

**Figure S2.** Gain or loss of Syd-1 does not increase satellite bouton number, and Cdc42 overexpression is not sufficient to prevent Rac1 from increasing NMJ bouton number.

(A) Quantification of the number of boutons on muscle 4 in abdominal segment 3 per muscle area. As expected, overexpressing Rac1<sup>wt</sup> in the motorneurons of wild-type animals increases bouton number (*BG380-Gal4*, *UAS-Rac1<sup>wt</sup>*: 46.2±2.4, n=11) compared to wild type (*BG380-Gal4* alone: 35.8±2.0, n=12, p=0.004). Coexpression of Cdc42<sup>wt</sup> with Rac1<sup>wt</sup> (*BG380-Gal4*, *UAS-Rac1<sup>wt</sup>*, *UAS-Cdc42<sup>wt</sup>*::45.8±2.8, n=10) does not cause a significant decrease in this number (p=0.45 compared to Rac1<sup>wt</sup> alone).

(B) Quantification of the number of satellite boutons on muscle 4 in abdominal segment 3. Satellite boutons are smaller boutons that bud off a larger, main bouton and are otherwise not connected to the linear chain of main boutons (e.g. Nahm et al. 2014). Overexpressing wild-type Syd-1 in motorneurons (*BG380-Gal4, UAS-syd-1<sup>wt</sup>*: 1.2±0.3, n=14) or entirely eliminating Syd-1 (*syd-1<sup>CD</sup>/syd-1<sup>w46</sup>*: 1.3±0.3, n=18) does not increase satellite bouton number compared to wild type (*BG380-Gal4* alone: 1.4.8±0.4, n=19, p=0.76 and p=0.68 respectively).