Supplemental Materials

Molecular Biology of the Cell

Seldin et al.

Online supplemental material

Figure S1 displays effective knockdown of LGN and NuMA proteins by shRNA and the effect of dynamitin-GFP expression on p150-glued localization. Figure S2 shows the effects of disrupting cytoskeletal elements or inhibiting myosin II activity on NuMA, $p150^{glued}$ and dynein intermediate chain cortical recruitment. Figure S3 shows the effects of extracellular matrix proteins on spindle orientation integrity in cultured keratinocytes. Supplemental Movie shows a time-lapse movie of a dividing NuMA-GFP primary cell isolated from K14: NuMA-GFP transgenic mouse backskin.

Supplemental Figure 1. Effects of dynamitin expression and myosin II inhibition on NuMA, p150glued and DIC localization. (A,B) Validation of protein loss upon knockdown of LGN and NuMA. (A) Western blot of NuMA on cell lysates prepared from control, LGN KD and NuMA KD cells. (B) Western blot of LGN on cell lysates prepared from control and LGN KD cells. (C,D) Immunofluorescence analysis of endogenous $p150^{glued}$ localization in control vs. Dynamitin-GFP transfected cells. (E) Quantitation of p150glued cortical localization in dynamitin expressing cells. n=25 cells, $p<0.0001$.

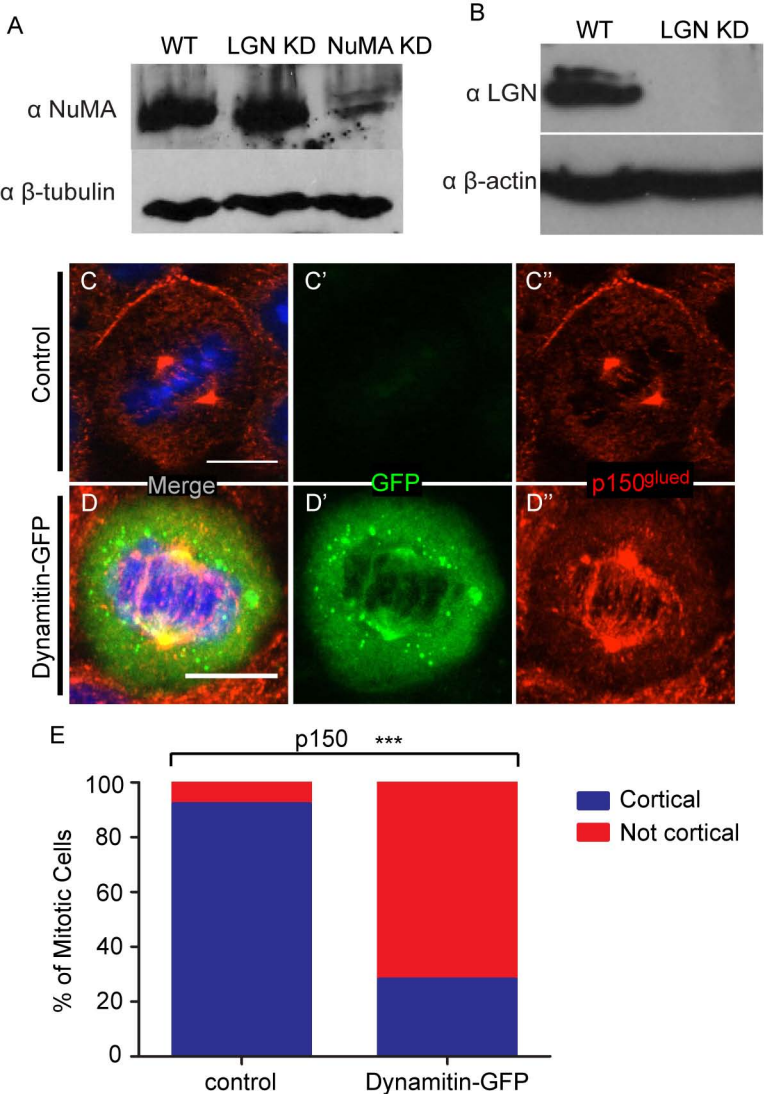
Supplemental Figure 2. Quantitation of cortical enrichment of spindle orientation proteins. (A) Sample images of cortical enrichment (left) and lack of cortical enrichment (right) with associated line scans. (B-G) Images on left and middle are line scans of 10 cells for each of the indicated conditions. The graphs

