Supplemental Figure 1. TGFβ blockade does not affect metastasis formation in vivo. A-C) Number of metastases in different metastatic sites for mice shown in Figure 1D, including lymph nodes (A), lungs (B), and livers (C). D) Correlation between primary tumor weight and number of metastases in isotype treated or αTGFβ treated groups. E) The correlation between primary tumor weight and number of metastases in all 6 groups was analyzed using a zero-inflated Poisson regression model. ***, $p < 0.001$; **, $p < 0.01$; ., $p < 0.1$. F) C57BL/6 mice were subcutaneously inoculated with 6694c2 WT or 6694c2-met cells as shown in Figure 1B. The percentage of mice containing at least 1 lung metastases upon harvest from the two groups was quantified. Data are combined from 3 independent experiments. Fisher's exact test was used for comparison between two groups.

Supplemental Figure 2. TGFβ blockade has minimal effect on the adjacent normal pancreas in KPC mice. LSL-KRASG12D/+;LSL-Trp53R172H/+; Ptf1aCre/+ mice with ultrasound-confirmed tumors of >2mm in diameter were treated with αTGFβ +/- chemotherapies as illustrated in Figure 5A. Pancreata were harvested after 9 days of treatment, embedded in FFPE, sectioned, and stained for H&E, Masson's Trichrome, and immunohistochemistry for aSMA, cleaved caspase 3, and Ki67. Representative images from the adjacent normal pancreas are shown. Images are 20x magnification.

Supplemental Figure 3. TGFβ blocking antibody can be detected in orthotopic tumors and spontaneous tumors by ELISA. A) 6694c2 parental cells were inoculated orthotopically into C57BL/6 mice. Mice were treated with αTGFβ +/- GnP as illustrated in Figure 2D. Mice treated with FOLFIRINOX (5mg/kg oxaliplatin, 75mg/kg leucovorin, 50mg/kg irinotecan, and 15mg/kg 5-FU) were dosed at D3, D6, D9 i.v.. For the positive control group (+αTGFβ inoculum) tumor cells were mixed with αTGFβ antibody at initial inoculation. Tumors were harvested on day 15 and homogenized with RIPA buffer. Protein lysates were analyzed by human IgG2 ELISA. n = 2 tumors per group. B) 6694c2 parental cells were inoculated orthotopically into C57BL/6 mice. Mice were treated with αTGFβ or isotype control every 2 days starting on day 4. Tumors were harvested at day 15. LSL-KRASG12D/+;LSL-Trp53R172H/+; Ptf1aCre/+ mice with ultrasound-confirmed tumors of >2mm in diameter were treated with αTGFβ +/- GnP as illustrated in Figure 2D. Mice treated with FOLFIRINOX (5mg/kg oxaliplatin, 75mg/kg leucovorin, 50mg/kg irinotecan, and 15mg/kg 5-FU) were dosed at D3, D6, D9 i.v.. Tumors were harvested after 9 days of treatment and homogenized with RIPA buffer. Protein lysates were analyzed by human IgG2 ELISA. C) LSL-KRASG12D/+;LSL-Trp53R172H/+; Ptf1aCre/+ mice with ultrasound-confirmed tumors of >2mm in diameter were treated with αTGFβ +/- chemotherapies as illustrated in Figure 5A. Pancreata were harvested after 9 days of treatment, embedded in FFPE, sectioned, and stained for H&E and Masson's Trichrome. Representative images from the tumor are shown. Images are 20x magnification.

Supplemental Figure 4. Anti-TGFβ +/- GnP variably increases T cell infiltrates in poorly immunogenic orthotopic pancreatic cancer. A) Representative plots and gating strategies for T cell infiltrates in tumors shown in Figure 2F-G. B) Representative immunohistochemistry staining with anti-CD3 antibody for tumors shown in Figure 2K. C) Quantification of CD3+ cell infiltration and PD1+ cell infiltration for tumors shown in Figure 2K.

Supplemental Figure 5. Single cell analysis of tumor microenvironment across different treatment groups. A) UMAP plot of single cell RNAseq data across different treatment groups. Corresponds to the combined UMAP plot in Figure 3B. B) CAF characterization across different treatment groups. UMAP plot of single cell RNAseq data across different treatment groups. Corresponds to the combined UMAP plot in Figure 6A. C) RNA velocity analysis of CAF cells separated by αTGFβ FFX group versus all other groups combined.

Supplemental Figure 6. **Body weight analysis of mice treated with different FOLFIRINOX regimens.** A) C57BL/6 mice inoculated orthotopically with 6694c2 WT cells were treated with αTGFβ or isotype control, +/- FOLFIRINOX (5mg/kg oxaliplatin, 75mg/kg leucovorin, 50mg/kg irinotecan, and 75mg/kg 5-FU; dosed i.v. at day 3 and day 6). Mouse body weights were analyzed over 15 days after tumor inoculation. B) Tumor weights were measured at day 15. Results are combined from three independent experiments. The FFX ISO group shown here is the same as the one in Figure 5C.

Supplemental Figure 7. Classical state score of tumor cells across different treatment groups from single cell analysis. A) UMAP plot of total cells analyzed by single cell RNAseq obtained after fibroblast enrichment described in Figure 6A. B) The classical state score of tumor cells across different treatment groups subclustered from the single cell RNAseq data shown in supplemental Figure 7A. C) Violin plots depicting log expression level of Tff2 across different treatment groups.

Supplemental Material and Methods

Cell lines
6694C2zsGreen cells were generated using parental 6694c2 cells engineered to express a zsGreen transgene. Cells were transfected with pUBC-zsGreen plasmid using Lipofectamine 2000 reagent and used according to the manufacturer's protocol. Cells were selected with hygromycin for successful transfection, and gene insertion was confirmed by flow cytometry and zsGreen bright cells were sorted to establish the 6694C2zsGreen line. Cells were cultured at 37 ^{o}C in a humidified incubator with 5% CO_2. RPMI media was supplemented with 10% FBS, 2 mmol/L L-glutamine, 1% penicillin/streptomycin, 1% MEM non-essential amino acids, 1 mmol/L sodium pyruvate, and 0.1 mmol/L β-mercaptoethanol. Cells used for *in vitro* experiments were cultured with 5ng TGFβ ligand, 20ug isotype or 20ug TGFβ blocking antibody. Cells used for *in vivo* experiments had been passaged for less than 2 months, were negative for known mouse pathogens, and were implanted at >95% viability.

Orthotopic pancreatic tumors
Orthotopic surgeries were performed. Mice were anesthetized with a ketamine/xylazine cocktail, shaved on the left flank, and the surgical site cleaned with ethanol and betadine. An incision was made in the skin and peritoneum, and the pancreas externalized with forceps. 6694c2, 6694c2zsGreen and KPC.1 cells were resuspended in PBS and mixed 1:1 by volume with matrigel (Corning) for a total of 50,000 cells per 30 µl. The cell suspension was kept on ice and drawn into a chilled insulin syringe. Cells were then injected into the tail of the pancreas, and a bubble was observed. Mice that showed signs of leakage were removed from the experiment. The pancreas was left external to the body cavity for 1 minute with the mice on a warming pad to solidify the matrigel. The pancreas was then reinserted, peritoneum sutured with one stitch of absorbable suture, and the skin stapled with a sterile wound clip. Mice were given analgesia (Meloxicam and ropivacaine) and monitored post-surgery according to protocols approved by the Dana-Farber IACUC. Mice were sacrificed 21 days post-surgery unless otherwise indicated. Tumors were weighed at the time of sacrifice.

Metastasis model
6694c2 parental line was subcutaneously inoculated, removed as detailed below, with the resulting spontaneous metastasis formation in the lung collected after 5 weeks. The metastasis was then isolated, cultured and passaged in 2D cultures for over 2 months to generate the 6694c2-met cell line. 6694c2-met were negative for known mouse pathogens. Low passage 6694c2-met tumor cells from a 70% to 90% confluent plate were implanted subcutaneously on the lower back of the mice for a total of 180,000 cells per 150ul. Primary tumors were surgically removed after 11 days post inoculation. Mice were treated with TGFβ blocking antibody and/or chemotherapies as indicated before surgical removal. Primary tumor weight was measured upon collection at time of surgery. Mice with tumor regrow at the primary site after surgical removal were eliminated from the study. Both macro- and micro- metastasis rates in the lung, liver, lymph nodes are counted by direct visualization and microscopic imaging with H&E staining at day 46 after initial inoculation.

Spontaneous tumor-bearing mice
LSL-KrasG12D;p53+/flox,p48-cre mice were bred in house as part of the Dana-Farber Cancer Institute Hale Center for Pancreatic Cancer Research. LSL-KrasG12D;p53+/flox,p48-cre mice were monitored weekly by palpation until tumor development was suspected. Mice were then shaved and received ultrasound monitoring to determine the size of the pancreatic tumor. Mice with ultrasound confirmed tumors >2mm in diameter were enrolled into experiments according to a prespecified enrollment schedule. Mice were treated for 9 days to observe potential effects on

the adjacent normal pancreas. Whole pancreata were embedded in FFPE, sectioned, and stained for H&E, Masson's Trichrome, and immunohistochemistry for aSMA, cleaved caspase 3, and Ki67. For some mice, tumors were excised, flash frozen and protein lysates prepared by homogenizing in RIPA buffer. Protein lysates were analyzed for human IgG2 levels by ELISA (Thermofisher, cat#: BMS2093TWO).

Agent names
Fluorouracil injection (Fresenius Kabi); Oxaliplatin injection (Sandoz); Leucovorin Calcium injection (Fresenius Kabi); Irinotecan Hydrochloride injection (Hikma); Gemcitabine Injection (NovaPlus); n(ab)paclitaxel (Abraxane, Celgene).

