[0249] IKZF1-mNeonGreen-P2A-mScarlet coding sequence (SEQ ID NO: 1):

ATGGATGCTGATGAGGGTCAAGACATGTCCCAAGTTTCAGGGAAGGAAAGC
CCCCCTGTAAGCGATACTCCAGATGAGGGCGATGAGCCCATGCCGATCCCCG
AGGACCTCTCCACCACCTCGGGAGGACAGCAAAGCTCCAAGAGTGACAGAG
TCGTGGCCAGTAATGTTAAAGTAGAGACTCAGAGTGATGAAGAGAATGGGC
GTGCCTGTGAAATGAATGGGGAAGAATGTGCGGAGGATTTACGAATGCTTG
ATGCCTCGGGAGAGAAAATGAATGGCTCCCACAGGGACCAAGGCAGCTCGG
CTTTGTCGGGAGTTGGAGGCATTCGACTTCCTAACGGAAAACTAAAGTGTGA
TATCTGTGGGATCATTTGCATCGGGCCCAATGTGCTCATGGTTCACAAAAGA
AGCCACACTGGAGAACGGCCCTTCCAGTGCAATCAGTGCGGGGCCTCATTCA
CCCAGAAGGGCAACCTGCTCCGGCACATCAAGCTGCATTCCGGGGAGAAGC
CCTTCAAATGCCACCTCTGCAACTACGCCTGCCGCCGGAGGGACGCCCTCAC
TGGCCACCTGAGGACGCACTCCGTTGGTAAACCTCACAAATGTGGATATTGT
GGCCGAAGCTATAAACAGCGAAGCTCTTTAGAGGAACATAAAGAGCGCTGC
CACAACTACTTGGAAAGCATGGGCCTTCCGGGCACACTGTACCCAGTCATTA
AAGAAGAAACTAATCACAGTGAAATGGCAGAAGACCTGTGCAAGATAGGAT
CAGAGAGATCTCTCGTGCTGGACAGACTAGCAAGTAACGTCGCCAAACGTA
AGAGCTCTATGCCTCAGAAATTTCTTGGGGACAAGGGCCTGTCCGACACGCC
CTACGACAGCAGCGCCAGCTACGAGAAGGAGAACGAAATGATGAAGTCCCA
CGTGATGGACCAAGCCATCAACAACGCCATCAACTACCTGGGGGCCGAGTCC
CTGCGCCCGCTGGTGCAGACGCCCCCGGGCGGTTCCGAGGTGGTCCCGGTCA
TCAGCCCGATGTACCAGCTGCACAAGCCGCTCGCGGAGGGCACCCCGCGCTC
CAACCACTCGGCCCAGGACAGCGCCGTGGAGAACCTGCTGCTGCTCTCCAAG
GCCAAGTTGGTGCCCTCGGAGCGCGAGGCGTCCCCGAGCAACAGCTGCCAA
GACTCCACGGACACCGAGAGCAACAACGAGGAGCAGCGCAGCGGTCTCATC
TACCTGACCAACCACATCGCCCCGCACGCGCGCAACGGGCTGTCGCTCAAGG
AGGAGCACCGCGCCTACGACCTGCTGCGCGCCGCCTCCGAGAACTCGCAGG
ACGCGCTCCGCGTGGTCAGCACCAGCGGGGAGCAGATGAAGGTGTACAAGT
GCGAACACTGCCGGGTGCTCTTCCTGGATCACGTCATGTACACCATCCACAT
GGGCTGCCACGGCTTCCGTGATCCTTTTGAGTGCAACATGTGCGGCTACCAC
AGCCAGGACCGGTACGAGTTCTCGTCGCACATAACGCGAGGGGAGCACCGC
TTCCACATGAGCGGTGGAGGCGGTTCAGGTGGCGGAGGAAGCGGGGGAGGT
GGAAGTATGGTGTCTAAAGGAGAAGAAGATAATATGGCTTCTCTGCCTGCTA
CACACGAACTGCATATTTTCGGATCTATTAATGGCGTGGATTTCGATATGGTG
GGACAGGGAACAGGAAACCCTAACGATGGATACGAAGAACTGAATCTGAAG
TCTACCAAAGGAGATCTGCAATTCTCTCCTTGGATCCTGGTCCCTCACATTGG
ATATGGATTCCATCAATATCTGCCTTACCCTGACGGAATGTCTCCTTTCCAAG