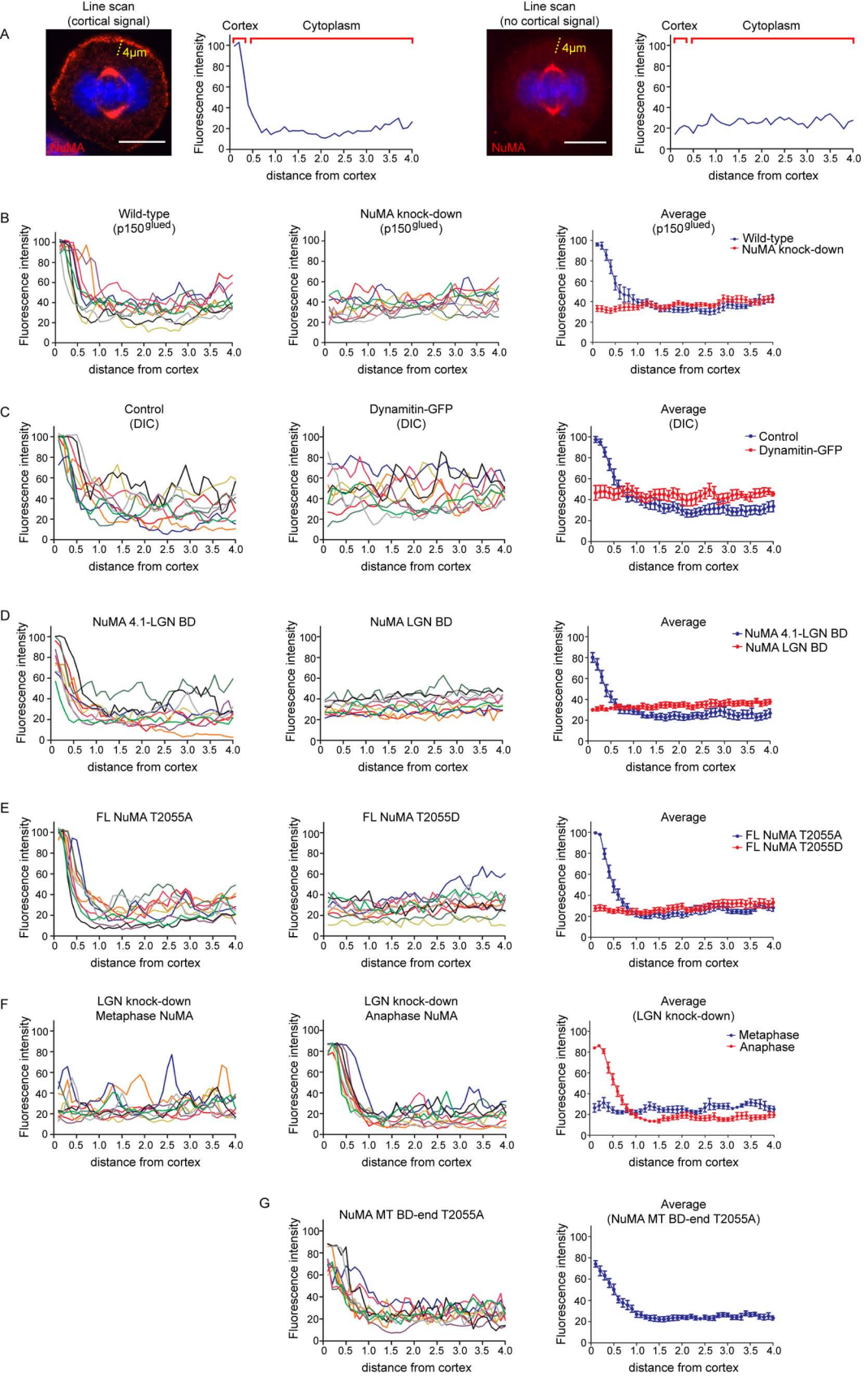
on the right plot the averages of these data with standard error bars. (B) Corresponds to Figure 1C-E, (C) corresponds to Figure 1H-J, (D) corresponds to Figure 2G,H,K, (E) corresponds to Figure 5J,K, (F) corresponds to Figure 6C-E, and (G) corresponds to Figure 6S,T.

