

Fig. S1. Identification of three segments in Sen1 that interact with Nrd1 CID. Related to Fig. 1. (a). Gel filtration profiles of Sen1 alone (blue), Nrd1-Nab3 (gray) and their mixture (red). The migration positions of Sen1 and Nrd1-Nab3 in the mixture are identical to those of them alone. There are no peaks for larger complexes in the mixture. The second peak for the Nrd1-Nab3 sample is excess Nab3. (b). SDS-PAGE of fractions from gel filtration on the Sen1 and Nrd1-Nab3 mixture shown in panel a. (c-e). Mixtures of other purified Sen1 and Nrd1-Nab3 proteins were run on a gel filtration column, and the indicated fractions were resolved by SDS-PAGE. Panels a and b are for *K. lactis* proteins, and all other panels for *S. cerevisiae* proteins.

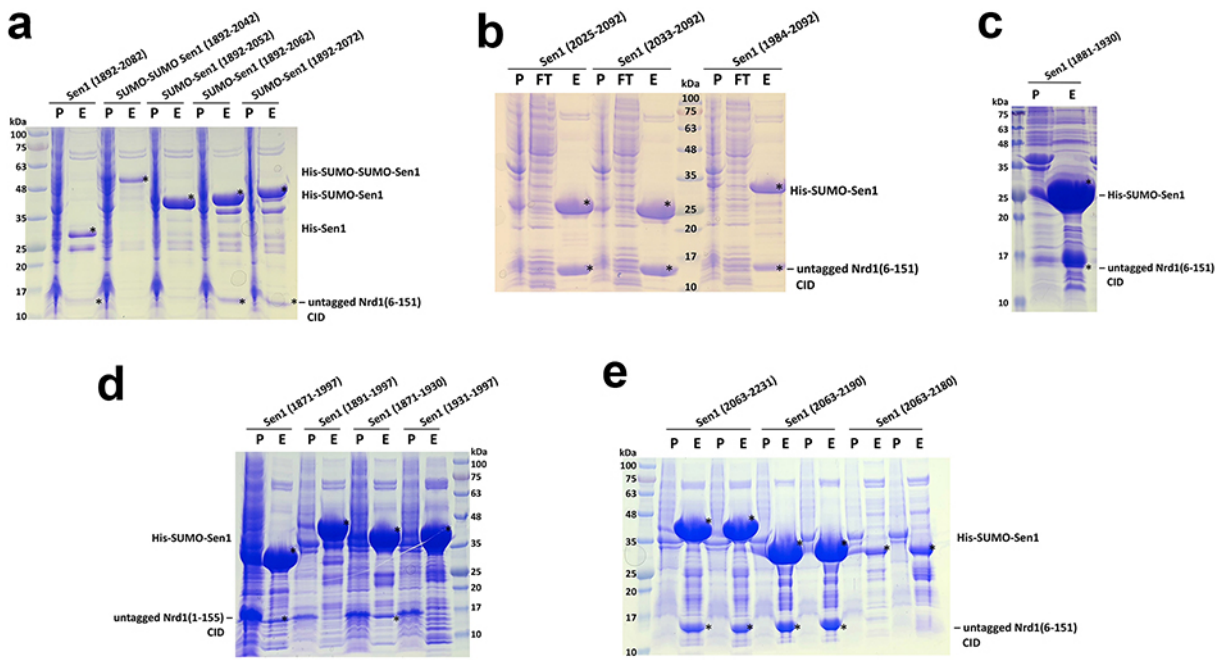


Fig. S2. Identification of three segments in Sen1 that interact with Nrd1 CID. Related to Fig. 1. (a-e). His-tagged Sen1 were co-expressed with untagged Nrd1 CID to map their regions of interaction. P: pellet, E: Ni column eluate, FT: flow through. Panel d is for *K. lactis* proteins, and all other panels for *S. cerevisiae* proteins.

