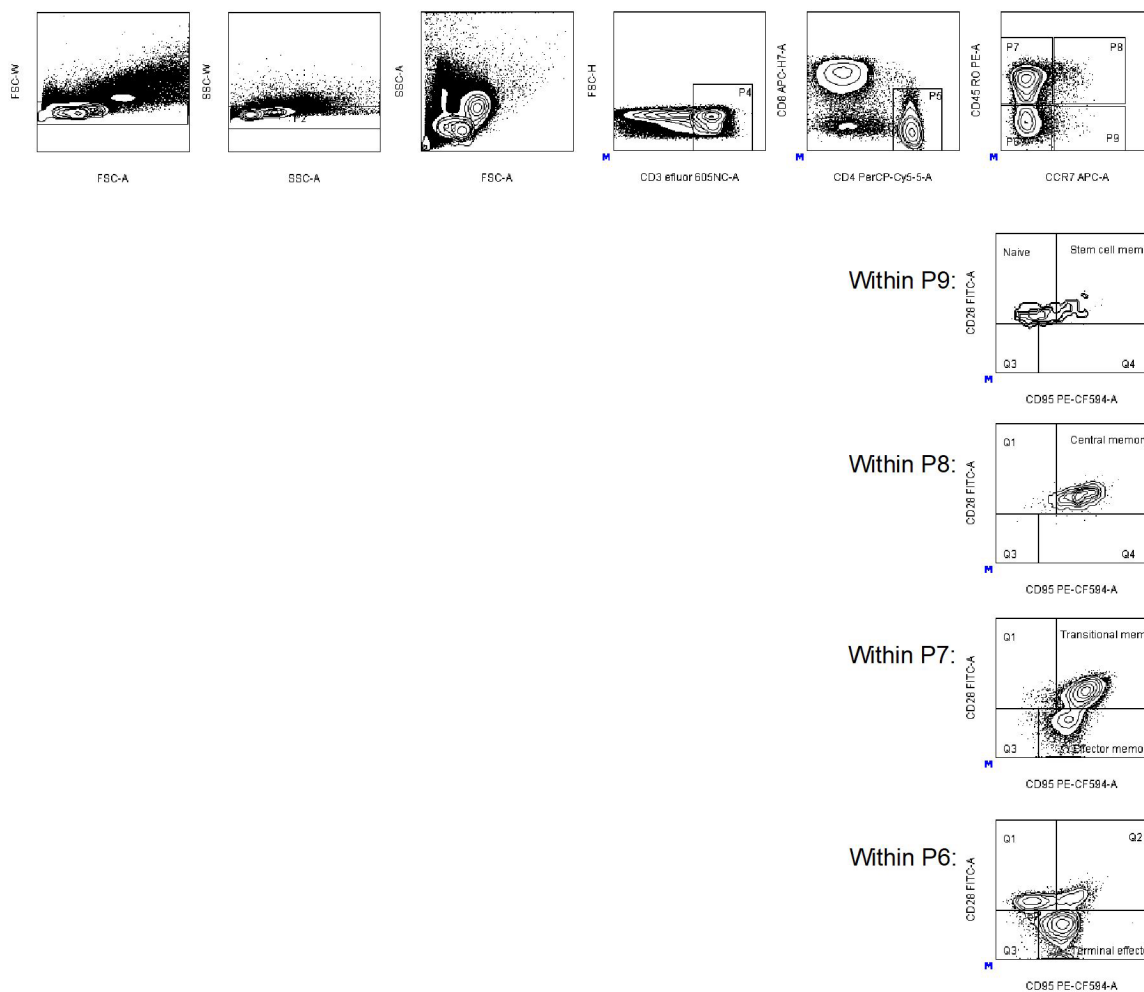
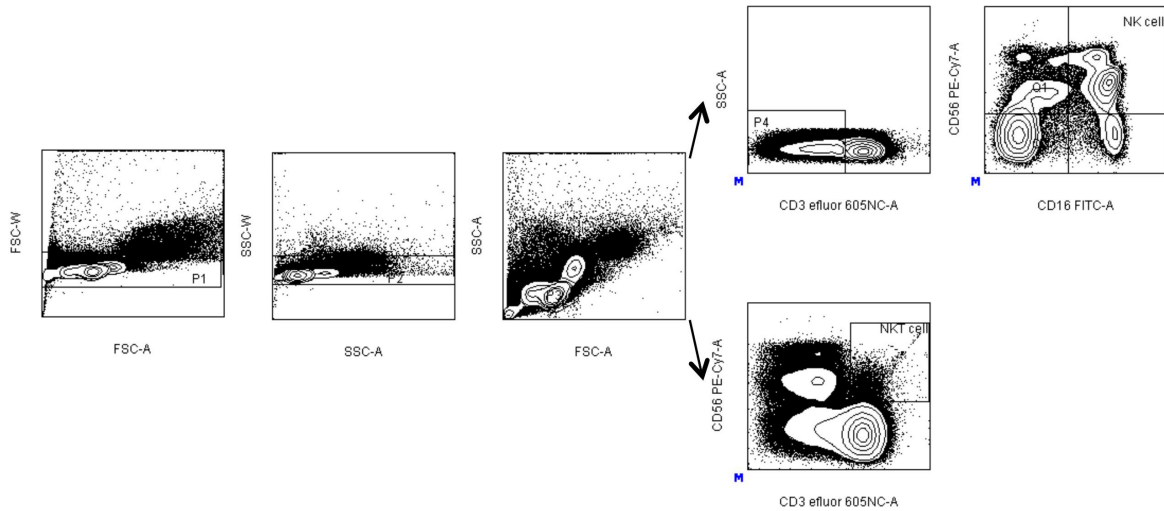


