Supporting Information

^{129}Xe NMR-Protein Sensor Reveals Cellular Ribose Concentration

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Table S1. Oligonucleotide primers used for RBP(L19A) insert amplification.

Forward primer	5' – TACTTCCAATCCAATGCAAAAGATACCATCGCGCTGGTCG – 3'
Reverse primer	5' – TTATCCACTTCCAATGTTATTACTGTTTGACAACCAGTTTCAGG – 3'

Table S2. Oligonucleotide primers used in RBP site-directed mutagenesis.

Q235A	Forward primer	5' – GCGACGATTGCAGCGCTGCCGGACCAG – 3'
	Reverse primer	5' – CTGGTCCGGCAGCGCTGCAATCGTCGC – 3'
T135A	Forward primer	5' – GGTATCGCGGGTGCCAGCGCTGCGCGC – 3'
	Reverse primer	5' – GCGCGCAGCGCTGGCACCCGCGATACC – 3'

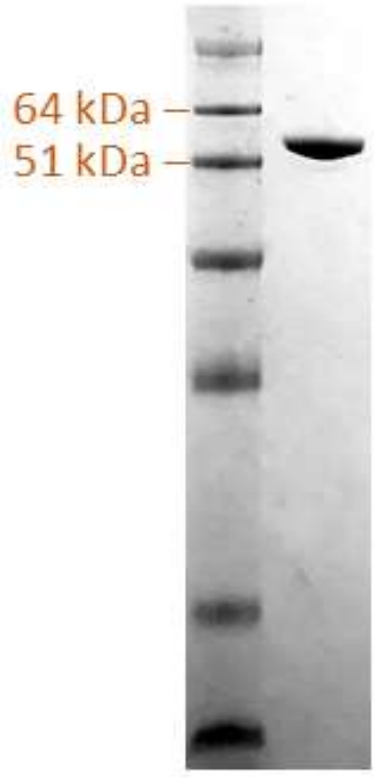

Figure S1. SDS-PAGE gel of purified GFP-RBP(L19A).

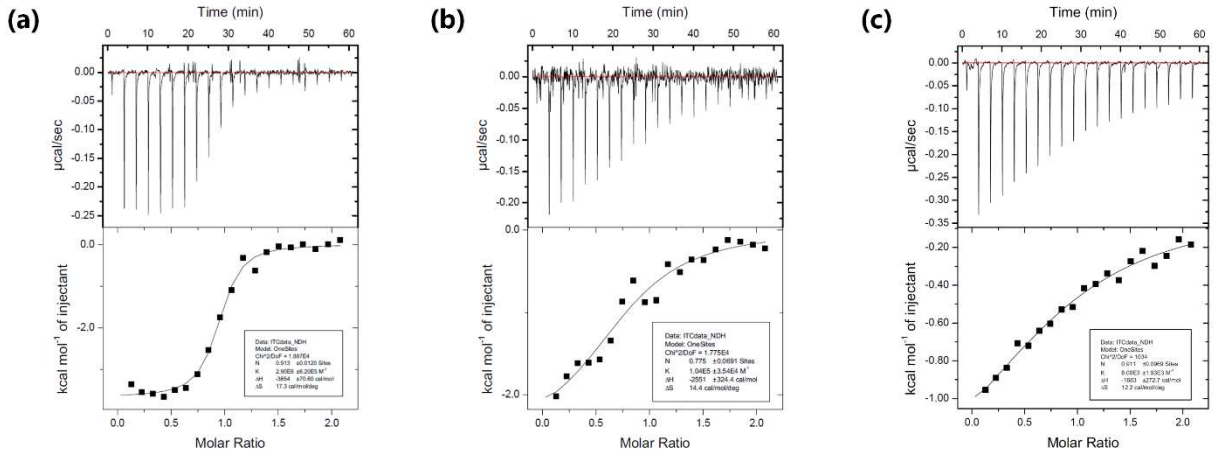

Figure S2. ITC enthalpograms for ribose binding for (a) GFP-RBP(L19A); (b) GFP-RBP(L19A/Q235A); (c) GFP-RBP(L19A/T135A/Q235A).