CTGCTATGGTCGATGGCTCTGGATATCAGGTCCACAGAACAATGCAATTTGA
AGATGGCGCCTCTCTGACAGTGAATTACAGATATACATACGAAGGCTCTCAT
ATTAAAGGCGAAGCCCAAGTGAAAGGCACAGGCTTTCCTGCCGACGGCCCT
GTGATGACCAACTCTCTGACCGCTGCCGATTGGTGCAGATCTAAGAAGACCT
ACCCTAATGATAAAACCATTATCTCTACCTTCAAATGGTCTTACACAACAGG
CAATGGCAAGAGATACAGATCTACCGCCAGAACCACCTATACCTTTGCTAAG
CCTATGGCTGCCAATTACCTGAAAAATCAGCCTATGTATGTGTTCAGAAAAA
CCGAACTGAAACACTCTAAAACCGAACTGAATTTCAAAGAATGGCAAAAGG
CTTTCACAGATGTGATGGGAATGGATGAACTGTACAAGGGAAGCGGAGCTA
CAAATTTTAGTCTTCTTAAACAAGCCGGTGACGTGGAGGAAAATCCCGGCCC
AATGGTGAGCAAGGGCGAGGCCGTGATCAAGGAGTTCATGCGCTTCAAGGT
GCACATGGAGGGCAGCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAA
GGGCGGCCCCCTGCCCTTCTCTTGGGACATCCTGAGCCCCCAGTTCATGTACG
GCAGCCGCGCCTTCACCAAGCACCCCGCCGACATCCCCGACTACTACAAGCA
GAGCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGG
CGGCGCCGTGACCGTGACCCAGGACACCAGCCTGGAGGACGGCACCCTGAT
CTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCCCCGACGGCCCCGTGATG
CAGAAGAAGACCATGGGCTGGGAGGCCAGCACCGAGCGCCTGTACCCCGAG
GACGGCGTGCTGAAGGGCGACATCAAGATGGCCCTGCGCCTGAAGGACGGC
GGCAGATACCTGGCCGACTTCAAGACCACCTACAAGGCCAAGAAGCCCGTG
CAGATGCCCGGCGCCTACAACGTGGACCGCAAGCTGGACATCACCAGCCAC
AACGAGGACTACACCGTGGTGGAGCAGTACGAGCGCAGCGAGGGCCGCCAC
AGCACCGGCGGCATGGACGAGCTGTACAAGTGA

[0250] IKZF2-mNeonGreen-P2A-mScarlet coding sequence (SEQ ID NO: 2):

ATGGAAACAGAGGCTATTGATGGCTATATAACGTGTGACAATGAGCTTTCAC
CCGAAAGGGAGCACTCCAATATGGCAATTGACCTCACCTCAAGCACACCCAA
TGGACAGCATGCCTCACCAAGTCACATGACAAGCACAAATTCAGTAAAGCTA
GAAATGCAGAGTGATGAAGAGTGTGACAGGAAACCCCTGAGCCGTGAAGAT
GAGATCAGGGGCCATGATGAGGGTAGCAGCCTAGAAGAACCCCTAATTGAG
AGCAGCGAGGTGGCTGACAACAGGAAAGTCCAGGAGCTTCAAGGCGAGGGA
GGAATCCGGCTTCCGAATGGTAAACTGAAATGTGACGTCTGTGGCATGGTTT
GCATTGGGCCCAATGTGCTTATGGTACATAAAAGGAGTCACACTGGTGAACG
CCCCTTCCACTGTAACCAGTGTGGAGCTTCTTTTACTCAGAAGGGCAACCTTC
TGAGACACATAAAGTTACACTCTGGAGAGAAGCCGTTCAAATGTCCTTTCTG
TAGCTACGCCTGTAGAAGAAGGGACGCCCTCACAGGACACCTCAGGACCCA

