Supplementary Materials

Kinetic Modeling

The simulation of the FRAP experiment in silico requires the duplication of the species shown in Fig. 5 so that molecules in the bleached and non-bleached regions of the nucleus can be treated separately so that the bleach pulse can be implemented (Fig S1). However, note that all rate constants for the corresponding binding reactions in the bleached and non-bleached compartments were identical, since the process of photobleaching does not significantly perturb the association and disassociation of molecules.

The model contains the following species/compartments; Unbound Swi6 (SWI6), Swi6 bound to non-methylated site (SWI6_H3), Swi6 bound to methylated H3 (fast phase) (SWI6_H3met_fast), Swi6 bound to methylated H3 (slow phase) (SWI6_H3met_slow), bleached unbound Swi6 (BSWI6), bleached Swi6 bound to non-methylated site (SWI6_H3), bleached Swi6 bound to methylated H3 (fast phase) (BSWI6_H3_fast) and bleached Swi6 bound to methylated H3 (slow phase) (BSWI6_H3_slow). P represents the relative proportion of methylated sites. The association and dissociation rate constants are given by k_{on} and k_{off}, respectively; k_{diff} is a pseudo diffusion constant that represents the movement of molecules from the non-bleached area to the bleached area. A lower limit of 60 s^{-1} was estimated for k_{diff} based upon the observation that recovery of free GFP was complete before the first image could be collected (within 62 ms). The molecules are portioned into the bleached and unbleached compartments by multiplying k_{on1} and k_{on2} by the fraction of the nucleus bleached (FB) and (1-FB), respectively, determined from experimental data and simulations, in all fits FB was constant at 0.25. In order to implement a bleach pulse a bleach constant was added to the compartments in the bleached region of interest, this is a time dependent rate constant that is 0 before and after the bleach pulse and high (>80 s^{-1}) during the bleach pulse. During the bleach pulse molecules are irreversibly removed from the

bleached compartment and therefore, no longer play a role in the reaction, as is the case for bleached Swi6-GFP in the actual experiment. The recovery to steady state post bleach is the summation of all species in the bleached region.

The differential equations describing the model are:

d/dt (SWI6) = - ((1-FB)*(1-P)*kon1*SWI6) + (koff1*SWI6_H3))- ((1-FB)*P*kon2*SWI6) + (koff2*SWI6_H3met_fast)) – ((FB*P*kon2*SWI6) + (koff2*BSWI6_H3met_fast))- ((FB*kdiff*SWI6) + (kdiff*BSWI6)) – ((FB*(1-P)*kon1*SWI6) + (koff1*BSWI6_H3))

d/dt (SWI6_H3) = ((1-FB)*(1-P)*kon1*SWI6) - (koff1*SWI6_H3))

d/dt (SWI6_H3met_fast) = ((1-FB)*P*kon2*SWI6) - (koff2*SWI6_H3met_fast)) – ((kon3*SWI6_H3met_fast) + (koff3*SWI6_H3met_slow))

d/dt (SWI6_H3met_slow) = (kon3*SWI6_H3met_fast) - (koff3*SWI6_H3met_slow)

d/dt (BSWI6) = (FB*kdiff*SWI6) - (kdiff*BSWI6) - (kbleach*BSWI6)

d/dt (BSWI6_H3) = (FB*(1-P)*kon1*SWI6) - (koff1*BSWI6_H3) - (kbleach*BSWI6_H3)

d/dt (BSWI6_H3met_fast) = (FB*P*kon2*SWI6) - (koff2*BSWI6_H3met_fast) - (kon3*BSWI6_H3met_fast) + (koff3*BSWI6_H3met_slow) - (kbleach*BSWI6_H3met_fast)

d/dt (BSWI6_H3met_slow) = (kon3*BSWI6_H3met_fast) - (koff3*BSWI6_H3met_slow) - (kbleach*BSWI6_H3met_slow)

Sensitivity Analysis

This single model is able to quantitatively describe several experimental data-sets, the range of values around the absolute values of the rate constants determined in fitting the data were determined by sensitivity analysis. Each individual rate constant was incrementally increased and decreased and the residuals from the original fit determined. A goodness of fit criteria of a sum of residuals < 10 over 400 points was chosen, the range of values for each parameter that provide a satisfactory fit are listed in Table S1 and shown in Fig. S3. The sensitivity analysis shows that that the fits to the experimental data are insensitive to the parameters k_{dif}, k_{on1}, k_{on2}, whilst it is sensitive to k_{off1}, k_{off2}, kon3 and k_{off3}.

