SUPPLEMENTARY MATERIAL

Supplemental Figures S1 and S2: generation of Sco2 mouse models

Sco2 (3 exons) resides in a region of mouse chromosome 15 between *Tymp* (10 exons; encoding thymidine phosphorylase, a protein involved in nucleotide metabolism (1); previously called *Ecgf1*) and *Ncaph2* (20 exons; encoding kleisin-β, a subunit of the condensin II complex of mitosis (2); previously called *C22.2*) **(Fig. S1A)**. *Tymp* and *Sco2* are transcribed in the same orientation (left to right in Fig. S1A), whereas *Ncaph2* is transcribed in the opposite orientation (right to left). To obtain the targeting vector, we screened hybridization filters spotted with a bacterial artificial chromosome (BAC) library of 129Sv/J mouse genomic DNA (Genome Systems) with a probe derived from a *Tymp* RT-PCR product radiolabeled with [α-^{32}P]dATP. One BAC, 65d3, was identified, from which a 5,075-bp *Kpn*I fragment containing the entire *Sco2* gene and flanking DNA, extending from the *Kpn*I site located in Intron 5 of *Tymp* (located at nt 89205407-89205412; numbering of the July, 2007 assembly of the mouse genome [http://genome.ucsc.edu/]) to the *Kpn*I site located in Exon 12 of *Ncaph2* (nt 89200332-89200337) **(Fig. S1A),** was isolated and then inserted into the *Kpn*I site of pBluescript II KS+ (Stratagene) to generate the targeting vector.

For the KO construct, we inserted a 2,074-bp *Pac*I-*Pac*I DNA cassette comprising a neomycin phosphotransferase resistance (Neo^R) gene flanked by two identical 34-bp *lox*P sites (loxP-Neo^R-loxP, or LNL) into a *Pac*I site that had been generated by *in vitro* mutagenesis of the endogenous *Hin*dIII site located 30 bp downstream of the start codon of *Sco2* (nt 8920853-8920858) **(Fig. S1A)**.

For the KI construct, we mutated *Sco2* codon 129 from GAG (Glu) to AAG (Lys) by *in vitro* mutagenesis, thereby creating an E129K substitution that is the mouse homolog of the human E140K mutation. The same LNL cassette was inserted into a *Pac*I site that had been generated by *in vitro* mutagenesis of an endogenous *Sma*I site located 643 bp downstream of the *Sco2* stop codon, within intron 18 of *Ncaph2* (nt 89201465-8920170) **(Fig. S1A)**.

Both constructs, verified by sequencing, were linearized at a unique *Not*I site in the plasmid and were electroporated into 129Sv/J mouse embryonic stem (ES) cells. G418-resistant clones and homologous recombinants were identified via a combination of long range PCR (Roche) and Southern blot analysis.

To confirm recombination of the KO targeting vector into the endogenous *Sco2* locus, we performed Southern blot analysis on the recombinant region. Ten μg of ES cell genomic DNA

were digested with *Bsm*I (**Fig. S1B**), electrophoresed through a 1% agarose gel, blotted onto a nitrocellulose filter, and hybridized separately with two probes (A and B) radiolabeled with [α-^{32}P]dCTP (PerkinElmer), using the 'Ready-To-Go' DNA labeling kit (Amersham). Probe A was a 292-bp PCR fragment corresponding to a region within *Tymp* located outside of the 5' end of the recombinant region, amplified using primers ScoproAF1 (5'-TGTCTGTCATAACTAATCGG-3') and ScoproAR1 (5'-GTTTCAGGTCACCTGTAATG-3'; nt 89205415-89205434). Probe B was a 525-bp PCR fragment corresponding to a region within *Ncaph2* located outside of 3' end of the recombinant region, amplified using primers ScoproBF1 (5'-AGACACAGCATGTGAAGGGG-3'; nt 89199541-89199560) and ScoproBR1 (5'-GGAACTAAAAGCTGGGAGCC-3'; nt 89199036-89199055). Using both probes, the *Sco2* WT allele was detected as a single 10.3-kb hybridizing band (**Fig. S1B**). However, because of the presence of an extra *Bsm*I site located within the Neo^{R} cassette, two bands were observed in heterozygous cells containing the recombinant allele, 10.3 kb (i.e. the WT allele) and 7.8 kb (i.e. the recombinant allele) in size using Probe A, and bands of 10.3 kb and 4.5 kb, respectively, using Probe B (**Fig. S1B**).

The mutated ES clones were injected into C57BL/6J blastocysts. We obtained seven chimeric KO and two chimeric KI mice. These animals were crossed with C57BL/6J wild-type mice to obtain germline transmission of the floxed allele.

