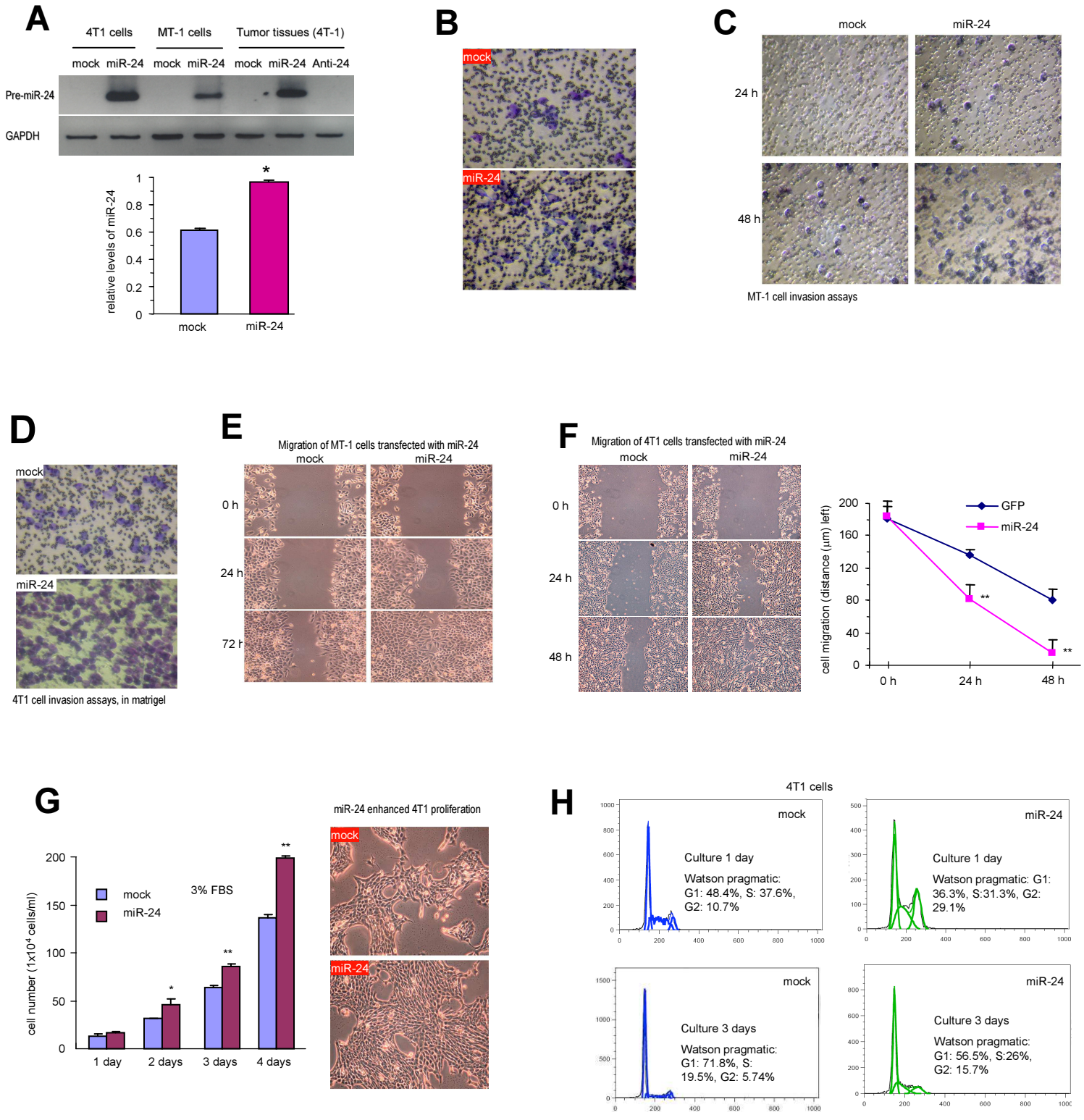

Fig S1. Expression of miR-24 enhanced breast cancer cell invasion, migration and proliferation. (**A**) Upper, DNAs isolated from 4T1 cells, MT-1 cells, and tumors expressing miR-24, anti-miR-24, or mock were subjected to PCR to confirm integration of miR-24 into the genome. Lower, RNAs were isolated from mock and miR-24 transiently transfected 4T1 cells and subjected to real-time PCR to measure mature miR-24 levels. Asterisks indicate significant differences. * $p<0.05$. Error bars, SD (n=3). (**B**) Mock- and miR-24-transfected 4T1 cells ($1x10^5$) were loaded into the insert with 100 ml serum-free DMEM medium and then incubated at 37° C for 12 hours. The migrated cells were stained blue and were counted in 6 randomly selected fields under a light microscope. (**C**) Mock- and miR-24-transfected MT-1 cells ($1x10^5$) suspended in 100 ml serum-free medium were loaded in the transwell insert containing Matrigel and incubated at 37° C for 24 hours for cell invasion assays. Expression of miR-24 promoted invasion. (**D**) Mock- and miR-24-transfected 4T1 cells ($1x10^5$) were subject to the same invasion assays. (**E**) Mock- and miR-24-transfected MT-1 cultures were wounded with a 200-μl pipette tip and cultured in 10% FBS/DMEM medium with 2 μM mytomycin for cell migration assay. Expression of miR-24 promoted migration. (**F**) Mock- and miR-24-transfected 4T1 cultures were subject to the same migration assay. (**G**) 4T1 cells stably transfected with miR-24 or mock were maintained in tissue culture dishes in DMEM with 3% FBS. Cell proliferation was determined by cell counting. **, $p< 0.01$, n=6. Right, typical photos of proliferating cells. (**H**) Mock- and miR-24transfected 4T1 cells ($5x10^5$) were analyzed for cell cycle progression. Fewer miR-24 cells were present in G1 phase than mock cells.

