**Supplementary data:**

**Suppl. Figure 1: Spontaneous motor activity of DM at ages of 3 to 18 months.**

Documentation of spontaneous open-field behaviour as the most sensitive and reliable measure of abnormal movements in PD mouse models revealed highly significant marked reductions of horizontal activity, vertical activity, and stereotype movements for the DM mice relative to their appropriate WT controls, already at the age of 3 months (columns at the left side). While this early onset low exploration activity of DM animals remained quite constant across their lifespan, the corresponding F1-hybrid controls showed later age-progressive reduction in spontaneous motor activity.

**Suppl. Figure 2: pSer129-SNCA immunohistochemistry of DM mice at ages 15-17 months.**

Bright field immunohistochemical stainings of pSer129-SNCA-immunoreactivity were performed in old DM mice with paralysis (A-C) or without paralysis (D/E). In paralytic DM animals, (A) thoracic / cervical levels of the spinal cord showed milder stages of inclusion formation (black arrows), (B) motor cortex showed discrete aggregation pathology (black arrows), while (C) striatum remained free of detectable pathology. In non-paralytic old DM mice, (D) the lumbar spinal cord showed mild inclusion pathology with only few immunopositive neurites (black arrow), and (E) the ventral tegmental area of the midbrain also showed only discrete aggregation (black arrow).

**Suppl. Figure 3: Immunohistochemistry of Pink1<sup>−/−</sup> mice at ages 15-17 months.**

Bright field immunohistochemical stainings were performed in age-matched Pink1<sup>−/−</sup> spinal cord as a control for Figure 4 spinal cord (upper panels) and for Figure 5 midbrain (lower panels). Protein aggregation was not detected by antibodies against pSer129-SNCA, p62 or ubiquitin.

**Suppl. Figure 4: Double immunofluorescence in DM midbrain beyond age 1 year demonstrates aggregates in non-dopaminergic cells.**

The colocalization with TH as a marker of midbrain dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNC) and the ventral tegmental area (VTA) was assessed. The 4B12 antibody detects human
alpha-synuclein selectively and demonstrates transgenic overexpression with nuclear staining throughout the midbrain DAergic neurons (A-C, white arrows). However, the pSer129-SNCA staining shows the protein aggregation to occur preferentially in proximity rather than in DAergic SN / VTA neurons (D-F). The p62 staining shows also protein aggregates again in neurons adjacent to DAergic cells (G-I). Similarly, ubiquitin staining in spite of high background immunoreactivity localizes the protein aggregates mostly outside the DAergic neurons (J-L). In low magnifications of all three stainings, the aggregates formed in a band of neurons and neurites just dorsal to the SN / VTA line of DAergic nerve cells.

**Suppl. Figure 5: Double immunofluorescence in DM midbrain beyond age 1 year demonstrates aggregates to occur not in astrocytes, but in neurons.**

The pSer129-SNCA staining in the VTA of aged DM mice was assessed by double immunofluorescence for colocalization with markers of glial and neuronal differentiation. GFAP (glial fibrillary acidic protein) as a marker of astrocytes clearly was not present in the cells affected by aggregates (A-C), while NeuN (neuronal nuclear antigen Rbfox3) as a general marker of neurons did colocalize with these cells (D-F, white arrows). Parvalbumin staining as a marker of interneurons produced immunoreactivity in multiple affected cells (G-I, white arrows), while GAD65 (glutamate decarboxylase encoded by GAD2) as a marker of GABAergic neurons were not present in the cells with aggregates (J-L), and VGLUT2 (vesicular glutamate transporter 2) as a marker of subcortical glutamatergic neurons was observed only in individual aggregate-containing cells (M-O, white arrows).

**Suppl. Figure 6: Global transcriptome GSEA downregulation for DM cerebellum at age 6 weeks.**

Details for the NOUSHMEHR_GBM_SILENCED_BY_METHYLATION gene set.

**Suppl. Figure 7: Global transcriptome GSEA upregulation for DM cerebellum at age 6 weeks.** Details for the NAGASHIMA_EGF_SIGNALING_UP gene set.

**Suppl. Figure 8: Global transcriptome GSEA upregulation for DM cerebellum at age 6 months.**

Details for the NAGASHIMA_EGF_SIGNALING_UP gene set.
Suppl. Figure 9: Global transcriptome GSEA downregulation for DM cerebellum at age 6 months. Details for the MITOCHONDRIAL_MATRIX gene set.

Suppl. Figure 10: Global transcriptome GSEA downregulation for DM midbrain at age 6 months. Details for the QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2 gene set.

Suppl. Figure 11: Global transcriptome GSEA upregulation for DM midbrain at age 6 months. Details for the PID_ATM_PATHWAY gene set.

Suppl. Movie 1: Progressive paralysis of DM mice beyond age 1 year.
The progressive phenotype of locomotor deficits was documented by video for several DM mice at ages beyond 1 year, before they had to be sacrificed to avoid animal suffering. Later their tissue was found to exhibit alpha-synuclein- / p62- / ubiquitin- positive aggregates in immunohistochemistry.

