Clinical & Experimental Metastasis

Integrin α3β1 regulates tumor cell responses to stromal cells and can function to suppress prostate cancer metastatic colonization

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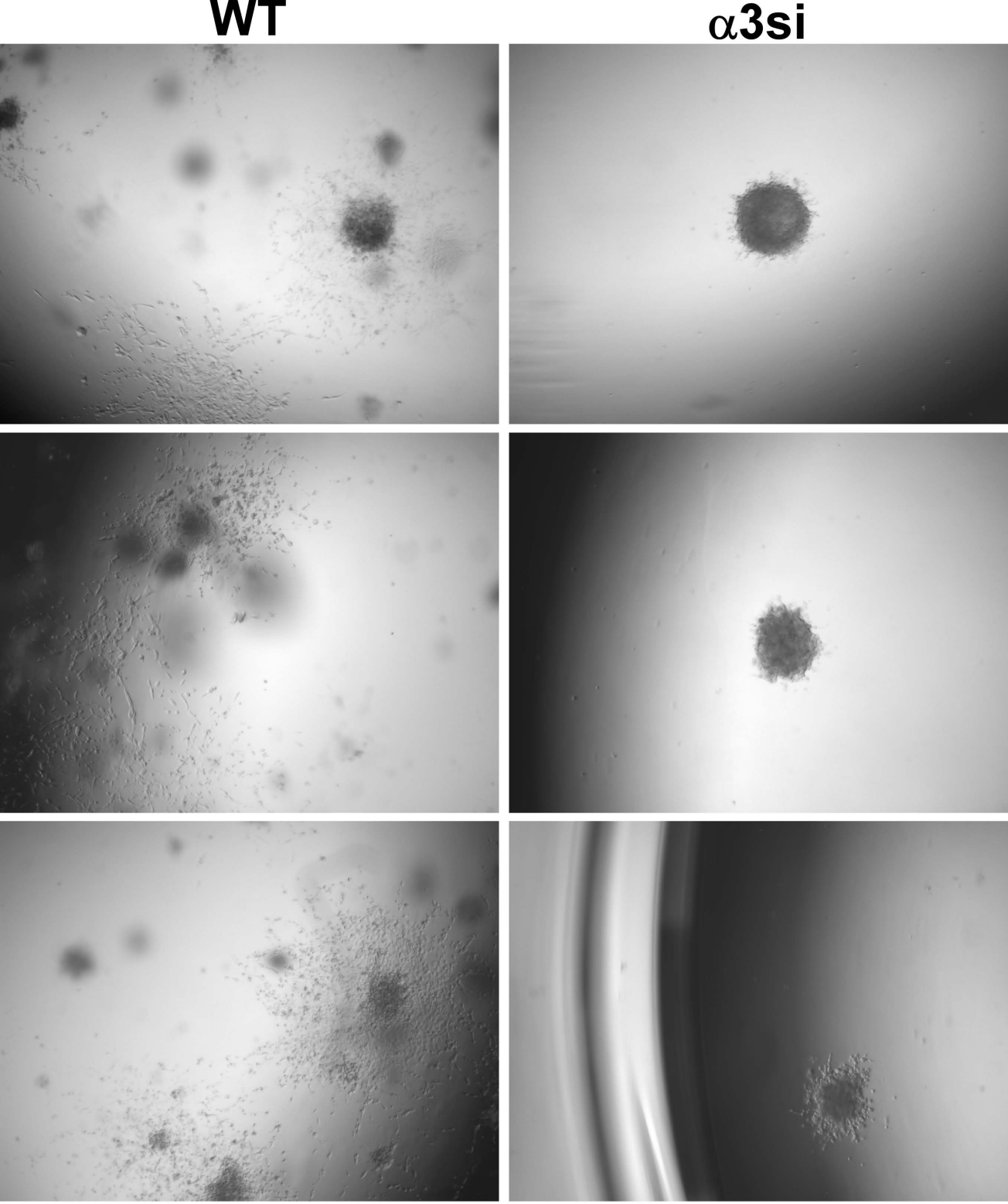
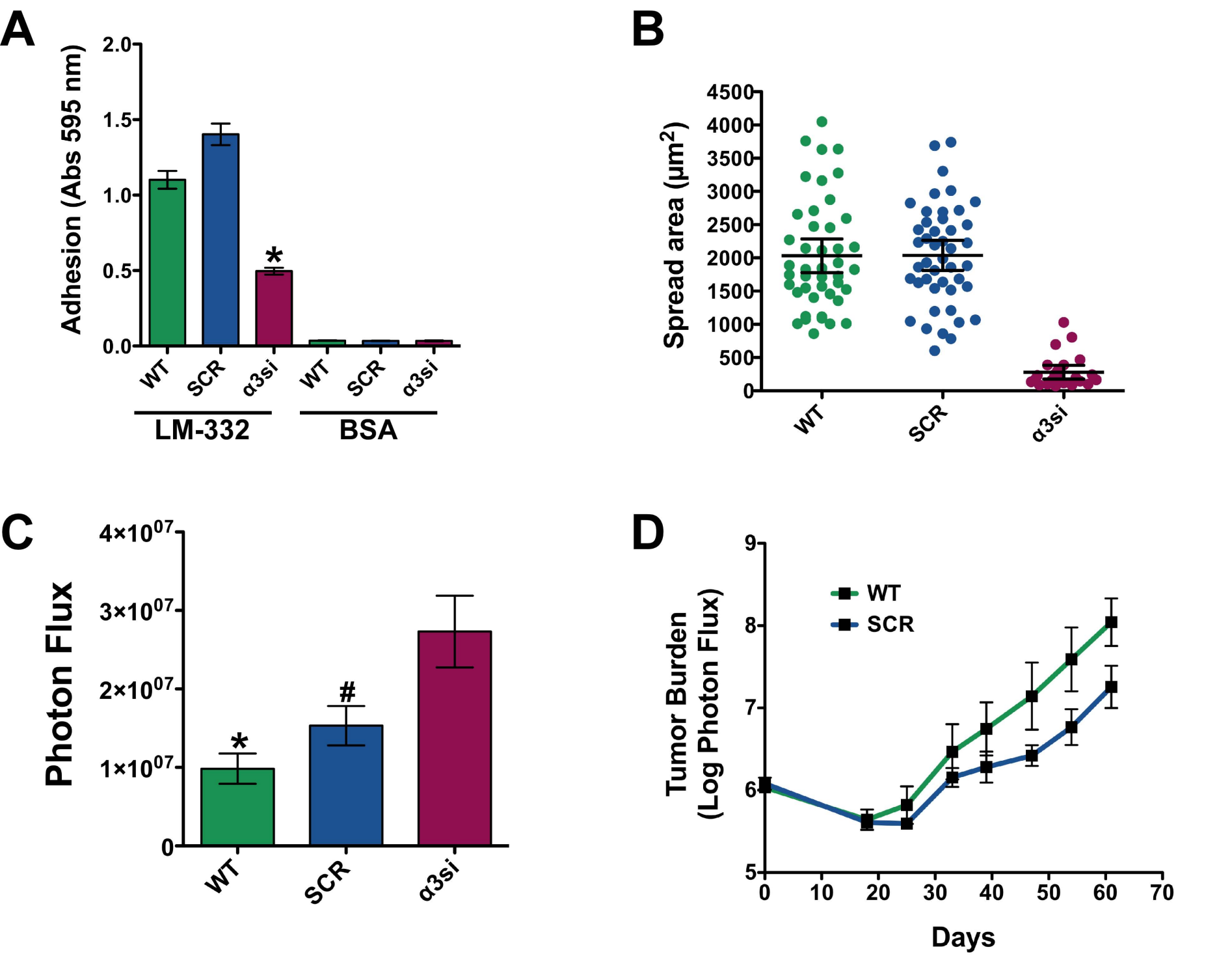
Supplementary Fig. 1 Severely impaired growth of α3-silenced cells in 3D Matrigel. 3000 wild type or α3-silenced cells per well were embedded in growth factor reduced 3D Matrigel and overlaid with serum-free medium. After 3 weeks, wells were photographed using a 4X objective, and colony formation was assessed by counting the number of colonies per field in 3 random fields from each of two wells per cell type. The wild type cells formed 10-fold more colonies per field than the α3-silenced cells ($p < 0.0001$, unpaired t test).

