Supplementary Data

Resource

No evidence of clonally selected somatic genomic alterations in cancer associated fibroblasts from human breast and ovarian carcinomas

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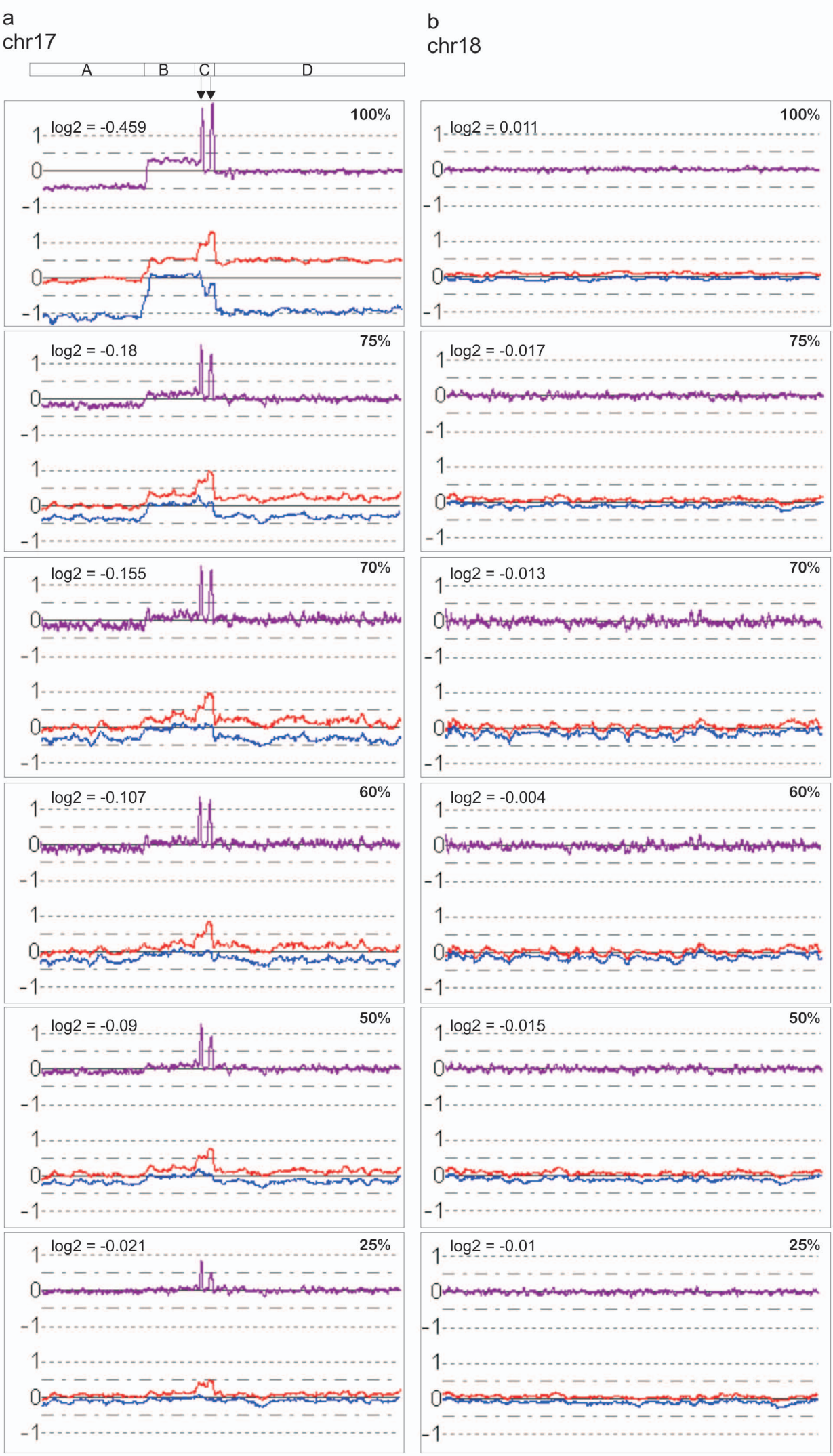
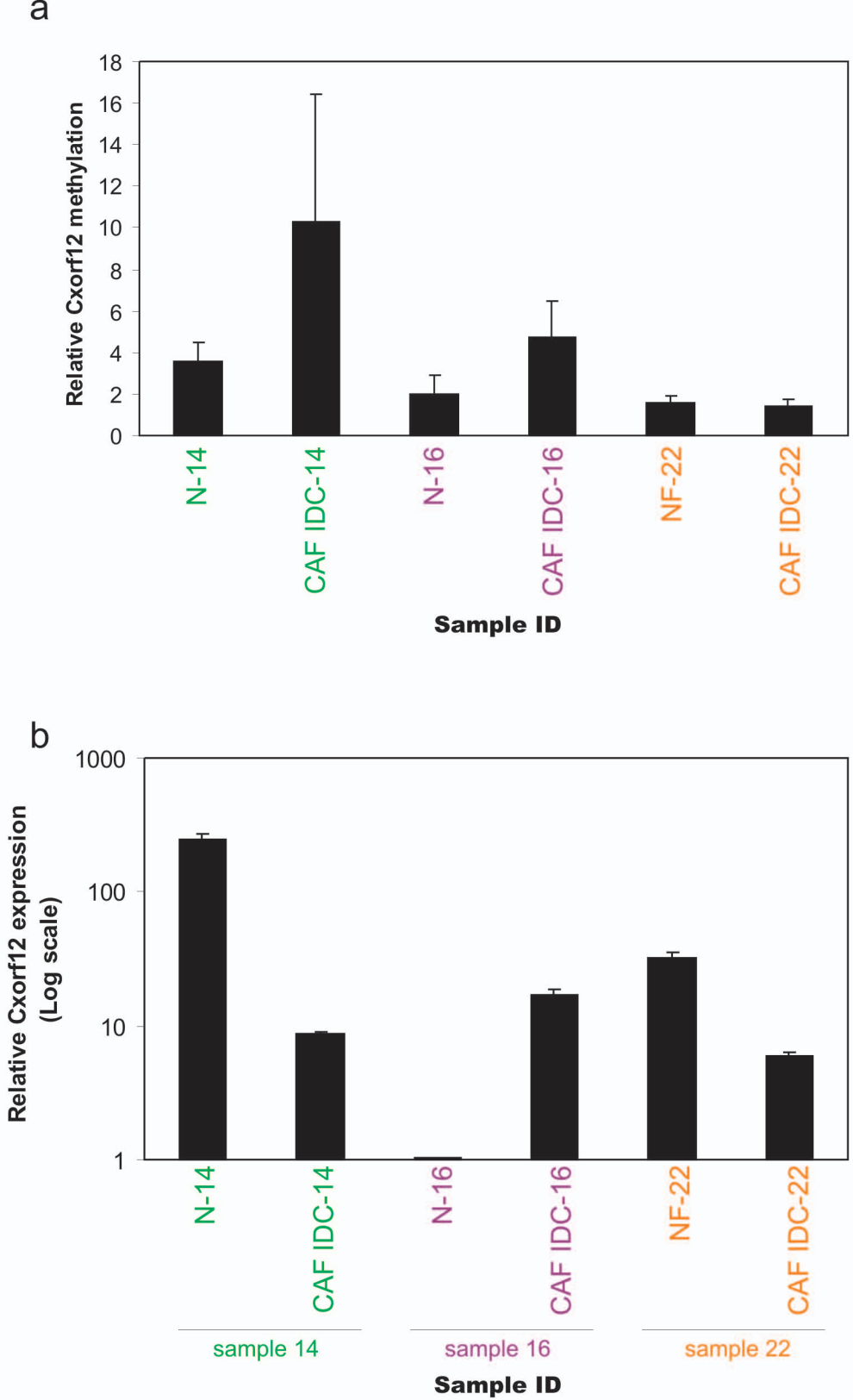


