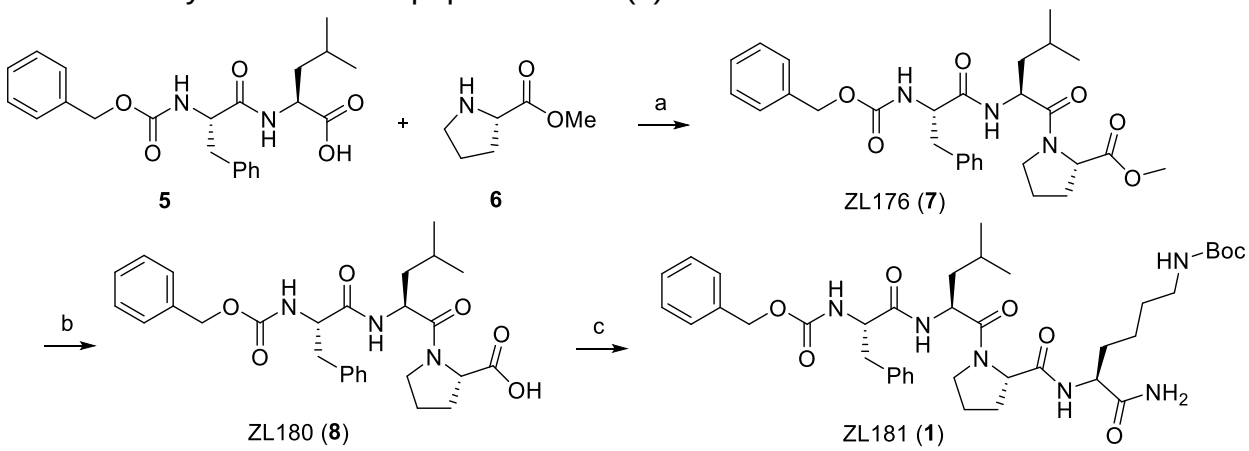
Supporting Information

Chemical Synthesis of Peptidomimetics

Scheme 1: Synthesis of tetrapeptide ZL181 (**1**).



Reagents and conditions: (a) HBTU, HOBt, DIEA, DCM, rt, quant.; (b) $LiOH{\cdot}H_2O$, CH_3OH/H_2O, rt, quant.; (c) *tert*-butyl (*S*)-(5,6-diamino-6-oxohexyl)carbamate (**9**), HBTU, HOBt, DIEA, DCM, rt, 69%.

Methyl ((benzyloxy)carbonyl)-*L*-phenylalanyl-*L*-leucyl-*L*-prolinate ZL176 (7).
To a solution of **5** (1000 mg, 2.43 mmol) and **6** (483 mg, 2.9 mmol) in 20 mL DCM, HBTU (2700 mg, 7.29 mmol), HOBt (328 mg. 2.43 mmol) and DIEA (1567 mg, 12 mmol) were added. The mixture was stirred at rt for 18 h. The solution was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (DCM/MeOH = 100/1 to 50/1) to obtain **ZL176** (1500 mg, quantitative yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 5H), 7.27 – 7.22 (m, 3H), 7.16 (dd, *J* = 7.3, 2.0 Hz, 2H), 6.70 (d, *J* = 8.1 Hz, 1H), 5.31 (d, *J* = 8.2 Hz, 1H), 5.13 – 5.02 (m, 2H), 4.78 (td, *J* = 8.6, 5.2 Hz, 1H), 4.48 (t, *J* = 10.9 Hz, 2H), 3.81 – 3.69 (m, 4H), 3.67 – 3.58 (m, 1H), 3.08 (d, *J* = 6.1 Hz, 2H), 2.29 – 2.15 (m, 1H), 2.12 – 1.94 (m, 3H), 1.70 – 1.41 (m, 3H), 0.98 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 172.34, 170.74, 170.55, 136.26, 129.38, 128.55, 128.50, 128.12, 128.02, 126.94, 67.02, 58.71, 52.21, 49.07, 46.85, 41.70, 28.98, 24.88, 24.49, 23.26, 21.92. ESI-MS $(M + H)^+$ m/z 524.3. HR ESI-MS $(M + H)^+$ m/z = 524.2753 (calcd for $C_{29}H_{38}N_3O_6$: 524.2761).

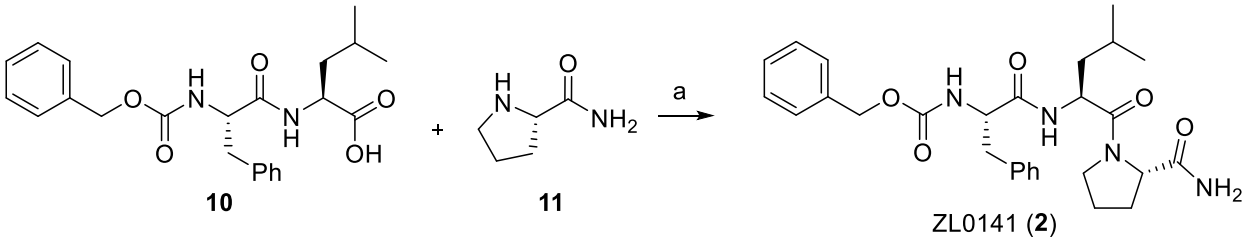
((Benzyloxy)carbonyl)-*L*-phenylalanyl-*L*-leucyl-*L*-proline ZL180 (8).
To a solution of ZL176 (1271 mg, 2.43 mmol) in 15 mL CH_3OH at 0 ˚C, $LiOH{\cdot}H_2O$ (162 mg, 3.85 mmol) in 5 mL H_2O was added. The solution was allowed to stir at 0 ˚C overnight, and then acidified to pH = 3 by 1 N $NaHSO_4$. The mixture was extracted by DCM and the organic layer was dried over Na_2SO_4. After concentration, **ZL180** (1340 mg, quantitative yield) was obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.30 (m, 5H), 7.25 – 7.10 (m, 5H), 5.67 (d, *J* = 8.0 Hz, 1H), 5.10 (d, *J* = 2.8 Hz, 2H), 4.80 (dd, *J* = 15.3, 7.1 Hz, 1H), 4.57 – 4.43 (m, 2H), 3.74 (dd, *J* = 16.3, 6.9 Hz, 1H), 3.60 (dd, *J* = 9.2, 6.4 Hz, 1H), 3.09 (tt, *J* = 13.7, 6.9 Hz, 2H), 2.30 – 1.98 (m, 4H), 1.55 (ddd, *J* = 20.8, 10.4, 6.5

Hz, 3H), 0.94 (d, *J* = 6.1 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H). ESI-MS (M + Na)$^+$ m/z 532.2. HR ESI-MS (M + Na)$^+$ m/z = 532.2423 (calcd for $C_{28}H_{35}N_3O_6Na$: 532.2424).

Benzyl ((*S*)-1-(((*S*)-1-((*S*)-2-(((*S*)-1-amino-6-((*tert*-butoxycarbonyl)amino)-1-oxohexan-2-yl)carbamoyl)pyrrolidin-1-yl)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (ZL181).

To a solution of ZL180 (790 mg, 1.55 mmol) and **9** (436 mg, 1.55 mmol) in 20 mL DCM, HBTU (1762 mg, 4.65 mmol), HOBt (209 mg, 1.55 mmol) and DIEA (1.4 mL, 7.75 mmol) were added. The mixture was stirred at rt overnight. The solution was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (DCM/MeOH = 50/1 to 20/1) to obtain **ZL181** (815 mg, 69% yield) as a white foam. ^{1}H NMR (300 MHz, MeOD) *δ* 7.40 – 7.09 (m, 10H), 5.02 (s, 2H), 4.78 – 4.25 (m, 4H), 3.85 – 3.55 (m, 2H), 3.19 – 2.99 (m, 3H), 2.85 (dd, *J* = 13.8, 9.5 Hz, 1H), 2.24 – 1.77 (m, 5H), 1.75 – 1.37 (m, 17H), 0.97 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (300 MHz, MeOD) *δ* 175.48, 172.84, 172.60, 171.68, 157.10, 156.74, 137.13, 136.77, 129.02, 128.06, 128.01, 127.53, 127.28, 126.34, 78.44, 66.13, 60.17, 56.13, 52.94, 49.50, 47.24, 39.96, 39.81, 37.73, 31.44, 29.11, 28.96, 27.46, 24.63, 24.34, 22.72, 22.38, 20.60. ESI-MS (M + H)$^+$ m/z 737.4. HR ESI-MS (M + H)$^+$ m/z = 737.4224 (calcd for $C_{39}H_{57}N_6O_8$: 737.4238).

