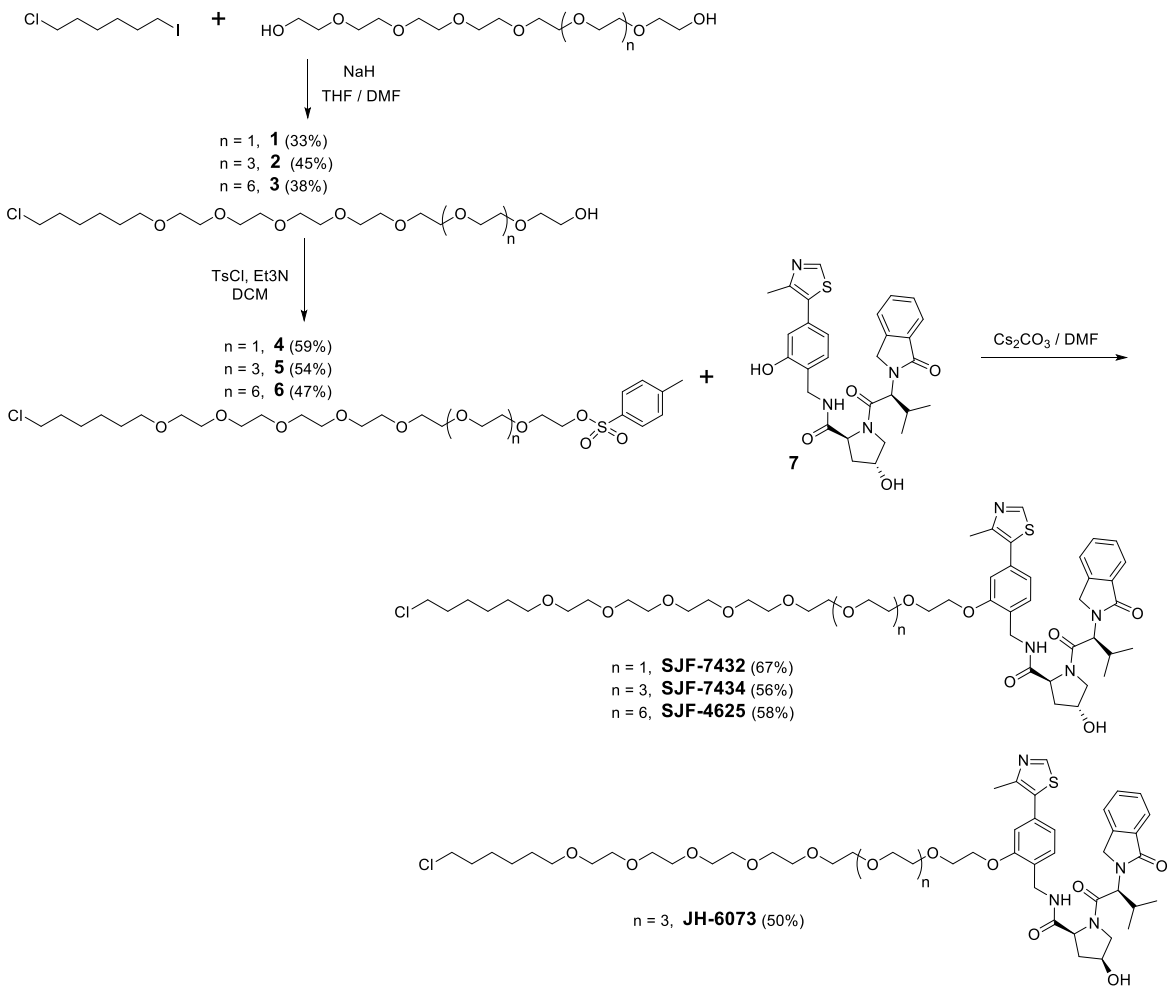
SUPPLEMENTAL INFORMATION

Methods S1. Chemical syntheses and characterization. Related to Figure 3.

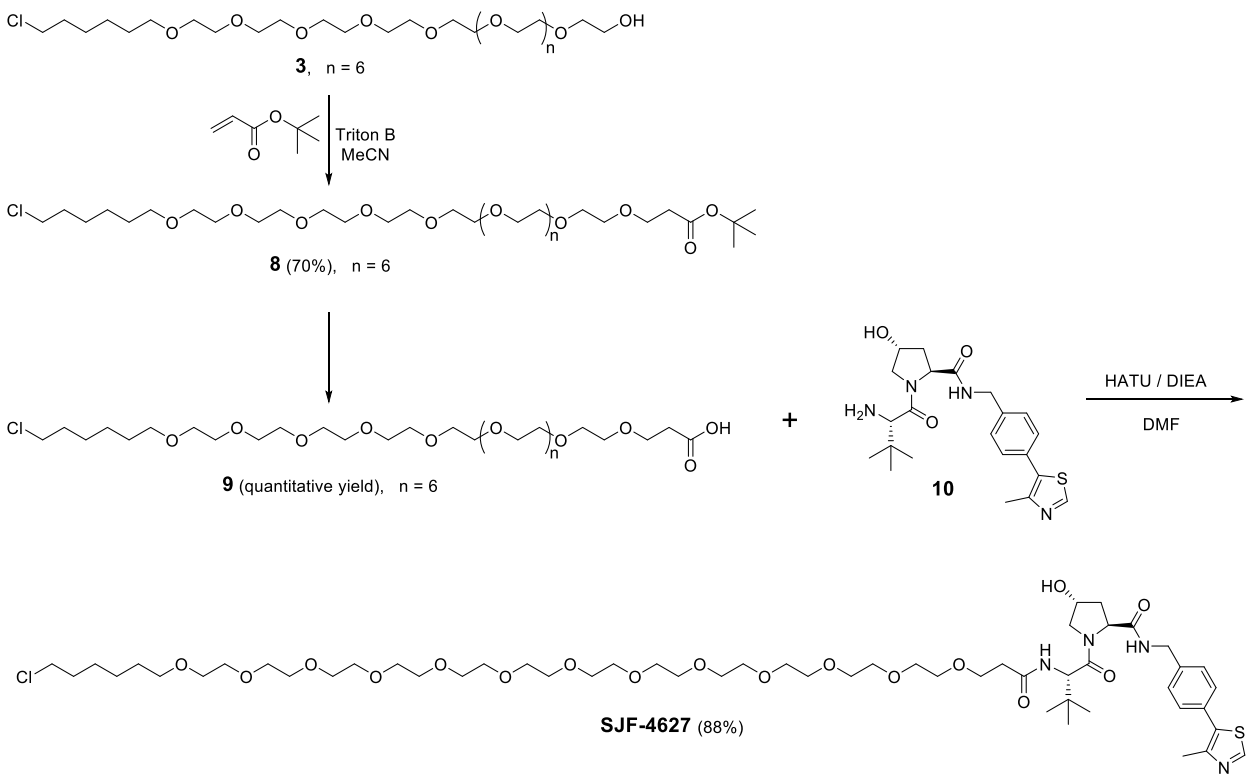
SJF-7432 = HP13
SJF-7434 = HP14
SJF-4625 = HP15
SJF-4627 = HP16
JH-6073 = HP17

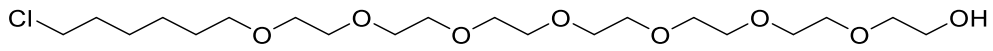
General comments. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. Tetrahydrofuran (THF), dimethylformamide (DMF), and Dichloromethane (CH_2Cl_2) were dried by a PureSolv^{TM} solvent drying system. Flash column chromatography was performed using silica gel 60 (230-400 mesh). Analytical (TLC) and preparative (PTLC) thin layer chromatography was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV or iodine. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent DD_2 500 (500 MHz ^{1}H; 125 MHz ^{13}C) or Agilent DD_2 600 (600 MHz ^{1}H; 150 MHz ^{13}C) or Agilent DD_2 400 (400 MHz ^{1}H; 100 MHz ^{13}C) spectrometer at room temperature. Chemical shifts were reported in ppm relative to the residual $CDCl_3$ (δ 7.26 ppm ^{1}H; δ 77.00 ppm ^{13}C), CD_3OD (δ 3.31 ppm ^{1}H; δ 49.00 ppm ^{13}C), or *d*^{6}-DMSO (δ 2.50 ppm ^{1}H; δ 39.52 ppm ^{13}C). NMR chemical shifts were expressed in ppm relative to internal solvent peaks, and coupling constants were measured in Hz. (bs = broad signal). Mass spectra were obtained using electrospray ionization (ESI) on a time of flight (TOF) mass spectrometer. **VHL** ligands **7** and **10** were prepared according with the literature or acquired commercially.

