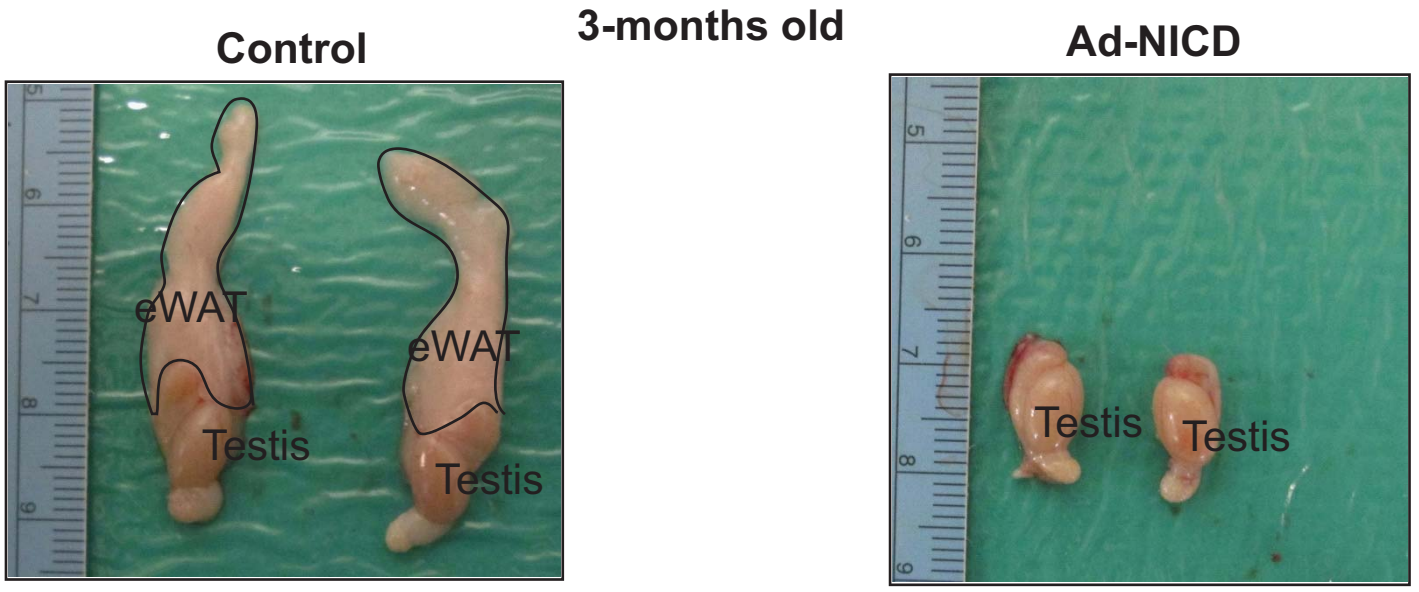
Supplemental data

Fig. S1

