SUPPLEMENTAL INFORMATION

Multiple DNA-binding modes for the ETS family transcription factor PU.1

August 7, 2017

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Table S1. **Apparent translational self-diffusion coefficients of PU.1/DNA complexes.** Diffusion coefficients ($\times 10^{-11}$ m^2 s^{-1}), as plotted in Figure 3 in the main text, of PU.1ΔN167 alone and in complex with 16-bp and 10-bp high-affinity (5'-GCAAGC**GGAA**GTGAGC-3'), low-affinity (5'-GCAAAA**GGAA**TGGAGC-3'), and nonspecific DNA (5'-GCAAGCGAGAGTGAGC-3'). The 10-bp DNA sites consist only of the underlined core sequences.

DNA to PU.1 molar ratio	*16-bp high-affinity*	*16-bp low-affinity*	*16-bp nonspecific*	*10-bp high-affinity*	*10-bp low-affinity*	*10-bp nonspecific*
0	9.11±0.17	9.00±0.15	9.40±0.13	9.08±0.17	8.90±0.59	9.29±0.24
1/6	8.15±0.25	7.80±0.25	7.73±0.15			8.26±0.33
1/4				8.30±0.24	8.03±0.45	
1/3	7.27±0.15	6.68±0.11	6.50±0.21			7.68±0.08
1/2	6.21±0.18	5.84±0.16	6.60±0.22	7.11±0.13	7.54±0.32	7.08±0.23
2/3	5.90±0.11	5.92±0.11	6.70±0.22			6.80±0.14
3/4				6.72±0.06	6.69±0.19	
5/6	6.53±0.14	6.56±0.13	6.59±0.14			6.49±0.25
1	7.00±0.14	7.38±0.12	6.70±0.10	6.27±0.10	6.68±0.18	6.88±0.18
1 1/6	7.72±0.27		6.46±0.27			
1 1/4				6.64±0.14	6.43±0.19	7.02±0.10
1 1/3	7.75±0.06		6.51±0.17			
1 1/2	7.53±0.23	7.79±0.08		6.30±0.14	6.62±0.16	7.00±0.18
1 3/4				6.81±0.10		
2	7.56±0.10	7.84±0.15			6.59±0.28	

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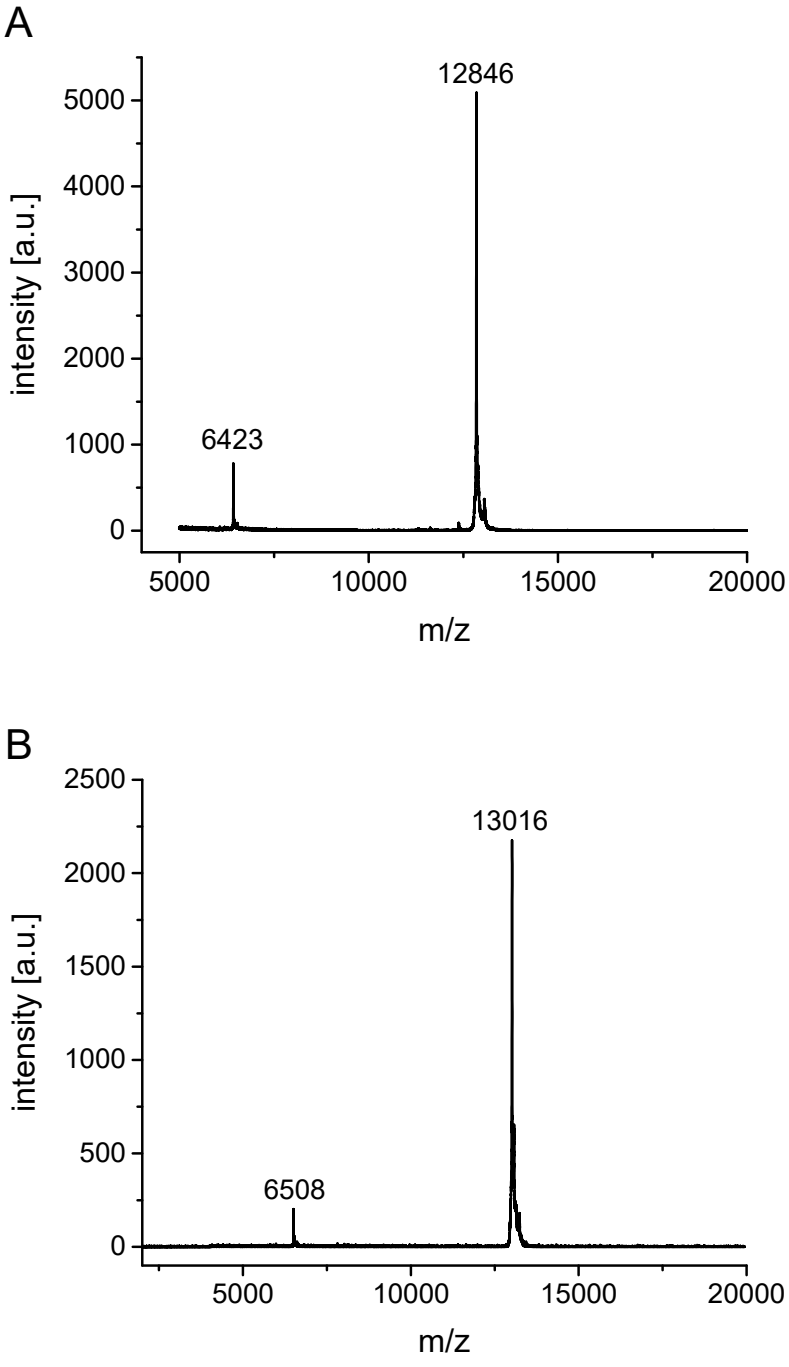

