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Supplemental Information

Cowchock Syndrome Is Associated with a Mutation in Apoptosis-Inducing Factor

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Patient           474 KPYWHQSMFWSDLGPDVGYVAIGLVDSSLPT   504
Homo sapiens      474 KPYWHQSMFWSDLGPDVGYEAIGLVDSSLPT   504
C. lupus          474 KPYWHQSMFWSDLGPDVGYEAIGLVDSSLPT   504
B. taurus         474 KPYWHQSMFWSDLGPDVGYEAIGLVDSSLPT   504
M. musculus       473 KPYWHQSMFWSDLGPDVGYEAIGLVDSSLPT   503
R. norvegicus     473 KPYWHQSMFWSDLGPDVGYEAIGLVDSSLPT   503
G. gallus         452 KPYWHQSMFWSDLGPDVGYEAIGLVDSTLPT   482
D. rerio          614 KPYWHQSMFWSDLGPDVGYEAIGIVDSSLPT   644
D. melanogaster   535 KPYQHQSMFWSDLGPEIGYEGIGLVDSSLPT   565
A. gambiae        556 KPYTHQSMFWSDLGPRISYEAIGLIDSSLPT   586
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Figure S1. Protein homology of AIF in various species. The mutation results in amino acid substitutions at Glu493 (asterisk), a highly conserved residue.

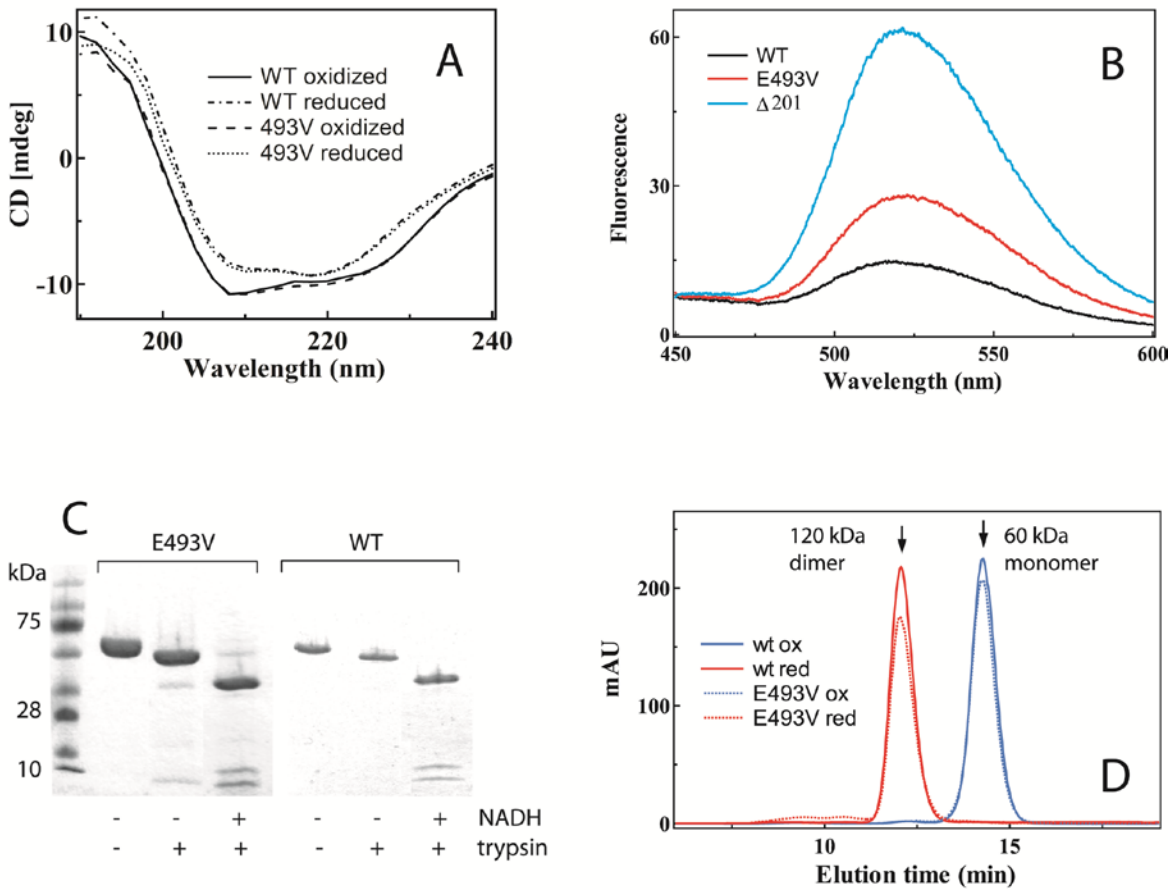

Figure S2. Effect of the E493V mutation on the molecular properties of AIF. (A) Circular dichroism (CD) spectra indicate that replacement of Glu493 with valine induces only subltle changes in the AIF structure. Far UV CD spectra were recorded in the absence and presence of a 20-fold excess of NADH.
(B) Flavin fluorescence in the wild type and mutant AIF. FAD is buried and virtually non-fluoresent in AIF^{wt}. 2- and 5-fold increases in flavin fluorescence in the E493V and Δ201 mutants, respectively, indicate higher solvent accessibility or partial exposure of FAD.
(C) Tryptic digest of oxidized and NADH-reduced AIF^{E493V} and AIF^{wt}. Oxidized AIF^{wt} undergoes proteolysis only at the Arg126-Ala127 site and runs on the SDS PAGE gel as a single band of ~50 kDa. The digest pattern indicates that, unlike in AIF^{wt}, the C-terminal regulatory insertion in the E493V mutant is partially unfolded and accessible to trypsin. (D) Redox-dependent monomer-dimer transition in AIF^{E493V} is not perturbed.

