SUPPLEMENTARY INFORMATION

Efficient discovery of anti-inflammatory small molecule combinations using evolutionary computing

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SUPPLEMENTARY METHODS

All procedures, protocols and methods were carried out under aseptic conditions where deemed necessary.

SINGLE REAGENT STUDIES

Preparation of primary peritoneal macrophages

Batches (4 per batch) of C57BL/6J male mice (6 - 8 weeks old) were sacrificed by rising carbon dioxide asphyxiation in accordance with schedule 1 of the Animals (Scientific Procedures) Act 1986 as issued by the Home Office, U.K. Slow intra-peritoneal (i.p) injection of pre-warmed (37°C, 8 mL) supplemented Dulbeccos Modified Eagles Medium (DMEM: penicillin and streptomycin (5 µg / mL); and foetal calf serum; 2.5%) was performed into the abdominal area of cadavers. Peritoneal lavage was carried out and an incision made into the abdominal surface to create a pocket to drain the lavage fluid. Lavage fluid was aspirated using a hypodermic syringe and titrated into a conical tube (15 mL).

A cell count of peritoneal macrophages was conducted using an Improved Neubauer haemocytometer. Macrophages were discriminated (large, circular, phase bright) from other cell types by their morphology. Lavage fluid was centrifuged (250g, 5 min) and the resultant pellet re-suspended in fresh, pre-warmed (37°C) DMEM to a cell concentration of 5.0 X 10^5 cells / mL. Aliquots (200 µL) of cell suspension were subsequently pipetted into 96-well plates (Costar, Corning; 3596) to ensure 100,000 cells / well. Plates of cells were maintained at 37°C in a humidified 95 % O_2 / 5 % CO_2 atmosphere for up to 96 h prior to use.

Preparation of single reagents and lipopolysaccharide (LPS) stimulation of macrophages for assessment of inhibiton of IL-1β expression

Stock reagents (Supplementary table 1) were prepared gravimetrically wherever possible, reconstituted to 20 mM in dimethylsulphoxide (DMSO) and serially diluted in $\log_{10}$ spacings in DMSO down to concentrations of 2 µM. The five stock concentrations of reagent were further diluted (1 : 20) into DMEM at 10 X their final concentration (0.1 µM - 1 mM). Peritoneal macrophages were exposed to reagents for 30 min prior to LPS stimulation. LPS (Sigma, serotype O26: B6) was diluted from a 1 mg / mL stock (in Phosphate Buffered Saline; PBS). LPS stimulated reactions were terminated after 4 h by addition of homogenising buffer (pH 7.6, 0.02 % NaN) supplemented with Triton X-100 (1 % v/v) and protease inhibitor cocktail (1 % v/v; Calbiochem). Plates were left on ice with regular agitation (to ensure maximal lysis of cells) for 20 min prior to freezing.

IBEA DIRECTED EVOLUTIONARY SEARCH OF COMBNATIONS INHIBITING IL-1β EXPRESSION

Multi-objective optimization of a chemical library and the implementation of an evolutionary algorithm: IBEA.

A multi-objective optimization problem (MOOP) of generic form can be defined as[1]:

Minimize

$$f(\vec{x}) = (f_1(\vec{x}), f_2(\vec{x}), \ldots, f_m(\vec{x})). \qquad \text{subject to } \vec{x} \quad \text{in } X \qquad (1)$$

where $\vec{x} = (x_1,\ldots,x_n)$ is a solution vector (here representing a particular combination of reagents) consisting of n decision variables (i.e. different reagents) for which values must be found and X the feasible search space. The function $f{:}X \rightarrow Y$ represents a mapping from X into the objective space Y in R^m. In general, a MOOP admits no single optimal solution, but rather a set of optimal trade-off solutions exist (also called Pareto-optimal solutions), none of which can be improved in all objectives simultaneously. The aim is to find this solution set, or an approximation of it, called the approximation set.

Our solution vectors represent whether or not to include a particular reagent in a combination or not (the concentration of reagents is fixed), and there are no constraints restricting the search space, hence the space X is binary or $\vec{x} \in \{0,1\}^n$ where n is the number of reagents. A 1 in the vector at position j means that the jth reagent is included in the cocktail, a 0 means do not include it. There are three objectives f_i, i

=1, 2, 3, to be minimized: IL-1β expression, LDH release and the number of component reagents in a given combination. The aim of the search process is to find reagent combinations that display the best trade-off between these objectives.

The field of evolutionary computation has proposed several techniques to solve MOOPs efficiently: after preliminary experimentation, we settled on using the indicator-based EA (IBEA) [2]. The aim of IBEA is find a set of Pareto-optimal solutions that that maximizes some unary performance measure (indicator), which here is the hypervolume of the objective space dominated by the approximation set. Our experimental run of IBEA used the parameters given in Supplementary Table 2

To monitor the progress of the evolutionary search, we use a population average rank measure (Figure 1(C)). This is a nonparametric statistic related to the familiar rank sum tests, Mann-Whitney U and Kruskal-Wallis. All reagent combinations evaluated up to the current generation *G* are labeled by the generation in which they were tested. These samples are then ranked by fitness, and, for each generation *g*=1 to *G*, the average of the ranks associated with generation *g* is computed. The standard error of these ranks is also shown in the plots. This ranking statistic is computed separately for each of the three objectives, and also for the IBEA indicator (i.e. the three objectives combined).

Construction, storage and maintenance of the chemical library

Reagents were purchased from either Sigma-Aldrich, Tocris, Chembridge Cheng-Du Biopurify Phytochemicals or Merck Chemicals and displayed purities ≥ 95 % as assessed by HPLC or TLC (except SIH; ≥ 90 % assessed by NMR). All compounds were prepared wherever possible gravimetrically and dissoluted into DMSO (Supplementary table 1) to a stock concentration of 15 mM. Subsequently, reagents were pipetted into a DMSO resistant, 2.0 mL, 96 deepwell plate (Starlab, E2896-0200) and sealed with a chemically resistant silicone mat (Starlab, E2896 3200) before being stored in an airtight plastic box partially filled with silica gel (Fisher Scientfic, S/0761/53) and kept in the dark at room temperature. Stock reagents were transferred periodically to new deep well plates to ensure their pharmacological integrity. A list of reagents and associated information (i.e. physicochemical properties) is given in Supplementary table 1.

Production of combinatorial chemical cocktails

The generation of poly-combinatorial mixtures of compounds was conducted using a Sciclone ALH 3000 Liquid Handling Workstation under the control of Maestro and HitPicking (v9.0) software (Both Caliper Life Sciences) running on a Windows XP platform on a personal computer. Compound combinations were specified in a 'Hitlist.csv' file; each line specifying the source and destination plate locations on the ALH3000 deck, source and destination well identities (e.g. D1, H12) and the volume (in µL) of compound required for transfer from the source to the destination plate. Populations of combinations and vehicle wells were constrained to 50 and 5 wells per plate respectively (with an additional 5 empty wells per plate) and randomly assigned to positions D1 – H12 across the plate to control for position-dependent plate effects (Supplementary figure 7) in accordance with the following equation (2).

$$P_i = (k - n)^{-1} \quad (2)$$

Where P_i is the probability of the i^{th} well position being assigned to one of the following treatments: a combination well, vehicle well or empty well. The total number of k wells available (i.e. in this instance; 60) and n the number of wells currently assigned to any treatment. Low volume (≥ 5 µL) dispense operations were executed after higher volumes of solvent (≥ 90 µL) had already been pipetted to ensure complete dispensing of these low volumes. Combinations were tested at a final concentration of 3 µM (The detail behind the serial dilutions of combinations and the % solvent at each step of the transfer are detailed in Supplementary figure 1).

Preparation of J774.A1 macrophages

J774.A1 macrophages were a kind gift from Prof. Anne-Marie Suprenant to DB's lab, used between passages 12 – 19 and maintained in Dulbeccos modified eagles medium (DMEM) supplemented with high serum (10%, FBS) and penicillin / streptomycin (5 µg / mL). Cells were split once 80 % confluency had been attained. Briefly, cells were removed from the base of a T75 flask (Costar) by aspirating existing media and replacing with a pre-warmed (37°C) aliquot (5 mL) of supplemented DMEM prior to using a cell scraper to remove the cell monolayer. After counting the resulting cell suspension was centrifuged (250 g, 5 min), the supernatant discarded and the resultant pellet re-suspended in fresh, pre-warmed (37°C) high serum DMEM to a cell concentration of 6.5 X 10^5 cells / mL. Aliquots (200 µL) of cell suspension were subsequently pipetted into 96-well plates (Costar, Corning; 3596) and were maintained at 37°C in a humidified 95 % O_2 / 5 % CO_2 atmosphere 24 h prior to use.

Treatment of J774.A1 macrophages with chemical combinations

Previously prepared J774.A1 macrophages were treated with either chemical combinations (all component compounds at 3 µM) or IKK inhibitor (100 µM) or DMSO (0.5 % v/v) alone for 10 minutes prior to stimulation with LPS (1 µg / mL) or PBS (0.1% v/v). Subsequently, cells were maintained at 37°C in a humidified 95 % O_2 / 5 % CO_2 atmosphere for 2h. After this time, triplicate wells on each plate were exposed to lysis buffer (20 µL, 1X final concentration, Promega) prior to being returned to the incubator for a remaining ten minutes. Immediately after this time, aliquots (50 µL) of well supernatants were taken and pipetted into a new 96-well plate

for the measurement of LDH release (see below). Termination of IL-1β expression and lysis of cells was conducted identically to that during the single reagent studies.

MEASUREMENT OF LDH & IL-1β EXPRESSION

Lactate dehydrogenase (LDH) cell viability measurements

This protocol is adapted from the recommended procedure provided with the CytoTox™ 96 Non-Radioactive Cytotoxicity Assay kit (Promega ; G1780). Well supernatants (50 µL aliquots) were pipetted into a fresh 96-well plate prior to the addition of aliquots (50 µL) of substrate mix and covering and wrapping of the plate with laboratory film (Parafilm) and foil respectively. The plate was placed on a mini-orbital shaker (50 rpm) for 30 minutes and subsequent addition of stop solution (acetic acid; 50 µL) added to each well prior to reading on a plate reader (Biotek Synergy HT; single reagent studies or FLUOstar Omega for combinatorial studies respectively) at an absorbance of 490 nm (A_{490nm}). During single reagent studies, treated wells (LPS and compounds) were compared to control wells (DMSO alone) to assess if there had been a reduction in cell viability. Assessment of treated wells during the combinatorial study was conducted by expressing the A_{490nm} of each well to that observed from the mean A_{490nm} (of triplicate wells) that had been exposed to lysis buffer and then normalising this value relative to that seen for positive control (quintuplicate) wells.

IL-1β ELISA

This protocol is adapted from the recommended procedure provided with the mouse IL-1β/IL-1F2 DuoSet ELISA development kit (R&D Systems; DY401). Coating of 96-well plates (Maxisorp) with aliquots (50 µL) of rat anti-mouse IL-1β capture antibody (cAb; 4.0 µg / mL) was conducted and the plates left on a mini orbital shaker

(50 rpm) overnight to ensure an even coating of cAb on all wells prior to the analysis of macrophage lysates.

Plates coated with cAb were aspirated of their cAb solution, washed three times with wash buffer (PBS with 0.05 % v/v Tween 20) after which any remaining well contents were blotted onto an absorbent paper towel until dry. This procedure was followed for all wash steps forthwith. Following this, blocking buffer (PBS and 1 % w/v BSA, Sigma) was aliquoted (50 µL) onto all wells, after which plates were covered with laboratory film and placed on a mini orbital shaker (50 rpm) until dilutions for the IL-1β standard curve had been made.

All IL-1β standards were made by reconstitution into lysis buffer (homogenising buffer supplemented with TX-100; 1% v/v). A recombinant mouse IL-1β standard (360 ng / mL, 3 µL) was diluted (537 µL) to produce a final concentration of 2 ng / mL of a top standard (S1), this was further serially diluted by halving its concentration, this procedure was repeated to produce 9 standards in total, descending to a concentration of 7.8 pg / mL (S9). The blocked plate was subsequently aspirated of the blocking buffer prior to the aliquoting (50 µL) of samples and standards onto the plate Plates were covered with laboratory film and placed on a mini orbital shaker (50 rpm) for 2 hours at room temperature (RT) prior to detection.

Detection of bound IL-1β was carried out by aspirating plate wells of their samples and standards. A biotinylated goat anti-mouse IL-1β detection antibody (dAb; 108 µg / mL) was diluted (10 µL) in blocking buffer (7.5 mL) to furnish a final concentration of (144 pg / mL) and aliquoted (50 µL) onto all wells prior to covering with

laboratory film and placing this plate on a mini orbital shaker (50 rpm) for 1 h. Subsequently, plates were aspirated of their dAb solution, Streptavidin-Horse Radish Peroxidase (Strep-HRP) solution (50 µL) was diluted into blocking buffer (10 mL) and aliquoted (50 µL) onto wells before the plate was covered with laboratory film and foil and placing on a mini-orbital shaker (50 rpm) for 20 mins. 3, 3', 5, 5' tetramethyl benzidine (TMB) substrate reagents A and B were mixed in equal volumes (2.5 mL : 2.5 mL) in a foil covered tube (15 mL), plates were aspirated of their Strep-HRP solution and aliquots (50 µL) of the mixed TMB substrate reagents added to all wells of the plate, covered with laboratory film and foil and left on a mini-orbital shaker for 20 min. Finally, plates were incubated with aliquots (50 µL) of dilute sulphuric acid (1M) and the resultant chromophore measured at an absorbance of 450 nm (A_{450nm}) and background subtracted for non-specific fluorescence by measuring at 570 nm (A_{570nm}) on a plate reader (Biotek Synergy HT).

Data analysis was conducted by normalizing absorbance readings to LPS (1 µg / mL) and DMSO (0.5 % v/v) positive controls in order to ascertain % inhibitions / potentiations of combinations. In instances where the majority of wells had not exceeded the IL-1β concentration of the top standard (2 ng / mL) measured, a standard curve was constructed and IL-1β concentrations interpolated for sample wells. However, in all cases % changes versus positive control responses are quoted.

REAGENT REMOVAL / ADDITION AND DATA ANALYSIS

Removal and replacement of reagents within the chemical library during the IBEA search

During the course of the evolutionary search it was necessary to remove and introduce reagents respectively into the chemical library. A simulation study revealed that it is possible to substitute small numbers of reagents (< 5) without undue effects on the course of the evolutionary search[3]. To this end, solutions where these single reagents were present after generation 3 were discarded from the retained elite solutions and a new generation (4) built. Similarly, Epigallocatechin gallate (ECGC) and Simvastatin were added to the chemical library from generation 4. Melatonin, was not introduced until generation 10, in order to compensate for this reagents late arrival into the library it was assigned a higher probability of selection (1 / 5) across all bits present.

Data analysis

Quantile-Quantile plots of the positive control (LPS & DMSO) responses plotted against theoretical quantiles drawn from a normal distribution confirmed the data were normally distributed (Supplementary Figure 8)[4].

Means and standard errors of the mean (SEM) of data are given as a function of the number of preparations of cells (where primary cells were used). That is 2 - 4 animals were used per preparation or plate of cells (n=1). Measurements were averaged across a minimum of triplicate wells per batch. Significance was assessed using a one-way ANOVA and appropriate post-hoc multiple comparison tests reporting significance at the $P<0.05$(*), $P<0.01$ (**) and $P<0.0001$ (***) levels.

Means and standard error of the mean of data are given as a function of the number of plates of cells per generation tested and were used for making inferences about the relative efficacy of combinations during the evolution directed and dose-matrix adaptive search strategies. A statistical measure of assay quality; Z'-factor [5] was calculated retrospectively for each generation of combinations tested in accordance with the following equation (3).

$$Z' = 1 - \frac{3(\hat{\sigma}_p + \hat{\sigma}_n)}{\left|\hat{\mu}_p - \hat{\mu}_n\right|} \tag{3}$$

Where *Z'* is an estimate of the size of the statistical effect, $\hat{\sigma}_p$ and $\hat{\sigma}_n$ are the sample standard deviations of the positive and negative controls respectively and $\hat{\mu}_p$ and $\hat{\mu}_n$ .the sample means for positive and negative controls.

SUPPLEMENTARY RESULTS

THE EFFECT OF SINGLE REAGENTS ON IL-1β EXPRESSION IN PERITONEAL MACROPHAGES

We characterized the potency and efficacy of single reagents selected to affect nodes (i.e. signalling proteins and processes respectively in this context)within the TLR4 signalling network and thought important for the expression of IL-1β[6] in LPS-stimulated murine peritoneal macrophages. In total, 24 different reagents were assessed over a 0.01 – 100 μM concentration range from the following pharmacological classes: NF-κB, MAPK and PI3kinase inhibitors, pro / anti-oxidants and iron chelators (which indirectly effect antioxidant activity[7,8]). In summary, VK-28, α-tocopherol succinate, PDTC, SB203580, LY294002, U0126 and IKK inhibitor III were effective inhibitors of IL-1β expression at concentrations between 1 and 100 μM (statistically significant at $P<0.05$ (*) and $P<0.01$ (**) levels by one-way ANOVA and Dunnett's test; see supplementary methods and Supplementary Fig. 2). Our subsequent experiments were designed to discover combinations of these reagents that have inhibitory effects at lower concentrations than when used in isolation. A fixed concentration of 3 μM was adopted, that was broadly sub-optimal in inhibiting IL-1β expression by any of these reagents used alone.

REAGENT & ASSAY QC / VALIDATION

Reagent physicochemical properties

In order to check whether there were common physicochemical parameters that may have influenced the biological activity of combinations within the search we attempted to mine these parameters for all reagents within the chemical library (Supplementary table 1). This was made possible by the recent provision of the publically accessible ChEMBL database of bioactive drug like small molecules (https://www.ebi.ac.uk/chembl/) AlogP is a calculated measure of the partition coefficient or lipophillic character of a drug molecule and is a ratio of a molecule's dispersion between organic solvent and aqueous phases. There was no obvious trend in AlogP for the prioritized reagents (i.e. SB203580; 3.88, SIH; 1.29 and Simvastatin; 4.63.) we derived from the IBEA search. However, all shared similar polar surface area (PSA) estimates, higher numbers of hydrogen bond accepting (HBA) moieties versus their hydrogen bond donating (HBD) counterparts and no rule of 5 violations. Unfortunately, owing to SKI-II's absence from the ChEMBL database we were unable to derive parameters for this molecule. Clearly, assessment of physicochemical parameters can form another criterion for proposing reagents to populate chemical libraries.

Assessment of assay discrimination

The ability of the IBEA[2] directed semi-automated robotic assay of chemical combinations generated to inhibit IL-1β expression was scrutinized retrospectively via calculation of the Z'-factor (see data analysis) statistic (Supplementary table 2 and Supplementary figure 6) to determine its ability to locate 'true' efficacious combinations and discriminate against trivial solutions. Although in two instances (at generations 2 and 5) this statistic fell beneath the acceptable range [9] across all other generations the assay was judged to have been of an acceptable or excellent standard and further to this the mean and median values for this statistic were 0.33 and 0.35 respectively, indicating overall that the assay was of an acceptable quality. In summary, this statistic in tandem with the canonical abilities of the IBEA to locate combinations in the presence of 80 % noise in IL-1β expression (see main paper; methods) point to a robust assay for determining novel combinatorial solutions

TABLES

Name	Supplier	Cat#	Purity (≥ %)	Lot#	Mwt	Pharmacological Class*	chEBI ID	Parent Mwt	AlogP	PSA	HBA	HBD	RO5
alpha-tocopherol	Sigma	T3251	96.0	078K1882	430.7	Antioxidant	103345	430.7	10.44	29.46	2	1	1
EGCG	ChengDu Biopurify Phytochemicals	E07018	99.3	E090715	458.4	Antioxidant	167987	458.4	3.10	197.36	11	8	2
alpha-tocopherol succinate	**Sigma**	**T3126**	**98.0**	**14H0063**	**530.8**	**Prooxidant**	**229103**	**530.8**	**10.30**	**72.83**	**5**	**1**	**2**
NAC	**Sigma**	**A9165**	**99.0**	**80K13495**	**163.2**	**Antioxidant**	**120881**	**163.2**	**-0.58**	**105.20**	**4**	**3**	**0**
Rotenone	Calbiochem	557368	98.0	D00055180	394.4	Antioxidant	102812	394.4	3.93	63.22	6	0	0
TEMPOL	**Tocris**	**3082**	**NA**	**1A/85506**	**172.2**	**Antioxidant**	**292703**	**173.3**	**0.32**	**43.70**	**3**	**2**	**0**
Calpain III	**Calbiochem**	**208722**	**95.0**	**B29649**	**382.5**	**Enzyme inhibitor**	**131775**	**382.5**	**3.53**	**84.50**	**4**	**2**	**0**
CA-074	*Sigma*	*C5732*	*99.0*	*NA*	*383.4*	*Enzyme inhibitor*	*560554*	*383.4*	*0.26*	*128.34*	*6*	*3*	*0*
NS-398	*Calbiochem*	*349254*	*98.1*	*D00017723*	*314.4*	*Enzyme inhibitor*	*439249*	*328.4*	*2.68*	*100.81*	*5*	*0*	*0*
GM-6001	*Calbiochem*	*364205*	*98.0*	*B69073*	*388.5*	*Enzyme inhibitor*	*123466*	*388.5*	*1.62*	*123.32*	*4*	*5*	*0*
Melatonin	Sigma	M5250	99.0	098K1117	232.3	Hormone	103012	232.3	1.56	54.12	2	2	0
Desferrioxamine mesylate	Sigma	D9533	100.0	128K1205	656.8	Iron chelator	330444	560.7	-0.89	205.83	9	6	2
SIH	**Chembridge**	**5109995**	**90.0**	**610065**	**225.3**	**Iron chelator**	**434094**	**241.3**	**1.29**	**74.58**	**4**	**2**	**0**
VK-28	**Sigma**	**V4264**	**97.0**	**034K46251**	**287.4**	**Iron chelator**	**NA**						
SR11302	Tocris	2476	99.3	1A/89800	376.5	MAPK pathway inhibitor	NA						
PD98059	*Calbiochem*	*513000*	*98.0*	*D00048465*	*267.3*	*MAPK pathway inhibitor*	*150222*	*267.3*	*2.37*	*61.55*	*4*	*1*	*0*
U0126 monoethanolate	**Calbiochem**	**662005**	**98.0**	**D00058335**	**403.5**	**MAPK pathway inhibitor**	**257660**	**380.5**	**2.18**	**202.26**	**8**	**4**	**0**
U0126 monoethanolate	**Tocris**	**1144**	**99.4**	**4A/93229**	**426.5**	**MAPK pathway inhibitor**	**257660**						
U0126 monoethanolate	**Tocris**	**1144**	**99.4**	**4A/97041**	**426.5**	**MAPK pathway inhibitor**	**257660**						
IRAK 1/4 inhibitor	**Calbiochem**	**407601**	**99.9**	**D00050155**	**395.4**	**MAPK pathway inhibitor**	**449907**	**395.4**	**2.60**	**105.21**	**6**	**1**	**0**
IRAK 1/4 inhibitor	**Calbiochem**	**407601**	**99.9**	**D00057214**	**395.4**	**MAPK pathway inhibitor**	**449907**						
SP600125	**Calbiochem**	**420119**	**98.0**	**D00002116**	**220.2**	**MAPK pathway inhibitor**	**431500**	**220.2**	**2.99**	**45.75**	**2**	**1**	**0**
SB203580	**Sigma**	**S8307**	**98.0**	**029K4619**	**377.4**	**MAPK pathway inhibitor**	**100250**	**377.4**	**3.88**	**77.84**	**3**	**1**	**0**

