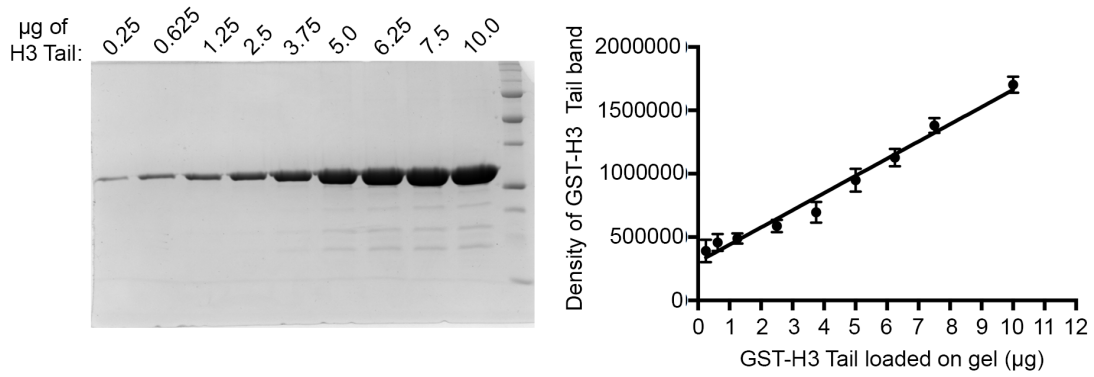
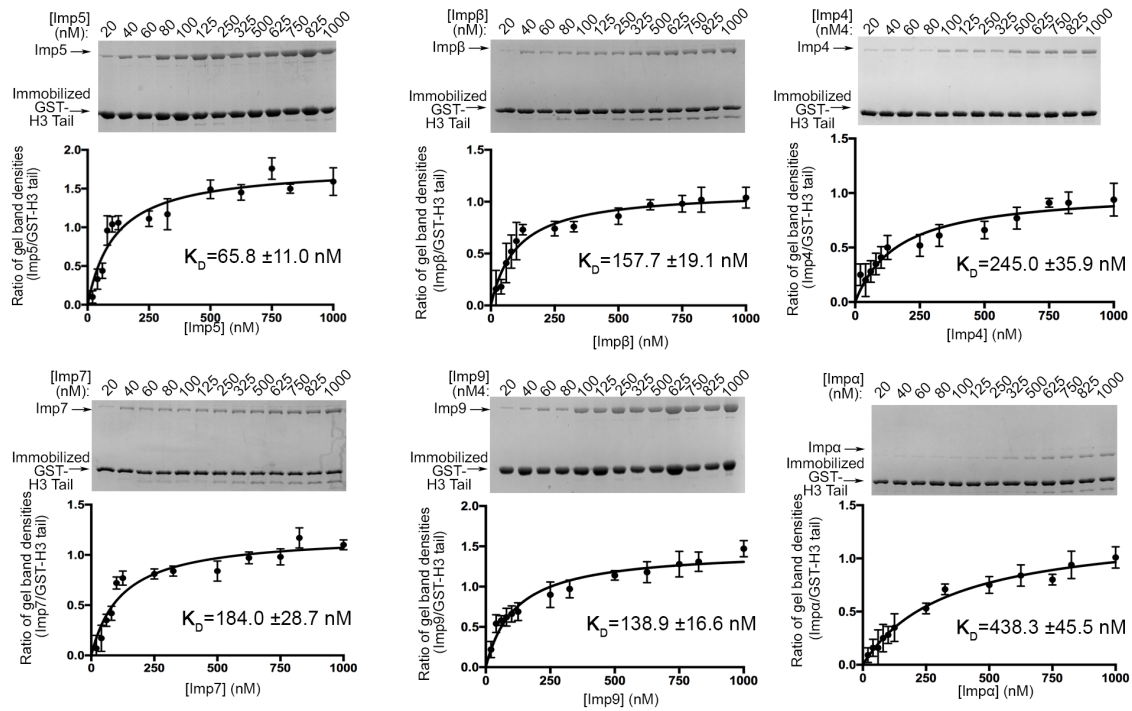


