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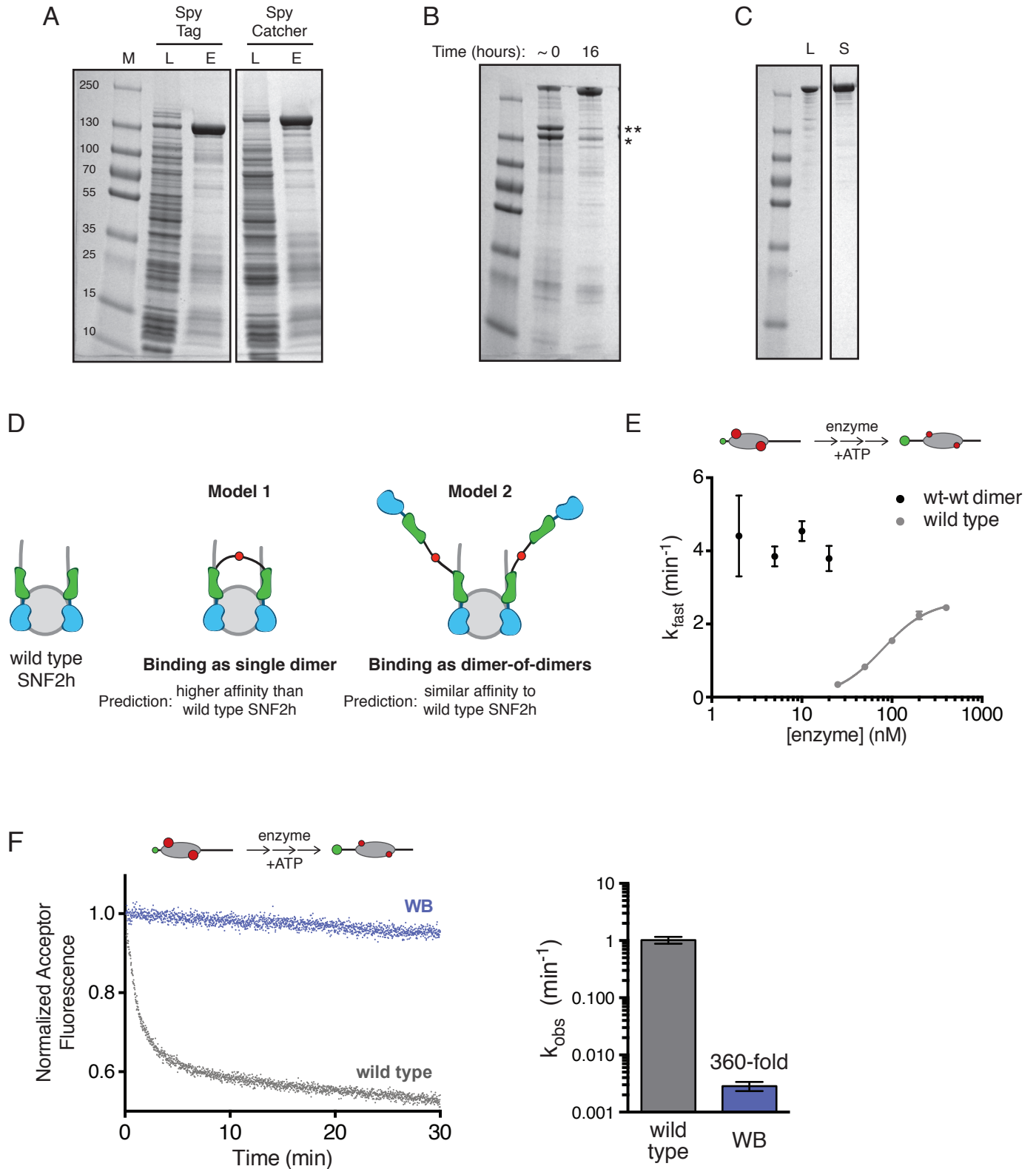
## **Supplemental Information**

### **A Nucleotide-Driven Switch Regulates Flanking DNA Length Sensing by a Dimeric Chromatin Remodeler**

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**Figure S1, related to Figure 1.**

**Preparation of covalent SNF2h dimers and nucleosome remodeling controls**



## Figure S1, related to Figure 1.

### Preparation of covalent SNF2h dimers and nucleosome remodeling controls

Purification and dimerization were performed as detailed in Supplemental Experimental Procedures.

(A) Affinity purification of SNF2h with C-terminal GGS linker and SpyTag or SpyCatcher. M = molecular weight marker (given in kDa); L = crude lysate; E = affinity resin elution.

(B) Dimerization reaction. SNF2h with C-terminal linker and SpyTag (\*) or SpyCatcher (\*\*\*) were mixed in equimolar amounts and incubated at 4°C for the time indicated. The presence of dimer band in the ~ 0 hour time point reflects rapid dimer formation occurring during the 3-5 minutes between mixing the two protein components and preparing the sample for SDS PAGE. Same molecular weight marker as in (A).

(C) Size exclusion chromatography (SEC) of SNF2h [wt]-[wt] dimer. L = SEC load; S = SEC elution peak fraction. Same molecular weight marker as in (A).

Gels in (A-C) were stained with SimplyBlue SafeStain (Life Technologies) and imaged on an Alpha Imager (Protein Simple).

