Supplemental Figure 1 Generation of purified ERK3 protein kinase. Myc-tagged ERK3 protein kinase was expressed in 293T cells, purified using anti-Myc beads, and eluted off the beads using Myc peptide. The purity of protein samples was examined by Coomassie staining (A) and Western blotting (B). Protein lysate of H1299 cells were used as a positive control for the expression of ERK3 and ERK1/2 proteins.

Supplemental Figure 2 Knockdown of SRC-3 and ERK3 inhibits lung cancer cell invasion. H441 **(A)** and H520 **(B)** cells were stably transduced with lentiviral non-targeting control shRNA (shCtrl), shSRC-3, shERK3, or both shSRC-3 and shERK3. Cell invasion was determined by transwell matrigel cell invasion assay. Depletion of SRC-3 or ERK3 inhibits the invasion of both H441 **(A)** and H520 **(B)** cells. Values are means ± s.e of four separate experiments. "*" indicates significant difference (Student's *t* test).

Supplemental Figure 3 ERK3 promotes H520 cell invasion in a SRC-3 dependent manner and S857 phosphorylation of SRC-3 is critical in promoting cell invasion. **(A).** Transwell matrigel cell invasion assay of H520 cells transiently transfected with either Myc-ERK3 or the empty vector, or together with either SRC-3 siRNA (siSRC-3) or non-targeting control siRNA (siCtrl). **(B).** Transwell matrigel cell invasion assay of H520 cells transiently transfected with either

lentiviral SRC-3Flag, SRC-3S857AFlag, or Myc-ERK3, or co-transfected with two of the constructs as indicated. Intact S857 is required for SRC-3 to promote cell invasion.

Supplemental Figure 4 Knockdown of SRC-3 or ERK3 does not change actin polymerization. Protein lysates were prepared from H1299 cells stably transduced with lentiviral non-targeting control shRNA (shCtrl), shSRC-3, or shERK3. Actin polymerization was measured at different time points and represented as relative fluorescence intensity normalized by protein content (cps/ g).

