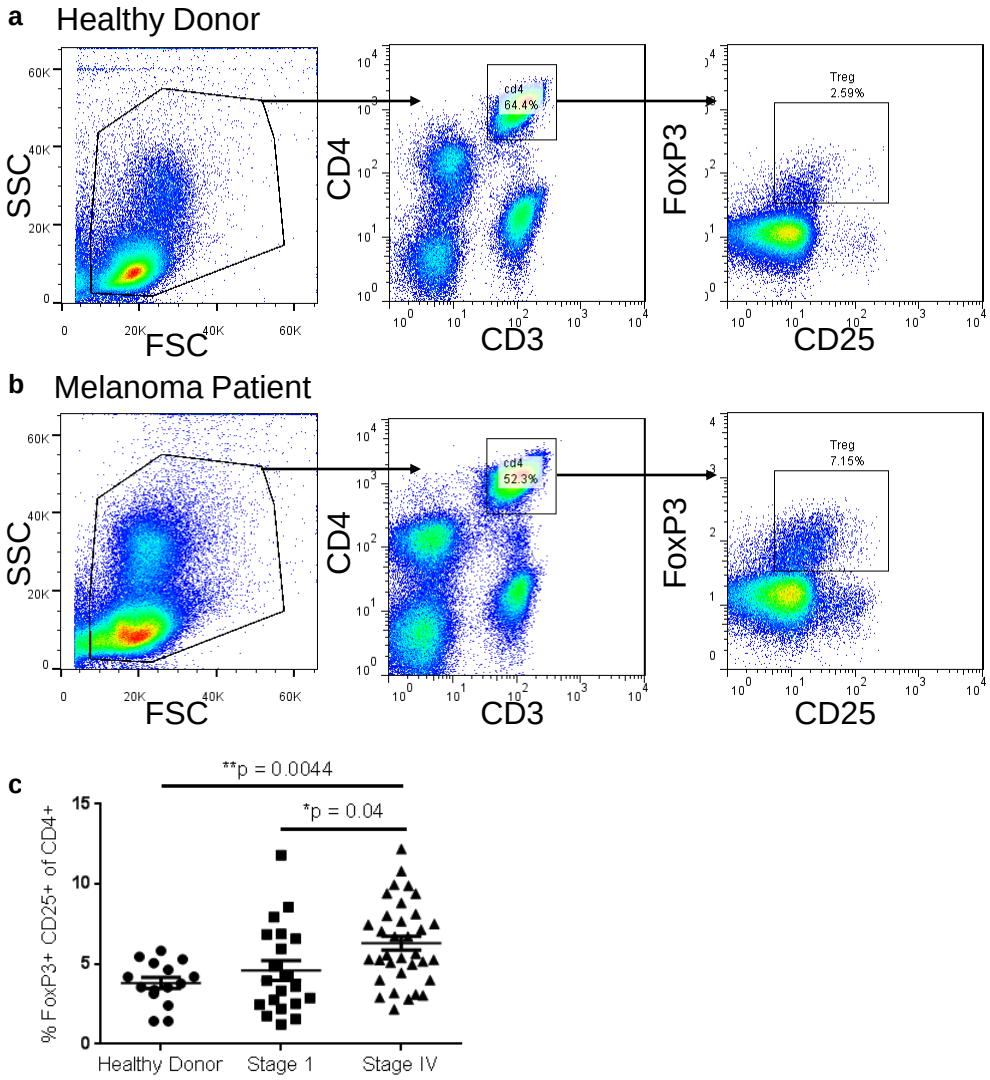
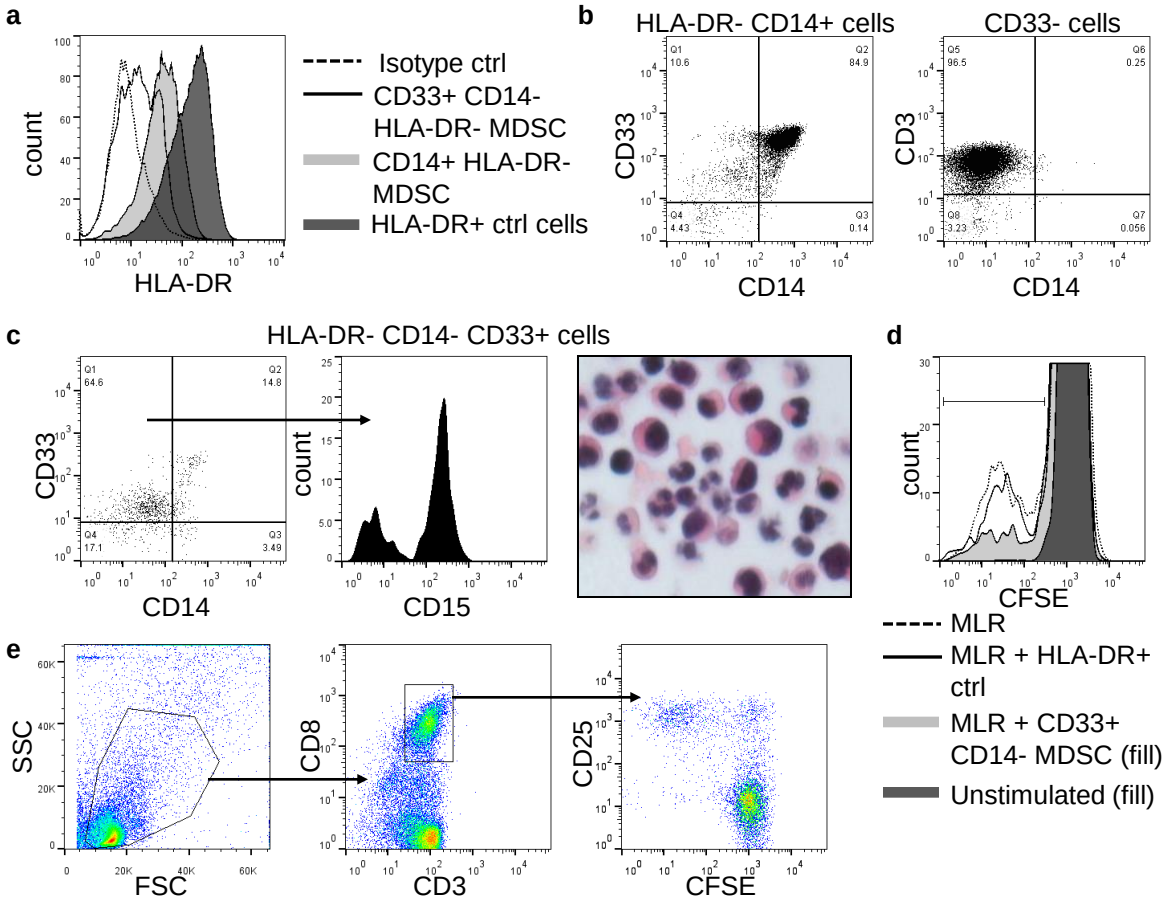
Online Resource 1. Clinical characteristics of patients in the functional analysis.

