Cell type-specific Nrf2 expression in MS lesions

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Supplementary Figure 1:

Slowly expanding (smoldering) lesion in primary progressive multiple sclerosis (MS21) stained for KEAP1 (immunohistochemistry). a) KEAP1 reactivity is seen in cells at the active lesion edge (left), while only few cells are present in the inactive lesion center (x 100); b-d) Higher magnification images from the same tissue sections showing immunoreactivity exclusively in the cytoplasm, but no reactivity within the nucleus. Reactivity is seen in different cells, including astrocytes (b) and oligodendrocytes and macrophages (c). In the normal appearing white matter, distant from active lesions immunoreactivity within cells is very low (d); x: 1000.
### Supplementary Table 1: Expression of Nrf2-responsive genes in multiple sclerosis

| Expression          | MS white matter | MS cortex |
|---------------------|-----------------|-----------|
|                     | Fold-change     | Fold-change |
|                     | Control/PPWM/C  | Initial/C/Active/C | MS/C/MS38/C |
| **Group 1**         |                 |             |             |
| **PGD**¹            | 11.1            | -1.10       | 20.62       | -1.35     | -2.00 | -1.38 | NM_002631 | Phosphogluconate dehydrogenase |
| **GNA2B**           | 6.0             | 2.27        | 12.60       | 3.25      | -1.32 | -1.67 | NM_002070 | Nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2, transcript variant 1 |
| **FTH1**³,⁴,⁵       | 8.8             | 1.45        | 6.04        | 1.41      | 1.40  | 2.23  | NM_002032 | Ferritin, heavy polypeptide 1 |
| **ERP29**³          | 6.4             | 2.87        | 4.41        | 4.76      | -1.99 | -2.31 | NM_006187 | Endoplasmic reticulum protein 29, transcript variant 1 |
| **TXN**³,⁴,⁵        | 7.2             | 1.60        | 4.02        | 1.23      | 7.09  | 17.71 | NM_003329 | Thioredoxin, transcript variant 1 |
| **ANKRD11**         | 5.9             | 2.21        | 3.91        | 1.01      | -1.57 | -1.08 | NM_013275 | Ankyrin repeat domain 11 |
| **GATA3**           | 12.5            | 1.25        | 3.41        | 1.93      | -3.71 | -4.21 | NM_01002295 | GATA binding protein 3, transcript variant 1 |
| **FTL**³,⁴,⁵        | 4.2             | 5.02        | 3.34        | 3.47      | -1.07 | 1.29  | NM_000146 | Ferritin, light polypeptide |
| **HBG1**           | 5.4             | 1.26        | 3.10        | 2.48      | -1.04 | -1.67 | NM_006559 | Hemoglobin, gamma A |
| **BACH1**          | 4.1             | -1.11       | 2.64        | -1.02     | -1.66 | -2.25 | NM_106866 | BTB and CNC homology 1, basic leucine zipper transcription factor 1, transcript variant 1 |
| **PPARG**          | 7.9             | -1.14       | 2.29        | -1.04     | -1.18 | -1.40 | NM_138711 | Peroxisome proliferator-activated receptor gamma, transcript variant 3 |
| **GCS**²           | 5.0             | 1.01        | 2.10        | 1.75      | -1.05 | -1.15 | NM_006366 | Gamma-glutamylcysteine synthetase |
| **PRDX1**³,⁴,⁵      | 10.4            | -1.12       | 2.06        | 1.43      | -2.52 | -3.03 | NM_002574 | Peroxiredoxin 1, transcript variant 1 |
| **UNKL**           | 10.0            | 4.00        | 1.96        | 1.42      | -1.52 | -1.35 | NM_01037125 | Unkempt homolog (Drosophila)-like, transcript variant 2 |
| **USP14**²          | 4.1             | 1.38        | 1.93        | 1.20      | -1.68 | -1.43 | NM_005151 | Ubiquitin specific peptidase 14 (RNA-guanine transglycosylase), transcript variant 1 |
| **TALDO1**         | 7.5             | -1.68       | 1.72        | -1.00     | 2.75  | 3.07  | NM_006755 | Transaldolase 1 |
| **FECH**           | 6.3             | 1.02        | 1.71        | 1.47      | 1.78  | 1.70  | NM_01012515 | Ferrochelatase (FECH), transcript variant 1 |
| **AMB1**           | 5.0             | 4.59        | 1.37        | 1.55      | -2.82 | -2.86 | NM_001633 | Alpha-1-microglobulin/bikunin precursor |

