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Supplementary Materials for

From hit to vial: Precision discovery and development of an imidazopyrimidine TLR7/8 agonist adjuvant formulation

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Materials

HTS Hit calling method.

All Data was log10-transformed (log10). CrossTalk Corrected luminescence data from columns E, and F was log-transformed (log10) in columns K, and L. Only experimental wells are evaluated, referencing column C with an "if" logic statement. An example of the calculation in column Q, log-transforming data from column E, is shown below:

=IF(C2 = "X",LOG10(E2), "")

A robust Z score is calculated for each experimental well with adjusted median absolute deviation (MAD) values. First, the plate median and MAD values are generated from the log-transformed data. The absolute deviation for each well value is calculated in columns M and N, an example of which is shown below:

=IF(C2 = "X", ABS(K2-I3),"") wherein K2 is a log transformed data point, and I3 is the median value for that plate for that readout.

The MAD is then calculated as the median of each of columns U-X multiplied by 1.4286, an example of which is shown below:

=MEDIAN(M2:M385)*1.4286

The robust Z score is then calculated as (well_value – median_plate) / (MAD_plate * 1.4286), an example calculation is shown below:

=IF($C2="X", ((K2-$I$3)/$I$5), "") wherein K2 is a log-transformed well value, I3 is the plate experimental median for that readout, and I5 is the plate experimental MAD for that readout.

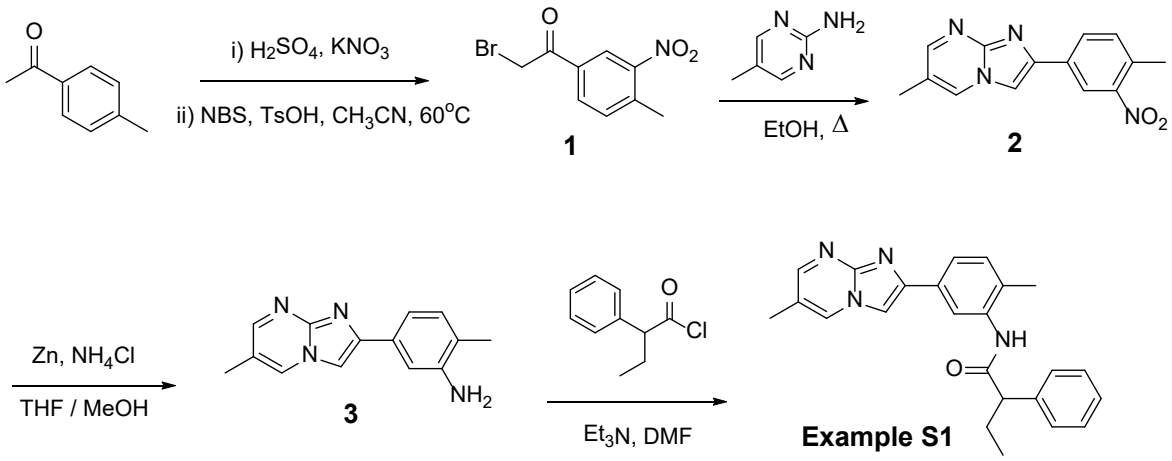
Any wells with robust Z score values of 2 or greater in both replicates are considered a hit. This is evaluated in column Q as shown in the example below:

=IF(AND((O2>I7), (P2>J7), ($C2="X")),TRUE,FALSE) wherein O2 and P2 are robust Z scores for replicates of the luminescence readout, while I7 and J7 represent the luminescence robust Z score threshold (2). “TRUE” will be returned in column Q if the compound meets these hit criteria.

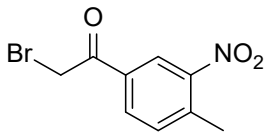
For any well that is determined to be a hit, the Plate:Well compound ID will be displayed in column R as shown in the example below:

=IF(Q2,CONCATENATE(A2,":",B2),"") in which Q2 is the TRUE/FALSE value determining the hit status of the compound while A2 is the plate ID and B2 is the well ID.

Preparation of example S1

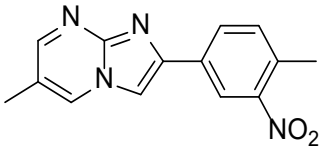


2-Bromo-1-(4-methyl-3-nitrophenyl)ethanone (1)



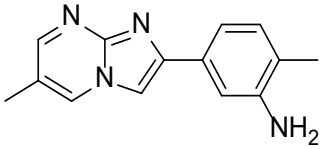
4'-Methylacetophenone (1 g, 7.5 mmol) was dissolved in conc. H_2SO_4 (10 mL) and the solution was cooled to 0 °C. KNO_3 (1 g, 9.9 mmol) was added in portions, and the mixture was stirred at 0 ºC for 2 hours, then poured onto crushed ice (100 g), and extracted with EtOAc (2 x 50 mL). The organic phase was washed with brine and concentrated to give 1.3 g of a yellow solid. The nitro intermediate (200 mg, 1.1 mmol) was dissolved in acetonitrile (2 mL). NBS (237 mg, 1.3 mmol) and p-TsOH (200 mg, 1.1) were added. The reaction mixture was heated at 60 ºC for 4 hours, then diluted with EtOAc (20 mL) and washed with sat. $Na_2S_2O_3$ solution and brine. The organic layer was concentrated to give the title compound (260 mg, yield 91%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.12 (d, 1H), 7.52 (d, 1H), 4.46 (s, 2H), 2.70 (s, 3H).

6-Methyl-2-(4-methyl-3-nitrophenyl)imidazo[1,2-a]pyrimidine (2)



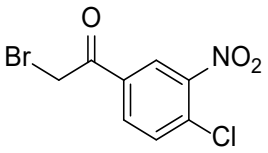
2-Bromo-1-(4-methyl-3-nitrophenyl)ethanone (300 mg, 1.16) and 2-amino-5-methylpyrimidine (200 mg, 1.83 mmol) were dissolved in EtOH (10 mL) and the solution refluxed overnight. The solution was diluted with DCM and washed with $NaHCO_3$ solution. The organic layer was concentrated and the residue purified by column chromatography to give the title compound (102 mg, yield 30%) as a yellow solid. MS m/z 269.9 $[M+H]^+$.

2-Methyl-5-(6-methylimidazo[1,2-a]pyrimidin-2-yl)aniline (3)



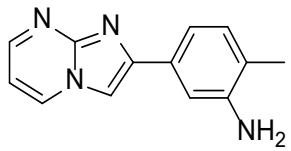
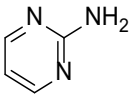
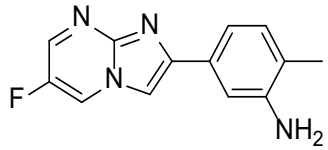
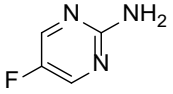
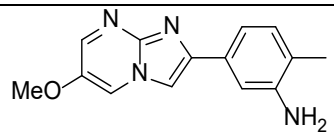
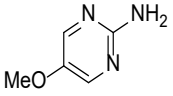
6-Methyl-2-(4-methyl-3-nitrophenyl)imidazo[1,2-a]pyrimidine (600 mg, 2.24 mmol) was dissolved in THF and methanol (3:1, 4 mL). Zinc powder (2.3 g, 35.4 mmol) and NH_4Cl (2 g, 37.0 mmol) were added to the solution and the mixture was stirred for 1 hour. The solid was filtered, and the filtrate was concentrated, dissolved in EtOAc, and then washed with water. The organic phase was concentrated to give compound the title compound (400 mg, 75% yield) as a yellow solid. MS m/z 238.7 $[M+H]^+$.

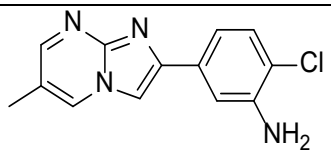
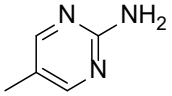
2-Bromo-1-(4-chloro-3-nitrophenyl)ethan-1-one (4)



2-Bromo-1-(4-chloro-3-nitrophenyl)ethan-1-one was prepared using a similar procedure to that described for **1**, from 4'-chloroacetophenone. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.62 (s, 1H), 8.26 (d, 1H), 8.00 (d, 1H), 5.02 (s, 2H).

