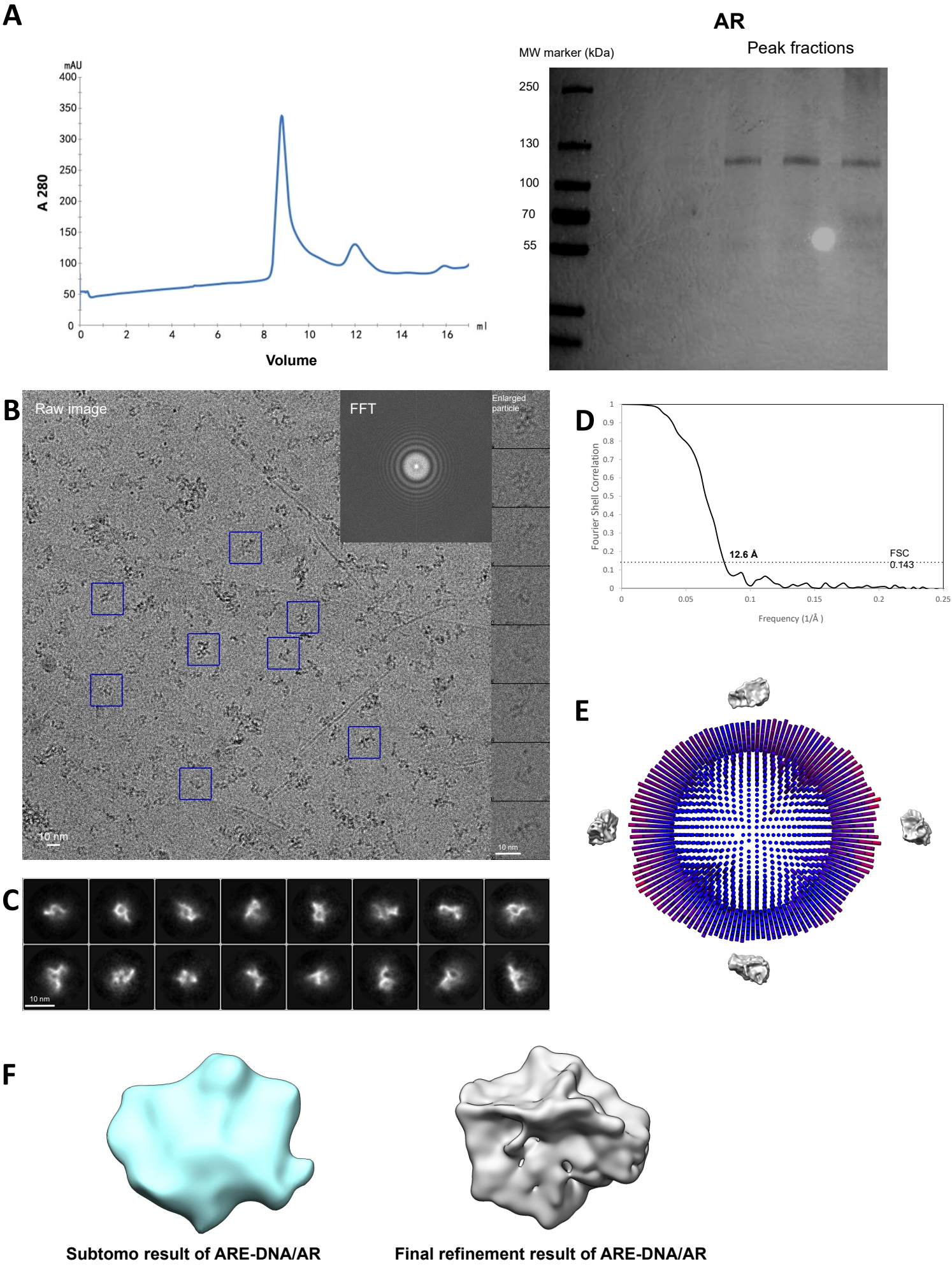
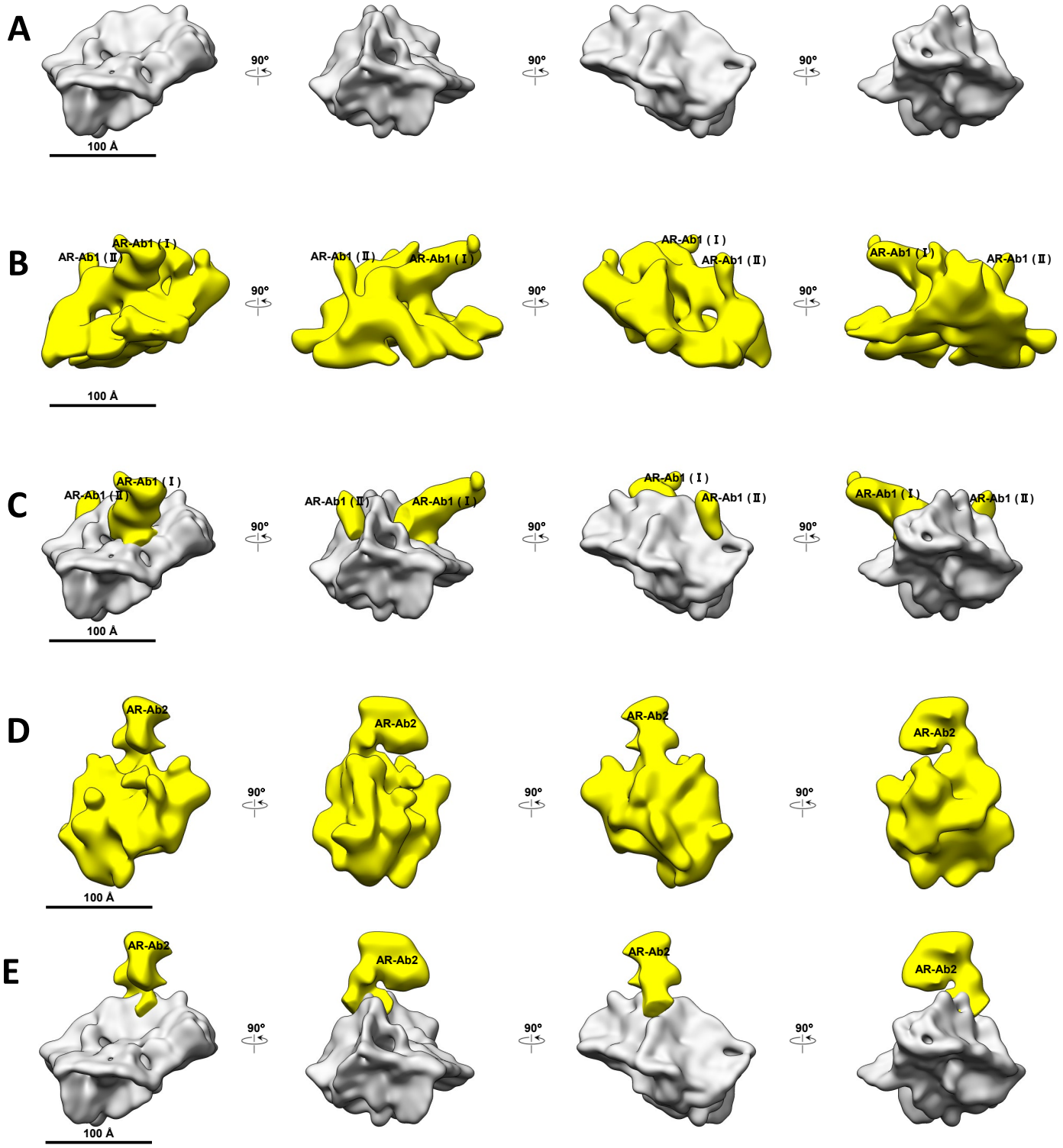
