$$\rho = \frac{P_{RG} - P_R P_G}{\sqrt{P_R(1 - P_R)\,P_G(1 - P_G)}}$$

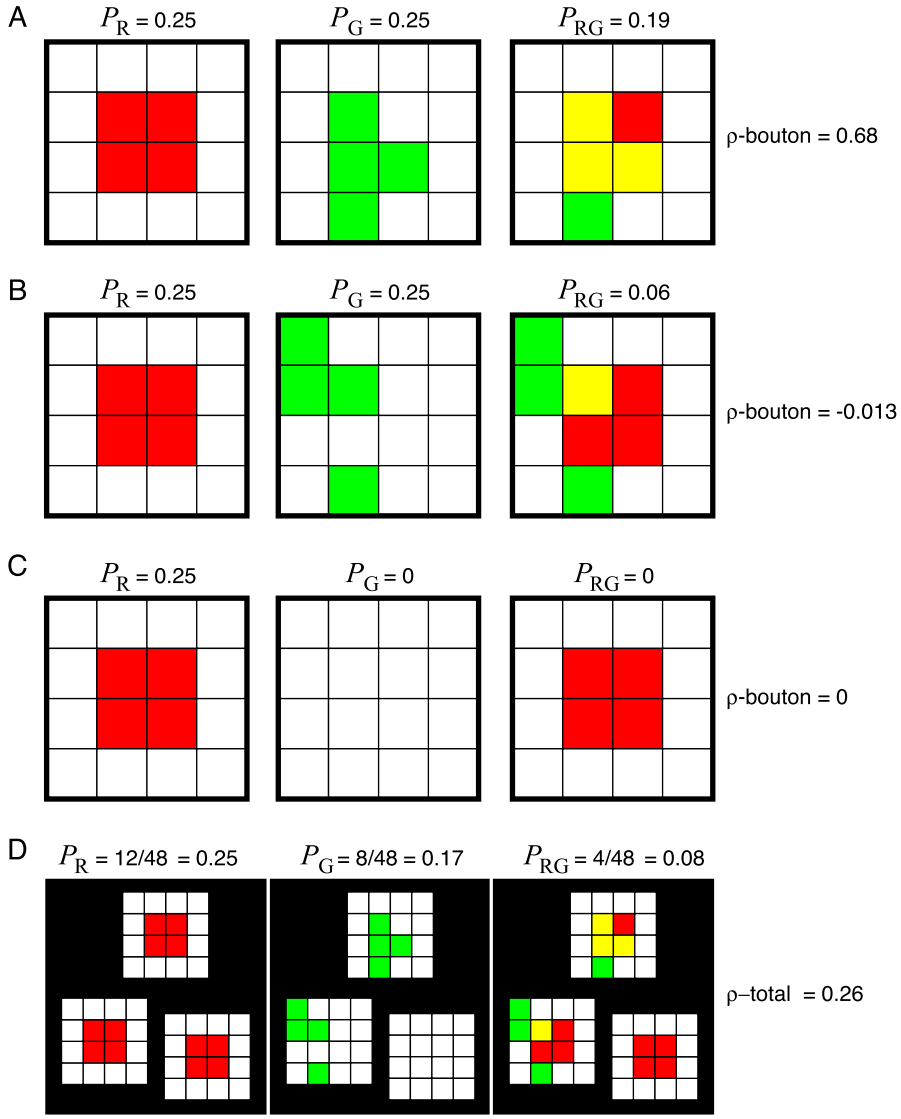

Figure S1. Possible experimental outcomes and calculation of ρ-bouton and ρ-total correlation coefficients. For each panel, P_R (P_G) is the probability of red (green) pixels occurring within the given region of interest (outer square) and P_{RG} is the probability of pixel overlap (yellow). Each box represents a single pixel and colored regions indicate pixel values greater than the intensity threshold (see Methods). (A) Red and green pixels are present within the region and exhibit significant overlap. (B) Red and green pixels are present within the region and exhibit minimal overlap. (C) Red pixels, but no green pixels, are selected resulting in zero probability of pixel overlap. This scenario can occur because regions of interest are pre-selected to include red pixels, blind to the presence or absence of VGluT2 (green pixels). In this case, the probability of overlap is zero (P_{RG} =0) and, by definition, ρ = 0. (D) ρ-total was calculated by combining the pixel counts from all regions (squares with grids) in a given image (indicated by the black box). The percentage of red, green and co-localized pixels (P_R, P_G and P_{RG}, respectively) within each image was calculated by dividing the sum total of colored pixels by the sum total of all pixels from all regions, ρ was then derived using these values.