An alternative model that contains the same number of states as the model in the main text is shown in Fig. S4. Fits to the model are shown in Fig S4. Note that especially for Swi6-GFP recovery at t>5secs fit to the model deviates from the experimental data.

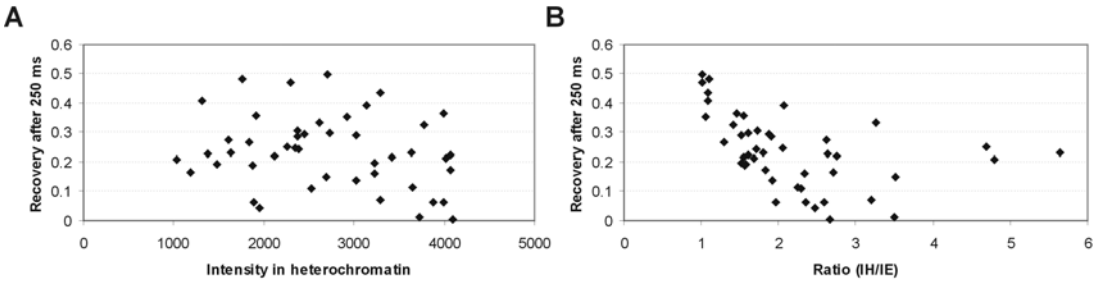

A
Recovery after 250 ms
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1000
2000
3000
4000
5000
Intensity in heterochromatin
B
Recovery after 250 ms
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
Ratio (IH/IE)

Fig. S1

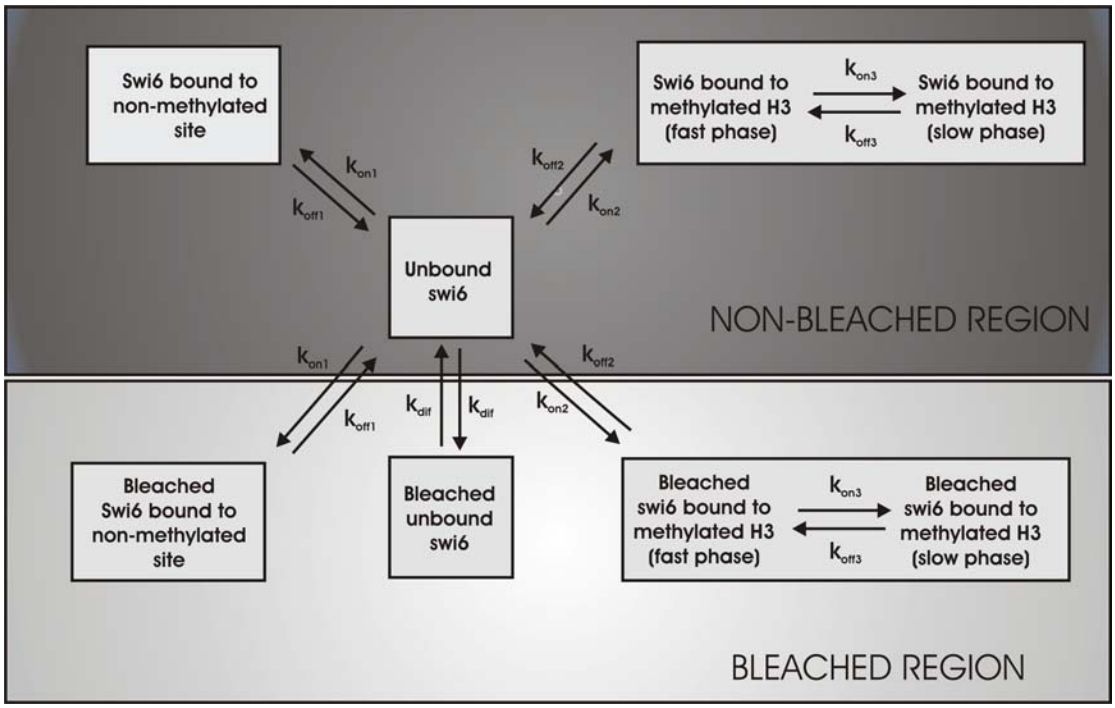

Swi6 bound to non-methylated site
Swi6 bound to methylated H3 (fast phase)
Swi6 bound to methylated H3 (slow phase)
Unbound swi6
NON-BLEACHED REGION
Bleached Swi6 bound to non-methylated site
Bleached unbound swi6
Bleached swi6 bound to methylated H3 (fast phase)
Bleached swi6 bound to methylated H3 (slow phase)
BLEACHED REGION

