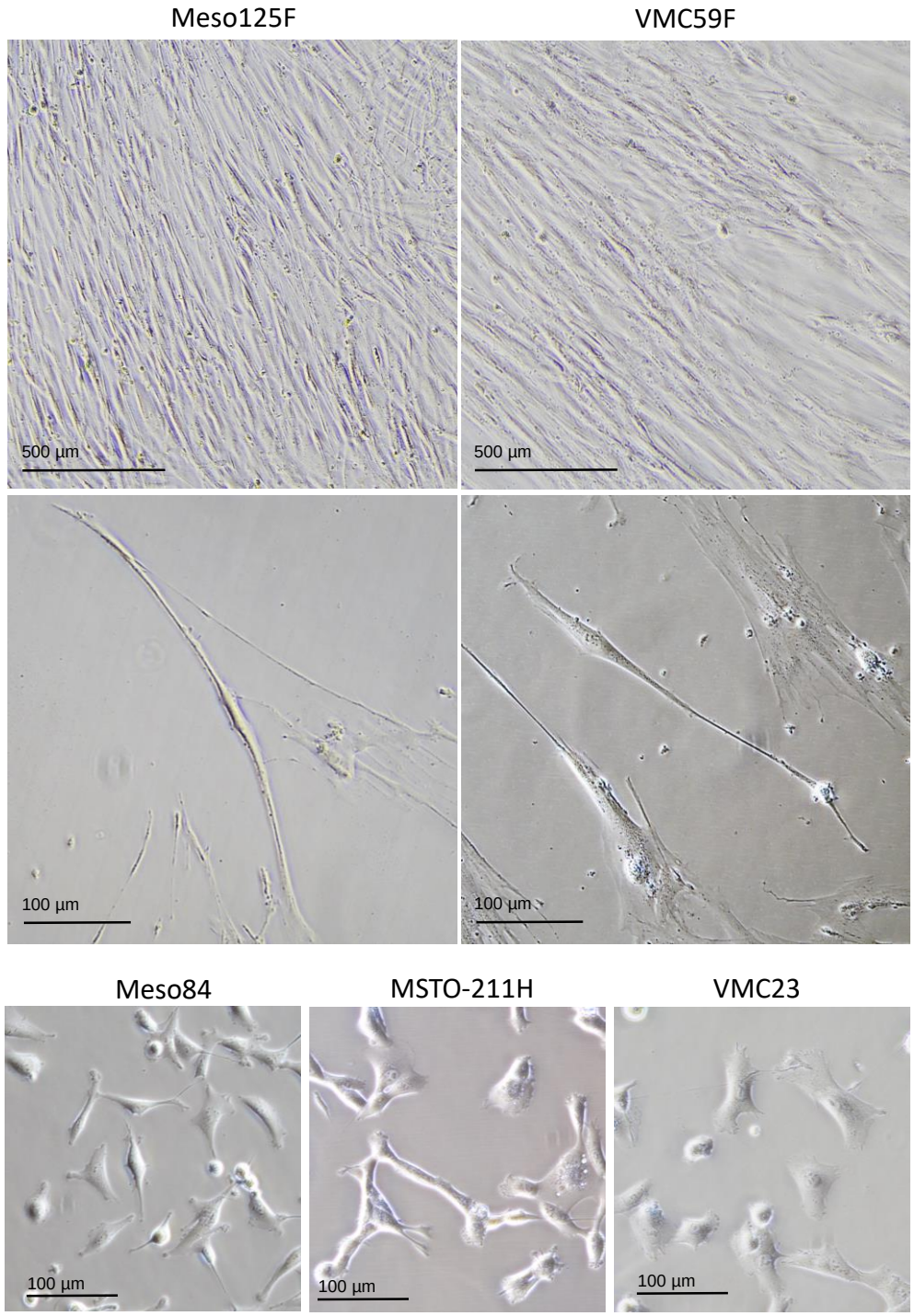
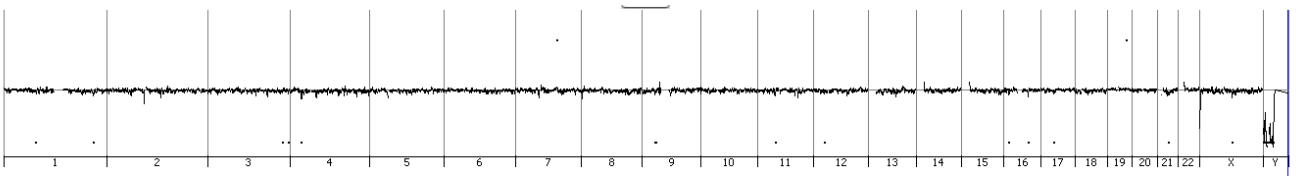
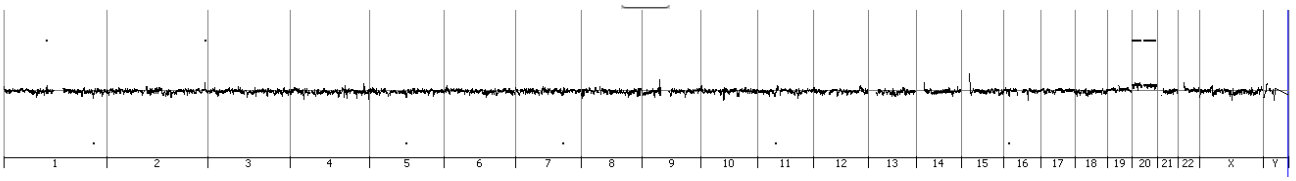


Supplementary Figure S1. Cell morphology of Meso-CAFs and PM cells. Micrographs of cultured Meso125F and VMC59F were taken at high (upper panels) and low (lower panels) cell density. Micrographs of Meso84 derived from sarcomatoid PM, MSTO-211H derived from biphasic PM and VMC23 derived from epithelioid PM were taken at low density.

