Supplementary Figure Legends

Supplementary Figure S1. The levels of three circulating miRNAs in the peripheral bloods from patients of five cancer types are detected with qRT-PCR assay.

A. Levels of miR-410-3p, miR-410-5p and pre-miR-410 in the serum from patients with five cancer types: ovarian cancer (n=12), prostate cancer (n=20), endometrial cancer (n=8), liver cancer (n=23) and renal cancer (n=13), in comparison with controls (n=7) *P<0.05,** P<0.01.

B. Levels of miR-410-3p, miR-410-5p and pre-miR-410 in cytoplasm (upper panel) and the culture medium (CM) (lower panel) of 7 cancer cell lines. Mean ±SD of three independent experiments. ** P<0.01.

C. Levels of miR-410-5p in the culture medium (CM) of DU145 and RM-1 cells treated with RNase A1. U6 was used as a positive control. Mean ±SD of three independent experiments, ** P<0.01.

D. Representative IP result of miR-410-5p in the protein complex. Upper panel: IP was performed with antibodies of AGO2, CD63 (common antibody for exosome) on the samples of both cytoplasm and the culture medium (CM) of DU145 and RM-1. Positive control: Cell lysis and culture medium (CM) of DU145 and RM-1, respectively. Lower panel: IP was performed with antibodies of AGO2 and CD63 on the serum samples of seven-month-old Pb-Cre^{+}&Pten$^{L/L}$ transgenic mice and patients with prostate cancer (PCa). 1, Positive control: serum of a PCa patient with a high level of miR410-5p. 2, Negative control: Protein A.

E. Left panel: Quantitative PCR of AGO2 mRNA level in the cytoplasm of prostate

normal cell RWPE1 and cancer cell lines DU145 and RM-1. Mean ± SD of three independent experiments, ** P< 0.01. Right panel: Immunostaining for AGO2 protein in the samples of the culture medium (CM) of prostate normal cell RWPE1 and cancer cell lines DU145 and RM-1.

Supplementary Figure S2. Dendritic cells (DCs) are the recipient cells of miR-410-5p produced by tumor cells.

A. Immunofluorescence (IF) assay with anti-AGO2 (upper, red) and anti-FLAG (lower, green) antibodies on the cultured cells of DU145 (left) and RM-1 (right) after transfected with pcDNA3.1-AGO2-FLAG (Ago2-FLAG) or blank control of backbone vector pcDNA3.1 (pcDNA3.1). Blue indicated the cell nuclei stained with DAPI. Scale bars represent 10μm.

B. Stable over-expressions from the constructed vector pcDNA3.1-AGO2-FLAG (Ago2-Flag) in transfected DU145 and RM-1 cells were proved by western blot (WB). DU145 and RM-1 cells transfected with backbone vector pcDNA 3.1(pcDNA3.1) were used as control.

C. miR-410-5p displayed in gel electrophoresis and AGO2 displayed in western blot (WB) after IP of Ago2-Flag protein complexes against Flag from samples of cytoplasm (upper) and the culture medium (CM) (lower) of both DU145 and RM-1 cells transfected with pcDNA3.1-Ago2-Flag.

Supplementary Figure S3. Dendritic cells (DCs) are the recipient cells of miR410-5P produced by tumor cells.

Immunofluorescence (IF) with double staining of anti-FLAG (middle) and anti-

CD11c (right) antibodies on the samples of prostate tumor (upper), lymph node (middle, arrowhead indicates representative cell with FLAG positive but CD11c negative) and spleen (lower) from the RM-1 tumor-bearing mice. The images were acquired by Leica confocal laser scanning microscope (Leica TCS MP). Blue indicated cell nuclei stained with DAPI. White Scale bars represent 10μm.

Supplementary Figure S4. The difference in the level of miRNAs in dendritic cells (DCs) under different tumor conditions.

A. Morphology of DCs from normal C57BL/6J mice, which were co-cultured with RM-1 cells (RM-1(C)) or with debris of RM-1cells (RM-1(D)) in assay of real-time imaging flow cytometry (Amnis image stream mkII）. DCs co-cultured with RM-1 cells did not have featured pseudopods, which is a morphological feature of normal DCs. Bright (grey): the co-cultured DCs; CD11c (red): a common marker for all DCs; CD80 (green) & CD86 (yellow): common markers only for matured DCs. Scale bars represent 10μm.

B. Clear distinction between the DCs co-cultured with RM-1cells (RM-1(C)) or with debris of RM-1 (RM-1(D)) for 48 miRNAs was displayed by bootstrapping hierarchical clustering analysis.

Supplementary Figure S5. Intratumoral injection of miR-410-3p down-regulates VEGFα level to inhibit tumor growth.

A. Results of qRT-PCR (left panel) and western blot (right panel) for samples from RM-1-tumor-bearing mice. Mean ± SD of three independent experiments, ** $P<0.01$.

B. Intratumoral injection of miR-410-3p inhibits the growth of tumors in both RM-

1-tumor-bearing mice (left panel) and DU145-tumor-bearing nude mice (right panel). Representative results from mice with intratumoral injection of miR-410-3p and miR-mock, respectively. Tumor size for each individual mouse was measured twice every week and graphed in analysis; each curve represents the tumor size of a single mouse (five mice in each group). The curve ends on the time point of mouse death.

Supplementary Figure S6. miRNA combination assessment using FRET assay.

A. Duplexes combination of miR-410-5p/miR-335-5p; miR-410-5p/Anti-miR-410-5p and miR-590-5p/ Anti-miR-410-5p.

B. Levels of miR-410-5p and miR-590-5p in the DCs co-cultured with RM-1 cells under non-contact condition and transfected with siRNA. Mean ± SD of three independent experiments, ** $P< 0.01$.

C. Representative results of in vivo cellular localization of FAM (Ex:488nm, Em:516nm) and Cy3 (Ex:508-532nm, Em:568nm) in the dendritic cells at the time after co-transfections with labeled miRNAs for 6 h, which were observed by real-time imaging flow cytometry（Amnis image stream mkII）. Scale bars represent 10 μm.

D. Mean fluorescence intensity (MFI) of both FAM and FRET（FAM→Cy3) in dendritic cells co-transfected with labeled miRNAs, which was analyzed with real-time imaging flow cytometry. Values of MFI are indicated. Mean±SD of three independent experiments.

Supplementary Figure S7. Diagram of miRNAs labeling and in vitro FRET

assay.

A. Schematic diagram of miRNA labeling and their combinations.

B. Time course of donor (FAM) and acceptor (Cy3) emission intensities, represented in the average values of in vitro micro-plate reader at each indicated time point.

