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Supplementary figures & tables

Low molecular weight inhibitors targeting the RNA-binding protein HuR

Benjamin Philipp Joseph [1,2,†], Verena Weber [1,2,†], Lisa Knüpfer [3], Alejandro Giorgetti [1,4], Mercedes Alfonso-Prieto [1], Sybille Krauß [3,*], Paolo Carloni [1,2,*] and Giulia Rossetti [1,5,6]

[1] Institute for Neuroscience and Medicine and Institute for Advanced Simulations (INM-9/IAS-5), Computational Biomedicine, Forschungszentrum Jülich, 52425 Jülich, Germany; b.joseph@fz-juelich.de (B.P.J.); v.weber@fz-juelich.de (V.W.); alejandro.giorgetti@univr.it (A.G.); m.alfonso-prieto@fz-juelich.de (M.A.-P.); g.rossetti@fz-juelich.de (G.R.)
[2] Faculty of Mathematics, Computer Science and Natural Sciences, RWTH Aachen University, 52062 Aachen, Germany
[3] Institute of Biology, University of Siegen, 57076 Siegen, Germany; lisa.knuepfer@uni-siegen.de
[4] Department of Biotechnology, University of Verona, 37134 Verona, Italy
[5] Jülich Supercomputing Centre (JSC), Forschungszentrum Jülich, 52425 Jülich, Germany
[6] Department of Neurology, RWTH Aachen University, 44517 Aachen, Germany
* Correspondence: sybille.krauss@uni-siegen.de (S.K.); p.carloni@fz-juelich.de (P.C.)
† These authors share first authorship as co-first authors.

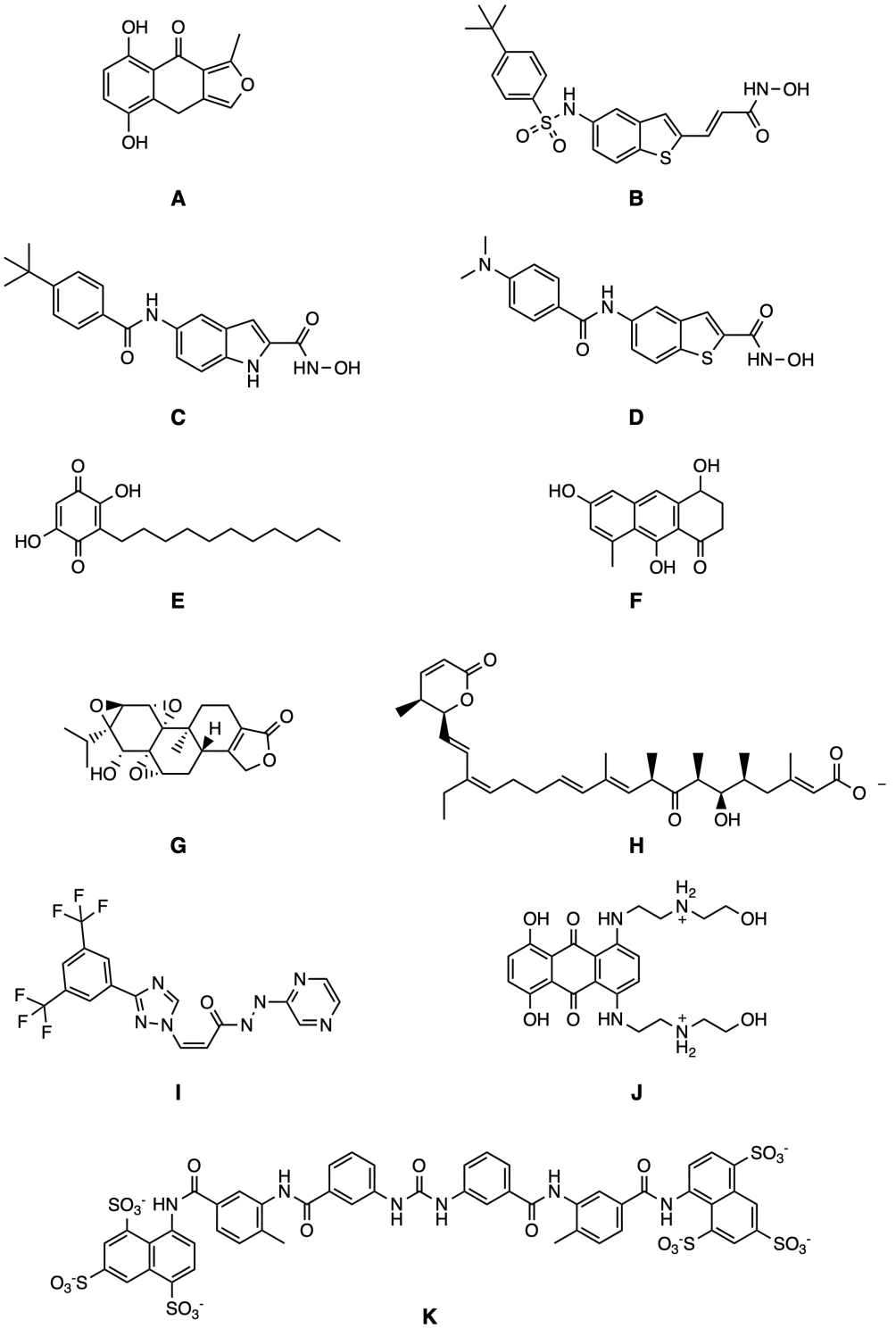


Figure S1. Molecular structures of the HuR inhibitors tested *in vivo*. **(A)** MS-444 (also under preclinical trials). **(B)** KH-3, **(C)** KH-3 derivative 1, **(D)** KH-3 derivative 2, **(E)** Embelin, **(F)** Okicenone, **(G)** Triptolide, **(H)** Leptomycin B, **(I)** Selinexor, **(J)** Mitoxantrone and **(K)** Suramin.

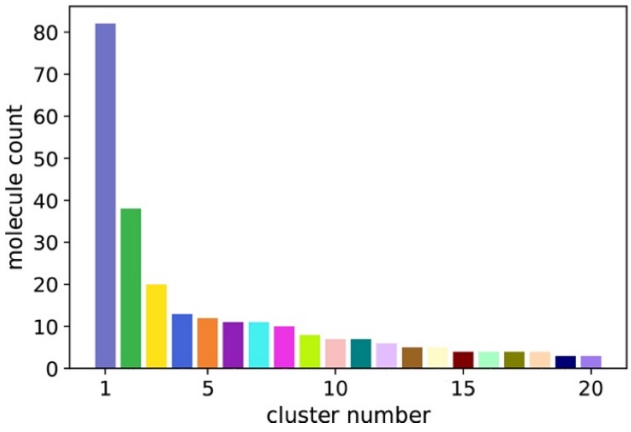


Figure S2. Bar plot representing the number of molecules of the first 20 most populated clusters from the HTVS experiment.

