Supplementary Materials

Fig. S1. Purified proteins used for the *in vitro* reconstitution experiments. Multiple preparations of each of the indicated purified proteins were used for the experiments documented in Figs. 1, 2, S2 and S3. Shown here are representative samples resolved on SDS polyacrylamide gels stained with Coommassie blue.

Fig. S2. Rac1 and Cdc42 bind IQGAP1. IQGAP1 binds with Rac1 and Cdc42 in their activated GTPγS-charged forms, but not with their GDP-charged forms or naked GST. Shown here are total and bound fractions of protein G beads saturated with polyclonal anti-IQGAP1 following their incubation with 200nM GST-tagged wild type Cdc42 or Rac1, along with 200nM IQGAP1.

Fig. S3. **Actin assembly monitored by spectrofluorometry**. Samples contained 2 μM total G-actin (5% labeled with pyrene), 50 nM N-WASP, 50 nM Arp2/3 complex, 45 nM IQGAP1 (homodimers) and the indicated concentrations of GTPγS-loaded GST-Cdc42 or GST-Rac1. These tracings provided the raw data for Fig. 2C.

Fig. S4. Spectra of the two triple-label Fusion Protein combinations (FPs) and their FRET interaction behaviors. (A,B) Each triple set meets FRET spectral pre-conditions in terms of spectral overlap. (C) Co-expression of Teal-N-WASP, Venus-IQGAP1, and mRFP-Cdc42 or mRFP1-Rac1 yielded FRET between all protein pairs. (D) In contrast, co-expression of CFP-Rac1 or CFP-Cdc42 plus Venus-IQGAP1 and mCherry-actin yielded FRET between Venus-IQGAP1 and each complementary fluorescent fusion protein, but not between either CFP-tagged small G protein and mCherry-actin.

Table. S1. **Comparison of scatter plot data in Figures 6 and 9 by T-test analysis**. The indicated pairs of the total population data sets from Figs. 6 and 9 were compared by Student's T-tests assuming unequal variances. Using the upper left panel of Fig. 6 as an example, the unfused Teal:Venus data points in the presence of unfused mRFP1 were compared with the Teal-N-WASP:Venus-IQGAP1 data points in the presence of mRFP1-Cdc42, and separately, with the

Teal-N-WASP:Venus-IQGAP1 data points in the presence of mRFP1-Rac1. Shown here are p-values for each comparison.