

Supplementary Materials for

**A potent and selective ENL degrader suppresses oncogenic gene expression
and leukemia progression**

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Supplementary Methods
Figs. S1 to S12
Original blots for Fig. 5A
Legends for tables S1 to S9

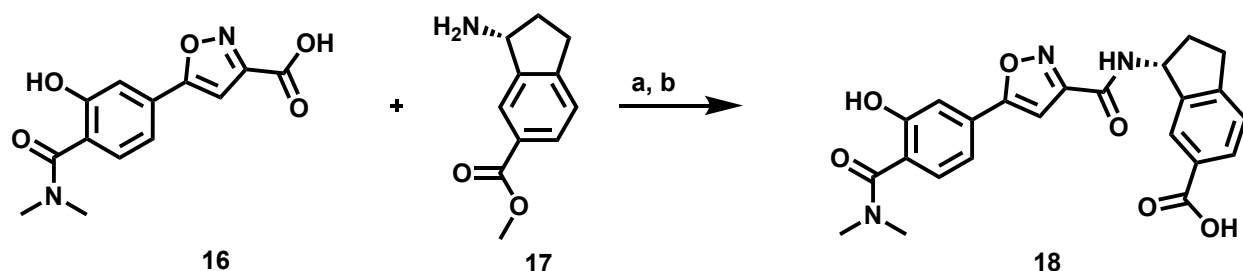
Other Supplementary Material for this manuscript includes the following:

Tables S1 to S9

Supplementary Methods

Synthesis of the ENL degraders and intermediates

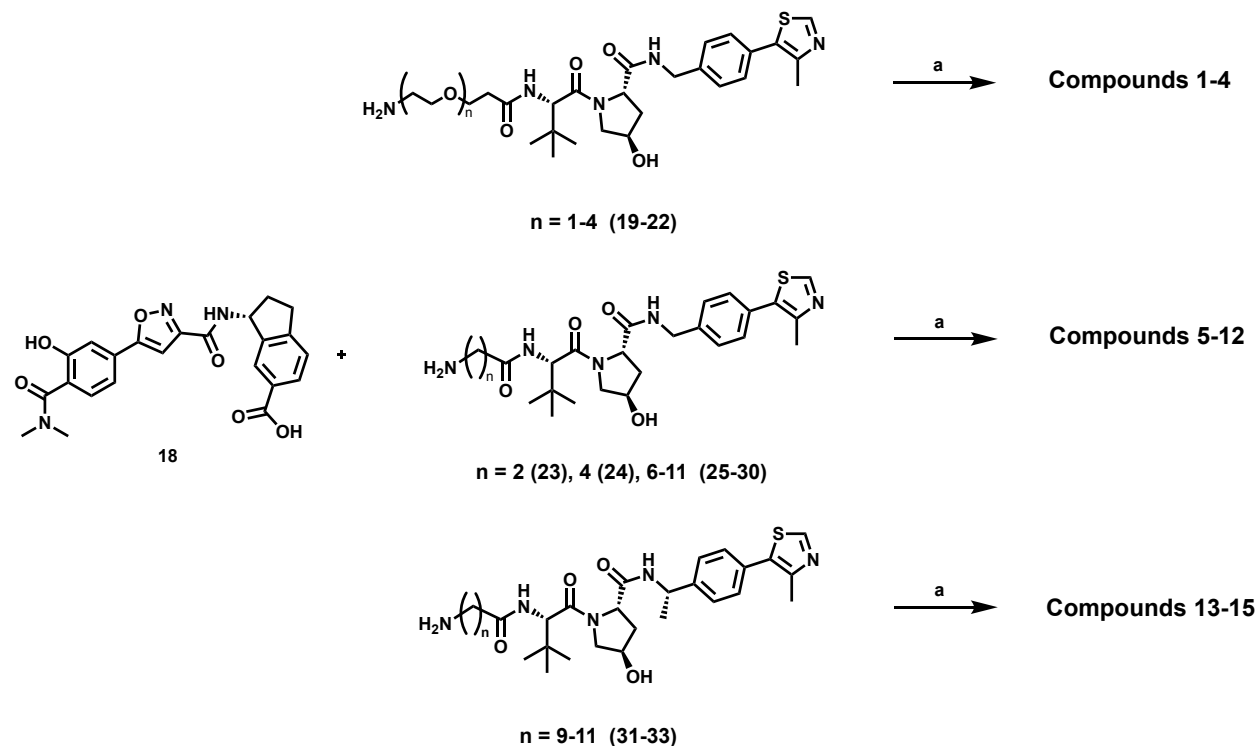
Synthesis of key intermediate 18



Reagent and conditions: a) EDCI, HOAt, NMM, DMSO, rt, 12h, 45% yield. d) LiOH, MeOH/H₂O, rt, 12 h, 90% yield;

(R)-3-(5-(4-(dimethylcarbamoyl)-3-hydroxyphenyl)isoxazole-3-carboxamido)-2,3-dihydro-1H-indene-5-carboxylic acid (18). To a solution of compound **16** (**36**) (570 mg, 2.06 mmol) in DMSO (20 mL) were added commercially available methyl (*R*)-3-amino-2,3-dihydro-1*H*-indene-5-carboxylate (**17**) (393 mg, 2.06 mmol), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide) (593 mg, 3.09 mmol), HOAt (1-hydroxy-7-azabenzotriazole) (420 mg, 3.09 mmol), and NMM (*N*-Methylmorpholine) (624 mg, 6.18 mmol). After being stirred overnight at room temperature, the resulting mixture was purified by reverse phase C18 column (10% - 100% methanol / 0.1% TFA in water) to afford the crude mixture. To a solution of this crude mixture in MeOH (6 mL), was then added the solution of LiOH (74 mg, 3.09 mmol) in H₂O (2 mL) dropwise. The mixture was stirred at rt overnight. Resulting crude mixture was purified by flash column chromatography by using reverse phase C18 column (10% - 100% methanol / 0.1% TFA in water) to yield title compound **18** as a white solid (403mg, two step yield: 45%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.88 – 7.80 (m, 2H), 7.32 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.29 – 7.20 (m, 3H), 7.04 (s, 1H), 5.58 (t, *J* = 7.9 Hz, 1H), 3.10 – 2.94 (m, 4H), 2.94 – 2.79 (m, 4H), 2.60 – 2.48 (m, 1H), 2.08 – 1.98 (m, 1H). MS (ESI) *m/z* [M + H]⁺ calcd for C₂₃H₂₂N₃O₆⁺ 436.1, found 436.1.

Synthesis of ENL degraders.



Reagent and conditions: a) EDCI, HOAt, NMM, DMSO, rt.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-N-((R)-6-((2-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)ethyl)carbamoyl)-2,3-dihydro-1H-inden-1-yl)isoxazole-3-carboxamide (1). To a solution of intermediate **18** (Scheme 1) (5 mg, 0.01 mmol) in DMSO (1 mL) were added (2S,4R)-1-((S)-2-(3-(2-aminoethoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**19**) (6.4 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv). After being stirred overnight at room temperature, the resulting mixture was purified by preparative HPLC (5%-60% acetonitrile / 0.1% TFA in H₂O) to afford compound **1** as a white solid (6.7 mg, 70%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 8.97 (s, 1H), 7.80 – 7.78 (m, 1H), 7.76 – 7.73 (m, 1H), 7.46 – 7.31 (m, 8H), 7.15 (s, 1H), 5.67 (t, *J* = 7.9 Hz, 1H), 4.64 – 4.60 (m, 1H), 4.58 – 4.46 (m, 3H), 4.33 (d, *J* = 15.6 Hz, 1H), 3.86 (d, *J* = 10.9 Hz, 1H), 3.80 – 3.69 (m, 3H), 3.65 – 3.51 (m, 4H), 3.16 – 2.90 (m, 8H), 2.67 – 2.59 (m,

1H), 2.57 – 2.44 (m, 5H), 2.24 – 2.17 (m, 1H), 2.14 – 2.03 (m, 2H), 0.99 (s, 9H). HRMS m/z [$M + H$]⁺ calcd for C₅₀H₅₉N₈O₁₀S⁺ 963.4069, found 963.4070.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-N-((R)-6-((2-(2-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)ethoxy)ethyl)carbamoyl)-2,3-dihydro-1H-inden-1-yl)isoxazole-3-carboxamide (2). Compound **2** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2S,4R)-1-(((S)-2-(3-(2-(2-aminoethoxy)ethoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**20**) (6.9 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **2** was obtained as a white solid (6.5 mg, 65%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.01 (s, 1H), 7.78 (s, 1H), 7.76 – 7.71 (m, 1H), 7.47 – 7.31 (m, 8H), 7.18 (s, 1H), 5.68 (t, *J* = 7.9 Hz, 1H), 4.63 (s, 1H), 4.58 – 4.46 (m, 3H), 4.33 (d, *J* = 15.5 Hz, 1H), 3.86 (d, *J* = 10.8 Hz, 1H), 3.77 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.72 – 3.66 (m, 2H), 3.64 – 3.57 (m, 6H), 3.55 – 3.49 (m, 2H), 3.16 – 2.90 (m, 8H), 2.67 – 2.59 (m, 1H), 2.53 – 2.40 (m, 5H), 2.21 (dd, *J* = 13.1, 7.8 Hz, 1H), 2.16 – 2.03 (m, 2H), 1.01 (s, 9H). HRMS m/z [$M + H$]⁺ calcd for C₅₂H₆₃N₈O₁₁S⁺ 1007.4332, found 1007.4335.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-N-((R)-6-(((S)-14-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-15,15-dimethyl-12-oxo-3,6,9-trioxa-13-azahexadecyl)carbamoyl)-2,3-dihydro-1H-inden-1-yl)isoxazole-3-carboxamide (3) Compound **3** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2S,4R)-1-(((S)-1-amino-14-(*tert*-butyl)-12-oxo-3,6,9-trioxa-13-azapentadecan-15-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**21**) (7.3 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **3** was obtained as a white solid (5.8 mg, 55%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.05 (s, 1H), 7.78 (s, 1H), 7.74 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.48 – 7.45 (m, 2H), 7.44 – 7.39 (m, 3H), 7.37 – 7.32 (m, 3H), 7.17 (s, 1H), 5.69 (t, *J* = 7.9 Hz, 1H), 4.63 (s, 1H), 4.59 – 4.51 (m, 2H), 4.50 – 4.47 (m, 1H), 4.34 (d, *J* = 15.5 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.78 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.70 – 3.50 (m, 14H), 3.16 – 2.91 (m,