C. Schematic diagram of double labeling of miR-410-3p, mmu-miR-1896-5p, miR-335-5p, miR-410-5p and miR-590-5p and schematic diagram of their combinations.

Supplementary Figure S8. miRNA degradation assessment using FRET assay.

A. Representative results of in vivo cellular localization of FAM (Ex:488nm, Em:516nm) and Cy3 (Ex:508-532nm, Em:568nm) in Dendritic cells at the time of 6 h post co-transfection with labeled miRNAs analyzed by real-time imaging flow cytometry（Amnis image stream mkII）. Scale bars represent 10μm.

B. Values of MFI are indicated. Mean ± SD of three independent experiments.

Supplementary Figure S9 Reduction of miR-410-5p suppresses growth of tumors in Pb-Cre^{+} &Pten$^{L/L}$ transgenic mice.

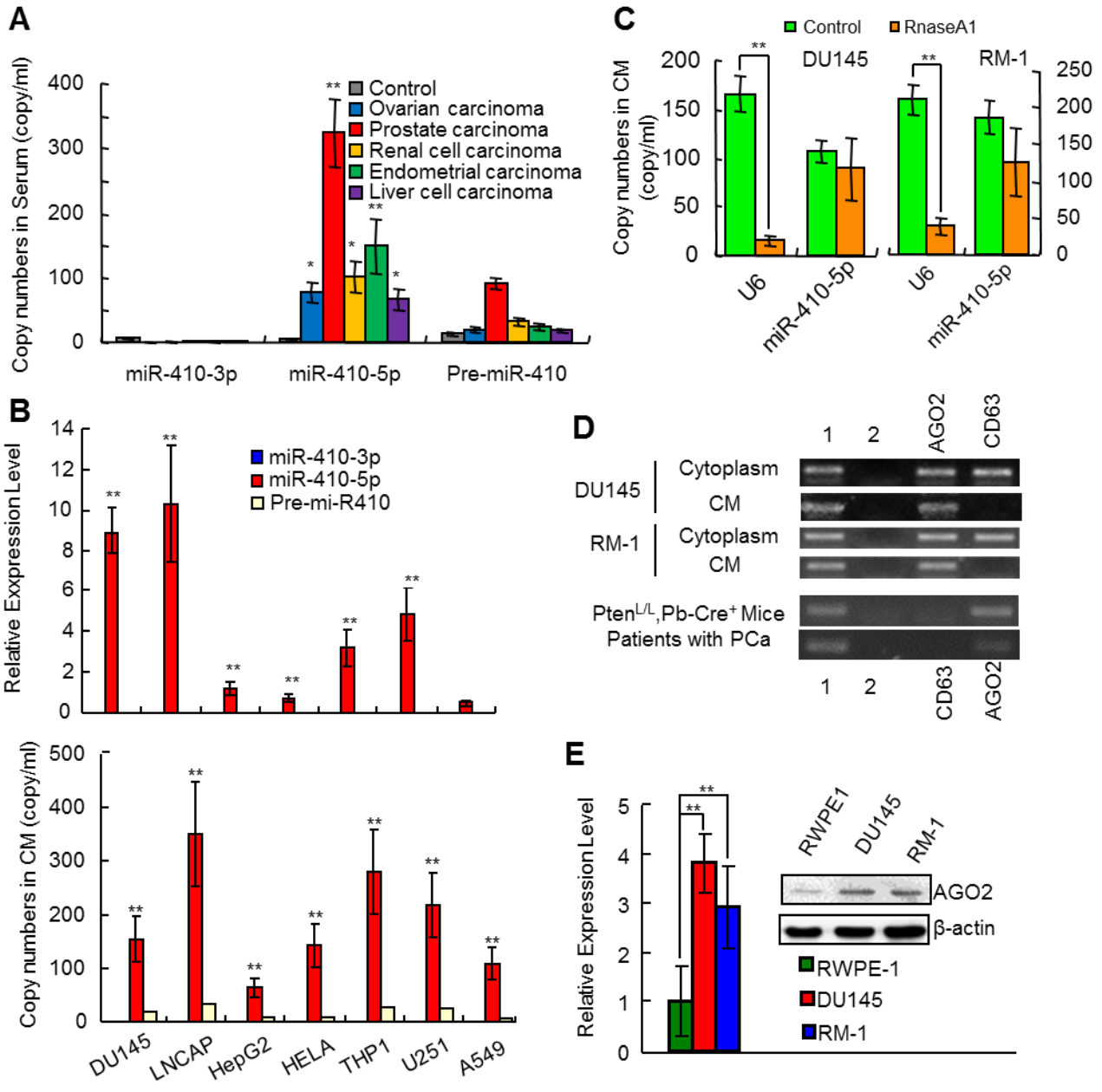
A. Genotype analysis to prove the used Pb-Cre^{+}&Pten$^{L/L}$ transgenic mice (upper panel) and the seven-month-old Pb-Cre^{+}&Pten$^{L/L}$ transgenic mice.

B. Results of immunofluorescence (IF) assay to analyze prostate tissues MVD of Pb-Cre+ & Pten$^{L/L}$ transgenic mice after treated with anti-mock and anti-miR-410-5p, respectively. DAPI (blue) represents nucleus; CD31 (red) represents blood vessel.

