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Supplemental Data

Structural and Molecular Evolutionary Analysis of Agouti and Agouti-Related Proteins

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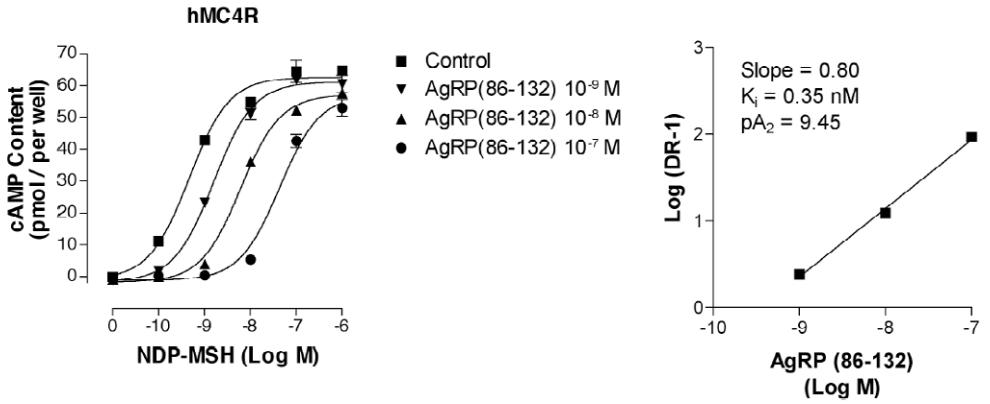

Figure S1. Inhibition of NDP-MSH stimulated cAMP generation at hMC4R by C-terminal AgRP, and corresponding Schild analysis, reveals competitive antagonism with a dissociation constant (K_i) of of 0.35 nM.

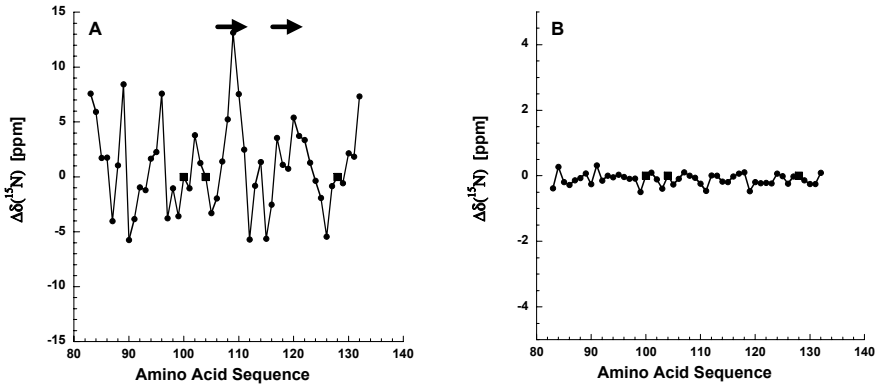

Figure S2 Analysis of AgRP(83-132) ^{15}N chemical shifts derived from the HSQC spectra of both the full-length protein and the isolated C-terminal domain. Panel A are the measured ^{15}N chemical shifts from AgRP(83-132), minus the consensus random coil

chemical shifts. The significant variation is consistent with a folded domain. Circles represent measured values; squares represent proline, which are not observable in the HSQC spectra. The arrows show the locations of β-strands adjacent to the RFF triplet (residues 111 – 113). Panel B shows the difference in chemical shifts, full-length minus C-terminal, for residues 83 – 132. Note that the vertical axis is expanded by a factor of three compared to panel A. The limited scatter is consistent with that observed for the ^{1}H chemical shifts, and demonstrates that the HSQC from residues 83 – 132 in full-length AgRP is essentially equivalent to the isolated C-terminal domain, AgRP(83-132).

Table S1. Retention Times for full-length AgRP and its derivatives
Column type: C18 vydac 4.6mm
Gradient: 25% acetonitrile-45% acetonitrile in 40min
Full-length AgRP, reduced: 28 minutes
Full-length AgRP, oxidized: 24 minutes
AgRP(83-132), oxidized: 14 minutes

Table S2. Backbone NOEs observed in the C-terminal domain of full-length AgRP
C94 NH -- Y118 HA
T107 NH -- R120 NH
Y109 NH -- Y118 NH
A106 NH -- C105NH
A106 NH -- P104 HA

Table S3. Backbone ^{15}N Chemical Shifts Determined from the HSQC Spectra

Ser83N	123.11
Ser84N	121.43
Arg85N	122.94
Arg86N	122.97
Cys87N	114.67
Val88N	120.35
Arg89N	129.63
Leu90N	116.66
His91N	114.26
Glu92N	119.26
Ser93N	114.31
Cys94N	120.37
Leu95N	124.69
Gly96N	115.09
Gln97N	116.73
Gln98N	119.47

Val99N	115.71
Pro100	NA
Cys101N	117.65
Cys102N	122.52
Asp103N	120.37
Pro104	NA
Cys105N	115.39
Ala106N	123.05
Thr107N	113.42
Cys108N	123.96
Tyr109N	134.06
Cys110N	126.25
Arg111N	123.68
Phe112N	115.00
Phe113N	119.89
Asn114N	120.37
Ala115N	119.36
Phe116N	118.18
Cys117N	122.25
Tyr118N	122.00
Cys119N	119.46
Arg120N	126.60
Lys121N	125.33
Leu122N	125.75
Gly123N	108.78
Thr124N	111.64
Ala125N	123.08
Met126N	114.85
Asn127N	118.18
Pro128	NA
Cys129N	118.14
Ser130N	117.66
Arg131N	123.05
Thr132N	119.33