Supplementary

Cell culture and reagents MM.1S (Dexamethasone sensitive), MM.1R (Dexamethasone-resistant), RPMI-8226, Doxorubicin (Dox)-resistant (Dox-40), and OPM2 human MM cell lines were cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine, as previously described (Chauhan et al., 2005). INA6-6 (IL-6-dependent) MM cell line was cutured as above in the presence IL-6 (2.5 ng/ml). For primary cells informed consent was obtained from all MM patients and normal donors in accordance with the Helsinki protocol. PB and BM MNCs from normal healthy donors and MM patients were isolated by Ficoll Hypaques density gradient centrifugation. Cells were then magnetically labeled by direct CD304 (BDCA-4) microbeads (for pDCs), CD1c (BDCA-1) microbeads (for mDCs) or CD138 microbeads (for MM cells), followed by separation by positive selection using Auto MACS magnetic cell sorter (Miltenyi Biotec Inc., Auburn, CA). Monocytes were isolated by positive selection using Auto MACS magnetic cell sorter (Miltenyi Biotec Inc., Auburn, CA). *Reagents:* Bortezomib and IKK-β inhibitor PS-1145 (Millennium Pharmaceuticals, Cambridge, MA), Dex (Sigma Chemical Co, St. Louis, MO), lenalidomide (Celgene, Inc, NJ), rhOPG, TACI-Fc, CD28-Fc (R&D Systems, Minneapolis, MN); small molecule inhibitor of BAFF (Amgen, Thousand Oaks, CA), and CpG ODNs (InVivoGen, San Diego, CA).

Antibodies for flow cytometry: Directly conjugated monoclonal Abs were purchased from Becton Dickinson (BD) Biosciences (San Jose, CA): CD123 PE-Cy5, HLA-DR Pacific Blue, BDCA-2 FITC, CD14-PE, CD20 PE, CD11c and CD3 APC antibodies.

Bone marrow stromal cell (BMSCs) culture BM aspirates from patients with MM were subjected to mononuclear cell separation by Ficoll-Hipaque density sedimentation and were cultured *in vitro* to establish long-term BMSC cultures, as described previously (Chauhan et al., 2006; Uchiyama et al., 1993). The adherent cell monolayer was harvested in HBSS containing 0.25% trypsin and 0.02% EDTA, washed, and collected by centrifugation.

Immunoblotting Immunoblot analysis was performed using antibodies to phospho-ERK, phospho-IκB-α (Cell Signaling, Beverly, MA), Actin or Tubulin (BD Bioscience Pharmingen, San Diego, CA), as previously described (Chauhan et al., 2005). Blots were then developed by enhanced chemiluminesence (ECL; Amersham, Arlington Heights, IL).

Immunohistochemistry (IHC) Four-micron thick sections of formalin fixed tissue were used for immunoperoxidase analysis after baking at 60°C for 1 h, deparaffinization and rehydration (100% xylene X4 for 3 mins each, 100% ethanol X4 for 3 mins each and running water for 5 mins). The sections were blocked for peroxidase activity with 3% hydrogen peroxide in methanol for 10 mins and washed under the running water for 5 mins. The sections with pressure cooked

(Biocare Medical) antigen retrieval were at 123°C in Citrate Buffer (DAKO Target Retrieval Solution, S1699). The slides were cooled for 15 minutes, and transferred to phosphate buffer saline (PBS). The sections with BDCA-2 1:400 incubated overnight in 4 degree. The secondary antibody was used DAKO Envision Poly (K4011)) 30 mins. incubation. All the incubations were carried out in a humid chamber at room temperature. The slides were rinsed with PBS in between incubation. The sections were developed using 3,3'-diaminobenzidine (DAB) (Sigma Chemical Company) as substrate and counter-stained with Mayer's Hematoxylin.

