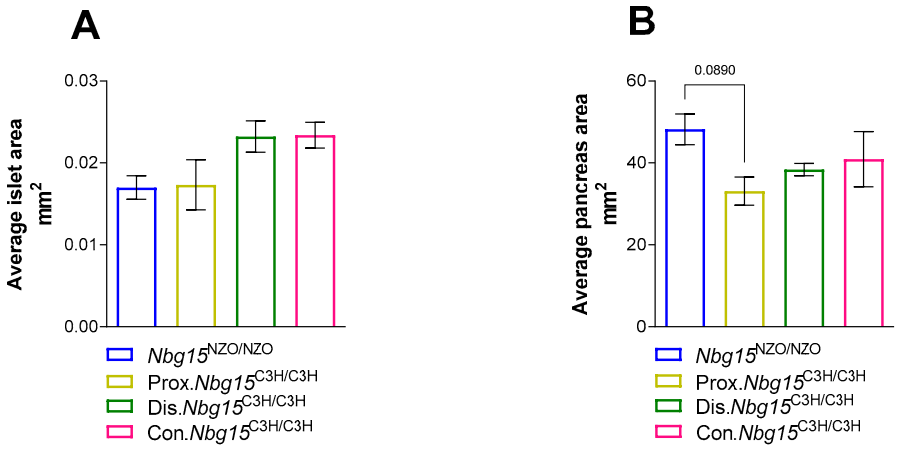
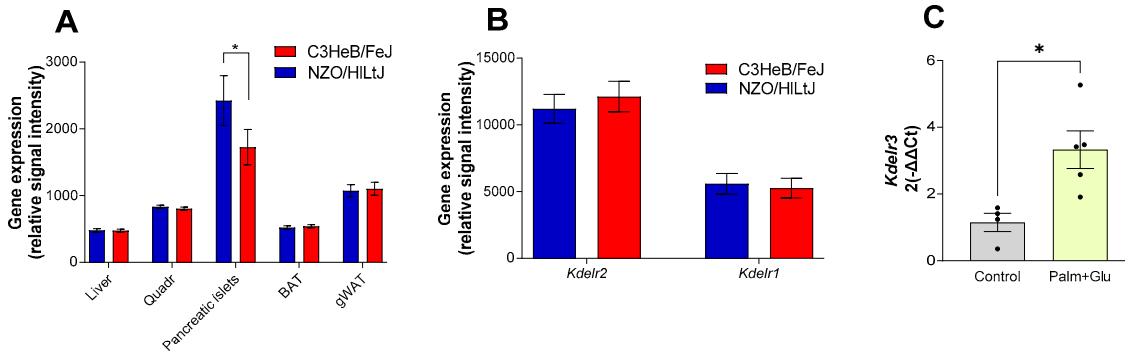
International Journal of
*Molecular Sciences*