Suppl. Table 1: Global ubiquitination changes of DM brain hemispheres at age 18 months.
(A) Primary UbiScan® results are represented. The average fold change is provided in column “/- TgA : Control”, while the fold change for each of the 3 DM:WT-F1-hybrid pairs is shown in the subsequent columns. Dysregulation of ubiquitination sites with increase above 2.0-fold were illustrated in green, while sites with decrease below -2.0-fold were highlighted in red (see a list in Excel Tab: Fold Change by Protein Type). Fold changes should be more accurate with higher maximal intensity values. Peak measurements reflect either area or height. An index number from the Details tab is used for easy lookup of peptides between tabs. Blue letters in Gene Names indicate siRNA products commercially available; in Protein Names they indicate site-specific antibodies commercially available. Within sequences in the peptide column, the ubiquitination site is indicated by *, while oxidized methionine is indicated by #. Count in Details refers to the number of MS/MS identifications in the Details tab for each protein/site. Average RT refers to average retention time for each peptide. In the Raw Intensity column, bold letters indicate intensities manually reviewed, while red letters indicate multiple identifications for 1 m/z measurement due
to ambiguous methylation site localization. The Normalized Log 2 Ratios were calculated by subtracting the median Log2 fold change for each comparison. The Details tab contains the MS/MS (blue box) and MS (red box) scan numbers, the Retention Time, the Xcorr values, the measured peptide mass accuracy, the DeltaCN value, the Rsp value, and the Peptide Prophet Probability (PP Probability) as a reference and measure of confidence to each peptide assignment. (B) Bioinformatics information is provided on the UbiScan® primary data.

Suppl. Table 2: Global transcriptome changes of DM brain hemispheres at ages 6 weeks and 6 months.

The columns from left show the microarray spot identifier, the transcript symbol, the transcript name, the average expression level, the adjusted p-value and then significant down-regulations (-1, green) and up-regulations (+1, red) for different organs and ages: This decision matrix assesses cerebellum (Cbll), midbrain+brainstem (MidB), striatum (Str), at the ages 6 weeks and 6 months, comparing in each column 4 DM versus 4 WT-F1-hybrid tissues, with a cutoff value of at least logarithmic fold changes of ±0.6.

Suppl. Table 3: Global transcriptome bioinformatics by Gene Set Enrichment Analysis.

The automatized output from the GSEA software for each of the three brain tissues (midbrain/brainstem = MB, striatum = ST, cerebellum = CB) at two ages (6 months = 6M, 6 weeks = 6W), separated for upregulation and for downregulation, is shown in twelve datasheets of this file, in parameters defined by the Guide To Interpret Results at the GSEA internet page.
**Table: GSEA Results Summary**

| Dataset                        | CB_6W_collapsed_to_symbols.general.cls#mut-versus_WT |
|-------------------------------|-------------------------------------------------------|
| Phenotype                     | general.cls#mut-versus_WT                             |
| Upregulated in class          | WT                                                    |
| GeneSet                       | NOUSHMEHR_GBM_SILENCED_BY_METHYLATION                 |
| Enrichment Score (ES)         | -0.6654536                                           |
| Normalized Enrichment Score (NES) | -2.131186                                      |
| Nominal p-value               | 0.0                                                   |
| FDR q-value                   | 0.016506651                                           |
| FWER p-Value                  | 0.034                                                 |

**Enrichment plot: NOUSHMEHR_GBM_SILENCED_BY_METHYLATION**

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

**Table: GSEA details**

| PROBE | GENE SYMBOL   | GENE_TITLE                                          | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|---------------|-----------------------------------------------------|-------------------|-------------------|------------|-----------------|
| FLNC  | FLNC          | filamin C, gamma (actin binding protein 280)        | 1296              | 0.809             | -0.0611    | No              |
| LDHA  | LDHA          | lactate dehydrogenase A                             | 1780              | 0.665             | -0.0722    | No              |
| EPHX2 | EPHX2         | epoxide hydrolase 2, cytoplasmic                    | 2676              | 0.482             | -0.1168    | No              |

