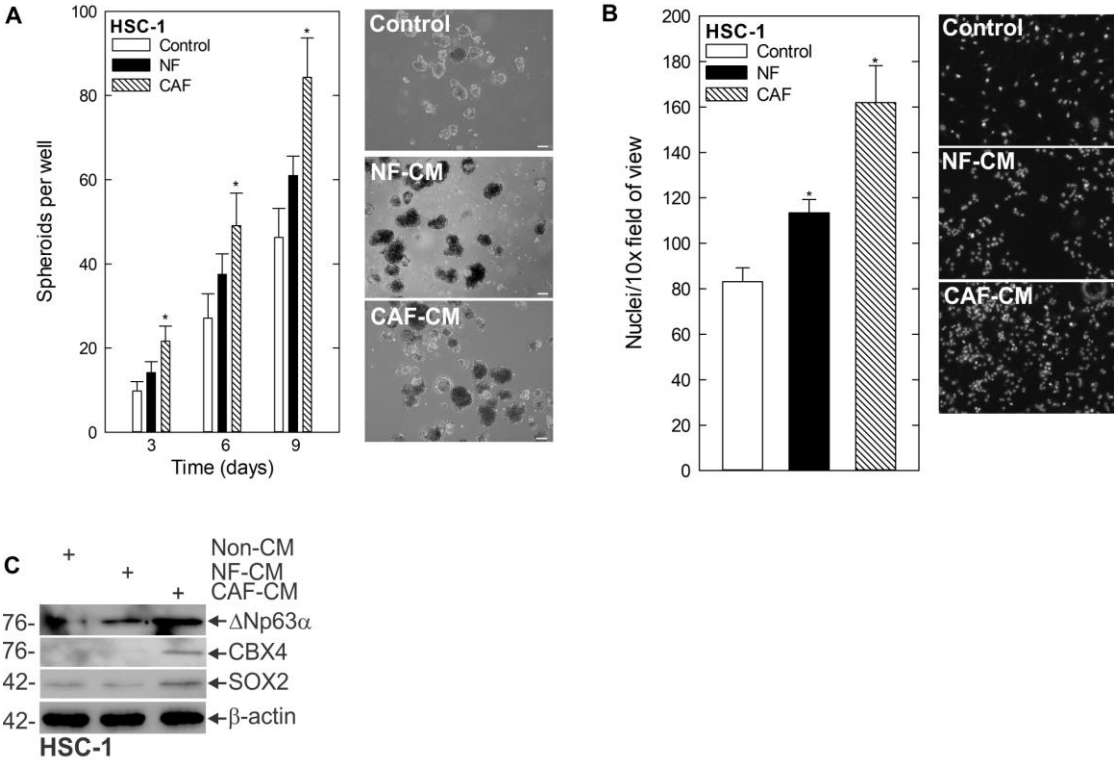
