# Supplementary material
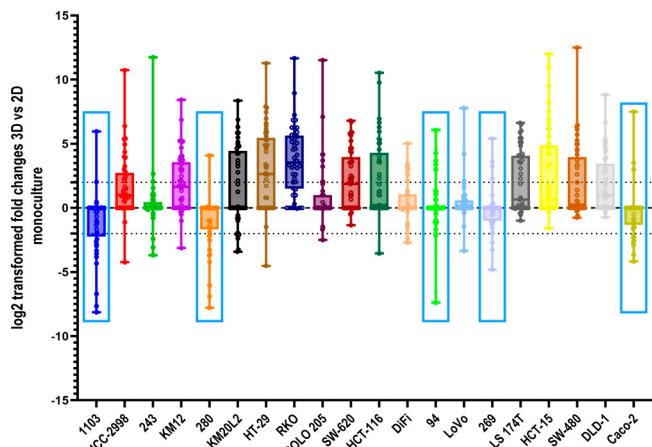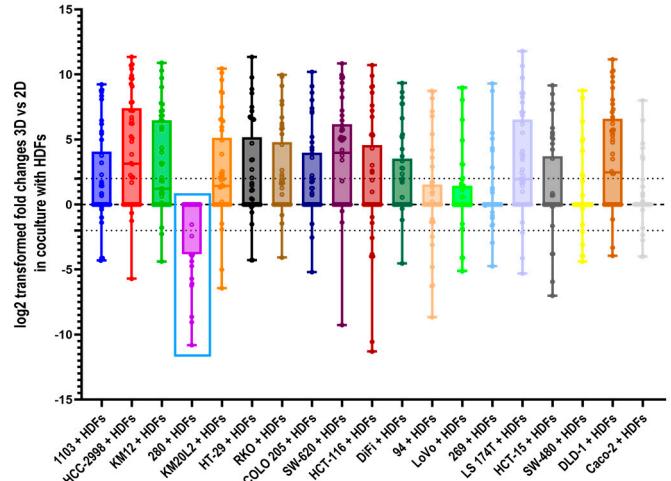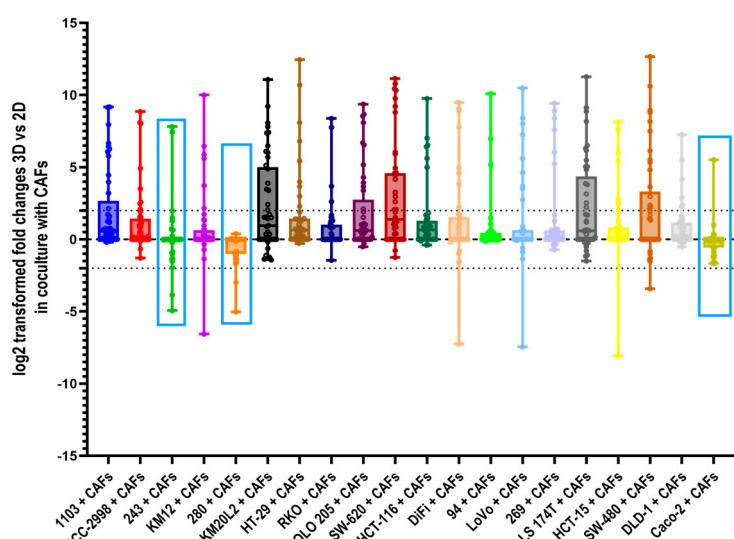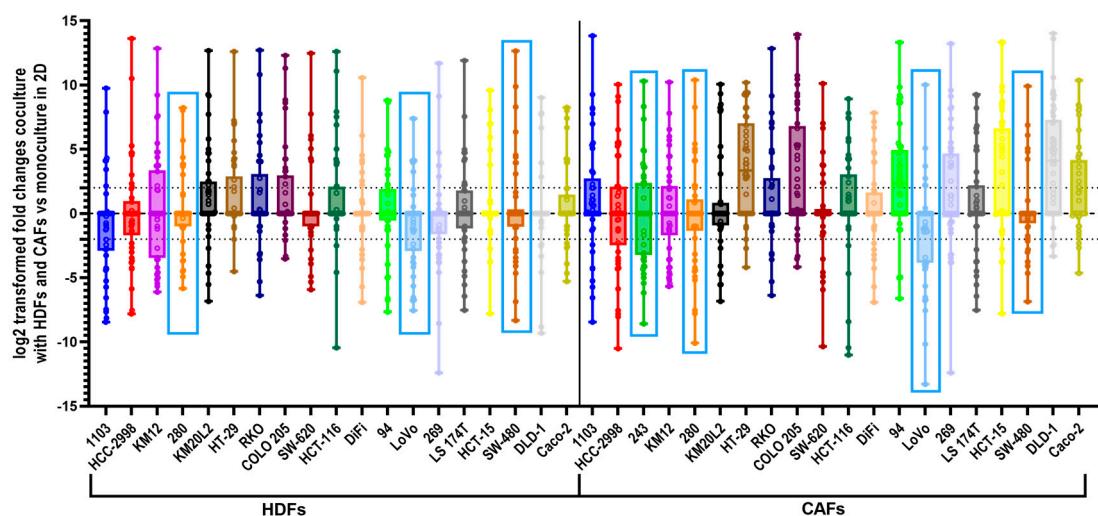
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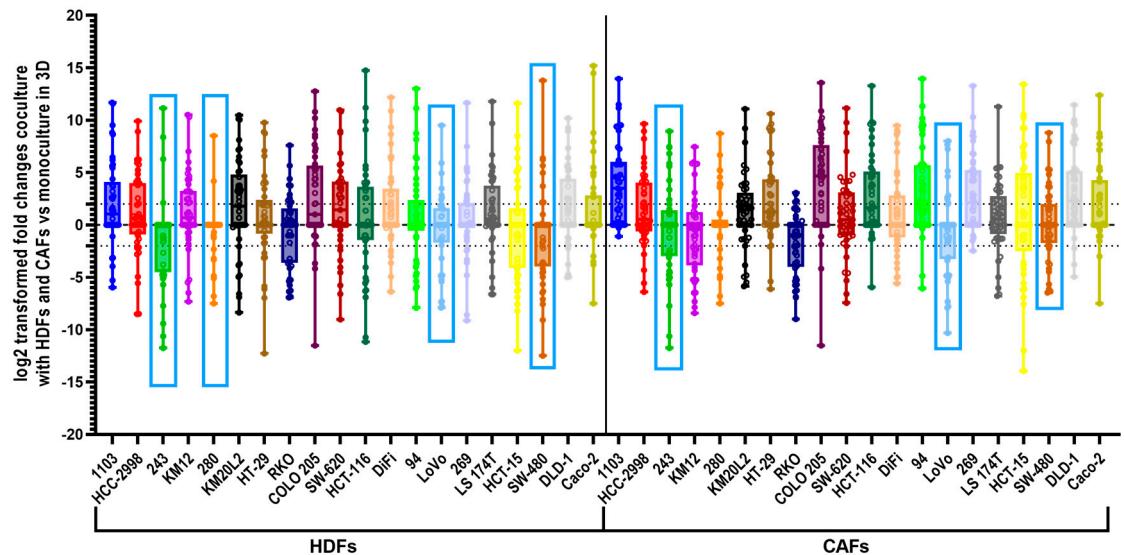
Figure S1. Calculation of fold changes between 3D and 2D settings and between coculture with fibroblasts and monoculture for each condition. A. Log2 transformed fold changes 3D vs 2D monoculture **B.** Log2 transformed fold changes 3D vs 2D coculture with HDFs. **C.** Log2 transformed fold changes 3D vs 2D coculture with CAFs. **D.** Log2 transformed fold changes coculture with HDFs and CAFs vs monoculture in 2D. E. Log2 transformed fold changes coculture with HDFs and CAFs vs monoculture in 3D.