For genotyping KO mice, tail genomic DNA was amplified using the Expand Long Template PCR System (Roche) in 50 μl of 1X #2 buffer, 10 μM dNTPs and 5 U/μl poly mix polymerase (Roche). An initial denaturation at 94°C for 2 min was followed by 94°C for 10 s, 65°C for 30 s, and 68°C for 10 min for 28 cycles, with a final extension at 68°C for 8 min. By using the forward primer TPE5F1 (5'-AGATGTGACAGCCACTGTGG-3'; nt 89205448-89205469) within *Tymp* and reverse primer C22.2E12F1 (5'-ACAGAAGCGCAAGAGGAAGG-3'; nt 89200282-89200301**)** within *Ncaph2*, corresponding to regions located outside the *Kpn*I sites in the recombination region, we amplified two PCR fragments, one of 5.2 kb (5,188 bp) corresponding to the *Sco2* WT allele, and the other of 7.2 kb (7,266 bp), corresponding to the *Sco2* KO allele containing the LNL cassette **(Fig. S2A)**.

To identify heterozygous KI mice, we used two sets of PCR amplifications. First, to identify the LNL cassette present in *Ncaph2* downstream of *Sco2*, and using the same long PCR system (Roche) as was done for KO mice, tail genomic DNA was amplified by using forward primer C22.2E16IF (5'-CTCTAGCAGCTCATGGTTGG-3'; nt 89201154-89201173) and reverse primer

C22.2E19R (5'-TGAAAAGGGACACCATTC-3'; nt 89201571-89201594), corresponding to regions located outside the LNL cassette, yielding two PCR fragments, one of 441 bp, corresponding to the *Sco2* WT allele, and the other of 2.5 kb (2,533 bp), corresponding to the *Sco2* KI allele containing the LNL cassette **(Fig. S2B)**. Second, to confirm the presence of the E129K mutation, tail DNA was amplified with forward primer mScoATG (5'-AGCTTCAGGTCCACGATGCTACTGGCTC-3'; nt 89202868-89202897) and reverse primer mScoE2R3 (5'-TTCCGCACCACCTGCACTAGCTTTTCAAGC-3'; nt 89202465-89202494) corresponding to a 433-bp region in the 5' end of *Sco2* and containing the putative G→A mutation at nt-385 in codon 129 (E129K). The reverse primer contained a mismatch (underlined in mScoE2R3), thereby creating an extra *Hin*dIII site only in E129K DNA. Digestion of the PCR products with *Hin*dIII yielded two diagnostic fragments, one of 390 bp, corresponding to the *Sco2* wt allele. and the other of 360 bp, corresponding to the E129K mutant allele (due to the presence of the extra *Hin*dIII site introduced at codon 129; **Fig. S2C)**. The presence of the E129 (WT) and K129 (KI) alleles were confirmed by sequencing of the PCR products.

Finally, to obtain $Sco2^{loxP}$ mice lacking the Neo^{R} cassette, we crossed heterozygous KO and KI mice with transgenic animals expressing Cre recombinase. The resulting $Sco2^{loxP}$ KO and KI heterozygotes were backcrossed with 129 Sv/J mice for more than 10 generations. Appropriate matings were then performed to generate homozygous KO/KO, homozygous KI/KI, and compound heterozygous KO/KI mice. To identify the KO allele containing a single LoxP site, we amplified the region within *Sco2* flanking the LNL and/or LoxP site, using forward primer MScoE2F (5'-ACGATGCTACTGGCTCTAGG-3'; nt 89202864-89202885) and reverse primer MScoE2R1 (5'-CTGTAGTATACGCGGTAGTT-3'; nt 89202285-89202304). PCR of the WT, KO (LNL) and KO (LoxP) alleles yielded the expected amplicons of 600-bp, 2680-bp, and 667-bp, respectively **(Fig. S2D)**. To identify the KI allele containing a single LoxP site, we amplified the region in *Ncaph2* with primers C22.2E16IF and C22.2E19R. PCR of the KI (LoxP) allele yielded the expected amplicon of 521 bp **(Fig. S2B)**. Finally, PCR of the WT, KO (LoxP), and KI alleles with primers mScoATG and mScoE2R3, followed by digestion with *Hin*dIII, yielded the expected diagnostic fragments of 390-bp, 500-bp, and 360-bp, respectively **(Fig. S2E)**.

