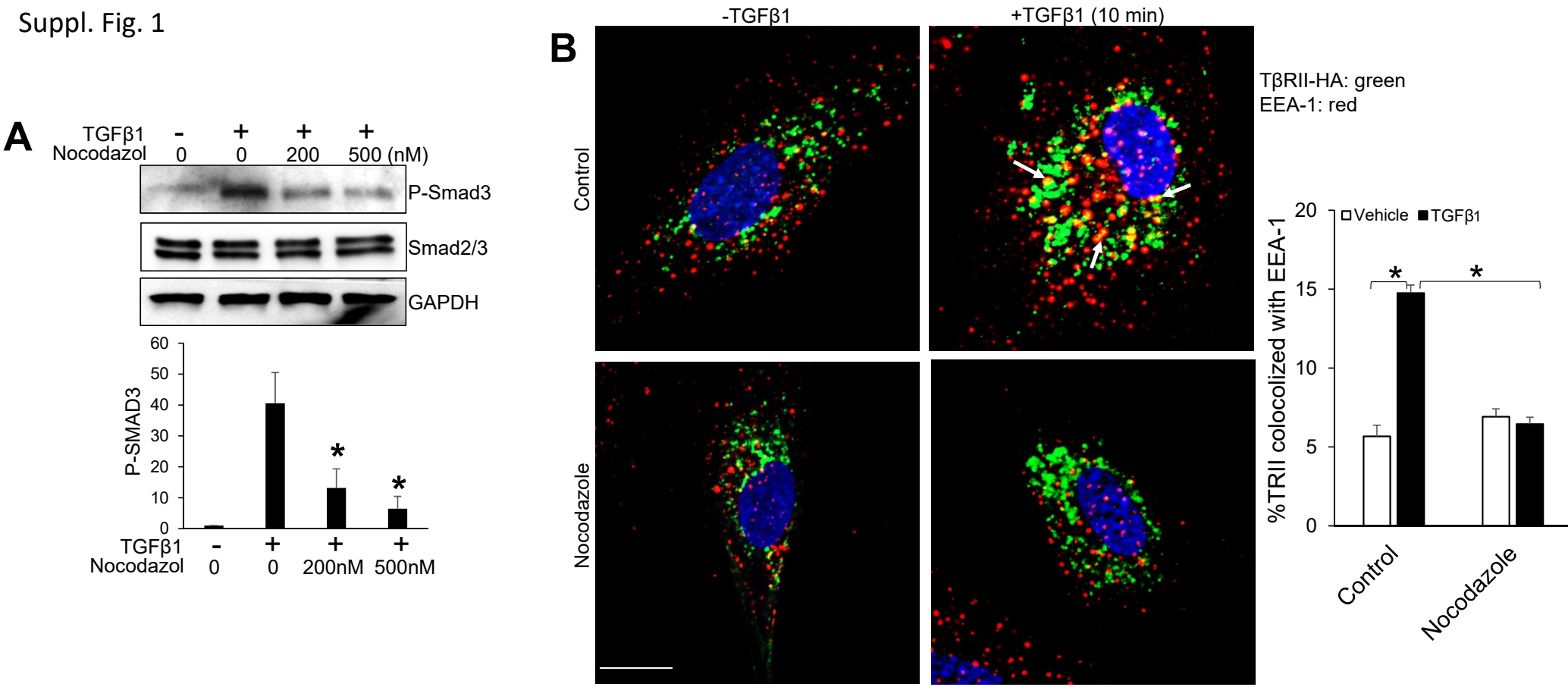
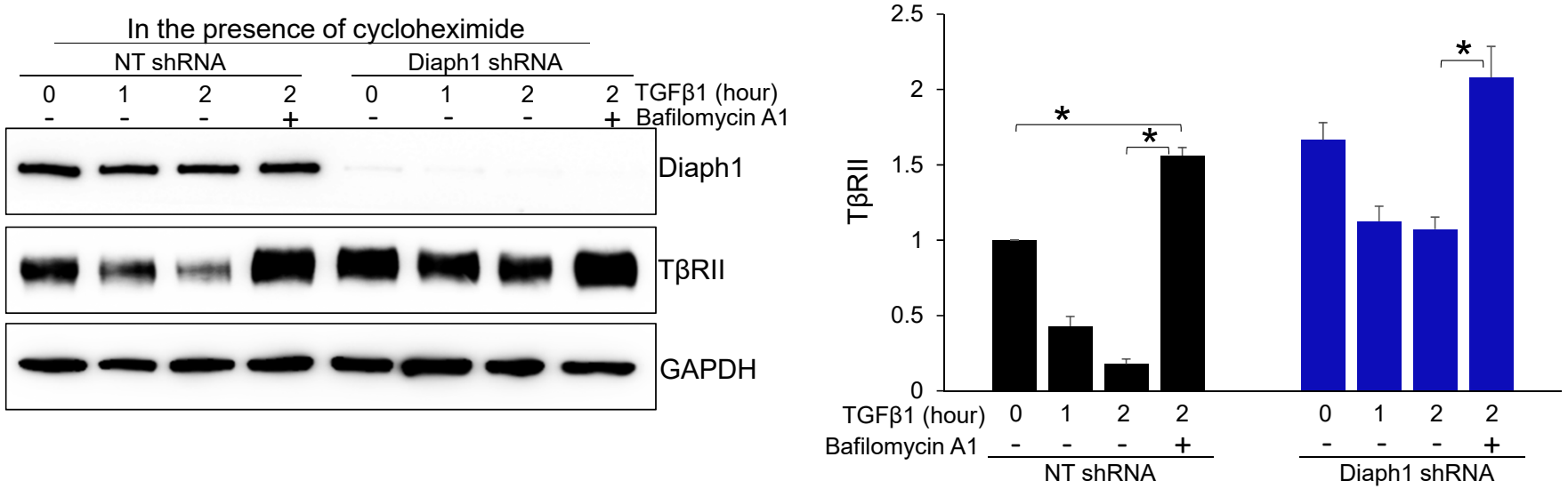
Suppl. Fig. 1