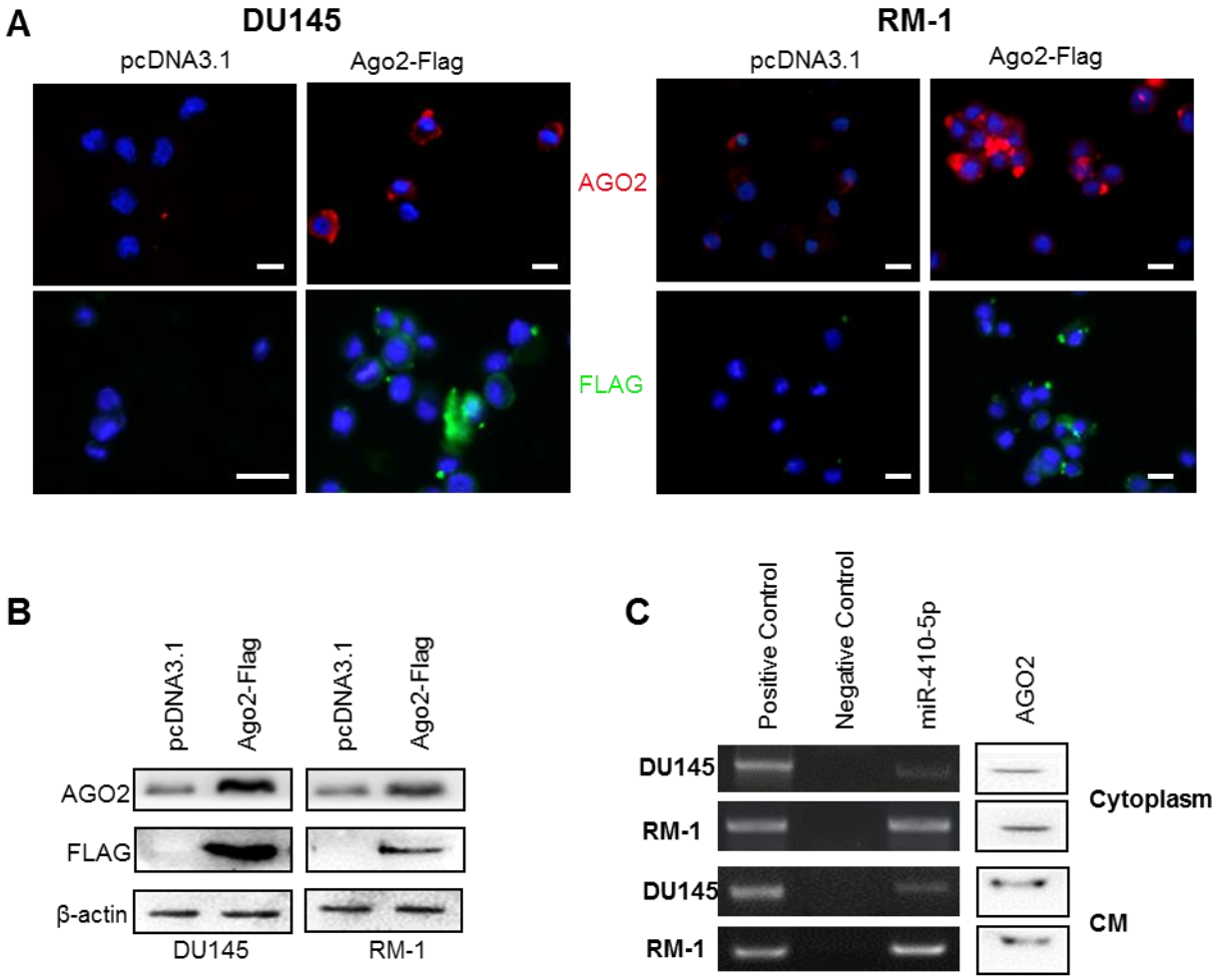
Scale bars for represents as indicated.

C. Representative results of prostates from twenty-eight Pb-Cre^{+} & Pten$^{L/L}$ transgenic mice (n=14) treated with pLKO-anti-mock (Anti-mock) (left) or pLKO-anti-miR-410-5p (Anti-miR-410-5p) (right) with intraperitoneal injection for three times a week during a total of four weeks. The pictures were photographed by camera（Olympus EM5）. Sv: seminal vesicle; Tu: tumor; Pt: prostate; Bd: bladder; Ur: urethra; Ts: testicle.

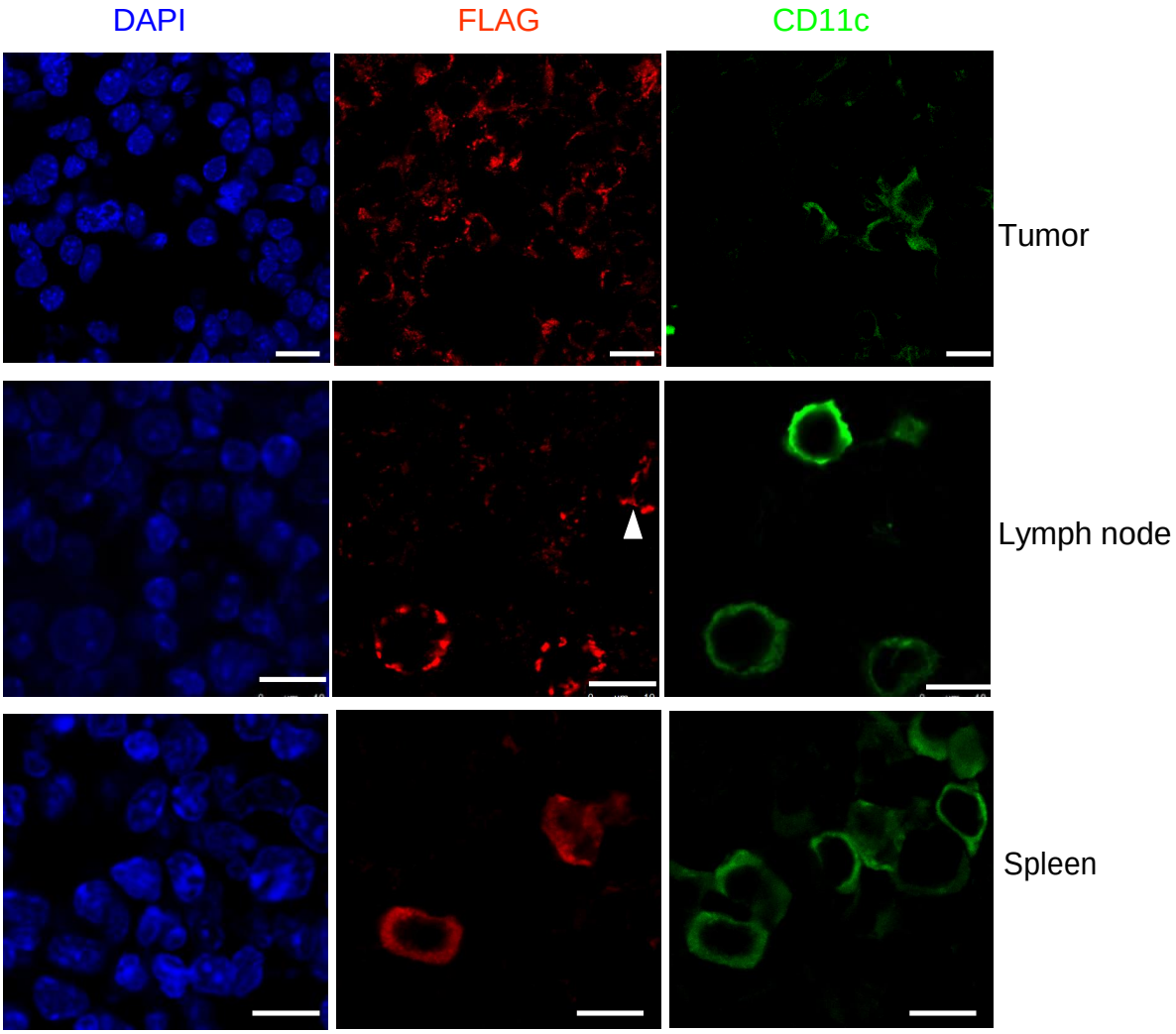
Supplementary Figure S1 Wang et al.