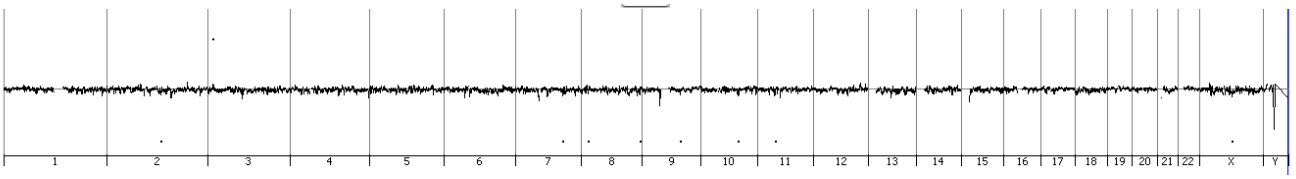
Meso125F



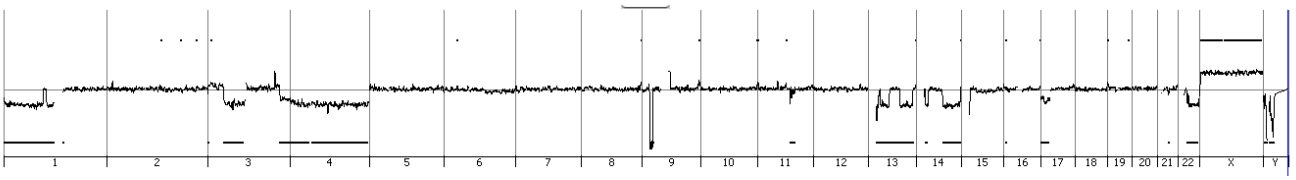
VMC59F



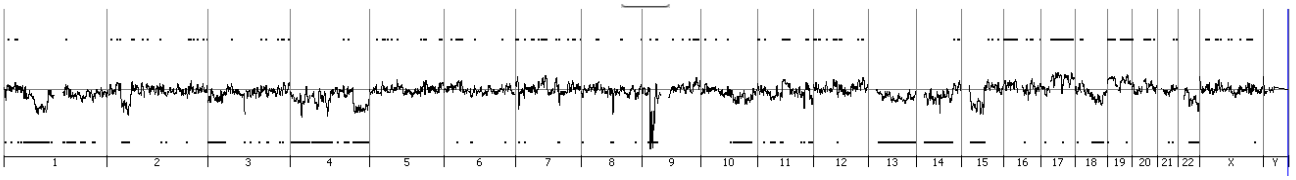
CAF-3



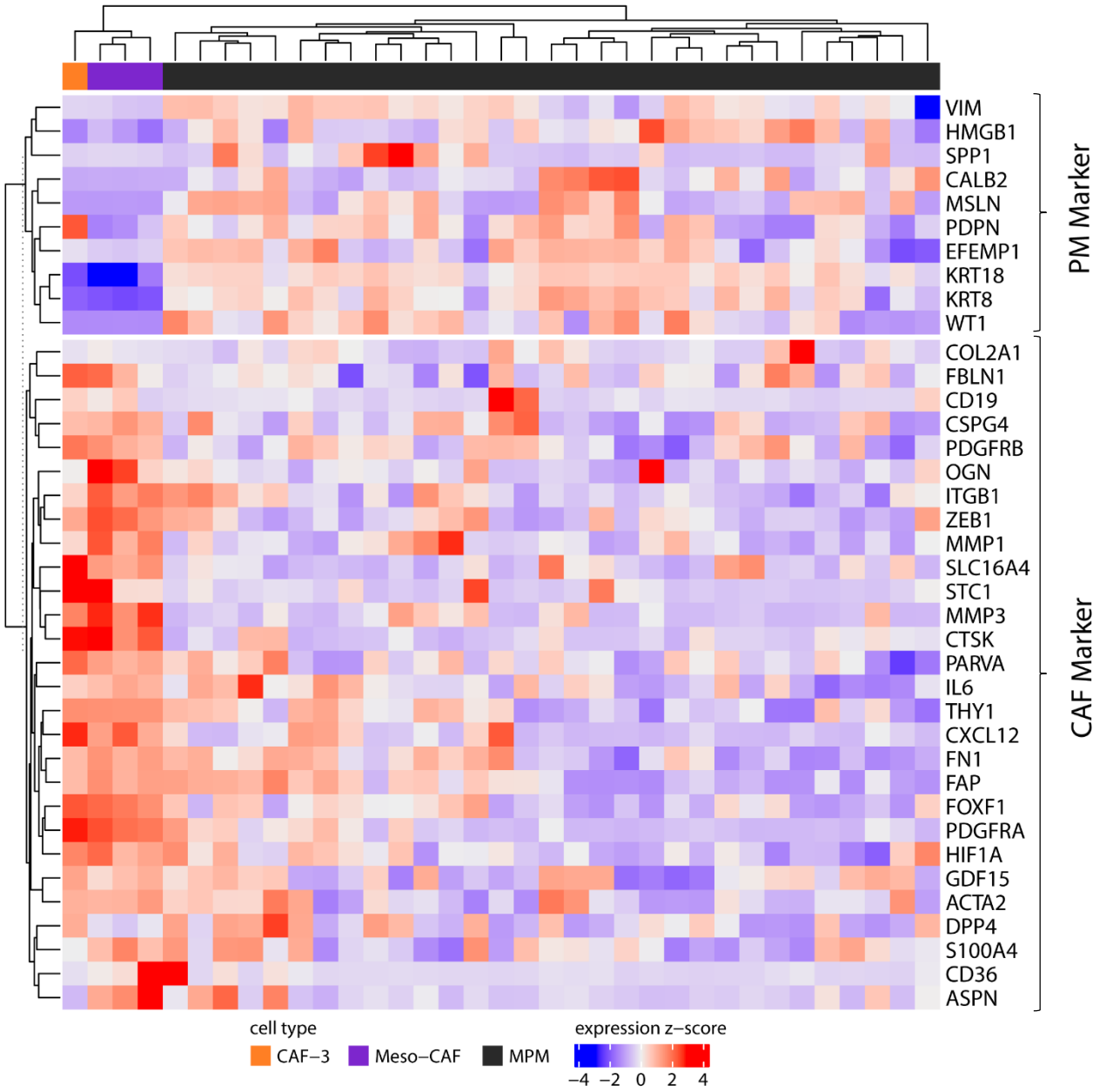
Meso84



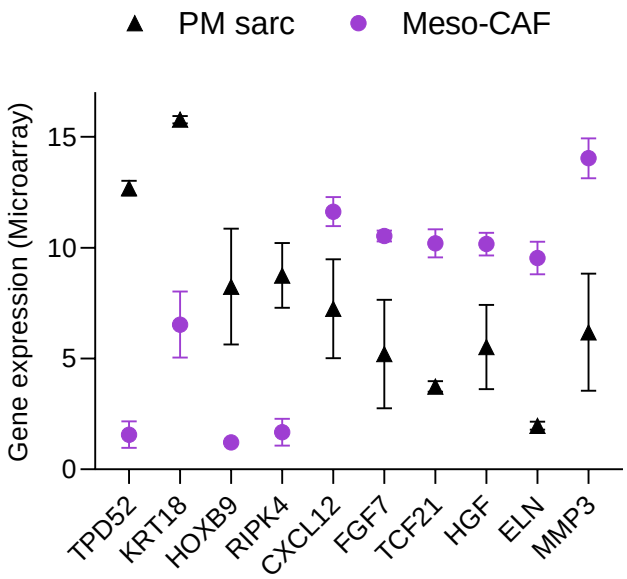
VMC23



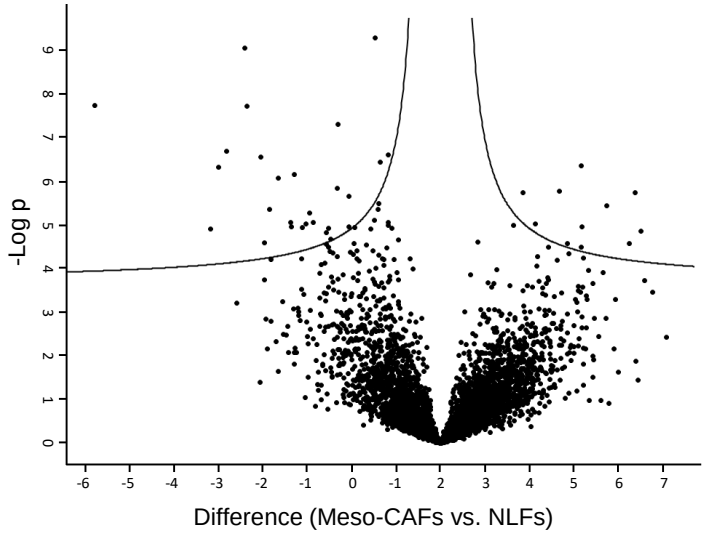
Supplementary Figure S2. Genomic profiles of CAFs and PM cells. Comparative genomic hybridization array (array CGH) profiles were established from Meso-CAFs (Meso125F, VMC59F), colon CAFs (CAF-3) and PM cell lines (Meso84, VMC23).



