

Supplementary Legends:

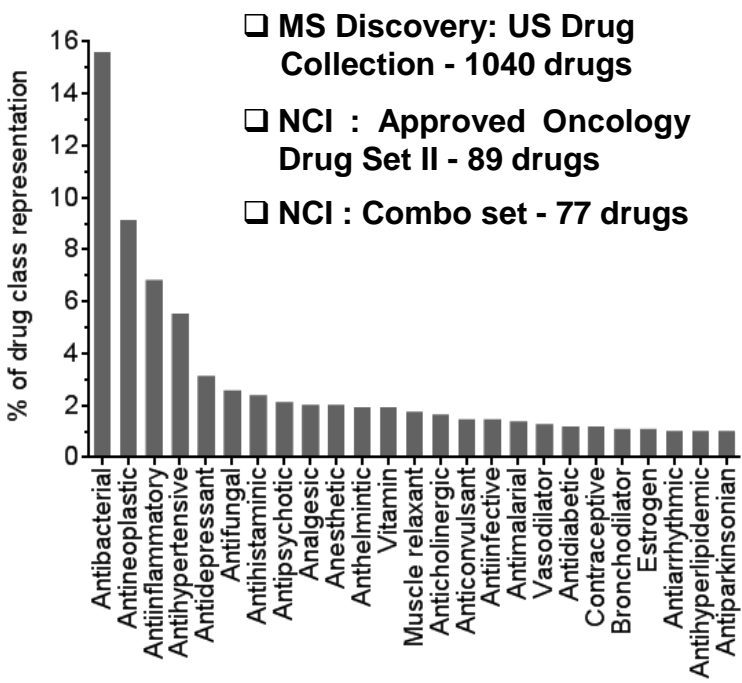
Supplementary Fig. S1: Percentage of drugs per drug class among drug libraries used in the HTS. Three libraries of FDA-approved drugs were used in this screen (MS Discovery US Drug Collection, NCI Approved Oncology Drug Set II and the NCI Combo Set).

Supplementary Fig. S2: Synergistic GFP reactivation in YB5 cells by HDACi TSA and positive hits identified in the HTS. A, Average values of all positive controls, included in each 96 well-plates (in quadruplicates), during HTS, with HDACi TSA (24h, 0.2 μ M) and decitabine (DAC, 72h, 50 nM) and their sequential combination. Statistical analysis was done by One-way ANOVA followed by the Tukey–Kramer Multiple Comparison Test ($P < 0.0001$). B, Validation experiment with digitoxin (24h treatment) in sequential combination after decitabine (72h treatment) and the doses indicated on the graph. GFP expression was measured immediately after digitoxin treatments by flow cytometry (n=3). C, Validation experiments with positive hits identified in the HTS in combination with decitabine (sequential and simultaneous) or with TSA (sequential).

Supplementary Fig. S3. Optimization of the treatment schedule of the decitabine and proscillaridin A combination. Time of exposure was modified to identify which schedule would induce the most potent GFP reactivation. Simultaneous combination of 24, 48, and 72h were compared with sequential combination with decitabine (48h, 100 nM) followed by proscillaridin A (24h and 48h at 50 nM).

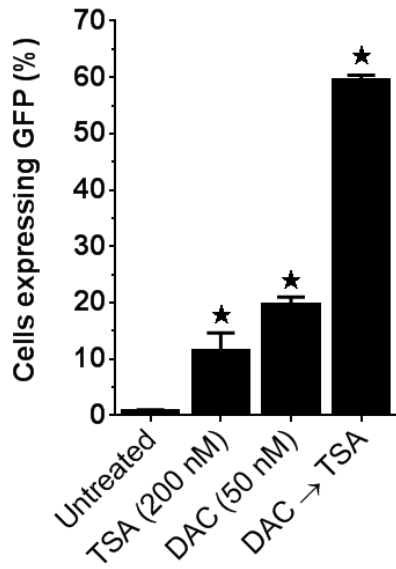
Supplementary Fig. S4. Transcriptome analysis using Metascape. A, Results of Metascape analysis in genes exclusively up-regulated with proscillaridin A treatment. B, Results of Metascape analysis in genes exclusively up-regulated by the combination treatment.