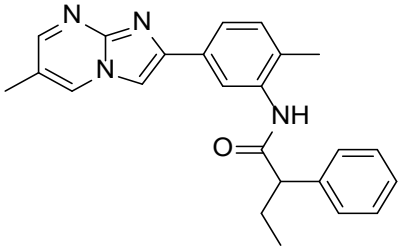
Anilines 5 - 8 were prepared by similar methods to **3**, from the corresponding aminopyrimidine and either **1** or **4**.

Aniline	Name	^{1}H NMR (d$_6$-DMSO)	*m/z* [M+1]$^+$	Starting materials
5	 5-(Imidazo[1,2-a]pyrimidin-2-yl)-2-methylaniline	(500MHz) 8.92 (m, 1H), 8.48 (m, 1H), 8.16 (s, 1H), 7.32 (d, 1H), 7.09 (d, 1H), 7.00 (m, 2H), 4.95 (s, 2H), 2.09 (s, 3H)	224.91	 and **1**
6	 5-(6-Fluoroimidazo[1,2-a]pyrimidin-2-yl)-2-methylaniline	(500MHz) 9.18 (m, 1H), 8.65 (d, 1H), 8.18 (s, 1H), 7.31 (d, 1H), 7.09 (m, 1H), 7.00 (d, 1H), 4.95 (s, 2H), 2.09 (s, 3H)	242.98	 and **1**
7	 5-(6-Fluoroimidazo[1,2-a]pyrimidin-2-yl)-2-methylaniline	(500MHz) 8.66 (d, 1H), 8.36 (d, 1H), 8.05 (s, 1H), 7.27 (d, 1H), 7.05 (m, 1H), 6.98 (d, 1H), 4.92 (s,	254.68	 and **1**

		2H), 3.85 (s, 3H), 2.08 (s, 3H)		
8	 2-Chloro-5-(6-methylimidazo[1,2-a]pyrimidin-2-yl)aniline		259.1	 and **4**

Example S1. Cpd-02-144-1

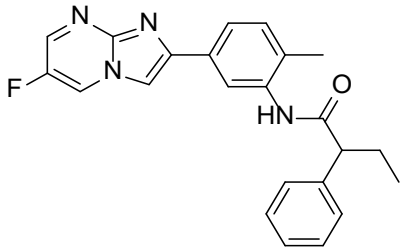
***N*-(2-Methyl-5-(6-methylimidazo[1,2-a]pyrimidin-2-yl)phenyl)-2-phenylbutanamide**



2-Methyl-5-(6-methylimidazo[1,2-a]pyrimidin-2-yl)aniline (75 mg, 0.32 mmol) was dissolved in DMF (5 mL). Triethylamine (0.09 mL, 0.64 mmol) and 2-phenylbutanoyl chloride (150 mg, 0.82 mmol) were added and the mixture was stirred for 1 hour. The reaction mixture was diluted with EtOAc (20 mL) and washed with sat. $NaHCO_3$ solution (20 mL). The organic phase was concentrated and purified by flash chromatography to give the title compound (26 mg, 21% yield). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.59 (s, 1H), 8.72 (s, 1H), 8.41 (d, 1H), 8.23 (s, 1H), 7.90 (s, 1H), 7.68 (d, 1H), 7.44 (m, 2H), 7.37 (m, 2H), 7.27 (m, 2H), 3.69 (m, 1H), 2.30 (s, 3H), 2.11 (s, 3H), 2.10 (m, 1H), 1.74 (m, 1H), 0.94 (t, 3H). MS m/z 385.3 $[M+H]^+$.

Example S2. Cpd-02-119

***N*-(5-(6-Fluoroimidazo[1,2-a]pyrimidin-2-yl)-2-methylphenyl)-2-phenylbutanamide**



2-Phenylbutanoic acid (16 mg, 0.099 mmol), diisopropylethylamine (29 uL, 0.165 mmol) and HATU (38 mg, 0.0099 mmol) were dissolved in DMF (6 mL). After 20 minutes, 5-(6-fluoroimidazo[1,2-a]pyrimidin-2-yl)-2-methylaniline (20 mg, 0.082 mmol) was added. After stirring for 18 hours, further portions of 2-phenylbutanoic acid (8 mg), diisopropylethylamine (15 uL) and HATU (18.5 mg) were added, and the mixture stirred for a further 4 hours. The mixture was diluted with DCM, washed with brine, concentrated and the residue purified by column chromatography on silica gel (0 to 5% MeOH in DCM) to give the title compound (19mg, 56% yield). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 9.57 (s, 1H), 9.18 (m, 1H), 8.69 (d, 1H), 8.33 (s, 1H), 7.93 (d, 1H), 7.70 (m, 1H), 7.45 (m, 2H), 7.36 (m, 2H), 7.27 (m, 2H), 3.70 (m, 1H), 2.12 (s, 3H), 2.09 (m, 1H), 1.74 (m, 1H), 0.94 (t, 3H). MS m/z 389.16 $[M+H]^{+}$.

Supp. Fig. 1

A

Donor / Blood Draw	A1	A2	A3
1st	~250x10⁶	~180x10⁶	~200x10⁶
2nd	~200x10⁶	~220x10⁶	~200x10⁶
3rd	~280x10⁶	~250x10⁶	~250x10⁶
4th	~200x10⁶	~250x10⁶	~270x10⁶
Total PBMCs Pool	**~930x10⁶**	**~900x10⁶**	**~920x10⁶**

B

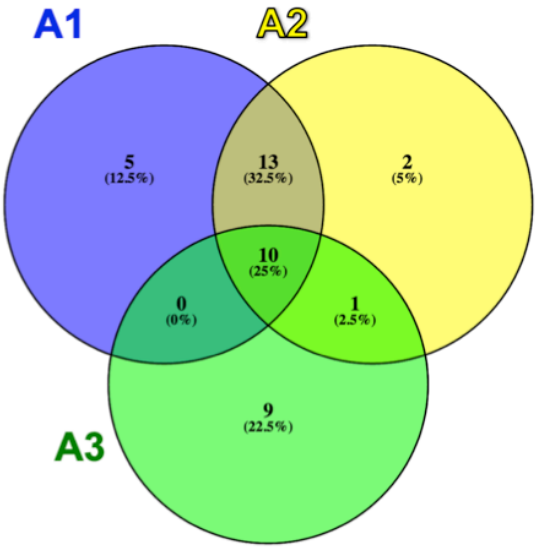
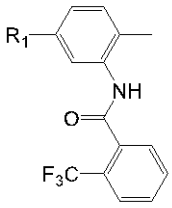


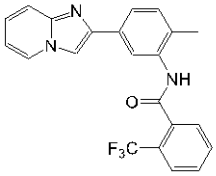
Fig. S1. Schematic representation of Human PBMCs Bio-bank created for the HTS screen and number of hits. A) A human bio-bank for PBMCs was created by drawing blood from 3 adult donors over months. A total of ~1 billion PBMCs were cryopreserved along with donor-matched platelet-poor plasma (PPP). B) Using statistical analyses ~250 potential hits were identified, among which ~25 were confirmed hits based on the results of ELISA-based titration assays. The Venn diagram represents number of compounds showing activity in each or multiple donors.

Supp. Fig. 2

A ***Core replacements***



Example: negative control



Cpd 2-139-1

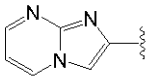
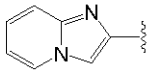
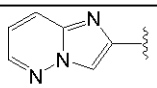
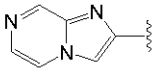
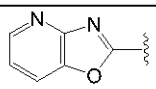
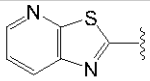
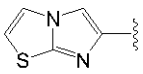
Compound ID	R	TNF Median @ 11 µM (PVP-037: 2.40 ng/ml)
Cpd 37.37		3.730
Cpd 2-139-1		0.051
Cpd 2-154-2		0.008
Cpd 2-208		0.049
Cpd 2-184-1		0.008
Cpd 3-086-1		0.040
Cpd 2-174-3		0.060

Fig. S2. Bicyclic core replacements for the imidazopyrimidine of Cpd 37.37. All series resulted in decreased activity, as demonstrated by the amount of TNF produced over 18 hrs in a human PBMCs stimulation assay (N = 5).

