SUPPLEMENTARY INFORMATION

ATP Binding Facilitates Target Search of SWR1 Chromatin Remodeler by Promoting One-Dimensional Diffusion on DNA

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Supplementary Figures 1-9

Supplementary Tables 1-3

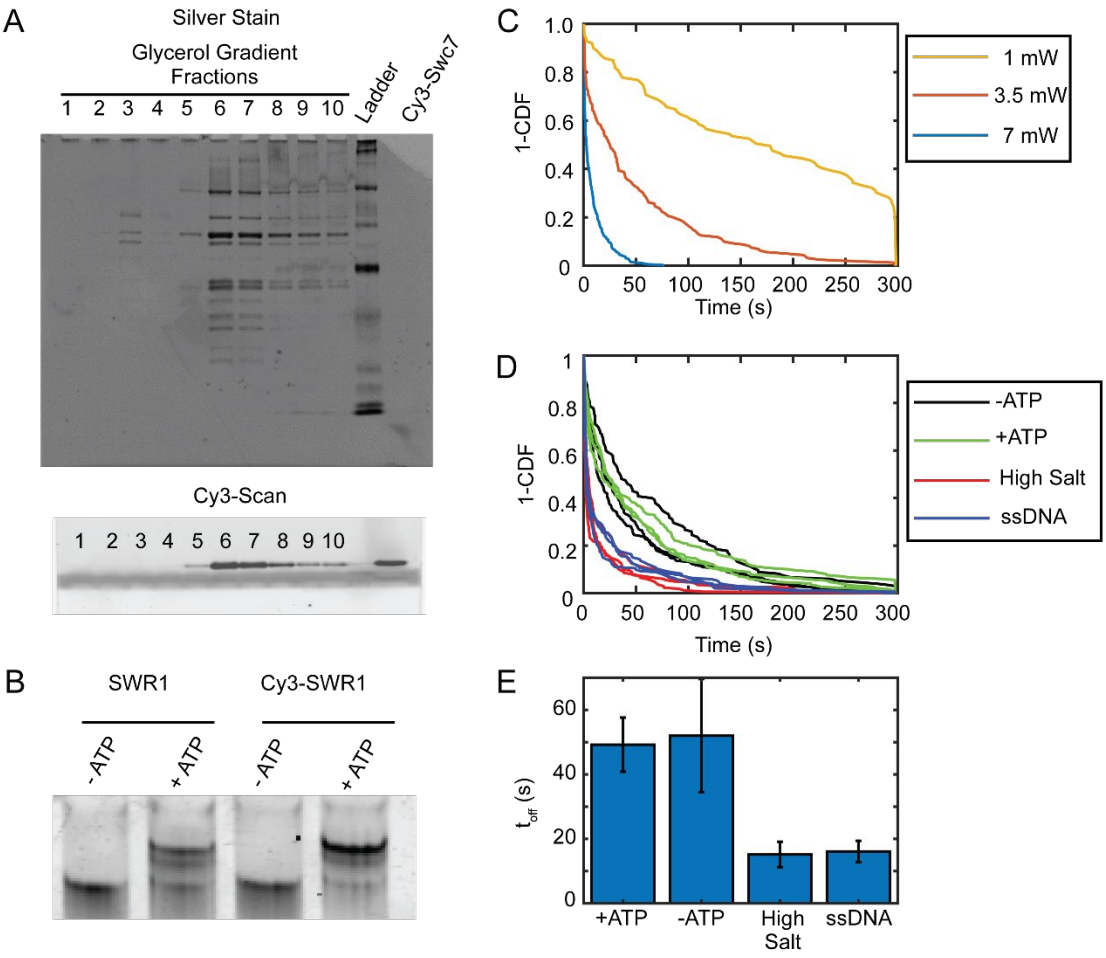

Figure S1. Cy3-SWR1 purification and DNA binding kinetics.
(A) A glycerol gradient purification of the Cy3-SWR1 complex after Cy3-Swc7 reincorporation. A silver stain (top image) shows that the SWR1 complex eluted in fractions 6 and 7 of the gradient. A Cy3 image of the same gel shows that Cy3-Swc7 also is found in fractions 6 and 7 (confirmed by a Cy3-Swc7 only control at the end of the gel). This demonstrates that the Cy3-Swc7 is incorporated into the SWR1ΔSwc7 complex. (B) A histone exchange assay that shows Cy3-SWR1 (right lanes) is as active as the wild type SWR1 complex. The gel-shift is caused by the incorporation of triple flag-tagged ZB dimers into the nucleosome. (C) t_{off} for Cy3-SWR1 bound to 150 bp DNA measured at different laser powers. These measurements show that the lifetime of Cy3-SWR1 bound to 150 bp DNA is