

Supplementary Table S1. Summary of immune checkpoint genes

Genes	Ligand	expressed	function	reference
B7-1 (CD80)	CD28, CTLA-4, PD-L1	dendritic cells, macrophages, B-cells, and other antigen-presenting cells	suppresses dendritic cells, also promotes suppressive effects of regulatory T cells. PD-L1 binds to CD80 in cis on APC cells, preventing CD80 from activating T cells via CD28.	29, 30
B7-2 (CD86)	CD28, CTLA-4	dendritic cells, macrophages, B-cells, and other antigen-presenting cells	impairs the co-stimulation necessary for proper T-cell activation.	31
HHLA2	TMIGD2	Tumor cells, monocytes	Inhibits the proliferation of T cells	32
VSIR (VISTA)	unknown	T cells and activated Treg, myeloid cells, mature APC	Increase threshold for TCR signaling, induce FOXP3 synthesis	33
PD-L1 (CD274)	PD-1, B7-1 (CD80)	dendritic cells, monocytes, macrophages, mast cells, T cells, B cells, NK cells	inhibits TCR-mediated activation of IL-2 production and T cell proliferation.	34
PDCD1LG2	PD-1	dendritic cells and macrophages	inhibits TCR/BCR-mediated immune cell activation	34
B7-H3 (CD276)	unknown	antigen-presenting cells	inhibits polyclonal or allogeneic CD4 and CD8 T cell activation, proliferation and effector cytokine production	35
VTCN1(B7-H4)	BTLA	tumor cells and tumor-associated macrophages	increase the number, function, and stability of tumor-associated Treg cells.	36
CD48	CD244, CD2	T cells, B cells, NK cells and thymocytes.	depletion of memory cells and inhibition of T cell activation	37
NECTIN2 (CD112, PVRL2)	CD226, PVRIG (CD112R)	antigen-presenting cells	diminishes IFN- γ production and cytotoxicity of NK cells	38
PVR (CD155)	CD226, TIGIT, CD96	antigen-presenting cells	suppresses interferon- γ production of NK cells and cytotoxic T cells	39, 40
LGALS9	TIM-3, CLEC7A, CD137, CD40	epithelial and stromal cells	Attenuates T-cell expansion and effectors function	41
HVEM (TNFRSF14)	BTLA, TRAF2, TNFSF14, TRAF5	T cells, B cells and immature dendritic cells	exert inhibitory function and reduce the proliferation ability of antigen-initiating T cells	42
CD200	CD200R	Neurons, epithelial cells, endothelial cells, fibroblasts, myeloid cells, and lymphoid cells	Suppresses memory T-cell and NK cell functions	43, 44
IDO1		cancer cells, stromal cells, and immune cells in the tumor microenvironment	limits T-cell function and engage mechanisms of immune tolerance.	45
CTLA4	B7-1 (CD80), B7-2 (CD86)	Activated T cells, Treg	Competitive inhibition of CD28 co-stimulation (binding of B7-1 and B7-2)	46, 47
PD-L1 (CD274)	PD-1, B7-1 (CD80)	dendritic cells, monocytes, macrophages, mast cells, T cells, B cells, NK cells	inhibits TCR-mediated activation of IL-2 production and T cell proliferation.	34
LAG3	MHC-II, LSECtin	Activated CD4 and CD8 T cells, NK cells, Treg	Competitive binding to MHC-II than CD4, negatively regulates cellular proliferation, activation, and homeostasis of T cells	48
B7-1 (CD80)	CD28, CTLA-4, PD-L1	dendritic cells, macrophages, B-cells, and other antigen-presenting cells	suppresses dendritic cells, also promotes suppressive effects of regulatory T cells. PD-L1 binds to CD80 in cis on APC cells, preventing CD80 from activating T cells via CD28.	29, 30
PDCD1	PD-L1, PD-L2	Activated T cells, NK cells, NKT cells, B cells, macrophages, DCs	Attenuate proximal TCR signaling, attenuate CD28 signaling	49
2B4 (CD244)	CD48	NK cell, T cell, eosinophils, mast cells and dendritic cells.	maintenance of an exhausted phenotype in NK cells and T cells	50
BTLA (CD272)	VTCN1, HVEM	Activated T cells	Binding to HVEM negatively regulates T cell immune response. Recruits SHP-1 and SHP-2 and inhibits signaling cascades.	36
CD160	HVEM	NK cells, T cells	inhibits CD4 T cell proliferation and cytokine production.	51
HAVCR2 (TIM3)	Galectin-9, PtdSer, HMGB1, CEACAM-1	Th1 CD4 and Tc1 CD8, Treg, DC, NK cells, monocytes	Negative regulation of proximal TCR components, inhibits NK cell cytotoxicity	52
CD96	PVR	T cells, NK cells	CD96 is co-expressed with PD-1 and TIGIT, Anti-CD96 therapy enhances suppression of primary tumor growth. Detailed mechanism unknown.	53
TIGIT	PVR (CD155), PVRL2 (CD112)	CD4, CD8 T cell, Treg, NK cells	Competitive inhibition of DNAM1, co-stimulation (binding of PVR), binding of DNAM1 in cis; cell-intrinsic ITIM-negative signaling	54
ADORA2A	Adenosine	T cells, macrophages, DCs and NK cells	suppresses immune cells, protecting tissue from inflammation	55
CD200R	CD200	myeloid cells, CD4+ T cells	Suppresses memory T-cell and NK cell functions	43, 44
PVRIG (CD112R)	NECTIN2	T cells	competes with CD226 to bind to CD112, diminishes IFN- γ production and cytotoxicity of NK cells	38