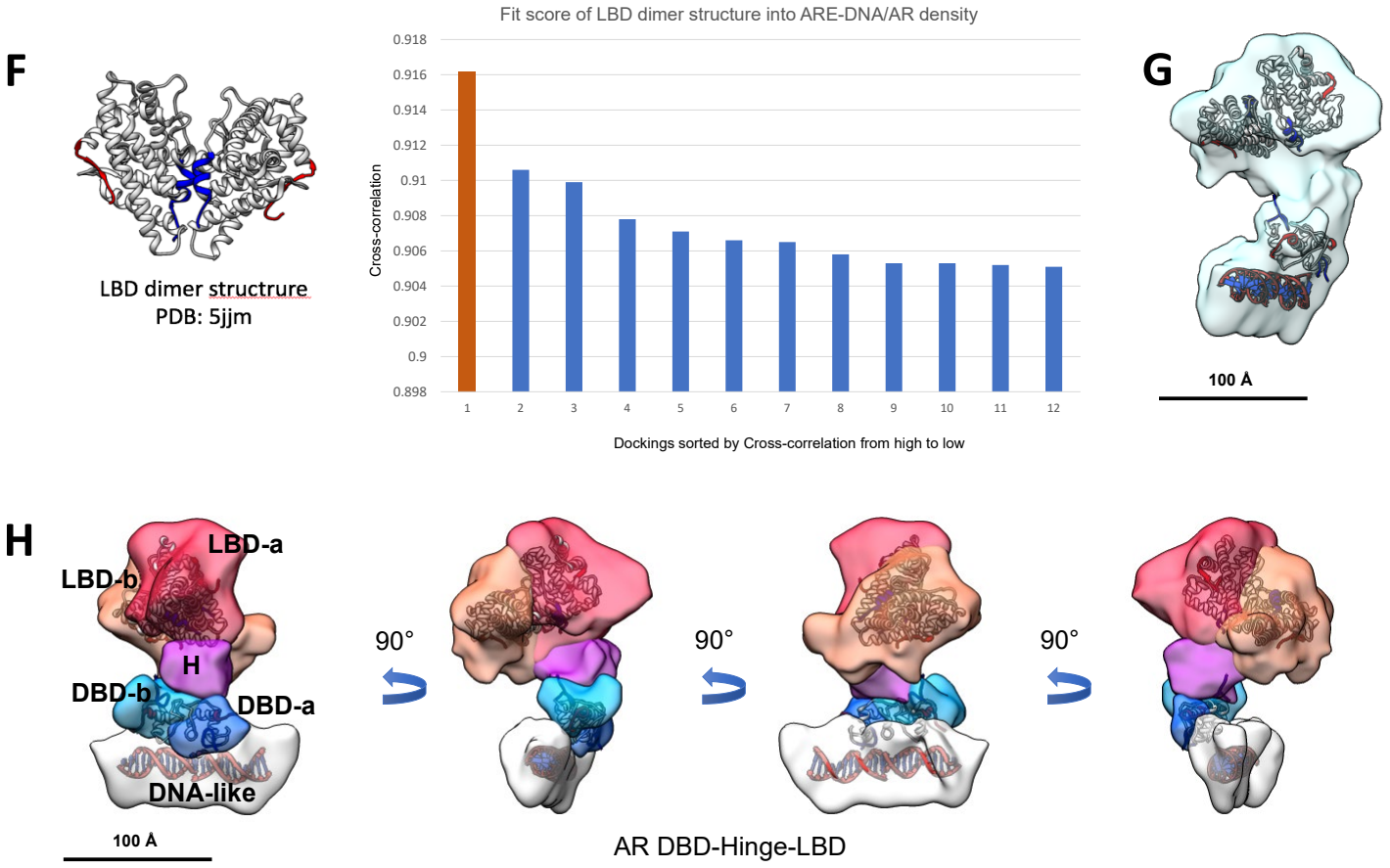
Supplemental Figure 1, related to Figure 1



