Supplementary figure 1. Protective effects of PSP against enzymatic degradation of FAM-siRNA in physiological fluids

PSPS (PSP/FAM-siRNA:1/5) complex was exposed to serum from healthy controls from 1 to 72 h at 37 ℃. **(A)** Gel shift assay and **(B)** Fluorescence intensity. Data are shown as the mean ± S.E. ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 2. PSP failed to bind with and internalize into PSMA- CRPC cells in vitro

(A). PSP failed to bind to PSMA- CRPC cells. PC-3 cells were incubated with 100nM PSP, PSMAb (positive control) or human IgG (negative control) for 1 h at 4℃ followed by the PE-conjugated anti-human IgG-Fc antibody. IF (x400). (**B).** PSP failed to internalize into PSMA- CRPC cells. PC-3 cells were incubated with 100nM PSP, PSMAb (positive control) or human IgG (negative control) for 3 h at 37℃ followed by the PE-conjugated anti-human IgG-Fc antibody. IF (x400). Scale bars = 20 mm. Representative results of 3 independent experiments are shown.

Supplementary figure 3. PSPS failed to Internalize into PSMA- CRPC cells.

PC-3 cells were incubated with 100nM PSP conjugated with FAM-siRNA for 3 h at 37℃. Cells transfected with FAM-siRNA by lipofectamine2000 were used as a positive control. Cells were fixed and stained with phalloidin to visualize the cytoskeleton and with DAPI to visualize the nuclei. IF (x400). Scale bars = 20 mm. Representative results of 3 independent experiments are shown.

Supplementary figure 4. PSP and PSPS failed to bind with and internalize into PSMA- CRPC cells in vivo

Dynamic distribution of ICG-labeled PSP in **(A)** whole-body and **(B)** major organs. ICG-labeled PSP was injected into PC-3 xenografted nude mice and its whole-body distribution was monitored by fluorescence imaging excited at 745 nm at indicated time points and the resected major organ

distribution was monitored 96 h after ICG-labeled PSP injection. BLI was acquired to identify the CRPC tumor tissues. Dynamic distribution of PSPS in vivo **(C)** whole-body and **(D)** major organs. PSPS complex was injected into PC-3 xenografted nude mice and its whole-body distribution was monitored by fluorescence imaging excited at 488 nm at indicated time points and the resected major organ distribution was monitored 96 h after PSPS injection. BLI was acquired to identify the CRPC tumor tissues. The presence of PSPS complexes in tumor and major organs in vivo. Tumors and major organs were resected 96 h after PSPS injection. Fluorescence images of FAM-siRNA in **(E)** tumors and **(F)** major organs. Cryosections were taken by LSCM. Scale bars = 20 μm. Representative results of 3 independent experiments are shown.

Supplementary figure 5. TRIM24 siRNA delivered by PSP failed to inhibit TRIM24 expression in PSMA- CRPC cells in vitro

Relative expression of **(A)** TRIM24 and **(B)** PSMA (B) in 492 patients of prostate adenocarcinoma (PRAD, red box) and 152 healthy controls (Black box). TCGA data analyzed by GEPIA (http://gepia.cancer-pku.cn/). **(C)**. Correlation of TRIM24 and PSMA expression in 492 PRAD patients. PSMAb-mediated RNAi failed to reduce TRIM24 target gene expression in the PSMA- CRPC cells. PC-3 cells were exposed to 100 nM of PSP-TRIM24-siRNA for 72 h at 37 °C. **(D)** Western blotting, **(E)** IF (Cells were fixed and stained with TRIM24 antibody (Red), phalloidin (Green) to visualize the cytoskeleton, and with DAPI (Blue) to visualize the nuclei.), and **(F)** qRT-PCR were performed for TRIM24. Scale bars = 20 μm. Data are shown as the mean ± S.E. ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 6. TRIM24 siRNA delivered by PSP failed to inhibit cell proliferation in PSMA- CRPC cells in vitro

(A). Proliferation of PC-3 cells treated as indicated were analyzed by the CCK-8 assay. **(B)**. Colony-forming ability of PC-3 cells treated as indicated was analyzed by the plate clone formation assay. **(D)** Apoptosis and **(F)** cell cycle of PC-3 cells treated as indicated were analyzed by flow cytometry. **(C), (E),** and **(G)**. Statistical analysis of B, D, and F, respectively from 3

independent experiments. Data are shown as the mean ± S.E. ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 7. TRIM24 siRNA delivered by PSP failed to inhibit cell migration and invasion in PSMA- CRPC cells in vitro

(A). The migration distance of PC-3 cells treated as indicated were analyzed by wound healing assay. Number of **(C)** migrative and **(E)** invasive cells PC-3 cells treated as indicated were analyzed by the Transwell assay. **(B), (D),** and **(F)**. Statistical analysis of A, C, and E, respectively from six randomly selected fields of paraffin-embedded tumor sections. Scale bars = 100 μm. Data are shown as the mean ± S.E. ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 8. TRIM24 siRNA delivered by PSP failed to inhibit tumor growth in PC-3 xenografts in vivo

(A). Tumor growth of different PC-3 xenografts treated as indicated was observed using BLI every week after the first treatment. **(B)**. Tumor volume analysis to show the growth of PC-3 tumors after receiving treatments as indicated. The arrows indicate the five treatments. **(C)**. Digital pictures of resected tumors after receiving treatments as indicated. **(D)**. Tumor weight analysis of PC-3 tumors after receiving treatments as indicated. **(E)**. Paraffin-embedded sections of resected PC-3 tumors from each group were stained with H&E. Scale bars = 50 μm. Data was shown as the mean ± S.E. for each group of mice (n=5). ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 9. TRIM24 siRNA delivered by PSP failed to inhibit TRIM24 expression and induce apoptosis in PC-3 xenografts in vivo

