negative control wells (MOCK) with uninfected $CD4^+$ cells and/or CD8+ cells
- ✓ For cell density control, the total number of cells per well remained constant



M. Zanoni, PLoS Pathogens 2020

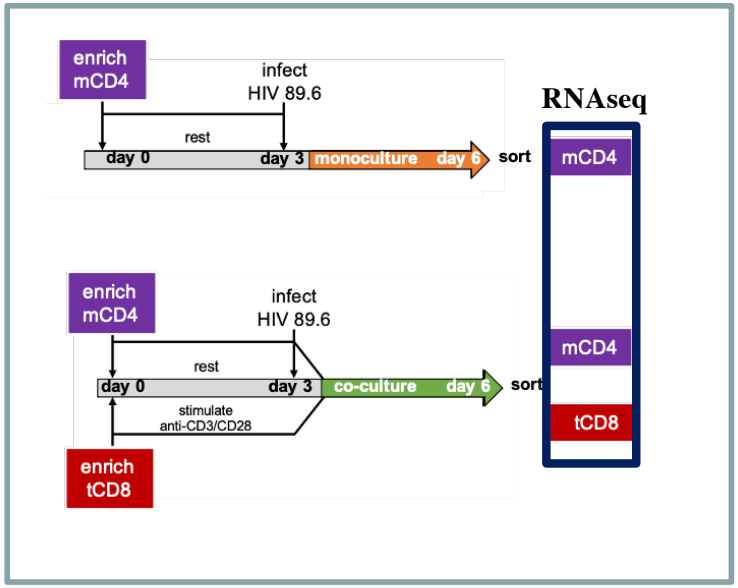
In vitro HIV infection of memory CD4+ T cells (mCD4) shows the impact of CD8+ T cell suppression activity

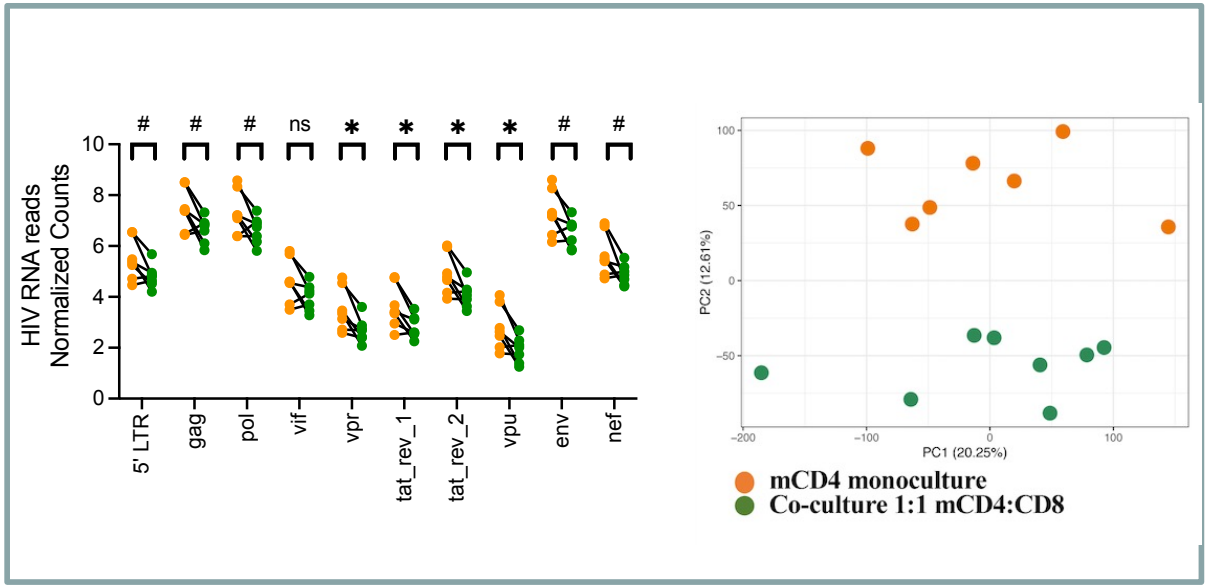


memory CD4= mCD4 total CD8 = tCD8

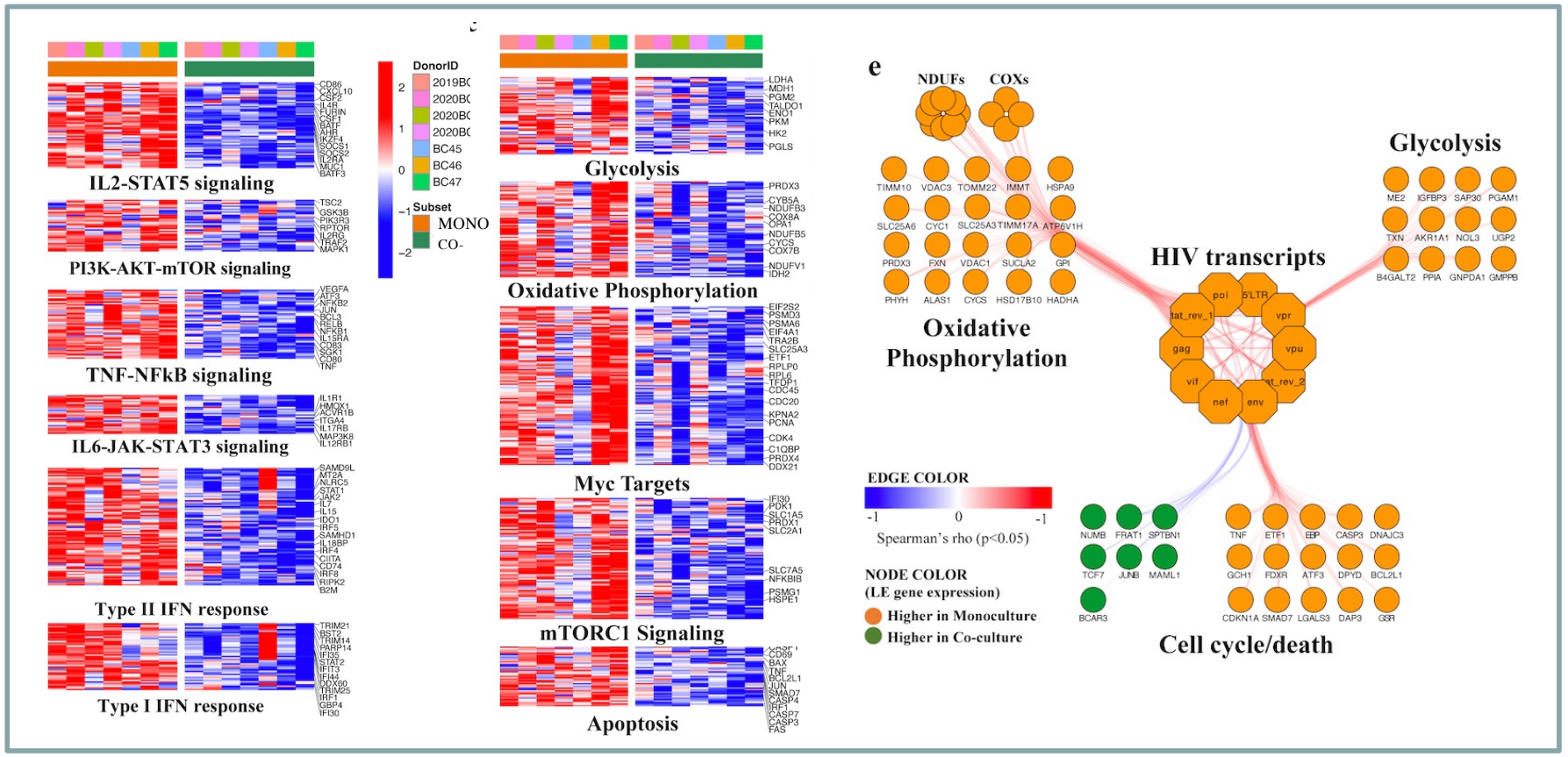
Franchitti et al., in preparation

Transcriptomics analyses showed reduced HIV RNA expression in sorted CD4+ T cells from *in vitro* CD8+ T cell co-cultures