Supplemental Figure 1. ARE DNA-bound AR dimer cryo-EM images. (A) AR protein purification. Gel filtration profile of AR (left panel) and SDS-PAGE analysis (right panel) of the peak fractions (Coomassie blue staining). The proteins were applied to Superdex 200 10/300 GL column. Shown on the left of the gel is a lane loaded with molecular weight markers. (B) Electron microscopy images of ARE DNA-bound AR. A representative raw image of ARE-DNA/AR complex is shown on the left. FFT (Fast Fourier Transform) image is shown at the right upper corner demonstrating the quality of data. Enlarged particles are shown on the right. (C) The reference free 2D class average results of boxed out ARE-DNA/AR particles. (D) The gold-standard FSC curve for the final cryo-EM 3D reconstruction generated using RELION. The overall resolution is estimated at 12.6 Å according to the 0.143 criteria. (E) Euler angle distribution of all particles used for the final 3D reconstruction. Each view is represented by a cylinder, for which the height is proportional to the number of particles for this specific view. (F) Side by side comparison between subtomogram averaged map (left) and the final ARE-DNA/AR density map (right) shows their agreement in the low resolution outfeatures.

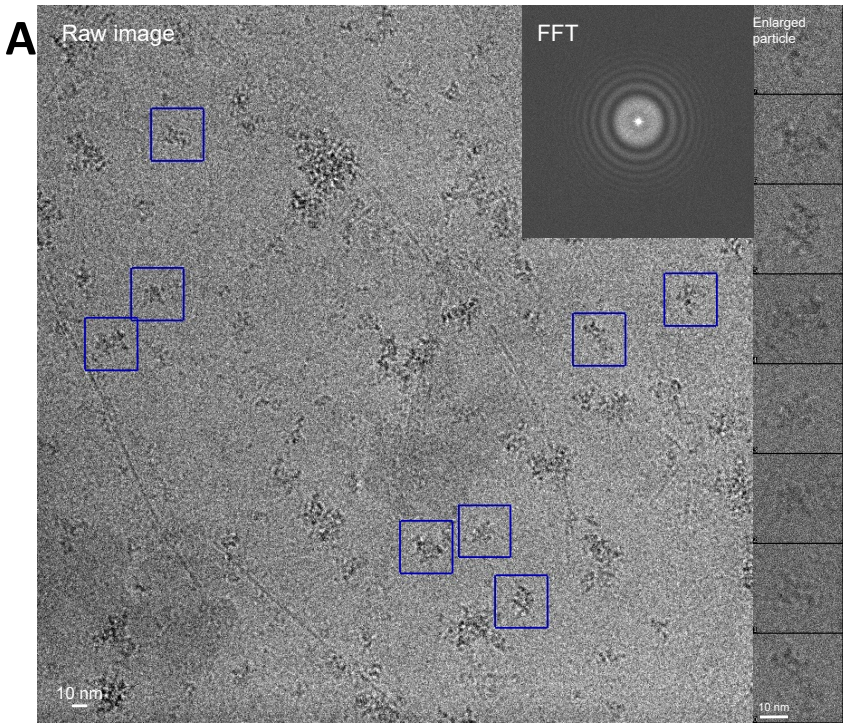
Supplemental Figure 2, related to Figure 2

