

Adapting AlphaLISA high throughput screen to discover a novel small-molecule inhibitor targeting protein arginine methyltransferase 5 in pancreatic and colorectal cancers

Supplementary Materials

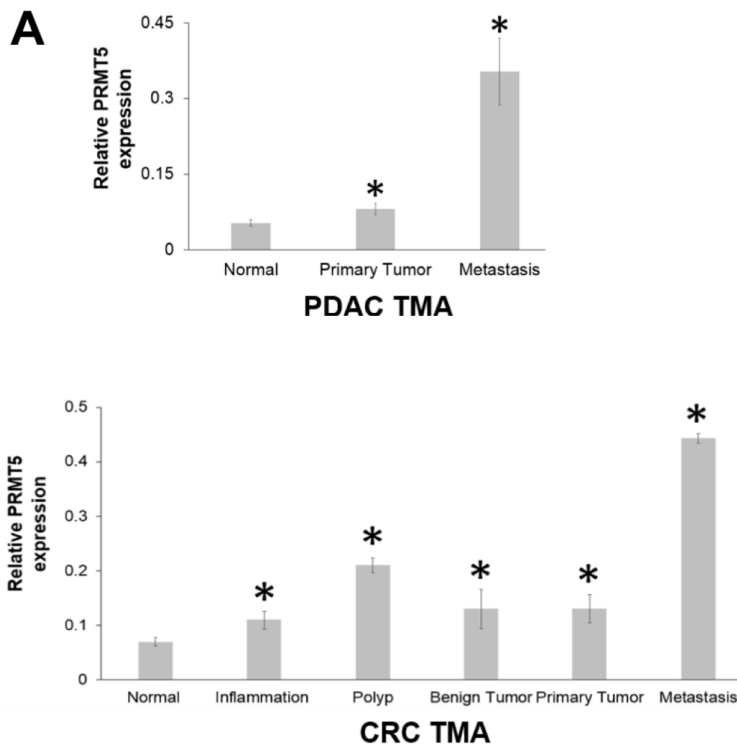
METHODS

Methyltransferase assay

The specificity of inhibition by PR5-LL-CM01 against the enzymatic activity of protein arginine methyltransferase family members was analyzed using the HotSpot radioisotope-based platform (Reaction Biology Corp) as described previously [35]. Briefly, PR5-LL-CM01 was incubated with a protein arginine methyltransferase, substrate, and tritium-labeled SAM. The PR5-LL-CM01 stock solution was prepared in DMSO at 10mM. PR5-LL-CM01 was tested at 1, 10, 25, 50 and 100 μ M. The methyltransferase inhibitor SAH (S-(5'-Adenosyl)-L-homocysteine) was used as a positive control. All reactions were carried out with 1 μ M tritium-labeled SAM and 5 μ M peptide substrate. The assays were performed using Reaction buffer (50mM Tris-HCl, pH 8.5, 5mM MgCl₂, 50mM NaCl, 0.01% Brij35, 1mM DTT). Reactions were performed for 1h at 30°C. Radiolabeled methylated product was detected using a filter-binding method. Curve fits and IC₅₀ determination were executed as described previously [35].

3D colony formation assay

500 cells per well were seeded in media containing 3% Reduced Growth Factor (RGF)-Matrigel (BD Biosciences) in a Corning® Costar® ultra-low attachment multiwell plate and allowed to form colonies. PR5-LL-CM01 stocks made in 100% DMSO were diluted using 3% RGF-Matrigel media and added to the respective wells, in a 2-fold serial dilution concentration range from 0.75 to 25 μ M on days 3 and 7 after seeding. On day 7 post first drug treatment, the cells were stained using Alamar blue dye (Fisher), and quantified using a Synergy H4 Multi-Mode Reader (BioTek Instruments Inc).



