Unbiased mapping of cereblon neosubstrate landscape by high-throughput proteomics

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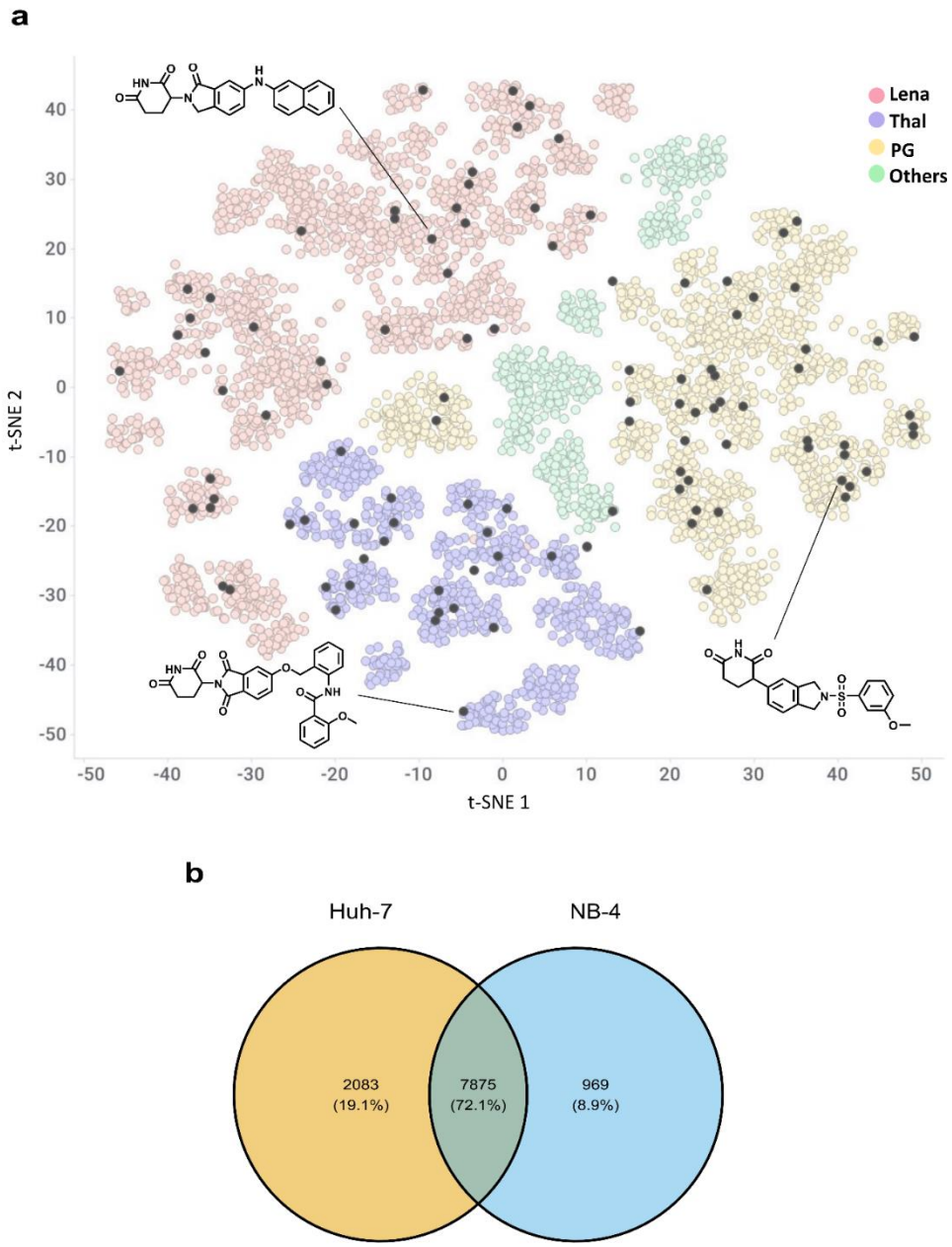
Supplementary Figures

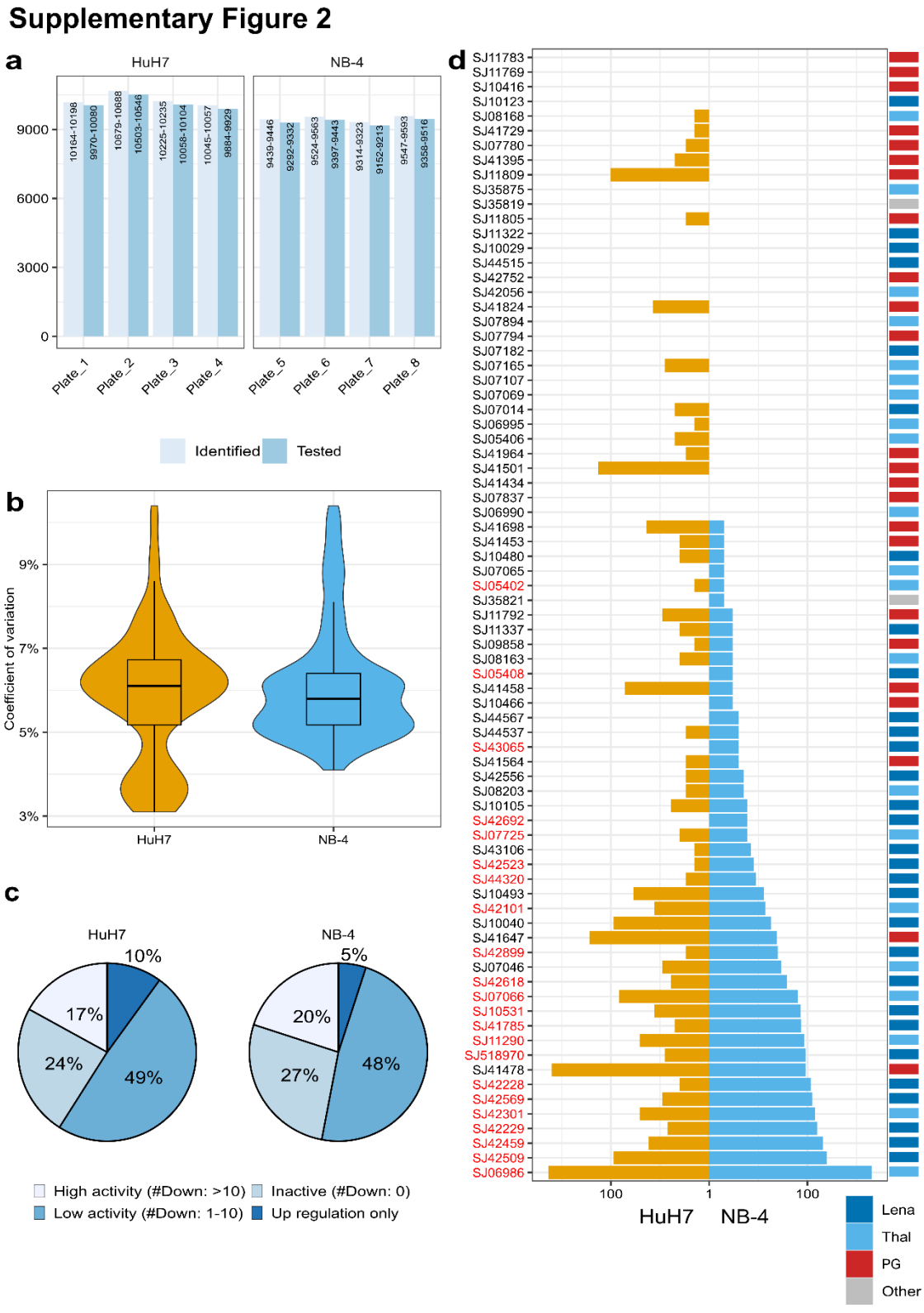
Supplementary Figure 1



Supplementary Figure 1: t-SNE plot representation of the molecular glue library comprising 5,000 compounds

a The clustering is based on molecular fingerprints and the colors are coded based on the CRBN-binding core diversity (lenalidomide, thalidomide, phenyl-glutarimide (PG), others). Each dot represents a compound, and the black dots denote the analogues selected for proteomics screening (100 compounds). Representative structures for lenalidomide-, pomalidomide- or PG-based cores are shown.

b Venn diagram illustrating overlapping and cell line-specific proteins quantified in Huh-7 and NB-4 cells.

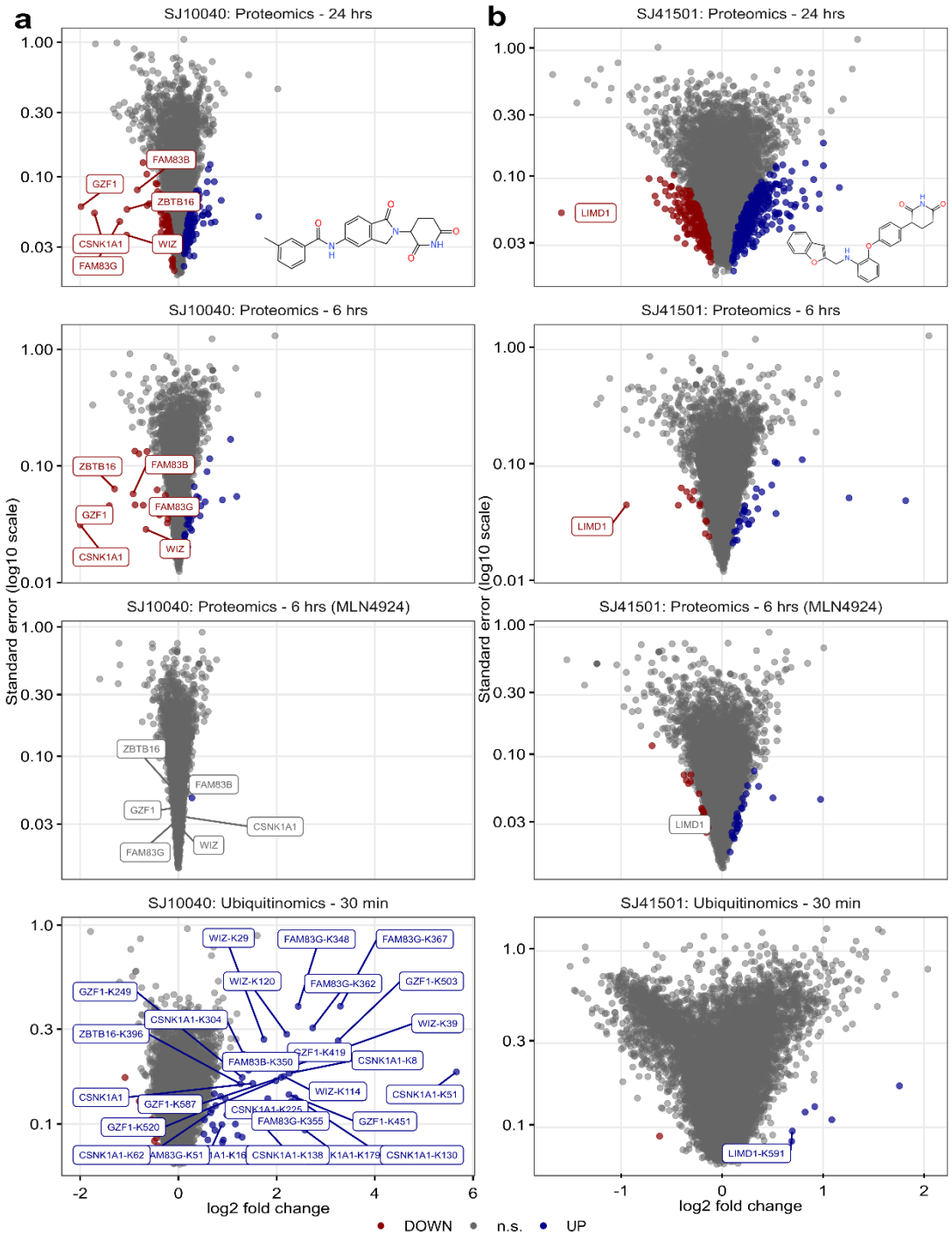


Supplementary Figure 2. Summary of proteomics screening data from Huh-7 and NB-4 cells

a Bar chart displaying numbers of all proteins quantified in Huh-7 and NB-4 cells (24-hour compound screening) after filtering for missing values (light blue) and the subset of proteins that underwent statistical testing (darker blue), which required 100% data completeness in the compound-treated samples.

b Violin plot showing distribution of protein coefficients of variation (%) in both cell lines. Bold horizontal lines in the violin plots demarcate the median and thin lines upper and lower quartiles.
c Pie chart displaying the activity of all screened compounds, defined by the number of induced significant (LIMMA[1], FDR=1%) protein regulations. Inactive = no significantly modulated protein; Low activity = number of regulated proteins <10; High activity = number of regulated proteins >10; In dark blue are compounds that did not induce any protein downregulations but instead significant protein upregulations.
d Number of significantly downregulated proteins in Huh-7 and NB-4 for the indicated compounds following 24 hours of compound treatment. Degraders of IKZF1 are labeled red. The labels on the right side indicate the core structures to which the different molecules belong: Phenyl-glutarimide (PG), Thalidomide (Thal) and Lenalidomide (Lena).

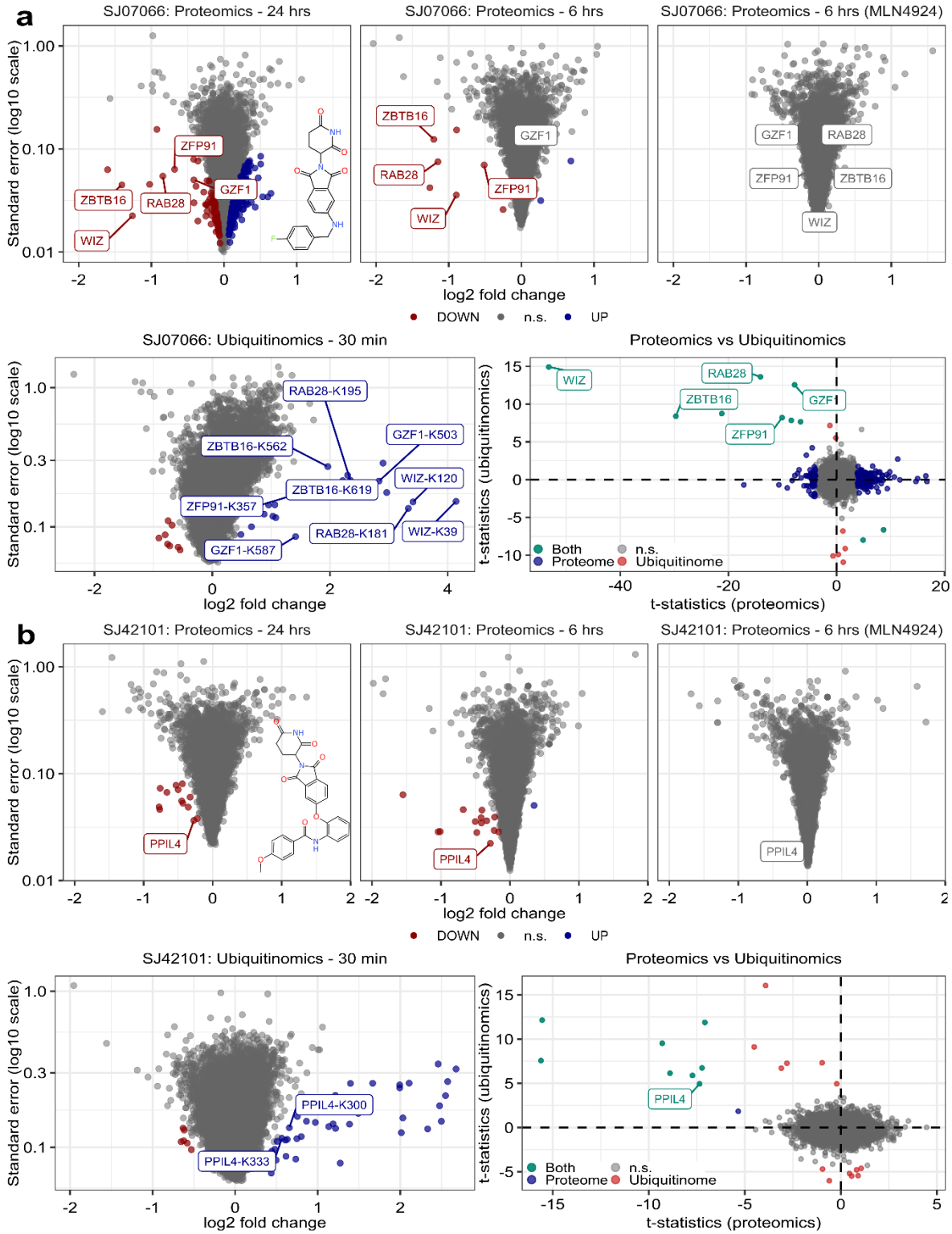
Supplementary Figure 3



Supplementary Figure 3. Examples of previously reported neosubstrates validated in our screen

Volcano plots of the proteomes after treatment with SJ10040 **a** and SJ41501 **b** for 24 hours (top), 6 hours (second from top), 6 hours in the presence of MLN4924 (second from bottom), and the ubiquitinome following 30 minutes of compound treatment (bottom). The x-axis shows log_2-transformed fold-changes and the y-axis the standard error on a log10 scale. Previously reported neosubstrates are highlighted, with significantly upregulated proteins (LIMMA[1], FDR=1%) marked in blue and downregulated proteins marked in red. n.s.= not significant.

Supplementary Figure 4

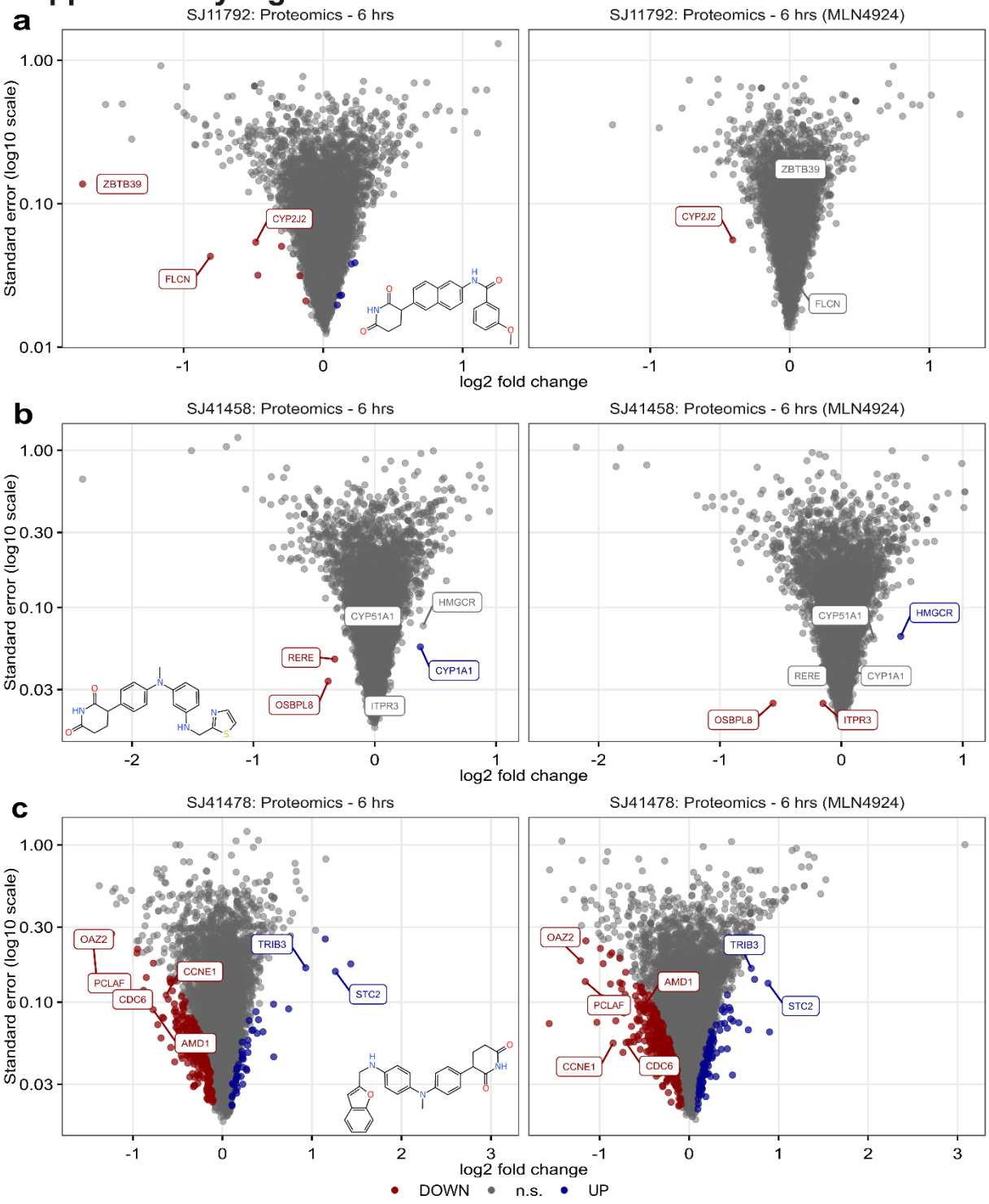


Supplementary Figure 4. Examples of previously reported neosubstrates validated in our screen

a and **b** Volcano plots of the proteomes are presented following treatment with the indicated compounds for 24 hours (top left), 6 hours (top middle), and 6 hours in the presence of MLN4924 (top right). The ubiquitinome following 30 minutes of compound treatment is shown in the bottom

left, while the bottom right displays a scatter plot comparing the proteomics and ubiquitinomics t-statistics. Previously reported neosubstrates are highlighted, and significantly upregulated and downregulated proteins (LIMMA[1], FDR = 1%) are marked in blue and red, respectively. The t-statistics of significantly upregulated ubiquitination sites mapping to the same proteins were averaged. In the t-statistic comparison plots, significantly modulated proteins are labeled in blue, ubiquitinated proteins in red, and proteins that are both significantly downregulated following a 24-hour compound treatment and harbor upregulated ubiquitination sites (30-minute compound treatment) are indicated in turquoise. n.s. = not significant.

