Stromal microenvironment in tumor development & progression

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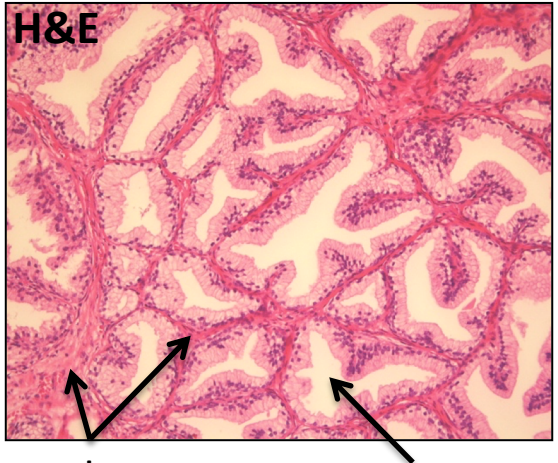
Overview

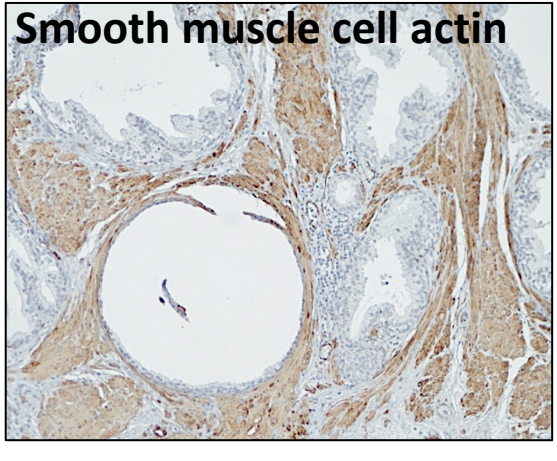
1. Background
   - Stromal tissue: definition, composition, function
   - Molecular/cellular changes in tumor microenvironment
   - Cancer-associated fibroblasts as major tumor-modulating entities in tumor microenvironment
2. Paper presentation
3. Take home message

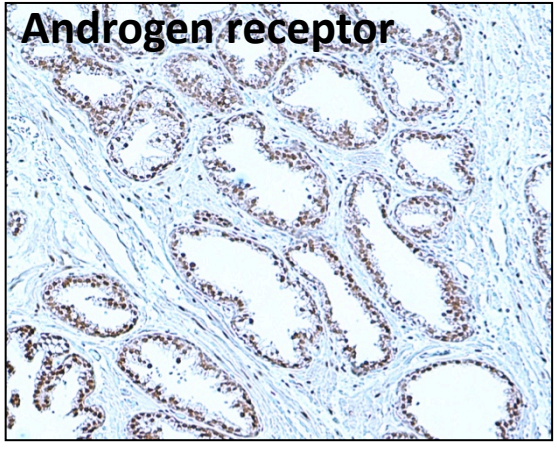
Stroma

Definition:

- the supportive framework or matrix of a cell or organ
- comes from Greek meaning **bed covering or mattress**







70% of prostate = stroma

Stroma: composition & origin

- stroma derives from embyronic mesenchyme

cellular component:
nerves
blood vessels
immune cells
fibroblasts
smooth muscle cells (SMCs)

non-cellular component:
connective tissue
extracellular matrix (ECM)

Stroma: function

1. provides structural support/rigidity to organ and tissue (connective tissue)

1. key regulator of tissue homeostasis & organ development:
   - reciprocal interactions between epithelium and stroma via paracrine-acting signaling molecules (growth factors and cytokines)
   - signaling cascades regulate epithelial and stromal cell proliferation, differentiation, ECM production, angiogenesis (i.e. key processes that contribute to cancer)

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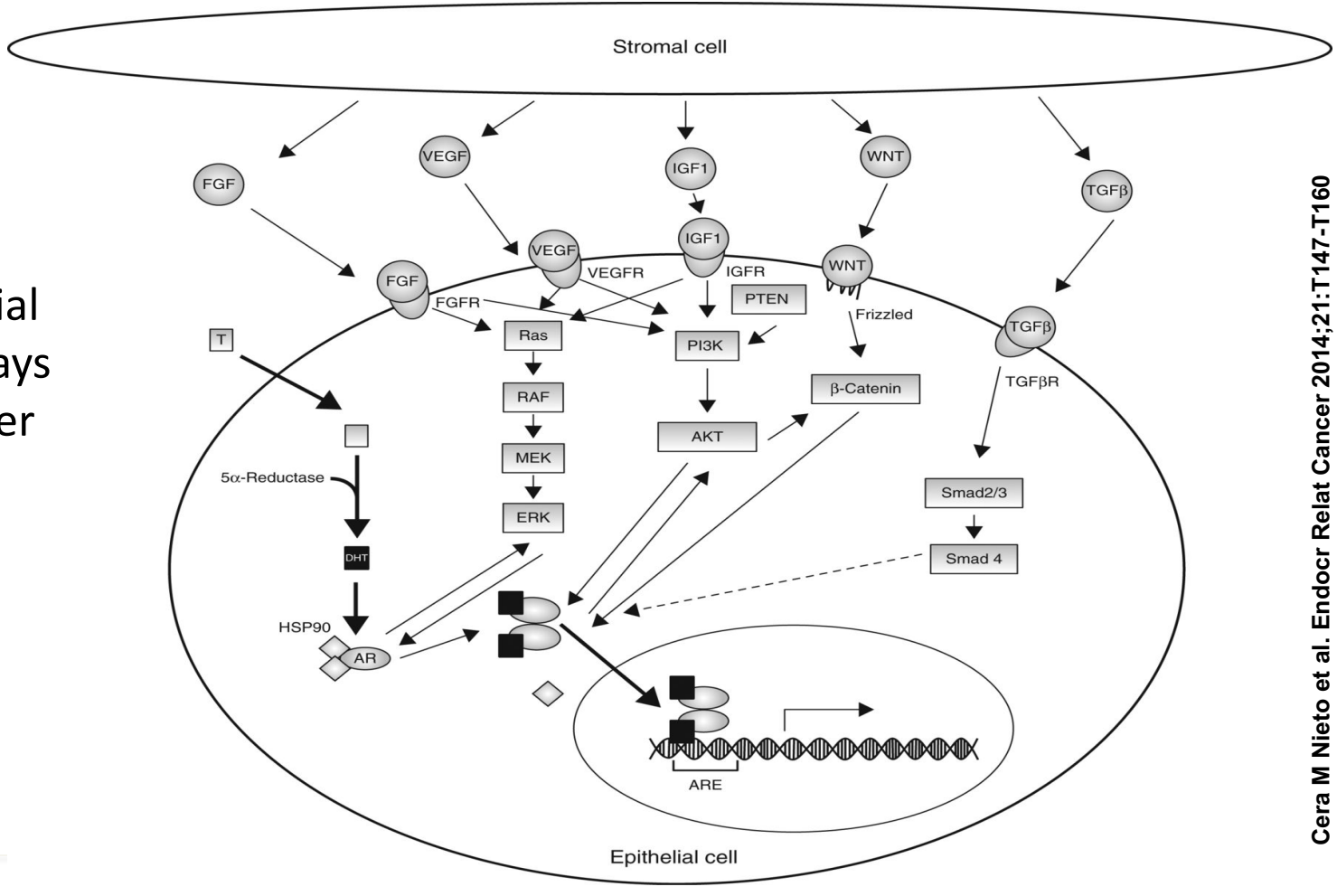
Stromal – epithelial interactions

EXAMPLE:
AR-regulated stromal–epithelial signaling pathways in prostate cancer



Cera M Nieto et al. Endocr Relat Cancer 2014;21:T147-T160

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Tumor stroma: historical perspective

1863: Rudolph Virchow observed leukocytes in stroma of neoplastic tissue. He hypothesized that malignancy originated at sites of chronic inflammation

1889: Paget noted that some tumor cells (the "seed") grow preferentially in the **microenvironment** of selected organs (the "soil") and that metastases only result when the appropriate seed is implanted in its suitable soil

1924: Max Borst wrote „with regards to the question of whether the epithelium or the connective tissue has the leading role in carcinogenesis, we think that asking "*either/or*" is bad."

Tumor-promoting microenvironment

Tumor stroma greatly enhances tumorigenicity

Inoculated cancer cells embedded in tumor stroma are 10 – 100 fold more tumorigenic than stroma-free suspensions of cancer cells

Tumor-adjacent stroma termed „reactive“ or „desmoplastic“ stroma

Table 1. *Tumorigenicity of Cancer Cells Inoculated into Normal Mice as Cell Suspensions or Fragments of Solid Tumors*

Tumor	Inoculum*	Tumor cells	Tumor outgrowth‡
		$\times 10^6$	
1591-PRO	Suspension	50	0/7§
	Suspension	10	1/8
	Fragments	15	11/15
	Fragments	3	10/12
	Fragments	1.5	8/12§
6134A-PRO	Suspension	50	0/5‖
	Suspension	10	0/16
	Fragments	15	9/11
	Fragments	3	8/12
	Fragments	1.5	7/12‖

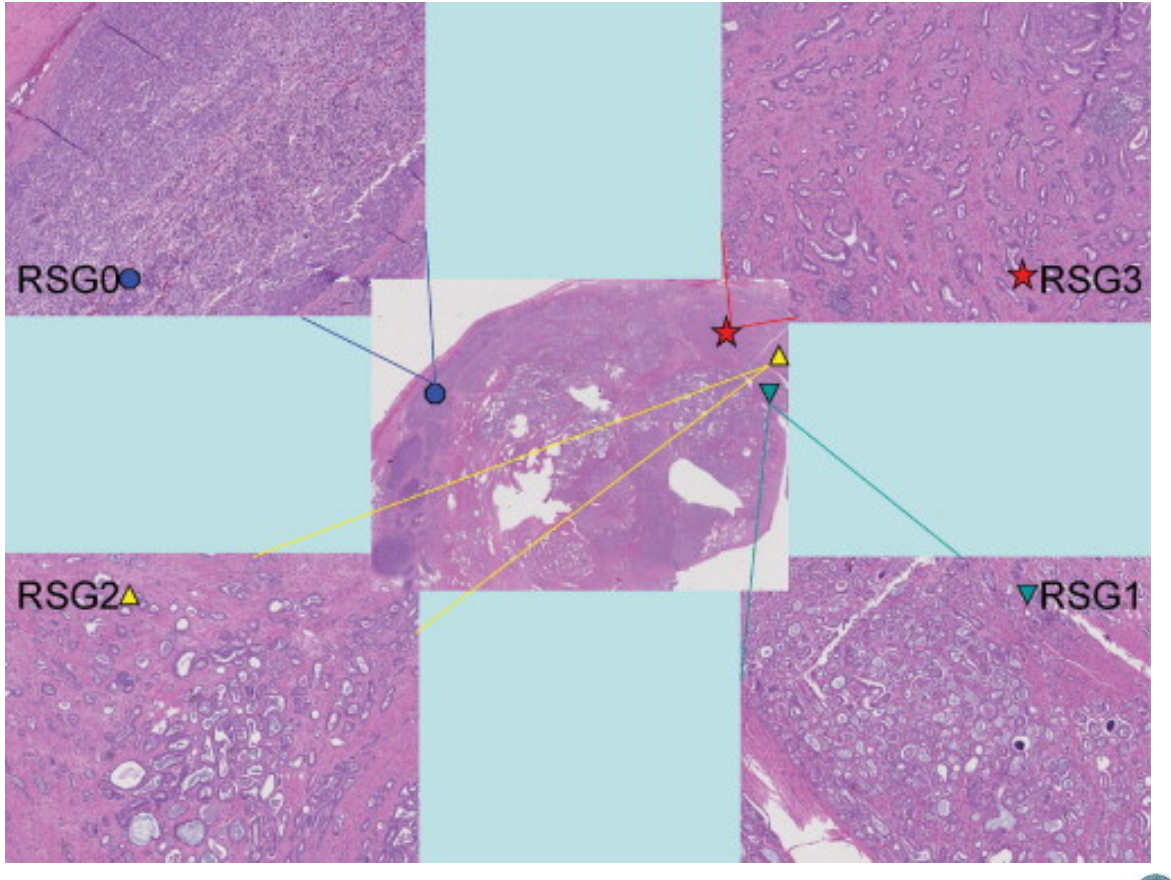
Singh et al. 1992 J Exp Med 175: 139

Reactive stroma grading

4 different reactive stromal grades (RSGs) depending on the <u>percentage of area of reactive stroma (RS) in the tumor</u>:

RSG 0: ≤5%
RSG 1: 6%– 15%
RSG 2: 16%–50%
RSG 3: at least a 1:1 ratio between reactive stroma and epithelial cancer

not routinely used in clinical diagnostics

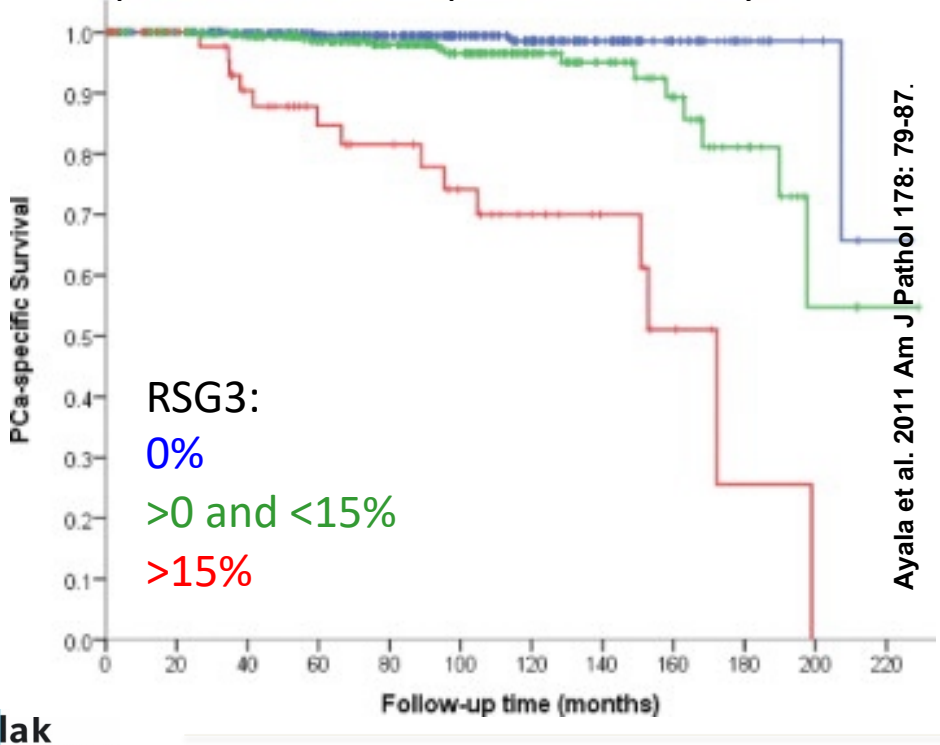


Ayala et al. 2011 Am J Pathol 178: 79-87.

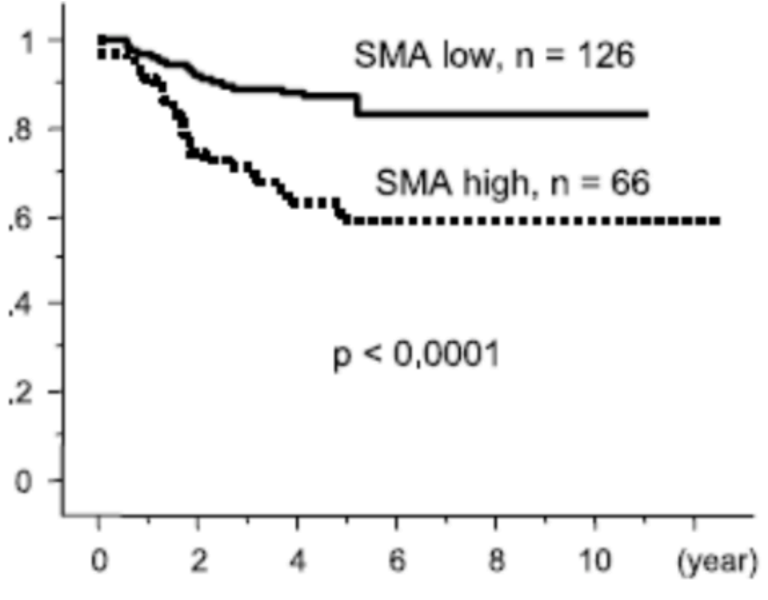
Reactive stroma: clinical relevance

stroma-enriched tumors are associated with poor prognosis

prostate cancer specific mortality



overall survival of colorectal cancer patients



(Tsujino et al., Clin. Cancer Res. 2007)

Tumor microenvironment

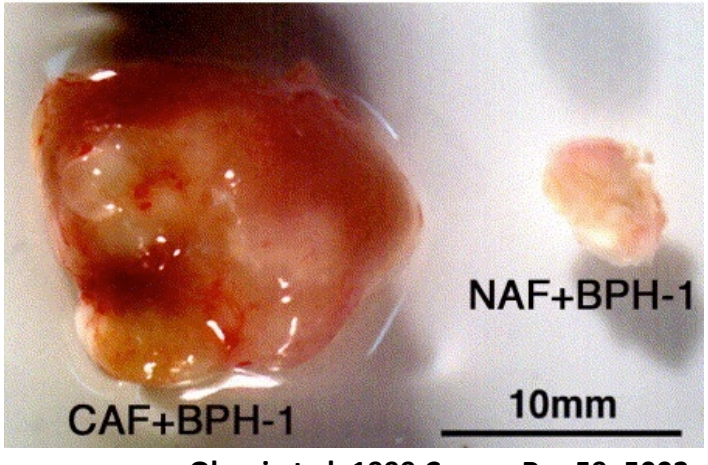
- reactive stroma exhibits histo-morphological hallmarks:
  1. presence of carcinoma-associated fibroblasts (CAFs, activated phenotype)
  2. increased deposition of altered ECM
  3. increased capillary density (aberrant structure/leaky vessels)
  4. immune cell infiltration

each required for tumor development

- changes apparent in pre-neoplastic lesions (early event in tumorigenesis)
- tumor-associated stroma changes (co-evolves) during tumor progression

Carcinoma Associated Fibroblasts

Fibroblast Cell Types	Epithelial Cell Types: Normal	Epithelial Cell Types: Initiated (BPH-1)
Normal	No tumor <10mg	No tumor <10mg
CAF	No tumor <10mg	Large tumors

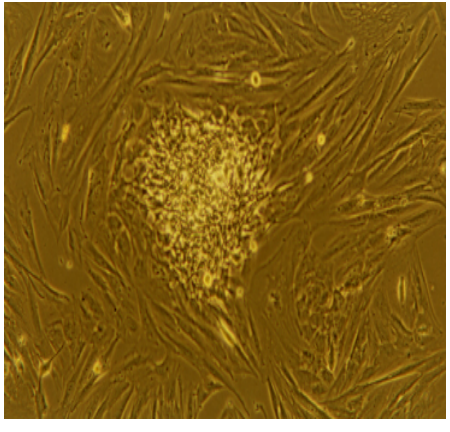


Olumi et al. 1999 Cancer Res 59: 5002

- Tumor-promoting capacity of stroma predominantly mediated by CAFs

CAFs

- persistently activated fibroblast-like cells in stroma adjacent to the tumor and at invasive front (spindle-like morphology)



- CAF phenotype proven by ability to promote tumorigenesis of initiated but non-tumorigenic epithelial cells

- Isolated from tumor biopsies via
  - (i) outgrowth from tissue slices in media containing serum
  - (ii) tissue digestion with collagenase and differential centrifugation/FACS

CAFs: molecular hallmarks

- exhibit widespread DNA hypomethylation
- no single molecular marker to define CAFs
- common markers include:

fibroblast activation protein (FAP)	platelet derived growth factor receptors
alpha smooth muscle actin (SMA)	Tenascin C
fibroblast specific protein (FSP1)	podoplanin
CD90/Thy1	↓ caveolin-1

- not all CAFs express these markers (different CAF subtypes) and these markers are not necessarily CAF-specific
- CAF subtypes also exhibit functional differences

CAF heterogeneity

- „CAF“ represents a heterogeneous mix of functionally-distinct cell types/cell states
- Cytokine/expression profiles may characterize different CAFs subtypes (tissue-specific)
- CAFs can promote or inhibit tumor progression:
  - *podoplanin$^+$ CAFs are prognostic indicator in lung adenocarcinoma, squamous cell carcinoma and breast cancer*
  - *FAP$^+$ CAFs associated with poor outcome in colon cancer*
  - *CD90 (Thy-1)$^+$ CAFs more tumor-promoting in prostate cancer*
  - *depleting FAP$^+$ CAFs promoted tumor progression in mouse PDAC model*
  - *inhibiting Shh signaling, depleted stromal content but promoted tumor aggressiveness in mouse PDAC model*

➢ Different subtypes of CAF exist, which are functionally/molecularly distinct - heterogeneity may reflect different activation stimuli (e.g. IL6 vs. TGFβ) and/or CAF cellular origin (i.e. local resident fibroblast vs. infiltrated BM-derived cell)

CAF tumor-promoting actions

Direct mechanisms

- secrete paracrine-acting soluble factors e.g. IL-6
- direct cell-cell contacts with tumor cells

Indirect mechanisms

- ECM remodeling
- angiogenesis
- modulate immune response
- metabolic reprogramming

Therapy resistance

- reduced chemotherapeutic efficacy
- endocrine/target resistance

➢ CAFs target for novel approach of stromal-targeted anti-cancer therapies?

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ARTICLE

CD10+GPR77+ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness

Shicheng Su[7], Jianing Chen[7], Herui Yao[7], Jiang Liu, Shubin Yu, Liyan Lao, Minghui Wang, Manli Luo, Yue Xing, Fei Chen, Di Huang, Jinghua Zhao, Linbin Yang, Dan Liao, Fengxi Su, Mengfeng Li, Qiang Liu, Erwei Song[8],

[7] These authors contributed equally

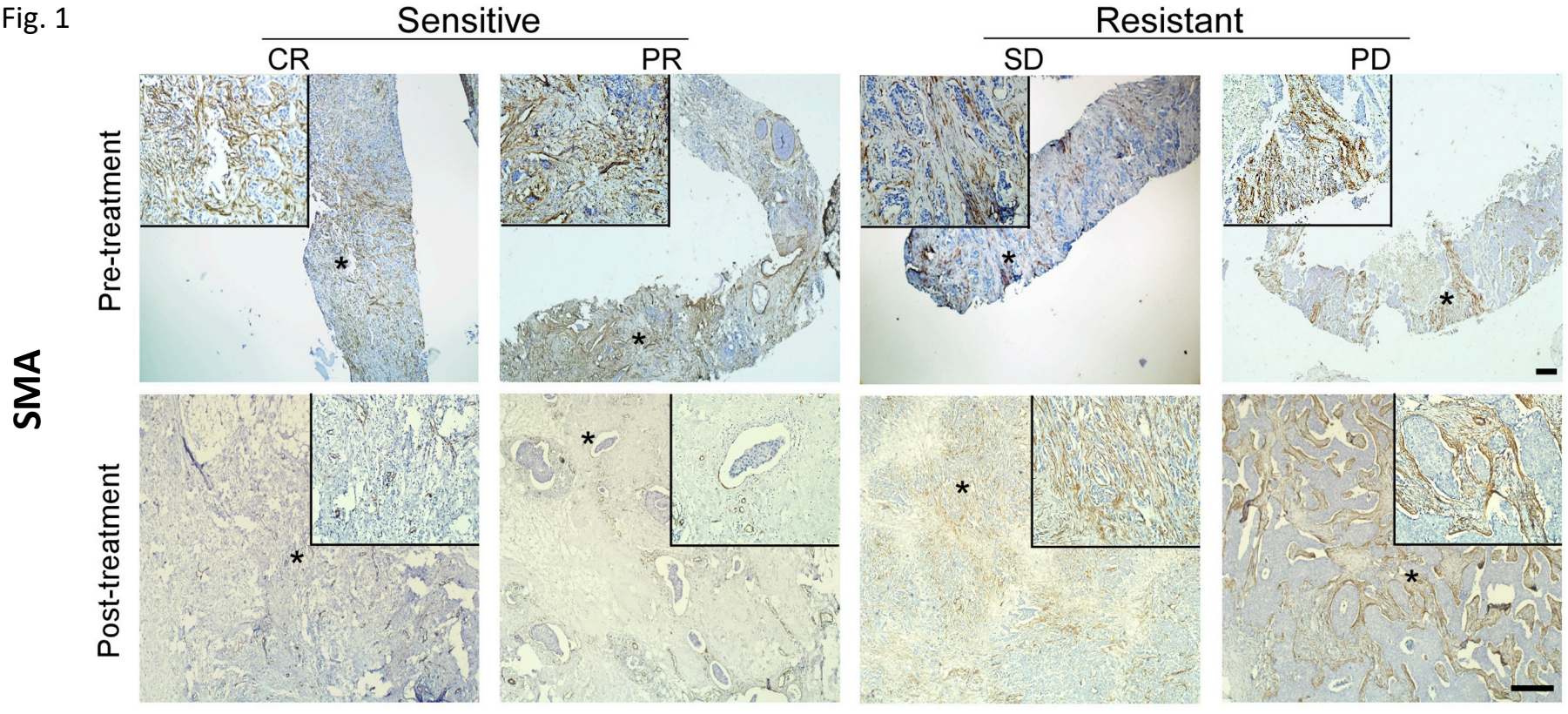
[8] Lead Contact

Aim

- Cancer stem cells (CSCs): population of highly tumorigenic & chemo-resistant cells
- CSC maintenance requires supportive niche