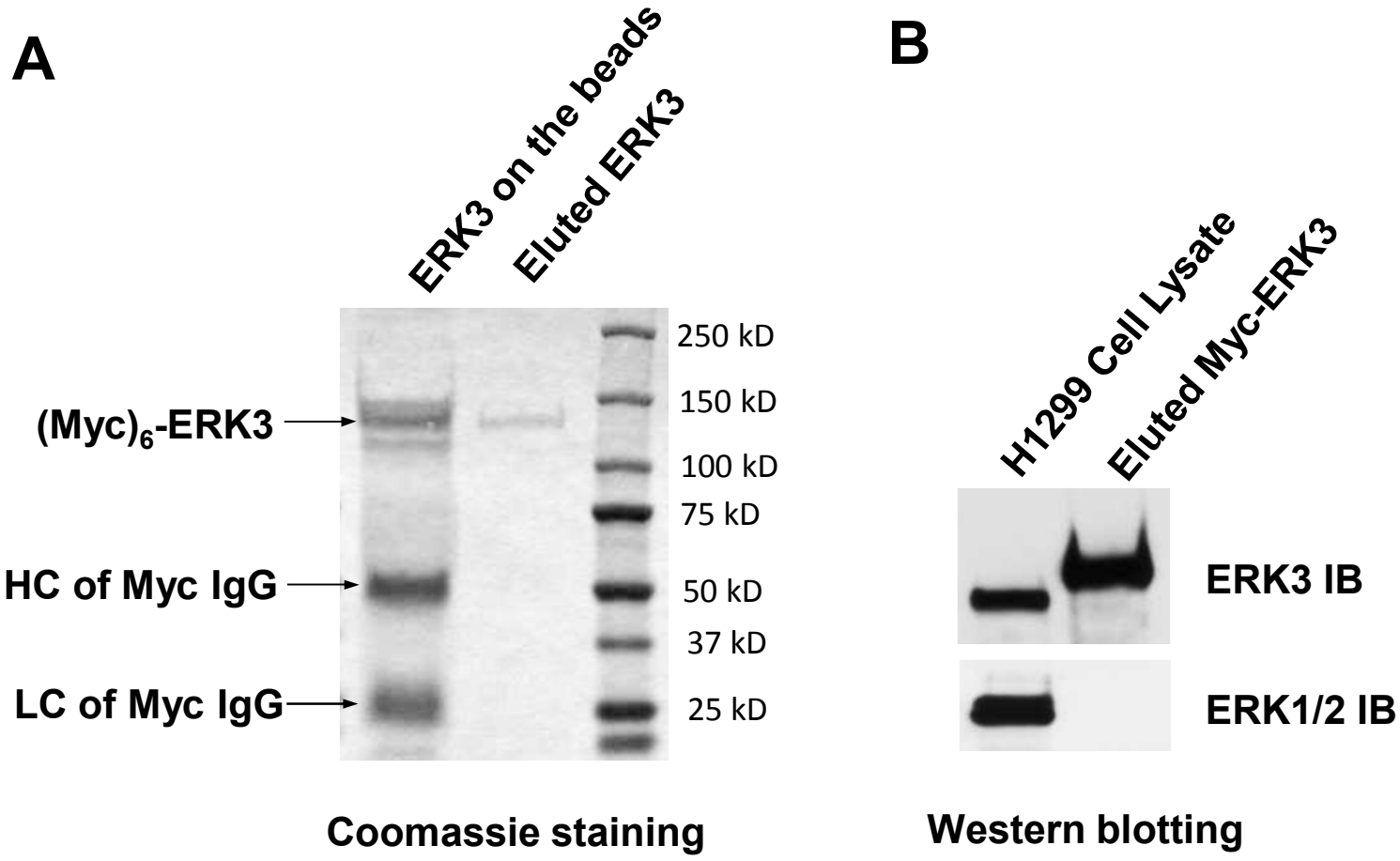
Supplemental Figure 5 ERK3 and SRC-3 cooperatively regulate MMP gene expression. **(A, B, and C)**. Depletion of SRC-3 or ERK3 significantly decreased MMP2 **(A and C)** and MMP10 **(B and D)** gene expression. MMP gene expression in A549 (**A and B**) and H441 (**C and D**) cell pools stably expressing shSRC-3, shERK3, or shCtrl was determined by RT-qPCR analysis. **(E).** SRC-3 and ERK3 cooperatively promote MMP2 promoter-driven luciferase activity, in which S857 of SRC-3 plays an important role. H1299 cells were singly- or co-transfected with the constructs as indicated. Luciferase activity is represented as relative luciferase units (RLU) on the Y-axis. **(F)**. Knockdown of SRC-3 significantly decreased ERK3-induced MMP2 promoter-driven luciferase activity. H1299 cells were transfected with either Myc-ERK3 or the empty vector, or together with either SRC-3 siRNA (siSRC-3) or non-targeting control siRNA (siCtrl). Values represent the means ± s.e of three separate experiments. "*" indicates a significant difference (Student's *t* test).

