Table S3 – Hb binding strengths

<u>rable 55</u> – 110 billuling strengths					
Model	3A3X, 2H	6B2H_lacZ <sup>b</sup>	6B1H_lacZ	$2x(6B1H)$ _lacZ <sup>e</sup>	4B1H_lacZ <sup>f</sup>
Experiment	WT	pThb5	pThb1,6,9	pThb8	pThb2
Position of	47	46	43	49	40
boundary (%EL)					
Sharpness (degrees)	83 (protein) 84 <sup>a</sup> (mRNA)	78	71	75	52
$1^{st}$ Hb bind $(k_3, M^{-1} s^{-1})$	4e6	4e6	4e6 <sup>d</sup>	4e6	4e6
$2^{\text{nd}}$ Hb $(k_6)$	1e8	1e8 <sup>c</sup>			

Unbinding rates,  $k_4$  and  $k_7$ , are set to 1/s.

<sup>&</sup>lt;sup>a</sup> WT protein sharpness is fit to data, mRNA sharpness is a model prediction. Hb diffusivity  $D_{Hb}$ =0.3 $\mu$ m<sup>2</sup>/s (equal to the nuclear-resolution value measured in [41]) gives the best fit to experimental sharpness.

<sup>&</sup>lt;sup>b</sup>Expression levels for simulations with Hb sites were higher, but never more than twice, that of 6B0H (Table S1).

<sup>&</sup>lt;sup>c</sup> The transcription rate ( $k_5$ ) was dropped from 3.1 (WT) to 0.4, to match the loss of sharpness observed in this construct. Sharpnesses for the other constructs are model predictions.

<sup>&</sup>lt;sup>d</sup> This value set by matching the posterior shift compared to  $hb^{14F}$ .

<sup>&</sup>lt;sup>e</sup> 6<sup>th</sup> Bcd binding ( $k_{26}$ ) set to 1e9, to match experimental posterior shift with construct doubling. <sup>f</sup> This construct has a truncated hb promoter: 1<sup>st</sup> Bcd (A<sub>1</sub>) binds at 2.8e7 ( $k_{11}$ ); 2<sup>nd</sup> Bcd (A<sub>2</sub>), 3.6e7 ( $k_{14}$ ); 3<sup>rd</sup> Bcd (X<sub>1</sub>), 2.5e7 ( $k_{17}$ ; slightly stronger than in 4X, to match position); 4<sup>th</sup> Bcd (X<sub>2</sub>), 1.4e8 ( $k_{20}$ ; as in 4X and WT).