photobleaching limited. (D) 1-CDF for Cy3-SWR1 bound to 150 bp DNA and exposed to ATP, 200 mM NaCl and 0.2 ug/mL of salmon sperm DNA (ssDNA). Three technical replicates are shown. (E) t_{off} for Cy3-SWR1 bound to 150 bp DNA in different conditions, same as those shown in panel (D).

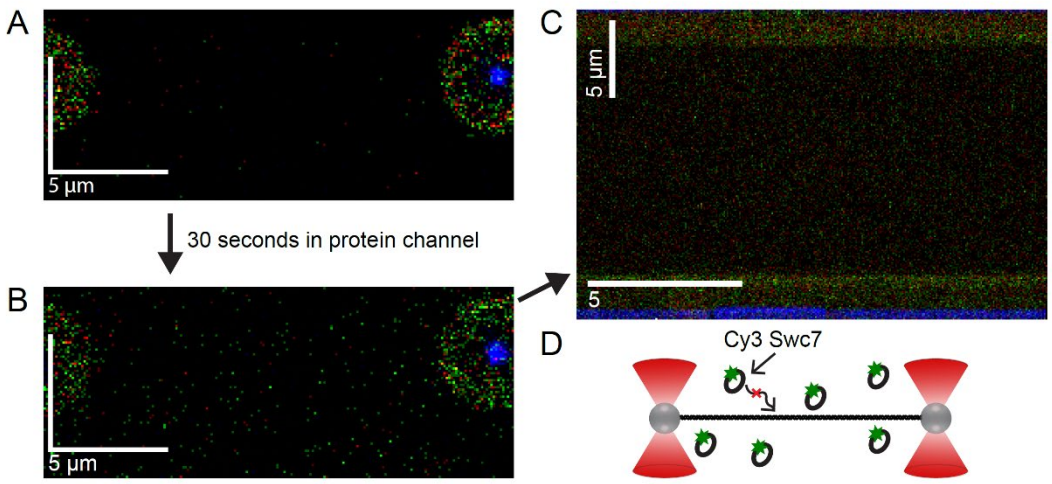

Figure S2. Swc7 cy3 does not bind DNA without the SWR1 complex.
(A) A scan of lambda DNA pulled to 5pN of tension in the absence of Cy3 labeled Swc7 imaged with green excitation. (B) A scan of lambda DNA in Cy3 Swc7 protein channel. Protein solution was flowed briefly to refresh the protein solution in the channel before and while DNA is introduced into the channel. The amount of Cy3 labeled Swc7 is equimolar to what is used for SWR1 sliding experiments. Apart from increased background, there is no specific interaction of Cy3 Swc7 with the DNA alone. (C) A kymograph along the length of the lambda DNA at a time resolution of 0.05 sec per line scan also shows no bound Cy3 Swc7. (D) Schematic summary: Cy3 Swc7 doesn't bind lambda DNA alone, even at the faster time resolution used to generate a kymograph.