Figure S1. **MALDI-ToF spectra of unlabeled and ^{15}N-labeled PU.1ΔN167.** The expected MW for the unlabeled (*A*) and ^{15}N-labeled (*B*) constructs were 12,847 and 13,018 (assuming 99% enrichment), respectively. Both the +1 and +2 ions were detected.

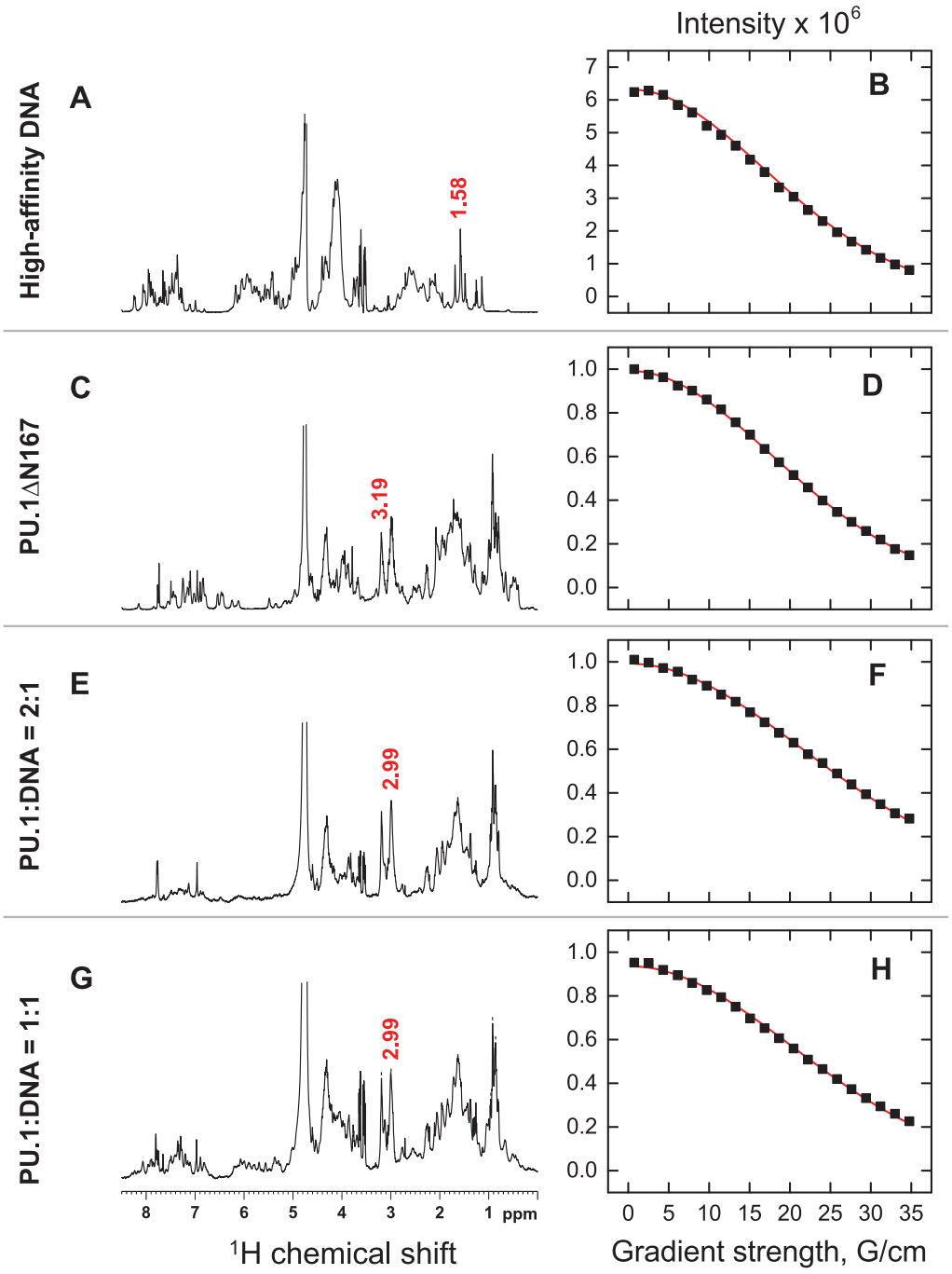

Figure S2. **Diffusion ordered NMR (DOSY) spectra of PU.1 ETS domain, target DNA, and their complexes.** Self-diffusion of unlabeled PU.1ΔN167, 16-bp high-affinity DNA, and mixtures of the two at 1:1 and 2:1 molar ratios was determined in solution using pulsed field gradients. Protein concentrations were 250 μM (*C*), 204 μM (*E*), and 173 μM (*G*), and the DNA concentrations were 562 μM (*A*), 102 μM (*E*), and 173 μM (*G*). At each gradient strength, the labeled peaks were individually fitted to Eq. (S1) to estimate the diffusion coefficient and then averaged. Fitted curves of intensity decay for representative peaks at the indicated chemical shifts are shown in Panels *B*, *D*, *F*, and *H*.

