Supplementary Information

Nonsynonymous Mutations in Linker-2 of the Pdr5 Multidrug Transporter Identify a New RNA Stability Element

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1 Supplementary Table

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Table S1

Primers used in the q-RT PCR experiments

Primer	***Sequence***
PDR5-F†	‘5-caaaactccactcaatcggcacccaac-3’
PDR5-R‡	‘5-agccatattcttaacccaggcggcac-3’
ALG9-F	‘5-cacggatagtggctttggtgaacaattac-3’
ALG9-R	‘5-tatgattatctggcagcaggaaagaacttggg-3’
TAF10-F	‘5-atattccaggatcaggtcttccgtagc-3’
TAF10-R	‘5-gtagtcttctcattctgttgatgttgttgttg-3’
GAPDH-F	‘5-cggtagatacgctggtgaagtttc-3’
GAPDH-R	‘5-tggaagatggagcagtgataacaac-3’

†F denotes forward primer for all the primer used

‡R denote Reverse primer for all the primer used

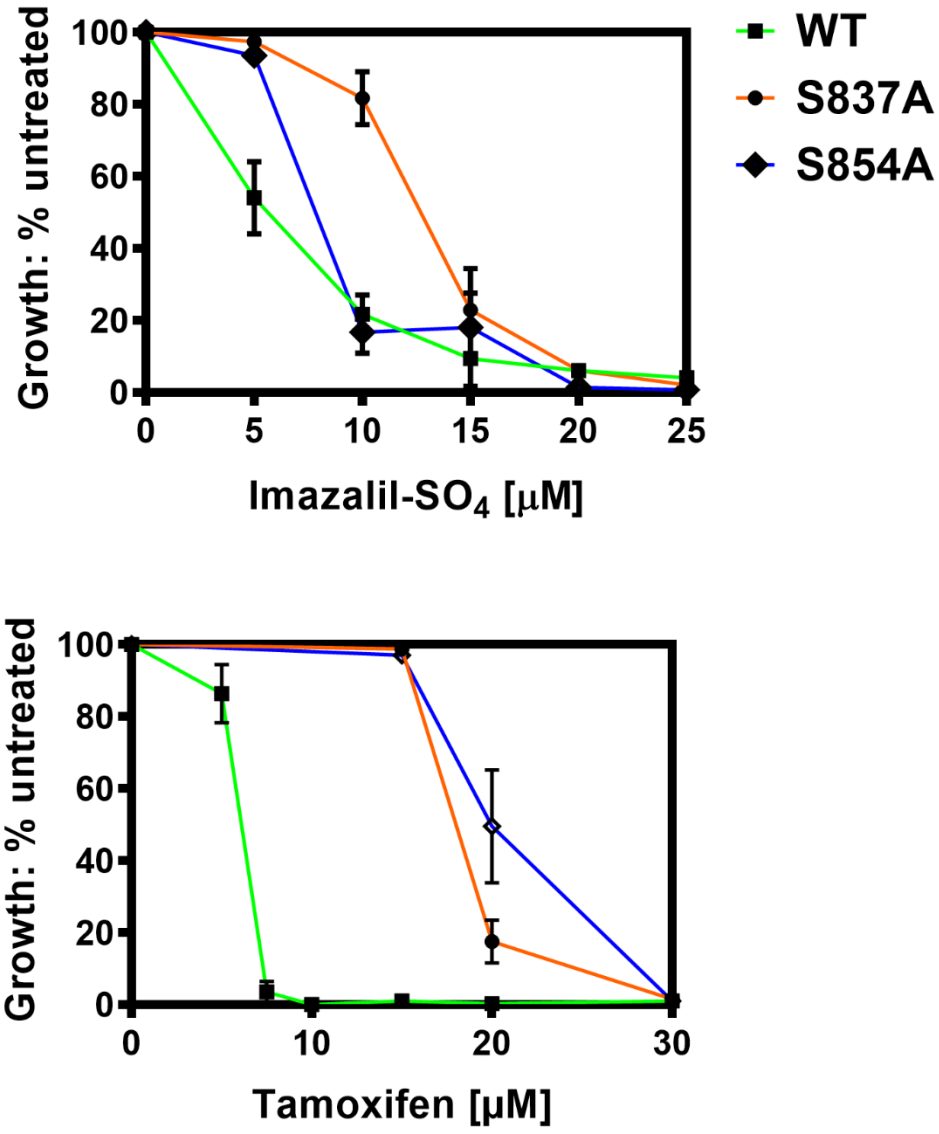

Figure S1. Both S837A and S854A showed enhanced resistance to imazalil-SO_4 and tamoxifen compare to WT. We investigated the relative resistance of S837A (JG2175), S854A (JG2181), and WT (JG2001) to imazalil-SO_4 and tamoxifen. In these experiments, n = 4. The WT, S837A and S854AA curves have green, orange and blue lines respectively.

