Supplementary Methods and Figure Legends

Generation of P₀-rtTA Transgenics and P₀-rtTA x TRE-caErb B₂ Bitransgenics—The cytomegalovirus immediate early promoter (Pcmv) was excised from pTet-On (Clontech, Mountain View, CA) by first digesting with Spe I, then filling in the ends using the Klenow fragment of DNA polymerase in the presence of dNTPs. The linearized vector was digested with Eco RI to remove the Pcmv promoter and the vector was gel purified. The rat P₀ promoter was generously provided by G. Lemke in the pBluescript KS (-) vector and the promoter was excised by first digesting with Xho I and creating blunt ends as described above. After purification, the linearized vector was digested with Eco RI, the 1.2 kb fragment was gel purified and ligated into the modified pTet-On vector creating P₀-rtTA. Four lines of founder mice were produced by the Transgenic and Knockout Mouse Facility at the University of Kansas in a B6C3F1/Hsd background. Backcrossing with C57BL/6 mates found that two lines stably transmitted the transgene. Of these two lines, one showed enriched expression of the rtTA transgene in sciatic nerve as determined by reverse transcriptase PCR using RNA isolated from various tissues of an adult animal (FIG. 4A).

To create the bitransgenics, the P₀-rtTA founder was bred with mice expressing the V664-E664 mutation of Erb B₂ under control of a tetracycline response element (TRE) linked to a minimal CMV promoter (1). The V664-E663 mutation leads to constitutive activity (caErb B₂) and these mice were generously provided by J. Kudlow (University of Alabama, Birmingham) in a mixed SJL-C57Bl/6 background. Bitransgenic mice were identified by genotyping of genomic DNA extracted from ear tissue obtained from 3 week old pups. The PCR strategy and identification of bitransgenic animals is shown in FIG. 4B. To identify the P₀-rtTA transgene from genomic DNA, we amplified a 341 bp sequence that spans the P₀-rtTA junction, (P1-forward: 5’-CCCAGAGTATACAATGCCCCTTC-3’, P2-reverse: 5’-GCCAATACAGTGTAGGCTGCTCT-3’). Amplification of a 136 bp sequence located in the P₀ promoter was used to identify the endogenous promoter (P1-forward and P3-reverse: 5’-GAGTGGTGTITTCTGGAACCTGC-3’). Primers directed against the caErb B₂ transgene (P4-forward: 5’-ATCCACGCTGTITTTGACCTC-3’; P5-reverse: 5’TCTGTCCGCTACACACTTG-3’) amplified a 300 bp product. Each 20 µl PCR reaction contained 0.2 mM dNTPs, 1× PCR buffer, 0.5 µM of PCR primers, 2 µl of genomic DNA, and 0.2 U of Deep Vent Taq polymerase (New England Biolabs, Ipswich, MA). The PCR conditions were: 94°C for 5 min, and 35 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec followed by a single 5 min extension at 72°C.

Assessment intra-epidermal nerve fiber density (iENFD). After measuring NCV, the animals were sacrificed and plantar foot pads were processed and stained with the pan-axonal marker, PGP9.5 (Chemicon, Temecula, CA) as described (2). iENFD was quantified by an observer blinded to treatment and was established by counting only fibers that traversed the epidermal-dermal border, excluding epidermal branches. Total fiber number per millimeter in each of several frames of view per section was averaged and the mean iENFD per animal was the average of six sections (3).
REFERENCES

**Supplementary FIG. 1.** Lack of Cav1 expression in lung and sciatic nerve from Cav1 KO mice. Lung and sciatic nerves were isolated from adult WT or Cav1 KO mice and 20 µg of protein was fractionated by SDS-PAGE. After transfer to nitrocellulose, immunoblot analysis for Cav1 and β-actin was performed on the lung samples. For the sciatic nerve, the myelin-associated enzyme 2’, 3’ cyclic nucleotide phosphodiesterase (CNPase) served as a loading control.

**Supplementary FIG. 2.** Effect of diabetes on iENDF in wild type or Cav1 knockout mice. A: Mice were treated with vehicle or rendered diabetic for 2 or 6 weeks. Foot pads were removed, fixed, prepared for immunohistochemistry using the pan-axonal marker PGP9.5 and iENDF was quantified by counting the number of PGP9.5 positive fibers that crossed the dermal/epidermal boundary. Results are from 4 animals per treatment per genotype. *, p< 0.05 compared to time-matched genotype control.
Supplementary FIG. 3. The DOX-diet does not alter weight gain, MNCV of WT mice or thermal sensitivity in bitransgenic mice. A: Mice were placed on the DOX-diet (n=6) or standard lab chow (n=4) for the indicated time and body weight was measured weekly. B: Wild type and bitransgenic mice were maintained on the DOX-diet (n=6) or standard chow (n=4) for 3 weeks and NCV was assessed. *, p < 0.05 compared to bitransgenic minus DOX-diet; ^ p < 0.01 compared to wild type plus DOX-diet. C: Bitransgenic mice (n=12) were assessed for baseline thermal sensitivity at week 0. Animals were place on standard chow (n=5) or the DOX diet (n=7) and thermal sensitivity measured weekly. Arrow indicates replacement of the DOX-diet with standard chow.
**Supplementary FIG. 4.** The compact myelin protein, P₀, did not decrease after 12 weeks of diabetes. The level of P₀ in lysates of sciatic nerve obtained from WT or Cav1 KO was determined by immunoblot analysis. The level of β-actin served as a loading control.