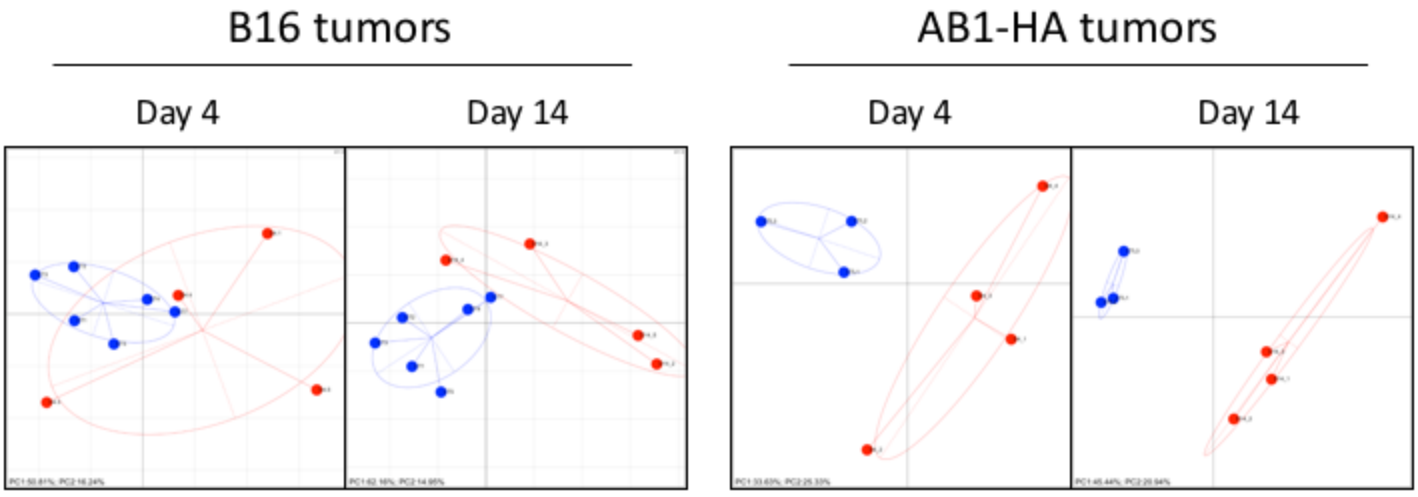
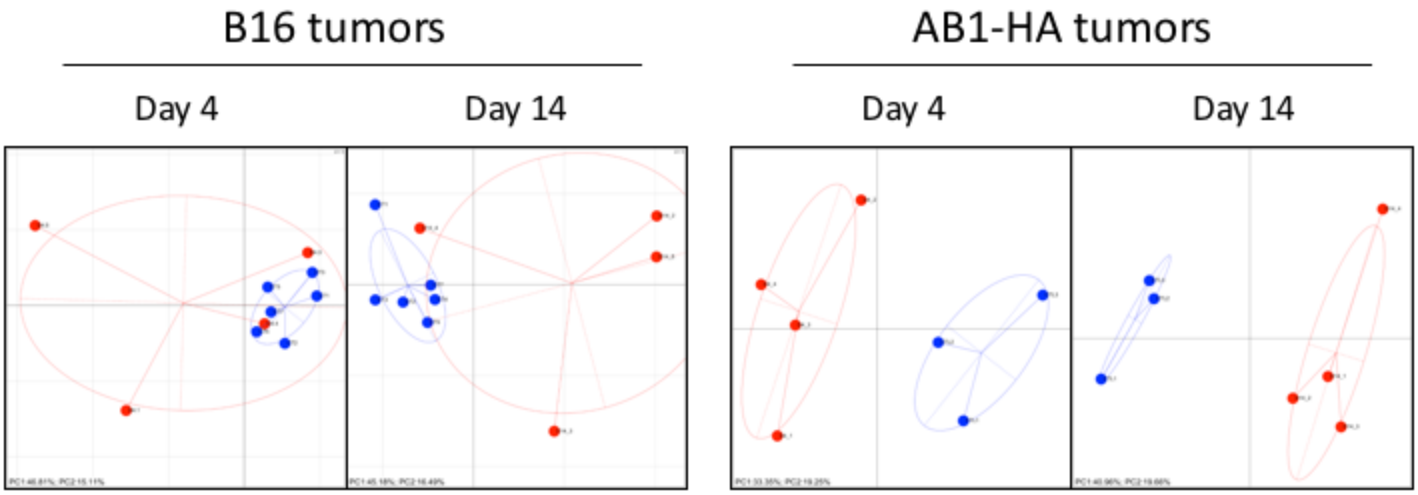
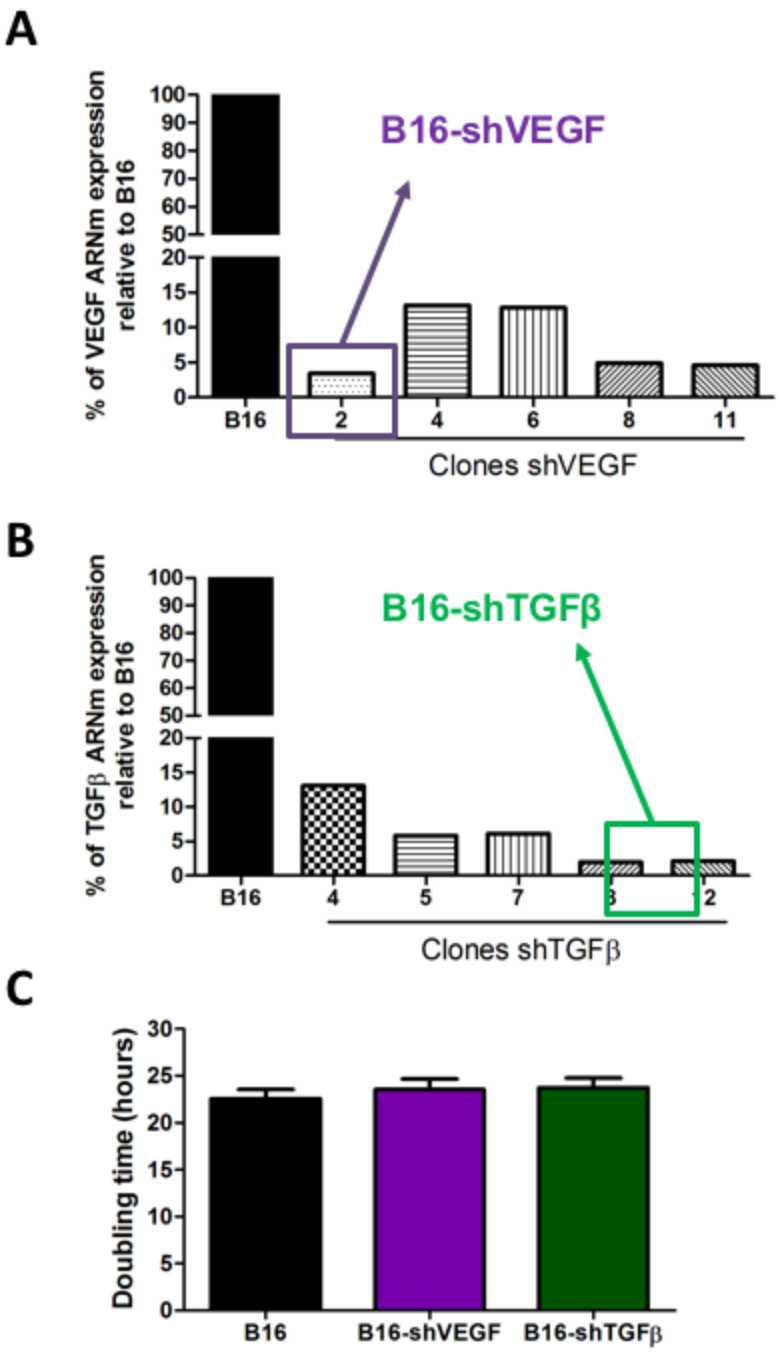
Vascular endothelial growth factor receptor signaling pathway: *18 genes*



