Supporting Information

Title: Discovery of Inhibitors for Proliferating Cell Nuclear Antigen Using Computational-Based Linked-Multiple-Fragment Screen.

Authors: Matthew D. Bartolowits[†], Jonathon M. Gast[†], Ashlee J. Hasler[†], Anthony M. Cirrincione[†], Rachel J. O'Connor[†], Amr H. Mahmoud[†‡], Markus A. Lill[†], Vincent Jo Davisson*[†]

[†] Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, IN 47907, United States

[‡] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt

*Corresponding author: davisson@purdue.edu

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Table S1. List of each peptoid-based ligand that was synthesized for this study. The calculated mass of each compound is shown, along with its experimentally determined mass by high resolution electrospray ionization or MALDI-TOF mass spectrometry. The yield for the synthesis of each ligand is also displayed.

Tables S2-S4. Descriptor statistics from the Bayesian classification method showing values for globularity, CW2, EDmin3 and IW4 for the full virtual set of tripeptoids, known inhibitors of protein-protein interactions or known non-inhibitors of protein-protein interactions, respectively.

Methods. Experimental procedures for the synthesis of non-commercially available primary amines and T2AA, as well as supporting experimental procedures for the synthesis and purification of His-tagged PCNA and FAM-PL peptide.

HPLC Traces.

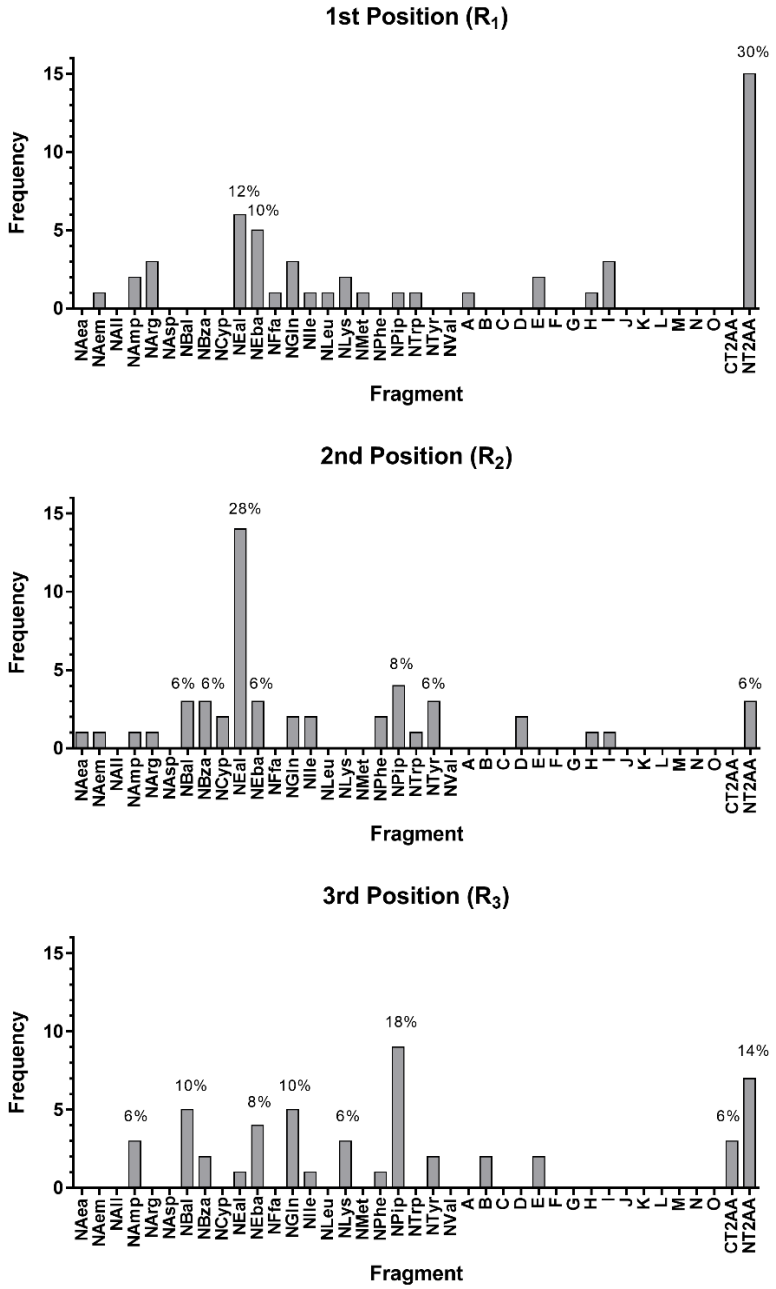


Figure S1. Glide docking-based frequency with which fragments appeared in the three substitution positions on a tripeptoid backbone containing T2AA as a fragment. A set of 37 fragments, in addition to two forms of T2AA (see Figure 1 of main text), were virtually combinatorially incorporated into a tripeptoid backbone, and were screened against the crystal structure of PCNA-T3 (PDB ID: 3VKX) *in silico* using the Glide HTVS, SP and XP docking algorithms. The frequency that respective fragments appeared in positions R_1, R_2 or R_3 were tallied for the top 50 hits. The percentage of the cumulative total of substitution frequency (out of a possible 50) at a given position is shown above columns.

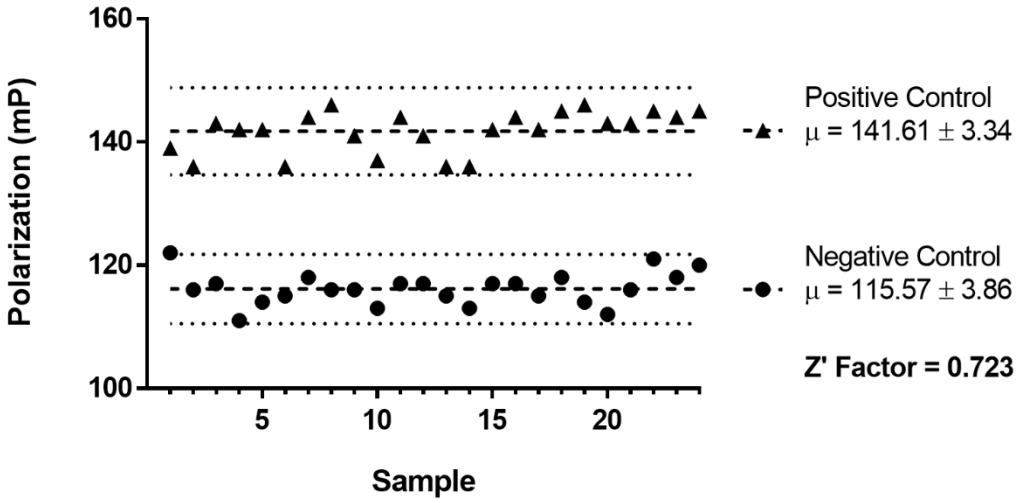


Figure S2. Z'-Factor analysis. Polarization values for 24 replicate samples of both positive and negative controls were used to evaluate the quality of the assay platform. 10 nM FAM-PL and 100 nM PCNA served as the positive control, while 10 nM FAM-PL in binding buffer served as the negative control. Dashed lines indicate the mean value of each set of controls, and dotted lines indicate the 95% prediction interval.

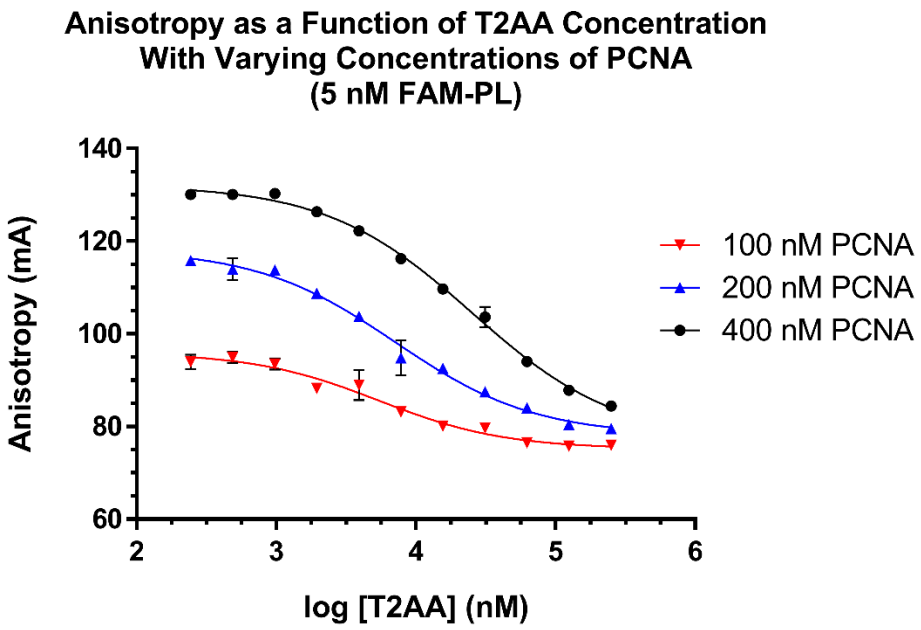


Figure S3. T2AA FP binding curves at different concentrations of PCNA. Using the same amount of FAM-PL peptide, the dynamic range of the FP assay increased significantly as additional PCNA was added to the assay. As can be seen here, increasing the concentration of PCNA from 100 nM to 400 nM increased the dynamic range from approximately 15 mA to more than 40 mA. Increasing the concentration of PCNA even higher would then theoretically continue to enhance the dynamic range.

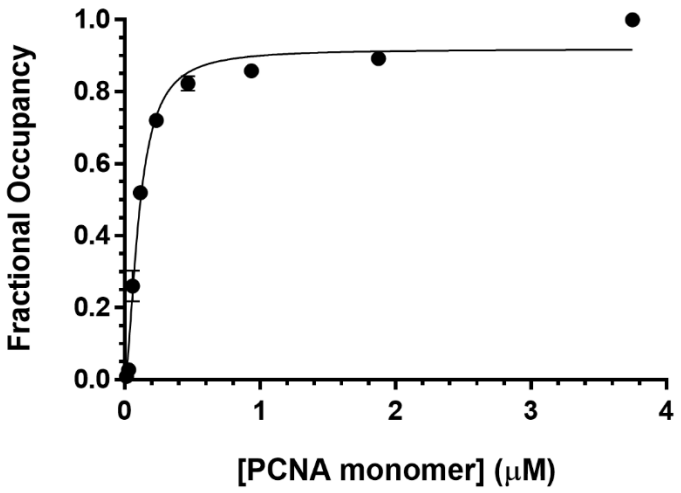


Figure S4. PCNA titration for determining affinity of FAM-PL. Increasing amounts of recombinant PCNA were added to a fixed concentration of the FAM-PL peptide (5 nM) in binding buffer. The data were fit to Equation 2.3 was used to determine the K_d value for the peptide (here, the calculated affinity was 107 nM) and the Hill slope of 1.745 =/-0.252.

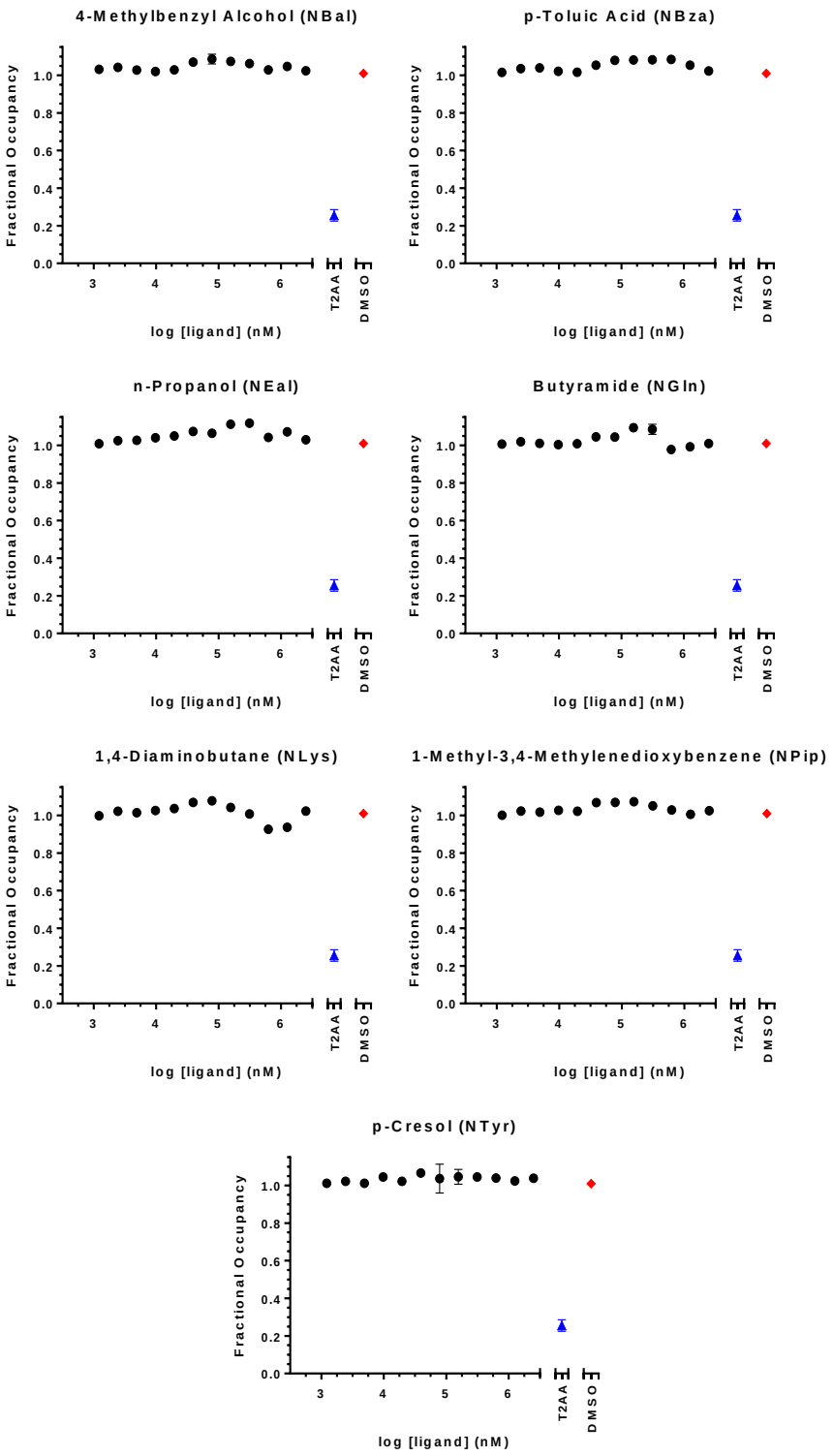


Figure S5. Dose response curves of individual peptoid fragments. The fragments present in hit tripeptoid molecules were screened individually in the FP assay to determine if they could individually disrupt the binding between PCNA and the PL peptide. None of the small fragments showed any evidence of inhibition. T2AA was selected as the positive control, and DMSO as the negative control. Error bars represent the standard error of the mean.

