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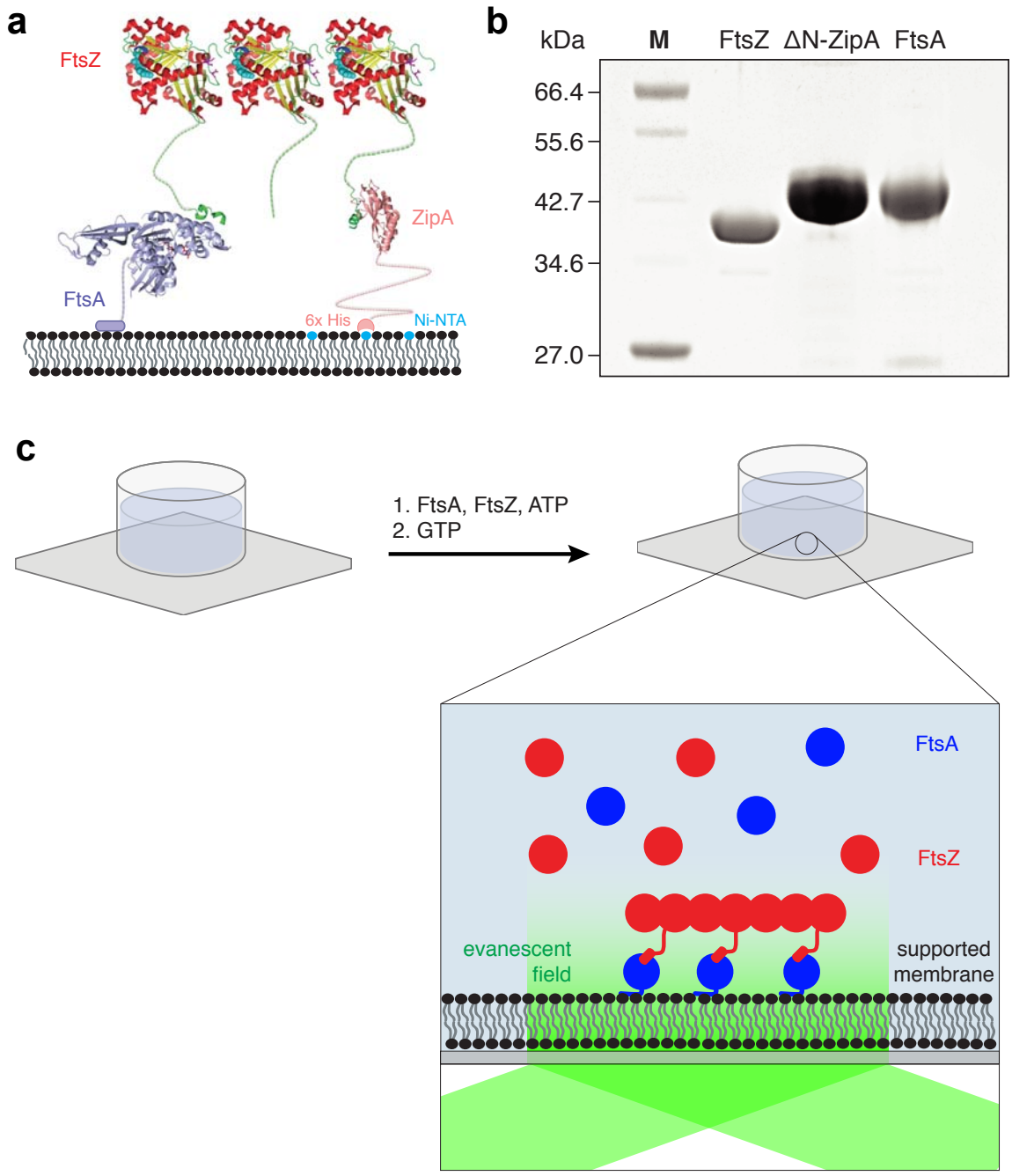
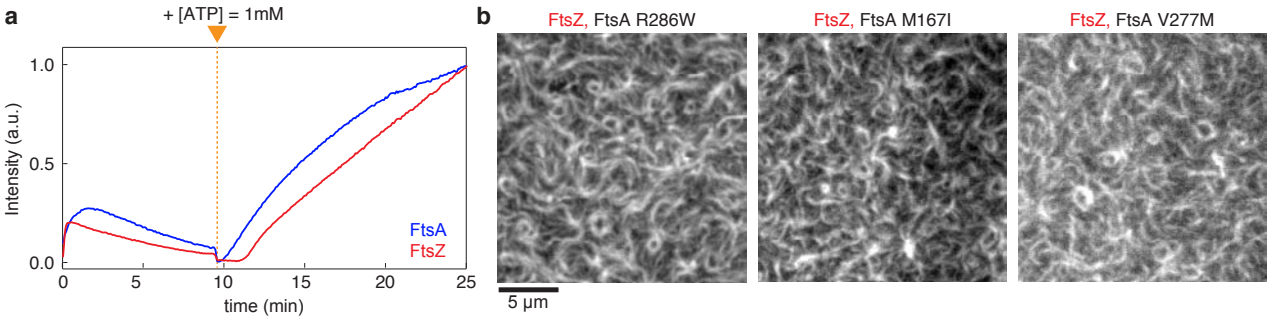
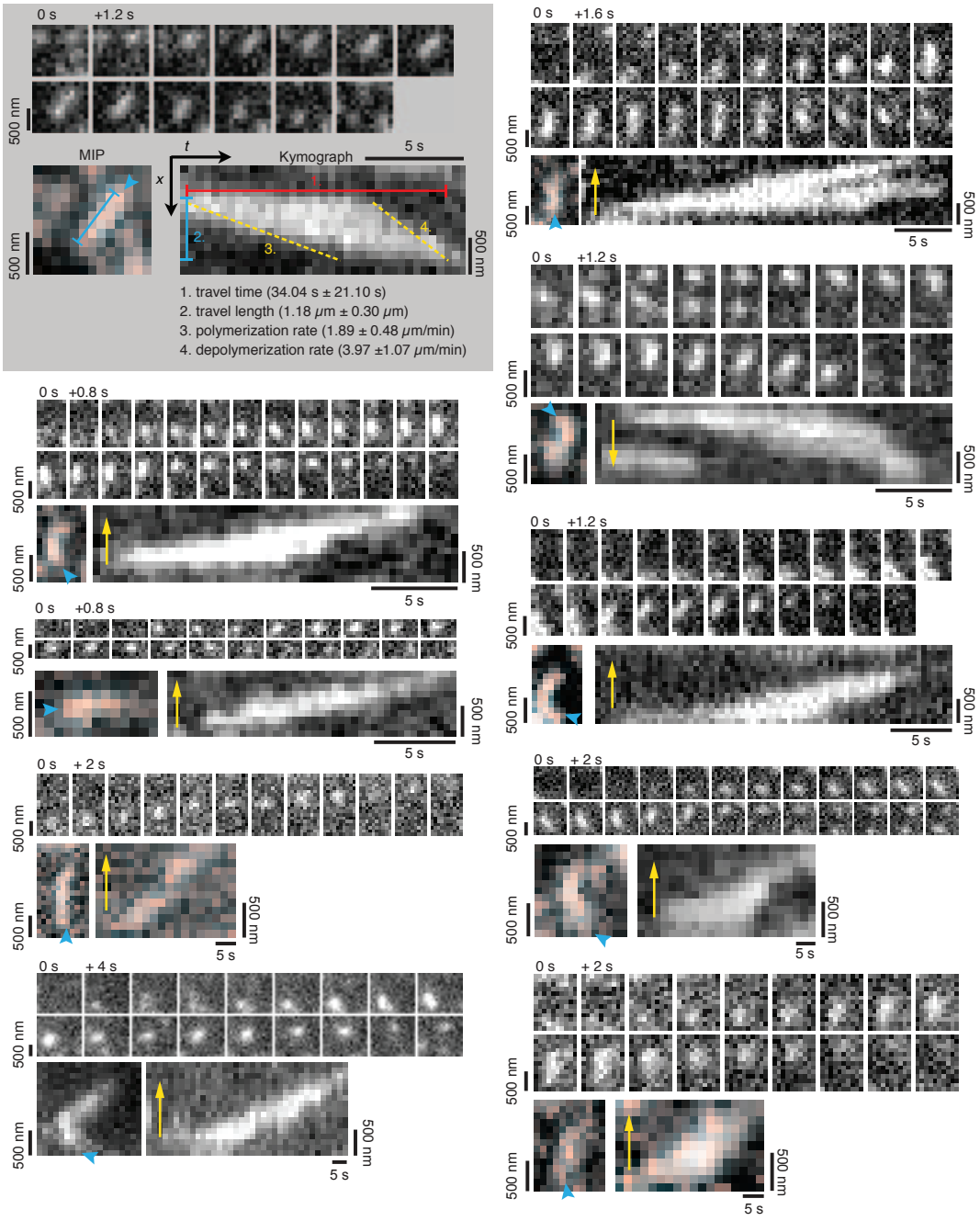


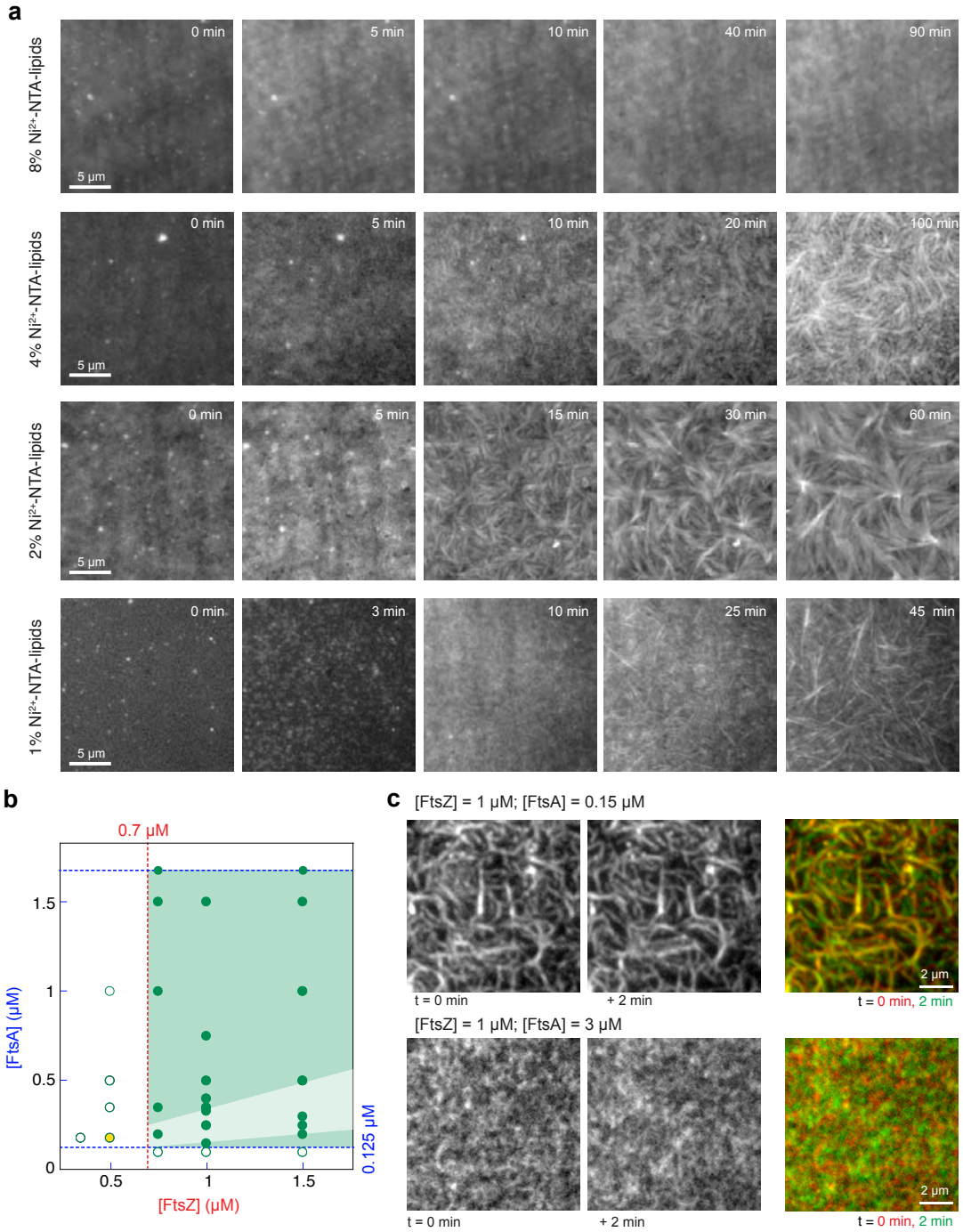
Supplementary Figure 1 Experimental assay. **(a)** Illustration of protein interactions lipids (adapted from ref. 20): FtsA and ZipA bind to same highly conserved C-tail of FtsZ (shown in green), which is connected to the rest of the protein by a flexible linker. FtsA binds to the membrane surface using an amphipathix