Suppl. Table 1: Differently expressed genes in CAFs

	Gene	Description
1.	AIMIL	Beta/gamma crystallin domain-containing protein 2
2.	ANXA3	Annexin A3
3.	BAMBI	BMP and activin membrane-bound inhibitor homolog
4.	BCYRN1	Brain Cystoplasmic RNA 1
5.	BICDL1	BICD Family-like cargo adaptor 1
6.	C10orf10	Protein DEPP1
7.	C1orf106	Innate immunity activator protein
8.	C1orf116	Specifically androgen-regulated gene protein
9.	CA2	Cabronic anhydrase 2
10.	CBLC	E3 ubiquitin-protein ligase CBL-C
11.	CCL20	C-C motif chemokine 20
12.	CCL5	C-C motif chemokine 5
13.	CDH23	Cadherin-23
14.	CDH3	Cadherin-3
15.	CDH7	Chromodomain-helicase-DNA-binding protein 7
16.	CHMP4C	Charged multivesicular body protein 7
17.	CLSTN2	Calsyntenin-2
18.	COL9A3	Collagen-alpha-3(IX) chain
19.	CXCL11	C-X-C motif chemokine 11
20.	DMKN	Dermokine
21.	DOK3	Docking protein 3
22.	DSC2	Desmocollin-2
23.	EDN1	Endothelin-1
24.	EFNA1	Ephrin-A1
25.	**EFNB2**	**Ephrin-B2**
26.	EPPK1	Epiplakin
27.	F11R	Junctional adhesion molecule A
28.	F12	Coagulation factor XII
29	FAM83H-ASI	FAM83H antisense RNA 1
30.	GJB2	Gap junction beta 2 protein
31.	GJB3	Gap junction beta 3 protein
32.	GRHL2	Grainyhead-like protein 2 homolog
33.	HAS3	Hyaluronan synthase 3
34.	HERC5	E3 ISG15-protein ligase HERC5
35.	HERC6	Portable E3 ubiquitin-protein ligase HERC6
36.	HORMADI	HORMA domain-containing protein 1
37.	IFI27	Interferon-alpha-inducible protein 27, mitochondrial
38.	IFI44L	Interferon-induced protein 44-like
39.	IFI6	Interferon-alpha-inducible protein 6
40.	IFIH1	Interferon-induced helicase C domain-containing protein 1
41.	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
42.	IFIT2	Interferon-induced protein with tetratricopeptide repeats 2
43.	IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
44.	IFNLR1	Interferon lambda receptor 1
45.	IGSF3	Immuniglobulin superfamily member 3
46.	INHBB	Ubiquitin beta B chain
47.	IRF6	Interferon regulatory factor 6
48.	ISG15	Ubiquitin-like protein ISG15
49.	ITGB4	Integrin beta-4
50.	ITGB8	Integrin beta-8

	Gene	Description
51.	KCNN4	Intermediate conductance calcium-activated potassium channel protein 4
52.	KDF1	Keratinocyte differentiation factor 1
53.	KLK10	Kallikrein-10
54.	KRT14	Keratin, type 1 cytoskeletal protein 14
55.	LAMC2	Laminin subunit gamma-2
56.	LRRC8B	Volume-regulated anion channel subunit LRRC8B
57.	LTB	Lymphotoxin-beta
58.	MIR205HG	MIR205 host gene
59.	MST1R	Macrophage-stimulating protein receptor
60.	NECTIN4	Nectin-4
61.	NKD1	Protein naked cutivle homolog 1
62.	NMU	Neuromedin-U
63.	OAS1	2´-5´-oligoadenylate synthase 1
64.	OAS2	2´-5´-oligoadenylate synthase 2
65.	PDE10A	cAMP and cAMP-inhibited cGMP 3´-5´-cyclic phosphodiesterase 10A
66.	PI3	Elafin
67.	PKP2	Plakophilin-2
68.	PLS1	Plastin-1
69.	PROSER2	Proline and serine-richt protein 2
70.	PTK6	Protein-tyrosine kinase 6
71.	PTPN6	Tyrosine-protein phosphate non-receptor type 6
72.	RBM47	RAN-binding protein 47
73.	RSAD2	Radical S-adenosyl methionine domain containing protein 2
74.	S100P	Protein S100-P
75.	SAA1	Serum amyloid A-1 protein
76.	SAA2	Serum amyloid A-2 protein
77.	SERPINB5	Serpin B5
78.	SH2D2A	SH2 domain containing protein 2A
79.	SMOC1	SPARC-related modular Calcium-binding protein 1
80.	SNX10	Sorting nexin-10
81.	TGFA	Protransforming growth factor alpha
82.	TMEM184A	Transmembrane protein 184A
83.	USP18	Ubl carboxyl-terminal hydrolase 18
84.	VAV3	VAV Guanine nucleotide exchange factor 3
85.	WFDC2	WAP four-disulphide core domain protein 2
86.	WNT6	Protein Wnt6
87.	WWC1	Protein KIBRA
88.	XIST	X inactive specific transcript
89	ZNF704	Zinc finger protein 704