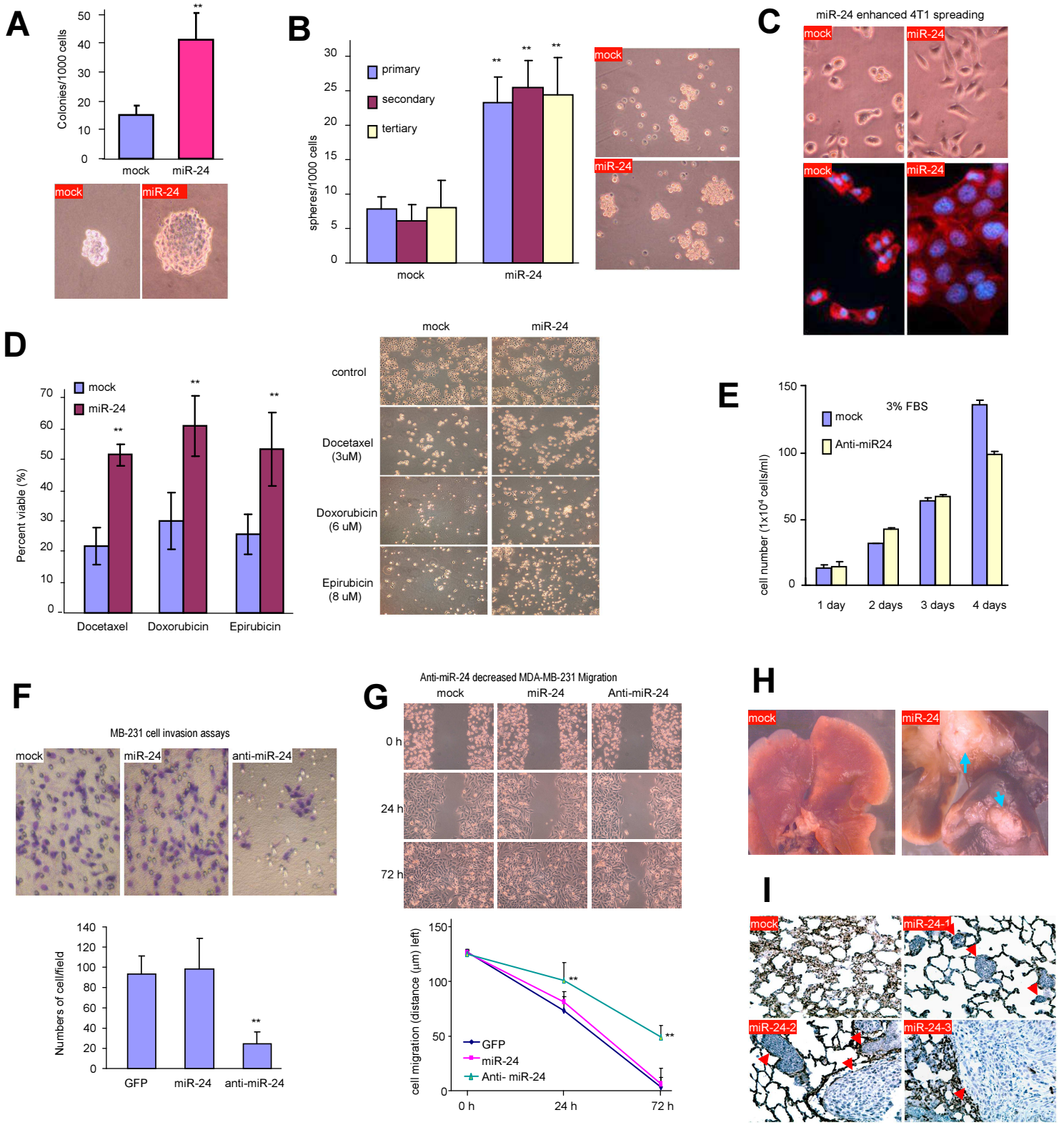

Fig S2. Expression of miR-24 enhanced colony formation, sphere formation, cell spreading, drug resistance, tumor invasion, and metastasis. (**A**) Mock- and miR-24-transfected MT-1 cells (103) were subject to colony formation assay. The number of colonies was counted in 6 fields of views for each plate. **, $p< 0.01$, $n = 6$. Lower, typical photos of colonies. (**B**) Mock- and miR-24-transfected MT-1 cells were subject to mammosphere formation assay. The numbers of mammospheres were counted in 6 randomly selected fields. **, $p< 0.01$, $n = 6$. Right, typical mammospheres after cultured in Petri dishes for 10 days. (**C**) 4T1 cells transfected with miR-24 or mock were maintained in tissue culture dishes in DMEM containing 3% FBS. Cell morphology was monitored with a light microscope (upper) or examined after immunostaining (lower) to detect actin filaments (red) and nuclei (blue). (**D**) Mock- and miR-24-transfected MT-1 cells ($1x10^4$ cells/well) were inoculated and cultured in 10% FBS/DMEM medium in 96-well culture dishes for 12 hours. After cell attachment, the medium was changed to 10% FBS/DMEM containing 3 μM Docetaxel, 6 μM Doxoeubicin or 8 μM Epirubicin for 24 hours. The cultures were incubated with 10 μl WST-1 reagent for 4 hours. The absorbance of the samples against a background blank control was measured by a microplate reader. **, $p< 0.01$, n=9. Expression of miR-24 led to acquired drug-resistance of the cells. Right, typical photos are shown. (**E**) Mock- and anti-miR-24-transfected 4T1 cells were subject to proliferation assay in DMEM supplemented with 3% FBS ($n = 6$). (**F**) Mock-, miR-24-, and anti-miR-24-transfected MDA-MB-231 cells (1x105) were subjected to invasion assays for 48 hours. miR-24 enhanced cell invasion but anti-miR-24 inhibited it. The invasive cells were counted for statistical analysis (lower). (**G**) Mock-, miR-24-, and anti-miR-24-transfected MDA-MB-231 cultures were subject to migration assays. Transfection with anti-miR-24 decreased cell migration. The migration distance was measured for statistical analysis (lower). (**H**) Mock- and miR-24-transfected MT-1 cells ($2×10^5$) were injected into NOD-SCID mice via tail vein, with 15 mice in each group. Six weeks after the injection, 3 mice in the miR-24 group developed cachexia, and 4 had lung metastasis in necroscopy. Typical metastatic lesions in the lungs are shown (arrows). (**I**) H&E staining of lungs from mock and miR-24 lung showed multi-focal metastasis lesions in the miR-24 lungs (arrows).

