

Supplemental Material

NPC1 is required for postnatal islet β cell differentiation by maintaining mitochondria turnover

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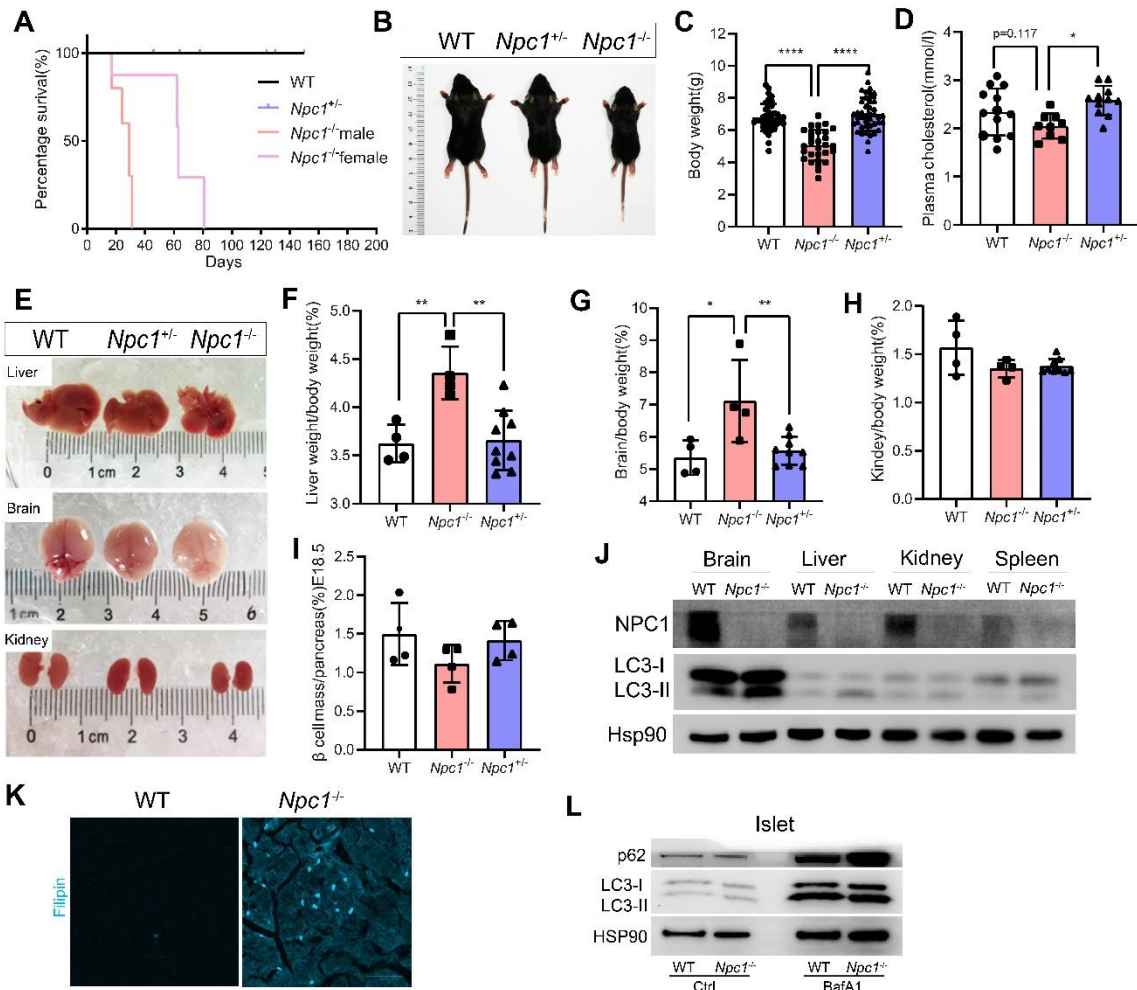
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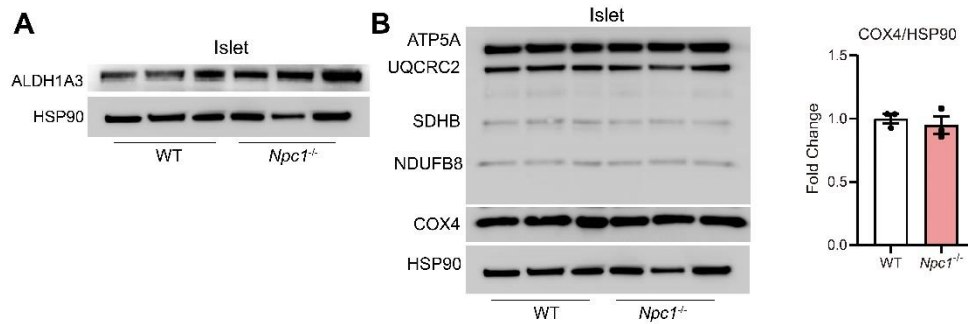
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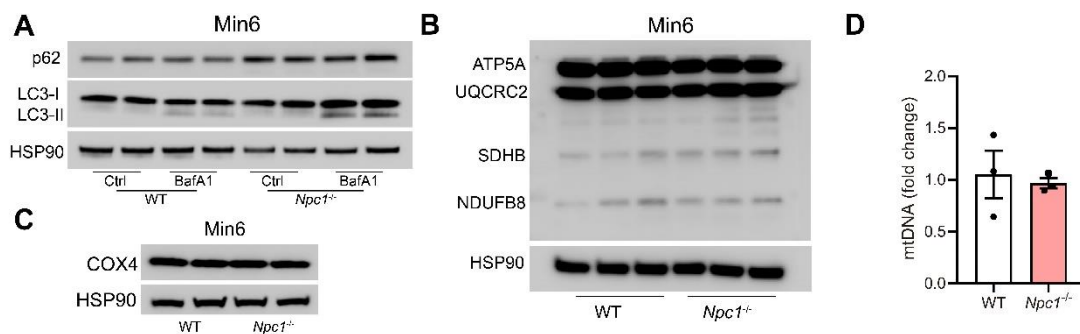
Supplemental Figure 1