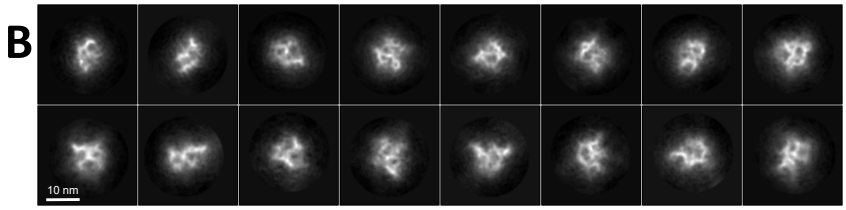


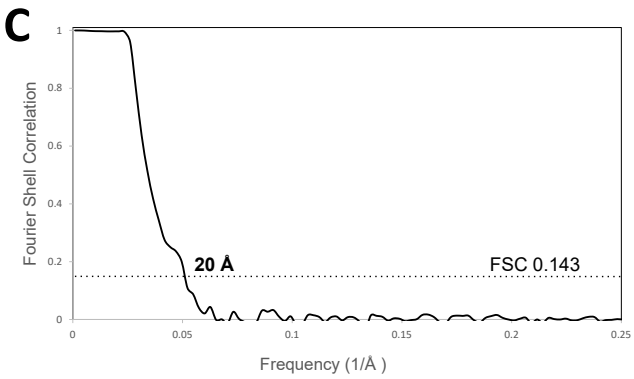


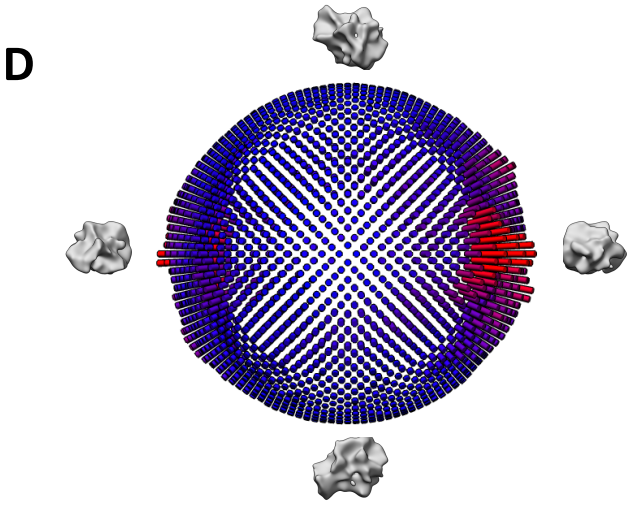
Supplemental Figure 2. Antibody labeling to locate the domains in the ARE-DNA/AR density. (A) The cryo-EM density map of ARE-DNA/AR. (B) AR-Ab1 recognizes residues 98-503 in the NTD of AR. The density map of ARE-DNA/AR/AR-Ab1 complex shows two AR-Ab1 binding densities. (C) The two AR-Ab1 locations on the ARE-DNA/AR density. The two AR-Ab1s are labeled as AR-Ab1 (Ⅰ) and AR-Ab1 (Ⅱ), respectively. AR-Ab1 (Ⅰ) density is stronger than the AR-Ab1 (Ⅱ) density. (D) AR-Ab2 labels the region close to the N-terminus of AR as well as the N/C interaction interface. The AR-Ab2 recognizes the residues 39-97 at the N-terminus. The density map of ARE-DNA/AR/AR-Ab2 complex is shown. Only one AR-Ab2 density is observed binding to the AR dimer. (E) The AR-Ab2 binding site is shown on ARE-DNA/AR. The position of AR-Ab2 is in proximity to the FXXLF motif, where the NTD contacts the LBD. (F) Docking of the LBD crystal structure into the ARE-DNA/AR density. Left panel, the published crystal structure of AR LBD dimer (PDBID: 5jjm). The N- and C- terminus of the LBD dimer are colored in Blue and Red, respectively. Right panel, the fitting score of the LBD dimer crystal structure into the ARE-DNA/AR cryo-EM map with global search in Chimera. Dockings sorted by Cross-Correlation are graphed from left to right, with the highest score labeled in brown color. The LBD position in the ARE-DNA/AR complex is based on the highest score fitting, which provides the basis for LBD segmentation in the ARE-DNA/AR density shown in Figure 1b. (G) Docking of the LBD crystal structure into ARE-DNA/AR density with the two NTDs omitted for a clear vision of the LBDs. Published crystal structures of the LBD dimer (PDBID: 5jjm), the DBD dimer (PDBID: 1r4i) and a 34bp DNA are fitted into the map. (H) Segmentation results of ARE-DNA/AR density without two NTDs are fitted well with known structures.

