Supplementary information

MCM double hexamer loading visualized with human proteins
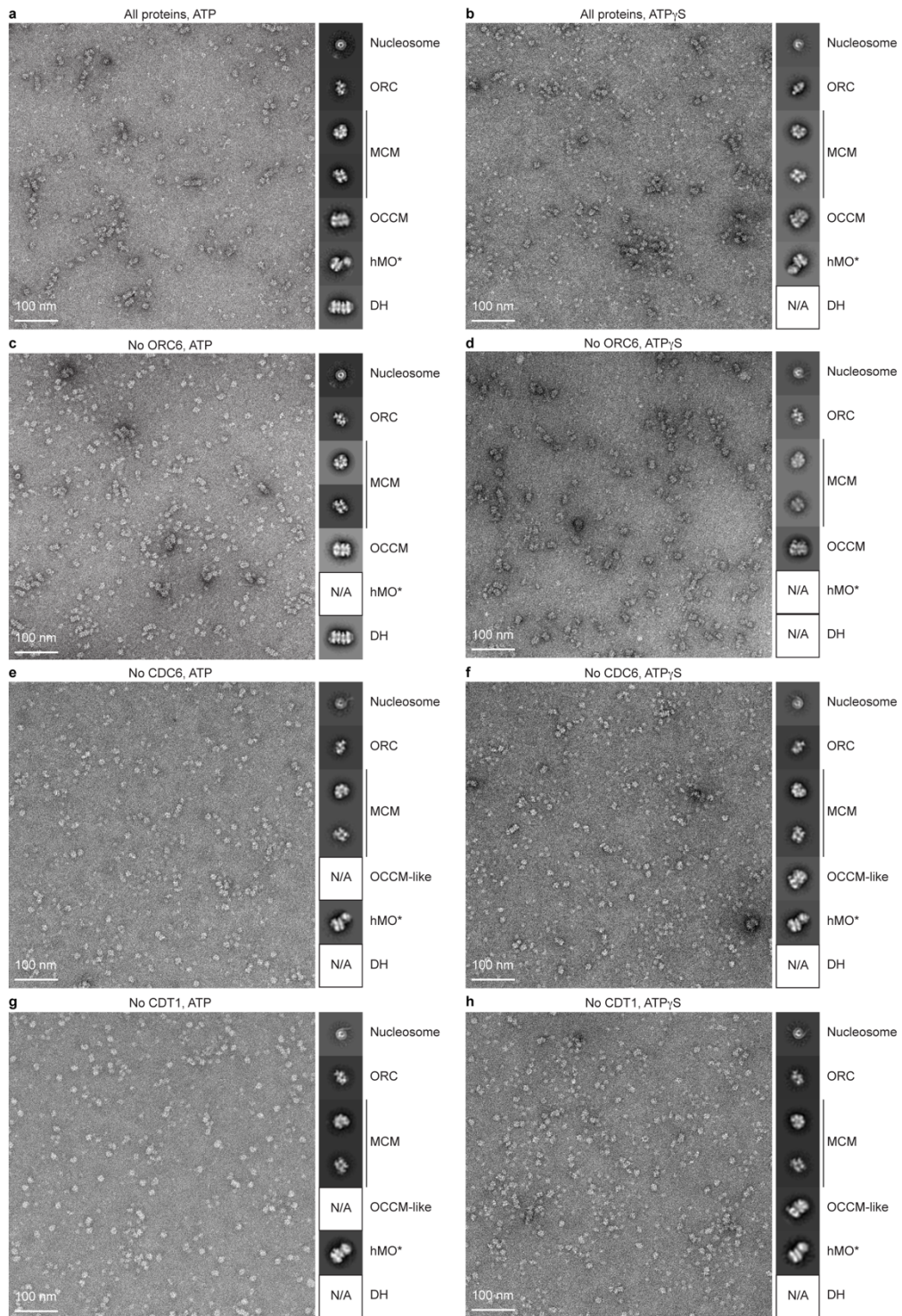