# Small Molecule Screen Identifies Non-Catalytic USP3 Chemical Handle

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## Table S1: Compounds smiles strings and catalog numbers

| Compound # | Catalog_Number | SMILES |
|---|---|---|
| 1 | STL102634 | C(C([O-])=O)c1cccnc1 |
| 2 | STK256632 | C(Cn1cccn1)C([O-])=O |
| 3 | STK661679 | C(Cc1ccno1)C([O-])=O |
| 4 | Z57127332 | C(Cc1ccccc1)C(O)=O |
| 5 | STK802016 | C(C([O-])=O)Nc1ncccn1 |
| 6 | IF_02_176 | C(Cc1cnccn1)C(O)=O |
| 7 | SY019802 | C(Cc1nccs1)C(O)=O |
| 8 | A0954 | C(C(O)=O)c1csc(N)n1 |
| 9 | STK891980 | C(COc1ccccc1)C([O-])=O |
| 10 | STL193409 | C(CN1C=CC=CC1=O)C([O-])=O |
| 11 | Z1171978885 | C(C([O-])=O)c1cc2ccccc2o1 |
| 12 | STL163377 | C(C([O-])=O)c1nc2ccccc2[nH]1 |
| 13 | Z1333043518 | C(C([O-])=O)n1cc2ccccc2n1 |
| 14 | F1913-0024 | C(C([O-])=O)c1nc2ccccc2o1 |
| 15 | STL124046 | C(C([O-])=O)NC(c1cccnc1)=O |
| 16 | STK526633 | C(CC(c1cccs1)=O)C([O-])=O |
| 17 | STL190846 | C(C([O-])=O)c1cccc2cccnc12 |
| 18 | STK695471 | C(Cc1cn2cccnc2n1)C([O-])=O |
| 19 | EN300-14584 | C(Cn1cnc2ccccc12)C(O)=O |
| 20 | EN300-70527 | C(C(O)=O)N1Cc2ccccc2C1=O |
| 21 | STK504141 | C(C([O-])=O)Nc1ccc2nncn2n1 |
| 22 | STK353430 | C(Cc1nc2ncccn2n1)C(O)=O |
| 23 | Z188924494 | C(Cc1ccc2c(c1)OCO2)C([O-])=O |
| 24 | IF_03_114 | C(CC(Nc1ccccc1)=O)C(O)=O |
| 25 | STK723995 | C(C([O-])=O)Nc1c2ccccc2ncn1 |
| 26 | MS-2504 | C(C([O-])=O)Oc1cnc2ccccc2n1 |
| 27 | STK392449 | C(C([O-])=O)N1C=Nc2ccccc2C1=O |
| 28 | EN300-188561 | C(C(O)=O)N1C=Cc2ccccc2C1=O |
| 29 | STK738252 | C(CN1C(=O)Oc2ccccc12)C([O-])=O |
| 30 | Z57983005 | C(C1C(Nc2ccccc2O1)=O)C(O)=O |
| 31 | STK447336 | Cc1cc(NC(CCC([O-])=O)=O)nn1C |
| 32 | CDS014372 | C1C[C@H](C(N)=O)N(C1)C(CCC([O-])=O)=O |
| 33 | Z126932466 | C(Cc1cnn(c1)c1ccccc1)C(O)=O |
| 34 | STK500480 | C(Cc1nc(c2ccccc2)no1)C([O-])=O |