A



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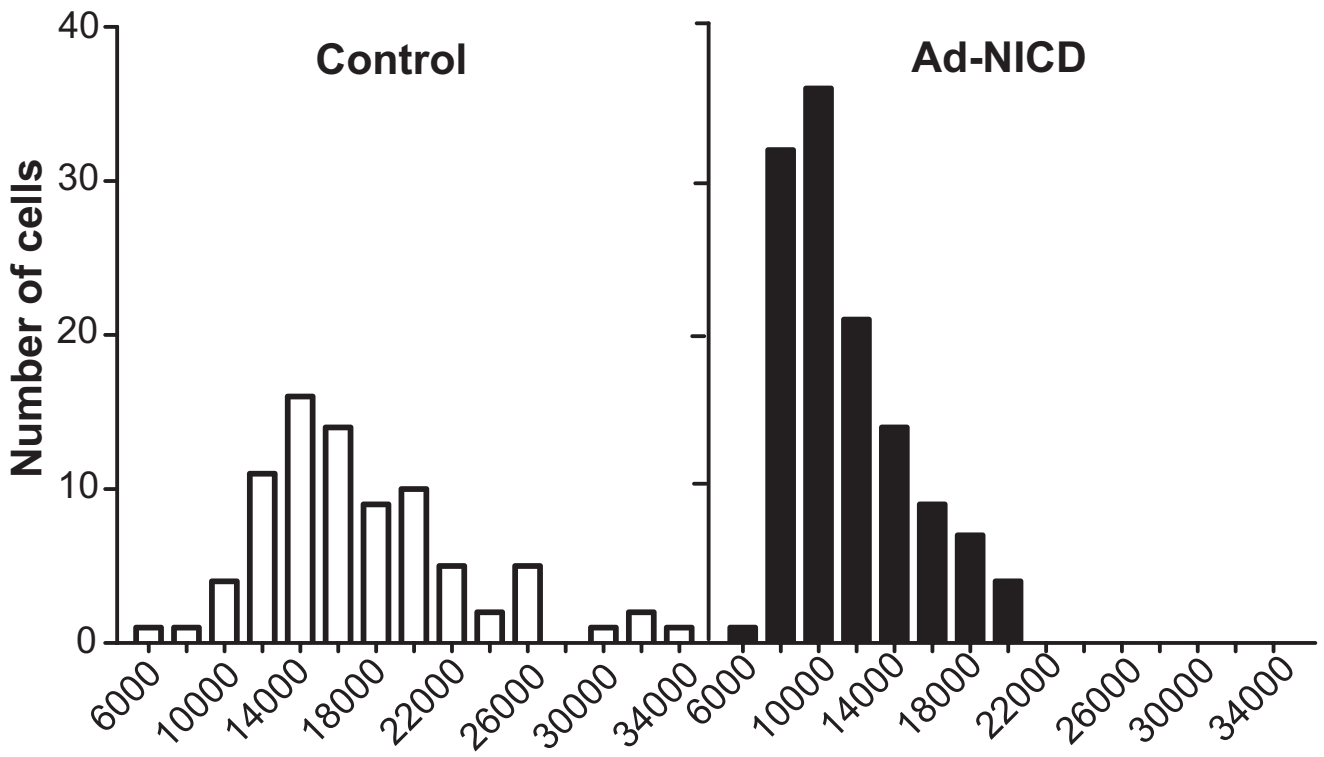
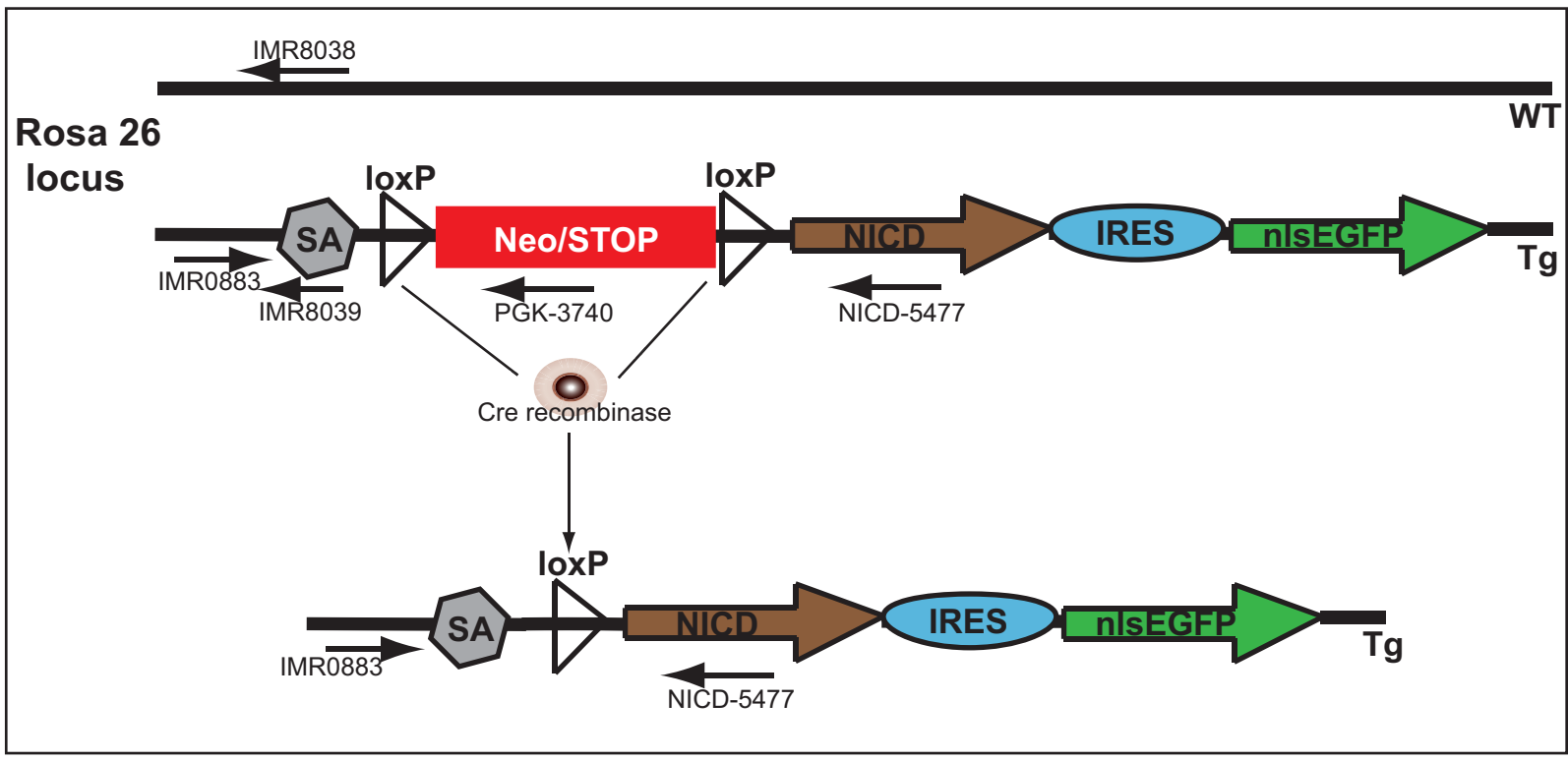