TTCTGTGGGTAAACCTCACAAGTGCAACTACTGTGGACGAAGCTACAAGCAG
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AAGATTGTAAGGAACAAGAGCCTATTATGGACAACAATATTTCTCTGGTGCC
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GGAGCTGAGGCCCTTCACCCTCTGATGCAGCACCCGCCAAGCACAATCGCTG
AAGTGGCCCCAGTTATAAGCTCAGCTTATTCTCAGGTCTATCATCCAAATAG
GATAGAAAGACCCATTAGCAGGGAAACTGCTGATAGTCATGAAAACAACAT
GGATGGCCCCATCTCTCTCATCAGACCAAAGAGTCGACCCCAGGAAAGAGA
GGCCTCTCCCAGCAATAGCTGCCTGGATTCCACTGACTCAGAAAGCAGCCAT
GATGACCACCAGTCCTACCAAGGACACCCTGCCTTAAATCCCAAGAGGAAAC
AAAGCCCAGCTTACATGAAGGAGGATGTCAAAGCTTTGGATACTACCAAGG
CTCCTAAGGGCTCTCTGAAGGACATCTACAAGGTCTTCAATGGAGAAGGAGA
ACAGATTAGGGCCTTCAAGTGTGAGCACTGCCGAGTCCTTTTCCTAGACCAT
GTCATGTACACCATTCACATGGGTTGCCATGGCTACCGGGACCCACTGGAAT
GCAACATCTGTGGCTACAGAAGCCAGGACCGTTATGAGTTTTCATCACACAT
TGTTCGAGGGGAGCACACATTCCACCTCGACGGTGGAGGCGGTTCAGGTGGC
GGAGGAAGCGGGGGAGGTGGAAGTATGGTGTCTAAAGGAGAAGAAGATAA
TATGGCTTCTCTGCCTGCTACACACGAACTGCATATTTTCGGATCTATTAATG
GCGTGGATTTCGATATGGTGGGACAGGGAACAGGAAACCCTAACGATGGAT
ACGAAGAACTGAATCTGAAGTCTACCAAAGGAGATCTGCAATTCTCTCCTTG
GATCCTGGTCCCTCACATTGGATATGGATTCCATCAATATCTGCCTTACCCTG
ACGGAATGTCTCCTTTCCAAGCTGCTATGGTCGATGGCTCTGGATATCAGGTC
CACAGAACAATGCAATTTGAAGATGGCGCCTCTCTGACAGTGAATTACAGAT
ATACATACGAAGGCTCTCATATTAAAGGCGAAGCCCAAGTGAAAGGCACAG
GCTTTCCTGCCGACGGCCCTGTGATGACCAACTCTCTGACCGCTGCCGATTGG
TGCAGATCTAAGAAGACCTACCCTAATGATAAAACCATTATCTCTACCTTCA
AATGGTCTTACACAACAGGCAATGGCAAGAGATACAGATCTACCGCCAGAA
CCACCTATACCTTTGCTAAGCCTATGGCTGCCAATTACCTGAAAAATCAGCCT
ATGTATGTGTTCAGAAAAACCGAACTGAAACACTCTAAAACCGAACTGAATT
TCAAAGAATGGCAAAAGGCTTTCACAGATGTGATGGGAATGGATGAACTGT
ACAAGGGAAGCGGAGCTACAAATTTTAGTCTTCTTAAACAAGCCGGTGACGT
GGAGGAAAATCCCGGCCCAATGGTGAGCAAGGGCGAGGCCGTGATCAAGGA
GTTCATGCGCTTCAAGGTGCACATGGAGGGCAGCATGAACGGCCACGAGTTC
GAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCC

AAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCTCTTGGGACATCCTGA
GCCCCCAGTTCATGTACGGCAGCCGCGCCTTCACCAAGCACCCCGCCGACAT
CCCCGACTACTACAAGCAGAGCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTG
ATGAACTTCGAGGACGGCGGCGCCGTGACCGTGACCCAGGACACCAGCCTG
GAGGACGGCACCCTGATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCC
CCGACGGCCCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCAGCACCG
AGCGCCTGTACCCCGAGGACGGCGTGCTGAAGGGCGACATCAAGATGGCCC
TGCGCCTGAAGGACGGCGGCAGATACCTGGCCGACTTCAAGACCACCTACA
AGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTGGACCGCAAGC
TGGACATCACCAGCCACAACGAGGACTACACCGTGGTGGAGCAGTACGAGC
GCAGCGAGGGCCGCCACAGCACCGGCGGCATGGACGAGCTGTACAAGTGA

[0251] IKZF3-mNeonGreen-P2A-mScarlet coding sequence (SEQ ID NO: 3):