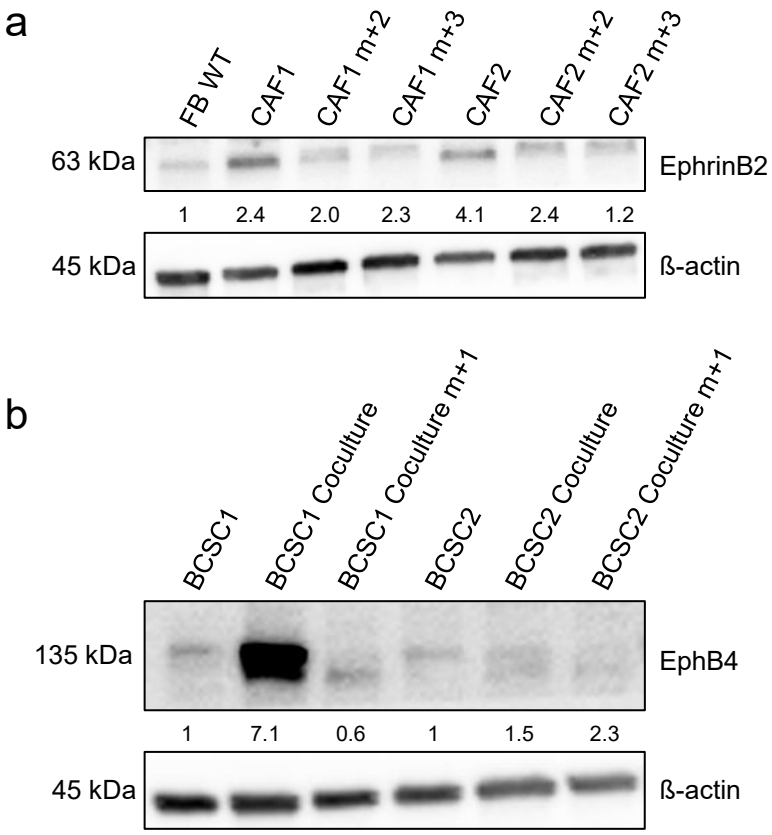
Significantly upregulated genes ($p \leq 0.01$; Fold change ≥ 5) in CAF1 and CAF2 after coculture with BCSC1 and BCSC2, FACS sort and then MACE-sequencing

Suppl. Table 2 Plasmids used for lentivirus production

	Cell type created	Plasmid used
1.	EFNB2 Knockdown (FB KD)	pLV[shRNA]-mCherry/Neo-U6>hEFNB2[shRNA#1] (Vectorbuilder, VB900120-6682jag)
2.	Control fibroblasts (FB Scr) [19]	pLV[shRNA]-mCherry/Neo-U6>Scramble_shRNA#1 (Vectorbuilder, VB010000-0012tsh)
3.	G418 resistant BCSCs (BCSCs were nuclear GFP tagged beforehand)	pLV[shRNA]-LacI:T2A:Neo-U6/2xLacO >Scramble[shRNA#1] (Vectorbuilder, VB260421-1579quq)