C



D

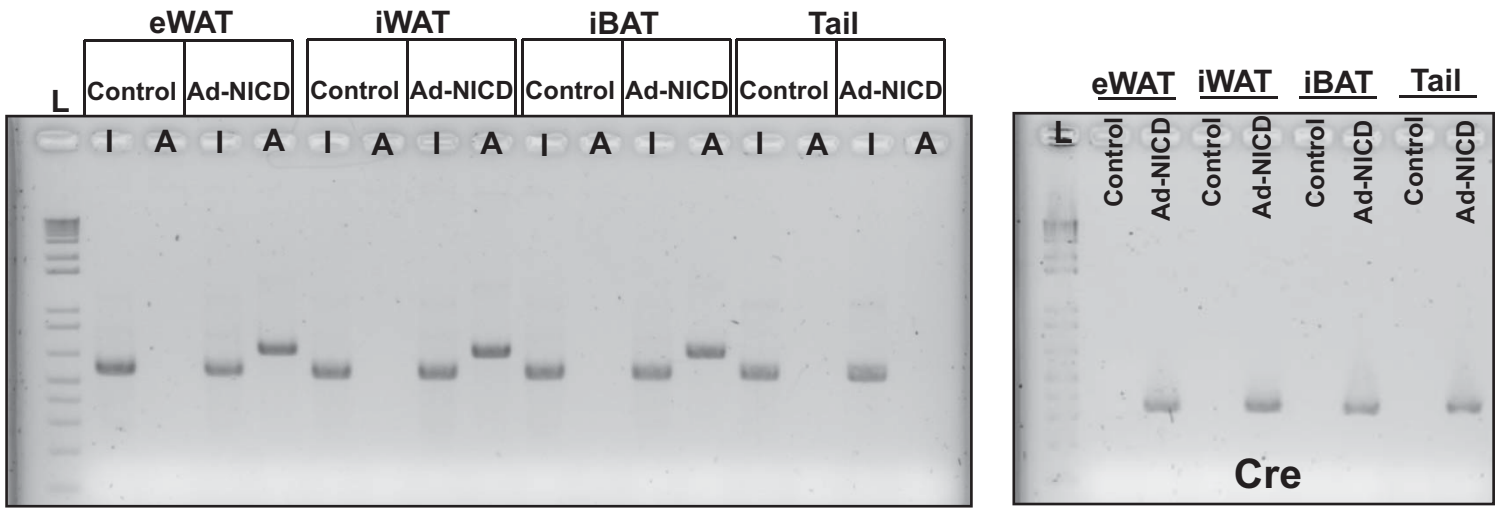


Figure S1.

A. Necropsy photos of the epididymal white adipose tissue (eWAT) in 3-month old male mice. Ruler values are in cm.

B. A representative histogram of the distribution of the adipocytes size in epididymal white adipose tissue of 1-month old male mice. Total number of samples processed n=3 per genotype.

C. A schematic representation of the $Rosa^{NICD/NICD}$ system. Arrows indicate the primers used for genotyping and for detection of the recombination. Primer sequences can be found in the supplemental experimental procedures.

D. Detection of the recombination in epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT) and interscapular brown adipose tissue (iBAT). Genomic DNA from these tissues was used as a template for the PCR. Tail DNA was used as a negative control for recombination. PCR products were run in a 2% agarose gel. Left panel: "I" in each lane indicates that the primer pair IMR0883 and the PGK-3740 was used for the detection of the "inactive" allele (absence of recombination). "A" in each lane indicates that the primer pair IMR0883 and NICD-5477 was used for the detection of the "active" allele (presence of recombination). Right panel; detection of the Cre transgene. The DNA ladder used is the 1kb plus ladder (Life Technologies).