Scheme 2: Synthesis of tripeptide ZL141 (**2**).

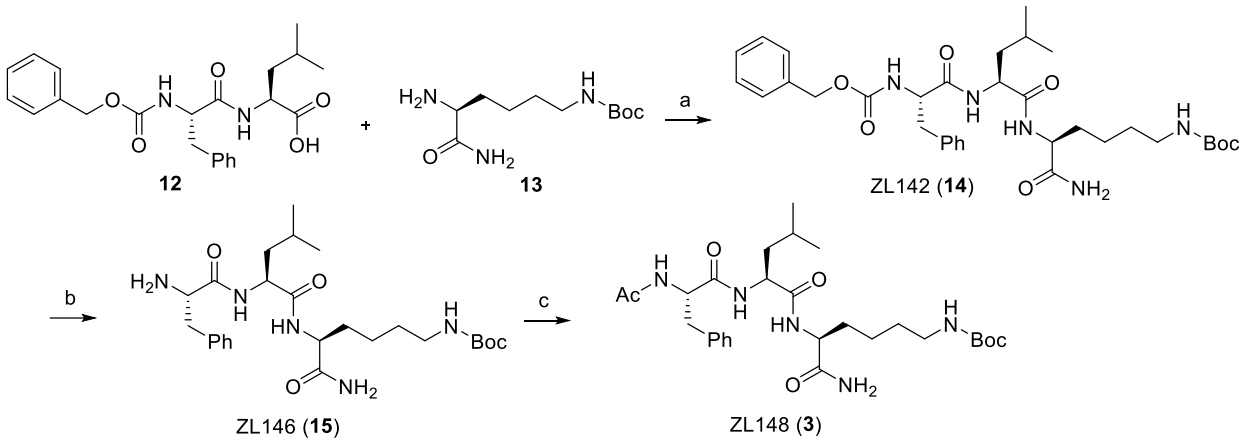


Reagents and conditions: (a) HBTU, HOBt, DIEA, DCM, rt.

Benzyl ((*S*)-1-(((*S*)-1-((*S*)-2-carbamoylpyrrolidin-1-yl)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (ZL141)

To a solution of **10** (206 mg, 0.5 mmol) and **11** (86 mg, 0.75 mmol) in 5 mL DCM, HBTU (569 mg, 1.5 mmol), HOBt (67 mg. 0.5 mmol) and DIEA (323 mg, 2.5 mmol) were added. The mixture was stirred at room temperature (rt) for 18 h. The solution was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (Hexane/EtOAc=10/1 to 7/1) to obtain **ZL141** (278 mg, quantitative yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 7.65 (s, 1H), 7.33 (s, 4H), 7.15 (d, J = 32.8 Hz, 6H), 6.87 (s, 1H), 5.57 – 5.32 (m, 2H), 5.11 – 4.96 (m, 2H), 4.86 (d, J = 4.8 Hz, 1H), 4.71 (s, 1H), 4.52 (s, 1H), 3.86 – 3.54 (m, 2H), 3.12 – 2.89 (m, 2H), 2.22 (s, 2H), 2.01 (s, 2H), 1.55 (d, J = 20.6 Hz, 3H), 0.93 (dd, J = 17.1, 6.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 173.74, 172.10, 170.94, 155.98, 136.48, 136.32, 129.37, 128.48, 128.09, 127.83, 126.79, 66.84, 59.53, 55.48, 49.01, 47.42, 41.63, 39.20, 38.61, 28.27, 25.03, 24.59, 23.28, 21.86. ESI-MS (M + H)$^+$ m/z 509.3. HR ESI-MS (M + H)$^+$ m/z = 509.2761 (calcd for $C_{28}H_{37}N_4O_5$: 509.2764).

Scheme 3: Synthesis of tripeptide ZL148 (**3**).



Reagents and conditions: (a) HBTU, HOBt, DIEA, DCM, rt, 61%; (b) 10% Pd/C, H_2, CH_3OH, rt, quant.; (c) CH_3COCl, NEt_3, DCM, rt, 29%.

Benzyl ((10*S*,13*S*,16*S*)-10-carbamoyl-13-isobutyl-2,2-dimethyl-4,12,15-trioxo-17-phenyl-3-oxa-5,11,14-triazaheptadecan-16-yl)carbamate ZL142 (14)

To a solution of **12** (206 mg, 0.5 mmol) and **13** (212 mg, 0.75 mmol) in 5 mL DCM, HBTU (569 mg, 1.5 mmol), HOBt (67 mg. 0.5 mmol) and DIEA (323 mg, 2.5 mmol) were added. The mixture was stirred at rt for 18 h. The mixture was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (Hexane/EtOAc=10/1 to 7/1) to obtain **ZL142** (196 mg, 61% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, MeOD + $CDCl_3$) δ 7.29 (dt, *J* = 14.7, 3.2 Hz, 10H), 5.04 (d, *J* = 5.0 Hz, 2H), 4.37 (ddd, *J* = 33.5, 9.0, 5.0 Hz, 3H), 3.21 – 2.84 (m, 4H), 1.90 – 1.48 (m, 6H), 1.45 – 1.33 (m, 11H), 0.93 (dd, *J* = 10.3, 5.5 Hz, 6H). ^{13}C NMR (75 MHz, MeOD + $CDCl_3$) δ 175.35, 173.10, 172.98, 157.11, 157.03, 136.99, 136.65, 129.00, 128.07, 127.59, 127.32, 126.38, 66.29, 56.45, 52.93, 52.09, 40.09, 37.43, 31.38, 29.12, 27.45, 24.37, 22.80, 22.14, 20.63. ESI-MS $(M + H)^+$ m/z 640.4. HR ESI-MS $(M + H)^+$ m/z = 640.3713 (calcd for $C_{34}H_{50}N_5O_7$: 640.3710).

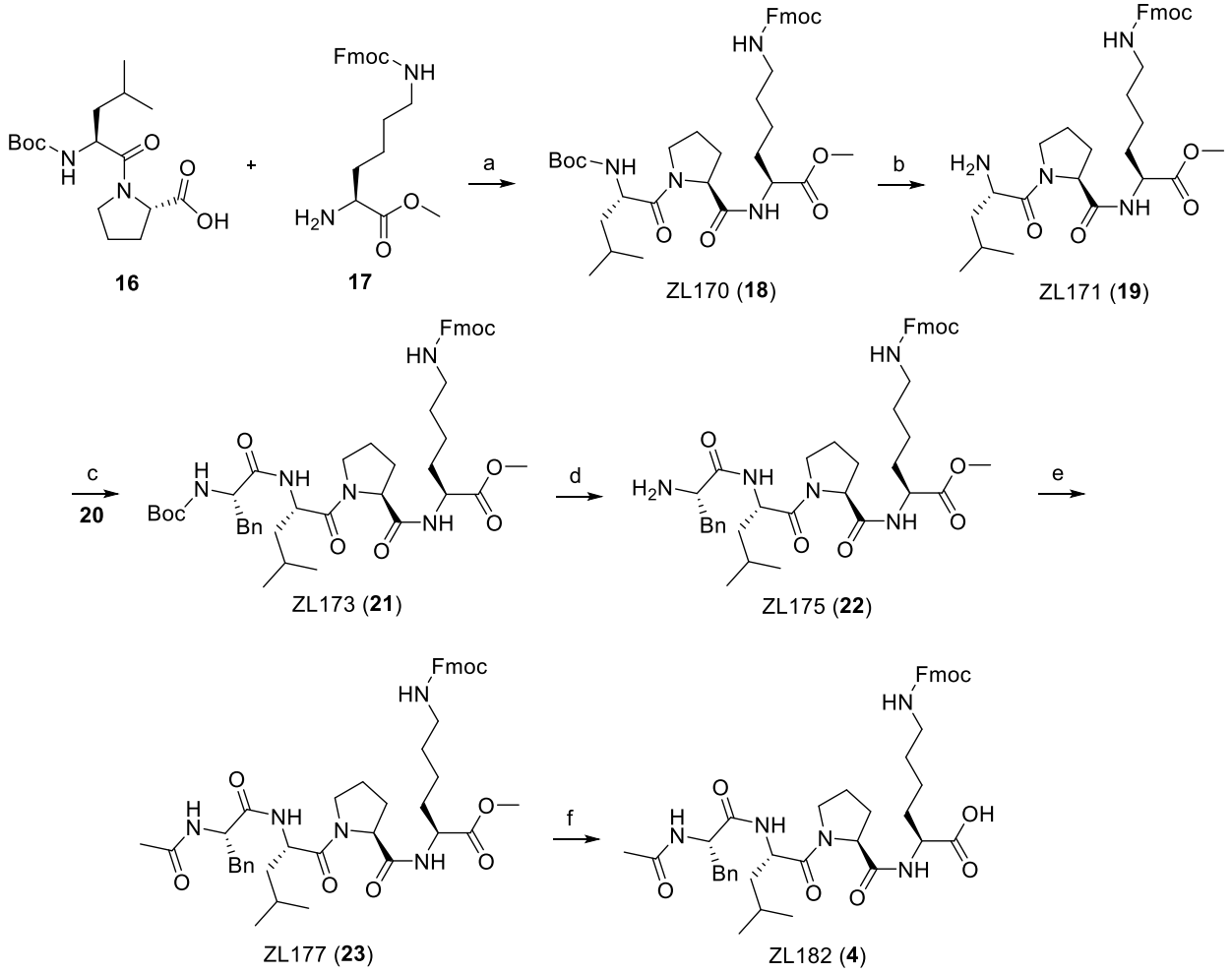
***tert*-Butyl ((*S*)-6-amino-5-((*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-4-methylpentanamido)-6-oxohexyl)carbamate ZL146 (15)**

