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Supplementary Materials for

miR-26a regulates extracellular vesicle secretion from prostate cancer cells via targeting SHC4, PFDN4, and CHORDC1

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This PDF file includes:

Figure S1.

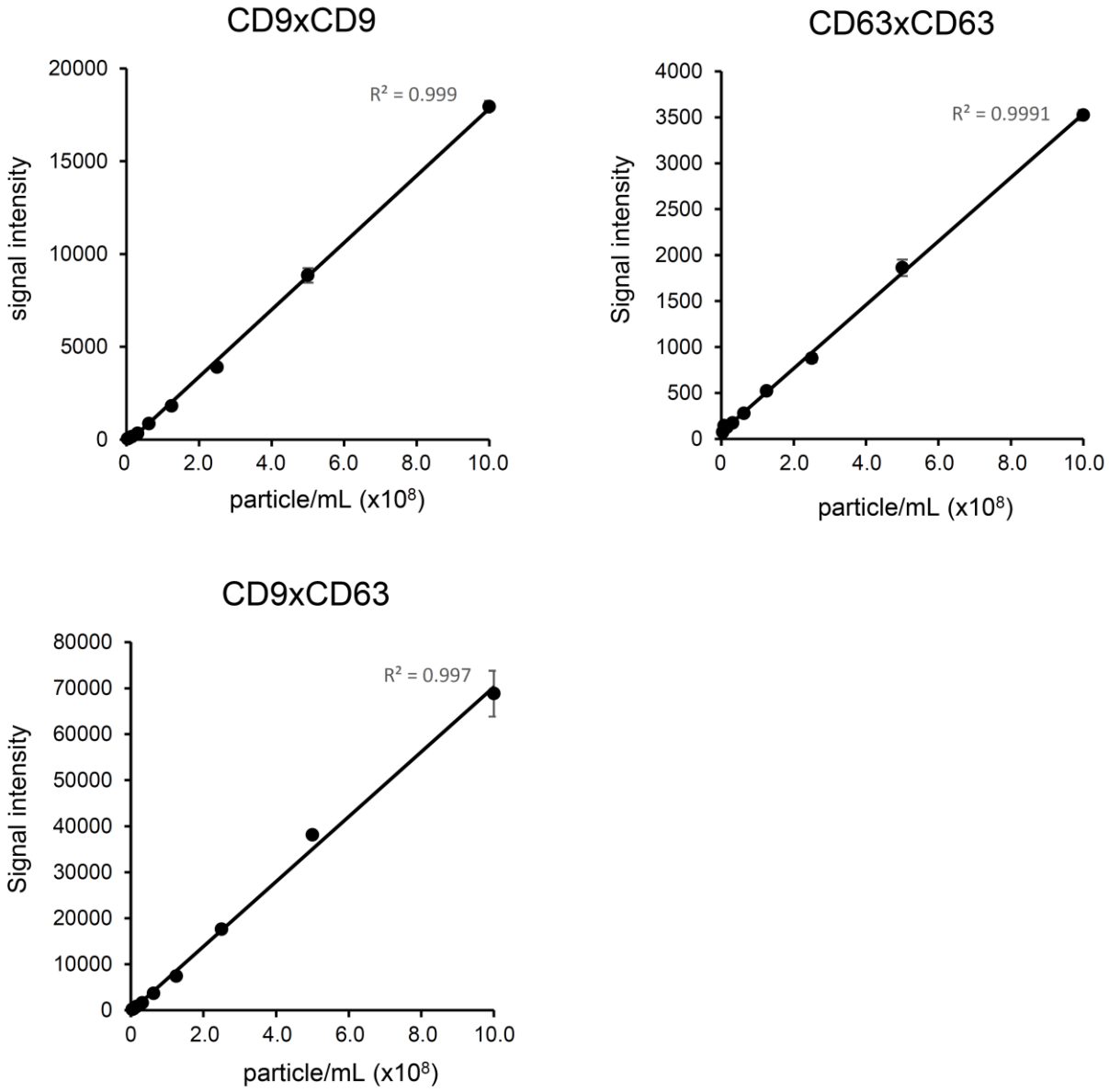

Fig. S1. Correlation between ExoScreen and NTA

Correlation between ExoScreen measurements for CD9 positive extracellular vesicles (EVs), CD63 positive EVs, or CD9/CD63 double positive EVs and particle number of EVs in a dilution series. Particle number was measured via NTA. EVs were collected from PC3M cells. The values are the mean ± SE (n = 3).

