A signal-based method for finding driver modules of breast cancer metastasis to the lung

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Running title: Find drivers of breast cancer metastasis to lung

Keywords: cancer metastasis, transcriptomic module, common signal, driver genome alterations.

Supplementary

Cell migration test for new drivers

Cell lines and cell culture: HCC1937, MDA-MB-231 and HCC1806 SPORE cell lines were from the American Type Culture Collection (Manassas, VA, USA). The MDA-MB-231 were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). HCC1937 and HCC1806 were maintained in RPMI 1640 supplemented with 10% FBS. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Antibodies: The specific antibody against *BCL2L11* and the secondary antibodies of Horseradish peroxidase–conjugated goat anti-mouse and anti-rabbit were from Cell Signaling (Beverly, MA). The *CDH9* antibody was from Thermo Fisher Scientific. The β-actin antibody was purchased from Sigma-Aldrich (St. Louis, MO).

SiRNA transfection:

Smartpool: on-targetplus *BCL2L11*, *CDH9* and control siRNA were purchased from Dharmacon.

Human *BCL2L11* siRNA - SMARTpool, L-004383-00-0005

Human *CDH9* siRNA - SMARTpool, L-013169-00-0005

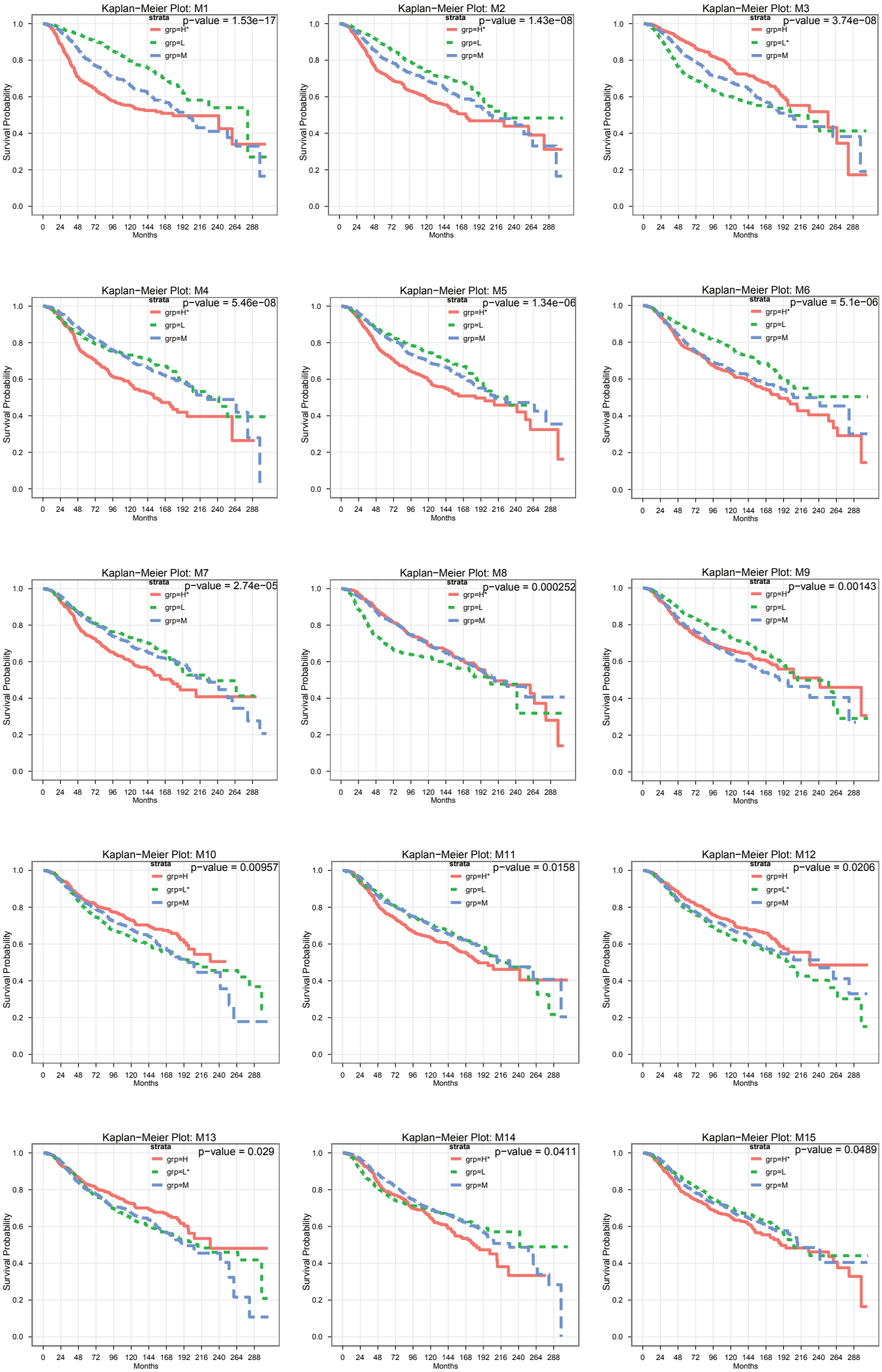
Non-targeting Pool, D-001810-10-05

SiRNAs were transfected into HCC1937, MDA-MB-231, and HCC1806 cells using Lipofectamine RNAiMAX Reagent (Life technologies).

Immunoblotting: The HCC1937, MDA-MB-231 and HCC1806 cells were washed with PBS and collected in a boiling sample buffer 3 days after siRNA transfection. Cellular proteins were resolved by SDS–PAGE (12% acrylamide) and transferred to PVDF membranes (Merck Millipore Ltd). After blocking with 5% non-fat milk in PBST (PBS and 0.1% Tween 20), the membranes were incubated overnight in a cold room with the primary antibodies and for 1 h with the horseradish peroxidase–conjugated secondary antibody. Bound antibodies were detected using Clarity Western ECL substrate (Bio-Rad).