Supplementary Table S2. Characteristics of breast cancer patients in the TCGA-BRCA cohort.

Characteristics	High miR-4759 (n=154)	Low miR-4759* (n=922)
Age	57.8 ± 12.4	59.4 ± 13.3
Gender, n (%)		
Female	150 (97.4%)	912 (98.9%)
Male	4 (2.6%)	10 (1.1%)
Histological type, n (%)		
Infiltrating ductal carcinoma	89 (57.8%)	676 (73.3%)
Infiltrating lobular carcinoma	56 (36.4%)	144 (15.6%)
Infiltrating carcinoma NOS	0 (0%)	1 (0.1%)
Medullary carcinoma	0 (0%)	6 (0.7%)
Metaplastic carcinoma	0 (0%)	9 (1.0%)
Mucinous carcinoma	2 (1.3%)	15 (1.6%)
Mixed histology	3 (1.9%)	25 (2.7%)
Other specify	3 (1.9%)	42 (4.6%)
Not available (NA)	1 (0.6%)	4 (0.4%)
Pathologic stage, n (%)		
Stage I	30 (19.5%)	150 (16.3%)
Stage II	85 (55.2%)	524 (56.8%)
Stage III	32 (20.8%)	213 (23.1%)
Stage IV	5 (3.2%)	15 (1.6%)
NA	2 (1.3%)	20 (2.2%)
ER status, n (%)		
Positive	133 (86.3%)	658 (71.4%)
Negative	13 (8.4%)	219 (23.8%)
NA	8 (5.2%)	45 (4.9%)
PR status, n (%)		
Positive	122 (79.2%)	563 (61.1%)
Negative	24 (15.6%)	311 (33.7%)
NA	8 (5.2%)	48 (5.2%)
HER2 status, n (%)		
Positive	75 (48.7%)	475 (51.5%)
Negative	7 (4.5%)	48 (5.2%)
NA	72 (46.8%)	399 (43.3%)

*The cutoff value of high and low expression was set as the average.

Supplementary Table S3.

Correlation analysis of miR-4759 and immune-related genes in BRCA datasets of TCGA.