Scheme 1.- Synthetic Approach for SJF-7432, SJF-7434, SJF-4625 and JH-6073



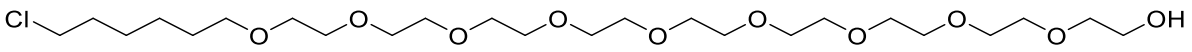
Scheme 2.- Synthetic Approach for SJF-4627



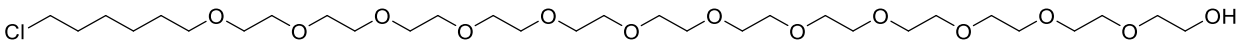


27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosan-1-ol (1). To a solution of 3,6,9,12,15,18-hexaoxaicosane-1,20-diol (1.06 mL, 3.65 mmol) in a mixture of DMF (5 mL) and THF (5 mL) was added NaH (60.0 %, 81.1 mg, 2.03 mmol) at room temperature under argon. After 40 minutes, 1-chloro-6-iodo-hexane (0.123 mL, 0.811 mmol) was added, and the mixture was stirred at room temperature for 16 h (overnight). The mixture was then quenched with diluted with 1 M HCl (50 mL) and extracted with Ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography (Gradient, DCM 100% to DCM:MeOH, 9:1) to give 120 mg of product as an oil (33% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 3.74 – 3.69 (m, 2H), 3.69 – 3.58 (m, 26H), 3.57 (t, J = 5.3 Hz, 2H), 3.44 (t, J = 6.6 Hz, 2H), 2.55 (bs, 1H), 1.81 – 1.71 (m, 2H), 1.58 (p, J = 6.8 Hz, 3H),

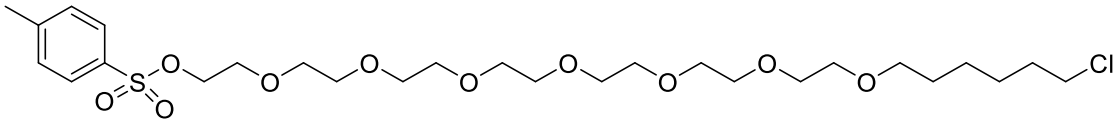
1.50 – 1.30 (m, 4H). ^{13}C NMR (151 MHz, Chloroform-d) δ 72.59, 71.35, 70.71, 70.71, 70.70, 70.69, 70.68, 70.67, 70.67, 70.66, 70.64, 70.44, 70.43, 70.21, 61.83, 45.18, 32.67, 29.58, 26.83, 25.55. LC-MS (ESI); m/z $[M+H]^+$; Calcd. $C_{20}H_{42}ClO_8$, 445.2568. Found 445.38.



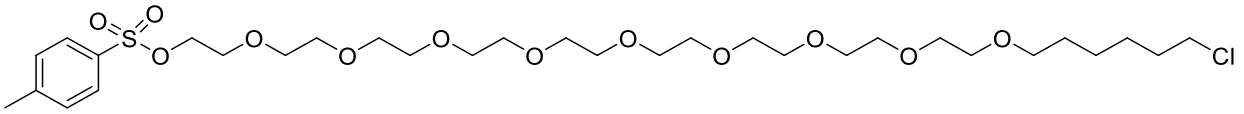
33-chloro-3,6,9,12,15,18,21,24,27-nonaoxatritriacontan-1-ol (**2**). To a solution of 3,6,9,12,15,18,21,24-octaoxahexacosane-1,26-diol (1.68 mL, 4.56 mmol) in a mixture of DMF (5 mL) and THF (5 mL) was added NaH (60.0 %, 150 mg, 3.75 mmol) at room temperature under argon. After 40 minutes, 1-chloro-6-iodo-hexane (0.154 mL, 1.01 mmol) was added, and the mixture was stirred at room temperature for 16 h (overnight). The mixture was then quenched with a mixture of diluted 1 M HCl (5 mL) and brine (15 mL), and then the reaction mixture was extracted with Ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography (Gradient, DCM 100% to DCM:MeOH, 9:1) to give 242 mg of pure product (45% yield).. ^{1}H NMR (500 MHz, Chloroform-d) δ 3.84 – 3.54 (m, 36H), 3.51 (t, J = 7.1 Hz, 2H), 3.44 (t, J = 6.6 Hz, 2H), 2.76 (bs, 1H), 1.76 (p, J = 7.0 Hz, 2H), 1.58 (p, J = 6.8 Hz, 2H), 1.48 – 1.30 (m, 4H). ^{13}C NMR (151 MHz, Chloroform-d) δ 72.66, 71.34, 70.72, 70.70, 70.66 (9C), 70.64, 70.62, 70.62, 70.40, 70.21, 61.81, 45.17, 32.66, 29.57, 26.82, 25.54. LC-MS (ESI); m/z $[M+H]^+$; Calcd. $C_{24}H_{50}ClO_{10}$, 533.3092. Found 533.45.



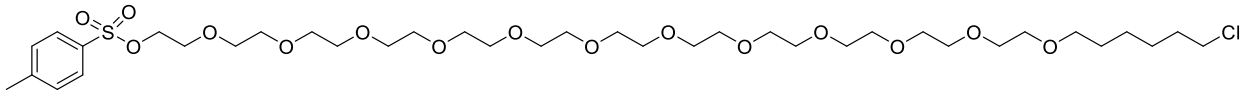
42-chloro-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxadotetracontan-1-ol (**3**). To a solution of 3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontane-1,35-diol (998 mg, 1.83 mmol) in a mixture of DMF (3 mL) and THF (3 mL) was added NaH (60.0 %, 48.7 mg, 1.22 mmol) at room temperature under argon. After 40 minutes, 1-chloro-6-iodo-hexane (0.0616 mL, 0.406 mmol) was added, and the mixture was stirred at room temperature for 16 h (overnight). The mixture was then quenched with a mixture of diluted 1 M HCl (5 mL) and brine (20 mL), and then the reaction mixture was extracted with Ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography (Gradient, DCM 100% to DCM:MeOH, 7:3) to give 104 mg of pure product as an oil (38% yield) ^{1}H (400 MHz, Chloroform-d) δ 3.93 – 3.55 (m, 48H), 3.51 (t, J = 6.7 Hz, 2H), 3.44 (t, J = 6.6 Hz, 2H), 2.56 (bs, 1H), 1.76 (pd, J = 6.7, 2.1 Hz, 2H), 1.64 – 1.50 (m, 2H), 1.50 – 1.27 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 72.63, 71.34, 70.72, 70.70, 70.68, 70.66 (15C), 70.63, 70.42, 70.42, 70.21, 61.82, 45.17, 32.65, 29.56, 26.81, 25.53. LC-MS (ESI); m/z $[M+H]^+$; Calcd. $C_{30}H_{62}ClO_{13}$, 665.3878. Found 665.54.



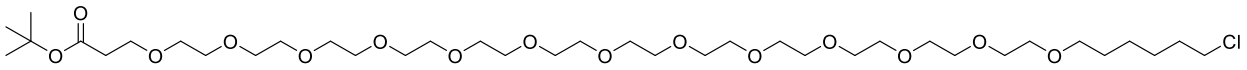
27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosyl 4-methylbenzenesulfonate (**4**). To a solution of 27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosan-1-ol (114 mg, 0.256 mmol) and TEA (0.214 mL, 1.54 mmol) in DCM (5 ml) was added toluene-4-sulfonyl chloride (63.5 mg, 0.333 mmol) at room temperature. The reaction mixture was stirred for 5 at the same temperature. Then the reaction mixture was poured into aqueous $NaHCO_3$ (sat. solution, 10 mL) and product was extracted with DCM (2x20 mL). Organic extracts were combined, dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by column chromatography (DCM 100% to DCM:MeOH, 95:5), to give 90 mg (58% yield) of product as an oil. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.79 (d, J = 10.1 Hz, 2H), 7.34 (d, J = 6.6 Hz, 2H), 4.15 (t, J = 6.1 Hz, 2H), 3.81 – 3.55 (m, 24H), 3.53 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 7.9 Hz, 2H), 2.44 (s, 3H), 1.77 (p, J = 9.2 Hz, 2H), 1.59 (p, J = 7.8, 6.3 Hz, 2H), 1.52 – 1.22 (m, 4H). ^{13}C NMR (151 MHz, Chloroform-d) δ 144.89, 133.13, 129.93, 128.10, 71.35, 70.87, 70.73, 70.71, 70.69 (6C), 70.68, 70.64, 70.22, 69.36, 68.80, 45.18, 32.67, 29.58, 26.82, 25.55, 21.77. LC-MS (ESI); m/z $[M+H]^+$; calcd. $C_{27}H_{48}ClO_{10}S$, 599.2656. Found 599.37.



