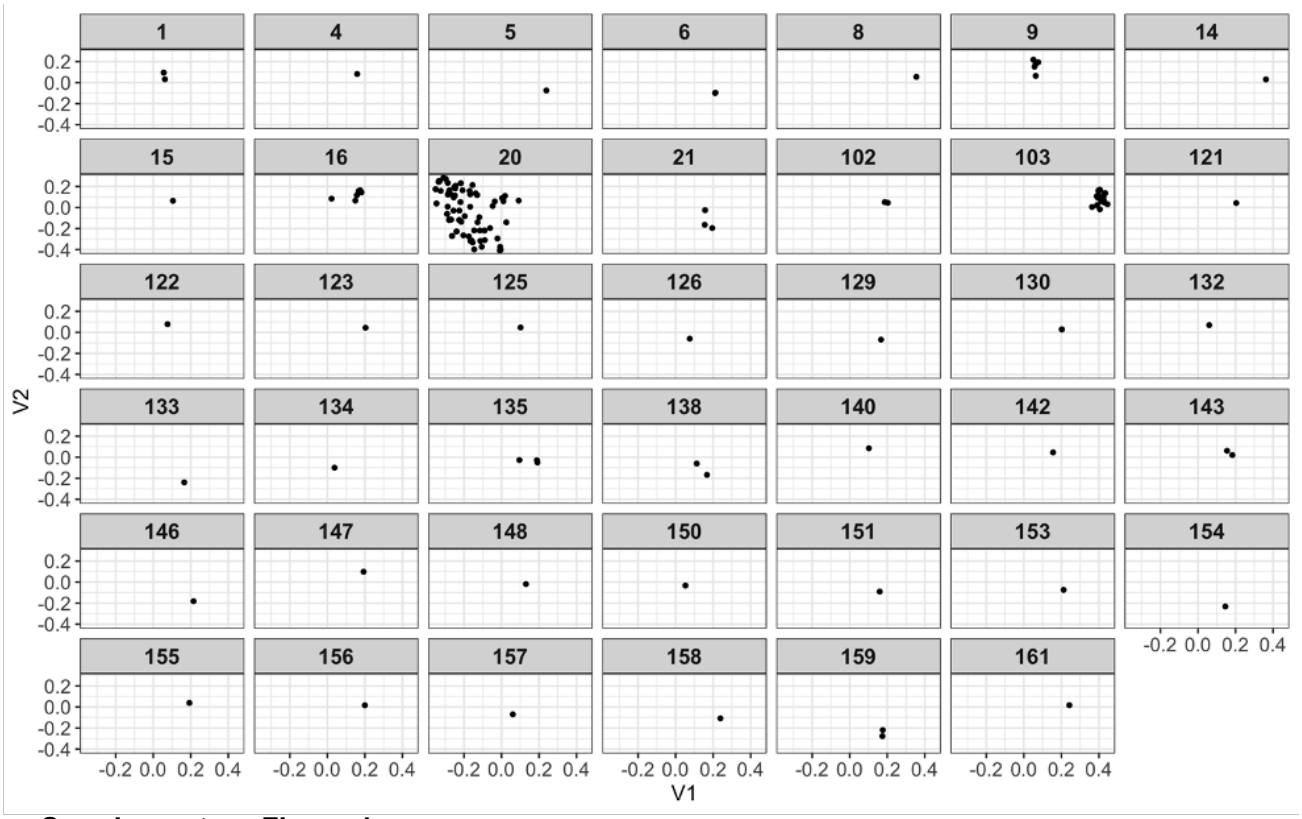
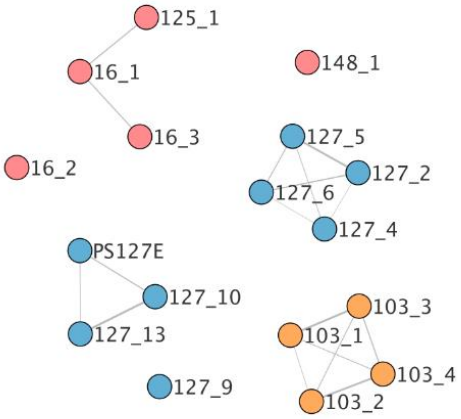
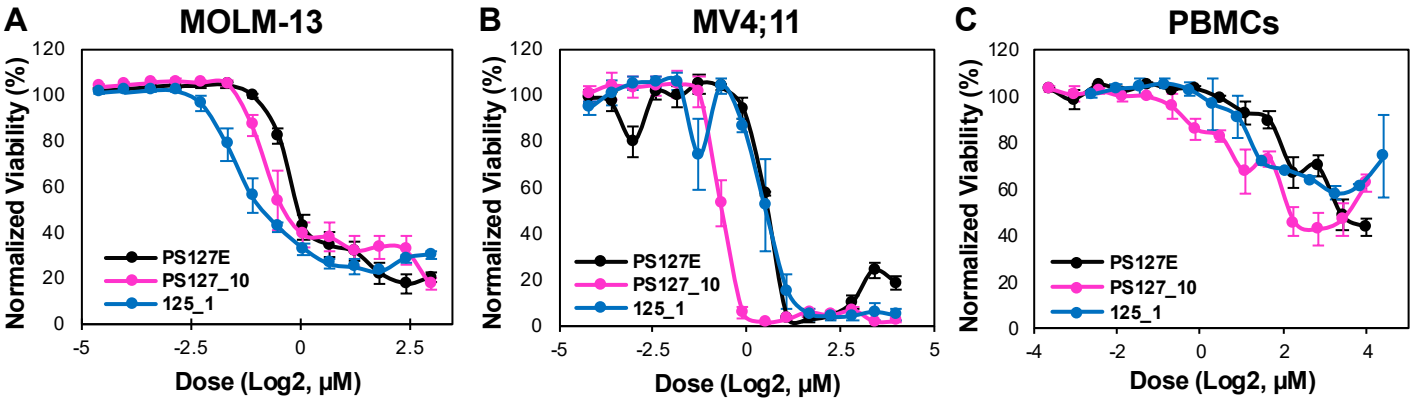


Supplementary Figure 1.



Supplementary Figure 2.

