Supporting Information

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Methods

Immunocytochemistry. MEFs were infected with lentivirus or transfected with pLKO.1 lentiviral constructs expressing shDHC1 or shMUT 72 h before fixation. Following 24 h serum starvation (0.5% calf serum) cells were either left untreated or treated with hTNFα (25 ng/ml) for 10, 20, or 40 min or Leptomycin B (LMB, 25 nM) for 30, 60, or 90 min before fixation and immunostaining. Cells were subsequently rinsed with 4% sucrose in PBS, fixed with 4% paraformaldehyde/4% sucrose in PBS for 30 min, permeabilized with 0.2% TritonX-100 in PBS for 20 min, and blocked with 10% BSA in PBS for 2 h at RT. Cells were then incubated with antibodies against p65 (1:200, SC-372 rabbit; SantaCruz Biotechnology) and GFP (1:500, mpA11120 mouse; Molecular Probes) in 1% BSA/PBS for 16 h at 4 °C. Bound primary antigen was detected by incubation with anti-rabbit Alexa Fluor 568 and anti-mouse Alexa Fluor 488 (1:1200, Molecular Probes) in 1% BSA/PBS for 1 h at RT. p65 antibody specificity was confirmed by lack of staining in MEFs from p65-deficient mice (Fig. S2*A*). Secondary antibody specificity was confirmed by lack of staining when primary antibody incubation was omitted (data not shown). Cells were mounted and subjected to confocal imaging as described in *Methods*. Alexa Fluor 488 was excited at 488 nm and emissions collected at 505–530 nm; Alexa Fluor 563 was excited at 543 nm and emissions collected above 560 nm. Laser power, gain and offset were adjusted to keep fluorescence signals within the dynamic range and were then held constant across all experiments to permit direct comparison of absolute fluorescence in all samples. Nuclear or cytoplasmic fluorescence was quantitated by ROI analysis (Pascal, Zeiss) in a representative area. The ratio of nuclear to cytoplasmic fluorescence for each time point was calculated by: nuclear ROI fluorescence/cytoplamsic ROI fluorescence.

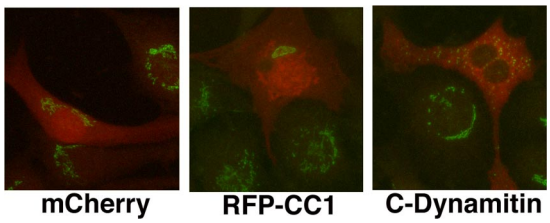

Fig. S1. Expression of fluorophore tagged components of the Dynactin Complex Results in Loss of Dynactin Function and Golgi Disruption. Confocal projections show Golgi structure (green) by immunostaining for the Golgi matrix (anti-GM1) in MEFs expressing either mCherry (normal golgi, *Left*), RFP-CC1 (condensed Golgi, *Middle*), or C-Dynamitin (dispersed Golgi, *Right*).

