Supplementary figures:

Figure S1

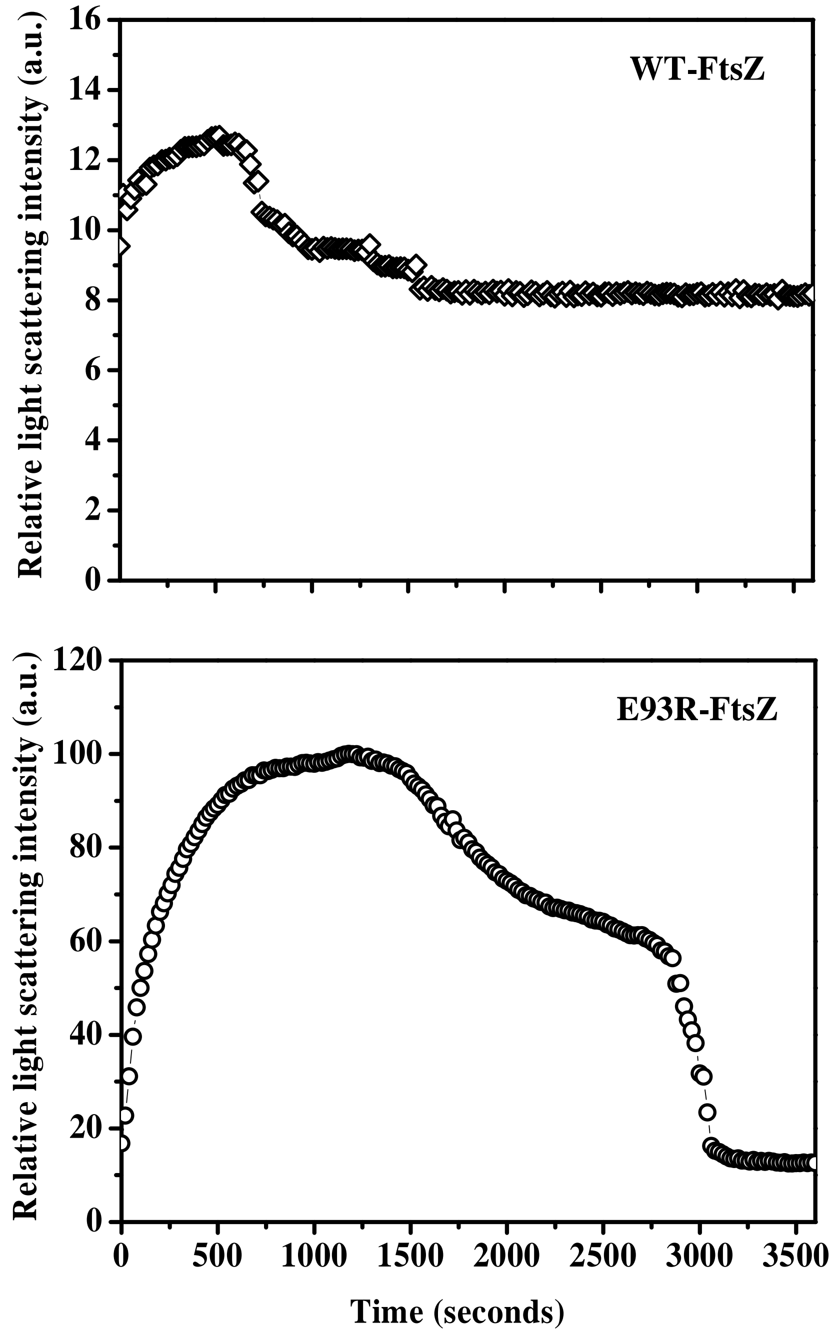

Figure S1: E93R mutation stabilized FtsZ polymers. WT and E93R FtsZ (14.4 μM) were polymerized at 37 ºC and the assembly kinetics was monitored through 90° light scattering at 500 nm.