Figure S2.

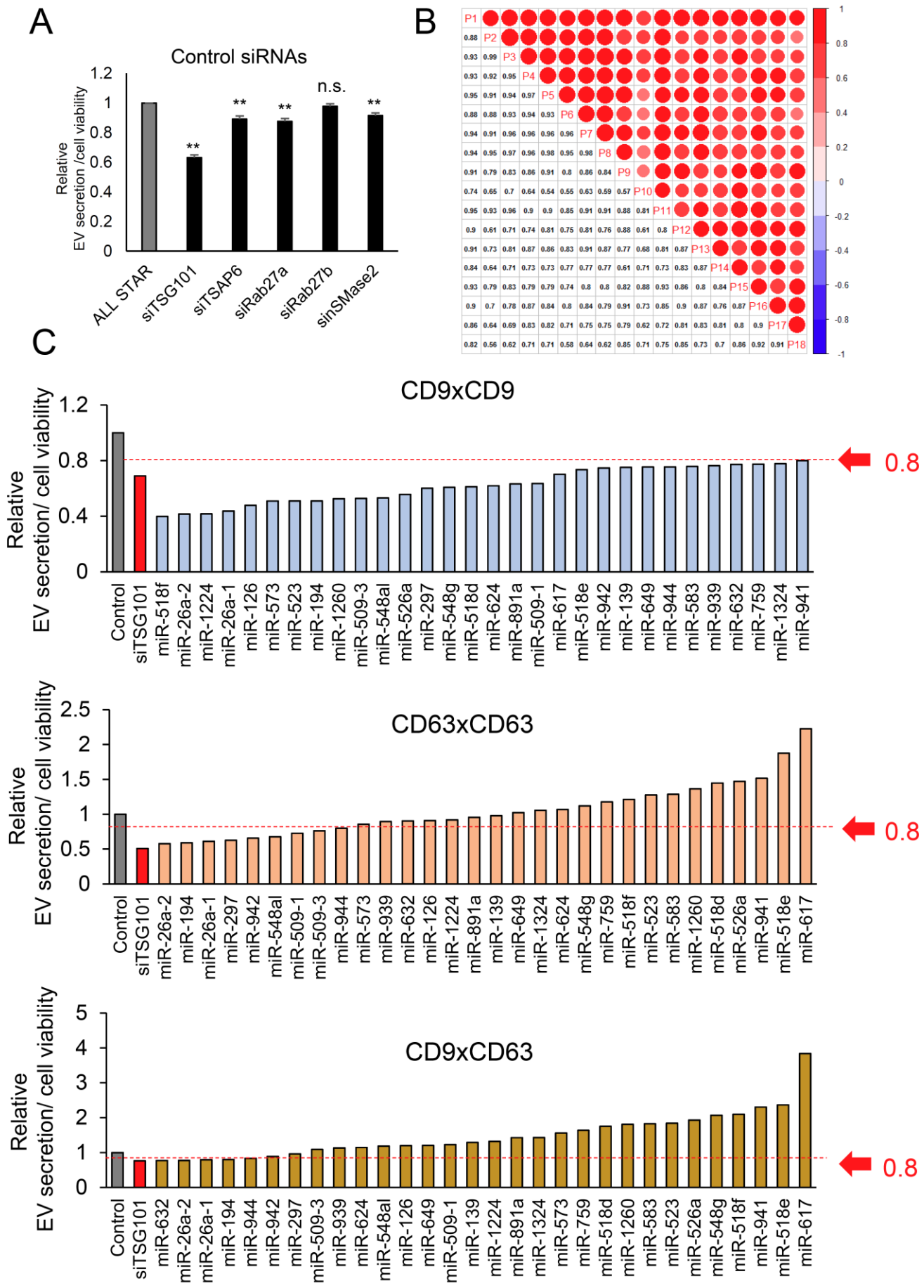
A
Control siRNAs
Relative EV secretion /cell viability
1.2
1
0.8
0.6
0.4
0.2
0
**
**
**
n.s.
**
ALL STAR
siTSG101
siTSAP6
siRab27a
siRab27b
siNSMase2
B
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
P18
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
C
CD9xCD9
Relative EV secretion/ cell viability
1.2
0.8
0.4
0
0.8
Control
siTSG101
miR-518f
miR-26a-2
miR-1224
miR-26a-1
miR-126
miR-573
miR-523
miR-194
miR-1260
miR-509-3
miR-548al
miR-526a
miR-297
miR-548g
miR-518d
miR-624
miR-891a
miR-509-1
miR-617
miR-518e
miR-942
miR-139
miR-649
miR-944
miR-583
miR-939
miR-632
miR-759
miR-1324
miR-941
CD63xCD63
Relative EV secretion/ cell viability
2.5
2
1.5
1
0.5
0
0.8
Control
siTSG101
miR-26a-2
miR-194
miR-26a-1
miR-297
miR-942
miR-548al
miR-509-1
miR-509-3
miR-944
miR-573
miR-939
miR-632
miR-126
miR-1224
miR-891a
miR-139
miR-649
miR-1324
miR-624
miR-548g
miR-759
miR-518f
miR-523
miR-583
miR-1260
miR-518d
miR-526a
miR-941
miR-518e
miR-617
CD9xCD63
Relative EV secretion/ cell viability
5
4
3
2
1
0
0.8
Control
siTSG101
miR-632
miR-26a-2
miR-26a-1
miR-194
miR-944
miR-942
miR-297
miR-509-3
miR-939
miR-624
miR-548al
miR-126
miR-649
miR-509-1
miR-139
miR-1224
miR-891a
miR-1324
miR-573
miR-759
miR-518d
miR-1260
miR-583
miR-523
miR-526a
miR-548g
miR-518f
miR-941
miR-518e
miR-617