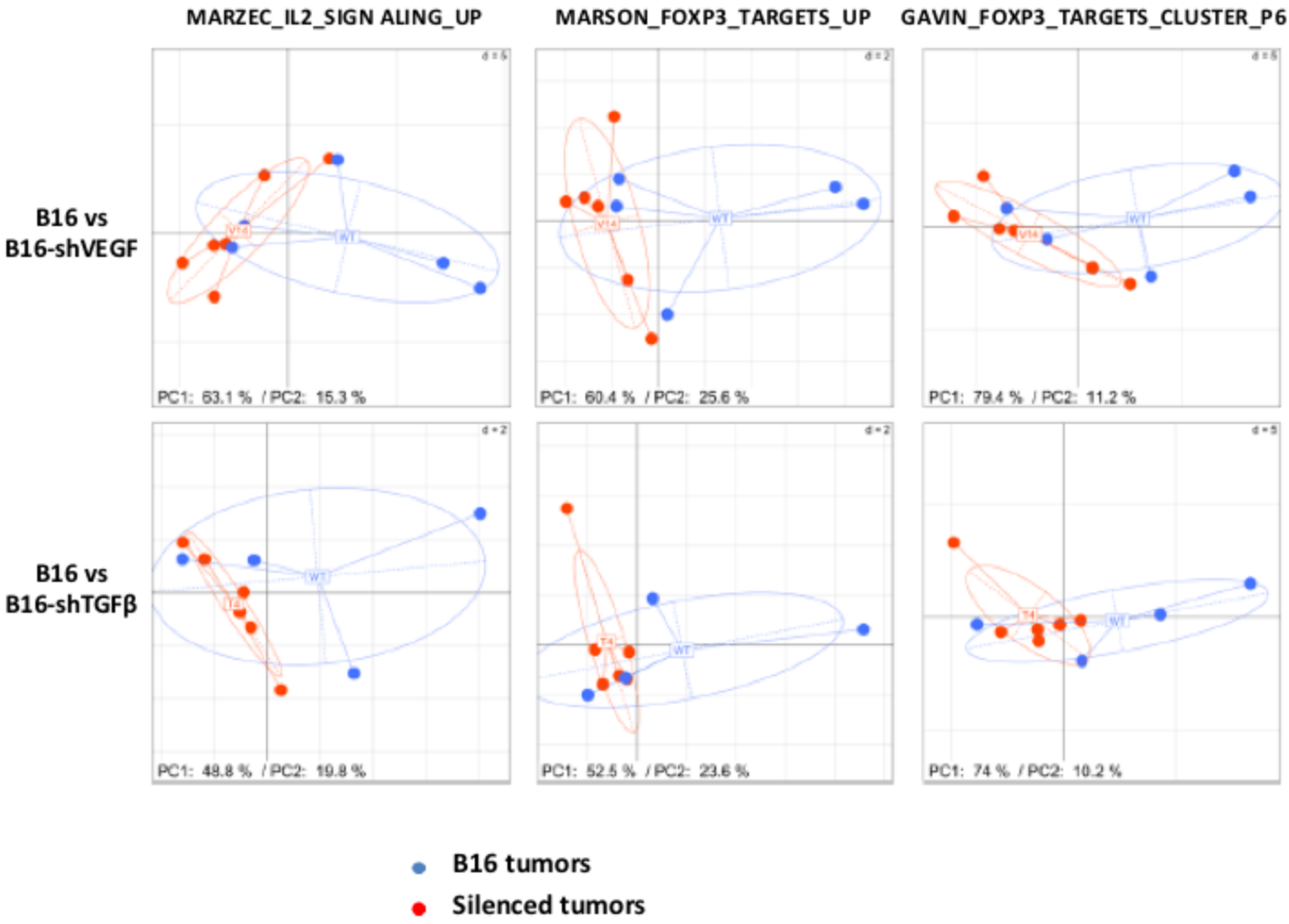
transforming growth factor beta receptor signaling pathway: 45 genes



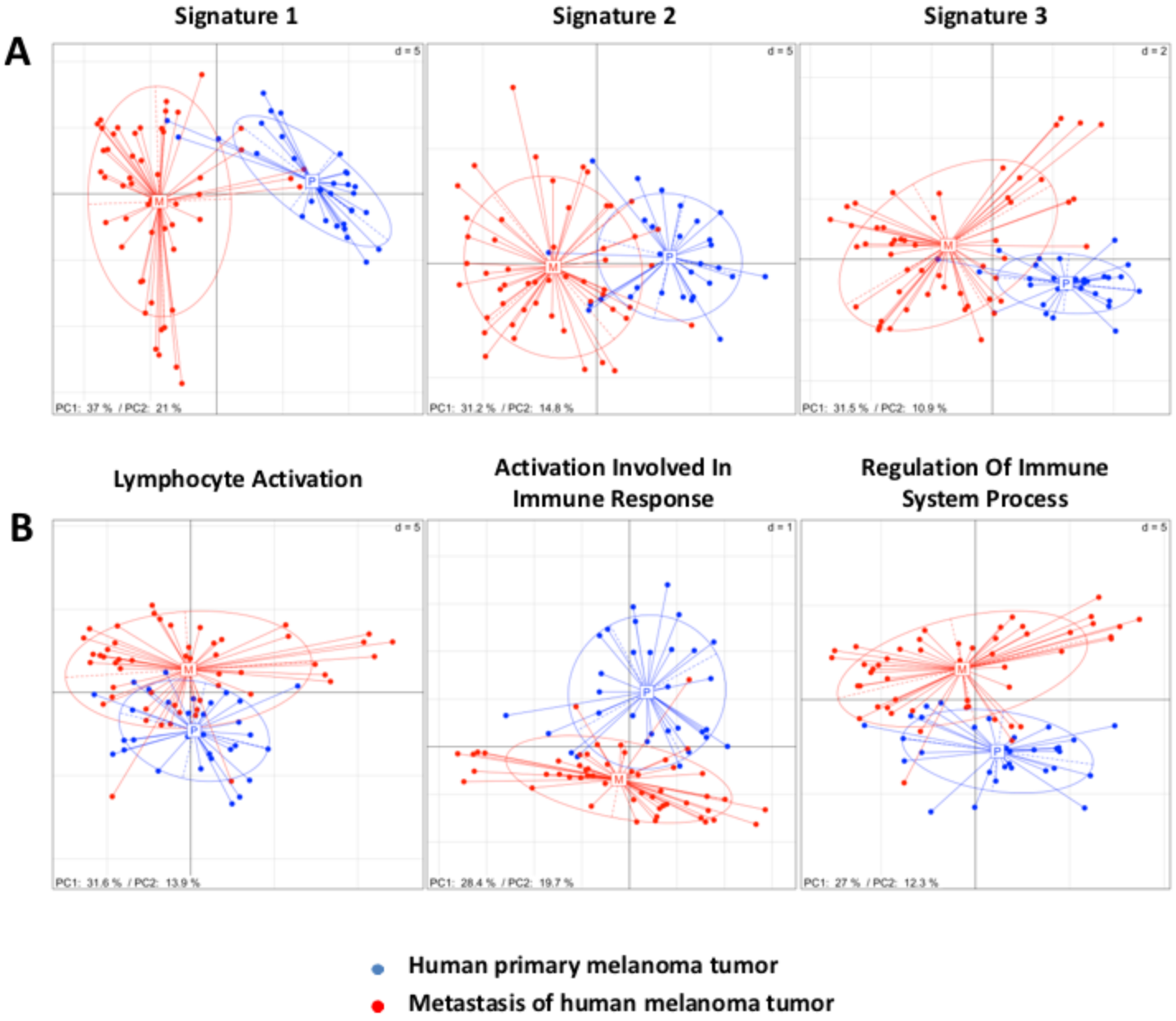
Supplementary Figure 1. Transcriptomic analysis of B16 and AB1-HA tumor environments reveals early modulation of VEGF and TGFβ. Principal Component Analysis (PCA) based on gene expression of "VEGF receptor signaling pathway" signature (upper panels) and "TGFβ receptor signaling pathways" signatures (lower panels). Control samples are plotted in red and tumor samples in blue at day-4 and day-14 after tumor inoculation.