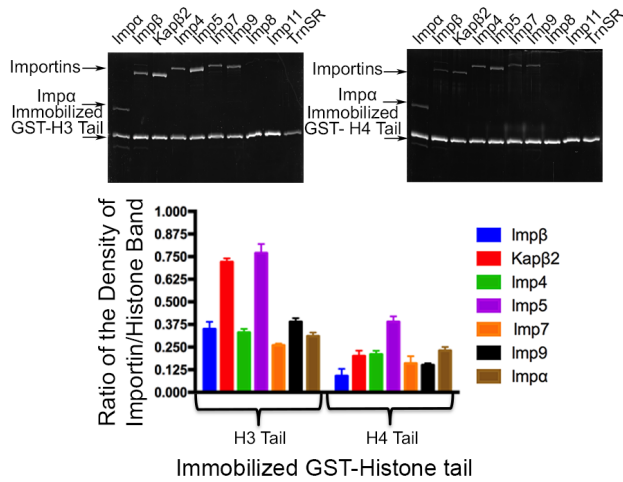
Supplementary Fig. 1. Control experiments to examine potential non-specific binding of Importins with Glutathione-sepharose beads. 40 μ L of glutathione-sepharose 4B beads were incubated with \sim 4 μ M of each purified Importin in TB buffer in total volumes of 100 μ L for 30 min at 4°C, followed by extensive washing with TB buffer. Bound proteins were visualized using SDS-PAGE/Coomassie blue.



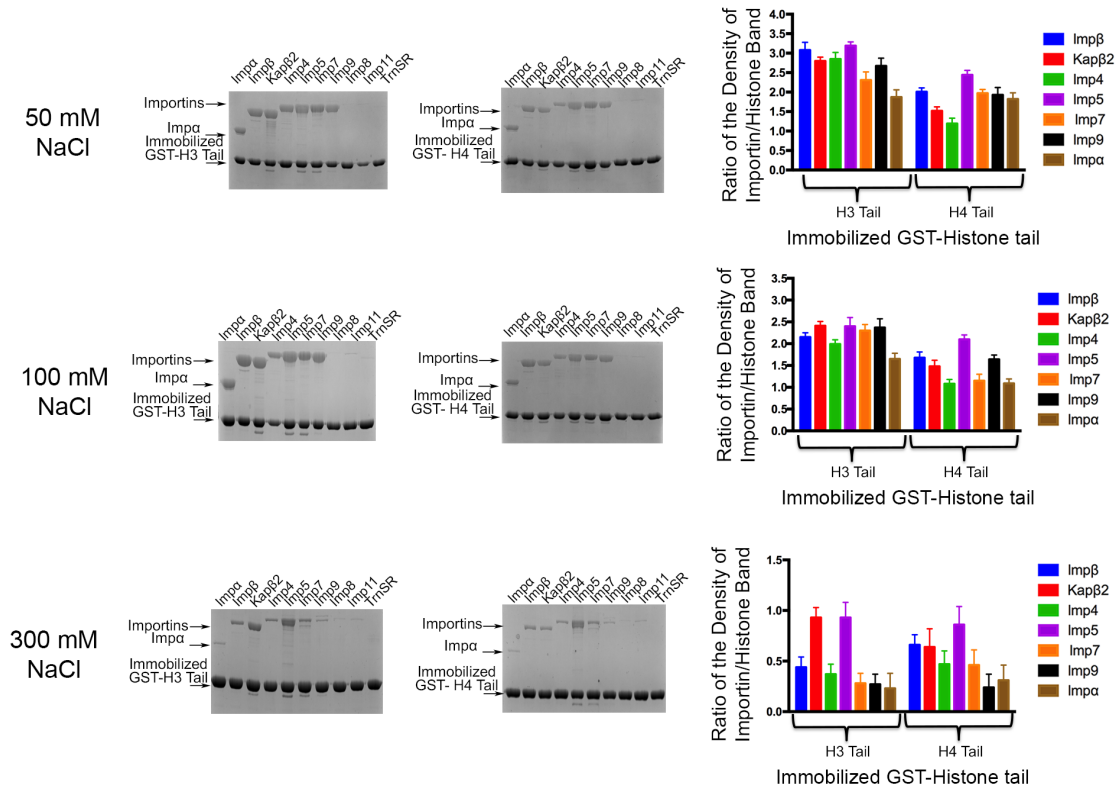
Supplementary Fig. 2. The linearity of Coomassie Blue staining. Concentrations of GST-H3 tail were first determined using two different methods: 1) absorbance at 280 nm and 2) quantitative Bradford protein assay (standard curve generated with BSA controls). 0.25 µg, 0.625 µg, 1.25 µg, 2.5 µg, 3.75 µg, 5.0 µg, 6.25 µg, 7.5 µg, and 10 µg of GST-H3 were loaded on SDS-PAGE gels and stained with Coomassie Blue. The experiment was performed three times and the densities of the protein bands (analyzed with ImageJ) are plotted.



