**Figure e-1. Heatmap representation of the multi-compartment miRNA screening performed in relapsing MS versus controls.**

Abbreviations: Ct = cycle threshold; hsa = Homo sapiens; PBMC = peripheral blood mononuclear cells; Rel = relapsing MS. Differences in miRNA expression in CSF, serum and PBMCs on a pool of ten relapsing MS patients versus ten controls were measured by two 84-plex PCR arrays for inflammatory response and autoimmunity (left) or T- and B-cell activation (right). miRNA species indicated in bold were subsequently measured by RT-qPCR in individual samples. △Ct corresponds to the difference in Ct (inversely related to the amount of target miRNA in the sample) between relapsing MS versus controls. They are indicated by a color graduation for absolute values ≥ 1.59, corresponding to a 3-fold change in expression (calculated as 2-△Ct). Significantly different △Ct values are indicated in numeric values in the graph. Underscored values correspond to miRNAs of low abundance with Cts > 30.



**Figure e-2. MiRNA expression levels in CSF, serum and PBMCs of relapsing/remitting MS and controls**

Abbreviations: HC = healthy controls; PBMCs = peripheral blood mononuclear cells; Rel = relapsing MS; Rem = remitting MS; SC = symptomatic controls.

Boxplots of relative expression for indicated miRNA are presented by Tukey’s method. p-value (\* ≤ 0.05; \*\* ≤ 0.01; \*\*\* ≤ 0.001) are calculated by Benjamini-Hochberg’s correction.

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**Table e-1. Baseline characteristics of patients and controls included in the several cohorts.**

Abbreviations: EDSS = expanded disability status score; FKLC = free kappa light chains; GEL = gadolinium-enhancing lesions; HC = healthy controls; Infect-ND = infectious neurological disorders; Inflam-ND = inflammatory neurological disorders; n = number; N/A = not applicable; OCB = oligoclonal bands; PBMCs = peripheral blood mononuclear cells; Rel MS = relapsing MS; Rem MS = remitting MS; RRMS = relapsing-remitting MS; SC = symptomatic controls; SD = standard deviation, % ♀ = percentage of female. Number of patients for which data is available is indicated by “n”. 1Barkhof criteria was assessed to reflect the lesion load (for definition see reference 13). 2IgG and IgM intrathecal fractions were calculated using Reiber’s formulas (see reference 12, normal value is 0%). All CSF RRMS patients were disease modifying treatment (DMT)-naïve. Only 7 out of 38 relapsing and 6 out of 35 remitting MS patients had stopped DMT three months to 13 years prior to serum or PBMCs sampling, with the remainder being treatment-naïve. Furthermore, none of the patients had received high dose intravenous methylprednisolone. According to the consensus definition (see reference 9), inflammatory neurological disease controls include all inflammatory neurological diseases that have abnormal CSF findings (pleocytosis and/or elevated QAlb) except if other findings support the inflammatory nature. Symptomatic controls (SCs) are patients with neurological symptoms, but without any objective clinical or paraclinical findings to support the diagnosis of a specific neurological disease, whereas healthy controls are subjects without any medical complaint or condition.

**Table 1-e.A: CSF, serum and PBMCs cohorts of miRNA arrays of relapsing MS vs SC/HC**

|  |  |  |
| --- | --- | --- |
|  | **CSF** | **Serum/PBMCs** |
| **MS vs SC array** | **MS vs HC array** |
| n (% ♀) | 20 (90.0) | 20 (90.0) |
| Mean age (±SD) | 31.1 (±8.8) | 33.3 (±8.9) (PBMCs)33.5 (± 9.6) (serum) |
|  | **Rel MS** | **SC** | **Rel MS** | **HC (serum)** | **HC (PBMCs)** |
| n (% ♀) | 10 (100) | 10 (80.0) | 10 (90.0) | 10 (90.0) | 10 (90.0) |
| Mean age (±SD) | 31.9 (±10.9) | 30.2 (±4.2) | 33.1 (±9.8) | 33.8 (± 9.9) | 33.4 (±8.3) |
| Disease duration in months: mean (±SD) | 5 (±11) | N/A | 14 (±31) | N/A | N/A |
| Relapse phenotype (%)1: Spinal2: Brainstem/cerebellum3: Optic neuritis4: Supratentorial5: Multifocal | 1: 20.0 | N/A | 1: 30.0 | N/A | N/A |
| 2: 10.0 | 2: 0.0 |
| 3: 40.0 | 3: 40.0 |
| 4: 10.0 | 4: 10.0 |
| 5: 20.0 | 5: 20.0 |
| EDSS: median [range] | 2.25 [2-3.5] | N/A | 2 [2-3.5] | N/A | N/A |
| Number of relapses since disease onset: median [range] | 1 [1-2] | N/A | 1 [1-2] | N/A | N/A |
| Proportion of patients with a 1st clinical event (%) | 70.0 | N/A | 70.0 | N/A | N/A |
| Barkhof criteria1 score 3 and 4 (%) | 90.0 | N/A | 90.0 | N/A | N/A |
| GELs on brain MRI: mean (±SD) | 2.6 (±1.7)*n=9* | N/A | 2.6 (±2.5) | N/A | N/A |
| Proportion of patients with spinal cord lesions on MRI (%) | 80.0 | N/A | 80.0 | N/A | N/A |
| GELs on spinal cord MRI: mean (±SD) | 0.3 (±0.5)*n=4* | N/A | 0*n=3* | N/A | N/A |
| Proportion of patients with at least one GEL on brain or spinal cord MRI (%) | 100 | N/A | 100 | N/A | N/A |
| Number of CSF cells/mm³: mean (±SD) [range] | 4.4 (±4.4) | 0.8 (±1.4) | 9.6 (±10.6) | N/A | N/A |
| [0-14] | [0-4] | [(0-34] |
| Proportion of patients with CSF-specific IgG OCBs or CSF FKLCs (%) | 100 | 10 | 100 | N/A | N/A |
| Proportion of patients with IgG intrathecal synthesis (%) | 90.0 | 0 | 90.0 | N/A | N/A |
| % IgG intrathecal synthesis2: mean (±SD) | 34.7 (±19.1) | 0 | 33.4 (±21.5) | N/A | N/A |
| Proportion of patients with IgM intrathecal synthesis (%) | 30.0 | 0 | 50.0 | N/A | N/A |
| % IgM intrathecal synthesis2:mean (±SD) | 16.6 (±27.6) | 0 | 25.4 (±30.9) | N/A | N/A |