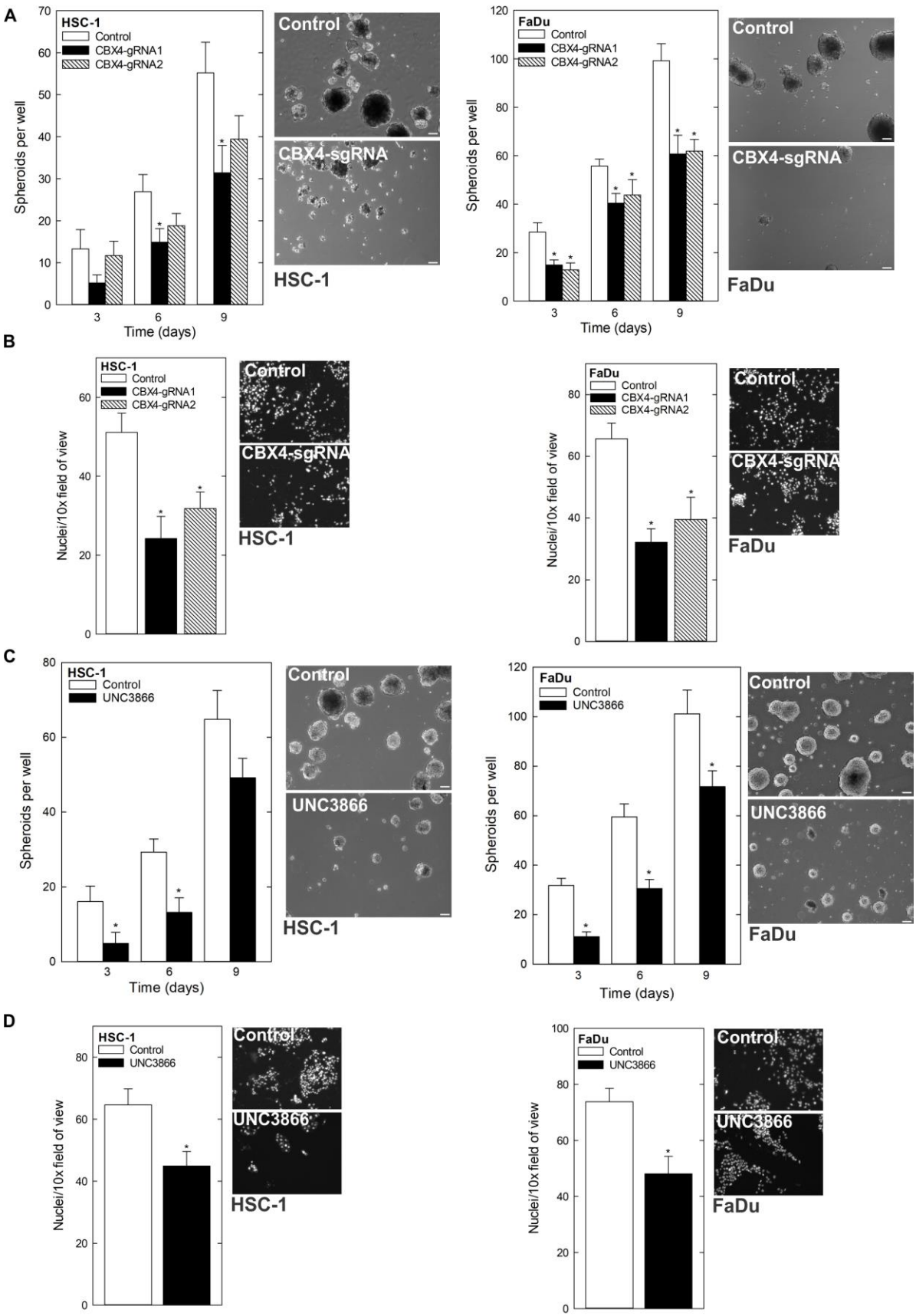
**Supplemental data**



