Zhang and Linden, Supplemental Figure Legends

Supplemental Figure 1: AP-evoked Ca^{2+} responses at PF boutons were heterogeneously attenuated by neuromodulators.

These are exemplar images and Ca^{2+} traces to correspond with the data shown in Figure 3. Representative Z projection images of the PFs used to examine the neuromodulator's effect on AP-evoked Ca^{2+} transient under different experimental conditions are shown on the left panels. Ca^{2+} imaging was performed in line scan mode on 3 boutons indicated by the dotted lines. The bouton with biggest volume among the 3 on the same fiber is indicated by the filled circle and the smallest is indicated by the open circle. The bouton with highest peak AP-evoked Ca^{2+} transient in the absence of agonist treatment is indicated by the filled square and the lowest is indicated by the open square. Single stimulus-evoked Ca^{2+} transients measured sequentially from 3 neighboring boutons on the same PF show significant variation in the amplitude and the decay time constant. The traces, shown on the right, are presented as the time plot of the $\%\Delta F/F$. The amplitudes of the AP-evoked Ca^{2+} transients in individual boutons were first measured in the absence (red) and then in the presence (blue) of neuromodulator receptor agonists. The average traces from 5 trials are shown and the Ca^{2+} responses were fitted with a single exponential decay curve, superimposed as black lines. The arrow indicates the onset of the single stimulation.

Supplemental Figure 2: Basal calcium levels of boutons were not changed by agonist addition.

The bouton basal fluorescence before the onset of the AP evoked calcium transient was quantified both before and after agonist addition for the various agonists. Control, n = 39 boutons; 2-CA, n = 36; L-AP4, n = 39; DMSO, n =36; WIN55212-2, n = 48. Error bars indicated the SEM.

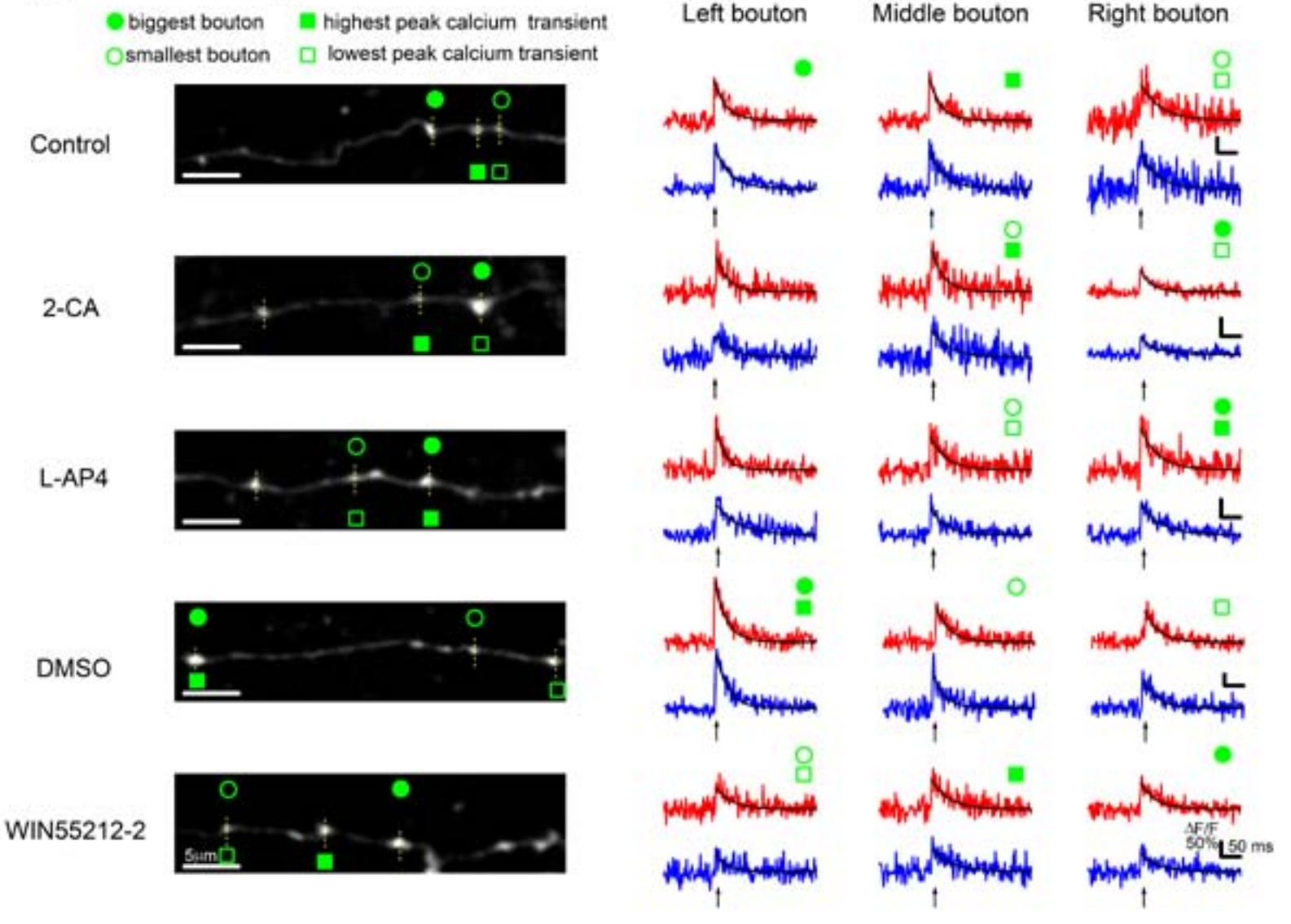
Supplemental Figure 3: Areas of the biggest and smallest boutons from within-fiber comparisons in the various experimental groups.

