Supporting Information

Human serum transferrin: Is there a link between autism, high oxalate and iron deficiency anemia?

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Supplementary Data

Supporting Information Table 1.

Kinetic rate constants for the control Fe_2hTF and the constructs with mutations that lock the lobes in the closed conformation preventing iron release in the absence of the sTFR.

Construct	k_1 (min^{-1})	k_2 (min^{-1})
Fe_2hTF	17.7 ± 2.2 (k_N)[a]	0.65 ± 0.06 (k_C)[a]
K206E/K534A Fe_2hTF	0.7 ± 0.3	0.09 ± 0.02
K206E/K534E Fe_2hTF	0.9 ± 0.4	0.15 ± 0.03
K206E/R632A Fe_2hTF	-	0.11 ± 0.05
K206E/R632E Fe_2hTF	0.6 ± 0.1	0.08 ± 0.003

[a]From reference 1.

Supporting Information Table 2.

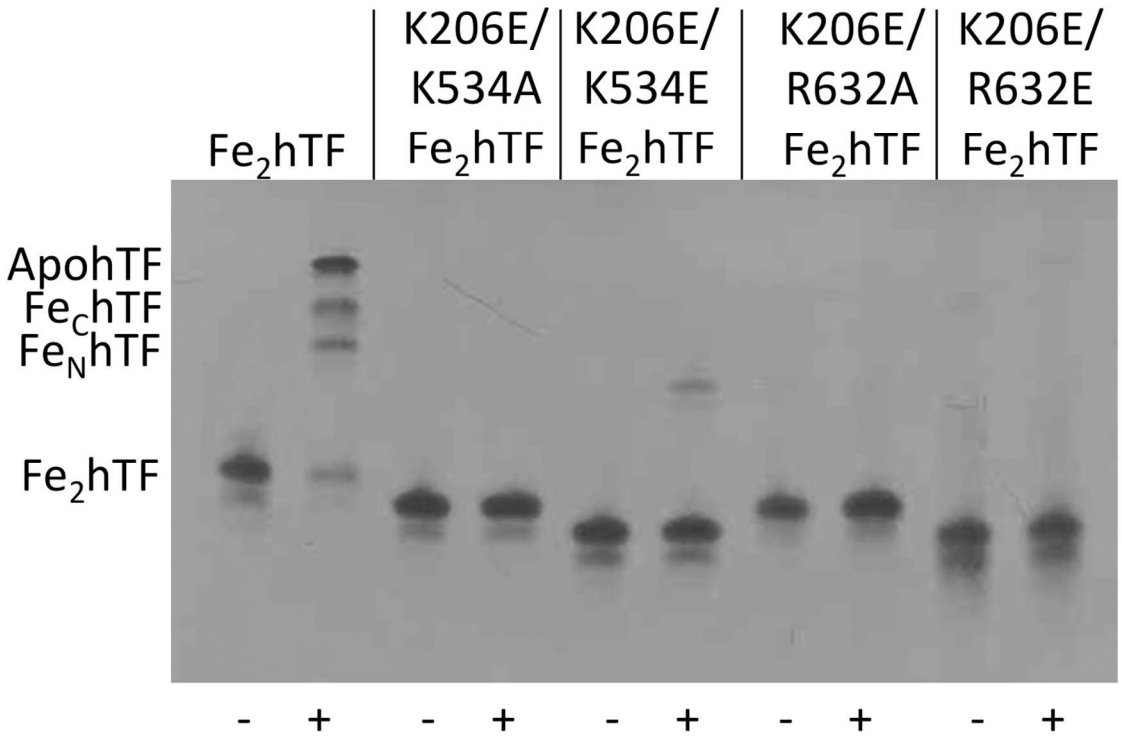
Kinetic rate constants for the control Fe_2hTF and the constructs with mutations that lock the lobes in the closed conformation preventing iron release in the presence of the sTFR.

hTF/sTFR complex	k_1 (min^{-1})	k_2 (min^{-1})
Fe_2hTF/sTFR	5.5 ± 0.9 (k_C)[a]	1.4 ± 0.2 (k_N)[a]
K206E/K534A Fe_2hTF/sTFR	-	2.3 ± 0.1
K206E/K534E Fe_2hTF/sTFR	-	3.0 ± 0.3
K206E/R632A Fe_2hTF/sTFR	-	2.0 ± 0.1
K206E/R632E Fe_2hTF/sTFR	32.3 ± 3.4	1.2 ± 0.05

[a]From reference 1.