MDPI

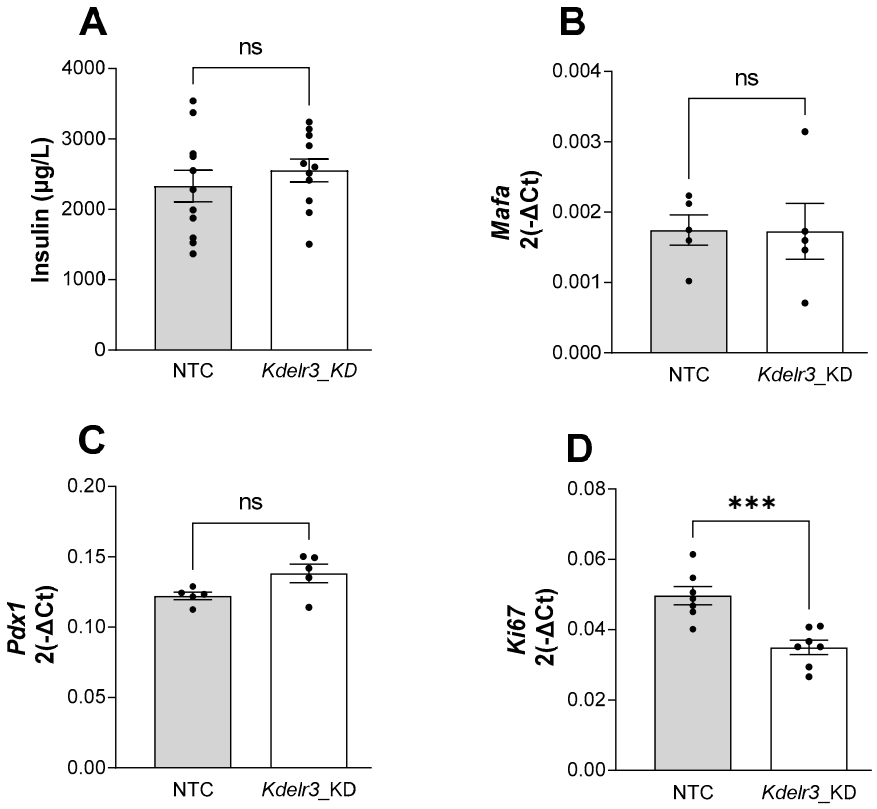
**Supplemental Material**




**Supplemental Figure S1.** Pancreata histological analysis of congenic strains for the *Nbg15* locus. Average islet area (A) and average pancreas area (B). Data are presented as mean ± SEM and were analyzed by one ANOVA followed by Bonferroni post-hoc-test, *p < 0.05; **p < 0.01; ****p < 0.0001 n=3-7.




**Supplemental Figure S2.** Microarray gene expression profiling of *Kdelr3* in liver, muscle (quadriceps), pancreatic islets, brown adipose tissue (BAT) and gonadal adipose tissue (gWAT) in parental C3H and NZO mice (A). Expression levels of *Kdelr2* and *Kdelr1* in pancreatic islets of parental C3H and NZO mice (B). Quantitative real-time PCR of *Kdelr3* in NZO pancreatic islets treated with 500 μM palmitate and 25 mM glucose for 24h (Palm+Glu) (C). Data are presented as mean ± SEM and were analyzed by Students *t* test, *p < 0.05; **p < 0.01; ****p < 0.0001 n=5-8 (A, B), n=4-5 (C).




**Supplemental Figure S3.** Total insulin content in *Kdelr3*-KD MIN6 cells versus control (A), quantitative real-time PCR of *Mafa* (B), *Pdx1* (C) and *Ki67* (D) in *Kdelr3* knockdown MIN6 cells. *Kdelr3* knockdown cells were generated using a pool of siRNA. Data are presented as mean ± SEM and were analyzed by Students *t* test, *p < 0.05; **p < 0.01; ****p < 0.0001 n=5-10 independent experiments.

**Supplemental Table S1: List of phenotyping and statistical testing of blood glucose development of *Nbg15* recombinant mouse strains.**

The table indicates the mean, standard deviation and number of animals analysed. Statistical comparison used two-way ANOVA followed by Bonferroni post-hoc-test. The results reported by 95% confidence interval and adjusted P value refer to the comparison of the respective recombinant group (Prox.*Nbg15*$^{C3H/C3H}$, Dis.*Nbg15*$^{C3H/C3H}$, Con.*Nbg15*$^{C3H/C3H}$) with the control group (*Nbg15*$^{NZO/NZO}$).

