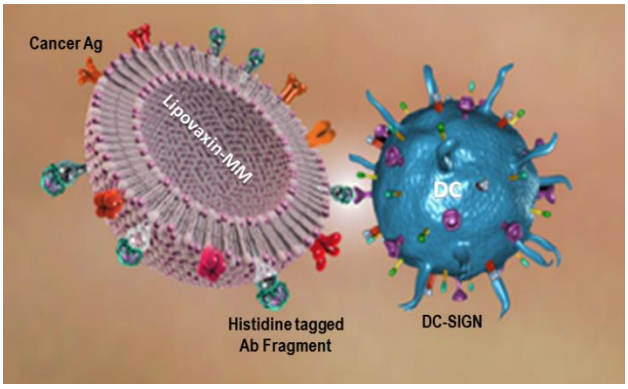
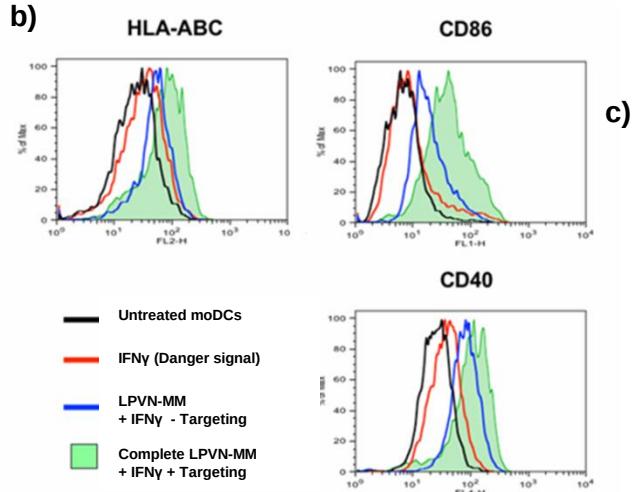
a)



Supplementary Figure 1. a) Lipovaxin-MM targets monocyte-derived DCs (moDCs) via DC-SIGN. **b)** MoDCs were cultured in vitro for 48 hours and left untreated, or treated with IFNγ (danger signal), Lipovaxin-MM lacking IFNγ, Lipovaxin-MM lacking the DC-SIGN targeting domain, or complete Lipovaxin-MM. Cells were harvested for flow cytometric analysis of cell surface human lymphocyte antigens (HLA) and activation markers CD86 and CD40, and **c)** culture supernatants were collected and analyzed for IL-12 secretion by ELISA. Please note that results for the Lipovaxin-MM lacking IFNγ group are not shown in panel b) because no significant change in surface marker expression was evident. **d)** MoDCs were incubated with fluorescent Lipovaxin-MM (green histogram) or left untreated (grey shaded histogram). Cells were analyzed by flow cytometry.

b)