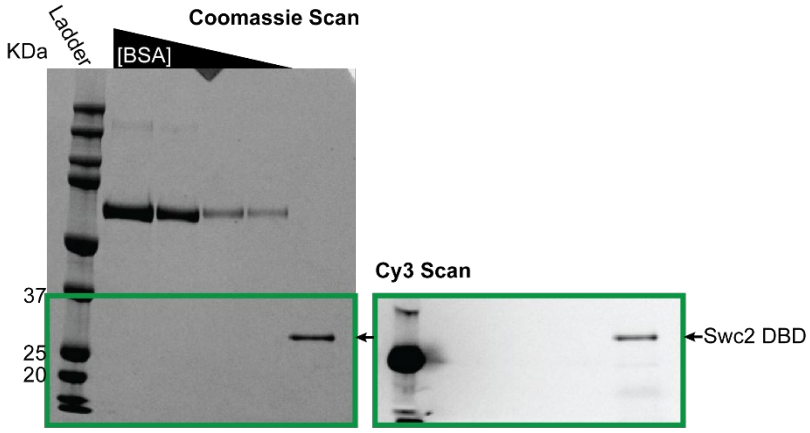

Figure S3. Purification and fluorophore labeling of the Swc2 DBD. Denaturing SDS PAGE gel of the Swc2 DNA binding domain (DBD) after nickel his-tag purification and labeling with Cy3-maleimide. A Cy3 scan of the gel (right) reveals that the Swc2 DBD (single band in Coomassie stain on the left) has been labeled with Cy3.

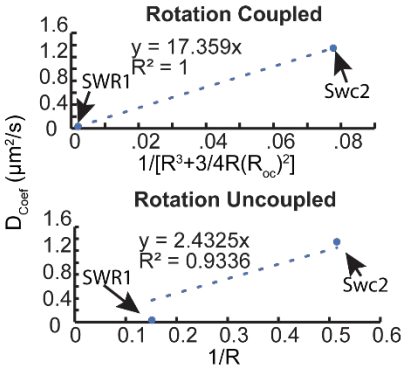

Figure S4. Rotation coupled vs uncoupled diffusion models and protein size effects on diffusion coefficient. Apparent diffusion coefficient as a function of two relationships to the radius of SWR1 or Swc2 DBD where the protein size is printed below the graph and the corresponding model is stated above. A line passing through the origin is fit to both relationships, and the Pearson correlation coefficient is printed within the graph showing a slightly better fit of the measured diffusion coefficients to the rotation coupled diffusion model.

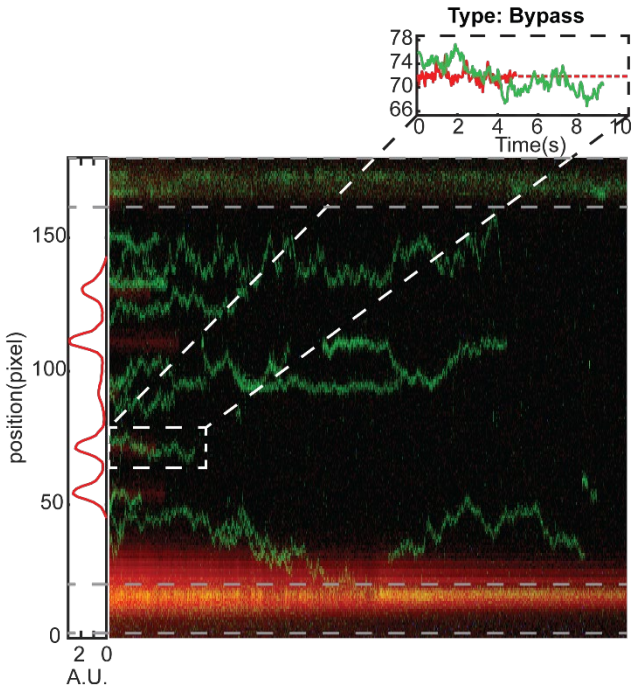

Figure S5. SWR1 bypassing dCas9 was a rare event. Of 107 total colocalization events, only 3 displayed some form of bypass event. Left of the representative kymograph is a sum of the red intensity, which is used to calculate the centroid of dCas9 for colocalization analysis.