Supplementary Figure 1

Supplementary Figure 1. Copy number and LOH plot of ovarian tumor IC257 containing varying ratios of normal DNA contamination. The copy number and LOH plots for chromosome 17 and 18 are shown in (**a)** and (**b)**, respectively. Tumor DNA for IC257 was mixed with corresponding normal DNA, in ratio of its matching normal DNA (extracted from peripheral blood lymphocytes) to prepare samples with 100%, 75%, 70%, 60%, 50% and 25% tumor DNA. The DNA was then analyzed with 250K *StyI* mapping array. As shown, tumor IC257 shows a complex pattern of LOH and copy number changes on chromosome 17 comprising a region of LOH coupled with copy number loss (region A), low level copy number gain (region B), high level copy number gain (arrows in region C) and copy number neutral LOH (region D). The $\log_2$ copy number value across region A is shown for each sample. The copy number and LOH plot for chromosome 18 is shown for comparison as this chromosome shows no evidence of copy number loss or LOH. As expected, the mean $\log_2$ copy number value across the chromosome 18 is close to zero for all tumor mixtures. Overall, the copy number and LOH changes on chromosome 17 observable with the 100% tumor sample are discernable at least down to 70% tumor content. For example, the high level amplification (region C) is easily observable even down to 25% tumor DNA. The copy number loss at region A is visible at 70% with a $\log_2$ value of -0.155 compared to the $\log_2$ value of -0.013 for the control chromosome 18. LOH across regions A and D are also discernable down to 70% tumor as evidence by the divergence of the allele specific copy number plots relative to the control chromosome 18. Purple line: 40-SNP moving average; Blue and red lines: allele-specific moving average.



Supplementary Figure 2

Supplementary Figure 2. qMSP and qRT-PCR analyses of three CAFs cultures that were tested in xenograft assays. (**a**) qMSP analysis of *Cxorf12* using bisulfate treated genomic DNA from the indicated samples. Each sample was analyzed in triplicate in one single experiment. Relative methylation levels normalized to *ACTB* (β-actin) are indicated on the y-axis. The error bar represents the variation of *Cxorf12* methylation between the triplicates of each sample, normalized to the average *ACTB* expression. (**b**) qRT-PCR analysis of *Cxorf12* in RNA prepared from the indicated samples. Each sample was analyzed in triplicate in one single experiment. Relative expression levels normalized to *RPL39* are indicated on the y-axis in logarithmic scale. The error bar represents the variation of *Cxorf12* expression between the triplicates of each sample, normalized to the average RPL39 expression. N: Normal bulk breast tissue; CAF: Cancer associated fibroblast culture; NF: Normal fibroblast culture. The qMSP and qRT-PCR results of each indicated sample is summarized in **Supplementary Table 3**.

Supplementary Table 1. Clinicopathological characteristics of ovarian cancers analyzed

Sample	Tumor subtype	Age	Grade	Stage	% of fibroblasts
32	serous	71	1	2	85
121	mucinous	77	1	3	80
128	endometrioid	56	2	1c	95
131	serous	74	3	3	80
135	serous	66	2	2	90
138	mucinous	47	1	1a	80
141	serous	56	3	3c	90
151	endometrioid	56	2	2c	60
277	serous	71	3	3c	80
281	serous	48	2	3b	50
292	mucinous	80	2	3	95
293	endometrioid	59	3	1	75
300	endometrioid	68	3	3	80
307	endometrioid	57	3		90
315	serous	82	3		50
318	serous	70	3	3	90
323	serous	54	2		60
374	endometrioid	80	2	3	85
400	mucinous	66	1	3	40
406	serous	76	3		70
477	serous	71	3	3	75
509	serous	66	2	3	85
533	endometrioid	87	3	4	80
551	serous	55	3	3	80
565	serous	63	2	2	85
	Number of samples	**average age**	**Grade 1/2/3**	**Stage 1/2/3/4/unknown**	**Number of samples with ≥70% Fibroblasts**
serous	14	65.9	1/5/8	0/3/8/0/3	10
endometrioid	7	66.1	0/3/4	2/1/2/1/1	7
mucinous	4	67.5	3/1/0	1/0/3/0/0	3
All types	25	66.5	4/9/12	3/4/13/1/4	20

Supplementary Table 2. Clinicopathological characteristics of breast cancers analyzed

Sample	Morphology	Age	Grade	Stage	% of fibroblasts
RMH06-204	Infiltrating duct carcinoma	60	3		75
7697	DCIS	82	0	0	80
7187	Infiltrating duct carcinoma	50	3	2b	95
5077	Medullary carcinoma	60	3	2a	95
1955	Infiltrating duct carcinoma	53	3	2a	80
8001	Ductal carcinoma	34	2	2a	85
7731	Ductal carcinoma	39	3	2b	90
2274	Infiltrating duct carcinoma	70	2	2a	90
1001	Mucinous adenocarcinoma	92	9	2a	85
1539	Infiltrating duct carcinoma	51	3	2b	75
	Number of samples	**average age**	**Grade 2/3/9unknown**	**Stage 2/unknown**	**Number of samples with ≥75% Fibroblasts**
All types	10	59.1	2/6/1/1	8/2	10