Figure S2

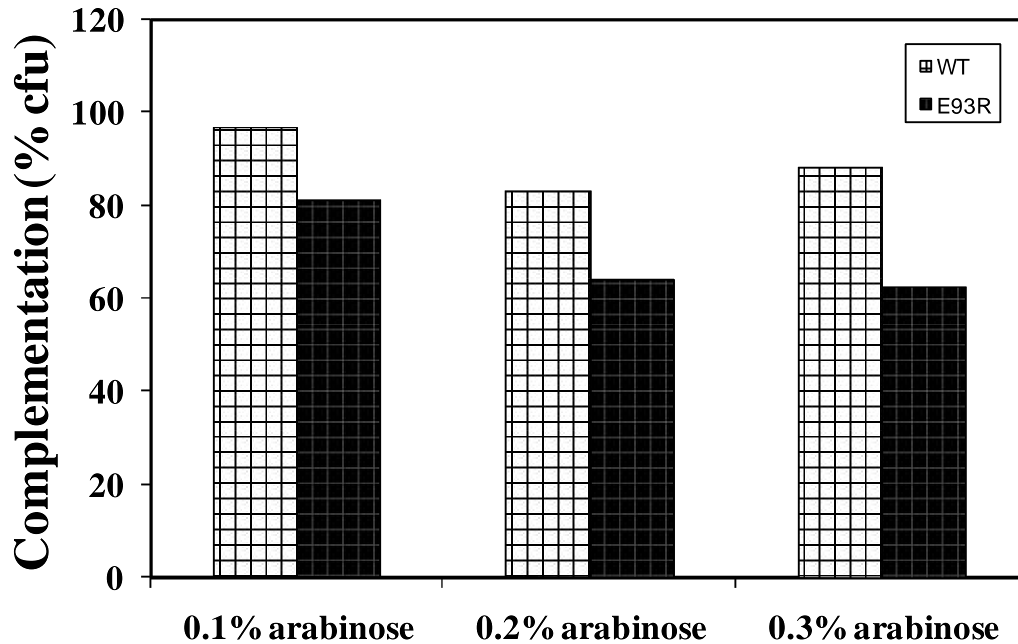

Figure S2: E93R mutation complements WT-FtsZ in JKD7-1/pKD3 cells. WT-FtsZ and E93R-FtsZ genes were inserted in complementation vector pJSB2. JKD7-1/pKD3 cells were transformed with the recombinant plasmids**.** Graph is showing the percent colonies formed in induction media (with 0.1%, 0.2% and 0.3% arabinose at 42 °C) calculated against the number of colonies formed in repression plates (with 0.5%, glucose at 30 °C). The experiment was done twice.

