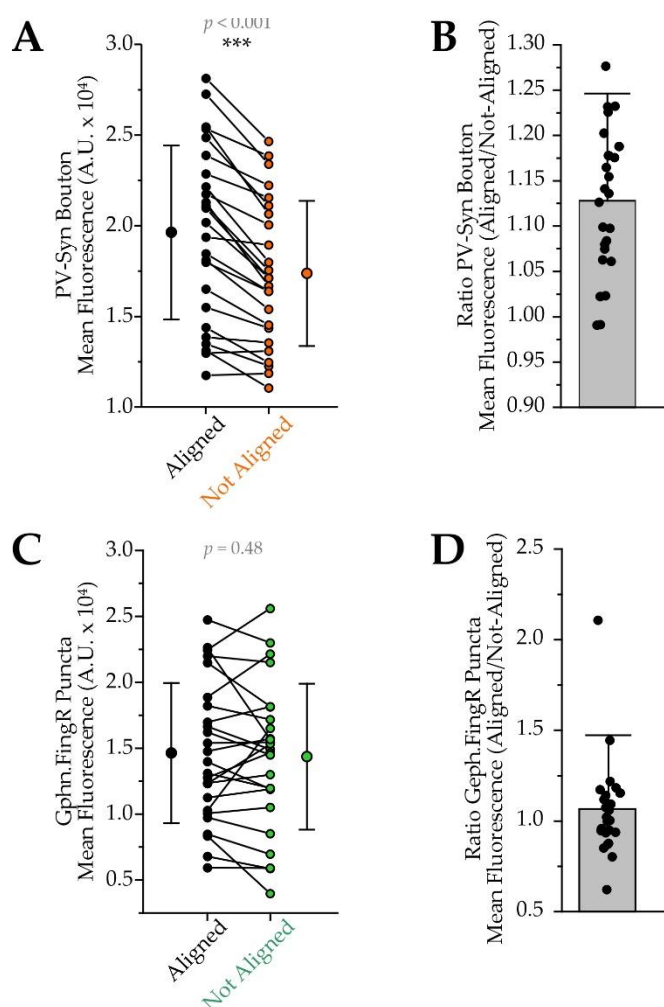
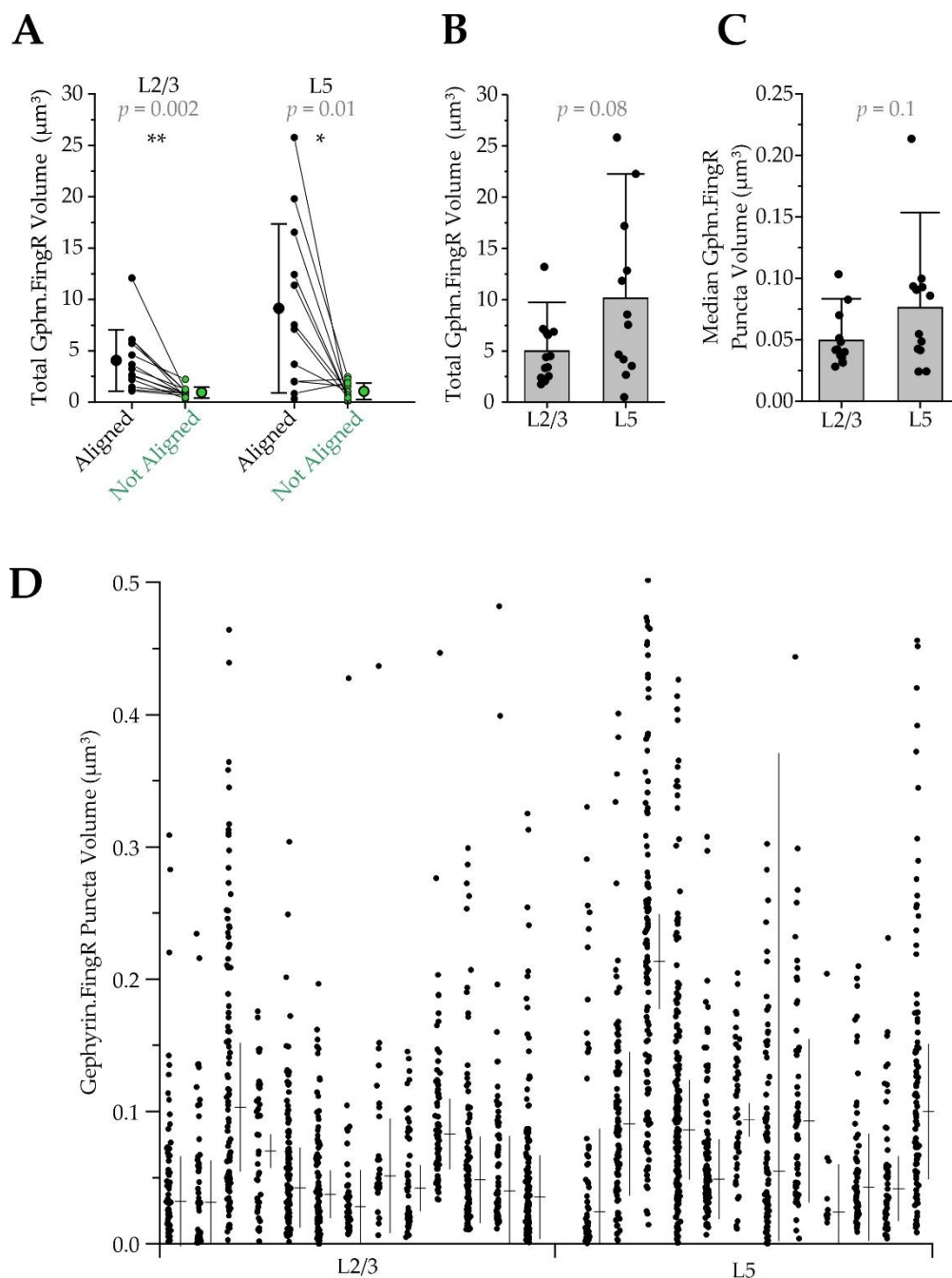