To a solution of ZL142 (100 mg) in MeOH, 10% Pd/C (10 mg) was added. Under H_2, the mixture was allowed to stir at rt for 2 hrs. The solution was filtrated and the filtrate was concentrated to get the crude product. The residue was purified by a silica gel column (Hexane/EtOAc = 10/1 to 5/1) to obtain **ZL146** (80 mg, quant.) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 7.1 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 2H), 7.23 – 7.13 (m, 3H), 6.73 (s, 1H), 6.08 (s, 1H), 4.96 (s, 1H), 4.44 – 4.32 (m, 2H), 3.70 (dd, *J* = 8.7, 3.9 Hz, 1H), 3.19 (dd, *J* = 13.7, 3.8 Hz, 1H), 3.08 (d, *J* = 5.9 Hz, 2H), 2.79 – 2.70 (m, 1H), 1.97 – 1.79 (m, 1H), 1.62 (dd, *J* = 25.0, 9.6 Hz, 4H), 1.43 (s, 13H), 0.91 (dd, *J* = 8.8, 6.2 Hz, 6H). ESI-MS $(M + H)^+$ m/z 506.3. HR ESI-MS $(M + H)^+$ m/z = 506.3339 (calcd for $C_{26}H_{44}N_5O_5$:506.3342).

***Tert*-butyl ((*S*)-5-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-4-methylpentanamido)-6-amino-6-oxohexyl)carbamate (ZL148).**

To a solution of ZL146 (0.078 mmol) in 5 mL DCM, Et_3N (40 mg, 0.4 mmol) and CH_3COCl (19 mg, 0.24 mmol) was added. The mixture was allowed to stir at rt overnight. The solution was washed with 1N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (Hexane/EtOAc = 10/1 to 7/1) to obtain **ZL148** (12 mg, 29% yield) as a white solid. ^1H NMR (300 MHz, MeOD) δ 4.63 (d, J = 15.4 Hz, 2H), 4.44 – 4.23 (m, 2H), 3.22 – 2.81 (m, 4H), 1.92 (s, 3H), 1.84 (d, J = 6.9 Hz, 1H), 1.75 – 1.59 (m, 4H), 1.44 (s, 13H), 0.95 (dd, J = 11.8, 5.9 Hz, 6H). ESI-MS $(M + H)^+$ m/z 548.3. HR ESI-MS $(M + H)^+$ m/z = 548.3437 (calcd for $C_{28}H_{46}N_5O_6$: 548.3448).

Scheme 4: Synthesis of tetrapeptide ZL182 (**4**).



Reagents and conditions: (a) HBTU, HOBt, DIEA, DCM, rt, quant.; (b) TFA, DCM, rt, 87%; (c) (*tert*-butoxycarbonyl)-*L*-phenylalanine (**20**), HBTU, HOBt, DIEA, DCM, rt, 81%; (d) TFA, DCM, rt, quant.; (e) CH_3COCl, Et_3N, DCM, rt, 91%; (f) $LiOH{\cdot}H_2O$, CH_3OH/H_2O, rt, 91%.

(*S*)-Methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-4-methylpentanoyl)pyrrolidine-2-carboxamido)hexanoate ZL170 (18).

To a solution of **16** (755 mg, 2.3 mmol) and **17** (964 mg, 2.3 mmol) in 5 mL DCM, HBTU (2615 mg, 6.9 mmol), HOBt (310 mg, 2.3 mmol) and DIEA (1483 mg, 11.5 mmol) were added. The mixture was stirred at rt for 18 h. The mixture was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column (Hexane/EtOAc=20/1 to 10/1) to obtain **ZL170** (1.6 g, quantitative yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.32 (d, J = 7.4 Hz, 2H), 7.03 (d, J = 7.6 Hz, 1H), 5.80 (s, 1H), 5.32 (d, J = 9.0 Hz, 1H), 4.50 (ddd, J = 41.9, 10.0, 4.0 Hz, 5H), 4.28 (t, J = 7.0 Hz, 1H), 3.77 (d, J = 16.4 Hz, 4H), 3.60 (t, J = 8.4 Hz, 1H), 3.30 – 3.06 (m, 2H), 2.21 (dd, J = 13.4, 7.8 Hz, 2H), 2.06 – 1.42 (m, 13H), 1.30 (s, 9H), 0.97 (dd, J = 11.8, 6.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.15, 172.57, 171.02, 156.56, 155.62, 144.07, 141.30, 127.62, 127.00, 125.16, 119.91, 79.59, 77.27, 66.65, 60.07, 52.37, 52.00, 50.29, 47.31, 42.21, 40.28, 31.58, 28.96, 27.84, 25.17, 24.59, 23.36, 21.91, 21.81. ESI-MS $(M + H)^+$ m/z 693.4. HR ESI-MS $(M + H)^+$ m/z = 693.3857 (calcd for $C_{38}H_{53}N_4O_8$: 693.3863).

(*S*)-Methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-1-((*S*)-2-amino-4-methylpentanoyl)pyrrolidine-2-carboxamido)hexanoate ZL171 (19).

To a solution of ZL170 (2.3 mmol) in 4 mL DCM, 1.0 mL of TFA was added. The mixture was allowed to stir at rt for 2 h. Then the solution was concentrated to get the crude product **ZL171** (1.47 g, 87% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, J = 7.4 Hz, 2H), 7.59 (d, J = 6.8 Hz, 2H), 7.40 (d, J = 7.4 Hz, 2H), 7.33 (d, J = 6.9 Hz, 2H), 7.15 (d, J = 8.7 Hz, 1H), 6.53 (s, 1H), 4.72 – 4.08 (m, 6H), 3.78 – 3.61 (m, 4H), 3.55 – 3.40 (m, 1H), 3.15 (s, 2H), 2.21 – 1.24 (m, 13H), 0.93 (t, J = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.65, 171.16, 169.16, 157.91, 156.74, 143.94, 141.29, 127.76, 127.08, 125.04, 120.00, 77.27, 67.47, 60.74, 52.32, 51.15, 50.46, 47.72, 47.11, 40.94, 39.79, 31.58, 29.31, 27.85, 25.08, 23.86, 22.99, 21.87. ESI-MS $(M + Na)^+$ m/z 615.3. HR ESI-MS $(M + Na)^+$ m/z = 615.3164 (calcd for $C_{33}H_{44}N_4O_6Na$: 615.3159).

(*S*)-Methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-1-((*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-4-methylpentanoyl)pyrrolidine-2-carboxamido)hexanoate ZL173 (21).