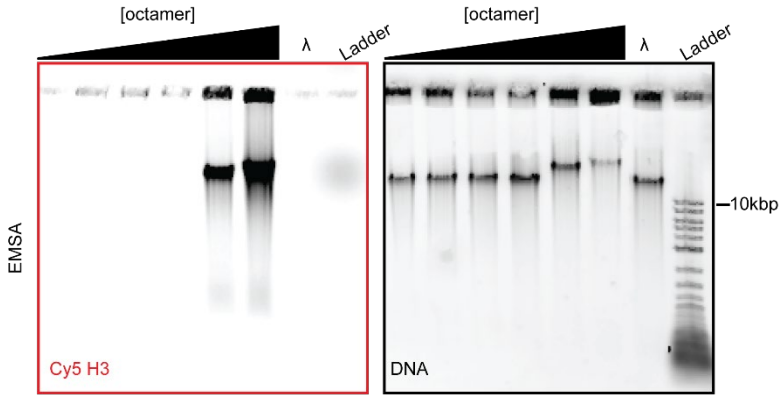

Figure S6. Lambda nucleosome array EMSA. Cy5 labeled H3 octamer (~20% labeling efficiency) was used in the reconstitution of nucleosomes onto lambda DNA via salt gradient dialysis. Typhoon imager scans (left Cy5 scan, right SYBR Gold scan). Octamer concentrations used are as follows reported as molar ratio of octamer to DNA, from left to right: 10:1, 50:1, 100:1, 200:1, 500:1, 700:1. Lambda DNA alone is shown for reference. The 700:1 condition was selected for use in experiments.

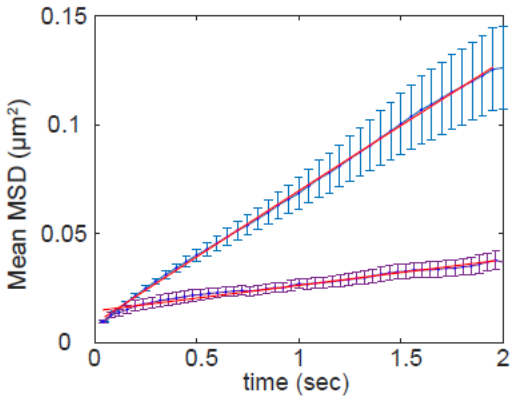

Figure S7. Fit to determine the length of confinement of SWR1 on nucleosome arrays. The length to which SWR1 is confined on the nucleosome array was determined using a fit to an exponential function. The fit lines are plotted as red lines. A fit to $y = m*(1-\exp(-b*x))+c$ produced a limit when x approaches infinity of $y = 0.054\ \mu m^2$ for SWR1 diffusing on a lambda nucleosome array, whereas the fit for SWR1 diffusing on lambda DNA produced a limit of $y = 2.92\ \mu m^2$. SWR1 on lambda DNA is plotted in blue, whereas SWR1 on lambda nucleosome array is plotted in purple. Fits are overlayed as red lines. Error bars on the Mean MSD represent SEM for averaged single particle MSDs.

