SUPPORTING INFORMATION

Determinants of Affinity and Activity of the Anti-Sigma Factor AsiA[†]

Joshua M. Gilmore[‡,§,#], Ramona J. Bieber Urbauer[‡,#], Leonid Minakhin[||], Vladimir Akoyev[⊥Ω], Michal Zolkiewski[⊥], Konstantin Severinov[||¶,Δ,Ø], and Jeffrey L. Urbauer *[,‡,∞]

[‡] Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA

[§] Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045, USA

[||] Waksman Institute of Microbiology, Piscataway, NJ 08854, USA

[⊥] Department of Biochemistry, Kansas State University, Manhattan, KS 66506, USA

[Ω] Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

[¶] Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

[Δ] Institute of Molecular Genetics, Russian Academy of Sciences, Moscow 123182, Russia

[Ø] Institute of Gene Biology, Russian Academy of Sciences, Moscow 117312, Russia

[∞] Department of Chemistry, University of Georgia, Athens, GA 30602, USA

[#] These authors contributed equally to this work

[†]This work was supported by the NIH (GM54998 to JLU) and the office of the Vice President for Research at the University of Georgia.

*To whom correspondence should be addressed: The University of Georgia, Department of Chemistry, Chemistry Building, Cedar Street Building 1001, Athens, GA 30602-2556, USA. Tel.: 706-542-7922; Fax: 706-542-1738; E-mail: urbauer@chem.uga.edu.

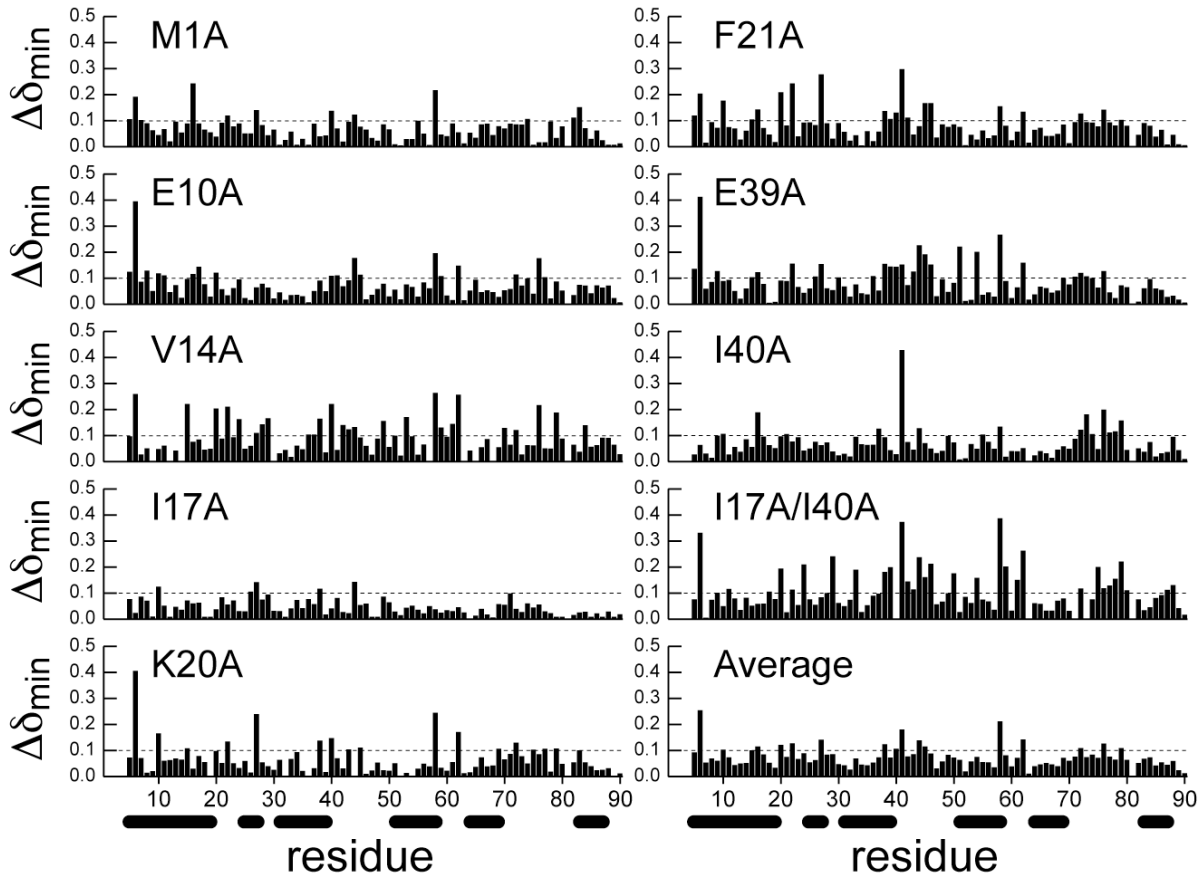

Figure S1. Minimal chemical shift perturbations for mutant AsiA proteins. The sequence-specific minimal chemical shift perturbations for main chain amide ^{1}H and ^{15}N pairs accompanying mutation compared to wild type are shown. This procedure is based on that described previously (Farmer, B. T., III, Constantine, K. L., Goldfarb, V., Friedrichs, M. S., Wittekind, M., Yanchunas, J., Robertson, J. G., and Mueller, L. (1996) *Nat. Struct. Biol.* **3,** 995–997) and provides a conservative estimate of chemical shift changes accompanying structural perturbation when chemical shift assignments are not known for one of the species, and thus can assist to localize structural changes. Chemical shift assignments for wild type AsiA permit calculation of minimal chemical shift changes accompanying mutation. For a given main chain amide $^{1}H_N$-^{15}N pair, the minimal chemical shift perturbation, $\Delta\delta_{min}$ (ppm), is defined as the minimal value for $((\Delta\delta\ ^{1}H_N)^2 + (\Delta\delta\ ^{15}N\ \alpha_N)^2)^{1/2}$, where α_N is a weighting factor that approximates the ratio of the relative chemical shift ranges for $^{1}H_N$ and ^{15}N. The ^{1}H, ^{15}N HSQC spectra shown in the text are the source of the data for the calculations. No data are available for residues 1-4 and 81. The cylinders in the figure represent the positions of the helices in AsiA as determined from the high resolution solution structure. The dashed lines at 0.1 ppm represent a qualitative estimate of an upper limit for a significant change, and are shown to guide the eye. An average over all mutants is also shown. Overall, the largest perturbations in most cases are at the ends of helices and between them. The results indicate the double mutant structure is somewhat more perturbed than each of the single mutants. The values for the I17A mutant are very small, indicating the chemical shifts of one of the conformers adopted by this mutant are very similar to wild type AsiA.