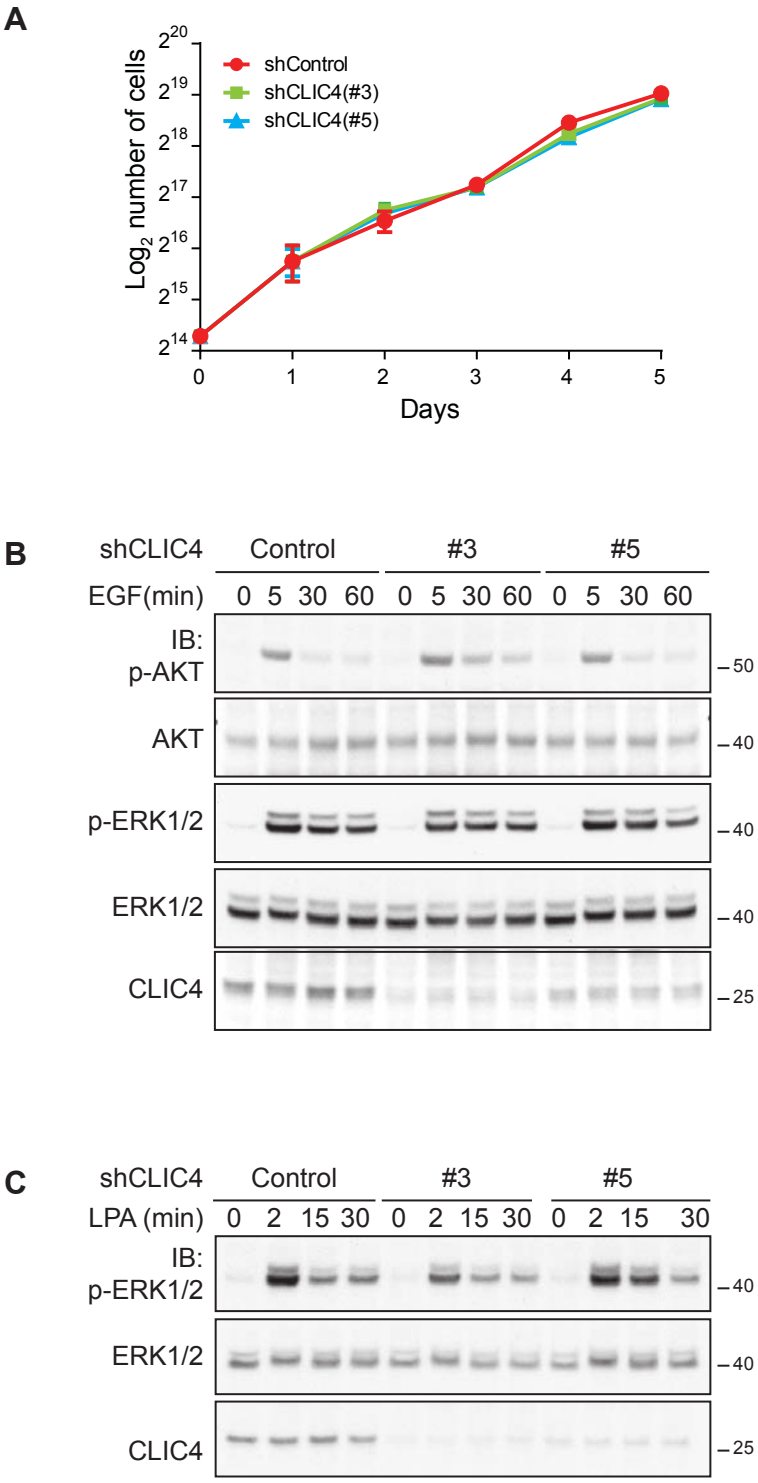

Figure S1. CLIC4 knockdown does not affect cell proliferation and growth factor signaling.
(A) Growth curves of shControl and shCLIC4 HeLa cells. (B-C) Growth factor-induced signaling. Serum starved shControl and shCLIC4 HeLa cells were left untreated or stimulated with 100 ng/ml EGF (B) or 5 μM LPA (C) for the indicated time points. AKT and ERK1/2 activation in time were monitored by immunoblot analysis of total cell lysates using anti-p-AKT and p-ERK1/2 antibodies. AKT and ERK were used as loading controls. Representative blots of one out of two experiments are shown.

