Supporting Information

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SI Materials and Methods

Lentiviral Infections and shRNA Sequences. Lentiviral shRNA constructs were obtained from the RNAi Consortium. Sequences of shRNAs are shown in Table S1. Details of lentiviral infections are described in the companion article.

Cell Fixation and Flow Cytometry Analysis. Cell fixation and flow cytometric analysis were carried out as described in the companion article. For cells expressing H2BGFP, cells were fixed in ethanol, washed once with PBS, and stained with anti-GFP at 1:400 in PBS for 1.5 h. Cells were pelleted and stained with AlexaFluor 488-conjugated donkey anti-chicken antibody at a ratio of 1:400 in PBS for 1 h. Cells were then pelleted, stained with propidium iodide in PBS with RNase, and analyzed.

Immunofluorescence Microscopy. For visualization of endogenous proteins, following one PBS wash, cells were fixed once with 0.5% paraformaldehyde in PBS for 10 min, followed by a second fixation with ice-cold 100% methanol for 5 min. Alternatively, cells were fixed in ice-cold 10% (vol/vol) trichloroacetic acid for 15 min on ice. Coverslips were washed three times with PBS plus 30 mM glycine and permeablized with 0.1% Triton X-100 for 30 s. Following one PBS wash, coverslips were blocked with 1% normal donkey serum in PBS (Equitech Bio). Coverslips were incubated overnight at 4 °C with primary antibody. Following three PBS washes, cells were incubated with the appropriate AlexaFluor-conjugated secondary antibodies (Invitrogen) for 1 h at room temperature. Cells were washed an additional three times in PBS, with the second wash containing 200 ng/mL propidium iodide where indicated (Sigma). Coverslips were mounted using Vectashield mounting media containing DAPI. Images were obtained using a Zeiss LSM 510 laser scanning microscope and a 100× objective. Approximately 50 confocal sections were obtained per mitotic cell at an interval of 0.37 μm.

Cell Lysis, Immunoprecipitation, and Western Blotting. Protein extracts were prepared with lysis buffer [50 mM Hepes (pH 7.8), 150 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, 1 mM DTT, and protease inhibitor mixture and phosphatase inhibitor mixture (both from Roche)]. Lysates were subjected to SDS/PAGE and transferred to nitrocellulose. Primary antibodies were used at a ratio of 1:1,000 in 5% (wt/vol) milk in TBS/0.1% Tween-20. For immunoprecipitation, 1 mL of cell lysate was precleared with protein G agarose beads for 30 min followed by incubation for 2 h with anti-Flag M2-conjugated agarose beads (Sigma). Beads were washed two times with lysis buffer followed by two washes with buffer D [20 mM Hepes (pH 7.8), 15% (vol/vol) glycerol, 150 mM KCl, 0.2 mM EDTA, 0.1% Nonidet P-40, and 1 mM DTT]. Beads were eluted with 500 μg/mL 3× FLAG peptide (Sigma) in buffer D. Immunoprecipitates were subjected to SDS/PAGE and Western blotting.

Quantitative RT-PCR. Details of RNA preparation and quantitative PCR (qPCR) analysis appear in the companion article. Probes used in this manuscript were no. 60 (GAPDH), no. 67 (GFP), no. 8 (RhoA), no. 80 (dynamin), and no. 37 (Cdc42). Primer sequences for qPCR are shown in Table S2.

Senescence-Associated β-Galactosidase Assay. HFK or HFK/E6 cells were infected with scrambled or NME1 shRNAs and were fixed and stained for acidic β-galactosidase activity as described (1).