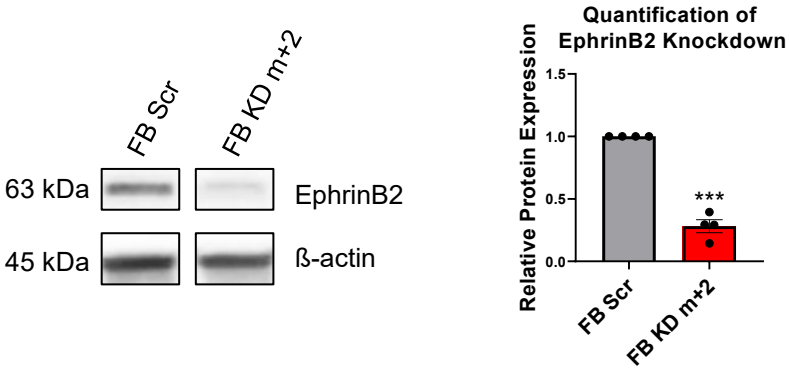
Following Plasmids were used for lentiviral transduction of shRNA. Lentivirus using plasmid no. 3 was created by Emma Spreemann

Suppl. Fig. 1: EphrinB2 and EphB4 upregulation reduced over time



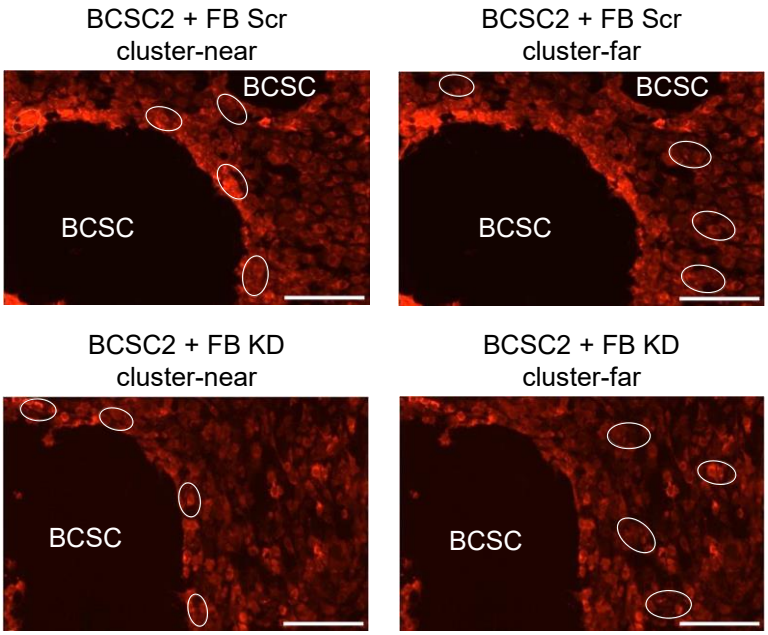
a) Western blot and quantification of EphrinB2 in fibroblasts after coculture with BCSC1 (CAF1) or BCSC2 (CAF2) directly after coculture and FACS sort as well as 2 passages (m+2) and 3 passages later (m+3), n=3 mean **b)** Western blot and quantification of EphB4 in BCSC1 and BCSC2 after coculture with fibroblasts directly after coculture and FAC sort as well as a passage later (m+1), n=3 mean

