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Supporting information for article:

Structural and biochemical insights into purine-based drug molecules in hBRD2 delineate a unique binding mode opening new vistas in the design of inhibitors of the BET family

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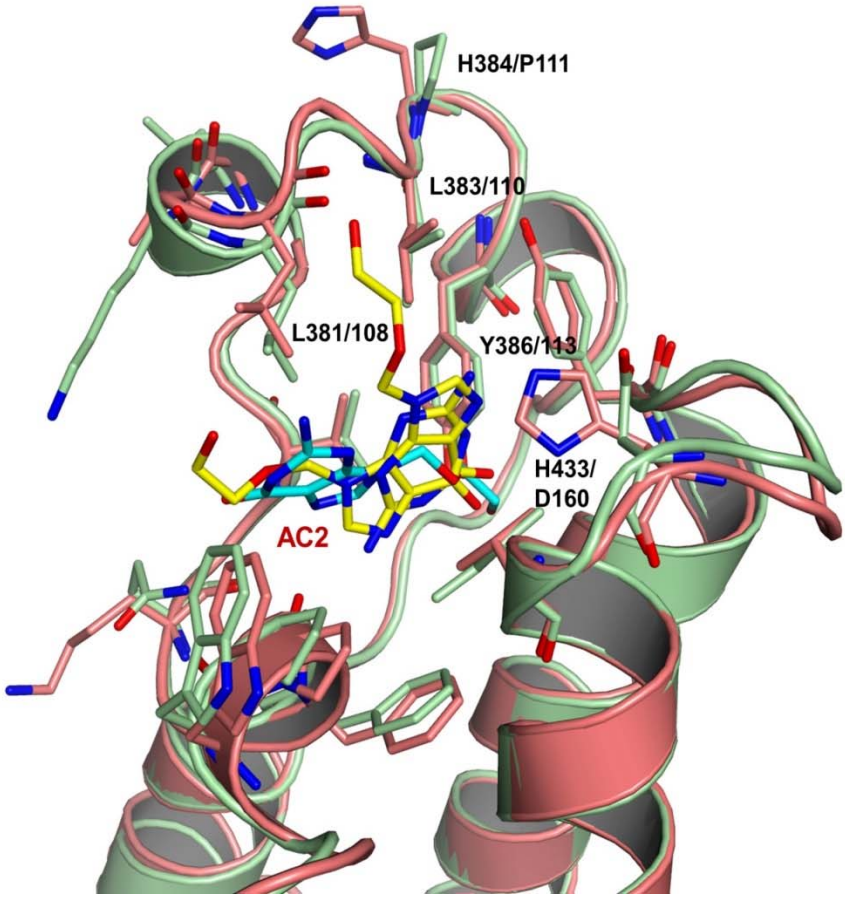


Figure S1 Superposition of the BD1-AC2 complex onto the BD2-AC2 complex. BRD2-BD1 and BRD2-BD2 complexes are represented in pale green and deep salmon, respectively. The AC2 molecules in BD1 and BD2 are shown in cyan and yellow sticks, respectively.

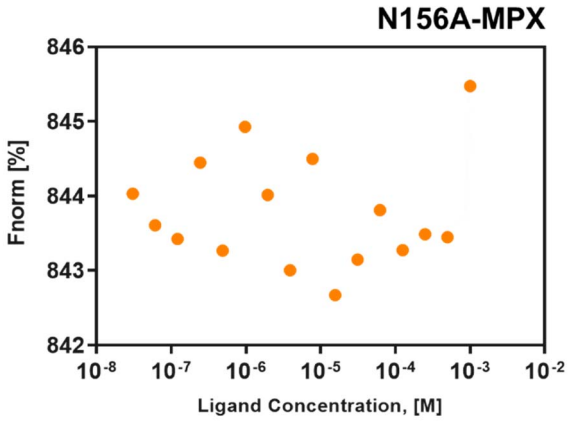


Figure S2 Quantitative binding studies of MPX. The N156A mutant abolishes the binding with MPX.

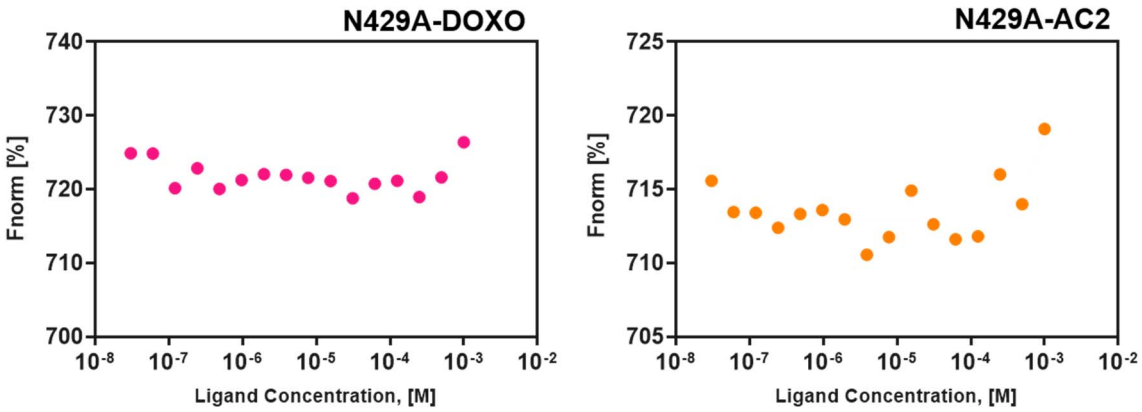


Figure S3 Quantitative binding studies of DOXO and AC2 with the BD2 mutant N429A**.** The N429A mutant abolishes the binding with these compounds.

