Supplementary Figure Legends

Supplementary Figure 1: AHIV plasma inhibits DCs stimulated with a range of TLR agonists

DCs were treated overnight with 10% control uninfected donor plasma or plasma from AHIV subjects. AHIV plasma corresponds to Fiebig stages 1-2 of infection when there is peak viremia. The next day, DCs were stimulated with 100ng/mL LPS + 500U/mL IFNγ, 2μg/mL poly I:C, 10μM R848, or 5μg/mL PGN and cytokine production was analyzed the following day (PGN-stimulated DCs do not produce substantial IL-12p70). The *p* values (unpaired student t test) for comparisons between groups are shown.

Supplementary Figure 2: AHIV plasma is inhibitory relative to plasma-free media

DCs were treated overnight with 10% control uninfected donor plasma, plasma from AHIV donors, or media only with no plasma. AHIV plasma corresponds to Fiebig stages 1-2 of infection when there is peak viremia. The next day, DCs were stimulated with poly I:C and cytokine production was analyzed the following day. Data is representative of at least 3 independent experiments. The *p* values (one way ANOVA with Tukey post-test) for indicated comparisons are shown.

Supplementary Figure 3: Inhibition of DCs by plasma from subjects at different stages of HIV infection

(A) DCs were treated overnight with 10% normal, uninfected donor plasma, plasma from AHIV donors, or chronic HIV or LTNP subjects. The next day, DCs were stimulated with poly I:C and cytokine production was analyzed the following day. Data is representative of at least 3 independent experiments. **(B)** IFNγ production from allogeneic CD4+ T cells co-cultured with

DCs treated as in **A**. Data is representative of at least 3 independent experiments. The *p* values (one way ANOVA with Tukey post-test) for indicated comparisons are shown.

Supplementary Figure 4: Elevated apoptotic MPs in AHIV plasma are CD41-

(A) Plasma from uninfected donors or AHIV donors (from Fiebig stages 1-2 of infection when there is peak viremia) was ultra-centrifuged at 100,000g for 30 mins and the pelleted fraction resuspended in plasma-free media. The pelleted fraction was analyzed for MPs by FACS, with background debris excluded by gating on a sample of media only. Shown are representative plots from uninfected plasma or AHIV plasma. (**B)** CD41 expression on MPs from uninfected plasma or AHIV plasma. Black line is CD41 stain and blue line is respective isotype control staining. (**C**) Uninfected plasma- and AHIV plasma-derived MPs were stained for CD41, and various lineage markers: CD3 (T cells), CD19 (B cells), CD14 (Monocytes), and CD16 (NK cells) and analyzed by FACS.

Supplementary Figure 5: Electron micrograph of apoptotic MPs

Transmission electron micrograph of apoptotic MP preparation from UV-irradiated PBMCs. Magnification is at 40,000x. Small membrane-bound fragments are observed ranging from 0.1-1 μm in size.

Supplementary Figure 6: Mass spectrometric analysis of MPs

Graphical representation of proteins associated with apoptotic MPs only, control MPs only, or both as analyzed by mass spectrometric analysis of three separate preparations of MPs. Isolates that were excluded from the overlapping circles graphic include HLA histocompatibility antigens due to the fact that MPs are derived from different allogeneic donor PBMCs, keratin (common contamination from human skin during protein preparation) and peptides that failed to correspond to a known protein. Selected proteins unique to either

apoptotic or control MPs are also indicated, and a full list of MP-associated proteins is provided in Supplementary Tables 1 A and B.

Supplementary Figure 7: Apoptotic MPs, not HIV-1, are responsible for DC inhibition

(**A**) Plasma from uninfected donors or acute HCV donors (at peak viremia) was ultra-centrifuged at 100,000g for 30 mins and the pelleted fraction containing MPs was resuspended in plasma-free media. DCs were then treated with MPs or plasma-free media control both with or without live HIV-1 (30 ng/mL of p24) and subsequently stimulated with poly I:C. IL-12p70 secretion was then assessed. (**B**) Uninfected or HIV-1 infected PBMCs were UV-irradiated to induce apoptosis and cell-free supernatant wase either filtered through a 0.2 μM filter (to remove MPs) or directly ultra-centrifuged to isolate MPs. Note: MPs removed from filtered supernatant but virus remains. Controls consisted of control MPs from uninfected or HIV-infected PBMCs (unfiltered). DCs were treated with above preparations and subsequently stimulated with poly I:C. IL-12p70 secretion was then assessed.

Supplementary Figure 8: CD44 is elevated on DCs from HIV-infected individuals during acute and early infection

PBMC from uninfected control subjects or HIV-infected individuals sampled at Fiebig stages 1-4 of AHIV or a time point in early infection (approx. 6 months post-seroconversion) were stained for identification of myeloid DCs, and CD44 expression was analysed on these cells. The *p* value (Mann-Whitney U-test) for comparison of the uninfected control and HIV-infected groups is shown.