The bouton areas of the biggest and smallest boutons in the various experimental groups are plotted. Control, n = 12 fibers; 2-CA, n = 10; L-AP4, n = 9; DMSO, n = 10; WIN55212-2, n = 10. Bouton areas were determined from the Z-projection of confocal images of the dye-filled bouton obtained after Ca^{2+} imaging. Error bars indicate the SEM.

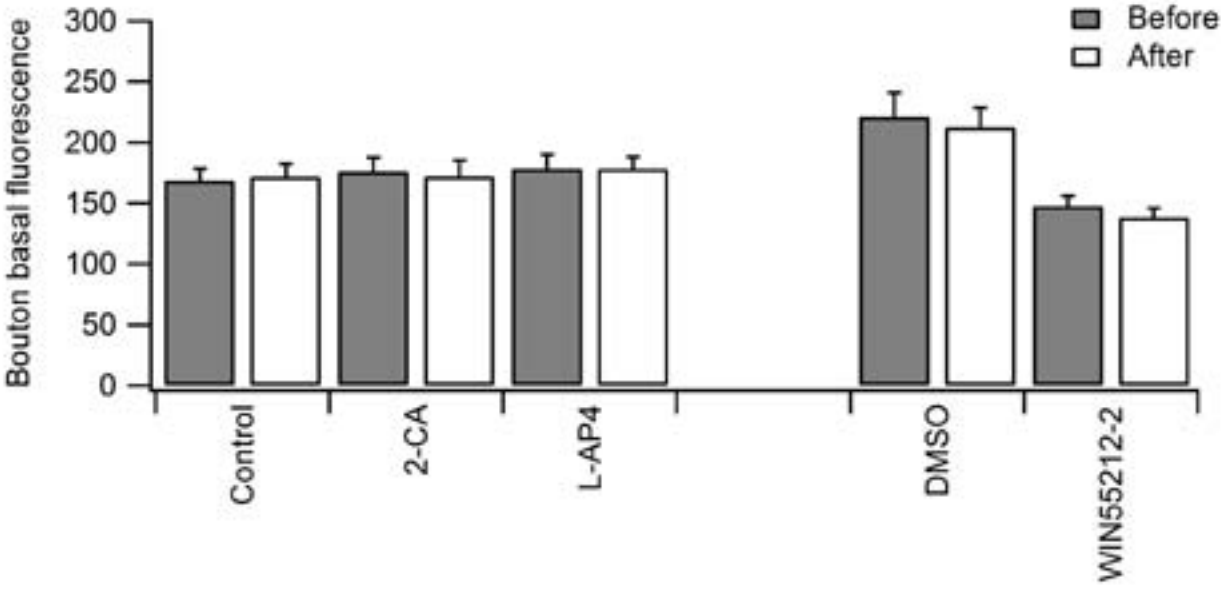
Supplemental Figure 4: Measurement of basal Ca^{2+} fluorescence in big and small boutons does not reveal differential bleaching across agonist treatment groups.

Bouton basal fluorescence before the onset of the AP evoked Ca^{2+} transient was measured before and after agonist treatment and the % change in this measure is plotted here. Control, n = 12 fibers; 2-CA, n = 10; L-AP4, n = 9; DMSO, n = 10; WIN55212-2, n = 10. Error bars indicate the SEM.

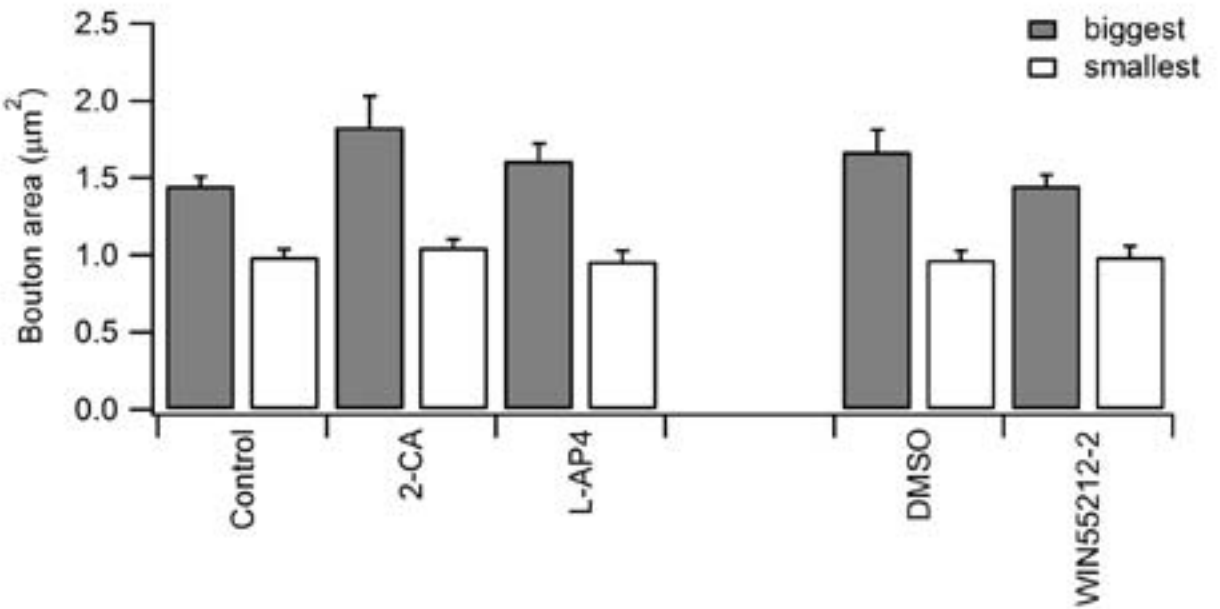
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4