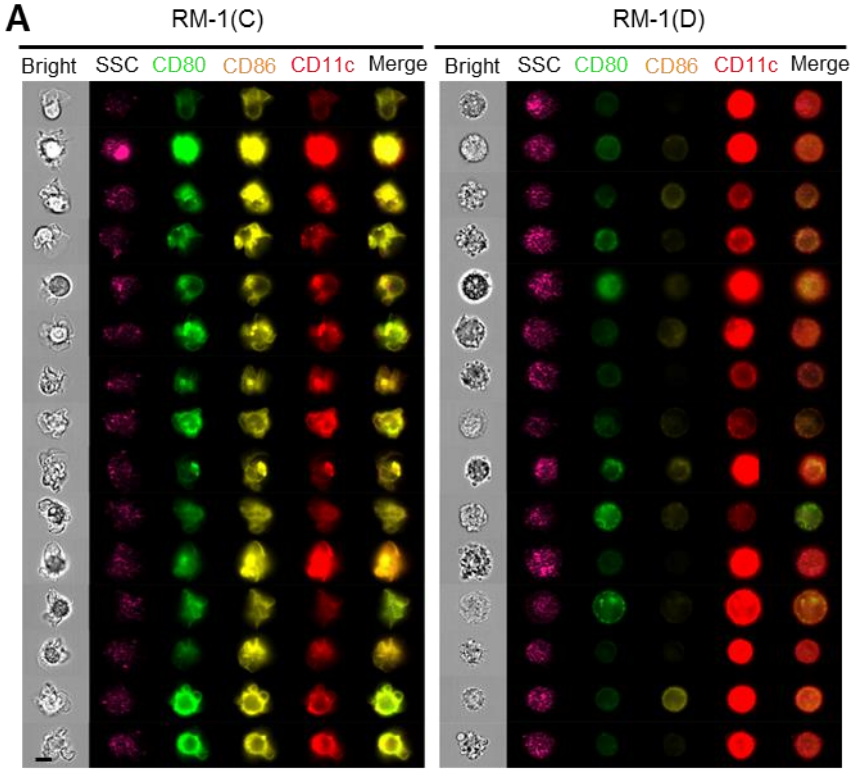
Supplementary Figure S2 Wang et al.



Supplementary Figure S3 Wang et al.



Supplementary Figure S4 Wang et al.

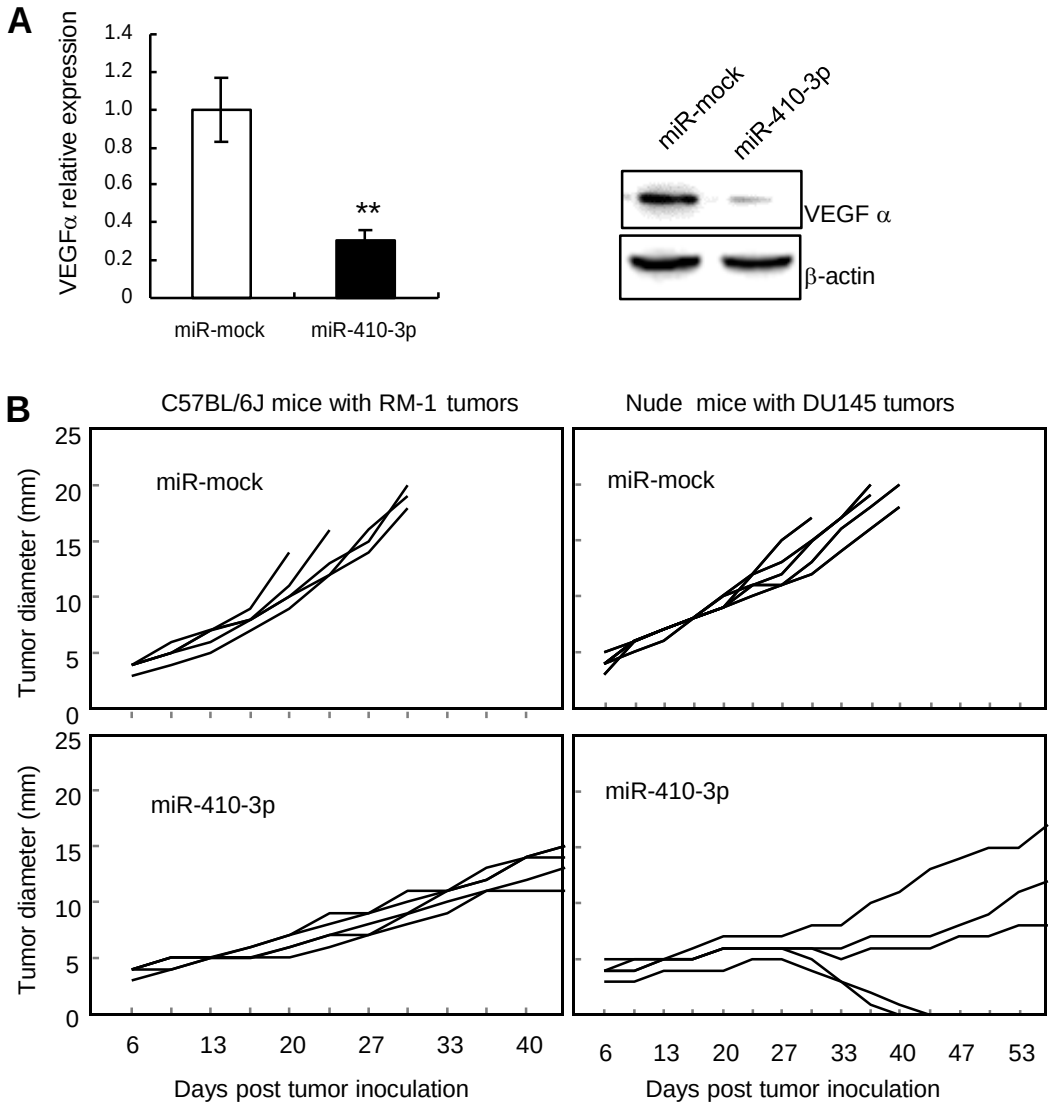


Wang et al.