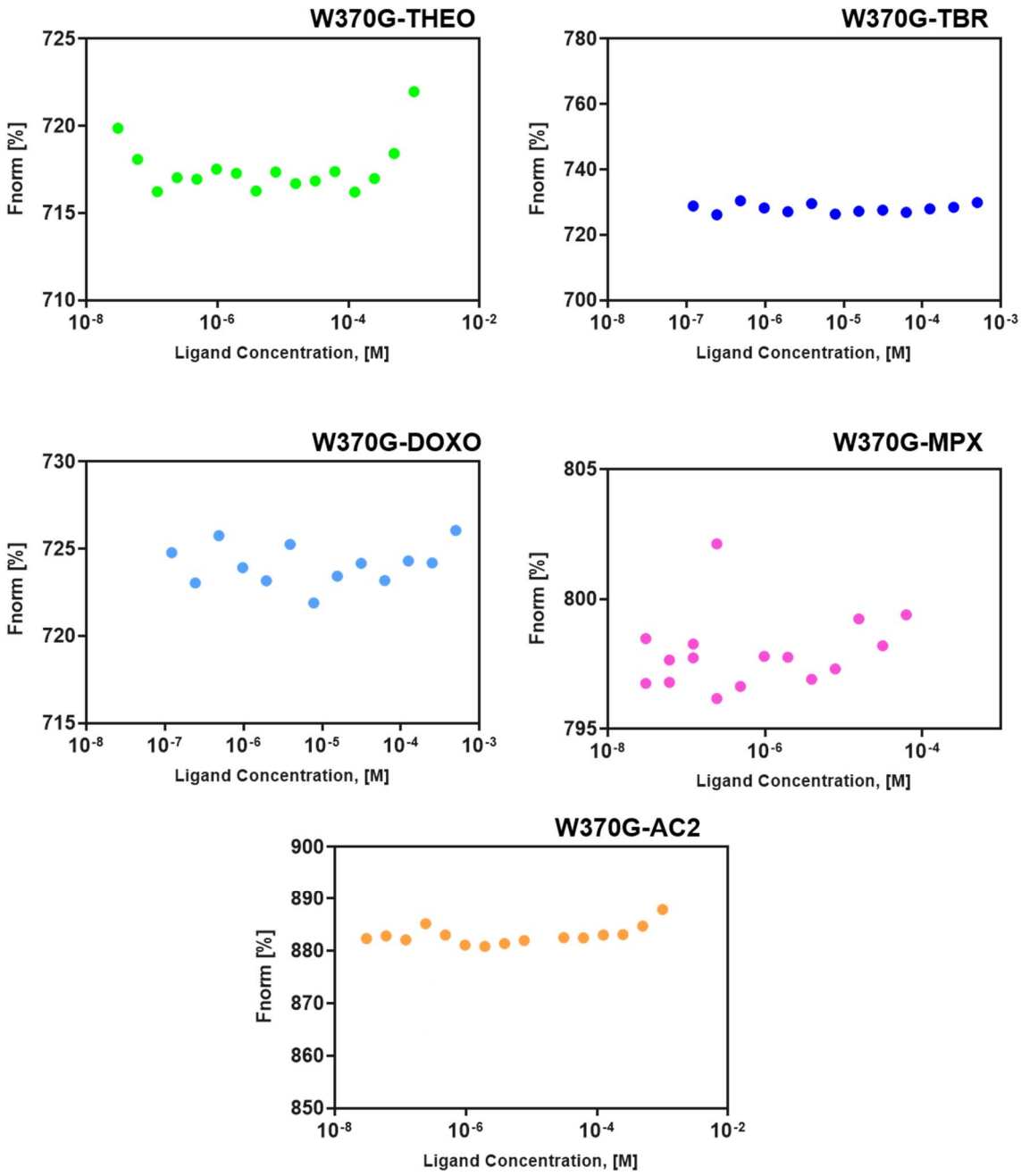


Figure S4 Quantitative binding studies of THEO, TBR, DOXO, MPX, and AC2 with BD2 mutant W370G. The W370G mutant shows no binding with these compounds.

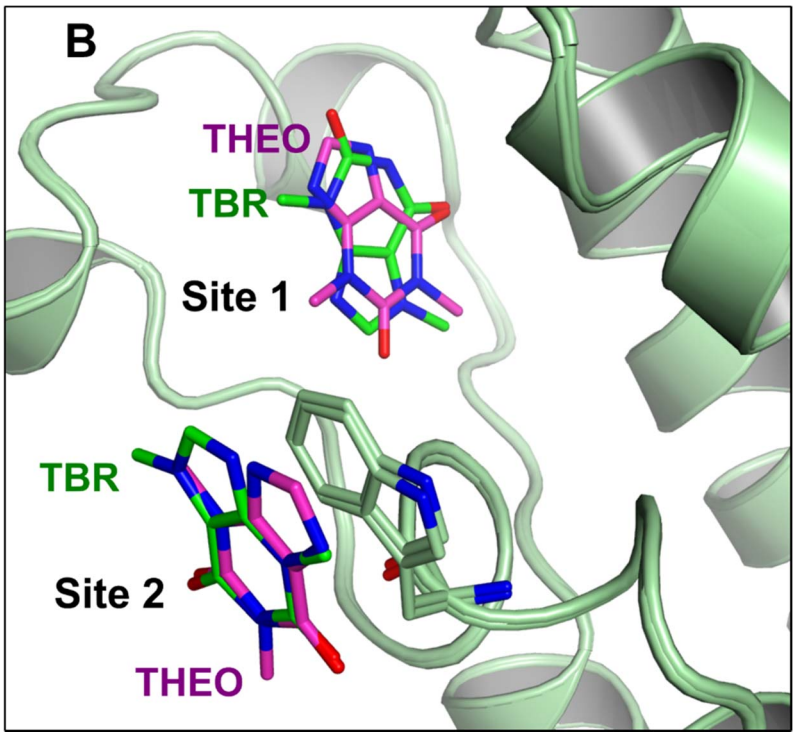


Figure S5 Superposition of the BD1-TBR complex onto the BD1-THEO complex. THEO and TBR are represented in purple and green sticks, respectively.