n=1,118 drugs

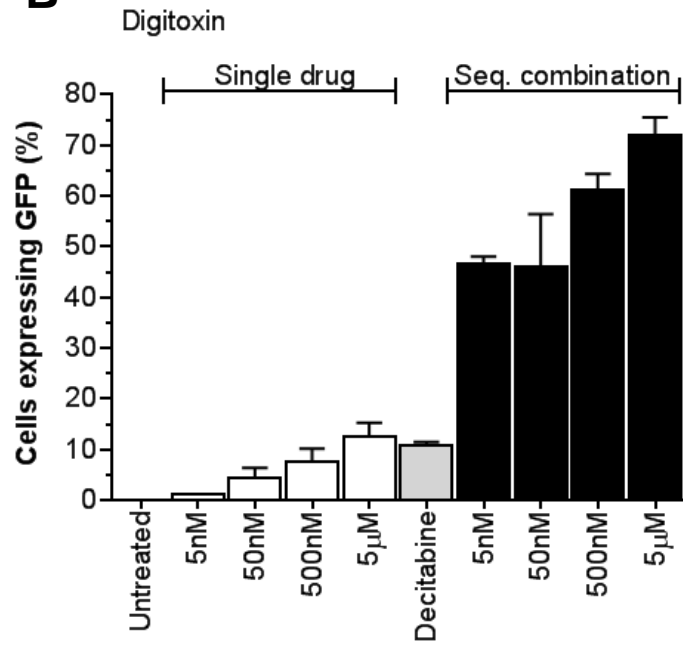


Supplementary Fig. S2.

