

Title: High throughput screening to identify inhibitors of the type I interferon – major histocompatibility complex class I pathway in skeletal muscle

Authors: Travis B. Kinder, Patricia K. Dranchak, James Inglese

SUPPORTING INFORMATION

HLA-ABC IF secondary assay. All aspiration and dispensing steps for immunostaining were conducted at ambient temperature with an EL406 (BioTek, Winooski, VT) with a 16x4 pin aspiration head and an 8 tip, 5 uL dispensing cassette. All solutions were prepared in PBS. Cells were fixed in 4.0% paraformaldehyde for 20 min, permeabilized in 0.1% Triton X-100 for 20 min, blocked in 10% goat serum for 30 min, incubated with 1:400 HLA-ABC antibody (clone W6/32, MA5-11723) + 2% goat serum + .05% tween-20 for 2.5 hrs, washed three times, incubated with 1:1000 Hoechst 33342 (H3570) + 1:1000 Alexa Fluor 488 goat anti-mouse antibody (A11001) + 1:5000 CellMask Red (H32712) for 1 hr, washed three times, and stored at 4 °C for later analysis

Western blotting. All lysates were stored at -80 °C for later analysis. Thawed samples were cleared of debris by centrifugation, and supernatant protein concentration was determined by BCA assay (23227).

For HiBit clones, 10 ug of protein/sample were prepared and reduced in NuPAGE LDS Sample buffer (NP00008) + 5% BME, then run on 1.0 mm, 12-well, 4-12% NuPAGE Bis-Tris gels in MOPS running buffer (NP0322 and NP0001) at 150 V for 80 min, and transferred to 0.45 um pore PVDF membrane (Bio-Rad, Hercules, CA, Immun-Blot LF, 1620263) at 150 mA for 1 hr in NuPAGE Transfer Buffer (NP0006) with 10% methanol. Lane 1 contained Amersham ECL Plex Fluorescent Rainbow Marker, Full Range (RPN850E). All blocking and immunoblotting was done with 5% non-fat milk powder in tris buffered saline + 0.05% tween-20 (TBST). Membranes were incubated with a 1:2000 mouse HLA-ABC antibody (HC-10, gift from Dr. Robert A. Colbert at National Institute of Arthritis and Musculoskeletal and Skin Diseases) + 1:1000 rabbit β -tubulin antibody (abcam, ab179513) overnight at 4 °C. After three washes in TBST, membranes were incubated with Alexa Fluor 647 goat anti-mouse antibody (A21235) for HLA-ABC and Alexa Fluor 488 goat anti-rabbit antibody

(A1108) for β -tubulin, both diluted 1:2000 for 1 hr at ambient temperature. After three washes in TBST, membranes were imaged on a Typhoon FLA 9500 (GE). Membranes were incubated with Restore Stripping Buffer (21059) for 15 min, washed 3 times in TBST, then HiBit was detected with the Nano-Glo HiBit Blotting System (Promega, N2410) according to the manufacturer's protocol using a 1 hr incubation with LgBit and chemiluminescent detection on a Bio-Rad Chemidoc Touch.

For active compound 30 min treatment, parental C25cl48 cells were pretreated with compounds for 1 hr prior to adding IFN- β for 30 min, whereas for the 24 hr treatment, compounds and IFN- β were added at same time. For both experiments, compounds were tested at their IC95 according to the HiBit CRCs (in μ M): Rux, Bari, and Tofa – 6.3, Pred – 1.0, Echi - 0.032, Givi – 18.2, Pano - 0.079, CUD – 1.0, Dina - 0.079, ARV – 0.13, Nemo – 0.32. For Western blot, 15 μ g of protein/sample were prepared and reduced in NuPAGE LDS Sample buffer (NP00008) + 5% BME, then run on 4-12% Bis-Tris gel, 10x8, 15-well (GenScript, Piscataway, NJ, M00654) at 150 V for 60 min, and transferred to 0.45 μ m pore nitrocellulose (Bio-Rad) at 150 mA for 1 hr in NuPAGE Transfer Buffer (NP00006) with 10% methanol. Lane 1 contained Amersham ECL Plex Fluorescent Rainbow Marker, Full Range. Membranes were blocked in 5% bovine serum albumin (BSA) in TBST, and antibodies were prepared in 1% BSA in TBST and incubated for 1 hr at ambient temperature except phospho-STAT1 was overnight at 4 °C. Antibodies used were 1:1000 rabbit phospho-STAT1 antibody (Cell Signaling Technology, Danvers, MA, 7649), 1:1000 rabbit STAT1 antibody (Cell Signaling Technology, 9172T), 1:1000 mouse HLA-ABC antibody (HC-10), and 1:1000 rabbit β -tubulin antibody. After three washes in TBST, membranes were incubated with either 1:5000 goat anti-rabbit stabilized peroxidase secondary antibody (Invitrogen 32460) or 1:5000 goat anti-mouse stabilized peroxidase secondary antibody (Invitrogen 32430) for 1 hr at ambient temperature. After three washes in TBST, membranes were imaged using Super Signal West Dura (34076) and a BioRad Chemidoc Touch. Membranes were stripped between each primary + secondary antibody pair as described above.

Whole Genome Sequencing. For WGS library preparation, an average of 200 ng of quality DNA was used. The sequencing libraries were made using the Twist library-enzymatic protocol according to

manufacturer’s instructions (Twist Biosciences, CA, USA). The fragmentation conditions were adapted to create fragments around 300-400bp followed by dual-SPRI size selection washing procedure. In summary gDNA was utilized for Fragmentation, End Repair, dA-tailing and Adapter Ligation. Twist Universal Adapter system consists of Twist Universal Adapters and Twist Unique Dual Indexed (UDI) Primers which provides high quality libraries. Two PCR cycles were applied after library addition to increase the yield and close the molecule ends for better quality control check on a Bionalyzer and qPCR. The duplicate rates were minimal and did not really impact the data with this library method. WES sequencing for 5 samples was performed on the Novaseq6000 (Illumina, La Jolla, CA, USA) using the S4 Flow cell with the paired-end 300 cycle protocol (2x150bp) following Illumina recommendations.

Raw sequencing data was outputted by the Illumina NovaSeq 6000 in the form of BCL (binary base call) files. The Illumina DRAGEN Bio-IT Platform (version 3.3.7) was used to generate a final output of VCF (variant call format) files for each sample. In more detail, the BCL files are first demultiplexed and converted into FASTQ files. DRAGEN then aligns these FASTQ files to the Homo sapiens GRCh37 genome (hs37d5) to generate CRAM alignment files. During this step, the alignment file was also sorted and duplicate reads marked. The final step performed by DRAGEN was the variant caller which generated a VCF file from the CRAM input and contained single-nucleotide variants (SNVs) and short insertions/deletions (indels). The HiBit insertion sites in the test samples were identified by comparing the short insertions in the VCF files from the control and test samples. The control cells have two heterozygous alleles for HLA-B (08:01 and 18:01). HiBit sequence was designed to target the HLA-B 08:01 allele. The HLA-B 08:01 allele contains a single-nucleotide variant (dbSNP:rs2308655 C>G) which is not present in HLA-B 18:01 and is 40bp away from the designed insertion site. The phase of the rs2308655 variant and the HiBit insertion site can be determined by checking whether they exist on the same short read (read-backed phasing).

