

**Supplementary Fig. 1:** (a,b) Dotplots depicting cell type markers and (b) selected PT markers (S1,S3 and S3T2). (c,d) Representative spatial featplot of PT markers resolved on uninjured (c) and injured (d) kidneys showing specific spatial localization of PT cells in uninjured and impairement under injury.



**Supplementary Fig. 2**: Spatial transcriptomics cluster annotation and cell type identification. (a, b) Probabilistic transfer of single-cell data to spatial transcriptomics, showing cluster prediction scores and specific marker expression features per cluster. (c) Representative cluster resolution on H&E stained kidney images and multimetric quality control analysis used for data curating and removal of low quality clusters (e.g. 2, 11 and 14) preformed on all spatial transcriptomics datasets.

![](_page_2_Figure_0.jpeg)

**Supplementary Fig. 3**: (a, b) Representative immunofluorescence images and quantification of Kim-1 protein expression showing increased injured proximal tubules in the renal cortices of  $\gamma$ GT-Cre;Tgfbr2<sup>m/n</sup> mice compared to their Tgfbr2<sup>m/n</sup> littermates 3 weeks after AA injury; n=6 (Tgfbr2<sup>m/n</sup>) and 6 ( $\gamma$ GT-Cre;Tgfbr2<sup>m/n</sup>) mice, *p*= 0.0302. Scale bar=50µm. (c) H&E injury quantification score showing increased tubular injury in  $\gamma$ GT-Cre;Tgfbr2<sup>m/n</sup> mice compared to their Tgfbr2<sup>m/n</sup> littermates 6 weeks after AA injury; n=11 (Tgfbr2<sup>m/n</sup>) and 12 ( $\gamma$ GT-Cre;Tgfbr2<sup>m/n</sup>) mice, *p*= 0.0389. Data are presented as mean values ±SEM. Statistical significance was determined by unpaired Student's *t* test (two groups) with p<0.05 considered statistically significant. The dots represents the number of animals per group (b). \* represents p<0.05. Source data are provided as a Source Data file.

![](_page_3_Figure_0.jpeg)

**Supplementary Fig. 4**: Proximal tubule T $\beta$ RII deletion affects mitochondrial homeostasis. (a) Integrative Genomics Viewer images of sequencing showing the deletion of T $\beta$ RII exon 2 in T $\beta$ RII<sup>-/-</sup> PT cells. (b) Volcano plot and heatmap of an RNAseq analysis of PT cells treated or not with H<sub>2</sub>O<sub>2</sub> showing 3359 genes deregulated genes (p<0.01 and fc ≥ 1.5) in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>flox/flox</sup> PT cells. (c) Overrepresentation analysis using EnrichR revealed mitochondrion as the top affected cellular component in T $\beta$ RII<sup>-/-</sup> PT cells as compared to T $\beta$ RII<sup>flox/flox</sup> PT cells. (d) Heatmap of T $\beta$ RII<sup>flox/flox</sup> PT cells treated or not with H<sub>2</sub>O<sub>2</sub> showing oxidative stress induction of mt-genome encoded mRNAs of electron transport chain subunits. (e) Heatmap of PT cells showing

basal induction of mt-genome encoded electron transport chain subunit mRNA in T $\beta$ RII<sup>-/-</sup> PT cells compared to T $\beta$ RII<sup>flox/flox</sup> PT cells. (f) Mitochondrial DNA synthesis measured by RT-qPCR using the mt-genome encoded gene (Nd1) and normalized to the nuclear genome encoded gene ( $\beta$ -actin) showing increased mitochondrial biogenesis in T $\beta$ RII<sup>-/-</sup> PT cells (n=3 independent experiments, *p*= 0.0038). Data are presented as mean values ±SEM. Statistical significance was determined by unpaired Student's *t* tests. \*\*p represents <0.01. Source data are provided as a Source Data file.

![](_page_5_Figure_0.jpeg)

**Supplementary Fig. 5:** Proximal tubule T $\beta$ RII deletion disrupts mitochondrial function and metabolism in vitro. (a-e) Seahorse analyses showing dose-dependent effect of TGF- $\beta$  on T $\beta$ RII<sup>nov/lox</sup> PT cell respiration (basal and maximal) and ATP production through OXPHOS (spare capacity and ATP-link OCR) (n=3 independent experiments). (f-I) Seahorse analyses showing decreased respiration (basal *p*= 0.0005 and *p*<0.0001 maximal), spare capacity (*p*= 0.0007) and ATP-linked OCR (*p*< 0.0001) and slightly increased extracellular acidification rate (ECAR) in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>fox/flox</sup> PT cells. Seahorse analyses of coupling efficiency (*p*<0.0001)

showing a decrease in T $\beta$ RII<sup>+/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n=3 independent experiments). (m) Representative graph of seahorse analysis of fatty acid oxidation showing decreased residual ability to metabolize fatty acid in T $\beta$ RII<sup>+/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n= 3 independent biological replicates). (n) Representative graph of seahorse analyses of substrate dependency indicating increased glycolytic dependency in T $\beta$ RII<sup>+/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n= 3 independent biological replicates). (o) Bioluminescence measurement of ATP production showing decreased production in T $\beta$ RII<sup>+/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n=3 independent experiments, *p*= 0.0014). (p) Bioluminescence measurement of lactate production showing increased production in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n=3 independent experiments, *p*= 0.0014). (p) Bioluminescence measurement of lactate production showing increased production in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>floc/flox</sup> PT cells (n=3 independent experiments, *p*= 0.0281). Data are presented as mean values ±SEM. Statistical significance was determined by unpaired Student's *t* test (two groups) with p<0.05 considered statistically significant.\* represents p<0.05; \*\* represents p<0.01; \*\*\*\* represents p<0.001; \*\*\*\* represents p<0.001. Source data are provided as a Source Data file.

![](_page_7_Figure_0.jpeg)

**Supplementary Fig. 6**: Supplementary Fig. 2: Seahorse analysis of fatty acid metabolism indicating impaired fatty acid oxidation in PT cells lacking T $\beta$ RII as reflected by respiration (basal and maximal), spare capacity and ATP-linked OCR (n=3 independent biological replicates). (C) *p*= 0.0012; (d) *p*= 0.0117 and (e) *p*= 0.0001. Data are presented as mean values ±SEM. Statistical significance was determined by 2 way ANOVA followed by Sidak's multiple comparisons test with *p*<0.05 considered statistically significant. Dots represent the number of animals per group. \* represents *p*<0.05; \*\* represents *p*<0.01 \*\*\**p*<0.001. Source data are provided as a Source Data file.

![](_page_8_Figure_0.jpeg)

**Supplementary Fig. 7**: Seahorse analyses of substrate dependency indicating increased glycolytic dependency in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>flox/flox</sup> PT cells as reflected by respiration (basal and maximal), spare capacity and ATP-linked OCR (n=3 independent biological replicates). Data are presented as mean values ±SEM. Statistical significance was determined by 2 way ANOVA followed by Sidak's multiple comparisons test with p<0.05 considered statistically significant. (b) Basal respiration (\*\**p*=0.0033). (c) Maximal respiration (\*\**p*=0.0004). (d) ATP-linked OCR (\*\*\**p*<0.001). (e) Spare capacity (\*\**p*=0.0066). \* represents p<0.05; \*\* represents p<0.01. Source data are provided as a Source Data file.

![](_page_9_Figure_0.jpeg)

**Supplementary Fig. 8**: (a-e) Seahorse analysis of substrate dependency indicating absence of glutamine oxidation dependency in PT cells as reflected by respiration (basal and maximal), spare capacity and ATP-linked OCR (n=3 independent biological replicates). Data are presented as mean values ±SEM. Statistical significance was determined by 2 way ANOVA followed by Sidak's multiple comparisons test with p<0.05 considered statistically significant. Source data are provided as a Source Data file.