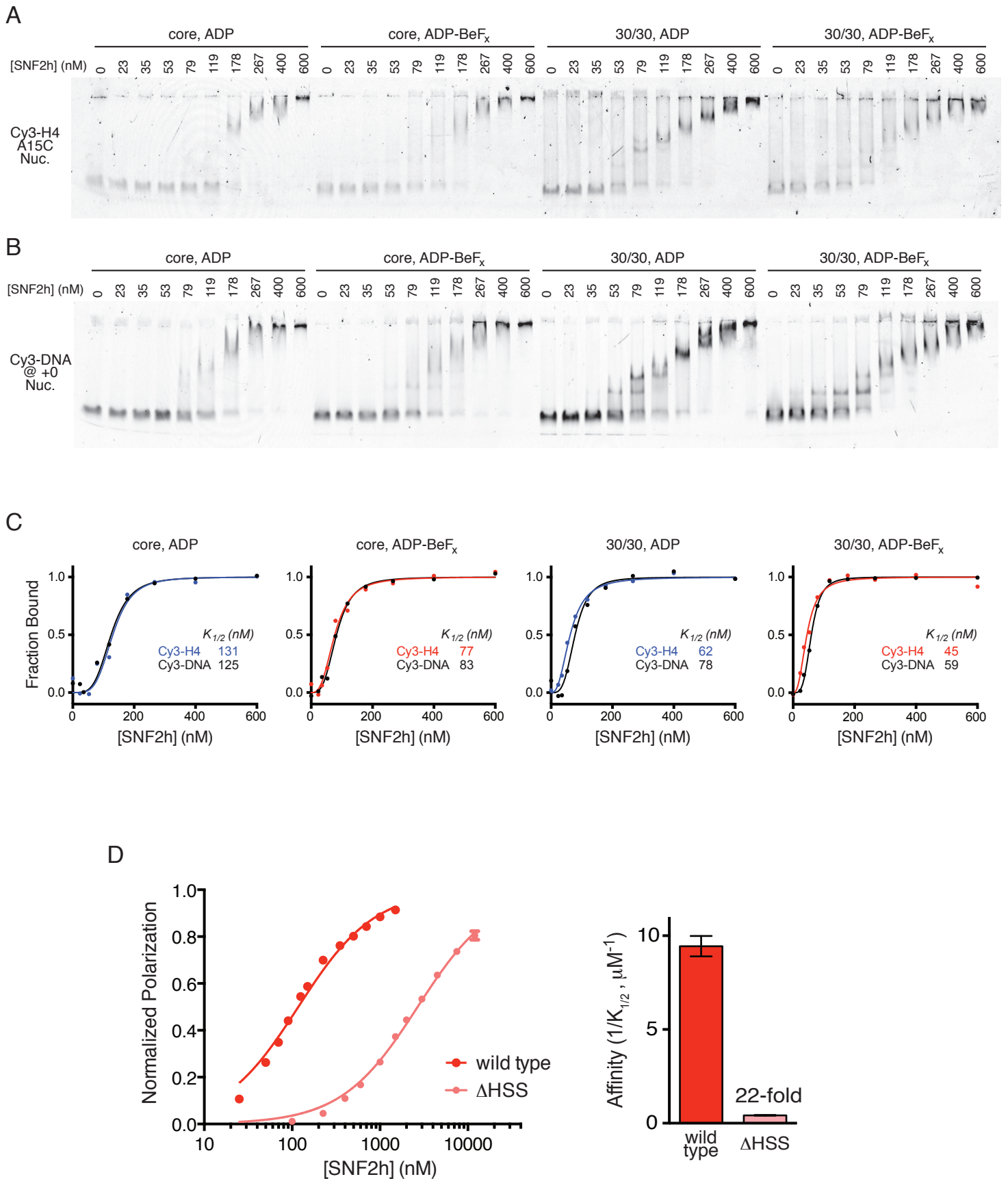
(D) Two possible models for the engagement of nucleosomes by [wt]-[wt] dimers (middle and right) as compared to wild type SNF2h (left). One possibility (Model 1) is that a single copy of the dimer may bind, with both protomers productively engaging the nucleosome. In this scenario, because both of the connected protomers participate in binding interactions, the observed affinity for nucleosomes should be tighter than that of unconnected wild type SNF2h. In a second scenario (Model 2), two copies of the dimer engage each nucleosome, with only one protomer from each dimer unit making binding contacts. Because the two protomers that bind to the nucleosome are not connected to each other, the observed affinity for nucleosomes in this scenario should be comparable to that of wild type SNF2h.

(E) FRET remodeling rate as a function of enzyme concentration. Varying concentrations of wild type SNF2h or [wt]-[wt] dimer were used to remodel 7.5 nM or 1 nM 0/60 nucleosomes,

respectively. The observed rate constants ( $k_{\text{fast}}$ ) obtained from kinetic experiments at each concentration of enzyme are plotted as mean  $\pm$  s.e.m. from three replicates. The concentration dependence of wild type SNF2h was fit to a cooperative Michaelis-Menten model, yielding the parameters  $K_{1/2} = 79$  nM and Hill coefficient = 1.7. The affinity of [wt]-[wt] dimer was too tight to measure. However, as no further increase in rate was observed between 2-20 nM enzyme, we conclude that  $K_{1/2} < 2$  nM for [wt]-[wt]. The  $> 40$ -fold higher affinity for nucleosomes of [wt]-[wt] relative to wild type SNF2h disfavors Model 2 and is consistent with Model 1.

(F) FRET nucleosome remodeling assay with wild type SNF2h and the SNF2h Walker B mutant E309A (WB). Reactions were performed under similar conditions to those detailed in Experimental Procedures, except reactions were carried out at 30°C with 100nM enzyme. Left, representative kinetic traces reporting loss of acceptor fluorescence from FRET-labeled nucleosomes remodeled by wild type (gray) or WB (blue) SNF2h. Right, observed rate constants, reported as mean  $\pm$  s.e.m. from three replicates.

**Figure S2, related to Figure 2.**  
**Nucleosome and DNA binding.**



## Figure S2, related to Figure 2.

### Nucleosome and DNA binding.

(A-C) Electrophoretic mobility shift assay (EMSA) comparing SNF2h binding to nucleosomes labeled at H4 A15C versus nucleosomes labeled on the DNA at the entry/exit site.

