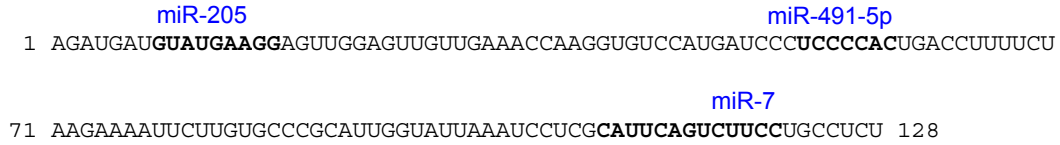
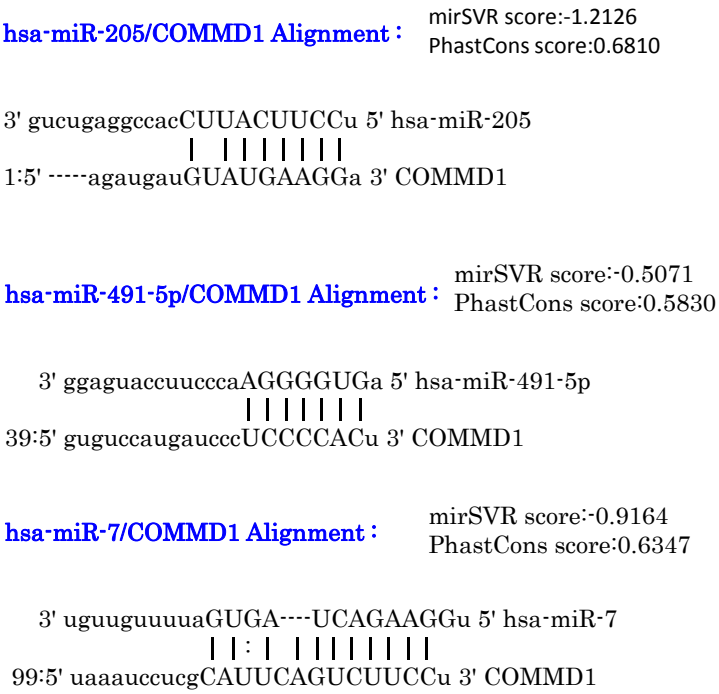
a

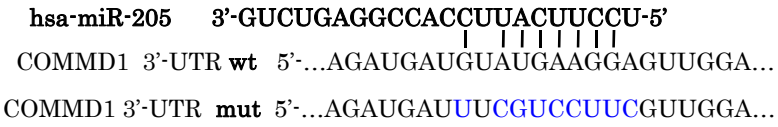


b



Supplementary Figure S1. Analysis of miRNA target sites on 3′-UTR of COMMD1 mRNA using the algorithm miRanda (http://www.microrna.org/). (**a**) Predicted miRNA target sites on the 3′-UTR of COMMD1. (**b**) Sequence alignment and mirSVR scores for the putative target sites.

a COMMD1 3'-UTR wild type and mutant construct schemes



b miR-205-sponge construct scheme

S1 5′-**ctaga**taaCAGACTCCGaccAATGAAGGAcgatCAGACTCCGaccAATGAAGGA**g**-3′

AS1 3′-**t**ATTGTCTGAGGCTGGTTACTTCCTGCTAGTCTGAGGCTGGTTACTTCCT**cgatc**-5′

S2 5′-**ctagc**ataaCAGACTCCGaccAATGAAGGAcgatCAGACTCCGaccAATGAAGGA**g**-3′

AS2 3′-**g**TATTGTCTGAGGCTGGTTACTTCCTGCTAGTCTGAGGCTGGTTACTTCCT**cttaa**-5′

S3 5′-**aattc**ataaCAGACTCCGaccAATGAAGGAcgatCAGACTCCGaccAATGAAGGA**g**-3′

AS3 3′-**g**TATTGTCTGAGGCTGGTTACTTCCTGCTAGTCTGAGGCTGGTTACTTCCT**cctag**-5′

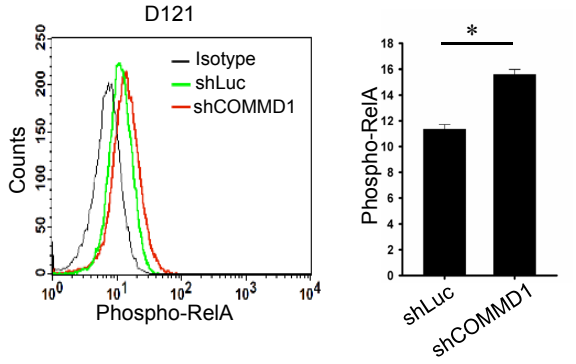
Supplementary Figure S2. Schematic illustration of (**a**) wild-type (wt) and mutant (mut) COMMD1 3′-UTR reporter constructs, and (**b**) miR-205-sponge construct containing six specific binding sites for miR-205.