| | | |
|---|---|---|
| 35 | TS-00478 | C(Cc1nnc(c2ccccc2)o1)C([O-])=O |
| 36 | STL310201 | C(CC([O-])=O)C1C(N2C=CC=CC2=NN=1)=O |
| 37 | AL-291/37197008 | C(Cn1nc(c2ccccc2)nn1)C(O)=O |
| 38 | STK099228 | C(CC(N1CCc2ccccc12)=O)C([O-])=O |
| 39 | Z166605066 | C(CN1C(c2ccccc2N=N1)=O)C(O)=O |
| 40 | STK946249 | C(CN1C(c2ccccc2S1)=O)C([O-])=O |
| 41 | EN300-00843 | CC1(C)Cc2cccc(c2O1)OCC(O)=O |
| 42 | EN300-35451 | CC1=C(CCC([O-])=O)C(NC(=N1)SC)=O |
| 43 | Z1259161843 | C(C([O-])=O)Nc1ccc(cc1)S(N)(=O)=O |
| 44 | EN300-10660 | Cc1c2C(N(CC([O-])=O)C=Nc2sc1C)=O |
| 45 | IF_04_062 | C(Cc1nc2cccc3cccc(c23)[nH]1)C(O)=O |
| 46 | IF_03_115 | C(CC(Nc1cccc2ccccc12)=O)C(O)=O |
| 47 | Z90120424 | C(C(O)=O)N1C=Nc2c3ccccc3oc2C1=O |
| 48 | EN300-137714 | C(CC(c1ccc2c(CCC(N2)=O)c1)=O)C(O)=O |
| 49 | Z102925466 | C(CN1C(c2ccccc2S1(=O)=O)=O)C(O)=O |
| 50 | IF_01_187 | C(Cc1ccccc1OCc1ccccn1)C(O)=O |
| 51 | STK925744 | CC1=CC(=O)Oc2cc(c(CCC([O-])=O)cc12)OC |
| 52 | STK682580 | C1CCn2c(C1)c(C#N)c1c2C(N(CC(O)=O)C=N1)=O |
| 53 | KM09821 | C(CC(O)=O)CN1CCC(=CC1)c1ccccc1.[Cl] |
| 54 | STK545027 | CC(C(O)=O)N1C(C(=Cc2ccc(cc2)OC)SC1=O)=O |
| 55 | Z1682077208 | C1CCN(C1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O |
| 56 | 79148212 | C(Cc1nc2ccccc2n1c1cc(C(N)=O)sc1)C(O)=O |
| 57 | MS-2054 | C(C(Nc1nnc(SCC(O)=O)s1)=O)Oc1ccccc1 |
| 58 | Z609944470 | C(Cc1nc2ccccc2c2nc(Cc3ccccc3)nn12)C([O-])=O |
| 59 | Z1373577255 [Enamine] | C(CC(c1cccc(c1)NS(c1c[nH]c2c1cccn2)(=O)=O)=O)C(O)=O |
| 60 | Z1413959119 | Cc1ccc(cc1S(Nc1cccc(c1)C(CCC(O)=O)=O)(=O)=O)C(O)=O |
| 61 | HGA-4028-0036-50 | C1CN(CCC1c1ccc(cc1)[Cl])C(c1ccc(C(NCC(O)=O)=O)nc1)=O |
| 62 | CAZ-0010-0660-20 | C1CN(CCC1N1C(=O)Oc2ccccc12)S(c1ccc(cc1)C(NCC(O)=O)=O)(=O)=O |