8H), 2.68 – 2.59 (m, 1H), 2.53 – 2.45 (m, 4H), 2.45 – 2.36 (m, 1H), 2.24 – 2.18 (m, 1H), 2.14 – 2.03 (m, 2H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{54}H_{67}N_8O_{12}S^+$ 1051.4594, found 1051.4594.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-(((*S*)-17-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-18,18-dimethyl-15-oxo-3,6,9,12-tetraoxa-16-azanonadecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (4). Compound **4** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-((*S*)-1-amino-17-(*tert*-butyl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecan-18-oyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**22**) (7.1 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **4** was obtained as a white solid (7.3 mg, 67%). 1H NMR (600 MHz, Methanol- d_4) δ 9.16 (s, 1H), 7.79 (s, 1H), 7.75 (dd, $J = 7.9, 1.7$ Hz, 1H), 7.50 – 7.46 (m, 2H), 7.44 – 7.40 (m, 3H), 7.38 – 7.32 (m, 3H), 7.16 (s, 1H), 5.69 (t, $J = 7.9$ Hz, 1H), 4.64 (s, 1H), 4.59 – 4.52 (m, 2H), 4.50 – 4.47 (m, 1H), 4.35 (d, $J = 15.5$ Hz, 1H), 3.91 – 3.85 (m, 1H), 3.79 (dd, $J = 11.0, 3.9$ Hz, 1H), 3.73 – 3.51 (m, 18H), 3.17 – 2.92 (m, 8H), 2.68 – 2.60 (m, 1H), 2.57 – 2.50 (m, 1H), 2.49 (s, 3H), 2.47 – 2.42 (m, 1H), 2.25 – 2.19 (m, 1H), 2.15 – 2.04 (m, 2H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{56}H_{71}N_8O_{13}S^+$ 1095.4856, found 1095.4853.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((3-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (5). Compound **5** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-((*S*)-2-(3-aminopropanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**23**) (6 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **5** was obtained as a white solid (6.2 mg, 68%). 1H NMR (600 MHz, Methanol- d_4) δ 9.07 (s, 1H), 7.98 – 7.92 (m, 1H), 7.76 – 7.72 (m, 1H), 7.49 – 7.31 (m, 8H), 7.16 – 7.15 (m, 1H), 5.66 (t, $J =$

7.6 Hz, 1H), 4.59 (s, 1H), 4.57 – 4.46 (m, 3H), 4.34 (d, $J = 15.5$ Hz, 1H), 3.90 (d, $J = 10.9$ Hz, 1H), 3.76 (dd, $J = 11.0, 3.9$ Hz, 1H), 3.67 – 3.53 (m, 2H), 3.18 – 2.88 (m, 8H), 2.68 – 2.52 (m, 3H), 2.48 (s, 3H), 2.20 (dd, $J = 13.3, 7.7$ Hz, 1H), 2.17 – 2.03 (m, 2H), 0.97 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{48}H_{55}N_8O_9S^+$ 919.3807, found 919.3798.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((5-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-5-oxopentyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (6).

Compound **6** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(5-aminopentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**24**) (5.7 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **6** was obtained as a white solid (6.1 mg, 64%). 1H NMR (600 MHz, Methanol- d_4) δ 9.16 (s, 1H), 7.77 (s, 1H), 7.75 – 7.72 (m, 1H), 7.48 (d, $J = 8.2$ Hz, 2H), 7.44 – 7.40 (m, 3H), 7.37 – 7.32 (m, 3H), 7.15 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 4.60 (s, 1H), 4.54 (dd, $J = 16.0, 8.4$ Hz, 2H), 4.50 – 4.46 (m, 1H), 4.35 (d, $J = 15.6$ Hz, 1H), 3.91 – 3.86 (m, 1H), 3.78 (dd, $J = 10.9, 3.9$ Hz, 1H), 3.39 – 3.33 (m, 2H), 3.16 – 2.90 (m, 8H), 2.67 – 2.59 (m, 1H), 2.49 (s, 3H), 2.37 – 2.26 (m, 2H), 2.20 (dd, $J = 13.2, 7.7$ Hz, 1H), 2.14 – 2.03 (m, 2H), 1.73 – 1.58 (m, 4H), 1.01 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{50}H_{59}N_8O_9S^+$ 947.4120, found 947.4125.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((7-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-7-oxoheptyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (7).

Compound **7** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(7-aminoheptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**25**) (5.9 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **7** was obtained as a white solid (6.5 mg, 67%). 1H NMR (600 MHz, Methanol- d_4) δ 9.10 (s, 1H), 7.76 (s, 1H), 7.73 – 7.71 (m, 1H), 7.47 (d, $J = 8.0$ Hz, 2H), 7.44 – 7.39 (m, 3H), 7.37 – 7.31 (m,

3H), 7.15 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 4.61 (s, 1H), 4.59 – 4.47 (m, 3H), 4.35 (d, $J = 15.5$ Hz, 1H), 3.92 – 3.87 (m, 1H), 3.79 (dd, $J = 10.9, 3.9$ Hz, 1H), 3.37 – 3.32 (m, 2H), 3.16 – 2.92 (m, 8H), 2.67 – 2.60 (m, 1H), 2.48 (s, 3H), 2.32 – 2.18 (m, 3H), 2.15 – 2.03 (m, 2H), 1.65 – 1.56 (m, 4H), 1.42 – 1.32 (m, 4H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{52}H_{63}N_8O_9S^+$ 975.4433, found 975.4428.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((8-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (8).

Compound **8** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(8-aminooctanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**26**) (6.7 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **8** was obtained as a white solid (7 mg, 71%). 1H NMR (600 MHz, Methanol- d_4) δ 9.04 (s, 1H), 7.76 (s, 1H), 7.72 (dd, $J = 7.7, 1.7$ Hz, 1H), 7.47 (d, $J = 8.0$ Hz, 2H), 7.43 – 7.40 (m, 3H), 7.37 – 7.31 (m, 3H), 7.15 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 4.62 (s, 1H), 4.59 – 4.47 (m, 3H), 4.35 (d, $J = 15.5$ Hz, 1H), 3.89 (d, $J = 10.9$ Hz, 1H), 3.79 (dd, $J = 10.9, 3.9$ Hz, 1H), 3.39 – 3.32 (m, 2H), 3.16 – 2.93 (m, 8H), 2.68 – 2.60 (m, 1H), 2.48 (s, 3H), 2.32 – 2.17 (m, 3H), 2.15 – 2.03 (m, 2H), 1.65 – 1.55 (m, 4H), 1.41 – 1.28 (m, 6H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{53}H_{65}N_8O_9S^+$ 989.4590, found 989.4589.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((9-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-9-oxononyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (9).

Compound **9** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(9-aminononanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**27**) (6.2 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **9** was obtained as a white solid (6.5 mg, 65%). 1H NMR (600 MHz, Methanol- d_4) δ 9.04 (s, 1H),

7.76 (s, 1H), 7.72 (dd, $J = 7.9, 1.7$ Hz, 1H), 7.49 – 7.45 (m, 2H), 7.44 – 7.40 (m, 3H), 7.37 – 7.32 (m, 3H), 7.16 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 4.62 (s, 1H), 4.59 – 4.47 (m, 3H), 4.35 (d, $J = 15.5$ Hz, 1H), 3.92 – 3.87 (m, 1H), 3.79 (dd, $J = 10.9, 3.9$ Hz, 1H), 3.37 – 3.32 (m, 2H), 3.18 – 2.92 (m, 8H), 2.68 – 2.60 (m, 1H), 2.48 (s, 3H), 2.32 – 2.18 (m, 3H), 2.16 – 2.03 (m, 2H), 1.65 – 1.54 (m, 4H), 1.40 – 1.28 (m, 8H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{54}H_{67}N_8O_9S^+$ 1003.4746, found 1003.4752.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((10-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-10-oxodecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (10). Compound **10** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-((*S*)-2-(10-aminodecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**28**) (6.9 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **10** was obtained as a white solid (6.8 mg, 67%). 1H NMR (600 MHz, Methanol- d_4) δ 9.03 (s, 1H), 7.76 (s, 1H), 7.74 – 7.69 (m, 1H), 7.47 (d, $J = 8.2$ Hz, 2H), 7.44 – 7.39 (m, 3H), 7.37 – 7.32 (m, 3H), 7.15 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 4.62 (s, 1H), 4.59 – 4.52 (m, 2H), 4.50 – 4.47 (m, 1H), 4.35 (d, $J = 15.5$ Hz, 1H), 3.89 (d, $J = 10.9$ Hz, 1H), 3.79 (dd, $J = 11.0, 3.9$ Hz, 1H), 3.36 – 3.31 (m, 2H), 3.15 – 2.93 (m, 8H), 2.68 – 2.61 (m, 1H), 2.48 (s, 3H), 2.30 – 2.18 (m, 3H), 2.15 – 2.03 (m, 2H), 1.62 – 1.53 (m, 4H), 1.39 – 1.26 (m, 10H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{55}H_{69}N_8O_9S^+$ 1017.4903, found 1017.4900.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((11-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (11). Compound **11** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-((*S*)-2-(11-aminoundecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**29**) (6.5 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0

equiv) in DMSO (1 mL). Compound **11** was obtained as a white solid (6.3 mg, 61%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.15 (s, 1H), 7.76 (s, 1H), 7.72 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.45 – 7.41 (m, 3H), 7.39 – 7.32 (m, 3H), 7.15 (s, 1H), 5.68 (t, *J* = 7.9 Hz, 1H), 4.62 (s, 1H), 4.59 – 4.52 (m, 2H), 4.51 – 4.47 (m, 1H), 4.36 (d, *J* = 15.5 Hz, 1H), 3.93 – 3.87 (m, 1H), 3.80 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.36 – 3.32 (m, 2H), 3.17 – 2.91 (m, 8H), 2.68 – 2.60 (m, 1H), 2.50 (s, 3H), 2.31 – 2.18 (m, 3H), 2.16 – 2.01 (m, 2H), 1.63 – 1.52 (m, 4H), 1.40 – 1.25 (m, 12H), 1.02 (s, 9H). HRMS *m/z* [M + H]⁺ calcd for C₅₆H₇₁N₈O₉S⁺ 1031.5059, found 1031.5056.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((12-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (12) Compound **12** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(12-aminododecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**30**) (7.4 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **12** was obtained as a white solid (6.3 mg, 60%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.16 (s, 1H), 7.76 (s, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.50 – 7.47 (m, 2H), 7.45 – 7.41 (m, 3H), 7.37 – 7.32 (m, 3H), 7.16 (s, 1H), 5.69 (t, *J* = 7.9 Hz, 1H), 4.63 (s, 1H), 4.59 – 4.53 (m, 2H), 4.51 – 4.48 (m, 1H), 4.38 – 4.34 (m, 1H), 3.90 (d, *J* = 11.0 Hz, 1H), 3.80 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.38 – 3.33 (m, 2H), 3.16 – 2.92 (m, 8H), 2.68 – 2.61 (m, 1H), 2.50 (s, 3H), 2.32 – 2.19 (m, 3H), 2.17 – 2.06 (m, 2H), 1.65 – 1.53 (m, 4H), 1.39 – 1.24 (m, 14H), 1.03 (s, 9H). HRMS *m/z* [M + H]⁺ calcd for C₅₇H₇₃N₈O₉S⁺ 1045.5216, found 1284.5206.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((10-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-10-oxodecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (MS41 (13)) **5-(4-(dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((10-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-10-oxodecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (MS41 (13))**. To a solution of intermediate **18** (1.306 g, 3.0

mmol) in DMSO (30 mL) were added the linker (2*S*,4*R*)-1-((*S*)-2-(10-aminodecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**31**) (1.841 g, 3.0 mmol), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide) (864 mg, 4.5 mmol), HOAt (1-hydroxy-7-azabenzotriazole) (612 mg, 4.5 mmol), and NMM (*N*-Methylmorpholine) (909 mg, 9 mmol). After being stirred overnight at room temperature, the resulting mixture was purified by preparative HPLC (5%-70% acetonitrile / 0.1% TFA in H₂O) to afford **MS41** as white solid in TFA salt form (1.454 g, yield: 47%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.97 (s, 1H), 7.78 (s, 1H), 7.75 – 7.69 (m, 1H), 7.47 – 7.36 (m, 5H), 7.36 – 7.30 (m, 3H), 7.14 (d, *J* = 3.9 Hz, 1H), 5.68 (t, *J* = 8.0 Hz, 1H), 4.99 (d, *J* = 7.0 Hz, 2H), 4.65 – 4.53 (m, 2H), 4.42 (s, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.73 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.34 (d, *J* = 7.2 Hz, 1H), 3.14 – 3.07 (m, 4H), 2.99 – 2.91 (m, 4H), 2.67 – 2.59 (m, 1H), 2.47 (d, *J* = 4.5 Hz, 3H), 2.31 – 2.17 (m, 3H), 2.13 – 2.06 (m, 1H), 1.97 – 1.90 (m, 1H), 1.58 (dt, *J* = 13.5, 6.9 Hz, 4H), 1.49 (d, *J* = 6.9 Hz, 3H), 1.30 (d, *J* = 14.1 Hz, 10H), 1.03 (s, 9H). ¹³C NMR (101 MHz, Methanol-*d*₄) δ 174.65, 171.86, 170.85, 170.45, 169.77, 168.54, 159.63, 159.27, 154.13, 151.96, 147.19, 144.55, 143.20, 133.20, 129.64, 129.08, 128.82, 128.71, 127.02, 126.30, 126.12, 125.99, 124.50, 122.60, 116.94, 112.29, 99.47, 69.57, 59.19, 57.61, 56.63, 54.33, 49.02, 48.75, 48.30, 48.09, 47.87, 47.66, 47.45, 47.23, 47.02, 39.69, 37.44, 35.22, 35.10, 32.66, 29.82, 29.14, 29.11, 28.99, 28.89, 26.70, 25.72, 25.65, 21.13, 14.20. HRMS *m/z* [M + H]⁺ calcd for C₅₆H₇₁N₈O₉S⁺ 1031.5059, found 1031.5083.