IHC with primary antibody specific for pDCs (CD123 at 1:400 dilution) and plasma cells/MM cells (CD138 at 1:500 dilution) was conducted using standardized automated methods (Carrasco et al., 2006). For expression detection, we employed the DAKO Envision system, which uses a peroxidase labeled synthetic polymer conjugated to rabbit anti-rat antibody with diaminobenzidine as the substrate to induce brown staining of pDCs. Sections were counterstained with hematoxylin to show MM cells in red color.

Transcriptional profiling Gene expression profiling analysis was performed in MM.1S cells cultured in the presence or absence of pDCs using HG-U133 plus 2.0 plus Affymetrix oligonucleotide microarrays (Chauhan et al., 2003) Data processing: The CEL files were obtained using Affymetrix Microarrays Suite 5.0 software. GeneChip 5.0 (Affymetrix, Santa Clara, CA) was utilized to scan, quantify and analyze

the scanned image. GeneChip software automatically calculates intensity values for each probe cell and mark a presence or absence call for each mRNA. Algorithms in the software use probe cell intensities to calculate an average intensity for each set of probe pairs representing a particular gene, which correlates with the amount of mRNA. Microsoft Excel are also used for data analysis. Expression patterns for MM.1S cells cultured with pDCs were compared with the control MM.1S cultured alone. In this analysis, >2-fold alteration in gene transcripts is considered significant. The raw microarray data is provided at the website Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). Accession Number "GSE17407".

References

Carrasco, D. R., Tonon, G., Huang, Y., Zhang, Y., Sinha, R., Feng, B., Stewart, J. P., Zhan, F., Khatry, D., Protopopova, M., *et al.* (2006). High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell *9*, 313-325.

Chauhan, D., Catley, L., Li, G., Podar, K., Hideshima, T., Velankar, M., Mitsiades, C., Mitsiades, N., Yasui, H., Letai, A., *et al.* (2005). A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell *8*, 407-419.

Chauhan, D., Hideshima, T., and Anderson, K. C. (2006). A novel proteasome inhibitor NPI-0052 as an anticancer therapy. Br J Cancer *95*, 961-965.

Chauhan, D., Li, G., Auclair, D., Hideshima, T., Richardson, P., Podar, K., Mitsiades, N., Mitsiades, C., Li, C., Kim, R. S., *et al.* (2003). Identification of genes regulated by 2-methoxyestradiol (2ME2) in multiple myeloma cells using oligonucleotide arrays. Blood *101*, 3606-3614.

Uchiyama, H., Barut, B. A., Mohrbacher, A. F., Chauhan, D., and Anderson, K. C. (1993). Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. Blood *82*, 3712-3720.

