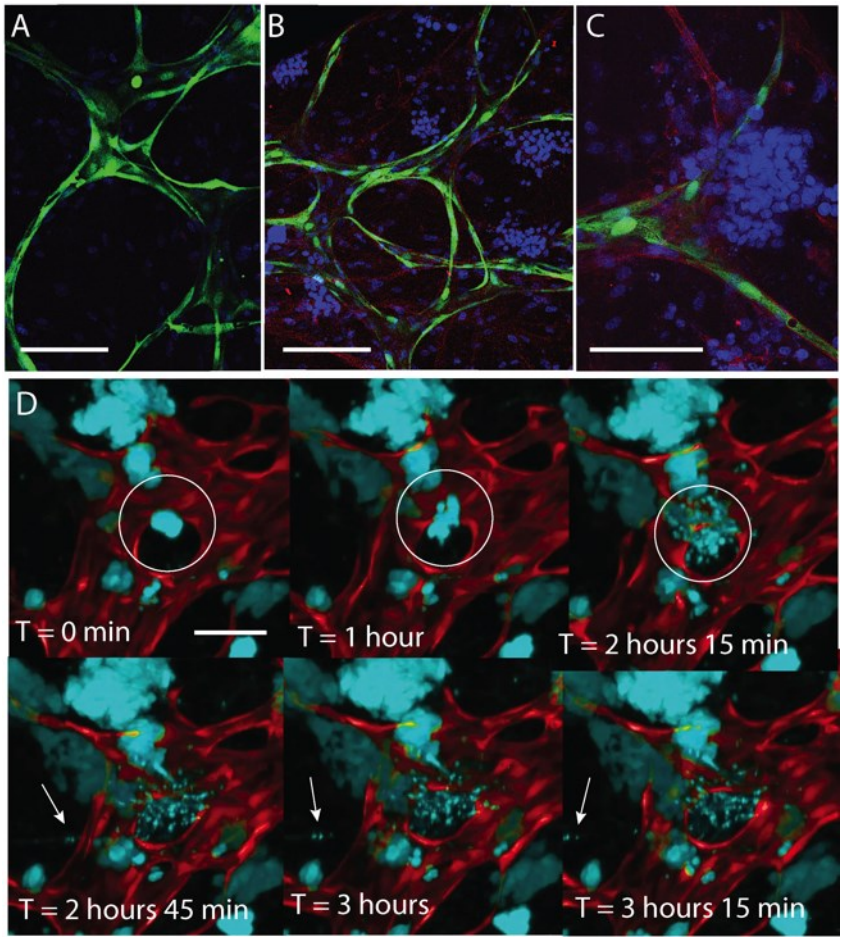
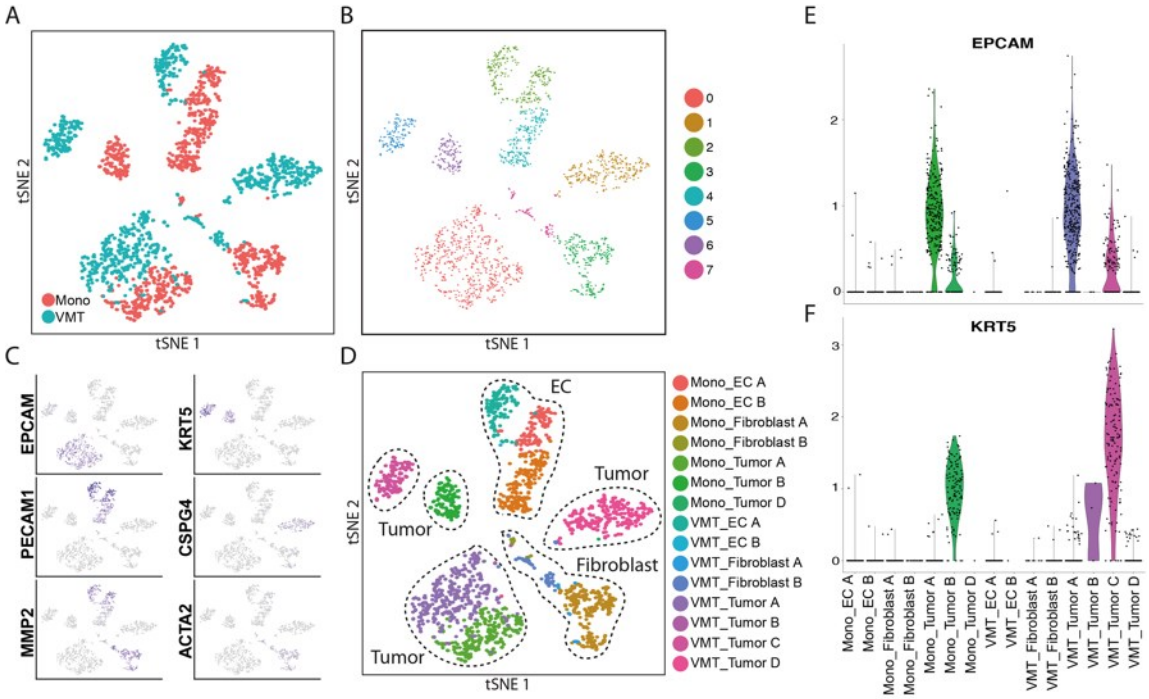
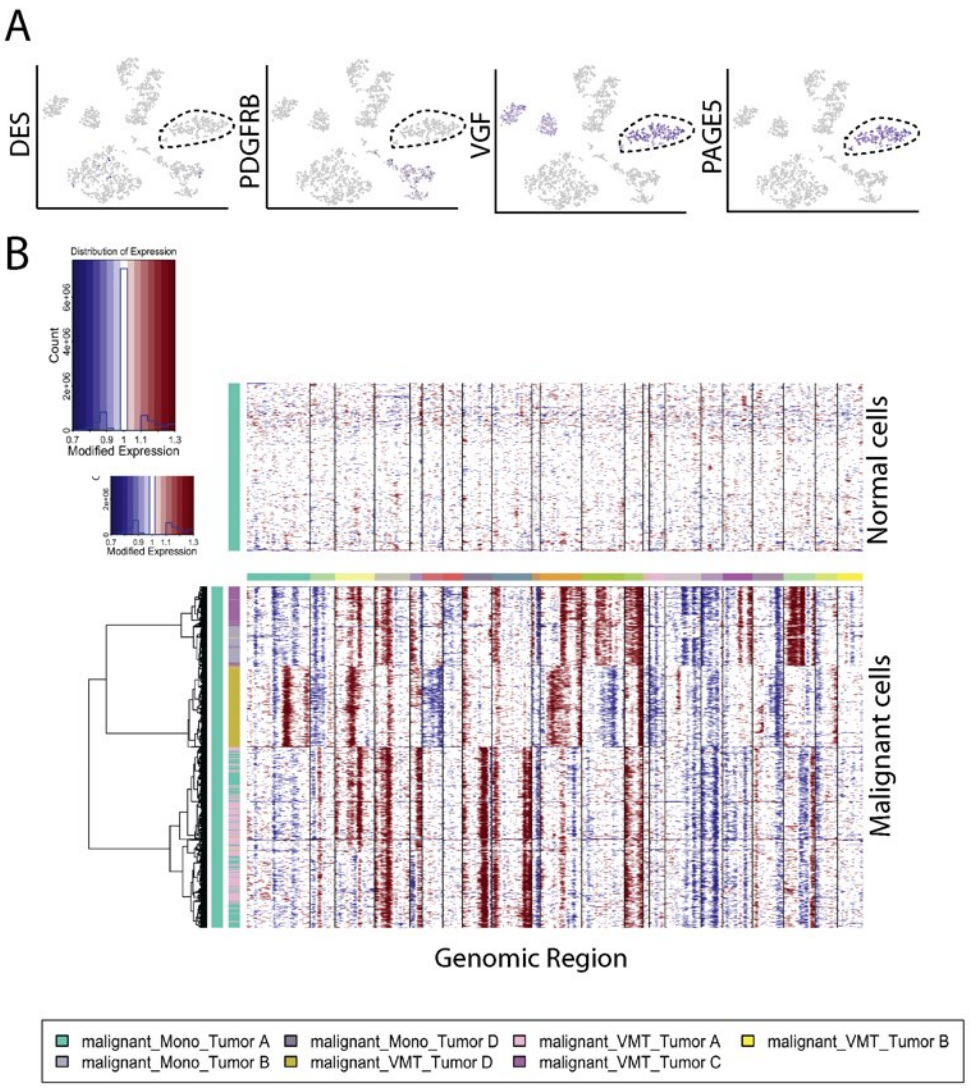
Electronic Supplementary Material (ESI) for Lab on a Chip.