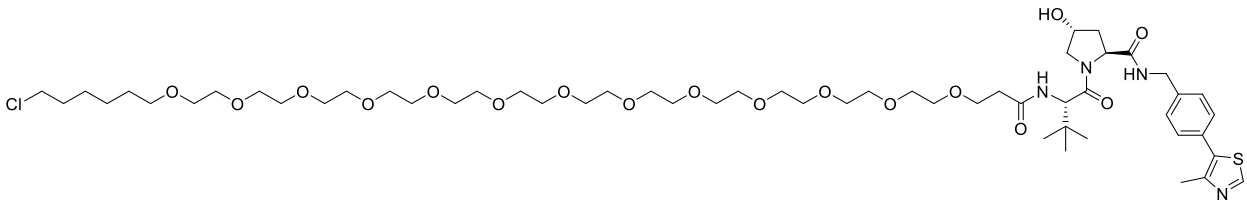
33-chloro-3,6,9,12,15,18,21,24,27-nonaoxatritriacontyl 4-methylbenzenesulfonate (**5**). To a solution of 33-chloro-3,6,9,12,15,18,21,24,27-nonaoxatritriacontan-1-ol (230 mg, 0.431 mmol) and TEA (0.361 mL, 2.59 mmol) in DCM (5 ml) was added Toluene-4-sulfonyl chloride (107 mg, 0.561 mmol) at room temperature. The reaction mixture was stirred for 5 at the same temperature. Then the reaction mixture was poured into aqueous $NaHCO_3$ (sat. solution, 10 mL) and product was extracted with DCM (2x20 mL). Organic extracts were combined, dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by column chromatography (DCM 100% to DCM:MeOH, 95:5), to give 161 mg (54% yield) of product. ^{1}H NMR (600 MHz, DMSO-d6) δ 7.78 (d, J = 7.6 Hz, 2H), 7.48 (d, J = 7.7 Hz, 2H), 4.11 (t, J = 5.2 Hz, 2H), 3.61 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 4.3 Hz, 2H), 3.47 (d, J = 32.9 Hz, 32H), 3.36 (t, J = 6.1 Hz, 2H), 2.42 (s, 3H), 1.70 (p, J = 6.7 Hz, 2H), 1.48 (p, J = 6.6 Hz, 2H), 1.38 (p, J = 7.1 Hz, 2H), 1.30 (p, J = 7.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d6) δ 144.86, 132.41, 130.11, 127.61, 70.16, 69.97, 69.81, 69.78 (6C), 69.75, 69.70, 69.65, 69.48, 67.88, 45.35, 32.02, 29.05, 26.11, 24.93, 21.08. LC-MS (ESI); m/z $[M+H]^+$; Calcd. $C_{31}H_{56}ClO_{12}S$, 687.3181. Found 687.44.



42-chloro-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxadotetracontyl 4-methylbenzenesulfonate (**6**). To a solution of 42-chloro-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxadotetracontan-1-ol (48.0 mg, 0.0722 mmol) and TEA (0.0603 mL, 0.433 mmol) in DCM (2 ml) was added Toluene-4-sulfonyl chloride (17.9 mg, 0.0938 mmol) at room temperature. The reaction mixture was stirred for 5h at the same temperature. Then the reaction mixture was poured into aqueous $NaHCO_3$ (sat. solution, 10 mL) and product was extracted with DCM (2x20 mL). Organic extracts were combined, dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1), to give 28mg (47% yield) of pure product. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.78 (d, J = 8.3 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 4.23 – 3.99 (m, 2H), 3.61 (t, J = 6.6 Hz, 2H), 3.59 – 3.40 (m, 46H), 3.36 (t, J = 6.5 Hz, 2H), 2.42 (s, 3H), 1.77 – 1.62 (m, 2H), 1.48 (p, J = 6.8 Hz, 2H), 1.43 – 1.17 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d6) δ 144.90, 132.42, 130.14, 127.64, 70.18, 69.99, 69.83, 69.79 (C18), 69.71, 69.67, 69.51, 67.89, 45.37, 32.04, 29.07, 26.13, 24.95, 21.10. LC-MS (ESI); m/z $[M+Na]^+$; Calcd. $C_{37}H_{67}ClO_{15}SNa$, 841.3787.

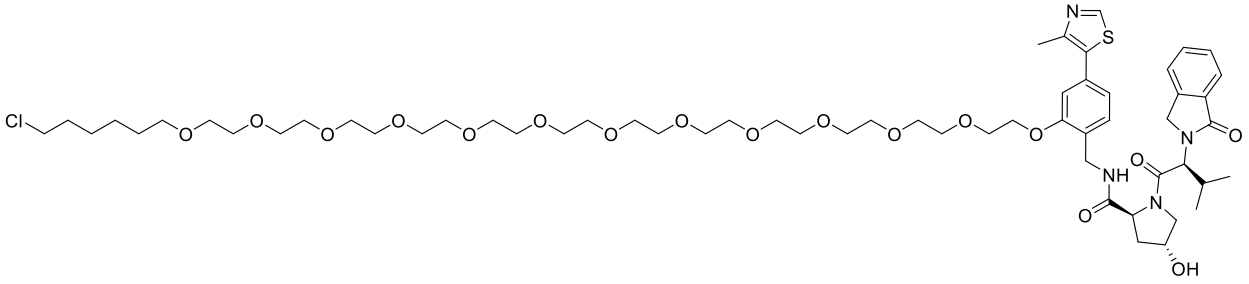


tert-butyl 46-chloro-4,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxahexatetracontanoate (**8**). To a solution of 42-chloro-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxadotetracontan-1-ol (**3**) (50.0 mg, 0.0752 mmol) in acetonitrile (2 mL) was added tert-butyl prop-2-enoate (0.218 mL, 1.50 mmol) followed by Triton B (40.0 %, 0.158 mL, 0.400 mmol, in 40% by weight in water). The mixture was stirred at room temperature for 48 hours. The mixture was concentrated under vacuum and crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1) to give 42 mg of product as an oil (70% yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 3.68 (t, J = 6.6 Hz, 2H), 3.65 – 3.54 (m, 48H), 3.51 (t, J = 6.7 Hz, 2H), 3.43 (t, J = 6.6 Hz, 2H), 2.48 (t, J = 6.6 Hz, 2H), 1.75 (p, J = 13.5, 6.2 Hz, 2H), 1.64 – 1.50 (m, 2H), 1.42 (s, 9H), 1.50 – 1.26 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d6) δ 170.42, 79.71, 70.19, 69.84, 69.81(20C), 69.72, 69.69, 69.52, 66.24, 45.38, 35.85, 32.04, 29.08, 27.75, 26.13, 24.95. LC-MS (ESI); m/z $[M+Na]^+$: Calcd. for $C_{37}H_{73}ClO_{15}Na$, 815.4535. Found 815.4527.



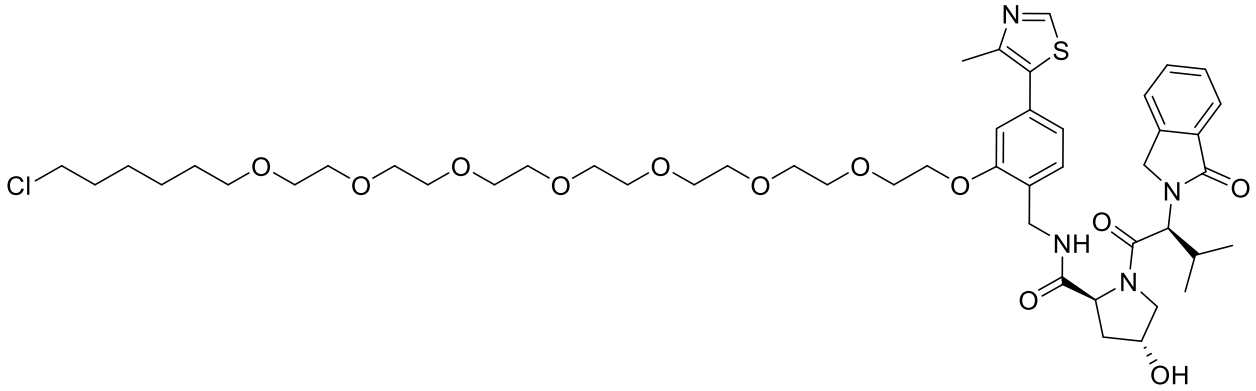
(2S,4R)-1-((S)-2-(tert-butyl)-49-chloro-4-oxo-7,10,13,16,19,22,25,28,31,34,37,40,43-tridecaoxa-3-azanonatetracontanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**SJF-4627**). A solution of tert-butyl 46-chloro-4,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxahexatetra contanoate (**8**) (18.0 mg, 0.0227 mmol) in a mixture of TFA (1 ml, 13.46 mmol) and Dichloromethane (2 ml) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h. Crude product (**9**) was used in the next step without any further purification (16.7 mg, quantitative yield). HRMS (ESI); m/z: $[M+H]^+$ Calcd. for $C_{33}H_{66}ClO_{15}$, 737.4090. Found 737.4090.

To a solution of 46-chloro-4,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxahexatetracontanoic acid (16.7 mg, 0.0226 mmol) and (2S,4R)-1-[(2S)-2-amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide;hydrochloride (12.7 mg, 0.0272 mmol) in DMF (2 ml) was added N,N-Diisopropylethylamine (0.298 mL, 1.71 mmol) and O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (12.9 mg, 0.0340 mmol) at room temperature. The reaction mixture was stirred for 12 h (overnight) at the same temperature. TLC (DCM:MeOH:NH_4OH, 1:1) shows no starting materials. Reaction mixture was diluted with EtOAc (10 mL), washed with water (4x10 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1), to 23 mg of product (88 % yield). ^1H NMR (500 MHz, DMSO-d6) δ 8.98 (s, 1H), 8.56 (t, J = 5.9 Hz, 1H), 7.91 (d, J = 9.3 Hz, 1H), 7.42 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 5.12 (d, J = 3.4 Hz, 1H), 4.55 (d, J = 9.4 Hz, 1H), 4.46 – 4.39 (m, 2H), 4.37 – 4.32 (m, 1H), 4.22 (dd, J = 15.8, 5.2 Hz, 1H), 4.00 – 3.42 (m, 54H), 3.37 (t, J = 6.5 Hz, 2H), 2.58 – 2.51 (m, 1H), 2.44 (s, 3H), 2.39 – 2.32 (m, 1H), 2.07 – 2.00 (m, 1H), 1.95 – 1.87 (m, 1H), 1.70 (dt, J = 14.3, 6.7 Hz, 2H), 1.48 (p, J = 13.8, 6.7 Hz, 2H), 1.42 – 1.25 (m, 4H), 0.94 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d6) δ 171.96, 169.96, 169.54, 151.48, 147.73, 139.53, 131.18, 129.65, 128.65, 127.44, 70.19, 69.84, 69.80, 69.73, 69.52, 69.50, 68.89 (20C), 66.97, 58.73, 56.40, 56.31, 45.39, 41.66, 37.96, 35.67, 35.39, 32.04, 29.08, 26.34, 26.13, 24.95, 15.96. HRMS (ESI); m/z $[M+H]^+$: Calcd. for $C_{55}H_{94}ClN_4O_{17}S$, 1149.6023. Found 1149.6023.