Figure legends

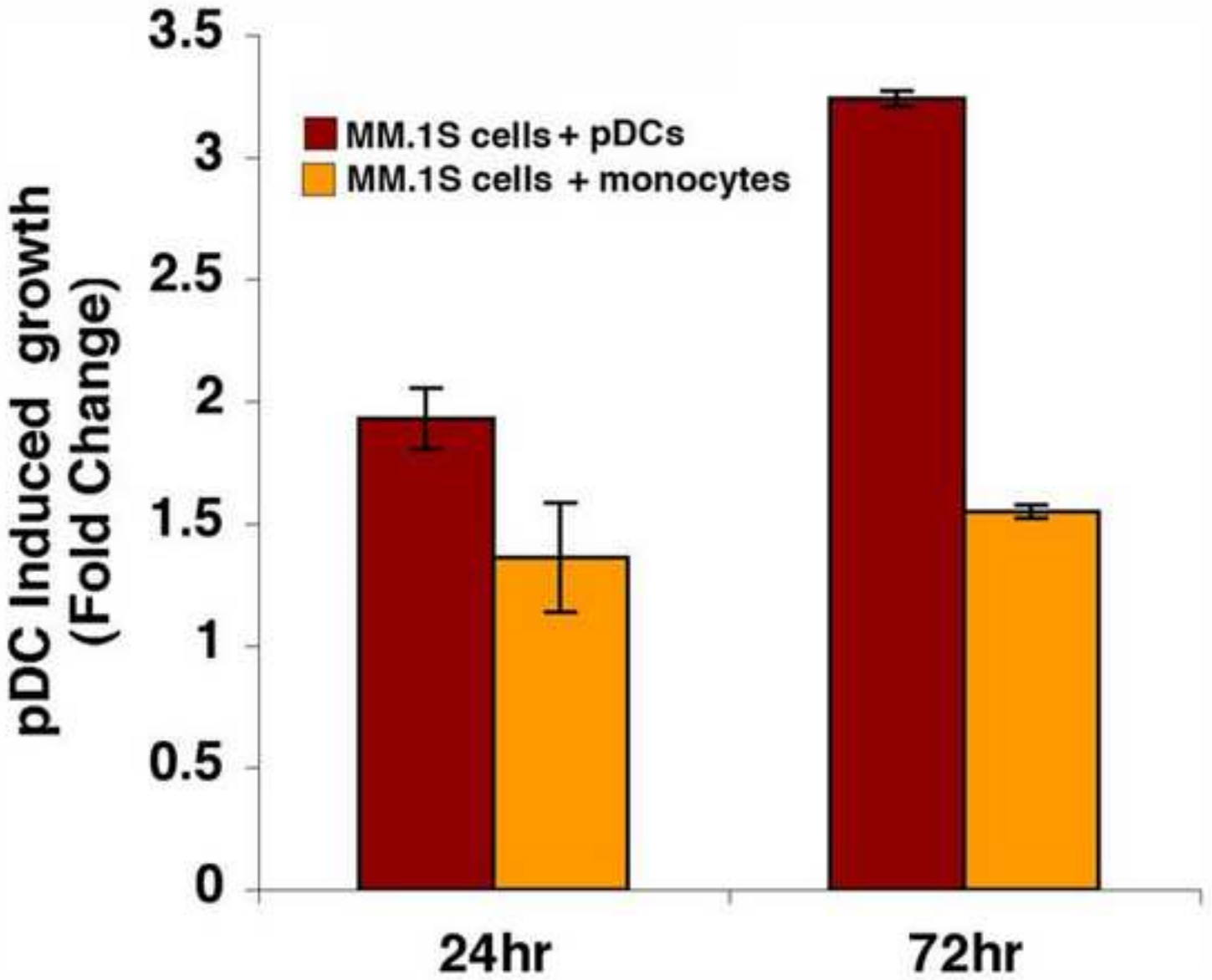
Supplementary Figure 1. **Comparative analysis of pDCs- or monocytes-induced MM.1S cell growth.** MM.1S cells, pDCs, or monocytes were cultured either alone or together at 1:5 (pDC/MM, or monocytes/MM) ratio for 3 days, and then analyzed for MM cell proliferation using WST proliferation assay (mean ± SD; $P < 0.005$, $n = 2$). Fold changes in the growth of MM.1S are shown. Error bars indicate standard deviation (SD).

Supplementary Figure 2. **Gene expression profiling of MM.1S cells cultured with or without pDCs.** MM.1S cells were cultured with pDCs for 72h; separated using CD138 Microbeads, and harvested. Poly RNA was subjected to microarray analysis using using HG-U133 plus 2.0 plus