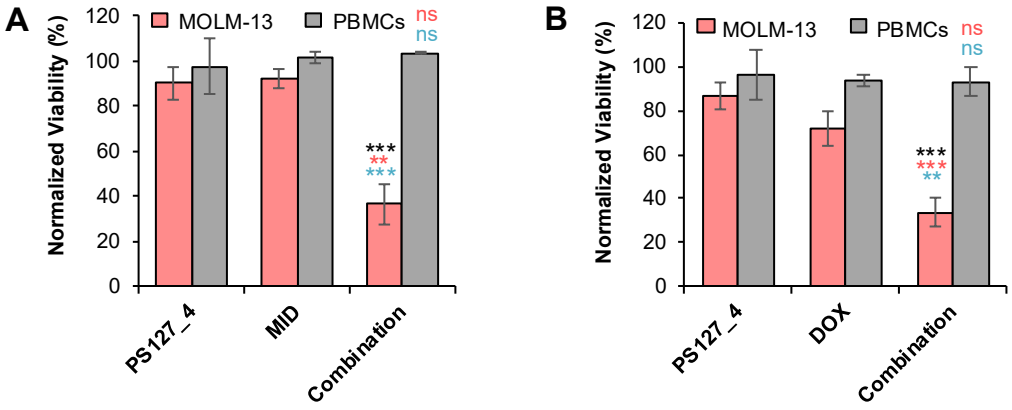


D

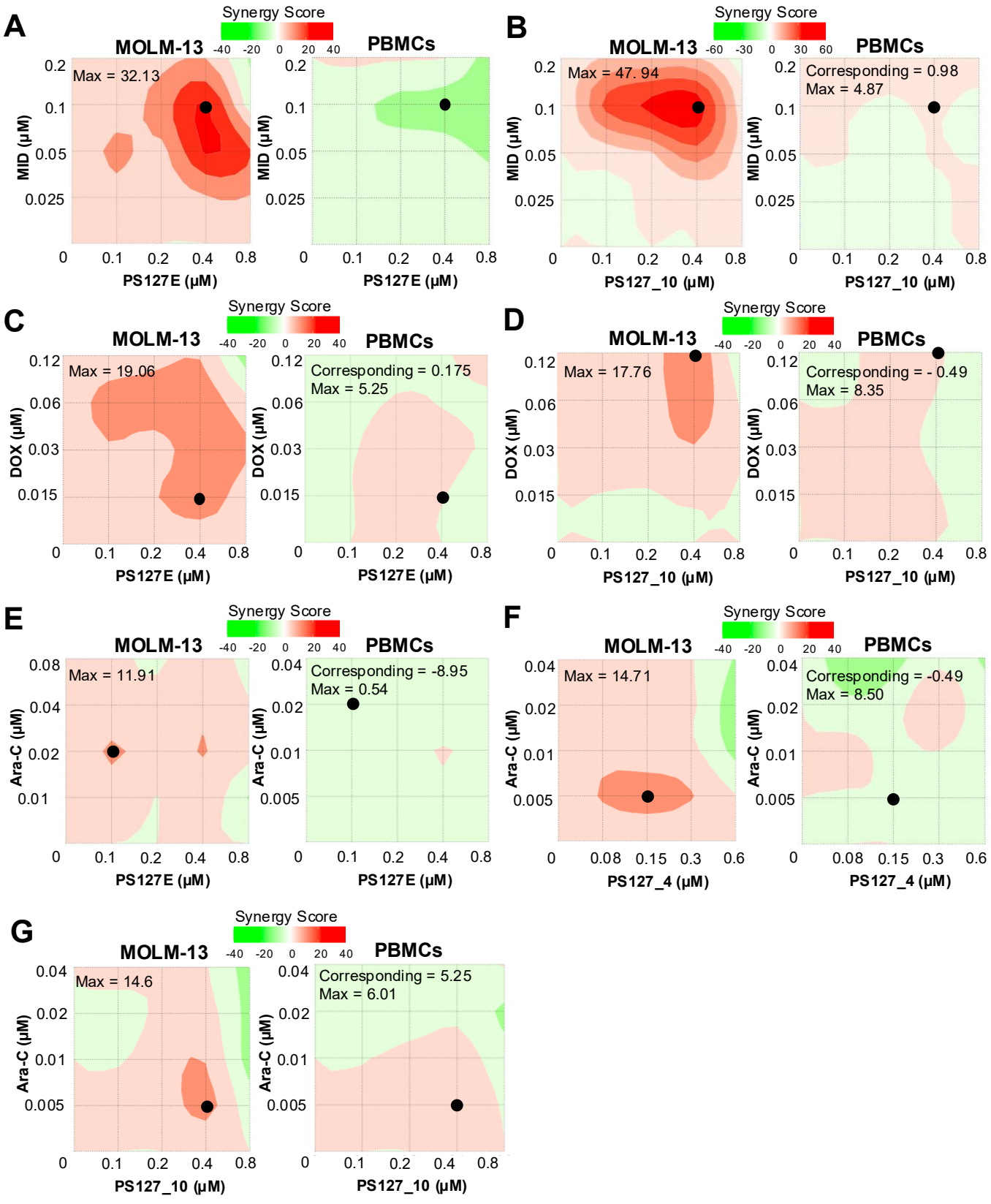
CC_{50} ± SEM (μM)

Compounds	MOLM-13	MV4;11	PBMCs	PBMCs/ MOLM-13	PBMCs/ MV4;11
PS127E	0.88 ± 0.04	1.42 ± 0.01	11.34 ± 1.94	12.89	7.99
PS127_10	0.63 ± 0.06	0.63 ± 0.03	8.59 ± 2.42	13.63	13.63
125_1	0.41 ± 0.04	1.45 ± 0.17	15.68 ± 0.75	38.24	10.81

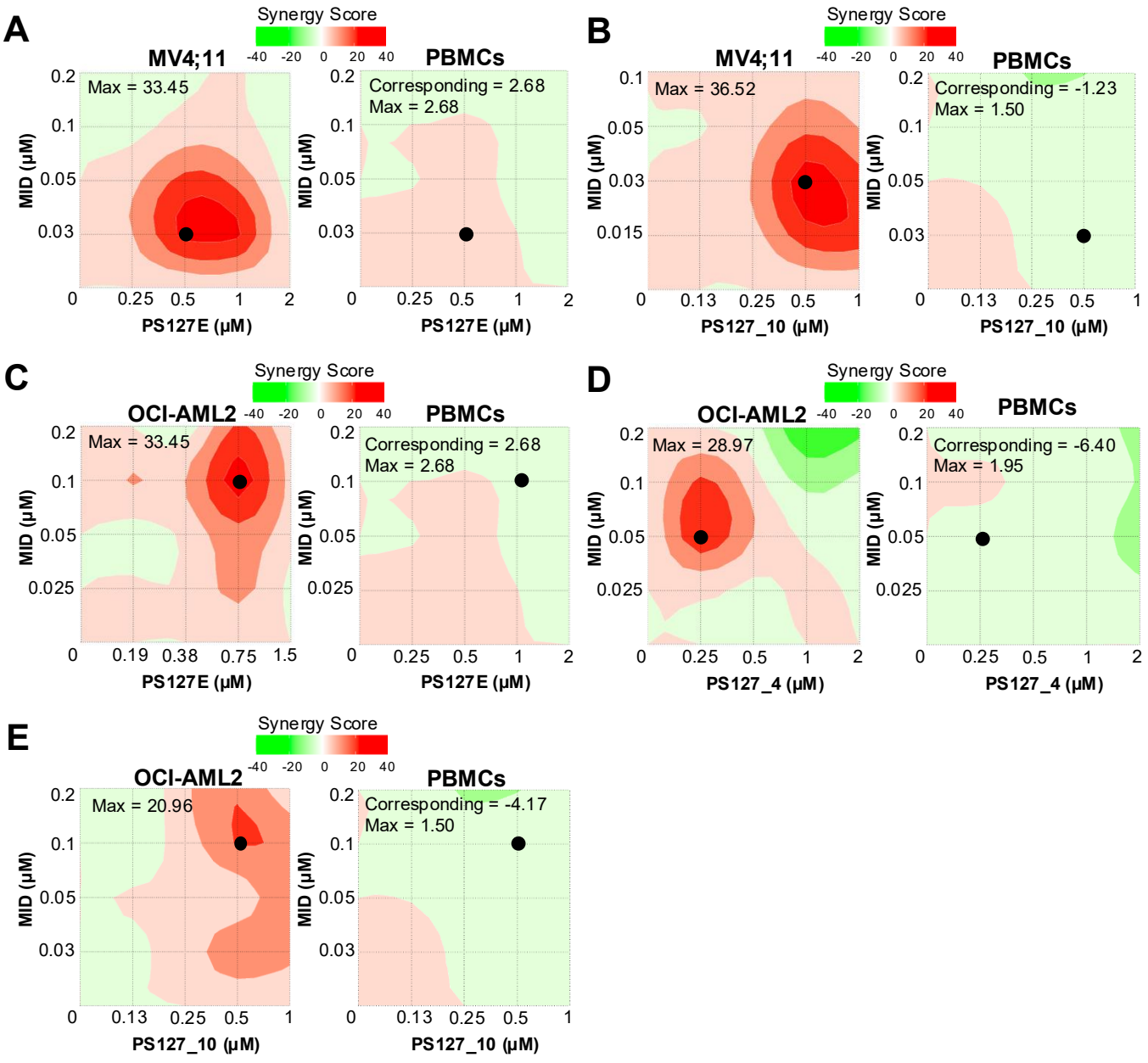
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