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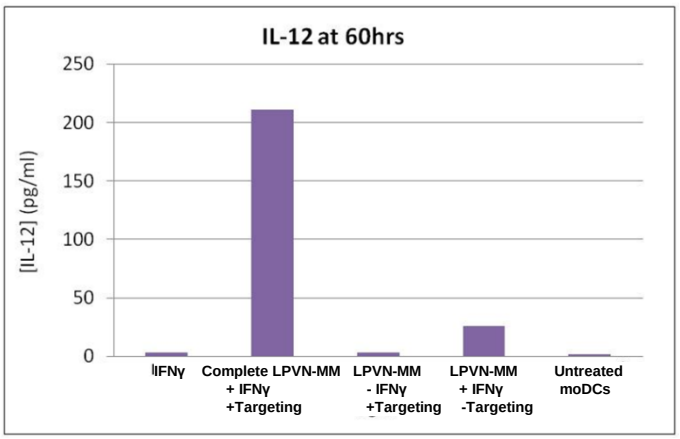
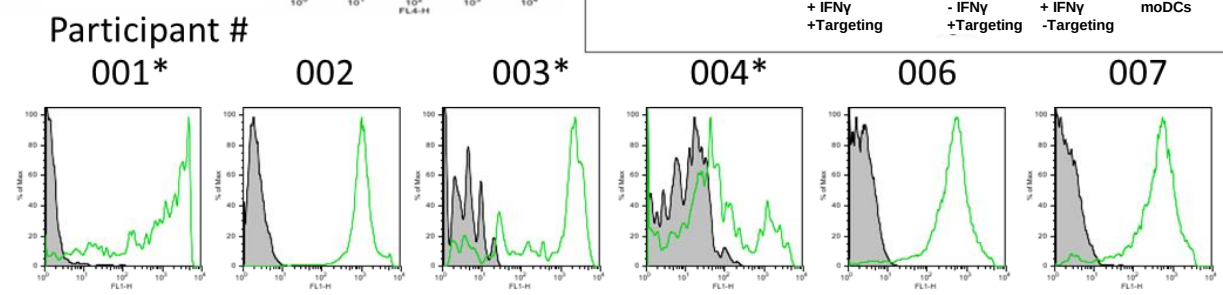
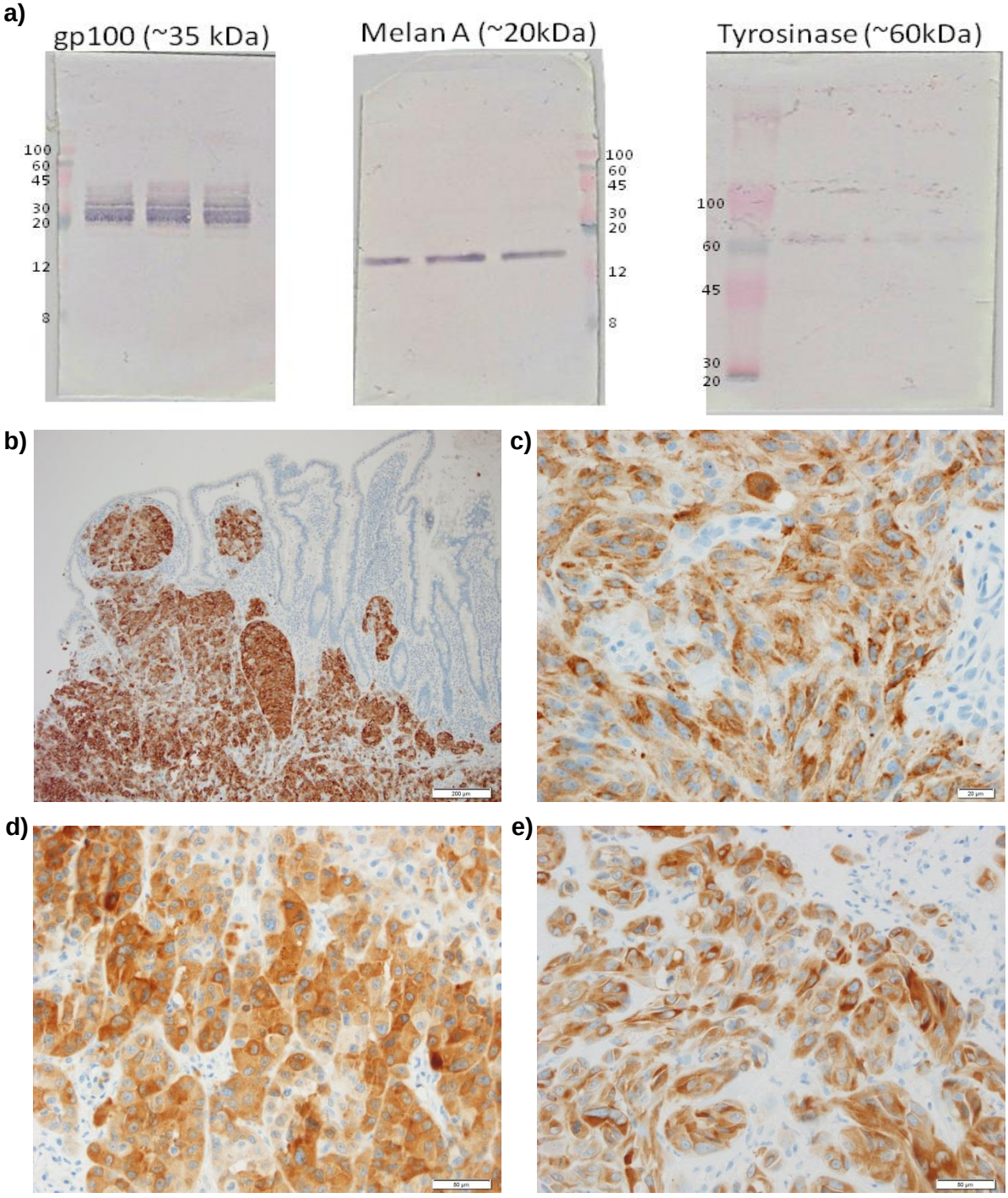