To a solution of **20** (838 mg, 3.16 mmol) and ZL171 (1.25 g, 2.11 mmol) in 20 mL DCM, HBTU (2.4 g, 6.3 mmol), HOBt (285 mg. 2.11 mmol) and DIEA (1.36 g, 10.55 mmol) were added. The mixture was stirred at rt for 18 h. The mixture was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column ($CHCl_3$/CH_3OH=50/1 to 20/1) to obtain **ZL173** (1.44 g, 81% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, J = 8.4 Hz, 1H), 7.78 (t, J = 6.3 Hz, 2H), 7.62 (d, J = 7.1 Hz, 2H), 7.41 (dd, J = 13.6, 6.7 Hz, 2H), 7.33 (d, J = 7.2 Hz, 2H), 7.25 – 7.04 (m, 5H), 6.87 (s, 1H), 6.69 (s, 1H), 5.42 (d, J = 9.0 Hz, 1H), 5.21 (s, 1H), 4.87 (d, J = 8.2 Hz, 1H), 4.70 – 4.19 (m, 6H), 3.87 (d, J = 8.5 Hz, 1H), 3.80 – 3.68 (m, 3H), 3.63 (dd, J = 14.4, 7.7 Hz, 1H), 3.36 – 3.03 (m, 3H), 2.89 (d, J = 8.4 Hz, 1H), 2.32 – 1.81 (m, 6H), 1.76 – 1.43 (m, 7H), 1.34 (d, J = 11.5 Hz, 9H), 1.02 –

0.83 (m, 6H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 172.88, 172.71, 172.61, 172.00, 171.86, 171.25, 171.05, 144.07, 141.34, 129.54, 129.35, 128.32, 128.07, 127.68, 127.05, 126.34, 125.14, 119.97, 77.28, 67.32, 66.59, 60.76, 59.89, 55.23, 52.45, 52.29, 51.84, 49.06, 47.64, 47.27, 31.67, 28.61, 28.27, 25.37, 24.49, 23.40, 21.80, 21.54. ESI-MS $(M + H)^+$ m/z 840.5. HR ESI-MS $(M + H)^+$ m/z = 840.4550 (calcd for $C_{47}H_{62}N_5O_9$: 840.4548).

(*S*)-Methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-1-((*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-4-methylpentanoyl)pyrrolidine-2-carboxamido)hexanoate ZL175 (22).

To a solution of ZL173 (1.38g, 1.64 mmol) in 4 mL DCM, 2.0 mL TFA was added. The mixture was allowed to stir at rt for 2 h. Then the solution was concentrated to get the crude product **ZL175** (1.4 g, quantitative yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.51 (s, 1H), 7.78 (d, *J* = 6.7 Hz, 2H), 7.60 (d, *J* = 4.4 Hz, 2H), 7.41 (t, *J* = 7.3 Hz, 2H), 7.35 – 7.30 (m, 2H), 7.27 – 7.00 (m, 5H), 6.75 (d, *J* = 7.9 Hz, 1H), 6.60 (s, 1H), 5.44 (s, 1H), 4.81 (dd, *J* = 13.5, 8.8 Hz, 1H), 4.63 – 4.20 (m, 5H), 3.89 (d, *J* = 8.8 Hz, 1H), 3.75 (d, *J* = 6.4 Hz, 3H), 3.62 (dd, *J* = 20.7, 9.2 Hz, 1H), 3.29 – 3.09 (m, 3H), 2.70 (dd, *J* = 13.0, 8.7 Hz, 1H), 2.10 (dd, *J* = 38.6, 9.0 Hz, 8H), 1.77 – 1.32 (m, 8H), 1.04 – 0.85 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 172.56, 171.01, 143.98, 141.30, 129.29, 128.63, 127.69, 127.05, 125.11, 119.98, 77.26, 66.58, 60.01, 52.37, 52.04, 48.73, 47.50, 47.31, 41.55, 40.48, 38.61, 31.65, 29.10, 25.19, 24.67, 23.30, 22.13, 21.86. ESI-MS $(M + H)^+$ m/z 740.4. HR ESI-MS $(M + H)^+$ m/z = 740.4022 (calcd for $C_{42}H_{54}N_5O_7$: 740.4023).

(*S*)-Methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-1-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-4-methylpentanoyl)pyrrolidine-2-carboxamido)hexanoate ZL177 (23).

To a solution of ZL175 (968 mg, 1.31 mmol) in 5 mL DCM, Et_3N (662 mg, 6.54 mmol) and CH_3COCl (308 mg, 3.93 mmol) were added. The mixture went on to stir at rt overnight. The solution was washed with 1 N $NaHSO_4$, saturated $NaHCO_3$ and brine. After drying over anhydrous Na_2SO_4, the solution was concentrated and purified with a silica gel column ($CHCl_3$/CH_3OH = 50/1 to 20/1) to obtain **ZL177** (930 mg, 91% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.58 (d, *J* = 8.6 Hz, 1H), 7.79 (t, *J* = 6.6 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.42 (dd, *J* = 13.4, 6.5 Hz, 2H), 7.33 (t, *J* = 6.9 Hz, 2H), 7.19 – 7.00 (m, 5H), 6.89 (t, *J* = 4.7 Hz, 1H), 6.71 (d, *J* = 8.1 Hz, 1H), 6.45 (t, *J* = 11.4 Hz, 1H), 5.32 (d, *J* = 5.6 Hz, 1H), 5.04 – 4.79 (m, 2H), 4.61 (d, *J* = 5.1 Hz, 1H), 4.49 – 4.17 (m, 4H), 3.92 – 3.80 (m, 1H), 3.74 (d, *J* = 14.1 Hz, 3H), 3.69 – 3.57 (m, 1H), 3.13 (ddd, *J* = 28.8, 14.3, 4.8 Hz, 3H), 2.93 (dd, *J* = 14.0, 7.7 Hz, 1H), 2.27 – 1.96 (m, 5H), 1.89 (d, *J* = 10.2 Hz, 3H), 1.70 – 1.26 (m, 8H), 1.02 – 0.81 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 172.69, 171.49, 171.08, 169.64, 143.97, 141.35, 129.44, 129.24, 128.26, 128.11, 127.77, 127.10, 126.54, 124.99, 120.02, 77.26, 67.25, 66.58, 60.72, 59.87, 53.78, 52.46, 51.89, 49.11, 47.66, 47.23, 41.09, 40.40, 38.70, 31.57, 28.66, 25.33, 24.47, 23.36, 23.12, 21.87, 21.61. ESI-MS $(M + H)^+$ m/z 782.4. HR ESI-MS $(M + H)^+$ m/z = 782.4125 (calcd for $C_{44}H_{56}N_5O_8$: 782.4129).

N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-acetyl-*L*-phenylalanyl-*L*-leucyl-*L*-prolyl-*L*-lysine (ZL182).

To a solution of ZL177 (600 mg, 0.77 mmol) in 10 mL CH_3OH at 0 ˚C, $LiOH{\cdot}H_2O$ (162 mg, 3.85 mmol) in 4 mL of H_2O was added. The solution was allowed to stir at 0 ˚C for 1 h, and then acidified to pH = 3 by 1 N $NaHSO_4$. The mixture was extracted by DCM and the organic layer was dried over Na_2SO_4. After concentration, **ZL182** (536 mg, 91% yield) was obtained as a white foam. ^{1}H NMR (300 MHz, MeOD) δ 8.20 (dd, J = 19.0, 7.6 Hz, 1H), 7.79 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 6.9 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.31 (d, J = 7.3 Hz, 2H), 7.21 (m, 5H), 4.78 – 4.63 (m, 2H), 4.54 – 4.30 (m, 4H), 4.18 (t, J = 6.6 Hz, 1H), 3.75 (dd, J = 15.8, 9.3 Hz, 2H), 3.68 – 3.57 (m, 1H), 3.12 (dd, J = 14.0, 5.0 Hz, 2H), 2.88 – 2.79 (m, 1H), 2.25 – 2.14 (m, 1H), 2.12 – 1.97 (m, 2H), 1.94 – 1.82 (m, 6H), 1.72 (dd, J = 13.3, 6.8 Hz, 2H), 1.64 – 1.56 (m, 2H), 1.49 (s, 3H), 0.96 (d, J = 5.8 Hz, 6H). ^{13}C NMR (75 MHz, MeOD) δ 173.94, 172.86, 172.23, 171.68, 171.45, 157.46, 143.95, 141.21, 137.08, 128.85, 127.96, 127.38, 126.75, 126.31, 124.79, 119.54, 67.46, 66.20, 59.87, 54.45, 52.13, 49.43, 49.35, 47.12, 40.14, 37.41, 28.95, 25.09, 24.52, 24.36, 22.56, 22.32, 20.98, 20.61. ESI-MS $(M + Na)^+$ m/z 790.4. HR ESI-MS $(M + Na)^+$ m/z = 790.3787 (calcd for $C_{43}H_{53}N_5O_8Na$: 790.3792).

