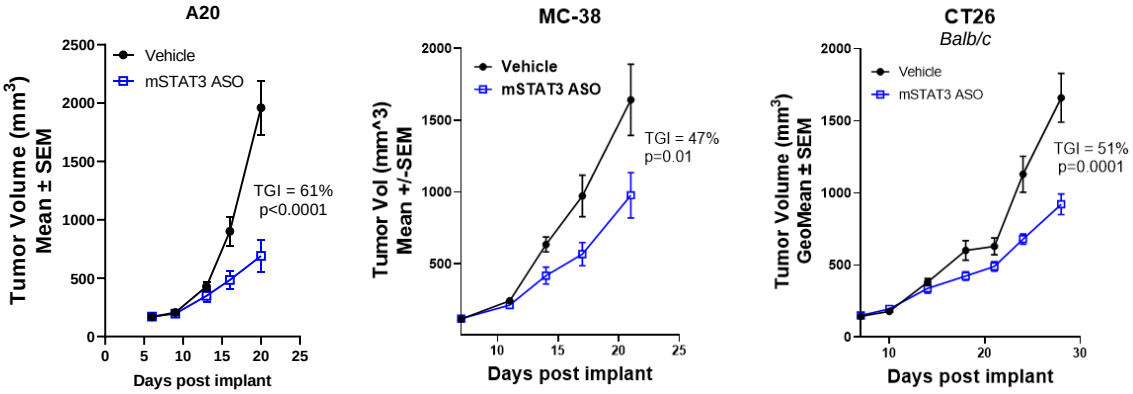
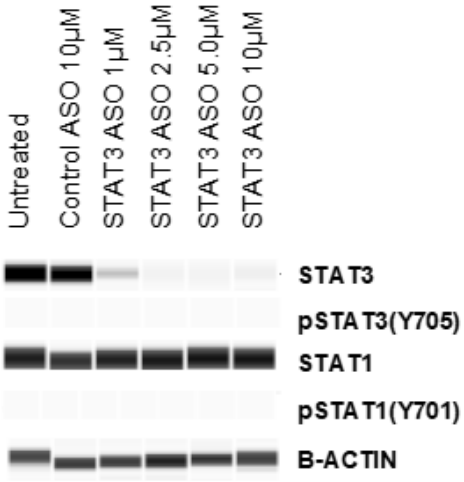
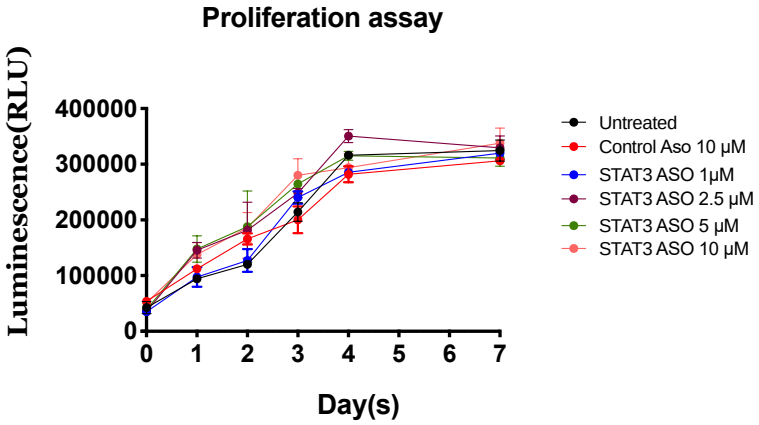
Supplemental Figure 3

A. Monotherapy STAT3 ASO activity



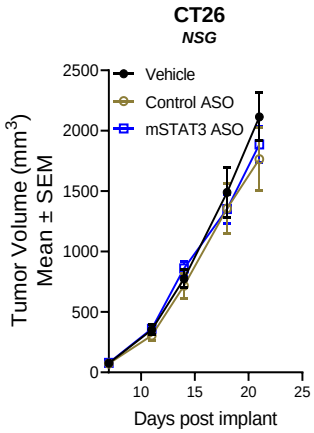
B. CT-26 proliferation assay results – 7 day assay





