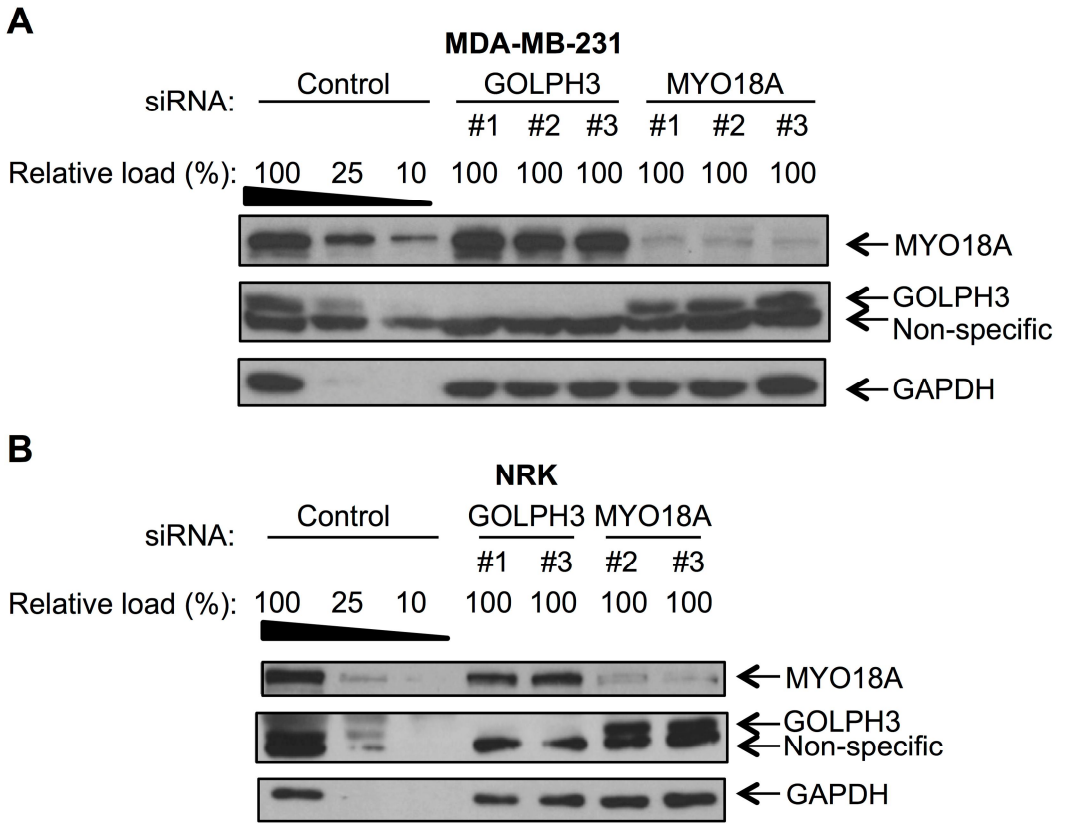
Supplemental Materials

Molecular Biology of the Cell

Xing et al.

