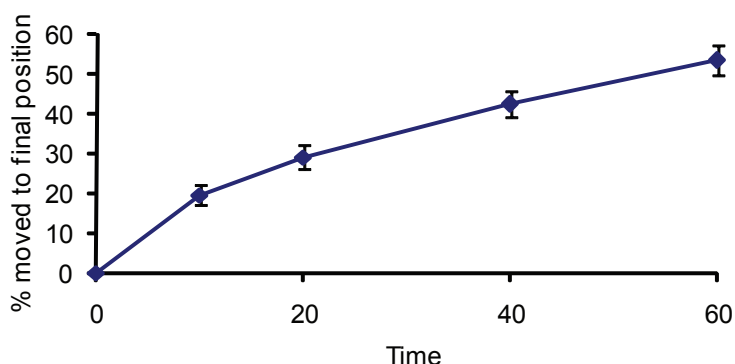
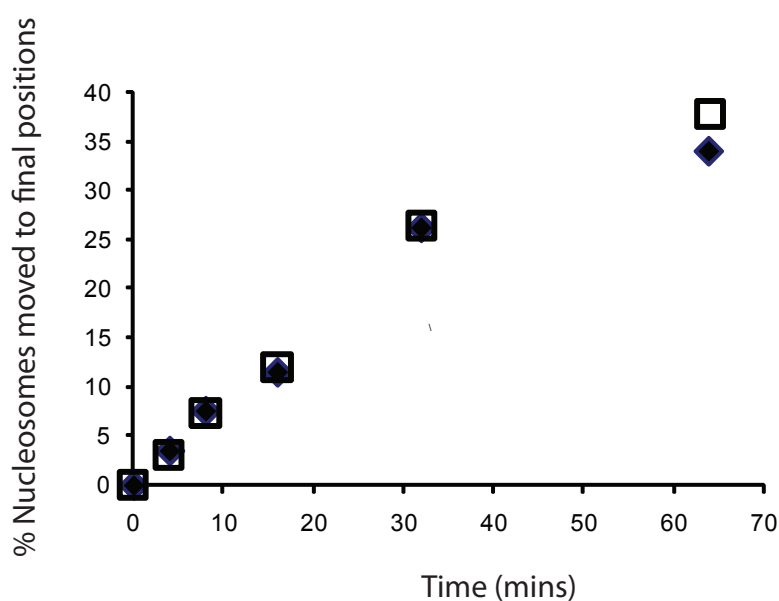