Tumor infiltrate analysis
Tumors were collected and digested as previously described [39]. Pancreatic tumors were excised, weighed, minced and incubated in digestion buffer [RPMI (Gibco), 2% FBS, 0.2 mg/mL Collagenase P (Roche), 0.2 mg/mL Dispase (Gibco), and 0.1 mg/mL DNase I (Roche) at 37 °C, followed by consecutive cycles of pipetting, and the supernatant containing freed cells was collected every 10 minutes and quenched at 4 °C in 50 mL conical tubes containing cold FACS buffer (PBS with 2% fetal calf serum and 0.5mM EDTA). Tumors were filtered through a 70-micron cell strainer, washed with PBS, and centrifuged. The resulting cell pellet containing tumor debris and infiltrating immune cells was resuspended in FACS buffer (PBS with 2% fetal calf serum and 0.5mM EDTA) and stained with a master mix of antibodies. Cells were incubated with staining mix for 15 minutes at 4ºC, washed once in PBS, and resuspended in 1% formalin prior to analysis on either a spectral flow cytometer (Sony SP6800) or a Fortessa cytofluorimeter (BD). Flow cytometry antibodies used in this study were purchased from Biolegend (αCD45[30-F11], αCD4[RM4-5], αCD8[53-6.7], αNK1.1[PK136], αCD103[2E7], αLy6C[1A8], αI-A/I-E[M5/114.5.2], αF4/80[BM8], αSiglecF[E50-2440], αCD11b[M1170] αCD11c[N418], αGR1[RB6-8C5, αH2-Kb[AF6-88.5]).

Immunoblotting
Protein was isolated using lab-made RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS) containing complete protease inhibitors (Roche) and phosphatase inhibitor cocktail (Cell Signaling Technology 5870S). Protein content was then quantified using the BCA Protein Assay Kit (Thermo Fisher 23235). Equal amounts of protein were then resolved on a 10% SDS-PAGE after five minutes at 95°C in Laemmli buffer. After transfer, the PVDF membrane was blocked in 5% BSA for one hour at room temperature and then exposed to primary antibody in 3% BSA overnight at 4°C. Membranes were imaged after exposure to HRP-conjugated secondary antibody and enhanced chemiluminescence substrate (PerkinElmer; catalog no. NEL104001EA).

Immunofluorescence
Tumors were resected from tumor-bearing mice and frozen on dry ice in optimal cutting temperature medium and stored at −80°C. To obtain tissue slides, the tissue was sectioned to 0.1 µm with a Leica 3000 cryostat. After sectioning, slides were dried at 37°C and incubated in fixation solution (DPBS containing 0.1 M MgSO4, 0.5 M PIPES, 0.1 M EGTA, 10% formaldehyde) for 20 mins, permeabilized for 2 min at room temperature with 0.1% Triton X-100 in DPBS before being blocked for 1 hour at room temperature with blocking solution (DPBS with 5% glycerol and 5% goat serum or 5% donkey serum). Tissue was stained with primary or conjugated antibodies at 4C overnight, including antibodies to PDGFR$\alpha\beta$ at 1:100 dilution (Abcam Ab32570), SMA-FITC at 1:25 dilution (Abcam Ab8211), PTX3 at 1:25 dilution (R&D BAF2166), Ly6c-BV785 at 1:50 dilution (Biolegend 128041), CC3 at 1:400 dilution (Cell Signaling 9664S). Secondary antibodies including goat anti-rabbit AF555 (Life technologies A21428 1:200 dilution), Streptavidin AF647

(Biolengend 405237 1:300 dilution). All tissues were stained with DAPI nuclear dye for 10 minutes at room temperature. Images were taken using a fluorescent microscope at the Dana-Farber Cancer Institute Imaging Core Facility. Fiji and Photoshop were used to interpret and display images.

Single-Cell RNA Sequencing

Cells excluding $CD45^-$ zsGreen+ were sorted by FACS from orthotopic 6694c2zsGreen pancreatic tumors implanted in treated with indicated groups in Figure 4. Stromal enrichment in Figure 6 was applied as previously described [14] with mouse FC block (Miltenyi #130-092-575), biotinylated CD31 (Biolegend 390, 1:50) and Thy1 (Biolegend 53-2.1, 1:50) antibodies, with the EasySep Selection Kit (Stemcell Technologies #18559). Cells were then washed in cold PBS, counted, and resuspended to approximately 10^6 for sequencing. A library was constructed from each sample using the Chromium Single-Cell 5′ Kit (10x Genomics; PN-1000006). Libraries were sequenced on an Illumina HiSeq system generating paired-end 150-bp reads. The 10× CellRanger pipeline (v3.0.2) was used to align reads to the mm10 reference genome and generate a single-cell feature count matrix for each library using default parameters. The count matrices were imported for downstream analysis into R using the "Seurat" package (v3.1.4). Genes expressed in fewer than 3 cells were discarded from further analysis. Barcodes were classified as cells if they satisfied the following criteria: reads detected in more than 100 distinct genes, percentage of mitochondrial reads less than 2 SDs from the mean, and total reads within 3 SDs of the mean. Then, counts were log-normalized, scaled, and subjected to dimensionality reduction using principal components analysis on the basis of the 2000 most variable genes. Unsupervised clusters were identified first by constructing a shared nearest neighbor graph based on each cell's 20 nearest neighbors and then applying modularity refinement with the Louvain algorithm. Another round of unsupervised clustering restricted to cells within the fibroblast cluster identified distinct subclusters that were manually labeled as such. Markers for each cluster were identified by comparing gene expression using model-based analysis of single-cell transcriptomic (MAST).

RNA Velocity

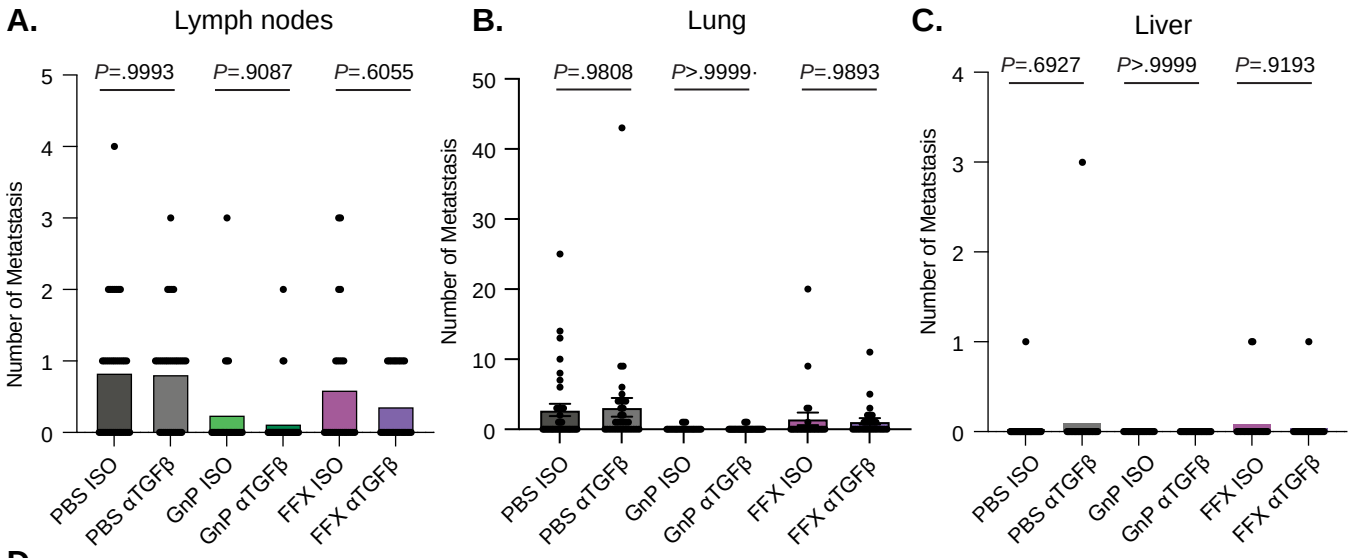
Alignment products (BAM files) obtained from the single- cell RNA libraries described above were analyzed by the Python (RRID:SCR_001658) tool "velocyto" (v0.17.17) to generate spliced and unspliced expression matrices. These data were then processed using the package "scVelo" (v0.1.25): genes with less than 30 counts in both spliced and unspliced reads were excluded, the top 2,000 most variable genes were selected, filtered counts were log-normal- ized, per-cell velocities were estimated, and finally averaged vector fields were visualized as a stream plot overlaid on the UMAP generated as described above.

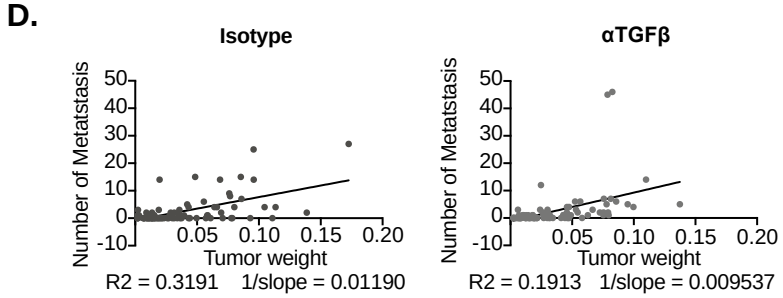
Bulk RNA sequencing and data analysis

$CD45^-$ zsGreen+ cells were sorted by FACS from orthotopic 6694c2zsGreen pancreatic tumors implanted in treated with indicated groups in Figure 7. Total RNA was prepared using Qiagen RNAeasy kit according to the manufacturer's protocol. Library construction and Illumina sequencing were performed by the DFCI Molecular Genomics Core Facility. Reads were aligned using STAR alignment to the mm10 genome. Transcript-level estimates were collapsed to gene-level estimates using the R package *tximport* and Ensembl transcript annotations (v79). Genes with less than 10 reads across all samples were discarded, and the remaining genes were analyzed for differential expression using the package *DESeq2*. Classical score was applied using ssGSEA as described previously [11].