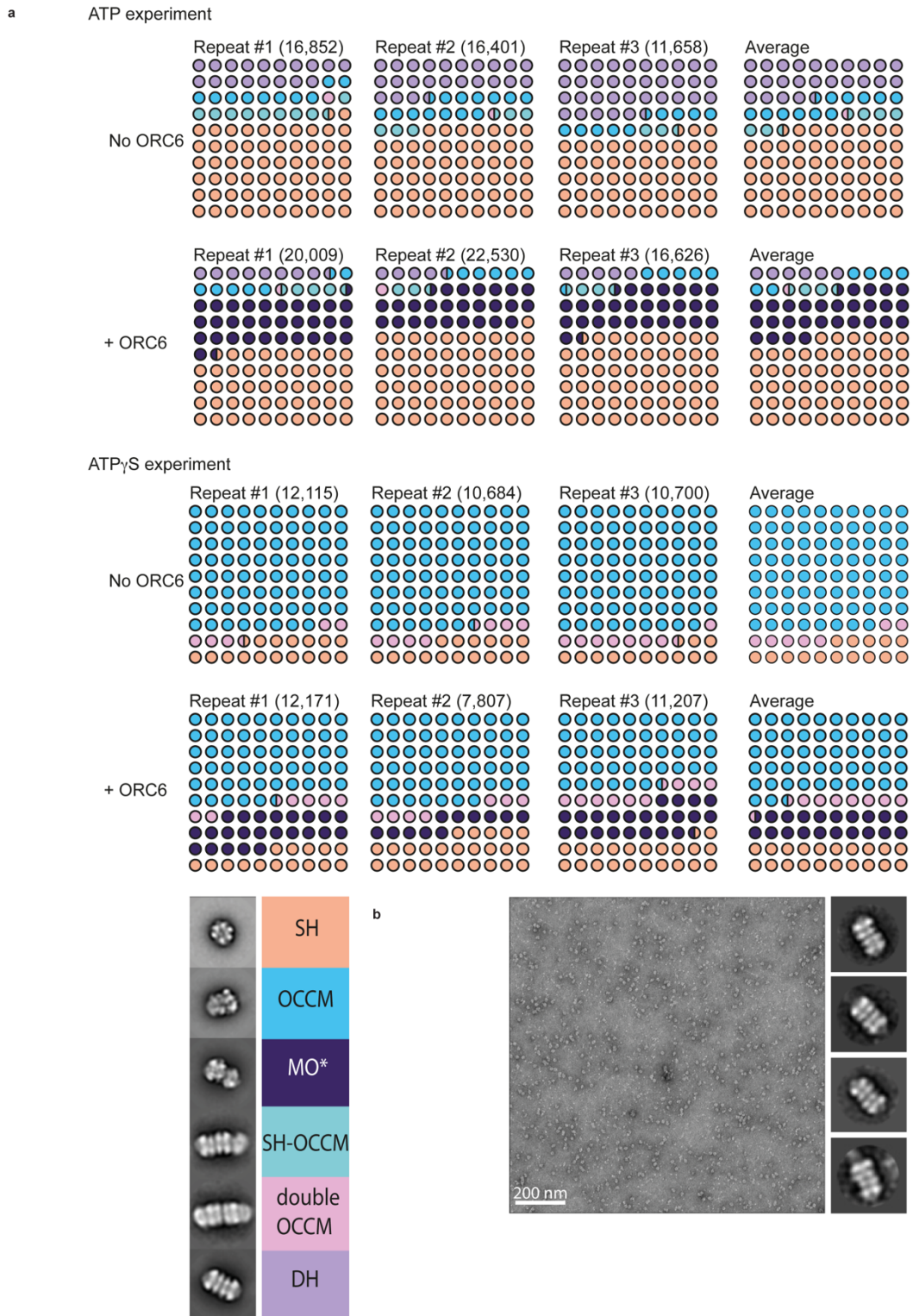
Supplementary Fig. 1 Representative negative-stain EM micrographs and 2D averages