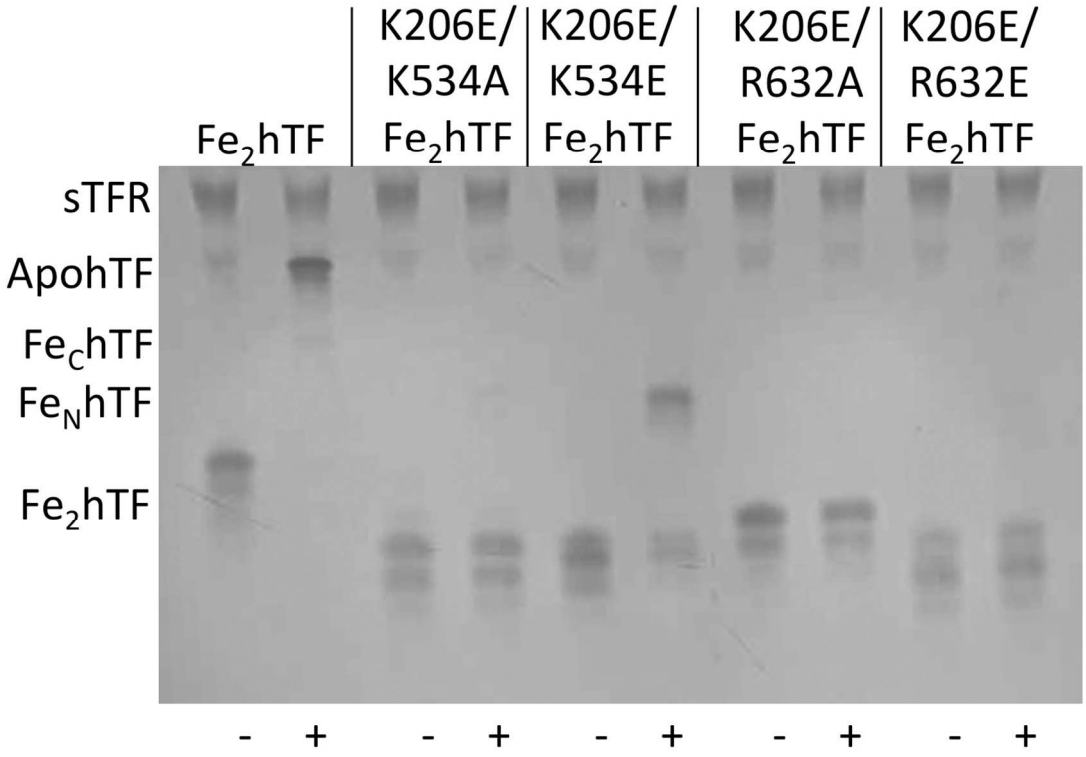
Supporting Information Figure 1.

Urea gel analysis of carbonate (CO_3^{-2}) containing Fe_2hTF and the four $Lock_N/Lock_C$ hTF constructs (K206E/K534A Fe_2hTF, K206E/K534E Fe_2hTF, K206E/R632A Fe_2hTF and K206E/R632E Fe_2hTF). Samples were electrophoresed before and after (+) incubation with iron removal buffer (100 mM MES, pH 5.6, containing 300 mM KCl and 4 mM EDTA) for 15 min.