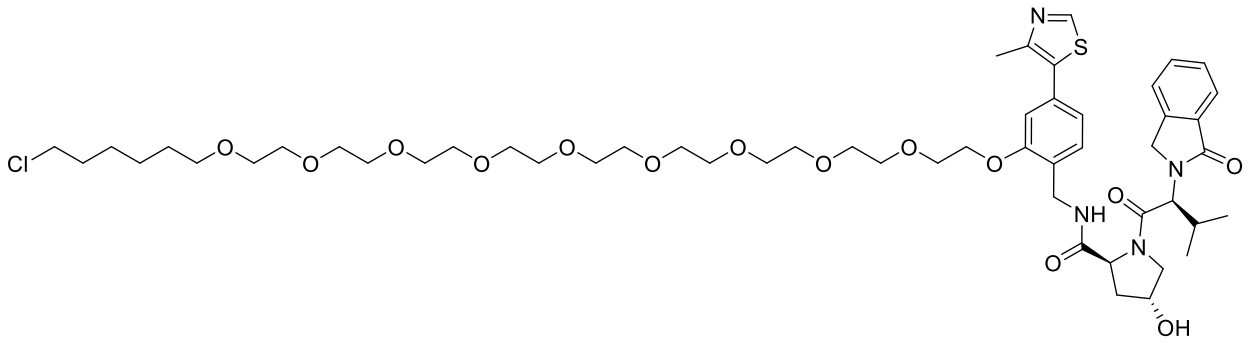
(2S,4R)-N-(2-((42-chloro-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxadotetracontyl)oxy)-4-(4-methyl -thiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl)pyrrolidine-2-carboxa -mide (**SJF-4625**). To a mixture of (2S,4R)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (22.0 mg, 0.0402 mmol) and 27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosyl 4-methylbenzenesulfonate (**6**) (23.0 mg, 0.0335 mmol) in DMF (1 mL) was added

Cs_2CO_3 (21.8 mg, 0.0669 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5x10 mL), organic phase was dried (Na_2SO_4, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1) to give 21 mg of product (58% yield). ^1H NMR (400 MHz, DMSO-d6) δ 8.99 (s, 1H), 8.38 (t, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.68 – 7.55 (m, 2H), 7.55 – 7.43 (m, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.05 (s, 1H), 7.01 (d, J = 7.8 Hz, 1H), 5.11 (d, J = 3.9 Hz, 1H), 4.71 (d, J = 10.8 Hz, 1H), 4.51 (dd, 2H), 4.42 – 4.13 (m, 5H), 4.10 – 3.25 (m, 50H), 2.47 (s, 3H), 2.39 – 2.26 (m, 1H), 2.11 – 1.99 (m, 1H), 1.97 – 1.86 (m, 1H), 1.69 (p, J = 14.4, 6.9 Hz, 2H), 1.48 (p, J = 6.8 Hz, 2H), 1.43 – 1.22 (m, 4H), 0.97 (d, J = 6.4 Hz, 3H), 0.74 (d, J = 6.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d6) δ 172.00, 168.53, 167.93, 156.30, 151.90, 148.35, 142.62, 132.02, 131.79, 131.69, 131.40, 128.34, 128.11, 127.60, 124.04, 123.45, 121.49, 112.55, 70.60, 70.51, 70.27, 70.24 (18C), 70.21, 69.92, 69.43, 69.05, 68.34, 59.14, 58.21, 55.84, 47.24, 45.80, 38.51, 37.50, 32.45, 29.48, 28.82, 26.54, 25.36, 19.30, 19.04, 16.44. HRMS (ESI); m/z: $[M+H]^+$ Calcd. for $C_{59}H_{92}ClN_4O_{17}S$, 1195.5866. Found 1195.5869.

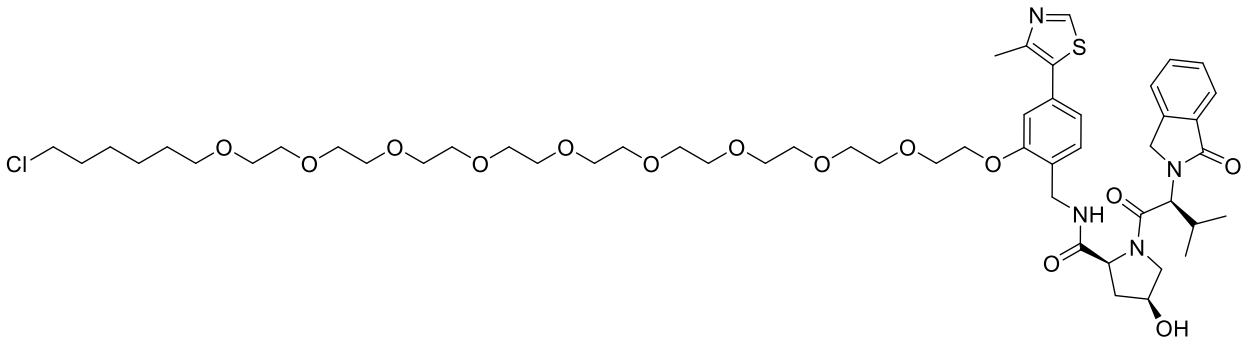


(2S,4R)-N-(2-((27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (**SJF-7432**). To a mixture of(2S,4R)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (25.3 mg, 0.0461 mmol) and 27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosyl 4-methylbenzenesulfonate (**4**) (23.0 mg, 0.0384 mmol) in DMF (1 mL) was added Cs_2CO_3 (25.0 mg, 0.0768 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5x10 mL), organic phase was dried (Na_2SO_4, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1) to give 25 mg of product (67% yield). ^1H NMR (500 MHz, DMSO-d6) δ 8.99 (s, 1H), 8.36 (t, J = 5.2 Hz, 1H), 7.71 (d, J = 7.4 Hz, 1H), 7.67 – 7.55 (m, 2H), 7.50 (t, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.05 (s, 1H), 7.01 (d, J = 7.8 Hz, 1H), 5.09 (d, J = 3.5 Hz, 1H), 4.72 (d, J = 10.7 Hz, 1H), 4.60 – 4.42 (m, 2H), 4.45 – 4.12 (m, 5H), 4.04 – 3.41 (m, 31H), 3.35 (t, J = 6.5 Hz, 2H), 2.47 (s, 3H), 2.34 (dq, J = 12.2, 6.6 Hz, 1H), 2.04 (dd, J = 12.7, 8.1 Hz, 1H), 1.92 (ddd, J = 12.9, 7.9, 4.7 Hz, 1H), 1.69 (dt, J = 13.1, 6.5 Hz, 2H), 1.47 (dt, J = 12.7, 6.4 Hz, 2H), 1.37 (dt, J = 14.2, 7.2 Hz, 2H), 1.34 – 1.22 (m, 2H), 0.97 (d, J = 6.1

Hz, 3H), 0.74 (d, J = 6.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d6) δ 171.53, 168.07, 167.45, 155.85, 151.45, 147.91, 142.19, 131.56, 131.37, 131.25, 130.96, 127.89, 127.67, 127.18, 123.61, 123.00, 121.05, 112.13, 70.16, 70.08, 69.84, 69.82, 69.80, 69.78 (6C), 69.48, 69.00, 68.61, 67.90, 58.70, 57.77, 55.41, 46.81, 45.36, 38.10, 37.06, 32.02, 29.05, 28.39, 26.11, 24.93, 18.88, 18.63, 16.03. HRMS (ESI); m/z: $[M+H]^+$ Calcd. for $C_{49}H_{72}ClN_4O_{12}S$, 975.4555. Found 975.4532.