***of human MCM loading reactions (ΔN) performed in EM buffer showing particles
observed under the indicated reaction conditions.***

a, Nucleosomes, ORC, MCM, OCCM, hMO* and DH classes were observed in the presence
of ATP and all proteins. **b**, All complexes apart from DH were present when the slowly
hydrolysable ATP analogue, ATPγS, was used. **c, d**, Without ORC6, DH classes were
visible in the presence of ATP (**c**). No hMO* had formed when ORC6 was omitted, either in
the presence of ATP (**c**) or ATPγS (**d**). **e,** No DH were observed in the absence of CDC6 in
ATP under these conditions. **f**, In ATPγS conditions, OCCM-like and hMO* class averages
were visible in the absence of CDC6. **g**, DH formation depends on CDT1 in ATP conditions.
h, Similar to the experiments without CDC6, OCCM-like and hMO* class averages were
detected when CDT1 was omitted in ATPγS conditions.

a ATP experiment

Repeat #1 (16,852) Repeat #2 (16,401) Repeat #3 (11,658) Average

No ORC6

Repeat #1 (20,009) Repeat #2 (22,530) Repeat #3 (16,626) Average

+ ORC6

ATPγS experiment

Repeat #1 (12,115) Repeat #2 (10,684) Repeat #3 (10,700) Average

No ORC6

Repeat #1 (12,171) Repeat #2 (7,807) Repeat #3 (11,207) Average

+ ORC6

SH

OCCM

MO*

SH-OCCM

double OCCM

DH

b

200 nm

Supplementary Fig. 2 Negative stain EM analysis of hDH loading without ORC6 and Cdc6.

