Supplementary Figure 4. Demonstration of CLIP-170 down-regulation by shRNAi.

NIH3T3 cells were transfected with pS-shCLIP170a, pS-shCLIP170b or pR-shIQGAP1 and were harvested 48 hours later for Western blot analysis. **Top panel:** Western blot of straight cell lysate showed a doublet, for which the lower band represented CLIP-170 protein that was down-regulated by CLIP-170 shRNAi. The level of CLIP-170 protein was little changed by shRNAi targeting IQGAP1. **Middle panel:** Pull-down using WASP-CRIB domain (WCD) beads demonstrated the presence of CLIP-170 in the complex. shRNAi targeting CLIP-170 decreased the amount of CLIP-170 in the WCD pull-down. Reducing levels of IQGAP1 with shRNAi reduced the amount of CLIP-170 found in the complex in the WCD pull-down, though it did little to the total level of CLIP-170. **Bottom panel:** Lis1 levels in the straight lysate provided a protein loading control.