

Supplementary Materials for

Invasive breast carcinoma cells from patients exhibit Mena^{INV} - and macrophage-dependent transendothelial migration

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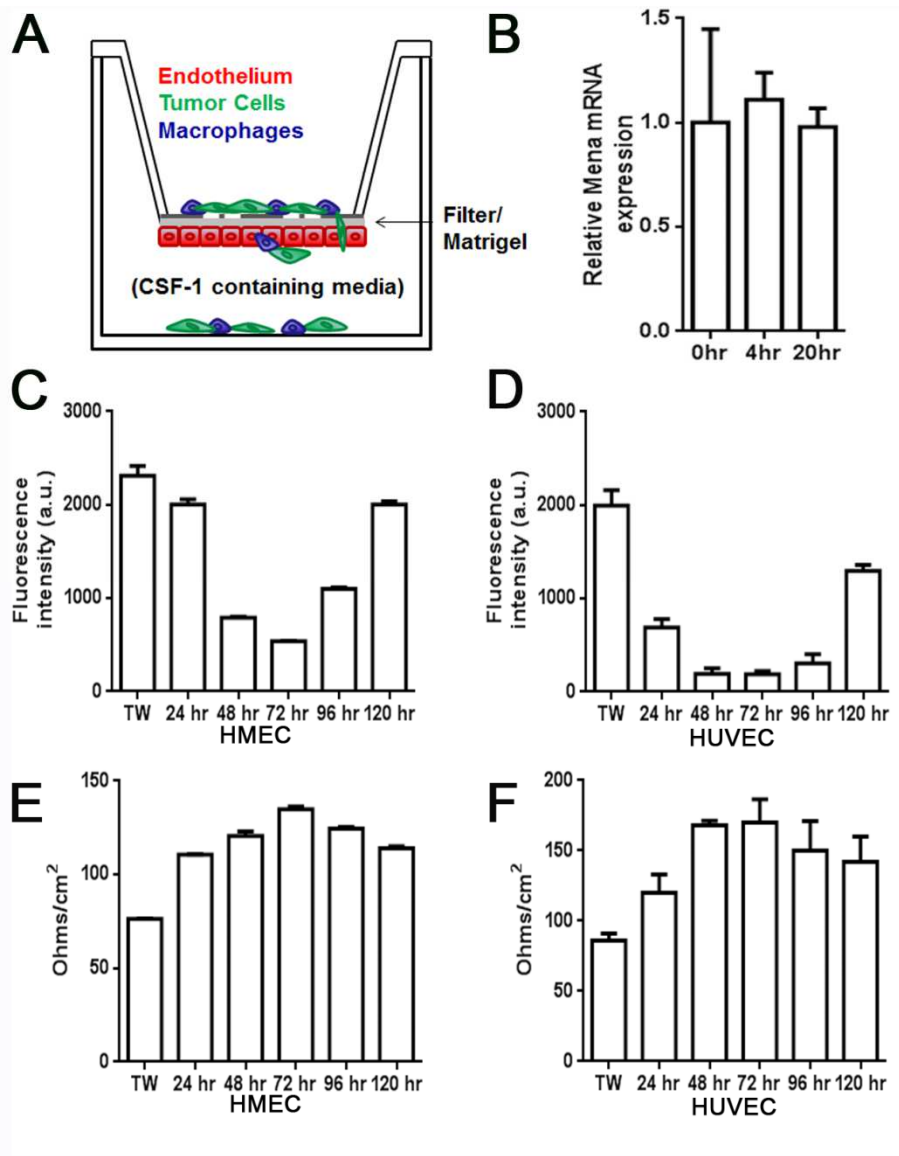


Figure S1. The experimental setup of the iTEM assay and the permeability of HMEC endothelium. (A) For in vitro iTEM assay, the bottom of a transwell is coated with Matrigel and an endothelial monolayer. Tumor cells and macrophages are added to the top of the transwell in serum-free medium, and medium containing CSF-1 is placed in the bottom well (the luminal side). **(B)** The effect of time in culture (hr: hours) on Mena^{INV} expression in FNA-obtained primary human breast cancer cells. **(C & D)** Fluorescence intensity measurements in bottom well 30 min after addition of 70 kD Texas Red dextran to upper well of HMEC and HUVEC monolayers. **(E & F)** Transendothelial electrical resistance measurements of HMEC (E) and HUVEC (F) monolayers. Data are means \pm SEM for 3 independent experiments. TW; uncoated transwell control.