## Supplemental Materials



**Figure S1.** Fluorescence intensity of PV boutons and gephyrin puncta across Pyr neurons by alignment category; **(A)** PV-Syn bouton mean fluorescence intensity for boutons aligned with postsynaptic Gephyrin.FingR (black) or not aligned (orange). Pairs of dots connected by a line represent the values for one Pyr neuron. Beside cell values are plotted means  $\pm$  SD for each alignment category; **(B)** the ratio of mean PV-Syn bouton fluorescence intensity for the median bouton for each Pyr cell that could be aligned with Gephyrin.FingR over not aligned; **(C)** Gephyrin.FingR puncta (Gphn.FingR) mean fluorescence intensity for puncta aligned with presynaptic PV-Syn (black) or not aligned (green). Pairs of dots connected by a line represent the values for one Pyr neuron. Beside cell values are plotted means  $\pm$  SD for each alignment category; **(D)** the ratio of mean Gephyrin.FingR fluorescence intensity for the median puncta for each Pyr cell that could be aligned with PV-Syn over not aligned; L2/3,  $n = 13$  cells; L5,  $n = 12$  cells;  $N = 2$  animals.



**Figure S2.** Total gephyrin volume and median gephyrin puncta sizes are similar for L2/3 and L5 Pyr neurons; (A) total Gephyrin.FingR puncta volume for L2/3 and L5 Pyr neuron somas based on whether puncta are aligned with presynaptic PV-Syn (black) or not aligned (green). Pairs of dots connected by a line represent the values for one Pyr neuron. Beside individual cell values are plotted means  $\pm$  SD for each alignment category; (B) total Gephyrin.FingR puncta volume for L2/3 and L5 Pyr neuron somas; (C) median Gephyrin.FingR puncta volume for L2/3 and L5 Pyr neuron somas; (D) all Gephyrin.FingR puncta volumes for individual L2/3 and L5 Pyr neuron somas, median  $\pm$  95% confidence interval plotted to the right of each cell's data; L2/3,  $n = 13$  cells; L5,  $n = 12$  cells;  $N = 2$  animals.