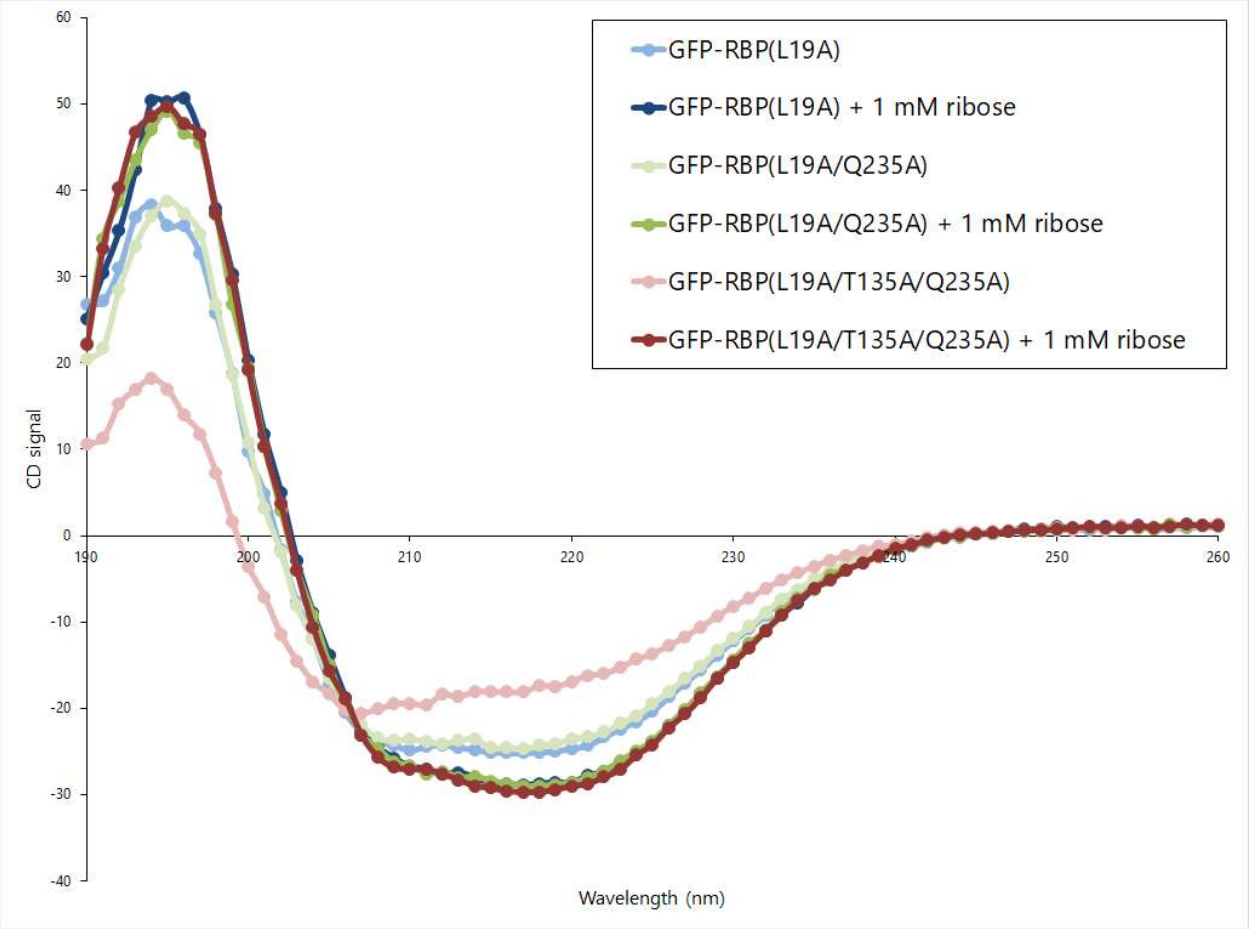

Figure S3. CD spectra of GFP-RBP(L19A) and mutants. Spectra taken from 5 μM protein in 10 mM sodium phosphate buffer (pH 8.0) at 20 °C.

