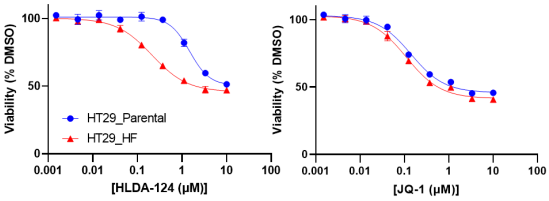
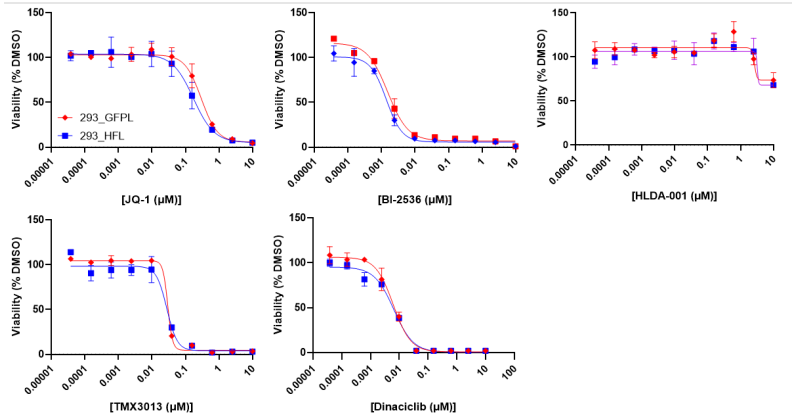
A)

Effector Ligand	BI-2536		Dinaciclib
Target	HaloTag	FKBP	HaloTag
RIPTAC	HLDA-131	HLDA-231	HLDA-119
293_GFPL GI_{50} (μM)	3.442	>10	4.090
293_HFL GI_{50} (μM)	0.007	0.069	0.021
Fold-Shift	492	145	195

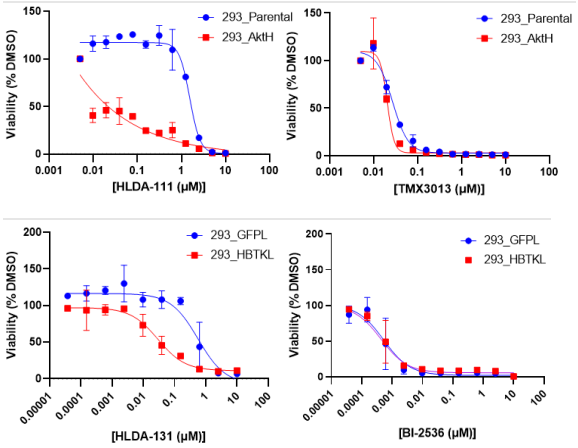
C)



B)

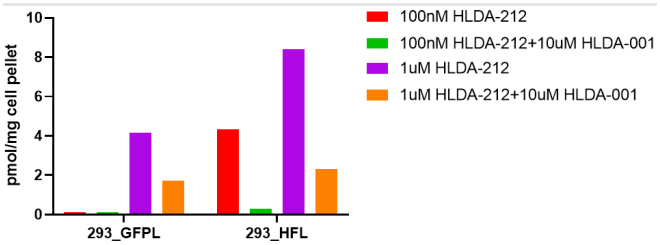


D)



Supplementary Fig. 1 a) Differential anti-proliferative activity of RIPTACs incorporating BI-2536 and dinaciclib ELs in 293_HFL cells under 4h pulse/7 day chase conditions in a CellTiterGlo assay. b) ELs and the FKBP ligand HLDA-001 show no differential anti-proliferative activity in 293_HFL cells in a 7 day CellTiterGlo assay. c) Differential biology observed with the covalent JQ1-CA RIPTAC HLDA-124 in a 3 day CellTiterGlo assay in HT29_HFL cells that express the HaloTag-FKBP target protein. d) Differential anti-proliferative activity observed in a 5 day CellTiterGlo assay with the TMX3013-CA RIPTAC HLDA-111 in 293_AktH cells overexpressing Akt1-HaloTag but not the TMX3013 EL. Similarly, differential anti-proliferative activity observed in a 5 day CellTiterGlo assay with the BI2536-CA RIPTAC HLDA-131, but not with BI2536 alone, in the HaloTag-BTK-overexpressing 293_HBTKL cells compared to 293_GFPL cells.