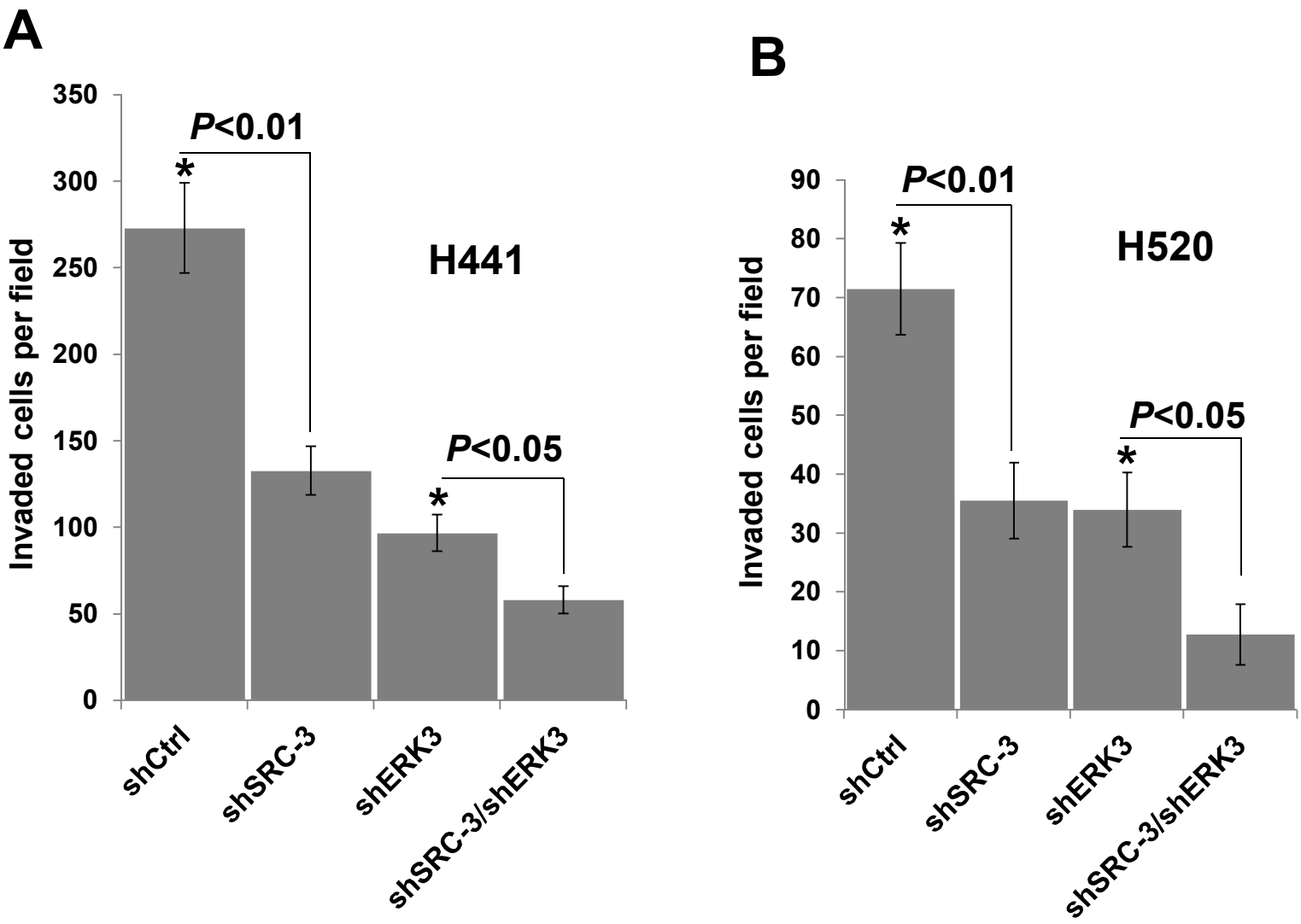
Supplemental Figure 6 ERK3 and SRC-3 regulate the activities of secreted MMP2 and MMP10. **(A and B)**. Depletion of SRC-3 or ERK3 significantly decreased the activities of secreted MMP2 **(A)** and MMP10 **(B)**. The activities of secreted MMP2 and MMP10 from H1299 cell pools stably expressing shSRC-3, shERK3, or shCtrl was determined using the SensoLyte fluorimetric

MMP2 and MMP10 activity assay kits (AnaSpec). MMP activity is represented as relative fluorescence units on the Y-axis. **(C).** SRC-3 and ERK3 cooperatively promote MMP2 activity, in which S857 of SRC-3 plays an important role. A549 cells were singly- or co-transfected with the constructs as indicated. Values represent the means ± s.e of three separate experiments. "*" indicates a significant difference (Student's *t* test).

