SUPPLEMENTAL FIGURE LEGENDS

Figure S1. DHC knockdown decreases dynactin patches at leading edge. NIH/3T3 cells were transfected with mock siRNA or with siRNA targeting DHC and were grown to confluency. The monolayer was wounded. Six hours after wounding, cells were fixed and stained for tubulin (green) and $p150^{Glued}$ (red). Bar, 10 microns. DHC knockdown cells had less $p150^{Glued}$ leading edge patches (arrowheads) than mock-transfected cells. Error bars indicate SEM; * indicates $p<0.05$.

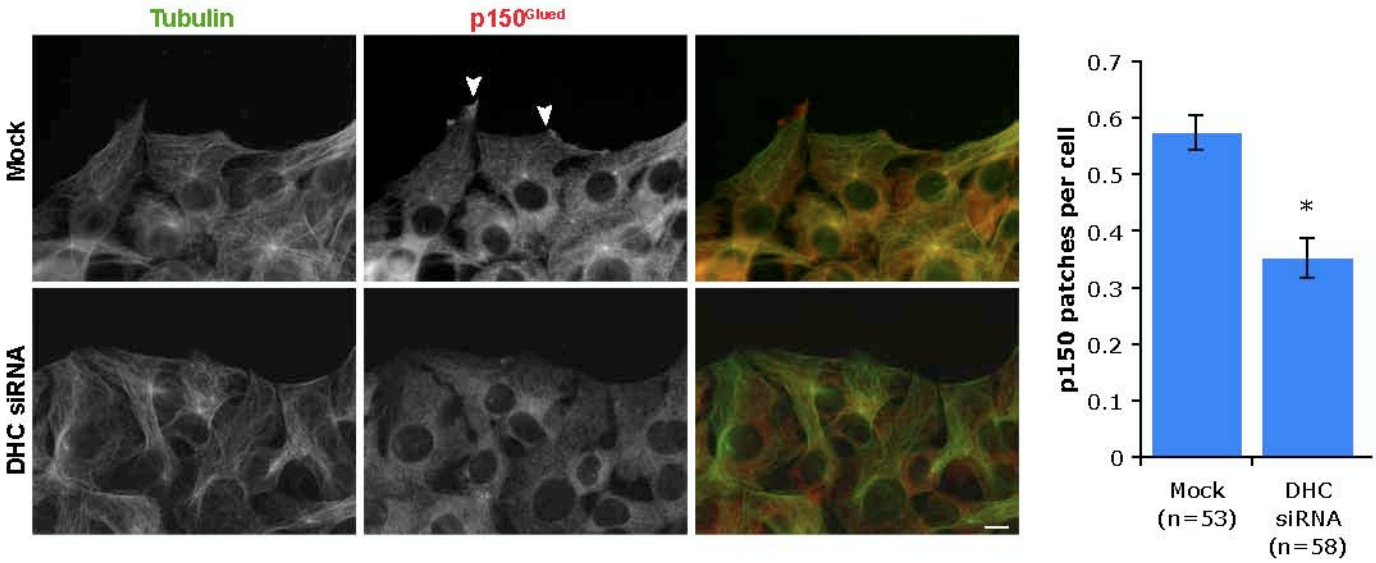
Figure S2. Multiple independent siRNA oligos induce similar phenotypes. Cells that are mock transfected or transfected with scrambled siRNA (scrambled siRNA corresponds to oligo p150 #1) show no difference in the velocity of nuclear rotations. Transfection of cells with alternate oligos against DHC and $p150^{Glued}$ show no difference when compared to one another, and all induce a significant reduction in angular velocity of rotations compared to mock-treated cells. siRNAs DHC #1, DHC #2, p150 #1, and p150 #2 all show similar inhibition of centrosome reorientation compared to either mock-treated or scrambled siRNA-transfected samples (data not shown). Error bars indicate SEM; ** indicates $p<0.005$; n.d. indicated no difference; n=10.