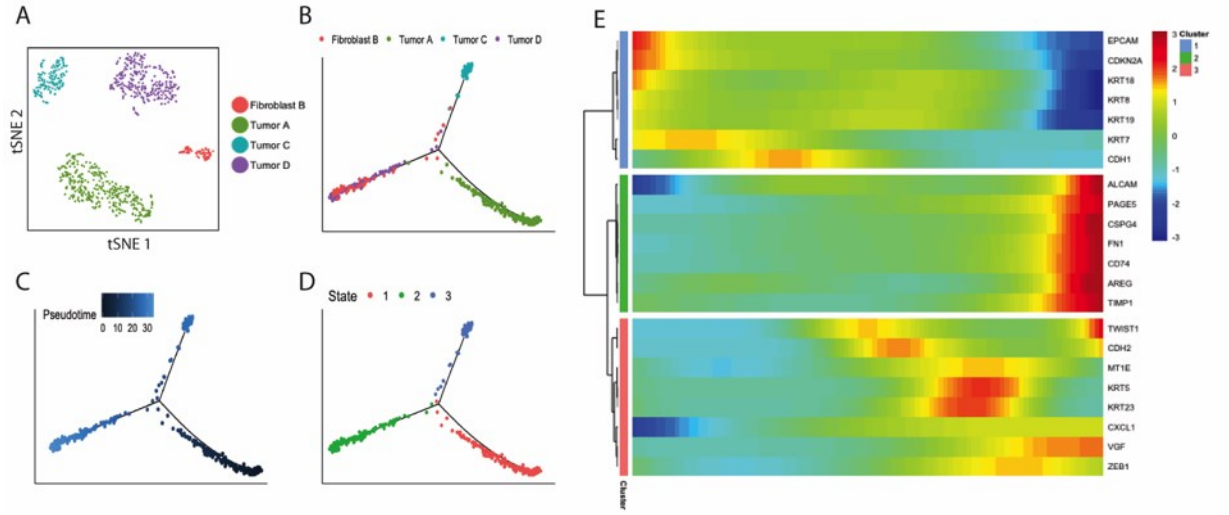
Supplemental Figure 1: Collagen tracks revealed within the VMT. (A) Collagen III staining (red) is not apparent in the VMO. EC are green. (B) Immunostaining for Collagen III (red) showing strong expression in the VMT. Cancer cells are blue. (C) Zoomed view reveals collagen fiber enrichment and 'tracks' in the VMT. (D) Time-lapse confocal imaging showing a likely EMT event within the VMT, characterized by collective and coordinated migration of tumor cells (blue). Vessels are red. Arrow shows movement of an individual CRC cell. Scale bars are 100 μm in A and B, and 50 μm in C and D.