ATGGAAGATATACAAACAAATGCGGAACTGAAAAGCACTCAGGAGCAGTCT
GTGCCCGCAGAAAGTGCAGCGGTTTTGAATGACTACAGTTTAACCAAATCTC
ATGAAATGGAAAATGTGGACAGTGGAGAAGGCCCAGCCAATGAAGATGAAG
ACATAGGAGATGATTCAATGAAAGTGAAAGATGAATACAGTGAAAGAGATG
AGAATGTTTTAAAGTCAGAACCCATGGGAAATGCAGAAGAGCCTGAAATCC
CTTACAGCTATTCAAGAGAATATAATGAATATGAAAACATTAAGTTGGAGAG
ACATGTTGTCTCATTCGATAGTAGCAGGCCAACCAGTGGAAAGATGAACTGC
GATGTGTGTGGATTATCCTGCATCAGCTTCAATGTCTTAATGGTTCATAAGCG
AAGCCATACTGGTGAACGCCCATTCCAGTGTAATCAGTGTGGGGCATCTTTT
ACTCAGAAAGGTAACCTCCTCCGCCACATTAAACTGCACACAGGGGAAAAA
CCTTTTAAGTGTCACCTCTGCAACTATGCATGCCAAAGAAGAGATGCGCTCA
CGGGGCATCTTAGGACACATTCTGTGGAGAAACCCTACAAATGTGAGTTTTG
TGGAAGGAGTTACAAGCAGAGAAGTTCCCTTGAGGAGCACAAGGAGCGCTG
CCGTACATTTCTTCAGAGCACTGACCCAGGGGACACTGCAAGTGCGGAGGCA
AGACACATCAAAGCAGAGATGGGAAGTGAAAGAGCTCTCGTACTGGACAGA
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GTGAGAAGCGCCACTGCTTTGATGTCAACTATAATTCAAGTTACATGTATGA
GAAAGAGAGTGAGCTCATACAGACCCGCATGATGGACCAAGCCATCAATAA
CGCCATCAGCTATCTTGGCGCCGAAGCCCTGCGCCCCTTGGTCCAGACACCG
CCTGCTCCCACCTCGGAGATGGTTCCAGTTATCAGCAGCATGTATCCCATAG
CCCTCACCCGGGCTGAGATGTCAAACGGTGCCCCTCAAGAGCTGGAAAAGA
AAAGCATCCACCTTCCAGAGAAGAGCGTGCCTTCTGAGAGAGGCCTCTCTCC
CAACAATAGTGGCCACGACTCCACGGACACTGACAGCAACCATGAAGAACG
CCAGAATCACATCTATCAGCAAAATCACATGGTCCTGTCTCGGGCCCGCAAT