| Blood glucose development | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Nbg15*$^{NZO/NZO}$ | | | Prox.*Nbg15*$^{C3H/C3H}$ | | | | | Dis.*Nbg15*$^{C3H/C3H}$ | | | | | Con.*Nbg15*$^{C3H/C3H}$ | | | | |
| **Age (weeks)** | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** | **95% CI of diff.** | **Adjusted P Value** | **Mean** | **SD** | **N** | **95% CI of diff.** | Adjusted P Value | Mean | SD | N | 95% CI of diff. | Adjusted P Value |
| 3 | 156.1 | 32.0 | 44 | 151.9 | 46.8 | 35 | -65.21 to 56.65 | >0.9999 | 172.8 | 27.9 | 35 | -44.27 to 77.59 | >0.9999 | 156.1 | 23.8 | 34 | -61.45 to 61.41 | >0.9999 |
| 4 | 216.0 | 28.0 | 31 | 214.2 | 33.5 | 20 | -80.73 to 64.62 | >0.9999 | 232.7 | 30.0 | 15 | -63.89 to 92.31 | >0.9999 | 225.1 | 27.1 | 22 | -72.13 to 70.29 | >0.9999 |
| 5 | 220.3 | 32.2 | 31 | 205.1 | 23.9 | 20 | -94.13 to 51.23 | >0.9999 | 244.9 | 32.5 | 15 | -55.98 to 100.2 | >0.9999 | 209.3 | 43.9 | 22 | -92.24 to 50.18 | >0.9999 |
| 6 | 238.4 | 73.7 | 38 | 205.6 | 40.7 | 25 | -101.2 to 33.15 | 0.6751 | 202.0 | 60.8 | 24 | -105.5 to 30.06 | 0.547 | 204.3 | 37.5 | 26 | -98.60 to 34.73 | 0.753 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 252.0 | 91.4 | 31 | 221.4 | 81.1 | 20 | -109.5 to 35.84 | 0.6736 | 248.1 | 36.0 | 15 | -84.52 to 71.68 | >0.9999 | 222.4 | 58.9 | 22 | -110.9 to 31.53 | 0.5455 |
| 8 | 277.6 | 114.8 | 31 | 255.4 | 110.0 | 20 | -101.1 to 44.29 | >0.9999 | 289.9 | 95.6 | 15 | -68.27 to 87.93 | >0.9999 | 240.0 | 77.1 | 22 | -118.9 to 23.52 | 0.3261 |
| 9 | 345.5 | 146.6 | 31 | 300.1 | 165.9 | 20 | -124.2 to 21.12 | 0.2678 | 296.8 | 78.8 | 15 | -129.3 to 26.93 | 0.3496 | 241.9 | 104.2 | 22 | -184.8 to -42.39 | 0.0004 |
| 10 | 368.0 | 139.1 | 44 | 319.5 | 139.8 | 35 | -109.4 to 12.42 | 0.1697 | 346.5 | 111.4 | 35 | -82.41 to 39.45 | >0.9999 | 255.4 | 112.8 | 34 | -174.1 to -51.21 | <0.0001 |
| 11 | 398.1 | 162.7 | 31 | 316.4 | 177.2 | 20 | -160.7 to -15.31 | 0.0113 | 328.9 | 116.4 | 15 | -149.8 to 6.385 | 0.0837 | 263.2 | 117.7 | 22 | -216.2 to -73.80 | <0.0001 |
| 12 | 417.8 | 170.9 | 31 | 312.7 | 161.4 | 20 | -184.1 to -38.72 | 0.0007 | 331.5 | 121.2 | 15 | -166.9 to -10.72 | 0.0195 | 264.2 | 117.2 | 22 | -234.9 to -92.46 | <0.0001 |
| 13 | 401.9 | 178.9 | 30 | 338.7 | 200.8 | 20 | -144.7 to 1.310 | 0.0562 | 342.2 | 145.0 | 15 | -142.9 to 13.95 | 0.1469 | 242.4 | 105.7 | 22 | -243.3 to -100.2 | <0.0001 |
| 14 | 393.7 | 184.0 | 29 | 338.3 | 187.6 | 20 | -140.9 to 5.754 | 0.082 | 256.9 | 141.6 | 15 | -224.0 to -66.55 | <0.0001 | 235.9 | 123.8 | 22 | -245.8 to -102.0 | <0.0001 |
| 15 | 361.8 | 187.0 | 32 | 307.9 | 201.3 | 24 | -135.5 to 3.138 | 0.0668 | 268.7 | 109.4 | 22 | -171.3 to -29.94 | 0.002 | 237.8 | 122.1 | 26 | -204.9 to -68.27 | <0.0001 |

**Supplemental Table S2.** List of phenotyping and statistical testing of body weight development of *Nbg15* recombinant mouse strains.