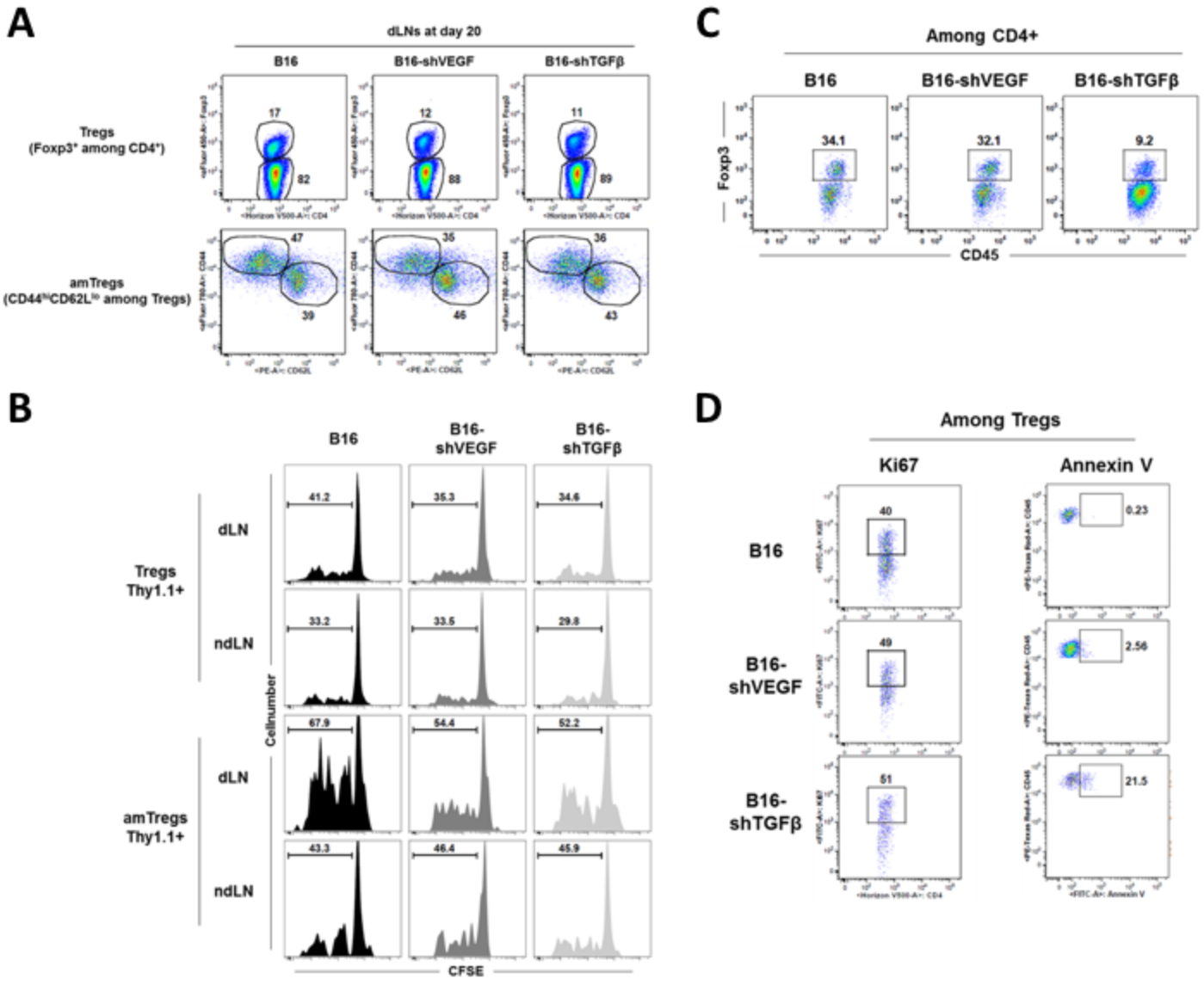
Supplementary Figure 2. VEGF or TGFβ silencing is efficiently achieved by shRNA and does not alter in vitro B16 doubling time. (A and B) qRT-PCR quantification of VEGF (A) and TGFβ (B) mRNA expression in B16 and B16-shVEGF / B16-shTGFβ tumor clones *in vitro*. The framed clones represent those that were chosen for the rest of the study. Data are shown as normalized percentages in a way that B16 expression reach 100%, n=1. (C) Tumor cells doubling time in vitro, shown in hours, n=10.