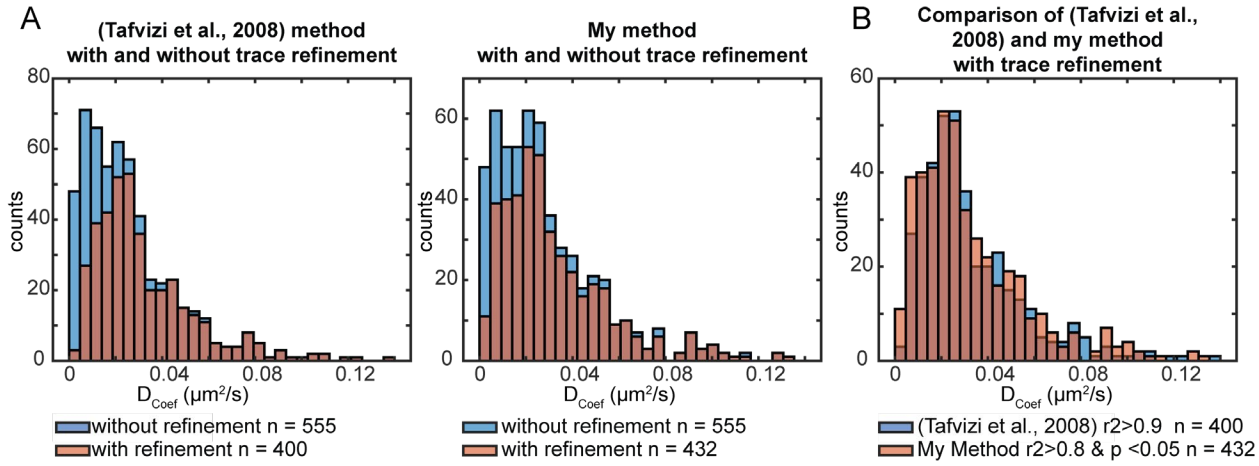

Figure S8. Validation of our method for calculating diffusion coefficients. Data taken from SWR1 diffusion in 70 mM KCl and 1 mM ATP. Linear fits to the MSD vs time plot are used to calculate diffusion coefficients for individual particles. The goodness of fit is assessed using Pearson's correlations coefficient (r^2) as well as the p-value of the linear fit, and in both methods traces are rejected based on the quality of the linear fit. (A) (Tafvizi et al., 2008) performs linear fitting on the first 10 points and refines the traces included by selecting for traces with $r^2 < 0.9$. The distribution of diffusion coefficients calculated using this method is shown in blue, and the refined distribution is shown in orange. This process selects against immobile particles and particles with poor signal to noise ratio. Our method fits a line to as many time-lags as possible such that the p-value is <0.1 and the r^2 is at a local maximum. This was done to consider the variation in the length of trajectories, and to optimize for the linear portion of the MSD curve. The distribution of diffusion coefficients before refinement is shown in blue. For refinement, traces with $r^2 < 0.8$ and p-values > 0.1 are rejected. The distribution after refinement is shown in orange. (B) Superimposed histograms of the post-refinement distribution of diffusion coefficients calculated using the method from (Tafvizi et al., 2008) in blue, versus my method in orange show that the distributions are almost identical.

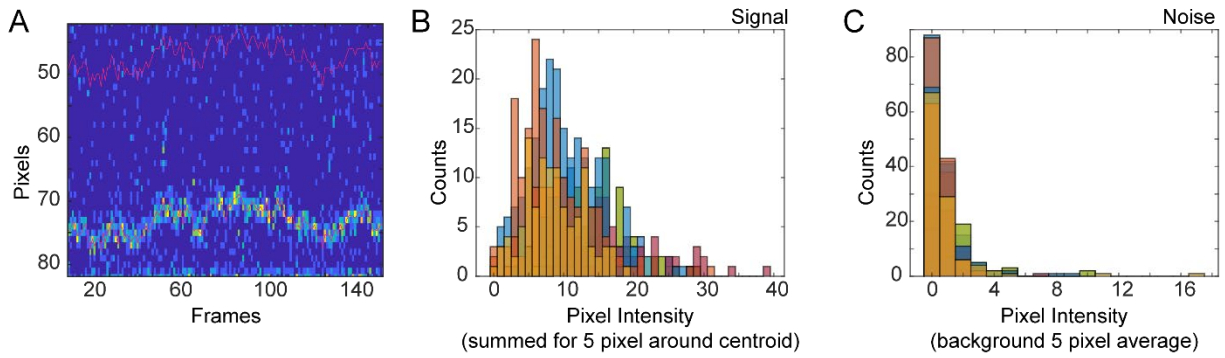

Figure S9. Signal to noise calculation. To determine the localization precision using **Equation 2** in **Materials and Methods**, an average signal intensity vs background intensity was determined. (A) Representative SWR1 particle with tracking information overlayed (lower) as well as tracking information overlayed on background (upper). (B) Histogram of signal intensity (summed over 5 pixels around the centroid) for SWR1 signal compared to (C) the background signal. An average signal value [12.9 photons] and average background value [0.8 photons] was used to solve for localization precision.