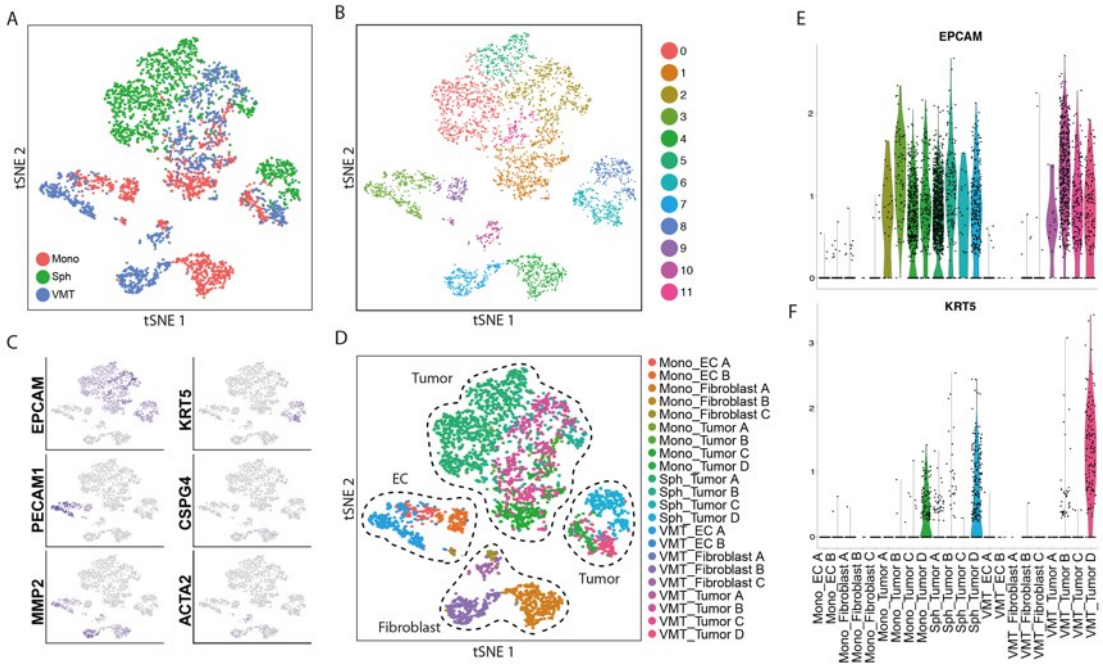
Supplemental Figure 2: Single cell RNA sequencing reveals distinct changes between 2D monocultures and the VMT. (A) tSNE plot reveals marked shifts in gene expression between VMT and monolayer samples for all cell types. (B) Unbiased clustering for HCT116 dataset. (C) Cell clusters are characterized into types by known markers, and differential gene expression is displayed by tSNE plot. (D) tSNE plot shows each cluster annotated by group (Mono vs VMT) and cell type (EC A, EC B, Fibroblast A, Fibroblast B, Tumor A, Tumor B, Tumor C, Tumor D). Note that tumor C and D are absent from the monolayer culture. (E) Violin plot for EPCAM expression. (F) Violin plot for KRT5 expression.



