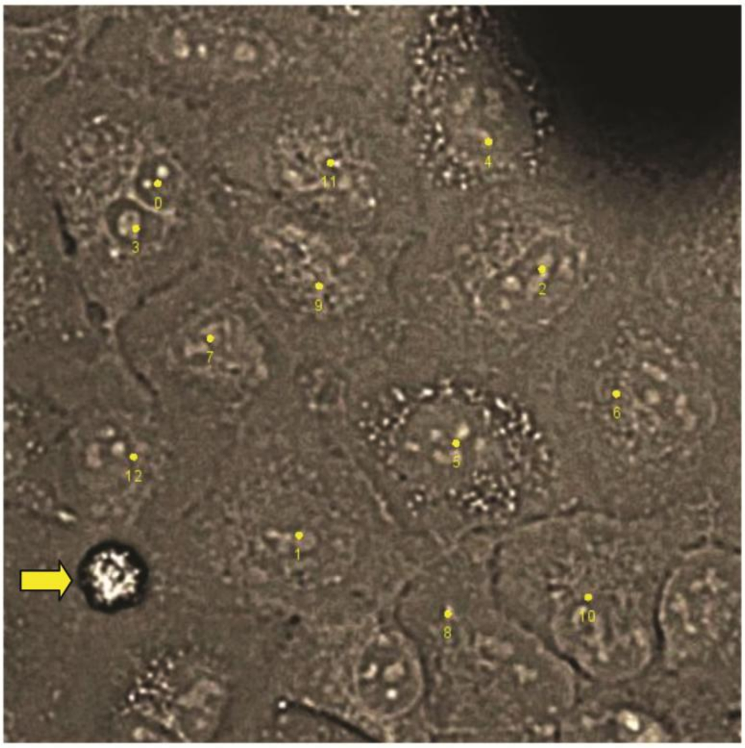
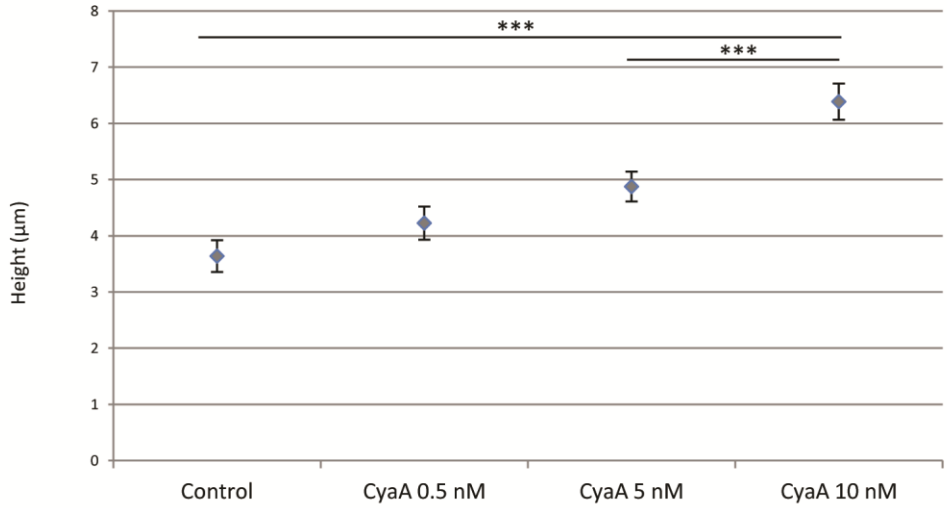
**Supplementary Material**

