Immunofluorescence

Cells were washed in PBS, resuspended for 10 minutes in PBS containing 2 mM ethylene glycol bis-N,N,N′,N′ – tetraacetic acid (EGTA) and cytocentrifuged on slides. Cells were then fixed in 0.5% glutaraldehyde/4% paraformaldehyde for 15 minutes and transferred to ice-cold methanol containing 1 mM EGTA for 20 minutes. After three washes in PBS, followed by blocking with 3% BSA, the slides were incubated with α-tubulin antibodies (Sigma, St Louis, MO) for 1 hour. Immunofluorescence for stathmin was performed using 3.7% paraformaldehyde /0.1% Triton X fixation and permeabilization followed by incubation with stathmin antibodies (BD Transduction Laboratories, Mountain View, CA) for 1 hour. The slides were washed and incubated for 1 hour with the secondary FITC-conjugated anti-mouse IgG antibodies (Sigma, St Louis, MO). After three washes in PBS, the cells were incubated with 1.5 μM Hoechst 33342 (Sigma, St Louis, MO) in PBS, rinsed in PBS and mounted under coverslips with Vectashield mounting solution (Vector Laboratories, Burlingame, CA). Image acquisition and analysis was performed using a Zeiss Axioplan2 microscope equipped with an AxioCam MRm camera and Zeiss AxioVision Software.

Electron microscopy

The cultured MK pellets were fixed in 2.5% glutaraldehyde with 0.2 M sodium cacodylate at pH 7.4. After overnight fixation, the fixative solution was removed and replaced with a phosphate buffer followed by 1% osmium tetroxide buffered with sodium cacodylate. After one hour the osmium was replaced with increasing concentrations of ethanol through propylene oxide and into embed 812. One micrometer plastic sections were cut, stained with methyl blue and azure II and observed by light microscopy. Representative areas were chosen for ultrathin sectioning and observed with a HitachiH-7650 transmission electron microscope (Hitachi High – Technologies Instrumentation, Tokyo, Japan) linked to a SIA (Scientific Instruments and Applications) digital camera controlled by Maxim CCD software.

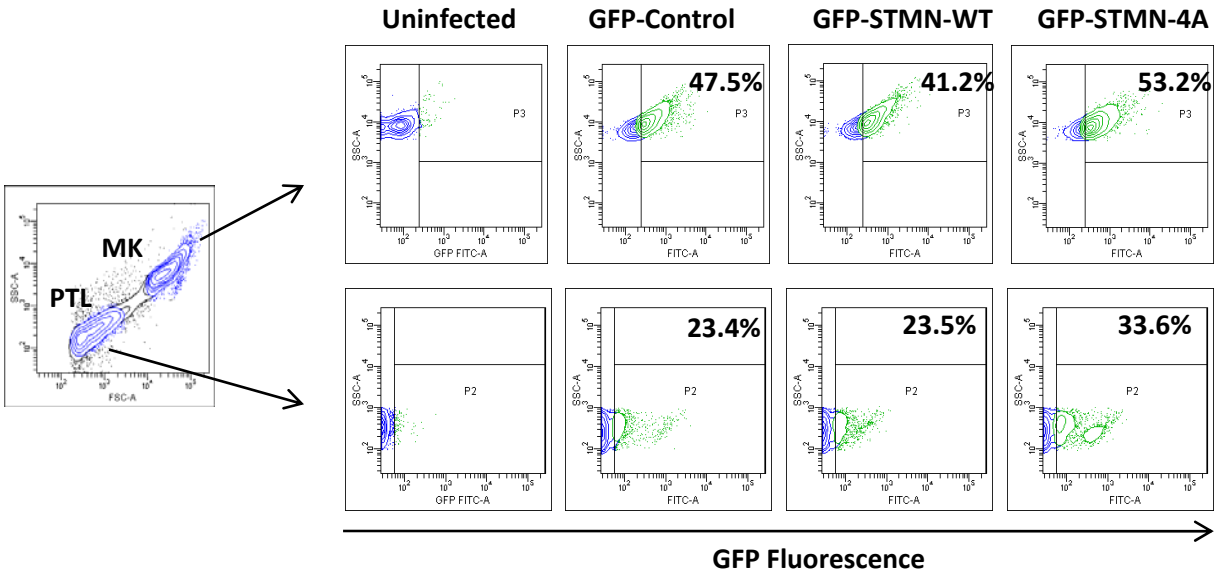

Figure S1. Flow cytometric analysis of lentiviral transduction efficiency in primary human MK. MK uninfected or infected with GFP or with GFP and stathmin-expressing lentiviruses (GFP-STMN) were generated as described in *Materials and Methods.* Following labeling with anti-CD41 antibodies, MK (upper panels) and platelets (PTL) (lower panels) were analyzed for GFP expression by flow cytometry. The numbers represent the percentage of GFP^+ MK or culture-generated platelets calculated from the total of number of $CD41^+$ cells in a representative experiment .