Name	Supplier	Cat#	Purity (≥ %)	Lot#	Mwt	Pharmacological Class*	chEBI ID	Parent Mwt	AlogP	PSA	HBA	HBD	RO5
SB203580	**Tocris**	**1202**	**98.7**	**4A/93107**	**377.4**	**MAPK pathway inhibitor**	**100250**						
2,4-dinitrophenol	**Sigma**	**D19,850-1**	**97.0**	**554589-308**	**184.1**	**mitochondrial uncoupler**	**110392**	**184.1**	**1.38**	**111.87**	**5**	**1**	**0**
CCCP	Sigma	C2759	97.0	088K1511	204.6	mitochondrial uncoupler	474035	204.6	2.47	71.97	4	1	0
FCCP	Tocirs	0453	99.0	2A/93205	254.2	mitochondrial uncoupler	615435	254.2	3.92	81.20	5	1	0
Apocynin	**Calbiochem**	**178385**	**99.9**	**D00055492**	**166.2**	**NADPH oxidase inhibitor**	**364242**	**166.2**	**1.31**	**46.53**	**3**	**1**	**0**
diphenylene iodonium (DPI)	Calbiochem	300260	100.0	D00057661	314.6	NADPH oxidase inhibitor	491098	279.1					
IKK inhibitor III (BMS-345541)	**Calbiochem**	**401480**	**96.0**	**D00034862**	**255.3**	**NF-kB pathway inhibitor**	**513905**	**255.3**	**1.00**	**68.23**	**4**	**2**	**0**
IKK inhibitor III (BMS-345541)	**Sigma**	**B9935**	**98.0**	**077K462Z2**	**291.8**	**NF-kB pathway inhibitor**	**513905**						
PDTC	**Sigma**	**P8765**	**99.0**	**1299391 / 106K1856**	**164.3**	**NF-kB pathway inhibitor**	**521017**	**147.3**	**2.48**	**74.13**	**2**	**1**	**0**
CAPE	*Calbiochem*	*211200*	*97*	*NA*	*284.3*	*tyrosine kinase inhibitor*	*271563*	*284.3*	*3.57*	*66.80*	*4*	*2*	*0*
Wortmanin	**Sigma**	**W1628**	**99.0**	**029K4036**	**428.4**	**PI3-Kinase inhibitor**	**111985**	**428.4**	**1.71**	**109.11**	**7**	**0**	**0**
LY294002.HCl	**Sigma**	**L9908**	**98.0**	**017K4616**	**343.8**	**PI3-Kinase inhibitor**	**257152**	**307.3**	**3.34**	**38.76**	**4**	**0**	**0**
LY294002.HCl	**Tocris**	**1130**	**99.4**	**3*A/93270**	**343.8**	**PI3-Kinase inhibitor**	**257152**						
NSC23766	Tocris	2161	99.0	2A/93470	558.0	Small GTPase inhibitor	525412	421.6	3.22	91.99	7	3	0
Y27632 dihydrochloride	Tocris	1254	98.0	12A/92215	329.3	Small GTPase inhibitor	150239	247.3	1.11	68.01	3	2	0
di-methyl sphingosine	Calbiochem	310500	98.0	D00033039	327.6	sphingosine kinase inhibitor	277963	327.6	5.79	43.70	3	2	1
SKI-II	**Calbiochem**	**567731**	**98.0**	**Unknown**	**339.2**	**sphingosine kinase inhibitor**	**NA**						
SKI-II	**Sigma**	**S5696**	**99.0**	**077K46181**	**302.8**	**sphingosine kinase inhibitor**	**NA**						
Mevastatin	**Sigma**	**M2537**	**95.0**	**0001423869**	**390.5**	**Statin**	**184814**	**390.5**	**3.97**	**72.83**	**5**	**1**	**0**
Simvastatin	Calbiochem	1965	98.2	2B/96951	418.6	Statin	238562	418.6	4.63	72.83	5	1	0
Genistein	**Sigma**	**G6649**	**98.0**	**018K1203**	**270.2**	**tyrosine kinase inhibitor**	**102658**	**270.2**	**2.14**	**86.99**	**5**	**3**	**0**
DMSO	Sigma	D8418	100.0	108K01864	78.1	Vehicle	110009	78.1	-0.32	36.28	1	0	0
TPEN	Sigma	P4413	99.0	078K1429	424.6	Zinc chelator	NA						

Supplementary Table 1. Identities and physicochemical properties of reagents populating the chemical libraries used in the assessment of the effects of single reagents and evolutionary (IBEA) directed combinations on LPS stimulated IL-1β expression in peritoneal macrophages and J774 macrophages respectively. Reagents highlighted in bold were utilized in both single reagent and combination studies. Those reagents highlighted in red and italicised (i.e. CA-074, NS-398, GM-6001, PD98059 and CAPE) were not pursued during the course of the evolution driven search for combinations inhibiting IL-1β expression. Discrepancies between 'Molecular Weight' (supplied by the manufacturer) and 'Parent Molecular Weight' (provided courtesy of the ChEMBL database) fields are attributable to the salt form of the compounds (i.e. NS-398, Desferrioxamine Mesylate, SIH, U0126, diphenylene iodonium, LY294002 hydrochloride, IKK inhibitor III, NSC23766, Y27632 dihydrochloride). * Pharmacological class is an arbitrary assignation defining biological targets / processes where we assume these molecules will exert their effects in the TLR4 stimulated macrophage IL-1β signalling network. Cat#; catalogue number, Mwt; Molecular weight, chEBI ID; Chemical Entity of Biological Interest (database) Identity, AlogP; calculated partition coefficient, PSA; polar surface area, HBA; number of hydrogen bond accepting moieties, HBD; number of hydrogen bond donating moieties, RO5; number of rule of five violations, NA; Not Identified.

Category	Parameter	Description
Assay	Type of assay	*In vitro*, evolution directed (IBEA) semi-automated robotic assay utilizing chemical combinations to inhibit LPS stimulated J774.A1 macrophage IL-1β expression.
	Target	IL-1β, UniProt (IL1B_MOUSE; P10749)
	Primary measurement	IL-1β expression measured via ELISA
	Key reagents	All antibodies and proteins were obtained from R&D systems. Capture Antibody (Part 840134, 1 vial) - 720 μg / mL of rat anti-mouse IL-1β when reconstituted with 1.0 mL of PBS. Detection Antibody (Part 840135, 1 vial) - 108 μg / mL of biotinylated goat anti-mouse IL-1β when reconstituted with 1.0 mL of blocking buffer (see supplementary methods for recipe). Standard (Part 840136, 1 vial) - 360 ng / mL of recombinant mouse IL-1β when reconstituted with 0.5 mL of blocking buffer. Streptavidin-HRP (Part 890803, 1 vial) - 1.0 mL of streptavidin conjugated to horseradish peroxidase.
	Assay protocol	This protocol is adapted from the recommended procedure provided with the mouse IL-1β/IL-1F2 DuoSet ELISA development kit (R&D Systems; DY401)
	Additional comments	see supplementary methods
Library	Library size	33 reagents in total, never exceeding 30 reagents during the course of the iterative, IBEA directed search.
	Library composition	Small molecule reagents: drug like inhibitor molecules of biological targets (e.g. NF-κB and MAPK inhibitors) and processes (e.g. redox reactions – antioxidants and iron chelators).
	Source	Sigma-Aldrich, Tocris, Chembridge Cheng-Du Biopurify Phytochemicals or Merck Chemicals
	Additional comments	see supplementary table 1
Screen	Format	96-well plates (Nunc; Maxisorp)
	Concentration(s) tested	All reagents evaluated either alone or in combinations at 3μM.
	Plate controls	Randomized quintuplicate positive control wells (LPS, 1μg / mL & DMSO, 0.5 % v/v) and negative control (C11) well (IKK inhibitor III, 100 μM)
	Reagent/ compound dispensing system	Sciclone ALH3000 (Caliper Life Sciences)
	Detection instrument and software	BioTek Synergy HT plate reader used for the measurement of the IL-1β ELISA absorbance (A_{450nm} – A_{570nm}) readings under the control of Gen 5 software. FLUOstar Omega (BMG LabTech) plate reader was used for the measurement of LDH reaction absorbance (A_{490nm}).
	Assay validation/QC	The median Z' factor for the assay was 0.35

		indicating the assay was acceptable. Plate edge effects were assessed by the heat mapping of randomized positive control wells (n = 3 minimum / data point) (see supplementary figure 7).
	Correction factors	NA
	Normalization	J774.A1 cell IL-1β expression data was expressed as a percentage of the measured IL-1β concentration (pg / mL) interpolated from the standard curve for a given well / plate to the mean IL-1β concentration (pg / mL) derived from five positive control wells for that plate. Populations of generations were assayed in plates in triplicate.
	Additional comments	See supplementary information; supplementary figure 6.
Post-HTS analysis	Hit criteria	Objective evaluation of all combinations across all generations (i.e. generation 1 – 11) was performed by measuring (post-hoc) the fitness contribution of reagents from combinations and deriving a ranked list.
	Hit rate	Reagents were selected from top five ranked reagents when assessed either singularly or as double or triple combinations in either the presence or absence of the dominant solution (i.e. SB203580).
	Additional assay(s)	Concentration-dependent adaptive dose matrix search of paired combinations from five selected reagents.
	Confirmation of hit purity and structure	Manufacturer specification and certificates of analysis were used as confirmatory evidence of hit purity and structure.
	Additional comments	See supplementary information, particularly supplementary table 1 (confirming reagent identities & physicochemical properties) and supplementary figure 7 detailing the variation in positive control responses across the plate.
IBEA parameter settings	Population size	50 reagent combinations were generated by IBEA at each generation.
	Parental selection	Solutions (parents) for reproduction were selected using binary tournament selection with replacement.
	Crossover probability	Offspring were generated by applying uniform crossover with a probability of 0.7 to the selected parents. In situations where crossover is not applied, offspring are simply copied versions of the parents.
	Per-bit mutation probability	Each decision variable (reagent) of an offspring (reagent combination) was mutated or flipped independently with a probability of 1/33 (i.e. on average one solution variable was flipped).
	Additional comments	Recall that the initial population was generated at random such that each reagent combination contains on average 3 reagents; i.e. any reagent of the library was included with a probability of 1/33 into a reagent combination. A more detailed explanation of IBEA including a pseudocode of the algorithm can be found in Reference 8. We use the adaptive version of IBEA from that reference. The value of the scaling parameter, κ, was 0.05, and the hypervolume reference point used was (2,2,2), as in the original source reference.

Supplementary Table 2. Summary of the screening and EA parameter settings for the assay of the evolution directed (IBEA) semi-automated robotic production of chemical combinations inhibiting LPS stimulated J774.A1 macrophage IL-1β expression.

Generation	Number of combinations per generation showing ≥ 95% inhibition of IL-1β expression	Mean number of component reagents per generation (rounded to integer)	Mean fractional LDH release of combinations per generation
2	1	3	0.96
4	4	5	1.01
6	8	5	1.08
7	3	4	0.99
8	1	4	1.00
9	27	3	1.05
10	3	5	1.00
11	4	4	0.97

Number of reagents per combination	Mean inhibition of IL-1β expression for combinations where inhibition was already ≥ 95 % of the positive control response	Number of combinations
7	98.51	4
6	99.10	2
5	99.09	8
4	99.20	16
3	98.93	13
2	100.00	5

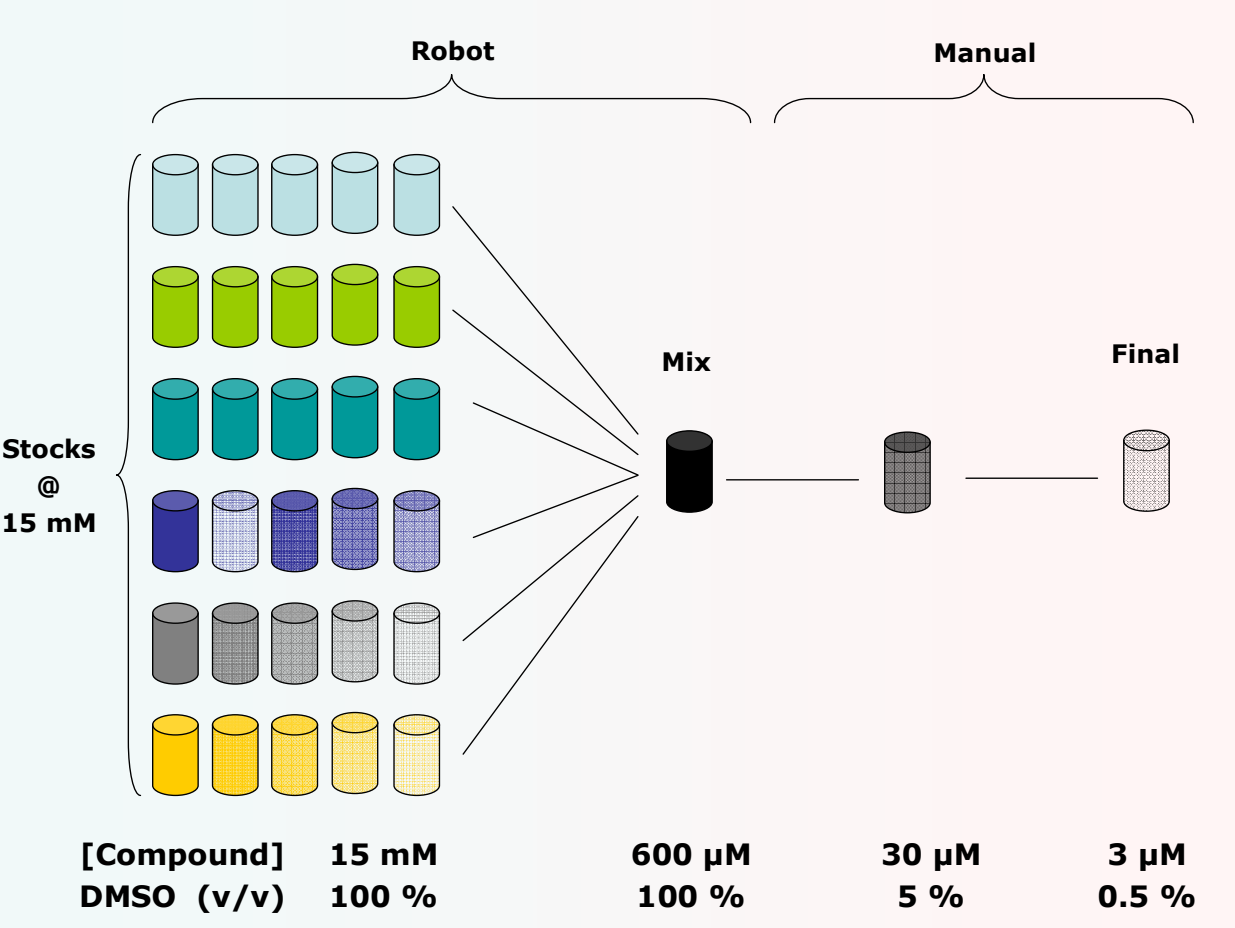
Supplementary Table 3. Characteristics of reagent combinations found from the IBEA directed search that inhibited IL-1β expression in the LPS stimulated J774 macrophages by ≥ 95% of positive control responses. Overall, successive generations of reagent combination yielded decreases in the number of component reagents and fractional LDH release (Top). Despite decreases in component reagent number the search still located five very efficacious dual combinations (Bottom).

Generation	Number of combinations per generation showing ≥ 70% inhibition of IL-1β expression	Mean number of component reagents per generation (rounded to integer)	Mean fractional LDH release of combinations per generation
1	8	4	1.06
2	5	5	1.03
3	32	4	1.02
4	12	5	1.02
5	18	4	1.01
6	29	4	1.04
7	27	4	1.06
8	24	4	1.04
9	28	4	1.05
10	32	3	1.01
11	18	3	0.98

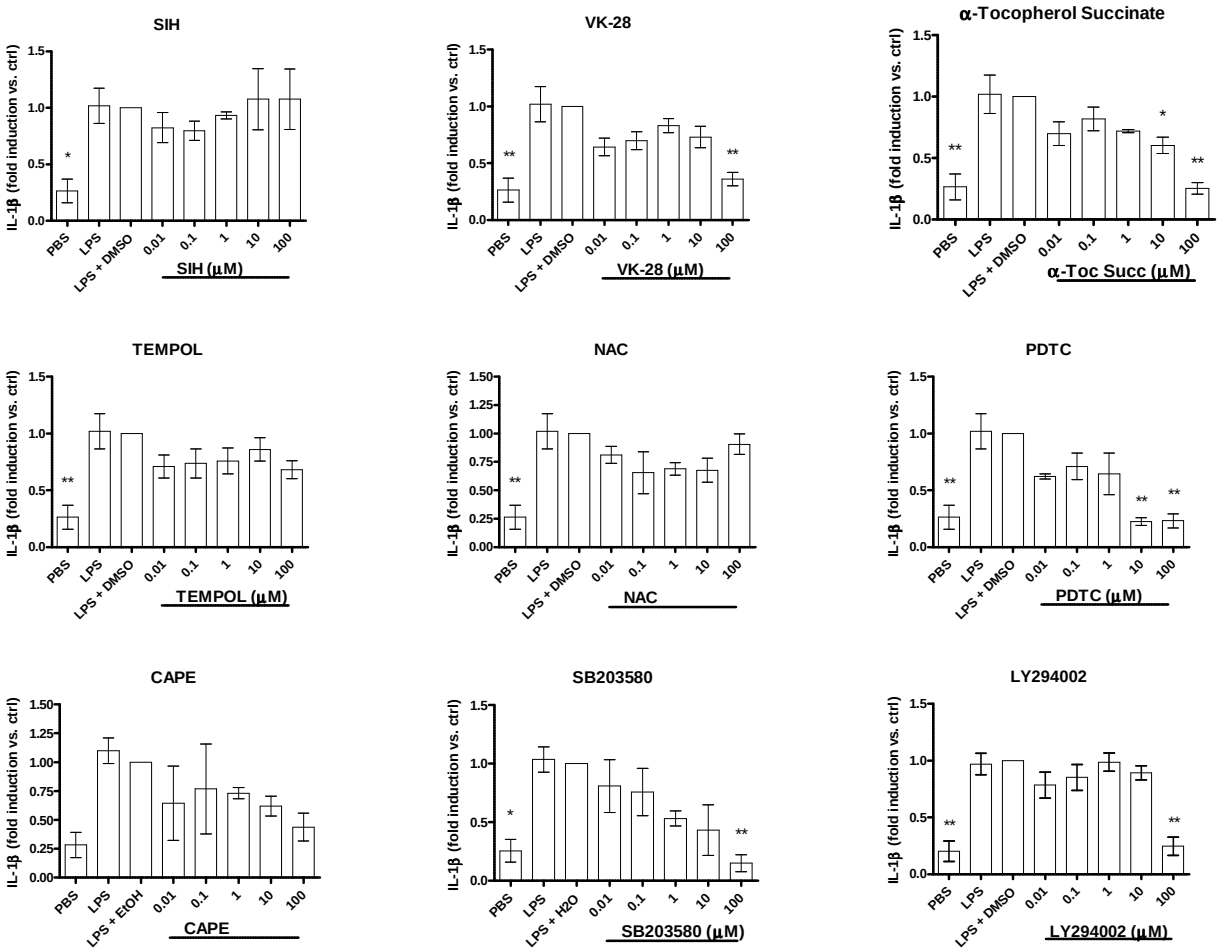
Number of reagents per combination	Mean inhibition of IL-1β expression for combinations where inhibition was already ≥ 70 % of the positive control response	Number of combinations
8	90.52	1
7	91.51	10
6	89.92	18
5	91.93	35
4	92.29	57
3	90.20	58
2	87.88	34

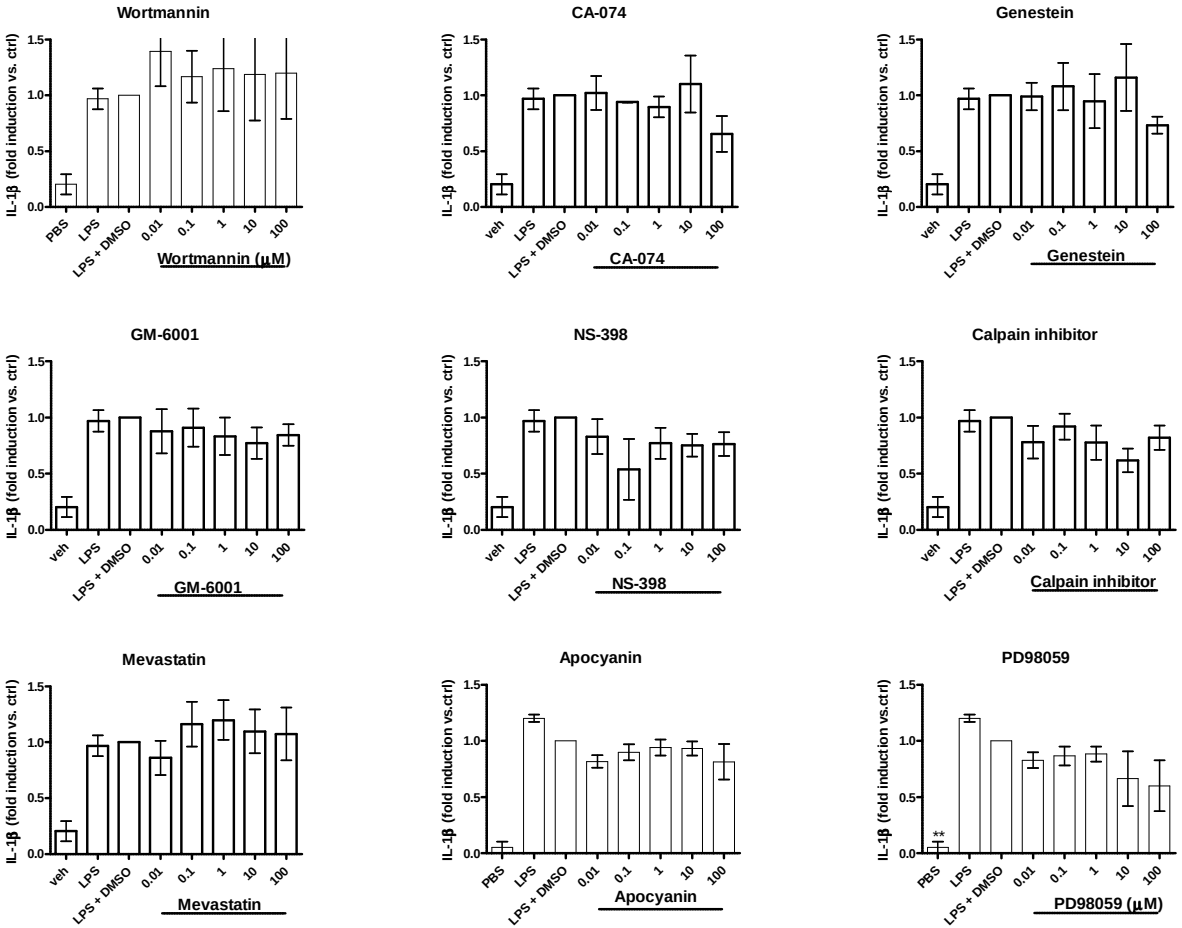
Supplementary Table 4. Characteristics of reagent combinations found from the IBEA directed search that inhibited IL-1β expression in the LPS stimulated J774 macrophages by ≥ 70% of positive control responses. Overall, successive generations of reagent combination yielded decreases in the number of component reagents and fractional LDH release (Top). Similarly, despite decreases in component reagent number the search still located thirty four dual combinations (Bottom).