**Supplementary Figure 1: Cell contact map**



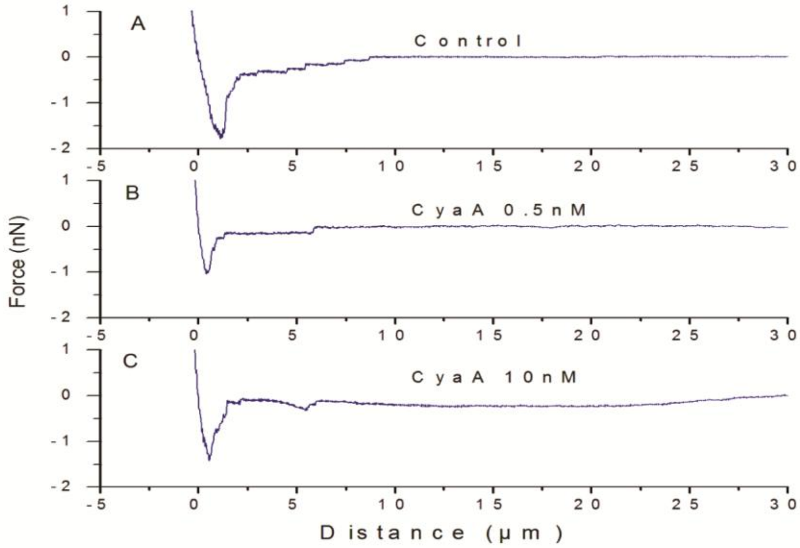
Legend: Optical transmission image obtained during a typical MFS experiment by AFM in control condition. This cell contact map shows A549 adherent cell monolayer grown on a petri-dish coated by human fibronectin. The cell spreading with net cell contours provides a cell-by-cell check (prior MFS) that tested cells is alive and adherent. By contrast, the yellow arrow shows a detached cell with a round shape which is characteristics of a dead cell. Points and numbers in yellow correspond to the positions of the contact points selected to perform the force-distance curves (only one per cell). Note that each position is located on the top of the cell above the nucleus of each cell where the distance from the substrate is maximal. The dark region on top right of the image is the shadow of the cantilever. The dimensions of this optical image are 150µm x 150µm.

