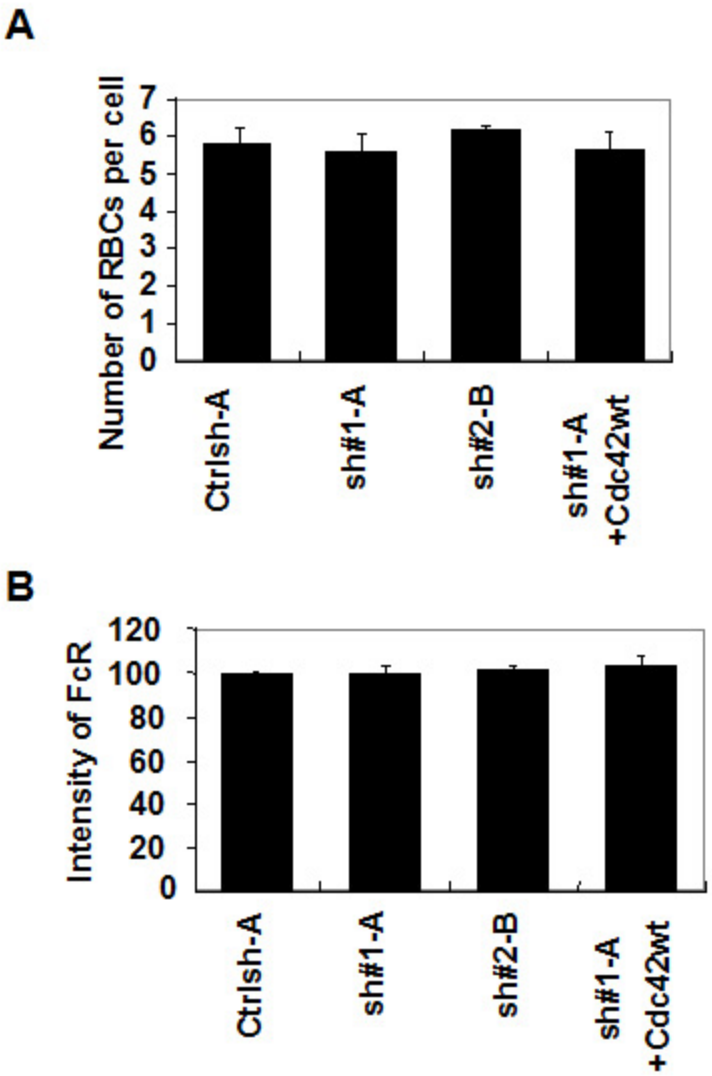
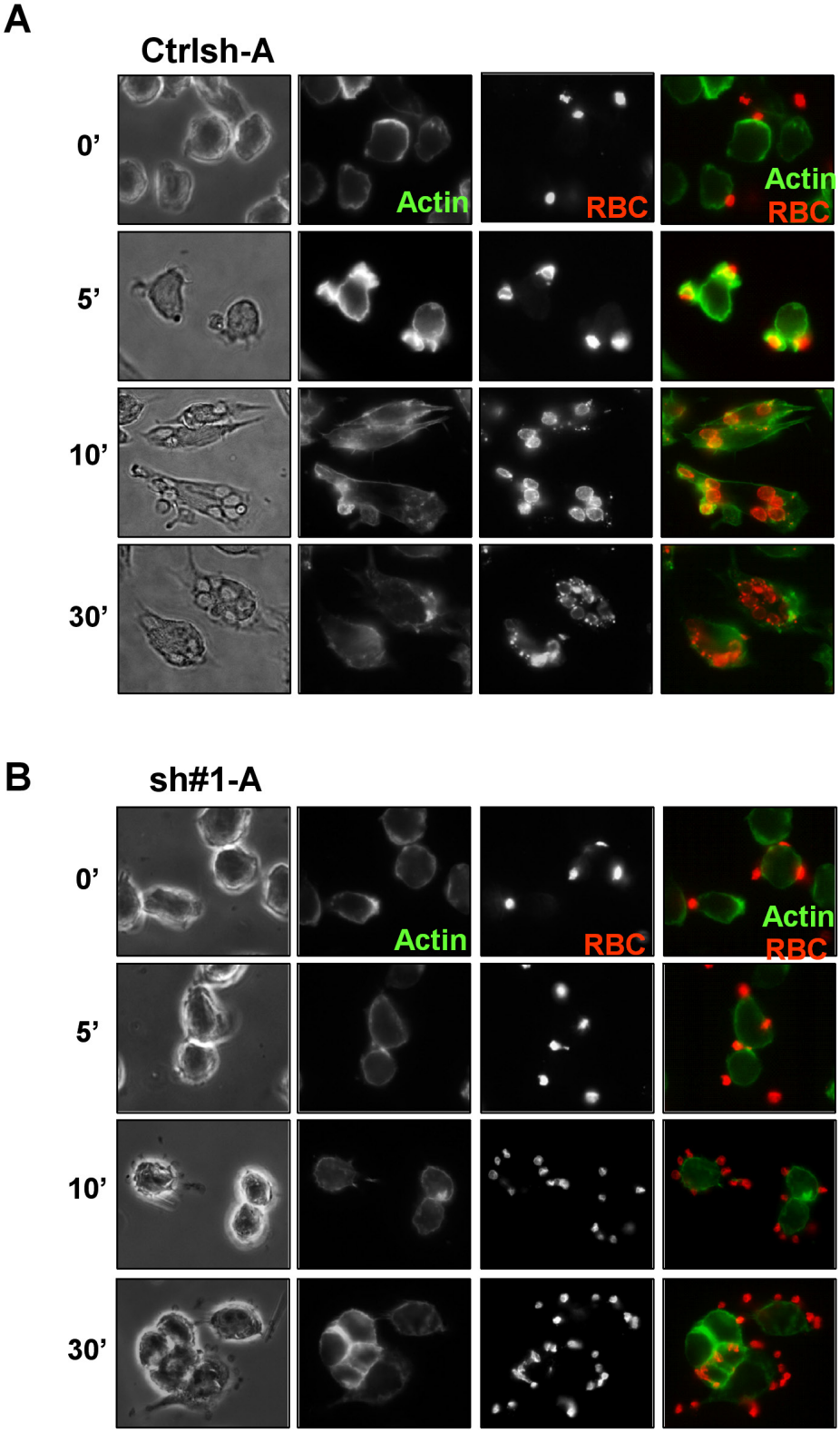
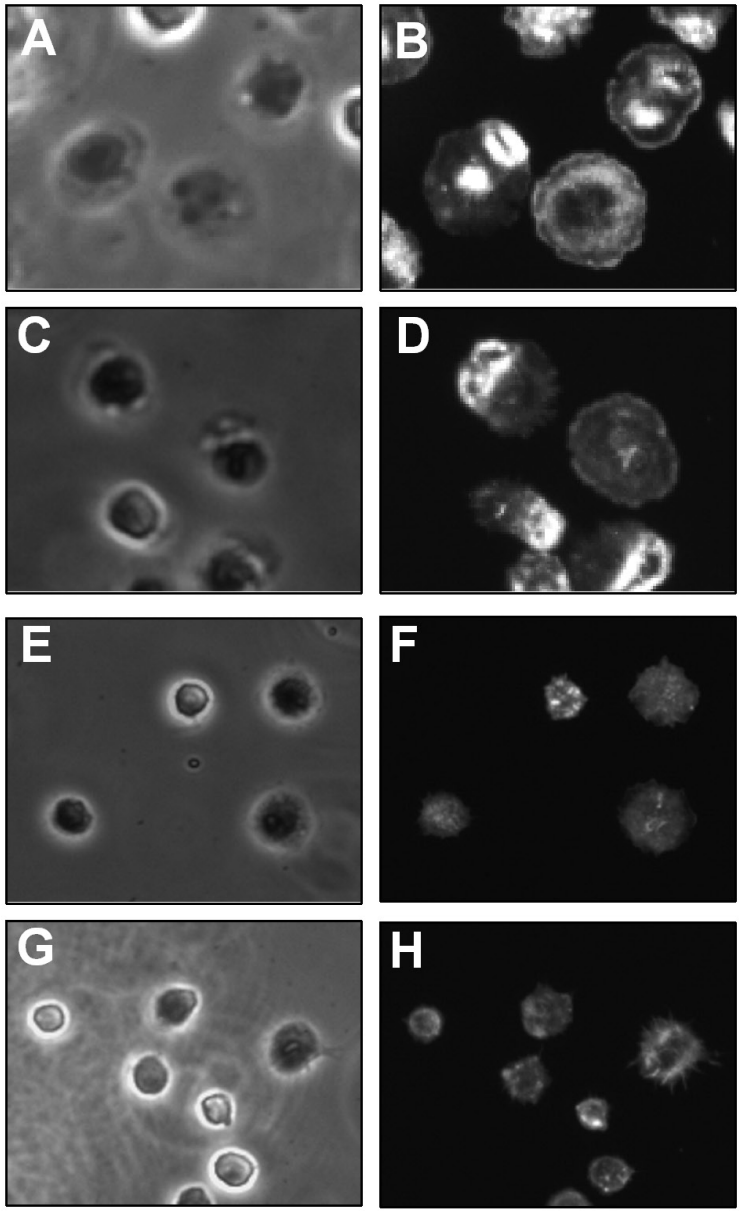


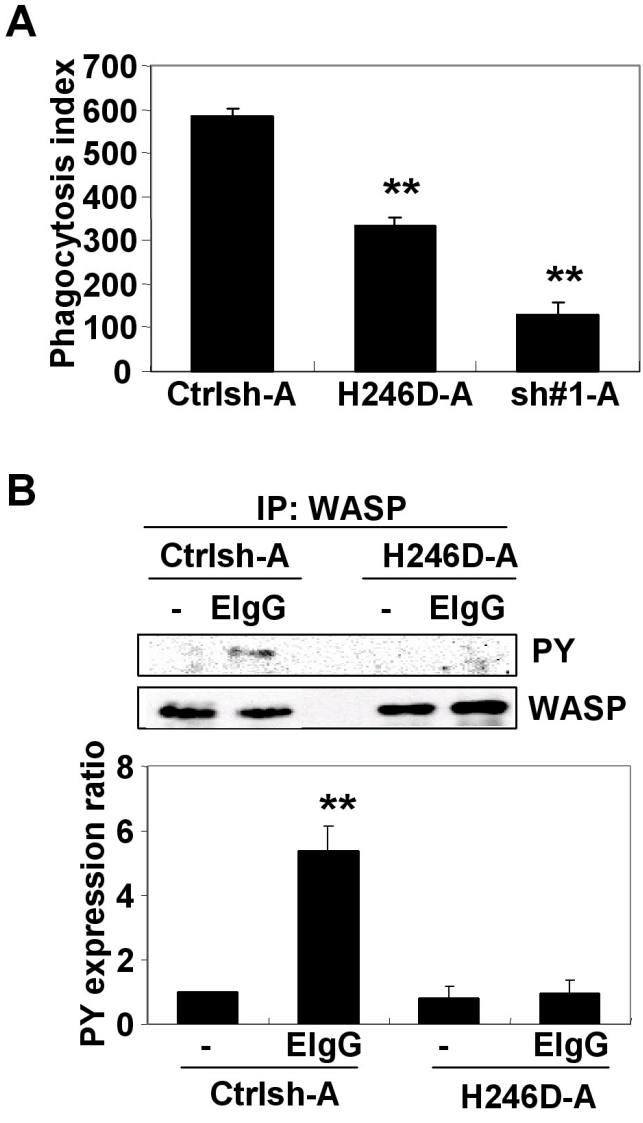
Figure S1. Expression of Cdc42 is not affect EIgG binding and FcγR intensity. (A) Controlsh-treated cells (Ctrlsh-A), two independent clones with reduced Cdc42 expression (sh#1-A or sh#2-B, see A) and re-introduction of Cdc42 wild-type protein in sh#1-A cells were incubated with EIgG for 15 min at 4°C before fixation and the ability of cells to bind EIgG was scored as the number of RBCs per cell ± SEM (n = 3 independent experiments). (B) Surface expression of FcγR was determined by immunofluorescence using CD16/CD32 antibody. Data, reported percent fluorescence intensity as compared to Ctrlsh-A cells, are expressed as mean ± SEM (n = 3 independent experiments).