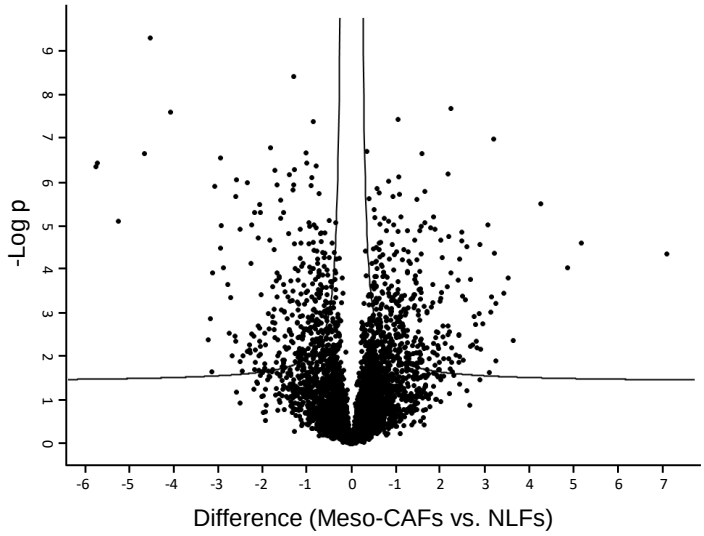
Supplementary Figure S3. Unsupervised clustering of CAFs and PM cells. Transcriptomic data of PM cell lines (n=31), Meso-CAFs (Meso109F, Meso125F, VMC59F) and colon CAFs (CAF-3) were used for unsupervised clustering using a list of PM and CAF marker genes.



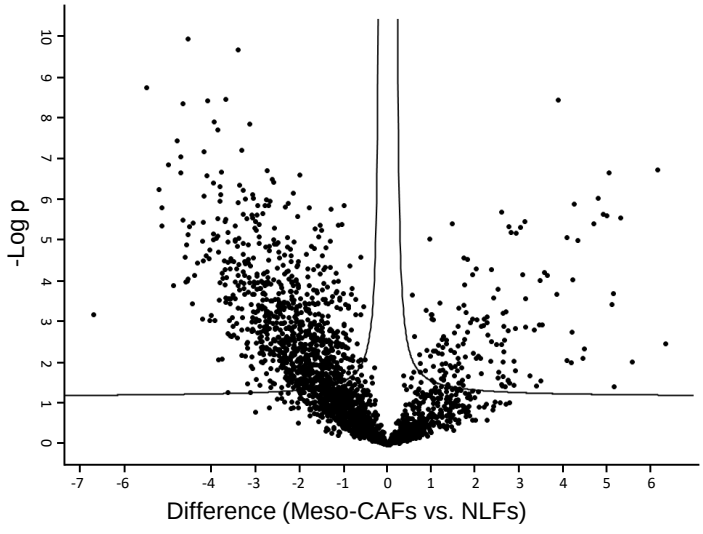
Supplementary Figure S4. Differentially expressed genes in Meso-CAFs versus sarcomatoid PM. Expression levels (log_2 transformed hybridization signals) of genes selected from whole genome gene expression microarrays are shown as means and SEM of 3 Meso-CAFs and 3 PM cell lines from sarcomatoid PM.



Nuclear fraction



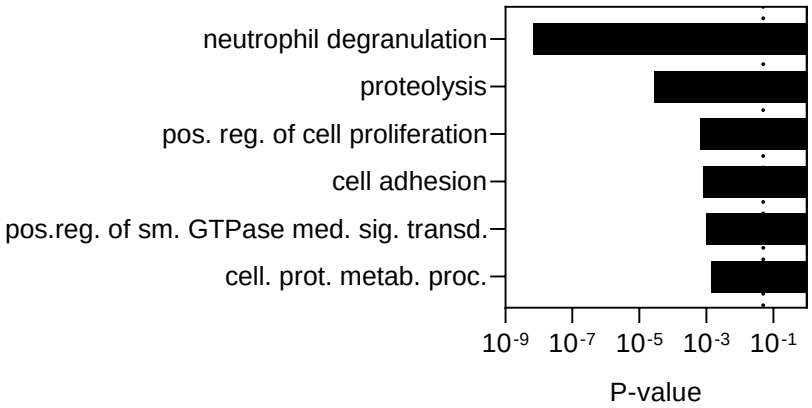
Cytoplasmic fraction



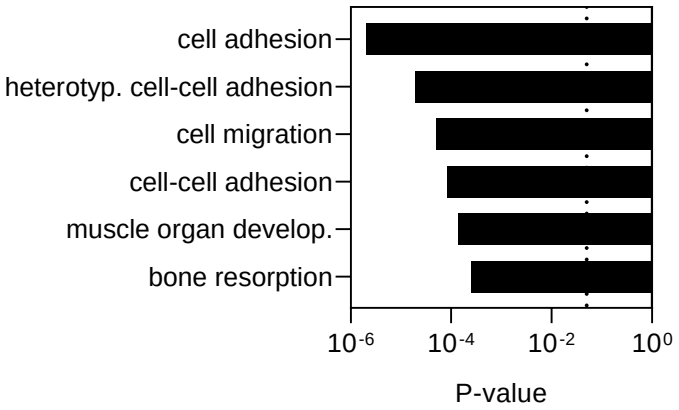
Supernatant fraction

Supplementary Figure S5. Volcano plots of differentially expressed proteins in Meso-CAFs versus normal lung fibroblasts (NLFs).

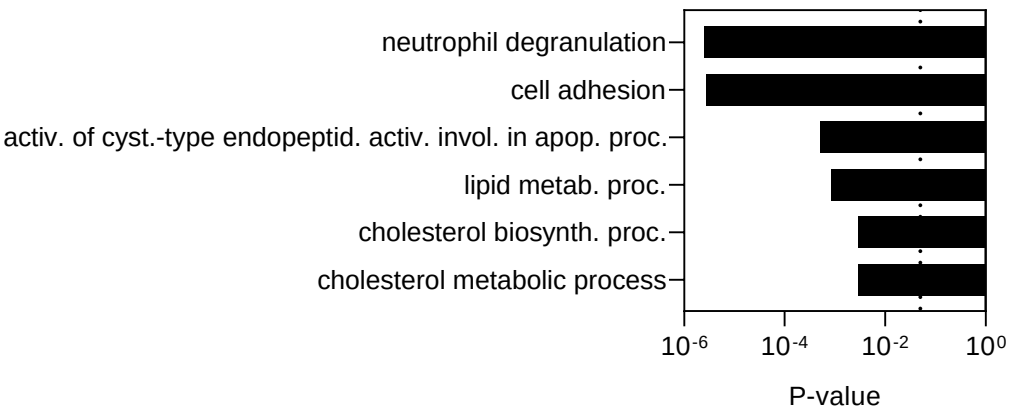
A


neutrophil degranulation
proteolysis
pos. reg. of cell proliferation
cell adhesion
pos.reg. of sm. GTPase med. sig. transd.
cell. prot. metab. proc.
10-9 10-7 10-5 10-3 10-1
P-value