A

B

C

D

E
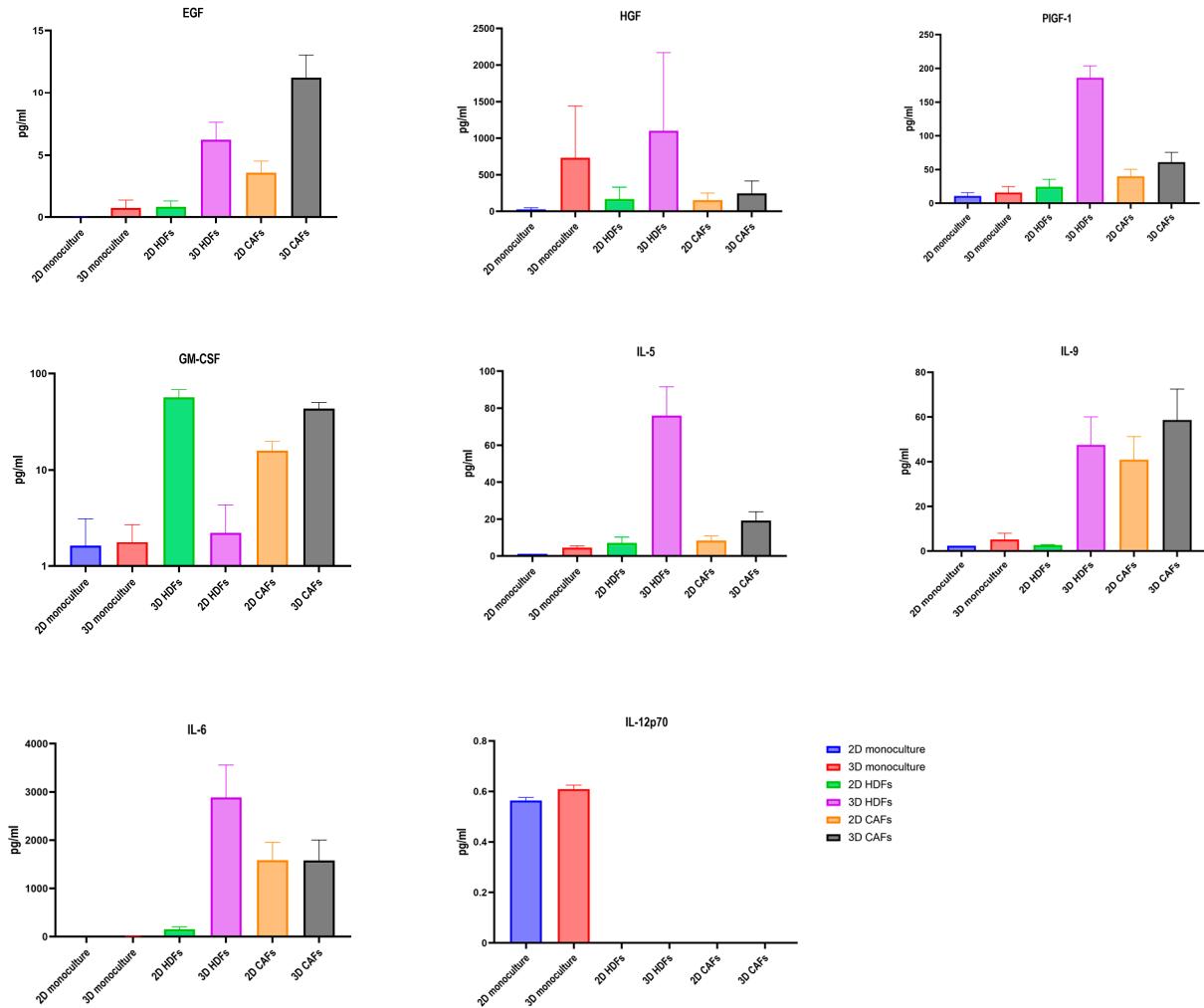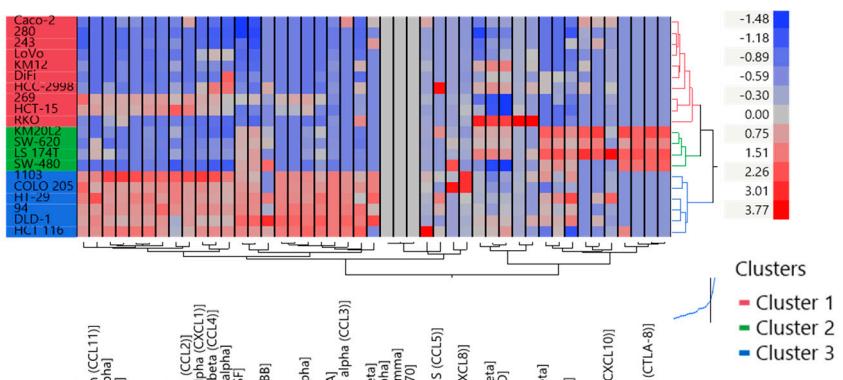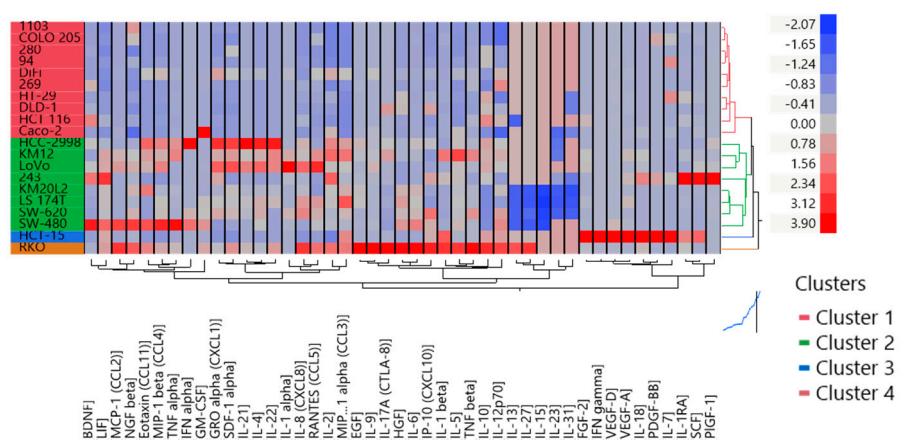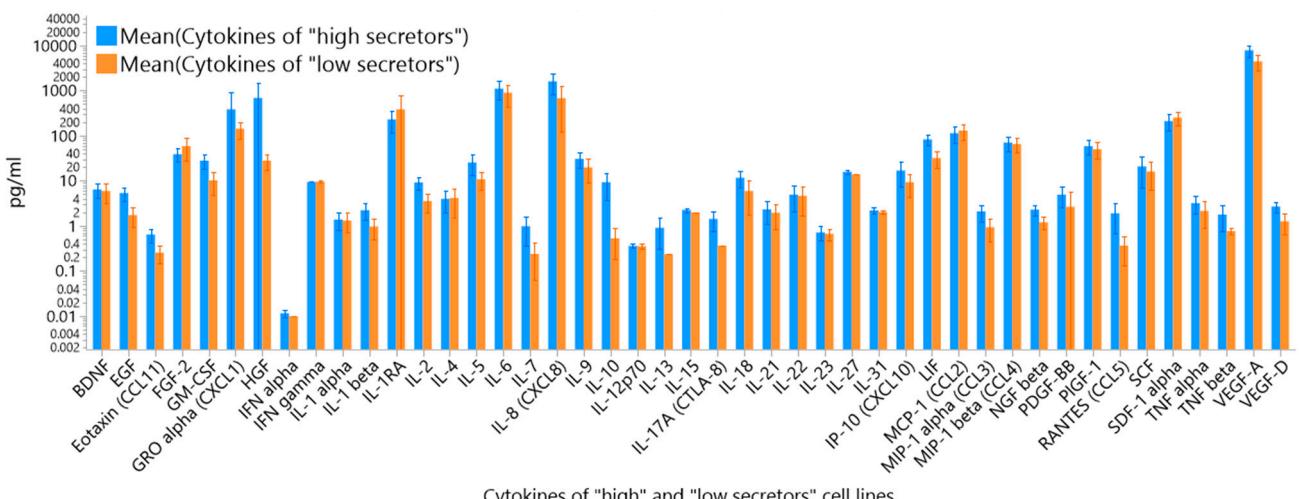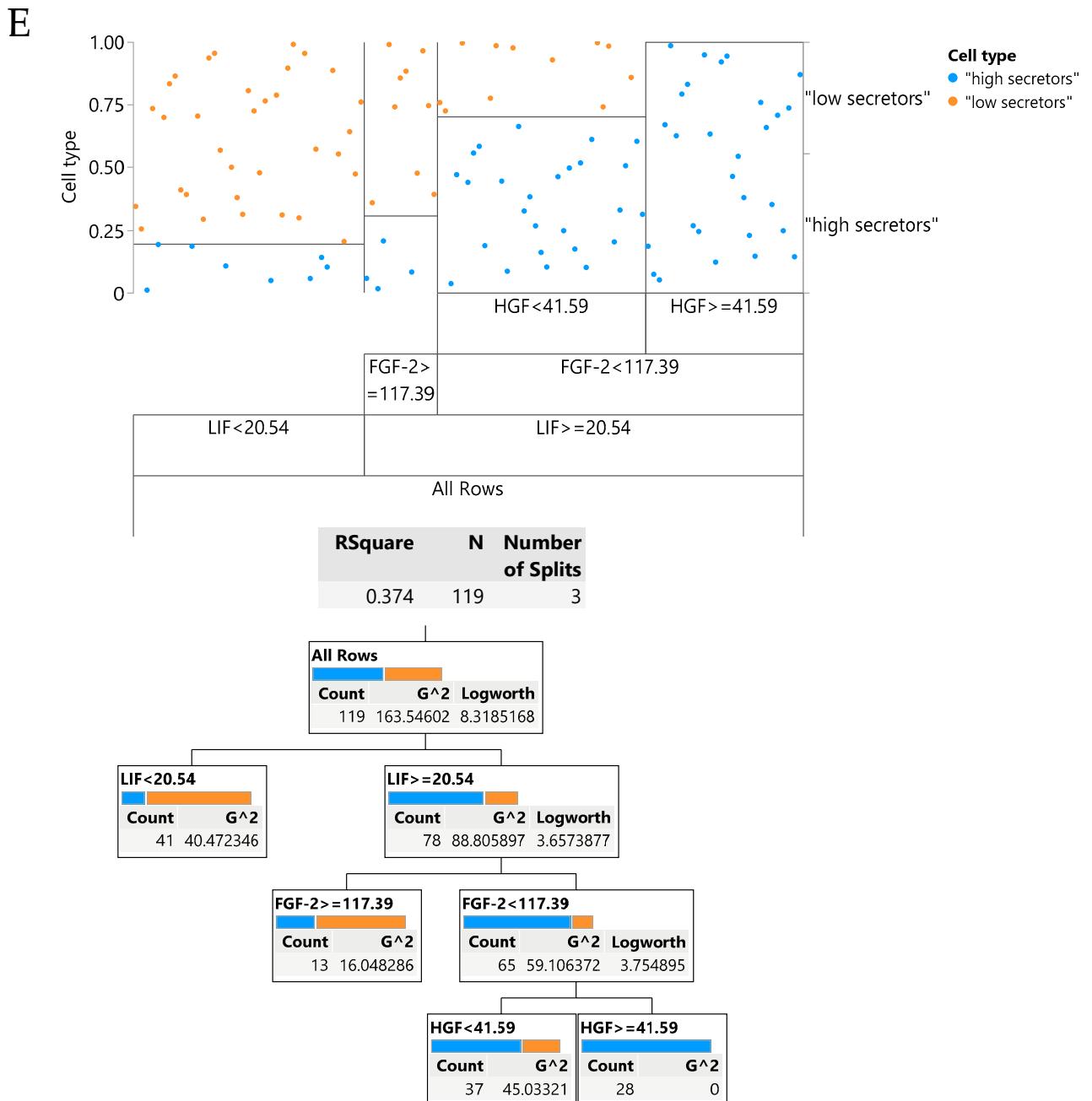
Figure S2. Cytokine profiles of 20 CRC cell lines in 3D spheroid cultures and in coculture with CAFs. A. GM-CSF, EGF, HGF, IL-5, IL-9, IL-6, IL-12p70, PIGF-1 pg/ml values are displayed in the bar graphs. **B.** Heat map of the cytokines levels (pg/ml) in 3D spheroids coculture with CAFs. **C.** Heat map of the fold changes coculture with CAFs vs 3D monoculture. Before clustering, B heatmaps values were standardized with values from Fig.2 A and B, while C heatmap values were standardized separately with values of Fig. 2 C. Hierarchical clustering (Method = Ward) is shown. n=2. **D.** Levels data (pg/ml) of cytokines in 2D and 3D in monoculture and coculture with HDFs and CAFs of "high and low secretor" CRC cell lines. Error bars represent Confidence intervals, Kruskal-Wallis test (Rank Sums) was carried out. P-values illustrating statistically significant difference can be found in Statistical analysis Fig. S2.pdf. Secreted factors are listed by goodness of fit. **E.** Partition plots and decision trees show the cytokines that explain at the most the differences between the "high and low secretor" cell lines.