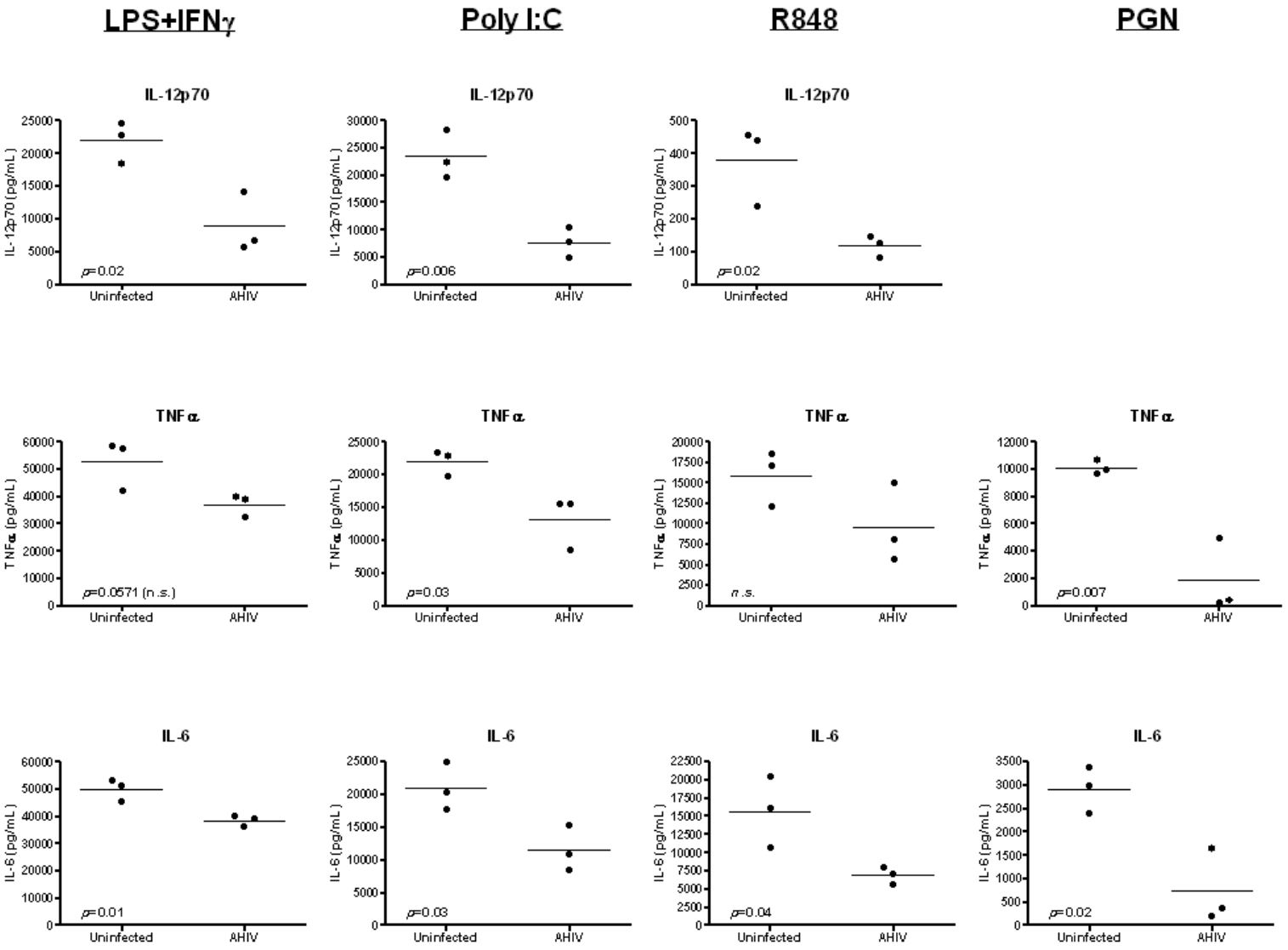
Supplementary Figure 9: Blockade with Annexin V or MFG-E8 fails to alleviate apoptotic MP-mediated DC inhibition

DCs were treated overnight with 50 μL preparation of control or apoptotic MPs that were pre-treated or not with 50 μg/mL Annexin V (from human plasma) or 50 μg/mL MFG-E8. DCs were also treated with 50 μL of media only with or without 50 μg/mL Annexin V or MFG-E8 (no MP control). The next day, DCs were stimulated with poly I:C and cytokine production analyzed the following day.

Supplementary Tables 1 A and B: MP-associated proteins identified by mass spectrometry

List of proteins found in apoptotic MPs (Supplementary Table 1A) or control MPs (Supplementary Table 1B) by mass spectrometric analysis of 3 separate MP preparations. Proteins identified are listed (numbers: column A) including the following information: column B: accession number (UniProtKB/Swiss-Prot); column C: protein description; column D: protein score (Mascot, www.matrixscience.com); column E: protein molecular mass; column F: MS/MS queries matching to this protein; column G: peptide sequences matching to this protein; column H: protein sequence coverage [%].

Supplementary Figure 1: AHIV plasma inhibits DCs stimulated with a range of TLR agonists