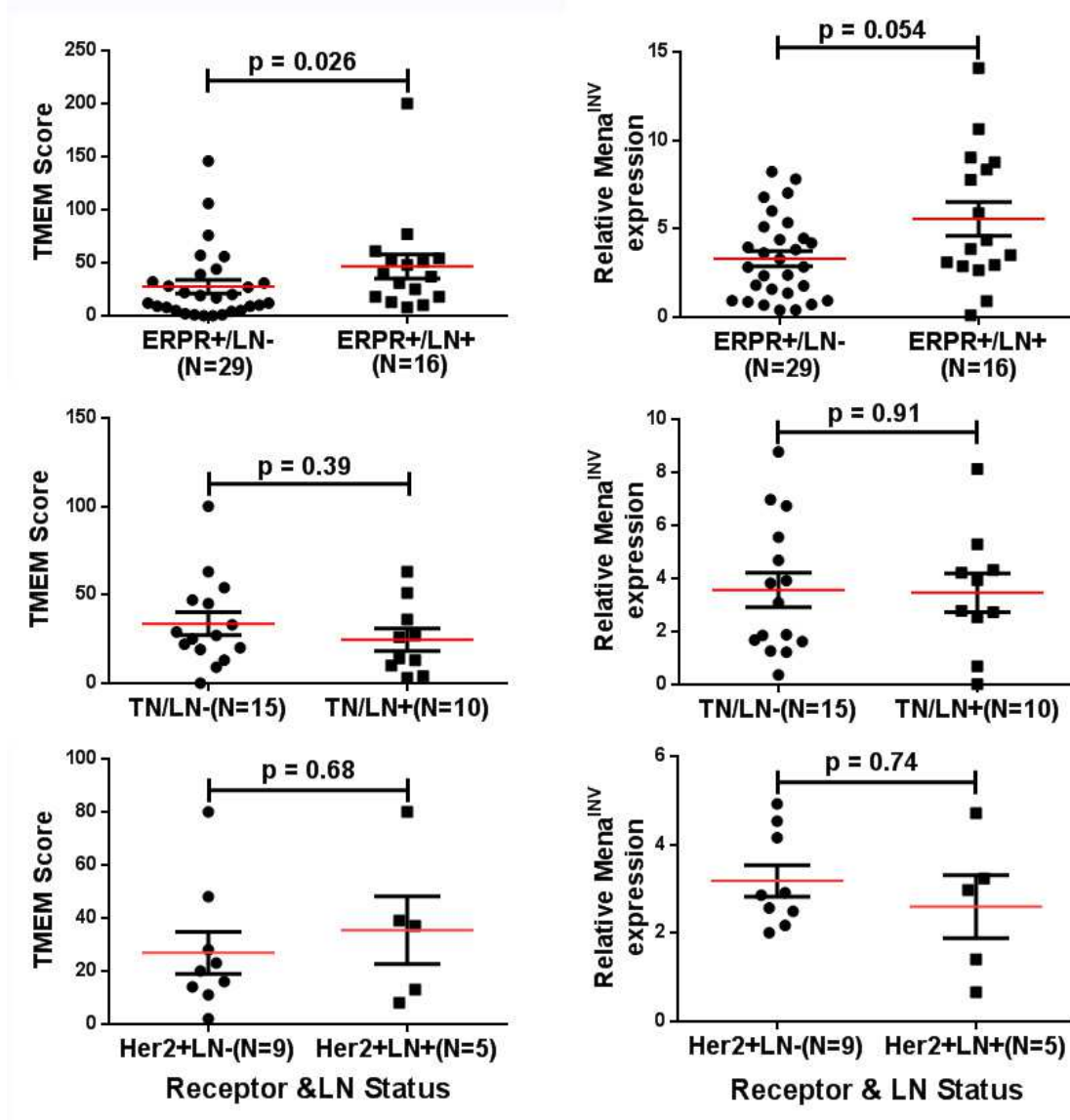


Figure S2. TMEM score and relative Mena^{INV} expression in LN-positive or LN-negative cases. TMEM score and relative Mena^{INV} expression are greater in lymph node-positive (LN+) compared with lymph node-negative (LN-) cases only in ERPR⁺/Her⁻ clinical subtype, ($P=0.026$ and 0.054 , respectively). However, this difference does not reach statistical significance (set at 0.008 ; see Materials and Methods). Data was analyzed with the Wilcoxon Mann-Whitney rank-sum test.

