**Supplementary Figure 3**

Differences between boutons in the number of active zone patches or the total quantity of an active zone marker cannot explain the proximal-distal gradient of transmission strength. (a) After imaging, active zones were identified with the Nc82 antibody (in green, SynapCam3.1 YFP in red). (b, c) The total quantity of active zone marker, Nc82 and the density of Nc82 puncta per bouton showed no significant correlation to the mean ∆FRET amplitude (respectively, $r = 0.121$, $p = 0.384$; $r = 0.225$, $p = 0.102$). For all panels, data consist of 55 postsynapses from 5 NMJs, each NMJ represented by a different symbol. For comparison, values were normalized within each NMJ dataset.