CAPTIONS FOR SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure 1. **Representative example of a purity check by flow cytometry of $CD4^+$ and $CD8^+$ T cells sorted from the spleen of mice treated with the TGFβ vaccine**. Four mice were vaccinated with all five TGFβ-derived peptides on days 0 and 7. On day 14, mice were sacrificed, spleens harvested and pulled. $CD4^+$ and $CD8^+$ T cells were sorted. The purity of the sorting was assessed by flow cytometry.

Supplementary Figure 2. **Pan02 cell line generates tumors with high *Tgfb1* expression.** Assessment of *Tgfb1* expression in Pan02, MC38, LL2, and B16 tumors that were subcutaneously inoculated in the right flank (0.5 million cancer cells). Tumors were harvested at endpoint and *Tgfb1* expression in whole tumor RNA was assessed by RT-qPCR relative to the housekeeping gene *Hprt1*. Dots represent individual mice (n=3 per group); a.u., arbitrary units. Data are presented as mean ± SD.

Supplementary Figure 3. Individual tumor growth curves for Pan02-tumor bearing mice treated with different combinations of TGFβ-derived peptides (data shown in Figure 2A). Individual tumor growth curves for Pan02-tumor bearing mice that were either (A) untreated or vaccinated with (B) a TGFβ-derived MHC-II predicted peptide (mTGFβ-18-32), (C) a pool of four MHC-I-restricted peptides (mTGFβ-4-11, 215-223, 282-289, and 334-342), or (D) vaccinated with a combination of all five peptides (referred to as "TGFβ vaccine") in a 4-dose regimen. Mice (n=5-8 mice per group) were vaccinated on days 10, 17, 24, and 35, as indicated by the arrows. Curves represent individual mice.

Supplementary Figure 4. TGFβ-derived peptides need to be combined with Montanide ISA 51 VG as an adjuvant to exert an anti-tumor immune response. (A) Average tumor growth in Pan02-tumor bearing mice that were either untreated, vaccinated with only Montanide, vaccinated

with unadjuvanted TGFβ-derived peptides or vaccinated with TGFβ-derived peptides emulsified with Montanide, as previously described in the paper (referred to as "TGFβ vaccine" in the graph). For Montanide-only treatments, each mouse received 100 uL of an emulsion containing 50 uL of H_2O and 50 uL of Montanide. For treatments with unadjuvanted TGFβ-derived peptides, each mouse was injected with two peptide solutions, one containing 100 μg of the murine TGFβ-derived MHC-II-restricted peptide (mTGFβ-18-32) and another containing 50 μg of each MHC-I-restricted peptide (mTGFβ-4-11, mTGFβ-215-223, mTGFβ-282-289 and mTGFβ-334-342). Peptide solutions were not emulsified with Montanide before vaccination. For treatments with the TGFβ vaccine, mice were treated as previously described in the paper (see method section). All vaccinations were performed subcutaneously at the base of the tail on day 10 and 17 post-inoculation, as indicated by the arrows. N=7 mice per group. Data are presented as mean ± SEM. (B) TGFβ vaccine-specific responses at endpoint (day 29) across the different treatment groups for the tumor study described in (A) assayed by IFNγ ELISPOT. n=5-9 mice per group. Data are presented as mean ± SEM. Dots represent individual mice. (C) Percentage change in body weight compared to day 0 in Pan02 tumor-bearing mice that were treated as described in (A). Mice (n=7 mice per group) were vaccinated on days 10 and 17, as indicated by the arrows. Data are presented as mean ± SEM. Ns, not significant; *p<0.05 according to TumGrowth software for (E) and unpaired two-tailed t test for (B) at each time point.

