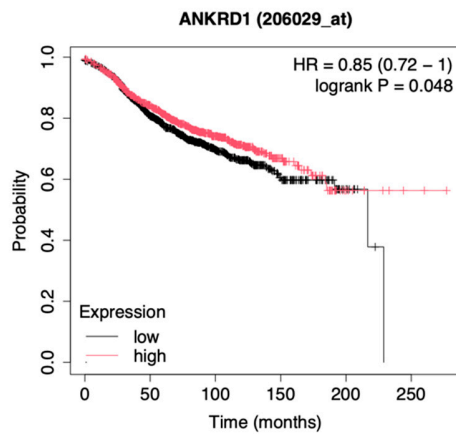
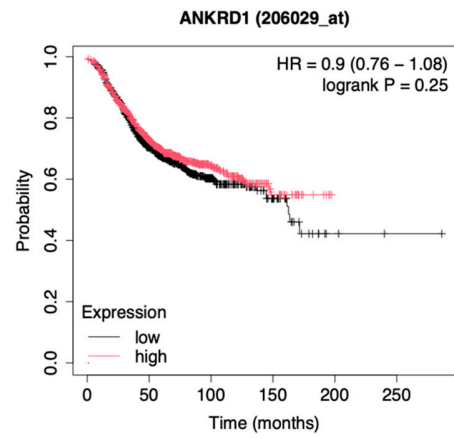
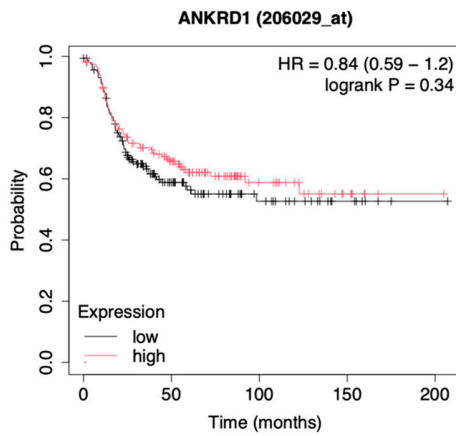
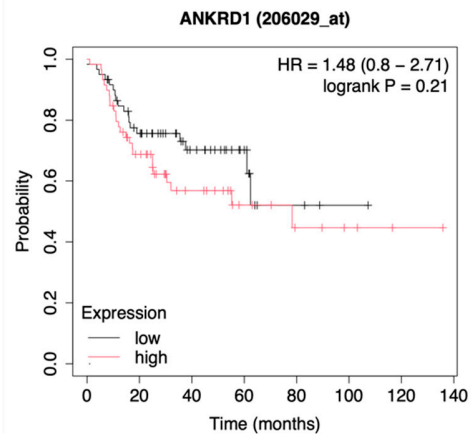
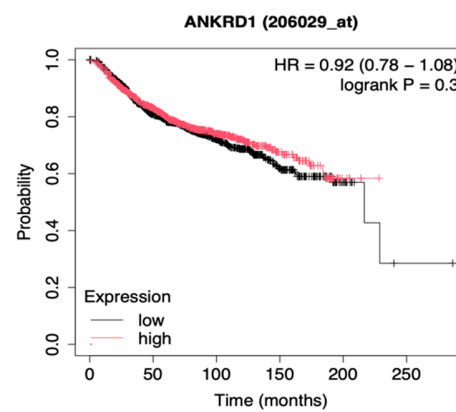
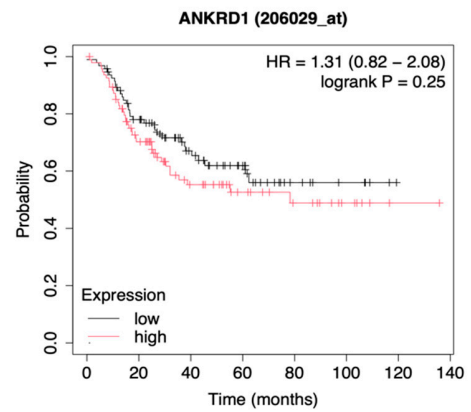