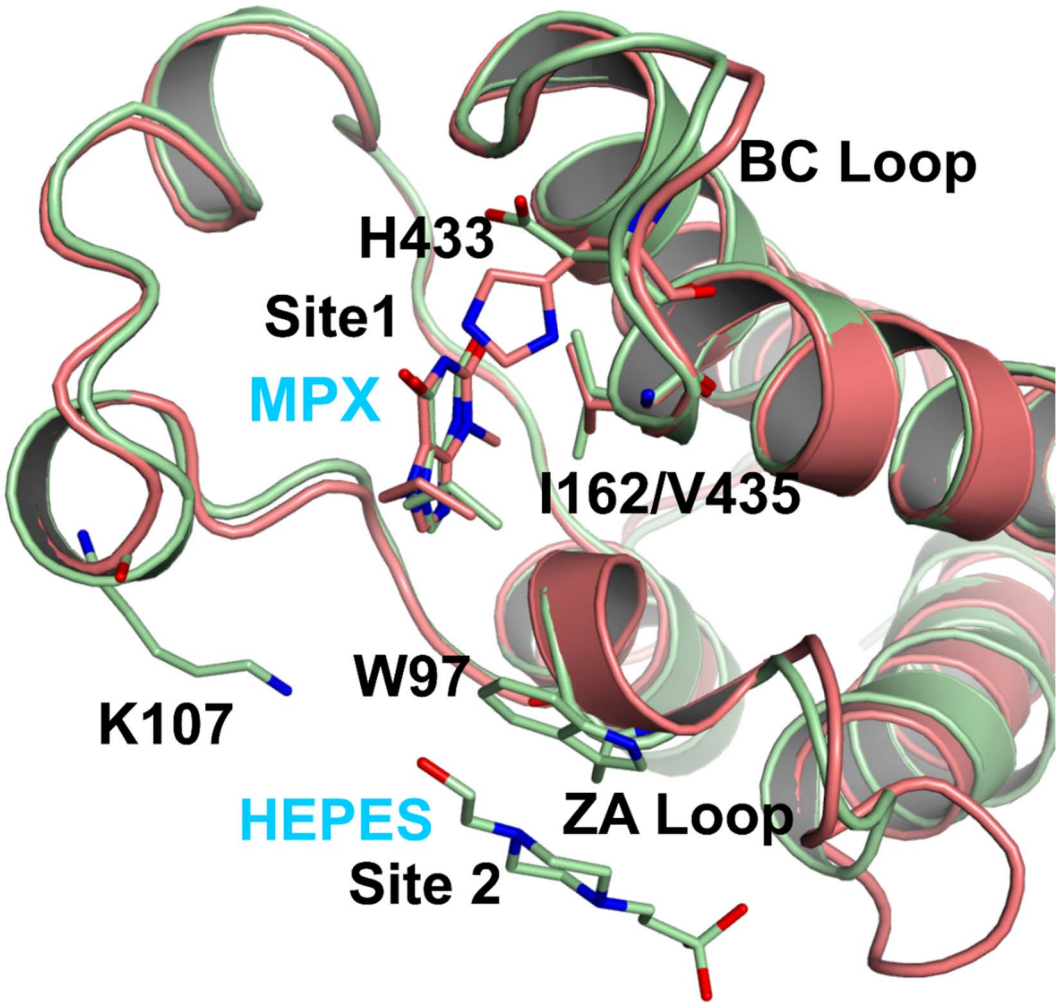
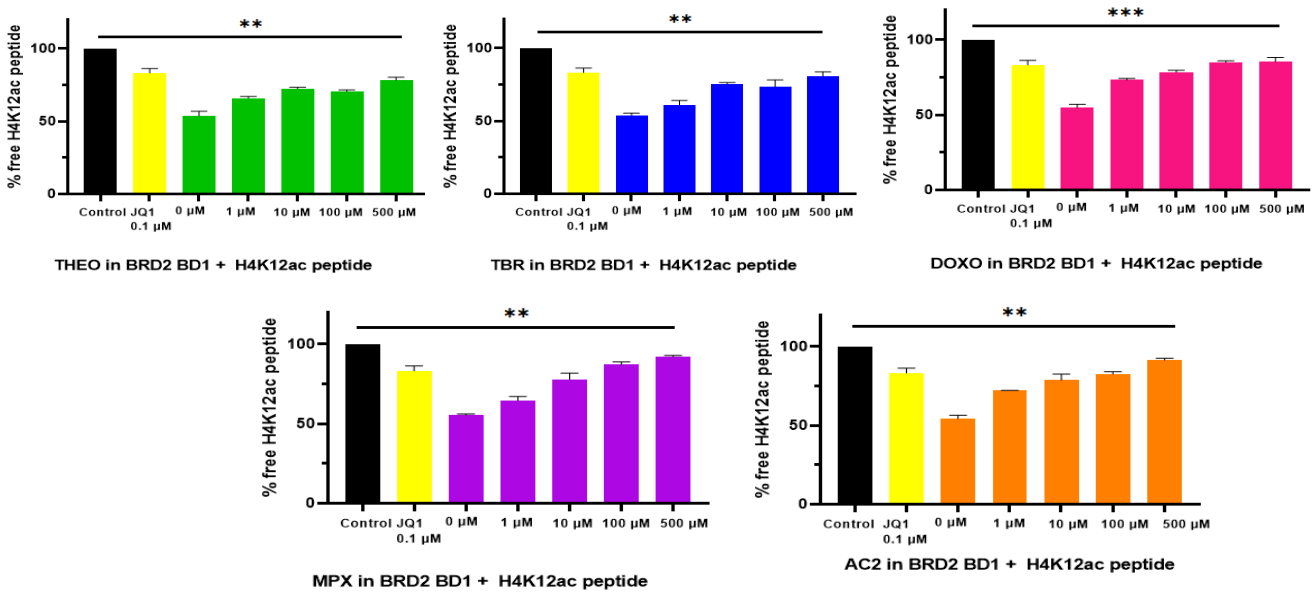
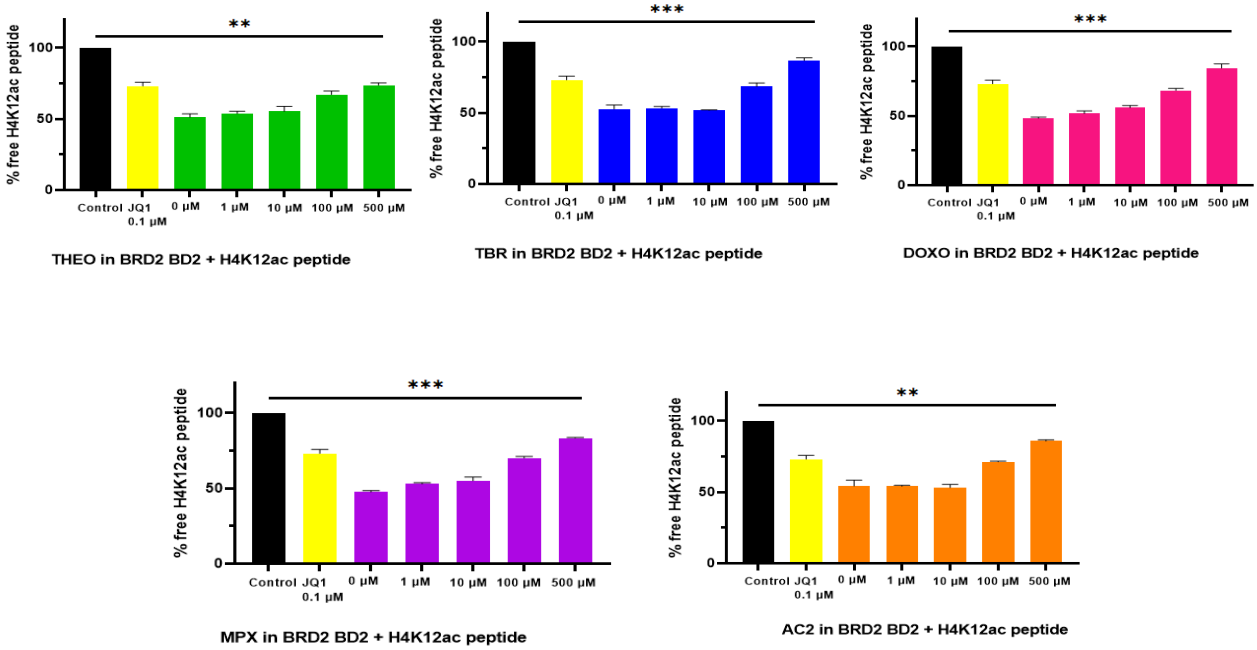


