Supplementary Information

The mechanism for autoinhibition and activation of the MORC3 ATPase

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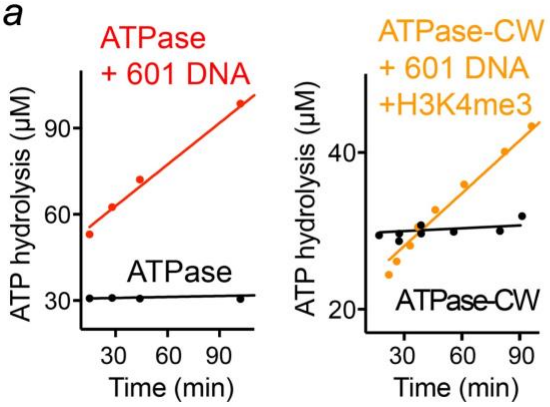
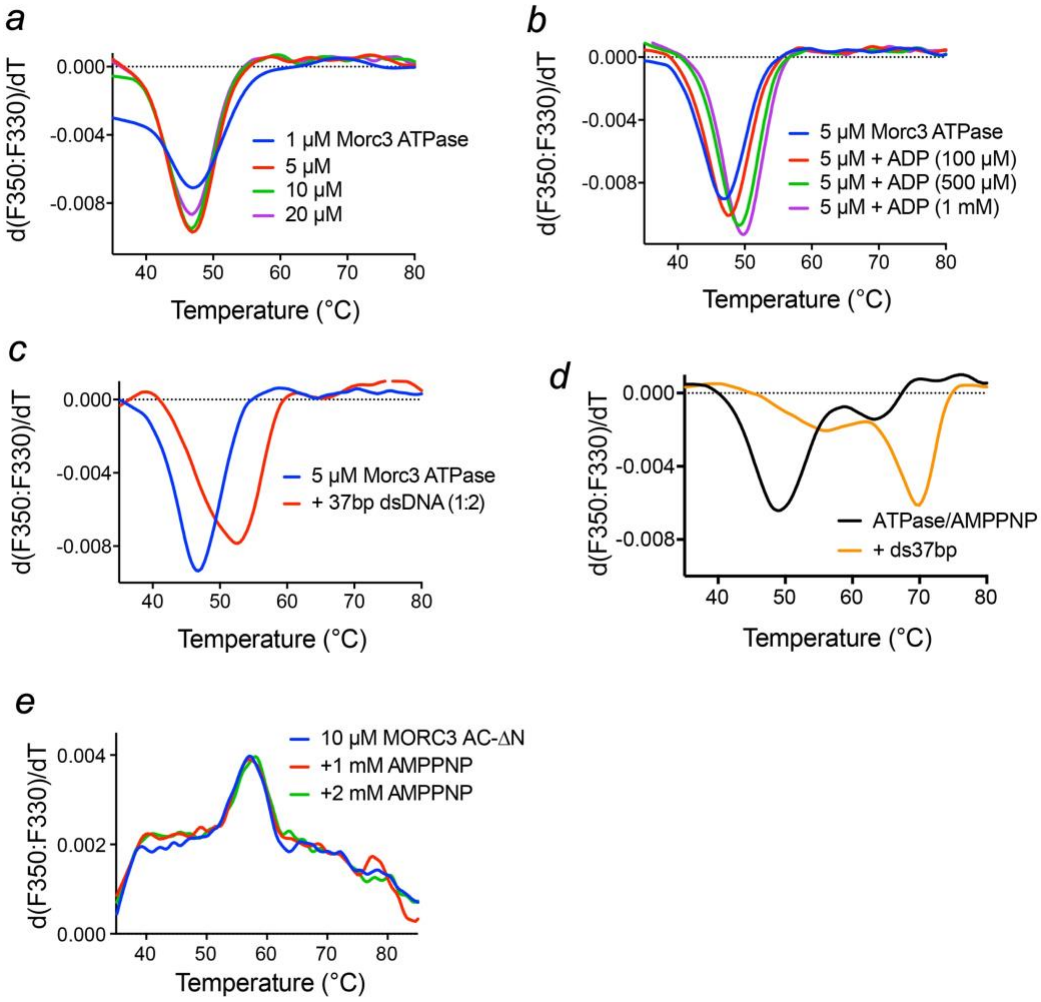
a



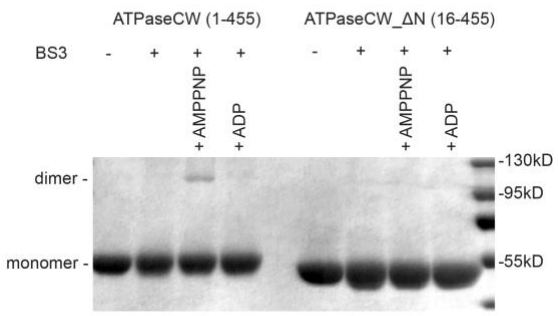
b

Rate of ATP hydrolysis (μM/min) per 1 μM enzyme	
ATPase	0.020 ± 0.013
ATPase + 601 DNA	0.481 ± 0.074
ATPase-CW	0.033 ± 0.007
ATPase-CW + H3	0.037 ± 0.011
ATPase-CW + 601 DNA	0.049 ± 0.018
ATPase-CW + 601 DNA + H3	0.175 ± 0.029
ATPase-CW + 601 DNA + H3K4me3	0.214 ± 0.006

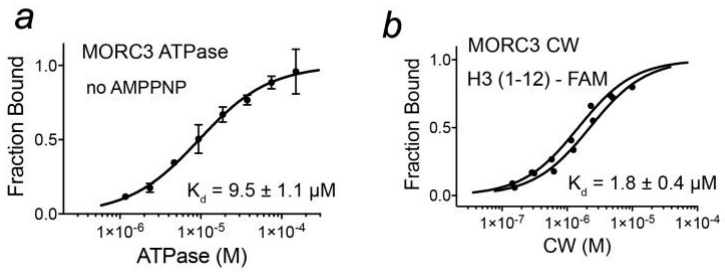
Supplementary Figure S1. (a) Representative plots of ATP hydrolysis by the ATPase domain of MORC3 (left) and the ATPase-CW cassette of MORC3 in the presence of 601 DNA and H3K4me3 peptide (right). (b) Rates of ATP hydrolysis by indicated domains of MORC3. Error represents S.D. of at least three separate experiments.