Supplemental Figure 3, related to Figure 3

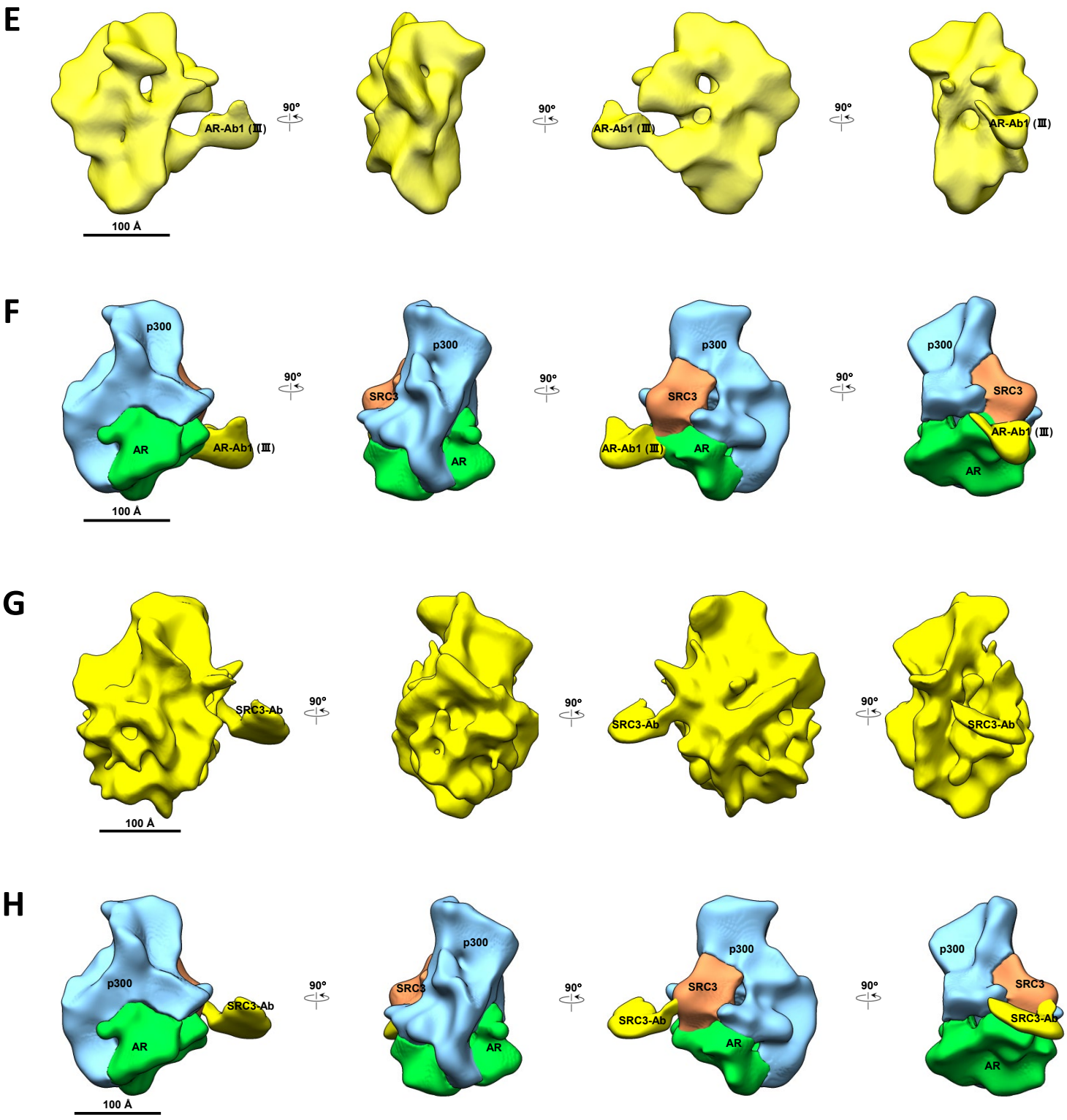






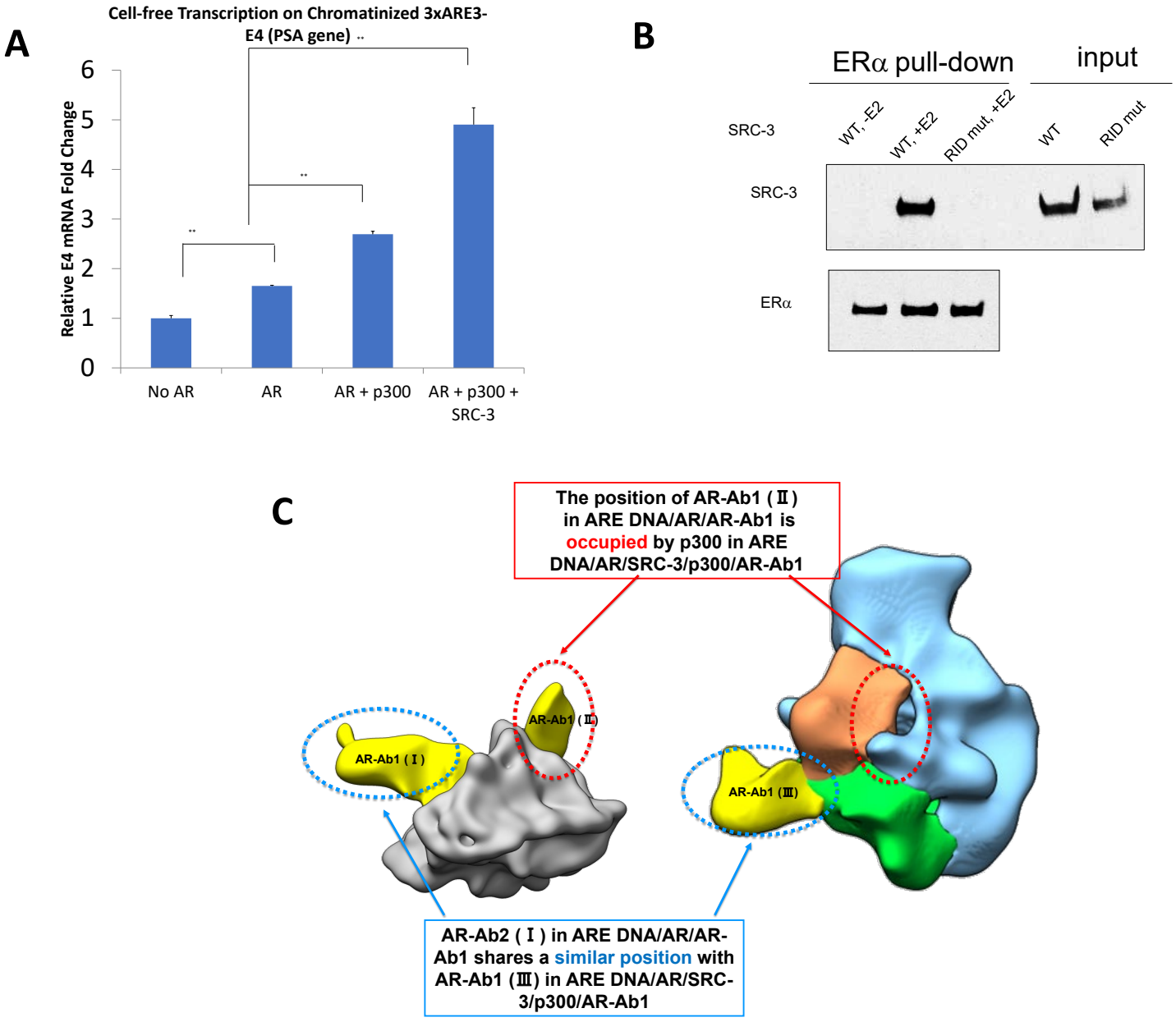


Supplemental Figure 3, related to Figure 3



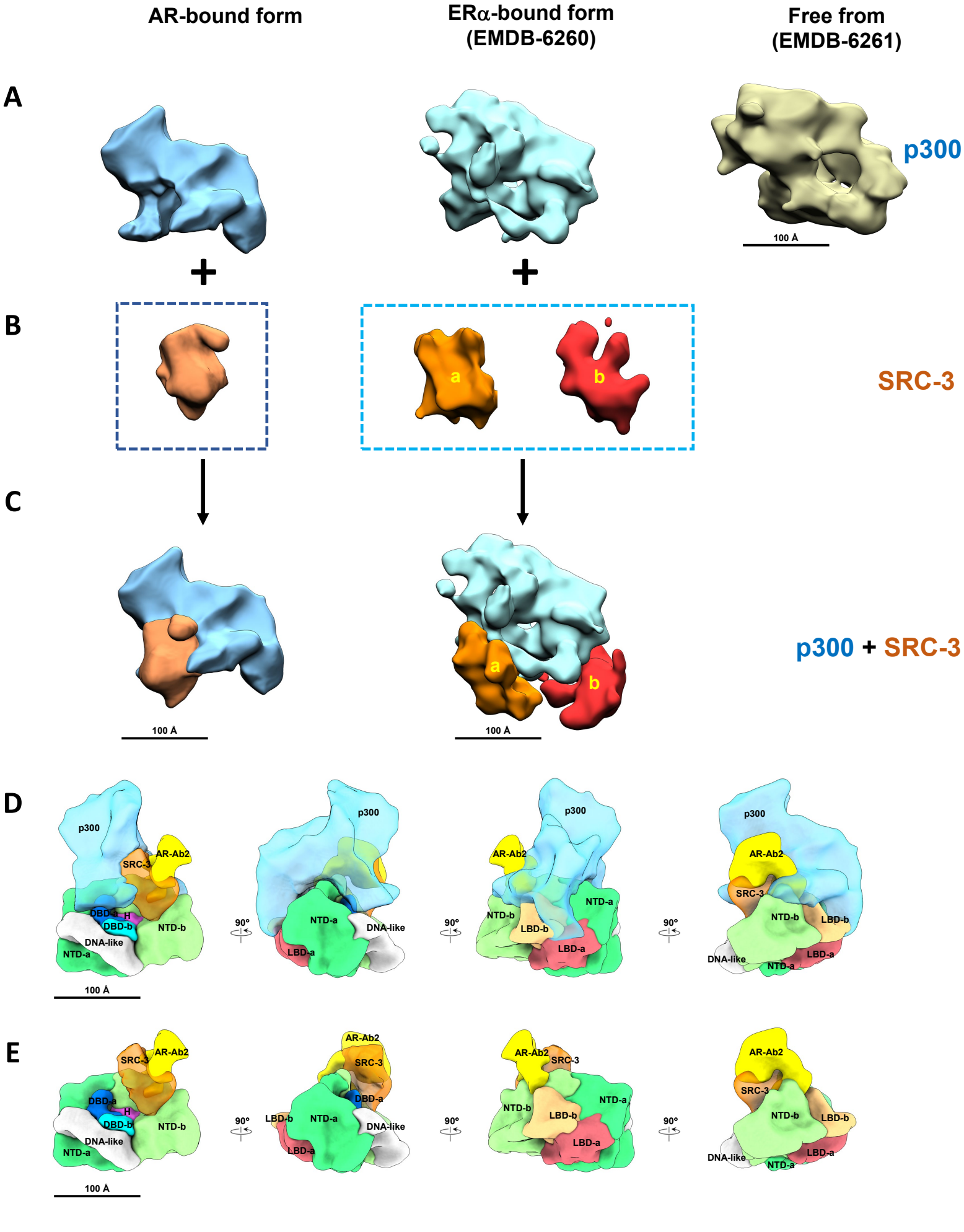
Supplemental Figure 3. ARE DNA-bound AR/SRC-3/p300 complex cryo-EM images and antibody labeling (A) Representative raw images of the ARE DNA-bound AR/SRC-3/p300 complex. The blue boxes represent raw images of the ARE-DNA/AR/SRC-3/p300 complex. The FFT (Fast Fourier Transform) image is shown at the right upper corner demonstrating the quality of data. Enlarged particles are shown on the right panel. (B) The reference free 2D class averages of boxed out ARE-DNA/AR/SRC-3/p300 particles. (C) The gold-standard FSC curve for the final cryo-EM 3D reconstruction generated using RELION. The overall resolution is estimated at 20 Å according to the FSC cut-off at 0.143. (D) Euler angle distribution of all particles used for the final 3D reconstruction. Each view is represented by a cylinder, for which the height is proportional to the number of particles for this specific view. (E) The density map of ARE-DNA/AR/SRC-3/p300/AR-Ab1 complex. (F) The AR-Ab1 density location in the segmented ARE-DNA/AR/SRC-3/p300 complex map. The AR-Ab1 is labeled as AR-Ab1 (Ⅲ). (G) The density map of ARE-DNA/AR/SRC-3/p300/SRC-3-Ab complex rotating in vertical direction. (H) SRC-3-Ab density location in the segmented ARE-DNA/AR/SRC-3/p300 complex map.