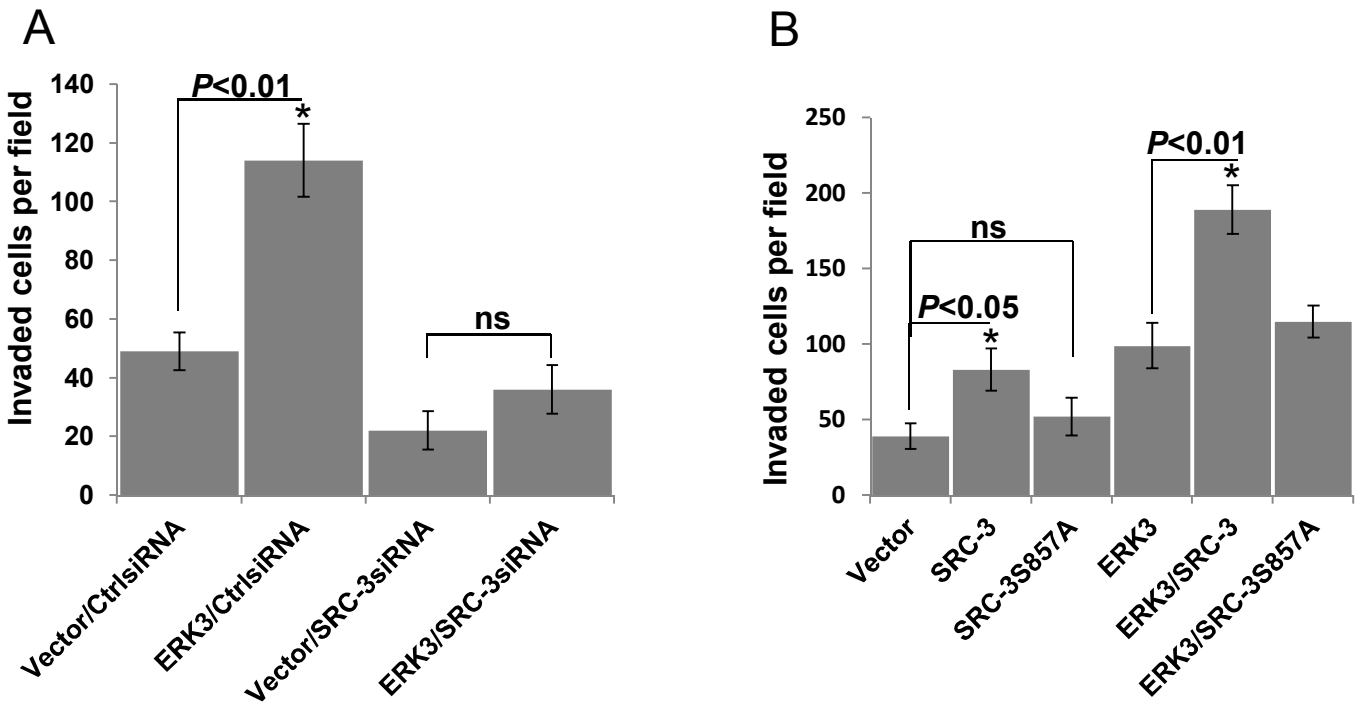
Supplemental Figure 7 Both SRC-3 and ERK3 are required for the invasiveness of H1299 lung cancer cells in vivo. 1 x 10^6 of H1299 cells with stable expression of the control shRNA (shCtrl), SRC-3 shRNA (shSRC-3), ERK3 shRNA (shERK3), or both shSRC3 and shERK3 were injected into SCID/Beige mice via tail vein. Tumor formation in the lungs was analyzed by hematoxylin/eosin (H/E) staining of paraffin-embedded tissue sections**.** Magnification: 50X.

Supplemental Figure 8 Representative images showing different levels of ERK3 immunostaining (red arrows) in squamous cell lung carcinomas (*A*-*C*) and lung adenocarcinomas (*D*-*F*). Magnification 200x.