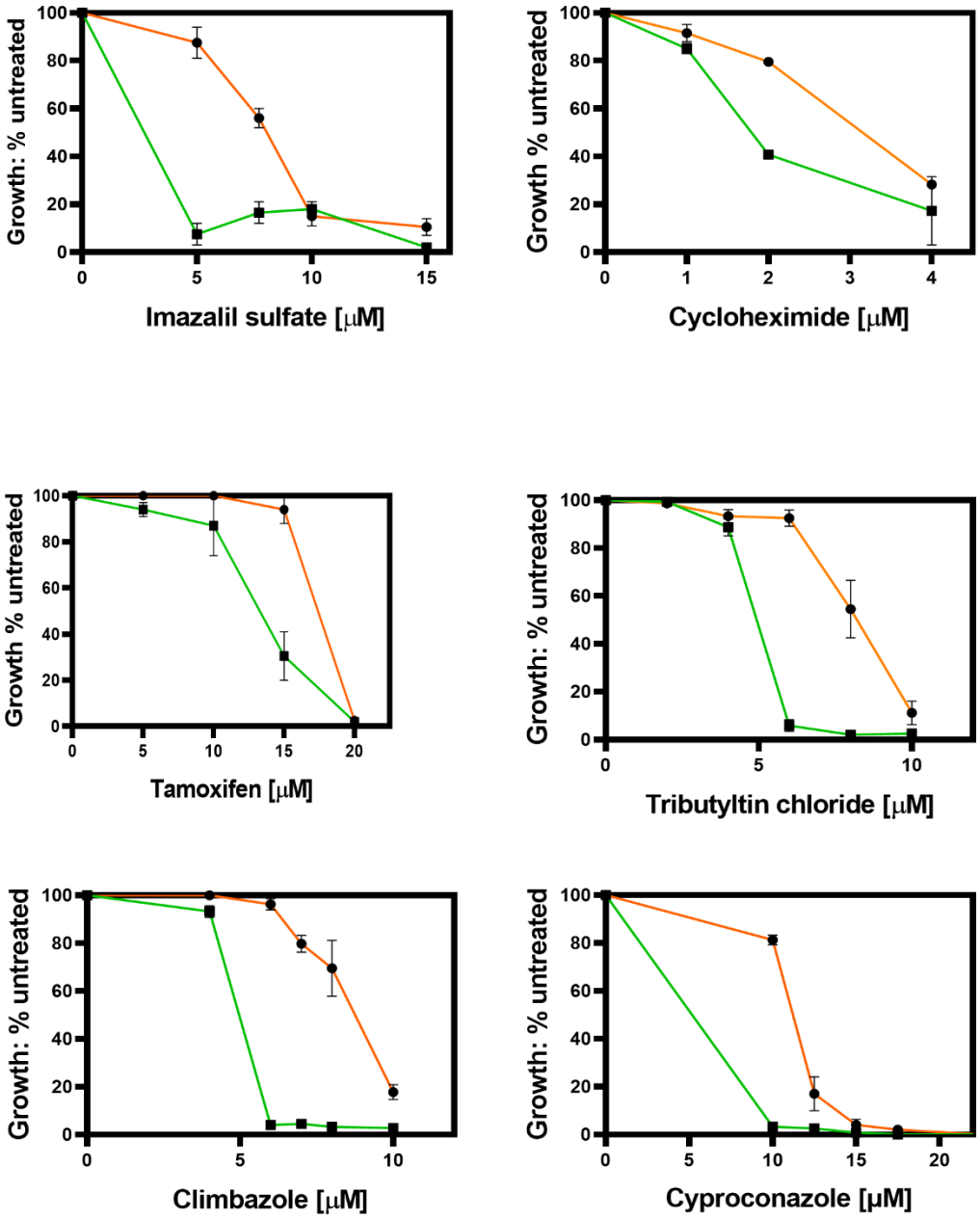

Figure S2. The S837A mutation results in enhanced resistance to transport substrates. The experiments are analogous to those described in Figure 3 except that the JG2015 WT strain (instead of the JG2001 strain) was used. In