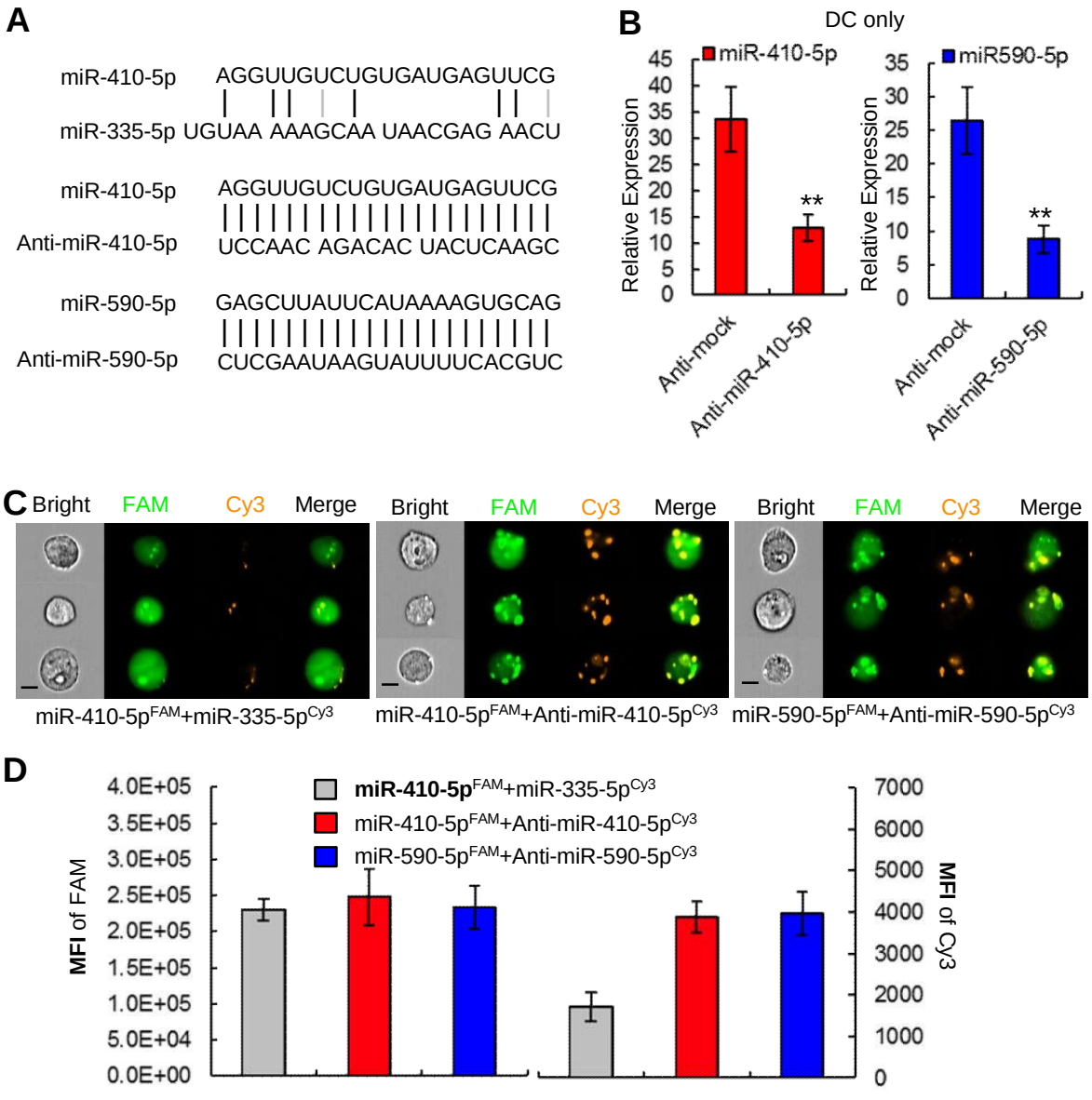
Wang et al.

Wang et al.

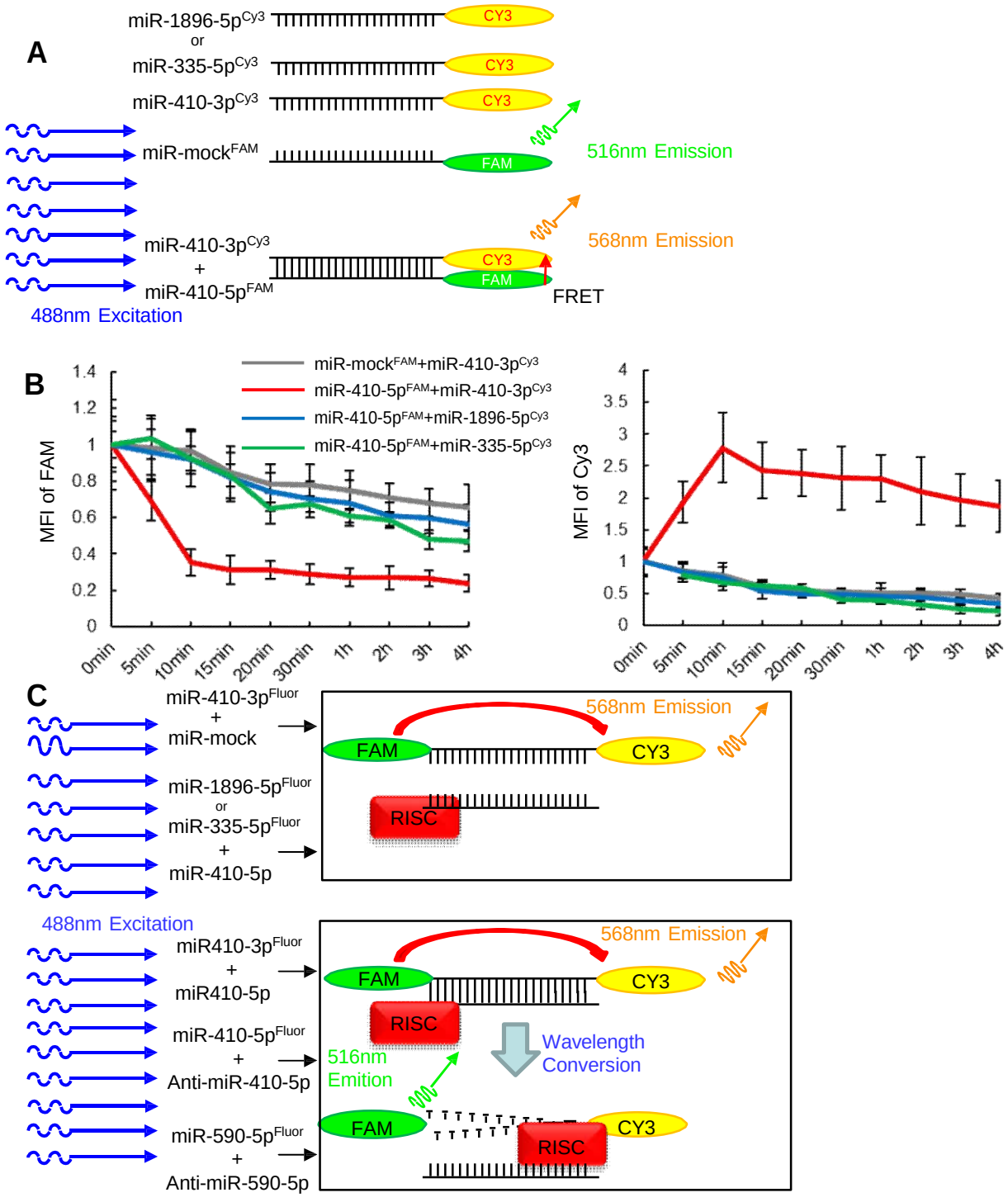
Supplementary Figure S5 Wang et al.



