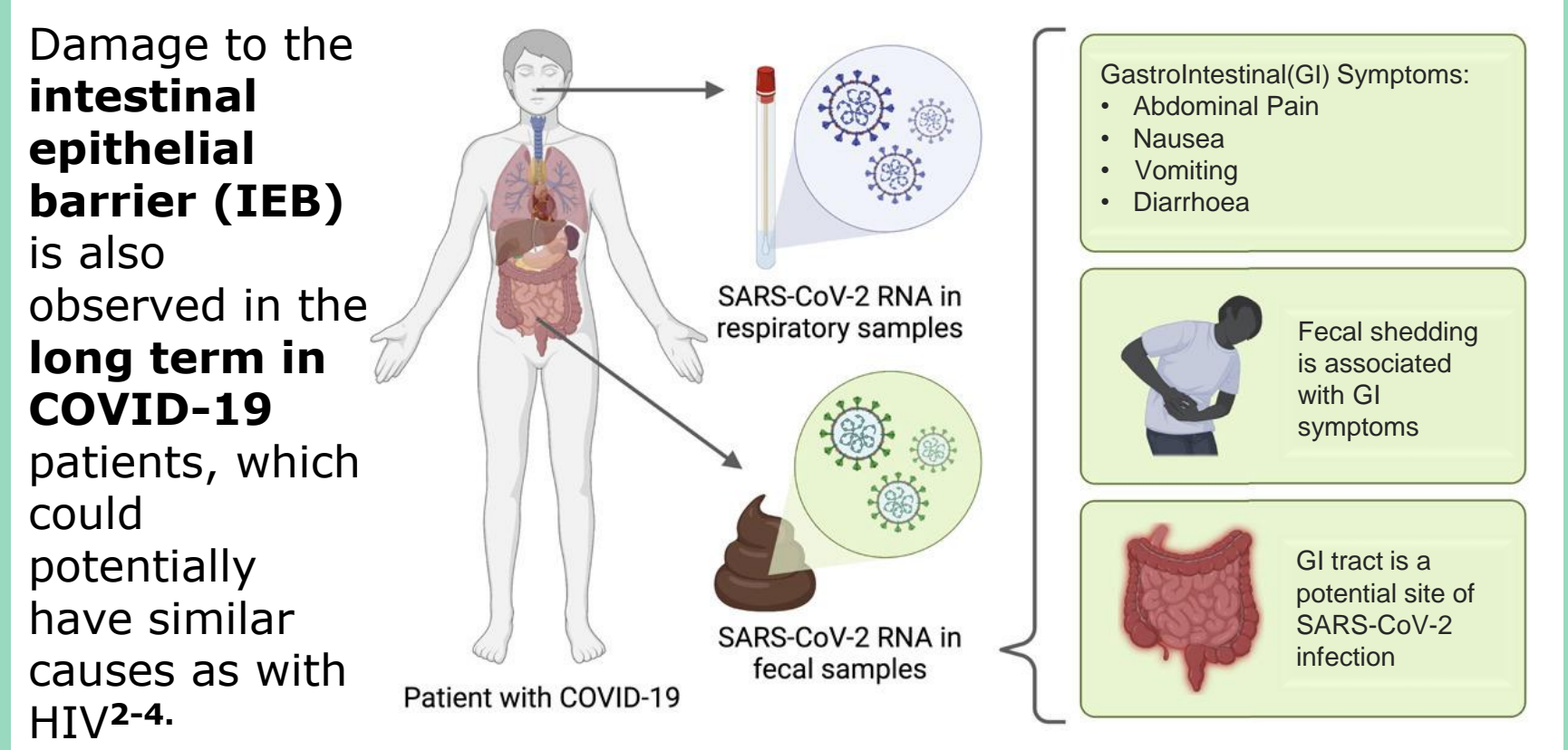