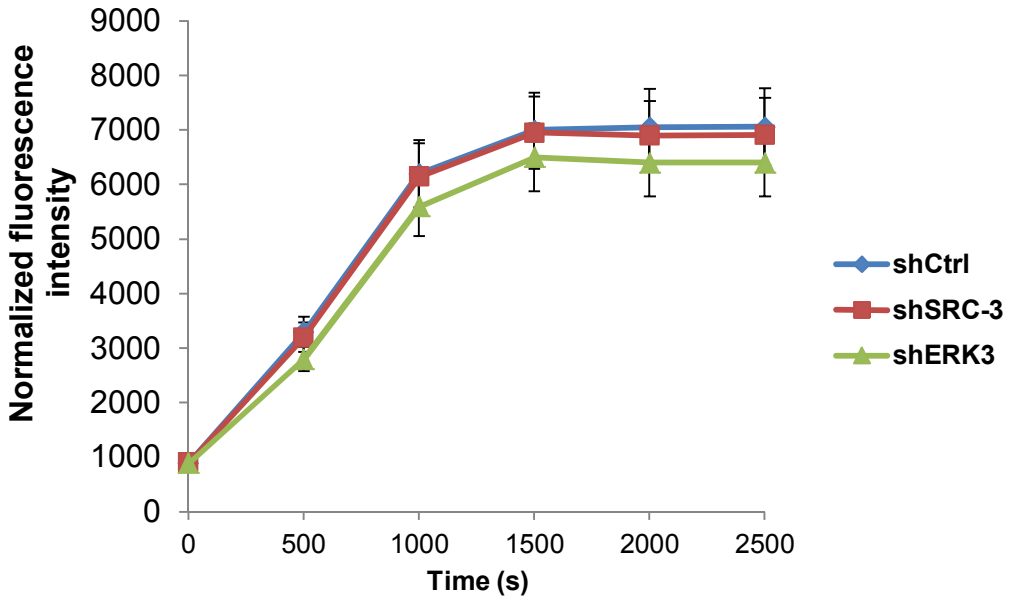

A
ERK3 on the beads
Eluted ERK3
(Myc)6-ERK3
HC of Myc IgG
LC of Myc IgG
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
Coomassie staining
B
H1299 Cell Lysate
Eluted Myc-ERK3
ERK3 IB
ERK1/2 IB
Western blotting


A
B
H441
H520
Invaded cells per field
P<0.01
P<0.05
*
shCtrl
shSRC-3
shERK3
shSRC-3/shERK3
0
50
100
150
200
250
300
350
10
20
30
40
60
70
80
90