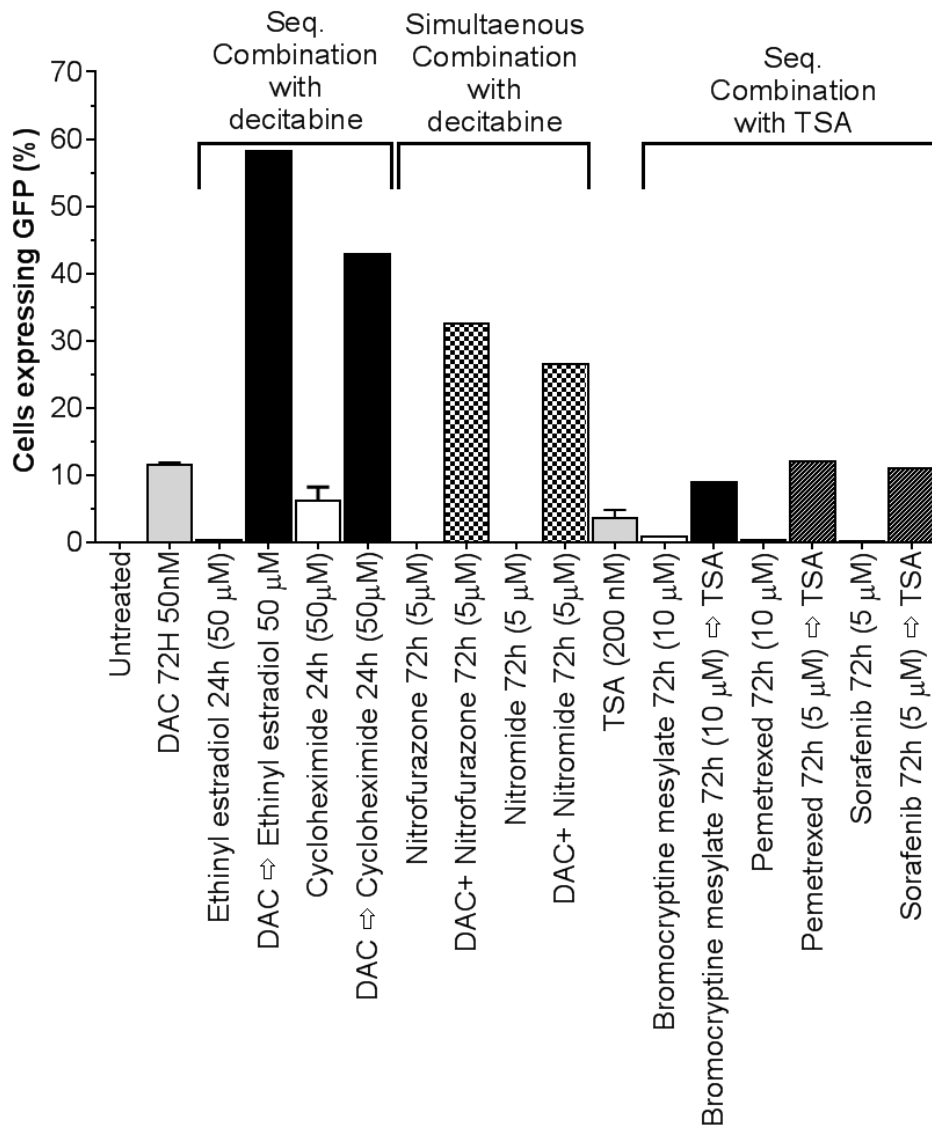
A



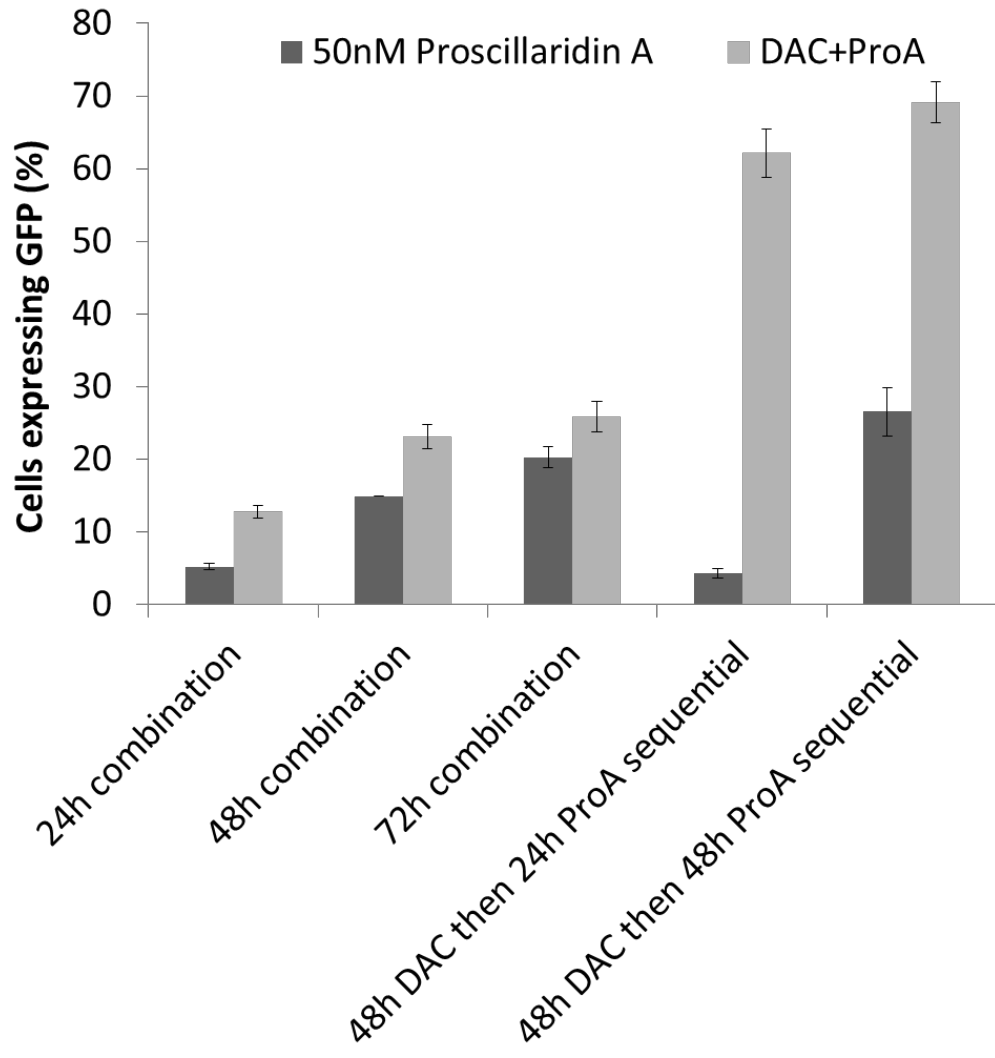
B