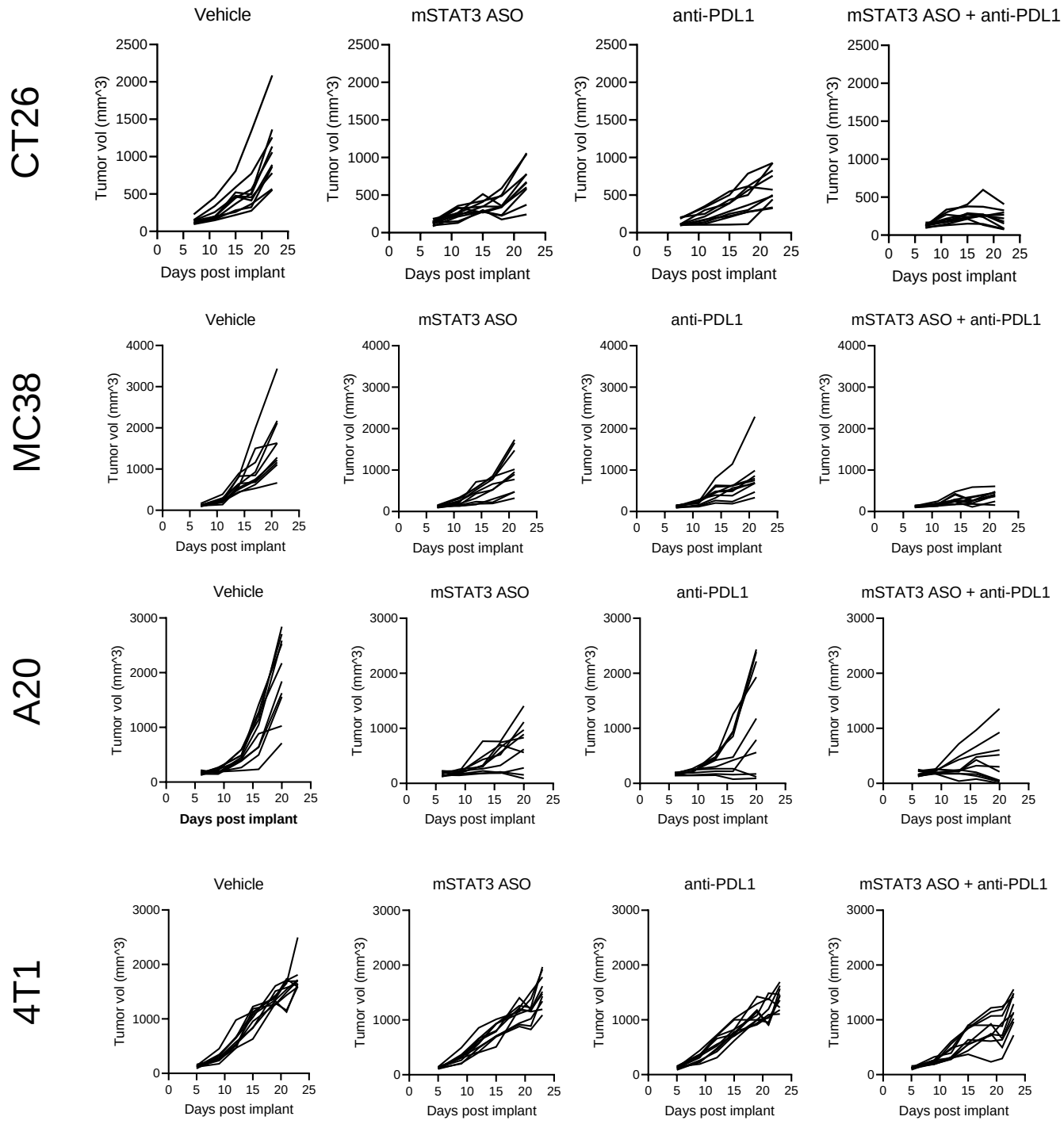
PS: Plated CT26WT 1x10^6 /100ul/well and 100ul of Cell titre glo was added and read using luminometer

C.