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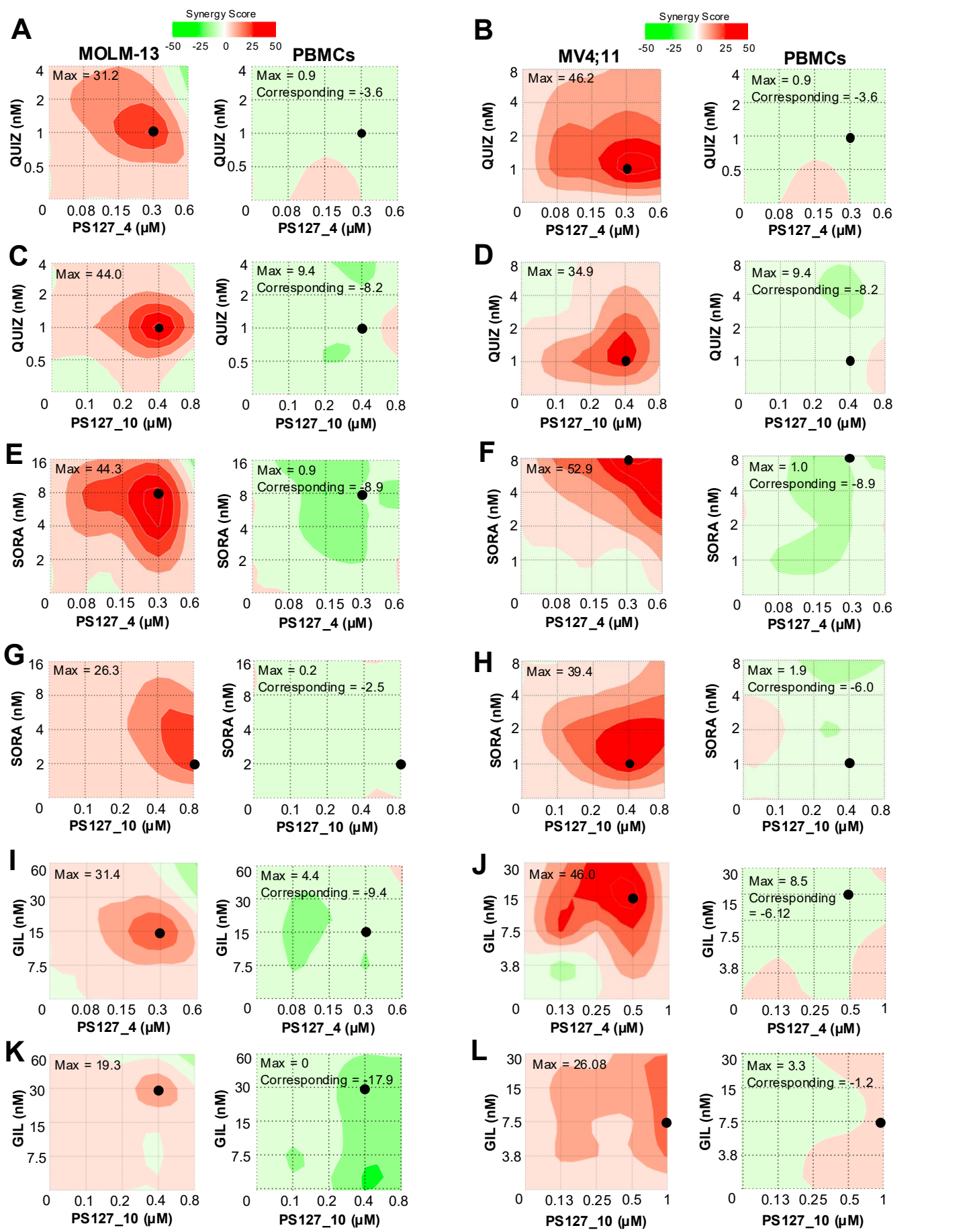

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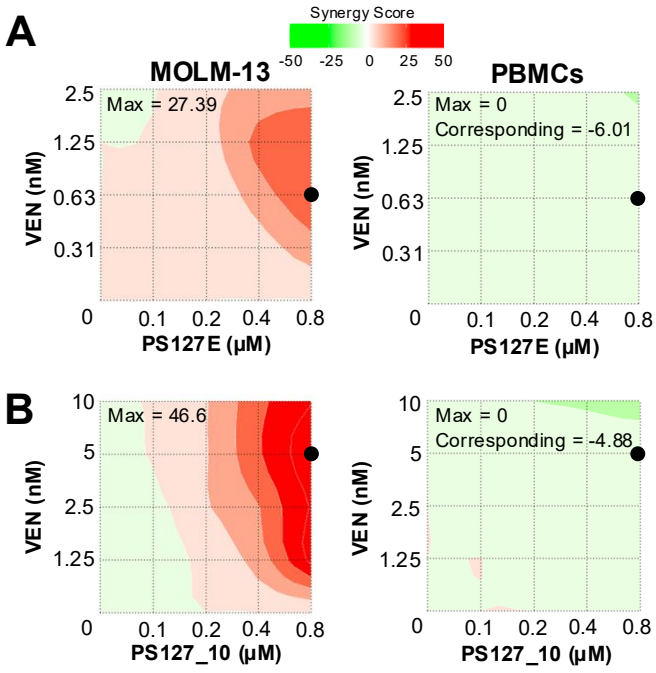

