

Appendix

A Dual Role for the Chromatin Reader ORCA/LRWD1 in Targeting the Origin Recognition Complex to Chromatin

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Table of Contents:

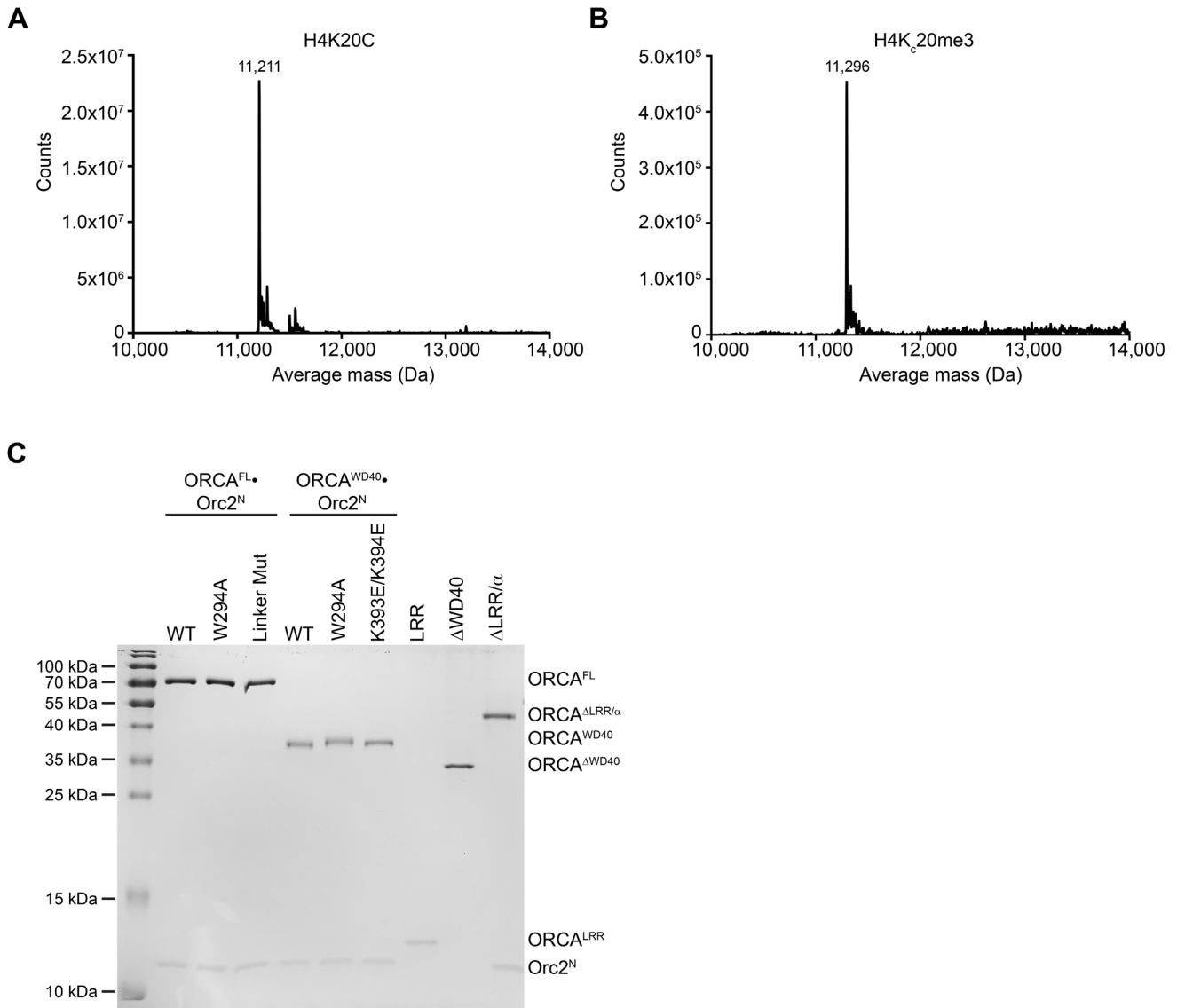
Appendix Figure S1	Page 2
Appendix Figure S2	Page 4
Appendix Figure S3	Page 5
Appendix Figure S4	Page 6
Appendix Figure S5	Page 7
Appendix Figure S6	Page 8
Appendix Figure S7	Page 9
Appendix References	Page 10