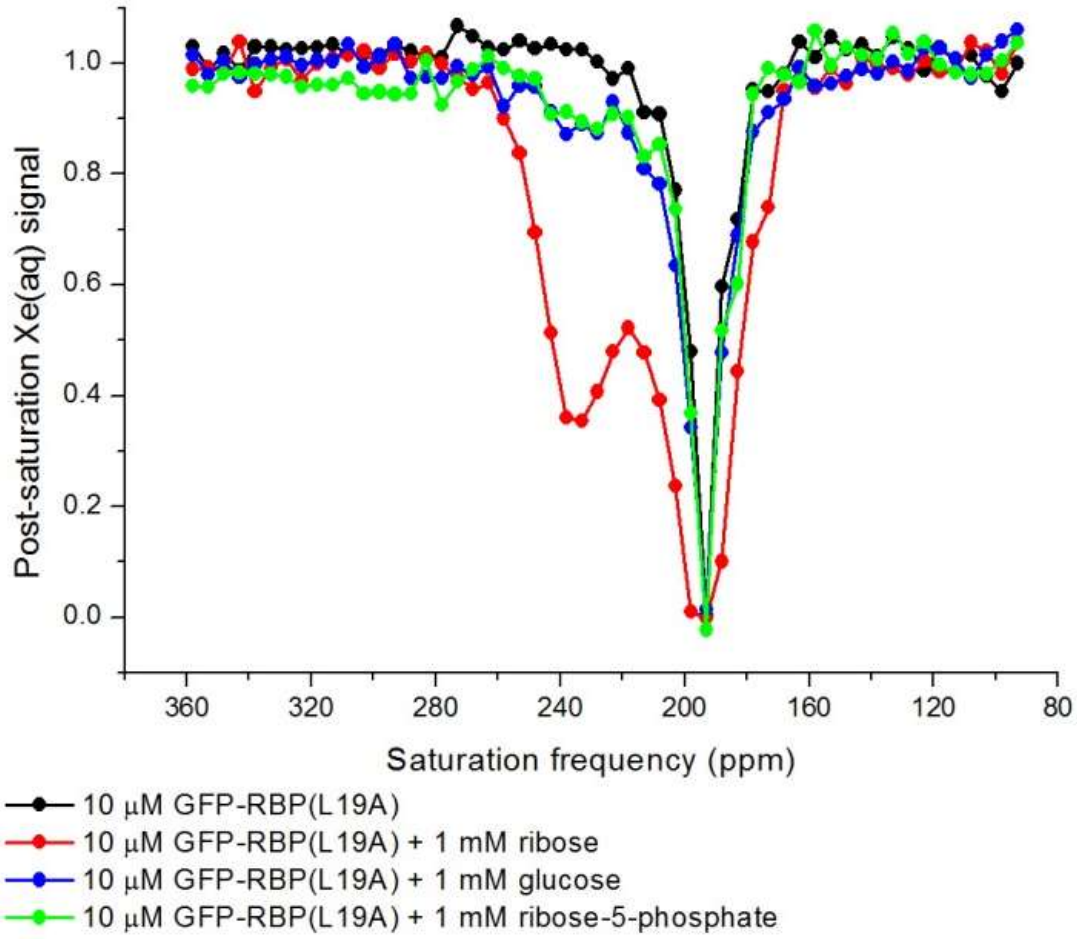

Figure S4. Hyper-CEST z-spectra of 10 μM GFP-RBP(L19A) in PBS. Data acquired with protein in its unliganded state and in the presence of indicated ligands in pH 7.2 buffer at 300 K. Data shown as an average of 3 trials.