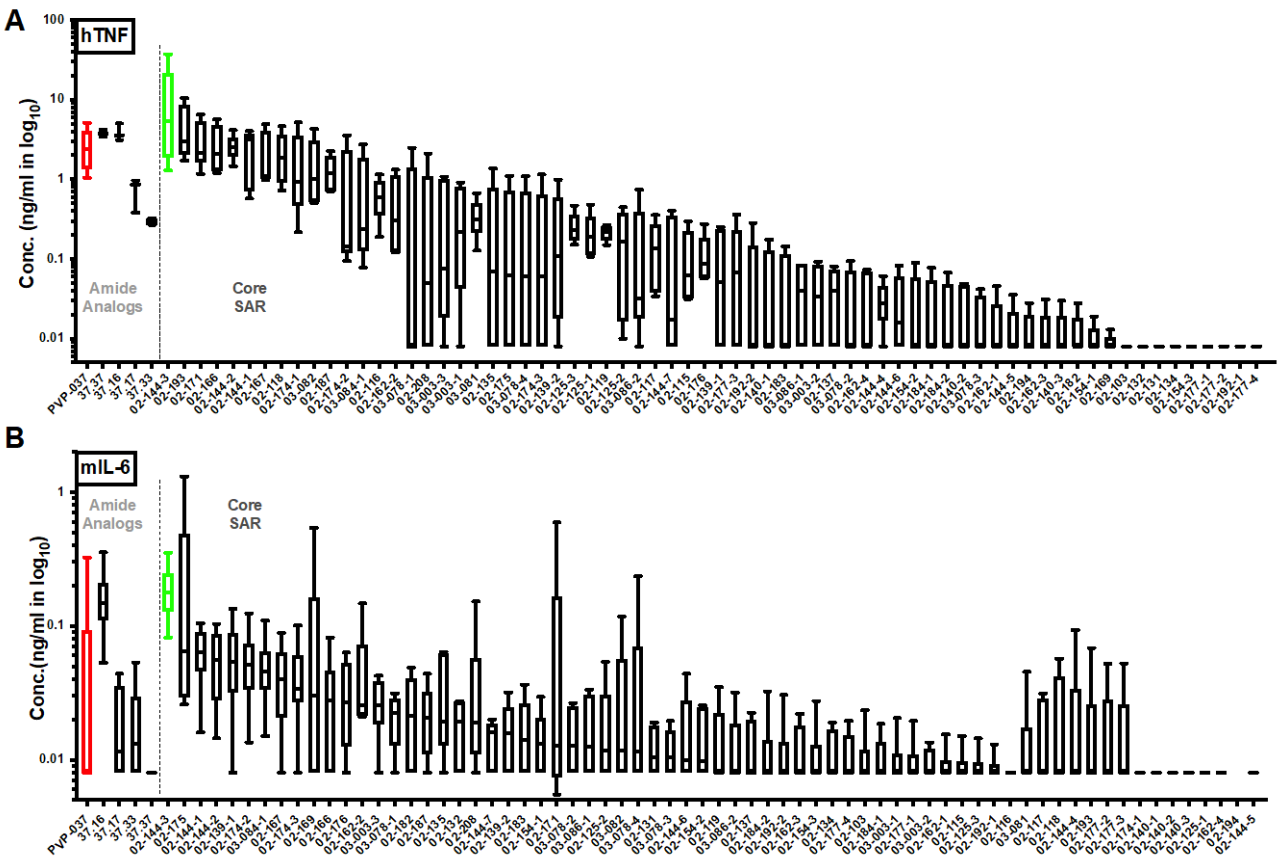


Fig. S3. PVP-037 analogs show similar rank order potency in human PBMC and murine splenocyte stimulation assays. Amide analogs of PVP-037 are shown left of dotted line in A and B. 37.37 was identified to possess enhanced activity (in human PBMCs), and further SAR was developed around core substituents and core replacements (right of dotted line in A and B). A**)** The graph depicts amount of TNF produced by representative analogs over 18 hrs in a human PBMCs stimulation assay. B**)** The graph depicts IL-6 production by analogs over 18 hrs in a murine splenocyte stimulation assay. Results are presented as box and whisker plots (N = 3 - 5). Cpd 2-144-3 referred to as PVP-037.1 was identified as the most potent molecule with an increased activity profile towards human and mouse leukocytes. Data and compound structures are included in Table S3.

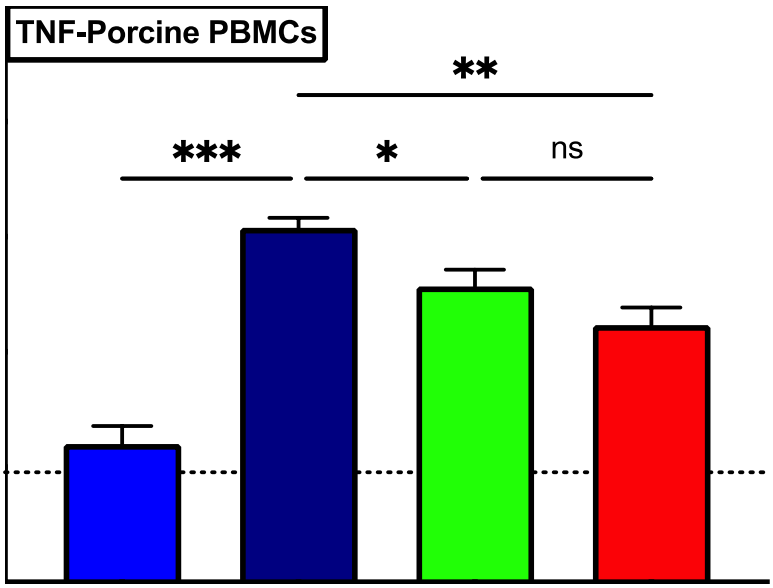


Fig. S4. Chemically optimized IMPs demonstrate enhanced activation of primary porcine PBMC *in vitro*. PBMCs were isolated from adult pigs and cryo-preserved prior to *in vitro* culture for 24 hrs with buffer control (DMSO), $PamCSK_4$ (100 µg/ml), LPS (100 ng/ml), R848 (10 µM), or analogs PVP-037 IMP family. Supernatants were collected for TNF ELISA (N = 5). Results represent mean ± SEM. For comparison at individual concentrations, repeated measures one-way ANOVA, *P < 0.05, **P < 0.01, **P<0.001. ns, not significant.

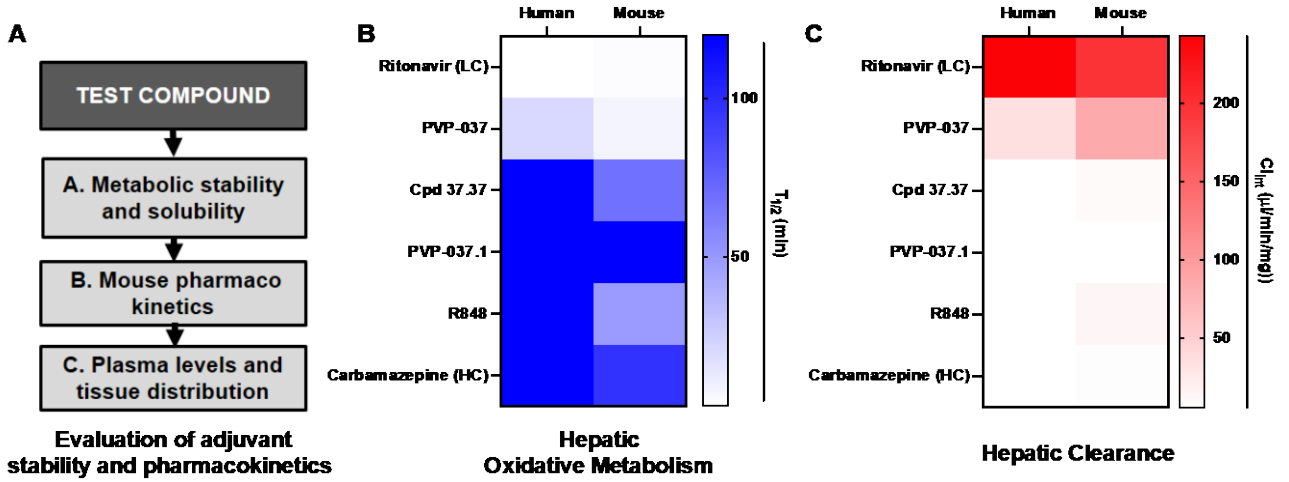


Fig. S5. PVP-037 based SAR improved molecular metabolic stability and pharmacokinetic profile. A) Steps for evaluation of adjuvant and pharmacokinectics to guide screening of adjuvant candidate with optimal PK/ADME profile. The ideal compound demonstrates enhanced resistance to oxidative hepatic metabolism as well as clearance rate to prevent systemic exposure B) Heat-map representative of half-life in human and mouse liver microsomal incubation. PVP-037.1 showed enhanced profile compared to PVP-037, demonstrating resistance to hepatic oxidative metabolism. **C)** Heat-map representative of clearance rate human and mouse liver microsomal incubation. PVP-037.1 demonstrated an enhanced clearance rate both in human and mouse microsomes, suggesting lower potential for systemic accumulation compared to PVP-037.