**Table 1-e.B: CSF cohort of Infect-/Inflam-ND and RRMS vs SC**

|  |  |
| --- | --- |
|  | **CSF** |
| **Infect- & Inflam-ND vs MS vs SC** |
| n (% ♀) | 75 (57.3) |
| Mean age (±SD) | 39.7 (±14.9) |
|  | **Infect-ND** | **Inflam-ND** | **Rel MS** | **Rem MS** | **SC** |
| n (% ♀) | 14 (57.1) | 12 (33.3) | 15 (73.3) | 9 (44.4) | 25 (64.0) |
| Mean age (±SD) | 41.2 (±15.0) | 32.8 (±20.2) | 32.5 (±10.6) | 40.1 (±10.8) | 46.3 (±13.1) |
| Disease duration in months: mean (±SD) | 0.7 (±0.5) | 7.7 (±13.4) | 33 (±65) | 38 (±60) | N/A |
| Relapse phenotype (%)1: Spinal2: Brainstem/cerebellum3: Optic neuritis4: Supratentorial5: Multifocal | N/A | N/A | 1: 53.3 | N/A | N/A |
| 2: 20.0 |
| 3: 6.7 |
| 4: 16.3 |
| 5: 6.7 |
| EDSS: median [range] | N/A | N/A | 2 [0-3] | 2 [1-3] | N/A |
| Number of relapses since disease onset: median [range] | N/A | N/A | 1 [1-6] | 2 [0-3] | N/A |
| Proportion of patients with a 1st clinical event (%) | N/A | N/A | 53.3 | 33.3 | N/A |
| Barkhof criteria1 score 3 and 4 (%) | N/A | N/A | 93.3 | 44.4 | N/A |
| GELs on brain MRI: mean (±SD) | N/A | N/A | 5.4 (±8.9)*n=13* | 0*n=6* | N/A |
| Proportion of patients with spinal cord lesions on MRI (%) | N/A | N/A | 85.7*n=14* | 75.0*n=8* | N/A |
| GELs on spinal cord MRI: mean (±SD) | N/A | N/A | 0.5 (±0.5)*n=10* | 0*n=3* | N/A |
| Proportion of patients with at least one GEL on brain or spinal cord MRI (%) | N/A | N/A | 100*n=14* | 0*n=8* | N/A |
| Number of CSF cells/mm³: mean (±SD) [range] | 80.9 (±133.5) | 23.8 (±30.5) | 6.8 (±6.4) | 4.4 (±3.7) | 1.2 (±1.8) |
| [0-470] | [2-102] | [0-18] | [0-11] | [0-6] |
| Proportion of patients with CSF-specific IgG OCBs or CSF FKLCs (%) | 46.2 *n=13* | 50 | 93.3 | 100 | 0 *n=24* |
| Proportion of patients with IgG intrathecal synthesis (%) | 30.8 *n=13* | 25.0 | 80.0 | 66.7 | 4.2 *n=24* |
| % IgG intrathecal synthesis2: mean (±SD) | 13.3 (±23.4) *n=13* | 3.7 (±7.0) | 32.0 (±26.8) | 17.4 (±21.1) | 0.3 (±1.4)*n=24* |
| Proportion of patients with IgM intrathecal synthesis (%) | 53.8 *n=13* | 25.0 | 33.3 | 22.2 | 0 *n=24* |
| % IgM intrathecal synthesis2:mean (±SD) | 24.8 (±29.8) | 4.5 (±11.2) | 14.7 (±23.2) | 7.6 (±15.5) | 0 *n=24* |

**Table 1-e.C: Serum and PBMCs cohorts of RRMS vs HC**

|  |  |  |
| --- | --- | --- |
|   | **Serum** | **PBMCs** |
| **MS vs HC** | **MS vs HC** |
| n (% ♀) | 59 (76.3) | 48 (83.3) |
| Mean age (±SD) | 40.8 (±11.2) | 41.5 (±13.3) |
|  | **Rel MS** | **Rem MS** | **HC** | **Rel MS** | **Rem MS** | **HC** |
| n (% ♀) | 20 (80.0) | 19 (79.0) | 20 (70.0) | 18 (77.8) | 16 (93.8) | 14 (78.6) |
| Mean age (±SD) | 32.8 (±7.8) | 47.3 (±8.9) | 42.7 (±11.6) | 31.8 (±10.4) | 50.9 (±10.5) | 43.3 (±11.4) |
| Disease duration in months: mean (±SD) | 51 (±60) | 173 (±115) | N/A | 52 (±77) | 133 (±97) | N/A |
| Relapse phenotype (%)1: Spinal2: Brainstem/cerebellum3: Optic neuritis4: Supratentorial5: Multifocal | 1: 45.0 | N/A | N/A | 1: 27.8 | N/A | N/A |
| 2: 15.0 | 2: 16.7 |
| 3: 15.0 | 3: 22.2 |
| 4: 15.0 | 4: 33.3 |
| 5: 10.0 | 5: 00 |
| EDSS: median [range] | 2.5 [0-3.5] | 2 [0-3.5] | N/A | 2.75 [0-3.5] | 2 [0-3.5] | N/A |
| Number of relapses since disease onset: median [range] | 2 [1-11] | 2 [1-9] *n=18* | N/A | 2 [1-9] | 1 [0-4] | N/A |
| Proportion of patients with a 1st clinical event (%) | 35.0 | 36.8 | N/A | 35.0 | 62.5 | N/A |
| Barkhof criteria1 score 3 and 4 (%) | 84.2*, n=19* | 84.2 | N/A | 82.4, *n=17* | 93.8 | N/A |
| GELs on brain MRI: mean (±SD) | 2.5 (±2.3)*n=17* | 0*n=11* | N/A | 3.6 (±4.5)*n=14* | N/A | N/A |
| Proportion of patients with spinal cord lesions on MRI (%) | 88.2*n=17* | 84.6*n=13* | N/A | 87.5*n=16* | 78.6*n=14* | N/A |
| GELs on spinal cord MRI: mean (±SD) | 0, *n=4* | N/A | N/A | 0.4 (±0.5) *n=5* | N/A | N/A |
| Proportion of patients with at least one GEL on brain or spinal cord MRI (%) | 93.8*n=16* | N/A | N/A | 92.9*n=14* | N/A | N/A |
| Number of CSF cells/mm³: mean (±SD) [range] | 11.3 (±9.3)  | N/A  | N/A | 5.64 (±7.1)  | N/A  | N/A  |
| [0-34]*n=13* | [0-22]*n=11* |
| Proportion of patients with CSF-specific IgG OCBs or CSF FKLCs (%) | 100*n=18* | 94.4*n=18* | N/A | 100 | 93.8 | N/A |
| Proportion of patients with IgG intrathecal synthesis (%) | 100*n=11* | N/A | N/A | 77.8*n=9* | N/A | N/A |
| % IgG intrathecal synthesis2: mean (±SD) | 34.9 (±20.0) *n=11* | N/A | N/A | 27.1 (±20.1)*n=9* | N/A | N/A |
| Proportion of patients with IgM intrathecal synthesis (%) | 45.5*n=11* | N/A | N/A | 33.3*n=9* | N/A | N/A |
| % IgM intrathecal synthesis2:mean (±SD) | 15.4 (± 22.6) *n=11* | N/A | N/A | 16.0 (±27.1)*n=9* | N/A | N/A |