Suppl. Fig. 2: EphrinB2 knockdown is stable over passages



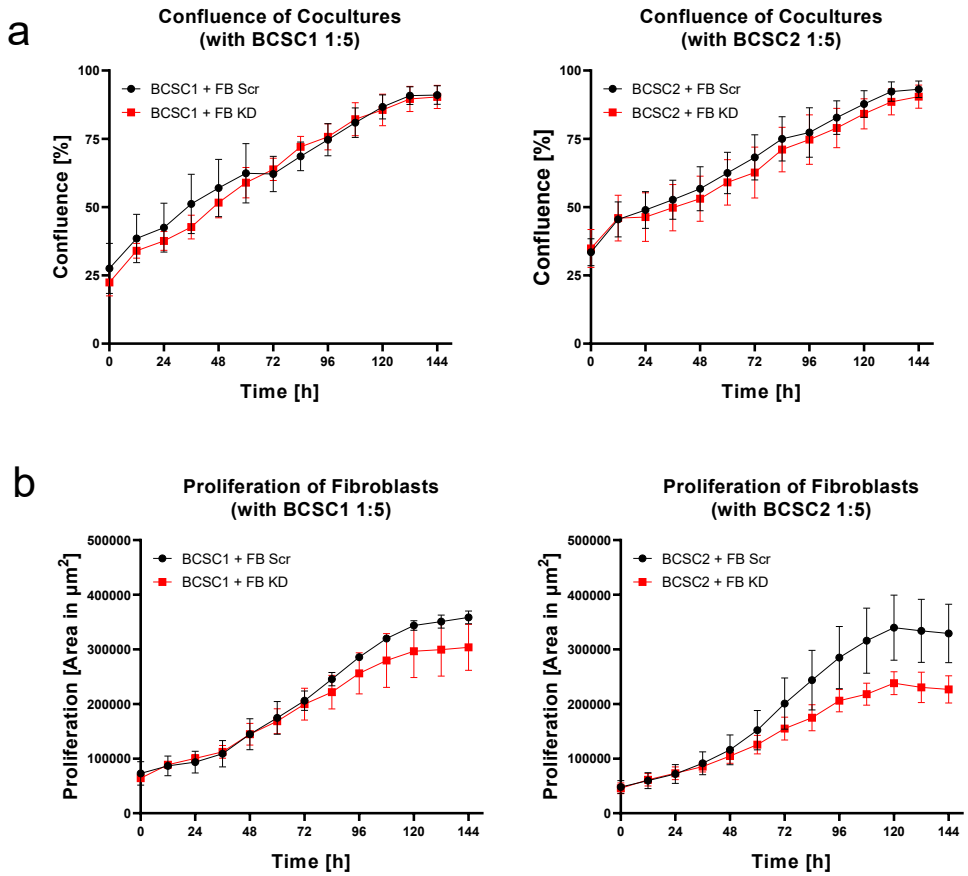
Western blot and quantification of EphrinB2 knockdown in fibroblasts two passages after transduction (m+2), n=4 mean ± SEM p=0.0008

Suppl. Fig. 3: Analysis of fibroblast density BCSC-cluster near vs. cluster far



Representative images showing the quantification of fibroblast density of BCSC-cluster near and BCSC-cluster far fibroblasts. Fibroblasts were cytoplasmatic mCherry-tagged. Using ImageJ, ellipses were placed randomly near or far from the BCSC cluster, then the fluorescence intensity/area was calculated. The intensity/area of the cluster near fibroblasts was compared to the intensity/area of the cluster far fibroblasts. The denser the fibroblasts are, the higher the fluorescence intensity in this area. The larger the difference in density between BCSC-cluster near and cluster far fibroblasts, the larger the difference in the fluorescence intensity between these areas. Scale bar represents 200µm.

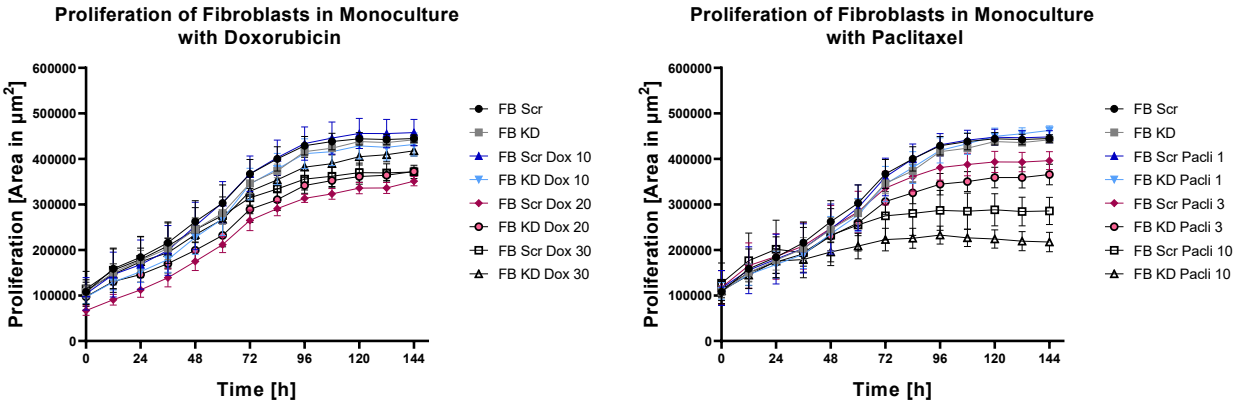
Suppl. Fig. 4: Knockdown-fibroblasts also proliferate slower in coculture with BCSCs in 1:5 ratio