Figure S3. Nucleoli remain in position relative to one another in migrating cells. (A) Tracks of the paths of 4 nucleoli in a single nucleus that is not rotating. Each path was normalized to the center of the nucleus to correct for migration of the nucleus towards the wound. Each nucleolus stays stationary relative to its position in the nucleus. Axis labels

are in microns, time is in minutes:seconds. (B) Tracks of the paths of 4 nucleoli in a single nucleus that is rotating while migrating into the wound. Each nucleolus follows a similar circular path in relation to the center of the nucleus, but all nucleoli do not change position relative to one another. Axis labels are in microns, time is in minutes:seconds.

Figure S4. Knockdown of dynein or dynactin does not alter actin distribution. Immunofluorescence of cells that were fixed 6 hours after monolayer wounding and stained with an antibody to α-tubulin (green) and TRITC-conjugated phalloidin (red). Bar, 10 microns.

Figure S1


Tubulin
p150Glued
Mock
DHC siRNA

p150 patches per cell
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
Mock
(n=53)
DHC
siRNA
(n=58)

Figure S2

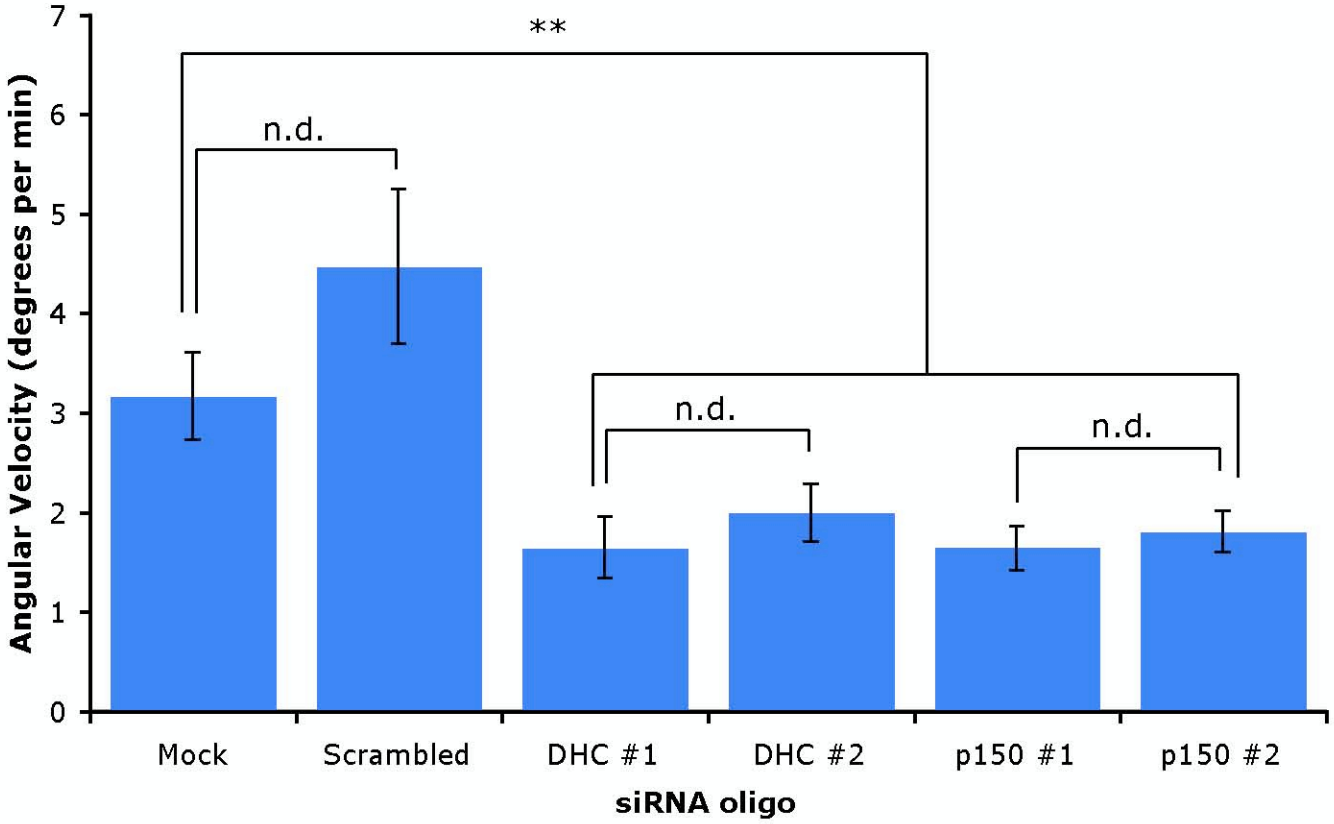

**
n.d.
n.d.
n.d.
Angular Velocity (degrees per min)
0
1
2
3
4
5
6
7
Mock
Scrambled
DHC #1
DHC #2
p150 #1
p150 #2
siRNA oligo