Fig 1: Enrichment plot: NOUSHMEHR_GBM_SILENCED_BY_METHYLATION

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List
| #  | Gene   | Entrez | Source | Description                                                                 | Score  | Log2 Fold Change | Influence |
|----|--------|--------|--------|------------------------------------------------------------------------------|--------|------------------|-----------|
| 4  | TOM1L1 |        |        | target of myb1-like 1 (chicken)                                             | 2843   | 0.457            | -0.1133   | No        |
| 5  | SEMA3E |        |        | sema domain, immunoglobulin domain (lg), short base domain, secreted, (semaphorin) 3E | 3037   | 0.427            | -0.1126   | No        |
| 6  | FABP5  |        |        | fatty acid binding protein 5 (psoriasis-associated)                         | 4221   | 0.271            | -0.1833   | No        |
| 7  | CBR1   |        |        | carbonyl reductase 1                                                         | 4389   | 0.253            | -0.1864   | No        |
| 8  | CA3    |        |        | carbonic anhydrase III, muscle specific                                      | 4473   | 0.245            | -0.1841   | No        |
| 9  | OCIAD2 |        |        | OCIA domain containing 2                                                    | 5023   | 0.187            | -0.2150   | No        |
| 10 | TRIP4  |        |        | thyroid hormone receptor interactor 4                                        | 5877   | 0.100            | -0.2690   | No        |
| 11 | G0S2   |        |        | G0/G1switch 2                                                                | 6786   | 0.017            | -0.3294   | No        |
| 12 | NMNAT3 |        |        | nicotinamide nucleotide adenyltransferase 3                                 | 7199   | -0.020           | -0.3564   | No        |
| 13 | MMP9   |        |        | matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) | 7309   | -0.032           | -0.3627   | No        |
| 14 | LGALS8 |        |        | lectin, galactoside-binding, soluble, 8 (galectin 8)                         | 8196   | -0.111           | -0.4187   | No        |
| 15 | CHI3L1 |        |        | chitinase 3-like 1 (cartilage glycoprotein-39)                               | 8699   | -0.156           | -0.4474   | No        |
| 16 | CHRDL2 |        |        | chordin-like 2                                                               | 9310   | -0.211           | -0.4815   | No        |
| 17 | RARRES1|        |        | retinoic acid receptor responder (tazarotene induced) 1                     | 10670  | -0.348           | -0.5616   | No        |
| 18 | SLC25A20|       |        | solute carrier family 25 (carnitine/acylcarnitine translocase), member 20   | 11064  | -0.391           | -0.5755   | No        |
| 19 | B3GNT5 |        |        | UDP-GlcNAc:betaGal beta-1,3-N-acetylgalcosaminyltransferase 5                | 11199  | -0.407           | -0.5714   | No        |
| 20 | FKBP5  |        |        | FK506 binding protein 5                                                       | 11914  | -0.508           | -0.6030   | No        |
| 21 | SLC39A12|       |        | solute carrier family 39 (zinc transporter), member 12                      | 12843  | -0.677           | -0.6436   | Yes       |
| 22 | RBP1   |        |        | retinol binding protein 1, cellular                                          | 13169  | -0.747           | -0.6415   | Yes       |
| 23 | PDPN   |        |        | podoplanin                                                                   | 13435  | -0.822           | -0.6330   | Yes       |
| 24 | FMOD   |        |        | fibromodulin                                                                 | 13637  | -0.882           | -0.6182   | Yes       |
|  | **Entrez** | **Source** | **Description** | **LogFC** | **Log2FoldChange** | **Adjusted p-value** | **Significance** |
|---|---|---|---|---|---|---|---|
| BIRC3 | BIRC3 | 13793 | 0.938 | 0.5986 | Yes |
| RARRES2 | RARRES2 | 13836 | 0.961 | 0.5706 | Yes |
| F3 | F3 | 14082 | 1.086 | 0.5522 | Yes |
| GJB2 | GJB2 | 14161 | 1.129 | 0.5213 | Yes |
| RAB36 | RAB36 | 14537 | 1.478 | 0.4991 | Yes |
| LGALS3 | LGALS3 | 14797 | 2.145 | 0.4478 | Yes |
| LECT1 | LECT1 | 14893 | 3.196 | 0.3518 | Yes |
| FZD6 | FZD6 | 14903 | 3.540 | 0.2390 | Yes |
| CTHRC1 | CTHRC1 | 14930 | 7.518 | 0.0001 | Yes |

Fig 2: NOUSHMEHR_GBM_SILENCED_BY_METHYLATION
Blue-Pink O' Gram in the Space of the Analyzed GeneSet
**Fig 3: NOUSHMEHR_GBM_SILENCED_BY_METHYLATION: Random ES distribution**

Gene set null distribution of ES for **NOUSHMEHR_GBM_SILENCED_BY_METHYLATION**
Table: GSEA Results Summary

| Dataset                          | CB_6W_collapsed_to_symbols.general.cls#mut_versus_WT |
|---------------------------------|-------------------------------------------------------|
| Phenotype                       | general.cls#mut_versus_WT                             |
| Upregulated in class            | mut                                                   |
| GeneSet                         | NAGASHIMA_EGF_SIGNALING_UP                             |
| Enrichment Score (ES)           | 0.6003276                                             |
| Normalized Enrichment Score (NES)| 2.117522                                              |
| Nominal p-value                 | 0.0                                                   |
| FDR q-value                     | 0.0585707                                             |
| FWER p-Value                    | 0.054                                                 |

Table: GSEA details

| PROBE | GENE SYMBOL | GENE TITLE                                                                 | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|-------------|----------------------------------------------------------------------------|-------------------|-------------------|------------|-----------------|
| 1     | NR4A1       | nuclear receptor subfamily 4, group A, member 1                            | 24                | 3.032             | 0.0923     | Yes             |