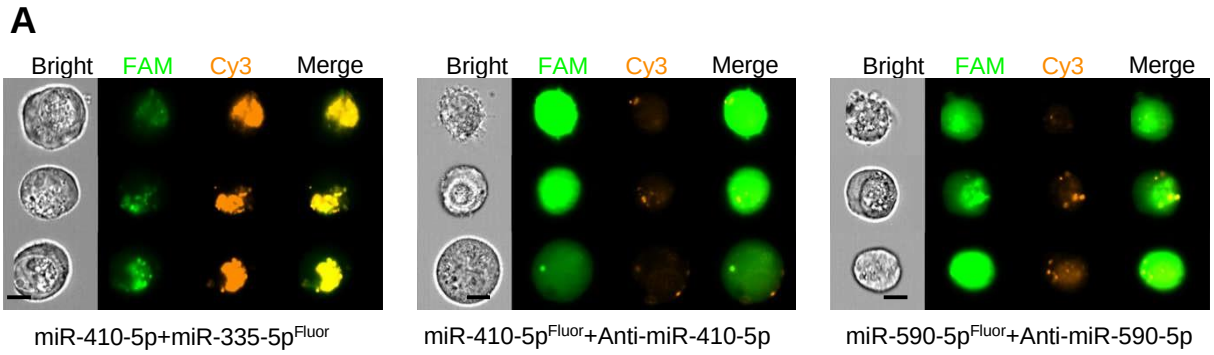
Supplementary Figure S6 Wang et al.

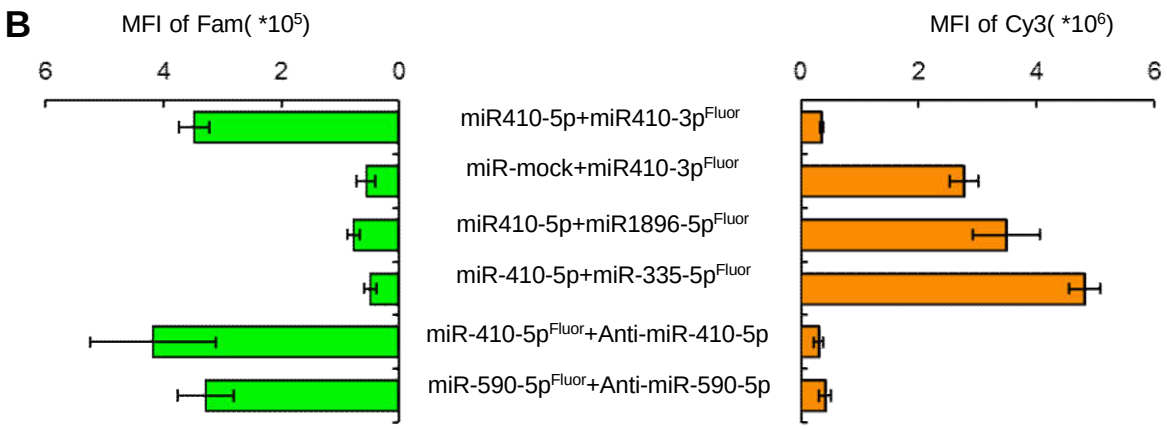


Supplementary Figure S7 Wang et al.



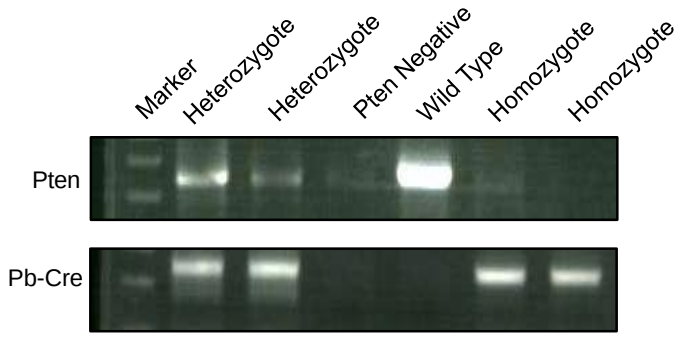
Supplementary Figure S8 Wang et al.





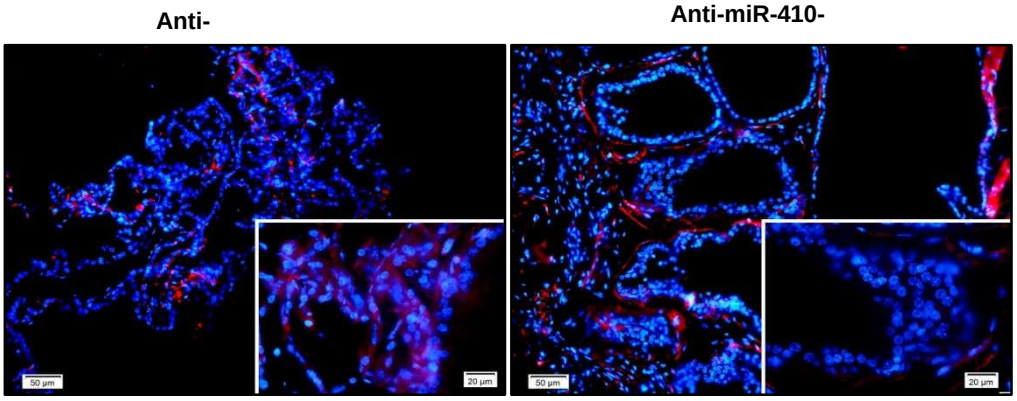
Supplementary Figure S9 Wang et al.

