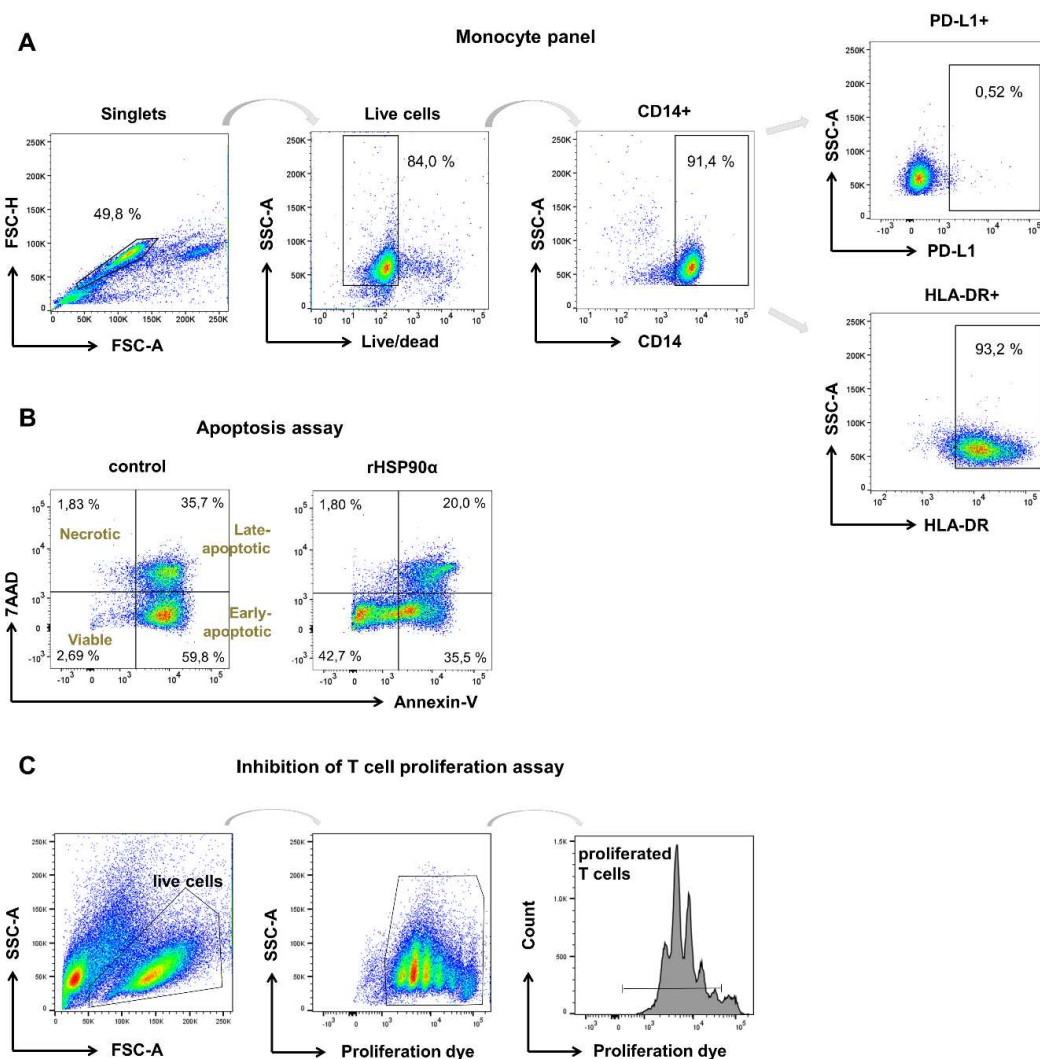
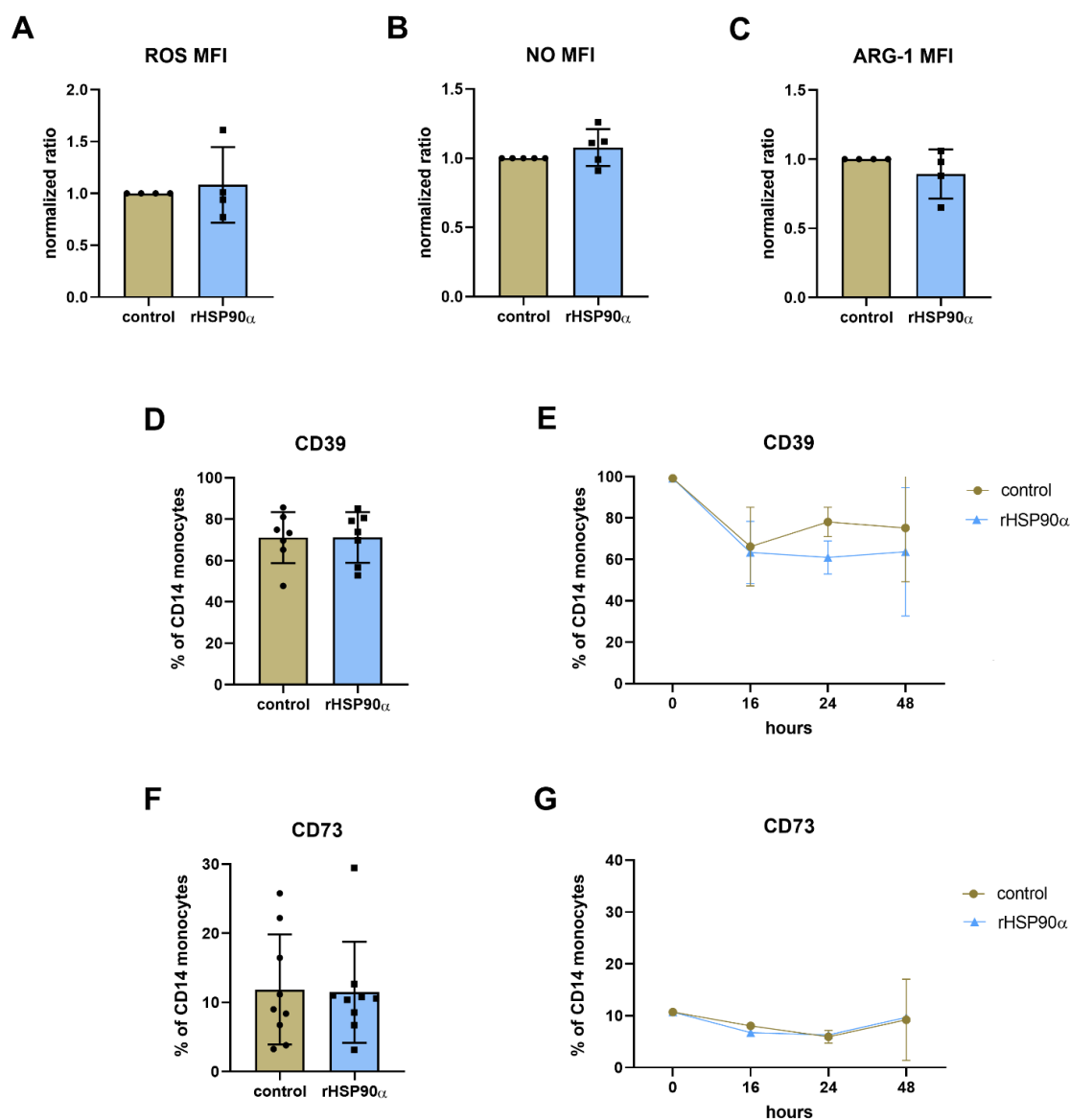




