

## **Blood endocan as a biomarker for breast cancer recurrence**

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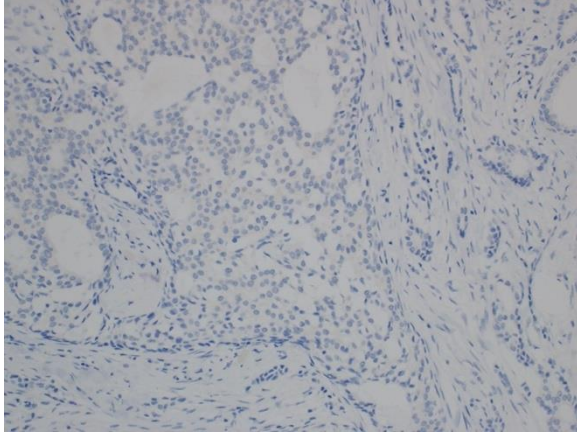
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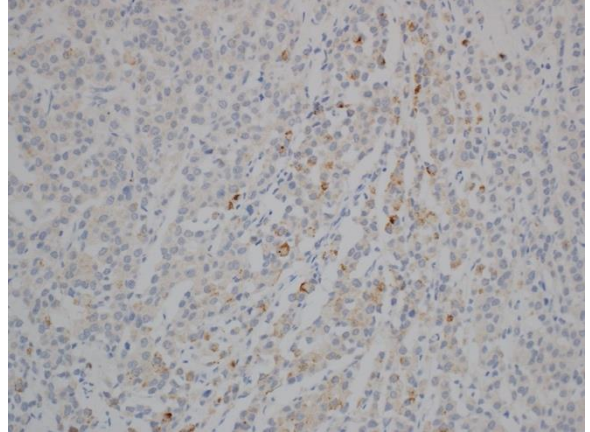
## Supplementary Information

### Supplementary Figures

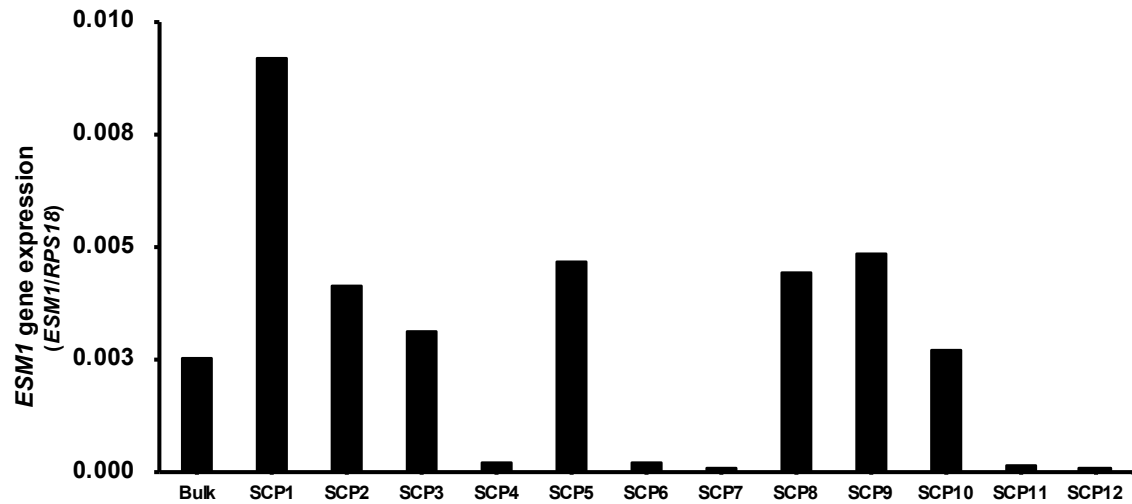
**a**



**b**



**Figure S1** Pathological assessment of endocan expression in primary breast tumors removed by the surgery. **a**, Representative tissue section from a breast cancer patient who showed blood endocan levels below the cutoff value (1.68 ng/mL). **b**, Representative tissue section from a breast cancer patient who tested positive for endocan by the blood test.



**Figure S2** Difference in *ESM1* expression of MDA-MB-231BR/*mVenus-Akaluc* obtained by limiting dilution cloning. Total RNA was extracted from twelve clones and bulk cells, and the expressions of the *ESM1* and *RPS18* were quantitated in real-time PCR. Each primer used in this study was as follows: *ESM1*-FW (5'-TTG CTA CCG CAC AGT CTC AG-3'), *ESM1*-RV (5'-GTG CCG TAG GGA CAG TCT TT-3'), *RPS18*-FW (5'-ATA CAG CCA GGT CCT AGC CA-3'), *RPS18*-RV (5'-AAG TGA CGC AGC CCT CTA TG-3').