helix, located at the end of a C-terminal flexible linker. ZipA is a transmembrane protein. The N-terminal transmembrane-domain has been substituted by a His-tag obtaining His-Δ22-ZipA, which was then permanently attached to the membrane using Ni^{2+}chelating. (**b**) Commassie-stained SDS-Page gel of purified proteins used for this study. (**c**) Schematic drawing of the experimental assay. A plastic ring was glued to a glass cover slip to create a reaction chamber and a supported lipid bilayer was formed on the glass surface. FtsA, FtsZ and ATP were added to the buffer before polymerization of FtsZ was initiated by adding GTP.



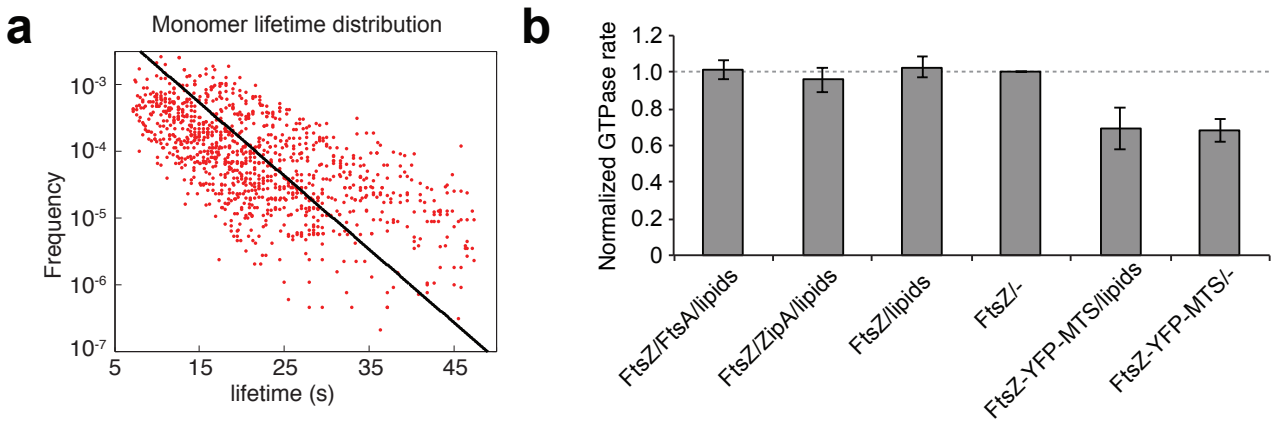
Supplementary Figure 2 Remodeling of cytoskeletal structures depends on the presence of ATP, but not on FtsA polymerization. (**a**) Representative intensity curve corresponding to Supplementary Video 4, showing the role of ATP for FtsZ-FtsA co-assembly: The initial transient binding of FtsZ to the membrane is likely due to residual amounts of ATP being co-purified with FtsA. After detachment of FtsZ and FtsA, the filament network reassembled as soon as fresh ATP is added (at orange arrowhead and dashed line). Similar intensity curves were obtained in 5 experiments. (**b**) FtsA polymerization is not important for the self-organization of FtsZ and FtsA in our *in vitro* system. When we used self-interaction mutants of FtsA (FtsA R286W (left), FtsA M167I (middle), FtsA V277M (right)), FtsZ formed the same cytoskeletal pattern as with the wildtype protein. Similar micrographs were obtained in n=10 experiments.