a

Human shCOMMD1-1 TRCN0000167145	Sense:5'-GATCCAAAGTCAACCAAATTCTGAACTCGAGTTCAGAATTTGGTTGACTTTGTTTTTG-3'
	Antisense:5'-AATTCAAAAACAAAGTCAACCAAATTCTGAACTCGAGTTCAGAATTTGGTTGACTTTG-3'
Human shCOMMD1-2 TRCN0000167998	Sense:5'-GATCCAAGCTGCTGTCATTTCCAAACTCGAGTTTGGAAATGACAGCAGCTTGTTTTTG-3'
	Antisense:5'-AATTCAAAAACAAGCTGCTGTCATTTCCAAACTCGAGTTTGGAAATGACAGCAGCTTG-3'
Human shCOMMD1-3 TRCN0000168056	Sense:5'-GATCCGAGGTCAAAGTCAACCAAATCTCGAGATTTGGTTGACTTTGACCTCGTTTTTG-3'
	Antisense:5'-AATTCAAAAACGAGGTCAAAGTCAACCAAATCTCGAGATTTGGTTGACTTTGACCTCG-3'
Human shCOMMD1-4 TRCN0000168045	Sense:5'-GATCGCTCAAATACACACACCTGTTCTCGAGAACAGGTGTGTGTATTTGAGCTTTTT-3'
	Antisense:5'-AATTAAAAAGCTCAAATACACACACCTGTTCTCGAGAACAGGTGTGTGTATTTGAGC-3'
Mouse shCOMMD1-1 TRCN0000197798	Sense:5'-GATCGTCTATTGCATCTGCAGACATCTCGAGATGTCTGCAGATGCAATAGACTTTTTG-3'
	Antisense:5'-AATTCAAAAAGTCTATTGCATCTGCAGACATCTCGAGATGTCTGCAGATGCAATAGAC-3'
Mouse shCOMMD1-2 TRCN0000350952	Sense:5'-GATCGATGAAGTTAAAGTCAAGCAACTCGAGTTGCTTGACTTTAACTTCATCTTTTTG-3'
	Antisense:5'-AATTCAAAAAGATGAAGTTAAAGTCAAGCAACTCGAGTTGCTTGACTTTAACTTCATC-3'
shLuc TRCN0000072249	Sense:5'-GATCGCGGTTGCCAAGAGGTTCCATCTCGAGATGGAACCTCTTGGCAACCGCTTTTTG -3'
	Antisense:5'-AATTCAAAAAGCGGTTGCCAAGAGGTTCCATCTCGAGATGGAACCTCTTGGCAACCGC -3'

b

Supplementary Figure S3. Development of short hairpin RNAs (shRNAs) for knockdown COMMD1 expression. Four shRNAs targeting different regions of the COMMD1 mRNA (shCOMMD1-1 to shCOMMD1-4) and a control shRNA targeting luciferase mRNA (shLuc) were designed and lentiviral expression vectors were constructed. (**a**) Sequences of sense and anti-sense oligonucleotides employed for generating these COMMD1-specific shRNAs. The shRNA sequences were obtained from the Public TRC Portal of the RNAi Consortium (www.broadinstitute.org/rnai/public/gene/search). The TRC identification numbers and oligonucleotide sequences are shown below the name of each shCOMMD1 and in the right column, respectively. **(b**) The efficiencies of these shRNAs were determined by achieving stable expression in SAS cells and analyzing COMMD1 expression by immunoblot analysis. The four COMMD1-specific shRNAs resulted in effective COMMD1 knockdown, while shLuc had no effect. shCOMMD1-4 (referred to as shCOMMD1 in this paper) was employed in experiments in this study. Samples were from COMMD1 over expressed cells for COMMD1 OE.



Supplementary Figure S4. COMMD1 regulates NF-κB activation in D121 cells. Phosphorylation levels of RelA in shLuc and shCOMMD1 stably transfected D121 cells were measured by flow cytometric analysis. Elevated levels of phosphorylated RelA (phospho-RelA) were detected in the COMMD1 knockdown cells. The left panel shows a representative histogram for the right bar figure for phospho-RelA levels in the shLuc and shCOMMD1 stably transfected D121 cells. Data represent mean ± SD from three independent experiments. *$P < 0.05$