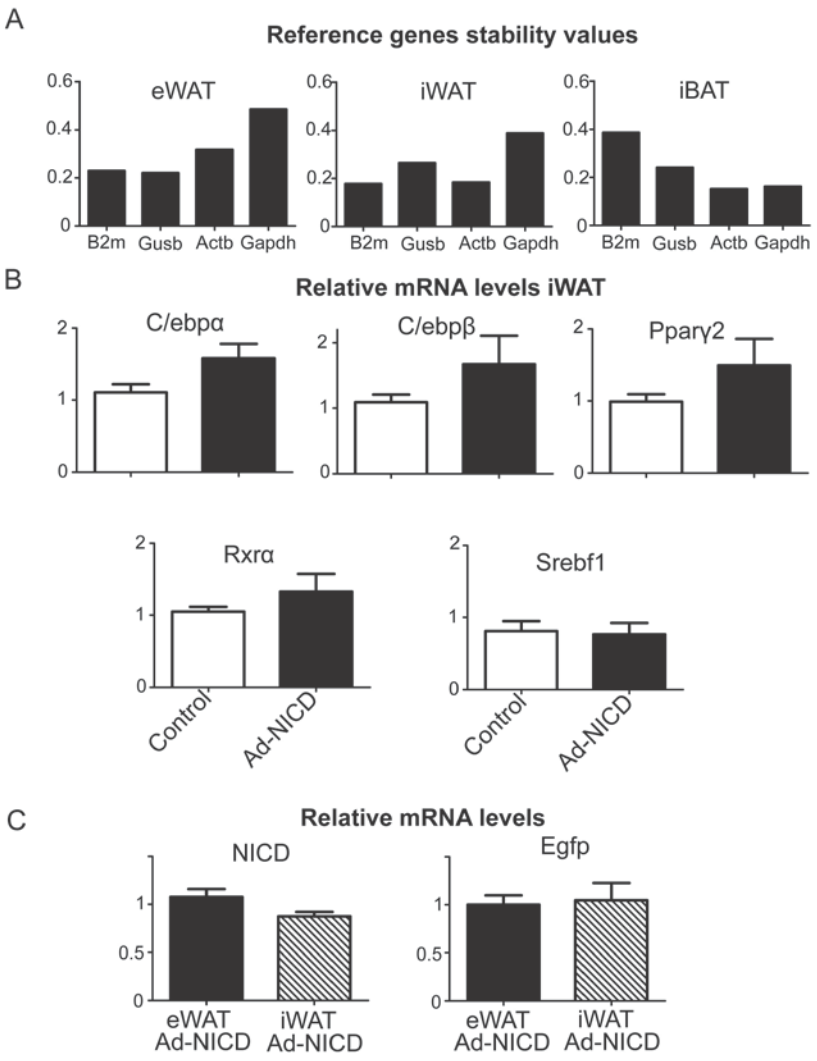


Figure S2.

A. Stability values of reference genes using the genorm algorithm. The geometric means of the two most "stable" genes (lower values) were selected to be used in the normalization of the results of the quantitative real-time PCR for each tissue. B2m; beta-2-microglobulin, Gusb; beta-glucoronidase, Actb; beta-actin, Gapdh; glyceraldehyde-3-phosphate dehydrogenase.

B. Relative mRNA levels of genes of interest in inguinal white adipose tissue (iWAT). n=8 for the control and n=9 for the Ad-NICD. Data are mean ± SE, *$p<0.05$.

C. Relative mRNA levels of NICD and Egfp in eWAT and iWAT of Ad-NICD mice. Egfp; enhanced green fluorescent protein. n=9 for each tissue. Data are mean ± SE.

Supplemental Experimental Procedures

Distribution of adipocyte size

To assess the size of the adipocytes in the white adipose tissue, photos from H&E sections of our samples were used and were analyzed in a computer with the NIS- Element AR software (Nikon instruments) that was kindly provided by the Center of Biological Imaging (CBI) in the University of Pittsburgh. A representative histogram of the adipocyte size distribution can be seen in Figure S1B.

Mouse models

The primer sequences used for genotyping the mouse models were based on our previous work [1] and are summarized below.

Primer	Sequence
IMR0883	AAAGTCGCTCTGAGTTGTTAT
IMR8038	TAAGCCTGCCCAGAAGACTC
IMR8039	GAAAGACCGCGAAGAGTTTG
PGK-3740	GATGTGGAATGTGTGCGAGGCCAGAGGC
NICD-5477	GATTGTCGTCCATCAGAGCACCATCTGAGG
Cre-1	ACGTTCACCGGCATCAACGT
Cre-2	CTGCATTACCGGTCGATGCA

A visual rough presentation of the localization of these primers on the wild-type or $Rosa^{NICD}$ allele can be found in Figure S1C.

In all PCR reaction 0.5µM of each primer was used. To genotype the experimental mice tail genomic DNA was used as a template. To detect the $Rosa^{NICD}$ allele the primers IMR0883, IMR8038 and IMR8039 were used in a single PCR reaction with the following cycling conditions;

Step #	Temperature (°C)	Time (sec)	Note
1	94	180	-
2	94	30	-
3	54	60	-
4	72	60	Go to step 2 35 times
5	72	120	
6	10	hold	

The PCR product from this reaction is expected to be 235bp in case of the wild-type allele and 320bp in case of the $Rosa^{NICD}$ allele.

To detect the presence or absence of the Cre transgene in our model mice the primers Cre1 and Cre2 were used in a single reaction using tail genomic DNA as template. The cycling conditions used were the following;

Step #	Temperature (°C)	Time (sec)	Note
1	94	60	-
2	94	30	-
3	60	30	-
4	72	30	Go to step 2 35 times
5	10	hold	

The expected product of this PCR in case of a Cre positive mouse is expected to be 355bp.