Data and code availability

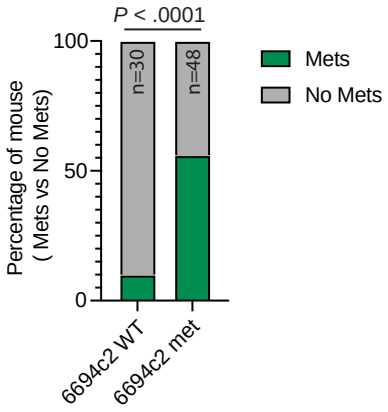
Bulk and single-cell RNA-sequencing data sets are available under accession #GSE224688. Code used to analyze data are available here: https://github.com/douganlab/

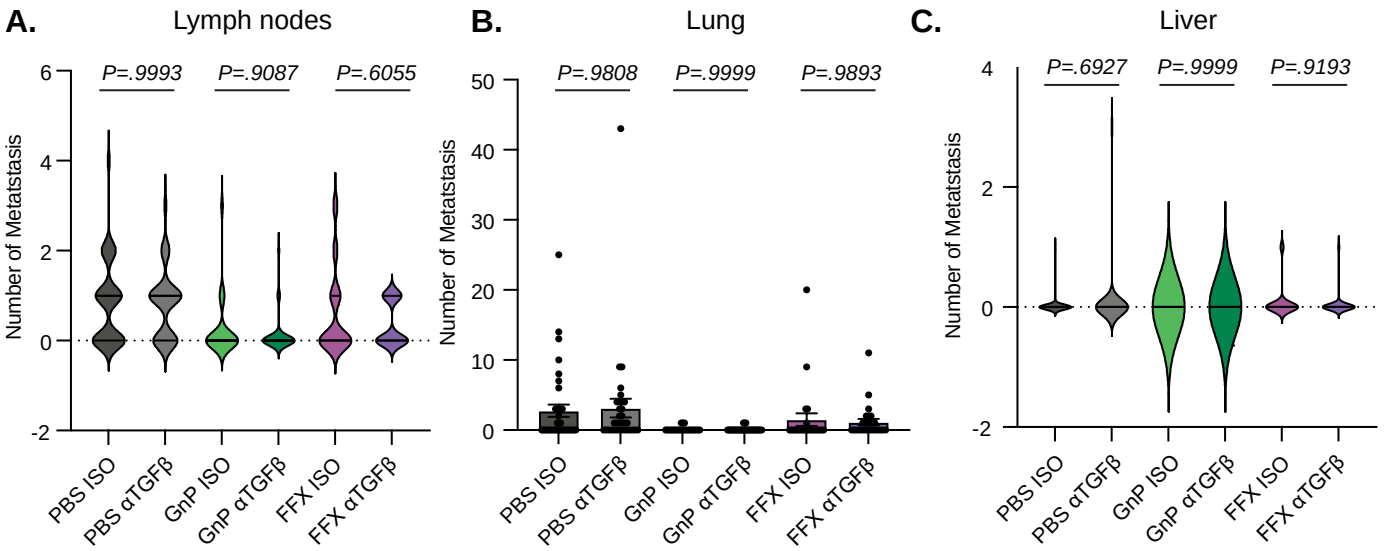


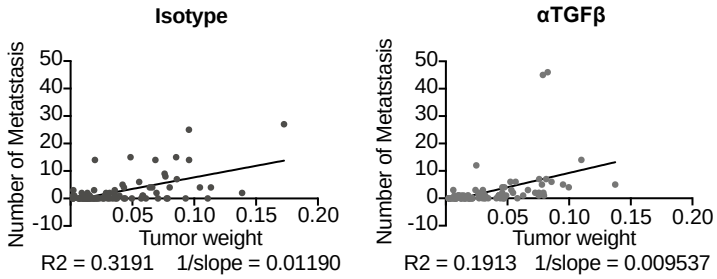


E.

```
Count model coefficients (negbin with log link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.2704     0.3749   0.721  0.47075
weight             19.6212     4.6726   4.199 2.68e-05 ***
treataTGFb          0.1599     0.3397   0.471  0.63770
treatFOL           -0.1823     0.4458  -0.409  0.68255
treatFOL_aTGFb     -0.6035     0.4136  -1.459  0.14452
treatGemAbx        -1.5958     0.5195  -3.072  0.00213 **
treatGemAbx_aTGFb  -2.0907     0.5354  -3.905 9.43e-05 ***
Log(theta)         -0.1873     0.2275  -0.824  0.41019

Zero-inflation model coefficients (binomial with logit link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.3309     1.1997   0.276    0.783
weight            -57.7276    32.4180  -1.781    0.075 .
treataTGFb         -0.4861     1.6475  -0.295    0.768
treatFOL            0.8168     1.3714   0.596    0.551
treatFOL_aTGFb     -0.1746     1.7239  -0.101    0.919
treatGemAbx       -22.2968 51951.8049   0.000    1.000
treatGemAbx_aTGFb -19.7148 32801.1741  -0.001    1.000
```

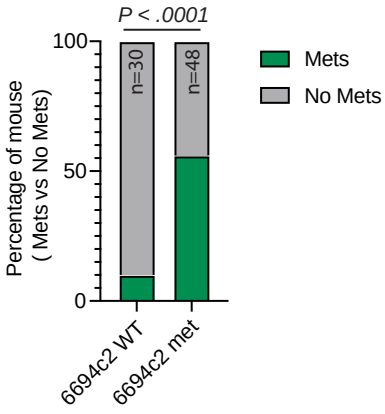


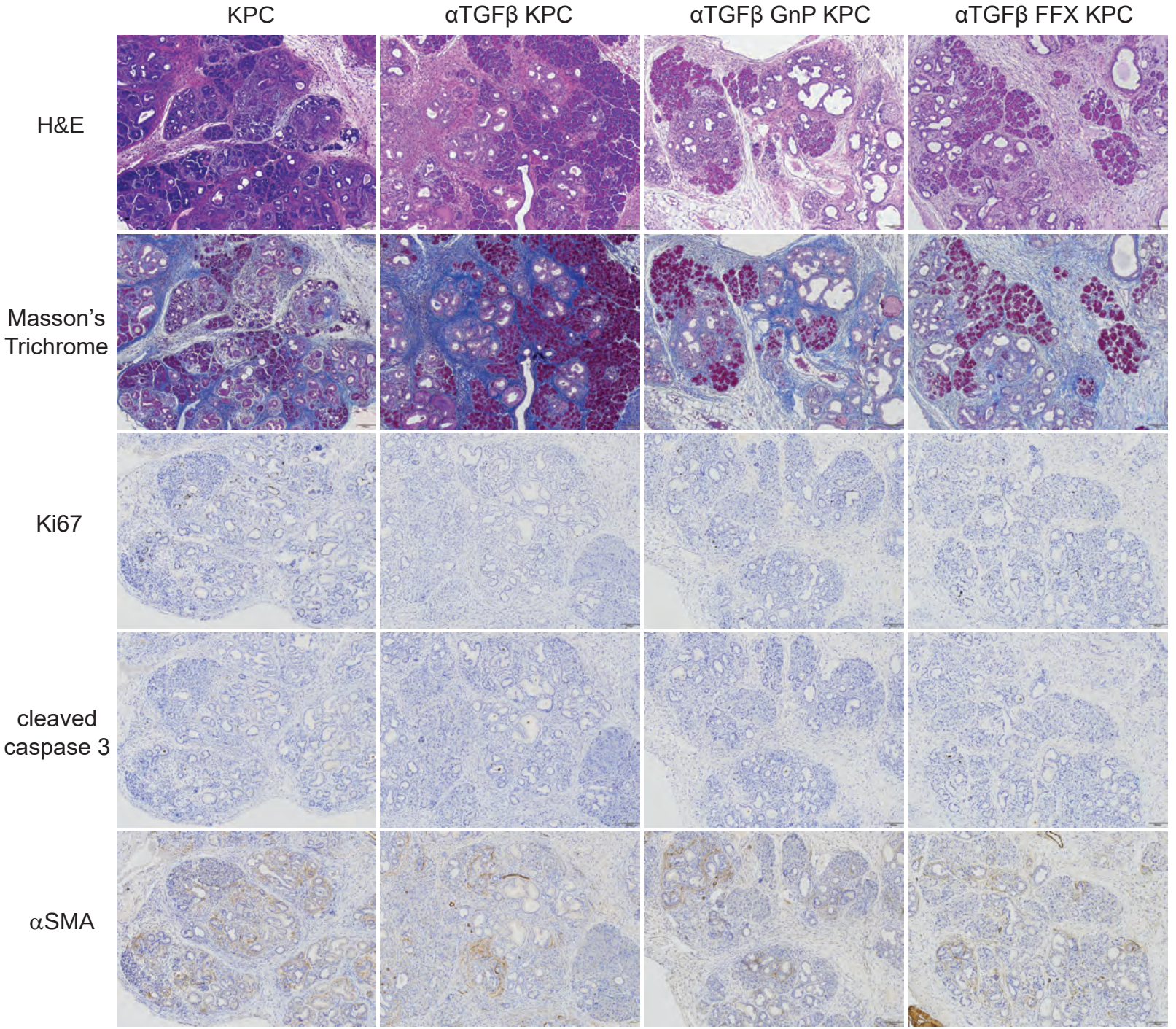


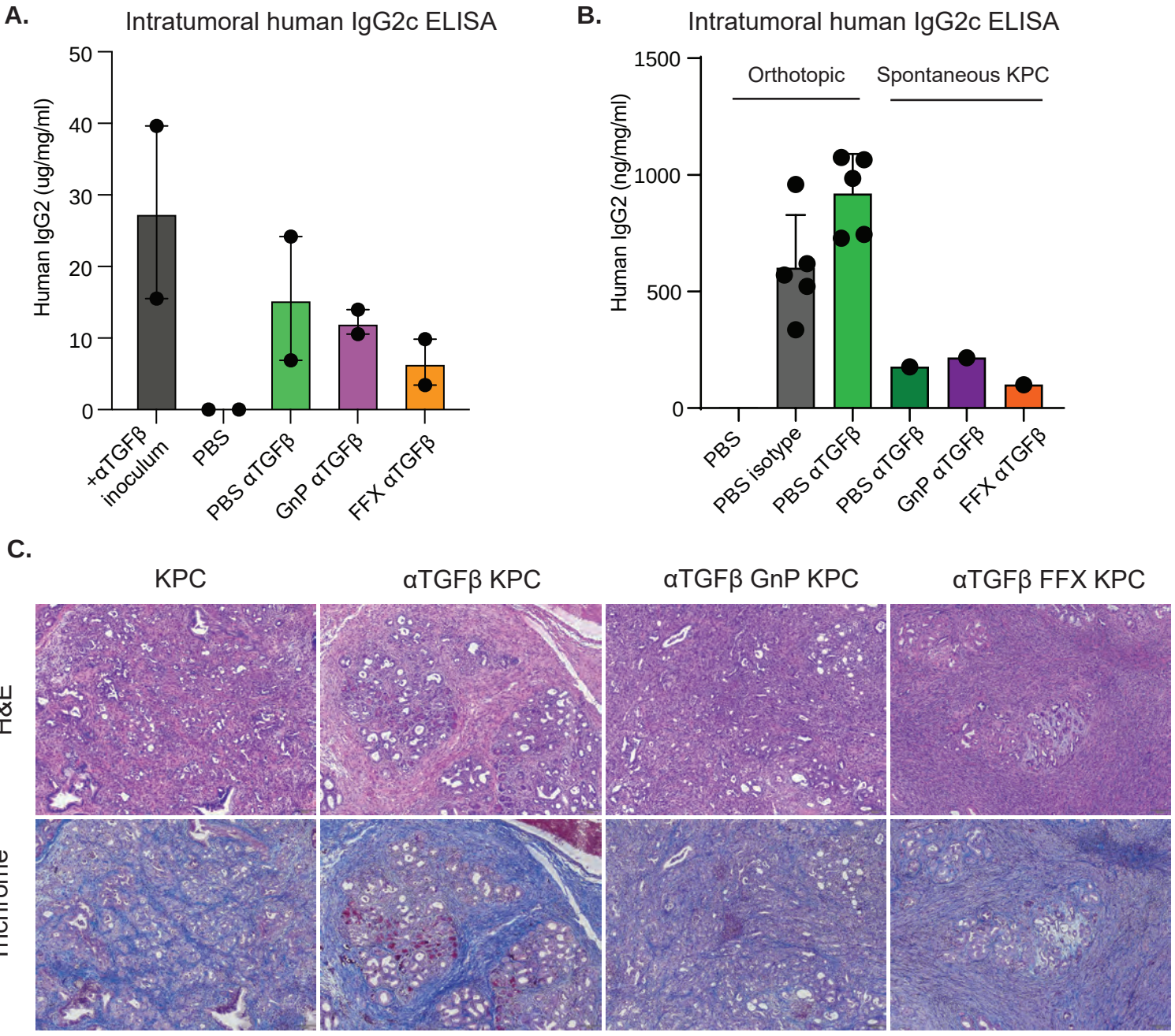


E.