Fig. S2. Results of screening to select miRNAs regulating EV secretion

(A) The average of EV secretion from the controls in the 1st screening. (B) Correlation matrix between the controls: positive correlations are shown in red, and negative correlations are shown in blue. The values are the mean ± SE (n = 18). **, $p < 0.01$; and n.s., not significant. (C) The results of the screening from 30 candidate miRNAs. The effect of 30 miRNAs and nonspecific miRNA mimic (control) on the secretion of EVs and cell viability. The secretion of EV was evaluated by ExoScreen, and the cell viability was measured using the MTS assay.

Figure S3.

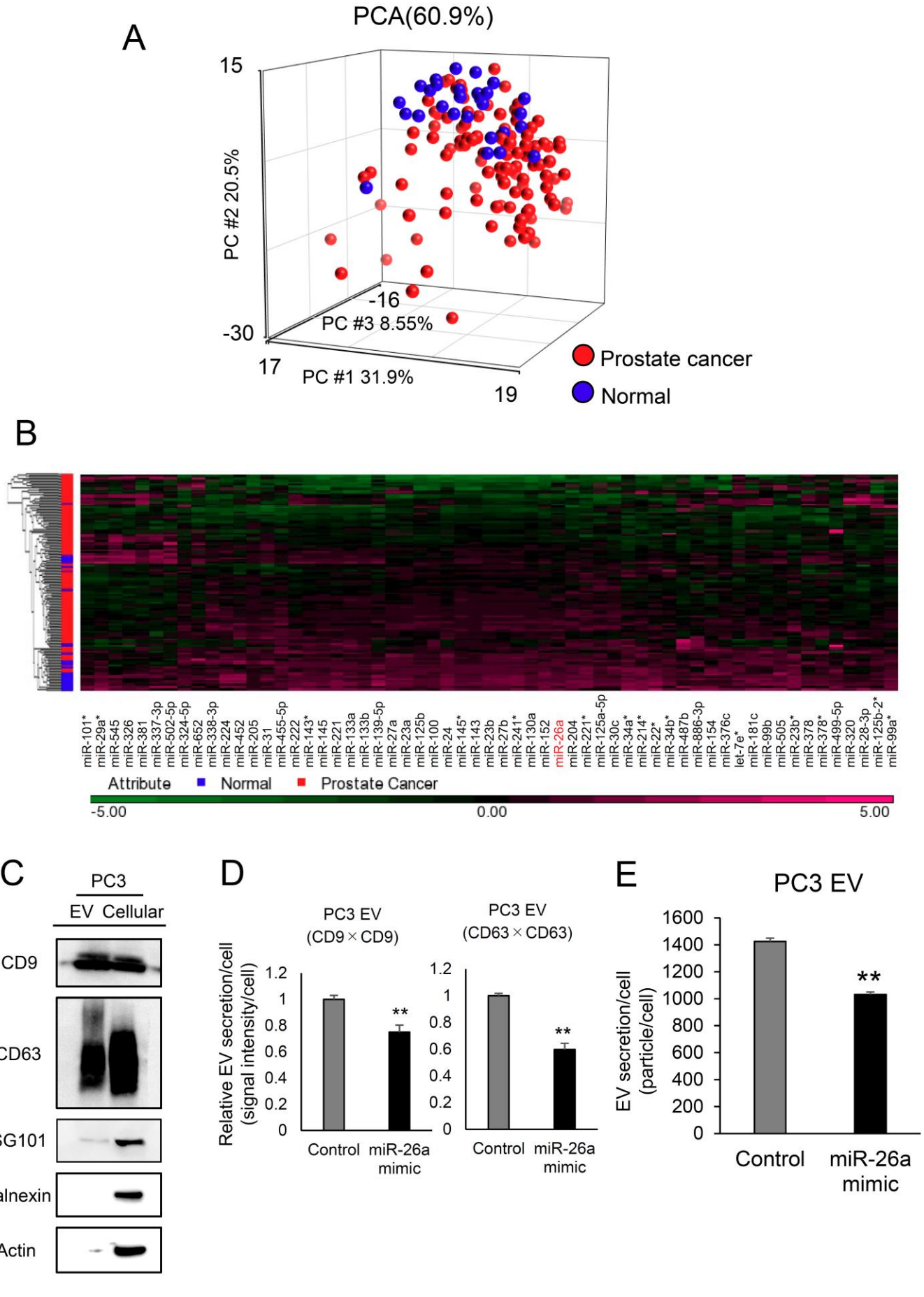
A
PCA(60.9%)
15
-30
PC #2 20.5%
-16
PC #3 8.55%
17
PC #1 31.9%
19
Prostate cancer
Normal
B
miR-101*
miR-29a*
miR-545
miR-326
miR-381
miR-337-3p
miR-502-5p
miR-324-5p
miR-652
miR-338-3p
miR-224
miR-452
miR-205
miR-31
miR-455-5p
miR-222
miR-143*
miR-145
miR-221
miR-133a
miR-133b
miR-139-5p
miR-27a
miR-23a
miR-125b
miR-100
miR-24
miR-145*
miR-143
miR-23b
miR-27b
miR-241*
miR-130a
miR-152
miR-26a
miR-204
miR-221*
miR-125a-5p
miR-30c
miR-34a*
miR-214*
miR-22*
miR-34b*
miR-487b
miR-886-3p
miR-154
miR-376c
let-7e*
miR-181c
miR-99b
miR-505
miR-23b*
miR-378
miR-378*
miR-499-5p
miR-320
miR-28-3p
miR-125b-2*
miR-99a*
Attribute
Normal
Prostate Cancer
-5.00
0.00
5.00
C
PC3
EV
Cellular
CD9