Supplementary Figure 1. Quantification for immunohistochemical analysis of the PDAC and CRC tumor microarray (TMA). A. Bar graph showing a significant increase in relative PRMT5 expression in primary stage tumor (n=19) and metastatic tumor patient tissue (n=6) vs. normal tissue (n=24) for the PDAC TMA. *p< 0.05, different samples vs. normal control. B. IHC quantification of CRC TMA show a similar increase in relative PRMT5 expression for the representative disease stages in the TMA, including inflammation (n=9), polyp (n=5), benign tumors (n=5), primary tumors (n=14) and metastatic tissue (n=20) vs. normal patient tissue (n=9). *p< 0.05, different samples vs. normal control.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	323582	402629	393541	381010	353979	369975	334891	358994	388971	369346	360397	320486	344740	343614	390927	390059	377026	361716	343854	333056	373423	383535	65808	59986
B	346832	373322	351102	318283	329922	340173	343065	334416	376670	339840	335268	359641	333788	343815	364203	323400	359823	315898	372138	318004	352947	375613	67424	59363
C	308367	303917	300671	276917	253907	360495	285150	269398	284290	274148	270131	317636	288616	334704	315090	333474	336685	311045	321043	220086	307934	290247	60417	54721
D	282473	268120	264842	282800	273662	304034	291648	307716	307016	241782	272700	280916	295388	292387	311880	281963	260531	238721	268432	273956	362169	329285	61529	53659
E	249892	309714	318268	294896	294293	281356	273678	292232	272096	286769	283507	290066	362414	306517	280492	311035	248941	278987	280271	305507	287185	311896	63149	52639
F	268271	290595	259357	279978	293950	283520	282908	275553	278959	258715	278535	278399	270206	303029	270469	305271	287064	266023	297394	291740	291014	310165	57826	57112
G	355745	356269	349170	342194	362764	384355	340500	347321	336589	351666	313594	325551	351914	313640	307315	331785	346261	327868	343248	329944	337880	330436	66710	56069
H	338355	350991	348699	317253	310469	297223	320999	318494	286383	299748	317752	338862	334128	292065	290994	282968	294753	311584	289708	329426	303255	372199	60555	55285
I	307420	337024	248008	287994	302264	298843	302220	303424	277085	294394	279113	233899	262422	285545	282716	262435	244186	263641	300808	282983	290488	284461	63715	57963
J	306084	313176	300637	296394	291826	272457	292859	313430	274189	264719	298608	292699	296263	269017	299814	282180	285756	255019	311731	296870	296927	279623	61835	54238
K	219066	292841	194327	184524	290547	311220	237136	241316	267578	237631	246134	255569	237802	255136	224442	232594	196679	217011	226301	253644	238477	251781	58662	52566
L	214476	248759	240270	244028	223208	194674	252876	229161	211925	234179	254771	238904	225187	223819	244762	244000	261181	183957	252067	259811	231824	286127	55459	48707
M	347585	325728	288251	370658	302381	339535	321321	257176	300071	308160	333782	321943	302070	329399	279517	293510	322115	318557	351170	316271	303447	362291	61175	58801
N	305643	287256	285817	276421	294651	284508	313770	304763	327945	303965	311674	274763	295645	292315	292707	348936	326083	270742	316942	320809	355276	316692	60351	56195
O	305774	298063	283843	299296	316577	291713	124624	294976	294044	295545	316668	277646	299810	318592	302531	330487	303757	333951	304400	312304	320092	315633	68722	55150
P	316628	317359	318554	306538	258866	291191	306502	271282	268188	246738	252050	309514	258059	273398	249178	294240	324296	321267	297234	292032	337714	336691	58756	64503

