Lewkowicz et al., http://www.jcb.org/cgi/content/full/jcb.200807023/DC1

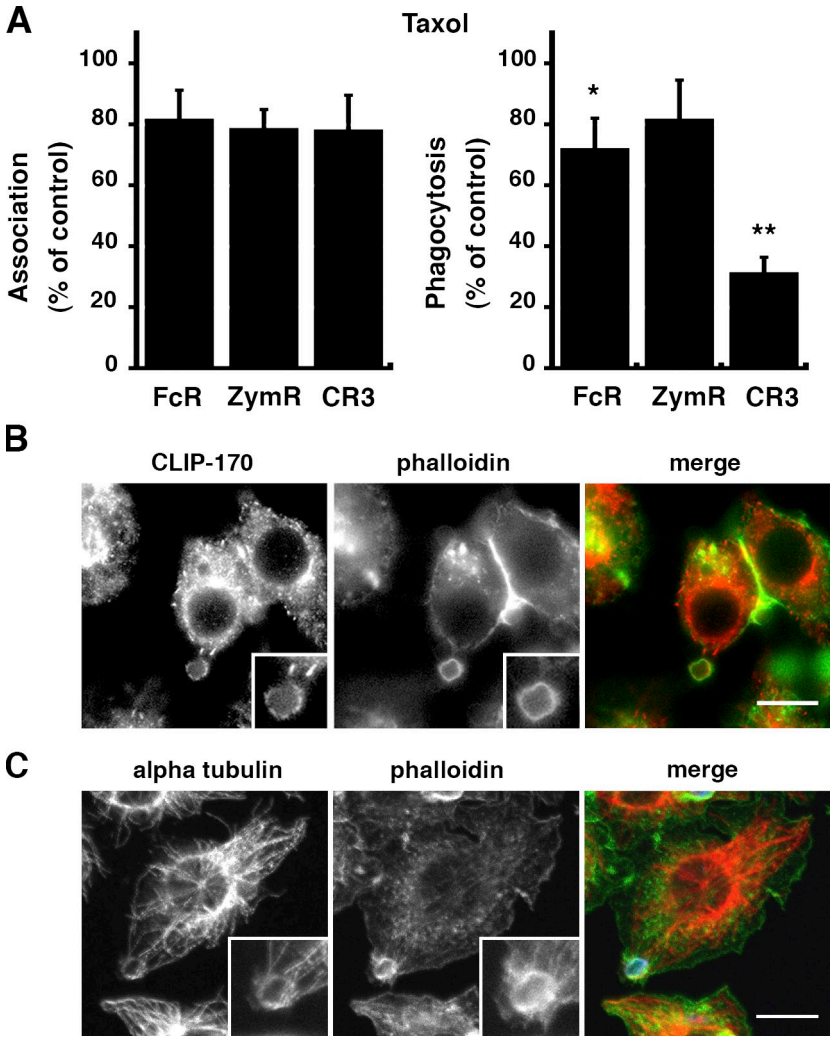

Figure S1. **Microtubule dynamics are necessary for CR3- but not FcR-mediated or zymosan phagocytosis.** (A) Dynamics of microtubules are important for CR3-mediated phagocytosis. RAW264.7 macrophages treated with 10 µM taxol or mock treated were allowed to phagocytose IgG-SRBCs, zymosans, or C3bi-SRBCs for 3 and 60 min at 37°C. The efficiencies of association (left) and phagocytosis (right) were scored in 50 taxol-treated and 50 control cells. Results are expressed as a percentage of control cells. The means ± SEM of four independent experiments are plotted. FcR- and CR3-mediated phagocytosis were significantly inhibited in taxol-treated cells (*, $P < 0.05$) compared with control cells (**, $P < 0.0001$), whereas zymosan uptake was not ($P > 0.1$). (B) Presence of CLIP-170 at phagocytic sites. RAW264.7 macrophages were incubated at 37°C with C3bi-SRBCs for 10 min before fixation. Cells stained with anti–CLIP-170 followed by Cy3–anti-mouse IgG and Alexa 488–phalloidin were observed by wide-field fluorescence microscopy. Bar, 10 µm. (C) Microtubules are recruited at sites of phagocytosis. RAW264.7 macrophages were incubated at 37°C with C3bi-SRBCs for 10 min. The cells were then fixed, permeabilized, and stained with anti–α-tubulin antibody followed by Cy3–anti-mouse IgG (red) and Alexa 350–phalloidin (green). Labeled cells were analyzed by confocal microscopy (a projection of three sections acquired with 0.2-µm step is shown). Bar, 10 µm.