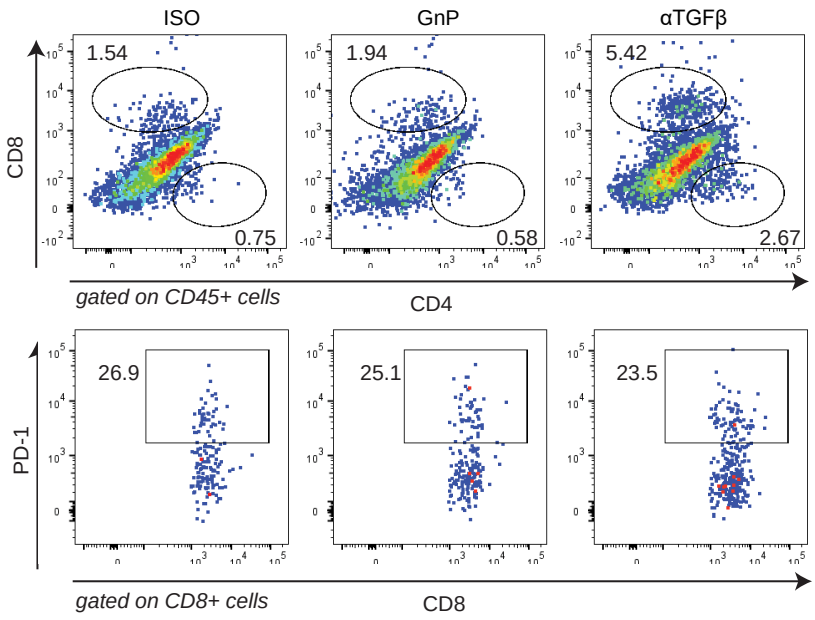
```
Count model coefficients (negbin with log link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.2704     0.3749   0.721  0.47075
weight             19.6212     4.6726   4.199 2.68e-05 ***
treataTGFb          0.1599     0.3397   0.471  0.63770
treatFOL           -0.1823     0.4458  -0.409  0.68255
treatFOL_aTGFb     -0.6035     0.4136  -1.459  0.14452
treatGemAbx        -1.5958     0.5195  -3.072  0.00213 **
treatGemAbx_aTGFb  -2.0907     0.5354  -3.905 9.43e-05 ***
Log(theta)         -0.1873     0.2275  -0.824  0.41019

Zero-inflation model coefficients (binomial with logit link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.3309     1.1997   0.276    0.783
weight            -57.7276    32.4180  -1.781    0.075 .
treataTGFb         -0.4861     1.6475  -0.295    0.768
treatFOL            0.8168     1.3714   0.596    0.551
treatFOL_aTGFb     -0.1746     1.7239  -0.101    0.919
treatGemAbx       -22.2968 51951.8049   0.000    1.000
treatGemAbx_aTGFb -19.7148 32801.1741  -0.001    1.000
```



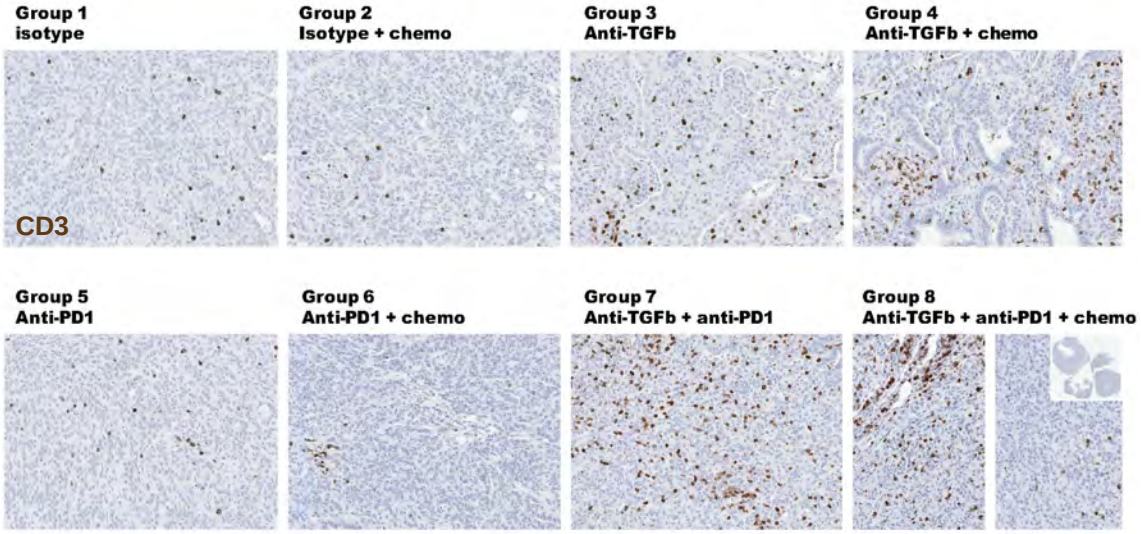

KPC
αTGFβ KPC
αTGFβ GnP KPC
αTGFβ FFX KPC
H&E
Masson's Trichrome
Ki67
cleaved caspase 3
αSMA


A.
Intratumoral human IgG2c ELISA
Human IgG2 (ug/mg/ml)
50
40
30
20
10
0
+αTGFβ inoculum
PBS
PBS αTGFβ
GnP αTGFβ
FFX αTGFβ
B.
Intratumoral human IgG2c ELISA
Human IgG2 (ng/mg/ml)
1500
1000
500
0
Orthotopic
Spontaneous KPC
PBS
PBS isotype
PBS αTGFβ
PBS αTGFβ
GnP αTGFβ
FFX αTGFβ
C.
KPC
αTGFβ KPC
αTGFβ GnP KPC
αTGFβ FFX KPC
H&E
Trichrome

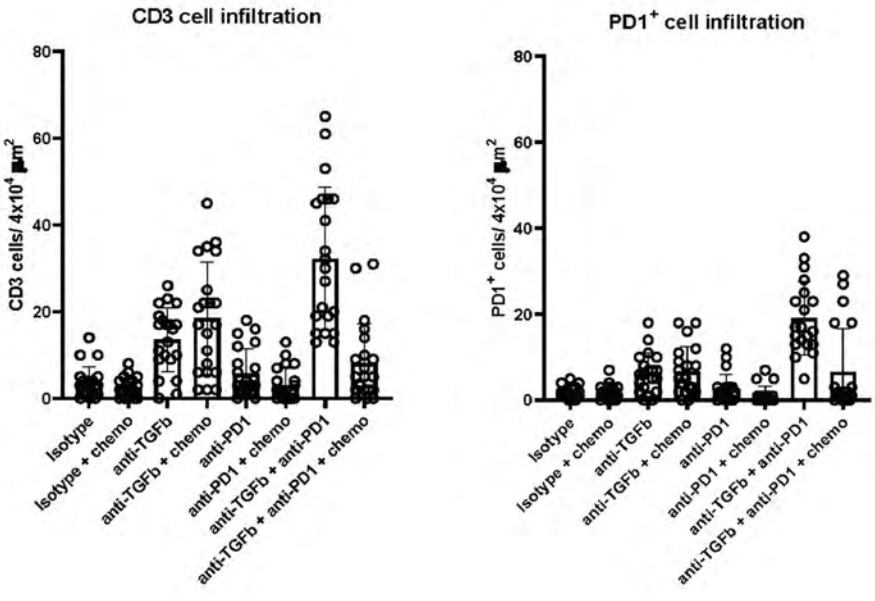
A.