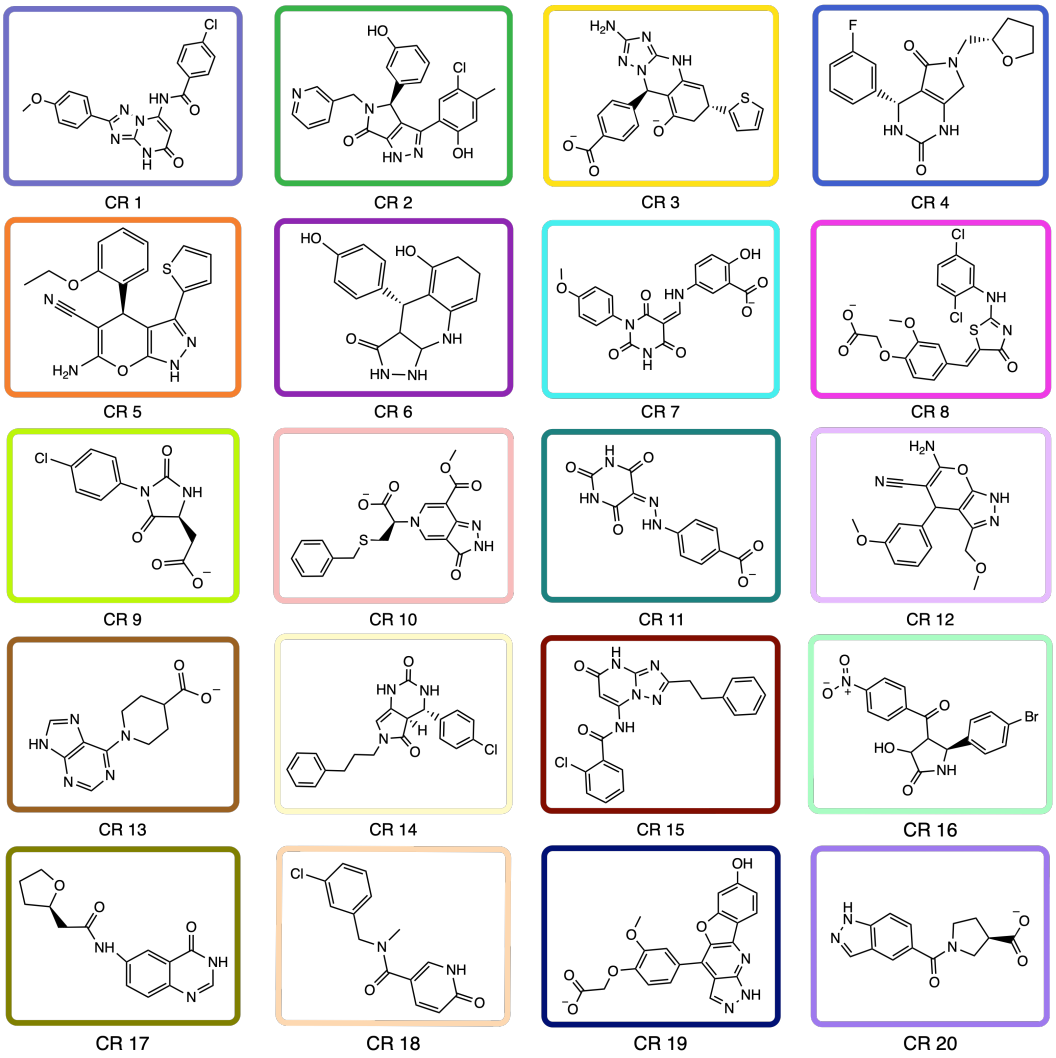
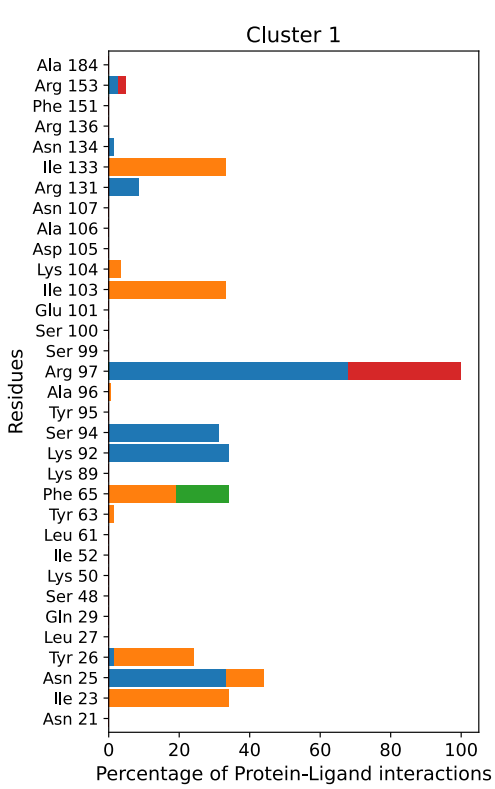


