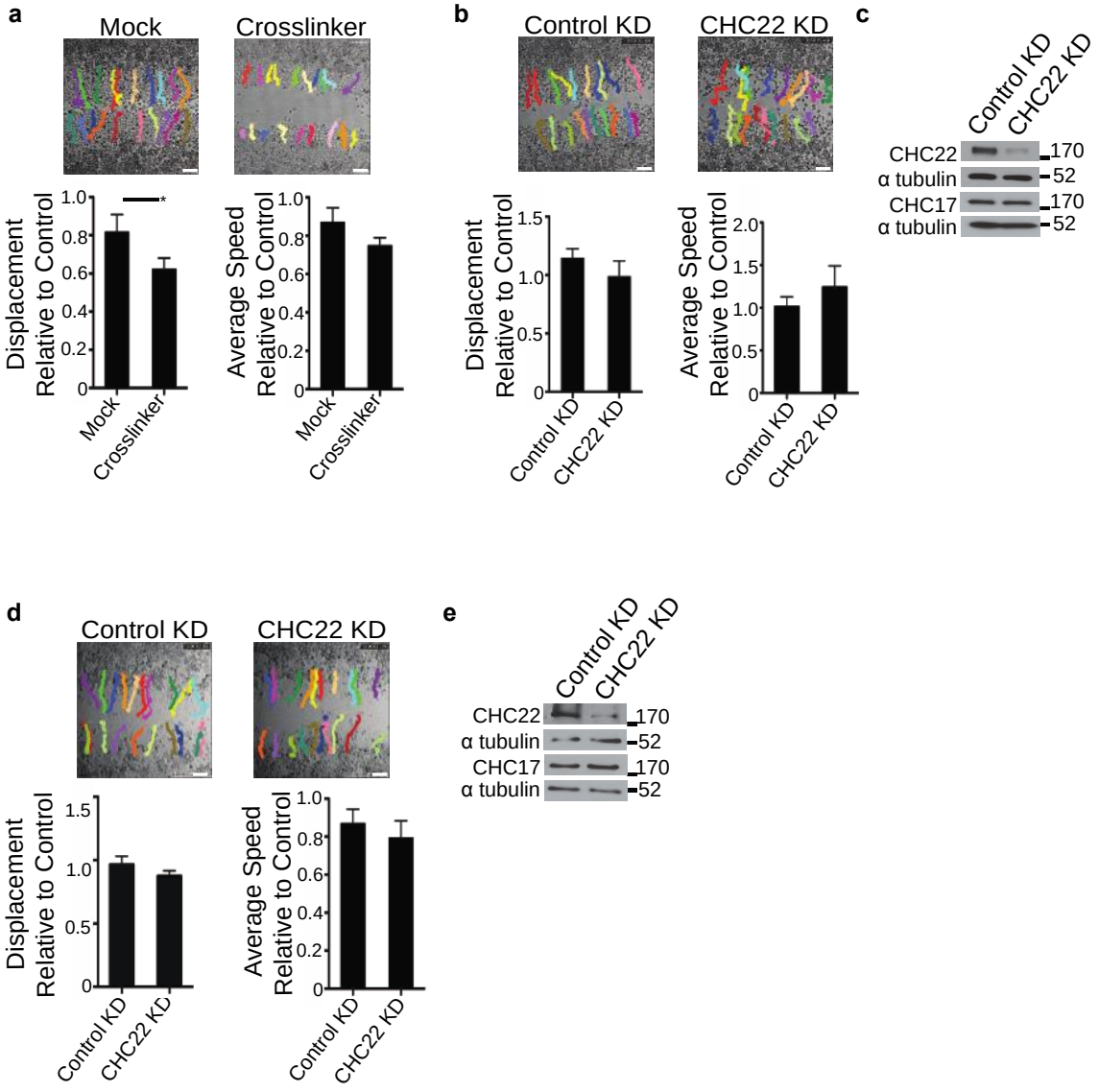
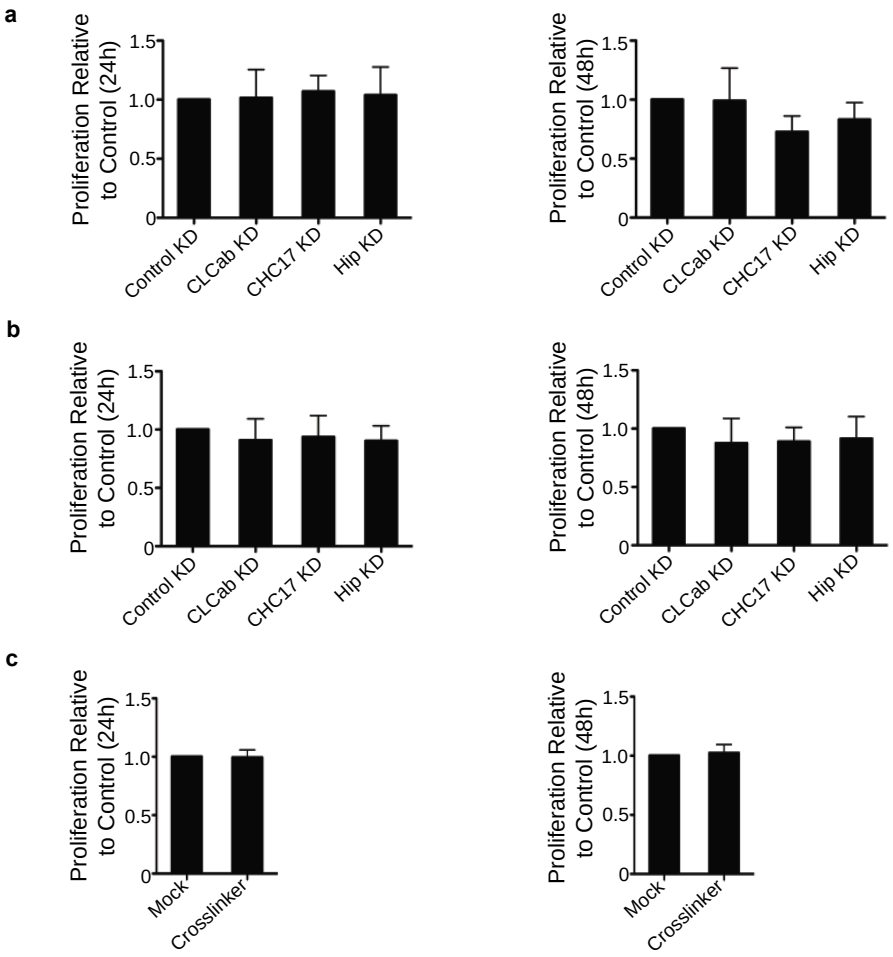
Supplementary Figures

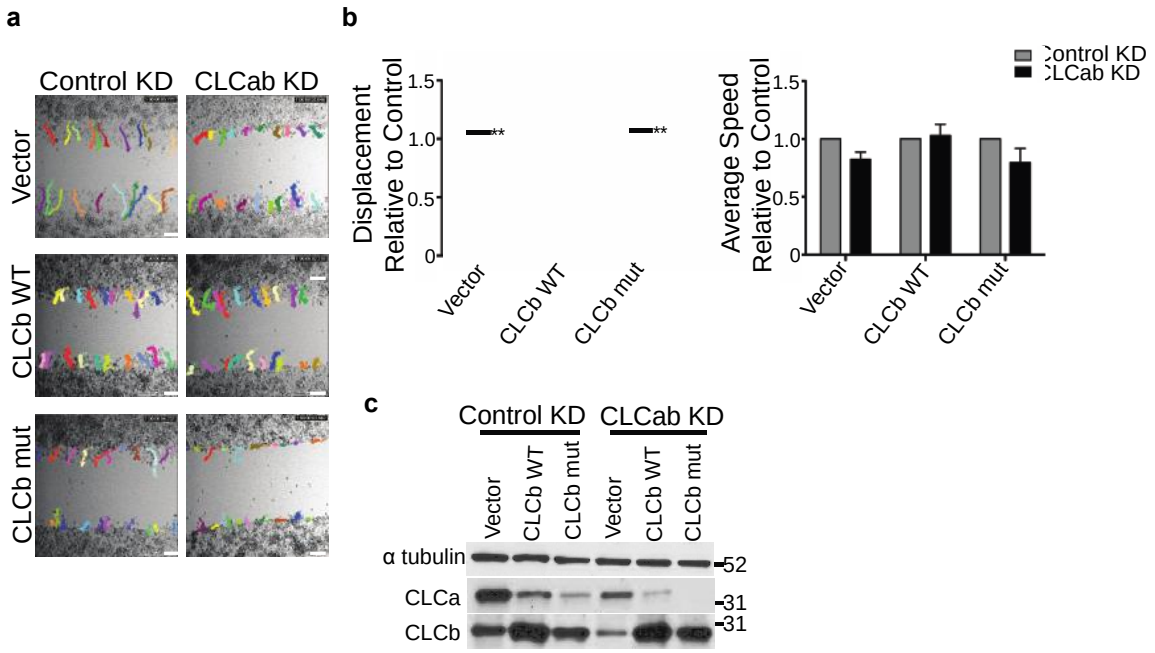


Supplementary Figure 1. Acute inactivation of CHC17 but not depletion of the CHC22 isoform of CHC affects cell migration. (a) HeLa-SNAP-CLCa cells were treated for 2 hours with either DMSO (mock) as a control, or with BG-GLA-BG (crosslinker), which acutely inactivates CHC17 by crosslinking the associated SNAP-tagged CLCa, and cell migration across a wound was assessed. Representative images of cell trajectories at end time points (24 hours) are shown in top panels. Quantitative analysis of net displacement (bottom left) and average speed relative to control, which were calculated as relative to the average control value

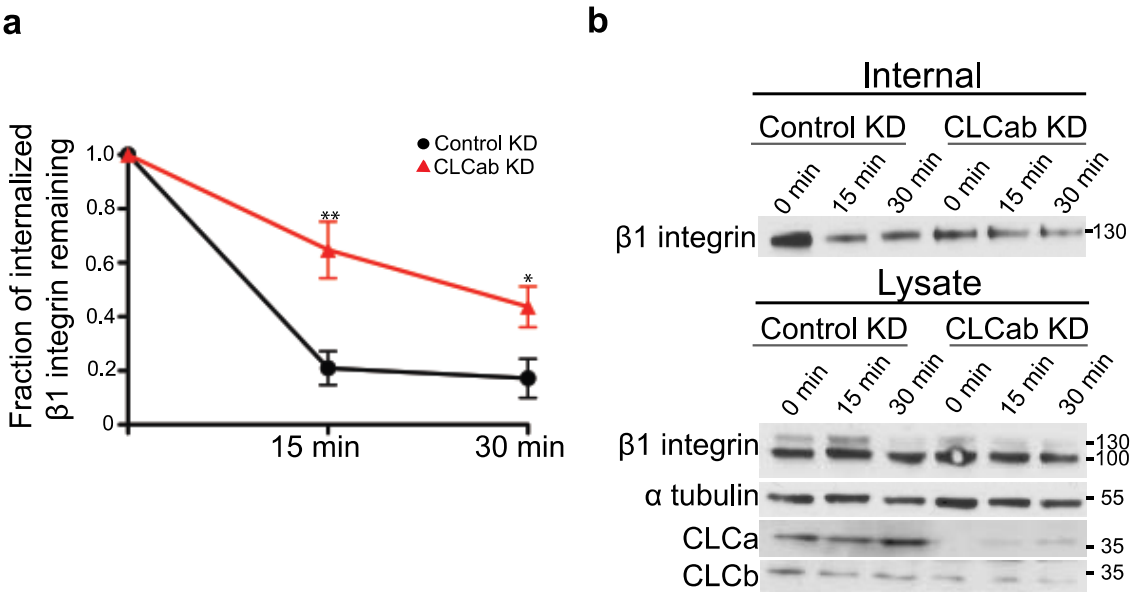
(bottom right; mean ± s.e.m. of 60 cells analyzed from three independent experiments; *P=0.0491; P value, Student's t-test). **(b-e)** Wound-healing assays were performed after 72 hours of the indicated siRNA treatment in HeLa or H1299 cells. Migration across the wound was imaged in the presence of medium containing 1% serum on tissue culture plastic plates using live-cell time-lapse