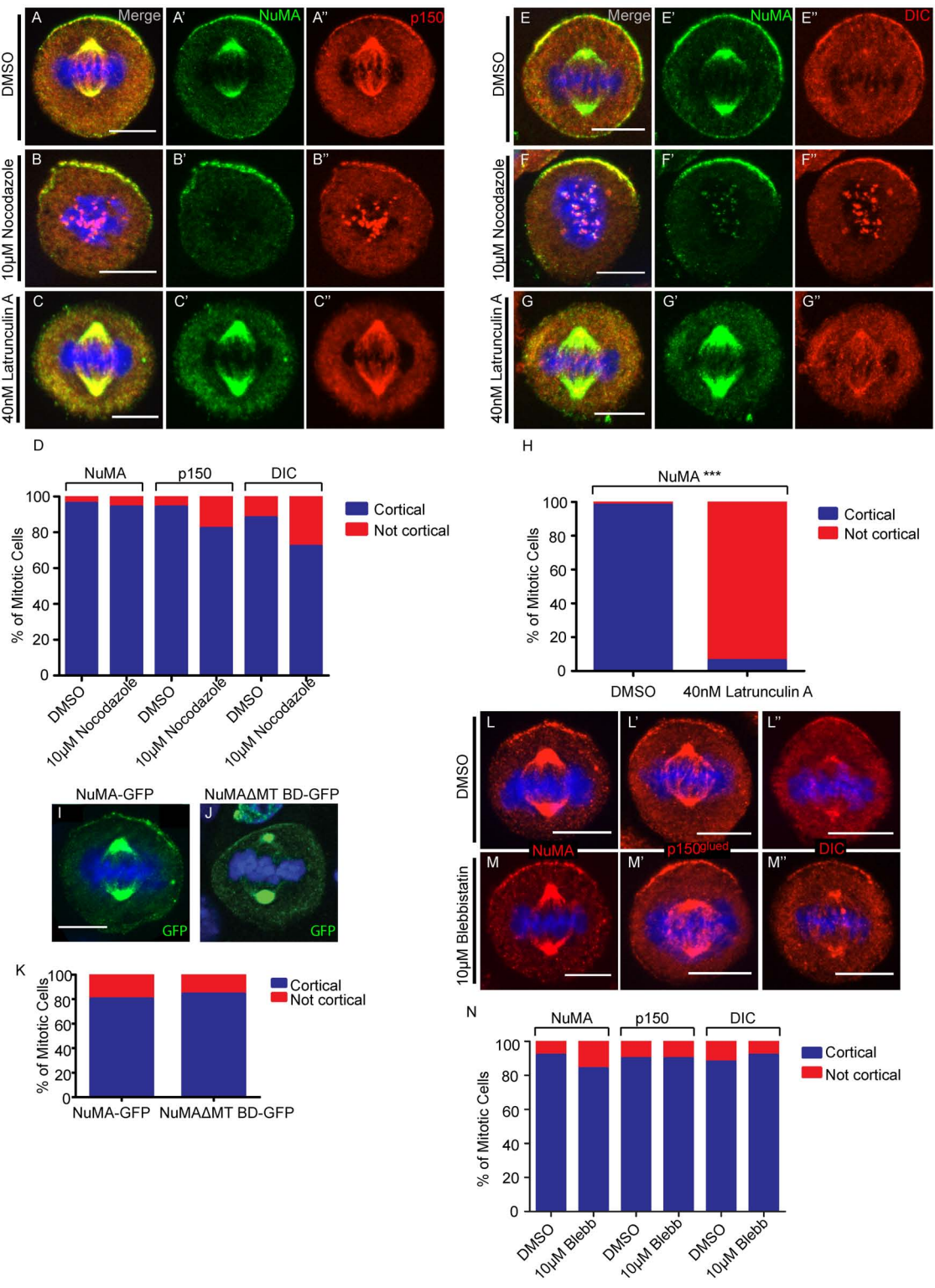
Supplemental Figure 3. Distinct cytoskeletal requirements for cortical and spindle pole NuMA recruitment. Immunofluorescence analysis of endogenous NuMA, $p150^{glued}$ and DIC localization in wild-type keratinocytes treated with DMSO (A, E), or 10μM Nocodazole to depolymerize microtubules (B, F) or 40nM Latrunculin A to depolymerize actin filaments (C, G). Drug treatments were for 10 minutes. (D) Quantitation of cortical localization of NuMA, p150 and DIC treated with different drugs. n=50 cells, p=1 for NuMA, p=0.12 for p150 and p=0.08 for DIC. (H) Quantitation of cortical localization of NuMA in latrunculin-treated cells. n=50, $p<0.0001$. (I,J) Full-length NuMA tagged to GFP (NuMA-GFP) and a GFP-tagged NuMA construct lacking the carboxy-terminal microtubule-binding domain (NuMAΔMT BD-GFP) were transfected into wild-type cells. Immunofluorescence analysis was performed to determine localization of these constructs. Scale bars: 10μm. (K) Quantitation of cells with cortical NuMA or mutant protein. n=25 cells, p=1. (L-M) Immunofluorescence analysis of endogenous NuMA, $p150^{glued}$ and DIC localization in wild-type keratinocytes treated with DMSO (L) or 100μM Blebbistatin (M) to inhibit Myosin II activity. Scale bars: 10μm. (N) Quantitation of cortical localization in blebbistatin treated cells. n=50 cells, $p>0.05$ for all.

