Supplementary Materials: Mmu-miR-1894-3p Inhibits Cell Proliferation and Migration of Breast Cancer Cells by Targeting Trim46

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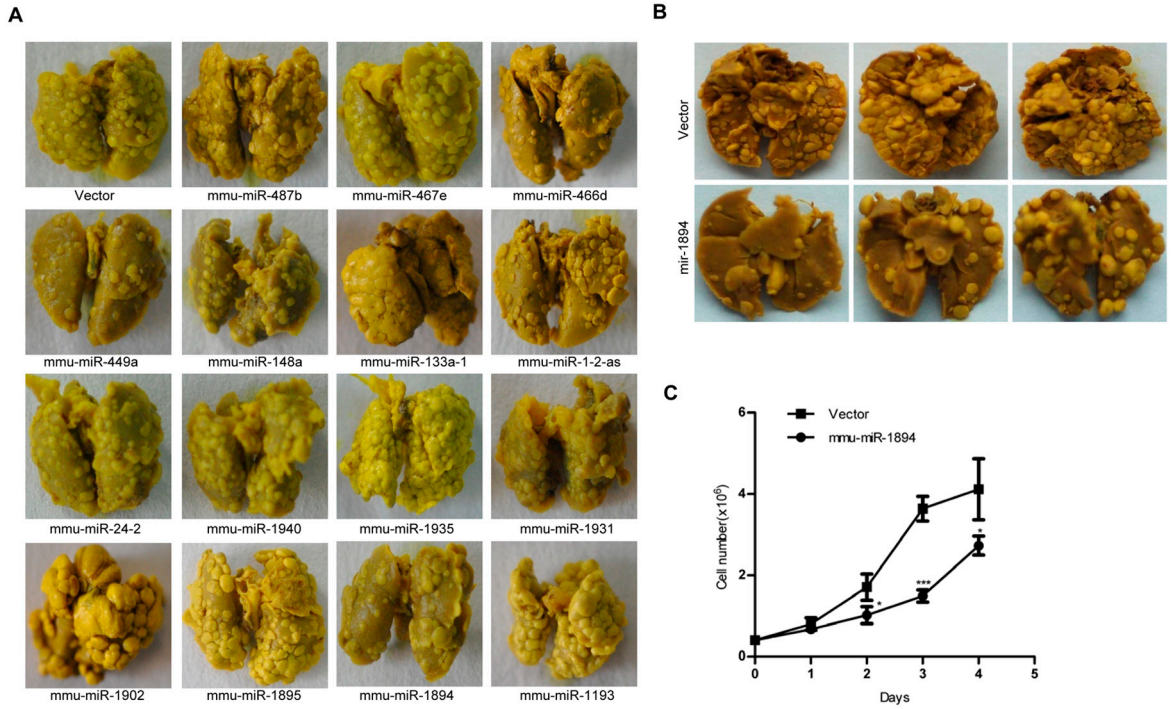

Figure S1. Fifteen mouse microRNAs and lung metastasis assay. (**A**) 4TO7 mouse breast cancer cells were transfected with empty vector or fifteen individual microRNA expression plasmids, including miR-487b, miR-467e, miR-466d, miR-449a, miR-148a, miR-133a-1, miR-1-2-as, miR-24-2, miR-1940, miR-1935, miR-1931, miR-1902, miR-1895, miR-1894, and miR-1193. Stable cell lines were generated and 1×10^6 cells were injected into the tail veins of Balb/c female mice ($n = 6$ for each group). Two weeks later, the mice were sacrificed; the lungs were fixed in Bouin's solution and photographed. Shown are the representative photos for lung metastasis nodules (one lung for each microRNA); (**B**) Representative photos for lung metastasis of 4TO7 cells expressing miR-1894 and the vector control; and (**C**) 4TO7 cells expressing miR-1894 and vector control were seeded into 24-well plates at 1×10^4 cells/well and counted using Trypan Blue after 24, 48, 72 and 96 h. Growth curves were made from data of cell counting for four days. * indicates $p < 0.05$; *** indicates $p < 0.001$ *versus* vector control.

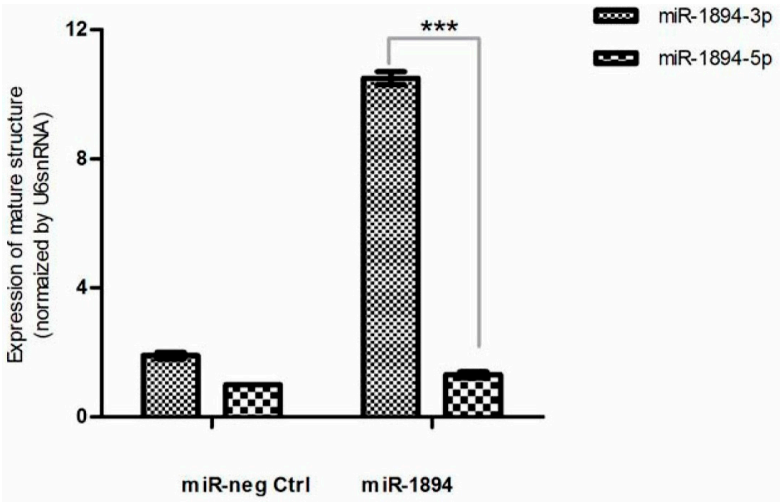

Figure S2. The expression level of two mature structures of miR-1894 in 4TO7 mouse breast cancer cells. miR-1894 produces two mature structure forms, miR-1894-3p and miR-1894-5p. The expression of miR-1894-3p and miR-1894-5p in 4TO7 cells expressing miR-1894 and the vector control was detected by real-time PCR. *** indicates $p < 0.001$ miR-1894-3p *versus* miR-1894-5p.