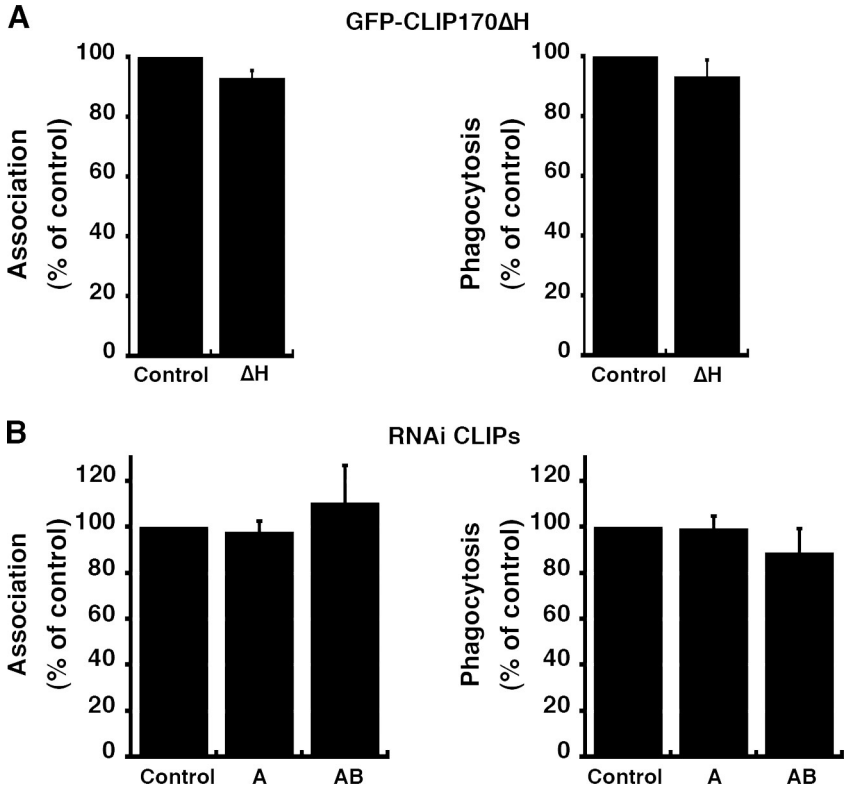

Figure S2. **Functional CLIP-170 is not required for FcR-mediated phagocytosis.** (A) Macrophages were transfected with pEGFP–CLIP-170ΔH or with pEGFP as a control. After 24 h, macrophages were allowed to phagocytose IgG-SRBCs for 3 and 60 min at 37°C, and then fixed and stained with Cy3–anti-rabbit IgG antibodies. Efficiencies of association (left) and phagocytosis (right) were scored in 50 cells expressing GFP–CLIP-170ΔH and 50 GFP-expressing control cells. Results are expressed as a percentage of control cells. The means ± SEM of at least three independent experiments are plotted. No significant difference was observed in the different conditions ($P > 0.05$). (B) RAW264.7 macrophages were transfected with pSUPER-A (directed against CLIP-170), pSUPER-AB (directed against CLIP-170 and CLIP-115), or pSUPER-G (directed against giantin) as a control. Cells were then allowed to phagocytose IgG-SRBCs for 3 and 60 min at 37°C, fixed and stained with Cy2–anti-rabbit IgG antibodies, and, after permeabilization, fixed and stained with the monoclonal anti–CLIP-170 antibody. The efficiencies of association (left) and phagocytosis (right) were calculated for 50 CLIP-170–depleted cells (A), 50 CLIP-170/CLIP-115–depleted cells (AB), and 50 control cells. Results are expressed as a percentage of control cells. The means ± SEM of three independent experiments are plotted. No significant difference was observed in the different conditions ($P > 0.1$).