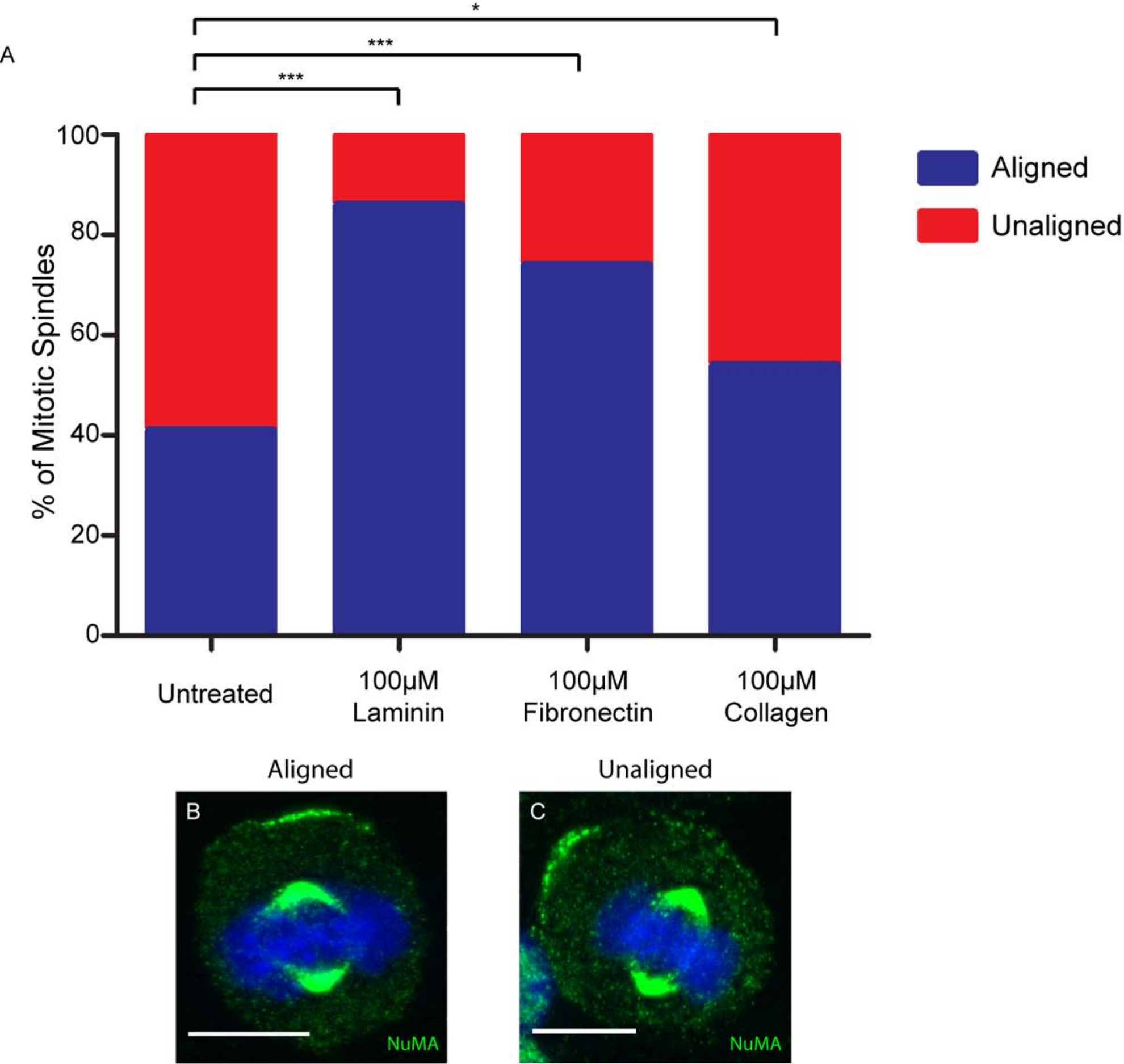
Supplemental Figure 4. Cultured keratinocytes require extracellular matrix components for proper spindle orientation. (A) Graph comparing mitotic spindle alignment with endogenous cortical NuMA crescents in wild-type cells plated on various extracellular matrix substrates: 100μM laminin, 100μM fibronectin and 100μM collagen. "Aligned" refers to spindles within 0-30º from the center of the NuMA crescent, and "Unaligned" refers to spindles within 30-90º from the center of the NuMA crescent. n=50 cell, p-values for untreated verses both laminin and fibronectin was <0.0001, for collagen it was p=0.03. (B-C) Sample immunofluorescence images of those analyzed for (A) of endogenous NuMA expression, representing both a spindle that is "aligned" (B) and a spindle that is "unaligned" (C) with the cortical NuMA crescent. Scale bars: 10μm.