PSMAb-mediated RNAi failed to reduce TRIM24 target gene expression in the PC-3 cells in vivo. **(A)** Western blot analysis, **(B)** IHC, and **(C)** qRT-PCR were performed for TRIM24 expression in paraffin-embedded sections of resected PC-3 xenografts after receiving five treatments as

indicated. and. Nude mice bearing PC-3 xenografts received five treatments as indicated, and paraffin-embedded sections of resected tumors from each group were stained for **(D)** TUNEL (x100, Scale bars = 50 μm) and **(E)** Ki67 (x200, Scale bars = 20 μm). **(F)** and **(G)**, statistical analysis of D and E, respectively, from six randomly selected fields of paraffin-embedded tumor sections. Data are shown as the mean ± S.E. ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary figure 10. In vivo toxicity of TRIM24 siRNA delivered by PSP in PC-3 xenografts

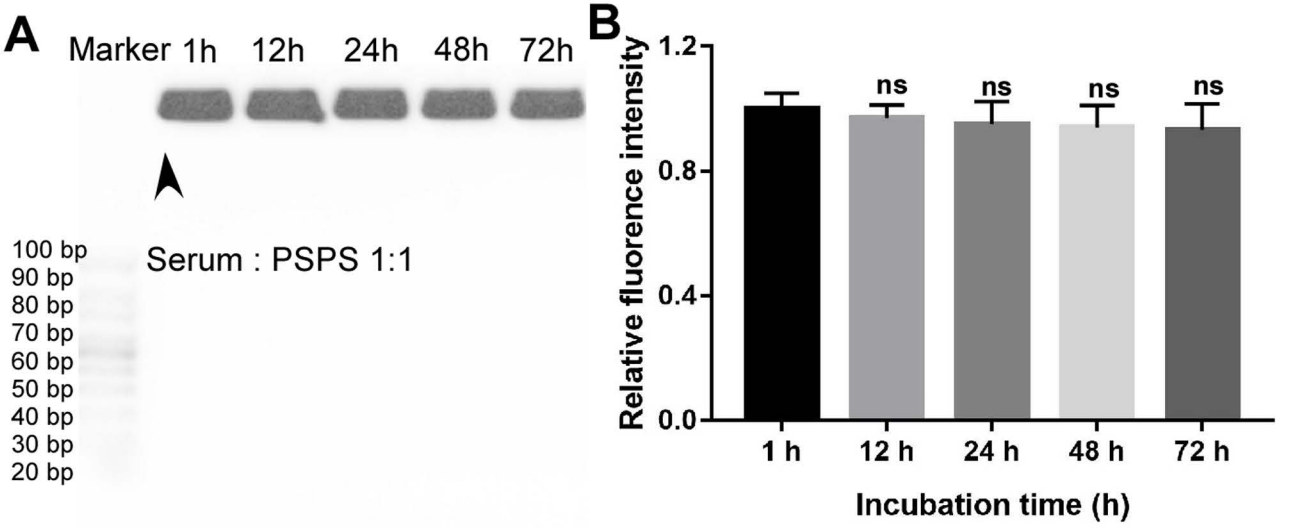
(A). Body weight analysis of each PC-3 group after receiving treatments as indicated. **(B).** The liver function and kidney function of each PC-3 group after receiving indicated treatments five times. **(C).** Paraffin-embedded sections of resected major organs from each PC-3 group receiving indicated treatments five times were stained with H&E and photographed (x200). Scale bars =20 μm. Data are shown as the mean ± S.E. (n=5). ns: not significant. Representative results of 3 independent experiments are shown.