A. Survival curve of studied mice (WT ♂4♀4, *Npc1*^{+/-} ♂4♀4, *Npc1*^{-/-} ♂6, *Npc1*^{-/-} ♀6) **B.** Representative images of whole 2-week-old mice **C.** Body weights of 2-week-old mice (n = 29-42) **D.** Plasma cholesterol levels in 2-week-old WT, *Npc1*^{-/-}, and *Npc1*^{+/-} mice (n = 9-13) **E.** Representative images of the liver, brain, and kidney of 2-week-old WT, *Npc1*^{-/-}, and *Npc1*^{+/-} mice (n = 4) **F-H.** Percentages of liver, brain, and kidney weights versus body weight of 2-week-old WT, *Npc1*^{-/-}, and *Npc1*^{+/-} mice (n = 4) **I.** Western blot depicts the expression of NPC1, LC3-I, and LC3-II in WT and *Npc1*^{-/-} mouse brain, liver, kidney, and spleen tissues **J.** Percentage of the insulin immunostaining area in the entire pancreas of E18.5 WT, *Npc1*^{-/-}, and *Npc1*^{+/-} mice (n = 4) **K.** Representative images of Filipin stain for pancreas section in WT and *Npc1*^{-/-} mice. **L.** Western blot of LC3 and p62 in WT and *Npc1*^{-/-} treated with and without BafA1(20 nM, 24 h) islets. Data are presented as the mean ± S.E.M, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Unpaired two-tailed Student's t test.