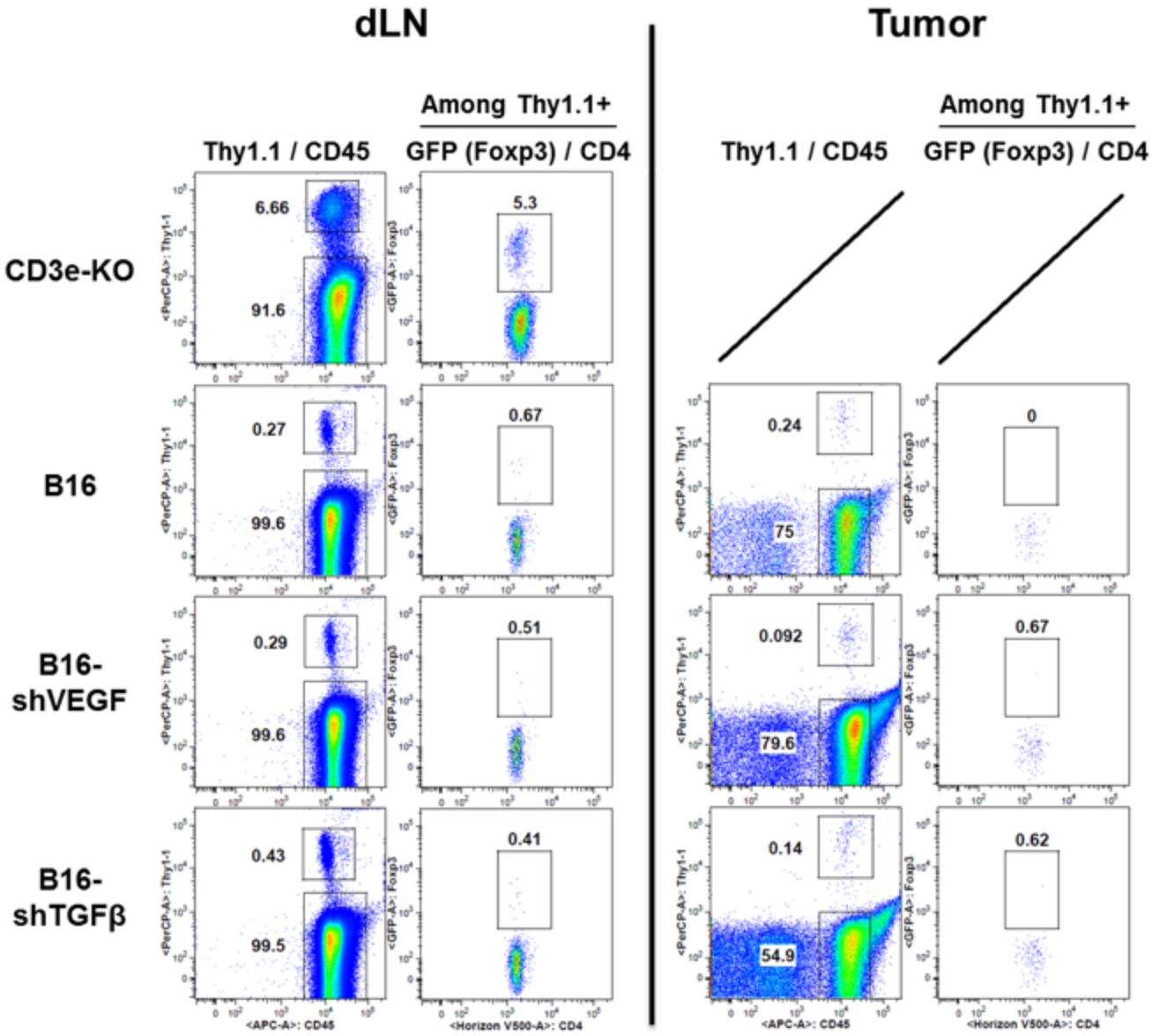
Supplementary Figure 3. PCA comparisons of B16 tumors (in blue) and VEGF- (top) or TGFβ - (bottom) silenced tumors (in red), based on three Treg-related signatures found in the literature (from Marzec et al., Cancer Res. 2008; Marson et al., Nature 2007; Gavin et al., Nature 2007).