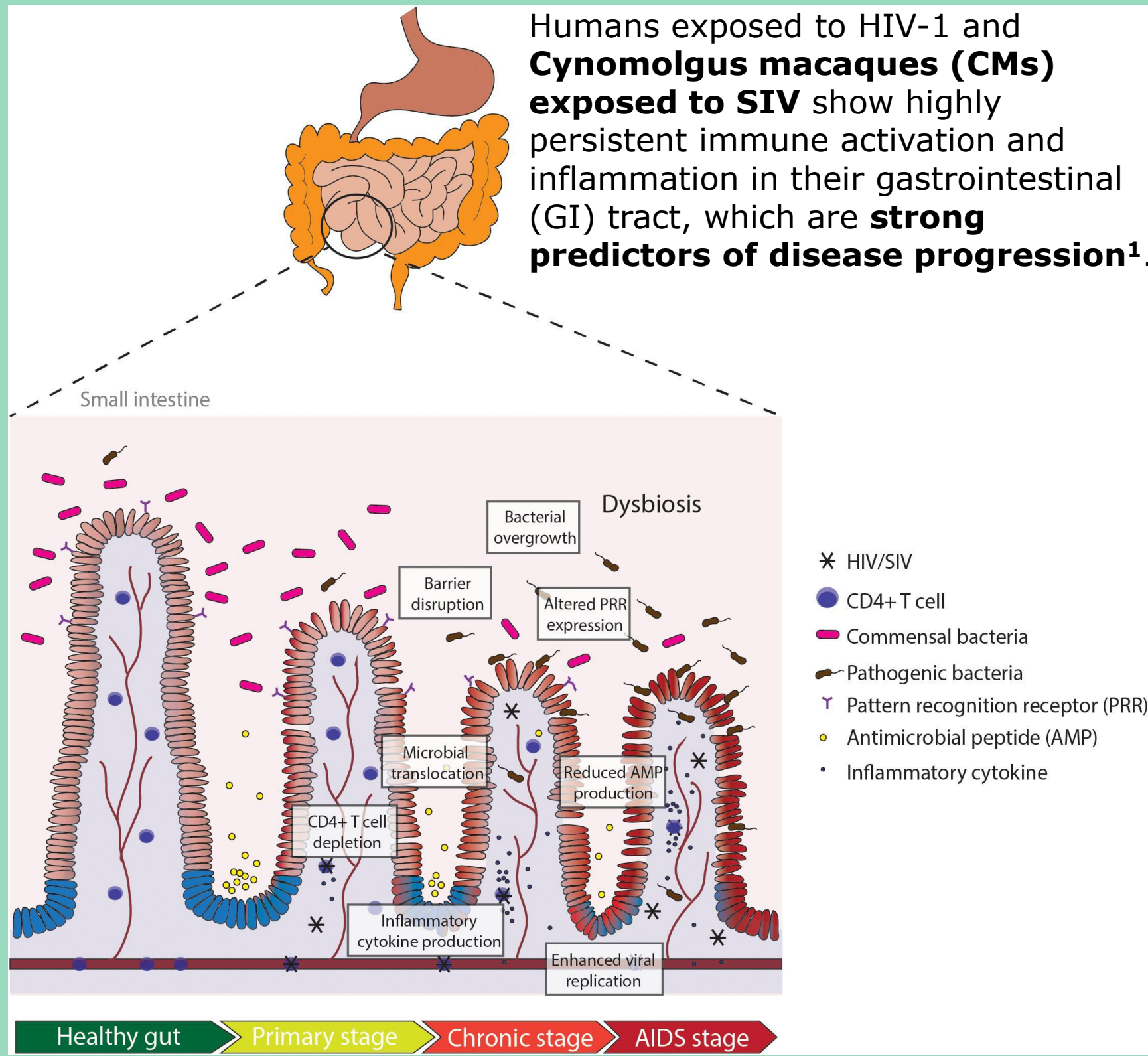


