# Figure S1



### Figure S1. Characterization of heterosynaptic spine shrinkage, Related to Figure 1.

(A) Extent of shrinkage of unstimulated spines plotted against relative initial volume of unstimulated spines (normalized to the mean spine size on the same dendritic segment). Both small and large unstimulated spines shrank following potentiation of several nearby spines on the same dendritic segment (31 cells; r = 0.033, p = 0.86).

(B) Unstimulated spines in stimulated (HFU, filled black bars; n = 31 spines) and shift-stimulated (shifted HFU, open black bars; n = 15 spines) groups were equally located on average from all uncaging spots (HFU,  $6.4 \pm 0.1$  uncaging events; shifted HFU,  $6.3 \pm 0.1$  uncaging events; p = 0.96), from the two close uncaging spots (p = 0.77), and from the closest one uncaging location (p = 0.59). (C) Total amount of structural potentiation of HFU-stimulated spines was inversely correlated with the extent of shrinkage of unstimulated spines (31 cells; r = -0.49, p = 0.004).

(D) Shrinkage of unstimulated spines was not observed (< 100, 8 cells, p = 0.8) on those cells for which HFU did not lead to strong potentiation; however, when the sum of HFU-induced structural potentiation of neighboring spines was higher than 100%, unstimulated spines shrank (100 - 240, 8 cells, p < 0.05; 240 - 335, 8 cells, p < 0.05; > 335, 7 cells, p < 0.01). Error bars represent s.e.m.; n.s., not significant.

## Figure S2



### Figure S2. Inverse correlations are found in both structural and functional heterosynaptic plasticity, Related to Figure 2.

(A) Relative expression level of SEP-GluA2 plotted against relative spine volume. Scatter plot showed SEP-GluA2 expression and spine volume are highly correlated (r = 0.8; p < 0.05; n = 68 spines, 8 cells). (B) An inverse correlation was found between the magnitude of structural potentiation of stimulated spines and the magnitude of shrinkage of unstimulated spines (11 cells; r = -0.69, p = 0.017).

(C) Homosynaptic increase in SEP-GluA2 fluorescence of stimulated spines was inversely correlated with heterosynaptic decrease in SEP-GluA2 fluorescence of unstimulated spines (11 cells; r = -0.46, p = 0.16).

## Figure S3



## Figure S4



### Figure S3. The magnitude of heterosynaptic spine shrinkage is not related to the extent of structural potentiation of the nearest stimulated spine, Related to Figure 3.

No correlation was observed between the magnitude of structural potentiation of the nearest stimulated spines (1 nearest stimulated spine / cell) and the magnitude of shrinkage of unstimulated spines (1 unstimulated spine / cell) on the same dendrites (31 cells; r = -0.0018, p = 0.99).

#### Figure S4. Neither KN62, FK506, Xestospongin C, nor MPEP and CPCCOEt alters the volume of distant unstimulated spine, Related to Figure 4. Blocking CaMKII, calcineurin, IP<sub>3</sub>R, or group I mGluR with

blocking Calvin, calcinedin, if sit, of group Finduct with bath-applied KN62, FK506, Xestospongin C, or MPEP and CPCCOEt, respectively, did not change the volume of distant unstimulated spines (KN62, n = 10 cells, p = 0.54; FK506, n = 9 cells, p = 0.49; Xesto C, n = 11 cells, p = 0.23; MP + CP, n = 15 cells, p = 0.16). Error bars represent s.e.m.; n.s., not significant.