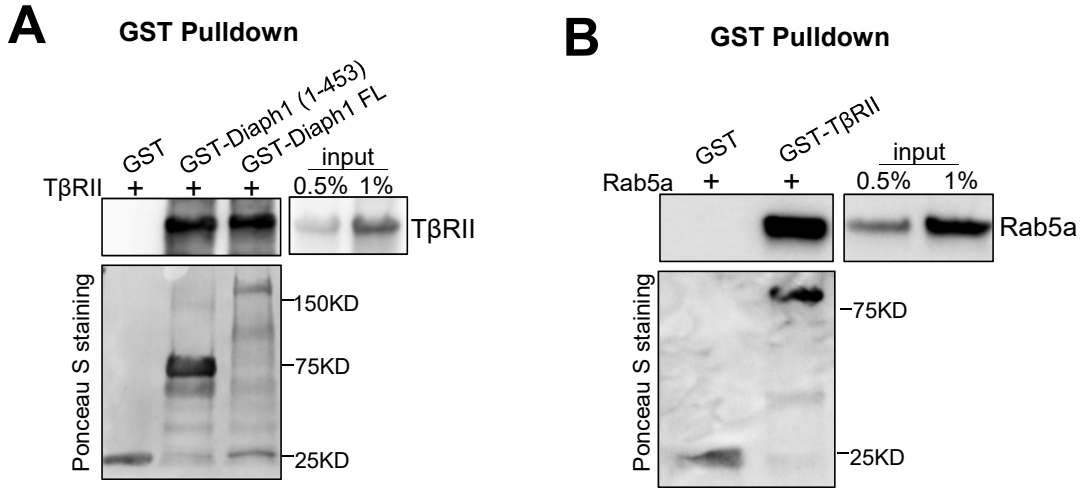
Suppl. Fig. 1. The phenotypes of Diaph1 knockdown HSCs are recapitulated by nocodozol treatment of HSCs. **A.** Serum-starved HSCs were stimulated with TGFβ1 in the presence or absence nocodazol and collected for Western blot. Nocodazol suppressed phosphorylation of SMAD3 induced by TGFβ1. *, $P<0.05$ by ANOVA, n=3 repeats. **B.** HSCs expressing TβRII-HA by retroviral transduction were pretreated with cycloheximide (40 μg/ml) and nocodazole (500 nM) followed by incubation with TGFβ1 for 10 minutes. Double IF for HA (green) and EEA-1(red) revealed that TGFβ1 increased colocalization of these 2 proteins (yellow) and this effect of TGFβ1 was inhibited by nocodazol. Quantitative data were shown on the right. *, $P<0.05$ by ANOVA, n=10 cells per group. Bar, 20 μm.