Protocol Tables for qHTS

HLAB HiBit Primary Screen: LOPAC			
Sequence	Parameter	Value	Description

1a	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 350 cells/well into nine Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK growth medium (GM) without dexamethasone into columns 1+2. Add metal lids with gas-exchange holes
1b	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 350 cells/well into nine Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK GM without dexamethasone + 4 ng/ml IFN- β into columns 3 – 48. Add metal lids with gas-exchange holes
2	Incubate	1 hr	Incubate at 37 C, 5% CO ₂ , 95% RH
3	Compounds	23 nl	Pin Tool (Wako) to transfer DMSO control, ruxolitinib IFN- β inhibitor control [High Concentration = 80 uM final, 16-pt, 1:2 in duplicate], and library compounds in DMSO
4	Incubate	24 hrs	Incubate at 37 C, 5% CO ₂ , 95% RH
5	Dispense	2.5 ul	BioRAPTR FRD (Beckman Coulter) to add HiBit lytic reagent
6	Incubate	10 min	Incubate in the dark at ambient temperature for lysis
7	Detector	5 sec	Measure luminescence with ViewLux 1430 Ultra HTS (Perkin Elmer). Settings: Exposure = 5 sec, Gain = High, Speed = Slow, Binning = 3X
Step	Notes		
1	Cells HLA-B HiBit myoblasts cultured in iHSMK growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 9, from three T175s at 90% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 70 cells/ul in GM - dexamethasone, and plated in 5 ul/well into Greiner 1536 well white/solid bottom, high base, TC plates (789173-F). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.		
3	Compounds Control plate contained DMSO in columns 1, 2, and 4, and ruxolitinib (Selleckchem, S1378) titration in DMSO in column 3. Ruxolitinib stock was reconstituted at 50 mM in DMSO and aliquots stored at -20 °C. 1,280 compounds were added in a 7-point, 1:4.3 titration with a high final concentration of 46 uM by Pintool from seven interplate titrations of library plates and two DMSO control plates at the beginning and end		
5	Dispense detection reagent HiBit lytic assay reagents stored at -30 °C and prepared fresh the day of according to the manufacturer's protocol. Dilute 739 ul substrate + 365 ul LgBit + 36.5 ml buffer to dispense with BioRAPTR		

HLAB HiBit Primary Screen: NPC				
Location	Sequence	Parameter	Value	Description
ADST Lab	1a	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 18 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK growth medium (GM) without dexamethasone into columns 1+2. Add metal lids with gas-exchange holes
	1b	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 18 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK GM without dexamethasone + 4 ng/ml IFN- β into columns 3 – 48. Add metal lids with gas-exchange holes
Kalypsys	2	Compound s	23 nl	Pin Tool (Wako) to transfer DMSO control, ruxolitinib IFN- β inhibitor control [High Concentration = 80 uM final, 16-pt, 1:2 in duplicate], and library compounds in DMSO
	3	Incubate	24 hrs	Incubate at 37 C, 5% CO ₂ , 95% RH

	4	Dispense	2.5 ul	Add HiBit lytic reagent (BioRaprt)
	5	Incubate	10 min	Incubate in the dark at ambient temperature for lysis
	6	Detector	5 sec	Measure luminescence with ViewLux 1430 Ultra HTS (Perkin Elmer). Settings: Exposure = 5 sec, Gain = High, Speed = Slow, Binning = 2X
Step	Notes			
1	Cells HLA-B HiBit myoblasts cultured in iHSM growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 10, from five T175s at 80% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 60 cells/ul in GM - dexamethasone, and plated in 5 ul/well into Greiner 1536 well white/solid bottom, high base, TC plates (789173-F). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.			
2	Compounds Control plate contained DMSO in columns 1, 2, and 4, and ruxolitinib (Selleckchem, S1378) titration in DMSO in column 3. Ruxolitinib stock was reconstituted at 50 mM in DMSO and aliquots stored at -20 °C. 2,816 compounds were added in an 8-point, 1:5 titration with a high final concentration of 46 uM by Pintool from 16 interplate titrations of library plates and two DMSO control plates at the beginning and end			
4	Dispense detection reagent HiBit lytic assay reagents stored at -30 °C and prepared fresh the day of according to the manufacturers protocol. Dilute 1.7 ml substrate + 830 ul LgBit + 83 ml buffer to dispense with BioRAPTR			

HLAB HiBit Primary Screen: MIPE5.0				
Location	Sequence	Parameter	Value	Description
ADST Lab	1a	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 24 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSM growth medium (GM) without dexamethasone into columns 1+2. Add metal lids with gas-exchange holes
	1b	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 24 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSM GM without dexamethasone + 4 ng/ml IFN-b into columns 3 – 48. Add metal lids with gas-exchange holes
Kalypsys	2	Compounds	23 nl	Pin Tool (Wako) to transfer DMSO control, ruxolitinib IFN-b inhibitor control [High Concentration = 80 uM final, 16-pt, 1:2 in duplicate], and library compounds in DMSO
	3	Incubate	24 hrs	Incubate at 37 C, 5% CO2, 95% RH
	4	Dispense	2.5 ul	Add HiBit lytic reagent (BioRaprt)
	5	Incubate	10 min	Incubate in the dark at ambient temperature for lysis
	6	Detector	5 sec	Use BioRAPTR FRD (Beckman Coulter) to add HiBit lytic reagent
Step	Notes			
1	Cells HLA-B HiBit myoblasts cultured in iHSM growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 10, from six T225s at 97% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 60 cells/ul in GM - dexamethasone, and plated in 5 ul/well into Greiner 1536 well white/solid bottom, high base, TC plates (789173-F). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.			
2	Compounds			

	Control plate contained DMSO in columns 1, 2, and 4, and ruxolitinib (Selleckchem, S1378) titration in DMSO in column 3. Ruxolitinib stock was reconstituted at 50 mM in DMSO and aliquots stored at -20 °C. 2,480 compounds were added in an 11-point, 1:3 titration with a high final concentration of 46 uM by Pintool from 22 interplate titrations of library plates and two DMSO control plates at the beginning and end
4	<u>Dispense detection reagent</u> HiBit lytic assay reagents stored at -30 °C and prepared fresh the day of according to the manufacturers protocol. Dilute 2 ml substrate + 1 ml LgBit + 100 ml buffer to dispense with BioRAPTR

HLAB HiBit Retest and CTG Counter Screen			
Sequence	Parameter	Value	Description
1a	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 16 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK growth medium (GM) without dexamethasone into columns 1+2. Add metal lids with gas-exchange holes
1b	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 300 cells/well into 16 Greiner 1536 well white/solid bottom, high base, TC plates in 5 ul/well of iHSMK GM without dexamethasone + 4 ng/ml IFN- β into columns 3 – 48. Add metal lids with gas-exchange holes
2	Compounds	23 nl	Pin Tool (Wako) to transfer DMSO control, 92 uM final digitonin cytotoxic control, ruxolitinib IFN- β inhibitor control [High Concentration = 80 uM final, 16-pt, 1:2 in duplicate], and library compounds in DMSO. Pin duplicate plates for two assays: HiBit and CellTiter Glo
3	Incubate	24 hrs	Incubate at 37 C, 5% CO ₂ , 95% RH
4	Dispense	2.5 ul	BioRAPTR FRD (Beckman Coulter) to add HiBit lytic reagent
5	Incubate	10 min	Incubate in the dark at ambient temperature for lysis
6	Detector	5 sec	Measure luminescence with ViewLux 1430 Ultra HTS (Perkin Elmer). Settings: Exposure = 5 sec, Gain = High, Speed = Slow, Binning = 2X
7	Dispense	2.5 ul	Use BioRAPTR FRD (Beckman Coulter) to add CellTiter Glo reagent
8	Incubate	10 min	Incubate in the dark at ambient temperature for lysis
9	Detector	1 sec	Measure luminescence with ViewLux 1430 Ultra HTS (Perkin Elmer). Settings: Exposure = 1 sec, Gain = High, Speed = Slow, Binning = 2X
Step	Notes		
1	<u>Cells</u> HLA-B HiBit myoblasts cultured in iHSMK growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 10, from four T225s at 95% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 60 cells/ul in GM - dexamethasone, and plated in 5 ul/well into Greiner 1536 well white/solid bottom, high base, TC plates (789173-F). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.		
2	<u>Compounds</u> Control plate contained DMSO in columns 1 and 4, digitonin in DMSO in column 2, and ruxolitinib (Selleckchem, S1378) titration in DMSO in column 3. Ruxolitinib stock was reconstituted at 50 mM in DMSO and aliquots stored at -20 °C. 650 compounds were added by Pintool in a 12-point, 1:3 titration with a high final concentration of 46 uM from six intraplate titrations of library plates and two DMSO control plates at the beginning and end		
4	<u>Dispense detection reagent</u> HiBit lytic assay reagents stored at -30 °C and prepared fresh the day of according to the manufacturers protocol. Dilute 740 ul substrate + 370 ul LgBit + 37 ml buffer to dispense with BioRAPTR		
7	<u>Dispense detection reagent</u>		