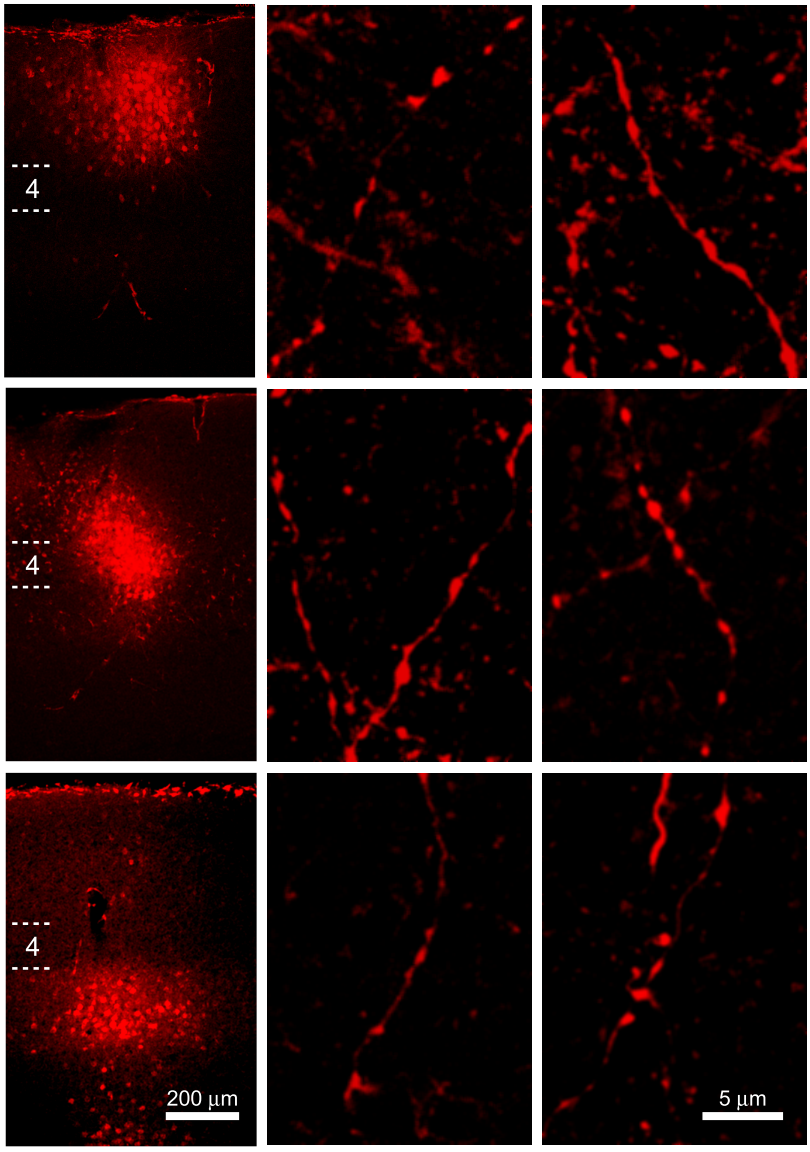

Figure S2. Cortical injections of micro-ruby and labeling of intracortical axons in layer 4. The images on the far left show low-magnification examples of three different injections with extracellularly filled cells located throughout the cortical layers (dashed lines mark layer 4 boundaries). To the right are examples of high-magnification images of filled axons acquired from within the bounds of layer 4.