A)



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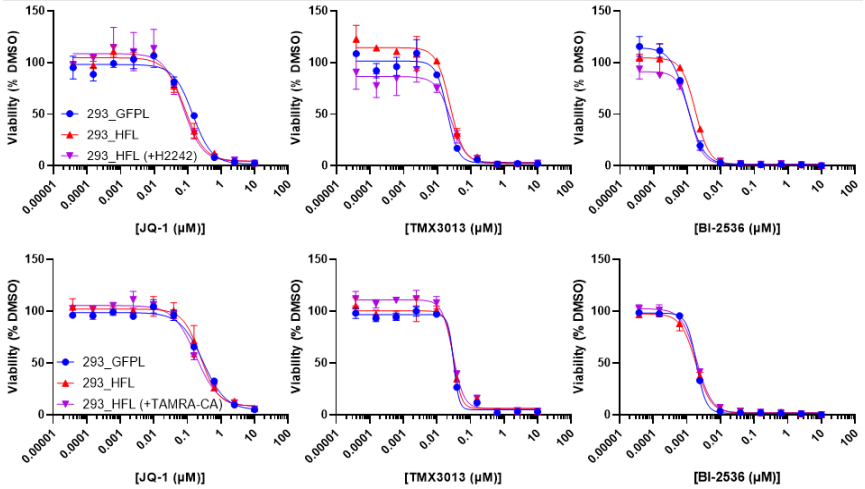

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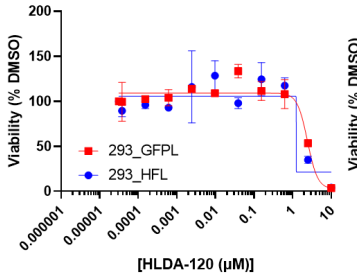

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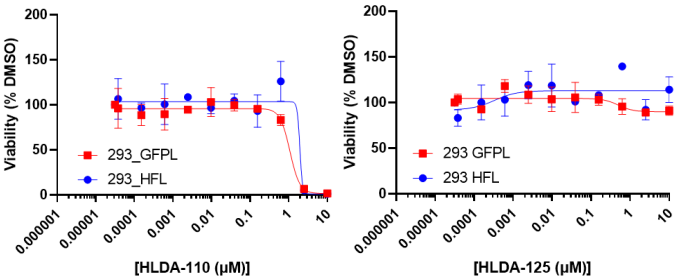
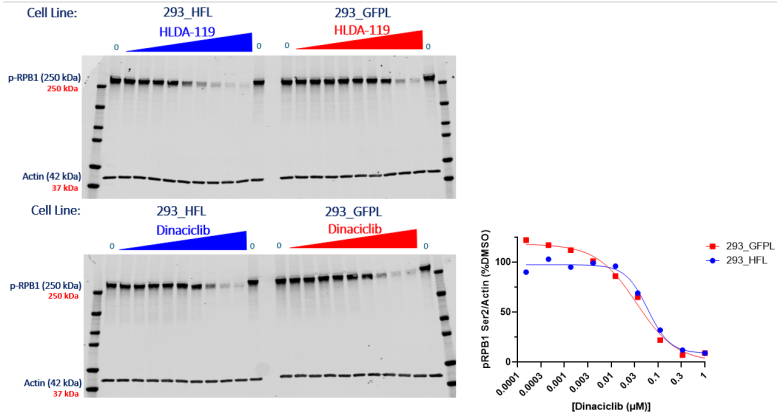
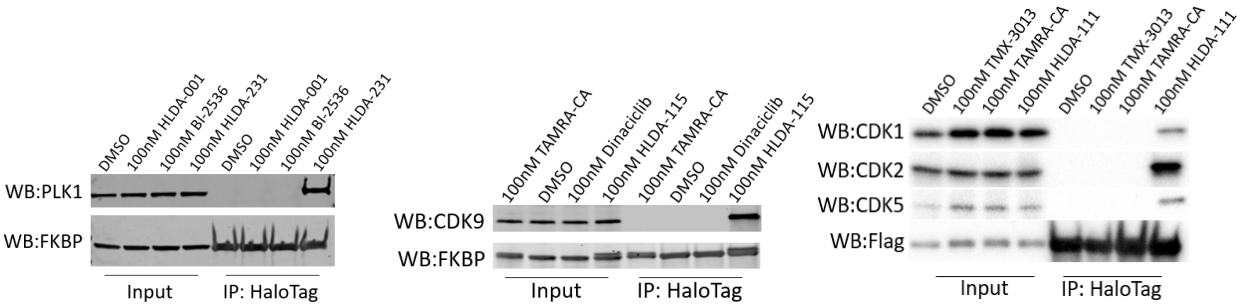


