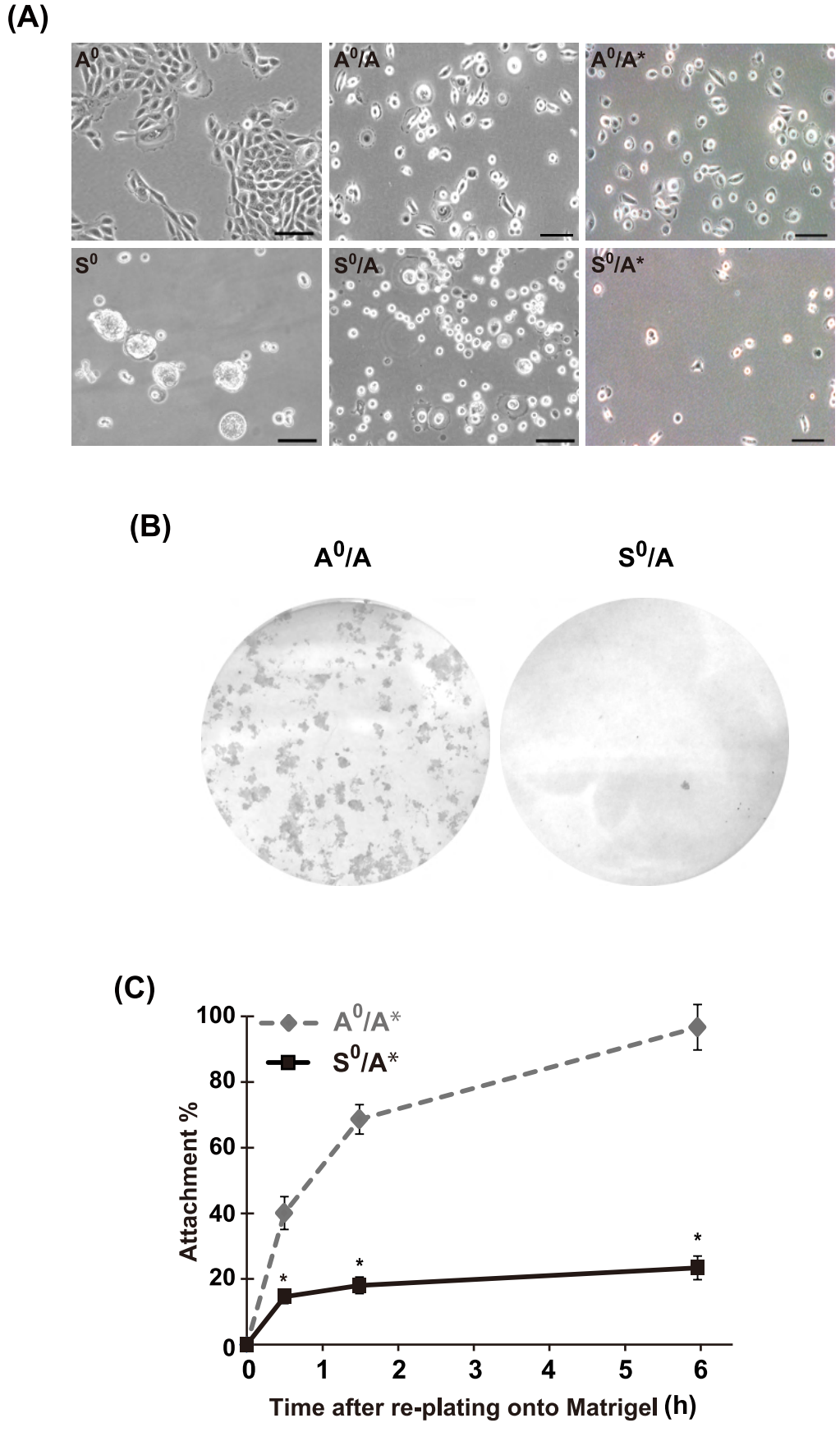

Fig. S1 Cell culture and colony formation under adherent and non-adherent conditions, as illustrated in Figure 1A. **A**, Representative images of HMECs under each culture condition. Scale bar, 100 μm. **B**, Colonies formed from HMECs preincubated under adherent (A^0/A) and non-adherent (S^0/A) conditions. **C**, Time course of reattachment of HMECs on Matrigel-coated plates after preincubation under adherent (A^0/A*) and non-adherent (S^0/A*) conditions. Attachment (%) was determined as in Figure 1D. Values represent mean ± S.D. from at least three independent experiments. *P < 0.01.