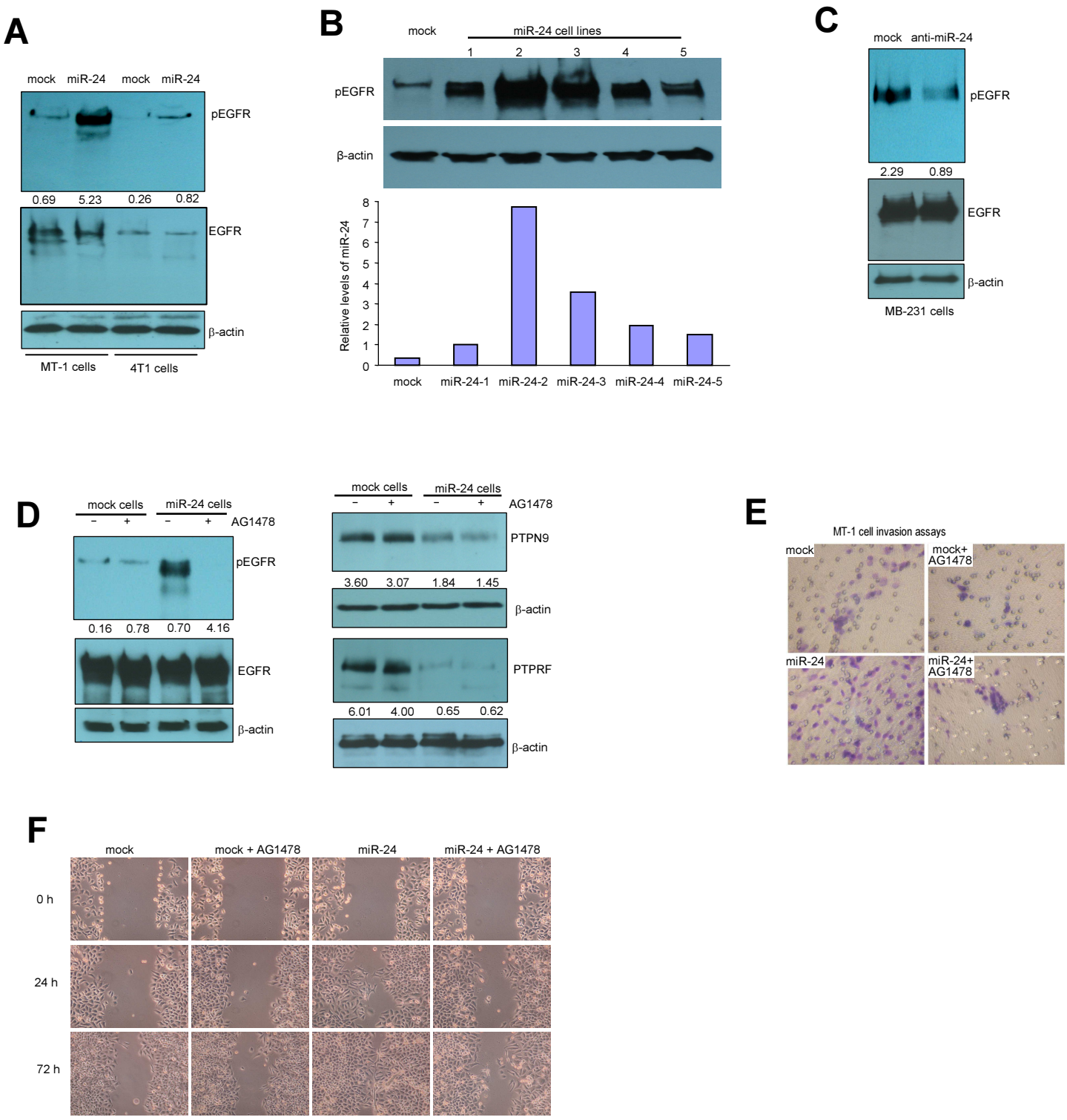

Fig S3. miR-24 affect pEGFR expression and functions. (**A**) Cell lysates from mock- and miR-24-transfected 4T1 and MT-1 cells were analyzed on Western blotting for levels of pEGFR, EGFR and b-actin. Expression of miR-24 increased pEGFR levels. (**A**) Single cell suspensions were plated on 96 well culture dishes. After culturing for an additional 2 days, single colony cells were moved to 6-well dishes and cultured to 70 % conference. Cell lysates were prepared and subjected to Western blot analysis for pEGFR expression. Higher levels of miR-24 were associated with higher levels of pEGFR. (**C**) Cell lysates prepared from vector- and anti-miR-24-transfected MDA-MB-231 cells were subjected to immunoblotting probed with antibodies against pEGFR, EGFR and b-actin. (**D**) Left, cell lysates prepared from mock- and miR-24-transfected MT-1 cells pre-treated with 2.0 μM AG 1478 for 24 hours were subjected to immunoblotting probed with antibodies against pEGFR, EGFR and b-actin. Increased levels of pEGFR by miR-24 expression was abolished by AG1478 treatment. Right, the lysates were also subjected to Western blot analysis for expression of PTPN9 and PTPRF. Treatment with AG1478 had little effect on expression of PTPN9 and PTPRF. (**E**) Mock- and miR-24-transfected MT-1 cells ($1x10^5$) were subject to invasion assays with or without 2.0 μM AG1478 treatment for 56 hours. The increased invasion due to miR-24 expression was abolished by AG1478 treatment. (**F**) Mock- and miR-24-transfected MT-1 cultures were treated with or without 2.0 μM AG1478 for migration assay. Increased cell migration acquired by miR-24 expression was abolished by AG1478 treatment.