Fig. S2

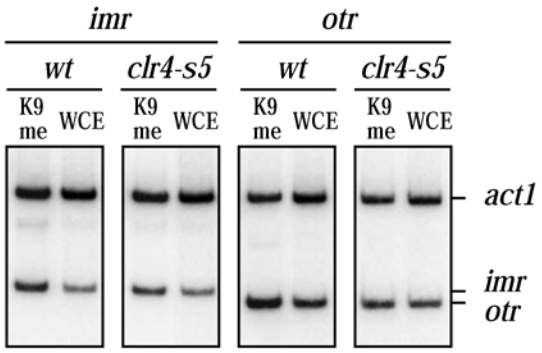

imr
otr
wt
clr4-s5
wt
clr4-s5
K9 me
WCE
K9 me
WCE
K9 me
WCE
K9 me
WCE
act1
imr
otr

Fig. S3

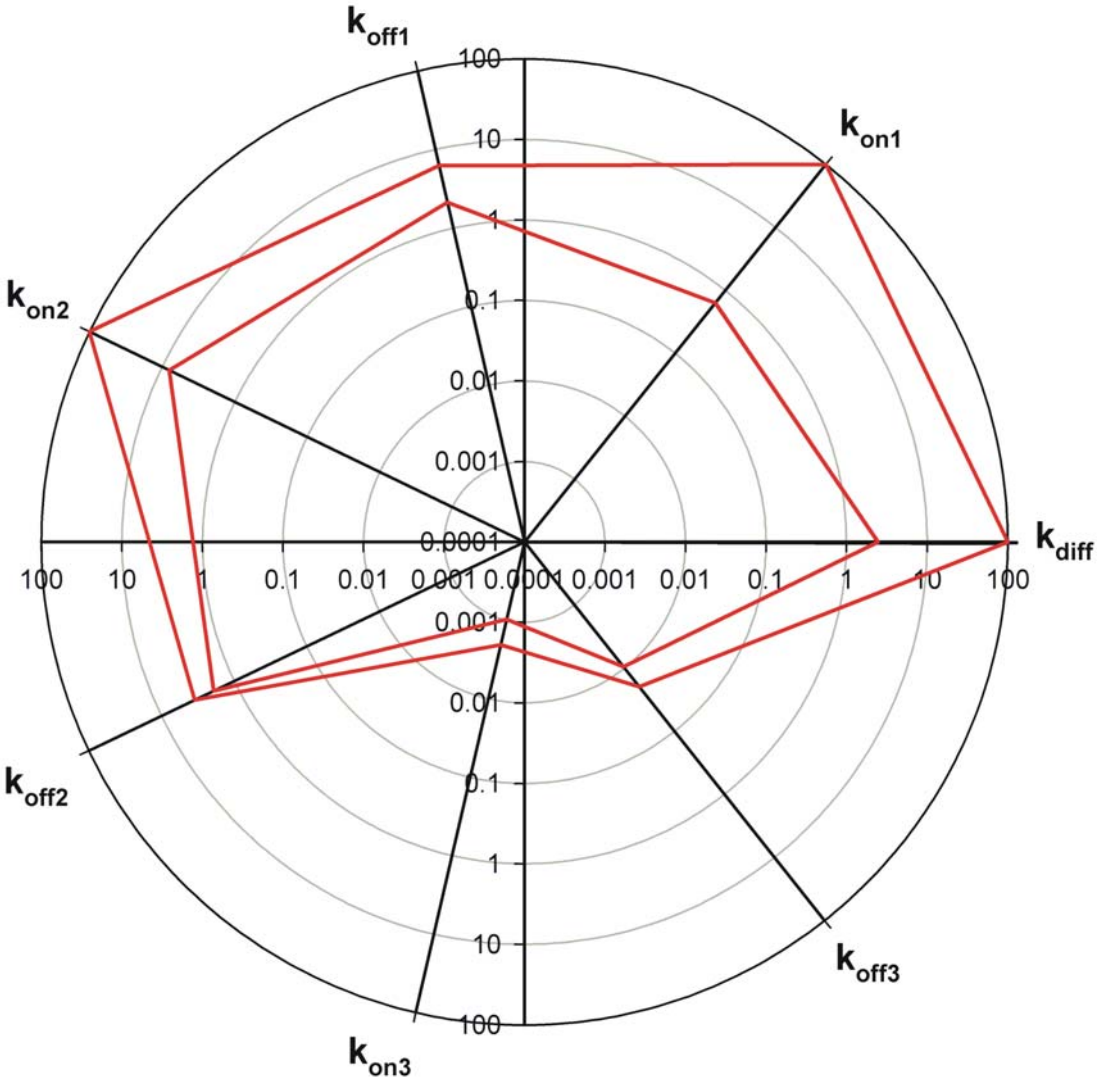
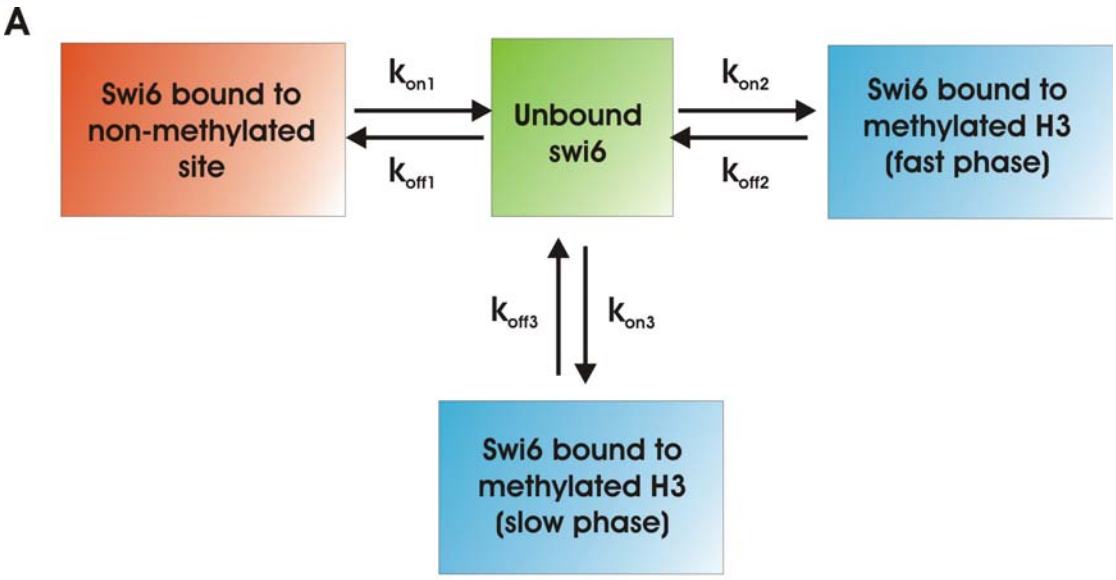

k_off1
k_on1
k_on2
k_diff
k_off2
k_off3
k_on3
100
10
1
0.1
0.01
0.001
0.0001

Fig. S4


A
Swi6 bound to non-methylated site
k on1
k off1
Unbound swi6
k on2
k off2
Swi6 bound to methylated H3 (fast phase)
k off3
k on3
Swi6 bound to methylated H3 (slow phase)