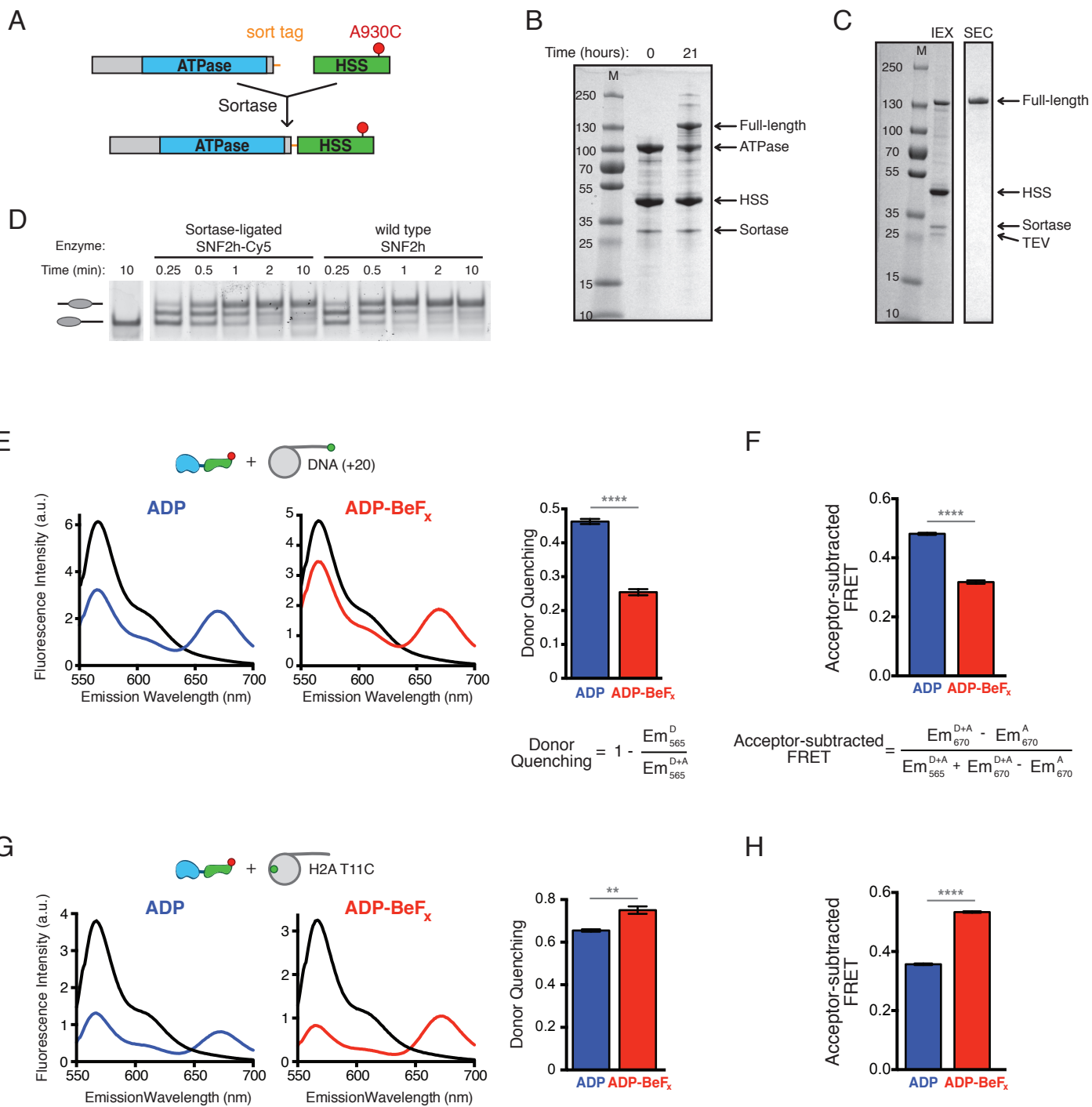
(A) EMSA measuring SNF2h binding to nucleosomes labeled with Cy3 on histone H4 A15C. Core or 30/30 nucleosomes (15 nM) were incubated with the indicated concentrations of SNF2h, separated on a non-denaturing gel, and imaged by Cy3 fluorescence (Supplemental Experimental Procedures).

(B) EMSA measuring SNF2h binding to nucleosome labeled with Cy3 on the nucleosomal DNA at the entry/exit site (+0), a probe location we have used previously (Racki et al., 2009). Same procedure as (A).

(C) Quantification of EMSA binding data from (A) and (B). Fraction bound was calculated from the disappearance of the unbound nucleosome band, plotted versus SNF2h concentration, and fit to a cooperative binding model (Supplemental Experimental Procedures). For all four conditions, the half-maximal binding concentrations of SNF2h ( $K_{1/2}$ ) for Cy3-H4- and Cy3-DNA-labeled nucleosomes were very similar (within 1.3-fold or less), indicating that the dye position has little or no effect on nucleosome binding.

(D) Wild type SNF2h and SNF2h  $\Delta$ HSS binding to a 30bp, double stranded DNA in the presence of ADP-BeF<sub>x</sub>. DNA labeled at its 5' end with fluorescein was incubated with various concentrations of protein under the same reaction conditions used to measure nucleosome binding in Figure 2. Fluorescence polarization was measured and data were fit similarly to nucleosome binding data (Supplemental Experimental Procedures), except that a non-cooperative binding model was used (Hill coefficient = 1). Left, binding curves fit to non-cooperative binding model. Points are mean  $\pm$  s.e.m. of three replicates. Right, affinities from fitting of data. Bars are mean  $\pm$  s.e.m.

**Figure S3, related to Figure 3**  
**Preparation of SNF2h-Cy5 and controls for HSS-nucleosome FRET experiments**



**Figure S3, related to Figure 3.**

**Preparation of SNF2h-Cy5 and controls for HSS-nucleosome FRET experiments.**

(A) Schematic of the method used to site-specifically label SNF2h at position A930C (Supplemental Experimental Procedures).

(B) Sortase-mediated ligation reaction containing affinity-purified Cy5-HSS and unlabeled ATPase after 0 or 21 hours. Bands corresponding to full-length Cy5-labeled SNF2h product (Full-length), ATPase and HSS fragments, and sortase enzyme are denoted with arrows. M = molecular weight marker (given in kDa).