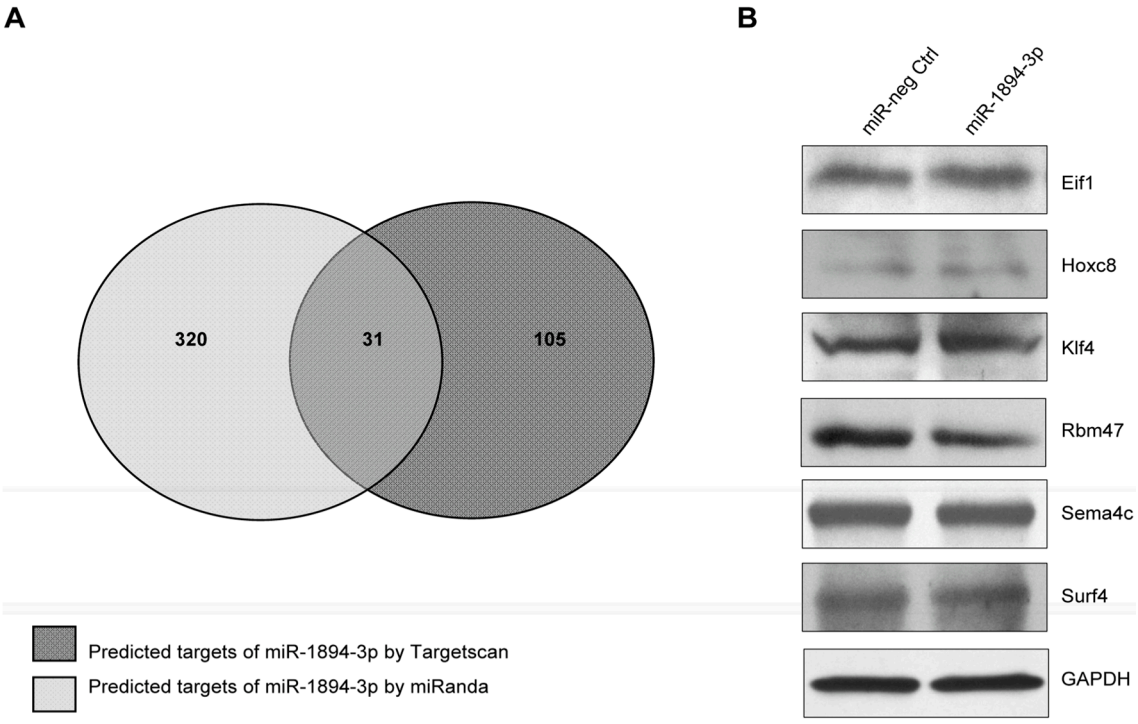

Figure S3. Identification of pontential target genes. (**A**) miR-1894-3p targets were predicted using the TargetScan and miRanda programs, and the lists of predicted targets were merged, resulting in 31 genes as candidates for direct targeting by miR-1894-3p.B. Seven genes (Eif1, Hoxc8, Klf4, Rbm47, Sema4c, Surf4 and Trim46) were selected as candidate targets of miR-1894-3p; and (**B**) Immunoblotting was carried out with antibodies specific for Eif1(1:200, Santa Cruz), HOXC8 (mAb; 1:1000; Abcam), KLF4 (1:1000, Abcam), Rbm47 (1:1000, Novus Biologicals), Sema4c (1:500, Bioss) SURF4-C-terminal (1:1000, Abcam), Trim46 (1:2000, Proteintech), Primary antibodies were visualized with anti-rabbit HRP conjugated secondary antibodies. GAPDH was used for normalization.

Table S1. Thirty-one potential targets predicted by TargetScan and MiRanda programs.

No.	Representative Transcript	Gene Name
1	NM_175123	*RIKEN cDNA 1110051M20* gene
2	NM_001081400	*RIKEN cDNA 1810013L24* gene
3	NM_002892	*AT rich interactive domain 4A (RBP1-like)*
4	NM_001126054	*Calcium/calmodulin-dependent serine protein kinase (MAGUK family)*
5	NM_001173982	*Carbohydrate (chondroitin 4) sulfotransferase 11*
6	NM_008176	*Chemoki ne (C-X- C motif) ligand 1*
7	NM_005801	*Eukaryotic translation initiation factor 1*
8	NM_001013415	*F-box and WD repeat domain containing 7*
9	NM_022658	*Homeobox C8*
10	NM_001034914	*Kcnip3 Kv channel interacting protein 3, calsenilin*
11	NM_000899	*KIT ligand*
12	NM_004235	*Kruppel-like factor 4 (gut)*
13	NM_001080774	*Myosin IC*
14	NM_002522	*Nptx1 neuronal pentraxin I*
15	NM_004161	*RAB1A, member RAS oncogene family*
16	NM_001098634	*RNA binding motif protein 47*
17	NM_172612	*Rnd1Rho family GTPase 1*
18	NM_017789	*Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C*
19	NM_001172700	*Shroom family member 1*
20	NM_001128225	*Solute carrier family 39 (zinc transporter), member 13*
21	NM_000342	*Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)*
22	NM_011512	*Surfeit* gene 4
23	NM_006950	*Synapsin I*
24	NM_015527	*TBC1 domain family, member 10B*
25	NM_001033422	*THO complex 2*
26	NM_001126339	*Thiamine triphosphatase*
27	NM_001039466	*Tripartite motif-containing 46*
28	NM_025692	*Ubiquitin-like modifier activating enzyme5*
29	NM_003680	*Tyrosyl-tRNA synthetase*
30	NM_152758	*YTH domain family, member 3*
31	NM_014827	*Zinc finger CCCH-type containing 11A*