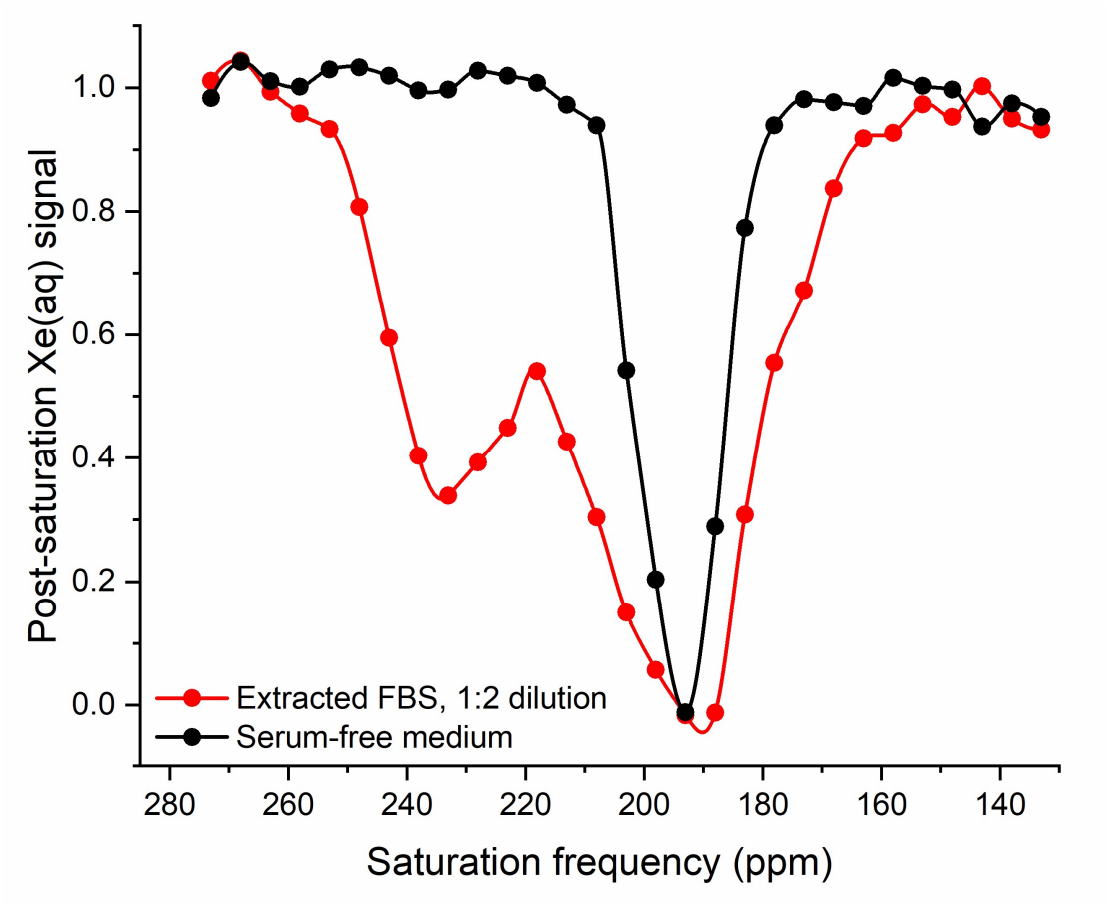

Figure S5. Hyper-CEST z-spectra of GFP-RBP(L19A) in FBS and serum-free medium. FBS was extracted with methanol following literature procedure[1] and then diluted 1:2 in PBS buffer. Pulse length, τ_{pulse} = 3.80 ms; field strength, $B_{1,max}$ = 77 μT. Experiments were performed at 300 K with 20 μM protein. Data shown as an average of 3 trials.

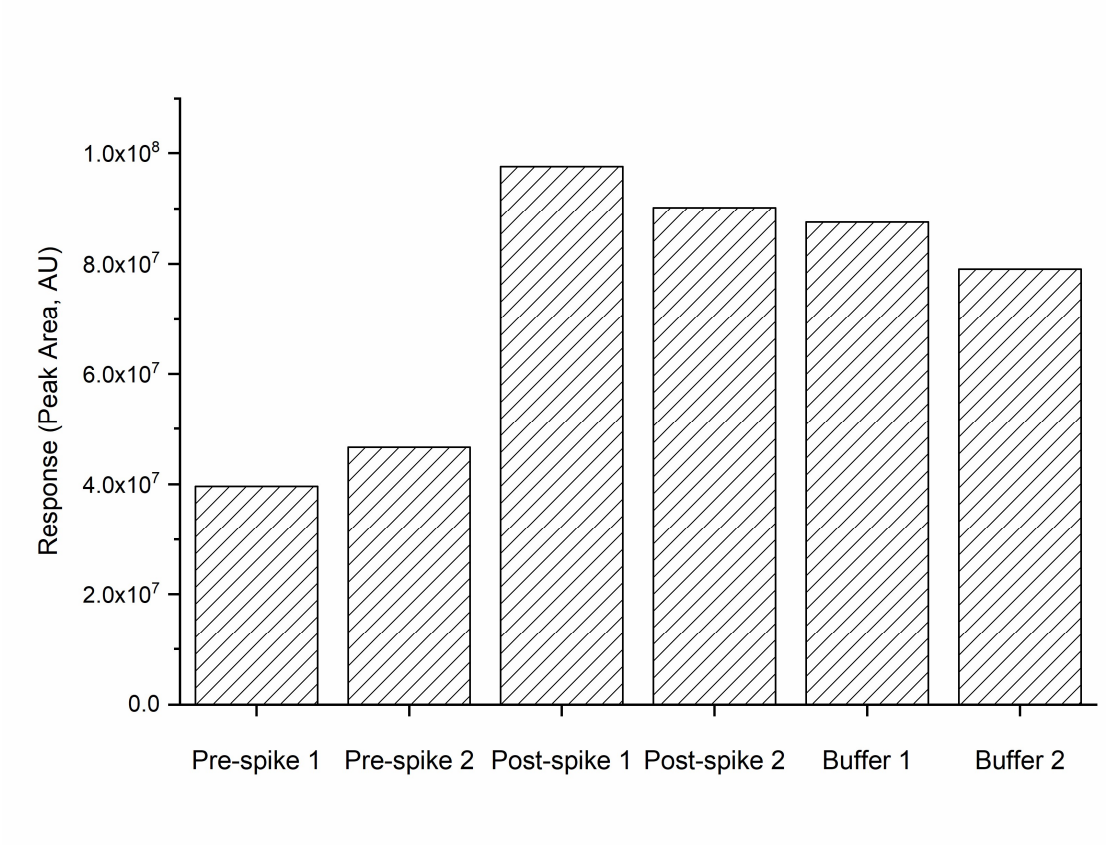

Figure S6. LC-MS response of [U-$^{13}C_5$] ribose spiked into extracted HeLa cell lysate samples. "Pre-spike" corresponds to samples of 2 million/mL lysed HeLa cell extract spiked pre-extraction with [U-$^{13}C_5$] ribose to a final concentration of 20 µM. "Post-spike" corresponds to samples of 2 million/mL lysed HeLa cell extract spiked post-extraction with [U-$^{13}C_5$] ribose to a final concentration of 20 µM. "Buffer" corresponds to controls of the extraction solution only (1:2 ddH_2O:MeOH v/v) spiked with [U-$^{13}C_5$] ribose to a final concentration of 20 µM. Each sample was injected twice. The acetate adduct peak was quantified. The peak areas for each sample are shown in Table S3.