## Supplementary Materials

**Table S1.** Primer list for real time PCR.

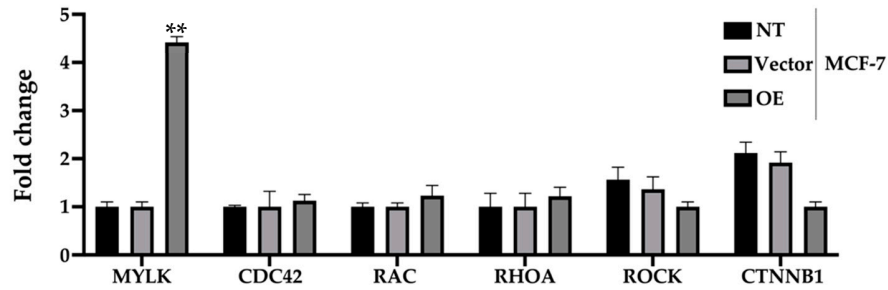
Gene name		Sequence (5'-3')
<i>Gapdh</i>	F R	CTTCAACAGCGACACCCACTCCTC GTCCACCACCCTGTTGCTGTAG
<i>ANKRD1</i>	F R	CGGAGCATCTTATCGCCTGT TTGAGATCCGCGCCATACAT
<i>ANKRD1_cloning</i>	F R	GACGTAAGATCTATGATGGTACTGAAAGTAGAGGAAC GGATCTGCGGCCGCTCAGAATGTAGCTATGCGAGAG
<i>E-cadherin</i>	F R	GAACGCATTGCCACATACAC ATTCGGGCTTGTTGTCATTC
<i>N-cadherin</i>	F R	AGGATCAACCCCATACACCA TGGTTTGACCACGGTGACTA
<i>Vimentin</i>	F R	GAGAACTTTGCCGTTGAAGC CTCAATGTCAAGGGCCATCT
<i>Snail1</i>	F R	TGCCCTCAAGATGCACATCCGA GGGACAGGAGAAGGGCTTCTC
<i>Twist</i>	F R	GTCCGCAGTCTTACGAGGAG TGAATCTTGCTCAGCTTGTC
<i>Fibronectin</i>	F R	AAAATGGCCAGATGATGAGC TGGCACCGAGATATTCCTTC
<i>CDC42</i>	F R	GCGATGGTGCTGTTGGTAAA TGTGGATAACTCAGCGGTCG
<i>MYLK</i>	F R	TGCCTCGTCACACATTTCCA TCCGAGGGGGGCAAAATGAAA
<i>ROCK1</i>	F R	TGAAGGTGATTGGTAGAGGTGC GGCAAAAGCCATGATGTCCC
<i>RAC</i>	F R	GGGAGACGGAGCTGTAGGTA CGGATAGGATAGGGGGCGTA
<i>RHOA</i>	F R	TCGTTAGTCCACGGTCTGGT CAGCCATTGCTCAGGCAAC
<i>CTNNB1</i>	F R	GCTGGGACCTTGCATAACCT CGCACTGCCATTTTAGCTCC
<i>SOX2</i>	F R	GGGAAATGGGAGGGGTGCAAAAGAGG TTGCGTGAGTGTGGATGGGATTGGTG
<i>Nanog</i>	F R	ACC AGT CCC AAA GGC AAA CA TCT GCT GGA GGC TGA GGT AT
<i>OCT4</i>	F R	CTT CCC TCC AAC CAG TTG CCC CAA AC GGG AAA TGG GAG GGG TGC AAA AGA GG
<i>KLF4</i>	F R	GGACACACGGGATGATGCTC TTGTGTAGGTTTTGCCGCAG
<i>CXCL1</i>	F R	TCACAGTGTGTGGTCAACAT AGCCCCTTTGTTCTAAGCCA
<i>CSF3</i>	F R	AGAGCTTCCTGCTCAAGTGC AGCTTGTAGGTGGCACACTC

