

## SUPPLEMENTARY DATA

### FIGURE LEGENDS

Supplementary Figure S1. **Sequence alignment of human HMGB1 and yeast Nhp6A, Nhp6B, and Hmo1 proteins.** Protein sequences were obtained from Genbank and sequence alignment was performed using ClustalW. The regions corresponding to HMG-box domains are underlined. In these regions the consensus HMG-box sequence is given below the alignment, as determined by Weir et al. (1), where “a” stands for aromatic residues and “h” stands for hydrophobic residues. The acidic stretches of Hmo1 and HMGB1 in their C-terminal region are displayed in italic, while the basic stretches of this region are displayed in bold.

Supplementary Figure S2. **Purification of HMG proteins.** A, SDS-PAGE analysis of WT HMG proteins, purified as His-tag fusion proteins. B, SDS-PAGE analysis of chimeric Nhp6A, Nhp6B and Hmo1 proteins, purified as His-tag fusion proteins. Hmo1 $\Delta$  corresponds to a C-terminal deletion mutant of Hmo1, lacking residues 212 to 246. Nhp6A-Ct corresponds to Nhp6A fused on its C-terminal end to these 35 residues (Ct). Nhp6B-Ct corresponds to Nhp6B fused on its C-terminal end to these 35 residues. The pictures show Coomassie staining of a 15% gel (12% for HMGB1). The identity of each sample is indicated at the top.

Supplementary Figure S3. **Restriction enzyme accessibility assay analyzing stimulation of SWI/SNF remodeling activity.** The assay used the *HhaI* enzyme, which restriction site is located at the middle of the nucleosomal DNA in the 216 bp nucleosome probe (see scheme on top). After the remodeling reaction in the presence of *HhaI*, the DNA content of the samples was purified and analyzed by electrophoresis in a non-denaturing polyacrylamide gel (4%, AA:Bis 40:1). The graph at the bottom of this figure corresponds to a quantification of the percentage of digested probe by *HhaI* under each condition (relative to lane 4, which corresponds to digestion of nucleosomal DNA in the absence of SWI/SNF and HMG proteins). The digestion extent corresponding to lane 5 was not included in the graph as it represents an excess (6x) of SWI/SNF complex. The assay used the 216 bp probe, at the form of naked DNA (lanes 1-2) or reconstituted mononucleosome (lanes 3-18).

Supplementary Figure S4. **Analysis of stimulation of SWI/SNF octamer transfer activity by Hmo1 and Hmo1 $\Delta$ .** The 147 bp DNA probe was incubated in the presence of oligonucleosomes. SWI/SNF and each of the HMG proteins were added to the reactions as depicted on the top of the gel picture. Conditions used in the assay were the same detailed in the legend of figure 3A. The values in the graph correspond to the fraction of naked DNA probe converted to nucleosomal DNA, relative to lane 3 (octamer transfer activity of SWI/SNF alone). The average values for the reactions corresponding to lanes 5, 6, 9, 11, 12 and 15 were obtained from 4 independent assays. Error bars represent one standard deviation. Asterisks denote a statistically significant difference (\* $p < 0.05$ ; \*\* $p < 0.01$ ), as deduced from the *t* test. Migration of the mononucleosome is represented schematically at the right of the picture. Lane 1 corresponds to the same probe reconstituted as a mononucleosome before performing the assay.

Supplementary Figure S5. **Restriction enzyme accessibility assay analyzing stimulation of SWI/SNF remodeling activity by Hmo1 and Hmo1 $\Delta$ .** The assay used the *BsrBI* enzyme; the recognition site for this enzyme is located in the nucleosomal DNA portion of the 216 bp nucleosome probe, at 25 bp from one of the nucleosome edges (see scheme in Figure 1A). After the remodeling reaction in the presence of *BsrBI*, the DNA content of the samples was purified and analyzed by electrophoresis in a non-denaturing polyacrylamide gel (5%, AA:Bis 40:1). The graph at the bottom of this figure corresponds to a quantification of the percentage of digested probe by *BsrBI* under each condition (relative to lane 4, which corresponds to digestion of nucleosomal DNA in the absence of SWI/SNF and HMG proteins).