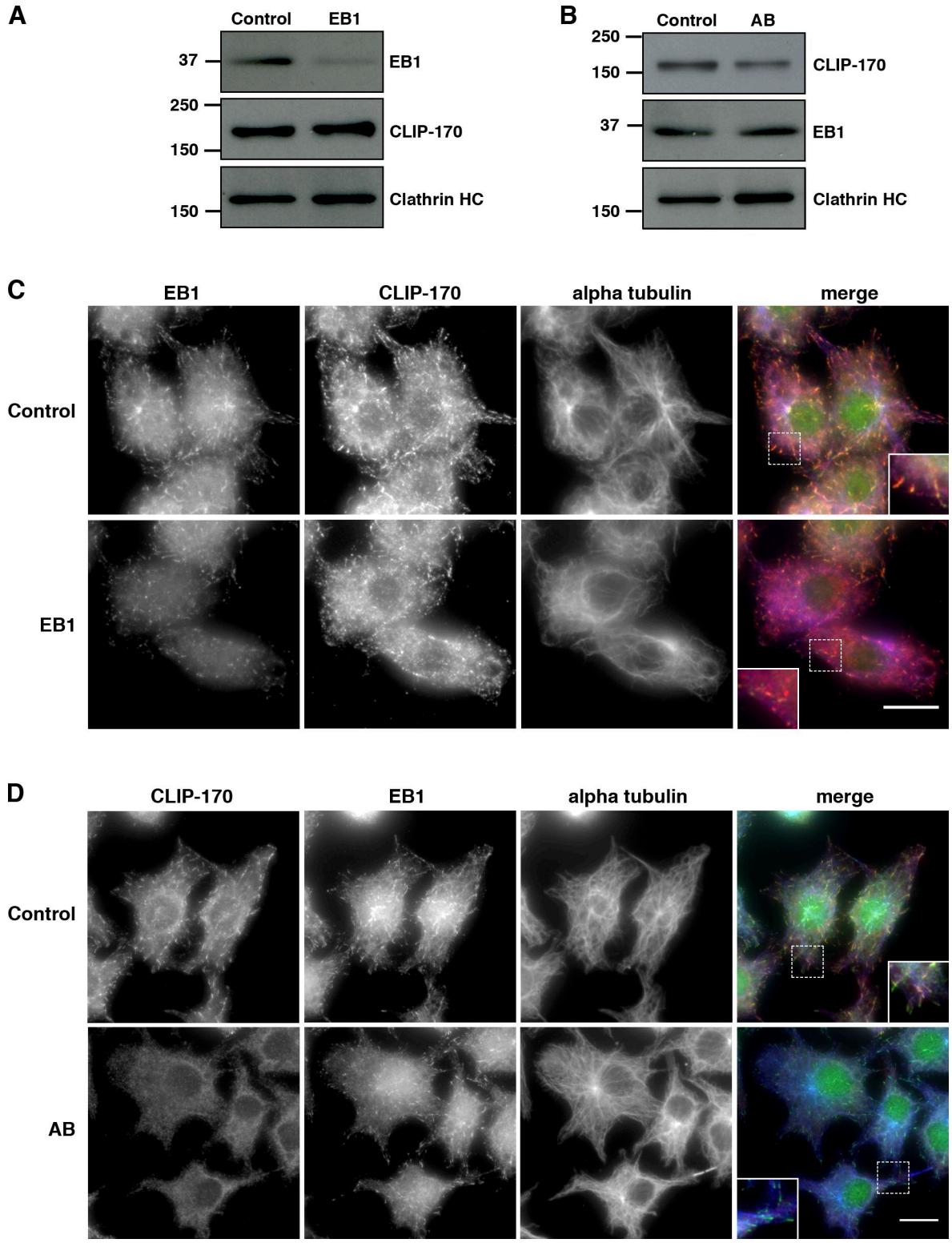

Figure S3. **EB1 siRNA does not impair the +Tip localization of CLIP-170/CLIP-115.** (A) RAW264.7 macrophages were transfected with siRNA directed against EB1 or GFP as a control, and after 24 h lysates were prepared and Western blotting was performed with anti-EB1 and anti–CLIP-170 (#2221) antibodies or with anti-clathrin HC as a loading control. (B) RAW264.7 macrophages were transfected with pSUPER-AB (directed against CLIP-170 and CLIP-115) or pSUPER-G (directed against giantin) as a control. After 48 h, lysates were prepared and Western blotting was performed as in A. (C) RAW264.7 cells were transfected with siRNA directed against EB1 (bottom) or GFP as a control (top), and after 24 h the cells were analyzed by immunofluorescence with anti-EB1, anti–CLIP-170/CLIP-115 (#2221 serum), and anti-tubulin (clone F2C) antibodies followed by Cy2–anti-mouse, Cy3–anti-rabbit, and Cy5–anti-human IgG, respectively. A z projection of three planes acquired with 0.2-µm step is shown. Insets show magnification of the region indicated in dotted line. Bar, 10 µm. (D) RAW264.7 macrophages were transfected with pSUPER-AB (directed against CLIP-170 and CLIP-115, lower panels; bottom) or pSUPER-G (directed against giantin) as a control (top). After 48 h, cells were fixed, permeabilized, and labeled as