ORCA-binding region

Species	Sequence	ORCA	Phylum
<i>Rattus norvegicus</i>	--LRLK--EAKVPSVQFVGGDDV-----LSHILD-----REGGTKLKKE	ORCA	Craniata
<i>Homo sapiens</i>	--PELK--EDKMLEVHFVGGDDV-----LNHILD-----REGGAKLKKE	ORCA	Craniata
<i>Pteropus vampyrus</i>	--LELK--EDKMLEVQFVGGDDV-----LNHILD-----REGGTKLKKE	ORCA	Craniata
<i>Phascoglossus cinereus</i>	--LKLQ--EVKMLQVQFVGGDDV-----LNHILD-----KEEGAKFRKD	ORCA	Craniata
<i>Ornithorhynchus anatinus</i>	--SKAK--EVKMLEVQFVGGDDV-----LNHILE-----KEEGAKLRRD	ORCA	Craniata
<i>Alligator sinensis</i>	--PALQ--GGRRLEATFVSDVDV-----LRHISE-----DAGAKVKRD	ORCA	Craniata
<i>Chelonia mydas</i>	--LELK--RSKVL EARFVAEDVDV-----LKHISD-----NGGVKVRKD	ORCA	Craniata
<i>Gekko japonicus</i>	--PDKR--KNRVL EVKFVAEDVDV-----LQHISE-----NAGPKVQKK	ORCA	Craniata
<i>Microcaecilia unicolor</i>	--RKMP--EAGALQVQFVGGDDV-----LDHIID-----KQEGVKIRKD	ORCA	Craniata
<i>Xenopus laevis</i>	--EAHN--GTLKFKVQFTPEEVDV-----LEHITD-----KQGVTPQSNT	ORCA	Craniata
<i>Cygnus olor</i>	--AERK--GGAAALQVKFVPEEAV-----LRHIAD-----DAGIKVRKD	ORCA	Craniata
<i>Gallus gallus</i>	--AECR--SAGALEVKFVPEEVDV-----LRHIAD-----DAGIKVQKG	ORCA	Craniata
<i>Spheniscus magellanicus</i>	--PEGK--GSAGALEVKFVSEEEV-----LKHAD-----GAGIKVRKD	ORCA	Craniata
<i>Alca torda</i>	--PEGK--GGGGLRVKFFVPEEVDV-----LKHAD-----DPGIKVRKD	ORCA	Craniata
<i>Columba livia</i>	--AGRR--ERGALEVKFVPEEQV-----LKHIAE-----DSGVKVRKD	ORCA	Craniata
<i>Clarias magur</i>	--KRTM--ESGALVRFVGGDDV-----LDHIVE-----KNEGSAKPPS	ORCA	Craniata
<i>Danio rerio</i>	-----T--QKPLVRFVPEEVDV-----RDHIVE-----KQDQTKAVNG	ORCA	Craniata
<i>Pungitius pungitius</i>	-----SLEVKFVIGDGDV-----LEHIVD-----KQEGVQSNSSG	ORCA	Craniata
<i>Oreochromis aureus</i>	-----SLEVKFVIGDGDV-----LEHIVD-----KQEGVQSNSSG	ORCA	Craniata
<i>Salmo salar</i>	-----SLEVKFVIGDGDV-----LEHIVD-----KQEDVQSGSHS	ORCA	Craniata
<i>Polypterus senegalus</i>	SRCPVT--ARRVLEVRVFGDENV-----IDHIFD-----KGEGLPLNKT	ORCA	Cephalo-chordata
<i>Protopterus annectens</i>	--KQLK--ENKIPVQFVGGDDV-----LEHID-----KREGVKAGKD	ORCA	Cephalo-chordata
<i>Chiloscyllium plagiosum</i>	-----MKVGDVRFVIGDEEV-----VEHITD-----KRHGINQKNN	ORCA	Cephalo-chordata
<i>Scyliorhinus canicula</i>	-----MRPRLRFVGGDDV-----VELIRE-----RRQGITQKNN	ORCA	Cephalo-chordata
<i>Branchiostoma belcheri</i>	--AAPM--SKGGVVKFVGGDDV-----LQHVVD-----KRPTLRSSNR	ORCA	Cephalo-chordata
<i>Branchiostoma floridae</i>	--AAPK--ANGSMEVQFVGGDDV-----AQHVVD-----KRPTLRSSNR	ORCA	Cephalo-chordata
<i>Branchiostoma lanceolatum</i>	--AAPM--AKRSMEVQFVGGDDV-----LQHVVD-----KRPTLRSSNR	ORCA	Cephalo-chordata
<i>Styela clava</i>	--SKTP--SRRSVLVKHFVGGDDV-----EHHVTMLGVK-----NQRKTRSSMG	ORCA	Cephalo-chordata
<i>Ciona intestinalis</i>	-----ENTSVIVYVGGDDV-----GKHVGVGP-----KKKAVAKGIA	ORCA	Cephalo-chordata
<i>Lamellibrachia satsuma</i>	--TLQ--CRKKVYVTFAGDDDV-----IQHIVPLKE-----RKQSAFSQKT	ORCA	Urochordata
<i>Owenia fusiformis</i>	--GGKME--PKKKVVKFVCGDEEV-----VEHIIHKDS-----SKHGKQGRKA	ORCA	Annelida
<i>Trichoplax sp. H2</i>	-----AKVDLSITFLGDDNV-----VDHIVNIAVGN-----KQNKTTSTRT	ORCA	Placozoa
<i>Priapulid caudatus</i>	--STNP--KTSIVTLQFVGGDDV-----AEHIIINITE-----RKASLRSACK	ORCA	Priapulida
<i>Saccoglossus kowalevskii</i>	-----AAS--GGKHVKIRFVGGDDV-----VEHLKDTLV-----RNYGLRQRKN	ORCA	Hemichordata
<i>Mercenaria mercenaria</i>	-----LRRRSVNTFAGDDDV-----LQHVVD-----KTTSKSRRKS	ORCA	Mollusca
<i>Crassostrea angulata</i>	--SGRP--RRKCVSVFVGGDDV-----IGHIVDVN-----KKYESRKQKK	ORCA	Mollusca
<i>Patella vulgata</i>	-----KTS--RLKVVSVTFAGDDNV-----VQHVPLEE-----KKTTRKHSR	ORCA	Mollusca
<i>Aplysia californica</i>	-----EEK--RKKSIRITEVSEDAV-----IQHILPLNDL-----TRVKRR	ORCA	Mollusca