A

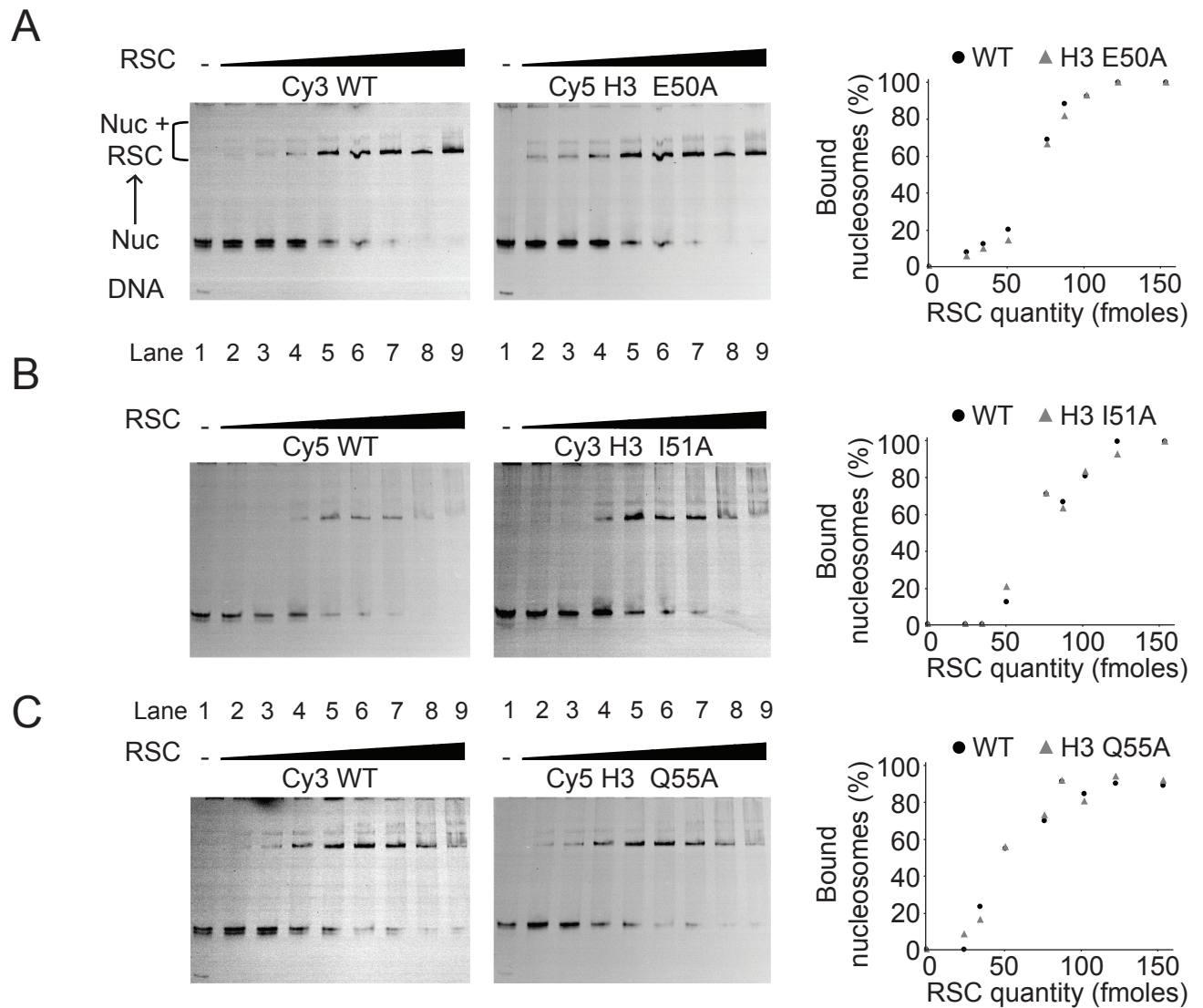


A) Graph showing the average rate of nucleosome sliding for 110 repeats of a thermal sliding assay carried out at 47°C for the indicated times. Error bars show the standard deviation, this is ranges from 7 to 13% of the overall value for each time point.

B



B) Graph showing illustrating reproducibility of remodelling reactions with RSC. Wild type nucleosomes were assembled on both cy3 (squares) and cy5 (diamonds) labelled DNA fragments and subject to remodelling with 0.44 fmoles RSC for the indicated times. The proportion of nucleosomes repositioned was calculated following native gel electrophoresis of the samples. Most of the points overlap indicating that the assay is sensitive enough to detect small changes in nucleosome sliding.



Supplementary Figure 2 Histone mutations do not affect the binding of RSC complex

A competitive binding reactions were set up in which RSC was incubated with wild type nucleosomes assembled on Cy3 labelled DNA and H3 E50A (A), I51A (B) and) Q55A (C) nucleosomes on cy5 labelled fragments. For each binding assay the quantity of RSC added per reaction is 0, 25, 35, 51, 77, 88, 102, 123, 154 fmoles, lanes 1-9, respectively. The quantification of the binding assay is shown in the graph to the right of each respective gel. WT, wild-type.

Supplementary method

Competitive binding assays

Wild-type and mutant nucleosomes were assembled using 0W0 DNA fragments that were end-labelled with cy3 or cy5 fluorescence. To quantitate nucleosome assembly, 2 pmoles of each reconstitution was resolved on a 5% native polyacrylamide gel and the band intensity measured relative to 1 pmole of 0W0 DNA. Each binding reaction was prepared on ice and contained 25 fmole of wild-type and mutant nucleosomes, 20 mM Tris pH 7.5, 20 mM NaCl, 3 mM MgCl₂, 3% (w/v) Ficoll-400, 1 X BSA and the quantities of RSC specified in Supplementary Figure 1. Samples were incubated on ice for 10 minutes before loading onto a binding assay gel (3.5% acrylamide:bis acrylamide (49:1 ratio), 1 X HI buffer (43 mM imidazole, 35 mM HEPES, pH 7.4), 0.1% APS, 0.1% TEMED). The gels were cast between 10 by 8 cm glass plates at 0.75 mm thickness (BioRad) and pre-run with 1 X HI buffer for 30 minutes at 170 Volts, followed by immediate loading and running for a further 35 minutes.