Fig 1: Enrichment plot: NAGASHIMA_EGF_SIGNALING_UP
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List
| # | Gene | Description | Entrez | Source | Fold Change | FDR | Status |
|---|------|-------------|--------|--------|-------------|-----|--------|
| 1 | JUNB | Jun B proto-oncogene | 28     | 2.903  | 0.1821      | Yes |
| 2 | EGR4 | Early growth response 4 | 211    | 1.682  | 0.2220      | Yes |
| 3 | IER3 | Immediate early response 3 | 256    | 1.566  | 0.2676      | Yes |
| 4 | FOS  | V-fos FBJ murine osteosarcoma viral oncogene homolog | 300    | 1.459  | 0.3099      | Yes |
| 5 | CTGF | Connective tissue growth factor | 328    | 1.415  | 0.3519      | Yes |
| 6 | EGR1 | Early growth response 1 | 412    | 1.296  | 0.3865      | Yes |
| 7 | PHLDA1 | Pleckstrin homology-like domain, family A, member 1 | 709    | 1.069  | 0.3998      | Yes |
| 8 | HBEFG | Heparin-binding EGF-like growth factor | 756    | 1.044  | 0.4291      | Yes |
| 9 | NAB2 | NGFI-A binding protein 2 | 813    | 1.015  | 0.4568      | Yes |
| 10| ID3  | Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | 835    | 1.005  | 0.4865      | Yes |
| 11| KLF10| Kruppel-like factor 10 | 839    | 1.002  | 0.5173      | Yes |
| 12| DUSP5| Dual specificity phosphatase 5 | 1136   | 0.875  | 0.5246      | Yes |
| 13| NR4A2| Nuclear receptor subfamily 4, group A, member 2 | 1210   | 0.845  | 0.5459      | Yes |
| 14| DUSP1| Dual specificity phosphatase 1 | 1362   | 0.784  | 0.5600      | Yes |
| 15| ARC  | Activity-regulated cytoskeleton-associated protein | 1455   | 0.755  | 0.5772      | Yes |
| 16| GEM  | GTP binding protein overexpressed in skeletal muscle | 1510   | 0.739  | 0.5965      | Yes |
| 17| HES1 | Hairy and enhancer of split 1, (Drosophila) | 1852   | 0.647  | 0.5936      | Yes |
| 18| DUSP2| Dual specificity phosphatase 2 | 2033   | 0.606  | 0.6003      | Yes |
| # | Gene Symbol | Gene Name | Description | #1 | #2 | #3 | #4 | Details |
|---|-------------|-----------|-------------|----|----|----|----|---------|
| 20 | TRIB1 | tribbles homolog 1 (Drosophila) | | 2435 | 0.525 | 0.5897 | No |
| 21 | EDN1 | endothelin 1 | | 2611 | 0.492 | 0.5932 | No |
| 22 | FOSB | FBJ murine osteosarcoma viral oncogene homolog B | | 2867 | 0.454 | 0.5901 | No |
| 23 | TNFRSF12A | tumor necrosis factor receptor superfamily, member 12A | | 3720 | 0.329 | 0.5431 | No |
| 24 | EGR2 | early growth response 2 (Krox-20 homolog, Drosophila) | | 4783 | 0.212 | 0.4783 | No |
| 25 | LIF | leukemia inhibitory factor (cholinergic differentiation factor) | | 5421 | 0.146 | 0.4401 | No |
| 26 | MYC | v-myc myelocytomatosis viral oncogene homolog (avian) | | 6559 | 0.039 | 0.3649 | No |
| 27 | TIPARP | TCDD-inducible poly(ADP-ribose) polymerase | | 6968 | 0.000 | 0.3375 | No |
| 28 | DLX2 | distal-less homeobox 2 | | 7206 | -0.021 | 0.3222 | No |
| 29 | MCL1 | myeloid cell leukemia sequence 1 (BCL2-related) | | 7478 | -0.046 | 0.3055 | No |
| 30 | MAFF | v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian) | | 7644 | -0.062 | 0.2963 | No |
| 31 | ID1 | inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | | 7860 | -0.081 | 0.2844 | No |
| 32 | KLF6 | Kruppel-like factor 6 | | 7906 | -0.085 | 0.2840 | No |
| 33 | IER2 | immediate early response 2 | | 8546 | -0.141 | 0.2455 | No |
| 34 | EPHA2 | EPH receptor A2 | | 9067 | -0.189 | 0.2164 | No |
| 35 | RYBP | RING1 and YY1 binding protein | | 9274 | -0.207 | 0.2090 | No |
| 36 | EGR3 | early growth response 3 | | 9709 | -0.248 | 0.1875 | No |
| 37 | DNAJB1 | DnaJ (Hsp40) homolog, subfamily B, member 1 | | 10485 | -0.330 | 0.1457 | No |
| 38 | SPRED2 | sploty-related, EVH1 domain containing 2 | 11647 | -0.469 | 0.0823 | No |
| 39 | ETS2 | v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) | 12973 | -0.704 | 0.0151 | No |
| 40 | NEDD9 | neural precursor cell expressed, developmentally down-regulated 9 | 13252 | -0.769 | 0.0202 | No |
| 41 | ZFP36 | zinc finger protein 36, C3H type, homolog (mouse) | 13351 | -0.797 | 0.0383 | No |
| 42 | CYR61 | cysteine-rich, angiogenic inducer, 61 | 14080 | -1.086 | 0.0231 | No |
| 43 | JUN | jun oncogene | 14109 | -1.099 | 0.0553 | No |

Fig 2: NAGASHIMA_EGF_SIGNALING_UP
Blue-Pink O’ Gram in the Space of the Analyzed GeneSet
Fig 3: NAGASHIMA_EGF_SIGNALING_UP: Random ES distribution
Gene set null distribution of ES for NAGASHIMA_EGF_SIGNALING_UP
### Table: GSEA Results Summary

|               | Value                      |
|---------------|---------------------------|
| Dataset       | CB_6M_collapsed_to_symbols.general.cls#mut_versus_WT |
| Phenotype     | general.cls#mut_versus_WT  |
| Upregulated in class | mut                     |
| GeneSet       | NAGASHIMA_EGF_SIGNALING_UP |
| Enrichment Score (ES) | 0.62230736                |
| Normalized Enrichment Score (NES) | 2.2834282                 |
| Nominal p-value | 0.0                      |
| FDR q-value   | 0.0018626772              |
| FWER p-Value  | 0.0030                    |

#### Table: GSEA details [plain text format]

| PROBE | GENE SYMBOL | GENE_TITLE                                      | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|-------------|-------------------------------------------------|-------------------|-------------------|------------|------------------|
| NR4A2 | NR4A2       | nuclear receptor subfamily 4, group A, member 2 | 99                | 2.157             | 0.0564     | Yes              |