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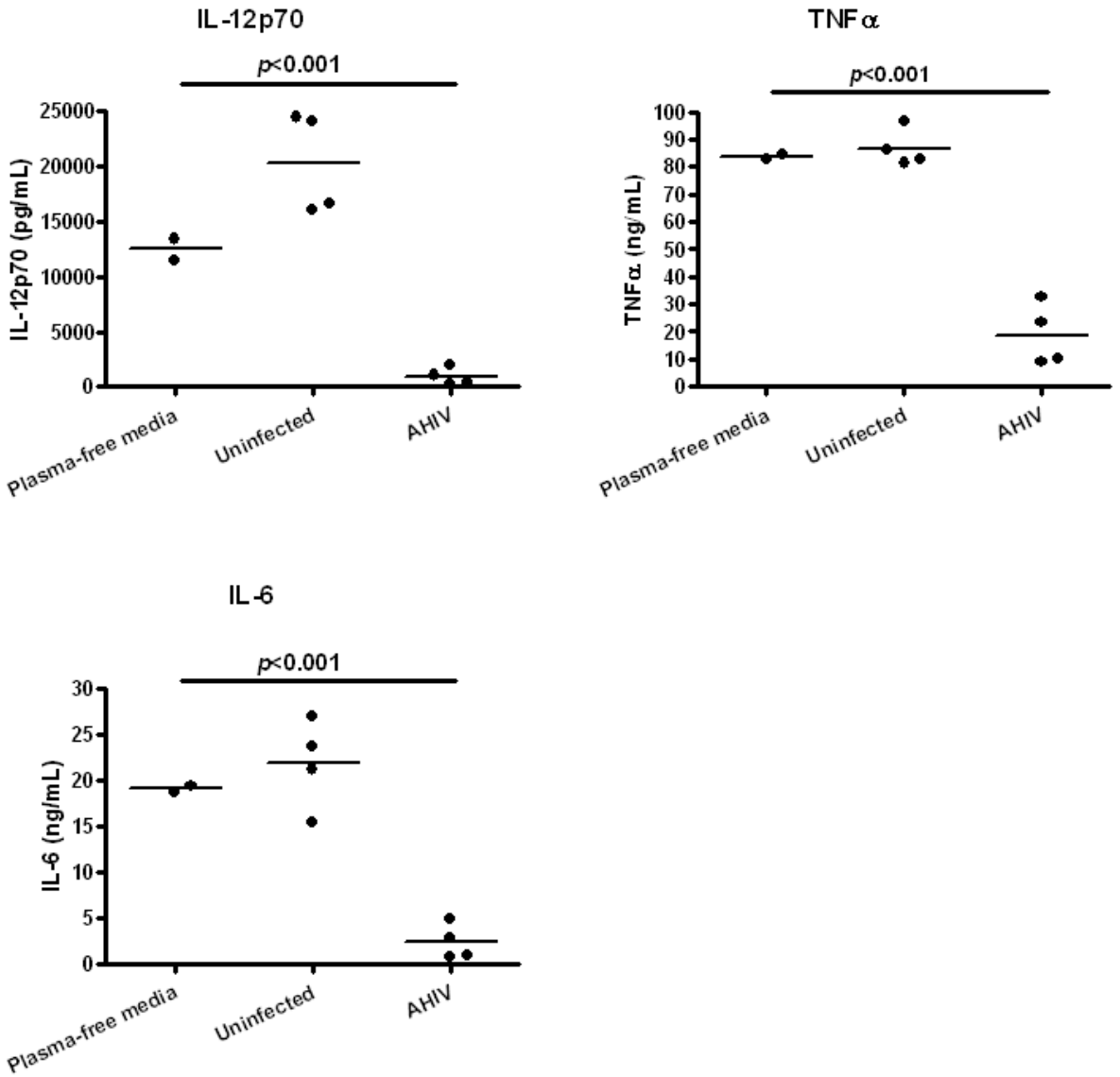
Supplementary Figure 2: AHIV plasma is inhibitory relative to plasma-free media



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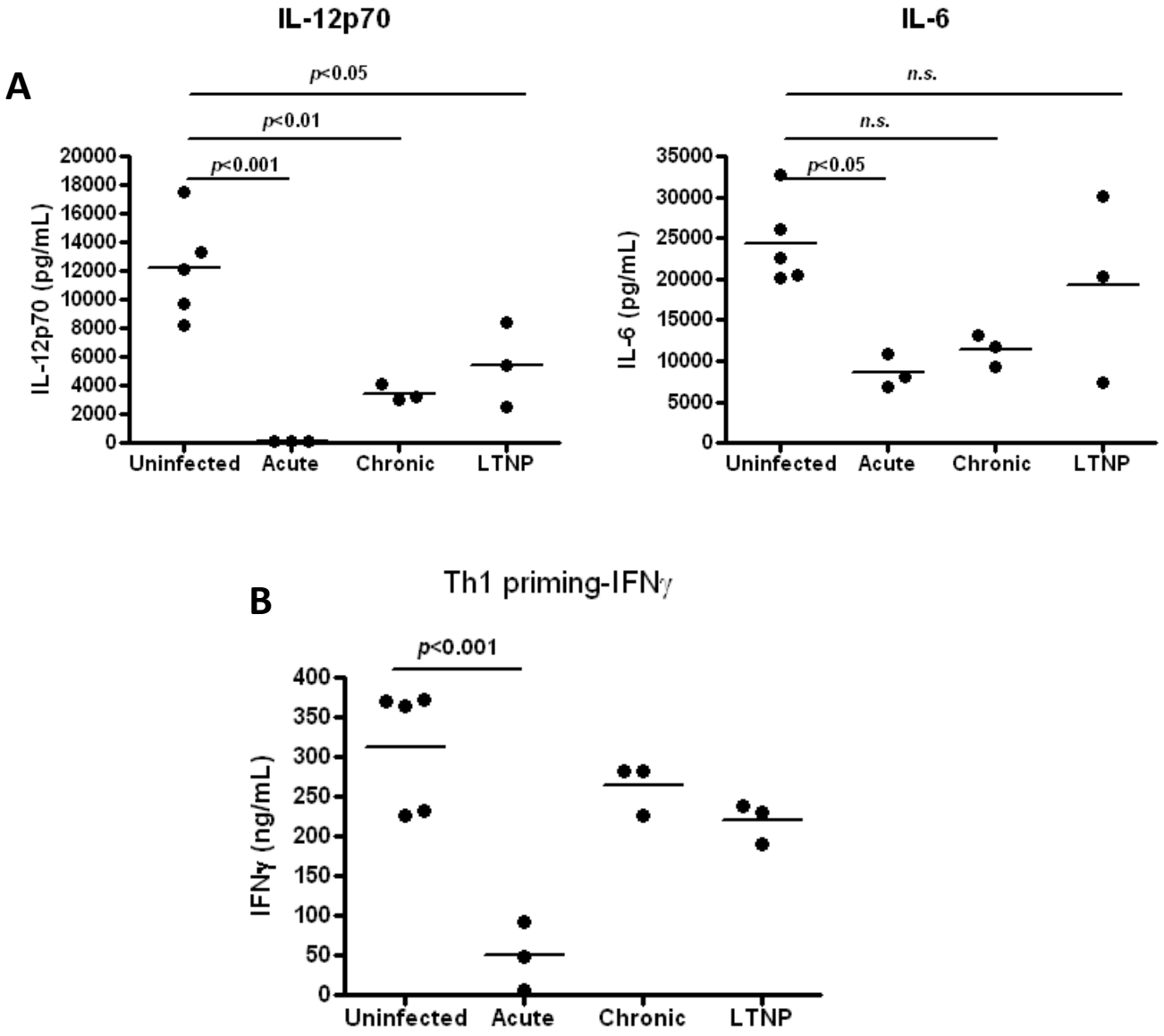
Supplementary Figure 3: Inhibition of DCs by plasma from subjects at different stages of HIV infection