(C) Purification of sortase-ligated, Cy5-labeled SNF2h. Gel of ligation products purified by cation exchange chromatography (IEX), then by size exclusion chromatography (SEC). Bands are labeled as in (B), with the band corresponding to TEV protease also indicated.

(D) Native gel mobility shift nucleosome remodeling assay with sortase-ligated SNF2h-Cy5 and wild type SNF2h. 50nM 0/60 nucleosomes were remodeled with saturating amounts (300nM) of each enzyme and stopped at various time points.

Gels in (B) and (C) were stained with SimplyBlue SafeStain (Life Technologies) and imaged on an Alpha Imager (Protein Simple). Gel in (D) was stained with SYBR Gold (Life Technologies) and imaged on a Typhoon imager (GE LifeSciences).

(E-H) Alternate methods for determination of FRET using probe sets from Figure 3. A more detailed description of reaction setup and calculations can be found in Supplemental Experimental Procedures.

(E) FRET determination by donor quenching for the DNA(+20) Cy3 position. Top left, schematic of label positions. Bottom left, representative emission spectra in the presence of ADP or ADP-BeF<sub>x</sub>. Colored curves are emission from complexes containing both labeled components (Cy3 nucleosomes + Cy5 SNF2h). Black curves are emission from complexes containing donor only (Cy3 nucleosomes + unlabeled SNF2h). FRET results in quenching of the donor peak

(~565nm) in the presence (colors) vs. absence (black) of the acceptor dye. Right, the equation used to calculate donor quenching and mean donor quenching values  $\pm$  s.e.m. from three replicates.

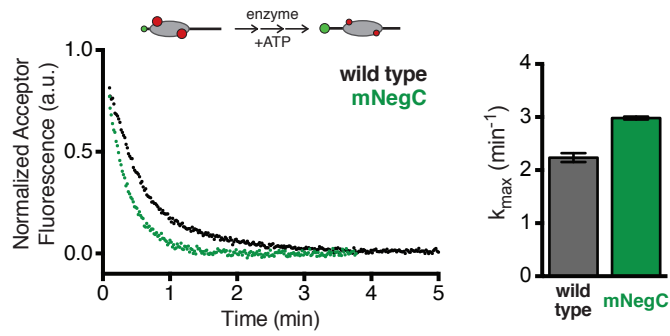
(F) Acceptor-subtracted FRET determination for the DNA(+20) Cy3 position. Direct acceptor fluorescence was determined by assembling complexes with acceptor only (unlabeled nucleosomes + Cy5 SNF2h) and exciting with the donor excitation wavelength (515 nm). This value was subtracted from the observed acceptor intensity as in the equation shown, and as described in Supplemental Experimental Procedures. Bars are mean  $\pm$  s.e.m. of three replicates.

(G) FRET determination by donor quenching for the H2A T11C Cy3 position. Same presentation as in (E).

(H) Acceptor-subtracted FRET determination for the H2A T11C Cy3 position. Same presentation as in (F).