**Fig 1: Enrichment plot: NAGASHIMA_EGF_SIGNALING_UP**

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List
| #  | Gene   | Description                                                                 | Score | Adjusted | Significant? |
|----|--------|-----------------------------------------------------------------------------|-------|----------|--------------|
| 2  | CTGF   | connective tissue growth factor                                              | 304   | 1.598    | Yes          |
| 3  | FOS    | v-fos FBJ murine osteosarcoma viral oncogene homolog                         | 347   | 1.544    | Yes          |
| 4  | NAB2   | NGFI-A binding protein 2 (EGR1 binding protein 2)                            | 414   | 1.480    | Yes          |
| 5  | NR4A1  | nuclear receptor subfamily 4, group A, member 1                              | 424   | 1.467    | Yes          |
| 6  | DUSP5  | dual specificity phosphatase 5                                               | 430   | 1.461    | Yes          |
| 7  | DNAJB1 | DnaJ (Hsp40) homolog, subfamily B, member 1                                  | 448   | 1.446    | Yes          |
| 8  | DUSP1  | dual specificity phosphatase 1                                               | 489   | 1.408    | Yes          |
| 9  | EPHA2  | EPH receptor A2                                                             | 654   | 1.289    | Yes          |
| 10 | IER3   | immediate early response 3                                                   | 710   | 1.249    | Yes          |
| 11 | IER2   | immediate early response 2                                                   | 742   | 1.230    | Yes          |
| 12 | EGR1   | early growth response 1                                                      | 770   | 1.213    | Yes          |
| 13 | KLF10  | Kruppel-like factor 10                                                       | 923   | 1.122    | Yes          |
| 14 | FOSB   | FBJ murine osteosarcoma viral oncogene homolog B                             | 944   | 1.113    | Yes          |
| 15 | PHLDA1 | pleckstrin homology-like domain, family A, member 1                          | 977   | 1.093    | Yes          |
| 16 | EDN1   | endothelin 1                                                                 | 990   | 1.090    | Yes          |
| 17 | TIPARP | TCDD-inducible poly(ADP-ribose) polymerase                                  | 1041  | 1.066    | Yes          |
| 18 | KLF6   | Kruppel-like factor 6                                                        | 1430  | 0.912    | Yes          |
| 19 | LIF    | leukemia inhibitory factor (cholinergic differentiation factor)              | 1883  | 0.778    | Yes          |
|   | Gene   | Description                                      | GeneID | FDR   | PA   | Regulator |
|---|---------|--------------------------------------------------|--------|-------|------|-----------|
| 20| EGR4    | early growth response 4                         | 2142   | 0.708 | 0.6000 | Yes       |
| 21| ARC     | activity-regulated cytoskeleton-associated protein | 2197   | 0.695 | 0.6166 | Yes       |
| 22| TRIB1   | tribbles homolog 1 (Drosophila)                 | 2398   | 0.654 | 0.6223 | Yes       |
| 23| HBEGF   | heparin-binding EGF-like growth factor           | 2915   | 0.546 | 0.6036 | No        |
| 24| SPRED2  | sprouty-related, EVH1 domain containing 2       | 3941   | 0.376 | 0.5457 | No        |
| 25| TNFRSF12A | tumor necrosis factor receptor superfamily, member 12A | 4064   | 0.358 | 0.5480 | No        |
| 26| RYBP    | RING1 and YY1 binding protein                    | 4342   | 0.319 | 0.5387 | No        |
| 27| GEM     | GTP binding protein overexpressed in skeletal muscle | 4516   | 0.296 | 0.5357 | No        |
| 28| ETS2    | v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) | 5212   | 0.211 | 0.4952 | No        |
| 29| CYR61   | cysteine-rich, angiogenic inducer, 61            | 5829   | 0.138 | 0.4579 | No        |
| 30| JUN     | jun oncogene                                     | 6104   | 0.107 | 0.4426 | No        |
| 31| MCL1    | myeloid cell leukemia sequence 1 (BCL2-related)  | 7147   | -0.008 | 0.3729 | No        |
| 32| DLX2    | distal-less homeobox 2                          | 7529   | -0.049 | 0.3487 | No        |
| 33| ZFP36   | zinc finger protein 36, C3H type, homolog (mouse) | 7970   | -0.099 | 0.3221 | No        |
| 34| EGR3    | early growth response 3                         | 8269   | -0.127 | 0.3058 | No        |
| 35| JUNB    | jun B proto-oncogene                             | 8290   | -0.130 | 0.3082 | No        |
| 36| MYC     | v-myc myelocytomatosis viral oncogene homolog (avian) | 8331   | -0.134 | 0.3094 | No        |
| 37| NEDD9   | neural precursor cell expressed, developmentally down-regulated 9 | 9193   | -0.228 | 0.2583 | No        |
|   | Gene ID | Gene Symbol | Source | Description | FC | P.Value | Status |
|---|---------|-------------|--------|-------------|----|---------|--------|
| 38 | MAFF   | MAFF        | Entrez, Source | v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian) | 11825 | -0.575 | No |
| 39 | EGR2   | EGR2        | Entrez, Source | early growth response 2 (Krox-20 homolog, Drosophila) | 12645 | -0.724 | No |
| 40 | HES1   | HES1        | Entrez, Source | hairy and enhancer of split 1, (Drosophila) | 12668 | -0.729 | No |
| 41 | ID3    | ID3         | Entrez, Source | inhibitor of DNA binding 3, dominant negative helix-loop-helix protein | 12777 | -0.750 | No |
| 42 | ID1    | ID1         | Entrez, Source | inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | 12818 | -0.758 | No |
| 43 | DUSP2  | DUSP2       | Entrez, Source | dual specificity phosphatase 2 | 13012 | -0.803 | No |

Fig 2: NAGASHIMA_EGF_SIGNALING_UP
Blue-Pink O' Gram in the Space of the Analyzed GeneSet