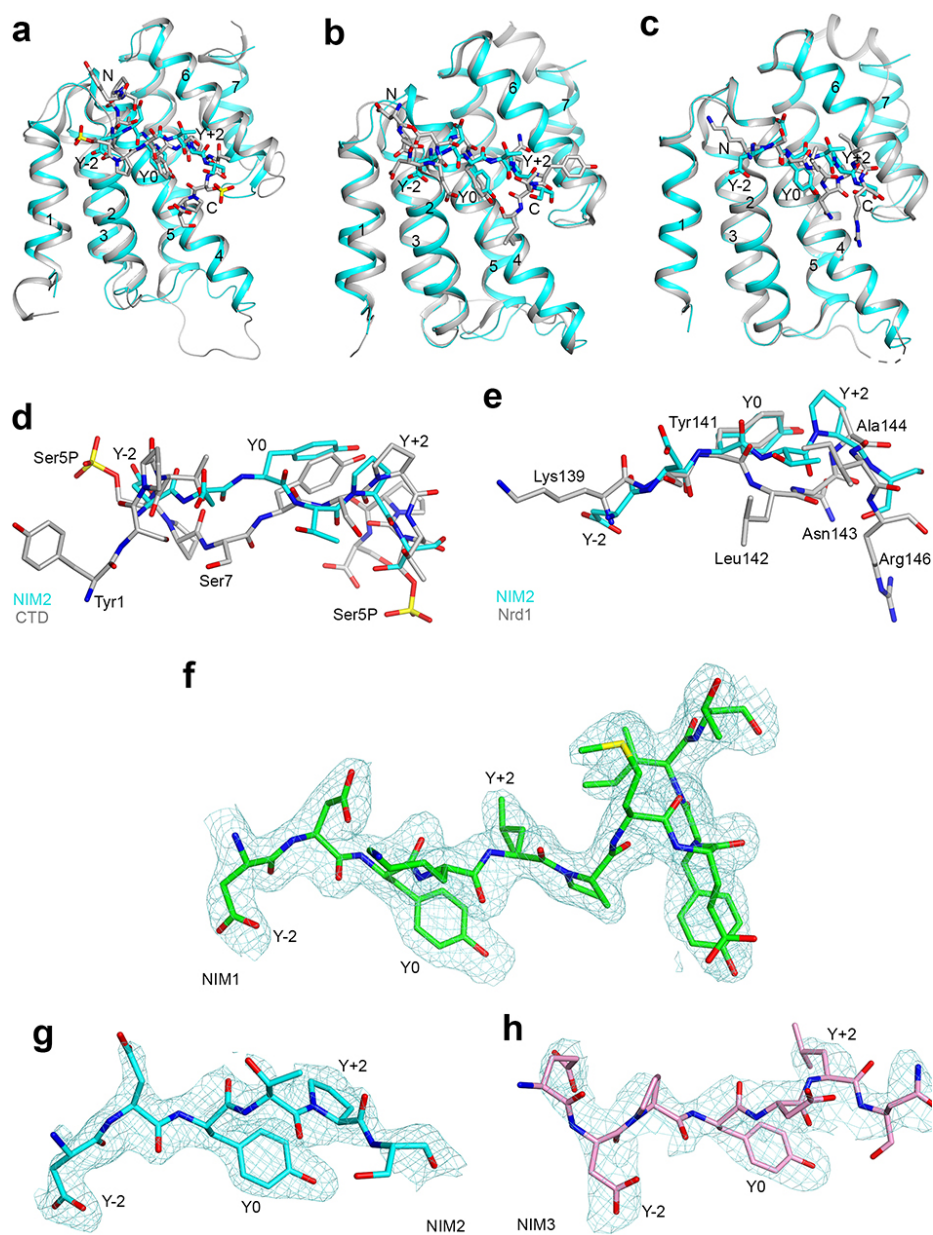


Fig. S3. Comparison of the binding modes of other sequence motifs to Nrd1 CID. Related to Fig. 1. (a). Overlay of the NIM2 complex (cyan) with the Ser5P CTD complex (gray). (b). Overlay of the NIM2 complex (cyan) with the Trf4 NIM complex (gray). (c). Overlay of the NIM2 complex (cyan) with the Nrd1 CID in complex with residues 139-146 from another molecule by crystal packing (gray). These residues are in helix $\alpha 7$, which has become partially unwound. (d). Overlay of the binding modes of NIM2 (cyan) with the Ser5P CTD (gray). (e). Overlay of the binding modes of NIM2 (cyan) with the Nrd1 CID residues 139-146 from crystal packing (gray). Omit Fo–Fc electron density map for (f) NIM1 at 2.1 Å resolution, contoured at 2.5σ ; (g) NIM2 at 2.0 Å resolution, contoured at 2.5σ ; (h) NIM3 at 2.8 Å resolution, contoured at 2σ .