Supplementary video 1. Bone loss in PBS group

Supplementary video 2. Bone loss in IgG-TRIM24 siRNA group

Supplementary video 3. Bone loss in PSP-NC group

Supplementary video 4. Bone loss in PSP-TRIM24 siRNA group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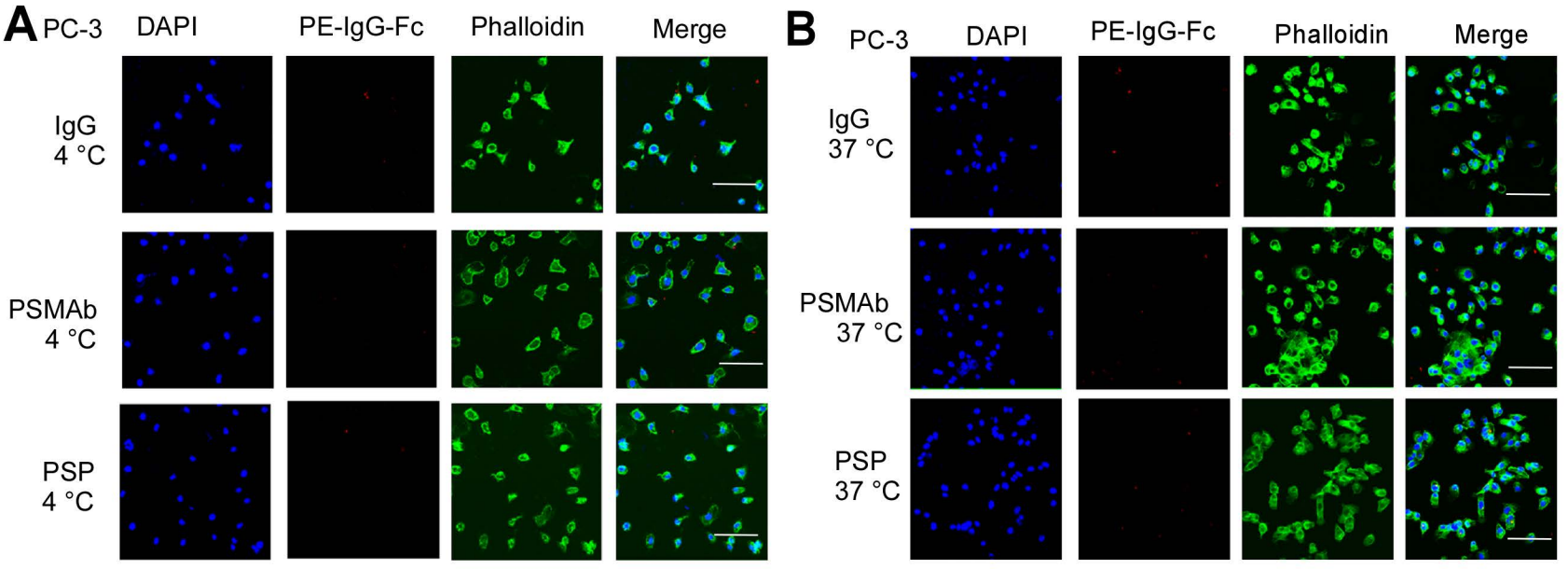
Marker 1h 12h 24h 48h 72h
Serum : PSPS 1:1
100 bp
90 bp
80 bp
70 bp
60 bp
50 bp
40 bp
30 bp
20 bp
B
Relative fluorence intensity
1.2
0.8
0.4
0.0
ns
ns
ns
ns
1 h
12 h
24 h
48 h
72 h
Incubation time (h)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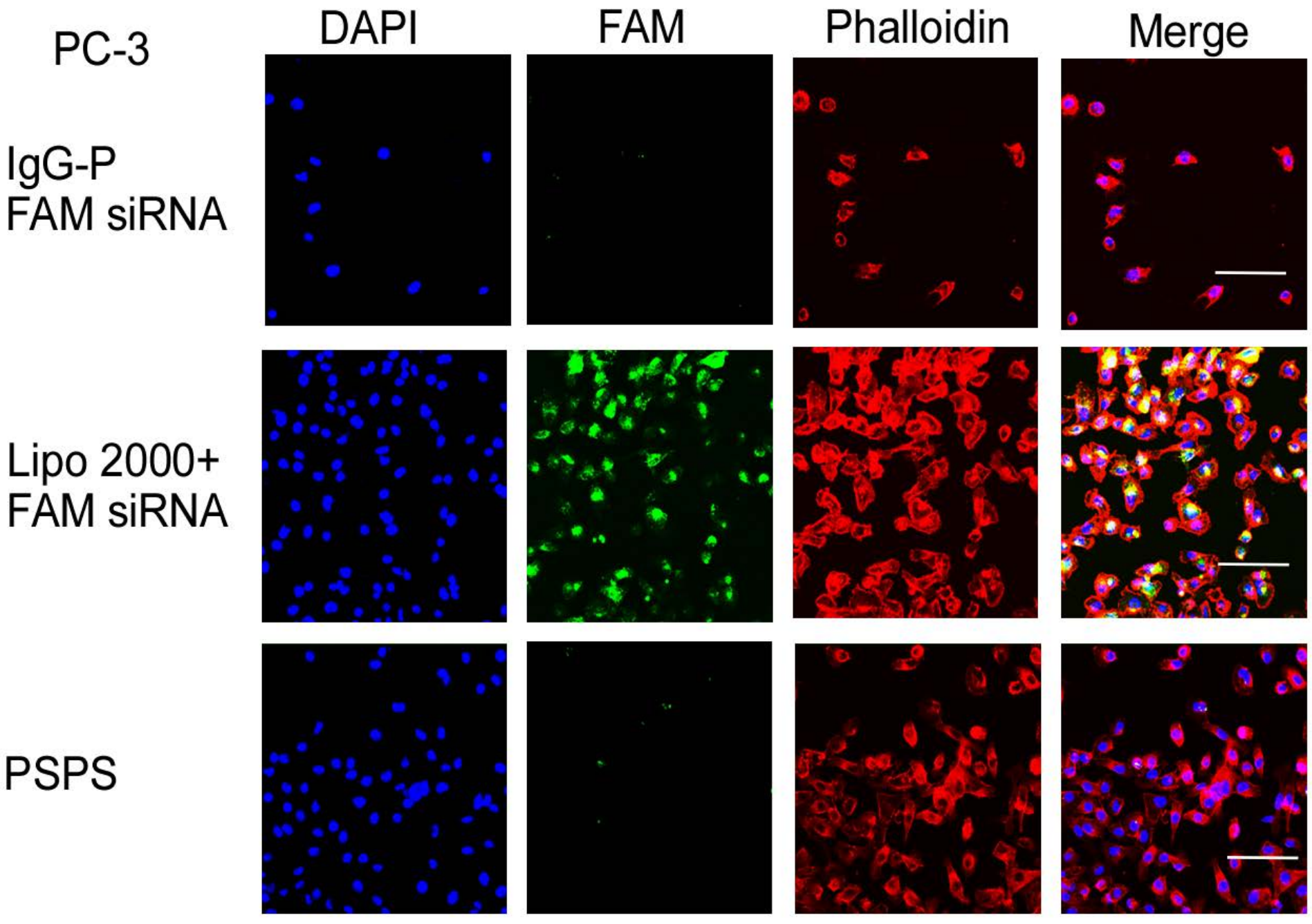
PC-3
DAPI
PE-IgG-Fc
Phalloidin
Merge
IgG
4 °C
PSMAb
4 °C
PSP
4 °C
B
PC-3
DAPI
PE-IgG-Fc
Phalloidin
Merge
IgG
37 °C
PSMAb
37 °C
PSP
37 °C