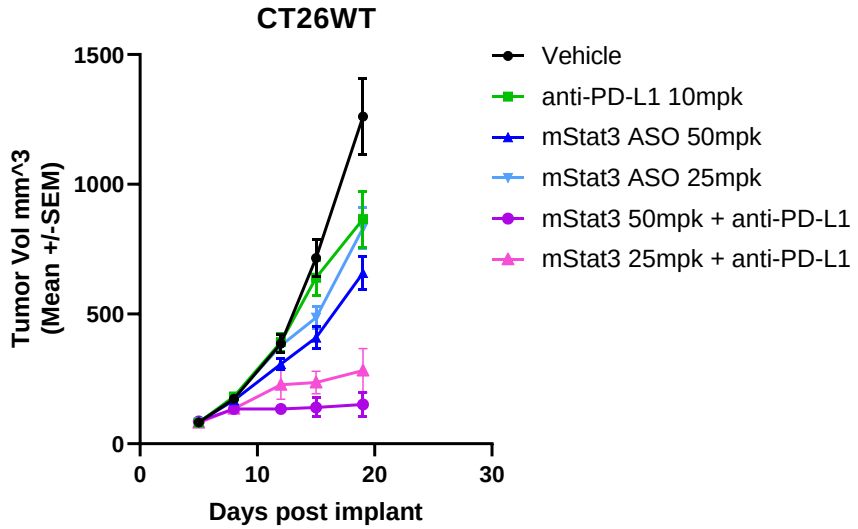
Supplemental Figure 3. **A.** CT26, A20, and MC-38 tumor bearing mice were treated with mSTAT3 ASO at 50mg/kg (5 on 2 off). **B.** CT26 cells were treated in culture with mSTAT3 ASO as indicated and harvested on day 7 for western blot analysis using the indicated antibodies. Proliferation was monitored using Cell Titer Glo. **C.** NSG mice bearing CT26 tumors were treated with mSTAT3 ASO or control ASO at 50 mg/kg (5 on 2 off).

Supplemental Figure 4. Individual animal curves of data shown in Figure 2A.



Supplemental Figure 5

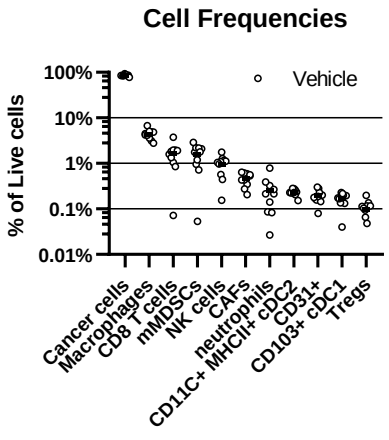
A. CT-26 in vivo dose response



B. Quantification of STAT3 reduction in immune/stromal cells in CT-26

	mSTAT3 ASO (25mpk) + anti-PD-L1 % STAT3 decrease	mSTAT3 ASO (50mpk) + anti-PD-L1: % STAT3 decrease
Cancer Cells	38.7	77.3
Cancer associated fibroblasts	57.1	85.8
CD31+ endothelial cells	96.7	98.9
Macrophages (F4/80+)	78.1	95.6
MHCII+ M1-like macrophages	80.8	96.2
MHCII+ CD206+ macrophages	93.8	99.0
CD206+ macrophages	76.8	95.0
MHCII- CD206- macrophages	44.6	76.6
CD11c+ MHCII+ dendritic cells (cDC2)	50.9	79.1
CD103+ dendritic cells (cDC1)	54.3	80.9
CD103- dendritic cells	43.4	79.2
Ly6C+ neutrophil-like cells	36.2	61.2
Ly6G+ neutrophil like cells	23.7	20.4
NK cells (NKP46+)	19.3	34.1
CD8+ T cells	5.7	25.0
CD4+ FOXP3- T cells	14.9	4.6
CD4+ FOXP3+ T regs	73.5	67.0