Supplementary Figure 5. **Representative dot plots showing the percentage of various cell subsets included in the flow cytometric analysis of the tumor-microenvironment of untreated or TGFβ-vaccinated Pan02-tumor bearing mice.** Representative dot plots showing (A) the percentage of cancer cells gated on $CD45^-$ $CD31^-$ cells, (B) the percentage of endothelial cells and leukocytes gated on live cells, (C) the percentage of T cells gated on $CD45^+$ cells, (D) the percentage of Tregs gated on $CD4^+$ T cells in the tumor of untreated or mice treated with the TGFβ

vaccine. Percentage of (E) polymorphonuclear MDSCs (PMN-MDSCs) gated as $CD11b^{+}$ $F4/80^{-}$ $Ly6G^{+}$ $Ly6C^{low}$ and (F) monocytic MDSCs (M-MDSCs) gated as $CD11b^{+}$ $F4/80^{-}$ $Ly6G^{-}$ $Ly6C^{high}$ in the tumor of untreated or mice treated with the TGFβ vaccine. Representative dot plots showing (G) the percentage of PMN-MDSC and M-MDSC gated on $CD11b^{+}$ $F4/80^{-}$ cells, (H) the percentage of macrophages gated on live cells and (I) the percentage of $CD26^{hi}$ $Ly6C^{hi}$ and $CD26^{lo}$ $Ly6C^{lo}$ gated on CAFs cells in the tumor of untreated or mice treated with the TGFβ vaccine.

Supplementary Figure 6. **Additional changes in the gene expression profiles of Pan02 tumors induced by the TGFβ vaccine.** Pan02 tumor-bearing mice were either left untreated or vaccinated with the TGFβ vaccine on days 10 and 17. Tumors were harvested (n=3-4 per group) on day 30, RNA extracted, and bulk RNAseq performed. (A) Principal component analysis (PCA) plot of RNA samples from Pan02 tumors from untreated (U1-U4) or TGFβ-vaccinated mice (T1-T3). (B) Box plots showing the expression level assessed by RNAseq and presented as VST-normalized counts of CD8 T cell-related genes (*Cd8a*, *Tnf*, *Gzmb* and *Cd69*) in Pan02 tumors from untreated or TGFβ-vaccinated mice. (C) Box plots showing the absolute immune score (AIS, arbitrary units) for different immune populations and immune score ratio for CD8/CD4, CD8/Treg, or M1/M2 immune populations calculated from bulk RNAseq using the ImmuCC model in Pan02 tumors from untreated or TGFβ-vaccinated mice. (D) Box plots showing the expression level assessed by RNAseq and presented as VST-normalized counts of angiogenesis-related genes (*Vegfa* and *Flt1*) in Pan02 tumors from untreated or TGFβ-vaccinated mice. Dots represent individual mice. ns = not significant, *= $p<0.05$ according to an unpaired two-tailed t test.

Supplementary Figure 7. **Classification of the biological processes associated with significantly upregulated in genes in Pan02 tumors from TGFβ-vaccinated mice compared to untreated tumors assessed by Gene Ontology (GO) analysis**. Pan02 tumor-bearing mice were either left untreated or vaccinated with the TGFβ vaccine on days 10 and 17. Tumors were harvested (n=3-4

per group) on day 30, RNA extracted, and bulk RNAseq performed. GO analysis for biological processes was performed using The Gene Ontology Resource software (http://geneontology.org/) with differentially upregulated genes as an input. The most specific subclasses according to hierarchy were selected. (A) Percentage of immune and non-immune-related biological processes enriched in the tumors of TGFβ-vaccinated mice compared to untreated mice. (B) Percentage of T cell, antigen presentation, cytotoxicity, cytokine, leukocyte, B cell, chemotaxis, innate, neuro-immunity, or pathogen immunity-related processes among the immune-related biological processes enriched in the tumors of TGFβ-vaccinated mice compared to untreated mice.

Supplementary Figure 8. **Gating strategy for the flow cytometry panel for general cell subsets in the tumor.** Cancer cells were gated as $CD45^-CD31^-FAP^-$. Leukocytes were gated as $CD45^+CD31$. Endothelial cells were gated as $CD45^-$ $CD31^+$. All populations were gated from singlet live cells.