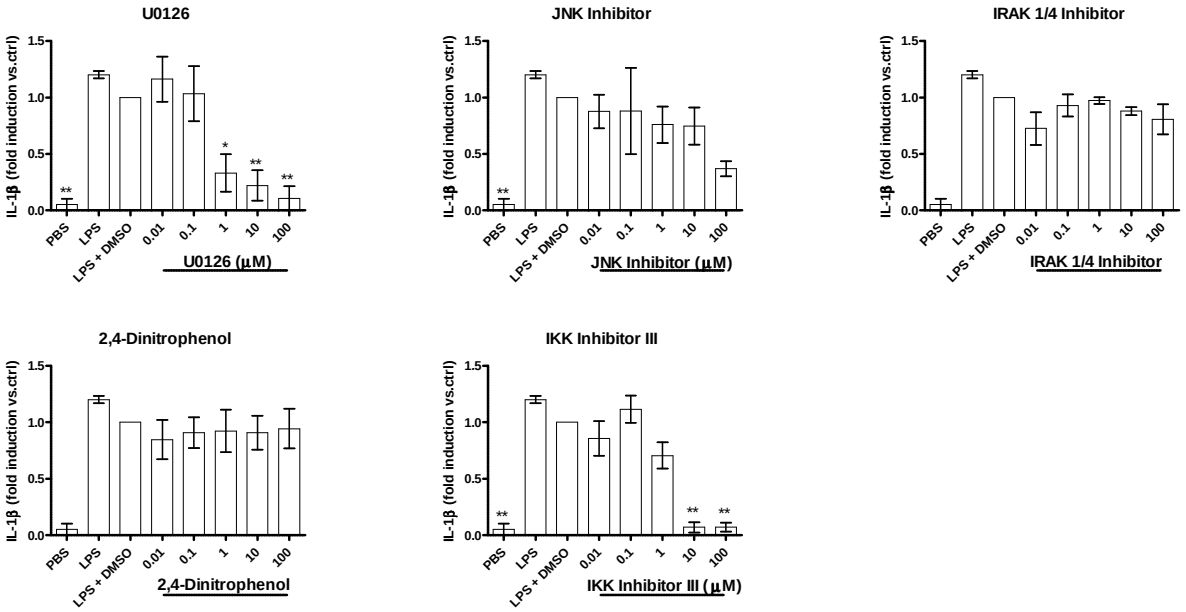
FIGURES



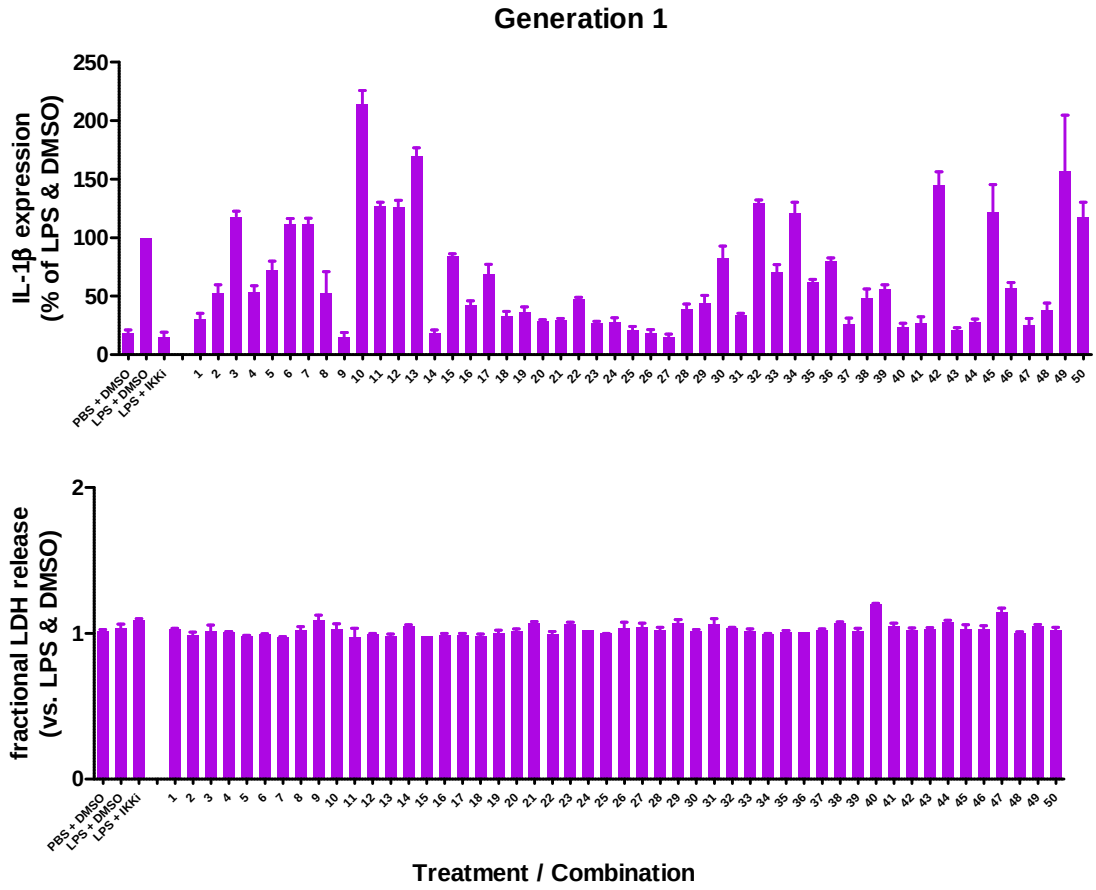
Supplementary Figure 1. Reagent dilutions and the percentage of DMSO in the semi-automated production of chemical combinations

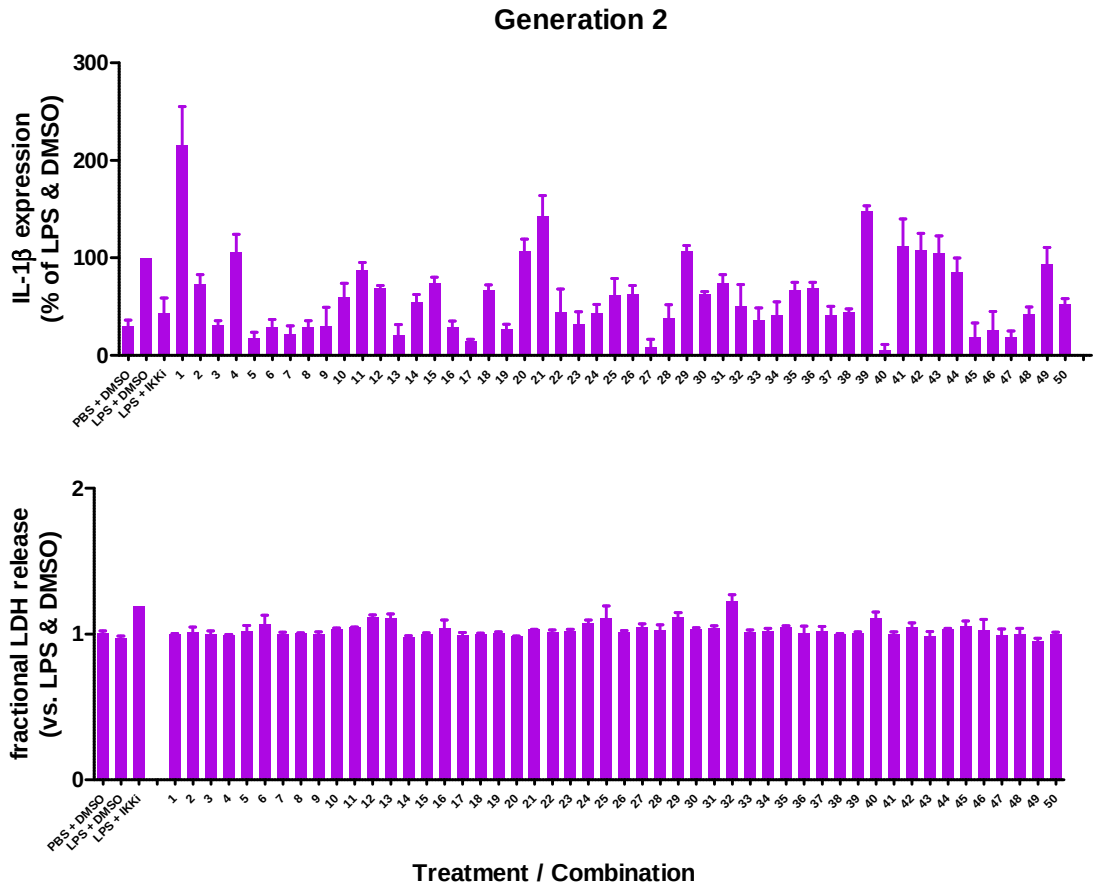

SIH
VK-28
α-Tocopherol Succinate
TEMPOL
NAC
PDTC
CAPE
SB203580
LY294002
IL-1β (fold induction vs. ctrl)
PBS
LPS
LPS + DMSO
LPS + EtOH
LPS + H2O
0.01
0.1
1
10
100
SIH (μM)
VK-28 (μM)
α-Toc Succ (μM)
TEMPOL (μM)
NAC
PDTC (μM)
CAPE
SB203580 (μM)
LY294002 (μM)

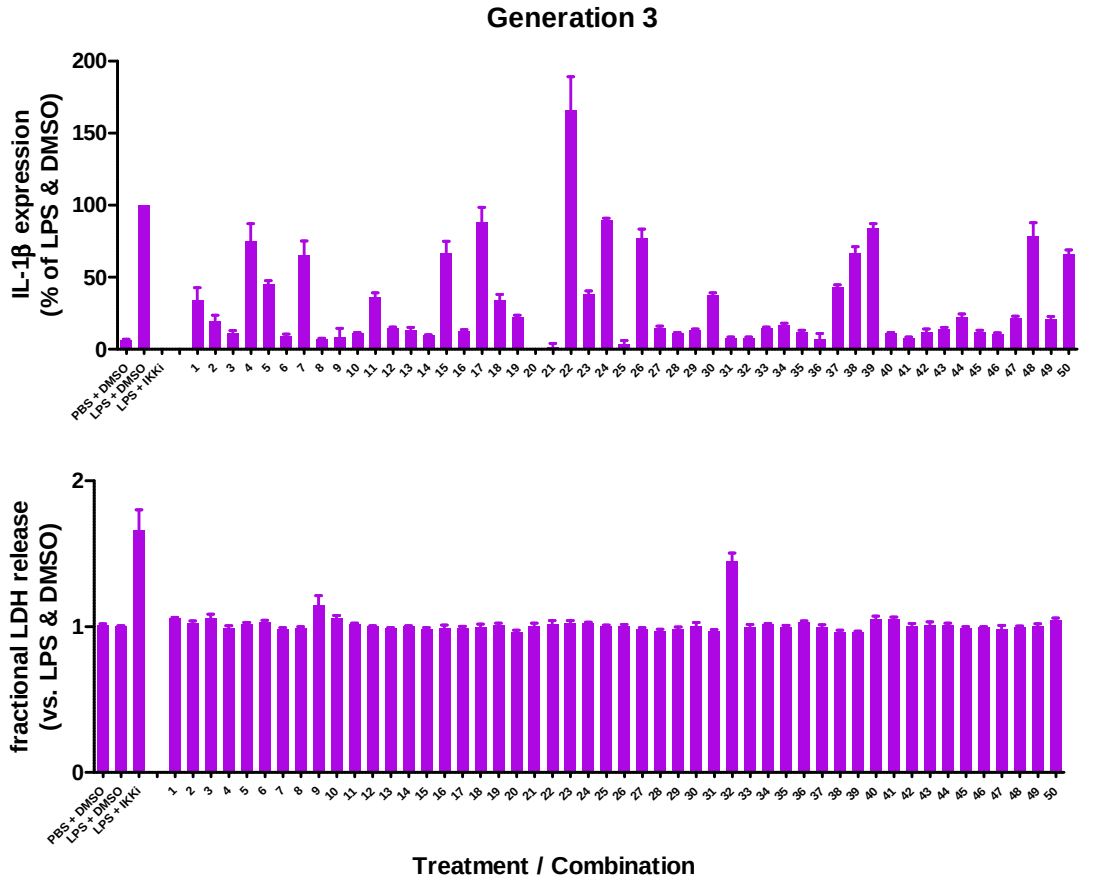

Wortmannin
IL-1β (fold induction vs. ctrl)
1.5
1.0
0.5
0.0
PBS
LPS
LPS + DMSO
0.01
0.1
1
10
100
Wortmannin (μM)
CA-074
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
CA-074
Genestein
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
Genestein
GM-6001
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
GM-6001
NS-398
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
NS-398
Calpain inhibitor
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
Calpain inhibitor
Mevastatin
IL-1β (fold induction vs. ctrl)
veh
LPS
LPS + DMSO
0.01
0.1
1
10
100
Mevastatin
Apocyanin
IL-1β (fold induction vs.ctrl)
PBS
LPS
LPS + DMSO
0.01
0.1
1
10
100
Apocyanin
PD98059
IL-1β (fold induction vs.ctrl)
**
PBS
LPS
LPS + DMSO
0.01
0.1
1
10
100
PD98059 (μM)

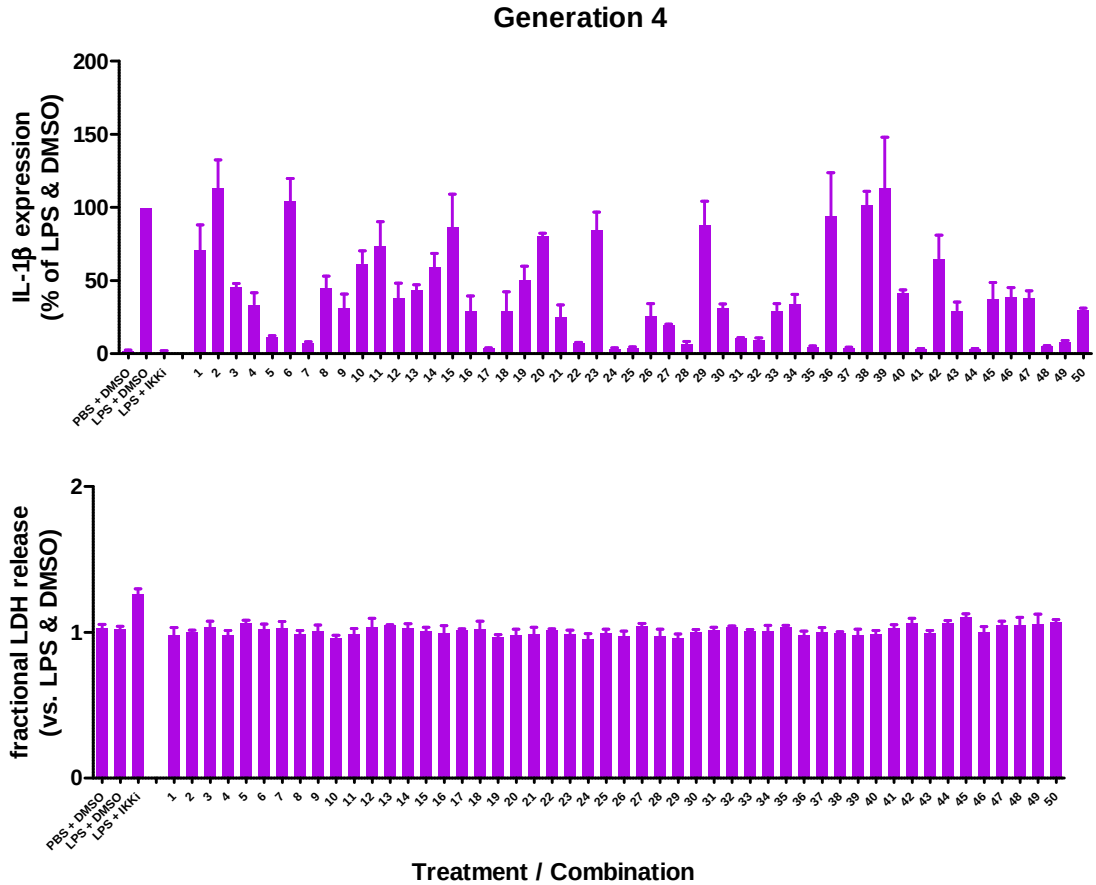


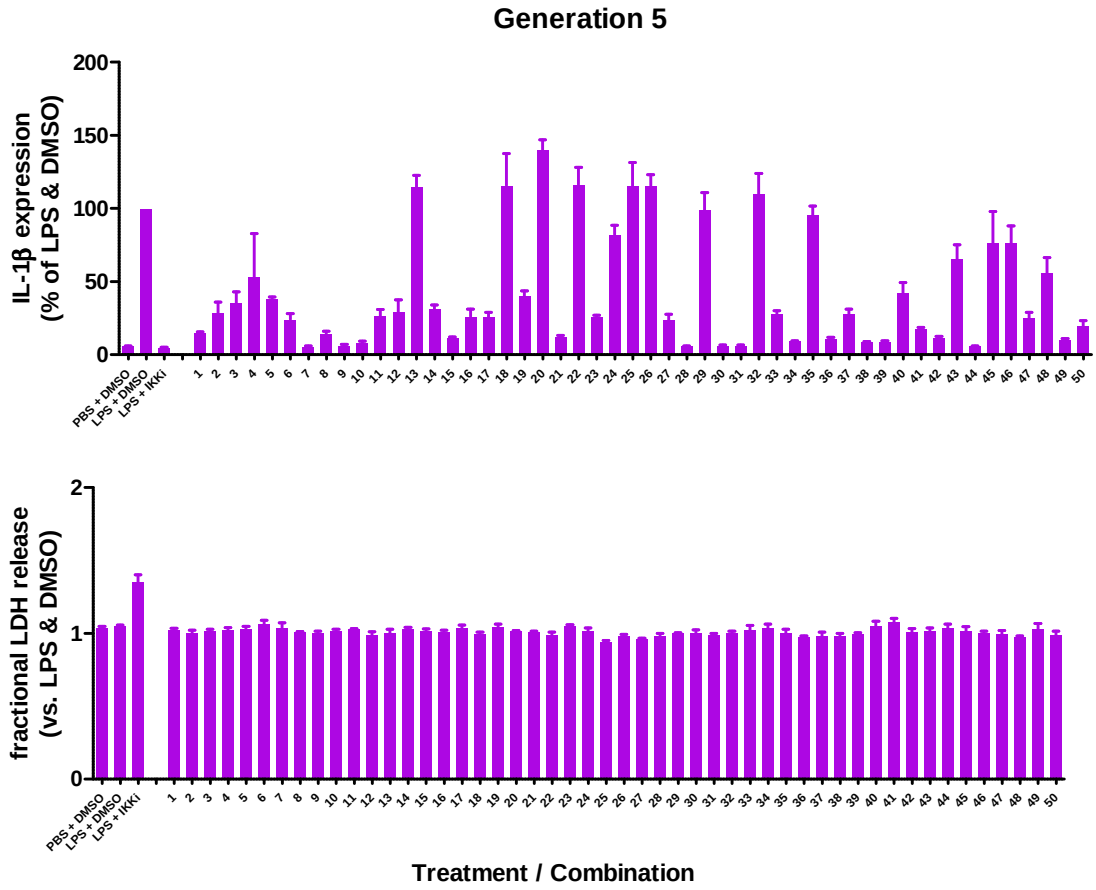
Supplementary Figure 2. Pharmacological activities of selected reagents on LPS (1μg / mL) stimulated IL-1β expression in mouse primary peritoneal macrophages. Comparison of different treatments to positive control (LPS and DMSO) was assessed by means of a one-way ANOVA and a post-hoc Dunnett's multiple comparison test reporting significance at the $P<0.05$ (*) and $P<0.01$ (**) levels. Data are expressed as mean ± SEM (n=3 preparations).

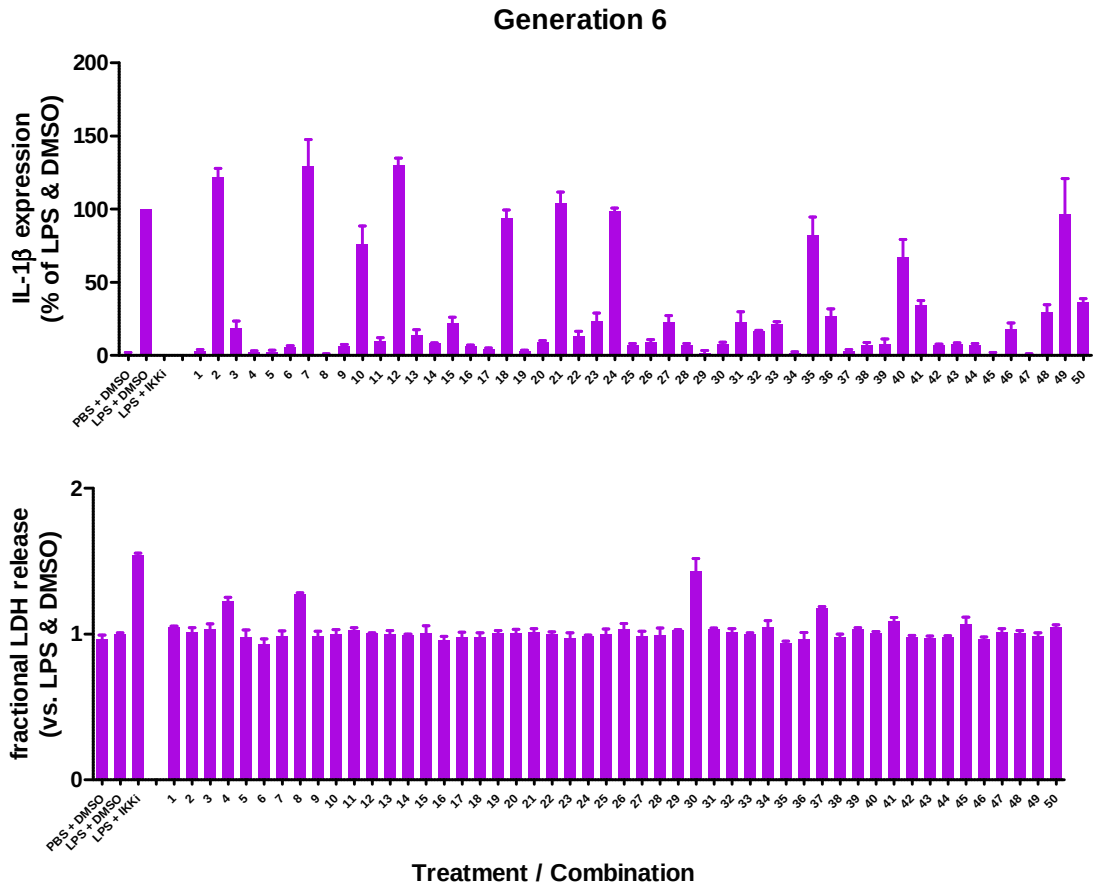

Generation 1
IL-1β expression
(% of LPS & DMSO)
250
200
150
100
50
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