Figure S3

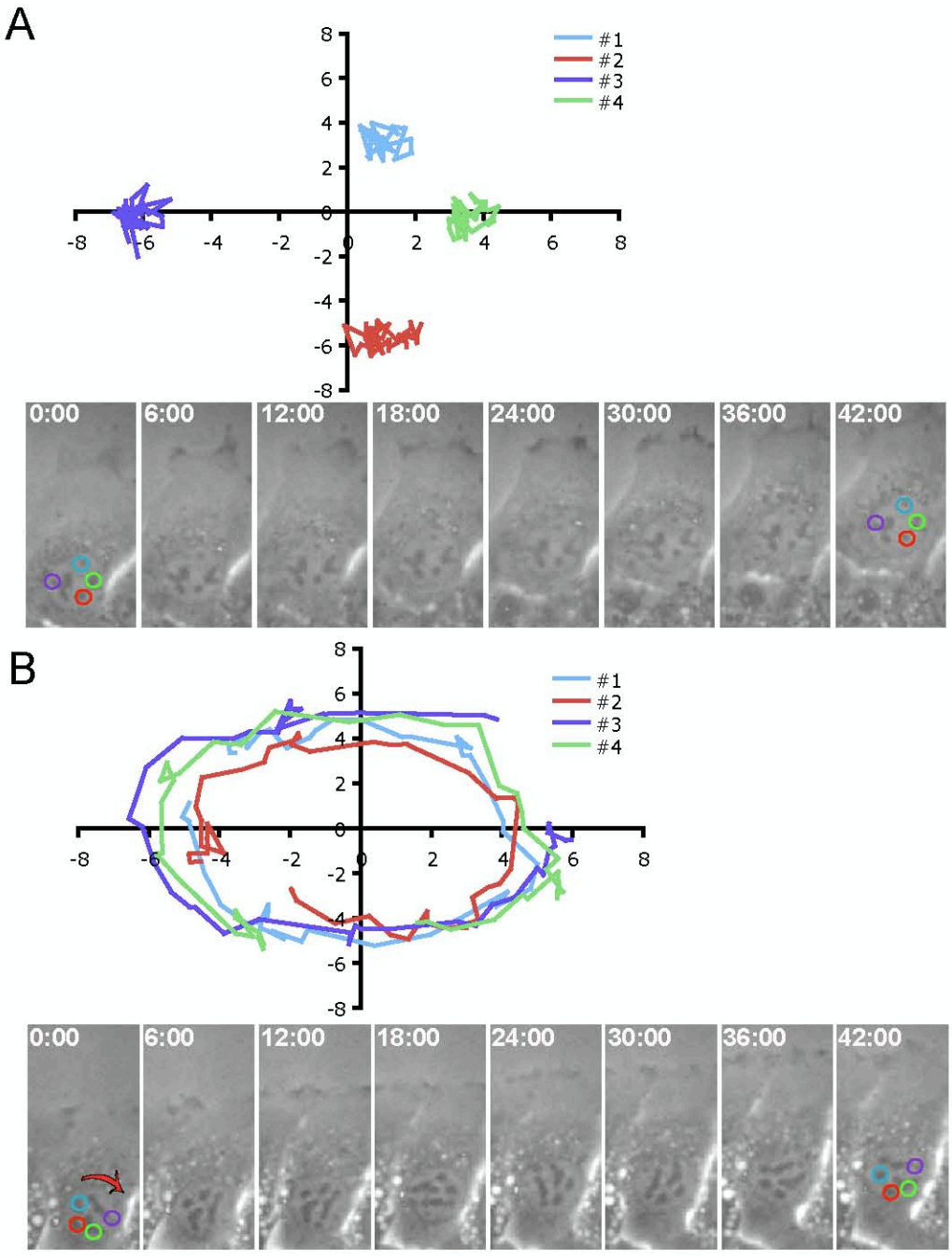