**Table e-2. Diagnoses of infectious and inflammatory neurological disorders.**

Abbreviations: HaNDL = headache and neurologic deficits with CSF lymphocytosis; HSV = Herpes Simplex Virus; Infect-ND = infectious neurological disorders, Inflam-ND = inflammatory neurological disorders.

|  |  |  |
| --- | --- | --- |
|   | **Diagnosis** | n |
| **Infect-ND** | Bacterial meningitis (unidentified pathogen) | 2 |
| Pneumococcal meningitis with subdural empyema | 1 |
| Presumptive tuberculous meningitis | 1 |
| Focal meningitis consecutive to otitis media (by H. *influenzae* and S. *pneumoniae*) | 1 |
| Unspecified meningitis | 2 |
| HSV meningo-encephalitis | 2 |
| HIV meningitis | 1 |
| Progressive Multifocal Leukoencephalopathy | 1 |
| Viral meningitis and cerebellitis | 1 |
| Meningo-encephalitis of unknown origin | 1 |
| Lepto-meningitis of unknown origin | 1 |
| **Inflam-ND** | Neuro-Behçet's disease | 3 |
| Neurosarcoidosis | 2 |
| Hashimoto’s encephalopathy | 1 |
| Susac syndrome | 1 |
| Idiopathic CNS vasculitis | 1 |
| Primary granulomatous CNS vasculitis | 1 |
| Post-infectious meningo-encephalitis | 1 |
| Isolated optical neuritis | 1 |
| HaNDL syndrome | 1 |

**Table e-3. List of miRNAs selected for individual amplification by quantitative PCR in the different cohorts.**

Abbreviations: HC = healthy controls; Infect-ND = infectious neurological disorders; Inflam-ND = inflammatory neurological disorders; PBMCs = peripheral blood mononuclear cells; SC = symptomatic controls. The miRNAs were selected based on the results of the screening arrays or identified from bibliographic search.They are grouped according to their involvement in immunity/inflammation, lipid metabolism or neurodegeneration. MiRNAs that could not be analyzed for technical reasons (qPCR melt curve with several peaks, heterogeneous melt curves, low miRNA levels resulting in a flat amplification curve) are indicated by °.

|  |  |  |
| --- | --- | --- |
| **CSF of MS vs SC** | **CSF of Infect- & Inflam-ND vs MS vs SC** | **Serum & PBMCs****of MS vs HC** |
| 64 targets | 57 targets | 24 targets |
| **Immunological (n=34)** | **Immunological (n=35)** | **Immunological (n=18)** |
| let-7a-5p​ | miR-133b​ | let-7a-5p | miR-145-5p | let-7e-5p​ |
| let-7b-5p​ | miR-145-5p​ | let-7b-5p | miR-146a-5p | let-7d-5p​ |
| let-7d-5p​ | miR-146a-5p​ | let-7e-5p | miR-150-5p | miR-15a-3p​ |
| let-7e-5p​ | miR-150-5p​ | let-7f-5p | miR-155-5p | miR-16-5p​ |
| let-7f-5p​ | miR-155-5p​ | miR-15a-3p | miR-181a-5p | miR-17-5p​ |
| miR-15a-3p​ | miR-181b-5p​ | miR-15b-5p | miR-181b-5p | miR-20a-5p​ |
| miR-15b-5p​ | miR-206​ | miR-16-5p | miR-210-3p | miR-24-3p​ |
| miR-16-5p​ | miR-210-3p​ | miR-17-5p | miR-222-3p | miR-29b-3p​ |
| miR-17-5p​ | miR-223-3p​ | miR-20a-5p | miR-223-3p | miR-34c-5p°​ |
| miR-18b-5p°​ | miR-326​ | miR-22-3p | miR-326 | miR-124-3p​ |
| miR-20a-5p​ | miR-342-3p​ | miR-24-3p | miR-342-3p | miR-126-3p​ |
| miR-24-3p​ | miR-423-5p​ | mir-25-3p | miR-423-5p | miR-145-5p​ |
| mir-25-3p​ | miR-96-5p​° | miR-29b-3p | let-7d-5p° | miR-146a-5p​ |
| miR-29b-3p​ | miR-181c-5p​° | miR-34c-5p | let-7i-5p° | miR-150-5p​ |
| miR-34c-5p​ | miR-493-3p​° | miR-92a-3p | miR-15a-5p° | miR-155-5p​ |
| miR-92a-3p​ | miR-599​° | miR-124-3p | miR-18b-5p° | miR-181c-5p​ |
| miR-124-3p°​ |  | miR-126-3p | miR-96-5p° | miR-210-3p​ |
| miR-126-3p​ |  | miR-133b |  | miR-223-3p​ |
| **Immunological & lipidic (n=11)** | **Lipidic (n=15)** | **Immunological & lipidic (n=8)** | **Lipidic (n=10)** | **Immunological & lipidic (n=3)** |
| miR-19b-3p​ | miR-33a-3p​ | miR-21-5p | miR-122-5p | miR-34a-5p |
| miR-21-5p​ | miR-122-5p​ | miR-27a-3p | miR-192-5p | miR-142-3p |
| miR-27a-3p​ | mir-124-5p​ | miR-27b-3p | miR-297 | miR-214-3p |
| miR-27b-3p​ | miR-125a-3p° | miR-34a-5p | miR-338-3p |  |
| miR-34a-5p​ | miR-147b° | miR-101-3p | miR-378a-5p | **Lipidic (n=1)**​ |
| miR-101-3p​ | miR-149-3p | miR-125b-5p | miR-422b (= miR-378a-3p) | miR-297 |
| miR-125a-5p​ | miR-192-5p | miR-142-3p | miR-32-5p° |  |
| miR-125b-5p​ | miR-297 | miR-214-3p | miR-33a-5p° |  |
| miR-142-3p​ | miR-338-3p |  | miR-138-5p° |  |
| miR-146b-5p​ | miR-378a-5p |  | miR-219a-5p° |  |
| miR-214-3p​ | miR-422b (= miR-378a-3p) |  |  |  |
|  | miR-32-5p° |  |  |  |
|  | miR-33a-5p° |  |  |  |
|  | miR-138-5p° |  |  |  |
|  | miR-219a-5p° |  |  |  |
| **Immunological & neuro-degenerative (n=3)**​ | **Neuro-degenerative (n=1)**​ | **Immunological & neuro- degenerative (n=3)** | **Neuro-degenerative (n=1)** | **Immunological & neuro-degenerative (n=2)** |
| miR-29a-3p | miR-107 | miR-29a-3p | miR-107 | miR-29c-3p |
| miR-29c-3p |  | miR-29c-3p |  | miR-191-5p |
| miR-191-5p |  | miR-191-5p |  |  |