<i>Octopus sinensis</i>	LTKLIA--TRKSVRVKFNDEDI-----VQHVHLKER-----KRGGPLHMER	ORCA	Mollusca
<i>Strongylocentrotus purpuratus</i>	--SKKS--SVKGVSVTFVGGDDV-----MNHIME-----EVADTKVRKS	ORCA	Mollusca
<i>Acanthaster planci</i>	-----SRVRSLEFVGGDDV-----VRHIVTVND-----VCSAKPPRRS	ORCA	Echinodermata
<i>Patiria miniata</i>	--MAAS--RGKHVALQFVGGDDV-----LKHIVAVDE-----VSSAKPPRRS	ORCA	Echinodermata
<i>Orbicella faveolata</i>	-----EVEFVSDDEDV-----PE-----LKKRRE	ORCA	Echinodermata
<i>Nematostella vectensis</i>	--RRSSRLQTRAVSRLTAAKPK-----DQHKLRDTRQ-----RRLPPRQPKI	ORCA	Cnidaria
<i>Xenia sp. Carnegie-2017</i>	--ANS--IEKDMPIVFDGDDV-----KQCFTP-----RRTGLRSARK	ORCA	Cnidaria
<i>Penaeus vannamei</i>	--ERRR--SKRFLSVTFVGGDDV-----EKVILGVEGGKVVVRP--LQSRGRTSSRI	ORCA	Crustacea
<i>Portunus trituberculatus</i>	-----MRARLRVKFLDANV-----EKVRD-----KEEGVVVRV	ORCA	Crustacea
<i>Eurytemora affinis</i>	-----RNMVVRVVFVGGDDV-----PEMNTTGDE-----ISRVLRTNS	ORCA	Crustacea
<i>Rhipicephalus microplus</i>	-----AQAARVRFVGGDDV-----IEHIVNLSASK-----KKNVAAKEA	ORCA	Crustacea
<i>Centruroides sculpturatus</i>	-----KDGVEVVVFVGGDDV-----FENIVHISLRE-----KKNVGVSTT	ORCA	Crustacea
<i>Dinotrombium tinctorium</i>	--LRGR--DNTKVDVIVDESYK-----HERVVDITE-----KKNVGVSTT	ORCA	Crustacea
<i>Melanaphis sacchari</i>	-----DKNEILVEFINQAV-----QNLIDVTQQ-----KNHSTKKQKS	ORCA	Crustacea
<i>Cloeon dipterum</i>	--KSDD--CEDYVVKVFCGDDV-----EKHLFIKGS-----KAEKVYAGSS	ORCA	Crustacea
<i>Chironomus tentans</i>	-----KRVIEIRSDDDV-----ETIKPLSVVIEDPK-----GARMTRSRNN	ORCA	Crustacea
<i>Daphnia atkinsoni</i>	-----RGSALPTIQESEEDS-----DHVIEDILRTSRKK-----TECRTPSKKN	ORCA	Crustacea
<i>Eubosmina coregoni</i>	-----QIRGQPASLDMHEDL-----NEVLEDVMRSRRK-----RDCQTPSKKN	ORCA	Crustacea
<i>Notodromas monacha</i>	-----VKTNNFDDGDN-----LDVRNVLPTTSKR-----KRSIYRNSI	ORCA	Crustacea
<i>Nymphon striatum</i>	-----LNGGSILDDCTGKEDS-----TKGRYSFRK-----RQSENRYDKS	ORCA	Crustacea
<i>Nephila pilipes</i>	-----RHEVVRVGGIRSRPEQGGQPQNPRARLNI MNKSKYAVSKSPGRNKERIKKSS	ORCA	Crustacea
<i>Sarcoptes scabiei</i>	-----PIKRTPLPLSTDEVV-----LKHIVAVDE-----VSSAKPPRRS	ORCA	Crustacea
<i>Drosophila melanogaster</i>	--GYKTP--RKENLMSIENLTNSEE-----ESEDLNTAMVGNVAV-----SQPKVTSRRS	no ORCA	Crustacea
<i>Ctenocephalides felis</i>	-----ENERAQ-----ADHLQ-----TPSRLRPRKS	no ORCA	Crustacea
<i>Temnothorax curvispinosus</i>	--LRRS--TRVTRVKYTEDSDD-----ADHLQ-----TPSRLRPRKS	no ORCA	Crustacea
<i>Timema douglasi</i>	-----KKCFKYVYDSSSEEDS-----VEI-----CKVSTRKNKN	no ORCA	Crustacea
<i>Folsomia candida</i>	-----PSSNYEPTDSEENL-----ADHLQ-----TPSRLRPRKS	no ORCA	Crustacea
<i>Opisthorchis felinus</i>	-----TSNSDLSEPNQDDI-----SRLA-----SRLA	ORCA	Platyhelminthes
<i>Paragonimus westermani</i>	-----FHGTQENLEPNDDI-----SRLA-----SRLA	ORCA	Platyhelminthes
<i>Schistosoma spindale</i>	-----FLESGIASEFSELNI-----SRLA-----SRLA	ORCA	Platyhelminthes
<i>Spirometra erinaceieuropaei</i>	-----GRSGKLVLEALSGMNI-----SRLA-----SRLA	ORCA	Platyhelminthes
<i>Macrostomum lignano</i>	-----PLEANRDDAI-----SRLA-----SRLA	ORCA	Platyhelminthes
<i>Brachionus calyciflorus</i>	--SAKDLVLAQVQSDV-----TDIIDKSY-----GGLKLVSSNKE	ORCA	Rotifera
<i>Adineta steineri</i>	-----TNRSPVIVVISENI-----PNKIRNIDDIIEYVQSFLPLKSTPSRKS	ORCA	Rotifera
<i>Didymodactylos carnosus</i>	-----NRPVKVLFIASENV-----PNHFVNIDDAEYINEQRQEQMEKMGKLS	ORCA	Rotifera
<i>Brugia pahangi</i>	-----SAKGVRLRSFERNQ--GISPGNALIFWYLFVTEEGRT-----PRPKILKRAT	no ORCA	Rotifera
<i>Caenorhabditis elegans</i>	-----PRPKILKRAT-----PRPKILKRAT	no ORCA	Nematoda
<i>Trichinella spiralis</i>	-----GDDDD-----PRPKILKRAT	no ORCA	Nematoda