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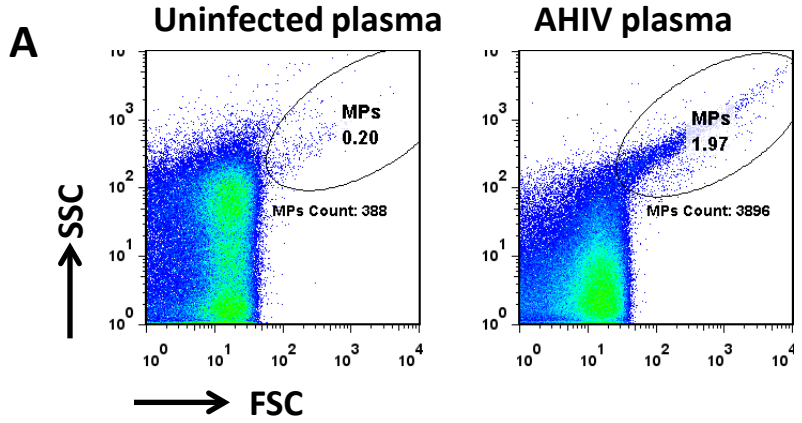
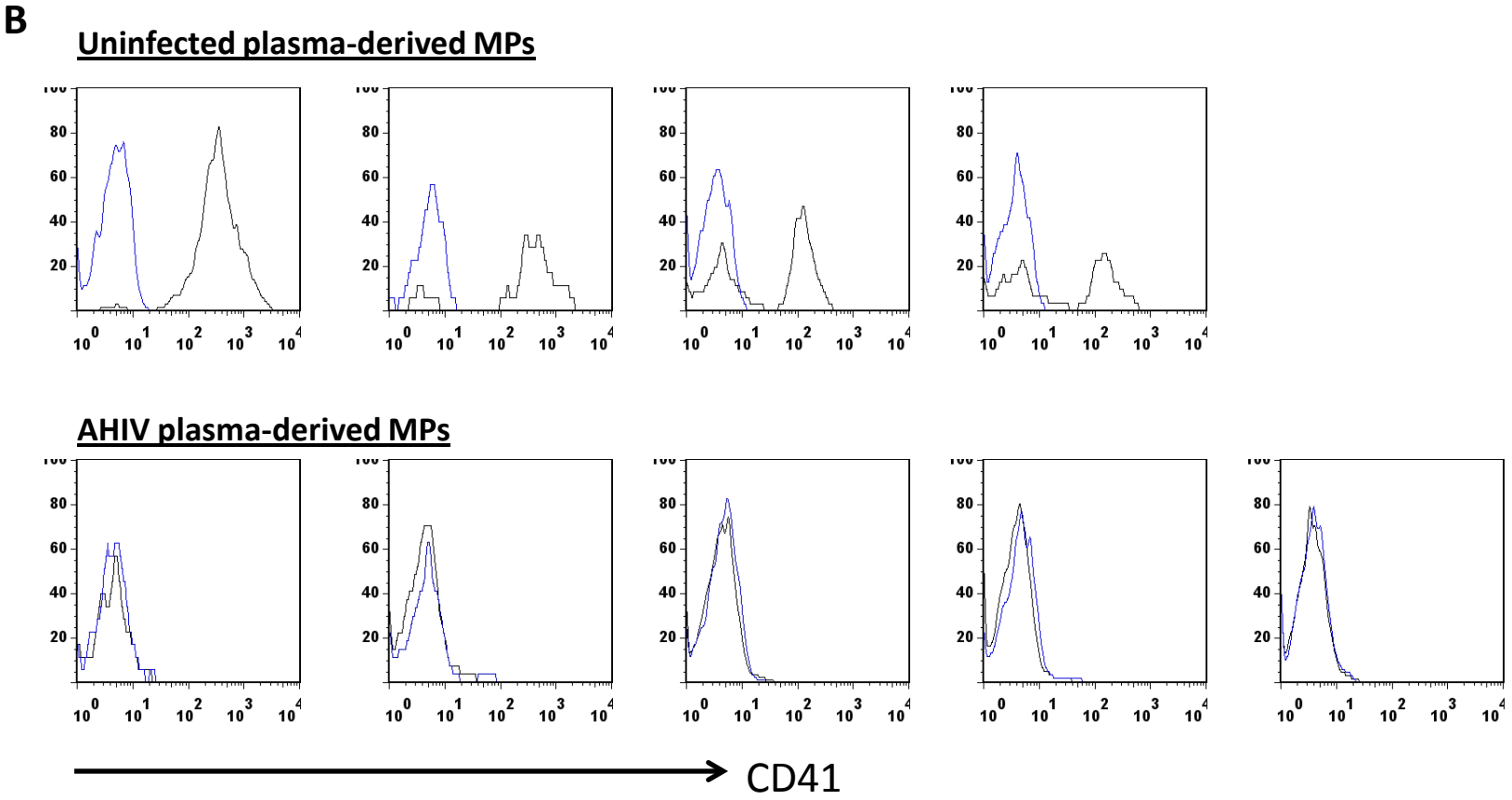
Supplementary Figure 4: Elevated apoptotic MPs in AHIV plasma are CD41-





