9*H*-Purine Scaffold Reveals Induced-Fit Pocket Plasticity of the BRD9 Bromodomain

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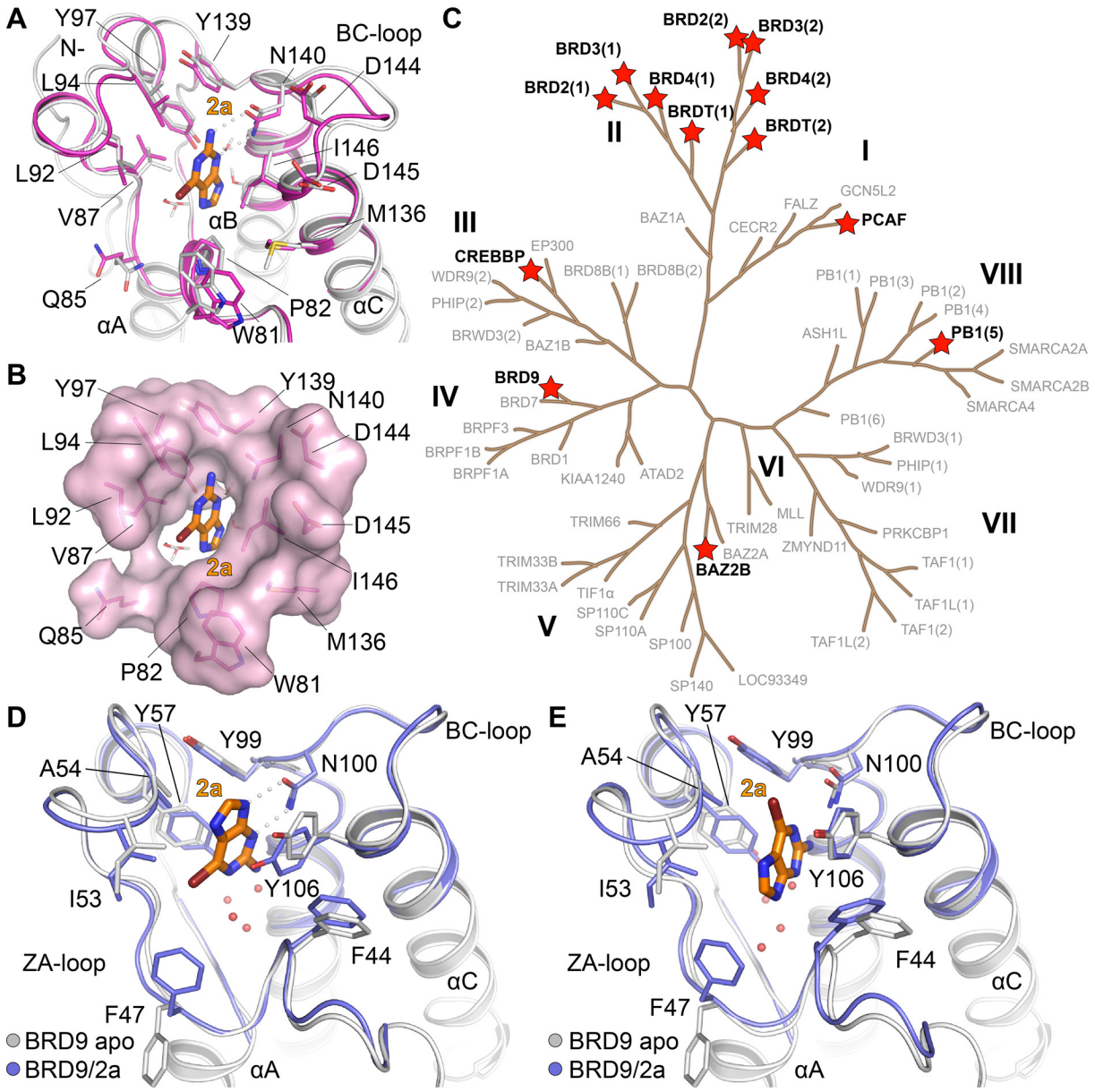
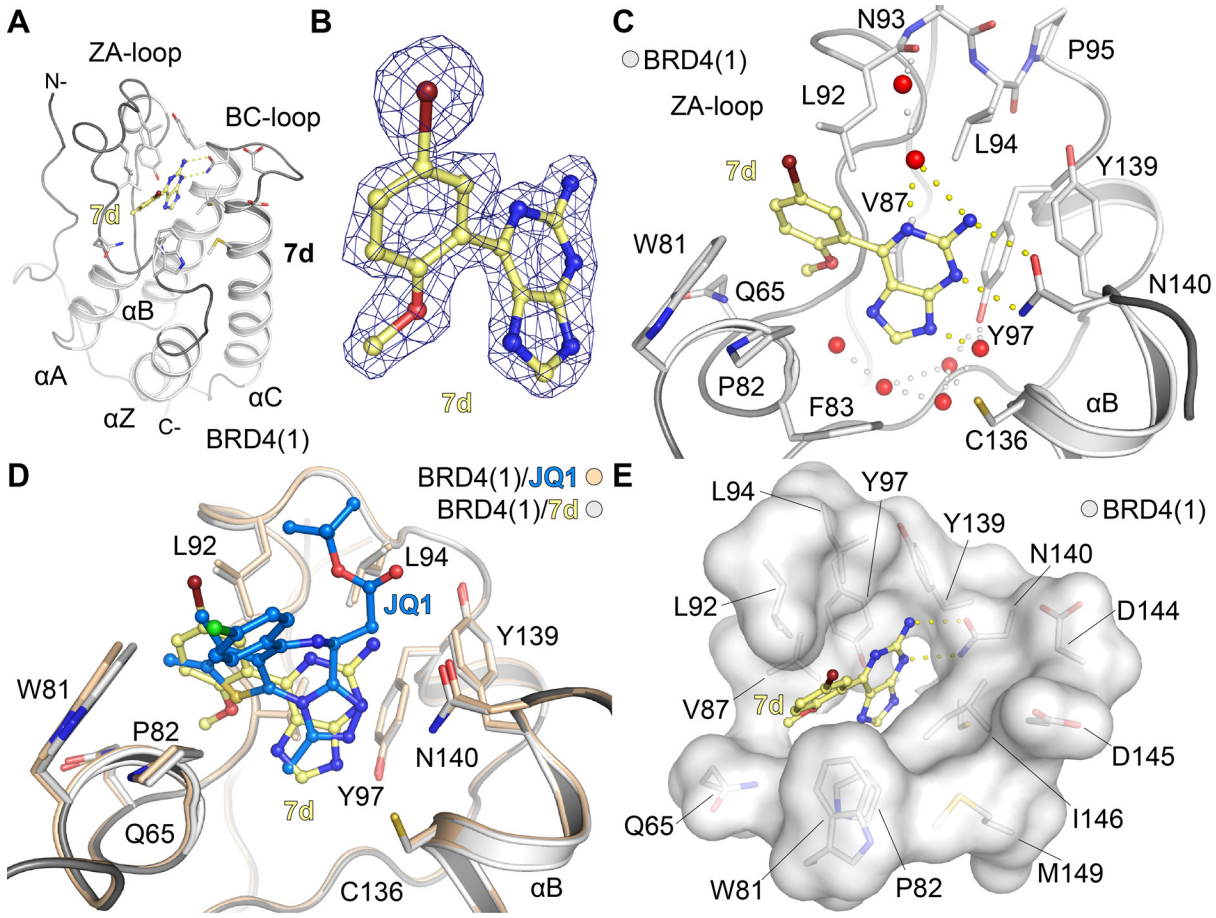