The digestion extent corresponding to lane 5 was not included in the graph as it represents an excess (6x) of SWI/SNF complex. The assay used the 216 bp probe, at the form of naked DNA (lanes 1-2) or reconstituted mononucleosome (lanes 3-10).

Supplementary Figure S6. **Stimulation of SWI/SNF sliding activity by WT and chimeric versions of Hmo1, Nhp6A and Nhp6B.** See legend of figure 2 for a general description of the sliding assay. Migration of naked DNA is indicated at right of the pictures, where migration of the reconstituted mononucleosome is indicated schematically. The term “Rem” stands for remodeled (slid) nucleosome. 6A-Ct and 6B-Ct stand for Nhp6A-Ct and Nhp6B-Ct, respectively. 3x corresponds to a remodeling complex concentration 3 times higher than in the rest of the reactions where the complex is present.

## REFERENCES

1. Weir, H.M., Kraulis, P.J., Hill, C.S., Raine, A.R., Laue, E.D., Thomas, J.O. (1993) Structure of the HMG box motif in the B-domain of HMG1. *EMBO J.* **12**, 1311-1319

# Figure S1

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Nhp6A (P11632) -----
Nhp6B (P11633) -----
Hmo1 (S49770) MTTDPSVKLKSADSLVSSLFELSKAANQTASSIVDFYNAIGDDEEEKIEAFTTLTESLQ 60
HMGB1 (NP002119.1) ---MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEK 54
                PKKPh-Aahha--E-R--h--E-P--h---Eh-K-hGE-W--h---K
                Box A

Nhp6A -----MVT-----PREPKK-----RTTRKKK--DPNAPKRALSAYMFFANEN 35
Nhp6B -----MAATKEAKQPKEPKK-----RTTRKKK--DPNAPKRRLSAYMFFANEN 41
Hmo1 TLTSGVNHHLGIISELVNPIDDDKDAIIAAPVKAVRRKIERDPNAPKKPLTVFFAYSAYV 120
HMGB1 GKFEDMA-----KADKARYEREMKTY-IPPKGETKKKFK--DPNAPKRPPSAFFLFCSEY 109
      -a----- -K-a-----a                PKRPh-Aahha--E-

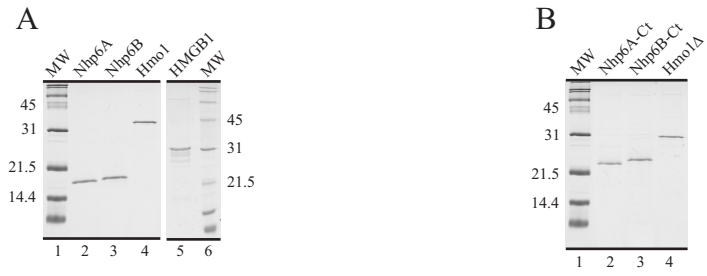
Nhp6A RDIVRSEN----PDITFGQVGKKLGEKWKALTPEEKQPYEAKAQADKKRYESEKELYNA 90
Nhp6B RDIVRSEN----PDVTFGQVGRILGERWKALTAEEKQPYESKAQADKKRYESEKELYNA 96
Hmo1 RQELREDRQKAGLPPLSSTEITQEI SKWKELSDNEKEKWKQAYNVELENYQREKSKYLE 180
HMGB1 RPKIKGEH----PGLS IGDVAKKLGEMWNNTAADDKQPYEKKAALKKEYEKDIAAYRA 164
      R--h--E-      P-h---Eh-K-hGE-W--h---K--a-----K--a-----a
                Box B

Nhp6A TLA----- 93
Nhp6B TRA----- 99
Hmo1 AKKNGTLPPASLENGPTHAPVPIPFSLQHAAEPPVEKRPHDDGSSLEKKKKKKKKDKKKD 240
HMGB1 KGKPDAAKGVVK-----AEKSKKKKEEEEEDEEDEEEDDEEEDDEEED 211