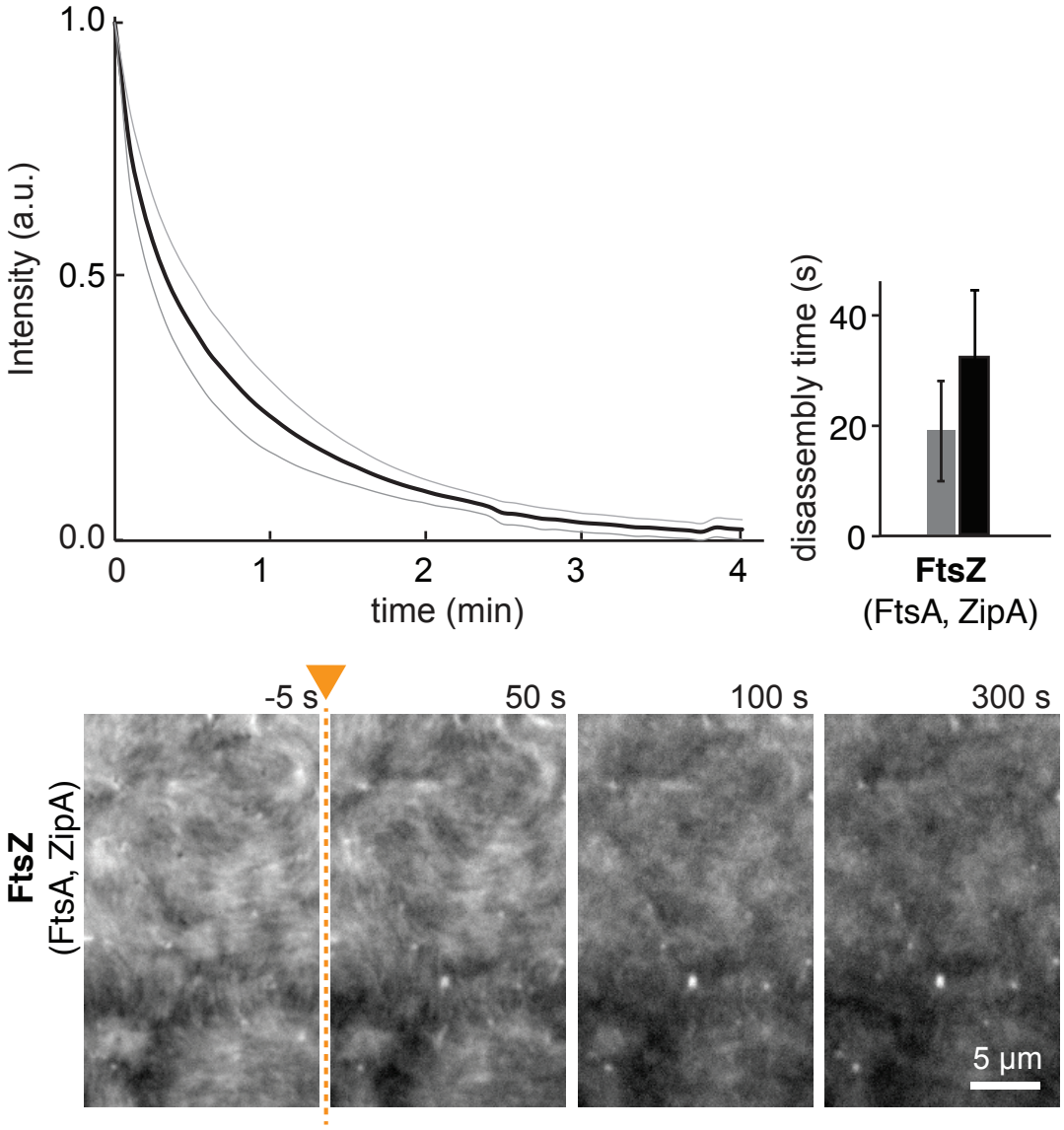
Supplementary Figure 3 Dynamics of single FtsZ filaments recruited to the membrane by FtsA. Snapshots, maximum intensity projections (MIP) and kymographs of single FtsZ filaments from movies acquired at a frame-rate of 2s or 400 ms (FtsZ = 0.45 μM with 30 mol-% FtsZ-Alexa488, FtsA = 0.2 μM). Cyan arrowheads in MIPs indicate the first binding of FtsZ to the membrane. Yellow arrows in kymograph show the polymerization direction. Top left, with grey background. Scale bars correspond to 500 nm or 5 s. Values in grey box (top left) represent average values of n=38 analyzed filaments from 5 independent experiments.