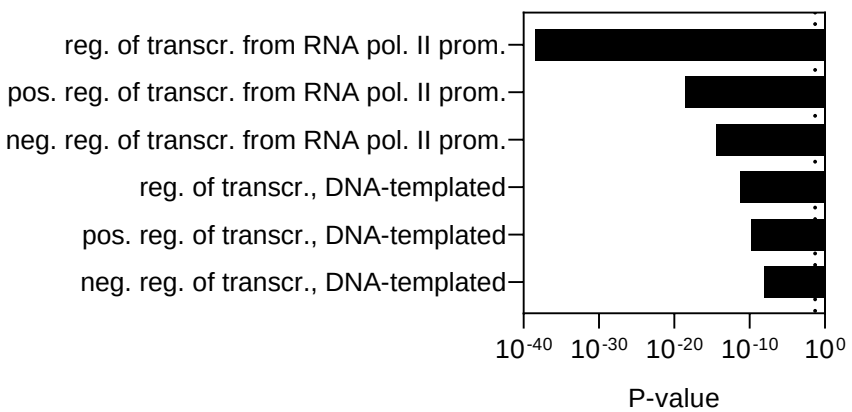
B


cell adhesion
heterotyp. cell-cell adhesion
cell migration
cell-cell adhesion
muscle organ develop.
bone resorption
10-6 10-4 10-2 100
P-value

C

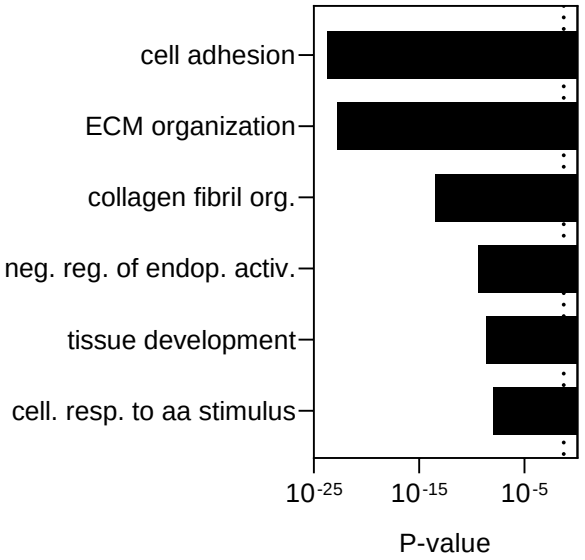

neutrophil degranulation
cell adhesion
activ. of cyst.-type endopeptid. activ. invol. in apop. proc.
lipid metab. proc.
cholesterol biosynth. proc.
cholesterol metabolic process
10-6 10-4 10-2 100
P-value

D

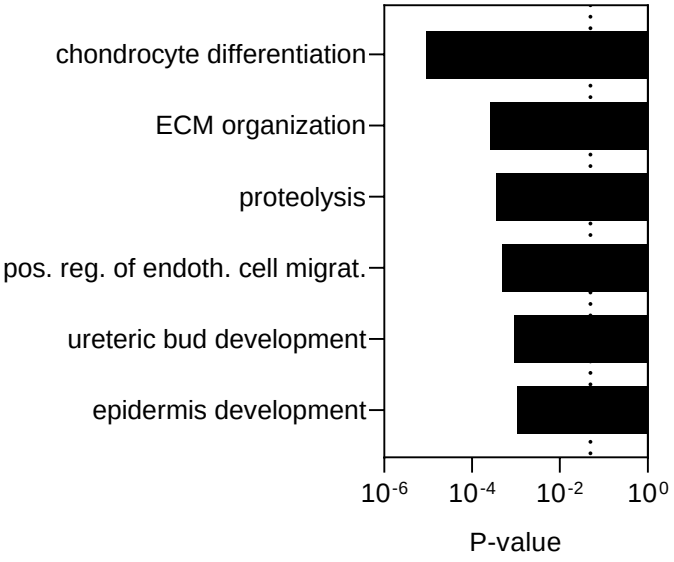

reg. of transcr. from RNA pol. II prom.
pos. reg. of transcr. from RNA pol. II prom.
neg. reg. of transcr. from RNA pol. II prom.
reg. of transcr., DNA-templated
pos. reg. of transcr., DNA-templated
neg. reg. of transcr., DNA-templated
10-40 10-30 10-20 10-10 100
P-value

Supplementary Figure S6. Biological processes associated with proteins differentially expressed between Meso-CAFs and normal lung fibroblasts (NLFs). (A) Biological processes associated with secreted proteins identified only in NLFs or significantly upregulated in NLFs compared to Meso-CAFs. (B) Biological processes associated with membrane proteins identified only in Meso-CAFs or significantly upregulated in Meso-CAFs compared to NLFs. (C) Biological processes associated with membrane proteins identified only in NLFs or significantly upregulated in NLFs compared to Meso-CAFs. (D) Biological processes associated with DNA-binding proteins identified only in Meso-CAFs or significantly upregulated in Meso-CAFs compared to NLFs. Due to the small number of DNA-binding proteins identified only in NLFs or significantly downregulated in NLFs compared to Meso-CAFs, no analysis was performed for this category.