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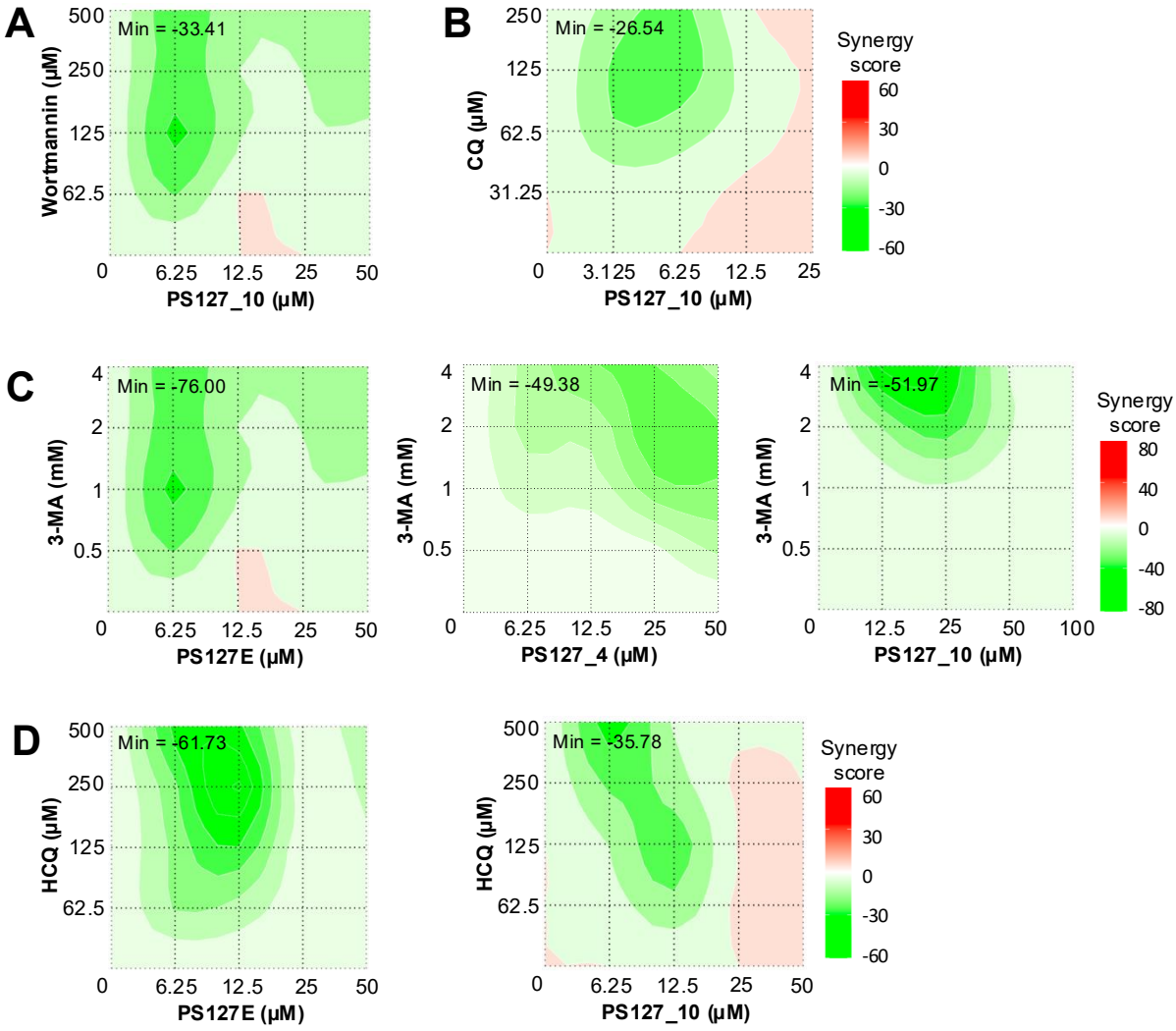
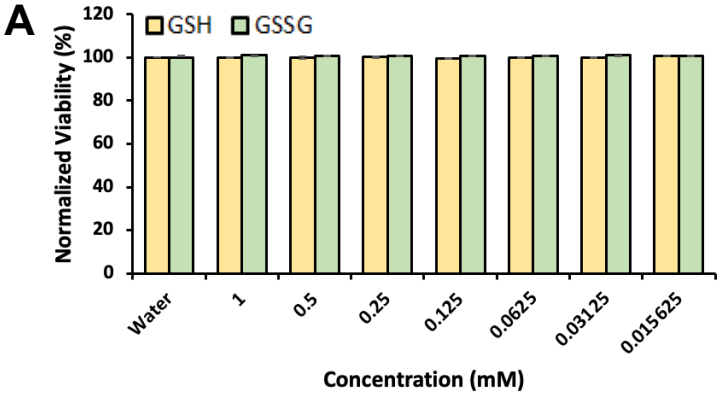
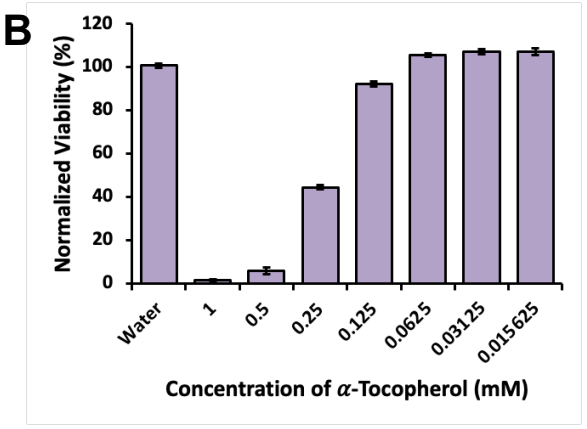
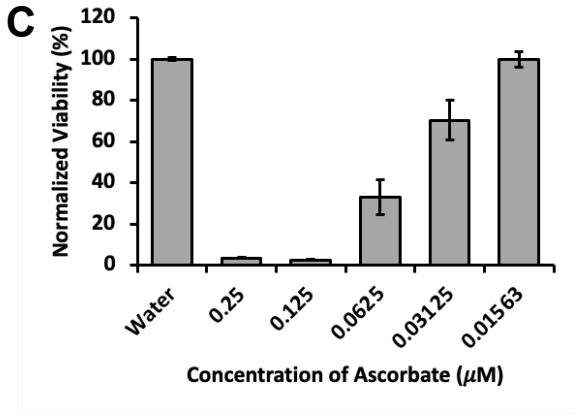


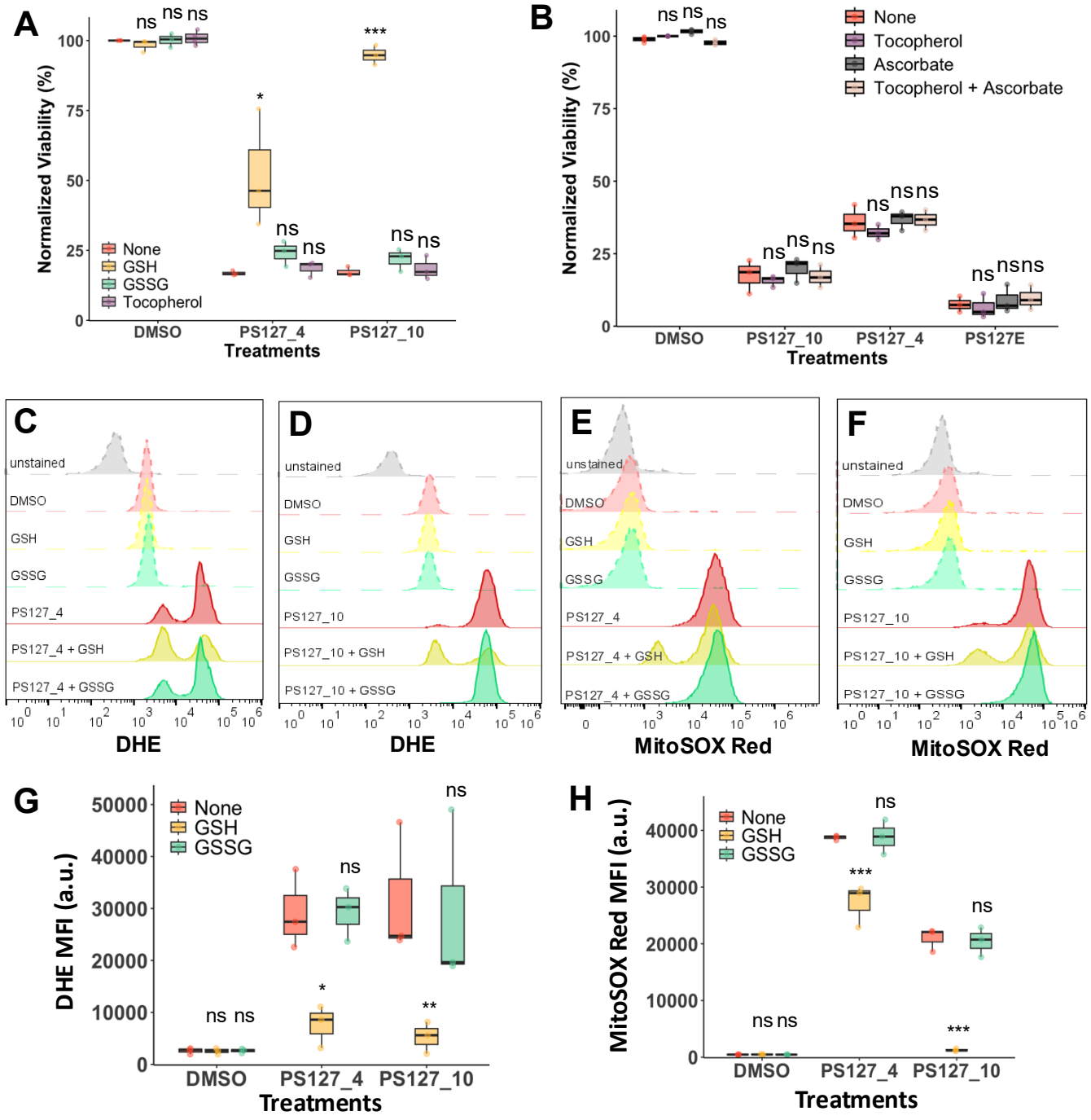
Supplementary Figure 9.