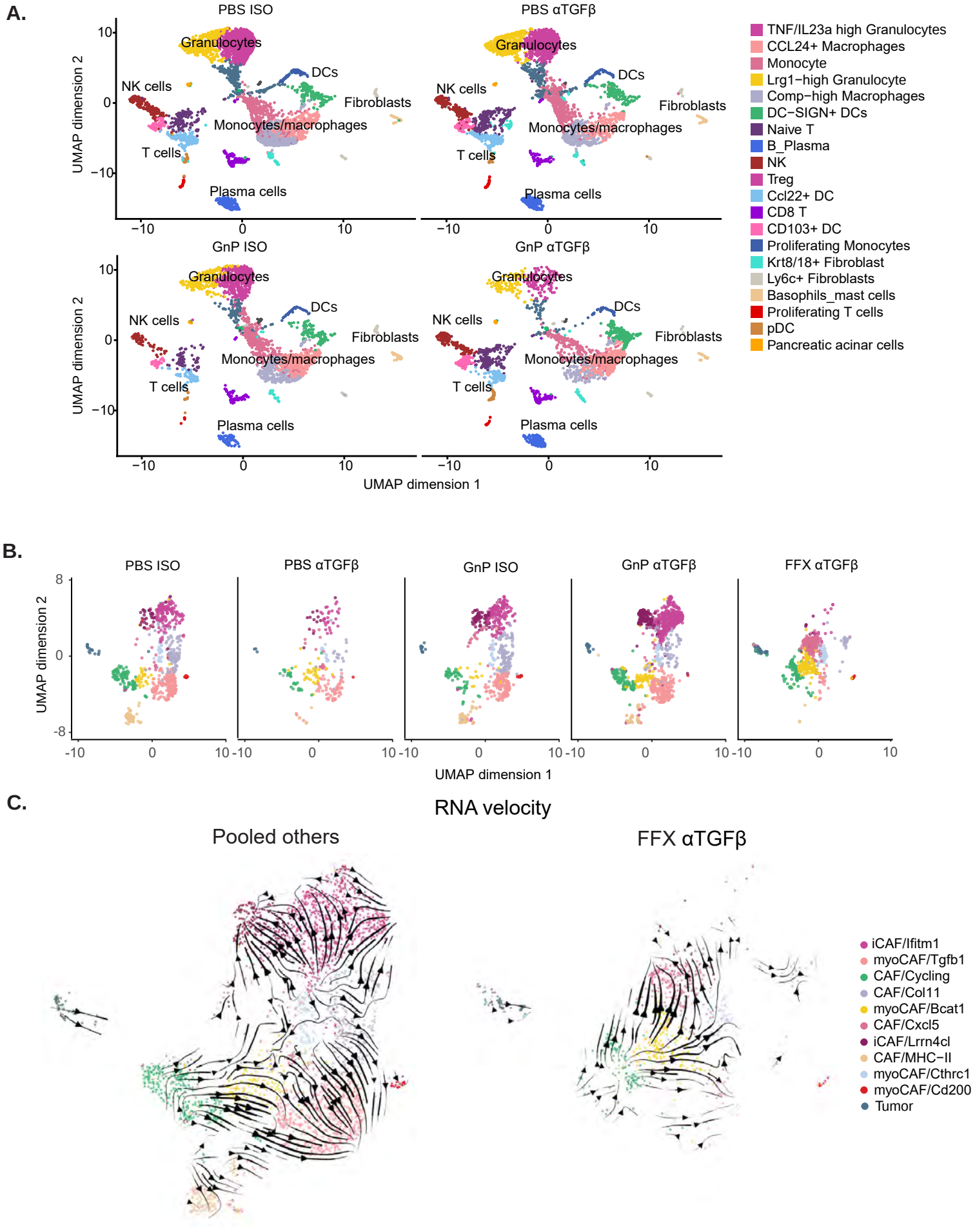

ISO
GnP
αTGFβ
1.54
0.75
1.94
0.58
5.42
2.67
CD8
gated on CD45+ cells
CD4
26.9
25.1
23.5
PD-1
gated on CD8+ cells
CD8

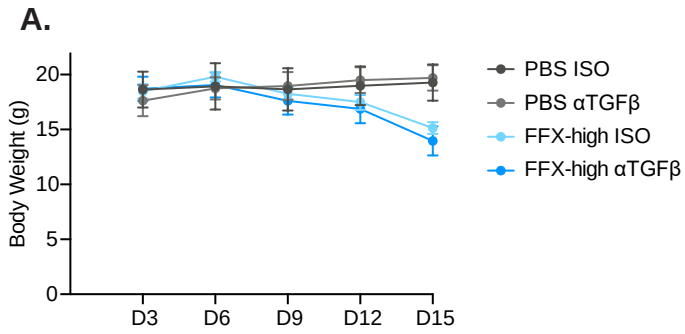
B.

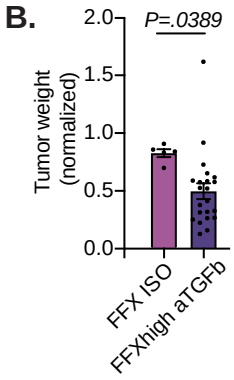

Group 1
isotype
Group 2
Isotype + chemo
Group 3
Anti-TGFb
Group 4
Anti-TGFb + chemo
CD3
Group 5
Anti-PD1
Group 6
Anti-PD1 + chemo
Group 7
Anti-TGFb + anti-PD1
Group 8
Anti-TGFb + anti-PD1 + chemo

C.

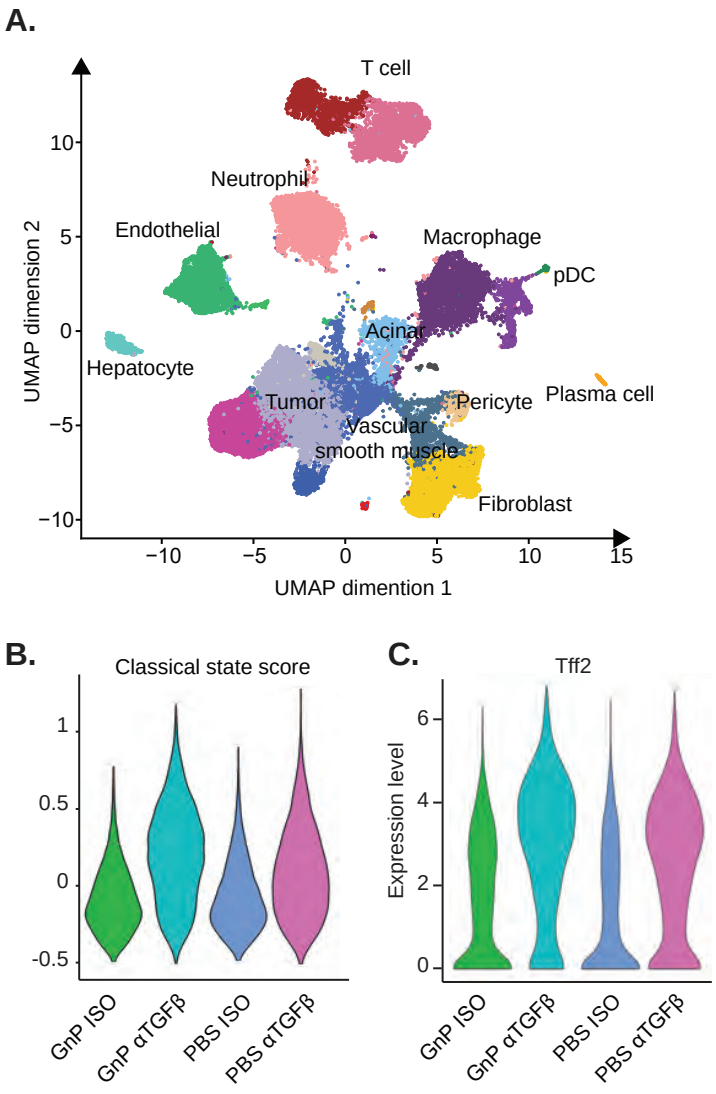

CD3 cell infiltration
CD3 cells/ 4x10⁴ μm²
PD1⁺ cell infiltration
PD1⁺ cells/ 4x10⁴ μm²
Isotype
Isotype + chemo
anti-TGFb
anti-TGFb + chemo
anti-PD1
anti-PD1 + chemo
anti-TGFb + anti-PD1
anti-TGFb + anti-PD1 + chemo


A.
PBS ISO
PBS αTGFβ
GnP ISO
GnP αTGFβ
Granulocytes
DCs
NK cells
Fibroblasts
Monocytes/macrophages
T cells
Plasma cells
UMAP dimension 2
UMAP dimension 1
TNF/IL23a high Granulocytes
CCL24+ Macrophages
Monocyte
Lrg1−high Granulocyte
Comp−high Macrophages
DC−SIGN+ DCs
Naive T
B_Plasma
NK
Treg
Ccl22+ DC
CD8 T
CD103+ DC
Proliferating Monocytes
Krt8/18+ Fibroblast
Ly6c+ Fibroblasts
Basophils_mast cells
Proliferating T cells
pDC
Pancreatic acinar cells
B.
PBS ISO
PBS αTGFβ
GnP ISO
GnP αTGFβ
FFX αTGFβ
UMAP dimension 2
UMAP dimension 1
C.
RNA velocity
Pooled others
FFX αTGFβ
iCAF/Ifitm1
myoCAF/Tgfb1
CAF/Cycling
CAF/Col11
myoCAF/Bcat1
CAF/Cxcl5
iCAF/Lrrn4cl
CAF/MHC−II
myoCAF/Cthrc1
myoCAF/Cd200
Tumor