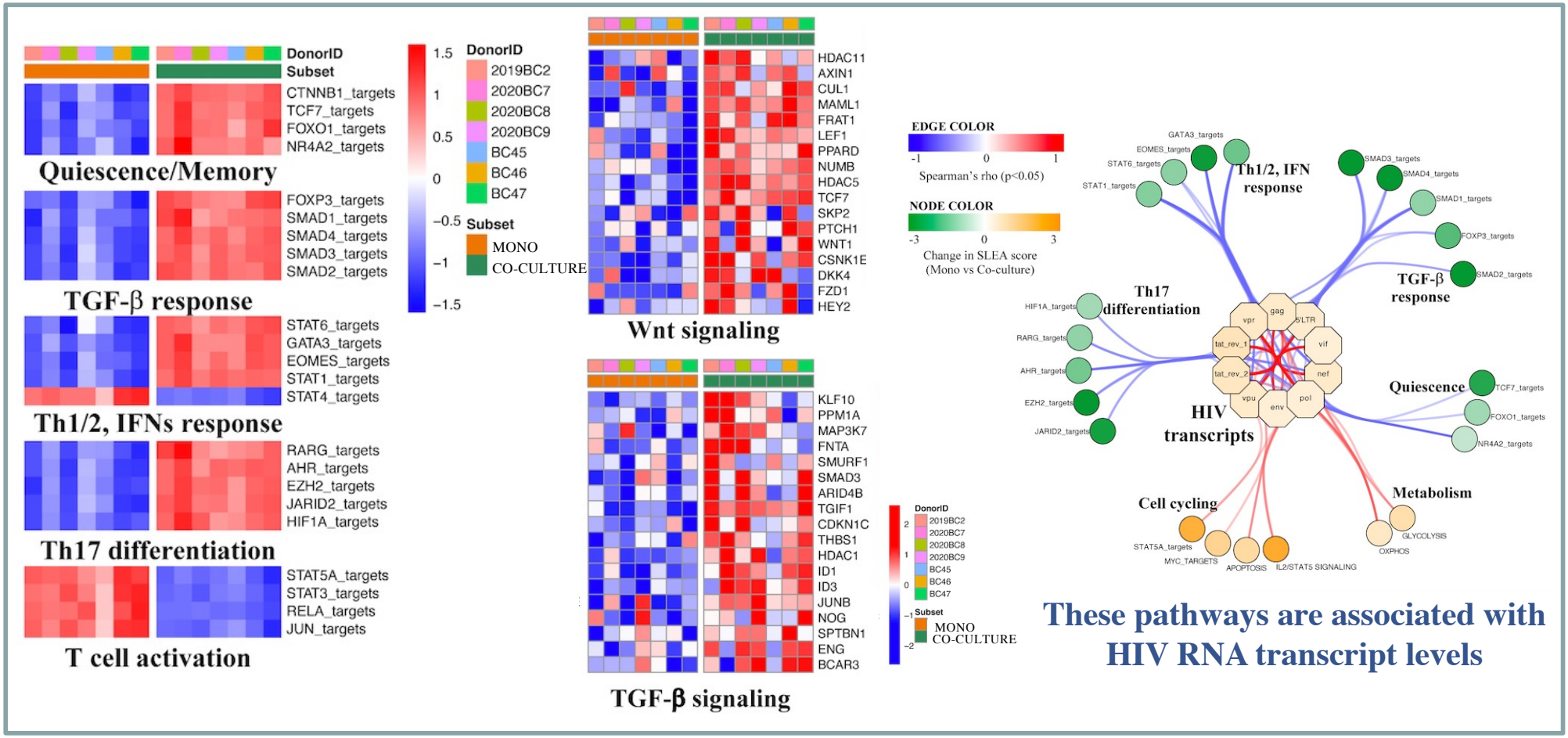




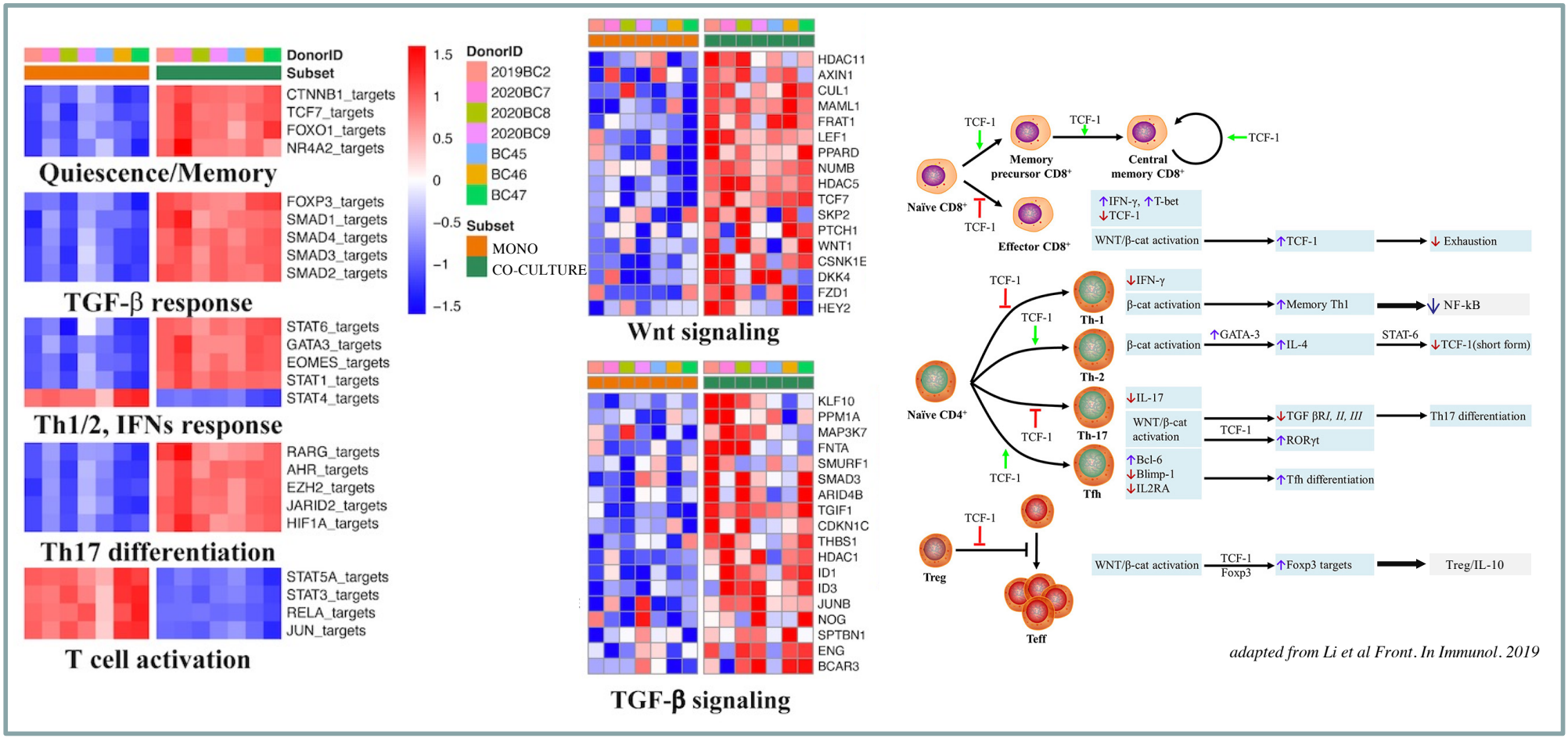
Metabolism, effector function and apoptosis pathways are downregulated in CD4+ T cells from CD4:CD8 co-cultures



Quiescence, memory maintenance pathways are upregulated in CD4+ T cells from CD4:CD8 co-cultures



Wnt signaling plays an important role in the regulation of lymphocyte differentiation, survival and stemness



adapted from Li et al Front. In Immunol. 2019

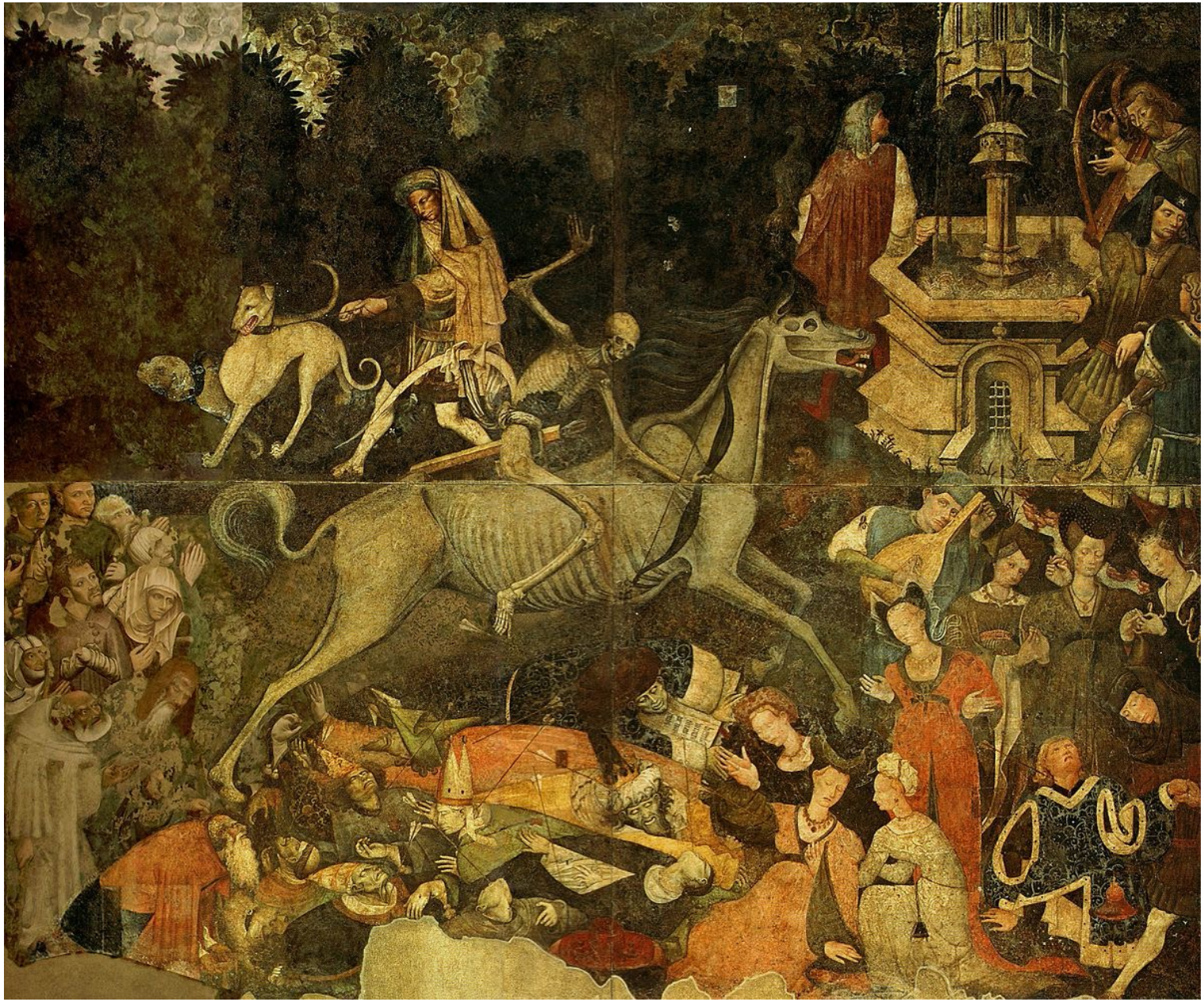
Conclusions

- *In vitro* data using peripheral blood from HIV-naïve individuals reveals a CD8- specific suppression of HIV expression in CD4+ T cells
- This reduction of HIV expression is not derived from a decrease in the frequency of infected cells, but reduced virus transcription
- CD8+ T cell modulation of the Wnt signaling axis (that includes NFKB/IL-10/Bcl-2/Dead-box) may play a key role in modulating stemness and pro-survival pathways that contribute to HIV persistence
- Ongoing experiments are centered on identifying and manipulating the Wnt/NFKB/IL-10/Bcl-2/Dead-box pathways