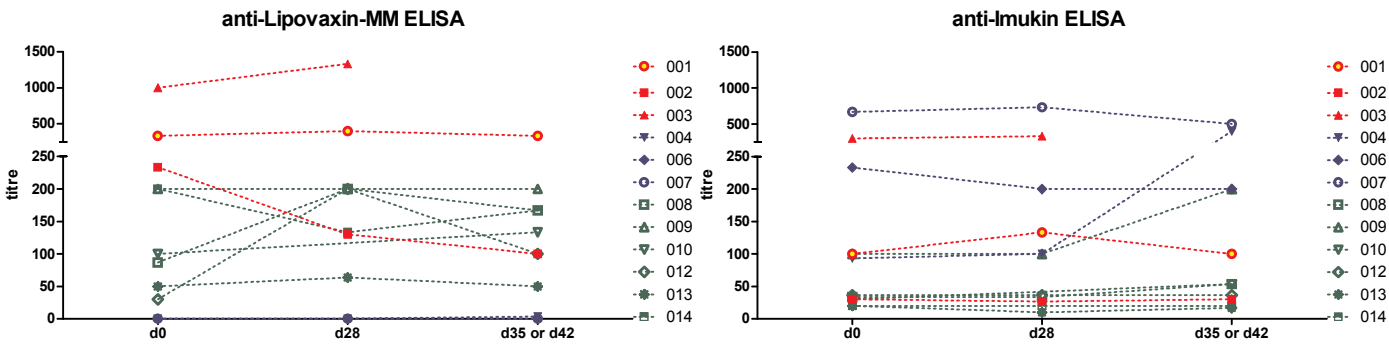
d)



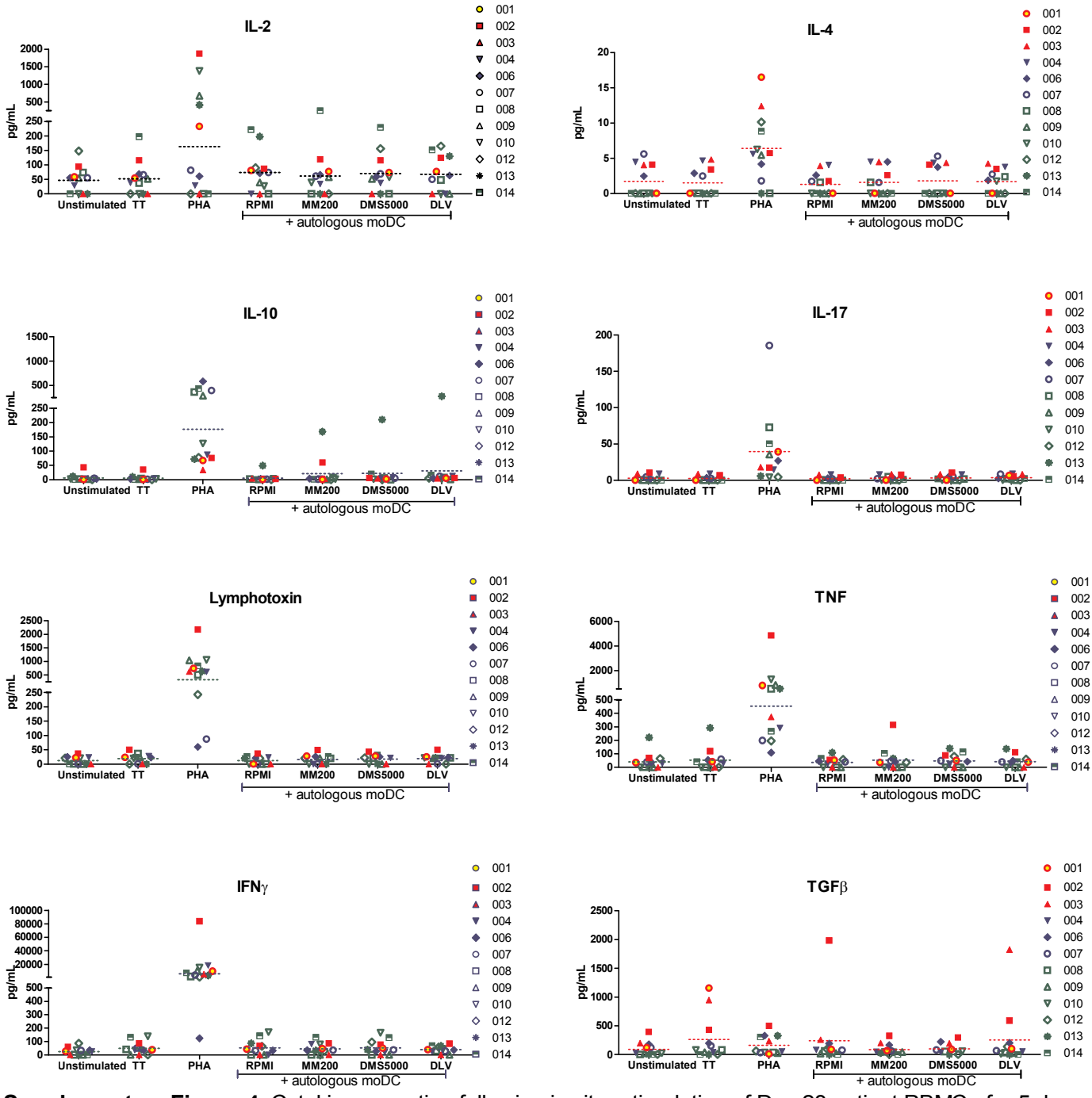
•Due to poor moDC yields fewer than 10^4 cells were used in targeting assay resulting in sub-optimal histograms