(a) ORC6 dropout experiment in ATP and ATPγS. Three individual MCM loading reactions were carried out per experimental condition (+/- ORC6 in ATP or ATPgS) and 2D classes of all MCM species were calculated. The amount of different MCM species (DH = violet, single OCCM = cyan, double OCCM = pink, SH-OCCM = turquois, MO* = dark blue, single MCM = peach) are represented as fraction of all well-averaging particles in a 10x10 dot plot (1 dot corresponding to 1% of well-averaging particles) for each repeat and as average per condition. Representative 2D classes are highlighted for each detected MCM species. **(b)** CDC6 dropout experiment. Representative negative-stain micrograph of a MCM double hexamer loading reaction in absence of CDC6 and ORC6 (scale bar = 200 nm). 2D class averages of the human MCM double hexamer were detected under these conditions.
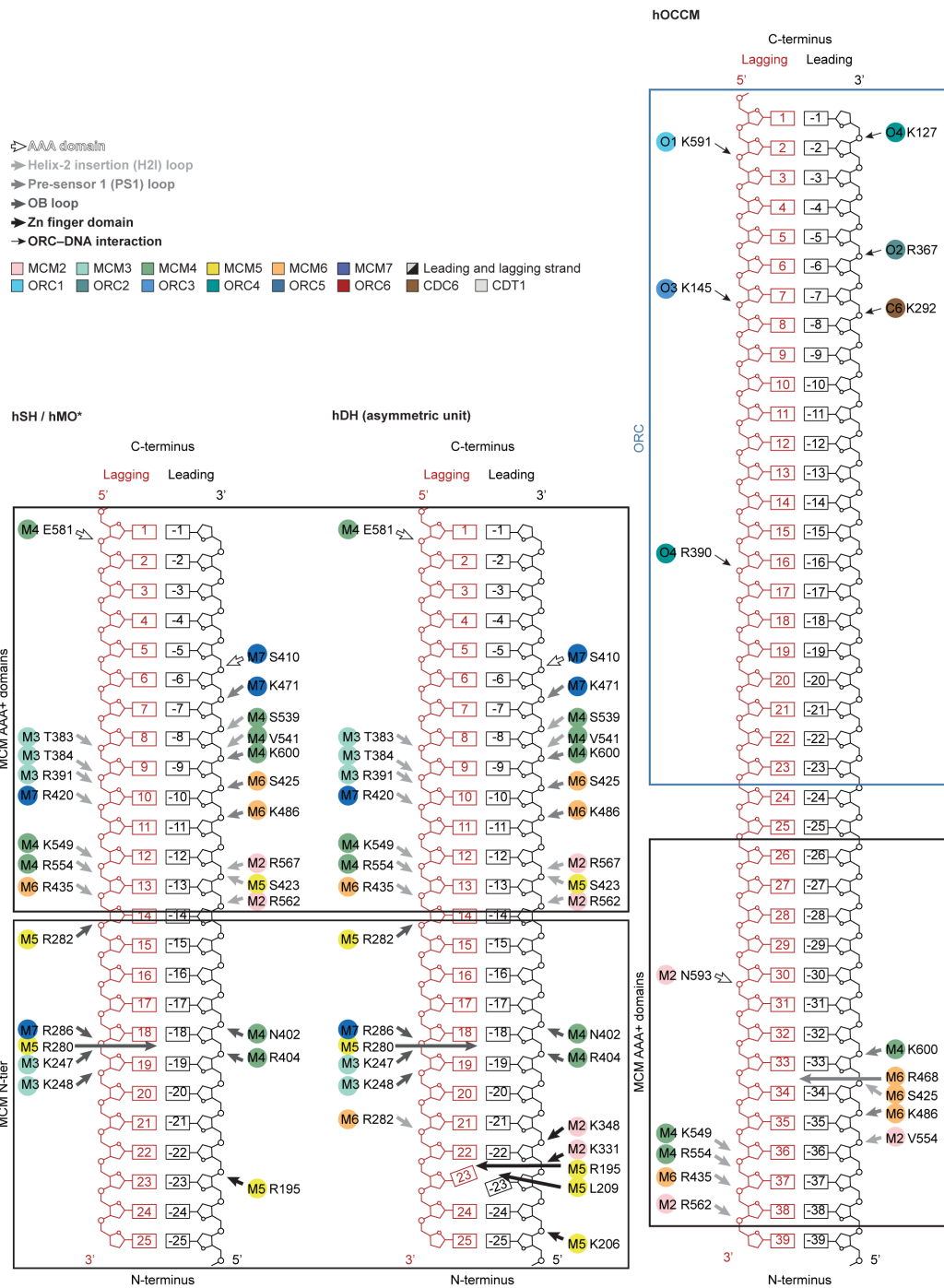
Supplementary Fig.3 Protein–DNA interactions within the hSH, hMO*, hDH (asymmetric unit) and hOCCM.