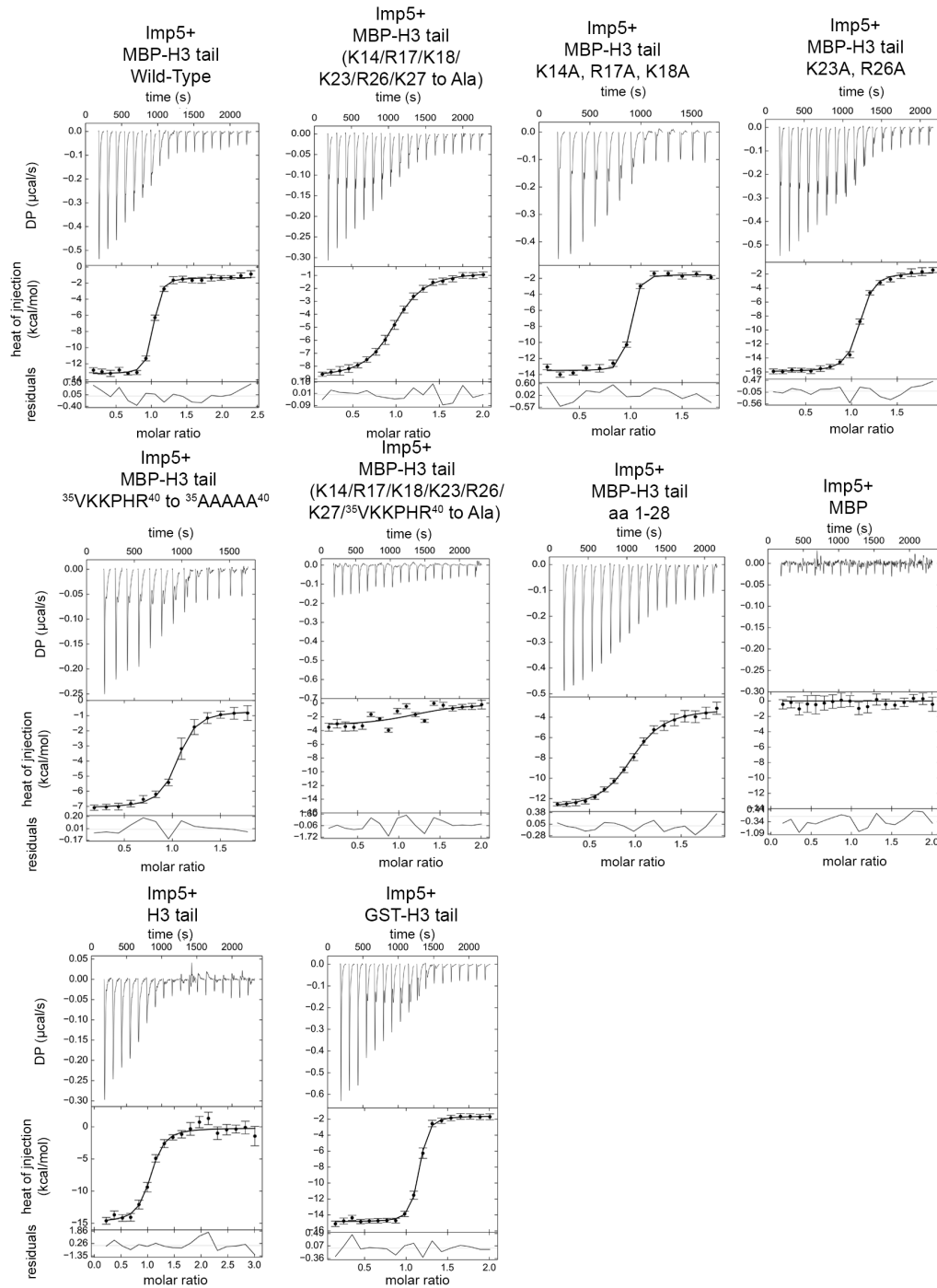
Supplementary Fig. 3. Estimation of Apparent K_D s for each Importin with the H3 tail from pull-down binding assay data. 20 nM to 1 μ M of each Importin in total volumes 0.4–15 ml (to ensure molar excess of Importins to the H3 tail) was titrated onto 0.2 nmole of GST-H3 tail immobilized on glutathione sepharose beads in a series of pull-down binding assays. Each Importin was mixed with the GST-H3 tail at 4°C for 12 hours, followed by washing of the beads and separation of bound proteins by SDS-PAGE (Coomassie blue stained). Relative densities of the gel bands from three separate experiments were measured using ImageJ. The data was fitted to a simple bimolecular equilibrium relationship in Prism Graphpad to obtain the apparent K_D .