GGGATGCCACTTCTGAAGGAGGTTCCCCGCTCTTACGAACTCCTCAAGCCCC
CGCCCATCTGCCCAAGAGACTCCGTCAAAGTGATCAACAAGGAAGGGGAGG
TGATGGATGTGTATCGGTGTGACCACTGCCGCGTCCTCTTCCTGGACTATGTG
ATGTTCACGATTCACATGGGCTGCCACGGCTTCCGTGACCCTTTCGAGTGTAA
CATGTGTGGATATCGAAGCCATGATCGGTATGAGTTCTCGTCTCACATAGCC
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GGCGGAGGAAGCGGGGGAGGTGGAAGTATGGTGTCTAAAGGAGAAGAAGA
TAATATGGCTTCTCTGCCTGCTACACACGAACTGCATATTTTCGGATCTATTA
ATGGCGTGGATTTCGATATGGTGGGACAGGGAACAGGAAACCCTAACGATG
GATACGAAGAACTGAATCTGAAGTCTACCAAAGGAGATCTGCAATTCTCTCC
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CTGACGGAATGTCTCCTTTCCAAGCTGCTATGGTCGATGGCTCTGGATATCAG
GTCCACAGAACAATGCAATTTGAAGATGGCGCCTCTCTGACAGTGAATTACA
GATATACATACGAAGGCTCTCATATTAAAGGCGAAGCCCAAGTGAAAGGCA
CAGGCTTTCCTGCCGACGGCCCTGTGATGACCAACTCTCTGACCGCTGCCGA
TTGGTGCAGATCTAAGAAGACCTACCCTAATGATAAAACCATTATCTCTACC
TTCAAATGGTCTTACACAACAGGCAATGGCAAGAGATACAGATCTACCGCCA
GAACCACCTATACCTTTGCTAAGCCTATGGCTGCCAATTACCTGAAAAATCA
GCCTATGTATGTGTTCAGAAAAACCGAACTGAAACACTCTAAAACCGAACTG
AATTTCAAAGAATGGCAAAAGGCTTTCACAGATGTGATGGGAATGGATGAA
CTGTACAAGGGAAGCGGAGCTACAAATTTTAGTCTTCTTAAACAAGCCGGTG
ACGTGGAGGAAAATCCCGGCCCAATGGTGAGCAAGGGCGAGGCCGTGATCA
AGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCAGCATGAACGGCCACG
AGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGA
CCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCTCTTGGGACAT
CCTGAGCCCCCAGTTCATGTACGGCAGCCGCGCCTTCACCAAGCACCCCGCC
GACATCCCCGACTACTACAAGCAGAGCTTCCCCGAGGGCTTCAAGTGGGAGC
GCGTGATGAACTTCGAGGACGGCGGCGCCGTGACCGTGACCCAGGACACCA
GCCTGGAGGACGGCACCCTGATCTACAAGGTGAAGCTGCGCGGCACCAACTT
CCCCCCCGACGGCCCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCAG
CACCGAGCGCCTGTACCCCGAGGACGGCGTGCTGAAGGGCGACATCAAGAT
GGCCCTGCGCCTGAAGGACGGCGGCAGATACCTGGCCGACTTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTGGACCG
CAAGCTGGACATCACCAGCCACAACGAGGACTACACCGTGGTGGAGCAGTA
CGAGCGCAGCGAGGGCCGCCACAGCACCGGCGGCATGGACGAGCTGTACAA
GTGA

[0252] IKZF1, IKZF2, and IKZF3 constructs were cloned into the UCOE Hygromycin expression vectors (Millipore Sigma). Reporter constructs were transfected using cationic lipid reagents into adherent HEK 293T cells and stable integrants were selected by treatment with 200 μg/mL hygromycin B. Clonal populations were obtained from the population of stable integrants either by limiting dilution or fluorescence activated cell sorting.

[0253] The clonal stable cell lines were maintained under constant 200 μg/mL hygromycin B selection while being passaged for use in the degradation assays. Flow analysis on a BD Accuri C6 showed the HEK 293T CMV-IKZF1 Clone 7 cell line to have an average Fluorescein isothiocyanate mean fluorescence intensity (FITC MFI) of 230,000 and phycoerythrin mean fluorescence intensity (PE MFI) of 33,000. HEK 293T EF1a-IKZF2 Clone 9 had an average FITC MFI of 150,000 and PE MFI of 26,000. HEK 293T EF1a-IKZF3 Clone 9 had an average FITC MFI of 400,000 and PE MFI of 60,000. The fluorescence intensity of the IKZF1/2/3-mNeonGreen (FITC channel) and mScarlet (PE channel) reporters were routinely analyzed by flow cytometry to confirm consistent expression levels between experiments.

IKZF1/2/3 Reporter Degradation Assay

[0254] The IKZF1/IKZF2/IKZF3 degradation assays were carried out by harvesting the HEK 293T reporter cell lines and resuspending the cells in media formulated for reduced background fluorescence (FluoroBrite; Thermo Fisher). The respective cell lines were seeded at a density of 4000 cells/well into black-walled 384-well optical grade assay tissue culture plates. The cells were incubated overnight at 37 °C to allow for attachment to the assay plate. Dilutions of the compounds were prepared in DMSO from 10 mM compound stocks. The assay plates were treated with appropriate concentrations of the compounds by dispensing the DMSO dilutions in quadruplicate wells with an upper limit of 0.5% final DMSO.