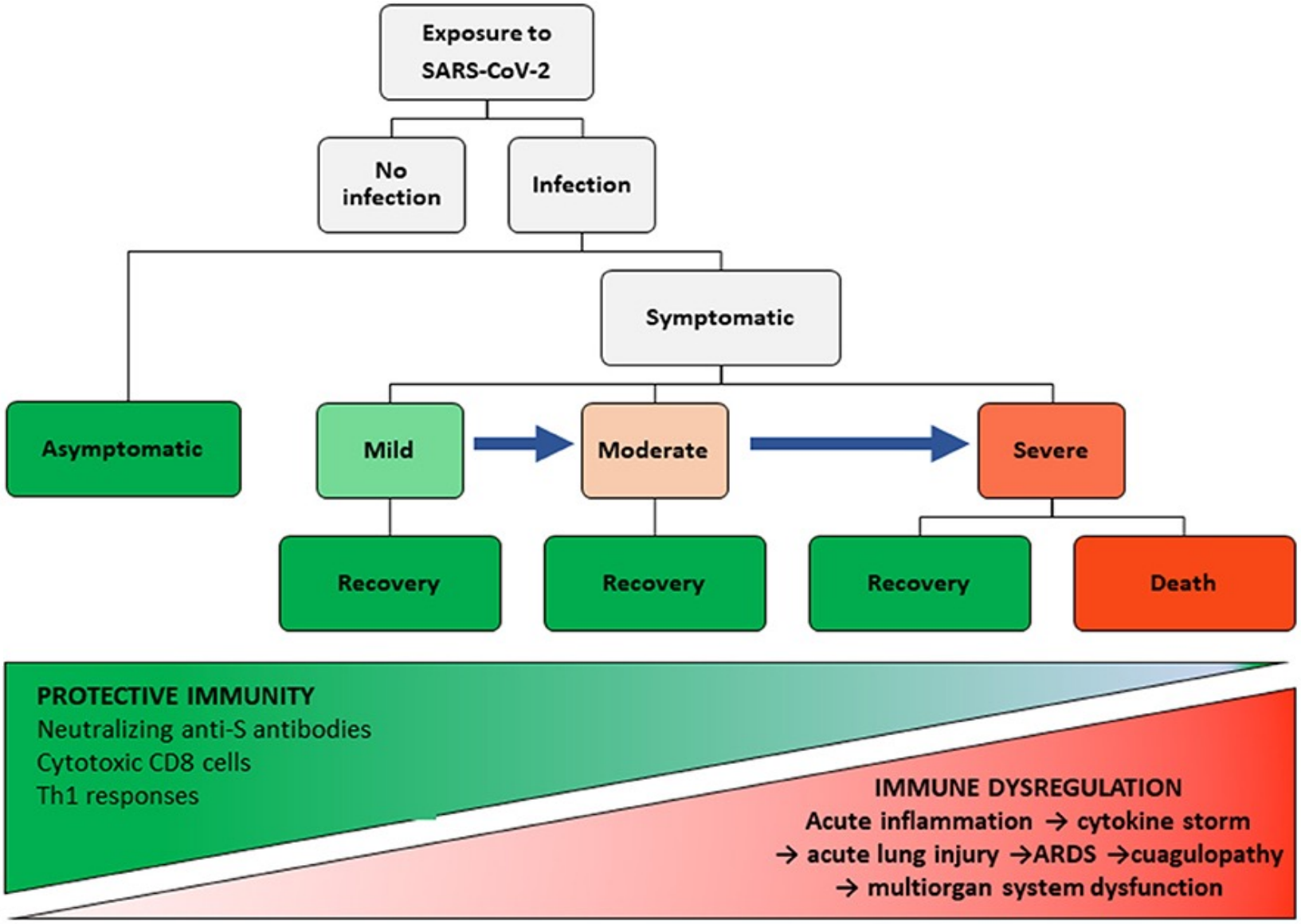
Antiviral activities of CD8+ T cells

	CTL activity	HIV silencing
Suppression of virus replication in absence of ART	**YES**	**YES**
MHC-I restriction	YES	NO
Present in HIV-neg subjects	NO	YES
Requires cell-cell contact	YES	Partly
Mediating factors	Perforin, granzyme B	Unknown
Subject to escape and exhaustion	YES	NO
Impact on virus reservoir under ART	**clearance**	**stabilization**
Impact on CD4 activation	**none**	**down-modulation**
Effect on latency reversal	**none**	**inhibition**

What about SARS-CoV-2 and COVID-19?



Immune responses & Immunopathology



Garcia et al., Front Immunol 2020

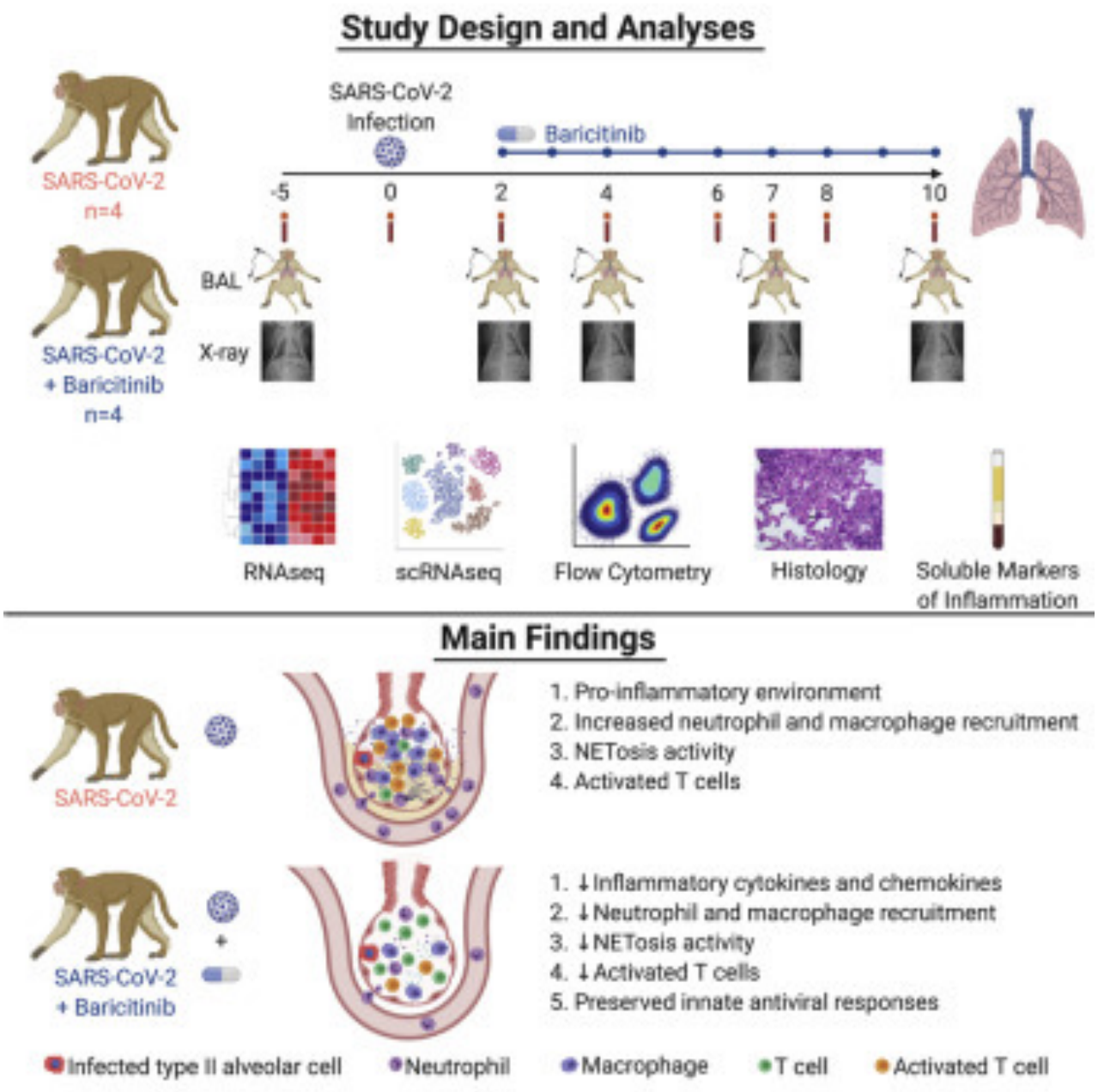
Antiviral and/or Immuno-modulatory Interventions