CD63
TSG101
Calnexin
Actin
D
PC3 EV
(CD9 × CD9)
PC3 EV
(CD63 × CD63)
Relative EV secretion/cell
(signal intensity/cell)
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
miR-26a
mimic
E
PC3 EV
EV secretion/cell
(particle/cell)
1600
1400
1200
1000
800
600
400
200
0
**
Control
miR-26a
mimic

Fig. S3. Analysis of expression of miR-26a in prostate cancer samples

(A) A principal component analysis (PCA) map for 99 prostate cancer (PCa) tissues and 28 normal adjacent benign prostate tissues with 373 miRNAs. (B) Heat map showing the differences in 59 miRNAs whose expression levels were repressed > 1.25-fold in PCa tissue relative to normal adjacent benign prostate tissue and p-value < 0.001. (C) Immunoblot analysis of the conventional EV markers. A 1 μg sample of EVs and a 10 μg sample of cell lysate from PC3 cells were loaded into each lane. (D) The effect of the miR-26a mimic on EV secretion per PC3 cell. The secretion of EVs per cell was evaluated by the signal intensity of ExoScreen per cell. The values are depicted as the fold-change relative to the nonspecific miRNA mimic (control). The values are the mean ± SE (n = 3). **, $p < 0.01$. (E) The effect of the miR-26a mimic on EV secretion per PC3 cell. The amount of EV secreted per cell was evaluated using a nanoparticle tracking system. The values are the mean ± SE (n = 3). **, $p < 0.01$.

