Figure S1

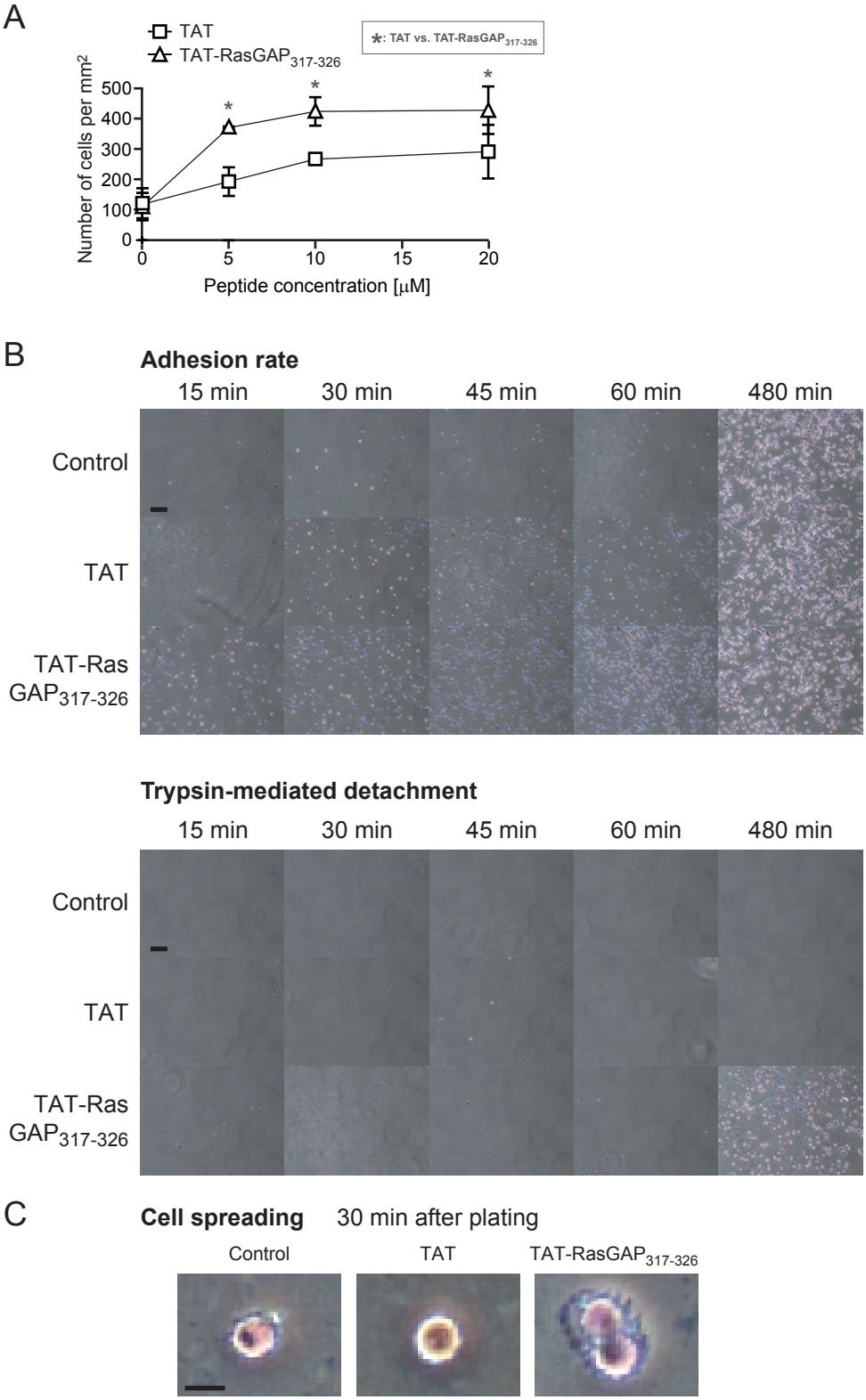


**Figure S1.** Kinetics of TAT-RasGAP$_{317-326}$-mediated adhesiveness increase.
**A**. U2OS cells were subjected to an adhesion assay for 30 minutes in presence of the indicated concentrations of TAT and TAT-RasGAP$_{317-326}$ (the statistical significance of the observed differences was assessed by one-way ANOVA).
**B**. Representative images of the adhesion assay performed in Figure 1E. Scale bar: 100 μm.
**C.** Higher magnification of cells 30 minutes after plating. Scale bar: 40 μm.