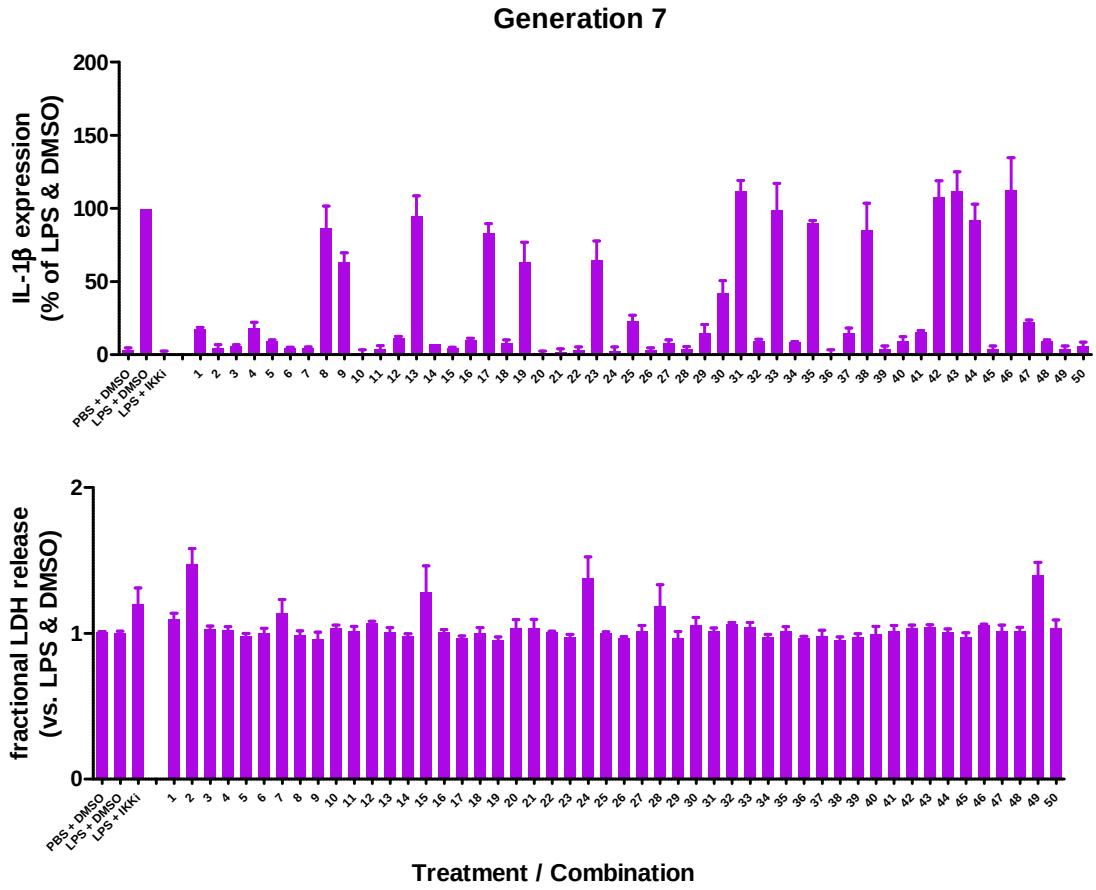

Generation 2
IL-1β expression
(% of LPS & DMSO)
300
200
100
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

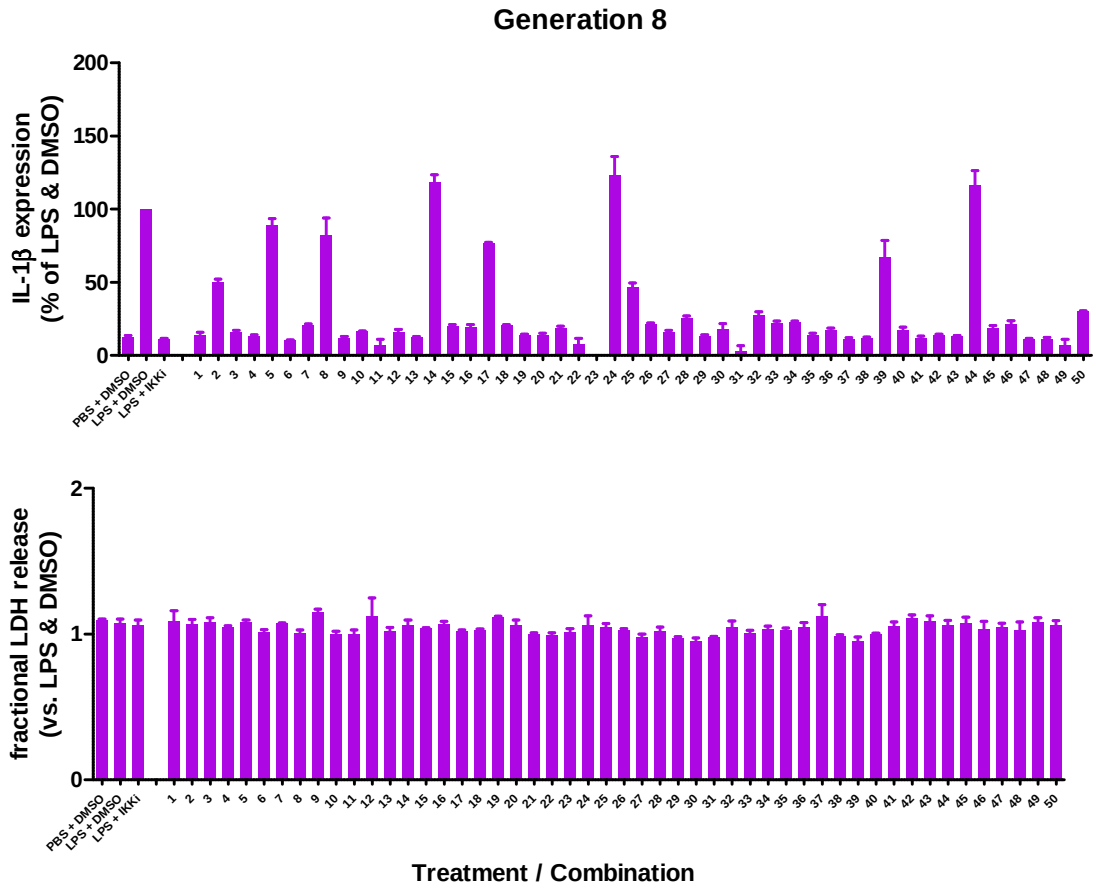

Generation 3
IL-1β expression
(% of LPS & DMSO)
200
150
100
50
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

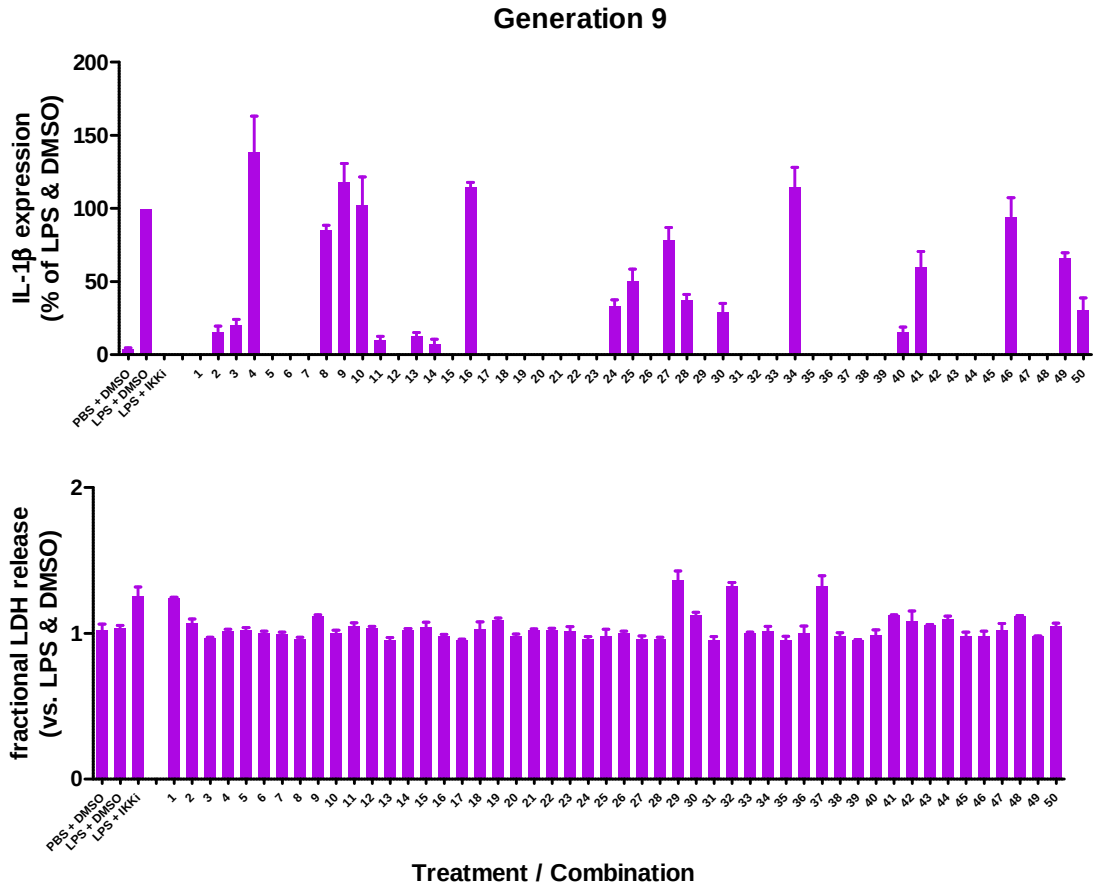

Generation 4
IL-1β expression
(% of LPS & DMSO)
200
150
100
50
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

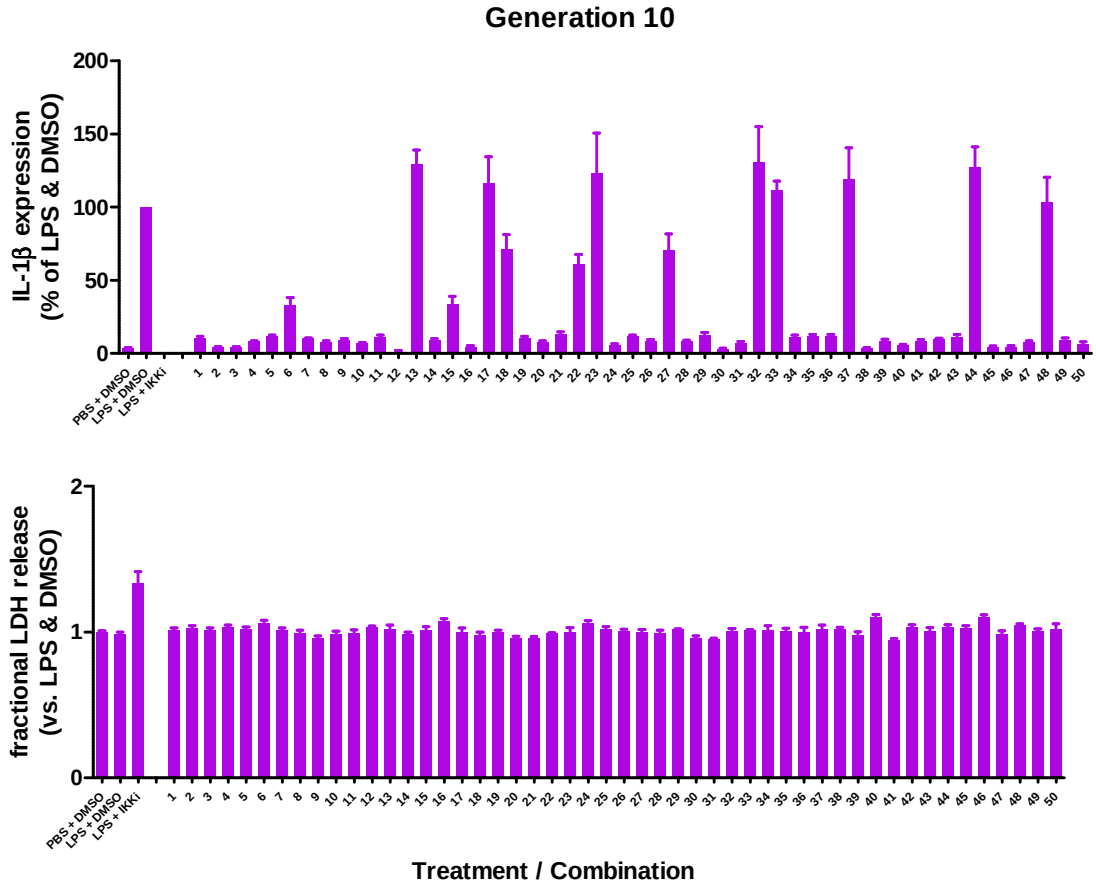

Generation 5
IL-1β expression (% of LPS & DMSO)
fractional LDH release (vs. LPS & DMSO)
PBS + DMSO
LPS + DMSO
LPS + IKKi
Treatment / Combination

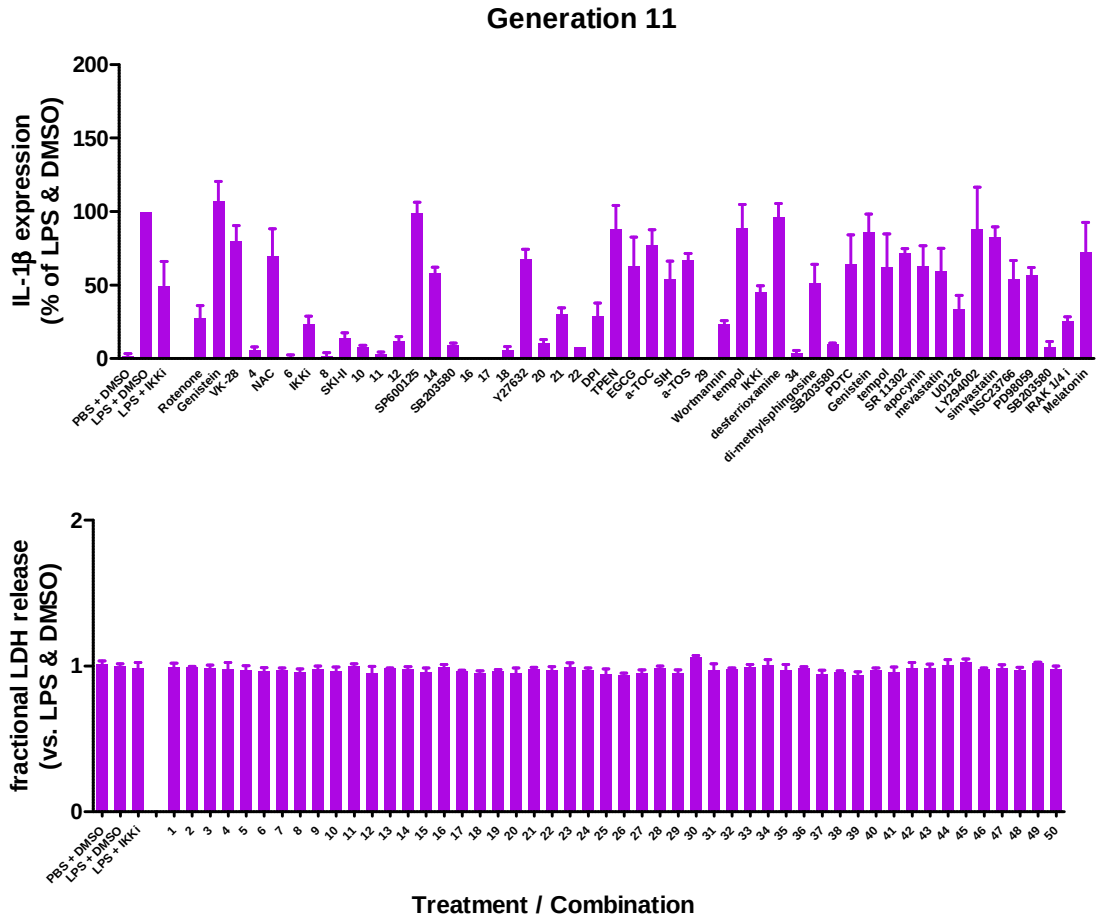

Generation 6
IL-1β expression (% of LPS & DMSO)
fractional LDH release (vs. LPS & DMSO)
PBS + DMSO
LPS + DMSO
LPS + IKKi
Treatment / Combination


Generation 7
IL-1β expression (% of LPS & DMSO)
fractional LDH release (vs. LPS & DMSO)
PBS + DMSO
LPS + DMSO
LPS + IKKi
Treatment / Combination

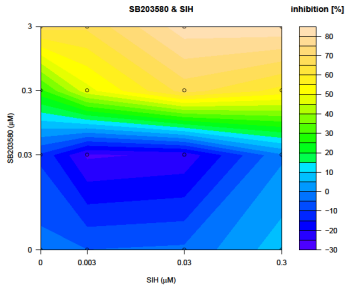

Generation 8
IL-1β expression (% of LPS & DMSO)
fractional LDH release (vs. LPS & DMSO)
PBS + DMSO
LPS + DMSO
LPS + IKKi
Treatment / Combination

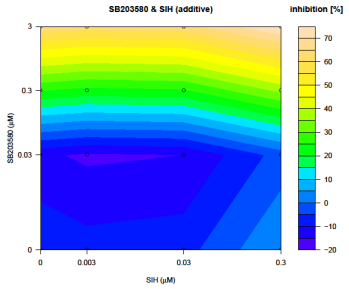

Generation 9
IL-1β expression
(% of LPS & DMSO)
200
150
100
50
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

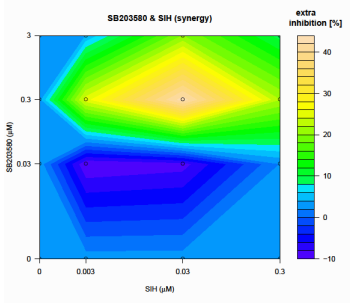

Generation 10
IL-1β expression
(% of LPS & DMSO)
200
150
100
50
0
fractional LDH release
(vs. LPS & DMSO)
2
1
0
PBS + DMSO
LPS + DMSO
LPS + IKKi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Treatment / Combination

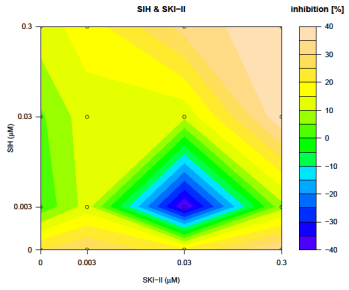


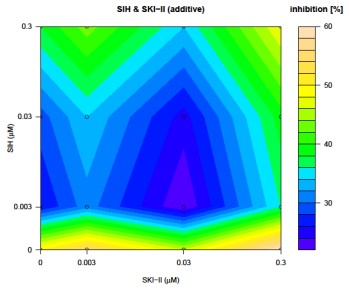
Supplementary Figure 3. Efficacy of chemical combinations with respect to LPS stimulated (1µg / mL) IL-1β expression (upper plot per generation) and LDH release (lower plot per generation) in J774.A1 macrophages for all tested generations (Gen 1 – Gen 11). Vehicle (PBS; 0.1 % v/v) and positive control (LPS; 1µg / mL & DMSO; 0.5 % v/v) responses were tested in triplicate or quintuplicate per plate respectively. Negative control (LPS; 1µg / mL & IKKi; 100 µM, excpt Gen 11 where IKKi; 3 µM data was substituted) responses were tested in single wells per plate. Fifty combinations were tested per plate and all data is expressed as n = 3 plates per generation ± SEM.

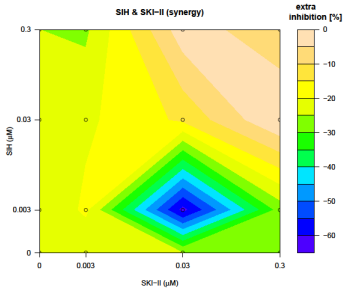

SB203580 & SIH
inhibition [%]
SB203580 (μM)
SIH (μM)

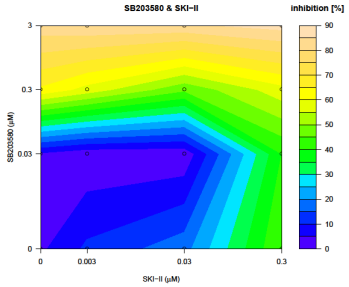

SB203580 & SIH (additive)
inhibition [%]
SB203580 (μM)
SIH (μM)

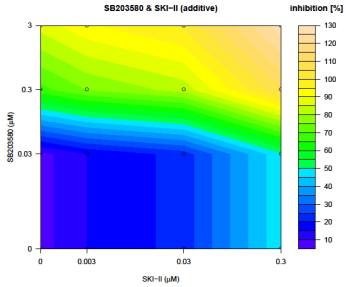

SB203580 & SIH (synergy)
extra inhibition [%]
SB203580 (μM)
SIH (μM)

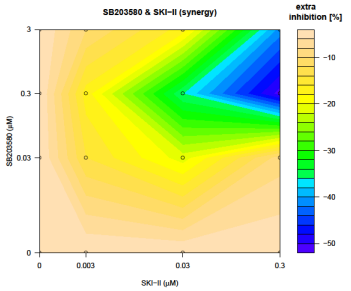

SIH & SKI–II
inhibition [%]
SIH (μM)
SKI–II (μM)

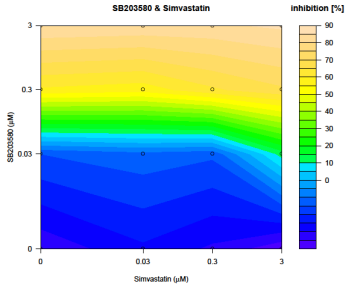

SIH & SKI–II (additive)
inhibition [%]
SIH (μM)
SKI–II (μM)

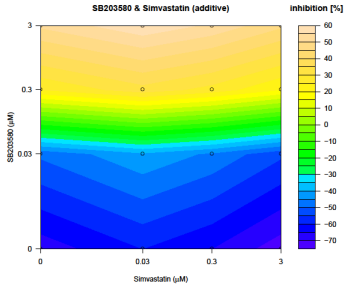

SIH & SKI–II (synergy)
extra inhibition [%]
SIH (μM)
SKI–II (μM)

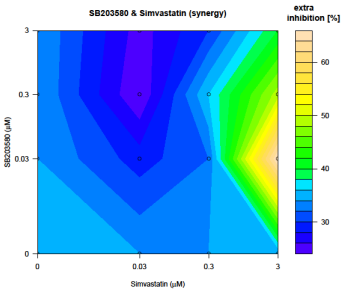

SB203580 & SKI–II
inhibition [%]
SB203580 (μM)
SKI–II (μM)

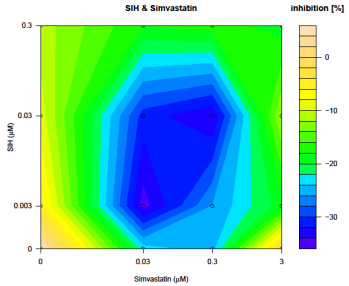

SB203580 & SKI–II (additive)
inhibition [%]
SB203580 (μM)
SKI–II (μM)

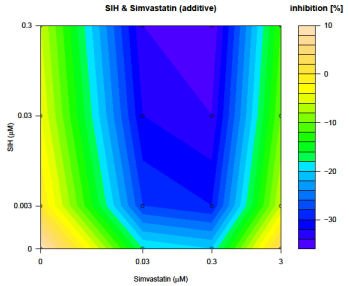

SB203580 & SKI–II (synergy)
extra inhibition [%]
SB203580 (μM)
SKI–II (μM)

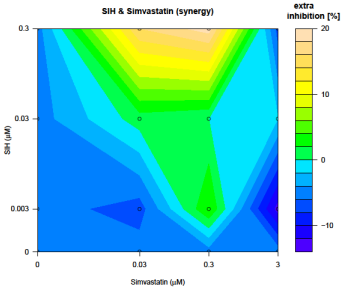

SB203580 & Simvastatin
inhibition [%]
SB203580 (μM)
Simvastatin (μM)

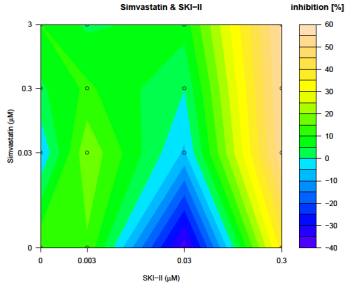

SB203580 & Simvastatin (additive)
inhibition [%]
SB203580 (μM)
Simvastatin (μM)