Source data
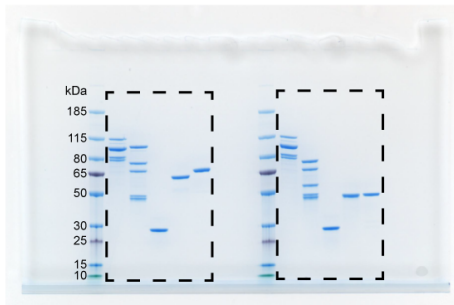
Fig. 1a (left)   Fig. 1a (right)
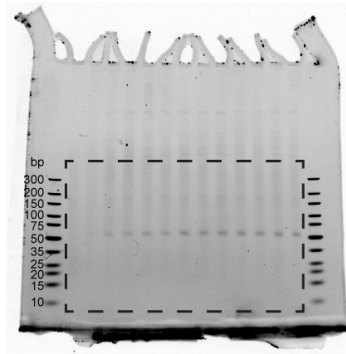
Fig. 1c (top)
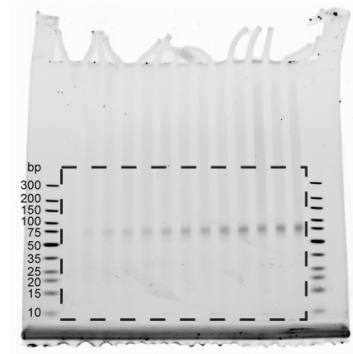
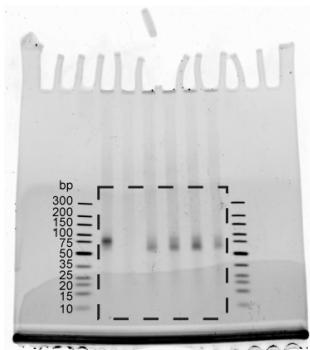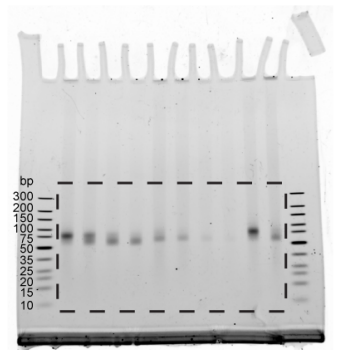
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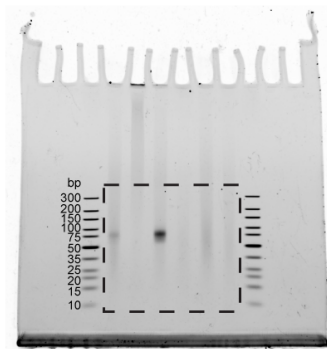
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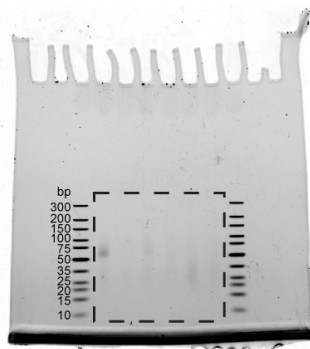
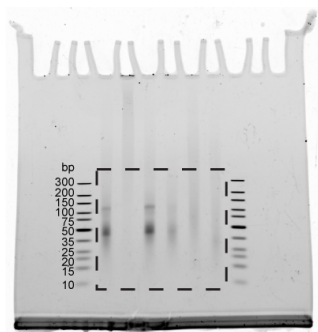Fig. 1e
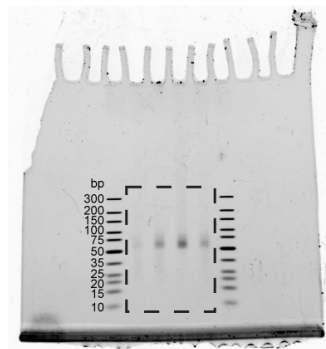
Fig. 1f
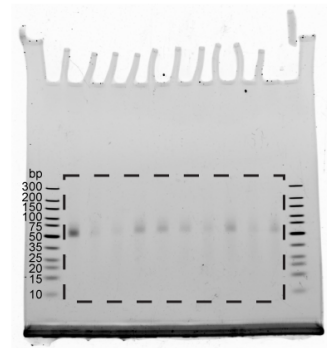
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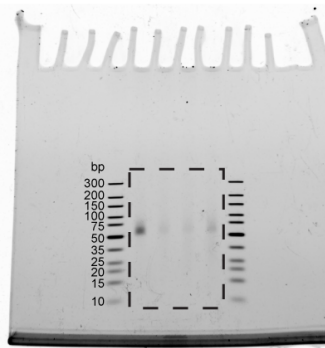
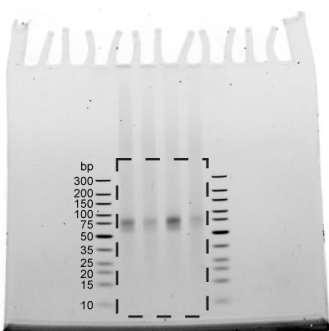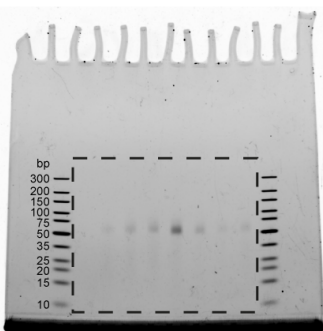Fig. 1h
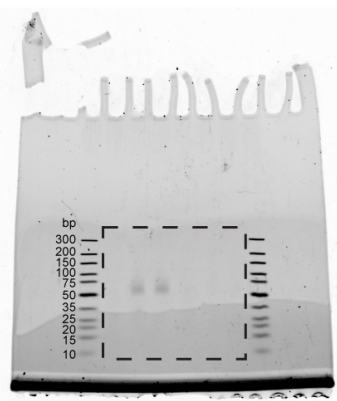
Fig. 1i
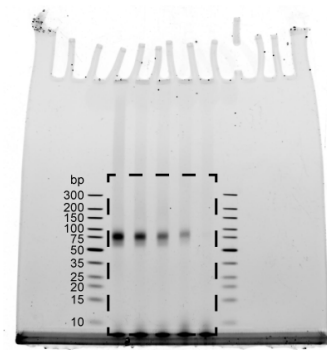
Fig. 1j