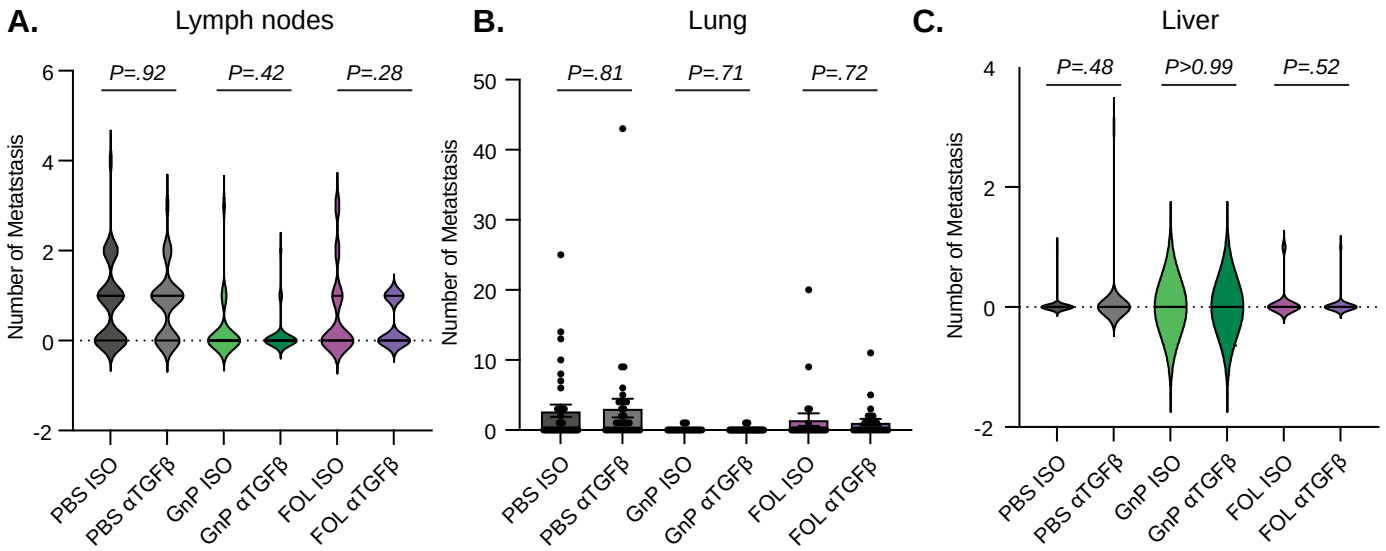

A.
Body Weight (g)
20
15
10
5
0
D3
D6
D9
D12
D15
PBS ISO
PBS αTGFβ
FFX-high ISO
FFX-high αTGFβ

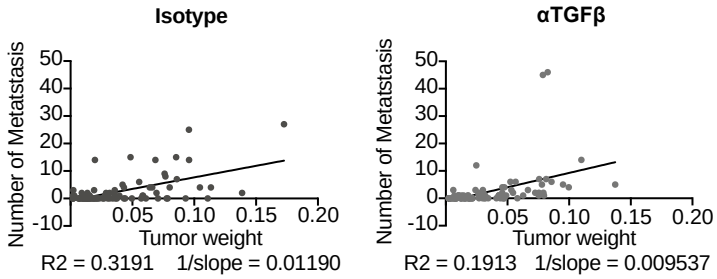

B.
P=.0389
Tumor weight (normalized)
2.0
1.5
1.0
0.5
0.0
FFX ISO
FFXhigh aTGFb

Supplemental Figure 7



Supplemental Figure 1

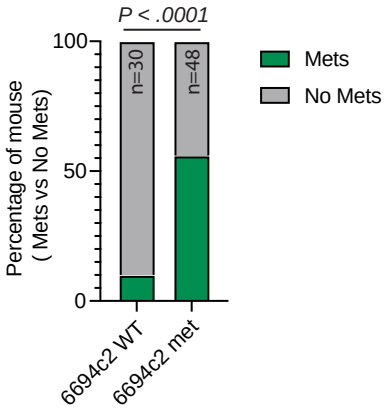


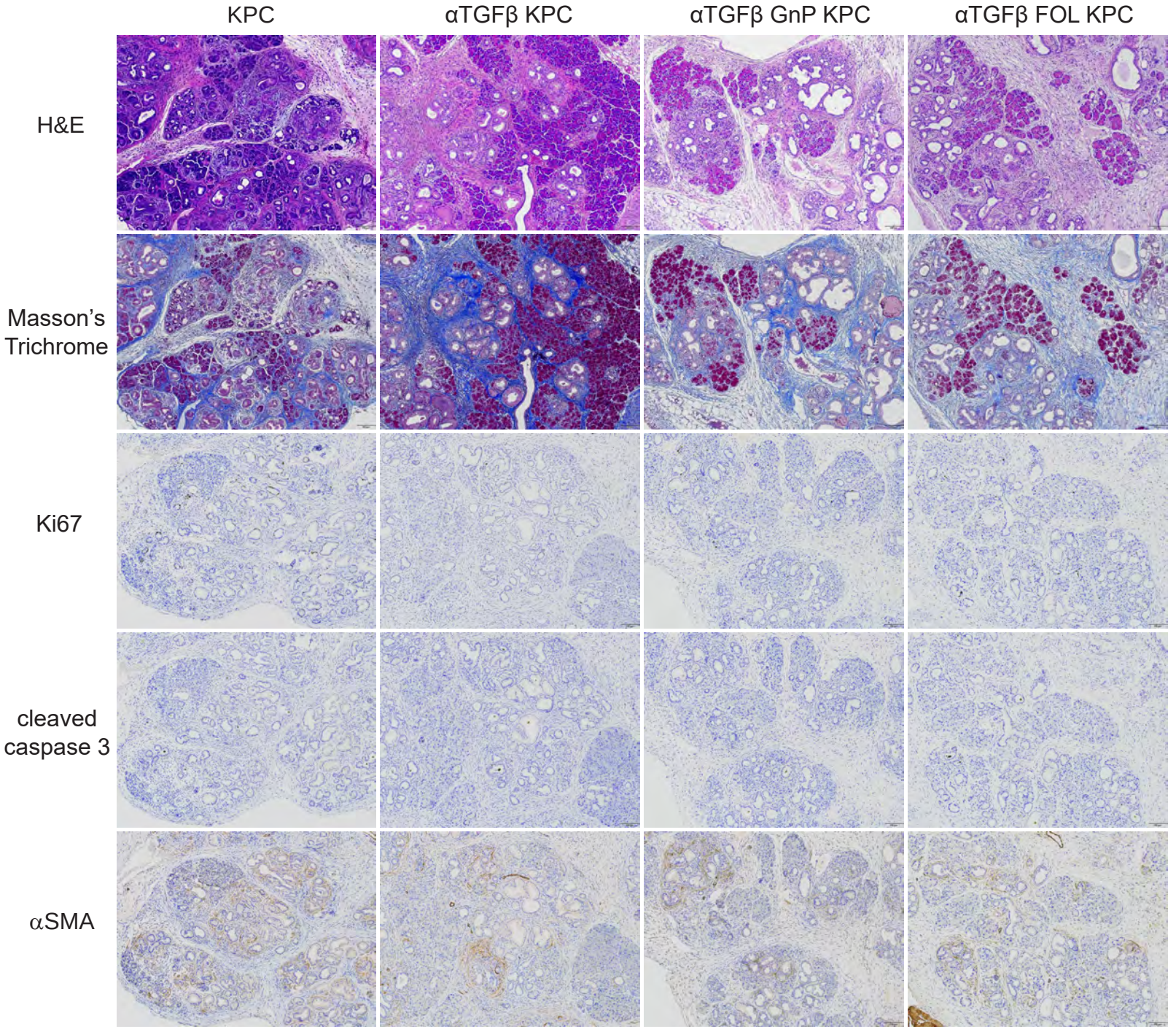


E.

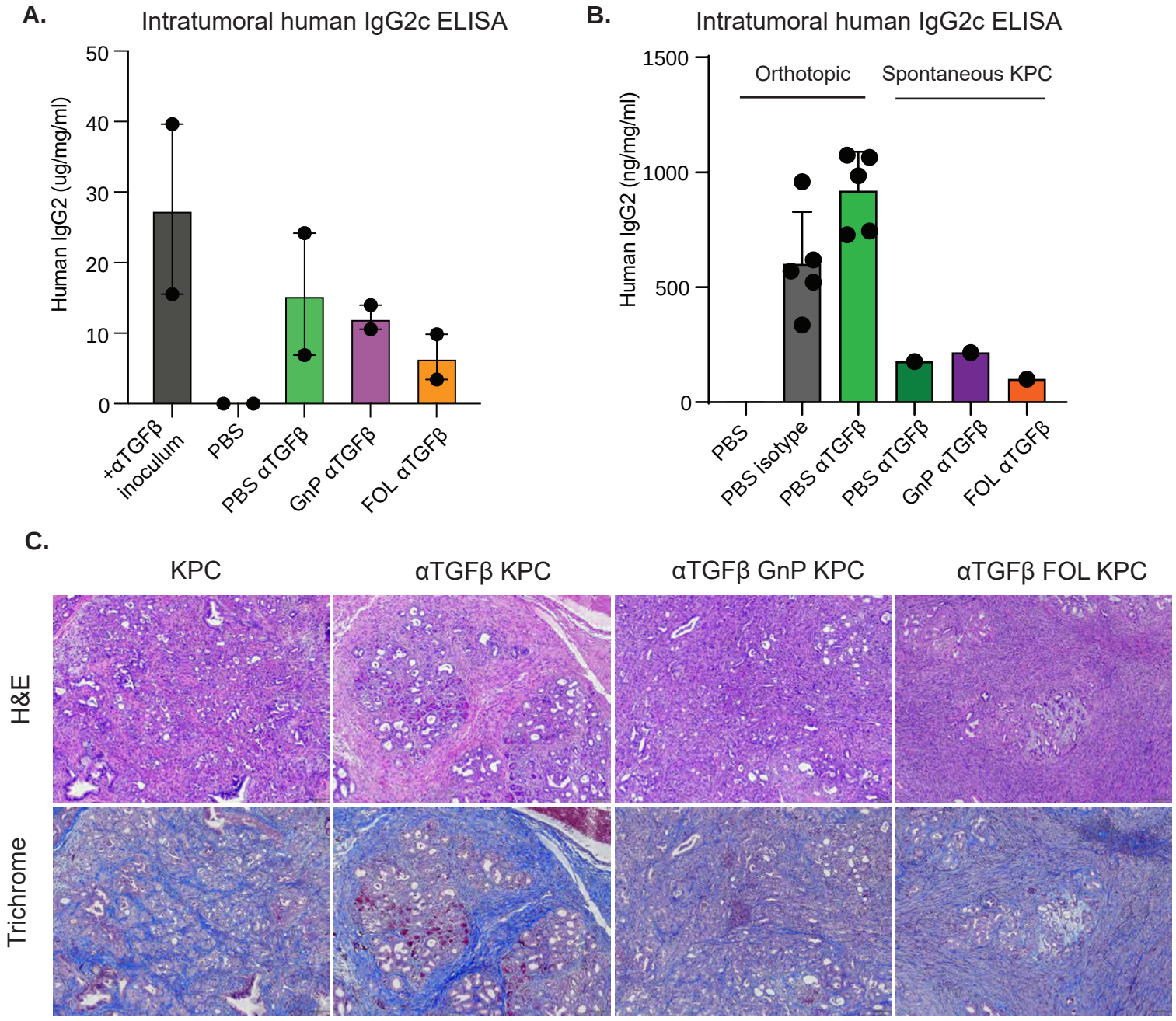
```
Count model coefficients (negbin with log link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.2704     0.3749   0.721  0.47075
weight             19.6212     4.6726   4.199 2.68e-05 ***
treataTGFb          0.1599     0.3397   0.471  0.63770
treatFOL           -0.1823     0.4458  -0.409  0.68255
treatFOL_aTGFb     -0.6035     0.4136  -1.459  0.14452
treatGemAbx        -1.5958     0.5195  -3.072  0.00213 **
treatGemAbx_aTGFb  -2.0907     0.5354  -3.905 9.43e-05 ***
Log(theta)         -0.1873     0.2275  -0.824  0.41019

Zero-inflation model coefficients (binomial with logit link):
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)         0.3309     1.1997   0.276    0.783
weight            -57.7276    32.4180  -1.781    0.075 .
treataTGFb         -0.4861     1.6475  -0.295    0.768
treatFOL            0.8168     1.3714   0.596    0.551
treatFOL_aTGFb     -0.1746     1.7239  -0.101    0.919
treatGemAbx       -22.2968 51951.8049   0.000    1.000
treatGemAbx_aTGFb -19.7148 32801.1741  -0.001    1.000
```

F.