**Table e-4. Differentially expressed miRNAs in MS and other inflammatory/infectious neurological disorders.**

Abbreviations: ND = neurological disorders; PBMCs = peripheral blood mononuclear cells; SC = symptomatic controls Median fold change and statistical significance are listed for miRNAs amplified from CSF, serum and PBMCs of relapsing or remitting MS vs. SC/HC (A) and in the CSF of inflammatory and infectious neurological diseases and relapsing or remitting MS vs. SC (B). Values significant according to Dunn’s post-test with (bold) or without (italic) confirmation by Benjamini-Hochberg’s correction are shown. qPCR inclusion criteria described in the material and methods section allowed the inclusion of over 90% of CSF samples, over 96% of serum samples and 100% of PBMCs samples in the analysis, except for (A) 188.2% and 286.4% and for (B) 374.7% and 481.3%.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A** |  | **Relapsing vs Remitting** | **Relapsing vs Controls** | **Remitting vs Controls** |
| **ID miRNA** | **ANOVA p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** |
| **CSF** |
| **miR-15a-3p** | 0,0215 | 1,25 | 0,9779 | 0,9779 | **-2,38** | **0,0071** | **0,0212** | **-2,94** | **0,0330** | **0,0494** |
| **miR-20a-5p** | 0,0077 | **4,03** | **0,0045** | **0,0076** | -1,01 | 0,6242 | 0,6242 | **-4,17** | **0,0051** | **0,0076** |
| **miR-21-5p** | 0,0231 | 1,29 | 0,4872 | 0,4872 | **1,76** | **0,0061** | **0,0182** | 1,37 | 0,1080 | 0,1619 |
| **miR-24-3p** | 0,0484 | **1,90** | **0,0158** | **0,0474** | 1,04 | 0,2764 | 0,2764 | -1,82 | 0,2446 | 0,2764 |
| **miR-27a-3p** | 0,0323 | **1,69** | **0,0092** | **0,0276** | 1,09 | 0,6875 | 0,6875 | -1,56 | 0,0610 | 0,0915 |
| **miR-27b-3p** | 0,0125 | **1,72** | **0,0032** | **0,0095** | 1,15 | 0,3247 | 0,3247 | -1,49 | 0,0892 | 0,1337 |
| **miR-29c-3p** | 0,0359 | **2,29** | **0,0102** | **0,0305** | 1,13 | 0,6645 | 0,6645 | -2,04 | 0,0650 | 0,0975 |
| **miR-33a-3p** | 0,0311 | 2,26 | 0,1047 | 0,1047 | -1,78 | 0,1006 | 0,1047 | **-4,00** | **0,0087** | **0,0260** |
| **miR-34c-5p** | 0,0269 | 1,13 | 0,9096 | 0,9096 | **-3,44** | **0,0108** | **0,0324** | **-3,85** | **0,0305** | **0,0457** |
| **mir-124-5p** | 0,016 | -1,28 | 0,3427 | 0,3427 | **-2,17** | **0,0041** | **0,0124** | -1,23 | 0,1513 | 0,2270 |
| **miR-125b-5p** | 0,0198 | **1,83** | **0,0067** | **0,0202** | 1,49 | 0,1656 | 0,2402 | -1,23 | 0,2402 | 0,2402 |
| **miR-145-5p** | 0,0257 | **2,28** | **0,0070** | **0,0209** | 1,07 | 0,6187 | 0,6187 | -2,13 | 0,0608 | 0,0911 |
| **miR-146a-5p**1 | 0,0018 | 1,75 | 0,0854 | 0,1281 | **2,04** | **0,0005** | **0,0016** | 1,17 | 0,1519 | 0,1717 |
| **miR-149-3p** | 0,0115 | **-2,00** | **0,0092** | **0,0138** | 1,06 | 0,4114 | 0,4114 | **2,12** | **0,0057** | **0,0138** |
| **miR-150-5p** | <0,0001 | 1,79 | 0,2007 | 0,2007 | **5,41** | **0,0000** | **0,0000** | **3,03** | **0,0116** | **0,0173** |
| **miR-155-5p** | 0,001 | -1,47 | 0,0659 | 0,0659 | **1,94** | **0,0081** | **0,0122** | **2,87** | **0,0002** | **0,0007** |
| **miR-214-3p** | 0,0035 | **1,69** | **0,0175** | **0,0340** | -1,47 | 0,0951 | 0,1225 | **-2,50** | **0,0008** | **0,0063** |
| **miR-297** | 0,0073 | 1,62 | 0,0930 | 0,0930 | **-1,96** | **0,0307** | **0,0460** | **-3,22** | **0,0020** | **0,0060** |
| **SERUM** |
| **miR-15a-3p** | 0,0175 | -1,11 | 0,9922 | 0,9922 | **-1,69** | **0,0140** | **0,0209** | **-1,54** | **0,0136** | **0,0209** |
| **miR-24-3p** | 0,0099 | 1,14 | 0,4974 | 0,4974 | **-1,75** | **0,0250** | **0,0374** | **-2,00** | **0,0038** | **0,0115** |
| **miR-126-3p** | 0,0069 | 1,14 | 0,8050 | 0,8050 | **-1,47** | **0,0043** | **0,0128** | **-1,69** | **0,0100** | **0,0150** |
| **miR-146a-5p** | 0,0010 | -1,10 | 0,7851 | 0,7851 | **-2,27** | **0,0007** | **0,0022** | **-2,04** | **0,0021** | **0,0032** |
| **miR-181c-5p**2 | 0,0192 | -1,16 | 0,5588 | 0,5588 | **-3,23** | **0,0074** | **0,0223** | **-2,78** | **0,0326** | **0,0488** |
| **miR-214-3p** | 0,0318 | -1,46 | 0,1093 | 0,1640 | **-1,64** | **0,0093** | **0,0279** | -1,11 | 0,3339 | 0,3339 |
| **PBMCs** |
| **miR-34a-5p** | 0,0013 | **-1,89** | **0,0003** | **0,0008** | -1,41 | 0,0621 | 0,0931 | 1,32 | 0,1086 | 0,1086 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **B** |  | **Infectious ND vs Inflammatory ND** | **Infectious ND vs** **Relapsing MS** | **Infectious ND vs** **Remitting MS** | **Infectious ND vs** **SC** | **Inflammatory ND vs Relapsing MS** |
| **ID miRNA** | **ANOVA p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** |
| **let-7a-5p** | 0,0019 | 1,72 | 0,0869 | 0,1538 | 1,51 | 0,1077 | 0,1538 | **2,47** | **0,0023** | **0,01132** | **2,16** | **0,0002** | **0,00186** | -1,14 | 0,8450 | 0,8836 |
