Supplemental Information:

Figure S1

Isothermal titrating calorimetry.

The DNA binding behavior of NKX3.1 is known to be affected by the N-terminal region (Gelmann et al., ref. 23), and the binding of NKX3.1 (1-184) has previously been measured to be approximately an order of magnitude weaker relative to the homeodomain construct NKX3.1 (114-184) (Zheng et al., ref. 6, and see figure S2 below). To test whether AD and SI interactions play a role in this reduction, isothermal calorimetry was performed, titrating the NK-2 consensus DNA 16-mer duplex into solutions containing either NKX3.1 (1-184) or (75-184) constructs. Previous NMR work has shown specific binding to this DNA sequence (6).

The ITC titrations were performed at 12 °C using a VP-ITC calorimeter (MicroCal, Northampton, MA). Proteins and DNA were dialyzed simultaneously in the same buffer as for CD spectroscopy. The consensus NK-2 16-mer duplex DNA ((+) strand 5'-TGTGTCAAGTGGCTGT-3') (Midland Certified Reagents, Midland, TX) at a concentration of 0.25 mM was loaded into the syringe and injected in 5 ml volumes at 300 s intervals into a 1.4 ml cell containing either NKX3.1 (1-184) or NKX3.1 (75-184) constructs at a concentration of 10mM. Data collection and analysis were performed with Origin software (OriginLab, Northampton, MA) using the model for a single class of binding sites.

Figure S1 shows A) NKX3.1 (1-184) and B) NKX3.1 (75-184) titrated with the consensus NK-2 16-mer duplex DNA at 12 °C, and the integrated data (circles) were fitted to a model for a single class of binding sites (solid line). The fitted thermodynamic

parameters are for A) NKX3.1 (1-184) K_d = 14 ± 5 nM, ΔH = -13.6 ± 0.1 kcal/mol, and for B) NKX3.1 (75-184) K_d = 9 ± 3 nM, ΔH = -13.0 ± 0.1 kcal/mol, with both binding at 1:1 stoichiometry. The K_d results for NKX3.1 (1-184) and (75-184) are the same within error.

Figure S2

EMSA Binding

The figure shows EMSA binding of NKX3.1 (1-184) and (114-184) to the NKX3.1 consensus DNA sequence (reproduced from parts of the multi-panel Figure 6 of ref. 6). The arrows indicate the band for the DNA complex. In Zheng et al. (6) the DNA binding constants of these two constructs were not reported, and so we present the analysis here. Determining approximate binding constants is mathematically straightforward using the expression for K_d

$$K_d = [A][B]/[AB] = (A_0 - fB_0)((1-f)B_0)/(fB_0)$$

Where [A], [B], and [AB] are free protein, free DNA, and protein-DNA complex concentrations, A_0, and B_0 are the initial concentrations of protein and DNA for each lane, and f is the fraction of DNA bound in the complex. Because for each lane B_0 (~0.1 nM) << A_0, $A_0 - fB_0$ is approximately equal to A_0, thus