Primers used in microRNA qRT-PCR

miR-205_RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCAGACT-3’
U6_RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAAAAATAT-3’
miR-205	forward 5’-GCATCATCCTTCATTCCACCGG-3’
U6	forward 5’-TTCCTCCGCAAGGATGACACGC-3’
Universal reverse primer	reverse 5’-GTGCAGGGTCCGAGGT-3’
Primary U6	forward 5’-CTCGCTTCGGCAGCACA-3’
	reverse 5’ -AACGCTTCACGAATTTGCGT-3’
Primary hsa-miR-205	forward 5’-ACAGGCTGAGGTTGACATGC-3’
	reverse 5’-CTCCTGAACTTCACTCCACTGAAA-3’
Primary mmu-miR-205	forward 5’-CTTCACTCCACTGAAATCTGGTTG-3’
	reverse 5’-CTACACGAGGAGGCTTAGTAGACA-3’

Synthetic oligonucleotide used to generate 3’ UTR reporter constructs

COMMD1 3’ UTR Wt	forward 5’-ATGTACGCGTAGATGATGTATGAAGGAGTTGGAGTTGTTG-3’
	reverse 5’-AGACAAGCTTAGAGGCAGGAAGACTGAATGCG-3’
COMMD1 3’ UTR Mut	forward 5’-ATGTACGCGTAGATGATTTCGTCCTTCGTTGGAGTTGTTGAA-3’

Synthetic oligonucleotide used to generate miR-205 sponge constructs

S1_Xba1	5’-CTAGATAACAGACTCCGACCAATGAAGGACGATCAGACTCCGACCAATGA AGGAG -3’
AS1_NheI	5’-CTAGCTCCTTCATTGGTCGGAGTCTGATCGTCCTTCATTGGTCGGAGTCTGTTAT-3’
S2_NheI	5’-CTAGCATAACAGACTCCGACCAATGAAGGACGATCAGACTCCGACCAATGAAGGAG-3’
AS2_EcoRI	5’-AATTCTCCTTCATTGGTCGGAGTCTGATCGTCCTTCATTGGTCGGAGTCTGTTATG-3’
S3_EcoRI	5’-AATTCATAACAGACTCCGACCAATGAAGGACGATCAGACTCCGACCAATGAAGGAG-3’
AS3_BamHI	5’-GATCCTCCTTCATTGGTCGGAGTCTGATCGTCCTTCATTGGTCGGAGTCTGTTATG-3’

Synthetic oligonucleotide used to generate precursor miR-205 construct

Pre hsa-miR-205	forward 5’-CATCGAATTCGGTCCTTGACATCTCCCAAA-3’
	reverse 5’-AGCAGGATCCCTGAAGAAGCACGCACACTC-3’

Supplementary Table S1. Sequences of primers employed for microRNA , RT-qPCR, and generation of COMMD1 3′-UTR reporter, miR-205-sponge, and miR-205 overexpression constructs.

COMMD1	forward 5’-CGGAGCCAGCTATATCCAGA-3’
	reverse 5’-TTGGAAATGACAGCAGCTTG-3’
ACTB	forward 5’-TCCCTGGAGAAGAGCTACGA-3’
	reverse 5’-AGCACTGTGTTGGCGTACAG-3’
GAPDH	forward 5’-GAGTCAACGGATTTGGTCGT-3’
	reverse 5’-GACAAGCTTCCCGTTCTCAG-3’
c-MYC	forward 5’-TTCGGGTAGTGGAAAACCAG-3’
	reverse 5’-CAGCAGCTCGAATTTCTTCC-3’
SOX-2	forward 5’-ACACCAATCCCATCCACACT-3’
	reverse 5’-GCAAACTTCCTGCAAAGCTC-3’
OCT-4	forward 5’-GAAGGATGTGGTCCGAGTGT-3’
	reverse 5’-GTGAAGTGAGGGCTCCCATA-3’
KLF-4	forward 5’-ACCCACACAGGTGAGAAACC-3’
	reverse 5’-ATGTGTAAGGCGAGGTGGTC-3’
NANOG	forward 5’-TTCCTTCCTCCATGGATCTG-3’
	reverse 5’-ATCTGCTGGAGGCTGAGGTA-3’
ALDH1	forward 5’-TGTTAGCTGATGCCGACTTG-3’
	reverse 5’-CTTCTTAGCCCGCTCAACAC-3’
ABCG2	forward 5’-GTGGCCTTGGCTTGTATGAT-3’
	reverse 5’-AACAATTGCTGCTGTGCAAC-3’
CD44	forward 5’-AAGGTGGAGCAAACACAACC-3’
	reverse 5’-AGCTTTTTCTTCTGCCCACA-3’
CD117	forward 5’-AGAGACTTGGCAGCCAGAAA-3’
	reverse 5’-AGGGGCTGCTTCCTAAAGAG-3’
CD133	forward 5’-GCCACCGCTCTAGATACTGC-3’
	reverse 5’-TGTTGTGATGGGCTTGTCAT-3’
TNF-α	forward 5’-AACCTCCTCTCTGCCATCAA-3’
	reverse 5’-CCAAAGTAGACCTGCCCAGA-3’
IL-1β	forward 5’-ACGATGCACCTGTACGATCA-3’
	reverse 5’-TCTTTCAACACGCAGGACAG-3’
IL-6	forward 5’-TACCCCCAGGAGAAGATTCC-3’
	reverse 5’-TTTTCTGCCAGTGCCTCTTT-3’
IL-8	forward 5’-GTGCAGTTTTGCCAAGGAGT-3’
	reverse 5’-CTCTGCACCCAGTTTTCCTT-3’
CXCL1	forward 5’-AGGGAATTCACCCCAAGAAC-3’
	reverse 5’-CACCAGTGAGCTTCCTCCTC-3’
CCL2	forward 5’-CCCCAGTCACCTGCTGTTAT-3’
	reverse 5’-TGGAATCCTGAACCCACTTC-3’
MMP9	forward 5’-CTCGAACTTTGACAGCGACA-3’
	reverse 5’-GCCATTCACGTCGTCCTTAT-3’
NOS2	forward 5’-ACAAGCCTACCCCTCCAGAT-3’
	reverse 5’-TCCCGTCAGTTGGTAGGTTC-3’