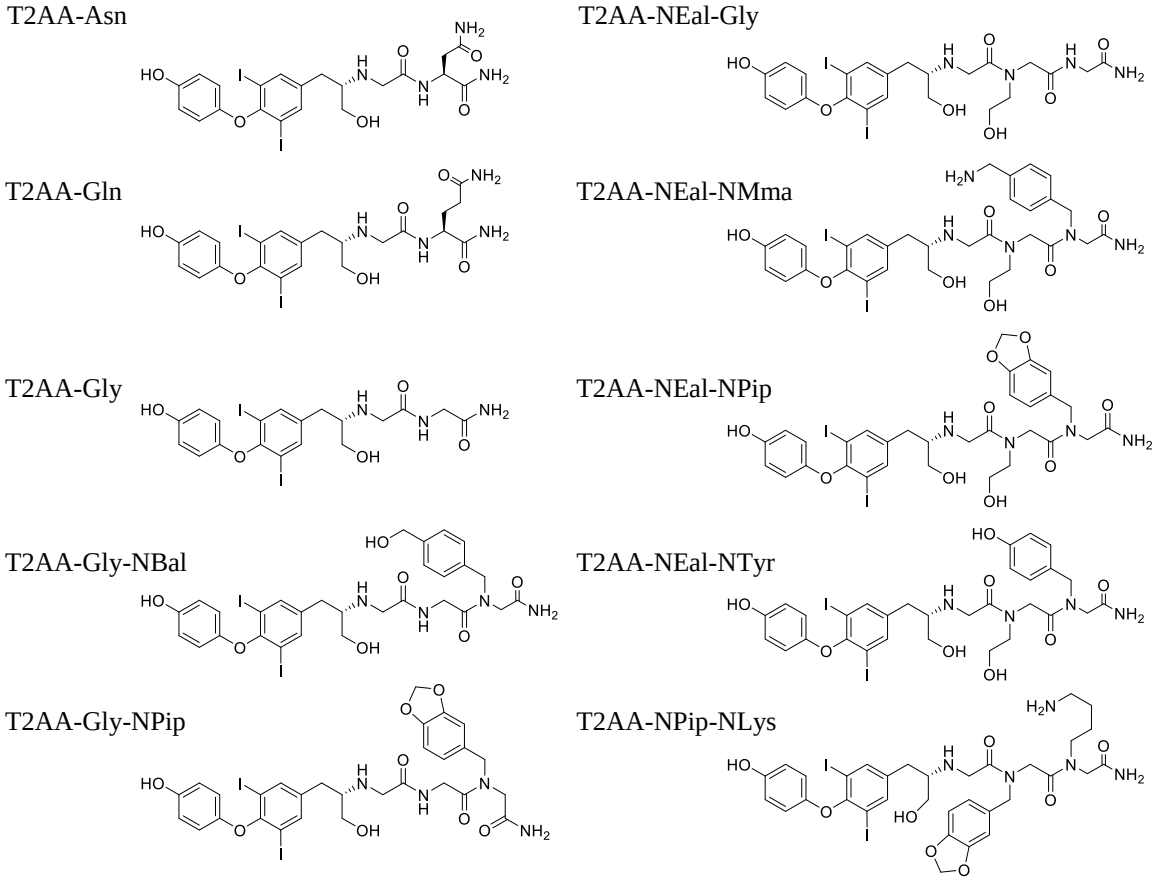


Figure S6. Structure of T2AA-conjugates. For second generation peptoid inhibitors of PCNA, T2AA was coupled to the N-terminus of peptoids or peptides anchored on solid phase resin to generate the molecules shown above.

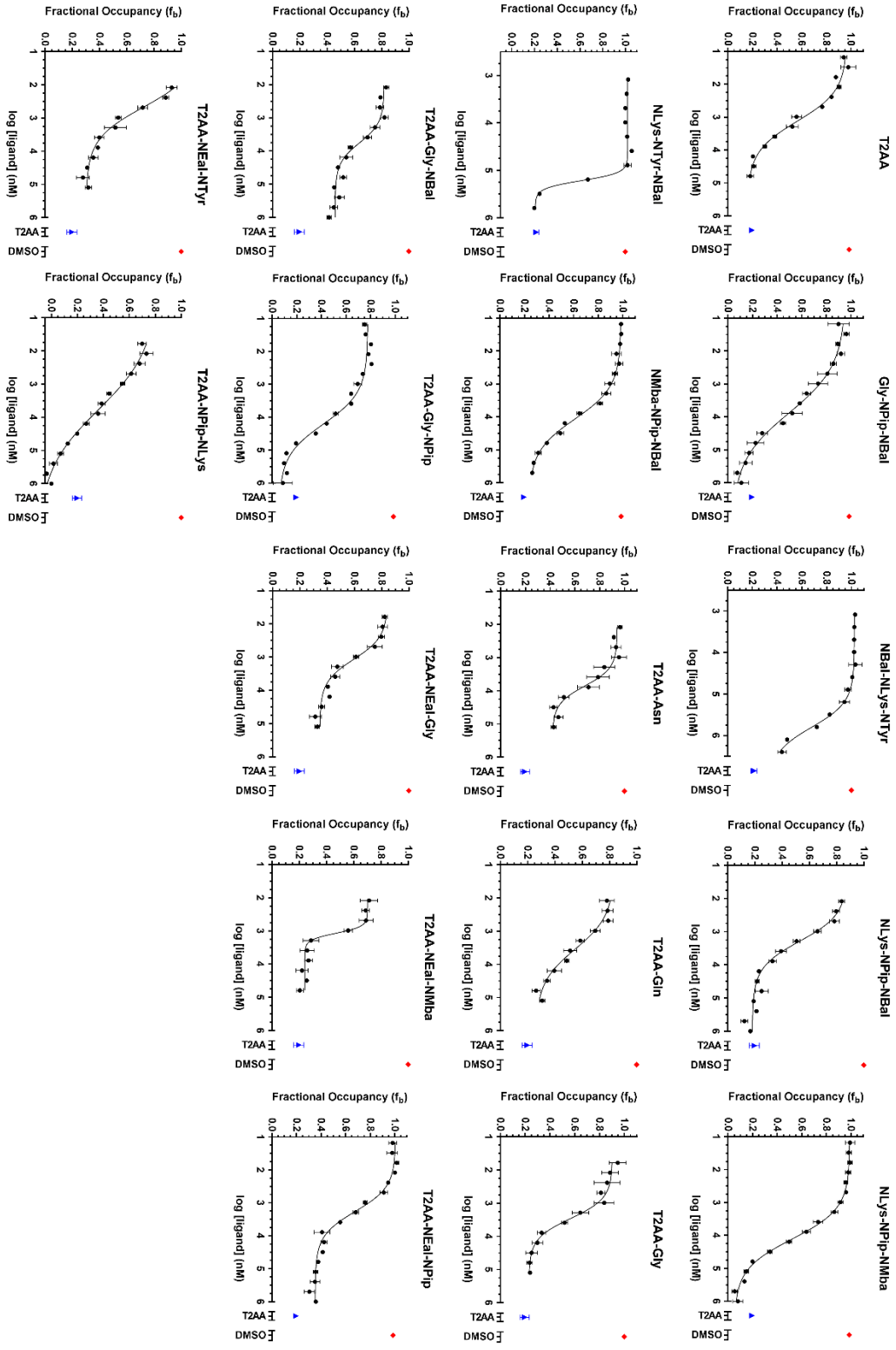
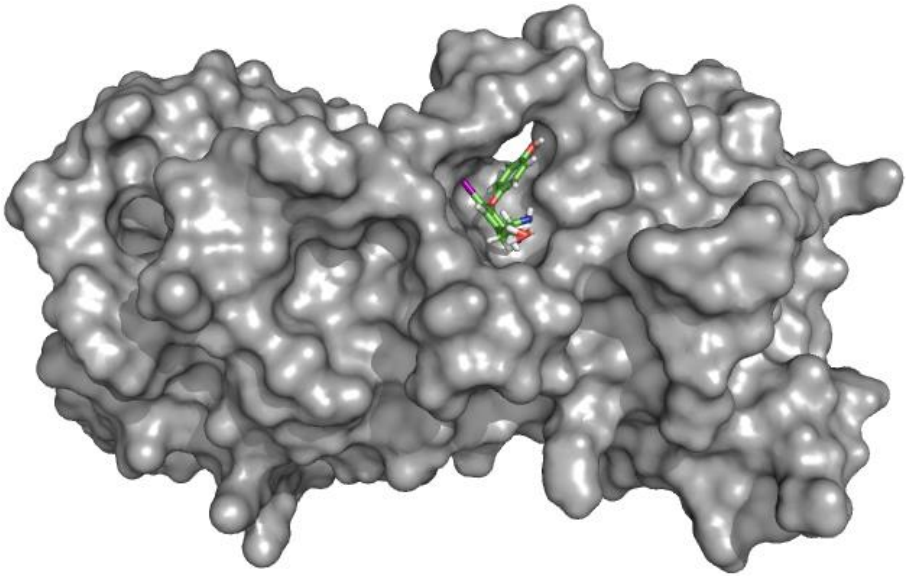
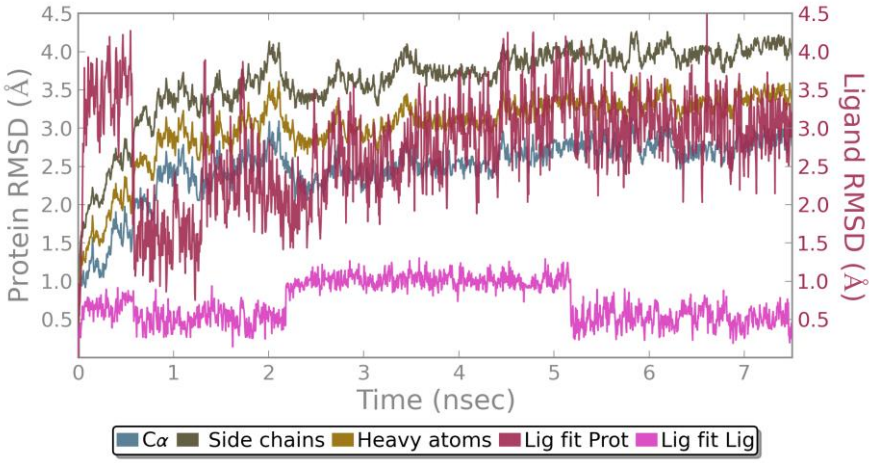


Figure S7. Dose response curves for all compounds listed in Table 1 of main text.

a)



b)



c)

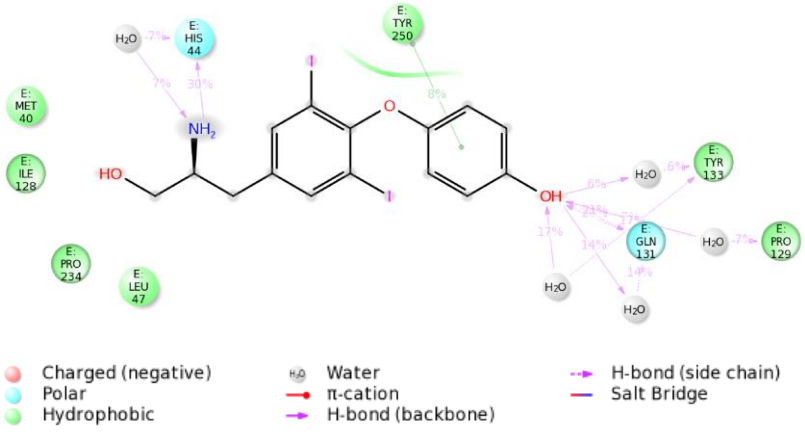


Figure S8. PCNA-T2AA MD simulation ouput. *(a)* Average structure of the final 50 frames of the PCNA-T2AA MD simulation. *(b)* RMSD diagram demonstrating simulation convergence. *(c)* Ligand interaction diagram showing molecular contacts over the course of the simulation.