![](_page_10_Figure_0.jpeg)

**Supplementary Fig. 9**: Proximal tubule T $\beta$ RII deletion increases susceptibility to AA induced mitochondrial dysfunction. (a-e) Treatment of T $\beta$ RII<sup>-/-</sup> PT cells with AA (10 or 20  $\mu$ M) decreased respiration (basal and maximal), spare capacity and ATP-linked OCR compared to T $\beta$ RII<sup>for/for</sup> PT cells (n=3 independent biological replicates). Data are presented as mean values ±SEM. Statistical significance was determined by 2 way ANOVA followed by Sidak's multiple comparisons test with p<0.05 considered statistically significant. (b) Basal respiration (\*\*p=0.0039 and \*\*\*\*p<0.0001). (c) Maximal respiration (\*p=0.0141 and \*\*\*\*p<0.0001). (d) ATP-linked OCR (\*\*p=0.0041 and \*\*\*\*p<0.0001). (e) Spare capacity (\*\*p=0.0052). \* represents p<0.05; \*\*\* represents p<0.001. Source data are provided as a Source Data file.

![](_page_11_Figure_0.jpeg)

**Supplementary Fig. 10:** Proximal tubule T<sub>β</sub>RII deletion impairs ubiquinone metabolism and mitochondrial complex I (a) Metacore overrepresentation pathway analysis of differentially expressed genes in PT clusters showing a decrease of complex I subunits in uninjured yGT-Cre;Tgfbr2<sup>n/n</sup> compared to Tgfbr2<sup>n/n</sup> PT cells. (b) AA injury-induced stress increased the mRNA levels of complex I subunits.

![](_page_12_Figure_0.jpeg)

**Supplementary Fig. 11**: FACS analysis plots and quantification ROS in PT cells treated or not with 10  $\mu$ M MitoQ. MitoQ treatment decreased ROS in T $\beta$ RII<sup>-/-</sup> PT cells to the level of T $\beta$ RII<sup>flox/flox</sup> PT cells, but did not affect basal ROS production in T $\beta$ RII<sup>flox/flox</sup> PT cells. Tert-Butyl hydroperoxide (TBHP) was used as positive control (3 biological replicates).Data are presented as mean values ±SEM. The dots represent biological replicates. Source data are provided as a Source Data file.

![](_page_13_Figure_0.jpeg)

**Supplementary Fig. 12:** Effect of proximal tubule TGF- $\beta$  signaling on Pgc1 $\alpha$ , Pol $\gamma$  and Pink1. (a) Dose-dependent effect of TGF- $\beta$  on T $\beta$ RII<sup>flox/flox</sup> PT cell Pgc1 $\alpha$  mRNA levels measured by RTqPCR and normalized to S12 mRNA levels (n=7 independent biological replicates, p<0.0001). (b) Immunoblotting and quantification showing the association of transient activation of TGF- $\beta$ signaling (p-Smad3) with transient repression of Pgc1 $\alpha$  protein induced by 2 ng/ml TGF- $\beta$ 1.  $\beta$ -Actin was used as loading and blotting control (n=3 independent experiments, \**p*= 0.0328 and \*\**p*= 0.0018). (c) Overrepresentation analysis using Metacore showing the top 10 significantly

affected in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>fox/fox</sup> PT cells. (d) RNAseq normalized Pgc1 $\alpha$  and Tfam signals were strikingly increased in T $\beta$ RII<sup>-/-</sup> (Pgc1 $\alpha$ ; minima= 395.6, center= 416.8, maxima= 465.2 and Tfam; minima=601.7, center= 673.1, maxima= 692.1) compared to TβRII<sup>flox/flox</sup> PT cells (Pqc1α; minima= 85.5, center= 86.1, maxima= 89.3 and Tfam; minima= 421.1, center= 484.4, maxima= 497); n=3 independent biological replicates. (e) Pgc1 $\alpha$  and Tfam mRNA levels measured by RT-qPCR and normalized to the mRNA levels of S12 showing basal increase in T $\beta$ RII<sup>-/-</sup> compared to T $\beta$ RII<sup>fox/flox</sup> PT cells (n=9 independent experiments for Pgc1 $\alpha$ , p= 0.0026) and (n=7 independent experiments for Tfam, p= 0.0308). (f) Immunoblotting and quantification showing basal increase of Pqc1 $\alpha$  protein expression in T $\beta$ RII<sup>--</sup> PT cells as compared to T<sub>β</sub>RII<sup>fox/fox</sup> PT cells (n=3 independent experiments, p=0.0112). (g) Immunoblotting and quantification showing basal decrease of Poly protein expression in T $\beta$ RII<sup>-/-</sup> compared to T<sub>β</sub>RII<sup>fox/fox</sup> PT cells (n=3 independent experiments, p=0.0022). (h) Immunoblotting and guantification showing basal decrease of Pink1 protein expression in TBRII<sup>-/-</sup> compared to TβRII<sup>fox/fox</sup> PT cells. β-Actin was used as loading and blotting control (n=3 independent experiments, p=0.0391). Data are presented as mean values ±SEM. Statistical significance was determined by unpaired Student's t test (two groups) with p<0.05 considered statistically significant. \* represents p<0.05; \*\* represents p<0.01; \*\*\*\* represents p<0.001. Source data are provided as a Source Data file.

![](_page_15_Figure_0.jpeg)

**Supplementary Fig. 13**: Proximal tubule  $T\beta$ RII deletion increases tubularmacrophage/dendritic cells interaction and infiltrates 3 weeks after AA injury. (a) Trajectory inference performed on *Myo/str. mixed* clusters reveals increased macrophages markers in the

dataset yGT-Cre;Tgfbr2<sup>#/#</sup> 3 weeks after AA injury compared to other conditions (3 weeks Tgfbr2<sup>1/1</sup>, 6 weeks  $\gamma$ GT-Cre;Tgfbr2<sup>1/1</sup> and 6 weeks Tgfbr2<sup>1/1</sup>). The trajectory diagram and heat map show macrophage marker expression at each injury time points. (b) Heat map of cluster interactions, analyzed with Cellchat database of LR pairs, showing increased S3T2 cells interaction with *Macro/Dend*. cells in *y*GT-Cre;Tgfbr2<sup>1/n</sup> compared to Tgfbr2<sup>1/n</sup> datasets 3 weeks after AA injury. (c) Representative immunofluorescence images and quantification of F4/80 showing increased macrophage infiltrates in renal cortices of *γ*GT-Cre;Tgfbr2<sup>t/f</sup> mice compared to their Tqfbr2<sup>1/1</sup> littermates 3 weeks after AA injury. Lotus tetragonolobus lectin (LTL) is used as proximal tubule's marker; n=7 (Tgfbr2<sup>1/n</sup>) and 6 (yGT-Cre;Tgfbr2<sup>1/n</sup>) mice, p=0.0307. (d) FACS analysis of renal interstitial cells showing increased numbers of dendritic cells in the kidneys of  $\gamma$ GT-Cre;Tgfbr2<sup>f/f</sup> mice compared to those from their Tgfbr2<sup>f/f</sup> littermates 3 weeks after AA injury (n=5 uninjured, 10 injured (Tgfbr2<sup>1/1</sup>) and 6 uninjured, 10 injured  $\gamma$ GT-Cre;Tgfbr2<sup>1/1</sup> mice, injured p=0.0002). Data are presented as mean values ±SEM. Statistical significance was determined by unpaired Student's t test (two groups) or 2 way ANOVA followed by Sidak's multiple comparisons test with p<0.05 considered statistically significant. Dots represent the number of animals per group. \* represents p<0.05; \*\*\*p<0.001. Source data are provided as a Source Data file.

![](_page_17_Figure_0.jpeg)

**Supplementary Fig. 14**: Myeloid and lymphoid cell FACS gating strategies. (a). Myeloid cells gating strategy depicting identification of dendritic cell and other cell types. (b) Lymphoid cells gating strategy showing identification of CD4+, CD8+ and their subpopulations.