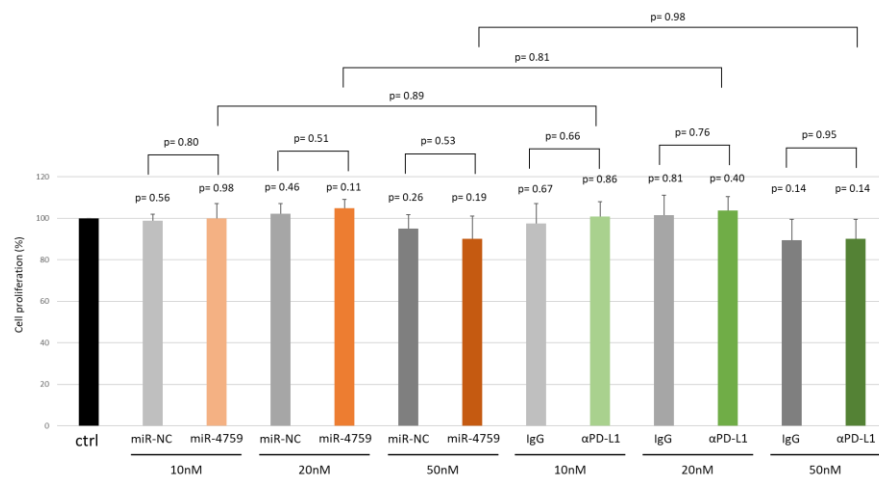
gene	spearman_cor ¹	spearman_pv ²	pearson_cor ³	pearson_pv ⁴	kendall_cor ⁵	kendall_pv ⁶	max_cor ⁷	Category
STAT1	-0.114806472	0.000164059	-0.121682179	6.44E-05	-0.090911873	0.000159142	0.121682179	tumor inflammation signature
CD80	-0.105988357	0.000505964	-0.11186659	0.000241002	-0.083798234	0.000499815	0.11186659	immune checkpoint genes
PVR	-0.117316333	0.00011729	-0.079696659	0.009009061	-0.091472599	0.000144904	0.117316333	immune checkpoint genes
CCL7	-0.154358614	3.76E-07	-0.081755925	0.007375037	-0.123183795	4.79E-07	0.154358614	cytokines and cytokine receptors
CCL8	-0.107368512	0.00042649	-0.090292251	0.003073643	-0.084858738	0.000423621	0.107368512	cytokines and cytokine receptors

¹spearman_cor=Spearman's rank correlation coefficient. ²spearman_pv=Spearman's rank correlation coefficient p value. ³pearson_cor=Pearson's rank correlation coefficient. ⁴pearson_pv=pearson's rank correlation coefficient p value. ⁵kendall_cor=kendall's rank correlation coefficient. ⁶kendall_pv=kendall's rank correlation coefficient p value. ⁷max_cor=maximum coefficient.