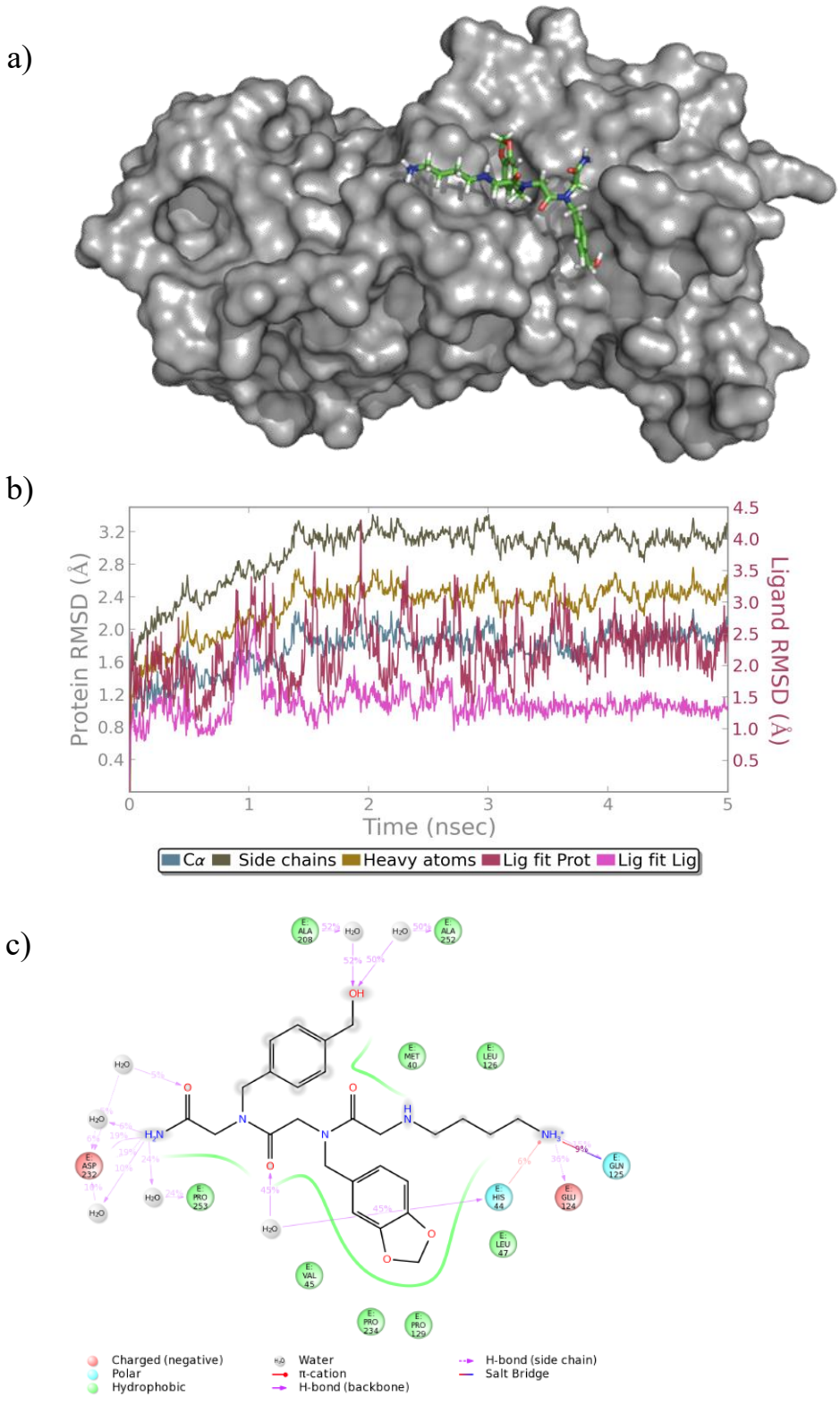


Figure S9. PCNA-NLys-NPip-NBal MD simulation ouput. *(a)* Average structure of the final 50 frames of the PCNA-NLys-NPip-NBal MD simulation. *(b)* RMSD diagram demonstrating simulation convergence. *(c)* Ligand interaction diagram showing molecular contacts over the course of the simulation.

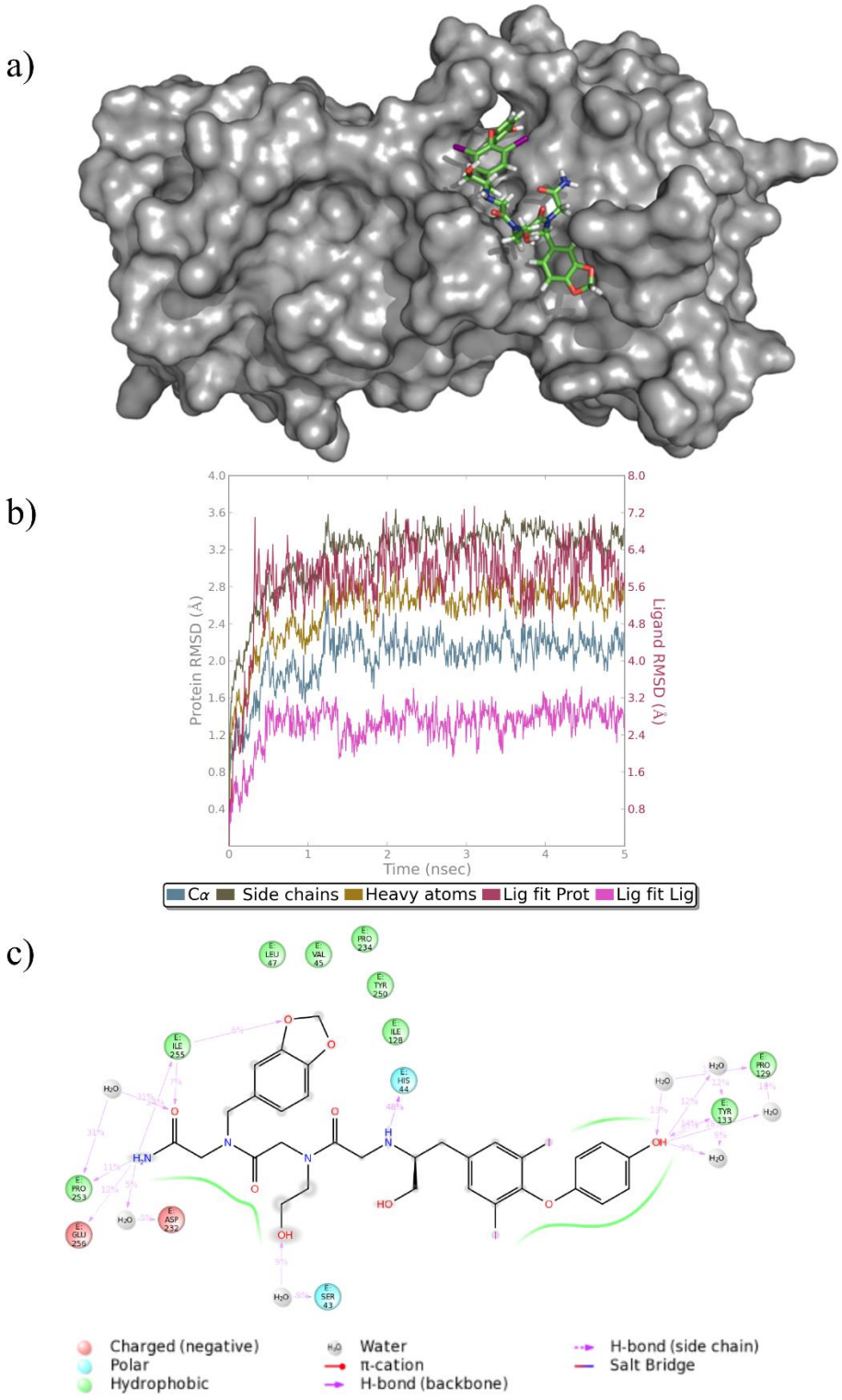


Figure S10. PCNA-T2AA-NEal-NPip MD simulation ouput. *(a)* Average structure of the final 50 frames of the PCNA-T2AA-NEal-NPip MD simulation. *(b)* RMSD diagram demonstrating simulation convergence. *(c)* Ligand interaction diagram showing molecular contacts over the course of the simulation.

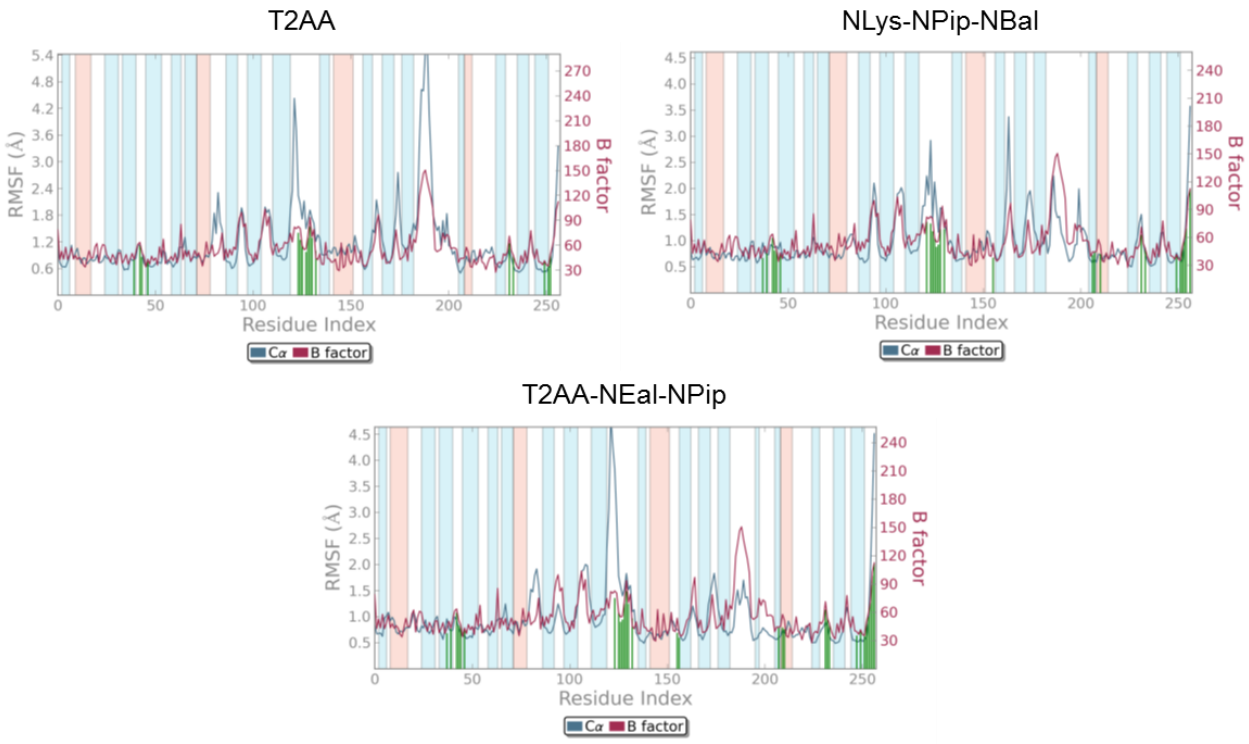


Figure S11. MD simulation Cα atom RMSD fluctuation by residue number. RMSD diagrams of each PCNA-peptoid MD simulation are shown. Peaks indicate areas of the protein that fluctuate the most during the simulation. Alpha helical and beta-strand regions are highlighted in orange and blue, respectively. Contacts between the ligand and PCNA residues are represented as green lines projecting upward from the x-axis.

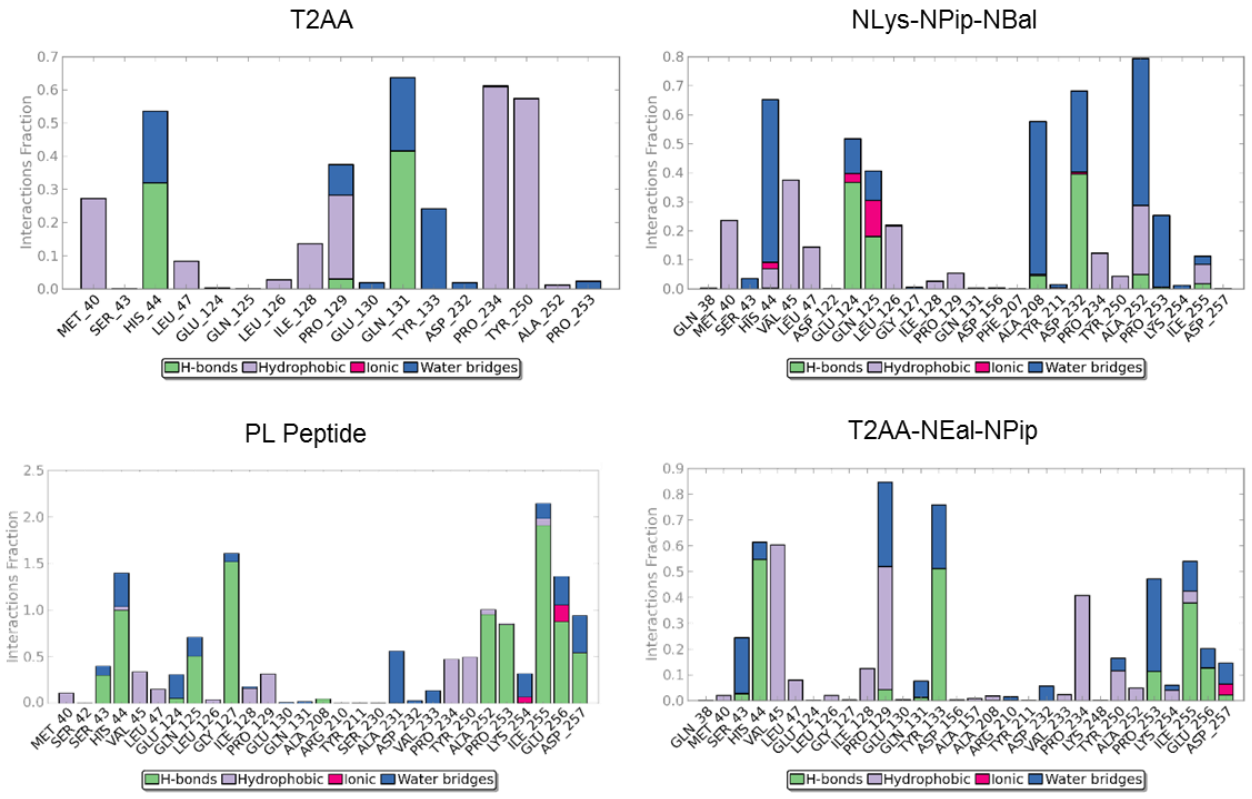


Figure S12. MD simulation interaction diagrams. Interactions between PCNA amino acids and peptoid ligands or the PL peptide were monitored over the course of each MD simulation. The number of trajectory frames in which an interaction occurred was recorded and listed as a fraction of the total number of possible simulation frames. Numbers larger than 1.0 indicate that during the simulation, two or more simultaneous interactions between a given protein residue and the ligand were taking place.

