Supporting Information:

Identification of tetrapeptides from a mixture based positional scanning library that can restore nM full agonist function of the L106P, I69T, I102S, A219V, C271Y, and C271R human melanocortin-4 polymorphic receptors (hMC4Rs)

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Table S1 Pipeline Pilot Biophysical Properties.

Table S2. Analytical Information for the single tetrapeptides synthesized and characterized in this study. Purity for these compounds is >95%.
Table S1. Summary of the Pipeline Pilot biophysical properties for the tetrapeptides examined in this study.

| Cmpd       | ALOGP  | Num_H_Acceptors | Num_Aromatic_Rings | Num_Rotatable_Bonds | Num_Arom_Rings | LogD | Molecular_Surface_Area | Energy   | Molecular 3D SASA |
|------------|--------|-----------------|--------------------|--------------------|----------------|------|------------------------|----------|------------------|
| NDP-MSH    | -5.886 | 22              | 118                | 51                 | 6              | 5    | -5.4220                | 168.54   | 153.66           |
| JRH887-9   | -0.576 | 8               | 50                 | 19                 | 4              | 4    | -0.1130                | 685.11   | 81.06            |
| EMH4-105   | 0.002  | 8               | 51                 | 19                 | 4              | 4    | 0.4650                 | 726.09   | 80.94            |
| 1981-2     | 4.642  | 5               | 52                 | 10                 | 6              | 4    | 4.6420                 | 725.56   | 12.99            |
| 1981-3     | 4.023  | 5               | 52                 | 7                  | 7              | 4    | 4.0230                 | 688.44   | 13.4             |
| 1981-4     | 1.67   | 9               | 53                 | 17                 | 4              | 3    | 1.6700                 | 747.21   | 44.24            |
| 1981-5     | 2.354  | 7               | 51                 | 16                 | 4              | 3    | 2.3540                 | 748.65   | 44.94            |
| 1981-6     | 1.735  | 7               | 51                 | 13                 | 5              | 3    | 1.7350                 | 711.53   | 45.93            |
| 1981-7     | 1.891  | 8               | 52                 | 14                 | 5              | 4    | 2.3550                 | 711.72   | 26.72            |
| 1981-8     | 2.575  | 6               | 50                 | 13                 | 5              | 4    | 3.0380                 | 713.16   | 29.91            |
| 1981-9     | 1.956  | 6               | 50                 | 10                 | 6              | 4    | 2.4200                 | 676.04   | 32               |
| 1981-10    | -0.397 | 10              | 51                 | 20                 | 3              | 3    | 0.066000               | 734.81   | 56.24            |
| 1981-11    | 0.287  | 8               | 49                 | 19                 | 3              | 3    | 0.750000               | 736.25   | 56.41            |
| 1981-12    | -0.332 | 8               | 49                 | 16                 | 4              | 3    | 0.131000               | 699.13   | 70.34            |
| 1981-13    | 1.67   | 9               | 53                 | 17                 | 4              | 3    | 1.6700                 | 747.21   | 25.89            |
| 1981-14    | 2.354  | 7               | 51                 | 16                 | 4              | 3    | 2.3540                 | 748.65   | 26.81            |
| 1981-15    | 1.735  | 7               | 51                 | 13                 | 5              | 3    | 1.7350                 | 711.53   | 32.11            |
| 1981-16    | -0.618 | 11              | 52                 | 23                 | 2              | 2    | -0.618000              | 760.31   | 53.51            |
| 1981-17    | 0.066  | 9               | 50                 | 22                 | 2              | 2    | 0.066000               | 771.74   | 50.98            |
| 1981-18    | -0.553 | 9               | 50                 | 19                 | 3              | 2    | -0.553000              | 734.62   | 57.03            |
| 2073-1     | -5.886 | 22              | 118                | 51                 | 6              | 5    | -5.4220                | 168.54   | 153.66           |
| 2073-2     | 0.066  | 9               | 50                 | 22                 | 2              | 2    | 0.066000               | 771.74   | 55.65            |
| 2073-3     | 4.045  | 7               | 54                 | 11                 | 6              | 4    | 4.0450                 | 706.21   | 11.06            |
| 2073-4     | 4.728  | 5               | 52                 | 10                 | 6              | 4    | 4.7280                 | 707.64   | 7.52             |
| 2073-5     | 4.11   | 5               | 52                 | 7                  | 7              | 4    | 4.1100                 | 670.52   | 11.42            |
| 2073-6     | 1.756  | 9               | 53                 | 17                 | 4              | 3    | 1.7560                 | 729.3    | 42.9             |
| 2073-7     | 2.44   | 7               | 51                 | 16                 | 4              | 3    | 2.4400                 | 730.73   | 42.41            |
| 2073-8     | 1.821  | 7               | 51                 | 13                 | 5              | 3    | 1.8210                 | 693.62   | 48.24            |
| 2073-9     | 1.977  | 8               | 52                 | 14                 | 5              | 4    | 2.4410                 | 693.81   | 26.79            |
| 2073-10    | 2.661  | 6               | 50                 | 13                 | 5              | 4    | 3.1250                 | 695.24   | 25.97            |
| 2073-11    | 2.042  | 6               | 50                 | 10                 | 6              | 4    | 2.5060                 | 658.12   | 31.78            |
| 2073-12    | -0.311 | 10              | 51                 | 20                 | 3              | 3    | 0.152000               | 716.9    | 58.71            |
| 2073-13    | 0.373  | 8               | 49                 | 19                 | 3              | 3    | 0.836000               | 718.33   | 56.71            |
| 2073-14    | -0.246 | 8               | 49                 | 16                 | 4              | 3    | 0.217000               | 681.22   | 69.33            |
| 2073-15    | 1.756  | 9               | 53                 | 17                 | 4              | 3    | 1.7560                 | 729.3    | 27.46            |
| 2073-16    | 2.44   | 7               | 51                 | 16                 | 4              | 3    | 2.4400                 | 730.73   | 25.89            |
| 2073-17    | 1.821  | 7               | 51                 | 13                 | 5              | 3    | 1.8210                 | 693.62   | 29.14            |
| 2073-18    | -0.532 | 11              | 52                 | 23                 | 2              | 2    | -0.532000              | 752.39   | 51.92            |
| 2073-19    | 0.152  | 9               | 50                 | 22                 | 2              | 2    | 0.152000               | 753.83   | 51.82            |
Table S2. Analytical data of synthesized peptides. All peptides were determined to be >95% pure by analytical RP-HPLC at 214nM.