1. **EARLY STAGES INTERVENTIONS: antivirals (remdesivir, molnupiravir, paxlovi); monoclonal antibodies.**

2. **LATE STAGES INTERVENTIONS: Immuno-modulatory interventions, blockade of IL-6, JAK/STAT, IL-1 etc.**

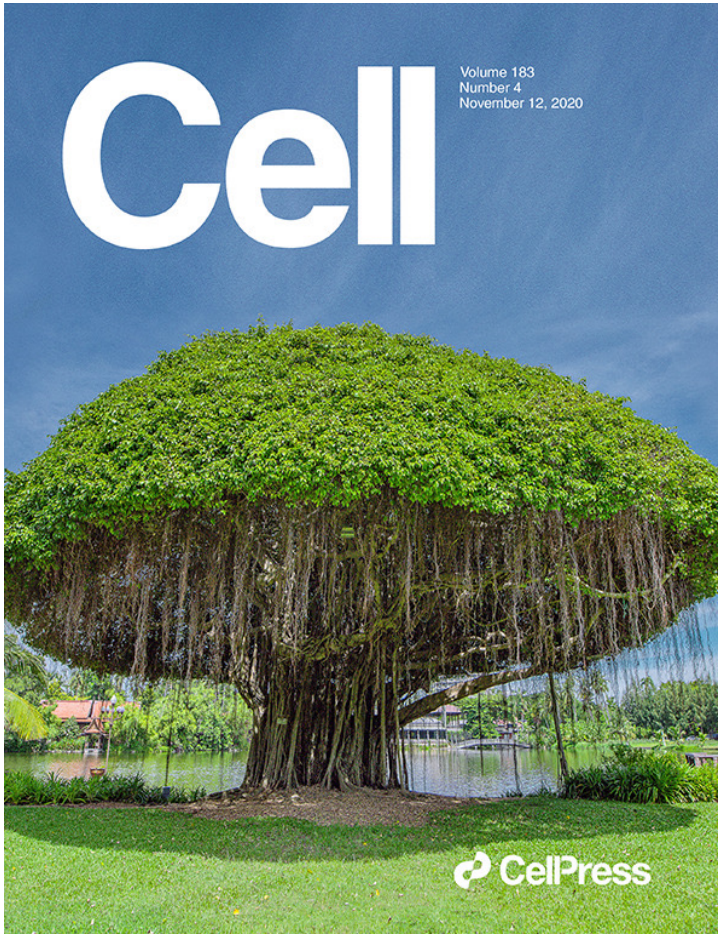
3. **The "therapeutic" role of vaccines – vaccinated individuals are better at early control of viremia**

Immuno-modulatory interventions

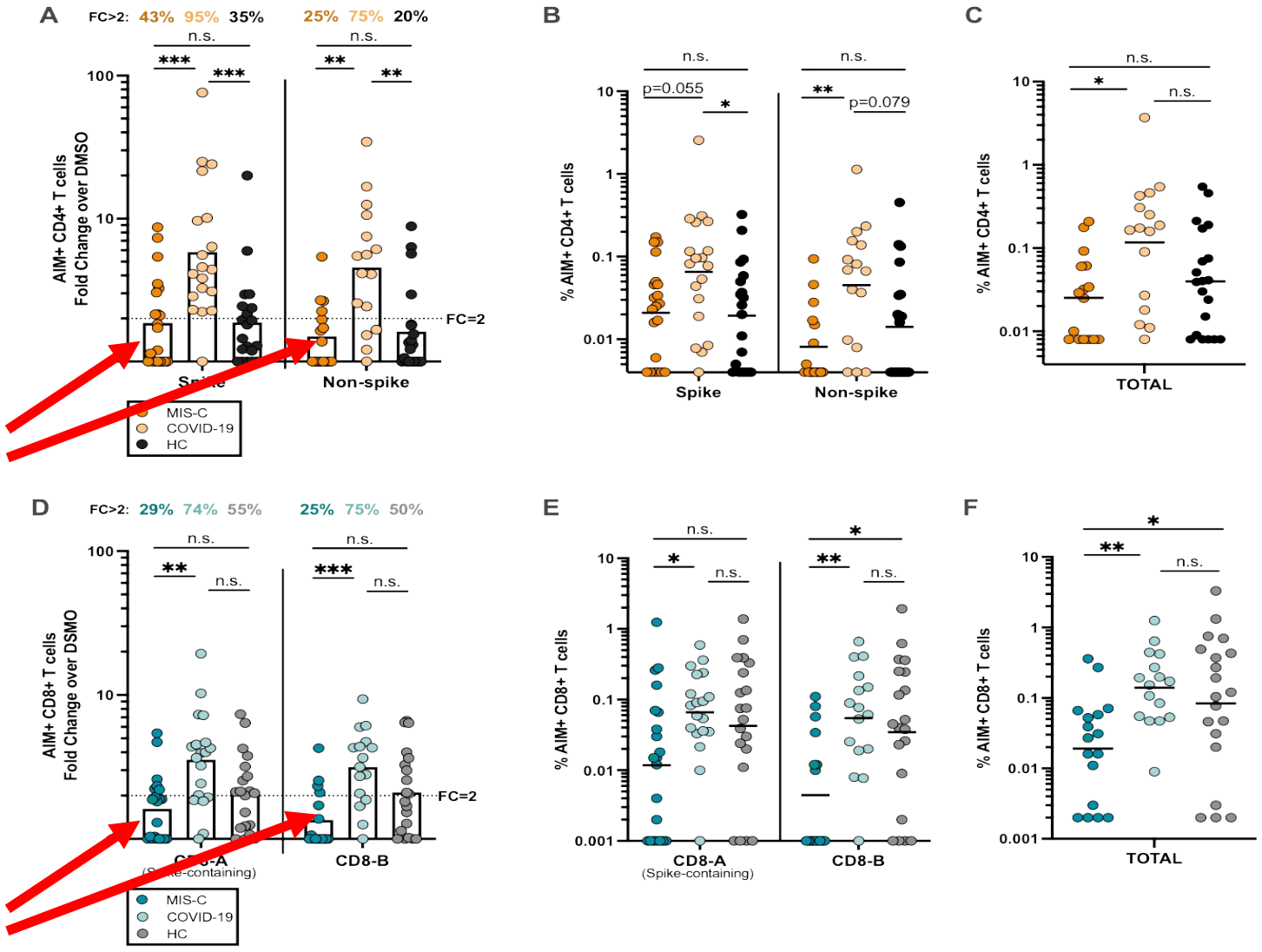


Immuno-modulatory Interventions

Hsoang T, … Silvestri G, Bosinger S, Paiardini M.
Baricitinib treatment resolves lower airway inflammation and neutrophil recruitment in SARS-CoV-2-infected rhesus macaques.



Reduced SARS-CoV-2 T cell responses in MIS-C



SARS-CoV-2-Specific T-cell Responses in MIS-C, convalescent COVID-19, and healthy children shown as AIM+ (CD4+OX40+41BB+ or CD8+CD69+41BB+ T-cells) in response to Spike and non-Spike peptide mega-pool stimulation.

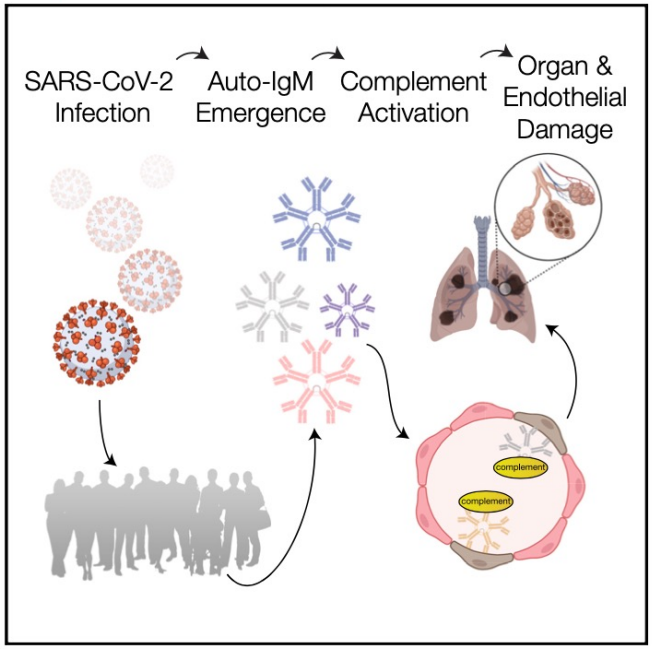
Singh V. et al., JCI insight

Auto-reactive IgM in severe COVID-19 patients

Cell Reports
Medicine

Broad auto-reactive IgM responses are common in critically ill patients, including those with COVID-19

Graphical abstract



Authors

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Correspondence

cheryl.maier@emory.edu

In brief

Critical illness can be associated with immune dysregulation; yet, mediators contributing to disease severity in COVID-19 are unclear. Wong et al. show a high percentage of critically ill patients possess auto-reactive IgM, which, in SARS-CoV-2 infection, are capable of binding diverse targets across key organs and inflicting complement-dependent cytotoxicity.