E)



Supplementary Fig. 2 a) Pre-treatment with 10μM FKBP ligand HLDA-001 abrogates intracellular accumulation of RIPTAC HLDA-212. b) ELs JQ1, TMX3013, and BI2536 are equipotent in 293_GFPL and 293_HFL cells and show no reduction in potency upon pre-treatment of 293_HFL cells with either 10μM FKBP ligand HLDA-001 or 300nM HaloTag ligand TAMRA-CA. c) HLDA-120 and HLDA-110, des-chloro negative controls for JQ1-CA HLDA-124 and TMX3013-CA HLDA-111 respectively, are equipotent in 293_GFPL and 293_HFL cells. d) HLDA-125, a negative control RIPTAC, that cannot bind BRD2/3/4, shows no differential potency in 293_HFL cells. e) Dinaciclib is equipotent in 293_GFPL and 293_HFL cells, as determined by its effect on phospho-RPB1 CTD Ser2 levels in cells.

A)



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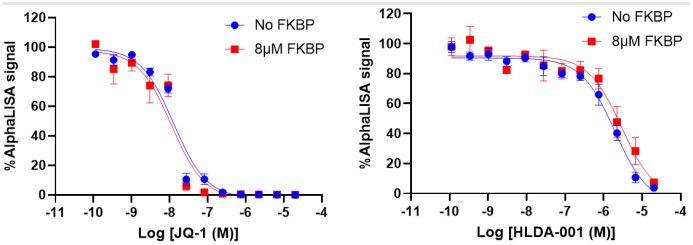

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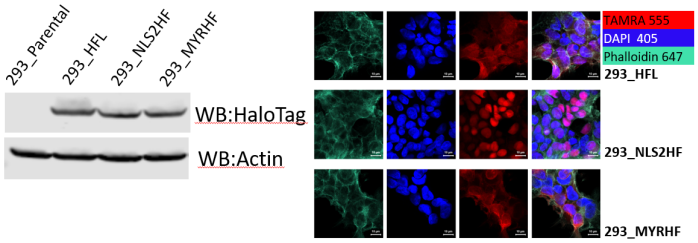