Supplementary Fig. 2 The RNAi silencing vector does not influence α3 integrin function and has minimal impact on tumor cell growth in vitro or in vivo. **(A)** GS689.Li wild type parental cells (WT), cells harboring a scrambled control shRNA (SCR), and α3 integrin-silenced cells (α3si) were plated in wells coated with LM-332 or BSA for an adhesion assay as in Fig. 1B. The SCR cells adhered to LM-332 just as well as the wild type cells, while the α3si cells showed significantly impaired adhesion compared to both WT and SCR cells (*$p<0.001$, ANOVA with Tukey post-test). **(B)** GS689.LI WT, SCR, and α3si cells were plated on LM-332-coated coverslips for a cell spreading assay as in Fig. 3. The α3si cell spread area was significantly less than the spread areas of the WT or SCR cells ($p<0.001$, ANOVA with Tukey post test). **(C)** GS689.LI WT, SCR, and α3si cells were plated on 3D collagen with embedded MRC-5 fibroblasts, as in Fig. 5, and tumor cell growth was monitored by bioluminescence (BLI). The α3si cell growth was significantly higher than both WT and SCR cells (*$p<0.01$ and $^{\#}p<0.05$, ANOVA with Tukey post test). The growth of SCR cells appeared modestly enhanced compared to WT, but the difference between them was not statistically significant. **(D)** GS689.Li WT and SCR cells were injected by tail vein into SCID mice, and in vivo growth was monitored by BLI as in Figure 2. The growth of the SCR cells was not enhanced compared to WT, and if anything appeared modestly reduced at later time points (*$p=0.0779$, unpaired t test for the final time point).



Supplementary Figure 1


A
Adhesion (Abs 595 nm)
2.0
1.5
1.0
0.5
0.0
*
WT
SCR
α3si
WT
SCR
α3si
LM-332
BSA
B
Spread area (μm²)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
WT
SCR
α3si
C
Photon Flux
4×10⁰⁷
3×10⁰⁷
2×10⁰⁷
1×10⁰⁷
0
*
#
WT
SCR
α3si
D
Tumor Burden
(Log Photon Flux)
9
8
7
6
5
WT
SCR
0
10
20
30
40
50
60
70
Days