![](_page_18_Figure_0.jpeg)

**Supplementary Fig. 15**: Cellchat analyses of differentially expressed genes involved in *S3T2-Macro/dend.* interactions. (a) Diagram showing factors mediating outgoing and incoming interactions between clusters. (b) RNAseq normalized signals of selected adaptive and maladaptive factors (Minima, center and maxima values are in Source Data file; n= 3 independent biological replicates). Numbers are 1: endothelial and glomerular cells (Endo/Glom); 2: S3 PT cells; 3: myofibroblasts and stromal cells (Myo/St. mixed); 4: distal convoluted tubular, connecting and intercalated cells (DCT.CNT.CD-IC); 5: S1 PT cells; 6: macrophages and dendritic cells (Macro/Dend.); 7: loop of Henle and principal cells (LOH/CD-

PC). Source data are provided as a Source Data file.

![](_page_20_Figure_0.jpeg)

**Supplementary Fig. 16**: (a, b) Potential adaptive factors,  $\beta$ -catenin (Ctnnb1) and Cystathionase (Cth) are decreased in T $\beta$ RII<sup>-/-</sup> (Ctnnb1; minima= 6969, center= 7027.9, maxima= 7066 and Cth; minima= 320.1, center= 323.6, maxima= 368.5) compared to T $\beta$ RII<sup>nox/flox</sup> PT cells (Ctnnb1; minima= 7880.6, center= 7919, maxima= 8211.5 and Cth; minima= 325.1, center= 360.2, maxima= 380.8) as assessed by RNAseq (n=3 independent biological replicates). Source data are provided as a Source Data file.

![](_page_21_Figure_0.jpeg)

**Supplementary Fig. 17**: Sub-clustering analysis of *Myo/Str. mixed* identified 3 subpopulations. Heatmap showing the 3 sub-populations (Strom/Myo\_0, Strom/Myo\_1 and Strom/Myo\_2) and significantly up and down-regulated markers per subgroups.

![](_page_22_Figure_0.jpeg)

**Supplementary Fig. 18**: (a-c) FACS analyses of renal leukocytes showing no significant difference of leukocytes 3 weeks after AA injury and significant increase of CD4+ T cell number in kidneys of  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice compared to those from their Tgfbr2<sup>n/n</sup> littermates 6 weeks after AA injury. CD8+ T cell numbers were increased in Tgfbr2<sup>n/n</sup> compared to  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> kidneys, but did not reach statistical significance 6 weeks after AA injury. Uninjured (n= 6 Tgfbr2<sup>n/n</sup> and 6  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup>), 3 weeks (n= 9 Tgfbr2<sup>n/n</sup> and 8  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup>) and 6 weeks (n=6 Tgfbr2<sup>n/n</sup> and 6  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup>, CD4+ T cells *p*=0.0205) mice. Data are presented as mean values ±SEM. Statistical significance was determined by 2 way ANOVA followed by Sidak's multiple comparisons test with p<0.05 considered statistically significant. The dots represent the number of animals per group. \*p represents <0.05. Source data are provided as a Source Data file.

![](_page_23_Figure_0.jpeg)

**Supplementary Fig. 19**: Proximal tubule T $\beta$ RII deletion decreases the number of Foxp3+ CD4 T cells after AA induced injury. (a) Representative image and quantification showing no difference in CD3+ cell infiltrate in renal cortices of  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> and floxed littermate mice; n=14 (Tgfbr2<sup>n/n</sup>) and 17 ( $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup>) mice. Scale bars represent 200  $\mu$ m. (b, c) FACS analyses of the Foxp3+ CD4 T cell percentage of the CD45+ cells 3 weeks (n=6 uninjured, 10 injured Tgfbr2<sup>n/n</sup> and 6 uninjured, 10 injured  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice, injured *p*<0.0001) (b) and 6 weeks (n=5 uninjured, 6 injured Tgfbr2<sup>n/n</sup> and 7 uninjured, 6 injured  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice, 6 injured Tgfbr2<sup>n/n</sup> mice, 6 injured Tgfbr2<sup>n/n</sup> and 7 uninjured, 7 uninjured, 7 uninjured  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice, 5 uninjured, 6 injured Tgfbr2<sup>n/n</sup> and 7 uninjured, 7 uninjured, 6 injured  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice, 6 injured  $\gamma$ GT-Cre;Tgfbr2<sup>n/n</sup> mice,

by Sidak's multiple comparisons test with p<0.05 considered statistically significant. The dots represent the number of animals per group. \* represents p<0.05; \*\*\*\* represents p<0.0001. Source data are provided as a Source Data file.

![](_page_25_Figure_0.jpeg)

**Supplementary Fig. 20**: (a-c) Differential gene expression analysis showing a significant increase of potential maladaptive factors (ANGPTL8, FGF5 and CXCL14) in CKD PT cells as compared to healthy PT cells from the snRNAseq online database. N= 5 CKD patients (eGFR<60) and 3 healthy controls. Differential gene expression was evaluated using the Wilcoxon Rank Sum test from the *FindMarkers* function. (a) p= 0.000003; (b) p= 0.00093617 and (c) p=0. Source data are provided as a Source Data file.

# Used Reagents and Antibodies

|  | Company                   | Cat. number    | Dilution |
|--|---------------------------|----------------|----------|
| AA Injury Models                         |                           |                |          |
| Aristolochic acid                        | Sigma                     | A9451          |          |
|  |                           |                |          |
| Staining                                 |                           |                |          |
| 10% Formalin                             | Sigma                     | HT501128       |          |
| Oil red O                                | Sigma                     | O0625          |          |
| Sirius red                               | Waldeck, Münster, Germany |                |          |
| H&E Staining Kit (Hematoxylin and Eosin) | Abcam                     | ab245880       |          |
| VECTASTAIN Elite ABC Kit                 | Vector Laboratories       | PK-6200        |          |
| H2O2                                     | Sigma                     | H1009          |          |
| DAB                                      | Vector Laboratories       | SK-4105-L120   |          |
| Citrate Buffer                           | Sigma                     | C9999          |          |
| Normal goat serum                        | Jackson Immuno research   | 005-000-121    |          |
| Normal donkey serum                      | Jackson Immuno research   | 017-000-122    |          |
| LowCross-Buffer                          | Candor                    | 100 050        |          |
| Antifade Monting medium with Dapi        | Vector Laboratories       | H-1800         |          |
| Spatial transcriptomics                  |                           |                |          |
| Visium Spatial Gene Expression           | 10X Genomics              |                |          |
| DNA 12000 Kit                            | Agilent                   | 5067-1508      |          |
| High Sensitivity D5000 ScreenTape        | Agilent                   | 5067-5592      |          |
| High Sensitivity D5000 Reagents          | Agilent                   | 5067-5593      |          |
| SPRIselect Reagent                       | Beckman Coulter           | 4700273718     |          |
| Flow cytometry                           |                           |                |          |
| Collagenase IV                           | Worthington               | LS004188       |          |
| Percoll                                  | Sigma-Aldrich             | GE17-0891      |          |
| Mitochondria fractionation               |                           |                |          |
| Cell mitochondria isolation kit          | ThermoFisher              | 89874          |          |
| Protease inhibitor coktail               | Roche                     | 11697498001    |          |
| Cell culture                             |                           |                |          |
| DMEM/F12                                 | Corning                   | 10-092-CV      |          |
| FBS                                      | Gibco                     | 10270-106      |          |
| PT supplements                           |                           |                |          |
| Hydrocortisone                           | Sigma Aldrich             | H0135          |          |
| ITS (insulin/transferrin/selenium)       | Sigma Aldrich             | 11884-1        |          |
| ТЗ                                       | Sigma Aldrich             | T5516          |          |
| Interferon-gamma                         | Sigma Aldrich             | 14777          |          |
| -  | -                         |                |          |
| TGF-beta 1 Protein                       | R&D Systems               | 101-B1-001/CF  |          |
|  |                           | 7754-BH-005/CF |          |