Migration assays: A wound healing assay was used to analyze the cell migration of transfectant cells. $4x10^5$ of cells were seeded in 35 mm dishes. Cells were transfected with siRNAs 24 hrs later and cultured for 2 days to a confluence of 90%. The cells were then starved with 0.1% FBS overnight and scratched with a sterile 200-μl micropipette tip to form a straight wound. The cells were washed three times with PBS and cultured in normal medium for an additional 24 h. An Olympus IX83 microscope was used to measure the wound closure. Images were recorded at the time points of 0, 6, 12 and 24 h after wounding. The distances invaded by the cells at the front of the wound were measured from the control and the experimental samples. Cell migration was assayed by calculating the migrated distance and comparing with time 0.

Statistic test: The differences between the control and treated groups was evaluated using a *t*-test. Statistical significance was calculated based on three experiments. A *p*-value of <0.05 was considered statistically significant.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Kaplan−Meier Plot: M2
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Kaplan−Meier Plot: M3
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Kaplan−Meier Plot: M4
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Kaplan−Meier Plot: M8
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Kaplan−Meier Plot: M9
p−value = 0.00143
Kaplan−Meier Plot: M10
p−value = 0.00957
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p−value = 0.0158
Kaplan−Meier Plot: M12
p−value = 0.0206
Kaplan−Meier Plot: M13
p−value = 0.029
Kaplan−Meier Plot: M14
p−value = 0.0411
Kaplan−Meier Plot: M15
p−value = 0.0489
Survival Probability
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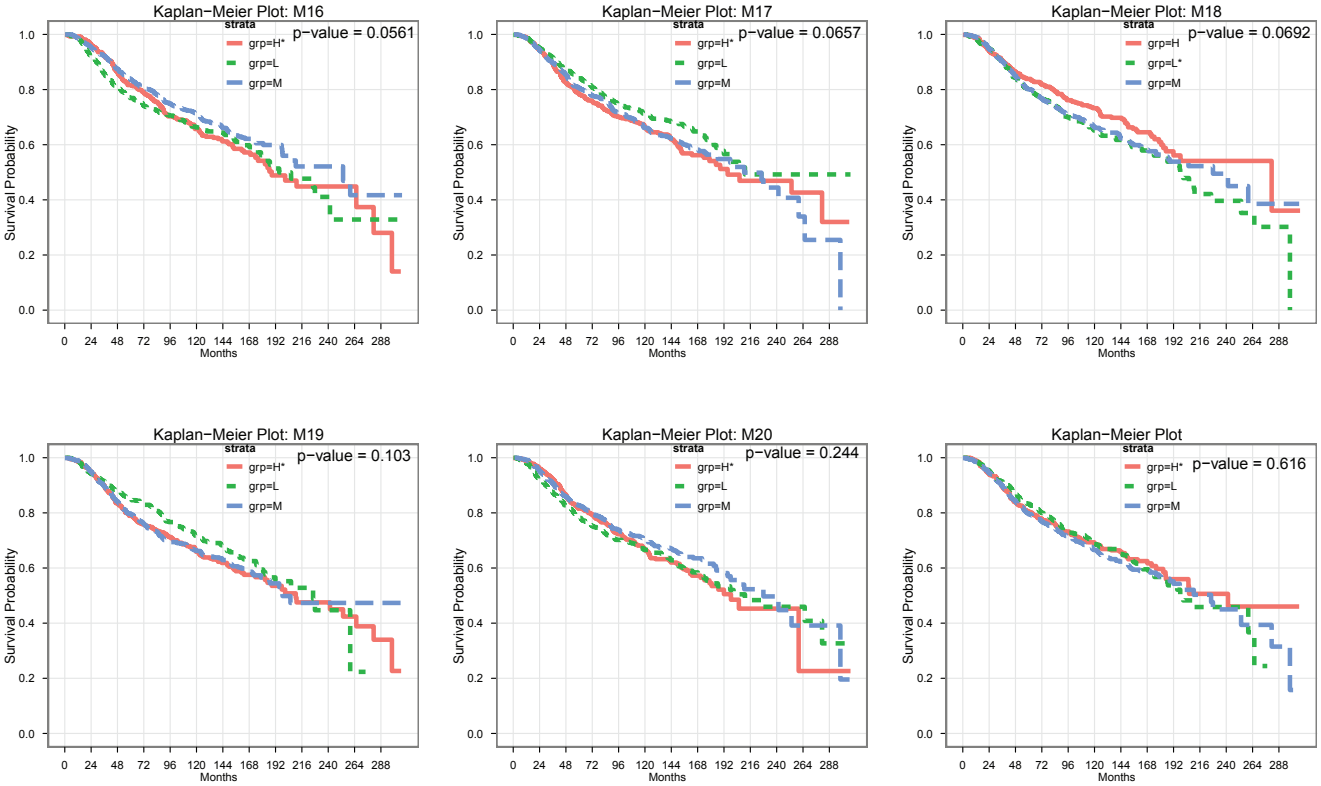