**Table S2: Matrix Screen results** (1000 means KD > 1000 μM)

| Compound # | KD (μM) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | USP3 | USP5 | USP13 | USP16 | USP20 | USP33 | USP39 | USP49 | USP51 | BRAP | HDAC6 |
| 1 | 121 | 1000 | 1000 | 96.1 | 707 | 115 | 180 | 283 | 1000 | 73.9 | 1000 |
| 2 | 1000 | 1000 | 98.2 | 1000 | 182 | 1000 | 1000 | 56 | 1000 | 74.4 | 1000 |
| 3 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 4 | 178 | 1000 | 1000 | 429 | 1000 | 1000 | 1000 | 172 | 1000 | 82.9 | 1000 |
| 5 | 855 | 1000 | 616 | 1000 | 574 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 7 | 173 | 1000 | 547 | 331 | 439 | 808 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 8 | 1000 | 1000 | 1000 | 1000 | 895 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 9 | 1000 | 1000 | 640 | 1000 | 523 | 724 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 10 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 11 | 77.1 | 1000 | 472 | 265 | 472 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 12 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 13 | 519 | 1000 | 1000 | 1000 | 715 | 268 | 665 | 1000 | 1000 | 411 | 1000 |
| 14 | 1000 | 1000 | 278 | 1000 | 403 | 368 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 15 | 63.2 | 1000 | 1000 | 198 | 1000 | 514 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 16 | 195 | 1000 | 194 | 1000 | 272 | 1000 | 1000 | 1000 | 1000 | 1000 | 555.4 |
| 17 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 18 | 1000 | 1000 | 833 | 1000 | 956 | 1000 | 1000 | 1000 | 1000 | 416 | 1000 |
| 19 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 20 | 758 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 525 | 1000 | 685 | 1000 |
| 21 | 1000 | 1000 | 780 | 1000 | 882 | 1000 | 1000 | 1000 | 1000 | 1000 | 869 |
| 22 | 1000 | 1000 | 118 | 1000 | 110 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 23 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 24 | 1000 | 1000 | 872 | 1000 | 817 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 25 | 191 | 1000 | 1000 | 603 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 26 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 885 | 1000 |
| 27 | 328 | 893 | 860 | 456 | 835 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 28 | 249 | 1000 | 228 | 1000 | 224 | 1000 | 1000 | 1000 | 1000 | 1000 | 698.4 |
| 29 | 817 | 1000 | 1000 | 981 | 1000 | 1000 | 1000 | 1000 | 1000 | 993 | 1000 |
| 30 | 1000 | 1000 | 306 | 1000 | 271 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 31 | 201 | 1000 | 660 | 193 | 777 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 32 | 165 | 265 | 1000 | 154 | 1000 | 1000 | 1000 | 399 | 1000 | 462 | 565 |
| 33 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 661 | 1000 | 1000 | 1000 |
| 34 | 1000 | 1000 | 128 | 1000 | 119 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 35 | 1000 | 831 | 220 | 1000 | 64.7 | 1000 | 1000 | 1000 | 1000 | 340 | 1000 |
| 36 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 37 | 146 | 1000 | 950 | 482 | 677 | 1000 | 1000 | 1000 | 1000 | 1000 | 231.8 |
| 38 | 1000 | 1000 | 294 | 1000 | 291 | 1000 | 1000 | 1000 | 1000 | 646 | 95.6 |
| 39 | 102 | 1000 | 1000 | 1000 | 1000 | 851 | 1000 | 1000 | 1000 | 1000 | 331.4 |
| 40 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 41 | 583 | 1000 | 1000 | 671 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 42 | 51.8 | 1000 | 1000 | 118 | 814 | 211 | 389 | 1000 | 1000 | 1000 | 1000 |
| 43 | 620 | 1000 | 1000 | 428 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 44 | 290 | 238 | 1000 | 579 | 674 | 1000 | 1000 | 1000 | 1000 | 315 | 187.6 |
| 45 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 66 | 198.8 |
| 47 | 1000 | 312 | 1000 | 344 | 426 | 1000 | 1000 | 1000 | 1000 | 253 | 247 |
| 48 | 140 | 532 | 1000 | 167 | 821 | 1000 | 1000 | 99.9 | 1000 | 126 | 103.6 |
| 49 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 50 | 49.5 | 1000 | 365 | 48.2 | 249 | 1000 | 1000 | 1000 | 1000 | 483 | 1000 |
| 51 | 354 | 1000 | 867 | 535 | 465 | 1000 | 1000 | 86.2 | 1000 | 71.6 | 343.2 |
| 52 | 603 | 207 | 1000 | 403 | 552 | 1000 | 1000 | 1000 | 1000 | 215 | 105.4 |
| 53 | 1000 | 561 | 594 | 1000 | 380 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 54 | 441 | 1000 | 1000 | 116 | 457 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 55 | 91.5 | 117 | 1000 | 139 | 798 | 1000 | 1000 | 1000 | 1000 | 340 | 51.54 |
| 56 | 1000 | 1000 | 754 | 1000 | 464 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 57 | 219 | 315 | 745 | 372 | 401 | 1000 | 1000 | 1000 | 1000 | 703 | 273.4 |
| 58 | 238 | 847 | 1000 | 285 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 187.4 |
| 59 | 19.1 | 168 | 1000 | 69.8 | 706 | 1000 | 1000 | 1000 | 1000 | 413 | 211.6 |
| 60 | 283 | 1000 | 1000 | 114 | 1000 | 537 | 942 | 187 | 1000 | 234 | 1000 |
| 61 | 344 | 71.1 | 642 | 436 | 430 | 1000 | 1000 | 1000 | 1000 | 537 | 415 |
| 62 | 216 | 127 | 1000 | 536 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 234.1 |
| Ub | 4.83 | 3.01 | 1000 | 8.55 | 1000 | 1000 | 1000 | 1000 | 1000 | 35 | 9.134 |
| Ub-RGGdel | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