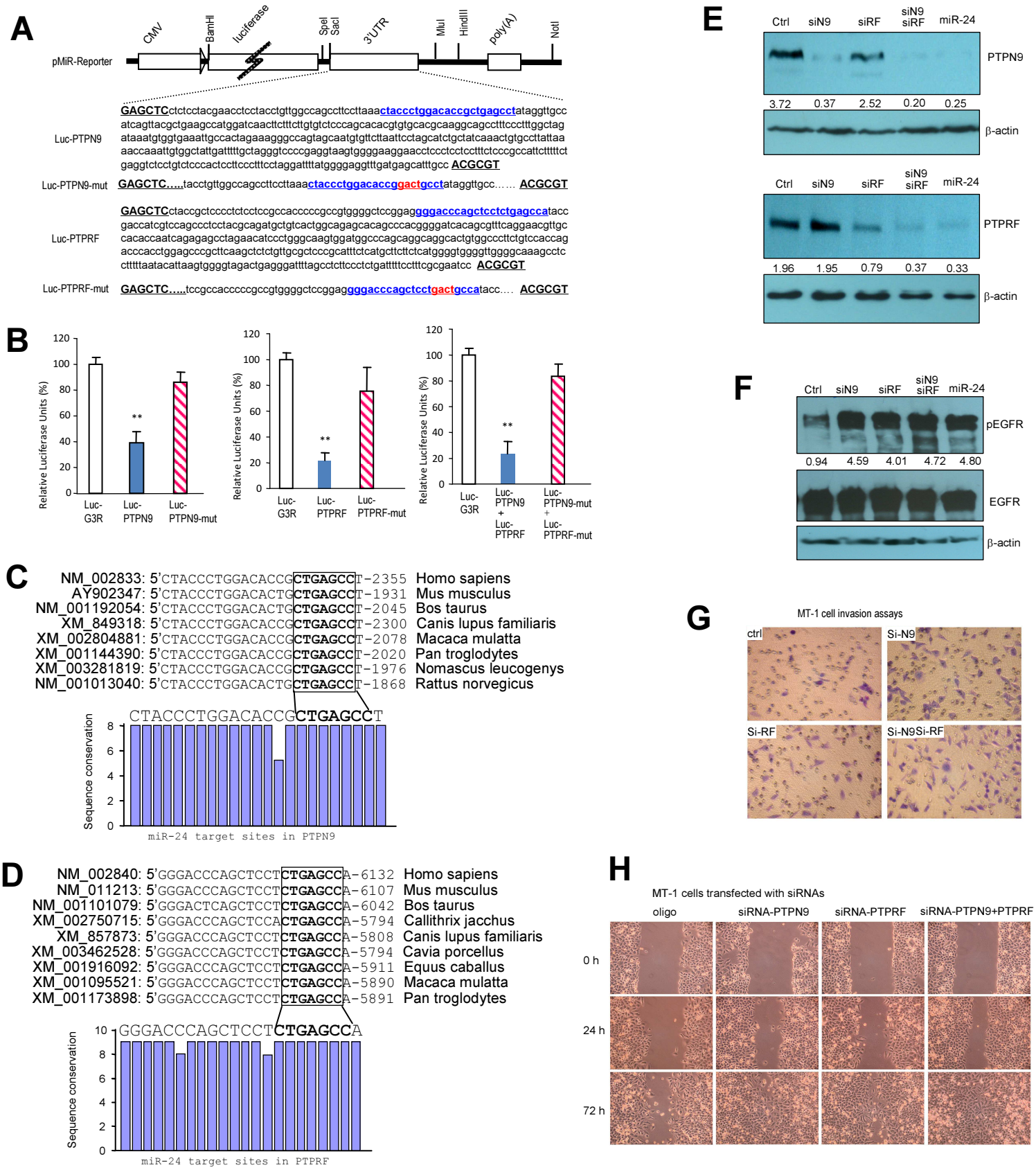

Fig S4. miR-24 targeting analyses. (**A**) Fragments of PTPN9 and PTPRF 3'UTRs were inserted into the luciferase report vector pMir-Report producing constructs Luc-PTPN9 and Luc-PTPRF. The potential miR-24 target sites were labeled in blue. Mutations labeled in red were generated in the miR-24 target sites producing mutant constructs Luc-PTPN9-mut and Luc-PTPN9-mut. (**B**) U343 cells were co-transfected with miR-24 and a luciferase reporter construct, as shown in the figure, for luciferase assays. Presence of the miR-24 target site reduced luciferase activities, which were reversed when the target site was mutated (n=9). (**C**) Alignment of the miR-24 target sites in PTPN9 across different species as shown. The miR-24/PTPN9 target sites are bolded. Conservation of the sequences is shown across all species. (**D**) Alignment of the miR-24 target sites in PTPRF across different species. The miR-24/PTPRF target sites are bolded. (**E**) Cell lysates prepared from MT-1 cells transfected with siRNAs against PTPN9 (siN9) and PTPRF (siRF) were subjected to Western blot analysis to confirm silencing of PTPN9 and PTPRF. (**F**) Treatment with 20 ng/ml EGF enhanced EGFR phospharylation, which had additive effect with PTPN9 and PTPEF silencing. (**G**) MT-1 cells (1x10^5) transfected with control oligo, siRNAs against PTPN9 and PTPRF as indicated were incubated at 37° C for 64 hours for invasion assays. Transfection with siN9 and siRF promoted cell invasion. (**H**) MT-1 cells (4x10^5) transfected with control oligo, siN9, and siRF were subject to migration assays. Transfection with siN9 and siRF promoted migration.

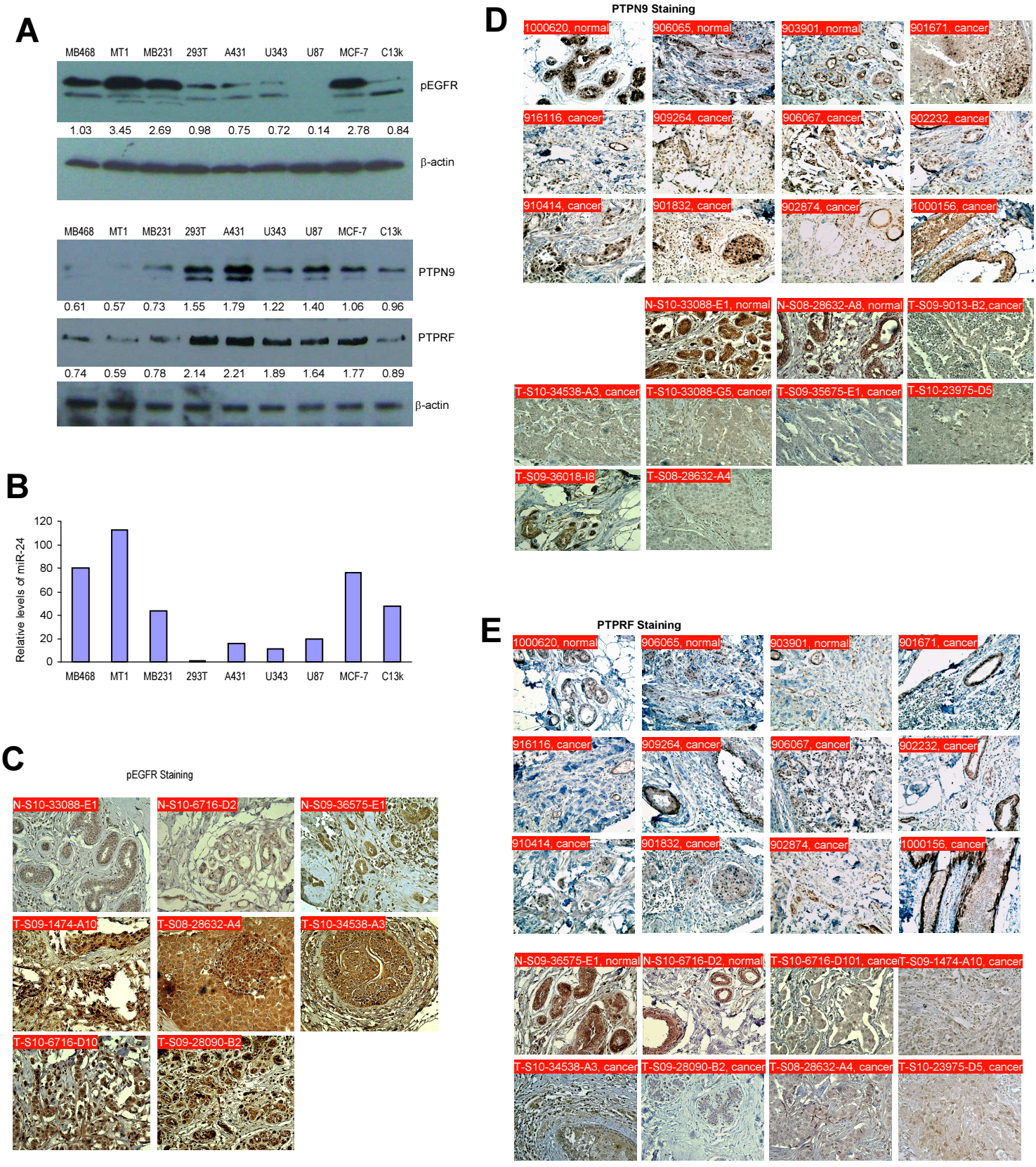

Fig S5. Relationship of the expression of pEGFR, PTPN9, PTPRF, and miR-24. (**A**) Cell lysates prepared from different cell lines were subjected to western blot analysis probed with anti-pEGFR, PTPN9, and PTPRF antibodies. While expression of pEGFR was high in the human breast cancer cell lines MB-468, MT-1, and MB-231, expression of PTPN9 and PTPRF was relatively low in these three cell lines. (**B**) Expression of miR-24 was analyzed on these cell lines with real-time PCR. The levels of miR-24 were relatively high in the three cell lines indicated above. (**C**) Human breast cancer tumors and normal breast tissues from The Affiliated People's Hospital of Jiangsu University were sectioned, and stained with antibody against pEGFR. Increased staining was detected in the tumor tissues. (**D**) Human breast cancer tumors and normal breast tissues obtained from (upper) The Affiliated People's Hospital of Jiangsu University and (lower) University of Iowa Carver College of Medicine were sectioned, and stained with antibody against PTPN9. Decreased staining was detected in the tumor tissues. (**E**) Human breast cancer tumors and normal breast tissues obtained from (upper) The Affiliated People's Hospital of Jiangsu University and (lower) University of Iowa Carver College of Medicine were sectioned, and stained with antibody against PTPRF. Decreased staining was detected in the tumor tissues.