Figure S4.

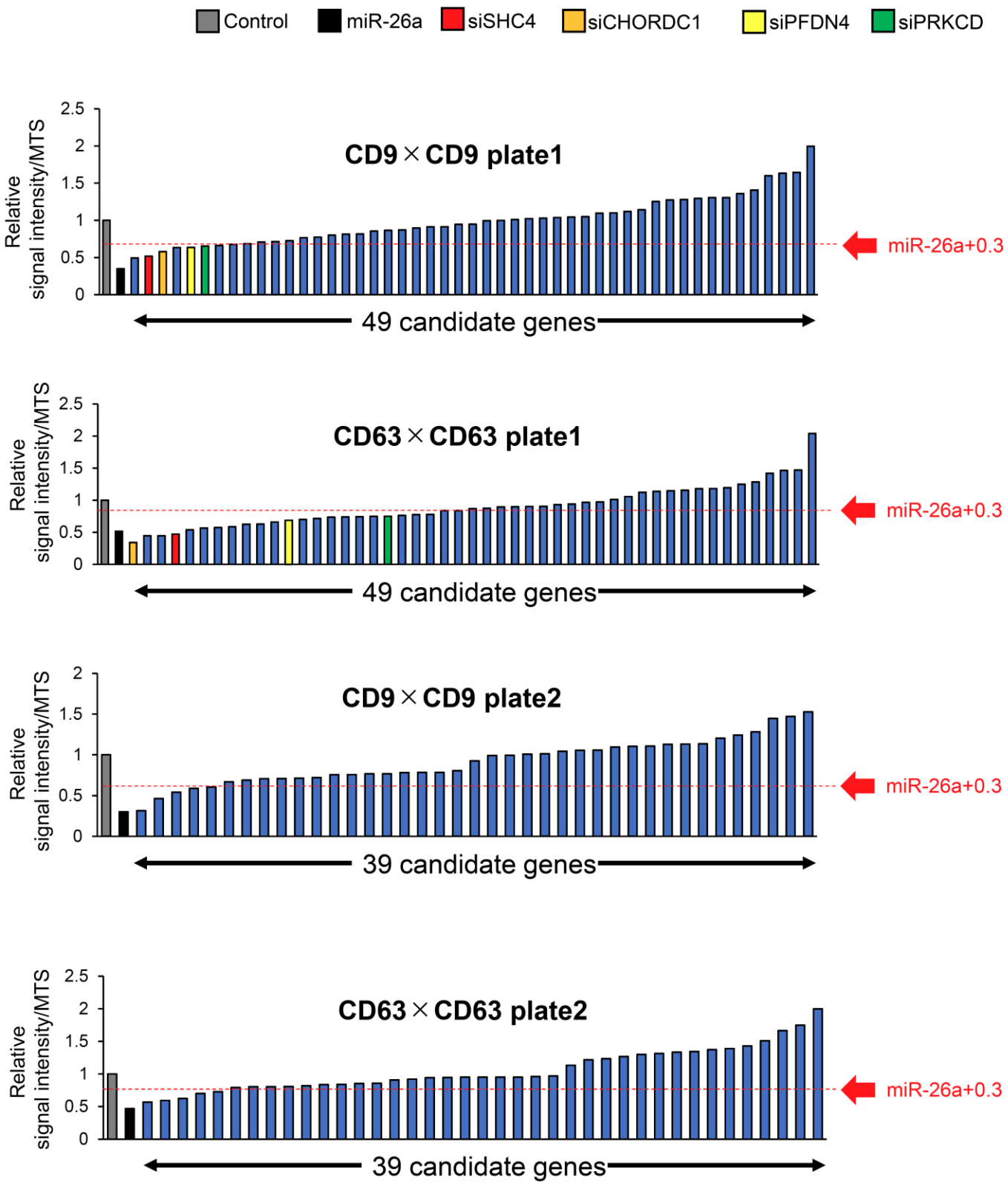

Fig. S4. Results of screening to select candidate genes regulating EV secretion

The effect of candidate gene siRNAs and negative control siRNA (control) on the secretion of EVs and cell viability. The secretion of EV was evaluated by ExoScreen, and the cell viability was measured using the MTS assay. A total of 88 candidate genes were separated into two plates, plate 1 and plate 2.