(2S,4R)-N-(2-((33-chloro-3,6,9,12,15,18,21,24,27-nonaoxatritriacontyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (**SJF-7434**). To a mixture of (2S,4R)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (22.0 mg, 0.0402 mmol) and 27-chloro-3,6,9,12,15,18,21-heptaoxaheptacosyl 4-methylbenzenesulfonate (**5**) (23.0 mg, 0.0335 mmol) in DMF (1 mL) was added Cs_2CO_3 (21.8 mg, 0.0669 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5x10 mL), organic phase was dried (Na_2SO_4, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1) to give 20 mg of product (56% yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.99 (s, 1H), 8.39 (t, J = 5.4 Hz, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.67 – 7.55 (m, 2H), 7.54 – 7.45 (m, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.05 (s, 1H), 7.01 (d, J = 7.8 Hz, 1H), 5.11 (d, J = 3.6 Hz, 1H), 4.71 (d, J = 10.8 Hz, 1H), 4.60 – 4.12 (m, 7H), 3.95 – 3.18 (m, 35H), 2.47 (s, 3H), 2.38 – 2.26 (m, 1H), 2.09 – 1.99 (m, 1H), 1.98 – 1.84 (m, 1H), 1.69 (p, J = 6.7 Hz, 2H), 1.47 (p, J = 6.6 Hz, 2H), 1.41 – 1.33 (m, 2H), 1.32 – 1.26 (m, 2H), 0.96 (d, J = 6.2 Hz, 3H), 0.73 (d, J = 6.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d6) δ 171.54, 168.08, 167.46, 155.86, 151.46, 147.92, 142.19, 131.57, 131.37, 131.25, 130.97, 127.90, 127.68, 127.18, 123.61, 123.01, 121.06, 112.13, 70.17, 70.09, 69.85, 69.83, 69.81, 69.78 (17C), 69.74, 69.73, 69.49, 69.00, 68.62, 67.91, 58.71, 57.78, 55.42, 54.93, 46.81, 45.37, 38.10, 37.07, 32.03, 29.06, 28.40, 26.12, 24.94, 18.88, 18.63, 16.04. HRMS (ESI); m/z: $[M+H]^+$ Calcd. for $C_{53}H_{80}ClN_4O_{14}S$, 1063.5080. Found 1063.5063



(2S,4S)-N-(2-((33-chloro-3,6,9,12,15,18,21,24,27-nonaoxatritriacontyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (**JH-6073**). To a mixture of (2S,4S)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (22.0 mg, 0.0402 mmol) and 2-[2-[2-[2-[2-[2-[2-[2-[2-(6-chlorohexoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl 4-methylbenzenesulfonate (23.0 mg, 0.0335 mmol) in N,N-Dimethylformamide (1 mL) was added Cs_2CO_3 (21.8 mg, 0.0669 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with EtOAcEtOAc (10 mL) and washed with water (5x10 mL), organic phase was dried (Na_2SO_4, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH, 9:1) to give 18 mg of product (56% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.83 (d, J = 7.7 Hz, 1H), 7.62 (t, J = 6.1 Hz, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.46 (dt, J = 7.5, 3.6 Hz, 2H), 7.32 (d, J = 7.7 Hz, 1H), 6.99 (d, J = 7.7 Hz, 1H), 6.92 (s, 1H), 4.84 (d, J = 11.0 Hz, 1H), 4.76 (d, J = 17.5 Hz, 1H), 4.55 (d, J = 9.0 Hz, 1H), 4.50 (t, J = 5.6 Hz, 2H), 4.47 – 4.36 (m, 2H), 4.22 (q, J = 4.9 Hz, 2H), 4.09 (dd, J = 11.4, 4.4 Hz, 1H), 3.99 – 3.89 (m, 3H), 3.74 (q, J = 4.4 Hz, 2H), 3.68 – 3.54 (m, 26H), 3.52 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 6.5 Hz, 3H), 2.53 (s, 3H), 2.41 – 2.03 (m, 4H), 1.77 (m, 5H), 1.59 (p, J = 6.9 Hz, 2H), 1.44 (t, J = 7.7 Hz, 2H), 1.45 – 1.31 (m, 2H), 0.89 (dd, J = 10.6, 6.5 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.68, 170.77, 169.14, 156.93, 150.52, 148.61, 142.17, 132.60, 131.93, 131.83, 129.88, 128.17, 126.35, 123.93, 123.03, 122.16, 114.64, 112.87, 71.37, 71.03, 70.94, 70.75, 70.72, 70.70, 70.67, 70.65, 70.24, 69.79, 68.05, 60.08, 58.33, 58.27, 47.26, 45.20, 39.55, 35.86, 32.68, 29.59, 29.05, 26.84, 25.56, 19.14, 18.91, 16.24. LC-MS (ESI); m/z $[M+Na]^+$; Calcd. $C_{53}H_{79}ClN_4NaO_{14}S$, 1085.4900. Found 1085.4894.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

A)

NFκB-TRAFTAC

5’
CGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCG
GUGUUUUU gctagc gggaatttccggggactttccgggaatttccggggactttccgggaatttccagatct 3’

Uppercase- crRNA sequence
Lowercase- DNA sequence
Green-NFkB binding site
Yellow- double stranded sequence

Reverse DNA

5’ gtccccggaaattcccggaaagt 3’

B) **Control-TRAFTAC**

5’
CGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUG
UUUUU atccgctcaactgatcgtacgtcgcag tcagtactag tactgtcaagcacgctgtacgcctcat 3’

Green- control double strand site
Red/green- DNA sequence
Yellow- double stranded control DNA sequence

Reverse control DNA

5’ acagtactagtactgactgcgac 3’

C)

5’ agatctggaaattcccggaaagtccccggaaattcccggaaagtccccggaaattcccgct-Fluorescein 3’

D)

allscrambled-TRAFTAC

5’
AUAGAAUAGAUGUACAACUAAGAUCAGUUGGCAAUUGUACAAUCGUAACGUAGAGCAUUAGUCAUUGUU
CGGUUAUCGCGG agcgtgtatggagcggcgt cgacggttagtctgccgactatcg ggttgtctctaacgtcatcta 3’

ReverseallSCRMB

5’ cgatagtcggcagactaaccgtcg 3’

Figure S1. Oligonucleotide sequences for TRAFTACs and reverse complements used in this study. Related to STAR methods.

A) Single stranded NFκB-TRAFTAC sequence.

B) Single stranded control-TRAFTAC sequence.

C) Reverse complement sequence covalently attached to fluorescein at the 5’ end of the sequence.

D) Single stranded sequences of allscrambled-TRAFTAC and reverse complement sequence used to generate the double stranded allscrambled-TRAFTAC.

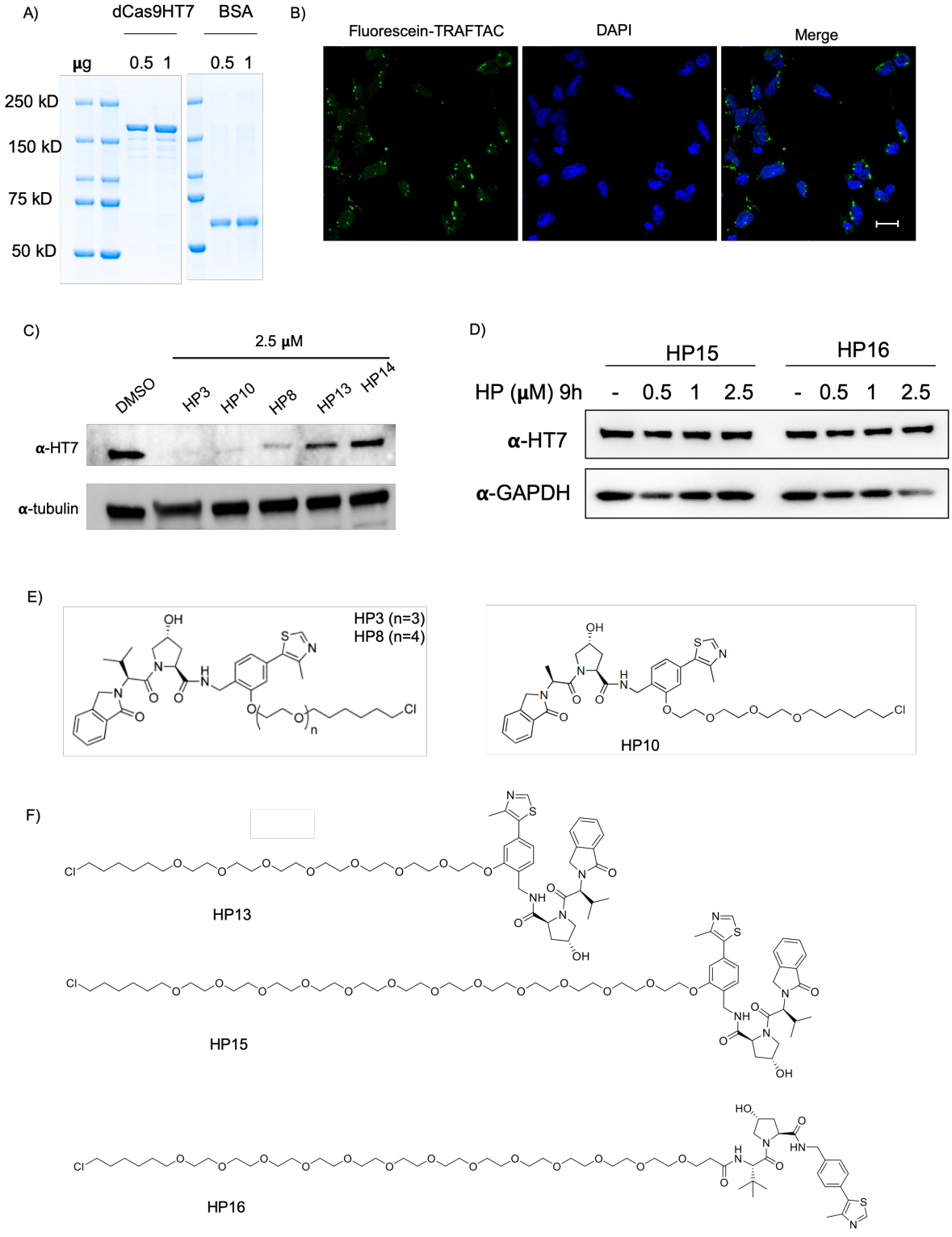

A)
dCas9HT7
BSA
µg
0.5 1
0.5 1
250 kD
150 kD
75 kD
50 kD
B)
Fluorescein-TRAFTAC
DAPI
Merge
C)
2.5 µM
DMSO
HP3
HP10
HP8
HP13
HP14
α-HT7
α-tubulin
D)
HP15
HP16
HP (µM) 9h
- 0.5 1 2.5
- 0.5 1 2.5
α-HT7
α-GAPDH
E)
HP3 (n=3)
HP8 (n=4)
HP10
F)
HP13
HP15
HP16

Figure S2. TRAFTAC transfection, HaloPROTAC screening and chemical structures used in the study. Related to Figure 3.