A
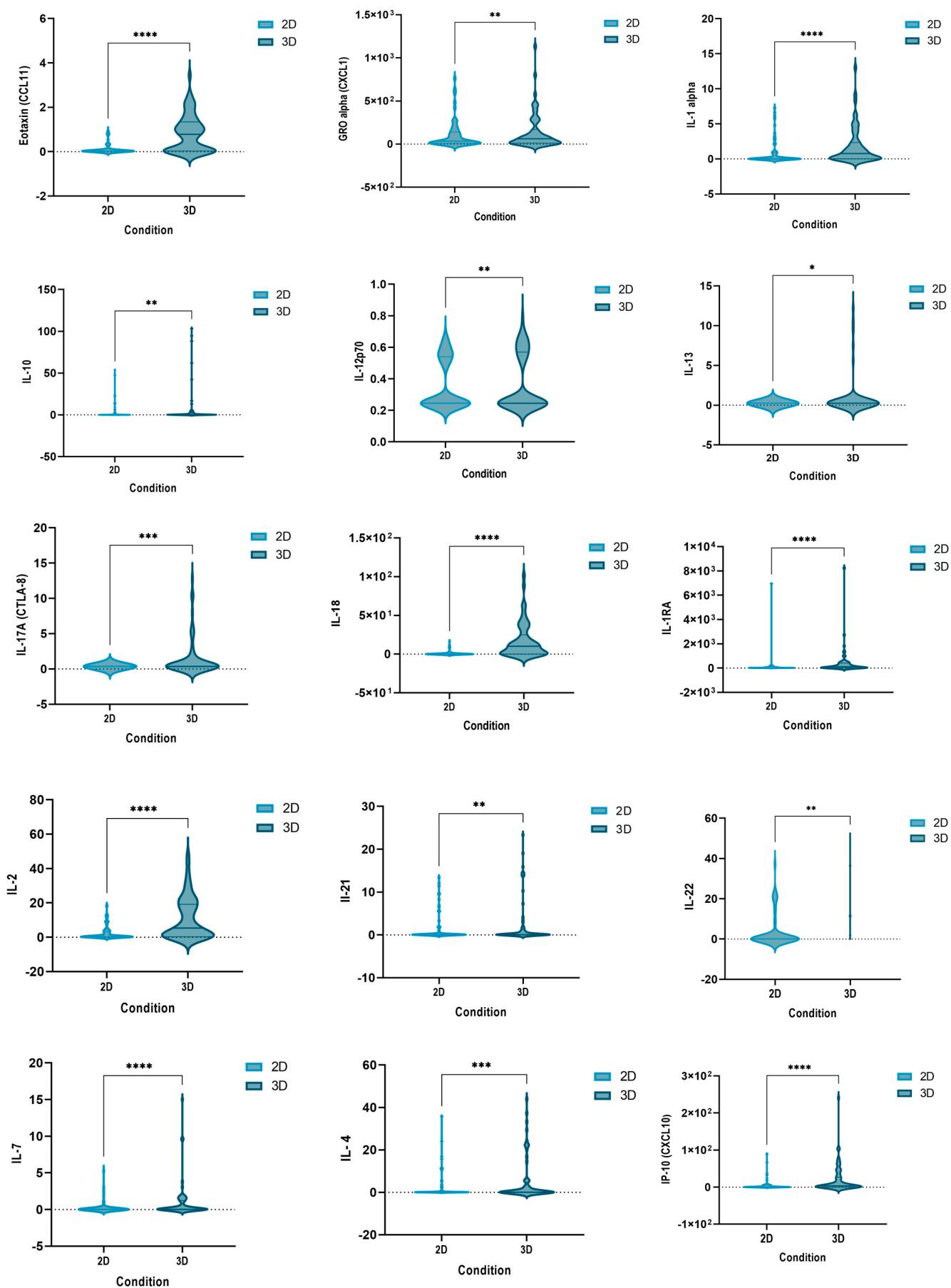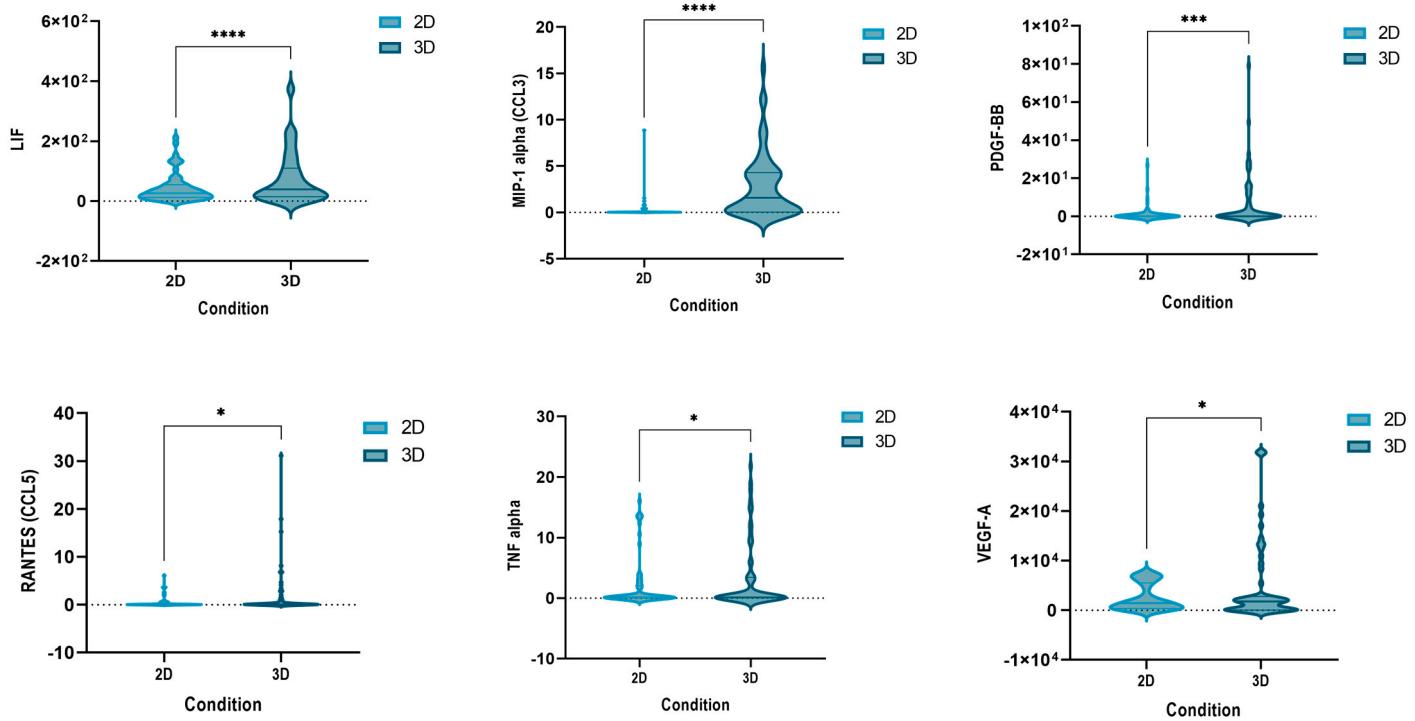
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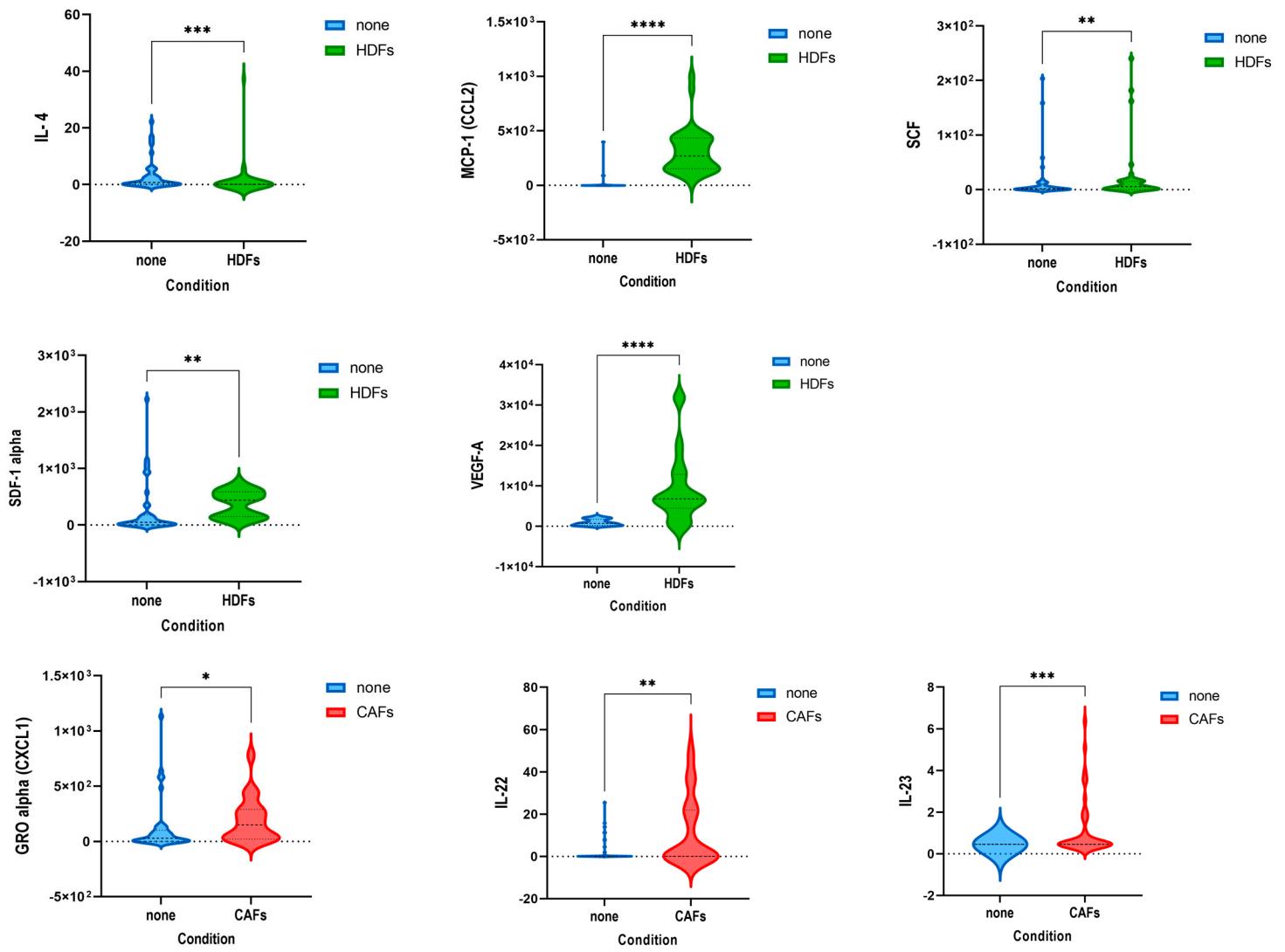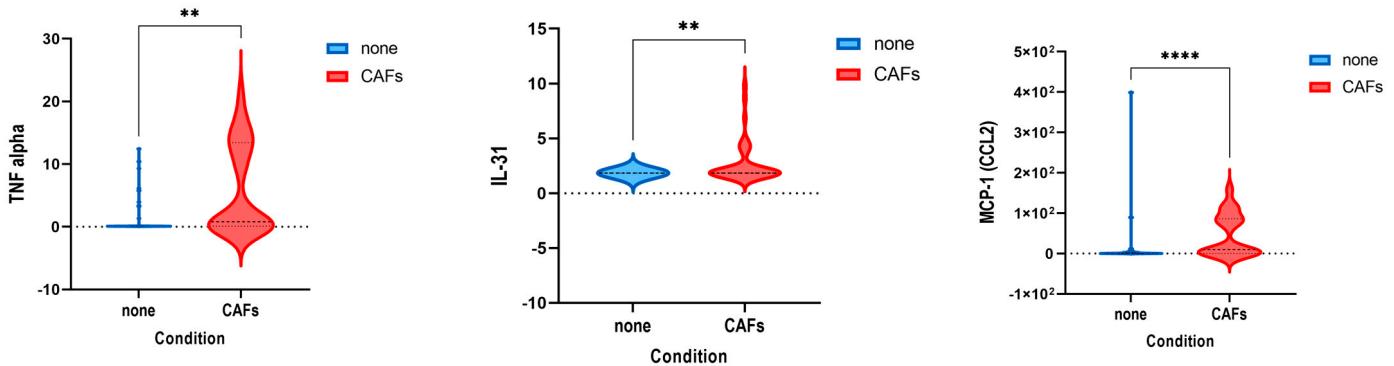
Figure S3. Cytokines levels in different settings and cell lines types. A. Violin plots depicting cytokines statistically significant upregulated in 3D when compared to 2D setting. **B.** Violin plots depicting cytokines statistically significant upregulated in coculture with HDFs and/or CAFs when compared to monoculture setting. P-values *< 0.05, **< 0.01, ***< 0.001, **** < 0.0001 of Wilcoxon matched-pairs signed rank test are illustrated.