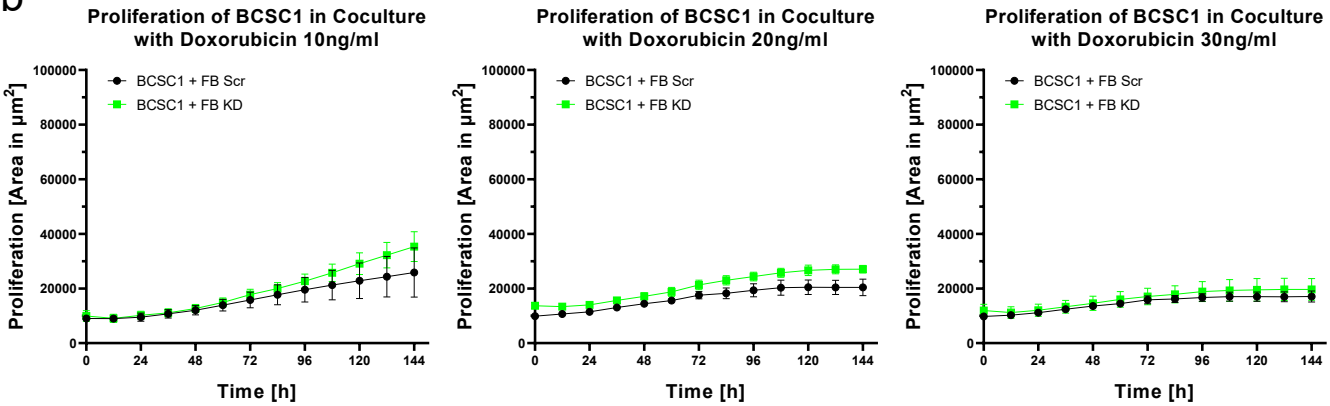
a) Total confluence over time of cocultures with BCSC1 and FB Scr or FB KD (left) and BCSC2 and FB Scr or FB KD (right). Ratio 1:5 n=3 mean ± SEM **b)** Proliferation of FB Scr and FB KD in coculture with BCSC1 (left) and BCSC2 (right). Ratio 1:5 n=3 mean ± SEM

Suppl. Fig. 5: Chemoresistance of BCSCs in coculture with FB Scr or FB KD is not affected