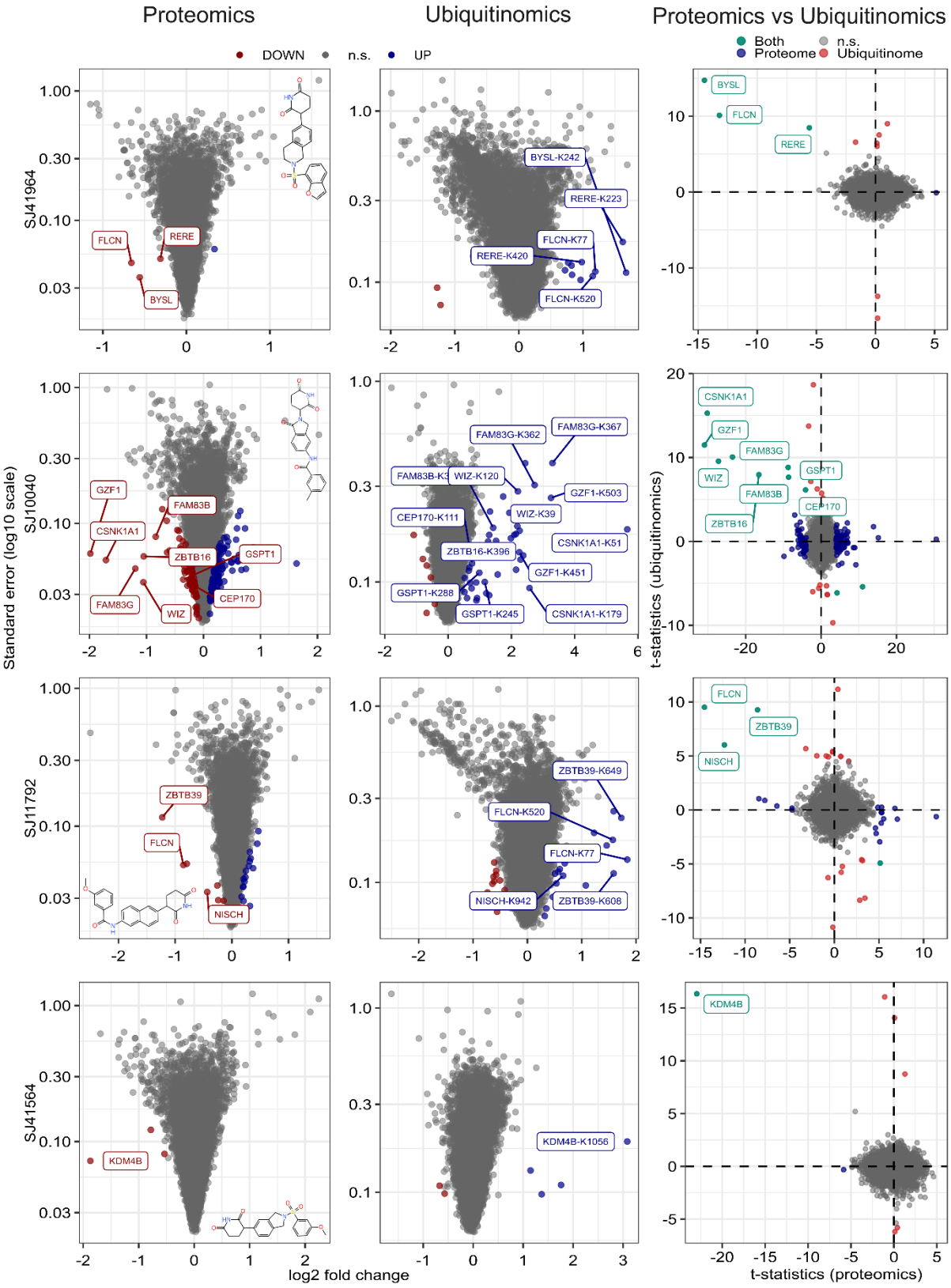
Supplementary Figure 5



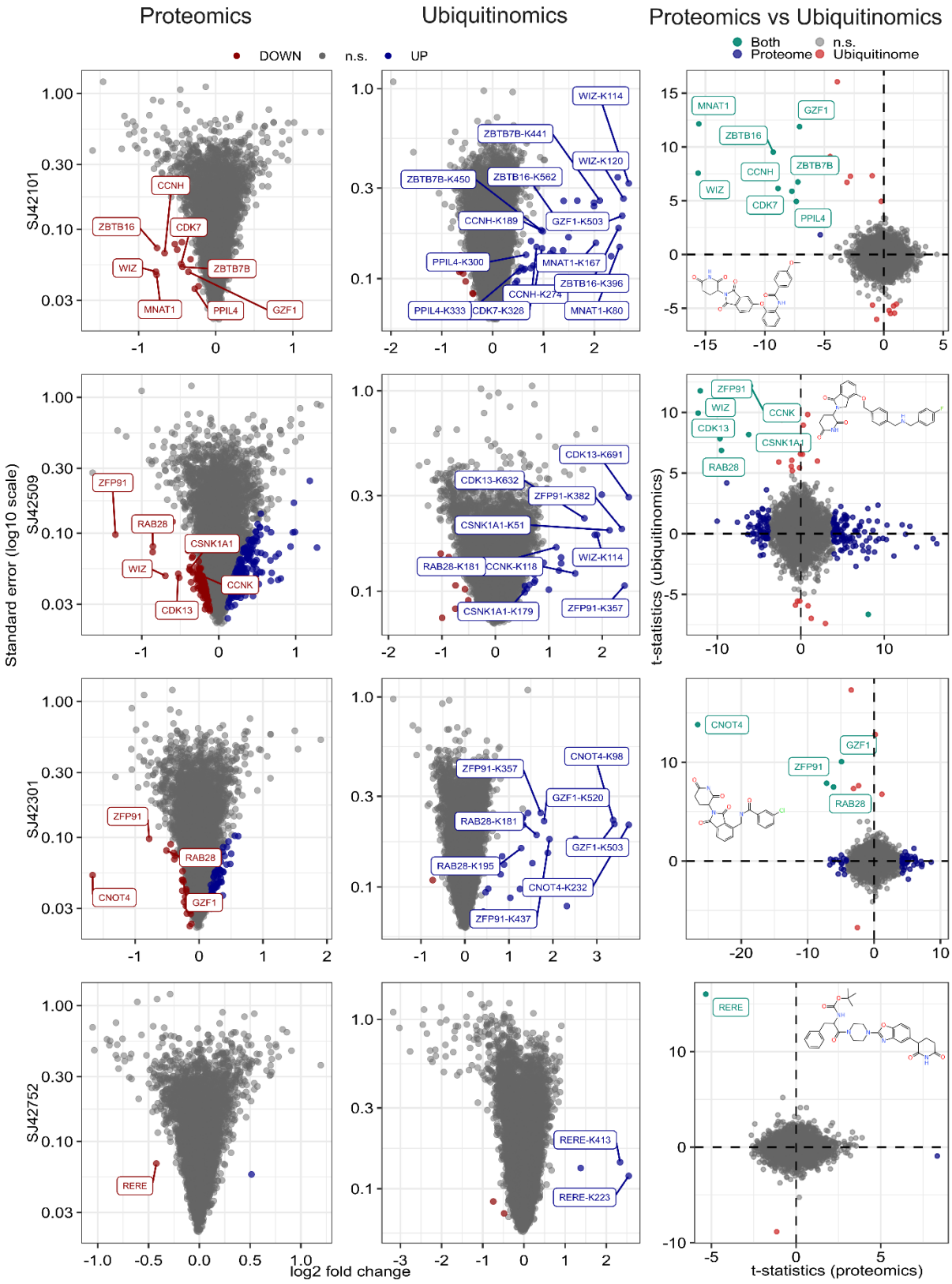
Supplementary Figure 5. Examples of MLN4924-independent protein regulations

a-**c** Volcano plots of the proteomes following treatment with the indicated compound for 6 hours, in the absence (left) or presence (right) of MLN4924. Significantly upregulated and downregulated proteins (LIMMA[1], FDR = 1%) are marked in blue and red, respectively. Selected proteins are highlighted to illustrate both neddylation-dependent and neddylation-independent regulatory events. n.s. = not significant.

Supplementary Figure 6



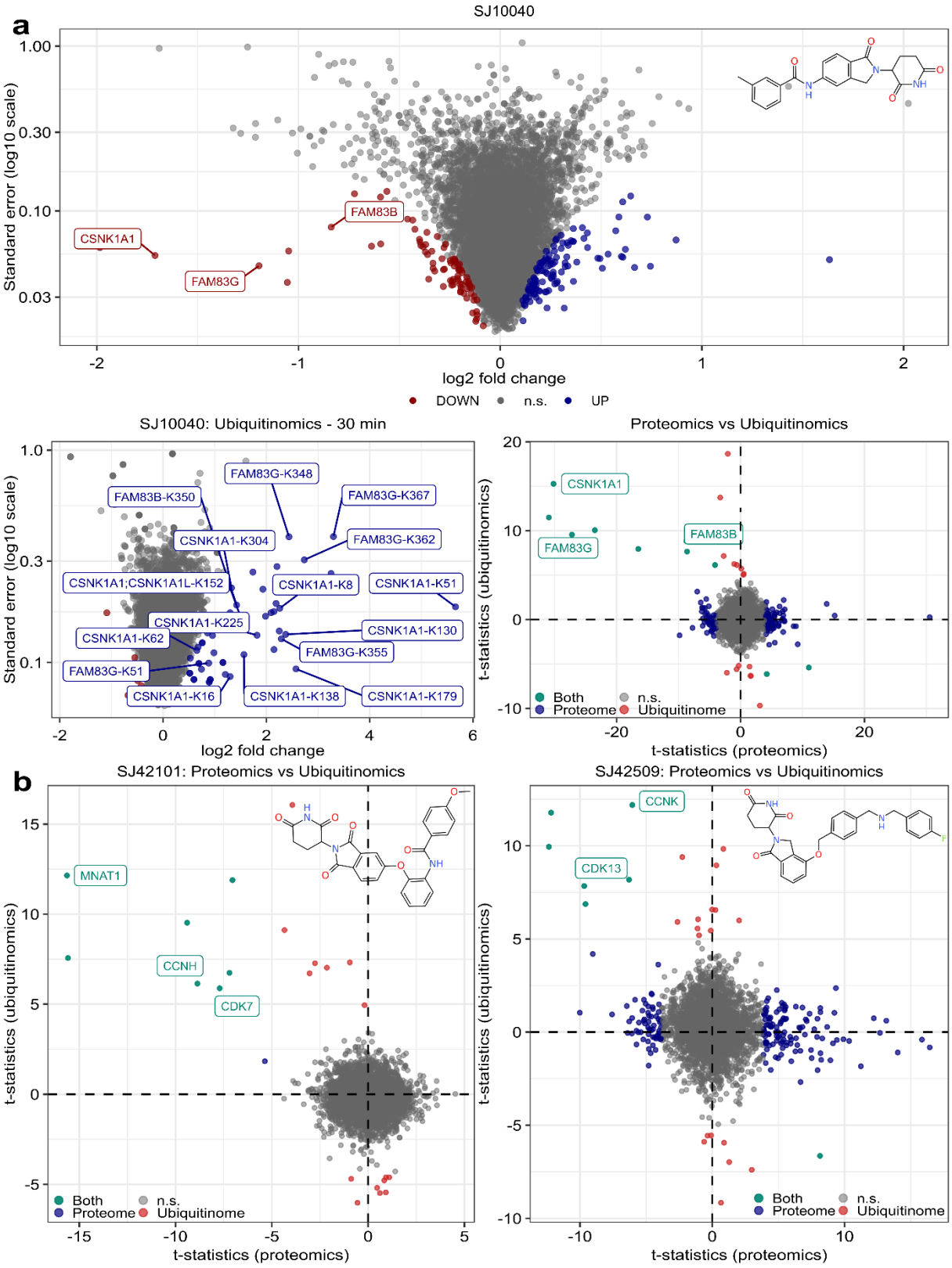
Supplementary Figure 6 (Cont.)



Supplementary Figure 6. Proteomics and ubiquitinomics screening results leading to the identification of 16 novel neosubstrates in Huh-7

The 24-hour proteomics screening results for each of the indicated compounds are presented on the left. The middle section displays the corresponding ubiquitinomics volcano plots (30-minute treatments), while the right section features a comparison plot of t-statistics between proteomics and ubiquitinomics. In the volcano plots, significantly upregulated proteins or ubiquitinated peptides are indicated in blue, and significantly downregulated proteins or ubiquitinated peptides are indicated in red (LIMMA[1], FDR = 1% and FDR=5%, for proteomes and ubiquitinomes, respectively). T-statistics for significantly upregulated ubiquitination sites mapping to the same proteins were averaged in the comparison plots. In these plots, significantly modulated proteins are labeled in blue, ubiquitinated proteins are labeled in red, and proteins that are both significantly downregulated and exhibit upregulated ubiquitination sites (or vice versa) are labeled in turquoise. n.s. = not significant.

Supplementary Figure 7



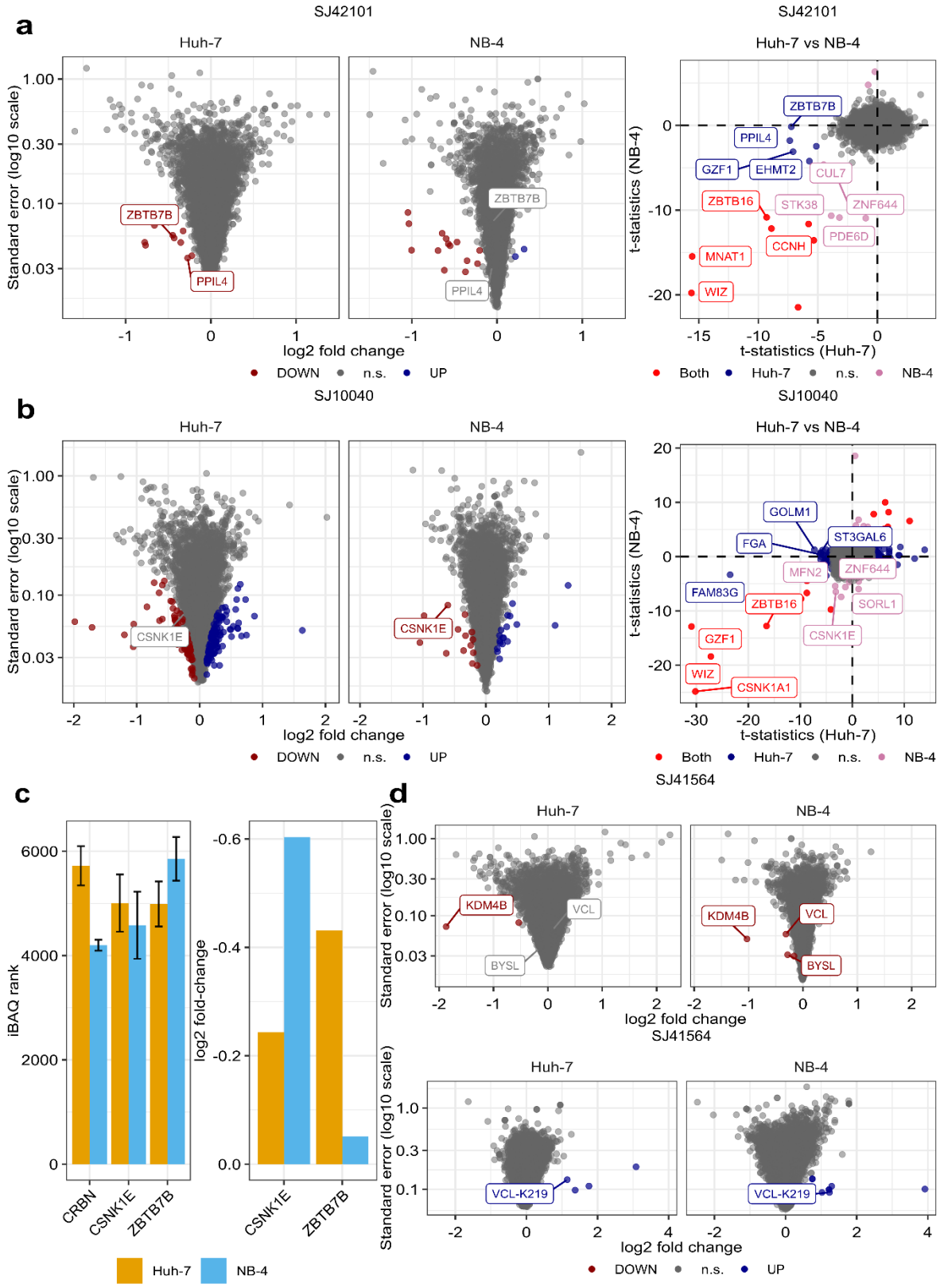
Supplementary Figure 7. Examples of neosubstrate protein complexes

a Volcano plot (top) of the proteome for SJ10040 following treatment for 24 hours, with CSNK1A1, FAM83G and FAM83B significantly downregulated (among others). For each protein, the $\log_2$-

transformed fold-change is depicted on the x-axis and its standard error (log_{10} scale) on the y-axis. Significantly up- and downregulated proteins are marked in blue and red, respectively (LIMMA[1], FDR = 1%). Volcano plot (bottom left) of the ubiquitinome for SJ10040 (30 minutes of treatment), with CSNK1A1, FAM83G and FAM83B ubiquitination sites significantly upregulated. Significantly up- and downregulated ubiquitinated peptides are marked in blue and red, respectively (LIMMA[1], FDR = 5%). On the bottom right, a proteomics vs. ubiquitinomics t-statistic comparison plot is shown. Significantly modulated proteins are labeled in blue, ubiquitinated proteins in red and proteins that are both significantly downregulated and harbor upregulated ubiquitination sites are in turquoise. n.s. = not significant.

b and **c** Proteomics vs. ubiquitinomics t-statistic comparison plots for SJ42101 **b** and SJ42509 **c**. T-statistics of significantly upregulated ubiquitination site mapping to the same proteins were averaged. Significantly modulated proteins (LIMMA[1], FDR = 1%, 24-hour treatment) are labeled in blue, ubiquitinated proteins (LIMMA[1], FDR = 5%, 30-minute treatment) in red and proteins that are both significantly downregulated and harbor upregulated ubiquitination sites (or vice versa) are in turquoise.