\*\*\*\*  $p < 0.0001$ , \*\*  $p < 0.01$

**Figure S4, related to Figure 4**  
**FRET nucleosome remodeling by SNF2h mNegC**

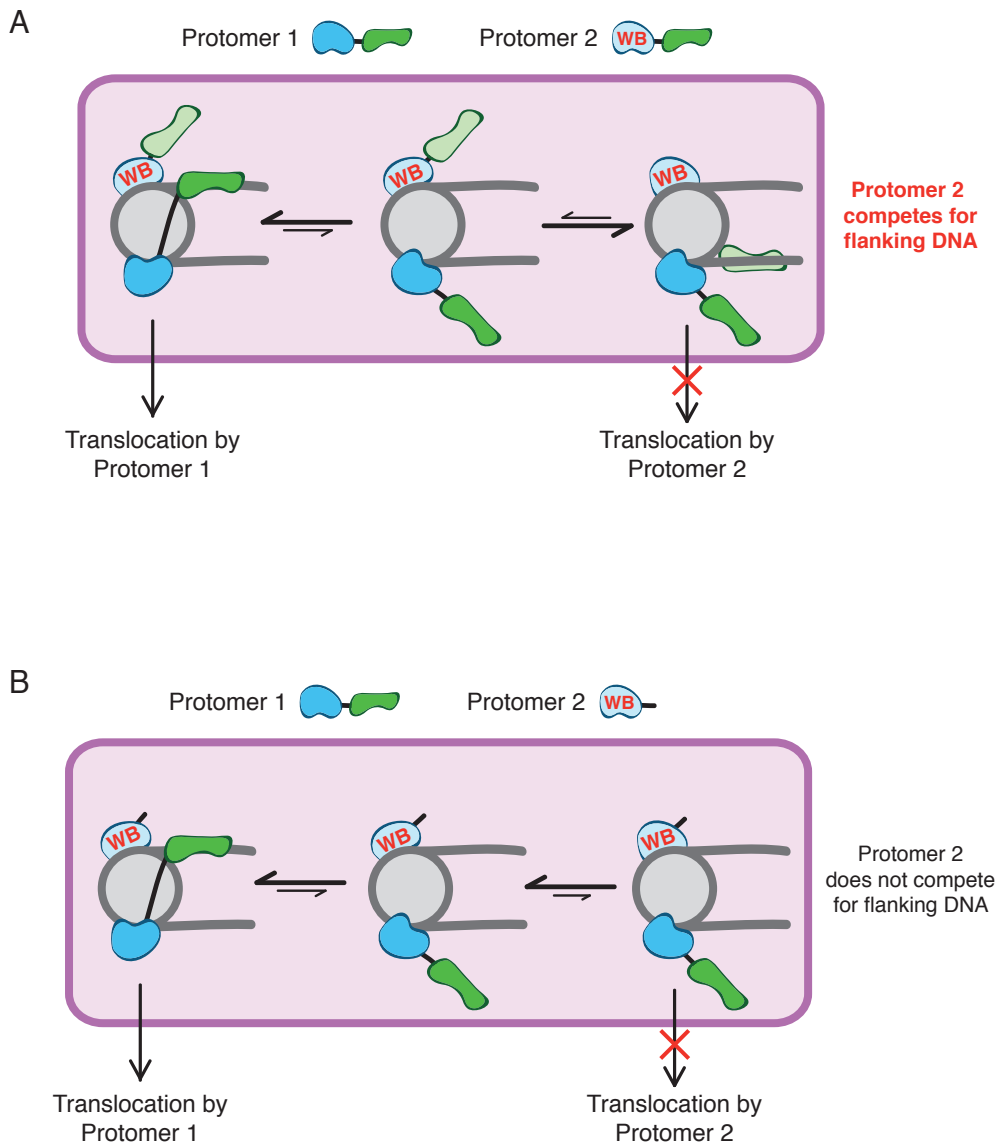


**Figure S4, related to Figure 4.**

**FRET nucleosome remodeling by SNF2h mNegC**

(A) FRET nucleosome remodeling assay comparing wild type SNF2h to SNF2h mNegC. Left, schematic of the assay setup (same as Figure 1C) and representative kinetic traces. Right, maximal rate constants from remodeling 7.5 nM FRET-labeled 0/60 nucleosomes with saturating concentrations (300 nM) of each enzyme. Bars are mean  $\pm$  s.e.m. of three replicates.

**Figure S5, related to Figure 5**  
**Supplemental models for SNF2h dimer action.**



**Figure S5, related to Figure 5.**

**Supplemental models for SNF2h dimer action.**

(A) When one protomer is catalytically compromised (WB), it can still compete with the wild type protomer for binding to flanking DNA. However, because it is impaired for ATP hydrolysis, the WB protomer does not proceed efficiently to translocation and instead remains bound to flanking DNA longer. This hinders DNA binding by the wild type protomer and thus slows the overall rate of nucleosome movement.

(B) When the HSS domain is deleted from the catalytically compromised protomer ( $\Delta$ HSS/WB), it can no longer compete for binding to flanking DNA. The wild type protomer is then unimpeded in binding to its DNA and proceeding to translocation.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Protein expression and purification

All SNF2h variants were cloned into pBH4, a pET3-based bacterial expression vector containing a TEV-cleavable N-terminal 6xHis tag and linker (Volkman et al., 2002). pBH4 was a kind gift from the Lim laboratory at UCSF. All constructs were expressed in Rosetta (DE3) *E. coli* (Novagen). An N-terminal epitope tag was necessary for expression in *E. coli* (data not shown). Cultures were grown to OD<sub>600</sub> ~0.3-0.5 at 37°C in 2X LB media with 1X NaCl, then switched to 18°C and induced at OD<sub>600</sub> ~0.65-0.75 with 0.4 mM IPTG. After ~18 hours, cells were harvested, resuspended in Prep Buffer (25 mM HEPES pH 8, 300 mM KCl, 7.5 mM imidazole, 10% v/v glycerol, 2 mM β-mercaptoethanol, 2 μg/mL aprotinin, 1 μg/mL peptstatin A, 3 μg/mL leupeptin, 1 mM PMSF) and lysed by high pressure with an Emulsiflex-C3 homogenizer (Avestin). Lysates were cleared by spinning at 30,000 x g for 30 minutes, and cleared lysates were passed by gravity flow over TALON cobalt resin (Clontech) equilibrated in Prep Buffer (3-4 mL resin per 1 L culture). Resin was washed with 15-20 bed volumes of Prep Buffer, then bound protein was eluted with 2-3 bed volumes of Elution Buffer (25 mM HEPES pH 7.0, 300 mM KCl, 400 mM imidazole, 2 mM β-mercaptoethanol, 2 μg/mL aprotinin, 1 μg/mL peptstatin A, 3 μg/mL leupeptin, 1 mM PMSF). Affinity-purified proteins were passed over HiTrap Q HP anion exchange columns (1 column per 3 L culture; GE Lifesciences) pre-equilibrated with SEC Buffer (25 mM HEPES pH 7.5, 300 mM KCl, 2 mM β-mercaptoethanol), to remove DNA. Anion exchange flowthrough (containing SNF2h) was concentrated, TEV protease was added to a final concentration of 0.075-0.15 mg/mL, and the mixture was dialyzed overnight into SEC Buffer with 5 mM β-mercaptoethanol. Dialyzed proteins were then purified by size exclusion chromatography on a HiLoad Superdex 200 26/60 column (320 mL bed volume; GE Lifesciences) in SEC Buffer. Relevant fractions were pooled, concentrated, dialyzed overnight

into Storage Buffer (25 mM HEPES pH 7.5, 210 mM KCl, 15% v/v glycerol, 2 mM  $\beta$ -mercaptoethanol), flash frozen in liquid N<sub>2</sub> and stored at -80°C.

### **Protein mutants and variants**

Full length,  $\Delta$ HSS, WB: The full length SNF2h sequence (aa 1-1052) was identical to NCBI Accession:NP\_003592, except that the N-termini of all constructs contained an additional Gly-Ser, left over from TEV protease cleavage. SNF2h  $\Delta$ HSS contained aa 1-736. SNF2h Walker B (WB) mutant was identical to full-length, with the single point mutation E309A.

SpyCatcher and SpyTag fusions: Dimerizable variants of SNF2h (wild type,  $\Delta$ HSS, WB, and WB/ $\Delta$ HSS) were constructed by appending directly to their C-termini a long, flexible linker (nine repeats of a Gly-Gly-Ser tripeptide; (GGG)<sub>9</sub>), followed by the SpyTag peptide or SpyCatcher domain (Zakeri et al., 2012). In order to avoid imposing steric constraints on SNF2h movement, the total length of the linker (split between two proteins) was designed to be greater than the diameter of the nucleosome: 2 x 27 aa linkers x 3.8Å/aa for a fully extended polypeptide of ~205Å, allowing for the fact that a randomly coiled peptide will be shorter than this. The amino acid sequences of the (GGG)<sub>9</sub> linker, SpyTag and SpyCatcher are:

(GGG)<sub>9</sub> linker:

GGSGGSGGSGGSGGSGGSGGSGGSGGS

SpyTag:

AHIVMVD**DAY**KPTK

SpyCatcher:

AMVDTLSGLSSEQGGSGGDMTIEEDSATHIKFS**KR**DEDGKELAGATMELRDSSGKTISTWISDGQVKDFYL  
YPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGD**AI**

SpyCatcher-mediated dimerization leaves all sequences intact, so dimerized products contained the indicated SNF2h variants, plus two (GGG)<sub>9</sub> linkers, one SpyTag and one SpyCatcher (linked by an isopeptide bond between the Asp and Lys residues shown in bold above).