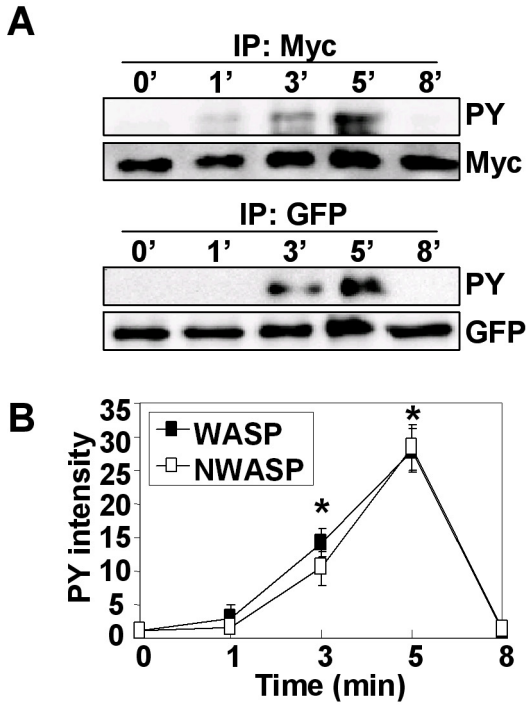
Supplemental Figure 2. Phagocytic cup formation is inhibited by reduced expression of Cdc42. (A) Control (Ctrlsh-A) and (B) Cdc42 shRNA-treated clones (sh#1-A) were incubated with EIgG for various times at 37°C before fixation. Immunofluorescence using Alexa Fluor 568 anti-rabbit IgG to identify EIgG and Alexa Fluor 488-phalloidin to stain for F-actin. Phagocytic cups were prevalent at 5 min in Control cells but were never seen in Cdc42 shRNA-treated cells.



Supplemental Figure 3. F-actin accumulation and cell spreading on IgG are inhibited by reduced expression of Cdc42. Control shRNA-treated cells (Ctrlsh-A, A-D) and cells with reduced Cdc42 expression (sh#1-A, E-H) were allowed to spread on human IgG-coated coverslips at 37°C for 10 min (A, B, E and F) or 30 min (C, D, G and H), and then fixed and stained for F-actin with Alexa Fluor 568-phalloidin.



Supplemental Figure 4. Phagocytosis was inhibited by expression of a Cdc42 binding deficient form of WASP (H246D). (A) Control (Ctrlsh-A), Cdc42 shRNA-treated cells (sh#1-A) and cells in which endogenous WASP has been replace by H246D WASP expression were incubated with EIgG for 30 min at 37°C before fixation. The phagocytic index was calculated as the average number of ingested particles for 100 cells ± SEM (n = 3 independent experiments). **: $p < 0.01$ compared to Ctrlsh-A. (B) Control (Ctrlsh-A) and clones expressing H246D WASP (H246D) were unstimulated (0') or incubated with EIgG for 5 min (5') at 37°C. WASP was immunoprecipitated using a WASP antibody, followed by western blotting with the HRP-PY and WASP antibodies. A representative western blot is shown. Blots were quantified by densitometry and normalized to WASP levels. Data is expressed as the fold increase as compared to EIgG unstimulated Ctrlsh-A. ± SEM ($n = 3$) *: $p < 0.05$



Supplemental Figure 5. WASP and N-WASP phosphorylation peaks at 5 min following FcγR ligation. RAW/LR5 cells were transiently transfected with myc-WASP and GFP-N-WASP and then incubated with EIgG for indicated time at 37°C. After cell lysis, WASP or N-WASP was immunoprecipitated using anti-myc or anti-GFP, respectively. (A) Samples were subjected to Western blotting with the indicated antibodies. (B) Blots were quantified by densitometry and normalized to myc or GFP. Data is expressed as the fold increase as compared to each 0'. ± SEM ($n = 3$) *: $p < 0.05$