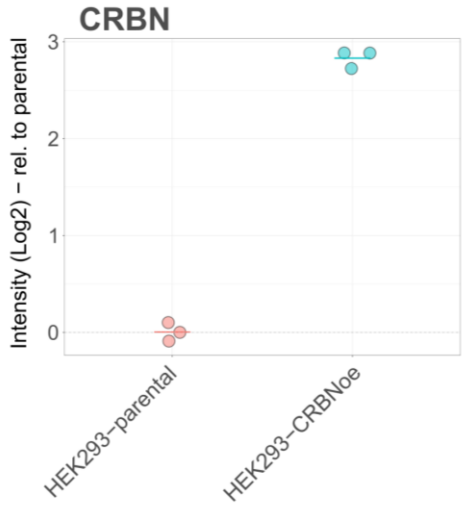
Supplementary Figure 8



Supplementary Figure 8. Comparison of proteomic profiles of Huh-7 and NB-4 cells.

a and **b** Volcano plots for the indicated 24-hour compound treatments in Huh-7 (left) and NB-4 cells (middle). For each protein, the log_2-transformed fold-change is depicted on the x-axis and its standard error (log_{10} scale) on the y-axis. Significantly up- and downregulated proteins (LIMMA[1], FDR = 1%) are marked in blue and red, respectively. A t-statistic comparison plot is shown on the right. Significantly modulated proteins in NB-4 cells are labeled in purple, significantly modulated proteins in Huh-7 cells in blue and proteins that are significantly modulated in both cell lines are marked in red. n.s. = not significant.
c Intensity-based absolute quantification-based protein ranks for CRBN, CSNK1E and ZBTB7B in NB-4 and in Huh-7 cells (values from multiple experiments were averaged, and error bar indicates Standard Error of the mean (SEM), shown on the left) and log2 fold-changes for CSNK1E (upon treatment with SJ10040) and ZBTB7B (upon treatment with SJ42101) on the right.
d Volcano plots of SJ41564-treated Huh-7 (left) and NB-4 (right) cells. The treatment was for 24 hours. A volcano plot depicting ubiquitinated peptides for both cell lines is shown below. One VCL site was significantly induced and is marked in blue. Significantly up- and downregulated ubiquitinated peptides (LIMMA[1], FDR = 5%) are marked in blue and red, respectively. n.s. = not significant.

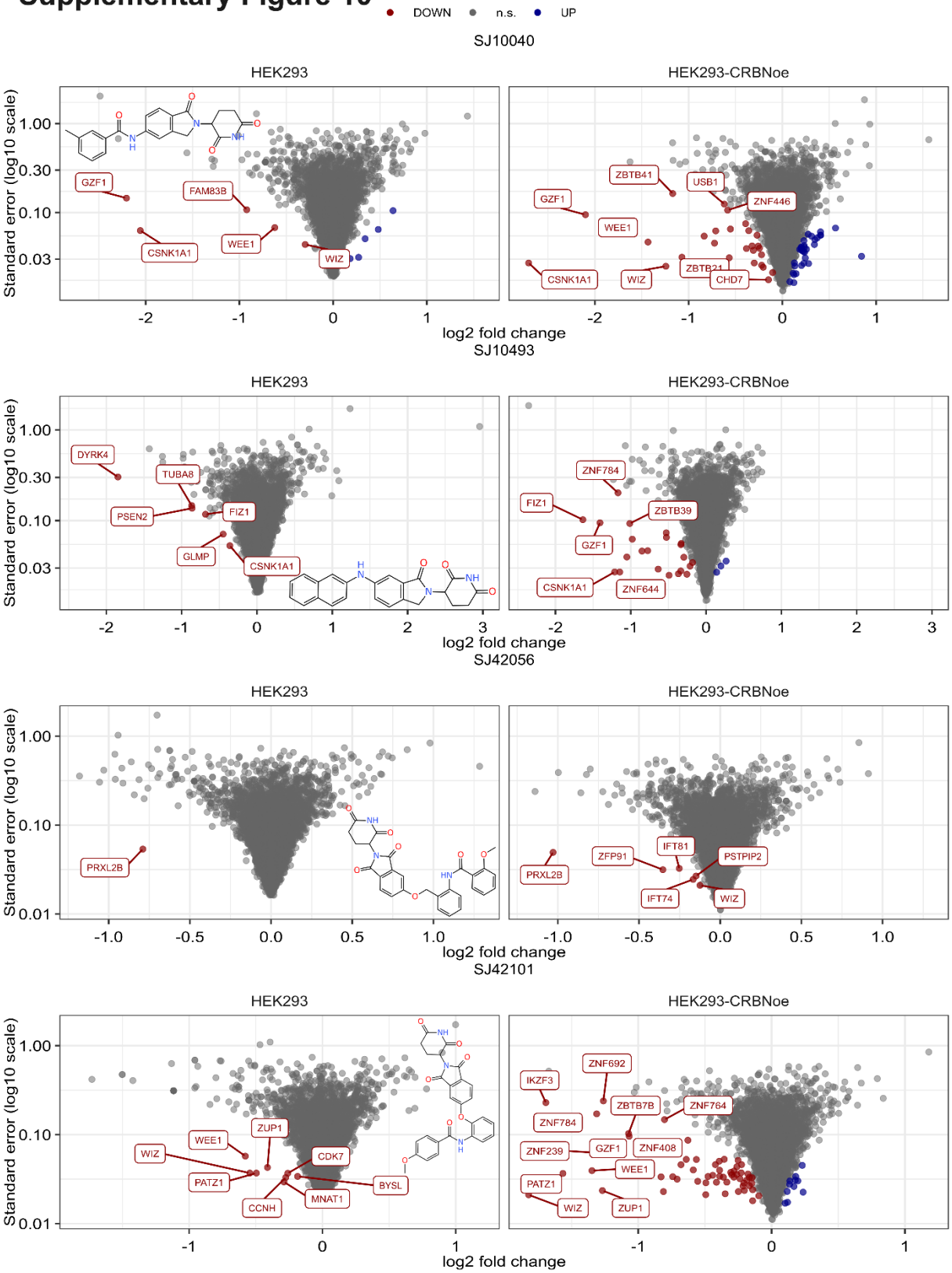
Supplementary Figure 9



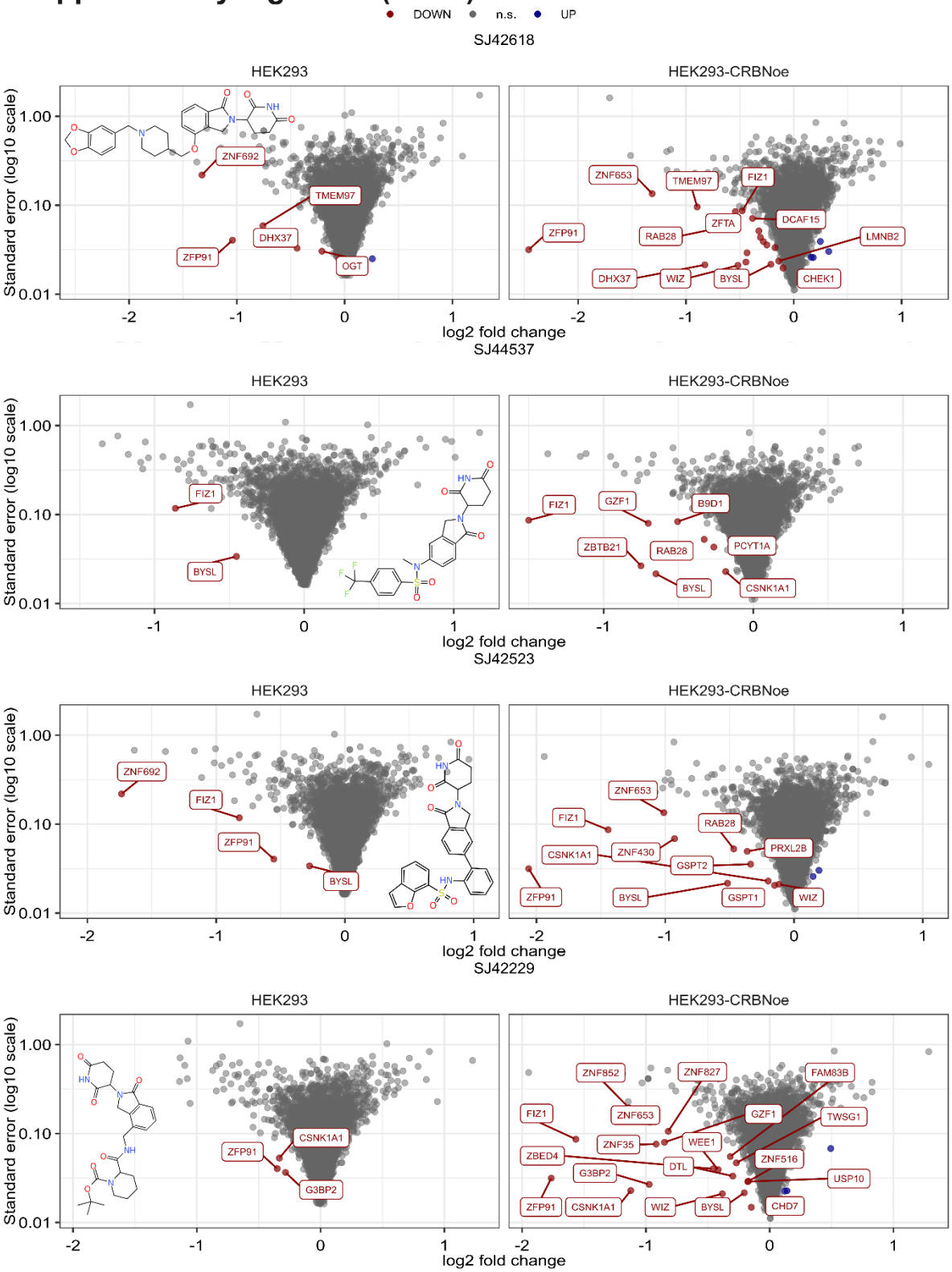
Supplementary Figure 9. MS-based quantification of CRBN levels in HEK293-CRBNoe cells

Scatter plot illustrating log_2-transformed CRBN levels quantified by mass spectrometry (MS)-based proteomics in HEK293 cells (parental) and HEK293 cells overexpressing CRBN (HEK293-CRBNoe). Three individual samples were processed for each cell line.

Supplementary Figure 10



Supplementary Figure 10 (Cont.)

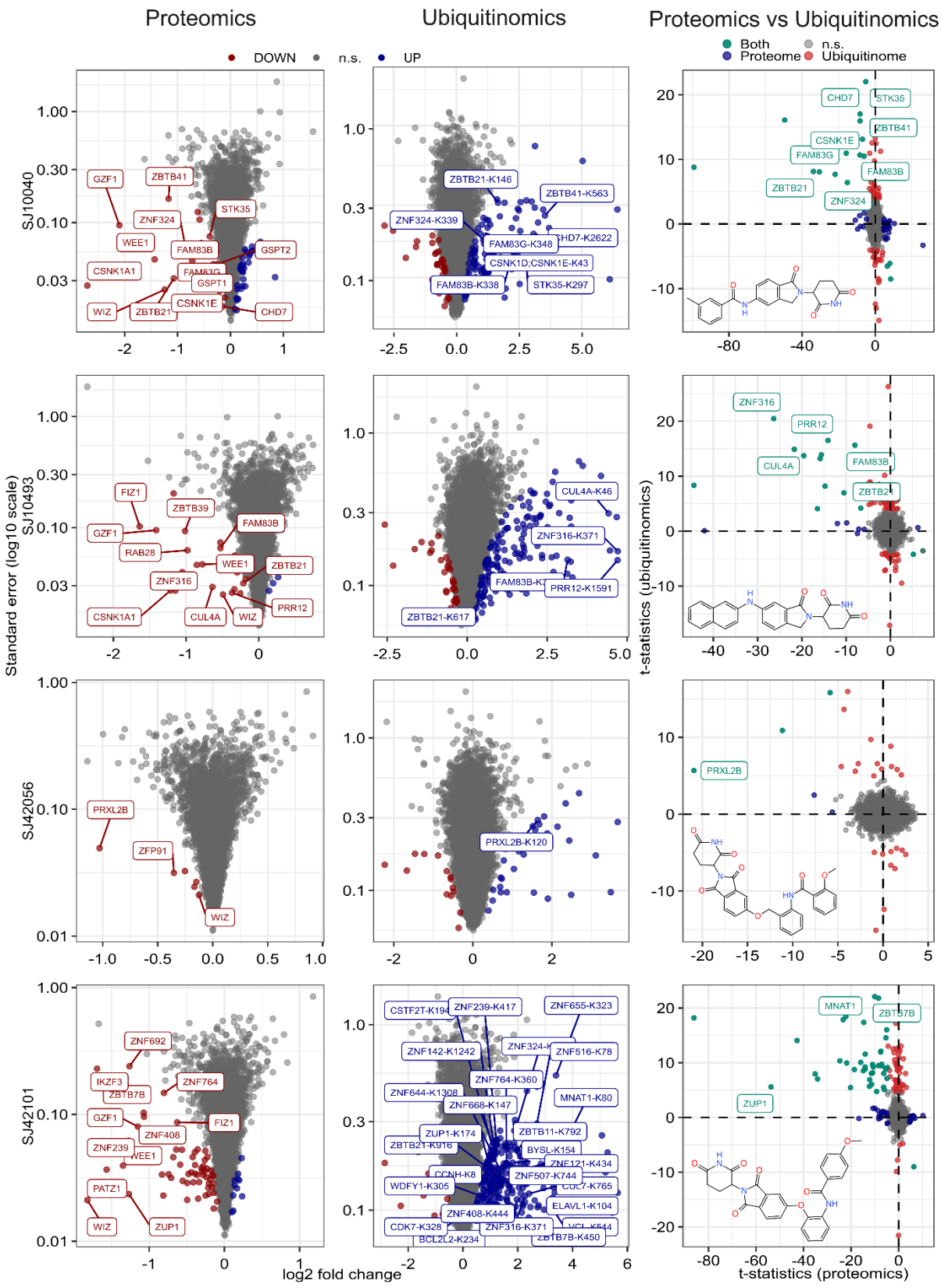


Supplementary Figure 10. Characterization of HEK293-CRBNoe cells for the responsiveness to MGDs

Volcano plot for the indicated 6-hour compound treatments in HEK293 (left) and HEK293-CRBNoe cells (right). The log_2-transformed fold-change is depicted on the x-axis and the standard

error (log_{10} scale) on the y-axis. Significantly up- and downregulated proteins (LIMMA, FDR = 1%) are marked in blue and red, respectively. n.s. = not significant.

Supplementary Figure 11



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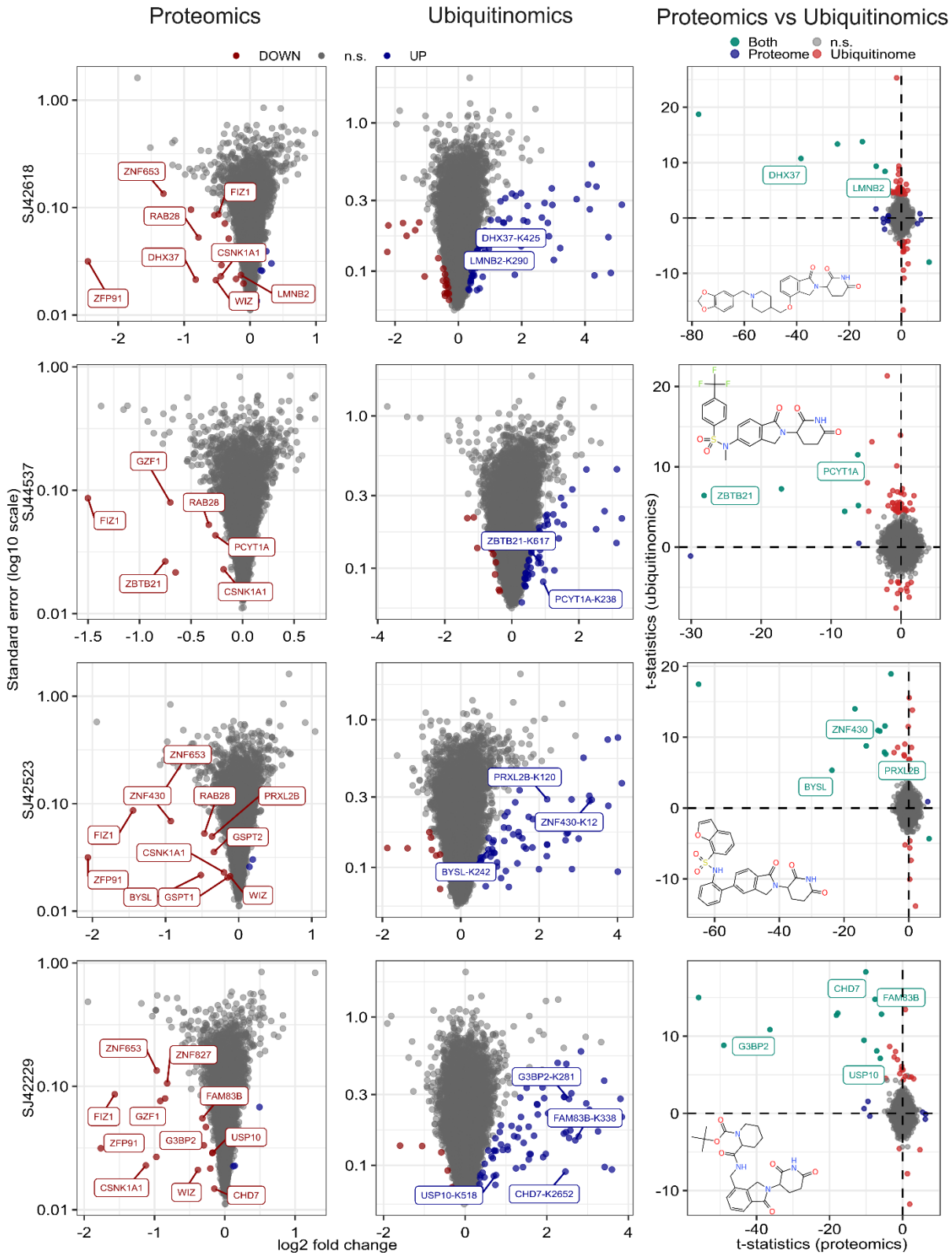
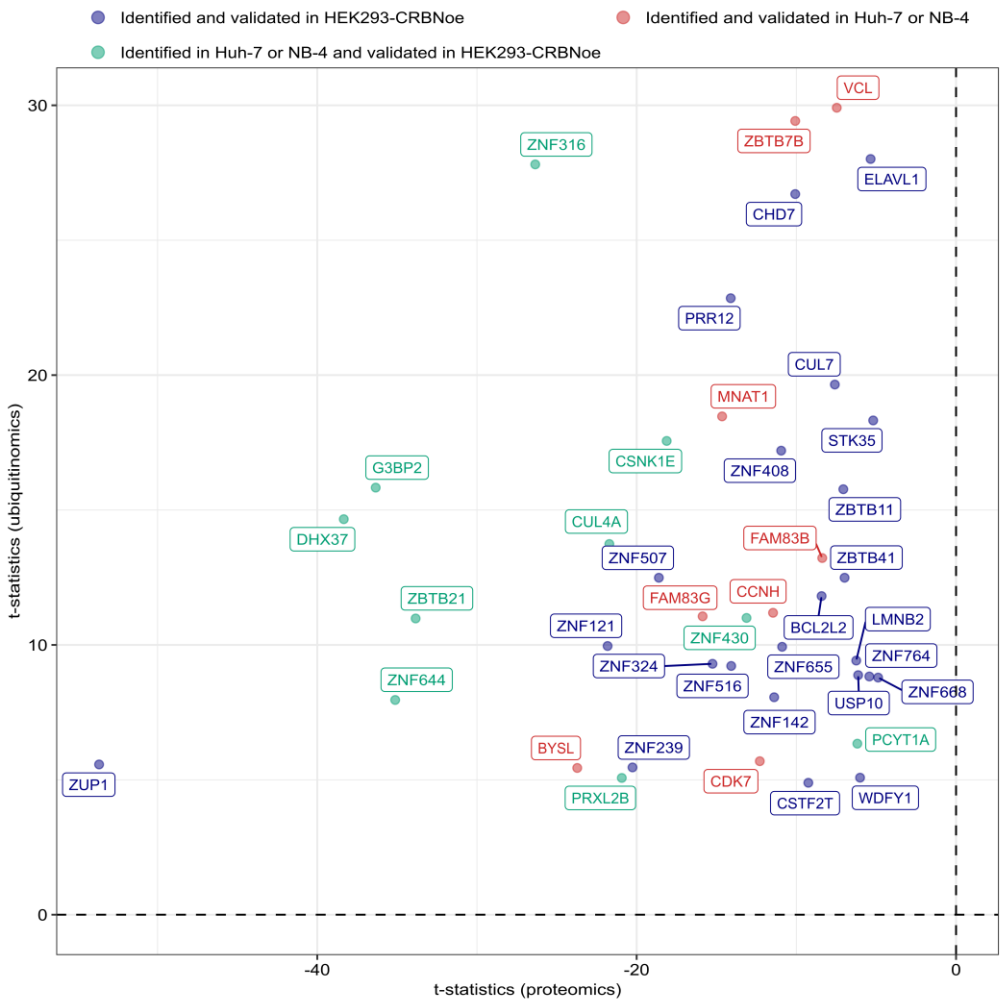


Supplementary Figure 11. Proteomics and ubiquitinomics of 8 degrader compounds in HEK293-CRBNoe cells

Volcano plots illustrating the proteomes (right panel, 6-hour treatments) and corresponding ubiquitinomes (middle panel, 30-minute treatments) for the indicated MGDs. The x-axis represents the $\log_2$-transformed fold change, while the y-axis displays the standard error on a $\log_{10}$ scale. Significantly upregulated proteins (LIMMA[1], FDR = 1%) are marked in blue, and significantly downregulated proteins are marked in red. Similarly, significantly ubiquitinated peptides (LIMMA[1], FDR = 5%) are also highlighted in blue and red, respectively. On the right, a t-statistic comparison plot for each tested compound is presented. Significantly modulated proteins are labeled in blue, ubiquitinated proteins in red, and proteins that are both significantly downregulated and exhibit upregulated ubiquitination sites (or vice versa) are colored in turquoise.