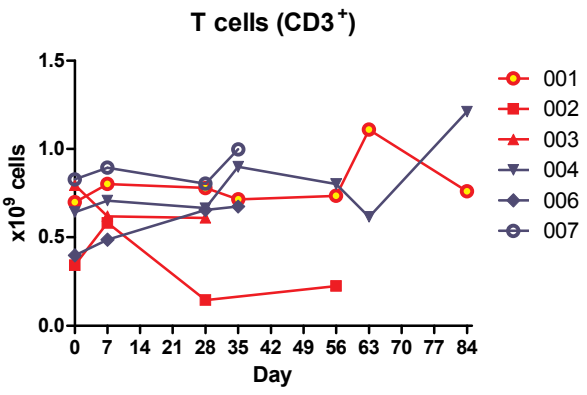
Supplementary Figure 2. a) The presence of key melanoma antigens was confirmed for every administered dose of Lipovaxin-MM by western blot. Representative image shows three doses of Lipovaxin MM. The molecular weight ladder (kDa) is indicated along the side of each blot. Sections of patient tumor blocks were assessed by immunohistochemistry for melanA/MART-1 antigen expression. Representative images are shown for two patients. **b)** Patient 001 **c)** Patient 008 **d)** Patient 006 **e)** Patient 004.



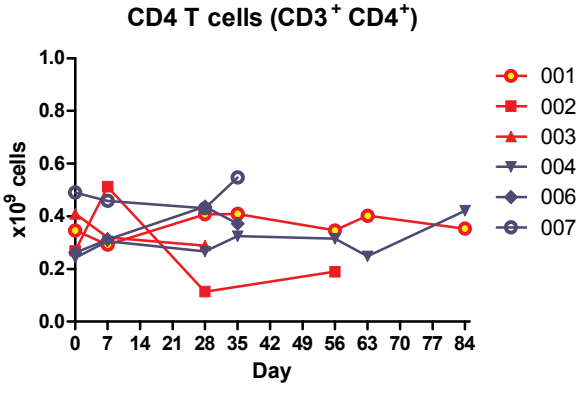
Supplementary Figure 3. ELISA was used to detect circulating antibodies in patient sera. Sera from Days 0, 28 and 35 (Patients 001-007) or Day 42 (Patients 008-014) were analyzed for antibodies against Lipovaxin-MM or interferon-gamma (Imukin). Cohort A (0.1mL) shown in red. Cohort B (1mL) shown in blue. Cohort C (3mL) shown in green. Only Patient 001 (red circle, yellow centre) had a response by RECIST criteria (v1.0).

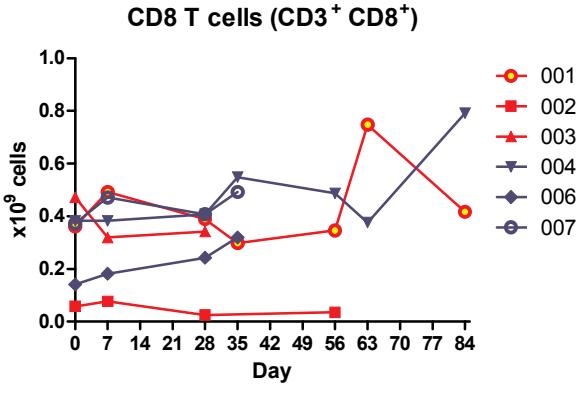


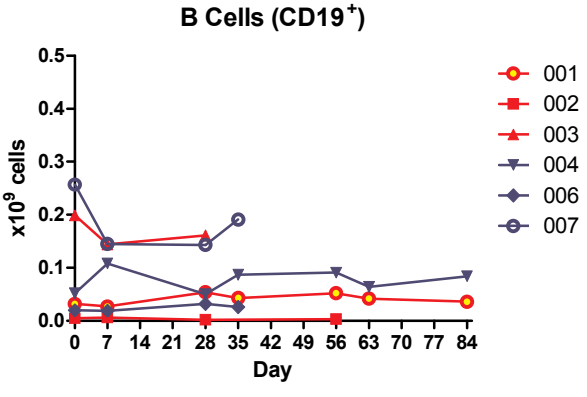
Supplementary Figure 4. Cytokine secretion following in vitro stimulation of Day 28 patient PBMCs for 5 days, as measured by cytokine bead array. Patient PBMCs were either unstimulated, or stimulated with tetanus toxoid (TT) or phytohemagglutinin (PHA) as positive controls, or with autologous patient-derived monocytic dendritic cells (moDCs) and media (RPMI) with or without the test antigens: MM200 melanoma cell membrane vesicles, DC-SIGN specific monoclonal antibody (DMS5000) or dummy Lipovaxin (DLV) formulated without interferon-gamma. Cohort A (0.1mL) shown in red. Cohort B (1mL) shown in blue. Cohort C (3mL) shown in green. Only patient 001 (red circle, yellow centre) had a response by RECIST criteria (v1.0)