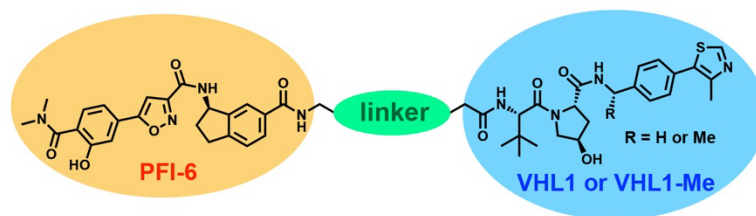
5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((11-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (14**)**. Compound **14** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-((*S*)-2-(11-aminoundecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**32**) (7.4 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **14** was obtained as a white solid (6.1 mg, 58%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.22 (s, 1H), 7.76 (s, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.48 – 7.40 (m, 5H), 7.36 – 7.31 (m, 3H), 7.14 (s, 1H), 5.68 (t, *J* = 7.9 Hz, 1H), 4.99 (q, *J* = 7.0 Hz, 1H),

4.60 (s, 1H), 4.56 (dd, $J = 10.1, 6.5$ Hz, 1H), 4.43 – 4.40 (m, 1H), 3.86 (d, $J = 11.1$ Hz, 1H), 3.73 (dd, $J = 11.0, 3.9$ Hz, 1H), 3.37 – 3.32 (m, 2H), 3.15 – 2.92 (m, 8H), 2.68 – 2.61 (m, 1H), 2.50 (s, 3H), 2.31 – 2.07 (m, 4H), 1.97 – 1.91 (m, 1H), 1.62 – 1.53 (m, 4H), 1.49 (d, $J = 7.0$ Hz, 3H), 1.38 – 1.26 (m, 12H), 1.02 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{57}H_{73}N_8O_9S^+$ 1045.5216, found 1045.5211.

5-(4-(Dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((12-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (15). Compound **15** was synthesized following the standard procedure used for synthesis of compound **1** starting from intermediate **18** (5 mg, 0.01 mmol), (2*S*,4*R*)-1-(((*S*)-2-(12-aminododecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**33**) (7.6 mg, 0.01 mmol, 1.0 equiv), EDCI (2.9 mg, 0.015 mmol, 1.5 equiv), HOAt (2.1 mg, 0.015 mmol, 1.5 equiv), and NMM (3.1 mg, 0.03 mmol, 3.0 equiv) in DMSO (1 mL). Compound **15** was obtained as a white solid (7 mg, 66%). 1H NMR (600 MHz, MeOD) δ 9.21 (s, 1H), 7.81 – 7.68 (m, 2H), 7.52 – 7.29 (m, 8H), 7.14 (s, 1H), 5.68 (t, $J = 7.9$ Hz, 1H), 5.04 – 4.97 (m, 2H), 4.67 – 4.52 (m, 2H), 4.42 (s, 1H), 4.02 – 3.93 (m, 1H), 3.91 – 3.84 (m, 1H), 3.78 – 3.68 (m, 1H), 3.18 – 2.92 (m, 8H), 2.70 – 2.58 (m, 2H), 2.50 (s, 3H), 2.37 – 2.04 (m, 4H), 2.01 – 1.90 (m, 1H), 1.66 – 1.47 (m, 6H), 1.42 – 1.22 (m, 14H), 1.03 (s, 9H). HRMS m/z $[M + H]^+$ calcd for $C_{58}H_{75}N_8O_9S^+$ 1059.5372, found 1059.5377.

5-(4-(dimethylcarbamoyl)-3-hydroxyphenyl)-*N*-((*R*)-6-((10-(((*S*)-1-((2*R*,4*S*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-10-oxodecyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)isoxazole-3-carboxamide (MS41N). To a solution of compound **18** (17.5 mg, 0.04 mmol) in DMSO (30 mL) were added the linker (2*S*,4*S*)-1-(((*S*)-2-(10-aminodecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**34**) (24.6 mg, 0.04 mmol), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide) (11.5 mg, 0.06 mmol), HOAt (1-hydroxy-7-azabenzotriazole) (8.2 mg, 0.06 mmol), and NMM (*N*-Methylmorpholine) (12.1 mg, 0.12 mmol). After being stirred overnight at room temperature, the resulting mixture was purified by preparative HPLC (5%-70% acetonitrile / 0.1% TFA in H_2O)

to afford **MS41N** as white solid in TFA salt form (20.1 mg, yield: 50%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 9.12 (s, 1H), 7.77 (s, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.46 – 7.38 (m, 3H), 7.38 – 7.30 (m, 3H), 7.14 (s, 1H), 5.69 (t, *J* = 7.9 Hz, 1H), 5.00 (d, *J* = 7.3 Hz, 2H), 4.55 (t, *J* = 7.4 Hz, 1H), 4.45 (d, *J* = 13.2 Hz, 2H), 3.93 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.68 (dd, *J* = 10.9, 3.4 Hz, 1H), 3.34 (d, *J* = 8.1 Hz, 1H), 3.15 – 3.08 (m, 4H), 3.00 – 2.92 (m, 4H), 2.67 – 2.60 (m, 1H), 2.49 (s, 3H), 2.30 – 2.06 (m, 5H), 1.57 – 1.50 (m, 4H), 1.43 (d, *J* = 7.0 Hz, 3H), 1.26 (d, *J* = 16.9 Hz, 10H), 1.05 (s, 9H). ¹³C NMR (101 MHz, Methanol-*d*₄) δ 175.04, 172.08, 170.82, 170.48, 169.81, 168.61, 159.69, 159.28, 154.12, 147.23, 144.56 (d, *J* = 1.7 Hz), 143.22, 133.21, 129.27, 129.00, 128.79, 128.73, 126.99, 126.52, 125.99, 124.50, 122.52, 116.92, 112.24, 99.40, 69.11, 59.32, 58.17, 55.49, 54.31, 39.61, 37.56, 35.08, 34.27, 32.64, 29.78, 29.06, 29.04, 28.95, 28.90, 28.85, 26.64, 25.64, 25.47, 21.24, 13.89. HRMS *m/z* [M + H]⁺ calcd for C₅₆H₇₁N₈O₉S⁺ 1031.5059, found 1031.5101.



Compound	Structure	Linker type	VHL ligand
1		1PEG	VHL1
2		2PEG	VHL1
3		3PEG	VHL1
4		4PEG	VHL1
5		2C	VHL1
6		4C	VHL1
7		6C	VHL1
8		7C	VHL1
9		8C	VHL1
10		9C	VHL1
11		10C	VHL1
12		11C	VHL1
13 (MS41)		9C	VHL1-Me
14		10C	VHL1-Me
15		11C	VHL1-Me

Fig. S1. Design of ENL PROTAC degraders. Top, schematic depiction of ENL degraders. Bottom, chemical structure of ENL PROTAC degraders 1-15.

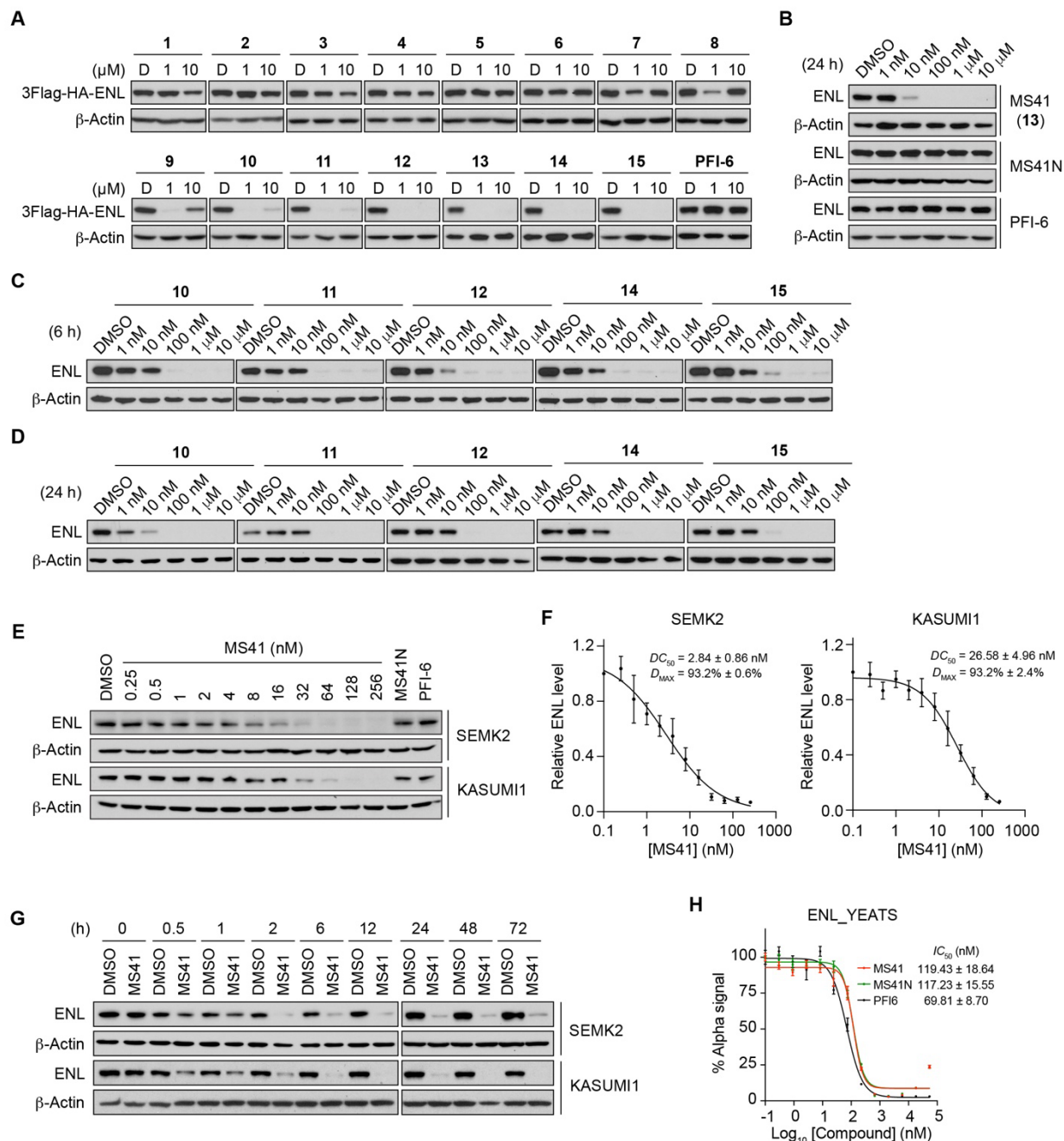


Fig. S2. Structure-activity relationship results of ENL PROTAC degraders. (A)