CX3CR1+ macrophages as guardians of the intestinal barrier: lessons learnt from non-human primates exposed to SIV or SARS-CoV-2.

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Introduction



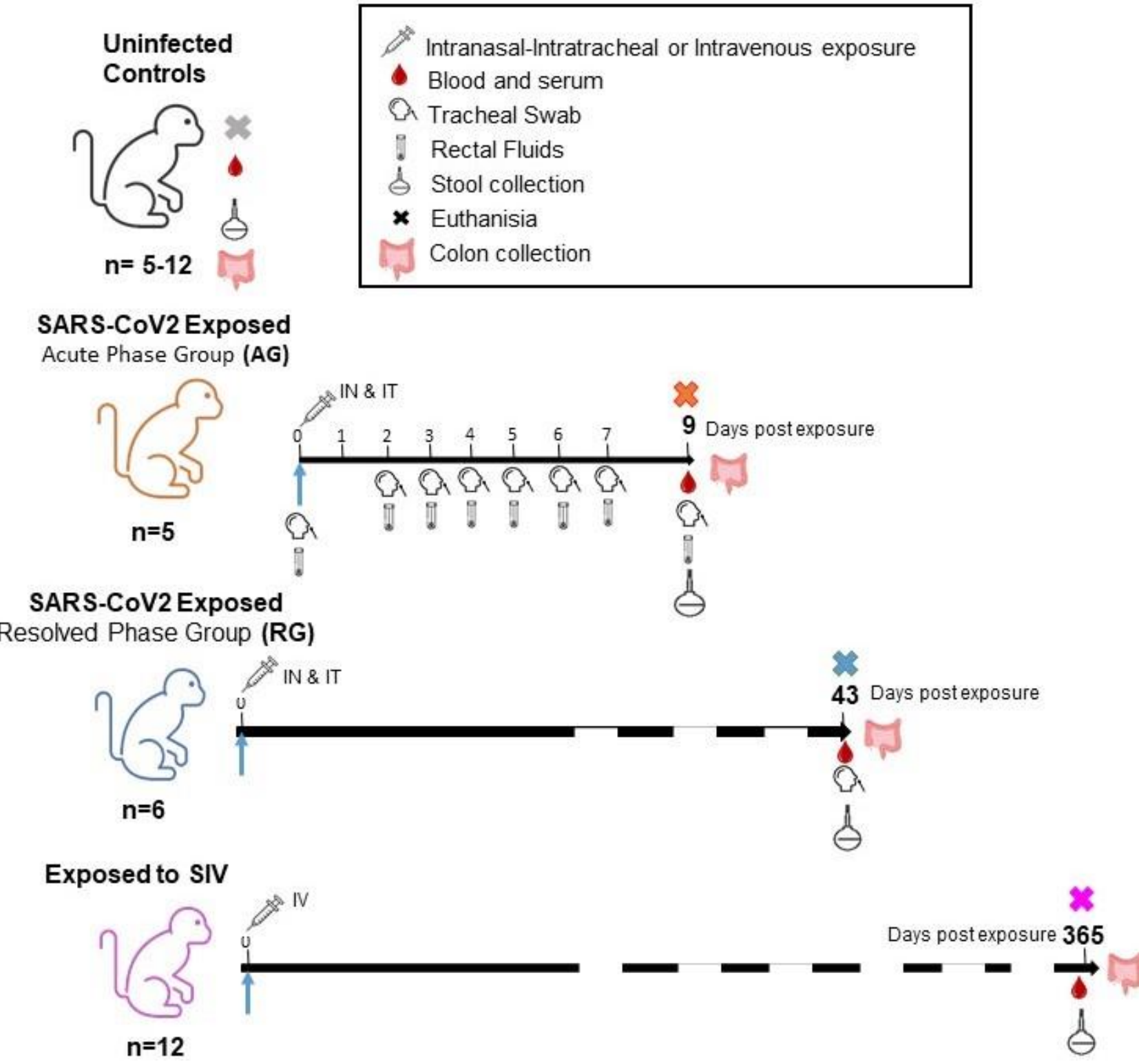
Role of CX3CR1+ macrophages (Mφ) in the GI tract⁵⁻⁹

	CX ₃ CR1 ^{high} Mφ	CX ₃ CR1 ^{low} Mφ
Phenotype	Anti-inflammatory	Proinflammatory
Products/Markers	IL-10, TGFβ, CCL17, CCL2, CD163, CD206, pSTAT3/6	TNFα, IL-1β, IL-6, IL-12, IL-23, CXCL10, pSTAT1, MMP9
Phagocytic activity	Low	High
Antigen presentation	Low	High
Functions	Induction & maintenance of T regulator activity	Induction of T effector cells
Responsiveness to TLR stimulation	Non responsive	Hyper-responsive

Hypothesis: Loss of intestinal CX3CR1+ Mφ homeostasis contributes to the IEB damage seen during SIV or SARS-CoV-2 Infection

Methods

The following groups were either uninfected controls, exposed to 10⁶-10⁵ plate-forming unit (pfu) of the BetaCoV SARS-CoV-2 strain or 1000 animal infectious dose (AID)₅₀ SIVmac251 for the specified time points.