C



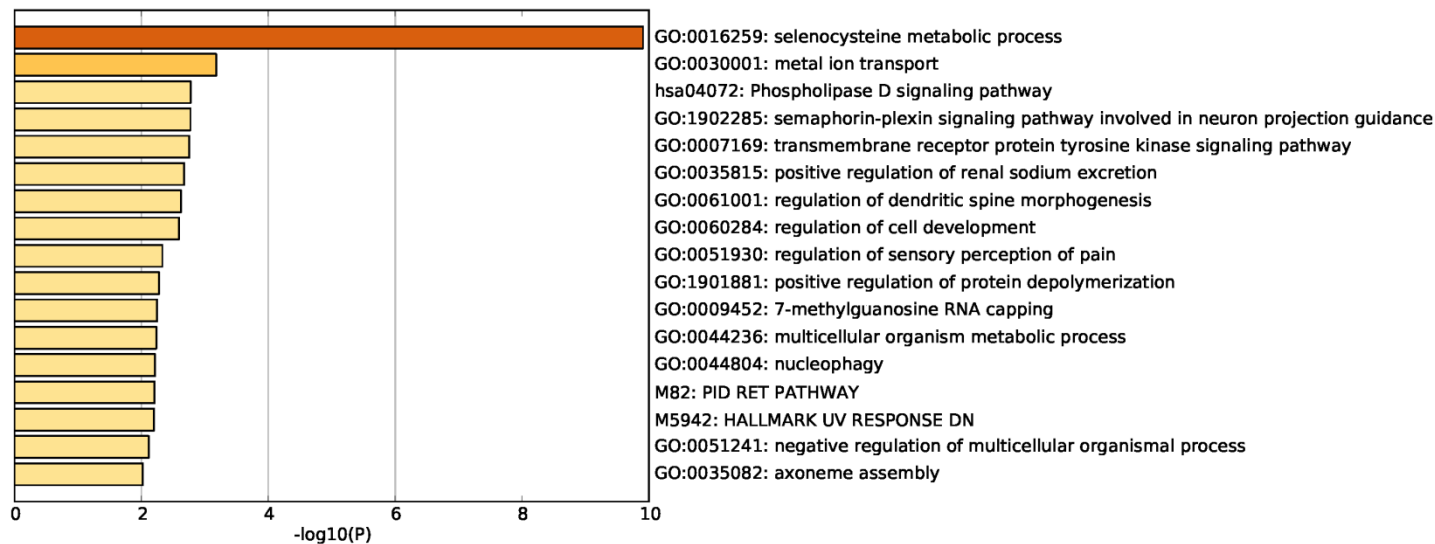
Supplementary Fig. S3.



Supplementary Fig. S4.