Immunoblots of stably expressed 3Flag-HA-ENL in MV4;11 cells treated with DMSO (D) or the indicated ENL degraders for 24 h. PFI-6 is shown for comparison. **(B)** Immunoblots for ENL in MV4;11 cells treated with the indicated concentrations of MS41 (compound **13**), MS41N, or PFI-6 for 24 h. **(C and D)** Immunoblots for ENL in MV4;11 cells treated with the indicated concentrations of ENL degraders **10**, **11**, **12**, **14**, and **15** for 6 h **(C)** or 24 h **(D)**. **(E)** Concentration-dependent ENL degradation mediated by MS41. Immunoblots for ENL in SEMK2 and KASUMI1 cells treated with DMSO, MS41 at the indicated concentrations, MS41N (256 nM), or PFI-6 (256 nM) for 24 h. **(F)** Measurement of DC_{50} and D_{max} values of MS41 in

SEMK2 and KASUMI1 cells based on ENL and β -Actin blots as described in (E). The band intensity is determined by Image J software. Values and error bars are presented as mean \pm SEM from three independent experiments. (G) Time-dependent ENL degradation mediated by MS41. Immunoblots for ENL in SEMK2 and KASUMI1 cells treated with DMSO or MS41 (100 nM) for the indicated time. (H) Measurement of IC_{50} values of MS41, MS41N, and PFI-6 for the inhibition of ENL_{YEATS}-H3K9ac interaction in AlphaScreen assay. Values and error bars are presented as mean \pm SEM from five replicates. β -Actin was used as loading control in immunoblots. All blots and data are representative of at least three independent experiments.

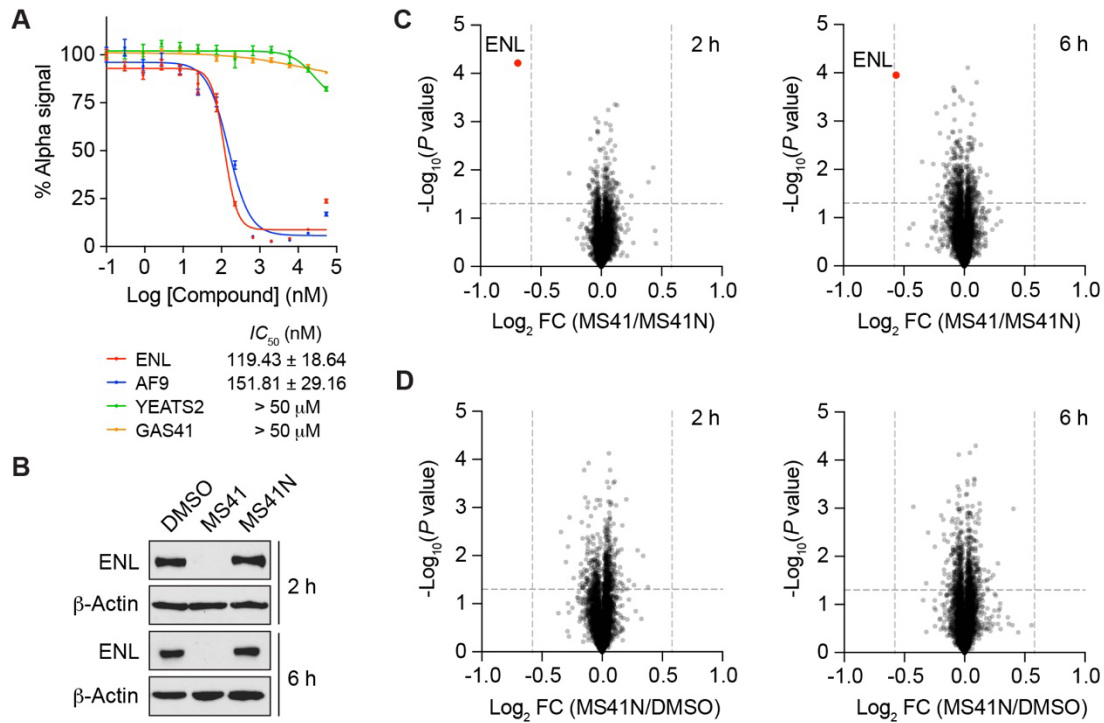


Fig. S3. MS41 induces specific degradation of ENL in cells. (A) Measurement of IC_{50} values of MS41 in the inhibition of ENL_{YEATS-H3K9ac}, AF9_{YEATS-H3K9ac}, GAS41_{YEATS-H3K14ac}, and YEATS2_{YEATS-H3K27cr} interaction in AlphaScreen assays. Values and error bars are presented as mean \pm SEM from five replicates. Data is representative of three independent experiments. (B) Immunoblots for ENL in MV4;11 cells treated with DMSO, MS41 (100 nM), or MS41N (100 nM) for 2 h or 6 h. β -Actin was used as loading control. (C) Quantitative proteomics analysis of MV4;11 cells treated with 100 nM MS41 vs. 100 nM MS41N for 2 h or 6 h. (D) Quantitative proteomics analysis of MV4;11 cells treated with 100 nM MS41N vs. DMSO for 2 h or 6 h. In (C) and (D), a total of 4,494 proteins were identified with ≥ 2 RazorUnique peptides in three biological replicates and quantified. The dash lines indicate cutoff of P value < 0.05 (y -axis) and fold change > 1.5 (x -axis) in three biological replicates.

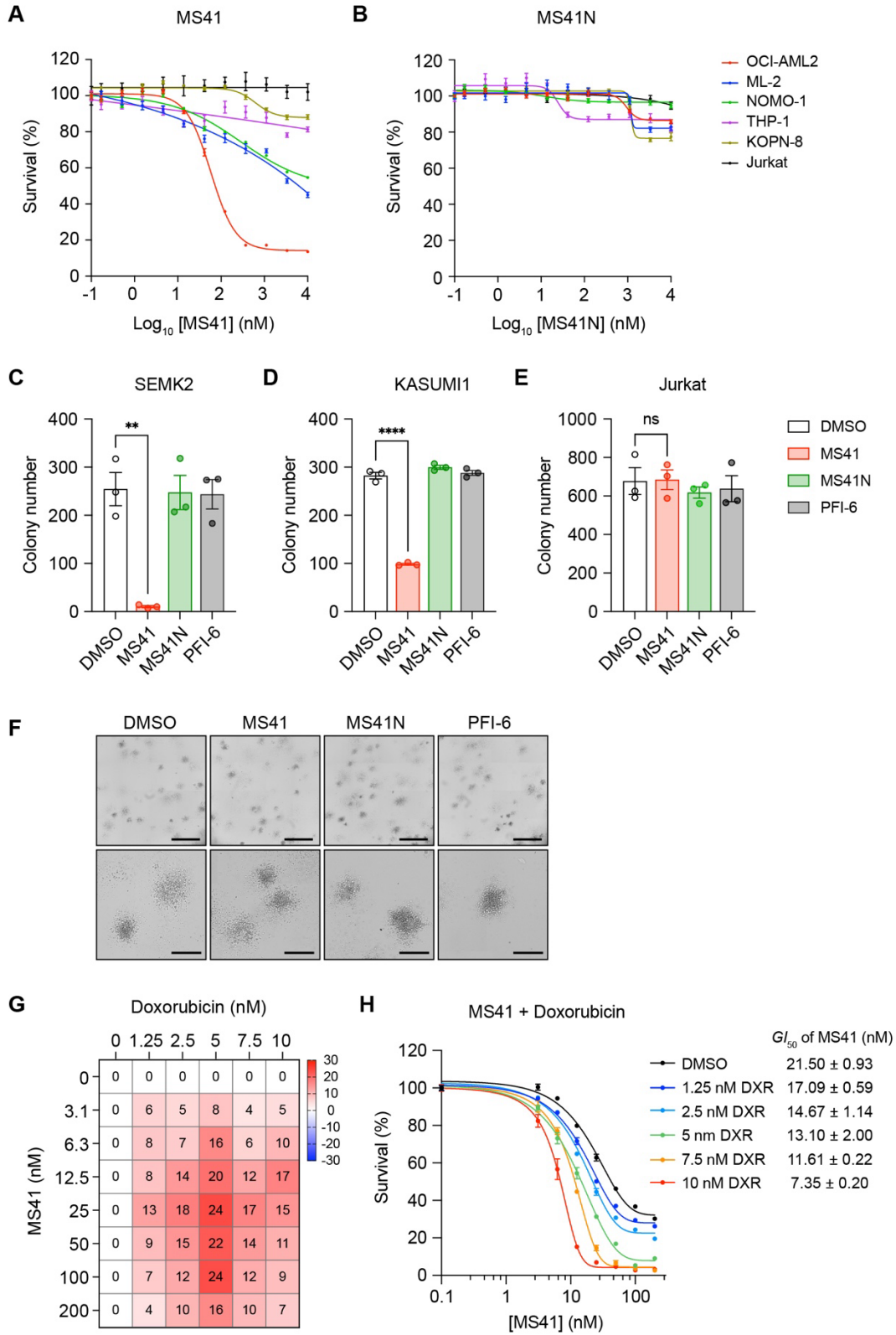


Fig. S4. MS41 suppresses the growth of human leukemia cells. (A to B) Growth inhibition curves of MS41 (A) and MS41N (B) in human leukemia cells, OCI-AML2, ML-2, NOMO-1,

THP-1, KOPN-8, and Jurkat. *Y*-axis shows the relative live cell ratio upon treatment with the indicated concentrations (*x*-axis) of compounds for 12 days, normalized to DMSO treated cells. Error bars represent mean \pm SEM, $n = 6$. (C to E) Quantification of colonies formed in SEMK2 (C), KASUMI1 (D), and Jurkat (E) cells treated with DMSO, MS41 (100 nM), MS41N (100 nM) or PFI-6 (100 nM) for 10 days. Error bars represent mean \pm SEM from three independent experiments. Student *t* test, ** $P < 0.01$, **** $P < 0.0001$; ns, not significant. (F) Representative colonies formed in Jurkat cells treated with DMSO, MS41 (100 nM), MS41N (100 nM) or PFI-6 (100 nM) for 10 days. Scale bars, 2 mm (top) and 500 μ m (bottom). (G) Synergistic effect between MS41 and doxorubicin in MV4;11 cells. Numbers are percentage of excess activity over that expected under the Bliss independence assumption for each dose combinations of MS41 and doxorubicin. Red indicates synergistic effect. (H) GI_{50} of MS41 when cotreated with the indicated concentrations of doxorubicin (DXR) in MV4;11 cells. *Y*-axis shows the relative live cell ratio upon treatment with the indicated concentrations (*x*-axis) of MS41 for 6 days, normalized to DMSO treated cells. Error bars represent mean \pm SEM, $n = 4$.