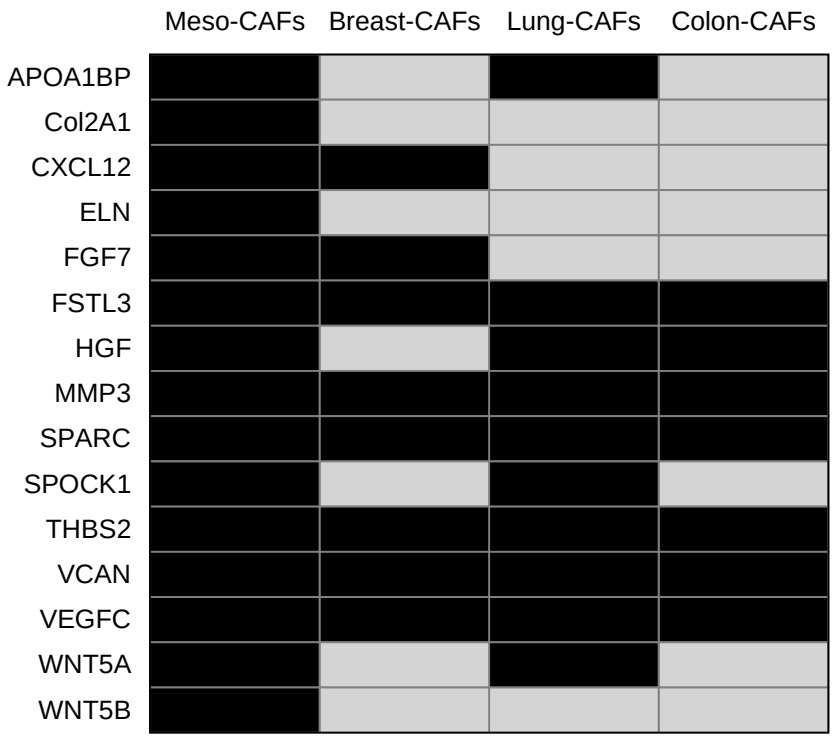
A



B

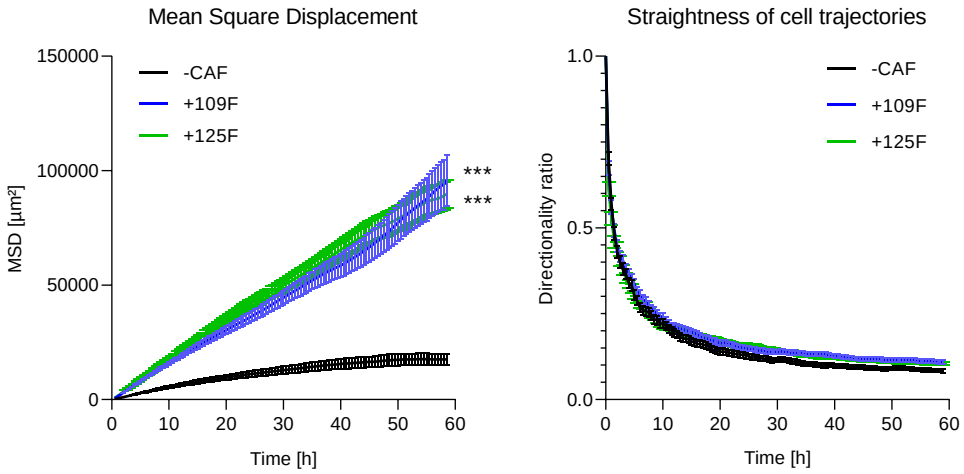


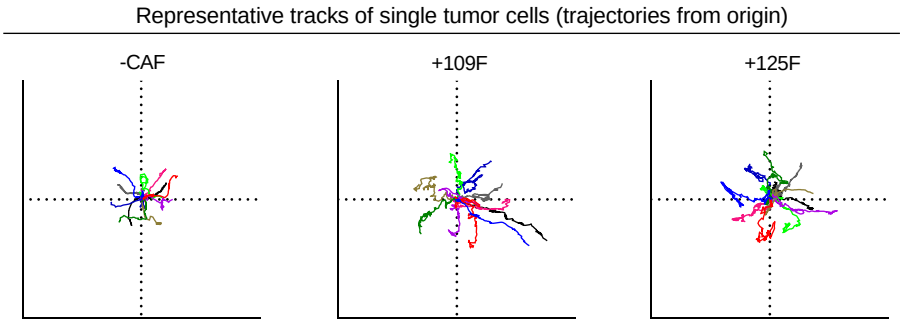
Supplementary Figure S7. Biological processes associated with secreted proteins found in CAFs from multiple cancers versus only in Meso-CAFs. (A) Biological processes associated with secreted proteins common to CAFs from PM, lung cancer, colon cancer and breast cancer. (B) Biological processes associated with secreted proteins found only in Meso-CAFs.



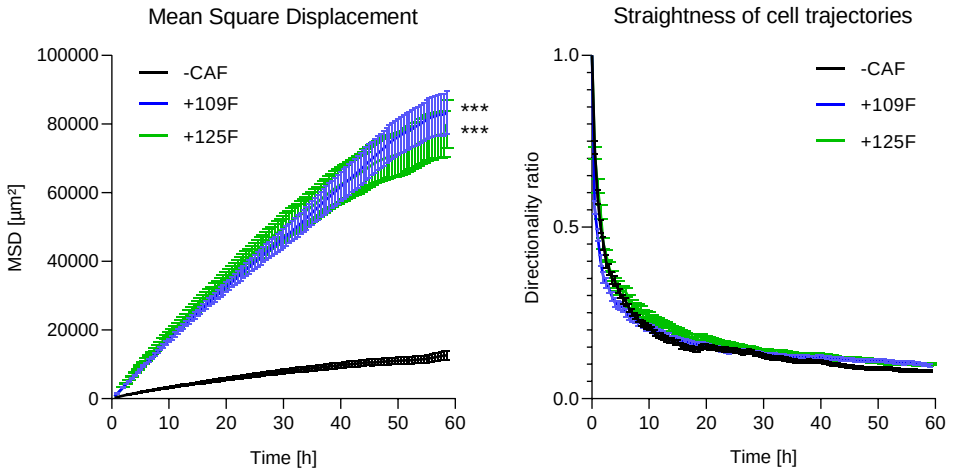
Supplementary Figure S8. Presence (black) or absence (gray) of selected proteins from the secretome of Meso-CAFs in the secretomes of CAFs from other cancers.

