We conclude that endogenous α1 integrin and TCPTP become physically associated in response to collagen or mitogenic stimuli in intact human cells.

To study the specificity of the interaction between TCPTP and collagen-binding integrins, we performed pull-down experiments with glutathione S-transferase (GST)-tagged α-cytoplasmic tails (GST–acyt).
Is TCPTP and integrin α1 cytoplasmatic tail interaction a direct interaction or an indirect interaction?

**Figure S1.** (d) Recombinant and purified TCPTP was incubated with GST or GST-fusion proteins with or without α1 integrin cytoplasmic domain peptide (1 µg/ml). Bound proteins (and TCPTP loading control, total) were probed for TCPTP and GST.
The interaction is sufficient for phosphatase stimulation of activity and is specific for TCPTP?

Figure S2. Adhesion to collagen and clustering of α1-integrin activate TCPTP. (a) Serum-starved HeLa cells were detached, plated on collagen or poly-L-lysine, subjected to immunoprecipitations (IP) and phosphatase activity (means ± SD, n=3) was determined. Half of the samples were immunoblotted for TCPTP. (b) Serumstarved HeLa cells were incubated with PBS, control IgG or anti-α1 mAb and clustering was induced with an anti-mouse secondary antibody for 30 min. Equal amounts of protein from lysates were assayed for phosphatase activity (means ± SD, n=3) in triplicates.
For **phosphatase assays**, immunoprecipitates were resuspended in phosphatase reaction buffer (25 mM Hepes at pH 7.4, 50 mM NaCl and 1 mM dithiothreitol) and 1/3 of the reaction subjected to western blot analysis and the remaining beads assayed for phosphatase activity in triplicate using diFMUP (6,8-difluoro-4-methylumbelliferyl phosphate; Molecular Probes, Eugene, OR) as a substrate and in the presence of a serine/threonine phosphatase inhibitor cocktail (Sigma), according to the manufacturer’s instructions. When indicated, synthetic peptides were pre-incubated with the immunoprecipitates for 15 min before the phosphatase assay reaction (α1 peptide, N-RPLKKKMEKRPLKKKMEK-C; α2 peptide, N-KLGFKKRYEKMTKNPDEIDETTELSS-C; gift from J. Heino).

For phosphatase assays with purified protein, full-length TCPTP, 37K TCPTP and SHP-2 were incubated in phosphatase reaction buffer in the presence or absence of GST-fusion TCPTP-deletion mutant proteins and synthetic integrin cytoplasmic tail peptides as indicated.

**SHP2** = non-receptor phospho-tyrosine phosphatase with SH2 domain

**Figure 2** The integrin α1 cytoplasmic tail activates TCPTP.

(a) HeLa cell lysates were immunoprecipitated (IP) with control (IgG), anti-TCPTP and anti-SHP2 antibodies. The phosphatase activity (means ± s.d., n = 3) was analysed using diFMUP as the substrate after treatments with vehicle (c), synthetic α1 (α1pep) and α2 (α2pep) cytoplasmic tail peptides. Half of the immunoprecipitates were resolved on SDS–PAGE gels and probed for TCPTP or SHP-2. All peptides were added at 1 mg ml⁻¹.
Which part of the TCPTP molecule is responsible for phosphatase activity and which is responsible for α1 interaction?

Figure 2 The integrin α1 cytoplasmic tail activates TCPTP.

(b) Recombinant, purified full-length TCPTP (45K), truncated TCPTP (37K) and SHP-2 (0.15 µg ml⁻¹) were incubated with different peptide concentrations and analysed for phosphatase activity (means ± s.d., n = 3).
TKRs are substrates for TCPTP: Studies with overexpression or knockout cells have shown that TCPTP has several plasma membrane-associated substrates, such as EGFR, platelet-derived growth factor (PDGF) β receptor, the insulin receptor and Janus kinases (JAKs) that regulate mitogen- and cytokine-induced signalling.

TCPTP autoinhibition: In vitro studies, using proteolytically cleaved fragments of TCPTP, have proposed that the catalytic activity of TCPTP is regulated by an intramolecular inhibition involving a carboxy-terminal segment of the 45K form of TCPTP. However, it has remained unclear whether such a regulatory mechanism would function in cells and how it would operate.

Association of TCPTP with α1 cyt could alleviate this autoinhibition and lead to activation of the phosphatase?
Figure 3 Integrin α1β1 ligation attenuates EGFR phosphorylation through activation of TCPTP.