| **let-7b-5p** | 0,0045 | 2,14 | 0,1699 | 0,2831 | 2,41 | 0,0539 | 0,1348 | *2,29* | *0,0207* | *0,10374* | **2,96** | **0,0002** | **0,00189** | 1,13 | 0,6490 | 0,6490 |
| **let-7e-5p** | 0,0066 | 1,24 | 0,4876 | 0,6095 | 1,24 | 0,3227 | 0,4610 | *2,10* | *0,0176* | *0,07410* | **2,27** | **0,0013** | **0,01301** | 1,00 | 0,7980 | 0,8867 |
| **let-7f-5p** | 0,006 | 1,16 | 0,8269 | 0,8269 | 1,32 | 0,3740 | 0,5342 | 2,56 | 0,0610 | 0,1525 | **2,76** | **0,0016** | **0,01569** | 1,13 | 0,5281 | 0,5868 |
| **miR-15b-5p** | <0,0001 | 2,45 | 0,2053 | 0,2933 | *2,96* | *0,0307* | *0,06144* | **3,80** | **0,0185** | **0,04635** | **8,31** | **0,0000** | **0,00002** | 1,21 | 0,4315 | 0,4795 |
| **miR-16-5p** | 0,0008 | 2,22 | 0,1193 | 0,2241 | 1,92 | 0,1344 | 0,2241 | *3,57* | *0,0157* | *0,05217* | **5,76** | **0,0000** | **0,00038** | -1,15 | 0,8839 | 0,8839 |
| **miR-17-5p** | 0,0147 | 1,59 | 0,4741 | 0,5926 | **3,29** | **0,0092** | **0,04596** | 2,04 | 0,0951 | 0,1902 | **3,12** | **0,0024** | **0,02395** | 2,07 | 0,0764 | 0,1902 |
| **miR-20a-5p**3 | 0,0067 | 4,84 | 0,0605 | 0,1513 | 2,09 | 0,1611 | 0,3223 | *14,43* | *0,0119* | *0,05937* | **14,42** | **0,0004** | **0,00384** | -2,31 | 0,5717 | 0,6352 |
| **miR-21-5p** | <0,0001 | 3,20 | 0,2286 | 0,3266 | **5,62** | **0,0010** | **0,00330** | **7,07** | **0,0003** | **0,00142** | **6,28** | **0,0000** | **0,00005** | 1,76 | 0,0527 | 0,0879 |
| **miR-22-3p** | 0,0163 | *8,67* | *0,0229* | *0,11468* | 3,62 | 0,0808 | 0,1347 | **14,38** | **0,0009** | **0,00906** | 4,93 | 0,0587 | 0,1347 | -2,39 | 0,4437 | 0,4930 |
| **miR-24-3p** | 0,0008 | *4,50* | *0,0472* | *0,09158* | *4,64* | *0,0168* | *0,05612* | **14,88** | **0,0001** | **0,00076** | **6,15** | **0,0005** | **0,00269** | 1,03 | 0,7553 | 0,7553 |
| **miR-25-3p** | 0,001 | 2,41 | 0,0798 | 0,1585 | *2,92* | *0,0393* | *0,13096* | **5,68** | **0,0011** | **0,00561** | **6,57** | **0,0001** | **0,00088** | 1,21 | 0,8427 | 0,8427 |
| **miR-27a-3p** | 0,0063 | 2,13 | 0,2912 | 0,4160 | **4,90** | **0,0026** | **0,01412** | **4,73** | **0,0042** | **0,01412** | **3,69** | **0,0042** | **0,01412** | 2,30 | 0,0689 | 0,1379 |
| **miR-27b-3p** | 0,0417 | -1,06 | 0,8956 | 0,8956 | *6,11* | *0,0303* | *0,10348* | *2,99* | *0,0233* | *0,10348* | *2,19* | *0,0493* | *0,10348* | 6,50 | 0,0517 | 0,1035 |
| **miR-29b-3p** | <0,0001 | 2,26 | 0,8830 | 0,8830 | 3,36 | 0,0782 | 0,1304 | *5,58* | *0,0258* | *0,06442* | **16,21** | **0,0000** | **0,00007** | 1,48 | 0,1235 | 0,1765 |
| **miR-29c-3p** | 0,0001 | 2,79 | 0,1858 | 0,2654 | 1,84 | 0,0619 | 0,1237 | **3,72** | **0,0048** | **0,01745** | **7,31** | **0,0000** | **0,00011** | -1,52 | 0,6301 | 0,6301 |
| **miR-92a-3p** | 0,0241 | 1,61 | 0,2168 | 0,4335 | *1,89* | *0,0358* | *0,17913* | 1,87 | 0,0750 | 0,2201 | **2,24** | **0,0012** | **0,01153** | 1,17 | 0,4477 | 0,5596 |
| **miR-125b-5p** | 0,0106 | 1,73 | 0,5982 | 0,6320 | *3,84* | *0,0319* | *0,06750* | **3,01** | **0,0074** | **0,03721** | **3,19** | **0,0041** | **0,03721** | 2,22 | 0,1277 | 0,2128 |
| **miR-142-3p** | <0,0001 | -1,27 | 0,5725 | 0,6361 | *1,95* | *0,0272* | *0,07703* | *5,39* | *0,0308* | *0,07703* | **11,47** | **0,0000** | **0,00014** | 2,47 | 0,1148 | 0,1640 |
| **miR-146a-5p**4 | 0,0001 | 3,16 | 0,0740 | 0,1234 | *4,47* | *0,0206* | *0,05324* | *3,69* | *0,0182* | *0,05324* | **10,34** | **0,0000** | **0,00002** | 1,41 | 0,6988 | 0,7764 |
| **miR-150-5p** | <0,0001 | 8,10 | 0,1990 | 0,2842 | *14,03* | *0,0380* | *0,06333* | **28,64** | **0,0184** | **0,03689** | **86,82** | **0,0000** | **0,00000** | 1,73 | 0,4824 | 0,5360 |
| **miR-181b-5p** | 0,0115 | 2,44 | 0,2004 | 0,2552 | 2,44 | 0,1185 | 0,2370 | **3,73** | **0,0090** | **0,04515** | **3,80** | **0,0011** | **0,01057** | 1,00 | 0,8435 | 0,9372 |
| **miR-191-5p** | 0,0275 | 1,02 | 0,8328 | 0,9099 | 1,37 | 0,1054 | 0,2109 | *1,91* | *0,0424* | *0,14121* | *2,32* | *0,0067* | *0,06718* | 1,35 | 0,1806 | 0,3009 |
| **miR-223-3p** | 0,0001 | 1,83 | 0,5104 | 0,5672 | *3,94* | *0,0379* | *0,07588* | **5,81** | **0,0046** | **0,01545** | **6,86** | **0,0000** | **0,00025** | 2,15 | 0,1859 | 0,2655 |
| **miR-297** | 0,033 | -1,20 | 0,7353 | 0,7910 | *2,28* | *0,0326* | *0,08500* | 2,53 | 0,1783 | 0,2972 | *1,84* | *0,0340* | *0,08500* | *2,73* | *0,0167* | *0,08334* |
| **miR-342-3p** | 0,0002 | 3,24 | 0,1737 | 0,2481 | **5,24** | **0,0126** | **0,03146** | **6,82** | **0,0072** | **0,02394** | **8,69** | **0,0000** | **0,00007** | 1,62 | 0,3005 | 0,3406 |
| **miR-423-5p** | 0,0003 | 1,72 | 0,0942 | 0,1569 | **2,13** | **0,0133** | **0,04430** | **2,15** | **0,0055** | **0,02749** | **3,91** | **0,0000** | **0,00010** | 1,24 | 0,4994 | 0,5280 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Inflammatory ND vs Remitting MS** | **Inflammatory ND vs** **SC** | **Relapsing MS vs****Remitting MS** | **Relapsing MS vs** **SC** | **Remitting MS vs** **SC** |
