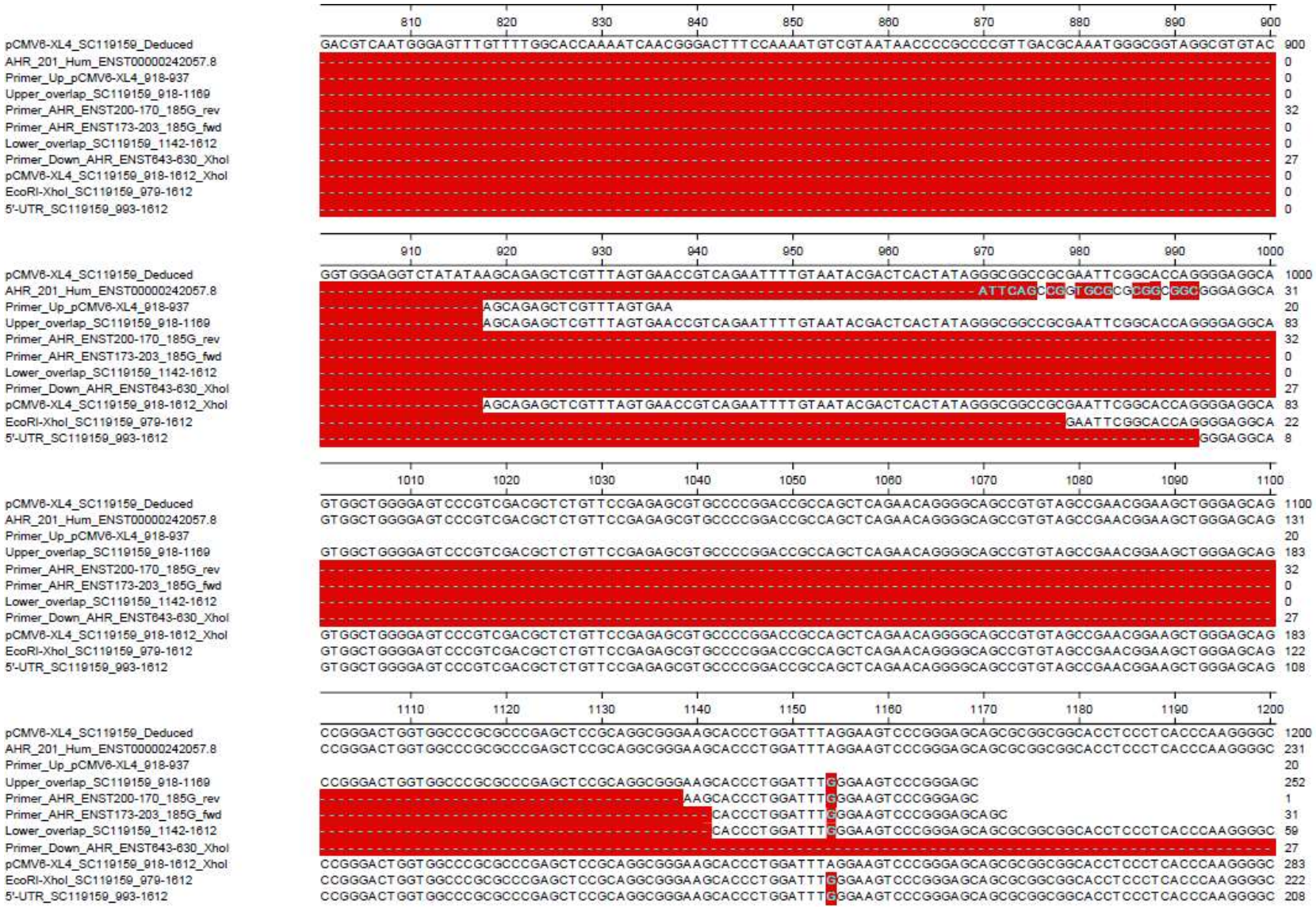
Supplementary Information SI7

Isolation and Mutation of the 5'-UTR Sequence from *AHR* cDNA Clone SC119159.

Alignment of the deduced sequence of template clone SC119159 purchased from Origene (Rockville MD 20850, USA), the modern human *AHR* mRNA reference sequence, primers, overlapping intermediate PCR fragments, final mutated PCR product, and insert for pcDNA3-EGFP generated to create the Neanderthal 185G variant (bottom track) of the *AHR* 5'-untranslated region (UTR) by overlap extension PCR (Heckman and Pease 2007). The wild-type (185A) and mutated (185G) 5'-UTR sequences were subsequently inserted into EGFP fusion protein expression vector pcDNA3-EGFP (see main text for details). The ruler above the alignment shows the position in the Origene construct carrying *AHR* cDNA clone SC119195 in vector pCMV6-XL4; numbers at the right of each alignment block refer to the position within the sequence shown on that specific line. Nucleotide positions deviating from the deduced sequence of SC119159 in pCMV6-XL4 are highlighted in red. Primer sequences and annealing temperatures are listed in Supplementary Information SI8.