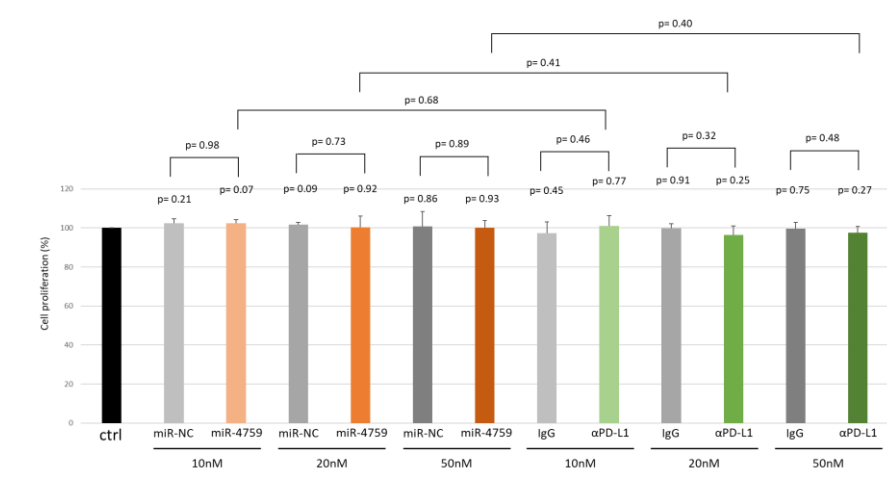
A

BT-549

24 hr

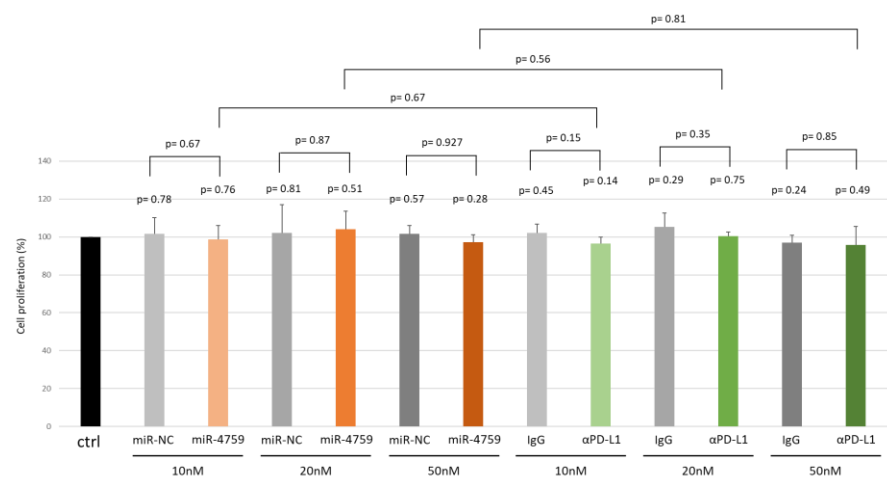
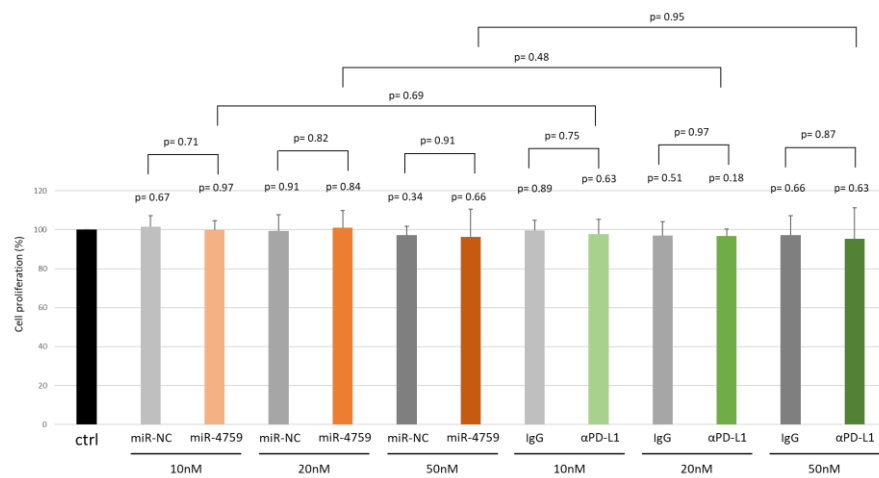