Figure S1

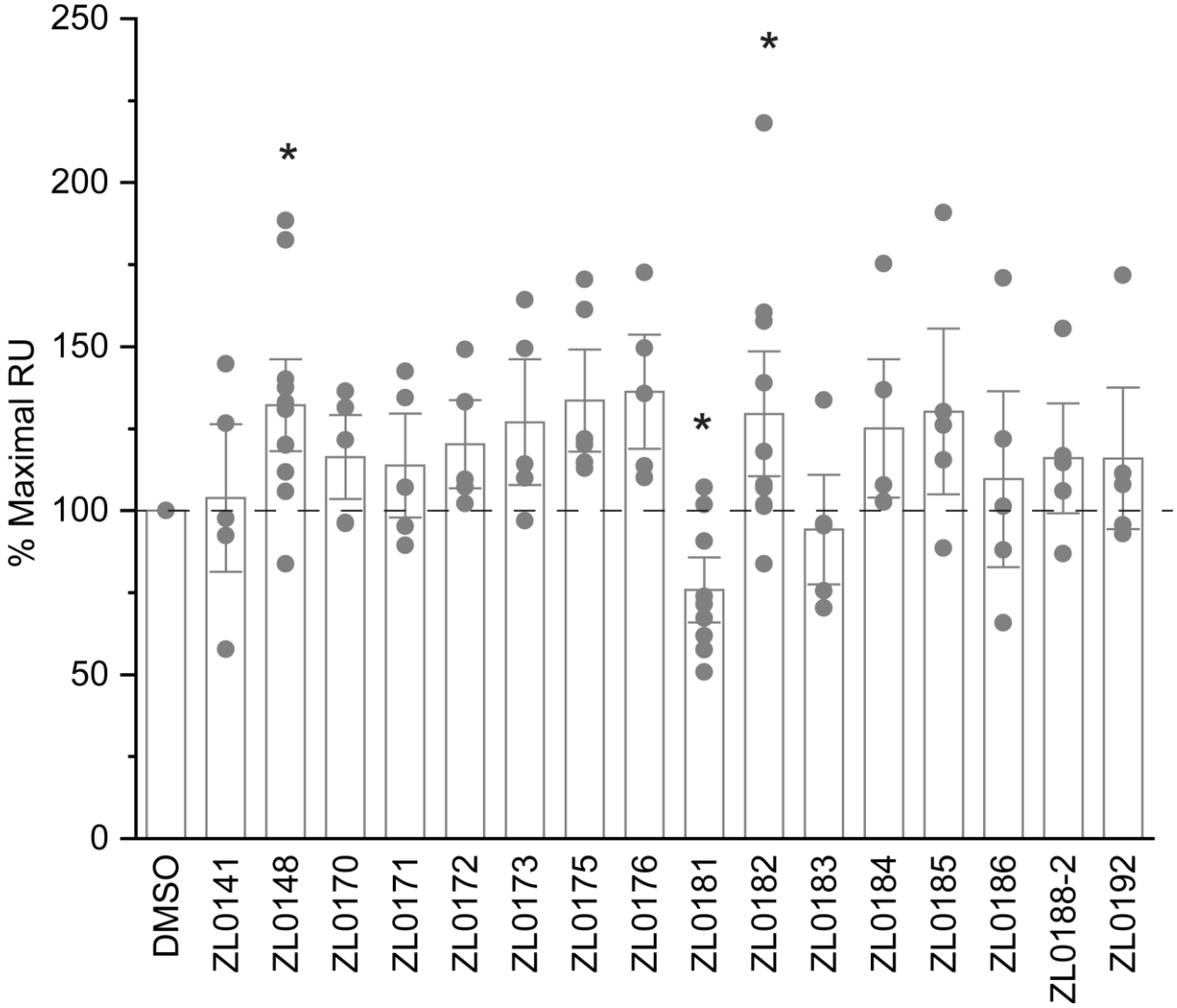


Figure S1. *In-cell* validation of peptidomimetics. (a) Luciferase complementation assay (LCA) in HEK293 cells expressing CLuc-FGF14 and CD4-Nav1.6-NLuc constructs were treated with the indicated peptidomimetics at 50 µM or DMSO (0.5%, control) and processed for LCA. Bar chart with data points overlap represents % maximal luminescence of treated compounds normalized to control; *p<0.05 or **p<0.01.

Figure S2

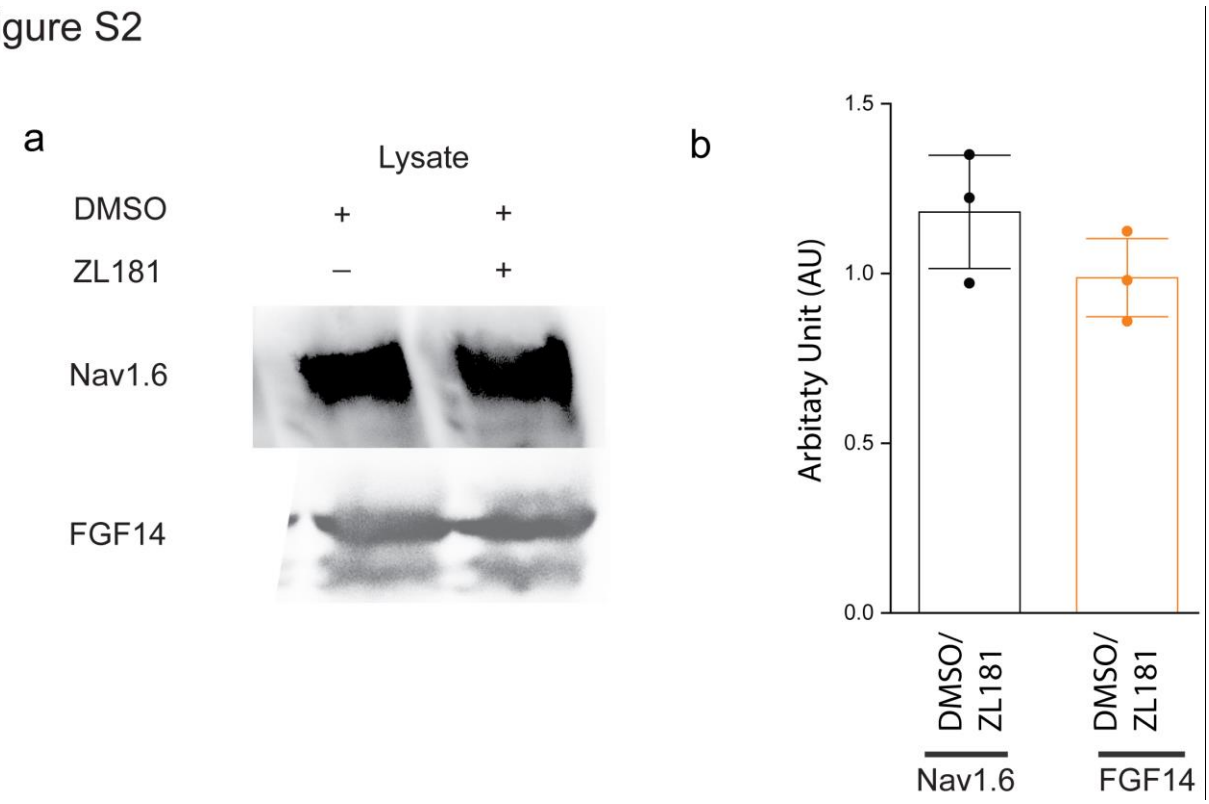


Figure S2. (a) Cell lysates from HEK-Nav1.6 cells were transiently transfected with FGF14-6xmyc and pretreated with 0.5% DMSO (control) or 50 μM ZL181. The immunoblot was detected with PanNav and anti-myc antibody. (b) Quantification of Panel a.