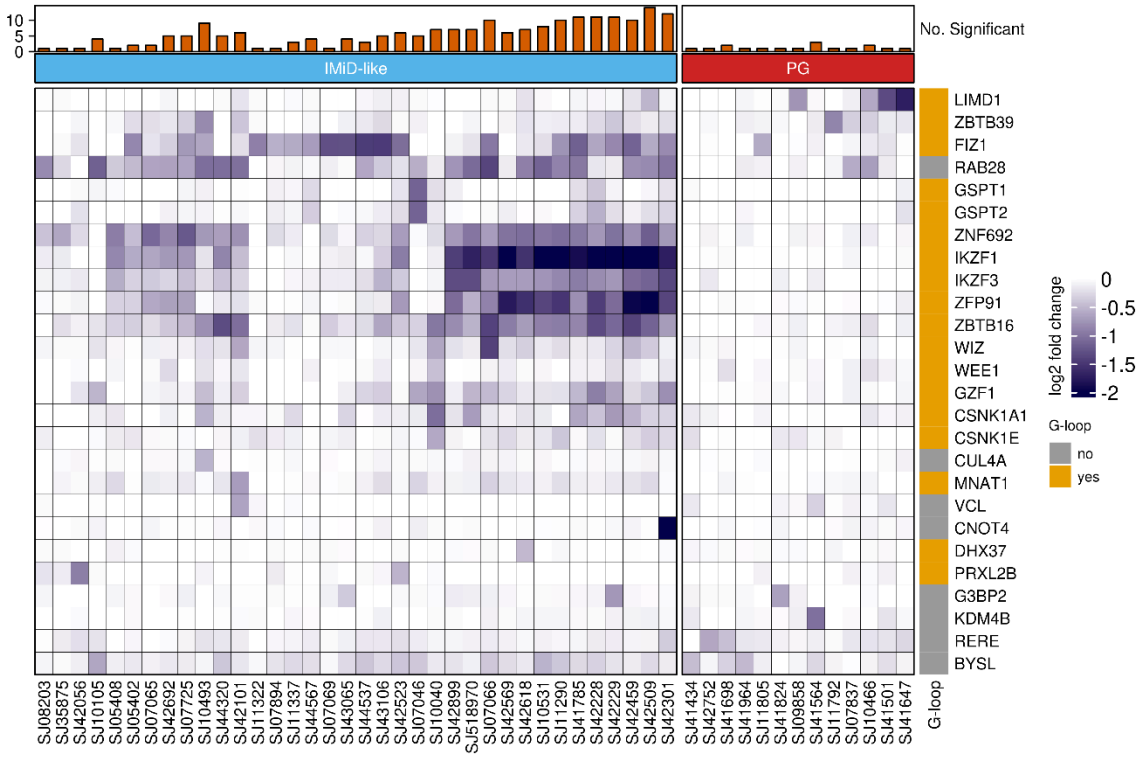
Supplementary Figure 12



Supplementary Figure 12. Neosubstrates validated in HEK293-CRBNoe cells

A proteomics versus ubiquitinomics t-statistic comparison plot for each newly validated neosubstrates in HEK293-CRBNoe is presented. Proteins identified and validated through ubiquitinomics specifically in HEK293-CRBNoe cells are shown in blue. Proteins identified and validated in HEK293-CRBNoe, Huh-7, or NB-4 cells are depicted in red. Neosubstrates identified in either NB-4 or Huh-7 cells, but subsequently validated in HEK293-CRBNoe cells, are represented in green.

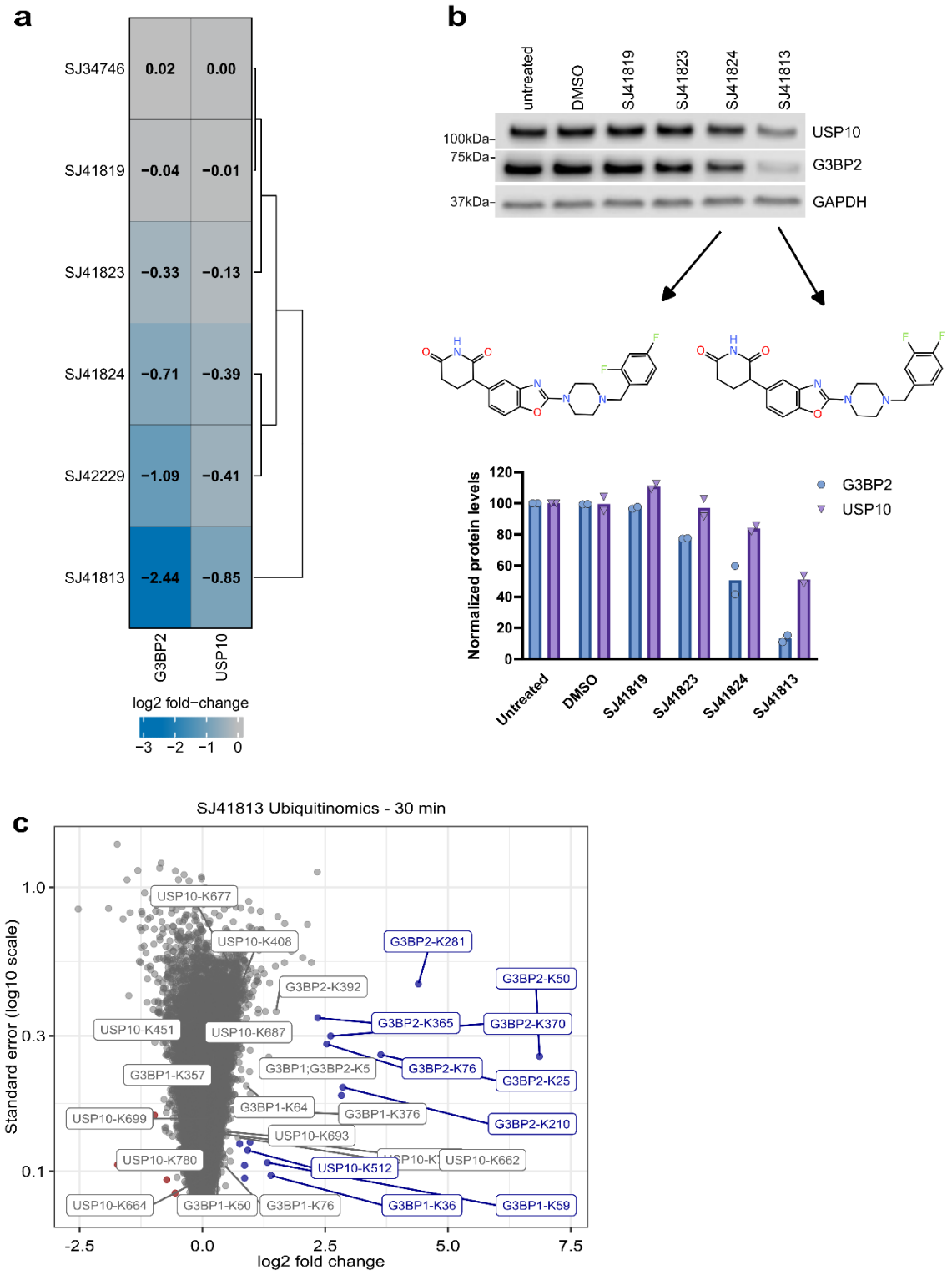
Supplementary Figure 13



Supplementary Figure 13. Heap map of selected targets identified in NB-4 cells

Heat map of the log_2-transformed fold changes of known and selected newly identified neosubstrates in NB-4. The compounds were arranged according to whether they contained an IMiD-like or a PG core structure. The bar chart on top shows the number of significantly downregulated neosubstrates for each compound. Proteins that contain a predicted G-loop motif are highlighted.

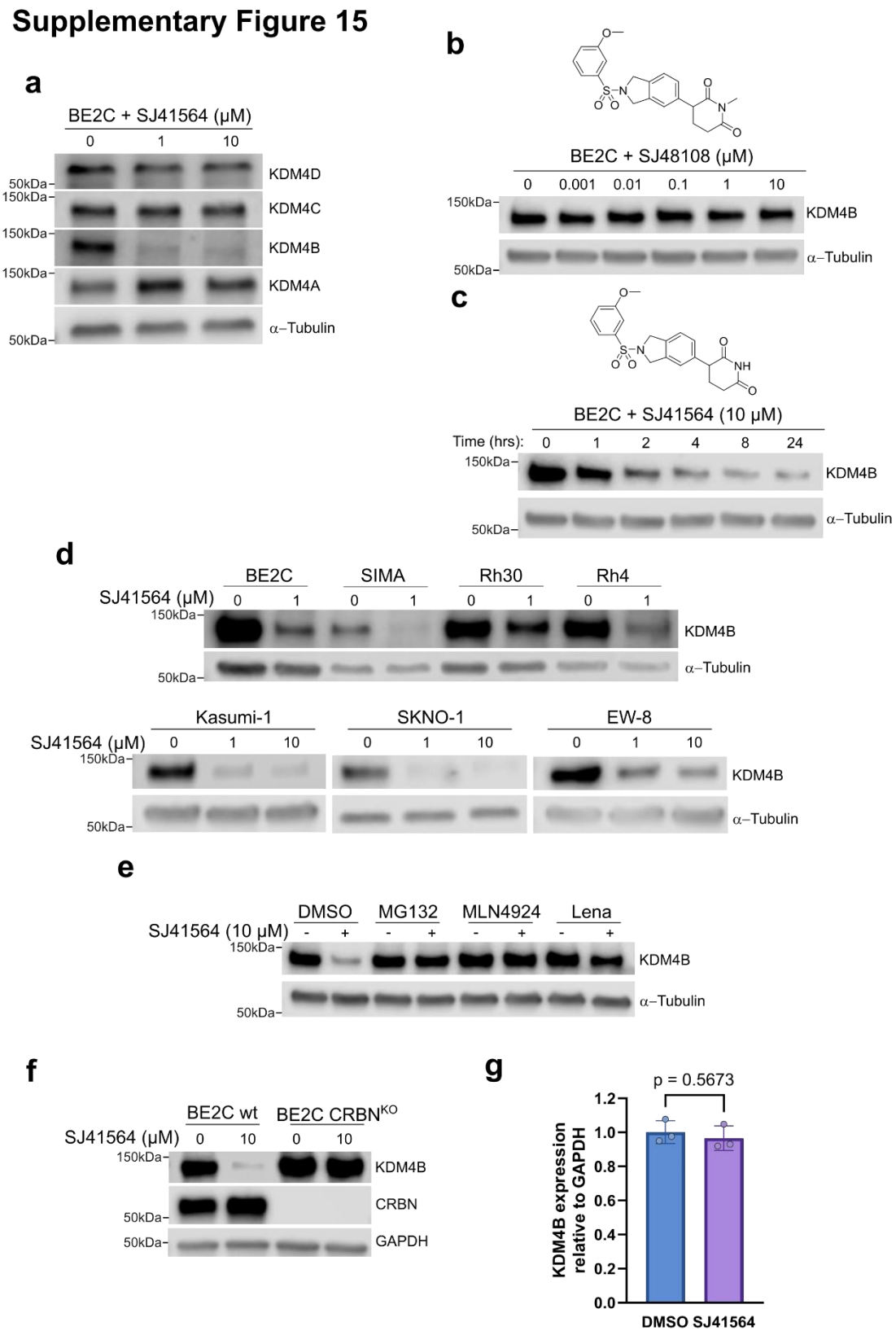
Supplementary Figure 14



Supplementary Figure 14. Characterization of G3BP2 degrader compounds

a Heat map showing proteomics-derived log_2 fold-changes for G3BP2 and USP10 following a 24-hour compound treatment with the indicated compounds.
b Western blot analysis for different analogues of the G3BP2 degrader SJ41824 in SK-N-BE(2)-C cells. Cells were treated with 10 µM of indicated compounds for 24 hours. Depicted blots are

representative of two independent experiments. The compound structures of SJ41824 and SJ41813 are shown.
c Volcano plot of ubiquitinated peptides upon treatment of HEK293-CRBNoe cells with SJ4813. The x-axis represents the log_2-transformed fold change, while the y-axis displays the standard error on a log_{10} scale. Significantly up- and downregulated ubiquitinated peptides (LIMMA[1], FDR = 5%) are marked in blue and red, respectively. Non-significant (n.s.) peptides are colored in grey.

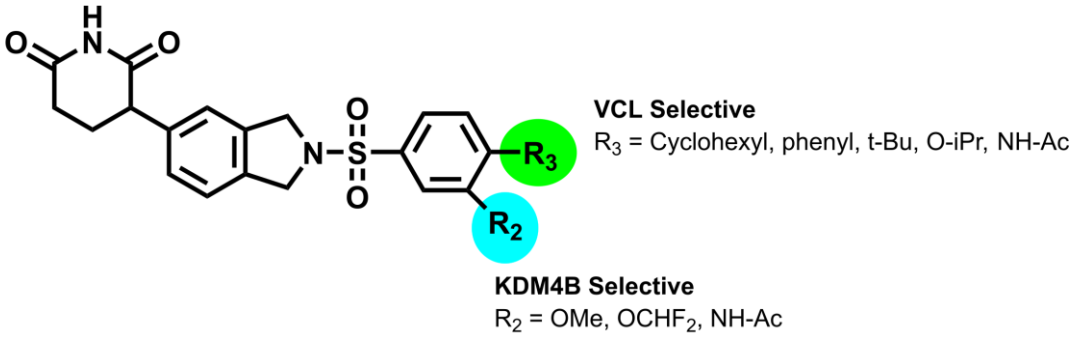


Supplementary Figure 15. Characterization of the KDM4B degrader SJ41564

a Western blot analysis for KDM4 family members upon treatment of SK-N-BE(2)-C cells (BE2C) with DMSO or the degrader SJ41564 for 24 hours (1 and 10 μM). Shown are the results of one experiment.

b Immunoblots for KDM4B protein after the treatment of SK-N-BE(2)-C cells with increasing concentrations of SJ48108 for 24 h. Depicted blots are representative of three independent experiments.
c Immunoblots for KDM4B protein after the treatment of SK-N-BE(2)-C cells with 10 μM of SJ41564 over 24 h, and proteins were harvested at 0, 1, 2, 4, 8, and 24 hours after treatment. Depicted blots are representative of three independent experiments. The chemical structure of both compounds is shown.
d Immunoblots for KDM4B protein after the treatment of neuroblastoma (SK-N-BE(2)-C (BE2C), SIMA), rhabdomyosarcoma (Rh30, Rh4), leukemia (Kasumi-1, SKNO-1), and Ewing sarcoma (EW-8) cells with 1 or 10 μM of SJ41564 for 24 h. Shown are the results of one experiment.
e Immunoblots for KDM4B protein after the treatment of SK-N-BE(2)-C cells with indicated compounds. Cells were pre-treated with MG132 (5 μM), MLN4924 (1 μM), and lenalidomide (20 μM) for 1 h prior to treatment with 10 μM of SJ41564 for 5 hrs. Shown are the results of one experiment.
f Western blot analysis for the indicated proteins of SK-N-BE(2)-C cells (wt and CRBN knockout (CRBNKO)) treated with DMSO or 10 μM of SJ41564 for 24 hours. Depicted blots are representative of three independent experiments.
g RT-qPCR analysis of KDM4B mRNA level after the treatment of SK-N-BE(2)-C cells with 10 μM of SJ41564 for 24 hours. Data represent mean ± S.D. of three technical replicates. Data are mean ± s.d. p= 0.5673 (two-tailed unpaired Student t test).

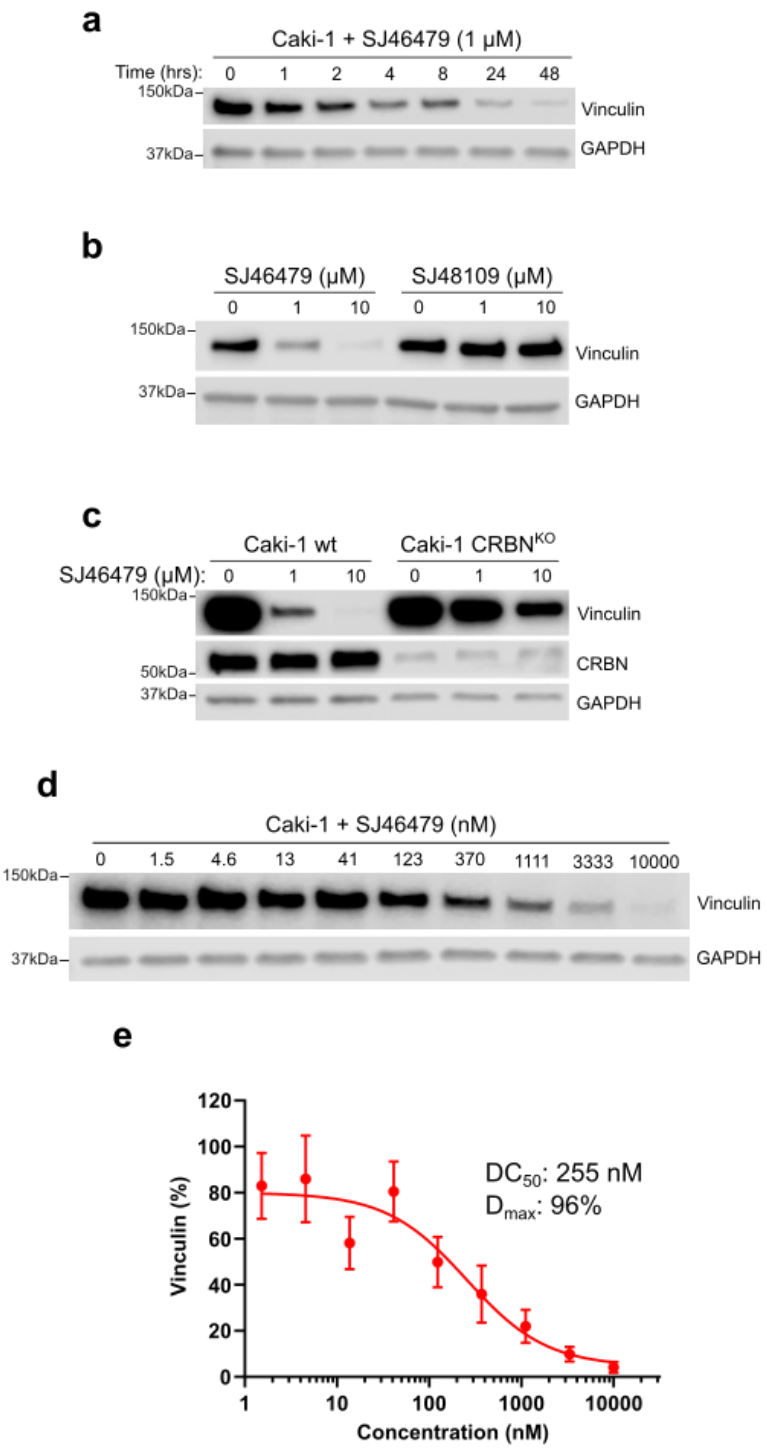
Supplementary Figure 16



Supplementary Figure 16: Chemical characterization of KDM4B degrader compounds

Structure-degradation relationship of scaffold illustrating substitution patterns associated with KDM4B and VCL degradation.