Gene name		Sequence (5'-3')
<i>EIF4EBP3</i>	F R	GTCCACTAGCTGCCCCGATTC CTCCAGCAGGAACTTTTCGGT
<i>IL1B</i>	F R	GTACCTGTCCTGCGTGTTGA GGGAACTGGGCAGACTCAAA
<i>IPO11</i>	F R	CAGGCCACCAGTCAGGATAC GCTACACGTCTCCAGTAGCG
<i>LRRC70</i>	F R	TTCTCTGCCTTGCCTACGAC GCAGAGCTGACAAACAGACG
<i>CXCL2</i>	F R	CTCAAGAATGGGCAGAAAGC CTTCAGGAACAGCCACCAAT
<i>CXCL3</i>	F R	TGTGAATGTAAGGTCCCCCG TTGGTGCTCCCCCTTGTTTCAG
<i>CSF2</i>	F R	GGGAGCATGTGAATGCCATC GGCTCCTGGAGGTCAAACAT
<i>MAGE-A6</i>	F R	CAGAGGAGTCAGCACTGCAA GACTCTGGGGAGGATCTGGT
<i>DST</i>	F R	TGCCAACAGAGTTCAGAGGG GCTGGCGTAAAAGGTTCTCAC
<i>IL-8</i>	F R	GGTGCAGTTTTGCCAAGGAG TTCCTTGGGGTCCAGACAGA

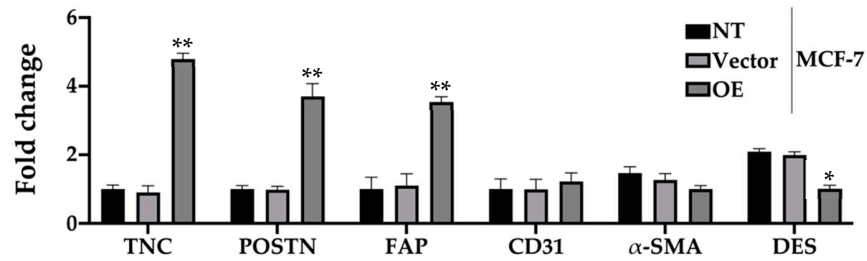
**A****B****C****D****E****F**

**Figure S1.** “ANKRD1” gene in TCGA on the database in breast cancer using Kaplan-Meier survival analysis (mRNA gene chip). **(A)** Survival rate of luminal A subtype. **(B)** Survival rate of luminal B subtype. **(C)** Survival rate of HER2 positive subtype. **(D)** Survival rate of triple negative subtype. **(E)** Survival rate of lymph node negative. **(F)** Survival rate of lymph node positive. ns > 0.01.