Figure S5.

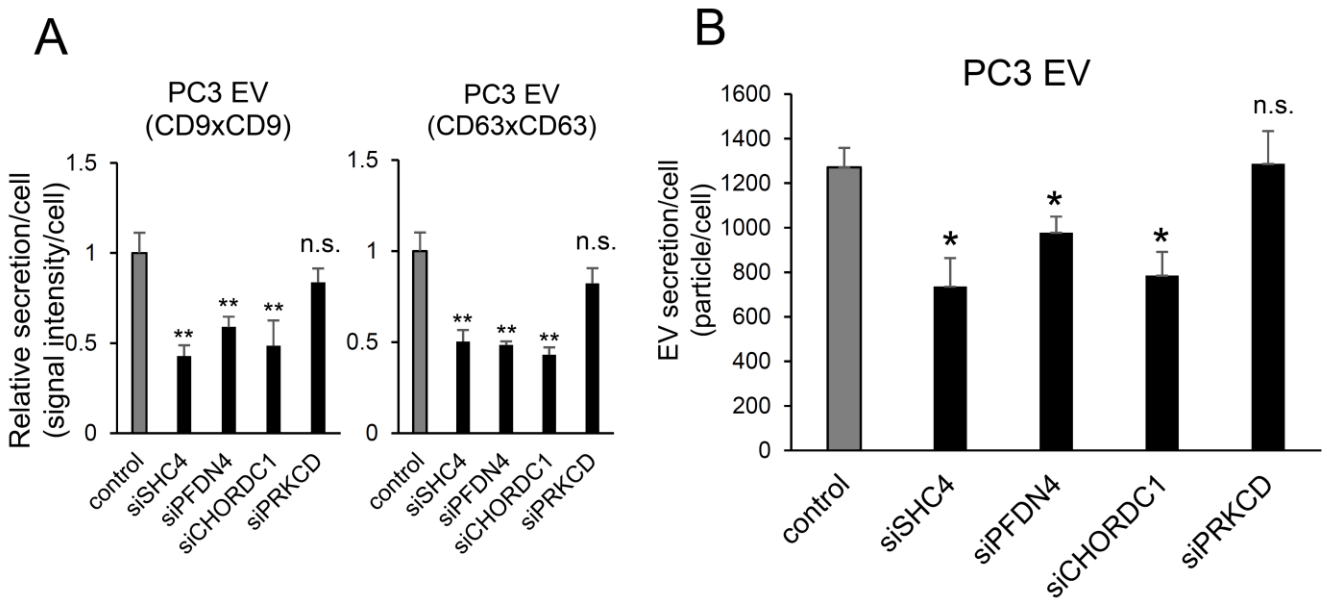

Fig. S5. Effect of siRNAs against the candidate genes on EV secretion

(A) The secretion of EVs was evaluated by the signal intensity of ExoScreen. The values are depicted as the fold-change relative to the nonspecific miRNA mimic (control). (B) The particle number of EVs was measured using a nanoparticle tracking system. The values are the mean ± SE (n = 3). *, $p < 0.05$; and n.s., not significant.

Figure S6.