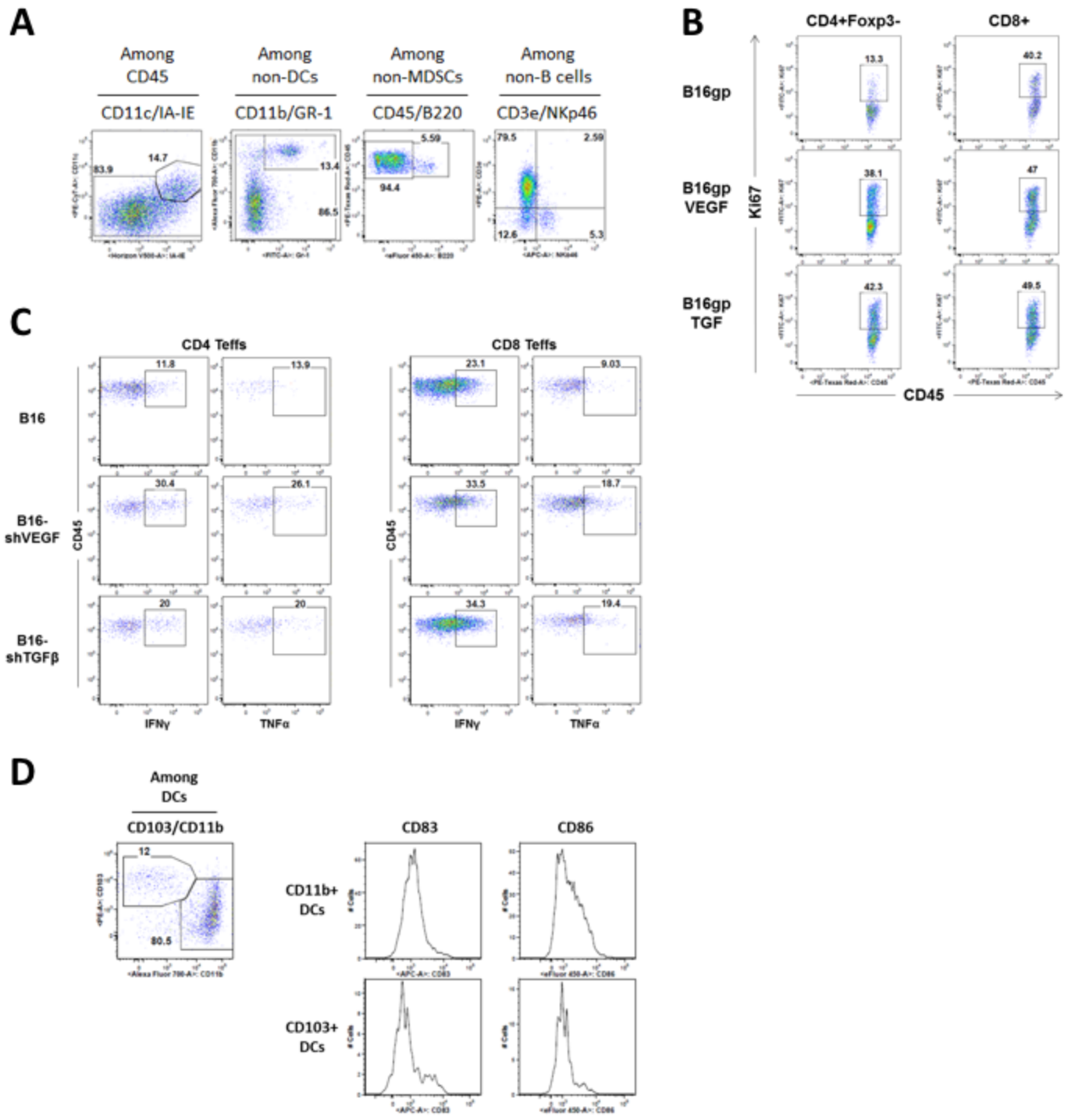
Supplementary Figure 4. The signatures identified by our mouse-based transcriptomic study are strongly relevant to human clinical specimens. PCA based on normalized genes separate metastasis patients (n=52; in red) from primary biopsies patients (n= 31; in blue). (A) ICA-extracted signatures. (B) Signatures from immune-related networks described in Figure.1.