[0255] After 24 hour incubation with the compounds, the assay plates were imaged on an ImageXpress Pico microscopy system (cells maintained at 37 °C during imaging) to obtain the fluorescent readouts. The assay plates were imaged in the FITC and Tetramethylrhodamine (TRITC) channels to obtain the mNeonGreen fluorescence intensity (reporter degradation data) and mScarlet fluorescence intensity (for cell segmentation). 293T-IKZF1 and 293T-IKZF3 reporter cell lines were imaged with exposures of 500 milliseconds (ms) for both FITC and TRITC channels, while the 293T-IKZF2 reporter cell line was imaged with exposures of 1000 ms for FITC and 1250 ms for TRITC. The resulting data was analyzed with Cell Reporter Xpress software using the 2-channel cell scoring analysis with a “percent positive” readout. The TRITC channel was selected for the “nuclei” segmentation with a threshold of 20 while the FITC channel was selected for the “Marker 1” segmentation and a threshold of 100 for the IKZF1 and IKZF3 reporter lines. The IKZF2 reporter line had a threshold of 120 set for the FITC channel, and 20 for TRITC. The minimum segmentation width was set to 6 micrometers and the maximum segmentation width was set to 15 micrometers for all cell lines. The DC_{50} calculations were determined by regression to best fit four-parameter logistic curves using GraphPad Prism.

[0256] Table 3 shows results from the assays described above.

TABLE 3

#	IKZF1 DC_{50} (μM)	IKZF2 DC_{50} (μM)	IKZF3 DC_{50} (μM)	IKZF2 % Degradation @ 1 μM
1	>30.0	0.024	>30.0	79.9
10	>30.0	0.006	>30.0	78.7
11	>30.0	>30.0	>30.0	-
13	>30.0	>30.0	>30.0	-
14	>30.0	0.292	>30.0	33.5
15	>30.0	0.004	>30.0	78.9
19	>30.0	0.004	>30.0	84.5
23	>30.0	0.005	>30.0	79.2
27	>30.0	0.005	>30.0	82.1
31	>30.0	0.0005	>30.0	83.3

[0257] Table 4 shows further results from the assays described above.

TABLE 4

#	CRBN Binding EC_{50} (μM)	IKZF2 % Degradation @ 1 μM
35	0.017	84.6
36	0.031	82.3
37	0.025	<15
38	0.052	84.9
39	0.008	84.6
40	0.011	<15
41	0.061	<15
42	0.043	<15
43	0.042	<15
44	0.065	85.6
45	0.022	88
46	0.022	<15
47	0.005	86.7
48	0.045	78.9
49	0.011	<15
50	0.004	73.6
51	0.018	74.2

52	0.011	90.5
53	0.029	86.9
54	0.089	<15
55	0.158	38.1
56	0.239	<15
57	0.006	85.8
58	0.071	85.8
59	0.011	32.7
60	0.015	84.9
61	0.014	36.3
62	0.046	87.5
63	0.015	44.9
64	0.011	76.3
65	0.052	75.1
66	0.024	74.8
67	0.017	37.8
68	0.026	42.5
69	0.025	<15
70	> 10.0	-
71	0.037	<15
72	0.007	38.2
73	0.005	63.6
74	0.02	63
75	0.012	36.8
76	0.022	73.7
77	> 10.0	-
78	> 10.0	-
79	0.012	<15
80	0.008	76.7
81	0.009	77.6
82	0.921	-
83	0.036	48.8
84	0.014	<15
85	> 10.0	-
86	> 10.0	-
87	> 10.0	-
88	0.0302	71.4

89	6.81	-
90	> 10.0	-
91	> 10.0	-
92	> 10.0	-
93	> 10.0	-
94	> 10.0	-
95	> 10.0	-
96	0.042	78.9
97	0.031	<15
98	0.032	<15
99	0.044	80.2
100	0.025	<15
101	0.033	79.2
102	0.024	-
103	0.015	73.4
104	0.018	71.2
105	> 10.0	-
106	> 10.0	-
107	> 10.0	-
108	0.058	71.3
109	0.037	<15
110	0.019	48.8
111	0.006	76.5
112	0.025	72.6
113	0.013	73.7
115	0.012	63.6
116	0.024	<15
117	0.031	<15
118	0.036	<15
119	0.008	63.8
120	0.011	78.8
121	0.006	75.9
122	0.02	<15
123	0.011	74.4
124	0.002	70.2
125	0.006	83.2
126	0.005	84.3

127	0.004	77.9
128	0.018	<15
129	0.017	83.8
130	0.004	84.8
131	0.011	92.3
132	0.011	77.2
133	0.014	56.4
134	0.012	75