Figure S1

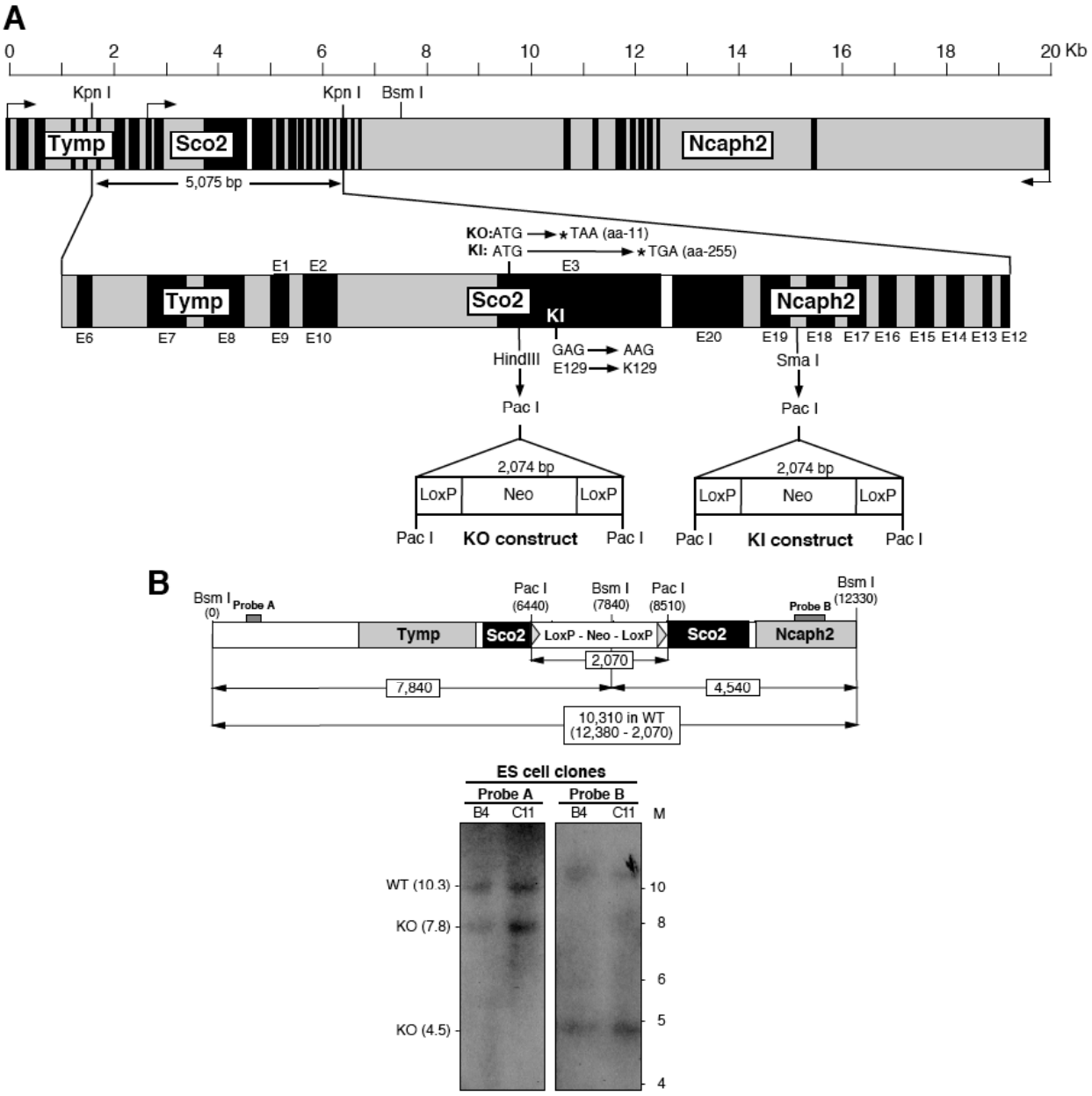

Figure S1. Generation of *Sco2* mouse models. **(A)** The region of mouse chromosome 15 showing *Sco2*, flanked by *Tymp* and *Ncaph2*, is indicated; black boxes, exons (also indicated with E's above and below the expanded 5.1-kb *Kpn*I region); gray boxes, introns; arrows mark transcription orientation. The indicated 5.1-kb *Kpn*I fragment was used to make the KO and KI targeting constructs. See text for details. **(B)** Map of the *Sco2* region (not to scale), with predicted sizes of hybridizing fragments in Southern blotting of ES cell clones B4 and C11 containing the KO construct, using the indicated radiolabelled probes. M, marker, in kb.