	CellTiter Glo assay reagents reconstituted, passed through a 0.22 um filter, aliquots stored at -30 °C, and thawed the day of. Thaw 37 ml reagent to dispense with BioRAPTR
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HLA-ABC IF Secondary Screen			
Sequence	Parameter	Value	Description
0	Compounds	25 nl	Follow-up compounds dispensed at 25 nl/well with Labcyte Echo 655 acoustic dispenser in triplicate Aurora ChemLib 1536K black/clear bottom, low base, TC 1536-well plates (00019324). Also dispensed 25 nl/well DMSO control, ruxolitinib IFN-b inhibitor control titration, and digitonin cytotoxic control
1a	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 125 cells/well into eight plates in 5 ul/well of iHSM growth medium (GM) without dexamethasone into columns 1+2. Add metal lids with gas-exchange holes
1b	Cells	5 uL	Multidrop Combi (Thermo Scientific) to seed 125 cells/well into eight plates in 5 ul/well of iHSM GM without dexamethasone + 4 ng/ml IFN-b into columns 3 – 48. Add metal lids with gas-exchange holes
2	Compounds	23 nl	Pin Tool (Wako) to transfer DMSO control, ruxolitinib IFN-b inhibitor titration, and 100 uM final digitonin to just two DMSO plates
3	Incubate	24 hrs	Incubate at 37 C, 5% CO ₂ , 95% RH
4	Aspirate & dispense	Z=22 & 5 ul	EL406 (Biotek) Aspirate Medium and add PBS with 5 ul cassette
5	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add 4.8% PFA in PBS with 5 ul cassette
6	Incubation	20 min	Incubate plates with lids at room temperature for fixation
7	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PFA and add PBS with syringe manifold
8	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add 0.12% Triton in PBS with 5 ul cassette
9	Incubation	20 min	Incubate plates with lids at room temperature for permeabilization
10	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate Triton and add PBS with syringe manifold
11	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add 10% Goat serum in PBS with 5 ul cassette
12	Incubation	30 min	Incubate plates with lids at room temperature for blocking
13	Aspirate & dispense	Z=22 & 3 ul	EL406 Aspirate blocking and add HLA-ABC primary antibody diluted 1:400 + 2% goat serum + .05% tween-20 in PBS with 5 ul cassette
14	Centrifuge	1000 rpm, 30 sec	Spin plates briefly to settle antibody solution
15	Incubation	2.5 hrs	Incubate plates with lids at room temperature with primary antibody
16	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate antibody and add PBS with syringe manifold
17	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add PBS with syringe manifold
18	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add PBS with syringe manifold
19	Aspirate & dispense	Z=22 & 3 ul	EL406 Aspirate PBS and add Alexa Fluor 488 goat anti-mouse secondary antibody 1:1000 + cell mask red 1:5000 + Hoechst 1:1000 in PBS with 5 ul cassette
20	Centrifuge	1000 rpm, 30 sec	Spin plates briefly to settle antibody solution

21	Incubation	1 hrs	Incubate plates with lids at room temperature with secondary antibody
22	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate antibody and add PBS with syringe manifold
23	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add PBS with syringe manifold
24	Aspirate & dispense	Z=22 & 5 ul	EL406 Aspirate PBS and add PBS with syringe manifold
25	Seal and store	4C	Apply plate seal and store at 4C protected from light until measurement
26	Read	405, 488, 561 nm	Scan plates on Acumen eX3 (TTP Labtech) running Cellista v4.3.4.0 software

Step	Notes
0	Compounds Two compound plates with 216 compounds in 11-point, 1:3 intraplate titrations with high concentration of 50 uM final. DMSO control into columns 1, 2, and 4, ruxolitinib IFN- γ inhibitor titration (Selleckchem, S1378) [High Concentration = 87 uM final, 8-pt, 1:4 in duplicate] in wells A3-P3, and 100 uM final digitonin cytotoxic control in wells Q3-AF3. Ruxolitinib stock was reconstituted at 50 mM in DMSO and aliquots stored at -20 °C.
1	Cells C25cl48 myoblasts cultured in iHSM growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 19, from one T175s at 70% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 25 cells/ul in GM - dexamethasone, and plated in 5 ul/well into Aurora ChemLib 1536K black/clear bottom, low base, TC 1536-well plates (00019324). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.
2	Compounds Control plate contained DMSO control in columns 1, 2, and 4, ruxolitinib IFN- γ inhibitor titration [High Concentration = 87 uM final, 8-pt, 1:4 in duplicate] in wells A3-P3, and 100 uM final digitonin cytotoxic control in wells Q3-AF3. Two DMSO plates were pinned to be processed first and last in the immunofluorescence protocol.
13	HLA-ABC primary antibody (clone W6/32, Thermo Fisher, MA5-11723) stored at 4 °C
19	Hoechst 33342 (Thermo Fisher, H3570) stored at 4 °C + Alexa Fluor 488 goat anti-mouse antibody (Thermo Fisher, A11001) stored at 4 °C + CellMask Red (Thermo Fisher, H32712) reconstituted at 5 mg/ml in DMSO and aliquots stored at -30 °C
26	Sample data type 'HLA-ABC' were quantified as number of composite objects positive for Hoechst & HLA-ABC & CellMask Red / number of objects positive for CellMask Red. Sample data type 'Viability' was quantified as number of objects positive for CellMask Red. For each compound plate, triplicate plates were assayed and averages were analyzed and plotted as concentration-response-curves