$\Rightarrow$ Identify/study the subpopulation of CAFs underlying breast cancer stemness/chemoresistance

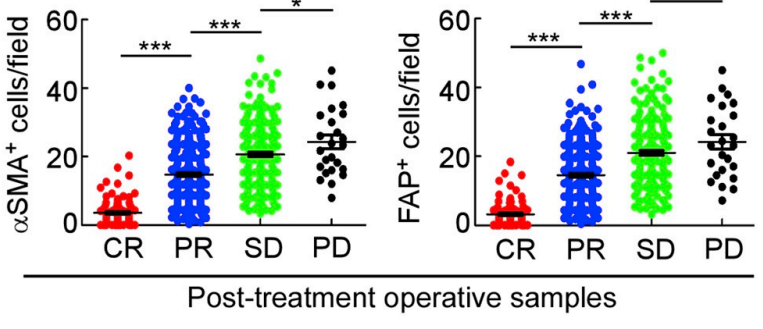
Fig. 1



<u>BEFORE treatment</u>: frequency of SMA$^+$ CAFs not different among patient groups

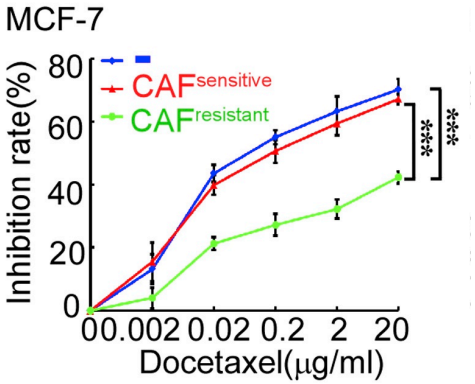
<u>AFTER treatment</u>: more SMA$^+$ CAFs in tissues of resistant patients compared to sensitive/responsive patients

$\Rightarrow$ Do heterogeneous CAFs contribute to chemoresistance?

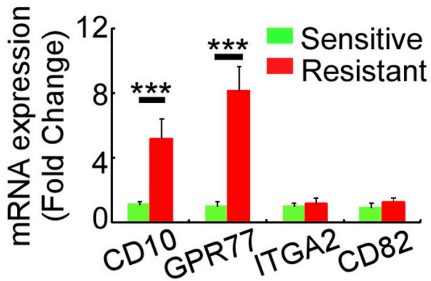


- isolated fibroblasts from chemoresistant/sensitive BrCa biopsies B4 chemotherapy
- co-culture with BrCa cell lines (MCF-7 and SK-BR3)
- challenged cells with chemotherapeutic drugs

⇒ **functionally distinct CAF subtypes in resistant *vs.* sensitive BrCA?**



- microarrays – cell surface markers
- abundance of CD10⁺GPR77⁺ CAFs associated with decreased survival



(CD10 = MME used in diagnosis of ALL)

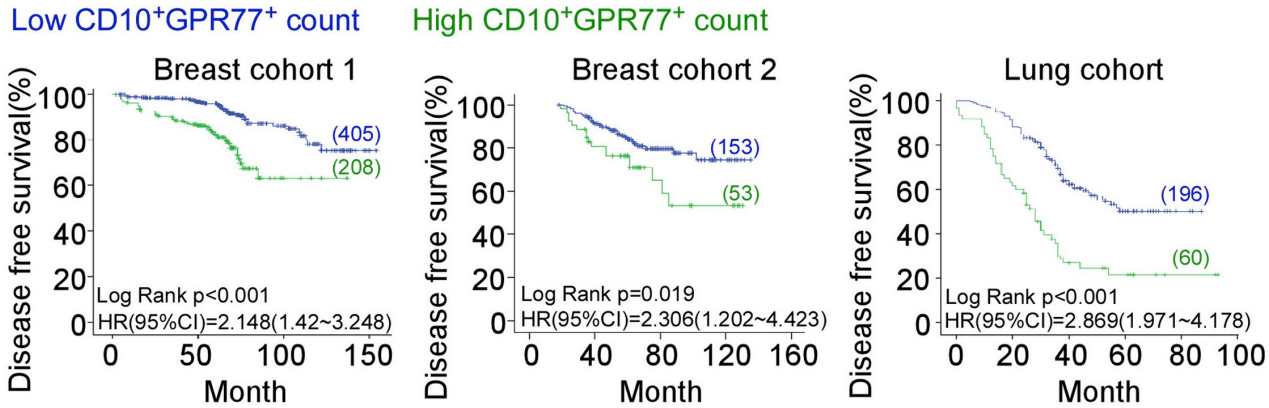
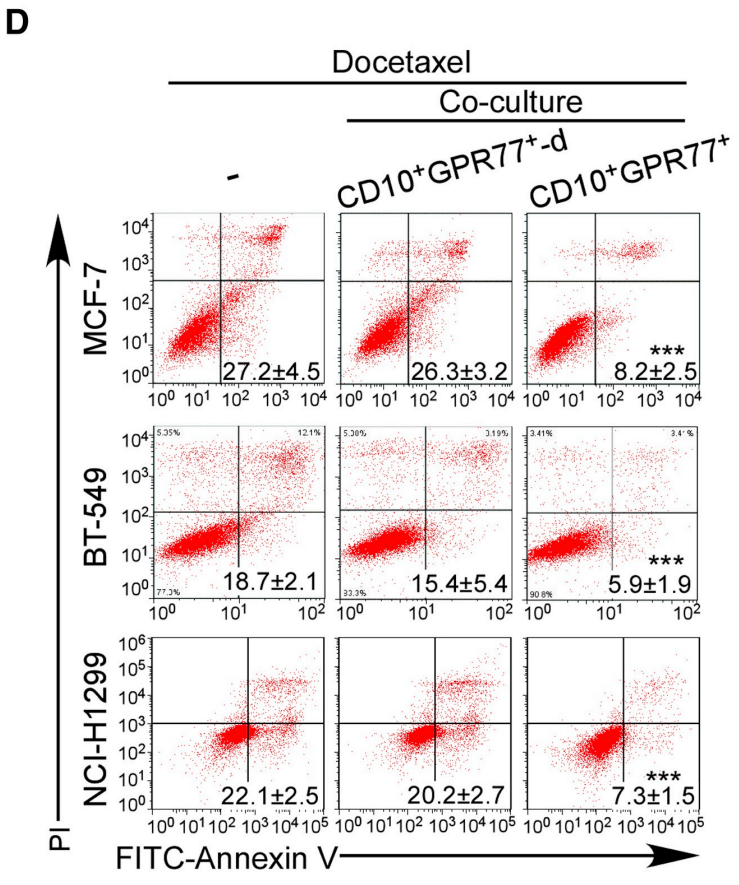
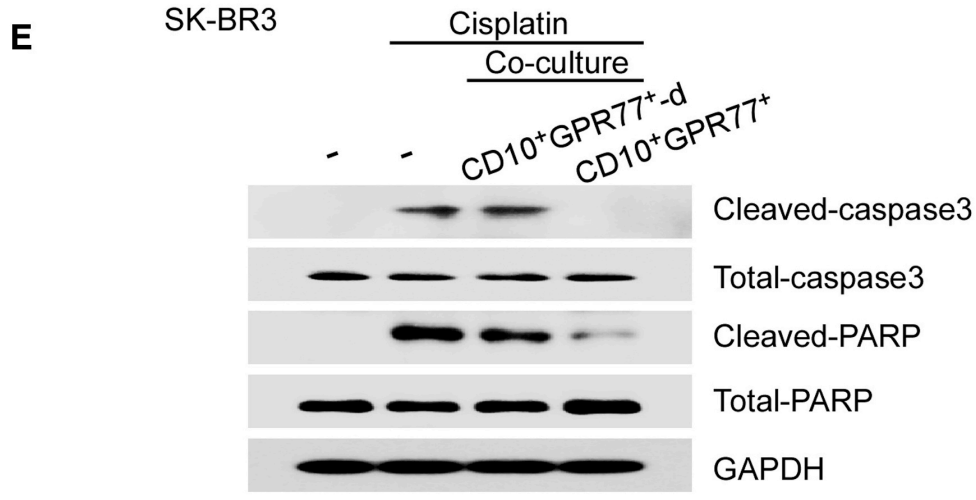


Fig. 1

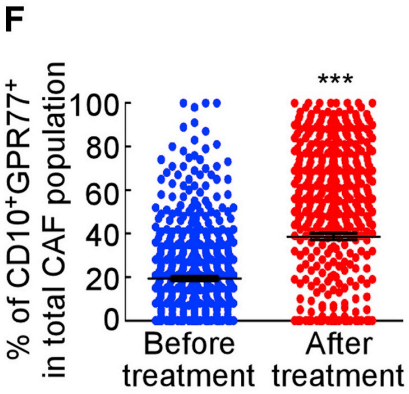
- A CAF subset with high CD10 and GPR77 expression correlates with chemoresistance and poor survival in breast and lung cancer patients

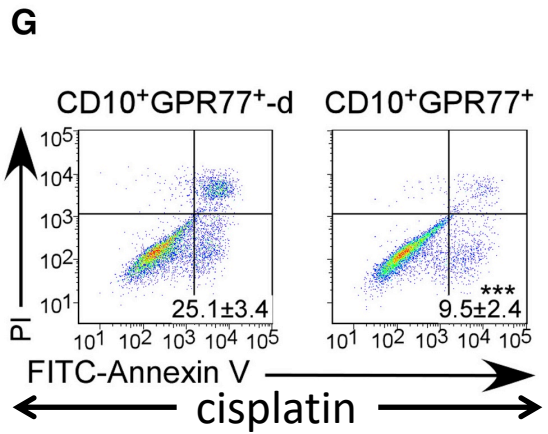
- What potential significance does this finding have for cancer patients/clinicians?