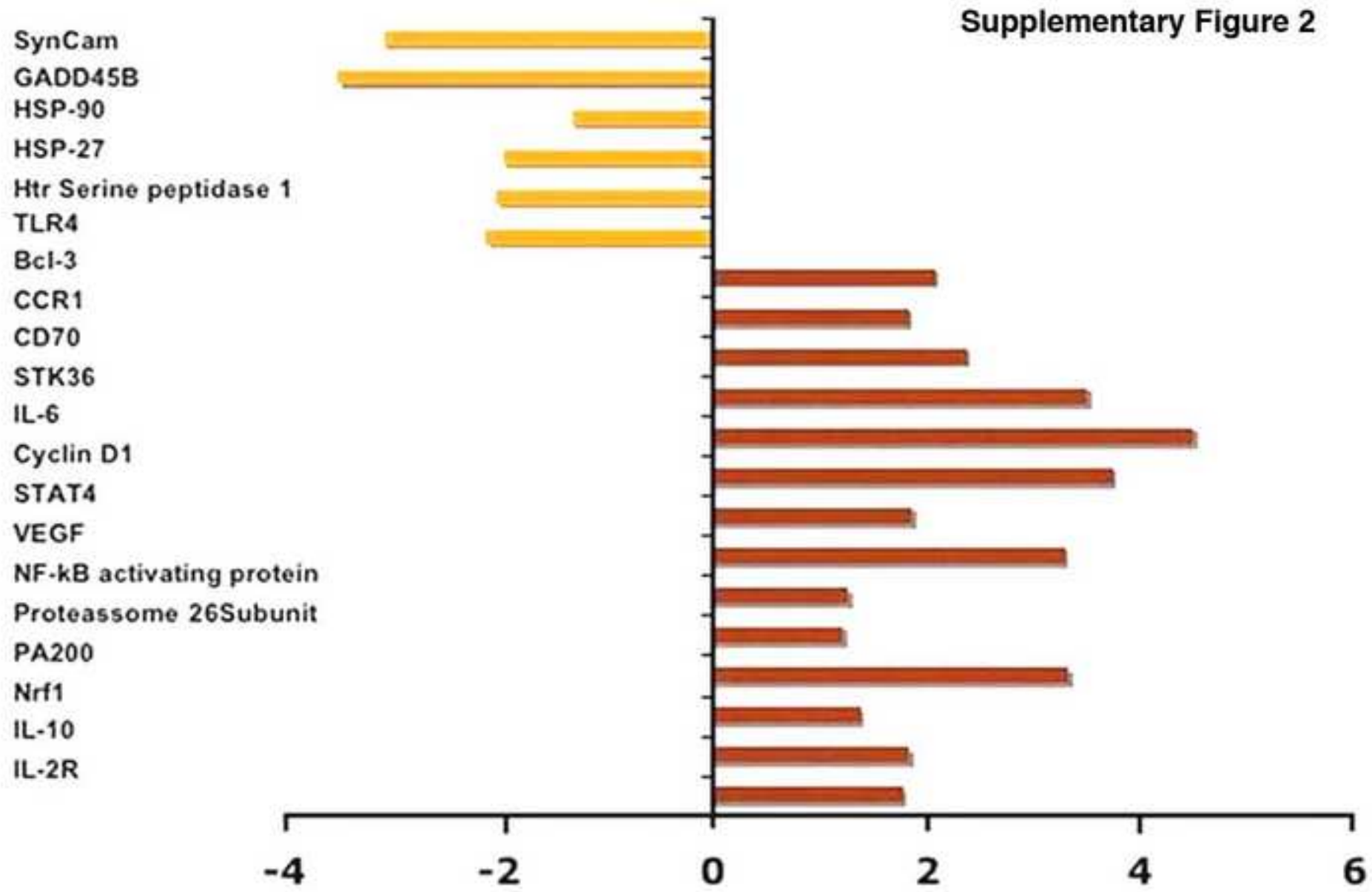
Affymetrix oligonucleotide microarrays. Shown are examples of transcripts that were significantly altered in MM.1S cells upon culture with pDCs (>2-fold alteration in gene transcripts is considered significant, CI > 95%).