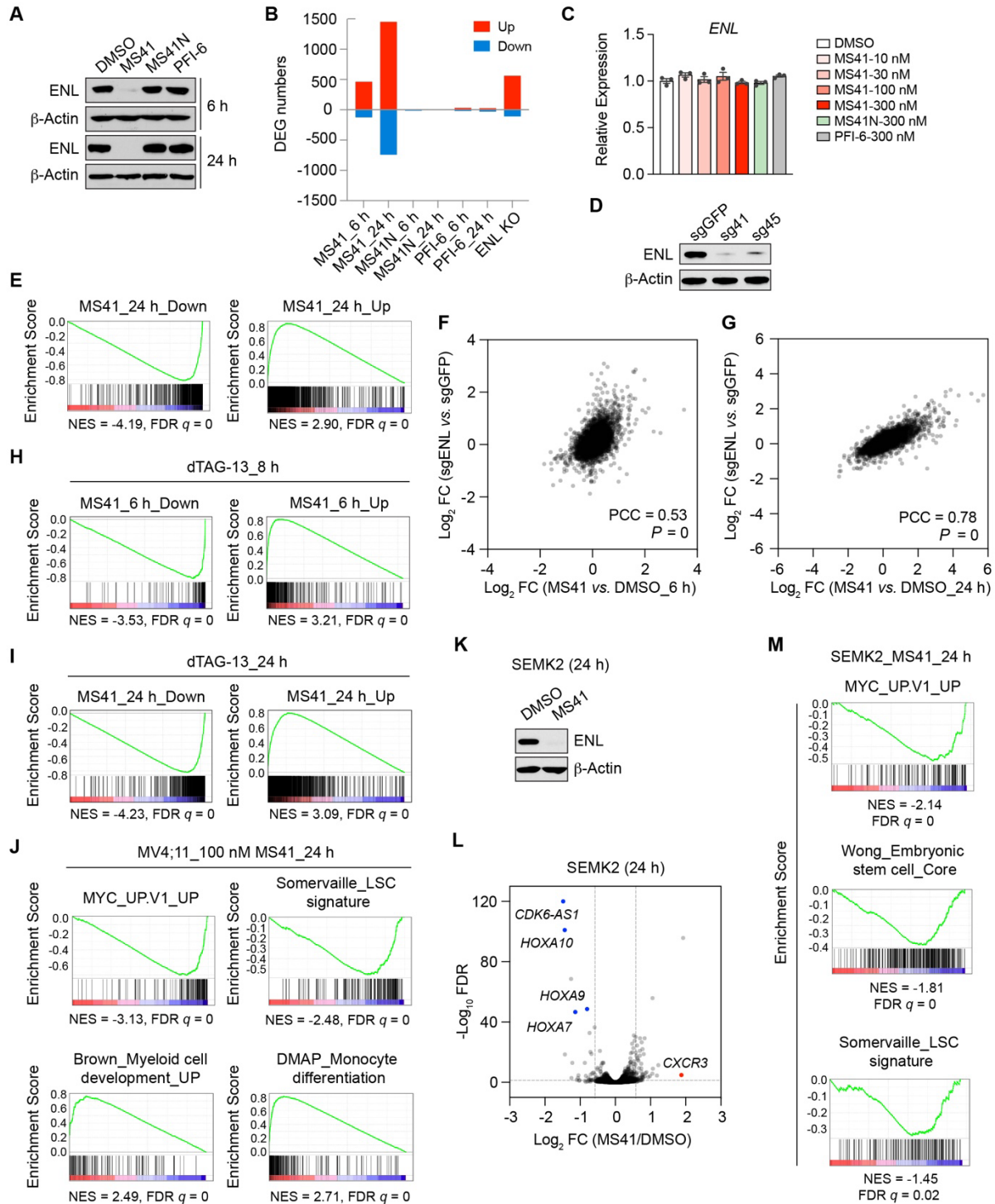


Fig. S5. MS41 suppresses ENL-dependent oncogenic gene expression in MV4;11 and SEMK2 cells. (A) Immunoblots for ENL and β -Actin in MV4;11 cells treated with DMSO, MS41 (100 nM), MS41N (100 nM), or PFI-6 (100 nM) for 6 h or 24 h. (B) Numbers of DEGs from cells treated with MS41, MS41N, PFI-6 to DMSO treated cells, and ENL KO cells to control cells. Up DEGs are plotted in red and down DEGs in blue color. (C) MS41 does not alter *ENL* gene expression. RT-qPCR of *ENL* gene expression in MV4;11 cells treated with DMSO or

the indicated concentrations of MS41, MS41N and PFI-6 for 24 h. Error bars represent mean \pm SEM from three independent experiments (Student's *t* test). **(D)** Immunoblots for ENL and β -Actin in ENL KO (sg41 and sg45) and the control sgGFP MV4;11 cells. **(E)** GSEA plots with ENL KO as ranking list and DEGs in cells treated with MS41 for 24 h as gene sets. **(F to G)** Correlation between gene expression changes of 6-h (F) or 24-h (G) MS41 treatment *vs.* DMSO and sgENL *vs.* sgGFP control in MV4;11 cells. **(H)** GSEA plots using MV4;11 cells expressing ENL-FKBP12(F36V) with 8-h dTAG-13 treatment *vs.* DMSO as ranking list and DEGs of 6-h MS41 treatment as gene sets. **(I)** GSEA plots using MV4;11 cells expressing ENL-FKBP12(F36V) with 24-h dTAG-13 treatment *vs.* DMSO as ranking list and DEGs of 24-h MS41 treatment as gene sets. **(J)** GSEA plots with 24-h MS41 *vs.* DMSO treated MV4;11 cells as ranking list and the selected curated gene sets. **(K)** Immunoblots for ENL and β -Actin in SEMK2 cells treated with DMSO or MS41 (100 nM) for 24 h. **(L)** Volcano plot of all expressed genes in SEMK2 cells treated with MS41 for 24 h. The *x*-axis is \log_2 fold change (\log_2 FC) of CPM values from MS41 *vs.* DMSO. The *y*-axis is $-\log_{10}$ transformed FDR values for each gene. **(M)** GSEA plots with 24-h MS41 *vs.* DMSO treated SEMK2 cells as ranking list and the selected curated gene sets.

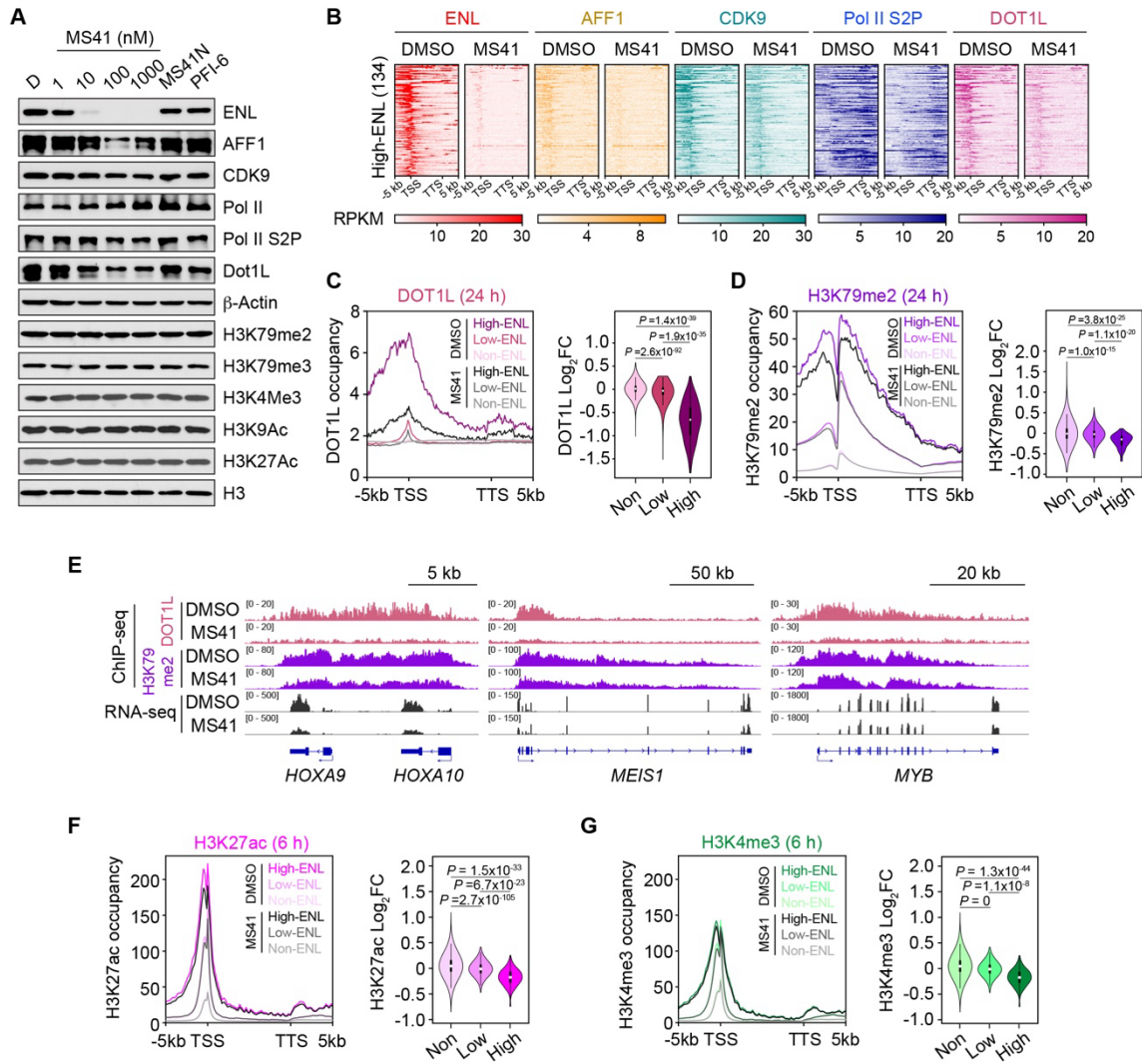


Fig. S6. MS41 reduces chromatin occupancy of SEC, DOT1L, and H3K79 methylation. (A) Immunoblots for the indicated proteins and histone modifications in MV4;11 cells treated with DMSO (D), the indicated concentrations of MS41, MS41N (1 μ M), or PFI-6 (1 μ M) for 24 h. β -Actin and H3 were used as loading controls. (B) Heatmaps of ENL, AFF1, CDK9, Pol II S2P, and DOT1L ChIP-seq densities on high ENL-bound genes in MV4;11 cells treated with DMSO or MS41 (100 nM) for 6 h. $n = 3$ biological replicates. RPKM, reads per kilobase million; TSS, transcription start site; TTS, transcription termination site. (C and D) Average profiles of DOT1L (C) and H3K79me2 (D) ChIP-seq densities on high, low, and non-ENL-bound genes in MV4;11 cells treated with DMSO or MS41 (100 nM) for 24 h (left). Violin plots of \log_2 FC of corresponding ChIP-seq densities in MS41 vs. DMSO from left panel (right). (E) Integrative Genomics Viewer (IGV) views of DOT1L and H3K79me2 ChIP-seq densities and RNA-seq reads on *HOXA9/10*, *MEIS1*, and *MYB* genes. (F and G) Average profiles of H3K27ac (F) and H3K4me3 (G) ChIP-seq densities on high, low, and non-ENL-bound genes in MV4;11 cells treated with DMSO or MS41 (100 nM) for 6 h (left). Violin plots of \log_2 FC of corresponding ChIP-seq densities in MS41 vs. DMSO from left panel (right). Two-tail t -test was used for calculating p -values in (C, D, F and G).