MDA-MB-231



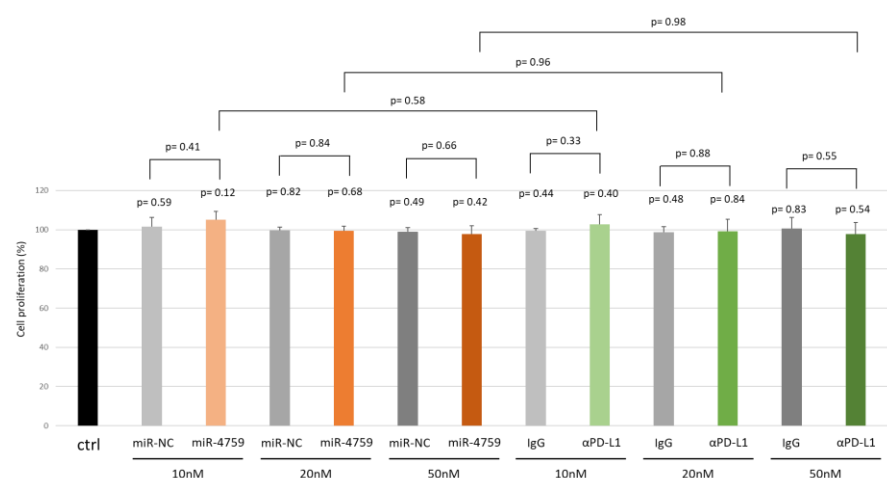
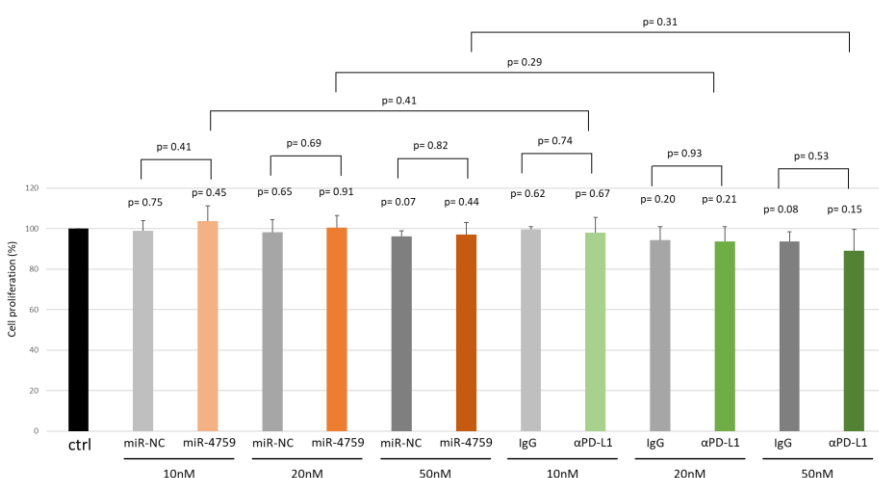
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48 hr