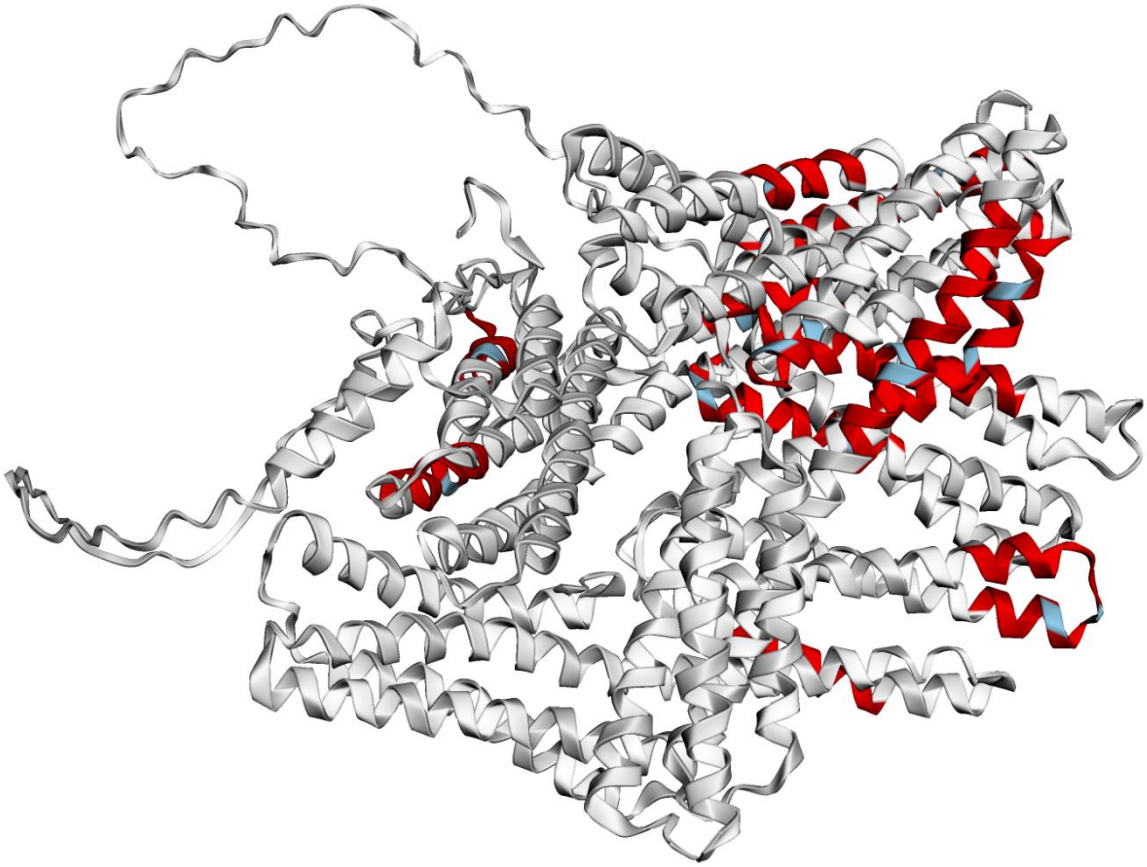
Supplementary Figure 17



Supplementary Figure 17: Characterization of the VCL degrader SJ46479

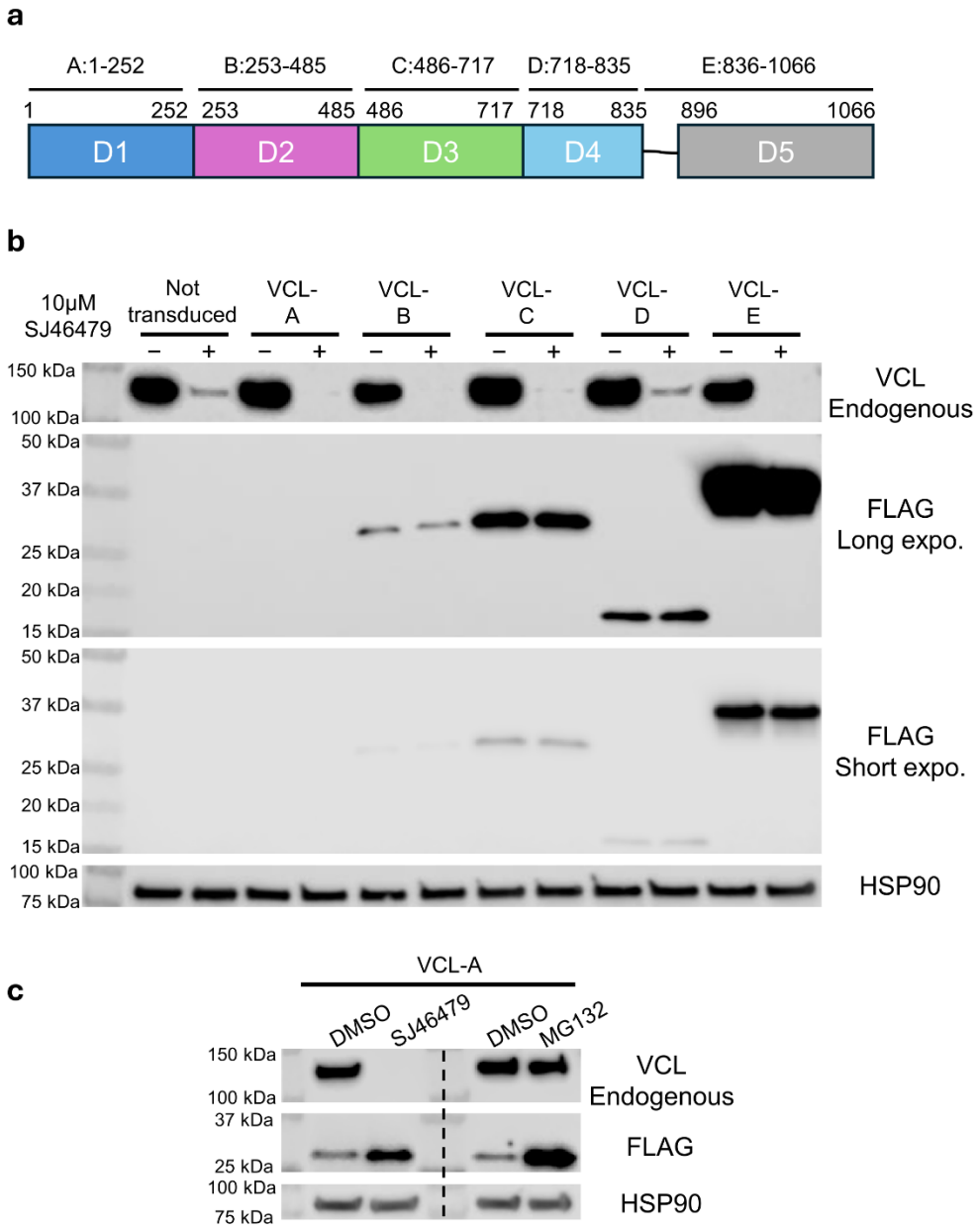
a Western blot analysis for vinculin (VCL) following treatment of Caki-1 cells with 1µM of SJ46479 for the indicated times. Depicted blots are representative of three independent experiments.

b Western blot analysis for vinculin (VCL) following treatment of Caki-1 cells with the indicated doses of SJ46479, and the negative control compound SJ48109 for 24 hours. Shown are the results of one experiment.
c Western blot analysis for vinculin (VCL) in Caki-1 cells (wt or CRBN knockout ($CRBN^{KO}$)) with the indicated doses of SJ46479 for 24 hours. Shown are the results of one experiment.
d Western blot analysis for vinculin (VCL) following treatment of Caki-1 cells with the indicated doses of SJ46479 for 24 hours. Depicted blots are representative of three independent experiments.
e Quantification of vinculin (VCL) from the Western blot shown in **d**. Plotted are mean values -/+ SD (n= 3).



Supplementary Figure 18: Structural model of VCL with mapped ubiquitination sites by SJ46479

A structural model of vinculin (VCL) predicted using AlphaFold2 (AF2)[2,3] is shown, with ubiquitinated peptides significantly upregulated upon SJ46479 treatment highlighted in red. Lysine residues identified as ubiquitination sites are indicated in light blue.



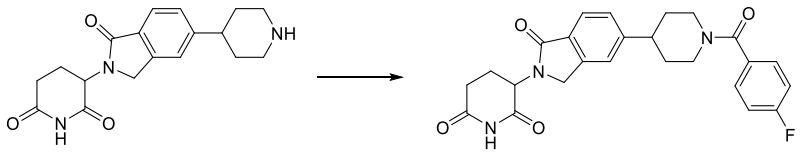
Supplementary Figure 19: SJ46479 selectively stabilized the domain D1 of vinculin

a Protein architecture of VCL and schematic of truncated VCL protein fragments.
b Western blot analysis of VCL, FLAG and HSP90 proteins following treatment of Caki-1 cells overexpressing Flag-tagged VCL fragments A-E with 10 μM of SJ46479 for 24 hours. Data shown are the results of one experiment.
c Western blot analysis of VCL, FLAG and HSP90 proteins following treatment of Caki-1 cells overexpressing VCL-A fragment with 10 μM of SJ46479 or 5 μM of MG132 for 24 hours. Data shown are the results of one experiment.

Supplementary Methods

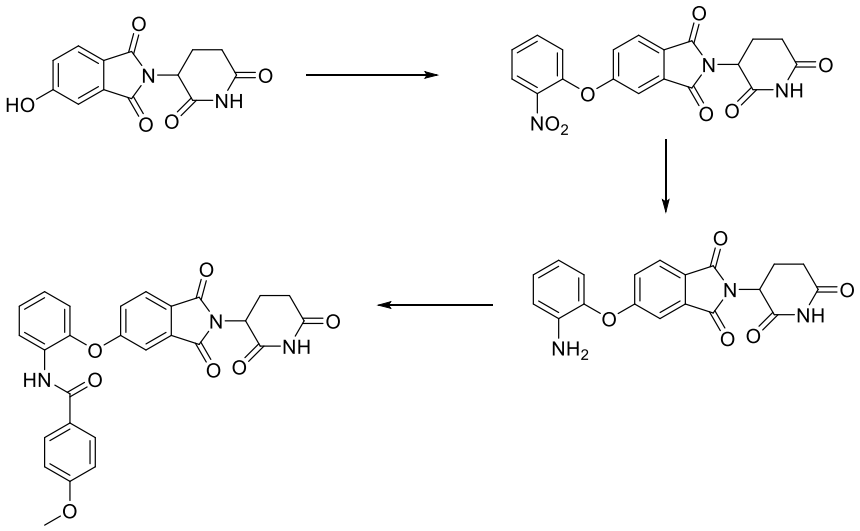
General Chemistry Methods and Materials

All reagents and solvents were obtained from commercially available sources and were used without further purification. SJ6986 and SJ10040 were synthesized as previously described. Reactions were set up in air and carried out under nitrogen atmosphere. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker NMR spectrometer at 500 MHz for 1H-NMR spectra and 125 MHz for 13C-NMR spectra. Chemical shifts (ppm) are reported relative to the solvent peak. Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quadruplet; m, multiplet. Coupling constants **j** are expressed in Hertz. The purity of final compounds was performed on an Acquity UPLC BEH C18 1.7 µm, 2.1 x 50 mm column (Waters Corporation, Milford, MA) using an Acquity ultra performance liquid chromatography system. The flow rate was 0.7 mL/min. The sample injection volume was 3 µL. The UPLC column was maintained at 50 °C and the gradient program started at 90% A (0.1% formic acid in MilliQ H2O), changed to 95% B (0.1% formic acid in Acetonitrile) over 2.5 min, held for 0.35 minutes, then to 90% A over 0.05 minutes.



To a vial containing 3-(1-oxo-5-(piperidin-4-yl)isoindolin-2-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in THF (0.75 mL, 0.1 M) was added 4-fluorbenzoyl chloride (0.15 mmoL, 2 equiv) followed by DIPEA (0.225 mmol, 3 equiv). The reaction mixture was allowed to stir at room temperature for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(5-(1-(4-fluorobenzoyl)piperidin-4-yl)-1-oxoisoindolin-2-yl)piperidine-

2,6-dione (SJ11322, 29.4 mgs, 87% yield). LCMS: 450.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C25H25FN3O4, 450.1824; found, 450.1821.

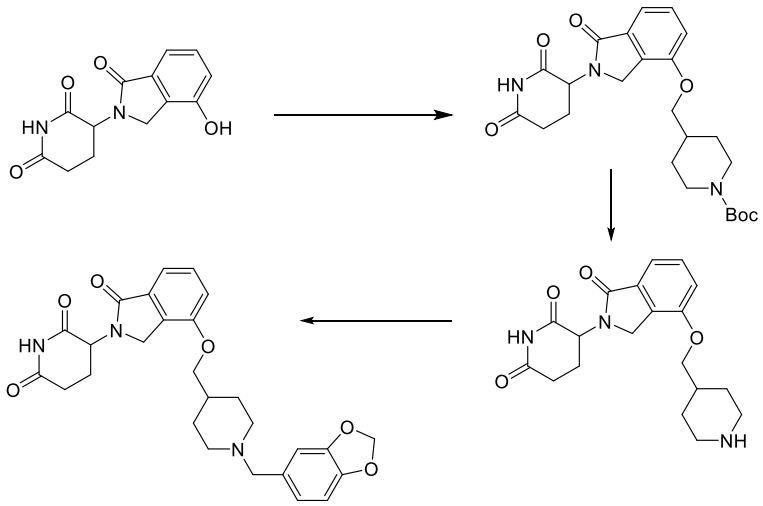


To a flask containing 2-(2,6-dioxopiperidin-3-yl)-5-hydroxyisoindole-1,3-dione (7.29 mmol, 1.00 eq) in DMF (20 mL, 0.35 M) was added O-fluoronitrobenzene (8.75 mmol, 1.20 equiv) followed by DMAP (1.07 g, 8.75 mmol, 1.20 equiv) at room temperature. The reaction was heated to 120 °C and stirred for 1h under nitrogen atmosphere. The reaction was cooled to room temperature and quenched by the H_2O. The aqueous layer was extracted with DCM. The organics were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography eluted with 50% of ethyl acetate in petroleum ether. Desired fractions were concentrated to give 2-(2,6-dioxopiperidin-3-yl)-5-(2-nitrophenoxy)isoindole-1,3-dione (1.70 g, 58.9% yield) as a brown solid. LCMS: 396 (M+1).

To a flask containing 2-(2,6-dioxopiperidin-3-yl)-5-(2-nitrophenoxy)isoindole-1,3-dione (4.30 mmol, 1.00 equiv) in EtOAc (43 mL, 0.1 M) was added $Pd(OH)_2/C$ (0.17 g, 1.21 mmol, 0.28 equiv) at room temperature. The resulting mixture was stirred for 4 h at room temperature under hydrogen atmosphere. The mixture was filtered through a pad of Celite and washed with EtOAc. The filtrate was concentrated to give 5-(2-aminophenoxy)-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (1.5156 g, 96.5% yield) as a yellow solid that was taken on without further purification. 1H NMR (300 MHz, DMSO-d6) δ 11.11 (s, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.34 – 7.22 (m, 1H), 7.17 (d, J = 2.3 Hz, 1H), 7.09 – 7.01 (m, 1H), 7.00 – 6.93 (m, 1H), 6.90 –

6.83 (m, 1H), 6.68 – 6.58 (m, 1H), 5.20 – 5.05 (m, 3H), 2.99 – 2.80 (m, 1H), 2.77 – 2.54 (m, 2H), 2.11 – 1.97 (m, 1H). LCMS: 366.15 (M+1).

To a vial containing 5-(2-aminophenoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (0.055 mmol, 1 equiv) in THF (0.55 mL, 0.1 M) was added 4-methoxybenzoyl chloride (0.11 mmoL, 2 equiv) followed by DIPEA (0.165 mmol, 3 equiv). The reaction mixture was allowed to stir at room temperature for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give N-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)oxy)phenyl)-4-methoxybenzamide (SJ42102, 5.3 mgs, 19% yield). LCMS: 500.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C27H22N3O7, 500.1453; found, 500.1451.



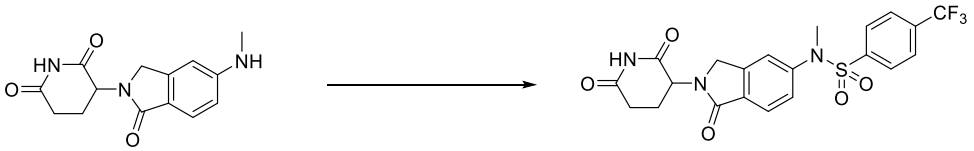
To a flask containing 3-(4-hydroxy-1-oxoisoindolin-2-yl)piperidine-2,6-dione (7.69 mmol, 1.00 equiv) in DMA (30 mL, 0.25 M) was added K_2CO_3 (23.1 mmol, 3.00 equiv) and tert-butyl 4-((tosyloxy)methyl)piperidine-1-carboxylate (9.22 mmol, 1.20 equiv) at 25 °C. The reaction was heated to 50 °C and stirred for 12 hrs. UPLC showed the complete consumption of starting material. The crude reaction mixture was filtered through a pad of Celite and washed with

EtOAc. The filtrate was concentrated then taken up in DMSO and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 20 min. Desired fractions were concentrated to give tert-butyl 4-(((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)oxy)methyl)piperidine-1-carboxylate (1.20 g, 2.62 mmol, 34.1% yield) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ = ppm 10.98 (s, 1H), 7.43 - 7.53 (m, 1H), 7.31 (d, J = 7.4 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.18-4.46 (m, 2H), 3.99 (br d, J = 6.4 Hz, 4H), 2.86-2.99 (m, 1H), 2.76 (br d, J = 5.5 Hz, 2H), 2.59 (br d, J = 18.1 Hz, 1H), 2.39-2.48 (m, 1H), 1.87 - 2.05 (m, 2H), 1.78 (br d, J = 12.6 Hz, 2H), 1.40 (s, 9H), 1.09 - 1.28 ppm (m, 2H).

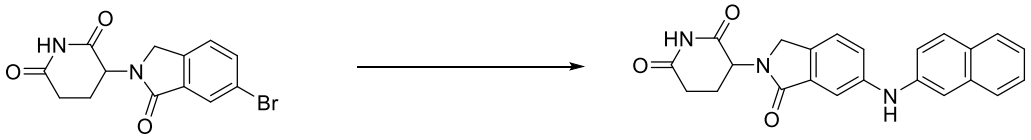
To a flask containing tert-butyl 4-(((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)oxy)methyl)piperidine-1-carboxylate (2.62 mmol, 1.00 equiv) was added HCl/EtOAc (4M, 30 mL, 45 equiv). The reaction mixture was stirred at 25 °C for 0.5 h. UPLC showed complete consumption of the starting material. The reaction mixture was filtered and washed with ACN. The filter cake was dried under high vac to give 3-(1-oxo-4-(piperidin-4-ylmethoxy)isoindolin-2-yl)piperidine-2,6-dione (0.925 g, 98% yield, 99.3% purity, HCl) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ = 10.99 (s, 1H), 9.14 (br d, J = 9.0 Hz, 1H), 8.86 (br d, J = 9.8 Hz, 1H), 7.40 - 7.55 (m, 1H), 7.20 - 7.38 (m, 2H), 5.12 (br dd, J = 13.3, 4.9 Hz, 1H), 4.32 - 4.46 (m, 1H), 4.13 - 4.30 (m, 1H), 4.00 (br d, J = 6.1 Hz, 2H), 3.27 (br d, J = 11.9 Hz, 2H), 2.78-3.04 (m, 3H), 2.59 (br d, J = 17.3 Hz, 1H), 2.42 (br dd, J = 13.1, 4.0 Hz, 1H), 1.85 - 2.17 (m, 4H), 1.55 ppm (q, J = 11.3 Hz, 2H). LCMS: 358.0 (M+H)

To a vial containing 3-(1-oxo-4-(piperidin-4-ylmethoxy)isoindolin-2-yl)piperidine-2,6-dione (0.060 mmol, 1 equiv) in MeOH:AcOH (100:1, 0.60 mL, 0.1 M) was added benzo[d][1,3]dioxole-5-carbaldehyde (0.060 mmoL, 1 equiv) followed by sodium cyanoborohydride (0.060 mmol, 1 equiv). The reaction mixture was heated to 50 °C for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then was

taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(4-((1-(benzo[d][1,3]dioxol-5-ylmethyl)piperidin-4-yl)methoxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (SJ42618, 5.3 mgs, 18% yield). LCMS: 492.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C27H30N3O6, 492.2129; found, 492.2130.