(a–c) Serum-starved HeLa cells maintained on plastic or plated on collagen I (CI) or fibronectin (Fn) were treated with EGF. EGFR phosphorylation was studied using phospho-specific antibodies. Densitometric analyses of three (a) experiments (means ± s.d.) are shown. Three asterisks denotes P < 0.001, Student’s t-test. Tubulin or EGFR were used as loading controls.

Cinetic analysis

(a–c) Serum-starved HeLa cells maintained on plastic or plated on collagen I (CI) or fibronectin (Fn) were treated with EGF.

Is that due to adhesion efficiency?
Adhesion of HeLa cells was studied on fibronectin and collagen I at different matrix concentrations. Cells were stained green with a live cell dye and the number of adherent cells was quantitated using Acumen laser-based cell scanner.

Figure S3. Integrin α1β1 is required for collagen-induced attenuation of EGFR signalling.

Comparable adhesion efficiency
(a) Adhesion of HeLa cells was studied on fibronectin and collagen I at different matrix concentrations. Cells were stained green with a live cell dye and the number of adherent cells was quantitated using Acumen laser-based cell scanner.

Different EGFR phosphorylation state

Integrin cell surface expression (FACS)

<table>
<thead>
<tr>
<th>Integrin</th>
<th>β1</th>
<th>α2</th>
<th>α3</th>
<th>α5</th>
<th>α6</th>
<th>β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>225</td>
<td>59</td>
<td>1</td>
<td>32</td>
<td>78</td>
<td>367</td>
</tr>
<tr>
<td>HT-1080</td>
<td>2</td>
<td>290</td>
<td>10</td>
<td>52</td>
<td>146</td>
<td>594</td>
</tr>
</tbody>
</table>

Integrins

<table>
<thead>
<tr>
<th></th>
<th>HeLa</th>
<th>HT1080</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CI CI FN FN</td>
<td>CI CI FN FN</td>
</tr>
<tr>
<td>160 -</td>
<td>- + - +</td>
<td>- + - +</td>
</tr>
<tr>
<td>50 -</td>
<td>EGFR(PY)1068</td>
<td>EGFR(PY)1068</td>
</tr>
<tr>
<td></td>
<td>Tubulin</td>
<td>Tubulin</td>
</tr>
</tbody>
</table>

5min EGF 50ng/ml
Figure S3. Integrin α1β1 is required for collagen-induced attenuation of EGFR signalling. α

HT1080 transfected with α1 cDNA

(c) HT1080 cells transiently transfected with α1-cDNA or mock-transfected were serum-starved, plated on collagen (CI) and treated with EGF. EGFR phosphorylation was analyzed as in b.

Clustering of α1 subunit in HeLa

(d) Serum-starved HeLa cells were treated as in Figure S2b to cross-link α1 receptors and EGFR phosphorylation was studied by immunoblotting.

finally, we studied the specificity by using wild-type and α1−/− mouse fibroblasts adhering to collagen
Integrin α1-/- fibroblasts from integrin α1 knockout animals

Integrin α1-/- fibroblasts from integrin α1 knockout animals are generated by targeting vector integration into the α1 integrin gene. The vector contains a cassette of DNA that results in the deletion of the α1 integrin gene in the fibroblasts. The targeting vector is used to introduce a mutation into the fibroblasts, resulting in the creation of mutant fibroblasts. The mutant fibroblasts are then used to generate a mouse model of integrin α1 deficiency.

The process involves the following steps:

1. **Targeting Vector Integration**: The targeting vector is introduced into the fibroblasts using a technique such as electroporation or transfection. The vector contains a cassette of DNA that results in the deletion of the α1 integrin gene.
2. **Selection for Homozygous Mutants**: The fibroblasts are screened for homozygous mutants by genotyping. The homozygous mutants are identified by the absence of the α1 integrin gene.
3. **Generation of Knockout Mice**: The homozygous mutants are then used to generate knockout mice by mating the mice to heterozygous mice. The knockout mice lack the α1 integrin gene and are used to study the role of integrin α1 in various biological processes.

The diagram illustrates the process of generating integrin α1 knockout mice. The targeting vector is shown to be integrated into the α1 integrin gene, resulting in the deletion of the gene. The knockout mice are then generated by mating the homozygous mutants with heterozygous mice. The knockout mice lack the α1 integrin gene and are used to study the role of integrin α1 in various biological processes.