Appendix Figure S1. Conservation of the ORCA-binding sequence in Orc2 across the metazoan kingdom. The presence of ORCA in different phyla and/or subphyla is indicated. Note that some orders in the phylum Arthropoda appear to encode an ORCA ortholog (as defined by a conserved

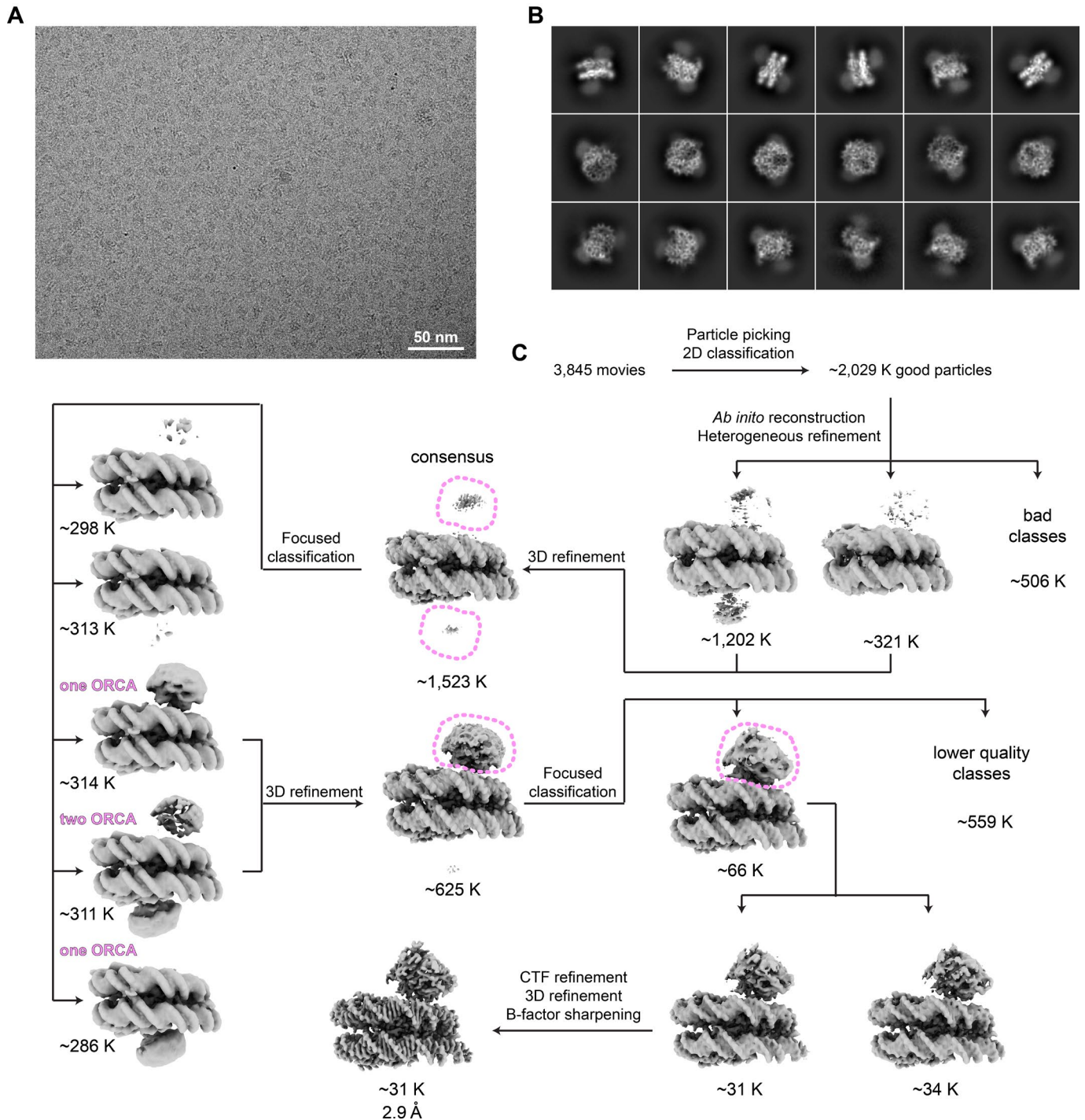
WD40 domain) while others do not, and that ORCA orthologs in the phylum Platyhelminthes are very long polypeptides and