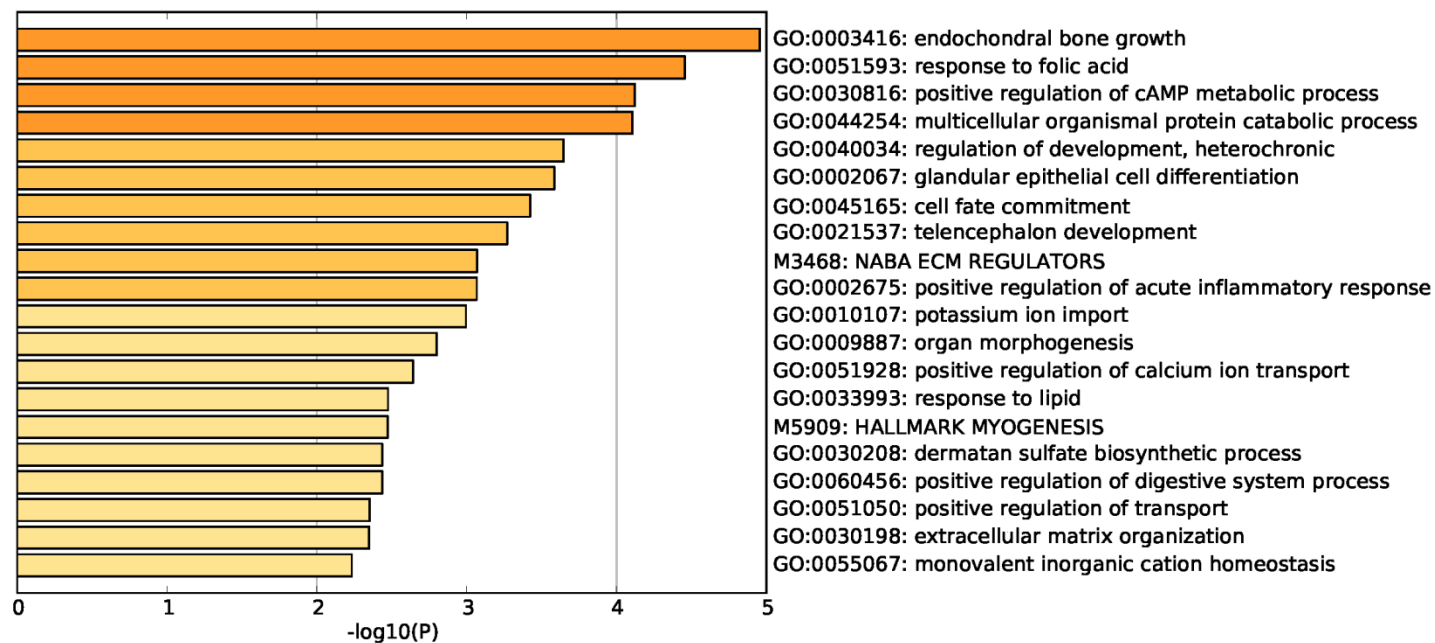
A

Up-regulated genes in Pros. A



B

Up-regulated genes in Combi.



Supplementary Table S1. List of GO-Terms identified by Metascape analysis in gene data sets exclusively up-regulated by proscillaridin A treatment and exclusively up-regulated by the combination treatment.

Treatment	GO-Term numbers	Descriptions
<u>Proscillaridin A</u> (non overlapping genes)	GO:0070509	Calcium Ion Import
	GO:0006816	Calcium Ion Transport
	GO:0006874	Cellular Calcium Ion Homeostasis
	GO:0051480	Regulation of Cytosolic Calcium Ion Concentration
<u>Combination</u> (non overlapping genes)	GO:0090280	Positive Regulation of Calcium Ion Import
	GO:0051928	Positive Regulation of Calcium Ion Transport
	GO:0051928	Positive Regulation of Calcium Ion Transport
	GO:0010524	Positive Regulation of Calcium Ion Transport Into Cytosol

Supplementary Table S2. List of GO-Terms identified by Metascape analysis among down-regulated genes shared by proscillaridin A and the combinatorial treatment with decitabine.

GO-Term numbers	Descriptions
GO:0031497	Chromatin Assembly
GO:0006333	Chromatin Assembly or Disassembly
GO:0006325	Chromatin Organization
GO:0006325	Chromatin Organization
GO:0006338	Chromatin Remodeling
GO:0031055	Chromatin Remodeling at Centromere
GO:0006342	Chromatin Silencing
GO:0000183	Chromatin Silencing at RDNA
GO:0032776	DNA Methylation on Cytosine
GO:0006304	DNA Modification
GO:0006335	DNA Replication-dependent Nucleosome Assembly
GO:0034723	DNA Replication-dependent Nucleosome Organization
GO:0006336	DNA Replication-independent Nucleosome Assembly
GO:0034724	DNA Replication-independent Nucleosome Organization
GO:0016458	Gene Silencing
GO:0043486	Histone Exchange
GO:0016570	Histone Modification
GO:0045814	Negative Regulation of Gene Expression, Epigenetic
GO:0006334	Nucleosome Assembly
GO:0034728	Nucleosome Organization
GO:0045815	Positive Regulation of Gene Expression, Epigenetic
GO:0040029	Regulation of Gene Expression, Epigenetic

Supplementary Table S3. Antagonistic hits identified in combination HTS with sequential decitabine treatment. Drug names, drug function and GFP ratio are shown.

