Supplementary Fig. 3. The R-Ras GAP activity of Plexin-A1
Measurement of GTPase activity in vitro was performed as described previously. Using the experimental system similar to that of Oinuma et al., we measured GTPase activity of R-Ras by using purified Plexin-A1-Cyt. Briefly, the purified Plexin-A1-Cyt proteins (0.5 mg) were treated with various combinations of reagents; in some experiment, Plexin-A1-Cyt proteins were clustered by anti-T7 antibodies, followed by incubation with an anti-mouse IgG. After the clustering reaction, the complex was incubated with recombinant Rnd1 (1 mg) for 30 min, and then 20 ng of R-Ras preloaded with [g-32P]GTP was added; and in other experiments, some of these reagents were omitted. The radioactivity bound to R-Ras in the indicated times was measured by a nitrocellulose filtration assay. GTPase activity of R-Ras is not altered by clustering of Plexin-A1-Cyt alone (yellow triangles). Rnd1-bound Plexin-A1-Cyt blocks the basal GTPase activity of R-Ras (blue circles), probably because of trapping of GTP-bound active R-Ras, and clustering of Rnd1-bound Plexin-A1 can dramatically stimulate hydrolysis of bound GTP of R-Ras (black squares). These results indicate that the clustering of the Rnd1-bound Plexin-A1 is necessary for displaying R-Ras GAP activity of Plexin-A1.

REFERENCE