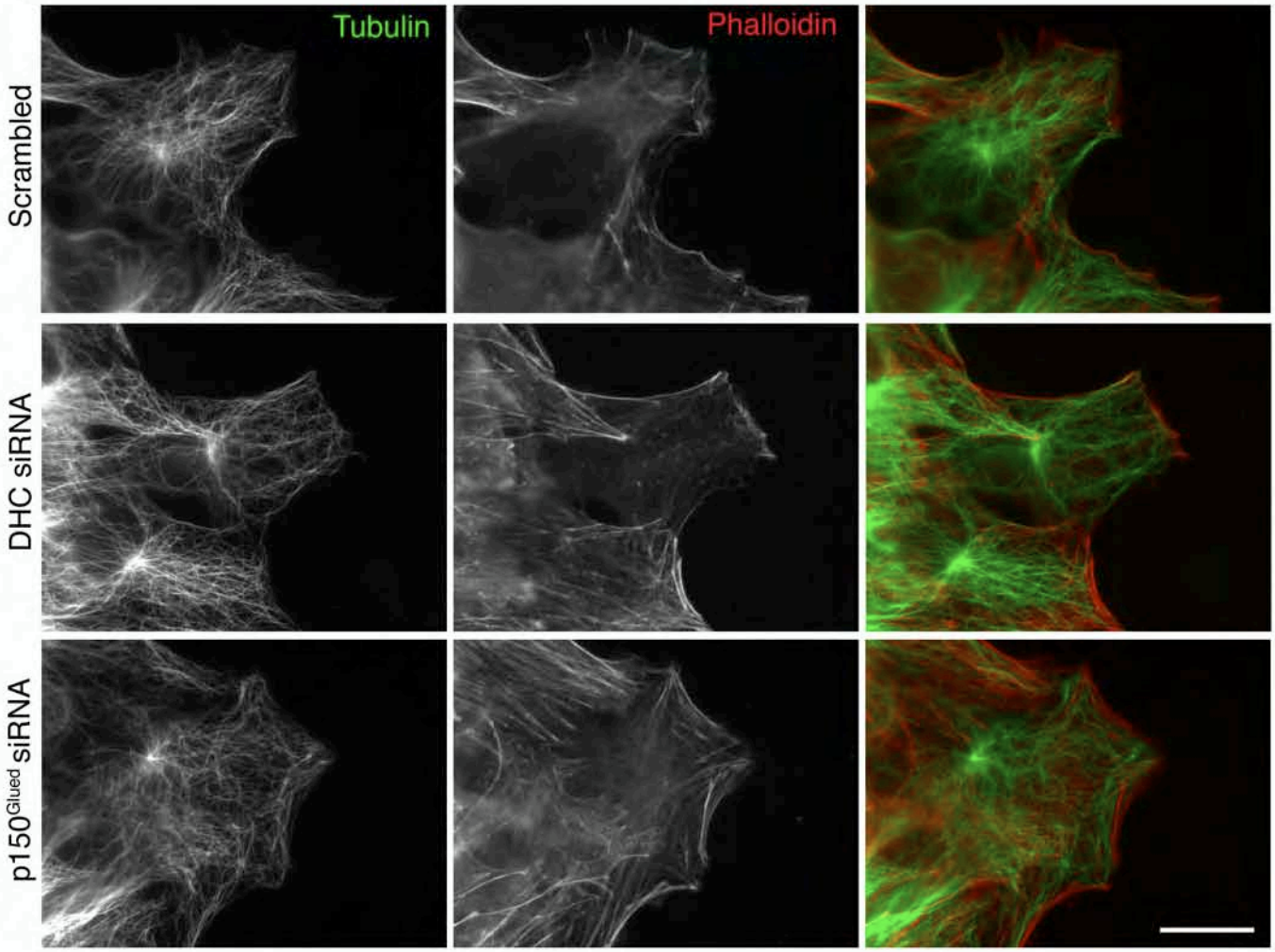