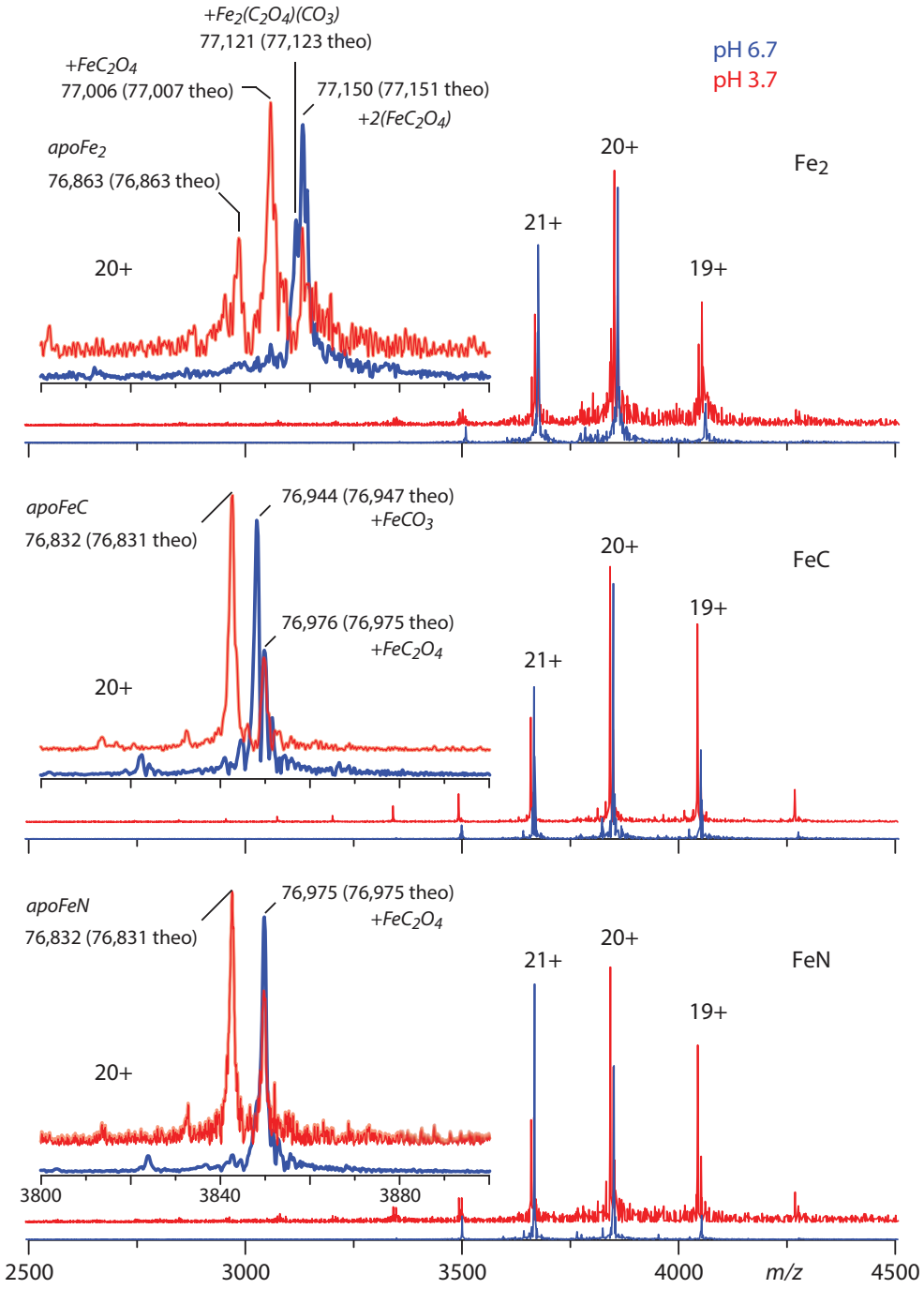


Supporting Information Figure 2.