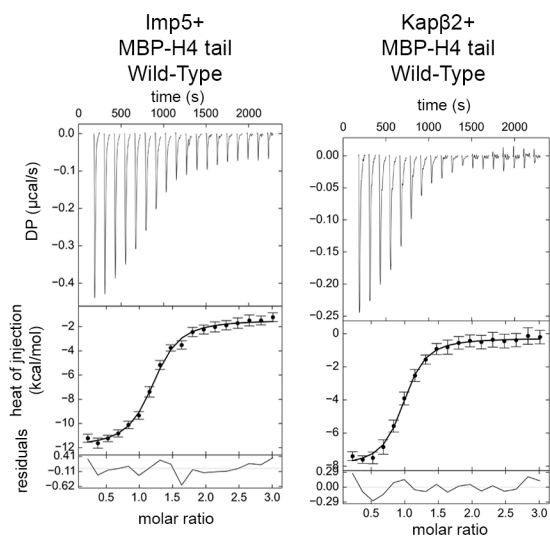
Supplementary Fig. 4. Importin-histone pull-down assays with 1/10 of the proteins. Pull-down binding assays were performed by incubating 20 pmole of immobilized GST-H3 tail or immobilized GST-H4 tail with 50 nM of each purified recombinant Importins in TB buffer. Bound proteins were separated by SDS-PAGE and stained with SYPRO Ruby protein gel stain. The protein bands were analyzed with ImageJ. The overall trend of Importin-histone binding using 10-fold less proteins is similar to the Importin-H3 tail affinity trend (Kapβ2, Imp5 > Impβ, Imp9, Impα > Imp4, Imp7) and the Importin-H4 tail affinity trend (Imp5 > Imp9, Impα > Impβ, Kapβ2, Imp4, Imp7) seen in Figure 1B.