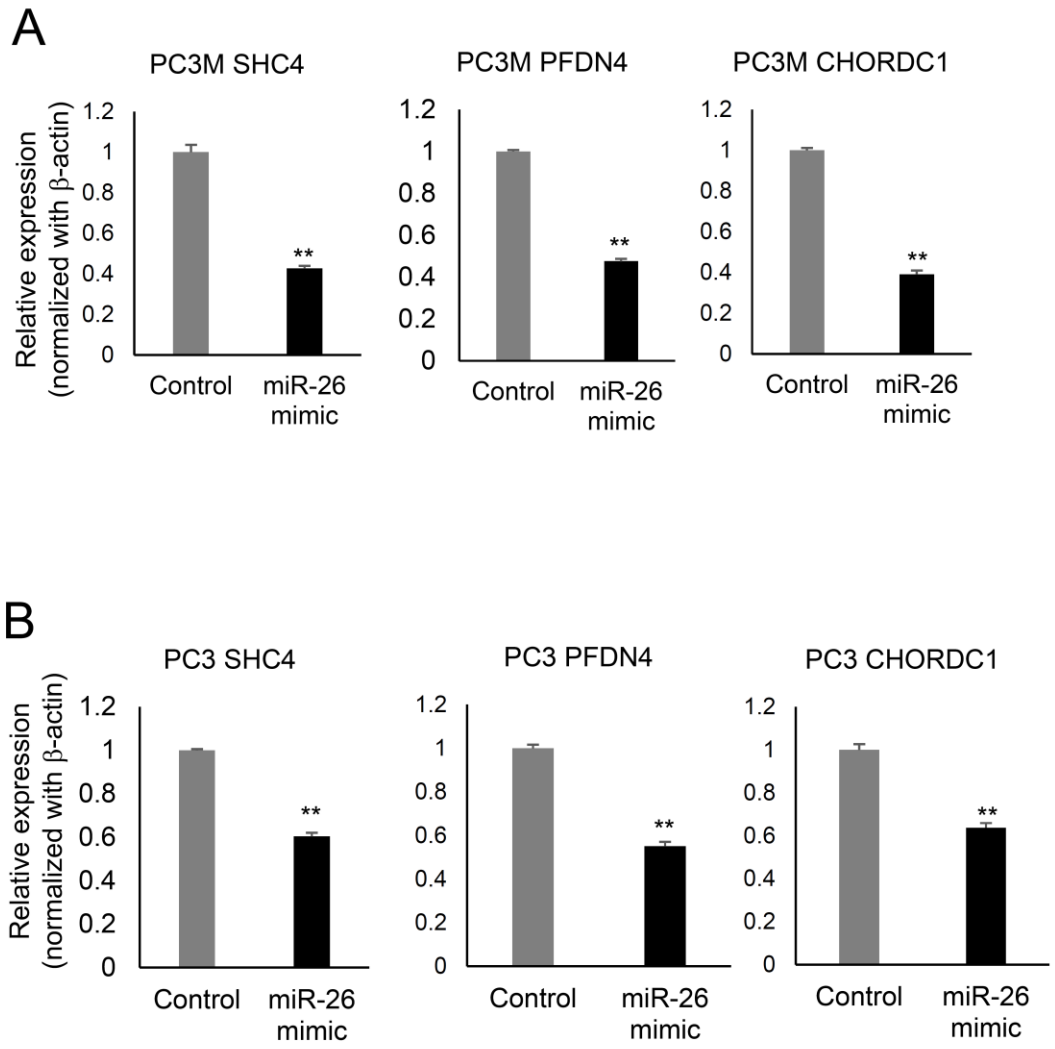

Fig. S6. Effect of miR-26a on the expression level of target genes

(A) The effect of miR-26a on the expression level of target genes in PC3M cells. The values are depicted as the fold-change relative to the nonspecific miRNA mimic (control). The values are the mean ± SE (n = 3). **, $p < 0.01$.

(B) The effect of miR-26a on the expression level of target genes in PC3 cells. The values are depicted as the fold-change relative to the nonspecific miRNA mimic (control). The values are the mean ± SE (n = 3). **, $p < 0.01$.

Figure S7.

