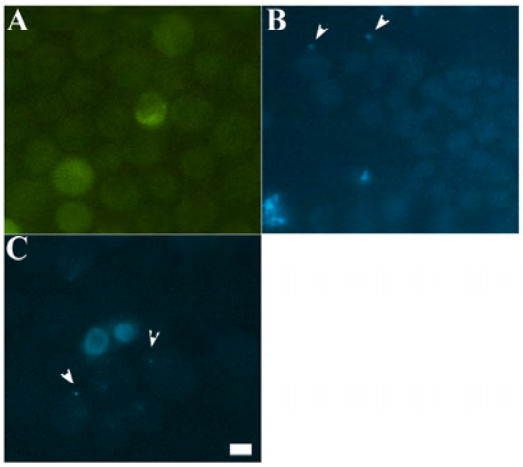


Fig. S1. Yeast cells expressing GFP- or CFP-μNS-protein fusions alone. **(A)** Cells expressing GFP-FtsZ show diffusely localized fluorescence 3-4 hours post induction of the fusion protein; **(B, C)** Cells expressing CFP-μNS-FtsZ or CFP-μNS-ZapD fusion proteins displayed characteristic fluorescent focal platforms (arrowheads). Scale bar = 2 μm.

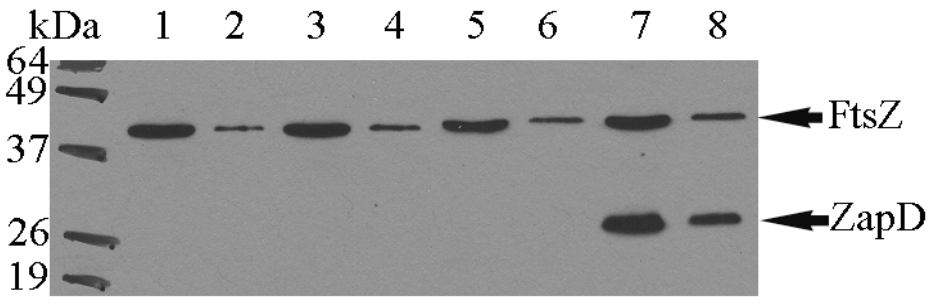
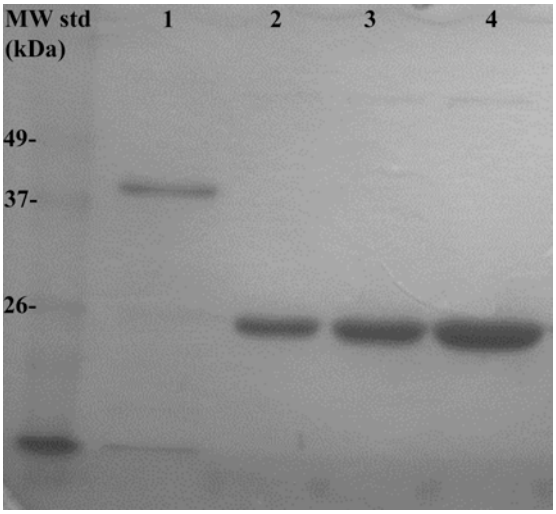


Fig. S2. Levels of FtsZ remain unchanged in cells lacking or overexpressing ZapD. Ten μls of undiluted and 10-fold diluted samples from whole cell protein preparations from wildtype (MG1655; lanes 1 and 2), *zapD* (JD310; lanes 3 and 4), pCA24N bearing wildtype cells (GF 73; lanes 5 and 6) and pCA24N-*zapD* bearing wildtype cells (JD381; lanes 7 and 8) were run on adjacent lanes in a 10% SDS-PAGE gel, blotted and probed for FtsZ with an anti-FtsZ rabbit polyclonal (Genscript) antibody. Intensity profiles were measured by ImageJ (NIH). In cells bearing pCA24N or pCA24N-*zapD* plasmids the blots were probed for ZapD with an anti-ZapD rabbit polyclonal (Genscript). Lanes 7 and 8 show leaky expression of ZapD from the pCA24N vector. In lanes 5 and 6 endogenous levels of ZapD were not detectable due to the low harvest volumes used for whole cell protein preparation in this experiment. Lanes 1 through 4 were not probed for ZapD.

