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Supplementary Materials for

Cancer-associated MSC drive tumor immune exclusion and resistance to immunotherapy, which can be overcome by Hedgehog inhibition

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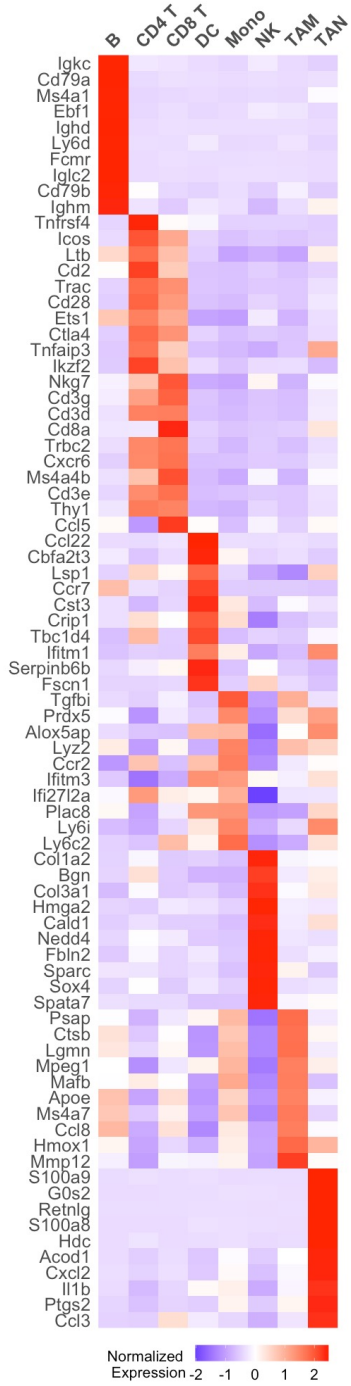

Fig. S1. Heatmap displaying normalized expression of top 10 differentially expressed genes in each cell type population.

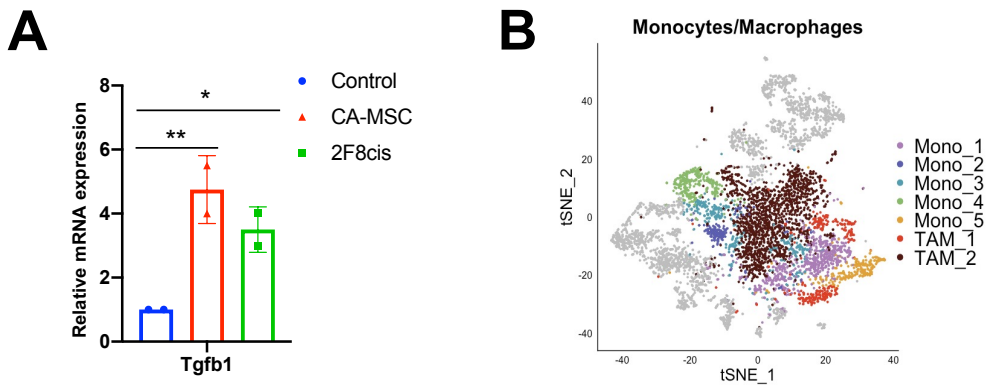

Fig. S2. Expression of *Tgfb1* gene expression in 2F8cis and CA-MSCs. A) RT-PCR was performed to calculate *Tgfb1* gene expression in CellTrace violet-labeled a-MSCs (CA-MSC) co-cultured with 2F8cis cells (2F8cis) and compared to 2F8cis cells cultured alone (Control). *Gapdh* was used as housekeeping gene. **B)** tSNE scRNAseq plot of monocytes and macrophages specific data from merged data of a-PD-L1-treated tumors.

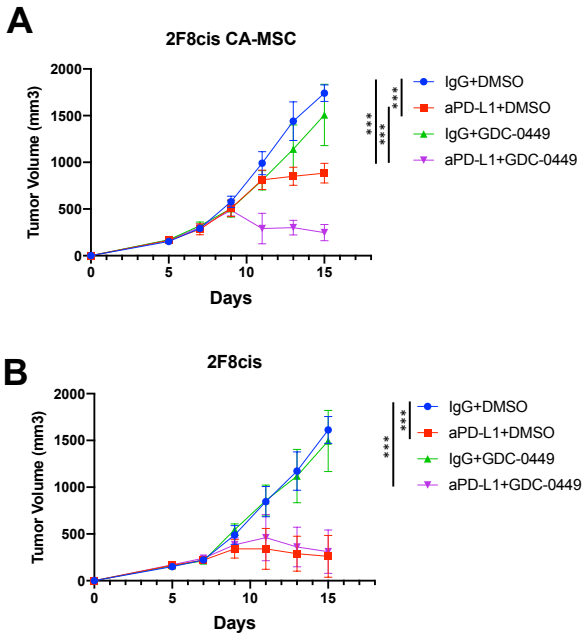

Fig. S3. GDC-0449 restores anti-PD-L1 therapy in CA-MSC+ tumors. A-B) Average tumor growth curves of 2F8cis/CA-MSC- (A) and 2F8cis- (B) tumor-bearing mice after receiving the indicated treatments. Statistical significances were calculated using two-way ANOVA. Error bars, SEM *p <0.05, ** p < 0.01, ** p<0.001, ***p<0.0001.