| **ID miRNA** | **ANOVA p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** | **Fold change** | **Dunn's p-value** | **Adjusted p-value** |
| **let-7a-5p** | 0,0019 | 1,44 | 0,1525 | 0,1906 | 1,26 | 0,1021 | 0,1538 | 1,64 | 0,0937 | 0,1538 | *1,43* | *0,0467* | *0,15381* | -1,14 | 0,8836 | 0,8836 |
| **let-7b-5p** | 0,0045 | 1,07 | 0,3096 | 0,4423 | *1,39* | *0,0443* | *0,13480* | -1,05 | 0,5192 | 0,5769 | 1,23 | 0,1045 | 0,2091 | 1,29 | 0,5062 | 0,5769 |
| **let-7e-5p** | 0,0066 | 1,69 | 0,0877 | 0,1753 | *1,83* | *0,0222* | *0,07410* | 1,69 | 0,1256 | 0,2094 | *1,83* | *0,0353* | *0,08837* | 1,08 | 0,9436 | 0,9436 |
| **let-7f-5p** | 0,006 | 2,20 | 0,1052 | 0,2104 | **2,37** | **0,0058** | **0,02888** | 1,95 | 0,2649 | 0,4415 | *2,10* | *0,0264* | *0,08809* | 1,08 | 0,5120 | 0,5868 |
| **miR-15b-5p** | <0,0001 | 1,55 | 0,2496 | 0,3120 | **3,40** | **0,0020** | **0,00996** | 1,28 | 0,6300 | 0,6300 | **2,81** | **0,0166** | **0,04635** | 2,19 | 0,1343 | 0,2239 |
| **miR-16-5p** | 0,0008 | 1,61 | 0,3411 | 0,4068 | *2,60* | *0,0288* | *0,07192* | 1,85 | 0,2585 | 0,3693 | *2,99* | *0,0117* | *0,05217* | 1,62 | 0,3661 | 0,4068 |
| **miR-17-5p** | 0,0147 | 1,28 | 0,3278 | 0,5464 | *1,96* | *0,0371* | *0,12379* | -1,61 | 0,5454 | 0,6060 | -1,06 | 0,8890 | 0,8890 | 1,53 | 0,4395 | 0,5926 |
| **miR-20a-5p**3 | 0,0067 | 2,98 | 0,5347 | 0,6352 | 2,98 | 0,2264 | 0,3235 | 6,90 | 0,2225 | 0,3235 | *6,89* | *0,0448* | *0,14943* | 1,00 | 0,6437 | 0,6437 |
| **miR-21-5p** | <0,0001 | **2,21** | **0,0146** | **0,02917** | **1,97** | **0,0029** | **0,00732** | 1,26 | 0,4383 | 0,4870 | 1,12 | 0,3671 | 0,4588 | -1,13 | 0,9339 | 0,9339 |
| **miR-22-3p** | 0,0163 | 1,66 | 0,3442 | 0,4917 | -1,76 | 0,4108 | 0,4930 | 3,97 | 0,0682 | 0,1347 | 1,36 | 0,9941 | 0,9941 | -2,92 | 0,0512 | 0,1347 |
| **miR-24-3p** | 0,0008 | *3,31* | *0,0330* | *0,08247* | 1,37 | 0,2704 | 0,3380 | 3,21 | 0,0549 | 0,0916 | 1,33 | 0,4259 | 0,4732 | -2,42 | 0,1571 | 0,2245 |
| **miR-25-3p** | 0,001 | 2,35 | 0,1109 | 0,1585 | 2,72 | 0,0774 | 0,1585 | 1,95 | 0,1376 | 0,1720 | 2,25 | 0,0962 | 0,1585 | 1,16 | 0,8313 | 0,8427 |
| **miR-27a-3p** | 0,0063 | 2,22 | 0,0664 | 0,1379 | 1,73 | 0,1245 | 0,2075 | -1,04 | 0,8033 | 0,8033 | -1,33 | 0,6136 | 0,6818 | -1,28 | 0,4874 | 0,6093 |
| **miR-27b-3p** | 0,0417 | *3,18* | *0,0374* | *0,10348* | 2,33 | 0,0843 | 0,1406 | -2,04 | 0,6965 | 0,7739 | -2,79 | 0,6759 | 0,7739 | -1,37 | 0,4406 | 0,6295 |
| **miR-29b-3p** | <0,0001 | *2,46* | *0,0425* | *0,08491* | **7,16** | **0,0000** | **0,00021** | 1,66 | 0,4794 | 0,5326 | **4,82** | **0,0098** | **0,03264** | 2,91 | 0,1606 | 0,2008 |
| **miR-29c-3p** | 0,0001 | 1,33 | 0,1055 | 0,1758 | **2,62** | **0,0052** | **0,01745** | 2,02 | 0,2151 | 0,2689 | **3,97** | **0,0177** | **0,04422** | 1,97 | 0,5430 | 0,6034 |
| **miR-92a-3p** | 0,0241 | 1,16 | 0,5333 | 0,5926 | 1,39 | 0,0880 | 0,2201 | -1,01 | 0,9633 | 0,9634 | 1,19 | 0,3504 | 0,5596 | 1,20 | 0,4040 | 0,5596 |
| **miR-125b-5p** | 0,0106 | *1,74* | *0,0337* | *0,06750* | *1,84* | *0,0327* | *0,06750* | -1,27 | 0,4116 | 0,5880 | -1,21 | 0,6242 | 0,6320 | 1,06 | 0,6320 | 0,6320 |
| **miR-142-3p** | <0,0001 | 6,84 | 0,1071 | 0,1640 | **14,57** | **0,0003** | **0,00164** | 2,77 | 0,8130 | 0,8130 | *5,90* | *0,0462* | *0,09243* | 2,13 | 0,1561 | 0,1951 |
| **miR-146a-5p**4 | 0,0001 | 1,17 | 0,5549 | 0,6936 | *3,27* | *0,0213* | *0,05324* | -1,21 | 0,8079 | 0,8079 | *2,31* | *0,0455* | *0,09093* | 2,80 | 0,1260 | 0,1800 |
| **miR-150-5p** | <0,0001 | 3,54 | 0,2496 | 0,3120 | **10,72** | **0,0000** | **0,00014** | 2,04 | 0,5763 | 0,5763 | **6,19** | **0,0002** | **0,00082** | **3,03** | **0,0133** | **0,03327** |
| **miR-181b-5p** | 0,0115 | 1,53 | 0,1653 | 0,2552 | 1,56 | 0,0932 | 0,2370 | 1,53 | 0,2042 | 0,2552 | 1,56 | 0,1162 | 0,2370 | 1,02 | 0,9542 | 0,9542 |
| **miR-191-5p** | 0,0275 | 1,88 | 0,0753 | 0,1883 | *2,28* | *0,0191* | *0,09558* | 1,39 | 0,5287 | 0,6609 | 1,69 | 0,3465 | 0,4949 | 1,21 | 0,9099 | 0,9099 |
| **miR-223-3p** | 0,0001 | *3,17* | *0,0311* | *0,07588* | **3,75** | **0,0011** | **0,00545** | 1,47 | 0,2984 | 0,3730 | 1,74 | 0,0520 | 0,0866 | 1,18 | 0,6136 | 0,6136 |
| **miR-297** | 0,033 | 3,03 | 0,1083 | 0,2167 | *2,20* | *0,0167* | *0,08334* | 1,11 | 0,6031 | 0,7910 | -1,24 | 0,7910 | 0,7910 | -1,38 | 0,7329 | 0,7910 |
| **miR-342-3p** | 0,0002 | 2,11 | 0,1589 | 0,2481 | **2,69** | **0,0043** | **0,02130** | 1,30 | 0,6015 | 0,6015 | 1,66 | 0,0620 | 0,1241 | 1,27 | 0,3065 | 0,3406 |
| **miR-423-5p** | 0,0003 | 1,25 | 0,2314 | 0,3306 | **2,27** | **0,0190** | **0,04749** | 1,01 | 0,5280 | 0,5280 | 1,83 | 0,0846 | 0,1569 | 1,82 | 0,4403 | 0,5280 |