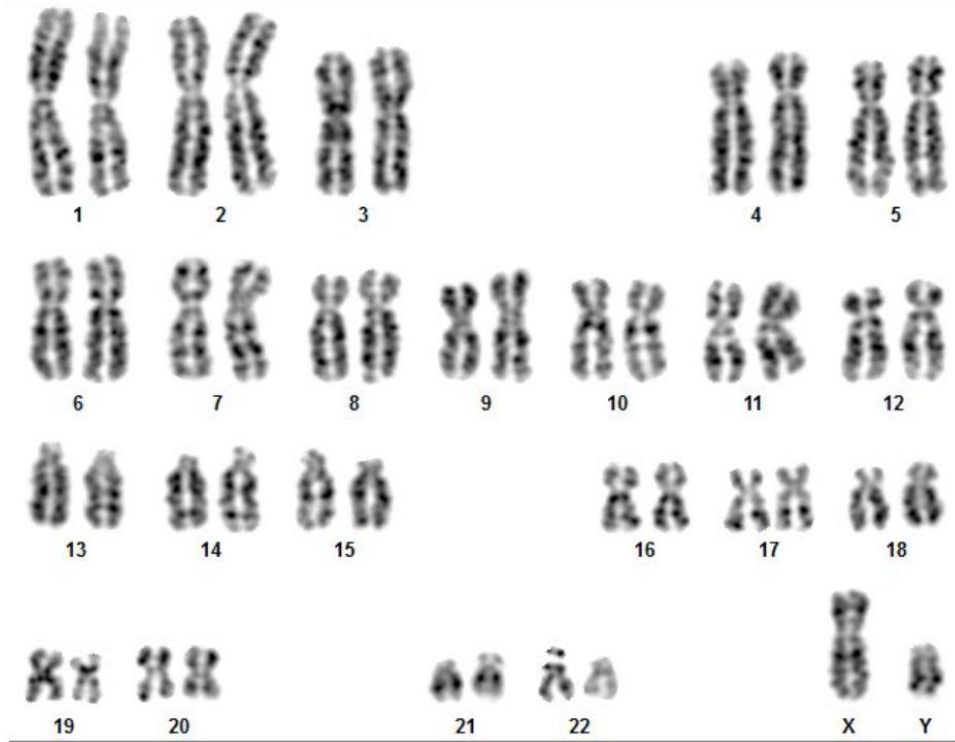
HLA-B RT-qPCR Tertiary Screen			
Sequence	Parameter	Value	Description
0	Compounds	150 nl	Follow-up compounds dispensed at 150 nl/well with Labcyte Echo 655 acoustic dispenser into one Corning white/clear bottom, high base TC 384-well plates (3765)
1a	Cells	30 uL	Using a p200 manual pipette, seed 780 cells/well into 1 plate in 30 ul/well of iHSM growth medium without dexamethasone into columns wells A1-A6.

1b	Cells	30 uL	Using a multichannel p200 pipette, seed 780 cells/well into 1 plate in 30 ul/well of iHSM growth medium without dexamethasone + 4 ng/ml IFN- β into columns all wells except A1-A6
2	Incubate	24 hrs	Incubate at 37 C, 5% CO ₂ , 95% RH
3	Aspirate by centrifugation	500 rpm, 30 sec	Aspirate medium by gently blotting upside down on paper towel, then centrifugation upside down on paper towel
4	Dispense	30 ul	PBS wash with Sartorius 12 channel e300 repeat pipette
5	Aspirate by centrifugation	500 rpm, 30 sec	Aspirate PBS by gently blotting upside down on paper towel, then centrifugation upside down on paper towel
6	Dispense	13 ul	Cells to Ct lysis buffer with Sartorius 12 channel e300 repeat pipette
7	Shake	750 rpm, 3 min	Shake to lyse cells on VWR microplate shaker
8	Dispense	1.3 ul	Stop solution with Sartorius 12 channel e10 repeat pipette, dispensed onto top, side of well above lysate
9	Centrifuge	1000 rpm 15 sec	Spin plate right side up to settle stop solution into lysate
10	Shake	750 rpm, 1 min	Shake to mix stop solution on VWR microplate shaker
11	Seal and store	-30 C	Add plate seal and store at -30 C until PCR
12	Dispense	14 ul	Thaw plate at 4C, prepare RT-qPCR master mix, then dispense master mix to 384 well PCR plate on ice, using a with Sartorius 12 channel e300 repeat pipette
13	Dispense	1.4 ul	Transfer lysate from cell culture plate to PCR plate using multichannel p20 pipette
14	Thermal cycle		Add clear plate seal, vortex, spin briefly, and thermal cycle with ViiA7 instrument (Life Technologies): 50 C – 5 min, 95 C – 20 sec, [95 C – 15 sec, 60 C - 1 min](40x)
Step	Notes		
1	<u>Cells</u> C25cl48 myoblasts cultured in iHSM growth medium (GM) - 4:1 ratio DMEM Glutamax (Thermo Fisher, 10566) : M199 (Thermo Fisher, 11150) + 10% FBS (Hyclone Standard) + 1% Pen/Strep + 50 ug/ml fetuin + 10 ng/ml hEGF + 1 ng/ml bhFGF + 10 ug/ml insulin + 0.4 ug/ml dexamethasone (supplements from Promocell, C-39360). Cells were trypsinized and collected at passage 19, from one T175s at 70% confluence, passed through a 40 um strainer, counted via trypan blue and Countess (Invitrogen), diluted to 26 cells/ul in GM - dexamethasone, and plated in 30 ul/well into Corning white/clear bottom, high base TC 384-well plates (3765). Recombinant human IFN- β (Peprotech, 300-02BC) was reconstituted in water, diluted in GM, and aliquots stored at -80 °C until day of use.		
6-14	<u>Cells-to-Ct</u> Cells were harvested and assayed using the Cells-to-CT 1-Step TaqMan Kit (A25602) and TaqMan assays for <i>HLA-B</i> (Hs00741005_g1) and <i>HPRT1</i> (Hs02800695_m1). <i>HLA-B</i> assay utilized FAM reporter, and <i>HPRT1</i> utilized VIC reporter for multiplexing. Thermal cycle on a ViiA7 instrument (Life Technologies) running ViiA7 software v1.0, and expression levels calculated using the $\Delta\Delta CT$ method relative to <i>HPRT1</i> .		

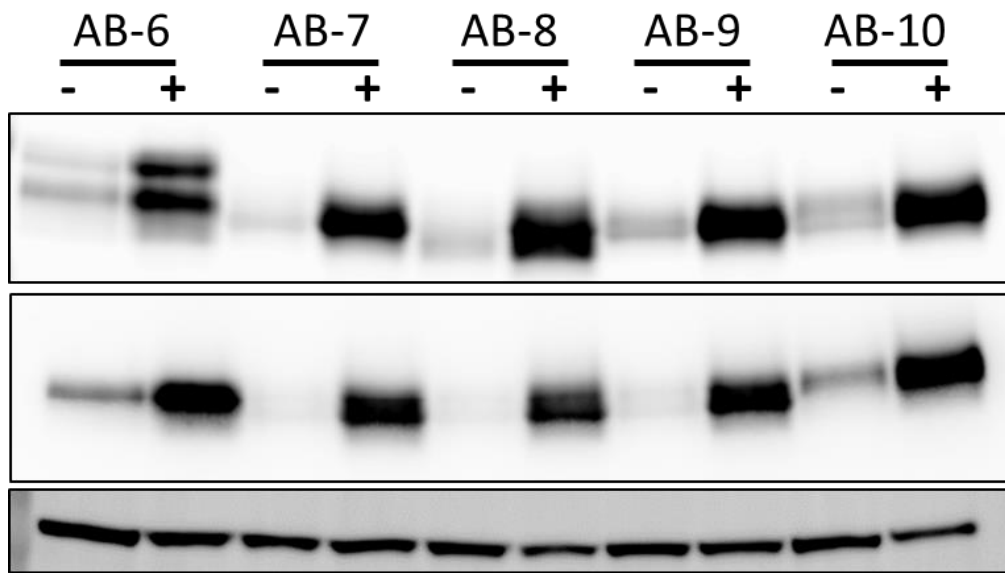
A

A	C	B	Bw	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1	DPB1
01:01 03:01	07:01 12:03	08:01 18:01	6 6	03:01 07:01	01:01	01:03N		05:01 02:01	02:01 03:03	04:01 04:02

B



Supplementary Figure 1. HLA genotyping and karyotyping for immortalized human myoblasts C25cl48. **A**, DNA was extracted from C25cl48 cells and HLA genotypes determined by next generation sequencing, high resolution HLA typing. C25cl48 cells were determined to be heterozygous at the *HLA-B* locus, and we targeted HLA-B*08:01 for genome-engineering. **B**, Karyotyping showed C25cl48 cells to be normal 46,XY.



Supplementary Figure 2. Western blot analysis of HLA-ABC, HiBit, and β -tubulin loading control in several additional genome-engineered clones treated + 8.0 ng/ml IFN- β for 24 hrs showed up-regulation of endogenous HLA-ABC and HiBit with IFN- β treatment.