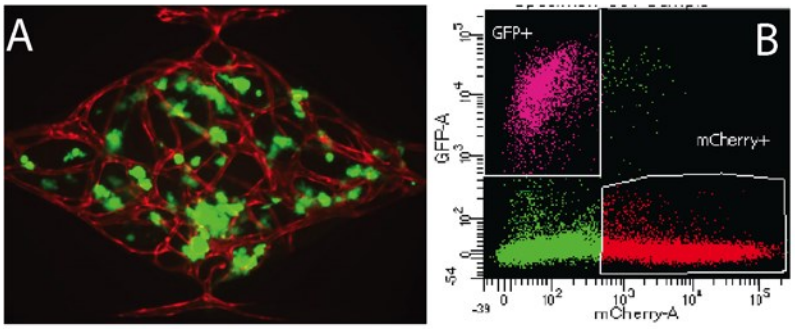
Supplemental Figure 3: Copy number variation analyses for HCT116. (A) tSNE plots showing marker gene expression for HCT116-derived VMT, highlighting the unique gene expression signature of tumor D (outlined). (B) CNV analyses showing that VMT-derived tumor D groups with known tumor-derived clusters A, B and C, whereas normal cells show greater chromosomal stability.



Supplemental Figure 4: Differential tumor states observed in VMT. (A) Clustering for HCT116 and fibroblasts. (B) Monocle trajectory shows that HCT116 tumor cells occupy three distinct states, and that fibroblasts co-segregate with tumor D. (C) Pseudotime plot of HCT116. (D) Cell state plot for HCT116. (E) Pseudotime heatmap for HCT116-derived VMT transition subset showing 3 differentially-expressed clusters across the pseudotime trajectory.



