**Fig. S1. Mitochondrial inner and outer membrane proteins have distinct numbers of molecules per μm^2.** Representative regions of 4×4 µm^2 from N-STORM single molecule localisation data were analysed for cluster properties by cluster analysis program DBSCAN for COXIV, Tim23, Tim44 and Tom20. (A) The number of molecules per μm^2 of selected regions from single molecule localisation. (B) The average number of molecules per cluster selected regions from single molecule localisation. n = 3 experiments, ≥ 33 regions from ≥ 9 cells per protein examined. Data are mean ± s.d.; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ (unpaired t test with Welch's correction).

A
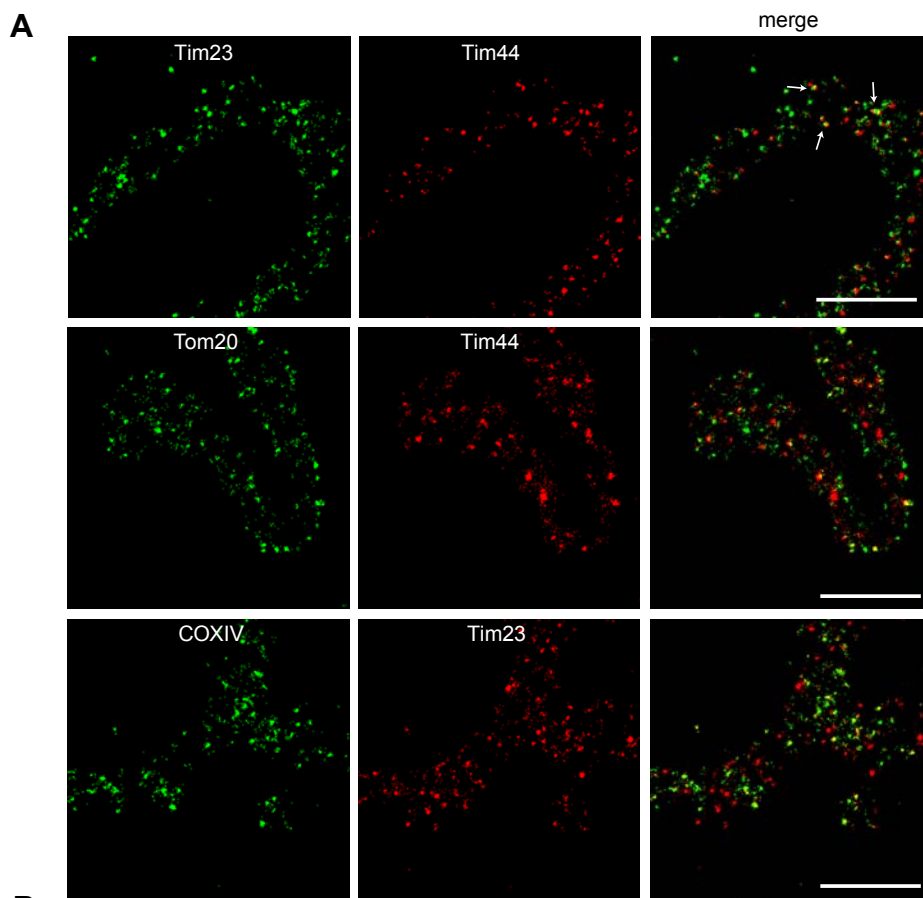
B
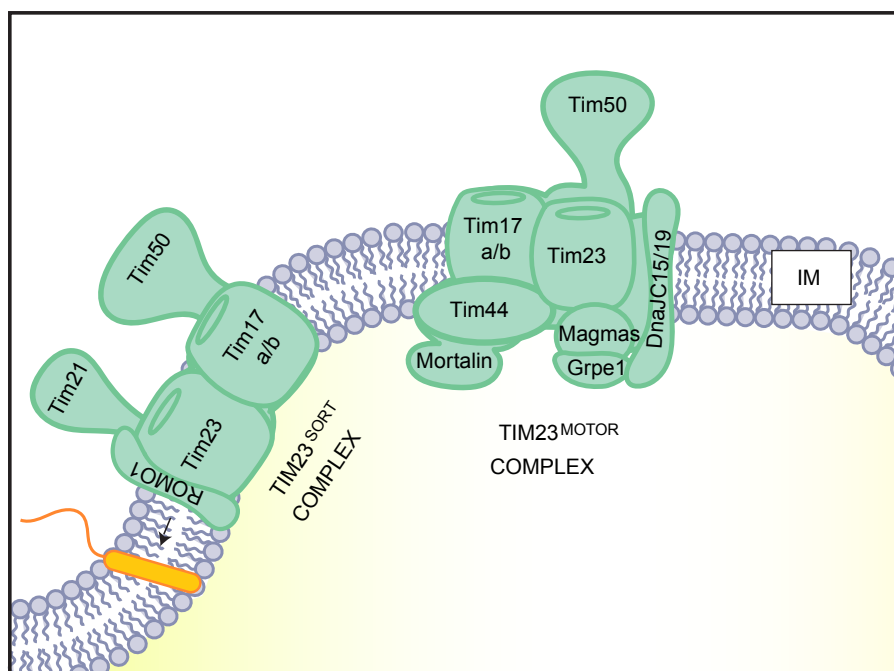
Fig. S2. Two colour STORM microscopy reveals arrangement of TIM23 complexes. (A) Schematic of the known TIM23 complex formations, with the Tim23 protein present in two distinct molecular machineries: the TIM23^{SORT} complex and the TIM23^{MOTOR} complex. (B) HeLa cells were immunostained for the proteins Tim23 and Tim44 (upper), Tom20 and Tim44 (middle) or COXIV and Tim23 (bottom) and visualised by STORM microscopy. Representative regions of STORM reconstructions are shown here. Scale bar = 1 μm. Arrows indicate representative points containing both Tim23 and Tim44.