

Supplementary Information

Table S1. Substance resources.

| Substance | Supplier & Order number | Comments |
|--|------------------------------------|--------------------------------|
| MEM-Non-Essential Amino Acids | Invitrogen 11140035 | |
| DMEM | Invitrogen 31966047 | |
| Penicillin/Streptomycin | Invitrogen 15140122 | |
| Fetal Bovine Serum (heat inactivated) | Invitrogen 10500064 | thaw, aliquot in 50 mL, freeze |
| Trypsin, 2.5% (10x) | Gibco 15090046 | |
| Trypsin Inhibitor (powder) | Sigma-Aldrich T-9253 | |
| Bovine serum albumin (powder) | Sigma-Aldrich A9418 – 10 g | |
| 0.05% Trypsin/EDTA | Gibco 25300-054 | |
| HBSS | Invitrogen 24020133 | |
| Neurobasal | Invitrogen 21103049 | |
| B27 – Supplement | Invitrogen 17504044 | |
| Glutamax-I Supplement | Invitrogen 35050038 | |
| HEPES (powder dissolved in miliQ water) | Sigma-Aldrich H4034 | freeze 3.5 ml aliquots |

Video S1: Tomographic reconstruction of a mature synapse (video corresponding to Figure 5D).

Video S2: Synaptic vesicle fusion and endocytosis in a mature synapse (video corresponding to Figure 5F).