pCMV6-XL4_SC119159_Deduced:	Relevant portion of the nucleotide sequence of *AHR* cDNA clone SC119159 in pCMV6-XL4 as deduced from the sequence information provided by Origene in combination with newly generated sequence information.
AHR_201_Hum_ENST00000242057.8:	Modern human *AHR* mRNA reference sequence retrieved from the Ensembl database.
Primer_Up_pCMV6-XL4_918-937:	Upper primer for generation of the upstream overlap PCR fragment.
Upper_overlap_SC119159_918-1169:	Upstream overlap PCR fragment.
Primer_AHR_ENST200-170_185G_rev:	Reverse mutagenesis primer to incorporate the G variant at position 185 of the *AHR* 5'-UTR sequence (position numbering of AHR_201_Hum_ENST00000242057.8). Note that the reverse complement of this primer as listed in SI8 is shown in the alignment.
Primer_AHR_ENST173-203_185G_fwd:	Forward mutagenesis primer to incorporate the G variant at position 185 of the AHR 5'-UTR sequence (position numbering of AHR_201_Hum_ENST00000242057.8).
Lower_overlap_SC119159_1142-1612:	Downstream overlap PCR fragment.
Primer_Down_AHR_ENST643-630_XhoI:	Lower primer for generation of the downstream overlap PCR fragment. Note that the reverse complement of this primer as listed in SI8 is shown in the alignment.
pCMV6-XL4_SC119159_918-1612_XhoI:	Complete SC119159 segment (185A) amplified and mutated into 185G by overlap extension PCR.
EcoRI-XhoI_SC119159_979-1612:	EcoRI – XhoI restriction subfragment inserted into EGFP fusion protein expression vector pcDNA3-EGFP (mutated 185G variant).
5'-UTR_SC119159_993-1612:	Complete *AHR* 5'-UTR sequence included in the inserted EcoRI – XhoI restriction fragment (mutated 185G variant).

Reference: Heckman KL, Pease LR. 2007. Gene splicing and mutagenesis by PCR-driven overlap extension. *Nat Protoc* 2(4):924-932.


pCMV6-XL4_SC119159_Deduced
AHR_201_Hum_ENST00000242057.8
Primer_Up_pCMV6-XL4_918-937
Upper_overlap_SC119159_918-1169
Primer_AHR_ENST200-170_185G_rev
Primer_AHR_ENST173-203_185G_fwd
Lower_overlap_SC119159_1142-1612
Primer_Down_AHR_ENST643-630_XhoI
pCMV6-XL4_SC119159_918-1612_XhoI
EcoRI-XhoI_SC119159_979-1612
5'-UTR_SC119159_993-1612

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                                          1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
pCMV6-XL4_SC119159_Deduced                CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  1300
AHR_201_Hum_ENST00000242057.8             CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  331
Primer_Up_pCMV6-XL4_918-937                                                                                                                       20
Upper_overlap_SC119159_918-1169                                                                                                                   252
Primer_AHR_ENST200-170_185G_rev                                                                                                                   1
Primer_AHR_ENST173-203_185G_fwd                                                                                                                   31
Lower_overlap_SC119159_1142-1612          CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  159
Primer_Down_AHR_ENST643-630_XhoI          ----------------------------------------------------------------------------------------------------  27
pCMV6-XL4_SC119159_918-1612_XhoI          CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  383
EcoRI-XhoI_SC119159_979-1612              CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  322
5'-UTR_SC119159_993-1612                  CGCGGCGACGGTCACGGGGCGCGGCGCCACCGTGAGCGACCCAGGCCAGGATTCTAAATAGACGGCCCAGGCTCCTCCTCCGCCCGGGCCGCCTCACCTG  308

                                          1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
pCMV6-XL4_SC119159_Deduced                CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  1400
AHR_201_Hum_ENST00000242057.8             CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  431
Primer_Up_pCMV6-XL4_918-937                                                                                                                       20
Upper_overlap_SC119159_918-1169                                                                                                                   252
Primer_AHR_ENST200-170_185G_rev                                                                                                                   1
Primer_AHR_ENST173-203_185G_fwd                                                                                                                   31
Lower_overlap_SC119159_1142-1612          CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  259
Primer_Down_AHR_ENST643-630_XhoI          ----------------------------------------------------------------------------------------------------  27
pCMV6-XL4_SC119159_918-1612_XhoI          CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  483
EcoRI-XhoI_SC119159_979-1612              CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  422
5'-UTR_SC119159_993-1612                  CGGGCATTGCCGCGCCGCCTCCGCCGGTGTAGACGGCACCTGCGCCGCCTTGCTCGCGGGTCTCCGCCCCTCGCCCACCCTCACTGCGCCAGGCCCAGGC  408