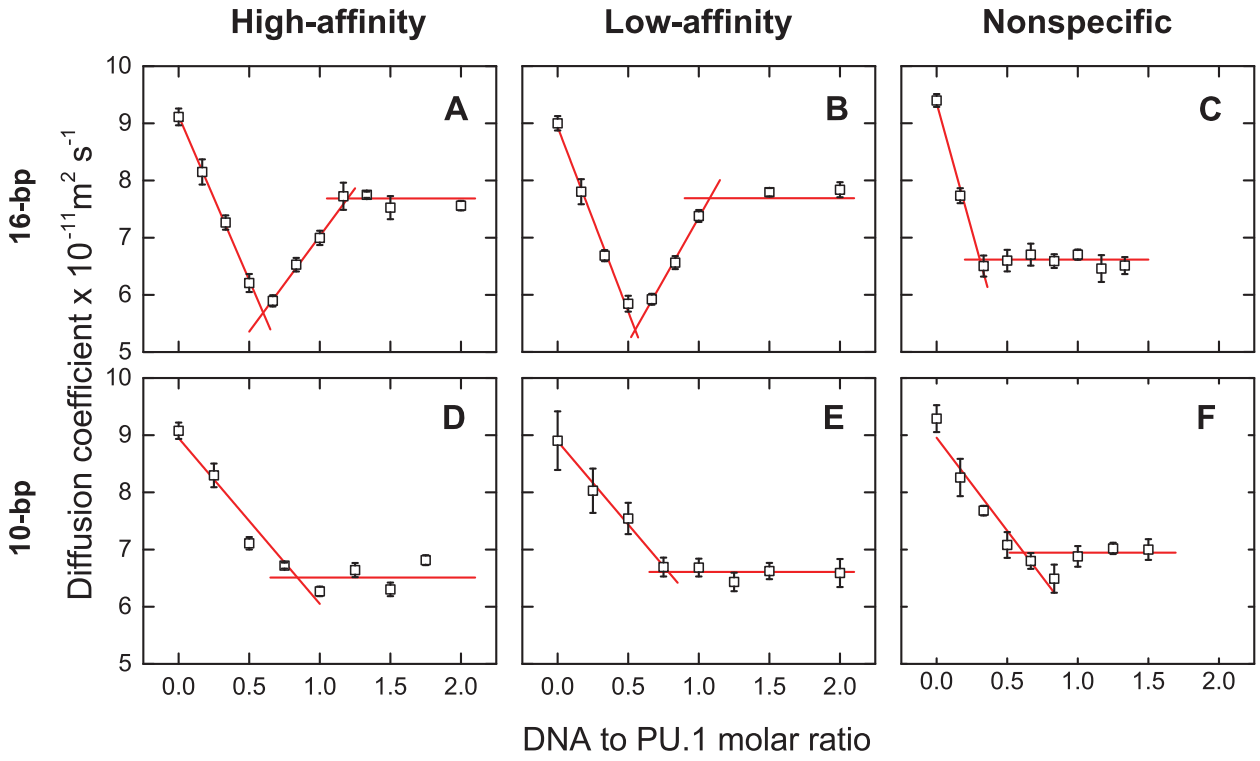

Figure S3. **DOSY titrations reveal site requirements for dimerization of DNA-bound PU.1 in solution.** Translational self-diffusion coefficients of PU.1ΔN167 alone and bound to a 16-bp high-affinity (*A*, 5'-GCAAGC**GGAA**GTGAGC-3'), low-affinity (*B*, 5'-GCAAAA**GGAA**TGGAGC-3'), or nonspecific DNA sequence (*C*, 5'-GCAAGCGAGAGTGAGC-3'). Measurements were repeated using 10-bp duplex sites harboring only the underlined sequences under the same solution conditions (*D* to *F*). *Lines* represent linear fits of the data in the indicated ranges. The diffusion coefficients of the 16- and 10-bp DNA alone were (10 ± 1) and (14 ± 1) × 10^{-11} m^2 s^{-1}, respectively. *Error bars*, S.D.

