

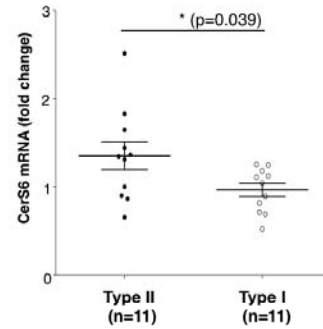
**A**

	Mesenchymal	Epithelial	Type I	Type II
1		BR:MCF7		
2	<b>BR:BT-549</b>			<b>BR:BT-549</b>
3	BR:HS578T			
4		<b>BR:T47D</b>	<b>BR:T47D</b>	
5	BR:MDA_MB_231			
6		CO:COLO205		
7	CNS:SF_268			
8	CNS:SF_295		CNS:SF_295	
9		CO:HCC_2998		CO:HCC_2998
10		CO:HCT_116		CO:HCT_116
11	CNS:SF_539			
12		CO:HCT_15		CO:HCT_15
13		CO:HT29		
14	CNS:SNB_19			
15	CNS:SNB_75			
16		CO:KM12		CO:KM12
17	CNS:U251			
18		LC:NCI_H322M		LC:NCI_H322M
19	CO:SW_620			
20		OV:OVCAR_3		LC:NCI_H460
21				
22	LC:HOP_62			
23		OV:OVCAR_4		
24	LC:HOP_92		LC:HOP_92	
25	LC:NCI_H226		LC:NCI_H226	
26	LC:NCI_H522			
27	<b>LE:CCRF_CEM</b>			<b>LE:CCRF_CEM</b>
28	LE:HL_60			
29				LE:RPMI_8226
30	LE:K_562			
31	LE:MOLT_4			
32	<b>LE:SR</b>			<b>LE:SR</b>
33	ME:LOXIMVI		ME:LOXIMVI	
34	ME:M14			
35	ME:MDA_MB_435			
36	ME:SK_MEL_2			
37	ME:SK_MEL_28			
38	<b>ME:UACC_62</b>			<b>ME:UACC_62</b>
39	OV:IGROV1		OV:IGROV1	
40	OV:NCI_ADR_RES			
41	OV:OVCAR_5			
42	OV:OVCAR_8			
43	PR:PC_3			
44	RE:786_0			
45	RE:A498		RE:A498	
46	RE:ACHN		RE:ACHN	
47	RE:CAKI_1		RE:CAKI_1	
48	RE:RXF_393			
49	RE:SN12C			
50	RE:TK_10		RE:TK_10	
51	RE:UO_31		RE:UO_31	