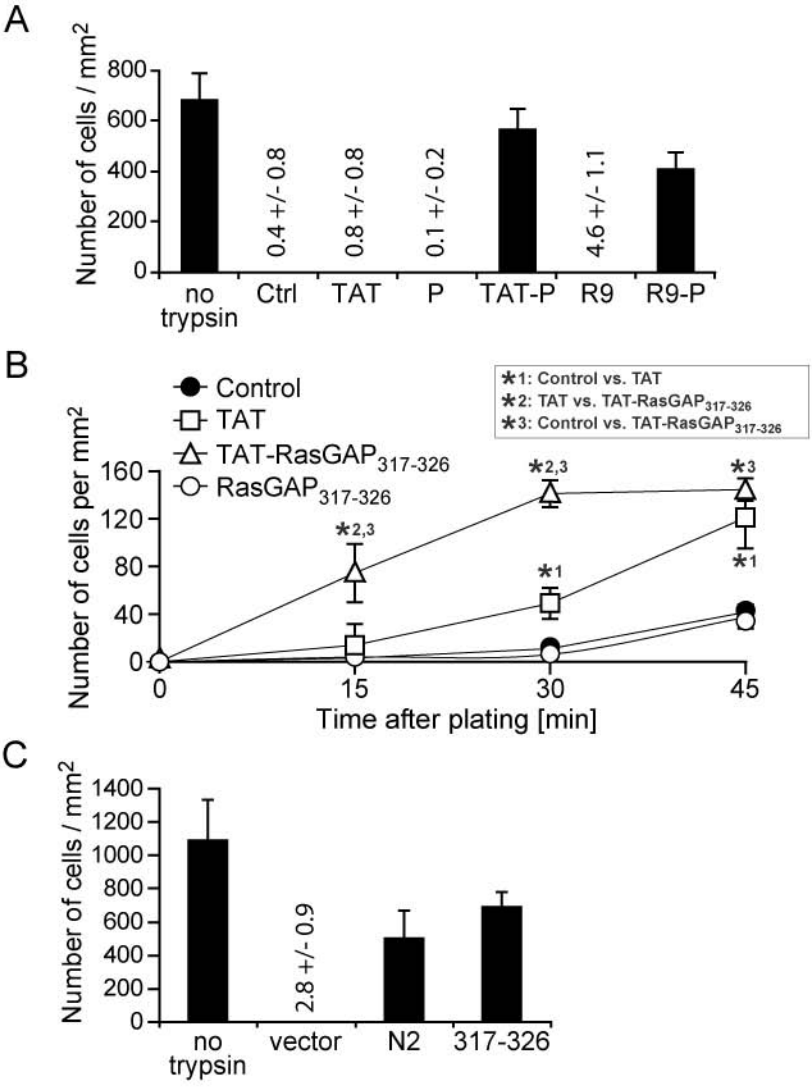


**Figure S2**. $RasGAP_{317-326}$ acts from inside cells.

**A**. U2OS cells were treated for 8 hours with 20 μM TAT, 20 μM $RasGAP_{317-326}$ (P), 20 μM TAT-$RasGAP_{317-326}$ (TAT-P), 20 μM R9, 20 μM R9-$RasGAP_{317-326}$ (R9-P) or left untreated (Ctrl). A trypsin/EDTA-mediated detachment assay was then performed. A control without trypsin was also performed.

