Saccharomyces cerevisiae rhodanese RDL2 uses the Arg residue of the active-site loop for thiosulfate decomposition

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Running title: *The loop-ending amino acids are critical for rhodaneses activities*

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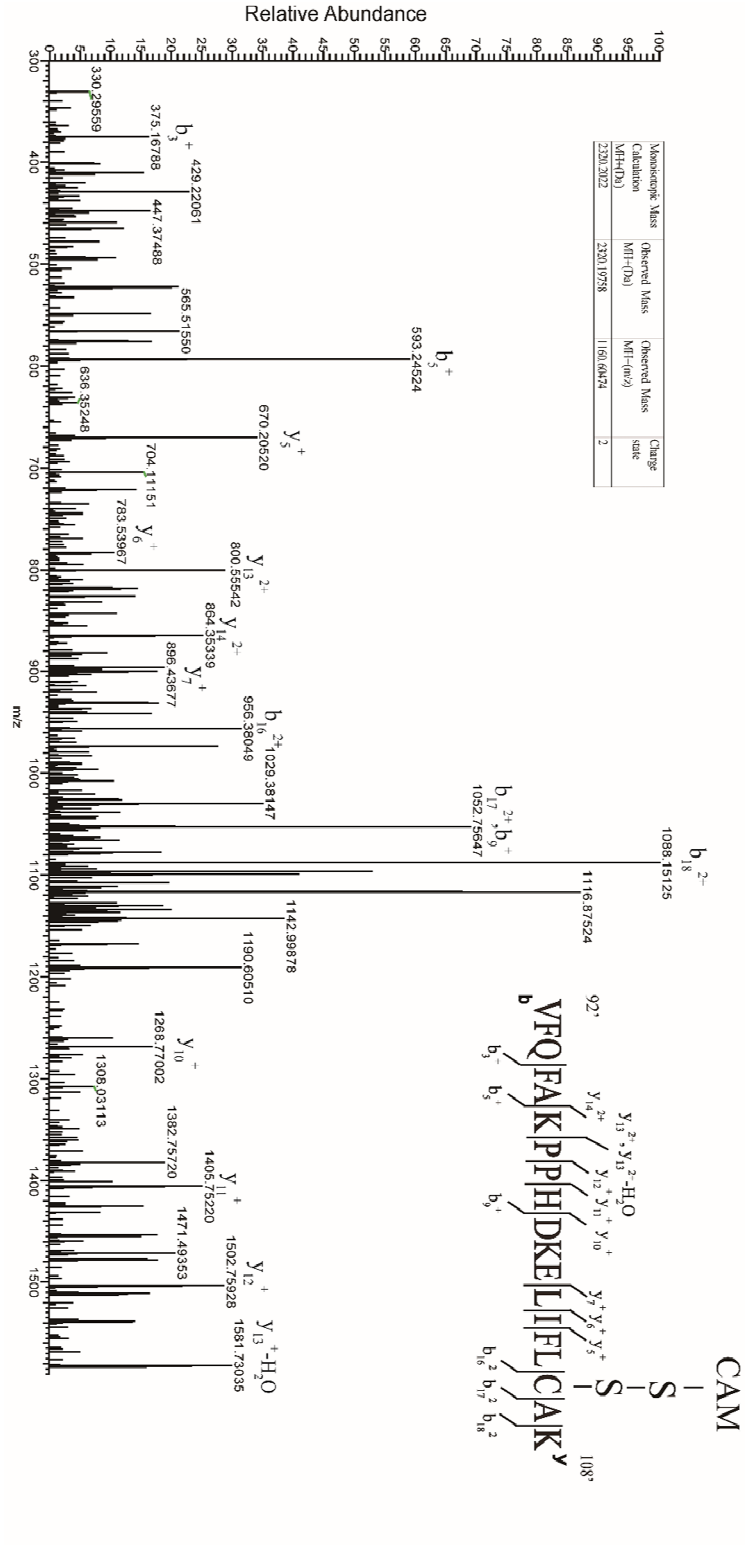

Figure S1. LTQ-Orbitrap tandem mass spectrometry analysis of Cys106 modification in thiosulfate-reacted RDL2.