Details for gene set NAGASHIMA_EGF_SIGNALING_UP [GSEA]
file:///server2/shared/Neurogenetik/ProjectsGrantsManuscripts/micro...
Fig 3: NAGASHIMA_EGF_SIGNALING_UP: Random ES distribution

Gene set null distribution of ES for NAGASHIMA_EGF_SIGNALING_UP
### Table: GSEA Results Summary

| Dataset                          | CB_6M_collapsed_to_symbols.general.cls#mut_versus_WT |
|---------------------------------|------------------------------------------------------|
| Phenotype                       | general.cls#mut_versus_WT                            |
| Upregulated in class            | WT                                                   |
| GeneSet                         | MITOCHONDRIAL_MATRIX                                 |
| Enrichment Score (ES)           | -0.66644275                                         |
| Normalized Enrichment Score (NES)| 2.174532                                            |
| Nominal p-value                 | 0.0                                                  |
| FDR q-value                     | 0.014642635                                         |
| FWER p-Value                    | 0.018                                                |

### Table: GSEA details [plain text format]

| PROBE | GENE SYMBOL | GENE TITLE                                                                 | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|-------------|---------------------------------------------------------------------------|-------------------|-------------------|------------|-----------------|
| 1     | NR3C1       | nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) | 5133              | 0.219             | -0.3321    | No              |
| 2     | MRPS22      | mitochondrial ribosomal protein S22                                       | 5613              | 0.164             | -0.3550    | No              |
| 3     | CASQ1       | calsequestrin 1 (fast-twitch, skeletal muscle)                            | 5732              | 0.150             | -0.3545    | No              |

### Fig 1: Enrichment plot: MITOCHONDRIAL_MATRIX

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List
| # | Gene  | Symbol  | Description                                                                 | Entrez | Source                      | Fold Change | Adj Fold Change | Significance |
|---|-------|---------|------------------------------------------------------------------------------|--------|-----------------------------|-------------|-----------------|--------------|
| 4 | MRPS11| Entrez  | mitochondrial ribosomal protein S11                                         | 5807   | Entrez                      | 0.141       | -0.3516         | No           |
| 5 | CS    | Entrez  | citrate synthase                                                            | 6927   | Entrez                      | 0.018       | -0.4257         | No           |
| 6 | POLG2 | Entrez  | polymerase (DNA directed), gamma 2, accessory subunit                        | 7290   | Entrez                      | -0.023      | -0.4487         | No           |
| 7 | NFS1  | Entrez  | NFS1 nitrogen fixation 1 (S. cerevisiae)                                    | 7731   | Entrez                      | -0.071      | -0.4742         | No           |
| 8 | PITRM1| Entrez  | pitrilysin metallopeptidase 1                                               | 8090   | Entrez                      | -0.110      | -0.4921         | No           |
| 9 | MRPS15| Entrez  | mitochondrial ribosomal protein S15                                         | 10064  | Entrez                      | -0.327      | -0.6061         | No           |
| 10| TIMM44| Entrez  | translocase of inner mitochondrial membrane 44 homolog (yeast)              | 10635  | Entrez                      | -0.401      | -0.6219         | No           |
| 11| NDUFAB1| Entrez | NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa          | 11300  | Entrez                      | -0.492      | -0.6389         | Yes          |
| 12| MRPL41| Entrez  | mitochondrial ribosomal protein L41                                         | 11553  | Entrez                      | -0.530      | -0.6260         | Yes          |
| 13| MRPS10| Entrez  | mitochondrial ribosomal protein S10                                         | 11689  | Entrez                      | -0.553      | -0.6041         | Yes          |
| 14| MRPS16| Entrez  | mitochondrial ribosomal protein S16                                         | 11735  | Entrez                      | -0.561      | -0.5756         | Yes          |
| 15| MRPL52| Entrez  | mitochondrial ribosomal protein L52                                         | 11972  | Entrez                      | -0.598      | -0.5579         | Yes          |
| 16| ATP5F1| Entrez  | ATP synthase, H+ transporting, mitochondrial F0 complex, subunit B1         | 12087  | Entrez                      | -0.617      | -0.5309         | Yes          |
| 17| MRPL55| Entrez  | mitochondrial ribosomal protein L55                                         | 12199  | Entrez                      | -0.638      | -0.5026         | Yes          |
| 18| BCKDHB| Entrez  | branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease) | 12567 | Entrez                      | -0.709      | -0.4875         | Yes          |
| 19| MRPS18A| Entrez | mitochondrial ribosomal protein S18A                                        | 12653  | Entrez                      | -0.726      | -0.4525         | Yes          |
| 20| MRPL10| Entrez  | mitochondrial ribosomal protein L10                                         | 12687  | Entrez                      | -0.732      | -0.4136         | Yes          |
| 21| ETFA  | Entrez  | electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)    | 12887  | Entrez                      | -0.773      | -0.3836         | Yes          |
| 22| MRPL40| Entrez  | mitochondrial ribosomal protein L40                                         | 12903  | Entrez                      | -0.778      | -0.3410         | Yes          |
| 23| MRPS21| Entrez  | mitochondrial ribosomal protein S21                                         | 13040  | Entrez                      | -0.810      | -0.3047         | Yes          |
|   | Gene Symbol | Description                                                                 | Score 1 | Score 2 | Fold Change | P-value | Expression |
|---|-------------|-----------------------------------------------------------------------------|---------|---------|-------------|---------|------------|
| 24 | BCKDHA      | branched chain keto acid dehydrogenase E1, alpha polypeptide                | 13063   | -0.815 | -0.2604     | -0.815 | Yes        |
| 25 | MRPL51      | mitochondrial ribosomal protein L51                                        | 13132   | -0.837 | -0.2181     | -0.837 | Yes        |
| 26 | DNAJA3      | DnaJ (Hsp40) homolog, subfamily A, member 3                                 | 13866   | -1.077 | -0.2068     | -1.077 | Yes        |
| 27 | DBT         | dihydrolipoamide branched chain transacylase E2                             | 14644   | -1.712 | -0.1630     | -1.712 | Yes        |
| 28 | ALDH4A1     | aldehyde dehydrogenase 4 family, member A1                                 | 14903   | -3.250 | 0.0019      | -3.250 | Yes        |