Suppl. Fig. 2



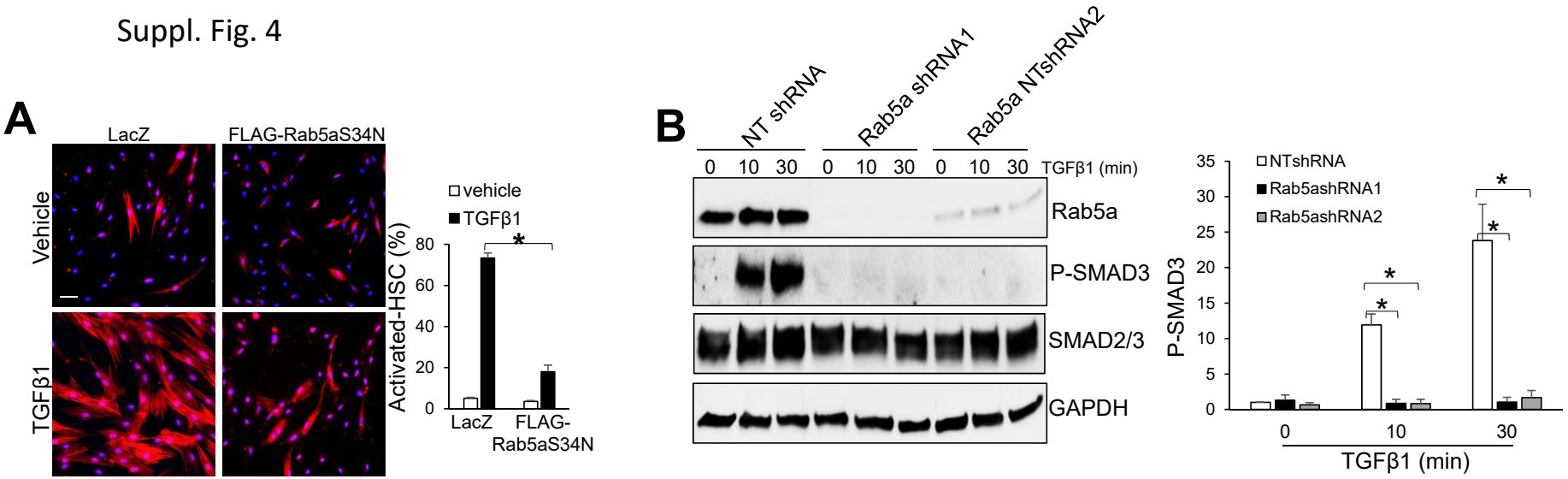
Suppl. Fig. 2. Bafilomycin A1 blocked TGFβ1-induced degradation of TβRII. HSCs preincubated with cycloheximide were stimulated with TGFβ1 for different times and collected for Western blot to quantitate TβRII. TβRII was downregulated much faster in control cells than in Diaph1 knockdown HSCs, which was prevented by Bafilomycin A1. *, $P<0.05$ by ANOVA, n = 3 repeats.

Suppl. Fig. 3



Suppl. Fig. 3. TβRII binds to Diaph1 and Rab5a directly in vitro. **A.** GST fusion proteins were purified from bacteria and used for GST pulldown assay, and Diaph1-bound TβRII was quantitated by WB. TβRII bound to full-length Diaph1 and Diaph1 (1-453). GST fusion proteins used for pulldown were shown by Ponceau S staining. **B.** GST pulldown assay showed that Rab5a and TβRII bound in vitro. GST fusion proteins were shown by Ponceau S staining.

Suppl. Fig. 4



Suppl. Fig. 4. Inactivation of Rab5a suppresses TGFβ-mediated activation of HSCs into myofibroblasts. **A.** HSCs transduced with retroviruses encoding LacZ (control) or FLAG-Rab5aS34N were serum-starved and stimulated with TGFβ1 for 24 hours. Cells were subjected to IF for α-SMA (red). FLAG-Rab5aS34N suppressed the formation of stress fibers in HSCs induced by TGFβ1. Bar, 50 μm. *, $P<0.05$ by ANOVA, n=6 randomly picked microscopic fields, each containing 100-200 cells. **B.** HSCs expressing NT shRNA (control) or Rab5a shRNA were stimulated with TGFβ1 for indicated times and cells were collected for WB for p-SMAD3. Rab5a shRNA suppressed TGFβ1-mediated phosphorylation of SMAD3 in HSCs. *, $P<0.05$ by ANOVA, n=3.