Figure S2

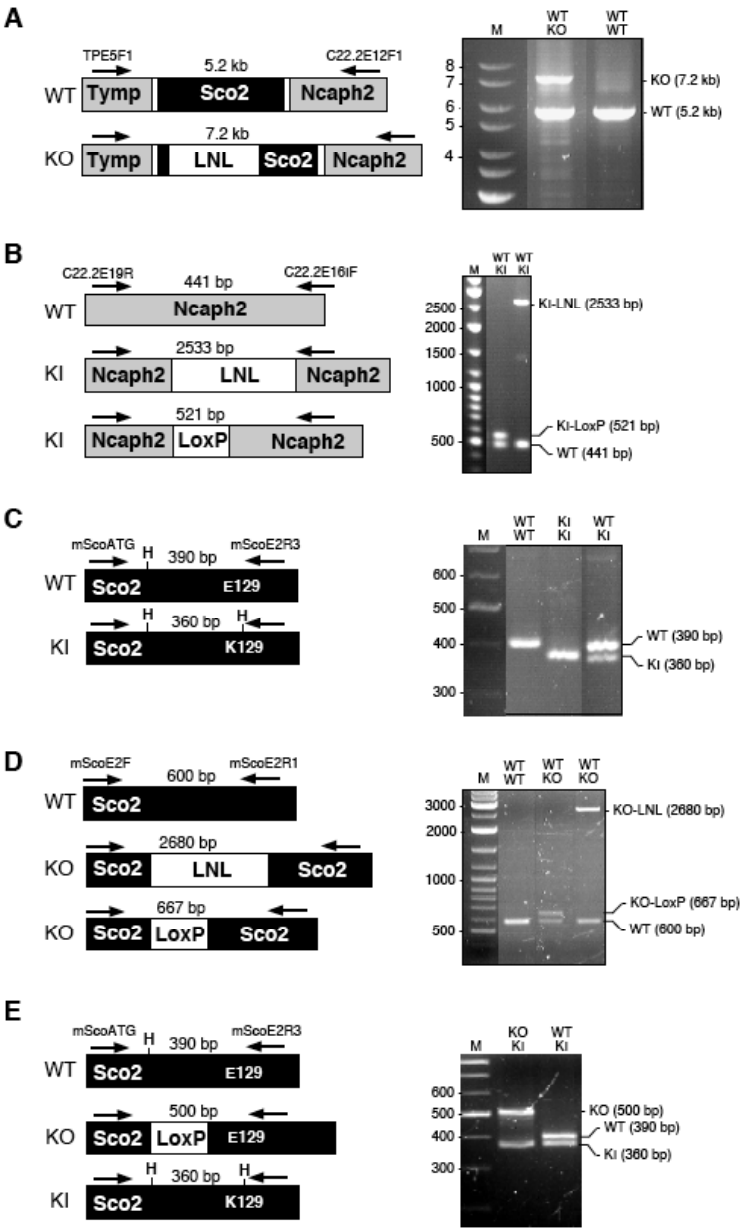

Figure S2. Representative PCR and PCR/RFLP analyses of *Sco2*-mutant progeny mice**.** Relevant maps, primers, and predicted sizes of PCR fragments (following *Hin*dIII digestion in panels C and E), are at left in each panel. **(A)** Detection of WT/KO heterozygous mice containing the KO-LNL construct. **(B)** Detection of WT/KI heterozygous mice containing the KI-LNL construct and the KI-LoxP construct after mating with *cre* mice. **(C)** Detection of the E129K mutation in WT/KI heterozygous and KI/KI homozygous mice, following digestion of the PCR products with *Hin*dIII (H). **(D)** Detection of WT/KO heterozygous mice containing the KO-LNL construct and the KO-LoxP construct after mating with *cre* mice. **(E)** Detection of WT/KI heterozygous and KI/KO compound heterozygous mice, following digestion of the PCR products with *Hin*dIII (H). M, size markers (GeneRuler DNA Ladder Mix; Fermentas #SM0331).