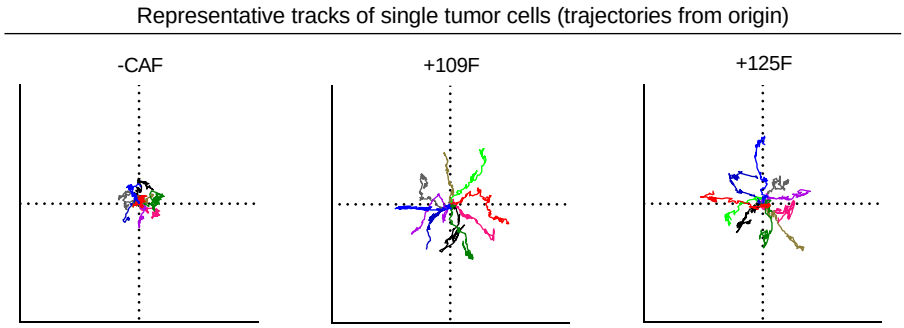
SPC212



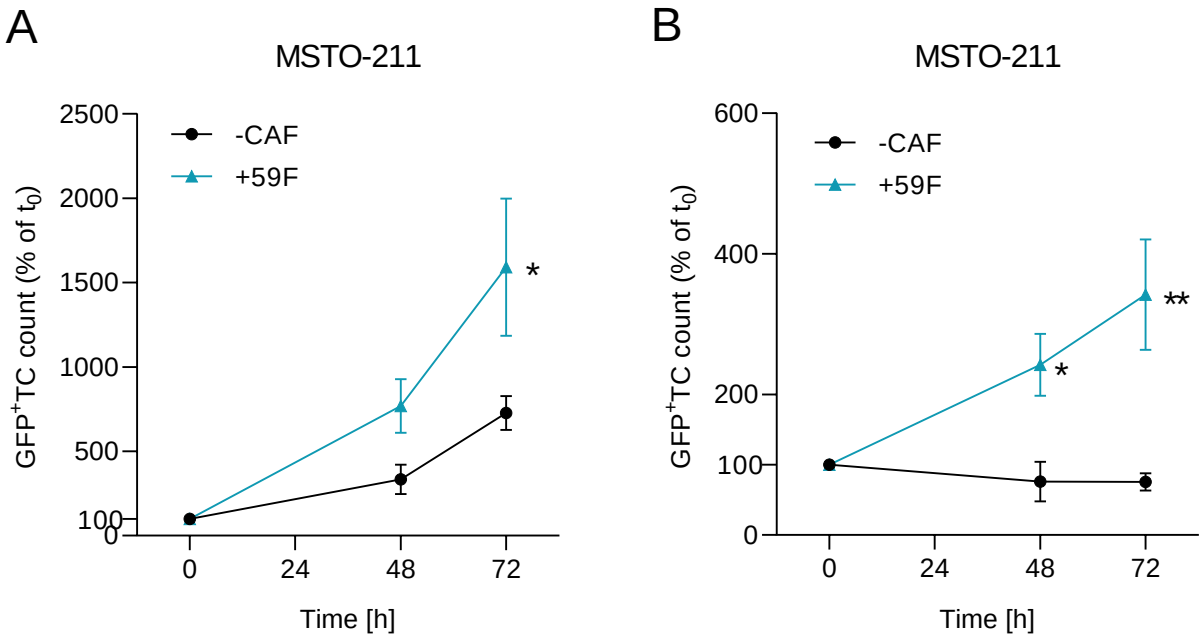


MSTO-211H

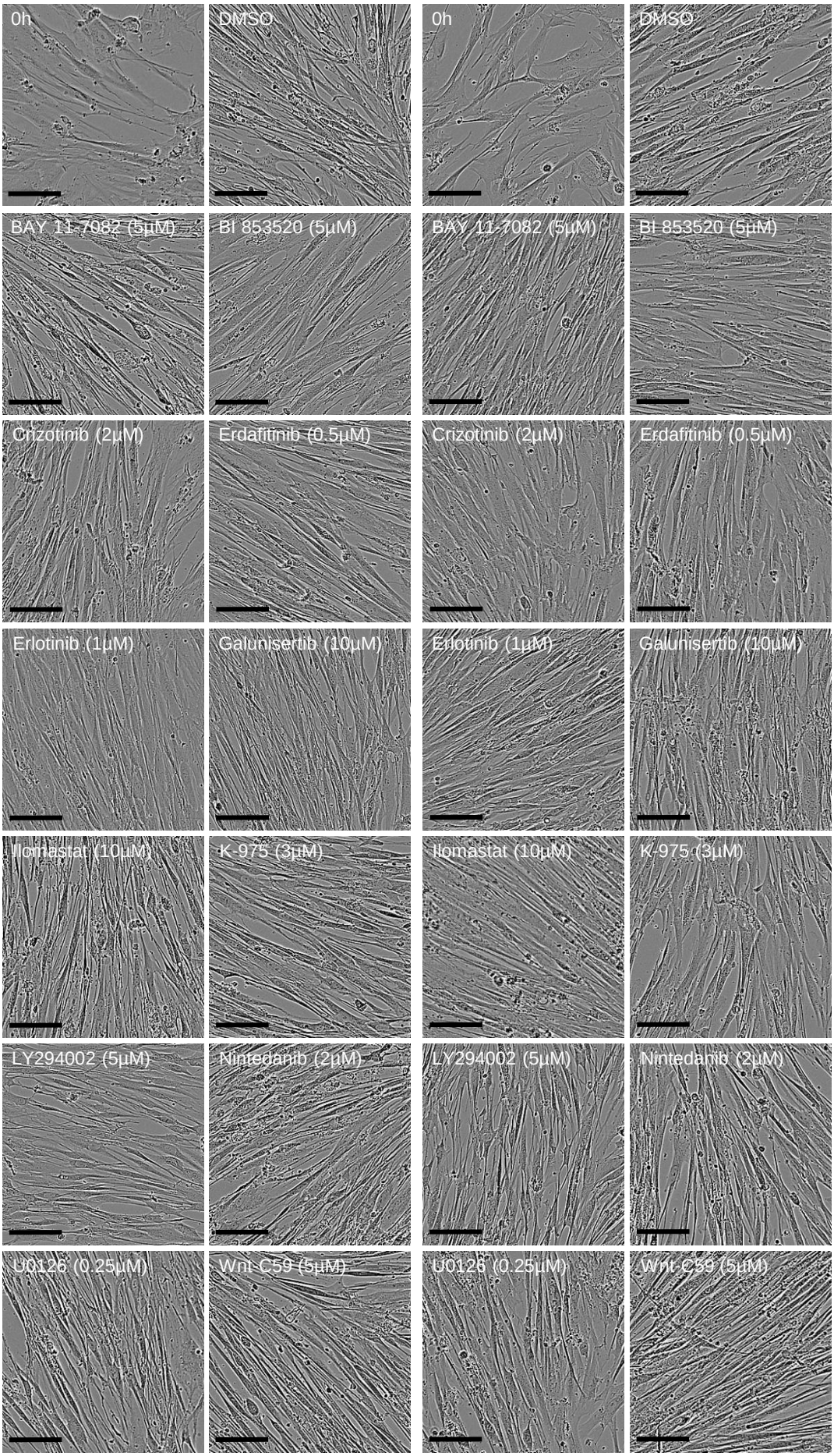




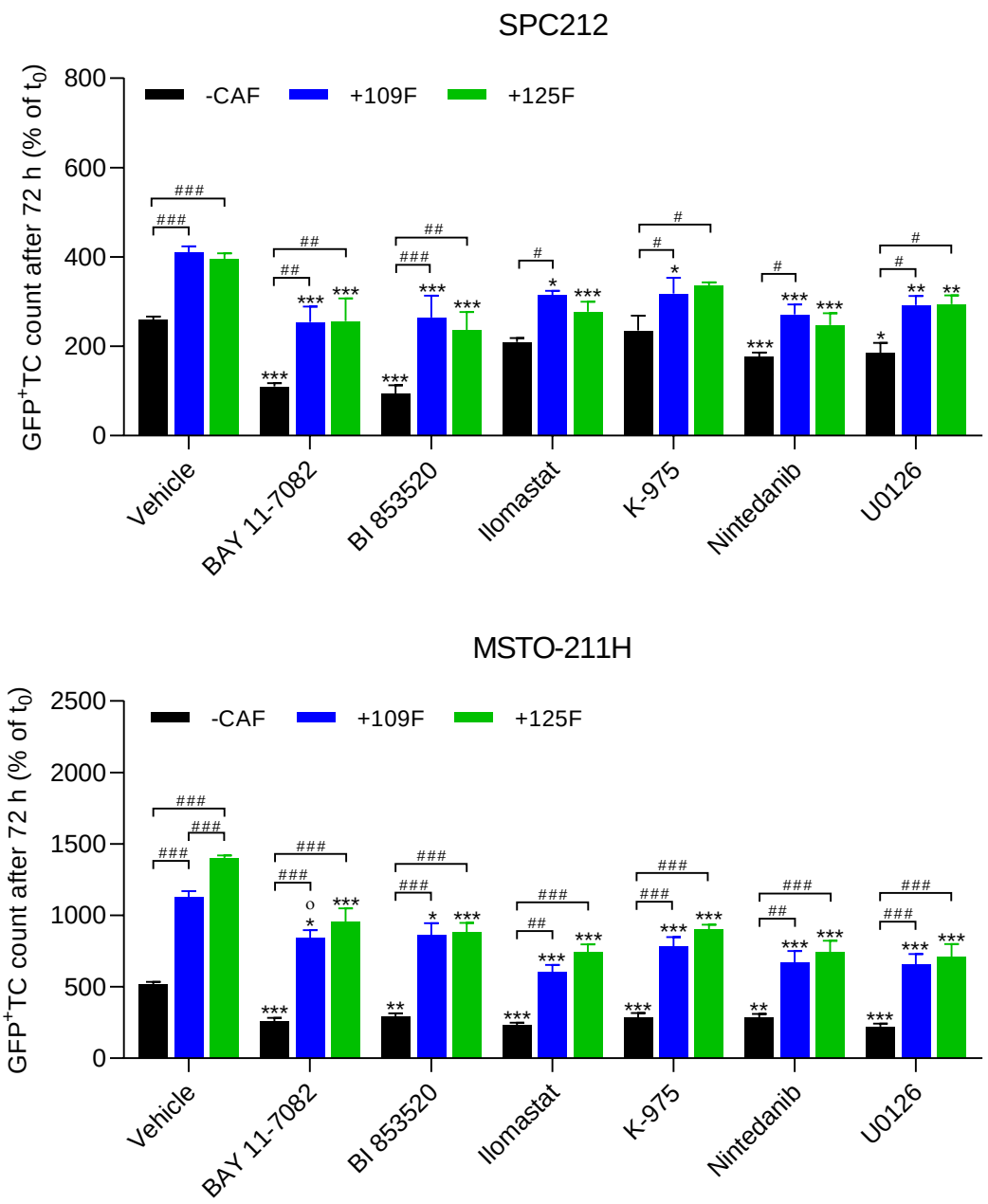
Supplementary Figure S9. Migration parameters of PM cells in the presence or absence of Meso-CAFs. GFP^+ SPC212 and GFP^+ MSTO-211H were cultured in the absence of CAFs (-CAF) or in the presence of Meso109F (+109F) or Meso125F (+125F). Individual tumor cells (n > 190 per condition) from three biological replicates were manually tracked for 60 h. Data for mean square displacement (MSD), straightness of cell trajectories and origin plots were generated with the DiPer migration tool for Microsoft Excel. *** $p<0.001$, MSD of TC in the presence of Meso-CAFs versus in the absence of Meso-CAFs at endpoints, one-way ANOVA with Dunnett's multiple comparisons test.