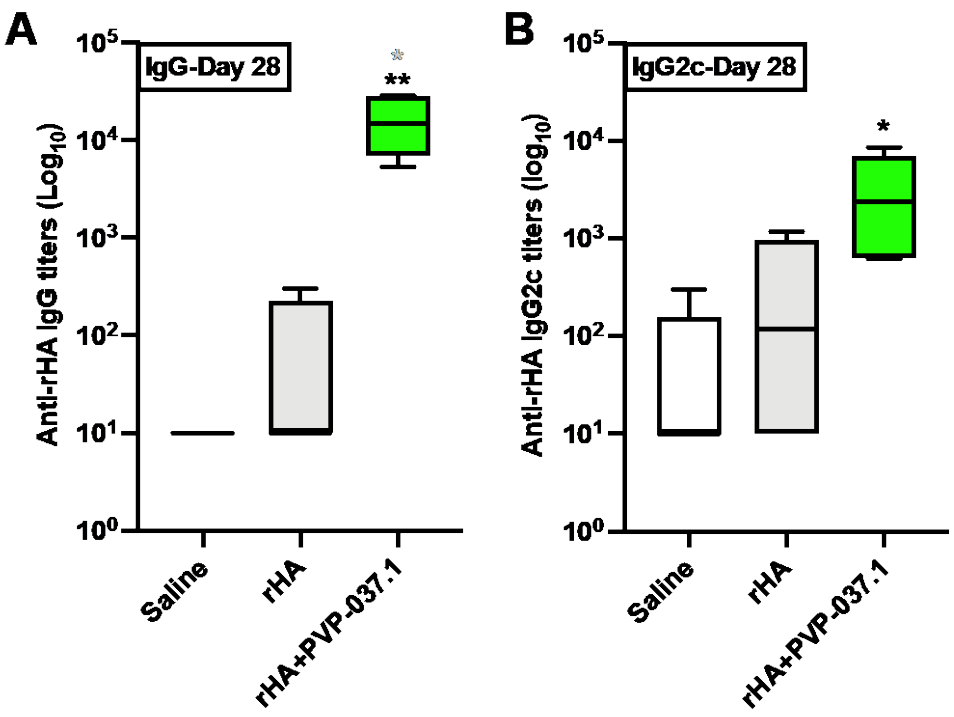


Fig. S6. PVP-037.1 demonstrated robust adjuvanticity upon subcutaneous immunization. 6-8 week old C57BL/6 adult mice were vaccinated subcutaneously (Day 0) with saline, rHA alone (1 µg antigen from Flublok 2017/18), rHA admixed with the PVP-037.1 (100 nmol). Ab titers for rHA-specific total IgG and subtypes were measured by ELISA 28 days post-immunization (N = 5 - 10). Results represent median ± SEM. For comparison at individual concentrations, repeated measures one-way ANOVA, *P < 0.05, **P < 0.01.

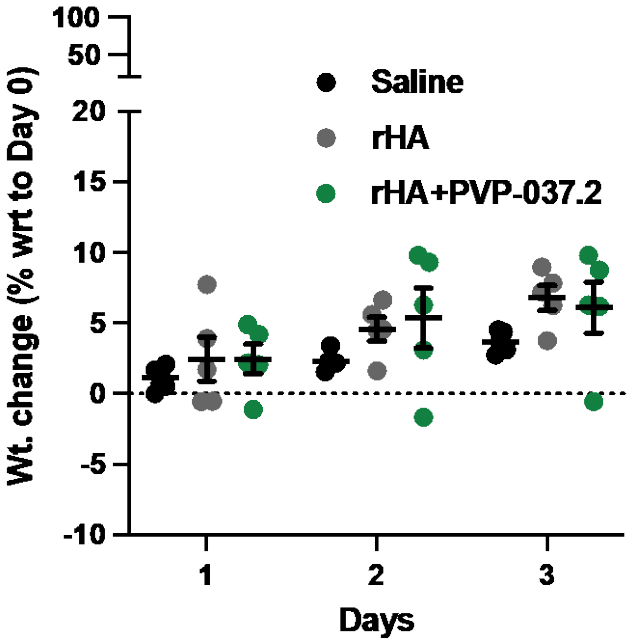


Fig. S7. Mice vaccinated with PVP-037.2 maintain normal weight gain. Young C57BL/6 adult mice (6-8 weeks old) were vaccinated IM (Day 0) with saline, rHA alone (1 µg antigen from Flublok 2017/18), rHA admixed with the PVP-037.2 (100 nmol). Body weight was measured 3 days post vaccination and presented as percent weight change as compared to day 0 (N = 5). Results represent mean ± SEM. No significant differences were observed between treatment groups.

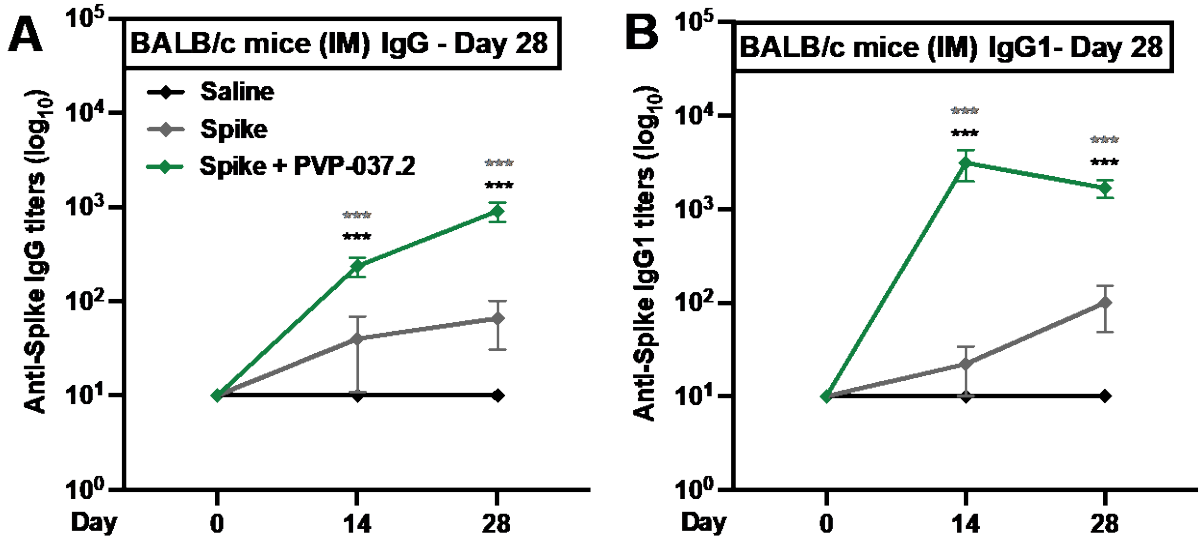


Fig. S8. PVP-037.2 demonstrates robust adjuvanticity across mouse strains. 6-8 weeks old BALB/c adult mice were vaccinated IM (Day 0) with saline, Spike alone (1 µg antigen from wild type SARS-CoV-2), Spike admixed with the PVP-037.2 (100 nmol). Ab titers for Spike-specific IgG (A) and IgG1 (B) were measured by ELISA on Days 0-, 14- and 28-days post-immunization (N = 10). Results represent mean ± SEM. For comparison at individual days, repeated measures two-way ANOVA, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