1. Dimri GP, et al. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 92:9363–9367.

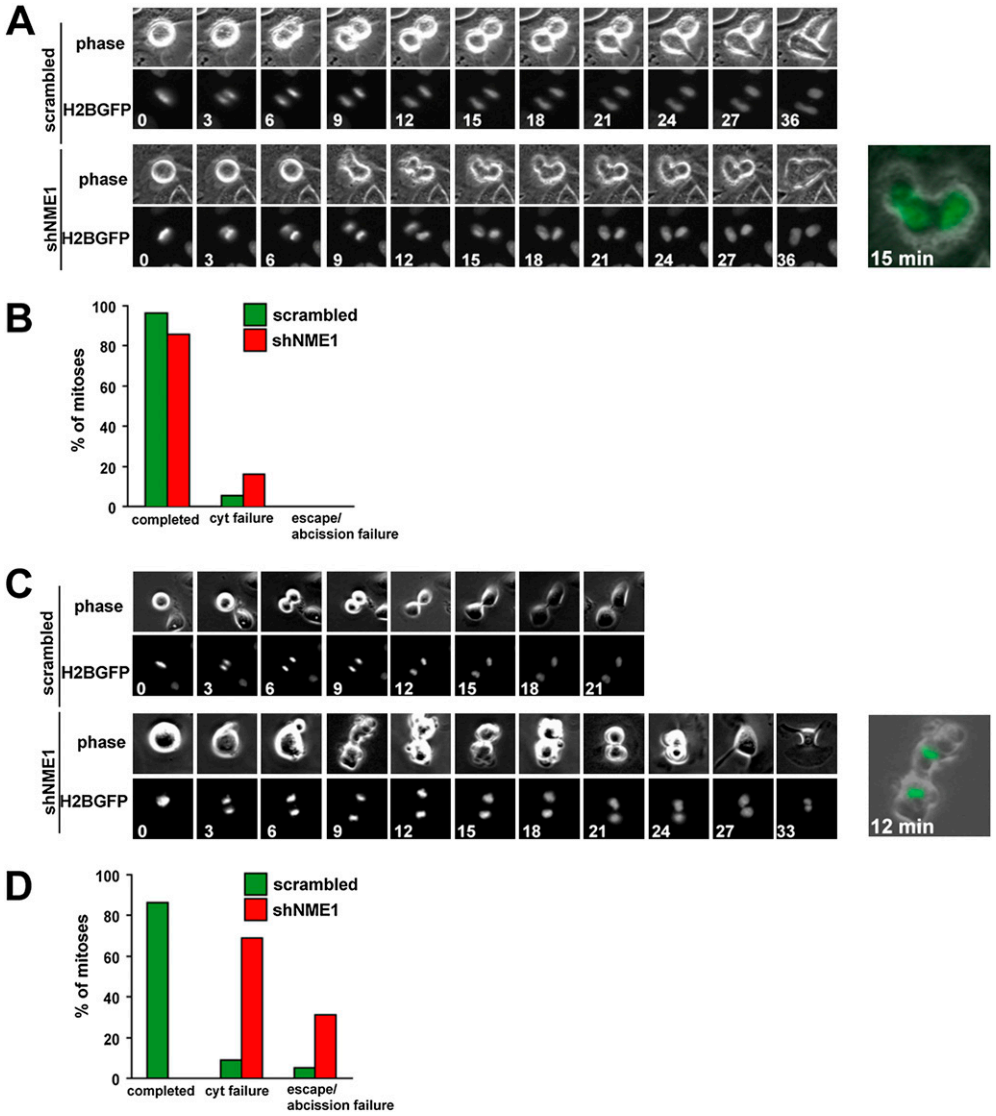

Fig. S1. Knockdown of NME1 causes cytokinesis failure in HeLa and HFK/E6 cells. (*A*) Examples of the late stages of mitosis in HeLa cells expressing a scrambled control or NME1 shRNA. shNME1, short hairpin NME1. HeLa cells were infected with H2BGFP-containing shRNA vectors, and time-lapse experiments were carried out as in Fig. 3. Images were captured at eight stage positions for each sample. (*B*) Quantitation of mitoses from one time-lapse experiment in HeLa cells as described in *A*. Each mitosis occurring during the course of the experiment was classified as completed, cytokinesis (cyt) failure, or escape or abscission failure. Scrambled, $n = 53$; shNME1, $n = 62$. (*C*) Examples of the late stages of mitosis in control or shNME1 primary HFKs expressing HFK/E6. Cells were infected and analyzed as described in *A*. Images were captured at eight stage positions for each sample. (*D*) Quantitation of mitoses from one time-lapse experiment in HFK/E6 cells as described in *C*. Analysis was carried out as in *B*. Scrambled, $n = 80$; shNME1, $n = 29$.