**Table S3: Eurofins Diversity panel on 58 kinases (Radiometric activity assay)**

| Kinase | Catalogue Number | % kinase activity in the presence of 10 µM of compound 59 |
|---|---|---|
| Abl(h) | 14-529KP | 96 |
| ALK(h) | 14-555KP | 98 |
| AMPKα1(h) | 14-840KP | 90 |
| ASK1(h) | 14-606KP | 91 |
| Aurora-A(h) | 14-511KP | 56 |
| CaMKI(h) | 14-663KP | 98 |
| CDK1/cyclinB(h) | 14-450KP | 111 |
| CDK2/cyclinA(h) | 14-448KP | 106 |
| CDK6/cyclinD3(h) | 14-519KP | 93 |
| CDK7/cyclinH/MAT1(h) | 14-476KP | 85 |
| CDK9/cyclin T1(h) | 14-685KP | 100 |
| CHK1(h) | 14-346KP | 108 |
| CK1γ1(h) | 14-711KP | 104 |
| CK2α2(h) | 14-689KP | 112 |
| c-RAF(h) | 14-352KP | 108 |
| DRAK1(h) | 14-668KP | 106 |
| eEF-2K(h) | 14-654KP | 106 |
| EGFR(h) | 14-531KP | 98 |
| EphA5(h) | 14-639KP | 99 |
| EphB4(h) | 14-554KP | 88 |

| | | |
|---|---|---|
| Fyn(h) | 14-441KP | 84 |
| GSK3β(h) | 14-306KP | 99 |
| IGF-1R(h) | 14-465KP | 125 |
| IKKα(h) | 14-461KP | 115 |
| IRAK4(h) | 14-599KP | 97 |
| JAK2(h) | 14-640KP | 108 |
| KDR(h) | 14-630KP | 87 |
| LOK(h) | 14-686KP | 103 |
| Lyn(h) | 14-510KP | 93 |
| MAPKAP-K2(h) | 14-337KP | 101 |
| MEK1(h) | 14-429KP | 107 |
| MLK1(h) | 14-690KP | 96 |
| Mnk2(h) | 14-664KP | 111 |
| MSK2(h) | 14-616KP | 108 |
| MST1(h) | 14-624KP | 96 |
| mTOR(h) | 14-769KP | 99 |
| NEK2(h) | 14-545KP | 105 |
| p70S6K(h) | 14-486KP | 99 |
| PAK2(h) | 14-481KP | 97 |
| PDGFRβ(h) | 14-463KP | 80 |
| Pim-1(h) | 14-573KP | 92 |
| PKA(h) | 14-440KP | 89 |
| PKBα(h) | 14-276KP | 104 |
| PKCα(h) | 14-484KP | 85 |
| PKCθ(h) | 14-444KP | 109 |
| PKG1α(h) | 14-688KP | 99 |
| Plk3(h) | 14-572KP | 104 |
| PRAK(h) | 14-334KP | 104 |
| ROCK-I(h) | 14-601KP | 112 |
| Rse(h) | 14-535KP | 118 |
| Rsk1(h) | 14-509KP | 110 |
| SAPK2a(h) | 14-251KP | 88 |
| SRPK1(h) | 14-564KP | 105 |
| TAK1(h) | 14-600KP | 106 |
| PI3 Kinase (p110β/p85α)(h) | 14-603KP | 98 |
| PI3 Kinase (p120γ)(h) | 14-558KP | 100 |
| PI3 Kinase (p110δ/p85α)(h) | 14-604KP | 96 |
| PI3 Kinase (p110α/p85α)(h) | 14-602KP | 98 |

**Table S4: ProteinLynx Global SERVER (PLGS) filtering parameters**

| **Parameter** | Min. intensity | Min. sequence length | Max. sequence length | Min. products per amino acid | Minimum score | Max. MH+ Error (ppm) |
|---|---|---|---|---|---|---|
| **Value** | 20000 | 4 | 26 | 0.12 | 7 | 5 |

**Figure S1: HDX sequence coverage of USP3$^{1-131}$**

A total of 59 peptides yielded 93.9% sequence coverage and a redundancy of 5.24. Black rectangles aligned to sequence represent peptides. Several peptides including 52-86 (length 35) and 53-86 (length 34) were manually added after PLGS filtering to obtain coverage from 53-60.