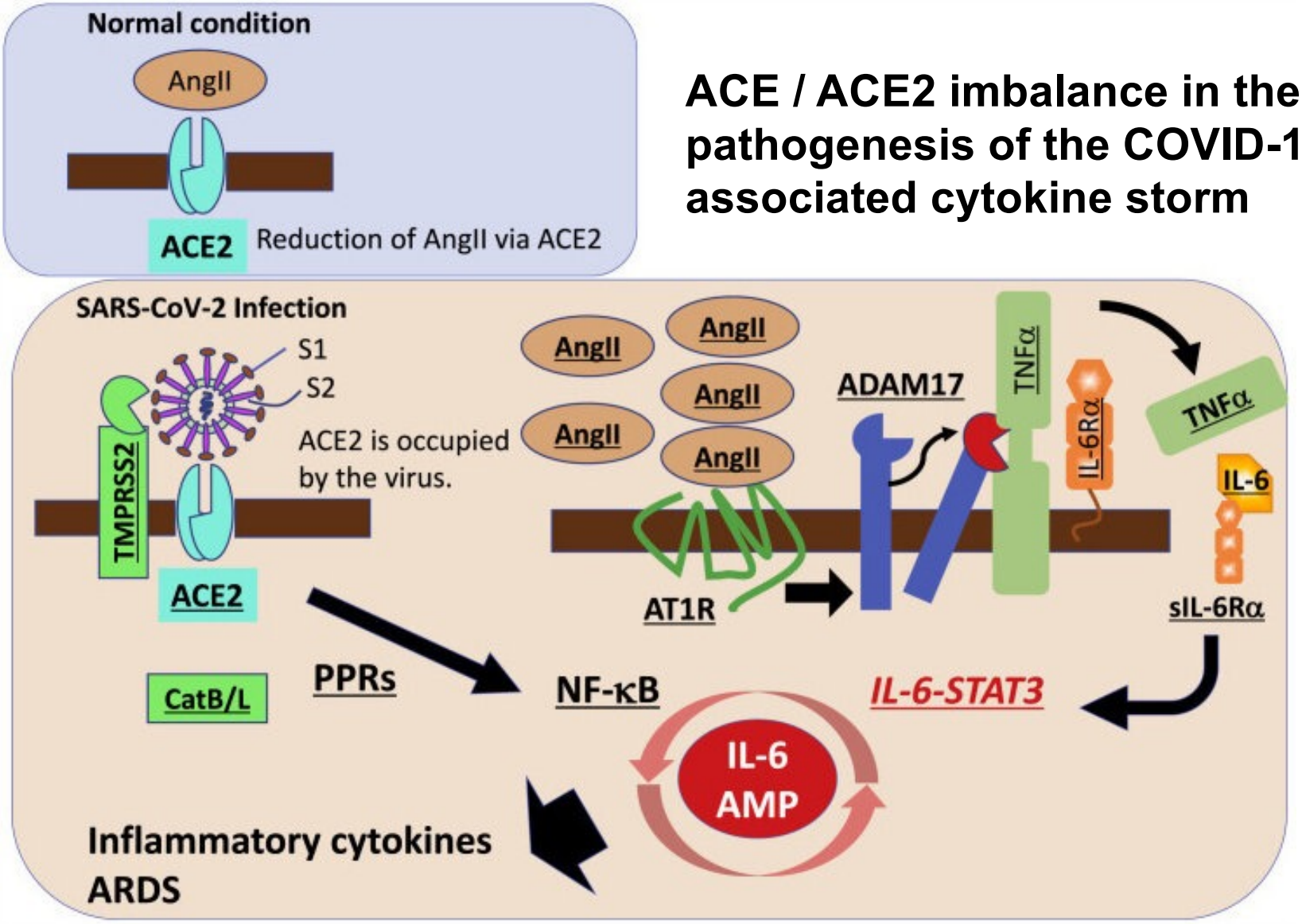
Highlights

- More than 90% of critically ill COVID-19 patients have auto-reactive IgM antibodies
- Auto-reactive IgM binds diverse targets across multiple organ types
- IgM and complement component C4d are abundant in COVID-19 non-survivor lung tissue
- COVID-19-associated auto-IgM fixes complement to induce cell death *in vitro*

Wong et al., 2021, Cell Reports Medicine 2, 100321
June 15, 2021
https://doi.org/10.1016/j.xcrm.2021.100321

Wong et al., Cell Reports Med 2021

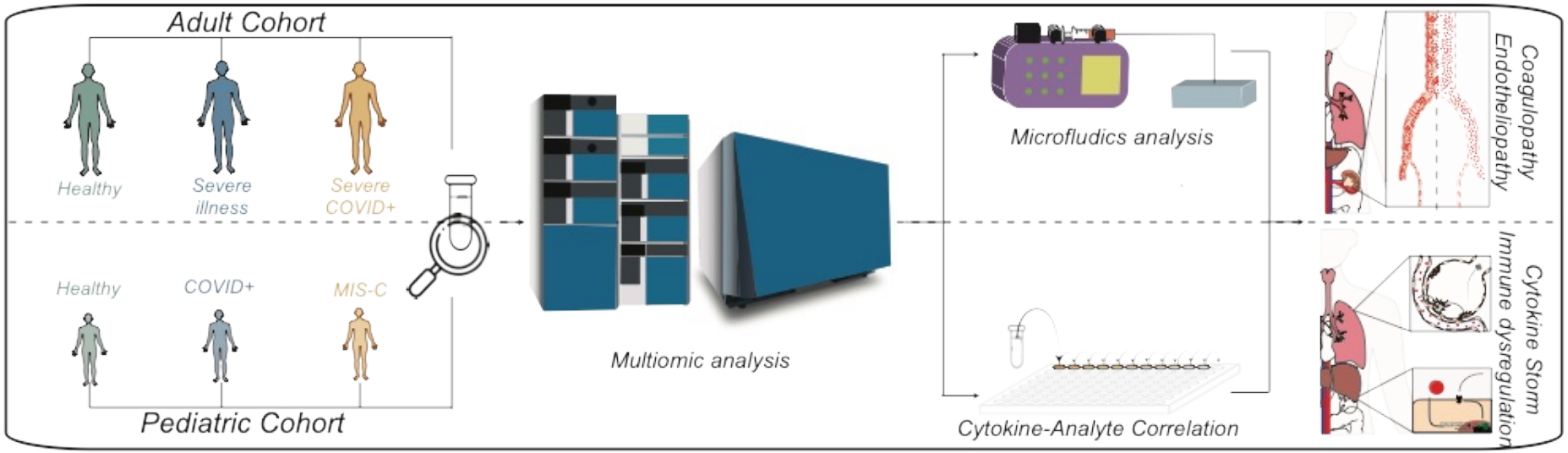
Severe COVID-19 as an endotheliopathy

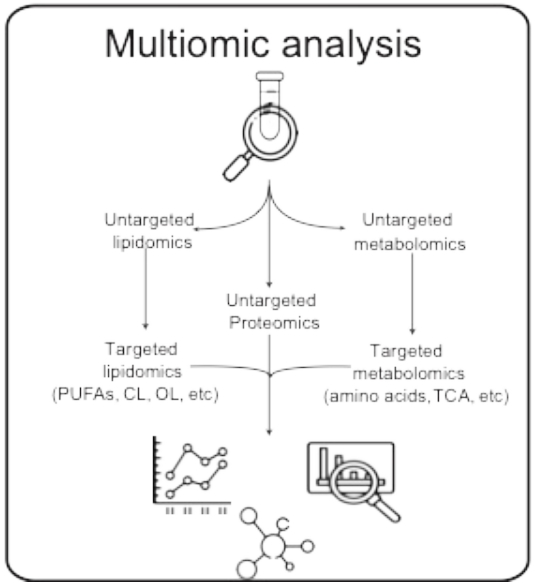


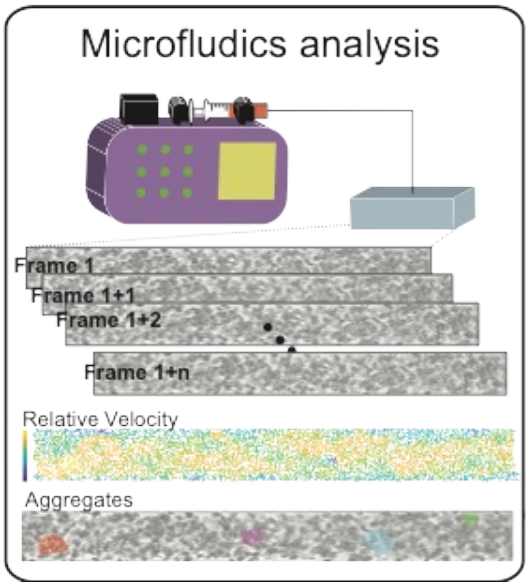
ACE / ACE2 imbalance in the pathogenesis of the COVID-19-associated cytokine storm