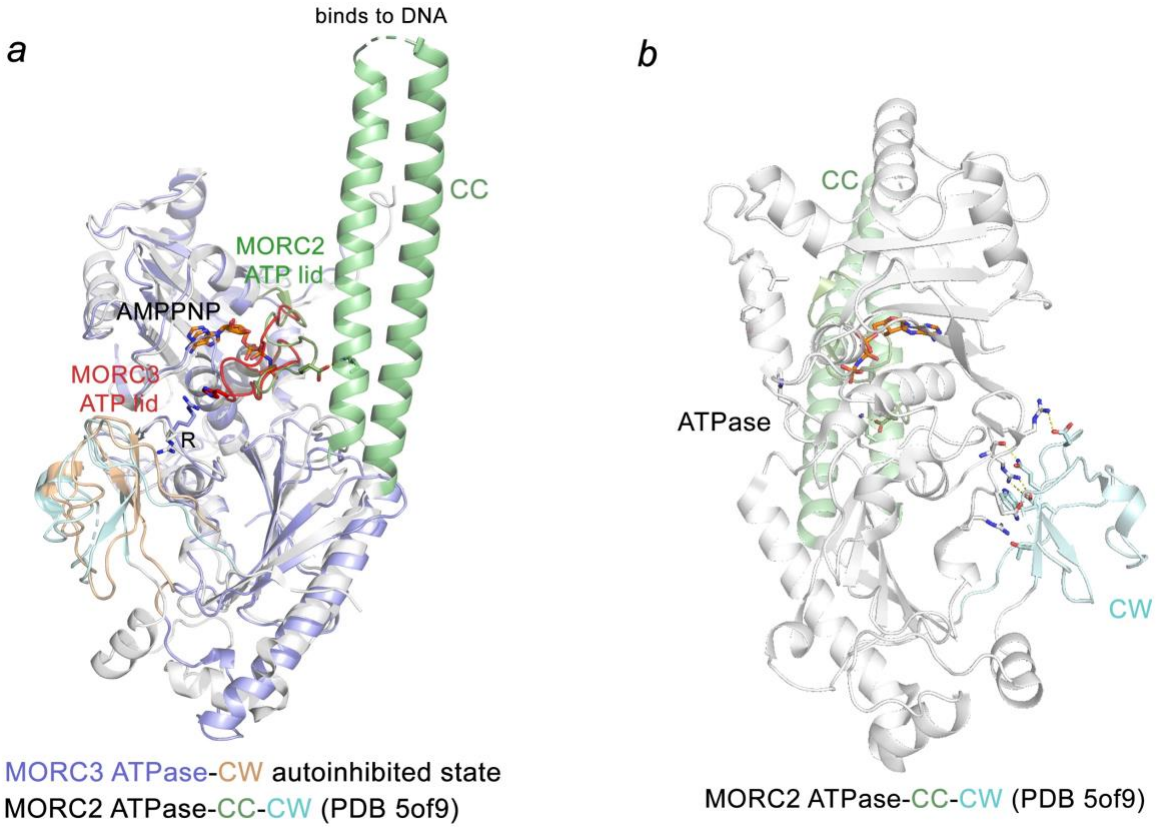
Supplementary Figure S2. (a) MORC3 ATPase stability vs. increased concentration monitored by DSF. (b) Binding of ADP to MORC3 ATPase monitored by DSF. (c, d) MORC3 ATPase binding to the 37-bp double stranded DNA fragments in the absence (c) or presence of AMPPNP (d) monitored by DSF. (e) Binding of AMPPNP to N-terminally truncated Morc3 ATPase-CW monitored by DSF.



Supplementary Figure S3. Cross-linking assays using WT or N-terminally truncated MORC3 ATPase-CW in the absence or presence of AMPPNP and ADP. Only AMPPNP induced dimerization.



Supplementary Figure S4. MST binding curves used to determine K_d for the MORC3 ATPase dimerization (a) and MORC3 CW binding to histone H3 (1-12) peptide (b). Error represents S.D. between three separate experiments; K_d values ± S.D. are shown.



Supplementary Figure S5. (a) MORC3 and MORC2 have distinctly different mechanisms of action. Structural overlay of the autoinhibited state of the MORC3 ATPase-CW cassette (this study, light blue and wheat) and the MORC2 ATPase-CC-CW region (PDB ID: 5of9, white, light green and cyan)[20]. In contrast to MORC3, the CW domain of MORC2 does not recognize histone tails, and the CC insertion of MORC2 binds to DNA and contacts the dimeric interface and the ATP lid region. An arginine that forms a salt bridge critical for the interaction of CW and ATPase in MORC2, is in close proximity to the ATP lid of MORC3. (b) A ribbon diagram of the monomer A of MORC2 ATPase-CC-CW (PDB ID: 5of9, white, light green and cyan)[20] in the same orientation as the MORC3 ATPase-CW cassette in Figure 2a. The ATPase:CW interface

residues are shown in stick representation. Dash lines indicate hydrogen bonds and salt bridges.