Supplementary Table 1. Summary statistics for HLA-B HiBit primary screen, LOPAC1280 library

Parameters	Assay Statistics	
1536-well plates	7 (+2 DMSO)	
Compounds tested	1,280	
Points per titration	7	
Data points	8,960	
Control condition	IFN- β (0.18 nM = 4.0 ng/ml)	
	DMSO	IFN- β
HiBit Luminescence	537.7 \pm 86.7	16,482.5 \pm 684.3
CV	15.7 \pm 5.1	4.1 \pm 0.7
S/B	32.5 \pm 9.3	
Z' factor	0.89 \pm 0.03	

S/B - Signal/Background, CV - Coefficient of variation

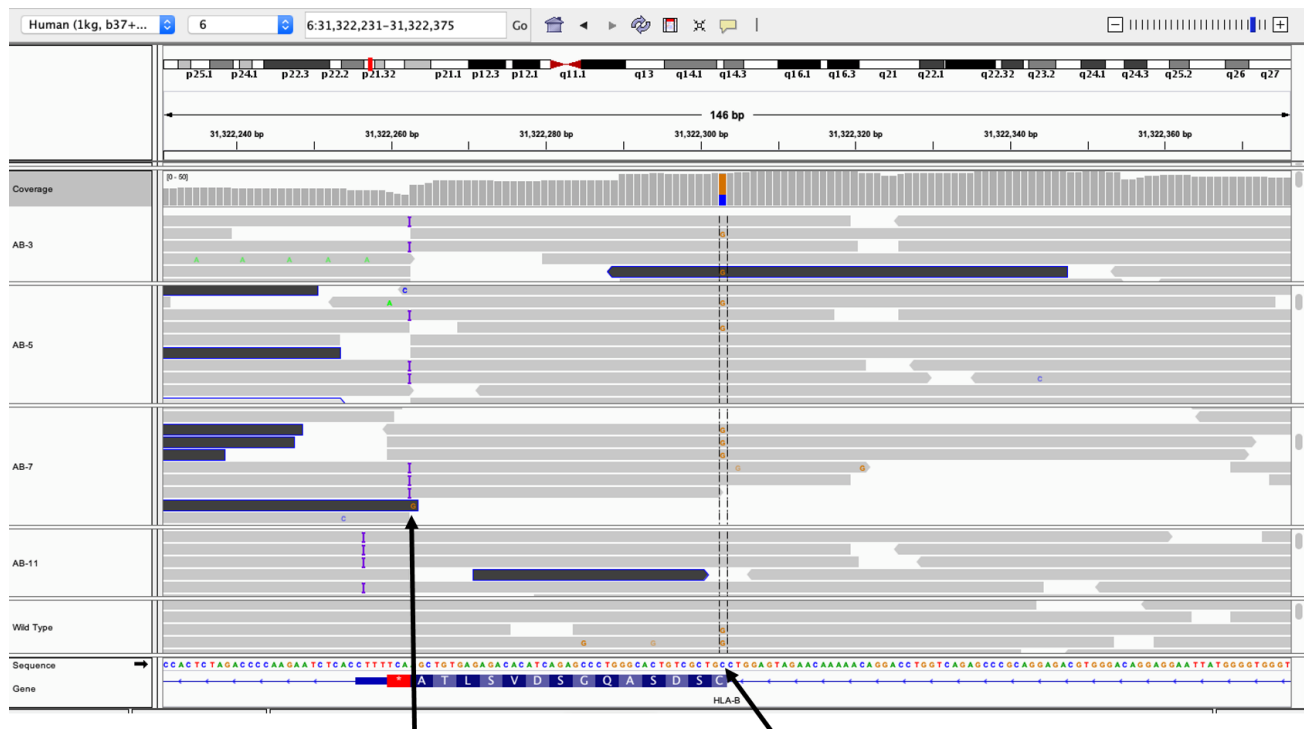
Supplemental Table 2. HLA-B
 HiBit clones whole genome
 sequencing coverage


Sample ID	Average depth of sequencing coverage
AB-3	38
AB-5	38
AB-7	31
AB-11	42
WT	48

Supplemental Table 3. HLA-B HiBit clones whole genome sequencing insert analysis

Sample ID	Insertion Location	Inserted Sequence	Note
AB-3	chr6:31322 263	GCTAATCTTCTTGAACAGCCGCCAGCCGCTCAC	Matches exact 33bp HiBit sequence
AB-5	chr6:31322 263	GCTAATCTTCTTGAACAGCCGCCAGCCGCTCAC	Matches exact 33bp HiBit sequence
AB-7	chr6:31322 261	TCCCCACCCACTCTAGACCCCAAGAATCTCACCT TTTCAGCTAATCTTCTTGAACAGCCGCCAGCCGC TCACAGCTGTGAGAGACACATCAGAGCCCTGGG CACTGTCGCTGCCTGGAGTAGAACAAAACA	HiBit sequence with duplicate neighboring sequence segments
AB-11	chr6:31322 261	TCAGCTAATCTTCTTGAACAGCCGCCAGCCGCTC ACAGCTGTGAGAGACACATCAGAGCCCTGGGCA CTGTCG	HiBit sequence with duplicate neighboring sequence segments

Red sequence corresponds to HiBit sequence



The HiBit insertion (shown as ) is on the same reads as the C allele.
Conclusion: HiBit is inserted into the B*08:01:01:01 allele.

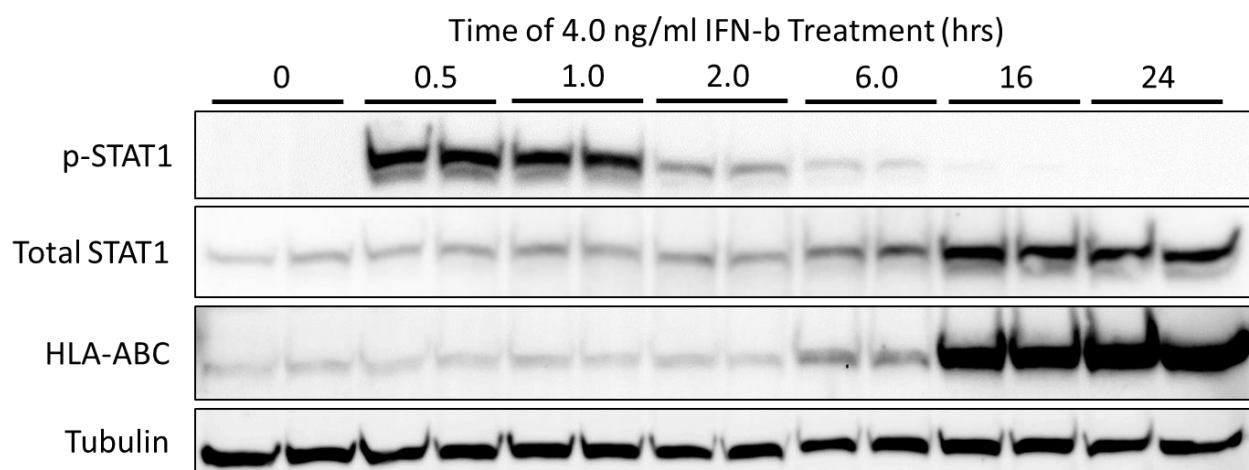
At 6:31322303, All 5 samples are het with C/G alleles
C is from B*08:01:01:01
G is from B*18:01:01:01

Supplementary Figure 4. Phasing analysis of whole genome sequencing for HLA-B HiBit clones showed insertion at the desired HLA-B*08:01:01:01 but not at the HLA-B*18:01:01:01 allele for all four clones AB-3, -5, -7, and -11.