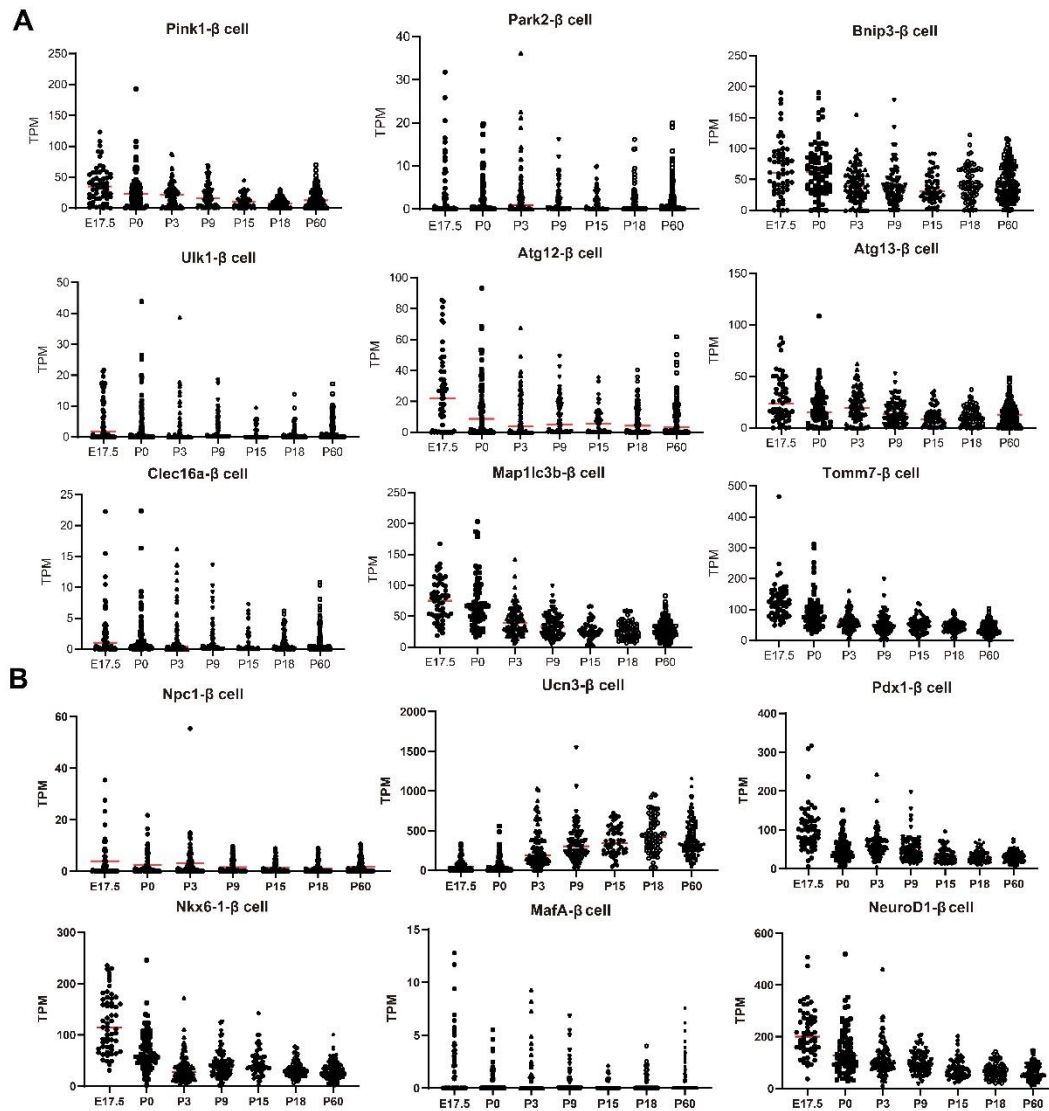
Supplemental Figure 2

A. Western blot of ALDH1A3 in WT and *Npc1^{-/-}* islets. **B.** Western blot of OXPHOS protein (ATP5A, UQCRC2, SDHB and NDUFB8) and COX4 (quantification) in WT and *Npc1^{-/-}* islets.



