

SUPPLEMENTAL MATERIAL

Altered expression of PiRNA in rat brain following transient focal ischemia

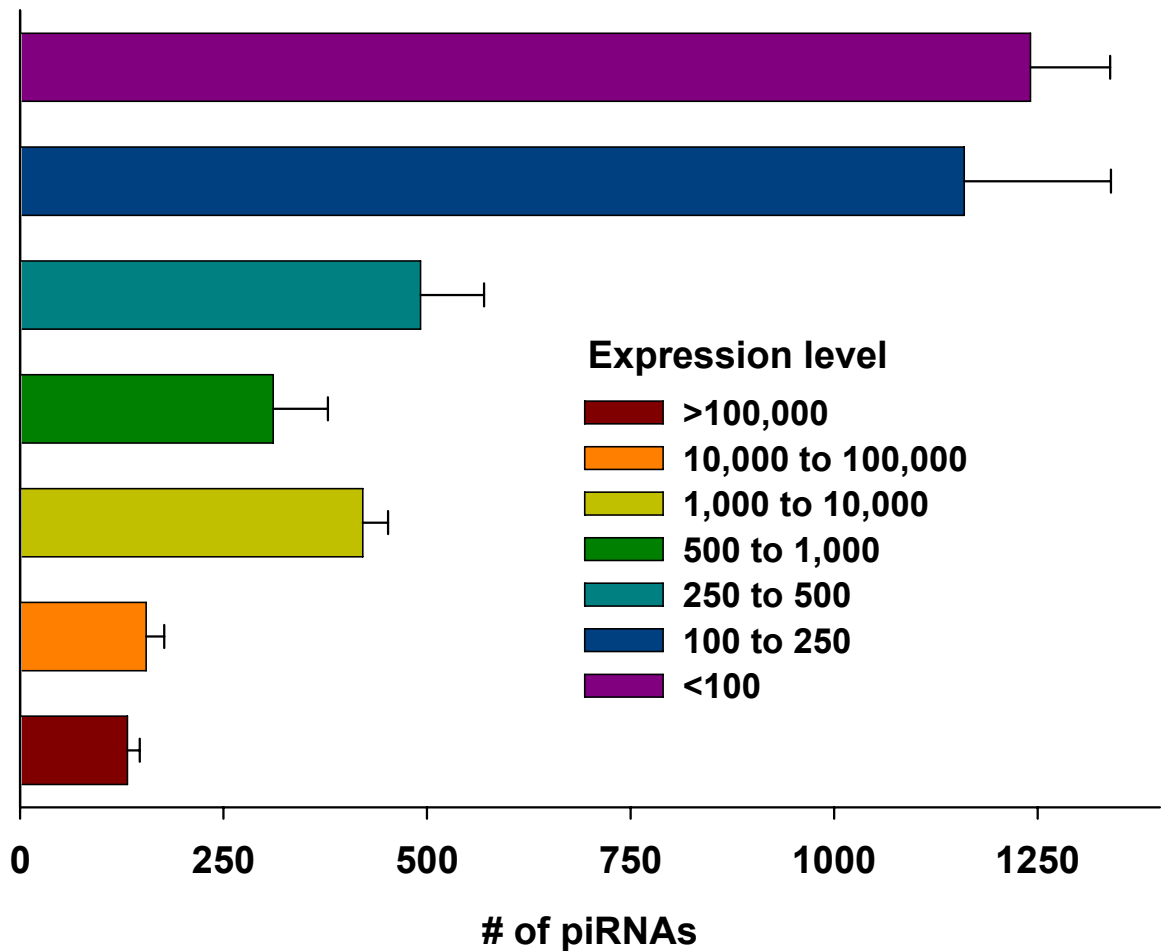
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Running Head: PiRNA in Stroke

Detailed method of focal ischemia: Adult, male, spontaneously hypertensive rats (SHR; 280-320g; Charles River, Wilmington, MA) used in these studies were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Services Publication number 86-23 (revised 1986). The Research Animal Resources and Care Committee of the University of Wisconsin-Madison approved the surgical procedures. Transient MCAO was induced with an intraluminal suture. In brief, a rat was anesthetized with halothane, placed in a stereotaxic frame fitted with a nose cone with 2% isoflurane anesthesia. A craniotomy (4 mm in diameter, 2-4 mm lateral and 1-2 mm caudal to bregma) was performed with extreme care over the MCA territory using a trephine. The dura was left intact and a laser Doppler flow-meter probe (model PD-434; Vasamedics, LLC, St Paul, MN, USA) was placed on the surface of the ipsilateral cortex and fixed to the periosteum with a 4-0 silk suture. The probe was connected to a laser flowmeter device (Laserflow blood perfusion monitor BPM 403A; TSI Inc., St Paul, MN) for continuous monitoring of regional cerebral blood flow (rCBF). The left femoral artery was cannulated for continuous monitoring of arterial blood pressure and to obtain the measurements of pH, P_{aO_2} , P_{aCO_2} , hemoglobin and blood glucose concentration (i-STAT; Sensor Devices, Waukesha, WI). The rectal temperature was controlled at $37.0 \pm 0.5^\circ\text{C}$ during surgery with a feedback-regulated heating pad. After a midline skin incision, the left external carotid artery (ECA) was exposed, and its branches were coagulated. A 3-0 surgical monofilament nylon suture, blunted at the end, was introduced into the ECA lumen and gently advanced to the internal carotid artery until rCBF was reduced to 10 to 16% of the baseline (recorded by laser Doppler flowmeter). After a 1h occlusion, the suture was withdrawn to restore the blood flow (confirmed by laser Doppler). Rats were killed at 24h of reperfusion. After suturing the wound, the rat was allowed to recover from anesthesia and returned to the cage with *ad libitum* access to food and water. During the MCAO, P_{aO_2} (100 to 200 mm Hg) and P_{aCO_2} (30 to 40 mm Hg) were maintained at physiological levels. Sham-operated rats served as control.