**Group 2**

| Expression          | MS white matter | MS cortex |
|---------------------|-----------------|-----------|
|                     | Fold-change     | Fold-change |
|                     | Control/PPWM/C  | Initial/C/Active/C | MS/C/MS38/C |
| **TXNRD2**          | 18.9            | 1.88       | 5.85        | 1.17      | -1.53 | -1.96 | NM_006440 | Thioridox reductase 2 |
| **CCT7**           | 18.1            | 3.70       | 5.45        | 14.30     | -5.54 | -2.98 | NM_006429 | Chaperonin containing TCP1, subunit 7 (eta), transcript variant 1 |
| **OSGIN1**          | 14.8            | -1.42      | 5.32        | -1.19     | -1.04 | -1.15 | NM_182981 | Oxidative stress induced growth inhibitor 1 |
| **GNPDA1**         | 28.1            | 6.61       | 5.10        | 3.38      | -3.69 | -4.46 | NM_005471 | Glucosamine-6-phosphate deaminase 1 |
| **HMOI**³,⁴,⁵       | 1689.4          | -2.44      | 3.85        | 2.17      | 129.23 | 254.91 | NM_002133 | Heme oxygenase (decycling) 1 |
| **BMP10**          | 22.6            | 2.64       | 2.70        | -1.15     | -1.09 | -1.70 | NM_014482 | Bone morphogenetic protein 10 |
| **GSTP1**³,⁴,⁵      | 22441.9         | -4.92      | 2.40        | 1.78      | 4.09  | 4.54  | NM_000852 | Glutathione S-transferase pi 1 |
| **SLC3A2**         | 124.2           | -12.69     | 2.34        | 2.77      | 2.96  | 3.54  | NM_001012662 | Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2, transcript variant 2 |
| **MT2A**           | 201.9           | -9.27      | 2.16        | 1.83      | 7.82  | 16.38 | NM_005953 | Metallothionein 2A |
| **ALDH2**²         | 14.6            | -1.86      | 2.05        | 1.53      | 1.11  | 1.39  | NM_000690 | Aldehyde dehydrogenase 2 family (mitochondrial), transcript variant 1 |
| **CYP1A1**         | 28.7            | 1.17       | 1.76        | 1.64      | -1.61 | -1.51 | NM_000499 | Cytochrome P450, family 1, subfamily A, polypeptide 1 |
| **SOD3**           | 71.0            | -1.96      | 1.63        | -1.01     | 3.08  | 8.01  | NM_003102 | Superoxide dismutase 3, extracellular |
| Gene                  | Description                                                                 | Fold Change | p-value | Fold Change |
|----------------------|-----------------------------------------------------------------------------|-------------|---------|-------------|
| KIF3 family member C3, transcript variant 1 | Kinesin family member C3, transcript variant 1 | 167.0       | 1.69    | 1.57        |
| VCP                  | Valosin containing protein                                                  | 23.1        | 1.05    | 1.43        |
| ME1 family member    | Malic enzyme, NADP(+)-dependent, cytosolic                                 | 90.7        | -2.12   | 1.26        |
| HERPUD1 family member| Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1, transcript variant 1 | 121.3       | -22.51  | 1.13        |
| IDH1                  | Isocitrate dehydrogenase 1 (NADP+), soluble                                 | 14.3        | 2.47    | 1.01        |
| MT1E                  | Metallothionein 1E                                                          | 208.0       | -3.20   | -1.07       |
| CLPP                  | ClpP caseinolytic peptidase, ATP-dependent, proteolytic subunit homolog (E. coli) | 86.1        | -5.26   | -1.15       |
| SOD1                  | Superoxide dismutase 1, soluble                                             | 102.9       | -3.47   | -1.24       |
| GPX2                  | Glutathione peroxidase 2 (gastrointestinal)                                 | 85.1        | 1.20    | -1.26       |
| STIP1                 | Stress-induced-phosphoprotein 1 (STIP1)                                     | 13.2        | -1.42   | -1.29       |
| UNKL                  | Unkempt homolog (Drosophila)-like, transcript variant 1                    | 157.5       | -1.43   | -1.35       |
| GSR                   | Glutathione reductase, transcript variant 1                                 | 10.2        | 1.02    | -1.41       |
| UBB                   | Ubiquitin B                                                                 | 184.2       | -1.12   | -1.45       |
| SQSTM1                 | Sequestosome 1, transcript variant 1                                        | 8.8         | 4.41    | -1.56       |
| RXRA                  | Retinoid X receptor, alpha                                                  | 61.4        | -1.97   | -1.59       |
| GSTA3                 | Glutathione S-transferase alpha 3                                           | 75.2        | -2.11   | -1.64       |
| GPI                   | Glucose-6-phosphate isomerase, transcript variant 2                         | 529.6       | -12.10  | -1.83       |
| FMO1                  | Flavin containing monoxygenase 1                                            | 403.4       | -1.40   | -1.85       |
| MT1A                  | Metallothionein 1A                                                          | 167.0       | -1.28   | -1.97       |
| ETSI family member    | V-ets erythroblastosis virus E26 oncogene homolog 1 (avian), transcript variant 2 | 10.3        | -1.48   | -2.00       |
| ABCG6                 | ATP-binding cassette, sub-family B (MDR/TAP), member 6                      | 25.0        | -2.15   | -2.07       |
| HBE1                  | Hemoglobin, epsilon 1                                                       | 46.5        | -1.48   | -2.08       |
| FKBP5                 | FK506 binding protein 5, transcript variant 1                              | 21.5        | -1.33   | -3.04       |
| TFE3                  | Transcription factor binding to IGHM enhancer 3                             | 908.7       | 1.20    | -7.41       |