may utilize alternative mechanisms to associate with Orc2 (see also [Table 2](#)).



Appendix Figure S2. Mass spectrometry of histone H4 containing the trimethyl-lysine analog (MLA) and SDS-PAGE of purified ORCA constructs.

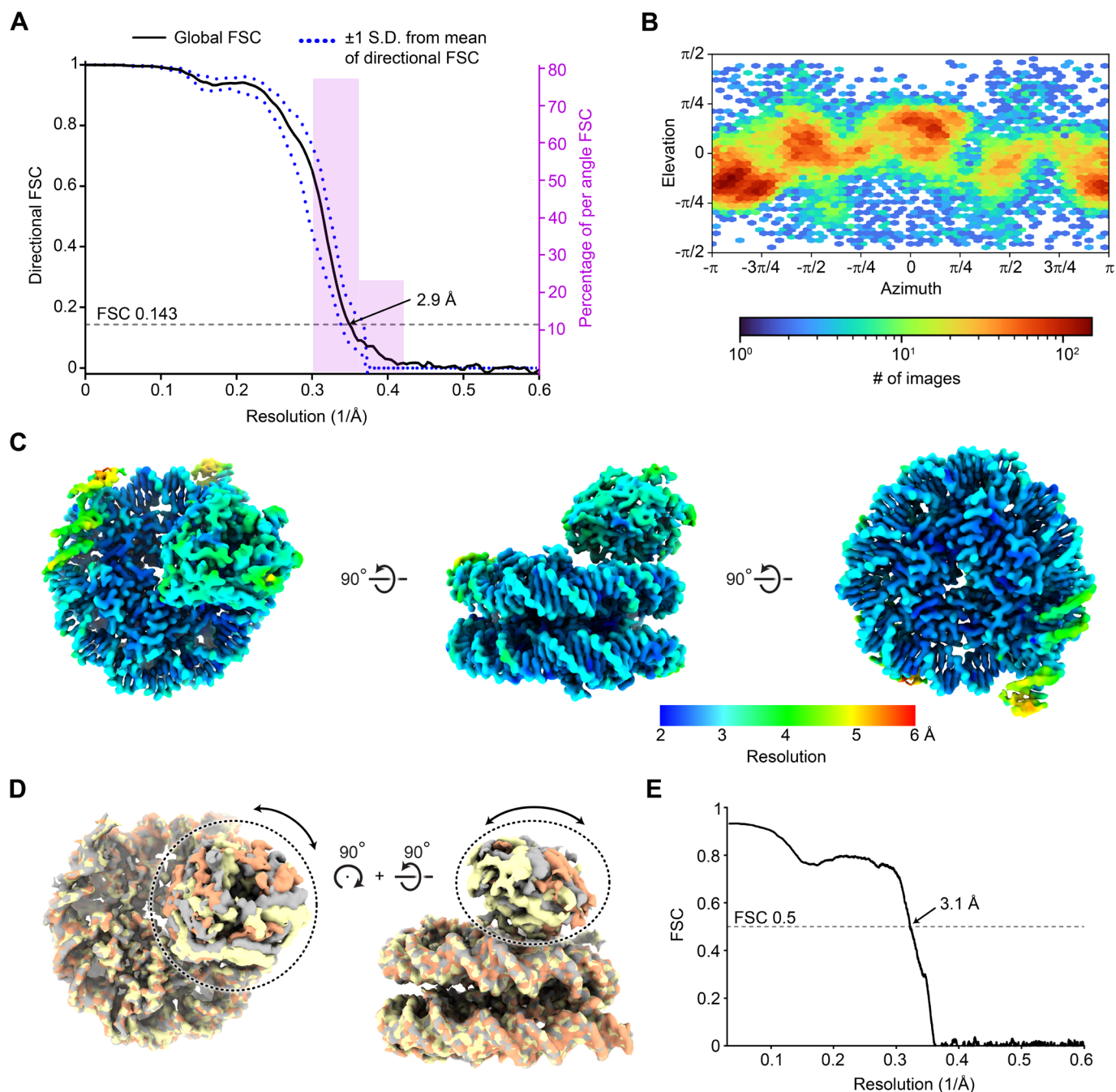
A-B. Intact mass spectrometry of H4 before and after MLA installation. The mass difference between H4K20C (in A) and H4K_c20me3 (in B) indicates successful MLA installation.