**B.** An adhesion assay was performed to assess the adhesion rate of U2OS cells in the presence of a $RasGAP_{317-326}$ peptide lacking the TAT cell permeable peptide. Treatment of cells with 20 μM TAT-$RasGAP_{317-326}$ increased significantly the speed of adhesion compared to cells treated with 20 μM TAT, 20 μM $RasGAP_{317-326}$ or not treated. The statistical significance of the observed differences (asterisks) was assessed by one-way ANOVA.

**C.** HEK 293T cells were transfected with pcDNA3 (vector) or pcDNA3 encoding the HA-tagged forms of the N2 fragment of RasGAP (N2) or the 317-326 RasGAP sequence (317-326). A trypsin-mediated detachment assay was then performed but without tapping the plates. No trypsin, cells transfected with pcDNA3 that were not trypsinized.

Figure S3

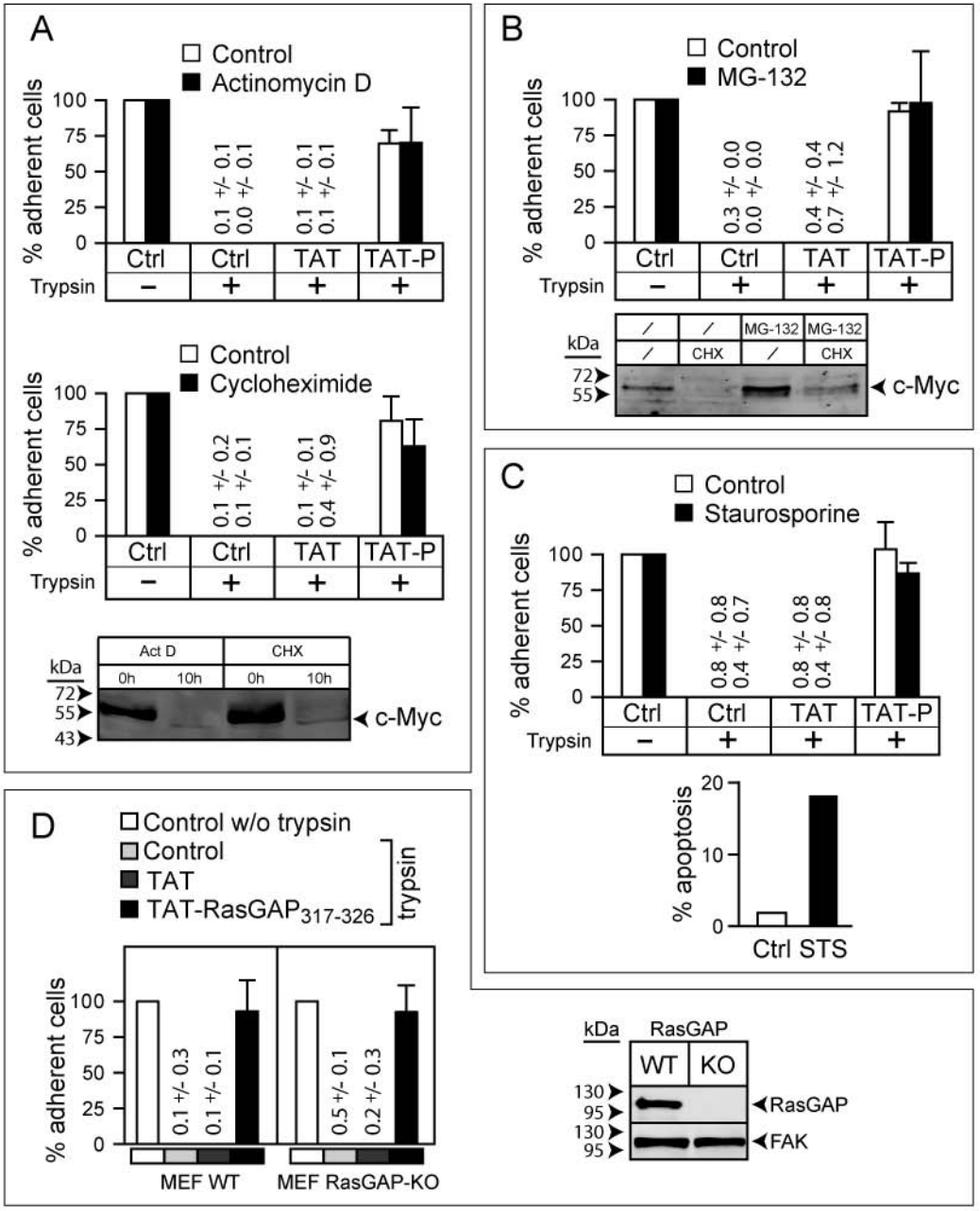


**Figure S3.** TAT-RasGAP$_{317-326}$ does not require transcription, translation, proteasome-mediated degradation, staurosporine-inhibitable kinases, and full-length RasGAP to increase adhesion.

**A-B-C.** U2OS cells were pre-incubated 2 hours with either 1 μg/ml actinomycin D (Act D) or 30 μg/ml cycloheximide (CHX) (panel A), with 10 μM MG-132 for 16 hours (panel B), with 10 nM staurosporin (STS) for 16 hours (panel C), then treated 8 hours, in the presence of the inhibitors, with 20 μM TAT (TAT), 20 μM TAT-RasGAP$_{317-326}$ (TAT-P) or left untreated (Ctrl). The cells were then subjected to a trypsin-mediated detachment assay. The lower panels in A and B depict c-Myc expression levels after Act D, CHX and MG-132 treatments (25 μg of loaded proteins). The activity of staurosporin was controlled by evaluating its capacity to induce apoptosis, determined by measuring the percentage of cells with pycnotic nuclei (panel C, lower graph).

**D.** Mouse embryonic fibroblasts (MEF) or RasGAP-knock-out MEF (KO) cells were treated for 8 hours with 20 μM TAT, 20 μM TAT-RasGAP$_{317-326}$ or left untreated. A trypsin-mediated detachment assay was performed as described for panel A-B. RasGAP expression was checked in wild-type and knock-out MEFs by immunoblotting (blot on the right-hand side of the panel).