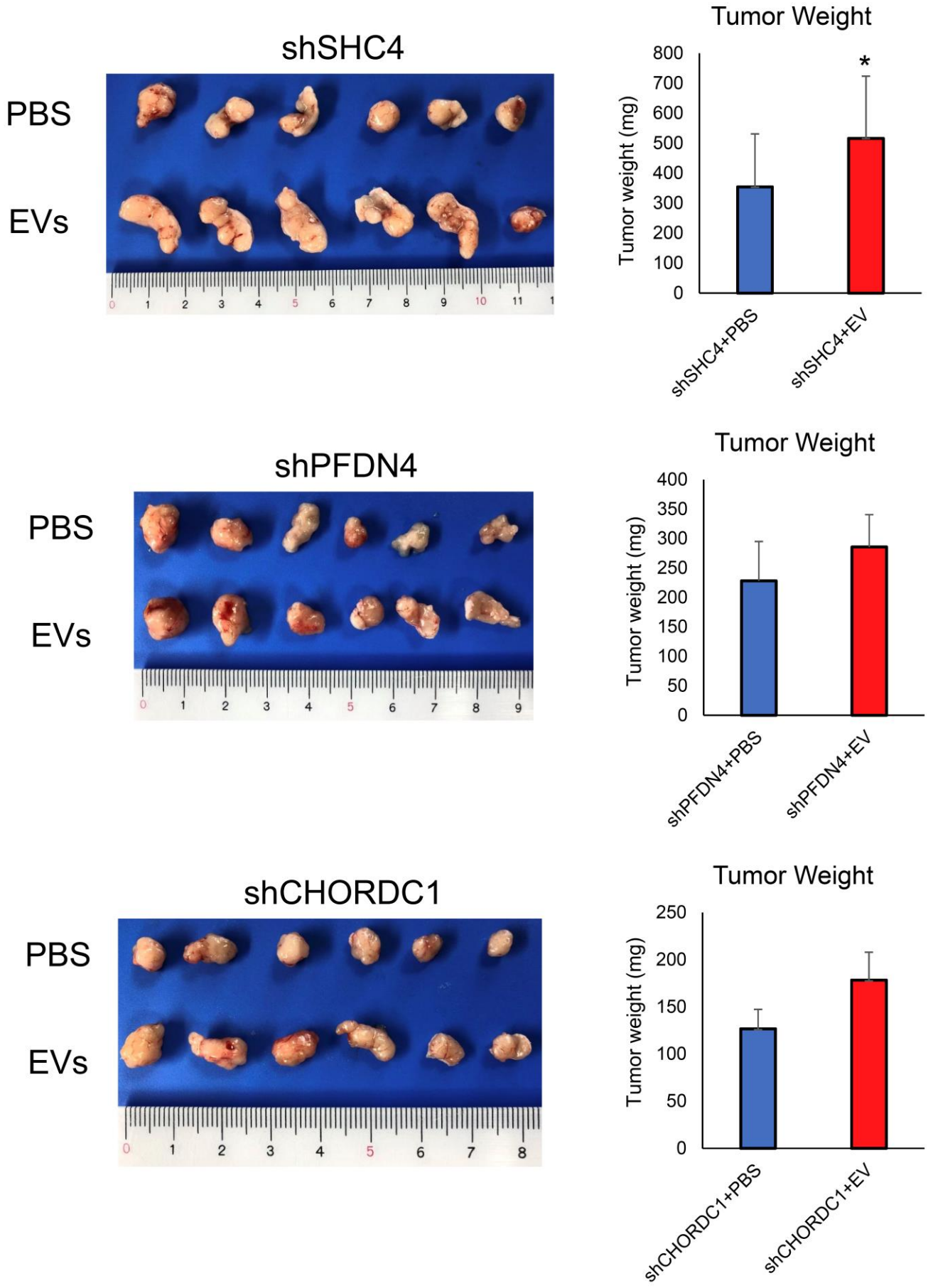
shSHC4
PBS
EVs
Tumor Weight
Tumor weight (mg)
800
700
600
500
400
300
200
100
0
*
shSHC4+PBS
shSHC4+EV
shPFDN4
PBS
EVs
Tumor Weight
Tumor weight (mg)
400
350
300
250
200
150
100
50
0
shPFDN4+PBS
shPFDN4+EV
shCHORDC1
PBS
EVs
Tumor Weight
Tumor weight (mg)
250
200
150
100
50
0
shCHORDC1+PBS
shCHORDC1+EV

Fig. S7. EVs partially rescued the tumor size and weight

The xenografts from nude mice injected with PBS or EVs. The tumor weights in nude mice at 31 days were determined. The values are the mean ± SE (n = 6). *, $p < 0.05$. (Photo credit: Fumihiko Urabe, The Jikei University School of Medicine)