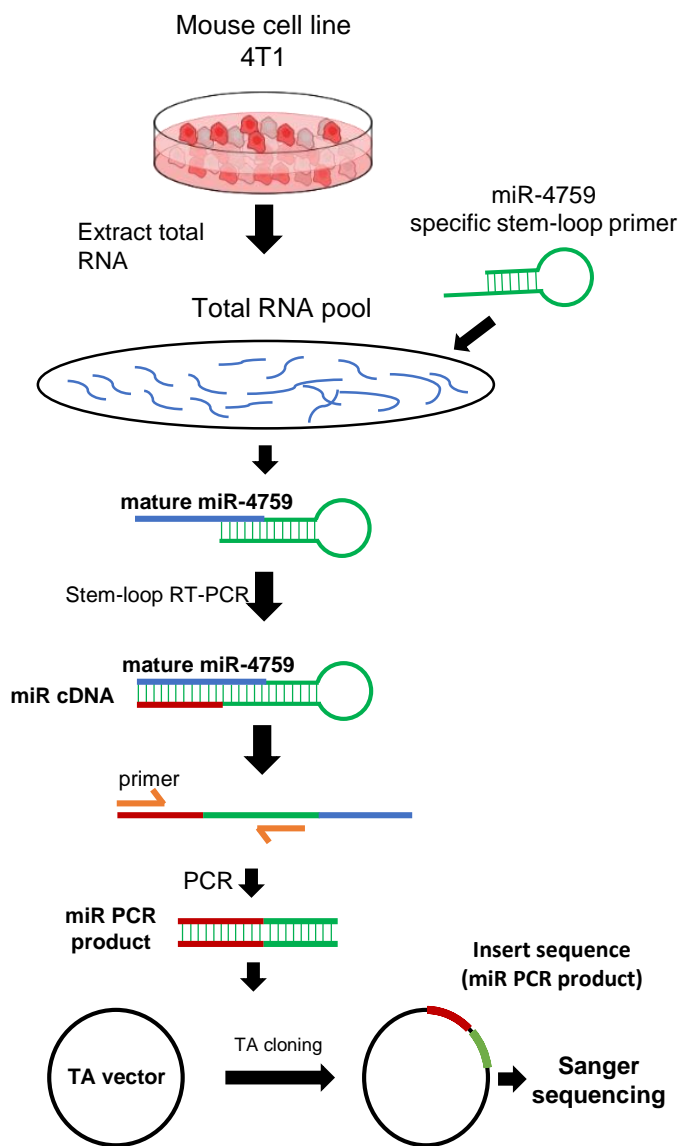
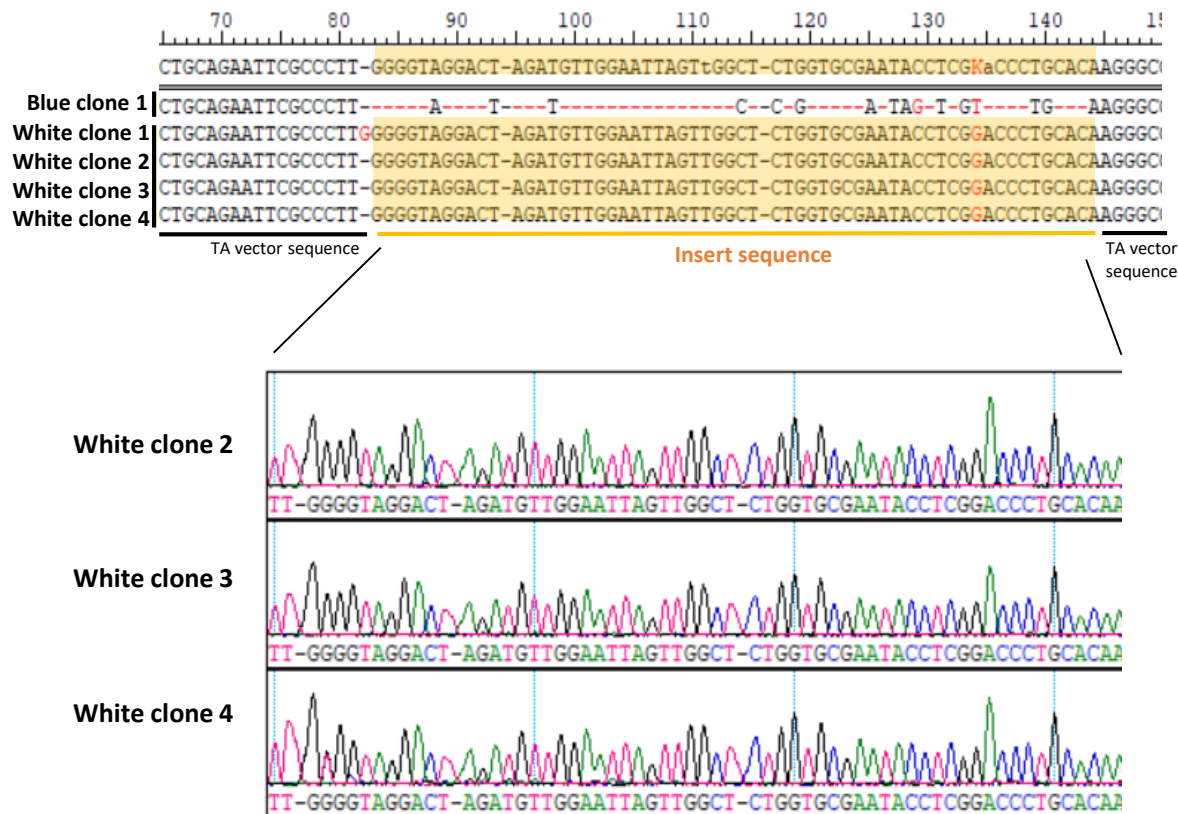