A) A fusion of HT7 and dCas9 proteins was purified using HIS-tag affinity purification and further purified by size exclusion chromatography. Purified fusion dCas9HT7 protein was ran in parallel with BSA.

B) HEK293 cells were transfected with 25 nM of fluorescein-labeled TRAFTAC and after 12 h cells were fixed, permeabilized and labeled with DAPI. Cells were analyzed for fluorescein and DAPI signal by confocal microscopy at 40X magnification. (scale bar: 25 μm).

C) and D) HaloPROTAC screening towards the degradation of HaloTag fusion protein, dCas9HT7.

E) and F) Chemical structures of HaloPROTACs (HP) used in this study.

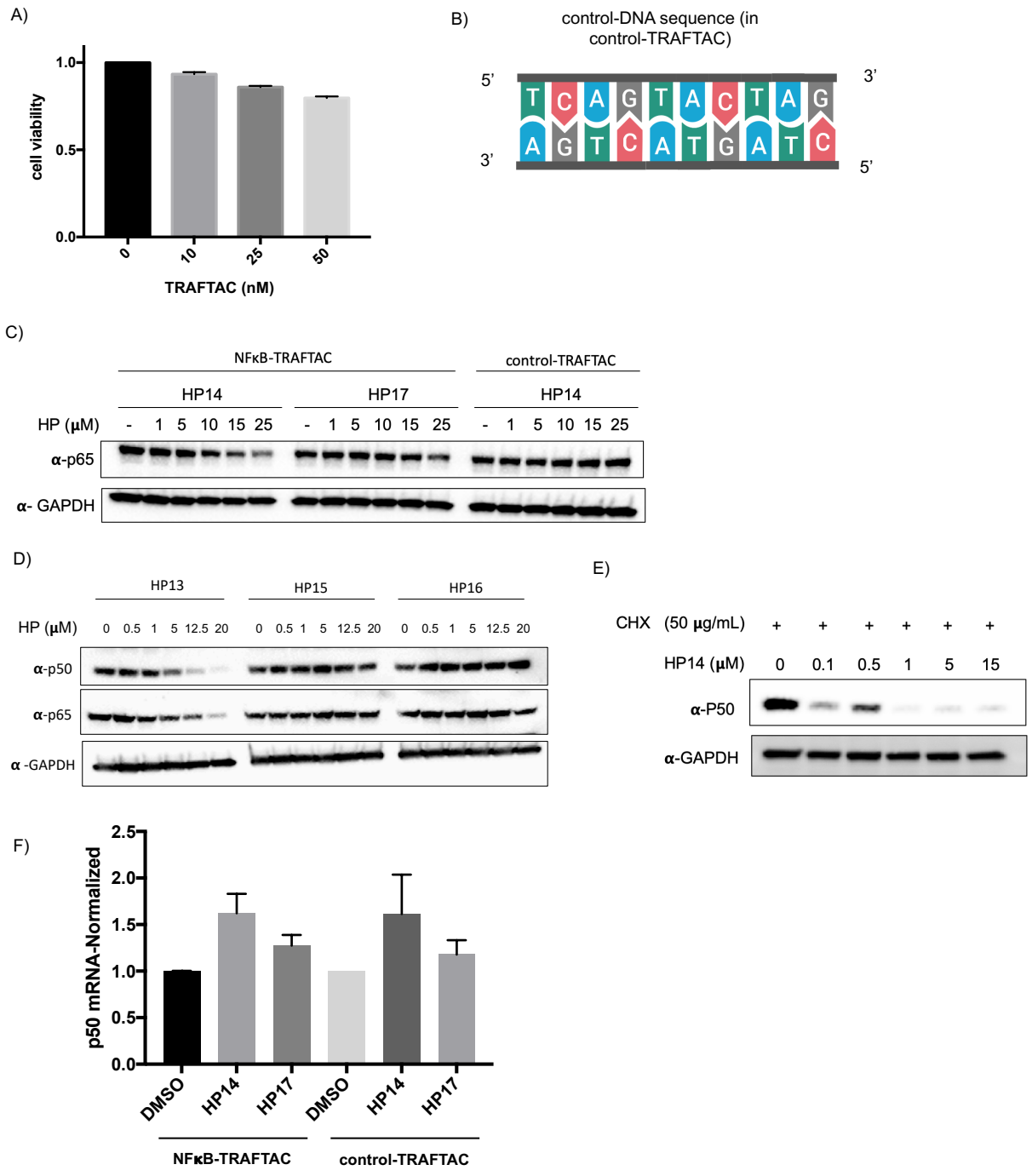

A)
cell viability
1.0
0.5
0.0
0
10
25
50
TRAFTAC (nM)
B)
control-DNA sequence (in control-TRAFTAC)
5'
3'
T C A G T A C T A G
A G T C A T G A T C
3'
5'
C)
NFκB-TRAFTAC
control-TRAFTAC
HP14
HP17
HP14
HP (μM) - 1 5 10 15 25 - 1 5 10 15 25 - 1 5 10 15 25
α-p65
α- GAPDH
D)
HP13
HP15
HP16
HP (μM) 0 0.5 1 5 12.5 20 0 0.5 1 5 12.5 20 0 0.5 1 5 12.5 20
α-p50
α-p65
α -GAPDH
E)
CHX (50 μg/mL) + + + + + +
HP14 (μM) 0 0.1 0.5 1 5 15
α-P50
α-GAPDH
F)
p50 mRNA-Normalized
2.5
2.0
1.5
1.0
0.5
0.0
DMSO
HP14
HP17
DMSO
HP14
HP17
NFκB-TRAFTAC
control-TRAFTAC

Figure S3. TRAFTACs induce NFκB degradation as a posttranslational event. Related to Figure 3.

A) Cell viability assay. Stable cells that express dCas9HT7 were transfected with increasing concentrations of NFκB-TRAFTAC and after 36 h, cells were subjected to MTS assay.

B) Double stranded, control oligonucleotide sequence used in the control-TRAFTAC.

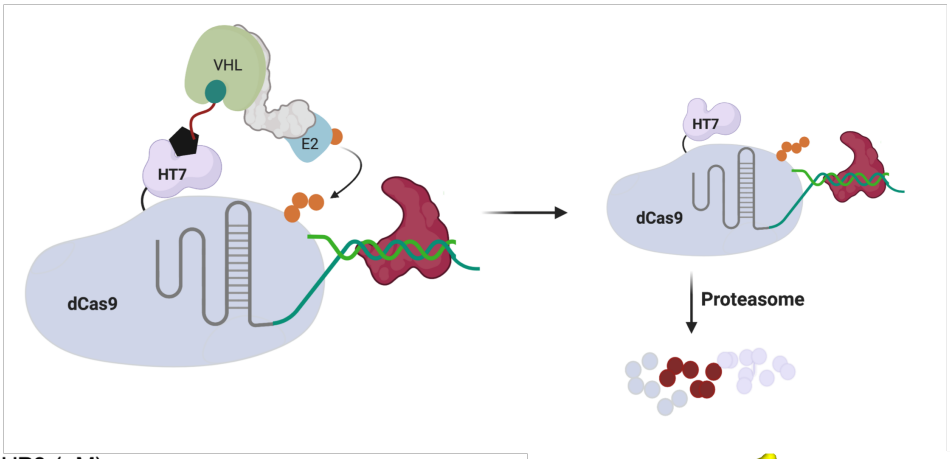
C) Stable cells overexpressing CT-dCas9HT7 were transfected with NFκB-TRAFTAC or control-TRAFTAC followed by HP and TNF-alpha treatment. Cells were lysed and analyzed for p65 and GAPDH levels. These data obtained from the same experiment discussed in the Figure 3D. Therefore, GAPDH blot in Figure 3D is the same as Figure S3C.

D) NFκB degradation by HP13, HP15 and HP16**.** Stable cells were transfected with NFκB-TRAFTAC followed by HP and TNF-alpha treatment. Cell lysates were probed as indicated.

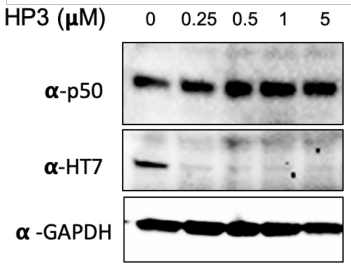
E) Cycloheximide (CHX) co-incubation with HP14/ TNF-alpha significantly enhanced p50 degradation levels in NFκB-TRAFTAC transfected cells.