C. Coomassie-stained SDS-PAGE gel of purified *RnORCA* proteins used in this study.



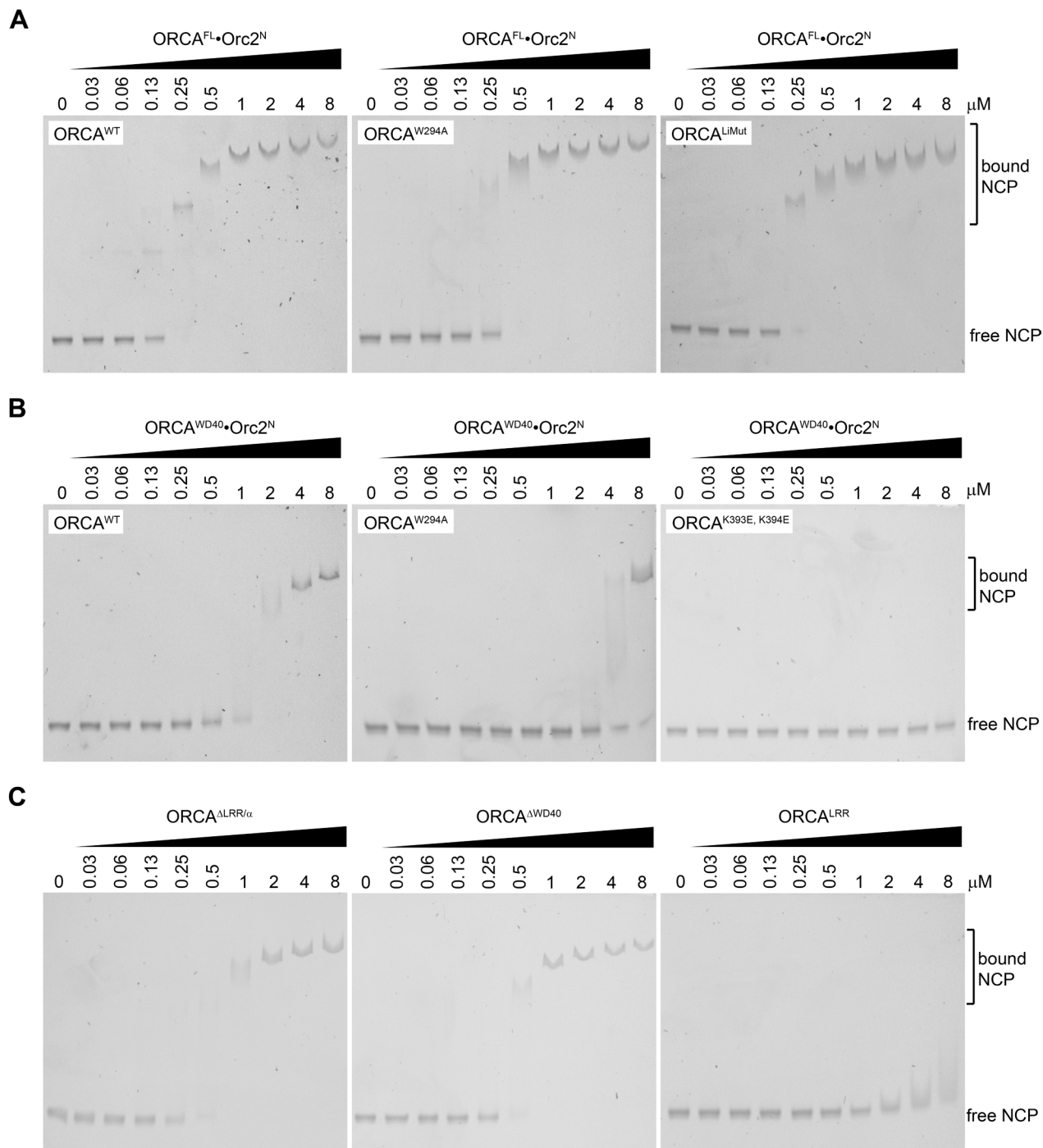
Appendix Figure S3. Summary of cryo-EM data processing workflow.

