In vitro characterization of a novel Isu homologue from *Drosophila melanogaster* for de novo Fe-S cluster formation.

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Figure S1: Rate of formation of FeS-fIscU under different Yfh1 concentrations (A). Initial rates of FeS-fIscU formation under differing Yfh1 concentrations (B, black). Gray line represents transition from stimulation to inhibition. Effect of Yfh1 on FeS-fIscU formation is concentration dependent with maximal Yfh1 stimulation occurs 5-10 μ M and minimal effect ~25 μ M. Reactions all done with 150 mM NaCl, 50 μ M fIscU, 10 μ M Nfs1-Isd11, and 75 μ M Fe.

Figure S2: Complete CD spectrum for FeS clusters formed after 40 minutes during iron concentration study. Spectra presented were formed at 75 uM Fe (green) and 100 uM Fe (black) concentrations, with the addition of 50 μ M flscU monomer and 10 μ M Nfs1-Isd11 monomer.

Supplemental Figure 1



Supplemental Figure 2



Table S1: Fe K-Edge energies and 1s-3d pre-edge energies for Fe-flscU and FeS-flscU in comparison to Fe(II) and Fe(III) standards.

Sample	Fe K-Edge Energy (eV)	1s-3d area (eV*100)
aq Fe(III) ₂ (SO ₄) ₃	7126.6	3.6
Fe(II)Cl ₂	7119.5	2.3
Fe(III)Cl ₃	7121.0	14.0
Fe-flscU	7122.7	4.8
FeS-fIscU	7120.1	21.7
FeS-Yah1	7119.7	29.1