| Seahorse                                   |                    |                |
|--|--------------------|----------------|
| Palmitate Oxidation Stress Test Kit        | Bucher             | 103693-100     |
| Mito Stress Test Kit                       | Bucher             | 103015-100     |
| Glucose/Pyruvate Oxidation Stress Test Kit | Bucher             | 103673-100     |
| Glutamine Oxidation Stress Test Kit        | Bucher             | 103674-100     |
| L-carnitine                                | Agilent            | 6496363        |
| Etomoxir                                   | Sigma              | E1905          |
| Oligomycin                                 | Sigma              | 75351          |
| FCCP                                       | Sigma              | C2920          |
| Rotenone/Antimycin                         | Sigma              | R8875          |
| BPTES                                      | Sigma              | SML0601        |
| UK5099                                     | Sigma              | PZ0160         |
| Passive lysis buffer                       | Promega            | E1941          |
| Bradford Assay reagents                    | Biorad             | 500002         |
| Pierce BCA Protein Assay A                 | Thermo Scientific  | 23228          |
| Pierce BCA Protein Assay B                 | Thermo Scientific  | 1859078        |
| ATP, Lactate and NAD+/NADH assay           |                    |                |
| ATP assay                                  | Promega            | G7570          |
| Lactate essay                              | Promega            | J5021          |
| NAD+/NADH essay                            | Promega            | G9071          |
| DCFDA assay                                |                    |                |
| MitoQ                                      | Focus Biomolecules | 10-1363-25     |
| NAD+                                       | Sigma Aldrich      | N8285          |
|  | Signa-Alunch       | N1636          |
| qPCR                                       |                    |                |
| Nucleospin RNA extraction kit              | Macherey Nagel     | 740955.25      |
| AffinityScript Multi-Temp RT kit           | Agilent            | 600107         |
| KAPA SYBR FAST                             | Roche              | KK4618         |
| Immunoblotting                             |                    |                |
| EDTA                                       | Sigma              | 27285          |
| NP-40 Substitute                           | Sigma              | 74385          |
| Na3VO4                                     | Sigma              | S6508          |
| Protease inhibitor cocktails               | Roche              | 11 836 170 001 |
| PMSF                                       | Sigma              | P 7626         |
| Aprotinin                                  | Sigma              | A1153          |
| Leupeptin                                  | Sigma              | L2884          |
| Pepstatin                                  | Sigma              | P 4265         |
| BSA  | Carl Roth          | 8076.4         |
| HRP substrate                              | PerkinElmer        | NEL113001EA    |

Antibodies IF/IHC

| Pgc1a (Monoclonal, 4C1.3)  | Merck               | ST1202       | 1:500   |
|--|---------------------|--------------|---------|
| CD3 (Monoclonal, SP7)  | Abcam               | ab16669      | 1:150   |
| F4/80 (Monoclonal, CI:A3-1)  | Abcam               | ab6640       | 1:150   |
| Kim-1 (Polyclonal)   | R&D Systems         | AF1817       | 1:200   |
| LTL  | Vector Laboratories | FL-1321      | 1:400   |
| Secondary AB, Alexa fluor  | Life technology     |              |         |
| Immunoblotting   |                     |              |         |
| Pink1 (Polyclonal)   | Novus               | BC100-494    | 1:1000  |
| LC3A (Monoclonal, D50G8)   | Cell Signaling      | 4599S        | 1:500   |
| Pgc1a (Monoclonal, 4C1.3)  | Merck               | ST1202       | 1:500   |
| Phospho-Smad3 (Polyclonal)   | Rockland            | 600-401-919  | 1:500   |
| Polg (Monoclonal, G-6)   | Santa Cruz          | sc-390634    | 1:200   |
| Tom20 (Polyclonal)   | Santa Cruz          | sc-11415     | 1:1000  |
| alpha-tubulin (Monoclonal, GT114)  | GenTex              | GTX628802-01 | 1:4000  |
| Beta-actin (Monoclonal, AC-15)   | Sigma               | A5441        | 1:20000 |
| Total OXPHOS Antibody Cocktail (5 mAbs)<br>Anti-NDUFB8 antibody [20E9DH10C12] (ab110242)<br>Anti-SDHB antibody [21A11AE7] (ab14714)<br>Anti-UQCRC2 antibody [13G12AF12BB11] (ab14745)<br>Anti-MTCO1 antibody [1D6E1A8] (ab14705)<br>Anti-ATP5A antibody [15H4C4] (ab14748) | Abcam               | ab110413     | 1:1000  |
| polyclonal goat anti-rabbit-HRP  | Pierce              | 31430        |         |
| polyclonal goat anti-mouse-HRP   | Pierce              | 31460        |         |

| List of antibo | dies used  | in FACS      |                      |            |                |
|----------------|------------|--------------|----------------------|------------|----------------|
|                |            | 1 .          |                      |            |                |
| Fluorochrome   | Target     | Clone        | Reseller             | Cat #      | Dilution (1/x) |
| BUV395         | CD45       | 30-F11       | BD Horizon           | 564279     | 300            |
| BV785          | CD11c      | N418         | BioLegend            | 117336     | 300            |
| BV711          | MHC II     | M5/114.15.2  | BioLegend            | 107643     | 1000           |
| BV650          | XCR1       | ZET          | BioLegend            | 148220     | 200            |
| BV605          | SA         | strepdavidin | BioLegend            | 405229     | 300            |
| BV510          | CD11b      | M1/70        | BioLegend            | 101263     | 100            |
| PerCP-Cy5.5    | MGL2       | URA1         | BioLegend            | 146810     | 300            |
| FITC           | Ly-6C      | HK1.4        | BioLegend            | 128006     | 300            |
| PE-Cy7         | CD103      | 2E7          | BioLegend            | 121426     | 200            |
| PE             | PDL2       | TY25         | BioLegend            | 107205     | 300            |
| APC-Cy7        | SiglecF    | E50-2440     | <b>BD</b> Pharmingen | 565527     | 300            |
| AF700          | Ly-6G      | 1A8          | BioLegend            | 127622     | 400            |
| APC            | CD64       | X54-5/7.1    | BioLegend            | 139306     | 100            |
| Biotin         | SIRPa      | P84          | BioLegend            | 144026     | 100            |
| BUV661         | CD3        | 145-2C11     | BD OptiBuild         | 750638     | 400            |
| BV785          | CD90.2     | 30-H12       | BioLegend            | 105331     | 1000           |
| BV711          | CD4        | RM4-5        | BioLegend            | 100550     | 100            |
| BV650          | CD19       | 6D5          | BioLegend            | 115541     | 300            |
| BV605          | CD25       | PC61         | BioLegend            | 102036     | 200            |
| BV421          | CD11b      | M1/70        | BioLegend            | 101251     | 300            |
| BV421          | CD11c      | N418         | BioLegend            | 117343     | 300            |
| BV421          | F4/80      | BM8          | BioLegend            | 123137     | 300            |
| BV421          | GR-1       | RB6-8C5      | BioLegend            | 108445     | 300            |
| BV421          | TER-119    | TER-119      | BioLegend            | 116234     | 300            |
| PerCP-eF710    | KLRG1      | 2F1          | Invitrogen           | 46-5893-82 | 300            |
| FITC           | Ki67       | 11F6         | Biolegend            | 151212     | 300            |
| PE-Cy7         | NKp46      | 29A1.4       | BioLegend            | 137618     | 200            |
| PE             | ST2        | DIH4         | BioLegend            | 146607     | 300            |
| APC-F750       | TCR gd     | GL3          | BioLegend            | 118136     | 150            |
| AF700          | TCR b      | H57-597      | BioLegend            | 109224     | 400            |
| APC            | DX5        | DX5          | BioLegend            | 108910     | 100            |
| Viabilitv dve  |            |              |                      |            |                |
| Zombie Red     | dead cells |              | BioLegend            | 423110     | 1500           |