**Table e-5. Strength of individual variables in representing population variability in the principal component analysis.**

Abbreviations: IF = intrathecal fractions; PCA = principal component analysis. A. PCA was applied between MS and controls, using the significantly upregulated CSF miRNAs in MS subjects and other quantitative CSF parameters (IgG or IgM IF, CSF pleocytosis). B. PCA was also applied using differentially expressed miRNAs between relapsing and remitting MS patients. Cos2 values, representing the strength of the individual variables in indicating population variability according to each dimension, are shown (see figure 4). Significant cos2 values (>0.5) are indicated in bold.

|  |  |
| --- | --- |
| **B** | Cos2 |
|  | Dimension 1 | Dimension 2 |
| miR-20a-5p | **0.546** | 0.006 |
| miR-24-3p | **0.896** | 0.006 |
| miR-145-5p | **0.821** | 0.001 |
| miR-29c-3p | 0.031 | **0.719** |
| miR-214-3p | 0.036 | **0.712** |
| miR-27a-3p | **0.884** | 0.006 |
| miR-27b-3p | **0.752** | 0.011 |

|  |  |
| --- | --- |
| **A** | Cos2 |
|  | Dimension 1 | Dimension 2 |
| Cells/mm3 | 0.088 | 0.113 |
| IgG IF | 0.133 | **0.507** |
| IgM IF | 0.020 | **0.742** |
| miR-146a-5p | **0.862** | 0.008 |
| miR-150-5p | **0.755** | 0.050 |
| miR-155-5p | **0.569** | 0.000 |
| miR-149-3p | 0.242 | 0.033 |
| miR-21-5p | **0.566** | 0.021 |

**Table e-6. Pathway enrichment analysis for differentially expressed miRNAs in all biological compartments associated to MS.**

Abbreviations: KEGG = Kyoto Encyclopedia of Genes and Genomes, MS = multiple sclerosis, PBMCs = peripheral blood mononuclear cells, Rel = relapsing MS, Rem = remitting MS.

