# Supporting Information

## Romano et al. 10.1073/pnas.1417320112

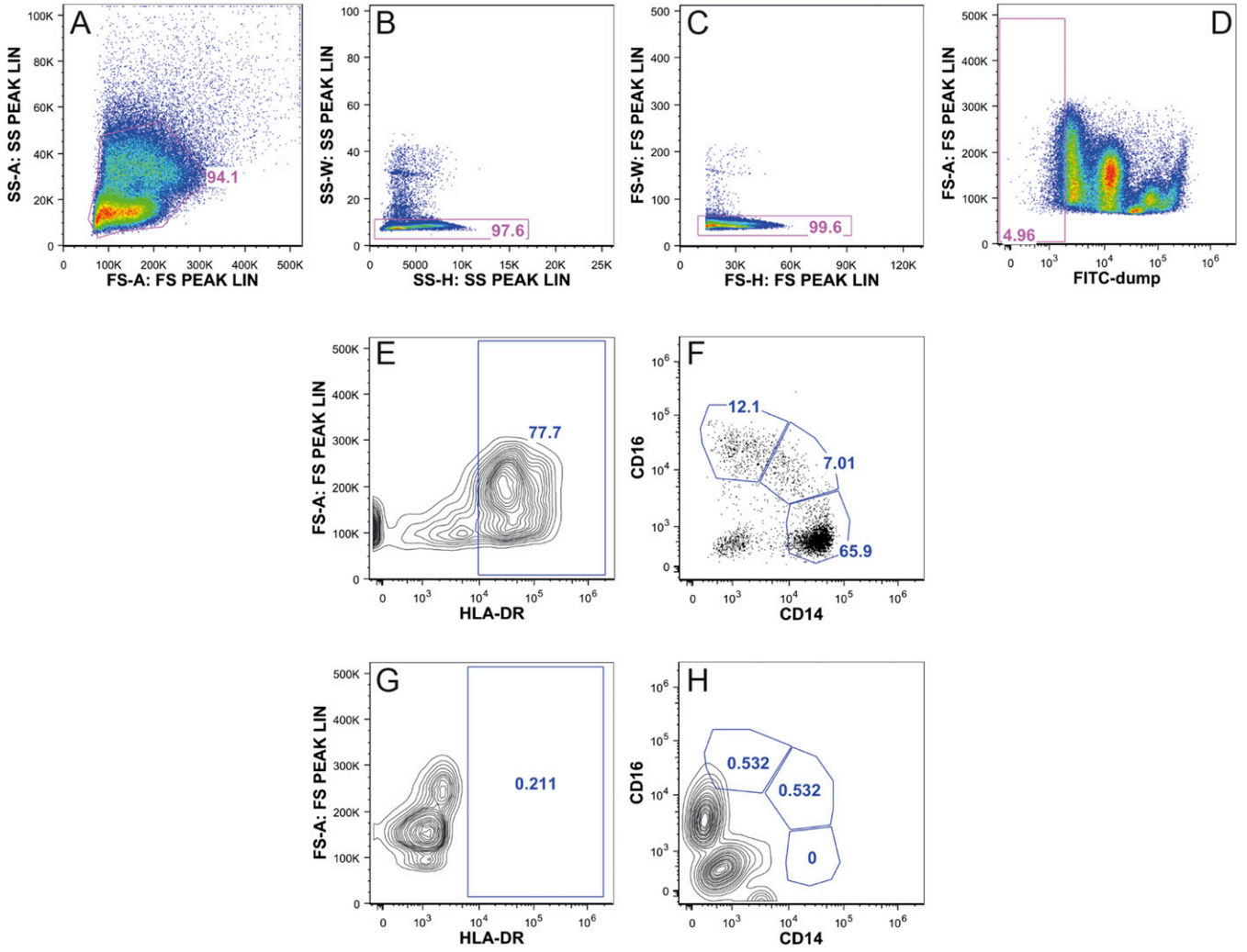


**Fig. S1.** Gating strategy for analysis of human monocytes. PBMC samples were stained with mAbs against human CD3/CD19/CD20/CD56/Vivid live/dead stain (dump channel), HLA-DR, CD14, CD16, CD11b, CD11c, and CD163. After gating on small and large cells in the SS-A/FS-A (*A*) and doublets (*B* and *C*), lineage-positive (FITC-dump) and dead cells (*D*) were excluded. From the live singlet cell population gated (*D*), FS-A was plotted against HLA-DR (*E*) and gates were drawn for monocytic lin-HLA-DR$^+$ cells based on isotype control (*G*). These cells were then analyzed for the expression of CD14 and CD16 (*F*) based on the isotype control (*H*). Each monocyte subset was then tested for expression of CD11b, CD11c, and CD163 relative to the isotype control (Fig. 1*B*).