## **Report of sequencing QC stats**

Name

o25586\_1\_1-wt-ctl o25586\_1\_2-ko-ctl o25586\_1\_3-wt-5w o25586\_1\_4-ko-5w o25586\_1\_6-ko3-8w o25586\_1\_7-wt5-8w

| Name   | Number of Spots Under Tissue                                 |
|--------|--|
| wt-ctl | 3146   |
| ko-ctl | 3411   |
| wt-5w  | 3515   |
| ko-5w  | 3872   |
| ko-8w  | 3347   |
| wt-8w  | 3320   |
|        | Name<br>wt-ctl<br>ko-ctl<br>wt-5w<br>ko-5w<br>ko-8w<br>wt-8w |

|                   | Name   | Number of Reads | Mean      | Reads per Spot |
|-------------------|--------|-----------------|-----------|----------------|
| o25586_1_1-wt-ctl | wt-ctl |                 | 256055078 | 81390.67959    |
| o25586_1_2-ko-ctl | ko-ctl |                 | 193407956 | 56701.24773    |
| o25586_1_3-wt-5w  | wt-5w  |                 | 214021389 | 60888.01963    |
| o25586_1_4-ko-5w  | ko-5w  |                 | 291406727 | 75260.00181    |
| o25586_1_6-ko3-8w | ko-8w  |                 | 224717809 | 67140.06842    |
| o25586_1_7-wt5-8w | wt-8w  |                 | 231795128 | 69817.80964    |

#### Mean Reads Under Tissue per Spot Fraction of Spots Under Tissue

| o25586_1_1-wt-ctl | wt-ctl | 51496.6691  | 0.630208333 |
|-------------------|--------|-------------|-------------|
| o25586_1_2-ko-ctl | ko-ctl | 36446.04134 | 0.683293269 |
| o25586_1_3-wt-5w  | wt-5w  | 50968.03812 | 0.704126603 |
| o25586_1_4-ko-5w  | ko-5w  | 60087.7079  | 0.775641026 |
| o25586_1_6-ko3-8w | ko-8w  | 46411.46011 | 0.670472756 |
| o25586_1_7-wt5-8w | wt-8w  | 51555.46024 | 0.665064103 |

|                   | Name   | Median Genes per Spot |        | Median UMI Counts per Spot |
|-------------------|--------|-----------------------|--------|----------------------------|
| o25586_1_1-wt-ctl | wt-ctl |                       | 4234.5 | 16670                      |
| o25586_1_2-ko-ctl | ko-ctl |                       | 3244   | 10801                      |
| o25586_1_3-wt-5w  | wt-5w  |                       | 4321   | 14337                      |
| o25586_1_4-ko-5w  | ko-5w  |                       | 5178   | 19197                      |
| o25586_1_6-ko3-8w | ko-8w  |                       | 3655   | 9007                       |
| o25586_1_7-wt5-8w | wt-8w  |                       | 3299.5 | 8285                       |

| Name   | Median Genes per Spot | Mec    | lian UMI Counts per Spot |
|--------|-----------------------|--------|--------------------------|
| wt-ctl |                       | 4234.5 | 16670                    |
| ko-ctl |                       | 3244   | 10801                    |
| wt-5w  |                       | 4321   | 14337                    |
| ko-5w  |                       | 5178   | 19197                    |
| ko-8w  |                       | 3655   | 9007                     |
| wt-8w  |                       | 3299.5 | 8285                     |
|        |                       |        |                          |

|                   | Name   | Median Genes per Spot | 1      | Median UMI Counts per Spot |
|-------------------|--------|-----------------------|--------|----------------------------|
| o25586_1_1-wt-ctl | wt-ctl |                       | 4234.5 | 16670                      |
| o25586_1_2-ko-ctl | ko-ctl |                       | 3244   | 10801                      |
| o25586_1_3-wt-5w  | wt-5w  |                       | 4321   | 14337                      |
| o25586_1_4-ko-5w  | ko-5w  |                       | 5178   | 19197                      |

| o25586_1_6-ko3-8w | ko-8w | 3655   | 9007 |
|-------------------|-------|--------|------|
| o25586_1_7-wt5-8w | wt-8w | 3299.5 | 8285 |

|                   | Name   | Valid Barcodes | Valid UMIs  |             |  |
|-------------------|--------|----------------|-------------|-------------|--|
| o25586_1_1-wt-ctl | wt-ctl |                | 0.96230914  | 0.99842514  |  |
| o25586_1_2-ko-ctl | ko-ctl |                | 0.963811225 | 0.998520304 |  |
| o25586_1_3-wt-5w  | wt-5w  |                | 0.963844684 | 0.998216459 |  |
| o25586_1_4-ko-5w  | ko-5w  |                | 0.963812864 | 0.999101421 |  |
| o25586_1_6-ko3-8w | ko-8w  |                | 0.960277741 | 0.999395193 |  |
| o25586_1_7-wt5-8w | wt-8w  |                | 0.957380895 | 0.999561354 |  |

|                   | Name   | Sequencing Saturation | Q30 Bases in Barcode |
|-------------------|--------|-----------------------|----------------------|
| o25586_1_1-wt-ctl | wt-ctl | 0.580787016           | 0.945740689          |
| o25586_1_2-ko-ctl | ko-ctl | 0.587929653           | 0.947285749          |
| o25586_1_3-wt-5w  | wt-5w  | 0.634684432           | 0.948436636          |
| o25586_1_4-ko-5w  | ko-5w  | 0.618744854           | 0.949343766          |
| o25586_1_6-ko3-8w | ko-8w  | 0.740219229           | 0.949567813          |
| o25586_1_7-wt5-8w | wt-8w  | 0.801069095           | 0.950086734          |

|                   | Name   | Sequencing Saturation | Q30 Bases in Barcode |
|-------------------|--------|-----------------------|----------------------|
| o25586_1_1-wt-ctl | wt-ctl | 0.580787016           | 0.945740689          |
| o25586_1_2-ko-ctl | ko-ctl | 0.587929653           | 0.947285749          |
| o25586_1_3-wt-5w  | wt-5w  | 0.634684432           | 0.948436636          |
| o25586_1_4-ko-5w  | ko-5w  | 0.618744854           | 0.949343766          |
| o25586_1_6-ko3-8w | ko-8w  | 0.740219229           | 0.949567813          |
| o25586_1_7-wt5-8w | wt-8w  | 0.801069095           | 0.950086734          |

o25586\_1\_1-wt-ctl o25586\_1\_2-ko-ctl o25586\_1\_3-wt-5w o25586\_1\_4-ko-5w o25586\_1\_6-ko3-8w o25586\_1\_7-wt5-8w

Name wt-ctl ko-ctl wt-5w ko-5w ko-8w wt-8w

| Q30 Bases in RNA Read | Q30 Bases in UMI   |
|-----------------------|--------------------|
| 0.9245                | 0.942876603        |
| 0.9245                | 62883 0.94432795   |
| 0.9231                | .23048 0.945344344 |
| 0.9286                | 0.946804633        |
| 0.9324                | 54876 0.947422186  |
| 0.9323                | 0.947881975        |

#### **RM= Reads Mapped**

|                   | Name   | RM to Genome |             | RM Confidently to Genome |
|-------------------|--------|--------------|-------------|--------------------------|
| o25586_1_1-wt-ctl | wt-ctl |              | 0.869175955 | 0.842248495              |
| o25586_1_2-ko-ctl | ko-ctl |              | 0.865237064 | 0.83669041               |
| o25586_1_3-wt-5w  | wt-5w  |              | 0.85405112  | 0.824711127              |
| o25586_1_4-ko-5w  | ko-5w  |              | 0.875439451 | 0.844984557              |
| o25586_1_6-ko3-8w | ko-8w  |              | 0.905486614 | 0.877937445              |
| o25586_1_7-wt5-8w | wt-8w  |              | 0.947664133 | 0.922604111              |

|                   | Name   | RM Confidently to Intergenic Regic RM | 1 Confid |
|-------------------|--------|---------------------------------------|----------|
| o25586_1_1-wt-ctl | wt-ctl | 0.019555988                           | 0.01     |

lently to Intronic Regions 13258667

| o25586_1_2-ko-ctl | ko-ctl | 0.020876085 | 0.014521254 |
|-------------------|--------|-------------|-------------|
| o25586_1_3-wt-5w  | wt-5w  | 0.0195584   | 0.015620135 |
| o25586_1_4-ko-5w  | ko-5w  | 0.020353013 | 0.015381831 |
| o25586_1_6-ko3-8w | ko-8w  | 0.021253077 | 0.012090604 |
| o25586_1_7-wt5-8w | wt-8w  | 0.022182662 | 0.012365346 |