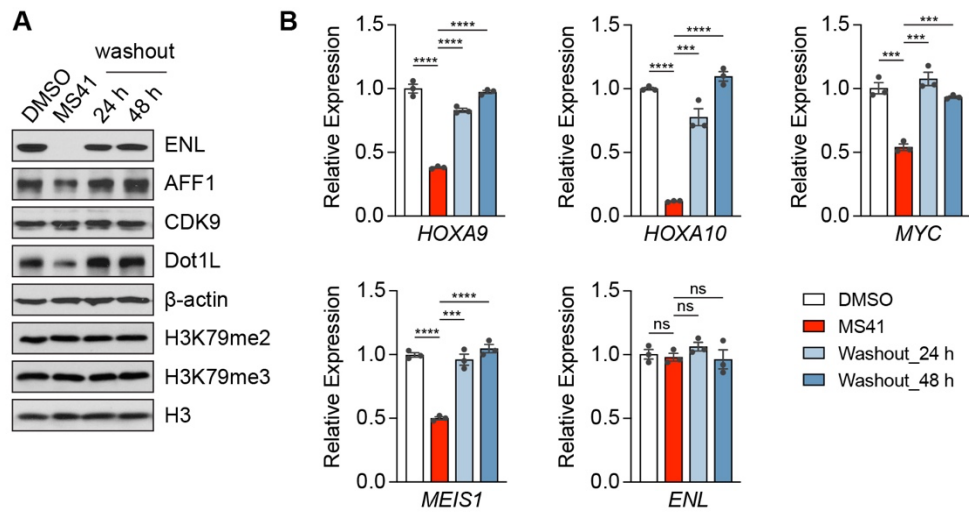


Fig. S7. The effects of MS41 are reversible. (A) Immunoblots for ENL, AFF1, CDK9, DOT1L, β -Actin, H3K79me2, H3K79me3, and H3 in MV4;11 cells treated with DMSO or 100 nM MS41 for 24 h, followed by washout for 24 and 48 h. (B) RT-qPCR analysis showing mRNA expression levels of selected ENL target genes in samples described in (A). Error bars represent mean \pm SEM, $n = 3$. Student's t test, ns, not significant, *** $P < 0.001$, **** $P < 0.0001$.

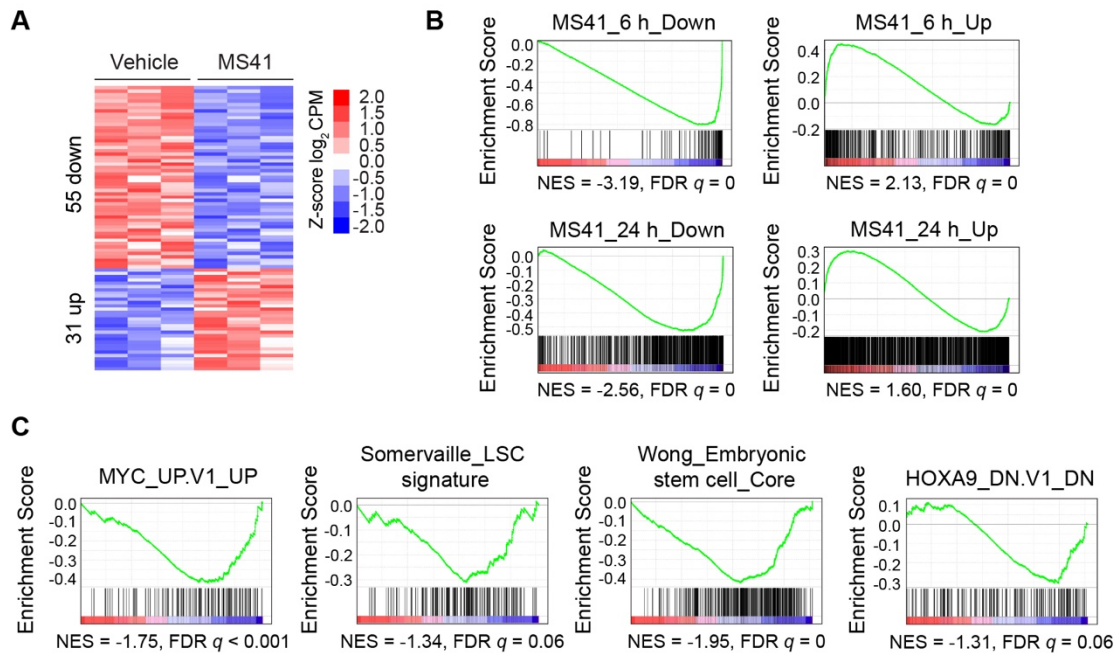


Fig. S8. Suppression of ENL-dependent oncogenic gene expression by MS41 *in vivo*. (A) Heatmap representation of differentially expressed genes in leukemia cells isolated from bone marrow of MV4;11 xenografted mice treated with MS41 vs. vehicle. $n = 3$ (B) GSEA plots with leukemia cells from xenograft mice treated with MS41 vs. vehicle as ranking list and Down and Up DEGs of 6-h (top) or 24-h (bottom) MS41 treatment *in vitro* as gene sets. (C) GSEA plots with leukemia cells from xenograft mice treated with MS41 vs. vehicle as ranking list and the selected curated lists as gene sets.

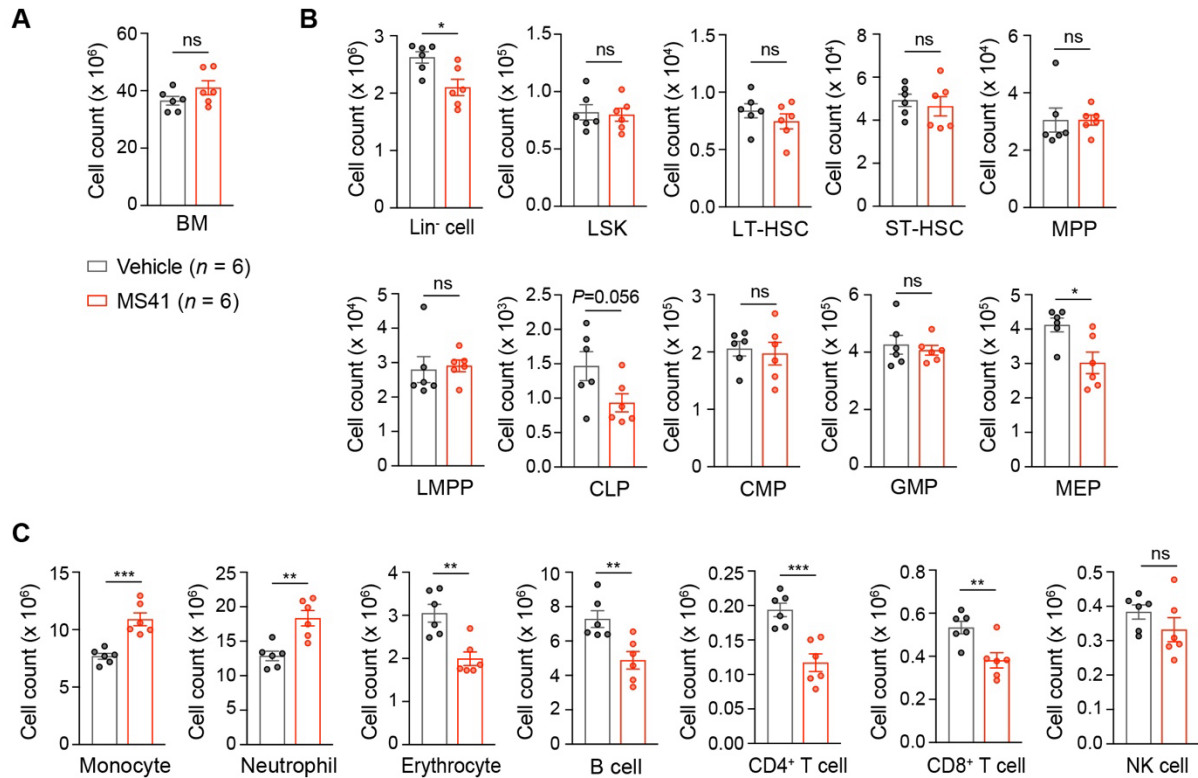


Fig. S9. MS41 has very mild effects on normal hematopoiesis. (A) Number of total bone marrow cells collected from mice at the end of 30 days treatment with vehicle and MS41 (50 mg/kg, once daily, i.p.). (B) Numbers of immature progenitor cells: lineage negative cell (Lin⁻), Lin⁻ cKit⁺ Sca1⁻ (LSK) cell, long-term hematopoietic stem cell (LT-HSC), short-term hematopoietic stem cell (ST-HSC), multiple progenitor (MPP), lymphoid-primed multipotent progenitor (LMPP), common lymphoid progenitor (CLP), common myeloid progenitor (CMP), granulocyte-monocyte progenitor (GMP), and megakaryocytic-erythroid progenitor (MEP) in bone marrow samples collected from mice as described in (A). (C) Numbers of differentiated blood cells, including monocyte (CD11b⁺, Ly6C⁺), neutrophil (CD11b⁺, Ly6G⁺), erythrocyte (Ter119⁺), B cell (B220⁺, CD19⁺), CD4⁺ T cell, CD8⁺ T cell, and NK cell (NK1.1⁺), in bone marrow samples collected from mice as described in (A). Error bars represent mean \pm SEM ($n = 6$). Unpaired two-tailed *t*-test was used for calculating *p*-values. ns, not significant.