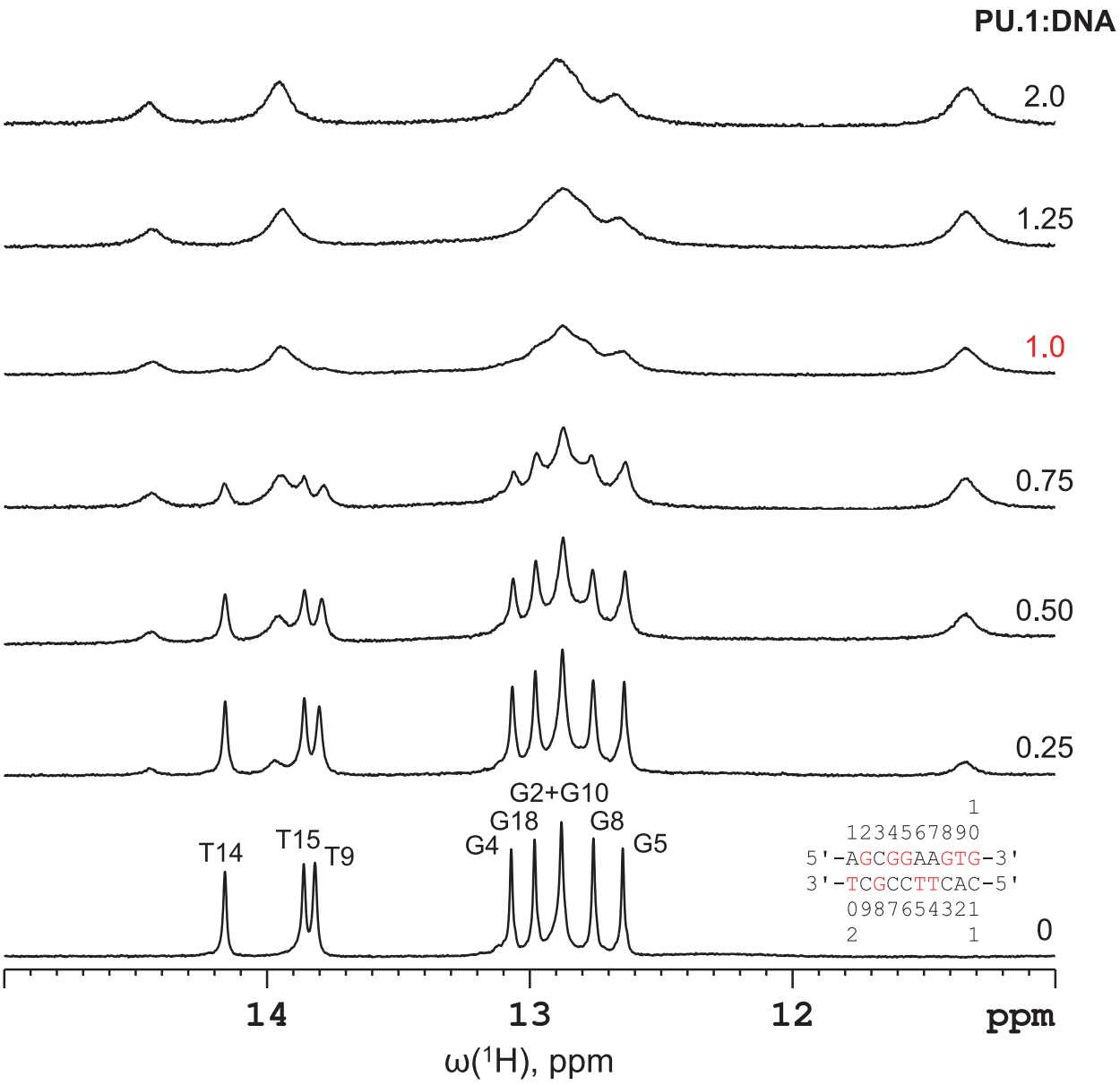

Figure S4. **NMR spectroscopic changes to 10-bp site-specific DNA upon titration by the ETS domain of PU.1.** Chemical shift perturbations of imino ^{1}H resonances were monitored upon titration with 250 μM PU.1ΔN167 at the indicated molar ratios at 20°C using 1-1 Jump and Return pulse sequence (600 MHz) (1). Each spectrum was referenced and normalized in intensity to DSS. Since the DOSY titration showed a single transition, resonances from unbound DNA would be expected to be persist up to the stoichiometric ratio of the complex. For the 10-bp high-affinity DNA, the observable imino ^{1}H peaks in the unbound 10-bp high-affinity DNA were fully exhausted by unit molar equivalence.