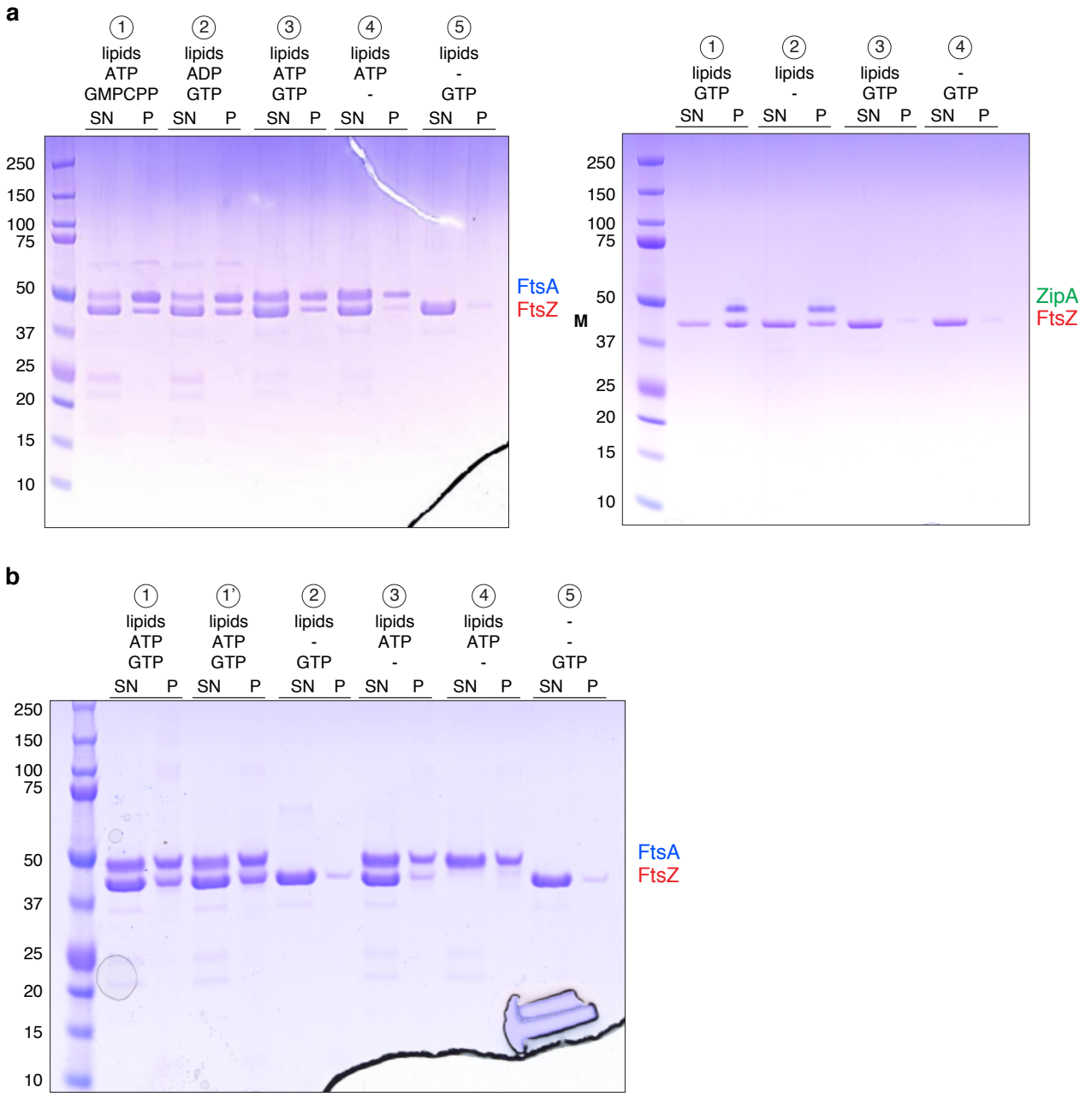
Supplementary Figure 4 Influence of different concentrations of membrane anchors on FtsZ filament patterns. (**a**) Representative snapshots of FtsZ filaments recruited to the membrane by ZipA at indicated time points after addition of GTP. The percentage of Ni^{2+}-chelating lipids (18:1 DGS-NTA-Ni^{2+}) defines the amount of ZipA immobilized and the amount of FtsZ recruited to the membrane. With 8% Ni^{2+}chelating lipids, FtsZ bundles densely covered the membrane. At lower concentrations (1%, 2% and 4%), bundles of FtsZ filaments start to appear after about 20 min. On membranes with 1% Ni^{2+}-chelating lipids, individual FtsZ filaments could briefly be resolved (see 3 min after addition of GTP), before the filament density became too high. Similar micrographs were obtained in 5 experiments. (**b**) Simplified phase diagram of FtsZ-FtsA filament networks on the membrane. Filled green dots represent experiments with protein concentrations allowing for the formation of a dynamic filament network (at protein concentrations of [FtsZ] > 0.7 µM and 0.125 µM < [FtsA] <1.7 µM). Empty dots represent experiments where FtsZ filaments were either too short for a continuous polymer network (with FtsZ < 0.7 µM) or where they did not show rapid rearrangements (with FtsA < 0.125 µM). Light green area represents the concentration ratio for FtsA and FtsZ found *in vivo*[26]. Yellow filled circle represents the concentration ratio used for single FtsZ filament experiments. (**c**) Representative snapshots and overlays of two individual frames separated by 2 min of a time-lapse movie. At low FtsA concentrations (top), FtsZ forms static, long filaments, whereas at high FtsA concentrations (bottom), filaments were short and dynamic, but did not form a continuous filament network. Similar micrographs were obtained in 5 experiments.