Supplementary Fig. 1: The piRNA microarray experiment showed that in the normal rat cerebral cortex an average of 3,885 (\pm 296) piRNAs of the ~40,000 piRNAs (9.7%) analyzed obtained a statistically significant present call. On a scale of 1 to 900,000 units, 132 piRNAs were expressed at a very high level (100,000 to 800,000 units), 155 at a high level (10,000 to 100,000 units), 421 at a moderate to high level (1,000 to 10,000 units), 311 at a moderate level (500 to 1,000), 492 at a low to moderate level (250 to 500), 1160 at a low level (100 to 250) and 1,241 at a very low level (<100 units).

Supplementary Table 1: Stroke-responsive piRNAs upregulated by >2.5 fold in rat cerebral cortex

| piRNA | NCBI # | Δ fold | piRNA | NCBI # | Δ fold |
|------------|----------|---------------|------------|----------|---------------|
| piR-177729 | DQ762407 | 36.01 | piR-77768 | DQ609656 | 3.31 |
| piR-143106 | DQ727784 | 31.26 | piR-80380 | DQ612268 | 3.28 |
| piR-173369 | DQ758047 | 12.9 | piR-64203 | DQ614091 | 3.26 |
| piR-64423 | DQ614312 | 8.78 | piR-63447 | DQ603335 | 3.23 |
| piR-176691 | DQ761369 | 6.83 | piR-87480 | DQ620368 | 3.19 |
| piR-64425 | DQ614313 | 6.23 | piR-62322 | DQ602210 | 3.11 |
| piR-71756 | DQ628212 | 5.14 | piR-173367 | DQ758045 | 3.08 |
| piR-87058 | DQ619946 | 5.06 | piR-87579 | DQ620467 | 3.04 |
| piR-62321 | DQ602209 | 4.98 | piR-153591 | DQ738269 | 3.04 |
| piR-62320 | DQ602208 | 4.43 | piR-64506 | DQ614394 | 2.98 |
| piR-88241 | DQ621129 | 4.43 | piR-87481 | DQ620369 | 2.93 |
| piR-62318 | DQ602206 | 4.29 | piR-74994 | DQ606882 | 2.89 |
| piR-78586 | DQ610474 | 4.25 | piR-66079 | DQ622535 | 2.83 |
| piR-154978 | DQ739656 | 4.18 | piR-64754 | DQ614642 | 2.80 |
| piR-173297 | DQ757975 | 4.17 | piR-156575 | DQ741253 | 2.77 |
| piR-74107 | DQ605995 | 4.10 | piR-65300 | DQ621756 | 2.74 |
| piR-64211 | DQ614099 | 3.96 | piR-144658 | DQ729336 | 2.73 |
| piR-62317 | DQ602205 | 3.73 | piR-62319 | DQ602207 | 2.67 |
| piR-151961 | DQ736639 | 3.70 | piR-63823 | DQ603711 | 2.66 |
| piR-156446 | DQ741124 | 3.69 | piR-74270 | DQ606158 | 2.64 |
| piR-77767 | DQ609655 | 3.67 | piR-75472 | DQ607360 | 2.60 |
| piR-159701 | DQ744379 | 3.56 | piR-177196 | DQ761874 | 2.58 |
| piR-142999 | DQ727677 | 3.49 | piR-78584 | DQ610472 | 2.57 |
| piR-177214 | DQ761892 | 3.45 | piR-165957 | DQ750635 | 2.55 |
| piR-64518 | DQ614406 | 3.43 | piR-83056 | DQ615944 | 2.54 |
| piR-64524 | DQ614412 | 3.38 | piR-80867 | DQ612755 | 2.53 |
| piR-64032 | DQ603920 | 3.31 | piR-84909 | DQ617797 | 2.49 |