Supplementary Table 3. Source and relevant properties of CAF cultures analyzed in the paper

CAF ID	tissue source	xenograft assay results[§]	Cxorf12 qMSP of cultured stroma	Cxorf12 qRT-PCR of cultured stroma
CAF IDC-14	invasive ductal breast carcinoma, ER+/PR+/HER2-	tested twice, promoted tumor growth	hypermethylated compared to matched normal bulk breast	lower level compared to matched normal bulk breast
CAF IDC-16	invasive ductal breast carcinoma, ER-/PR-/HER2+	tested twice, promoted tumor growth-but less efficiently than the other two	hypermethylated compared to matched normal bulk breast	low level, but no matching normal fibroblast to compare, Little expression in normal bulk breast
CAF IDC-1819	invasive ductal breast carcinoma, ER+/PR+/HER2-	not tested	hypomethylated, but no matching normal to compare	modest level, but no matching normal to compare
CAF IDC-22	invasive ductal breast carcinoma, ER-/PR-/HER2-	tested twice, promoted tumor growth	hypomethylated compared to matched normal bulk breast, no significant difference to matched normal stroma	lower level compared to matched normal stroma
CAF IDC-24	invasive ductal breast carcinoma	not tested	slightly hypomethylated compared to matched normal stroma, no significant difference to matched blood	lower level compared to matched normal stroma
CAF IDC-34	invasive lobular breast carcinoma, ER+/PR-/HER2-	not tested	hypomethylated compared to matched normal bulk breast	lower level compared to matched normal bulk breast
NR-1	invasive ductal breast carcinoma	tested four times, promoted tumor growth four to five fold over normal fibroblast culture	not tested	not tested

[§] Full details of the xenograft assay for CAF IDC 14, 16, 1819, 22, 24 and 34 are reported in M. Hu et al.

REGULATION OF *IN SITU* TO INVASIVE BREAST CARCINOMA TRANSITION
Min Hu, Jun Yao, Danielle K. Carroll, Stanislawa Weremowicz, Haiyan Chen, Daniel Carrasco, Andrea Richardson, Shelia Violette, Tatiana Nikolskaya, Yuri Nikolsky, Erica L. Bauerlein, William C. Hahn, Rebecca S. Gelman, Craig Allred, Mina J. Bissell, Stuart Schnitt, Kornelia Polyak.

(Cancer Cell, In press)

Xenograft assay data for NR-1 are from unpublished observation, by I. Haviv et al.

Supplementary Table 4. Primer sequences used in the paper

Chromosome 22 microsatellite markers (MSMs) (Figure 4d)

MSM		Primer sequence 5'-3'	Annealing °C
D22S1169	**s**	GCACACACATGCACATAATC	49
	as	AACAACTTCCAGCAGACG	
D22S1174	**s**	GAATCACTAGGGGCCTTCA	49
	as	TGAGGCTATGTGCCCAG	

MSMs (Figure 6)

MSM		Primer sequence 5'-3'	Annealing °C
D3S1613	**s**	TGTGATAAGGACCAAGGC	55
	as	GAGCAAATTGCAGAATGAG	
D3S3640	**s**	GATCGCGTGACATTCC	55
	as	TGCTACTTGCTATTTATCAGACC	
D3S1744	**s**	TTTAAGCGGAAGGAAGTGTG	55
	as	CTGGCCCCATCTCTCTCTAT	
D11S1999	**s**	TACATGGCAGCAGGCATATA	55
	as	GAGTAAACAAGATTGCTAGATAGGC	
D11S2365	**s**	TTCACATGCATATGTCTTTATGG	55
	as	CTGTCTAGTGTCCTGAGCTGC	
D11S2002	**s**	CATGGCCCTTCTTTTCATAG	55
	as	AATGAGGTCTTACTTTGTTGCC	

qMSP primer (Supplementary Figure 2a)

Gene		Primer sequence 5'-3'	Annealing °C
Cxorf12	**s**	GTCGGCGTTGTCGCGC	61
	as	CGAAAACGTAAAAACTCGCCCG	57.1

RT-PCR primer (Supplementary Figure 2b)

Gene		Primer sequence 5'-3'	Annealing °C
Cxorf12	**s**	ACCTGCTGAAAACCGATGAC	55.5
	as	CTCAGGAAAGGGCAGTCTTG	55.6