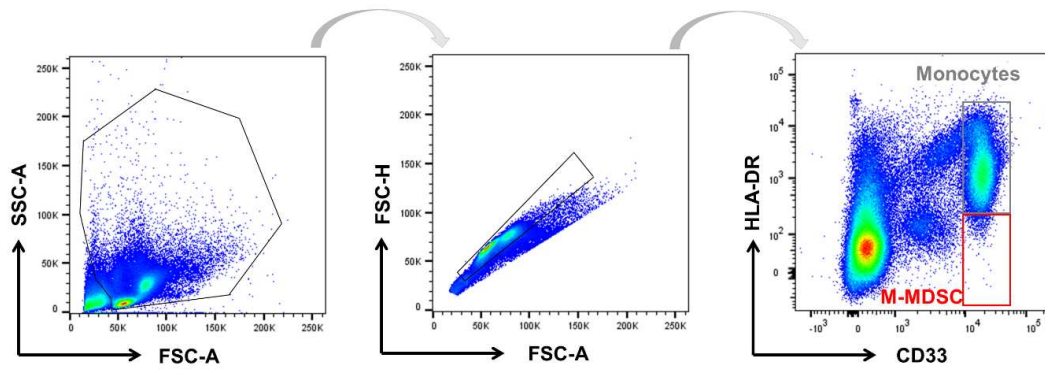
**Fig. S1 Digestion of rHSP90 $\alpha$  with trypsin.** rHSP90 $\alpha$  was incubated with immobilized trypsin for 18 h at 37°C. Then trypsin-beads were pelleted, and the supernatant was tested by Western Blot.