**Supplementary Figure 2: CyaA effect on cell height**



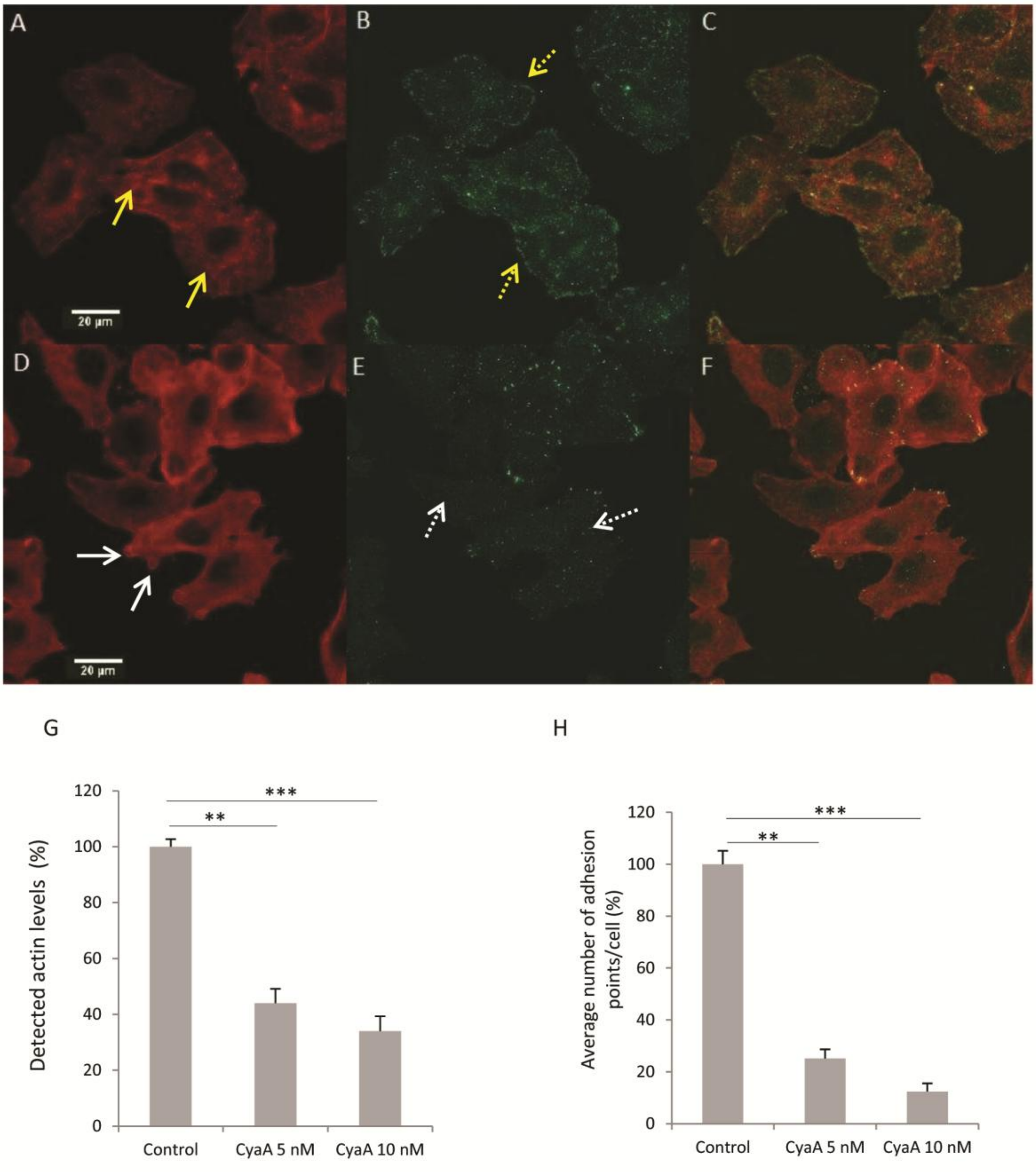

Legend: Height (in µm) measured from the distance separating the basal and apical planes in a z-stack confocal images of A549 cells in control condition (n=14) and after 30 min of exposure to CyaA toxin at different CyaA concentrations (n=14). The increase in height resulting from CyaA exposure reflects a cell rounding effect which increases with CyaA concentration. Error bar is ±SEM. * $p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

**Supplementary Figure 3: Force-distance curves for control and 2 CyaA concentrations**



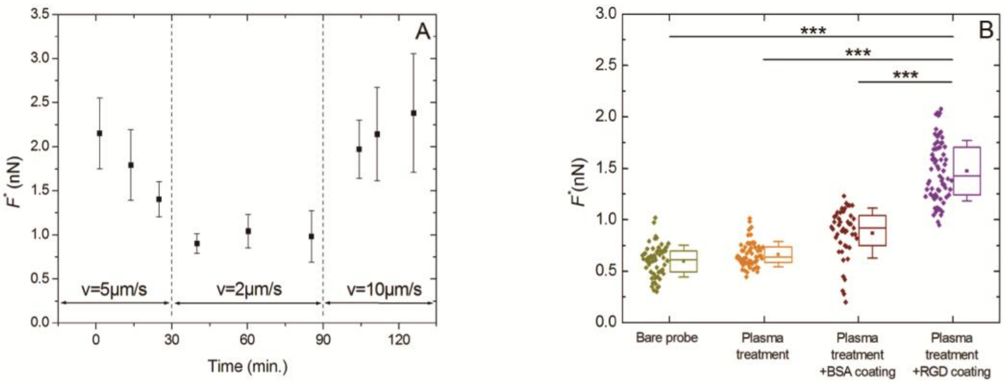

Legend: Typical records of force-distance curves obtained for 3 different condition: (**A**) control conditions (DMEM-1%BSA); (**B**) CyaA toxin at 0.5 nM (toxin + CyaA activation buffer); (**C**) CyaA toxin at 10 nM (toxin + CyaA activation buffer). These force-distance curves show a decrease in the global adhesion between the probe and cell as CyaA concentration increases. At the highest concentration of CyaA toxin (10 nM), force-distance curves are altered by the presence of some detached cells in medium.