SNF2h sortase substrates: We designed modified SNF2h fragments that, when ligated by *S. aureus* sortase A, reconstitute a full-length SNF2h protein with minor internal mutations. SNF2h aa 725-729 were chosen for replacement with the sortase cognate sequence because this site is between the HSS and ATPase domains and is poorly conserved between homologs of SNF2h. Ligation by sortase requires a C-terminus with the sequence LPETG and an N-terminus with one or more N-terminal Gly residues. We therefore created a variant of SNF2h aa1-730 with the following mutations:

wild type SNF2h  
723 QK**IAFTE**-WI 731

SNF2h 1-725sort  
723 QK**LPETGG**-WI 732

The fragment containing the HSS domain (aa731-1052) was modified such that TEV protease cleavage left Gly-Gly at its N-terminus, rather than Gly-Ser, making it a better nucleophile for the sortase reaction. Ligation with sortase results in replacement of the Gly residues in SNF2h 1-725sort with those from the HSS-containing fragment. Thus, the product of this sortase ligation is a full-length SNF2h protein with the same mutations as those present in SNF2h 1-725sort above. For experiments with fluorescently labeled HSS domain, the following substitutions were made in the HSS-containing fragment: C891S, C957S, C976S, C1001S, A930C.

SNF2h mNegC: The region of SNF2h homologous to the NegC region of *Drosophila ISWI* (Clapier and Cairns, 2012) – aa617-648 in dISWI; aa669-700 in SNF2h – was identified by Clustal sequence alignment in VMD (Humphrey et al., 1996) and replaced with a flexible linker of equivalent length:

wild type SNF2h:

664 SKESEITDDEDIDGILERGAKKTAEMNEKLSKMGESSLRNFT 704

SNF2h mNegC:

664 SKESEGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSRNFT 704

### **SpyCatcher covalent dimerization**

In the SpyCatcher method (Zakeri et al., 2012), two fragments of a bacterial adhesin (SpyTag and SpyCatcher) are expressed as fusion proteins to the dimerization targets of interest (Figure 1A, left). Upon mixing, the fragments associate and rapidly form a covalent isopeptide bond between two side chains, irreversibly linking the two proteins of interest. SpyCatcher dimers can be formed in any orientation (N-to-N, N-to-C, or C-to-C), making this a highly flexible method for systems with steric constraints.

We tested all three possible orientations for SNF2h and found that, while N-to-N and N-to-C linkages formed with very poor efficiency (data not shown), C-to-C dimerization was highly efficient (Figures 1A, S1B). We therefore used the C-to-C orientation for all dimeric SNF2h constructs.

SNF2h SpyCatcher and SpyTag constructs (described in the previous section) were expressed and purified similarly to other SNF2h variants, with a few modifications (Figure S1A). After anion exchange, SNF2h SpyCatcher and SpyTag fusions were mixed in equimolar ratios (~20  $\mu$ M of each protein), TEV protease was added to the mixed proteins and they were dialyzed overnight as described above (Figure S1B). Dimers were separated from monomers by size exclusion chromatography (Figure S1C), and purified dimers were concentrated, dialyzed into Storage Buffer and frozen as described above.

## Labeling and sortase-mediated transpeptidation of SNF2h

Because of the large number of endogenous cysteines in SNF2h, we developed an alternate approach for site-specific labeling (Figure S3A); we used the sortase A transpeptidase from *Staphylococcus aureus* to ligate Cy5-labeled HSS domain to the rest of the protein (Popp et al., 2007; 2009). The sortase expression plasmid was a gracious gift from Hidde Ploegh. Sortase A was expressed recombinantly in *E. coli* and purified by affinity chromatography.

The HSS fragment containing a single cysteine (A930C, described above) was labeled with Cy5 maleimide as follows: HSS was dialyzed overnight at 4°C into 25 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM TCEP. 1 mg Cy5-maleimide (Lumiprobe) was dissolved in 70 µL DMSO. The HSS protein (~100 µM) was removed from dialysis and brought to room temperature, then reconstituted Cy5-maleimide was added to a final concentration of ~500 µM (5:1 molar ratio dye:protein). This mixture was incubated for 15-20 minutes at room temperature, then quenched with 5 mM β-mercaptoethanol. To remove free dye, the labeled protein was buffer exchanged on a 7k MWCO Zeba Desalting Spin Column (Thermo Scientific) pre-equilibrated in dialysis buffer. The protein was then filtered using a 0.22 µm spin filter (Millipore) to remove precipitate.

Sortase-mediated ligation was performed by mixing ~75 µM SNF2h 1-725sort, ~150 µM Cy5-labeled HSS (2-fold molar excess over SNF2h 1-725sort), 50 µM sortase A, 150 mM KCl, 10 mM CaCl<sub>2</sub> and incubating 18-24 hours at 4°C (Figure S3B). Unligated SNF2h 1-725sort, sortase and undesired products were removed by ion exchange chromatography as follows: the reaction was diluted at least 10-fold into a mixture of ion exchange buffers (30 mM HEPES pH 7.0, 2 mM β-mercaptoethanol, and 50 mM KCl [Buffer A] or 1.5 M KCl [Buffer B]) containing 7% Buffer B (the remaining % was made up by Buffer A). Diluted protein was bound to a 5 mL HiTrap SP column (GE Lifesciences) pre-equilibrated in 7% Buffer B. The column was washed

with 2 column volumes of 7% Buffer B, then the protein was eluted with 2 column volumes of 20% Buffer B (Figure S3C). The ion exchange elution (which contained the full-length product and the labeled HSS fragment) was then concentrated and separated by size exclusion chromatography on either a Superdex 200 10/300 GL column or a HiLoad 26/60 Superdex 200 column (GE Lifesciences) in 25 mM HEPES pH 7.5, 300 mM KCl, 2 mM  $\beta$ -mercaptoethanol (Figure S3C). Fractions eluting after the void volume and containing full-length, labeled SNF2h were concentrated, dialyzed into 25 mM HEPES pH 7.5, 300 mM KCl, 15% glycerol, 2 mM  $\beta$ -mercaptoethanol, flash frozen in liquid N<sub>2</sub> and stored at -80°C.