Stage of disease at blood draw[a]	# of patients	Average age (range)[b]	Gender (M/F)
Healthy donor	11	45.9 (28-67)	4/7
I	10	58.6 (42-73)	3/7
IV	11	54.8 (38-87)	8/3

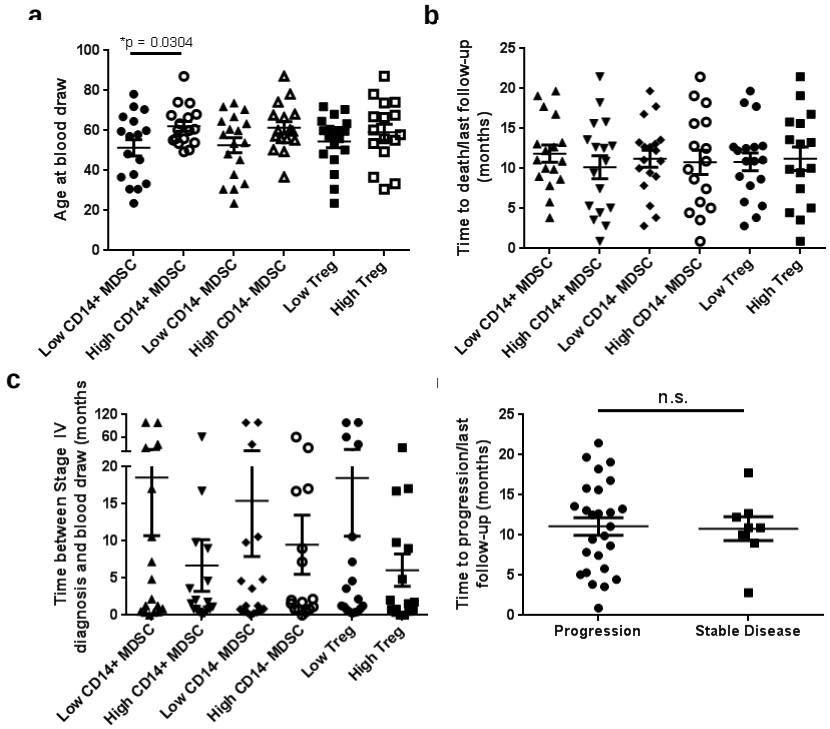
Eligible patients were diagnosed with Stage I or Stage IV melanoma.
[a]All patients enrolled in the study. [b]Age at blood draw.



