

## *Supplementary Material*

### **Targeting the essential transcription factor HP1043 of *Helicobacter pylori*: a drug repositioning study**

Federico Antoniciello<sup>1</sup>, Davide Roncarati<sup>1</sup>, Annamaria Zannoni<sup>1,#</sup>, Elena Chiti<sup>1</sup>, Vincenzo Scarlato<sup>1</sup>, Federica Chiappori<sup>2\*</sup>

#### **1 Supplementary Tables**

##### **1.1 Table S1 Plasmids used in this study**

<b>Plasmid</b>	<b>Description</b>	<b>Source/Reference</b>
pTrc::1043	Derivative of pTrcHisA expressing the HP1043 response regulator; Amp <sup>r</sup>	(Delany et al., 2002)
pGEMt-P1227 WT	pGEMt-Easy derivative, containing a 61 bp probe corresponding to the region from 1.290.664 to 1.290.725 of <i>H. pylori</i> G27 genome. The region corresponds to the promoter of <i>HPG27_RS06145</i> gene ( <i>hp1227</i> according to <i>H. pylori</i> 26695 strain annotation)	(Pellicciari et al., 2017)

##### **1.2 Table S2 Oligonucleotides used in this study**

<b>Primer</b>	<b>Sequence</b>	<b>Source/Reference</b>
Php1227_EMSA_F	GCCAAAACGCCTAAAGCC	(Zannoni et al., 2021)
Php1227_EMSA_R	TTGAAAGCGCAATAACCGC	(Zannoni et al., 2021)
16SRTF	GGAGTACGGTCGCAAGATTA	(Pellicciari et al., 2017)
16SRTR	CTAGCGGATTCTCTCAATGTCAA	(Pellicciari et al., 2017)

### 1.3 Table S3 Docking comparison to literature

Docking box have dimension limits, so to include the whole structure, two docking boxes were designed, HP1043\_site1 and Hp1043\_site 2. Boxes overlap at dimer interface.

		HP1043-DNA			HP1043 site1			HP1043 site2		
		binding energy	# cluster	Ki	binding energy	# cluster	Ki	binding energy	# cluster	Ki
nifedipine	ZINC85205448	-6.15	25	31.28 uM	-8.25	9	899.53 nM	-6.17	14	29.83 uM
nicardipine	ZINC19796087	-8.94	10	277.87 nM	-8.70	13	419.39 nM	-6.69	13	12.41 uM
	ZINC84400879	-8.39	10	707.68 nM	-7.10	2	6.29 uM	-7.81	12	1.90 uM
nisoldipine	ZINC591993	-5.53	13	88.97 uM	-6.80	6	10.36 uM	-6.84	22	9.70 uM
	ZINC19632706	-8.60	4	497.04 nM	-7.19	12	5.33 uM	-7.27	21	4.67 uM
nimodipine	ZINC19632713	-5.80	16	55.67 uM	-6.35	28	22.10 uM	-7.08	21	6.41 uM
	ZINC19632718	-5.03	11	205.41 uM	-5.83	11	53.39 uM	-8.05	8	1.26 uM
nitrendipine	ZINC84403367	-5.72	24	63.63 uM	-7.36	26	4.03 uM	-6.53	39	16.44 uM
	ZINC100001908	-7.70	10	2.29 uM	-7.69	93	2.32 uM	-7.62	46	2.58 uM
lercandipine	ZINC19685788	-8.04	7	1.27 uM	-7.34	4	4.15 uM	-7.82	1	1.85 uM
	ZINC19685790	-10.07	1	41.87 nM	-9.02	1	243.13 nM	-8.84	1	333.09 nM
chrysin	ZINC3872070	-8.80	83	353.26 nM	-8.70	34	416.11 nM	-9.14	9	201.10 nM
apigenin	ZINC3871576	-9.16	65	192.69 nM	-8.35	12	762.31 nM	-8.21	11	960.09 nM
luteolin	ZINC18185774	-8.73	88	397.61 nM	-10.10	5	39.33 nM	-8.84	17	331.86 nM
hesperetin (yso2)	ZINC39092	-8.98	92	260.60 nM	-7.72	72	2.19 uM	-7.56	89	2.86 uM
kaempferol	ZINC3869768	-8.46	16	627.90 nM	-7.86	10	1.72 uM	-7.70	47	2.28 uM
quercitin (meletin)	ZINC3869685	-8.77	34	371.41 nM	-8.09	10	1.17 uM	-9.44	9	121.28 nM
myricetin	ZINC3874317	-9.64	42	85.18 nM	-7.41	24	3.71 uM	-8.15	46	1.06 uM