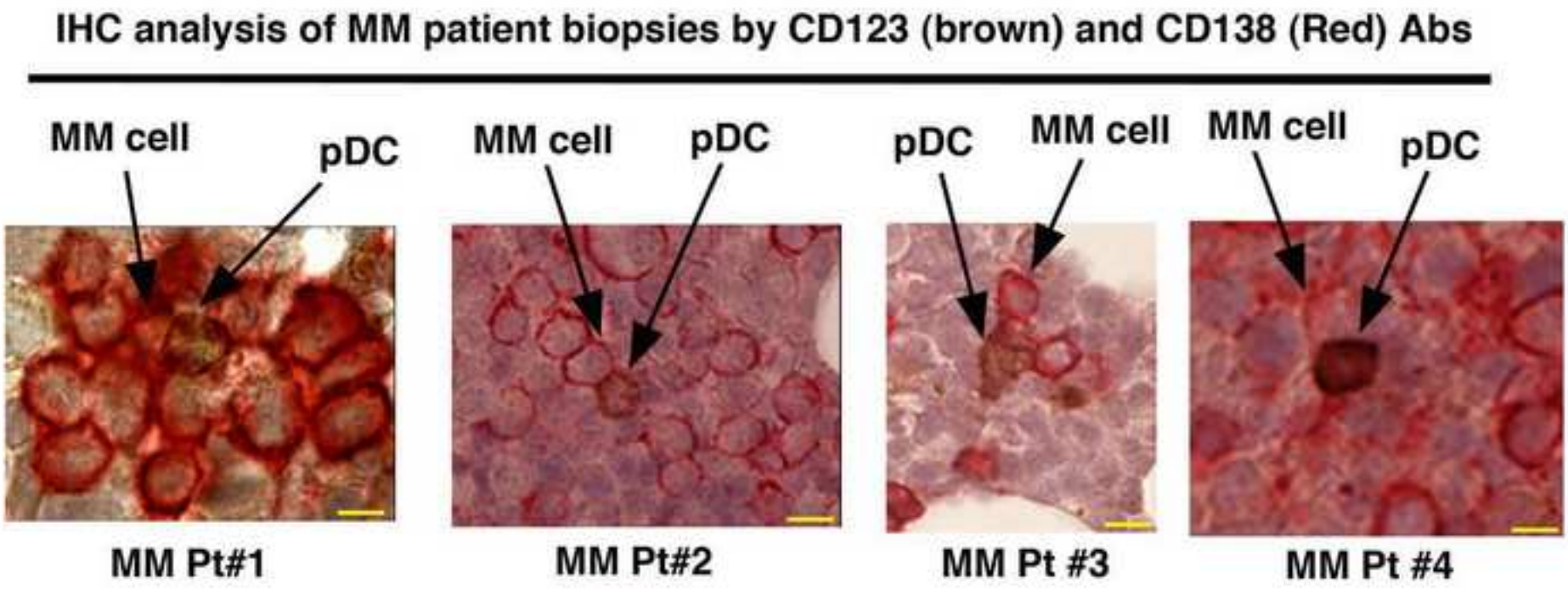
Supplementary Figure 3. **Micrographs show pDCs in proximity of MM cells.** Four MM patient BM biopsies were subjected to IHC analysis with Abs specific for MM cells (CD138 Ab, cell stained in red color) and for pDCs (CD123 Ab, cells stained in brown color). Data shown is representative of 12 samples analyzed with similar results. Scale bars, 10 μM.

Movie S1. **Chemotaxis of MM cells and pDCs (Movie)** pDCs were labeled with MitoFluor Red 594 (1:1000; Invitrogen Cat# M22422) and cultured with GFP-positive MM.1S in a glass-bottom culture dish (MatTek Inc, Ashland, MA), and live cell images were taken using an Apochromat 40x/1.2W objective lens on a LSM-510 confocal system. The movie were processed using Volocity software (Improvision Inc, Waltham, MA). Formation of filopodia from MM cells (green) demonstrate chemotactic activity between pDCs and MM cells.



Supplementary Figure 1


Supplementary Figure 2
SynCam
GADD45B
HSP-90
HSP-27
Htr Serine peptidase 1
TLR4
Bcl-3
CCR1
CD70
STK36
IL-6
Cyclin D1
STAT4
VEGF
NF-kB activating protein
Proteassome 26Subunit
PA200
Nrf1
IL-10
IL-2R
-4
-2
0
2
4
6



Supplementary Figure 3