Figure S3

Figure S3A

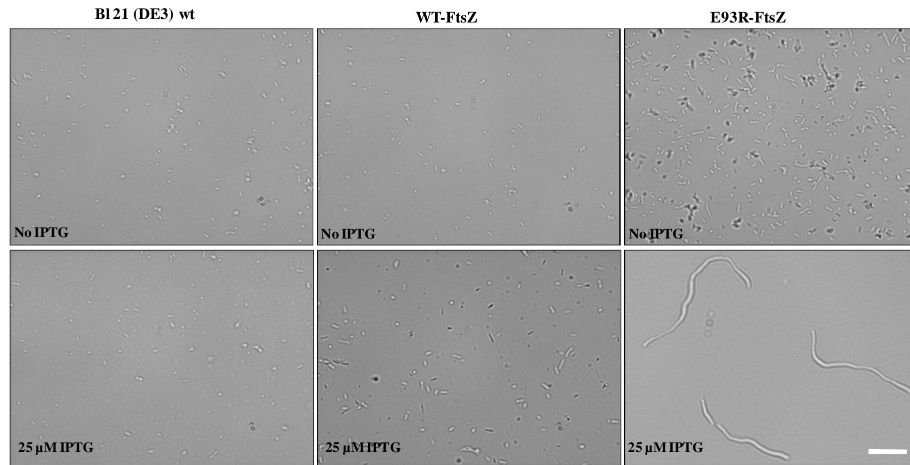

Figure S3B

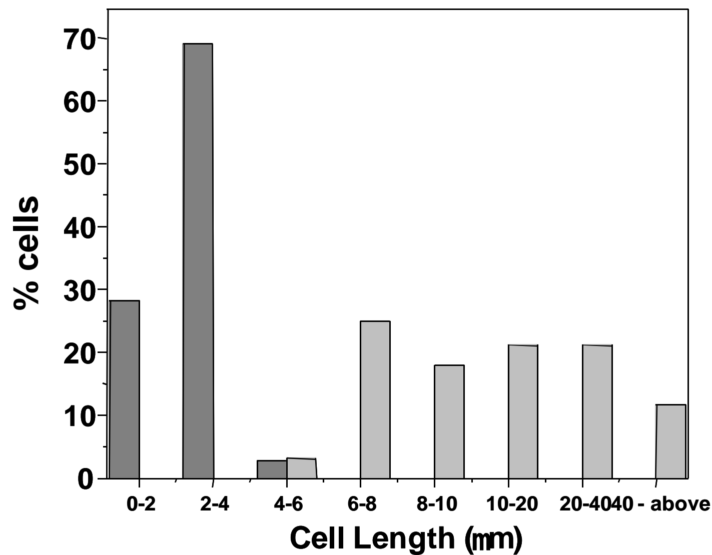

Figure S3: (A) E93R-FtsZ overexpression induced filamentation in *E. coli* cells. Cells harboring WT FtsZ or E93R mutant plasmid were grown for 3 hours in the absence and presence of 25 µM IPTG and observed under differential interference contrast microscope. Shown are the wild type *E. coli* BL21 (DE3) cells containing no vector, *E. coli* BL21 (DE3) cells containing plasmid for WT-FtsZ and *E. coli* BL21 (DE3) cells containing plasmid for E93R-FtsZ in the absence and presence of IPTG (upper and lower panels), respectively. Scale bar is 10 µm. (**B**) **Distribution of cell lengths in cells containing WT-FtsZ and E93R-FtsZ plasmids.** Percent of cells in total population was calculated as a function of cell length. Dark grey bars denote cells containing WT-FtsZ and light grey bars show cells containing E93R-FtsZ grown in the presence of 25 µM IPTG.