**Fig 2: MITOCHONDRIAL_MATRIX**

Blue-Pink O’ Gram in the Space of the Analyzed GeneSet
Fig 3: MITOCHONDRIAL_MATRIX: Random ES distribution
Gene set null distribution of ES for MITOCHONDRIAL_MATRIX
**Table: GSEA Results Summary**

| Dataset | MB_6M_collapsed_to_symbols.general.cls#mut_versus_WT |
|---------|-----------------------------------------------------|
| Phenotype | general.cls#mut_versus_WT |
| Upregulated in class | mut |
| GeneSet | QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2 |

| Enrichment Score (ES) | 0.71504146 |
| Normalized Enrichment Score (NES) | 2.1899784 |
| Nominal p-value | 0.0 |
| FDR q-value | 0.014409064 |
| FWER p-Value | 0.015 |

**Fig 1: Enrichment plot: QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2**

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

**Table: GSEA details [plain text format]**

| PROBE | GENE SYMBOL | GENE_TITLE | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|-------------|------------|-------------------|-------------------|------------|------------------|
| 1     | ATN1        | Entrez,    | atrophin 1        | 218               | 1.533      | 0.1012           |
|       |             | Source     |                   |                   |            | Yes              |
| 2     | BCL2L1      | Entrez,    | BCL2-like 1       | 404               | 1.251      | 0.1832           |
|       |             | Source     |                   |                   |            | Yes              |
|   | Gene ID | Gene Name | Description | Gene Rank | Log2Ratio | P-Value | Fold Change | Differential Expression Status |
|---|---------|-----------|-------------|-----------|-----------|---------|-------------|-----------------------------|
| 3 | LBH     | Entrez    | -           | 434       | 1.222     | 0.2735  | Yes         |                             |
| 4 | PRSS35  | Entrez    | protease, serine, 35 | 900       | 0.917     | 0.3115  | Yes         |                             |
| 5 | MPP2    | Entrez    | membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2) | 945       | 0.898     | 0.3764  | Yes         |                             |
| 6 | MARK2   | Entrez    | MAP/microtubule affinity-regulating kinase 2 | 974       | 0.887     | 0.4415  | Yes         |                             |
| 7 | CNOT3   | Entrez    | CCR4-NOT transcription complex, subunit 3 | 1444      | 0.736     | 0.4656  | Yes         |                             |
| 8 | PLOD2   | Entrez    | procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 | 1485      | 0.725     | 0.5176  | Yes         |                             |
| 9 | HK1     | Entrez    | hexokinase 1 | 1636      | 0.685     | 0.5593  | Yes         |                             |
|10 | AES     | Entrez    | amino-terminal enhancer of split | 1835      | 0.633     | 0.5938  | Yes         |                             |
|11 | MYBBP1A | Entrez    | MYB binding protein (P160) 1a | 1934      | 0.611     | 0.6334  | Yes         |                             |
|12 | CARM1   | Entrez    | coactivator-associated arginine methyltransferase 1 | 2039      | 0.590     | 0.6709  | Yes         |                             |
|13 | DUSP10  | Entrez    | dual specificity phosphatase 10 | 2045      | 0.589     | 0.7150  | Yes         |                             |
|14 | SRM     | Entrez    | spermidine synthase | 4261      | 0.270     | 0.5868  | No          |                             |
|15 | FSCN1   | Entrez    | fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus) | 4314      | 0.263     | 0.6032  | No          |                             |
|16 | COL6A2  | Entrez    | collagen, type VI, alpha 2 | 4314      | 0.249     | 0.6122  | No          |                             |
|17 | ABCF2   | Entrez    | ATP-binding cassette, sub-family F (GCN20), member 2 | 4850      | 0.209     | 0.6019  | No          |                             |
|18 | SEPW1   | Entrez    | selenoprotein W, 1 | 6823      | 0.042     | 0.4728  | No          |                             |
|19 | RNF126  | Entrez    | ring finger protein 126 | 6904      | 0.035     | 0.4701  | No          |                             |
|20 | ITGA5   | Entrez    | integrin, alpha 5 (fibronectin receptor, alpha polypeptide) | 7597      | -0.018    | 0.4250  | No          |                             |
|21 | FN1     | Entrez    | fibronectin 1 | 7895      | -0.042    | 0.4082  | No          |                             |
|   | Gene   | Entrez | Source                                                                 | GeneID | Score 1 | Score 2 | Presence |
|---|--------|--------|------------------------------------------------------------------------|--------|---------|---------|----------|
| 22| LSM2   |        | LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae)          | 8210   | -0.067  | 0.3922  | No       |
| 23| CITED2 |        | Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal | 9073   | -0.139  | 0.3449  | No       |
| 24| ACTN4  |        | actinin, alpha 4                                                       | 9929   | -0.215  | 0.3038  | No       |
| 25| CTNND1 |        | catenin (cadherin-associated protein), delta 1                         | 11781  | -0.420  | 0.2114  | No       |