### Fits of fluorescent binding data

ADP and apo state data were fit to a cooperative binding model:

$$B = (B_{\max} - B_{\min}) \frac{X^h}{\left( \left( \frac{1}{A} \right)^h + X^h \right)} + B_{\min}$$

where  $B$  is the observed fluorescence intensity,  $B_{\max}$  and  $B_{\min}$  are the fluorescence intensity when 100% and 0% of nucleosomes are bound, respectively,  $X$  is the concentration of protein in  $\mu$ M,  $h$  is the Hill coefficient, and  $A$  is the observed association affinity constant (or  $1/K_{1/2}$ ) in  $1/\mu$ M. During fitting, the constraint  $h \geq 1$  was applied.

Binding curves in the presence of ADP-BeF<sub>x</sub> exhibited an early, rapid increase in intensity, followed by a pseudo-plateau with a slow, linear increase in intensity. This behavior is consistent with low affinity, non-specific binding and was fit with an added linear component:

$$B = (B_{\max} - B_{\min}) \frac{X^h}{\left( \left( \frac{1}{A} \right)^h + X^h \right)} + NS * X + B_{\min}$$

where  $NS$  is a linear slope dependent on protein concentration ( $X$ ).

Nonlinear regression in all nucleotide states was performed with weighting by  $1/B^2$  to account for the increase in noise with increased signal intensity.

### **Behavior of nucleotide-free (apo) state**

We have repeatedly observed that incubation of SNF2h and nucleosomes in the absence of nucleotide prior to activity assays resulted in a large decrease in remodeling activity (data not shown). The magnitude of the decrease is proportional to the duration of the incubation, and this decrease in activity is not observed when SNF2h and nucleosomes are incubated separately, or when incubated together in the presence of ADP or ADP-BeF<sub>x</sub> (data not shown). For this reason, we exclusively considered nucleotide-bound states for the majority of our experiments. Furthermore, we removed the SNF2h-nucleosome pre-incubation step from all of our activity assays; we started our assays instead with the addition of nucleosomes to pre-incubated SNF2h-ATP.

### **Electrophoretic Mobility Shift Assays (EMSA)**

We have previously measured nucleosome binding under equilibrium conditions using a DNA label at the entry/exit site (Racki et al., 2009). However, we could not use this labeling position for the experiments in Figure 2 because it was insensitive to binding of SNF2h  $\Delta$ HSS (data not shown). By contrast, a label at H4 A15C showed a similar degree of signal change for both full length and  $\Delta$ HSS SNF2h constructs (data not shown).

To determine whether attachment of a Cy3 probe to histone H4 A15C influenced the affinity of SNF2h for nucleosomes, we compared SNF2h binding to nucleosomes labeled at H4 A15C versus nucleosomes labeled on the DNA at the entry/exit site. H4 A15C-labeled nucleosomes

were prepared as described in Experimental Procedures. DNA-labeled nucleosomes were prepared using a Cy3-labeled primer in the nucleosomal DNA PCR – a 5' end label for core (Integrated DNA Technologies) or an internally modified dT at the same location for 30/30 (IBA Life Sciences)

EMSA shown in Figures S2A-S2B were performed as follows: nucleosomes (15 nM) were incubated with varying concentrations of SNF2h in reaction buffer containing 20 mM HEPES pH 7.5, 70 mM KCl, 0.02% (v/v) Igepal (NP-40), 6mM MgCl<sub>2</sub>, 3 mM ADP, ~6% (v/v) glycerol. Reactions with ADP-BeF<sub>x</sub> also contained 3 mM BeCl<sub>2</sub> and 15 mM NaF. Complexes were incubated at room temperature for 30 minutes, then loaded (8 μL) onto a 3% polyacrylamide, 0.5X TBE native gel. Samples were separated by electrophoresis for 50 minutes at 100 V, and then imaged by Cy3 fluorescence on a Typhoon Imager (GE Lifesciences).

The intensity of the unbound nucleosome band was quantified using ImageJ (Schneider et al., 2012) and fraction unbound was calculated using the following equation:

$$B = (B_{\max} - B_{\min}) \left( 1 - \frac{X^h}{((K_{1/2})^h + X^h)} \right) + B_{\min}$$

where  $B$  is the observed band intensity,  $B_{\max}$  and  $B_{\min}$  are the intensities when 100% and 0% of nucleosomes are bound, respectively,  $X$  is the concentration of SNF2h (nM) and  $K_{1/2}$  is the dissociation affinity constant (nM). Raw intensity data were then converted to Fraction Unbound by normalizing such that  $B_{\min} = 0$  and  $B_{\max} = 1$ . Fraction Unbound was then converted to Fraction Bound:

$$\text{Fraction Bound} = 1 - \text{Fraction Unbound}$$

and Fraction Bound was then fit to a standard cooperative binding equation to obtain the data presented in Figures S2A-S2B.

$$\text{Fraction Bound} = \frac{X^h}{\left( (K_{1/2})^h + X^h \right)}$$

### Fits of kinetic data

Initial rates for gel shift remodeling experiments with excess, saturating nucleosomes (Figure 1D) were obtained by quantifying the centered product band (top) using ImageJ (Schneider et al., 2012) and fitting points with less than 20% centered product to a straight line.