Figure S3

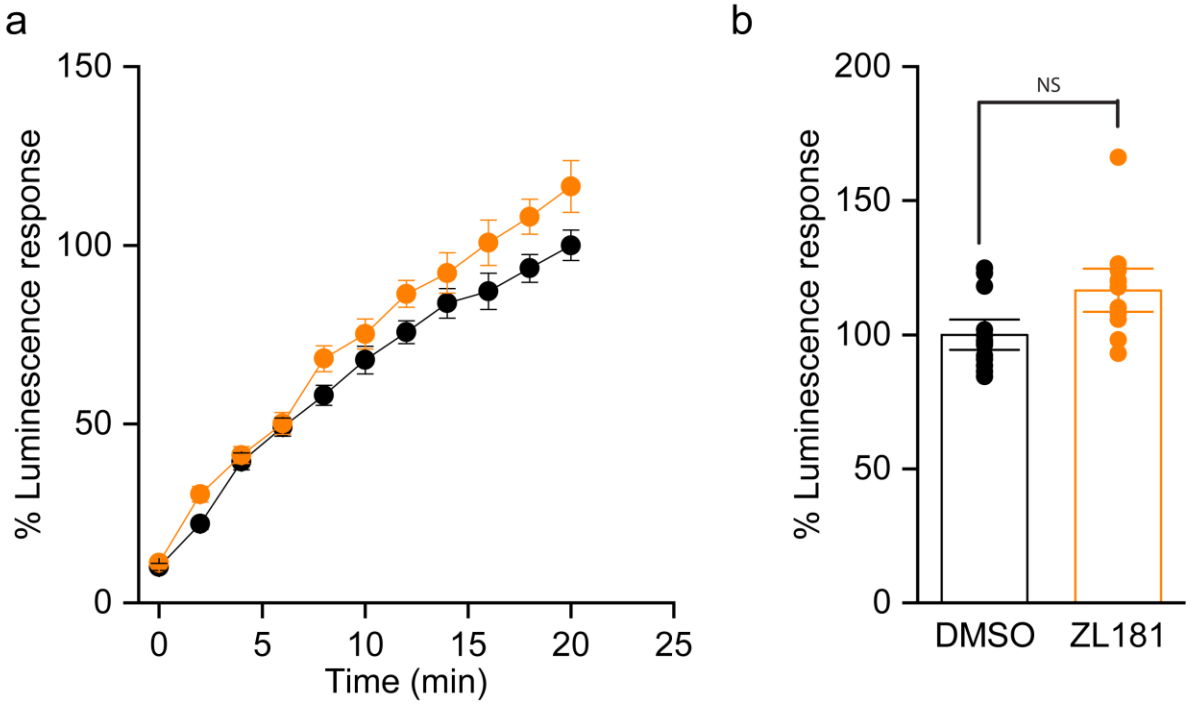


Figure S3. *In-cell* validation of ZL181 against the FGF13-Nav1.6 complex**.** (a) Luciferase complementation assay (LCA) in HEK293 cells expressing CLuc-FGF13 and CD4-Nav1.6-NLuc constructs were treated with ZL181 (50 µM) or DMSO (0.5%, control) and processed for LCA. (b) Bar chart with data points overlap represents % maximal luminescence of treated compounds normalized to control.

Figure S4

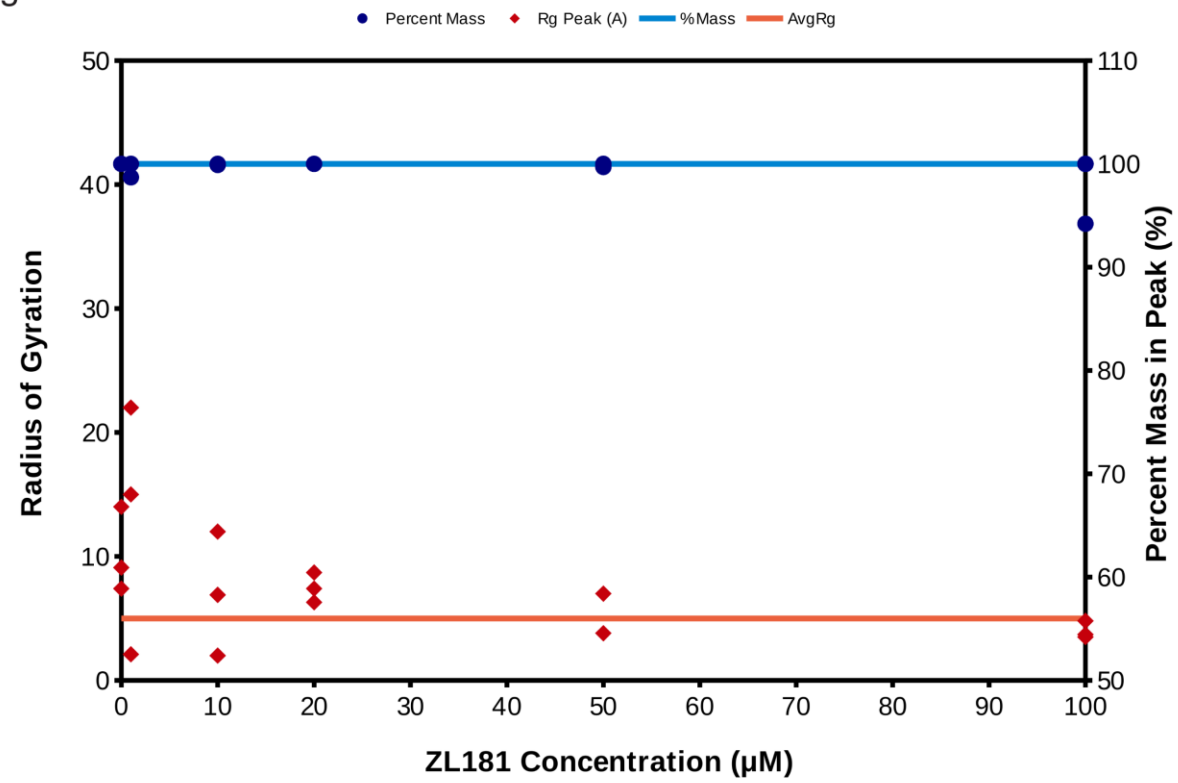


Figure S4. Dynamic light scattering (DLS) data for ZL181. The radius of gyration (Rg) of the major component, by mass (red diamonds, lower red line), was determined using a Malvern Zetasizer µV Dynamic Light Scattering system. The percentage of mass in the peptide peak (blue dots, upper blue 100% line) is also shown. No self-aggregation for the ZL181 peptide was observed up to 100 µM. The final concentration of DMSO was 0.2% in 1 µM, 10 µM, 20 µM, 50 µM, and 100 µM of ZL181 working solution.

Figure S5

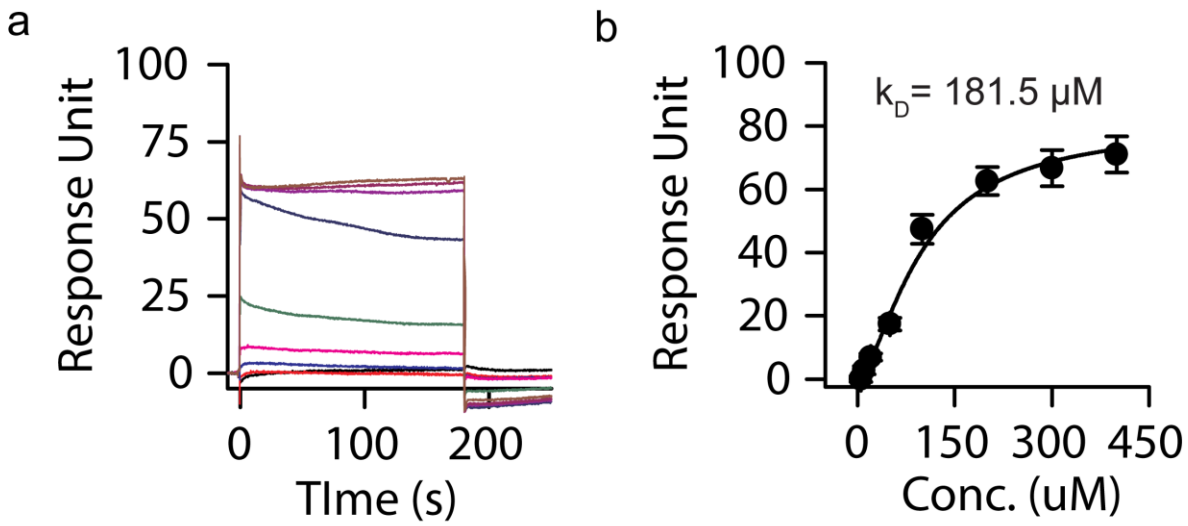


Figure S5. V160 in FGF14 is required for the activity of ZL181**.** (a)The SPR sensorgram of ZL181 (0-400 μM) to $FGF14^{V160A}$ and (b) the fitted saturation binding curves.