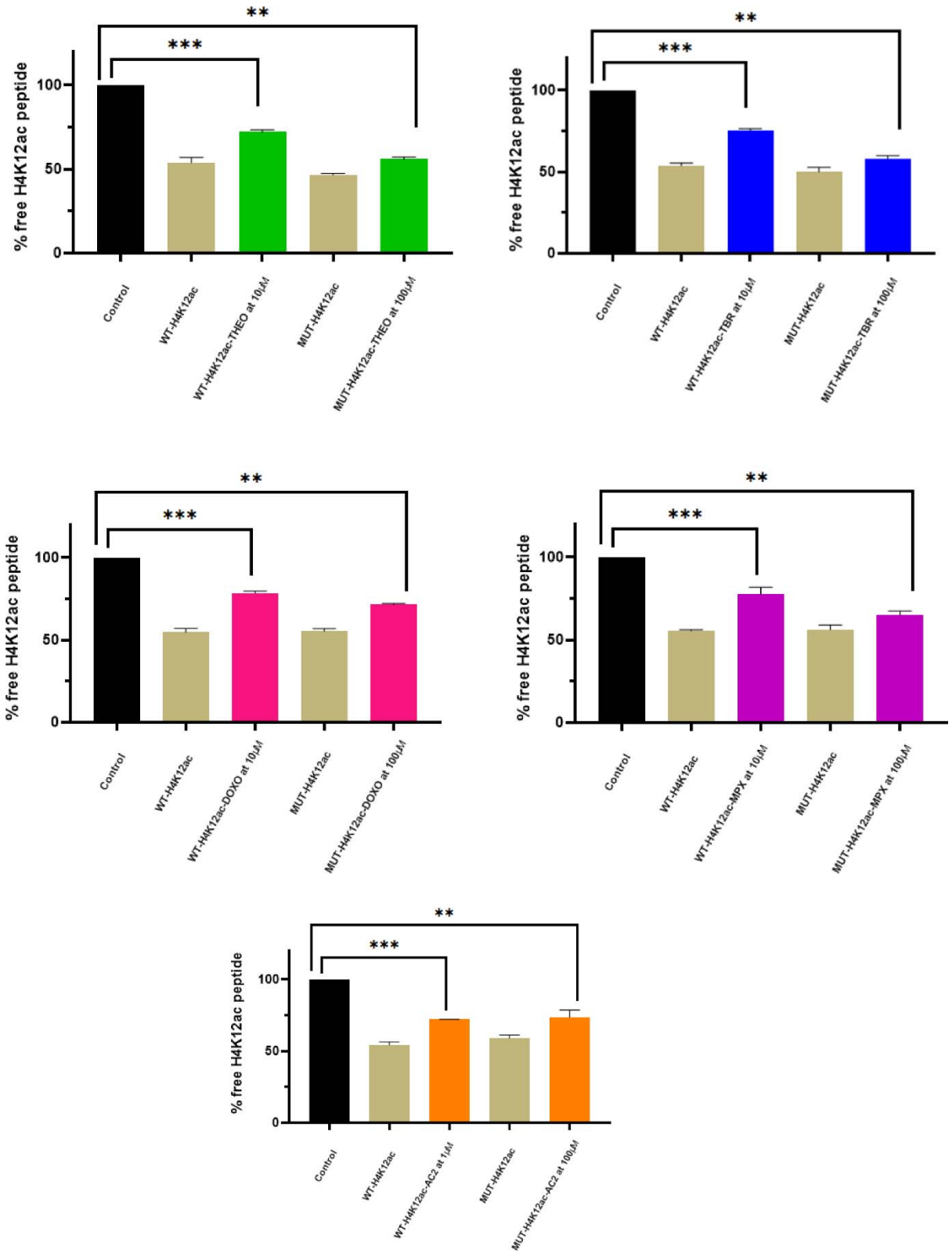
Figure S6 Superposition of the BD1-MPX complex onto the BRD2-BD2-MPX complex. BRD2-BD1 and BRD2-BD2 complexes are represented in pale green and deep salmon, respectively. The HEPES molecules stacking with W97 in the BD1 complex are also shown


% free H4K12ac peptide
100
50
0
**
Control JQ1 0.1 µM
0 µM
1 µM
10 µM
100 µM
500 µM
THEO in BRD2 BD1 + H4K12ac peptide
TBR in BRD2 BD1 + H4K12ac peptide
***
DOXO in BRD2 BD1 + H4K12ac peptide
MPX in BRD2 BD1 + H4K12ac peptide
AC2 in BRD2 BD1 + H4K12ac peptide

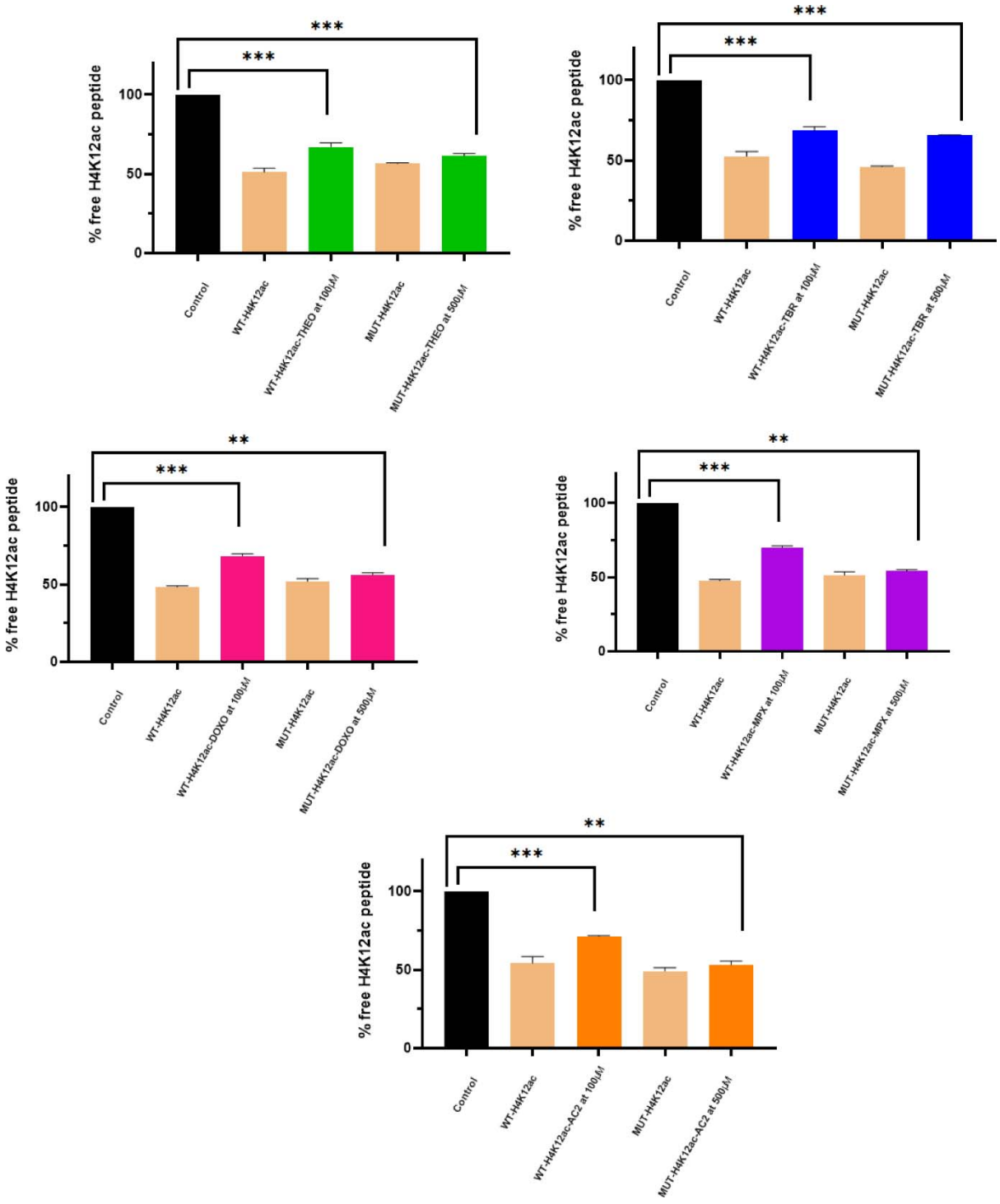
(a)


% free H4K12ac peptide
100
50
0
**
Control JQ1 0.1 µM
0 µM
1 µM
10 µM
100 µM
500 µM
THEO in BRD2 BD2 + H4K12ac peptide
***
TBR in BRD2 BD2 + H4K12ac peptide
***
DOXO in BRD2 BD2 + H4K12ac peptide
***
MPX in BRD2 BD2 + H4K12ac peptide
**
AC2 in BRD2 BD2 + H4K12ac peptide

(b)