Supplementary Table S2. Sequences of forward and reverse primers employed for PCR amplification of human genes.

Commd1	forward 5’-GCTCAAACCAAAAAGCAAGG-3’
	reverse 5’-GTTGAGTGCCGTGACTGAGA-3’
Actb	forward 5’-AGCCATGTACGTAGCCATCC-3’
	reverse 5’-CTCTCAGCTGTGGTGGTGAA-3’
Gapdh	forward 5’-ACCCAGAAGACTGTGGATGG-3’
	reverse 5’-CACATTGGGGGTAGGAACAC-3’
c-Myc	forward 5’-ACACGGAGGAAAACGACAAG-3’
	reverse 5’-TCGTCTGCTTGAATGGACAG-3’
Sox-2	forward 5’-AAGGGTTCTTGCTGGGTTTT-3’
	reverse 5’-AGACCACGAAAACGGTCTTG-3’
Oct-4	forward 5’-AAGCCCTCCCTACAGCAGAT-3’
	reverse 5’-CTGGGAAAGGTGTCCCTGTA-3’
Klf-4	forward 5’-GCAGTCACAAGTCCCCTCTC-3’
	reverse 5’-CTGTGTGAGTTCGCAGGTGT-3’
Nanog	forward 5’-CCAGTGGAGTATCCCAGCAT-3’
	reverse 5’-GAAGTTATGGAGCGGAGCAG-3’
Aldh1	forward 5’-GCACTCAATGGTGGGAAAGT-3’
	reverse 5’-TTTGGCCACACACTCCAATA-3’
Abcg2	forward 5’-CCATTCATCAGCCTCGGTAT-3’
	reverse 5’-AATCCGCAGGGTTGTTGTAG-3’
Cd44	forward 5’-TGGATCCGAATTAGCTGGAC-3’
	reverse 5’-AGCTTTTTCTTCTGCCCACA-3’
Cd117	forward 5’-GGGCTAGCCAGAGACATCAG-3’
	reverse 5’-AGGAGAAGAGCTCCCAGAGG-3’
Cd133	forward 5’-GAAAAGTTGCTCTGCGAACC-3’
	reverse 5’-TCTCAAGCTGAAAAGCAGCA-3’
Tnf-α	forward 5’-AGCCCCCAGTCTGTATCCTT-3’
	reverse 5’-CTCCCTTTGCAGAACTCAGG-3’
Il-1β	forward 5’-CAGGCAGGCAGTATCACTCA-3’
	reverse 5’-AGCTCATATGGGTCCGACAG-3’
Il-6	forward 5’-AGTTGCCTTCTTGGGACTGA-3’
	reverse 5’-TCCACGATTTCCCAGAGAAC-3’
Il-8	forward 5’-CGTCCCTGTGACACTCAAGA-3’
	reverse 5’-TAATTGGGCCAACAGTAGCC-3’
Cxcl1	forward 5’-GCTGGGATTCACCTCAAGAA-3’
	reverse 5’-CTTGGGGACACCTTTTAGCA-3’
Ccl2	forward 5’-CAGGTCCCTGTCATGCTTCT-3’
	reverse 5’-TCTGGACCCATTCCTTCTTG-3’
Mmp9	forward 5’-GAAGGCAAACCCTGTGTGTT-3’
	reverse 5’-AGAGTACTGCTTGCCCAGGA-3’
Nos2	forward 5’-CACCTTGGAGTTCACCCAGT-3’
	reverse 5’-ACCACTCGTACTTGGGATGC-3’

Supplementary Table S3. Sequences of forward and reverse primers employed for PCR amplification of mouse genes.