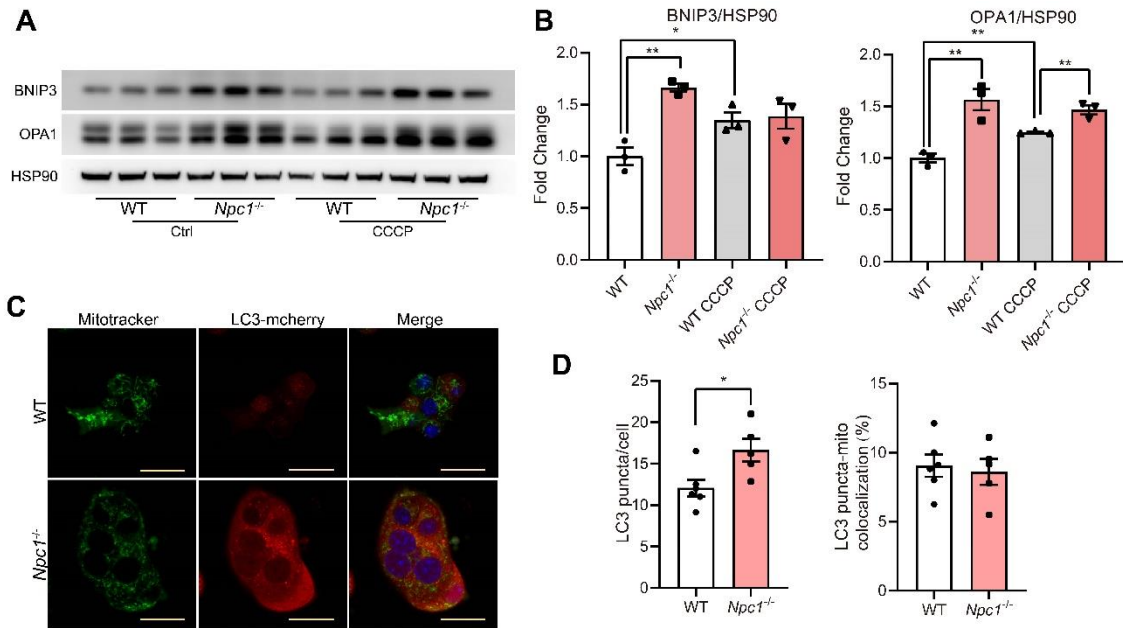
Supplemental Figure 3

A. Western blot of LC3, p62 in WT and *Npc1^{-/-}* cells treated Min6 with and without BafA1 (20 nM, 24 h). **B-C.** Western blot OXPHOS protein (ATP5A, UQCRC2, SDHB and NDUFB8) in WT and *Npc1^{-/-}* Min6 cells. **D.** MtDNA content in WT and *Npc1^{-/-}* Min6 cells. Data are presented as the mean \pm S.E.M,