Figure S3. Cluster representatives for the 20 most populated clusters.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Residues
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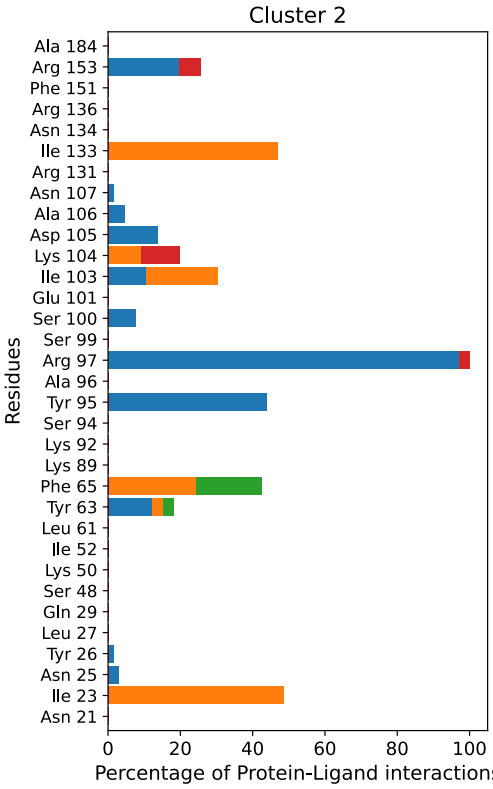
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions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Ile 133
Arg 131
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Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
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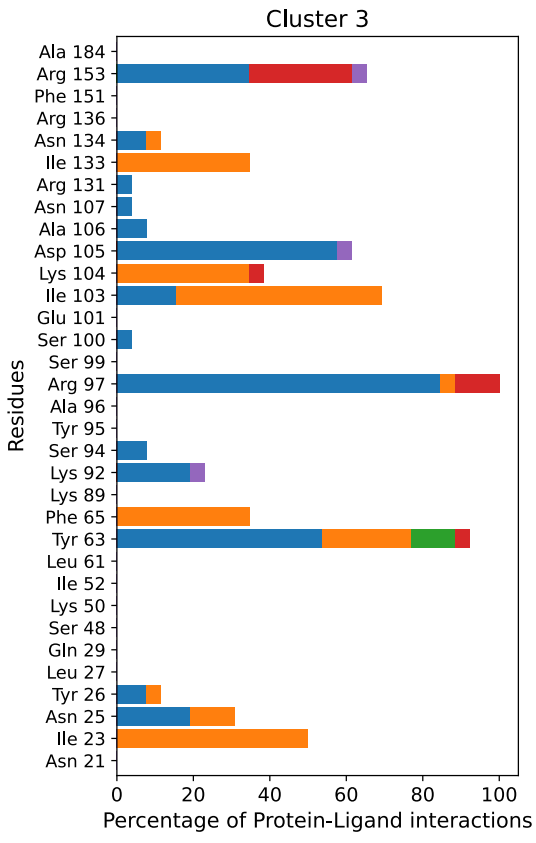
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions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Arg 131
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Asp 105
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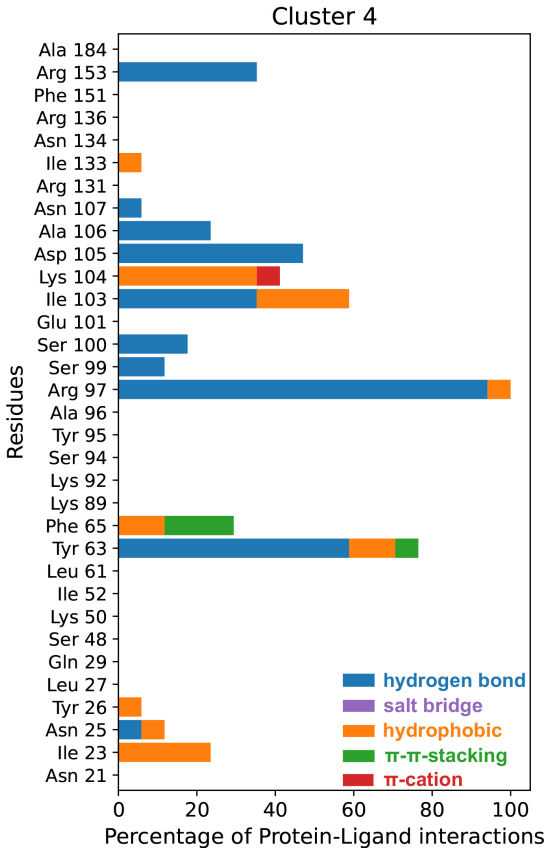
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions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Arg 131
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Asp 105
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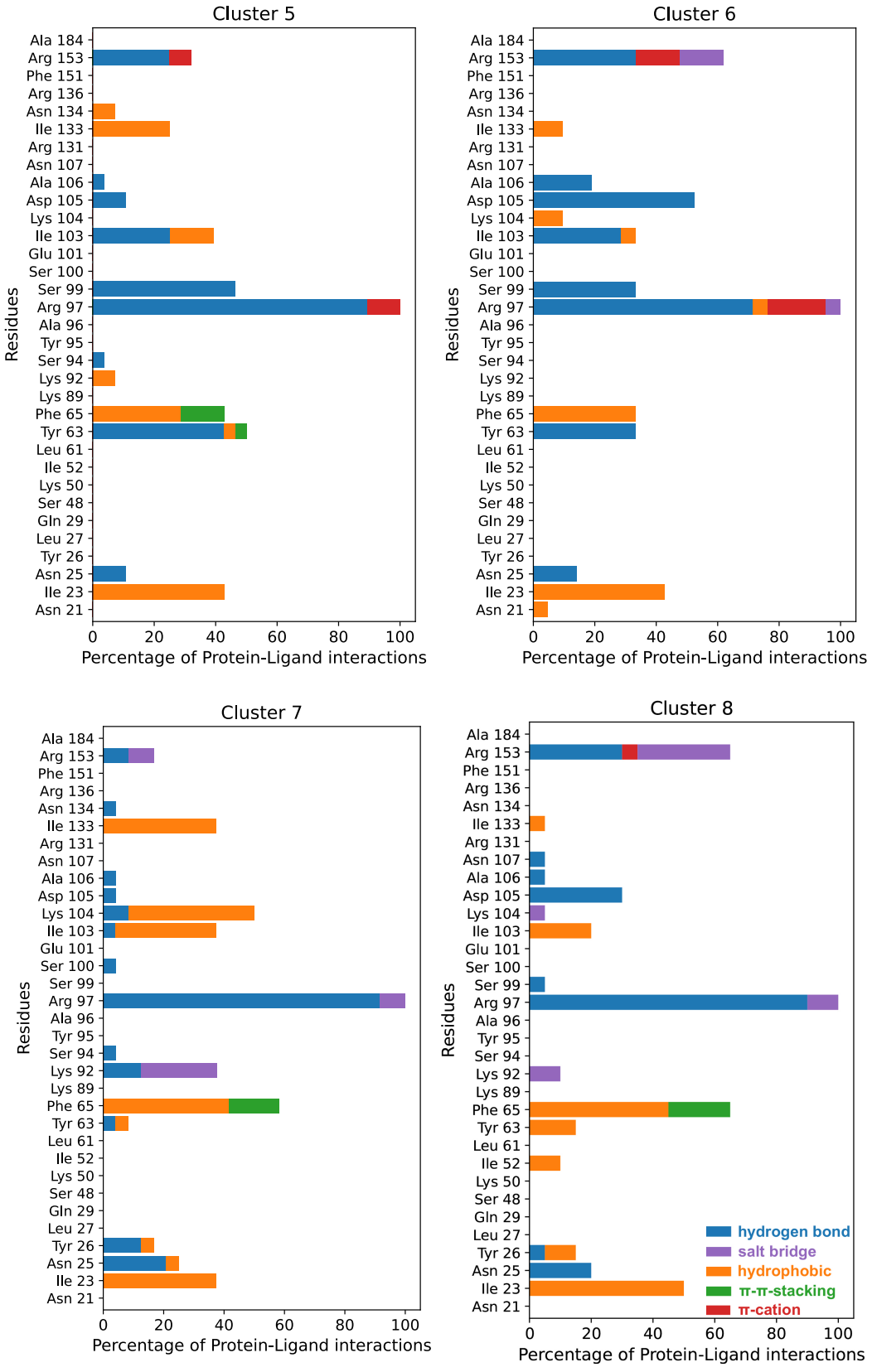
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation
0
20
40
60
80
100
Percentage of Protein-Ligand interactions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Cluster 6
Cluster 7
Cluster 8
Residues
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Arg 136
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Ile 133
Arg 131
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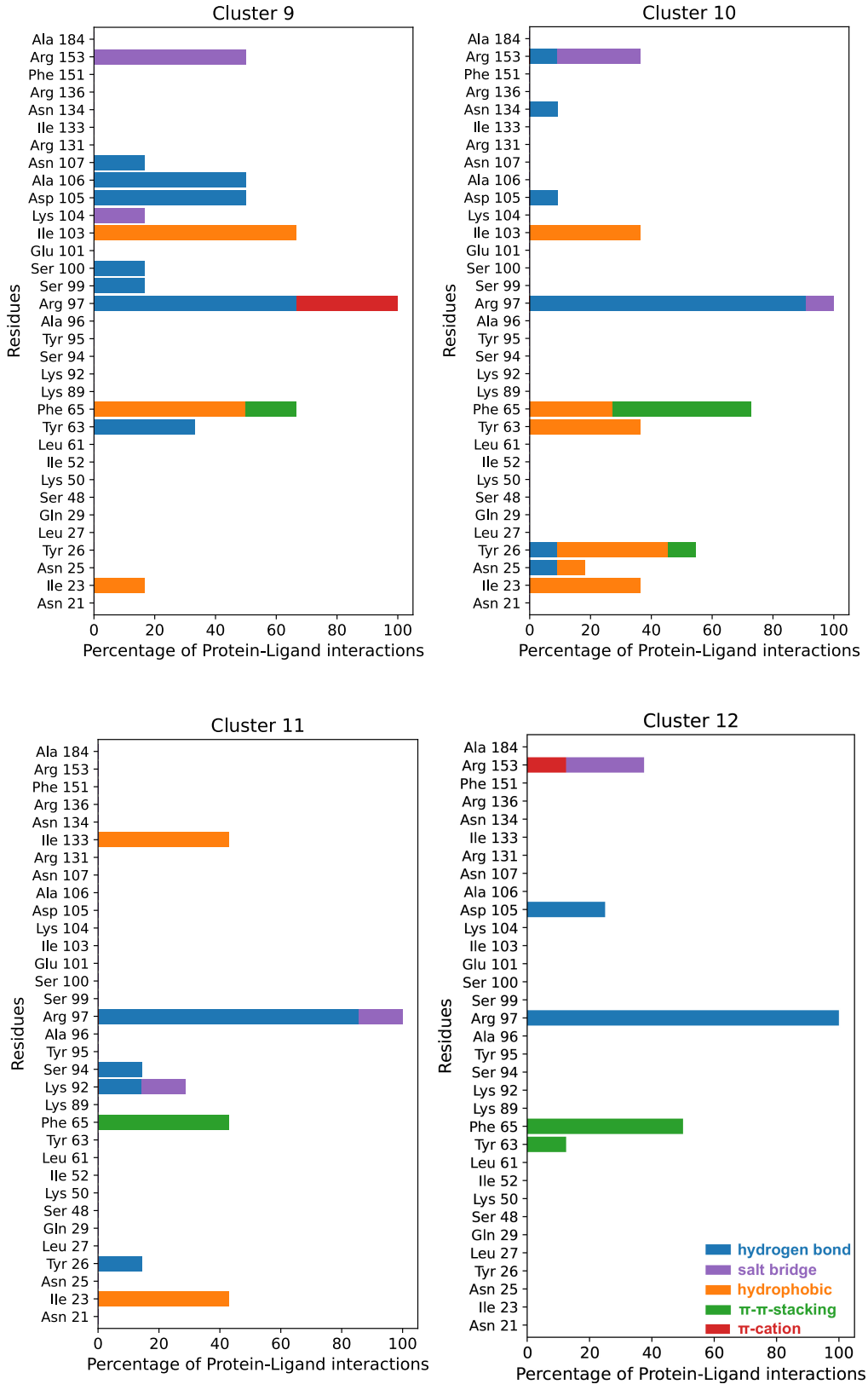
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation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Cluster 10
Cluster 11
Cluster 12
Residues
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Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
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Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
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40
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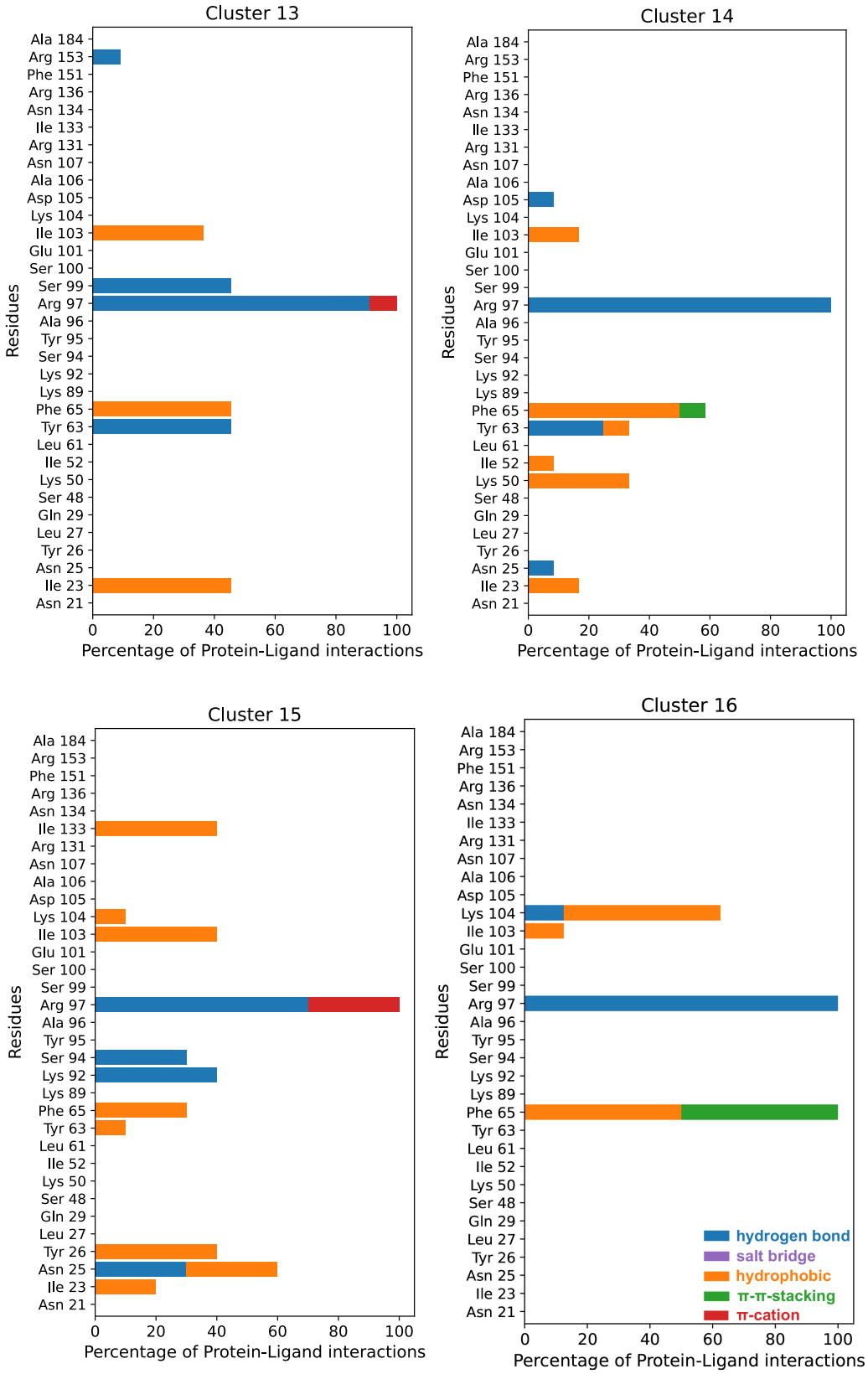
80
100
Percentage of Protein-Ligand interactions
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation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Cluster 14
Cluster 15
Cluster 16
Residues
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Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
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Leu 27
Tyr 26
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Percentage of Protein-Ligand interactions
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation

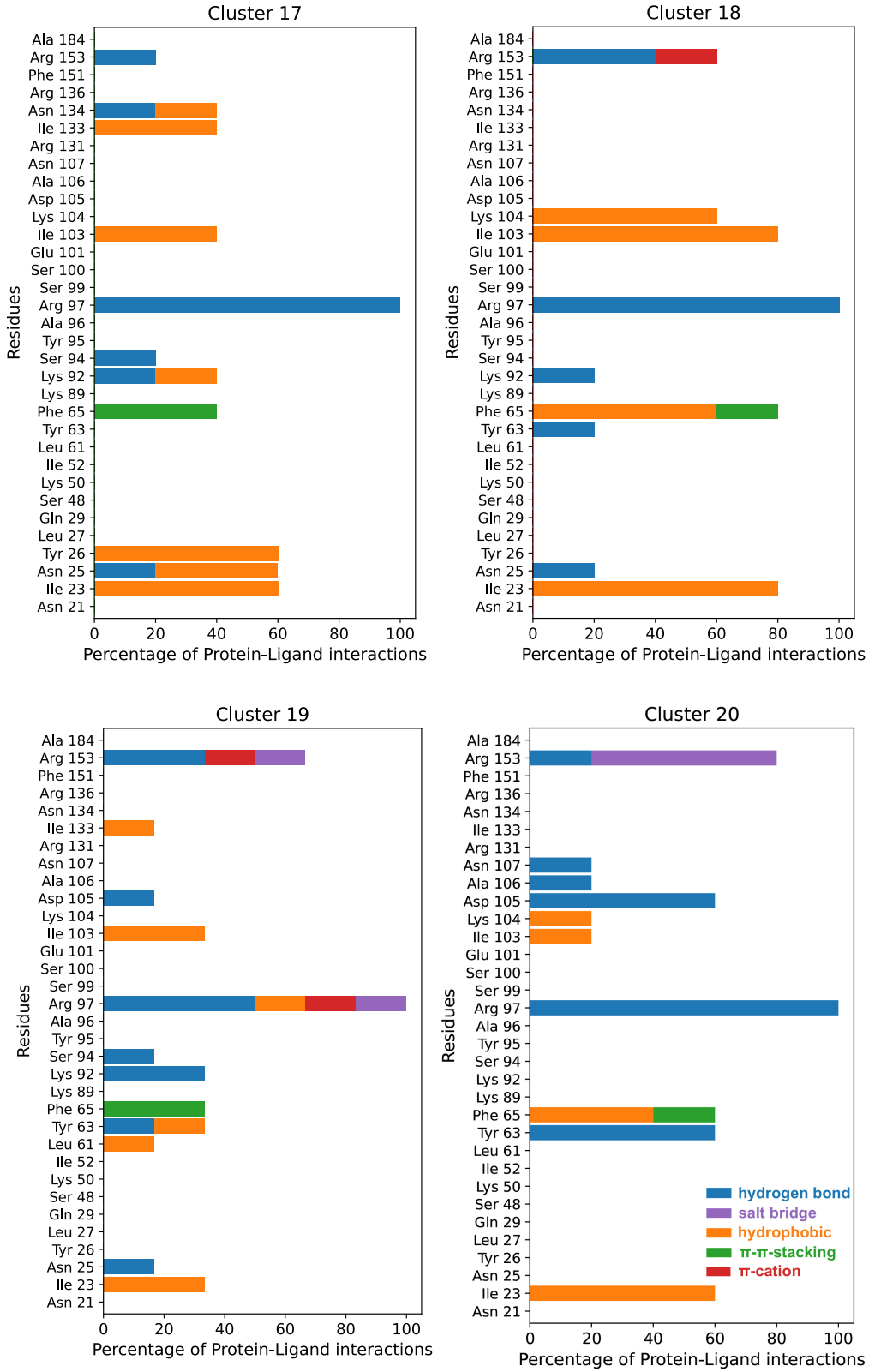


Figure S4. Protein-ligand interaction fingerprints for the 20 most populated clusters.