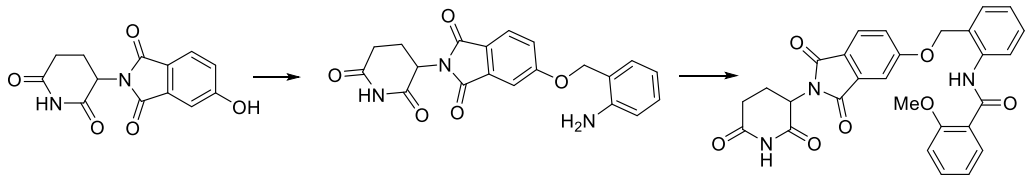


To a vial containing 3-(5-(methylamino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in pyridine (0.750 mL, 0.1 M) was added 4-(trifluoromethyl)benzenesulfonyl chloride (0.150 mmol, 2 equiv.) The reaction was heated to 80 °C and stirred overnight under nitrogen. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)-N-methyl-4-(trifluoromethyl)benzenesulfonamide (SJ44537, 13.7 mgs, 38% yield). LCMS: 482.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H19F3N3O5S, 482.0992; found, 482.099.



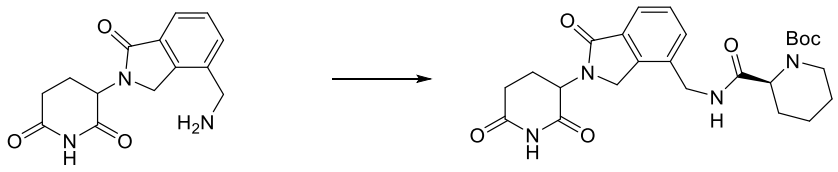
To a vial containing 3-(6-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in 1,4-Dioxane (0.750 mL, 0.1 M) was added naphthalen-2-amine (0.113 mmol, 1.5 equiv) followed by BrettPhos Pd G3 (0.0075 mmol, 0.1 equiv), BrettPhos (0.0075 mmol, 0.1 equiv), and K_2CO_3 (0.225 mmol, 3 equiv). The reaction was heated to 80 °C and stirred overnight under nitrogen. UPLC showed the complete consumption of the starting material.

The crude reaction mixture was filtered through Celite, and washed with EtOAc. The filtrate was concentrated then taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(6-(naphthalen-2-ylamino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (SJ10493, 10.9 mgs, 38% yield). LCMS: 386.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H20N3O3, 386.1499; found, 386.1499.

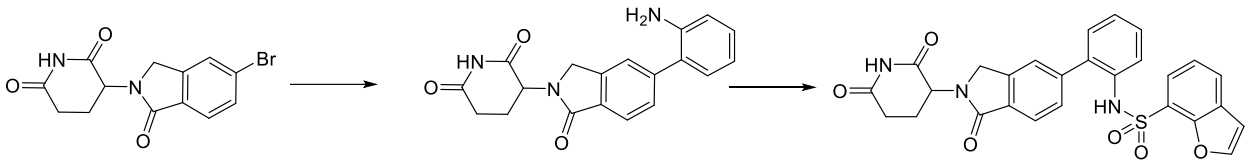


To a flask containing 2-(2,6-dioxopiperidin-3-yl)-5-hydroxy-2,3-dihydro-1H-isoindole-1,3-dione (6.00 g, 21.80 mmol, 1.00 equiv) in THF (300 mL, 0.72 M) was added (2-aminophenyl)mehtanol (26.0 mmol, 1.2 equiv) followed by PPh_3 (32.6 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 15 minutes then cooled to 0 ^oC and DIAD (32.6 mmol, 1.5 equiv) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 1 h. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through and pad of Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 5-((2-aminobenzyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (1.53 g, 18.4% yield) as a white solid. 1H NMR (300 MHz, DMSO-d6) δ 11.12 (s, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H), 7.44 (dd, J = 8.3, 2.3 Hz, 1H), 7.26 – 7.15 (m, 1H), 7.06 (td, J = 7.6, 1.6 Hz, 1H), 6.70 (dd, J = 8.1, 1.2 Hz, 1H), 6.57 (td, J = 7.4, 1.2 Hz, 1H), 5.20 – 5.07 (m, 5H), 2.90 (m, 1H), 2.67 – 2.52 (m, 2H), 2.11 – 1.99 (m, 1H). LCMS: 380.1 (M+1).

To a vial containing 5-((2-aminobenzyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (0.053 mmol, 1 equiv) in DMF (0.55 mL, 0.1 M) was added 2-methoxybenzoyl chloride (0.053 mmoL, 1 equiv) followed by DIPEA (0.265 mmol, 5 equiv). The reaction mixture was allowed to stir at room temperature for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give N-(2-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)oxy)methyl)phenyl)-2-methoxybenzamide (SJ42056, 4.5 mgs, 17% yield). LCMS: 514.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C28H24N3O7, 514.1609; found, 514.1606.



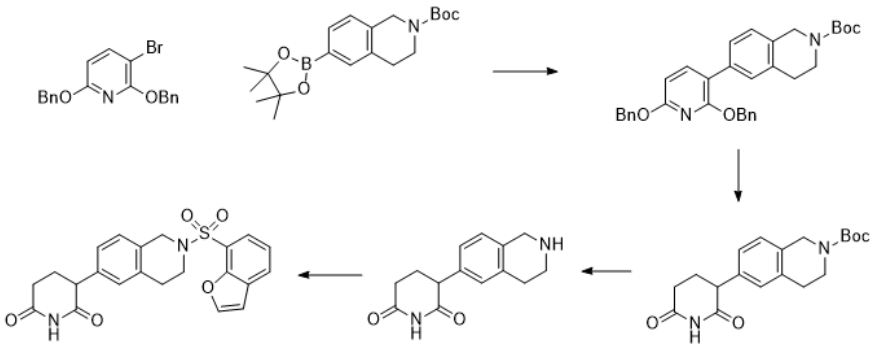
To a vial containing 3-(4-(aminomethyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in DMF (0.75 mL, 0.1 M) was added HBTU (0.098 mmol, 1.3 equiv), (S)-1-(tert-butoxycarbonyl)piperidine-2-carboxylic acid (0.090 mmoL, 1.2 equiv) followed by DIPEA (0.375 mmol, 5 equiv). The reaction mixture was allowed to stir at room temperature overnight. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give tert-butyl (2S)-2-(((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)methyl)carbamoyl)piperidine-1-carboxylate (SJ42229, 18.5 mgs, 51% yield). LCMS: 485.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C25H33N4O6, 485.2395; found, 485.2380.



To a flask containing 3-(5-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15.5 mmol, 1.00 equiv) in 1,4-Dioxane:H_2O (30:1) (100 mL, 0.15 M) was added (2-aminophenyl)boronic acid (20.1 mmol, 1.30 equiv) followed by Na_2CO_3 (40.4 mmol, 2.61 equiv) and $Pd(dppf)Cl_2$ (774 umol, 0.05 equiv). The flask was degassed and purged with N_2 for 3 times at 25 °C, and then the mixture was stirred at 100 °C for 12 h under N_2 atmosphere. UPLC showed the complete consumption of the starting material. The reaction mixture was allowed to cool to room temperature and was then filtered. The filter cake was washed with ACN (100 mL) and THF (100 mL). Then the solid was dissolved in H_2O (150 mL) in a 500 mL single-necked round bottom flask, filtered and the filter cake was dried. The filter cake was washed with ACN (100 mL) and dried to give 3-(5-(2-aminophenyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (4.11 g, 76.4% yield) as a gray solid. 1H NMR (400 MHz, DMSO-d6) δ = ppm 11.00 (s, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.65 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.00 - 7.13 (m, 2H), 6.79 (d, J = 7.5 Hz, 1H), 6.61-6.71 (m, 1H), 5.15 (dd, J = 13.3, 5.1 Hz, 1H), 4.92 (s, 2H), 4.26 - 4.59 (m, 2H), 2.82 - 3.04 (m, 1H), 2.56 - 2.67 (m, 1H), 2.36 - 2.48 (m, 1H), 1.93 - 2.10 ppm (m, 1H). LCMS: 336.0 (M+H).

To a vial containing 3-(5-(2-aminophenyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (0.060 mmol, 1 equiv) in pyridine (0.600 mL, 0.1 M) was added benzofuran-7-sulfonyl chloride (0.120 mmol, 2 equiv.) The reaction was stirred overnight at room temperature under nitrogen. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give N-(2-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)phenyl)benzofuran-7-sulfonamide (SJ42523,

11.3 mgs, 37% yield). LCMS: 516.4 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C27H22N3O6S, 516.1224; found, 516.1224.

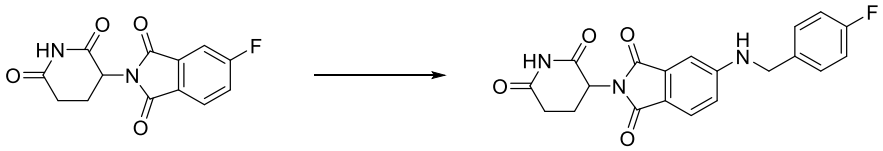


To a vial containing 2,6-bis(benzyloxy)-3-bromopyridine (0.278 mmol, 1 equiv) in 1,4-Dioxane:H_2O (6:1) (1.3 mL, 0.2 M) was added tert-butyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (0.278 mmol, 1 equiv) followed by K_3PO_4 (0.696 mmol, 2.5 equiv) and Pd(dppf)Cl_2 (0.028 mmol, 0.1 equiv). The reaction was heated to 100 ^oC and stirred for 2 hrs. UPLC showed complete comsumption of the starting material. The reaction mixture was cooled to room temperature, filtered through a plug of Celite and washed with EtOAc. The filtrate was concentrated then purified by silica gel chromatography eluted with petroleum ether/ethyl acetate = 20:1 ~ 4:1. Desired fractions were concentrated to give tert-butyl 6-(2,6-bis(benzyloxy)pyridin-3-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (100 mg, 69% yield) as a white solid. LCMS: 523 (M+1).

To a vial containing tert-butyl 6-(2,6-bis(benzyloxy)pyridin-3-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (0.153 mmol, 1 equiv) in THF (1.53 mL, 0.05 M) was added 10% Pd/C (0.153 mmol, 1 equiv). The reaction mixture was degassed then purged with H_2 three times. The reaction was stirred under H_2 for 18 hrs. UPLC showed complete consumption of the starting material. The crude material was filtered through a plug of Celite and washed with EtOAc. The filtrate was concentrated to give tert-butyl 6-(2,6-dioxopiperidin-3-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (50 mgs, 95% yield) as a red oil and was carried on without further purification. LCMS: 345 (M+1).

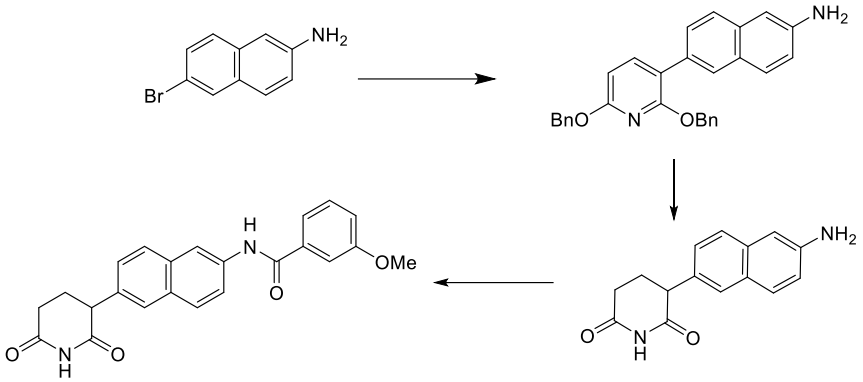
To a vial containing tert-butyl 6-(2,6-dioxopiperidin-3-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (0.145 mmol, 1 equiv) in DCM (1.53 mL, 0.05 M) was added TFA (0.59 mL, 50 equiv). The reaction mixture was stirred at room temperature for 1 h. UPLC showed complete consumption of the starting material. The crude reaction mixture was concentrated to dryness to give 3-(1,2,3,4-tetrahydroisoquinolin-6-yl)piperidine-2,6-dione (30 mg, 85% yield) as a red solid. The crude product was taken on without further purfication. LCMS: 245 (M+1).

To a vial containing 3-(1,2,3,4-tetrahydroisoquinolin-6-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in DMF (0.750 mL, 0.1 M) was added benzofuran-7-sulfonyl chloride (0.113 mmol, 1.5 equiv.) followed by triethylamine (0.375 mmol, 5 equiv). The reaction was stirred overnight at room temperature under nitrogen. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite, washed with DMSO (0.5 mL), and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(2-(benzofuran-7-ylsulfonyl)-1,2,3,4-tetrahydroisoquinolin-6-yl)piperidine-2,6-dione (SJ41964, 7.1 mgs, 22% yield). LCMS: 425.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C22H21N2O5S, 425.1166; found, 425.1166.



To a vial containing 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (0.100 mmol, 1 equiv) in NMP (0.350 mL, 0.5 M) was added (4-fluorophenyl)methanamine (0.120 mmol, 1.2 equiv.) followed by DIPEA (0.200 mmol, 2 equiv). The reaction was heated to 90 °C overnight under nitrogen. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite, washed with DMSO (0.5 mL), and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 2-(2,6-dioxopiperidin-3-yl)-5-((4-

fluorobenzyl)amino)isoindoline-1,3-dione (SJ07066, 5.2 mgs, 14% yield) as a yellow solid. LCMS: 382.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H17FN3O4, 382.1198; found, 382.1190.

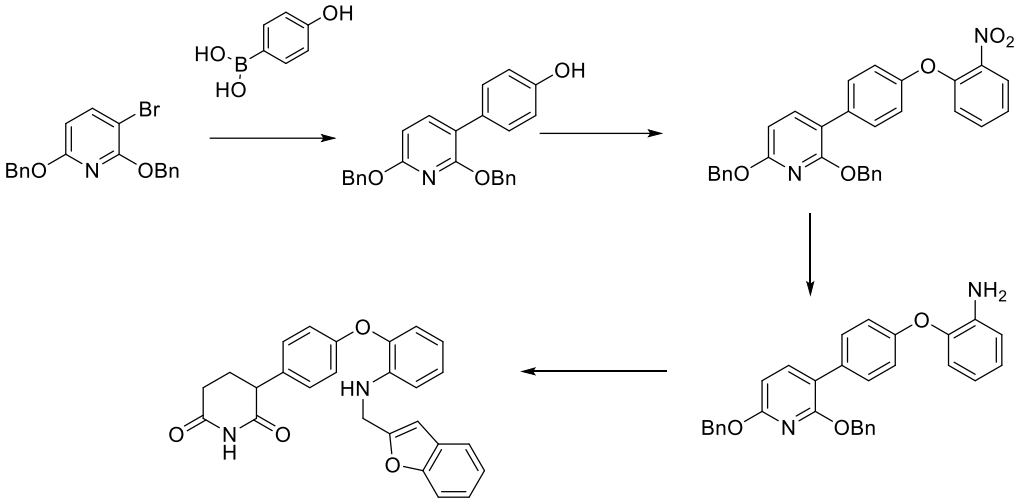


To a flask containing 6-bromonaphthalen-2-amine (13.5 mmol, 1.00 equiv), in 1,4-Dioxane:H_20 (5:1) (36 mL, 0.35 M) was added 2,6-bis(benzyloxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (13.5 mmol, 1.00 equiv), followed by Na_2CO_3 (28.4 mmol, 2.10 equiv) and Pd(dppf)Cl_2 (0.683 mmol, 0.05 equiv). The reaction mixture was heated to 90 °C and stirred for 3 hrs. UPLC showed complete consumption of the starting material. The reaction mixture was cooled to room temperature and was filtered through a pad of Celite and washed with EtOAc. The filtrate was concentrated then purified by silica gel chromatography eluted with petroleum ether/ethyl acetate = 20:1 ~ 4:1. Desired fractions were concentrated to give 6-(2,6-bis(benzyloxy)pyridin-3-yl)naphthalen-2-amine (3.50 g, 59.9% yield) as a light yellow solid. LCMS: 433.3 (M+1).

To a flask containing 6-(2,6-bis(benzyloxy)pyridin-3-yl)naphthalen-2-amine (8.32 mmol, 1.00 equiv) in THF:MeOH (10:1) (44 mL, 0.2M) was added 10% Pd/C (8.32 mmol, 1 equiv). The reaction mixture was degassed then purged with H_2 three times. The reaction was heated to 50 °C and stirred under H_2 for 18 hrs. UPLC showed complete consumption of the starting material. The crude material was cooled to roomt temperature, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated then triturated with EtOAc at room temperature for 4 hrs. The mixture was filtered and the solid was dried to give 3-(6-aminonaphthalen-2-yl)piperidine-2,6-dione (1.20 g, 57% yield) as a gray solid. 1HNMR (400

MHz, DMSO-d6) δ 10.84 (s, 1 H), 7.54 (d, J = 8.68 Hz, 1 H), 7.41-7.50 (m, 2 H), 7.13 (dd, J = 8.50, 1.65 Hz, 1 H). LCMS: 255 (M+1).

To a vial containing 3-(6-aminonaphthalen-2-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in DMF (0.75 mL, 0.1 M) was added 3-methoxybenzoyl chloride (0.075 mmoL, 1 equiv) followed by DIPEA (0.15 mmol, 2 equiv). The reaction mixture was allowed to stir at room temperature for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give N-(6-(2,6-dioxopiperidin-3-yl)naphthalen-2-yl)-3-methoxybenzamide (SJ11792, 6.4 mgs, 22% yield) as a white solid. LCMS: 389.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H21N2O4, 389.1496; found, 389.1497.



To a flask containing 2,6-bis(benzyloxy)-3-bromopyridine (2.70 mmol, 1 equiv) in 1,4-Dioxane:H_2O (6:1) (14 mL, 0.2 M) was added (4-hydroxyphenyl)boronic acid (4.05 mmol, 1.5 equiv) followed by K_3PO_4 (6.75 mmol, 2.5 equiv) and $Pd(dppf)Cl_2$ (0.27 mmol, 0.1 equiv). The reaction was heated to 100 ^oC and stirred for 2 hrs. UPLC showed complete consumption of the starting material. The reaction mixture was cooled to room temperature, filtered through a

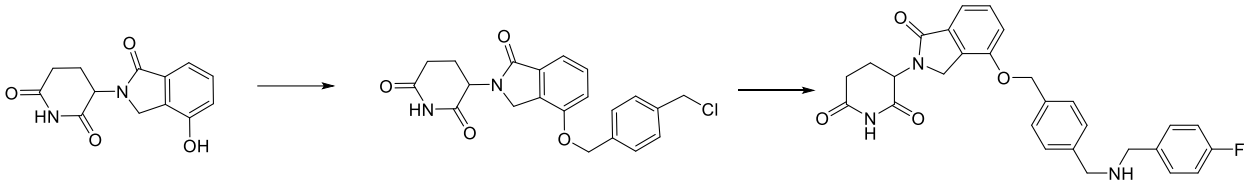
plug of Celite, and washed with EtOAc. The filtrate was concentrated then purified by silica gel chromatography eluting in hexanes/EtOAc 20:1 ~ 1:1. Desired fractions were concentrated to give 4-(2,6-bis(benzyloxy)pyridin-3-yl)phenol (680 mg, 66% yield) as a white solid. LCMS: 384 (M+1).