Figure S4

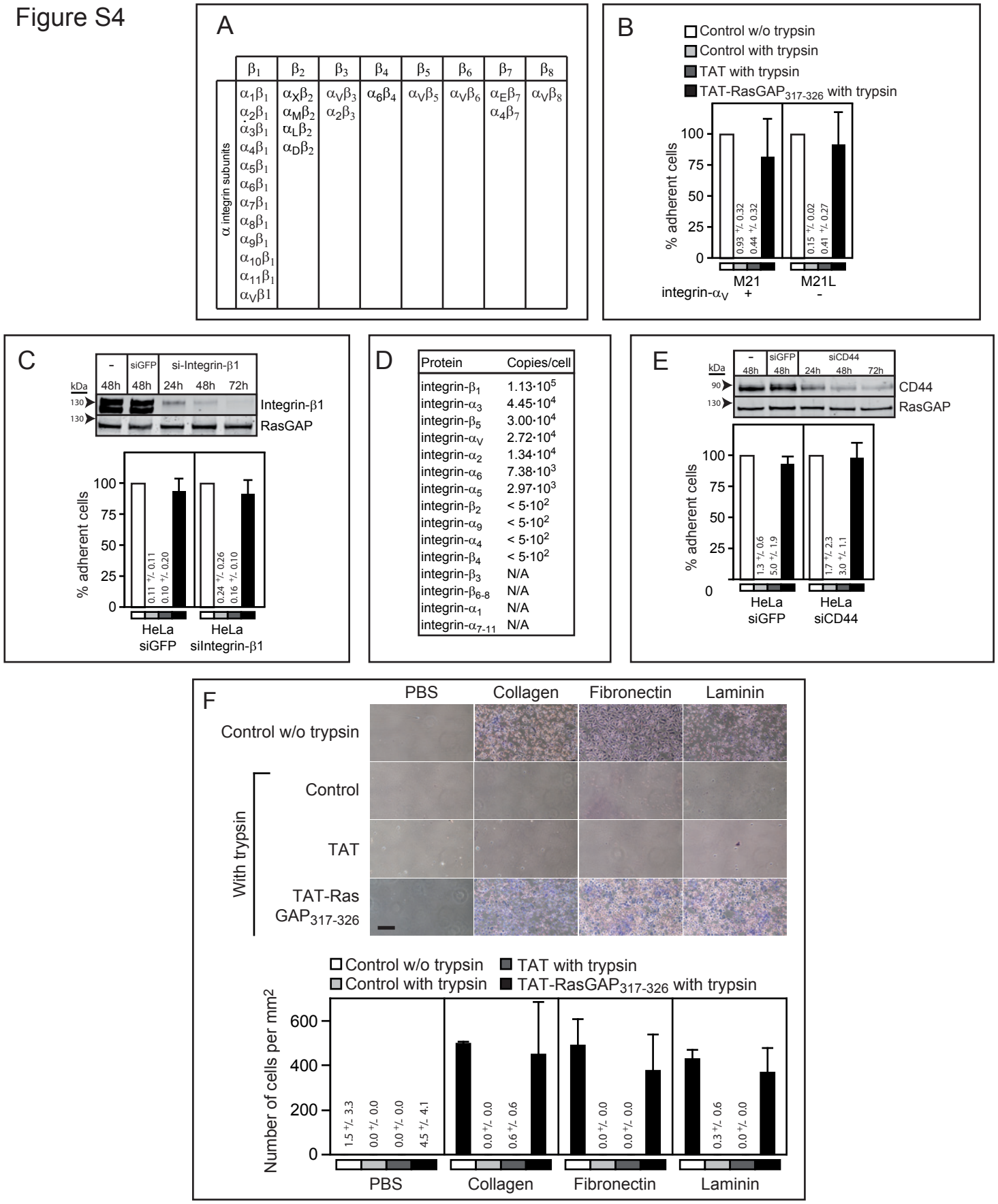


**Figure S4.** Integrins, CD44 and caspases are not required for TAT-RasGAP$_{317-326}$-mediated adhesion increase.

**A.** The twenty-four known integrin heterodimers are represented (8).

**B.** Wild-type (M21) or $\alpha_V$-integrin-null (M21L) melanoma cells were treated 8 hours with 20 μM TAT, 20 μM TAT-RasGAP$_{317-326}$ or left untreated. The cells were then subjected to a trypsin-mediated detachment assay.

**C.** HeLa cells were transfected with siRNAs targeted against GFP (siGFP) or β1-integrin (siIntegrin-β1). Forty-eight hours later, the cells were incubated 8 hours with 20 μM TAT, 20 μM TAT-RasGAP$_{317-326}$ or left untreated and were subjected to a trypsin-mediated detachment assay. The upper panel depicts the loss of β1-integrin expression after siRNA transfection.

**D.** Expression levels of the indicated integrin family members in U2OS cells [data taken from (9)].

**E.** CD44 was silenced in HeLa cells with siRNAs. Forty-eight hours later, the cells were incubated 8 hours with 20 μM TAT, 20 μM TAT-RasGAP$_{317-326}$ or left untreated. The cells were then subjected to a trypsin-mediated detachment assay. The upper panel depicts the loss of CD44 expression after siRNA transfection.

**F.** U2OS cells were grown overnight on bacteriological Petri dishes that had been coated beforehand with collagen, fibronectin or laminin. The cells were then treated or not with 20 μM TAT or 20 μM TAT-RasGAP$_{317-326}$ for 8 hours. A trypsin-based detachment assay was finally performed. Representative images of the cells remaining attached to the plates after trypsin treatment are shown. Scale bar: 100 μm.