these experiments, n = 4. The WT and S837A (JG2175) curves have green and orange lines respectively.

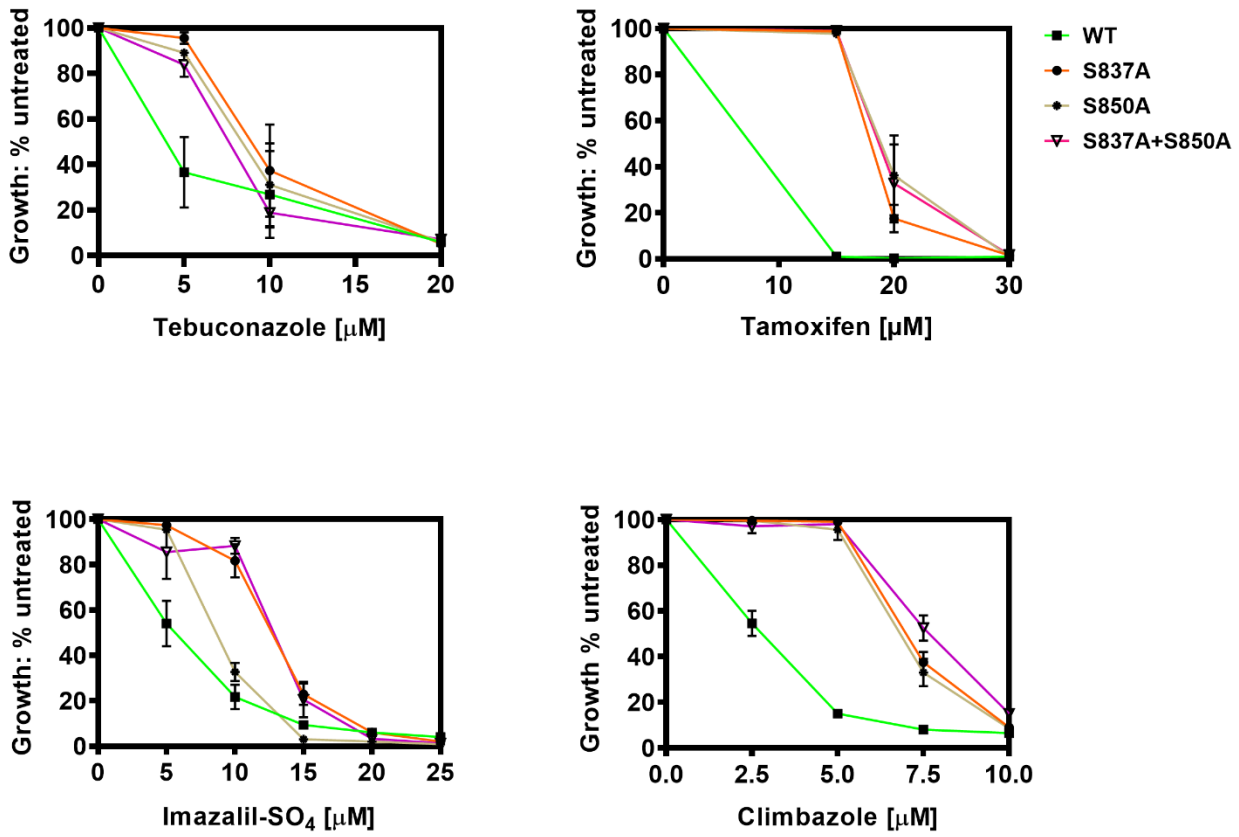

Figure S3. An S837A, S850A double mutant has similar drug resistance phenotype as with their single mutants counterpart.