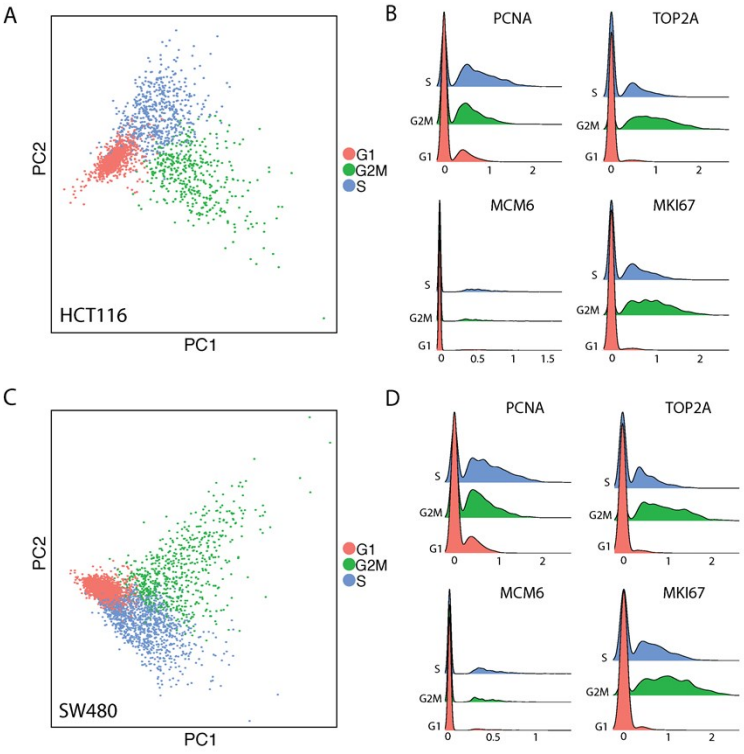
Supplemental Figure 5: Single cell RNA sequencing reveals distinct differences between 2D monoculture, 3D monocultures (spheroids) and the VMT. (A) tSNE plot reveals marked shifts in gene expression between VMT , monolayer and spheroid samples for all cell types. (B) Unbiased clustering for SW480 dataset. (C) Cell clusters are characterized into types by known markers, and differential gene expression is displayed by tSNE plot. (D) tSNE plot shows each cluster annotated by group (Mono vs spheroid vs VMT) and cell type (EC A, EC B, Fibroblast A, Fibroblast B, Fibroblast C, Tumor A, Tumor B, Tumor C, Tumor D). Note that tumor C and D are absent from the monolayer culture (only 1 cell in tumor D). (E) Violin plot for EPCAM expression. (F) Violin plot for KRT5 expression.