Figure S5

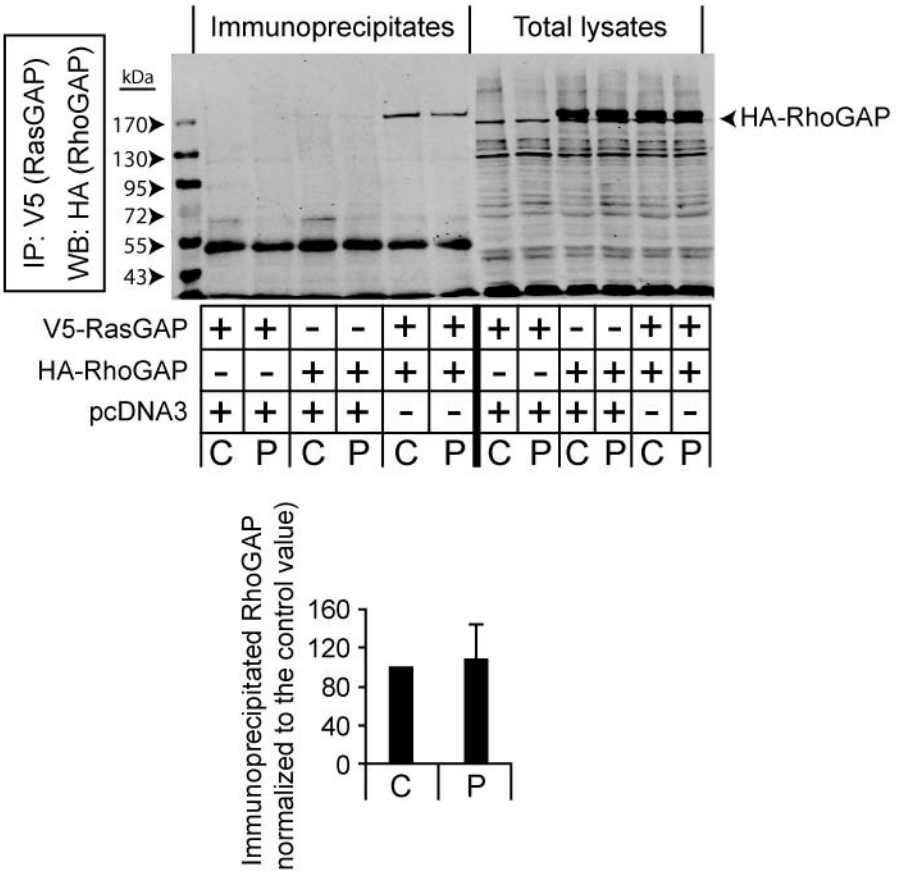


**Figure S5.** TAT-RasGAP$_{317\text{-}326}$ does not disrupt p120RasGAP:p190RhoGAP binding.
HEK 293T cells were transfected with the indicated combinations plasmids encoding V5-RasGAP or HA-RhoGAP. Seven hundred μg of lysates from cells treated with 20 μM TAT-RasGAP$_{317\text{-}326}$ for 24 hours (P) or left untreated (C) were immuno-precipitated with an anti-V5 antibody to precipitate exogenous p120RasGAP. Western blot against the HA-tag was performed to detect pull-down p190RhoGAP. Forty μg of total lysates were loaded as control. The graph represents the quantitation of the immuno-precipitated p190RhoGAP (normalized to the total lysate).