Here we used S837A, S850A double mutant (JG2183) to test for resistance to xenobiotic agents with the IC_{50} protocol as described in the materials and methods. Cells were cultured in YPD broth for 48 h at 30 °C. In these experiments, $n \geq 3$. In all panels, the S837A (JG2175, orange line), S850A ((JG2180, light brown), and double mutant (pink line) were compared to each other and to the WT control (JG2001).

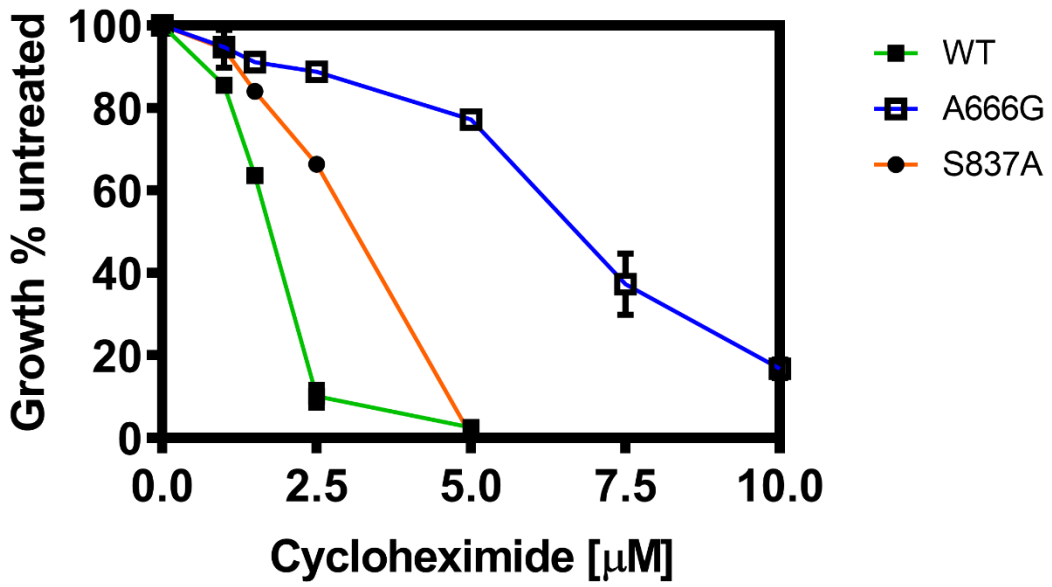

Figure S4. The A666G (JG2133) mutant exhibits stronger hyperresistance to cycloheximide than the S837A (JG2175) mutant or the WT strain (JG2015). Growth of each strain was measured in liquid culture as described in Figure 3 (n=3).