Supplementary Table 2: Stroke-responsive piRNAs down-regulated by >2.5 fold in rat cerebral cortex

| piRNA | NCBI # | Δ fold | piRNA | NCBI # | Δ fold |
|------------|----------|---------------|------------|----------|---------------|
| piR-169523 | DQ754201 | -13.41 | piR-153626 | DQ738304 | -3.90 |
| piR-70903 | DQ627359 | -11.07 | piR-63713 | DQ603601 | -3.75 |
| piR-88428 | DQ621316 | -9.20 | piR-160391 | DQ745069 | -3.72 |
| piR-64621 | DQ614509 | -9.18 | piR-147004 | DQ731682 | -3.71 |
| piR-182498 | DQ767176 | -8.78 | piR-154203 | DQ738881 | -3.55 |
| piR-177543 | DQ762221 | -8.67 | piR-74656 | DQ606544 | -3.33 |
| piR-170937 | DQ755615 | -8.49 | piR-78358 | DQ610246 | -3.24 |
| piR-148170 | DQ732848 | -8.24 | piR-72952 | DQ604840 | -3.22 |
| piR-167731 | DQ752409 | -8.18 | piR-164431 | DQ749109 | -3.19 |
| piR-176643 | DQ761321 | -7.76 | piR-144266 | DQ728944 | -3.14 |
| piR-82548 | DQ615436 | -6.93 | piR-164579 | DQ749257 | -3.12 |
| piR-168069 | DQ752747 | -6.19 | piR-166439 | DQ751117 | -3.09 |
| piR-176687 | DQ761365 | -5.98 | piR-149616 | DQ734294 | -3.07 |
| piR-71808 | DQ628264 | -5.81 | piR-177367 | DQ762045 | -2.96 |
| piR-151446 | DQ736124 | -5.71 | piR-144043 | DQ728721 | -2.89 |
| piR-75840 | DQ607728 | -5.52 | piR-168048 | DQ752726 | -2.80 |
| piR-177411 | DQ762089 | -4.73 | piR-151551 | DQ736229 | -2.76 |
| piR-168978 | DQ753656 | -4.20 | piR-63162 | DQ603050 | -2.67 |
| piR-70579 | DQ627035 | -4.13 | piR-157563 | DQ742241 | -2.65 |
| piR-163442 | DQ748120 | -4.12 | piR-168937 | DQ753615 | -2.62 |
| piR-67691 | DQ624147 | -4.07 | piR-165546 | DQ750224 | -2.58 |
| piR-151308 | DQ735986 | -4.03 | piR-168485 | DQ753163 | -2.54 |
| piR-71768 | DQ628224 | -4.00 | piR-153572 | DQ738250 | -2.53 |
| piR-159005 | DQ743683 | -3.98 | piR-66673 | DQ623129 | -2.52 |
| piR-67146 | DQ623602 | -3.93 | piR-83599 | DQ616487 | -2.51 |
| piR-174902 | DQ759580 | -3.93 | | | |

Supplementary table 3: Comprehensive list of all TFs and TF families showing hits on the 10 piRNA promoters

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| TF matrix | TF matrix description | # of TF hits | No of TF family hits | TF family | TF family description |
|--------------|---|--------------|----------------------|-----------|--|
| ZNF219.01 | Kruppel-like zinc finger protein 219 | 3 | 25 | ZF | Zinc binding protein factors |
| ZF9.01 | Core promoter-binding protein (CPBP) with 3 Krueppel-type zinc fingers | 2 | | | Zinc binding protein factors |
| ZBP89.01 | Zinc finger transcription factor ZBP-89 | 1 | | | Zinc binding protein factors |
| ZNF35.01 | Human zinc finger protein ZNF35 | 1 | | | Zinc finger protein ZNF35 |
| ZF5.01 | Zinc finger / POZ domain transcription factor | 3 | | | ZF5 POZ domain zinc finger |
| SIP1.01 | Smad-interacting protein | 2 | | | Two-handed zinc finger homeodomain transcription factors |
| ZBRK1.01 | Transcription factor with 8 central zinc fingers and an N-terminal KRAB domain | 1 | | | Zinc finger proteins |
| MAZ.01 | Myc associated zinc finger protein (MAZ) | 2 | | | Myc associated zinc fingers |
| MAZR.01 | MYC-associated zinc finger protein related transcription factor | 2 | | | Myc associated zinc fingers |
| MZF1.01 | Myeloid zinc finger protein MZF1 | 2 | | | Myeloid zinc finger 1 factors |
| MZF1.02 | Myeloid zinc finger protein MZF1 | 2 | | | Myeloid zinc finger 1 factors |
| MZF1.03 | Myeloid zinc finger protein MZF1 | 2 | | | Myeloid zinc finger 1 factors |
| GLI1.01 | Zinc finger transcription factor GLI1 | 1 | | | GLI zinc finger family |
| GLI1.02 | Glioma-associated oncogene homolog 1 | 1 | | | GLI zinc finger family |
| ATF.02 | Activating transcription factor | 2 | 13 | CREB | cAMP-responsive element binding proteins |
| CREB.02 | cAMP-responsive element binding protein | 2 | | | cAMP-responsive element binding proteins |
| CREB1.01 | cAMP-responsive element binding protein 1 | 2 | | | cAMP-responsive element binding proteins |
| ATF.01 | Activating transcription factor | 1 | | | cAMP-responsive element binding proteins |
| ATF2.01 | Activating transcription factor 2 | 1 | | | cAMP-responsive element binding proteins |
| ATF6.02 | Activating transcription factor 6, member of b-zip family, induced by ER stress | 1 | | | cAMP-responsive element binding proteins |
| CJUN_ATF2.01 | c-Jun/ATF2 heterodimers | 1 | | | cAMP-responsive element binding proteins |
| CREB2.01 | cAMP-responsive element binding protein 2 | 1 | | | cAMP-responsive element binding proteins |