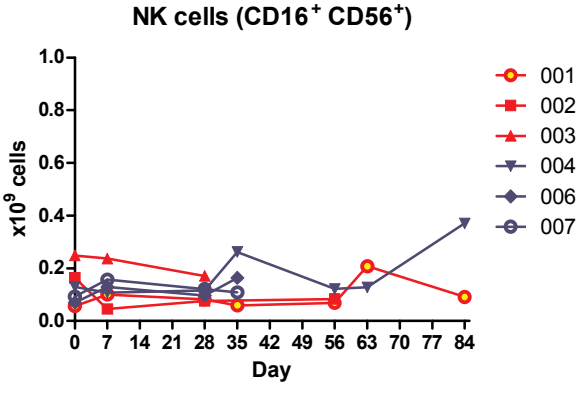


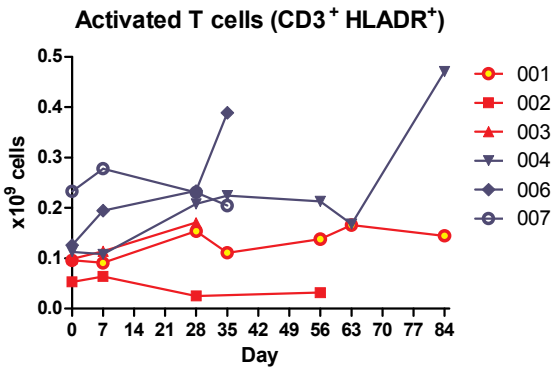
Supplementary Figure 5. Circulating leukocyte populations in peripheral blood. Leukocyte markers were assessed by flow cytometry and total numbers of leukocyte subsets were enumerated based on WBC/L counts. The sample for Patient 008-014 was not analyzed. Patients 002, 003, 006, 007 withdrew from study before the final timepoint on Day 84. Cohort A (0.1mL) shown in red. Cohort B (1mL) shown in blue. Only Patient 001 (red circle, yellow centre) had a response by RECIST criteria (v1.0). Data for the CD14+ macrophage/monocyte/DC population was initially recorded but is missing from this analysis.