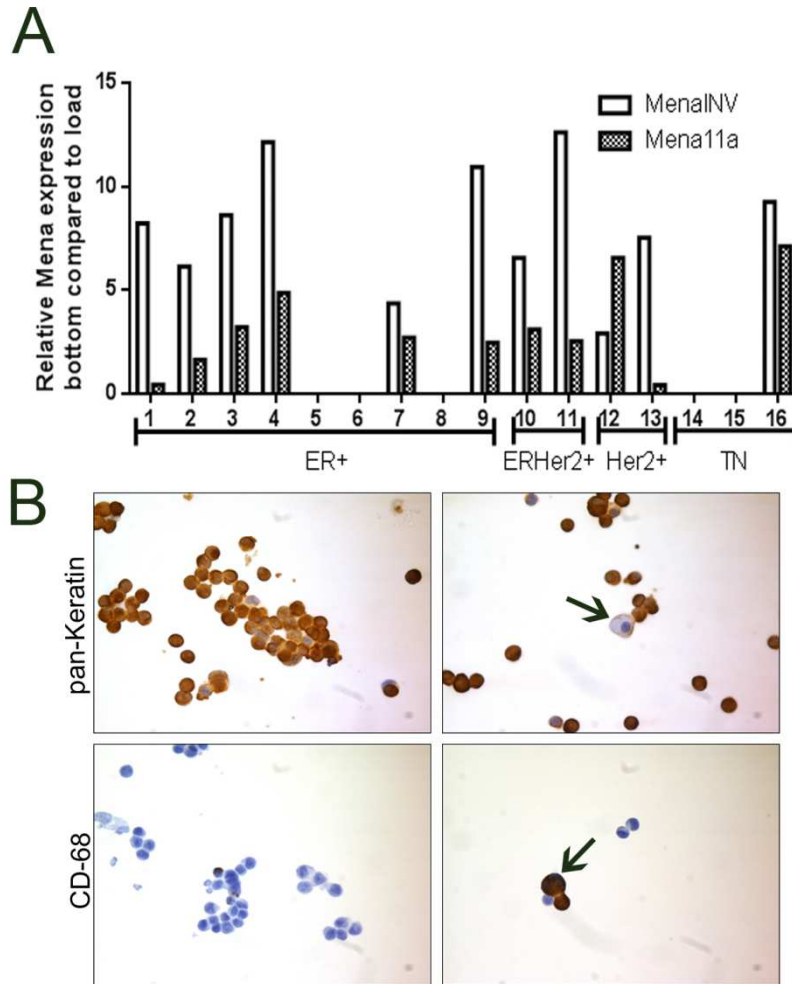


Figure S3. iTEM assays engineered with HMEC-1 endothelium performed with human IDC cells obtained by FNA. (A) RT-PCR for *MENA* isoform expression. Cells from 11 out of 16 cases obtained from patient breast cancer FNA biopsies were capable of crossing an in vitro engineered endothelium. In 10 out of the 11 FNA samples that showed crossing ability, the cells in the bottom well of the intravasation assay were enriched for Mena^{INV}-expressing cells. **(B)** Representative images of immunochemically stained human IDC cells used in iTEM assays. The cells in the upper panels are stained with pan-keratin antibody AE1:3, while the cells in the lower panels are stained with CD68 antibody. The % of tumor cells and macrophages was assessed on the whole cytopsin filed (314 μ m). About 97% of the cells present in FNA sample are keratin-positive, CD68-negative. Macrophages represent about 3% of the cells in FNA samples and they were keratin-negative (arrow in upper right) and CD68-positive (arrow in bottom right).