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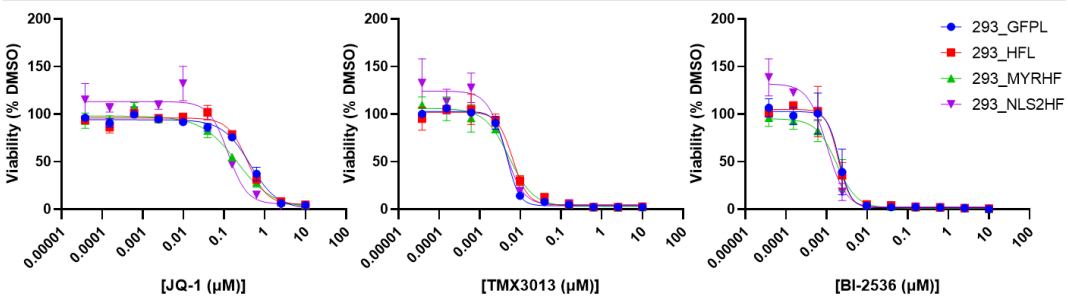
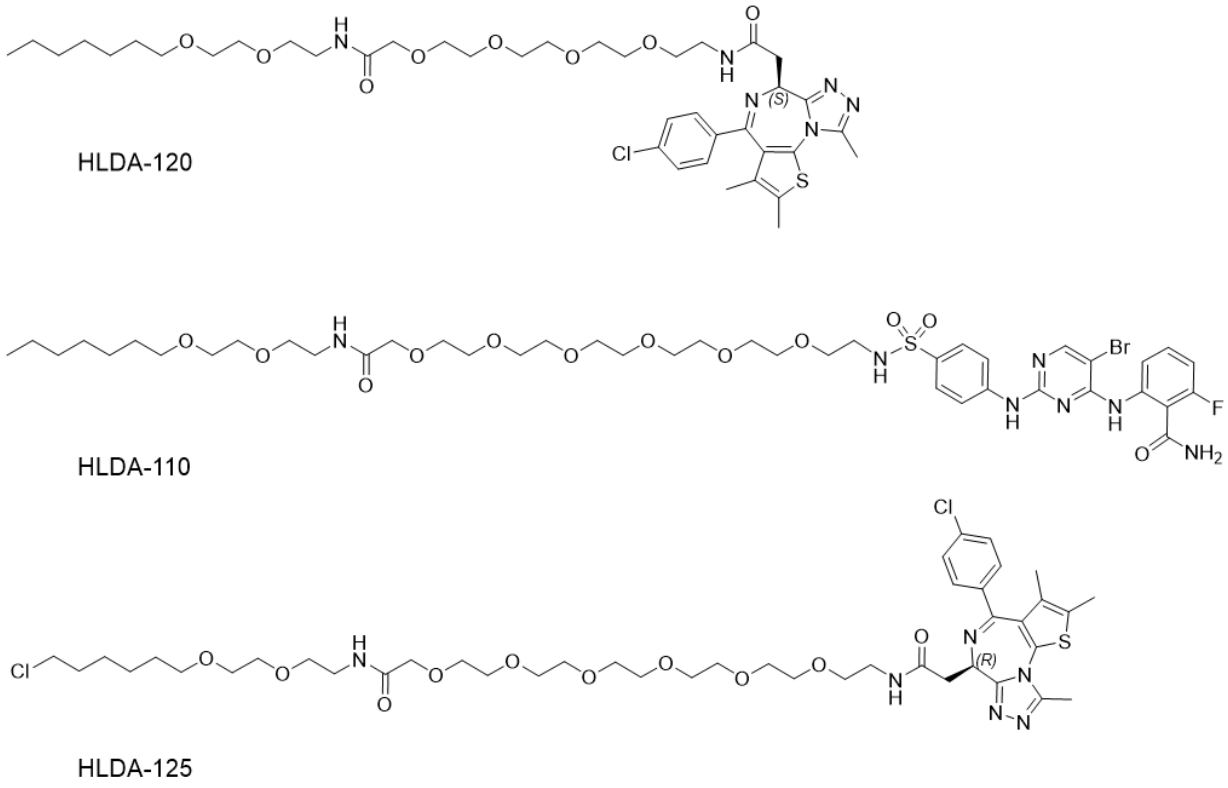


Supplementary Fig. 3 a) Ternary complex formation observed with PLK1 by treating 293_HFL cells with the BI2536 containing non-covalent RIPTAC HLDA-231, with CDK9 by treating with the dinaciclib-CA RIPTAC HLDA-115, and with CDK1/2/5 with the TMX3013-CA RIPTAC HLDA-111. No complex formation is observed with EL and TL controls. b) No positive co-operativity observed in AlphaLISA with TL HLDA-001 or EL JQ1. c) Expression levels of HaloTag-FKBP by immunoblotting and localization by confocal microscopy in 293_NLS2HF and 293_MYRHF cells compared to 293_HFL cells. d) ELs are equipotent across 293_HFL, 293_NLS2HF, and 293_MYRHF cells in a 7-day CellTiter Glo assay.



Supplementary Fig. 4 a) Structures of negative control compounds HLDA-120, HLDA-110, and HLDA-125