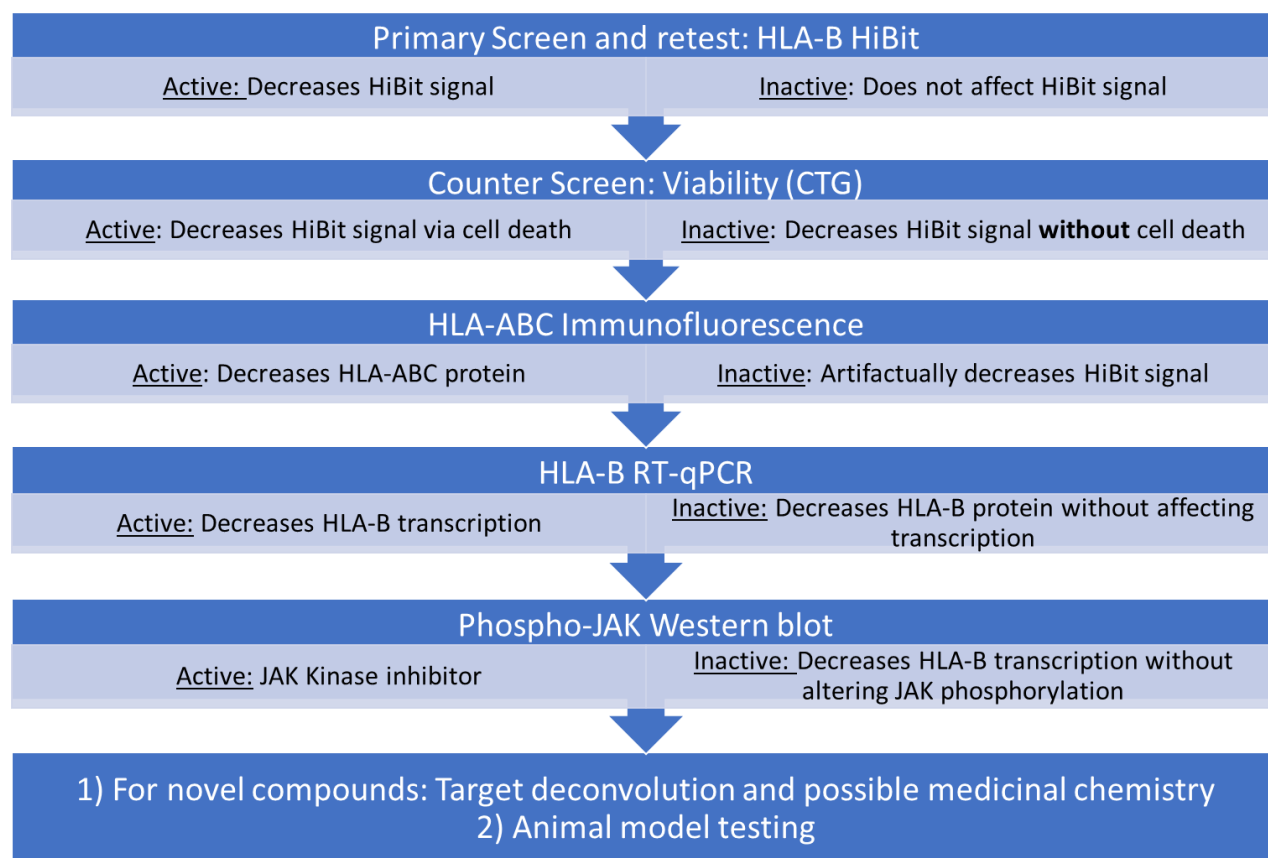
Supplementary Table 4. Primary libraries screened

Library	Compounds	Titirations	Dilution Factor	Z'-factor
LOPAC	1,280	7	1:4.3	0.89
NPC	2,816	8	1:5	0.82
MIPE5.0	2,480	11	1:3	0.80
Sum	6,576			
Unique	4,679			

LOPAC - Library of pharmacologically active compounds, NPC - NCATS pharmaceutical collection, MIPE5.0 - Mechanism interrogation plate version 5.0



Supplementary Figure 5. Western blot analysis of phospho-STAT1 (p-STAT1), total STAT1, HLA-ABC, and tubulin loading control for C25cl48 cells treated with 4.0 ng/ml IFN- β for 0.5, 1.0, 2.0, 6.0, 16 and 24 hrs. Stimulation with IFN- β caused phosphorylation of STAT1 by 0.5 hrs of treatment, began to reduce at 2.0 hrs, and was back to baseline by 24 hrs. Whereas stimulation with IFN- β caused total STAT1 and HLA-ABC to be up-regulated starting at 6.0 hrs and greatly increasing by 16 hrs. Duplicate adjacent lanes represent duplicate treated wells from 6 well plates.



Supplementary Figure 6. Flow chart showing the series of follow-up assays for screening compounds and inferences drawn from results at each stage. This strategy will reduce assay artifacts, ensure biological pharmacology of active compounds, and help elucidate mechanisms of action. Compounds identified in primary screening are retested by HLA-B HiBit assay as well as the cytotoxicity CTG assay to eliminate compounds that are cytotoxic. Minimally toxic actives are then assayed by IF of endogenous HLA-ABC protein in unedited C25cl48 cells to eliminate compounds that artifactually decrease HiBit signal. Next, actives are assayed by RT-qPCR, where inactivity at this stage represents compounds that decrease HLA-B protein without affecting transcription, and activity at this stage indicate inhibitors of HLA-B transcription. Testing for JAK phosphorylation shows compounds that inhibit transcription via inhibition of JAK activation or some other mechanism such as epigenetic regulation. Compounds with unknown targets or mechanisms need to be deconvoluted and medicinal chemistry can optimize leads. Finally, leads need to be tested in animal models to show safety and efficacy before clinical trials.

Supplementary Table 5. JAK inhibitors

Sample Name	Activity				Cytotoxicity				Approval	Targets/Mechanism
	Data Type	IC50 (nM)	CC	Efficacy	Data Type	IC50 (nM)	CC	Efficacy		
AZD-1480	HitBit	1,400	-1a	-100	CTG	7,000	-2b	-65		Jak1/2 Inhibitor
	HLA-ABC	650	-1a	-100	Cell count	29,000	-2b	-75		
	HLAB	4,100	-2b	-77						
Baricitinib	HitBit	1,600	-1a	-100	CTG		4	0	FDA	Jak1/2 Inhibitor
	HLA-ABC	1,100	-1a	-100	Cell count		4	0		
	HLAB	1,500	-1a	-100						
CEP-33779	HitBit	5,200	-2b	-100						Jak1/2/3 Inhibitor
	HLA-ABC	1,800	-1a	-100	Cell count		4	0		
	HLAB	8,200	-2a	-146						
Cerdulatinib	HitBit	2,000	-1a	-100	CTG		4	0		TYK2 Inhibitor
	HLA-ABC	1,300	-1a	-100	Cell count	16,000	-2b	-56		
	HLAB	5,800	-2a	-105						
Filgotinib	HitBit	17,000	-2b	-77						Jak1/2 Inhibitor
	HLA-ABC	1800	-1a	-100	Cell count		4	-23		
	HLAB	18,000	-2a	-141						
Itacitinib	HitBit	1,600	-1a	-100	CTG		4	0		Jak1 Inhibitor
	HLA-ABC	1,300	-1a	-128	Cell count		4	0		
	HLAB	3,400	-2a	-118						
Lestaurtinib	HitBit	700	-1a	-100	CTG		4	-16		Jak/Tyk/Flt Inhibitor
	HLA-ABC	650	-1a	-128	Cell count		4	0		
	HLAB	2,600	-2a	-118						
Mometinib	HitBit	4,400	-1a	-100	CTG		4	0		Jak1/2 Inhibitor
	HLA-ABC	1,600	-1a	-100	Cell count	46,000	-3	-57		
	HLAB	23,000	-2a	-168						
Peficitinib	HitBit	2,200	-1a	-100	CTG		4	0	PMDA	Jak3
	HLA-ABC	460	-1a	-100	Cell count		4	0		
	HLAB	1,600	-1a	-103						
Pyridone 6	HitBit	2,500	-1a	-100	CTG		4	0		Jak1/2/3 Inhibitor
	HLA-ABC	510	-1a	-100	Cell count		4	-17		
	HLAB	1,600	-1b	-65						
RO495	HitBit	2,800	-1a	-100	CTG		4	0		Tyk 2 Inhibitor
	HLA-ABC	2,000	-1a	-100	Cell count		4	0		
	HLAB	4,600	-2a	-110						
Ruxolitinib	HitBit	1,100	-1a	-100	CTG		4	0	FDA	Jak1/2 Inhibitor
	HLA-ABC	880	-1a	-100	Cell count		4	0		
	HLAB	1,600	-1a	-115						
Tofacitinib	HitBit	3,100	-1a	-100	CTG		4	0	FDA	Jak1/2/3 Inhibitor
	HLA-ABC	3,300	-1a	-100	Cell count		4	0		
	HLAB	2,900	-1a	-83						