                                          1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
pCMV6-XL4_SC119159_Deduced                AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  1500
AHR_201_Hum_ENST00000242057.8             AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  531
Primer_Up_pCMV6-XL4_918-937                                                                                                                       20
Upper_overlap_SC119159_918-1169                                                                                                                   252
Primer_AHR_ENST200-170_185G_rev                                                                                                                   1
Primer_AHR_ENST173-203_185G_fwd                                                                                                                   31
Lower_overlap_SC119159_1142-1612          AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  359
Primer_Down_AHR_ENST643-630_XhoI          ----------------------------------------------------------------------------------------------------  27
pCMV6-XL4_SC119159_918-1612_XhoI          AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  583
EcoRI-XhoI_SC119159_979-1612              AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  522
5'-UTR_SC119159_993-1612                  AGCTCACCTGTACTGGCGCGGGCTGCGGAAGCCTGCGTGAGCCGAGGCGTTGAGGCGCGGCGCCCACGCCACTGTCCCGAGAGGACGCAGGTGGAGCGGG  508

                                          1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
pCMV6-XL4_SC119159_Deduced                CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  1600
AHR_201_Hum_ENST00000242057.8             CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  631
Primer_Up_pCMV6-XL4_918-937                                                                                                                       20
Upper_overlap_SC119159_918-1169                                                                                                                   252
Primer_AHR_ENST200-170_185G_rev                                                                                                                   1
Primer_AHR_ENST173-203_185G_fwd                                                                                                                   31
Lower_overlap_SC119159_1142-1612          CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  459
Primer_Down_AHR_ENST643-630_XhoI          ----------------------------------------------------------------------------------------------------GT  25
pCMV6-XL4_SC119159_918-1612_XhoI          CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  683
EcoRI-XhoI_SC119159_979-1612              CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  622
5'-UTR_SC119159_993-1612                  CGCGGCTTCGCGGAACCCGGCGCCGGCCGCCGCAGTGGTCCCAGCCTACACCGGGTTCCGGGGACCCGGCCGCCAGTGCCCGGGGAGTAGCCGCCGCCGT  608
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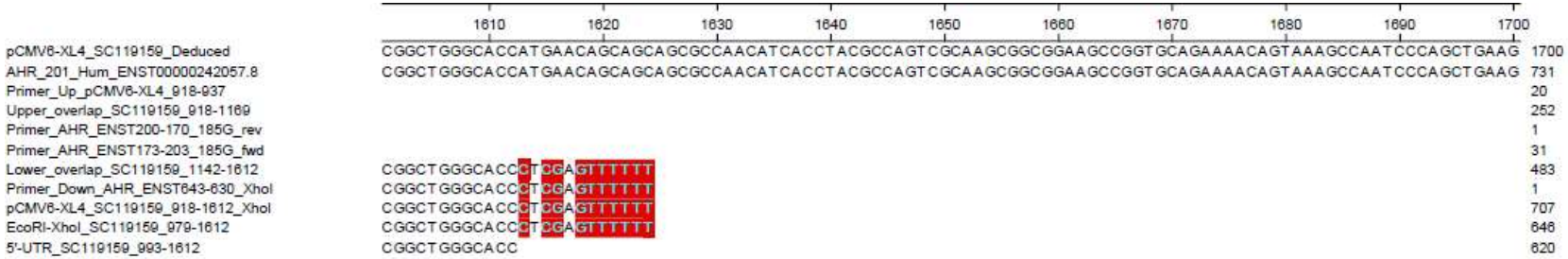
1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
pCMV6-XL4_SC119159_Deduced CGGCTGGGCACCATGAACAGCAGCAGCGCCAACATCACCTACGCCAGTCGCAAGCGGCGGAAGCCGGTGCAGAAAACAGTAAAGCCAATCCCAGCTGAAG 1700
AHR_201_Hum_ENST00000242057.8 CGGCTGGGCACCATGAACAGCAGCAGCGCCAACATCACCTACGCCAGTCGCAAGCGGCGGAAGCCGGTGCAGAAAACAGTAAAGCCAATCCCAGCTGAAG 731
Primer_Up_pCMV6-XL4_918-937 20
Upper_overlap_SC119159_918-1169 252
Primer_AHR_ENST200-170_185G_rev 1
Primer_AHR_ENST173-203_185G_fwd 31
Lower_overlap_SC119159_1142-1612 CGGCTGGGCACCCTCGAGTTTTTT 483
Primer_Down_AHR_ENST643-630_XhoI CGGCTGGGCACCCTCGAGTTTTTT 1
pCMV6-XL4_SC119159_918-1612_XhoI CGGCTGGGCACCCTCGAGTTTTTT 707
EcoRI-XhoI_SC119159_979-1612 CGGCTGGGCACCCTCGAGTTTTTT 646
5'-UTR_SC119159_993-1612 CGGCTGGGCACC 620