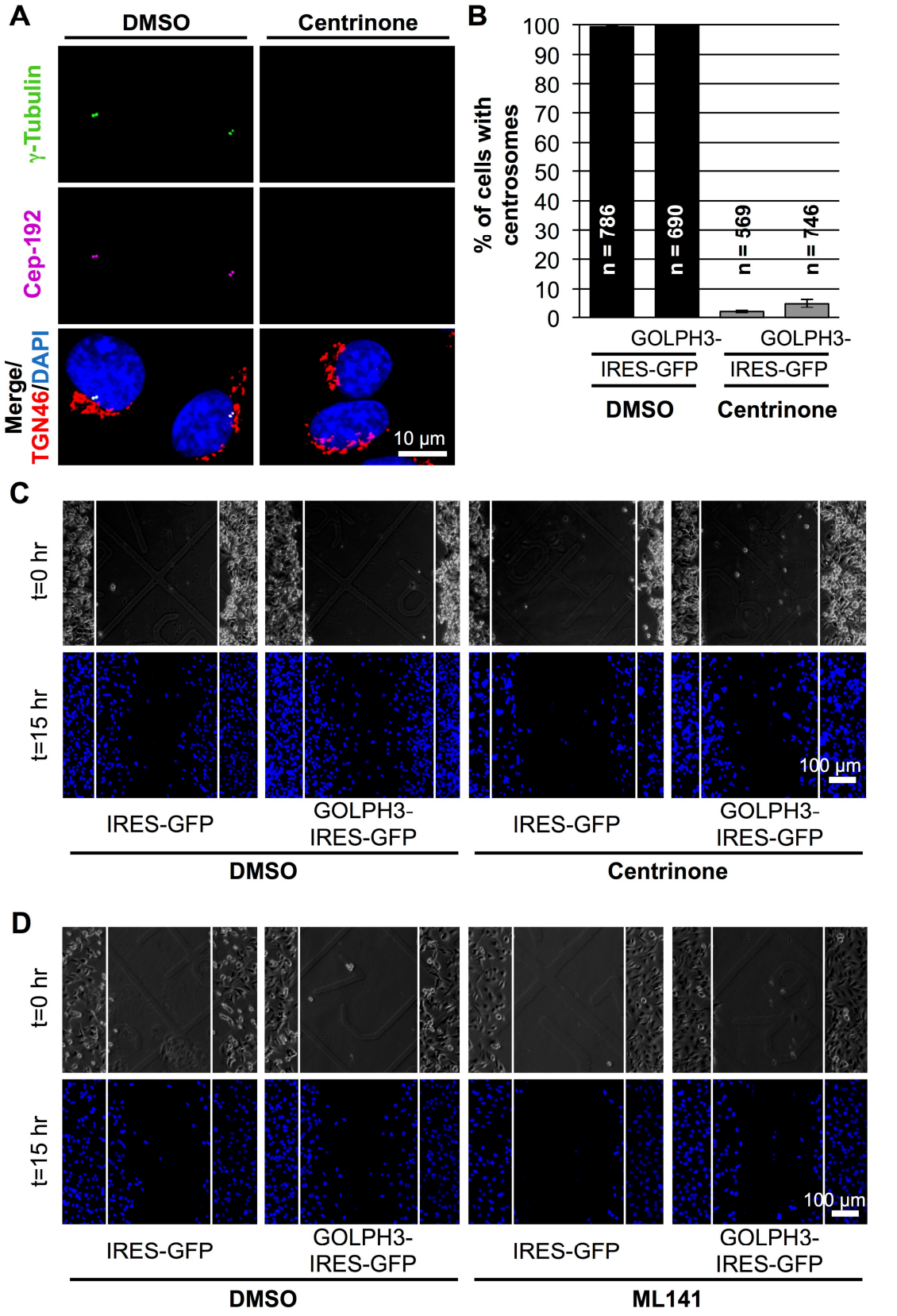
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1 **Xing et al.**