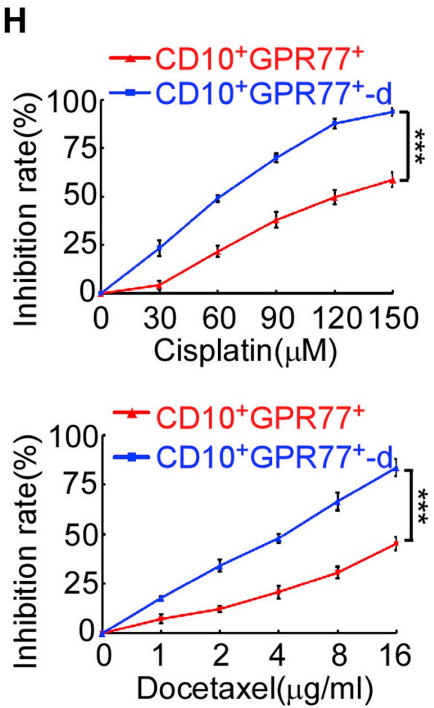




- Tumor cell survival enhanced when co-cultured with $CD10^{+}GPR77^{+}$ CAFs:





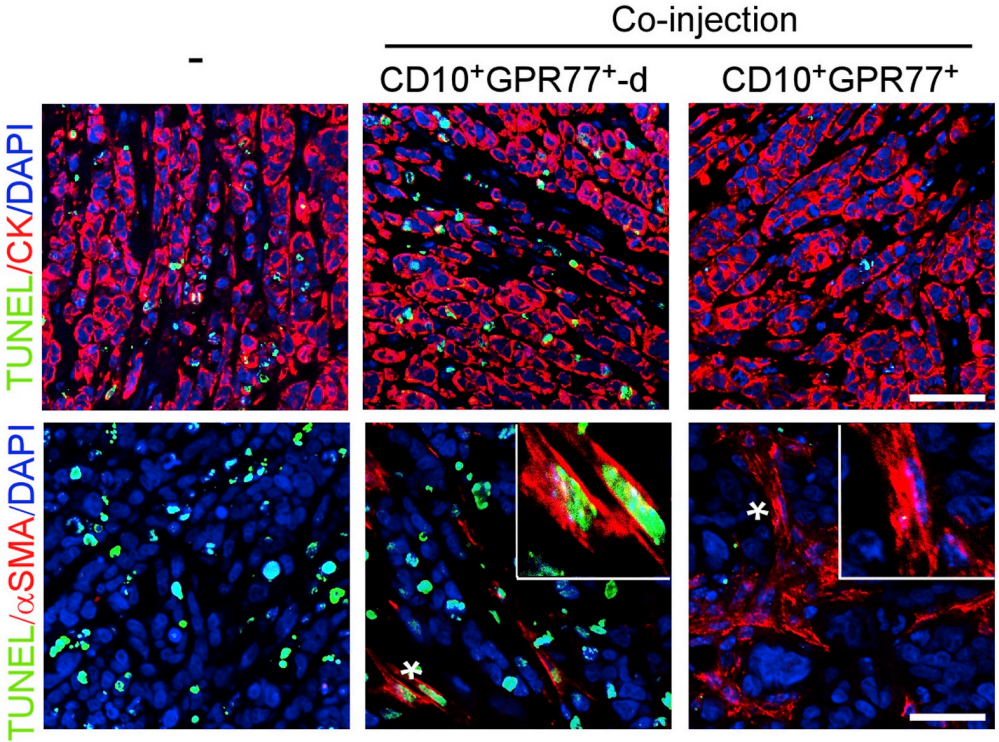


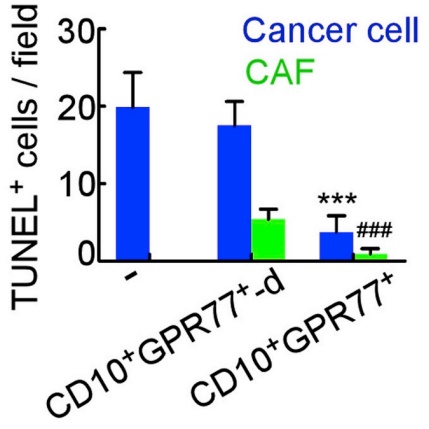
Proportion of CD10⁺GPR77⁺ CAFs increased after neo-adjuvant chemotherapy

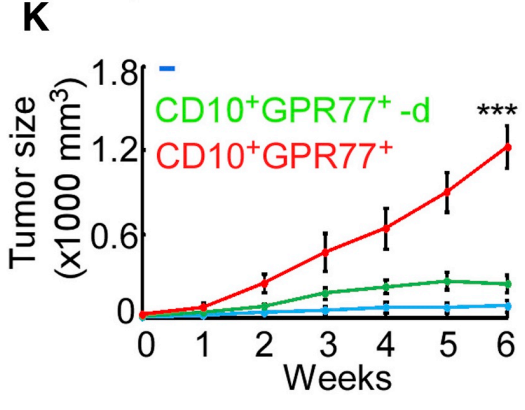
CD10⁺GPR77⁺ CAFs show greater resistance to chemotherapeutics *in vitro*

(tumor cells co-cultured with CD10⁺GPR77⁺ CAFs express elevated ABCG2, which rendered tumor cells more resistant to chemotherapeutics)

co-injected CAFs and BrCa cells into mammary fat pads of nude mice treated with docetaxel:







⇒ data suggest that CD10⁺GPR77⁺ CAFs are not only chemoresistant but can also convey chemoresistance to tumor cells in their microenvironment

Fig. 2

- $CD10^{+}GPR77^{+}$ CAFs induce chemoresistance of tumor cells and are chemoresistant themselves

  - When co-inoculated into nude mice, human CAFs are rapidly lost and replaced by infiltrating mouse fibroblasts ("host stromal response"). The authors stained the stromal component in their xenografts using anti-smooth muscle actin antibody.

  ⇒ How could the authors have investigated the human/mouse origin of the stromal cells?

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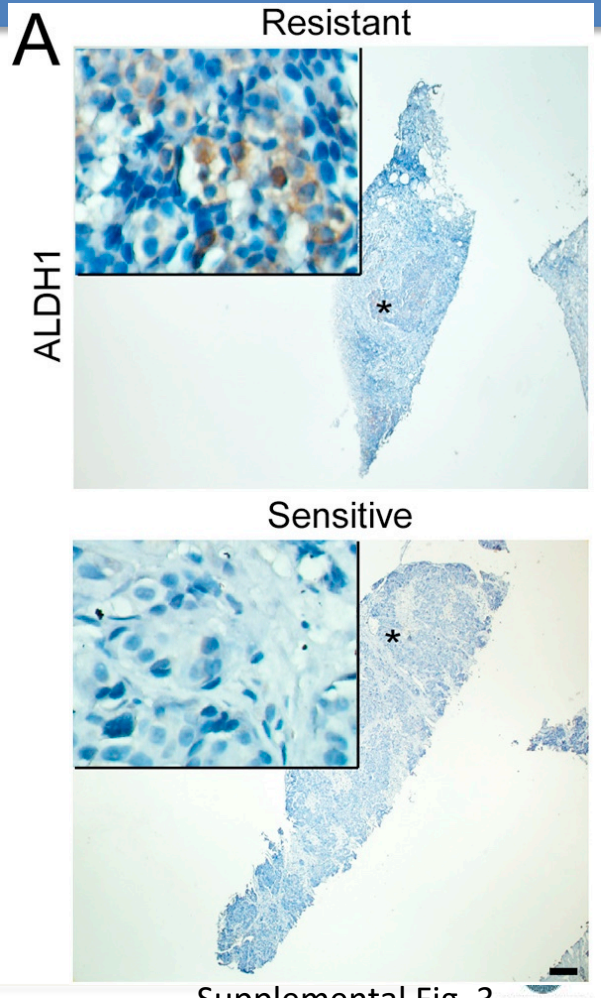
Cancer Stem Cells (CSCs)

- highly-tumorigenic & chemo-resistant cells
- CSC markers typically non-specific/unclear (ALDH1)
- like normal stem cells, maintenance of CSCs requires supportive niche
- fibroblasts are main components of CSC niches
- CAFs isolated from only a fraction of BrCa patients could enrich CSCs

➢ heterogeneous capacity of CAFs in supporting CSCs?

➢ could $CD10^{+}GPR77^{+}$ CAF subtype be supporting CSCs and thus lead to chemoresistance?

➢ proportion of $ALDH1^{+}$ tumor cells correlates with chemoresistance



Supplemental Fig. 3

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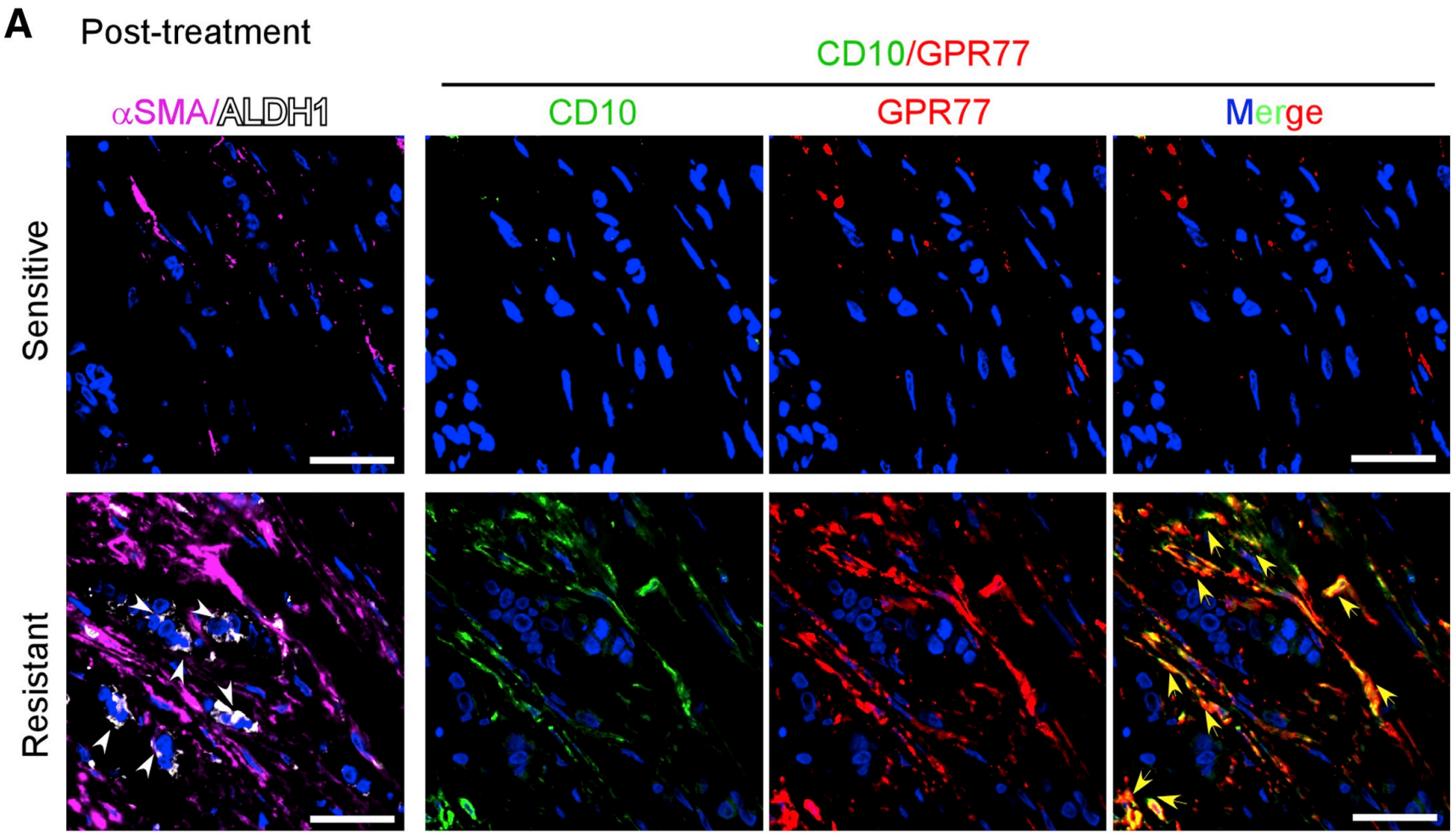
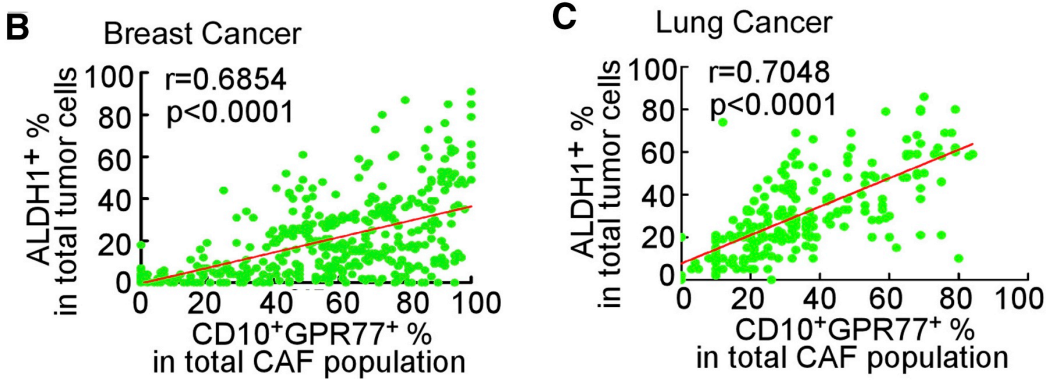
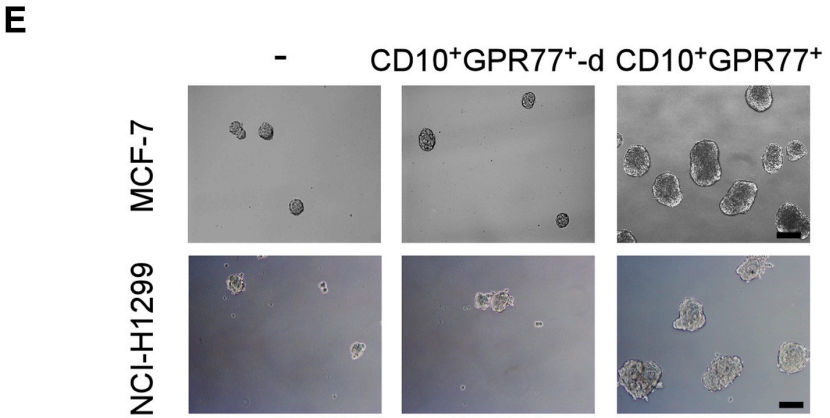