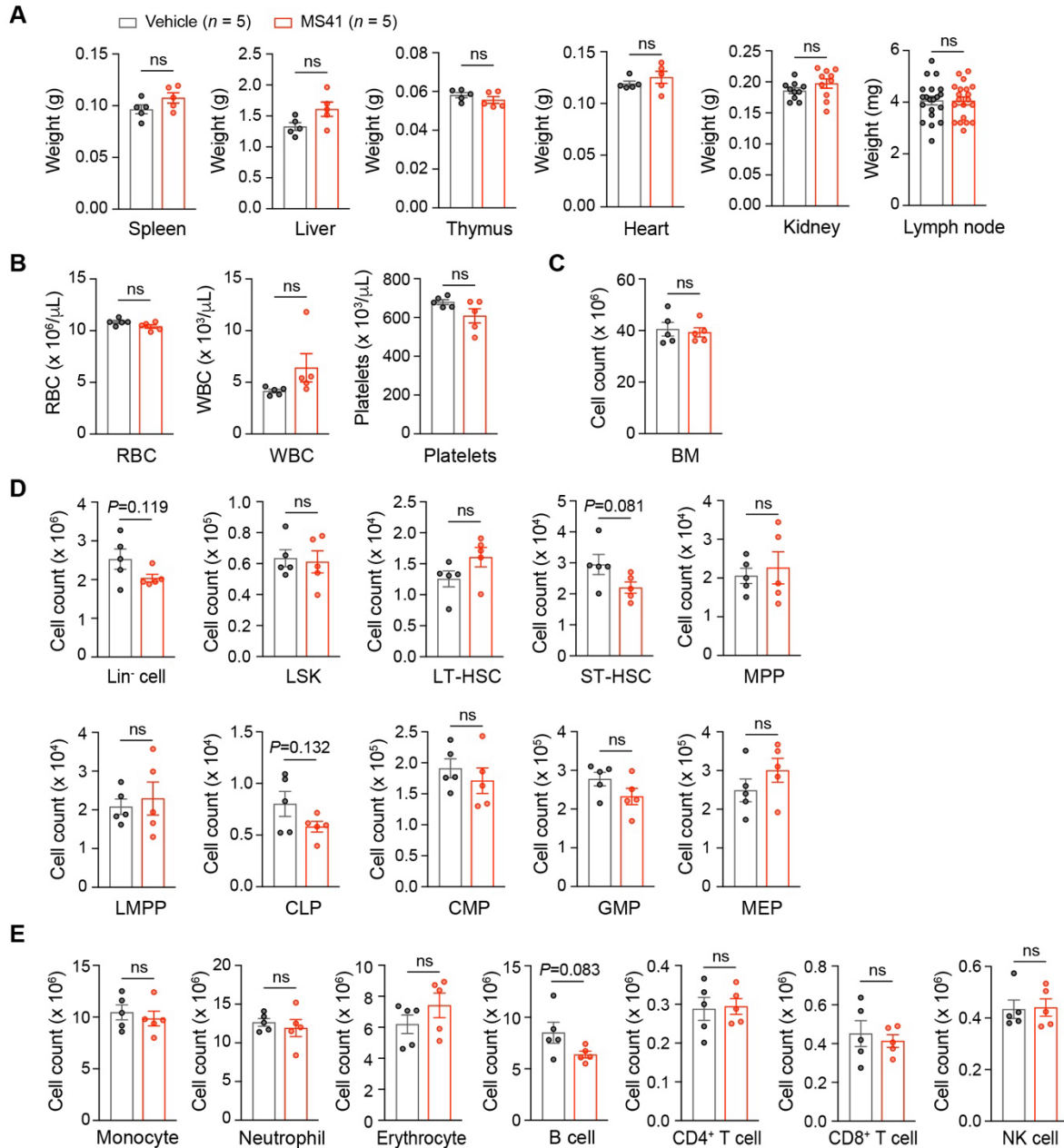
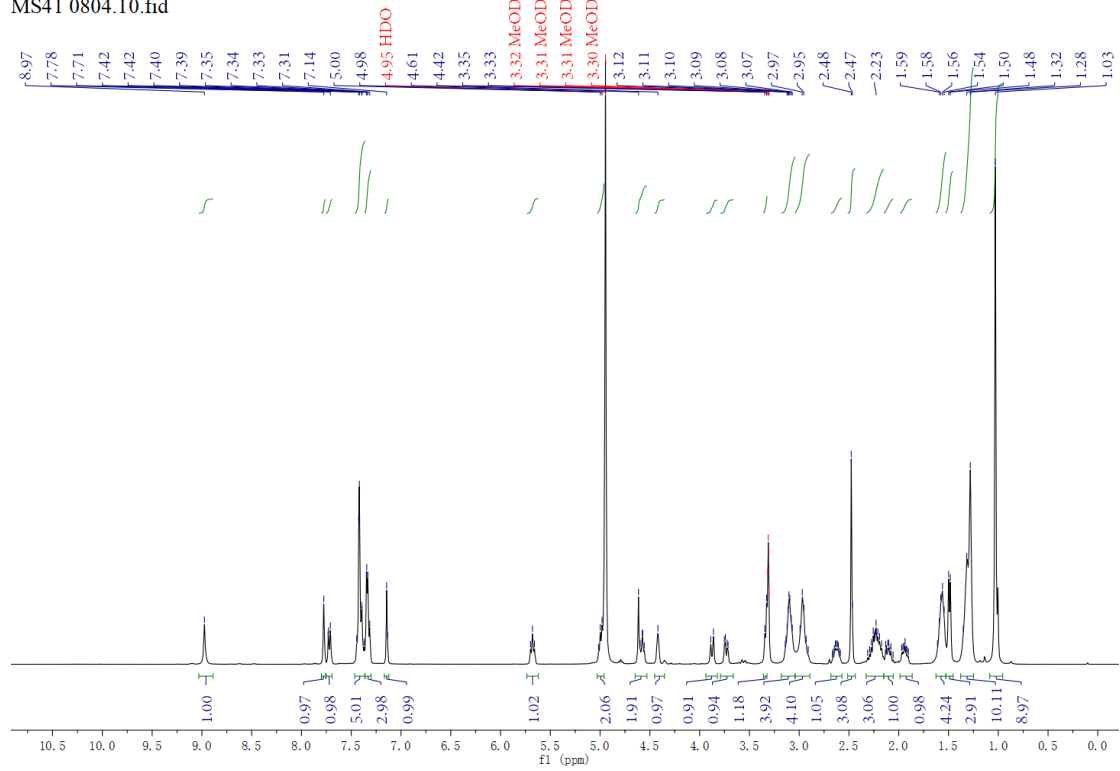


Fig. S10. MS41-induced mild effects on normal hematopoiesis are fully recoverable after the cessation of treatment. (A) Weight quantification of spleen, liver, thymus, heart, kidney, and lymph node collected from mice 90 days after completing treatment with vehicle and MS41 (50 mg/kg, once daily, i.p.). (B) Complete blood count analyses (WBC, RBC, and platelets) of peripheral blood collected from mice as described in (A). (C and D) Numbers of total cells (C) and immature progenitor cells (Lin⁻, LSK, LT-HSC, ST-HSC, MPP, LMPP, CLP, CMP, and GMP cells (D) collected in bone marrow samples from mice as described in (A). (E) Numbers of differentiated blood cells (monocyte, neutrophil, erythrocyte, B cell, CD4⁺ T cell, CD8⁺ T cell and NK cell) in bone marrow samples as described in (A). In all panels, error bars represent mean \pm SEM ($n = 5$). Unpaired two-tailed t -test was used for calculating p -values. ns, not significant.

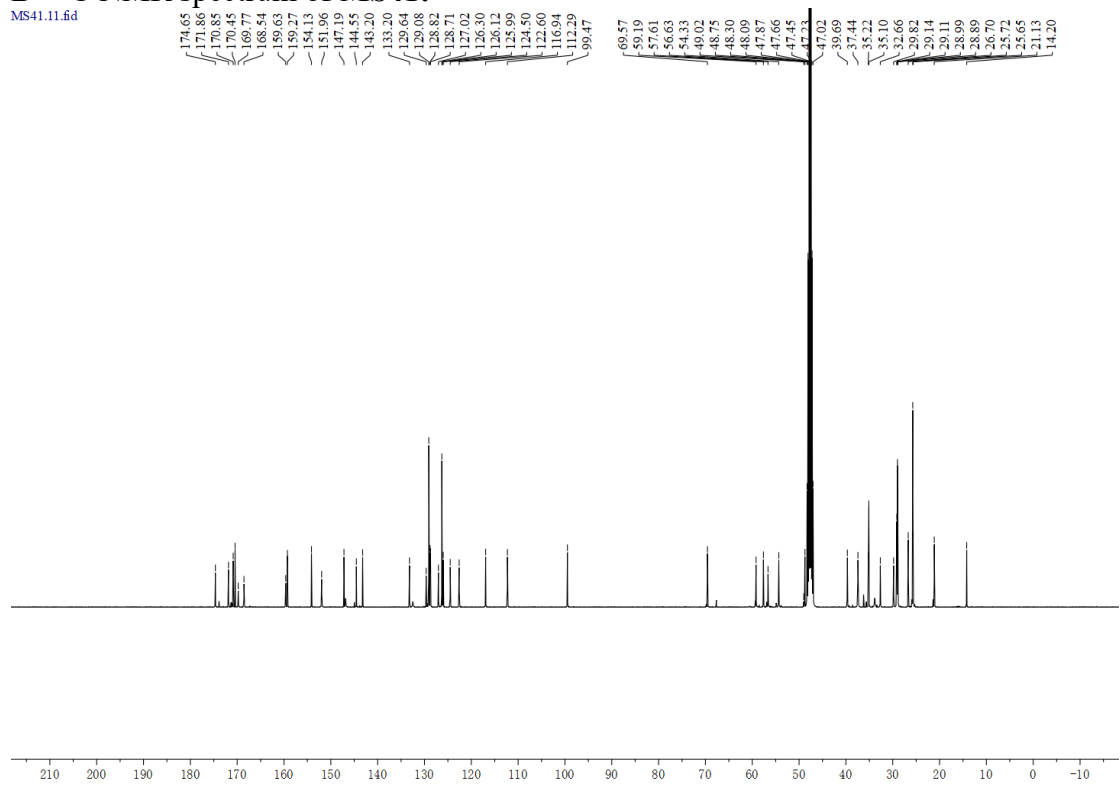
A ¹H NMR spectrum of MS41:

MS41 0804.10.fid



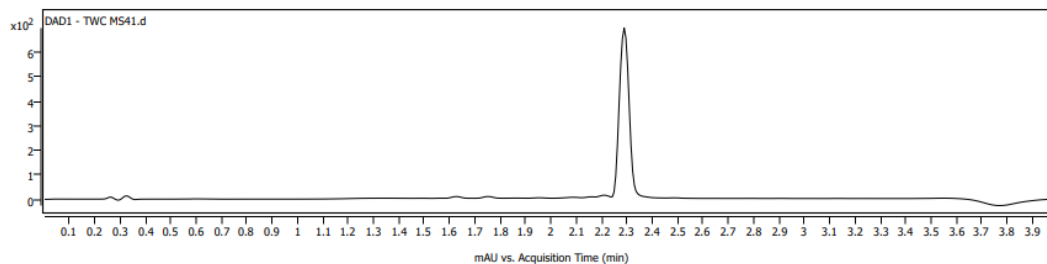
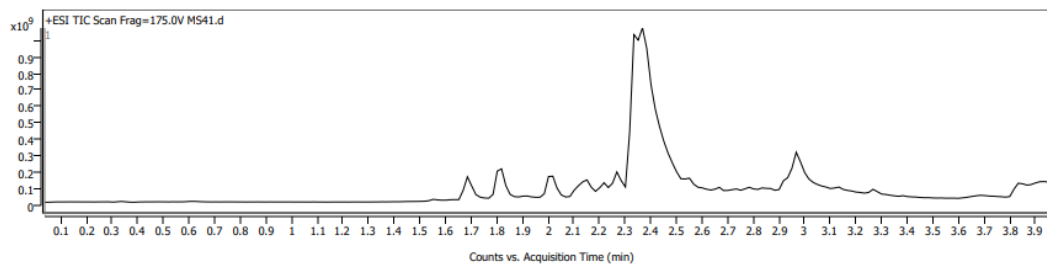
B ¹³C NMR spectrum of MS41:

MS41.11.fid



C LC-MS spectrum of MS41:

Sample Chromatograms



Sample Spectra

+ Scan (rt: 2.302-2.469 min)