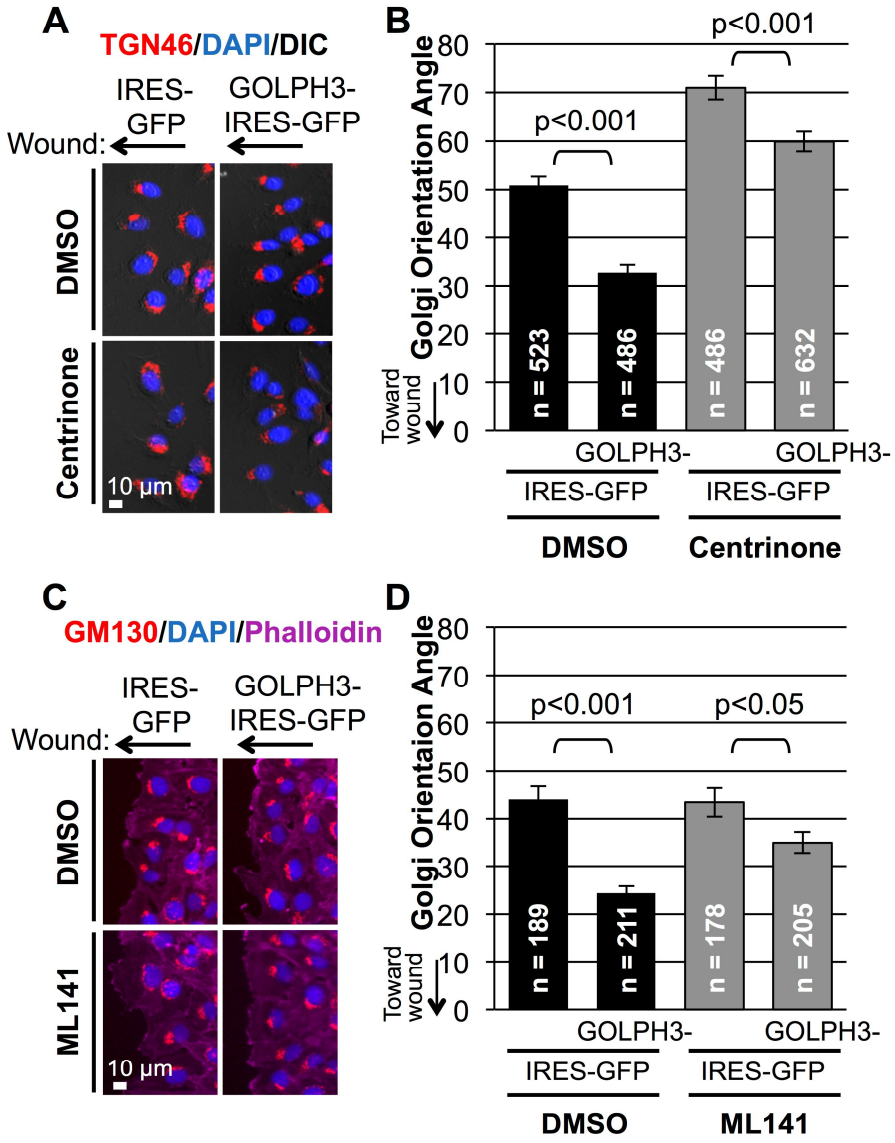
SUPPLEMENTAL FIGURE 1. Efficient knockdown of GOLPH3 and MYO18A. (A) MDA-MB-231 and (B) NRK cells were transfected with control siRNA, siRNAs specific to GOLPH3, and siRNAs specific to MYO18A as indicated. Lysates were Western blotted for MYO18A and GOLPH3 with GAPDH as a loading control. Lysates from control siRNA-treated cells were loaded at different amounts to allow semiquantitative assessment of knockdown efficiency. Transfection with each of the GOLPH3 or MYO18A siRNA oligos results in at least 90% knockdown.