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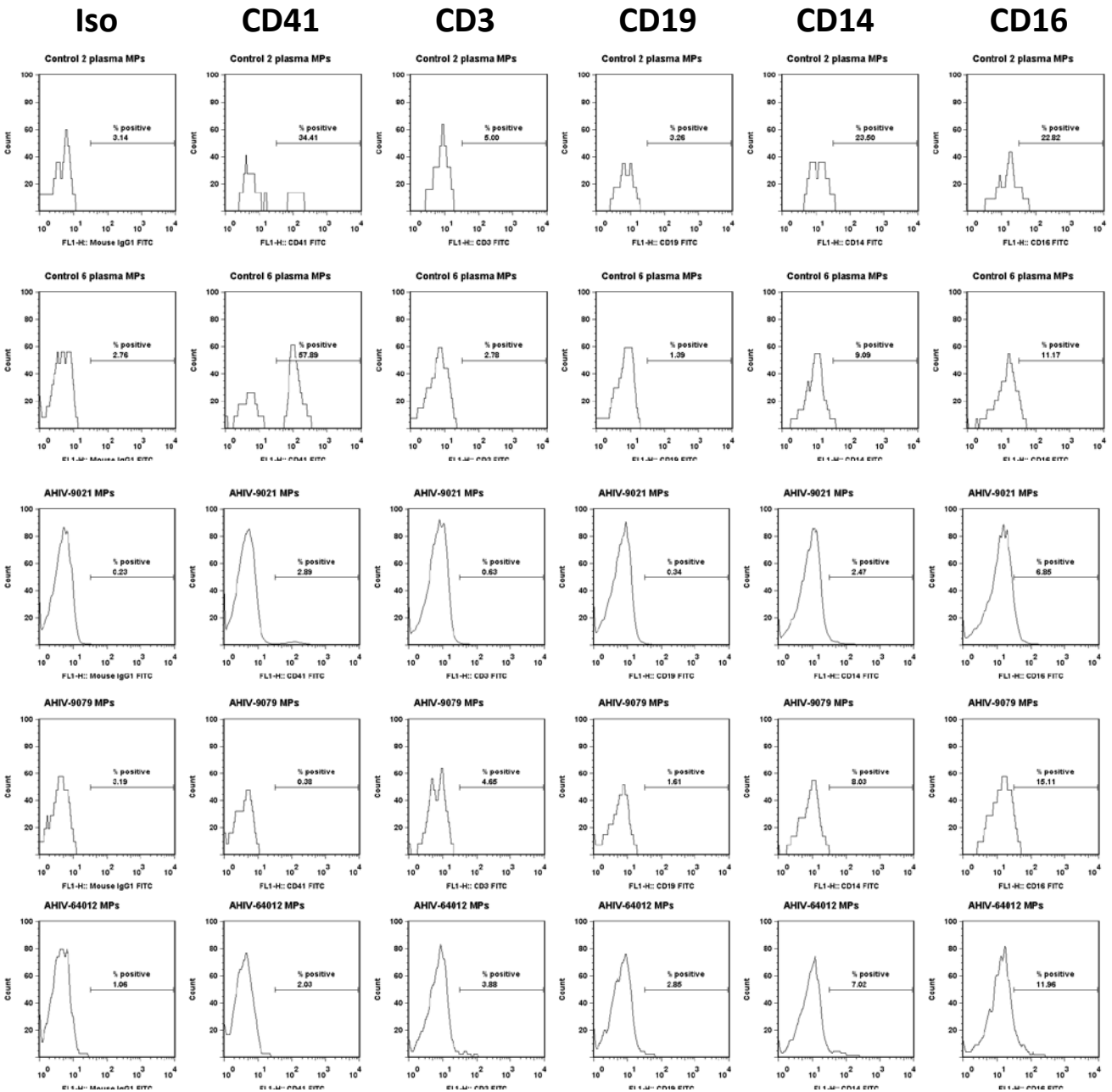
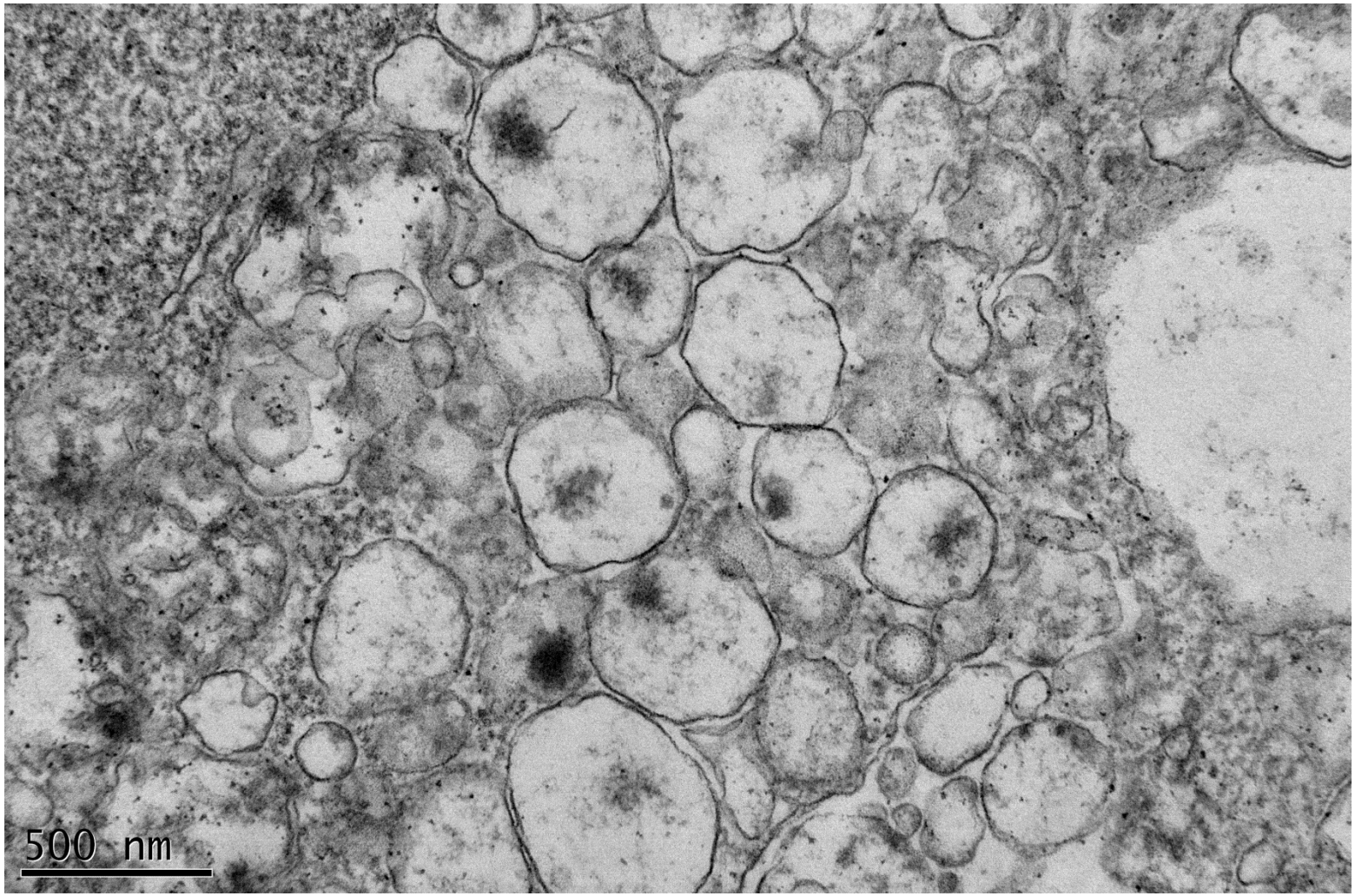