described in C. Cells were analyzed by wide-field fluorescence microscopy. A z projection of three planes acquired with 0.2-µm step is shown. Insets show magnification of the region indicated in dotted line. Bar, 10 µm.

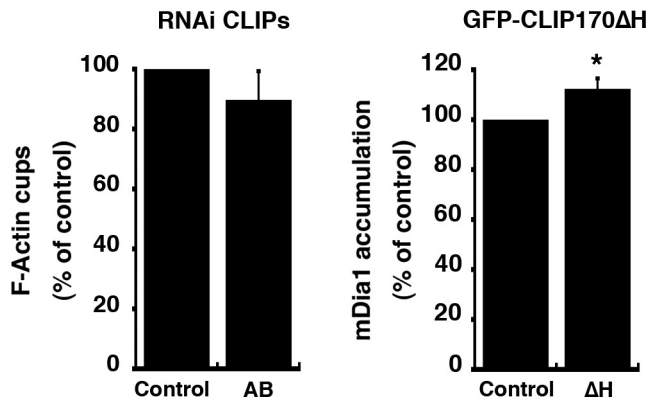

Figure S4. **CLIP-170 function is not important for actin polymerization and mDia1 recruitment during FcR-mediated phagocytosis.** RAW264.7 macrophages were transfected with pSUPER-AB (directed against CLIP-170 and CLIP-115; left) or pEGFP–CLIP-170ΔH (right) and then allowed to phagocytose IgG-SRBCs for 10 or 60 min at 37°C. The cells were fixed and stained with AMCA– or Cy2–anti-rabbit IgG to detect the external particles, phalloidin–Alexa 488 or –Alexa 350 to stain F-actin and anti-mDia1 antibodies followed by Cy3–anti-mouse IgG. 50 CLIP-170–inhibited cells and 50 control cells were scored for the presence or absence of F-actin (left) or mDia1 (right) accumulation around bound particles. Results are expressed as a percentage of control cells. Means ± SEM of three independent experiments are plotted. F-actin enrichment in cells treated with shRNA against CLIP-170 and CLIP-115 was not significantly impaired compared with control cells ($P > 0.1$). mDia1 enrichment was increased in cells expressing CLIP-170ΔH (112 ± 4% as compared with control cells; *, $P < 0.05$).