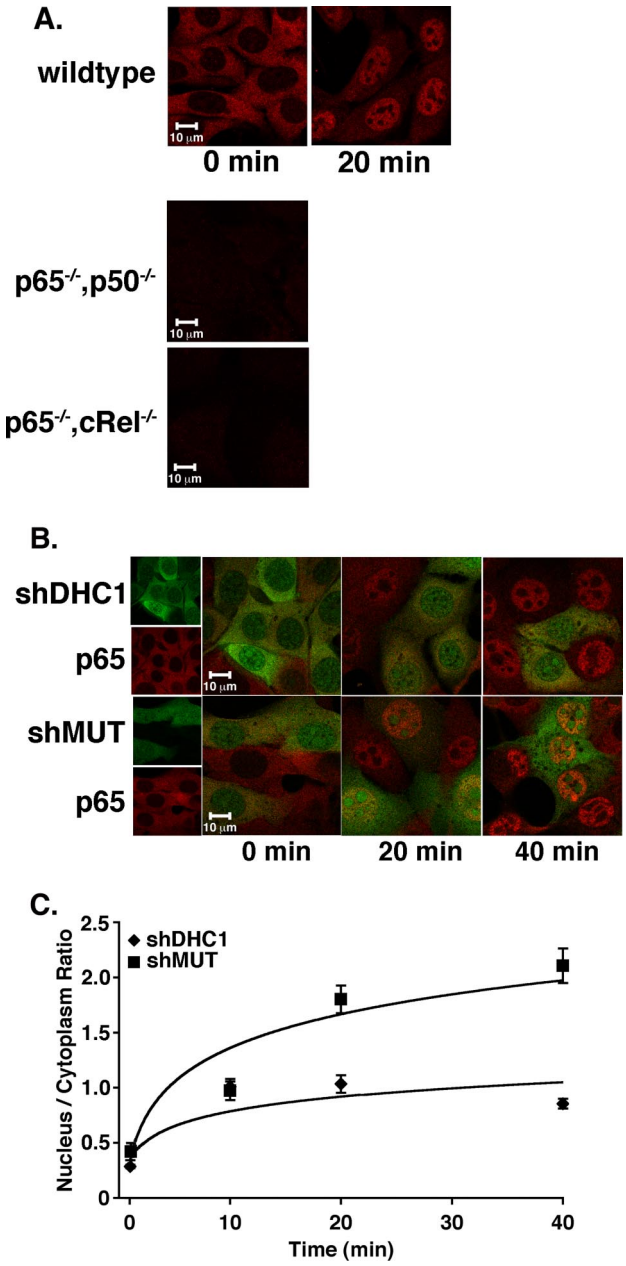

Fig. S2. Loss of Dynein Reduces the Rate and Amplitude of Stimulated Nuclear Translocation of Endogenous NF-κB. (*A*) Antibody specificity for the p65 subunit of murine NF-κB is verified by assessing the nuclear translocation response (*Upper*) 20 min following TNFα addition (at 0 min), and by assessing staining in MEFs from p65-deficient mice: $p65^{-/-}p50^{-/-}$ (*Middle*), $p65^{-/-}$,$cRel^{-/-}$ (*Lower*). (*B*) Representative confocal projections of TNFα-stimulated nuclear translocation of endogenous p65 in fixed immunostained MEFs infected with lentivirus expressing shDHC1 or shMUT. Green (GFP co-expressed from pLKO.1 viral constructs) and Red (p65) channels are overlayed in the large panels (*Right*); small panels (*Left*) show each channel separately. Overlayed images show inhibition of stimulated 65 nuclear accumulation at 20 and 40 min in cells expressing shDHC1 relative to shMUT. (*C*) Quantitated change in the nucleus/cytoplasm fluorescence ratio from p65 (see *SI Methods*) is averaged across all experiments for each time point and demonstrates significant inhibition of TNFα-stimulated p65 nuclear accumulation in MEFs expressing shDHC1 compared to shMUT at all time points ≥20 min ($n = 50–80$ cells for each time point, $P \leq 0.001$). Nuclear levels of the untagged GFP were unchanged during the time course. Error bars represent one s.e.m.

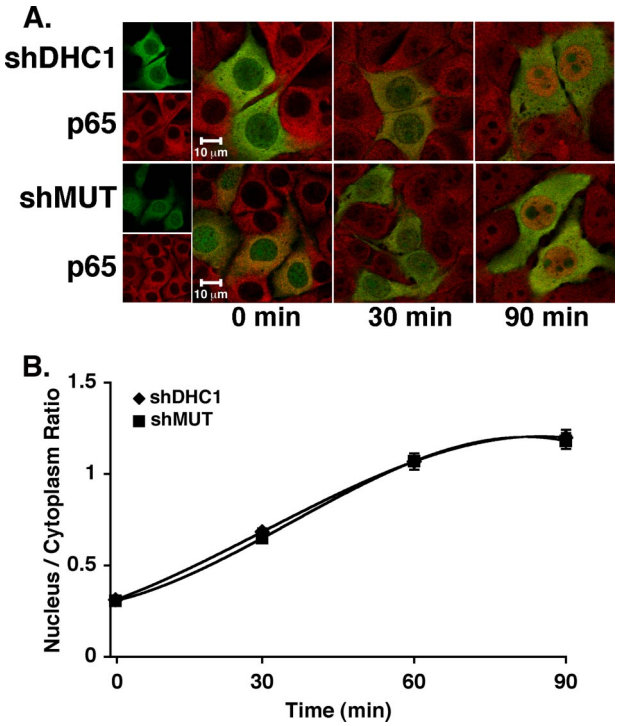

Fig. S3. Shuttling of Endogenous NF-κB is Not Altered by Loss of Dynein. (*A*) Representative confocal projections of endogenous p65 nuclear translocation in fixed immunostained MEFs infected with lentivirus expressing shDHC1 or shMUT and treated with LMB at time = 0 min. Overlayed images (*Right*) show nuclear accumulation of endogenous p65 (red) by 90 min post-LMB in cells with dynein knockdown (shDHC1) as well as control cells (shMUT). Nuclear levels of untagged GFP (green), also produced by pLKO.1 infection, are unchanged. (*B*) Quantitated change in the nucleus/cytoplasm fluorescence ratio from p65 (see *SI Methods*) in LMB-treated MEFs is averaged across all experiments for each time point and demonstrates no significant difference in p65 nuclear accumulation in MEFs expressing shDHC1 compared to shMUT at any time points (n = 40–65 cells for each time point). Nuclear levels of the untagged GFP were unchanged during the time course. Error bars represent one s.e.m.