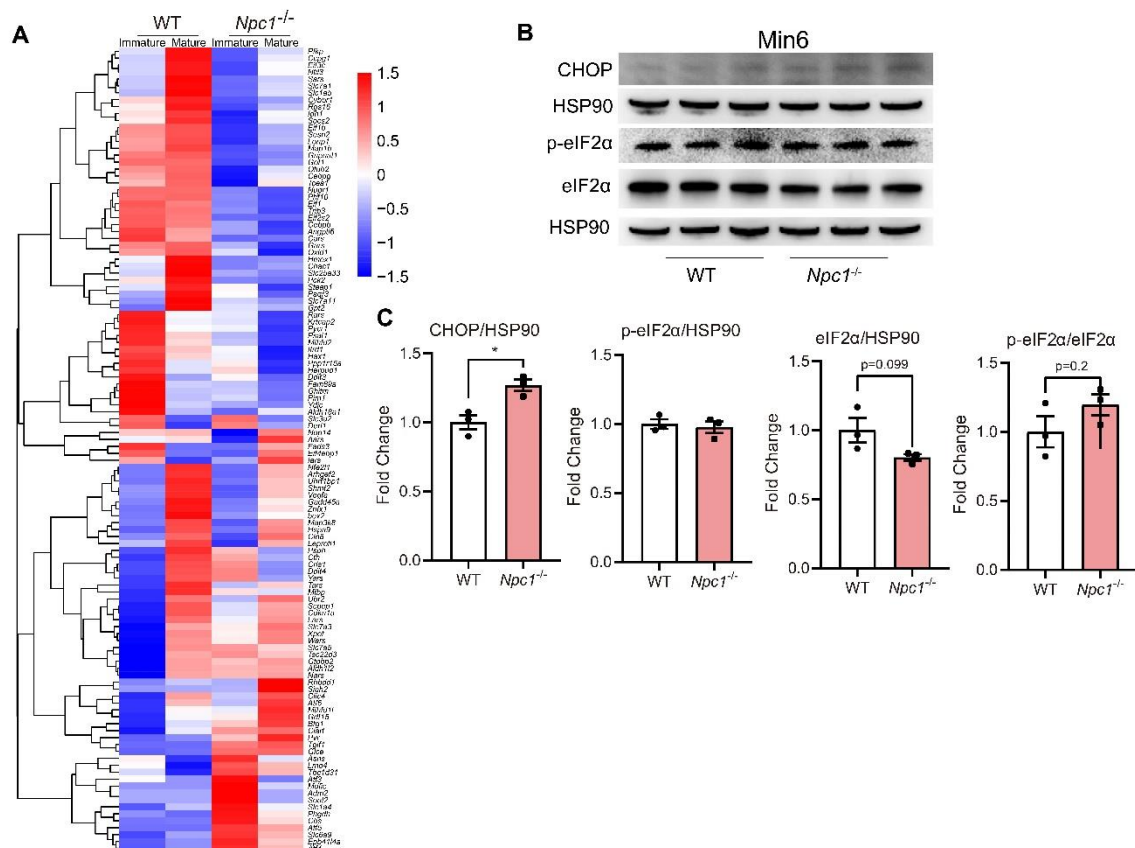
Supplemental Figure 4

A. Main mitophagy gene expression in embryonic 17.5 days to postnatal day 60. **B.** *Npc1* and islet maturation gene expression in embryonic 17.5 days to postnatal day 60. (scRNA-seq data from the GEO database GSE87375)



Supplemental Figure 5

A-B. Western blot and quantification of BNIP3 and OPA1 in WT and *Npc1*^{-/-} treated with CCCP (1 μ M, 3 h) and control vehicle on Min6 cells. **C-D.** Representative images and quantification of LC3-puncta (red) and its colocalization with Mitotracker (green) staining in WT and *Npc1*^{-/-} Min6 cells. Scale bars, 20 μ m. Data are presented as the mean \pm S.E.M, * $P < 0.05$, ** $P < 0.01$, Unpaired two-tailed Student's t test.



Supplemental Figure 6

A. Heatmap of the average ISR gene expression in scRNA seq data from mature and immature WT and *Npc1^{-/-}* β cells. **B-C.** Western blot and quantification of ISR key factors (CHOP, eIF2 α , p-eIF2 α) in WT and *Npc1^{-/-}* Min6 cells. Data are presented as the mean \pm S.E.M, * $P < 0.05$, $P < 0.01$. Unpaired two-tailed Student's t test.