#### 1.4 Table S4 docking results

ligand	binding energy (kcal/mol)	run	Ki	
<b>ZINC000022443609_HP1043-DNA</b>	<b>-23.84</b>	<b>38</b>	<b>3.37 aM</b>	
ZINC000013492579_HP1043-DNA	-16.79	117	494.39 fM	Not for sale
ZINC000003938681_HP1043-DNA	-16.41	66	937.24 fM	DNA
<b>ZINC000019363537_HP1043-DNA</b>	<b>-15.57</b>	<b>81</b>	<b>3.86 pM</b>	
<b>ZINC000001566899_HP1043-DNA</b>	<b>-15.41</b>	<b>98</b>	<b>5.06 pM</b>	
ZINC000004214772_HP1043-DNA	-15.31	147	5.96 pM	DNA
ZINC000100088802_HP1043-DNA	-14.99	156	10.26 pM	DNA
ZINC000150338708_HP1043-DNA	-14.39	11	28.4 pM	Expensive
ZINC000013492553_HP1043-DNA	-14.3	190	33.17 pM	Not for sale
ZINC000028823338_HP1043-DNA	-14.14	124	43.44 pM	Not for sale
ZINC000004097448_HP1043-DNA	-14.05	43	50.02 pM	DNA
ZINC000004097404_HP1043-DNA	-13.99	79	55.55 pM	DNA
ZINC000222508879_HP1043-DNA	-13.99	75	55.58 pM	Not for sale
ZINC000028823530_HP1043-DNA	-13.93	60	61.88 pM	Not for sale
ZINC000013494070_HP1043-DNA	-13.92	42	63.08 pM	Not for sale
<b>ZINC000019364225_HP1043-DNA</b>	<b>-13.84</b>	<b>70</b>	<b>71.87 pM</b>	
ZINC000013494068_HP1043-DNA	-13.73	46	86.02 pM	Not for sale
ZINC000043206370_HP1043-DNA	-13.56	176	115.23 pM	DNA
ZINC000001612996_HP1043-DNA	-13.32	199	171.12 pM	DNA
ZINC000013494069_HP1043-DNA	-13.1	10	250.98 pM	Not for sale
<b>ZINC000036701290_HP1043-DNA</b>	<b>-13.08</b>	<b>103</b>	<b>256.35 pM</b>	
ZINC000028823491_HP1043-DNA	-12.92	139	339.8 pM	Not for sale
ZINC000087666889_HP1043-DNA	-12.92	22	335.64 pM	Not for sale
<b>ZINC000098023177_HP1043-DNA</b>	<b>-12.89</b>	<b>92</b>	<b>356.1 pM</b>	
ZINC000150598297_HP1043-DNA	-12.82	121	401.34 pM	Not for sale
ZINC000004097383_HP1043-DNA	-12.81	40	406.52 pM	
ZINC000028134101_HP1043-DNA	-12.79	153	421.91 pM	Not for sale
ZINC000028823512_HP1043-DNA	-12.79	70	420.99 pM	Not for sale
ZINC000087666882_HP1043-DNA	-12.79	140	420.91 pM	Not for sale
<b>ZINC000072316335_HP1043-DNA</b>	<b>-12.7</b>	<b>128</b>	<b>487.81 pM</b>	
ZINC000022448983_HP1043-DNA	-12.66	167	526.94 pM	
ZINC000028823490_HP1043-DNA	-12.56	47	616.87 pM	Not for sale
ZINC000028823510_HP1043-DNA	-12.53	9	652.73 pM	Not for sale
<b>ZINC000003978083_HP1043-DNA</b>	<b>-12.46</b>	<b>143</b>	<b>737.86 pM</b>	
ZINC000030690433_HP1043-DNA	-12.46	46	740.65 pM	Expensive