Figure S6

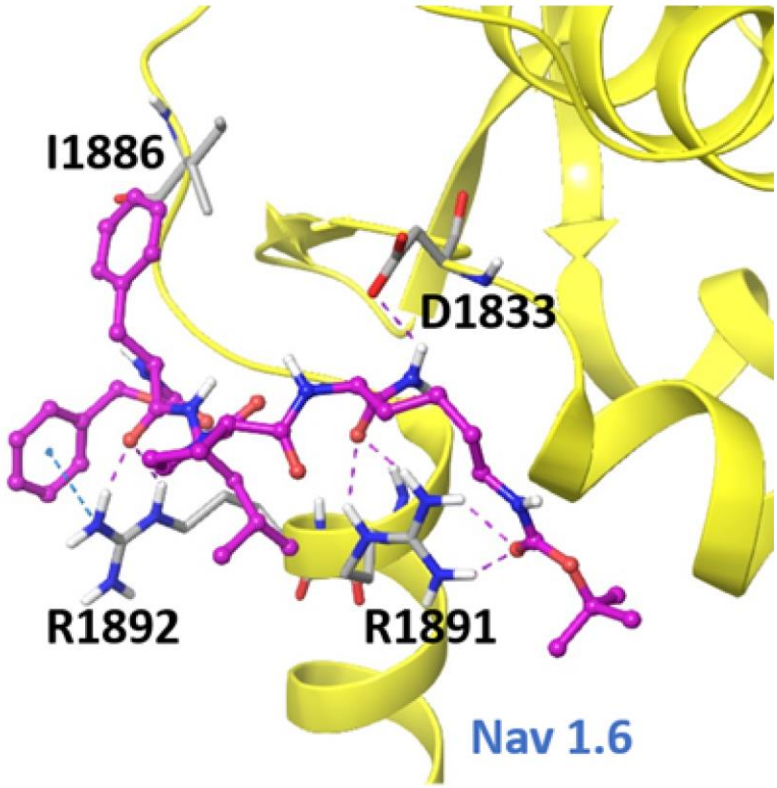


Figure S6. Ribbon presentation of ZL181 (purple) docking on Nav1.6 c-tail (yellow) homology model. Hydrogen bonds are shown as purple dotted lines, and π cation interaction as blue dotted line.

Figure S7

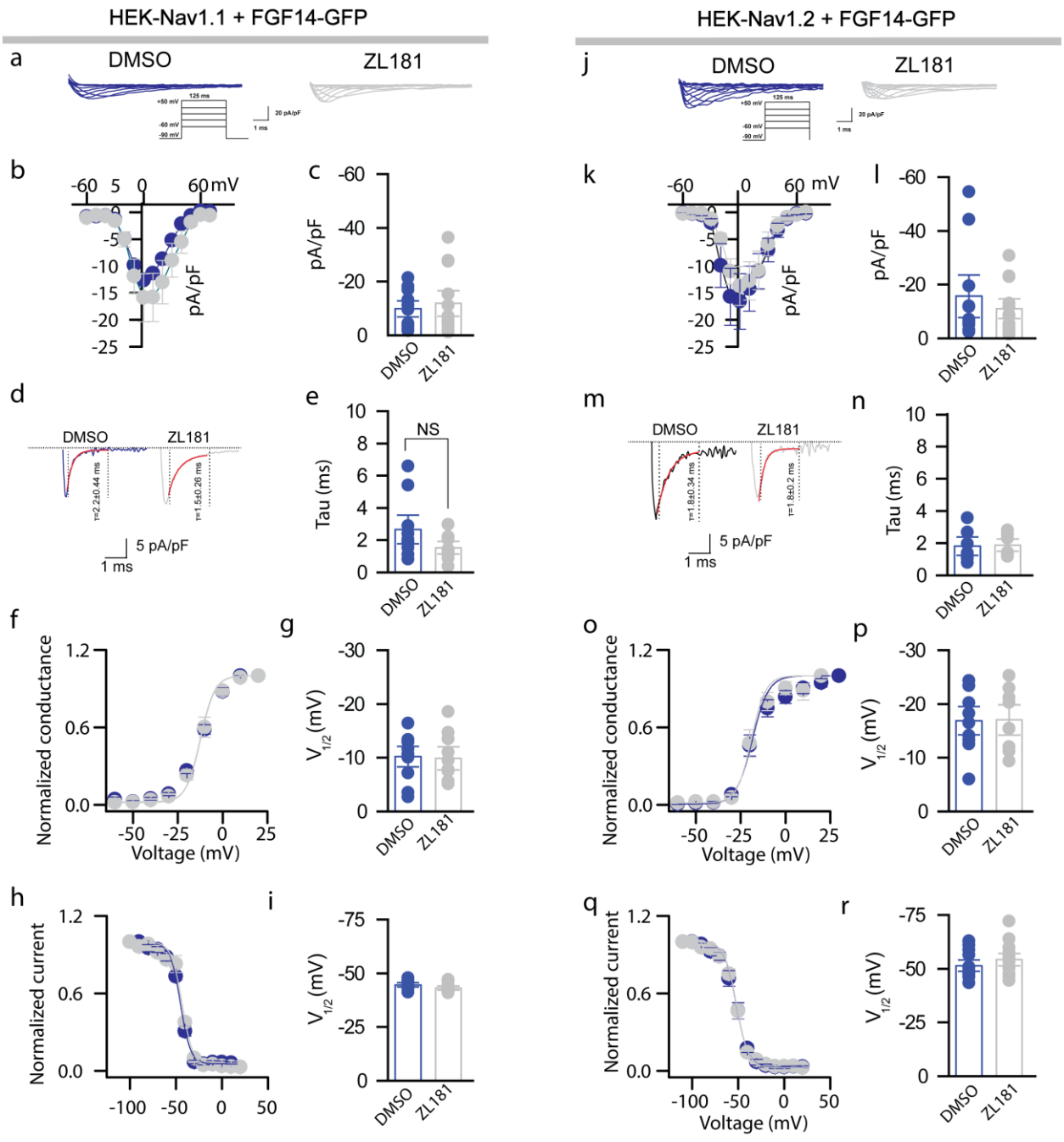


Figure S7. The functional role of ZL181 on Nav1.1- and the Nav1.2-mediated currents in the presence of FGF14 (a, j). Representative traces of Na^+ transient currents (I_{Na+}) recorded from HEK-Nav1.1 or HEK-Nav1.2 cells transiently expressing the indicated constructs in response to depolarizing voltage steps (inset). FGF14-GFP expressing cells were treated with either 0.2% DMSO (blue traces) or 20 μM ZL181 (gray traces). (b,k) Current-voltage relationships of I_{Na+} from the experimental groups described in panel a and j respectively. (c,l) Bar chart with data points overlap represent peak current densities derived from panel b and k respectively. (d, m) Representative traces of

experimental groups described in panel a and j respectively to illustrate tau (τ) of I_{Na+}. (e, n) Summary bar chart with data points overlap of τ from the indicated experimental groups. Voltage dependence of I_{Na+} activation (f,o) and steady-state inactivation (h,q) are plotted as a function of the membrane potential (mV). Bar chart with data points overlap summary of $V_{½}$ of activation (g,p) and steady-state inactivation) (i, r) in the indicated experimental groups. Data are mean ± S.E.M; *p<0.05; p**<0.01. The fitted parameters are provided in **Table S3.**

Table S1. Voltage-gated Na^+ currents in HEK-Nav1.6

Condition for Nav1.6	Peak density	Activation	K_{act}	Inactivation	K_{inact}	Tau (τ)
	pA/pF	mV	mV	mV	mV	ms
GFP (DMSO)	−73.8 ± 13.6 (12)	−21.5 ± 1.3 (11)	4.8 ± 0.4 (10)	−64.1 ± 1.6 (12)	6.9 ± 0.5 (12)	0.9±0.05 (11)
GFP (ZL181)	−20.9 ± 3.4 (15)[a]	-19.4±0.9 (12)	4.8±0.4 (12)	−66.3 ± 1.6 (14)	7.2±0.4 (14)	1.0±.08(14)
FGF14-GFP (DMSO)	−18.1 ± 3.8 (20)[b]	-16.9±0.9 (19)[d]	5.3 ± 0.4 (19)	−50.7 ± 1.6 (14)[e]	7.2±0.6 (14)	1.5±0.1(13)[g]
FGF14-GFP (ZL181)	−7.4 ± 4.4 (19)[c]	-16.6±1.4 (11)	6.6 ± 0.5 (11)	−57.6 ± 1.6 (14)[f]	7.9±1.2 (14)	2.3±0.4(9)[h,I,j]