Supplementary Figure 9. **Gating strategy for the T cell flow cytometry panel.** $CD3^+$ T cells were gated as $CD45^+CD3^+$. $CD8^+$ T cells were gated $CD45^+CD3^+$ $CD8^+$ $CD4^-$. $CD4^+$ T cells were gated as $CD45^+CD3^+CD8^-CD4^+$. Tregs were gated as $CD45^+CD3^+CD8^-$ $CD4^+$ $CD25^+$ $FoxP3^+$. All populations were gated from singlet live cells.

Supplementary Figure 10. **Gating strategy for the myeloid flow cytometry panel.** Polymorphonuclear MDSCs (PMN-MDSCs) were gated as $CD11b^+$ $F4/80^-$ $Ly6G^+$ $Ly6C^{low}$. Monocytic MDSCs (M-MDSCs) were gated as $CD11b^+$ $F4/80^-$ $Ly6G^-$ $Ly6C^{high}$. Macrophages were gated as $CD11b^+$ $F4/80^+$. M1 macrophages were gated as $CD11b^+$ $F4/80^+$ mannose receptor $(MR)^-$ and M2 macrophages as $CD11b^+$ $F4/80^+$ MR^+. All populations were gated from singlet live cells.

Supplementary Figure 11. Gating strategy for the cancer-associated fibroblast (CAF) flow cytometry panel. CAFs were gated as $CD45^-CD31^-CD90^+$ $PDPN^+$ cells. Two CAF subsets were

identified as Ly6C^{hi} CD26^{hi} and as Ly6C^{lo} CD26^{lo}, as previously described[38]. Mean fluorescence intensity (MFI) of αSMA was assessed in CAFs and in the two CAF subsets. All populations were gated from singlet live cells.

Supplementary Figure 12. **Gating strategy for the T cell proliferation flow cytometry panel.** CD3$^+$ T cells were gated as singlet live CD3$^+$ cells. Proliferation was evaluated by examining CFSE intensity in the CD3$^+$ population.

Supplementary Figure 13. Gating strategy for the macrophage flow cytometry panel. **M**acrophages were gated as CD11b$^+$ F4/80$^+$. M1 macrophages were gated as CD11b$^+$ F4/80$^+$ mannose receptor (MR)$^-$ and M2 macrophages as CD11b$^+$ F4/80$^+$ MR$^+$. Mean fluorescence intensity (MFI) of PD-L1 and Arg1 was assessed in macrophages. All populations were gated from singlet live cells.

SUPPLEMENTARY TABLES

Supplementary Table 1. **Differentially expressed genes in Pan02 tumors from TGFβ vaccine-treated mice compared to Pan02 tumors from untreated mice (cut off p-adjusted value <0.05 and absolute log2 fold change >0.585).**

Supplementary Table 2. **Gene Ontology biological processes associated with the differentially upregulated genes in Pan02 tumors from TGFβ vaccine-treated mice compared to Pan02 tumors from untreated mice**

Supplementary Table 3. **Gene lists used for the generation of heatmaps**. Gene lists related to antigen presentation, lymphocyte activation, cytokines and chemokines, inflammation, interferon signaling, TLR pathway, phagocytosis, immune response to tumors, apoptosis, pro-fibrotic fibroblast, inflammatory fibroblast and fibroblast-derived collagens. All gene lists were obtained from nanoString panel gene lists, except for i) the cytokine and chemokine gene list, which was

118 self-generated, ii) the pro-fibrotic fibroblast and inflammatory fibroblast gene lists, which were
119 retrieved from[27] (gene list named "TGFß1_up[g]" and "TNFα_up[g]") and iii) the fibroblast-derived
120 collagen gene list, which was retrieved from[28].

121 **Supplementary Table 4. Population schemes used for the ImmuCC algorithm.**

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