CC - curve class, IC50 - half max inhibitory concentration

Supplementary Table 6. Other kinase inhibitors

Sample Name	Activity				Cytotoxicity				Approval	Targets/Mechanism
	Data Type	IC50 (nM)	CC	Efficacy	Data Type	IC50 (nM)	CC	Efficacy		
A-443654	HitBit	1,100	-1a	-100	CTG	4,400	-2b	-37		AKT Inhibitor
	HLA-ABC	230	-1a	-83	Cell count	26,000	-2a	-100		
	HLAB	210	-1a	-109						
APY29	HitBit	1,400	-1a	-100	CTG	2,000	1b	25		IRE1 Inhibitor
	HLA-ABC	650	-1a	-100	Cell count	23,000	-2b	-49		
	HLAB	650	-1a	-121						
CGP-60474	HitBit	130	-1a	-100	CTG		4	0		CDK1/2, SMG1, mTOR Inhibitor
	HLA-ABC	81	-1a	-100	Cell count		4	0		
	HLAB	120	-1a	-121						
CUDC-101	HitBit	2,500	-1a	-100	CTG		4	0		EGFR, HDAC Inhibitor
	HLA-ABC	290	-1a	-100	Cell count		4	0		
	HLAB	410	-1b	-68						
Dinaciclib (SCH727965)	HitBit	20	-1a	-100	CTG		4	0		CDK1/2/5/9 Inhibitor
	HLA-ABC	17	-1a	-100	Cell count		4	0		
	HLAB	21	-1a	-117						
NCB-1026	HitBit	560	-1a	-100	CTG		4	0		TNIK Inhibitor, Wnt Signaling Inhibitor
	HLA-ABC	1,000	-1a	-128	Cell count		4	0		
	HLAB	650	-1a	-136						
OTS-167	HitBit	99	-1a	-100	CTG		4	-15		MELK Inhibitor
	HLA-ABC	46	-1a	-118	Cell count	15,000	-2a	-100		
	HLAB	120	-1a	-130						
OTS964	HitBit	79	-1a	-100	CTG		4	0		PDZ-binding kinase Inhibitor
	HLA-ABC	81	-1a	-100	Cell count		4	0		
	HLAB	52	-1a	-136						
PF-3758309	HitBit	63	-1a	-100	CTG		4	0		PAK1/4 Inhibitor
	HLA-ABC	58	-1a	-100	Cell count		4	0		
	HLAB	1,800	-1a	-135						
PIK-75	HitBit	50	-1a	-100	CTG		4	0		CDK9, PI3K Inhibitor
	HLA-ABC	160	-1a	-128	Cell count		4	0		
	HLAB	230	-1a	-136						
RGB-286147	HitBit	35	-1a	-100	CTG		4	26		CDK1/2/3/7/9 Inhibitor
	HLA-ABC	23	-1a	-100	Cell count		4	0		
	HLAB	37	-1a	-90						
SR-3306	HitBit	2,500	-1a	-100	CTG		4	0		JNK 1/2/3 Inhibitor
	HLA-ABC	1,800	-1a	-100	Cell count		4	0		
	HLAB	1,500	-1b	-64						

CC - curve class, IC50 - half max inhibitory concentration

Supplementary Table 7. Transcriptional and epigenetic modulators

Sample Name	Activity				Cytotoxicity				Approval	Targets/Mechanism
	Data Type	IC50 (nM)	CC	Efficacy	Data Type	IC50 (nM)	CC	Efficacy		
Aldoxorubicin hydrochlorid	HitBit	2,800	-1a	-100	CTG		4	0		DNA topoisomerase II inhibitor (pro-Doxorubicin)
	HLA-ABC	1,000	-1a	-100	Cell count		4	0		
	HLAB	4,600	-1a	-143						
CBL-0137	HitBit	1,100	-1a	-100	CTG		4	0		NF-kappaB Activation Inhibitor
	HLA-ABC	650	-1a	-100	Cell count		4	0		
	HLAB	1,800	-1a	-136						
CUDC-907	HitBit	28	-1a	-86	CTG	3,500	-1b	-40		PI3K/HDAC Inhibitor
	HLA-ABC	6	-1a	-100	Cell count		4	0		
	HLAB	23	-1b	-73						
Dacinostat	HitBit	200	-1a	-86	CTG		4	0		HDAC Inhibitor
	HLA-ABC	36	-1a	-100	Cell count		4	0		
	HLAB	120	-1b	-55						
Doxorubicin	HitBit	500	-1a	-100	CTG		4	-17	FDA	DNA Topoisomerase II Inhibitor
	HLA-ABC	230	-1a	-100	Cell count		4	0		
	HLAB	1,000	-1a	-156						
Echinomycin	HitBit	11	-1a	-100	CTG		4	0		HIF-1 seq specific DNA intercalater
	HLA-ABC	4	-1a	-120	Cell count		4	0		
	HLAB	3	-1a	-112						
Givinostat hydrochloride	HitBit	1,000	-1a	-82	CTG		4	-30		HDAC Inhibitor
	HLA-ABC	260	-1a	-100	Cell count	51,000	-3	-52		
	HLAB	4,100	-2a	-100						
Nemorubicin	HitBit	150	-1a	-100	CTG		4	0	EMA	DNA Topoisomerase II Inhibitor
	HLA-ABC	70	-1a	-107	Cell count		4	0		
	HLAB	92	-1a	-133						
Panobinostat	HitBit	63	-1a	-86	CTG		4	-100	FDA	HDAC Inhibitor
	HLA-ABC	28	-1a	-111	Cell count		4	0		
	HLAB	29	-1b	-68						
Parthenolide	HitBit	7,000	-1a	-100	CTG		4	0		Adiponectin receptor protein 2, HDAC, Microphthalmia-associated transcription factor inhibitor, NF-kappaB Activation Modulator
	HLA-ABC	7,300	-1a	-100	Cell count	12,000	-1a	-100		
	HLAB	16,000	-2a	-126						
Quisinostat	HitBit	160	-1a	-100	CTG	1,400	-1b	-30		HDAC Inhibitor
	HLA-ABC	9	-1a	-100	Cell count		4	0		
	HLAB	23	-1b	-56						
TPCA-1	HitBit	1,800	-1a	-100	CTG		4	-38		IKK beta Inhibitor
	HLA-ABC	1,400	-1a	-121	Cell count		4	0		
	HLAB	10,000	-2a	-117						
Triptonide	HitBit	47	-1a	-100	CTG		4	0		XPB transcription factor inhibitor, Immunosuppressant
	HLA-ABC	190	-1a	-100	Cell count		4	0		
	HLAB	500	-1a	-113						
Vorinostat (SAHA)	HitBit	3,500	-1a	-100	CTG		4	0	FDA	HDAC Inhibitor
	HLA-ABC	910	-1a	-100	Cell count		4	0		
	HLAB	1,800	-1b	-69						
Withaferin A	HitBit	880	-1a	-100	CTG	12,000	-2b	-100		Glu [NMDA] receptor, HSP90, PANC-1, NF-kappaB Activation Inhibitor
	HLA-ABC	730	-1a	-100	Cell count	2,000	-1a	-100		
	HLAB	1,300	-1a	-85						