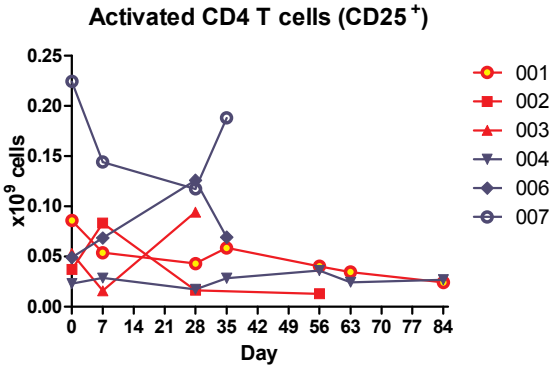


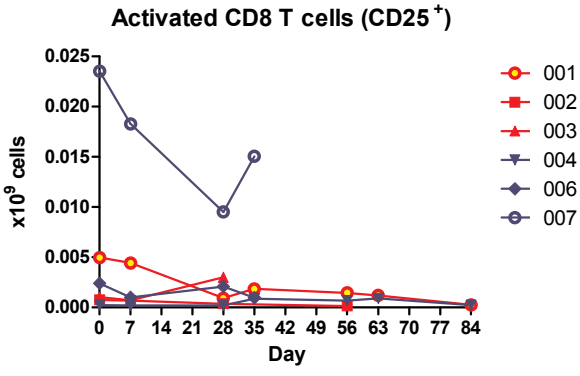


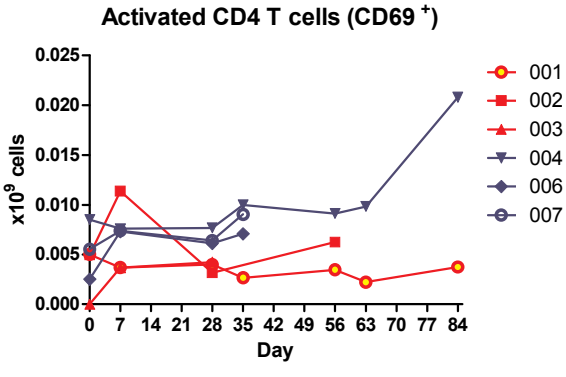


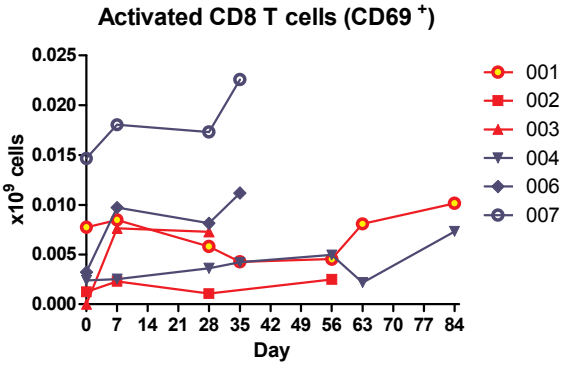


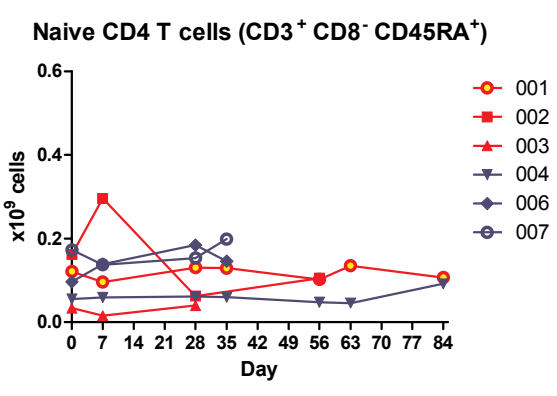
Supplementary Figure 6. Activation and memory phenotype of T cells circulating in peripheral blood. Activation markers (HLADR, CD25 and CD69) and naive/memory markers (CD45RA/CD45RO) were assessed by flow cytometry and total numbers were enumerated based on WBC/L counts. The sample for Patient 008-014 was not analyzed. Patients 002, 003, 006 and 007 withdrew from the study before the final timepoint on Day 84. Cohort A (0.1mL) shown in red. Cohort B (1mL) shown in blue. Only Patient 001 (red circle, yellow centre) had a response by RECIST criteria (v1.0).