Supplementary Figure 5: Electron micrograph of apoptotic MPs



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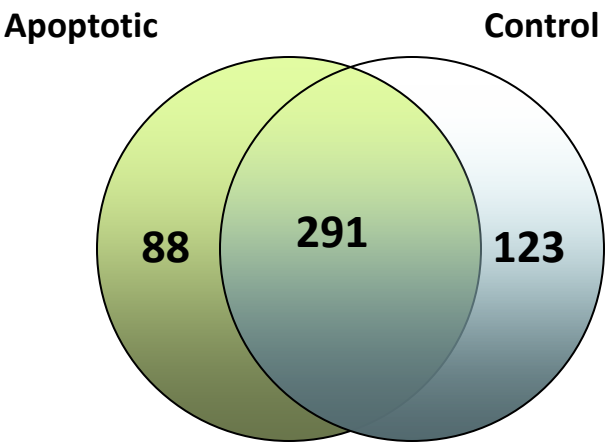
Supplementary Figure 6: Mass spectrometric analysis of MPs

Apoptotic MPs only:

Arp2/3 complex
Calpastatin
Cathepsin S
Endoplasmic reticulum aminopeptidase
ERO1-L-α
ERp44
Ficolin-1
hASC
HIP-1α
HSP70
KIR 3DP1, 2DP1, 2DS5
Multimerin-1
Plasminogen
Plexin C-1
PP2A
Rab-1a, Rab-2a, Rab-11b
Reticulon-4
Septin-7
Stathmin
Stomatin-like protein-2
Stress-induced phosphoprotein 1
Syntaxin binding protein II
VAMP-2, 3
CD67
CD81
CD158
CDw328 (Siglec-7)