Primers:
GAPDH F: CAT GAG AAG TAT GAC AAC AGC CT
GAPDH R: AGT CCT TCC ACG ATA CCA AAG T
Mena 11a F: CAA CCT GTT GTC AAA AAC AAT CT
Mena 11a R: GGA CCT GTT GTC AAA AAC AAT CT
Mena INV F: AGA GGA TGC CAA TGT CTT CG
Mena INV R: TTA GTG CTG TCC TGC GTA GC

Table S1. *MENA* and *GAPDH* primer sequences. F, forward sequence 5'–3'; R, reverse sequence 5'–3'.

Clinical Subtype	N	Mena ^{INV}		Mena11a	
All	100	$r = 0.57$	$p = 10^{-6}$	$r = -0.20$	$p = 0.04$
ERPR ⁺ /Her2 ⁻	47	$r = 0.72$	$p = 10^{-6}$	$r = -0.34$	$p = 0.02$
Triple Negative	25	$r = 0.55$	$p = 4^{-3}$	$r = -0.20$	$p = 0.34$
ER ⁺ /PR ⁻ /HER2 ⁻	14	$r = 0.28$	$p = 0.34$	$r = -0.22$	$p = 0.44$
Her2 ⁺	14	$r = 0.42$	$p = 0.07$	$r = 0.04$	$p = 0.90$

Table S2. Correlation coefficients for TMEM score and relative expression of *MENA* isoforms in IDCs, overall and by clinical subtype. The values represented in the table are based on TMEM score and Mena isoform analyses from the 100 IDCs used to generate data in Figures 1 and 2. The statistically significant correlations indicated in red were calculated using Pearson's correlation coefficient.

	Mena^{INV}	Mena 11a	TMEM
Tumor Characteristics	Median (5 th , 95 th percentile)	Median (5 th , 95 th percentile)	Median (5 th , 95 th percentile)
BR 3, 4 & 5 N = 9	0.93 (0.39, 4.02)	0.15 (0.02, 1.11)	5.00 (0.80, 27.60)
BR 6 & 7 N = 40	3.28 (0.67, 8.78)	0.37 (0.01, 1.65)	18 (1.00, 78.45)
BR 8 & 9 N = 51	3.63 (0.96, 8.28)	0.31 (0.01, 1.75)	27 (3.05, 111.00)
< 2 cm N = 59	3.30 (0.67, 7.83)	0.51 (0.01, 1.62)	10.50 (1.00, 107.60)
> 2 < 5 cm N = 35	2.95 (0.59, 8.76)	0.21 (0.01, 1.75)	13.50 (1.70, 76.30)
> 5 cm N = 6	4.19 (0.59, 12.83)	0.02 (0.01, 0.22)	31.00 (10.00, 162.75)
LN+ N = 34	3.32 (0.47, 9.59)	0.19 (0.01, 1.64)	33.5 (3.65, 78.05)
LN- N = 64	2.91 (0.67, 7.98)	0.34 (0.01, 1.66)	22.00 (1.00, 105.1)
ER+ N = 70	3.63 (0.67, 8.61)	0.23 (0.01, 1.68)	25.00 (1.00, 114.80)
ER- N = 30	2.49 (0.50, 7.60)	0.56 (0.01, 1.68)	25.00 (3.45, 63.00)
PR+ N = 55	3.70 (0.58, 8.85)	0.22 (0.01, 1.66)	28.50 (1.1, 89.10)
PR- N = 46	2.78 (0.66, 8.10)	0.53 (0.01, 1.65)	22 (2.00, 91.25)
HER2/Neu+ N = 14	2.91 (1.50, 4.78)	0.42 (0.02, 1.72)	23 (5.90, 80.00)
HER2/Neu- N = 86	3.30 (0.47, 8.68)	0.32 (0.01, 1.66)	25 (1.00, 104.50)
Triple Negative N = 25	2.78 (0.43, 7.90)	0.59 (0.01, 1.62)	27 (3.2, 63.00)

Table S3. TMEM scores and relative Mena isoform expression for tumors with different clinical and pathological variables. Median, 5th and 95th percentile of relative expression of Mena^{INV} or Mena11a and TMEM counts for (i) well-differentiated

[Bloom-Richardson score (BR) 3-5], moderately differentiated (BR 6 & 7), or poorly differentiated (BR 8 & 9) IDCs; (ii) tumors that were < 2cm, between 2 and 5 cm, or > 5 cm in diameter; (iii) tumors with positive (+) or negative (-) expression of the genes encoding estrogen (ER), progesterone (PR), or HER2/Neu receptor. The only statistically different values (by Mann-Whitney test) were those observed for the fold change in Mena^{INV} and TMEM scores between well-differentiated and poorly differentiated IDCs (indicated in red).