F) Cells were transfected either with NFκB-TRAFTAC or control-TRAFTAC followed by HP14 or HP17 and TNF-alpha treatment. The pool of isolated RNA was subjected to one-step qRT-PCR using primers specific to p50 and GAPDH and normalized data presented as a bar graph.

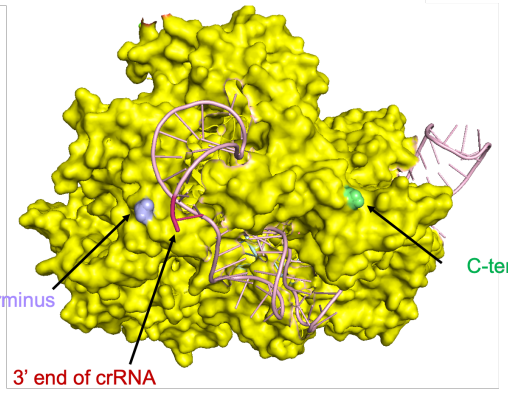
A)



B)

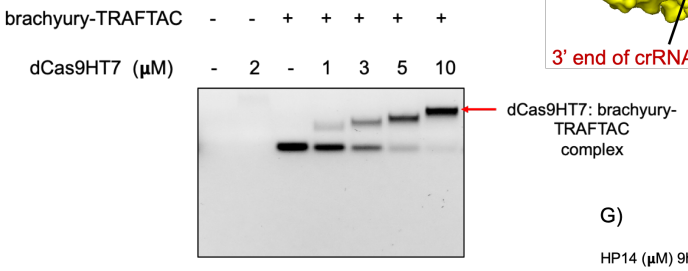


C)

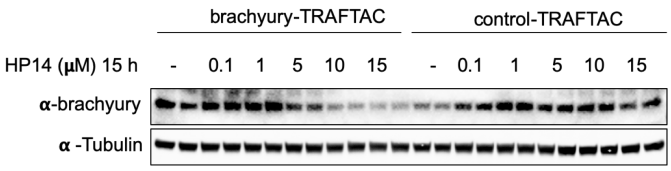


PDB ID:4OO8

D)



E)



F)

brachyury-TRAFTAC

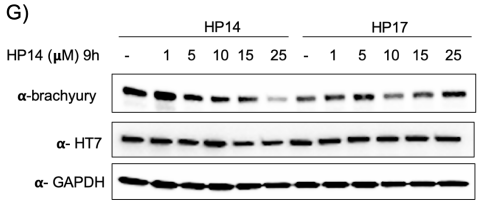
5'
CGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU
UGAAAAAGUGGCACCGAGUCGGUGUUUUU gctagcgggaatttccgggg actttcc
aatttcacacctaggtgtgaaatt ggggac tttccgggaatttccagatct 3'

Uppercase- crRNA sequence
Lowercase- DNA sequence
Green- Brachyury binding site

RssDNA

5' gtccccaatttcacacctaggtgtgaaattggaaagt 3'

G)



H)

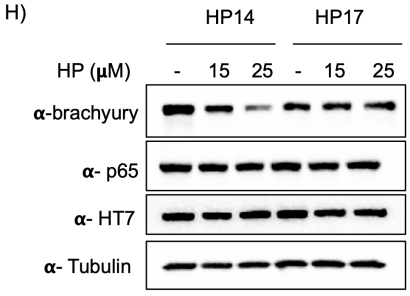


Figure S4. TRAFTAC mediated NFκB degradation did not result from dCas9HT7 degradation. Related to Figure 4.

A) Schematic representation of possible HP3 mediated degradation of dCas9HT7:TRAFTAC:p50 complex.

B) Stable cells were transfected with NFκB-TRAFTAC and treated with HP3 followed by TNF-alpha. Lysates were probed as indicated in the figure.

C) Crystal structure of *S. pyogenes* Cas9 in complexed with gRNA (PDB ID:4OO8). Both N- and C-terminal amino acids of the Cas9 protein are located in the space such that N- or C-terminally tagged fusion HT7 can access the 3' end of the Cas9 bound gRNA.

D) Fusion protein of dCas9HT7 forms the binary complex with brachyury-TRAFTACs *in vitro* as seen by EMSA.

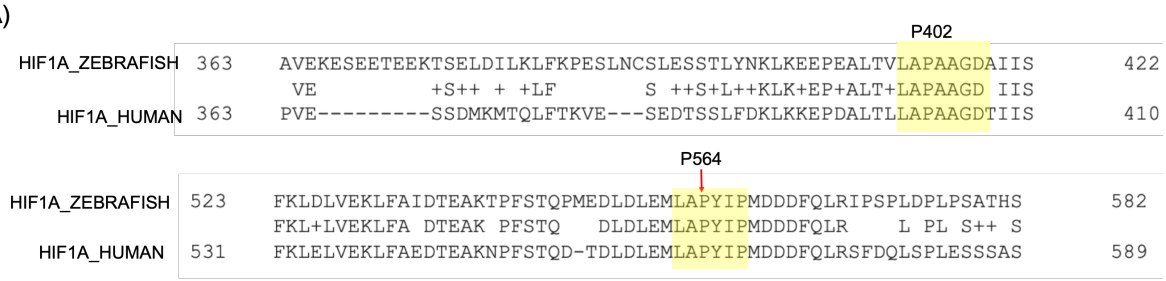
E) Cells were transfected with brachyury-TRAFTAC or control-TRAFTAC. Then cells were treated with HP14 for 15 and cell lysates were subjected to western blot analysis as shown in the figure.

F) The chimeric oligo sequence of the single stranded brachyury-TRAFTAC and reverse complement sequence of brachyury targeting DNA sequence (shown in green).

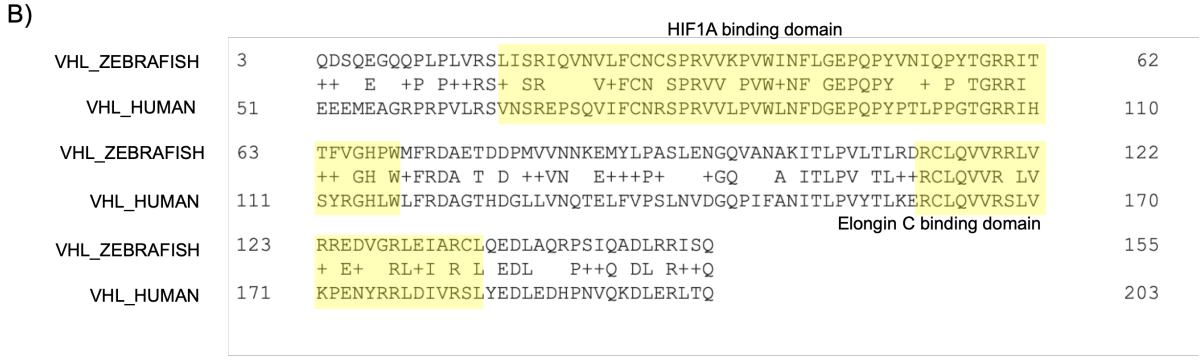
G) After transfection of brachyury-TRAFTAC, cells were treated with HP14 and HP17 for 9 h before cell lysis. Cell lysates were analyzed by western blot using antibody against brachyury, HT7and GAPDH.

H) Brachyury targeting TRAFTAC did not induce degradation of other transcription factors. Cells were transfected with brachyury-TRAFTAC followed by HP14 and HP17 treatment. After 9 h, cells were lysed and lysates were analyzed for brachyury-GFP, p65, HT7 and tubulin.

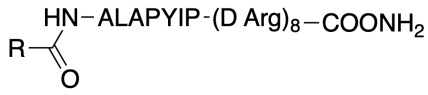
A)


P402
HIF1A_ZEBRAFISH 363 AVEKESEETEEKTSELDILKLFKPESLNCSLESSTLYNKLKEEPEALTVLAPAAGDAIIS 422
VE +S++ + +LF S ++S+L++KLK+EP+ALT+LAPAAGD IIS
HIF1A_HUMAN 363 PVE---------SSDMKMTQLFTKVE---SEDTSSLFDKLKKEPDALTLLAPAAGDTIIS 410
P564
HIF1A_ZEBRAFISH 523 FKLDLVEKLFAIDTEAKTPFSTQPMEDLDLEMLAPYIPMDDDFQLRIPSPLDPLPSATHS 582
FKL+LVEKLFA DTEAK PFSTQ DLDLEMLAPYIPMDDDFQLR L PL S++ S
HIF1A_HUMAN 531 FKLELVEKLFAEDTEAKNPFSTQD-TDLDLEMLAPYIPMDDDFQLRSFDQLSPLESSSAS 589

B)


HIF1A binding domain
VHL_ZEBRAFISH 3 QDSQEGQQPLPLVRSLISRIQVNVLFCNCSPRVVKPVWINFLGEPQPYVNIQPYTGRRIT 62
++ E +P P++RS+ SR V+FCN SPRVV PVW+NF GEPQPY + P TGRRI
VHL_HUMAN 51 EEEMEAGRPRPVLRSVNSREPSQVIFCNRSPRVVLPVWLNFDGEPQPYPTLPPGTGRRIH 110
VHL_ZEBRAFISH 63 TFVGHPWMFRDAETDDPMVVNNKEMYLPASLENGQVANAKITLPVLTLRDRCLQVVRRLV 122
++ GH W+FRDA T D ++VN E+++P+ +GQ A ITLPV TL++RCLQVVR LV
VHL_HUMAN 111 SYRGHLWLFRDAGTHDGLLVNQTELFVPSLNVDGQPIFANITLPVYTLKERCLQVVRSLV 170
Elongin C binding domain
VHL_ZEBRAFISH 123 RREDVGRLEIARCLQEDLAQRPSIQADLRRISQ 155
+ E+ RL+I R L EDL P++Q DL R++Q
VHL_HUMAN 171 KPENYRRLDIVRSLYEDLEDHPNVQKDLERLTQ 203

C)


HN−ALAPYIP-(D Arg)8−COONH2
R
O

D)

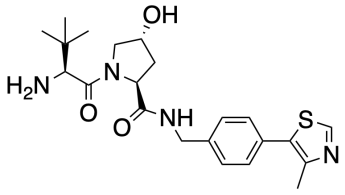

OH
H2N
N
O
O
NH
S
N

Figure S5. HIF1A and VHL proteins in human and zebrafish is highly conserved. Related to Figure 6.