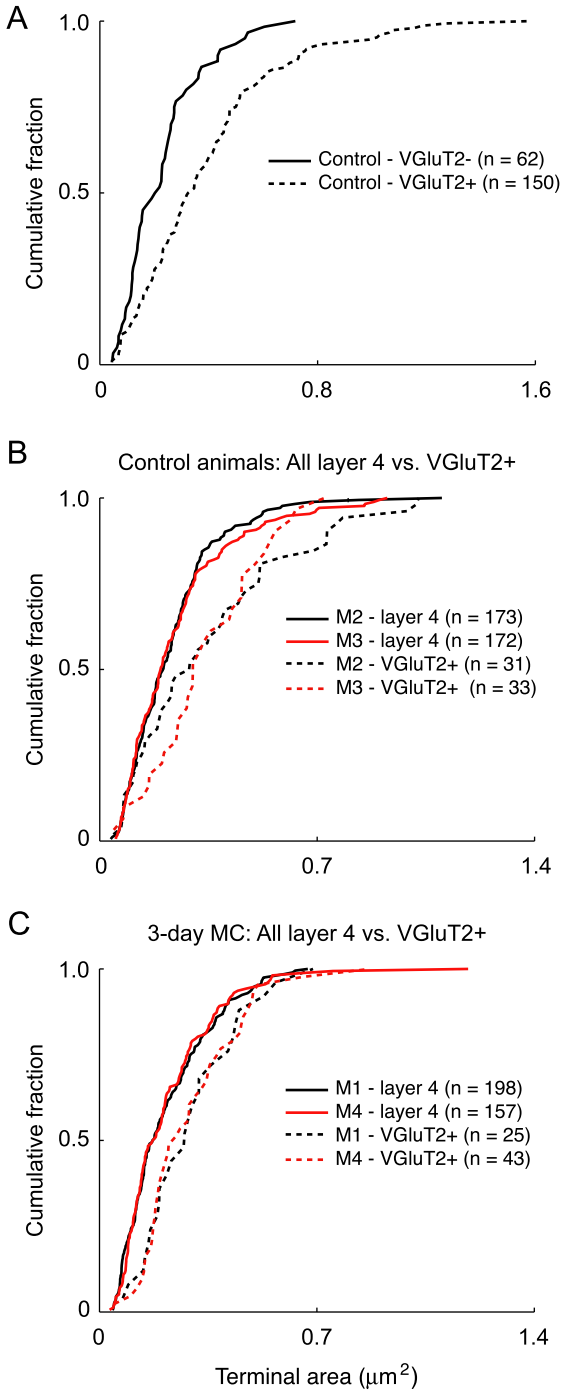

Figure S3. VGluT2-labeled synaptic terminals represent a unique subpopulation of synapses in layer 4 of binocular V1. (A) Cumulative probability histograms comparing size distributions of VGluT2-labeled (+) and unlabeled (-) terminals sampled near the tissue-resin interface ($P < 10^{-9}$, Kolmogorov-Smirnov [K-S] test). (B-C) Cumulative probability histograms comparing size distributions of VGluT2+ and all layer 4 terminals. Terminal size distributions are shown for (B) two control animals (cases M2 and M3) and (C) two 3-day MC animals (cases M1 and M4). There was no significant difference in the distribution of terminal sizes between individual animals ($P > 0.1$ for M2 vs. M3 layer 4 and VGluT2+ terminal populations; $P > 0.1$ for M1 vs. M4 layer 4 and VGluT2+ terminal populations, K-S tests). In all cases, VGluT2-labeled terminals constitute a larger-sized subpopulation ($P < 0.05$ for all layer 4 vs. VGluT2+ terminals, K-S test).

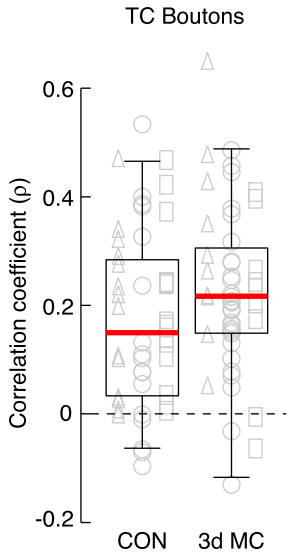

Figure S4. VGluT2 expression in TC boutons is not affected by MC. Summary of co-localization analysis for TC boutons from control ($n = 3$ animals, 47 boutons) and 3-day MC ($n = 3$ animals, 48 boutons) tissue. The box plots show the distribution of pooled correlation coefficients calculated for each group. Horizontal lines mark the 25th, 50th (red) and 75th percentiles and the ends of the whiskers mark the 2.5th and 97.5th percentiles. The two distributions were not statistically different from each other ($P = 0.07$, Kolmogorov-Smirnov test). Correlation coefficients for individual boutons are plotted as gray symbols; data from each animal are represented by different shapes. All 3-day MC tissue and images were prepared and acquired blind to condition and yoked to control tissue (control data are the same as shown in Figure 2C).