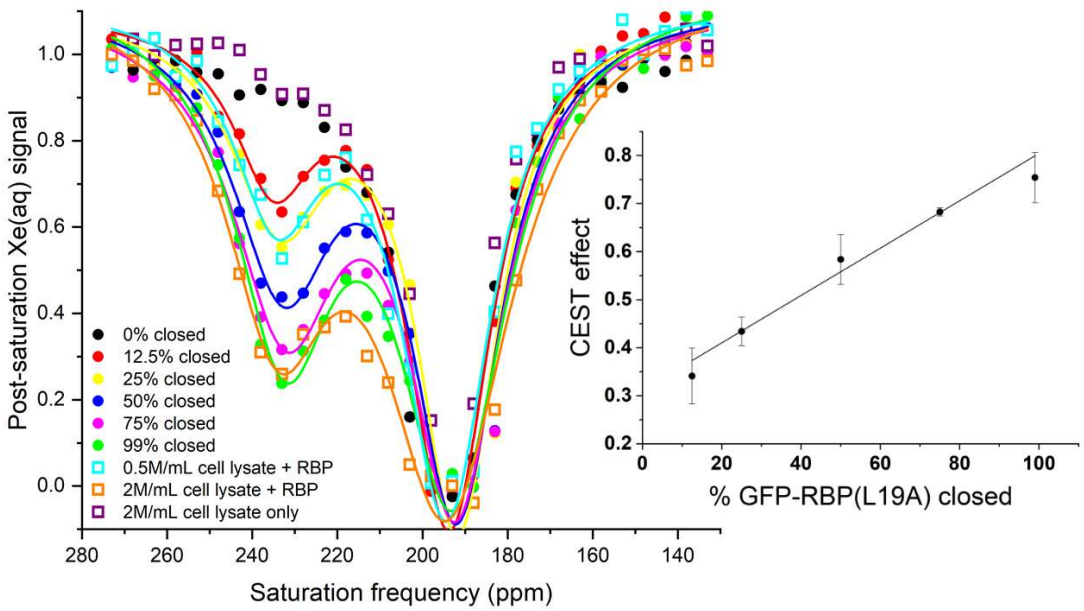

Figure S7. Hyper-CEST z-spectra of GFP-RBP(L19A) in FBS-supplemented HeLa cell lysate. Z-spectra of varying percentages of GFP-RBP(L19A) in the closed conformation ([ribose] = 0, 2.5, 5.1, 10.3, 15.9, 49.5 μM), and with cell lysate from HeLa cells grown in FBS-supplemented medium are shown. The z-spectrum of cell lysate only is shown for reference. All z-spectra were obtained with 20 μM GFP-RBP(L19A) in pH 7.2 PBS at 300 K. Pulse length, τ_{pulse} = 3.80 ms; field strength, $B_{1,max}$ = 77 μT. Data shown as an average of 3 trials. Inset: the magnitude of the CEST effect from each z-spectrum plotted against the percentage of closed RBP. R^2 = 0.982.