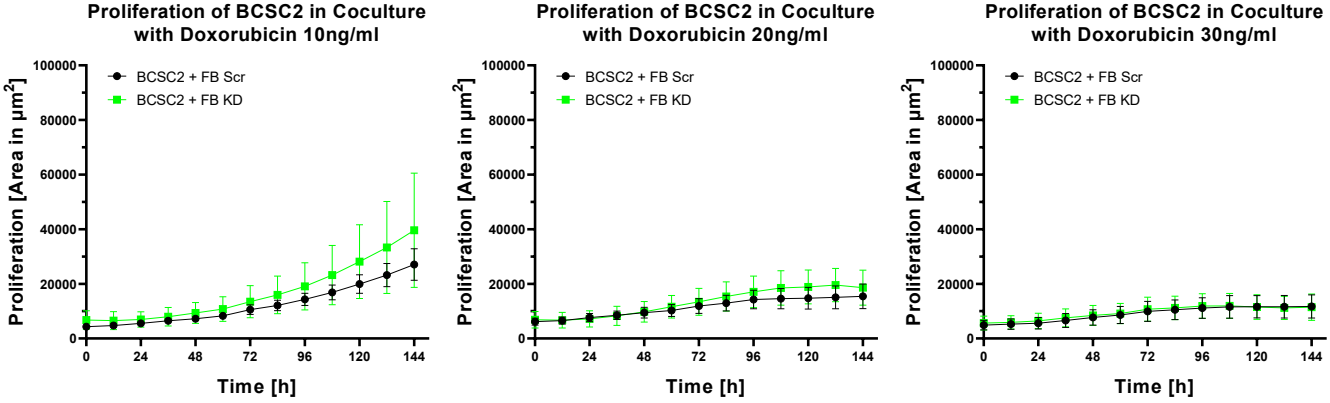
a



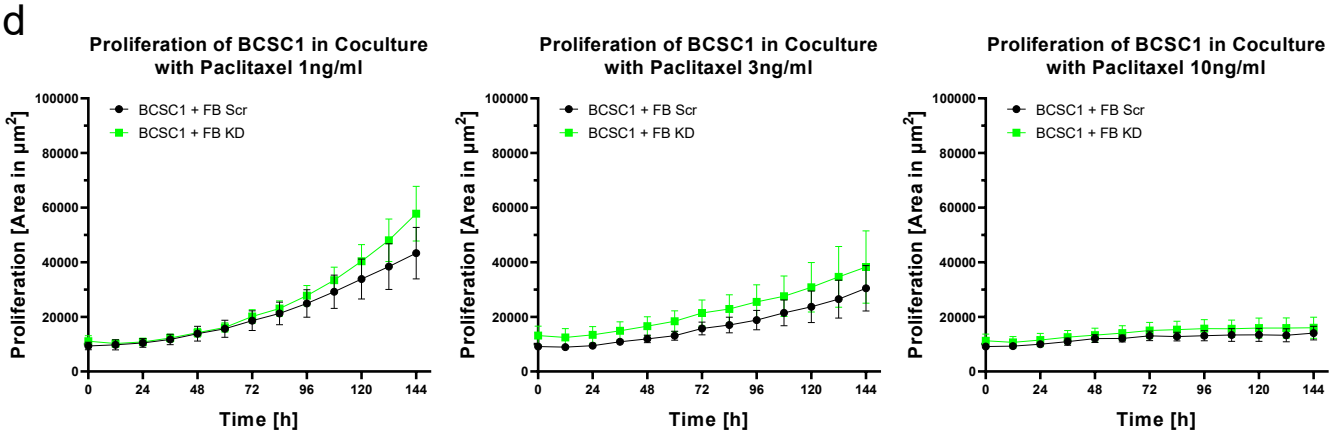
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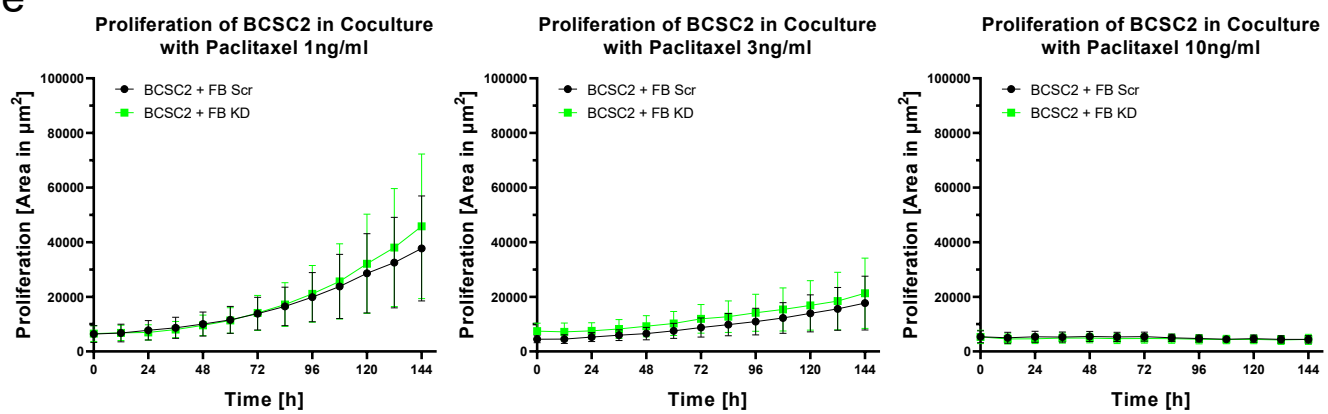
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d



