Supplemental Figure 1. Treg frequencies and TIGIT expression in T cell subsets in healthy donors and melanoma patients. (A) Summary data from all donors showing ex vivo frequencies of CD25^{hi}Foxp3^{+} CD4^{+} Tregs within total CD4^{+} T cells in PBMCs of healthy donors (HDs) and melanoma patients (MPs) and in metastatic melanoma (MM) tumor-infiltrating lymphocytes (TILs). n = 20. (B) Summary data showing ex vivo percentages and mean fluorescence (MFI) of TIGIT expression by total CD8^{+} T cells, total CD4^{+} T cells and CD25^{hi}Foxp3^{+} CD4^{+} Tregs in MM TILs (n = 12) Horizontal bars depict the mean percentages. Error bars indicate s.e.m. P values were obtained by unpaired t-test. *P < 0.05; **P < 0.01; ***P < 0.001.
Supplemental Figure 2. TIGIT+ Treg suppression in transwell experiments and pro-inflammatory cytokine production by Tregs. (A and B) Flow cytometric analysis from one representative experiment (A) and summary data (B) showing the suppression of proliferation of responder CD8+ T cells that were stimulated for 6 days with autologous antigen presenting cells (APCs) and anti-CD3 mAbs in the presence of TIGIT- CD4+ Teffs or TIGIT+ CD4+ Tregs. TIGIT- CD25- CD127-/+ CD4+ Teffs or TIGIT+ CD25hi CD127- CD4+ Tregs isolated from PBMCs of melanoma patients (MPs) were cultured either in direct contact with responder T cells in the bottom chamber of a transwell plate (No transwell) or in the top chamber of a transwell plate (Transwell) with allogeneic APCs and anti-CD3 mAbs (n = 3). P values were obtained by paired t-test. (C) Pooled data showing the production of IFN-γ and IL-2 by TIGIT- and TIGIT+ CD25- CD127-/+ CD4+ Teffs and CD25hi CD127- CD4+ Tregs isolated from PBMCs of MPs after a 6-day in vitro stimulation with aCD3/CD28 beads (n = 20). Results represent the mean of independent experiments. Error bars indicate s.e.m. P values were obtained by unpaired t-test. **P < 0.01; ***P < 0.001.
Supplemental Figure 3. TIGIT expression in Foxp3+ and Foxp3- CD4+ T cell subsets in melanoma patients. (A and B) Flow cytometry dot plots from 2 different melanoma patients (MPs) (A) and summary data from all MPs (B) showing ex vivo TIGIT expression [% and mean fluorescence intensity (MFI)] by Foxp3hi CD45RA- activated CD4+ Tregs (Fr. II), Foxp3lo CD45RA+ resting CD4+ Tregs (Fr. I) and non-suppressive Foxp3lo CD45RA- CD4+ T cells (Fr. III), as well as Foxp3- CD45RA- and Foxp3- CD45RA+ CD4+ T cells in PBMCs and tumor-infiltrating lymphocytes (TILs) of MPs (n = 20). Horizontal bars depict the mean percentage or MFI. Error bars indicate s.e.m. P values were obtained by unpaired t-test and repeated-measures ANOVA followed by Tukey’s test. *P < 0.05; **P < 0.01; ***P < 0.001.
Supplemental Figure 4. Treg suppression upon TIGIT or CD226 blockade in melanoma patients. (A) Flow cytometric analysis from representative experiments and summary data showing the suppression of responder CD8+ T cell proliferation after a 6-day in vitro stimulation (IVS) with autologous antigen presenting cells and anti-CD3 mAbs in the absence or presence of TIGIT+ CD25hiCD127− CD4+ Tregs isolated from PBMCs of melanoma patients (MPs) and with blocking aTIGIT or aCD226 mAbs or IgG control mAbs (n = 10). (B) Flow cytometric analysis from one representative experiment and summary data showing the suppression of responder CD8+ T cell proliferation after a 6-day IVS as in A but in the absence or presence of total CD25− CD127+/− CD4+ Teffs or CD25hiCD127− CD4+ Tregs isolated from PBMCs of MPs and with blocking aTIGIT mAbs or IgG control mAbs (n = 7). (C) Flow cytometric analysis from one representative experiment and summary data showing the suppression of responder CD8+ T cell proliferation after a 6-day IVS as in A but in the absence or presence of total CD25− CD127+/− CD4+ Teffs or CD25hiCD127− CD4+ Tregs isolated from metastatic melanoma (MM) tumor-infiltrating lymphocytes (TILs) and with blocking aTIGIT or aCD226 mAbs or IgG control mAbs (n = 10). The ratios of CD4+ T cells to responder CD8+ T cells are indicated. (D) Representative histograms and summary data showing PVR/CD155 expression on CD14+ CD11c+ monocytes and CD14− CD11c+ DCs in non-CD3 cells from some samples used in stimulations of A, B and C (n = 10). Solid grey histogram indicates isotype staining. Results represent the mean of independent experiments. Error bars indicate s.e.m. P values were obtained by paired t-test. n.s., non significant.
Supplemental Figure 5. PVR expression by circulating antigen presenting cells (APCs) upon treatment with human PVR-Fc and by APCs in the tumor microenvironment. (A) Representative histograms and summary data showing ex vivo PVR/CD155 expression on CD14+ CD11c+ monocytes and CD14- CD11c+ DCs in metastatic melanoma (MM). n = 10. Solid grey histogram indicates isotype staining. (B-C) Flow cytometric analysis from one representative experiment (B) and summary data showing PVR expression [% and mean fluorescence intensity (MFI)] (C) on CD14+ CD11c+ monocytes and CD14- CD11c+ DCs in PBMCs of donors after 2 hours of incubation in the presence of soluble PVR-Fc with or without blocking antibodies against Fcγ receptors CD16, CD32 and CD64 (aFcgRs) and/or IgG control mAbs. At the end of incubation, cells were stained with APC-labeled anti-PVR mAbs or APC-labeled IgG isotype control mAbs. n = 6. Results represent the mean of independent experiments. Error bars indicate s.e.m. P values were obtained by repeated-measures ANOVA followed by Tukey’s test. **P < 0.01.
Supplemental Figure 6. Cytokine production by TIGIT+ Tregs from melanoma patients upon TIGIT and/or CD226 blockade and PVR binding. (A-B) Summary data showing the production of IFN-γ (A) and FGL2 and TGF-β1 (B) by TIGIT+ CD25hi CD127− CD4+ Tregs isolated from PBMCs of melanoma patients (MPs) (n = 15 for IFN-γ, n = 14 for FGL2 and TGF-β1) and total CD25hi CD127− CD4+ Tregs isolated from metastatic melanoma (MM) tumor-infiltrating lymphocytes (TILs) (n = 27 for IFN-γ, n = 20 for FGL2, n = 19 for TGF-β1) after a 6-day IVS with aCD3/CD28/IgG or aCD3/CD28/PVR beads in the presence of aTIGIT and/or aCD226 blocking mAbs and/or IgG control mAbs. Error bars indicate s.e.m. P values were obtained by repeated-measures ANOVA followed by Tukey’s test. *P < 0.05; **P < 0.01; ***P < 0.001.