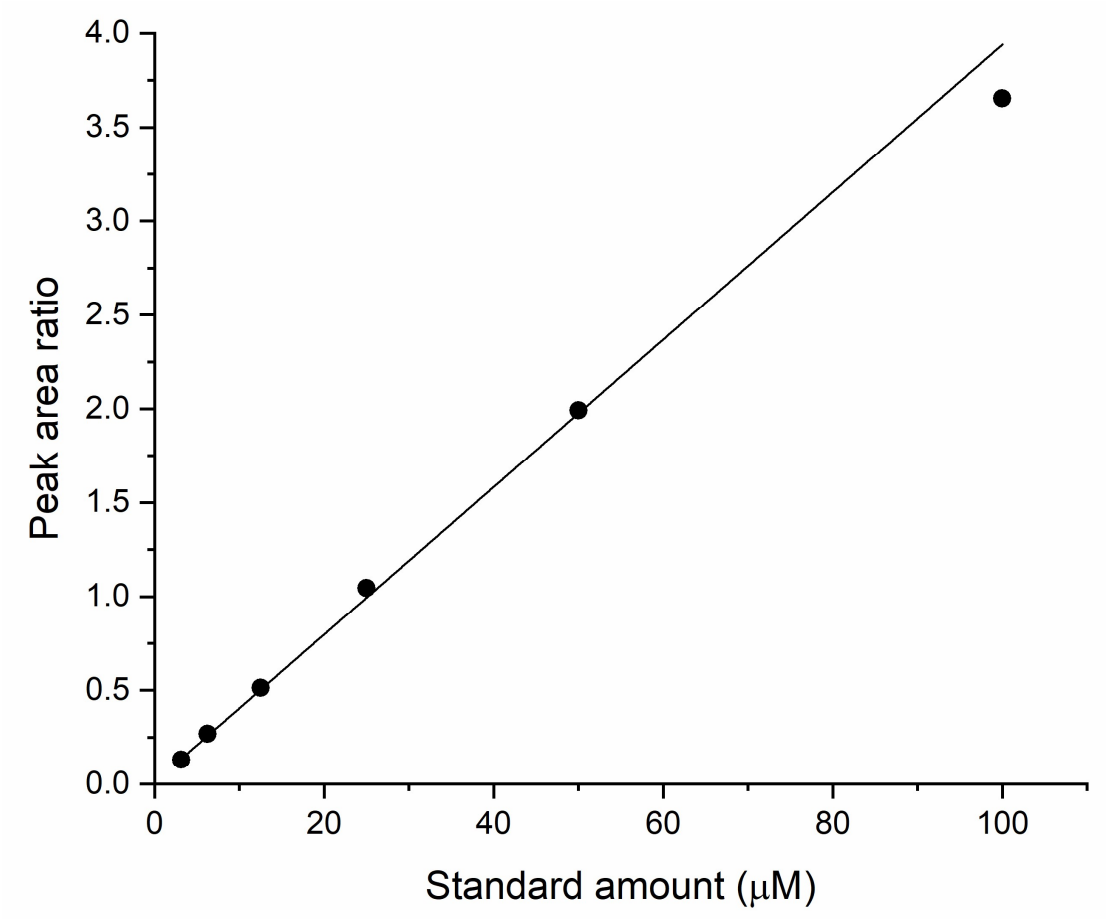

Figure S8. Ribose calibration curve for LC-MS analysis. Ribose was quantified as the ratio of the ribose standard peak area to the [U-$^{13}C_5$] ribose internal standard peak area. The ribose standard concentrations used were 3.13, 6.25, 12.5, 25, 50, and 100 μM, with 20 μM [U-$^{13}C_5$] ribose internal standard. $R^2 = 0.995$, slope = 0.0393 ± 0.001, y-intercept = 0.0121 ± 0.008. The acetate adduct peak was quantified. The peak areas for each sample are shown in Table S3.

Table S3. Peak areas and calculated ribose concentrations from LC-MS analysis.[a]

	3.13 µM ribose	6.25 µM ribose	12.5 µM ribose	25 µM ribose	50 µM ribose	100 µM ribose	Pre-spike 1	Pre-spike 2	Post-spike 1	Post-spike 2	Buffer 1	Buffer 2
Ribose peak area	1.85 x 10^7	4.37 x 10^7	7.73 x 10^7	1.49 x 10^8	2.73 x 10^8	4.73 x 10^8	6.39 x 10^7	6.75 x 10^7	6.23 x 10^7	6.27 x 10^7	N/F	N/F
[U-$^{13}C_5$] ribose peak area	1.41 x 10^8	1.63 x 10^8	1.51 x 10^8	1.42 x 10^8	1.37 x 10^8	1.29 x 10^8	3.96 x 10^7	4.67 x 10^7	9.76 x 10^7	9.02 x 10^7	8.75 x 10^7	7.90 x 10^7
Light/ heavy ratio	0.13	0.27	0.51	1.05	1.99	3.66	1.61	1.45	0.64	0.70	N/A	N/A
Calculated ribose (µM)	3.02	6.54	12.7	26.3	50.4	92.7	**40.7**	**36.5**	**15.9**	**17.4**	N/A	N/A

[a]All samples spiked with [U-$^{13}C_5$] ribose to a final concentration of 20 µM. The "pre-spike", "post-spike", and "buffer" terms refer to the same conditions as described in Figure S6. The acetate adduct peak was quantified.