Supplementary Figure 5 The membrane anchor does not influence the GTPase rate and lifetime of FtsZ monomers. (**a**) With ZipA, the average lifetime of FtsZ was slightly longer than with FtsA. Linear-log plot of FtsZ lifetime distribution with ZipA as membrane anchor (filled red circles) about 30 min after addition of GTP. Black line represents the averaged linear fit to individual lifetime distributions with $<t> = 10.68\ s \pm 2.33\ s$ (s.d. n = 24 videos with 1500 particles obtained from 5 independent experiments), which is slightly longer than for FtsZ/FtsA (p=0.0174). (**b**) FtsZ GTPase activity was not affected by the membrane anchor. Bar graph shows GTPase activity of FtsZ (5 μM) or FtsZ-YFP-MTS (5 μM) and the influence of membrane anchors (His-ZipA or FtsA, 3 μM) and phospholipids (1 mg/ml). The corresponding rates were normalized to the GTP activity of FtsZ alone (0.083/s). We found that the GTPase activity of FtsZ-YFP-MTS to be about 30% lower than of wildtype FtsZ. GTPase activities were determined using the EnzChek Phosphate Assay Kit (Molecular Probes). Error bars correspond to s.d, from n=3 independent experiments.



Supplementary Figure 6 FtsA destabilizes the FtsZ filament network also in presence of ZipA. Left, mean intensity traces for FtsZ depolymerization upon rapid dilution for FtsZ filaments recruited to the membrane by ZipA and FtsA. Right: mean disassembly times obtained from double-exponential fits, error bars and thin lines correspond to s.e.m, (n=3 independent experiments). Bottom: Representative snapshots showing disassembly of FtsZ filaments after dilution (time point of dilution is indicated by orange arrowhead and dashed line). In the presence of both anchors, ZipA and FtsA, no thick bundles of FtsZ persist on the membrane. Fluorescence intensity of each frame was normalized.



Supplementary Figure 7 Uncropped Commassie-stained SDS-Page gels corresponding to Figs. 3c (**a**) and 4d (**b**). P = Pellet, SN = Supernatant; 1 and 1' in **b** indicate replicates using identical experimental conditions.