C

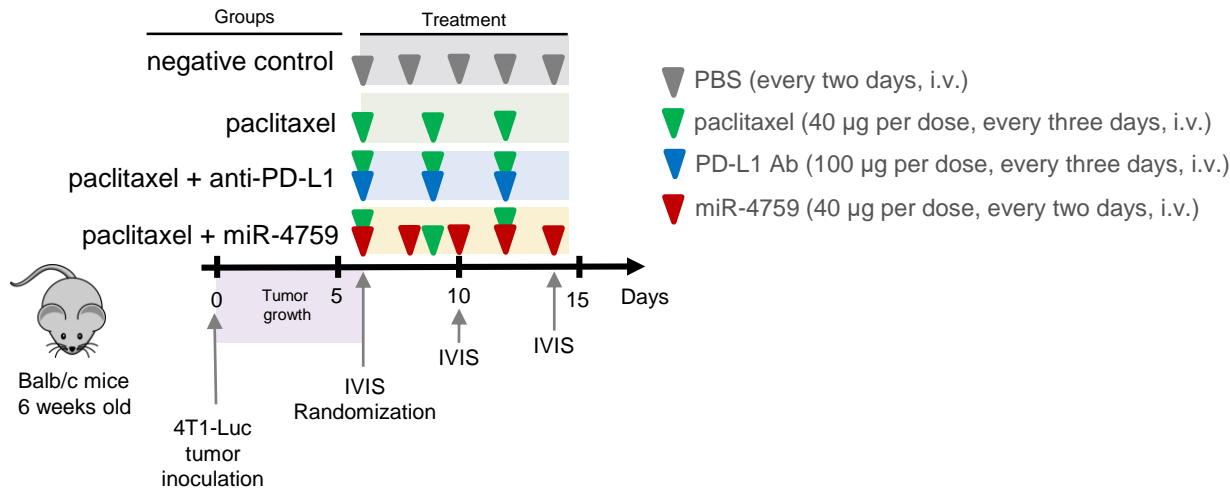
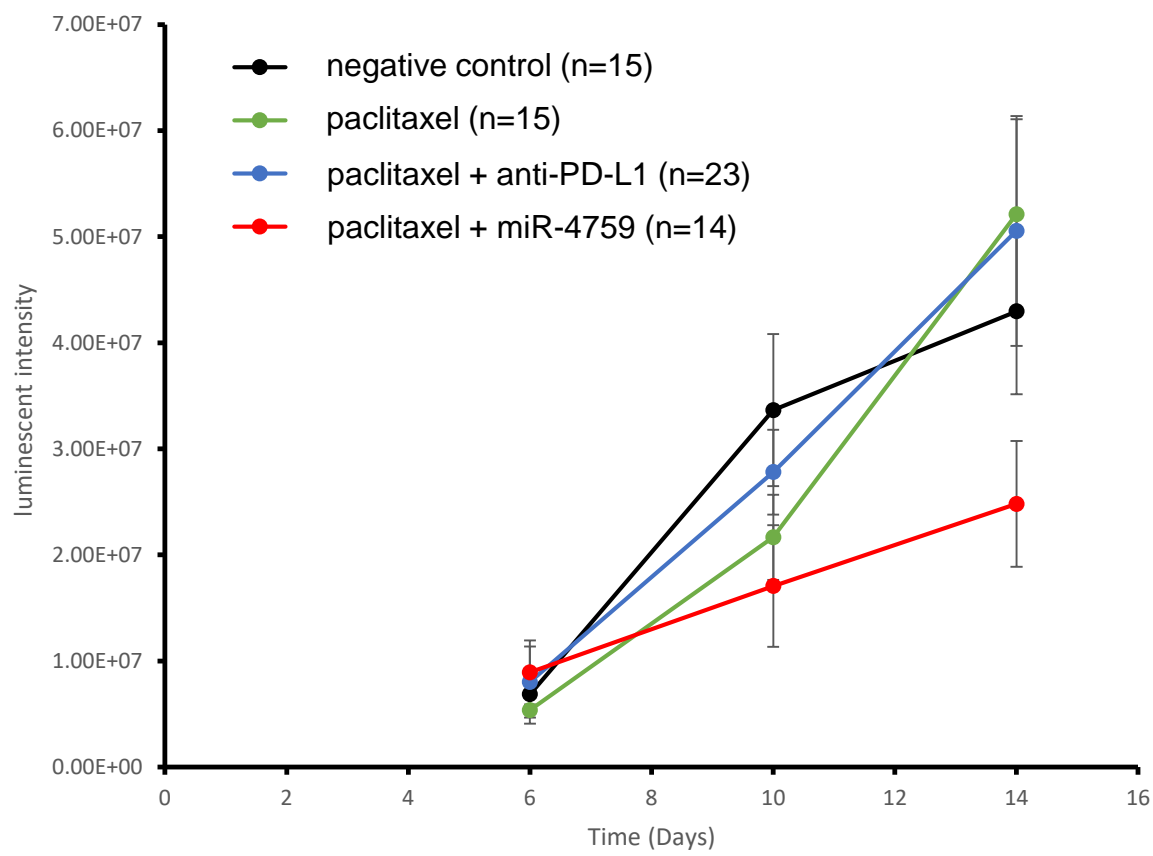
72 hr



