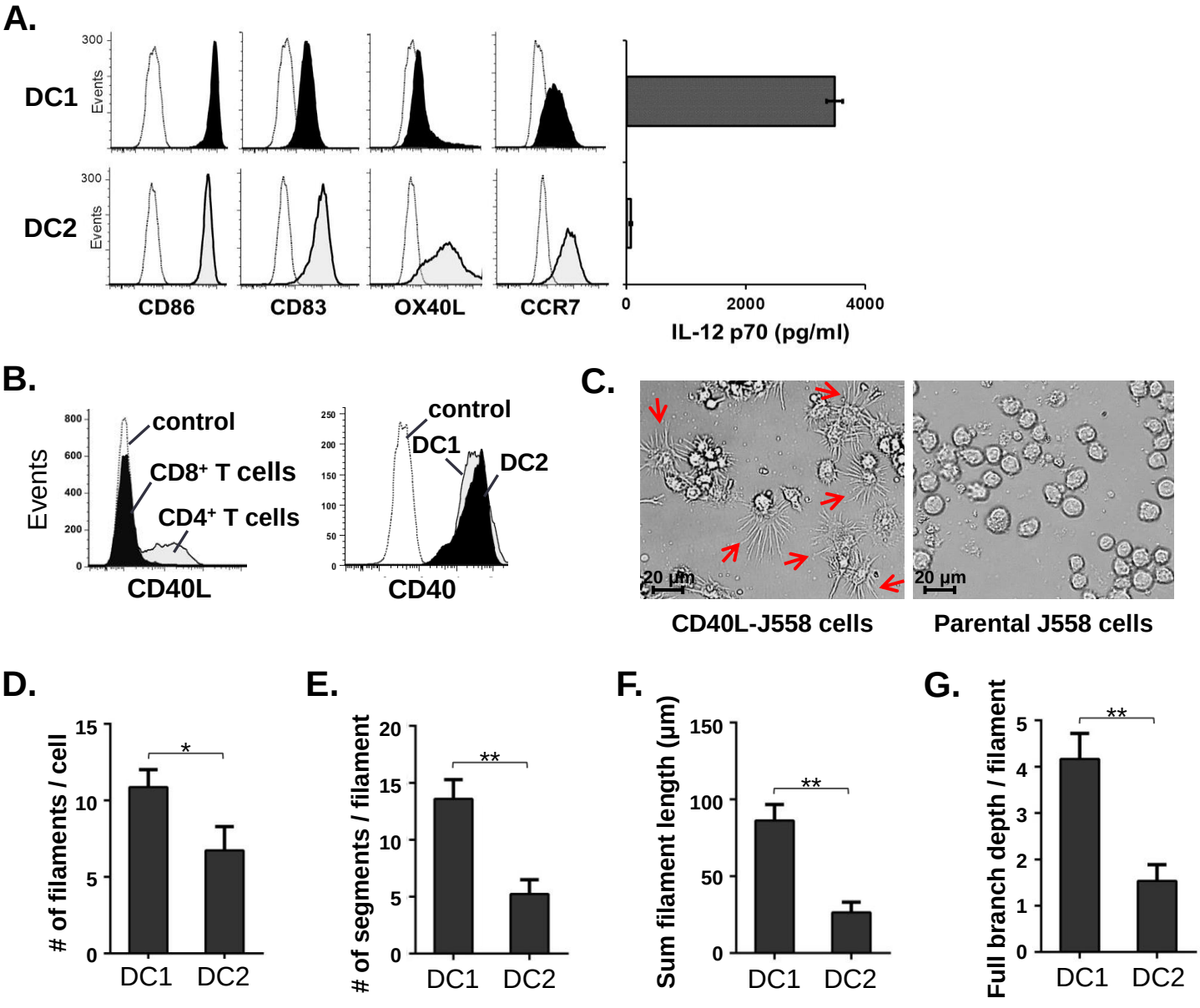
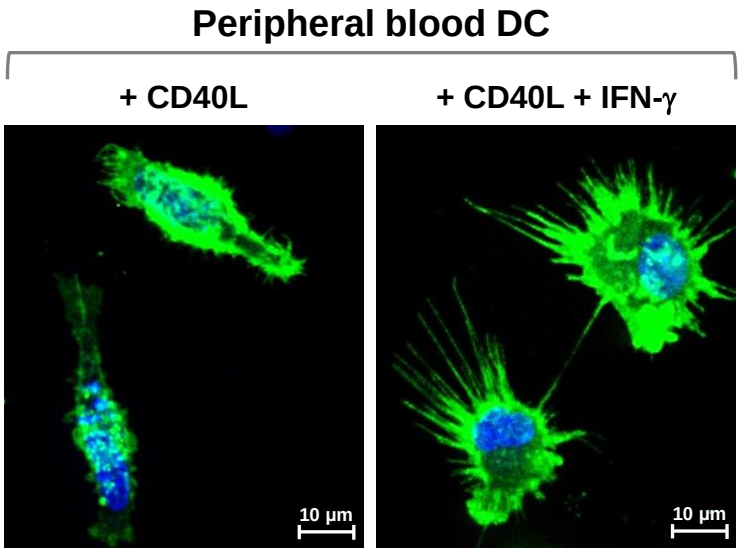
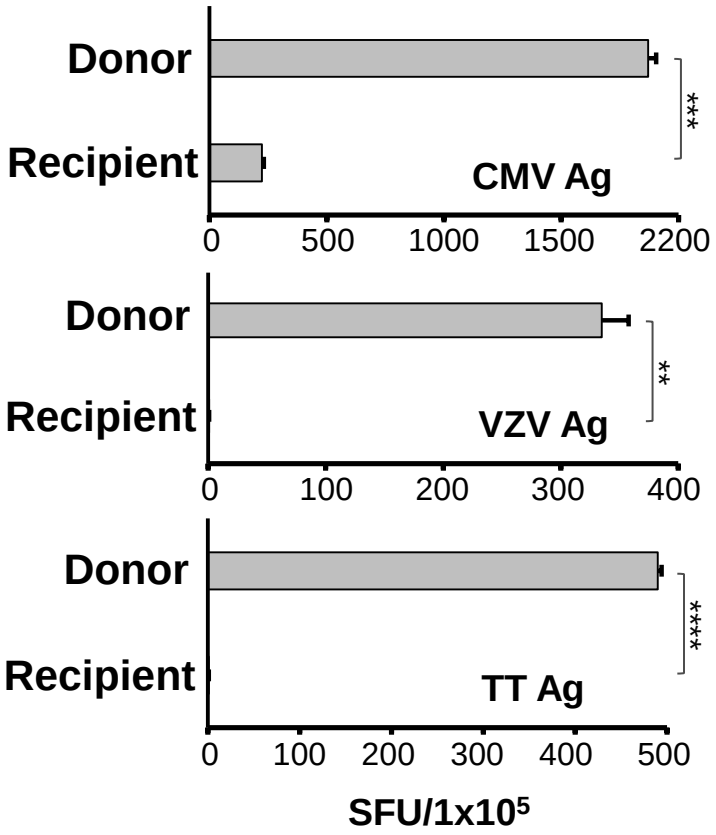
Supplemental materials