- $CD10^{+}GPR77^{+}$ CAFs surround $ALDH1^{+}$ CSCs

Fig. 3

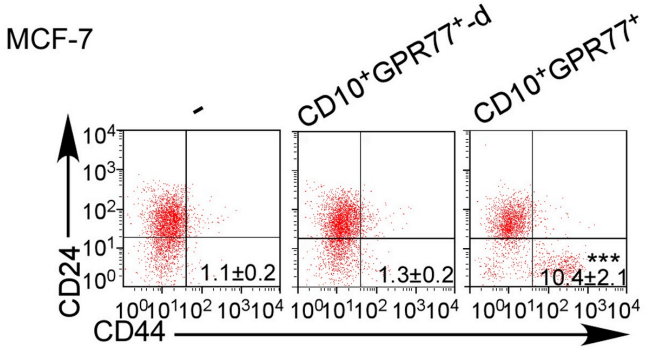


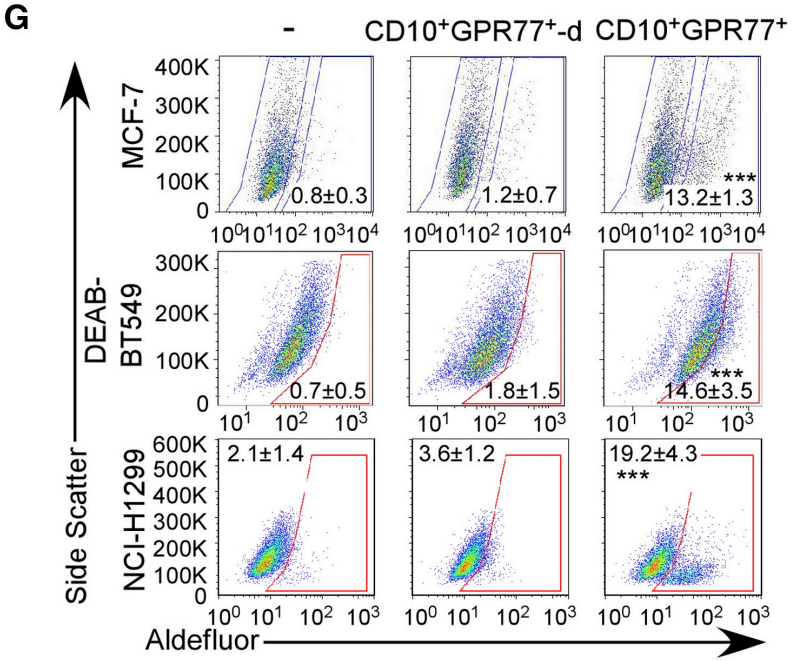
- $CD10^{+}GPR77^{+}$ CAFs positively correlate with abundance of $ALDH1^{+}$ CSCs



- more mammospheres formed when tumor cell lines co-cultured with $CD10^{+}GPR77^{+}$ CAFs

Fig. 3



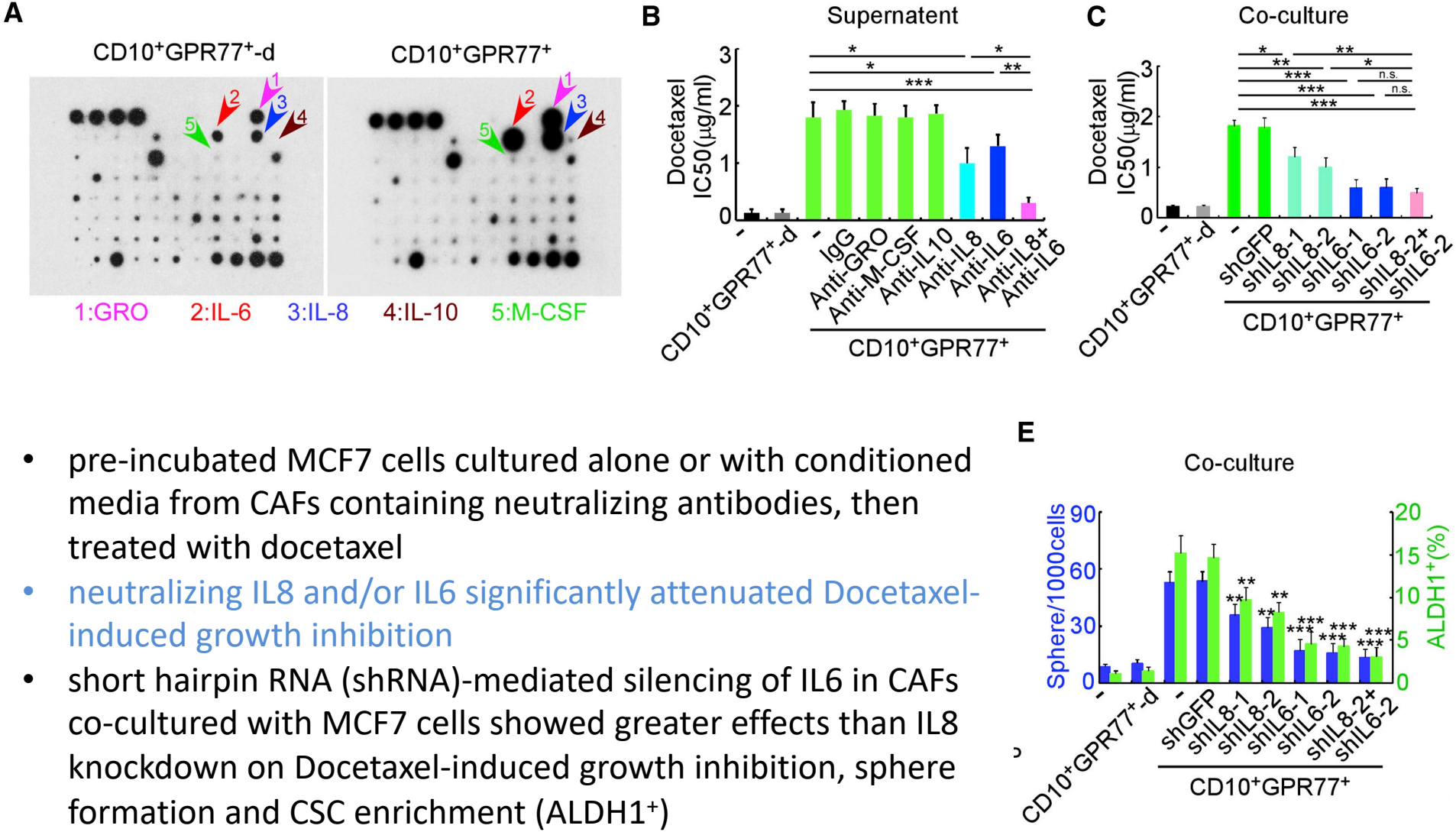


- proportion of CD24⁺CD44⁺ and ALDH1⁺ breast cancer CSCs increased upon co-culture with CD10⁺GPR77⁺ CAFs

Fig. 3

- $CD10^{+}GPR77^{+}$ CAFs constitute a supporting niche for CSCs

  - What is the significance of the experiments using mammospheres?
  - Indirect co-culture of CAFs with tumor cells is sufficient to mediate their onco-supportive effects. What does this tell us about the likely effector molecules?



- pre-incubated MCF7 cells cultured alone or with conditioned media from CAFs containing neutralizing antibodies, then treated with docetaxel
- neutralizing IL8 and/or IL6 significantly attenuated Docetaxel-induced growth inhibition
- short hairpin RNA (shRNA)-mediated silencing of IL6 in CAFs co-cultured with MCF7 cells showed greater effects than IL8 knockdown on Docetaxel-induced growth inhibition, sphere formation and CSC enrichment (ALDH1+)

Fig. 4

IL6 and IL8 abundantly expressed in CD10^{+}GPR77^{+} CAFs in chemoresistant BrCa tissues

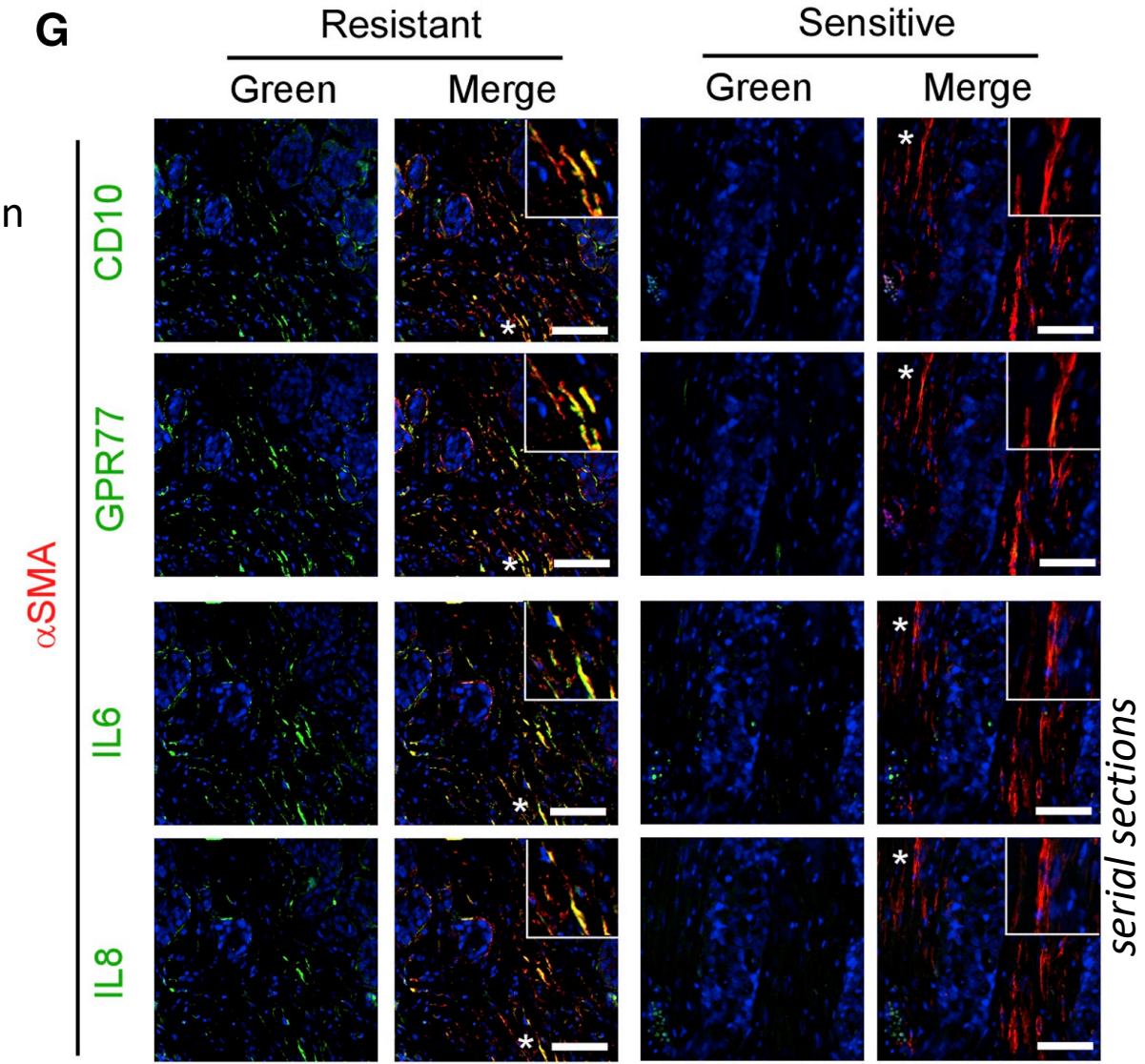
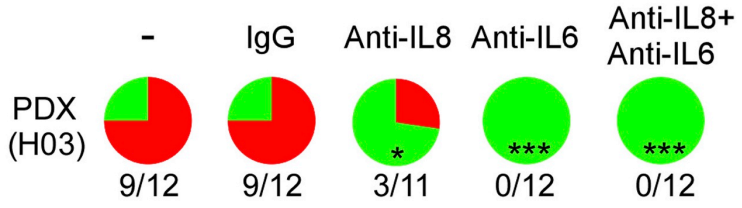
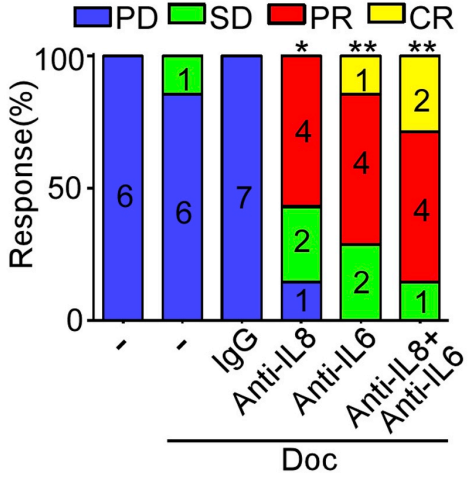
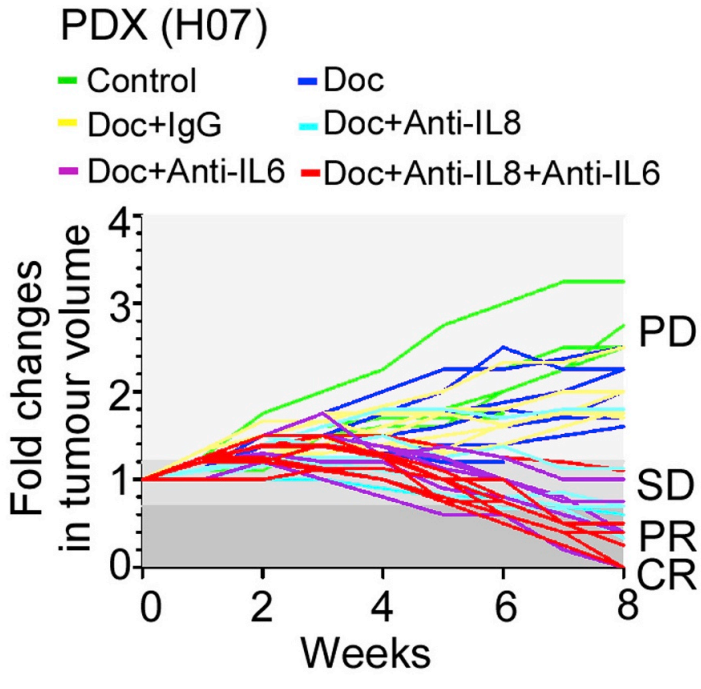