| | | | | | |
|--------------|--|---|----|----------------|--|
| CREB2CJUN.01 | CRE-binding protein 2/c-Jun heterodimer | 1 | | | cAMP-responsive element binding proteins |
| E4BP4.01 | E4BP4, bZIP domain, transcriptional repressor | 1 | | | cAMP-responsive element binding proteins |
| NKX24.01 | NK2 homeobox 4, NKX 2 DELTA | 2 | 10 | NKXH | NKX homeodomain factors |
| NKX25.04 | NK2 homeobox 5, cardiac-specific homeo box 1 (Csx1) | 2 | | | NKX homeodomain factors |
| NKX26.01 | NK2 homeobox 6, Csx2 | 2 | | | NKX homeodomain factors |
| TTF1.02 | Thyroid transcription factor-1, NK2 homeobox 1 (Nkx2-1) | 2 | | | NKX homeodomain factors |
| BAPX1.01 | Bagpipe homeobox homolog 1 (homeodomain protein Nkx-3.2) | 1 | | | NKX homeodomain factors |
| NKX25.01 | Homeo domain factor Nkx-2.5/Csx, tinman homolog, high affinity sites | 1 | | | NKX homeodomain factors |
| KLF6.01 | Kruppel-like factor 6 | 3 | 9 | Kruppel | Krueppel like transcription factors |
| KKLF.01 | Kidney-enriched kruppel-like factor, KLF15 | 2 | | | Krueppel like transcription factors |
| BTEB3.01 | Basic transcription element (BTE) binding protein, BTEB3, FKLF-2 | 1 | | | Krueppel like transcription factors |
| GKLF.01 | Gut-enriched Krueppel-like factor | 1 | | | Krueppel like transcription factors |
| MOK2.02 | Ribonucleoprotein associated zinc finger protein MOK-2 (human) | 2 | | | Mouse Krueppel like factor |
| HOXA10.01 | Homeobox A10 / Hox1.8 | 1 | 7 | ABDB | Abdominal-B type homeodomain transcription factors |
| HOXA9.02 | Homeobox A9 / Hox1-gamma | 1 | | | Abdominal-B type homeodomain transcription factors |
| HOXB9.02 | Homeobox B9 | 1 | | | Abdominal-B type homeodomain transcription factors |
| HOXC11.01 | Homeobox C11/ Hox-3H | 1 | | | Abdominal-B type homeodomain transcription factors |
| HOXC12.01 | Homeobox C12/ Hox-3phi | 1 | | | Abdominal-B type homeodomain transcription factors |
| HOXC9.01 | Homeobox C9 / Hox-3beta | 1 | | | Abdominal-B type homeodomain transcription factors |
| HOXD10.01 | Homeobox D10 | 1 | | | Abdominal-B type homeodomain transcription factors |
| SP1.03 | Stimulating protein 1, ubiquitous zinc finger transcription factor | 2 | 7 | SP1F | GC-Box factors SP1/GC |
| GC.01 | GC box elements | 1 | | | GC-Box factors SP1/GC |

| | | | | | |
|-------------|---|---|---|-------------|---|
| SP1.01 | Stimulating protein 1, ubiquitous zinc finger transcription factor | 1 | | | GC-Box factors SP1/GC |
| SP1.02 | Stimulating protein 1, ubiquitous zinc finger transcription factor | 1 | | | GC-Box factors SP1/GC |
| SP2.01 | Sp2, member of the Sp/XKLF transcription factors with three C2H2 zinc fingers in a conserved carboxyl-terminal domain | 1 | | | GC-Box factors SP1/GC |
| TIEG.01 | TGFbeta-inducible early gene (TIEG) / Early growth response gene alpha (EGRalpha) | 1 | | | GC-Box factors SP1/GC |
| E2F.03 | E2F, involved in cell cycle regulation, interacts with Rb p107 protein | 3 | 7 | E2FF | E2F-myc activator/cell cycle regulator |
| E2F4_DP1.01 | E2F-4/DP-1 heterodimeric complex | 3 | | | E2F-myc activator/cell cycle regulator |
| E2F.02 | E2F, involved in cell cycle regulation, interacts with Rb p107 protein | 1 | | | E2F-myc activator/cell cycle regulator |
| IRX3.01 | Iroquois homeobox 3 | 2 | 7 | IRXF | Iroquois homeobox transcription factors |
| IRX4.01 | Iroquois homeobox 4 | 2 | | | Iroquois homeobox transcription factors |
| IRX6.01 | Iroquois homeobox 6 | 2 | | | Iroquois homeobox transcription factors |
| IRX2.01 | Iroquois homeobox 2 | 1 | | | Iroquois homeobox transcription factors |
| PAX2.01 | Zebrafish PAX2 paired domain protein | 1 | 7 | PAX | PAX-2 binding sites |
| PAX3.01 | Pax-3 paired domain protein, expressed in embryogenesis, mutations correlate to Waardenburg Syndrome | 1 | | | PAX-3 binding sites |
| PAX5.01 | B-cell-specific activator protein | 2 | | | PAX-5 B-cell-specific activator protein |
| PAX5.02 | B-cell-specific activator protein | 1 | | | PAX-5 B-cell-specific activator protein |
| PAX2.02 | Paired box protein 2 | 1 | | | PAX-2/5/8 binding sites |
| PAX9.01 | Zebrafish PAX9 binding sites | 1 | | | PAX-9 binding sites |
| CKROX.01 | Collagen krox protein (zinc finger protein 67 - zfp67) | 3 | 6 | EGR | EGR/nerve growth factor induced protein C & related factors |
| WT1.01 | Wilms Tumor Suppressor | 2 | | | EGR/nerve growth factor induced protein C & related factors |
| EGR1.02 | EGR1, early growth response 1 | 1 | | | EGR/nerve growth factor induced protein C & related factors |
| PTX1.01 | Pituitary Homeobox 1 (Ptx1, Pitx-1) | 2 | 5 | BCDF | Bicoid-like homeodomain transcription factors |
| CRX.01 | Cone-rod homeobox-containing transcription factor / otx-like homeobox gene | 1 | | | Bicoid-like homeodomain transcription factors |
| DMBX1.01 | Diencephalon/mesencephalon homeobox 1 | 1 | | | Bicoid-like homeodomain transcription factors |