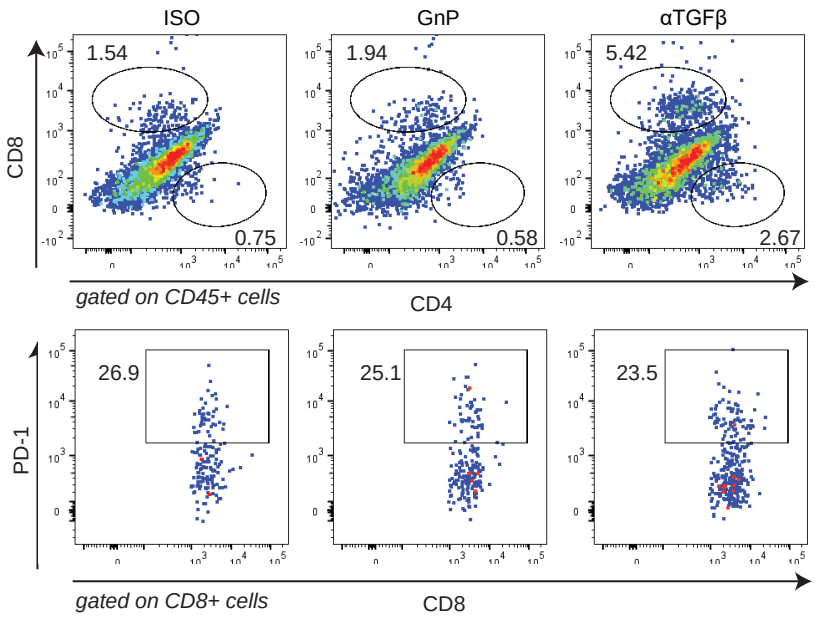
Supplemental Figure 2



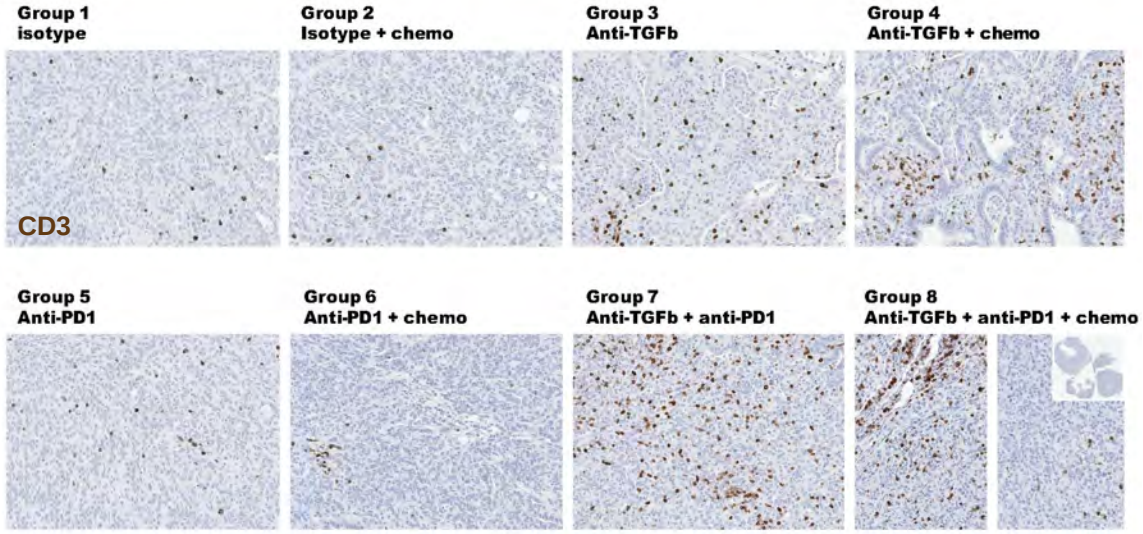
Supplemental Figure 3

Supplemental Figure 4

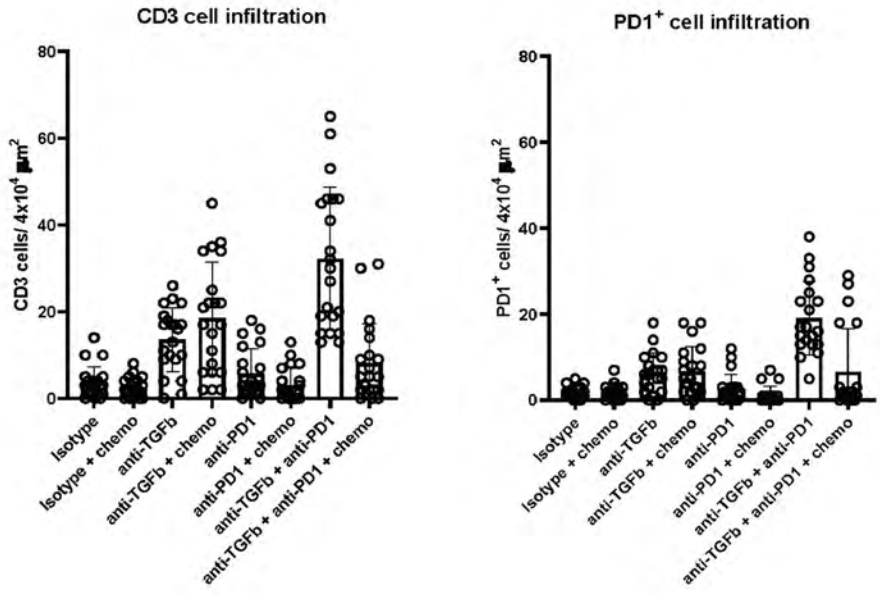
A.



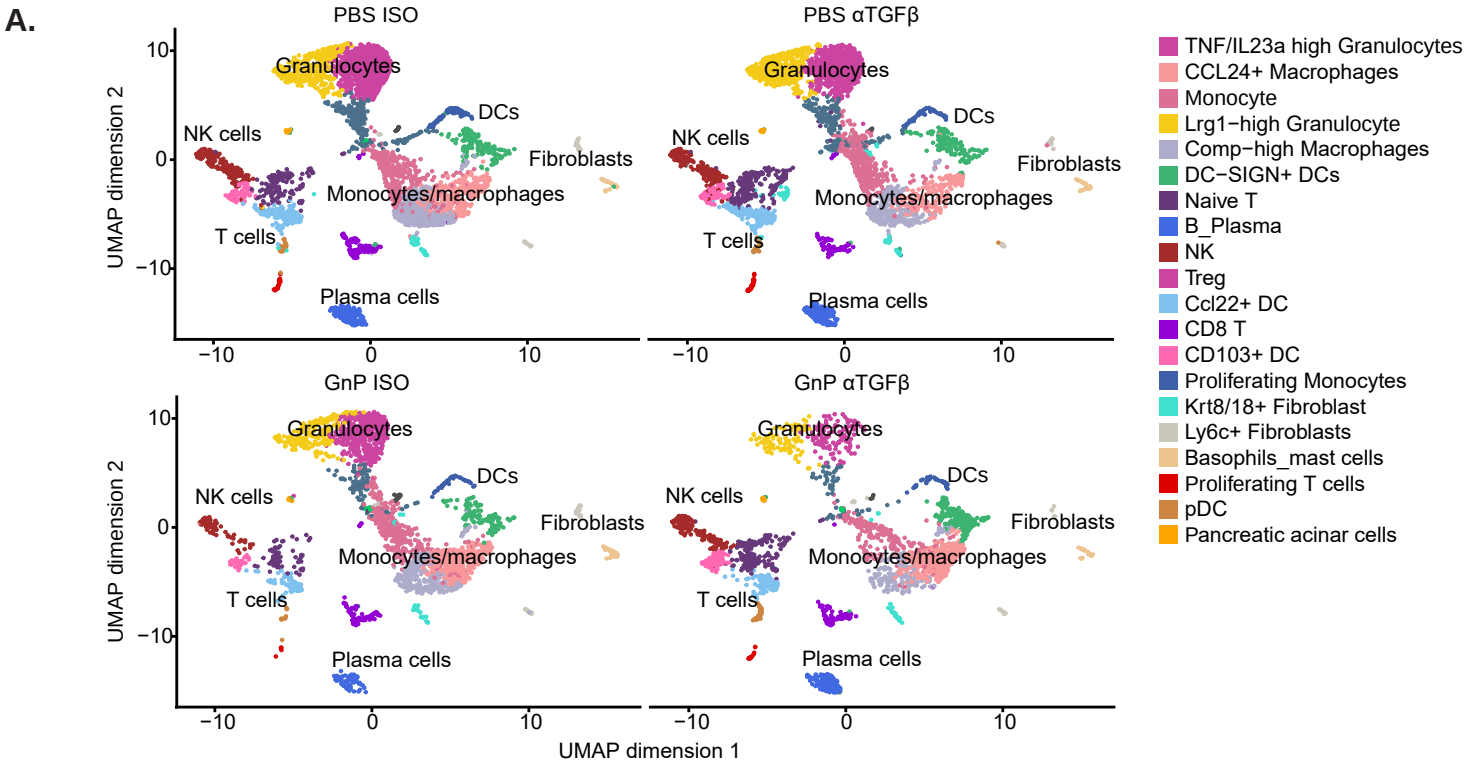
B.