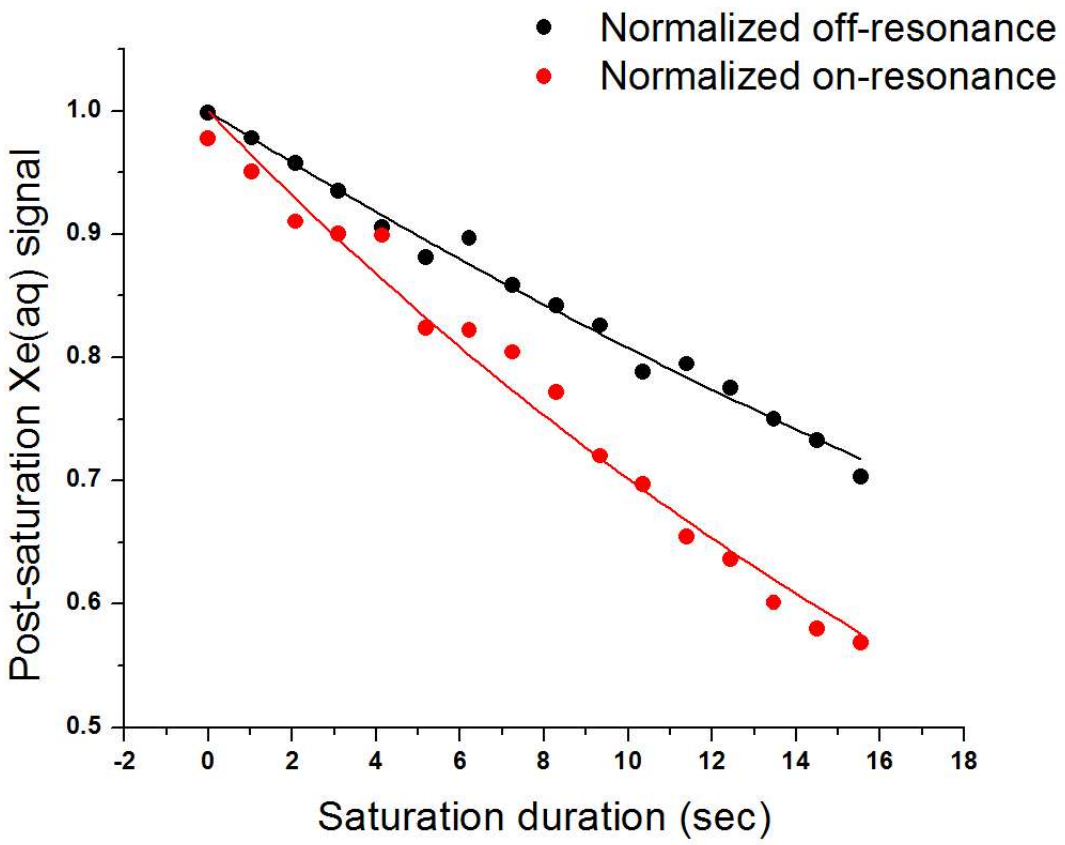

Figure S9. Time dependent saturation curves with GFP-RBP(L19A). Saturation contrast for 100 nM GFP-RBP(L19A) with 1 mM ribose at 300 K in pH 7.2 PBS buffer was determined to be 0.30 ± 0.01. T_{1on} = 28 ± 1 s and T_{1off} = 47 ± 1 s. Pulse length, τ_{pulse} = 1.727 ms; field strength, $B_{1,max}$ = 170 μT. Data shown as an average of three trials.