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|---------------|---|---|---|-------------|--|
| GSC.02 | Vertebrate bicoid-type homeodomain protein Goosecoid | 1 | | | Bicoid-like homeodomain transcription factors |
| PBX1_MEIS1.02 | Binding site for a Pbx1/Meis1 heterodimer | 2 | 5 | PBXC | PBX1 - MEIS1 complexes |
| PBX1_MEIS1.03 | Binding site for a Pbx1/Meis1 heterodimer | 2 | | | PBX1 - MEIS1 complexes |
| PBX1_MEIS1.01 | Binding site for a Pbx1/Meis1 heterodimer | 1 | | | PBX1 - MEIS1 complexes |
| FHXB.01 | Fork head homologous X binds DNA with a dual sequence specificity (FHXA and FHXB) | 1 | 4 | FKHD | Fork head domain factors |
| HFH2.01 | HNF-3/Fkh Homolog 2 (FOXD3) | 1 | | | Fork head domain factors |
| HFH3.01 | HNF-3/Fkh Homolog 3 (FOXI1, Freac-6) | 1 | | | Fork head domain factors |
| HFH8.01 | HNF-3/Fkh Homolog-8 (FOXF1) | 1 | | | Fork head domain factors |
| STAT6.01 | Signal transducer and activator of transcription 6 | 2 | 4 | STAT | Signal transducer and activator of transcription |
| STAT3.01 | Signal transducer and activator of transcription 3 | 1 | | | Signal transducer and activator of transcription |
| STAT3.02 | Signal transducer and activator of transcription 3 | 1 | | | Signal transducer and activator of transcription |
| ATF6.01 | Member of b-zip family, induced by ER damage/stress, binds to the ERSE in association with NF-Y | 3 | 4 | EBOX | E-box binding factors |
| NMYC.02 | v-myc myelocytomatosis viral related oncogene, neuroblastoma derived | 1 | | | E-box binding factors |
| PTATA.02 | Plant TATA box | 1 | 3 | TBP | Plant TATA binding protein factor |
| ATATA.01 | Avian C-type LTR TATA box | 1 | | | Vertebrate TATA binding protein factor |
| SPT15.01 | TATA-binding protein, general transcription factor that interacts with other factors to form the preinitiation complex at promoters | 1 | | | Yeast TATA binding protein factor |
| CDX1.02 | Caudal type homeo box 1 | 1 | 3 | CDXF | Vertebrate caudal related homeodomain protein |
| VCDX2.01 | Cdx-2 mammalian caudal related intestinal transcr. factor | 1 | | | Vertebrate caudal related homeodomain protein |
| CDX2.02 | Caudal type homeobox transcription factor 2 | 1 | | | Vertebrate caudal related homeodomain protein |
| P53.04 | Tumor suppressor p53 | 1 | 3 | P53 | p53 tumor suppressor |
| P53.05 | Tumor suppressor p53 | 1 | | | p53 tumor suppressor |
| P53.06 | Tumor suppressor p53 | 1 | | | p53 tumor suppressor |
| GRHL1.01 | Grainyhead-like 1 (LBP32, MGR, TFCP2L2) | 2 | 3 | GRHL | Grainyhead-like transcription factors |
| GRHL3.01 | Grainyhead-like 3 (sister-of-mammalian grainyhead - SOM) | 1 | | | Grainyhead-like transcription factors |