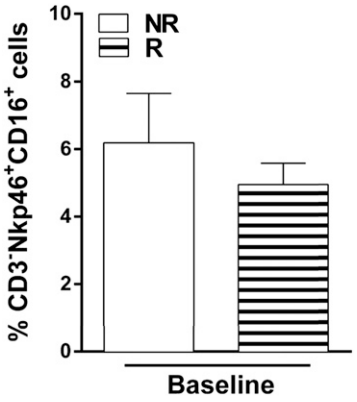


**Fig. S2.** No difference in baseline peripheral $CD3^{-}NKp46^{+}CD16^{+}$ NK cell frequencies in responding vs. nonresponding patients with melanoma. PBMC samples were stained with mAbs against human CD19/CD20/Vivid live/dead stain (dump channel) and CD3, CD335 (NKp46), HLA-DR, CD14, and CD16. At baseline, there was no significant difference in peripheral frequencies of $CD3^{-}NKp46^{+}CD16^{+}$ NK cells in responding vs. nonresponding patients with melanoma.

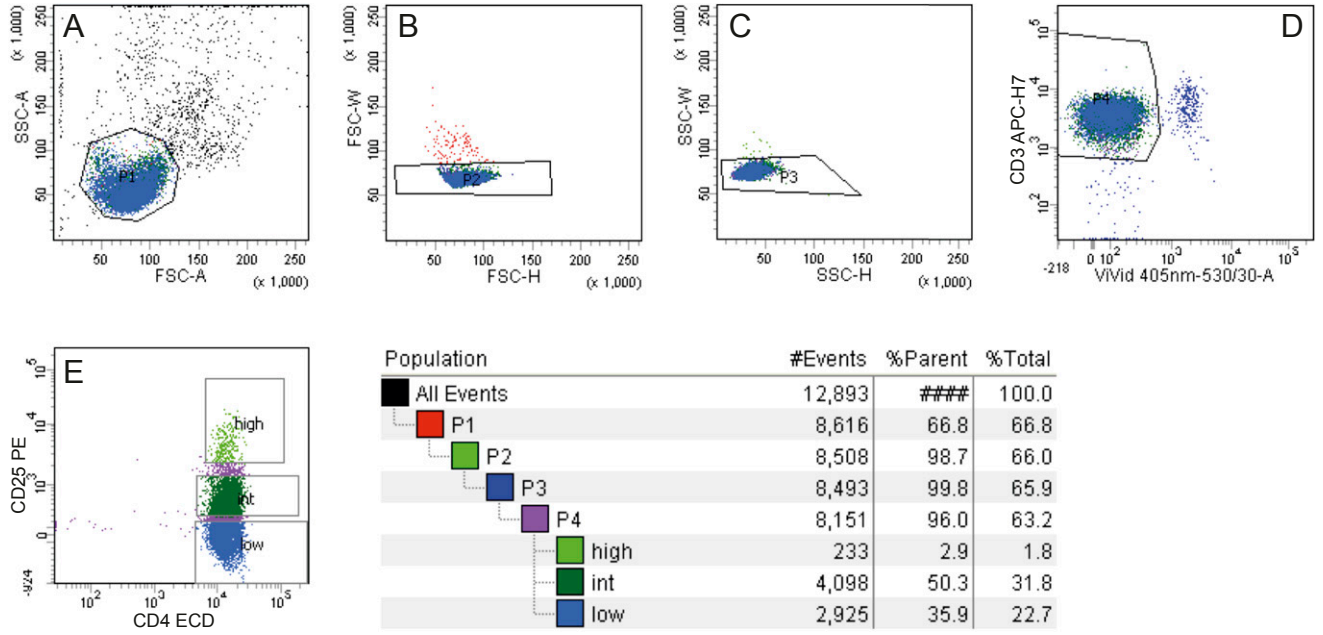


**Fig. S3.** Gating strategy for sorting of human $CD3^{+}CD4^{+}$ T cells according to CD25 expression. Before staining, $CD4^{+}CD3^{+}$ T cells were enriched from PBMCs. T cells were then stained with mAbs against human CD3/CD4/CD25/AmCyan live/dead stain. After gating on SS-A/FS-A (*A*) and doublets (*B* and *C*), dead cells (*D*) were excluded. From the live singlet $CD3^{+}CD4^{+}$ cell population gate (*D*), CD25 was plotted against CD4 and gates were drawn for $CD4^{+}$ cells with different expression level of CD25 (*E*). Based on this gating strategy, three different cell subpopulations were sorted: $CD3^{+}CD4^{+}CD25^{bright}$, $CD3^{+}CD4^{+}CD25^{int}$, and $CD3^{+}CD4^{+}CD25^{neg}$.