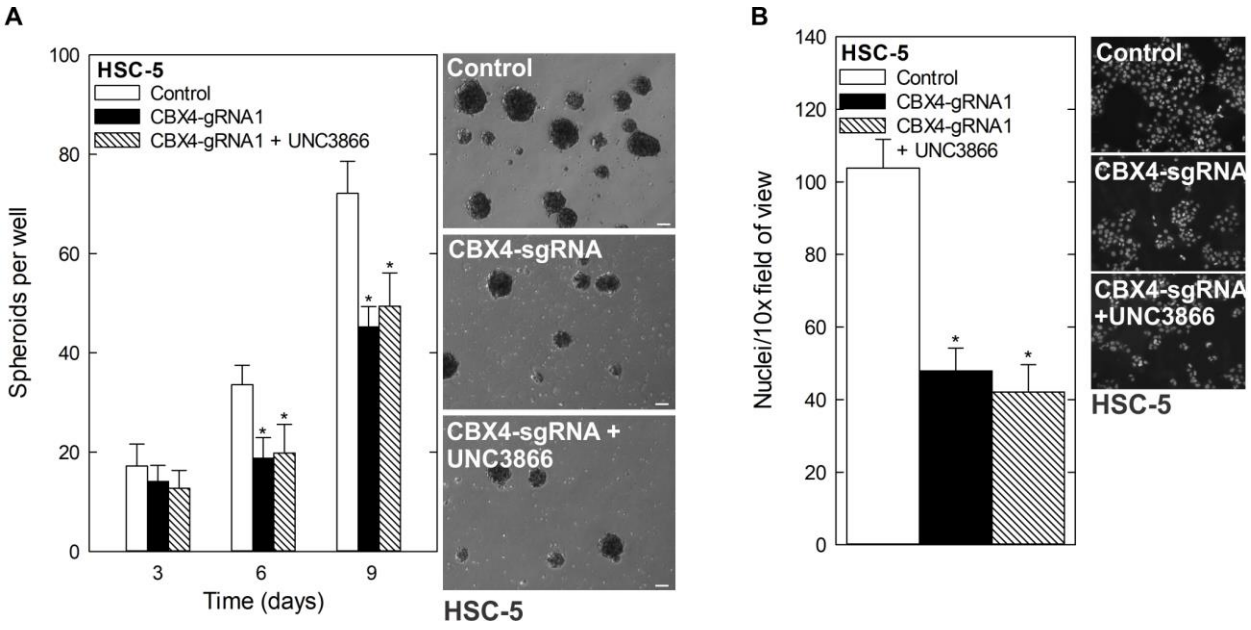

**Supplemental fig. 1 CAFs stimulate CSC phenotype and CBX4 expression**

**A** HSC-1 monolayer cultures maintained in growth medium were harvested and plated at $4x10^4$ cells per well in spheroid growth conditions with normal or cancer associated fibroblast conditioned media and spheroid number monitored for 9 days (left). Representative images on day 9 of growth are shown (right). Scale bars, 200 μm. The values are mean +/- SEM. The asterisks indicate significant difference compared with control. **B** spheroids were trypsinized and single-cell suspensions seeded onto Matrigel-coated membranes in Millicell chambers for invasion assays with normal or cancer associated fibroblast conditioned media in the bottom chamber **C** After 10 days of spheroid growth in normal or cancer associated fibroblast conditioned media, lysates were electrophoresed for detection of the indicated proteins.


A
HSC-1
Control
CBX4-gRNA1
CBX4-gRNA2
Spheroids per well
Time (days)
Control
CBX4-sgRNA
HSC-1
FaDu
Control
CBX4-gRNA1
CBX4-gRNA2
Spheroids per well
Time (days)
Control
CBX4-sgRNA
FaDu
B
HSC-1
Control
CBX4-gRNA1
CBX4-gRNA2
Nuclei/10x field of view
Control
CBX4-sgRNA
HSC-1
FaDu
Control
CBX4-gRNA1
CBX4-gRNA2
Nuclei/10x field of view
Control
CBX4-sgRNA
FaDu
C
HSC-1
Control
UNC3866
Spheroids per well
Time (days)
Control
UNC3866
HSC-1
FaDu
Control
UNC3866
Spheroids per well
Time (days)
Control
UNC3866
FaDu
D
HSC-1
Control
UNC3866
Nuclei/10x field of view
Control
UNC3866
HSC-1
FaDu
Control
UNC3866
Nuclei/10x field of view
Control
UNC3866
FaDu