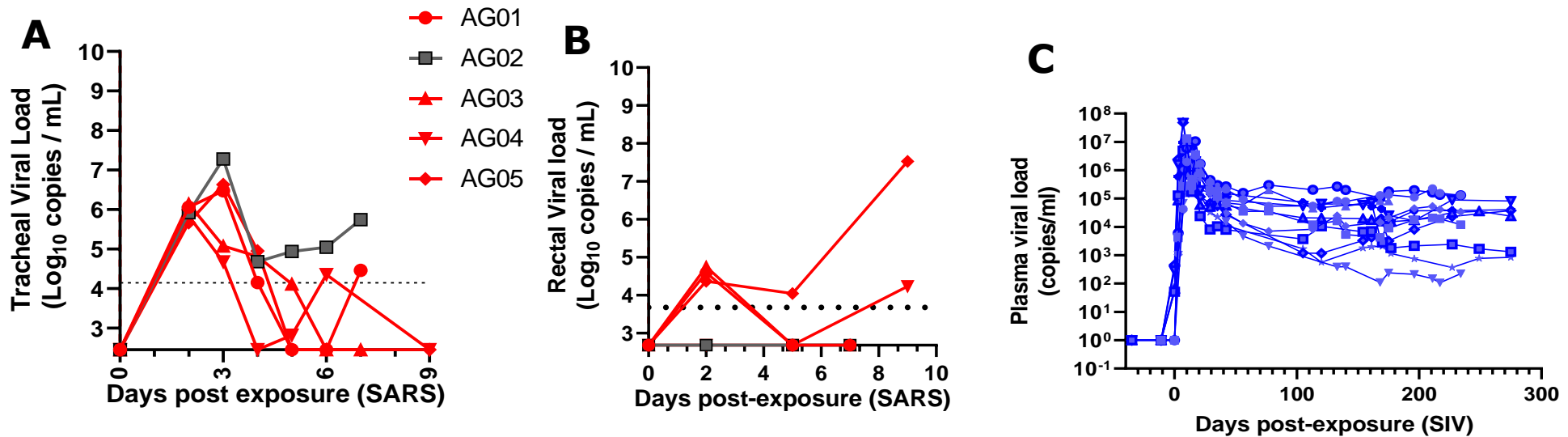
-Each **colon** were processed to **lamina propria mononuclear cells (LPMC)** on euthanasia and stained to analyse the Mφ populations by **flow cytometry** (data in Fig2 and Fig3).

-For the **SARS-CoV-2 exposed AG**, the **viral load** was estimated till euthanasia from tracheal swabs and rectal fluid. Viral load was estimated for SIV exposed CM from plasma (data in Fig1).

-**Fractalkine**, **sCD14** and **iFABP** levels were estimated from **serum**, while **calprotectin** was assessed from **stool** (data in Fig4).