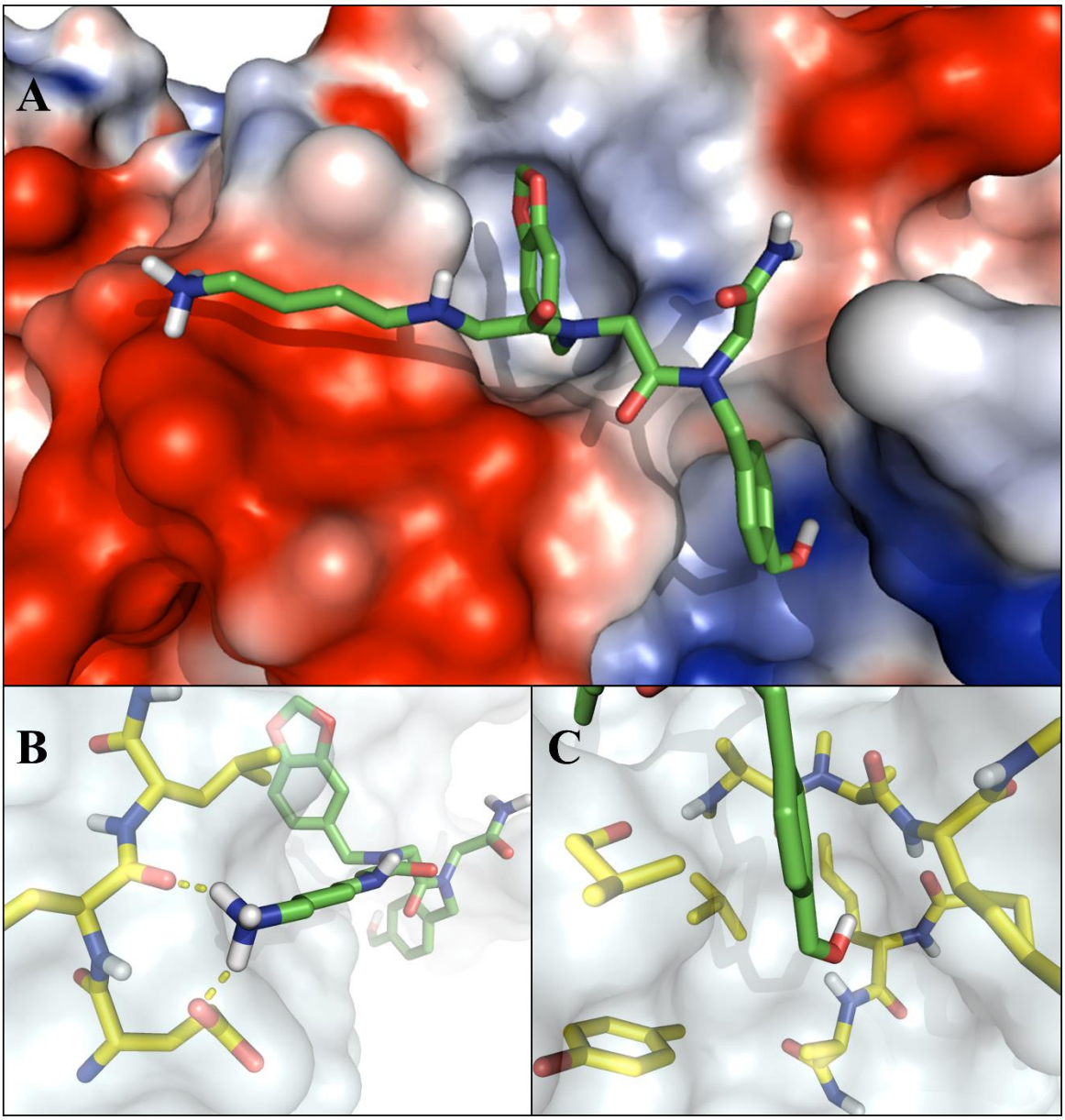
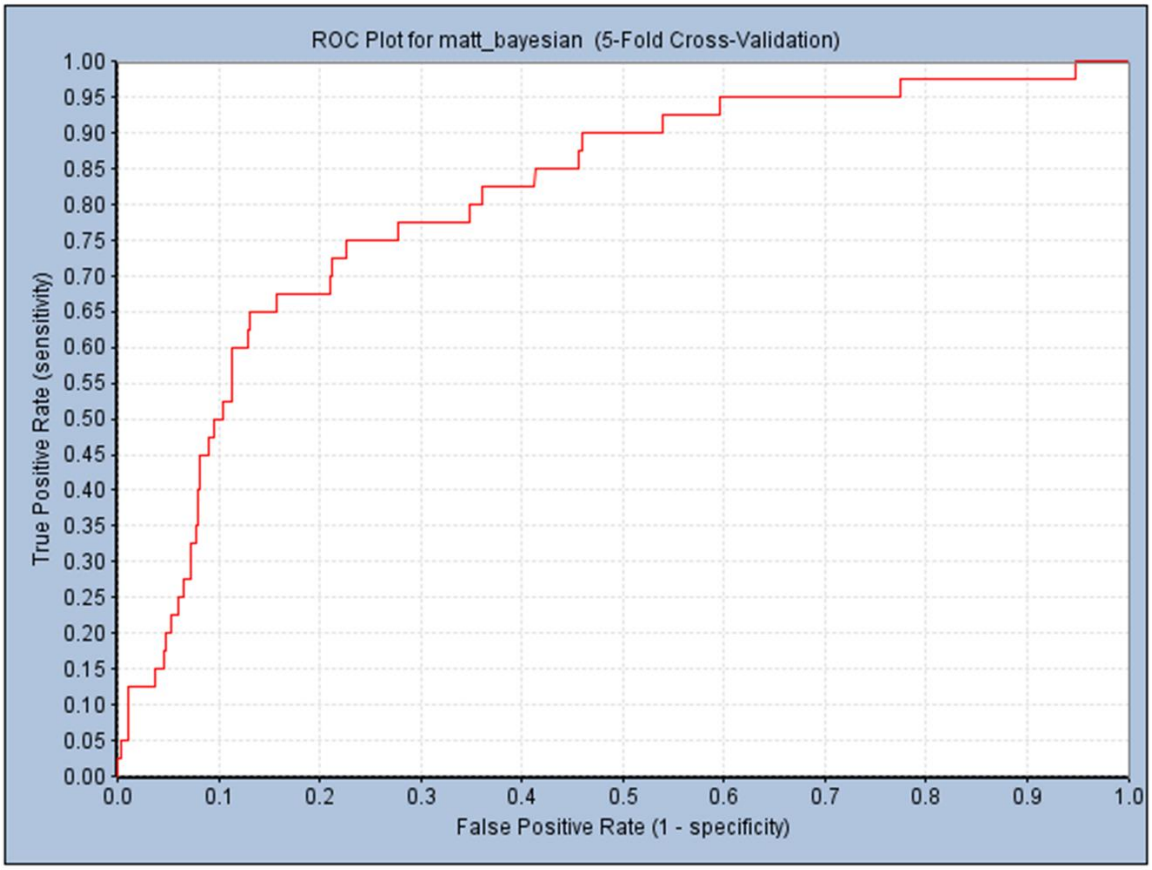


Figure S13. Predicted PCNA surface electrostatic potential and specific molecular contacts between NLys-NPip-NBal and PCNA. (A) The average MD output structure of NLys-NPip-NBal is shown with the predicted vacuum electrostatic potential (ranges from red [negative] to blue [positive], with gray being neutral) of PCNA shown as a surface. (B) The positively charged primary amine of the inhibitor is predicted to form hydrogen bonding and electrostatic contacts with residues Gln125 and Glu124 of PCNA. (C) The benzyl alcohol fragment of NLys-NPip-NBal is predicted to bind in a shallow surface pocket defined by mostly hydrophobic contacts, while the hydroxyl group has the potential to interact with PCNA residues Ala252, Pro253 or Lys254 through hydrogen bonding or water bridging.

XV ROC AUC	Best Split	TP/FN FP/TN	# in Category
0.876	-0.188	36/4 249/769	40

5-Fold Cross-Validation Result									
Model Name	ROC Score	ROC Rating	True Positive	False Negative	False Positive	True Negative	Sensitivity	Specificity	Concordance
matt_bayesian	0.806	Good	38	2	344	674	0.950	0.662	0.673



Category %	1%	5%	10%	25%	50%	75%	90%	95%	99%
3.781%	5%	35%	52.5%	85%	95%	100%	100%	100%	100%

Figure S14. Enrichment plot for Bayesian model prediction of the 2P2I Hunter compound set. A leave-one-out cross-validation of the Bayesian model for the 1058-ligand 2P2I Hunter set was performed, an enrichment plot was generated, and the percentages of true category members captured at particular cutoff percentages were listed. From this, a best split value was calculated as -0.188 for the Bayesian score.

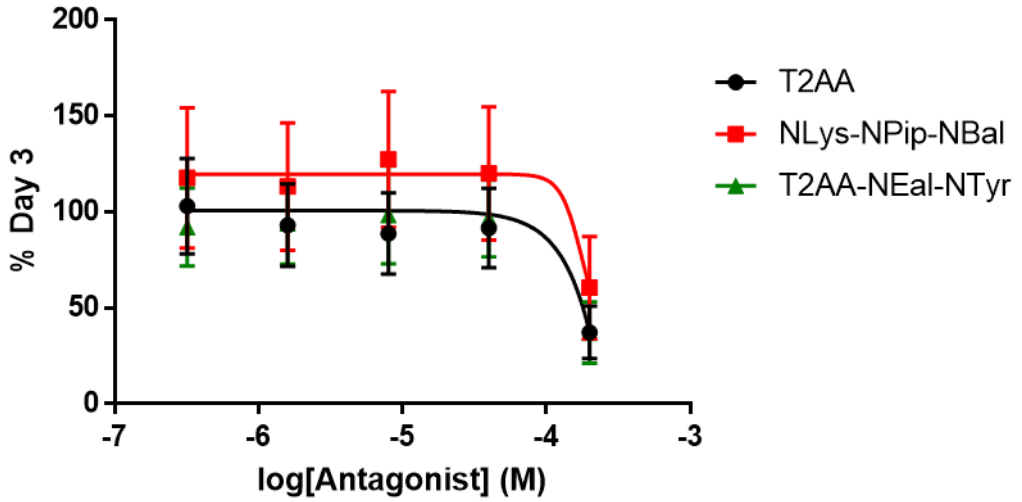


Figure S15. PCNA Antagonist Effects on Cell Growth and Viability: T2AA, T2AA-NEal-NTyr, and NLys-NPip-NBal effects on MDA-MB-231, breast tumor cell line. Their effects on cell viability and growth showed that these agents were non-toxic at concentrations up to 50 μM.

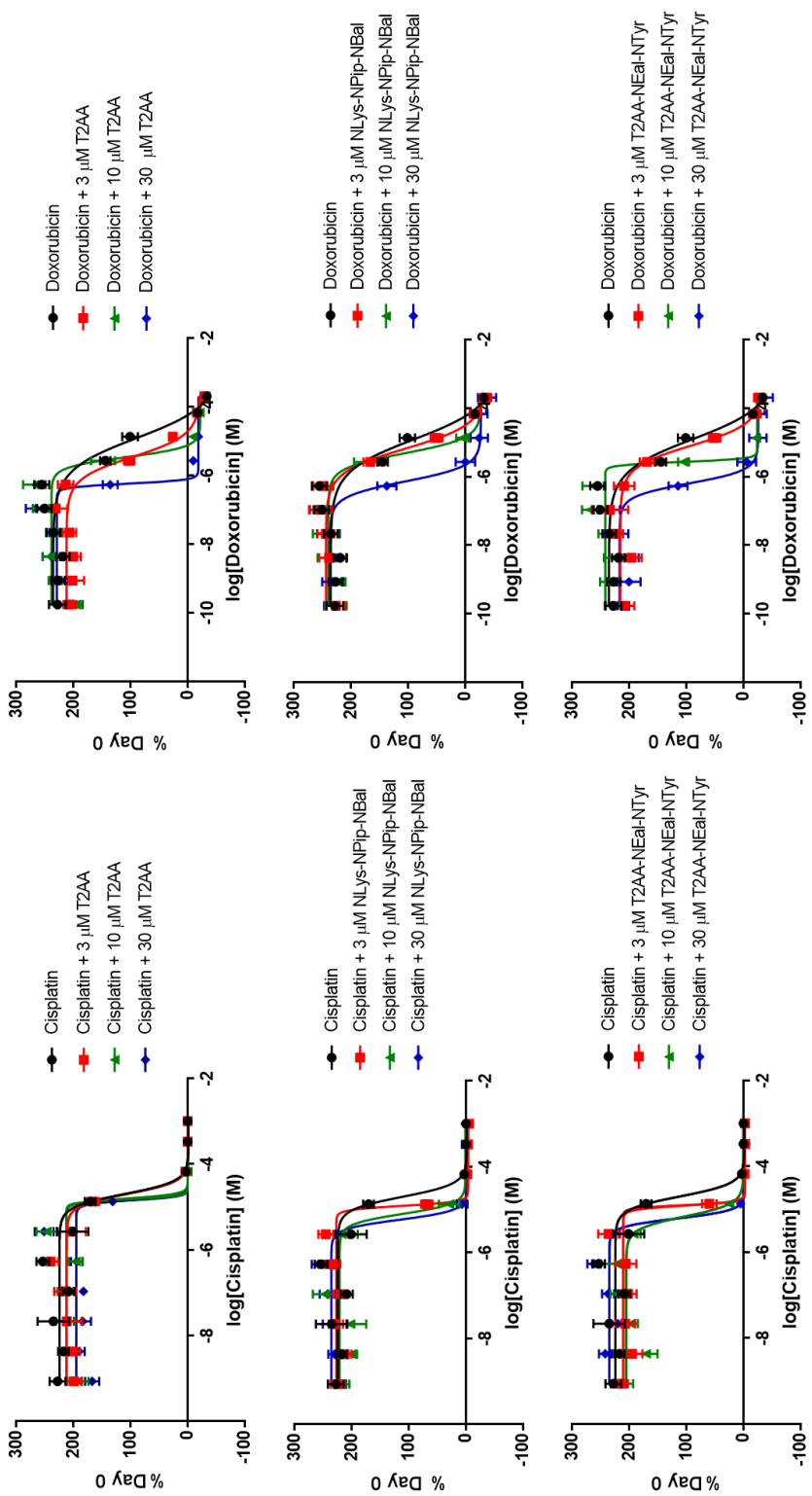


Figure S16. Cisplatin and Doxorubicin Combination in A549: T2AA, T2AA-NEal-NTyr, and NLys-NPip-NBal were used in combination with cisplatin, a single strand DNA break promoting agent, and doxorubicin, a double-strand DNA break promoting agent. The dosing of the second agents were 3 μM, 10 μM, and 30 μM in A549, lung tumor cell line.