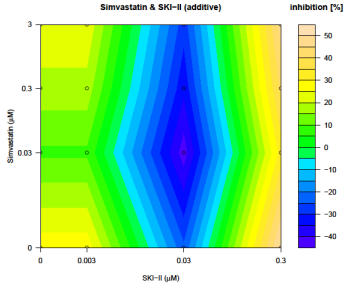

SB203580 & Simvastatin (synergy)
extra inhibition [%]
SB203580 (μM)
Simvastatin (μM)

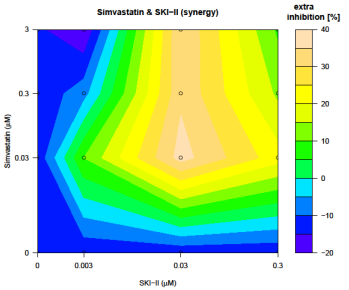

SIH & Simvastatin
inhibition [%]
SIH (μM)
Simvastatin (μM)

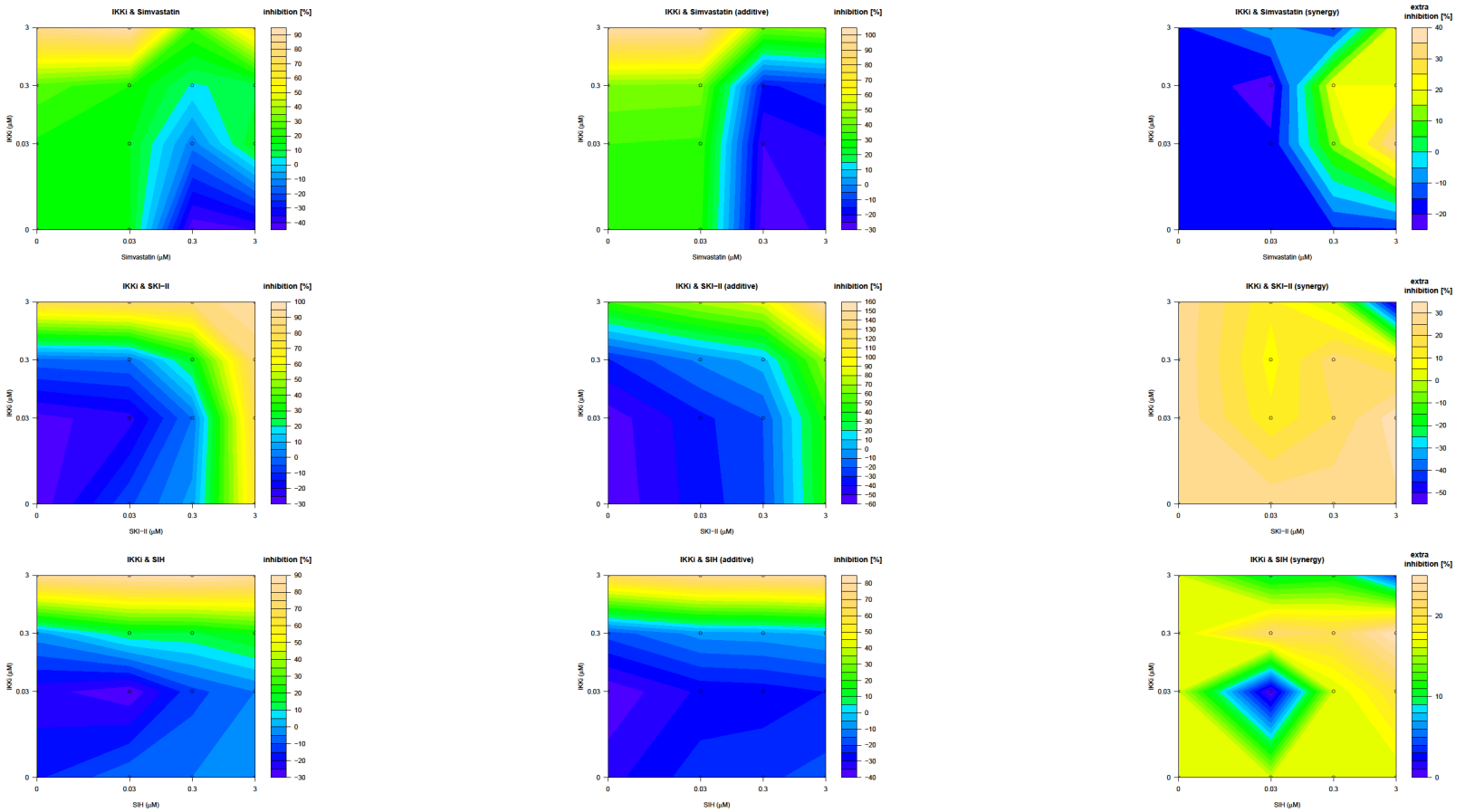

SIH & Simvastatin (additive)
inhibition [%]
SIH (μM)
Simvastatin (μM)


SIH & Simvastatin (synergy)
extra inhibition [%]
SIH (μM)
Simvastatin (μM)


Simvastatin & SKI−II
inhibition [%]
Simvastatin (μM)
SKI−II (μM)

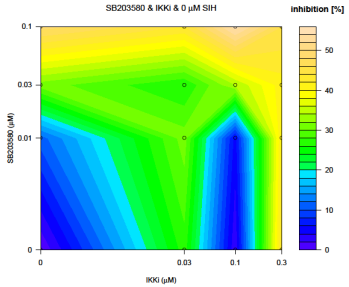

Simvastatin & SKI−II (additive)
inhibition [%]
Simvastatin (μM)
SKI−II (μM)

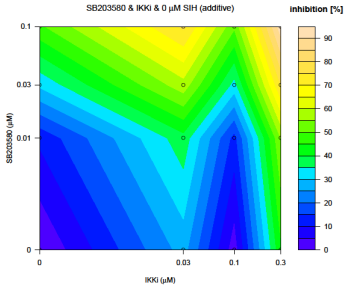

Simvastatin & SKI−II (synergy)
extra inhibition [%]
Simvastatin (μM)
SKI−II (μM)

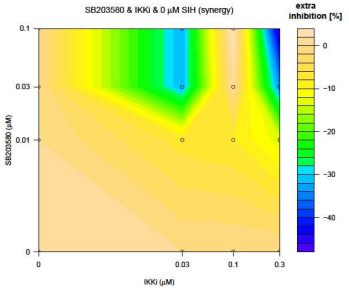


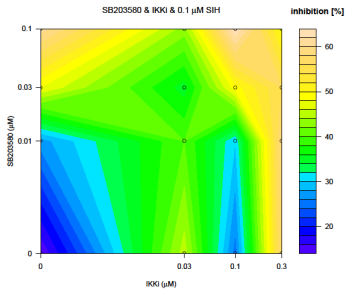
Supplementary Figure 4. Adaptive dose-matrix search of paired reagent combinations for potential synergy in their inhibition of IL-1β expression. Combination response shape plots shown are for a fully enumerated search of all possible pair wise combinations of the five reagents prioritized from the post-hoc analysis of the IBEA search (i.e. SB203580 & SIH, SIH & SKI-II, SB203580 & SKI-II, SB203580 &

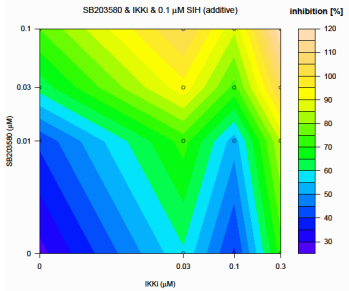
Simvastatin, SIH & Simvastatin, Simvastatin & SKI-II, IKKi &. Simvastatin, IKKi &. SKI-II, IKKi &. SIH). Three plots are shown (from left to right) representing: the experimental data; simple additive effects of the combination calculated from single reagent data in the absence of the other reagent; and detection of synergy in the paired combination by subtraction of additive effects from the experimental data. Pseudocolor mappings were performed by linear interpolation between samples. All data are expressed as n = 3 - 4 plates.

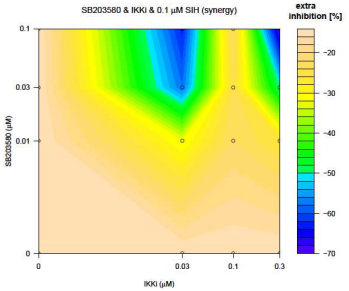

SB203580 & IKKi & 0 µM SIH
inhibition [%]
SB203580 (µM)
IKKi (µM)

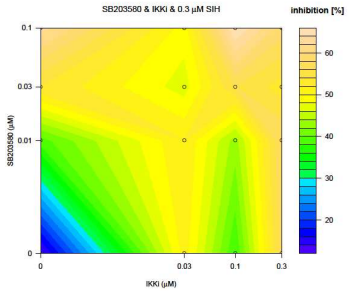

SB203580 & IKKi & 0 µM SIH (additive)
inhibition [%]
SB203580 (µM)
IKKi (µM)

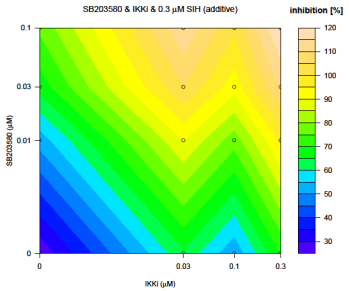

SB203580 & IKKi & 0 µM SIH (synergy)
extra inhibition [%]
SB203580 (µM)
IKKi (µM)

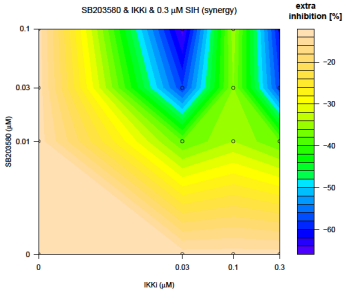

SB203580 & IKKi & 0.1 µM SIH
inhibition [%]
SB203580 (µM)
IKKi (µM)

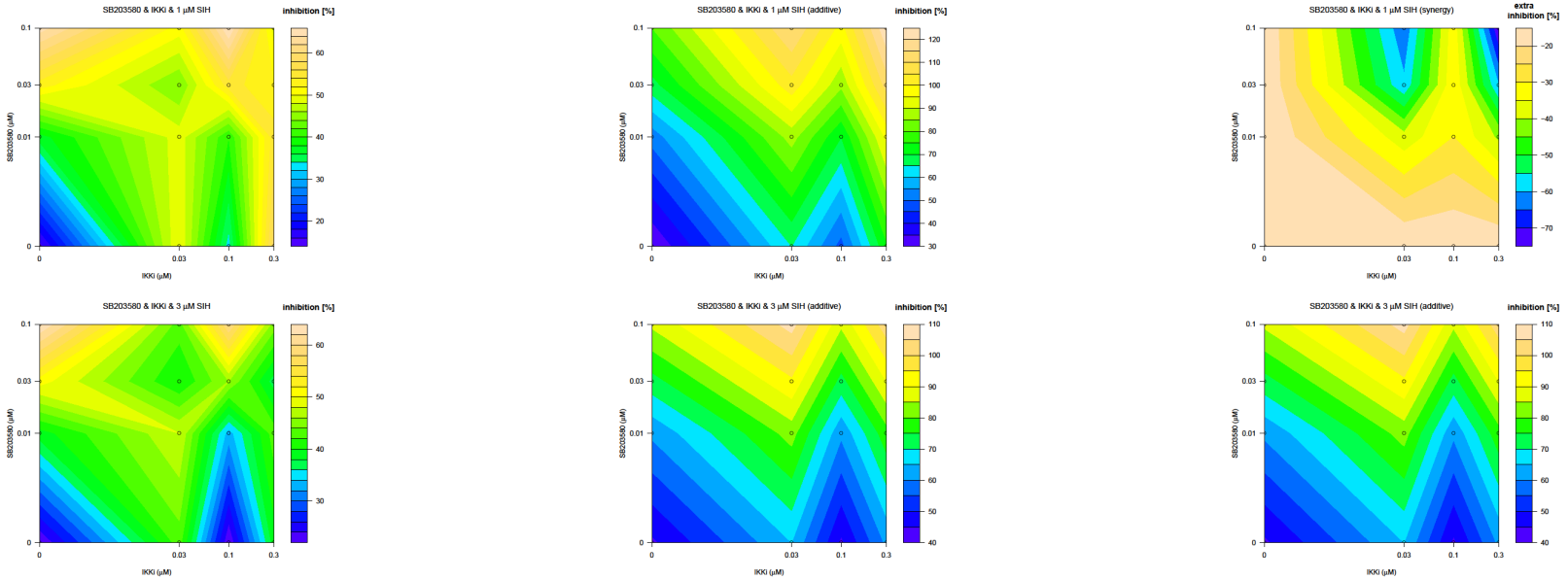

SB203580 & IKKi & 0.1 µM SIH (additive)
inhibition [%]
SB203580 (µM)
IKKi (µM)


SB203580 & IKKi & 0.1 µM SIH (synergy)
extra inhibition [%]
SB203580 (µM)
IKKi (µM)

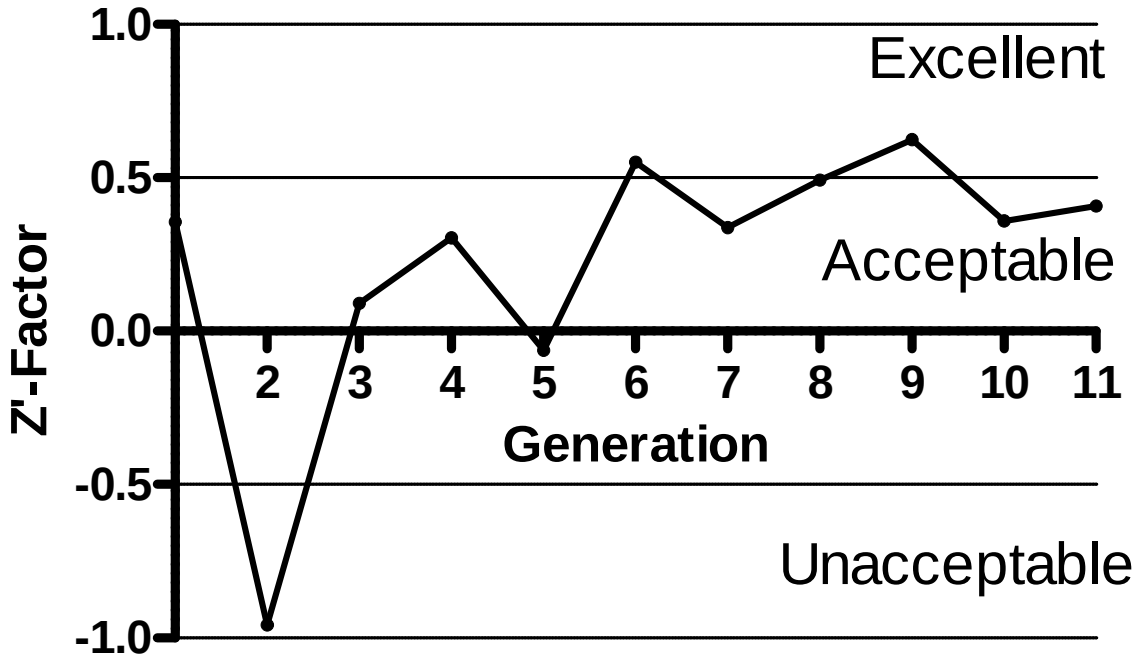

SB203580 & IKKi & 0.3 µM SIH
inhibition [%]
SB203580 (µM)
IKKi (µM)


SB203580 & IKKi & 0.3 µM SIH (additive)
inhibition [%]
SB203580 (µM)
IKKi (µM)

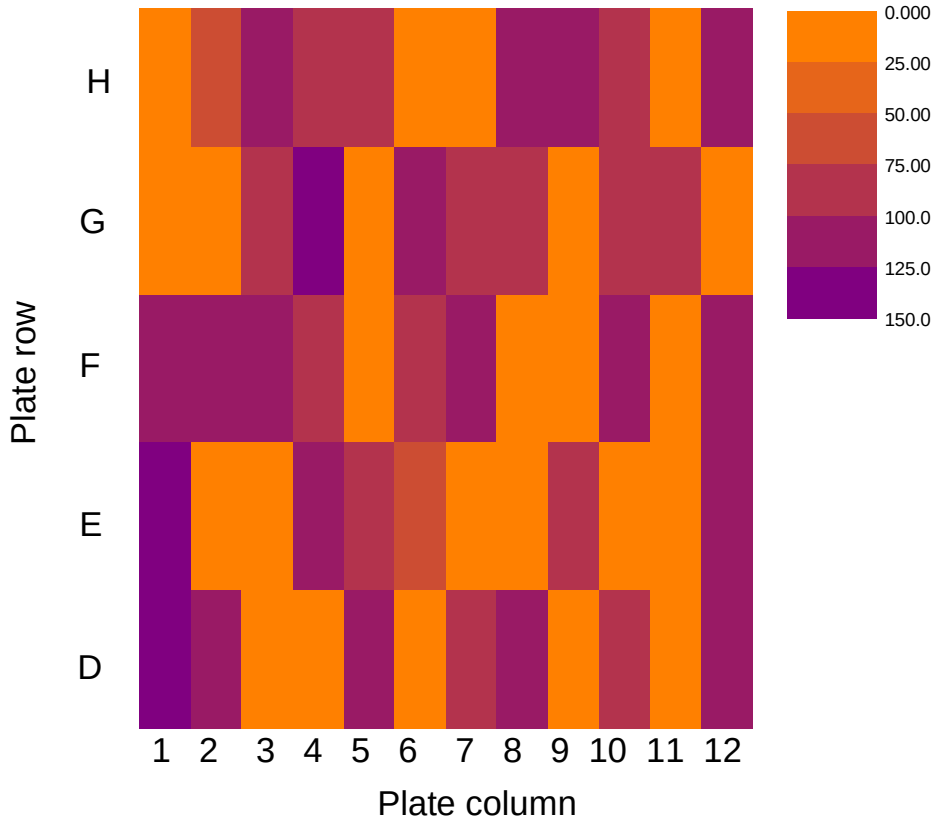

SB203580 & IKKi & 0.3 µM SIH (synergy)
extra inhibition [%]
SB203580 (µM)
IKKi (µM)



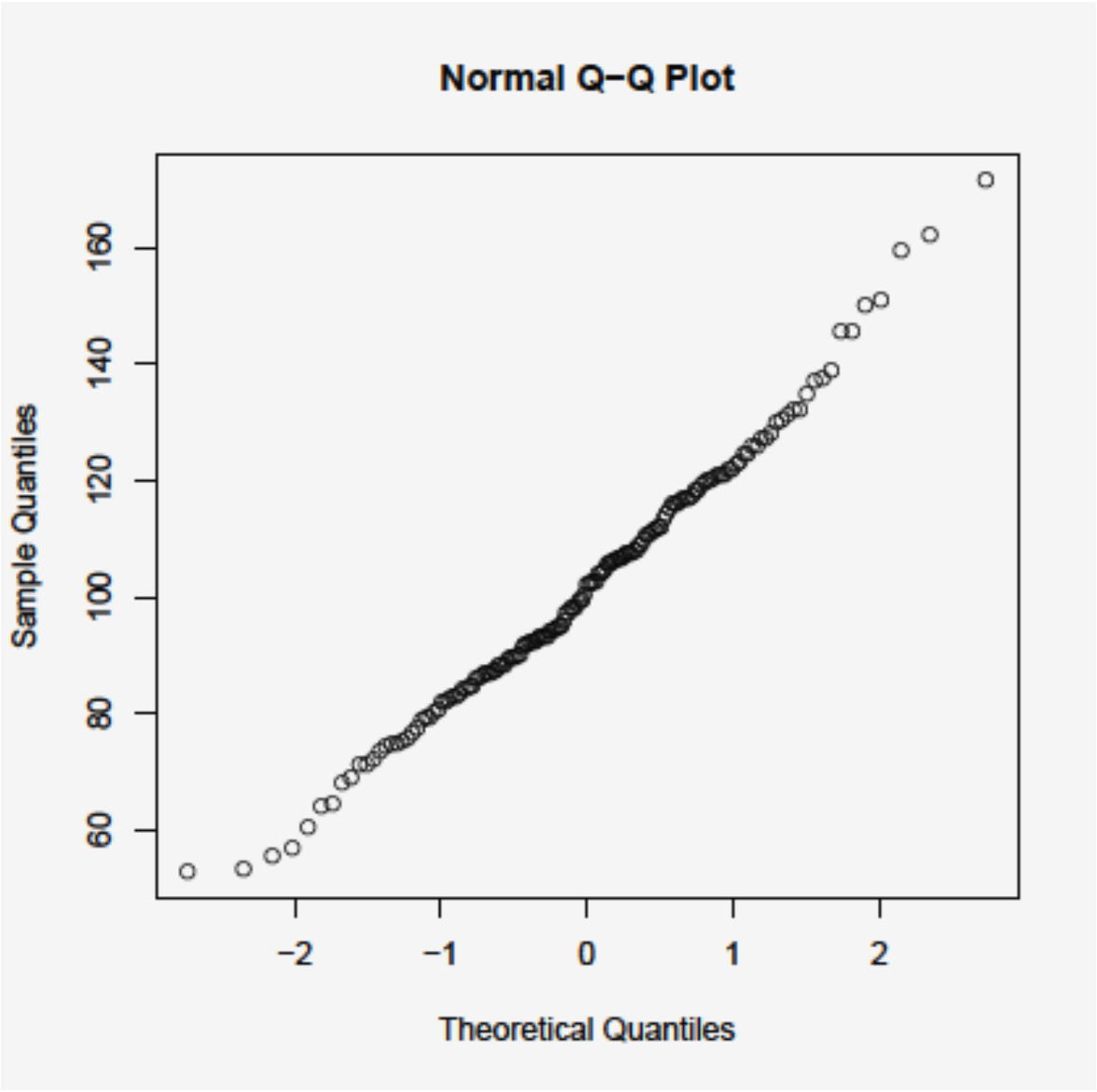
Supplementary Figure 5. Dose-matrix search of the triple reagent combination (SB203580, IKKi and SIH) for potential synergy in its inhibition of IL-1β expression. Combination response shape plots show the effect of increasing concentration (by row) of SIH (0, 0.1, 0.3, 1 and 3 µM) Three plots are shown (from left to right) representing: the experimental data; simple additive effects of the combination calculated from single reagent data in the absence of the other reagent; and detection of synergy in the paired combination by subtraction of additive effects from the experimental data. Pseudocolor mappings were performed by linear interpolation between samples. All data are expressed as n = 5 plates.



Supplementary Figure 6. Calculation of the Z'-Factor statistic retrospectively for the IBEA directed search revealed mean and median values of 0.33 and 0.35 respectively indicating overall that the assay was of an acceptable quality. The Z'-Factor was determined by four parameters (see data analysis); positive and negative control, mean and standard deviation respectively. At each generation there were 15 positive control wells and 3 negative control wells.



Supplementary Figure 7. Pseudocolor heat mapping of positive control (LPS (1µg / mL) & DMSO (0.5 % v/v)) responses (% IL-1β expression) to plates of J774.A1 macrophages during the assay of chemical combinations from all generations (Gen 1 – Gen 11). Quintuplicate positive control responses were randomly assigned across wells (D1 – H12) for each generation tested. Data are expressed as a % of the mean positive control response of the plate they were assayed on (n=5 wells) with purple colors representing high positive control responses to mauve representing lower responses. Orange space represented wells that were not sampled.



Supplementary Figure 8. Quantile-Quantile plots of the positive control (LPS & DMSO) responses plotted against theoretical quantiles drawn from a normal distribution confirmed the data were normally distributed.