Unless otherwise indicated, FRET remodeling data were fit with a two-phase exponential decay model:

$$B = B_{\min} + \text{frac}_{\text{fast}} * (B_{\max} - B_{\min}) * e^{-k_{\text{fast}} * t} + (1 - \text{frac}_{\text{fast}}) * (B_{\max} - B_{\min}) * e^{-k_{\text{slow}} * t}$$

where  $B$  is the observed fluorescence intensity,  $B_{\max}$  and  $B_{\min}$  are the maximum and minimum fluorescence intensities, respectively,  $\text{frac}_{\text{fast}}$  is the fraction of the total fluorescence change attributed to the fast phase,  $k_{\text{fast}}$  and  $k_{\text{slow}}$  are rate constants for the fast and slow phases ( $\text{min}^{-1}$ ), and  $t$  is time (min). For all FRET remodeling experiments, the fast phase accounted for >70% of the observed fluorescence change, so  $k_{\text{fast}}$  values are reported.

For the Walker B mutant data in Figure S1F, FRET change was too slow to be fit to an exponential decay model. Instead, the total fractional fluorescence change from wild type traces was used to estimate the final fluorescence intensity of WB traces. These traces were then fit to a single exponential decay model, with the ending fluorescence intensity fixed to the predicted value.

Restriction enzyme accessibility (REA) data were fit using a single exponential model:

$$Y = Y_{\max} (1 - e^{-kt})$$

where  $Y$  is the fraction of DNA cut,  $Y_{max}$  is the maximal fraction of DNA cut,  $k$  is the observed rate constant ( $\text{min}^{-1}$ ) and  $t$  is time (min).

### **FRET between HSS and nucleosomes**

For Cy3 DNA(+20) and Cy3 DNA(-25) labels: 1  $\mu\text{M}$  Cy5-labeled SNF2h was incubated with 200 nM 20/0 Cy3-labeled nucleosomes in: 7 mM HEPES pH 7.5, 70 mM KCl, 0.02% (v/v) Igepal (NP-40), 7% (v/v) glycerol, 6 mM  $\text{MgCl}_2$ , 3 mM ADP.

Samples were incubated in a quartz cuvette for 5-10 min in the presence of ADP at 25°C, then an emission spectrum was measured with excitation at the donor excitation wavelength (515 nm). 4  $\mu\text{L}$   $\text{BeF}_x$  mixture (60 mM  $\text{BeCl}_2$ , 300 mM NaF) was added (to  $\sim 3$  mM final), followed by mixing. Either donor (565 nm) or acceptor (670 nm) emission was monitored over time until no further fluorescence change was observed ( $\sim 10$  minutes). Then another emission spectrum was measured in the presence of ADP- $\text{BeF}_x$ .

H2A T11C-Cy3 nucleosomes were treated the same as above, but reactions contained 5 mM  $\text{MgCl}_2$ , 2 mM ADP (such that the concentration of free  $\text{Mg}^{2+}$  is the same as above), and 2.67  $\mu\text{L}$   $\text{BeF}_x$  mixture (same as above) was added to a final concentration of  $\sim 2$  mM.

For the control reactions in Figure S3, mock FRET experiments were performed similar to above, but with SNF2h-nucleosome complexes containing either unlabeled enzyme (Figures S2E, S2G) or unlabeled 20/0 nucleosomes (Figures S2F, S2H). The analysis of these data and the various FRET calculations used are described below.

## FRET calculations

FRET Ratios reported in Figure 3 were calculated as follows:

$$\text{FRET Ratio} = \frac{\text{Em}_{670}}{\text{Em}_{565} + \text{Em}_{670}}$$

where  $\text{Em}_{565}$  and  $\text{Em}_{670}$  are the maximal donor and acceptor emission, respectively, of samples excited at the donor excitation wavelength of 515 nm.

To control for any intrinsic change in donor fluorescence as a function of nucleotide state, we also calculated FRET efficiency based on the degree of donor quenching in the presence vs. absence of the acceptor dye (Figures S3E, S3G).

$$\text{Donor Quenching} = 1 - \frac{\text{Em}_{565}^{\text{D}}}{\text{Em}_{565}^{\text{D+A}}}$$

Here,  $\text{Em}_{565}^{\text{D}}$  is the donor emission of SNF2h-nucleosome complexes labeled with donor dye only (i.e. unlabeled SNF2h plus Cy3-labeled nucleosomes).  $\text{Em}_{565}^{\text{D+A}}$  is the donor emission of complexes containing both donor and acceptor labels (i.e. Cy5-labeled SNF2h plus Cy3-labeled nucleosomes). Because the conditions are otherwise identical, a decrease in the donor emission of the donor+acceptor complex relative to the donor only complex reflects energy transfer due to FRET.

Finally, to correct for the possibility of nucleotide state-dependent changes in direct excitation of the acceptor dye at the donor excitation wavelength, we also calculated a corrected FRET Ratio by subtracting direct acceptor excitation (Figures S3F, S3H).

$$\text{Acceptor - Subtracted FRET} = \frac{\text{Em}_{670}^{\text{D+A}} - \text{Em}_{670}^{\text{A}}}{\text{Em}_{565}^{\text{D+A}} + \text{Em}_{670}^{\text{D+A}} - \text{Em}_{670}^{\text{A}}}$$

$\text{Em}_{670}^{\text{D+A}}$  is the acceptor emission from SNF2h-nucleosome complexes containing both donor and acceptor.  $\text{Em}_{670}^{\text{A}}$  is the acceptor emission from complexes containing only the acceptor dye (i.e.

Cy5-labeled SNF2h plus unlabeled nucleosomes).  $Em_{565}^{D+A}$  is the donor emission from complexes containing both donor and acceptor. All measurements were taken with excitation at the donor excitation wavelength (515 nm).

### **Statistical analysis and data fitting**

For binding affinity measurements, standard errors and P values were calculated during nonlinear regression analysis. All other reported p-values were calculated using Welch's t-test. All data fitting and statistical tests were conducted using Prism (GraphPad Software).

## **SUPPLEMENTAL REFERENCES**

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