Figure S1. Serum-starved fibroblasts from $\alpha_1^{-/-}$ and $^{+/+}$ mice were stimulated with 10% FBS for 30 min. (FBS) or plated on collagen I (CI) for 1h. The cells were surface biotinylated and $\beta_1$-integrins were immunoprecipitated (IP $\beta_1$). Biotin, TCPTP and SHP-2 (72 kD) were immunoblotted.

Integrin $\alpha_1^{-/-}$ fibroblasts from integrin $\alpha_1$ knockout animals

Figure S3. Integrin $\alpha_1\beta_1$ is required for collagen-induced attenuation of EGFR signalling.

Integrin $\alpha_1^{-/-}$ fibroblasts from integrin $\alpha_1$ knockout animals

(e) Fibroblasts from $\alpha_1^{-/-}$ and $^{+/+}$ mice were plated on collagen I and immunostained for integrin $\alpha_1$ (green) and TCPTP (red). Pixel intensity for green and red (means $\pm$ SEM, n=12) were analyzed starting from the cell edge with confocal microscope software.
(e) Serum-starved fibroblasts from \( \alpha_{1}^{-/-} \) (two separate isolates) and \( \alpha_{1}^{+/+} \) mice were plated on collagen I or fibronectin, treated with EGF and resolved on SDS–PAGE gels (7% for \( \alpha_{1}^{+/+} \) and \( \alpha_{1}^{-/-} \) isolate1; 10% for \( \alpha_{1}^{-/-} \) isolate2. Note that the top band in isolate 2 is intact EGFR) and immunoblotted. Values are densitometric quantifications normalized to the tubulin in the same panel.

**Figure 3** Integrin \( \alpha_{1} \beta_{1} \) ligation attenuates EGFR phosphorylation through activation of TCPTP.

Low level of EGFR phosphorylation on collagen is due to TCPTP activity?

**Knockdown:** siRNAs
Figure 3 Integrin α1β1 ligation attenuates EGFR phosphorylation through activation of TCPTP.

(d) HeLa cells transfected with two siRNAs specific for TCPTP or SHP-2 (or scramble control) were plated on collagen I and treated with EGF. Extracts were immunoblotted for the indicated proteins. A representative of three experiments with similar results is shown.

Figure S4. α1 cytoplasmic tail peptide induces phosphatase activity in vivo

(a) HeLa cells were microinjected (asterisks) with fluorescein diphosphate (FDP) and integrin cytoplasmic tail peptides. Fluorescence intensity was monitored for 30 min. (representative images at 30 min. are shown) and mean intensity in individual cells (means ± SEM, n=12) was measured. ***, p-value <0.001, NS, not significant, Kruskal-Wallis followed by Dunn’s multiple comparisons test.

FDP: non fluorescent di phosphofluorescein

Dephosphorylation
(not specific for TCPTP)
Figure S4. α1 cytoplasmic tail peptide induces phosphatase activity in vivo

**TCPTP knockdown: Short hairpin RNA**

(b) HeLa cells with shRNA mediated down-regulation of TCPTP (HeLaRNA-U6.1TCPTP) and control cells (HeLaRNA-U6.1) were plated on collagen and microinjected with fluorescein diphosphate (FDP) and integrin cytoplasmic tail peptides as in Figure 4A. Fluorescence intensity was monitored for 30 min. and mean intensity in individual cells (means ± SEM, n=5) was measured. Western-blotting was performed to validate TCPTP down-regulation in HeLaRNA-U6.1TCPTP (2) cells. Tubulin was used as a loading control.

**Induction of phosphatase activity may influence tumorigenicity of cells** in a manner similar to overexpression of TCPTP. Therefore, we used cell-permeable fluorescein isothiocyanate (FITC)-conjugated peptides fused to the TAT sequence to deliver α1cyt peptide into cells for proliferation and tumorigenicity assays. α1-TAT peptide and a scramble control peptide both entered the cells efficiently (Fig. 4a), fluorescence was sustained over 24 h and no detachment of cells was observed (data not shown). In HeLa and tumorigenic B104-1-1 cells growing in suspension, 200 nM α1-TAT peptide specifically inhibited serum-induced cell growth (Figs 4a, c). Furthermore, α1-TAT peptide was efficient at inhibiting EGF-induced cell growth (Fig. 4a; also see Supplementary Information, Fig. S4e), HeLaRNA-U6.1TCPTP cells were no longer sensitive to growth inhibition by α1-TAT peptide (Fig. 4b) and treatment of HeLa cells with α1-TAT peptide resulted in attenuated EGFR phosphorylation (see Supplementary Information, Fig. S4c), suggesting that the effects of the peptide are most probably mediated through TCPTP.
Figure 4 α1 cytoplasmic tail peptide induces phosphatase activity in vivo and inhibits anchorage-independent and EGF-induced cell growth. (a) FITC labelled TAT-α1 cytoplasmic tail fusion peptide (α1-TAT) and TAT-scramble control fusion peptide (Scr-TAT) both enter HeLa cells. Their effect on the number of: (a) live non-adherent HeLa cells in serum,

α1-TAT peptide and a scramble control peptide both entered the cells efficiently.

Anchorage-independent growth

(b) HeLa cells with shRNA-mediated downregulation of TCPTP (HeLapRNA-U6.1TCPTP) and control cells (HelapRNA-U6.1) in serum

Inhibition of TCPTP = less survival of non adherent cells
Figure S4. α1 cytoplasmic tail peptide induces phosphatase activity in vivo

(d) Integrin α1 cytoplasmic peptide does not affect EGF-independent proliferation of matrix-adherent cells. HeLa cells were cultured in fibronectin-coated wells in 5% serum in the presence or absence of 200 nM TAT peptides and 50 ng/ml EGF and the numbers of live cells (means ± SD, n=3) at various time points were analysed.
(d) HeLa cells were grown for 9 days in agarose with or without the TAT-peptides. Representative phase-contrast images taken double-blindly and analyses of colony sizes are shown.

In vivo induction of phosphatase activity by α1cyt peptide causes identical effects to those achieved by overexpressing TCPTP in tumorigenic cells. Moreover, the peptide can be transduced to living cells, and it efficiently blocks anchorage-independent, EGF-induced proliferation of malignant cells.
Integrin signaling

α<sub>1</sub>, α<sub>2</sub>, α<sub>5</sub>  
↓  
Cav  
↓  
shc  
↓  

β  
↓  
FAK  
↓↓↓

α<sub>1</sub>  
↓  
TCPTP  
↓↑↓

?  
↓  

Integrin family members α<sub>1</sub>, α<sub>2</sub>, α<sub>5</sub> are involved in signaling through Cav and shc. FAK is also implicated in this process.