microscopy. **(b)** Representative images of cell trajectories at end time points (24 hours) for migration analyses performed in control and CHC22 siRNA-treated HeLa cells in top panels. Quantitative analysis of relative net displacement (bottom left) and average speed (bottom right; mean ± s.e.m. of 60 cells analyzed from three independent experiments; P=not significant; P value, Student's t-test). **(c)** Representative immunoblots of siRNA treatments in (b). **(d)** Representative images of cell trajectories at end time points (15 hours) for migration assays performed in control and CHC22 siRNA treated H1299 cells in top panels. Quantitative analysis of net displacement (bottom left) and average speed relative to control (bottom right; mean ± s.e.m. of 60 cells analyzed from three independent experiments; P=not significant; P value, Student's t-test). **(e)** Representative immunoblots of siRNA treatments in (d). Migration positions of molecular mass markers are indicated at the right of immunoblot panels in kDa. Scale bars, 100 μm.



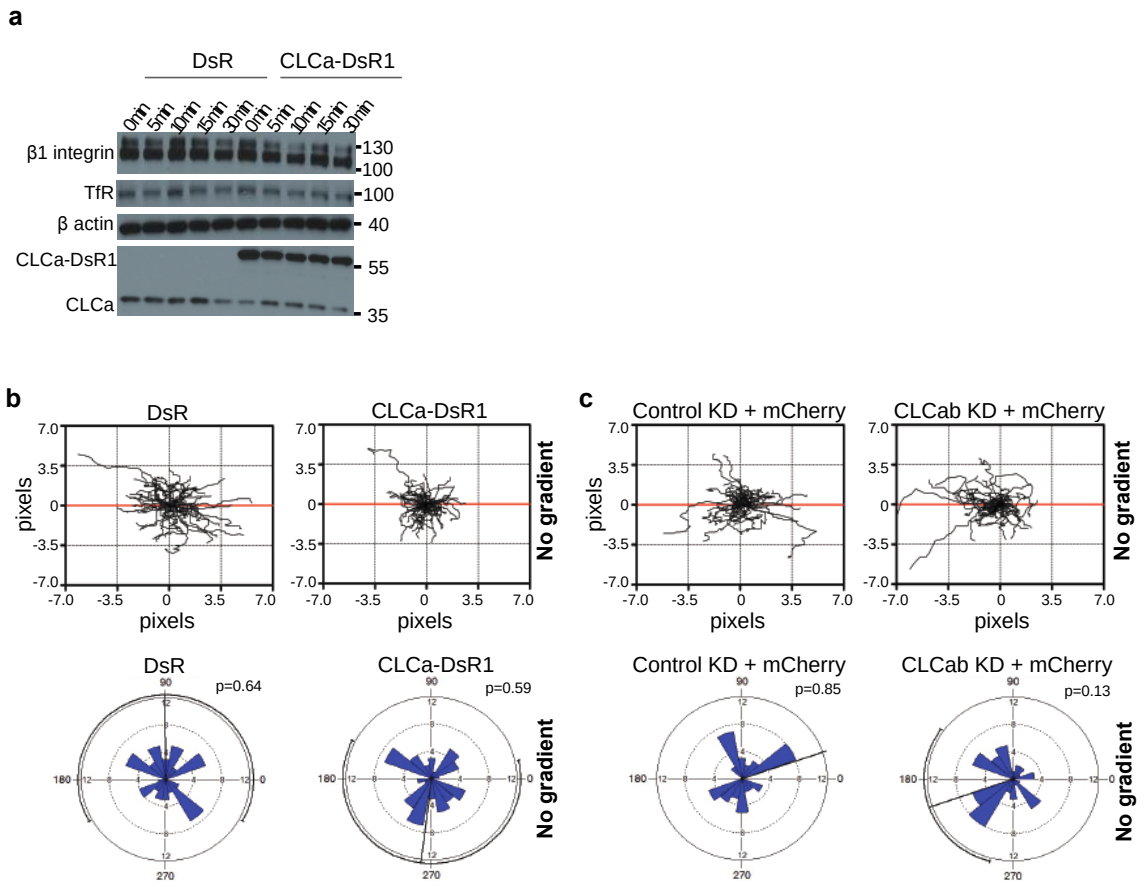
