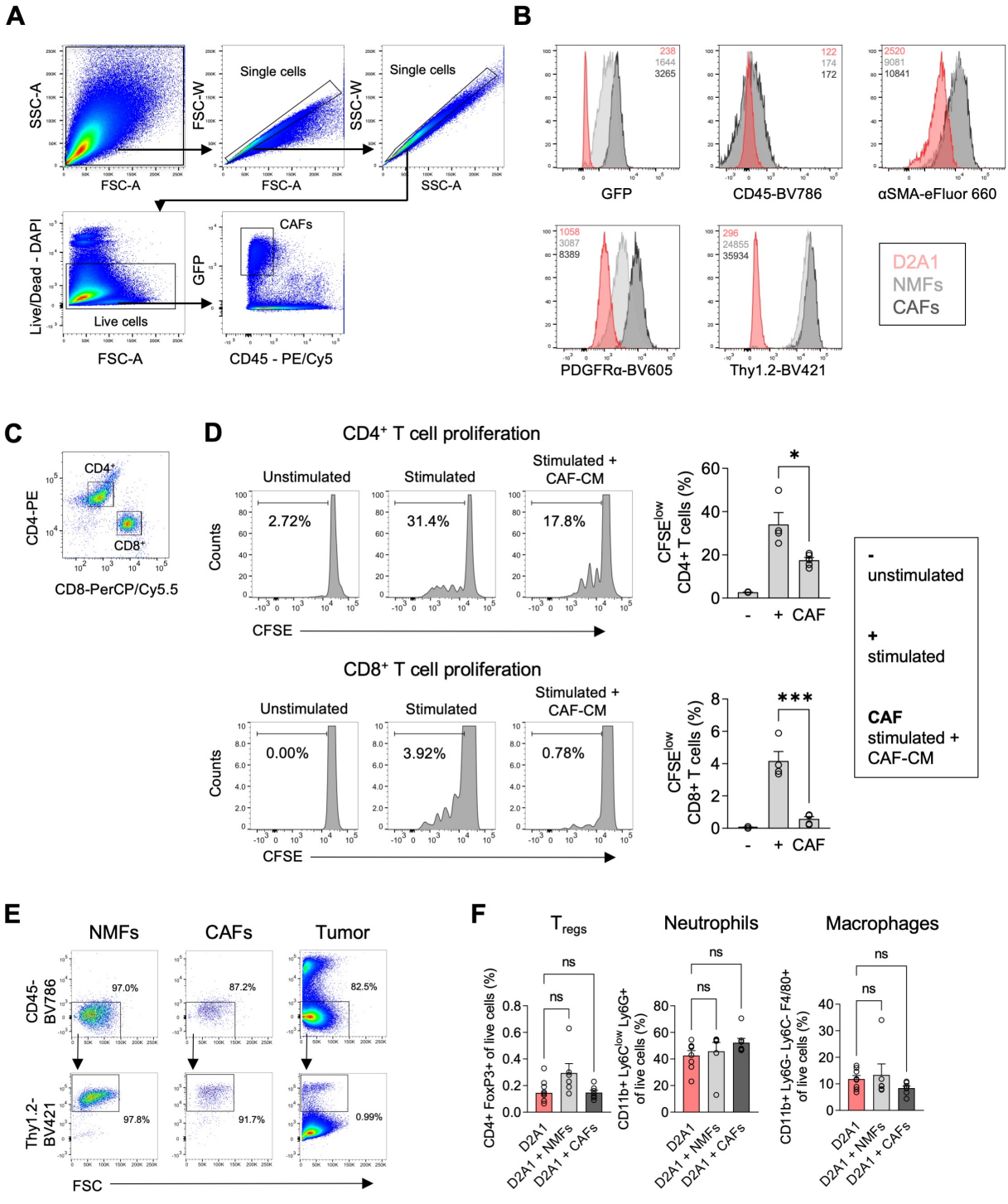
A
SSC-A
FSC-A
Single cells
FSC-W
FSC-A
Single cells
SSC-W
SSC-A
Live/Dead - DAPI
Live cells
FSC-A
GFP
CAFs
CD45 - PE/Cy5
B
238
1644
3265
GFP
122
174
172
CD45-BV786
2520
9081
10841
αSMA-eFluor 660
1058
3087
8389
PDGFRα-BV605
296
24855
35934
Thy1.2-BV421
D2A1
NMFs
CAFs
C
CD4⁺
CD8⁺
CD4-PE
CD8-PerCP/Cy5.5
D
CD4⁺ T cell proliferation
Unstimulated
Stimulated
Stimulated + CAF-CM
Counts
2.72%
31.4%
17.8%
CFSE
CFSE^low CD4+ T cells (%)
*
- + CAF
CD8⁺ T cell proliferation
0.00%
3.92%
0.78%
CFSE^low CD8+ T cells (%)
***
- unstimulated
+ stimulated
CAF stimulated + CAF-CM
E
NMFs
CAFs
Tumor
CD45-BV786
97.0%
87.2%
82.5%
Thy1.2-BV421
97.8%
91.7%
0.99%
FSC
F
T_regs
Neutrophils
Macrophages
CD4+ FoxP3+ of live cells (%)
CD11b+ Ly6C^low Ly6G+ of live cells (%)
CD11b+ Ly6G- Ly6C- F4/80+ of live cells (%)
ns
D2A1
D2A1 + NMFs
D2A1 + CAFs

Supplementary Figure S4

CAF isolation and analysis. Associated with Fig. 5A-D. **A,** 4T1 cells were implanted orthotopically into Ub-GFP BALB/c mice. Mice were culled at day 17. Shown are representative plots of the gating strategy employed for the isolation of CAFs. Live cells were identified using DAPI. CAFs were identified as GFP+ CD45- cells. **B,** D2A1 tumor cells, normal BALB/c mouse mammary gland fibroblasts (NMFs) and CAFs isolated from 4T1 tumors (CAFs) were stained with BV786-conjugated αCD45, BV605-conjugated αPDGFRα and BV421-conjugated αThy1.2 (CD90.2) antibodies. Shown are representative flow cytometry plots with MFI values. **C,** Gating strategy for identification of CD4+ and CD8+ T cells in the experiment described in panel D. **D,** Splenocytes isolated from naive 6 - 8 week female BALB/c mice were labelled with 1 mM CFSE and cultured with 5 µg/mL anti-CD28 antibody and 10 ng/mL IL2 in normal complete medium or CAF conditioned medium (CAF-CM) in uncoated (unstimulated, *n*=3) or anti-CD3 coated (stimulated, *n*=4 or 5) 96-well plates. After 4 days, cells were stained with DAPI and αCD45, αCD8 and αCD4 antibodies. Bar charts show the % $CFSE^{low}$ CD4+ (upper panel) and CD8+ (lower panel) T cells assessed via flow cytometry (mean values ± SEM; unpaired *t*-test). **E,** Flow cytometry plots showing gating strategy for identification of CD45-/Thy1.2+ cells in cultured NMFs, cultured CAFs and a representative D2A1 tumor sample from the experiment described in Fig. 5A-D. **F,** Quantification of T_{regs} (left panel), neutrophils (middle panel) and macrophages (right panel) in the experiment described in Fig. 5A-D (mean values ± SEM, one-way ANOVA).