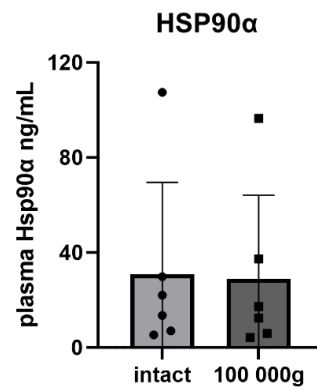
**Fig. S2 Gating strategy for FACS analysis.** (A) Gating strategy for surface marker expression of monocytes. Representative dot plots are shown for freshly isolated human CD14 monocytes. Monocytes were gated after exclusion of doublets and dead cells. (B) Gating strategy for apoptosis of monocytes treated with rHSP90 $\alpha$  for 24 h. Dot plots show Annexin V<sup>+</sup>7AAD<sup>-</sup> live cells, Annexin V<sup>+</sup>7AAD<sup>-</sup> early apoptotic cells, and Annexin V<sup>+</sup>7AAD<sup>+</sup> late apoptotic cells. (C) Representative dot plots and histograms for proliferated T cells stimulated for 96 h.



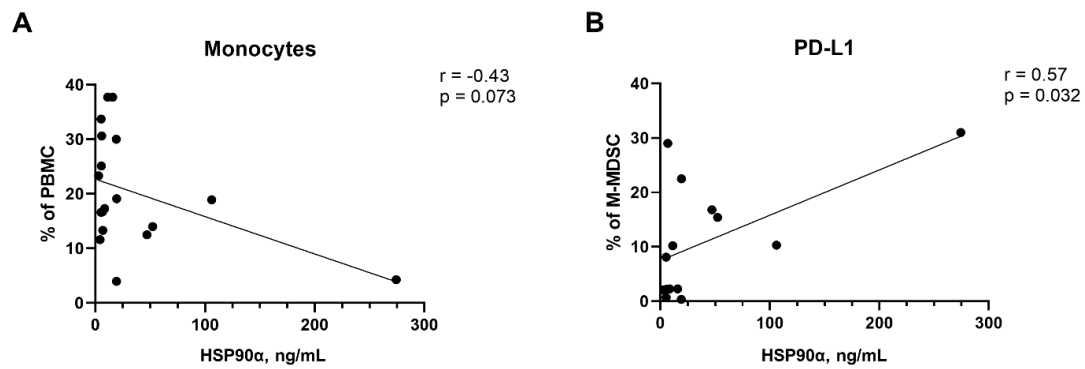
**Fig. S3 Effect of rHSP90 $\alpha$  on production of ROS and NO as well as expression of Arg-1, CD39 and CD73 in monocytes.** Monocytes were treated with 2 $\mu$ g/mL rHSP90 $\alpha$  or PBS (control). Production of ROS (A), NO (B) and expression of ARG-1 (C) is presented as normalized mean fluorescence intensity (MFI) (mean $\pm$ SD; n=4). Expression of CD39 (D, E), and CD73 (F,G) is shown as the percentage of CD39<sup>+</sup> or CD73<sup>+</sup> monocytes among total monocytes (mean $\pm$ SD; n=3-9).



**Fig. S4 Gating strategy for sorting of monocytes and M-MDSC via FACS.** After exclusion of cell debris and doublets, monocytes and M-MDSC were sorted from PBMC according to the expression of HLA-DR and CD33.



**Fig. S5 Impact of ultracentrifugation on the level of HSP90 $\alpha$  in plasma of melanoma patients.** Concentration of HSP90 $\alpha$  was measured by ELISA in plasma before or after the ultracentrifugation at 100,000 g for 16 h. Data are expressed as ng/mL.



**Fig. S6 Effect of soluble HSP90 $\alpha$  on myeloid cells in melanoma patients.** Monocytes and M-MDSC were sorted from the PBMC of melanoma patients at the baseline or during the therapy and analyzed by flow cytometry. The concentration of HSP90 $\alpha$  was detected in plasma of the same patients by ELISA. The frequency CD14 monocytes within PBMC (A; n=18) or PD-L1<sup>+</sup> M-MDSC among total MDSC (A; n=14) were plotted against the levels of HSP90 $\alpha$  expressed in ng/mL. The correlation was evaluated by a linear regression analysis.