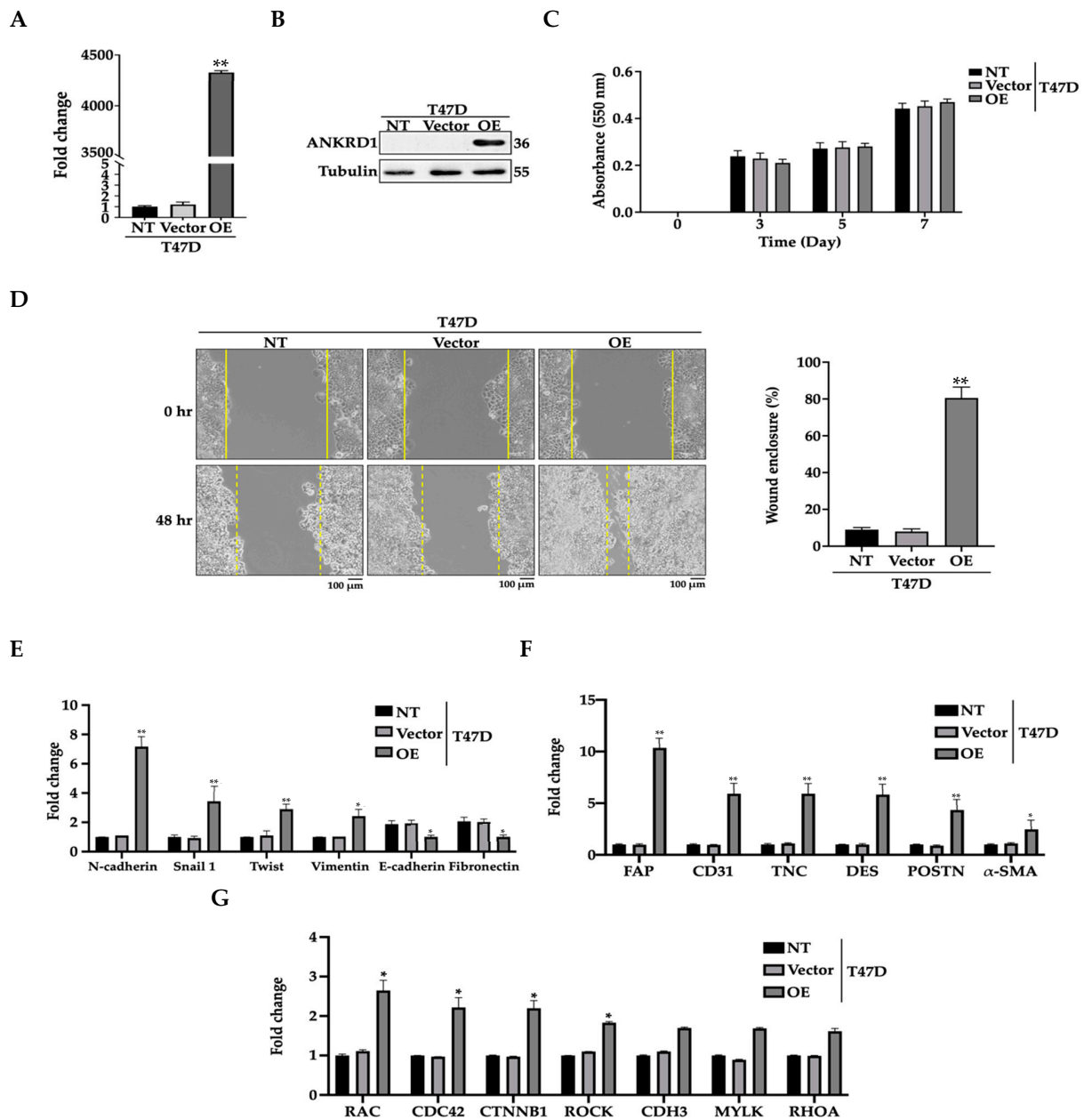
**A**



**B**

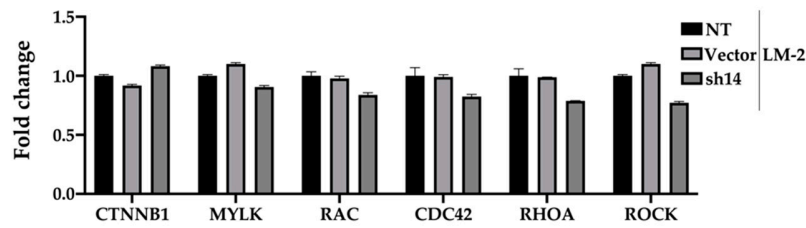


**Figure S2.** Comparison of migration and adhesion and cancer associated fibroblasts markers. **(A)** Real time PCR results showing fold change of migration and adhesion markers. The data shows upregulation of MYLK and downregulation of CTNNB1 in OE cells compared to NT and Vector. **(B)** Real time PCR results showing fold change of cancer associated fibroblasts (CAFs) markers. The data shows upregulation of TNC, POSTN, FAP and downregulation of DES in OE cells compared to NT and Vector. NT: MCF-7 parental, Vector: MCF-7-empty vector and OE: MCF-7-ANKRD1 overexpression. All experiments were performed in triplicate. \*\* $\leq 0.001$  and \* $\leq 0.05$ .