Parameters:
Ions score cut off: 30
Minimum peptide length: 6 AAs
$p < 0.05$

Control MPs only:

Apolipoprotein A2, A4, C2, E
Copine-1, 3
Cytochrome Bc-1, C
Elfin
Ezrin
Flotillin-2
Grancalcin
HMGB-1
Hyaluronan synthase 1
ILT-2, 4
Matrin-3
Plastin-1
Prohibitin-2
Radixin
Septin-2
Serotransferrin
SH3BP-1
Sorcin
Syntaxin-7
Testilin
TGF-β1
VAMP-8
CD47
CD54
CD59
14-3-3 protein sigma

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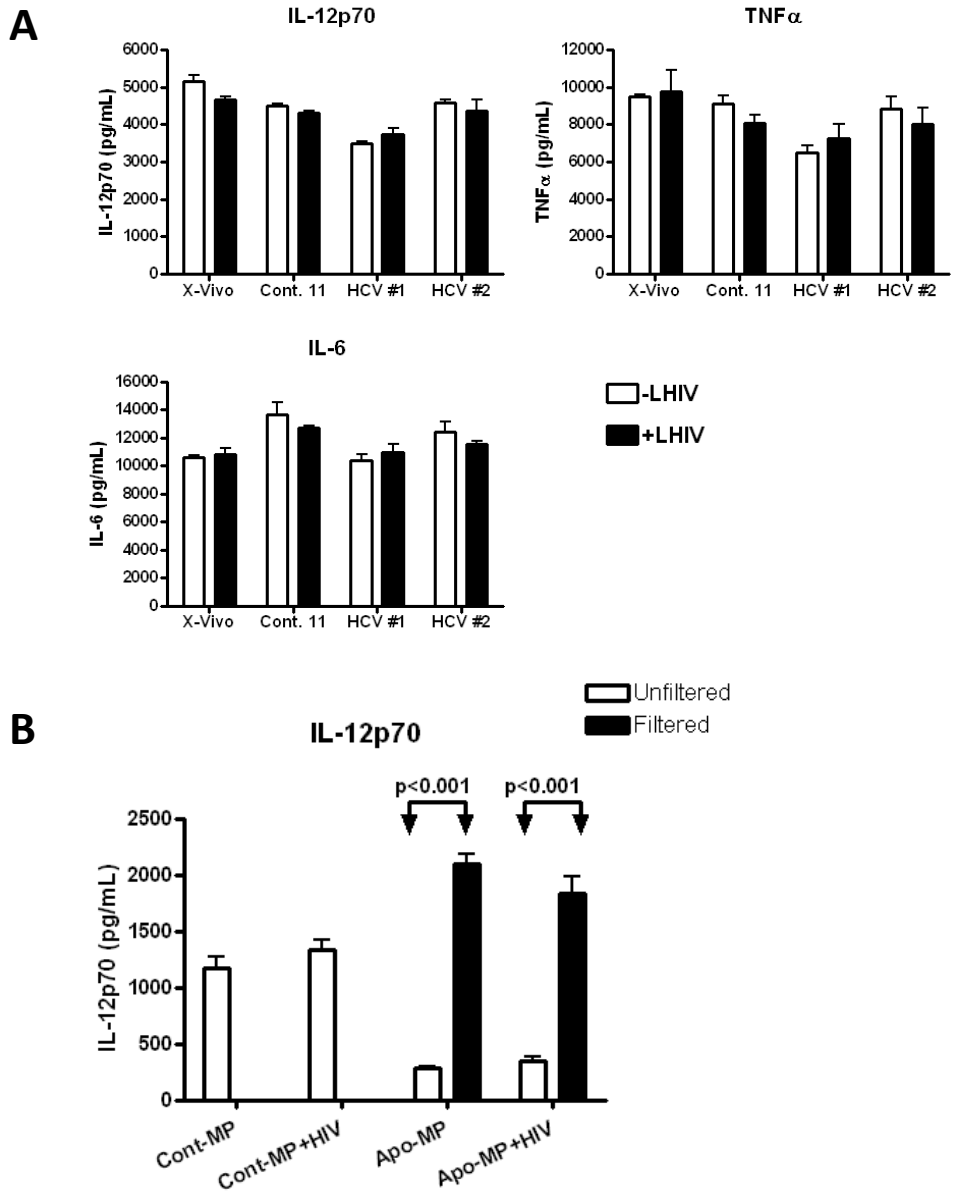