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|---|---|---|---|------|--|
| VMYB.01 | v-Myb | 2 | 3 | MYBL | Cellular and viral myb-like transcriptional regulators |
| VMYB.04 | v-Myb, AMV v-myb | 1 | | | Cellular and viral myb-like transcriptional regulators |
| All transcription factors below show no family affiliation, or if a family affiliation is present they show <3 total family hits: | | | | | |
| PLAG1.01 | Pleomorphic adenoma gene (PLAG) 1, a developmentally regulated C2H2 zinc finger protein | 4 | 4 | | Pleomorphic adenoma gene |
| CTCF.01 | CCCTC-binding factor | 2 | 2 | | CTCF and BORIS gene family, transcriptional regulators with 11 highly conserved zinc finger domains |
| E4F.01 | GLI-Krueppel-related transcription factor, regulator of adenovirus E4 promoter | 2 | 2 | | Ubiquitous GLI - Krueppel like zinc finger involved in cell cycle regulation |
| GTF3R4.01 | GTF2I-like repeat 4 of GTF3 | 2 | 2 | | GTF2IRDI upstream control element |
| HSF1.01 | Heat shock factor 1 | 2 | 2 | | Heat shock factors |
| NRF1.01 | Nuclear respiratory factor 1 (NRF1), bZIP transcription factor that acts on nuclear genes encoding mitochondrial proteins | 2 | 2 | | Nuclear respiratory factor 1 |
| PURALPHA.01 | Purine-rich element binding protein A | 2 | 2 | | Pur-alpha binds both single-stranded and double-stranded DNA in a sequence-specific manner |
| BRE.01 | Transcription factor II B (TFIIB) recognition element | 1 | 1 | | RNA polymerase II transcription factor II B |
| XCPE1.01 | X gene core promoter element 1 | 1 | 1 | | Activator-, mediator- and TBP-dependent core promoter element for RNA polymerase II transcription from TATA-less promoters |
| AHR.01 | Aryl hydrocarbon / dioxin receptor | 1 | 1 | | AHR-arnt heterodimers and AHR-related factors |
| AP1.03 | Activator protein 1 | 1 | 1 | | AP1, Activating protein 1 |
| BRN5.01 | Brn-5, POU-VI protein class (also known as emb and CNS-1) | 1 | 1 | | Brn-5 POU domain factors |
| KAISO.01 | Transcription factor Kaiso, ZBTB33 | 1 | 1 | | BTB/POZ (broad complex, TramTrack, Bric-a-brac/pox viruses and zinc fingers) transcription factor |
| NFY.02 | Nuclear factor Y (Y-box binding factor) | 1 | 1 | | CCAAT binding factors |
| PHOX2.01 | Phox2a (ARIX) and Phox2b | 1 | 1 | | Cart-1 (cartilage homeoprotein 1) |

| | | | | |
|--------------|--|---|---|--|
| CDP.02 | Transcriptional repressor CDP | 1 | 1 | CLOX and CLOX homology (CDP) factors |
| DMRT3.01 | Doublesex and mab-3 related transcription factor 3 | 1 | 1 | DM domain-containing transcription factors |
| DMP1.01 | Cyclin D-interacting myb-like protein, DMTF1 - cyclin D binding myb-like transcription factor 1 | 1 | 1 | Cyclin D binding myb-like transcription factor |
| ETS1.01 | c-Ets-1 binding site | 1 | 1 | Human and murine ETS1 factors |
| GABP.01 | GABP: GA binding protein | 1 | 1 | Human and murine ETS1 factors |
| GAGA.01 | GAGA-Box | 1 | 1 | GA-boxes |
| GATA1.01 | GATA-binding factor 1 | 1 | 1 | GATA binding factors |
| GATA2.02 | GATA-binding factor 2 | 1 | 1 | GATA binding factors |
| GCM1.01 | Glial cells missing homolog 1, chorion-specific transcription factor GCMA | 1 | 1 | Chorion-specific transcription factors with a GCM DNA binding domain |
| AML1.01 | AML1/CBFA2 Runt domain binding site | 1 | 1 | Human acute myelogenous leukemia factors |
| HAND2_E12.01 | Heterodimers of the bHLH transcription factors HAND2 (Thing2) and E12 | 1 | 1 | Twist subfamily of class B bHLH transcription factors |
| HEN1.02 | HEN1 | 1 | 1 | Twist subfamily of class B bHLH transcription factors |
| GSH1.01 | Homeobox transcription factor Gsh-1 | 1 | 1 | Homeobox transcription factors |
| HDBP1_2.01 | Huntington's disease gene regulatory region-binding protein 1 and 2 (SLC2A4 regulator and papillomavirus binding factor) | 1 | 1 | Huntington's disease gene regulatory region binding proteins |
| HES1.01 | Drosophila hairy and enhancer of split homologue 1 (HES-1) | 1 | 1 | Vertebrate homologues of enhancer of split complex) |
| MTBF.01 | Muscle-specific Mt binding site | 1 | 1 | Human muscle-specific Mt binding site |
| HMBOX.01 | Homeobox containing 1 | 1 | 1 | Hepatic Nuclear Factor 1 |
| TCF2.01 | Hepatocyte nuclear factor 1 beta (HNF1B) | 1 | 1 | Hepatic Nuclear Factor 1 |
| HNF6.01 | Liver enriched Cut - Homeodomain transcription factor HNF6 (ONECUT) | 1 | 1 | Onecut homeodomain factor HNF6 |
| HMX2.02 | Hmx2/Nkx5-2 homeodomain transcription factor | 1 | 1 | Homeodomain transcription factors |
| HOX1-3.01 | Hox-1.3, vertebrate homeobox protein | 1 | 1 | Paralog hox genes 1-8 from the four hox clusters A, B, C, D |
| HOMEZ.01 | Homeobox and leucine zipper encoding transcription factor | 1 | 1 | Homeodomain-leucine zipper transcription factors |
| IK3.01 | Ikaros 3, potential regulator of lymphocyte differentiation | 1 | 1 | Ikaros zinc finger family |
| INSM1.01 | Zinc finger protein insulinoma-associated 1 (IA-1) functions as a transcriptional repressor | 1 | 1 | Insulinoma associated factors |