Fig. 4

- implanted breast tumor samples containing high proportions of CD10^{+}GPR77^{+} CAFs (>30%) into nude mice and administered IL6 and/or IL8 neutralizing antibodies:
  - IL8 Ab retarded PDX establishment
  - IL6 Ab alone or in combination with IL8 Ab completely blocked PDX growth







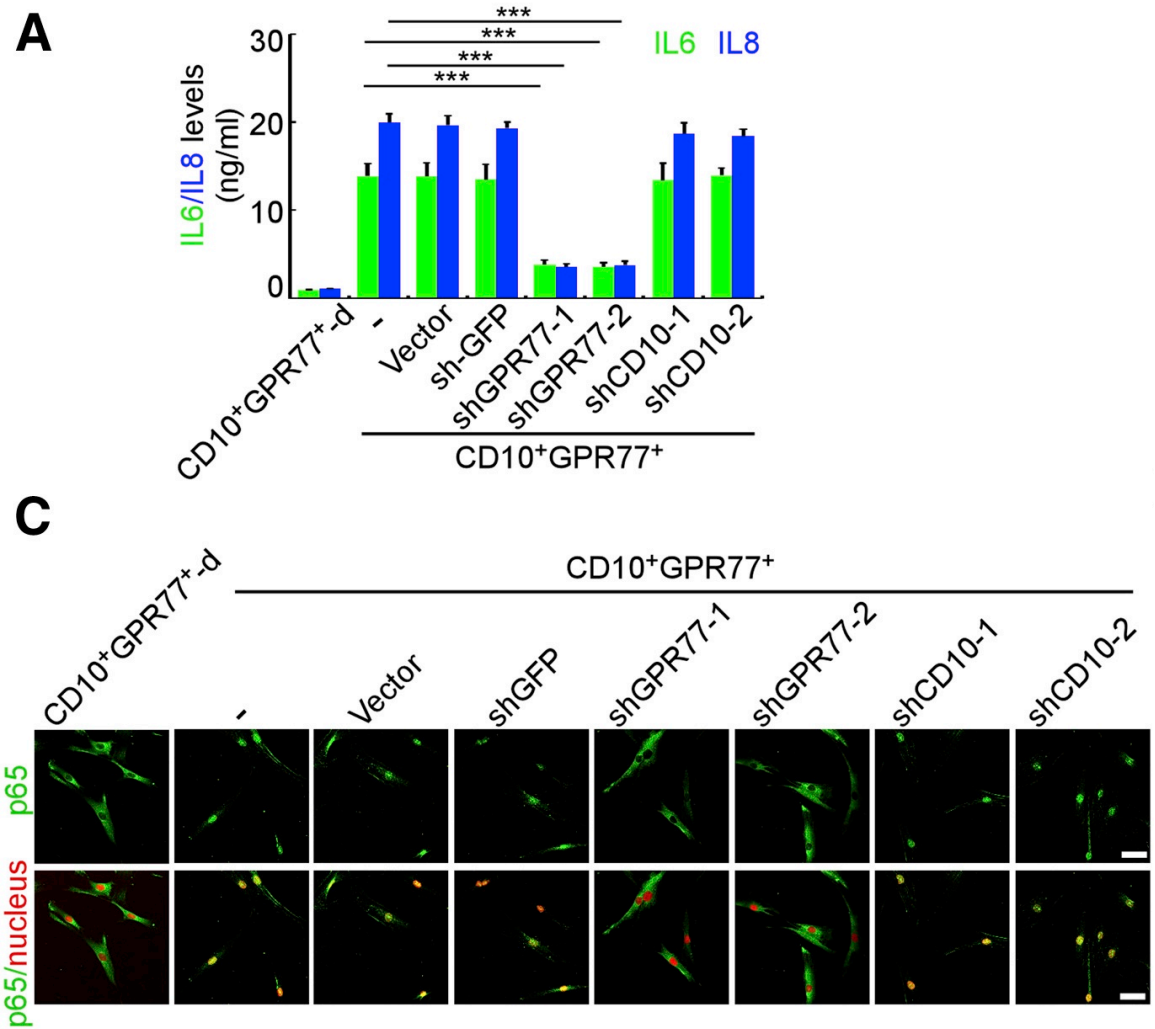
- combined IL8/docetaxel treatment improved IL8 treatment response
- combined IL6/docetaxel treatment almost eradicated PDXs

Fig. 4

- $CD10^{+}GPR77^{+}$ CAFs induce CSC enrichment and chemoresistance by secreting IL-6 and IL-8

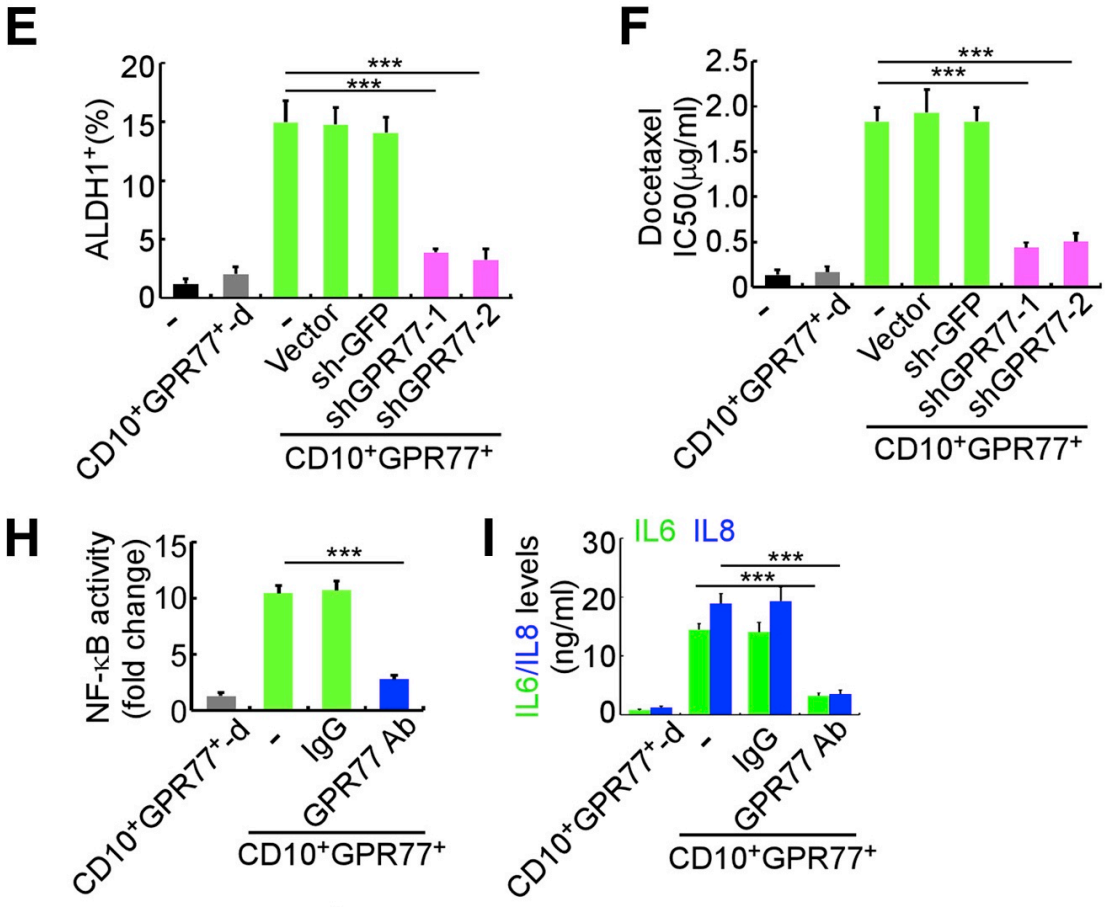
- *Do CD10 or GPR77 play role in maintaining CAF subset functions/signaling pathways?*

Fig. 6



- *Do CD10 or GPR77 play role in maintaining CAF subset functions/signaling pathways?*

Fig. 6



GPR77 depletion/nAb reduces CSC enrichment and chemoresistance of MCF7 breast cancer cells

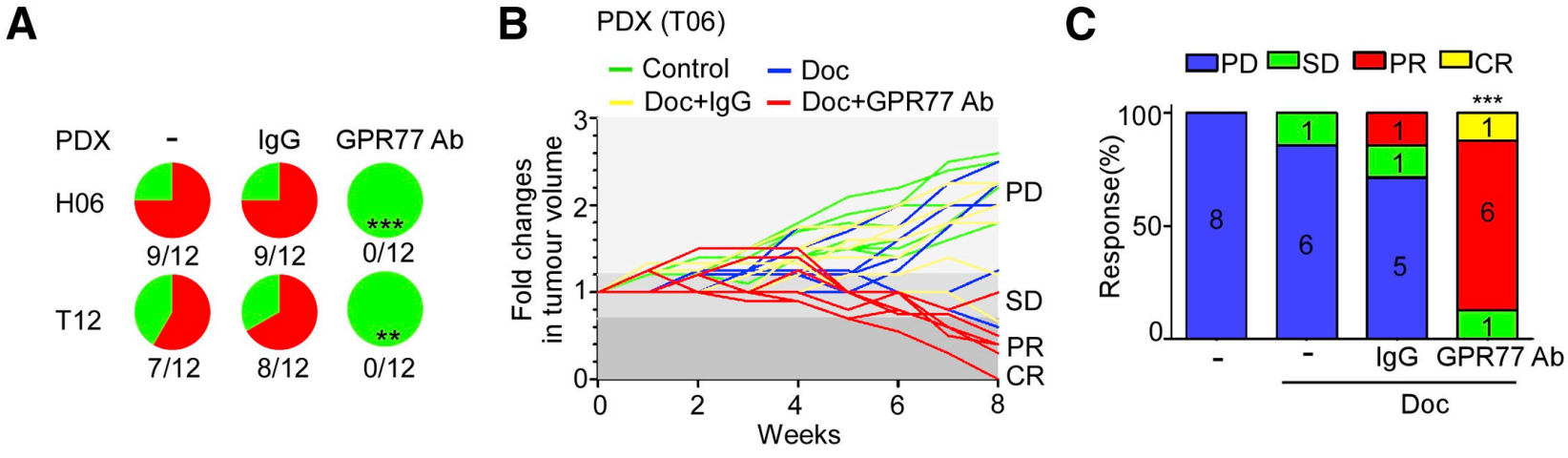
(GPR77 is a known receptor for complement C5a. C5a nAb recapitulated effects of GPR77 knockdown. CAFs may produce complement to self sustain GPR77 signaling.