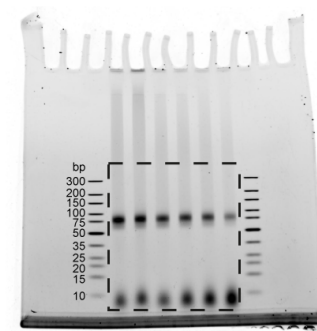Fig. 2e

Fig. 2g

Fig. 3a
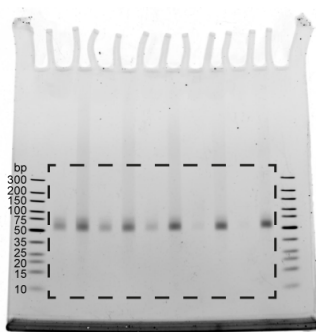
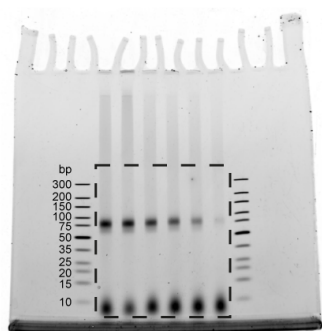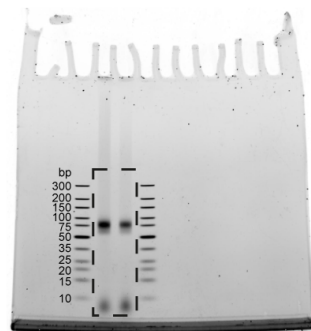Fig. 3f
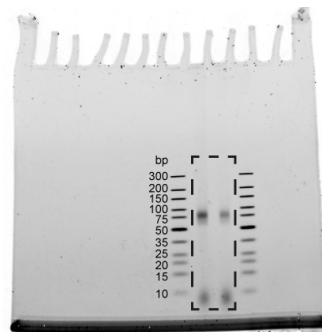
Fig. 3g
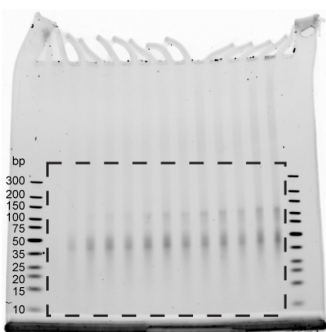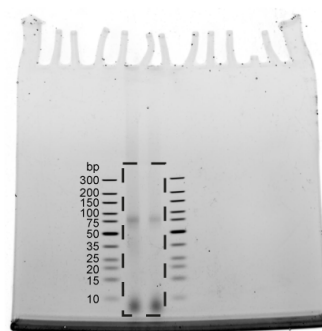
Fig. 3i
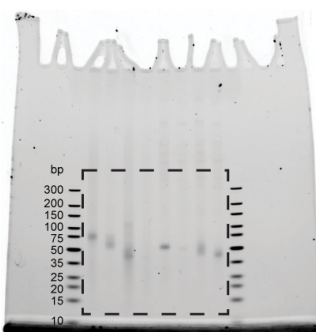
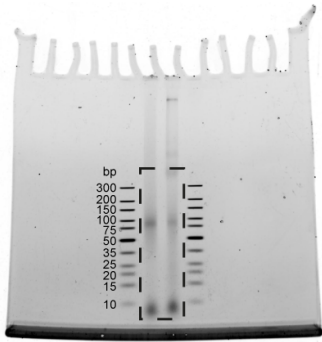Fig. 3k
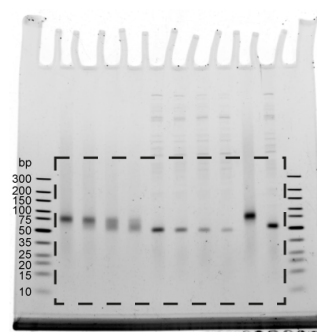
ED Fig. 1b
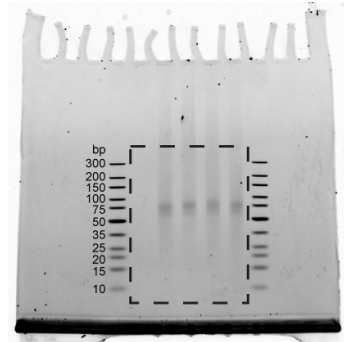
ED Fig. 1c

