Video S1. Live-cell single-cell tracking of HeLa cells co-overexpressing GFP-tagged Nup88 and mCherry-tagged truncated Kif7. Live-cell single-cell tracking was performed as described in the Materials and methods. A total of 24 h of time-lapse images, captured every 10 min, were edited into a 10 sec video. The data are presented in Fig. 2C. The scale bar indicates 20 μm. Nup88, nucleoporin 88.

Video S2. Live-cell single-cell tracking of HeLa cells over-expressing GFP-tagged Nup88 following Gli1 depletion by siRNA transfection. Live-cell single-cell tracking was performed as described in the Materials and methods. A total of 24 h of time-lapse images , captured every 10 min, were edited into a 10 sec video. The data are presented in Fig. 5B. The scale bar indicates 20 μm. Gli1, glioma-associated oncogene homolog 1; Nup88, nucleoporin 88.

Figure S1. Analysis of the involvement of PI3K-AKT-mTOR and TGF-β signaling in the increased expression of Gli1 in Nup88 overexpressing cells. Analysis of AKT activation. Immunoblotting was performed to assess the activation status of AKT by examining the phosphorylation state of (A) threonine 308 (pT308) and (B) serine 473 (pS473) in cells overexpressing GFP or GFP-tagged Nup88 (Nup88-GFP). No difference in the phosphorylation status of AKT was observed between the two cell lines. α-Tubulin was used as a loading control. Analysis of mTOR activation. Immunoblotting was carried out to assess the activation status of mTOR by measuring the phosphorylation state of (C) serine 2448 (pS2448) and (D) serine 2481 (pS2481) in cells overexpressing GFP or Nup88-GFP. No difference in the phosphorylation status of mTOR was observed between the two cell lines. α-Tubulin was used as a loading control. Findings in (A-D) suggested that PI3K-AKT-mTOR signaling may not be involved in the increased expression of Gli1 in Nup88-overexpressing cells. Analysis of Smad2/3 activation. (E) Cytosolic and nuclear fractions were prepared from cells overexpressing GFP or Nup88-GFP. (F) Nuclear translocation of Smad2/3 was analyzed by immunoblotting. The same cell lysates in (E) were loaded onto a separate gel. Detection of Lamin A/C in the nuclear fraction confirmed that the fractionation procedure was performed properly. β-actin was used as a loading control. Translocation of Smad2/3 into the nucleus was not observed in either cell line, suggesting that TGF-β signaling may not be involved in the increase in Gli1 expression. PI3K-AKT-mTOR, phosphoinositide 3-kinase-Protein kinase B-mechanistic target of rapamycin; Gli1, glioma-associated oncogene homolog 1; Nup88, nucleoporin 88; Cyt., cytosolic; Nu., nuclear