The table indicates the mean, standard deviation and number of animals analysed. Statistical comparison used two-way ANOVA followed by Bonferroni post-hoc-test. The results reported by 95% confidence interval and adjusted P value refer to the comparison of the respective recombinant group (Prox.*Nbg15*$^{C3H/C3H}$, Dis.*Nbg15*$^{C3H/C3H}$, Con.*Nbg15*$^{C3H/C3H}$) with the control group (*Nbg15*$^{NZO/NZO}$).

| Body Weight development | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Nbg15*$^{NZO/NZO}$ | | | Prox.*Nbg15*$^{C3H/C3H}$ | | | | | Dis.*Nbg15*$^{C3H/C3H}$ | | | | | Con.*Nbg15*$^{C3H/C3H}$ | | | | |
| Age (weeks) | Mean | SD | N | Mean | SD | N | 95% CI of diff. | Adjusted P Value | Mean | SD | N | 95% CI of diff. | Adjusted P Value | Mean | SD | N | 95% CI of diff. | Adjusted P Value |
| 3 | 13.2 | 0.4 | 44 | 15.1 | 0.6 | 35 | -0.3117 to 4.062 | 0.1201 | 14.7 | 0.6 | 35 | -0.6889 to 3.685 | 0.3024 | 14.0 | 0.3 | 34 | -1.380 to 3.029 | >0.9999 |
| 4 | 22.3 | 0.6 | 31 | 21.3 | 1.0 | 20 | -3.160 to 2.050 | >0.9999 | 20.1 | 0.9 | 15 | -5.213 to 0.3834 | 0.1163 | 20.8 | 0.6 | 22 | -3.993 to 1.114 | 0.5304 |
| 5 | 31.4 | 0.6 | 31 | 29.4 | 0.9 | 20 | -4.214 to 0.9965 | 0.4171 | 30.3 | 0.8 | 15 | -4.152 to 1.445 | 0.7393 | 30.4 | 0.5 | 22 | -3.586 to 1.521 | 0.9974 |
| 6 | 38.0 | 0.5 | 38 | 36.2 | 0.5 | 25 | -3.738 to 1.079 | 0.5579 | 37.8 | 0.8 | 24 | -2.589 to 2.275 | >0.9999 | 36.8 | 0.5 | 26 | -3.514 to 1.269 | 0.7821 |
| 7 | 42.8 | 0.5 | 31 | 41.0 | 0.5 | 20 | -4.062 to 1.148 | 0.5408 | 43.3 | 0.6 | 15 | -2.575 to 3.021 | >0.9999 | 42.2 | 0.7 | 22 | -3.139 to 1.967 | >0.9999 |
| 8 | 47.4 | 0.4 | 31 | 44.9 | 0.5 | 20 | -4.629 to 0.5813 | 0.1884 | 49.1 | 0.4 | 15 | -1.297 to 4.300 | 0.5961 | 45.9 | 0.7 | 22 | -4.022 to 1.085 | 0.5045 |
| 9 | 49.9 | 0.4 | 31 | 47.8 | 0.3 | 20 | -4.250 to 0.9602 | 0.391 | 51.8 | 0.5 | 15 | -1.120 to 4.477 | 0.4523 | 49.3 | 0.8 | 22 | -3.124 to 1.982 | >0.9999 |
| 10 | 52.5 | 0.5 | 44 | 50.2 | 0.4 | 35 | -4.456 to -0.08225 | 0.039 | 54.7 | 0.7 | 35 | 0.02069 to 4.395 | 0.047 | 53.0 | 0.7 | 34 | -1.651 to 2.759 | >0.9999 |
| 11 | 53.4 | 0.7 | 31 | 50.0 | 0.6 | 20 | -5.594 to -0.3835 | 0.0181 | 58.0 | 0.6 | 15 | 1.528 to 7.125 | 0.0007 | 54.4 | 1.1 | 22 | -1.527 to 3.580 | >0.9999 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 54.4 | 0.8 | 31 | 51.3 | 0.8 | 20 | -5.374 to -0.1632 | 0.0329 | 60.2 | 0.7 | 15 | 2.725 to 8.322 | <0.0001 | 57.5 | 1.2 | 22 | 0.5909 to 5.697 | 0.0096 |
| 13 | 54.6 | 1.0 | 30 | 51.5 | 1.1 | 20 | -5.408 to -0.1739 | 0.0321 | 60.9 | 1.1 | 15 | 3.159 to 8.777 | <0.0001 | 58.3 | 1.2 | 22 | 1.031 to 6.160 | 0.0024 |
| 14 | 53.6 | 1.3 | 29 | 52.6 | 1.3 | 20 | -3.279 to 1.979 | >0.9999 | 60.3 | 1.1 | 15 | 3.625 to 9.266 | <0.0001 | 60.0 | 1.2 | 22 | 3.830 to 8.985 | <0.0001 |
| 15 | 54.6 | 1.2 | 32 | 53.0 | 1.3 | 24 | -3.525 to 1.445 | 0.948 | 62.5 | 1.5 | 22 | 5.588 to 10.66 | <0.0001 | 61.3 | 1.3 | 26 | 4.326 to 9.226 | <0.0001 |