Supplementary Figure 2. CLCab or CHC17 depletion does not cause significant changes in cell proliferation after 24 or 48 hours. (a) HeLa cells treated with the indicated siRNA for 72 hours were plated in triplicate and allowed to adhere for 24 hours (left), or were treated with media containing 1% serum for an additional 24 hours (right), after which cell proliferation was measured by uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (mean ± s.d.; n=3; P= not significant; *P* value, One-way ANOVA). **(b)** Cell proliferation was measured by MTT assay in H1299 cells treated with the indicated siRNA as described above at 24 hours (left) and 48 hours (right) (mean ± s.d.; n=3; P= not significant; *P* value, One-way ANOVA). **(c)** Cell proliferation was measured by MTT assay in a HeLa cell subclone in which clathrin was acutely inactivated after 24 hours (right) and 48 hours (left) (mean ± s.d.; n=3; P= not significant; *P* value, Student's t-test).



Supplementary Figure 3. Hip binding by CLC is required to rescue the migration defect induced by CLC depletion in HeLa cells. Wound-healing assays were performed in HeLa clones that stably expressed empty vector, siRNA-resistant WT CLCb or siRNA-resistant mutant CLCb after 72 hours of control or CLCab siRNA treatment to deplete endogenous CLCs. Migration across the wound was imaged in the presence of medium containing 1% serum using live-cell time-lapse microscopy for 24 hours. **(a)** Representative cell trajectories at end time points are shown. The MtrackJ plugin of ImageJ was used to manually trace cell tracks, marked in color. **(b)** Quantitative analysis of relative net displacement (left) and relative average speed (right) were quantified from track plots (mean ± s.e.m. of 60 cells from three independent experiments; $^{**}P<0.01$; *P* value, Two-way ANOVA followed by Bonferroni post test) in (a). **(c)** Representative immunoblots of siRNA treatments in (a). Migration positions of molecular mass markers are indicated at the right in kDa. Scale bars, 100 μm. We note that the track lengths suggest that over-expression of mutant CLCb in this particular clone reduces the cells' migratory capacity, even under control conditions.



Supplementary Figure 4. CLC depletion reduces recycling of β1 integrin at 15 and 30 min. (a) Recycling of β1 integrin in control and CLCab-siRNA treated HeLa cells assessed by biotinylation as in Fig. 4d (mean ± s.e.m.; n=3; **P*<0.05, ***P*<0.01 *P* value, Two-way ANOVA followed by Bonferroni post test). **(b)** Representative immunoblot of one experiment in (a). Migration positions of molecular mass markers are indicated at the right in kDa.



Supplementary Figure 5. Control experiments for Fig 6 showing protein levels in cell lysate and migration tracks of transfected H1299 cells in the absence of a chemotactic gradient. **(a)** Immunoblot of lysate from samples analyzed in Fig 6f. Migration positions of molecular mass markers are indicated at the right in kDa. **(b)** The Dunn chamber migration tracks (over 3 hours) of H1299 cells transiently transfected with DsR or CLCa-DsRed1 in the presence of control media with circular histograms (below) depicting the proportion of cells whose final position was within each of 18 equal 20° sectors, generated as in Fig. 6g. **(c)** The Dunn chamber migration tracks (over 3 hours) of H1299 cells transfected with mCherry plus either control or CLCab-targeting siRNA in the presence of control media with circular histograms (below) as in (b) In b and c: mean and 95% confidence interval are indicated by black line; at least 50 cells were analyzed from 4 independent experiments for each condition, *P* values, Raleigh uniformity test).