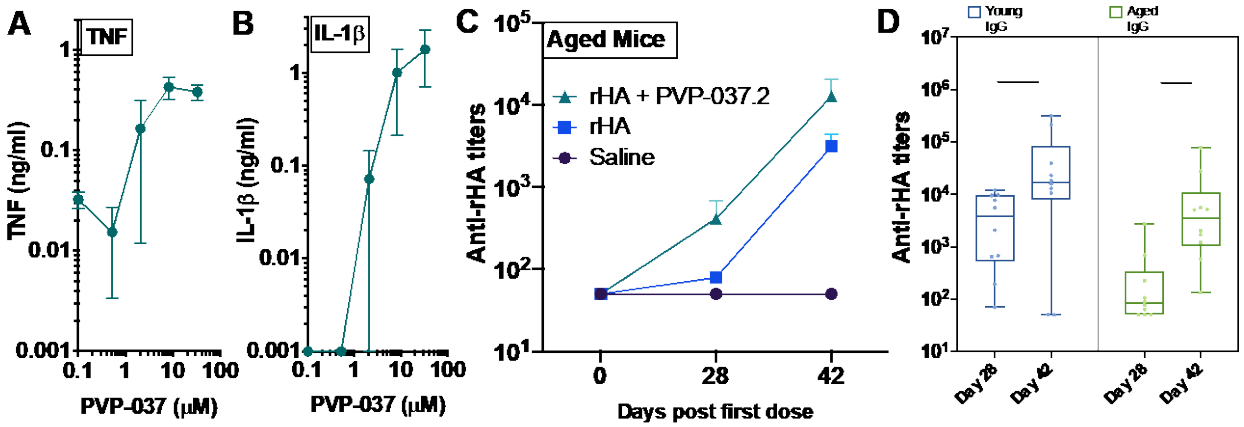


Fig. S9. PVP-037.2 demonstrates robust adjuvanticity in elder human PBMCs *in vitro* and aged mice *in vivo*. PVP-037 is active in elder human PBMCs *in vitro*, as indicated by titratable TNF (**A**) and IL-1β (**B**) responses measured by ELISA after 24hrs stimulation. Results represent mean ± SEM (N = 5). (**C** & **D**) 6-8 weeks and 56 weeks old BALB/c adult mice were vaccinated IM (Day 0) with saline, rHA alone (1 µg antigen from Flublok 2019/20) or rHA admixed with the PVP-037.2 (100 nmol). Ab titers for rHA-specific total IgG were measured by ELISA on Days 0-, 28- and 42-days post-immunization (N = 10 mice per group). Anti-rHA total IgG titers are shown as mean ± SEM (C) and median with IQR (D). Repeated measures one-way ANOVA was applied for intra-group comparison for post-first versus post-second dose (i.e., day 42 versus 28, *$p < 0.05$, ***$p < 0.001$).

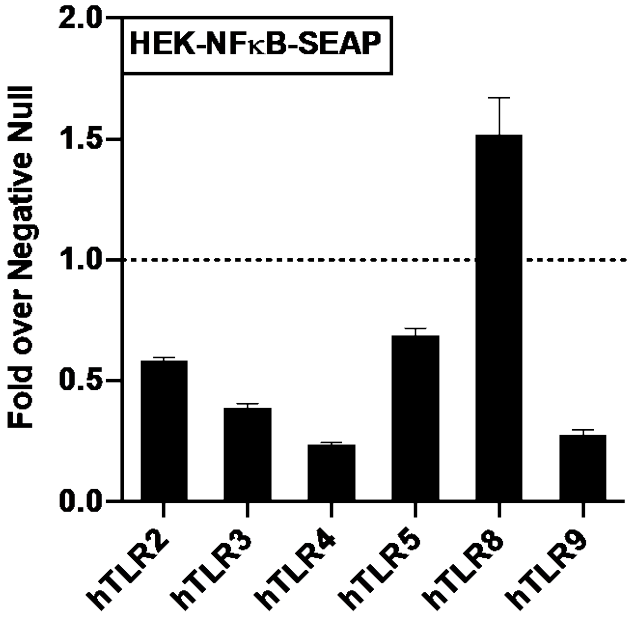


Fig. S10. PVP-037.2 demonstrated TLR8-dependent activity *in vitro*. HEK-293 cells transfected with human TLRs and an NF-kB-driven reporter SEAP gene were stimulated for 18–24 h with PVP.037.2 at 33 μM. Bar graphs represents the mean of triplicate culture wells, and is representative of two separate experiments. Both were transfected and non-transfected parental Null cell for TLR2, -3, -4, -5, or -9 HEK293 were stimulated with PVP.037.2, and graphed as fold- change of the TLR competent cell response over the negative Null cell.

Table S1.

Library	Plate	Library	Plate	Library	Plate	Library	Plate
ChemDiv1	587	ChemBridge3	1592	ChemDiv6	1846	ChemDiv7	3467
ChemDiv1	588	ChemBridge3	1593	ChemDiv6	1847	ChemDiv7	3468
ChemDiv1	589	ChemBridge3	1594	ChemDiv6	1848	ChemDiv7	3469
ChemDiv1	590	ChemBridge3	1595	ChemDiv6	1849	ChemDiv7	3470
ChemDiv1	591	ChemBridge3	1596	ChemDiv6	1850	ChemDiv7	3471
ChemDiv1	592	ChemBridge3	1597	ChemDiv6	1851	ChemDiv7	3472
ChemDiv1	593	ChemBridge3	1598	ChemDiv6	1852	ChemDiv7	3473
ChemDiv1	594	ChemBridge3	1599	ChemDiv6	1853	ChemDiv7	3474
ChemDiv1	595	ChemBridge3	1600	ChemDiv6	1854	ChemDiv7	3475
ChemDiv1	596	ChemBridge3	1601	ChemDiv6	1855	ChemDiv7	3476
ChemDiv1	597	ChemBridge3	1602	ChemDiv6	1856	ChemDiv7	3477
ChemDiv1	598	ChemBridge3	1603	ChemDiv6	1857	ChemDiv7	3478
ChemDiv1	599	ChemBridge3	1604	ChemDiv6	1858	ChemDiv7	3479
ChemDiv1	600	ChemBridge3	1605	ChemDiv6	1859	ChemDiv7	3480
ChemDiv1	601	ChemBridge3	1606	ChemDiv6	1860	ChemDiv7	3481
ChemDiv1	602	ChemDiv4	1607	ChemDiv6	1861	ChemDiv7	3482
ChemDiv1	603	ChemDiv4	1608	ChemDiv6	1862	ChemDiv7	3483
ChemDiv1	604	ChemDiv4	1609	ChemDiv6	1863	ChemDiv7	3484
ChemDiv1	605	ChemDiv4	1610	ChemDiv6	1864	ChemDiv7	3485
ChemDiv1	606	ChemDiv4	1611	ChemDiv6	1865	ChemDiv7	3486
ChemDiv1	607	ChemDiv4	1612	ChemDiv6	1866	ChemDiv7	3487
ChemDiv1	608	ChemDiv4	1613	ChemDiv6	1867	ChemDiv7	3488
ChemDiv1	609	ChemDiv4	1614	ChemDiv6	1868	ChemDiv7	3489
ChemDiv1	610	ChemDiv4	1619	ChemDiv6	1869	ChemDiv7	3490
ChemDiv1	611	ChemDiv4	1620	ChemDiv6	1870	ChemDiv7	3491
ChemDiv1	612	ChemDiv4	1621	ChemDiv6	1871	ChemDiv7	3492
ChemDiv1	613	ChemDiv4	1622	ChemDiv6	1872	ChemDiv7	3493
ChemDiv1	614	ChemDiv4	1623	ChemDiv6	1873	ChemDiv7	3494
ChemDiv1	615	ChemDiv4	1624	ChemDiv6	1874	ChemDiv7	3495
ChemDiv1	616	ChemDiv4	1625	ChemDiv6	1875	ChemDiv7	3496
ChemDiv1	617	ChemDiv4	1626	ChemDiv6	1876	ChemDiv7	3497
ChemDiv1	618	ChemDiv4	1627	ChemDiv6	1877	ChemDiv7	3498
ChemDiv1	619	ChemDiv4	1628	ChemDiv6	1878	ChemDiv7	3499
ChemDiv1	620	ChemDiv4	1629	ChemDiv6	1879	ChemDiv7	3500
ChemDiv1	621	ChemDiv4	1630	ChemDiv6	1880	ChemDiv7	3501
ChemDiv1	622	ChemDiv4	1631	ChemDiv6	1881	ChemDiv7	3502
ChemDiv1	623	ChemDiv4	1632	ChemDiv6	1882	ChemDiv7	3503