- Representative image of summed movie frames.
- 2D class averages of the ORCA^{FL}•Orc2^N•H4K20me3-nucleosome complex.
- Data processing scheme used to obtain the 2.9 Å map of the ORCA^{FL}•Orc2^N•H4K20me3 nucleosome complex. Dotted lines indicate masks used for focused 3D classification. Note that one of the two ORCA WD40 domains bound to a nucleosome is averaged out during 3D reconstruction due to substoichiometric occupancy and/or flexibility (see also [Appendix Fig S4D](#)).



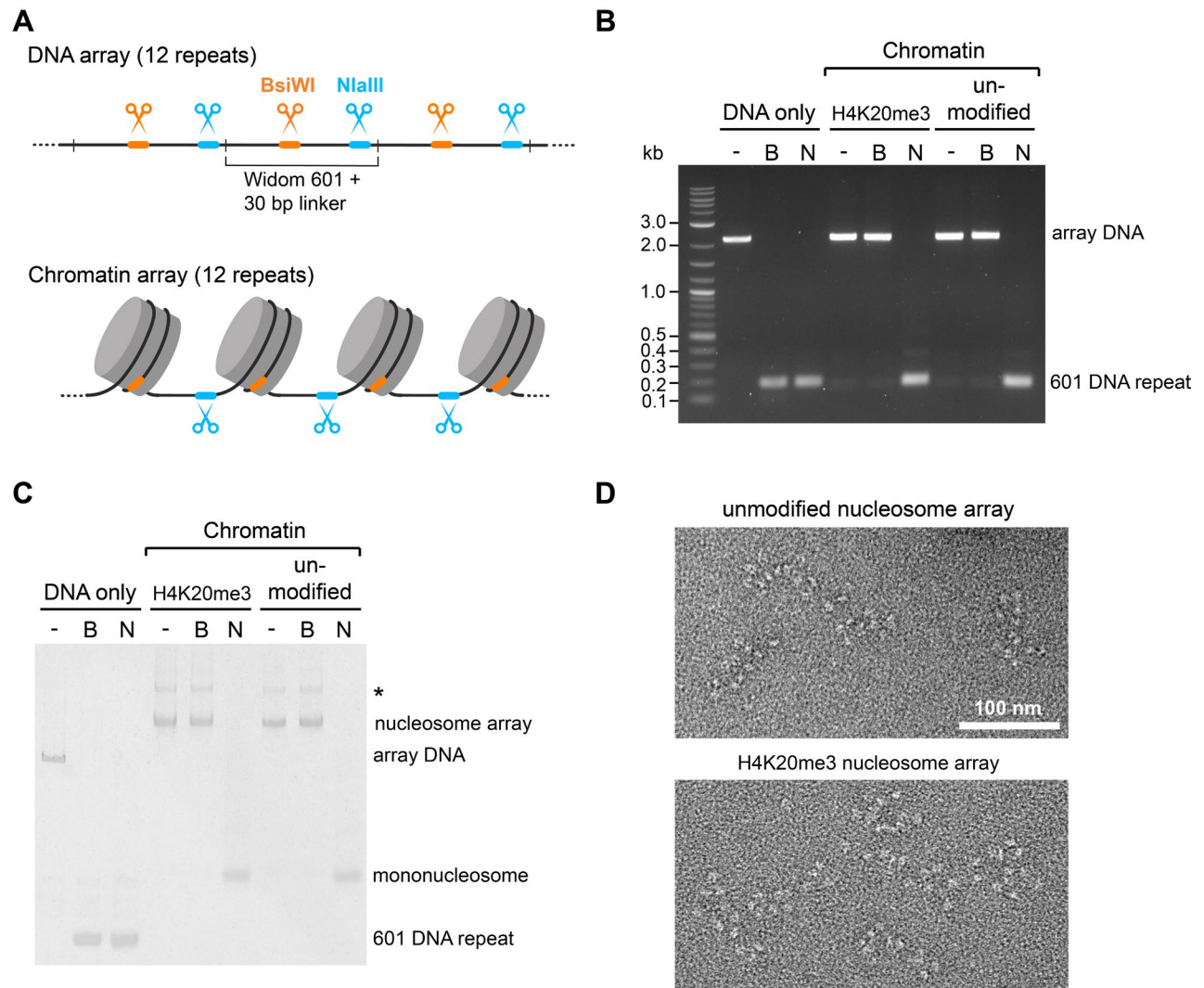
Appendix Figure S4. Resolution and angular particle distributions for the ORCA^{FL}•Orc2^N•H4K20me3-nucleosome cryo-EM structure.