C

P value = 0.7370

P value = 0.3285

D

P value = 0.3079

P value = 0.2947

E

P value = 0.0388*

P value = 0.7649

F

P value = 0.2424

P value = 0.6636

G
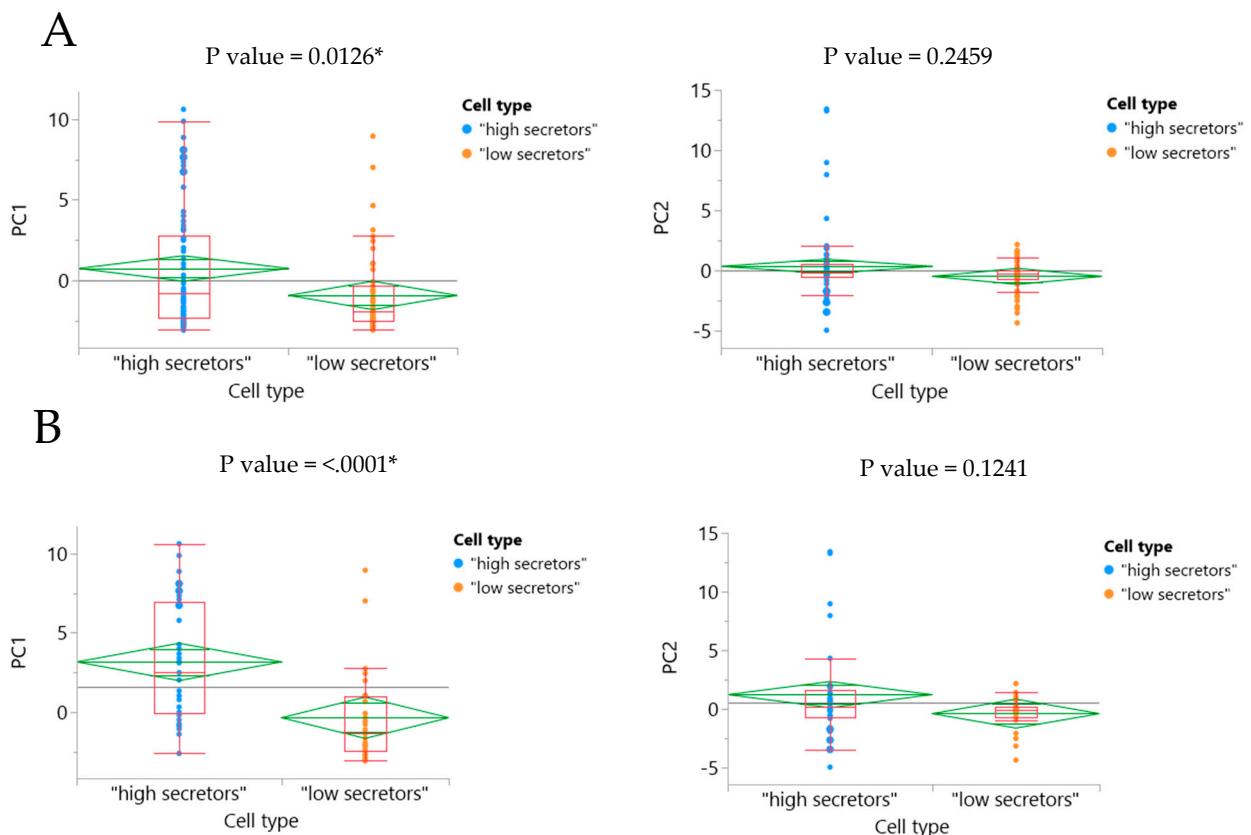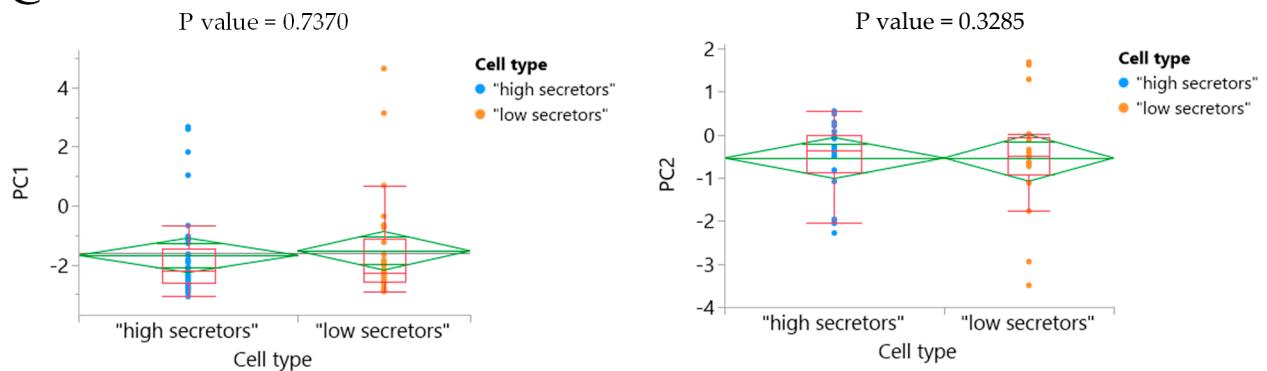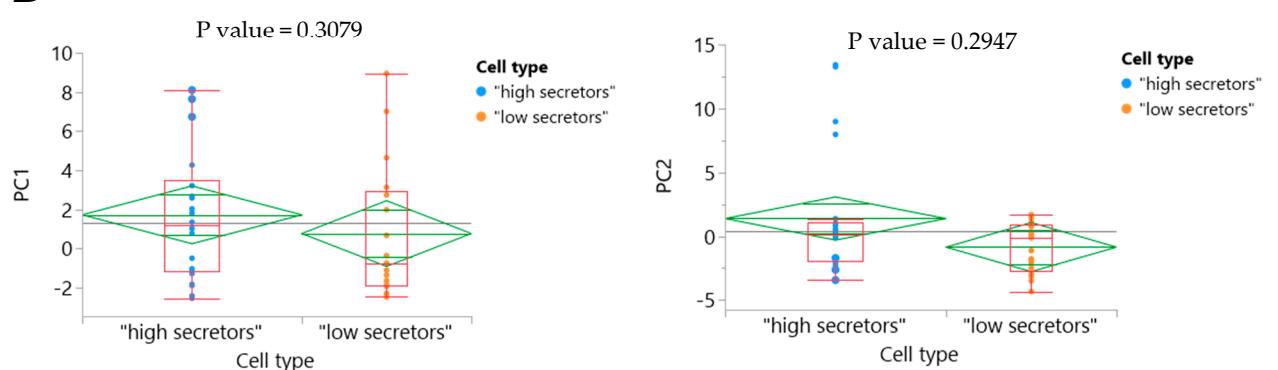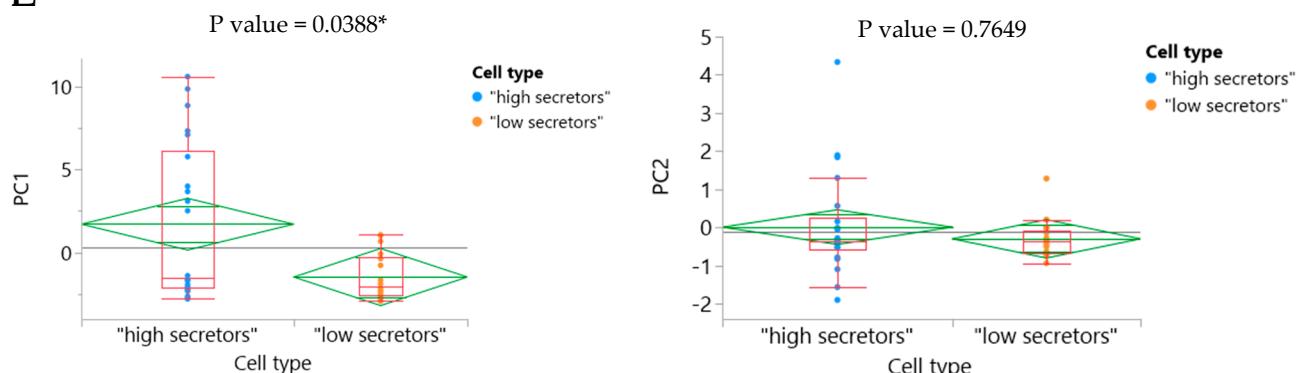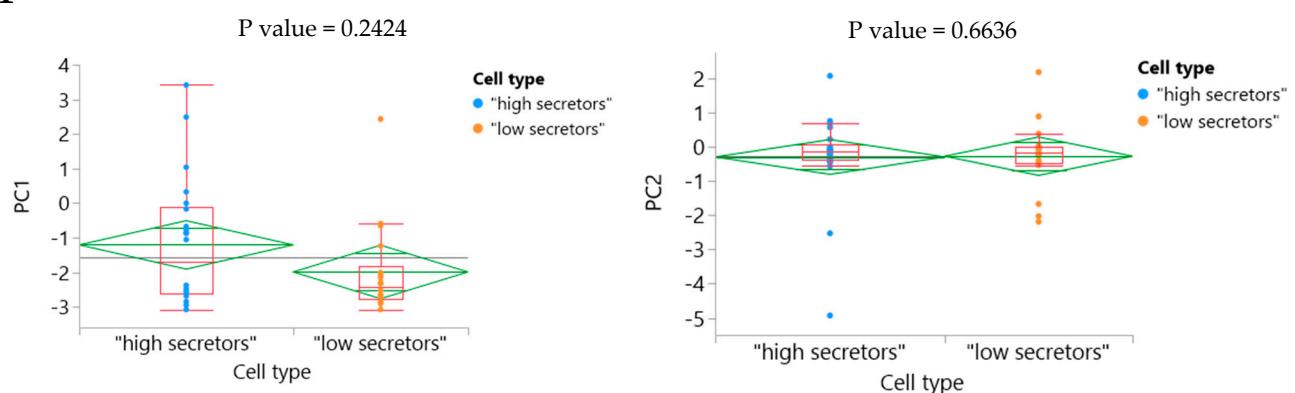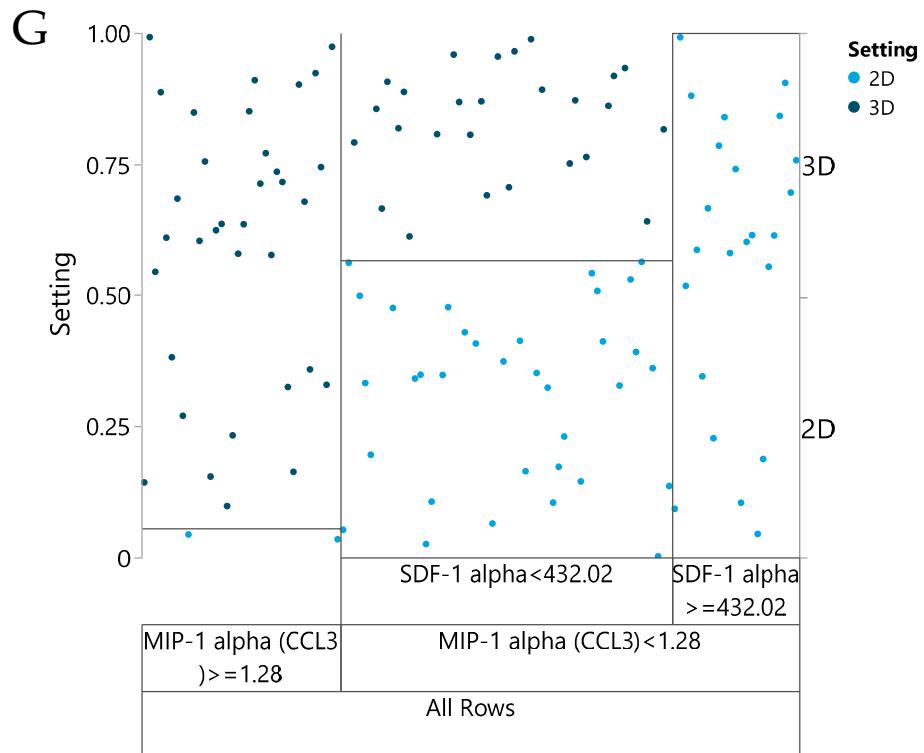
RSquare	N	Number of Splits
0.402	119	2
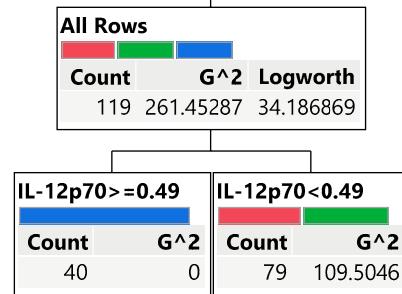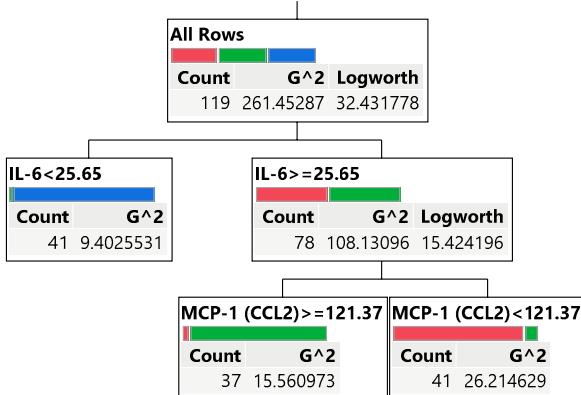
Figure S4. The cytokine profile of the "high secretor" lines is positively influenced by the 3D culture setting and the presence of fibroblasts. **A.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between the "high and low secretor" cell lines in 2D and 3D settings. **B.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between "high and low secretor"cell lines in 3D setting. **C.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between "high and low secretor" cell lines in 2D setting. **D.