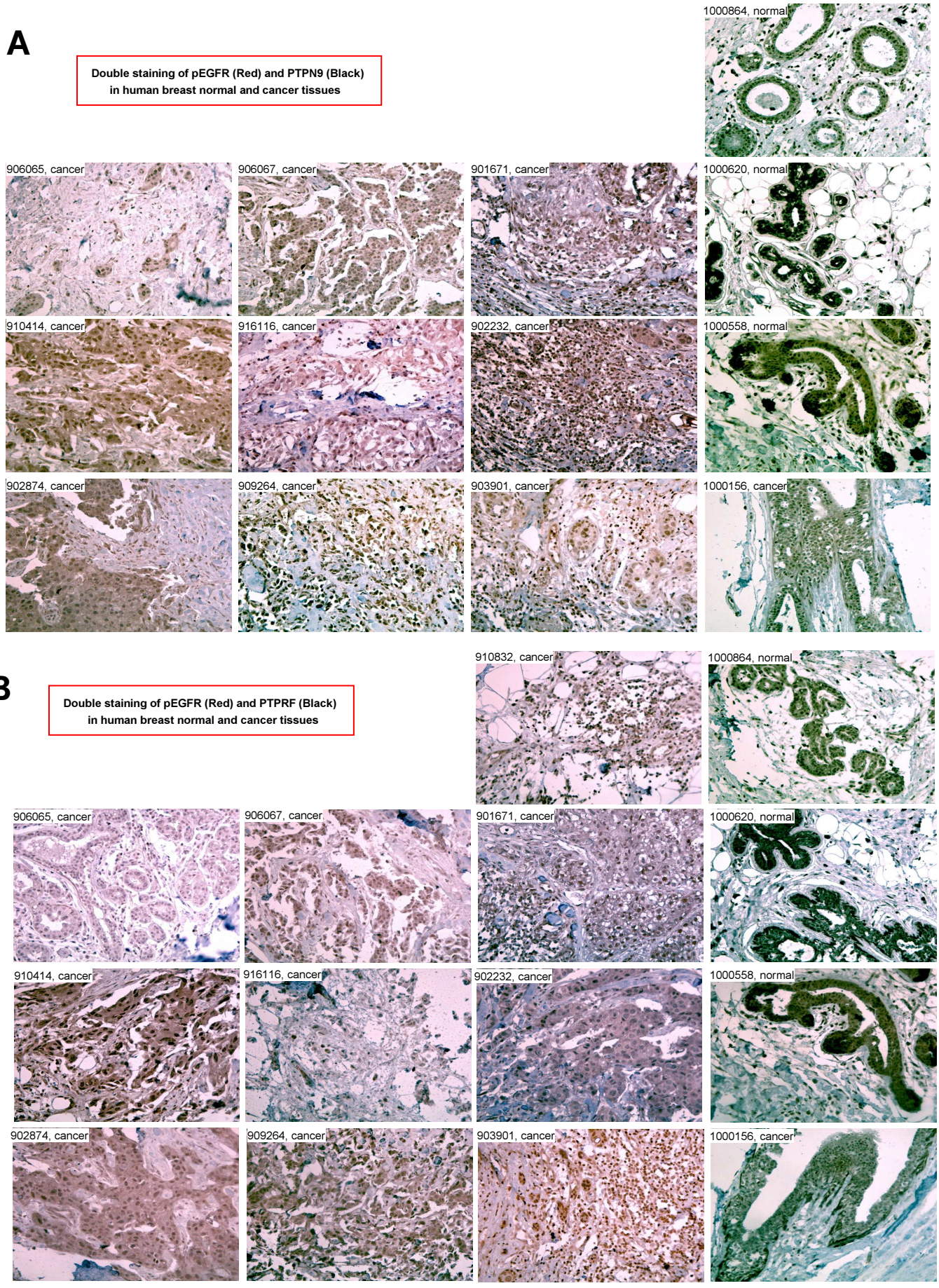

Fig S6. Double staining of pEGFR with PTPN9 or PTPRF. (**A**) Human breast cancer tumors and normal breast tissues were sectioned, and incubated with antibody to pEGFR, and stained with ACE (Red, SK-4200). After blocking with avidin/biotin blocking kit (SP-2001), all samples were incubated with antibody to PTPN9, and then stained with DAB (Black, SK-4100). (**B**) The sections were also double stained with anti-pEGFR antibody (Red) and anti-PTPRF (Black) antibodies.