Fig 2: QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2
Blue-Pink O' Gram in the Space of the Analyzed GeneSet
**Fig 3: QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2: Random ES distribution**

Gene set null distribution of ES for QI_HYPOXIA_TARGETS_OF_HIF1A_AND_FOXA2
### Table: GSEA Results Summary

|                  | Value                                      |
|------------------|--------------------------------------------|
| Dataset          | MB_6M_collapsed_to_symbols.general.cls#mut_versus_WT |
| Phenotype        | general.cls#mut_versus_WT                  |
| Upregulated in class | mut                                       |
| GeneSet          | PID_ATM_PATHWAY                            |
| Enrichment Score (ES) | 0.5320049                                   |
| Normalized Enrichment Score (NES) | 1.6343192                                  |
| Nominal p-value  | 0.006342495                                |
| FDR q-value      | 1.0                                        |
| FWER p-Value     | 1.0                                        |

### Fig 1: Enrichment plot: PID_ATM_PATHWAY

Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

### Table: GSEA details [plain text format]

| PROBE | GENE SYMBOL | GENE_TITLE                                      | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|-------|-------------|-------------------------------------------------|-------------------|-------------------|------------|-----------------|
| 1     | BRCA1       | breast cancer 1, early onset                     | 66                | 2.166             | 0.2090     | Yes             |
| 2     | BID         | BH3 interacting domain death agonist             | 713               | 1.018             | 0.2660     | Yes             |
| 3     | RFWD2       | ring finger and WD repeat domain 2              | 1002              | 0.874             | 0.3328     | Yes             |
| Gene Code | Gene Name    | Source                              | Description                                                                 | PID | FDR 1 | FDR 2 | Pathway   |
|-----------|--------------|-------------------------------------|-----------------------------------------------------------------------------|-----|-------|-------|-----------|
| RAD50     | RAD50 homolog (S. cerevisiae) | Entrez, Source                      |                                                                             | 1495| 0.723 | 0.3711| Yes       |
| CDC25A    | cell division cycle 25A        | Entrez, Source                      |                                                                             | 1692| 0.673 | 0.4242| Yes       |
| H2AFX     | H2A histone family, member X   | Entrez, Source                      |                                                                             | 2094| 0.580 | 0.4545| Yes       |
| ABL1      | v-abl Abelson murine leukemia viral oncogene homolog 1 | Entrez, Source                      |                                                                             | 2374| 0.523 | 0.4873| Yes       |
| RAD17     | RAD17 homolog (S. pombe)       | Entrez, Source                      |                                                                             | 2498| 0.501 | 0.5284| Yes       |
| XRCC4     | X-ray repair complementing defective repair in Chinese hamster cells 4 | Entrez, Source                      |                                                                             | 3053| 0.414 | 0.5320| Yes       |
| YWHAB     | tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, beta polypeptide | Entrez, Source                      |                                                                             | 3739| 0.329 | 0.5185| No        |
| RBBP8     | retinoblastoma binding protein 8 | Entrez, Source                      |                                                                             | 4954| 0.199 | 0.4567| No        |
| RNF8      | ring finger protein 8          | Entrez, Source                      |                                                                             | 5376| 0.161 | 0.4443| No        |
| ATM       | ataxia telangiectasia mutated (includes complementation groups A, C and D) | Entrez, Source                      |                                                                             | 5809| 0.126 | 0.4277| No        |
| BLM       | Bloom syndrome                | Entrez, Source                      |                                                                             | 6187| 0.093 | 0.4116| No        |
| TRIM28    | tripartite motif-containing 28 | Entrez, Source                      |                                                                             | 6499| 0.068 | 0.3974| No        |
| CTBP1     | C-terminal binding protein 1   | Entrez, Source                      |                                                                             | 6630| 0.058 | 0.3944| No        |
| MDC1      | mediator of DNA damage checkpoint 1 | Entrez, Source                      |                                                                             | 6807| 0.043 | 0.3869| No        |
| RAD9A     | RAD9 homolog A (S. pombe)      | Entrez, Source                      |                                                                             | 7948| -0.046| 0.3150| No        |
| SMC1A     | structural maintenance of chromosomes 1A | Entrez, Source                      |                                                                             | 8491| -0.091| 0.2877| No        |
| CHEK2     | CHK2 checkpoint homolog (S. pombe) | Entrez, Source                      |                                                                             | 8729| -0.112| 0.2828| No        |
| FANCD2    | Fanconi anemia, complementation group D2 | Entrez, Source                      |                                                                             | 8839| -0.120| 0.2873| No        |
| TOP3A     | topoisomerase (DNA) III alpha  | Entrez, Source                      |                                                                             | 10252| -0.244| 0.2166| No        |
| SMC3      | structural maintenance of chromosomes 3 | Entrez, Source                      |                                                                             | 10393| -0.258| 0.2326| No        |
| Rank | Gene Symbol | Gene Name | Entrez | Source | Pearson Correlation Coefficient | p-value | Clustering | Description |
|------|-------------|-----------|--------|--------|---------------------------------|---------|------------|-------------|
| 24   | UBE2N       | ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast) | 11028  | -0.327 | 0.2223 | No |
| 25   | MRE11A      | MRE11 meiotic recombination 11 homolog A (S. cerevisiae) | 11632  | -0.401 | 0.2214 | No |

Fig 2: PID_ATM_PATHWAY
Blue-Pink O’ Gram in the Space of the Analyzed GeneSet

Fig 3: PID_ATM_PATHWAY: Random ES distribution
Gene set null distribution of ES for PID_ATM_PATHWAY