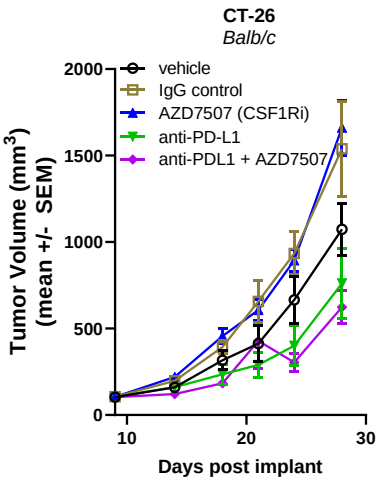
C. Quantification of cell frequencies in CT-26



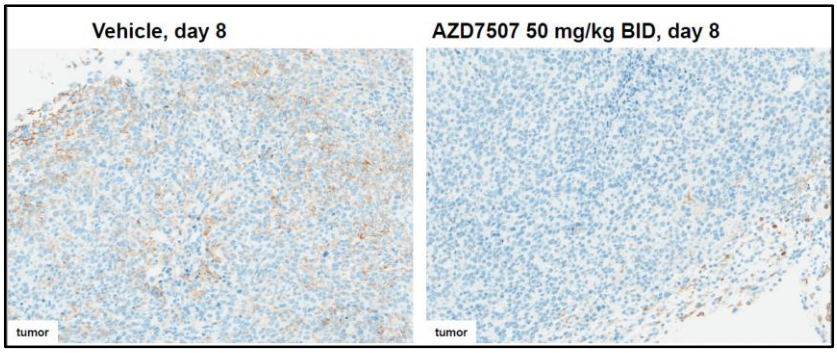
Supplemental Figure 5. **A.** CT26 tumor bearing balb/c mice were treated with mSTAT3 ASO at 50mg/kg or 25mg/kg, 5 on 2 off, +/- anti-PD-L1. **B.** Quantification of % STAT3 decrease at each dose level compared to average vehicle control treated mice. **C.** Quantification of cell frequencies of different stromal and immune populations in vehicle treated mice.

Supplemental Figure 6. CSF1R inhibition with AZD7507 in CT26 has minimal activity as monotherapy or in combination with anti-PD-L1

A. CT-26 efficacy



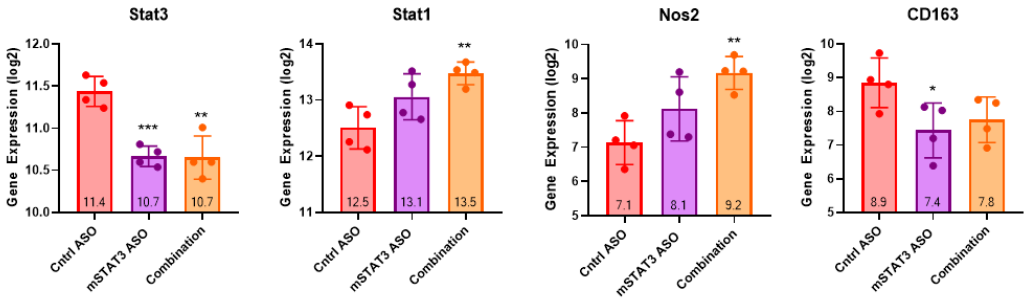
B. AZD7507 treatment in CT26 reduces F4/80+ macrophages



Supplemental Figure 6. A. CT26 tumor bearing mice were treated with AZD7507 at 50mg/kg, BID for ~3 weeks +/- anti-PD-L1. **B.** CT26 tumor bearing mice were treated with AZD7507 for 8 days and harvested for immunohistochemistry analysis of F4/80 to demonstrate on target depletion of macrophages.

Supplemental Figure 7. Nanostring analysis of formalin fixed paraffin embedded (FFPE) CT-26 tumor sections

A. Nanostring analysis of FFPE CT-26 tumors



B. Heatmap of Nanostring analysis of FFPE CT-26 tumors