To a vial containing 4-(2,6-bis(benzyloxy)pyridin-3-yl)phenol (1.77 mmol, 1 equiv) in DMF (20 mL, 0.08 M) was added 1-fluoro-2-nitrobenzene (1.77 mmol, 1 equiv) followed by K_2CO_3 (3.55 mmol, 2 equiv). The reaction mixture was heated to 100 ^oC and stirred for 18 hrs. UPLC showed complete consumption of starting material. The reaction mixture was cooled to room temperature and was quenched with H_2O. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organics were dried of Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography eluting in hexanes/EtOAc 100:0 ~ 70:30. Desired fractions were concentrated to give 2,6-bis(benzyloxy)-3-(4-(2-nitrophenoxy)phenyl)pyridine (680 mg, 76 % yield) as a while solid. LCMS: 505 (M+1).

To a flask containing 2,6-bis(benzyloxy)-3-(4-(2-nitrophenoxy)phenyl)pyridine (1.150 mmol, 1 equiv) in EtOAc (15 ml) was added 10% Pd/C (0.115 mmol, 0.1 equiv). The reaction mixture was degassed then purged with H_2 three times. The reaction was heated to 50 ^oC and stirred under H_2 for 18 hrs. UPLC showed complete consumption of the starting material. The crude material was cooled to room temperature, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated to give 3-(4-(2-aminophenoxy)phenyl)piperidine-2,6-dione (270 mg, 79 % yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.87 (s, 1H), 7.26 – 7.19 (m, 2H), 6.98 (td, J = 7.6, 1.5 Hz, 1H), 6.93 – 6.88 (m, 2H), 6.86 (ddd, J = 7.9, 3.3, 1.6 Hz, 2H), 6.61 (td, J = 7.6, 1.6 Hz, 1H), 4.96 (s, 2H), 3.87 (dd, J = 11.7, 4.9 Hz, 1H), 2.72 (ddd, J = 17.2, 11.9, 5.3 Hz, 1H), 2.53 (t, J = 4.2 Hz, 1H), 2.22 (dtd, J = 13.2, 11.9, 4.4 Hz, 1H), 2.12 – 2.03 (m, 1H). LCMS: 297 (M+1).

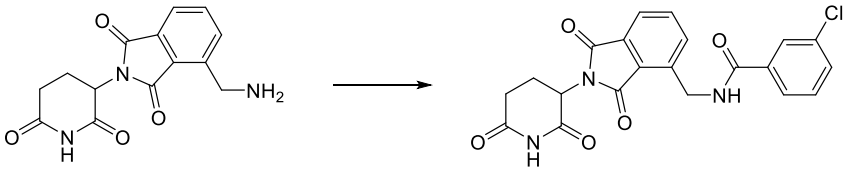
To a vial containing 3-(4-(2-aminophenoxy)phenyl)piperidine-2,6-dione (0.070 mmol, 1 equiv) in MeOH:AcOH (100:1, 0.70 mL, 0.1 M) was added benzofuran-2-carbaldehyde (0.070 mmoL, 1 equiv) followed by sodium cyanoborohydride (0.070 mmol, 1 equiv). The reaction mixture

was heated to 50 ºC for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then was taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(4-(2-((benzofuran-2-ylmethyl)amino)phenoxy)phenyl)piperidine-2,6-dione (SJ41501, 18.7 mgs, 63% yield) as a white solid. LCMS: 427.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C26H23N2O4, 427.1653; found, 427.1654.

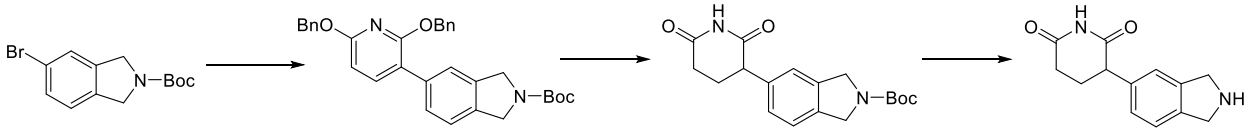


To a flask containing 3-(4-hydroxy-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15.37 mmol, 1.00 equiv) in ACN (100 mL, 0.15 M) was added 1,4-bis(chloromethyl)benzene (46.11 mmol, 3.00 equiv) followed by K_2CO_3 (15.37 mmol, 1.00 equiv). The reaction mixture was heated to 50 °C and stirred for 12 hrs. UPLC showed the complete consumption of the starting material. The reaction mixture was cooled to room temperature, filtered through a plug of Celite and washed with EtOAc. The filtrate was concentrated then taken up in DMSO and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(4-((4-(chloromethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (1.10 g, 17.64% yield) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ = ppm 10.98 (s, 1H), 7.55 - 7.43 (m, 5H), 7.33 (dd, J = 5.7, 7.6 Hz, 2H), 5.27 (s, 2H), 5.12 (dd, J = 5.1, 13.3 Hz, 1H), 4.78 (s, 2H), 4.50 - 4.37 (m, 1H), 4.34 - 4.20 (m, 1H), 3.00 - 2.83 (m, 1H), 2.58 (br d, J = 17.8 Hz, 1H), 2.49 - 2.40 (m, 1H), 2.49 - 2.40 (m, 1H), 2.05 - 1.90 (m, 1H). LCMS: 399.0 (M+H).

To a vial containing 3-(4-((4-(chloromethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (0.050 mmol, 1 equiv) in DMF (1.00 mL, 0.05 M) was added (4-fluorophenyl)methanamine (0.150 mmol, 3 equiv). The reaction mixture was heated to 50 °C and stirred for 48 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(4-((4-(((4-fluorobenzyl)amino)methyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (SJ42509, 6.6 mgs, 27% yield) as a white solid. LCMS: 488.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C28H27FN3O4, 488.1980; found, 488.1980.



To a vial containing 4-(aminomethyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (0.070 mmol, 1 equiv) in DMF (0.700 mL, 0.1 M) was added 3-chlorobenzoyl chloride (0.070 mmoL, 1 equiv) followed by DIPEA (0.21 mmol, 3 equiv). The reaction mixture was allowed to stir at room temperature for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-chloro-N-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)methyl)benzamide (SJ42301, 6.7 mgs, 22% yield) as a white solid. LCMS: 426.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H17ClN3O5, 426.0851; found, 426.0851.

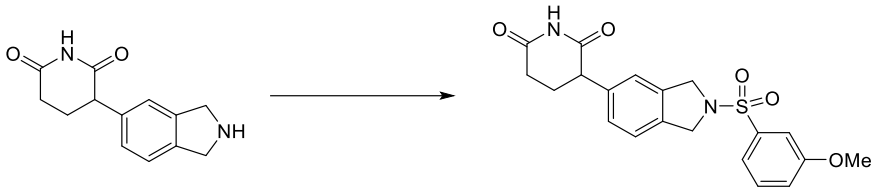


To a flask containing tert-butyl 5-bromoisoindoline-2-carboxylate (40.24 mmol, 1 equiv) in 1,4-Dioxane:H_2O (10:1) (132 mL, 0.3M) was added 2,6-dibenzyloxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (44.27 mmol, 1.1 equiv) followed by K_3PO_4 (120.73 mmol, 3 equiv) and Pd(dppf)Cl_2 (2.01 mmol, 0.05 equiv). The reaction mixture was heated to 110 °C for 12 hrs. UPLC showed the complete consumption of the starting material. The reaction mixture was cooled to room temperature, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated then purified by silica gel chromatography hexane/EtOAc 100:0 ~ 30:1. Desired fractions were concentrated to give tert-butyl 5-(2,6-bis(benzyloxy)pyridin-3-yl)isoindoline-2-carboxylate (20 g, 98% yield) was obtained as yellow oil. LCMS: 509 (M+1).

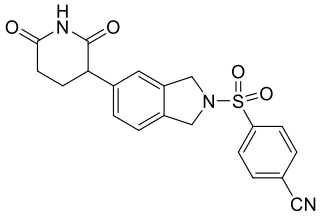
To a flask containing tert-butyl 5-(2,6-bis(benzyloxy)pyridin-3-yl)isoindoline-2-carboxylate (19.66 mmol, 1 equiv) in THF:IPA (3:1) (400 mL, 0.05 M) was added 10% Pd/C (4.92 mmol, 0.25 equiv). The reaction mixture was degassed then purged with H2 three times. The reaction was heated to 50 ^oC and stirred under H_2 for 18 hrs. UPLC showed complete consumption of the starting material. The crude material was cooled to room temperature, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated the purified by silica gel chromatography eluting with hexanes/EtOAc 100:0 ~ 2:1. Desired fractions were concentrated to give tert-butyl 5-(2,6-dioxopiperidin-3-yl)isoindoline-2-carboxylate (2.6 g, 40% yield) was obtained as white solid. LCMS: 331 (M+1)

To a flask containing tert-butyl 5-(2,6-dioxopiperidin-3-yl)isoindoline-2-carboxylate (7.87 mmol, 1 equiv) was added HCl/EtOAc (4 M) (26.00 mL, 13.22 equiv). The reaction mixture stirred at room temperature for 2 h. UPLC showed the complete consumption of the starting material. The reaction mixture was concentrated the triturated in EtOAc at room temperature for 1 h. The solids were filtered and the filter cake was dried under vacuum to give 3-(isoindolin-5-yl)piperidine-2,6-dione (2.01 g, 95.42% yield) was obtained as a gray solid. LCMS: 231 (M+1).

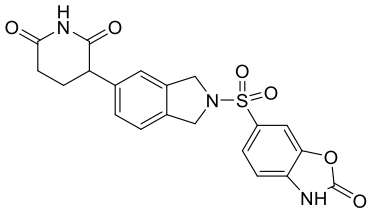
General Procedure A



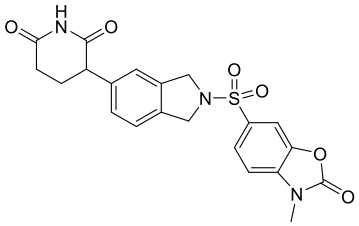
To a vial containing 3-(isoindolin-5-yl)piperidine-2,6-dione (0.085 mmol, 1 equiv) in DMF (0.600 mL, 0.1 M) was added triethylamine (0.425 mmoL, 5 equiv) followed by 3-methoxybenzenesulfonyl chloride (0.102 mmol, 1.2 equiv). The reaction mixture was allowed to stir at room temperature for 18 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite, rinsed with DMSO (0.5 mL), and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(2-((3-methoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ41564, 17.9 mgs, 52.6% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.83 (s, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.44 (dt, J = 7.7, 1.3 Hz, 1H), 7.30 (t, J = 2.1 Hz, 1H), 7.28 – 7.19 (m, 2H), 7.14 – 7.08 (m, 2H), 4.57 (s, 2H), 3.83 (s, 3H), 2.70 – 2.59 (m, 2H), 2.46 (t, J = 4.1 Hz, 1H), 2.20 – 2.08 (m, 2H), 1.98 (dq, J = 13.4, 4.9 Hz, 2H). 13C NMR (126 MHz, DMSO) δ 174.61, 173.84, 160.11, 139.35, 137.43, 136.38, 134.93, 131.27, 128.67, 123.40, 123.20, 119.90, 119.61, 112.65, 56.10, 53.92, 53.78, 47.63, 31.85, 26.40. LCMS: 401.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H21N2O5S, 401.1166; found, 401.1165.



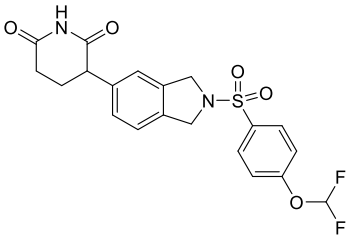
See General Procedure A. 4-((5-(2,6-dioxopiperidin-3-yl)isoindolin-2-yl)sulfonyl)benzonitrile (SJ47879, 10.4 mgs, 31% yield) as a white solid. LCMS: 396.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H18N3O4S, 396.1013; found, 396.1012.



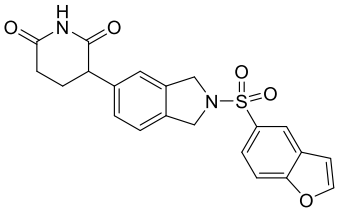
See General Procedure A. 3-(2-((2-oxo-2,3-dihydrobenzo[d]oxazol-6-yl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47469, 21.1 mgs, 58% yield) as a white solid. LCMS: 428.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H18N3O6S, 428.0911; found, 428.0912.



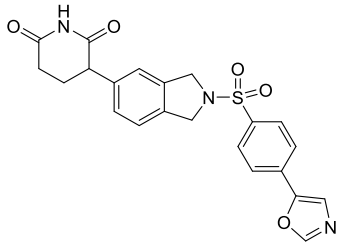
See General Procedure A. 3-(2-((3-methyl-2-oxo-2,3-dihydrobenzo[d]oxazol-6-yl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47471, 11.1 mgs, 30% yield) as a white solid. LCMS: 442.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H20N3O6S, 442.1068; found, 442.1066.



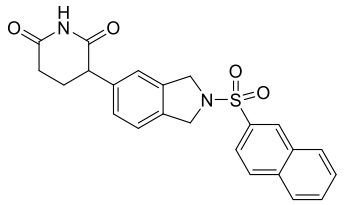
See General Procedure A. 3-(2-((4-(difluoromethoxy)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47483, 9.2 mgs, 25% yield) as a white solid. LCMS: 437.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H19F2N2O5S, 437.0977; found, 437.0976.



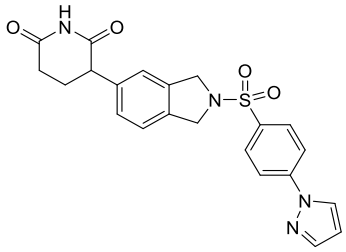
See General Procedure A. 3-(2-(benzofuran-5-ylsulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47491, 7.7 mgs, 22% yield) as a white solid. LCMS: 411.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H19N2O5S, 411.1009; found, 411.1009.



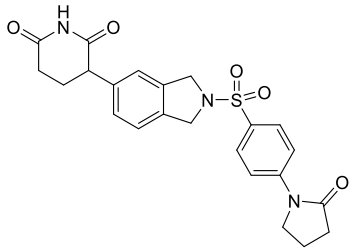
See General Procedure A. 3-(2-((4-(oxazol-5-yl)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47489, 7.0 mgs, 19% yield) as a white solid. LCMS: 438.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C22H20N3O5S, 438.1118; found, 438.1121.



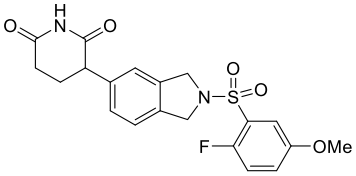
See General Procedure A. 3-(2-(naphthalen-2-ylsulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47877, 12.4 mgs, 35% yield) as a white solid. LCMS: 421.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H21N2O4S, 421.1217; found, 421.1216.



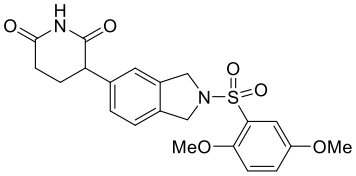
See General Procedure A. 3-(2-((4-(1H-pyrazol-1-yl)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47486, 8.7 mgs, 23% yield) as a white solid. LCMS: 437.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C22H21N4O4S, 437.1278; found, 437.1280.



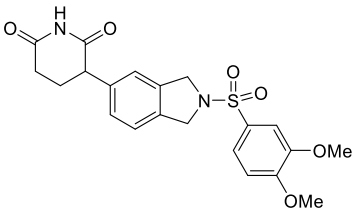
See General Procedure A. 3-(2-((4-(2-oxopyrrolidin-1-yl)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47490, 3.1 mgs, 8% yield) as a white solid. LCMS: 454.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H24N3O5S, 454.1431; found, 454.1433.



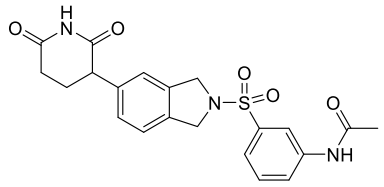
See General Procedure A. 3-(2-((2-fluoro-5-methoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47475, 12 mgs, 34% yield) as a white solid. LCMS: 419.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H20FN2O5S, 419.1072; found, 419.1073.



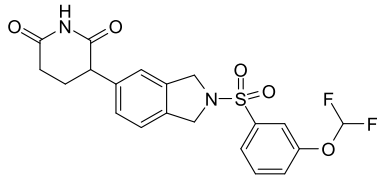
See General Procedure A. 3-(2-((2,5-dimethoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47473, 13.4 mgs, 37% yield) as a white solid. LCMS: 431.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H23N2O6S, 431.1272; found, 431.1274.



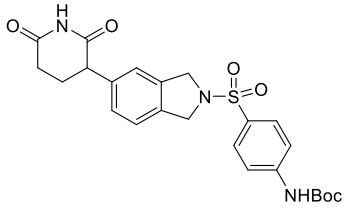
See General Procedure A. 3-(2-((3,4-dimethoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47468, 13.9 mgs, 38% yield) as a white solid. LCMS: 431.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H23N2O6S, 431.1272; found, 431.1266.



See General Procedure A. N-(3-((5-(2,6-dioxopiperidin-3-yl)isoindolin-2-yl)sulfonyl)phenyl)acetamide (SJ47487, 10.9 mgs, 30% yield) as a white solid. LCMS: 428.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H22N3O5S, 428.1275; found, 428.1274.

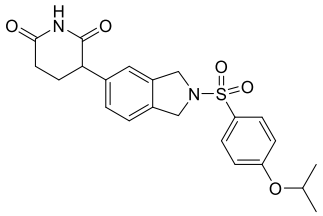


See General Procedure A. 3-(2-((3-(difluoromethoxy)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47474, 13.4 mgs, 36% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.83 (s, 1H), 7.76 (dt, J = 7.8, 1.4 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.60 (t, J = 2.1 Hz, 1H), 7.55 – 7.47 (m, 1H), 7.38 (s, 1H), 7.25 – 7.19 (m, 1H), 7.12 (dt, J = 4.5, 1.9 Hz, 2H), 4.59 (s, 4H), 3.84 (dd, J = 11.7, 5.0 Hz, 1H), 2.65 (ddd, J = 17.2, 12.0, 5.3 Hz, 1H), 2.51 – 2.44 (m, 1H), 2.20 – 2.06 (m, 1H), 2.02 – 1.93 (m, 1H). 13C NMR (126 MHz, DMSO) δ 174.62, 173.84, 151.76 (t, J = 3.8 Hz), 139.39, 138.06, 136.28, 134.84, 132.08, 128.71, 124.54, 123.98, 123.40, 123.20, 117.54, 116.66 (t, J = 260 Hz), 53.90, 53.77, 47.63, 31.86, 26.39. LCMS: 437.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C20H19F2N2O5S, 437.0977; found, 437.0978.

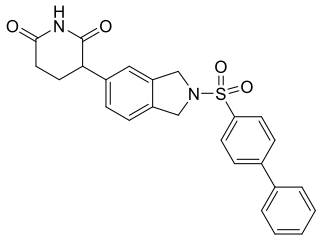


See General Procedure A. tert-butyl (4-((5-(2,6-dioxopiperidin-3-yl)isoindolin-2-yl)sulfonyl)phenyl)carbamate (SJ47485, 25.2 mgs, 61% yield) as a white solid. LCMS: 486.2

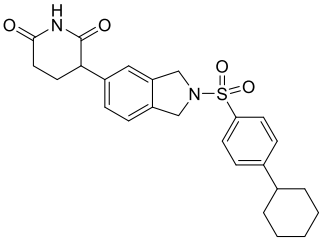
(M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C24H28N3O6S, 486.1694; found, 486.1697.



See General Procedure A. 3-(2-((4-isopropoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47481, 9.8 mgs, 34.2% yield) as a white solid. LCMS: 429.1 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C22H25N2O5S, 429.1479; found, 429.1477.

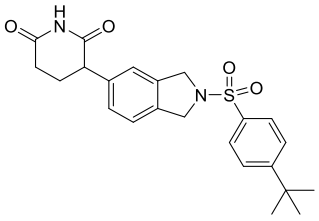


See General Procedure A. 3-(2-([1,1'-biphenyl]-4-ylsulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ48037, 29 mgs, 75% yield) as a white solid. LCMS: 447.3 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C25H23N2O4S, 447.1373; found, 447.1371.