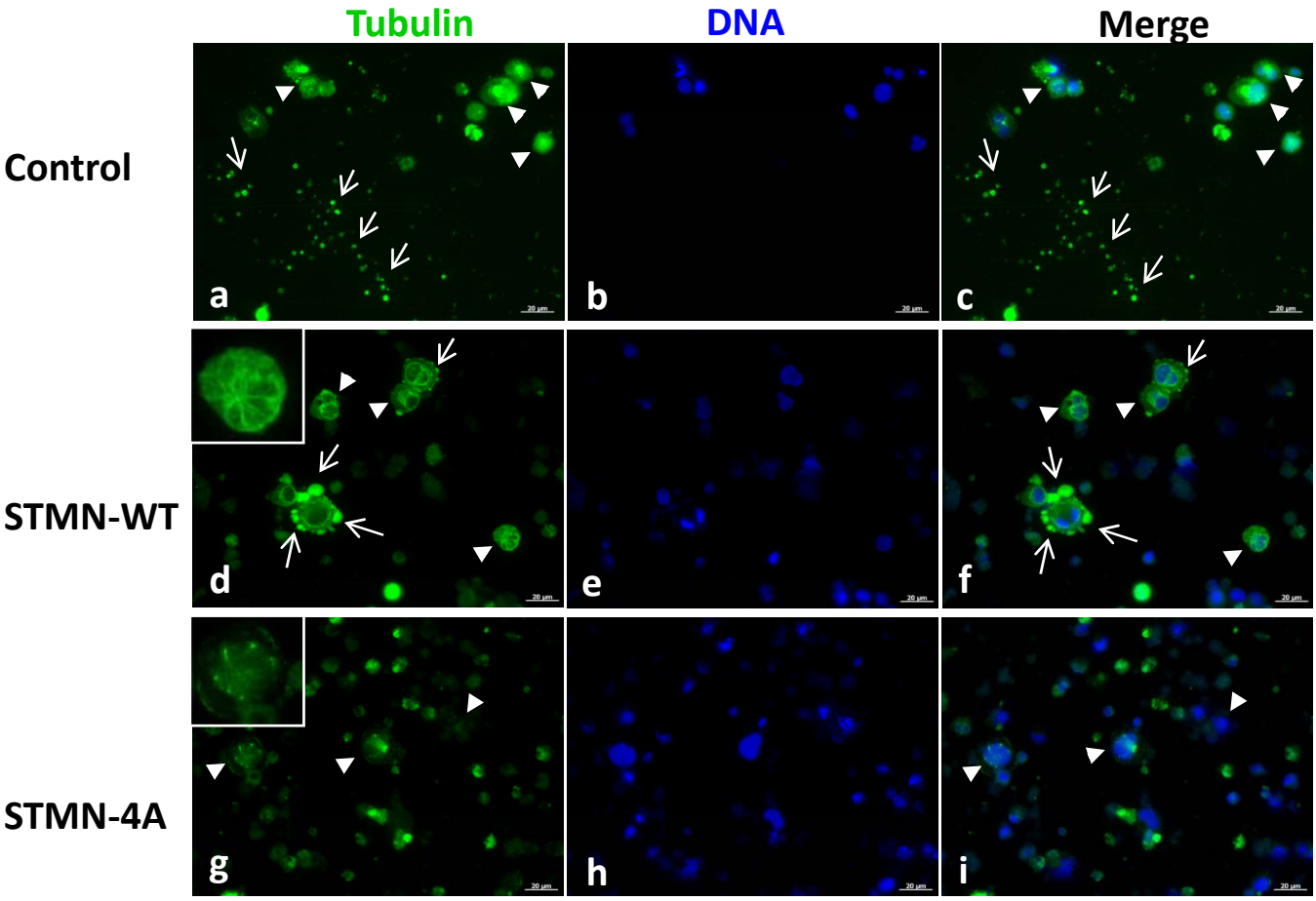

Figure S2. Microtubule organization in primary human MK transduced with control and stathmin-expressing lentiviruses. MK generated in the presence of control, wild-type (STMN-WT) or constitutively active (STMN-4A) stathmin-expressing lentiviruses were labeled with anti-α-tubulin primary antibodies followed by FITC-conjugated secondary antibodies to visualize microtubules (*green* fluorescence) and with Hoechst 33342 to visualize DNA and nuclear morphology (*blue* fluorescence). Both microtubule networks and cytoplasmic tubulin are present in control and STMN-WT-transduced MK (*arrow heads* in a, c, d and f and inset in d). Short bundles of microtubules and less cytoplasmic tubulin immunofluorescence are observed in STMN-4A-transduced MK (*arrow heads* in g and i and inset in g). Of note, platelet-sized cells with intense tubulin fluorescence and lacking nuclei are observed in control cultures (*arrows* in a and c). STMN-WT-transduced MK show cytoplasmic blebs suggestive of platelets with variable size and diffuse tubulin immunofluorescence (*arrows in d and f*). Magnification 63x/oil.