Suppl. Fig. 5

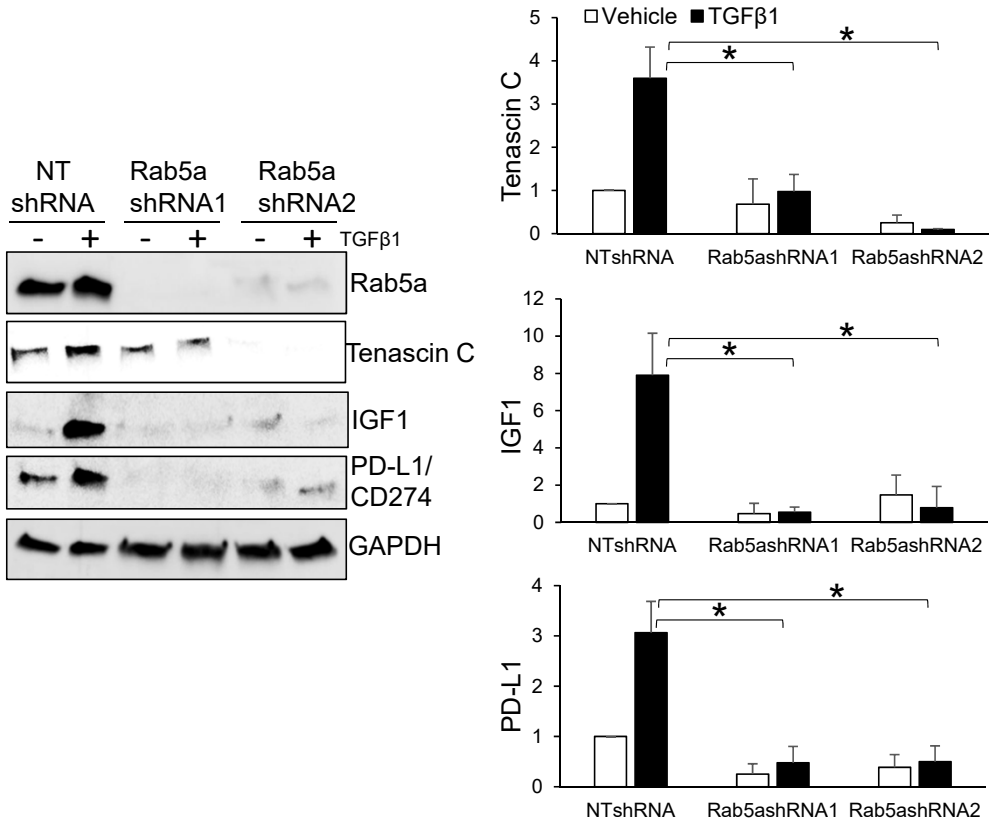
A

Gene expression value (Gene Expression Omnibus GSE127964)

Gene	DMSO 1	DMSO 2	DMSO 3	DMSO Mean	TGFβ1 1	TGFβ1 2	TGFβ1 3	TGFβ1 Mean	
TNC	7073	8967	7224	**7754.667**	46847	40340	33532	**40239.67**	*
IGF1	2	6	0	**2.666667**	314	181	173	**222.6667**	*
CTGF	6812	8962	7089	**7621**	283620	235415	181253	**233429.3**	*
CD274	74	82	75	**77**	329	291	199	**273**	*

