

# *Tbc1d1* mutation in lean mouse strain confers leanness and protects from diet-induced obesity

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**We previously identified *Nob1* as a quantitative trait locus for high-fat diet-induced obesity and diabetes in genome-wide scans of outcross populations of obese and lean mouse strains. Additional crossbreeding experiments indicated that *Nob1* represents an obesity suppressor from the lean Swiss Jim Lambert (SJL) strain. Here we identify a SJL-specific mutation in the *Tbc1d1* gene that results in a truncated protein lacking the TBC Rab-GTPase-activating protein domain. TBC1D1, which has been recently linked to human obesity, is related to the insulin signaling protein AS160 and is predominantly expressed in skeletal muscle. Knockdown of TBC1D1 in skeletal muscle cells increased fatty acid uptake and oxidation, whereas overexpression of TBC1D1 had the opposite effect. Recombinant congenic mice lacking TBC1D1 showed reduced body weight, decreased respiratory quotient, increased fatty acid oxidation and reduced glucose uptake in isolated skeletal muscle. Our data strongly suggest that mutation of *Tbc1d1* suppresses high-fat diet-induced obesity by increasing lipid use in skeletal muscle.**

Dietary fat intake is considered a major factor in the development of obesity and insulin resistance. In mice, sensitivity to dietary fat is strain specific and consequently has a genetic basis<sup>1–3</sup>. To identify dietary fat-responsive genetic loci in mice, we carried out crossbreeding experiments using the New Zealand obese (NZO) and the lean SJL mouse strains. The NZO strain presents features of the metabolic syndrome, with early-onset polygenic obesity, dyslipidemia, hypertension and type 2-like diabetes mellitus<sup>4–8</sup>. Moreover, NZO mice are highly susceptible to weight gain when fed a high-fat diet, resulting in the development of morbid obesity, with fat depots exceeding 40% of total body weight<sup>7,9,10</sup>. In contrast, the lean SJL strain is resistant to high-fat diet-induced obesity and diabetes<sup>1,9</sup> (Fig. 1a).

The propensity of NZO mice for high-fat diet-induced body weight gain was retained in the NZO × F<sub>1</sub> (SJL × NZO) backcross popula-

tion (Fig. 1a). Although backcross and parental NZO mice had similar mean body weights on a low-fat diet, the high-fat diet-induced weight gain of the backcross population was lower (Fig. 1a). In a subsequent genome-wide scan of the backcross population, we identified *Nob1*, a major quantitative trait locus (QTL) on chromosome 5 for high-fat diet-induced obesity (body weight, body mass index and total body fat; logarithm (base 10) of odds (LOD) score 7.9; Fig. 1b)<sup>9,11,12</sup>. In addition to its interaction with dietary fat<sup>9</sup>, *Nob1* showed strong epistatic interaction with *Nidd/SJL*, a QTL for pancreatic islet cell destruction<sup>12</sup>. Carriers of the NZO allele of *Nob1* (*Nob1*<sup>NZO</sup>) showed a pronounced acceleration of diabetes development when fed a high-fat diet<sup>9,13</sup>.

In the NZO × SJL backcross population, heterozygous carriers of the SJL allele of *Nob1* (*Nob1*<sup>SJL</sup>) and the corresponding homozygous carriers of the *Nob1*<sup>NZO</sup> allele were not different in body weight when fed a low-fat diet (Fig. 1c). In contrast, *Nob1*<sup>NZO/NZO</sup> mice showed a markedly increased body weight on a high-fat diet compared with heterozygous *Nob1*<sup>NZO/SJL</sup> mice, indicating that the diet-induced weight gain was either increased by the *Nob1*<sup>NZO</sup> allele or decreased by the *Nob1*<sup>SJL</sup> allele. To assess the net effect of the *Nob1*<sup>NZO</sup> allele, we conducted additional crossbreeding experiments with NZO, the closely related nonobese New Zealand black (NZB) strain and C57BL/6J laboratory mice. In the corresponding NZO × C57BL/6J backcross and NZO × NZB intercross, there was no correlation between *Nob1* genotype and high-fat diet-induced body weight gain, and no linkage of diet-induced body weight to chromosome 5, suggesting that *Nob1* represents an obesity suppressor gene from the lean SJL strain (Fig. 1c and Supplementary Fig. 1 online). To validate the obesity-suppressive effect of *Nob1*<sup>SJL</sup> on a different genetic background and to narrow down the crucial region of the *Nob1* QTL, we generated a recombinant congenic mouse strain (B6.SJL-*Nob1*.24) harboring a 24-Mb fragment (53.6–77.5 Mb) of the *Nob1* region from SJL on a C57BL/6J background (Fig. 1b). Introgression of the *Nob1*.24 segment from SJL into a mixed NZO × C57BL/6J background replicated the lean phenotype of SJL mice (Fig. 1d). On a

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