Supplementary materials

Orphan nuclear receptor RORα regulates enzymatic metabolism of cerebral 24S-hydroxycholesterol through CYP39A1 intronic response element activation

Hiroshi Matsuoka, Miyu Katayama, Ami Ohishi, Kaoruko Miya, Riki Tokunaga, Sou Kobayashi, Yuya Nishimoto, Kazutake Hirooka, Akiho Shima and Akihiro Michihara

Table S1. Primers used in this study.

Primer	Sequence (5′–3′)
EMSA (Mutated sequences are underlined)	
CYP39A1-RORE1wt-sense	CGTTTTTAAAAAGGTCATCCTC
CYP39A1-RORE1wt-antisense	GAGGATGACCTTTTTAAAAACG
CYP39A1-RORE2wt-sense	TGTGGTGACCCATTTTCCATCT
CYP39A1-RORE2wt-antisense	AGATGGAAAATGGGTCACCACA
CYP39A1-RORE1mt-sense	CGTTTTT<u>GG</u>AA<u>CC</u>GTCATCCTC
CYP39A1-RORE1mt-antisense	GAGGATGAC<u>GG</u>TT<u>CC</u>AAAAACG
CYP39A1-RORE2mt-sense	TGTGGTGAC<u>GG</u>AT<u>CC</u>TCCATCT
CYP39A1-RORE2mt-antisense	AGATGGA<u>GG</u>AT<u>CC</u>GTCACCACA
Ikb-RORE-sense	GATCCAATGTAGGTCACATG
Ikb-RORE-antisense	CATGTGACCTACATTGGATC
ChIP-PCR	
ChIP-CYP39A1-RORE1-FW	AGTAGTCGATGCTCTGTGAG
ChIP-CYP39A1-RORE1-RV	CTTCCCTTAGGCATGTGCTG
ChIP-CYP39A1-RORE2-FW	AGTAATCCTCCTTGTAGTAGATG
ChIP-CYP39A1-RORE2-RV	CAGTACAGCTGGTGTGATATTC
Luciferase reporter cloning (Mutated sequences are underlined)	
pCYP39A1-RORE1wt-MluI-FW	ACACGCGTCGTTTTTAAAAAGGTCATCCTC
pCYP39A1-RORE1mt-MluI-FW	ACACGCGTCGTTTTT<u>GG</u>AA<u>CC</u>GTCATCCTC
pCYP39A1-RORE2wt-KpnI-FW	AATGGTACCTGTGGTGACCCATTTTCCATCTGGTTAGC
pCYP39A1-RORE2mt-KpnI-FW	AATGGTACCTGTGGTGAC<u>GG</u>AT<u>CC</u>TCCATCTGGTTAGC
pCYP39A1(−235)-MluI-FW	CTAACGCGTAGCTGGAGTGGTCCAGCTGCC
pCYP39A1(+1264)-MluI-RV	TAAAACGCGTGTACAGCTGGTGTGATATTC
pCYP39A1(+86)-XhoI-RV	GCAACTCGAGCAGCTCTCCTTCGTAACTGTAG
PGVB2-FW	GCCCAAGCTACCATGATAAG
PGVB2-RV	TCATAGCTTCTGCCAACCGAAC
qRT-PCR	
rtRORA-FW	TCCATGCAAGATCTGTGGAG
rtRORA-RV	ACAGCATCTCGAGACATCCC
rtCYP39A1-FW	TTGGACACTTGCATACGTCC
rtCYP39A1-RV	CTAGTAATGACACCAGGAGC
rtBMAL1-FW	GTTCTTCTATTCTTGGTGAGAAC

rtBMAL1-RV	ACAGCCATTGCTGCCTCATC
rt18S rRNA-FW	CGATAACGAACGAGACTCTGG
rt18S rRNA-RV	TAGGGTAGGCACACGCTGAGC

SiRNA experiments	(Synthesised siRNA sequences are underlined)
siRORA258-S-u	GATCACTAATACGACTCACTATAGGG<u>GTCAGAAGAACTGTTTGAT</u>TT
siRORA258-S-d	AA<u>ATCAAACAGTTCTTCTGAC</u>CCCTATAGTGAGTCGTATTAGTGATC
siRORA258-AS-u	GATCACTAATACGACTCACTATAGGG<u>ATCAAACAGTTCTTCTGAC</u>TT
siRORA258-AS-d	AA<u>GTCAGAAGAACTGTTTGAT</u>CCCTATAGTGAGTCGTATTAGTGATC
siRORA1388-S-u	GATCACTAATACGACTCACTATAGGG<u>CTAATGGCATTTAAAGCAA</u>TT
siRORA1388-S-d	AA<u>TTGCTTTAAATGCCATTAG</u>CCCTATAGTGAGTCGTATTAGTGATC
siRORA1388-AS-u	GATCACTAATACGACTCACTATAGGG<u>TTGCTTTAAATGCCATTAG</u>TT
siRORA1388-AS-d	AA<u>CTAATGGCATTTAAAGCAA</u>CCCTATAGTGAGTCGTATTAGTGATC
siGFP-S-u	GATCACTAATACGACTCACTATAGGG<u>CAAGCTGACCCTGAAGTTC</u>TT
siGFP-S-d	AA<u>GAACTTCAGGGTCAGCTTG</u>CCCTATAGTGAGTCGTATTAGTGATC
siGFP-AS-u	GATCACTAATACGACTCACTATAGGG<u>GAACTTCAGGGTCAGCTTG</u>TT
siGFP-AS-d	AA<u>CAAGCTGACCCTGAAGTTC</u>CCCTATAGTGAGTCGTATTAGTGATC