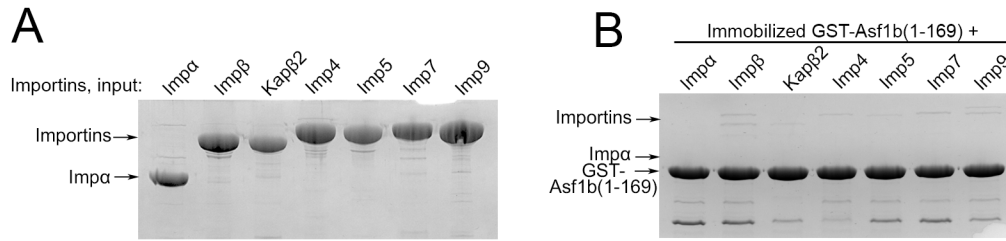
Supplementary Fig. 5. Importin-histone interactions are salt-dependent. Pull-down binding assays of immobilized GST-H3 tail (250 nM) and immobilized GST-H4 tail (250 nM) with 500 nM of each recombinant Importins in TB buffer with 50 mM, 100 mM, and 300 mM NaCl (SDS-PAGE/Coomassie Blue). Relative densities of the gel bands from three separate experiments are plotted in histograms. High salt (300 mM NaCl) decreased binding to all seven Importins to while salt concentrations (50 mM and 100 mM) increased Importin binding compared to experiments shown in Figure 1B, which were performed at 150 mM NaCl. These results are consistent with the dominance of electrostatic interactions in Importin-H3 tail binding, shown by the crystal structure of Kapβ2-H3 tail and by mutagenic analysis (Soniati and Chook, 2016).



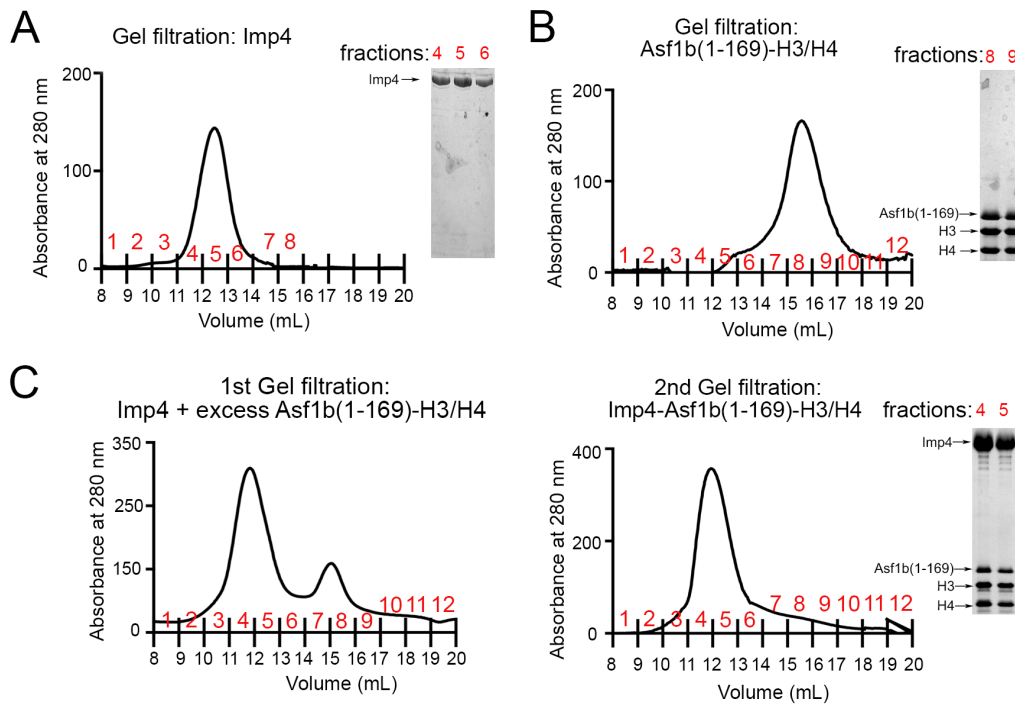
Supplementary Fig. 6. Representative ITC measurements of MBP-H3 tail, MBP, and H3 tail (no MBP tag) binding to Imp5. After dialysis against the same buffer, 200-400 μM H3 proteins were titrated into a sample cell containing 20-40 μM recombinant Imp5. ITC experiments were performed at 20°C with 19 rounds of 4- μl injections. Data were plotted and analyzed using NITPIC and Sedphat. Data was visualized by GUSLI.