ED Fig. 1d

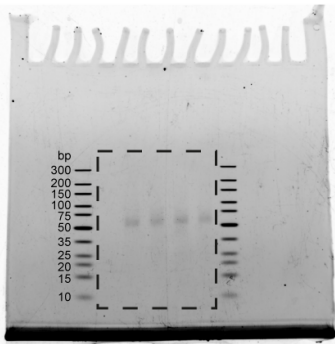ED Fig. 1e

ED Fig. 1f

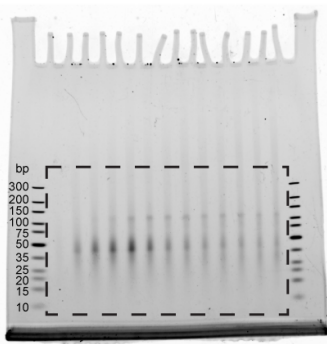ED Fig. 1g (gel 1)

ED Fig. 1g (gel 2)
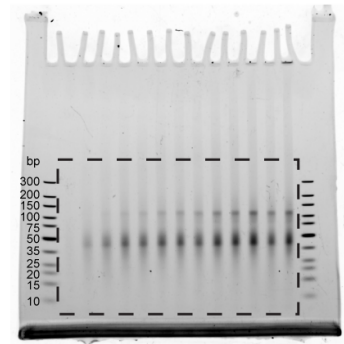
ED Fig. 1g (gel 3)

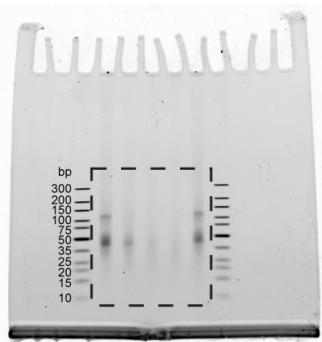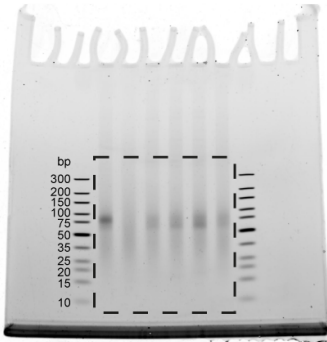ED Fig. 1g (gel 4)

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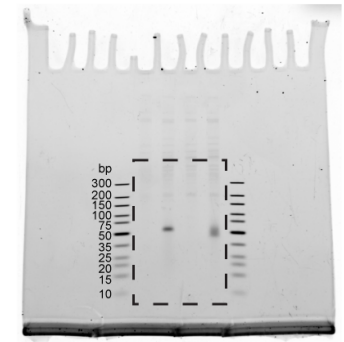
ED Fig. 1i

ED Fig. 1m (top)

ED Fig. 1m (bottom)

ED Fig. 1n

ED Fig. 3d

ED Fig. 4a

Supplementary Fig. 4 Source data.