

## Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Long time-lapse (30 min) SMLM of lysosomes in a live HeLa cells applying photoactivation pulses (405 nm, 2.6 W cm<sup>-2</sup>, 20 ms) every 10 min and imaging at 561 nm (0.25 kW cm<sup>-2</sup>, 20 ms).

File Name: Supplementary Movie 2

Description: Time-lapse (13 s) SMLM of synaptic vesicles in live neurons derived from neuroblastoma cells. Imaging conditions: single photoactivation pulse (405 nm, 2.6 W cm<sup>-2</sup>, 20 ms), imaging at 561 nm (0.25 kW cm<sup>-2</sup>, 20 ms).

File Name: Supplementary Movie 3

Description: Time-lapse (19 s) SMLM of a synaptic vesicle hopping between hotspots in a projection of a live neuron. Imaging conditions: single photoactivation pulse (405 nm, 2.6 W cm<sup>-2</sup>, 20 ms), imaging at 561 nm (0.25 kW cm<sup>-2</sup>, 20 ms).

File Name: Supplementary Movie 4

Description: Time-integrated (3 s), 3D reconstruction of two hotspots (lower blobs) connected by a track through which synaptic vesicles transit. The unit cell is 1.5 μm x 1.5 μm x 1.5 μm and labeled with ticks of 0.5 μm. Imaging conditions: single photoactivation pulse (405 nm, 2.6 W cm<sup>-2</sup>, 20 ms), imaging at 561 nm (0.25 kW cm<sup>-2</sup>, 20 ms).