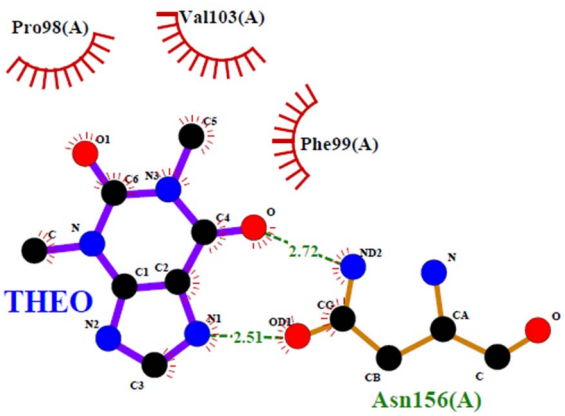
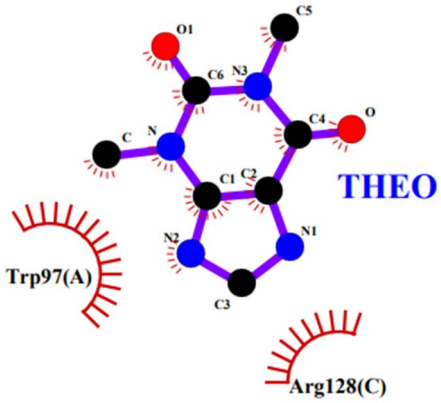
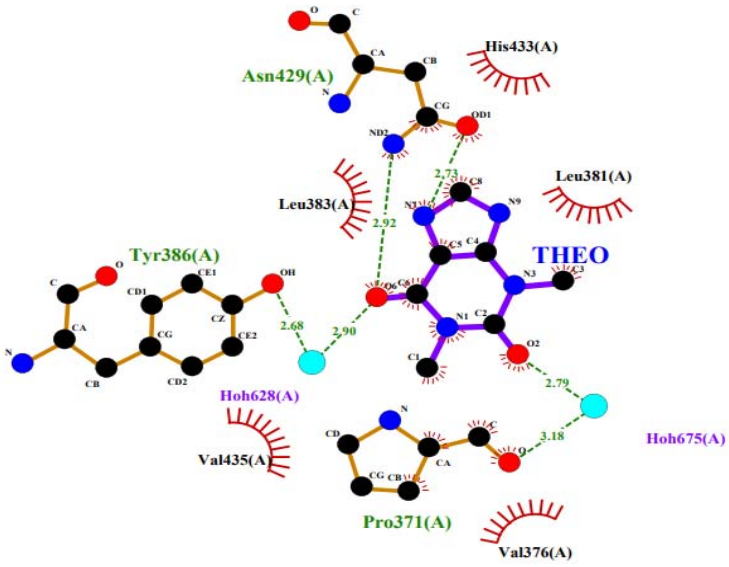

**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-THEO at 10µM
MUT-H4K12ac
MUT-H4K12ac-THEO at 100µM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-TBR at 10µM
MUT-H4K12ac
MUT-H4K12ac-TBR at 100µM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-DOXO at 10µM
MUT-H4K12ac
MUT-H4K12ac-DOXO at 100µM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-MPX at 10µM
MUT-H4K12ac
MUT-H4K12ac-MPX at 100µM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-AC2 at 1µM
MUT-H4K12ac
MUT-H4K12ac-AC2 at 100µM

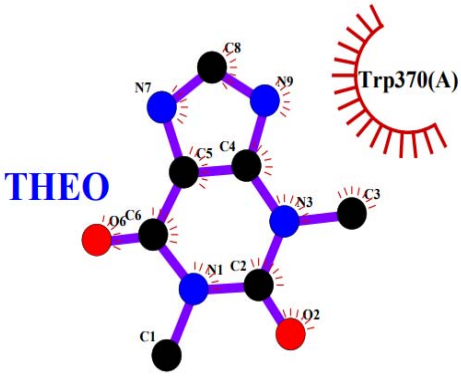
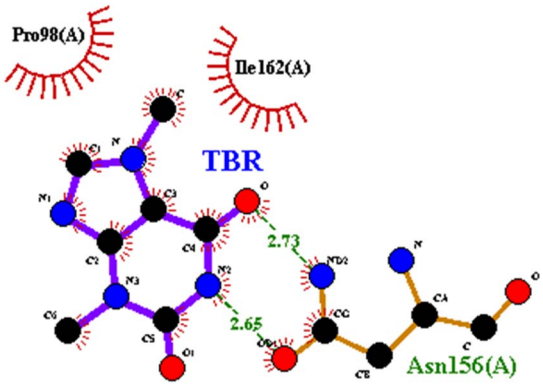
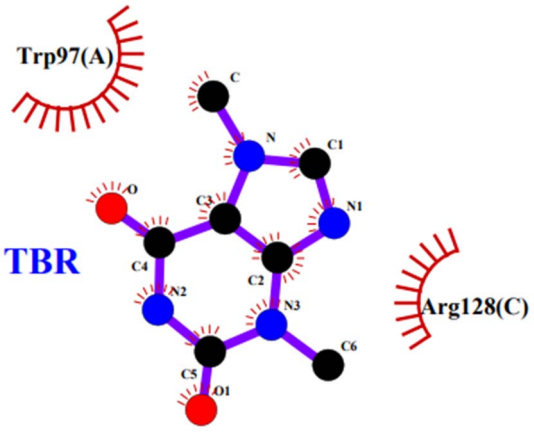
(c)

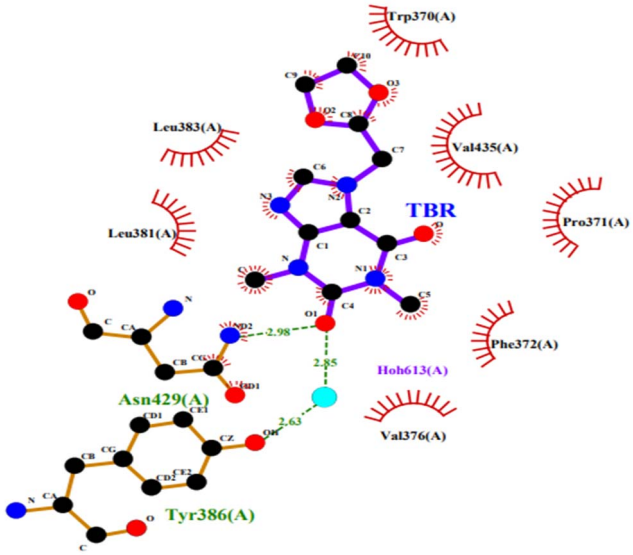
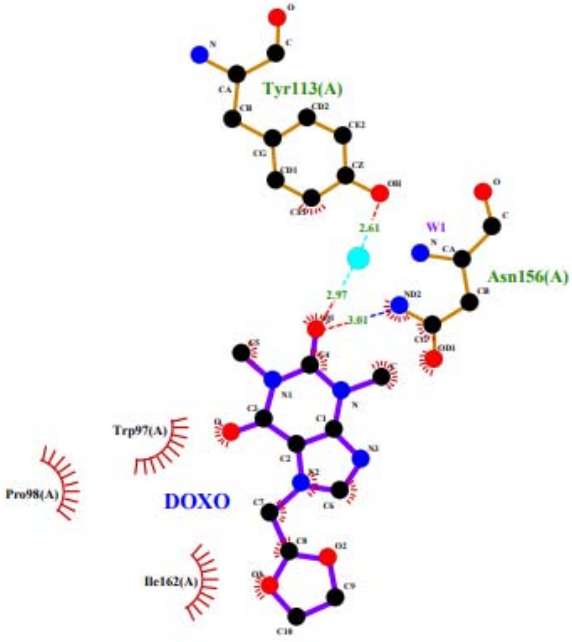
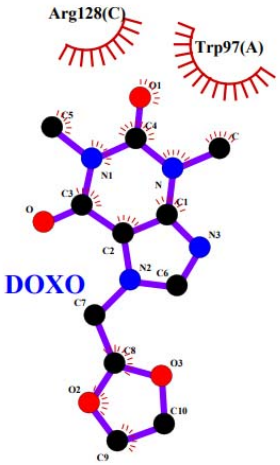