Case#	Mena ^{INV}	Mena11a	Size	Grade	LN	LVI	ER %	PR %	Her2/Neu	Age
1	8.22	0.43	1.3	7	0/4	+	99	99	-	78
2	6.13	1.65	4	6	3/10	-	90	60	-	45
3	8.61	3.21	0.9	6	0/5	-	90	30	-	57
4	12.14	4.86	1.2	6	0/11	-	95	95	-	77
5	0.00	0.00	2.6	9	1/11	-	100	99	-	86
6	0.00	0.00	1.1	6	0/11	-	90	5	-	48
7	4.36	2.71	1.3	8	0/2	-	90	90	-	82
8	10.93	2.45	1.9	5	0/1	-	90	80	-	58
9	6.55	3.09	1.5	5	0/5	-	90	80	-	60
10	12.61	2.53	1.5	9	0/7	-	98	2	+	58
11	0.00	0.00	4	8	0/15	+	80	70	+	45
12	2.91	6.54	2.4	7	0/11	-	<1	<1	+	62
13	7.53	0.42	2.3	9	0/6	-	<1	<1	+	57
14	0.00	0.00	1.6	8	0/2	-	<1	<1	-	48
15	0.00	0.00	3.5	9	0/4	-	<1	<1	-	55
16	9.24	7.12	1	7	0/5	-	<1	<1	-	53

Table S4. Clinical and pathological data pertaining to iTEM data in fig. S3A. Mena values represent relative expression of Mena isoforms in the cells collected from the bottom well of iTEM assay compared to that in cells loaded in the top well. Tumor size is represented as the largest diameter in centimeters. Tumor grade is expressed as Bloom-Richardson score. LN: lymph node status, represented as the number that were positive out of the total number examined. LVI: lymphovascular invasion, represented as present (+) or absent (-). ER and PR expression is represented as percentage of positive cells. Her2/Neu abundance was examined by IHC and was considered positive if the score was 3+; any 2+ score was analyzed for copy number change by FISH and considered positive if the copy number was > 2.2 per nucleus.

Case#	Mena ^{INV}	Mena11a	CSF-1R	Size	Grade	LN	LVI	ER (%)	PR (%)	Her2/Neu	Age
1	2.88	0.04	2.36	3.5	6	0/2	-	90	95	-	54
2	3.01	0.53	2.99	1.5	6	0/5	-	100	60	-	62
3	6.96	0.23	1.00	2	7	1/6	+	90	90	-	58
4	7.01	0.004	0.83	2.3	6	0/7	-	100	100	-	70
5	9.7	0.45	0.05	1.8	6	0/4	-	80	<1	-	70
6	10.13	ND	0.95	1.2	5	0/6	-	90	<1	-	70
7	10.63	ND	3.58	2.2	6	0/3	-	100	90	-	73
8	6.931	0	-	5.2	6	17/2	-	90	30	-	87
9	5.42	ND	4.29	1.9	9	1/2	-	90	90	-	67
10	13.11	ND	11.45	6	8	0/4	-	70	55	+	49
11	3.73	1.49	7.2	1.8	8	0/3	-	30	<1	+	52
12	8.65	0.74	11.16	8	9	0/6	-	<1	<1	-	95
13	4.66	ND	14.51	1	8	0/6	-	<1	<1	-	69
14	15.37	ND	14.83	1.6	9	0/1	-	<1	<1	-	79
15	16.56	0.11	4.46	2.5	9	1/7	-	<1	<1	-	40
16	7.39	ND	9.82	2.2	9	0/6	-	<1	<1	-	53

Table S5. Clinical and pathological data pertaining to iTEM data in Figs. 4 and 6. Mena and CSF-1R values represent relative expression of Mena isoforms and CSF-1R in the cells collected from the bottom well of iTEM assays compared to that from cells loaded in the top well. Details and abbreviations as described for table S4.