Fig. S1: **The impact of expression statuses of gene transcriptomic modules to the patients' survival**. Patient groups with high, middle, low expressions of a transcriptomic module are represented as H, M, L, respectively. H* means that the module is up-regulated in cell lines with high metastatic activities. L* means that the module is down-regulated in cell lines with high metastatic activities.

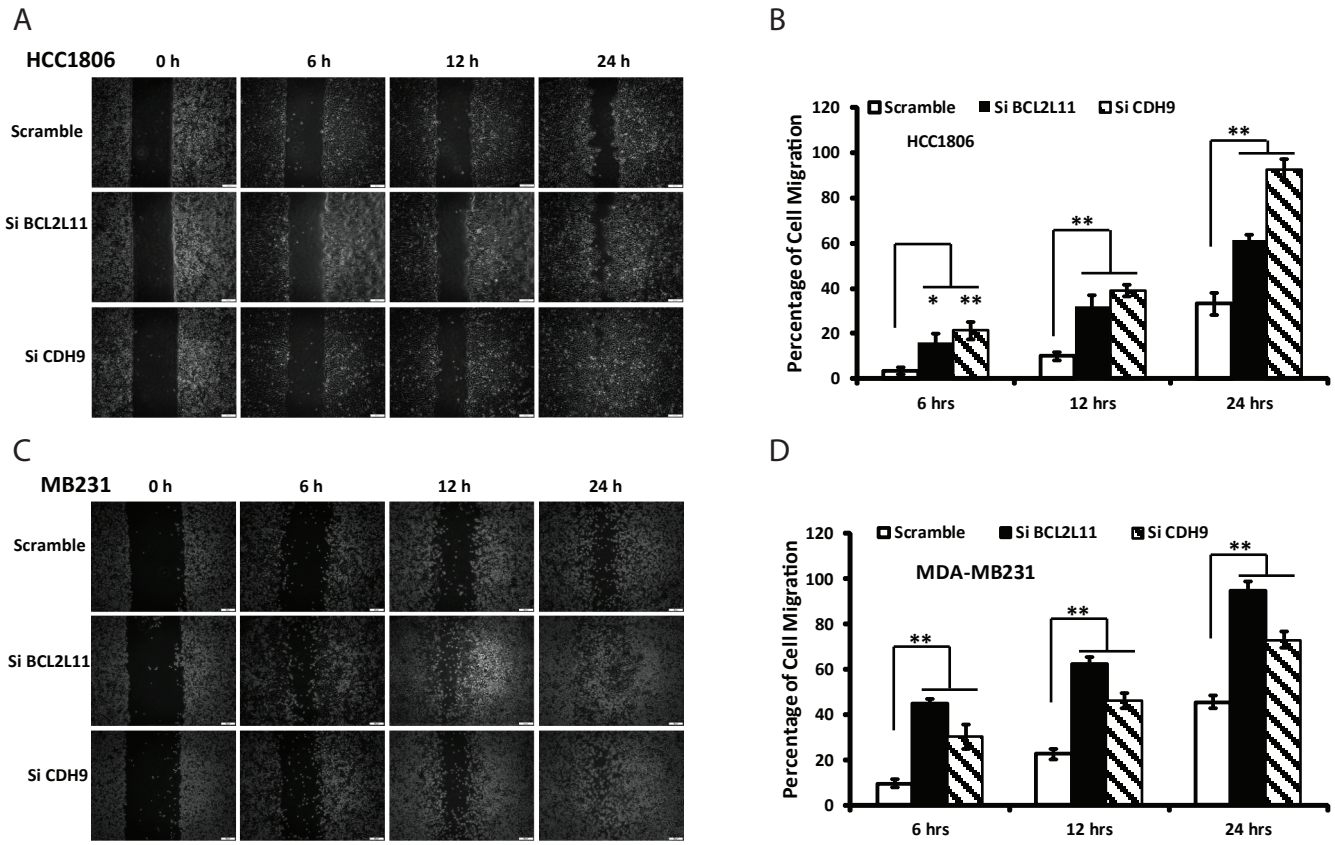

Fig. S2: Wound healing assays of HCC1806 and MDA-MB-231 cell lines. A B) siBCL2L11, siCDH9 of HCC1806 cell line promote EMT significantly. C D) siBCL2L11, siCDH9 of MDA-MB-231 cell line promote EMT significantly.

Table S1: Literature search reveals that many genes in our driver modules are related to metastasis

Modu ID	References	Count
1	LSR[1-3], PTP4A3[4-7], S100A14[8-10], SMCP[11], TP53[12-16], ZWINT	5
2	ABCF3, ATG7[17,18], ERBB2[19-22], NME1[23-26], ODF2[27,28], VPS72	4
3	FAM49B, NME1[23-26], STARD7[29], TMEM189, ZFYVE16, ZNF596	2
4	C20orf201, ERBB2[19-22], FBN1, HOOK1[30], RP9, ZBTB7B	2
5	COIL, GRB7[31-34], IL20[35-37], PYCR2, SH2B1[38], TPCN2	3