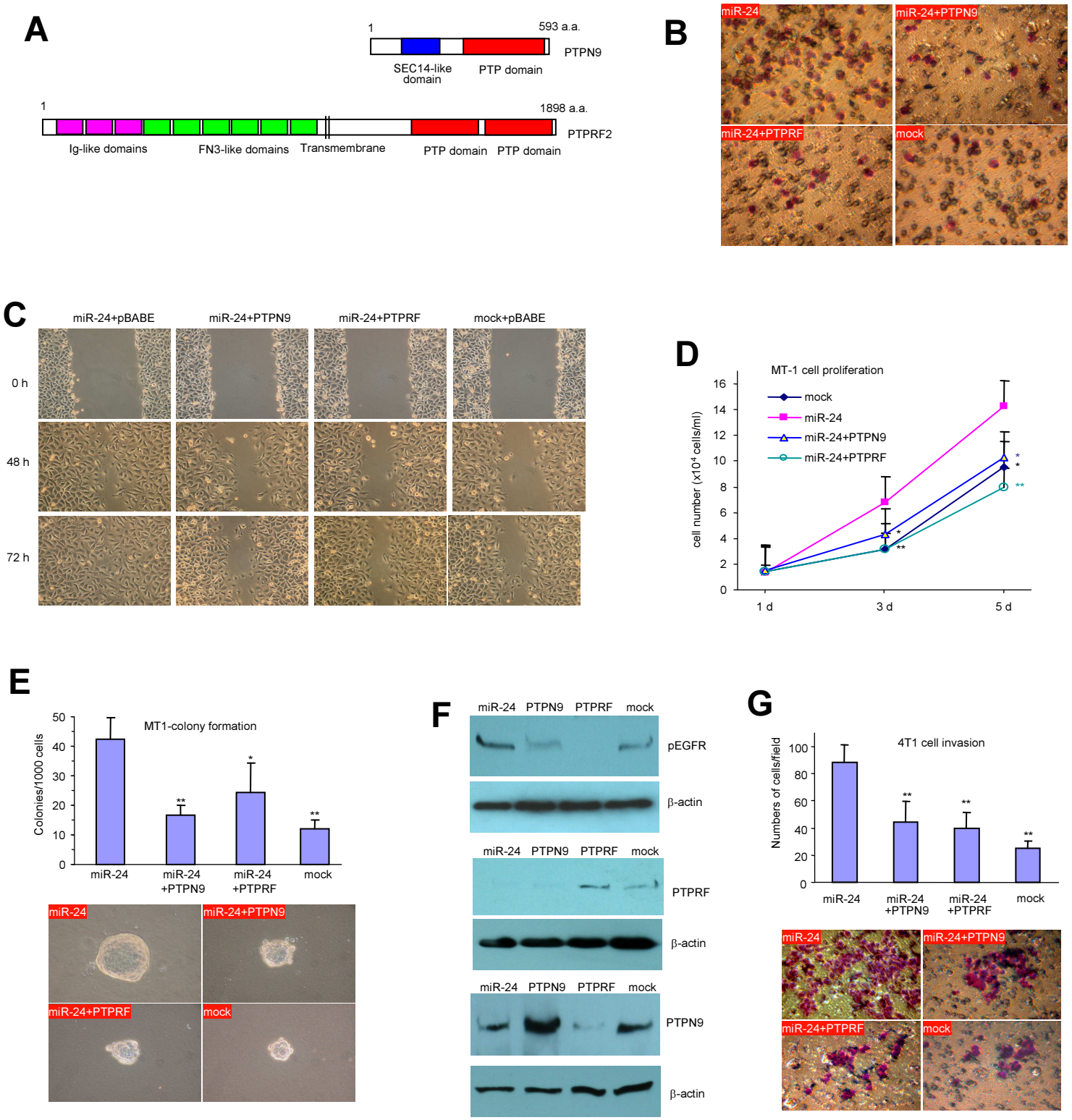

Fig S7. Reversed effects of PTPN9 and PTPRF on cell activities. (**A**) Constructs of PTPN9 and PTPRF. (**B**) Mock-, miR-24-, miR-24 and PTPN9-, or miR-24 and PTPRF-transfected MT-1 cells ($1x10^5$) were incubated at 37° C for 48 hours for invasion assays. Enhanced cell invasion by miR-24 expression was abolished by transfection with PTPN9 and PTPRF. (**C**) The cells ($1x10^3$) were also subject to migration assay. Enhanced cell migration by miR-24 expression was abolished by transfection with PTPN9 and PTPRF. (**D**) MT-1 cells transfected with mock, miR-24, miR-24 and PTPN9, or miR-24 and PTPRF were maintained in tissue culture dishes in DMEM containing 1.25% FBS for 1, 3, and 5 days for proliferation assay. Increased proliferation by miR-24 expression was abolished by transfection with PTPN9 and PTPRF. *, $p< 0.05$; **, $p< 0.01$, $n = 4$. (**E**) Mock-, miR-24-, miR-24 and PTPN9-, or miR-24 and PTPRF-transfected MT-1 cells ($1x10^3$) were subject to colony formation assays. Increases in colony formation as a result of miR-24 expression was abolished by transfection with PTPN9 and PTPRF ($n = 4$). Typical photos are shown (lower). (**F**) Cell lysates prepared from 4T1 cells transfected with mock, miR-24, miR-24 and PTPN9, or miR-24 and PTPRF were subjected to Western blot analysis for expression and activation of PTPN9, PTPRF, and EGFR. Transfection with PTPN9 and PTPRF increased their expression respectively, which resulted in decrease in pEGFR levels. (**G**) The mock- and miR-24-transfected 4T1 cells ($1x10^3$) were transfected with PTPN9 and PTPRF and incubated for 48 hours for invasion assays. PTPN9 and PTPRF partially reversed the effect of miR-24 on cell invasion ($n = 8$). Lower, typical photos of cell invasion.