| Compound Reference | Calculated Molecular Weight | MS+1 Observed Molecular Weight | Analytical RP-HPLC k’ in Solvent 1* | Analytical RP-HPLC k’ in Solvent 2* |
|--------------------|-----------------------------|-------------------------------|-------------------------------------|-------------------------------------|
| EMH4-90            | 685.78                      | 686.61                        | 3.6                                 | 6.2                                 |
| EMH4-91            | 704.82                      | 705.22                        | 3.7                                 | 6.6                                 |
| EMH4-92            | 734.85                      | 735.38                        | 6.4                                 | 10.5                                |
| EMH4-93            | 711.81                      | 712.42                        | 5.2                                 | 9.0                                 |
| EMH4-94            | 704.82                      | 705.53                        | 3.8                                 | 6.5                                 |
| EMH4-99            | 707.82                      | 708.47                        | 6.6                                 | 10.4                                |
| EMH4-100           | 707.82                      | 708.42                        | 6.6                                 | 10.4                                |
| EMH4-101           | 730.26                      | 731.44                        | 6.9                                 | 11.0                                |
| EMH4-102           | 821.71                      | 822.43                        | 7.3                                 | 11.4                                |
| EMH4-103           | 837.71                      | 838.47                        | 7.2                                 | 11.2                                |
| EMH4-95            | 694.79                      | 695.73                        | 2.2                                 | 5.0                                 |
| EMH4-104           | 720.22                      | 720.52                        | 4.3                                 | 7.4                                 |
| EMH4-105           | 811.67                      | 812.49                        | 4.7                                 | 8.1                                 |
| EMH4-96            | 657.76                      | 658.67                        | 3.6                                 | 5.0                                 |
| EMH4-106           | 688.31                      | 711.46 (+23.15)               | 6.8                                 | 10.6                                |
| EMH4-107           | 658.75                      | 659.44                        | 3.8                                 | 6.7                                 |
| EMH4-97            | 681.18                      | 681.46                        | 4.3                                 | 7.6                                 |
| EMH4-98            | 681.18                      | 681.45                        | 6.2                                 | 7.2                                 |
| EMH4-108           | 772.64                      | 773.20                        | 4.6                                 | 8.3                                 |
| EMH4-109           | 691.74                      | 692.14                        | 3.5                                 | 11.5                                |
| EMH4-110           | 696.80                      | 697.33                        | 4.5                                 | 8.1                                 |
| 1981-2             | 923.58                      | 946.68 (+23)                  | 11.8                                | 14.4                                |
| 1981-3             | 809.69                      | 809.69                        | 9.4                                 | 12.2                                |
| 1981-4             | 839.68                      | 840.23                        | 7.7                                 | 10.0                                |
| 1981-5             | 920.58                      | 920.89                        | 7.7                                 | 11.9                                |
| 1981-6             | 806.69                      | 807.24                        | 7.7                                 | 11.0                                |