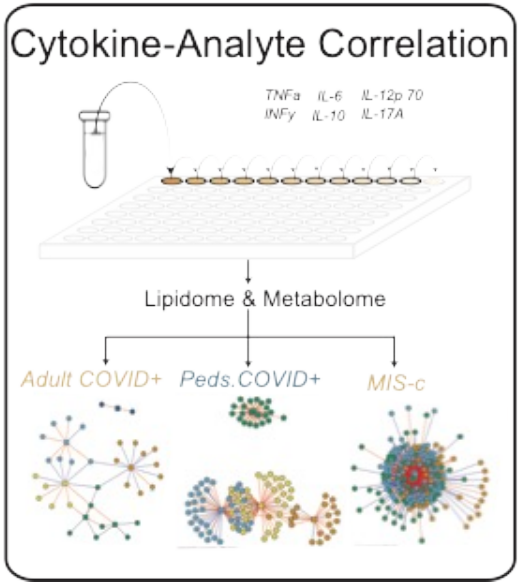
Hirano & Murakami, Immunity 2020

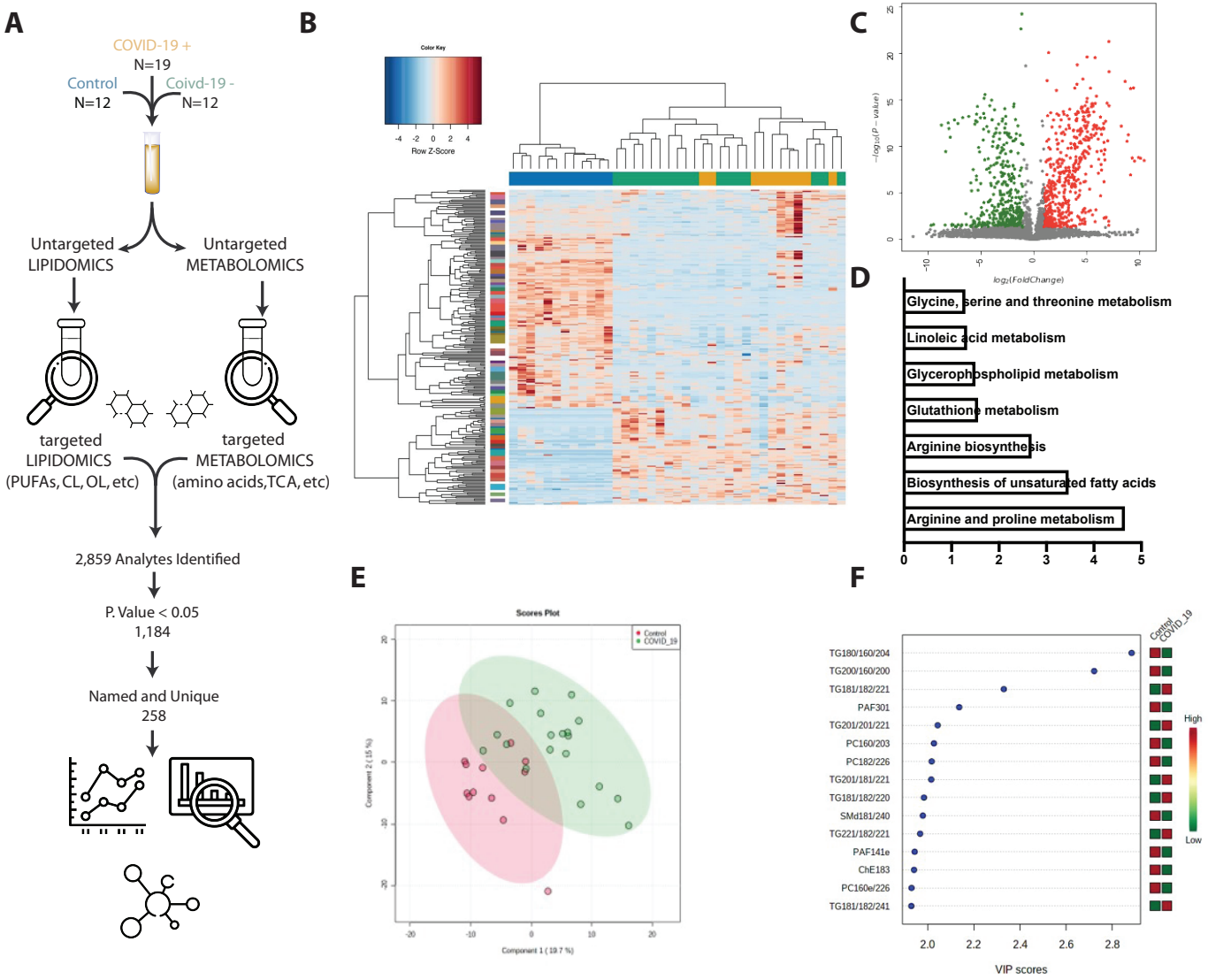
Severe COVID-19 as an endotheliopathy





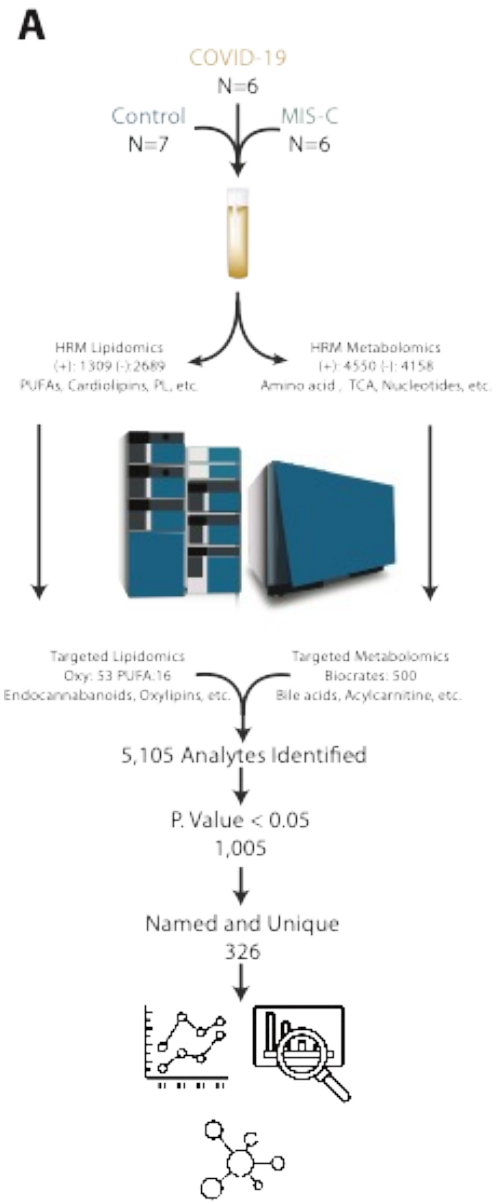


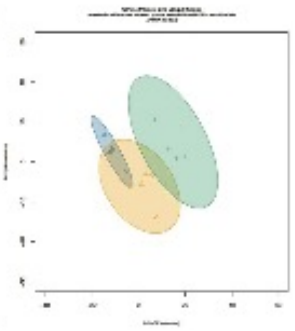


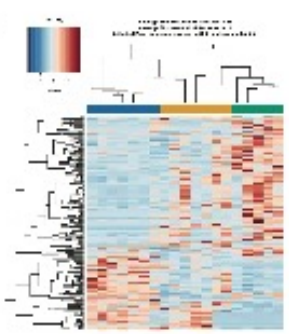


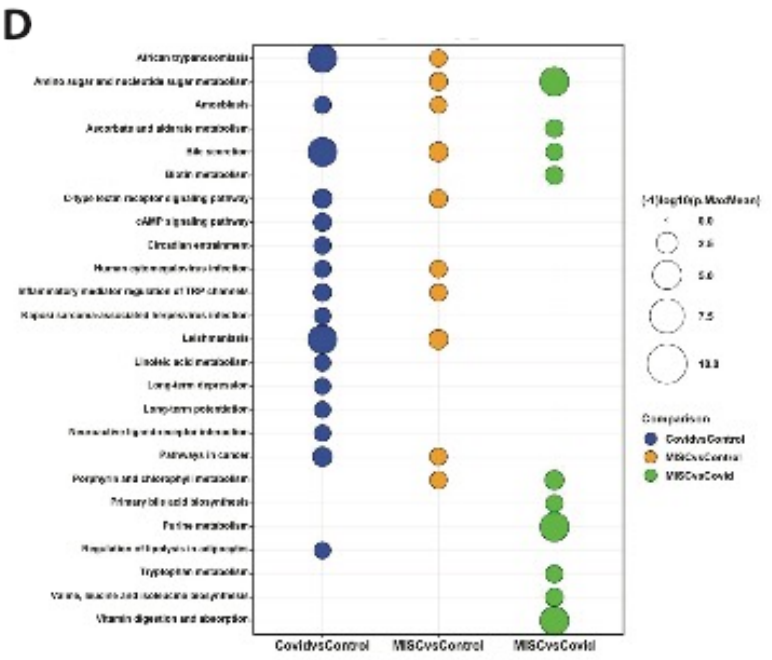
Lipidomics/metabolomics analysis in plasma from severe COVID patients reveals changes of the following pathways: arginine and prolin metabolism; arginine biosynthesis; glycin, serine, and threonine metabolism; biosynthesis of unsaturated fatty acids, linoleic acid and glycerophospholipids; glutathione metabolism.