### Name RM Confidently to Exonic Regions RM Confidently to Transcriptome

| wt-ctl | 0.80943384   | 0.788727588   |
|--------|--|---|
| ko-ctl | 0.801293071  | 0.780388972   |
| wt-5w  | 0.789532592  | 0.769288643   |
| ko-5w  | 0.809249712  | 0.78880806  |
| ko-8w  | 0.844593763  | 0.822598239   |
| wt-8w  | 0.888056103  | 0.863456608   |
|        | wt-ctl<br>ko-ctl<br>wt-5w<br>ko-5w<br>ko-8w<br>wt-8w | wt-ctl0.80943384ko-ctl0.801293071wt-5w0.789532592ko-5w0.809249712ko-8w0.844593763wt-8w0.888056103 |

|                   | Name   | RM Antisense to Gene | Fraction Reads in Spots Under Tissue |
|-------------------|--------|----------------------|--------------------------------------|
| o25586_1_1-wt-ctl | wt-ctl | 0.006559706          | 0.656598495                          |
| o25586_1_2-ko-ctl | ko-ctl | 0.007157177          | 0.654459588                          |
| o25586_1_3-wt-5w  | wt-5w  | 0.007004842          | 0.870071277                          |
| o25586_1_4-ko-5w  | ko-5w  | 0.006450812          | 0.825810692                          |
| o25586_1_6-ko3-8w | ko-8w  | 0.006733752          | 0.722138807                          |
| o25586_1_7-wt5-8w | wt-8w  | 0.009899992          | 0.773334206                          |

|                   | Name   | Total Genes Detected |       |
|-------------------|--------|----------------------|-------|
| o25586_1_1-wt-ctl | wt-ctl |                      | 18293 |
| o25586_1_2-ko-ctl | ko-ctl |                      | 17715 |
| o25586_1_3-wt-5w  | wt-5w  |                      | 18690 |
| o25586_1_4-ko-5w  | ko-5w  |                      | 19061 |
| o25586_1_6-ko3-8w | ko-8w  |                      | 17909 |
| o25586_1_7-wt5-8w | wt-8w  |                      | 17598 |

### After QC analysis and additional filtering

| o25586_1_1-wt-ctl-space wt-ctl | 2187 |
|--------------------------------|------|
| o25586_1_2-ko-ctl-space ko-ctl | 2423 |
| o25586_1_3-wt-5w-spac( wt-5w   | 2770 |
| o25586_1_4-ko-5w-spaceko-5w    | 2934 |
| o25586_1_6-ko3-8w-spacko-8w    | 2261 |
| o25586_1_7-wt5-8w-sparwt-8w    | 2549 |

Total number of spots: 15124 Median UMIs: 14774 Median genes: 4374 Median mitochondrial percentage: 13.050

| MetaCore Data |           |  |             |  | S3T2 Ko v<br>ctl_avg_lo | vs WT<br>og2FC |
|---------------|-----------|--|-------------|--|-------------------------|----------------|
| #             | Input IDs | Network Object<br>Name   | Gene Symbol | Description  | Signal                  | p-value        |
| 1             |           | 2-Decaprenyl-6-<br>methoxy-1,4-<br>benzoquinone<br>intracellular<br>anatomical<br>structure              |             | 2-Decaprenyl-6-<br>methoxy-1,4-<br>benzoquinone                                  |                         |                |
| 2             |           | 2-Decaprenyl-6-<br>methoxyphenol<br>intracellular<br>anatomical<br>structure                             |             | 2-Decaprenyl-6-<br>methoxyphenol   |                         |                |
| 3             |           | 2-decaprenyl-3-<br>methyl-6-<br>methoxy-1,4-<br>benzoquinone<br>intracellular<br>anatomical<br>structure |             | 2-decaprenyl-3-<br>methyl-6-<br>methoxy-1,4-<br>benzoquinone                     |                         |                |
| 4             |           | <u>2.1.1</u>   |             |  |                         |                |
| 5             |           | 3-Decaprenyl-<br>4.5-<br>dihydroxybenzoa<br>te intracellular<br>anatomical<br>structure                  |             | <u>3-Decaprenyl-</u><br><u>4.5-</u><br><u>dihydroxybenzoa</u><br><u>te</u>       |                         |                |
| 6             |           | 3-Decaprenyl-4-<br>hydroxy-5-<br>methoxybenzoat<br>e intracellular<br>anatomical<br>structure            |             | <u>3-Decaprenyl-4-</u><br><u>hydroxy-5-</u><br><u>methoxybenzoat</u><br><u>e</u> |                         |                |

# MetaCore Analysis Report of Complex I in S3T2 PT

| 8  |         | 3-Hexaprenyl-4-<br>hydroxy-benzoic<br>acid intracellular<br>anatomical<br>structure<br>4-Hydroxy-<br>benzoic acid<br>intracellular<br>anatomical<br>structure |             | 3-Hexaprenyl-4-<br>hydroxy-benzoic<br>acid<br>4-Hydroxy-<br>benzoic acid                          |           |   |
|----|---------|---|-------------|---|-----------|---|
| 9  |         | <u>COQ3</u>   | <u>Coq3</u> | <u>Ubiquinone</u><br><u>biosynthesis O-</u><br><u>methyltransferas</u><br><u>e, mitochondrial</u> |           |   |
| 10 |         | <u>COQ6</u>   | <u>Coq6</u> | <u>Ubiquinone</u><br><u>biosynthesis</u><br><u>monooxygenase</u><br><u>COQ6,</u><br>mitochondrial |           |   |
| 11 |         | Coenzyme-Q10<br>intracellular<br>anatomical<br>structure  |             | Coenzyme-Q10  |           |   |
| 12 | Ndufa12 | <u>DAP13</u>  | Ndufa12     | NADH_<br>dehydrogenase<br>[ubiquinone] 1_<br>alpha_<br>subcomplex                                 | -0.490431 | 0 |
| 13 |         | <u>H('+) +</u>  |             |   |           |   |
|    |         | <u>Coenzyme-Q10</u><br><u>+ NADH =</u><br><u>Ubiquinol-10 +</u><br><u>NAD('+)</u>   |             |   |           |   |

| 15 |         | H('+) + NAD(P)H<br>+ p-<br>Hydroxyphenylp<br>yruvic acid =<br>NADP('+) + p-<br>Hydroxyphenylla<br>ctic acid<br>L-Aspartic acid +<br>p- |            |   |           |   |
|----|---------|--|------------|---|-----------|---|
|    |         | <u>Hydroxyphenylp</u><br><u>yruvic acid = L-</u><br><u>Tyrosine +</u><br><u>Oxaloacetic acid</u>                                       |            |   |           |   |
| 17 |         | L-Tyrosine<br>intracellular<br>anatomical<br>structure   |            | <u>L-Tyrosine</u>   |           |   |
| 18 | mt-Nd5  | <u>MT-ND5</u>  | <u>ND5</u> | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 5</u>  | -0.500391 | 0 |
| 19 | mt-Nd1  | MTND1  | <u>ND1</u> | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 1</u>  | -0.312574 | 0 |
| 20 | mt-Nd2  | MTND2  | <u>ND2</u> | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 2</u>  | -0.345866 | 0 |
| 21 | mt-Nd3  | <u>MTND3</u>   | <u>ND3</u> | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 3</u>  | -0.253271 | 0 |
| 22 | mt-Nd4  | MTND4  | ND4        | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 4</u>  | -0.37974  | 0 |
| 23 | mt-Nd4l | MTND4L   | ND4L       | <u>NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase</u><br><u>chain 4L</u> | -0.431259 | 0 |

