

Nikonenko et al., <http://www.jcb.org/cgi/content/full/jcb.200805132/DC1>

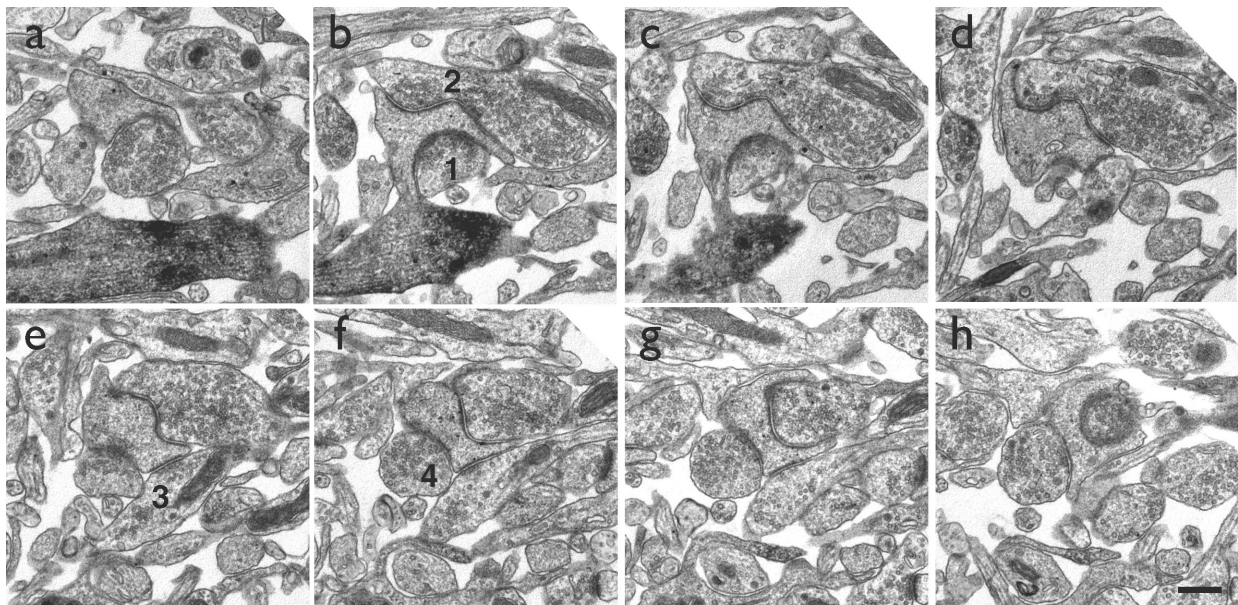


Figure S1. **Serial sections of an MIS from a PSD-95-transfected cell.** Note the dark-labeled dendritic shaft on a–c and the neck of the MIS on b and c. The four axon terminals contacting this spine are labeled as 1–4. Bar, 0.5  $\mu$ m.

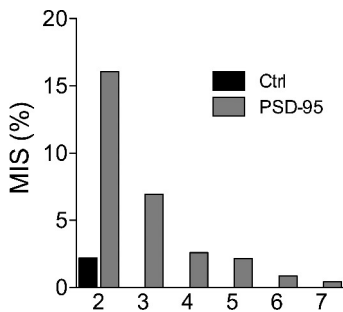


Figure S2. **Distribution of the number of presynaptic partners in MISs.** Data were obtained from the 3D analysis of 16 and 67 MISs observed in a pool of 615 and 234 spines from control and PSD-95-transfected neurons, respectively, and are expressed as a percentage of the total number of spines.