Supplemental Movie. Simultaneous cortical NuMA enrichment and spindle pole NuMA depletion during anaphase. A time-lapse movie imaged in wide-field of a dividing NuMA-GFP primary cell isolated from a K14: NuMA-GFP transgenic mouse. Images were acquired every 45 seconds over a 50 minute time period. The frame rate of this movie is 7 frames per second.


A
WT
LGN KD
NuMA KD
α NuMA
α β-tubulin
B
WT
LGN KD
α LGN
α β-actin
C
C'
C''
Control
Merge
GFP
p150glued
D
D'
D''
Dynamitin-GFP
E
p150
***
100
80
60
40
20
0
% of Mitotic Cells
Cortical
Not cortical
control
Dynamitin-GFP


A
Line scan
(cortical signal)
4μm
NuMA
Cortex
Cytoplasm
Fluorescence intensity
distance from cortex
Line scan
(no cortical signal)
4μm
NuMA
Cortex
Cytoplasm
Fluorescence intensity
distance from cortex
B
Wild-type
(p150glued)
NuMA knock-down
(p150glued)
Average
(p150glued)
Wild-type
NuMA knock-down
C
Control
(DIC)
Dynamitin-GFP
(DIC)
Average
(DIC)
Control
Dynamitin-GFP
D
NuMA 4.1-LGN BD
NuMA LGN BD
Average
NuMA 4.1-LGN BD
NuMA LGN BD
E
FL NuMA T2055A
FL NuMA T2055D
Average
FL NuMA T2055A
FL NuMA T2055D
F
LGN knock-down
Metaphase NuMA
LGN knock-down
Anaphase NuMA
Average
(LGN knock-down)
Metaphase
Anaphase
G
NuMA MT BD-end T2055A
Average
(NuMA MT BD-end T2055A)
Fluorescence intensity
distance from cortex


A
Merge
A'
NuMA
A''
p150
DMSO
B
B'
B''
10μM Nocodazole
C
C'
C''
40nM Latrunculin A
E
Merge
E'
NuMA
E''
DIC
DMSO
F
F'
F''
10μM Nocodazole
G
G'
G''
40nM Latrunculin A
D
NuMA
p150
DIC
% of Mitotic Cells
Cortical
Not cortical
DMSO
10μM Nocodazole
H
NuMA ***
% of Mitotic Cells
Cortical
Not cortical
DMSO
40nM Latrunculin A
NuMA-GFP
NuMAΔMT BD-GFP
I
J
GFP
K
% of Mitotic Cells
Cortical
Not cortical
NuMA-GFP
NuMAΔMT BD-GFP
L
L'
L''
DMSO
M
NuMA
M'
p150glued
M''
DIC
10μM Blebbistatin
N
NuMA
p150
DIC
% of Mitotic Cells
Cortical
Not cortical
DMSO
10μM Blebb


A
*
***
***
100
80
60
40
20
0
% of Mitotic Spindles
Aligned
Unaligned
Untreated
100μM Laminin
100μM Fibronectin
100μM Collagen
Aligned
Unaligned
B
C
NuMA
NuMA