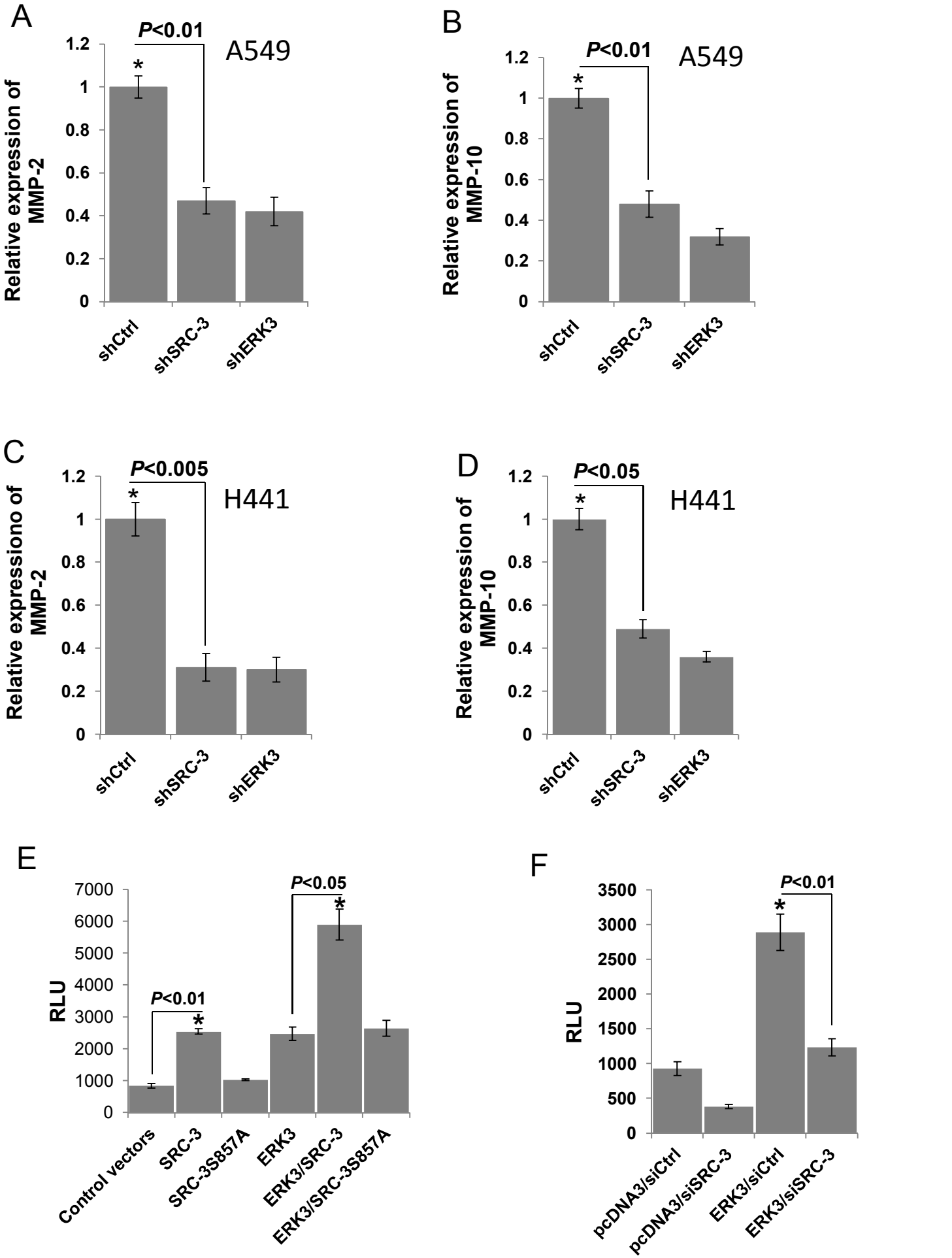
Supplemental Figure 3



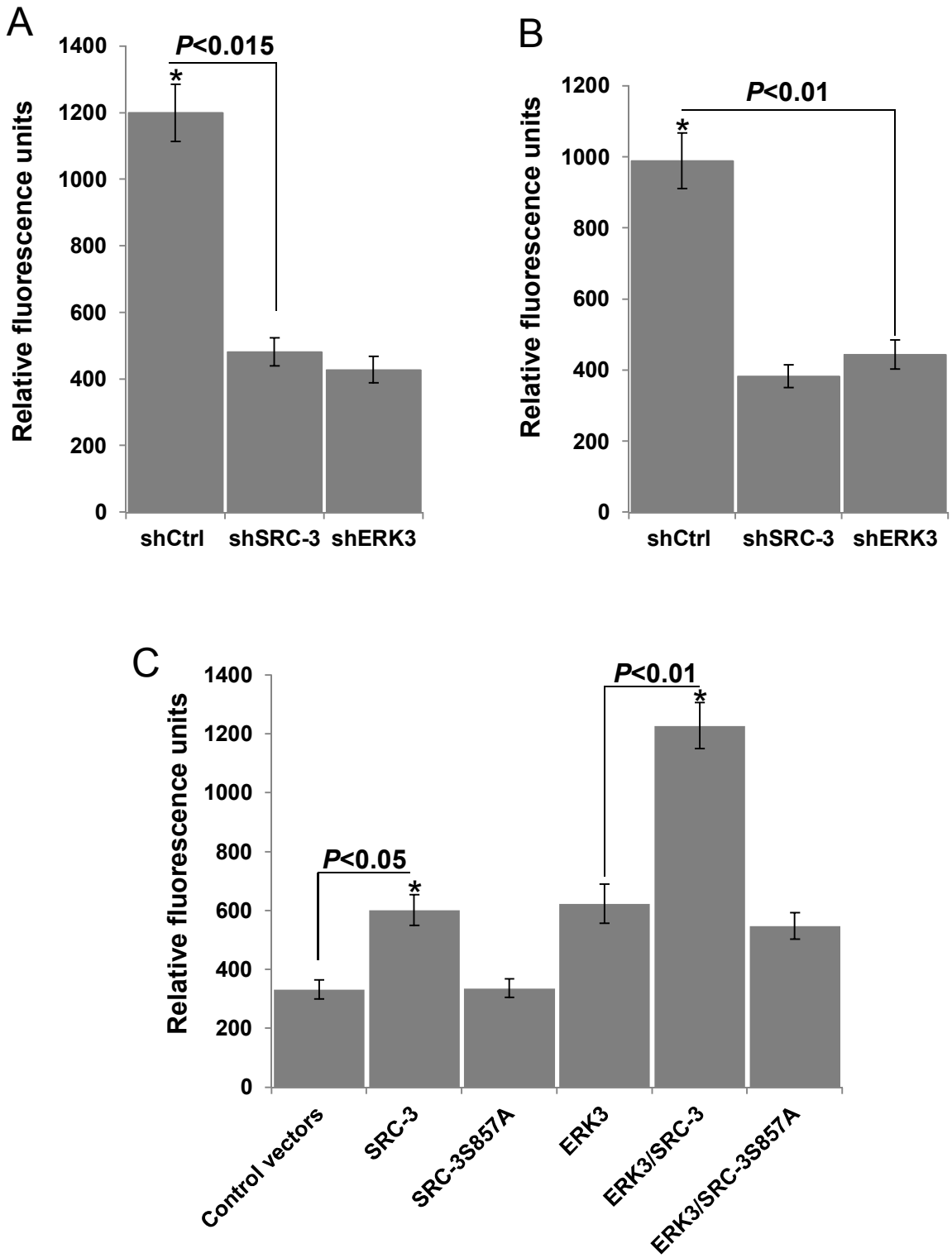