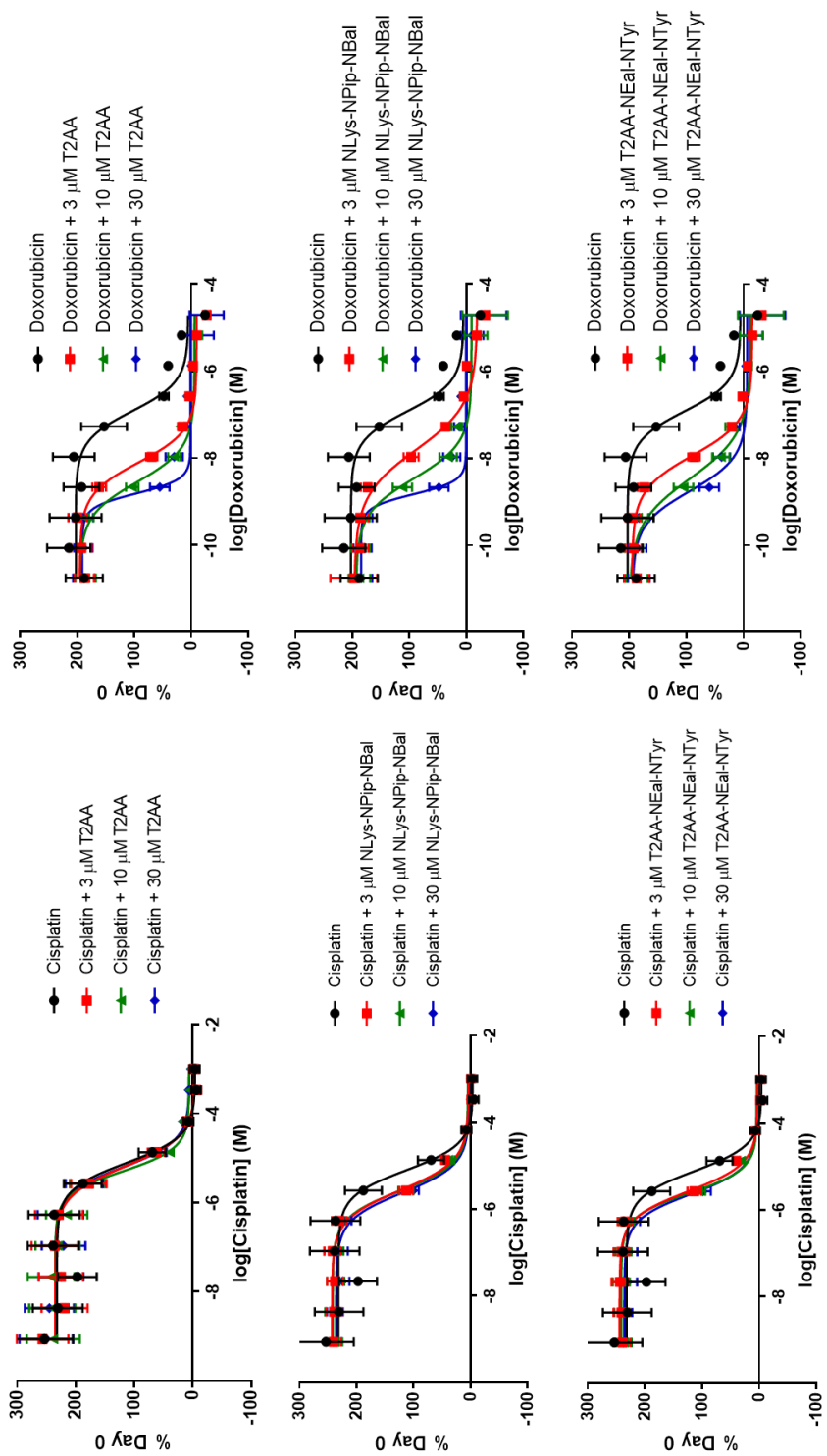


Figure S17 Cisplatin and Doxorubicin Combination in MDA-MB-231: T2AA, T2AA-NEal-NTyr, and NLys-NPip-NBal were used in combination with cisplatin, a single strand DNA break promoting agent, and doxorubicin, a double-strand DNA break promoting agent. The dosing of the second agents were 3 μM, 10 μM, and 30 μM in breast tumor cell line.

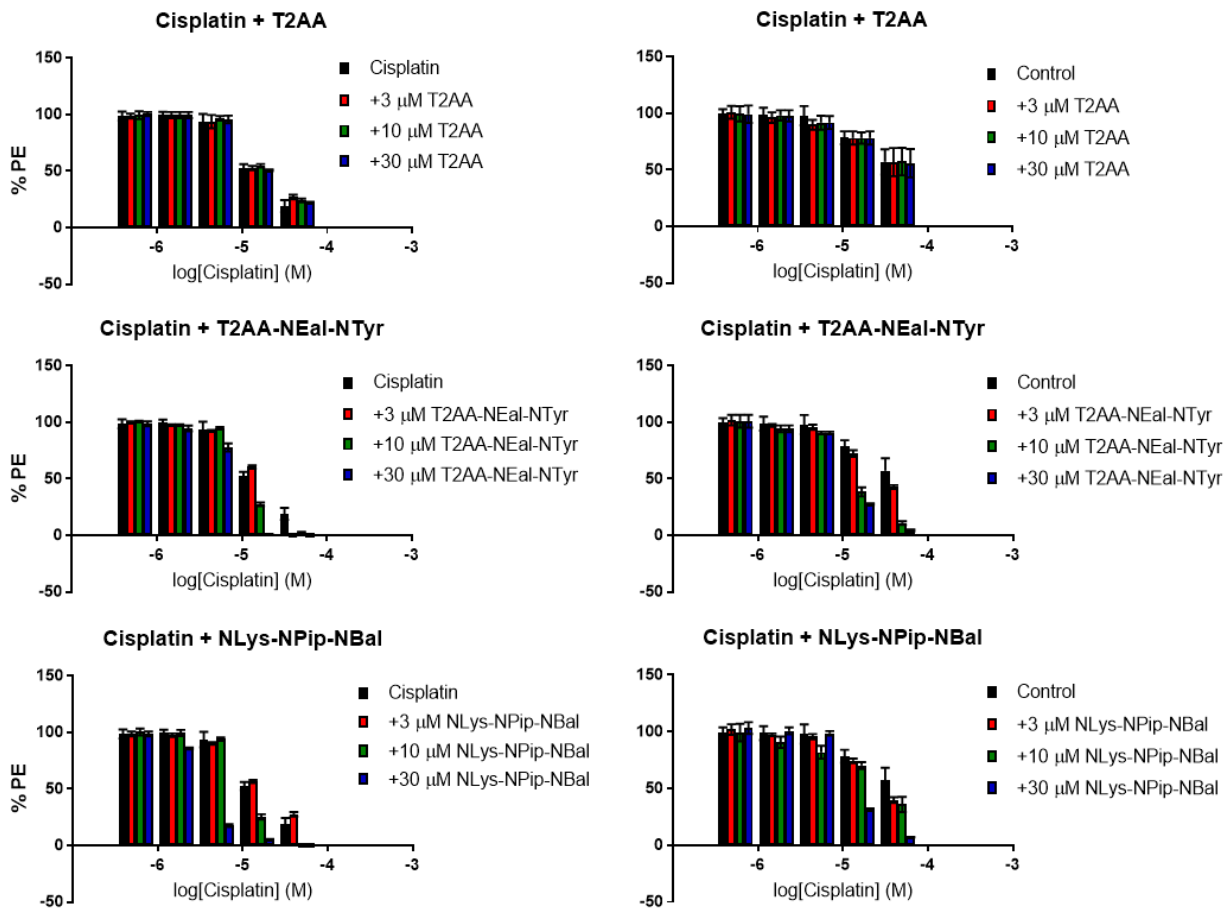


Figure S18: PCNA inhibitor synergism with cisplatin in A549 (left) and MDA-MB-231 (right). T2AA synergized with doxorubicin, but not cisplatin. Both T2AA-NEal-NTyr and NLys-NPip-NBal were able to synergize with both doxorubicin and cisplatin. Cell counts were normalized to the plating efficiency value as 100%.

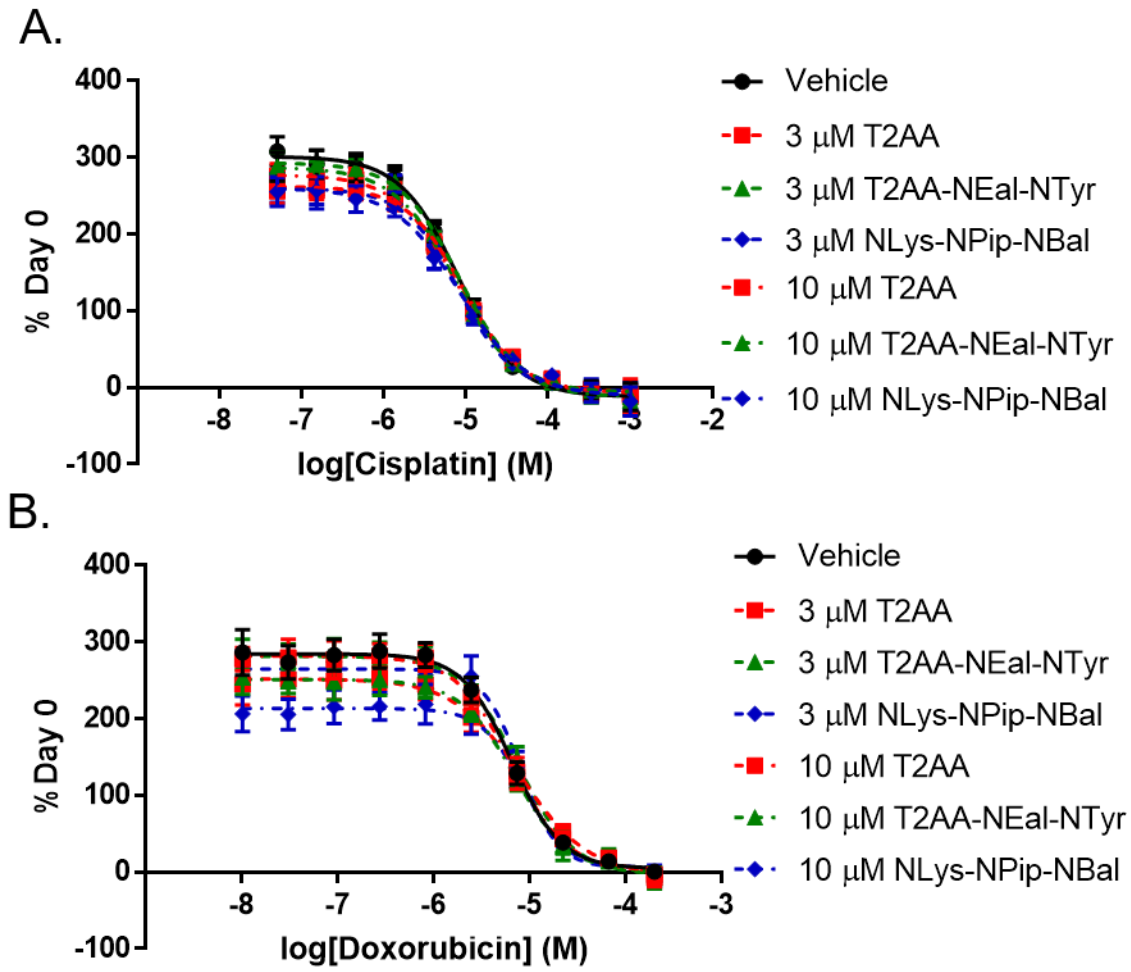


Figure S19: PCNA Inhibitors with DNA damage agents in HEK293 cells. HEK293 cells were dosed with (A) cisplatin or (B) doxorubicin and the concentration of a PCNA antagonist noted in the legend. All GI_{50} values of the combinations are consistent with the DNA damage agent alone showing no amount of synergy.