| 24 |         | <u>MTND6</u> | <u>ND6</u>     | NADH-<br>ubiquinone<br>oxidoreductase<br>chain 6                                   |           |   |
|----|---------|--------------|----------------|--|-----------|---|
| 25 | Ndufa1  | NDUFA1       | <u>Ndufa1</u>  | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 1        | -0.638382 | 0 |
| 26 | Ndufa10 | NDUFA10      | <u>Ndufa10</u> | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 10,      | -0.526248 | 0 |
| 27 | Ndufa11 | NDUFA11      | <u>Ndufa11</u> | NADH_<br>dehydrogenase_<br>[ubiquinone] 1_<br>alpha_<br>subcomplex_<br>subunit 11_ | -0.400389 | 0 |
| 28 | Ndufa13 | NDUFA13      | <u>Ndufa13</u> | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 13       | -0.500268 | 0 |
| 29 | Ndufa2  | NDUFA2       | <u>Ndufa2</u>  | NADH_<br>dehydrogenase_<br>[ubiquinone] 1_<br>alpha_<br>subcomplex_<br>subunit 2   | -0.645568 | 0 |
| 30 | Ndufa3  | NDUFA3       | <u>Ndufa3</u>  | NADH_<br>dehydrogenase<br>[ubiquinone] 1_<br>alpha_<br>subcomplex_<br>subunit 3    | -0.633872 | 0 |
| 31 | Ndufa4  | NDUFA4       | <u>Ndufa4</u>  | Cytochrome c<br>oxidase subunit<br>NDUFA4  | -0.647713 | 0 |

| 32 | Ndufa5  | NDUFA5        | <u>Ndufa5</u>  | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 5                   | -0.469147 | 0 |
|----|---------|---------------|----------------|---|-----------|---|
| 33 | Ndufa6  | NDUFA6        | <u>Ndufa6</u>  | NADH_<br>dehydrogenase<br>[ubiquinone] 1_<br>alpha_<br>subcomplex_<br>subunit 6               | -0.687919 | 0 |
| 34 | Ndufa7  | NDUFA7        | <u>Ndufa7</u>  | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 7                   | -0.55122  | 0 |
| 35 | Ndufa8  | <u>NDUFA8</u> | <u>Ndufa8</u>  | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 8                   | -0.409007 | 0 |
| 36 | Ndufa9  | NDUFA9        | <u>Ndufa9</u>  | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>alpha<br>subcomplex<br>subunit 9,<br>mitochondrial | -0.503337 | 0 |
| 37 | Ndufab1 | NDUFAB1       | <u>Ndufab1</u> | <u>Acyl carrier</u><br>protein,<br>mitochondrial  | -0.534754 | 0 |
| 38 | Ndufb10 | NDUFB10       | Ndufb10        | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 10                   | -0.461899 | 0 |

| 39 | Ndufb2<br>Ndufb3 | NDUFB2 | Ndufb2<br>Ndufb3 | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 2,<br>mitochondrial<br>NADH | -0.498395<br>-0.4932 | 0 |
|----|------------------|--------|------------------|--|----------------------|---|
|    |                  |        |                  | dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 3                                   |                      |   |
| 41 | Ndufb4           | NDUFB4 | <u>Ndufb4</u>    | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 4                           | -0.581655            | 0 |
| 42 | Ndufb4           | NDUFB4 | <u>Ndufb4b</u>   | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 4                           | -0.581655            | 0 |
| 43 | Ndufb5           | NDUFB5 | <u>Ndufb5</u>    | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 5,<br>mitochondrial         | -0.53372             | 0 |
| 44 | Ndufb6           | NDUFB6 | <u>Ndufb6</u>    | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 6                           | -0.519488            | 0 |
| 45 | Ndufb7           | NDUFB7 | <u>Ndufb7</u>    | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 7                           | -0.374883            | 0 |

| 46 | Ndufb8 | NDUFB8        | <u>Ndufb8</u> | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 8,<br>mitochondrial | -0.523241 | 0 |
|----|--------|---------------|---------------|--|-----------|---|
| 47 | Ndufb9 | <u>NDUFB9</u> | <u>Ndufb9</u> | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>beta<br>subcomplex<br>subunit 9                   | -0.530734 | 0 |
| 48 | Ndufc1 | NDUFC1        | <u>Ndufc1</u> | NADH<br>dehydrogenase<br>[ubiquinone] 1<br>subunit C1,<br>mitochondrial                      | -0.655074 | 0 |
| 49 | Ndufc2 | NDUFC2        | Ndufc2        | NADH_<br>dehydrogenase<br>[ubiquinone] 1_<br>subunit C2                                      | -0.485233 | 0 |
| 50 | Ndufs1 | NDUFS1        | <u>Ndufs1</u> | NADH-<br>ubiquinone<br>oxidoreductase<br>75 kDa subunit,<br>mitochondrial                    | -0.641851 | 0 |
| 51 | Ndufs2 | <u>NDUFS2</u> | <u>Ndufs2</u> | NADH<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 2,<br>mitochondrial            | -0.57924  | 0 |
| 52 | Ndufs3 | NDUFS3        | Ndufs3        | NADH<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 3,<br>mitochondrial            | -0.438247 | 0 |

| 53 | Ndufs4 | NDUFS4        | <u>Ndufs4</u> | NADH<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 4,<br>mitochondrial  | -0.477776 | 0 |
|----|--------|---------------|---------------|--|-----------|---|
| 54 | Ndufs5 | NDUFS5        | <u>Ndufs5</u> | NADH<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 5                    | -0.413575 | 0 |
| 55 | Ndufs6 | <u>NDUFS6</u> | <u>Ndufs6</u> | NADH<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 6,<br>mitochondrial  | -0.567075 | 0 |
| 56 | Ndufs7 | <u>NDUFS7</u> | <u>Ndufs7</u> | NADH_<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 7,<br>mitochondrial | -0.442479 | 0 |
| 57 | Ndufs8 | NDUFS8        | <u>Ndufs8</u> | NADH_<br>dehydrogenase<br>[ubiquinone] iron-<br>sulfur protein 8,<br>mitochondrial | -0.432103 | 0 |
| 58 | Ndufv1 | NDUFV1        | <u>Ndufv1</u> | NADH<br>dehydrogenase<br>[ubiquinone]<br>flavoprotein 1,<br>mitochondrial          | -0.613443 | 0 |
| 59 | Ndufv2 | NDUFV2        | <u>Ndufv2</u> | NADH<br>dehydrogenase<br>[ubiquinone]<br>flavoprotein 2,<br>mitochondrial          | -0.561687 | 0 |
| 60 | Ndufv3 | NDUFV3        | <u>Ndufv3</u> | NADH<br>dehydrogenase<br>[ubiquinone]<br>flavoprotein 3,<br>mitochondrial          | -0.53266  | 0 |

| 61 |         | $\frac{O(.2) + 2}{Decaprenyl-6}$<br>methoxyphenol<br>= H(.2)O + 2-<br>Decaprenyl-6-<br>methoxy-1,4-<br>benzoquinone |            |           |   |
|----|---------|---|------------|-----------|---|
| 62 | mt-Nd1  | <u>Respiratory</u><br><u>Complex I</u><br>(NADH-<br><u>ubiquinone</u><br><u>oxidoreductase</u> )                    | <u>ND1</u> | -0.312574 | 0 |
| 63 | mt-Nd2  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)   | <u>ND2</u> | -0.345866 | 0 |
| 64 | mt-Nd3  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)   | <u>ND3</u> | -0.253271 | 0 |
| 65 | mt-Nd4  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)   | <u>ND4</u> | -0.37974  | 0 |
| 66 | mt-Nd4l | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)   | ND4L       | -0.431259 | 0 |
| 67 | mt-Nd5  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)   | <u>ND5</u> | -0.500391 | 0 |