**Group 3**

| Gene                  | Description                                                                 | Fold Change | p-value | Fold Change |
|----------------------|-----------------------------------------------------------------------------|-------------|---------|-------------|
| SH3TC1 family variant 1 | SH3 domain and tetratricopeptide repeats 1                                 | 4.0         | 1.02    | 1.48        |
| CBR1 family member    | Carbonyl reductase 1                                                         | 4.6         | 2.05    | 1.45        |
| NUP62 family member   | Nucleoporin 62kDa, transcript variant 1                                      | 3.8         | 2.34    | 1.39        |
| CAT family member     | Catalase                                                                    | 5.0         | 1.60    | 1.37        |
| NDUFAD1 family member 1 | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4     | 4.2         | -1.03   | 1.27        |
| CPEB3 family member   | Cytoplasmic polyadenylation element binding protein 3, transcript variant 1 | 3.9         | 1.14    | 1.27        |
| DNAJC1 family member 1 | DNA (Hsp40) homolog, subfamily A, member 1                                   | 12.9        | 1.11    | 1.31        |
| HST1H4H family member | Histone cluster 1, H4h                                                       | 4.0         | 1.10    | 1.30        |
| NQO2 family member    | NAD(P)H dehydrogenase, quinone 2                                             | 3.8         | -1.05   | 1.29        |
| PPARGClB family member| Peroxisome proliferator-activated receptor gamma, coactivator 1 beta, transcript variant 1 | 4.1         | 1.05    | 1.29        |
| RBX1 family member    | Ring-box 1, E3 ubiquitin protein ligase                                      | 5.2         | 2.04    | 1.23        |
| GCLC family member    | Glutamate-cysteine ligase, catalytic subunit, transcript variant 1           | 3.8         | 1.02    | 1.20        |

Note: The values indicate fold change and p-value, with negative values indicating a decrease and positive values indicating an increase. The p-values indicate the significance of the change, with values below 0.05 considered significant.
| Gene Symbol | Fold Change | Expression | Gene Symbol | Fold Change | Expression |
|-------------|-------------|------------|-------------|-------------|------------|
| GSTA5       | 3.7         | 1.21       | CPEB2       | 5.2         | -1.07      |
| CPEB2       | 4.1         | -1.01      | G6PD        | 5.7         | -1.36      |
| G6PD        | 8.2         | -1.18      | AIFM2       | 4.1         | 1.08       |
| AIFM2       | 3.8         | 1.18       | UNKL        | 3.6         | -1.01      |
| UNKL        | 4.2         | 1.20       | GSTA1       | 3.7         | 1.12       |
| GSTA1       | 3.6         | 1.05       | PIR         | 3.7         | 1.25       |
| PIR         | 4.2         | 1.04       | GDP2        | 3.7         | 1.25       |
| GDP2        | 3.7         | 1.04       | PPLAD1      | 5.9         | -1.12      |
| PPLAD1      | 3.7         | 1.11       | NQO1        | 3.9         | -1.02      |
| NQO1        | 4.2         | 1.01       | HTATIP2     | 3.9         | -1.05      |
| HTATIP2     | 4.2         | -1.00      | CUL3        | 4.8         | 1.07       |
| CUL3        | 4.5         | -1.05      | BACH1       | 4.5         | 1.07       |
| BACH1       | 4.5         | -1.03      | IAKR        | 4.5         | -1.04      |
| IAKR        | 4.1         | -1.13      | KEAP1       | 4.5         | -1.04      |
| KEAP1       | 5.5         | -1.23      | PSMA3       | 5.5         | -1.34      |
| PSMA3       | 10.2        | 1.02       | GSTA2       | 5.9         | 1.11       |
| GSTA2       | 7.0         | -1.57      | PPARGC1A    | 5.6         | 1.01       |
| PPARGC1A    | 6.6         | -1.63      | HTATIP1     | 6.6         | 1.04       |
| HTATIP1     | 7.0         | -1.57      | EPHX1       | 7.0         | -1.57      |

The first column of the table gives the official gene symbols. Additionally, the respective literature is referenced in superscript numbers. In the second column, the mean basal expression levels within the normal white matter of control cases is depicted. The following three columns show the calculated gene expression fold-changes within the periplaque white matter (PP), initial (prephagocytic) lesions (I) and active demyelinating lesions (A) in comparison with the normal white matter of controls. Thereafter, the calculated gene expression fold-changes within active cortical lesions from all three evaluated MS cases (All) or a single MS case (MS15) in comparison with the normal grey matter of control cases are shown.

The Nrf2-responsive genes are categorized according to their basal expression in the NWM of controls and expression fold-changes in MS lesions in comparison with the respective control tissue. **Group 1** represents Nrf2-responsive genes that show a low basal expression in the NWM of controls, but were up-regulated in the zones of initial demyelination, while their expression was lower in the NAWM and the demyelinated part of the active lesion. The second pattern (**Group
2) consists of genes, which are expressed in high levels in the NWM of controls. Genes within group 3 show a very low basal expression in the NWM of controls and do not show any dynamic expression changes in MS lesions.
Supplementary References:

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