A.



B.

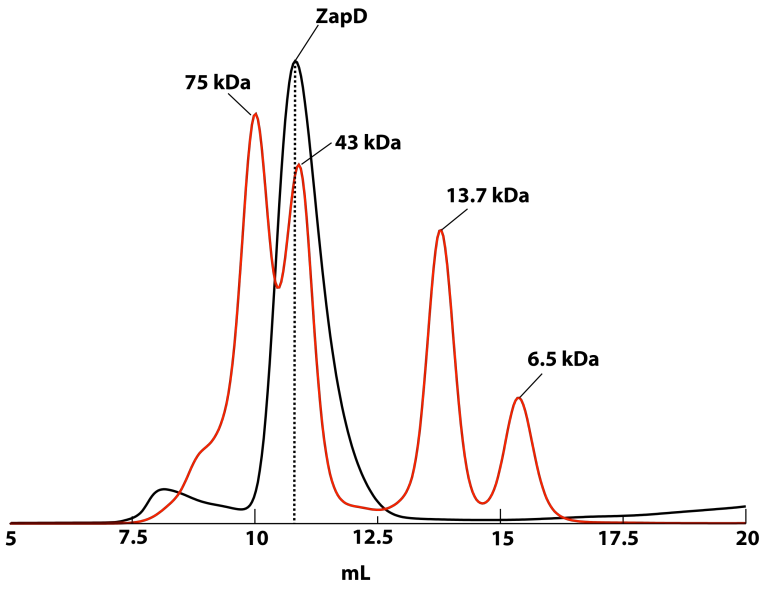


Fig. S3. Purified tag-free ZapD is mostly dimeric in solution. **(A)** Two-fold serial dilutions of purified and concentrated ZapD obtained from JD160 cells were run in lanes 2, 3 and 4 and compared to His_{10}-Smt3-ZapD fusion protein in lane 1. Conservative estimates of band intensities by ImageJ (NIH) analysis indicate ZapD to be ≥95% pure. **(B)** Elution profile of purified tag-free ZapD separated through a Sephadex75 (GE Healthcare) gel filtration column indicates ZapD (black line) to be mostly dimeric in solution with some higher order oligomers likely present in the solution.