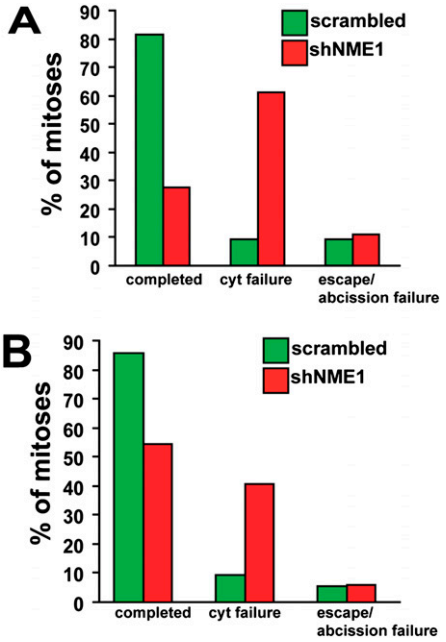

Fig. S2. Knockdown of NME1 causes cytokinesis (cyt) failure in HBE135 and HFK cells. (*A*) Quantification of time-lapse imaging in Fig. 2*A* for HBE135 cells. Categories are as described for Fig. S1*B*. Scrambled, $n = 38$; short hairpin NME1 (shNME1), $n = 92$. (*B*) Quantification of time-lapse imaging in Fig. 2*A* for HFK cells. Categories are as described for Fig. S1*B*. Scrambled, $n = 41$; shNME1, $n = 63$.

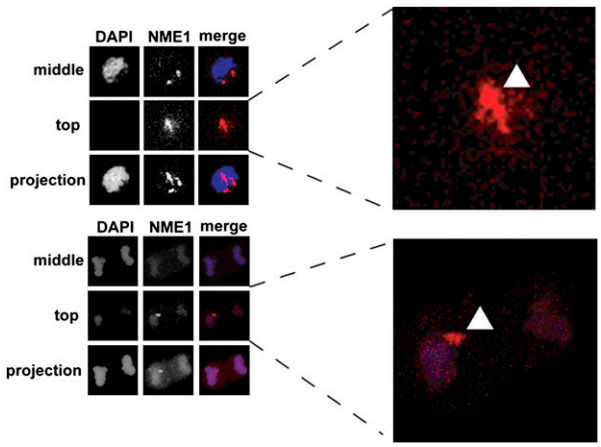

Fig. S3. NME1 localizes to the cortex in primary HFK cells. NME1 localizes to the cortex of primary HFK cells in prometaphase and late anaphase. HFK cells were processed for immunofluorescence as in Fig. 4 and stained with anti-NME1 (red) and DAPI (blue). Sections are as described in Fig. 4. (*Right*) Arrowheads in the zoomed images indicate cortical NME1 (red).

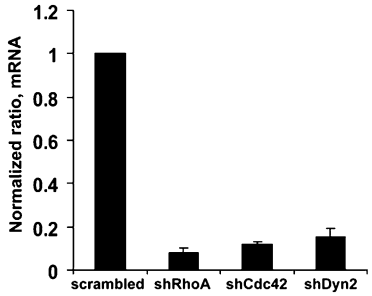

Fig. S4. shRNAs targeting RhoA, Cdc42, and dynamin efficiently knock down their targeted mRNA. HBE135 cells were transduced with lentiviral scrambled shRNAs or shRNAs targeting RhoA (shRhoA), Cdc42 (shCdc42), or dynamin (shDyn2). After 3 d, total RNA was prepared and reverse-transcribed and quantitative PCR was performed. Normalized ratios were calculated by normalizing mRNA levels to those of GAPDH and comparing the ratios with the scrambled control cells. Error bars represent SEM of two replicates.

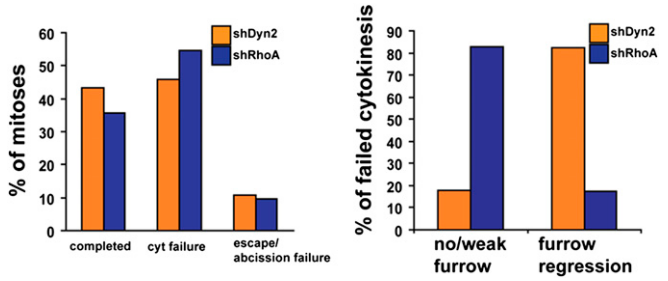

Fig. S5. Knockdown of dynamin phenocopies knockdown of NME1. (*Left*) Quantification of one time-lapse experiment as described in Fig. 4. The results of mitoses were classified as in Fig. 3*B* [*n* = 42 (shRhoA) or 37 (shDyn2)]. cyt, cytokinesis. (*Right*) Cytokinesis failure resulting from dynamin-2 knockdown follows furrow regression. The percentage of cells failing cytokinesis that demonstrated furrow ingression and regression for shRhoA or shDyn2 knockdown cells is shown.