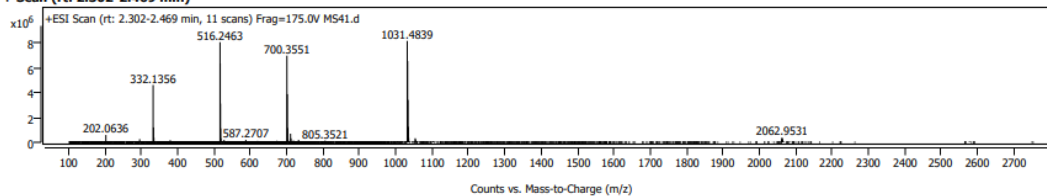
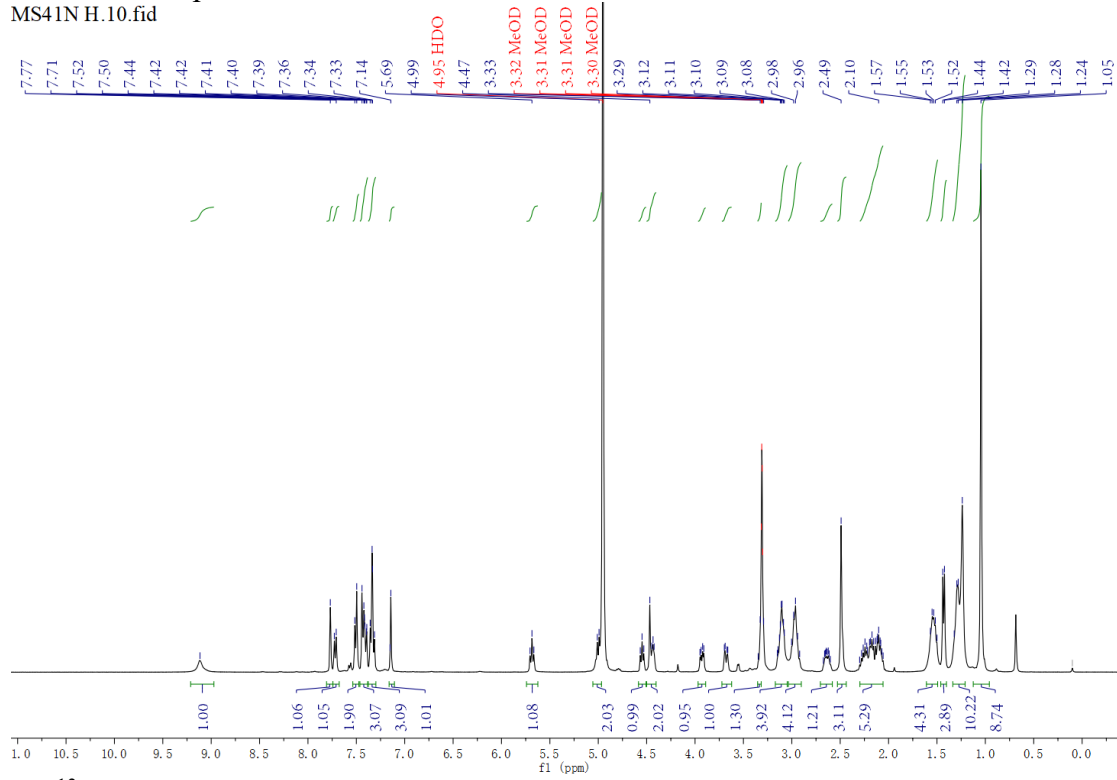
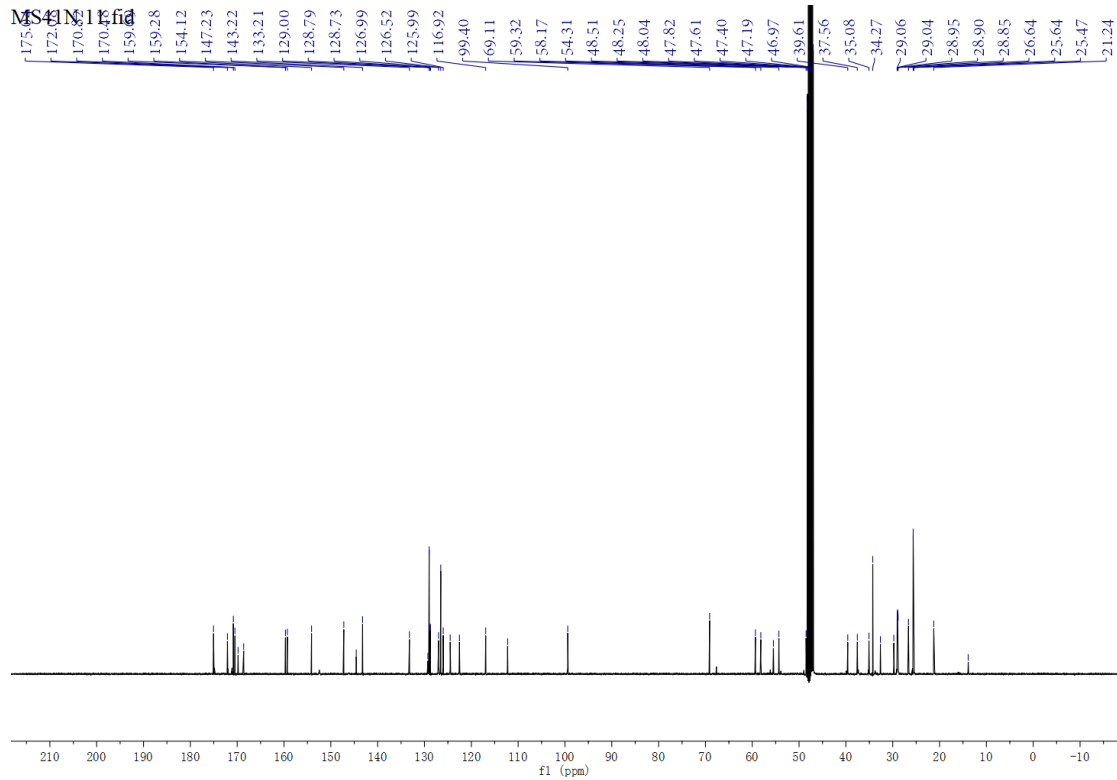


Fig. S11. Characterization of MS41. (A) ^1H NMR spectrum of MS41. (B) ^{13}C NMR spectrum of MS41. (C) LC-MS spectrum of MS41.

A ^1H NMR spectrum of MS41N:
MS41N.H.10.fid

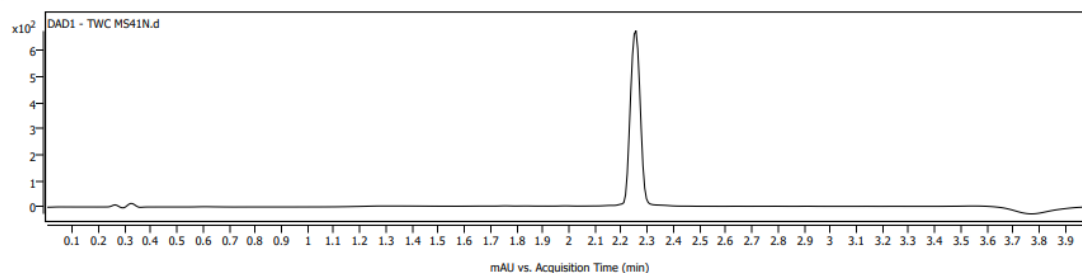
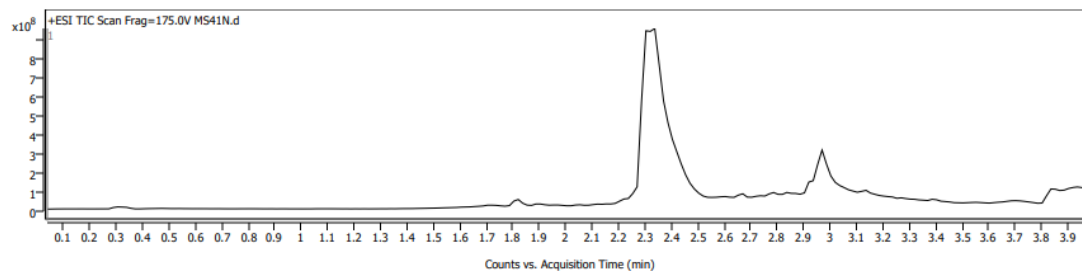


B ^{13}C NMR spectrum of MS41N:



C LC-MS spectrum of MS41N:

Sample Chromatograms



Sample Spectra

+ Scan (rt: 2.271-2.487 min)

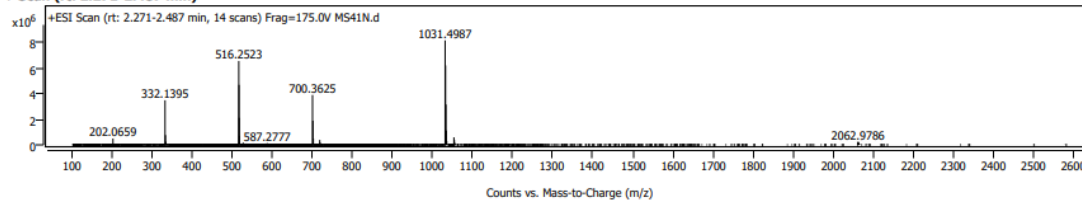


Fig. S12. Characterization of MS41N. (A) ^1H NMR spectrum of MS41N. (B) ^{13}C NMR spectrum of MS41N. (C) LC-MS spectrum of MS41N.

Supplementary Tables (combined in an Excel file)

Table S1. Global proteomics analysis in MV4;11 cells treated with MS41.

Table S2. Genes differentially expressed in MV4;11 cells treated with MS41.

Table S3. Genes differentially expressed in ENL knockout MV4;11 cells.

Table S4. GSEA gene sets used in this study.

Table S5. Genes differentially expressed in SEMK2 cells treated with MS41.

Table S6. ENL ChIP peaks in MV4;11 cells treated with DMSO or MS41 for 6 h.

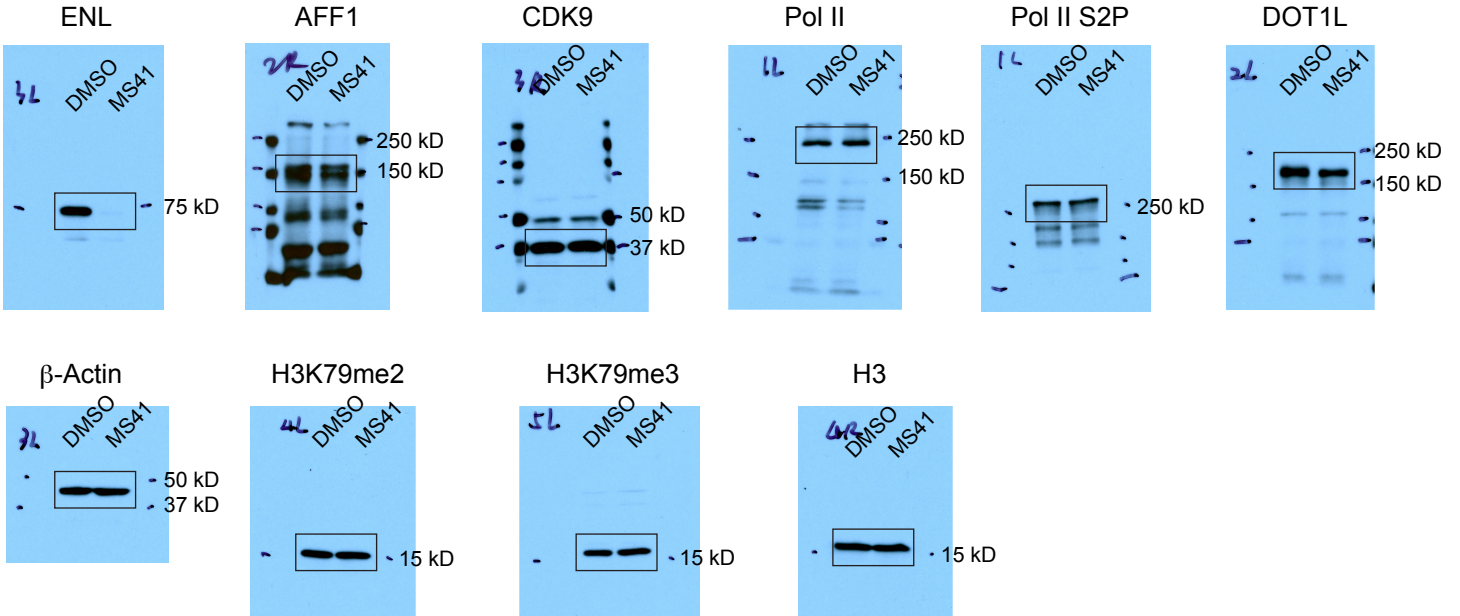
Table S7. Genes differentially expressed in xenografted MV4;11 cells treated with MS41 *in vivo*.

Table S8. Oligos used in this study.

Table S9. Antibodies used in this study.

Original blots for Fig. 5A

6 h treatment



24 h treatment