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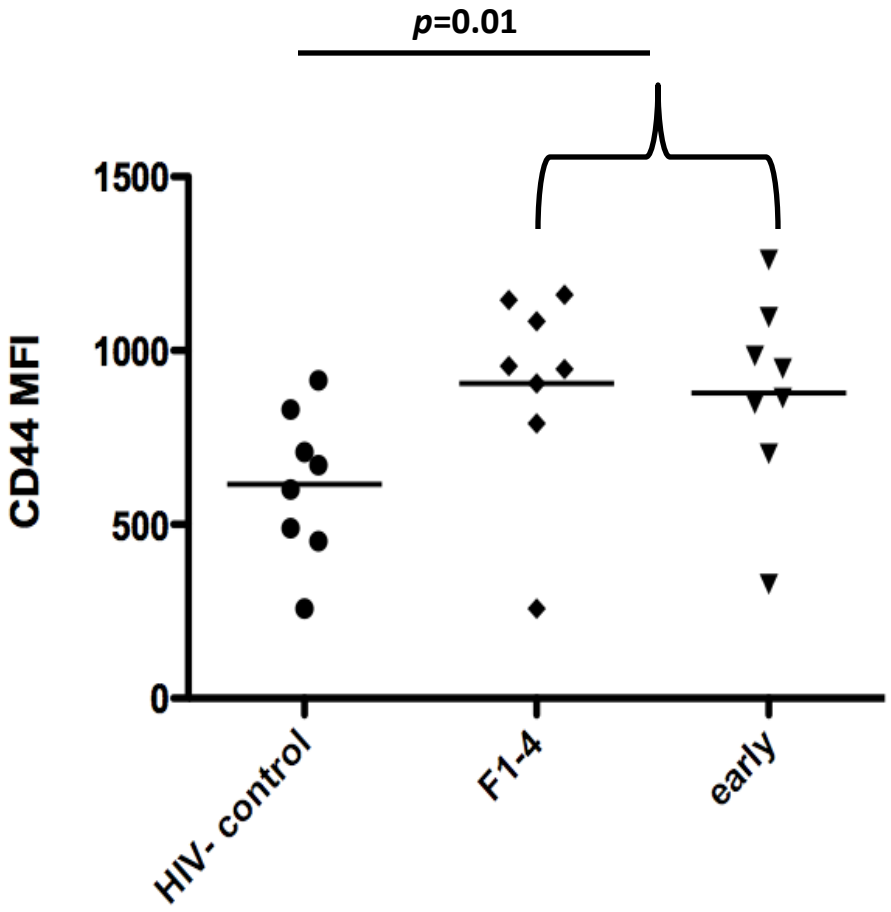

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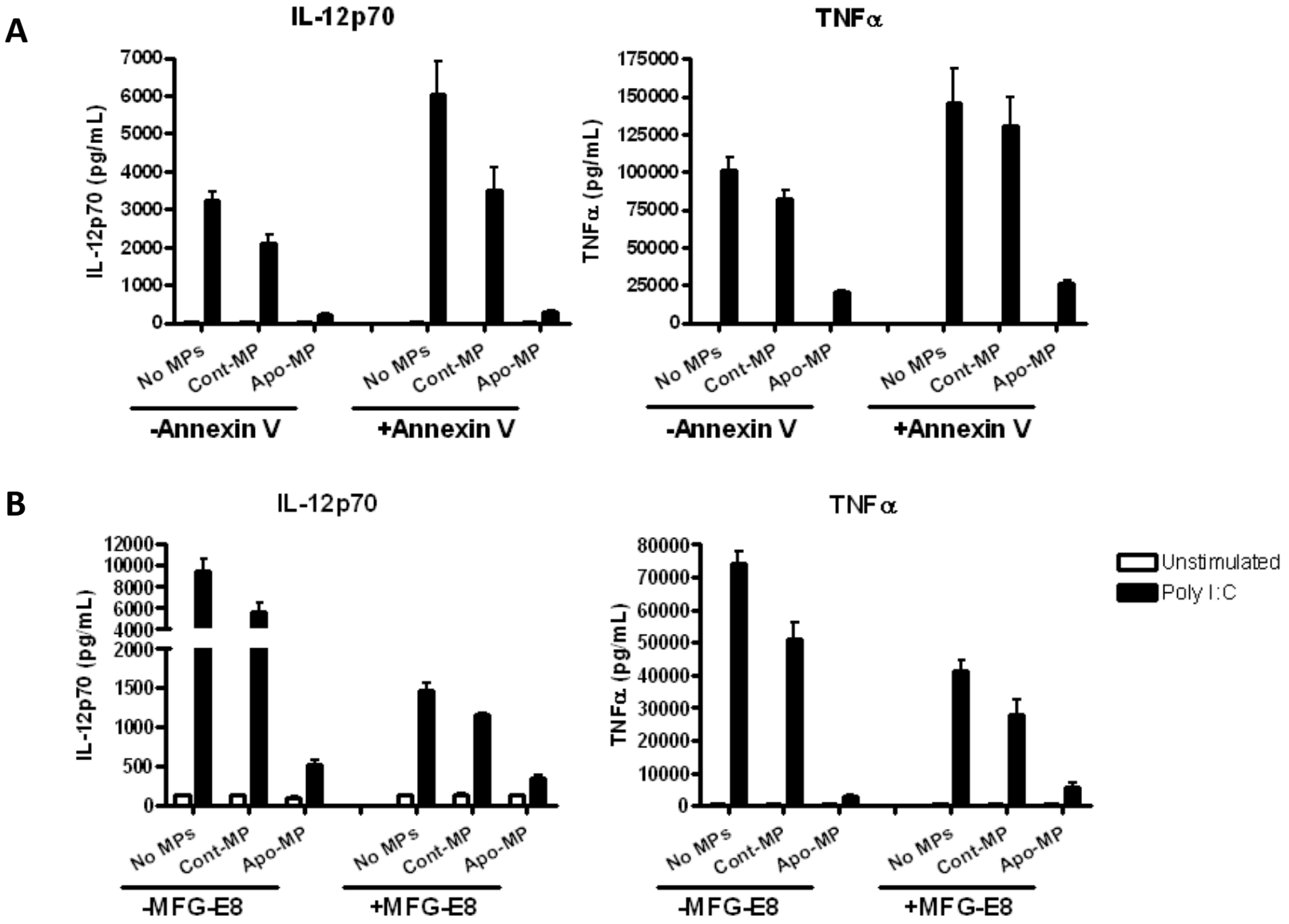

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