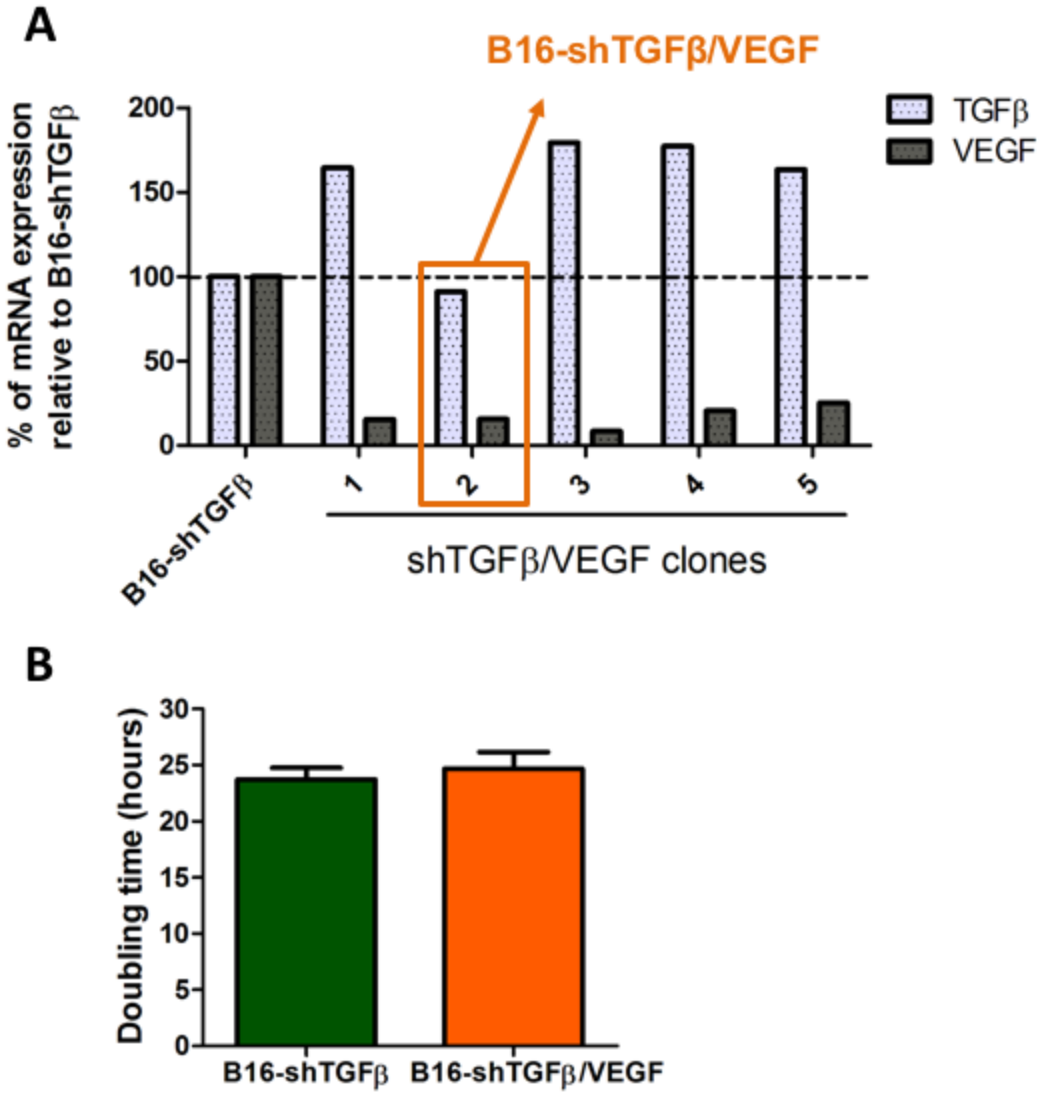
Supplementary Figure 5. VEGF or TGFβ silencing abolishes Treg and amTreg accumulation and proliferation in the tumor-draining lymph nodes, but only TGFβ silencing affects tumor-infiltrating Tregs. Flow cytometry gating strategies relative to the results presented in Figure 4. A for Figure 4A, B for Figure 4B, C for Figure 4C and D for Figure 4D-E.



Supplementary Figure 6. VEGF or TGFβ silencing does not affect in vivo peripheral Tregs induction from naïve CD4+CD25- naïve cells. Naïve CD3ε-KO or 1-day WT / silenced tumor-bearing WT C57BL/6 Thy1.2+ mice received 2 millions FACS-sorted Thy1.1+CD4+CD25-GFP- T cells i.v obtained from naive Thy1.1+Foxp3eGFP mice. Twenty days after cell transfer, we measured the GFP expression by Thy1.1+CD4+ T cells present in dLNs and tumor masses.



Supplementary Figure 7. VEGF or TGFβ silencing dramatically impacts the composition and activation status of the tumor immune cell infiltrates. Flow cytometry gating strategies relative to the results presented in Figure 5. A for Figure 5B, B for Figure 5C, C for Figure 45D-E and D for Figure 4F-G.



Supplementary Figure 8. VEGF and TGFβ cosilencing is efficiently achieved by shRNA and do not alter in vitro B16 doubling time. (A) qRT-PCR quantification of VEGF and TGFβ mRNA expression in B16-shTGFβ and B16-shTGFβ/VEGF tumor clones *in vitro*. The framed clone represent the one that has been chosen for the rest of the study. Data are shown as normalized percentages in a way that B16-shTGFβ expression reach 100%, n=1. (B) Tumor cells doubling time in vitro, shown in hours, n=10.