**Supplemental Table S3.** List of *Nbg15* scored genes according to variant effect predictor analysis.

Genes carrying potential disruptive mutations were subjectively scored by the variant effect predictor analysis (VEP_Ensembl) as "high" and "moderate". Highly impacting genes were scored by the presence of stop lost/gain predicted mutations. Furthermore, polymorphisms scored with moderate impact were further ranked using the Sorting Intolerant From Tolerant tool (SIFT- Open source tool) that predicts the impact of amino acid substitution according to sequence homology of the given amino acid exchange. Highly disruptive mutations were scored when SIFT <0.05. Considering a percentage of false positive/negative errors, all genes ranked by SIFT within the range of 0.05-0.5 were further considered for analysis. *gene not covered by microarray analysis in the analysed tissue. Sequence reference: **REL-1303 - GRCm38. SNP with low coverage (©).**

| Chr.15 position (Mbp) | Gene symbol | dbSNP | C3H.HeJ | NZO.HlLtJ | Protein position | Amino acids | Codons | IMPACT | SIFT |
|---|---|---|---|---|---|---|---|---|---|
| 71.806 | *Col22a1** | rs46351738 | C | T | 1461 | K/E | Aag/Gag | Moderate | 0.00 |
| 63.825 | *Gsdmc2* | rs252605414 | C | G | 407 | L/V | Ctc/Gtc | Moderate | 0.01 |
| 78.349 | *Csf2rb* | rs4230845 | C | A | 742 | S/R | Agt/Cgt | Moderate | 0.01 |
| 74.540 | *Adgrb1/Bai1* | rs51566550 | A | G | 379 | R/H | cGc/cAc | Moderate | 0.01 |
| 76.705 | *Recql4* | rs215408973 | G | A | 967 | A/V | gCt/gTt | Moderate | 0.02 |
| 77.718 | *Apol7e** | rs107983511 | C | - | 220 | M/V | Atg/Gtg | Moderate | 0.02 |
| 77.051 | *Apol6* | rs261533897 | G | C | 122 | E/D | gaG/gaC | Moderate | 0.04 |
| 79.919 | *Cbx7* | rs36397331 | T | C | 153 | R/H | cGc/cAc | Moderate | 0.05 |
| 52.040 | *Aard* | rs31595794 | T | A | 103 | T/S | Acg/Tcg | Moderate | 0.07 |
| 76.421 | *Mroh1* | rs214800774 | G | C | 404 | D/H | Gat/Cat | Moderate | 0.08 |
| 78.908 | *Sh3bp1* | rs13463449 | T | C | 515 | P/L | cCg/cTg | Moderate | 0.08 |
| 79.525 | *Kdelr3* | rs256912165 | A | T | 96 | E/V | gAg/gTg | Moderate | 0.08 |
| 77.389 | *Apol7a* | rs229171902 | G | A | 272 | T/I | aCc/aTc | Moderate | 0.10 |
| 78.961 | *Triobp* | rs31569891 | A | G | 168 | T/A | Act/Gct | Moderate | 0.10 |
| 78.938 | *Nol12* | rs13466090 | T | C | 92 | A/V | gCg/gTg | Moderate | 0.11 |
| 71.464 | *Fam135b* | rs49135881 | C | T | 485 | V/I | Gtc/Atc | Moderate | 0.17 |
| 75.896 | *Eef1d* | rs227346636 | C | A | 155 | K/N | aaG/aaT | Moderate | 0.18 |
| 57.936 | *Tbc1d31* | rs265189026 | G | C | 247 | G/R | Ggg/Cgg | Moderate | 0.22 |
| 79.828 | *Cbx6* | rs227221465 | C | G | 309 | Q/H | caG/caC | Moderate | 0.22 |
| 76.888 | *Zfp7* | rs31575067 | T | G | 72 | E/D | gaG/gaT | Moderate | 0.24 |
| 79.357 | *Maff* | rs252068181 | T | A | 25 | S/T | Tcg/Acg | Moderate | 0.25 |
| 76.596 | *Cpsf1** | rs248187700 | G | A | 1439 | L/F | Ctc/Ttc | Moderate | 0.26 |
| 57.251 | *Slc22a22* | rs31794878 | A | T | 344 | L/M | Ttg/Atg | Moderate | 0.27 |
| 79.892 | *Apobec3* | rs108537160 | A | C | 6 | L/M | Ctg/Atg | Moderate | 0.27 |
| 77.481 | *Apol10a** | rs31475931 | © | C | 7 | L/S | tTa/tCa | Moderate | 0.34 |
| 66.542 | *Tmem71* | rs256104481 | A | T | 214 | D/V | gAt/gTt | Moderate | 0.35 |
| 58.874 | *Trmt12* | rs36461259 | A | G | 342 | S/N | aGc/aAc | Moderate | 0.36 |
| 76.870 | *Zfp251* | rs47788016 | A | G | 78 | L/F | Ctt/Ttt | Moderate | 0.38 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 79.738 | *Sun2* | rs232461033 | T | C | 165 | R/G | Agg/Ggg | Moderate | 0.38 |
| 75.712 | *Rhpn1* | rs229443499 | G | A | 386 | V/I | Gtc/Atc | Moderate | 0.46 |
| 76.329 | *Exosc4* | rs13465162 | T | C | 69 | */R | Tga/Cga | High | – |
| 79.637 | *Fam227a* | rs37552463 | A | G | 1 | M/T | aTg/aCg | High | – |
| 79.900 | *Apobec3* | rs46309510 | A | G | 11 | W/* | tgG/tgA | High | – |
| 79.934 | *Cbx7* | rs47269926 | T | A | 24 | L/* | tTg/tAg | High | – |