<u>Combination</u>	<u>Drug name</u>	<u>Drug function</u>	<u>GFP Ratio</u>
<u>Sequential with decitabine</u>	Fluphenazine hydrochloride	Antipsychotic	0.00
	Penfluridol	Antipsychotic	0.00
	Toremiphen citrate	Anticancer	0.00
	Hexylresorcinol	Antiseptic	0.00
	Gentian violet	Antibacterial	0.00
	Econazole nitrate	Antifungal	0.01
	Tamoxifen citrate	Anticancer	0.01
	Thioridazine hydrochloride	Antipsychotic	0.01
	Selamectin	Anthelmintic	0.02
	Sertraline hydrochloride	Antidepressant	0.02
	Perhexiline maleate	Coronary vasodilator	0.02
	Gramicidin A	Antibacterial	0.02
	Mefloquine	Antimalarial	0.04
	Dioxybenzone	Sunscreen	0.04
	Estradiol valerate	Estrogen	0.05
	Perphenazine	Antipsychotic	0.05
	Cetylpyridinium chloride	Antiinfective	0.06
	Fluoxetine	Antidepressant	0.09
	Norgestimate	Contraceptive	0.09
	Diphenhydramine hydrochloride	Antihistaminic	0.10
	Thiothixene	Antipsychotic	0.11
	Triclosan	Antiinfective	0.15
	Astemizole	Antihistaminic	0.17
	Nitrogen mustard	Anticancer	0.19
	Mepartricin	Antifungal	0.21
	Nylidrin hydrochloride	Vasodilator	0.22
	Mechlorethamine	Anticancer	0.23
	Pyrithione Zinc	Antibacterial	0.24
	Ethinodiol Diacetate	Contraceptive	0.27
	Phenolphthalein	Cathartic	0.29
	Clotrimazole	Antifungal	0.30
	Mitoxantrone hydrochloride	Anticancer	0.32
	Estradiol cypionate	Estrogen	0.32
	Acrisorcin	Antifungal	0.33
Dactinomycin	Anticancer	0.40	
Methicillin sodium	Antibacterial	0.44	
Candesartan cilextil	Antihypertensive	0.45	

Supplementary Table S4. Antagonistic hits identified in combination HTS with simultaneous decitabine treatment. Drug names, drug function and GFP ratio are shown.

<u>Combination</u>	<u>Drug name</u>	<u>Drug function</u>	<u>GFP Ratio</u>
<u>Simultaneous with decitabine</u>	Methylbenzethonium chloride	Antiinfective	0.00
	Valrubicin	Anticancer	0.00
	Benzethonium chloride	Antiseptic	0.00
	Gentian violet	Antibacterial	0.00
	Dactinomycin	Anticancer	0.00
	Epirubicin hydrochloride	Anticancer	0.01
	Mitomycin	Anticancer	0.01
	Nitrogen mustard	Anticancer	0.01
	Nilotinib	Anticancer	0.02
	Gemcitabine hydrochloride	Anticancer	0.03
	Plicamycin	Anticancer	0.03
	Cytarabine	Anticancer	0.03
	Mitoxantrone hydrochloride	Anticancer	0.03
	Rapamycin	Anticancer	0.03
	Cladribine	Anticancer	0.03
	Mechlorethamine	Anticancer	0.03
	Topotecan hydrochloride	Anticancer	0.05
	Dipyridamole	Coronary vasodilator	0.05
	Everolimus	Anticancer	0.07
	Pyrrithione zinc	Antibacterial	0.07
	Clofarabine	Anticancer	0.09
	Dasatinib	Anticancer	0.09
	Amsacrine	Anticancer	0.10
	Teniposide	Anticancer	0.12
	Ciclopirox olamine	Antifungal	0.12
	Erlotinib hydrochloride	Anticancer	0.13
	Thimerosal	Antiinfective	0.14
	Fuchsin N	Anthelmintic	0.14
	Tyrothricin	Antibacterial	0.15
	Cetylpyridinium chloride	Antiinfective	0.15
	Triethylenemelamine	Anticancer	0.16
	Gefitinib	Anticancer	0.16
	Vinorelbine tartrate	Anticancer	0.18
	Cycloheximide	Antipsoriatic	0.18
	Methylene blue	Antimethemoglobinemic	0.19
	Hexachlorophene	Antiinfective	0.19
	Etoposide	Anticancer	0.19
	Benzalkonium chloride	Antiinfective	0.20
	Fluorouracil	Anticancer	0.21
	Ixabepilone	Anticancer	0.22
Bortezomib	Anticancer	0.25	
Paclitaxel	Anticancer	0.28	
Aminacrine	Antiseptic	0.29	