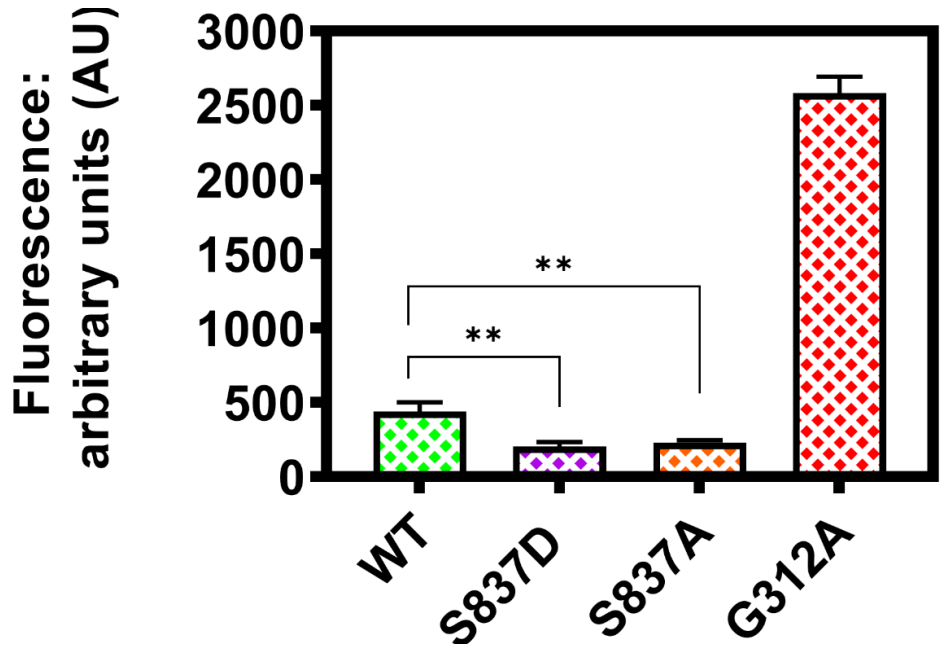

Figure S5. The S837D mutant strain exhibits enhanced R6G transport. R6G transport was performed in whole cells of WT (JG2015), S837A (JG2075), S837D (JG2076) or the phenotypically null mutation G312A (JG2063) as described in the Materials and Methods. In these experiments, n =3.