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between "high and low secretor" cell lines in coculture with CAFs. **E.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between "high and low secretor" cell lines in coculture with HDFs. **F.** Wilcoxon Two-Sample Test analysis of Principal component 1 (PC1) and 2 (PC2) between "high and low secretor" cell lines in monoculture. **G, H, J.** Partition plots and decision trees show the cytokines that explain at most the differences between the culture settings (G: 2D vs 3D; H, J: coculture with HDFs and CAFs vs monoculture).
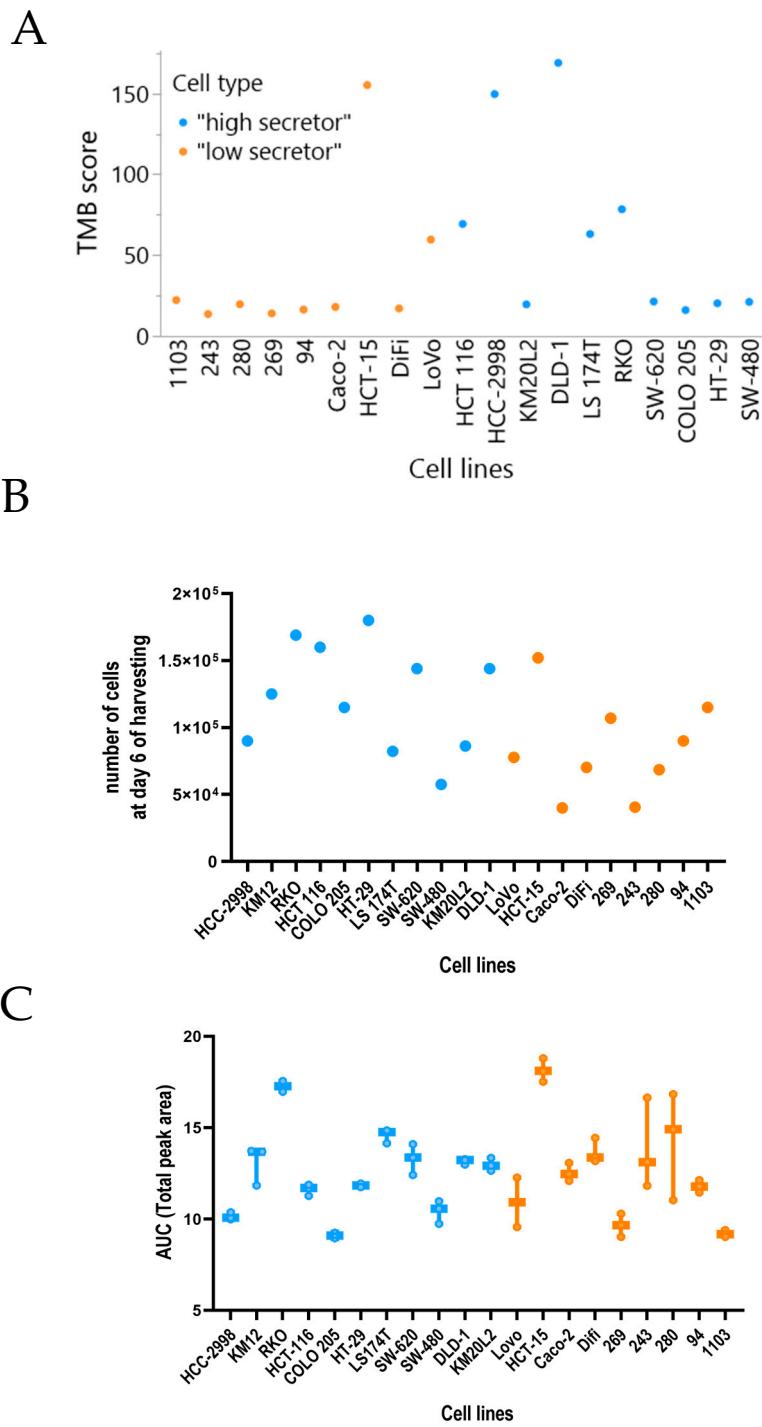
Figure S5: TMB scores and proliferation rate of the CRC cell lines. **A.** TMB scores of the CRC lines. **B.** Proliferation rate of the cancer cells in the 2D culture setting. The number of cells at day 6, i.e. day of harvesting of the supernatant for the cytokine analysis, are shown for the 20 CRC cell lines. **C.** Proliferation rate of the cancer cells in the 3D spheroids culture setting. AUC of the largest brightfield object areas (μm²) of the spheroids in monoculture for all 20 CRC cell lines are shown.