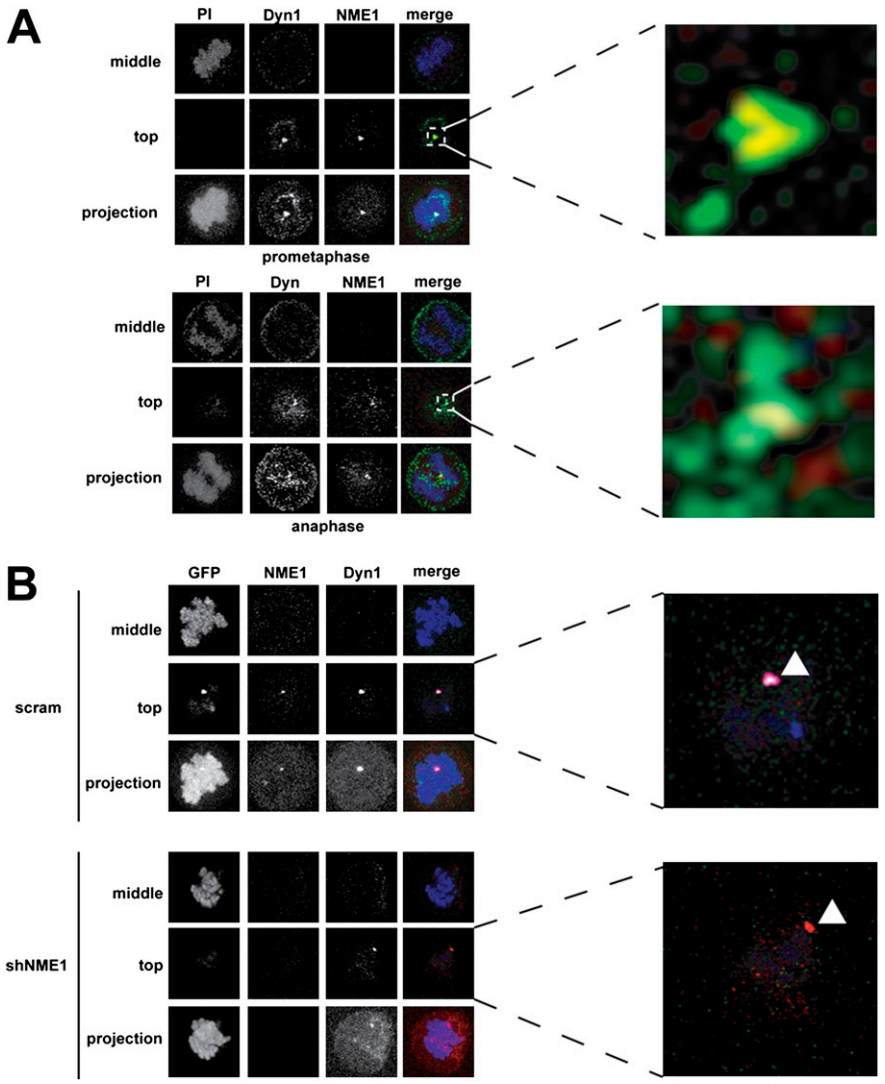

Fig. S6. Dynamin and NME1 colocalize. (*A*) NME1 and dynamin (Dyn1) colocalize at the cortex in prometaphase and anaphase. HBE135 cells were fixed and stained with anti-NME1 (red) and anti-dynamin1/2 (green). Nuclei were stained with propidium iodide (PI, blue). Confocal sections were obtained as in Fig. 6*C*. (*Right*) Zoomed images are of the merged top sections; arrowheads indicate colocalization (yellow) of NME1 and dynamin. (*B*) NME1 is not required for cortical localization of dynamin. HBE135 cells were transduced with H2BGFP-containing shRNA vectors containing scrambled or NME1 shRNAs. After 2 d, cells were fixed and stained with anti-GFP (false-colored blue), anti-NME1 (green), or anti-dynamin 1/2 (red). Confocal sections were obtained as in Fig. 6*C*. (*Right*) Zoomed images show dynamin colocalized with NME1 (scrambled) or in the absence of NME1. shNME1, short hairpin NME1.