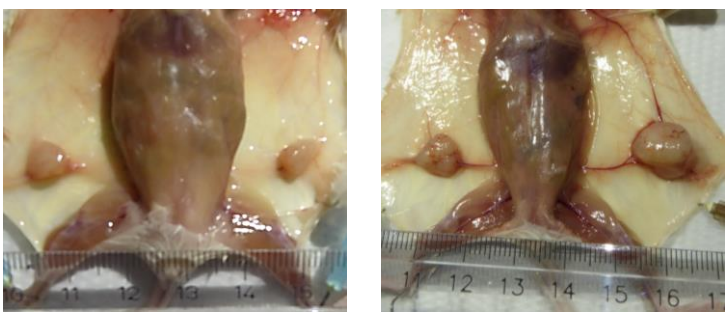
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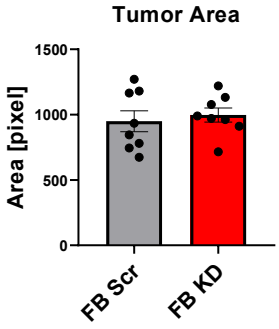
a) Proliferation of FB Scr and FB KD in monoculture and doxorubicin (Dox; left) and paclitaxel (Pacli; right) in increasing concentrations (in ng/ml). n≥3 mean ± SEM **b), c), d), e)** Proliferation of BCSC1 and BCSC2 in coculture with FB Scr or FB KD with increasing concentrations of doxorubicin (10, 20, 20 ng/ml) and paclitaxel (1, 3, 10 ng/ml). n≥3 mean ± SEM

Suppl. Fig. 6: In-vivo experiment with orthotopically transplanted BCSC1 and FB Scr or FB KD

a

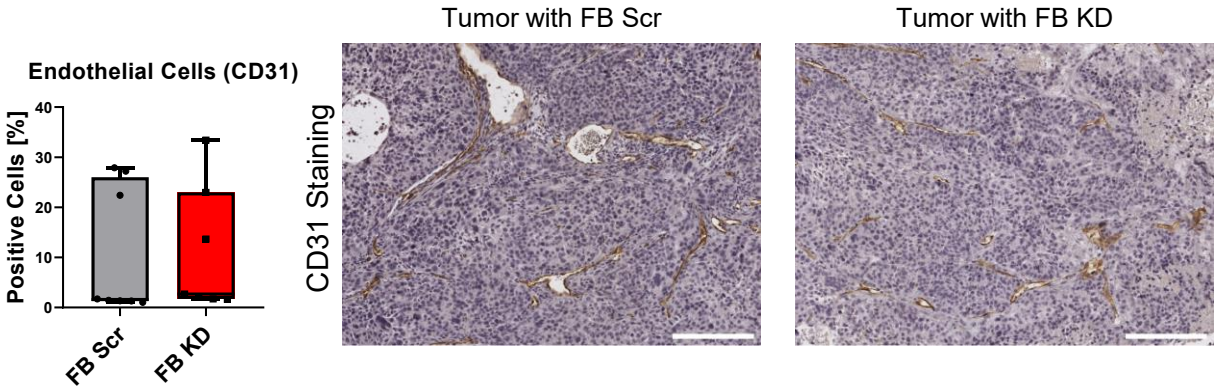


b



a) Representative images of mice autopsies with tumors in both mammary fat pads. Tumors consist of BCSC1 and FB Scr or FB KD in a ratio of 1:5 **b)** Area of the tumors at their widest circumference after autopsy of the mice, measured using ImageJ n=8 mean ± SEM

Suppl. Fig. 7: No differences in vasculature of the tumors observed



Representative images and quantification of immunohistochemistry staining of CD31, so of the endothelial cells in murine tumors consisting of BCSC1 and FB Scr or FB KD n=8. Scalebar represents 200µm