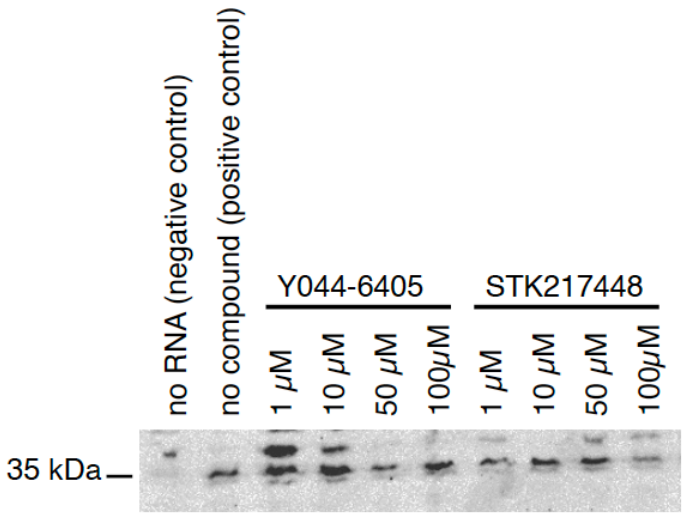
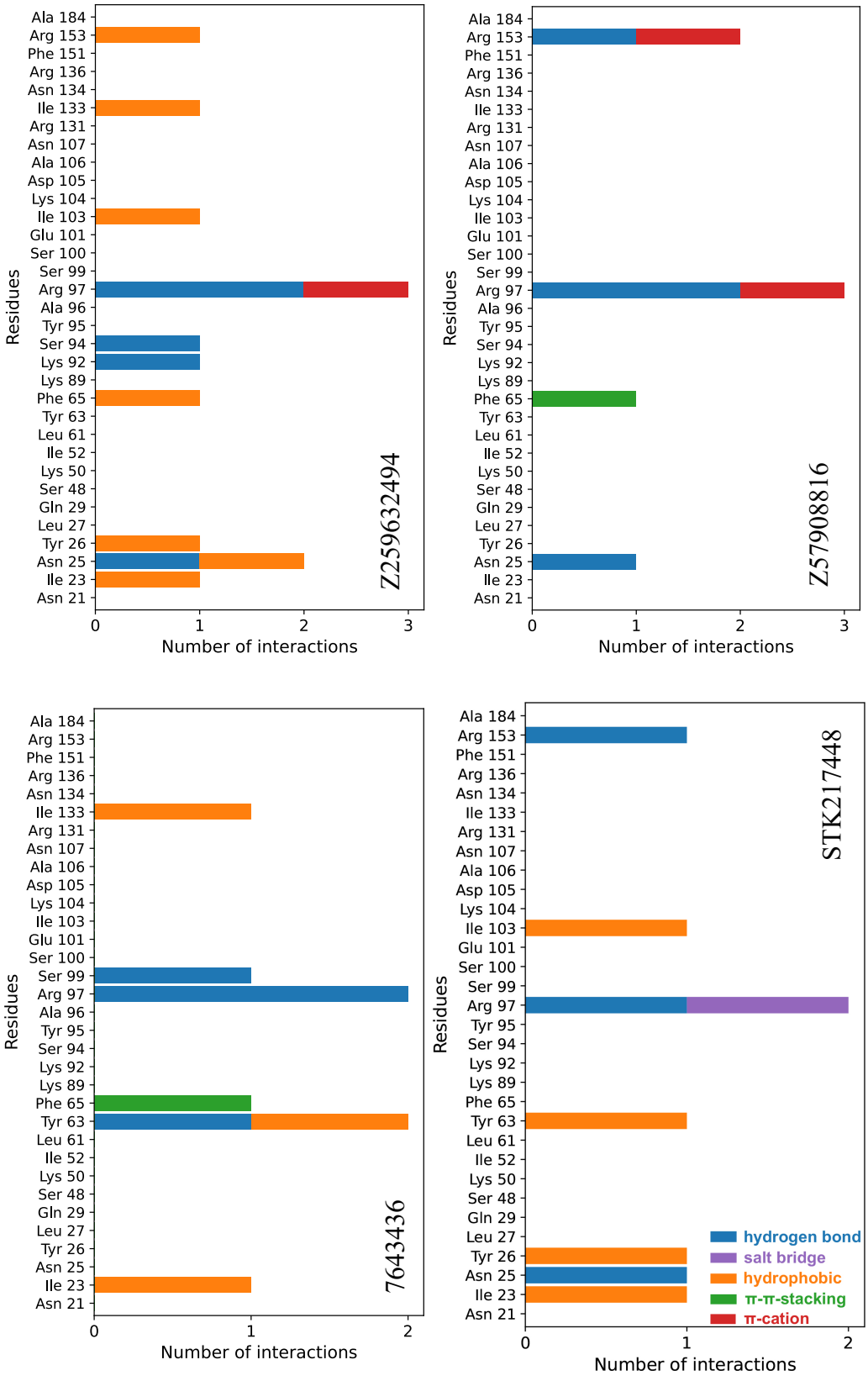
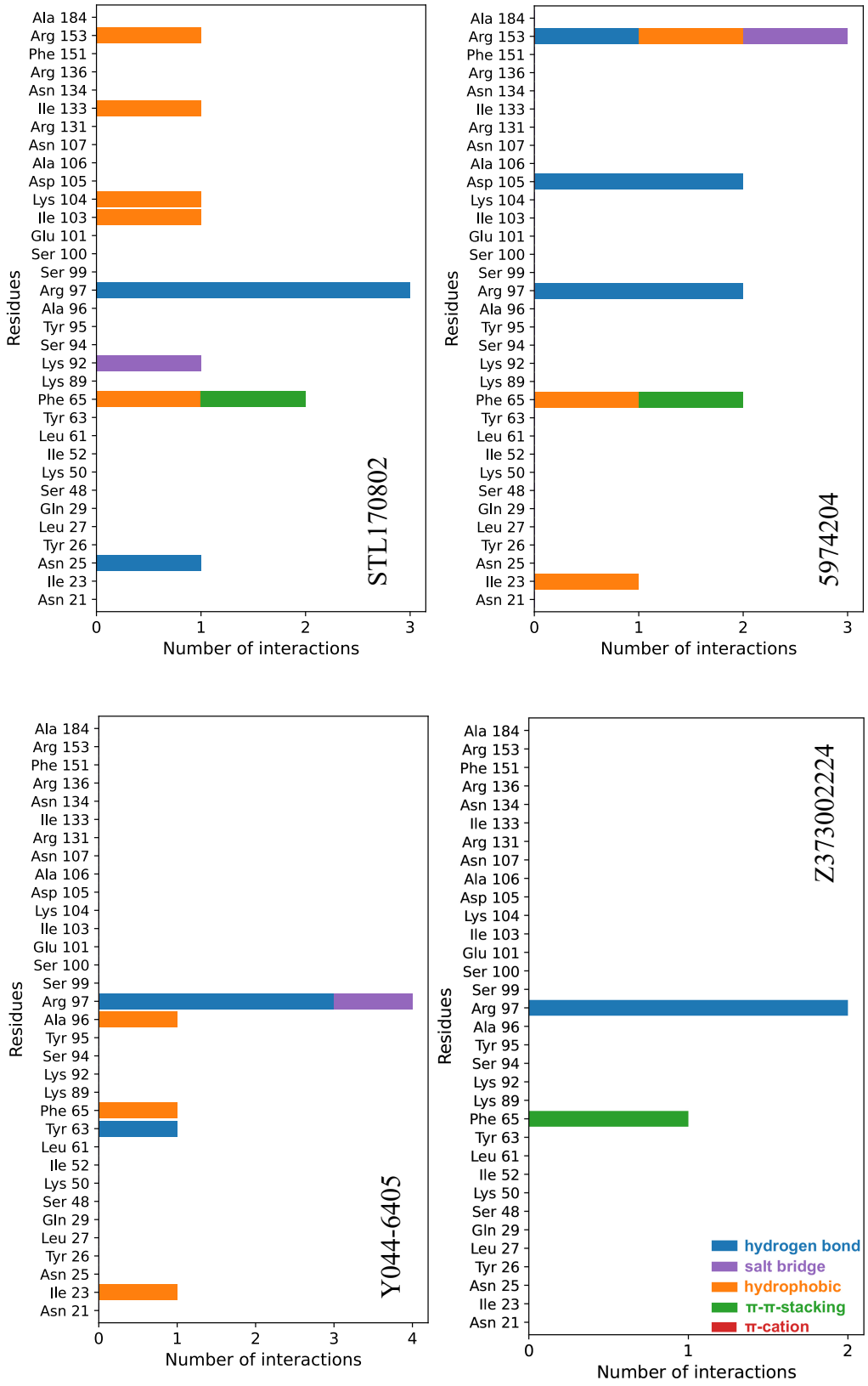


Figure S5. RNA–protein pull-down of HuR as described in Figure 4(A) of the main text with different concentrations of the compounds Y044-6405 and STK217448.


Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
1
2
3
Number of interactions
Z259632494
Z57908816
7643436
STK217448
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation


Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
Residues
0
1
2
3
4
Number of interactions
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Y044-6405
Z373002224
hydrogen bond
salt bridge
hydrophobic
π-π-stacking
π-cation

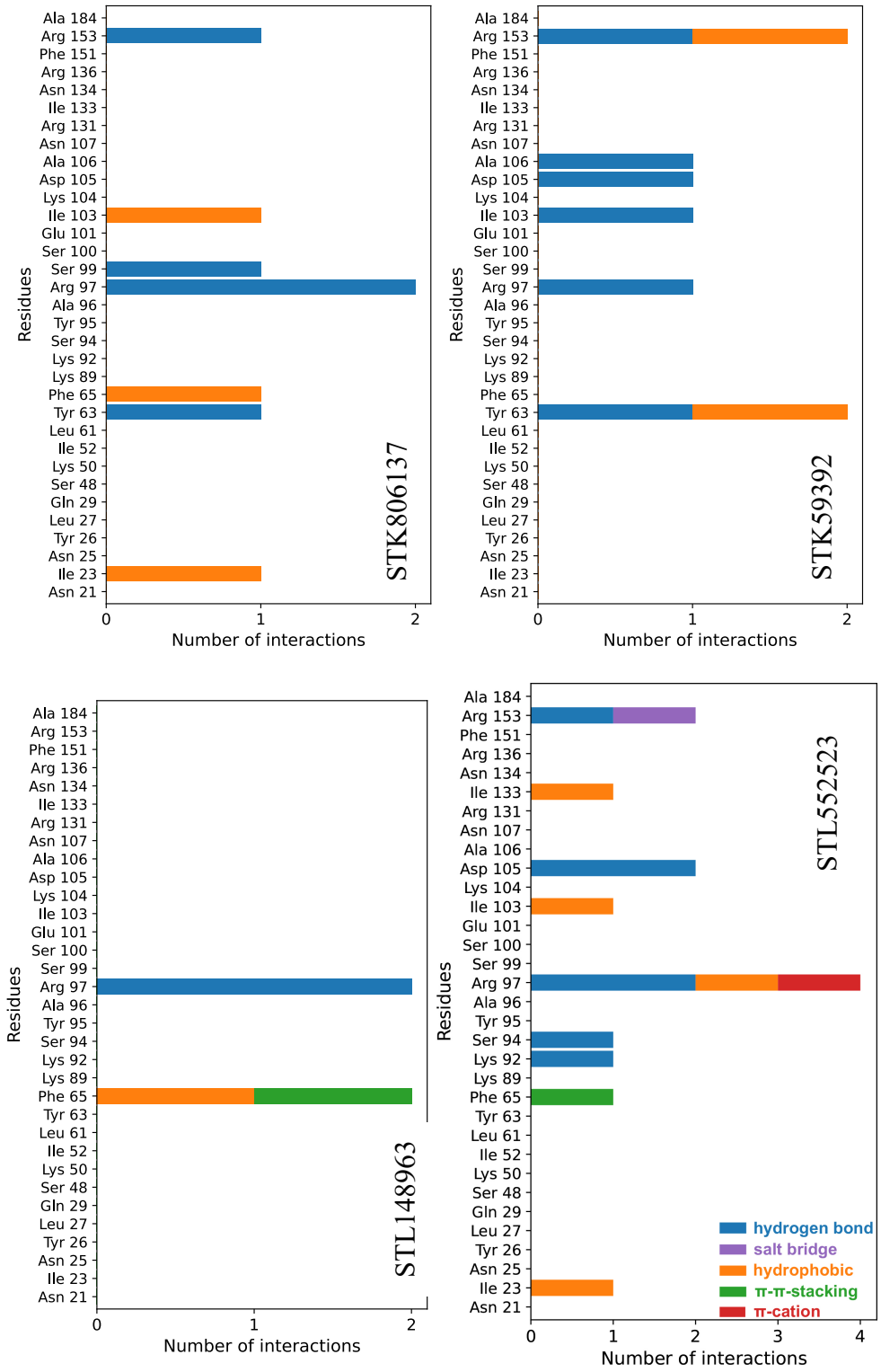

Figure S6. PLIFs of the other 12 bought compounds (C1) derivative, Z259632494; (C2) derivative, Z57908816; (C5) representative, 7643436; (C6) derivative, STK217448; (C7) member, STL170802; (C8) derivative, 5974204; (C9) member, Y044-6405; (C11) representative, STK018404; (C12) derivative, Z373002224; (C13) derivative, STK806137; (C15) derivative, STK593921; (C16) derivative, STL148963; (C19) representative, STL522523.

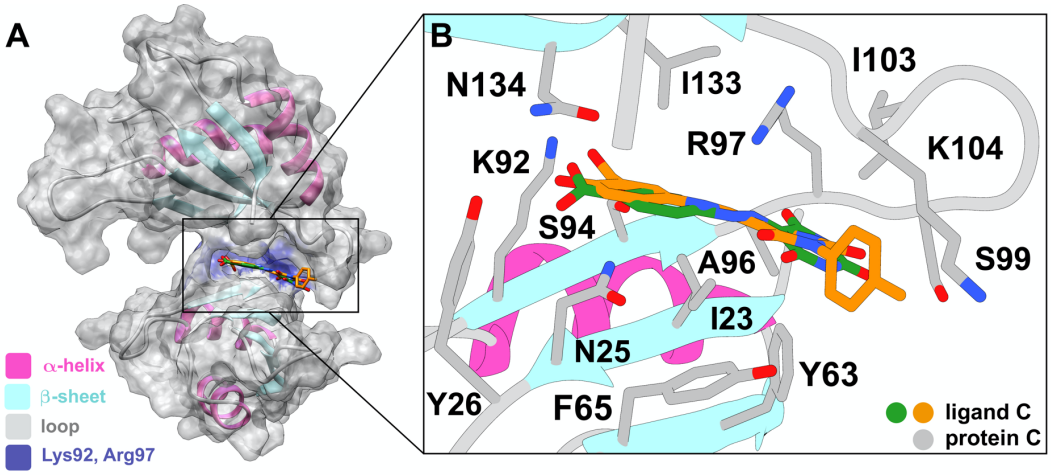

Figure S7. (**A**) Surface representation of HuR RRM1/2 (PDB code 4ED5) with the two docked ligands STK018404 and STL170802. (**B**) Docking pose in licorice fashion of compound STK018404 and STL170802 (with labeled residues within 4 Å of the ligands.

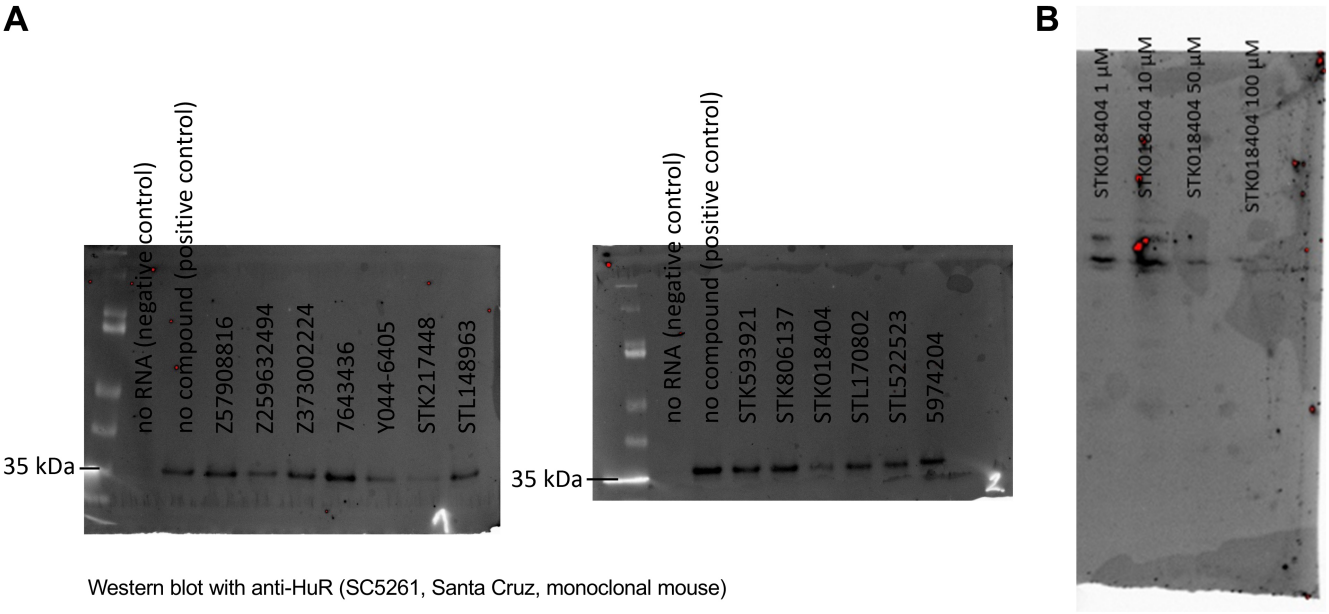

Figure S8. Uncropped western blot gels corresponding to the RNA pull-down assays shown in Figure 4 in the main text. (**A**) RNA–protein pull-down of HuR with its target RNA motif (AUUUUUAUUUU) in the absence (positive control) or presence of the respective compounds (final concentration 100µM). RNA-bound proteins were analyzed on western blots detecting HuR. A negative control that does contain RNA and a positive control with no compound were included. (**B**) RNA–protein pull-down of HuR as described in (A) with different concentrations of the compound STK018404.