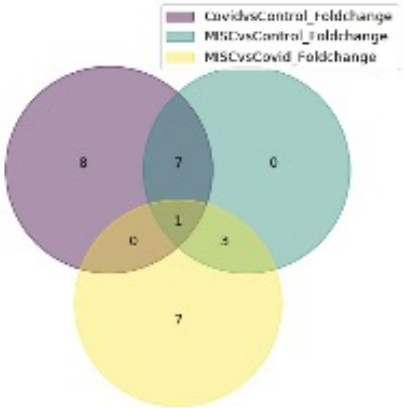
Severe COVID-19 as an endotheliopathy: lipidomics and metabolomics analysis in MIS-C

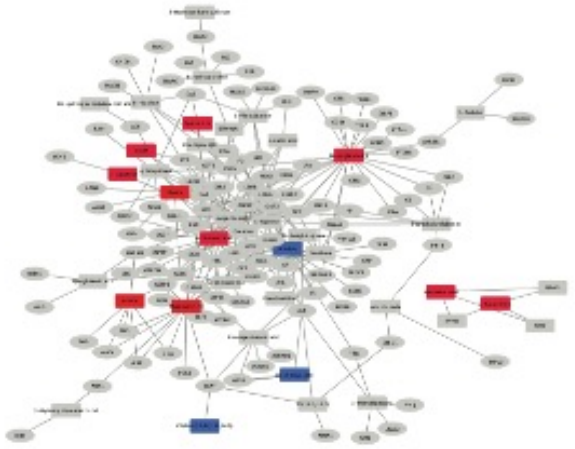


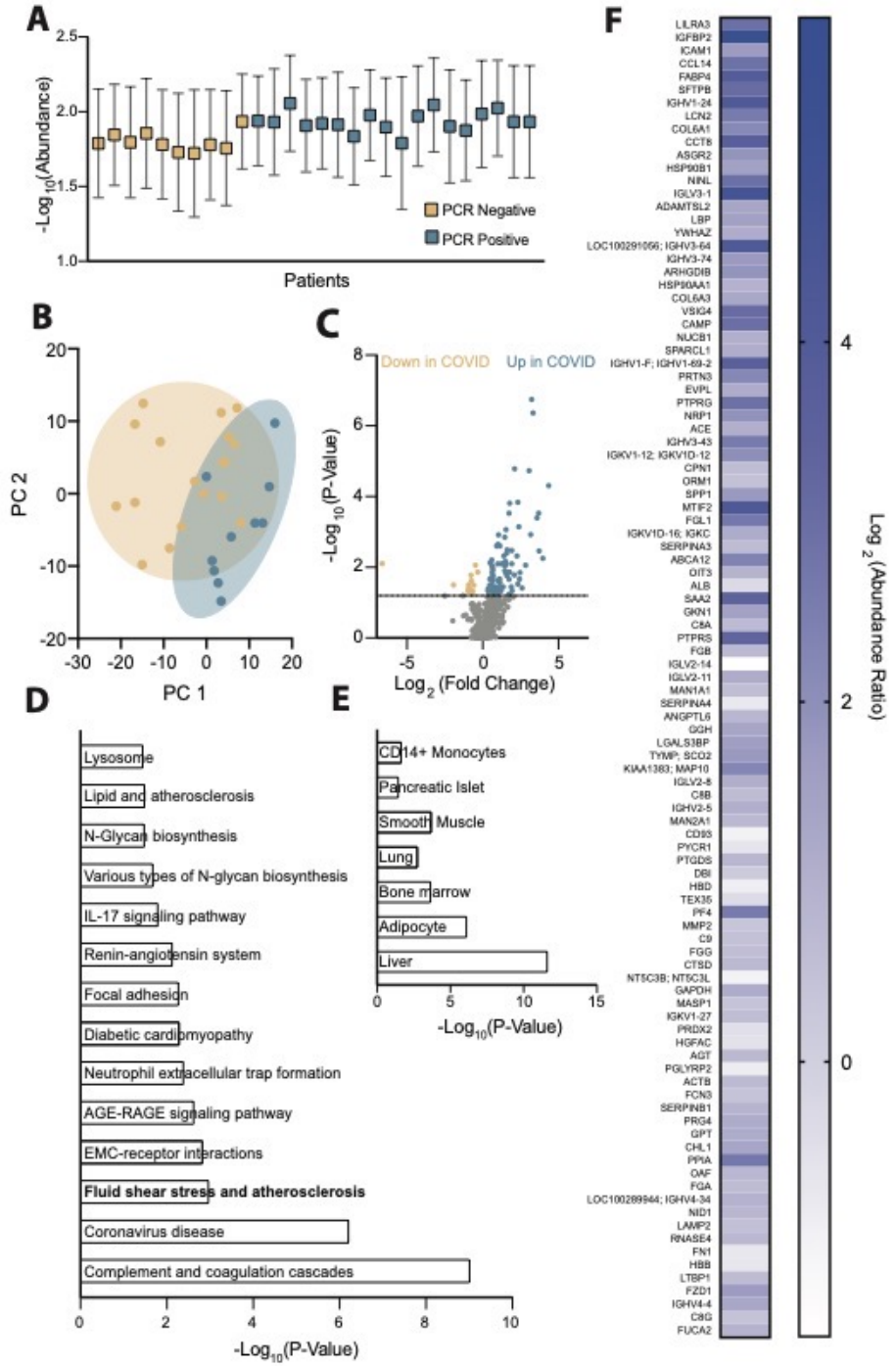










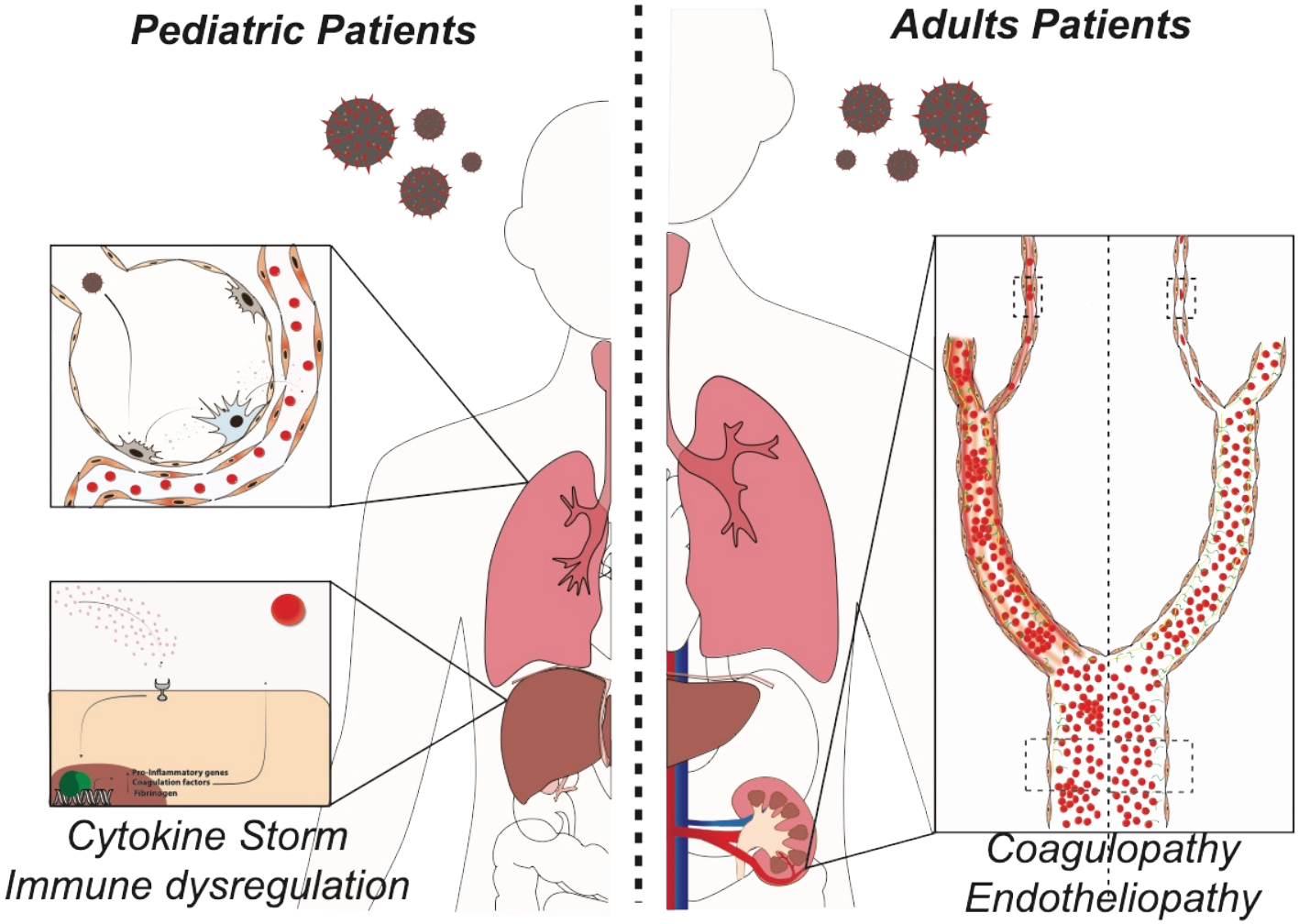


Severe COVID-19 as an endotheliopathy: proteomics analysis of the PLASMA of COVID patients (severe vs non severe), non-COVID ICU patients, and controls.