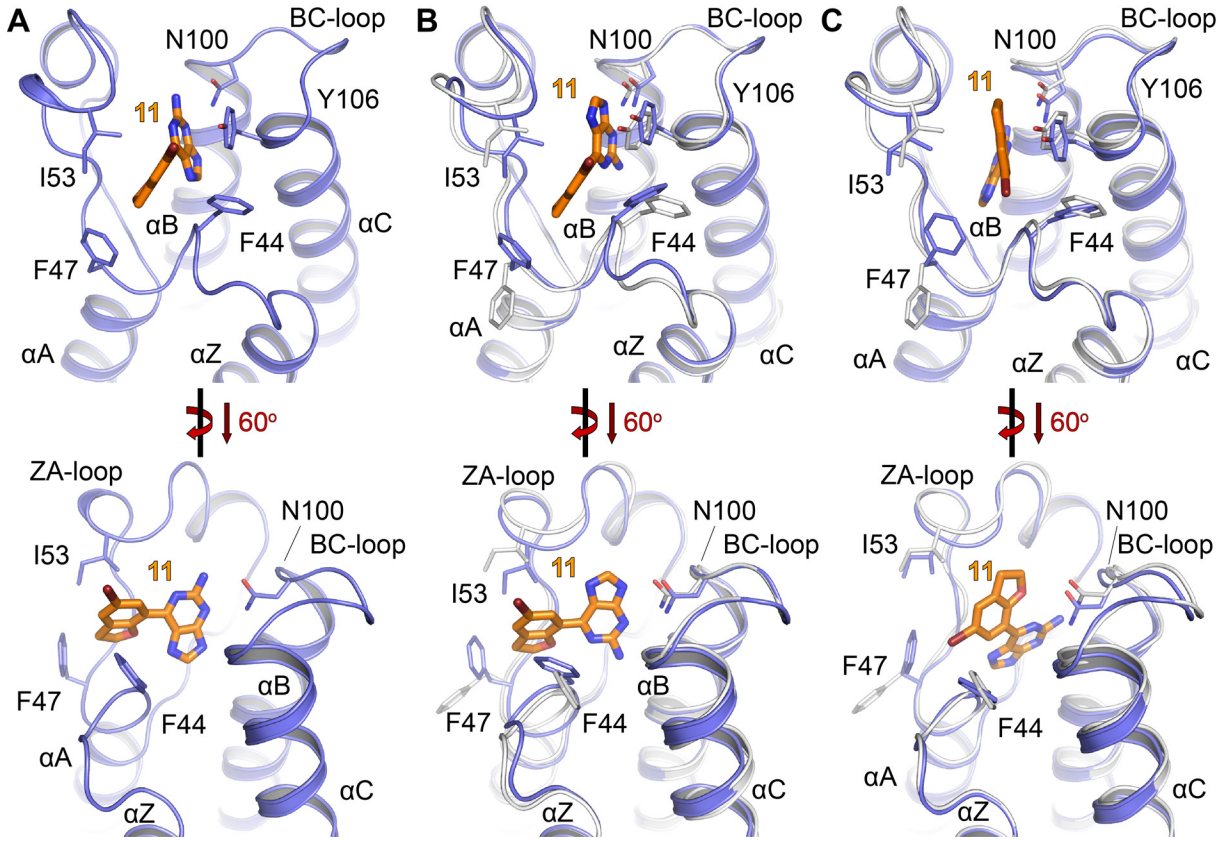
Supplemental Figure 1 (**A**) Alternative docking detail of compound **2a** (orange sticks) onto BRD4(1). The ligand adopts a Kac-mimetic pose within the BRD cavity, with the amine group directly engaging the conserved asparagine (N140) and the 6-Br substituent vector orienting between the ZA-loop W81 and L92 channel. The protein is shown in white (apo BRD4(1) structure) or in magenta (docked model) with residues making up the binding cavity shown in stick representation. (**B**) Surface representation of the binding cavity of BRD4(1) with compound

