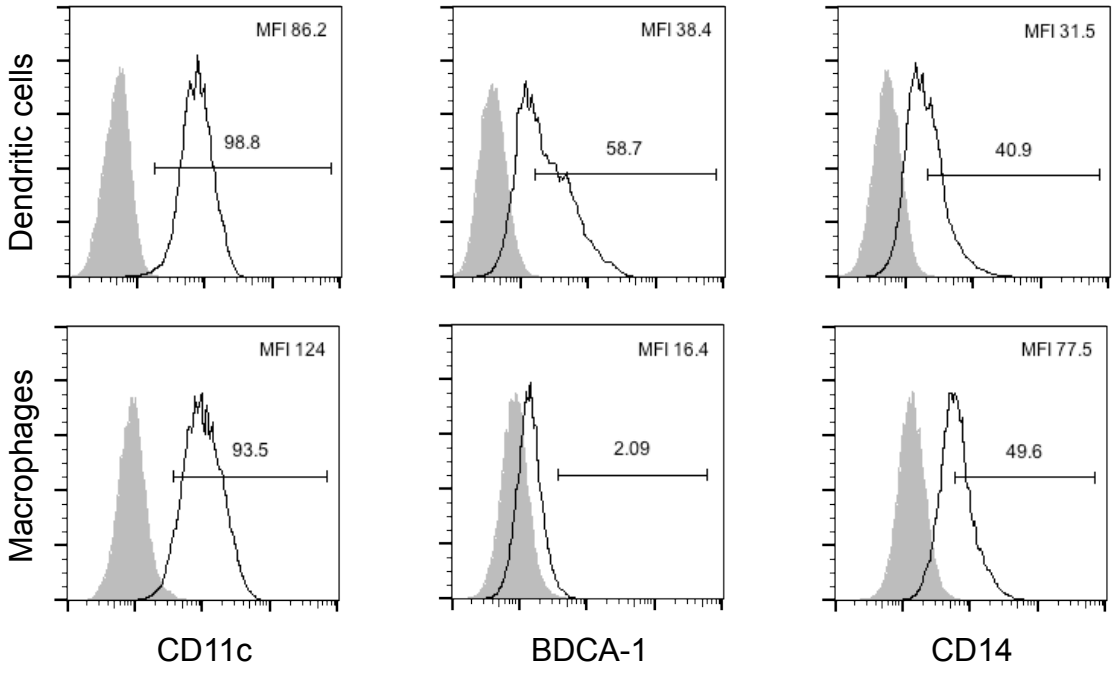

Supplemental figure 1. Phenotypic analysis of dendritic cell and macrophage populations. Immature DCs or macrophages were phenotypically analyzed by flow cytometry. Cells were stained (solid line) with antibodies for CD11c, BDCA-1 and CD14 or isotype controls (filled area) to assess the purity of each population. The level of surface expression is indicated as mean fluorescence intensity (upper right corner).