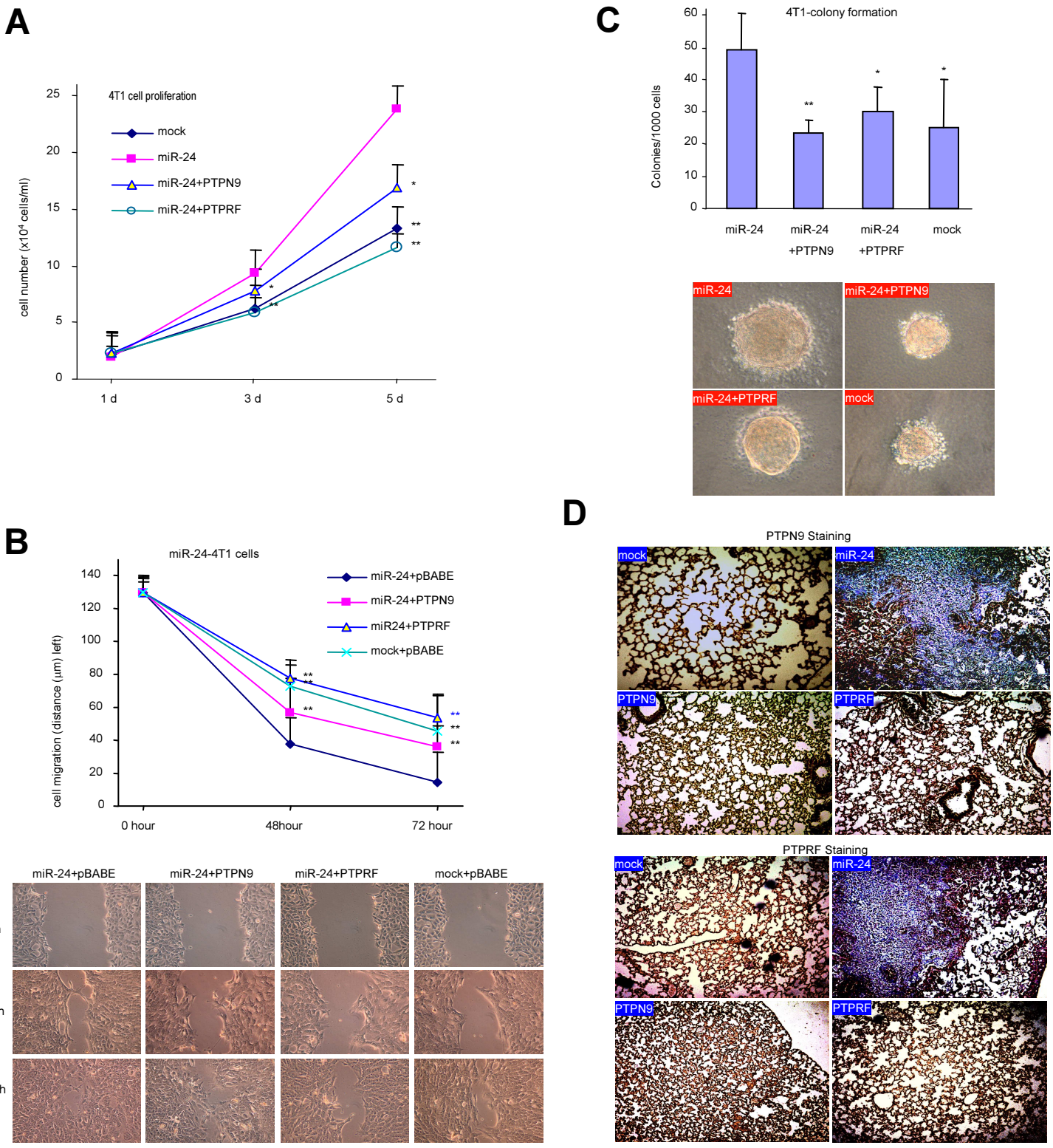

Fig S8. Reversed effects of PTPN9 and PTPRF on proliferation, migration, colony formation, and metastasis. (**A**) 4T1 cells transfected with mock, miR-24, miR-24 and PTPN9, or miR-24 and PTPRF were maintained in tissue culture dishes in DMEM with 1.25% FBS for proliferation assay (n=4). (**B**) The mock- and miR-24-transfected 4T1 cells ($1x10^3$) were transfected with PTPN9 and PTPRF as indicated for cell migration assays. PTPN9 and PTPRF reversed the effect of miR-24 on cell migration (n = 10). Lower, typical photos of cell migration. (**C**) The mock- and miR-24-transfected 4T1 cells ($1x10^3$) were transfected with PTPN9 and PTPRF and subject to colony formation assays. PTPN9 and PTPRF reversed the effect of miR-24 on the formation of colonies (n = 4). Right, typical photos of colonies. (**D**) The mock- and miR-24 lung tissues were subject to immuno-staining with anti-PTPN9 and anti-PTPRF antibodies. Increased expression of pEGFR and decreased expression of PTPN9 and PTPRF were detected in the tumor tissues.

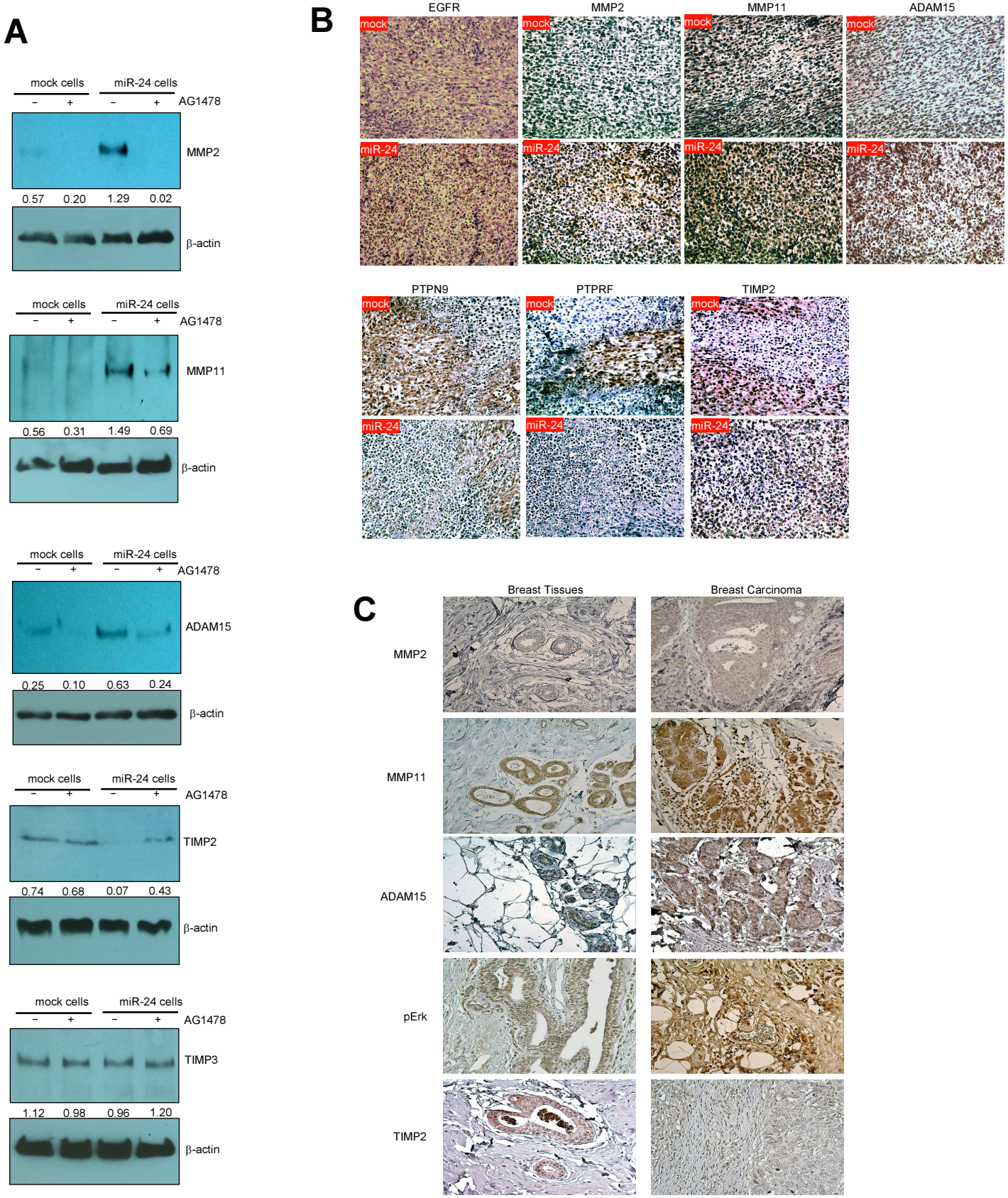

Fig S9. Effects of miR-24 on other signaling molecules. (**A**) The mock- and miR-24- transfected MT-1 cells were treated with 2.0 μM AG 1478 for 24 hours, followed by analysis on Western blot for expression of MMP2, MMP11, ADAM15, TIMP2, and TIMP3. Treatment with AG1478 down-regulated MMP2, MMP11, and ADAM15 but up-regulated TIMP2 levels in the miR-24-transfected cells. (**B**) The mock- and miR-24 tumor tissues were subjected to immunohistochemistry probed with antibodies against pEGFR, PTPN9, PTPRF, MMP2, MMP11, ADAM15, and TIMP2. Increased expression of pEGFR, MMP2, MMP11, and ADAM15 was detected, while decreased expression of PTPN9, PTPRF, and TIMP2 was detected in the tumor tissues. (**C**) Human breast carcinoma specimens were subject to immunohistochemistry probed with antibodies against MMP2, MMP11, ADAM15, pErk, and TIMP2. Increased expression of MMP2, MMP11, ADAM15, and pErk was detected, while expression of TIMP2 was decreased in the tumor tissues.

