Expanded View Figures

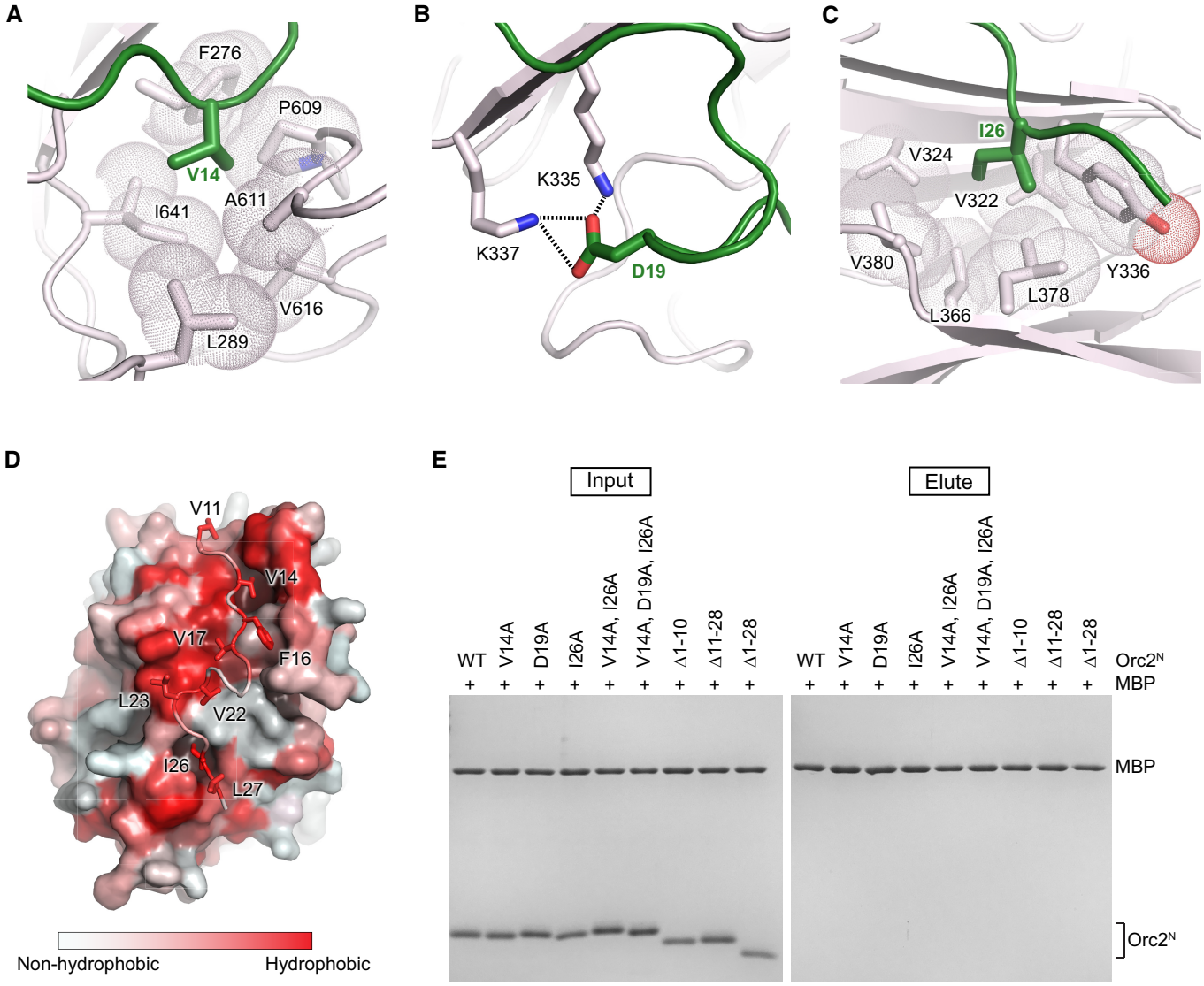

Figure EV1. Conserved Orc2 residues mediate the interaction with ORCA^{WD40}.