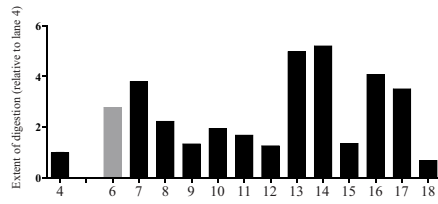
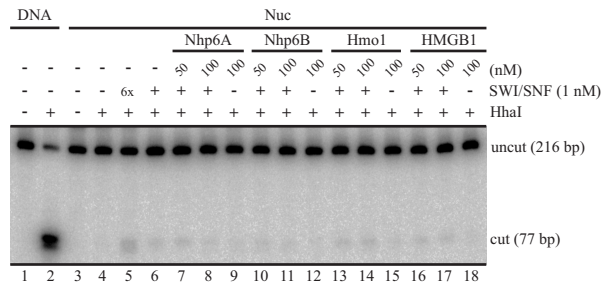
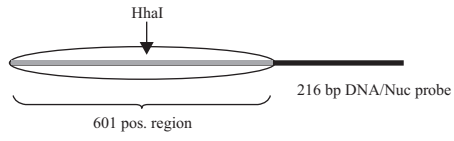
Nhp6A -----
Nhp6B -----
Hmo1 KSNSSI 246
HMGB1 DDDE-- 215

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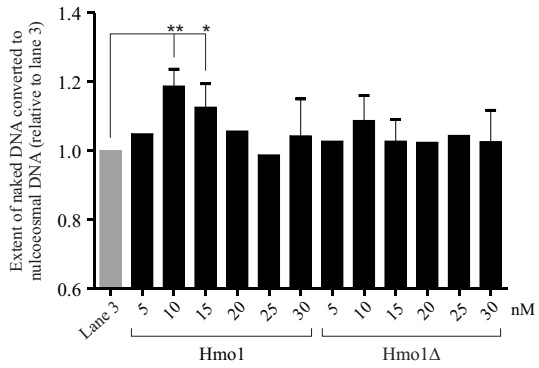
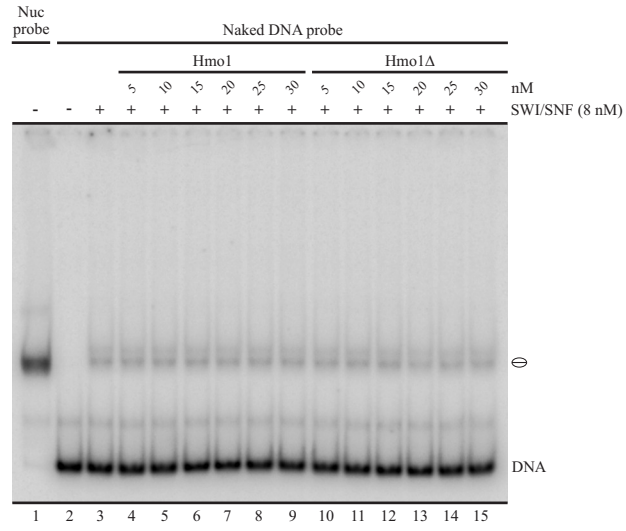
# Figure S2



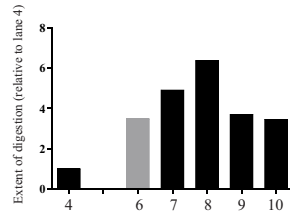
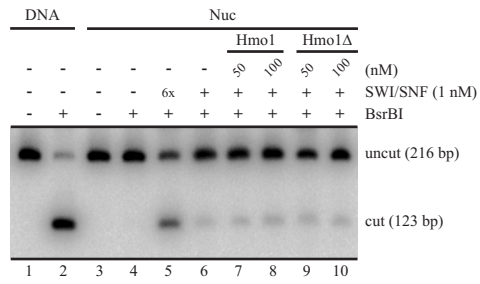
# Figure S3



# Figure S4



# Figure S5



# Figure S6