Supplementary Table 4 (continued).

<u>Combination</u>	<u>Drug name</u>	<u>Drug function</u>	<u>GFP Ratio</u>
<u>Simultaneous</u>	Oxaliplatin	Anticancer	0.31
<u>With decitabine</u>	Bromocriptine mesylate	Antiparkinsonian	0.32
	Sirolimus	Anticancer	0.32
	Azacitidine	Anticancer	0.33
	Avermectin B1	Antiparasitic	0.33
	Ouabain	Heart failure treatment	0.33
	Irinotecan hydrochloride	Anticancer	0.33
	Fenbendazole	Anthelmintic	0.34
	Mebendazole	Anthelmintic	0.34
	Oxyphenbutazone	Antiinflammatory	0.34
	Thiostrepton	Antibacterial	0.36
	Monensin A	Antibacterial	0.37
	Selamectin	Anthelmintic	0.38
	Tacrolimus	Immunosuppressant	0.39
	Clioquinol	Antiseptic	0.40
	Mycophenolic acid	Immunosuppressant	0.40
	Acrisorcin	Antifungal	0.40
	Nonoxynol-9	Contraceptive	0.43
	Floxuridine	Anticancer	0.45
	Podofilox	Anticancer	0.48
	Doxazosin mesylate	Antihypertensive	0.50
	Bleomycin B2	Anticancer	0.52
	Hycanthone	Anthelmintic	0.57
	Uracil mustard	Anticancer	0.58
	Docetaxel	Anticancer	0.59
	Vinblastine sulfate	Anticancer	0.59

Supplementary Table S5. Antagonistic hits identified in combination HTS with sequential TSA treatment. Drug names, drug function and GFP ratio are shown.

<u>Combination</u>	<u>Drug name</u>	<u>Drug function</u>	<u>GFP Ratio</u>
<u>Sequential with TSA</u>	Valrubicin	Anticancer	0.00
	Epirubicin hydrochloride	Anticancer	0.00
	Dactinomycin	Anticancer	0.00
	Plicamycin	Anticancer	0.02
	Mitoxantrone	Anticancer	0.02
	Teniposide	Anticancer	0.09
	Penicillin V potassium	Antibacterial	0.11
	Cycloheximide	Antipsoriatic	0.15
	Oxymetazoline hydrochloride	Nasal decongestant	0.17
	Bortezomib	Anticancer	0.17
	Fuchsin N	Anthelmintic	0.18
	Mechlorethamine	Anticancer	0.19
	Nitrogen mustard	Anticancer	0.19
	Mepenzolate bromide	Anticholinergic	0.20
	Benzethonium chloride	Antiseptic	0.23
	Ivermectin	Antiparasitic	0.23
	Methscopolamine bromide	Anticholinergic	0.24
	Mebendazole	Anthelmintic	0.24
	Novobiocin sodium	Antibacterial	0.25
	Fenbendazole	Anthelmintic	0.25
	Tyrothricin	Antibacterial	0.25
	Nylidrin hydrochloride	Vasodilator	0.26
	Nifedipine	Antihypertensive	0.27
	Mafenide hydrochloride	Antibacterial	0.27
	Lincomycin hydrochloride	Antibacterial	0.31
	Minocycline hydrochloride	Antibacterial	0.32
	Minoxidil	Antihypertensive	0.32
	Menadione	Prothrombogenic agent	0.33
	Gramicidin A	Antibacterial	0.36
	Noscapine hydrochloride	Antitussive	0.36