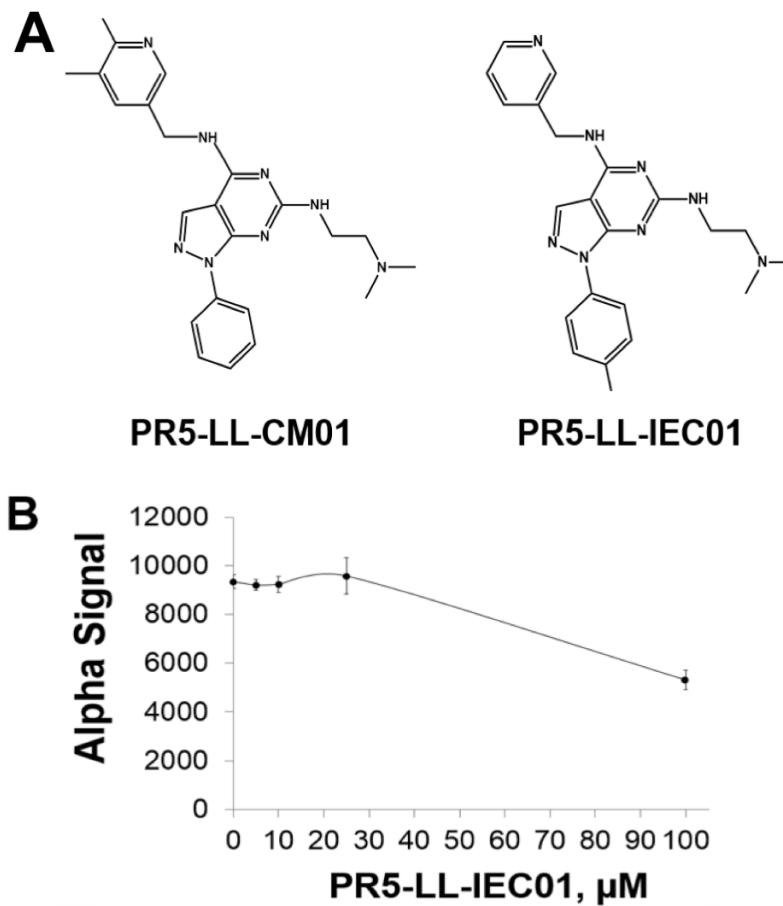
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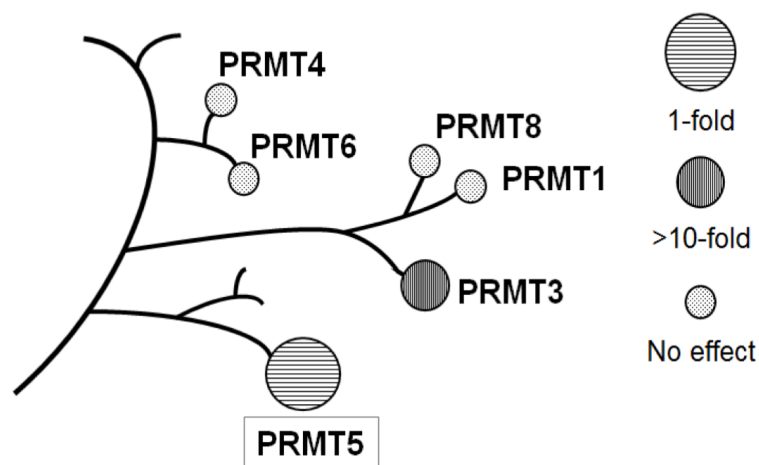
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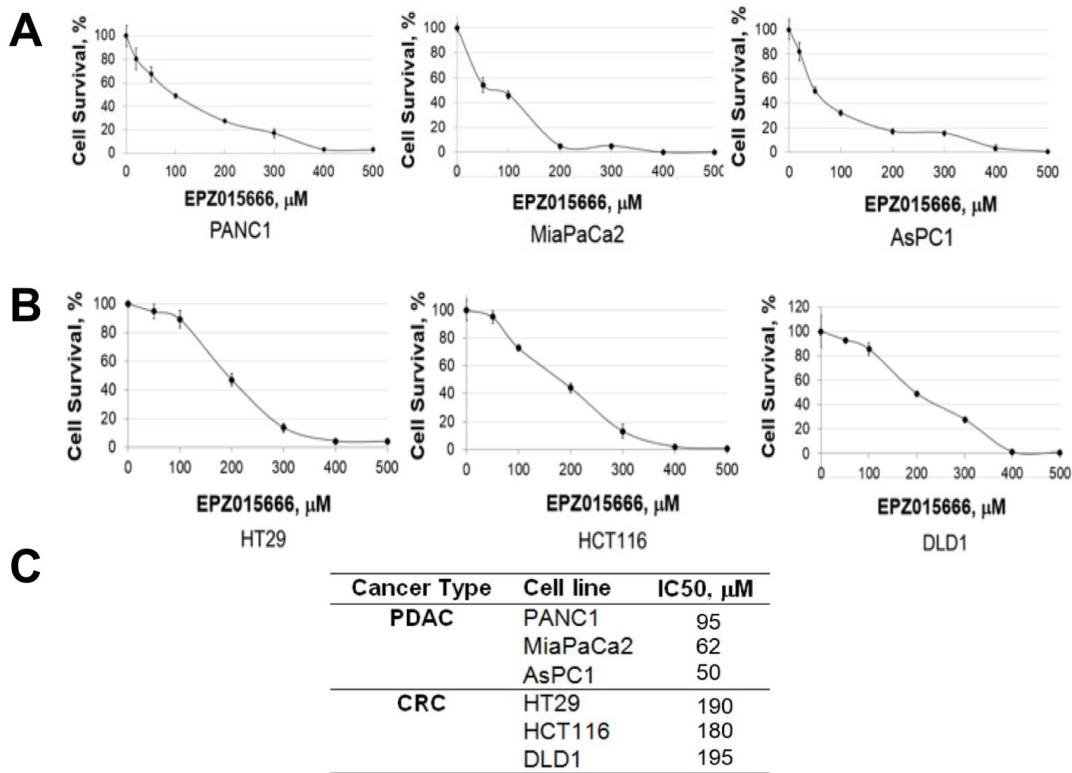
Supplementary Figure 2. Representation of a 384-well plate used in the HTS, with positive control (no inhibitor, with enzyme), test compounds (inhibitor and enzyme present) and negative controls (no inhibitor, no enzyme). A potential hit with decreased AlphaLISA signal is indicated with a red arrow.