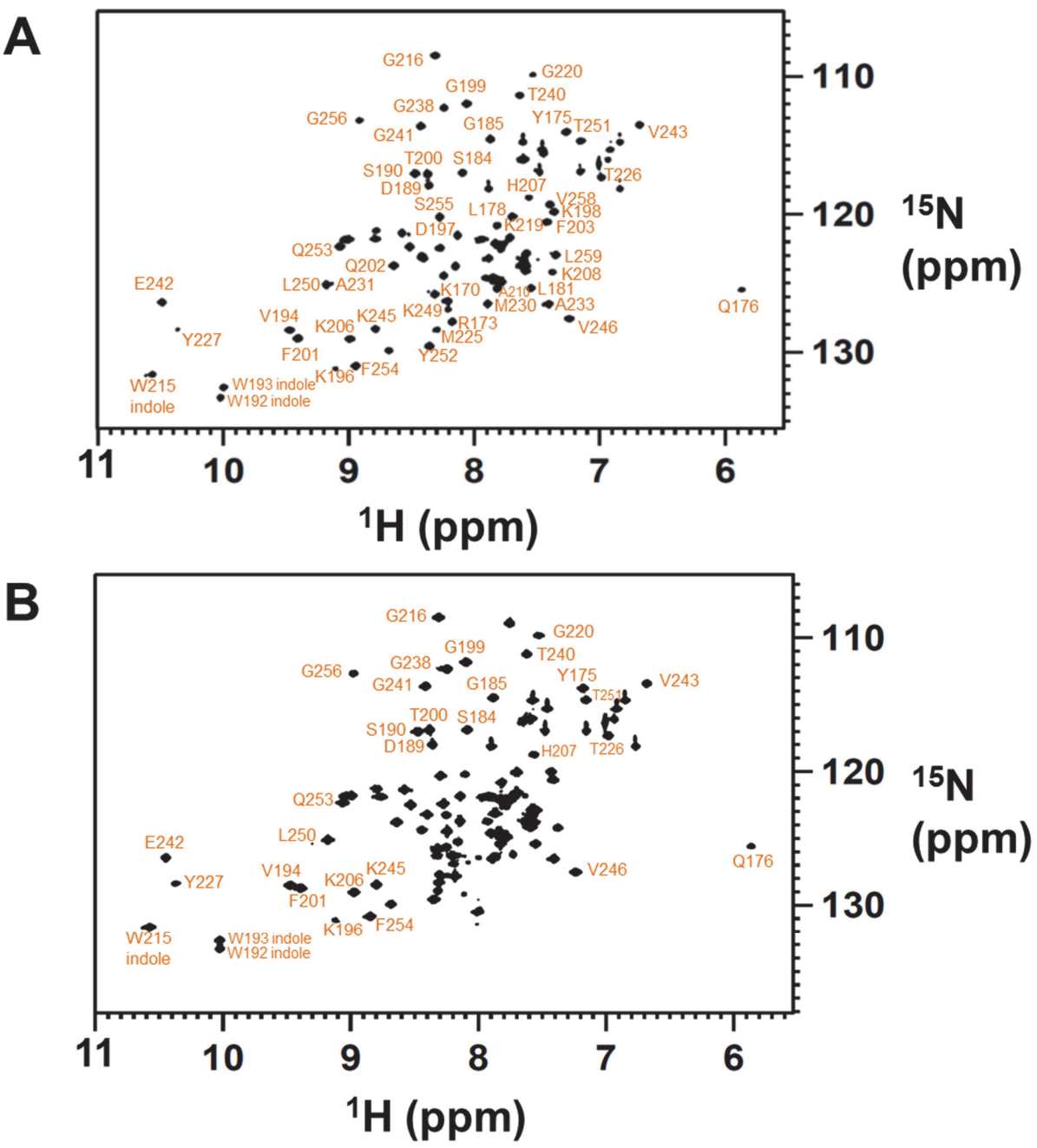

Figure S5. **^{1}H-^{15}N HSQC spectra of unbound murine PU.1(167-260) and PU.1ΔN167.** A truncated PU.1 ETS construct without the final 12 residues in the PU.1ΔN167 used in the experiments described in the main text was cloned and over-expressed as a uniformly ^{15}N-labeled protein in E. coli similarly as PU.1ΔN167, and purified on Sepharose SP. The ^{1}H-^{15}N HSQC of PU.1(167-260) (*A*) closely matched a previously reported spectrum of Jia et al. (2) which in turn allowed assignment of many resonances in PU.1ΔN167 (*B*).

SUPPLEMENTAL METHODS

Pulsed field gradient diffusion-ordered NMR (DOSY). NMR experiments were performed on Bruker Avance 500 and 600 MHz spectrometers, equipped with a 5 mm TBI and QXI probe, respectively (Bruker). Purified PU.1ΔN167 (~250 μM) and DNA (~600 μM high-affinity, low-affinity and non-specific duplexes) were extensively co-dialyzed against 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.4) containing 150 mM NaCl, lyophilized, and re-dissolved to their previous volumes with 99.996% D_2O. The pH* (meter reading) of the reconstituted samples was 7.6. Protein was titrated with DNA to the indicated ratios in the text. In the case of low-affinity and nonspecific DNA, turbidity was observed at ratios below DNA:protein = 1:3 immediately after titration that resolved overnight at room temperature. A 1D pre-saturation (zgpr) spectrum was measured for each