This table shows the most commonly targeted KEGG pathways and genes by the miRNAs reaching statistical significance among the different groups and biological compartments. Up- and down-regulated miRNAs were grouped as sets according to the biological compartment and the disease category. These miRNA sets were then introduced into two databases, namely miRNet and mirPath. Only KEGG pathways significant in both databases for at least two miRNA sets have been selected (top part of the table). MiRNAs identified by both databases are shown in the middle part of the table, miRNAs common in at least two miRNA sets are indicated by ‘X’, those appearing in only one miRNA set by ‘o’. Genes from top enrichedcanonical pathways identified by both databases and targeted at least (i) by two miRNAs, (ii) in one database and (iii) in two miRNA sets are shown in the lower part of the table. Genes have been ranked from top to bottom based on the number of miRNAs targeting them in both databases. Genes indicated in bold are common to four or five pathways.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| KEGG pathways | Cell cycle | ErbB signaling | mTOR signaling | Insulin signaling | p53 signaling | TGFβ signaling | Adherens junction | Endocytosis | Neurotrophin signaling | Chronic Myeloid Leukemia | Glioma |
| miRNA sets |  |
| CSF | Up Rel MS | X | X | X | X | X | X | X |  | X | X | X |
| Down Rem MS | X | X | X | X | X | X | X | X | X | X | X |
| Down Rel MS |  |  |  |  |  |  |  |  |  |  | X |
| Serum | Down Rel MS | X | X |  |  |  |  |  | X |  | X | X |
| Down Rem MS | X | X |  |  |  |  |  | X |  | X | X |
| PBMCs | Down Rel MS |  |  |  |  | X |  | X |  |  | X | X |
| miRNAs |  |
| hsa-miR-20a-5p | X | X | X | o | X | X | o | o | o | X | X |
| hsa-miR-21-5p | o | o | o |  | o | o |  |  |  | o | o |
| hsa-miR-24-3p | X | o |  |  | o | o | o | X |  | X | o |
| hsa-miR-27a-3p | o | o | o | X | o | X | o |  | o | o | o |
| hsa-miR-27b-3p | o |  | o |  |  | o |  |  | o | o | o |
| hsa-miR-29c-3p |  |  | o |  |  |  |  |  | o | X | o |
| *hsa-miR-33a-3p°* |  |  |  |  |  |  | o |  |  |  |  |
| *hsa-miR-34a-5p°* | o |  |  |  | o |  | o |  |  | o | o |
| *hsa-miR-34c-5p°* |  |  |  |  |  |  |  |  |  |  | o |
| hsa-miR-125b-5p | o | X | o | o | o |  | o |  | o | o | o |
| hsa-miR-126-3p |  |  |  |  |  |  |  |  |  | X | X |
| hsa-miR-145-5p |  | o |  |  |  |  | o |  |  |  | o |
| hsa-miR-146a-5p | X | X |  |  |  |  |  |  | o |  |  |
| *hsa-miR-150-5p°* |  |  | o |  |  |  |  |  |  |  |  |
| *hsa-miR-155-5p°* | o |  | o | o |  | o | o |  |  |  |  |
| hsa-miR-181c-5p |  |  |  |  |  |  |  |  |  |  | X |
| hsa-miR-214-3p |  |  |  |  | X |  |  |  | o | X | o |
| Targeted genes |  |
|  |  | **MYC** | **MYC** | VEGFA | MKNK2 | **TP53** | **MYC** | CTNND1 |  | **TP53** | **TP53** | **TP53** |
|  |  | **TP53** | NRAS | HIF1A | FOXO1 | PTEN | BMPR2 | EGFR |  | BCL2 | **MYC** | **CDK6** |
|  |  | E2F3 | **CDKN1A** | RPS6KA3 | GSK3B | THBS1 | TGFBR2 | SMAD2 |  |  | E2F3 | E2F1 |
|  |  | **CDK6** | ERBB3 |  | PRKAR1A | **CDK6** | THBS1 | **SMAD4** |  |  | **CDK6** | NRAS |
|  |  | E2F1 | EGFR |  |  | SESN1 | **SMAD4** | CTNNB1 |  |  | E2F1 | PTEN |
|  |  | **SMAD4** | AKT1 |  |  | **CCND1** | SP1 | SSX2IP |  |  | TGFBR2 | E2F3 |
|  |  | E2F2 | CDKN1B |  |  | CCNG1 | TGFBR1 |  |  |  | NRAS | **CCND1** |
|  |  | CCNA2 |  |  |  | **CDKN1A** | SMAD2 |  |  |  | E2F2 | MDM2 |
|  |  | **CCND1** |  |  |  | RRM2 | ID4 |  |  |  | RUNX1 | **CDKN1A** |
|  |  | CDKN2A |  |  |  | MDM4 | SMAD5 |  |  |  | **CDKN1A** | EGFR |
|  |  | WEE1 |  |  |  | CCNB1 |  |  |  |  | MAPK1 | CDK4 |
|  |  | CDK4 |  |  |  |  |  |  |  |  | **CCND1** | AKT1 |
|  |  | **CDKN1A** |  |  |  |  |  |  |  |  | TGFBR1 | KRAS |
|  |  | GSK3B |  |  |  |  |  |  |  |  | CDKN2A | PIK3R1 |
|  |  | CCNB1 |  |  |  |  |  |  |  |  | **SMAD4** |  |
|  |  | SMAD2 |  |  |  |  |  |  |  |  | CDK4 |  |
|  |  | YWHAZ |  |  |  |  |  |  |  |  | NFKBIA |  |
|  |  | CDKN1B |  |  |  |  |  |  |  |  | AKT1 |  |
|  |  | TGFB1 |  |  |  |  |  |  |  |  | CDKN1B |  |
|  |  |  |  |  |  |  |  |  |  |  | CRKL |  |
|  |  |  |  |  |  |  |  |  |  |  | NFKBIA |  |
|  |  |  |  |  |  |  |  |  |  |  | TGFB1 |  |