PC-3
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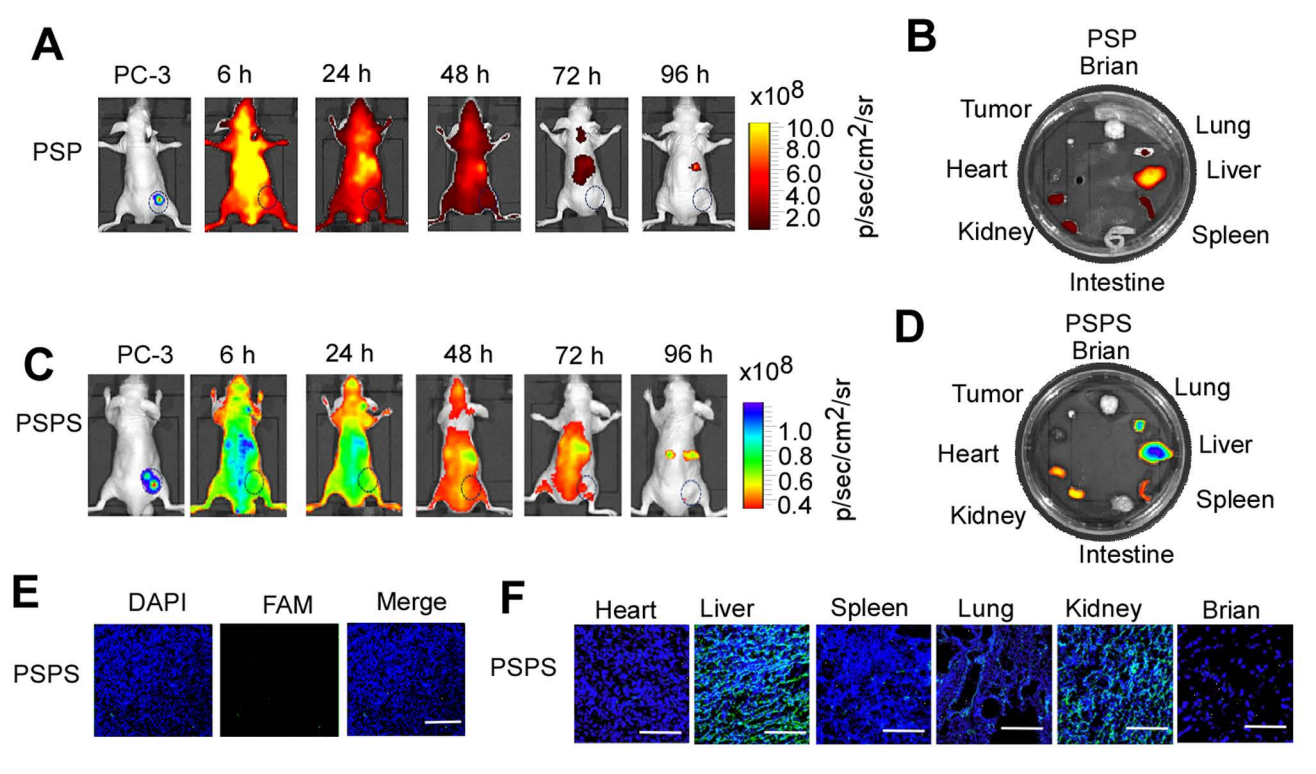
FAM
Phalloidin
Merge
IgG-P
FAM siRNA
Lipo 2000+
FAM siRNA
PSPS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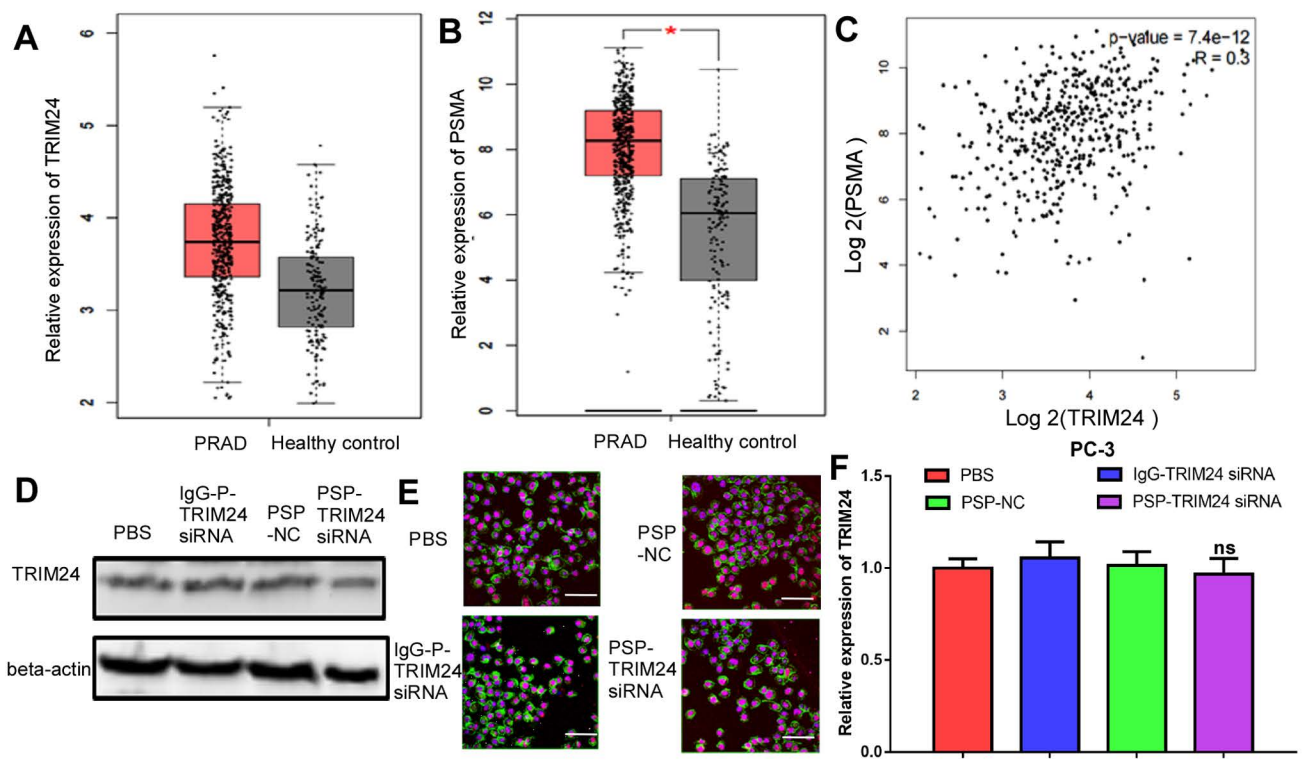
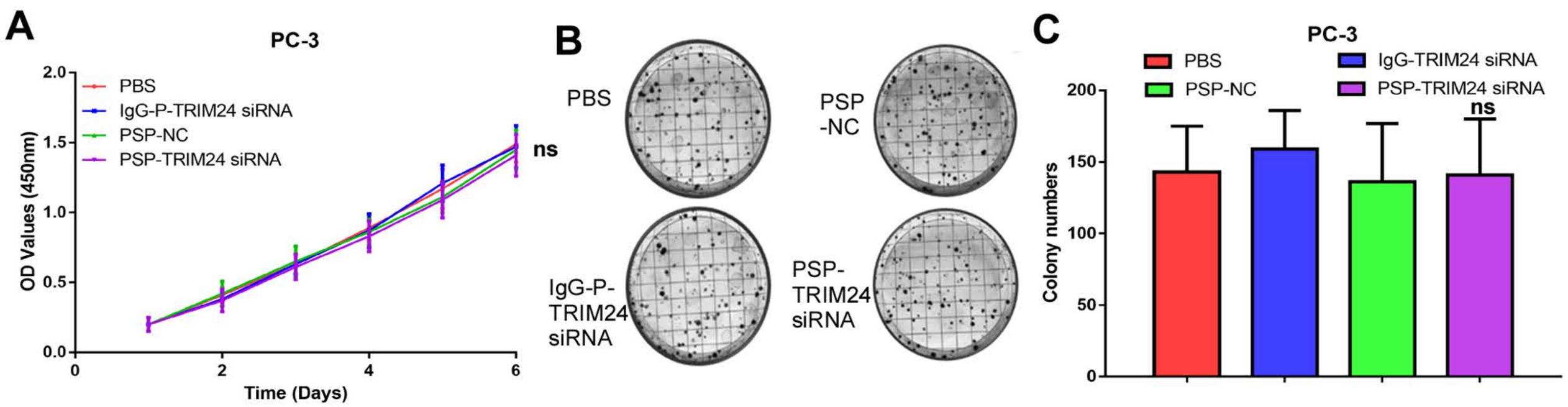
PC-3
6 h
24 h
48 h
72 h
96 h
PSP
x10^8
10.0
8.0
6.0
4.0
2.0
p/sec/cm2/sr
B
PSP
Brian
Tumor
Lung
Heart
Liver
Kidney
Spleen
Intestine
C
PC-3
6 h
24 h
48 h
72 h
96 h
PSPS
x10^8
1.0
0.8
0.6
0.4
p/sec/cm2/sr
D
PSPS
Brian
Tumor
Lung
Heart
Liver
Kidney
Spleen
Intestine
E
DAPI
FAM
Merge
PSPS
F
Heart
Liver
Spleen
Lung
Kidney
Brian
PSPS