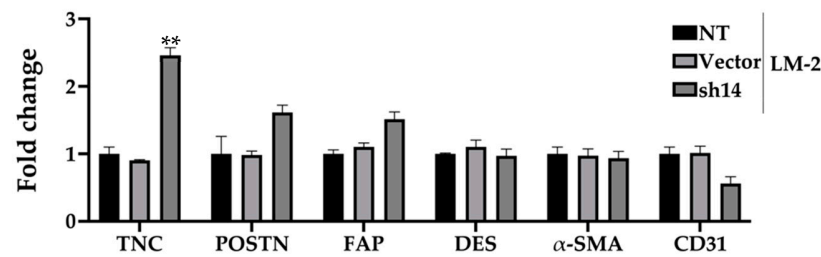


**Figure S3.** Overexpression of ANKRD1 induces cell migration and invasion in T47D, a non-metastatic breast cancer (**A-B**) Results of real time PCR and Western blot assay showing the gene and protein expression in the ANKRD1 overexpressed cell lines (T47D-ANKRD1 OE, OE) (**C**) Cell proliferation of ANKRD1 overexpressing cells compared to vector and normal T47D was determined by EZ-Cytos assay. (**D**) Representative images depicting wound healing quantified as a percentage of the healed wound area and analyzed by Image JS. (**E-G**) Real time PCR showing the gene expression involved in EMT, migration-adhesion and CAFs. OE cells showed reduced expression of E-cadherin and increased expression of N-cadherin, Snail1, Twist, FAP, CD31, TNC, DES, POSTN,  $\alpha$ -SMA, RAC, CDC42, CTNNB1, ROCK, CDH3, MYLK and RHOA compared to Vector. NT: T47D parental, Vector: T47D-empty vector and OE: T47D-ANKRD1 overexpression. All experiments were performed in triplicate. \*\* $\leq 0.001$  and \* $\leq 0.05$ .

**A**

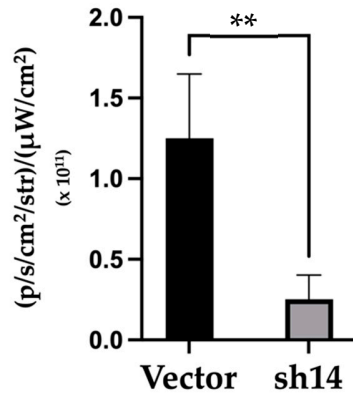


**B**

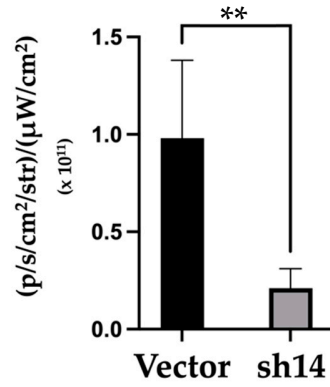


**Figure S4.** Comparison of migration and adhesion and cancer associated fibroblasts markers **(A)** Real time PCR results showing fold change of migration and adhesion markers. The data shows downregulation of RHOA and ROCK in sh14 cells compared to NT and Vector. **(B)** Real time PCR results showing fold change of cancer associated fibroblasts (CAFs) markers. The data shows upregulation of TNC, POSTN and FAP and downregulation of CD31 in sh14 cells compared to NT and Vector. NT: LM-2 parental, Vector: LM-2 with empty vector and sh14: LM-2-ANKRD1 knockdown with sh14. All experiments were performed in triplicate. \*\* $\leq 0.001$  and \* $\leq 0.05$ .

A

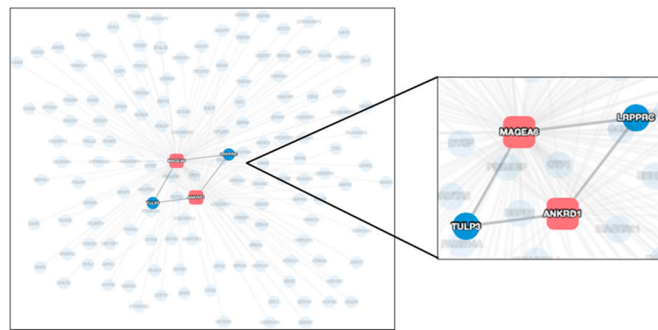


B



**Figure S5.** Comparison of the radiant efficacy of different organs at day 28 *in vivo* metastasis models **(A)** Radiant efficacy of lung. **(B)** Radiant efficacy of liver. \*\* $\leq 0.001$  and \* $\leq 0.05$ .

A



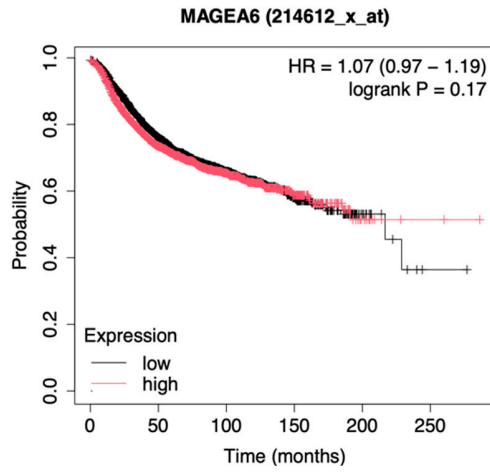
**Figure S6.** Protein-protein interaction **(A)** Protein interaction between ANKRD1 and MAGE-A6 using <http://omics.bjccancer.org>.