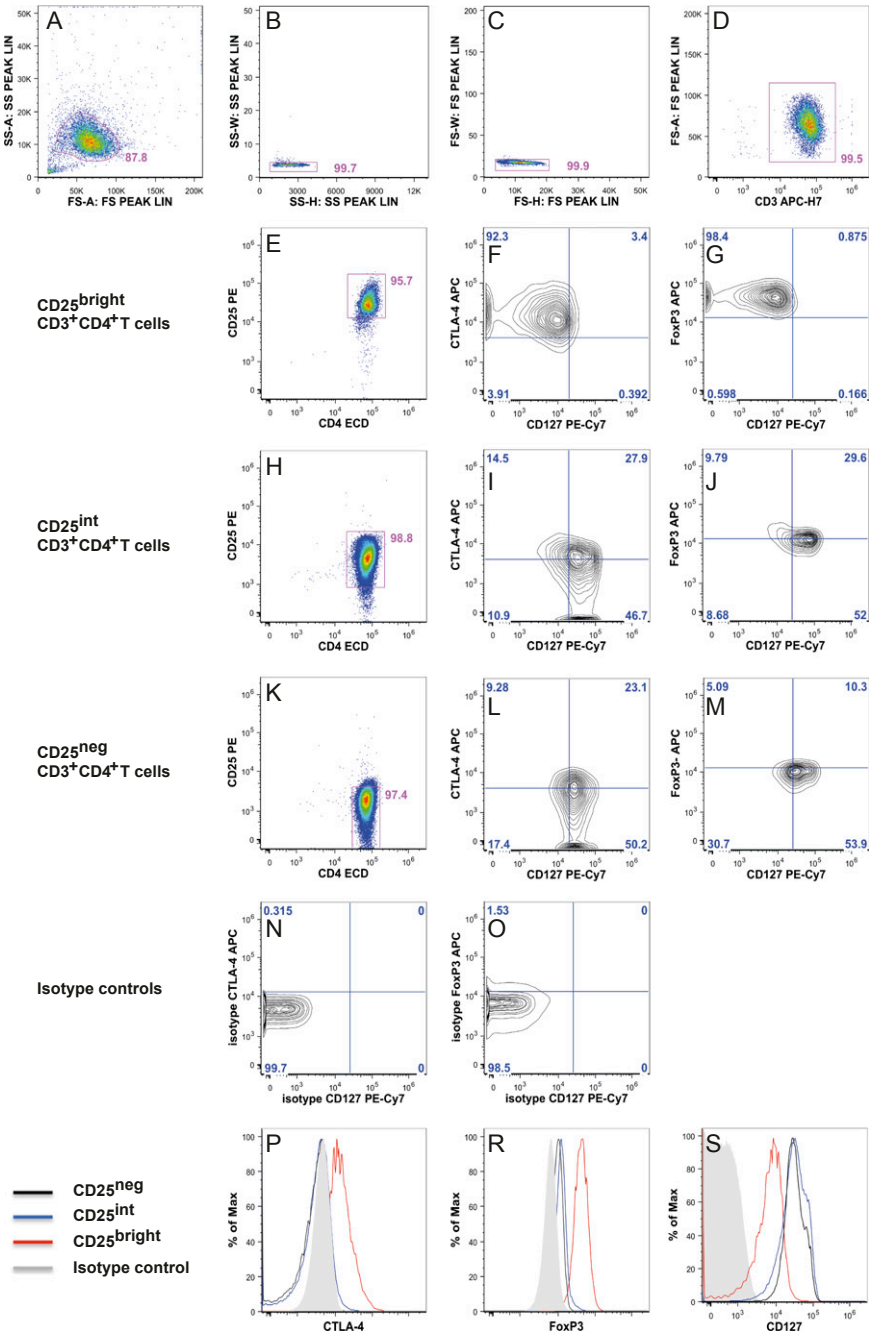


**Fig. S4.** CTLA4, Foxp3, and CD127 staining on sorted $CD3^+CD4^+CD25^{bright}$, $CD25^{int}$, or $CD25^{neg}$ T cells. Three sorted, live T-cell subpopulations with different expression levels of CD25—$CD3^+CD4^+CD25^{bright}$, $CD3^+CD4^+CD25^{int}$, and $CD3^+CD4^+CD25^{neg}$—were stained with monoclonal antibodies against human CTLA-4, Foxp3, and CD127. After gating on cells in SS-A/FS-A (*A*), doublets (*B* and *C*), and $CD3^+CD4^+$ (*D*), expression level of CD25 was checked once again (*E*, *H*, and *K*). Then, CTLA-4 or Foxp3 were plotted against CD127 (*F*, *G*, *I*, *J*, *L*, and *M*) and the expression of CTLA-4 (*P*), Foxp3 (*R*), and CD127 (*S*) was analyzed based on the isotypes controls (*N* and *O*).