To confirm that the expected recombination by Cre recombinase has occurred in the target tissues in our mouse models, genomic DNA from eWAT, iWAT, iBAT and tail was used as a template. For detection of the "inactive allele" (absence of recombination) the the primer pair IMR0883 and the PGK-3740 was used. For detection of of the "active" allele (presence of recombination) the primer pair IMR0883 and NICD-5477 was used. The cycling conditions for both these PCR reactions were the following;

Step #	**Temperature (°C)**	**Time (sec)**	**Note**
1	95	60	-
2	95	30	-
3	66	30	-
4	72	20	Go to step 2 35 times
5	10	hold	

The expected PCR products from these reactions are 550bp for the "inactive allele" and 650bp for the "active allele" and can be seen in Figure S1D.

Quantitative real-time PCR

The table in the following page summarizes the primer sequences used in quantitative real-time PCR (qPCR). The source of the primers sequences was PrimerBank [2] with the following exceptions; *C/ebpβ* [3], *Pparγ2* [4], *Pgc-1α* and *Prdm16* [5], *Dio2* [6], *P2rx5* and *Pat2*[7]. *NICD* and *Egfp* primers were custom designed. Primers were used at a final concentration of 0.5μM in each PCR reaction with the following cycling conditions; 95°C for 10 sec, 61°C for 30 sec. To confirm specificity of the amplified products a melt curve was run for each PCR reaction.

To select the most suitable reference genes for the qPCR, the geNorm algorithm was used as described in the main text. The following on-line source was used for calculation of the stability value for each reference gene; http://www.leonxie.com/referencegene.php?type=reference. In figure S2A the stability values diagrams for the reference genes(*B2M, Gusb, ActB, GapdhI)* are shown for each tissue. The two most "stable" genes (the ones with the lower values) were selected and their geometric means was used for normalization of the qPCR data. Specifically, in the case of eWAT *B2M* and *Gusb*, in the case of iWAT *B2M and ActB* and in the case of iBAT *ActB* and *Gapdh*.

Gene	Primer 1 (forward)	Primer 2 (reverse)
Hes-1	ATAGCTCCCGGCATTCCAAG	GCGCGGTATTTCCCCAACA
Lep	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Acacb	CCTTTGGCAACAAGCAAGGTA	AGTCGTACACATAGGTGGTCC
C/ebpα	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
C/ebpβ	GGACTACGCAACACACGTGTAACT	ACAAAACCAAAAACATCAACAACC
*Pppar*γ*2*	CTCTGTTTTATGCTGTTATGGGTGA	GGTCAACAGGAGAATCTCCCAG
Rxrα	ATGGACACCAAACATTTCCTGC	CCAGTGGAGAGCCGATTCC
Srebf1	GATGTGCGAACTGGACACAG	CATAGGGGGCGTCAAACAG
Hsl	GATTTACGCACGATGACACAGT	ACCTGCAAAGACATTAGACAGC
Adipsin	CATGCTCGGCCCTACATGG	CACAGAGTCGTCATCCGTCAC
Pgc-1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Dio2	CGCCCCAGTGTCAAGTTGT	CCCGTAAGCTACGTTGGCATT
Ucp-1	CACTCAGGATTGGCCTCTACG	GGGGTTTGATCCCATGCAGA
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
B2m	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC
Gusb	GTGGTATGAACGGGAAGCAAT	AACTGCATAATAATGGGCACTGT
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Emr1	TGACTCACCTTGTGGTCCTAA	CTTCCCAGAATCCAGTCTTTCC
Cd68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
Csf1r	TGTCATCGAGCCTAGTGGC	CGGGAGATTCAGGGTCCAAG

P2rx5	CTGCAGCTCACCATCCTGT	CACTCTGCAGGGAAGTGTCA
Pat2	GTGCCAAGAAGCTGCAGAG	TGTTGCCTTTGACCAGATGA
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
NICD	TGAGACAGGCAACAGTGAAG	CAGCATCTGAACGAGAGTATCG
Egfp	ATCATGGCCGACAAGCAGAA	TCTCGTTGGGGTCTTTGCTC

Supplemental References

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