Supplemental Figure 2

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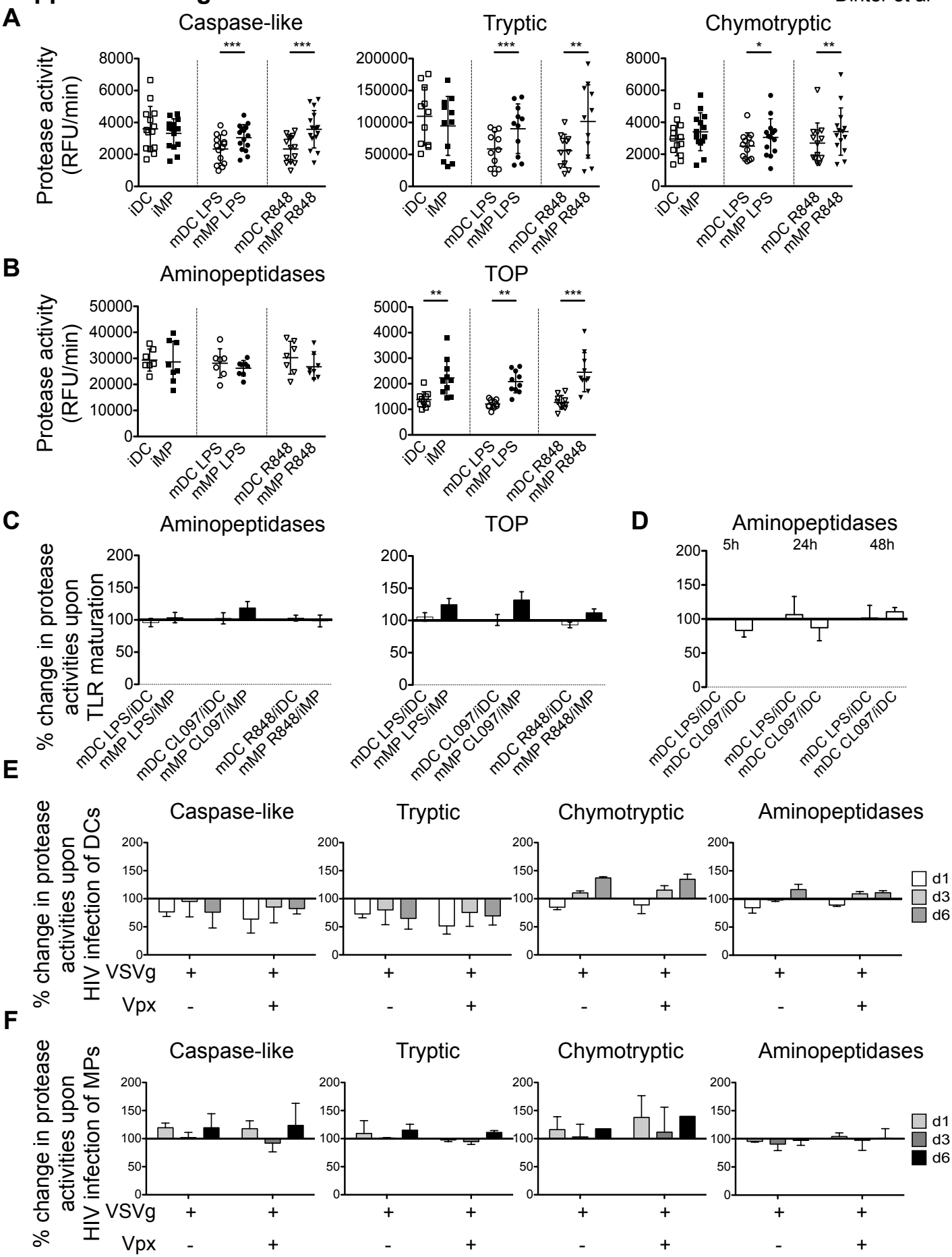

Supplemental figure 2. Antigen processing activities in human monocyte-derived DCs and MPs change differentially upon TLR ligand-induced maturation or HIV infection

A. Proteasomal caspase-like, tryptic, and chymotryptic activities were measured iDC (□), iMP (■), mDC LPS (○), mMP LPS (●), mDC R848 (▽) and mMP R848 (▼) using protease-specific fluorogenic substrates. n>10 healthy donors.

B. Post-proteasomal activities for aminopeptidases and TOP were measured as described in (A). n>7 healthy donors. For (A) and (B), paired t-tests were performed (*p<0.05, **p<0.01, ***p<0.001), and mean ± SD are shown.

C. Ratios of aminopeptidases and TOP activities in TLR-matured cells over immature cells from the same donor were calculated and represented as mean ± SD for DCs (open bars) and MPs (solid bars). n>10 healthy donors. For (A) to (C) maturation was induced for 48h.

D. Ratios of aminopeptidases activities in TLR-maturing DCs over immature cells of the same donor were calculated and represented as mean ± SD at the indicated time points post stimulation. n=2 healthy donors.

Immature DCs (E) and MPs (F) were infected with VSVg-DeltaEnv NL4.3 alone or in combination with Vpx for the indicated time points. At each time point proteasomal caspase-like, tryptic and chymotryptic activities as well as post-proteasomal aminopeptidases activities were measured using protease-specific fluorogenic substrates. Ratios of activities in infected cells over non-infected cells from the same donor were calculated and represented as mean ± SD. n=2 different donors.