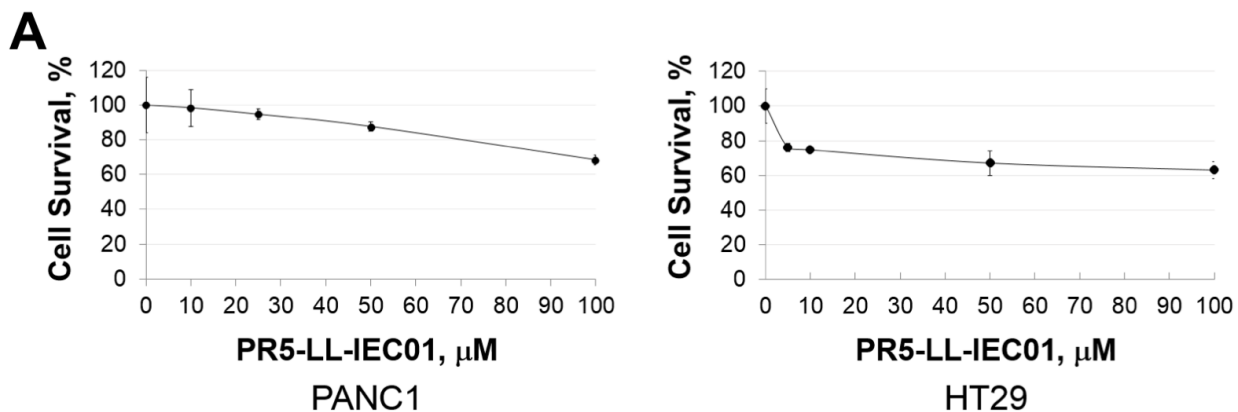
Supplementary Figure 3. PR5-LL-IEC01, a structural analog of PR5-LL-CM01. A. Structure of PR5-LL-IEC01, in side-by-side comparison to the lead compound, PR5-LL-CM01. B. Calculation of IC_{50} of PR5-LL-IEC01 using AlphaLISA, with IC_{50} calculated to be $\sim 118 \mu\text{M}$, ~ 16 -fold higher than the lead compound PR5-LL-CM01.