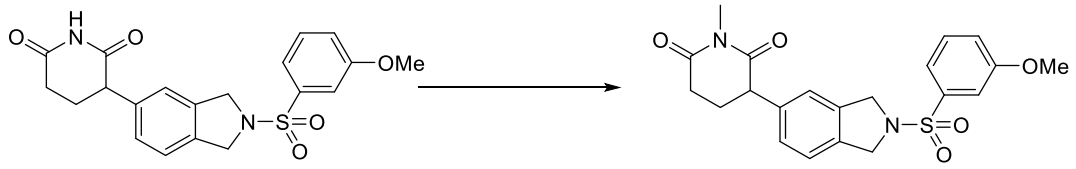


See General Procedure A. 3-(2-((4-cyclohexylphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ46479, 30 mgs, 61% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.83 (s, 1H), 7.81 – 7.76 (m, 2H), 7.47 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.3 Hz, 1H), 7.12-7.09 (m, 2H), 4.54 (s, 4H), 3.83 (dd, J = 11.7, 4.9 Hz, 1H), 2.70 – 2.55 (m, 2H), 2.51 – 2.43 (m, 1H), 2.14 (qd, J = 12.2, 4.5 Hz, 1H), 1.98 (dq, J = 13.7, 4.8 Hz, 1H), 1.80 – 1.74 (m, 4H), 1.69 (d, J = 12.8 Hz, 1H), 1.45 – 1.29 (m, 4H), 1.22 (q, J = 11.7 Hz, 1H). 13C NMR (126 MHz, DMSO) δ 174.61, 173.83, 153.59, 139.36, 136.38, 134.94, 133.80, 128.68, 128.32, 128.10, 123.43,

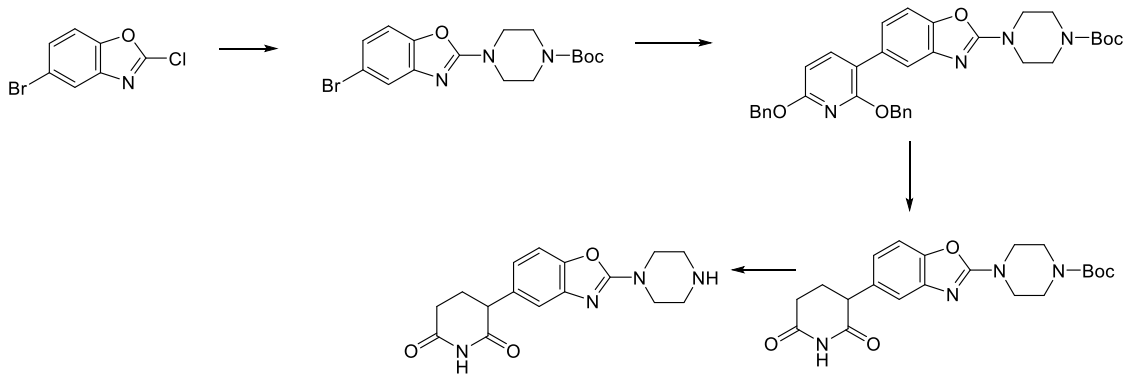
123.22, 53.84, 53.70, 47.63, 44.00, 33.88, 31.85, 26.62, 26.40, 25.90. LCMS: 453.3 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C25H29N2O4S, 453.1843; found, 453.1838.



See General Procedure A. 3-(2-((4-(tert-butyl)phenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (SJ47878, 11.3 mgs, 31% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.83 (s, 1H), 7.83 – 7.77 (m, 2H), 7.68 – 7.61 (m, 2H), 7.24 – 7.19 (m, 1H), 7.12 (s, 1H), 7.10 (d, J = 1.6 Hz, 1H), 4.55 (s, 4H), 3.83 (dd, J = 11.7, 4.9 Hz, 1H), 2.70 – 2.59 (m, 1H), 2.51 – 2.44 (m, 1H), 2.20 – 2.08 (m, 1H), 2.02 – 1.93 (m, 1H), 1.28 (s, 9H). 13C NMR (126 MHz, DMSO) δ 174.62, 173.83, 156.70, 139.39, 136.37, 134.93, 133.47, 128.70, 127.88, 126.90, 123.44, 123.24, 53.85, 53.71, 47.63, 35.37, 31.87, 31.19, 26.40. LCMS: 427.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H27N2O4S, 427.1686; found, 427.1684.



To a vial containing 3-(2-((3-methoxyphenyl)sulfonyl)isoindolin-5-yl)piperidine-2,6-dione (0.045 mmol, 1 equiv) in DMF (0.450 mL, 0.1 M) was added iodomethane (0.054 mmoL, 1.2 equiv) followed by K_2CO_3 (0.054 mmol, 1.2 equiv). The reaction mixture stirred at room temperature for 1 hr. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite, washed with DMSO (0.5 mL), and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated using a TurboVap® LV evaporator to afford 3-(2-((3-methoxyphenyl)sulfonyl)isoindolin-5-yl)-1-methylpiperidine-2,6-dione (SJ48108, 8.6 mgs, 46% yield) as a white solid. LCMS: 415.5 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C21H23N2O5S, 415.1322; found, 415.1319.



To a flask containing 5-bromo-2-chlorobenzo[d]oxazole (17.2 mmol, 1.00 equiv) in DMF (50 mL 0.35 M) was added tert-butyl piperazine-1-carboxylate (20.7 mmol, 1.20 equiv) followed by K_2CO_3 (34.4 mmol, 2.00 equiv). The reaction mixture was heated to 120 ºC and stirred for 2 hrs. UPLC showed the complete consumption of the starting material. The reaction mixture was cooled to room temperature and H_2O was added. The crude mixture was filtered and the solids were then dried under vacuum to give crude tert-butyl 4-(5-bromobenzo[d]oxazol-2-yl)piperazine-1-carboxylate (6 g, 92% yield) as a white solid. LCMS: 382 (M+1)

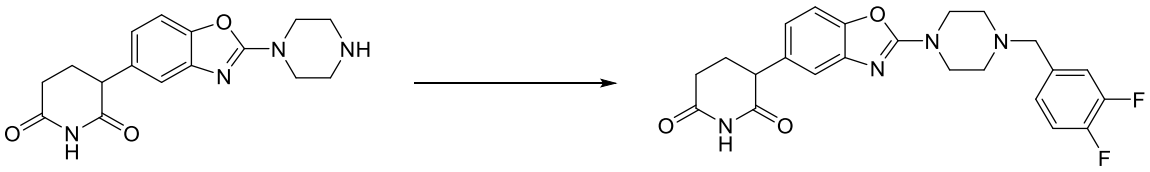
To a flask containing tert-butyl 4-(5-bromobenzo[d]oxazol-2-yl)piperazine-1-carboxylate (6.00 g, 15.7 mmol, 1.00 eq) in 1,4-Dioxane:H_2O (4:1) (50 mL, 0.3 M) was added K_3PO_4 (47.1 mmol, 3.00 equiv), 2,6-dibenzyloxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (17.3 mmol, 1.10 equiv) and Pd(dppf)Cl_2 (1.57 mmol, 0.100 equiv). The reaction mixture was heated to 90 ºC for 2 hrs. UPLC showed the complete consumption of the starting material. The reaction was allowed to cool to room temperature then H_2O and EtOAc were added. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organics were dried over Na_2SO_4, filtered, and concentration. The crude product was purified by silica gel chromatography eluting with hexanes/EtOAc 100:0 ~ 50:50. Desired fractions were concentrated to give tert-butyl 4-(5-(2,6-bis(benzyloxy)pyridin-3-yl)benzo[d]oxazol-2-yl)piperazine-1-carboxylate (6.57 g, 65.19% yield) as a white solid. LCMS: 593 (M+1).

To a flask containing tert-butyl 4-(5-(2,6-bis(benzyloxy)pyridin-3-yl)benzo[d]oxazol-2-yl)piperazine-1-carboxylate 6.50 g, 11.0 mmol, 1.00 eq) in THF (50 mL, 0.2 M) was added 10 % Pd/C (11.0 mmol, 1 equiv). The reaction mixture was degassed then purged with H_2 three

times. The reaction was heated to 50 °C and stirred under H_2 for 18 hrs. UPLC showed complete consumption of the starting material. The crude material was cooled to room temperature, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated to give tert-butyl 4-(5-(2,6-dioxopiperidin-3-yl)benzo[d]oxazol-2-yl)piperazine-1-carboxylate (4.5 g, 98% yield crude) as a white solid. LCMS: 415 (M+1).

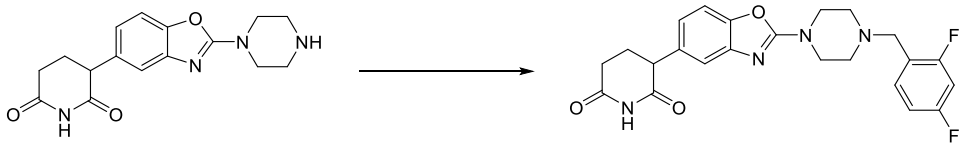
To a flask containing tert-butyl 4-(5-(2,6-dioxopiperidin-3-yl)benzo[d]oxazol-2-yl)piperazine-1-carboxylate (10.0 mmol, 1 eq) was added HCl/EtOAc (4 M, 40 mL, 15.98 eq). The reaction was stirred at 15 °C for 1 hr. UPLC showed the complete conversion to product. The reaction mixture was filtered and the filter cake was dried under reduced pressure. The crude product was taken up in DMSO and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(2-(piperazin-1-yl)benzo[d]oxazol-5-yl)piperidine-2,6-dione (2.34 g, 62.15% yield, 95.68% purity, FA) as a white solid.

General Procedure B

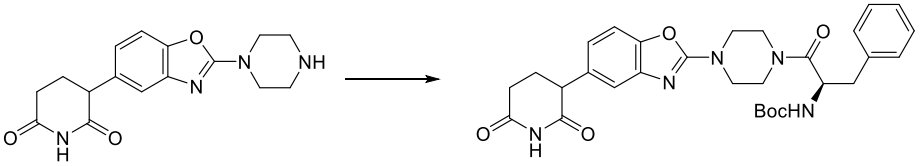


To a vial containing 3-(2-(piperazin-1-yl)benzo[d]oxazol-5-yl)piperidine-2,6-dione (0.111 mmol, 1 equiv) in MeOH:AcOH (100:1, 1.100 mL, 0.1 M) was added 3,4-difluorobenzaldehyde (0.111 mmoL, 1 equiv) followed by sodium cyanoborohydride (0.111 mmol, 1 equiv). The reaction mixture was heated to 50 °C for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then was taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(2-(4-(3,4-difluorobenzyl)piperazin-1-

yl)benzo[d]oxazol-5-yl)piperidine-2,6-dione (SJ41813, 11.0 mgs, 22% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.82 (s, 1H), 7.45 – 7.36 (m, 2H), 7.34 (d, J = 8.2 Hz, 1H), 7.23 – 7.17 (m, 1H), 7.15 (d, J = 1.7 Hz, 1H), 6.87 (dd, J = 8.2, 1.8 Hz, 1H), 3.88 (dd, J = 11.6, 4.9 Hz, 1H), 3.64 – 3.59 (m, 4H), 3.54 (s, 2H), 2.67 (ddd, J = 17.2, 11.9, 5.2 Hz, 1H), 2.49 (s, 5H), 2.29 – 2.18 (m, 1H), 2.05 (dp, J = 13.6, 5.1 Hz, 1H). 13C NMR (126 MHz, DMSO) δ 174.94, 173.93, 162.58, 150.37 (dd, J = 102, 13 Hz), 148.42 (dd, J = 101, 13 Hz), 147.79, 143.50, 136.35, 135.54 (dd, J = 5.8, 3.1 Hz), 125.88 (dd, J = 6.6, 3.3 Hz), 121.49, 117.81 (dd, J = 36, 17 Hz), 116.44, 108.92, 60.94, 52.03, 47.85, 45.80, 31.95, 26.74. LCMS: 441.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H23F2N4O3, 441.1733; found, 441.1731.

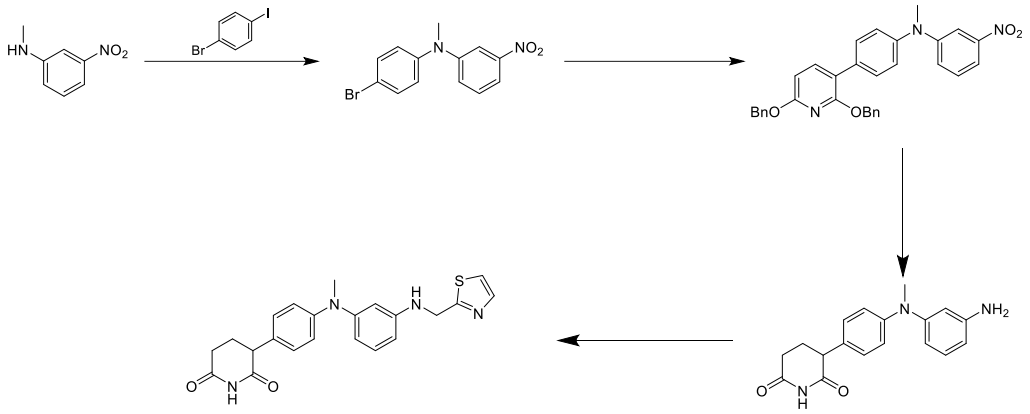


See General Procedure B. 3-(2-(4-(2,4-difluorobenzyl)piperazin-1-yl)benzo[d]oxazol-5-yl)piperidine-2,6-dione (SJ41824, 9.8 mgs, 34.2% yield) as a white solid. 1H NMR (500 MHz, DMSO) δ 10.82 (s, 1H), 7.49 (td, J = 8.5, 6.7 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.23 (td, J = 9.7, 2.6 Hz, 1H), 7.14 (d, J = 1.7 Hz, 1H), 7.13 – 7.06 (m, 1H), 6.87 (dd, J = 8.2, 1.8 Hz, 1H), 3.88 (dd, J = 11.6, 4.9 Hz, 1H), 3.59 (dd, J = 10.8, 5.7 Hz, 6H), 2.67 (ddd, J = 17.2, 11.9, 5.3 Hz, 1H), 2.52-2.46 (s, 5H), 2.29 – 2.18 (m, 1H), 2.05 (qd, J = 10.2, 5.5 Hz, 1H). 13C NMR (126 MHz, DMSO) δ 174.93, 173.93, 162.61 (dd, J = 60, 12 Hz), 162.57, 160.66 (dd, J = 97, 12 Hz), 147.79, 143.49, 135.54, 133.25 (dd, J = 9.8, 6.2 Hz), 121.50, 121.06 (dd, J = 15, 3.7 Hz), 116.44, 111.78 (dd, J = 21, 3.3 Hz), 108.92, 104.20 (t, J = 26 Hz), 54.49, 51.90, 47.85, 45.78, 31.95, 26.74. LCMS: 441.2 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C23H23F2N4O3, 441.1733; found, 441.1735.



To a vial containing 3-(2-(piperazin-1-yl)benzo[d]oxazol-5-yl)piperidine-2,6-dione (0.075 mmol, 1 equiv) in DMF (0.75 mL, 0.1 M) was added HBTU (0.098 mmol, 1.3 equiv), (tert-

butoxycarbonyl)-D-phenylalanine (0.090 mmoL, 1.2 equiv) followed by DIPEA (0.375 mmol, 5 equiv). The reaction mixture was allowed to stir at room temperature overnight. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with DMSO (0.5 mL). The crude material was purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give tert-butyl ((2R)-1-(4-(5-(2,6-dioxopiperidin-3-yl)benzo[d]oxazol-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (SJ42752, 16.4 mgs, 39% yield) as a white solid. LCMS: 562.3 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C30H36N5O6, 562.266; found, 562.2656.



To a solution of N-methyl-3-nitroaniline (66 mmol, 1 *equiv*) in 1,4-Dioxane (150 mL) were added 1-bromo-4-iodo-benzene (72 mmol, 1.1 *equiv*), Cs_2CO_3 (164 mmol, 2.5 *equiv*), BINAP (8 mmol, 0.12 *equiv*) and $Pd(OAc)_2$ (5.2 mmol, 0.08 *equiv*). The reaction was stirred at 110 °C under the protection of N_2 overnight. UPLC showed the complete consumption of the starting material. The solution was concentrated under reduced pressure to give a residue. H_2O (400 mL) was added, the product was extracted with EtOAc (150 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 1/0 to 4/1) to give N-(4-bromophenyl)-N-methyl-3-nitroaniline (20 g, 99% yield) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (br d, J = 8.0 Hz, 1H), 7.64 -

7.56 (m, 3H), 7.51 (br t, *J* = 8.0 Hz, 1H), 7.34 (br d, *J* = 7.8 Hz, 1H), 7.19 (br d, *J* = 8.0 Hz, 2H), 3.35 (s, 3H).

To a solution of N-(4-bromophenyl)-N-methyl-3-nitroaniline (37.4 mmol, 1 *equiv*) in 1,4-Dioxane:H_2O (5:1 240 mL) were added 2,6-dibenzyloxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (44.9 mmol, 1.20 *equiv*), Pd(dppf)Cl_2 (3.74 mmol, 0.10 *equiv*), and K_2CO_3 (74.9 mmol, 2 *equiv*). The reaction was stirred at 80 °C overnight under N_2. UPLC showed the complete consumption of the starting material. The solution was concentrated under reduced pressure to give a residue. H_2O (300 mL) was added, the product was extracted with EtOAc (150 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography on silica gel (hexanes/EtOAc = 1/0 to 4/1) to give N-(4-(2,6-bis(benzyloxy)pyridin-3-yl)phenyl)-N-methyl-3-nitroaniline (19 g, 98.04% yield) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (br d, *J* = 8.2 Hz, 1H), 7.70 - 7.56 (m, 4H), 7.50 - 7.23 (m, 14H), 6.57 (br d, *J* = 8.0 Hz, 1H), 5.45 (s, 2H), 5.39 (s, 2H), 3.36 (s, 3H).

To a solution of 10% Pd/C (17.4 mmol, 1.00 *equiv*) in THF (200 mL) was added N-(4-(2,6-bis(benzyloxy)pyridin-3-yl)phenyl)-N-methyl-3-nitroaniline (17.4 mmol, 1.00 *equiv*) and the suspension was degassed and purged with H_2 for three times. The reaction was stirred at 50 °C for 12 h under H_2 (50 psi) atmosphere. UPLC showed the complete consumption of the starting material. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by *prep*-HPLC column: FLM Titank C18 Bulk 250 * 70mm 10u; mobile phase: [H_2O (0.02% FA) - MeCN]; gradient: 10%-40% B over 18.0 min to give 3-(4-((3-aminophenyl)(methyl)amino)phenyl)piperidine-2,6-dione (2.5 g, 46.4% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (br s, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.98 - 6.86 (m, 3H), 6.27 (d, *J* = 1.8 Hz, 1H), 6.24 - 6.17 (m, 2H), 5.01 (br s, 2H), 3.76 (dd, *J* = 11.2, 4.8, Hz, 1H), 3.18 (s, 3H), 2.64 (dt, *J* = 11.6, 5.6 Hz, 1H), 2.46 (br t, *J* = 4.4 Hz, 1H), 2.23 - 2.08 (m, 1H), 2.07 - 1.94 (m, 1H)

To a vial containing 3-(4-((3-aminophenyl)(methyl)amino)phenyl)piperidine-2,6-dione (0.060 mmol, 1 equiv) in MeOH:AcOH (100:1, 0.60 mL, 0.1 M) was added thiazole-2-carbaldehyde (0.060 mmoL, 1 equiv) followed by sodium cyanoborohydride (0.060 mmol, 1 equiv). The reaction mixture was heated to 50 ^{o}C for 3 hrs. UPLC showed the complete consumption of the starting material. The crude reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was concentrated then was taken up in DMSO (0.8 mL) and purified by the Waters purification/analytical LC/UV/ELSD system and the gradient program started at 95% (0.1% formic acid in MilliQ H_2O), changed to 90% (0.1% formic acid in Acetonitrile) over 14 min. Desired fractions were concentrated to give 3-(4-(methyl(3-((thiazol-2-ylmethyl)amino)phenyl)amino)phenyl)piperidine-2,6-dione (SJ41458, 4.3 mgs, 17.6% yield). LCMS: 407.15 (M+1), purity > 95%; HRMS (m/z): [M+H]+ calcd. for C22H23N4O2S, 407.1536; found, 407.1533.

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