Table S1

Characterization of Screened Tripeptoids

Peptoid Sequence [a]	Calculated Mass $[M+H]^{1+}$	Observed Mass [b] (m/z)	% Yield [c]
Gly-NPip-NBal	443.1931	443.5391	7.19%
NAem-NArg-NPip	535.2948	535.2581	47.49%
NAem-NArg-NTyr	507.2999	507.3044	22.44%
NAem-NEal-NBal	466.2621	466.2658	15.51%
NAem-NLys-NPip	507.2887	507.3019	32.08%
NAem-NLys-NTyr	479.2938	479.2624	33.93%
NAem-NPip-NBal	556.2772	556.2767	22.40%
NAem-NPip-NBza	570.2520	570.2553	34.71%
NAem-NPip-NPip	570.2520	570.3911	73.95%
NAem-NPip-NTyr	542.2570	542.2764	50.28%
NAem-NTyr-NPip	542.2615	542.3094	40.60%
NAem-NTyr-NTyr	514.2621	514.3411	7.16%
NArg-NArg-NPip	521.2904	521.4318	32.30%
NArg-NIle-NBal	464.2986	464.2994	9.40%
NArg-NLys-NBza	493.2843	493.2887	32.32%
NArg-NLys-NPip	493.2843	493.5698	34.09%
NArg-NPip-NBal	542.2683	542.2703	29.86%
NArg-NPip-NBza	556.2475	556.1994	13.39%
NArg-NPip-NPip	556.2475	556.2516	8.29%
NArg-NPip-NTyr	528.2526	528.2560	3.31%
NArg-NTyr-NBal	514.2778	514.2761	10.11%
NArg-NTyr-NTyr	500.2577	500.2616	13.73%
NBal-NArg-NBal	528.2890	528.2910	12.43%
NBal-NEal-NBza	487.2193	487.2186	29.94%
NBal-NLys-NPip	514.2621	514.2656	29.10%
NBal-NLys-NTyr	486.2672	486.3338	10.92%
NBal-NPip-NBal	563.2462	563.1897	5.32%
NBal-NPip-NBza	577.2254	577.2296	19.10%
NBal-NPip-NTyr	549.2305	549.2338	46.74%

NBal-NTyr-NBal	535.2512	535.2536	12.01%
NBal-NTyr-NPip	549.2350	549.2787	26.26%
NBza-NArg-NBal	542.2683	542.2712	20.25%
NBza-NArg-NPip	556.2475	556.2506	49.91%
NBza-NLys-NBza	528.2414	528.2452	23.80%
NBza-NLys-NPip	528.2414	528.2443	49.19%
NBza-NLys-NTyr	500.2465	500.2509	27.46%
NBza-NPip-NBal	577.2254	577.2283	14.47%
NBza-NPip-NBza	591.2047	591.2075	14.18%
NBza-NTyr-NBal	549.2305	549.2157	10.93%
NBza-NTyr-NPip	563.2098	563.4002	17.49%
NEal-NEal-NBza	411.1836	411.3020	10.34%
NEal-NEal-NPip	411.1836	411.1878	22.09%
NEal-NEal-NTrp	420.2203	420.2237	28.15%
NEal-NLys-NPip	438.2308	438.2353	33.02%
NEal-NPip-NBza	501.1941	501.1979	1.71%
NEal-NPip-NPip	501.1941	501.1972	37.39%
NEal-NPip-NTrp	510.2353	510.2364	11.03%
NLys-NArg-NPip	493.2843	493.4769	29.45%
NLys-NBal-NPip	514.2621	514.2684	35.56%
NLys-NEal-NBza	438.2308	438.2353	7.50%
NLys-NEal-NPip	438.2308	438.2351	34.86%
NLys-NIle-NBal	437.2958	437.4813	0.82%
NLys-NLys-NPip	465.2781	465.5013	28.13%
NLys-NPip-NBal	514.2621	514.2656	8.62%
NLys-NPip-NBza	528.2414	528.3454	36.49%
NLys-NPip-NMma	513.2826	513.6709	6.75%
NLys-NPip-NPip	528.2414	528.3143	27.24%
NLys-NTyr-NBal	486.2672	486.2708	20.16%
NLys-NTyr-NBza	500.2465	500.2403	21.69%
NLys-NTyr-NPip	500.2465	500.2713	10.44%
NMma-NPip-NBal	562.2666	562.6433	23.68%
NPip-NPip-NPip	591.2047	591.8057	3.97%
NTyr-NArg-NBal	514.2734	514.3009	11.59%

NTyr-NArg-NPip	528.2526	528.3313	3.44%
NTyr-NEal-NBal	459.2199	459.2245	16.40%
NTyr-NIle-NBal	471.2563	471.3852	12.99%
NTyr-NLys-NPip	500.2509	500.2503	47.78%
NTyr-NLys-NTyr	472.2516	472.1906	4.57%
NTyr-NPip-NBal	549.2350	549.6621	0.75%
NTyr-NPip-NBza	563.2098	563.2132	27.46%
NTyr-NPip-NPip	563.2098	563.2128	23.04%
NTyr-NPip-NTyr	535.2193	535.2186	45.90%
NTyr-NTyr-NPip	535.2149	535.2551	23.10%
T2AA-Asn	682.9864	682.9854	5.78%
T2AA-Gln	697.0021	697.0003	8.15%
T2AA-Gly	625.9649	625.9629	0.81%
T2AA-Gly-NBal	803.0439	803.0422	9.83%
T2AA-Gly-NPip	817.0232	817.0266	4.51%
T2AA-NEal-Gly	727.0126	727.0109	3.81%
T2AA-NEal-NBal	847.0701	847.0695	1.67%
T2AA-NEal-NMma	846.0861	846.8161	13.13%
T2AA-NEal-NPip	861.0450	861.0513	5.95%
T2AA-NEal-NTyr	833.0545	833.0562	15.00%
T2AA-NPip-NLys	888.0967	888.0983	9.41%
T2AA-NPip-NPip	951.0560	951.0586	4.95%

[a] N-substituted side chains listed from N-terminus to most C-terminal

[b] as determined by high resolution electrospray ionization or MALDI-TOF

[c] based on dry mass recovery after HPLC purification

Table S2
Peptoid Descriptor Statistics from Bayesian Model

Statistic	Globularity	CW2	EDmin3	IW4
Mean	0.123096	2.19173	-2.62519	1.99723
Median	0.11273	2.204385	-2.59537	1.91726
Minimum	0.027005	1.58044	-4.37756	0.059068
Maximum	0.654219	2.76792	-1.89029	6.11639
Skew	1.691743	-0.33117	-0.6282	0.488549

Table S3
iPPI Descriptor Statistics from Bayesian Model

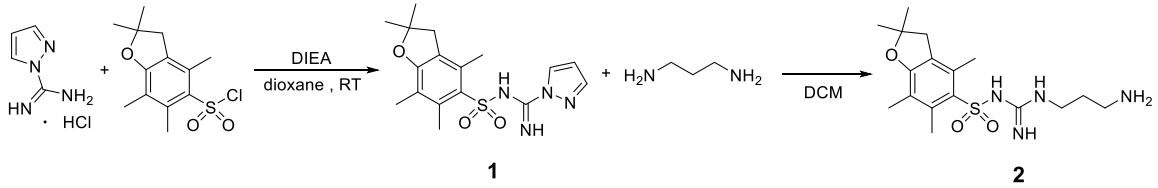
Statistic	Globularity	CW2	EDmin3	IW4
Mean	0.11331	1.953635	-2.84006	2.699039
Median	0.079983	1.92631	-2.81911	2.54485
Minimum	0.013987	1.54225	-3.30688	0.525021
Maximum	0.45662	2.37198	-2.4452	5.5549
Skew	1.613415	0.248895	-0.48162	0.282107

Table S4
Non-iPPI Descriptor Statistics from Bayesian Model

Statistic	Globularity	CW2	EDmin3	IW4
Mean	0.055457	2.082103	-2.48144	2.579951
Median	0.037349	2.05015	-2.45079	2.440295
Minimum	0	1.26745	-3.51407	0
Maximum	0.356383	3.04432	-1.70217	8.53636
Skew	1.820263	0.377792	-0.55035	0.525307

Methods

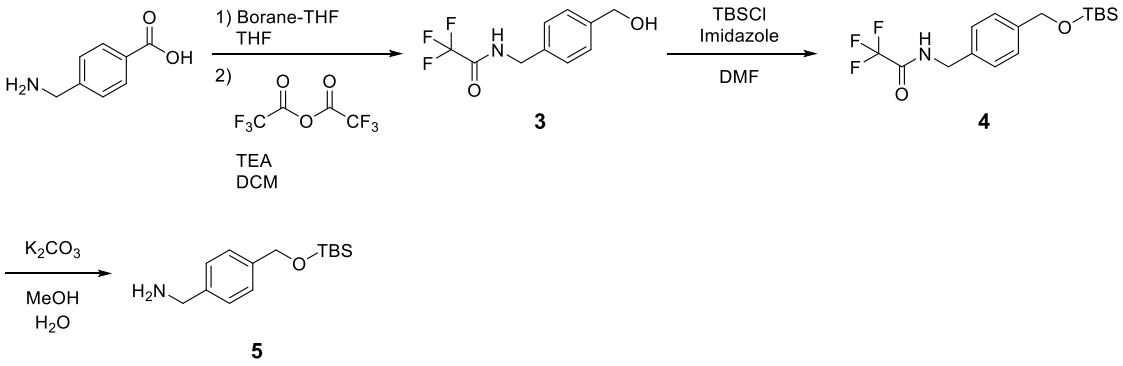
Synthesis of Non-Commercially Available Primary Amines and T2AA



Scheme 1
Synthesis of **NArg**

*Synthesis of N-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-1H-pyrazole-1-carboximidamide (**1**).* 16.16 g (55.96 mmol) of 2,2,5,7,8-pentamethylchroman-6-sulfonyl chloride (Combi-Blocks, Inc., San Diego, CA) was dissolved in dioxane (200 mL), and 9.07 g (61.88 mmol) of 1H-pyrazole-1-carboxamidine HCl dissolved in dioxane (200 mL) was added to the solution followed by 22 mL (2 eq.) of DIEA. The reaction mixture was stirred at room temperature for 48 hours, at which time all of the 1H-pyrazole-1-carboxamidine HCl had been consumed as confirmed via TLC. The dioxane was evaporated in vacuo and the remaining brown oil was redissolved into 200 mL DCM. The organic layer was washed with water (3 x 200 mL), dried over sodium sulfate and evaporated to give a brown solid. The crude material was recrystallized three times from ethanol to give 13.17 g (64.9% yield) of **1** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.67 (dd, *J* = 1.6, 0.7 Hz, 1H), 6.40 (dd, *J* = 2.9, 1.6 Hz, 1H), 2.98 (s, 2H), 2.62 (s, 3H), 2.56 (s, 3H), 2.11 (s, 3H), 1.47 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 12.34, 17.86, 19.16, 28.48, 42.99, 86.67, 109.47, 117.74, 124.83, 128.92, 130.88, 132.98, 139.13, 143.44, 148.66, 159.41. HRMS (ESI): calculated mass ($C_{17}H_{23}N_4O_3S$) $[M+H]^{1+}$: 363.1491, mass found *m/z*: 363.1510 $[M+H]^{1+}$.

*Synthesis of N-(N-(3-aminopropyl)carbamimidoyl)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide (**2** ; "**NArg**").* 5.88 g (17.5 mmol) of **1** was dissolved into DCM (100 mL) and was added dropwise to 6.75 g (91.1 mmol; 5.2 equiv.) of 1,3-diaminopropane dispersed in DCM (100 mL) at room temperature while stirring. The reaction was allowed to stir for 24 hours, at which time TLC showed that all of **1** had been consumed. The reaction mixture was washed with water (3 x 100 mL) and brine (100 mL), the organic layer was dried over sodium sulfate, and the organics were evaporated in vacuo to give an off-white solid. The product was recrystallized using ethyl acetate/hexanes to give 3.57 g (59.7% yield) of **2** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.30 (t, *J* = 6.09 Hz, 2H), 3.25 (q, *J* = 6.20 Hz, 1H, NH), 3.20 (q, *J* = 6.65 Hz, 1H, NH), 2.93 (s, 2H), 2.88 (t, *J* = 7.63 Hz, 2H), 2.54 (s, 3H), 2.47 (s, 3H), 2.07 (s, 3H), 1.82 – 1.70 (m, 2H), 1.66 (t, *J* = 6.66 Hz, 1H, NH), 1.45 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.71, 138.15, 132.32, 132.12, 124.62, 117.50, 109.22, 86.35, 43.10, 39.78, 37.98, 28.50, 19.23, 17.85, 12.39. HRMS (ESI): calculated mass ($C_{17}H_{29}N_4O_3S$) $[M+H]^{1+}$: 369.1961, mass found *m/z*: 369.1980 $[M+H]^{1+}$.



Scheme 2
Synthesis of **NBal**

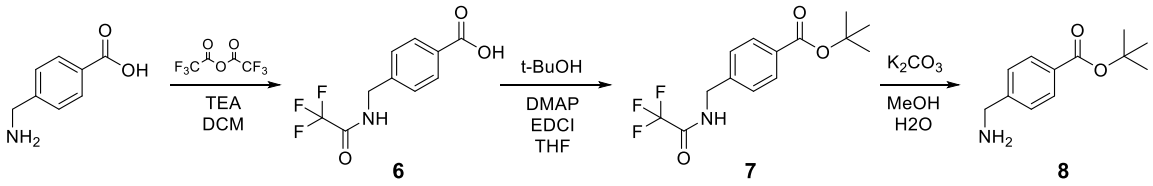
*Synthesis of 2,2,2-trifluoro-N-(4-(hydroxymethyl)benzyl)acetamide (**3**).* 20.0 g (132 mmol) of 4-(aminomethyl)benzoic acid was dispersed in 100 mL of anhydrous THF, and 350 mL (2.65 equiv.) of 1 M borane-THF in THF was added dropwise. The reaction mixture was heated to reflux for eight hours, and then allowed to cool to room temperature. 100 mL of MeOH was added to quench the remaining borane-THF, and the reaction was stirred for an additional 15 minutes. The reaction solution was then filtered over celite and evaporated in vacuo to give a light yellow solid. 250 mL of DCM was then added to the reaction flask containing the crude intermediate product, followed by 37 mL (2 equiv.) of triethylamine. The reaction was cooled to 0°C, 21 mL (1.1 equiv.) of trifluoroacetic anhydride was added dropwise and the reaction was allowed to stir overnight, allowing it to gradually reach room temperature. After 22 hours of reaction time, all of the starting material had been consumed as evidenced by TLC. 250 mL of water was added to the reaction and the organic layer was separated. The aqueous layer was extracted once more with 250 mL of DCM, the combined organics were washed with water (100 mL), and were dried over sodium sulfate, filtered and evaporated in vacuo to give a thick yellow oil. This oil was purified via automated flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp., Yodogawa-Ku Osaka, Japan) using DCM/MeOH as the eluents. The fractions containing the desired product were combined and

evaporated to give 15.87 g (51.44% yield for both steps) of **3** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.17 Hz, 2H), 7.26 (d, J = 8.16 Hz, 2H), 6.86 (s, br, 1H), 4.66 (s, 2H), 4.49 (d, J = 5.86 Hz, 2H), 2.02 (s, 1H).