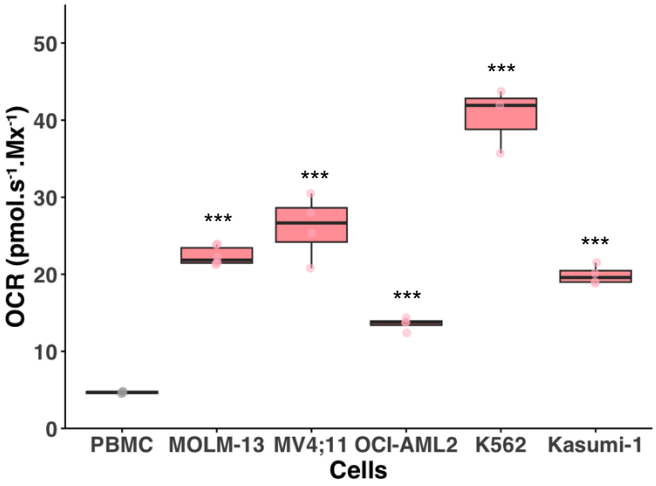




Supplementary Figure 10.



Supplementary Figure 11.



Supplementary Figure 12.

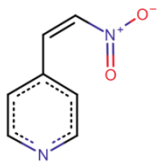
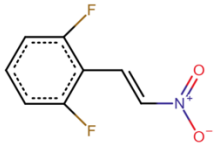
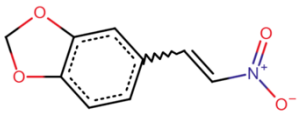
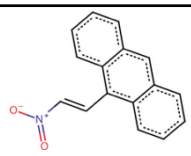
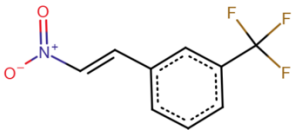
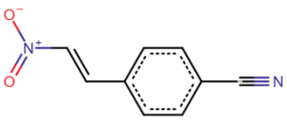
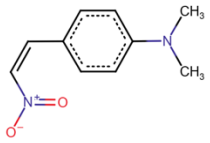
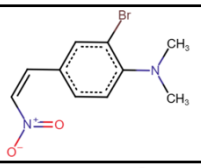
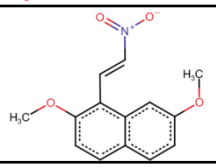
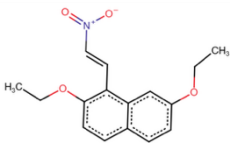
Supplementary Table 1.

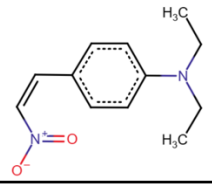
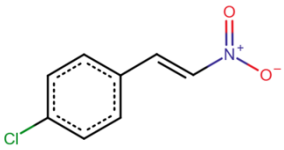
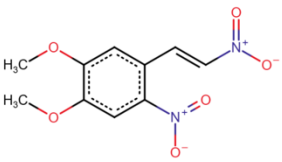
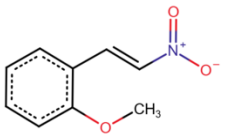
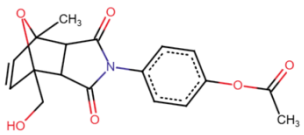
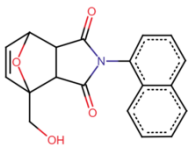
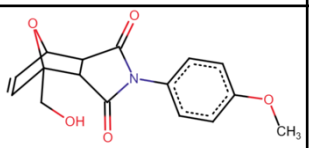
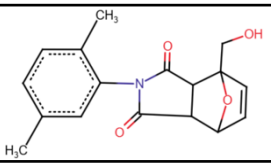
Patient ID	Cohort	Age	Organ	Cell Count ($x10^6$)	Blast Count ($x10^6$)	WBC ($x10^6$)	Cytogenetics
AML-1	FLT3+	60	Bone Marrow	13.7			
AML-2	FLT3+	46	Bone Marrow	14.5			
AML-3	FLT3+	58	Blood	4	31	2.8	46,XY,-15,+mar[1]/46,XY[19]
AML-4	FLT3+	59	Blood	4	13	1.7	46,XX[20]
AML-5	FLT3+	65	Blood	4	8	1.7	42,XY,-6,-11,-12,-16,-17,+mar[1]/46,XY[19]
AML-6	FLT3+	67	Blood	11	26	18	46,XX,inv(16)(p13.1q22)[20]
AML-7	FLT3+	66	Blood	10	70	18.6	46,XY[20]
AML-8	FLT3+	52	Blood	15	30	10.5	46,XY[20]
AML-9	FLT3+	68	Blood	20	89	77.3	47,XY,+8[3]/49,idem,+11,+13[7]/46,XY,+1,der(1;14)(q10;q10)[3]/46,XY,+1,der(1;13)(q10;q10)[2]/46,XY,+1,der(1;15)(q10;q10)[2]/47,XY,+mar[1]/46,XY[2]
AML-10	FLT3+	76	Blood	20	39	45.1	47,XY,+mar[1]/46,XY[19]
AML-11	FLT3-	89	Bone Marrow	10			
AML-12	FLT3-	73	Bone Marrow	4.94			
AML-14	FLT3-	74	Bone marrow	5.50	54	33.9	46,XY,del(5)(q11.2)[1]/46,idem,add(12)(p13)[16]/46,idem,der(7)add(7)(p13)add(7)(q32),add(12)(p13)[3]
AML-15	FLT3-	68	Bone marrow	15	21	3.7	46,XX[20]
AML-16	FLT3-	76	Blood	20	40	15.8	45,X,-Y[14]/46\R\49,XY,+Y,+Y,+13,i(17)(q10)[cp6]
AML-17	FLT3-	78	Blood	20	95	23.7	46,XX[20]

Supplementary Table 2.

Donor ID	Gender	Age	Cell count ($x10^5$)	Organ	CD34	Purity (%)
Primary-1	Male	21	8	Bone marrow	+	98
Primary-2	Male	21	7	Bone marrow	+	96
Primary-3	Male	25	9	Bone marrow	+	92

Supplementary Table 3.

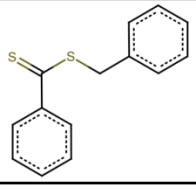
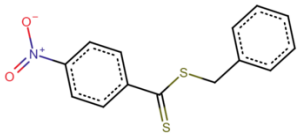
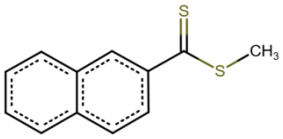
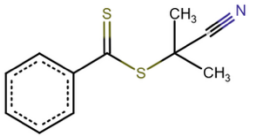
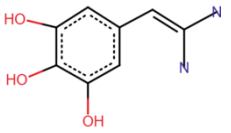
No	PS compound	MolPort ID	MW (g/mol)	Structure	CC_{50}, μM, MOLM-13
1.	127_1	Molport-002-717-654	150.137		>8
2.	127_2	Molport-019-923-348	185.13		1.62
3.	127_3	Molport-009-653-856	193.158		2.41
4.	127_4	Molport-002-918-210	249.269		0.33
5.	127_5	Molport-002-918-866	217.147		1.15
6.	127_6	Molport-023-299-362	174.159		1.05
7.	127_8	Molport-002-951-542	192.218		6.03
8.	127_9	Molport-030-040-762	271.114		0.59
9.	127_10	Molport-003-827-524	259.261		0.63
10.	127_13	Molport-003-826-996	287.315		2.15

No	PS compound	MolPort ID	MW (g/mol)	Structure	CC_{50}, µM, MOLM-13
11.	127_14	Molport-019-686-955	220.272		3.68
12.	127_20	Molport-000-153-349	183.59		1.41
13.	127_21	Molport-002-918-274	254.198		>8
14.	127E	Molport-000-156-857	179.175		0.88
Other Tested Compounds					
15.	103_1	Molport-003-185-960	343.335		>10
16.	103_2	MolPort-001-002-648	321.332		>10
17.	103_3	MolPort-003-874-156	301.298		>10
18.	103_4	MolPort-002-807-280	299.326		>10

Supplementary Table 4.