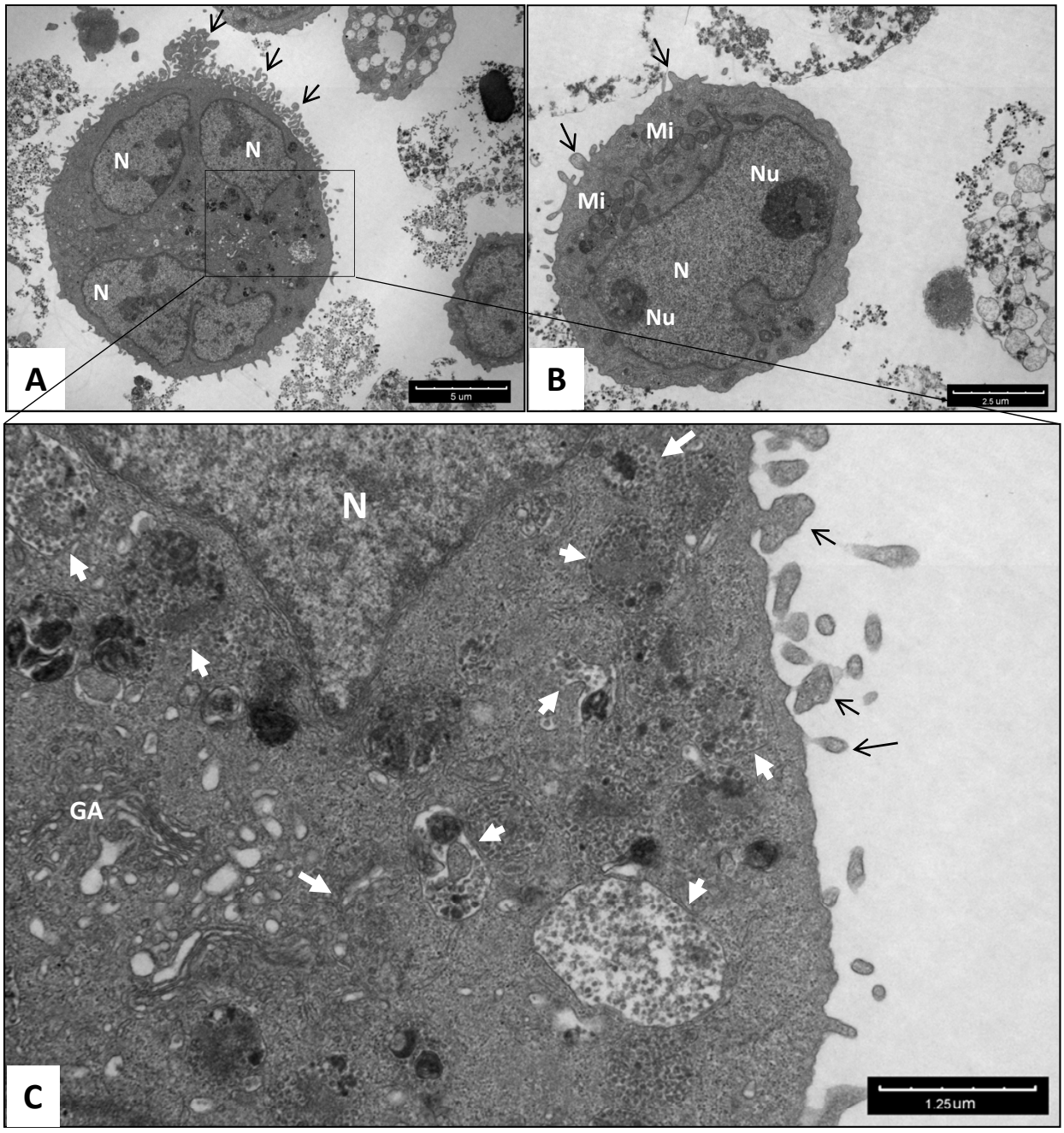

Figure S3. Transmission electron microscopy of MK. Representative electron microscopy microphotographs of MK infected with wild-type stathmin lentiviruses (A) or constitutively active stathmin (B). Note, multilobulated nucleus (N), abundant Golgi apparatus (GA) multivesicular bodies (*white arrows* in C) and numerous cytoplasmic blebs suggestive of microparticles (*black arrows* in A and C); Immature nucleus (N) with nucleoli (Nu), numerous mitochondria (Mi) and less frequent cytoplasmic blebs (*black arrows*) are observed in B. Magnification: 2000X in A, 4000X in B and 8000X in inset C.