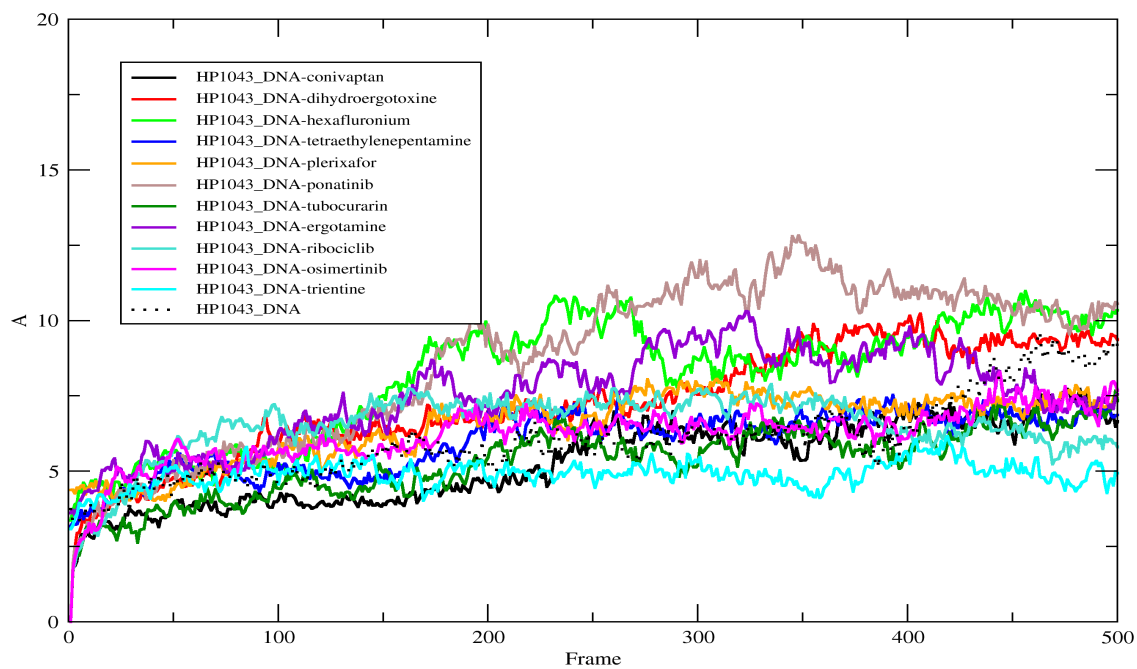
<b>ZINC000052955754_HP1043-DNA</b>	<b>-12.46</b>	<b>31</b>	<b>736.06 pM</b>	
<b>ZINC000012503187_HP1043-DNA</b>	<b>-12.42</b>	<b>134</b>	<b>791.48 pM</b>	
<b>ZINC000014880002_HP1043-DNA</b>	<b>-12.39</b>	<b>15</b>	<b>827.22 pM</b>	
ZINC000087666886_HP1043-DNA	-12.38	137	837.75 pM	Not for sale
ZINC0000222508821_HP1043-DNA	-12.32	63	936.79 pM	Not for sale
ZINC000028823531_HP1043-DNA	-12.29	149	985.83 pM	Not for sale
<b>ZINC000022443609_HP1043</b>	<b>-17.65</b>	<b>35</b>	<b>116.01 fM</b>	
<b>ZINC000019363537_HP1043</b>	<b>-13.68</b>	<b>21</b>	<b>94.13 pM</b>	
<b>ZINC000019363537_HP1043</b>	<b>-12.86</b>	<b>20</b>	<b>373.37 pM</b>	
<b>ZINC000003934128_HP1043</b>	<b>-12.23</b>	<b>146</b>	<b>1.09 nM</b>	
<b>ZINC000003934128_HP1043</b>	<b>-11.18</b>	<b>102</b>	<b>6.41 nM</b>	
ZINC000029059664_HP1043	-10.82	134	11.73 nM	Not for sale
ZINC000028824061_HP1043	-10.67	124	15.01 nM	Not for sale
ZINC000072126305_HP1043	-10.2	118	33.25 nM	Not for sale
ZINC000072126306_HP1043	-10.18	137	34.59 nM	Not for sale
ZINC000042804032_HP1043	-10.04	102	43.55 nM	Not for sale
<b>ZINC000000004724_HP1043</b>	<b>-9.94</b>	<b>103</b>	<b>51.39 nM</b>	
ZINC000044352037_HP1043	-9.91	178	54.83 nM	Not for sale
ZINC000029060547_HP1043	-9.7	127	76.97 nM	Not for sale
ZINC000029060386_HP1043	-9.55	108	99.38 nM	Not for sale