Online Resource 2. Regulatory T cells are increased in melanoma patients. PBMCs from healthy donors (**a**) or melanoma patients (**b**) were stained with antibodies specific for CD3, CD4, CD8, CD25, and FoxP3 and analyzed by flow cytometry. The gating scheme used to analyze T_{regs} is shown. Gates were set using isotype control antibodies. **c.** The frequency of T_{regs} was compared between healthy donors, Stage I melanoma patients, and Stage IV melanoma patients using one-way ANOVA ($p = 0.0026$) followed by a Tukey's Multiple Comparison test (p values shown).



Online Resource 3. Magnetic separations of MDSCs and gating scheme for T cell functional assays. **a**. Expression of HLA-DR on magnetically separated cells used in the T cell suppression assays. **b**. Post-sort analysis of HLA-DR- CD14+ cells (80-90% CD14+) and CD33- CD14- HLA-DR- cells (greater than 90% T cells) used in the suppression assays. **c**. CD33+ CD14- HLA-DR- MDSCs used in suppression assays are a mixed population of CD15- and granulocytic CD15+ cells, analyzed by flow cytometry (left, 60-70% CD14- CD33+ MDSCs), and morphologically by hematoxylin and eosin staining of paraffin imbedded cells (right). **d.** CFSE-labeled CD33- cells (shown in **b**) from fresh PBMC samples were incubated with dendritic cells from an unrelated healthy donor in a mixed lymphocyte reaction (MLR). After 4 days, the frequency of divided CD3+ CD8+ T cells was determined using the gating scheme shown in (**e**) in the presence or absence of HLA-DR+ control cells or MDSCs. **e.** Gating scheme used to determine the frequency of divided cells and the CD25 MFI in the T cell suppression assays.



Online Resource 4. Patients with "Low" or "High" frequencies of CD14- MDSCs have similar clinical characteristics and follow-up times. **a**. The average age of patients with a high or average/low frequency of MDSCs and Tregs were compared using a Student's *t* test. **b.** The average follow-up time was compared for patients with a high or low frequency of MDSCs and Tregs. **c**. The average time between Stage IV diagnosis and blood draw was compared between patients with a high or low frequency of MDSCs. **d**. The average follow-up time was compared between patients that progressed during the study and those with stable disease.

Online Resource 5. A high frequency $CD14^-$ MDSCs independently predicts a significant increase in the risk of death and disease progression in Stage IV melanoma patients.

Overall Survival

Variable	Univariate Analysis[a] HR (95% CI) *p* value	Mean follow-up time (m)
CD14-		
High	4.83 (1.34 – 17.5) 0.016	10.8
Low	1.0	11.2

Time to Progression

Variable	Multivariate Analysis[b] HR (95% CI) *p* value	Mean follow-up time (m)
CD14-		
High	2.39 (1.04 – 5.56) 0.039	5.5
Low	1.0	7.8
CD14+		
High	2.10 (0.9 – 4.80) 0.089	5.3
Low	1.0	8.0

Overall survival and time to progression were analyzed for all Stage IV patients. [a]Gender, categorical age (<58 or ≥ 58), prior treatments (chemo/radiation, immunotherapy, biologic therapy), frequency of $CD14^+$ MDSCs and T_{regs}, stage of initial diagnosis, time from Stage IV diagnosis to blood draw, and BRAF mutation status were not univariately significant and therefore not included in a multivariate model for overall survival. [b]Gender, categorical age (<58 or ≥ 58), prior treatments, frequency of T_{regs}, stage of initial diagnosis, time from Stage IV diagnosis to blood draw, and BRAF mutation status were not univariately significant and therefore not included in a final model for time to progression.