**Supplementary Figure 4: Co-staining of F-actin and of focal adhesion sites in fixed A549 cells exposed to CyaA**




Legend: Co-staining of F-actin (phalloidin, red) and of focal adhesion (phosphotyrosin Py99, green) in fixed A549 cells: (**A,D**) F-actin staining, (**B,E**) focal adhesion staining, (**C,F**) merge, (**A-C**) Cells after 30 min of exposure to CyaA toxin concentrated at 5 nM (n=13), (**D-F**) Cells after 30 min of exposure to CyaA concentrated at 10 nM (n=13), (**G**) Quantification of fluorescent F-actin for control group and after 30 min of exposure to CyaA concentrated at 5 and 10 nM, (**H**) Percentage of average number of focal adhesions per cell in control conditions and after 30 min of exposure to CyaA concentrated at 5 and 10 nM . Compared to the control group (see Fig. 3A-C of manuscript), cells exposed to CyaA toxin concentrated at 5 and 10 nM have lost stress fibers and the F-actin network appears more diffuse (yellow arrows) which is confirmed by the significant decay in fluorescent F-actin. In cells exposed for 30 min to CyaA toxin at a concentration of 10 nM, blebs are clearly visible (blue arrows) which is a mark of cell death. Compared to control group (see Fig. 3A-C of manuscript), cells exposed to CyaA toxin at a concentration of 5nM maintain focal adhesions but their number is significantly decreased compared to the control group. In the case of cells exposed to CyaA toxin at a concentration of 10nM, the number of focal adhesions drastically decreases and cells have a tendency to die (white arrows) which initiates a delamination phenomenon. Scale bar is 20 µm. Error bar is ±SEM. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

**Supplementary Figure 5: Stability and specificity of RGD coating**




Legend: In (**A**) Time evolution of global detachment force $F^*$ obtained at 3 different retraction speeds (v= 5, 2, and 10 µm/s) using the integrin specific coating (RGD) (1 data point per cell). In (**B**) Global detachment force obtained for 4 different coating types: From left to right, are presented 3 non specific coatings (i) Bare probe (green diamonds), (ii) Plasma treatment (orange diamond), (iii) Plasma treatment with BSA coating (brown diamonds), and 1 integrin-specific coating: (iv) Plasma treatment with RGD coating (purple diamonds). These results show that RGD coating leads to relatively stable results (in terms of $F^*$ and in the limit of intercellular variability) through times representative of typical AFM experiments $F^*$. It appears that global detachment forces $F^*$ with integrin-specific coating are significantly increased compared to values obtained with non specific coating. Dots in box mark the mean values, whisker bars the ±SD values, and horizontal bars in box give the median values.

**Supplementary Table 1: Values of global adhesion and mechanical parameters obtained at 3 different concentrations of CyaA**

| Values obtained for v = 5µm/s | | | |
|---|---|---|---|
| | ***Mean*** | ***SD*** | ***n*** |
| **Detachment force $F^*$ (nN)** | | | |
| Control | 1.70 | 0.53 | 120 |
| CyaA 0.5nM | 1.19 | 0.36 | 68 |
| Control | 2.13 | 1.27 | 77 |
| CyaAE5 0.5nM | 2.04 | 1.04 | 74 |
| Control | 2.17 | 0.52 | 31 |
| CyaA 5nM | 1.21 | 0.41 | 27 |
| Control | 2.19 | 0.58 | 45 |
| CyaA 10nM | 1.25 | 0.39 | 39 |
| **Work of adhesion $W_{adh}$ (nN × µm)** | | | |
| Control | 3.53 | 2.03 | 120 |
| CyaA 0.5nM | 1.62 | 0.85 | 68 |
| Control | 4.14 | 2.32 | 77 |
| CyaAE5 0.5nM | 3.98 | 2.20 | 74 |
| Control | 6.52 | 2.73 | 31 |
| CyaA 5nM | 1.28 | 0.42 | 27 |
| Control | 3.87 | 1.44 | 45 |
| CyaA 10nM | 1.35 | 0.61 | 39 |
| **Young's modulus $E_{cell}$ (Pa)** | | | |
| Control | 529 | 337 | 120 |
| CyaA 0.5nM | 855 | 470 | 68 |
| Control | 587 | 359 | 77 |
| CyaAE5 0.5nM | 580 | 325 | 74 |
| Control | 491 | 200 | 31 |
| CyaA 5nM | 773 | 558 | 27 |
| Control | 620 | 440 | 45 |
| CyaA 10nM | 1044 | 821 | 39 |