A–C Zoomed views of the Orc2·ORCA-binding site highlighting contacts between conserved Orc2 residues (in green) V14 (in A), D19 (in B), and I26 (in C) with the ORCA β-propeller circumference (in light pink). V14 and I26 in Orc2 bind hydrophobic pockets in ORCA, while Orc2-D19 forms salt bridges with ORCA lysines.

D View of the ORCA^{WD40}·Orc2^{N}-binding site colored according to the Eisenberg hydrophobicity scale (Eisenberg *et al*, 1984). ORCA is rendered as surface and Orc2 as cartoon with side chains in stick representation.

E *In vitro* pulldown (using amylose beads) of MBP with wild-type (WT) or mutant Orc2^{N}. This pulldown is a control for the one shown in Fig 1F. The lack of interactions between MBP and Orc2 seen here supports the specific association of ORCA with Orc2^{N} in Fig 1F.

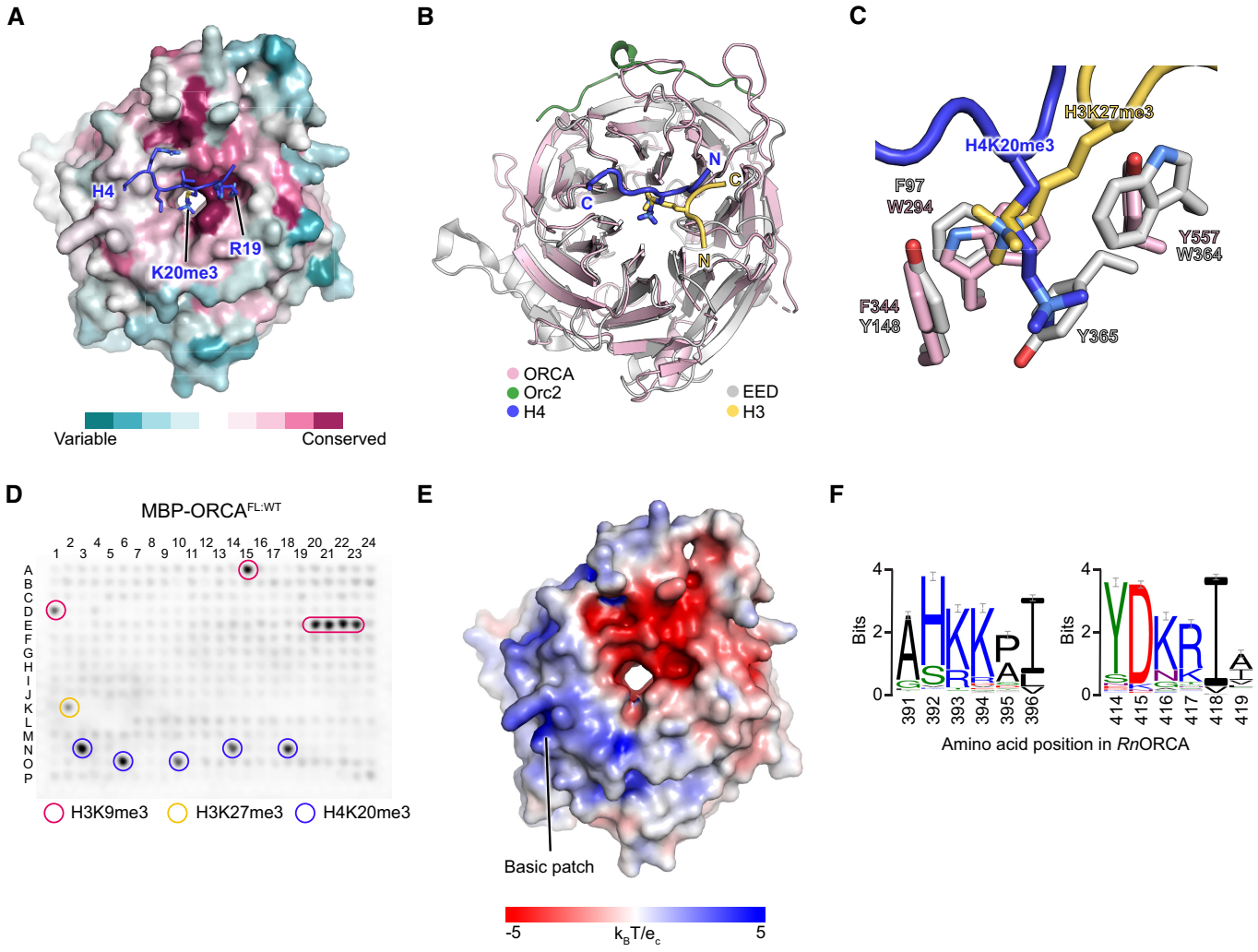

Figure EV2. ORCA binds nucleosomes using two surfaces on the WD40 domain.

A The H4-binding site constitutes a conserved region of the ORCA β-propeller. The ORCA^{WD40} surface is colored according to ConSurf conservation scores.

B, C ORCA's WD40 domain is structurally similar to that of EED. (B) Structural superposition of the WD40 domains of ORCA (this study) and EED (PDB 3IIW; Margueron *et al*, 2009) with bound H4 and H3 peptides, respectively. (C) Zoomed view of the aromatic cages in ORCA and EED.

D Histone peptide array binding experiment for full-length (FL) ORCA. A similar set of modified histone peptides is bound by the full-length protein as by the WD40 domain alone (compared to Fig 2E).

E, F Positively charged residues form a basic patch on the surface of ORCA's WD40 domain. (E) View of the nucleosome-facing surface of ORCA's WD40 domain colored by electrostatic potential. (F) Sequence logo of DNA-binding residues (K393, K394, and R417) in ORCA's basic patch reveals conservation of chemical side chain properties among ORCA orthologs.