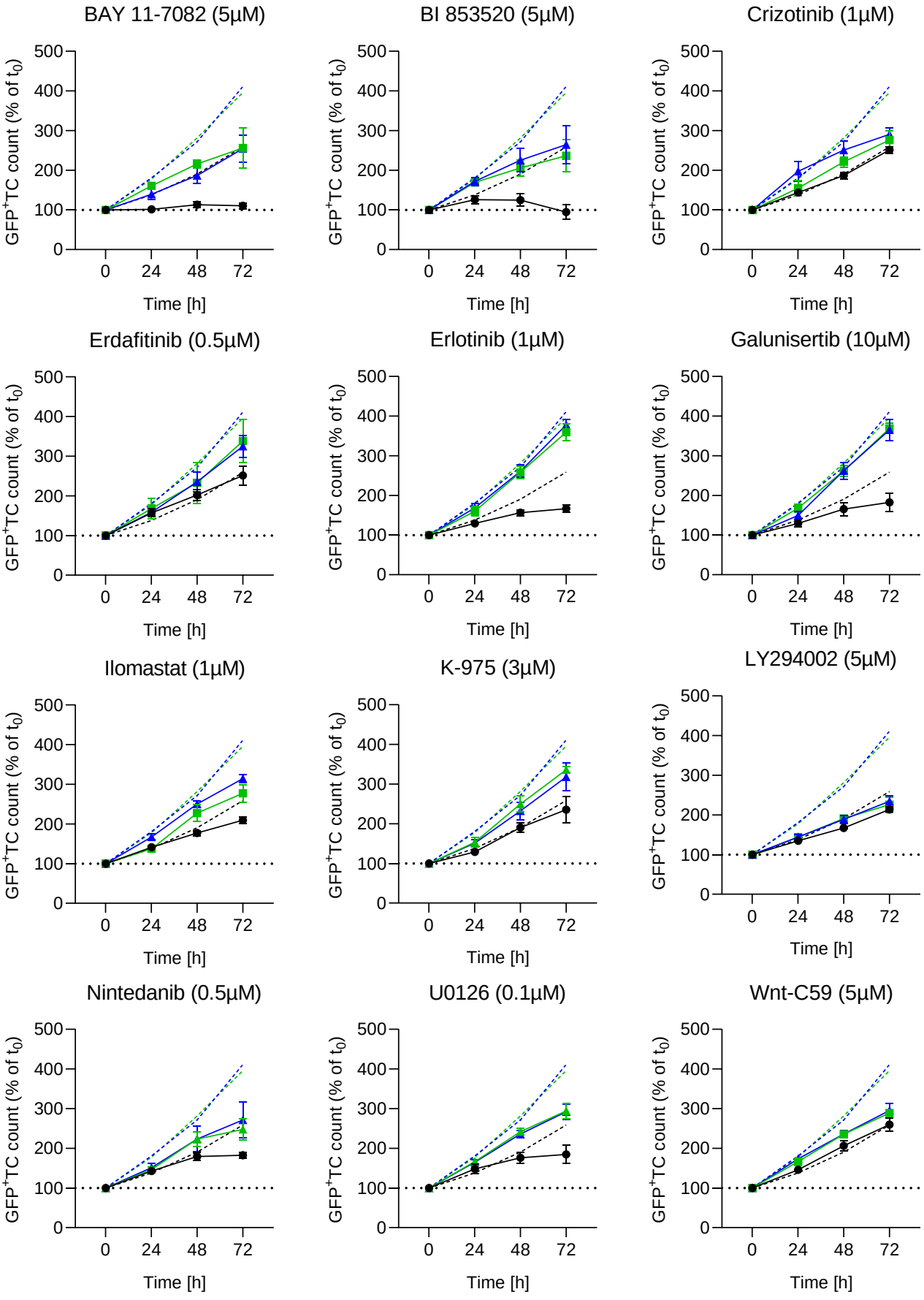
Supplementary Figure S10. Stimulation of MSTO-211H growth by VMC59F. Green fluorescent protein tagged (GFP^+) MSTO-211H were incubated in the presence and absence of VMC59 as 2D monolayers (A) or in 3D collagen gels (B). Micrographs were taken after 48 and 72 h and numbers of GFP^+ tumor cells (TC) were determined by automated image analysis. * $p<0.05$, ** $p<0.01$, TC number in the presence of VMC59F (+59F) versus TC number in the absence of VMC59F (-CAF), two-way ANOVA with Tukey's multiple comparisons test.


