Supplemental data

Supplemental Figure 1
NMII proteins and siRNA treated cells used in this study. *A*, Purified NMII rod fragments were separated by SDS-PAGE and Coomassie stained. *B*, Western blot depicting the different NMII rod chimeras as detected by isoform specific antibodies. *C*, Western blot depicting the different full length NMII chimeras expressed in COS-7 cells. Note that GFP-NMII-A and GFP-NMII-B are larger than the HA tagged NMII-C constructs. COS-7 cells express NMII-B and NMII-C endogenously. Western blot depicting the expression levels of the three NMII isoforms after cell infection with NMII siRNA Lentivirus particles. *D*, NMII-A specific siRNA treated MEF B^-/B^- cells. *E*, NMII-C specific siRNA treated A549 cells.

Supplemental Figure 2
The tailpiece determines NMII-C paracrystal morphology. Full length coiled-coil NMII-C (amino acids 856-2000), tailless full length coiled-coil NMII-C (amino acids 856-1953), and NMII-C rod fragment (amino acids 1288-2000) used in this study, were dialyzed against low NaCl buffer and stained with uranyl acetate as described in Experimental Procedures. Note the similar morphology between the full length coiled-coil NMII-C and NMII-C rod fragments indicating that the effect is due to the tailpiece. Bar=50nm.

Supplemental Figure 3
Focal adhesion in NMII-C siRNA treated cells. A549 cells depleted of NMII-C were stained for Vinculin to detect focal adhesion complexes. Red: Actin, Green: Vinculin, Blue: siRNA marker.

Supplemental Figure 4
In-vitro self-assembly of tail-swapped NMII rod fragments. 0.1 mg/ml rod fragments, were expressed and purified from *E. coli* as described in Experimental Procedures. Proteins were dialyzed against different NaCl concentrations and the extent of assembly was calculated as the percentage of rod fragment remaining in the supernatant after high speed centrifugation. Results are averages +/- S.D of at least three independent experiments.

Supplemental Table 1

NMII Rod striation

Striation of paracrystals was measured from images as in Fig. 3 using Image-Pro plus software on calibrated electron micrographs at X88000 magnification.

Rod	Length (nm)	+/- SD
NMII-A wild type	15.28	1.66
NMII-A-tailB	15.08	0.94
NMII-A-tailC	14.93	1.39
NMII-B wild type	15.49	1.18
NMII-B-tailA	13.36	1.23
NMII-B-tailC	12.77	1.68
NMII-C wild type	14.35	1.76
NMII-C-tailA	14.56	1.80
NMII-C-tailB	16.47	0.78

Supplemental Table 2

Mapping of PKC and CKII phosphorylation sites on NMII-C

Peptide data obtained by mass spectrometry of phosphorylated NMII-C

MS/MS fragment	Amino Acid	Mr (Calc)	Mr (observed)	Delta	MASCOT ion score
GPLTF**p**TTR**p**TVR	1952-1968	1407.6312	704.9539	0.2620	46
ELEDV**p**TESAESMNR	1927-1940	1687.7124	845.5539	1.3809	80
LEEGVA**p**SDEEEAEGAEPGSAPGQEPEAPPPATPQ	1967-2000	3452.4304	1164.1252	-0.0484	83
LEEGVASDEEEAEGAEPG**p**SAPGQEPEAPPPATPQ	1967-2000	3452.4304	1151.4952	-0.9666	49