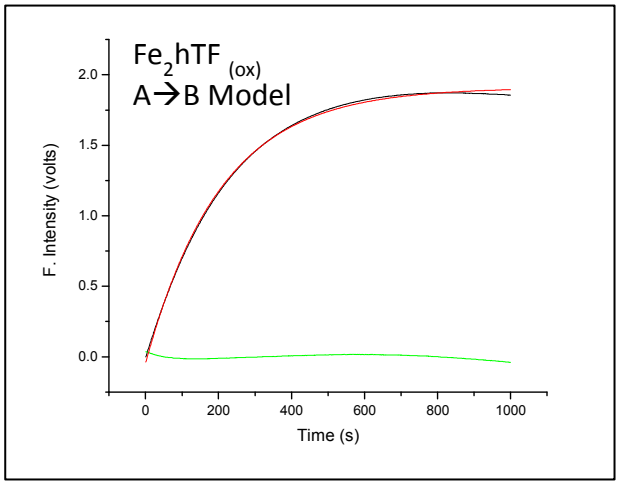
Urea gel analysis of carbonate (CO_3^{-2}) containing Fe_2hTF/sTFR and the four $Lock_N/Lock_C$ hTF/sTFR (K206E/K534A Fe_2hTF, K206E/K534E Fe_2hTF, K206E/R632A Fe_2hTF and K206E/R632E Fe_2hTF) complexes. Samples were electrophoresed before and after (+) incubation with iron removal buffer (100 mM MES, pH 5.6, containing 300 mM KCl and 4 mM EDTA) for 15 min.

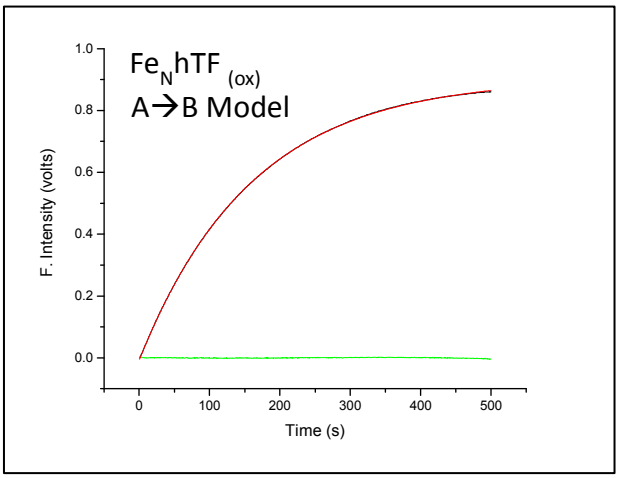


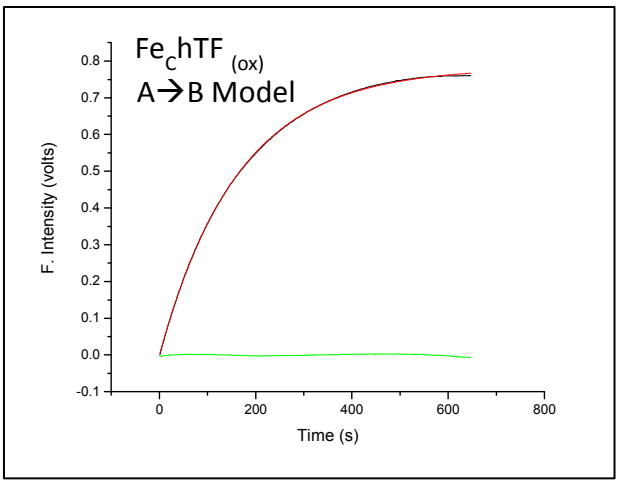
Supporting Information Figure 3. ESI MS analysis of the iron variants of hTF showing incomplete exchange of anion. Spectra for hTF and each monoferric variant (Fe_C, and Fe_N) were recorded at pH 6.7 and then at 3.7. The presence of carbonate was clearly evident in these samples.