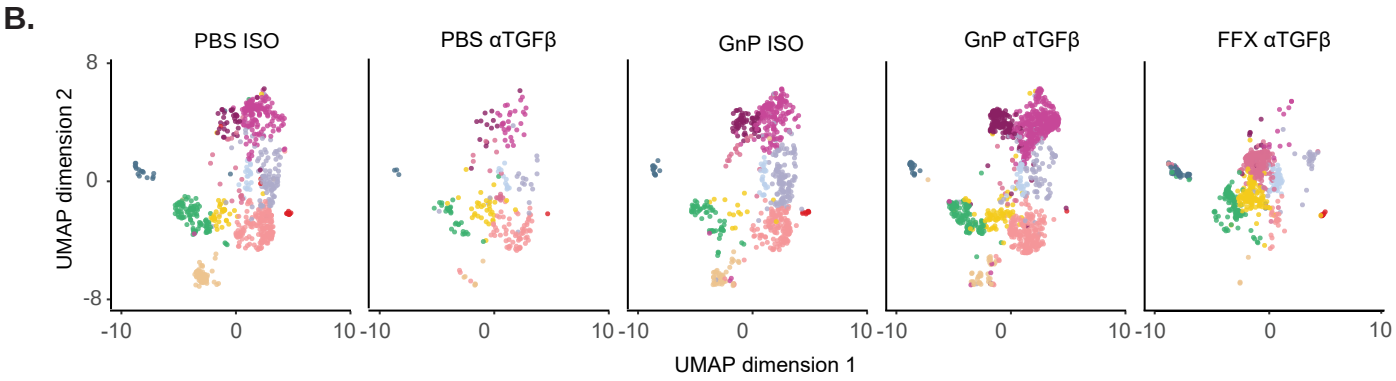


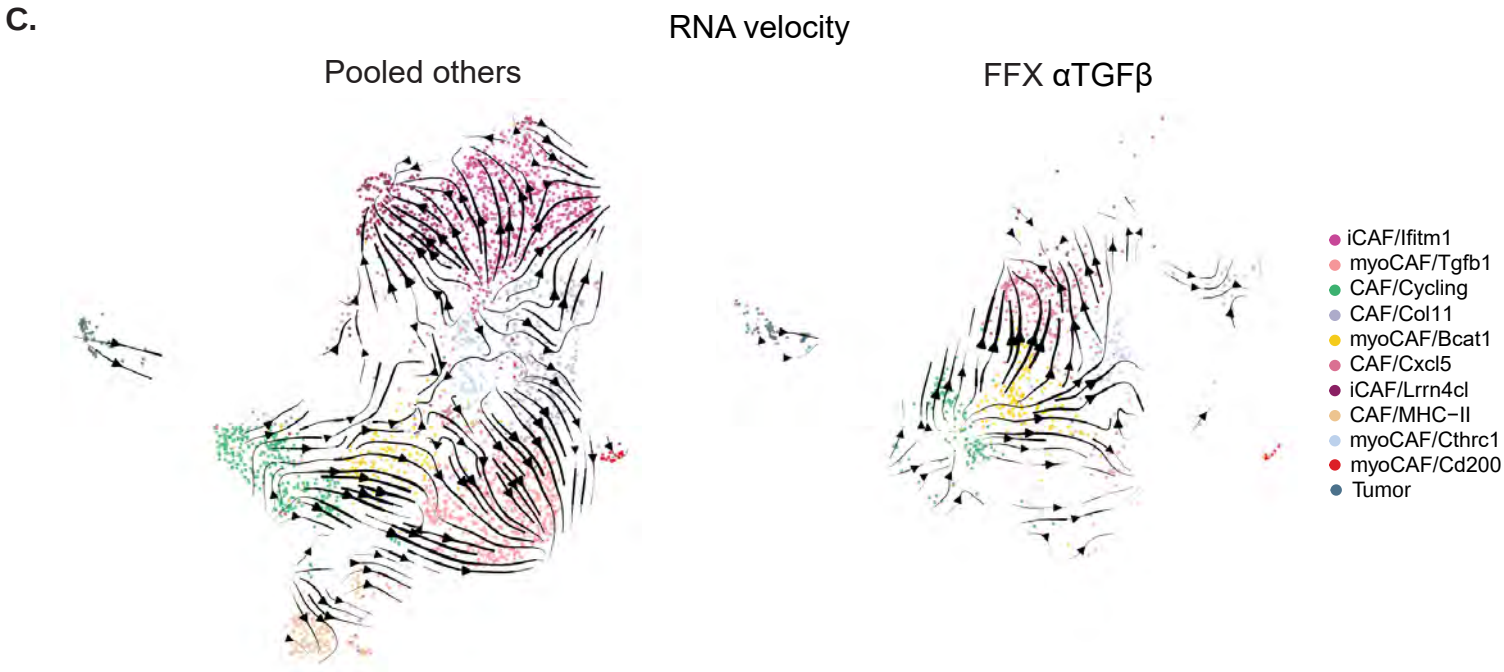
C.



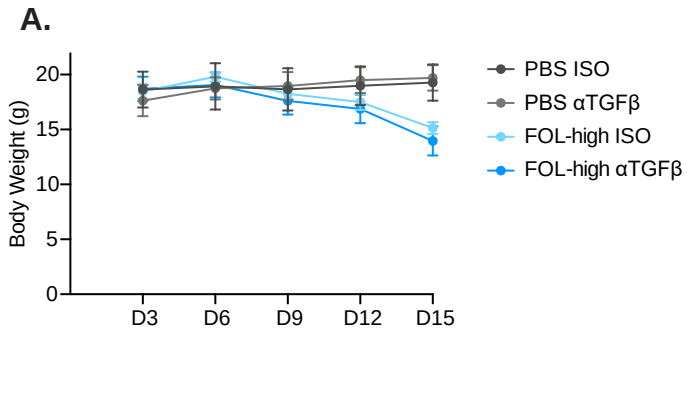
Supplemental Figure 5

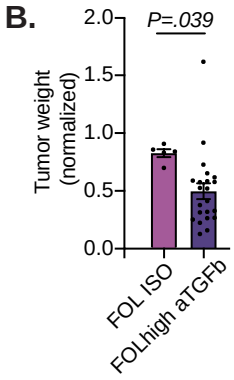




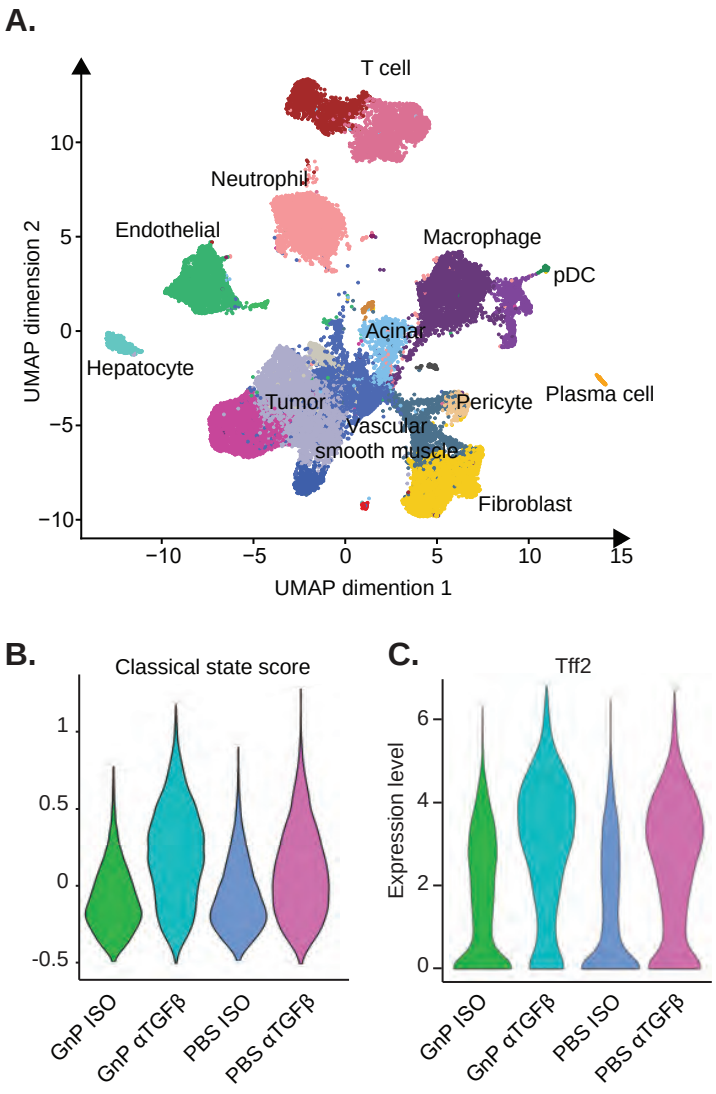


Supplemental Figure 6





Supplemental Figure 7



What you need to know

BACKGROUND AND CONTEXT

TGFβ plays pleiotropic roles in pancreatic cancer including promoting metastasis, attenuating CD8 T cell activation, and enhancing myofibroblast differentiation and deposition of extracellular matrix. In other cancer types, TGFb blockade reprograms fibroblasts and induces relocalization of CD8 T cells to the tumor interior. However, single-agent TGFβ inhibition has shown limited efficacy against pancreatic cancer in mice or humans.

NEW FINDINGS

We evaluated the TGFβ blocking antibody NIS793 in combination with either gemcitabine/n(ab)-paclitaxel or FOLFIRINOX chemotherapy in orthotopic pancreatic cancer models. Blockade of TGFβ with chemotherapy reduced tumor burden in poorly immunogenic pancreatic cancer, without affecting the metastatic rate of cancer cells. Surprisingly, we were unable to demonstrate a role for T cells or fibroblasts in the efficacy of TGFβ blockade in murine PDAC models. Instead, the primary effect of TGFβ blockade in mouse pancreatic tumors is to polarize malignant cells to a more classical expression state. This mechanism of action supports combination of TGFβ blockade with chemotherapy, as classical tumors display increased sensitivity to chemotherapy both in vitro and in clinical trials. We demonstrate in mice that blockade of TGFβ increases sensitivity to either gemcitabine/n(ab)paclitaxel or FOLFIRINOX despite highly divergent effects of these two chemotherapy regimens on immune and stromal cells in the microenvironment.

LIMITATIONS

One limitation of our current study is that we evaluated effects in mouse models, all of which have intact TGFβ signaling pathways. In humans, SMAD4 status may affect clinical response to TGFβ blockade. Although tumor cell intrinsic effects were dominant in mouse models, it is possible that TGFβ signaling in fibroblasts, T cells, or macrophages may be more critically important in human PDAC.

CLINICAL RESEARCH RELEVANCE

NIS793 in combination with gemcitabine/n(ab)paclitaxel is currently being tested in phase II and phase III trials of first line metastatic PDAC (NCT04390763, NCT04935359), and based on the results shown here, trials in combination with FOLFIRINOX have now started in both resectable and first line metastatic PDAC (NCT05546411, NCT05417386). Comprehensive profiling of on-treatment samples from these clinical trials and subgroup analysis of SMAD4 mutant tumors will help identify the definitive role of TGFβ in human PDAC.

Lay Summary

Blockade of the cytokine TGFβ enhances pancreatic tumor cell chemotherapy sensitivity by promoting a basal-like to classical cell state transition. TGFβ blockade and combination chemotherapies are currently in clinical trials.