Figure S3. AIF immunodetection in fibroblasts. Western blot analysis of patient (Mut) and control (Ct) skin cultured fibroblasts, immunodetected with an antibody against AIF (Chemicon). Antibodies against subunit A of complex II (SDHA, Invitrogen) and ß-tubulin (Sigma) were used as loading controls.

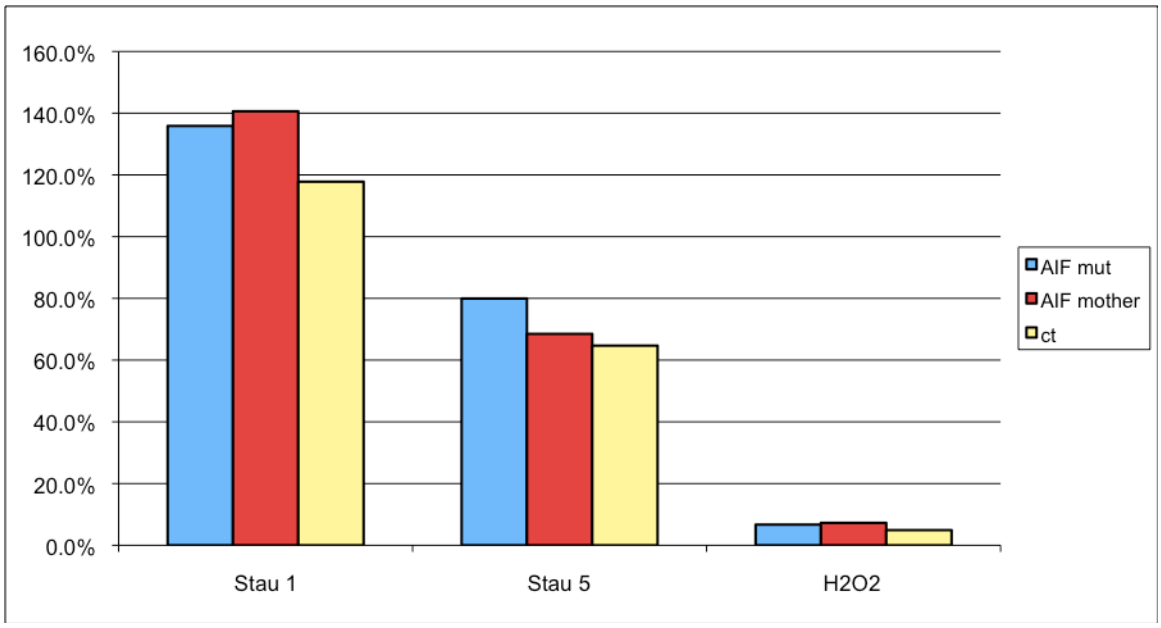

Figure S4. Viability assay. The viability of fibroblasts from affected (AIF mut), carrier (AIF mother) and control (ct) subject was assessed using CyQUANT Direct Cell Proliferation Assay (Invitrogen). Cells were treated for 2 hours with staurosporine 1µM (Stau 1), 5 µM (Stau 5), or hydrogen peroxide (H2O2) 15 mM.

Table S1. Data collection and refinement statistics

Data statistics	
Space group	C2
Unit cell parameters	*a* = 102 Å, *b* = 63 Å, *c* = 102 Å;
	α, β = 90°; γ = 119°
Resolution range	39.5 – 2.4 (2.40 – 2.46)[a]
Total reflections	148410 (1427)
Unique reflections	20005 (1308)
Redundancy	6.8 (6.8)
Completeness	94.2 (88.7)
Average $I/\sigma I$	11.3 (3.1)
R_{merge}	0.075 (0.45)
Refinement statistics	
Molecules per asymmetric unit	1
R/R_{free}[b]	25.5/29.7
r.m.s. deviations	
Bond lengths, Å	0.007
Bond angles, °	1.2

[a] Values in brackets are for the highest resolution shell.

Supplemental Experimental Procedures

Crystallization of AIF^{E493V}. AIF^{E493V} was crystallized by a microbatch method under oil. The protein (0.6 µl, 12 mg⁄ml) in 100 mM BisTris propane buffer, pH 7.5, was mixed with 0.6 µl of the crystallization solution containing 30% polyethylene glycol 1500, 0.1 M Tris pH 8.5, and 4% acetone, and then covered with 10 µl of paraffin oil. Crystals grew within several days at room temperature. X-ray diffraction data were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) beamline 7-1 using paratone-N as a cryoprotectant. The structure was solved by molecular replacement with AIF^{wt} (PDB code 1M6I) as a search model.