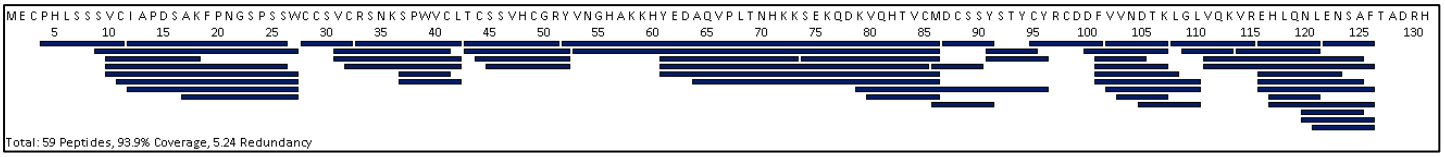


**Figure S2: Differential HDX heatmaps**

Differential fractional uptake is displayed as a per-residue averaged heatmap aligned to USP3 primary sequence for USP3 Zf-UBD in complex with **59** at 1:10 (top) and 1:20 (bottom) . The highest magnitude D decreases (-5 %) are blue and D increases (+5%) are red. A limitation of this heatmap illustration (DynamX 3.0) is that is does not factor in the associated error for each residue. Please refer to Figure 4 for a peptide-level differential HDX bar-plot with associated error.

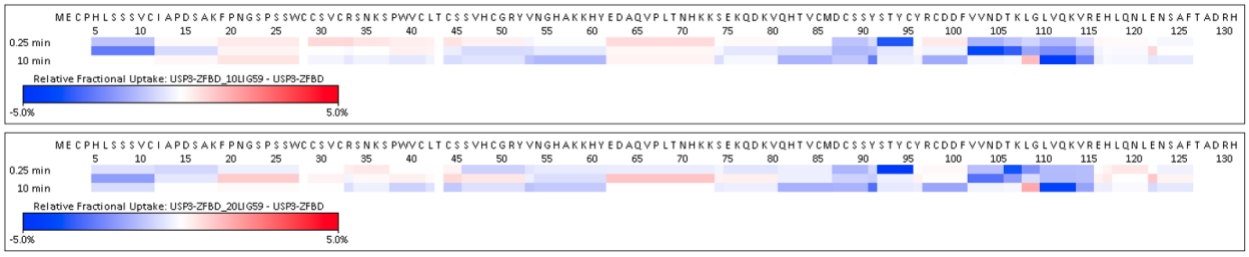

**Figure S3: Diversity of sidechains lining the binding pocket of 59**. Residues lining the pocket are highlighted in blue. Positions where a specific side-chain is unique to USP3 are highlighted with red arrows.

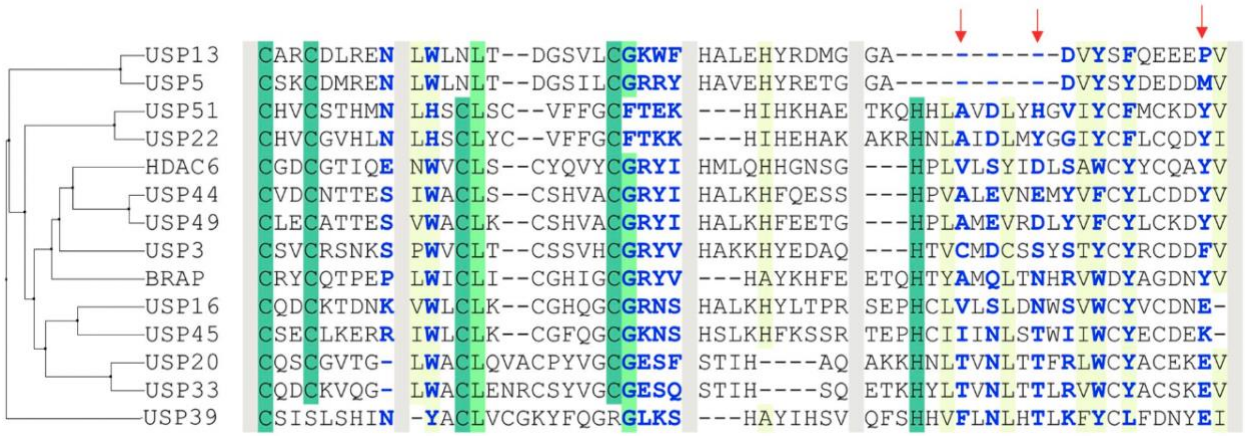


**Figure S4: Ligand RMSD over 100 ns MD simulation**