Table S1. Pathology information of patient specimens

	Age	Breast	Surgery	Histology	Grade	Tumor size	Axillary lymph nodes	ER	PR	HER2	Tumor Block	Benign Block
23975-10	27	Left	Lumpectomy	Invasive ductal carcinoma	3	3.4	Positive (1/3) Micrometastasis	Positive	Negative	Negative	D5	D8
34538-10	69	Right	Mastectomy	Invasive ductal carcinoma	2	1.5	Positive (5/10) Macrometastases	Positive	Negative	Negative	A3	A12
28090-09	33	Right	Mastectomy	Invasive ductal carcinoma	3	1.2	Positive (2/11) Macrometastases	Positive	Positive	Negative	B26	
35675-09	83	Right	Lumpectomy	Invasive ductal carcinoma	1	1.1	Positive (1/3) Macrometastases	Positive	Positive	Negative	E13	
6716-10	70	Left	Lumpectomy	Invasive ductal carcinoma	2	6.3	Positive (1/2) Macrometastases	Positive	Negative	Negative	D10	
36018-09	53	Left	Lumpectomy	Invasive ductal carcinoma	2	1.1	Positive (1/11) Macrometastases	Positive	Negative	Negative	I8	
11777-11	57	Right	Lumpectomy	Invasive ductal carcinoma	1	2.6	Positive (2/14) Macrometastases	Positive	Positive	Negative	A1	A7
33088-10	49	Right	Lumpectomy	Invasive ductal carcinoma	3	2.5	Positive (1/3) Macrometastases	Positive	Negative	Negative	G5	
28632-08	58	Left	Mastectomy	Invasive ductal carcinoma	3	4	Positive (5/12) Macrometastases	Negative	Negative	Positive	A4	A8
9013-09	45	Left	Lumpectomy	Invasive ductal carcinoma	3	1.4	Positive (1/35) Macrometastases	Negative	Negative	Negative	B26	E3
9223-10	48	Left	Lumpectomy	Invasive ductal carcinoma	2	1.1	Negative (0/2)	Positive	Positive	Negative	B2	B4
24019-10	51	Right	Mastectomy	Invasive ductal with mucinous	2	1.6	Negative (0/4)	Positive	Positive	Negative	E7	E13
21822-10	56	Left	Lumpectomy	Invasive ductal carcinoma	3	1.1	Negative (0/4)	Negative	Negative	Negative	C3	G7
28219-10	68	Left	Lumpectomy	Mucinous carcinoma	1	1.9	Negative (0/3)	Positive	Positive	Negative	C4	C6
30677-10	59	Right	Lumpectomy	Invasive ductal carcinoma	2	3.1	Negative (0/3)	Positive	Positive	Negative	C3	C8
30727-10	55	Left	Mastectomy	Invasive ductal carcinoma	3	1.9	Negative (0/4)	Negative	Negative	Negative	E4	E8
35350-10	50	Left	Lumpectomy	Invasive ductal carcinoma	3	2.6	Negative (0/1)	Positive	Positive	Positive	C3	B5
11917-11	60	Left	Lumpectomy	Invasive ductal carcinoma	2	2.9	Negative (0/2)	Positive	Positive	Negative	A1	B1
12259-11	69	Right	Lumpectomy	Invasive ductal carcinoma	1	0.4	Negative (0/4)	Positive	Positive	Negative	A18	A2
25438-10	64	Left	Mastectomy	Invasive ductal carcinoma	3	2	Negative (0/7)	Negative	Negative	Positive	D7	D19
15222-10	61	Right	Mastectomy	Invasive ductal carcinoma	2	3.8	Positive (7/14) Macrometastases	Positive	Positive	Negative	C1	D5
15195-10	65	Right	Mastectomy	Invasive ductal carcinoma	2	3.5	Positive (32/36) Macrometastases	Positive	Negative	Negative	B15	B22
21311-10	44	Left	Mastectomy	Invasive ductal carcinoma	2	2.1	Positive (1/17) Macrometastasis	Positive	Positive	Negative	A2	A1
23893-10	49	Right	Mastectomy	Invasive ductal carcinoma	2	2	Positive (1/3) Macrometastasis	Positive	Positive	Negative	D9	D12
27206-10	48	Left	Mastectomy	Invasive ductal carcinoma	2	3.1	Positive (2/12) Macrometastasis	Negative	Negative	Negative	E6	D4
27707-10	39	Right	Mastectomy	Invasive lobular carcinoma	2	0.9	Positive (1/5) Macrometastasis	Positive	Positive	Negative	B3	C8
11556-11	45	Right	Lumpectomy	Invasive ductal carcinoma	2	3.4	Positive (4/7) Macrometastases	Positive	Positive	Negative	A13	A49
1474-09	57	Left	Mastectomy	Invasive ductal carcinoma	3	2	Positive (1/11) Macrometastasis	Positive	Positive	Positive	A14	A8
25055-08	60	Left	Mastectomy	Invasive ductal carcinoma	3	0.4	Positive (12/13) Macrometastases	Negative	Negative	Positive	G2	G10
19057-08	52	Left	Mastectomy	Invasive ductal carcinoma	3	1.9	Positive (4/8) Macrometastases	Negative	Negative	Negative	B1	B16
17862-11	52	Left	Mastectomy	Invasive ductal carcinoma	3	7.8	Positive (18/18) Macrometastases	Negative	Negative	Negative	C7	
19378-11	63	Right	Mastectomy	Invasive ductal carcinoma	2	3.5	Positive (1/3) Macrometastasis	Positive	Positive	Negative	C1	
29075-11	77	Left	Mastectomy	Invasive ductal carcinoma	1	3.8	Positive (1/4) Micrometastasis	Positive	Positive	Negative	D12	
31466-11	50	Left	Mastectomy	Invasive ductal carcinoma	3	4.4	Positive (1/12) Macrometastasis	Negative	Negative	Negative	A4	
36798-11	57	Right	Lumpectomy	Invasive ductal carcinoma	3	1.7	Positive (6/19) Macrometastasis	Negative	Negative	Positive	I12	
37033-11	48	Right	Lumpectomy	Invasive ductal carcinoma	3	2.3	Positive (1/2) Macrometastasis	Negative	Negative	Negative	A5	
39338-11	42	Left	Mastectomy	Invasive ductal carcinoma	3	8	Positive (7/22) Macrometastases	Negative	Negative	Negative	A1	
20572-10	50	Left	Mastectomy	Invasive ductal carcinoma	3	6.1	Positive (2/13) Macrometastases	Negative	Negative	Negative	I4	
32212-10	57	Right	Mastectomy	Invasive ductal carcinoma	3	3.9	Positive (7/11) Macrometastases	Positive	Positive	Negative	B3	
36598-10	40	Left	Lumpectomy	Invasive ductal carcinoma	3	3.5	Positive (7/11) Macrometastases	Negative	Negative	Negative	A4	
30703-11	83	Right	Mastectomy	Invasive ductal carcinoma	1	2.5	Negative (0/3)	Positive	Positive	Negative	D6	
25800-11	74	Left	Mastectomy	Invasive ductal carcinoma	3	2.8	Negative (0/4)	Positive	Positive	Positive	D8	
19287-11	45	Right	Mastectomy	Invasive ductal carcinoma	3	3	Negative (0/2)	Positive	Positive	Positive	C2	
13853-11	66	Right	Lumpectomy	Invasive ductal carcinoma	2	2.6	Negative (0/3)	Positive	Negative	Negative	A3	
36126-10	52	Left	Mastectomy	Invasive ductal carcinoma	3	5.9	Negative (0/2)	Negative	Negative	Negative	C6	