- GPR77 is required for sustained NF-κB activation in $CD10^{+}GPR77^{+}$ CAFs

- Do $CD10^{+}GPR77^{+}$ CAFs represent a stable, self-sustained population and can they be dynamically reversed to the CAF "ground state" (e.g. to the $CD10^{-}$ $GPR77^{-}$ population)?

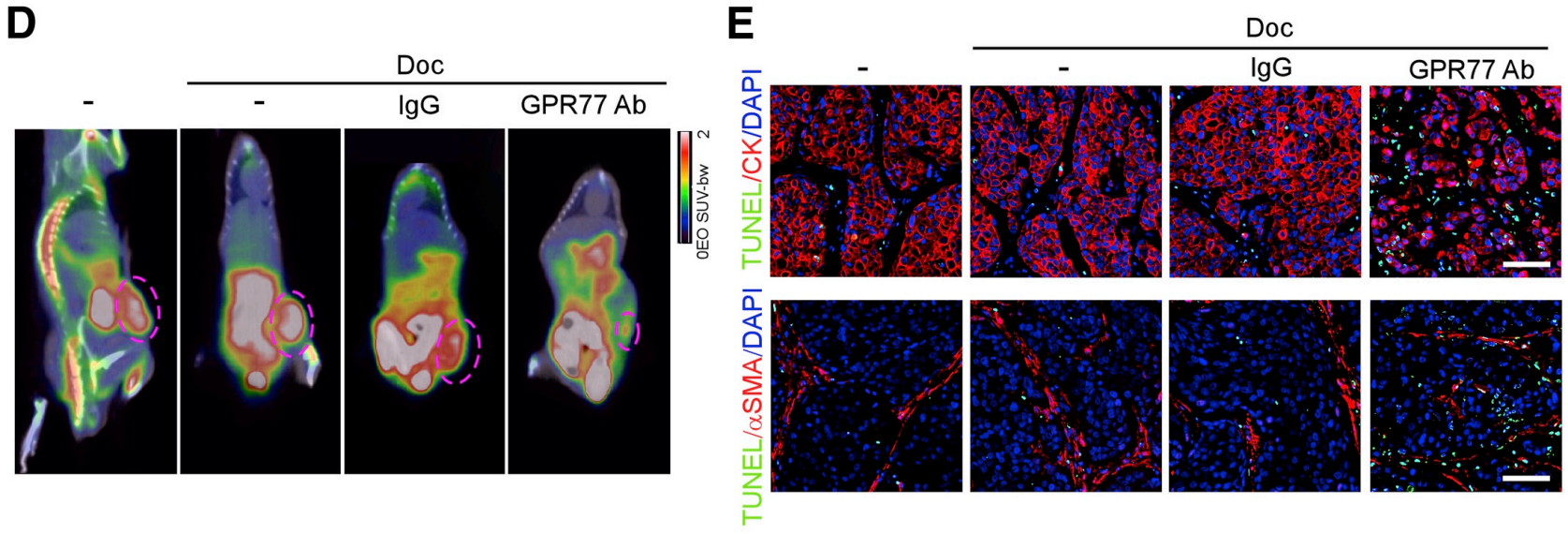
- *Therapeutic potential of anti-GPR77 neutralizing Ab?*

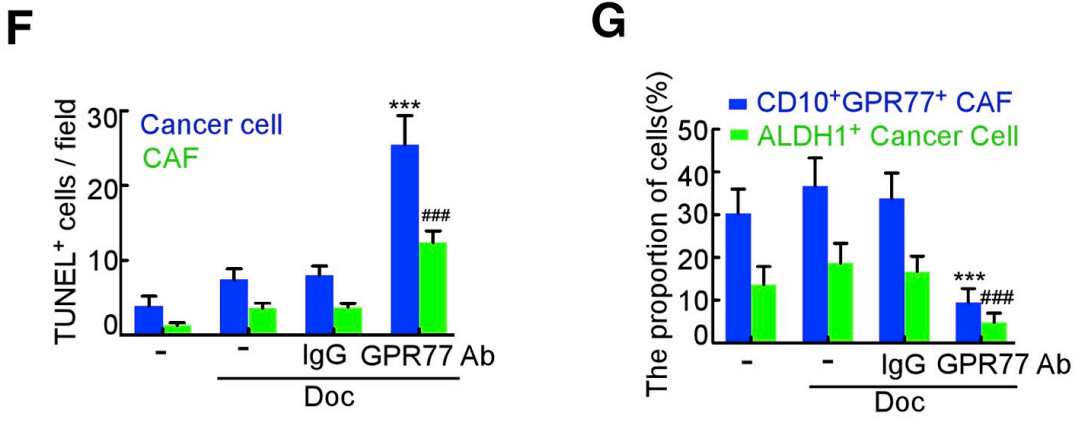
Fig. 7



anti-GPR77 Ab almost abolished PDX establishment

blocking GPR77 reverses chemoresistance in breast cancer with high infiltration of $CD10^{+}GPR77^{+}$ CAFs





- Combined treatment with anti-GPR77 Ab enhanced apoptosis of both tumor cells and CAFs
- reduced infiltration of CD10+GPR77+ CAFs and proportion of ALDH1+ breast CSCs in PDXs

- Treatment of breast cancer PDXs with anti-GPR77 inhibits tumorigenesis and enhances chemotherapeutic effects

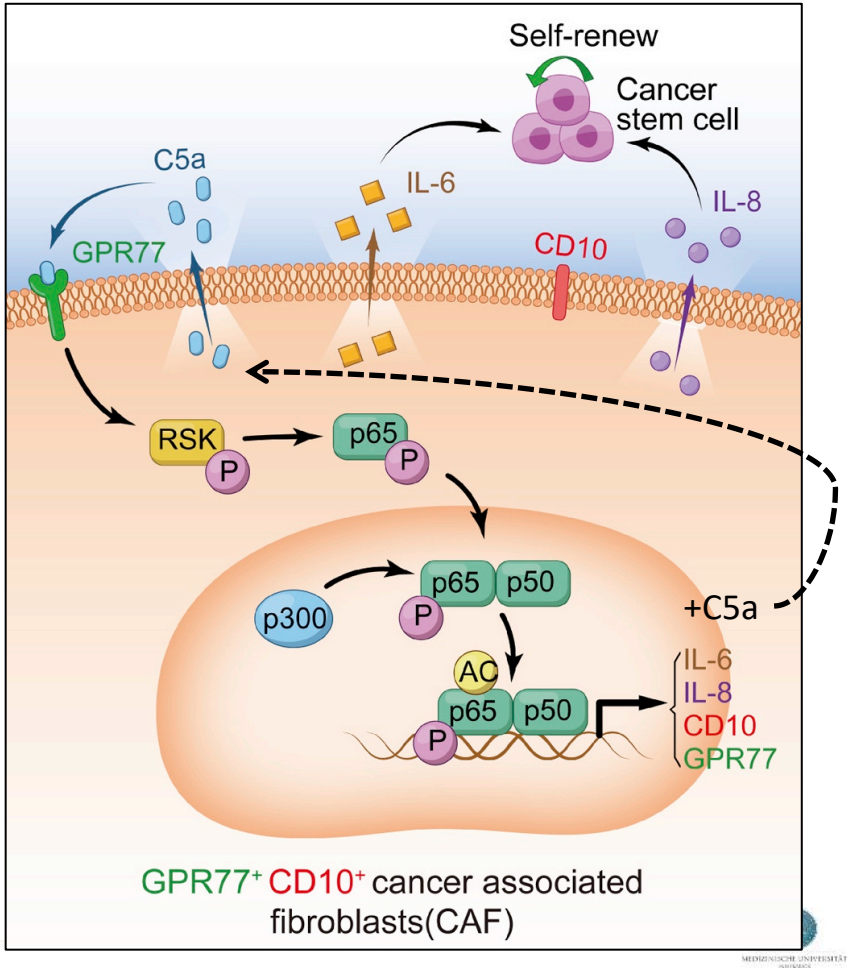
Summary

- CD10/GPR77 co-expression defines a human CAF subset that provides a niche for CSCs and protects them from chemotherapy-induced cell death
- $CD10^{+}GPR77^{+}$ CAFs themselves are also chemo-resistant
- niches formed by $CD10^{+}GPR77^{+}$ CAFs provide constant source of IL-6 and IL-8 for the CSCs due to persistent NF-κB signaling maintained by p65 phosphorylation/acetylation
- GPR77 (a C5a receptor) plays a crucial functional role in maintaining p65 post-translational modification and sustained NF-κB signaling (autocrine C5a-NF-κB loop)

- CSCs difficult to target (no defined marker and may be replenished by non-CSCs in presence of supporting niche

- targeting the supportive niche alternative option?

- <u>proof-of-principle:</u>
  GPR77 neutralizing Ab, eradicates $CD10^{+}GPR77^{+}$ CAFs & CSCs, retards tumor formation and reverses chemoresistance in PDX mouse models



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Questions to be addressed

1. What is the prevalence and functional importance of this CAF subtype across other tumors?

2. What is the origin of these CAFs?
3. How does the $CD10^{+}GPR77^{+}$ CAF subset form? They are already present prior to neo-adjuvant therapy so there must be an inducing event linked to but not necessarily derived directly from the cancer cells

1. How is C5 activated in the tumor microenvironment?
2. Is C5a ligand supplied within bona fide tumors in an autocrine or paracrine fashion?

3. Clearly there are other CAF subtypes in these tumors (e.g. $CD10^{-}GPR77^{-}$). Are these tumor promoting or tumor inhibiting or even both?

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TAKE HOME MESSAGES

- Stromal microenvironment is dynamic and integral part of solid tumors that plays key role in tumor development/progression, immune suppression, therapy resistance and clinical outcome

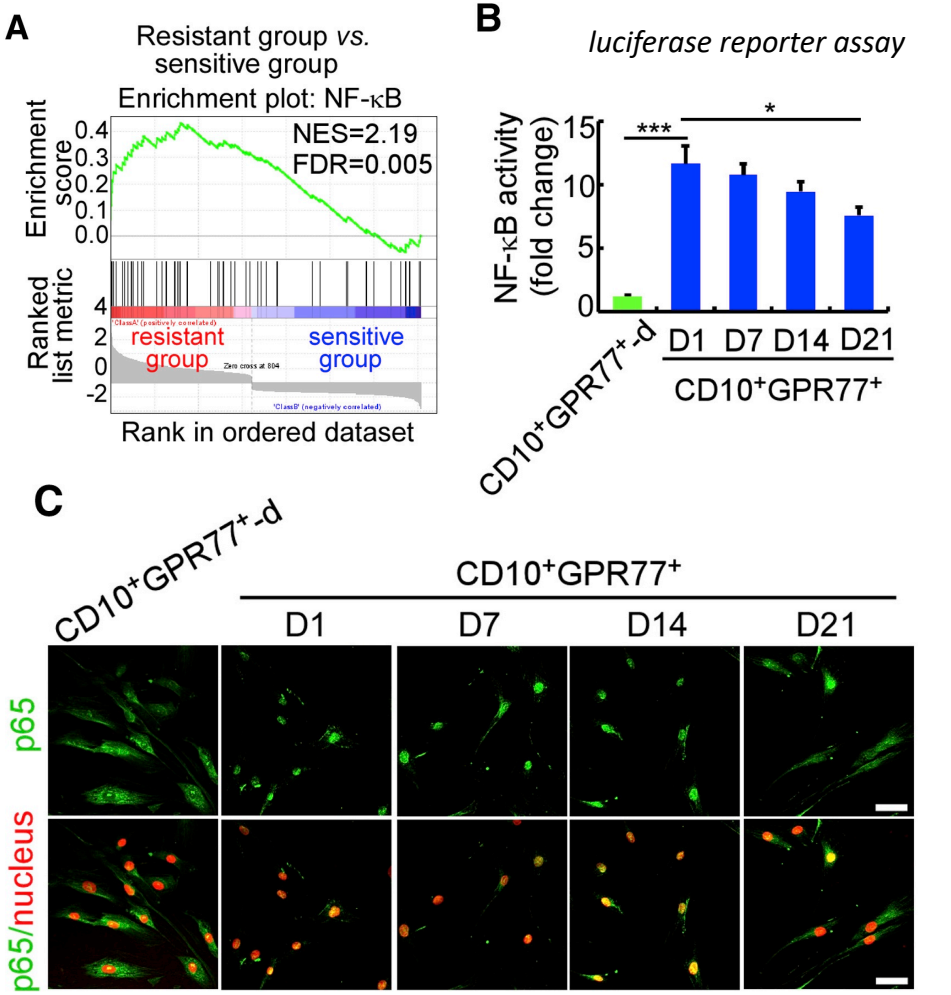
- Tumor stroma not just a innocent bystander but an active driver of tumor progression

- Altered paracrine signaling by CAFs is major effector mechanism underlying tumor-promoting actions of the tumor-associated stroma (effects on tumor cells, ECM remodeling, angiogenesis and immune suppression)

- Restoring paracrine signaling networks between stromal components/eradicating specific CAF subpopulations may represent anti-cancer therapeutic strategy

(subsequent slides for additional info only)

➢ Which signaling pathways sustain IL-6/IL-8 production in CD10⁺GPR77⁺ CAFs?