*Synthesis of N-(4-(((tert-butyldimethylsilyl)oxy)methyl)benzyl)-2,2,2-trifluoroacetamide (**4**).* 15.68 g (67.24 mmol) of **3** was dissolved in DMF (200 mL), followed by the addition of 9.17 g (2 equiv.) of imidazole and 11.29 g (1.11 equiv.) of tert-butyl dimethylchlorosilane. The reaction was allowed to stir overnight at room temperature. After 20 hours of reaction time, the reaction mixture was evaporated in vacuo to half of its original volume. 100 mL of water was added, and the solution was extracted with ethyl acetate (2 x 200 mL), the combined organics washed with water (200 mL) and brine (100 mL), dried over sodium sulfate, filtered and evaporated to give clear, yellow oil. This oil was purified via automated flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp.) using ethyl acetate/hexanes as the eluents. The fractions containing the desired product were combined and evaporated to give 20.9 g (89.5% yield) of **4** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.26 Hz, 2H), 7.25 (d, J = 8.17 Hz, 2H), 6.83 (s, br, 1H), 4.74 (s, 2H), 4.49 (d, J = 5.80 Hz, 2H), 0.95 (s, 9H), 0.11 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.52, 141.83, 134.51, 128.03, 126.72, 117.90, 64.65, 43.81, 26.04, 18.53, -5.17. HRMS (ESI): calculated mass ($C_{16}H_{25}F_3NO_2Si$) $[M+H]^{1+}$: 348.1607, mass found *m/z*: 348.1597 $[M+H]^{1+}$.

*Synthesis of (4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanamine (**5**; "**NBal**").* 20.8 g (59.9 mmol) of **4** was dissolved in methanol (100 mL), followed by the addition of a 2 M aqueous solution of potassium carbonate (27.1 g in 100 mL water). The reaction was heated to reflux for seven hours, and then allowed to cool to room temperature. Methanol was evaporated from the

reaction mixture in vacuo, the remaining aqueous solution was transferred to a separatory funnel, and was extracted with DCM (2 x 400 mL). The combined organics were washed with water (100 mL), dried over sodium sulfate, filtered and evaporated in vacuo to give a yellow oil. The crude product was purified via automated flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp.) with an increasing gradient of DCM/MeOH w/ 1% TEA. The fractions containing the desired material were combined and evaporated to give 12.35 g (82.0% yield) of **5** as a clear oil, which solidified upon storage at -20°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.58 Hz, 2H), 7.25 (d, J = 8.14 Hz, 2H), 4.72 (s, 2H), 4.43 (s, br, 1H), 3.81 (s, 2H), 0.95 (s, 9H), 0.10 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.85, 139.83, 126.87, 126.18, 64.70, 46.16, 29.35, 25.90, -5.29. HRMS (ESI): calculated mass ($C_{17}H_{27}NOSi$) $[M+H]^{1+}$: 252.1784, mass found *m/z*: 252.1785 $[M+H]^{1+}$.



Scheme 3
Synthesis of **NBza**

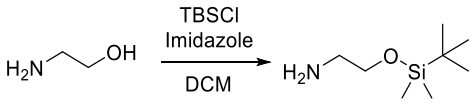
*Synthesis of 4-((2,2,2-trifluoroacetamido)methyl)benzoic acid (**6**).* 21.16 g (140 mmol) of 4-(aminomethyl) benzoic acid was suspended in 450 mL of dichloromethane, followed by 42.0 mL (301 mmol) of triethylamine. The reaction was then cooled in an ice bath, and 60.44 g (2.06 eq.) of trifluoroacetic anhydride in 50 mL of dichloromethane was added dropwise over the course of 1 hour. The reaction was stirred for an additional three hours while being allowed to gradually warm to room temperature. 500 mL of aqueous saturated sodium bicarbonate solution was then slowly added to the reaction mixture in portions, and the solution was acidified with 4 N HCl (pH

< 3). The resultant precipitate was collected via filtration, and the filter cake was washed three times with water and twice with ice-cold ether. The solid was dissolved in ethyl acetate, dried over sodium sulfate, filtered, transferred to a round bottom flask and evaporated to give an off-white solid. The product was recrystallized from ethyl acetate/hexanes three times to give 24.63 g (71.19% yield) of **6** as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 10.15 (t, J = 5.91 Hz, 1H), 8.13 (d, J = 8.36 Hz, 2H), 7.51 (d, J = 8.38 Hz, 2H), 4.52 (d, J = 5.97 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) δ 167.97, 162.96, 145.92, 131.66, 130.49, 128.91, 127.95, 43.27.

*Synthesis of tert-butyl 4-((2,2,2-trifluoroacetamido)methyl)benzoate (**7**).* 12.55 g (50.77 mmol) of **6** was dissolved in anhydrous THF (150 mL), and 135 mL of t-butanol and 6.20 g (50.75 mmol) DMAP were added. The reaction was cooled to 0°C under argon, and 29.2 g (152 mmol) of EDCI was added followed by an additional 50 mL of anhydrous THF to wash down the insides of the reaction flask. The reaction was sealed and stirred under argon overnight, allowing it to gradually reach room temperature. After 16 hours of reaction time, 200 mL of water was added to the reaction and the organics were evaporated in vacuo. The aqeuous solution was extracted with DCM (2 x 200 mL), and the subsequent combined organics were washed with 5% HCl (2 x 200 mL), 200 mL of water and 200 mL of brine. The organic layer was dried over sodium sulfate and evaporated to give a yellow oil. The product was purified using normal phase flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp.) with an increasing gradient of ethyl acetate/hexanes (10:90 to 100:0 over 60 minutes). The fractions containing the desired product were combined and evaporated to give 10.78 g (70.0% yield) of **7** as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 1.53 (s, 9H), 4.46 (d, J = 5.94 Hz, 2H), 7.39 (dt, J = 1.78, 8.33 Hz, 2H), 7.89 (dt, J = 1.83, 8.33 Hz, 2H), 10.09 (t, J = 6.11 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 27.75, 42.36,

80.63, 116.22, 127.40, 129.31, 130.45, 142.54, 156.56, 164.70. HRMS (ESI): calculated mass ($C_{14}H_{17}F_3NO_3$) $[M+H]^{1+}$: 304.1161, mass found *m/z*: 304.1170 $[M+H]^{1+}$.

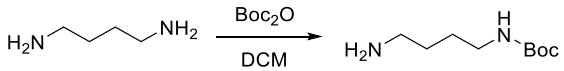
*Synthesis of tert-butyl 4-(aminomethyl)benzoate (**8**; "**NBza**").* 10.75 g (35.5 mmol) of **7** was dissolved in methanol (45 mL), and 12.25 g (2.5 eq.) of potassium carbonate dissolved in water (45 mL) was added in one portion. The reaction was sealed and stirred overnight at room temperature. After 18 hours of reaction time, methanol was evaporated from the reaction mixture and the remaining aqueous solution was adjusted to pH > 10 with 4 N NaOH. The aqueous layer was extracted with DCM (3 x 200 mL), the combined organics washed with water (50 mL) and brine (50 mL), and the organic layer dried over sodium sulfate. The organics were evaporated to give 7.10 g (96.7% yield) of **8** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.30 Hz, 2H), 7.31 (d, *J* = 8.32 Hz, 2H), 3.86 (s, 2H), 1.69 (s, 1H), 1.55 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.57, 147.58, 130.47, 129.60, 126.71, 80.76, 46.02, 28.12. HRMS (ESI): calculated mass ($C_{12}H_{18}NO_2$) $[M+H]^{1+}$: 208.1338, mass found *m/z*: 208.1334 $[M+H]^{1+}$.



9

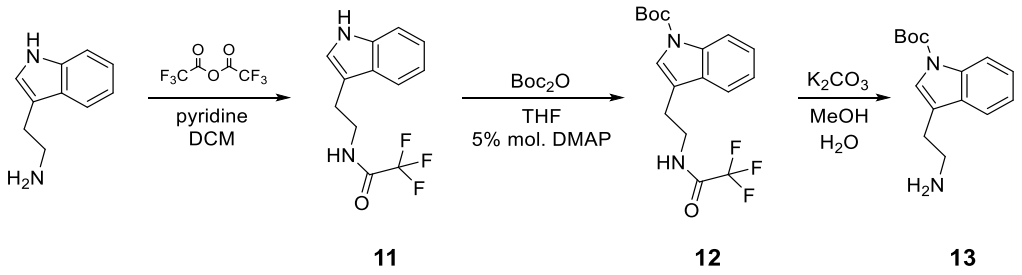
*Synthesis of 2-((tert-butyldimethylsilyl)oxy)ethan-1-amine (**9**; "**NEal**").* 6.11 g (100 mmol) of ethanolamine and 13.62 g (2 equiv.) of imidazole were dissolved in DCM (100 mL) in a 500 mL round bottom flask. 15.83 g (105 mmol) of tert-butyldimethylchlorosilane dissolved in DCM (50 mL) was added dropwise over the course of 20 minutes, and the reaction mixture was stirred for one hour at room temperature. At that time, all of the starting material had been consumed as

confirmed by TLC; 100 mL of water was added and the layers were separated. The aqueous layer was extracted twice with DCM (2 x 100 mL), and the combined organics washed with water (50 mL), dried over sodium sulfate and evaporated to give 13.68 g (77.9% yield) of **9** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (t, *J* = 5.36 Hz, 2H), 2.71 (t, *J* = 5.25 Hz, 2H), 2.08 (s, 2H, NH_2), 0.84 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.13, 44.27, 25.96, 18.35, -5.27. HRMS (ESI) calculated mass ($C_9H_{22}NOSi$) $[M+H]^{1+}$: 188.1471, mass found *m/z*: 188.1495 $[M+H]^{1+}$.



10

*Synthesis of tert-butyl (4-aminobutyl)carbamate (**10**; "**NLys**").* 50.02 g (567.4 mmol) of 1,4-diaminobutane was dissolved in chloroform (600 mL) and was cooled to 0°C. 13.17 g (6.03 mmol) of di-tert-butyl dicarbonate dissolved in chloroform (300 mL) was added drop-wise over the course of two hours and the reaction was stirred overnight, allowing it to reach room temperature. After 21 hours of reaction time, the entire reaction mixture was transferred to a separatory funnel and was washed with water (8 x 200 mL), dried over sodium sulfate and evaporated in vacuo to give 10.71 g (94.3% yield) of **10** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.84 (s, 1H), 3.04 (t, 2H), 2.64 (t, *J* = 6.7 Hz, 2H), 1.46 – 1.38 (m, 4H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.93, 78.82, 41.70, 40.30, 30.77, 28.32, 27.37. HRMS (ESI) calculated mass ($C_9H_{21}N_2O_2$) $[M+H]^{1+}$: 189.1603, mass found *m/z*: 189.1601 $[M+H]^{1+}$.



Scheme 4
Synthesis of **NTrp**

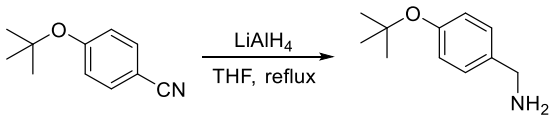
*Synthesis of N-(2-(1H-indol-3-yl)ethyl)-2,2,2-trifluoroacetamide (**11**).* 19.99 g (124.8 mmol) of tryptamine (AK Scientific, Inc., Union City, CA) was dissolved in DCM (300 mL) followed by the addition of 11.1 mL (1.1 equiv.) of pyridine. This solution was cooled to 0°C and 19.4 mL (1.1 equiv.) of trifluoroacetic anhydride was added dropwise. After 22 hours of reaction time, all of the starting material had been consumed, as evidenced by TLC; the reaction mixture was washed with 2 N HCl (3 x 250 mL), water (100 mL) and brine (100 mL), dried over sodium sulfate and evaporated to give a brown solid. This solid was dissolved in a mixture of acetone and DCM and was absorbed onto silica gel. This was dry loaded into an empty flash column, and the product was purified via normal phase flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp.) using an increasing solvent gradient of ethyl acetate/hexanes (20:80 to 100:0 over 60 minutes). The fractions containing the desired product were combined and evaporated to give 24.1 g (75.3% yield) of **11** as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 10.86 (s, 1H, NH), 9.55 (t, *J* = 5.56 Hz, 1H), 7.53 (d, *J* = 7.72 Hz, 1H), 7.34 (dd, *J* = 1.05, 7.99 Hz, 1H), 7.16 (d, *J* = 2.35 Hz, 1H), 7.07 (td, *J* = 1.25, 7.55, 8.09 Hz, 1H), 6.98 (ddd, *J* = 1.13, 7.10, 7.90 Hz, 1H), 3.45 (q, *J* = 6.82 Hz, 2H).

*Synthesis of tert-butyl 3-(2-(2,2,2-trifluoroacetamido)ethyl)-1H-indole-1-carboxylate (**12**).* 24.0 g (93.7 mmol) of **11** was dissolved in THF (200 mL), followed by 30.76 g (1.5 equiv.) of di-tert-

butyl dicarbonate with an additional 50 mL of THF to wash down the sides of the flask. 0.59 g (0.052 equiv.) of DMAP was then added and the reaction was heated to 40°C for two hours. At that time, TLC showed that all of the starting material had been consumed, so 250 mL of DCM was added to the reaction and the organic solution was washed with water (2 x 100 mL), dried over sodium sulfate and evaporated in vacuo to give a viscous brown oil. The product was purified via normal phase flash chromatography (EPCLC W-Prep 2XY, Yamazen Corp.) using an increasing gradient of ethyl acetate/hexanes (10:90 to 100:0 over 100 minutes). The fractions containing the desired product were combined and evaporated in vacuo to give 17.27 g (51.7% yield) of **12** as a white solid. ^{1}H NMR (300 MHz, DMSO) δ 9.57 (t, *J* = 5.78 Hz, 1H), 8.05 (d, *J* = 8.20 Hz, 1H), 7.62 (d, *J* = 7.48 Hz, 1H), 7.50 (s, 1H), 7.33 (td, *J* = 1.39, 7.77, 8.28 Hz, 1H), 7.25 (td, *J* = 1.17, 7.44 Hz, 1H), 3.49 (q, *J* = 6.72 Hz, 2H), 2.91 (t, *J* = 6.98 Hz, 2H), 1.61 (s, 9H). ^{13}C NMR (75 MHz, DMSO) δ 156.51, 156.03, 149.02, 134.78, 130.13, 124.42, 123.18, 122.52, 119.07, 117.40, 114.74, 83.48, 39.07, 27.67, 23.53.