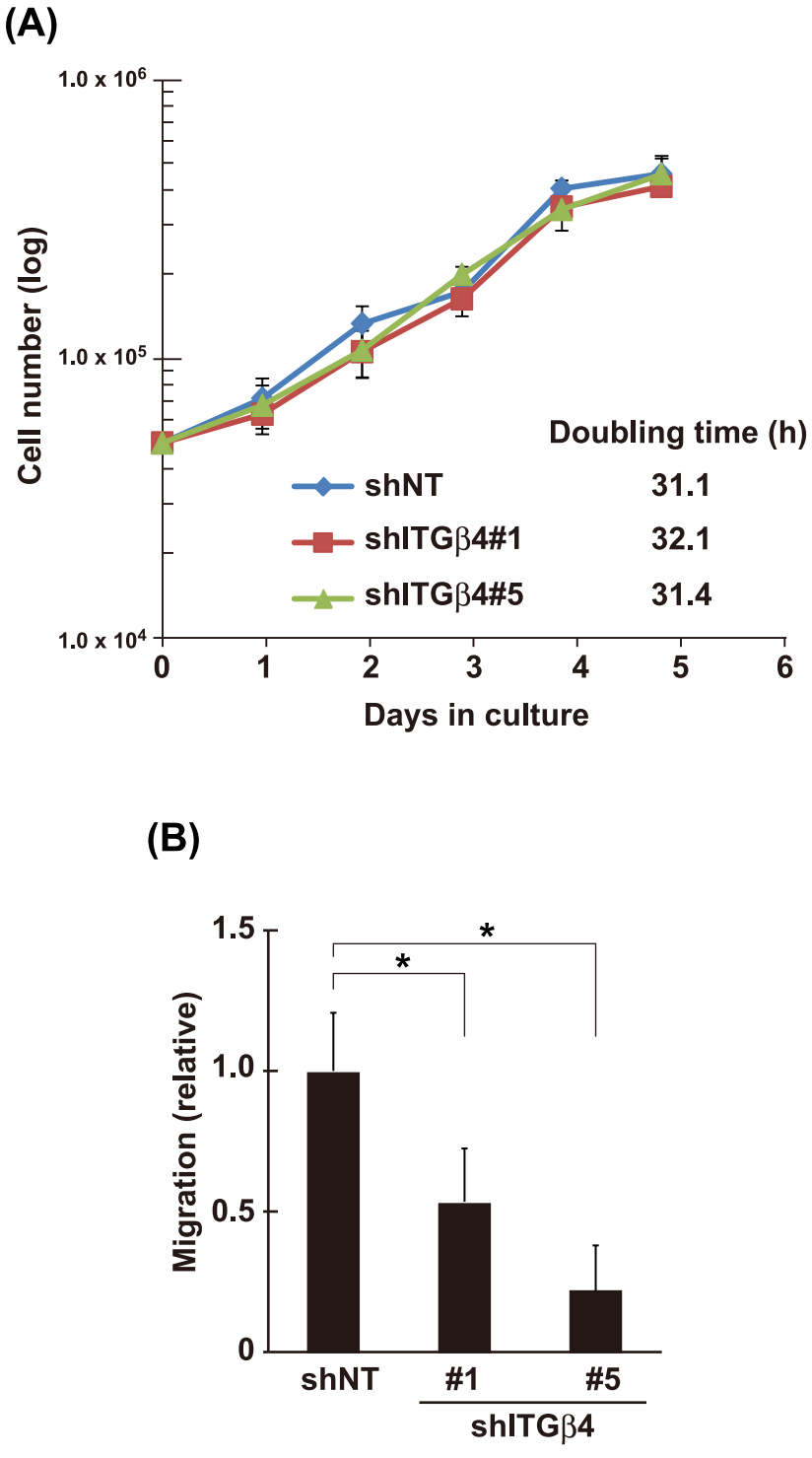

Fig. S2 Cell proliferation and migration of shRNA [shITGβ4#1, #5, and control (shNT)]-expressing MDA-MB-231 cells. **A**, A total of 5 × 10^4 cells were seeded, and viable cells were counted each day. Doubling time was calculated from the slope of the growth curve. **B**, Cell migration was assessed using transwell chambers as previously described (Mori K, *et al.*, FEBS. J. 2019; 286: 459-478). Values represent mean ± S.D. from at least three independent experiments. *$P < 0.01$.