## Supplementary Tables

**Table S1** Clinical characteristics of breast cancer patients whose blood was collected before and after the surgery.

Clinicopathological features	Total patients, <i>N</i> ( <i>N</i> = 16)	Endocan negative population, <i>N</i> (%) ( <i>N</i> = 8)		Endocan positive population, <i>N</i> (%) ( <i>N</i> = 8)	
<u>Age at blood collection</u>					
Median (years)	53	52		52	
(Minimum-Maximum)	(42-83)	(46-83)		(42-76)	
<u>Menopausal status</u>					
Pre-menopausal	9	4	(44.4%)	5	(55.6%)
Post-menopausal	7	4	(57.1%)	3	(42.9%)
<u>BMI</u>					
BMI<25%	12	6	(50.0%)	6	(50.0%)
BMI≥ 25%	4	2	(50.0%)	2	(50.0%)
<u>ER expression</u>					
<90%	0				
≥ 90%	16	8	(50.0%)	8	(50.0%)
<u>PgR expression</u>					
<10%	2	1	(50.0%)	1	(50.0%)
10% ≤ PgR <90%	4	2	(50.0%)	2	(50.0%)
≥ 90%	10	5	(50.0%)	5	(50.0%)
<u>Tumor size</u>					
Tis*	1	1	(100.0%)	0	(0.0%)
T1	9	5	(55.6%)	4	(44.4%)
T2	6	2	(33.3%)	4	(66.7%)
<u>Lymph node metastasis</u>					
N-	11	6	(54.5%)	5	(45.5%)
N+	5	2	(40.0%)	3	(60.0%)
<u>Pathological Stage</u>					
0	1	1	(100.0%)	0	(0.0%)
I	7	4	(57.1%)	3	(42.9%)
II	8	3	(37.5%)	5	(62.5%)
<u>Ki67 labeling index</u>					
<20%	11	6	(54.5%)	5	(45.5%)
≥ 20%	4	2	(50.0%)	2	(50.0%)
Unknown	1	0	(0.0%)	1	(100.0%)

\* Tis: Ductal carcinoma in situ (DCIS) or Paget's disease with no associated tumor mass.

**Table S2** Clinical characteristics of breast cancer patients with postoperative recurrence.

Clinicopathological features	Total patients, <i>N</i> ( <i>N</i> = 20)	Endocan negative population, <i>N</i> (%) ( <i>N</i> = 7)		Endocan positive population, <i>N</i> (%) ( <i>N</i> = 13)	
<u>Age at blood collection</u>					
Median (years)	55	63		55	
(Minimum-Maximum)	(35-78)	(35-78)		(41-70)	
<u>ER expression</u>					
<90%	9	3	(33.3%)	6	(66.7%)
≥ 90%	11	4	(36.4%)	7	(63.6%)
<u>PgR expression</u>					
<10%	11	4	(36.4%)	7	(63.6%)
10% ≤ PgR <90%	5	1	(20.0%)	4	(80.0%)
≥ 90%	4	2	(50.0%)	2	(50.0%)
<u>HER2 expression</u>					
≤ 1+	16	7	(43.8%)	9	(56.3%)
2+	3	0	(0.0%)	3	(100.0%)
≥ 3+	1	0	(0.0%)	1	(100.0%)
<u>Subtype</u>					
Luminal	12	5	(41.7%)	7	(58.3%)
HER2	1	0	(0.0%)	1	(100.0%)
Triple-negative	7	2	(28.6%)	5	(71.4%)
<u>Ki67 labeling index</u>					
<20%	6	2	(33.3%)	4	(66.7%)
≥ 20%	14	5	(35.7%)	9	(64.3%)

**Table S3** Effect of the interval between the surgery and the blood collection on changes in blood endocan levels.

Patient #	Pre-surgical endocan level (ng/mL)	Post-surgical endocan level (ng/mL)	UP or DOWN	Interval of time between the surgery and the blood collection (Days)
1	5.97	10.90	UP	29
2	5.66	1.03	DOWN	32
3	12.45	1.47	DOWN	32
4	17.31	5.60	DOWN	46
5	5.60	4.38	DOWN	59
6	4.41	2.20	DOWN	64
7	2.24	14.51	UP	67
8	19.64	10.86	DOWN	83

**Table S4** Endocan, CEA, and CA15-3 concentrations in the blood samples of patients with postoperative breast cancer recurrence.

Patient #	Endocan level (ng/mL)	CEA level (ng/mL)	CA15-3 level (U/mL)
1	<0.156	5.6	23.9
2	0.16	2.8	12.4
3	0.30	4.8	8.1
4	0.43	3.1	85.9
5	0.61	4.6	17.4
6	0.89	2.7	14.6
7	1.22	1.5	14.2
8	2.35	9.4	189.9
9	3.88	1.7	5.8
10	4.48	0.9	5.5
11	5.20	1.4	25.6
12	5.78	0.6	18.1
13	5.80	1.3	4.6
14	6.21	23.9	8.6
15	6.63	1.7	27.2
16	9.13	10.2	95.7
17	9.45	6.4	30.2
18	10.34	2.1	6.5
19	10.75	23.4	34.7
20	10.82	4.1	9.0

Red numbers indicate positive for the respective biomarker.