Supplementary Figure S1. MTT assay was conducted to measure the cell growth in breast cancer cells with miR-NC/miR-4759 transfection or IgG/anti-PD-L1 treatment for (A) 24 , (B) 48 and (C) 72 hours.

A**B****C.**

Supplementary Figure S2. Identification of miR-4759 in murine cell line 4T1. (A) Schematic diagram of the strategy to identify miR-4759 in 4T1 cells. (B) the alignment and original traces of TA cloning samples sequenced by Sanger method. (C) the miR-4759 sequence is identified by the alignment of sequencing data and PCR product sequence.

A**B****C**

Comparison	P value
NC vs. paclitaxel	0.44
NC vs. paclitaxel + anti-PD-L1	0.61
NC vs. paclitaxel + miR-4759	0.12
paclitaxel vs. paclitaxel + anti-PD-L1	0.92
paclitaxel vs. paclitaxel + miR-4759	0.03
paclitaxel + anti-PD-L1 vs. paclitaxel + miR-4759	0.11

Supplementary Figure S3. miR-4759 systemic treatment combined with chemotherapy in 4T1-Luc tumor-bearing mice. (A) Schematic diagram illustrating the protocol of combination treatment of paclitaxel and miR-4759 in BALB/c mice bearing 4T1-Luc tumors. (B) 4T1-Luc tumor growth in BALB/c mice treated with different treatments. (C) Comparison of tumor growth in 4T1-luc tumor bearing Balb/c mice received with different treatments at Day 14.

Supplementary Table S4. Characteristics of breast cancer patients in the TMA cohort.

Characteristics	High miR-4759 (n=50)	Low miR-4759* (n=131)
Age	49.1 ± 11.9	46.5 ± 10.5
Gender, n (%)		
Female	50 (100%)	131 (100%)
Male	0 (0.0%)	0 (0.0%)
Pathologic stage, n (%)		
Stage I	3 (6.0%)	9 (6.9%)
Stage II	26 (52.0%)	34 (26.0%)
Stage III	7 (14.0%)	13 (10.0%)
Stage IV	0 (0.0%)	0 (0.0%)
NA	14 (28%)	75 (57.2%)
ER status, n (%)		
Positive	32 (64.0%)	87 (66.4%)
Negative	17 (34.0%)	42 (32.1%)
NA	1 (2.0%)	2 (1.5%)
PR status, n (%)		
Positive	25 (50.0%)	70 (53.4%)
Negative	24 (48.0%)	60 (45.8%)
NA	1 (2.0%)	1 (0.8%)
HER2 status, n (%)		
Positive	32 (64.0%)	58 (44.3%)
Negative	18 (36.0%)	70 (53.4%)
NA	0 (0.0%)	3 (2.3%)
Site, n (%)		
Primary	40 (80.0%)	110 (84.0%)
Metastasis	10 (20.0%)	21 (16.0%)

*The cutoff value of high and low expression was set as the average.