REFERENCES

1. Deb, K. *Multi-objective optimization using evolutionary algorithms*, (John Wiley & Sons, New York, 2001).
2. Zitzler, E. & Kunzli, S. Indicator based selection in multi-objective search. in *Parallel problem solving from nature - PPSN VIII : 8th international conference, Birmingham, UK, September 18-22, 2004 : proceedings* (ed. Yao, X.) 832 - 842 (Springer, Berlin ; [London], 2004).
3. Allmendinger, R. & Knowles, J. Evolutionary Optimization on Problems Subject to Changes of Variables. in *Parallel Problem Solving from Nature – PPSN XI*, Vol. 6239 (eds. Schaefer, R., Cotta, C., Kolodziej, J. & Rudolph, G.) 151-160 (Springer Berlin / Heidelberg, 2010).
4. Crawley, M.J. *Statistics : an introduction using R*, xiii, 327 p. (John Wiley & Sons, Chichester, 2005).
5. Zhang, J.H., Chung, T.D. & Oldenburg, K.R. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen* **4**, 67-73 (1999).
6. Oda, K. & Kitano, H. A comprehensive map of the toll-like receptor signaling network. *Mol Syst Biol* **2**, 2006 0015 (2006).
7. Kell, D. Iron Behaving Badly: Inappropriate Iron Chelation as a Major Contributor to the Aetiology of Vascular and Other Progressive Inflammatory and Degenerative Diseases. *BMC Med Genomics* **2**, 2 (2009).
8. Kell, D.B. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Archives of toxicology* (2010).
9. Sui, Y. & Wu, Z. Alternative statistical parameter for high-throughput screening assay quality assessment. *Journal of biomolecular screening : the official journal of the Society for Biomolecular Screening* **12**, 229-34 (2007).

Generation #	Combination #	Combination reagent composition									Objective 1_%IL-1b			Objective 2_cell death			Objective 3_# component reagents
		Reagent_1	Reagent_2	Reagent_3	Reagent_4	Reagent_5	Reagent_6	Reagent_7	Reagent_8	Reagent_9	P1	P2	P3	P1	P2	P3	
1	1	Diphenylene	FCCP								22.60	31.89	38.34	1.02	1.04	1.03	2
1	2	TPEN	N-acetyl cyst	tempol	SR 11302	CCCP					39.13	57.11	61.97	1.02	0.96	0.99	5
1	3	N-acetyl cyst									110.53	125.92	118.35	1.00	0.96	1.09	1
1	4	rotenone	SR 11302	PDTC	2-4-dinitrophe	mevastatin	NSC23766 (				43.64	59.31	58.66	1.00	1.00	1.02	6
1	5	alpha-tocoph	U0126	PD98059							61.30	71.90	85.43	0.99	0.98	0.98	3
1	6	VK-28	NSC23766 (								110.53	104.65	120.11	0.99	1.00	0.99	2
1	7	DMSO									116.89	101.55	115.70	0.97	0.97	0.98	2
1	8	alpha-tocoph	N-acetyl cyst	U0126	mevastatin	Y27632 dihy					27.01	41.16	88.83	1.06	0.97	1.03	5
1	9	SIH	wortmannin	IRAK 1	FCCP	NSC23766 (					9.52	22.03	13.80	1.16	1.06	1.05	5
1	10	tempol	apocynin	LY294002 hy							193.03	224.10	227.19	1.00	1.10	0.99	3
1	11	SIH	tempol	SR 11302	NSC23766 (						130.53	129.92	121.00	0.87	0.97	1.08	4
1	12	desferrioxam									116.18	131.53	132.59	1.00	1.00	0.98	1
1	13	alpha-tocoph	LY294002 hy	mevastatin							172.93	180.36	155.31	1.00	0.99	0.96	3
1	14	alpha-tocoph	wortmannin	CCCP							13.23	22.73	19.27	1.06	1.03	1.06	3
1	15	di-methyl sph									81.43	83.93	87.99	0.98	0.98	0.98	1
1	16	U0126	PDTC	NSC23766 (							35.92	46.93	44.79	1.00	1.00	0.96	3
1	17	rotenone									58.00	61.52	86.28	0.97	0.99	1.01	1
1	18	tempol	PDTC	SKI-II							25.75	40.45	31.93	1.01	0.96	0.97	3
1	19	SKI-II									28.91	41.89	39.95	0.99	0.97	1.04	1
1	20	desferrioxam	SB203580								30.18	29.77	27.95	1.02	1.04	0.99	2
1	21	desferrioxam	wortmannin								28.91	32.60	27.95	1.07	1.05	1.09	2
1	22	TPEN	alpha-tocopl	SKI-II							50.78	45.48	45.59	1.03	0.97	0.98	3
1	23	wortmannin									23.23	29.77	27.16	1.06	1.09	1.04	1
1	24	LY294002 hy	PD98059	genistein	FCCP						20.72	33.31	29.54	1.02	1.02	1.02	4
1	25	SIH	apocynin	SB203580							15.72	25.54	22.42	0.99	1.00	1.00	3
1	26	N-acetyl cyst	LY294002 hy	IRAK 1	SKI-II	di-methyl spl	2-4-dinitrophe				11.99	21.33	21.63	1.10	0.96	1.05	6
1	27	alpha-tocoph	U0126	IRAK 1	CCCP						11.99	19.94	13.80	1.06	0.99	1.08	4
1	28	apocynin	IRAK 1	Y27632 dihy							32.09	42.60	43.97	1.05	0.98	1.03	3
1	29	LY294002 hy	IRAK 1	di-methyl spl							32.72	48.38	52.92	1.05	1.12	1.03	3
1	30	alpha-tocoph	tempol	Diphenylene	SR 11302						61.96	86.20	98.28	1.00	1.02	1.03	4
1	31	apocynin	SKI-II								32.09	35.44	35.12	1.00	1.04	1.14	2
1	32	DMSO									126.20	134.75	127.23	1.03	1.05	1.02	2
1	33	alpha-tocoph	tempol	U0126	PD98059						82.79	68.18	61.14	1.04	1.02	0.99	4
1	34	DMSO									109.11	116.40	138.89	1.00	0.98	1.00	2
1	35	alpha-tocoph	LY294002 hy	IRAK 1							65.29	63.73	58.66	1.02	0.98	1.02	3
1	36	PD98059	di-methyl spl	genistein							76.02	83.17	82.04	1.01	1.01	1.01	3
1	37	U0126	SR 11302	CCCP							21.34	19.94	36.73	1.02	1.00	1.04	3
1	38	Diphenylene	SP600125	SKI-II	genistein						43.64	63.00	40.75	1.09	1.05	1.06	4
1	39	IKK inhibitor									54.71	51.27	62.79	1.03	0.98	1.04	1
1	40	alpha-tocoph	Diphenylene	wortmannin	di-methyl spl						16.96	24.84	28.75	1.18	1.20	1.21	4
1	41	PDTC	di-methyl spl	genistein	FCCP						18.21	35.44	28.75	1.03	1.03	1.09	4
1	42	TPEN	genistein								134.16	133.95	167.37	1.00	1.02	1.05	2
1	43	U0126	PDTC	SKI-II	2-4-dinitrophe						18.21	22.03	24.00	1.04	1.04	1.01	4
1	44	SIH	alpha-tocopl	rotenone	Diphenylene	IRAK 1	SR 11302	PDTC			27.01	32.60	25.58	1.10	1.06	1.07	7
1	45	desferrioxam									78.03	132.34	156.24	1.09	1.00	1.00	1
1	46	U0126	di-methyl spl	genistein							52.09	53.46	66.11	1.08	1.00	1.00	3
1	47	VK-28	wortmannin								16.34	34.74	25.58	1.19	1.15	1.10	2
1	48	tempol	CCCP								27.01	44.76	43.17	1.00	0.98	1.02	2
1	49	LY294002 hy	NSC23766 (								114.06	105.43	252.46	1.07	1.05	1.03	2
1	50	genistein									110.53	142.05	101.74	1.01	0.99	1.06	1
2	1	LY294002 hy									289.30	202.58	156.58	0.99	1.01	0.98	1
2	2	U0126	PDTC	NSC23766 (							91.26	69.62	59.74	0.97	1.08	0.99	3
2	3	N-acetyl cyst	IRAK 1		SKI-II		2-4-dinitrophe				35.74	35.50	22.13	0.98	1.04	0.97	4
2	4	desferrioxam									142.39	82.81	92.67	1.00	1.00	0.98	1
2	5	alpha-tocoph	alpha-tocopl	U0126	IRAK 1		CCCP	mevastatin			23.26	24.66	7.51	0.96	1.08	1.03	6
2	6	diphenylene i	wortmannin	mevastatin							28.44	42.79	17.50	1.04	1.19	0.98	3
2	7	tempol	U0126	PDTC	SKI-II						20.15	37.32	8.28	1.03	0.99	0.98	4
2	8	wortmannin		2-4-dinitrophe							23.26	41.87	22.90	1.00	1.01	1.00	2

2	9	alpha-tocoph	U0126	SKI-II	di-methyl spl	genistein	2-4-dinitrophe				65.35	25.56	0.00	1.02	1.01	0.98		6
2	10	diphenylene i	di-methyl spl								69.62	78.08	31.44	1.03	1.01	1.05		2
2	11	alpha-tocoph	rotenone	apocynin							102.21	85.65	74.11	1.05	1.05	1.05		3
2	12	alpha-tocoph	wortmannin								69.62	65.88	73.31	1.08	1.14	1.11		2
2	13	SIH	LY294002 hy	wortmannin	IRAK 1		di-methyl spl	genistein	FCCP	NSC23766 (	36.78	25.56	0.00	1.14	1.14	1.06		8
2	14	apocynin	IRAK 1		Y27632 dihy						69.62	51.05	42.38	0.97	1.00	0.96		3
2	15	alpha-tocoph	U0126	PD98059	SR 11302						85.81	71.50	65.32	0.99	1.02	0.98		4
2	16	wortmannin	genistein	NSC23766 (							25.33	40.96	22.13	1.01	1.15	0.97		3
2	17	alpha-tocoph	SB203580	PDTC	SKI-II						13.97	18.38	12.11	1.02	1.00	0.95		4
2	18	di-methyl sph									73.92	70.55	56.58	0.98	1.01	0.99		1
2	19	SR 11302	IKK inhibitor	di-methyl spl							20.15	37.32	22.90	0.99	1.02	1.00		3
2	20	DMSO									121.02	117.45	83.76	0.98	0.99	0.99		0
2	21	desferrioxam									125.49	184.83	117.24	1.03	1.03	1.04		1
2	22	tempol	LY294002 hy	PDTC	SKI-II	di-methyl spl	FCCP				90.16	31.87	12.11	1.04	1.02	0.99		6
2	23	U0126	PDTC	FCCP	NSC23766 (						55.75	28.26	12.88	1.01	1.04	1.02		4
2	24	desferrioxam	rotenone	wortmannin							57.88	45.53	26.78	1.05	1.12	1.05		3
2	25	diphenylene i	U0126	genistein		2-4-dinitrophe					92.34	60.29	32.22	1.00	1.04	1.28		4
2	26	di-methyl sph									70.70	72.43	45.53	1.00	1.02	1.02		1
2	27	diphenylene i	IRAK 1		SKI-II						0.00	24.66	0.00	1.06	1.08	1.01		3
2	28	tempol	U0126	CCCP	mevastatin						15.00	64.02	34.56	0.98	1.10	1.01		4
2	29	TPEN	rotenone	SR 11302	CCCP						115.46	109.65	95.93	1.15	1.14	1.06		4
2	30	alpha-tocoph	U0126	PD98059	SP600125	di-methyl spl	mevastatin				66.41	59.37	64.52	1.06	1.01	1.02		6
2	31	IRAK 1									91.26	69.62	60.54	1.03	1.01	1.07		1
2	32	tempol	wortmannin	IRAK 1		CCCP					42.03	92.32	15.96	1.15	1.24	1.30		4
2	33	alpha-tocoph	N-acetyl cys	PDTC	genistein	FCCP					57.88	35.50	17.50	1.04	1.00	1.00		5
2	34	IRAK 1		SR 11302	NSC23766 (						67.48	33.68	23.68	0.99	1.06	1.00		3
2	35	rotenone	PDTC		2-4-dinitrophe						82.56	62.16	55.78	1.02	1.06	1.06		3
2	36	U0126	PD98059								63.20	80.92	62.93	0.98	1.10	0.95		2
2	37	desferrioxam	alpha-tocopl	diphenylene	U0126						45.18	54.74	24.45	1.07	1.04	0.96		4
2	38	VK-28	U0126	NSC23766 (							50.46	43.70	40.82	1.01	1.00	1.00		3
2	39	alpha-tocoph	tempol	apocynin	LY294002 hy						159.49	144.13	138.89	0.99	1.02	1.01		4
2	40	desferrioxam	wortmannin	SB203580	genistein	FCCP					0.00	16.59	0.00	1.12	1.03	1.18		5
2	41	desferrioxam	NSC23766 (	Y27632 dihy							166.38	94.23	75.71	1.02	1.01	0.97		3
2	42	N-acetyl cyst	PDTC								142.39	88.50	91.87	1.01	1.10	1.03		2
2	43	SIH	tempol								137.86	100.00	77.32	0.99	1.04	0.93		2
2	44	alpha-tocoph	tempol	diphenylene	SR 11302	genistein					107.70	90.42	58.16	1.04	1.04	1.02		5
2	45	VK-28	SIH	alpha-tocopl	U0126	SR 11302	di-methyl spl	mevastatin	NSC23766 (	Y27632 dihy	0.00	47.37	9.81	0.99	1.12	1.05		9
2	46	SB203580	PDTC	di-methyl spl	FCCP						12.95	64.02	0.00	1.01	1.16	0.91		4
2	47	alpha-tocoph	alpha-tocopl	U0126	PDTC	SKI-II	CCCP				21.19	28.26	6.74	1.07	0.98	0.92		6
2	48	N-acetyl cyst	LY294002 hy	SR 11302	FCCP		2-4-dinitrophe				30.52	40.96	55.78	1.00	0.93	1.07		5
2	49	SIH	desferrioxam	tempol	SR 11302	NSC23766 (					68.55	127.25	84.57	0.99	0.94	0.94		5
2	50	tempol	CCCP								51.51	63.08	43.17	0.97	1.02	1.00		2
3	1	TPEN	U0126								48.64	34.47	19.66	1.06	1.05	1.06		2
3	2	tempol	CCCP								27.13	13.59	19.10	1.00	1.05	1.02		2
3	3	apocynin	PDTC	SKI-II							13.90	8.61	11.92	1.11	1.03	1.03		3
3	4	DMSO									91.37	82.76	52.14	1.02	0.98	0.97		0
3	5	wortmannin		2-4-dinitrophe							42.34	42.85	49.81	1.04	0.99	1.02		2
3	6	tempol	SP600125	SKI-II	CCCP						9.55	7.38	10.83	1.03	1.01	1.05		4
3	7	alpha-tocoph	U0126	PD98059							69.08	80.04	48.06	0.98	1.00	0.96		3
3	8	VK-28	PDTC	FCCP							7.93	7.38	6.46	0.98	1.01	0.98		3
3	9	VK-28	wortmannin	PDTC	IKK inhibitor	SKI-II					5.77	0.00	19.66	1.10	1.08	1.28		5
3	10	tempol	apocynin	U0126	PDTC	FCCP					11.18	11.10	11.92	1.05	1.05	1.09		5
3	11	SIH	apocynin	IRAK 1							31.60	35.12	41.70	1.02	1.00	1.03		3
3	12	SIH	tempol	apocynin	SB203580	PDTC					15.54	13.59	14.13	1.00	1.01	1.00		5
3	13	SB203580	PDTC								17.18	12.35	11.38	0.98	1.00	0.99		2
3	14	desferrioxam	N-acetyl cys	SP600125	SR 11302	SKI-II	CCCP		2-4-dinitrophe	NSC23766 (	9.55	8.61	10.28	1.00	1.00	1.01		8
3	15	apocynin	U0126	PDTC		2-4-dinitrophe	Y27632 dihy				70.26	78.67	50.97	0.99	0.99	0.97		5
3	16	desferrioxam	tempol	SB203580							13.36	12.35	13.58	1.00	0.95	1.02		3
3	17	DMSO									76.23	78.67	108.86	0.97	1.00	1.01		0
3	18	rotenone									34.41	40.91	28.05	1.00	1.03	0.97		1

3	19	wortmannin		2-4-dinitrophe					22.69	23.64	20.21	1.03	1.02	0.98	2
3	20	SIH	SB203580	SKI-II					0.00	0.00	0.00	0.97	0.98	0.93	3
3	21	SB203580	PDTC	di-methyl spl	genistein	FCCP			0.00	0.00	6.46	0.98	1.00	1.04	5
3	22		2-4-dinitrophe						121.42	185.91	191.90	0.99	1.01	1.06	1
3	23	VK-28	wortmannin	NSC23766 (					33.29	38.98	41.70	1.02	1.00	1.06	3
3	24	tempol	U0126	PDTC					87.71	88.93	91.88	1.03	1.00	1.03	3
3	25	alpha-tocoph	SB203580	U0126	PD98059	SKI-II	genistein		5.77	0.00	5.92	1.02	0.99	1.00	6
3	26	di-methyl sph							79.23	65.22	86.95	1.00	0.98	1.02	1
3	27	tempol	wortmannin	SB203580	genistein				14.99	16.72	13.03	0.98	1.00	0.97	4
3	28	SIH	alpha-tocopl	SB203580	SR 11302	PDTC	NSC23766 (		11.72	9.86	11.38	0.97	0.95	0.99	6
3	29	SIH	SB203580						12.81	13.59	14.13	0.98	0.96	1.01	2
3	30	SIH	alpha-tocopl	wortmannin					38.37	40.26	35.41	1.00	1.05	0.97	3
3	31	SIH	tempol	U0126	PDTC	SKI-II	di-methyl spl		8.47	8.61	7.55	0.97	0.99	0.96	6
3	32	TPEN	alpha-tocopl	wortmannin	SKI-II				9.01	6.76	8.09	1.39	1.56	1.40	4
3	33	alpha-tocoph	SB203580	PDTC		2-4-dinitrophe			13.90	13.59	16.33	1.00	0.97	1.02	4
3	34	VK-28	desferrioxam	tempol	LY294002 hy	SKI-II	Y27632 dihy		18.28	16.09	17.99	1.02	1.02	1.02	6
3	35	SKI-II							12.27	11.10	13.58	1.00	0.98	1.01	1
3	36	alpha-tocoph	apocynin	wortmannin	PD98059	CCCP	mevastatin		0.00	9.86	11.92	1.03	1.05	1.01	6
3	37	wortmannin	genistein						40.63	42.85	46.32	1.00	0.97	1.02	2
3	38	desferrioxam	N-acetyl cys	tempol	NSC23766 (				62.59	75.29	62.74	0.95	0.99	0.95	4
3	39	SIH	tempol	SR 11302					88.92	84.81	78.37	0.95	0.97	0.97	3
3	40	IRAK 1		SKI-II	NSC23766 (				10.63	12.35	11.38	1.04	1.02	1.09	3
3	41	tempol	U0126	PDTC	SKI-II	NSC23766 (			6.85	8.00	8.64	1.04	1.05	1.07	5
3	42	SKI-II	Y27632 dihy						14.45	13.59	7.55	1.01	1.03	0.97	2
3	43	VK-28	SIH	alpha-tocopl	SKI-II				13.36	14.22	15.78	0.97	1.00	1.05	4
3	44	CCCP							18.83	24.28	25.24	0.98	1.03	1.02	1
3	45	alpha-tocoph	PD98059	SKI-II					11.72	11.10	13.58	1.00	0.98	1.00	3
3	46	tempol	FCCP						9.55	10.48	11.92	1.00	0.99	1.00	2
3	47	SR 11302	CCCP						24.35	20.49	21.89	1.00	1.02	0.94	2
3	48	U0126	PDTC						82.85	91.68	59.78	0.99	1.01	0.98	2
3	49	U0126	SR 11302	CCCP					21.04	23.64	19.10	1.02	1.02	0.98	3
3	50	rotenone	SP600125						66.12	70.57	61.56	1.05	1.02	1.06	2
4	1	TPEN	alpha-tocopl	U0126					45.45	102.61	65.41	0.87	1.03	1.03	3
4	2	PD98059							107.75	148.58	83.08	0.97	1.02	1.01	1
4	3	apocynin	SKI-II	EGCG					44.99	50.34	42.37	1.01	1.12	0.98	3
4	4	tempol	SKI-II						18.64	35.98	45.59	0.92	1.02	1.00	2
4	5	SIH	desferrioxam	diphenylene	LY294002 hy	PD98059	IRAK 1	SKI-II	11.14	13.23	9.95	1.05	1.11	1.02	7
4	6	VK-28	TPEN	alpha-tocopl	EGCG				73.19	126.38	112.61	0.95	1.09	1.01	4
4	7	VK-28	desferrioxam	SB203580					5.25	8.97	7.21	0.99	1.12	0.97	3
4	8	U0126	SKI-II						61.11	37.25	36.97	1.03	0.98	0.95	2
4	9	SIH	TPEN	apocynin	IRAK 1		SKI-II		19.05	49.79	25.07	0.95	1.09	0.99	5
4	10	apocynin	PD98059	SR 11302	genistein				47.76	78.04	59.10	0.91	0.99	0.98	4
4	11	SIH	desferrioxam	tempol	SR 11302	NSC23766 (	Y27632 dihy		56.18	107.14	57.38	1.04	1.01	0.92	6
4	12	diphenylene i	di-methyl spl						22.13	55.74	37.29	0.91	1.10	1.09	2
4	13	tempol	rotenone	SR 11302	EGCG				50.13	42.78	36.89	1.04	1.05	1.06	4
4	14	SR 11302	IKK inhibitor	di-methyl spl					71.03	65.83	40.93	1.02	1.08	0.96	3
4	15	DMSO							44.72	121.99	93.24	0.96	1.06	1.00	0
4	16	apocynin	PDTC	SKI-II					13.16	48.37	27.42	0.92	1.10	0.97	3
4	17	wortmannin	SB203580	SKI-II	genistein				3.32	4.32	3.66	1.04	0.99	1.01	4
4	18	PDTC	SKI-II						9.75	54.14	23.89	0.93	1.13	1.00	2
4	19	desferrioxam	N-acetyl cys	tempol	di-methyl spl	NSC23766 (	simvastatin		35.18	67.47	50.42	0.92	1.00	0.97	6
4	20	SIH	desferrioxam	PDTC					80.31	78.18	83.73	1.04	0.99	0.92	3
4	21	alpha-tocoph	apocynin	SKI-II	simvastatin				12.85	41.24	21.50	0.90	1.05	1.01	4
4	22	SIH	TPEN	N-acetyl cys	SB203580	PD98059	SR 11302		7.82	7.42	6.93	1.03	1.01	1.01	6
4	23	alpha-tocoph	PD98059						70.33	108.51	73.77	0.99	1.04	0.95	2
4	24	SIH	alpha-tocopl	SB203580	PDTC	SKI-II			2.02	3.97	3.93	0.88	0.99	0.99	5
4	25	SIH	tempol	SB203580	SR 11302	SKI-II	NSC23766 (		4.47	5.46	3.45	1.00	1.04	0.95	6
4	26	apocynin	PDTC	SKI-II					10.83	40.94	25.01	0.91	1.04	0.97	3
4	27	desferrioxam	rotenone	SKI-II	genistein				19.38	20.67	18.08	1.08	1.02	1.03	4
4	28	tempol	SB203580	PDTC	simvastatin				3.81	10.10	6.38	0.90	1.07	0.95	4