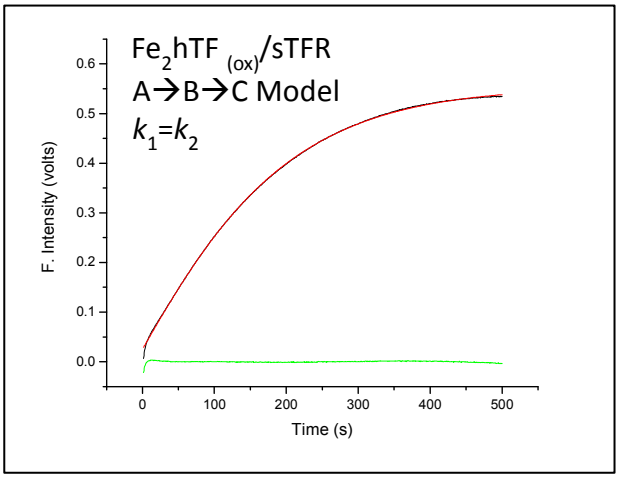
Supporting Information Figure 4. Iron release curves from $Fe_2hTF_{(OX)}$, $Fe_NhTF_{(OX)}$ and $Fe_ChTF_{(OX)}$ (black line) and fit (red line). The residuals are indicated in green.

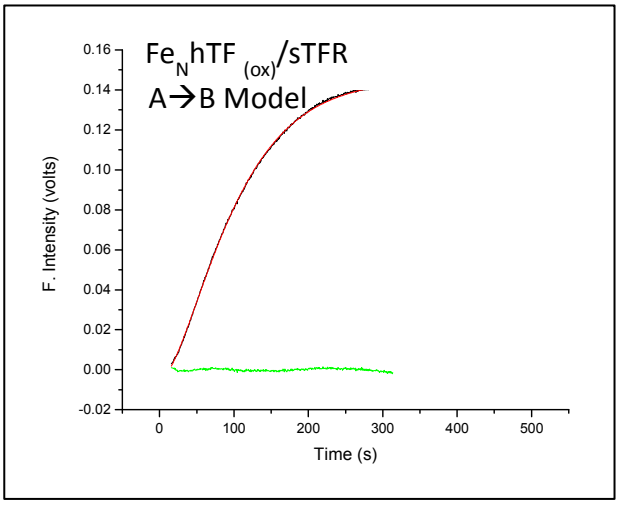


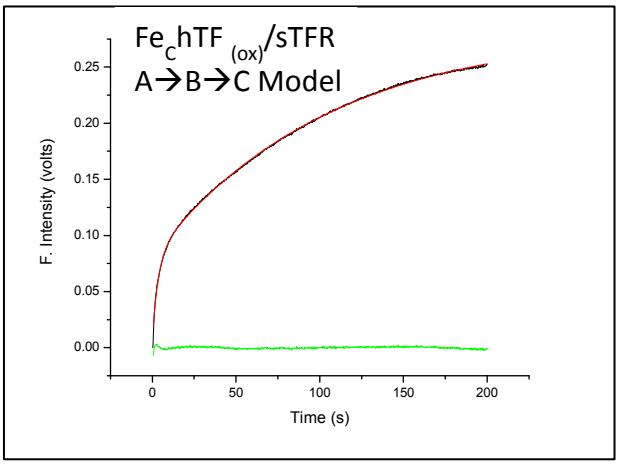




Supporting Information Figure 5. Iron release curves from $Fe_2hTF_{(OX)}$/sTFR, $Fe_NhTF_{(OX)}$/sTFR and $Fe_ChTF_{(OX)}$/sTFR complexes (black line) and fit (red line). The residuals are indicated in green.







REFERENCES

1. Byrne, S. L., Chasteen, N. D., Steere, A. N. & Mason, A. B. (2010). The unique kinetics of iron-release from transferrin: The role of receptor, lobe-lobe interactions and salt at endosomal pH. *J. Mol. Biol. 396*, 130-140.