## **Supporting Information**

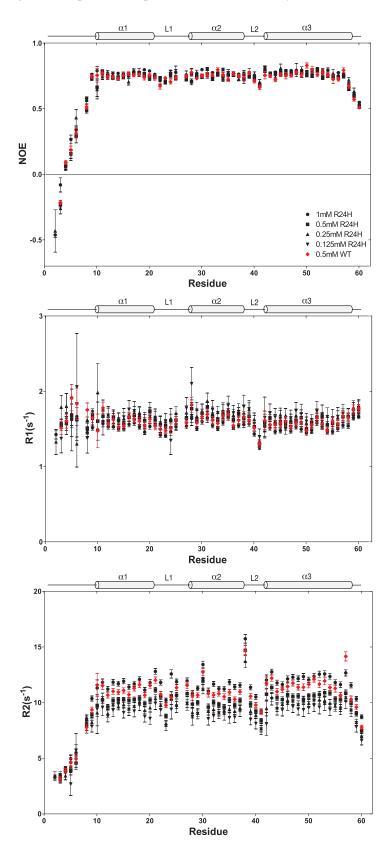
Structural and Biophysical Insights into the Ligand-Free Pitx2 Homeodomain and

a Ring Dermoid of the Cornea Inducing Homeodomain Mutant

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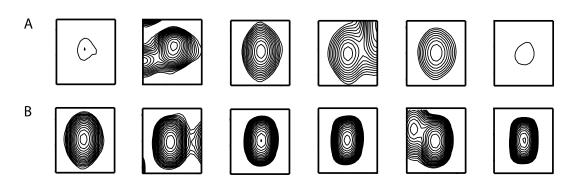
## Supplementary Figure 1

<sup>15</sup>N-relaxation rates for the Pitx2 wild-type homeodomain (red) and R24H mutant (black). The concentration series for the mutant protein establishes the concentration dependence of the  $R_1$  and  $R_2$  measurements, but clearly demonstrates the increased flexibility of the N-terminal arm and the L1 and L2 loop regions independent of protein concentration. Cylinders indicate the positions of the  $\alpha$ -helices.



## **Supplementary Figure 2**

Amide backbone chemical shift resonances in a standard  ${}^{1}\text{H}{}^{15}\text{N}$  HSQC experiment. **A.** N-terminal resonances 1-7 (left to right) with the second box to the left including overlapped residues 2 and 3. **B.** C-terminal tail of the expression system resonances 61-66 (left to right)



**Supplementary Figure 3** 2D <sup>1</sup>H-<sup>15</sup>N HSQC spectra of the wild-type (top), R24H mutant (middle) and V45L (bottom) Pitx2 homeodomains. Data was recorded at 600 MHz and a temperature of 295K.