***
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-THEO at 100μM
MUT-H4K12ac
MUT-H4K12ac-THEO at 500μM
***
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-TBR at 100μM
MUT-H4K12ac
MUT-H4K12ac-TBR at 500μM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-DOXO at 100μM
MUT-H4K12ac
MUT-H4K12ac-DOXO at 500μM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-MPX at 100μM
MUT-H4K12ac
MUT-H4K12ac-MPX at 500μM
**
***
% free H4K12ac peptide
100
50
0
Control
WT-H4K12ac
WT-H4K12ac-AC2 at 100μM
MUT-H4K12ac
MUT-H4K12ac-AC2 at 500μM

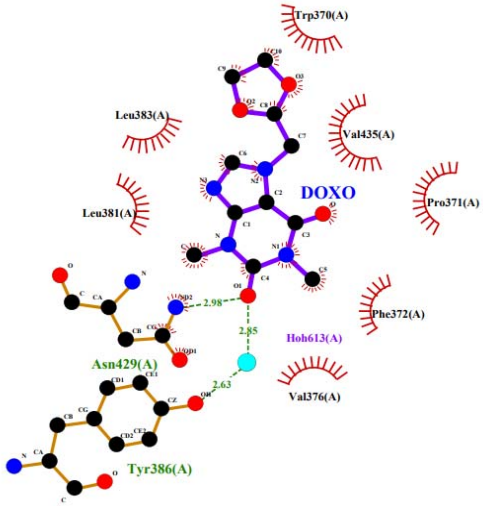
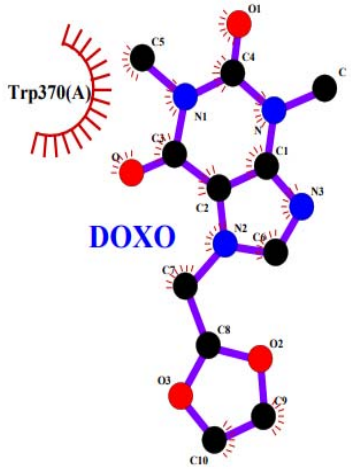
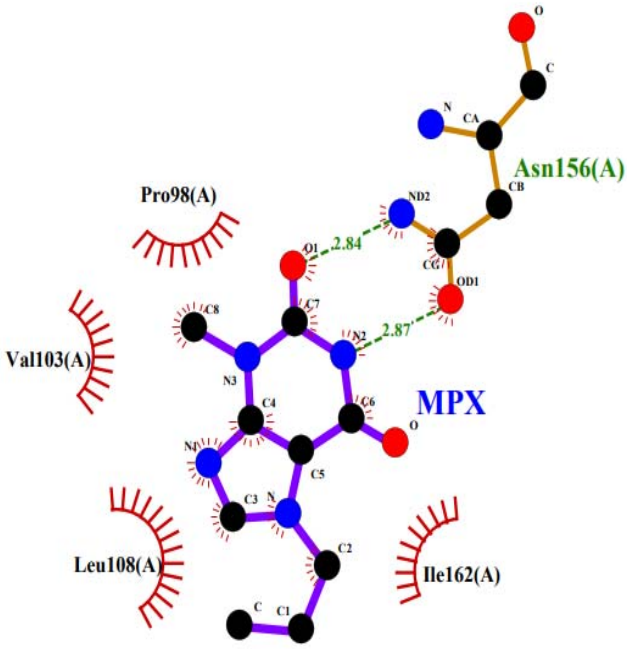
(d)

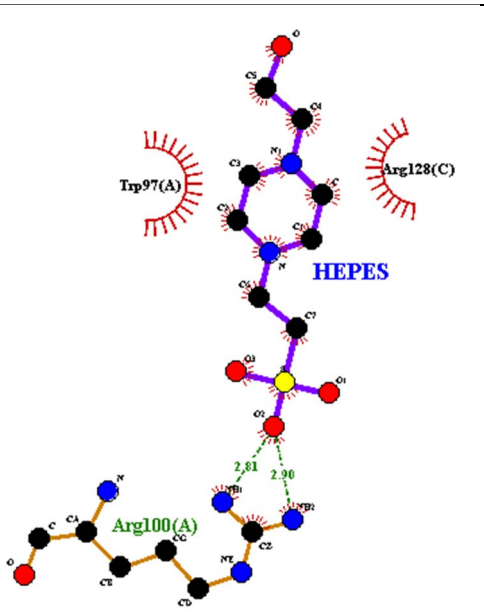
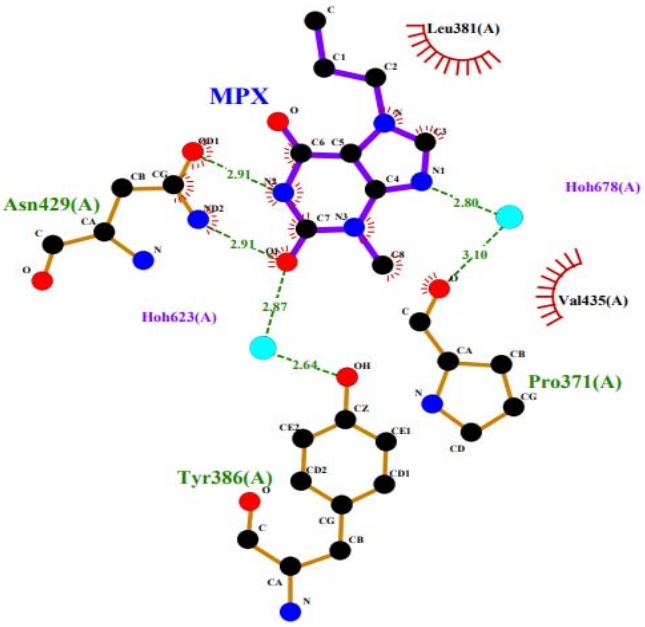
Figure S7 The competitive binding assay. ***(a)*** **and** ***(b)*** Competitive binding assay between the ligands and the H4K12ac peptide against the BRD2 BD1 and BD2 bromodomains. The FITC-tagged H4K12ac peptide bound with the bromodomain was displaced by the ligands. The percentage of free H4K12ac peptide was calculated and plotted. The values are normalized to the percentage of free H4K12ac peptide; data are the means of triplicate experiments ± SD; the p-value was calculated using Friedman non-parametric test using GraphPad Prism, which revealed a statistically significant difference between experimental ligands and control $p < 0.05$. ***(c)*** **and** ***(d)*** Competitive binding assay between ligands and the H4K12ac peptide against wild-type Bromodomain BRD2 BD1 and BD2 along with Mutants W97A and W370G were shown in Fig 7c & d The FITC-tagged H4K12ac peptide bound with Bromodomain were displaced by ligands. The percentage of free H4K12ac peptide was calculated and plotted. The values are normalized to the percentage of free H4K12ac peptide; data are the means of triplicate experiments ± SD; One sample t-test revealed a statistically significant difference between experimental ligands and control with $p<0.05$ considered as significant.