A

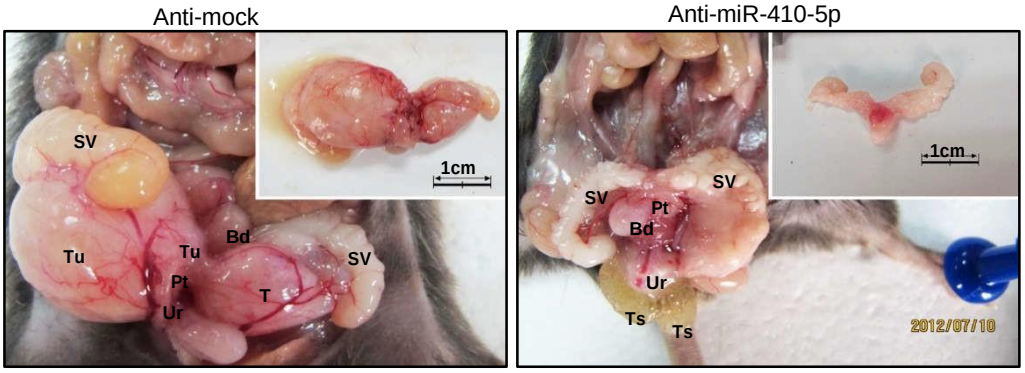


Genotype	Pten	Pb-Cre
Homozygote	-	+/
Wild Type	+/+	-/-
Pten Negative	-/	-
Heterozygote	+/+ or +/-	+/- or +/+

B



C



Supplementary Table 1. Oligonucleotides

Name	Sequence (5’ → 3’)
miR-mock	UUGUACUACACAAAAGUACUG
miR-410-3p	AAUAUAACACAGAUGGCCUGU
miR-410-5p	AGGUUGUCUGUGAUGAGUUCG
miR-mock^{FAM}	FAM-UUGUACUACACAAAAGUACUG
miR-410-3p^{Cy3}	AAUAUAACACAGAUGGCCUGU-Cy3
miR-410-5p^{FAM}	FAM-AGGUUGUCUGUGAUGAGUUCG
mmu-miR-1896-5p^{Cy3}	cucucugaugugggugaggag-Cy3
miR-410-3p^{Fluor}	FAM-AAUAUAACACAGAUGGCCUGU-Cy3
miR-1896-5p^{Fluor}	FAM-cucucugaugugggugaggag-Cy3
miR-590-5p^{FAM}	FAM-GAGCUUAUUCAUAAAAGUGCAG
miR-590-5p^{Fluor}	FAM-GAGCUUAUUCAUAAAAGUGCAG-Cy3
anti-miR-590-5p^{Cy3}	CUCGAAUAAGUAUUUUCACGUC-Cy3
anti-miR-590-5p	CUCGAAUAAGUAUUUUCACGUC
anti-miR-410-5p^{Cy3}	UCCAACAGACACUACUCAAGC-Cy3
anti-miR-410-5p^{Flour}	FAM-UCCAACAGACACUACUCAAGC-Cy3
anti-miR-410-5p	UCCAACAGACACUACUCAAGC
miR-335-5p^{Cy3}	UGUAAAAAGCAAUAACGAGAACU-Cy3
miR-335-5p^{Fluor}	FAM-UGUAAAAAGCAAUAACGAGAACU-Cy3

Supplementary Table

2. Primers

Name	Sequence(5’ → 3’)	Application
miR-139-5p-Forward	TCTACAGTGCACGTGTCTCCAGT	qPCR
miR-15a-Forward	TAGCAGCACATAATGGTTTGTG	qPCR
miR-15b-Forward	TAGCAGCACATCATGGTTTACA	qPCR
miR-200b-Forward	TAATACTGCCTGGTAATGATGA	qPCR
miR-410-3p-Forward	AATATAACACAGATGGCCTGT	qPCR,
miR-590-5p-Forward	GAGCTTATTCATAAAAGTGCAG	qPCR,
miR-424-Forward	CAGCAGCAAUUCAUGUUUUGAA	qPCR
miR-429-Forward	UAAUACUGUCUGGUAAAACCGU	qPCR
miR-410-5p-Forward	AGGUUGUCUGUGAUGAGUUCG	qPCR RNaseI-enzyme hydrolysis
Pre-miR-410-Forward	GUCUGUGAUGAGUUCGCUUUUA	qPCR,
Human-ACTB-Forward	ATGATGATATCGCCGCGCTC	RNaseI-enzyme hydrolysis
Human-ACTB-Reverse	CCACCATCACGCCCTGG	RNaseI-enzyme hydrolysis
Mouse-Actb-Forward	AGCTCCTTCGTTGCCGGTCC	qPCR,
Mouse-Actb-Reverse	TCCTCAGGGGCCACACGCA	qPCR,
Mouse-Vegfa-Forward	TATTCAGCGGACTCACCAGC	qPCR,
Mouse-Vegfa-Reverse	AACCAACCTCCTCAAACCGT	qPCR,
Human-U6-Forward	CTCGCTTCGGCAGCACA	qPCR + RNase A1 hydrolysis
Human-U6-Reverse	AACGCTTCACGAATTTGCGT	qPCR + RNase A1 hydrolysis
Flag-Forward	CTAGAGATTACAAGGATGACGACGATAAGTAA	Plasmid construction/Transfection
Flag-Reverse	TTACTTATCGTCGTCATCCTTGTAATCT	Plasmid construction/Transfection
Human-Ago2-a-Forward	GCCACCATGTACTCGGGAGC	Plasmid construction/Transfection
Human-Ago2-a-Reverse	GGCAGGATGACCACCACCAG	Plasmid construction/Transfection
Human-Ago2-b-Forward	GGGCATCGAGATCAAGGTGT	Plasmid construction/Transfection
Human-Ago2-b-Forward	CATATTTCCTATGACATTGGGTTC	Plasmid construction/Transfection
Human-Ago2-a-Forward-2	GATC AAGCTT GCCACCATGTACTCGGGAGC	Plasmid construction/Transfection
Human-Ago2-b-Forward-2	GATC GAATTC GGGCATCGAGATCAAGGTGT	Plasmid construction/Transfection
Human-Ago2-b-Reverse-2	GATCTCTAGA ATATTTCCTATGACATTGGGTTC	Plasmid construction/Transfection
Anti-miR410-5p-Forward	CCGGTCGAACTCATCACAGACAACCT CGAACTCATCACAGACAACCT CGAACTCATCACAGACAACCT G	Plasmid construction/Transfection
Anti-miR410-5p-Reverse	AATTCAGGTTGTCTGATGAGTTCG AGGTTGTCTGATGAGTTCG AGGTTGTCTGATGAGTTCG A	Plasmid construction/Transfection
Anti-mock-Forward	CCGGT UUGUACUACACAAAAGUACUG G	Plasmid construction/Transfection
Anti-mock-Reverse	AATTC UUGUACUACACAAAAGUACUG A	Plasmid construction/Transfection
Transgenic Mice-Pb-Cre-Forward	AATGCTTCTGTCCGTTTGC	Transgenic Mice identification
Transgenic Mice-Pb-Cre-Reverse	ACCAGAGTCATCCTTAGCG	Transgenic Mice identification