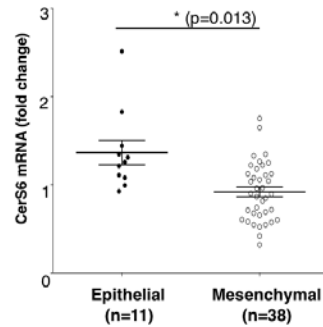
## Supporting Figure 1

**B**

Type I / Type II cells

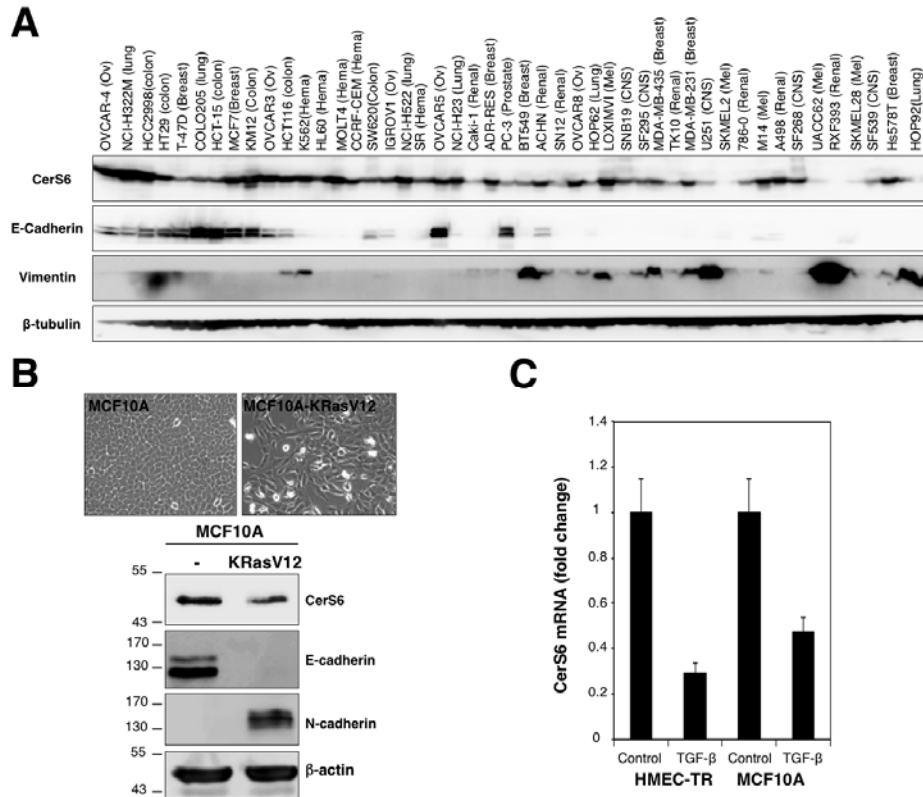


Epithelial vs Mesenchymal Cells



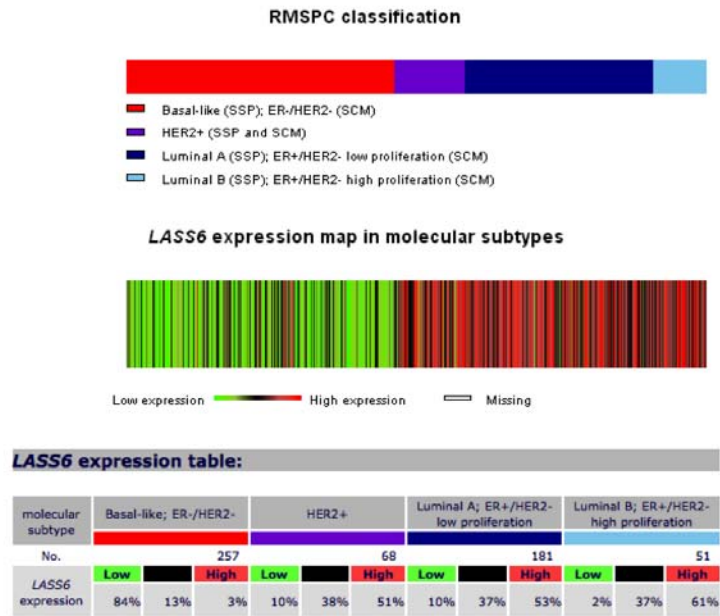
**Supporting Figure 1. Comparative analysis of CerS6 mRNA expression between Type I versus Type II cell types and Mesenchymal versus Epithelial cell lines.** **A.** Mesenchymal versus Epithelial classification (according to Vimentin and E-cadherin expression levels from Park SM et al.<sup>2</sup>) and Type I versus Type II cell classification (according to DISC formation and cell death response to the CD95L called S2 by Algeciras et al.<sup>1</sup>). Cell lines in red show a different classification between the two studies whereas cell lines in bold display similar classification. **B.** Based on table A, a comparative analysis of CerS6 expression was carried out using transcriptomic data from Ross et al.<sup>3</sup>. Statistical analyses were performed using the non-parametric Mann-Whitney U test. Values represent means  $\pm$  SEM of n cell lines analyzed (see table A).

## Supporting Figure 2



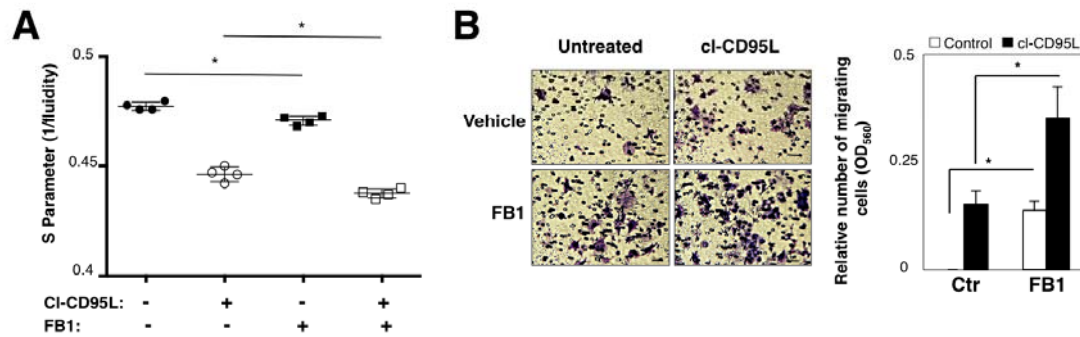
**Supporting Figure 2. CerS6 expression is lower in mesenchymal-like than in epithelial tumor cells.** **A.** Western blot analysis of 47 NCI60 cell lines for E-cadherin, Vimentin and CerS6 expression. Each cell line was lysed and 30  $\mu$ g of protein was loaded per lane.  $\beta$ -Tubulin was used as a loading control. Data are representative of three independent experiments. *CNS*: Central nervous system; *Ov*: Ovary; *Mel*: Melanoma. **B.** CerS6 expression in the human mammary epithelial cell line MCF10A and its K-Ras<sup>V12</sup>-expressing counterpart. **Upper panel:** Phase-contrast images ( $\times 10$  magnification) of the indicated cells. **Lower panel:** Cells were lysed and 30  $\mu$ g of protein was loaded per lane. Levels of CerS6, E-cadherin, and N-cadherin were determined by immunoblotting.  $\beta$ -Actin was used as a loading control. **C.** CerS6 mRNA expression was determined by qRT-PCR in MCF10A and HMEC-TR cultured in the absence or presence of TGF $\beta$  (5 ng/mL) for 24 h. Values were normalized against the level of HPRT mRNA and are expressed relatively to the values measured in non-treated cells. Data are means  $\pm$  S.D. of 3 independent experiments.