| 68 |         | Respiratory   | ND6             |           |   |
|----|---------|---|-----------------|-----------|---|
| 08 |         | <u>Complex I</u><br>(NADH-<br>ubiquinone<br>oxidoreductase)         |                 |           |   |
| 69 | Ndufa1  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa1</u>   | -0.638382 | 0 |
| 70 | Ndufa10 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa10</u>  | -0.526248 | 0 |
| 71 | Ndufa11 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa11b</u> | -0.400389 | 0 |
| 72 | Ndufa12 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa12</u>  | -0.490431 | 0 |
| 73 | Ndufa13 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufa13         | -0.500268 | 0 |
| 74 | Ndufa2  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa2</u>   | -0.645568 | 0 |
| 75 | Ndufa3  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufa3</u>   | -0.633872 | 0 |

| 76 | Ndufa4  | Respiratory<br>Complex I  | Ndufa4         | -0.647713 | 0 |
|----|---------|---|----------------|-----------|---|
|    |         | <u>(NADH-</u><br><u>ubiquinone</u><br><u>oxidoreductase)</u>                                    |                |           |   |
| 77 | Ndufa5  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufa5</u>  | -0.469147 | 0 |
| 78 | Ndufa6  | <u>Respiratory</u><br><u>Complex I</u><br>(NADH-<br><u>ubiquinone</u><br><u>oxidoreductase)</u> | <u>Ndufa6</u>  | -0.687919 | 0 |
| 79 | Ndufa7  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufa7</u>  | -0.55122  | 0 |
| 80 | Ndufa8  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufa8</u>  | -0.409007 | 0 |
| 81 | Ndufa9  | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufa9</u>  | -0.503337 | 0 |
| 82 | Ndufab1 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufab1</u> | -0.534754 | 0 |
| 83 | Ndufb10 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase)                             | <u>Ndufb10</u> | -0.461899 | 0 |

| 84 | Ndufb11 | <b>Respiratory</b>           | Ndufb11 | -0.53213     | 0 |
|----|---------|------------------------------|---------|--------------|---|
|    |         | Complex I                    |         |              |   |
|    |         | (NADH-                       |         |              |   |
|    |         | <u>ubiquinone</u>            |         |              |   |
|    |         |                              |         |              |   |
| 85 | Ndufb2  | Respiratory                  | Ndufb2  | -0.498395    | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | (NADH-                       |         |              |   |
|    |         | <u>ubiquinone</u>            |         |              |   |
|    |         |                              |         |              |   |
| 86 | Ndufb3  | Respiratory                  | Ndufb3  | -0.4932      | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | (NADH-                       |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |
| 87 | Ndufb4  | Respiratory                  | Ndufb4  | -0.581655    | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | <u>(NADH-</u>                |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |
| 88 | Ndufb4  | Respiratory                  | Ndufb4b | -0.581655    | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | <u>(INADH-</u><br>ubiquipope |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |
| 89 | Ndufb5  | Respiratory                  | Ndufb5  | <br>-0.53372 | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | (NADH-                       |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |
| 90 | Ndufb6  | Respiratory                  | Ndufb6  | -0.519488    | 0 |
|    |         | Complex I                    |         |              |   |
|    |         | (NADH-<br>ubiquipope         |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |
| 91 | Ndufb7  | Respiratory                  | Ndufb7  | -0.374883    | 0 |
|    |         | (NADH-                       |         |              |   |
|    |         | ubiquinone                   |         |              |   |
|    |         | oxidoreductase)              |         |              |   |
|    |         |                              |         |              |   |

| 92 | Ndufb8 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufb8        | -0.523241 | 0 |
|----|--------|---|---------------|-----------|---|
| 93 | Ndufb9 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufb9        | -0.530734 | 0 |
| 94 | Ndufc1 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufc1</u> | -0.655074 | 0 |
| 95 | Ndufc2 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufc2        | -0.485233 | 0 |
| 96 | Ndufs1 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs1</u> | -0.641851 | 0 |
| 97 | Ndufs2 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs2</u> | -0.57924  | 0 |
| 98 | Ndufs3 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufs3        | -0.438247 | 0 |
| 99 | Ndufs4 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | Ndufs4        | -0.477776 | 0 |

| 100 | Ndufs5 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs5</u> | -0.413575 | 0 |
|-----|--------|---|---------------|-----------|---|
| 101 | Ndufs6 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs6</u> | -0.567075 | 0 |
| 102 | Ndufs7 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs7</u> | -0.442479 | 0 |
| 103 | Ndufs8 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufs8</u> | -0.432103 | 0 |
| 104 | Ndufv1 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufv1</u> | -0.613443 | 0 |
| 105 | Ndufv2 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufv2</u> | -0.561687 | 0 |
| 106 | Ndufv3 | Respiratory<br>Complex I<br>(NADH-<br>ubiquinone<br>oxidoreductase) | <u>Ndufv3</u> | -0.53266  | 0 |

| 107 | <u>S-Adenosyl-L-</u><br><u>methionine + 2-</u><br><u>Decaprenyl-6-</u><br><u>methoxy-1,4-</u><br><u>benzoquinone =</u><br><u>S-Adenosyl-L-</u><br><u>homocysteine +</u><br><u>2-decaprenyl-3-</u><br><u>methyl-6-</u><br><u>methoxy-1,4-</u><br><u>benzoquinone</u> |            |   |  |
|-----|---|------------|---|--|
| 108 | <u>S-Adenosyl-L-</u><br><u>methionine + 3-</u><br><u>Decaprenyl-4,5-</u><br><u>dihydroxybenzoa</u><br><u>te = S-Adenosyl-</u><br><u>L-homocysteine</u><br><u>+ 3-Decaprenyl-</u><br><u>4-hydroxy-5-</u><br><u>methoxybenzoat</u><br><u>e</u>                        |            |   |  |
| 109 | S-Adenosyl-L-<br>methionine +<br>demethyl-<br>Coenzyme-Q10<br>= Coenzyme-<br>Q10 + S-<br>Adenosyl-L-<br>homocysteine  |            |   |  |
| 110 | <u>TAT</u>  | <u>Tat</u> | <u>Tyrosine</u><br><u>aminotransferas</u><br><u>e</u> |  |
| 111 | Ubiquinol-10<br>intracellular<br>anatomical<br>structure  |            | <u>Ubiquinol-10</u>                                   |  |

|     |      |   |             | 1  |           |   |
|-----|------|---|-------------|--|-----------|---|
| 112 |      | all-trans-<br><u>Hexaprenyl</u><br><u>diphosphate + 4-</u><br><u>Hydroxy-benzoic</u><br><u>acid =</u><br><u>Pyrophosphate</u><br><u>+ 3-Hexaprenyl-</u><br><u>4-hydroxy-</u><br><u>benzoic acid</u><br>all-trans- |             | all-trans-   |           |   |
|     |      | Hexaprenyl<br>diphosphate<br>intracellular<br>anatomical<br>structure   |             | <u>Hexaprenyl</u><br>diphosphate                                     |           |   |
| 114 | Coq2 | <u>coenzyme Q2</u><br><u>homolog,</u><br><u>prenyltransferas</u><br><u>e (yeast)</u>  | <u>Coq2</u> | <u>4-</u><br>hydroxybenzoat<br><u>e</u><br>polyprenyltransf<br>erase | -0.364377 | 0 |
| 115 |      | demethyl-<br>Coenzyme-Q10<br>intracellular<br>anatomical<br>structure   |             | <u>demethyl-</u><br><u>Coenzyme-Q10</u>                              |           |   |
| 116 |      | p-Coumaric acid<br>extracellular<br>region  |             | <u>p-Coumaric acid</u>   |           |   |
| 117 |      | <u>p-</u><br><u>Hydroxyphenylla</u><br><u>ctic acid</u><br>intracellular<br>anatomical<br><u>structure</u>  |             | <u>p-</u><br><u>Hydroxyphenylla</u><br><u>ctic acid</u>              |           |   |
| 118 |      | <u>p-</u><br><u>Hydroxyphenylp</u><br><u>yruvic acid</u><br><u>intracellular</u><br><u>anatomical</u><br><u>structure</u>   |             | <u>p-</u><br><u>Hydroxyphenylp</u><br><u>yruvic acid</u>             |           |   |