



**Additional file 12. Gating strategy for CD8<sup>+</sup>, non-Treg CD4<sup>+</sup> and regulatory (Treg) T-cell populations.** Characterization was done according to our previously publications on Treg and Tbet gating [31]. Gating strategy is depicted for a representative VSCC tumor sample. (a) Following doublet and dead cell exclusion, CD3<sup>+</sup> T cells were selected, which were further divided on the basis of CD4 and CD8 expression. Total CD8<sup>+</sup> T cells are gated in green. Within the CD4<sup>+</sup> T cells, CD25<sup>+</sup>CD127<sup>-</sup> cells (red) were selected for further Foxp3 gating (Foxp3<sup>+</sup> Tregs) and the non CD25<sup>+</sup>CD127<sup>-</sup> CD4<sup>+</sup> T cells for further non-Treg CD4 gating (in blue). (b) Expression of the transcription factor Tbet (top panel), and the checkpoint molecules Tim-3 (middle panel) and Lag-3 (bottom panel) within CD3<sup>+</sup>CD8<sup>+</sup> T cells (left), non-Treg CD3<sup>+</sup>CD4<sup>+</sup> T cells (middle) and CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-</sup>Foxp3<sup>+</sup> T cells (right).