Online supplemental material

Figures S1-S4 show transmission electron microscopy of stratifying immortalized wt and P0 keratinocytes, P1c-MT cosedimentation, P1c−/− keratinocytes transfected with P1c variants lacking the IF-binding domain and full-length P1c without drug challenge, and glucose uptake measured after MT disruption by colchicine, respectively. Figure S5 presents inhibition of MAP-MT interaction using plectin fragment p16-21. Details about the primers used for RT-PCR analysis are shown in Table S1. Video 1 shows time-lapse images of EGFP-tubulin-transfected wt and P0 keratinocytes. Video 2 shows a survey view of GFP-EB1 comets in wt keratinocytes. Video 3 shows a survey view of GFP-EB1 comets in P0 keratinocytes. Video 4 shows a survey view of GFP-EB1 comets in P1c−/− keratinocytes. Video 5 shows a comparison of wt, P0, and P1c−/− MT growth rates by tracing GFP-EB1. Video 6 shows detail captures of GFP-EB1 comets in wt keratinocytes. Video 7 shows detail captures of GFP-EB1 comets in P0 keratinocytes. Video 8 shows detail captures of GFP-EB1 comets in P1c−/− keratinocytes.
Figure S1. Transmission electron microscopy of stratifying (1.8 mM Ca\(^{2+}\); 3h) immortalized wt and P0 keratinocytes. Sections were cut parallel to the cell layers and close to the cellular periphery (a and b). In wt cells sectioned underneath and parallel to the plasma membrane, only a few MTs were identifiable (a), whereas in corresponding sections of P0 cells numerous MTs were visualized (b). In sections cut perpendicularly to the cell layer, densely packed filamentous structures resembling longitudinally- and cross-sectioned MTs, surrounded by electron dense material of unknown nature, could be seen in P0, but not in wt cells (c and d). In section corresponding to (d), typical (25 nm) MT structures could clearly be identified at higher magnification (e); longitudinally (parallel lines) and cross-sectioned (circles) MTs detected are outlined in (f). Scale bars, 500 nm (a-d); 150 nm (e).
**Figure S2.** Partial cosedimentation of keratinocyte P1c with taxol-stabilized brain MTs. Cell homogenates prepared from immortalized wt keratinocytes were separated into high-speed supernatant (HS-SN) and pellet (HS-P) fractions. Aliquots of the HS-SN fractions were then incubated with (+MT) or without (-MT) taxol-stabilized brain MTs, and centrifuged to yield pellet (P) and supernatant (SN) fractions. Immunoblots of the various fractions using isoform-specific antibodies to P1c and P1a are shown. Note partial (~30%) cosedimentation of endogenous P1c, but not P1a with pre-assembled MTs.
Figure S3. Neither P1c variants lacking the IF-binding domain, nor full-length P1c-EGFP effect MT depolymerization without drug challenge. Primary P1c−/− keratinocytes transfected with full-length P1c-EGFP (upper row), P1c8-EGFP (middle row), and P1c-30-EGFP (lower row) were immuno-labeled using antibodies to α-tubulin. Scale bar, 15 µm.
Figure S4. Influence of colchicine on glucose uptake of keratinocytes. Primary P0 keratinocytes were incubated with 2-NBDG in the presence and absence of 10 µM colchicine for 30 min. Fluorescence intensities of cells were measured as in Fig. 7 (n=3; 200 cells/experiment). Error bars, ± SEM. Scale bar, 1 mm.
Figure S5. Plectin's SH3 domain compromises MAP-MT interaction. (A) Inhibition of MAP2c-promoted in vitro assembly of MTs by fragment p16-21. MTs were assembled in vitro from purified samples of tubulin and recombinant MAP2c in the presence of fragment p16-21 (at concentrations indicated) and sedimented by centrifugation. Resulting pellet (p) fractions (containing polymerized MTs and MT-bound MAP2c), and supernatant (s) fractions (containing soluble tubulin and unbound MAP2c, as well as p16-21) were analyzed by SDS-PAGE. Coomassie-stained gel bands corresponding to MAP2c and tubulin in s and p fractions were quantified (bar graph). Error bars, ± SEM (n=8). (B) Far UV circular dichroism (CD) spectrum of purified fragment p16-21 (concentration 0.11 mg/ml) measured in 2 mM sodium phosphate, pH 6.8 and 4 mM NaCl at 25°C. Note, the CD spectrum is typical for an α-helical protein, confirming that protein fragment p16-21 adopts a well defined structure under in vitro conditions.
**Table S1** Primers used for RT-PCR analysis