COVID-associated proteomic changes involve pathways such as:

(i) C' and coagulation cascades
(ii) IL-6/IL-6R pathway
(iii) IL-17 pathway
(iv) CD4 monocytes
(v) neutrophil trap formation
(vi) renin-angiotensin system
(vii) fluid shear stress and athero-sclerosis
(viii) focal adhesion
(ix) N-glycan byosinthesis
(x) AGE-RAGE pathway
(xi) EMC-receptor interactions

Multiplatform analyses reveal coagulopathy and endotheliopathy as key drivers of systemic pathogenesis in severe COVID-19



Druzek S, Iffrig E et al., in revision

Key unknowns about COVID-19 pathogenesis

1. **What is the role of genetic factors in influencing transmission and/or pathogenesis?**

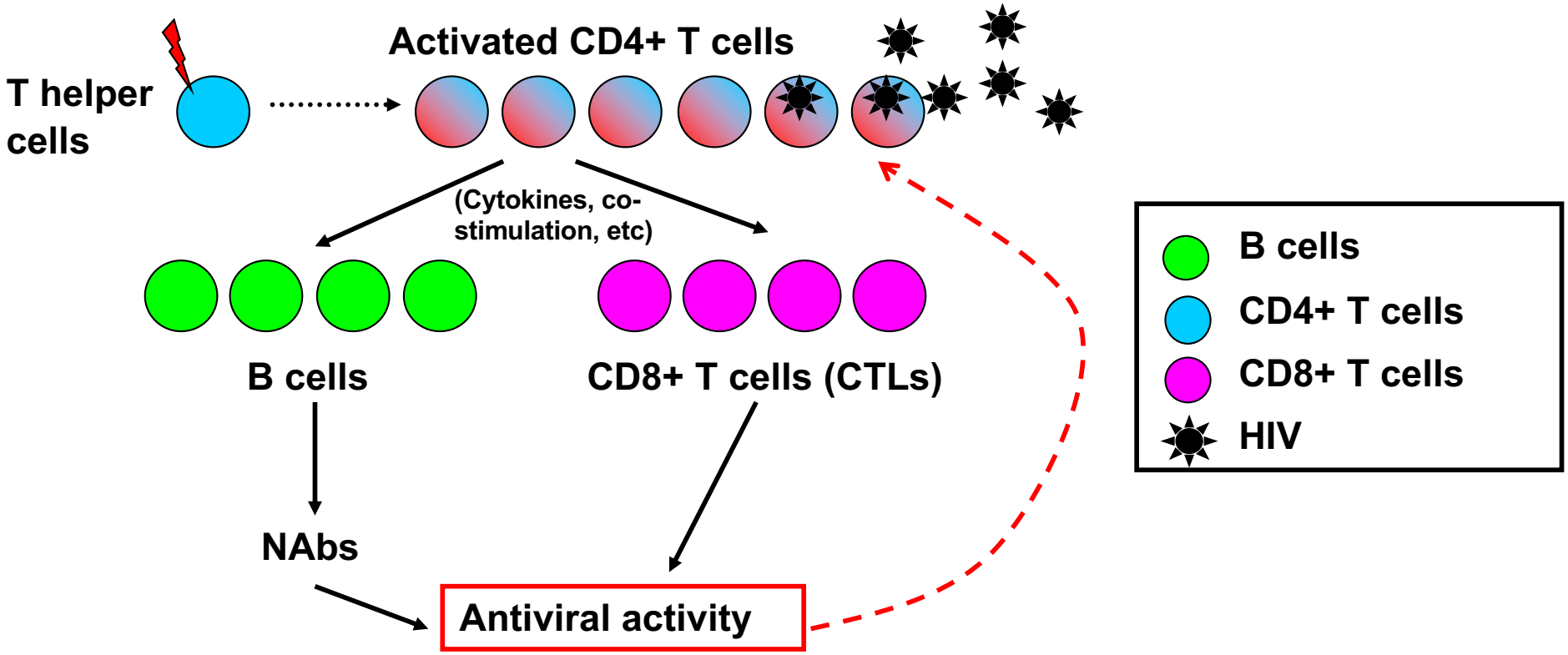
2. **What is the role of pre-existing cross-reactive immunity against other human Coronaviruses?**

3. **Why does COVID-19 have a disproportionate impact on the elderly and people with specific pre-existing conditions?**

4. **How can severe COVID-19 be treated from the point of view of patho-physiology?**

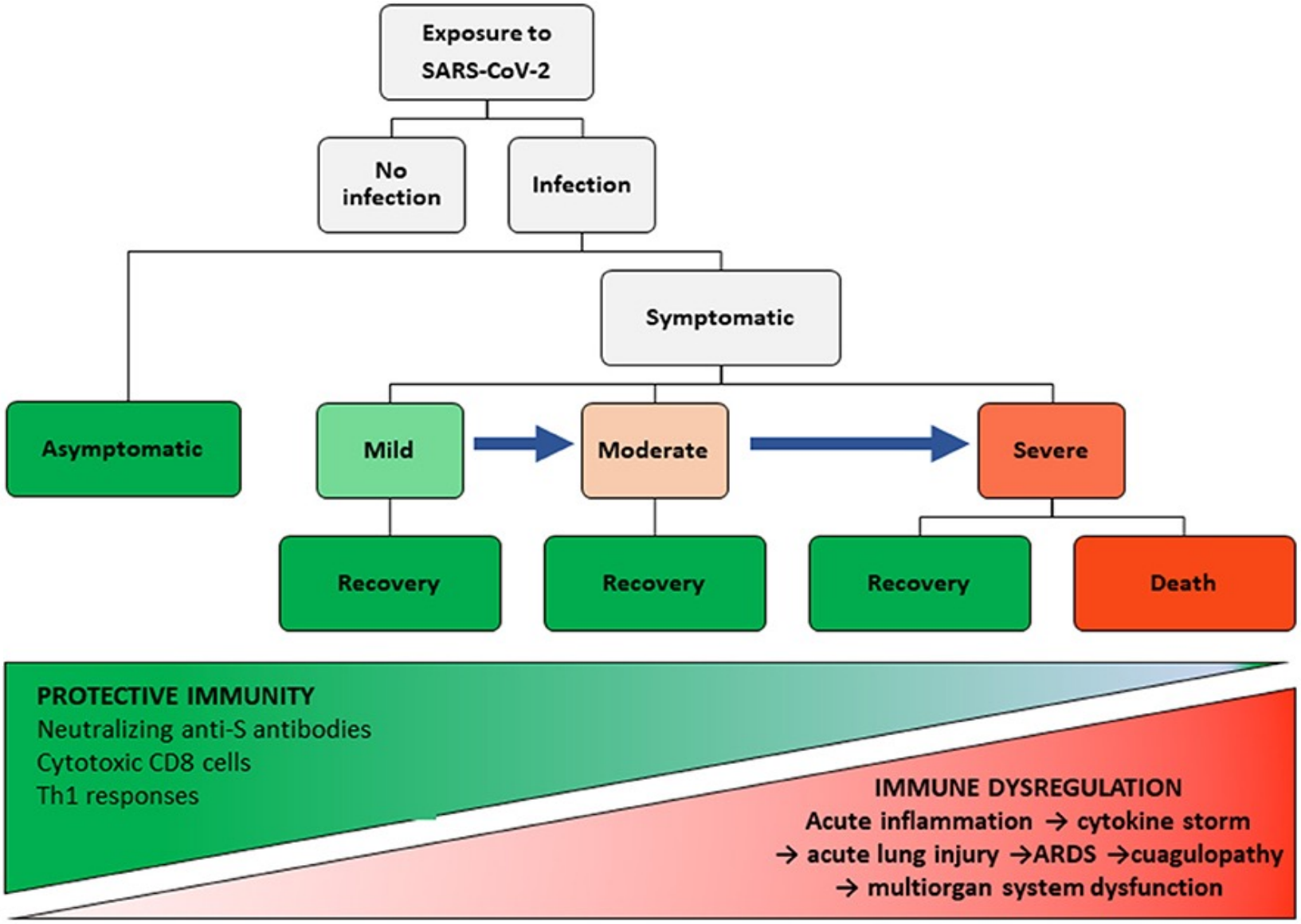
Viral pathogenesis is a COMPLICATED business!

Immune responses to HIV: a fine balancing act



The key for an immunization strategy that successfully controls HIV transmission and/or early replication is to fully exploit the antiviral role of CD4+ T cells without the side effect of creating additional target cells for the virus.

Immune responses & Immunopathology



Garcia et al., Front Immunol 2020

Immune responses & Immunopathology

Other examples of viruses that are less pathogenic in natural hosts "endemic" vs. non-natural "recent" hosts include Hantaviruses, Rabies, Nepah viruses, Ebola, Marburg, and many others.

Seal et al., Evolution of pathogen tolerance and emerging infections: A missing experimental paradigm. Elife 2021

"Researchers worldwide are repeatedly warning us against future zoonotic diseases resulting from humankind's insurgence into natural ecosystems. The same zoonotic pathogens that cause severe infections in a human host frequently fail to produce any disease outcome in their natural hosts. What precise features of the immune system enable natural reservoirs to carry these pathogens so efficiently? To understand these effects, we highlight the importance of tracing the evolutionary basis of pathogen tolerance in reservoir hosts, while drawing implications from their diverse physiological and life-history traits, and ecological contexts of host-pathogen interactions. Long-term co-evolution might allow reservoir hosts to modulate immunity and evolve tolerance to zoonotic pathogens, increasing their circulation and infectious period. Such processes can also create a genetically diverse pathogen pool by allowing more mutations and genetic exchanges between circulating strains, thereby harboring rare alive-on-arrival variants with extended infectivity to new hosts (i.e., spillover). Finally, we end by underscoring the indispensability of a large multidisciplinary empirical framework to explore the proposed link between evolved tolerance, pathogen prevalence, and spillover in the wild."

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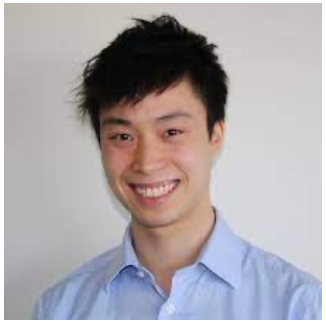




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