Supplemental Figure 2 **Xing et al.**



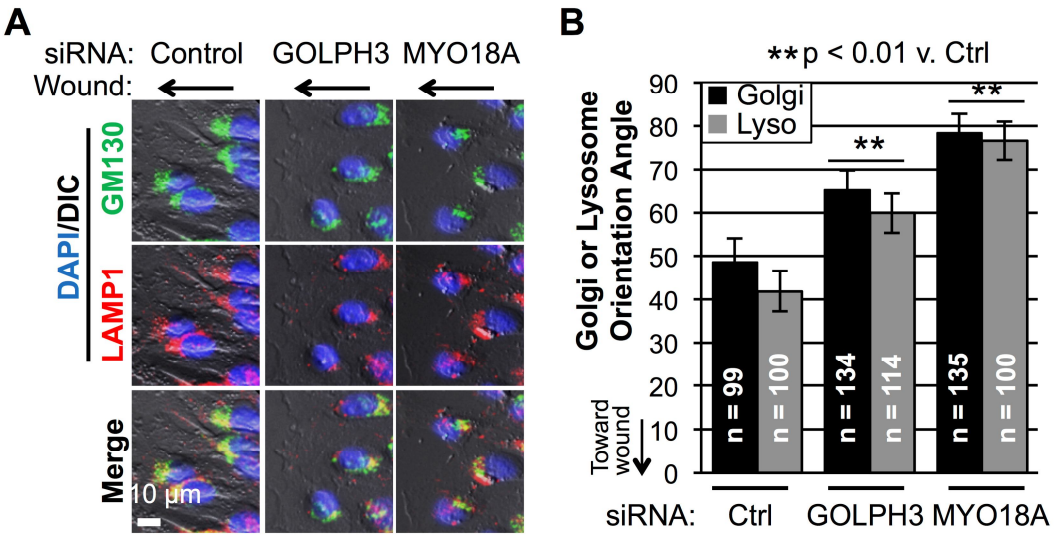
SUPPLEMENTAL FIGURE 2. GOLPH3 overexpression enhances scratch wound healing independently from centrosomes and Cdc42. (A) Representative IF images of GOLPH3-IRES-GFP overexpressing MDA-MB-231 cells grown in DMSO (vehicle) or 100 nM centrinone for >2 weeks. Centrosomes are identified by γ-Tubulin (green) and Cep-192 (magenta) with the Golgi marked by TGN46 (red) and the nucleus by DAPI (blue). (B) Percentage of cells with centrosomes was measured for IRES-GFP (control) and GOLPH3-IRES-GFP cells grown for >2 weeks in DMSO (vehicle control) or 100nM centrinone. Treatment with centrinone results in nearly complete loss of centrosomes. Graphed are mean and SEM, pooling data from two independent experiments, with number of cells scored (n) indicated. (C) Representative images of scratch wound healing by MDA-MB-231 cells grown in DMSO (vehicle) or 100 nM centrinone for >2 weeks or (D) pre-treated for 1 hour and after monolayer wounding with DMSO (vehicle) or 10 μM ML141. Top row, images of random fields at the scratch were taken at time of wounding (t=0 hr), with the scratch area indicated by the white box. Bottom row, the same fields after 15 hr, fixed and stained with DAPI for cell counting (t=15 hr). See Figure 3, D and E, for quantification.

Supplemental Figure 3 **Xing et al.**



SUPPLEMENTAL FIGURE 3. GOLPH3 drives Golgi reorientation independently from centrosomes or Cdc42. (A) Representative IF images of MDA-MB-231 cells at wound edge grown in the presence of DMSO (vehicle) or 100 nM centrinone for 2 weeks with Golgi labeled by TGN46 (red) and the nucleus by DAPI (blue). (B) Quantification of Golgi angles from (A). (C) Representative IF images of MDA-MB-231 cells at wound edge pre-treated for 1 hour and after monolayer wounding with DMSO (vehicle) or 10 μM ML141 and fixed 5 hrs after wounding. (D) Quantification of Golgi orientation angles from (C). (B) and (D) graphed are mean ± SEM pooled from two independent experiments, with number of cells measured (n) and p-values (t-test with Holm-Bonferroni correction) indicated.

Supplemental Figure 4 **Xing et al.**



SUPPLEMENTAL FIGURE 4. GOLPH3 and MYO18A are required for Golgi and lysosome reorientation at cell culture wound edge in Hela cells. (A) Representative IF images of Hela cells at wound edge. Cells were transfected with control siRNA or siRNA targeting GOLPH3 or MYO18A 48 hrs prior to wounding and fixed 5 hrs after wounding. The Golgi is labeled in green (GM130), lysosome in red (LAMP1), and the nucleus in blue (DAPI). (B) Quantification of Golgi and lysosome orientation angles from (A). Reorientation of the Golgi and lysosome toward the leading edge upon wounding is significantly impaired by depletion of GOLPH3 or MYO18A. Graphed are mean ± SEM pooled from two independent experiments, with number of cells (n) measured and p-values (t-test with Holm-Bonferroni correction) indicated.