Figure S4

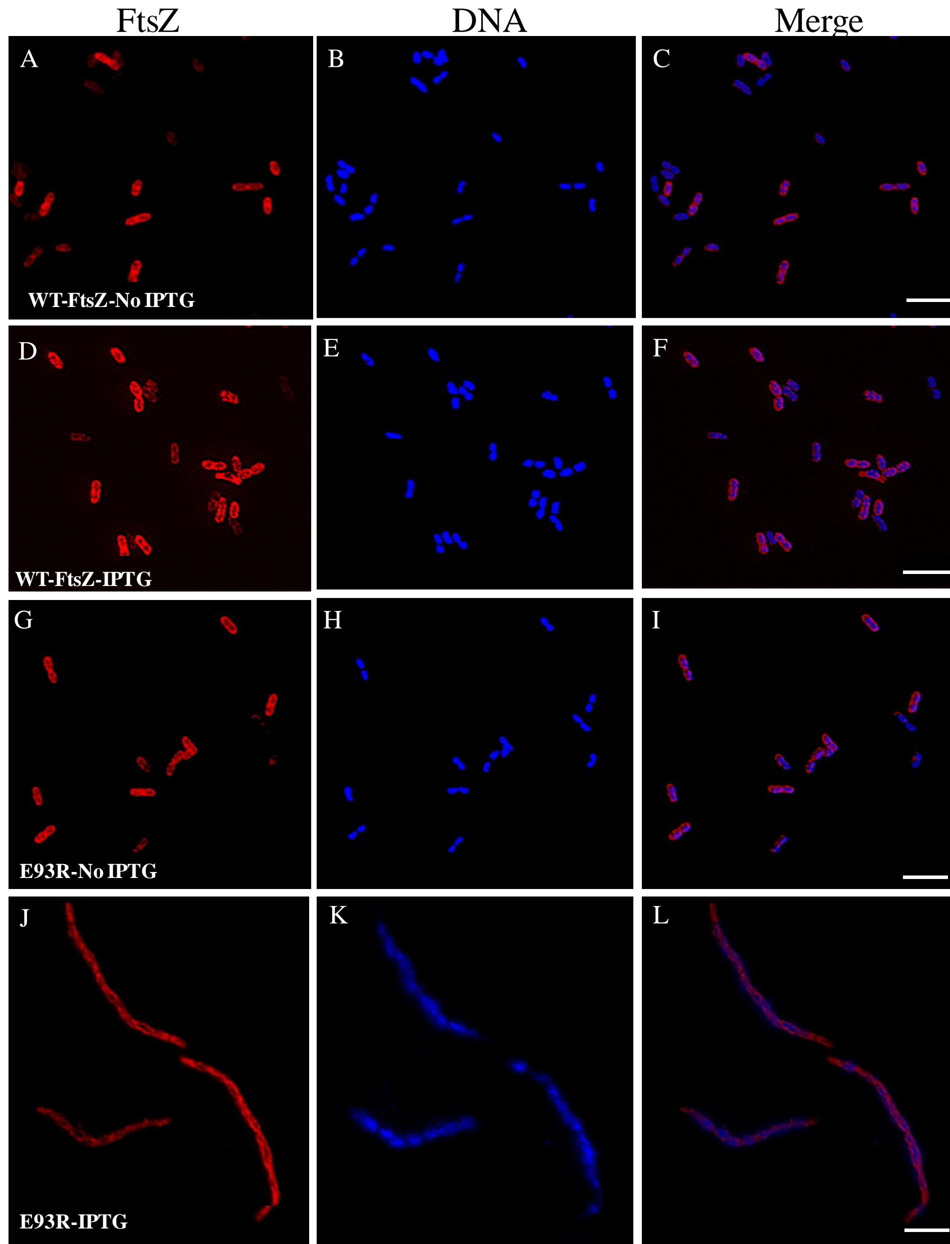

Figure S4: Effect of E93R mutation on the Z-ring assembly and nucleoid segregation. Z-ring and DNA of bacterial cells containing WT-FtsZ and E93R-FtsZ mutant plasmids were grown in the absence or presence of 25 µM IPTG. Z-rings (red) were immunostained with anti FtsZ antibody and DNA (blue) was stained with DAPI. Shown are the cells containing WT-FtsZ in the absence (A, B, and C) and presence of IPTG (D, E, and F) and E93R mutant cells in the absence (G, H, and I) and presence of IPTG (J, K, and L). Experiment was performed three times. Scale bar is 10 µm.

Figure S5

Figure S5A

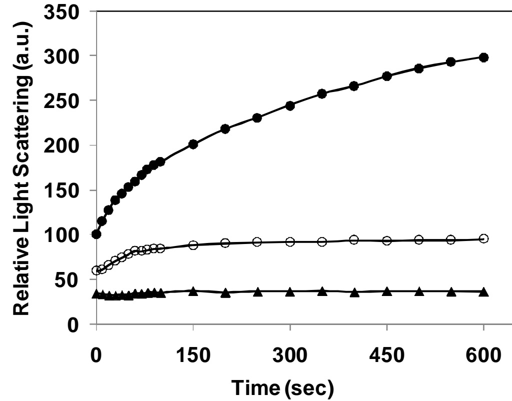

Figure S5B

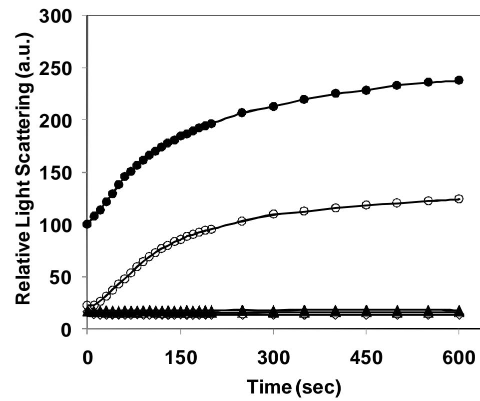

Figure S5C

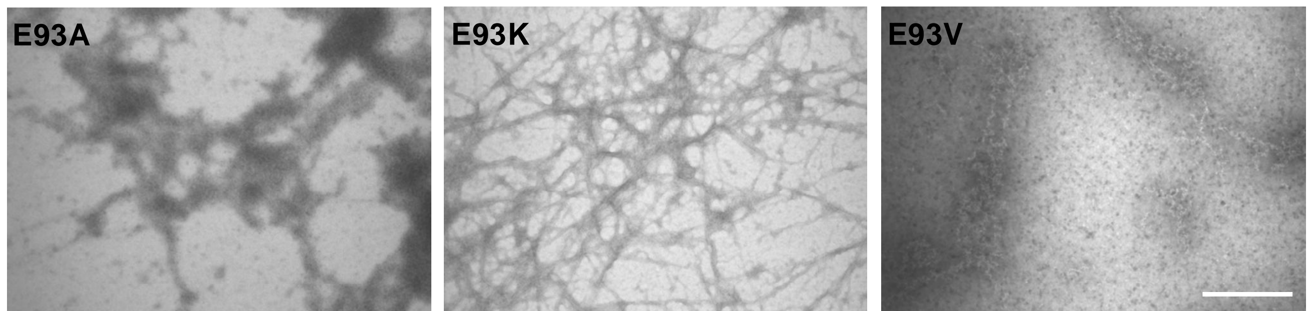

Figure S5: Effect of substitution of E93 to alanine, lysine, or valine on the assembly of FtsZ. The assembly kinetics of native and mutant FtsZ proteins were analyzed by light scattering at 500 nm (Panel A and B). WT, E93R, E93A, E93K, E93V (14.4 µM) were polymerized at 37 °C in the assembly buffer containing 25 mM PIPES pH 6.8, 50 mM KCl, 10 mM $MgCl_2$ and 1mM GTP. Shown are the **(A)** Light scattering traces of WT (▲), E93R (●) and E93K (○) FtsZ. **(B)** Light scattering traces of WT (▲), E93R (●), E93A (○), and E93V (Δ) FtsZ. **(C)** Electron micrographs of E93A, E93K and E93V FtsZ polymers are shown. 7.2 µM of E93A, E93K and E93V FtsZ proteins were polymerized as described in the experimental procedures. Scale bar is 1000 nm.