| MAP2 primer set          | Tau primer set                                         |
|-------------------------|--------------------------------------------------------|
| mMAP2\_fw               | mM Tau/exon9\_fw                                      |
| 5’- GGAAAGATGAAGGAAAAGGCAACCA  | 5’ CACCAAAAATCCGGAGAACGA                                 |
| mMAP2\_rev              | mM Tau/exon11\_rev                                     |
| 5’- GGCTGGTCTTTTGATTTGGGCTTCC | 5’ CTGGCTCAGGTCCACCAGGC                                   |
| Amplification product: 344 bp | Amplification products: 390 bp (exon 10+), 297 bp (exon 10-) |
**Video legends**

**Video 1.** Time-lapse images of EGFP-tubulin-labeled MTs in immortalized wt and P0 keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV) at 2 s intervals. (Left panel) The shrinking of two MTs (outlined in Fig. 4A) in a wt cells is traced by black and red arrowheads (appearing one after the other). Note that the MTs, having grown perpendicular to the cell margin, undergo catastrophe at 0 s and 24 s, respectively. A blue arrowhead (appearing at 44 s) indicates a MT that stays at the membrane until it undergoes catastrophe at time point 76 s. (Right panel) Tracing of two MTs (outlined in blue in Fig. 4A) that fail to undergo catastrophe in P0 cells. Note that after having reached the plasma membrane, the MTs have bent and stay parallel to the cell margins (red and black arrowheads).

**Video 2.** Time-lapse images of GFP-EB1 comets in immortalized wt keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV) at 2 s intervals. Squares indicate the frames selected for Video 6 and Fig. 4F.

**Video 3.** Time-lapse images of GFP-EB1 comets in immortalized P0 keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV) at 2 s intervals. Note higher numbers of GFP-EB1 comets per cell area in P0 keratinocytes than in wt cells (Video 2). Squares indicate the frames selected for Video 7 and Fig. 4F.

**Video 4.** Time-lapse images of GFP-EB1 comets in primary P1c−/− keratinocytes. Images were collected using an inverted microscope (Olympus, Delta Vision®) at 2 s intervals. Note higher numbers of GFP-EB1 comets per cell area in P1c−/− keratinocytes than in wt cells (Video 2). Squares indicate the frames selected for Video 8 and Fig. 4F.

**Video 5.** Time-lapse images of GFP-EB1 comets in immortalized wt (upper panel), immortalized P0 (lower panel), and primary P1c−/− (lower panel) keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV or Olympus, Delta Vision®) at 2 s intervals. Note that within the same period of time, the EB1 comets traced in P0 and P1c−/− cells (red and yellow arrowheads) cover larger distances than the one in the wt cell (white arrowhead).
**Video 6.** Time-lapse images of GFP-EB1 comets at the cell margins of immortalized wt keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV) at 2 s intervals. Note that wt MTs approach the membrane perpendicularly, pause once they reach it, and then disappear (colored arrows, appearing in succession).

**Video 7.** Time-lapse images of GFP-EB1 comets at the cell margins of immortalized P0 keratinocytes. Images were collected using an inverted microscope (Zeiss, Axiovert S100TV) at 2 s intervals. Note that MTs in P0 cells, upon reaching the cell margins, start bending and continue growing (right panel, three arrows appearing in succession).

**Video 8.** Time-lapse images of GFP-EB1 comets at the cell margins of primary P1c<sup>−/−</sup> keratinocytes. Images were collected using an inverted microscope (Olympus, Delta Vision<sup>®</sup>) at 2 s intervals. Note that MTs in P1c<sup>−/−</sup> cells, grow parallel to the membrane (first three colored arrows appearing in succession) or start bending and continue growing upon reaching the cell margins (fourth arrow in black).