CC - curve class, IC50 - half max inhibitory concentration

Supplementary Table 8. Other actives

Sample Name	Activity				Cytotoxicity				Approval	Targets/Mechanism
	Data Type	IC50 (nM)	CC	Efficacy	Data Type	IC50 (nM)	CC	Efficacy		
BMS-214662	HitBit	1,300	-1a	-100	CTG		4	-24		Farnesyltransferase (geranylgeranyl pyrophosphate synthase) Inhibitor
	HLA-ABC	580	-1a	-100	Cell count	20,000	2b	45		
	HLAB	650	-1b	-74						
Bruceantin	HitBit	88	-1a	-100	CTG		4	0		MYC Expression Inhibitor, Apoptosis Inducers
	HLA-ABC	36	-1a	-122	Cell count		4	0		
	HLAB	370	-1b	-52						
Fluocinonide	HitBit	4	-1b	-25	CTG		4	0	FDA	Glucocorticoid steroid
	HLA-ABC	13	-1b	-28	Cell count		4	0		
	HLAB	15	-1b	-18						
Methylprednisolone	HitBit	14	-1b	-23	CTG		4	0	FDA	Glucocorticoid steroid
	HLA-ABC	10	-1b	-25	Cell count		4	0		
	HLAB	24	-1b	-20						
Prednisolone	HitBit	35	-1b	-20	CTG		4	0	FDA	Glucocorticoid steroid
	HLAB	29	-1b	-14						
Selinexor	HitBit	630	-1b	-63	CTG		4	0	FDA	Exportin-1 Antagonist
	HLA-ABC	320	-1a	-100	Cell count		4	0		
	HLAB	26,000	-2b	-60						
WS-3	HitBit	440	-1a	-100	CTG		4	0		beta Cell Proliferation Inducer
	HLA-ABC	65	-1a	-100	Cell count	16,000	-2b	-100		
	HLAB	146	-1a	-104						

CC - curve class, IC50 - half max inhibitory concentration

Supplementary Table 9. Selective activity for HiBit and HLA-ABC protein

Sample Name	Activity				Cytotoxicity				Approval	Targets/Mechanism
	Data Type	IC50 (nM)	CC	Efficacy	Data Type	IC50 (nM)	CC	Efficacy		
ARV-771	HitBit	390	-1a	-100	CTG		4	-15		BRD4 inhibitor
	HLA-ABC	150	-1a	-100	Cell count	29,000	-3	-144		
	HLAB	160	-1b	-72						
ARV-825	HitBit	97	-1b	-71	CTG		4	-20		BRD4 inhibitor
	HLA-ABC	91	-1a	-82	Cell count		4	0		
	HLAB	65	-1b	-43						
AZD-5153 6-Hydroxy-2-naphthoic acid	HitBit	130	-1b	-62	CTG		4	0		BRD4 inhibitor
	HLA-ABC	14	-1b	-63	Cell count		4	0		
	HLAB	230	-2b	-69						
Bortezomib	HitBit	31	-1a	-81	CTG	31	-1b	-37	FDA	Proteasome Inhibitor
	HLA-ABC	26	-1b	-78	Cell count		4	0		
	HLAB	820	-2b	-68						
Carfilzomib	HitBit	88	-1a	-100	CTG	50	-1b	-38	FDA	Proteasome Inhibitor
	HLA-ABC	65	-1a	-120	Cell count	46,000	-3	-52		
	HLAB	1,800	-2b	-99						
CC-885	HitBit	8	-1a	-100	CTG		4	-12		Cereblon (CRBN) Modulator
	HLA-ABC	8	-1a	-114	Cell count		4	0		
	HLAB		4	0						
CPI-203	HitBit	560	-1b	-58	CTG		4	0		BRD4 inhibitor
	HLA-ABC	160	-1b	-60	Cell count		4	0		
	HLAB	13	-1b	-47						
Ixazomib citrate	HitBit	310	-1b	-74	CTG		4	-29		Proteasome Inhibitor
	HLA-ABC	810	-1a	-100	Cell count		4	0		
	HLAB	6,500	-2b	-39						
OTX-015	HitBit	880	-1b	-63	CTG		4	0		BRD2/4 inhibitor
	HLA-ABC	230	-1b	-71	Cell count		4	0		
	HLAB	37,000	-2b	-40						
Ouabain	HitBit	56	-1a	-83	CTG	50	-1b	-49	PMDA	Na ⁺ /K ⁺ -ATPase Inhibitors
	HLA-ABC	65	-1a	-81	Cell count		4	0		
	HLAB		4	-34						
Proscillaridin	HitBit	12	-1a	-81	CTG	20	-1b	-51	PMDA	Na ⁺ /K ⁺ -ATPase Inhibitors
	HLA-ABC	36	-1a	-100	Cell count		4	0		
	HLAB		4	10						
SNX-2112	HitBit	70	-1b	-66	CTG	560	-1b	-40		HSP90 Inhibitor
	HLA-ABC	32	-1b	-73	Cell count		4	0		
	HLAB	23	-1b	-43						
Tanespimycin	HitBit	70	-1b	-68	CTG	560	-1b	-40		HSP90 Inhibitor
	HLA-ABC	13	-1b	-70	Cell count		4	0		
	HLAB		4	0						
TAS-116	HitBit	630	-1b	-63	CTG	7,000	-1b	-51		HSP90 Inhibitor
	HLA-ABC	260	-1a	-83	Cell count		4	0		
	HLAB	370	-1b	-30						
Thapsigargin	HitBit	8	-1a	-100	CTG	4	-1b	-37		SERCA inhibitor
	HLA-ABC	81	-1b	-76	Cell count	14	-3	-22		
	HLAB	29,000	-3	-128						

CC - curve class, IC50 - half max inhibitory concentration

Supplementary Table 10. Primary HLA-B HiBit screening activities of small molecule myositis therapeutics

Drug Name	Activity	IC50 (uM)	Efficacy
Ruxolitinib	Active	1.1	100%
Baricitinib	Active	1.6	100%
Tofacitinib	Active	3.1	100%
Methylprednisolone	low efficacy active	0.014	24%
Prednisolone	low efficacy active	0.035	20%
Methotrexate	Inactive		
Azathioprine	Inactive		
Mycophenolate mofetil	Inactive		
Mycophenolic acid	Inactive		
Cyclophosphamide	Inactive		
Cyclosporine A	Inactive		
Tacrolimus (FK-506)	Inactive		
Sirolimus (rapamycin)	Inactive		
Chloroquine	Inactive		
Hydroxychloroquine sulfate	Inactive		
Quinacrine	Inactive		
Lenalidomide (revlimid)	Inactive		
Chlorambucil	Inactive		
Pamidronic acid (Pamidronate)	Inactive		
(+)-Diltiazem	Inactive		
Diltiazem hydrochloride	Inactive		
Thalidomide	Inactive		
Auranofin	Inactive		
colchicine	Inactive		
Dapsone	Inactive		
Fingolimod hydrochloride	Inactive		