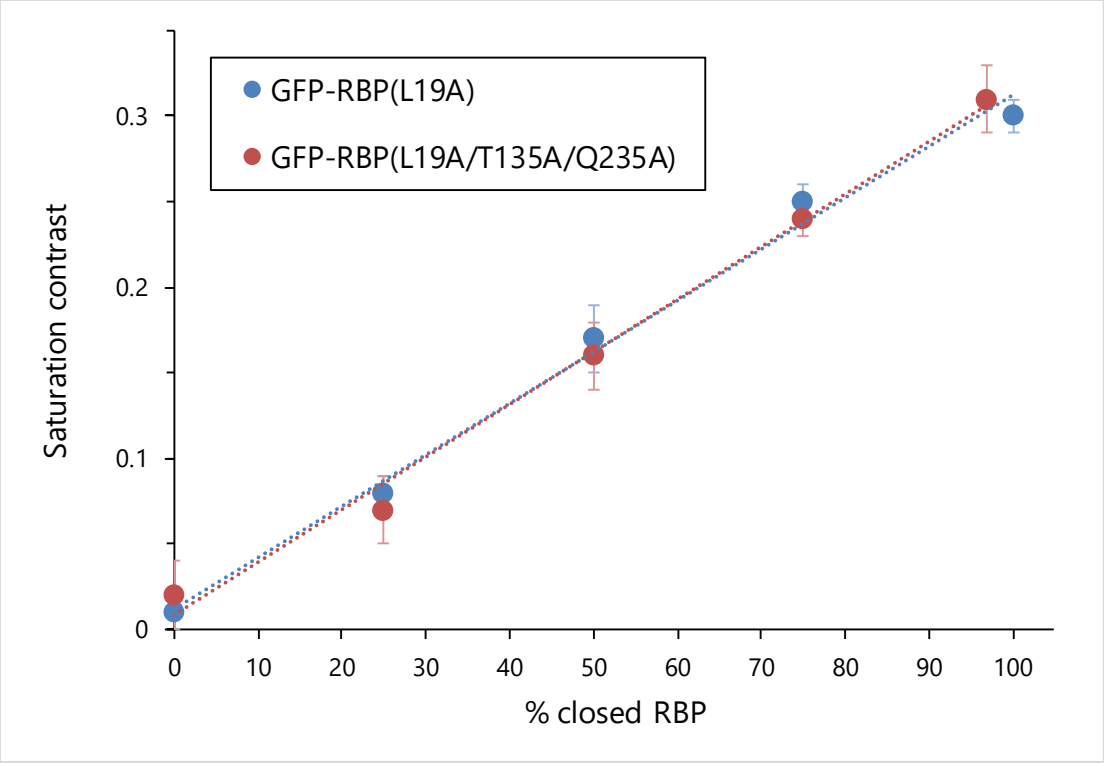

Figure S10. Saturation contrast as a function of ribose-bound RBP. Saturation contrast for 100 nM GFP-RBP(L19A) and 100 nM GFP-RBP(L19A/T135A/Q235A) at 300 K in pH 7.2 PBS buffer as a function of percent protein in the ribose-bound, closed conformation. For GFP-RBP(L19A), (K_d = 0.3 μM) [ribose] = 0, 0.15, 0.35, 0.975, 1000 μM. For GFP-RBP(L19A/T135A/Q235A), (K_d = 130 μM) [ribose] = 0, 43, 130, 390, 4000 μM. Pulse length, τ_{pulse} = 1.727 ms; field strength, $B_{1,max}$ = 170 μT. Data shown as an average of three trials. Linear regressions for both sets of data reported R^2 values > 0.99.

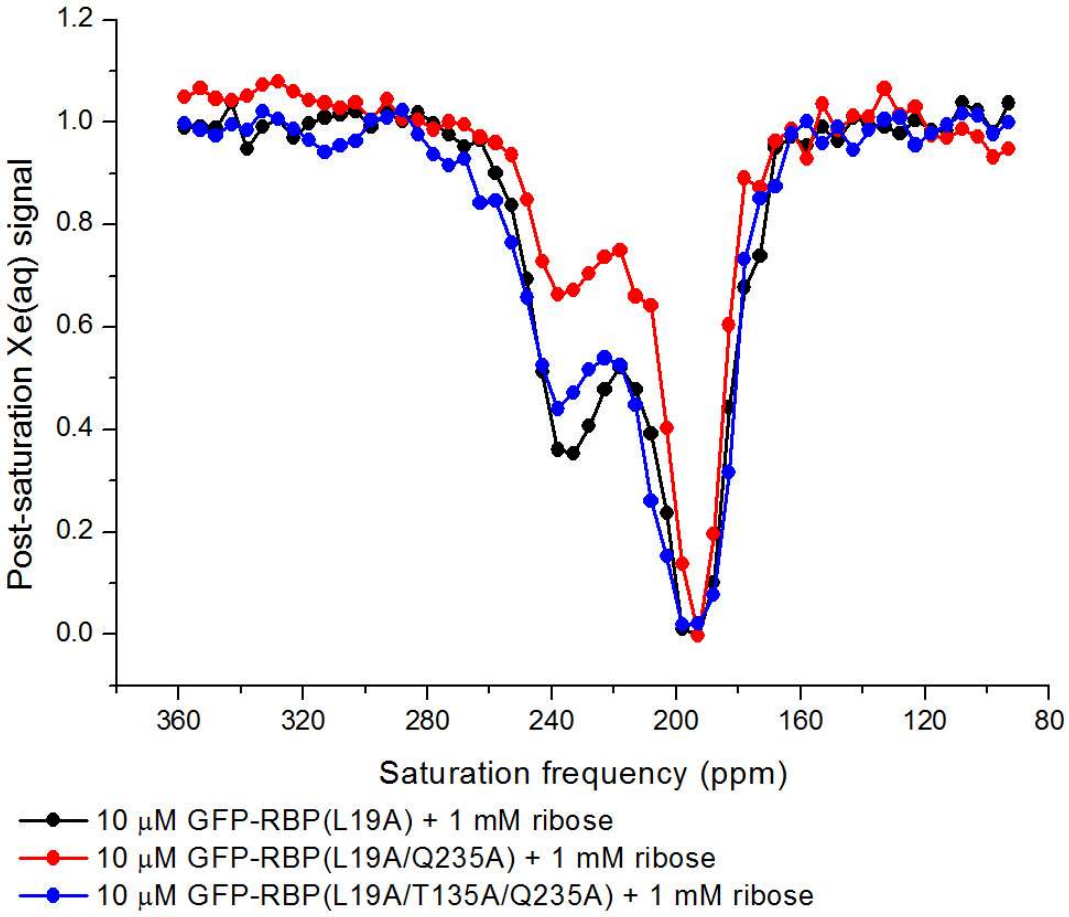

Figure S11. Hyper-CEST z-spectra of RBP mutants. Data acquired in pH 7.2 PBS at 300 K. Pulse length, τ_{pulse} = 3.80 ms; field strength, $B_{1,max}$ = 77 μT. Data shown as an average of 3 trials.

References

(1) Nagana Gowda, G. A.; Djukovic, D.; Bettcher, L. F.; Gu, H.; Raftery, D. *Anal. Chem.* **2018**, *90* (3), 2001–2009.