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|----------|--|---|---|--|
| IRF4.01 | Interferon regulatory factor (IRF)-related protein (NF-EM5, PIP, LSIRF, ICSAT) | 1 | 1 | Interferon regulatory factors |
| IRF7.01 | Interferon regulatory factor 7 (IRF-7) | 1 | 1 | Interferon regulatory factors |
| MEF2.07 | Myocyte-specific enhancer factor 2 | 1 | 1 | MEF2, myocyte-specific enhancer binding factor |
| E47.01 | MyoD/E47 and MyoD/E12 dimers | 1 | 1 | Myoblast determining factors |
| COUP.02 | Chicken ovalbumin upstream promoter (COUP-TF), DR0 sites | 1 | 1 | Nuclear receptor subfamily 2 factors |
| OCT1.01 | Octamer-binding factor 1 | 1 | 1 | Octamer binding protein |
| OCT1.05 | Octamer-binding factor 1 | 1 | 1 | Octamer binding protein |
| VBP.01 | PAR-type chicken vitellogenin promoter-binding protein | 1 | 1 | PAR/bZIP family |
| PDX1.01 | Pdx1 (IDX1/IPF1) pancreatic and intestinal homeodomain TF | 1 | 1 | Pancreatic and intestinal homeodomain transcription factor |
| PLZF.01 | Promyelocytic leukemia zinc finger (TF with nine Krueppel-like zinc fingers) | 1 | 1 | C2H2 zinc finger protein PLZF |
| PPARG.01 | Pal3 motif, bound by a PPAR-gamma homodimer, IR3 sites | 1 | 1 | Peroxisome proliferator activated receptor homodimers |
| PRDM5.01 | PR domain containing 5 | 1 | 1 | PRDI-BF1 and RIZ homologous (PR) domain proteins (PRDM) |
| RREB1.01 | Ras-responsive element binding protein 1 | 1 | 1 | Ras-responsive element binding protein |
| SATB1.01 | Special AT-rich sequence-binding protein 1, predominantly expressed in thymocytes, binds to matrix attachment regions (MARs) | 1 | 1 | Special AT-rich sequence binding protein |
| SOX5.01 | Sox-5 | 1 | 1 | SOX/SRY-sex/testis determining and related HMG box factors |
| SPZ1.01 | Spermatogenic Zip 1 transcription factor | 1 | 1 | Testis-specific bHLH-Zip transcription factors |
| SRF.03 | Serum response factor | 1 | 1 | Serum response element binding factor |
| SRF.04 | Serum response factor | 1 | 1 | Serum response element binding factor |
| STAF.01 | Se-Cys tRNA gene transcription activating factor | 1 | 1 | Selenocysteine tRNA activating factor |
| STAF.02 | Se-Cys tRNA gene transcription activating factor | 1 | 1 | Selenocysteine tRNA activating factor |
| TEF1.01 | TEF-1 related muscle factor | 1 | 1 | TEA/ATTS DNA binding domain factors |
| WHN.01 | Winged helix protein, involved in hair keratinization and thymus epithelium differentiation | 1 | 1 | Winged helix binding sites |

| | | | | |
|--------|---|---|---|---|
| YB1.01 | Y box binding protein 1, has a preference for binding ssDNA | 1 | 1 | Y-box binding transcription factors, multifunctional proteins involved in transcriptional and translational regulation, mRNA splicing, DNA replication and repair |
| | | | | |

The TF matrix, TF family and TF family descriptions are presented according to the nomenclature of MatBase (Genomatix, GmbH). The number of hits for each transcription factor is presented in addition to the total number of hits for each TF family, which is obtained by combining the number of hits for each individual transcription factor in the family.

Supplementary Table 4: The top 20 TF families showing three or more promoter hits per family in a redundant manner

| TF family | # of piRNA promoter hits | # of individual piRNAs |
|--------------------|---------------------------------|-------------------------------|
| Zinc Finger family | 25 | 7 |
| CREB family | 10 | 3 |
| ABDB family | 7 | 3 |
| NKXH family | 6 | 4 |
| PAX family | 6 | 3 |
| SP1F family | 6 | 4 |
| Kruppel family | 5 | 7 |
| BCDF family | 4 | 2 |
| FKHD family | 4 | 2 |
| IRXF family | 4 | 2 |
| EBOX family | 4 | 4 |
| TBP family | 3 | 2 |
| CDXF family | 3 | 1 |
| E2FF family | 3 | 5 |
| EGR family | 3 | 4 |
| P53 family | 3 | 3 |
| PBXC family | 3 | 2 |
| STAT family | 3 | 3 |
| GRHL family | 3 | 2 |
| MYBL family | 3 | 3 |

The TF family names are presented according to the nomenclature of MatBase (Genomatix, GmbH). The number of piRNA promoter hits for each family corresponds to the number of hits of all the family members combined, for all 10 promoters that were scanned. The third column corresponds to the total number of promoters out of 10 that showed hits by all members combined of a particular family. The highest and lowest number of promoters targeted by a single TF family is 7 and 1 respectively.