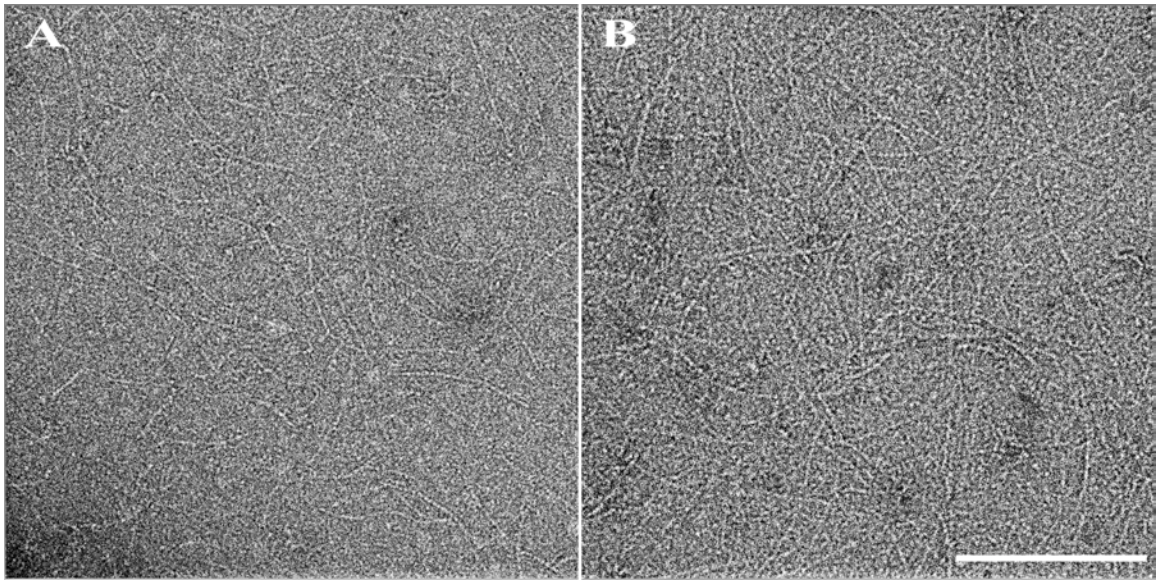


Fig. S4. Electron microscopy images of sub-stoichiometric levels of ZapD with respect to FtsZ show occasional bundling of FtsZ protofilaments. **(A)** Negative stained TEM images of FtsZ (5 μM) in MOPS pH 6.5, 50 mM KCl, 2.5 mM $MgCl_2$ buffer in the presence of 1 mM GTP forms characteristic single protofilaments and **(B)** upon addition of ZapD (1 μM) occasional loosely bundled FtsZ protofilaments are visualized. Scale bar = 200 nm

Table S1. DNA oligonucleotides used in the study.

ftsZ 3P GW	GGGGACAACTTTGTACAAGAAAGTTGGTTAATCAGCTTGCTTACGCAGGAAT
ftsZ-372 3P GW	GGGGACAACTTTGTACAAGAAAGTTGGTTACAGATAATCCGGCTCTTTCGCA
ftsZ-314 3P GW	GGGGACAACTTTGTACAAGAAAGTTGGTTACCTGTCGCAACAACGGTTACG
ftsZ 5P GW	GGGGACAACTTTGTACAAAAAAGTTGGCATGTTTGAACCAATGGAACTTACCAATG
ftsZ-289-383 5P GW	GGGGACAACTTTGTACAAAAAAGTTGGCATGAACGCGACTGTGGTTATCGGTACTT
ftsZ-364-383 5P GW	GGGGACAACTTTGTACAAAAAAGTTGGCATGCAAACTGCGAAAGAGCCGGATTATC
ftsZ-374-383 5P GW	GGGGACAACTTTGTACAAAAAAGTTGGCATGATCCCAGCATTCCTGCGTAAGCAAG
new YacF tst 5P	TGCTCAGGCAACGCGCGAAGCCCGCCTTGC
new YacF tst 3P	TTCAGCAGCCCATTCTCCGAGGTCGATCAG
SalI yacF 5P	ACGCGTCGACAGGAGGGCCAGCATGCAGACCCAGGTC
SalI yacF 3P	AAAAGTCGACGCAACAGGCCAGTTCGAA
SUMO-5 YacF-BamHI	CGCGGATCCATGCAGACCCAGGTCCTTTTTG

SUMO-3 YacF-HindIII	CCCAAGCTTTTAGCAACAGGCCAGTTCGAA
yacF 5P GW	GGGGACAACTTTGTACAAAAAAGTTGGCATGCAGACCCAGGTCCTTTTTGAACAT
yacF 3P GW	GGGGACAACTTTGTACAAGAAAGTTGGTTAGCAACAGGCCAGTTCGAAATCC
yacF 3P GW-C	GGGGACAACTTTGTACAAGAAAGTTGGGCAACAGGCCAGTTCGAAATCCAGA
YcbW FP tst	GCGAAGCGACAATGG
YcbW RP tst	CACCATTCTTTGCTG
ZapA-tstFP	GCCCTGGTGGACCAG
ZapA-tstRP	CAACCGCGGAGCGCC