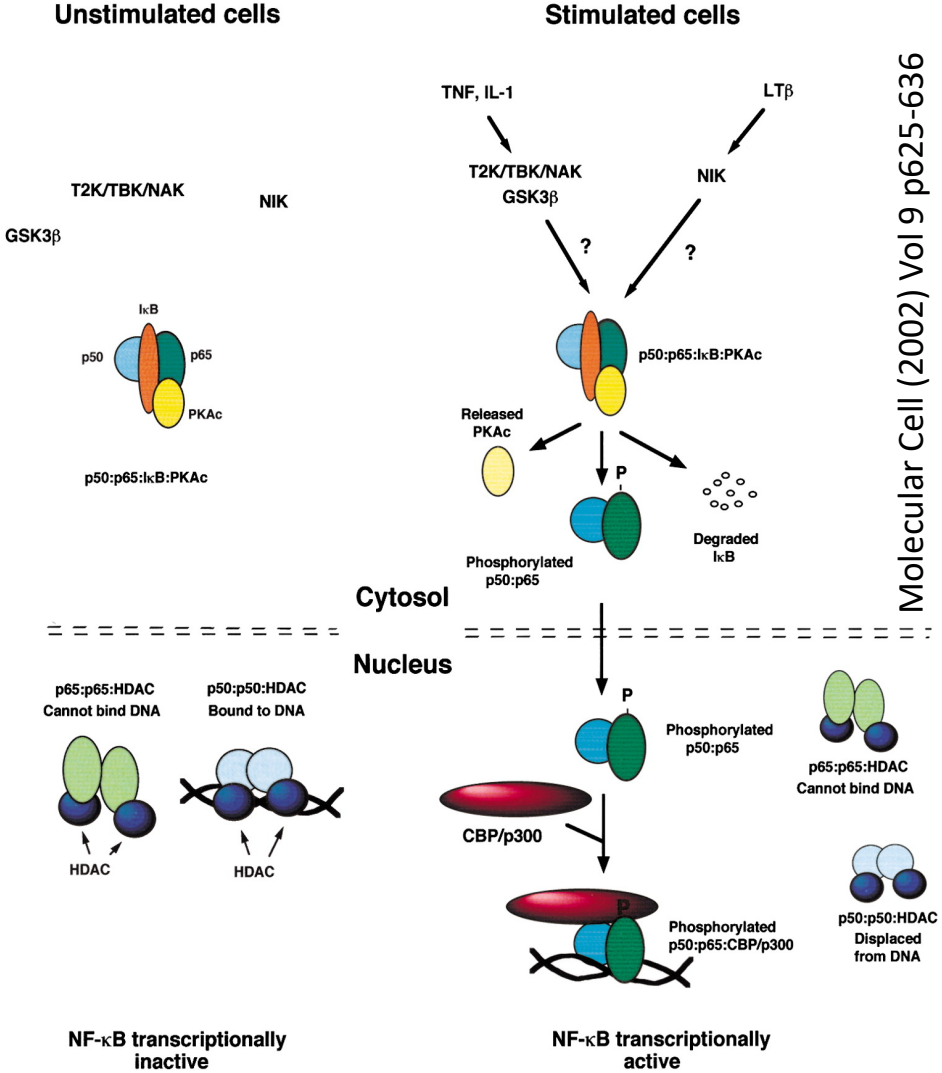
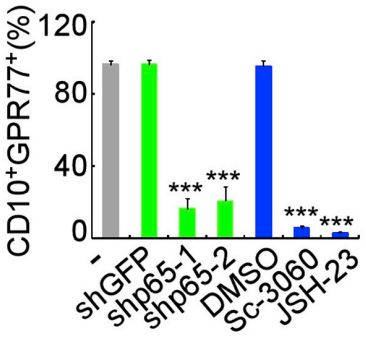


Fig. 5

D



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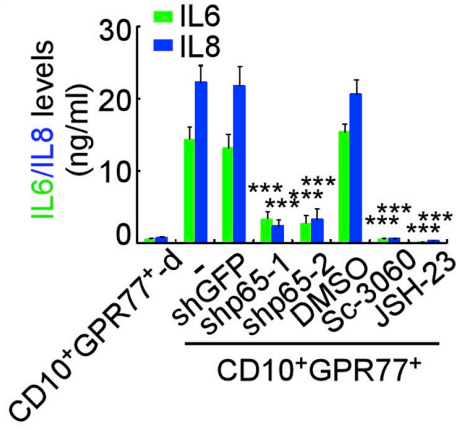
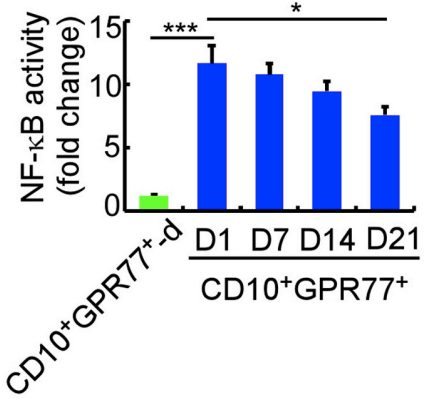


- NF-κB signaling essential for CD10⁺GPR77⁺ CAF phenotype and function

can NF-κB signaling sustain CD10⁺GPR77⁺ CAF phenotype?

B

luciferase reporter assay



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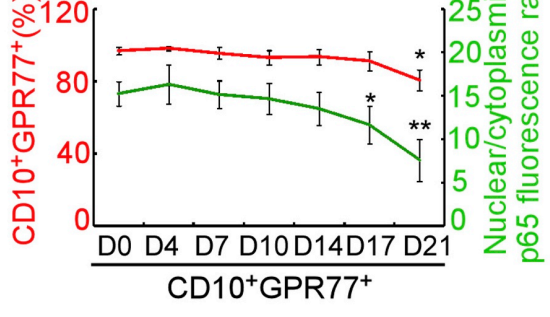
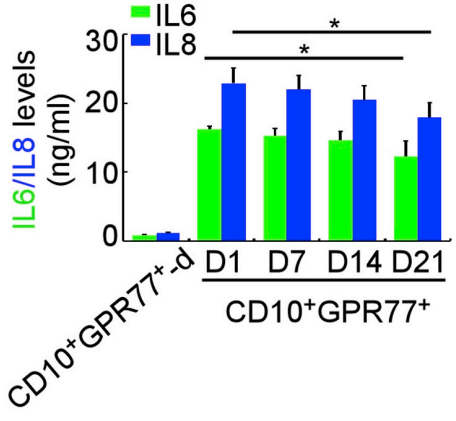




NF-κB transcriptional activity, CD10/GPR77 expression and IL-6/-8 production sustained in CD10⁺GPR77⁺ CAFs

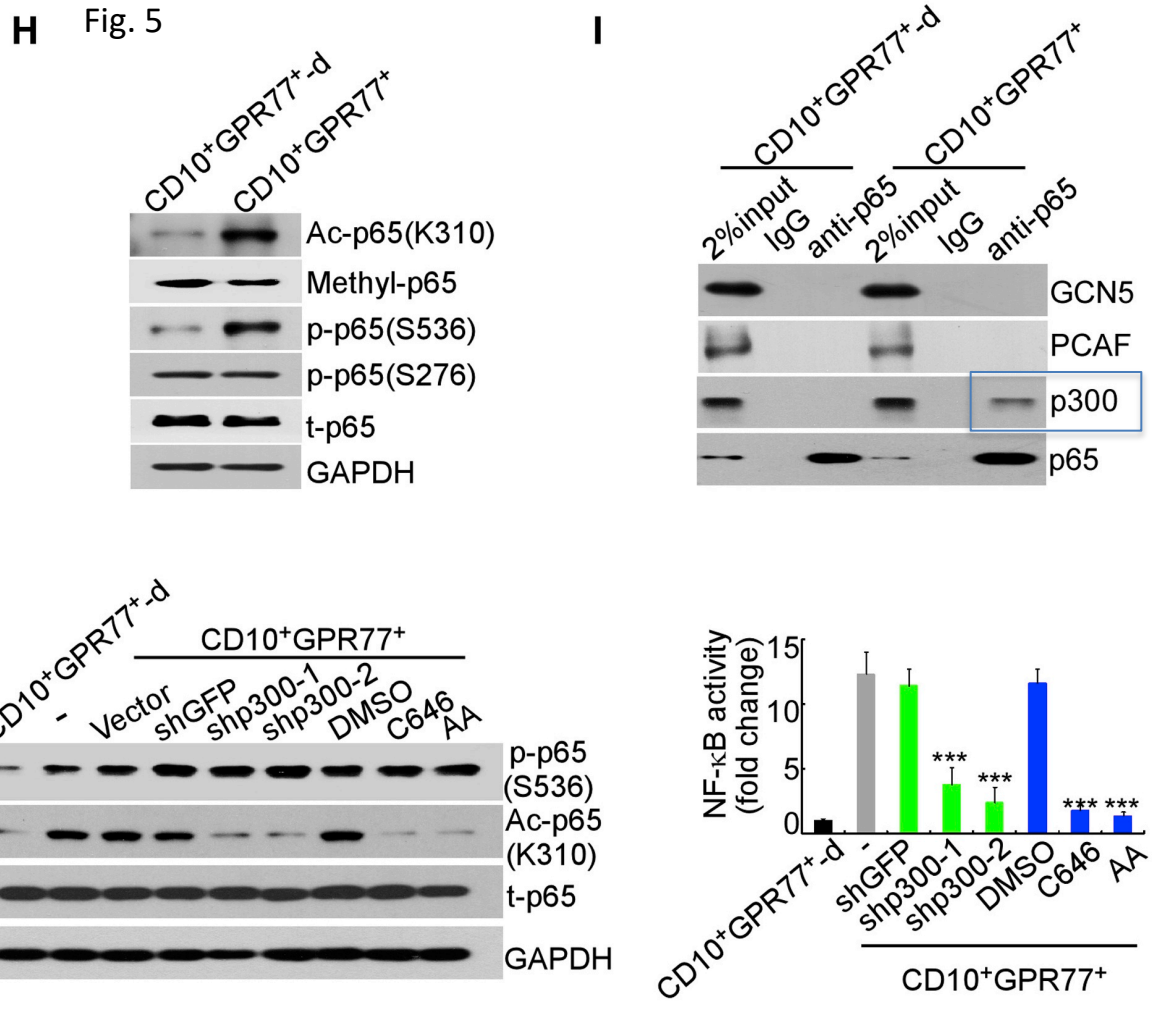
- *$CD10^+GPR77^+$ CAFs display low IKK/IκBα phosphorylation levels (sustained p65 nuclear retention independent?). Other mechanisms?*

- elevated p65 acetylation (K310) and phosphorylation (S536) in $CD10^+GPR77^+$ CAFs

- co-IP revealed p65 interacted with the histone acetyltransferase p300 in $CD10^+GPR77^+$ CAFs

- p300 silencing/inhibition reduced NF-kB activity, p65 acetylation and nuclear accumulation but not p65 phosphorylation

Fig. 5



➢ interaction of p65 with p300 leads to its acetylation at K310 but does not influence its phosphorylation in these CAFs

- Prolonged NF-κB activation (via p300-mediated p65 acetylation) maintains the phenotypes and functions of $CD10^{+}GPR77^{+}$ CAFs