Supplementary Table 5: List of individual TFs that bind each of the 10 piRNA promoters

| piR-177369 DQ758047 | | piR-87058 DQ-619946 | piR-64423 DQ614312 | piR-71756 DQ628212 |
|-------------------------------|-------------|-------------------------------|------------------------------|------------------------------|
| ZF5.01 | KKLF.01 | NKX25.04 | ZF5.01 | E4BP4.01 |
| MAZ.01 | SP1.03 | BAPX1.01 | ZF9.01 | HOXC12.01 |
| ZNF219.01 | E2F4DP1.01 | NKX26.01 | SIP1.01 | IRX3.01 |
| MZF1.02 | GSC.02 | HOXA10.01 | ATF6.02 | IRX4.01 |
| ZNF35.01 | PTX1.01 | HOXD10.01 | CREB.02 | IRX6.01 |
| MAZR.01 | ATF6.01 | HOXA9.02 | ATF.02 | HFH2.01 |
| MZF1.03 | GRHL1.01 | HOXC11.01 | CREB1.01 | HFH3.01 |
| MZF1.01 | GABP.01 | HOXB9.02 | CJUN_ATF2.01 | HFH8.01 |
| ATF.01 | PRDM5.01 | HOXC9.01 | CREB2.01 | ATATA.01 |
| CREB.02 | HEN1.02 | E2F4DP1.01 | ATF2.01 | ATATA.01 |
| ATF.02 | IK3.01 | IRX3.01 | CREB2CJUN.01 | P53.05 |
| CREB1.01 | GTF3R4.01 | IRX6.01 | TTF1.02 | VMYB.04 |
| KLF6.01 | GCM1.01 | IRX2.01 | NKX24.01 | GSH1.01 |
| GKLF.01 | INSM1.01 | IRX4.01 | E2F.03 | HOMEZ.01 |
| SP1.01 | HAND2E12.01 | PAX2.01 | PAX5.02 | BRN5.01 |
| GC.01 | PHOX2.01 | FHXB.01 | PAX5.01 | VBP.01 |
| E2F.03 | | PTATA.02 | PAX2.02 | SATB1.01 |
| CKROX.01 | | SPT15.01 | PBX1_MEIS1.03 | TCF2.01 |
| WT1.01 | | CDX2.01 | PBX1_MEIS1.02 | HNF6.01 |
| CRX.01 | | CDX1.02 | STAT3.02 | DMRT3.01 |
| DMBX1.01 | | CDX2.02 | XCPE1.01 | HOX1-3.01 |
| PLAG1.01 | | HMX2.02 | TEF1.01 | |
| ETS1.01 | | SOX5.01 | E4F.01 | |
| YB1.01 | | HMBOX.01 | E47.01 | |
| E4F.01 | | IRF4.01 | | |
| NRF1.01 | | HSF1.01 | | |
| AHR.01 | | PLZF.01 | | |
| RREB1.01 | | PDX1.01 | | |
| SRF.04 | | IRF7.01 | | |
| SRF.03 | | AP1.03 | | |

| piR-77768 | piR-64032 | piR-64518 | piR-64524 | piR-74107 | piR-77767 |
|------------------|------------------|------------------|------------------|------------------|------------------|
| DQ609656 | DQ603920 | DQ614406 | DQ614412 | DQ605995 | DQ609655 |
| ZNF219.01 | ZF5.01 | NKX26.01 | MZF1.03 | SIP1.01 | BTEB3.01 |
| ZF9.01 | ZBRK1.01 | TTF1.02 | MZF1.01 | MOK2.02 | STAT3.01 |
| ZBP89.01 | KLF6.01 | NKX24.01 | ZNF219.01 | TIEG.01 | STAT6.01 |
| MAZ.01 | SP2.01 | NKX25.04 | MZF1.02 | E2F4DP1.01 | NMYC.02 |
| MAZR.01 | E2F.03 | MOK2.02 | NKX25.01 | CKROX.01 | VMYB.01 |
| KKLF.01 | E2F.02 | PTX1.01 | KLF6.01 | P53.06 | STAF.02 |
| SP1.03 | NRF1.01 | PBX1_MEIS1.01 | CKROX.01 | VMYB.01 | STAF.01 |
| SP1.02 | GAGA.01 | PBX1_MEIS1.03 | STAT6.01 | GATA1.01 | CDP.02 |
| PAX5.01 | DMP1.01 | PBX1_MEIS1.02 | GRHL1.01 | MEF2.07 | GATA2.02 |
| PAX9.01 | MTBF.01 | P53.04 | GRHL3.01 | PLAG1.01 | |
| PAX3.01 | PURALPHA.01 | NFY.02 | PPARG.01 | SPZ1.01 | |
| EGR1.02 | WHN.01 | KAISO.01 | PLAG1.01 | | |
| WT1.01 | ATF6.01 | COUP.02 | | | |
| HDBP1_2.01 | AML1.01 | HSF1.01 | | | |
| BRE.01 | HES1.01 | OCT1.05 | | | |
| PURALPHA.01 | CTCF.01 | OCT1.01 | | | |
| GLI1.01 | PLAG1.01 | | | | |
| GTF3R4.01 | | | | | |
| ATF6.01 | | | | | |
| CTCF.01 | | | | | |
| GLI1.02 | | | | | |

The TF family names are presented according to the nomenclature of MatBase (Genomatix, GmbH).