Supplemental Figure 4, related to Figure 3 and 4



Supplemental Figure 4. Functional assay and comparison of the AR-Ab1 binding sites in AR or coactivator-bound AR (A) AR, SRC-3 and p300 form an active transcriptional complex. Cell free transcription activity of 3XARE-driven E4 reporter gene in the absence or presence of purified AR, p300, or SRC-3. Shown is the E4 mRNA level transcribed in vitro. * represents p<0.01., n=3. (B) LXXLL motif mutation at the SRC-3 RID abolished the interaction between SRC-3 and ERα. Cell lysates expressing flag-tagged SRC-3 or its RID mut (mutant) were incubated with purified recombinant ERα protein in the absence or presence of 1μM 17β-estradiol (E2) followed by co-immunoprecipitation using an ERα-specific antibody. (C) The two AR-Ab1s in ARE /AR/AR-Ab1 complex are labeled as AR-Ab1 (Ⅰ) and AR-Ab1 (Ⅱ), while the AR-Ab1 in ARE-DNA/AR/SRC-3/p300/AR-Ab1 complex is labeled as AR-Ab1 (Ⅲ). As shown in the blue circle, the AR-Ab1 (Ⅰ) shares a similar position with AR-Ab1 (Ⅲ). The binding site of AR-Ab1 (Ⅱ) (red circle) is blocked by p300 in the ARE DNA/AR/SRC-3/p300 complex.

Supplemental Figure 5, related to Figure 3



Supplemental Figure 5. The topological position of AR-Ab2 binding site related with p300 and SRC-3 binding sites in AR, and AR or ERα-bound coactivator structural comparisons (A-C) Comparison of SRC-3 and p300 structures in AR- or ERα-bound state. (A) The density maps of p300 in an AR-bound, ERα-bound (EMDB-6260) or free form (EMDB-6261) (B) The density maps of SRC-3 in different states. SRC-3a and SRC-3b in ERα complex were labeled as a and b, respectively.(C) The density maps of SRC-3/p300 complex in AR or ERα complex (D) Docking of AR-Ab2 labeled AR dimer structure into AR/coactivator complex structure. (E) SRC-3 binding site is in close proximity to AR-Ab2 recognized region on NTD-b with a small density overlap. The p300 density is hided from the complex for a better view of the positions of SRC-3 and AR-Ab2.

Supplemental Table 1, related to Figure 1 and 3

Image number and particle number table

	#1	#2	#3	#4	#5	#6
	ARE-DNA/AR	ARE-DNA/AR/SRC-3/p300	ARE-DNA/AR/AR-Ab1	ARE-DNA/AR/AR-Ab2	ARE-DNA/AR/SRC-3/p300/AR-Ab1	ARE-DNA/AR/SRC-3/p300/SRC3-Ab
	(EMDB-22079)	(EMDB-22080)	(EMDB-22079)	(EMDB-22079)	(EMDB-22080)	(EMDB-22080)
Data collection and processing						
Magnification	105,000	20,000	20,000	20,000	20,000	20,000
Voltage (kV)	300	300	300	300	300	300
Electron exposure (e–/Å^2)	1	0.55	0.55	0.55	0.55	0.55
Defocus range (μm)	-1.0 to -2.5	-1.2 to -2.8	-1.2 to -2.8	-1.2 to -2.8	-1.2 to -2.8	-1.2 to -2.8
Pixel size (Å)	1.39	1.74	1.74	1.74	1.74	1.74
Symmetry imposed	2.7	4.7	4.7	4.7	4.7	4.7
Initial particle images (no.)	2	2	2	2	2	2
Final particle images (no.)	50	50	50	50	50	50
Movie stack number	1,958	5,741	1,035	3,146	1,373	3,360
Particle numbers (boxed out)	223,299	869,561	134,460	512,352	118,151	510,788
Particle numbers (refinemnet)	30,958	53,198	12,947	62,722	15,155	100,,666
Map resolution (Å)	12.6	20	23.7	20.9	26.8	16.6
FSC threshold	0.143	0.143			0.143	0.143
Map resolution range (Å)	10.0 to 14.0	17.0 to 22.0			17.0 to 21.0	17.0 to 21.0
Initial model used (PDB code)	N/A	N/A	N/A	N/A	N/A	N/A