Supplemental Materials

Movie S1

CCF4-based real-time translocation assay: Increased CCF4 hydrolysis in CNF-Y treated HeLa cells

GST- or GST-CNF-Y treated HeLa cells were pre-loaded with CCF4-AM substrate and then infected with WA-314-pMK-bla. Hydrolysis of CCF4 substrate by translocated beta-lactamase was monitored by live cell imaging. Excitation at 409 nm resulted in green fluorescence emission (520 nm) of the intact substrate and in blue fluorescence emission (447 nm) of the cleaved hydrolysis product. Frames were acquired every 10 min and recorded for 100 min. The movie shows GST-treated cells on the left and CNF-Y-treated cells on the right.

Figure S1

No effect of CNF-Y treatment or RacL61 expression on number of cell-associated bacteria

(A) HeLa cells were treated with GST or GST-CNF-Y for 2 h and subsequently infected with WA-314 for 1 h. Cells were then thoroughly washed with cold PBS and subsequently lysed. Cell-associated bacteria were quantified by serial dilution and culture on LB agar. (B) HeLa cells were transfected with myc-Rac1L61 or left untreated. After 16 h cells were infected with WA-314 for 1h and analyzed as stated above. Bars represent the mean ± SD (error bars) of three independent experiments.

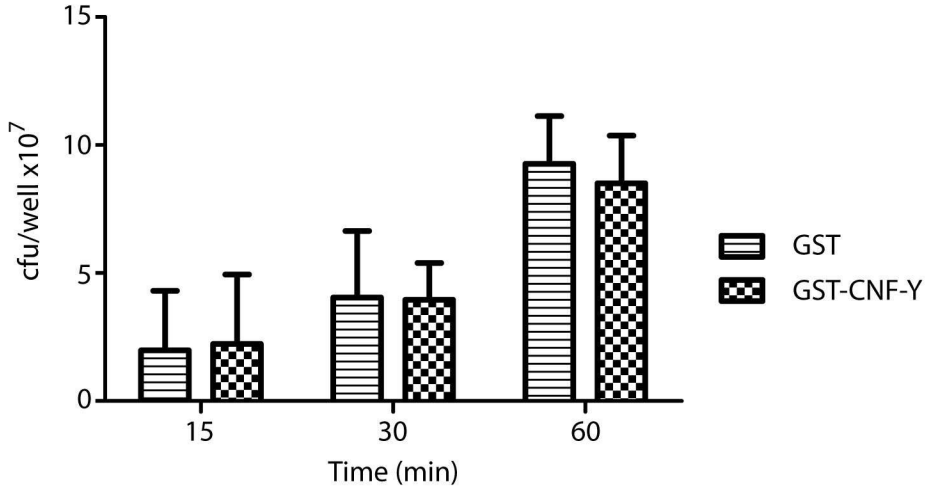
Figure S2

Time course of CNF-Y mediated Rac1 activation

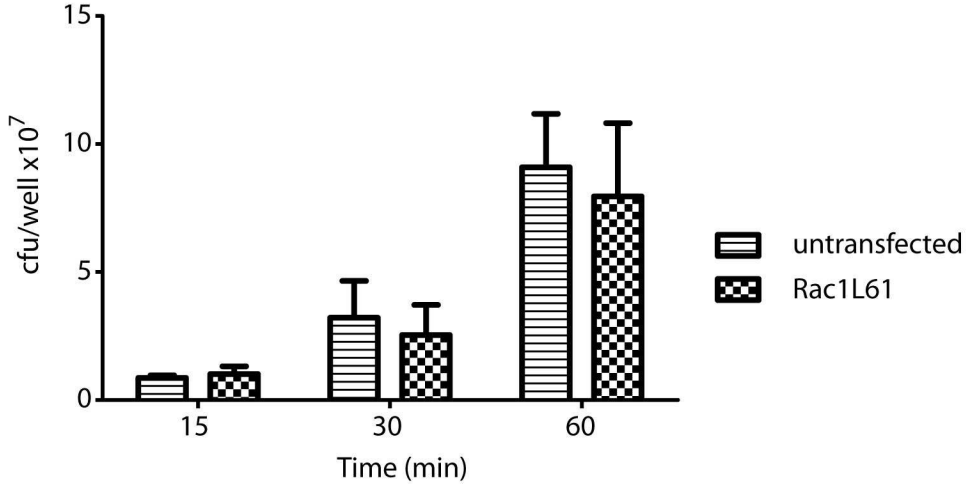
HeLa cells were treated with GST-CNF-Y as indicated. Active Rac1 was precipitated with GST-PAK-CRIB and analyzed by western blotting. The input represents approximately 4% of the cell lysate used for the respective pull down at the indicated time point. Immunoblots of pull down assays and inputs were investigated with the same antibodies. Data are representative of three independent experiments.

Wolters et al. Fig. S1

A



B



Wolters et al. Fig. S2