A
#1
#2
#3
#4
0:00
6:00
12:00
18:00
24:00
30:00
36:00
42:00
B
#1
#2
#3
#4
0:00
6:00
12:00
18:00
24:00
30:00
36:00
42:00

Figure S4



VIDEO LEGENDS

Video 1. Migration of NIH/3T3 cells after wounding. Cells were either mock transfected (left), transfected with siRNA targeting DHC (center), or transfected with siRNA targeting $p150^{Glued}$ (right). Cell were then grown to confluence, and scratch wounded. Acquisition rate was 1 frame every 40 seconds for 45 min.

Video 2. 3T3 cells in an unwounded monolayer. Cells were grown to confluence and imaged. Acquisition rate was 1 frame every 40 seconds for 45 min.

Video 3. ER dynamics during nuclear rotation. 3T3 cells were transfected with dsRed2-ER, grown to confluence, and scratch wounded. The cells were then imaged using fluorescence to visualize dsRed2-ER and phase to visualize the nucleus. Acquisition rate was 1 frame every 30 seconds for 20 min.

Video 4. Microtubule dynamics during nuclear rotation. 3T3 cells were transfected with the microtubule marker 3XGFP-EMTB, grown to confluence, and scratch wounded. The cells were then imaged using fluorescence to visualize microtubules and phase to visualize the nucleus. Acquisition rate was 1 frame every 30 seconds for 20 min.

Video 5. Centrosome localization during nuclear rotation. 3T3 cells were transfected with dsRed1-Centrin2, grown to confluence, and scratch wounded. The cells were then imaged using fluorescence to visualize dsRed1-Centrin2 (overlay in green) and phase to visualize the nucleus. Acquisition rate was 1 frame every 30 seconds for 30 min.

Video 6. 3T3 cells treated with microtubule, myosin II, and Golgi inhibitors during migration. (Far left) Cells were grown to confluence, scratch wounded, and treated with a high dose of nocodazole to depolymerize MTs. Acquisition rate was 1 frame every 40 seconds. (Second from left) 3T3 cells treated with blebblistatin during migrations. Cells were grown to confluence, scratch wounded, and treatead with blebbistatin to inhibit myosin II activity. Acquisition rate was 1 frame every 40 seconds for 45 min. (Second from right) 3T3 cells treated with low doses of nocodazole during migration. Cells were grown to confluence, scratch wounded, and treated with a low dose of nocodazole to inhibit microtubule dynamics and dynactin accumulation at the leading edges of wound-edge cells. Acquisition rate was 1 frame every 40 seconds for 45 min. (Far right) 3T3 cells treated with BFA during migration. Cells were grown to confluence, scratch wounded, and treated with BFA to disrupt the Golgi complex. Acquisition rate was 1 frame every 40 seconds for 45 min.