4	29	SIH	tempol							57.00	111.81	94.79	0.89	0.99	0.99	2
4	30	IRAK 1		NSC23766 (						33.16	25.38	34.82	1.04	0.98	0.97	2
4	31	SIH	apocynin	SB203580						10.53	10.63	9.71	1.06	0.99	0.99	3
4	32	apocynin	SB203580	SP600125	PDTC					8.87	11.61	5.55	1.01	1.06	1.03	4
4	33	apocynin	PD98059	SKI-II						31.04	36.66	20.90	1.00	1.03	0.99	3
4	34	SIH	rotenone							21.57	38.34	43.40	0.92	1.04	1.06	2
4	35	SIH	SB203580	SKI-II						3.76	6.11	3.66	1.02	1.06	1.01	3
4	36	N-acetyl cyst	apocynin							53.15	151.72	76.99	0.92	1.03	0.97	2
4	37	SIH	SB203580	SKI-II						2.56	5.11	3.45	0.93	1.05	1.01	3
4	38	SIH								113.26	109.03	82.95	1.01	0.97	0.99	1
4	39	TPEN	tempol	U0126	Y27632 dihy					58.66	177.34	104.52	0.93	1.07	0.94	4
4	40	wortmannin	U0126	IRAK 1						45.45	36.66	42.28	1.03	0.96	0.98	3
4	41	SIH	SB203580	SKI-II						2.94	3.76	3.18	1.07	0.99	1.02	3
4	42	IKK inhibitor								49.75	97.44	47.65	1.00	1.13	1.05	1
4	43	VK-28	alpha-tocopl	SKI-II	EGCG					38.58	19.17	31.19	1.00	0.97	1.03	4
4	44	SB203580	PD98059	SKI-II	di-methyl spl	NSC23766 (	simvastatin	EGCG		2.51	3.62	2.81	1.03	1.09	1.07	7
4	45	diphenylene i								20.26	58.66	32.03	1.07	1.15	1.10	1
4	46	SKI-II								37.74	49.57	28.01	0.98	1.08	0.95	1
4	47	desferrioxam	alpha-tocopl	U0126	PD98059	IKK inhibitor	simvastatin			36.66	30.41	46.58	1.11	1.00	1.03	6
4	48	SIH	SB203580	SP600125	IRAK 1					3.98	6.11	4.52	1.08	1.13	0.94	4
4	49	tempol	wortmannin	SB203580						9.70	5.82	7.83	1.19	0.99	0.98	3
4	50	rotenone								30.34	32.36	28.30	1.10	1.04	1.07	1
5	1	apocynin	SB203580	SR 11302						16.47	15.44	13.58	1.01	1.05	1.01	3
5	2	SIH	SKI-II	di-methyl spl						35.99	35.58	13.67	0.99	1.04	0.98	3
5	3	SIH	tempol	SKI-II						48.78	35.28	22.38	0.98	1.03	1.02	3
5	4	tempol	wortmannin	PDTC	simvastatin					111.97	28.97	19.28	0.99	1.06	1.01	4
5	5	tempol	SP600125	PDTC	SKI-II	di-methyl spl				38.77	39.81	35.51	0.97	1.05	1.05	5
5	6	PDTC	IKK inhibitor	SKI-II						26.77	28.53	16.17	1.02	1.12	1.03	3
5	7	alpha-tocoph	diphenylene	SB203580	PDTC	SKI-II	Y27632 dihy			5.64	5.61	4.46	0.99	1.02	1.10	6
5	8	TPEN	tempol	apocynin	SB203580	IRAK 1		SR 11302	genistein	16.82	15.04	9.72	1.00	1.02	0.99	7
5	9	SB203580	SKI-II							7.08	6.51	4.63	1.03	1.00	0.98	2
5	10	alpha-tocoph	tempol	SB203580	PDTC	genistein	simvastatin			9.66	8.44	7.37	1.01	0.99	1.04	6
5	11	SKI-II	mevastatin							26.89	34.24	17.95	1.01	1.04	1.02	2
5	12	SIH	tempol	SR 11302	SKI-II	Y27632 dihy				36.78	38.44	12.49	0.98	1.03	0.95	5
5	13	apocynin	PD98059	SR 11302	genistein					126.67	116.96	100.00	0.95	1.03	1.03	4
5	14	SIH	PD98059	SKI-II	genistein					30.81	35.58	26.78	1.00	1.04	1.04	4
5	15	VK-28	desferrioxam	SB203580	SR 11302	Y27632 dihy				12.39	12.24	9.11	0.99	1.05	1.01	5
5	16	SKI-II								29.41	33.35	15.89	1.00	1.03	0.98	1
5	17	SKI-II	di-methyl spl							22.94	31.59	21.89	0.98	1.06	1.05	2
5	18	LY294002 hy	di-methyl spl							149.98	122.44	74.77	1.00	1.02	0.97	2
5	19	TPEN	SKI-II							34.03	42.73	44.41	1.05	1.01	1.08	2
5	20	U0126	SR 11302	EGCG						151.80	129.18	139.91	1.00	1.03	1.01	3
5	21	SIH	apocynin	SB203580						11.59	14.37	10.70	0.99	1.02	1.00	3
5	22	tempol								105.71	139.67	103.44	0.98	1.03	0.96	1
5	23	tempol	SR 11302	SKI-II	di-methyl spl	EGCG				23.67	27.96	25.87	1.02	1.04	1.07	5
5	24	wortmannin	PD98059	di-methyl spl						87.12	90.33	69.38	0.99	1.06	1.00	3
5	25	DMSO								104.04	146.92	94.82	0.96	0.91	0.93	0
5	26	tempol								101.45	122.88	122.55	0.97	1.00	0.98	1
5	27	SKI-II								30.17	24.83	16.07	0.96	0.98	0.94	1
5	28	SB203580	SKI-II							6.41	6.12	4.63	1.00	0.99	0.96	2
5	29	alpha-tocoph	mevastatin							100.36	77.72	118.70	0.99	1.01	0.99	2
5	30	SIH	SB203580	PD98059	SKI-II					5.97	6.76	4.63	0.99	1.05	0.95	4
5	31	SIH	SB203580	SKI-II	Y27632 dihy					6.30	6.76	5.31	0.97	0.99	1.01	4
5	32	DMSO								83.39	132.18	113.64	0.97	1.03	0.99	0
5	33	SIH	SKI-II							25.27	26.96	32.50	0.99	0.97	1.09	2
5	34	tempol	SB203580	PDTC	simvastatin					9.10	10.39	8.50	1.01	1.09	1.03	4
5	35	desferrioxam	alpha-tocopl							86.95	92.65	107.48	0.95	1.01	1.04	2
5	36	wortmannin	SB203580	simvastatin						10.22	12.50	8.93	0.98	0.98	0.96	3
5	37	tempol	SKI-II							22.57	34.24	26.68	0.92	1.00	1.02	2
5	38	SIH	SB203580	PDTC						9.43	8.70	7.89	0.94	1.01	0.98	3

5	39	SIH	SB203580	SP600125	SKI-II				9.66	8.57	6.93	1.00	0.98	1.01	4
5	40	rotenone	wortmannin						55.95	33.79	36.93	1.04	1.00	1.11	2
5	41	rotenone	PD98059	SKI-II					17.53	18.56	16.45	1.06	1.05	1.13	3
5	42	TPEN	wortmannin	SB203580					10.00	11.58	13.22	0.97	1.00	1.05	3
5	43	alpha-tocoph	tempol	U0126	PDTC				46.68	67.72	80.82	0.99	1.06	0.98	4
5	44	SB203580	SKI-II						5.53	5.99	5.31	1.02	1.09	1.00	2
5	45	apocynin	SR 11302	genistein					47.10	65.12	118.13	0.99	0.97	1.08	3
5	46	VK-28							55.08	75.72	96.63	0.98	1.02	1.01	1
5	47	SIH	SKI-II						19.67	33.06	21.99	0.94	1.02	1.02	2
5	48	PDTC	NSC23766 (						46.40	44.44	76.52	0.96	0.97	0.99	2
5	49	SIH	SB203580						8.87	7.92	11.95	1.01	0.96	1.10	2
5	50	wortmannin	SKI-II	genistein	Y27632 dihy				26.64	16.39	16.35	0.97	0.96	1.04	4
6	1	wortmannin	SB203580	SKI-II					0.00	4.87	2.66	1.06	1.04	1.03	3
6	2	N-acetyl cyst	NSC23766 (						119.95	133.27	112.91	1.02	1.06	0.96	2
6	3	IKK inhibitor (	SKI-II						17.10	27.56	11.38	1.08	1.05	0.96	2
6	4	desferrioxam	tempol	wortmannin	SB203580	PD98059	Y27632 dihy	simvastatin	0.00	2.97	2.66	1.27	1.23	1.18	7
6	5	SIH	alpha-tocopl	SB203580	PDTC	SKI-II			3.10	4.39	0.00	0.98	1.07	0.89	5
6	6	SIH	SB203580	SP600125	SKI-II				6.78	6.46	5.02	0.93	1.00	0.86	4
6	7	desferrioxam	tempol	LY294002 hy	Y27632 dihy				132.94	159.43	95.96	0.96	1.06	0.93	4
6	8	wortmannin	SB203580	PD98059	simvastatin				0.00	0.00	2.39	1.26	1.29	1.26	4
6	9	SB203580	simvastatin						5.30	8.06	6.56	1.00	1.04	0.93	2
6	10	N-acetyl cyst	NSC23766 (						74.83	97.98	55.48	1.04	1.02	0.93	2
6	11	SIH	alpha-tocopl	SB203580	SP600125	simvastatin			7.52	14.39	7.82	1.04	1.05	0.99	5
6	12	VK-28							131.21	137.57	122.23	1.01	1.01	1.00	1
6	13	wortmannin	SKI-II	simvastatin					16.64	18.20	7.54	1.02	1.03	0.94	3
6	14	SB203580							7.52	7.89	8.81	0.99	1.00	0.98	1
6	15	desferrioxam	SKI-II						25.01	27.21	14.26	1.03	1.08	0.92	2
6	16	SIH	desferrioxan	tempol	SB203580				7.08	7.09	5.30	0.99	0.98	0.91	4
6	17	SB203580	di-methyl spl	Y27632 dihy					3.98	5.18	5.16	0.97	1.04	0.93	3
6	18	VK-28	tempol						103.08	83.38	95.16	0.93	0.97	1.04	2
6	19	SIH	alpha-tocopl	SB203580	PDTC	SKI-II			2.96	3.60	2.25	0.99	1.04	1.00	5
6	20	SIH	LY294002 hy	SB203580	PDTC				9.62	9.83	7.12	1.03	1.03	0.96	4
6	21	tempol	wortmannin						98.69	95.31	118.73	1.01	0.98	1.06	2
6	22	alpha-tocoph	SB203580	genistein					10.22	19.54	10.23	1.01	1.02	0.96	3
6	23	SIH	tempol	SR 11302	SKI-II				24.37	32.63	12.82	1.00	1.01	0.91	4
6	24	VK-28	SP600125						96.31	102.71	95.76	1.00	0.98	0.98	2
6	25	SIH	SB203580	PDTC					7.97	8.22	4.88	1.05	1.02	0.93	3
6	26	rotenone	IRAK 1		SKI-II				9.32	12.10	5.58	1.09	1.05	0.97	3
6	27	SIH	tempol	SR 11302	PDTC	SKI-II			23.56	29.99	12.67	0.99	1.04	0.92	5
6	28	SIH	SB203580						6.48	8.22	8.39	0.94	0.95	1.09	2
6	29	SB203580	SKI-II	simvastatin					0.00	4.55	0.00	1.01	1.03	1.03	3
6	30	SIH	tempol	wortmannin	SKI-II				7.23	9.83	7.12	1.52	1.52	1.26	4
6	31	SKI-II	di-methyl spl						9.92	32.98	26.01	1.03	1.05	1.01	2
6	32	tempol	SKI-II	NSC23766 (					15.09	17.04	17.33	1.04	1.03	0.96	3
6	33	apocynin	SKI-II						23.72	22.41	17.62	1.00	1.01	0.99	2
6	34	VK-28	SIH	tempol	diphenylene	SB203580	SKI-II		0.00	3.13	2.25	1.07	1.11	0.97	6
6	35	SIH	alpha-tocopl	tempol					102.63	83.81	59.98	0.92	0.96	0.94	3
6	36	wortmannin	SKI-II						22.77	37.26	20.43	0.94	1.05	0.91	2
6	37	wortmannin	SB203580	mevastatin	simvastatin				0.00	2.97	4.60	1.18	1.15	1.20	4
6	38	desferrioxam	tempol	SB203580					6.93	9.50	5.44	1.00	0.99	0.94	3
6	39	SIH	SB203580	SR 11302	SKI-II				2.52	14.23	6.56	1.01	1.05	1.04	4
6	40	tempol	SP600125	SR 11302	PDTC	NSC23766 (			83.29	75.22	43.72	1.01	1.02	0.99	5
6	41	rotenone	NSC23766 (						29.40	37.80	36.77	1.07	1.05	1.14	2
6	42	SIH	desferrioxan	tempol	SB203580	SR 11302	PDTC		7.23	6.77	8.39	0.96	0.99	0.99	6
6	43	SIH	tempol	SB203580	SR 11302	genistein			6.04	8.54	8.39	0.99	0.98	0.94	5
6	44	tempol	SB203580	PDTC	Y27632 dihy	simvastatin			4.72	7.74	7.68	0.99	0.99	0.96	5
6	45	SIH	N-acetyl cys	SB203580	U0126	SKI-II			0.00	3.45	0.00	1.12	1.11	0.97	5
6	46	SKI-II	Y27632 dihy	EGCG					14.17	26.00	15.42	0.99	0.97	0.93	3
6	47	SB203580	U0126	PDTC	simvastatin				2.38	0.00	0.00	1.02	0.96	1.05	4
6	48	LY294002 hy	wortmannin	SKI-II					35.21	33.69	18.51	1.03	1.01	0.98	3

6	49	IRAK 1							108.88	131.03	49.03	1.01	1.01	0.95	1
6	50	SKI-II	EGCG						33.87	34.93	40.63	1.03	1.02	1.09	2
7	1	tempol	SP600125	SKI-II					16.06	15.77	20.31	1.15	1.13	1.01	3
7	2	wortmannin	SB203580	simvastatin					4.04	0.00	9.03	1.32	1.41	1.69	3
7	3	SIH	SB203580	SP600125	SKI-II	NSC23766 (			4.91	3.57	8.39	0.99	1.02	1.07	5
7	4	SKI-II							25.64	13.44	15.13	1.03	0.97	1.06	1
7	5	apocynin	SB203580						10.75	7.83	8.39	1.01	0.98	0.96	2
7	6	SB203580	SKI-II	simvastatin					4.91	3.57	4.58	1.05	1.02	0.94	3
7	7	alpha-tocoph	wortmannin	SKI-II	di-methyl spl				4.04	4.22	6.48	1.09	1.32	0.99	4
7	8	Y27632 dihyc							90.13	58.00	110.88	0.98	0.93	1.04	1
7	9	genistein							76.11	54.82	59.01	1.06	0.89	0.92	1
7	10	SB203580	SKI-II						0.00	0.00	5.21	1.05	1.06	1.00	2
7	11	SB203580	SKI-II						4.04	0.00	8.07	0.99	0.96	1.08	2
7	12	rotenone	SB203580	Y27632 dihy					9.58	8.82	14.48	1.05	1.05	1.10	3
7	13	tempol							90.13	73.77	121.18	0.99	0.96	1.07	1
7	14	SIH	SB203580	SP600125	SKI-II				7.24	6.85	7.43	1.00	1.00	0.93	4
7	15	alpha-tocoph	wortmannin	SB203580	simvastatin				5.49	4.22	4.89	1.59	1.29	0.97	4
7	16	SB203580	Y27632 dihy						9.58	8.82	11.91	0.99	1.00	1.04	2
7	17	SIH	apocynin	SP600125	SKI-II	Y27632 dihy	EGCG		82.07	94.74	72.37	0.96	1.00	0.93	6
7	18	SIH	SB203580	SP600125	SKI-II	NSC23766 (			6.65	6.19	11.91	1.01	0.92	1.06	5
7	19	SKI-II	NSC23766 (						89.79	52.00	49.35	1.00	0.93	0.92	2
7	20	diphenylene i	SB203580	IKK inhibitor	SKI-II	simvastatin			0.00	0.00	3.94	1.16	0.97	0.97	5
7	21	VK-28	SIH	desferrioxam	alpha-tocopl	diphenylene	SB203580	SKI-II	0.00	0.00	6.48	0.99	0.98	1.15	7
7	22	apocynin	SB203580	SKI-II					3.46	0.00	7.43	0.99	1.02	1.01	3
7	23	apocynin	mevastatin	simvastatin					63.39	43.97	88.22	0.97	0.93	1.01	3
7	24	SIH	wortmannin	IKK inhibitor	SKI-II				0.00	0.00	8.39	1.09	1.52	1.53	4
7	25	SKI-II							16.36	22.79	29.50	0.99	0.99	1.02	1
7	26	IRAK 1		SKI-II	di-methyl spl				0.00	4.22	4.89	0.98	0.97	0.95	3
7	27	SIH	rotenone	SB203580					6.95	5.21	12.23	1.01	0.95	1.09	3
7	28	LY294002 hy	wortmannin	SB203580	SKI-II				6.36	0.00	4.89	1.48	1.05	1.03	4
7	29	SKI-II	di-methyl spl						25.94	8.49	10.95	1.06	0.90	0.92	2
7	30	tempol	rotenone						42.48	28.19	56.58	1.03	0.97	1.16	2
7	31	SKI-II							103.79	125.69	105.62	1.03	1.05	0.96	1
7	32	SIH	SB203580	PDTC	SKI-II				7.82	7.50	11.59	1.04	1.07	1.08	4
7	33	EGCG							86.42	75.58	134.79	1.02	1.00	1.11	1
7	34	SB203580	di-methyl spl						8.41	8.82	9.35	1.00	0.99	0.93	2
7	35	apocynin							90.47	92.51	86.76	1.03	1.06	0.95	1
7	36	SIH	SB203580	SKI-II	mevastatin	simvastatin			0.00	0.00	4.58	0.97	0.99	0.93	5
7	37	alpha-tocoph	SB203580	SKI-II					10.46	14.11	20.97	0.98	0.90	1.05	3
7	38	tempol	mevastatin						121.69	68.35	65.31	0.99	0.90	0.96	2
7	39	SIH	SB203580	PDTC	IKK inhibitor				6.07	0.00	5.53	1.02	0.94	0.96	4
7	40	SIH	desferrioxam	alpha-tocopl	tempol	SB203580	Y27632 dihy		7.53	5.21	14.81	0.99	0.91	1.09	6
7	41	SIH	LY294002 hy	SB203580	IRAK 1		SKI-II		14.29	16.10	17.39	1.06	1.05	0.92	5
7	42	apocynin	SKI-II						95.21	98.49	129.70	1.01	1.04	1.07	2
7	43	desferrioxam							97.61	99.62	137.56	1.04	1.02	1.07	1
7	44	desferrioxam	SKI-II	genistein	Y27632 dihy	EGCG			86.76	112.54	76.29	1.02	1.04	0.96	5
7	45	SIH	alpha-tocopl	SB203580	PDTC	SKI-II			5.78	0.00	5.53	0.99	1.01	0.91	5
7	46	DMSO							86.76	93.26	156.78	1.04	1.07	1.06	0
7	47	LY294002 hy	SR 11302	SKI-II	Y27632 dihy				21.73	25.49	18.69	1.08	1.02	0.94	4
7	48	SB203580	PDTC						10.46	7.50	10.31	1.06	1.01	0.96	2
7	49	TPEN	wortmannin	SB203580	SKI-II	simvastatin			5.78	0.00	5.85	1.54	1.42	1.23	5
7	50	SIH	desferrioxam	tempol	SB203580	IKK inhibitor			8.11	0.00	9.03	1.02	0.95	1.14	5
8	1	SB203580	SP600125	SKI-II	mevastatin				17.42	12.03	13.51	1.23	1.04	1.00	4
8	2	N-acetyl cyst	diphenylene	Y27632 dihy					47.47	51.12	52.55	1.09	1.11	1.01	3
8	3	SIH	LY294002 hy	SB203580	PDTC	SKI-II	genistein		17.42	17.11	14.47	1.13	1.08	1.02	6
8	4	tempol	SB203580	SKI-II					14.48	11.53	13.51	1.06	1.04	1.04	3
8	5	tempol	PD98059	simvastatin					96.73	88.58	80.62	1.09	1.10	1.05	3
8	6	SIH	SB203580	SP600125	IKK inhibitor	SKI-II	di-methyl spl	mevastatin	10.97	9.51	10.15	0.99	1.01	1.04	7
8	7	SB203580	PDTC	genistein					22.15	20.18	21.23	1.08	1.07	1.07	3
8	8	SR 11302	genistein						60.43	83.51	102.17	0.99	0.99	1.05	2

