Figure 9. AVDCs resist viral infection. A) Barcoding for flow cytometry analysis. Cells were stained with different combinations of 0, 0.3, 1, 4 or 15 μg/ml Pacific Blue–NHS, 0, 1.25, 5 or 20 μg/ml Alexa 350–NHS and 0, 4 or 20 μg/ml Alexa 750–NHS to enable 60 different conditions in one FACS run. B) Time course results for NDV-RFP expression following infection in naïve DC and AVDC. C) Time course for expression of MHC-I, MHC-II and CD86 surface markers in the same cells studied in A and B. The results shown are representative of two independent experiments using cells from different donors.

Figure 10. Schematic of the possible role of AVDCs in developing adaptive immunity. The paracrine signals released by DCs that are first infected by virus generate AVDCs that are primed to resist virus and to develop into antigen presenting cells.

Supplementary Figure Legends:

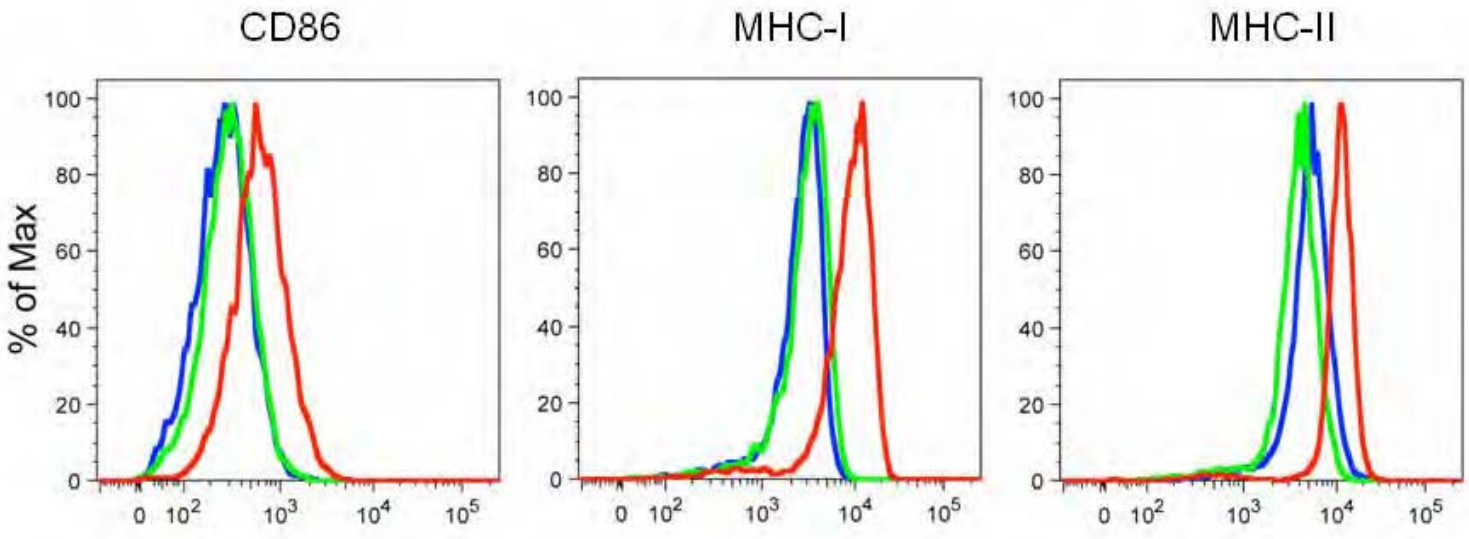
Figure. S1. Effects of proteinase K in the generation of AVDCs. Three surface markers were studied, CD86, MHC-I and MHC-II. Red: effects of supernatant conditioned by NDV-infected DCs in one trans-well-chamber on naïve DCs in the other chamber. Green: effects of supernatant conditioned by NDV-infected DC subsequently digested with proteinase K in one transwell-chamber on naïve DCs in the other chamber. Blue: naïve DCs with unconditioned media in the other trans-well chamber. The data shown are representative of two replicate experiments using two different donors that showed similar results.

Figure. S2. Paracrine effects of virus-infected human epithelial fibroblasts on naïve DCs. Fibroblasts were seeded in the lower compartment of the trans-well system and were infected,

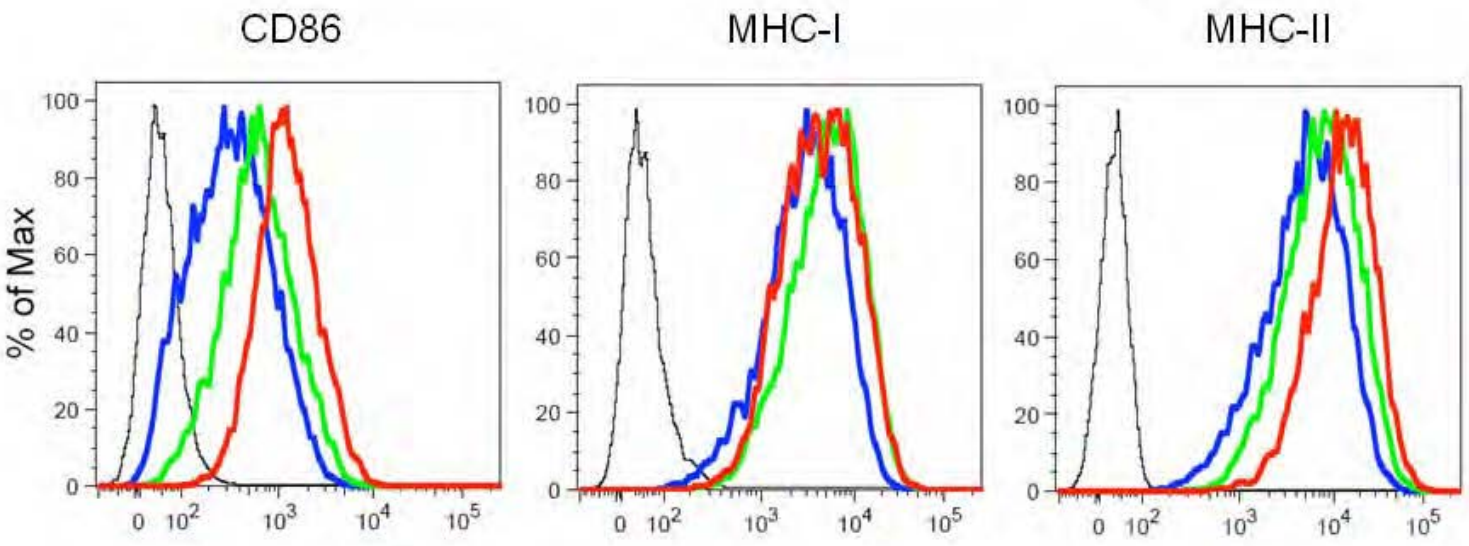
(green), or not infected, (blue), with NDV to see if they were capable of generating the same maturation surface markers pattern as AVDCs (red). Fluorescence minus one control (FL -1) is shown in black. The data shown are representative of three different experiments using three different donors that showed similar results.

Figure. S3. Effect of extended trans-well culturing on naïve DCs after 18, 24 and 48 hours, (red, blue and green respectively). FL-1 control is shown in black. The data shown are representative of three different experiments using three different donors that showed similar results.

Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3