- A. ThreeD Fourier shell correlation (FSC) analysis (Tan et al, 2017) of the reconstructed cryo-EM map.
- B. Angular distribution of particles contributing to the final reconstruction.
- C. Sharpened cryo-EM map of the ORCA^{FL}•Orc2^N•H4K20me3-nucleosome complex colored by local resolution.
- D. Superposition of three different 3D class volumes (low-pass filtered to 4 Å) illustrates flexibility of ORCA bound to a nucleosome, limiting the local resolution of ORCA. Despite the flexibility, similar ORCA•nucleosome contacts are formed.
- E. FSC curve calculated using the final refined model and cryo-EM map.



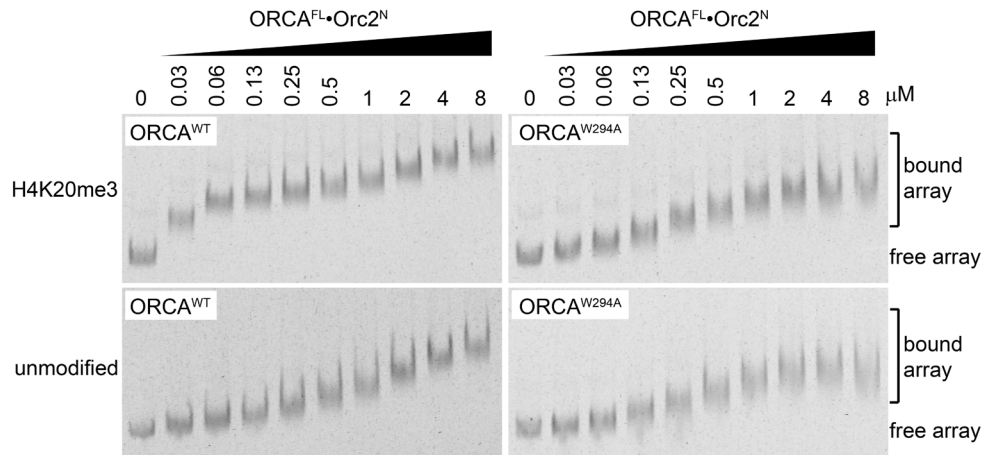
Appendix Figure S5. Examples of EMSA gels for H4K20me3-monomucleosome binding by ORCA. See [Figs 2H, 3B, and 5B](#) for binding curves.

- EMSA gels for wildtype or mutant full-length ORCA binding to H4K20me3-monomucleosomes.
- EMSA gels for wildtype or mutant ORCA-WD40 domain binding to H4K20me3-monomucleosomes.
- EMSA gels for binding of other truncated ORCA constructs to H4K20me3-monomucleosomes.



Appendix Figure S6. Reconstitution of nucleosome arrays.

- DNA arrays comprising 12 repeats of the Widom 601 nucleosome positioning sequence separated by 30 bp linkers were used to reconstitute chromatin arrays. Successful reconstitution was validated by restriction digest with BsiWI and NlaIII. BsiWI only cleaves free DNA since the recognition site in the Widom 601 sequence is protected upon nucleosome formation. The NlaIII recognition site is located in the linker region and is cleaved both in free DNA and nucleosome arrays.
- Agarose gel electrophoresis of free array DNA and reconstituted H4K20me3- and unmodified chromatin arrays after BsiWI (B), NlaIII (N), and mock (-) restriction digestion followed by proteinase K treatment.
- Native agarose-polyacrylamide gel electrophoresis of free array DNA and reconstituted H4K20me3- and unmodified chromatin arrays after BsiWI (B), NlaIII (N), and mock (-) restriction digestion (without proteinase K treatment). Asterisk denotes a chromatin oligomer observed in some reconstitutions.
- Electron micrograph of negatively stained unmodified and H4K20me3-modified nucleosome arrays.



Appendix Figure S7. The aromatic cage in ORCA's WD40 domain is required for high-affinity chromatin binding. Native agarose-polyacrylamide gels of EMSA assays comparing binding of full-length, wildtype ORCA and that harboring the W294A mutation in the aromatic cage to nucleosome arrays. The aromatic cage mutation abolishes ORCA's ability to distinguish H4K20me3- and unmodified arrays. The gels for ORCA^{WT} are reproduced from [Fig 3D](#) for direct comparison.

Appendix References

Tan YZ, Baldwin PR, Davis JH, Williamson JR, Potter CS, Carragher B, Lyumkis D (2017) Addressing preferred specimen orientation in single-particle cryo-EM through tilting. *Nat Methods* **14**: 793-796