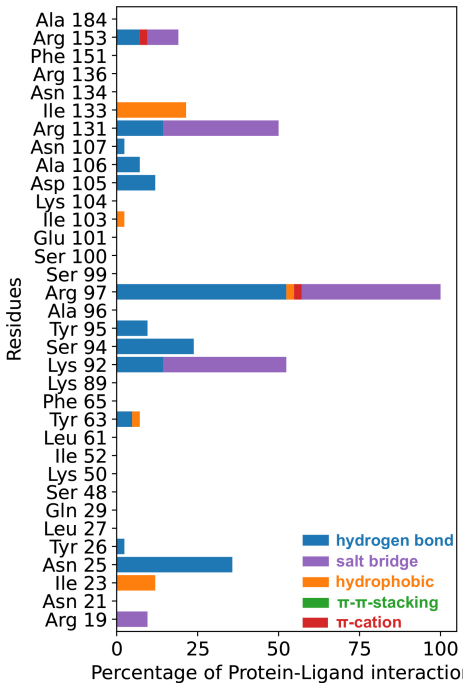

Figure S9. PLIF of the twenty-three poses obtained upon docking of the decoys generated with the DUDE-Z webserver [1].

Table S1. List of previously reported small molecule inhibitors of the HuR protein as of January 2023.

#	Compound name	Status	IC_{50} [μM]	PubChem ID	Ref.
1	MS-444	*In vivo*	2.1-40.7	132904	[2]
2	Dehydromutactin	*In vitro*	39.0-130.0	54685140	[2]
3	Okicenone	*In vitro*	0.53-2.9	132015	[2]
4	Quercetin	*In cell*	5.78-31.04	5280343	[3]
5	6n	*In cell*	0.015	137652621	[4]
6	24	*In vitro*	-	-	[5]
7	CMLD-1	*In vitro*	5.98	16060621	[6]
8	CMLD-4	*In vitro*	5.22	24868003	[6]
9	CMLD-5	*In vitro*	9.13	16746465	[6]
10	CMLD-6	*In vitro*	8.88	16746321	[6]
11	CMLD-3	*In vitro*	7.11	16746461	[6]
12	31	*In vitro*	44.4	265580	[7]
13	32	*In vitro*	21.7	137651476	[7]
14	23	*In vitro*	41.0	66672	[7]
15	30	*In vitro*	41.9	54677920	[7]
16	MPT0B098	*In vitro*	0.08-0.51	50898338	[8]
17	SP600125	*In vitro*	0.12-16.0	8515	[9]
18	Trichostatin A	*In vitro*	-	444732	[9]
19	5-aza-2-deoxycitidine	*In vivo*	0.01-0.05	460487	[9]
20	N-Benzylcantharidinamide	*In vitro*	50.0-100.0	-	[10]
21	Triptolide	*In vivo*	0.01-0.03	107985	[11]
22	Leptomycin B	*In vivo*	0.01	6917907	[12]
23	Selinexor	*In vivo*	0.12-0.22	71481097	[13]

24	Latrunculin A	*In vitro*	0.33-0.76	445420	[14]
25	Blebbistatin	*In cell*	0.43-22.8	3476986	[14]
26	Mitoxantrone	*In vivo*	0.01-1.13	4212	[5]
27	Suramin	*In vivo*	732	5361	[15]
28	NSC 5836	*In vitro*	14.7	3034178	[7]
29	NSC 7572	*In vitro*	41.0	66672	[7]
30	NSC 44750	*In vitro*	26.9	239577	[7]
31	NSC 50648	*In vitro*	97.4	242249	[7]
32	NSC 62685	*In vitro*	16.7	247668	[7]
33	NSC 123418	*In vitro*	69.9	276150	[7]
34	NSC 143491	*In vitro*	21.7	9575999	[7]
35	NSC 227186	*In vitro*	41.9	54706138	[7]
36	NSC 651084	*In vitro*	17.4	6713252	[7]
37	Myricetin	*In vitro*	1.0	5281672	[3]
38	b-40	*In cell*	0.38	1806245	[16]
39	Ellagic acid	*In vitro*	0.6	5281855	[3]
40	(-)-Epicatechin gallate	*In vitro*	0.2	107905	[3]
41	Rhamnetin	*In vitro*	1.2	5281691	[3]
42	Cryptotanshinone	*In cell*	6.0	160254	[17]
43	(-)-Epigallocatechin gallate	*In vitro*	0.2	65064	[3]
44	Dihydrotanshinone (DHTS)	*In vivo*	0.84-1.2	5316743	[17]
45	6a	*In cell*	0.0128	132251303	[4]
46	b-41	*In cell*	6.21	58739563	[16]
47	b-68	*In cell*	-	1801337	[16]
48	a-63	*In cell*	-	6161395	[16]

49	Azaphilone-9	*In vitro*	51.9-67.6	57326411	[18]
50	CMLD-2	*In vitro*	4.44	16746438	[6]
51	Tanshinone I	*In cell*	-	114917	[17]
52	HuR inhibitor 5	*In cell*	15-27	3634762	[19]
53	1	*In vitro*	-	156010691	[20]
54	2	*In vitro*	-	155560371	[20]
55	3	*In vitro*	-	155517914	[20]
56	4	*In vitro*	-	155540762	[20]
57	Hyperoside	*In vitro*	-	5281643	[21]
58	Rutin	*In vitro*	-	5280805	[21]
59	Isovitexin	*In vitro*	-	162350	[21]
60	Vitexin-2-O-rhamnoside	*In vitro*	-	5282151	[21]
61	Aesculin	*In vitro*	-	5281417	[21]
62	Novobiocin	*In vitro*	-	54675769	[21]
63	Chlorogenic acid	*In vitro*	-	1794427	[21]
64	7-hydroxymatairesinol	*In vitro*	-	44147426	[21]
65	Colchicoside	*In vitro*	-	92763	[21]
66	Asiaticoside	*In vitro*	-	11954171	[21]
67	Embelin	*In cell*	-	3218	[22]
68	KH-3	*In cell*	3.34	45138018	[23]
69	KH-3 derivative 1 (1c)	*In cell*	4.74	155343380	[23]
70	KH-3 derivative 2 (7c)	*In cell*	1.31	11187381	[23]

Table S2. Docking scores of the thirteen experimentally tested ligands (grey cell) and the known HuR inhibitors.

Compound name	Docking score [kcal/mol]	Compound name	Docking score [kcal/mol]
Z259632494	*-8.646*	NSC 44750	-5.949
Z57908816	*-4.494*	NSC 50648	-5.507
7643436	*-8.618*	NSC 62685	-6.261
STK217448	*-8.287*	NSC 123418	-2.736
STL170802	*-8.623*	NSC 143491	-5.803
5974204	*-8.561*	NSC 227186	-5.485
Y044-6405	*-9.002*	NSC 651084	-4.937
STK018404	*-10.154*	Myricetin	-5.630
Z373002224	*-6.083*	b-40	-5.897
STK806137	*-8.681*	Ellagic acid	-5.427
STK593921	*-5.014*	(-)-Epicatechin gallate	-6.149
STL148963	*-3.350*	Rhamnetin	-2.508
STL522523	*-9.201*	Cryptotanshinone	-3.978
MS-444	-5.226	(-)-Epigallocatechin gallate	-6.212
Dehydromutactin	-5.403	Dihydrotanshinone (DHTS)	-3.963
Okicenone	-5.514	6a	-5.253
Quercetin	-6.475	b-41	-4.969
6n	-4.151	b-68	-5.149
24	-4.590	a-63	-4.498
CMLD-1	-4.586	Azaphilone-9	-3.784
CMLD-4	-3.667	CMLD-2	-3.965
CMLD-5	-3.661	Tanshinone I	-4.690