Impact of lenalidomide maintenance on the immune environment of multiple myeloma patients with low tumor burden after autologous stem cell transplantation

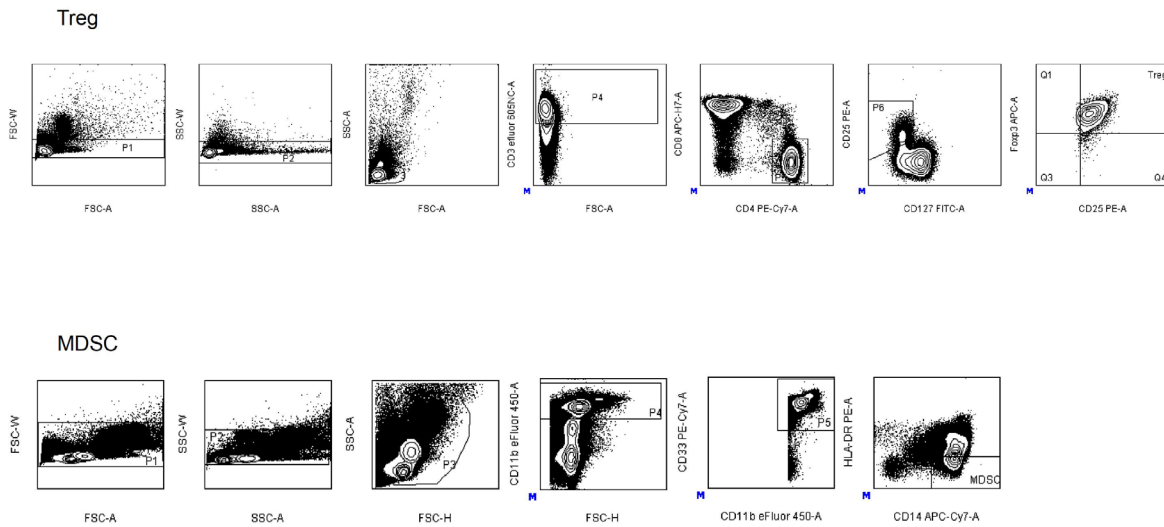
SUPPLEMENTARY MATERIALS



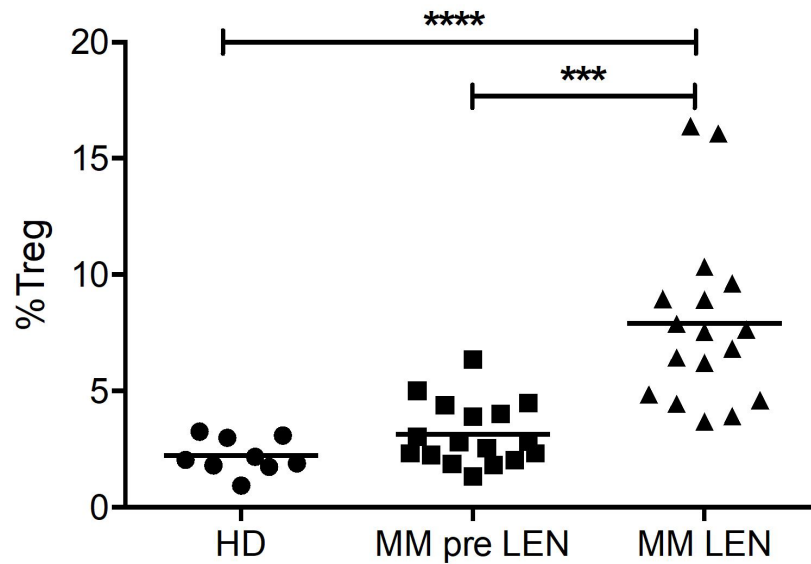
Supplementary Figure 1: The flow cytometry plots show the T cell subset gating strategy. First, we gated on single cells using FSC-A/FSC-W and SSC-A/SSC-W plots. Next, we gated on the lymphocytes using FSC-A/SSC-A plots. Within the CD3⁺ population, we gated on CD4⁺ and CD8⁺ T cells. Next, we identified 4 populations based on CCR7 and CD45RO expression. Within CD45RO⁻ CCR7⁺ population (P9): naïve (CD28⁺ CD95⁻) and stem cell memory (CD28⁺ CD95⁺) T cells were identified. Within the CD45RO⁺ CCR7⁺ population (P8) the central memory T cells (CD28⁺ CD95⁺) T cells were identified. Within the CD45RO⁻ CCR7⁺ population (P7), the transitional memory (CD28⁺ CD95⁺) and the effector memory T cells (CD28⁻ CD95⁺) were identified. Finally, within the CD45RO⁻ CCR7⁻ population (P6), the terminal effector T cells (CD28⁻ CD95⁻) were identified.