| 1981-7             | 820.63                      | 821.30                        | 7.1                                 | 11.0                                |
| 1981-8             | 903.55                      | 924.25                        | 8.1                                 | 12.3                                |
| 1981-9             | 787.65                      | 810.51 (+23)                  | 6.7                                 | 10.6                                |
| 1981-10            | 817.63                      | 818.55                        | 4.3                                 | 7.3                                 |
| 1981-11            | 898.53                      | 899.48                        | 5.1                                 | 8.8                                 |
| 1981-12            | 784.65                      | 785.49                        | 4.4                                 | 7.6                                 |
| 1981-13            | 839.68                      | 840.41                        | 7.1                                 | 10.9                                |
| 1981-14            | 920.58                      | 921.16                        | 8.2                                 | 12.2                                |
| 1981-15            | 806.69                      | 807                           | 6.7                                 | 10.4                                |
| 1981-16            | 836.68                      | 837.26                        | 4.4                                 | 7.4                                 |
| 1981-17            | 917.58                      | 918.38                        | 5.2                                 | 9.2                                 |
| 1981-18            | 803.69                      | 804.51                        | 4.6                                 | 8.2                                 |
| 2073-1             | 842.68                      | 864.95 (+22)                  | 4.9                                 |                                     |
| 2073-6             | 751.23                      | 751.05                        | 4.8                                 |                                     |
| 2073-7             | 832.13                      | 831.95                        | 5.3                                 |                                     |
| 2073-8             | 718.24                      | 739.90 (+22)                  | 4.8                                 |                                     |
| 2073-9             | 748.23                      | 748.00                        | 2.8                                 |                                     |
| 2073-10            | 829.13                      | 830.40                        | 2.8                                 |                                     |
| 2073-11            | 715.24                      | 715.00                        | 2.7                                 |                                     |
|       |       |       |   |
|-------|-------|-------|---|
| 2073-12 | 729.18 | 729.40 | 2.7 |
| 2073-13 | 812.1  | 809.90 | 3.0 |
| 2073-14 | 696.19 | 695.95 | 2.7 |
| 2073-15 | 726.18 | 726.05 | 1.8 |
| 2073-16 | 807.08 | 807.00 | 2.0 |
| 2073-17 | 693.2  | 693.05 | 1.9 |
| 2073-18 | 748.23 | 748.05 | 2.8 |
| 2073-19 | 829.13 | 829.00 | 3.0 |
| 2073-20 | 715.24 | 715.10 | 2.7 |
| 2073-21 | 745.23 | 745.15 | 1.9 |
| 2073-22 | 826.13 | 826.10 | 2.1 |
| 2073-23 | 712.24 | 712.10 | 1.9 |

*For the EMH and TPI1981 compounds, the HPLC k’ value equals [(peptide retention time – solvent retention time)/ solvent retention time]. Two different solvent systems were used. Solvent system 1 equals (10% acetonitrile in 0.1% trifluoroacetic acid/H$_2$O and a gradient to 90% acetonitrile over 35 min). Solvent system 2 equals (10% methanol in 0.1% trifluoroacetic acid/ H$_2$O and a gradient to 90% methanol over 35 min). The analytical Vydac C18 column (Vydac 218TP104) in combination with a flow rate of 1.5 ml/min was used for analytical characterization. The peptide purity was determined by HPLC at a wavelength of 214 nm. The TPI2073 compounds were characterized in only Solvent system 1, consisting of 5% acetonitrile in 0.1% formic acid/H$_2$O and a gradient to 95% acetonitrile over 6 min using a Phenomenex C18-100a 50x4.6µM 5 micron analytical column. The peptide purity was determined by HPLC at a wavelength of 214 nm.