*Synthesis of tert-butyl 3-(2-aminoethyl)-1H-indole-1-carboxylate (**13**; "**NTrp**").* 17.20 g (48.27 mmol) of **12** was dissolved in methanol (60 mL) followed by the addition of 16.75 g (2.511 equiv.) of potassium carbonate dissolved in water (60 mL). The reaction flask was covered and the reaction was allowed to stir overnight at room temperature. After 16 hours of reaction time, little progress was seen with the reaction, so it was heated to reflux for seven hours. At that time, TLC showed that all starting material had been consumed. Methanol was evaporated in vacuo, and the remaining aqueous solution was adjusted to pH >10 with 4 N NaOH. The product was extracted with DCM (3 x 200 mL), and the combined organics were washed with water (100 mL) and brine (100 mL), dried over sodium sulfate, filtered and evaporated in vacuo to give 7.05 g (56.1% yield) of **13** as a

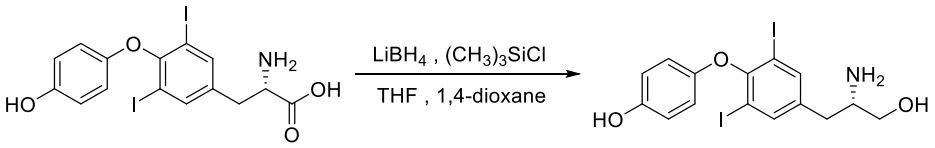
yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 – 7.46 (m, 1H), 7.46 – 7.36 (m, 1H), 7.34 – 7.17 (m, 3H), 3.54 (t, *J* = 7.74 Hz, 2H), 3.03 (t, *J* = 7.11 Hz, 2H), 1.66 (s, 9H), 1.64 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.81, 135.55, 130.74, 124.17, 123.05, 122.72, 122.26, 118.98, 115.15, 83.27, 51.28, 28.17, 26.50. HRMS (ESI) calculated mass ($C_{15}H_{21}N_2O_2$) $[M+H]^{1+}$: 261.1603, mass found *m/z*: 261.1598 $[M+H]^{1+}$.



14

*Synthesis of (4-(tert-butoxy)phenyl)methanamine (**14**; "**NTyr**").* 175 mL of 1M (175 mmol; 2.6 eq.) lithium aluminum hydride in THF was added to a round bottom flask with a stir bar and was cooled to 0°C. A solution of 11.83 g (67.5 mmol) of 4-(tert-butoxy)benzonitrile (Alfa Aesar, Ward Hill, MA) in 50 mL of anhydrous THF was added to the stirring solution dropwise over the course of 30 minutes. The reaction was then fitted with a reflux condenser and was heated to reflux for six hours, followed by stirring overnight under argon, allowing the reaction to cool to room temperature. After 22 hours of total reaction time, the reaction mixture was cooled to 0°C and was quenched with 7 mL of water, followed by 6 mL of 15% NaOH (aq) and an additional 17 mL of water. The resulting emulsion was filtered over celite, with the filter cake being washed with methanol (2 x 50 mL) and DCM (2 x 50 mL). The filtrate was evaporated, and the resulting dark yellow oil was dissolved into 75 mL of water. The solution was transferred to a separatory funnel and was extracted with ethyl acetate (4 x 150 mL). The combined extractions were washed with water (100 mL) and brine (100 mL), dried over sodium sulfate, and evaporated in vacuo to give a dark yellow oil. The oil was separated using basic alumina chromatography and a solvent system of ethyl acetate/hexanes (20:80 to 50:50). The fractions containing the product (as evidenced by

TLC) were combined and evaporated to give 5.71 g (47.2% yield) of **14** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (dd, *J* = 2.26, 6.46 Hz, 2H), 6.94 (dd, *J* = 2.32, 6.46 Hz, 2H), 4.38 (s, 2H), 1.31 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.80, 135.32, 128.26, 124.27, 78.17, 54.93, 28.75. LRMS (EI) calculated mass ($C_{11}H_{18}NO$) $[M+H]^{1+}$: 180.1388, mass found *m/z*: 180.1404 $[M+H]^{1+}$.



15

*Synthesis of (S)-4-(4-(2-amino-3-hydroxypropyl)-2,6-diiodophenoxy)phenol (**15**; "**T2AA**").* 7.55 mL of 1 M (15.1 mmol) lithium borohydride in THF was added to a 50 mL round bottom flask followed by 5.2 mL of anhydrous THF and 5 mL of anhydrous dioxane. The solution was cooled to 0°C under argon, and 3.85 mL (30.3 mmol) of chlorotrimethylsilane was slowly added. The resulting solution was stirred for 15 minutes at 0°C, and 0.90 g (1.71 mmol) of 3,5-diiodo-L-thyronine (Combi-Blocks, Inc.) was added in one portion with the aid of an additional 5.2 mL of anhydrous THF and 5 mL of anhydrous dioxane. The flask was sealed and the reaction was stirred overnight under argon while being allowed to slowly warm to room temperature. After 18 hours of reaction time, the reaction was poured into 25 mL of ice-water, adjusted to pH >9 with 4 N NaOH and was extracted with ethyl acetate (3 x 50 mL). The combined organic extractions were dried over sodium sulfate, filtered and evaporated to give a light brown solid. This solid was dissolved in a mixture of ACN/H_2O (75:25) and was purified on an Agilent ZORBAX SB-C18 reverse phase semi-preparative column on a System Gold 166 (Beckman Coulter) HPLC system,

using a gradient of ACN (0.1% TFA)/H_2O (0.1% TFA) 0:100 to 100:0 over 30 minutes with detection at 254 nm. The fractions containing the purified product were combined, frozen and lyophilized to give 460 mg (52.5% yield) of **15** as a fluffy white solid. ^{1}H NMR (300 MHz, DMSO) δ 9.12 (s, 1H), 7.84 (s, 5H), 6.68 (dt, *J* = 2.30, 3.84, 9.08 Hz, 2H), 6.54 (dt, *J* = 2.30, 3.61, 8.98 Hz, 2H), 5.39 (t, *J* = 4.49 Hz, 1H), 3.56 (dd, *J* = 4.86, 7.97 Hz, 1H), 3.40 (dq, *J* = 5.48, 9.94 Hz, 2H), 2.87 – 2.69 (m, 2H), 2.07 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 152.65, 152.26, 148.83, 140.67, 137.48, 115.86, 115.82, 92.48, 60.07, 53.27, 40.02, 39.85, 39.78, 39.69, 39.52, 39.35, 39.19, 39.02, 33.06. HRMS (ESI) calculated mass ($C_{15}H_{16}I_2NO_3$) $[M+H]^{1+}$: 511.9220, mass found *m/z*: 511.9678 $[M+H]^{1+}$.

Ligation Independent Cloning of N-terminal His-tag PCNA Construct. Procedure was adopted from Pedley, *et al.*[29] Ligation independent cloning compatible expression vector pEV-L8 containing an N-terminal His-tag and TEV protease recognition site was linearized by digestion with Ssp1 (New England Biolabs), purified by gel filtration, and treated with T4 DNA polymerase (Novagen) in the presence of dGTP (New England Biolabs) for 30 minutes at 22°C, followed by heat inactivation at 75°C for 20 minutes. The PCNA fragment was amplified by PCR from a template plasmid (Genecopeia) using a high-fidelity polymerase Platinum *Pfx* DNA polymerase (Invitrogen). The resulting PCR products were treated with T4 DNA polymerase in the presence of dCTP to generate 5' overhangs necessary for annealing. A total of 0.2 pmol of each insert was incubated with 0.01 pmol of pEV-L8 vector in 3 µL reaction mix at 22°C for 10 minutes followed by addition of 1 µL of 25 mM EDTA at 22°C for 5 minutes. Annealing reaction products were transformed into X10Gold competent cells (Strategene) and plated on LB agar containing 50 µg/mL kanamycin. Individual colonies were grown and the constructs were assessed by PCR for insert size and verified by sequencing before propagating the plasmid.

Expression, Induction and Purification of N-terminal His-tagged PCNA. Procedure was adapted and modified from Pedley, *et al.*[29] 10 µL aliquots of chemically competent BL21 (DE3) E. Coli cells (Agilent) were transformed via heat shock with 1 µL of purified plasmid encoding the fusion protein, N-terminal $(His)_6$-PCNA for 30 seconds at 42°C. The cells were then immediately placed on ice for 2 minutes, and 140 µL of SOC medium was added. Transformed cells were allowed to grow for 1 hour at 37°C before streaking on a LB agar plate containing 50 µg/mL kanamycin. Single isolated colonies were picked and grown at 37°C to an OD of 0.7-1.0 in the presence of 50 µg/mL kanamycin. Transformed cells were induced with 0.4 mM IPTG for 4 hours at 37°C. Transformed cells were pelleted at 4,000 x *g* for 20 minutes at 4°C, and stored at -80°C until lysis.

Two pellets of transformed BL21 (DE3) E. Coli cells were each resuspended in 20 ml of ice-cold lysis buffer (50 mM Tris HCl at pH 8.0, 0.15 mM NaCl), lysed by sonication at a 30% amp output for 3 minutes (20 second pulses), and centrifuged at 4,000 x *g* for 20 minutes at 4°C. Each supernatant was decanted and combined. Recombinant $(His)_6$-PCNA fusion protein was then purified from the soluble fraction by affinity column chromatography using Ni-NTA resin at 4°C. After charging the column resin with the entire soluble protein fraction, the column was washed with 20 mM imidazole in Tris buffer at pH 8.0 to remove nonspecific binding protein. $(His)_6$-PCNA was then eluted with 10 mL of 1M imidazole in Tris buffer at pH 8.0. The eluted protein was diluted two-fold with dialysis buffer (25 mM HEPES at pH 7.4, 10% glycerol, 0.01% Triton X-100), DTT and EDTA were added to a final concentration of 2 mM, and the entire solution was diluted two-fold with 2 M ammonium sulfate in 25 mM Tris buffer at pH 8.0 to give a final $(NH_4)_2SO_4$ concentration of 1 M. The solution was agitated for 1 hour at 4°C, and during that time

$(His)_6$-PCNA precipitated from solution. The precipitated protein was pelleted by centrifugation (5,000 x *g* for 10 minutes), the supernatant decanted, and the protein pellet dissolved into 10 mL of dialysis buffer. The protein concentration was immediately assessed via measuring its absorbance at 280 nm (using an extinction coefficient of 16,000 $M^{-1}cm^{-1}$), and the protein solution was diluted as necessary to give a stock concentration of 4 µM. This was then dialyzed for 24 hours, swapping the dialysis buffer twice with fresh 25 mM HEPES at pH 7.4, 10% glycerol, 0.01% Triton X-100. After dialysis, the protein concentration was re-confirmed by measurement of its absorbance at 280 nm.

Synthesis of Fluorescein-labeled POGO Ligase Peptide (FAM-PL). POGO Ligase peptide (sequence: SAVLQKKITDYFHPKK) was synthesized by GenScript USA Inc. (Piscataway, NJ) and provided uncleaved on Rink Amide MBHA resin. 0.10 mmol of this resin was transferred to a glass fritted peptide reaction vessel and swelled in DMF for 30 minutes, followed by washing with DCM (3x). Sufficient deprotection (using 20% piperidine in DMF) of the final amino acid residue (N-terminal serine) was confirmed by a ninhydrin (Kaiser's) test for primary amines. An aminohexanoic acid linker was added (1 mmol Fmoc-6-Ahx-OH, 2.1 ml of 0.45 M HCTU, 500 µL of 4 M DIEA in NMP; 2 hours at room temperature) to separate the fluorescent dye from the peptide sequence. Following subsequent washing of the resin (DMF (6x) and DCM (3x)) and Fmoc deprotection (20% piperidine in DMF; 30 minutes at room temperature), the resin was again washed (DMF (6x) and DCM (3x)) and then transferred to a glass scintillation vial wrapped in foil. A solution of 75.3 mg of 5-FAM, 80.7 mg of HCTU and 46 mg of DIEA in 2 mL of DMF was added, and the resin was then placed on an orbital shaker overnight at room temperature. After 20 hours of incubation time, the resin was washed with DMF (6x) and DCM (3x), dried over vacuum, transferred to a glass scintillation vial and cleaved from resin using a solution of

trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/water (95:2.5:2.5) for 3 hours at room temperature in the dark. The 5-FAM-labeled POGO ligase peptide (FAM-PL) was then precipitated into ice cold diethyl ether and collected by centrifugation at 4,000 x *g* for 10 minutes at 4°C. It was purified via HPLC (Beckman Coulter System Gold 166 or 168) using an increasing gradient of acetonitrile (ACN)/water with 0.1% TFA (5:95) to (100:0) over 30 minutes on an Agilent ZORBAX SB-C18 reverse phase semi-preparative column. The molecular mass and sequence were validated via MALDI-TOF/TOF and LCMS. Purity was determined by HPLC using absorbences at 219 and 280 nm. HRMS (LCMS): calculated mass ($C_{116}H_{164}N_{25}O_{29}$) $[M-H]^{1-}$: 2372.6980, mass found *m/z*: 2372.7221 $[M-H]^{1-}$.