2a bound as shown in (A). The starting model for docking used in panels A and B was PDB ID: 4MEN. (**C**) Phylogenetic tree of the human bromodomain family. Structural sub-families are annotated with roman numerals. Domains tested for compound binding are annotated in bold typeface and are highlighted with a red star, covering most subfamilies of human BRDs. (**D**) Docking of compound **2a** (shown as orange sticks) onto the bromodomain of BRD9 (from PDB ID: 3HME). The ligand adopts an acetyl-lysine mimetic pose as in the case of BRD4(1), directly engaging the protein at the conserved asparagine (Asn100) via N3 and N9 while sterically packing between the ZA-loop Ile53/Ala54 and F47/F44 at the front of the BRD cavity. The protein is colored white (apo structure) or light blue (docked model) with key residues shown in stick representation and annotated. (**E**) Alternative docking mode of **2a** in the BRD9 cavity inserting the primary function towards the conserved asparagine (Asn100) while retaining the steric packing within the ZA-loop residues. The protein/ligand are colored as in (E) and the model used for docking was PDB ID: 3HME.



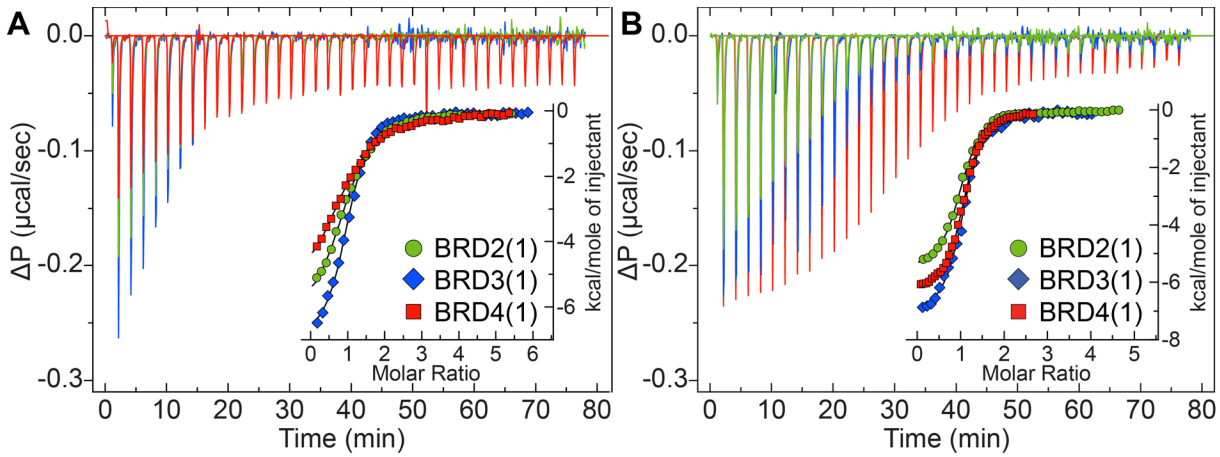
Supplemental Figure 2 – *Binding of compound **7d** to BRD4(1)* (**A**) Overall fold of BRD4(1)/**7d** complex. (**B**) 2FcFo map of **7d** in complex with BRD4(1) contoured at 2σ. (**C**) Similar to the BRD9 complex, **7d** occupies the acetyllysine binding cavity of the BRD4(1) bromodomain module initiating direct interactions with the conserved asparagine (N140) and packing onto the ZA-loop hydrophobic backbone (L92/L94) and the front part of the ZA-channel (W81/P82). The conserved water network is also preserved in this structure and the ligand initiates hydrogen bonds that further stabilize binding. As in the case of the BRD9 complex the ligand engages two water molecules located on the top of the BRD cavity in a network of hydrogen bonds to the backbone of N93. (**D**) The mode of **7d** binding to BRD4(1) is similar to that of JQ1 (PDB ID: 3MXF) with the five member ring of the purine core mimicking the methyl-tiazolo function of

JQ1. (**E**) Surface representation of the binding cavity of BRD4(1) in complex with the **7d** highlighting the steric fit of the bromo-methoxyphenyl substituent between the ZA-loop L92 and the ZA-channel W81. The model and structure factors of the BRD4(1)/**7d** complex have been deposited to the PDB with ascension code 4XY9.