$$K_d \approx A_0(1-f)/f$$

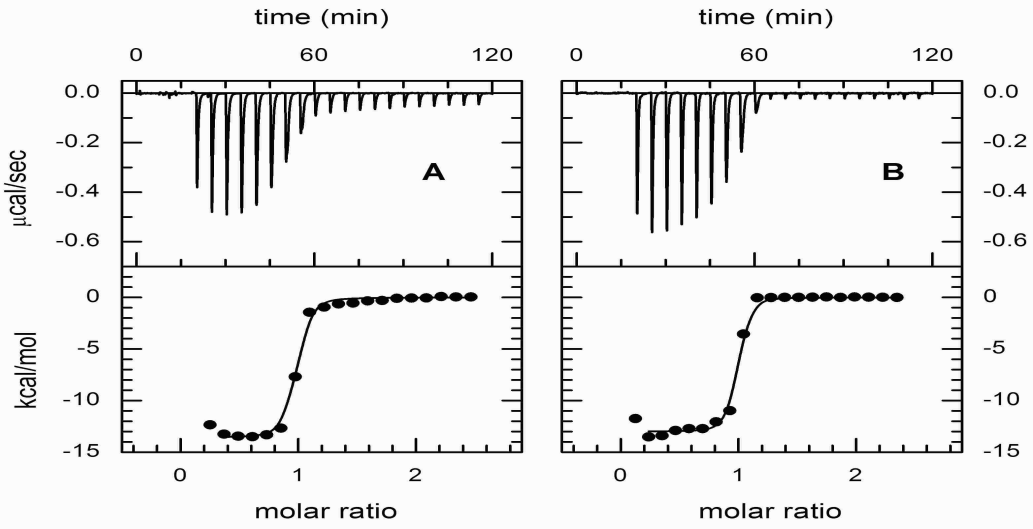
When f = 0.5, $K_d \approx A_0$, and by fitting intensity scans of the NKX3.1 (1-184) EMSA (6), this occurs at a concentration of approximately 30 ± 5 nM.

For NKX3.1 (114-184), no significant intensity reduction is apparent in the band for the DNA complex as the protein concentration is lowered, and no absolute binding constant can be determined from this data. However, obtaining an approximate upper bound for the binding constant is straightforward. If we assume at the lowest concentration (14 nM) at least 80% ($f > 0.8$) of the DNA is still bound, then we get an upper limit for K_d

$$K_d < A_0(1-0.8)/0.8, \quad K_d < 3.5 \text{ nM}$$

Thus, the binding of NKX3.1 (1-184) is at least an order of magnitude weaker than NKX3.1 (114-184). Taken with the ITC results (Figure S1), it appears the region including residues 75-114, which encompass the AD and SI motifs, is responsible for the weaker binding of the NKX3.1 (1-184) construct.

FigureS1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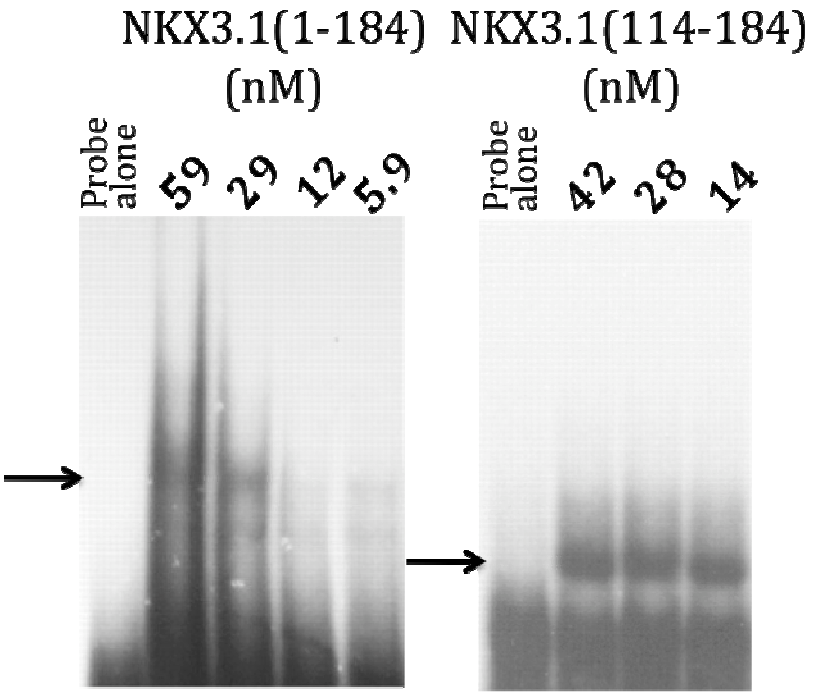
0
60
120
0.0
-0.2
-0.4
-0.6
µcal/sec
A
B
0
-5
-10
-15
kcal/mol
0
1
2
molar ratio

Figure S2


NKX3.1(1-184)
(nM)
Probe alone
59
29
12
5.9
NKX3.1(114-184)
(nM)
Probe alone
42
28
14