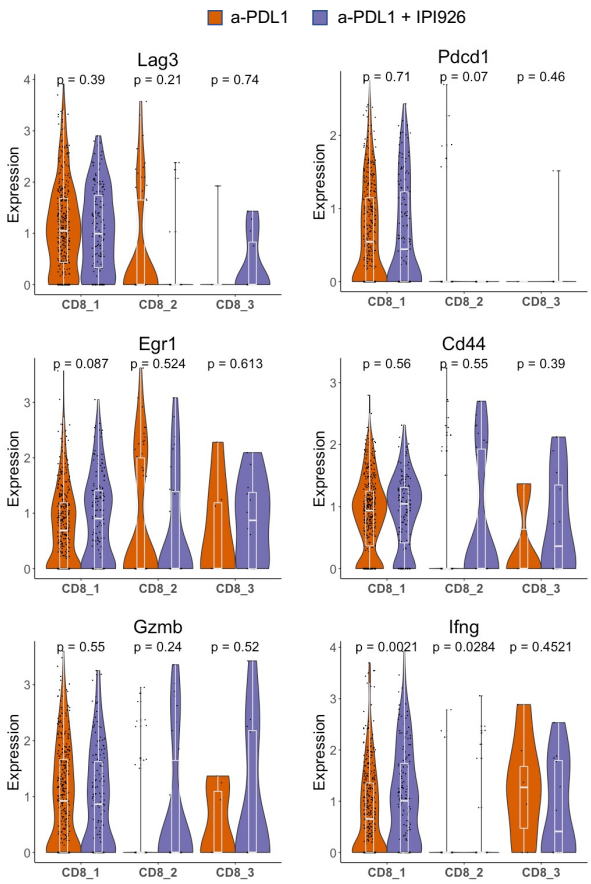

Fig. S4. Gene expression in CD8 T cells isolated from a-PDL1- and a-PDL1 + IPI-926-treated CA-MSC+ tumors. Violin plots showing the expression of *Lag3*, *Pdcd1*, *Erg1, Cd44, Gzmb* and *Ifng* in each CD8 T cell cluster of the indicated a-PD-L1- and a-PD-L1 + IPI-926-treated 2F8cis/CA-MSC tumors. Results were analyzed using two-way ANOVA or Wilcoxon sign rank test. Error bars, SEM *p <0.05, ** p < 0.01, ** p<0.001.

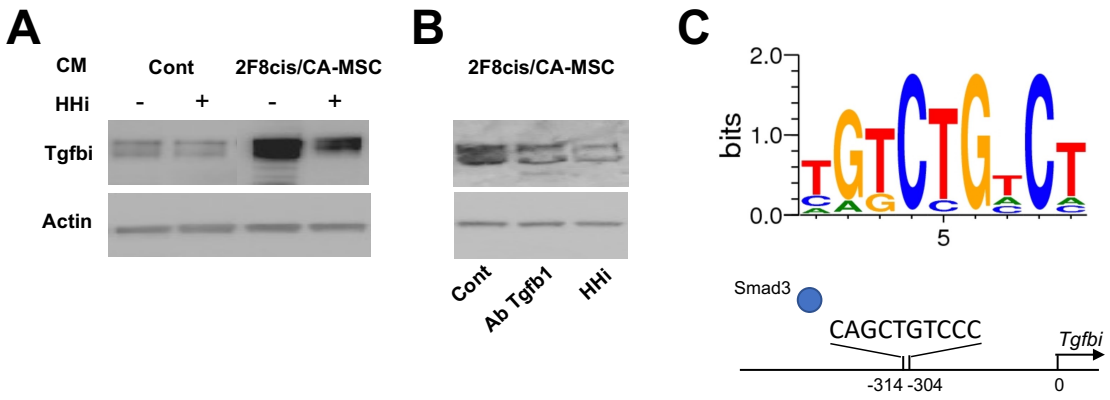

Fig. S5. CA-MSC-secreted Tgf-b1 modulate Tgfbi expression in macrophage. A-B) Western blotting showing the abundance of Tgfbi in BM-derived macrophages cocultured with the CM of 2F8cis/CA-MSCs pre-treated or not with HHi. BM-derived macrophages cultured in DMEM were used as control (Cont). Actin was used as loading control. **C)** The *Tgfbi* promoter region contains a putative SMAD3-binding site, detected by MotifMap.

Table S1. Clinical characteristics of PD-1/PD-L1 ICI treated OvCa patients

Characteristic	N=14
Histology	
clear cell	3 (25%)
serous	8 (67%)
Serous	1 (8.3%)
Unknown	2
Stage	
I	1 (7.7%)
II	2 (15%)
III	5 (38%)
IV	5 (38%)
NA	1
MSI	
Stable	7 (50%)
NA	7 (50%)
Age	63 (57, 71)
Toxicity	6 (46%)
NA	1
Resp	
Prog	7 (50%)
Resp	4 (29%)
Stable	3 (21%)
PFSMonth	6 (3, 9)
NA	1
OSMonth	10 (5, 27)
NA	1
Death	9 (64%)

[1] Statistics presented: n (%); Median (IQR)