ChemDiv1	624	ChemDiv4	1633	ChemDiv6	1883	ChemDiv7	3504
ChemDiv1	625	ChemDiv4	1634	ChemDiv6	1884	ChemDiv7	3505
ChemDiv1	626	ChemDiv4	1635	ChemDiv6	1885	ChemDiv7	3506
ChemDiv1	627	ChemDiv4	1636	ChemDiv6	1886	ChemDiv7	3507
ChemDiv1	628	ChemDiv4	1637	ChemDiv6	1887	ChemDiv7	3508
ChemDiv1	629	ChemDiv4	1638	ChemDiv6	1888	ChemDiv7	3509
ChemDiv1	630	ChemDiv4	1639	ChemDiv6	1889	ChemDiv7	3510
ChemDiv1	631	ChemDiv4	1640	ChemDiv6	1890	ChemDiv7	3511
ChemDiv1	632	ChemDiv4	1641	ChemDiv6	1891	ChemDiv7	3512
ChemDiv1	633	ChemDiv4	1642	ChemDiv6	1892	ChemDiv7	3513
ChemDiv1	634	ChemDiv4	1643	ChemDiv6	1893	ChemDiv7	3514
ChemDiv1	635	ChemDiv4	1644	ChemDiv6	1894	ChemDiv7	3515
ChemDiv1	636	ChemDiv4	1645	ChemDiv6	1895	ChemDiv7	3516
ChemDiv1	637	ChemDiv4	1646	ChemDiv6	1896	ChemDiv7	3517
ChemDiv1	638	ChemDiv4	1647	ChemDiv6	1897	ChemDiv7	3518
ChemDiv1	639	ChemDiv4	1648	ChemDiv6	1898	ChemDiv7	3519
ChemDiv1	640	Asinex1	1671	ChemDiv6	1899	ChemDiv7	3520
ChemDiv1	641	Asinex1	1672	ChemDiv6	1900	ChemDiv7	3521
ChemDiv1	642	Asinex1	1673	ChemDiv6	1901	ChemDiv7	3522
ChemDiv1	643	Asinex1	1674	ChemDiv6	1902	ChemDiv7	3523
ChemDiv1	644	Asinex1	1675	ChemDiv6	1903	ChemDiv7	3524
ChemDiv1	645	Asinex1	1676	ChemDiv6	1904	ChemDiv7	3525
ChemDiv1	646	Asinex1	1677	ChemDiv6	1905	ChemDiv7	3526
ChemDiv1	647	Asinex1	1678	ChemDiv6	1906	ChemDiv7	3527
ChemDiv1	648	Asinex1	1679	ChemDiv6	1907	ChemDiv7	3528
ChemDiv1	649	Asinex1	1680	ChemDiv6	1908	ChemDiv7	3529
ChemDiv1	650	Asinex1	1681	ChemDiv6	1909	ChemDiv7	3530
ChemDiv1	651	Asinex1	1682	ChemDiv6	1910	ChemDiv7	3531
ChemDiv1	652	Asinex1	1683	ChemDiv6	1911	ChemDiv7	3532
ChemDiv1	653	Asinex1	1684	ChemDiv6	1912	ChemDiv7	3533
ChemDiv1	654	Asinex1	1685	ChemDiv6	1913	ChemDiv7	3534
ChemDiv1	655	Asinex1	1686	ChemDiv6	1914	ChemDiv7	3535
ChemDiv1	656	Asinex1	1687	ChemDiv6	1915	ChemDiv7	3536
ChemDiv1	657	Asinex1	1688	ChemDiv6	1916	ChemDiv7	3537
ChemDiv1	658	Asinex1	1689	ChemDiv6	1917	ChemDiv7	3538
ChemDiv1	659	Asinex1	1690	ChemDiv6	1918	ChemDiv7	3539
ChemDiv1	660	Asinex1	1691	ChemDiv6	1919	ChemDiv7	3540
ChemDiv1	661	Asinex1	1692	Gray1	2043	ChemDiv7	3541
ChemDiv1	662	Asinex1	1693	Biomol4	2089	ChemDiv7	3542
ChemDiv1	663	Asinex1	1694	Biomol4	2090	ChemDiv7	3543

ChemDiv1	664	Asinex1	1695	Microsource1	2091	ChemDiv7	3544
ChemDiv1	665	Asinex1	1696	Microsource1	2092	ChemDiv7	3545
ChemDiv1	666	Asinex1	1697	Microsource1	2093	ChemDiv7	3546
ChemDiv1	667	Asinex1	1698	Microsource1	2094	ChemDiv7	3547
ChemDiv1	668	Asinex1	1699	Prestwick2	2095	ChemDiv7	3548
ChemDiv3	1473	Asinex1	1700	Prestwick2	2096	ChemDiv7	3549
ChemDiv3	1474	Asinex1	1701	Prestwick2	2097	ChemDiv7	3550
ChemDiv3	1475	Asinex1	1702	Prestwick2	2098	ChemDiv7	3551
ChemDiv3	1476	Asinex1	1703	EMD1	3229	ChemDiv7	3552
ChemDiv3	1477	Asinex1	1704	LOPAC1	3260	ChemDiv7	3553
ChemDiv3	1478	Asinex1	1705	LOPAC1	3261	ChemDiv7	3554
ChemDiv3	1479	Asinex1	1706	LOPAC1	3262	ChemDiv7	3555
ChemDiv3	1480	Enamine2	1716	LOPAC1	3263	ChemDiv7	3556
ChemDiv3	1481	Enamine2	1717	MSDiscovery1	3264	ChemDiv7	3557
ChemDiv3	1482	Enamine2	1718	LINCS1	3265	ChemDiv7	3558
ChemDiv3	1483	Enamine2	1719	LINCS2	3295	ChemDiv7	3559
ChemDiv3	1484	ChemDiv6	1795	NCC2-2012	3392	ChemDiv7	3560
ChemDiv3	1485	ChemDiv6	1796	BiomolICCBL-2012	3402	ChemDiv7	3561
ChemDiv3	1486	ChemDiv6	1797	BiomolICCBL-2012	3403	ChemDiv7	3562
ChemDiv3	1487	ChemDiv6	1798	CB GPCR	3407	ChemDiv7	3563
ChemDiv3	1488	ChemDiv6	1799	CB IONCore	3408	ChemDiv7	3564
ChemDiv3	1489	ChemDiv6	1800	CB NHRBCore	3409	ChemDiv7	3565
ChemDiv3	1490	ChemDiv6	1801	CB KINACore	3410	ChemDiv7	3566
ChemDiv3	1491	ChemDiv6	1802	Tocris2	3411	ChemDiv7	3567
ChemDiv3	1492	ChemDiv6	1803	Tocris2	3412	CMLD-BU-July2013	3568
ChemDiv3	1493	ChemDiv6	1804	Tocris2	3413	CMLD-BU-July2013	3569
ChemDiv3	1494	ChemDiv6	1805	Tocris2	3414	CMLD-BU-July2013	3570
ChemDiv3	1495	ChemDiv6	1806	LINCS3	3426	CMLD-BU-July2013	3571
ChemDiv3	1496	ChemDiv6	1807	ChemDiv7	3428	CMLD-BU-July2013	3572
ChemDiv3	1497	ChemDiv6	1808	ChemDiv7	3429	CMLD-BU-July2013	3573
ChemDiv3	1498	ChemDiv6	1809	ChemDiv7	3430	CMLD-BU-July2013	3574