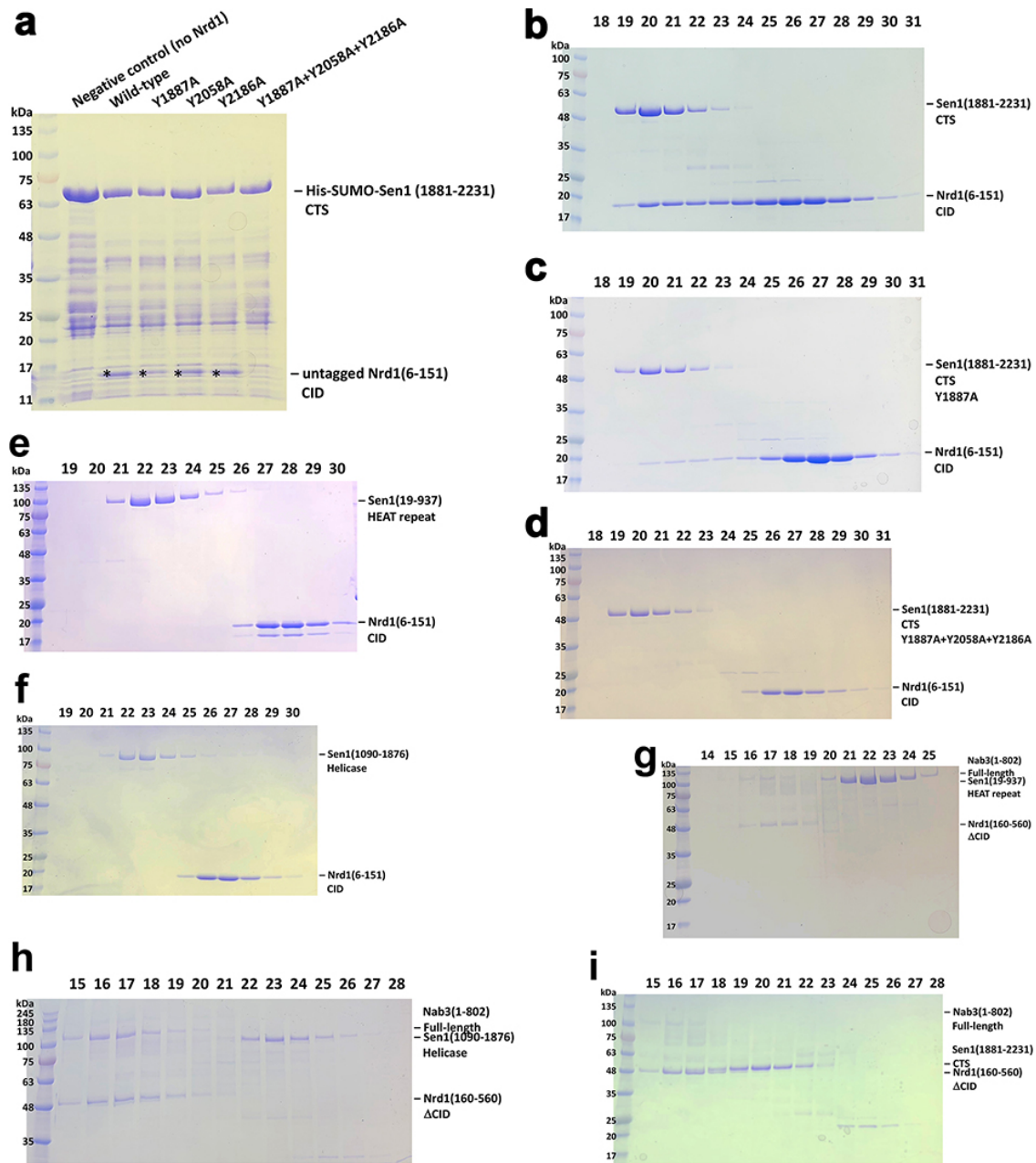


Fig. S4. Mutation of all three NIMs is necessary to block Sen1-Nrd1 interaction and Sen1 interacts with Nrd1 only through the CID. Related to Fig. 1. (a). His-tagged Sen1 CTS wild-type and various NIM mutants were co-expressed with untagged Nrd1 CID. The Ni column eluate was then resolved on SDS-PAGE. **(b-d).** Mixing experiments with purified Sen1 CTS (wild-type and indicated mutants) with Nrd1 CID. **(e-i).** Mixtures of purified Sen1, Nrd1 and Nab3 proteins were run on a gel filtration column, and the indicated fractions were resolved by SDS-PAGE. **(e).** No interaction between Sen1 HEAT repeat domain and Nrd1 CID. **(f).** No interaction between Sen1 helicase domain and CID. **(g).** No ternary complex among full-length Nab3, Sen1 HEAT repeat domain, and Nrd1 lacking the CID. **(h).** No ternary complex among full-length Nab3, Sen1 helicase domain, and Nrd1 lacking the CID. **(i).** No ternary complex among full-length Nab3, Sen1 CTS, and Nrd1 lacking the CID.