Identity	Sequence
Cas9 crRNA sequence “lambda 1”	5'- /AltR 1 /rGrGrC rGrCrA rUrArA rArGrA rUrGrA rGrArC rGrCrG rUrUrU rUrArG rArGrC rUrArU rGrCrU / AltR2/ -3'
Cas9 crRNA sequence “lambda 2”	5'- / AltR 1 /rGrUrG rArUrA rArGrU rGrGrA rArUrG rCrCrA rUrGrG rUrUrU rUrArG rArGrC rUrArU rGrCrU / AltR2/ -3'
Cas9 crRNA sequence “lambda 3”	5'- / AltR 1 /rCrUrG rGrUrG rArArC rUrUrC rCrGrA rUrArG rUrGrG rUrUrU rUrArG rArGrC rUrArU rGrCrU / AltR2/ -3'
Cas9 crRNA sequence “lambda 4”	5'- /AltRl /rCrArG rArUrA rUrArG rCrCrU rGrGrU rGrGrU rUrCrG rUrUrU rUrArG rArGrC rUrArU rGrCrU / AltR2/ -3'
Cas9 crRNA sequence “lambda 5”	5'- /AltR 1 /rGrGrC rArArU rGrCrC rGrArU rGrGrC rGrArU rArGrG rUrUrU rUrArG rArGrC rUrArU rGrCrU / AltR2/ -3'
3x-biotin-cos1 oligo	5' - /5Phos/ AGG TCG CCG CCC TT/iBiodT/TT/iBiodT/TT/3BiodT/-3'
3x-biotin-cos2 oligo	5'- /5Phos/ GGG CGG CGA CCT TT/iDigN/TT/iDigN/TT/3DigN/-3'

Supplementary Table 1. crRNA sequences for dCas9 binding and custom oligos sequences for DNA tethering.

Identity	Sequence
Swc2 DNA binding domain (italicized) with added cysteine (bolded)	HHHHHHSSGLEVLFQGPH**C***IRRQELLSRKKRNKRLQKGPV VIKKQKPKPKSGEAIPRSHHTHEQLNAETLLLNTRRTSKRSS VMENTMKVYEKLSKAEKKRKIIQERIRKHKEQESQHMLTQE ERLRIAKETEKLNILSLDKFKEQEVWKKENRLALQKRQKQK FQPNETILQFLSTAWLMTPAMELEDRKYWQEQLNKRDKKK KKYPRKPKKNLNLGKQDASDDKKRE*

Supplementary Table 2. Sequences of protein constructs.

Condition	n before	criteria for linear fit cutoff	n after	median D $\mu m^2/sec$	SEM*$\sqrt{(\pi/2)}$ $\mu m^2/sec$
SWR1: 70mM KCl no ATP	345	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	245	0.013	0.002
SWR1: 70mM KCl + 1mM ATP	555	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	462	0.024	0.001
SWR1: 70mM KCl + 1mM ADP	476	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	313	0.011	0.002
SWR1: 70mM KCl + 1mM ATP-gamma-S	367	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	283	0.026	0.002
SWR1: 25mM KCl + 1mM ATP	171	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	157	0.015	0.001
SWR1: 200mM KCl + 1mM ATP	136	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	131	0.041	0.003
SWR1: 70mM KCl + 1mM ATP on lambda nucleosome array	301	pvalue<0.1, rsquared>0.8 and dcoef < 0.14	100	0.009	0.003
Swc2: 25mM KCl no ATP	200	pvalue<0.1, rsquared>0.8 and dcoef < 5	152	0.719	0.069
Swc2: 70mM KCl no ATP	143	pvalue<0.1, rsquared>0.8 and dcoef < 5	115	1.038	0.088
Swc2: 150mM KCl no ATP	98	pvalue<0.1, rsquared>0.8 and dcoef < 5	79	1.549	0.125
Cy3 dCas9	44	none	44	$2.7x10^{-4}$	$3.7x10^{-4}$

Supplementary Table 3. Summary of median diffusion coefficients as well as rejection criteria implemented per condition for particle refinement.