# Supporting Figure 3



**Supporting Figure 3. The mRNA level of CerS6 is lower in basal breast tumors (mesenchymal-like) than in luminal breast tumors (epithelial-like).** *In vivo* prognostic value of CerS6 was assessed in normal, luminal, HER2+, and basal molecular breast cancer subtypes using <http://bcgenex.centregauducheau.fr/BC-GEM>. **Abbreviations:** RMSPC (Robust Molecular Subtype Predictors Classification), patients classified in the same molecular subtype according to the six molecular subtype predictors (MSP); SSP (Single Sample Predictors); SCM (Subtype Clustering Models); ER (estrogen receptor).

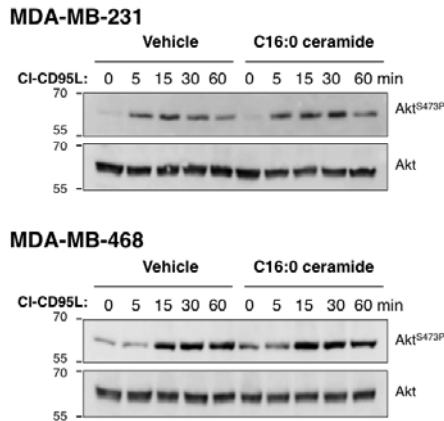
## Supporting Figure 4



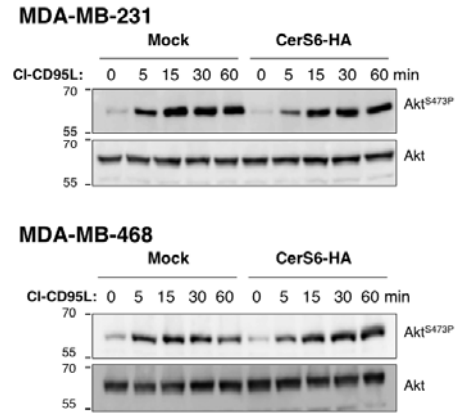
**Supporting Figure 4. Inhibition of CerS activity by FB1 promotes membrane fluidity and cell migration in the epithelial breast tumor cell line MCF7.** MCF7 cells were pre-incubated with or without a non-cytotoxic dose of FB<sub>1</sub> (50  $\mu$ M) for 72 h, and were then incubated with or without cl-CD95L (100 ng/mL) for 30 min. **A.** Membrane fluidity was assessed by EPR. Values are means  $\pm$  SEM of four independent experiments (\*,  $p < 0.05$ ). **B.** *Left panel:* Cell migration was assessed in the presence or absence of cl-CD95L (100 ng/mL) over 24 h using the Boyden Chamber assay. Bars=70  $\mu$ m. *Right panel:* Giemsa-stained cells that migrated to the lower side of the membrane were lysed and absorbance at 560 nm was recorded. Values represent the means  $\pm$  SEM of three independent experiments (\*  $p < 0.05$ ).

## Supporting Figure 5

**A**



**B**



**Supporting Figure 5. Treatment of mesenchymal breast tumor cells with C16-ceramide does not alter CD95-driven Akt phosphorylation.** **A.** MDA-MB-468 and MDA-MB-231 cells were pre-incubated with vehicle or a non-cytotoxic dose (1  $\mu$ M) of C16:0 ceramide (C16-Cer) for 60 min, and then cells were stimulated with cl-CD95L (100 ng/mL) for the indicated amount of time. Cells were lysed and 100  $\mu$ g of protein was loaded per lane. Indicated proteins were analyzed by immunoblotting. Blots are representative of three independent experiments. **B.** MDA-MB-468 and MDA-MB-231 cells transduced with a retroviral vector encoding CerS6-HA or with a control vector (Mock) were stimulated for the indicated amount of time with cl-CD95L (100 ng/mL). Cells were lysed and 100  $\mu$ g of protein was loaded per lane. Indicated proteins were analyzed by immunoblotting. Blots are representative of three independent experiments.

## References :

1. Algeciras-Schimmich A, Pietras EM, Barnhart BC, Legembre P, Vijayan S, Holbeck SL *et al.* Two CD95 tumor classes with different sensitivities to antitumor drugs. *Proc Natl Acad Sci U S A* 2003; **100**: 11445-11450.
2. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 2008; **22**: 894-907.
3. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P *et al.* Systematic variation in gene expression patterns in human cancer cell lines. *Nat Genet* 2000; **24**: 227-235.