**Supplemental data**

**Failure to repair endogenous DNA damage in β-cells causes adult-onset diabetes in mice**

Running Title: DNA damage in β-cells causes diabetes

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**Supplemental Figure 1. Schematic diagram of gene targeting strategy.**

The *Ercc1* WT allele is indicated above with numerals over the vertical line noting the 10 exons of *Ercc1*. The null allele of *Ercc1* (*Ercc1-*) was generated by disrupting exon 7 via insertion of a neomycin resistance (neor) cassette shown in green [58]. For the floxed allele (*Ercc1fl*, USA), exons 7-10 were replaced by a cDNA cassette (grey rectangle) containing a fusion of exons 7-10 and a neor cassette, flanked by *LoxP* sites. Expression of Cre drives deletion of the 3’ end of the transcript, which destabilizes the mRNA and prevents ERCC1 interaction with XPF, destabilizing the holoenzyme. The floxed allele of *Ercc1* used in NL has *LoxP* sites in intron 2 and 5 [59]. Expression of Cre deletes exons 3-5.

**Supplementary Figure 2. *Ins2-cre Ercc1fl/-* mice show elevated blood glucose levels.**

(A) Blood glucose levels of fasted male mice by age (n=5-19; NL).

(B) Oral glucose tolerance test on 4-5-month-old female *Ins2-cre Ercc1fl/-* mice and age-matched controls (n=6-12; NL).

(C) Blood glucose levels in 3-4-month-old non-fasted male *Ins2-cre Ercc1fl/-* mice and littermate controls (n=23-49; USA).

(D) Oral glucose tolerance test on male (left panel, 10 – 20-week-old, n=8-11) and female (right panel, 20 – 30-week-old, n=6-9) *Ins1-cre Ercc1fl/-* mice and age-matched controls (USA).

Data represent mean ± SD, unpaired Student’s *t*-test or repeated measurement two-way ANOVA. p<0.05 \*, p<0.01 \*\*, p<0.001 \*\*\*, p<0.0001 \*\*\*\*.

**Supplementary Figure 3. Body weight in *Ins2-cre Ercc1fl/-*** **mice and littermate controls at different ages.** (blue = USA, n=7-31, both sexes; red = NL; n=6-27, male mice).

Data represent mean ± SD, repeated measurement two-way ANOVA. p<0.05 \*, p<0.01 \*\*, p<0.001 \*\*\*, p<0.0001 \*\*\*\*.

**Supplementary Figure 4. Glucose homeostasis is similar between all genetic control groups.**

(A) Blood glucose levels of non-fasted and (B) fasted 3-month-old male *Ins2-cre Ercc1fl/-* mice and different control groups (n=6-12; NL).

(C) Blood glucose levels during the oGTT and (D) plasma insulin levels before and 15 min after the oral glucose bolus in 3-month-old male *Ins2-cre Ercc1fl/-* mice and genetic controls (n=6-8; NL)

(E) Random blood glucose levels and (F) blood glucose levels during the oGTT in 10-20-week-old *Ins2-cre Ercc1fl/-* mice and age-matched controls (both sexes) (n=4-20; USA)

Data represent mean ± SD, one-way ANOVA or repeated measurement two-way ANOVA. p<0.01 \*\*, p<0.001 \*\*\*, p<0.0001 \*\*\*\*.

**Supplementary Figure 5. Expression of *Ercc1* and measures of genotoxic stress are not altered in the hypothalamus of *Ins2-cre Ercc1fl/-* mice.** Expression of *Ercc1*, *p21*, and *Gadd45a* was measured by qPCR on RNA isolated from hypothalamic tissues from 3-4-month-old mutant and control male mice (n=6; NL).

Data represent mean ± SD, unpaired Student’s *t*-test.