Supplemental Table 1. Primer sequences

Gene	Forward	Reverse
<i>Npc1</i>	TGTTTGGTATGGAGAGTGTGGA	GTCACAGCAGAGACTGACATTG
<i>Ins1</i>	CACTTCCTACCCCTGCTGG	ACCACAAAGATGCTGTTTGACA
<i>Ins2</i>	GCTTCTTCTACACACCCATGTC	AGCACTGATCTACAATGCCAC
<i>Ucn3</i>	GCTGTGCCCTCGACCT	TGGGCATCAGCATCGCT
<i>MafA</i>	TTCAGCAAGGAGGAGGTCAT	CTCTGGAGCTGGCACTTCTC
<i>Pdx1</i>	CCCCAGTTTACAAGCTCGCT	CTCGGTTCCATTCGGGAAAGG
<i>Slc2a2</i>	CAGCAGCACTCCACTATGATTG	CGTACACCGTCTCCCTACAA
<i>Nkx6.1</i>	TCTGGACAGCAAATCTTCGCCC	ACTTGGTCCTGCGGTTCTGGAA
<i>Slc30a8</i>	CAGAGAACTTCGACAGAAGCC	CTTGCTTGCTCGACCTGTT
<i>Gck</i>	TGAGCCGGATGCAGAAGGA	GCAACATCTTACACTGGCCT
<i>Txnip</i>	TCTTTTGAGGTGGTCTTCAACG	GCTTTGACTCGGGTAACTTCACA
<i>Ldha</i>	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGGA
<i>Aldob</i>	GAAACCGCCTGCAAAGGATAA	GAGGGTCTCGTGAAAAGGAT
<i>Olfm1</i>	CACCGAACTCACCCAAGTGTT	CACTGTGCAGATACACCTGCC
<i>Fgf1</i>	CCCTGACCGAGAGGTTCAAC	GTCCCTTGTCATCCACG
<i>DIk1</i>	AGTGCGAAACCTGGGTGTC	GCCTCCTTGTGAAAGTGGTCA
<i>Ngn3</i>	AGTGCTCAGTTCCAATTCCAC	CGGCTTCTTCGCTTTTTGCTG
<i>Aldh1a3</i>	GGGTACACTGGAGCTAGGA	CTGGCCTCTTCTTGGCGAA
<i>Hmgcr</i>	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
<i>Srebf2</i>	GCAGCAACGGGACCATTCT	CCCCATGACTAAGTCCTTCAACT
<i>Ldlr</i>	TGACTCAGACGAACAAGGCTG	ATCTAGGCAATCTCGGTCTCC
<i>Npc2</i>	AGGACTGCGGCTCTAAGGT	AGGCTCAGGAATAGGGAAGGG
<i>Lamp1</i>	CAGCACTCTTTGAGGTGAAAAAC	ACGATCTGAGAACCATTGCA
<i>Lamp2</i>	TGTATTTGGCTAATGGCTCAGC	TATGGGCACAAGGAAGTTGTC
<i>Ctsd</i>	GCTTCCGGTCTTTGACAACCT	CACCAAGCATTAGTTCTCCTCC
<i>Ctsb</i>	TCCTTGATCCTTCTTTCTTGCC	ACAGTGCCACACAGCTTCTTC
<i>Cox6a2</i>	CTGCTCCCTTAACTGCTGGAT	GATTGTGGAAAAGCGTGTGGT
<i>Uqcrc2</i>	AAAGTTGCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
<i>Uqcrc1</i>	AGACCCAGGTCAGCATCTTG	GCCGATTCTTTGTTCCCTTGA
<i>Atp5a1</i>	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
<i>Atp4a</i>	GATGGAGATTAACGACCACCAG	ACGGGCAAACCTTCACATACTC
<i>Pink1</i>	TTCTTCCGCCAGTCGGTAG	CTGCTTCTCCTCGATCAGCC
<i>Bnip3</i>	TCCTGGGTAGAACTGCACTTC	GCTGGGCATCCAACAGTATTT
<i>Clec16a</i>	AGACCTTCTCTGGATATGGTG	GAGGGCGTGATAGTCATCGTC
<i>Dnm1L</i>	CAGGAATTGTTACGGTTCCCTAA	CCTGAATTAACCTGTCCCCTGA
<i>Huwe1</i>	TCTTCCACTAGAGATTCTGCCG	TGATACCAGCAAGGGGATCTTC
<i>Tomm7</i>	ATCCGCTGGGGCTTTATTCC	CGACGGTTCAGGCATTCCA
<i>Uba52</i>	GGCCAAGATCCAAGACAAGGA	CTGACGAAGGGATGGCTCAA
<i>Ulk1</i>	AAGTTCGAGTTCTCTCGCAAG	CGATGTTTTCTGCTTTAGTTCC
<i>Casp3</i>	ATGGAGAACAACAAAACCTCAGT	TTGCTCCCATGTATGGTCTTTAC
<i>Actb</i>	GGCTGTATCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