Supplemental Figure S3: Histochemical analysis of tissues in *Sco2*-mutant mice

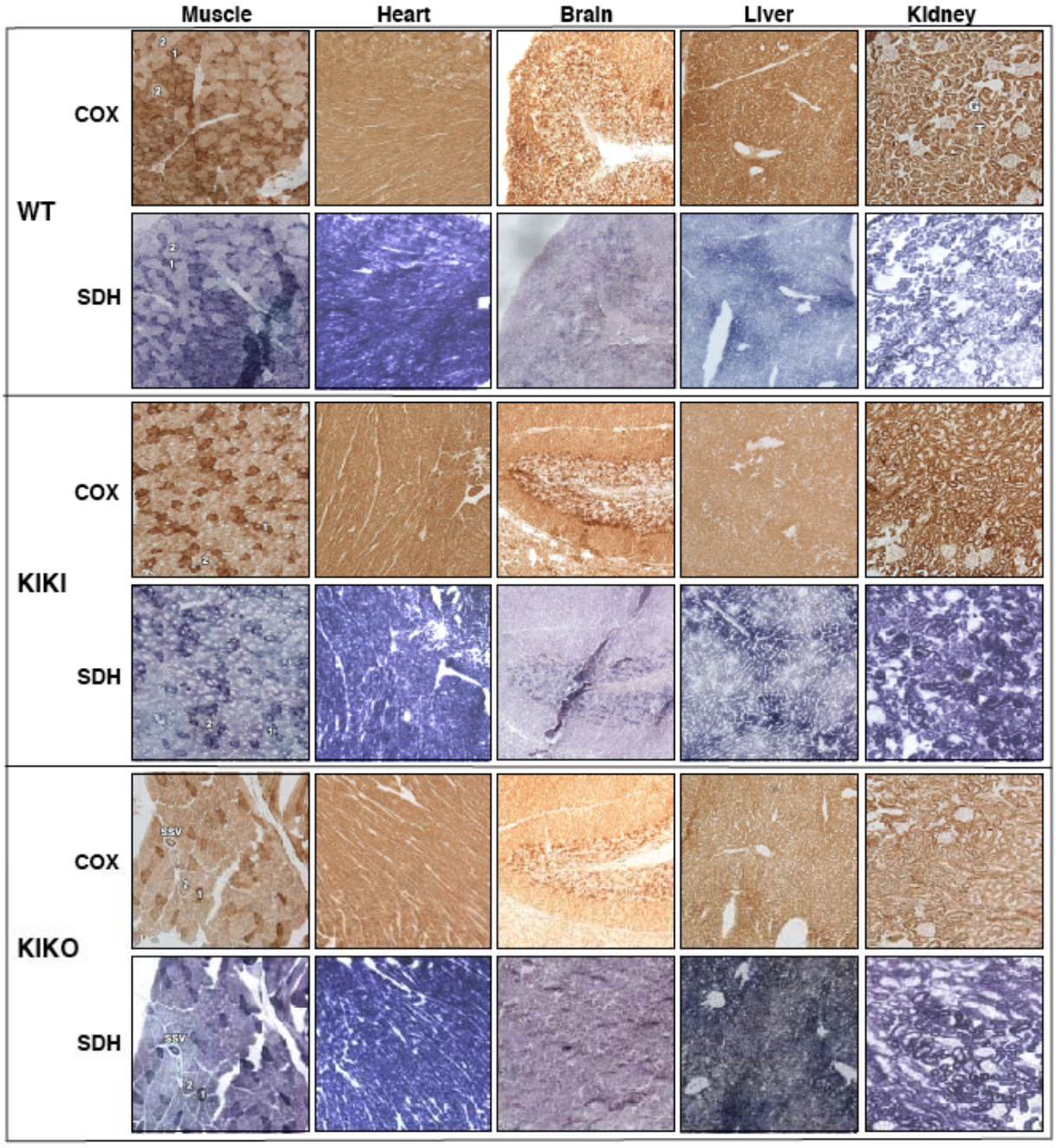

Figure S3. Enzyme histochemistry in mouse tissues. Histochemistry to detect COX and SDH in the indicated issues from 4-month-old female mice. Note the reduction in the intensity and distribution of the COX stain, most notably in muscle and brain, in the KIKO mice. G, glomerulus; T, tubule; 1, 2, type I and II muscle fibers, respectively; SSV, strong SDH-reactive blood vessels (3), indicative of mitochondrial proliferation. x20. Similar results were obtained in male mice (not shown).

References for Supplemental Material

1 Nishino, I., Spinazzola, A. and Hirano, M. (1999) Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science*, **283**, 689-692.

2 Gosling, K.M., Makaroff, L.E., Theodoratos, A., Kim, Y.H., Whittle, B., Rui, L., Wu, H., Hong, N.A., Kennedy, G.C., Fritz, J.A. *et al.* (2007) A mutation in a chromosome condensin II subunit, kleisin beta, specifically disrupts T cell development. *Proc. Natl. Acad. Sci. USA*, **104**, 12445-12450.

3 Hasegawa, H., Matsuoka, T., Goto, Y. and Nonaka, I. (1993) Cytochrome *c* oxidase activity is deficient in blood vessels of patients with myoclonus epilepsy with ragged-red fibers. *Acta Neuropathol.*, **85**, 280-284.