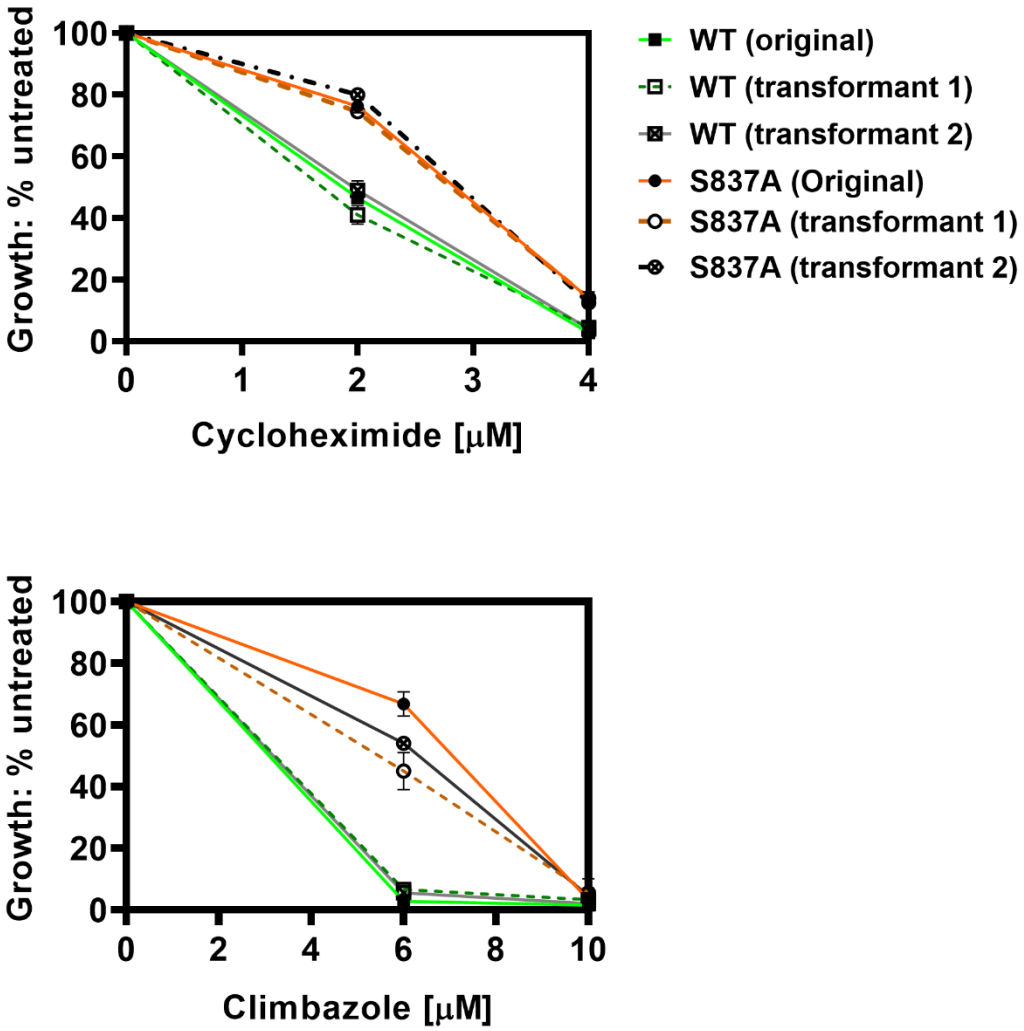

Figure S6. Recreation of the WT and S837A mutant strains recreated their phenotypic differences. (A) Resistance to cycloheximide and climbazole was measured in liquid culture at several concentrations of each drug (n=4) as described in the Materials and Methods. The original strains and two transformants of R-1 with the WT (JG2015*) and mutant allele (JG2075*) were tested. (B) Q-RT PCR was performed with RNA extracted from one WT and one S837A transformant as described in the Materials and Methods. The ΔC_T values for the *ALG9* and *TAF10* references genes were determined and these values were used to obtain the $\Delta\Delta C_T$ between the WT and mutant strains for each reference gene. The times difference was calculated from the formula: fold change = $2^{\Delta\Delta C_T}$.

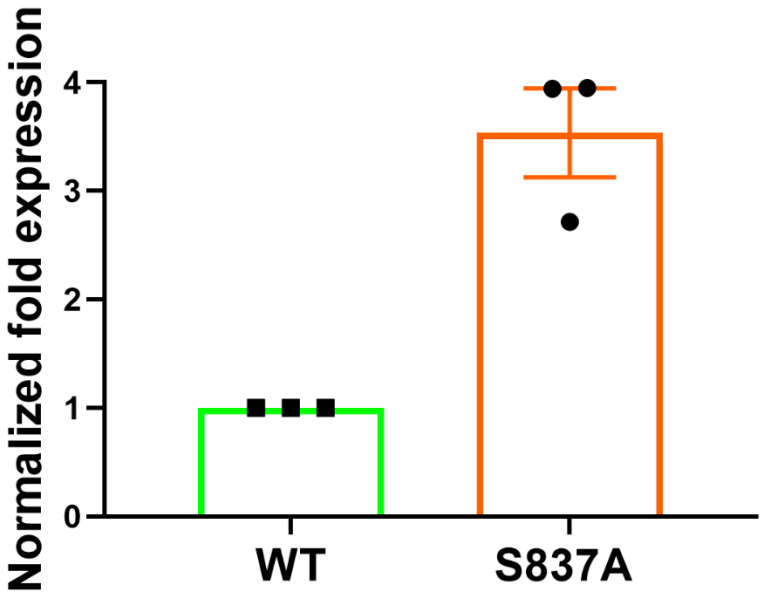

Figure S7: mRNA expression level of Pdr5 from the WT and S837A transformants. Here using q-RT PCR technique we quantified the whole cells expression of Pdr5 transcripts from the transformants. The fold expression for both strains is normalized to two reference genes. S837A (JG2075*, ●, orange bar graph) showed significantly higher expression level than WT (JG2015*,■, green bar). In this experiment, n=3.