Supplemental Figure 3

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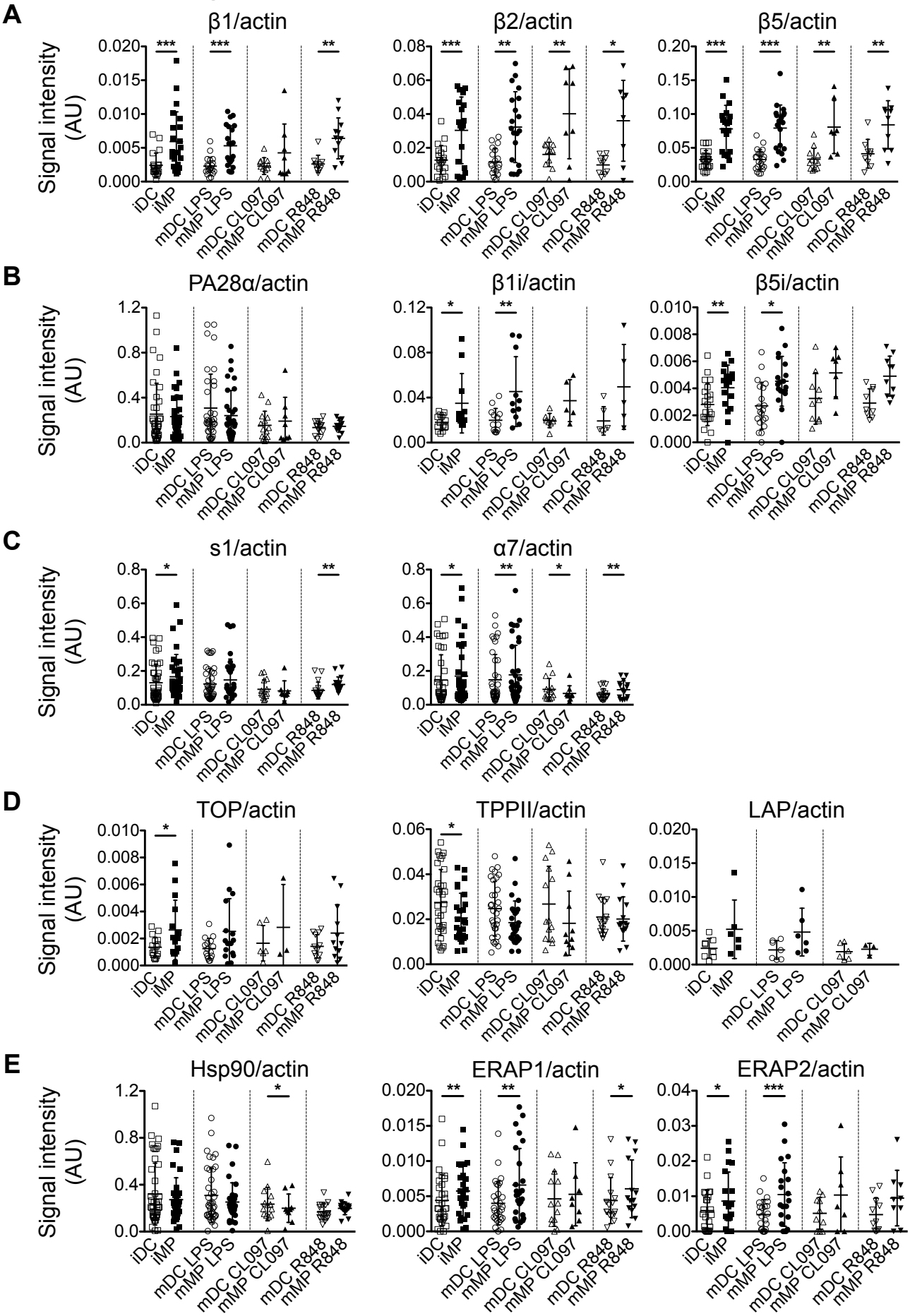
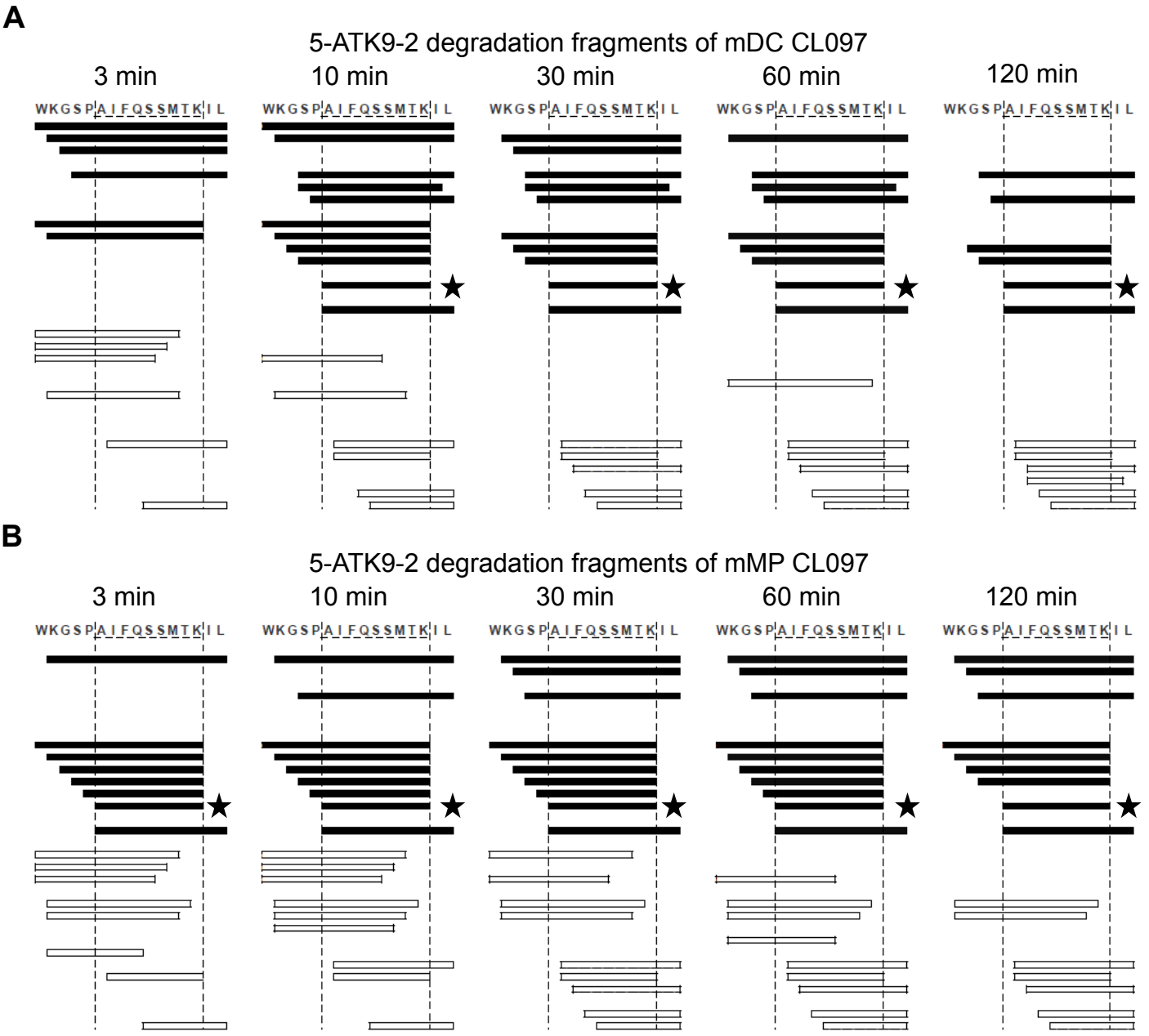