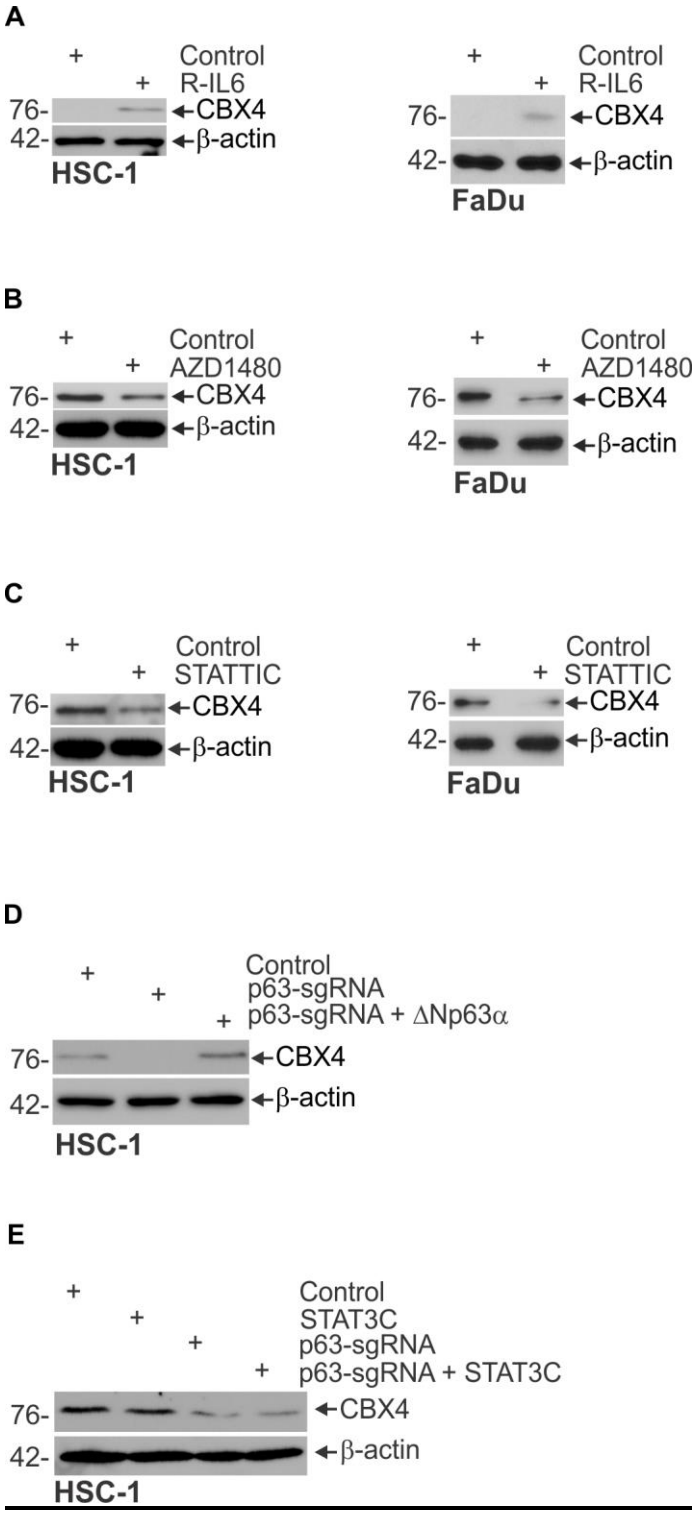
**Supplemental fig. 2 CBX4 regulates the CSC phenotype**

**A** HSC-1 or FaDu control empty vector (EV) and CBX4 CRISPR-depleted cells were seeded in spheroid growth conditions and spheroid number monitored for 9 days (left). Representative images on day 9 of growth are shown (right). **B** HSC-1 or FaDu control and CBX4 CRISPR-depleted cells were seeded in Matrigel-coated membranes in Millicell chambers for invasion assays. **C** HSC-1 or FaDu cells ($4x10^4$) were plated in non-adherent 6-well plates, grown for 9 days in spheroid medium, with or without UNC3866 and spheroid numbers were counted over 9 days. The values are mean +/- SEM. The asterisks indicate significant difference compared with control. Representative spheroid images following a 9-day treatment with 0 or 20 mmol/L UNC3866 are shown. **D** HSC-1 or FaDu spheroids were trypsinized to form single-cell suspensions and reseeded for invasion assays +/- UNC3866. The values are mean +/- SEM. The asterisks indicate significant difference compared with control, $P < 0.05$.




**Supplemental fig. 3 UNC3866 phenotype is independent of CBX7**

**A** HSC-5 control empty vector (EV) and CBX4 CRISPR-depleted cells were seeded in spheroid growth conditions +/- UNC3866 and spheroid number monitored over 9 days of growth. **B** HSC-5 control or CBX4 CRISPR depleted spheroids were trypsinized to form single cell suspensions and then seeded for invasion assays +/- UNC3866. The values are mean +/- SEM. The asterisks indicate significant difference compared with control, $P < 0.05$.




**Supplemental fig. 4 IL-6/JAK/STAT pathway regulates CBX4 expression**

**A** HSC-1 or FaDu spheroids were grown for 8 days then IL-6 was added to the media for 48 h and lysates collected for immunoblot. **B** HSC-1 or FaDu Spheroids were grown in CAF conditioned media and treated with 1 μM of the JAK inhibitor AZD1480 and lysates were collected for protein detection of the indicated epitopes. **C** HSC-1 or FaDu Spheroids were

grown in CAF conditioned media and treated with 5 μM of the STAT3 inhibitor STATTIC and lysates were collected for western blots of the indicated proteins. **D** HSC-1 Control, p63-sgRNA, and p63-sgRNA + ΔNp63α–expressing cells were harvested for protein for western blot. **E** HSC-1 Control, STAT3C, p63-sgRNA and STAT3C + p63-sgRNA expressing cells were harvested for protein for western blotting of the indicated proteins.