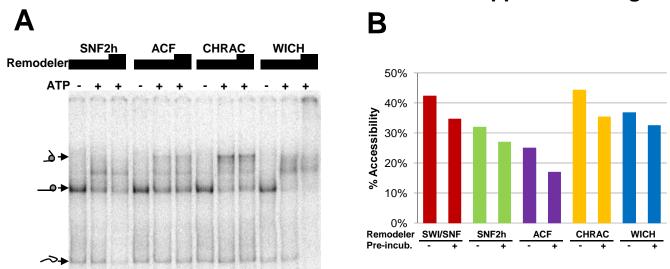
Supplemental Fig. 1



Supplemental Figure 1. (A) Mixed template EMSA assay for complete remodeling. Mononucleosomes were assembled onto radiolabeled linear templates bearing the 601 positioning sequence, along with 120 bp of flanking sequence DNA on one side (manuscript ref. (30)). We then combined our polynucleosomal template chromatin (at the standard final concentration) with a tracer amount of this labeled mononucleosome probe template, incubated each template mix with remodeler and/or ATP, separated the resulting products by PAGE and measured nucleosome positions on the probe fragment by EMSA. Because the NPS is at one edge of the probe template, the unremodeled mononucleosome has a tear-drop shape that runs quickly through the gel (-ATP lanes). As expected from prior studies (manuscript refs. (24,30,42)) remodeling of these mononucleosome probe templates by SNF2h, hACF, CHRAC or WICH, in the presence of ATP, results in movement of the histone octamer away from the 601 NPS and towards the center of the DNA fragment, producing a "C"-shaped nucleosomal product that runs slowly through the gel (+ATP lanes). In order to establish complex concentrations sufficient for complete remodeling (of both the polynucleosomal plasmid template and the probe mononucleosomal template), we titrated each SNF2h complex into the reaction until higher concentrations of remodeler no longer altered the nucleosome positions observed by EMSA. We then used a concentration at least two fold above the minimum required to give complete remodeling. Note that, while each complex shifts the histone octamer away from the edge of the template (slower migration), the pattern of bands does differ, which might indicate either differential sequence specificity for remodeling or differential effects of DNA ends on the remodeling outcome. (B) Remodelers remain active during a two-hour pre-incubation. Complexes were pre-incubated with ATP but without template for 2 hours, before measuring Pstl accessibility (as per Fig. 4B). The similarity in percent cutting (up from a non-remodeled level of ~5%) indicates that the complex remains active for the full 2 hour incubation used in our mapping studies.