Figure S6

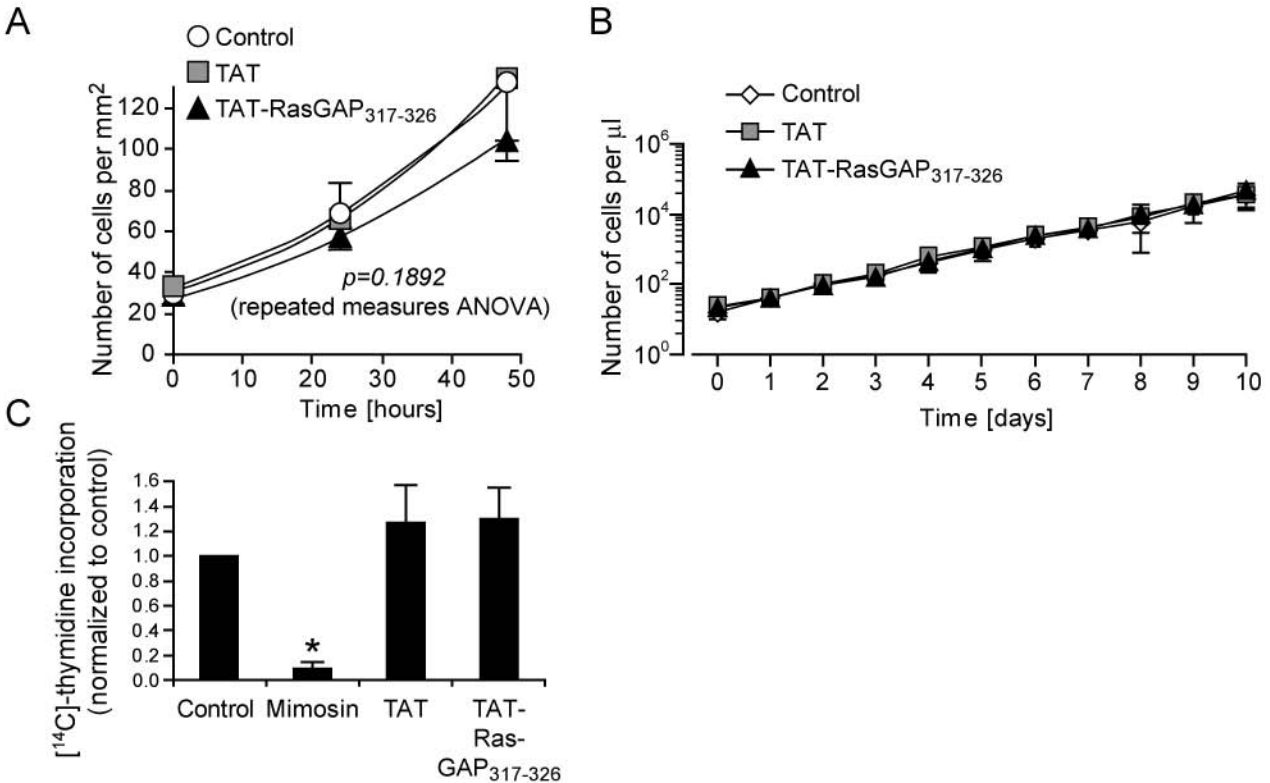


**Figure S6.** TAT-RasGAP317-326 does not affect cell proliferation

**A.** U2OS cells were treated with 20 µM TAT, 20 µM TAT-RasGAP$_{317-326}$ or left untreated. The cell density was determined 0, 24 and 48 hours later. The curves were not found to differ significantly as assessed by repeated measures ANOVA.

**B.** Jurkat cells were treated with 20 µM TAT, 20 µM TAT-RasGAP$_{317-326}$ or left untreated, and were tested for their ability to proliferate in suspension for 10 days. The graph displays the number of cells per ml (cell splitting was performed when appropriate).

**C.** U2OS cells were treated 16 hours with 400 µM mimosine, 20 µM TAT, 20 µM TAT-RasGAP$_{317-326}$ or left untreated, and were tested for their ability to incorporate radioactive thymidine during 2.5 hours. Mimosine significantly altered DNA synthesis, while TAT and TAT-RasGAP$_{317-326}$ did not.