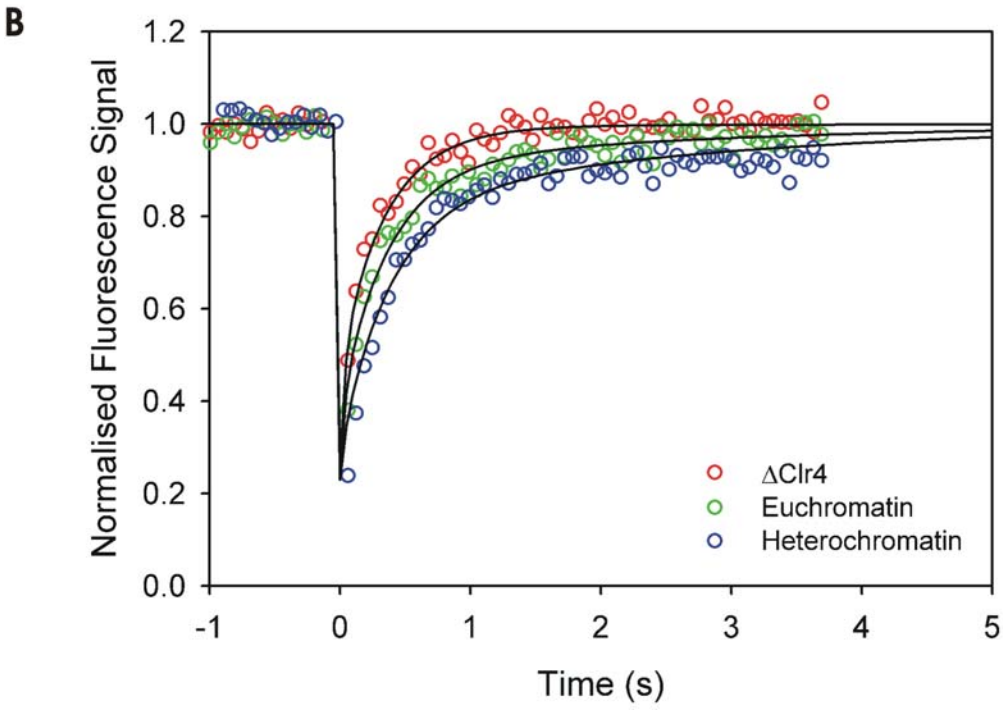

B
Normalised Fluorescence Signal
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-1
0
1
2
3
4
5
Time (s)
ΔClr4
Euchromatin
Heterochromatin

Fig. S5

Figure S1

(A) Scatter plot showing no correlation between the intensity of GFP-Swi6 in heterochromatin and the mobility of GFP-Swi6. (B) Scatter plot showing a correlation between the ratio of the intensity of GFP-Swi6 in heterochromatin vs. euchromatin and the mobility of GFP-Swi6.

Figure S2

Full kinetic model used to fit the experimental FRAP data. Duplication of species partitions molecules into the region of the nucleus bleached and the remaining area of the nucleus. The association and dissociation rate constants are given by k_{on} and k_{off}, respectively, k_{diff} is a pseudo diffusion constant that represents the movement of molecules from the non-bleached area to the bleached area.

Figure S3

ChIP analysis as described in Noma et al. were performed to study the relative enrichment of histone H3 methylated on K9 at the *imr* and *otr* loci in both wt cells and the *clr4-S5* strain. For both loci, we observed a ~3-fold reduction of H3 methylated on K9 in *clr4-S5* compared to wt strain.

Figure S4

Sensitivity analysis of parameters determined from fitting the FRAP data to the model. Range of values, which produce satisfactory fits with a sum of residuals < 10 lie within the boundaries of the two lines. All radial axis show log rate constants (s^{-1}).

Figure S5

Fits to data for an alternative model describing Swi6 binding to chromatin**.** (A) Alternative model used to describe the kinetics of Swi6 binding, the model has the same number of species/compartments as the model described in Fig. 5 and Fig. S1. k_{on} and k_{off} depicts association and dissociation rate con-

stants. (B) Best fits to the data using the model described in (A) for wt Swi6 recovery in *clr4-S5* cells, wt cells in euchromatin and heterochromatin. Note significant deviation from the experimental data for heterochromatin in wt cells t>3.5secs.

Table S1 Sensitivity analysis of kinetic parameters for Swi6 binding model.

parameter	Lower Limit	Upper Limit
k_{on1} (s^{-1})	0.63	100
k_{off1} (s^{-1})	2.11	6.2
k_{on2} (s^{-1})	7.94	100
k_{off2} (s^{-1})	1.91	3.47
k_{on3} (s^{-1})	0.00097	0.00205
k_{off3} (s^{-1})	0.0095	0.0198
k_{diff} (s^{-1})	2.45	100
P (*clr4-S5*, eu)	0.01	0.05
P (wt, eu)	0.06	0.27
P (wt, het)	0.36	0.99