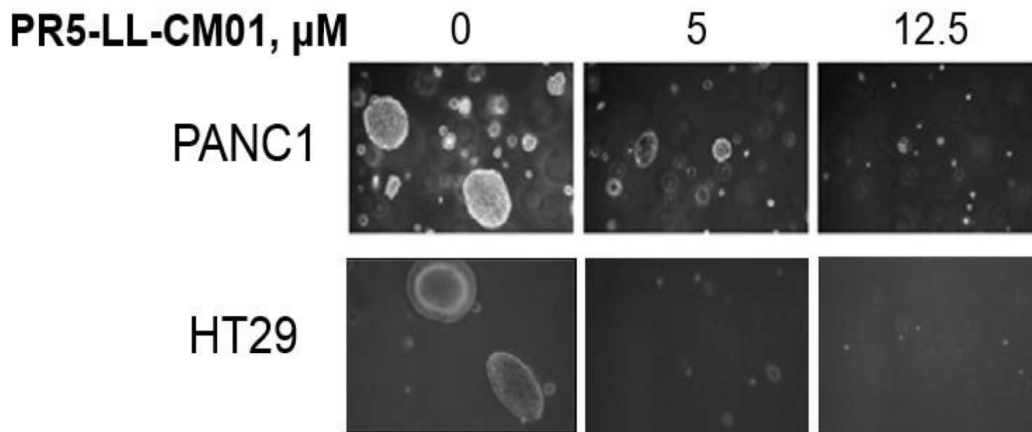
Supplementary Figure 4. Methyltransferase assay. The specificity of inhibition by PR5-LL-CM01 against the enzymatic activity of protein arginine methyltransferase family members was analyzed using the HotSpot radioisotope-based platform (Reaction Biology Corp) [35]. PR5-LL-CM01 showed high specificity to PRMT5, while showed either zero effect or at least a 10-fold higher IC_{50} to other PRMT family members than that of PRMT5.



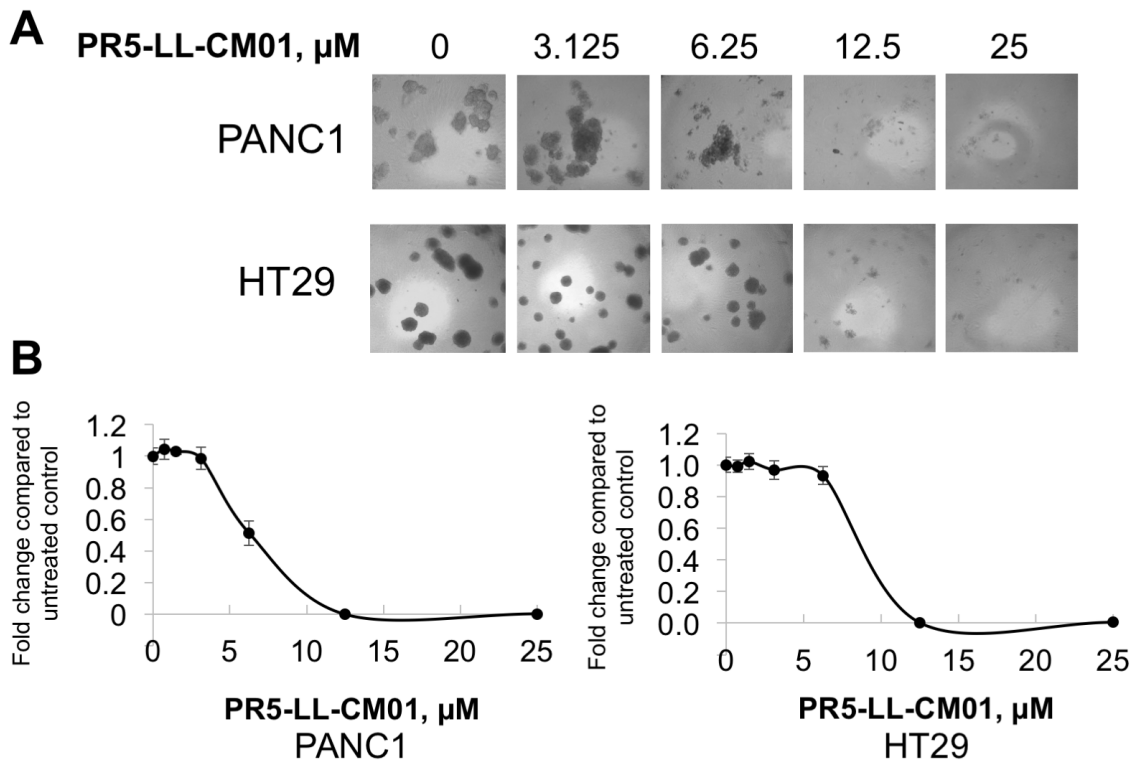
Supplementary Figure 5. Effect of EPZ015666 on PDAC and CRC lines. A. MTT assay, showing that in PDAC cells (PANC1, MiaPaCa2 and AsPC1) EPZ015666 decreased cell viability, however had a lower efficacy to decrease cell viability than that of PR5-LL-CM01 (Figure 4A). B. MTT assay, showing that in CRC cells (HT29, HCT116, and DLD1), EPZ015666 decreased cell viability, but had lower efficacy to decrease cell viability than that of PR5-LL-CM01 (Figure 4B). The data represent the means \pm S.D. for three independent experiments. * $P < 0.05$ vs. Ctrl group. C. Table summarizing the IC₅₀ values for EPZ015666 in PDAC and CRC cell lines, respectively.