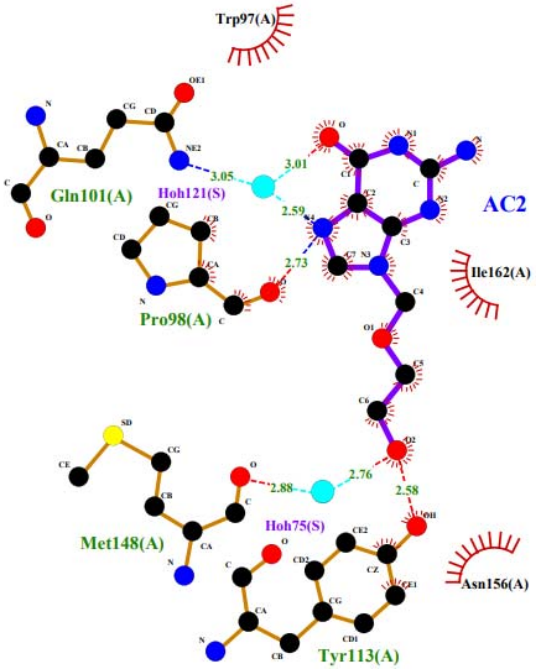
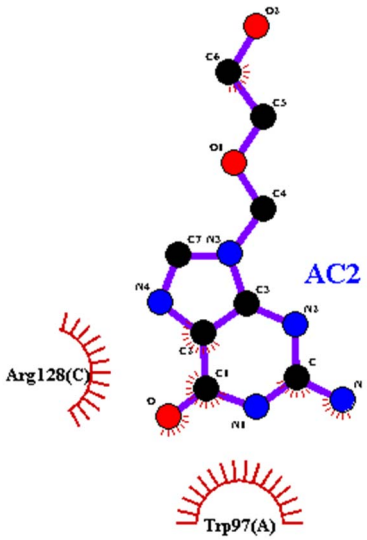
BD1-THEO-Site 1 **PDB ID: 7VRK**	
BD1-THEO-Site 2 **PDB ID: 7VRK**	
BD2-THEO-Site 1 **PDB ID: 7VRM**	

BD2-THEO-Site 2 **PDB ID: 7VRM**	
BD1-TBR-Site 1 **PDB ID: 7VRO**	
BD1-TBR-Site 2 **PDB ID: 7VRO**	

BD2-TBR-Site 1 **PDB ID: 7VRQ**	
BD1-DOXO-Site 1 **PDB ID: 7VRZ**	
BD1-DOXO-Site 2 **PDB ID: 7VRZ**	

BD2-DOXO-Site 1 **PDB ID: 7VS0**	
BD2-DOXO-Site 2 **PDB ID: 7VS0**	
BD1-MPX-Site 1 **PDB ID: 7VSF**	

BD1-HEPES-Site 2 **PDB ID: 7VSF**	
BD2-MPX-Site 1 **PDB ID: 7VS1**	

BD1-AC2-Site 1 **PDB ID: 7VRH**	
BD1-AC2-Site 2 **PDB ID: 7VRH**	

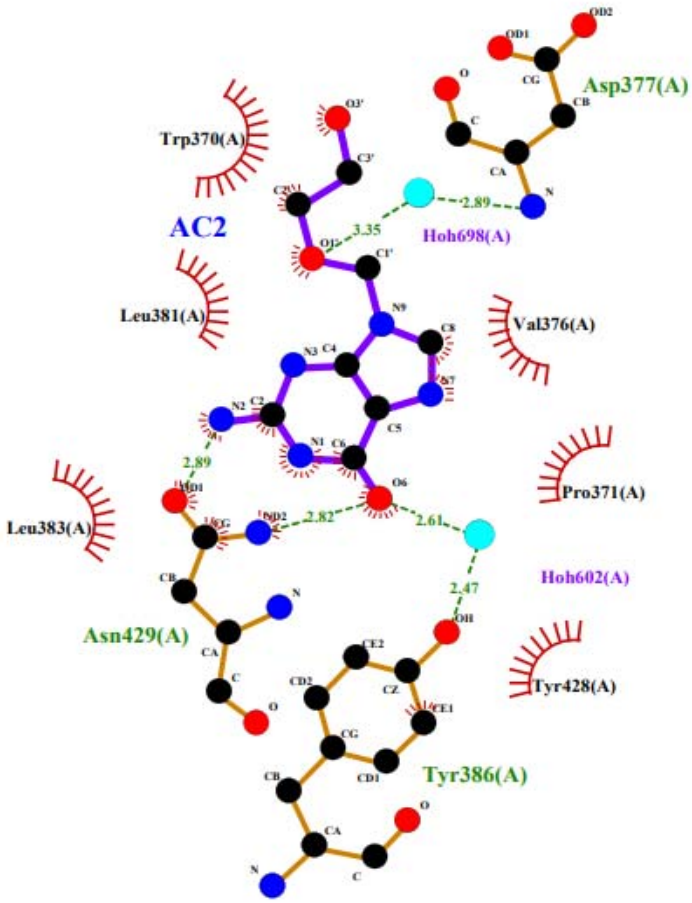
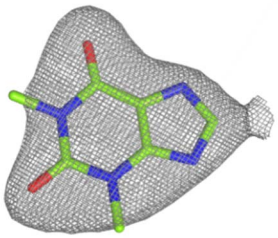
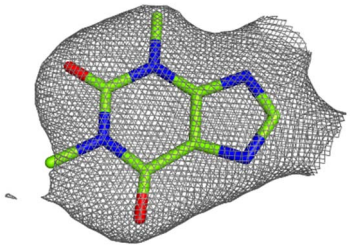
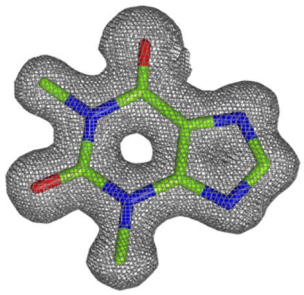
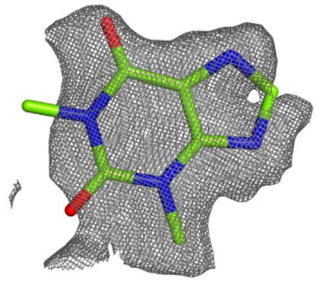
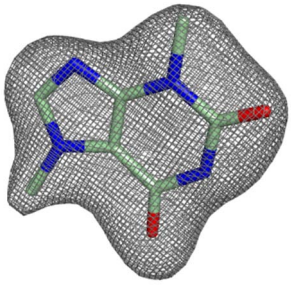
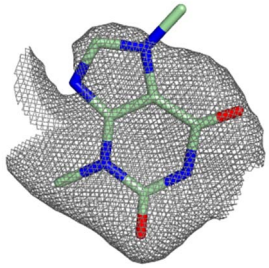
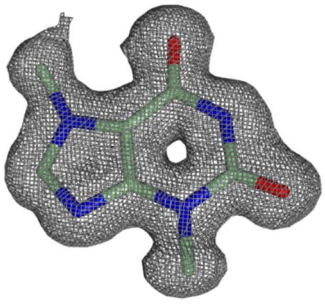
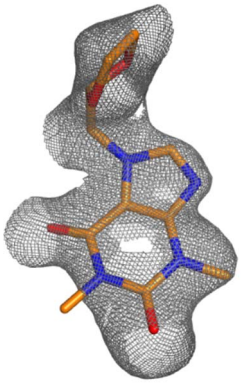
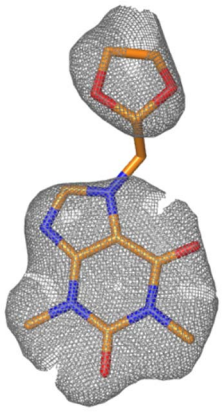
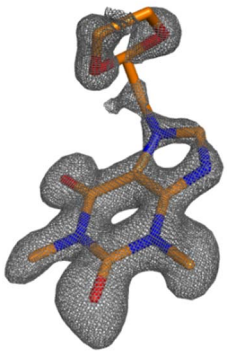
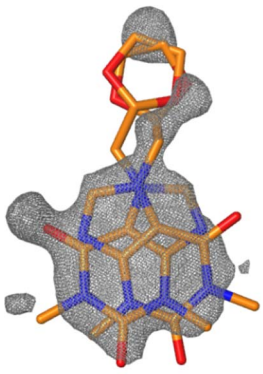
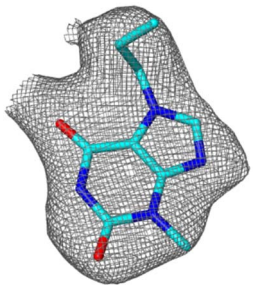
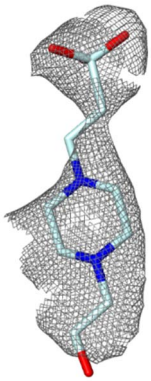
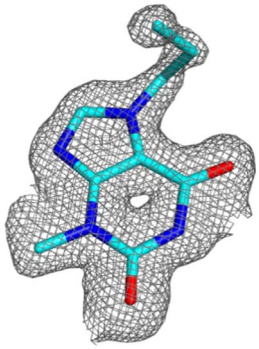
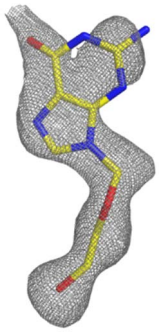
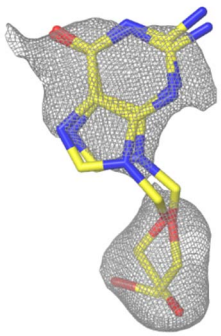
BD2-AC2-Site 1 **PDB ID: 7VRI**	