**Supplemental Table S4.** Chemicals and buffers.

| Compound | Supplier |
|---|---|
| **Anticoagulant coktail:** | |
| **EDTA 0.5 M** | Sigma Aldrich, Steinheim, Germany |
| **Aprotinin 0.15 M** | Sigma Aldrich, Steinheim, Germany |
| **Heparin 10.000U/Ml** | Biochrom, Berlin, Germany |
| **Diprotin A** | Bachem, Bubendorf, Switzerland |
| Trizol | Qiagen, Crawley, U.K. |
| Chloroform (pure) | AppliChem, Darmstadt, Germany |
| Hexanucleotide primer | Roche, Mannheim, Germany |
| Insulin Actrapid® HM Penfill® | Novo Nordisk Pharma GmbH, Mainz, Germany |
| Isopropanol (≥ 99. 5 %) | AppliChem, Darmstadt, Germany |
| QIAzol® reagent | QIAGEN, Hilden, Germany |
| Sterile glucose 20 % | Braun (Melsungen, Germany) |
| Microvette CB 300 µL Lithium-Heparin | Sarstedt, Nümbrecht, Germany |
| PCR-Plates (FrameStar® 384-Well) | 4titude, Berlin, Germany |
| Rotilabo microtest plates (96 well) | Carl Roth, Karlsruhe, Germany |
| Krebs-Ringer HEPES | 15 mM HEPES, 5 mM KCl, 120 mM NaCl, 24 mM NaHCO3, 1 mM MgCl2, 2 mM CaCl2, and 1 mg/ml BSA |
| Lysis buffer | 20 mmol/L Tris-HCl, 150 mmol/L sodium chloride, 1 mmol/L EGTA, 1 mmol/L EDTA, 1%(v/v) Triton-X-100 and a proteinase inhibitor and a phosphatase inhibitor cocktail |