	103_1	103_2	103_3	103_4	PS127_2	PS127_4	PS127_5	PS127_6	PS127_9	PS127_10	PS127_13	PS127E	16_1	16_2	16_3	125_1	148_1
103_1																	
103_2	0.764																
103_3	0.944	0.807															
103_4	0.799	0.928	0.833														
PS127_2	0.314	0.360	0.327	0.367													
PS127_4	0.312	0.412	0.324	0.377	0.753												
PS127_5	0.309	0.354	0.322	0.360	0.933	0.787											
PS127_6	0.307	0.352	0.320	0.375	0.848	0.758	0.817										
PS127_9	0.415	0.457	0.431	0.474	0.604	0.548	0.588	0.596									
PS127_10	0.476	0.449	0.486	0.410	0.540	0.627	0.565	0.523	0.437								
PS127_13	0.508	0.457	0.495	0.426	0.515	0.611	0.538	0.500	0.421	0.952							
PS127E	0.489	0.409	0.509	0.424	0.644	0.581	0.626	0.620	0.500	0.833	0.794						
16_1	0.278	0.346	0.290	0.327	0.593	0.573	0.573	0.567	0.430	0.420	0.401	0.456					
16_2	0.376	0.450	0.391	0.432	0.615	0.596	0.598	0.593	0.659	0.456	0.439	0.492	0.680				
16_3	0.279	0.368	0.290	0.341	0.537	0.684	0.568	0.546	0.411	0.489	0.467	0.434	0.831	0.593			
125_1	0.305	0.342	0.317	0.357	0.635	0.561	0.614	0.663	0.471	0.424	0.406	0.500	0.836	0.634	0.744		
148_1	0.434	0.397	0.443	0.403	0.466	0.417	0.455	0.513	0.366	0.574	0.551	0.578	0.424	0.382	0.394	0.491	

Supplementary Table 5.

No	Compound	MolPort ID	MW (g/mol)	Structure	CC_{50}, µM, MOLM-13
19.	16_1	Molport-020-171-975	244.37		ND, <10
20.	16-2	Molport-003-005-421	289.37		ND, <10
21.	16-3	MolPort-003-800-321	218.33		ND, >10
22.	125_1	MolPort-006-727-986	221.34		0.41
23.	148_1	MolPort-003-850-809	202.169		ND, >10

ND = not determined

Supplementary Figure 1. MDS clustering of screened compounds for apoptosis induction and T/GR inhibition. Clustering of 161 primary screen hits using MDS algorithm. Two large clusters, 20 and 103, were identified. Cluster 20 contained PS127-family molecules.

Supplementary Figure 2. Structural similarity of compounds selected for wet-lab validation. Clustering is based on Tanimoto coefficient generated using PubChem algorithm. Compounds with similarity coefficients at or above 0.75 were connected by line and placed into the same cluster.

Supplementary Figure 3. Dose-dependent cytotoxic concentration of lead compounds in FLT3-ITD AML cells. A-C) Dose-response curve of lead compounds in MOLM-13 (A), MV4;11 (B), and PBMCs (C). Cells were treated with a range of concentrations of compound for 72 hours. **D**) Summary of CC_{50} values (μM) derived from dose-response curve. Ratio of CC_{50} in PBMC to the respective leukemic cells was calculated to show selectivity. Error bars show SEMs from three biological replicates.

Supplementary Figure 4. Synergistic cytotoxicity of PS127_4 in combination with MID and DOX. A) Viability of MOLM-13 cells (pink bars) and healthy PBMCs (grey bars) following treatment with PS127_4, MID, or their combination. **B**) Viability of MOLM-13 cells and healthy PBMCs following treatment with PS127_4, DOX, or their combination. Error bars show SEM from three biological replicates. Black asterisks indicate comparison of MOLM-13 cells vs. healthy PBMCs under the same combinatorial treatment conditions. Pink asterisks indicate significantly lower survival under combinatorial treatment compared to single PS127-family compound treatment; Blue asterisks indicate significantly lower survival under combinatorial treatment compared to a single MID treatment. Significance was assessed via Student's *t*-test. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$; ns: not significant ($p>0.05$).

Supplementary Figure 5. Synergistic effects of PS127-family compounds with commercial chemotherapeutics in MOLM-13. Representative synergy landscape of MOLM-13 cells (left) or PBMCs (right) with PS127E, PS127_4 or PS127_10 with MID (A, B), DOX (C, D), and ara-C (E-G). Black dots represent the maximum synergy point in MOLM-13 and its correspondence in PBMCs.

Supplementary Figure 6. Synergistic effect of PS127-family compounds with MID in other AML cell lines. Representative synergy landscape of MV4;11 with PS127E or PS127_10 and MID (A,B). Representative synergy landscape of OCI-AML2 with lead PS127-family compounds (C-E). Black dots represent the highest synergy score in AML cell lines (right) and its corresponding in PBMCs (left).

Supplementary Figure 7. Synergistic effect of PS127-family compounds with other FLT3 inhibitors in leukemic cell lines. Representative synergy landscape of PS127-family compounds and QUIZ in MOLM-13 (A, C) or MV4;11 (B, D). Representative synergy landscape of PS127-family compounds and SORA in MOLM-13 (E, G) or MV4;11 (F, H). Representative synergy landscape of PS127-family compounds and GIL in MOLM-13 (I, K) or MV4;11 (J, L). Black dot represents the highest Bliss score in leukemic cell lines (left) and its correspondence in PBMCs (right).

Supplementary Figure 8. Synergistic effect of PS127-family compounds with VEN in MOLM-13 cell lines. Representative synergy landscape of PS127E (A) or PS127_10 (B) and VEN. Black dot represents the highest Bliss score in MOLM-13 cell lines (left) and its correspondence in PBMCs (right).

Supplementary Figure 9. Antagonistic effect of PS127-family compounds with various autophagy inhibitors in MOLM-13 cell lines. Representative synergy landscape of lead PS127-family compounds with Wortmannin (A), CQ (B), 3-MA (C), and HCQ (D). Minimum Bliss scores are shown for each combination.

Supplementary Figure 10. Cytotoxicity of various redox metabolites after 72-hour treatment. A) Neither GSH (yellow, n = 3) nor GSSG (green, n = 3) were toxic to AML cells. **B**) α-tocopherol (purple, n = 3) and **C**) ascorbate (green, n = 3) showed cytotoxicity at high concentrations. Cell viability was normalized to solvent-treated as negative control. The highest concentration of α-tocopherol or ascorbate with minimal toxicity was used to assess their impact on compound-induced cytotoxicity. Bar graphs represent the mean normalized viability from three biological replicates, error bars indicate SEM.

Supplementary Figure 11. Evaluation of PASS-predicted activities in other top PS127-family compounds and their mechanistic activity to induce cell death. A) The effect of redox-active compounds (GSH [n = 3], GSSG [n = 3], and α-tocopherol [n = 3]) on PS127-induced cytotoxicity in MOLM-13. **B**) The effect of ascorbate or α-tocopherol supplementation either individually or in combination on cell viability treated with lead PS127-family compounds, **C-D**) Representative histograms of DHE^+ population upon PS127-family treatment in the presence or in absence of redox metabolites. **E-F**) Representative histogram of MitoSOX Red^+ population upon PS127-family treatment in the presence or in absence of redox metabolites. Peaks with dashed lines display control treatment or in absence of PS127-molecule, while solid lines represent treatment with PS127-molecule. **G)** Quantification of total ROS upon PS127-family treatment in the presence of glutathione redox metabolites. ROS level was indicated by MFI of DHE (n = 3). **H**) Quantification of mitochondrial ROS level in the presence of redox metabolites. ROS level was indicated by MFI of MitoSOX Red (n = 3). Biological replicates are shown by data points. Statistical significance of apoptotic cells and ROS level was assessed via ANOVA using *post hoc* Dunnett's test, comparing each treatment against the no redox metabolite group. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$; ns: not significant ($p>0.05$).