A
Relative expression of TRIM24
PRAD
Healthy control
B
Relative expression of PSMA
*
PRAD
Healthy control
C
p-value = 7.4e-12
R = 0.3
Log 2(PSMA)
Log 2(TRIM24)
D
PBS
IgG-P-TRIM24 siRNA
PSP -NC
PSP-TRIM24 siRNA
TRIM24
beta-actin
E
PBS
PSP -NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
F
PC-3
PBS
PSP-NC
IgG-TRIM24 siRNA
PSP-TRIM24 siRNA
Relative expression of TRIM24
ns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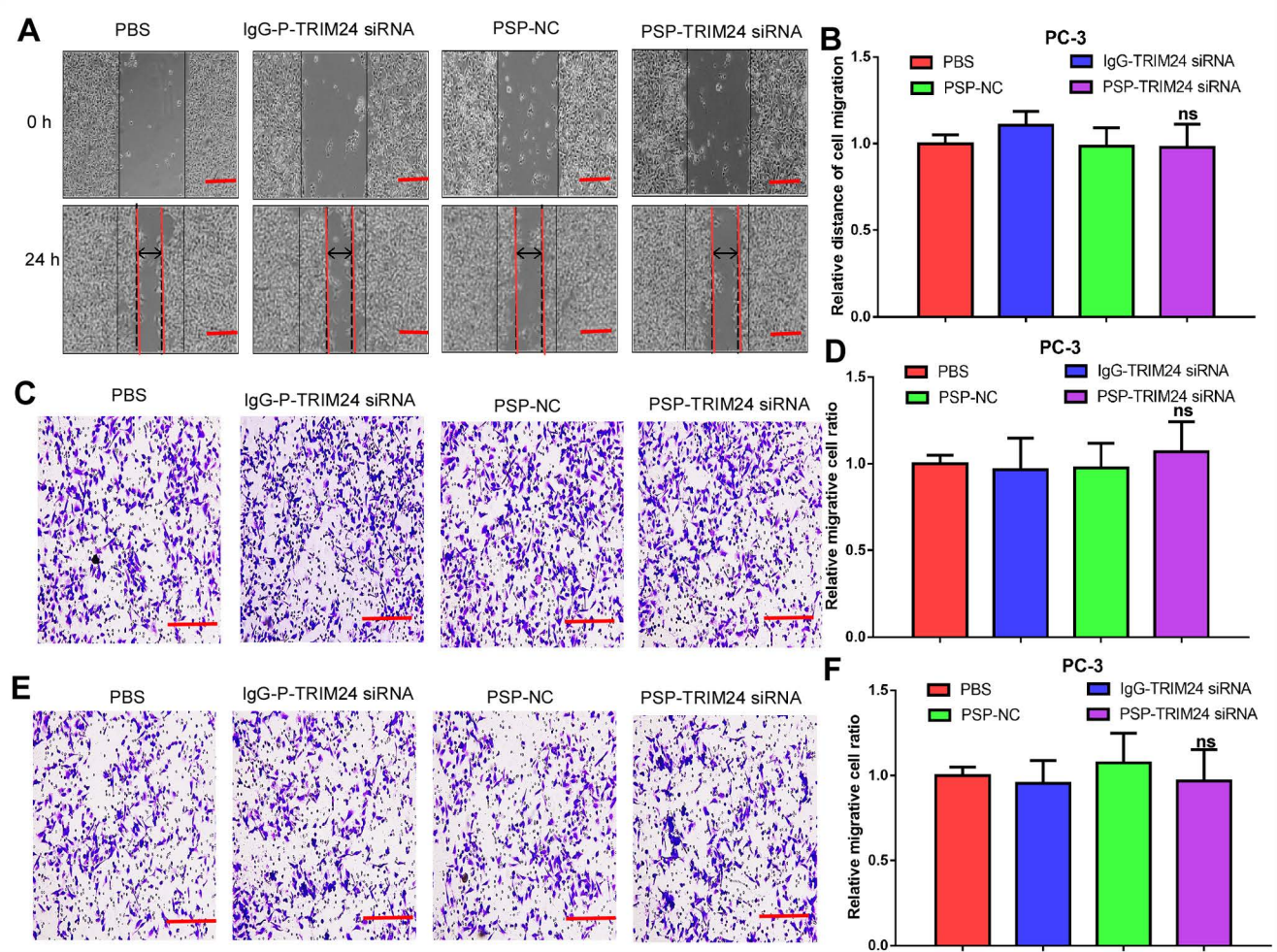
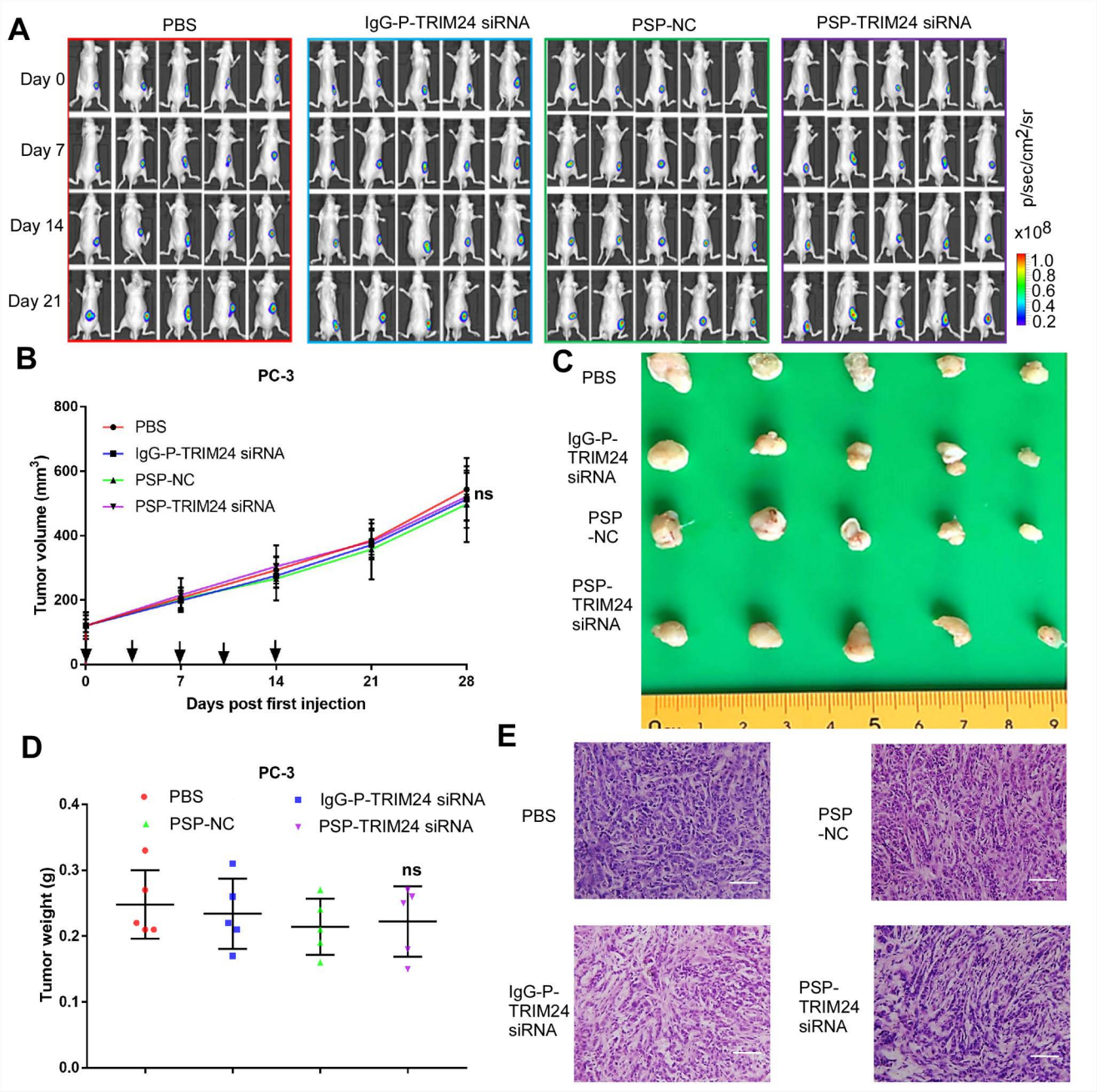
PC-3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
OD Values (450nm)
Time (Days)
ns
B
PBS
PSP -NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
C
PC-3
PBS
IgG-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Colony numbers
ns
D
PBS
PSP -NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
PI
Annexin V-FITC
B1 0.4% B2 2.2% B3 93.5% B4 3.9% 3.94
B1 0.4% B2 1.2% B3 93.6% B4 4.8% 4.85
B1 0.5% B2 1.9% B3 93.4% B4 4.1% 4.16
B1 1.3% B2 2.4% B3 91.7% B4 4.7% 4.73
E
PC-3
PBS
IgG-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Percent of early apoptotic cells
ns
F
PBS
PSP -NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
Numbers
G1:51.24 G2:10.69 S:38.07
G1:47.95 G2:13.34 S:38.71
G1:52.27 G2:12.31 S:35.42
G1:53.48 G2:10.79 S:35.73
2N
4N
G
PC-3
PBS
PSP-NC
IgG-TRIM24 siRNA
PSP-TRIM24 siRNA
Cell cycle distribution
ns