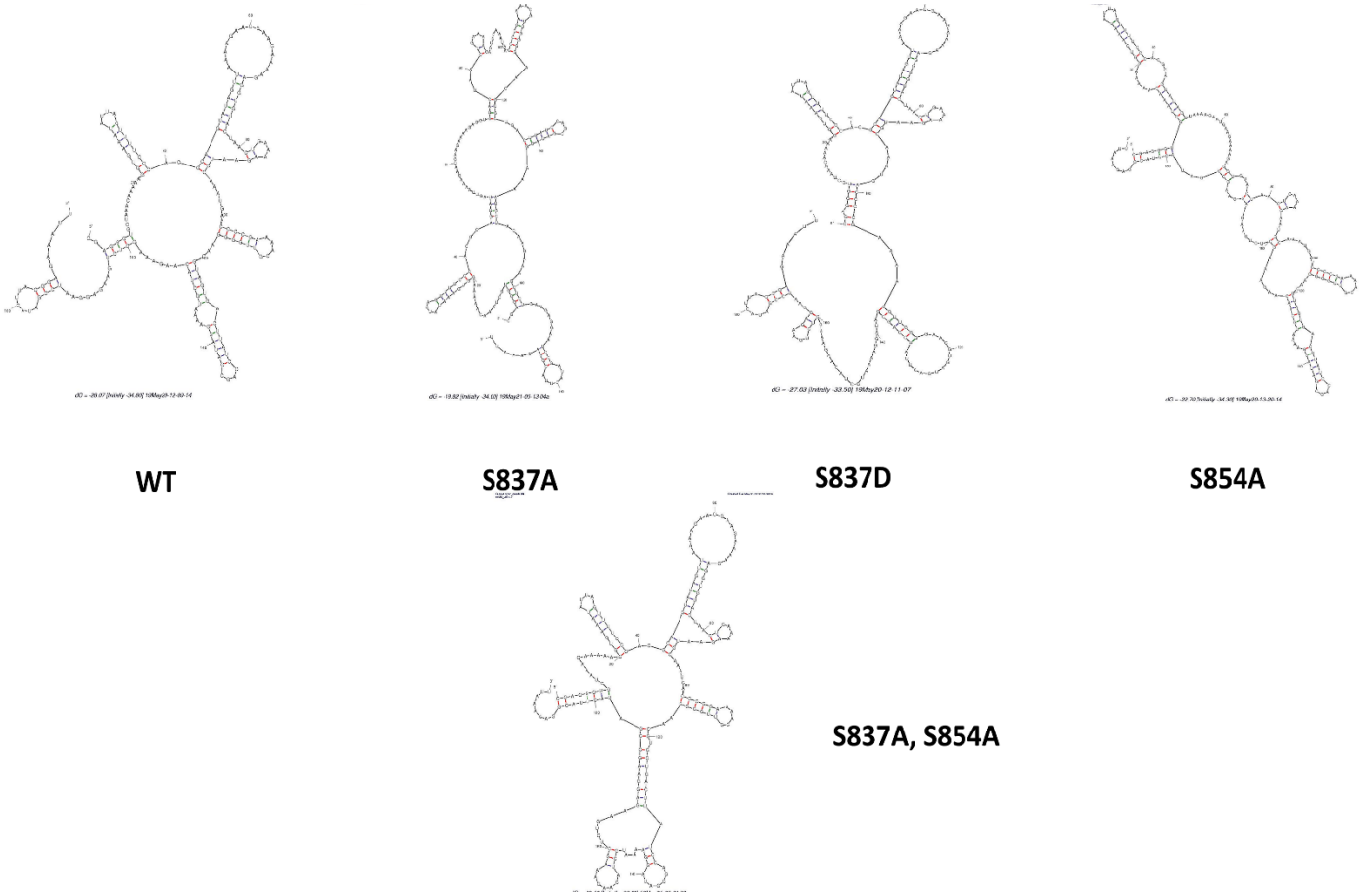

Figure S8. Local changes in the secondary structure of linker-2 RNA as a result of one (JG2181, S854A), two (JG2075, S837A), and three (JG2182, S837A, S854A) nucleotide substitutions. The structures were made using the complete nucleotide sequence of linker-2 (Rutledge *et al.* 2011) and the online software available from The RNA Institute (Albany, NY).

Supplementary Information Reference:

Rutledge, R.M., Esser, L., Ma, J., and Xia, D. (2011) Toward understanding the mechanism of action of the yeast multidrug transporter Pdr5p: A molecular modeling study (2011) *Journal of Structural Biology*, **173,** 333-344.