Supplementary Figure 12. Basal OCR of PBMCs and various leukemic cells. OCR was normalized to the number of live cells (in millions, Mx) per chamber volume (n = 4). OCR of leukemic cells were statistically compared to PBMCs using *post hoc* Dunnett's test. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$; ns: not significant ($p>0.05$).

Supplementary Table 1. Sample information of primary AML patients.

Supplementary Table 2. Sample information of $CD34^+$ samples.

Supplementary Table 3. Structures of tested compounds from first *in silico* screen selection criteria.

Supplementary Table 4. Pairwise Tanimoto coefficient matrix of representative compounds with three predicted activities

Supplementary Table 5. Structures of *in silico* hits from non-PS127-family clusters

SUPPLEMENTAL METHODS

SM1 Cell culturing of AML cell lines, PBMCs, cardiomyocytes, AML patient samples, and CD34+ progenitor cells

AML cell cultures were maintained in standard culture media (RPMI-1640 medium supplemented with 2 mM L-glutamine and sodium bicarbonate [Sigma-Aldrich], 10% Corning™ Regular Fetal Bovine Serum [FBS] [Heat Inactivated] and 1% penicillin-streptomycin solution [P/S] [Sigma-Aldrich]) in a 37°C humidified atmosphere with 5% CO_2. MOLM-13 was used as an AML cell line model, unless specified otherwise. H9C2 cardiomyocytes were subcultured in similar condition via trypsinization at 70% confluence prior to experimental treatment. A new tube was thawed after about 50 passages.

Peripheral blood mononuclear cells (PBMCs) were obtained from the Gulf Coast Regional Blood Centre (Houston, TX, USA). PBMCs were isolated using Ficoll®-Paque PREMIUM (Cytiva) according to manufacturer's instructions. PBMCs were maintained overnight (at least 16 hours) at a minimum density of 10^6 cells/mL before experimental use.

Cryopreserved AML patient samples were anonymized to maintain patient confidentiality **(Supplementary Table 1)**. Frozen cells were thawed and transferred into thawing media (RPMI-1640 medium with 20% FBS, 1 mM $MgSO_4$, and 1 mM EDTA). The vials were washed twice with thawing media and spun down for 5 minutes at 400 g. The samples were washed with RPMI-1640 medium (10% FBS, 1% P/S) and spun down twice more. Samples were then cultured in 20% FBS, 1% P/S RPMI-1640 at a 10^6 cells/mL the day prior to experimental set-up.

All assays with patient samples used patient-experimental media (RPMI-1640, 2.5% FBS, 1% P/S). AML patient cells were seeded at 30,000 cells per well for synergy experiments with PS127_10 and MID or DOX (depending on sample size), as well as supplementation assays of

cell treated with 4 μM of compound and 500 μM of GSH. Experimental plates were imaged after 72 hours. Routine respiration of AML patient cells ($pmol \cdot s^{-1} \cdot Mx^{-1}$) was measured in the 0.5 mL chamber of the Oroboros in patient-experimental medium at 20^6 cells/mL. Routine respiration was also measured for MOLM-13 (10^6 cell/mL) and PBMCs (20^6 cells/mL) in the 0.5 mL chamber to parallel conditions. Measurements were normalized to the number of live million cells (Mx) per mL.

SM2 *In silico* screen

To identify selective cytotoxic against AML cells, we screened a commercially available library of approximately 4.2 million compounds (MolPort [https://www.molport.com/shop/index]) using PASS software version 2022. PASS is a ligand-based cheminformatics approach that is target-agnostic and predicts biological activity solely from chemical structure. Library was filtered for molecules exhibiting three biological activities previously linked to selective cytotoxicity in AML cells: (1) apoptotic agonism, (2) thioredoxin/glutathione reductase (T/GR) inhibition, and (3) autophagic induction. PASS uses a training set comprising thousands of known bioactive compounds to predict over 6,000 types of biological activities based on molecular structure descriptors. For each activity, it calculates two probabilities: Pa (probability of being active, ranging from 0 to 1) and Pi (probability of being inactive, ranging from 0 to 1). In the primary screening, a compound was considered a hit if the difference ΔP (Pa – Pi) for each of the three target functions (apoptotic agonism, T/GR inhibition, and autophagy induction) was ≥ 0.7. This stringent threshold was chosen to minimize false positives.

Because the primary screen alone is typically insufficient, we conducted an additional

refinement of the hit list. Based on our previous findings highlighting the critical role of autophagy in the mechanism of PS127-family compounds, we applied a secondary filter requiring a Pa value ≥ 0.7 specifically for the "autophagy induction" activity. This refinement step further reduced the final hit list. The 93 final hits were clustered by structural similarity using the multidimensional scaling algorithm implemented in ChemMine Tools (https://chemminetools.ucr.edu/), with a similarity cut-off of 0.4 for cluster formation.

To visualize and analyze structural relationships among the hits, pairwise Tanimoto coefficients were calculated using PubChem fingerprints, and the resulting network was visualized in Cytoscape 3.10.3 (https://cytoscape.org/).

Tanimoto coefficients calculated as:

$$T(a,b) = \frac{N_c}{N_a + N_b - N_c}$$

N_c – number of features or descriptors common to both molecules

N_a – number of features or descriptors of the first molecule in the pair

N_b – number of features or descriptors of the second molecule in the pair

To validate the output of the *in silico* screen, a representative subset of compounds was selected for experimental testing. The selection strategy was designed to achieve two primary goals: (1) to sample the chemical space of the most populated cluster, and (2) to test the core hypothesis that the predicted bioactivity profile serves as a generalizable predictor of biological effect, independent of any specific chemical scaffold.

The identified hits were first partitioned into distinct structural classes using molecular similarity-based clustering. From the predominant structural class, which comprised analogs of the initial lead compound PS127E, a representative set of molecules was selected. This selection prioritized maximal structural diversity within the class rather than adherence to a single

quantitative metric, thereby ensuring a comprehensive exploration of its chemical space.

To decouple the observed biological effects from the primary chemical scaffold, a separate panel of compounds was chosen from the remaining structural classes. These compounds were intentionally selected for their structural dissimilarity to the main cluster (i.e., their distinct core scaffolds) but were predicted by the PASS algorithm to share the same trio of high-probability biological activities. This design enabled direct testing of whether the predicted functional profile - rather than structural similarity itself - served as the critical determinant of the desired phenotypic outcome.

SM3 Cell viability assays

1. Cytotoxicity assay

Each *in silico*-identified compounds and commercial chemotherapeutics were prepared as a 10 mM stock solution in DMSO, aliquoted, and stored long-term at -20°C and 4°C for short-term use. Cells were treated with compounds or solvent controls in experimental media (RPMI-1640 with 1% FBS, 1% P/S) and stained with Hoechst 33342 and propidium iodide (PI) to detect total and dead cells, respectively. Plates were spun down for 5 minutes at 1,000 rcf in the Hermile Z446 Benchmark. Stained cells were then analyzed on a BioTek plate reader (Cytation 5 Cell Imaging Multi-Mode Reader) with Gen5 software.

Cells were seeded in 96-well plates (5,000 AML cells/well or 50,000 PBMCs/well) and immediately treated with screened compound or the solvent control, DMSO in experimental media (RPMI-1640, 1% FBS, 1% P/S). AML patient samples were seeded at 30,000 cells/well in patient-experimental media (RPMI-1640, 2.5% FBS, 1% P/S). Cells were treated with compounds or solvent controls for 72 hours in experimental media. Preliminary cytotoxicity

assays assessed compound efficacy at 10 μM of compound. The concentration of 50% cytotoxicity (CC_{50}) for each compound was determined by fitting dose-response models using the dose-response curve (drc) package in R (https://cran.r-project.org/package=drc). CC_{50} assays were performed using 2-fold serial dilutions. The highest concentration of solvent control was used, never exceeding 0.5% (v/v). Cell viability was normalized to the solvent-control viability. Three technical replicates were performed per dose with at least three biological replicates.