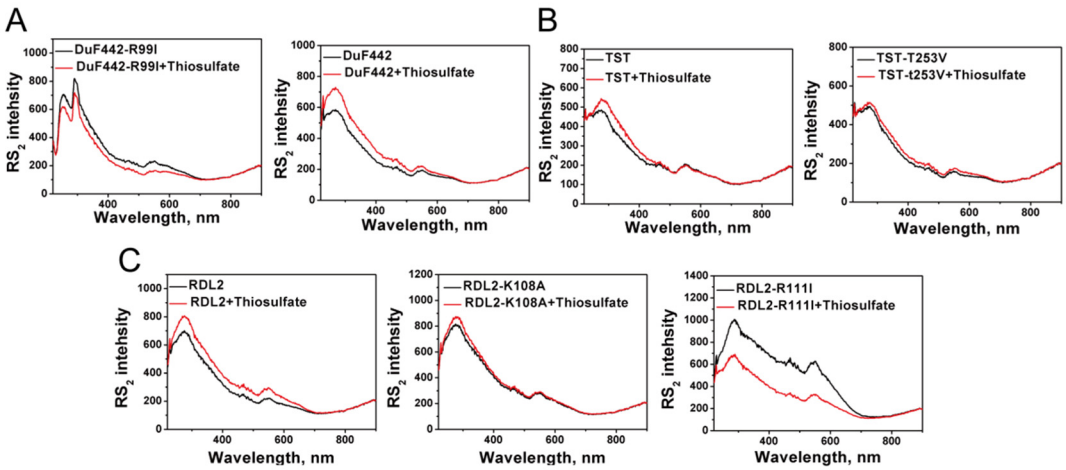

Figure S2. RS_2 analysis of unreacted- and thiosulfate-reacted rhodanese. ΔRS_2 at a specific wavelength was calculated by $\Delta RS_2{}^{w}_{\text{reacted}}$ -$\Delta RS_2{}^{w}_{\text{unreacted}}$ (w is the wavelength). Total ΔRS_2 was calculated by adding up all ΔRS_2 values at 240 nm~550 nm range.

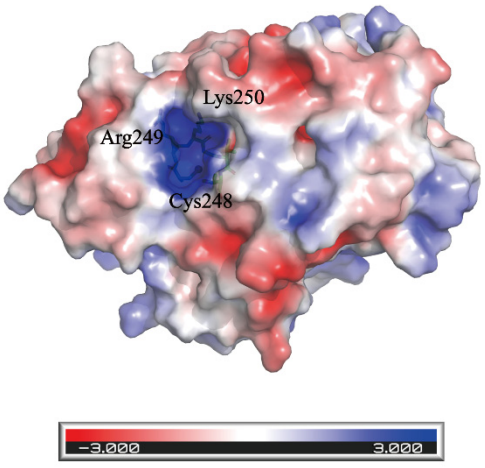

Figure S3. **Modeled 3D structure of TST.** The structure of DUF442 was generated by SWISS-MODEL using the *Bos taurus* rhodanese as a template (PDB entry:1DP2) (90.7% sequence similarity). The 3D homology modeling structure of TST showed that its active

center is located in a pocket with positive electrostatic field. Lys_{250} and Arg_{249} on the active site loop are major contributors to the positive electrostatic field.

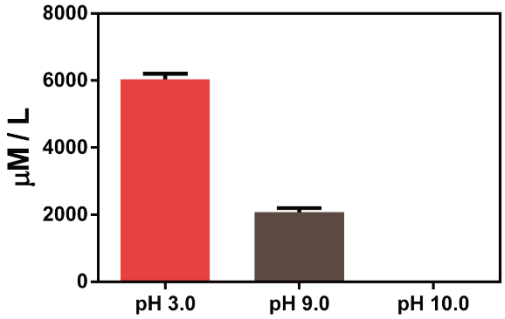

Figure S4. HPLC quantification of S_8 produced from decomposition of thiosulfate. Thiosulfate produces more S_8 at pH 3.0 and does not decompose at pH 10.0.