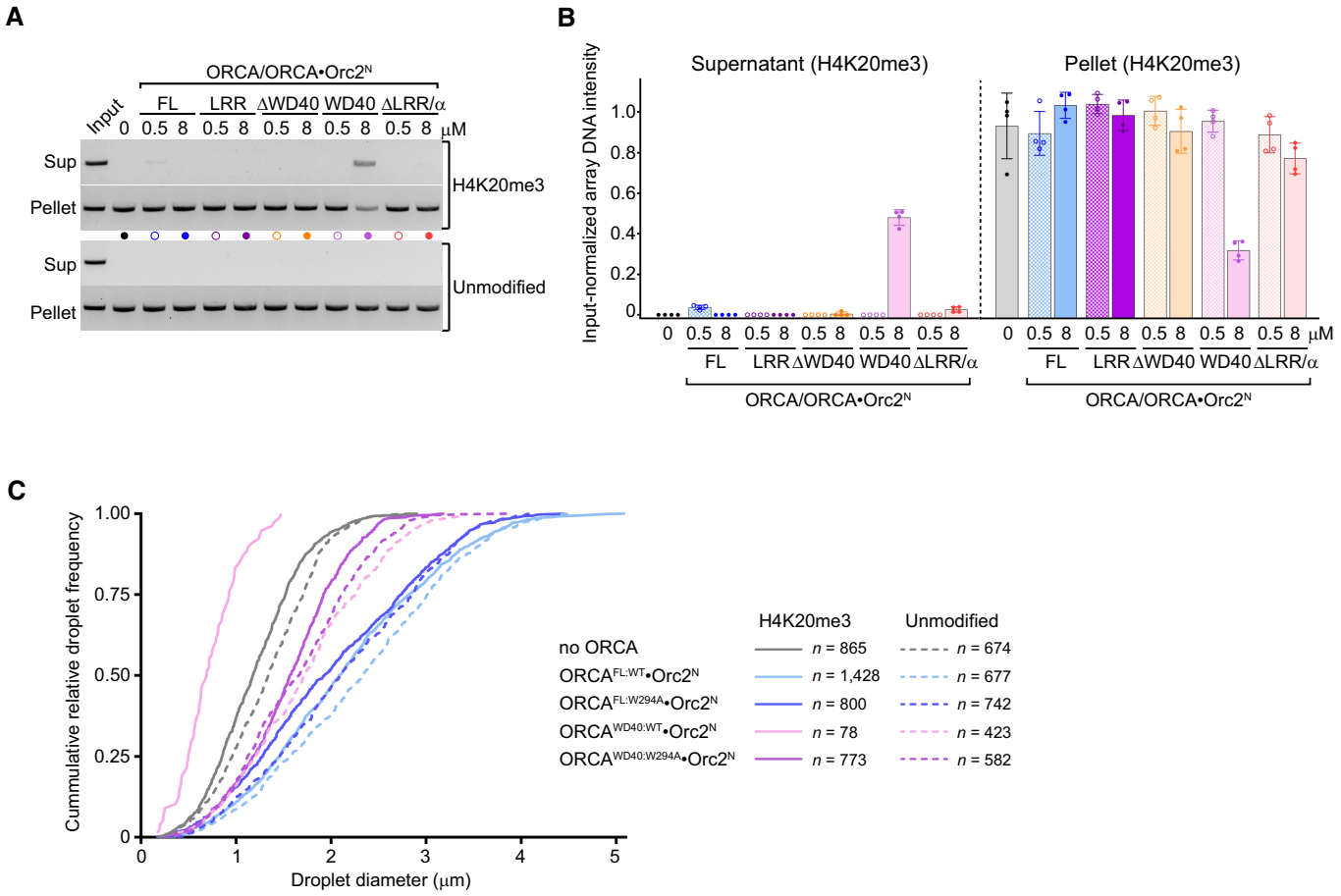

Figure EV3. Binding of ORCA to nucleosome arrays alters inter-nucleosomal interactions.

A Nucleosome array pelleting assays in the absence and presence of full-length ORCA and indicated ORCA deletion constructs. Agarose gels of array DNA from supernatants and pellet fractions are shown. Input refers to nucleosome array without pelleting that is loaded for comparison.

B Quantification (mean and s.d.) of DNA band intensities from four independent experiments as done in A.

C Cumulative frequency distributions of chromatin droplet diameters in the absence and presence of wild-type or mutant ORCA proteins (see Fig 4G). Number of droplets measured is listed.