2. **Synergy assay**

Cell viability upon combinatorial treatment was assessed by following previously described guidelines [22]. Commercially available AML chemotherapeutics (Doxorubicin [DOX] [Ark Pharm Inc.], cytarabine [ara-C] [Accela Chembio Inc], midostaurin [MID] [MedChemExpress], or venetoclax [VEN] [ChemieTek]) or other FLT3 inhibitors (gilteritinib [GIL] [Thermo Scientific Chemicals], quizartinib [QUIZ] [Targetmol Chemicals], sorafenib [SORA] [Medchemexpress]) were used.

Cells were seeded in 96-well plates and immediately treated for 72 hours. Single drug treatments and combinations were assessed in technical duplicates for at least three biological replicates. The highest DMSO concentration (not more than 0.5%) was used as the solvent-control. Viability was measured as in cytotoxicity assay to determine synergy for each combinatorial treatment using SynergyFinder package in R (https://synergyfinder.fimm.fi). Synergy scores were calculated using Bliss model. Bliss synergy scores indicate that scores larger than 10 are synergistic; scores between -10 and 10 are additive, and scores below -10 are antagonistic. We further categorized synergistic strength as follows: low synergy (10-20), medium synergy (>20-30), and high synergy (>30). Synergy plots were created using the same

package and visualized in a 2D format. Synergistic potential of combinations was determined by taking the average of maximum synergy (± standard error of the mean [SEM]) scores from at least three biological replicates. Corresponding PBMC synergy matrices were performed to assess potential synergy in healthy cells.

SM4 Evaluation of T/GR inhibition activity

1. Thioredoxin reductase activity (TrxR) assay

To assess TrxR activity, MOLM-13 cells seeded at density of 10^6 cells/mL were treated with PS127-compound at $CC_{50,\ 72hours}$ for 48 hours. DMSO was used as solvent-control. Cell lysates were prepared using sonication. TrxR activity was assessed using the Thioredoxin Reductase Colorimetric Assay Kit at 414 nm according to manufacturer's instructions. PS127-treated sample, rat liver TrxR as positive control, and solvent-control were assessed in duplicate.

2. GSH-to-GSSG ratio assay

The ratio of GSH to GSSG was assessed using the EnzyChrom™ Glutathione GSH/GSSG Assay Kit. MOLM-13 cells were treated with similar conditions as above. Samples were prepared following manufacturer's instructions for GSSG and total glutathione measurements. The amount of GSSG and total glutathione was determined from OD_{412nm} measurements at 0 and 10 minutes. GSH or GSSG concentration was normalized to total protein concentration, determined by Pierce™ BCA Protein Assay Kit and solvent-control.

3. Supplementation of exogenous redox metabolites

GSH (Thermo Scientific Chemicals), GSSG (TCI America™), N-acetylcysteine (NAC)

(Thermo Scientific Chemicals), α-tocopherol (Thermo Scientific Chemicals), and freshly prepared ascorbate (TCI Chemicals) were dissolved in sterile water. Redox metabolites were added as is into experimental media. MOLM-13 cells were simultaneously treated with redox metabolites and tested compounds at their 4X CC_{50} for 72 hours to assess rescue of cell viability. Cell viability was assessed as per cytotoxicity assay.

4. Glutathione reductase (GR) activity assay

Cells were treated with lead compounds (2 μM PS127E, 1 μM PS127_10, or 2 μM 125_1) or solvent control for 24 hours. Cells were lysed using sonication in ice and assessed using DetectX® Glutathione Reductase Fluorescence Activity Kit. Human GR was used as a reference. GR activity upon treatment was normalized to total protein concentration measured using Pierce™ BCA Protein Assay Kit.

SM5 Thermal shift assay

Differential scanning fluorimetry (DSF) was performed by combining 2.5 μM of human GR (Sigma-Aldrich), 20 μM of compound, and 5X of SYPRO® Orange Protein Gel Stain (ThermoFisher) in 20 mM HEPES, pH 7.4, 100 mM NaCl with 1% DMSO buffer solution [30]. The following controls were used: no protein controls, compound-fluoresce interference, and autofluorescence plate controls. The known GR inhibitor, 2-AAPA (Sigma-Aldrich), was used as a positive experimental control. Sealed PCR plates were continuously heated with gradual increase in temperature from 25 to 95°C at 1-minute intervals using the PREP BioRad qPCR instrument. RFU was then determined through the FRET channel. Melting temperature was determined by the derivative of relative fluorescence units (dRFU) using the open access tool,

DSFworld [31]. The thermal shift analysis was performed with the same materials as described above with a concentration gradient of PS127_10 from 2.5 to 40 μM. Analysis was performed in Thermott, using dRFU as the binding parameters and using their pre-determined thermodynamic parameters for the monomeric oligomer sequence (522 amino acids) [32]. Three technical replicates per four biological replicates were inputted into the binding curve to determine dissociation constant (K_d).

SM6 Bioenergetic measurements

1. **ROS and apoptosis evaluation**

Total and mitochondrial ROS were measured using dihydroethidium (DHE) (Invitrogen™) or MitoSOX™ Red (Invitrogen™) staining, respectively, as described in manufacturer's protocols. Annexin V-FITC (Thermo Fisher Scientific)/PI/Hoechst staining was used to detect live, apoptotic, and dead cells as previously described [13]. MOLM1-13 cells were seeded at a density of 10^6 cells/mL and treated with 8 μM of lead compound or 4 μM of PS127_10 for 24 hours. DMSO was used as solvent-control. Flow cytometry was performed on SONY MA900 Cell Sorter. Mean fluorescence intensities (MFIs) were analyzed in FlowJo software 10.8.1.

2. Oxygen Consumption Rate (OCR)

Instrumental background calibration with MiR05 (Oroboros) and oxygen air calibration with experimental RPMI-1640 media were performed according to standardized protocols at 37ºC in the NextGen-O2k instrument (Oroboros Instrument, Innsbruck, Austria).

MOLM-13 cells were treated for 3 hours with 2 μM of compound or DMSO at 10^6 cells/mL and transferred into the 2-mL Duran® glass chambers maintained at 37°C. Oxygen

concentration and negative slope were measured every 2 seconds and stirred at 750 rpm. Cell concentration and viability were measured with the Invitrogen Countess II Automated Cell Counter using Trypan-Blue (0.4%). OCR ($pmol \cdot s^{-1} \cdot Mx^{-1}$) was calculated from the average of oxygen negative slope over time ($pmol \cdot s^{-1}$) of a stable 5 min interval (~10 to 15 minutes after experimental start), normalized to the number of live million cells (Mx) per mL.

3. ATP measurements

To assess ATP levels, cells were seeded per well into an opaque 96-well plate and incubated with either 2 µM compound or corresponding volume of solvent control (DMSO) for 3 hours at a final volume of 100 µL in experimental RPMI-1640 medium. Each biological replicate was analyzed in three technical replicates. Viability was confirmed to be at least 90% based on Hoechst/PI staining before proceeding with cell lysis. After imaging, 50 µL of media was removed from each well. Subsequently, 50 µL of CellTiter-Glo® 2.0 (Promega) solution was added to each well. Plate was then kept in the dark, gently shaken for 1 minute to induce cell lysis, and incubated at room temperature for 10 minutes to allow luciferase activation. The luciferase signal was measured via luminescence on the BioTek plate reader with Gen5 software. The total ATP production was then calculated by dividing the luminescence signal by the total number of live cells per well.