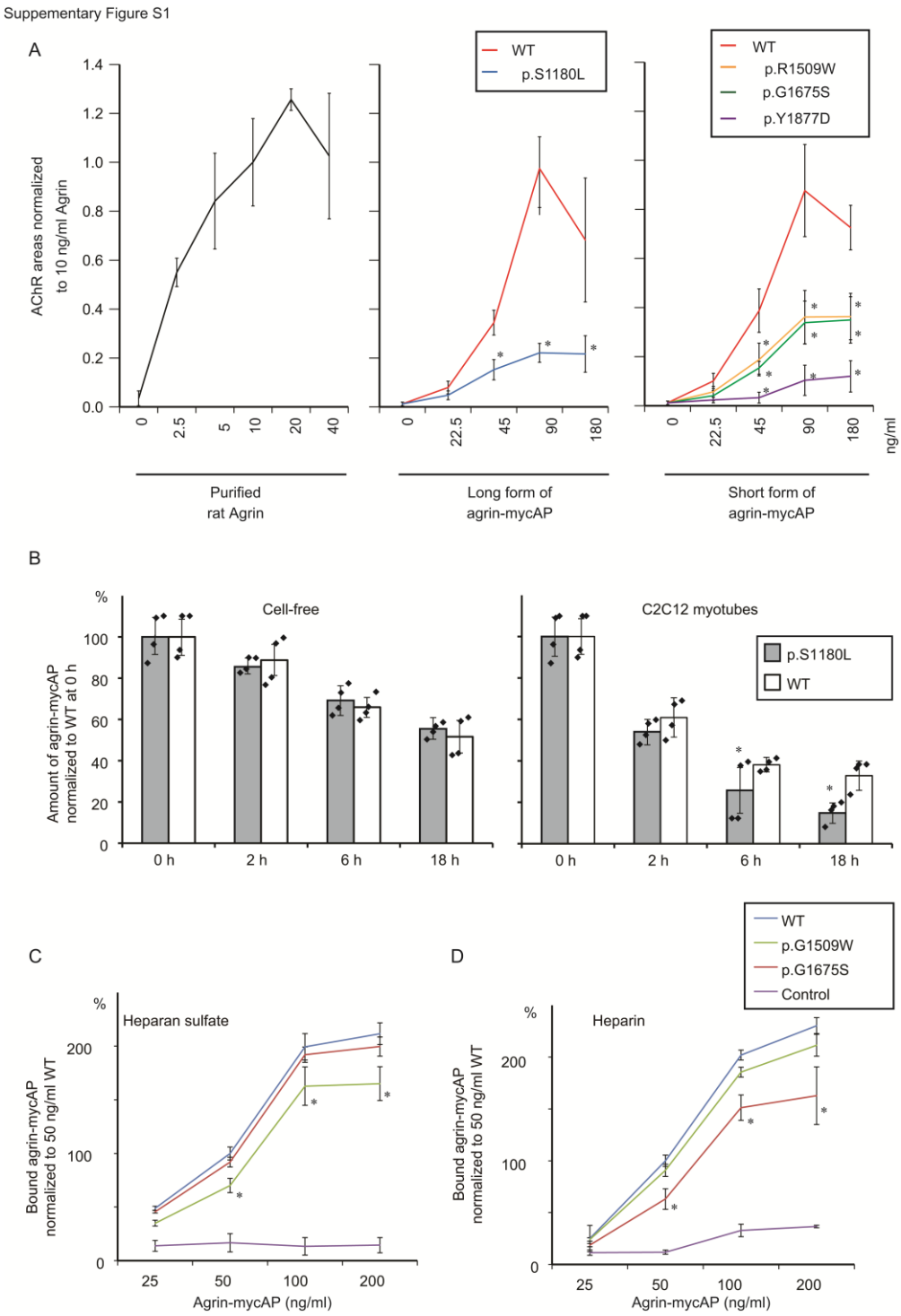

Supplemental Figure 1. Quantitative analysis of the effects of wild-type and mutant agrin on AChR clustering on C2C12 myotubes, protein degradation, and binding to heparin or heparan sulfate.

(A) C2C12 myotubes were cultured with control conditioned medium (0 ng/ml), or conditioned medium containing indicated concentrations of wild-type (WT) or mutant agrin-mycAP for 18 h.

The AChR signal area was blindly quantified with Metamorph. The AChR signal area was normalized for the myotube area and also for the ratio of 10 ng/ml purified rat agrin. Mean and SD are indicated (n = 3 images each in 3 wells). Estimation of the concentration of agrin-mycAP in the conditioned medium is described in Materials and Methods, **(B)** Degradation assay of WT and p.S1180L-mutant agrin-mycAP. Purified WT or p.S1180L agrin-mycAP was added to the medium culturing C2C12 myotubes, and the amount of agrin-mycAP was quantified by measuring the AP activity at 0, 2, 6 and 18 h. The AP activity of agrin-mycAP was normalized for that at 0 h. Mean and SD (n = 4 wells) are indicated. One-way ANOVA followed by post-hoc Tukey test. *P < 0.05. Half lives of WT and p.S1180L-mutant agrin-mycAP were 2.3 h and 2.6 h, respectively. **(C, D)** Plate-binding assay of purified WT, p.G1509W, and p.G1675S agrin-mycAP to heparan sulfate **(C)** and heparin **(D)**. Mean and SD (n = 3 wells) are indicated. One-way ANOVA followed by post-hoc Tukey test. *P < 0.05.