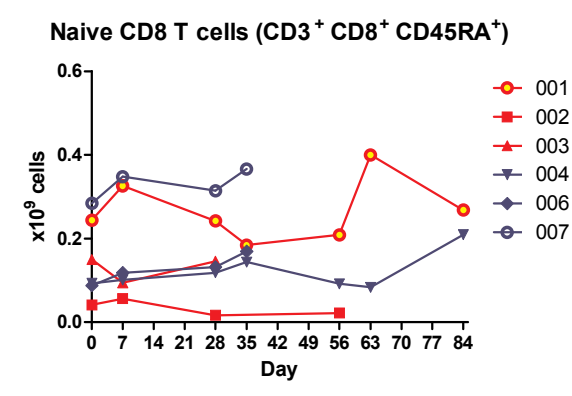


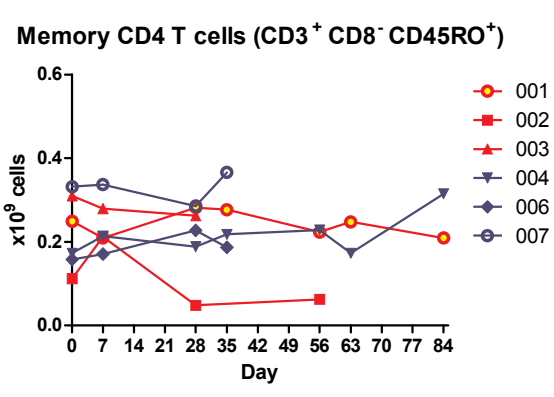


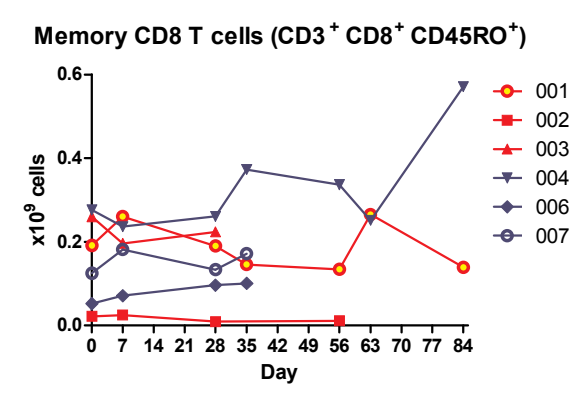


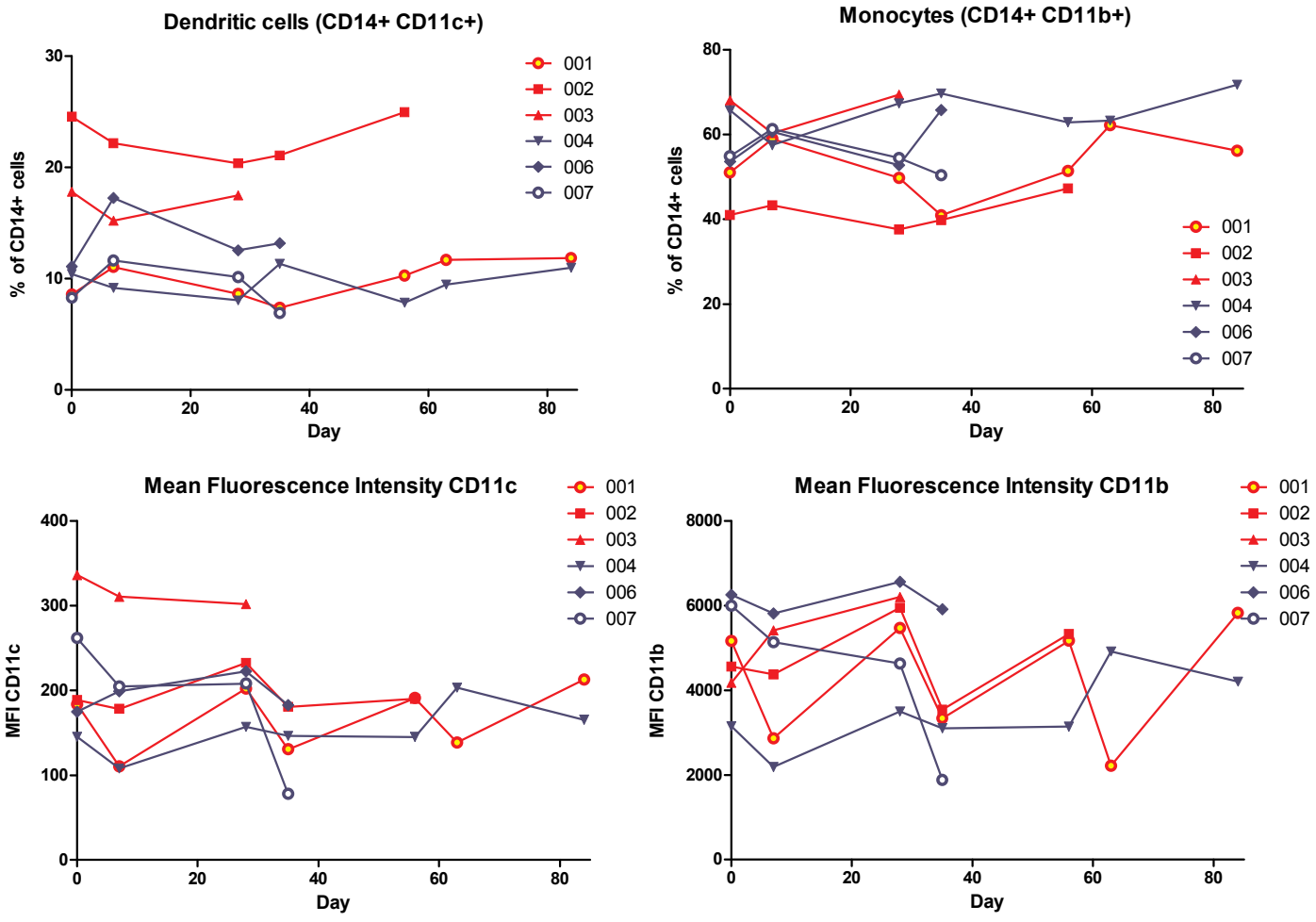




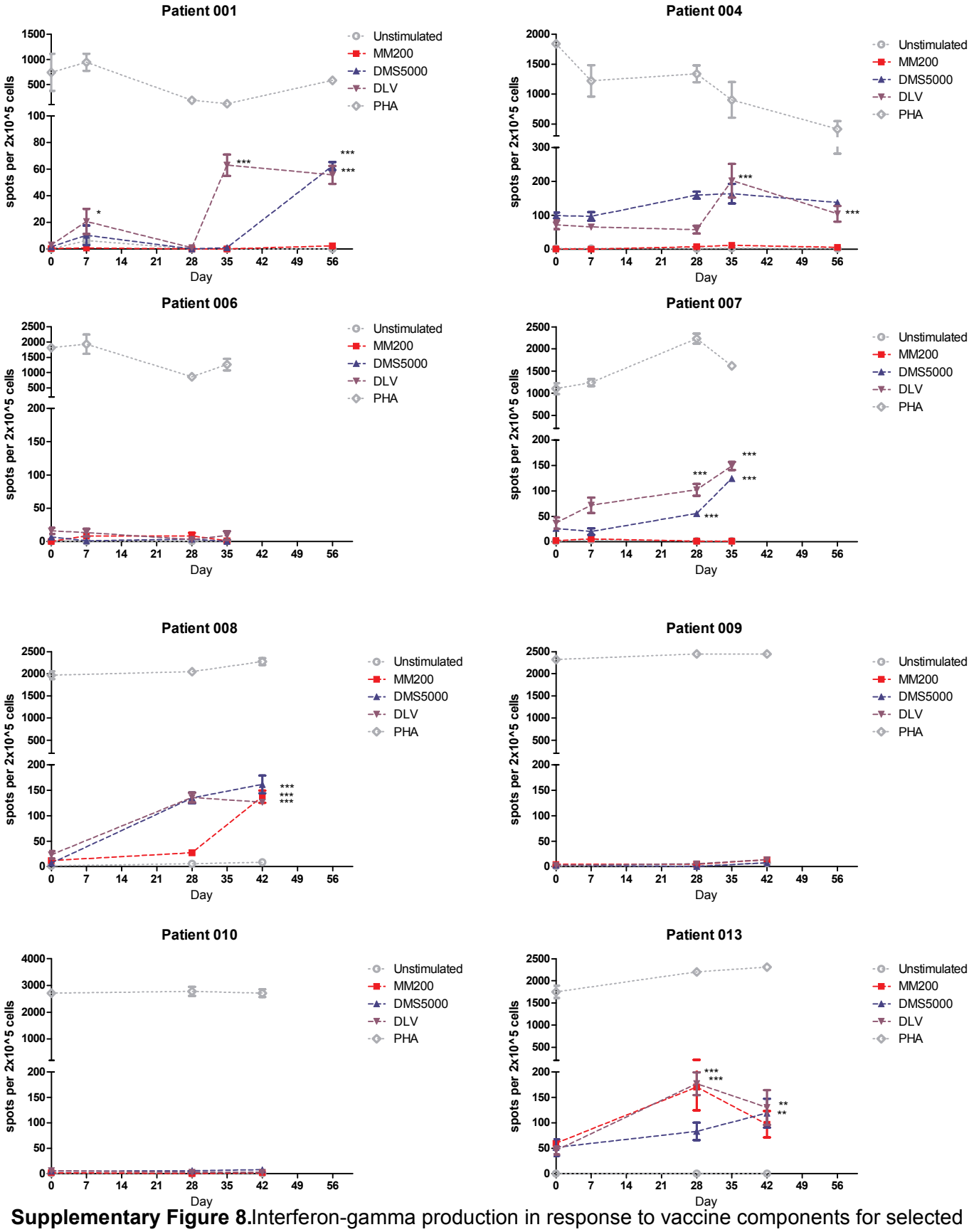








Supplementary Figure 7. Proportions of CD11b+ and CD11c+ cells within the parent CD14+ population. Mean fluorescence intensity of CD11b and CD11c on CD14+ cells. Expression of CD11b and CD11c is activation dependent, and upregulates following activation. The sample for Patient 008-014 was not analyzed. Patients 002, 003, 006 and 007 withdrew from the study before the final timepoint on Day 84. Cohort A (0.1mL) shown in red. Cohort B (1mL) shown in blue. Only Patient 001 (red circle, yellow centre) had a response by RECIST criteria (v1.0).



Supplementary Figure 8. Interferon-gamma production in response to vaccine components for selected patients. Patient peripheral blood lymphocytes were collected on the indicated days and pre-sensitized with antigen and moDCs, as described in Figure 3, for 96-120 hours in culture. Cells were then harvested and cultured in ELISPOT plates for 20-24 hours in the presence of PHA or the indicated test antigens: MM200 melanoma cell membrane vesicles, DC-SIGN specific monoclonal antibody (DMS5000) or dummy Lipovaxin (DLV) formulated without interferon-gamma. Additional positive controls: tetanus toxoid, RPMI + anti-CD3 and DLV + anti-CD3 (not shown) and additional negative controls RPMI (not shown). Patients 002, 003, 012, 014 were excluded from analysis because of insufficient PBMC or failure of PHA control stimulation. Statistical analysis was a two-way ANOVA with Bonferonni post-test comparing to values at Day 0. Significance is represented on graphs as * < 0.05, ** < 0.01 and *** < 0.001.