**Supplemental Material**

**Supplementary Figure 1**

**Afbeelding met diagram, tekst, lijn

Automatisch gegenereerde beschrijving**

**Supplementary Figure 2**

**Afbeelding met tekst, diagram, lijn

Automatisch gegenereerde beschrijving**

**Supplementary Figure 3**

**Afbeelding met tekst, diagram, lijn, Perceel

Automatisch gegenereerde beschrijving**

**Supplementary Figure 4**

**Afbeelding met tekst, diagram, Plan, Technische tekening

Automatisch gegenereerde beschrijving**

**Supplementary Figure 5**

Afbeelding met tekst, diagram, schermopname, Lettertype

Automatisch gegenereerde beschrijving

**Supplementary Table 1**

|  |  |
| --- | --- |
| **Primer** | **Sequence** |
| Ercc1 (USA) | Fwd 5’-AAAAGCTGGAGCAGAACT-3’ |
|  | Rev 5’-AAGAGCTGTTCCAGGGAT-3’ |
| Ercc1 (NL) | Fwd 5’-ATTCGGTGAGGTGATTCCCG-3’ |
|  | Rev 5’-GTAGCGGAGGCTGAGGAAAA-3’ |
| Cdkn1a (p21Cip1) (USA) | Fwd 5’-GTCAGGCTGGTCTGCCTCCG-3’ |
|  | Rev 5’-CGGTCCCGTGGACAGTGAGCAG-3’ |
| Cdkn1a (p21Cip1) (NL) | Fwd 5’-TTGTCGCTGTCTTGCACTCTG-3’ |
|  | Rev 5’-GCTTGGAGTGATAGAAATCTGTCAG-3’ |
|  | Probe 5’-CTGCCTCCGTTTTCGGCCCTG-3’ |
| Gadd45a | Fwd 5’-TGGTGACGAACCCACATTCA-3’ |
|  | Rev 5’-CTGCTTCACCACCTTCTTGA-3’ |
| Ins1 | Fwd 5’-CCATCAGCAAGCAGGTCATT-3’ |
|  | Rev 5’-GGCTCCCAGAGGGCAAG-3’ |
|  | Probe 5’-CCCTGTTGGTGCACTTCCTACCCC-3’ |
| Ins2 | Fwd 5’-GGAGGACCCACAAGTGGCA-3’ |
|  | Rev 5’-CAAGGTCTGAAGGTCACCTGCT-3’ |
|  | Probe 5’-CCGGGCCTCCACCCAGCTCC-3’ |
| Gck | Fwd 5’-CCTGGGCTTCACCTTCTCCTT-3’ |
|  | Rev 5’-GAGGCCTTGAAGCCCTTGGT-3’ |
|  | Probe 5’-CACGAAGACATAGACAAGGGCATCCTGCTC-3’ |
| Pc | Fwd 5’-GCCAGAGGCAGGTGTTCTTT-3’ |
|  | Rev 5’-CATGGCCTGGGTGTCTTTAAC-3’ |
| Pcsk2 | Fwd 5’-GGCAGCTGGCGTGTTTGCATT-3’ |
|  | Rev 5’-AGCTGGTTCCGCTTGGAGGTGA-3’ |
| Glut2 | Fwd 5’-GCTGTCTCTGTGCTGCTTGT-3’ |
|  | Rev 5’-CGTAACTCATCCAGGCGAAT-3’ |
| MafA | Fwd 5’-CAGCTGGTATCCATGTCCGT-3’ |
|  | Rev 5’-CCGCTTCTGTTTCAGTCGGA-3’ |
| Pdx1 | Fwd 5’-CTCCCTTTCCCGTGGATGAAA-3’ |
|  | Rev 5’-TAAGCACCTCCTGCCCACTG-3’ |
| Nkx6.1 | Fwd 5’-GCACGCTTGGCCTATTCTCT-3’ |
|  | Rev 5’-TGGAACCAGACCTTGACCTGA-3’ |
| HK | Fwd 5’-CACCGGCAGATTGAGGAAAC-3’ |
|  | Rev 5’-CTCAGCCCCATTTCCATCTCT-3’ |
|  | Probe 5’-TCCCACTTCCGCCTCAGCAAGC-3’ |
| Ldha1 | Fwd 5’-TCTGTGGCAGACTTGGCTGA-3’ |
|  | Rev 5’-GGGACACTGAGGAAGACATCCT-3’ |
|  | Probe 5’-CCTGAGGCGGGTGCATCCCATTTC-3' |
| Gapdh | Fwd 5’-AAGGTCATCCCAGAGCTGAA-3’ |
|  | Rev 5’-CGGGAGATTAATCACGGGCA-3’ |

**Supplementary Table 2**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype | Females | Males | Expected  # | Observed  # | Expected  % | Observed  % |  |
| *Ins2-cre Ercc1+/+* | 13 | 11 | 22.6 | 24 | 12.5 | 13.3 |  |
| *Ins2-cre Ercc1+/-* | 10 | 9 | 22.6 | 19 | 12.5 | 10.5 |  |
| *Ins2-cre Ercc1fl/+* | 9 | 5 | 22.6 | 14 | 12.5 | 7.7 |  |
| *Ins2-cre Ercc1fl/-* | 10 | 14 | 22.6 | 24 | 12.5 | 13.3 |  |
| *Ercc1+/+* | 15 | 10 | 22.6 | 25 | 12.5 | 13.8 |  |
| *Ercc1+/-* | 11 | 9 | 22.6 | 20 | 12.5 | 11.1 |  |
| *Ercc1fl/+* | 15 | 11 | 22.6 | 26 | 12.5 | 14.4 |  |
| *Ercc1fl/-* | 16 | 13 | 22.6 | 29 | 12.5 | 16 |  |
| Total | 99 | 82 | 181 | 181 | 100 | 100 | p=0.44 |