ChemDiv3	1499	ChemDiv6	1810	ChemDiv7	3431	CMLD-BU-July2013	3575
ChemDiv3	1500	ChemDiv6	1811	ChemDiv7	3432	HME1	3576
ChemDiv3	1501	ChemDiv6	1812	ChemDiv7	3433	NCC1-2013	3577
ChemDiv3	1502	ChemDiv6	1813	ChemDiv7	3434	NCC1-2013	3578
ChemDiv3	1503	ChemDiv6	1814	ChemDiv7	3435	NCC2-2013	3579
ChemDiv3	1504	ChemDiv6	1815	ChemDiv7	3436	SYNthesis2	3580
ChemDiv3	1505	ChemDiv6	1816	ChemDiv7	3437	eMolecules 2014	3604
ChemDiv3	1506	ChemDiv6	1817	ChemDiv7	3438	Cayman Biolipid 1	3648
ChemDiv3	1507	ChemDiv6	1818	ChemDiv7	3439	Cayman Biolipid 1	3649
ChemDiv3	1508	ChemDiv6	1819	ChemDiv7	3440	Cayman Biolipid 1	3650
ChemDiv3	1509	ChemDiv6	1820	ChemDiv7	3441	Selleck-10mM	3651
ChemDiv3	1510	ChemDiv6	1821	ChemDiv7	3442	Selleck-10mM	3652
ChemDiv3	1511	ChemDiv6	1822	ChemDiv7	3443	Selleck-10mM	3653
ChemDiv3	1512	ChemDiv6	1823	ChemDiv7	3444	Selleck-10mM	3654
ChemDiv3	1513	ChemDiv6	1824	ChemDiv7	3445	Selleck-10mM	3655
ChemDiv3	1514	ChemDiv6	1825	ChemDiv7	3446	Selleck-10mM	3656
ChemDiv3	1515	ChemDiv6	1826	ChemDiv7	3447	Selleck-10mM	3657
ChemDiv3	1516	ChemDiv6	1827	ChemDiv7	3448	Selleck-3.33mM	3658
ChemDiv3	1517	ChemDiv6	1828	ChemDiv7	3449	Selleck-3.33mM	3659
ChemDiv3	1518	ChemDiv6	1829	ChemDiv7	3450	Selleck-3.33mM	3660
ChemDiv3	1519	ChemDiv6	1830	ChemDiv7	3451	Selleck-3.33mM	3661
ChemBridge3	1577	ChemDiv6	1831	ChemDiv7	3452	Selleck-3.33mM	3662
ChemBridge3	1578	ChemDiv6	1832	ChemDiv7	3453	Selleck-3.33mM	3663
ChemBridge3	1579	ChemDiv6	1833	ChemDiv7	3454	Selleck-3.33mM	3664
ChemBridge3	1580	ChemDiv6	1834	ChemDiv7	3455	Selleck-1.11mM	3665
ChemBridge3	1581	ChemDiv6	1835	ChemDiv7	3456	Selleck-1.11mM	3666

ChemBridge3	1582	ChemDiv6	1836	ChemDiv7	3457	Selleck-1.11mM	3667
ChemBridge3	1583	ChemDiv6	1837	ChemDiv7	3458	Selleck-1.11mM	3668
ChemBridge3	1584	ChemDiv6	1838	ChemDiv7	3459	Selleck-1.11mM	3669
ChemBridge3	1585	ChemDiv6	1839	ChemDiv7	3460	Selleck-1.11mM	3670
ChemBridge3	1586	ChemDiv6	1840	ChemDiv7	3461	Selleck-1.11mM	3671
ChemBridge3	1587	ChemDiv6	1841	ChemDiv7	3462	LINCS4	3672
ChemBridge3	1588	ChemDiv6	1842	ChemDiv7	3463	LINCS4	3673
ChemBridge3	1589	ChemDiv6	1843	ChemDiv7	3464	LINCS4	3674
ChemBridge3	1590	ChemDiv6	1844	ChemDiv7	3465		
ChemBridge3	1591	ChemDiv6	1845	ChemDiv7	3466		

Table S1. **List of libraries used in NF-κB-induced Luminescence HTS Assay**. The 574 chemical libraries plate screened are listed, included known bioactive, academic collections and commercial libraries from various sources. All libraries were provided by the Institute of Chemistry and Cell Biology (ICCB)-Longwood, Harvard Medical School. Data related to high-throughput screening may be accessed per the ICCB-Longwood Screening Facility User Agreement.

Table S2.

Library	Plate	Library	Plate
ChemBridge3	1581	ChemDiv6	1833
ChemBridge3	1582	ChemDiv6	1836
ChemBridge3	1583	ChemDiv6	1864
ChemBridge3	1585	ChemDiv6	1865
ChemBridge3	1588	ChemDiv6	1896
ChemBridge3	1593	ChemDiv6	1897
ChemBridge3	1594	ChemDiv6	1901
ChemBridge3	1596	ChemDiv7	3432
ChemDiv6	1825	ChemDiv7	3437
ChemDiv6	1831	HME1	3576
ChemDiv6	1832		

Table S2. List of libraries used in AlphaLISA HTS Assay. The 21 down selected chemical libraries plate screened via counter TNF AlphaLISA assay are listed. All libraries were provided by the Institute of Chemistry and Cell Biology (ICCB)-Longwood, Harvard Medical School. Data related to high-throughput screening may be accessed per the ICCB-Longwood Screening Facility User Agreement.

Table S3.

Compound ID	TNF (Median)	TNF % (Median)	# replicates	Series	Mol. Wt. (Daltons)	SMILES
02-144-2	2.5245	104.32	5	6-Me Imidazopyrimidine	376.84	CC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2Cl)=C1
02-144-3 (PVP-037.1)	5.397	99.81	5	6-Me Imidazopyrimidine	410.40	CC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2C(F)(F)F)=C1
02-193	2.997	91.73	5	Imidazopyrimidine	372.43	CC1=CC=C(C=C1NC(=O)C(CO)C1=CC=CC=C1)C1=CN2C=CC=NC2=N1
37.16	3.5625	90.17	3	Imidazopyrimidine	407.27	O=C(C1=C(Br)C=CC=C1)NC2=C(C)C=CC(C3=CN4C=CC=NC4=N3)=C2
37.37	3.73	71.12	3	Imidazopyrimidine	396.37	O=C(C1=C(C(F)(F)F)C=CC=C1)NC2=C(C)C=CC(C3=CN4C=CC=NC4=N3)=C2
Cpd 37	2.403	69.79	5	Imidazopyrimidine	370.46	O=C(C(C1=CC=CC=C1)CC)NC2=C(C)C=CC(C3=CN4C=CC=NC4=N3)=C2
02-171	2.1615	65.93	5	6-F Imidazopyrimidine	428.39	CC1=CC=C(C(=O)NC2=CC(=CC=C2C)C2=CN3C=C(F)C=NC3=N2)C(=C1)C(F)(F)F
02-144-1	3.144	55.24	5	6-Me Imidazopyrimidine	384.48	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=C(C)C=NC2=N1)C1=CC=CC=C1
02-166	2.0745	53.91	5	6-F Imidazopyrimidine	432.35	CC1=CC=C(C=C1NC(=O)C1=CC(F)=CC=C1C(F)(F)F)C1=CN2C=C(F)C=NC2=N1
02-167	1.101	51.62	5	6-F Imidazopyrimidine	432.35	CC1=CC=C(C=C1NC(=O)C1=CC=C(F)C=C1C(F)(F)F)C1=CN2C=C(F)C=NC2=N1
02-118	1.8795	43.53	5	6-F Imidazopyrimidine	414.36	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(

						F)(F)F)C1=CN2C=C(F)C=NC2=N1
03-082	1.008	41.65	5			
02-174-1	0.924	35.71	5	6-Me Imidazopyrimidine	404.90	CCC(C(=O)NC1=CC(=CC=C1Cl)C1=CN2C=C(C)C=NC2=N1)C1=CC=CC=C1
02-187	1.191	31.32	5	6-F Imidazopyrimidine	390.42	COC1=CC=C(C)C(=C1)C(=O)NC1=CC(=CC=C1C)C1=CN2C=C(F)C=NC2=N1
37.33	0.288	22.94	4	Imidazopyrimidine	362.82	O=C(C1=C(Cl)C=CC=C1)NC2=C(C)C=CC(C3=CN4C=CC=NC4=N3)=C2
37.17	0.865	20.64	3	Imidazopyrimidine	342.40	O=C(C1=C(C)C=CC=C1)NC2=C(C)C=CC(C3=CN4C=CC=NC4=N3)=C2
02-174-2	0.1455	13.99	5	6-Me Imidazopyrimidine	430.82	CC1=CN2C=C(N=C2N=C1)C1=CC=C(Cl)C(NC(=O)C2=CC=CC=C2C(F)(F)F)=C1
03-003-1	0.219	9.05	5	Thiazolopyridine	387.50	CCC(C(=O)NC1=C(C)C=CC(=C1)C1=NC2=CC=CN=C2S1)C1=CC=CC=C1
02-116	0.594	8.48	5	6-F Imidazopyrimidine	425.26	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Br)C1=CN2C=C(F)C=NC2=N1
03-081	0.3195	7.51	5			
02-162-2	0.309	6.34	5	6-Me Imidazopyrimidine	356.43	CC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2C)=C1
03-084-1	0.24	6.00	5			
02-125-3	0.234	5.47	5	Imidazopyridine	341.41	CC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=CN2C=CC=CC2=N1
02-139-2	0.109	4.50	5	Imidazopyridine	369.47	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=CC=CC2=N1)C1=CC=CC=C1