Supplementary Figure 2: The flow cytometry plots show the NK and NKT cell subset gating strategy. First, we gated on single cells using FSC-A/FSC-W and SSC-A/SSC-W plots. For the NK cells, we first gated on CD3⁻ cells. Within the CD3⁻ population, NK cells were defined as CD56⁺ CD16⁺ cells. NKT cells were defined as CD3⁺ CD56⁺ cells.



Supplementary Figure 3: The flow cytometry plots show the gating strategy for immune suppressive cell types. For the Treg stainings, we first gated on single cells using FSC-A/FSC-W and SSC-A/SSC-W plots. Next, we gated on the lymphocytes using FSC-A/SSC-A plots. Within the CD3⁺ population, we gated on CD4⁺ T cells. Next, we gated on the CD25^{high} CD127^{low} population (P6). Within P6, we gated on the Foxp3^{high} cells. For the MDSC stainings, we first gated on single cells using FSC-A/FSC-W and SSC-A/SSC-W plots. Next, we gated on the viable cells using FSC-A/SSC-A plots. Within the CD11b⁺ CD33⁺ population, we gated on CD14⁺ HLA-DR^{low} cells.



Supplementary Figure 4: The graphs shows the Treg frequency in healthy donors compared to MM patients before or during lenalidomide maintenance therapy. The Kruskal–Wallis test was performed to evaluate statistical significant differences, $***p < 0.001$, and $****p < 0.0001$.