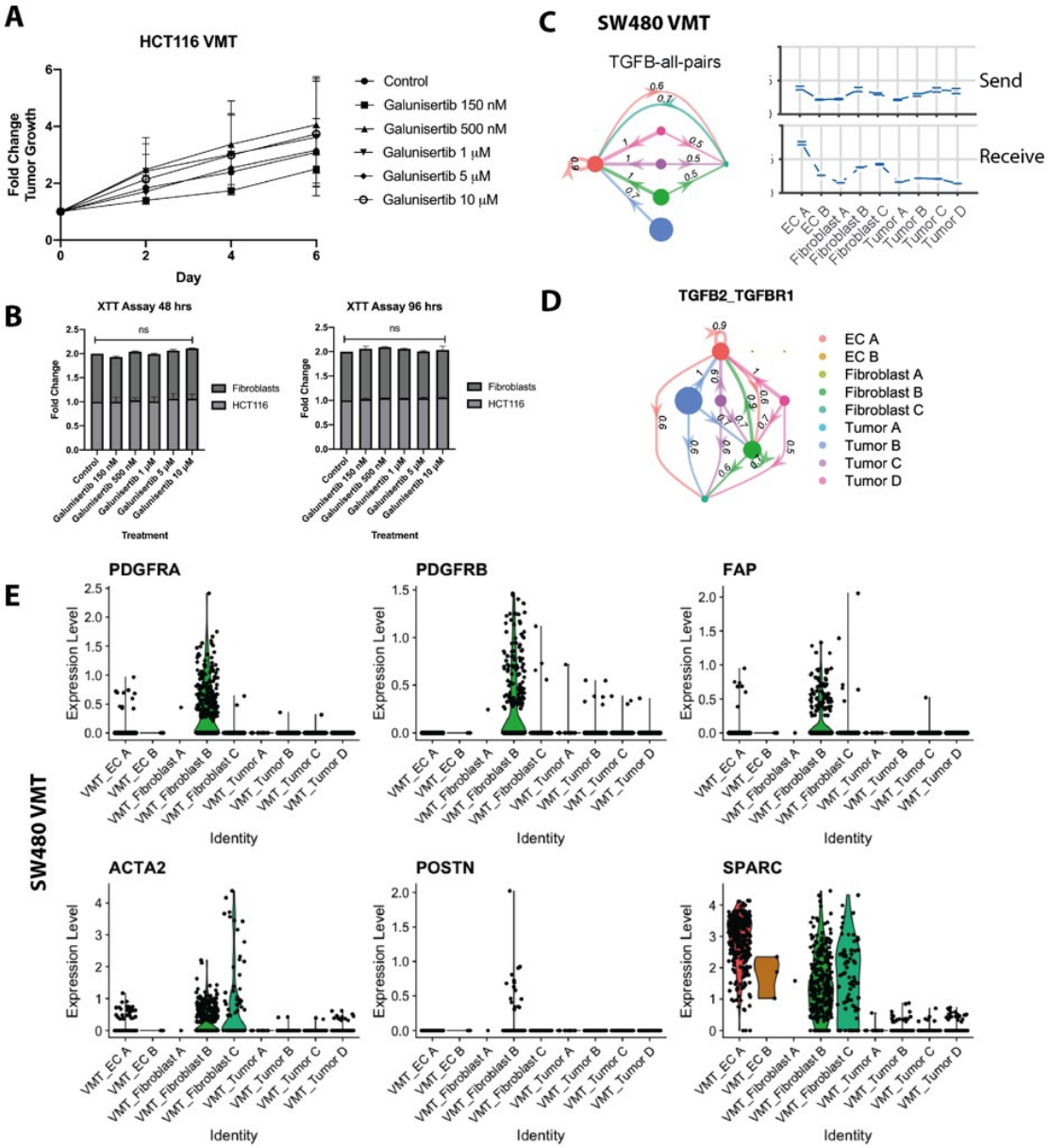
C

Population	#Events	%Parent	%Total
All Events	202,224	####	100.0
Scatter	36,554	18.1	18.1
Single cells	27,019	73.9	13.4
GFP+	3,061	11.3	1.5
mCherry+	10,367	38.4	5.1

Supplemental Figure 6: Cells in the VMT can be isolated via fluorescence activated cell sorting (FACS). (A) Representative image of SW480 VMT. CRC cells shown in green and vessels in red. Note that mCherry labeled CRC cells are pseudocolored green here for consistency with other figures. (B) Gating strategy for FACS. (C) Table showing proportions of isolated cells.



Supplemental Figure 7: Cell cycle gene visualization for HCT116 and SW480. (A) Principal Component (PC) plot of HCT116 cells in G1, G2M, or S phase. (B) Representative cell cycle genes showing marginal upregulation across the HCT116 samples, with highest peak expression centered at 0 (no expression) and normal distribution with mean approximately 0.5 for expressing cell populations. (C) PC plot of SW480 cells in G1, G2M, or S phase. (D) Representative cell cycle genes showing marginal upregulation across the SW480 samples, with highest peak expression centered at 0 (no expression) and normal distribution with mean approximately 0.5 for expressing cell populations.



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Pathway	# of genes	Adjusted p-value	Combined score
TGF-β regulation of extracellular matrix	76	1.05e-34	521.94
IL-1 regulation of extracellular matrix	33	1.56e-24	757.70
Collagen biosynthesis and modifying enzymes	19	1.50e-14	499.06

Pathway Enrichment: SW480 Fibroblast B

Supplemental Figure 8: Cancer-associated fibroblast (CAF) population in SW480-VMT is likely galunisertib target. (A) HCT116 growth in the VMT was not significantly inhibited by galunisertib. (B) XTT assay for fibroblasts and HCT116 CRC cells grown in monolayer shows no significant difference between control and galunisertib treated conditions at 48 hours of treatment and at 96 hours (48 hours post-treatment). (C) Cell-cell interaction plot for SW480-VMT shows EC A, Fibroblast B and Fibroblast C populations are receiving TGF-β signals from all populations. (D) Interaction plot focusing on TGF-β2 and TGFBR1. (E) Violin plots for several CAF-associated genes showing upregulation in the SW480-VMT derived Fibroblast B population. (F) Pathway analysis for Fibroblast B population shows enrichment for collagen biosynthesis and matrix remodeling.