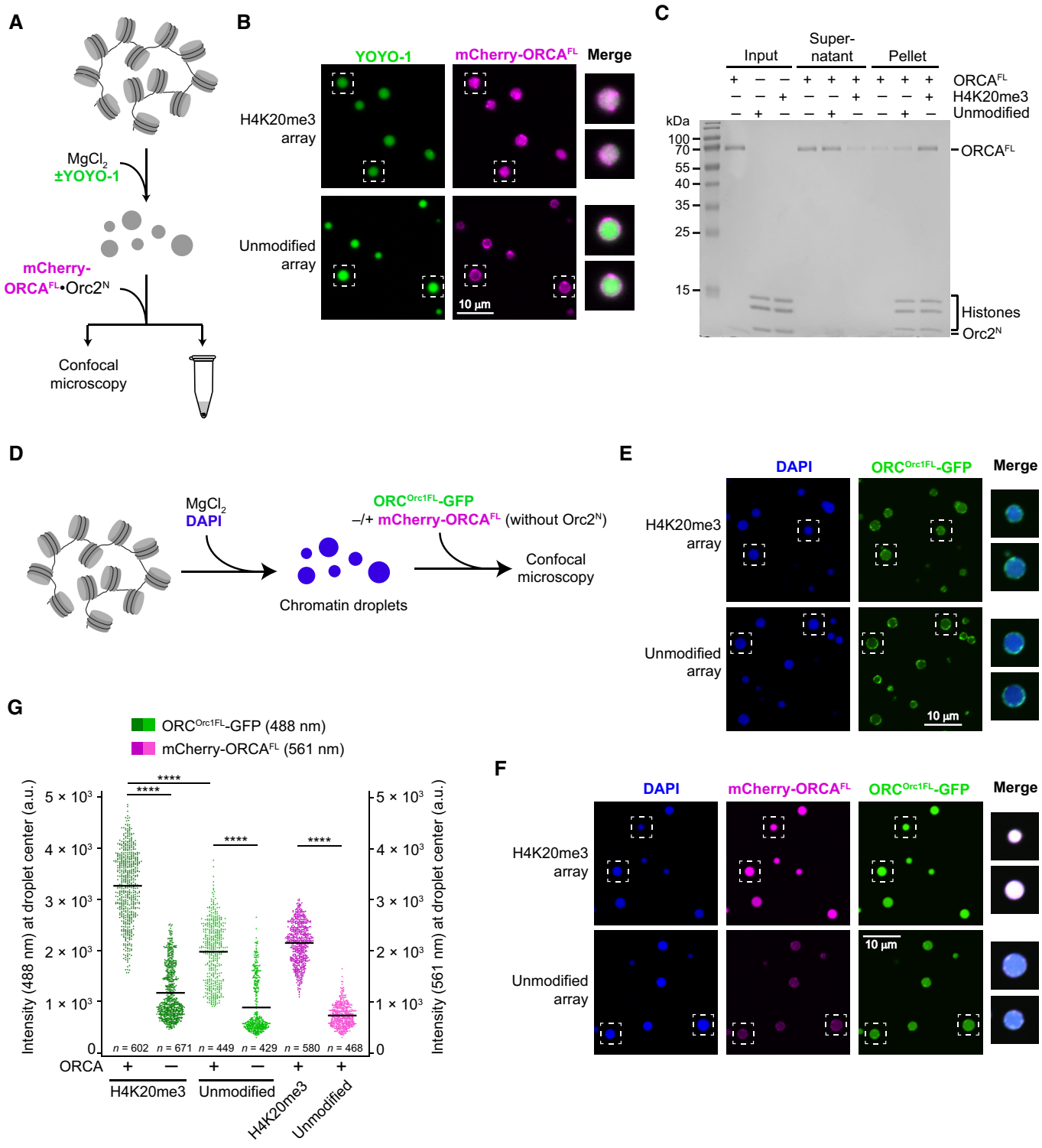

Figure EV4.

Figure EV4. ORCA and ORC are recruited to nucleosome arrays in a histone modification-specific manner.

A–C ORCA is recruited into preformed H4K20me3-chromatin but not unmodified chromatin condensates. (A) Experimental setup. The endpoint is either confocal imaging of chromatin droplets or pelleting of nucleosome arrays by centrifugation. (B) Confocal microscopy images of unmodified and H4K20me3-modified chromatin droplets after addition of mCherry-labeled ORCA. Merged images for YOYO-1 and mCherry channels are shown for boxed droplets. (C) Coomassie-stained SDS–PAGE gel of histones and ORCA partitioning into supernatants and pellet fractions in nucleosome array pelleting assays. The difference between A-C in this figure and Fig 6A–D is that here nucleosome arrays were first allowed to oligomerize prior to addition of ORCA.

D–G ORCA enriches full-length ORC in condensed nucleosome arrays in a histone modification-dependent manner. (D) Schematic of experimental setup. ORCA and ORC are added to preformed chromatin droplets. (E) Confocal microscopy images of chromatin droplets with GFP-labeled full-length ORC in the absence of mCherry-labeled ORCA. (F) Confocal microscopy images of chromatin droplets with GFP-labeled full-length ORC in the presence of mCherry-labeled ORCA. (G) Quantification of ORC-GFP and mCherry-ORCA intensities at droplet centers. Statistical significance according to the Kruskal–Wallis test and Dunn's multiple comparisons test is indicated by asterisks (****$P < 0.0001$). The higher background recruitment of ORC to unmodified arrays as compared to Fig 6H is likely caused by interactions of the Orc1-IDR with nucleosome array DNA.