## 2 Reference

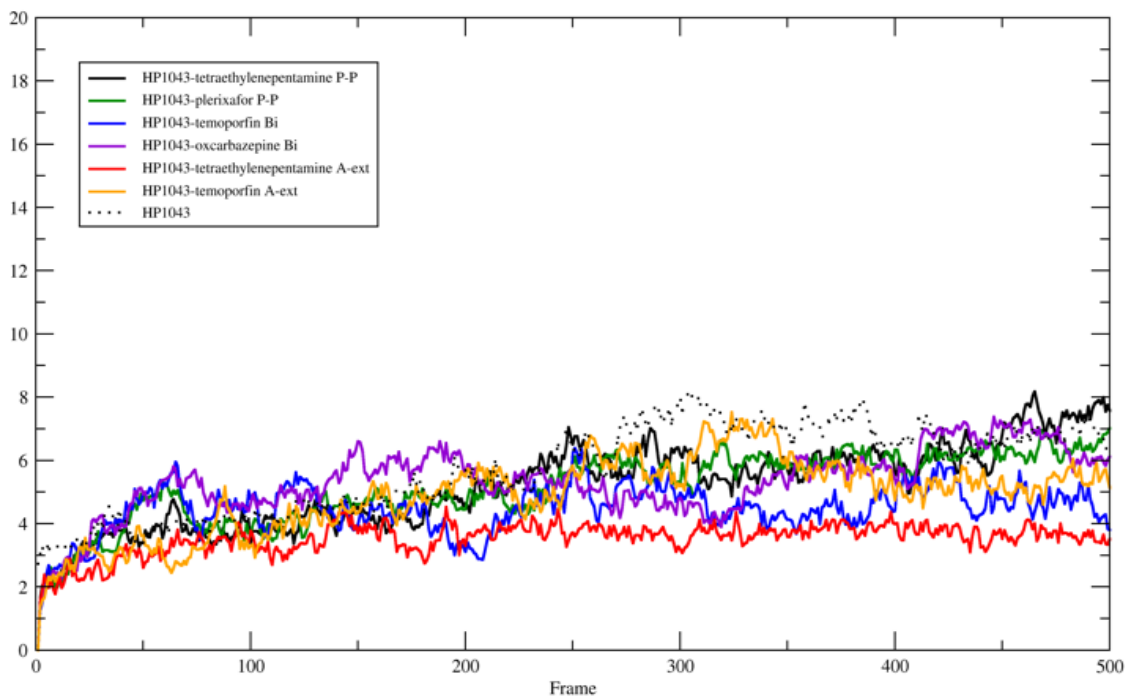
- Delany, I., Spohn, G., Rappuoli, R., and Scarlato, V. (2002). Growth phase-dependent regulation of target gene promoters for binding of the essential orphan response regulator HP1043 of *Helicobacter pylori*. *J. Bacteriol.* 184. doi:10.1128/JB.184.17.4800-4810.2002.
- Pellicciari, S., Pinatel, E., Vannini, A., Peano, C., Puccio, S., Bellis, G. De, et al. (2017). Insight into the essential role of the *Helicobacter pylori* HP1043 orphan response regulator: Genome-wide identification and characterization of the DNA-binding sites. *Sci. Rep.* 7. doi:10.1038/srep41063.
- Zannoni, A., Pellicciari, S., Musiani, F., Chiappori, F., Roncarati, D., and Scarlato, V. (2021). Definition of the Binding Architecture to a Target Promoter of HP1043, the Essential Master Regulator of *Helicobacter pylori*. *Int. J. Mol. Sci. Artic.* doi:10.3390/ijms22157848.