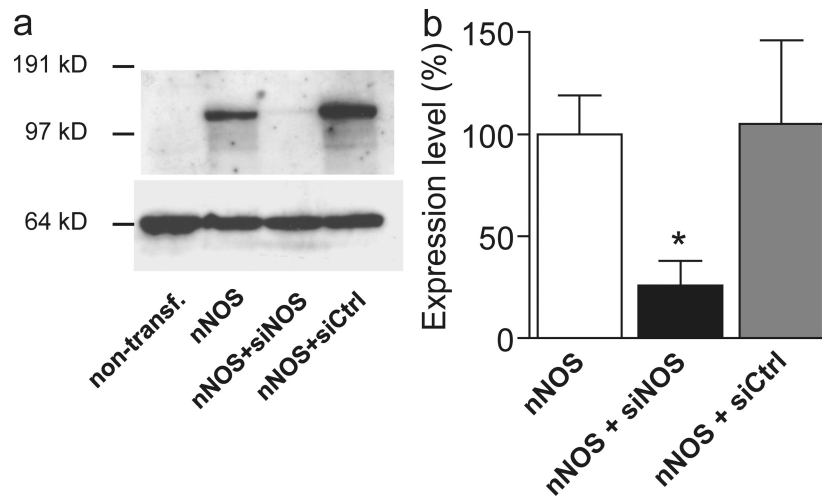


Figure S3. **siNOS activity in fibroblasts.** (a) Western blot showing the level of nNOS expression (top bands) and actin (bottom bands) in nontransfected fibroblasts and in cells transfected with nNOS, nNOS + siNOS, and nNOS + siCtrl. (b) Quantitative analysis, shown as a percentage, of nNOS expression in cells transfected with nNOS, nNOS + siNOS, and nNOS + siCtrl. Data are mean  $\pm$  SEM (error bars) of three experiments (\*,  $P < 0.05$ ).

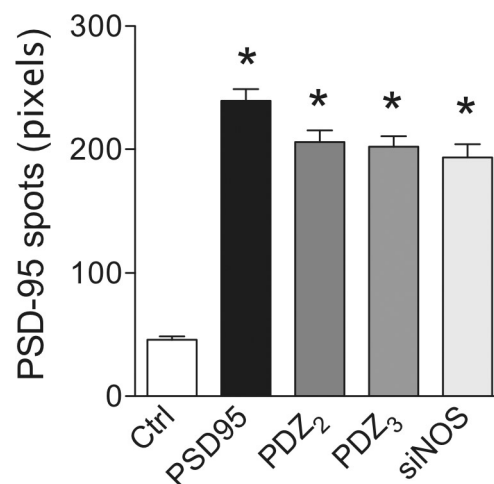


Figure S4. **Level of expression of PSD-95 in transfected neurons.** The expression of PSD-95 at excitatory synapses of transfected neurons was assessed by measuring the size of PSDs revealed by PSD-95 immunostaining or PSD-95-EGFP. The size of PSD-95 spots, expressed as the number of immunostained pixels per spot, was analyzed under control conditions (nontransfected neurons) and in cells transfected with PSD-95, PDZ<sub>2</sub> mutant or PDZ<sub>3</sub> mutant PSD-95, and PSD-95 together with siNOS. Data are mean  $\pm$  SEM (error bars) of an analysis of 68–92 spots from three to four slice cultures per condition (\*,  $P < 0.01$ ). Note that the PSD-95-immunostained spots in all transfected cells are four to five times larger than under control conditions, reflecting the increased level of PSD-95 expression.

Table S1. **Summary of morphometric analyses**

Groups	Number of cells analyzed	Number of dendritic segments analyzed	Total length of dendritic segments	Number of dendritic spines analyzed	Spine density
			$\mu\text{m}$		$\text{spines}/\mu\text{m}$
Nontransfected control	8	8	134	145	$1.08 \pm 0.12$
EGFP control	4	4	184.6	164	$0.96 \pm 0.29$
PSD-95	7	19	492.5	234	$0.63 \pm 0.12$
PDZ <sub>2</sub> mutation of PSD-95	3	7	184.8	56	$0.39 \pm 0.15$
PDZ <sub>3</sub> mutation of PSD-95	3	10	197.9	86	$0.43 \pm 0.06$
PSD-95 + L-NAME	4	7	185.5	128	$0.68 \pm 0.14$
PSD-95 + siNOS	5	14	252.9	127	$0.52 \pm 0.09$
L-NAME	8	8	99.2	59	$0.65 \pm 0.11$
DETA NONOate	10	10	96.8	104	$1.06 \pm 0.10$
8-Br-cGMP	13	13	78.5	95	$1.23 \pm 0.11$
PDZ <sub>2</sub> + DETA NONOate	4	4	44.3	19	$0.45 \pm 0.08$
PSD-95 + ODQ	3	9	148.6	97	$0.64 \pm 0.03$
Total	72	113	2,099.6	1,314	NA

NA, not applicable. Data indicate the number of cells, dendritic segments, total segment length, number of spines, and spine density measured through 3D reconstruction under the various conditions analyzed.



Video 1. **3D reconstruction of an MIS contacted by six different axons.** This video shows the 3D reconstruction of a spine (gray) of a PSD-95-transfected cell with the PSDs indicated in red and the different axons and terminals contacting it illustrated in transparent blue colors (16 frames/s).



Video 2. **3D reconstruction of two MISs on the same dendritic segment.** The spines for a PSD-95-transfected cell are shown in gray with their PSDs in red and the contacting axons in transparent blue (10 frames/s).