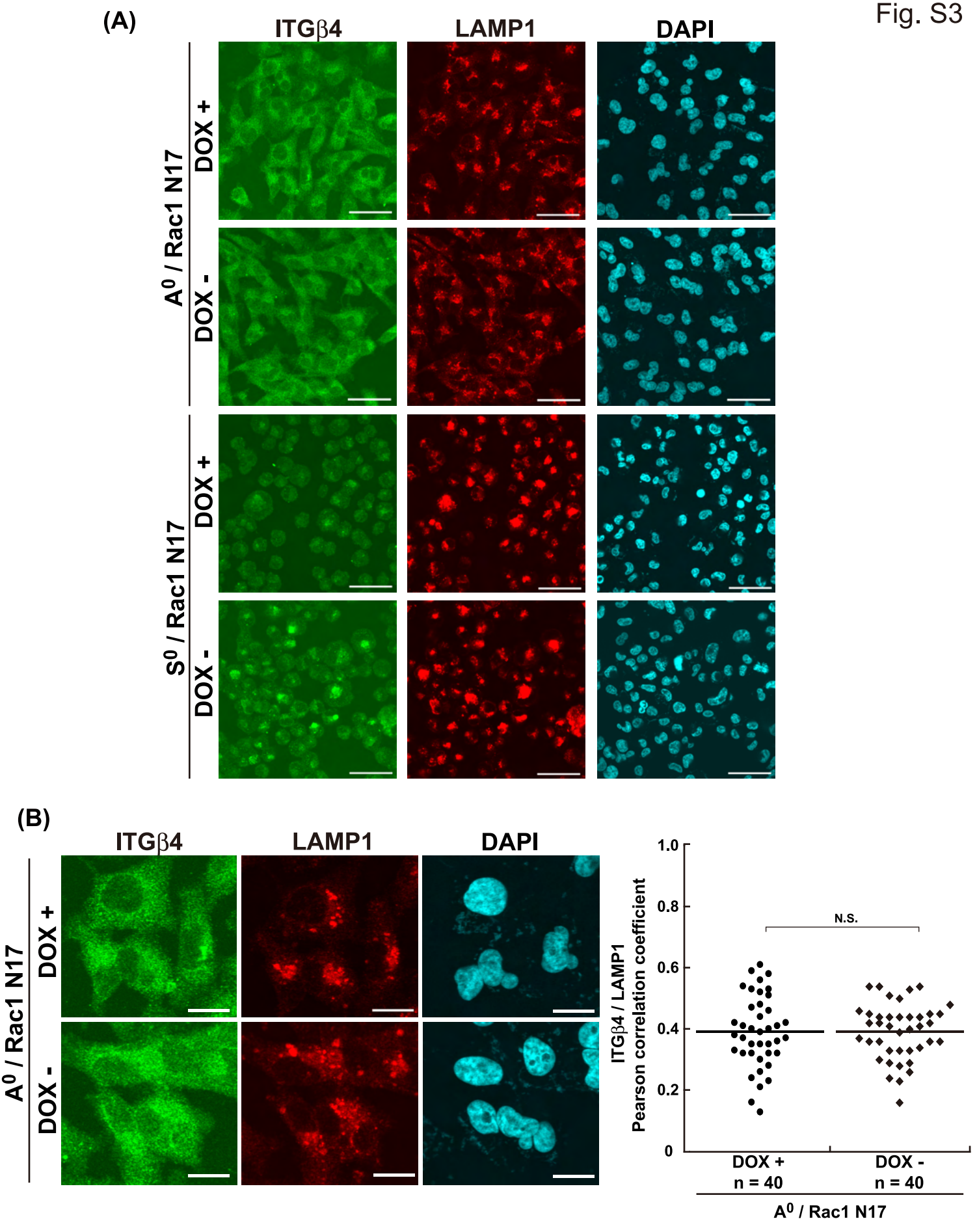

Fig. S3 Immunocytochemical analysis of MDA-MB-231 cells expressing Rac1 N17. Cells expressing Rac1 N17 incubated under DOX+ or DOX− as in Figure 5A were cultured under A^0 or S^0 and examined by immunocytochemistry as in Figure 5E. **A**, Representative images at low magnification are shown. Scale bar, 50 μm. **B**, High-magnification images of cells incubated under adherent (A^0) condition. Scale bar, 10 μm. Pearson' s correlation coefficient for colocalization values of β4 integrin with LAMP1 was calculated using Coloc 2 plugin and plotted. Horizontal lines indicate the means from the indicated number of cells. N.S., not significant.

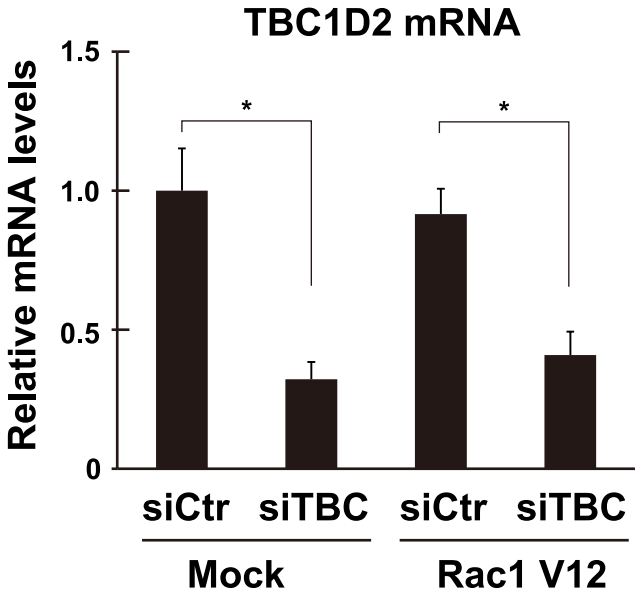

Fig. S4 Knockdown of TBC1D2 with siRNA. HMECs infected with lentiviral expression vectors (Mock; empty vector, Rac1 V12; Flag-tagged CA mutant of Rac1) were transfected with siRNA against TBC1D2 (siTBC) or control (siCtr). After 48 h, mRNA of TBC1D2 was quantified by qPCR. The values were normalized with TATA-box binding protein and shown as relative to the control (Mock/siCtr). *$P < 0.01$.