Supplemental Figure 1. Phenotypic characteristics of differentially polarized DC and their functional responsiveness to CD40L. (**A**) DC1 and DC2 were harvested and analyzed by flow cytometry for the following cell surface markers: CD83, CD86, CCR7, and OX40L (left panel). IL-12p70 production of DC1 and DC2 in response to secondary rhCD40L stimulation was determined by MSD electrochemiluminescence (right panel). (**B**) Flow cytometric analysis showing the differential surface expression of CD40L on 24 h CD3/CD28-activated $CD4^+$ and $CD8^+$ T cells (left panel), and comparable surface expression of the receptor CD40 on DC1 and DC2 (right panel). (**C**) DC1 co-cultured with either a J558-CD40L cell line (left panel) or the control parental CD40L-deficient J558 cell line (right panel) for 20 h and analyzed by bright field microscopy (400X) for the presence of induced TNT-like protrusions (arrows). (A-C) Data are representative of 6 independent experiments using cells from 3 healthy donors. (**D**) Graphical depiction of the mean number of filaments per cell in reticulation positive DC1 compared to DC2. (**E**) Graph of the mean number of segments per filament in individual reticulating DC1 versus DC2. (**F**) Mean filament length per cell, defined as the sum length (µm) of its segments, graphically depicted in reticulating DC1 compared to DC2. (**G**) Graph of the mean full branch depth of filaments per cell, delineated as the maximum number of bifurcations from the origin point to a terminal point in an individual filament, in reticulation positive DC1 versus DC2. (D-G) Complex membrane filaments, displayed by 100% of individual CD40L-activated DC1 and 16.7% of DC2, were analyzed using IMARIS. Data are represented as mean ± SD from independent experiments of 3 healthy donors, and p-values <0.0001, <0.001, <0.01 and <0.05 are represented by ****, ***, **, and *, respectively.



Supplemental Figure 2. IFN-γ-dependent reticulation of peripheral blood-isolated DC in response to CD40L. Z plane reconstruction image (1000X) of TNF-α-treated, human peripheral blood-isolated myeloid DC (lineage$^-$, HLA-DR$^+$ and CD1c$^+$) following 20 h activation with rhCD40L alone or in combination with IFN-γ. Confocal mages are representative of 3 healthy donors independently tested.



Supplemental Figure 3. Ag exchange between reticulating DC1 is contact dependent. IFN-γ ELISPOT assays measuring Ag-specific recall responses of cultured T cells following their in vitro sensitization with YG bead- and Ag-containing donor DC1 or recipient DC1 separated from reticulating donor DC1 by a trans-well system. Data are represented as mean ± SD of 2 replicate experiments and p-values <0.0001, <0.001, <0.01 and <0.05 are represented by ****, ***, **, and *, respectively.