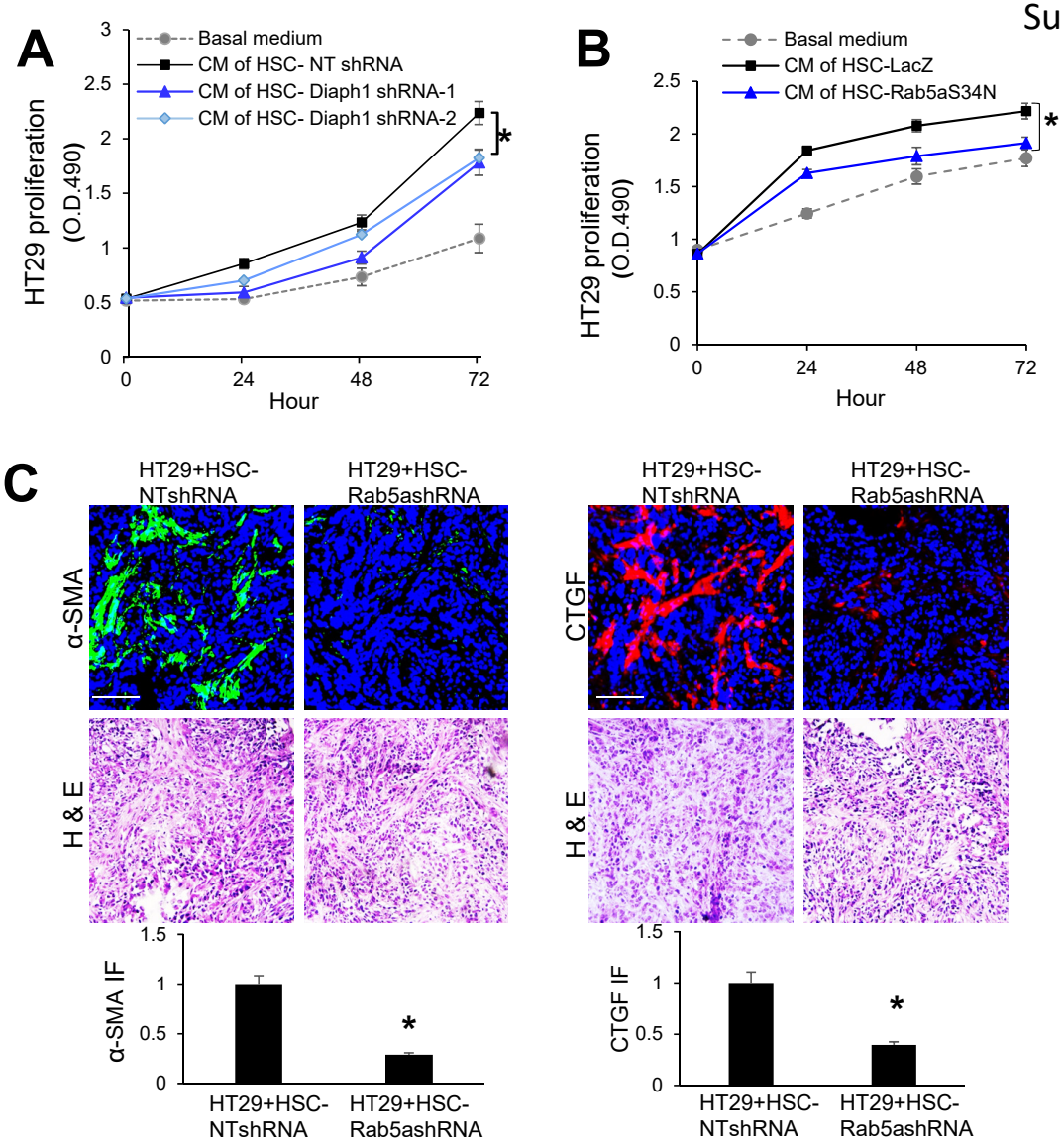
*, *P*<0.05 by ANOVA

B



Suppl. Fig. 5. Knockdown of Rab5a suppresses HSC-derived tumor-promoting factors. **A.** HSCs incubated with or without TGFβ1 for 24 hours were subjected to RNA sequencing and data were deposited to Gene Expression Omnibus (GSE127964). TNC, IGF1, CTGF, and CD274 genes were turned on for transcription by TGFβ1. **B.** HSCs expressing NT shRNA (control) or Rab5a shRNA were stimulated with TGFβ1 and collected for WB. TGFβ1 stimulated HSC to produce tenascin C, IGF1, and PD-L1 and this effect of TGFβ1 was abolished by Rab5a shRNA. *, *P*<0.05 by ANOVA, n=3 repeats.

Suppl. Fig. 6



Suppl. Fig. 6. Inactivation of Diaph1 or Rab5a suppresses the tumor-promoting effect of HSCs in vitro and in mice. **A.** and **B.** Conditioned media were collected from HSCs expressing NT shRNA, Diaph1 shRNA, LacZ, or Rab5aS34N mutant and their effects on HT29 proliferation were analyzed by a nonradioactive cell proliferation assay. Diaph1 shRNA (left) or Rab5aS34N mutant (right) reduced the effect of HSCs on HT29 proliferation. *, $P<0.05$ by ANOVA, n=5. **C.** IF was performed with tumors arising from HSC/HT29 coinjections. IF densities of α-SMA and CTGF were reduced in the tumors arising from HT29/HSC-Rab5ashRNA coinjections compared to the tumors arising from control coinjections. *, $P<0.05$ by *t*-test, n=4. Bar, 50 μm.