Supplementary Fig. 7. Representative ITC measurements of MBP-H4 tail binding to Imp5 or Kap β 2. After dialyzed against the same buffer, 200-400 μ M H4 tail proteins were titrated into a sample cell containing 20–40 μ M recombinant Imp5 or Kap β 2. ITC experiments were performed at 20°C with 19 rounds of 4- μ l injections. Data were plotted and analyzed using NITPIC and Sedphat. Data was visualized by GUSSI.



Supplementary Fig. 8. A) 5 μ L samples of the purified recombinant Importins (8 μ M) were loaded in each lane of the gel. The amount of protein in each lane is \sim 10% of the protein used in Asf1b(1-169)-H3/H4 binding assays (**Figures 6A-C**). **B)** Pull-down binding assays of immobilized GST-Asf1b(1-169) with all seven Importins. No binding is observed between GST-Asf1b(1-169) and any of the Importins.



Supplementary Fig. 9. Assembly and Purification of the Imp4-Asf1b(1-169)-H3/H4 complex. **A**) Gel filtration (Superdex 200, GE Healthcare) chromatogram of purified Imp4. The SDS/PAGE gel of fractions 4-6 was Coomassie-stained. Imp4 elutes at 12.5 ml, consistent with a MW of 118 kDa **B**) Gel filtration (Superdex 200) chromatogram of the Asf1b(1-169)-H3/H4 complex. The SDS/PAGE gel of fractions 8 and 9 was Coomassie-stained. Elution volume of The Asf1b(1-169)-H3/H4 complex elutes at 15.6 ml, consistent with MW 46 kDa of a 1:1:1 heterotrimer. The Asf1b(1-169)-H3/H4 complex was prepared by mixing purified *X. laevis* H3/H4 tetramers with GST-Asf1b(1-169) that was immobilized on GSH sepharose beads (1:3 molar ratio) followed by extensive washing to remove the excess histones. The immobilized GST-Asf1b(1-169)-H3/H4 complex was cleaved with Tev protease to release Asf1b(1-169)-H3/H4 from the beads. The chaperone-histone complex was then subjected to gel filtration chromatography. **C**) Two consecutive gel filtration chromatography steps to purify the Imp4-Asf1b(1-169)-H3/H4 complex. The 4-protein complex was first assembled by mixing purified Imp4 with molar excess of GST-Asf1b(1-169)-H3/H4 that is immobilized on GSH sepharose beads. After washing, the Imp4-GST-Asf1b(1-169)-H3/H4 complex was cleaved with TEV protease to release Imp4-Asf1b(1-169)-H3/H4 and excess Asf1b(1-169)-H3/H4 from the beads. The mixture of complexes was separated in the first gel-filtration (Superdex 200; chromatogram on the left). Fractions 4 and 5 containing Imp4-Asf1b(1-169)-H3/H4 were pooled and subjected to a second round of gel filtration (Superdex 200; chromatogram on the right) to ensure homogeneity and stability of the complex. The SDS/PAGE gel of fractions 4-5 from the 2nd column was Coomassie-stained. The Imp4-Asf1b(1-169)-H3/H4 complex elutes at 11.9 ml, consistent with MW 164 kDa of a 1:1:1:1 heterotetrameric complex.

References

Soniat, M., and Chook, Y.M. (2016). Karyopherin- β 2 recognition of a PY-NLS variant that lacks the proline-tyrosine motif. *Structure*. In Press