Supplemental Figure 4



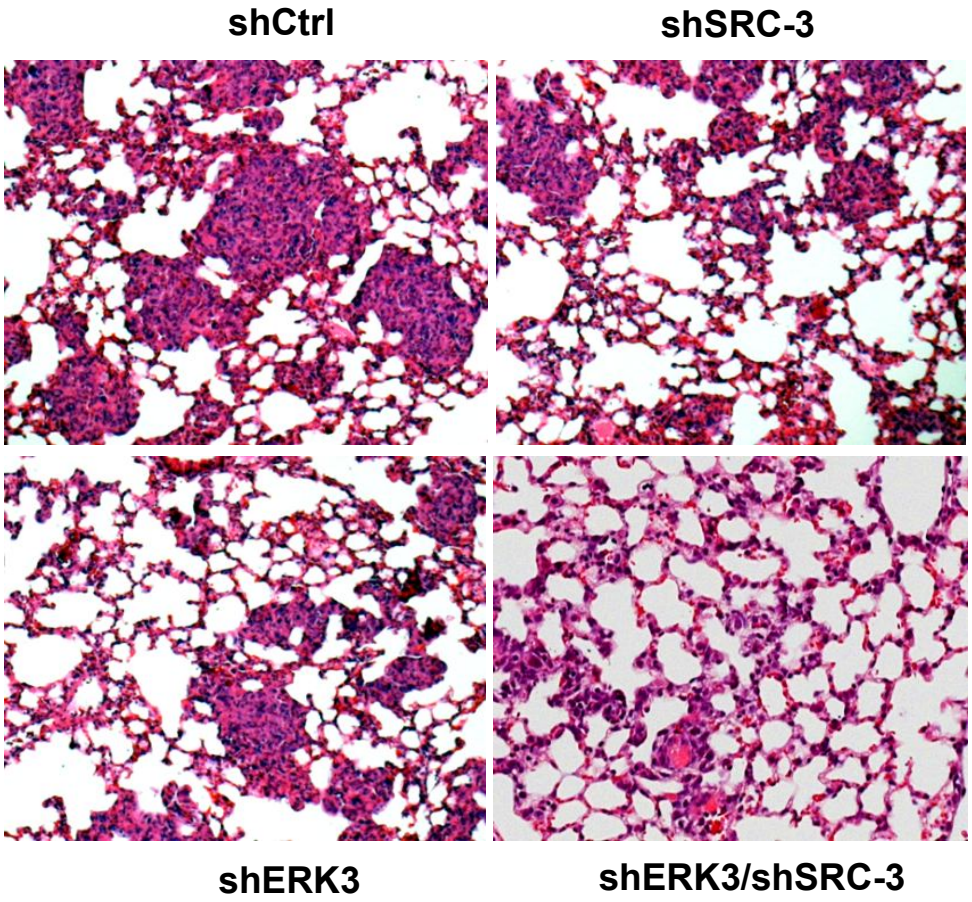
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8