titration prior to diffusion measurements. Diffusion experiment parameters (Δ, δ and gradient strength) were first optimized by running 1D diffusion experiments (stebpgp1sd) at 2 and 95% gradient strengths with 100 ms and 5 ms, Δ and δ diffusion times, respectively for ~10% signal retention. Using these parameters, a pseudo-2D DOSY experiment using stimulated echo with bipolar gradient pulses (stebpgp1s) was acquired with 16k × 20 data points with a spoil gradient of 1.1 ms and 4.0 s relaxation delay from 2 to 95% gradient strength with a linear ramp. Data was processed with Bruker Topspin T1/T2 software using manual peak picking. Care was taken to avoid NMR peaks that potentially overlap with free DNA at 1:1 (protein:DNA) and excess DNA titrations. The intensity I of each picked peak was fitted to the following equation as a function of field gradient strength g:

$$I(g) = I_0 e^{-DQg^2} \tag{S1}$$

where I_0 is the reference (unattenuated) intensity, D the diffusion coefficient, and Q is a constant consisting of fixed parameters specific to the experimental configuration.

SUPPLEMENTAL REFERENCES

1. Clore, G., Kimber, BJ, Gronenborn, AM. (1983) The 1-1 Hard Pulse: A Simple and Effective Method of Water Resonance Suppression in FT ^{1}H NMR. *J. Magn. Reson.* **54**, 170-173
2. Jia, X., Lee, L. K., Light, J., Palmer, A. G., 3rd, and Assa-Munt, N. (1999) Backbone dynamics of a short PU.1 ETS domain. *J Mol Biol* **292**, 1083-1093