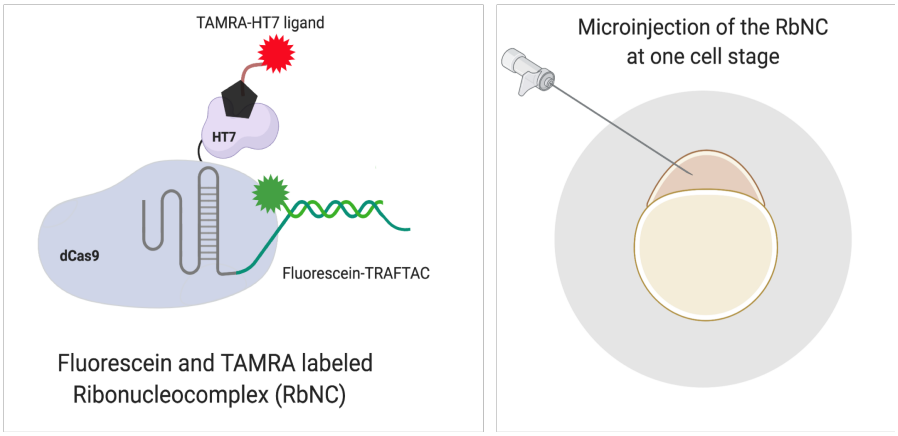
A) Sequence alignment for human and zebrafish HIF1A protein. VHL binding sequence in both species are highly conserved (highlighted in yellow).

B) HIF1A and elongin C binding domains are conserved in zebrafish and human VHL proteins (highlighted in yellow).

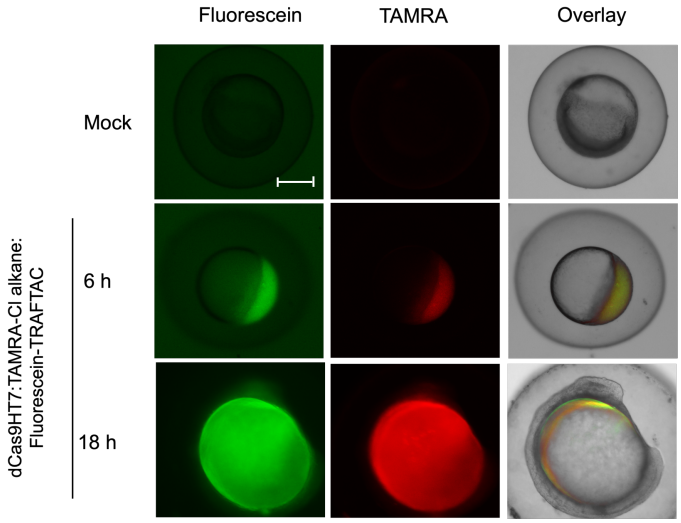
C) VHL ligand that derived from VHL-binding HIF1A peptide sequence (LAPYIP).

D) small molecule based VHL ligand is derived from HIF1A peptide sequence which is conserved in zebrafish and human.

A)


TAMRA-HT7 ligand
HT7
dCas9
Fluorescein-TRAFTAC
Fluorescein and TAMRA labeled
Ribonucleocomplex (RbNC)
Microinjection of the RbNC
at one cell stage

B)


Fluorescein
TAMRA
Overlay
Mock
6 h
18 h
dCas9HT7:TAMRA-Cl alkane:
Fluorescein-TRAFTAC

C)

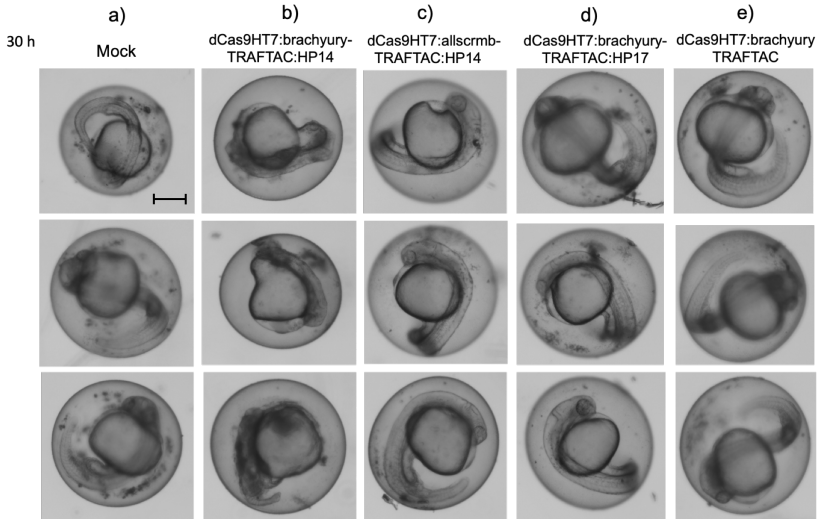

30 h
a)
Mock
b)
dCas9HT7:brachyury-
TRAFTAC:HP14
c)
dCas9HT7:allscrmb-
TRAFTAC:HP14
d)
dCas9HT7:brachyury-
TRAFTAC:HP17
e)
dCas9HT7:brachyury
TRAFTAC

Figure S6. Microinjection of ribonucleocomplex into zebrafish embryos. Related to Figure 7.

A) Schematic representations of the ribonucleocomplex and an embryo at one cell stage.

B) Embryos were injected with either mock (buffer solution; a) or ribonucleocomplex consists with dCas9HT7, TAMRA-HT7 ligand and fluorescein labelled TRAFTAC. Injected embryos were incubated at 29 degrees and fluorescence images were captured after 6 and 18 h of post injection. (scale bar: 100 μm).

C) Brachyury targeting ribonucleocomplex (dCas9HT7:brachyury-TRAFTAC:HP14) induced severe tail deformation. Embryos were injected with mock (a) and all combinations of (b-e) ribonucleocomplexes at single cells stage. After 30 hpf, embryos in each group were analyzed using a microscope for any defects in tail formation. (scale bar: 100 μm).

Table S1. List of oligonucleotde sequences used to generate plasmids. Related to STAR methods.

Name	Sequence (5'--3')
F-NTHT7	ACGTACCTGACTATGCTGGAGCAGAAAUCGGTACTGGCTTTCCATTCG
R-NTHT7	ATCAGCGGGTACCGGAAATCUCCAGAGTAGACAGC
F-NTPCDNA5	AGATTTCCGGTACCCGCTGAUCAGCCTCG
R-NTPCDNA5	ATTTCTGCTCCAGCATAGTCAGGTACGUCATAAGGG
F-NTdCas9	ATTTCCGGTGGTGGCTCCAGAUCTGTGGATAAGAAATACTCAATAGGCTT AGCTATCGGC
R-NTdCas9	AGCGGGTTTAGTCACCTCCTAGCUGACTCAAATCAATGC
F-NTPCDNA5	AGCTAGGAGGTGACTAAACCCGCUGATCAGCCTCG
R-NTPCDNA5	ATCTGGAGCCACCACCGGAAAUCTCCAGAGTAGACAGC
F-PNLGFP	AGCAAGGGCGAGGAGCUG
R-PNLGFP	AGTCGCGGCCTTACTTGTCGUCATCGTCTTTGTAGTCTGAGTTTGTATCT CGAGCCTTGTACAGCTC
F-flagPNL	ACGACAAGTAAGGCCGCGACUCTAGAGTCGG
R-flagPNL	AGCTCCTCGCCCTTGCUCACCGCCAGAATGCGTTCGCAC
F-Cas9CTNLSN	GATTTGAGTCAGCTAGGAGGTGAC AAAAGGCCGGCGGCCACGAAAAA GGCCGGCCAGGCAAAAAAGAAAAAG TAA ACC CGC TGA TCA GCC
R-Cas9CTNLSN	GGCTGATCAGCGGGTTTACTTTTTCTTTTTTGCCTGGCCGGCCTTTTTCGT GGCCGCCGGCCTTTTGTCACCTCCTAGCTGACTCAAATC