Transgenic Mice-Pten-Forward	AATTGAAAGCTCAGGGTAGC	Transgenic Mice identification
Transgenic Mice-Pten-Forward	ATCTGAACACTTCATCGGGA	Transgenic Mice identification
miRNA-RT-a	CACTGTCATGCCGTTAGGTAGCGTATCGTTGACAGC TTTTTTTTTTTTTTTA	miRNA Reverse Transcription
miRNA-RT-c	CACTGTCATGCCGTTAGGTAGCGTATCGTTGACAGC TTTTTTTTTTTTTTTC	miRNA Reverse Transcription
miRNA-RT-g	CACTGTCATGCCGTTAGGTAGCGTATCGTTGACAGC TTTTTTTTTTTTTTTG	miRNA Reverse Transcription
R-miRNA-R1	TCATGCCGTTAGGTAGCGTA	miRNA qPCR

Supplementary Table 3.
Antibodies

Name	Cat.No.	Application	Source	Species reactivity
Anti-AGO2antibody-ChIP Grade	ab32381	WB/IF/IP /FCM	Rabbit	Human/Mouse
Anti-CD63 antibody [EPR5702]	ab134045	RIP	Rabbit	Human
Anti-DDDDK (Flag) antibody	ab1257	WB/IF/RIP	Goat	
Donkey anti-Goat IgG H&L (PE)	ab7004	IF	Donkey	
Donkey Anti-Goat IgG H&L (FITC)	ab6881	IF	Donkey	
Donkey Anti-Rabbit IgG H&L (PE)	ab7007	IF	Donkey	
Goat Anti-Rabbit IgG H&L (HRP)	ab6721	WB	Goat	
Goat Anti-Rabbit IgG H&L (FITC)	ab6717	IF	Goat	
Rabbit Anti-Goat IgG H&L (HRP)	ab6741	WB	Rabbit	
Anti-beta Actin antibody	ab8226	WB	Mouse	Human
Anti-beta Actin antibody	ab129348	WB	Rabbit	Mouse
Anti-CD11c antibody	ab11029	IF	Rabbit	Human/Mouse
Anti-Fluorescein antibody	ab6213	RIP	Mouse	
Anti-Cy3 / Cy5 antibody	ab52060	RIP	Mouse	
Anti-VEGFA antibody	ab51745	WB	Rabbit	Human/Mouse
Anti-CD31 antibody	ab81289	IF	Rabbit	Human/Mouse
Anti-CD11c antibody (APC)	130-102-800	Flow Cytometry		Mouse
Anti-CD80 antibody (FITC)	130-102-882	Flow Cytometry		Mouse
Anti-CD86 antibody (PE)	130-102-604	Flow Cytometry		Mouse
isotype-PE	130-098-845	Flow Cytometry		Mouse
isotype-FITC	130-098-847	Flow Cytometry		Mouse

Supplementary Table 4. Shapiro-Wilk examination

Shapiro-Wilk examination for Figure. 1A

Tests of Normality[a]							
Team		Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	2	0.232	7	.200*	0.881	7	0.231
*. This is a lower bound of the true significance.							
a. Data is constant when Team = 1.00. It has been omitted.							
b. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 2A Up

Tests of Normality[a,b]							
Team		Kolmogorov-Smirnov[c]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	3	0.194	6	.200*	0.934	6	0.586
	4	0.265	6	0.147	0.901	6	0.335
	5	0.21	6	.200*	0.964	6	0.849
	6	0.175	6	.200*	0.916	6	0.436
*. This is a lower bound of the true significance.							
a. Data is constant when Team = 1.00. It has been omitted.							
b. Data is constant when Team = 2.00. It has been omitted.							
c. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 2A Down

Tests of Normality[a,b]							
Team		Kolmogorov-Smirnov[c]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	3	0.205	6	.200*	0.946	6	0.697
	4	0.153	6	.200*	0.963	6	0.846
	5	0.202	6	.200*	0.886	6	0.255
	6	0.192	6	.200*	0.924	6	0.497
*. This is a lower bound of the true significance.							
a. Data is constant when Team = 1.00. It has been omitted.							
b. Data is constant when Team = 2.00. It has been omitted.							
c. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 3A Left

Tests of Normality							
Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	1	0.155	6	.200*	0.982	6	0.967
	2	0.114	6	.200*	0.997	6	1

Value	3	0.114	6	.200*	0.997	6	1
	4	0.141	6	.200*	0.974	6	0.925
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 3A Right

Tests of Normality							
Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	1	0.137	6	.200*	0.992	6	0.996
	2	0.245	6	.200*	0.884	6	0.245
	3	0.169	6	.200*	0.923	6	0.496
	4	0.191	6	.200*	0.967	6	0.875
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 3C Right

Tests of Normality							
Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	1	0.135	9	.200*	0.963	9	0.848
	2	0.314	9	0.036	0.82	9	0.064
	3	0.272	9	0.126	0.832	9	0.084
	4	0.228	9	.200*	0.95	9	0.732
	5	0.214	9	.200*	0.918	9	0.454
	6	0.13	9	.200*	0.977	9	0.942
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 4A Left

Tests of Normality[a]							
Team		Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	2	0.184	10	.200*	0.944	10	0.678
	3	0.145	10	.200*	0.967	10	0.875
	4	0.164	10	.200*	0.932	10	0.572
*. This is a lower bound of the true significance.							
a. Data is constant when Team = 1.00. It has been omitted.							
b. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 4B Right

Tests of Normality

Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	1	0.156	9	.200*	0.988	9	0.989
	2	0.195	9	.200*	0.944	9	0.678
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 4D Right

Tests of Normality							
Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	1	0.156	18	.200*	0.988	18	0.989
	2	0.212	12	.200*	0.968	12	0.883
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 4E Left

Tests of Normality[a]							
Team		Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	2	0.187	28	.200*	0.959	28	0.811
	3	0.155	28	.200*	0.99	28	0.993
	4	0.291	28	0.075	0.873	28	0.199
*. This is a lower bound of the true significance.							
a. Data is constant when Team = 1.00. It has been omitted.							
b. Lilliefors Significance Correction							

Shapiro-Wilk examination for Figure. 4E Right

Tests of Normality							
Team		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Value	1	0.333	42	0.018	0.843	42	0.107
	2	0.243	42	.200*	0.88	42	0.228
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							