Figure S8 A two-dimensional schematic representation of the protein residues' interactions with the respective ligands, generated using LigPlot+ v.2.2.8 (Laskowski and Swindells, 2011). The water molecules are shown as spheres (cyan). Hydrogen bonds are shown by dashed lines (green), and hydrophobic interactions are shown by spiked arcs between the ligands and the protein. Default criteria were used for determining hydrogen bonds and hydrophobic interactions (Wallace et al., 1995).

Structure name **PDB ID**	**Site 1**	**Site 2**
BRD2-BD1THEO **7VRK**	 contoured at 3σ	 contoured at 2.7σ
BRD2-BD2THEO **7VRM**	 contoured at 3σ	 contoured at 3σ
BRD2-BD1TBR **7VRO**	 contoured at 3σ	 contoured at 2.7σ

BRD2-BD2TBR **7VRQ**	 contoured at 3σ	-
BRD2-BD1DOXO **7VRZ**	 contoured at 3σ	 contoured at 2.7σ
BRD2-BD2DOXO **7VS0**	 contoured at 3σ	 contoured at 2.7σ

BRD2-BD1MPX **7VSF**	 contoured at 3σ	 contoured at 2.7σ
BRD2-BD2MPX **7VS1**	 contoured at 3σ	-
BRD2-BD1AC2 **7VRH**	 contoured at 3σ	 contoured at 2.7σ

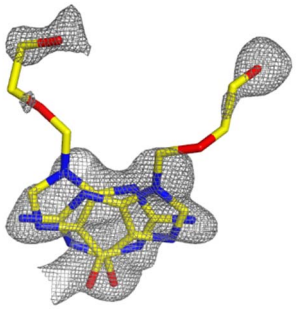
BRD2-BD2AC2 **7VRI**	 contoured at 2.7σ	-

Figure S9 The Polder difference Fourier maps of the ligands bind to the respective bromodomains, BD1 and BD2. The aromatic ring plane of the ligands oriented in the plan of page for clarity.

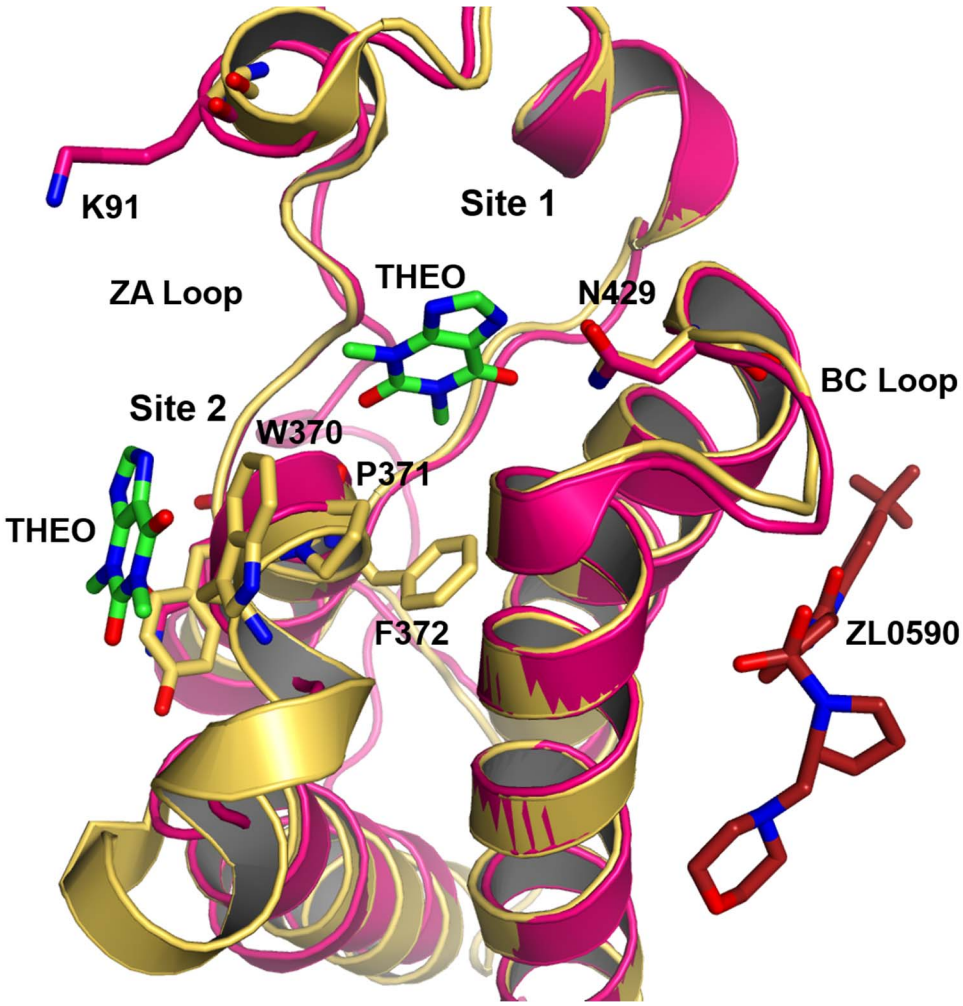


Figure S10 Superposition of the BD2-THEO complex onto the BRD4-BD1-ZL0590 complex (PDB Id: 6U0D). THEO and ZL0590 are represented in green and brown sticks, respectively.