Table S1: Metadata about CRC cell lines: name, 2D and 3D assay doubling times, type of cell line based on cluster analysis, patient histology, gender, patient age at surgery, pathogenic status, site of origin.

Cell lines	2D doubling time [h]	3D assay doubling time[h]	high/low secretor	Patient histology	Gender	Patient Age at Surgery	Pathogenic status	Site of origin
COLO 205	25	9	"high secretor"	adeno carcinoma	male	70	Primary	Colon
DLD-1	20	13	"high secretor"	adeno carcinoma	male	20	Primary	Colon
HCC-2998	32	10	"high secretor"	adeno carcinoma	n.k.	n.k.	Primary	Colon
HCT 116	18	12	"high secretor"	adeno carcinoma	male	n.k.	Primary	Colon
HT-29	16	12	"high secretor"	adeno carcinoma	female	44	Primary	Colon
KM12	23	13	"high secretor"	adeno carcinoma	n.k.	n.k.	Metastasis	Lymph Node
KM20L2	33	13	"high secretor"	adeno carcinoma	n.k.	n.k.	Metastasis	Lung
LS 174T	35	15	"high secretor"	adeno carcinoma	female	58	Primary	Colon
RKO	17	17	"high secretor"	squamous carcinoma	n.k.	n.k.	Primary	Colon
SW-480	50	10	"high secretor"	adeno carcinoma	male	51	Metastasis	Colon
SW-620	20	13	"high secretor"	adeno carcinoma	male	50	Primary	Lymph Node
94	32	12	"low secretor"	adeno carcinoma	male	70	Primary	Colon
243	71	14	"low secretor"	adeno carcinoma	male	45	Metastasis	Liver
269	27	10	"low secretor"	adeno carcinoma	male	56	Primary	Colon
280	42	14	"low secretor"	adeno carcinoma	female	55	Metastasis	Skin
1103	25	9	"low secretor"	adeno carcinoma	male	55	Metastasis	Skin
Caco-2	72	13	"low secretor"	adeno carcinoma	male	72	Primary	Colon
DiFi	41	14	"low secretor"	adeno carcinoma	female	46	Metastasis	Ascites
HCT-15	19	18	"low secretor"	adeno carcinoma	male	n.k.	Primary	Colon
LoVo	37	11	"low secretor"	adeno carcinoma	male	56	Metastasis	metastatic nodule