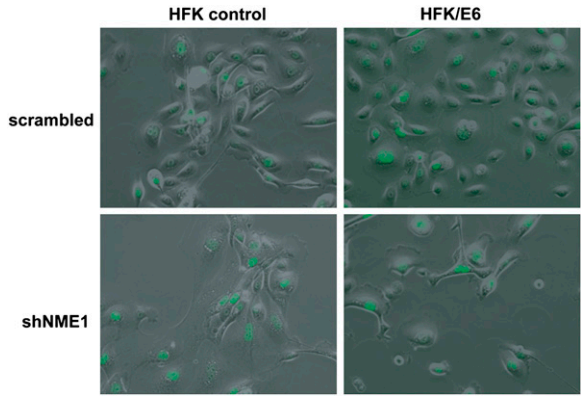

Fig. S7. Loss of NME1 results in morphological changes in primary cells. Primary HFK or HFK/E6 cells were transduced with H2BGFP-containing scrambled or NME1 shRNA vectors. Images were obtained 96 h following infection. shNME1, short hairpin NME1.

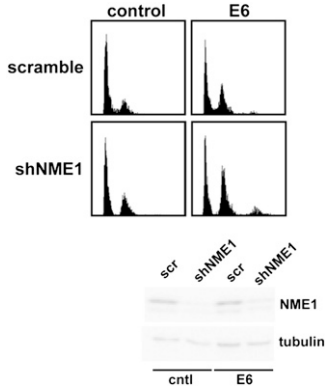

Fig. S8. NME1 knockdown in HFK and HFK/E6 cells. HFK and HFK/E6 cells were infected with scrambled or short hairpin NME1 (shNME1) lentivirus and fixed for FACS analysis after 4 d. Lysates were also prepared to assess NME1 and tubulin protein levels. cntl, control, scr, scrambled.

Table S1. Sequences of shRNAs

Gene	Name	Accession	Position	Region	Target 21-mer
NME1	shRNA 1	NM_000269	NM_000269.x-182s1c1	CDS	TCCGCCTTGTTGGTCTGAAAT
NME1	shRNA 2	NM_000269	NM_000269.x-183s1c1	CDS	CCGCCTTGTTGGTCTGAAATT
NME1	shRNA 3	NM_000269	NM_000269.x-452s1c1	CDS	TGGAGAGTGCAGAGAAGGAGA
NME1	shRNA 4	NM_000269	NM_000269.x-99s1c1	5′-UTR	GCGTACCTTCATTGCGATCAA
NME1	shRNA 5	NM_000269	NM_000269.x-270s1c1	CDS	CGGCCTGGTGAAATACATGCA
RhoA		NM_001664.1	NM_001664.1-300s1c1	CDS	GAAAGCAGGTAGAGTTGGCTT
Cdc42		NM_001791.2	NM_001791.2-193s1c1	CDS	CGGAATATGTACCGACTGTTT
Dynamin-2		NM_004945	NM_004945.1-3242s1c1	3′-UTR	CCTGAGGTGTACATAGTCCTT
N/A	Scrambled		Mouse genomic		CCTAAGGTTAAGTCGCCCTCG

Sequences of shRNAs used in the study are shown, along with the targeted region of the mRNA. The sense 21-mer is shown. N/A, not applicable.

Table S2. Sequences of qRT-PCR primers

Gene	Primer	Sequence
GADPH	Left	agccacatcgctcagacac
GAPDH	Right	gcccaatacgaccaaatcc
NME1	Left	cagccggagttcaaaccta
NME1	Right	gcaatgaagg tacgctcaca
GFP	Left	gaagcgcgatcacatggt
GFP	Right	ccatgccgagagtgatcc
RhoA	Left	ggagctagccaagatgaagc
RhoA	Right	gccaatcctgtttgccata
Cdc42	Left	tctgcagaagacgctgaatc
Cdc42	Right	ggccgaaagttcttgtactcc
Dynamin-2	Left	tggagtgttctgcacttacaca

Primer sequences are listed 5′-3′.