8	9	VK-28	SIH	alpha-tocopl	wortmannin	SB203580			14.48	10.52	11.11	1.17	1.17	1.12	5
8	10	SIH	SB203580	mevastatin	simvastatin				16.25	17.11	15.91	0.97	1.04	0.99	4
8	11	SIH	desferrioxam	SB203580	IKK inhibitor				12.14	0.00	10.15	1.06	0.97	0.96	4
8	12	IRAK 1		SKI-II					19.19	13.05	15.91	1.37	1.03	0.98	2
8	13	SIH	SB203580	SKI-II					12.72	12.03	12.55	1.01	1.07	0.97	3
8	14	SP600125	di-methyl spl						127.24	118.64	109.33	1.13	1.03	1.01	2
8	15	SB203580	SP600125	di-methyl spl					22.15	18.65	19.29	1.05	1.02	1.04	3
8	16	apocynin	SB203580	PDTC	NSC23766 (				22.15	19.67	16.87	1.10	1.07	1.04	4
8	17	SIH	PD98059						75.51	75.68	78.50	1.03	1.01	1.03	2
8	18	SB203580	genistein						19.78	21.21	20.74	1.03	1.04	1.01	2
8	19	diphenylene i	wortmannin	genistein					13.90	11.53	15.43	1.12	1.11	1.12	3
8	20	diphenylene i	SB203580						15.07	14.57	12.55	1.12	1.06	1.01	2
8	21	N-acetyl cyst	tempol	SB203580	simvastatin				19.19	20.69	16.87	0.99	1.00	1.01	4
8	22	SB203580	SKI-II	genistein	NSC23766 (				10.97	0.00	11.59	1.02	0.97	0.99	4
8	23	SB203580	IRAK 1		simvastatin				0.00	0.00	0.00	1.00	0.98	1.06	3
8	24	SIH	tempol						147.76	105.76	116.01	1.19	1.03	0.97	2
8	25	IKK inhibitor							52.38	45.77	41.94	1.10	1.03	1.02	1
8	26	SB203580	genistein						23.34	21.21	21.23	1.05	1.02	1.00	2
8	27	VK-28	SIH	TPEN	SB203580	SP600125	SKI-II	mevastatin	16.25	17.62	14.47	1.01	0.95	0.98	7
8	28	apocynin	LY294002 hy	SB203580					28.11	24.81	23.66	1.07	1.00	0.99	3
8	29	VK-28	SB203580	SP600125	SR 11302	SKI-II			14.48	12.03	14.47	0.96	0.98	0.98	5
8	30	SB203580	di-methyl spl						24.53	14.57	15.43	0.99	0.95	0.92	2
8	31	SIH	SB203580	SKI-II					0.00	0.00	10.15	0.98	0.99	0.98	3
8	32	apocynin	SB203580						32.30	25.33	25.13	1.13	1.03	0.98	2
8	33	SIH	apocynin	SB203580					20.97	25.33	19.77	0.98	1.05	0.99	3
8	34	SB203580							23.34	21.72	23.66	1.07	1.02	1.02	1
8	35	tempol	SB203580	IRAK 1					13.90	11.02	15.91	1.05	1.00	1.02	3
8	36	alpha-tocoph	SB203580	simvastatin					20.38	17.11	14.95	1.11	1.04	0.99	3
8	37	SIH	SB203580	IRAK 1		SKI-II			12.14	9.00	11.59	1.28	1.06	1.03	4
8	38	SB203580	SKI-II						13.31	11.02	11.59	0.96	1.01	0.98	2
8	39	genistein	simvastatin						89.60	55.42	57.15	1.01	0.93	0.91	2
8	40	VK-28	N-acetyl cys	rotenone	SKI-II	Y27632 dihy	EGCG		16.25	15.59	21.23	0.98	1.02	0.99	6
8	41	SB203580	SKI-II						14.48	11.53	11.11	1.11	1.04	1.02	2
8	42	rotenone	apocynin	SB203580	Y27632 dihy				13.31	15.08	14.47	1.12	1.14	1.06	4
8	43	SB203580	SKI-II						13.31	14.06	13.03	1.16	1.05	1.05	2
8	44	TPEN	genistein						103.27	109.83	135.82	1.12	1.03	1.04	2
8	45	apocynin	SB203580	PD98059					21.56	18.14	16.39	1.13	1.10	0.99	3
8	46	N-acetyl cyst	SB203580	NSC23766 (					26.32	18.14	20.26	1.14	0.98	0.99	3
8	47	SB203580	SKI-II	simvastatin					12.14	10.52	11.11	1.09	1.06	1.00	3
8	48	SIH	desferrioxam	tempol	SB203580	SKI-II			12.72	9.51	11.11	1.14	0.98	0.96	5
8	49	SIH	SB203580	PDTC	IKK inhibitor				11.55	9.51	0.00	1.14	1.06	1.04	4
8	50	tempol	SKI-II						31.10	28.95	30.03	1.12	1.02	1.04	2
9	1	diphenylene i	SB203580	IKK inhibitor	SKI-II	simvastatin			0.00	0.00	0.00	1.24	1.25	1.24	5
9	2	VK-28	alpha-tocopl	tempol	PD98059	IKK inhibitor	mevastatin	simvastatin	19.22	19.77	8.23	1.02	1.13	1.06	7
9	3	IKK inhibitor	NSC23766 (						27.95	16.48	16.85	0.98	0.96	0.95	2
9	4	tempol							141.29	179.90	94.85	1.03	1.03	0.98	1
9	5	SB203580	PDTC	di-methyl spl					0.00	0.00	0.00	1.03	0.99	1.05	3
9	6	SB203580	SP600125	di-methyl spl	Y27632 dihy				0.00	0.00	0.00	1.01	1.02	0.97	4
9	7	alpha-tocoph	tempol	SB203580	SP600125	NSC23766 (	simvastatin		0.00	0.00	0.00	0.98	0.98	1.02	6
9	8	PDTC							78.24	87.64	88.52	0.94	0.95	0.99	1
9	9	VK-28	SIH	tempol					97.08	141.14	115.94	1.11	1.10	1.13	3
9	10	DMSO							97.08	138.29	73.50	0.98	1.05	0.98	0
9	11	SKI-II	NSC23766 (						6.33	11.45	14.36	1.01	1.08	1.07	2
9	12	SIH	desferrioxam	SB203580					0.00	0.00	0.00	1.03	1.03	1.06	3
9	13	IRAK 1		Y27632 dihy					14.20	15.58	8.47	0.98	0.96	0.92	2
9	14	SKI-II	NSC23766 (						10.13	0.00	11.40	1.02	1.00	1.04	2
9	15	IKK inhibitor	SKI-II	NSC23766 (					0.00	0.00	0.00	1.10	1.05	0.98	3
9	16	DMSO							108.61	121.23	112.90	0.97	0.96	1.00	0
9	17	SB203580	genistein						0.00	0.00	0.00	0.97	0.94	0.95	2
9	18	SIH	tempol	SB203580	IKK inhibitor				0.00	0.00	0.00	1.04	1.11	0.94	4

9	19	tempol	SB203580	PD98059	SR 11302						0.00	0.00	0.00	1.09	1.07	1.10	4
9	20	SB203580									0.00	0.00	0.00	1.00	0.99	0.95	1
9	21	SB203580	Y27632 dihy								0.00	0.00	0.00	1.01	1.04	1.00	2
9	22	apocynin	SB203580	SP600125	SKI-II	mevastatin					0.00	0.00	0.00	1.00	1.05	1.01	5
9	23	SB203580									0.00	0.00	0.00	0.98	1.08	0.99	1
9	24	TPEN	tempol	U0126							34.40	40.12	27.02	0.98	0.98	0.91	3
9	25	SP600125	SKI-II	genistein							57.99	59.30	35.40	1.03	1.02	0.88	3
9	26	SIH	alpha-tocopl	SB203580	EGCG						0.00	0.00	0.00	1.02	0.99	1.00	4
9	27	genistein									85.13	88.71	62.02	1.00	0.97	0.90	1
9	28	U0126	genistein								44.85	34.15	33.02	0.99	0.94	0.94	2
9	29	N-acetyl cyst	wortmannin	SB203580	SKI-II	di-methyl spl					0.00	0.00	0.00	1.43	1.42	1.23	5
9	30	SIH	LY294002 hy	PD98059	IRAK 1		simvastatin				17.46	36.02	34.08	1.12	1.10	1.14	5
9	31	SB203580									0.00	0.00	0.00	0.94	1.00	0.92	1
9	32	desferrioxam	alpha-tocopl	wortmannin	SKI-II	Y27632 dihy					0.00	0.00	0.00	1.31	1.30	1.37	5
9	33	SB203580	di-methyl spl	Y27632 dihy							0.00	0.00	0.00	0.99	1.02	0.98	3
9	34	apocynin									86.53	123.56	131.95	0.99	1.08	0.97	1
9	35	SB203580	SR 11302								0.00	0.00	0.00	0.98	0.98	0.90	2
9	36	tempol	SB203580	SKI-II	simvastatin						0.00	0.00	0.00	1.05	1.05	0.90	4
9	37	wortmannin	SKI-II	Y27632 dihy							0.00	0.00	0.00	1.41	1.18	1.38	3
9	38	SIH	SB203580								0.00	0.00	0.00	1.00	1.00	0.92	2
9	39	TPEN	alpha-tocopl	SB203580	Y27632 dihy						0.00	0.00	0.00	0.96	0.94	0.95	4
9	40	SKI-II	NSC23766 (	EGCG							16.37	21.27	9.93	0.98	1.05	0.94	3
9	41	IKK inhibitor	EGCG								39.81	70.12	70.81	1.12	1.13	1.12	2
9	42	diphenylene i	SB203580	IKK inhibitor	SKI-II	simvastatin					0.00	0.00	0.00	1.11	1.19	0.95	5
9	43	SIH	SB203580	U0126	SP600125						0.00	0.00	0.00	1.06	1.06	1.05	4
9	44	apocynin	SB203580	IRAK 1							0.00	0.00	0.00	1.12	1.12	1.05	3
9	45	SB203580	di-methyl spl								0.00	0.00	0.00	1.02	0.99	0.92	2
9	46	genistein	Y27632 dihy								87.65	118.90	76.51	1.00	1.03	0.92	2
9	47	SIH	SB203580	IRAK 1		di-methyl spl	genistein				0.00	0.00	0.00	1.08	1.05	0.92	5
9	48	alpha-tocoph	rotenone	SB203580	IRAK 1		SKI-II	di-methyl spl	simvastatin		0.00	0.00	0.00	1.13	1.11	1.12	7
9	49	N-acetyl cyst	tempol	PDTC	genistein	simvastatin					72.05	67.39	59.14	0.98	0.97	0.98	5
9	50	rotenone	NSC23766 (								25.46	46.84	19.11	1.03	1.09	1.02	2
10	1	SIH	SB203580								9.23	11.38	11.83	0.98	1.02	1.03	2
10	2	SB203580	SKI-II	di-methyl spl							2.97	5.47	4.32	1.00	1.06	1.02	3
10	3	SB203580	U0126								3.42	4.47	5.01	1.00	0.99	1.04	2
10	4	SB203580	PDTC	di-methyl spl	NSC23766 (						7.84	8.99	7.82	1.03	1.06	1.00	4
10	5	SB203580									11.57	12.58	9.67	1.01	1.05	1.01	1
10	6	TPEN	diphenylene	Y27632 dihy							23.43	39.88	35.80	1.04	1.09	1.06	3
10	7	SIH	SB203580	Y27632 dihy							9.69	10.86	9.67	1.00	1.05	0.99	3
10	8	SIH	SB203580	NSC23766 (							9.00	8.49	6.98	0.99	1.03	0.96	3
10	9	SB203580	SR 11302								9.92	10.18	7.26	0.95	0.99	0.93	2
10	10	TPEN	alpha-tocopl	SB203580	PD98059	Y27632 dihy					6.01	6.97	7.97	0.96	0.98	1.02	5
10	11	SB203580									9.23	14.48	9.67	0.99	1.03	0.96	1
10	12	SB203580	U0126	IRAK 1		genistein	Y27632 dihy	EGCG	Melatonin		0.00	0.00	3.34	1.02	1.02	1.05	7
10	13	PD98059	Y27632 dihy	EGCG	Melatonin						109.94	142.32	135.89	0.98	1.07	1.02	4
10	14	TPEN	alpha-tocopl	SB203580	SP600125	Y27632 dihy					10.98	8.15	8.96	0.95	1.00	0.99	5
10	15	alpha-tocoph	apocynin	IKK inhibitor							22.79	41.04	37.41	0.97	1.06	1.01	3
10	16	rotenone	SB203580	di-methyl spl							3.76	5.80	3.90	1.07	1.11	1.04	3
10	17	genistein									82.38	143.45	124.22	0.96	1.04	1.01	1
10	18	SR 11302	Melatonin								88.68	70.37	55.36	1.00	1.00	0.93	2
10	19	alpha-tocoph	SB203580								8.65	12.58	9.25	0.98	1.03	0.97	2
10	20	SB203580	di-methyl spl	Y27632 dihy							8.65	8.32	7.26	0.97	0.97	0.93	3
10	21	LY294002 hy	SB203580	di-methyl spl							12.87	15.52	10.82	0.96	0.98	0.93	3
10	22	TPEN	alpha-tocopl	Y27632 dihy							55.66	53.75	74.05	0.97	0.99	1.00	3
10	23	TPEN	SR 11302	genistein							125.38	169.99	75.97	0.99	1.06	0.95	3
10	24	TPEN	diphenylene	SB203580	genistein						3.98	5.63	6.70	1.03	1.10	1.05	4
10	25	SIH	TPEN	SB203580							10.39	12.92	12.41	0.99	1.05	1.02	3
10	26	TPEN	alpha-tocopl	SB203580	Y27632 dihy						7.50	10.86	7.12	1.01	1.03	0.98	4
10	27	alpha-tocoph	U0126								47.71	86.05	76.93	0.97	1.02	1.01	2
10	28	SB203580									8.88	8.83	6.70	1.02	1.01	0.95	1

10	29	SB203580	genistein				10.16	15.69	11.68	1.02	1.03	1.01	2
10	30	SIH	SB203580	IKK inhibitor			3.20	4.30	3.21	0.96	0.99	0.92	3
10	31	TPEN	SB203580	di-methyl spl	Y27632 dihy	EGCG	7.27	8.83	6.41	0.96	0.96	0.94	5
10	32	di-methyl sph					136.40	170.29	86.99	1.01	1.04	0.97	1
10	33	EGCG					105.90	105.38	123.99	1.00	1.02	1.01	1
10	34	TPEN	SB203580	SR 11302	PDTC	Y27632 dihy	8.65	13.44	11.83	0.97	1.07	1.01	5
10	35	SB203580	di-methyl spl	genistein			10.51	13.96	9.67	1.00	1.04	0.98	3
10	36	SB203580	SP600125	genistein	Y27632 dihy	simvastatin	8.65	13.44	12.55	0.95	1.06	1.00	5
10	37	genistein					118.25	156.73	81.61	1.01	1.07	0.97	1
10	38	SB203580	U0126	di-methyl spl	simvastatin		2.53	4.14	3.76	1.00	1.04	1.03	4
10	39	VK-28	SB203580	Melatonin			8.30	11.03	6.27	0.98	1.02	0.93	3
10	40	SIH	apocynin	wortmannin	SKI-II		4.43	7.31	5.01	1.09	1.14	1.08	4
10	41	SIH	SB203580				7.15	10.69	6.70	0.95	0.96	0.92	2
10	42	SIH	SB203580				9.23	8.83	10.53	1.02	1.02	1.07	2
10	43	SB203580	SR 11302				10.27	15.00	8.39	0.98	1.06	0.97	2
10	44	DMSO					109.94	154.67	117.11	1.00	1.07	1.03	0
10	45	alpha-tocoph	wortmannin	SB203580	Y27632 dihy		4.77	5.13	3.48	1.01	1.06	1.00	4
10	46	wortmannin	SB203580	di-methyl spl	NSC23766 (		4.09	5.63	3.48	1.09	1.14	1.07	4
10	47	desferrioxam	alpha-tocopl	SB203580	Y27632 dihy		7.96	9.16	6.84	0.98	1.03	0.95	4
10	48	SIH	genistein				73.88	131.82	105.36	1.03	1.07	1.04	2
10	49	SB203580	PDTC	di-methyl spl			7.61	12.41	6.55	1.01	1.03	0.98	3
10	50	TPEN	alpha-tocopl	SB203580	Y27632 dihy		5.67	9.16	5.29	1.08	1.03	0.95	4
11	1	rotenone					10.89	36.18	36.16	0.94	0.99	1.04	1
11	2	genistein					116.62	123.79	81.31	0.99	0.99	1.00	1
11	3	VK-28					92.99	58.81	88.46	1.02	0.94	0.99	1
11	4	SB203580	di-methyl spl	Y27632 dihy			0.00	8.69	7.01	0.90	1.05	0.99	3
11	5	N-acetyl cyst					37.72	68.07	103.46	0.92	0.96	1.03	1
11	6	VK-28	SIH	SB203580	IKK inhibitor		0.00	0.00	4.43	0.94	0.95	1.01	4
11	7	IKK inhibitor					33.87	17.73	19.32	1.00	0.97	0.94	1
11	8	IRAK 1		SKI-II			0.00	0.00	5.90	0.93	0.95	1.00	2
11	9	SKI-II					10.89	21.21	11.08	0.99	1.01	0.93	1
11	10	LY294002 hy	SB203580				5.98	10.40	7.75	0.93	1.02	0.95	2
11	11	SIH	TPEN	SB203580	SP600125	IKK inhibitor	0.00	5.28	4.43	1.01	1.02	0.96	5
11	12	VK-28	TPEN	SB203580	SR 11302	genistein	5.98	14.70	14.81	0.89	0.93	1.04	5
11	13	SP600125					95.80	113.29	87.62	0.98	0.98	0.99	1
11	14	alpha-tocoph	PDTC	mevastatin			64.34	50.58	59.51	1.00	0.94	0.99	3
11	15	SB203580					5.98	10.40	10.71	0.91	0.95	1.01	1
11	16	N-acetyl cyst	SB203580	U0126			0.00	0.00	0.00	0.95	1.00	1.02	3
11	17	SIH	SB203580	IKK inhibitor	NSC23766 (		0.00	0.00	0.00	0.97	0.95	0.97	4
11	18	SIH	SB203580	genistein			0.00	6.13	9.97	0.93	0.94	0.98	3
11	19	Y27632 dihyd					57.25	68.07	78.80	0.95	0.99	0.95	1
11	20	N-acetyl cyst	SB203580				5.98	13.41	13.32	0.90	0.93	1.02	2
11	21	VK-28	IRAK 1		Y27632 dihy		33.45	35.73	22.35	0.96	0.99	0.99	3
11	22	SB203580	PDTC	mevastatin			7.61	8.26	8.49	1.00	0.93	0.99	3
11	23	diphenylene i					12.53	43.79	29.98	0.96	1.05	0.97	1
11	24	TPEN					61.23	86.48	117.07	0.95	0.97	1.00	1
11	25	EGCG					32.17	59.26	98.71	0.89	0.94	1.01	1
11	26	alpha-tocoph					94.86	58.81	77.55	0.96	0.94	0.92	1
11	27	SIH					33.02	50.58	77.13	0.92	0.95	0.99	1
11	28	alpha-tocoph					60.79	76.04	64.38	0.96	0.99	1.01	1
11	29	SB203580	PD98059	SR 11302	di-methyl spl		0.00	0.00	0.00	0.92	0.99	0.95	4
11	30	wortmannin					20.82	27.77	21.59	1.06	1.05	1.08	1
11	31	tempol					57.69	95.15	112.65	0.91	0.94	1.06	1
11	32	IKK inhibitor					48.49	50.58	37.32	0.96	0.98	0.99	1
11	33	desferrioxam					111.36	77.92	98.71	1.03	0.96	0.98	1
11	34	wortmannin	SB203580	di-methyl spl			0.00	4.85	5.90	0.95	1.07	1.01	3
11	35	di-methyl sph					35.15	42.44	77.13	0.91	0.97	1.04	1
11	36	SB203580					8.43	9.97	11.45	0.99	1.00	0.96	1
11	37	PDTC					30.48	62.03	99.57	0.89	0.96	0.98	1
11	38	genistein					92.99	61.57	103.03	0.96	0.97	0.95	1

11	39	tempol	25.00	56.97	103.90	0.89	0.96	0.96		1
11	40	SR 11302	67.46	71.80	76.71	0.98	0.99	0.95		1
11	41	apocynin	35.57	81.72	70.93	0.91	1.02	0.95		1
11	42	mevastatin	28.36	74.15	76.30	0.92	0.99	1.05		1
11	43	U0126	15.83	45.14	41.22	0.93	1.03	0.99		1
11	44	LY294002 hy	34.30	100.98	129.60	0.94	1.01	1.07		1
11	45	simvastatin	85.61	92.72	69.70	1.03	1.06	0.99		1
11	46	NSC23766 (h	29.63	61.57	70.52	0.96	0.99	0.98		1
11	47	PD98059	66.57	51.94	51.90	1.03	0.96	0.97		1
11	48	SB203580	0.00	9.12	14.07	0.93	0.98	1.00		1
11	49	IRAK 1	18.74	28.21	28.83	1.02	1.02	1.03		1
11	50	Melatonin	33.87	101.47	82.98	0.95	1.02	0.96		1
1		DMSO	116.89	101.55	115.70	0.97	0.97	0.98		
1		DMSO	126.20	134.75	127.23	1.03	1.05	1.02		
1		DMSO	109.11	116.40	138.89	1.00	0.98	1.00		
1		IKK inhibitor (BMS-345541)	0.00	21.00	17.00	1.08	1.06	1.11		
2		DMSO	64.27	81.86	53.41	0.94	1.01	1.02		
2		DMSO	129.97	86.61	92.67	1.02	1.00	1.01		
2		DMSO	116.57	131.21	125.52	0.98	1.06	0.96		
2		DMSO	87.99	106.75	112.29	1.01	0.96	0.97		
2		DMSO	105.51	97.12	119.73	1.05	0.96	1.03		
2		IKK inhibitor (BMS-345541)	57.97	28.74	23.74	1.19	NA	NA		
3		DMSO	128.48	150.32	162.43	1.06	1.05	1.07		
3		DMSO	120.13	97.90	122.99	1.03	0.97	1.04		
3		DMSO	78.63	84.12	56.83	1.00	1.01	0.95		
3		DMSO	80.44	71.25	96.87	0.96	0.96	0.97		
3		DMSO	93.21	97.90	68.11	0.96	1.01	0.96		
3		IKK inhibitor (BMS-345541)	0.00	0.00	0.00	1.65	1.45	1.91		
4		DMSO	137.18	130.31	113.27	1.06	1.03	1.00		
4		DMSO	132.36	118.28	115.09	1.05	0.99	0.98		
4		DMSO	104.05	94.44	81.93	0.97	1.00	1.03		
4		DMSO	71.27	89.11	82.83	0.98	0.98	0.98		
4		DMSO	72.11	74.91	111.63	0.94	1.01	1.01		
4		IKK inhibitor (BMS-345541)	1.00	2.00	2.00	1.24	1.33	1.22		
5		DMSO	159.47	121.78	92.20	1.02	1.01	1.02		
5		DMSO	124.41	171.31	95.81	0.99	1.03	0.93		
5		DMSO	117.22	74.10	121.97	1.01	0.97	1.03		
5		DMSO	64.48	75.17	84.32	0.97	1.06	0.97		
5		DMSO	53.20	73.20	108.73	1.01	0.93	1.05		
5		IKK inhibitor (BMS-345541)	5.00	5.00	4.00	1.45	1.32	1.29		
6		DMSO	92.68	111.47	107.84	0.99	1.01	1.01		
6		DMSO	105.51	110.53	107.64	1.00	0.99	1.01		
6		DMSO	104.62	99.55	75.87	1.02	1.01	0.98		
6		DMSO	120.88	111.94	106.38	1.01	0.99	0.99		
6		DMSO	77.62	68.88	103.69	0.98	1.00	1.01		
6		IKK inhibitor (BMS-345541)	0.00	0.00	0.00	1.51	1.55	1.56		
7		DMSO	86.08	84.73	88.22	1.01	0.99	1.07		
7		DMSO	107.95	121.02	92.97	1.03	1.04	0.92		
7		DMSO	89.45	106.42	55.54	1.00	1.00	0.92		
7		DMSO	92.15	89.91	116.97	0.97	1.00	1.03		
7		DMSO	124.53	98.87	150.71	0.98	0.96	1.07		
7		IKK inhibitor (BMS-345541)	0.00	0.00	0.00	1.43	1.07	1.09		
8		DMSO	111.18	145.72	109.88	0.94	1.03	1.00		
8		DMSO	99.34	91.98	106.02	1.02	0.98	1.02		
8		DMSO	79.33	86.33	94.01	0.99	0.99	1.00		
8		DMSO	89.60	93.13	94.55	1.01	0.99	0.97		
8		DMSO	122.52	84.62	95.09	1.05	1.01	1.01		
8		IKK inhibitor (BMS-345541)	12.00	11.00	11.00	1.12	1.05	1.00		
9		DMSO	107.41	102.60	94.22	1.00	1.06	1.03		
9		DMSO	80.70	87.28	90.09	0.99	1.07	1.01		

9	DMSO	102.34	107.45	118.34	0.97	0.95	0.97
9	DMSO	100.29	86.92	83.23	0.98	0.96	1.00
9	DMSO	110.42	116.20	115.94	1.06	0.96	0.99
9	IKK inhibitor (BMS-345541)	0.00	0.00	0.00	1.20	1.19	1.38
10	DMSO	145.60	98.06	87.99	1.00	1.00	0.98
10	DMSO	102.73	92.35	132.02	1.09	0.97	1.00
10	DMSO	87.05	101.94	60.34	0.96	0.97	0.92
10	DMSO	91.45	89.76	120.52	0.97	1.05	1.07
10	DMSO	79.04	119.60	105.57	0.99	1.02	1.03
10	IKK inhibitor (BMS-345541)	0.00	0.00	0.00	1.48	1.33	1.21
11	DMSO	86.06	82.66	104.33	1.02	0.99	0.99
11	DMSO	114.22	137.53	121.07	1.02	1.01	0.99
11	DMSO	76.48	82.19	87.20	0.96	0.98	0.97
11	DMSO	106.61	127.34	94.85	1.00	1.04	1.03
11	DMSO	119.04	74.62	91.86	1.00	0.99	1.02
11	IKK inhibitor (BMS-345541)	18.74	75.09	54.69	0.94	1.06	0.96