Meso109F
Meso125F
0h
DMSO
0h
DMSO
BAY 11-7082 (5μM)
BI 853520 (5μM)
BAY 11-7082 (5μM)
BI 853520 (5μM)
Crizotinib (2μM)
Erdafitinib (0.5μM)
Crizotinib (2μM)
Erdafitinib (0.5μM)
Erlotinib (1μM)
Galunisertib (10μM)
Erlotinib (1μM)
Galunisertib (10μM)
Ilomastat (10μM)
K-975 (3μM)
Ilomastat (10μM)
K-975 (3μM)
LY294002 (5μM)
Nintedanib (2μM)
LY294002 (5μM)
Nintedanib (2μM)
U0126 (0.25μM)
Wnt-C59 (5μM)
U0126 (0.25μM)
Wnt-C59 (5μM)

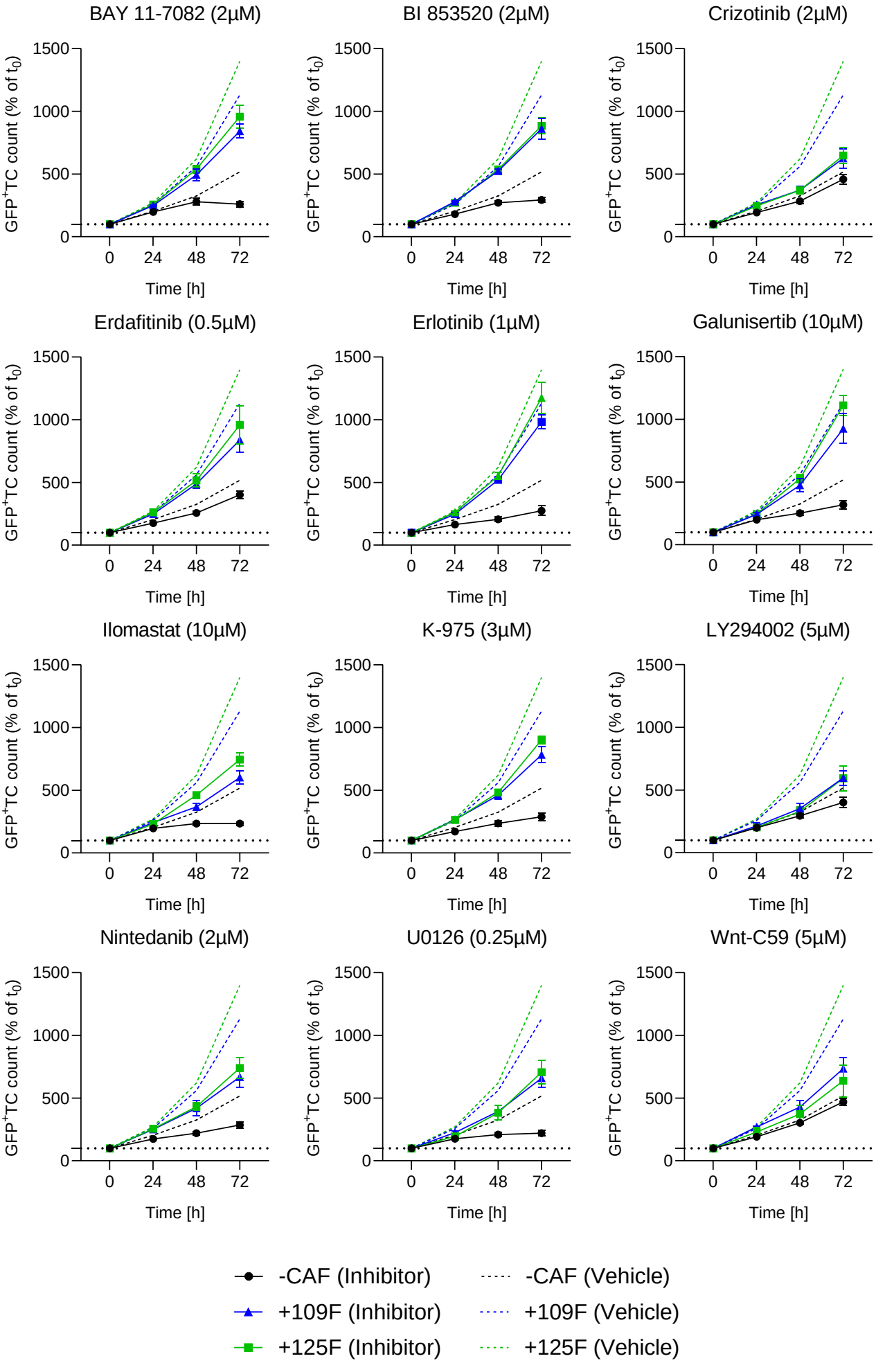
Supplementary Figure S11. Absence of inhibitor-induced cytotoxicity in Meso-CAFs. Micrographs were taken after 72 h of inhibitor treatment at the indicated concentrations. DMSO was used as vehicle in all cases and is shown for comparison. Meso-CAFs before treatment start (0 h) are also shown. Scale bar = 100 µm.



Supplementary Figure S12. Response of PM cells to signaling pathway inhibitors in the presence or absence of Meso-CAFs. GFP⁺ SPC212 (upper panel) or GFP⁺ MSTO-211H (lower panel) were cultured in the absence of CAFs (-CAF) or in the presence of Meso109F (+109F) or Meso125F (+125F) and treated with BAY 11-7082, BI 853520, ilomastat, K-975, nintedanib, U0126 or vehicle (DMSO). Numbers of GFP⁺ TC after 72 h were calculated from micrographs by automated image analysis. ** p<0.01, *** p<0.001 inhibitor treated versus vehicle treated, # p<0.05, ## p<0.01, ### p<0.001 growth in presence of Meso-CAFs versus growth in absence of Meso-CAFs, ° p<0.05, °° p<0.01, °°° p<0.001 percent inhibition in presence of Meso-CAFs versus percent inhibition in absence of Meso-CAFs, one-way ANOVA with Tukey's multiple comparisons test.


BAY 11-7082 (5µM)
BI 853520 (5µM)
Crizotinib (1µM)
Erdafitinib (0.5µM)
Erlotinib (1µM)
Galunisertib (10µM)
Ilomastat (1µM)
K-975 (3µM)
LY294002 (5µM)
Nintedanib (0.5µM)
U0126 (0.1µM)
Wnt-C59 (5µM)
GFP+TC count (% of t0)
Time [h]
0
24
48
72
100
200
300
400
500
-CAF (Inhibitor)
-CAF (Vehicle)
+109F (Inhibitor)
+109F (Vehicle)
+125F (Inhibitor)
+125F (Vehicle)

Supplementary Figure S13. Time course of SPC212 cell response to inhibitors in the presence or absence of Meso-CAFs. GFP^+ SPC212 were cultured in the absence of CAFs (-CAF) or in the presence of Meso109F (+109F) or Meso125F (+125F) and treated with the indicated inhibitors or vehicle (DMSO). Micrographs were taken every 24 h and numbers of GFP^+ tumor cells (TC) were calculated by automated image analysis. Statistical evaluation at endpoint (72 h) is shown in Figure 5 and Supplementary Figure 12.


BAY 11-7082 (2µM)
BI 853520 (2µM)
Crizotinib (2µM)
Erdafitinib (0.5µM)
Erlotinib (1µM)
Galunisertib (10µM)
Ilomastat (10µM)
K-975 (3µM)
LY294002 (5µM)
Nintedanib (2µM)
U0126 (0.25µM)
Wnt-C59 (5µM)
GFP+TC count (% of t0)
Time [h]
0
500
1000
1500
0
24
48
72
-CAF (Inhibitor)
+109F (Inhibitor)
+125F (Inhibitor)
-CAF (Vehicle)
+109F (Vehicle)
+125F (Vehicle)

Supplementary Figure S14. Time course of MSTO-211H cell response to inhibitors in the presence or absence of Meso-CAFs. GFP^+ MSTO-211H were cultured in the absence of CAFs (-CAF) or in the presence of Meso109F (+109F) or Meso125F (+125F) and treated with the indicated inhibitors or vehicle (DMSO). Micrographs were taken every 24 h and numbers of GFP^+ tumor cells (TC) were calculated by automated image analysis. Statistical evaluation at endpoint (72 h) is shown in Figure 5 and Supplementary Figure 12.