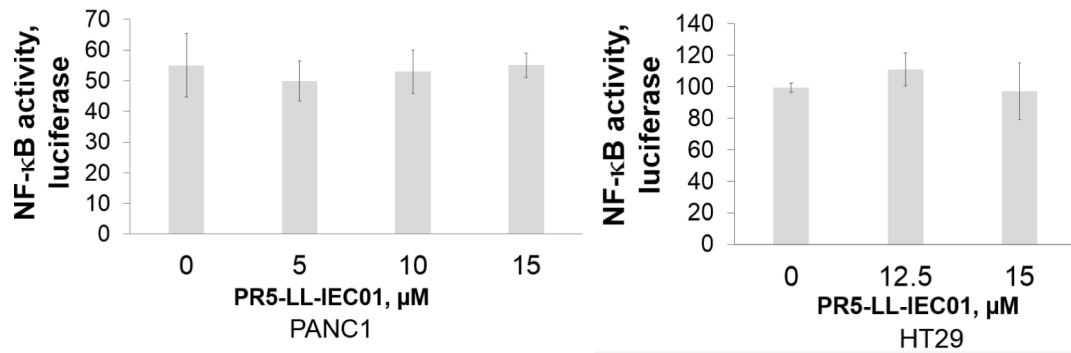
Supplementary Figure 6. Effect of PR5-LL-IEC01 on cell viability of PDAC and CRC lines. A. MTT assay in PDAC (PANC1) and CRC (HT29), showing a high IC₅₀ for PR5-LL-IEC01 in both the cell lines. B. Table, summarizing the IC₅₀ values for PR5-LL-IEC01 in PANC1 and HT29 cells, respectively.



Supplementary Figure 7. Effect of PR5-LL-CM01 on anchorage-independent growth of PDAC and CRC lines. Anchorage-independent assay, showing that with increasing concentrations of PR5-LL-CM01, there was a significant decrease in the anchorage-independent growth ability in both PANC1 as well as HT29 cells.



Supplementary Figure 8. Effect of PR5-LL-CM01 on 3D colony formation of PDAC and CRC lines. 3D colony formation assay, showing that with increasing concentrations of PR5-LL-CM01, there was a significant decrease in the 3D colony formation ability in both PANC1 as well as HT29 cells. A. Representative pictures in 4X magnification. B. Quantification for the fold change in 3D colony formation, upon treatment with increasing concentrations of PR5-LL-CM01, as compared to the untreated control.



Supplementary Figure 9. Treatment with PR5-LL-IEC01 had no significant effect on NF- κB activation in PDAC and CRC cells. NF- κB luciferase assay, showing that upon treatment with increasing concentrations of PR5-LL-IEC01, there was no significant change in NF- κB activation in PANC1 and HT29 cells.

Supplementary Table 1: Table comparing the cancer cell lines with their respective normal control cells. The normal cells had a much higher cell survival rate at the IC_{50} observed in their cancer cell line counterparts.

Cancer	Cancer Cells	IC_{50} , μM	% survival in control cells (treated with IC_{50} of respective cancer cell line) PDAC normal control: HPNE CRC normal control: FHC
PDAC	PANC1	4	80%
	MiaPaCa2	2	90%
	AsPC1	4	80%
CRC	HT29	10	80%
	HCT116	10	80%
	DLD1	11	80%