6	CGN, GNA13[39-41], GRHL2[42-44], ITM2B, MTL5, TNFRSF10B[45]	3
7	EPPK1, ERBB2[19-22], IMPG2, KIAA0195, POLDIP2[46,47], PYGB	2
8	ANO1[48-50], ATAD5, CLCF1, IARS, PIK3CA[51-55], TRIM58	2
9	BCL2L11[56-58], DISP2, IL2RA[59-62], PLEKHG6[63-65], SMC1B, TMEM105	3
10	APOA1BP, MRPL55, RBM4B, SHARPIN[66,67], SMARCD2, XPO7[68]	2
11	CCNI, DPT[69,70], LIMD2, LRCH4, LY96[71,72], RUSC2	2
12	GNRHR2, LRRC42, PPP1R3B, SDHAP1, STMN3[73,74], TRPS1[75-77]	2
13	ARHGEF10, CBWD1, EIF4A3, RECQL4[78], RUSC1, TRPS1[75-77]	2
14	ATP1B4, ERLIN2, IFT88, RNFT1, SLC7A13, VIL1[79]	1
15	DOCK11, GRB7[31-34], MAP3K13, SLC39A11, TAB2[80,81], ZCCHC11[82,83]	3
16	C8orf86, CACNG4, CHAF1A[84], FAM5B, SMYD3[85-87], ZNF835	2
17	CAPN8, CDH9, COQ10B, HOXB3[88-90], IPPK, SGK494	1
18	BAIAP2[91], EEF1A2[92-94], GUK1[95], PPP1R3B, TEX2, TRPS1[75-77]	4
19	ADCK5, CHMP6, NDUFS6, RAB13[96-98], TDRD12, TRMT1	1
20	PIK3CA[51-55], RGS16[99-101], STARD3[102,103], TANC2, TGFB2[104-106], TRIM58	4
21	C1S[107-109], LY96[71,72], PLP2[110-112], PPP3CA[113], SLAMF8, ZBP1[114-117]	5

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