Figure S6

Figure S6A

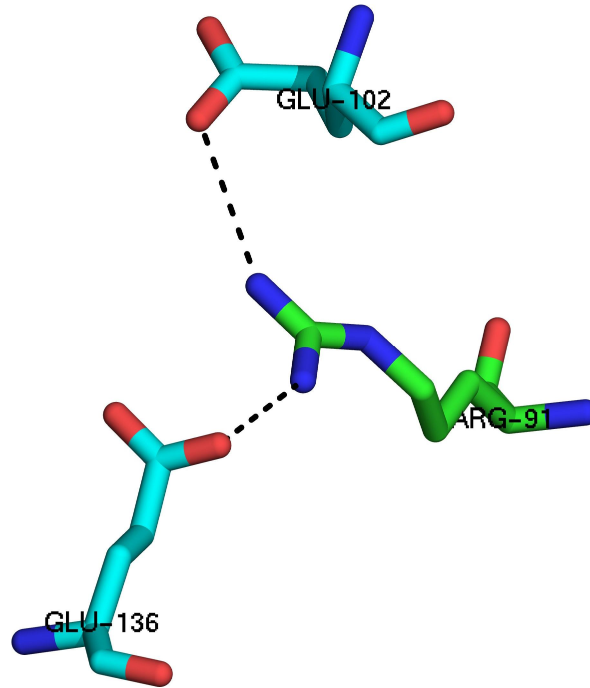

Figure S6B

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FTSZ_MYCTU   1   MTPPHNYL--AVIKVVGIGGGGVNAVNRMIEQGLKGVEFIAINTDAQALLMSDADVKLDVGRDSTRGLGA
FTSZ_ECOLI   1   MFEPMELTNDAVIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDAQALRKTAVGQTIQIGSGITKGLGA

FTSZ_MYCTU  69   GADPEVGRKAAEDAKDEIEELLRGADMVFVTAGEGGGTGTGGAPVVASIARKLGALTVGVVTRPFSFEGK
FTSZ_ECOLI  71   GANPEVGRNAADEDRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEVAKDLGILTVAVVTKPFNFEGK
                                       *          *                                 *
FTSZ_MYCTU  139  RRSNQAENGIAALRESCDTLIVIPNDRLLQMGDAAVSLMDAFRSADEVLLNGVQGITDLITTPGLINVDF
FTSZ_ECOLI  141  KRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLLDAFGAANDVLKGAVQGIAELITRPGLMNVDF

FTSZ_MYCTU  209  ADVKGIMSGAGTALMGIGSARGEGRSLKAAEIAINSPLLEAS-MEGAQGVLMSIAGGSDLGLFEINEAAS
FTSZ_ECOLI  211  ADVRTVMSEMGYAMMGSGVASGEDRAEEAAEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGN

FTSZ_MYCTU  278  LVQDAAHPDANIIFGTVIDDSLGDEVRVTVIAAGFDVSGPGRKPVMGETGGAHRIESAKAGKLTSTLFEP
FTSZ_ECOLI  281  TIRAFASDNATVVIGTSLDPDMNDELRVTVVATGIGMDKRPEITLVTNKQVQQPVMDRYQQHGMAPLTQE

FTSZ_MYCTU  348  VDAVSVPLHTNGATLSIGGDDDDVDVPPFMRR---
FTSZ_ECOLI  351  QKPVAKVVNDNAPQTAK--EPDYLDIPAFLRKQAD
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Figure S6: **(A)** Salt bridges at the interface in the crystal structure of *Mtb*FtsZ (1RQ7). Arg91 is from chain A and Glu102 and Glu136 are from chain B. The snapshot was created using PyMol *(36)*. **(B)** Alignment of *Ec*FtsZ (FTSZ_ECOLI; Uniprot id: P0A9A6) and *Mtb*FtsZ (FTSZ_MYCTU; Uniprot id: P64170) was obtained using ClustalW (S1). Identical and similar residues are displayed in black and grey backgrounds, respectively, using Bioedit (S2). The residues forming salt bridge in crystal structure of *Mtb*FtsZ are shown with asterisk (*) below the alignment. The numbering on the left of sequence shows the residue numbering.

References:

S1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-4680

S2. Hall, T. A. (1999) *Nucleic Acids Symp. Series* 41, 95-98