[a] p =0.04, Kruskal-Wallis, Kruskal-Wallis statistic=33.07, post hoc Dunn test compared with GFP (DMSO); data are mean ± S.E.
[b] p =0.0019, Kruskal-Wallis, Kruskal-Wallis statistic=33.07, post hoc Dunn test compared with GFP (ZL181); data are mean ± S.E.
[c] p = 0.0132 (two-tailed), unpaired *t* tests compared to FGF14-GFP (DMSO); data are mean ± S.E.
[d] p = 0.0404 (two-tailed), Mann Whitney test compared with GFP (DMSO), Mann-Whitney U=44; data are mean ± S.E.
[e] p < 0.001, Kruskal-Wallis, Kruskal-Wallis statistic=33.91, post hoc Dunn test compared to GFP (DMSO); data are mean ± S.E.
[f] p = 0.0307 (two-tailed), Mann-Whitney test compared to FGF14-GFP (DMSO), Mann-Whitney U=63.
[g] p =0.00325, Kruskal-Wallis, Kruskal-Wallis statistic=25.37, post hoc Dunn test to GFP (DMSO); data are mean ± S.E.
[h] p =0.0002, Kruskal-Wallis, Kruskal-Wallis statistic=25.37, post hoc Dunn test to GFP (DMSO); data are mean ± S.E.
[i] p =0.0017, Kruskal-Wallis, Kruskal-Wallis statistic=25.37, post hoc Dunn test to GFP (Zl181); data are mean ± S.E.
[j] p =0.0392 (two-tailed), Student t-test compared to FGF14-GFP (DMSO); data are mean ± S.E.

The number of independent experiments is shown in parentheses.

Table S2. The role of ZL181 on the modulation of voltage-gated Na^+ currents in HEK-Nav1.6 cells co-expressing with GFP or FGF14-ΔNT proteins

Condition	Peak density	Activation ($V_{1/2}$)	K_{act}	Inactivation ($V_{1/2}$)	K_{inact}	Tau (τ)
	pA/pF	mV	mV	mV	mV	ms
GFP (DMSO)	−59.0 ± 8.9 (13)	−19.4 ± 1.5 (12)	4.9 ± 0.3 (12)	−61.1 ± 1.4 (10)	6.9 ± 0.4 (10)	1.1±0.06
GFP (ZL181)	−18.6 ± 3.9 (12)[a]	−18.6 ± 1.1 (9)	4.8 ± 0.6 (9)	−64.7 ± 1.5 (11)	7.2 ± 0.4 (11)	1.0±0.08
FGF14-ΔNT (DMSO)	−99.8 ± 13.9 (18)[b]	−18.3 ±0.9 (17)	4.9 ± 0.3 (17)	−68.2 ± 1.6 (14)[e]	6.0 ± 0.3 (14)	1.0±.07
FGF14-ΔNT (ZL181)	−25.4 ± 6.9 (10)[c]	−11.2 ±1.4 (10)[d]	6.1 ± 0.5 (10)	−67.5 ± 1.1 (9)	7.6 ± 0.5 (9)	1.0±.14

[a] $p < 0.05$, One-way ANOVA, post hoc Holm-Sidak's multiple test compared with GFP (DMSO); data are mean ± S.E.

[b] $p < 0.05$, One-way ANOVA, post hoc Holm-Sidak's multiple test compared with GFP (DMSO); data are mean ± S.E.

[c] $p < 0.001$, One-way ANOVA, post hoc Holm-Sidak's multiple test compared with FGF14-ΔNT (DMSO); data are mean ± S.E.

[d] $p < 0.001$, One-way ANOVA, post hoc Tukey's multiple comparisons test compared with FGF14-ΔNT (DMSO); data are mean ± S.E.

[e] $p < 0.01$, one-way ANOVA, post hoc Tukey's multiple comparisons test compared with GFP (DMSO); data are mean ± S.E.

The number of independent experiments is shown in parentheses.

Table S3. Voltage-gated Na^+ currents in HEK-Nav1.1 and HEK-Nav1.2

Condition	Peak density	Activation	K_{act}	Inactivation	K_{inact}	Tau (τ)
	pA/pF	mV	mV	mV	mV	ms
Nav1.1+ FGF14-GFP (DMSO)	−9.83 ± 1.9 (12)	-10.1±1.2 (11)	6.1 ± 0.6 (11)	−44.6 ± 0.6 (10)	5.6±1.1 (10)	2.2±0.44 (10)
Nav1.1+ FGF14-GFP (ZL181)	−11.8 ± 3.1 (11)	-8.5±1.8 (11)	6.8 ± 0.7 (11)	−43.3 ± 0.6 (10)	5.9±1.0 (10)	1.52±0.26 (10)
Nav1.2+ FGF14-GFP (DMSO)	−17.7 ± 5.2 (11)	-16.9±1.7 (11)	5.6 ± 0.7 (11)	−51.4 ± 1.7 (11)	6.3±0.8 (11)	1..81±0.34 (7)
Nav1.2+ FGF14-GFP (ZL181)	−11.0 ± 2.4 (15)	-17.0±1.8 (10)	4.1 ± 0. (10)	−54.2 ± 1.8 (15)	7.3±0.7 (15)	1.84±0.20 (7)

Table S4. Active and passive properties of medium spiny neurons in control (DMSO) and ZL181 (50 µM) from FGF14^{WT} mice.

	MRP (mV)	V_{trh} (mV)	I_{trh} (pA)	max rise (mV/msec)	max decay (mV/msec)	Cm (pF)	Rin (mΩ)	Tau (msec)	Latency to first AP (msec)	half-width (msec)
DMSO control	-79.61±1.83 (8)	-35.89±2.25 (8)	72.5±11.46 (8)	243.66±20.73 (8)	-70.41±6.81 (8)	61.6±5.6 (8)	137.2±15.18 (8)	8.34±1.02 (8)	253.18±39 (8)	1.04±0.07 (8)
ZL 181	-78.17±2.19 (10)	-27.75±1.99[a] (10)	135±12.5[b] (10)	211.39±18.04 (10)	-74.15±4.05 (10)	67.04±7.78 (10)	115.75±8.21 (10)	7.82±1.12 (10)	392.5±68.46 (10)	0.95±0.05 (10)
p value	0.62	0.016	0.002	0.26	0.65	0.58	0.24	0.73	0.09	0.32

[a] p=0.0156, Student *t*-test compared to control (DMSO); data are mean ± S.E.
[b] p=0.0024, Student *t*-test compared to control (DMSO); data are mean ± S.E.
The number of independent experiments is shown in parentheses.

Table S5. Active and passive properties of medium spiny neurons in control (DMSO) and ZL181 (50 µM) from FGF14^{KO} mice.

	MRP (mV)	V_{trh} (mV)	I_{trh} (pA)	max rise (mV/msec)	max decay (mV/msec)	Cm (pF)	Rin (mΩ)	Tau (msec)	Latency to fist AP (msec)	half-width (msec)
DMSO control	-79.27±2.31 (6)	-29.5±3.35 (6)	116.7±25.25 (6)	252.22±15.93 (6)	-85.12±6.65 (6)	57.1±12.6 (6)	143.26±22.87 (6)	7.8±1.68 (6)	581.55±84.23 (6)	0.86±0.06 (6)
ZL 181	-68.14±3.9 (6)	-21.82±2.27 (6)	110±19.66 (6)	208.66±35.17 (6)	-68.54±10.87 (6)	51.6±13.11 (6)	199.07±35.1 (6)	8.18±1.56 (6)	258.04±47.22 (6)	1.01±0.09 (6)
p value	0.03[a]	0.09	0.84	0.28	0.22	0.77	0.21	0.87	0.007	0.24

[a] $p<0.05$, Student *t*-test compared to control (DMSO); data are mean ± S.E.

MRP – membrane resting potential, V_{trh} – voltage threshold, I_{trh} – current threshold, max rise – action potential repolarization maximal speed, max decay – action potential depolarization maximal speed, Cm – membrane capacitance, R_{in} – input resistance, Tau – membrane constant, latency to first AP – latency to first spike induced with I_{trh}, half-width – action potential half-width. All *p*-values obtained with Student *t*-test. The number of independent experiments is shown in parentheses.