Legend: Mean ±SD values of adhesion ($F^*$ and $W_{adh}$) and mechanical ($E_{cell}$) parameters determined in « *n* » different cells and at the same predetermined speed ( v=5µm/s ) for indentation and retraction, during control and exposition to CyaA toxin at 0.5 nM concentration. These values correspond to data plotted in Fig 4A, B, and C.

**Supplementary Table 2: Values of global and local parameters for cell adhesion and cell mechanics obtained for the 3 speeds tested and the lowest CyaA concentrations (0.5 nM)**

| Values for CyaA 0.5 nM | | | | | | |
|---|---|---|---|---|---|---|
| | v=2µm/s | | v=5µm/s | | v=10µm/s | |
| Global | (n=42) | | (n=68) | | (n=77) | |
| | Mean | SD | Mean | SD | Mean | SD |
| Detachment force $F^*$ (nN) | 0.60 *(0.84)** | 0.25 *(0.27)* | 1.19 *(1.7)* | 0.36 *(0.53)* | 1.34 *(1.71)* | 0.41 *(0.53)* |
| Work of adhesion $W_{adh}$ (nN x µm) | 0.54 *(1.68)* | 0.36 *(1.1)* | 1.62 *(3.53)* | 0.85 *(2.03)* | 2.00 *(4.17)* | 1.31 *(1.7)* |
| Young's modulus $E_{cell}$ (Pa) | 870 *(394)* | 491 *(284)* | 855 *(528)* | 470 *(337)* | 741 *(438)* | 434 *(256)* |
| Characteristic distance δ (µm) | 0.90 *(1.96)* | 0.55 *(1.02)* | 1.37 *(2.18)* | 0.63 *(1.27)* | 1.50 *(2.58)* | 0.87 *(1.14)* |
| Global loading rate $r_f$ (pN/s) | 1.44E+03 *(1.42E+03)* | 1.07E+03 *(1.13E+03)* | 5.21E+03 *(5.02E+03)* | 2.81E+03 *(3.19E+03)* | 1.07E+04 *(1.02E+03)* | 8.60E+03 *(5.81E+0.3)* |
| | | | | | | |
| Local | (n=10) | | (n=24) | | (n=16) | |
| | Mean | SD | Mean | SD | Mean | SD |
| Rupture force $f^*$ (pN) | 69 *(59.1)* | 23 *(35.8)* | 88 *(77.3)* | 32 *(35.4)* | 149 *(114.6)* | 62 *(40.6)* |
| Local loading rate $r_f$ (pN/s) | 392 *(263.3)* | 223 *(229.7)* | 460 *(309.6)* | 348 *(225.3)* | 1358 *(931.9)* | 1451 *(755.3)* |

**Control values are in parenthesis*

Legend: The upper part of the table represents mean ±SD values of global adhesion ($F^*$ and $W_{adh}$) and mechanical parameters ($E_{cell}$) determined in "*n*" different cells and at 3 different predetermined indentation/separation speeds: v=2, 5 and 10 µm/s during exposure to CyaA toxin at 0.5 nM concentration. A characteristic distance of cell deformation can be obtained from $\delta = W_{adh}/F^*$ in each cell (mean ±SD values of δ are given). The global loading rate $r_f$ is calculated cell-by-cell from the product $r_f = E_{cell} \times \delta \times$ v. The second part of the table represents mean values ±SD of local rupture force ($f^*$) and the local loading rate $r_f$ estimated at 3 different predetermined separation speeds: v=2, 5 and 10 µm/s during exposure to CyaA toxin at 0.5 nM concentration. The local loading rates are calculated from the local stiffness $k_{local}$ (the slope of the force-distance curve preceding each rupture event) from the expression: $r_f = k_{local} \times$ v. Mean ±SD values of $k_{local}$ are given.