02-119	0.219	4.35	5	6-F Imidazopyrimidine	388.45	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=C(F)C=NC2=N1)C1=CC=CC=C1
03-003-3	0.075	3.97	5	Imidazopyrazine	370.46	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=CN=CC2=N1)C1=CC=CC=C1
02-176	0.087	3.81	5	6-F Imidazopyrimidine	390.42	COC1=CC=C(C(=O)NC2=CC(=CC=C2C)C2=CN3C=C(F)C=NC3=N2)C(C)=C1
02-139-1	0.051	3.62	5	Imidazopyridine	395.39	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(F)(F)F)C1=CN2C=CC=CC2=N1
03-078-4	0.0615	3.25	5	Pyrazolopyrimidine	370.46	CCC(C(=O)NC1=CC(=CC=C1C)C1=NN2C=CC=NC2=C1)C1=CC=CC=C1
02-125-1	0.1915	3.13	5	Imidazopyridine	361.83	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=CN2C=CC=CC2=N1
02-117	0.138	3.06	5	6-F Imidazopyrimidine	360.39	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C)C1=CN2C=C(F)C=NC2=N1
02-174-3	0.06	2.73	5	Imidazothiazole	401.41	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(F)(F)F)C1=CN2C=CSC2=N1
02-115	0.0625	2.42	5	6-F Imidazopyrimidine	380.81	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=CN2C=C(F)C=NC2=N1
02-125-2	0.1675	2.27	5	Imidazopyridine	341.41	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C)C1=CN2C=CC=CC2=N1
02-177-3	0.0675	1.79	5	Imidazothiazole	375.49	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=CSC2=N1)C1=CC=CC=C1
02-135	0.0705	1.60	5	6-MeO Imidazopyrimidine	426.40	COC1=CN2C=C(N=C2N=C1)C1=CC=C(C)

						C(NC(=O)C2=CC=CC=C2C(F)(F)F)=C1
02-208	0.0495	1.56	5	Imidazopyrazine	396.37	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(F)(F)F)C1=CN2C=CN=CC2=N1
02-175	0.063	1.43	5	6-F Imidazopyrimidine	432.35	CC1=CC=C(C=C1NC(=O)C1=C(F)C=CC=C1C(F)(F)F)C1=CN2C=C(F)C=NC2=N1
03-086-2	0.0325	1.34	5			
02-137	0.04	1.27	5	6-MeO Imidazopyrimidine	400.48	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2C=C(OC)C=NC2=N1)C1=CC=CC=C1
03-003-2	0.034	1.02	5	Oxazolopyridine	343.39	CC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=NC2=NC=CC=C2O1
02-162-4	0.008	0.77	5	Imidazopyridazine	362.82	CC1=CC=CC=C1C(=O)NC1=CC(=CC=C1Cl)C1=CN2N=CC=CC2=N1
02-154-2	0.008	0.77	5	Imidazopyridazine	396.37	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(F)(F)F)C1=CN2N=CC=CC2=N1
02-183	0.008	0.77	5	Imidazopyrimidine	372.43	COC1=CC=C(C)C(=C1)C(=O)NC1=CC(=CC=C1C)C1=CN2C=CC=NC2=N1
02-144-4	0.028	0.75	5	Imidazopyridine	389.88	CCC(C(=O)NC1=CC(=CC=C1Cl)C1=CN2C=CC=CC2=N1)C1=CC=CC=C1
02-144-7	0.0175	0.72	5	Imidazopyridazine	390.87	CCC(C(=O)NC1=CC(=CC=C1Cl)C1=CN2N=CC=CC2=N1)C1=CC=CC=C1
02-140-2	0.008	0.62	5	Imidazopyridazine	342.40	CC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=CN2N=CC=CC2=N1
02-184-2	0.008	0.57	5	Oxazolopyridine	371.44	CCC(C(=O)NC1=CC(=CC=C1C)C1=NC2=NC=CC=C2O1)C1=CC=CC=C1

03-086-1	0.04	0.55	5			
02-144-6	0.016	0.52	5	Imidazopyridine	361.83	CC1=CC=CC=C1C(=O)NC1=CC(=CC=C1Cl)C1=CN2C=CC=CC2=N1
02-162-1	0.008	0.42	5	6-Me Imidazopyrimidine	356.43	CC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)CC2=CC=CC=C2)=C1
02-154-1	0.008	0.42	5	Imidazopyridine	415.80	FC(F)(F)C1=CC=CC=C1C(=O)NC1=CC(=CC=C1Cl)C1=CN2C=CC=CC2=N1
03-078-1	0.008	0.42	5	Thiazolopyridine	379.86	CC1=CC=C(C=C1NC(=O)C1=C(Cl)C=CC=C1)C1=NC2=CC=CN=C2S1
03-078-2	0.008	0.42	5	Thiazolopyridine	359.45	CC1=C(NC(=O)CC2=CC=CC=C2)C=C(C=C1)C1=NC2=CC=CN=C2S1
03-078-3	0.008	0.42	5	Thiazolopyridine	359.45	CC1=CC=CC=C1C(=O)NC1=C(C)C=CC(=C1)C1=NC2=CC=CN=C2S1
02-182	0.008	0.39	5	Imidazopyrimidine	372.43	COC1=CC=C(C(=O)NC2=CC(=CC=C2C)C2=CN3C=CC=NC3=N2)C(C)=C1
02-192-2	0.008	0.33	5	Oxazolopyridine	363.80	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=NC2=NC=CC=C2O1
02-162-3	0.008	0.33	5	Imidazopyridazine	362.82	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=CN2N=CC=CC2=N1
02-177-1	0.008	0.33	5	Imidazothiazole	347.44	CC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=CN2C=CSC2=N1
02-177-2	0.008	0.33	5	Imidazothiazole	347.44	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C)C1=CN2C=CSC2=N1
02-192-1	0.008	0.33	5	Oxazolopyridine	343.39	CC1=CC=C(C=C1NC(=O)C1=C(C)C=CC=C1)C1=NC2=NC=CC=C2O1

02-184-1	0.008	0.33	5	Oxazolopyridine	397.36	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C(F)(F)F)C1=NC2=NC=CC=C2O1
02-177-4	0.008	0.33	5	Imidazothiazole	367.85	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=CN2C=CSC2=N1
02-140-1	0.008	0.20	5	Imidazopyridazine	370.46	CCC(C(=O)NC1=CC(=CC=C1C)C1=CN2N=CC=CC2=N1)C1=CC=CC=C1
02-144-5	0.008	0.20	5	Imidazopyridine	361.83	ClC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=CN2C=CC=CC2=N1
02-103	0.008	0.18	5	Imidazopyrimidine	342.40	CC1=CC=C(C=C1NC(=O)CC1=CC=CC=C1)C1=CN2C=CC=NC2=N1
02-169	0.008	0.18	5	6-F Imidazopyrimidine	448.81	CC1=CC=C(C=C1NC(=O)C1=CC=C(Cl)C=C1C(F)(F)F)C1=CN2C=C(F)C=NC2=N1
02-132	0.008	0.18	5	6-MeO Imidazopyrimidine	437.30	COC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2Br)=C1
02-131	0.008	0.18	5	6-MeO Imidazopyrimidine	392.84	COC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2Cl)=C1
02-134	0.008	0.18	5	6-MeO Imidazopyrimidine	372.43	COC1=CN2C=C(N=C2N=C1)C1=CC=C(C)C(NC(=O)C2=CC=CC=C2C)=C1
02-140-3	0.008	0.18	5	Imidazopyridazine	342.40	CC1=CC=C(C=C1NC(=O)C1=CC=CC=C1C)C1=CN2N=CC=CC2=N1
02-154-3	0.008	0.18	5	Imidazopyridine	382.24	ClC1=CC=C(C=C1NC(=O)C1=CC=CC=C1Cl)C1=CN2C=CC=CC2=N1
02-194	0.008	0.18	5	6-F Imidazopyrimidine	390.42	CC1=CC=C(C=C1NC(=O)C(CO)C1=CC=CC=C1)C1=CN2C=C(F)C=NC2=N1

Table S3. ***In vitro*** **efficacy of PVP-037 analogs as benchmarked to R848**. TNF production measured by ELISA after stimulation of adult peripheral blood mononuclear cells for PVP-037 analogs or R848 at 33 μM for ~18 hours. Analogs are ranked top to bottom by median percentage TNF production (TNF %) as compared to R848. Compounds inducing TNF > 15% of R848 are indicated in green. N = 3 - 5.

Data S1. (multiple sheets): Data generated in this study; each datasheet labeled with the figure number.