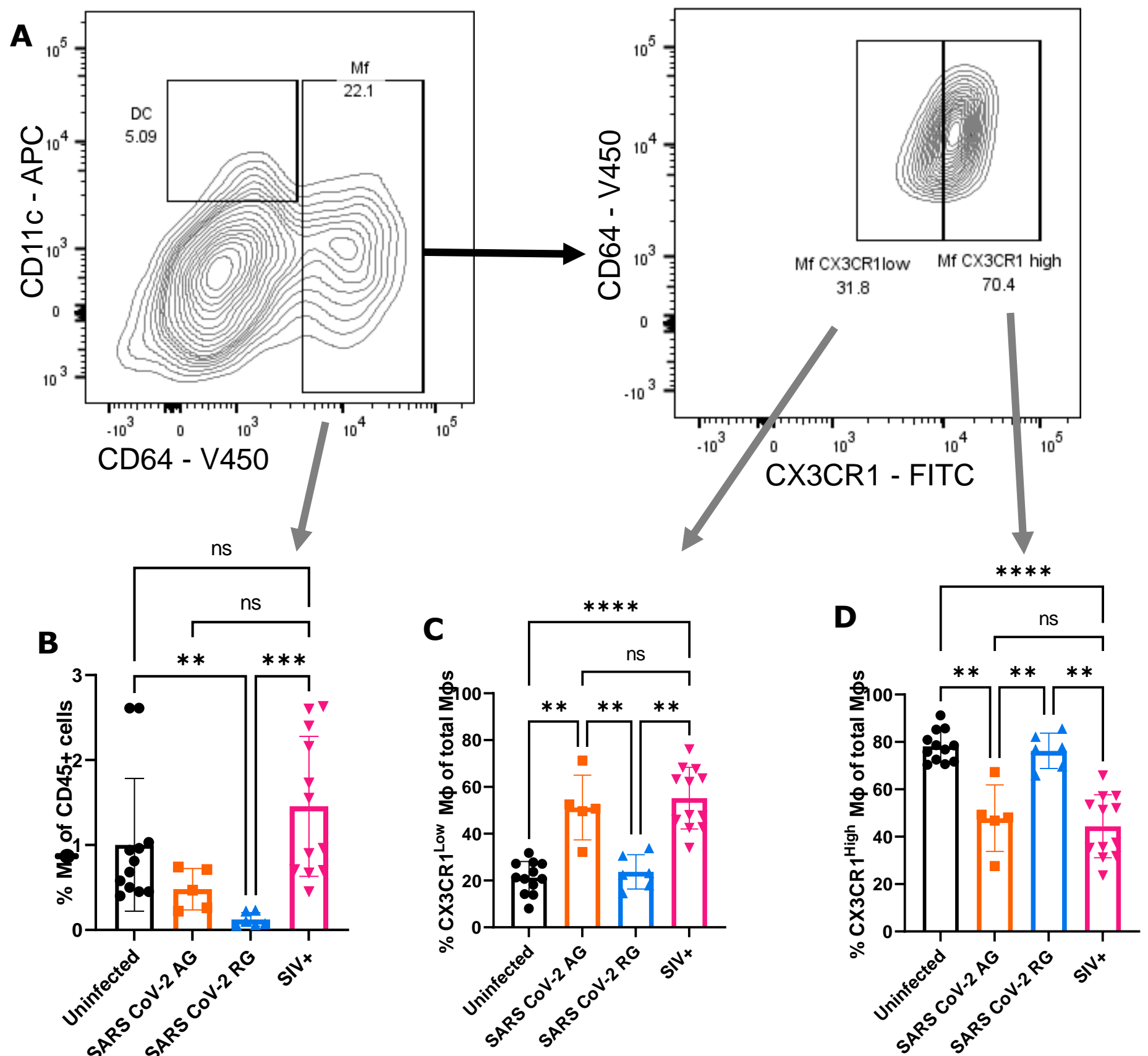
Results

Fig 1. Viral load detection in SARS-CoV-2 and SIV infected animals



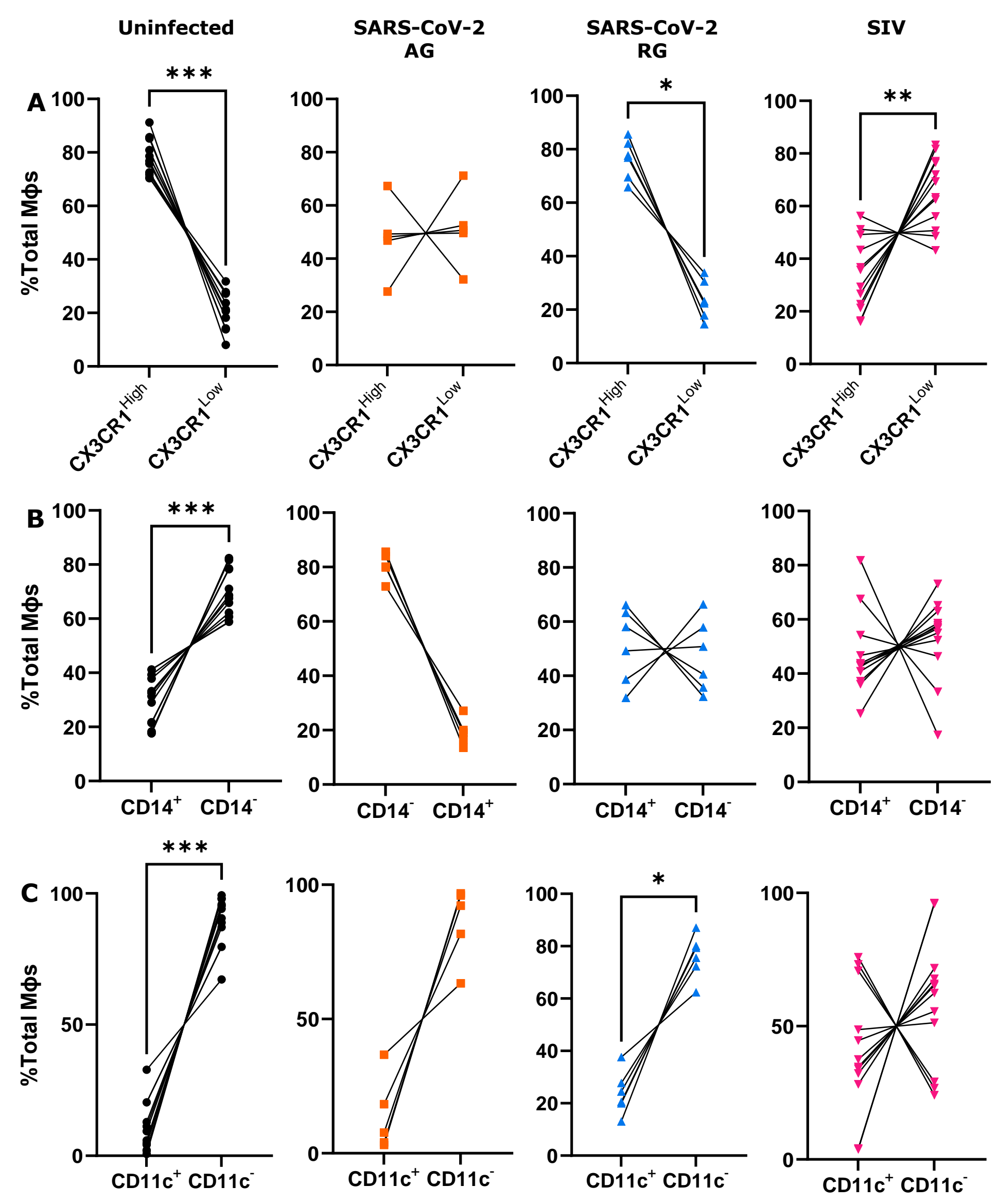
Detectable rectal viral load in 4/5 macaques (Fig1B) up to 9 days post exposure to SARS-CoV-2 in AG (while all have detectable tracheal viral load, including RG, Fig1A and data not shown), however by 43 days no virus is detected in RG (not shown). Meanwhile, SIV infected CMs have a steady plasma viral load for the year post exposure (Fig1C).

