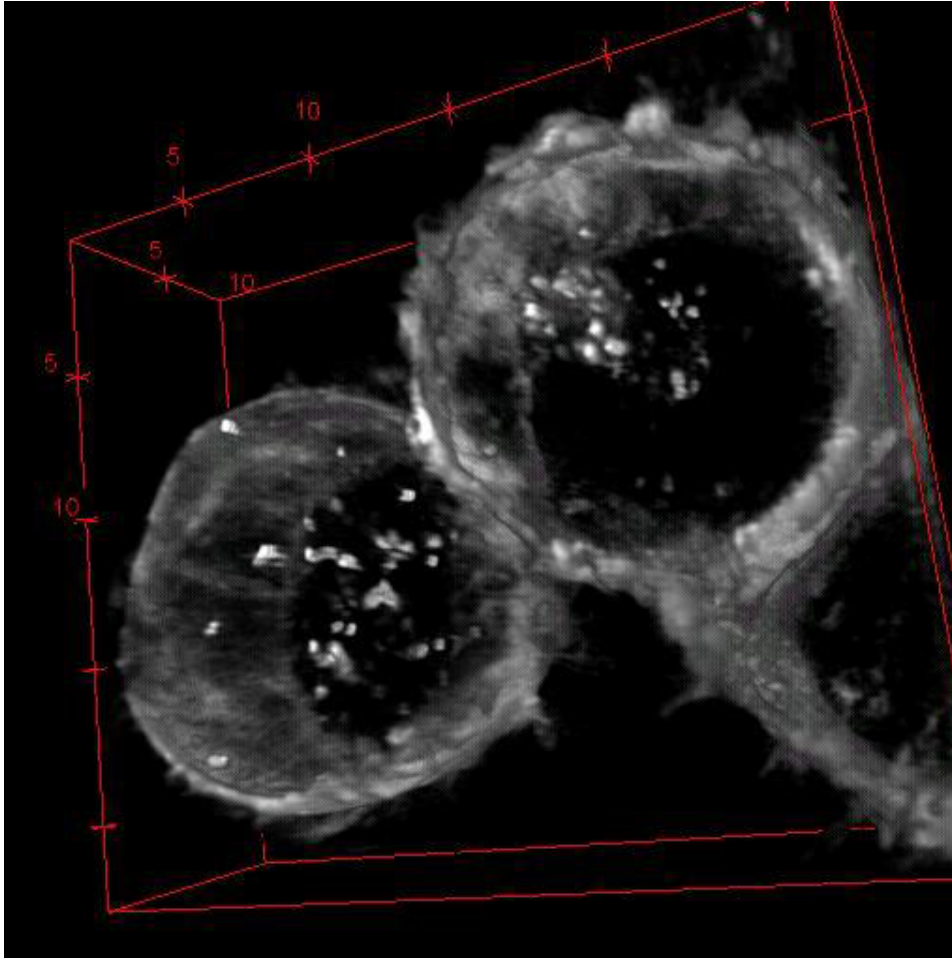
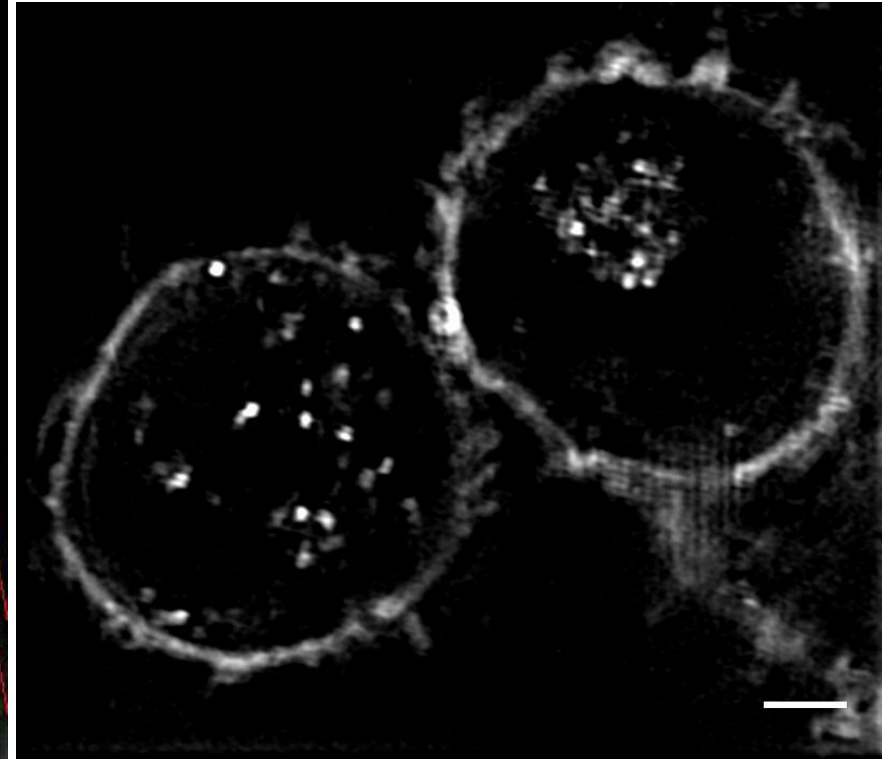


**Figure S1:** 3D reconstruction and 3D video of SIM analysis of  $N_3$ - $C_6$ -cer in Jurkat T cells

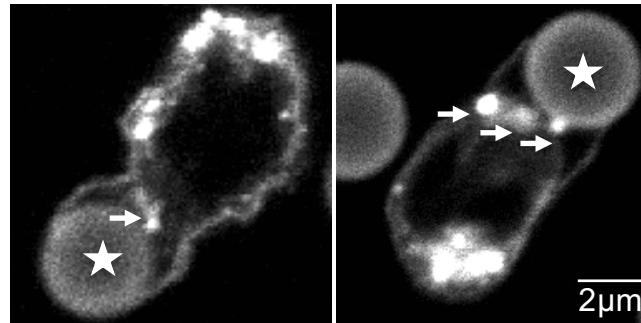
Collenburg et al., 2016, supplementary Fig. 1



3D reconstruction of a SIM z-stack of living Jurkat cells fed with  $25 \mu\text{M}$   $N_3$ - $C_6$  cer and labeled with DBCO-Sulfo-Cy5. An  $8.4 \mu\text{m}$  stack of the center of the cells were recorded with an interval of  $100 \text{ nm}$ . Unit of the coordinate system:  $\mu\text{m}$ .



3D SIM video of living Jurkat cells incubated with  $N_3$ - $C_6$ -cer and visualized with copper-free click chemistry. The z-stack comprises  $8.4 \mu\text{m}$  of the central region of a cell (in z dimension) and the distance between each slice is  $100 \text{ nm}$ . Scale bar:  $5 \mu\text{m}$ .



**Figure S2:** Clicked  $N_3$ - $C_6$ -cer clusters in subsynaptic vesicles in dynabead-stimulated primary T cells.

$N_3$ - $C_6$ -cer was applied to human T cells pre-activated by co-stimulatory beads (marked by stars) for 30 min (two additional examples to that shown in Fig. 7C are shown) (subsynaptic vesicles are marked by arrows).