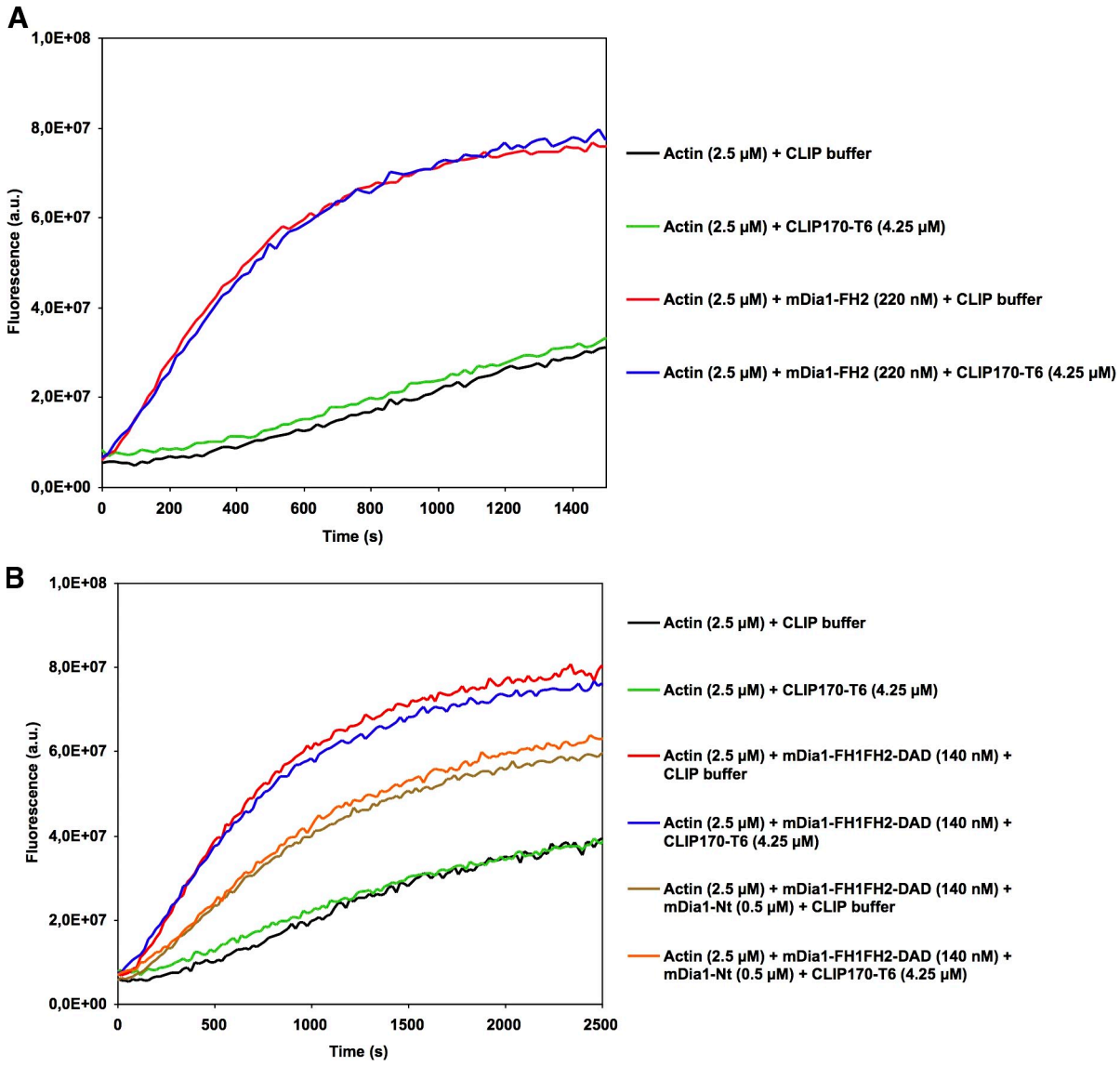

Figure S5. **CLIP-170–T6 does not affect the nucleation of actin by mDia1.** Based on our estimation of the affinity of GST–CLIP-170–T6 for mDia1 (K_d = 0.8 µM), we used 4.25 µM of GST–CLIP-170–T6, which saturates most of the 220 nM of mDia1-FH1FH2-DAD and 140 nM mDia1-FH2 constructs. (A) Effect of 4.25 µM CLIP-170–T6 or the same volume of CLIP buffer on assembly of 2.5 µM MgATP–G-actin, 10% pyrenyl labeled, in the presence of 220 nM mDia1-FH2. (B) Effect of 4.25 µM CLIP-170–T6 or the same volume of CLIP buffer on assembly of 2.5 µM MgATP–G-actin, 10% pyrenyl labeled, in the presence of 140 nM mDia1-FH1FH2-DAD and 0.5 µM mDia-Nt . As a control, we also show that the addition of 5 µM CLIP-170–T6 and 0.5 µM mDia-Nt does not affect actin assembly.