Supplemental figure 3. The expression of the antigen processing machinery in DCs and MPs varies upon TLR maturation.
Densitometric quantification normalized to actin was performed for the expression of (A) constitutive proteasomal catalytic subunits β1, β2, and β5; (B) immunoproteasomal activator PA28α, and for immunoproteasome catalytic subunit β1i and β5i expression; (C) proteasomal lid S1 and proteasomal core α7; (D) post-proteasomal cytosolic proteases TOP, TPPII, and LAP; (E) cytosolic chaperone Hsp90, and ER-resident aminopeptidases, ERAP1 and ERAP2.
For (A) to (E), paired t-tests were performed between matched DCs and MPs in their immature or TLR-matured state. Maturation was induced for 48h with the indicated TLR ligand. Mean ± SD is shown.



Supplemental figure 4. Degradation patterns of a HIV-1 RT 16-mer fragment in cytosolic extracts from CL097-matured DCs and MPs

Four nmol of 5-ATK9-2 (aa 153-168 in HIV-1 RT) were degraded with 30µg of cytosolic extracts from CL097-matured DCs (A) or MPs (B) for 3, 10, 30, 60, 120, and 210 minutes. Peptides containing (black bars) or lacking intact ATK9 (white bars) were identified by mass spectrometry. Optimal ATK9 (★) is indicated. The data are representative of three independent experiments from different donors.