GSPT1 degradation assay

Generation of Stable Cell Lines

[0258] HEK293_hGSPT1_HiBiT-tagged cells were generated using CRISPR-Cas12a technology. Briefly, ~400,000 HEK293 cells were transiently co-transfected with precomplexed ribonuclear proteins (RNPs) consisting of 80 pmol of crRNA (IDT), 62 pmol of Cas12a protein (IDT), 3 μg of ssODN donor (IDT; AltRTM modifications), 78 pmol of electroporation enhancer (IDT), and 200 ng of pMaxGFP (Lonza). The transfection was performed via nucleofection (Lonza, 4D-Nucleofector X-unit) using solution P3 and program CM-130 in a (20 μL) cuvette. Five days post-nucleofection, cells were single-cell-sorted for GFP+ (transfected) cells by FACs in 96-well plates and clonally selected. Clones were screened and verified for the desired modification via targeted deep sequencing using gene-specific primers with partial Illumina adapter overhangs as previously described. In brief, clonal cell pellets were harvested, lysed, and used to generate gene-specific amplicons with partial Illumina adapters in PCR#1. Amplicons were indexed in PCR#2 and pooled with other targeted amplicons for other loci to create sequence diversity. Additionally, 10% PhiX sequencing control V3 (Illumina) was added to the pooled amplicon library prior to running the sample on a Miseq Sequencer System (Illumina) to generate paired 2 × 250 bp reads. Samples were demultiplexed using the index sequences, fastq files were generated, and NGS analysis was performed using CRIS.py. Final clones were authenticated using the PowerPlex fusion system (Promega) and tested negative for mycoplasma by the MycoAlertTMPlus mycoplasma detection kit (Lonza).

[0259] Editing construct sequences and screening primers are outlined below (sequence from 5′ to 3′). hGSPT1Cas12acrRNA, CAGE635.GSPT1.g1: TTTCTCTGGAACCAGTTTCAGAACT(SEQ ID NO: 4); CAGE635.g1.anti.ssODN: ttcctcacagtattgtgcagggtcatcaagaaaatgcttaGCTAATCTTCTTGAACAGCCGC CAGCCGCTCACgtcCttctctggaaccagtttcagaacttttccaattgcaatggtcttacctagaaatgaaattttaa (HiBiT tag and silent blocking modifications to prevent Cas12a recutting after integration are in upper case) (SEQ ID NO: 5); CAGE635.hGSPT1.DS.F: GGTTTGGCAGTAAAGCTAGTTAAT (SEQ ID NO: 6); CAGE635.hGSPT1.DS.R: GTGAA GTAGGCTTCTGCAGTC (SEQ ID NO: 7).

GSPT1 Reporter Degradation Assay

[0260] The GSPT1 degradation assay was carried out by harvesting the HEK 293T reporter cell lines and resuspending the cells in media formulated for reduced background fluorescence (FluoroBrite; Thermo Fisher). The respective cell lines were seeded at a density of 8,000 cells/well into white-opaque 384-well optical grade assay tissue culture plates (Greiner 781080-20). The cells were incubated overnight at 37 °C to allow for attachment to the assay plate. Dilutions of the compounds were prepared in DMSO from 10 mM compound stocks. Test compounds were added in a 10-point dilution series (typically 10 μM to 100 pM) using a TECAN D300e Digital Dispenser with an upper limit of 0.5% final DMSO. Plates were centrifuged at 320x g for 2 minutes at room temperature, and subsequently incubated at 37 °C.

[0261] After 24 hour incubation with the compounds, the plates were allowed to cool to room temperature for 10 minutes. 30 μL of HiBiT lytic buffer + 1:50 HiBiT substrate Solution were added to each well. Plates were centrifuged at 320x g for 2 minutes at room temperature and then incubated with shaking at room temperature for 10 minutes covered with foil. Plates were read on a CLARIOstar microplate reader (BMG LabTech), measuring at 450 nm (donor emission) and 610 nm or 630 nm (acceptor emission). The DC_{50} values were determined by regression to best fit four-parameter logistic curves using GraphPad Prism.

[0262] Table 5 shows results from the assays described above for certain compounds described herein demonstrating selectivity.

TABLE 5

#	**GSPT1 Degradation DC_{50} (μM)**	**GSPT1 % Degradation @ 1 μM (%)**
1	> 30.0	-
10	> 30.0	-
23	> 30.0	-
35	> 30.0	-
36	> 30.0	-
50	> 30.0	-
52	> 30.0	-
64	> 30.0	-
65	> 30.0	-
66	> 30.0	-
100	> 30.0	-