A
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
0 h
24 h
B
PC-3
PBS
IgG-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Relative distance of cell migration
1.5
1.0
0.5
0.0
ns
C
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
D
PC-3
PBS
IgG-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Relative migrative cell ratio
1.5
1.0
0.5
0.0
ns
E
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
F
PC-3
PBS
IgG-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Relative migrative cell ratio
1.5
1.0
0.5
0.0
ns


A
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Day 0
Day 7
Day 14
Day 21
p/sec/cm2/sr
x10^8
1.0
0.8
0.6
0.4
0.2
B
PC-3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Tumor volume (mm3)
800
600
400
200
0
ns
0
7
14
21
28
Days post first injection
C
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
D
PC-3
PBS
PSP-NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
Tumor weight (g)
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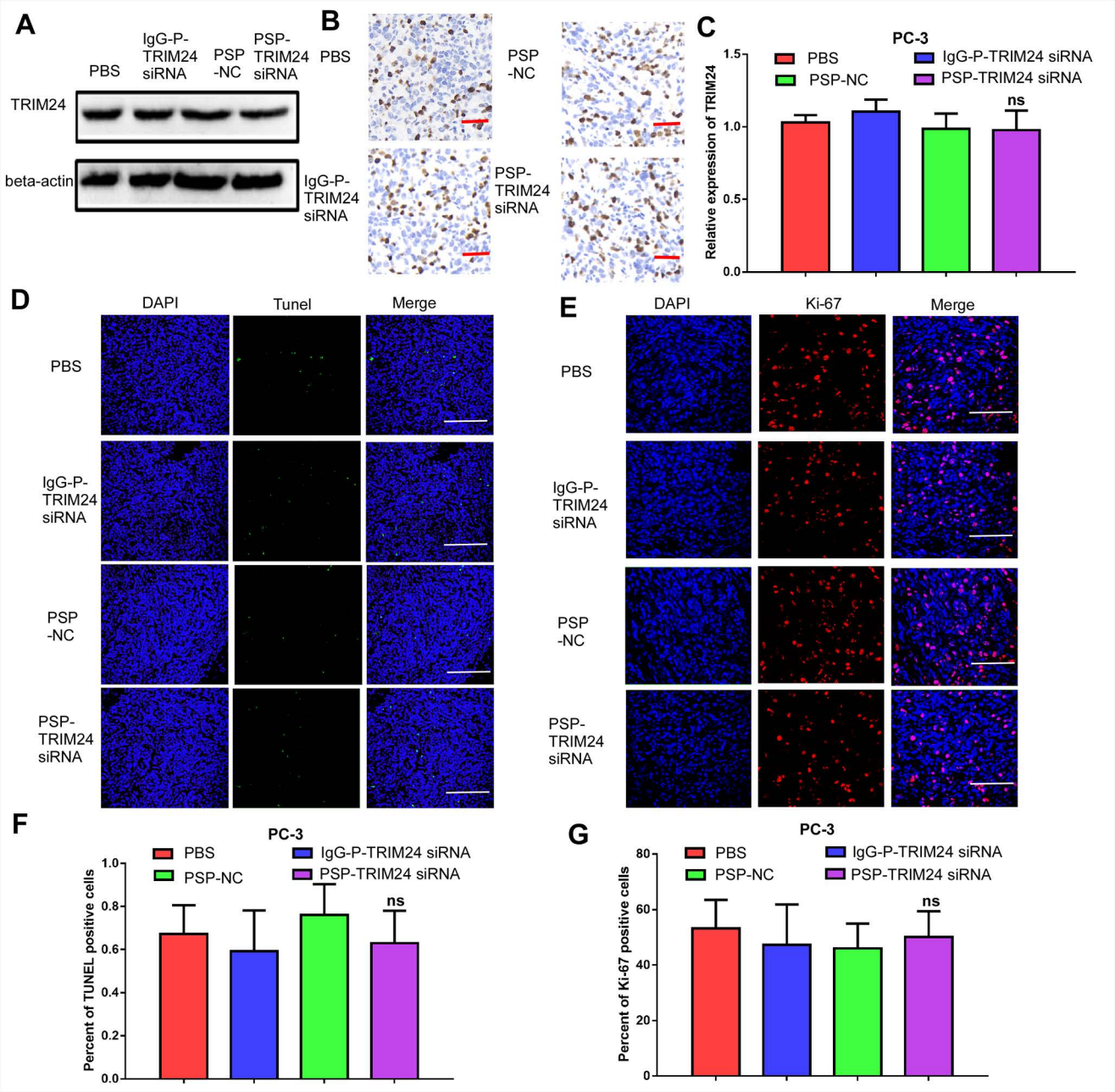
0.3
0.2
0.1
0.0
ns
E
PBS
PSP-NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA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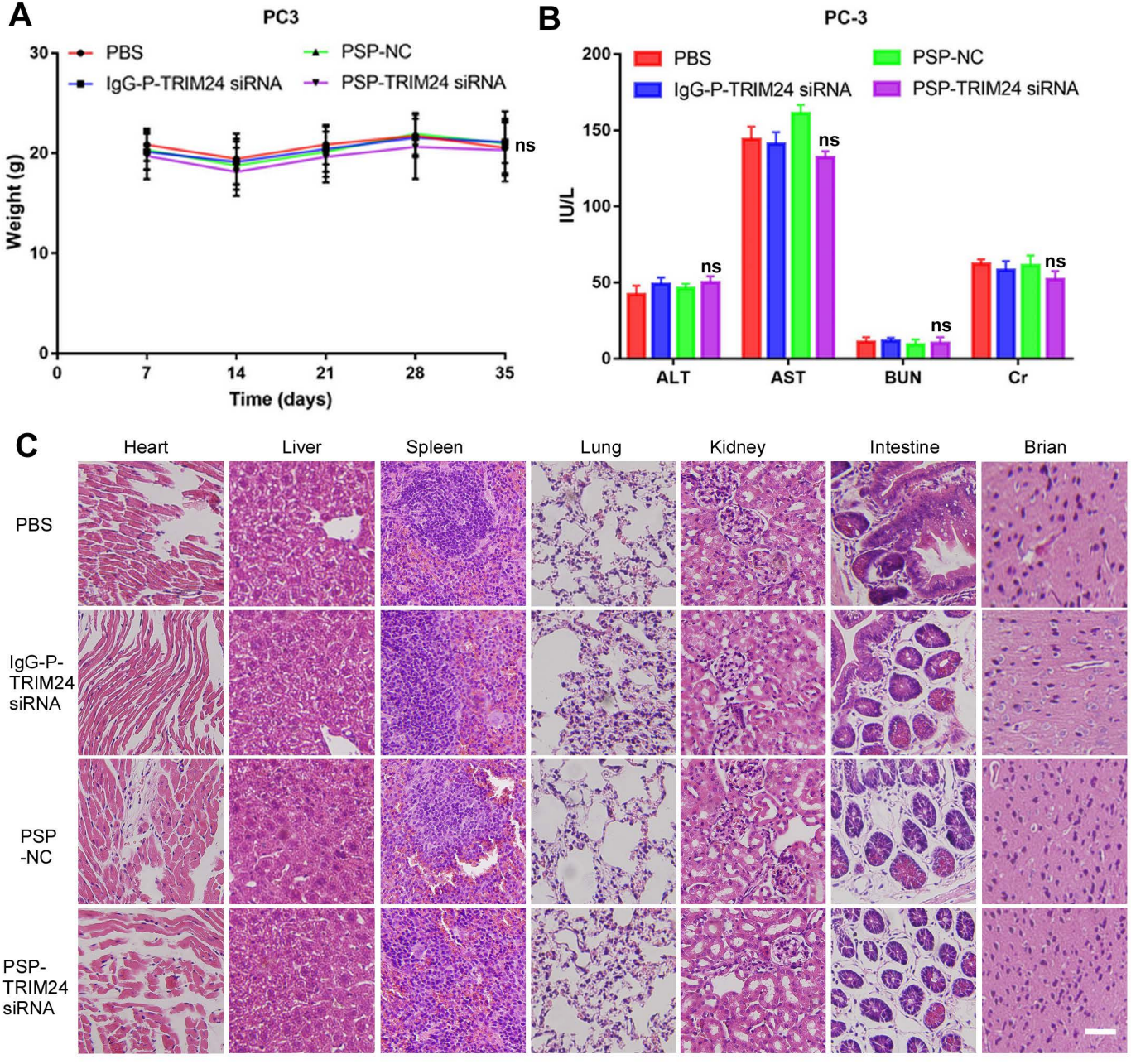
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
TRIM24
beta-actin
B
PBS
PSP-NC
IgG-P-TRIM24 siRNA
PSP-TRIM24 siRNA
C
PC-3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Relative expression of TRIM24
ns
D
DAPI
Tunel
Merge
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
E
DAPI
Ki-67
Merge
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
F
PC-3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Percent of TUNEL positive cells
ns
G
PC-3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Percent of Ki-67 positive cells
ns


A
PC3
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA
Weight (g)
Time (days)
ns
B
PC-3
IU/L
ALT
AST
BUN
Cr
C
Heart
Liver
Spleen
Lung
Kidney
Intestine
Brian
PBS
IgG-P-TRIM24 siRNA
PSP-NC
PSP-TRIM24 siRNA