CMLD-6	-4.045	HuR inhibitor 5	-4.320
CMLD-3	-3.684	1	-3.163
31	-3.209	2	-4.940
32	-5.874	3	-6.945
23	-5.249	4	-5.393
30	-5.132	Hyperoside	-4.485
MPT0B098	0.090	Rutin	-6.708
SP600125	-7.062	Isovitexin	-5.854
Trichostatin A	-3.380	Vitexin-2-O-rhamnoside	-6.320
5-aza-2-deoxycitidine	-4.140	Aesculin	-5.816
N-Benzylcantharidinamide	-4.437	Novobiocin	-5.038
Triptolide	-2.731	Chlorogenic acid	-6.284
Leptomycin B	-5.892	7-hydroxymatairesinol	-6.507
Selinexor	-5.084	Colchicoside	-5.767
Latrunculin A	-4.269	Asiaticoside	-6.402
Blebbistatin	-4.754	Embelin	-2.156
Mitoxantrone	-4.978	KH-3	-3.387
Suramin	-8.556	KH-3 derivative 1 (1c)	-5.417
NSC 5836	-3.913	KH-3 derivative 2 (7c)	-4.980
NSC 7572	-5.249		

Table S3. Predicted off-targets classified by the estimated binding probability (in %) for the 32 representatives of the 70 known HuR known inhibitors (Table S1) and our thirteen experimentally tested molecules, including our identified hit STK18404. Ligands are colored according to the probability range with the largest number of predicted off-targets.

	Target Probability in 100 entries [%]							
Drug	0*	1-9	10-15	16-20	21-39	40-50	51-80	81-100
1	0	0	100	0	0	0	0	0
DHTS	41	0	53	0	0	0	0	6
6a	0	0	100	0	0	0	0	0
b-40	32	0	68	0	0	0	0	0
Quercetin	0	0	0	1	9	6	16	69
Hyperoside	78	0	10	1	0	2	1	8
Chlorogenic acid	62	0	34	0	2	0	1	1
Mitoxantrone	85	0	0	0	0	0	0	15
CMLD-2	0	0	99	0	0	0	0	1
KH-3 d1	65	0	35	0	0	0	0	0
NSC 62685	99	0	1	0	0	0	0	0
Azaphilone-9	56	0	44	0	0	0	0	0
Embelin	47	0	50	0	3	0	0	0
MPT0B098	0	0	100	0	0	0	0	0
Latrunculin	100	0	0	0	0	0	0	0
Leptomycin B	0	0	100	0	0	0	0	0
MS-444	no off-target found#							
N-Benzylcantharidinamide	46	0	54	0	0	0	0	0
Selinexor	24	0	76	0	0	0	0	0
SP600125	57	43	0	0	0	0	0	0
Suramin	100	0	0	0	0	0	0	0
Dehydromutactin	78	0	22	0	0	0	0	0
Okicenone	no off-target found#							

23	32		68	0	0	0	0	0
30	100		0	0	0	0	0	0
31	100		0	0	0	0	0	0
32	81		19	0	0	0	0	0
Trichostatin A	0	0	100	0	0	0	0	0
5-aza-2-deoxycitidine	94	6	0	0	0	0	0	0
Triptolide	no off-target found#							
Blebistatin	3		97	0	0	0	0	0
HuR inhibitor 5	0		100	0	0	0	0	0
5974204	31		69	0	0	0	0	0
7643436	0		100	0	0	0	0	0
STK018404	93		7	0	0	0	0	0
STL170802	100		0	0	0	0	0	0
STK217448	0	0	100	0	0	0	0	0
STK593921	0	0	100	0	0	0	0	0
STK806137	0	0	100	0	0	0	0	0
STL148963	62	0	38	0	0	0	0	0
STL522523	8	0	92	0	0	0	0	0
Y044-6405	65	1	34	0	0	0	0	0
Z57908816	0	0	99	0	0	1	0	0
Z373002224	88	0	12	0	0	0	0	0
Z259632494	no off-target found#							

* Probability equal to 0% means that the confidence of the off-target prediction is very low, due to limited similarity between the query ligand and the known actives present in the SwissTargetPrediction database. In this regard, the webserver combines 2D and 3D similarity measures between the query and the database molecules, with different thresholds [24]. This might result in predicted off-targets with a certain number of know actives similar in 3D to the query ligand, but no known active similar in 2D, in which case the probability of the investigated but no known active similar in 2D, in which case the probability of the investigated

"No off-target found" indicates that there are no active molecules in the SwissTargetPrediction database similar to the query molecule and thus no off-target was predicted.

Table S4. Protein class of the predicted top off-targets for the 32 representatives of the known HuR known inhibitors and the thirteen experimentally tested ligands. Color code for the ligands is the same as in Table S3.

Drug	Top Targets			
1	Kinase	Family A GPCR		
DHTS	Aldose reductase	Acetylcholinesterase		
6a	Kinase	Family A GPCR		
b-40	Kinase	Family A GPCR		
Quercetin	Kinase	Lyase	Protease	
Hyperoside	Aldose reductase	Carbonic anhydrases 2, 4, 7, 12	NADPH oxidase 4	Adrenergic receptor alpha-2
Chlorogenic acid	Aldose reductase	Aldo-keto reductase family 1 member B10		
Mitoxantrone	Electrochemical transporter	Kinase	Family A GPCR	
CMLD-2	Eukaryotic translation inhibition factor 4H	Family A GPCR	Kinase	Protease
KH-3 d1	Eraser	Protease		
NSC 62685	5′-nucleotidase			
Azaphilone-9	Kinase	Family A GPCR		
Embelin	Histone Acetyltransferase	Macrophage-expressed gene 1 protein	Arachidonate 5-lipoxygenase	
MPT0B098	Family A GPCR	Kinase		
Latrunculin				
Leptomycin B	Family A, B, C GPCR	Eraser	Protease	
MS-444	no off-target found			
N-Benzylcantharidinamide	Family A GPCR	Protease	Reader	
Selinexor	Kinase	Protease	Family A GPCR	
SP600125	Kinase	Family A GPCR		
Suramin				
Dehydromutactin	Oxidoreductase	Voltage-gated ion channel		
Okicenone	no off-target found			
23	Kinase	Lyase	Family A GPCR	

30	Kinase	Protease		
31				
32	Protease	Family A GPCR		
Trichostatin A	Eraser	Protease	Family A GPCR	
5-aza-2-deoxycitidine	Protease	Hydrolase		
Triptolide	no off-target found			
Blebistatin	Kinase	Protease	Family A GPCR	
HuR inhibitor 5	Kinase	Protease	Family A GPCR	
5974204	Kinase	Family A GPCR		
7643436	Kinase	Lyase		
STK018404	Oxidoreductase	Transferase	Kinase	
STL170802	Eraser	Phosphodiesterase	Lyase	
STK217448	Family A GPCR	Kinase	Voltage-gated ion channel	
STK593921	Kinase	Potease	Eraser	Family A GPCR
STK806137	Protease	Lyase	Kinase	
STL148963	Protease	Family A GPCR	Fatty acid protein binding family	Family A GPCR
STL522523	Protease	Kinase		
Y044-6405	Eraser	Kinase	Lyase	
Z57908816	Adenosine alpha 2a receptor			
Z373002224	Kinase	Family A GPCR		
Z259632494	no off-target found			

Table S5. Docking scores of the twenty-three successfully docked decoys.

Compound name	Docking score [kcal/mol]	Compound name	Docking score [kcal/mol]
ZINC000008220417	-6.929	ZINC000005112560	-5.484
ZINC000005340116	-6.782	ZINC000712777334	-5.352
ZINC000006827695	-6.687	ZINC000008214677	-5.351
ZINC000000895180	-6.388	ZINC000005828408	-5.340
ZINC000002044203	-5.933	ZINC000013542400	-5.269
ZINC000001542148	-5.930	ZINC000089222544	-5.150
ZINC000004096278	-5.929	ZINC000206744254	-5.082
ZINC000004096089	-5.866	ZINC000136274150	-5.044
ZINC000001529331	-5.856	ZINC000003869232	-5.033
ZINC000020157024	-5.795	ZINC000005840872	-4.999
ZINC000029391179	-5.487	ZINC000006090886	-4.492
ZINC000001850486	-5.484		

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