Fig 2. Flow gating and frequency of CX3CR1 (high/low) Mφ populations



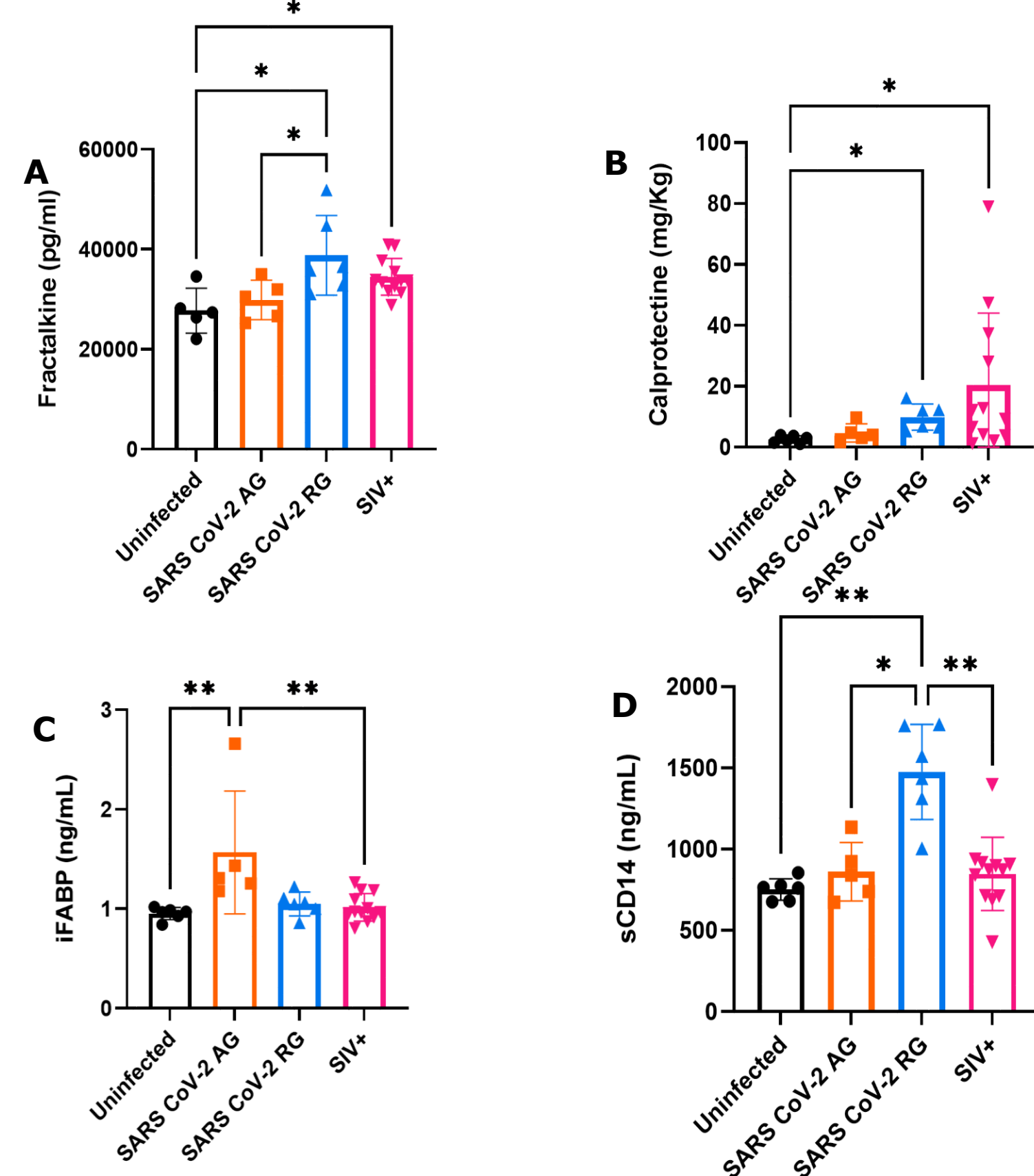
Mφ were identified among live, single cells, CD45⁺ cells as lineage negative (CD3⁻, CD20⁻ and CD8⁻), HLA-DR⁺ and CD64⁺ cells. While total Mφs are reduced in SARS-CoV-2 exposed RG, their frequency is similar to uninfected in chronic SIV exposed CM (Fig2B). However, in both SARS-CoV-2 AG and SIV+ animals, Mφs are mostly CX3CR1^{Low} inflammatory cells (Fig2C).