**Supplemental Table S5.** Genotyping primers for the recombinant congenic strains.

Mouse DNA was isolated from tail tips using the Invisorb® Genomic DNA Kit II (STRATEC Molecular GmbH, Berlin, Germany) according to manufacturer's instructions. For genotyping the PCR-based KASP (Kompetitive Allele-Specific PCR) method from LGC genomics (LGC group, Teddington, United Kingdom).

| Primer | SNP ID | Position (Mbp) | C3HeB/FeJ | NZO/HlLtJ |
|---|---|---|---|---|
| 15-005994001-M | rs3715343 | 6.12 | C | A |
| 15-033125499-M | rs3720676 | 32.96 | G | A |
| 15-041919640-N | rs3090057 | 41.56 | C | T |
| 15-049283927-M | rs3719583 | 48.89 | A | C |
| 15-059192855-M | rs3653368 | 58.6 | G | A |
| 15-063976267-N | rs3023419 | 63.34 | T | A |
| 15-071973707-N | rs3088506 | 71.35 | G | T |
| 15-074947367-M | rs3724474 | 74.31 | T | A |
| 15-080641980-N | rs4230879 | 79.74 | A | G |
| 15-087100507-M | rs3690689 | 86.13 | G | A |
| 15-093195380-M | rs3707104 | 92.14 | T | C |
| 15-098621793-N | rs3023427 | 97.54 | T | C |
| 15-102788257-N | rs3023429 | 101.64 | T | G |

**Supplemental Table S6.** Sequence information of *Kdelr3* siRNA pool of oligonucleotides.

| Gene ID | Target sequence | Supplier |
|---|---|---|
| *Kdelr3* | 1 GUAGUGUCUGGAGUUGUUC<br>2 CUGAGGUGCUAAAUAGAAU<br>3 GCACCAUCUUGGGAUAAAC<br>4 CUAAGAACCACCAAUGUAG | Dharmacon (Lafayette, CO, USA), #E-052192-00-0005, Accell Mouse Kdelr3 (105785) siRNA SMART pool |

**Supplemental Table S7.** List of SYBR green primers for quantitative real-time PCR.

Primers were designed using primer designing tool from NCBI and synthesized by Eurogentec (Seraing, Belgium) and diluted at a final concentration of 10 nM. F: forward; R: reverse.

| Gene symbol | Primer ID | Primer sequence (5 →3′) |
|---|---|---|
| *Kdelr3* | Kdelr3_F | GGCGGGCGGGACCAT |
| | Kdelr3_R | GAAAACCACCTTCATCACTGTGT |
| *Kdelr1* | Kdelr1_F | TTGACCTCATCGCCATCGTT |
| | Kdelr1_R | TTCCCCTTCAGGACTTTGGTG |
| *Kdelr2* | Kdelr2_F | TGGTGTCGTCCAGACCATTC |
| | Kdelr2_R | GCTTCTTCCCTTTGAGTACTTTTG |
| *Ins2* | Ins2-F | CCCTGCTGGCCCTGCTCTT |
| | Ins2-R | AGGTCTGAAGGTCACCTGCT |
| *Cbx6* | Cbx6 _F | GCCGAATCCATCATTAAACG |
| | Cbx6 _R | TTGGGTTTAGGTCCCCTCTT |
| *Fam135b* | Fam135b_F | TAGAGACCTGAGACAGCTCG |
| | Fam135b_R | TGATCTTTTTGGTTCATTACCTGTC |
| *Znt8* | Znt8_F | TGCCAAGTGGAGACTCTGTG |
| | Znt8_R | CTGCTCGATACCACCCAAAT |
| *Pcsk1* | Pcsk1_F | TCTGGTTGTCTGGACCTCTGAGT |
| | Pcsk1_R | CATCAAGCCTGCCCCATTCTTT |
| *Pcsk2* | Pcsk2_F | TCTGACTGTGCTCACCTCCAA |
| | Pcsk2_R | AGGACTCCGTAGCCAAAGAGG |
| *Chop* | Chop_F | CCACCACACCTGAAAGCAGAA |
| | Chop_R | AGGTGAAAGGCAGGGACTCA |
| *Atf3* | Atf3_F | CTCTGCGCTGGAGTCAGTT |
| | Atf3_R | GCCTCCTTTTCCTCTCAT |