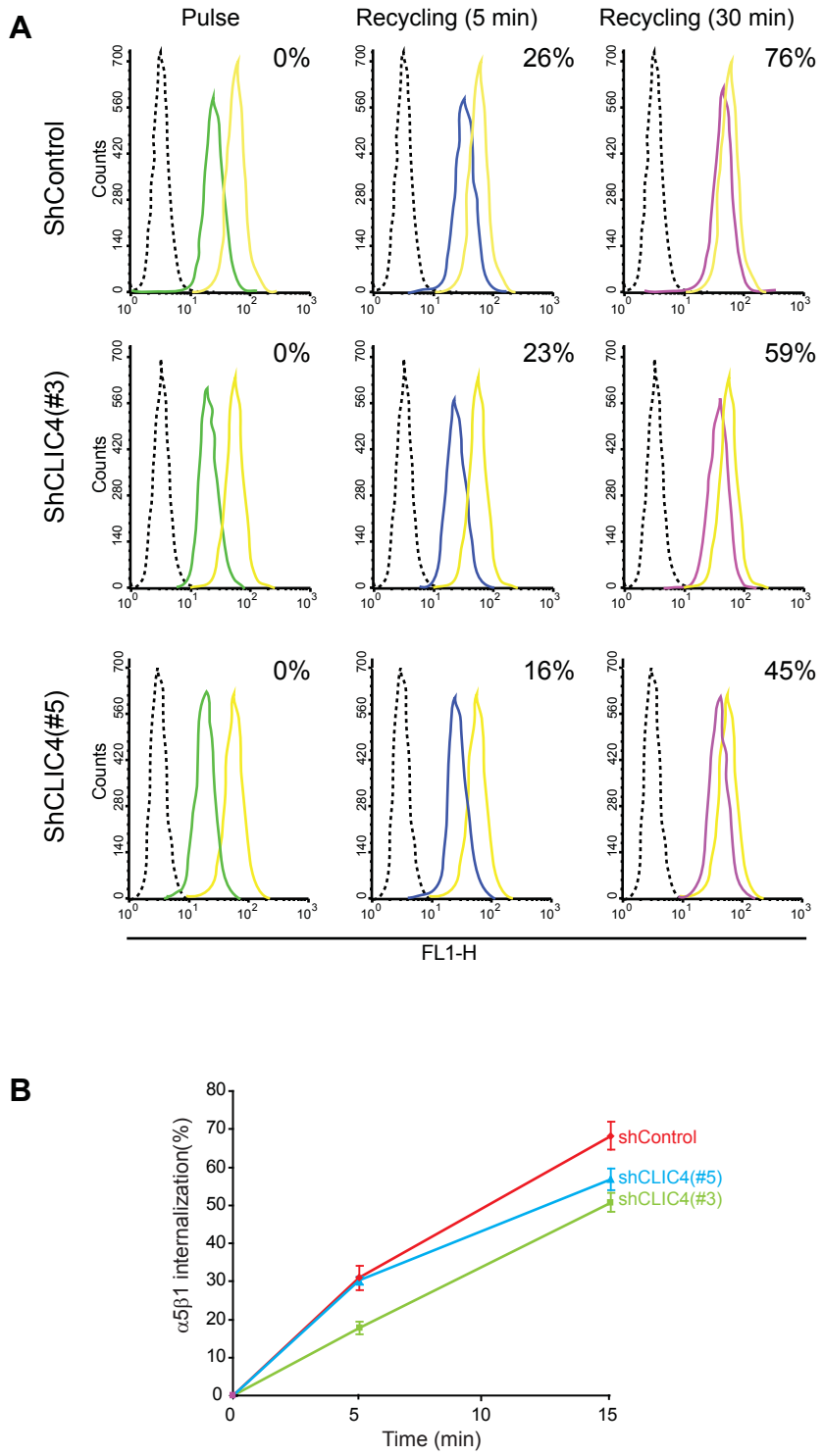

Figure S2. CLIC4 knockdown reduces recycling and internalization of α5β1 integrin

(A) FACS-based recycling assay. Serum starved shControl and shCLIC4 HeLa cells were incubated for 1 hour with anti-β1 antibody TS2/16 on ice. Cells were shifted at 37°C for 30 min (pulse), washed with an acidic buffer to strip the anti-β1 antibody at the plasma membrane and further incubated in presence of 10%FBS and FITC-conjugated secondary antibody for 5 and 30 min (recycling). Cells were gently scraped and analyzed by flow cytometry. Representative histograms of one out of two experiments are shown. Negative control= dashed black line, surface β1integrin = yellow line, Pulse= green line, 5 minutes recycling= blue line, 30 minutes recycling= magenta line. Percentage of recycled β1 integrin is shown. 50000 cells were analyzed for each time point.

(B) Biotin-based internalization assay. shContol and shCLIC4 HeLa cells were surface labeled with NHS-S-S-Biotin. Internalization was allowed to proceed for the indicated time points at 37°C. Cell surface biotin was reduced with MesNa at 4°C and the amount of internalized, biotin-labeled integrins was determined by capture ELISA using an anti-α5 integrin antibody. Data represent mean ± SEM of three independent experiments.

Table S1. CLIC4 knockdown does not affect expression of integrin α/β subunits.
Table showing expression of integrin α/β subunits. Cells were trypsinized, incubated with the indicated antibody against α- and β- integrin subunits and analyzed by flow cytometry. Staining with secondary antibody only was used as negative control. Geometric means ± SD from three independent experiments are shown.

	shControl		shCLIC4(#3)		
	Geo Mean	**SD**	**Geo Mean**	**SD**	**p-value**
FITC	3.69	0.57	3.12	0.44	0.059
alpha2	17.07	3.88	10.37	3.12	0.004
alpha3	38.84	13.77	36.20	11.79	0.856
alpha5	10.59	1.00	9.84	1.55	0.621
alpha6	30.53	7.93	29.51	12.77	0.932
alphav	23.17	1.96	18.09	0.26	0.068
beta1	45.23	9.10	39.95	11.85	0.407
beta3	3.93	0.67	3.20	0.57	0.058
beta4	8.67	1.53	7.30	1.44	0.456