Fig 3. Expression CX3CR1, CD11c and CD14 by total colonic Mφs



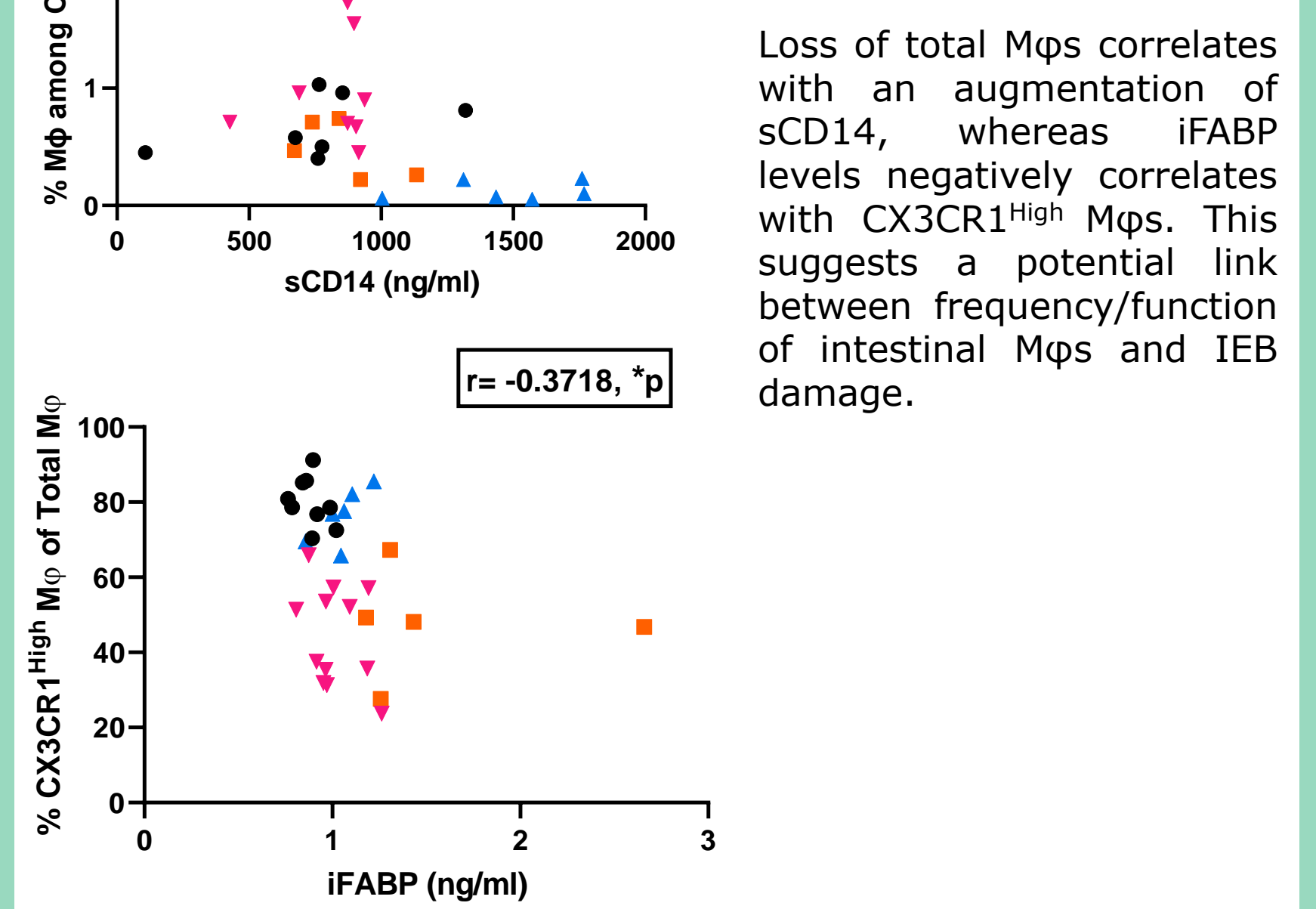
Uninfected CMs are predominantly expressing CX3CR1^{High}, CD14⁻ and CD11c⁻ (mature) Mφs (Fig3A-C). Mφs from SARS-CoV-2 exposed RG are CX3CR1^{High}, CD11c⁻ Mφs (Fig3A,C). However, despite one year of SIV exposure, Mφs are predominantly CX3CR1^{Low} (inflammatory) cells (Fig3A).

Fig 4. Soluble factors changes after SARS-CoV-2 and SIV infection



Intestinal damage/inflammation factors calprotectin and fractalkine are upregulated in SARS-CoV-2 RG and SIV infected CMs (Fig4A-B). Meanwhile, Intestinal Fatty Acid Binding Protein (iFABP) is upregulated only in the SARS-CoV-2 exposed AG (Fig4C), while soluble CD14 (sCD14) is upregulated in the SARS-CoV-2 exposed RG (Fig4D). Thus, some signs of IEB damage observed post SARS-CoV-2 exposure, are similar to those observed in SIV exposed CMs.

Fig 5. Spearman correlation analysis performed to investigate the relationship between Mφs and soluble factors



Loss of total Mφs correlates with an augmentation of sCD14, whereas iFABP levels negatively correlates with CX3CR1^{High} Mφs. This suggests a potential link between frequency/function of intestinal Mφs and IEB damage.

Conclusions

Uninfected	SARS-CoV-2 AG	SARS-CoV-2 RG	SIV+
Predominant anti-inflammatory, resident and mature Mφs	CX3CR1 ^{Low} Mφs	CX3CR1 ^{High} and mature Mφs but total Mφs	CX3CR1 ^{Low} Mφs
Very little expression of soluble factors	iFABP	Calprotectin, Fractalkine, sCD14	Calprotectin, Fractalkine

Our results suggest that the **maintenance of Mφ homeostasis is vital to the preservation of the GI barrier** and the **short and long-term response to viral exposures**, be it with HIV-1 or SARS-CoV-2.

Future work will try to cover more conditions such as interactions with T/B cells (to mitigate adaptive responses) or to assess changes post treatment, especially with long term combination Anti-Retroviral therapy (cART).

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