**Table S2.** List of siRNAs used in the study.

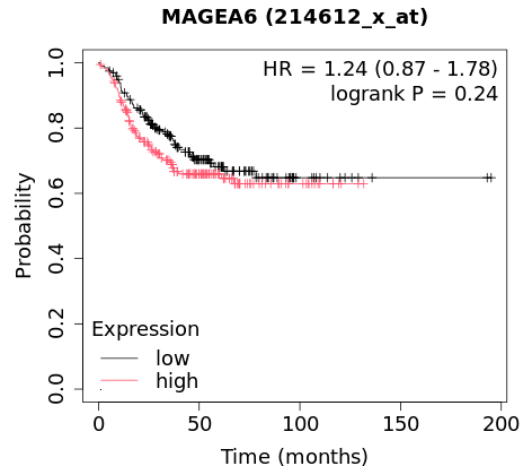
siRNA name	Duplex Sequence (5'-3')	
MAGE-A6	Duplex 1	GAUGGUUGAAUGAGCGUCAdTdT
	Duplex 2	GGUAAAGAUCAGUGGAGGAdTdT
NF- $\kappa$ B #1	Sense	UUUCUCAUCCCAUCUUUGAUU
	Anti-Sense	UCAAAGAUGGGAUGAGAAAUU
NF- $\kappa$ B #2	Sense	GUGACAAGGUGCAGAAAGAUU
	Anti-Sense	UCUUUCUGCACCUUGUCACUU
NF- $\kappa$ B #3	Sense	GACAUUGAGGUGUAUUUCAUU
	Anti-Sense	UGAAAUACACCUCAAUGUCUU
NF- $\kappa$ B #4	Sense	ACAU AUGAGACCUUCAAGAUU
	Anti-Sense	UCUUGAAGGUCUCAUAUGUUU
Negative control	Sense	CCUCGUGCCGUUCCAUCAGGUAGUU
	Anti-Sense	CUACCUGAUGGAACGGCACGAGGUU



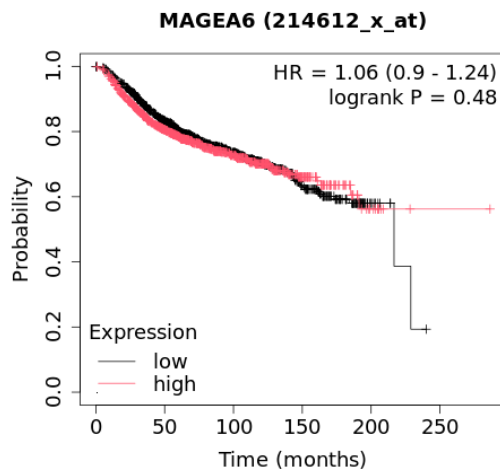
**A**  
Uncategorized



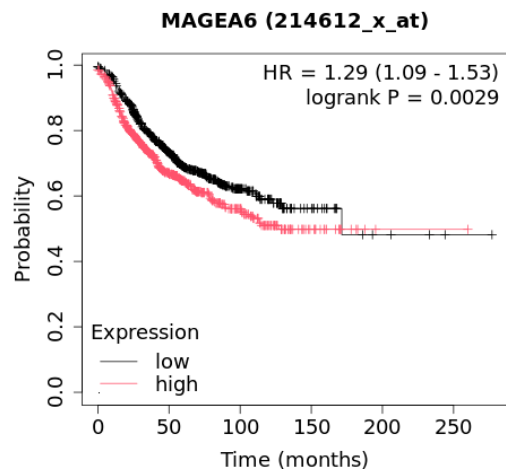
**B**  
All three negative (TNBC)



**C**  
Lymph node negative



**D**  
Lymph node positive



**Figure S7.** “MAGEA6” gene in TCGA on the database in breast cancer using Kaplan-Meier survival analysis (mRNA gene chip) **(A)-(B)** Comparison of Kaplan-Meier survival analysis between receptor types of breast cancer. **(C)-(D)** Comparison of Kaplan-Meier survival analysis lymph node system of breast cancer. ns > 0.01.