### 3 Supplementary Figures

#### RMSD HP1043\_DNA

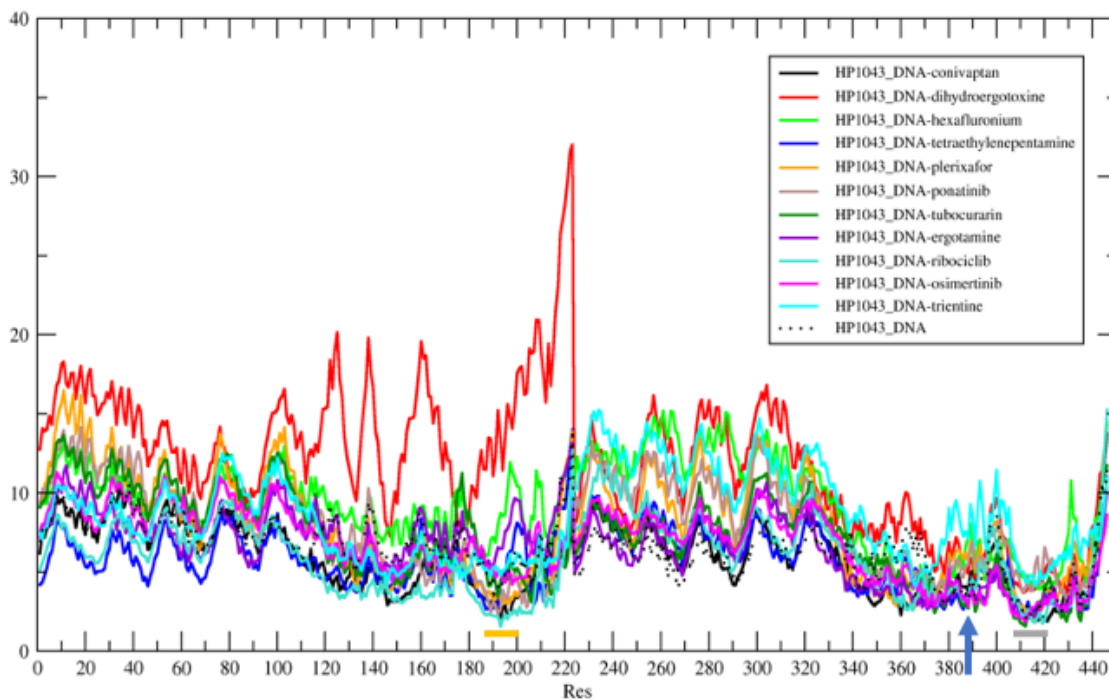


#### RMSD HP1043

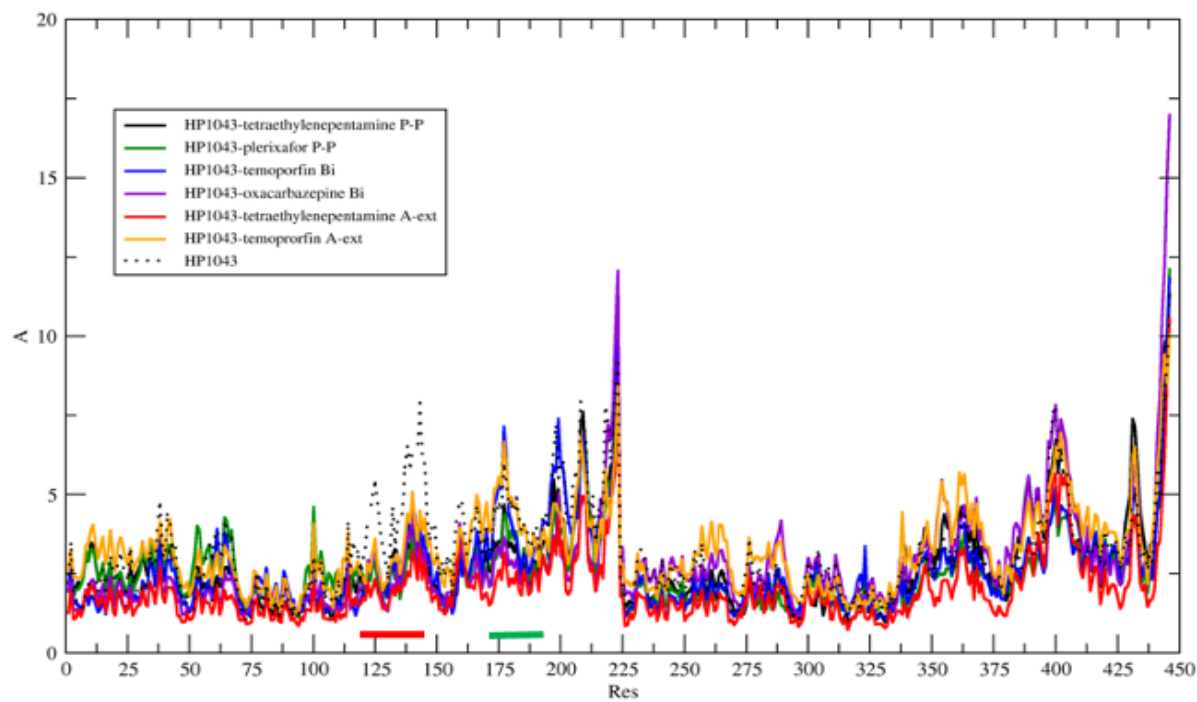


**Supplementary Figure S1.** C $\alpha$  RMSD. On the top HP1043 bound to DNA complexes, bottom HP1043 free complexes.

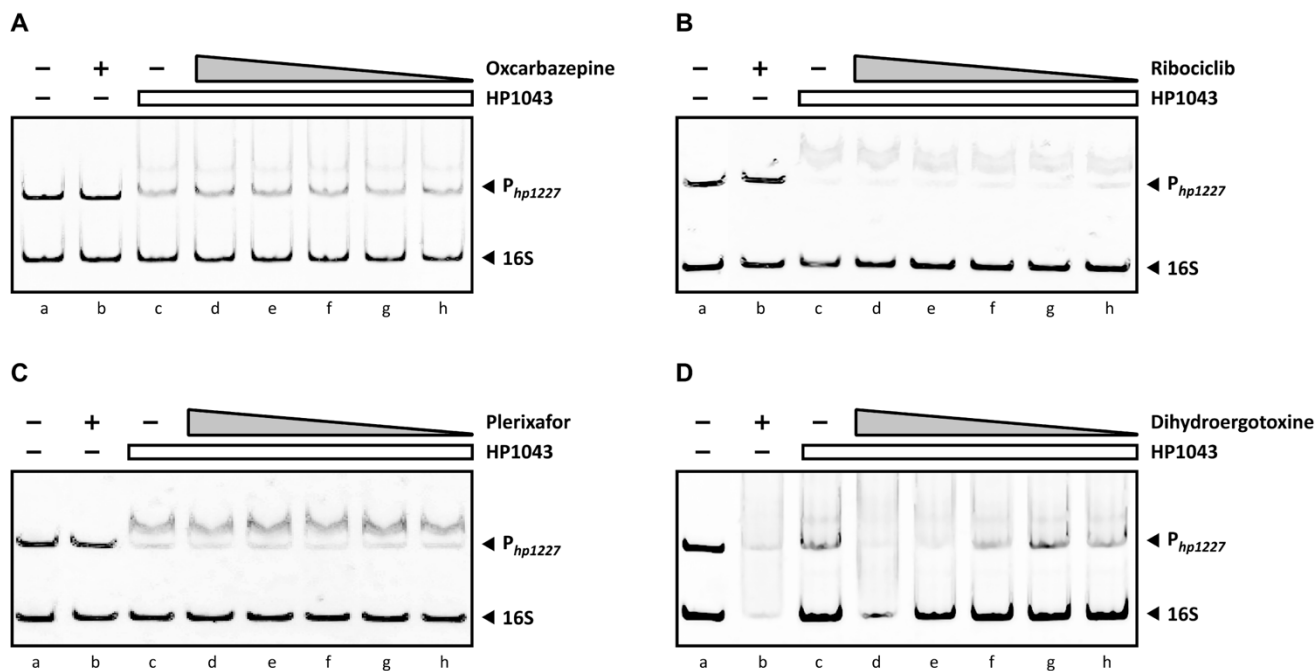
## RMSF HP1043\_DNA



## RMSF HP1043



**Supplementary Figure S2.** RMSF per-residue. On the top HP1043 bound to DNA complexes, bottom HP1043 free complexes.



**Supplementary Figure S3.** EMSAs in the presence of putative ligands. All EMSAs show the same amount of specific ( $P_{hp1227}$ ) and non-specific (16S rRNA gene) DNA probes and the same samples order: lane a) DNA probes control, protein- and ligand-free; b) compound control at 1 mM (indicated by a “+”) without the protein; c) DNA binding control in the presence of HP1043; lane d) to h) show samples containing a fixed amount of HP1043 monomeric protein (4  $\mu$ M) with a decreasing concentration of the ligand, respectively 1, 0.5, 0.2, 0.1, 0.05 mM; the absence of protein and compound is indicated by a “-”; the compound concentration is depicted as a grey triangle, while a white rectangle is used for the HP1043 protein fixed concentration. **A)** Oxcarbazepine did not cause a significant loss of the mobility shift if compared with the protein control (lane c). As well as **B)** Ribociclib and **C)** Plerixafor, whose presence in the reaction mix did not affect the DNA binding. **D)** Dihydroergotamine interacted with both DNA probes at higher concentrations causing a shift in a protein-free reaction (lane b). Therefore, a putative inhibition was not assessable.