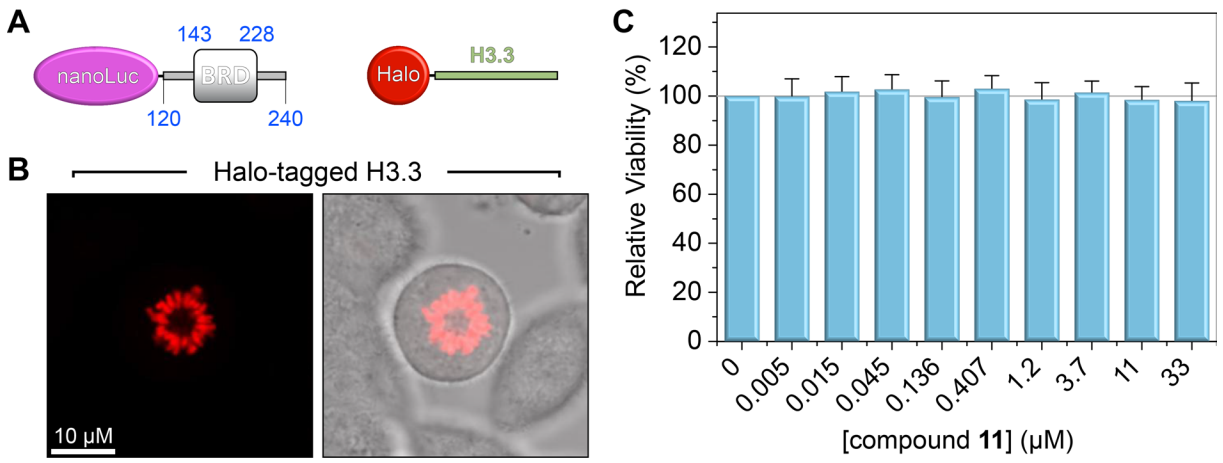


Supplemental Figure 3 – *Docking of compound **11** to BRD9.* (A) Rigid docking of compound **11** onto the complex structure of BRD9/**7d** results in a minimal energy pose that resembles the **7d**/BRD9 complex. PDB ID: 4XY8 was used as a starting point for docking. (**B**) Induced fit of compound **11** into the cavity of the BRD9/**7d** complex (taken from PDB ID: 4XY8) results in an orientation of the ligand that inverts its primary amine function, without affecting the side-chains of the residues within the binding site of BRD9. (**C**) Induced fit docking of compound **11** into the apo site of BRD9 (PDB ID: 3HME) results in a re-arrangement of the binding site residues in a similar mode to that observed in the case of compound **7d**, however the ligand rotates its 6-aryl substituent by 180 degrees. In all cases the apo protein is colored white and the resulting docking model is colored in blue with the ligand shown as orange sticks and the bottom panel showing a

clockwise 60 degree rotation of the structures, highlighting the tilt of the ligand poses with respect to each other.



Supplemental Figure 4 – *Binding of compounds **7d** and **11** to BET bromodomains* Binding of compound (**A**) **7d** or (**B**) **11** to BET bromodomains was evaluated by isothermal titration calorimetry and was found to be in the low micro-molar range. Raw injection heats are shown for the reverse titrations of protein into a solution of the ligand. The inset shows the normalized binding enthalpies corrected for the heat of protein dilution as a function of binding site saturation (symbols as indicated in the figure). Solid lines represent a nonlinear least squares fit u sing a single-site binding model. ITC titrations were carried out in 50 mM HEPES pH 7.5 (at 25 °C), 150 mM NaCl and 15 °C while stirring at 1000 rpm.



Supplemental Figure 5 – *In cell validation of compound **11***. (**A**) NanoLuc fusion construct of the bromodomain of BRD9 (UniProt: Q9H8M2, residues 120-240) used to probe binding to Halo-tagged histone H3.3 in a BRET assay. (**B**) Confocal images of Halo-tagged histone H3.3 transfected into HEK293 cells demonstrating incorporation into the nucleus. (**C**) Cytotoxicity assay demonstrating that compound **11** is not toxic to HEK293 cells in the concentration range used for the BRET assay in Figure 5.