**Supplemental Data**

**Supplemental Tables and Figures**

**Supplemental Table 1. Summarized miRNA sequence counts and percent per total.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Average miRNA sequence counts*** | | | | | | | |
| **Sample** | **NS** | **CN** | **PCM** | **CMELL** | **CMELM** | **CMELD** | **Melanoma cell lines\*** |
| hsa-miR-451a | 668.0 | 52.5 | 80.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| hsa-miR-144-3p | 216.0 | 6.0 | 31.2 | 0.0 | 8.3 | 0.0 | 0.0 |
| hsa-miR-144-5p | 4.0 | 0.0 | 1.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total miRNAs | 26391.8 | 111455.0 | 14315.6 | 13617.0 | 18193.0 | 15572.0 | 9962.7 |
|  | | | | | | | |
| ***Average percent/total miRNAs*** | | | | | | | |
| **Sample** | **NS** | **CN** | **PCM** | **CMELL** | **CMELM** | **CMELD** | **Melanoma cell lines\*** |
| hsa-miR-451a | 2.1 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| hsa-miR-144-3p | 0.6 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| hsa-miR-144-5p | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

\*Melanoma cell lines were A2058, A375P, A375SM, C32, WM35 and WM1552C. CMELL, cultured melanocytes of light skin color. CMELM, cultured melanocytes of medium skin color. CMELD, cultured melanocytes of dark skin color.

**Supplemental Table 2. TaqMan qRT-PCR primer assays for miR-451a isomiRs.**

|  |  |
| --- | --- |
| >hsa-mir-451a MI0001729  (miRBase v18) | CUUGGGAAUGGCAAGGAAACCGUUACCAUUACUGAGUUUAGUAAUGGUAAUGGUUCUCUUGCUAUACCCAGA |
| >hsa-mir-451a MI0001729  (miRBase v17) | ………………..……………AAACCGUUACCAUUACUGAGUUU………………… |
| >has-mir-451a  qRT-PCR assay | ……………………………..AAACCGUUACCAUUACUGAGUUU………………… |
| >has-mir-451a.1  qRT-PCR assay | ……………………………..AAACCGUUACCAUUACUGAGU…………… |

The underlined denotes the sequence for miR-451a in miRBase.

**Supplemental** **Table 3. Clinicopathological characteristics of patient specimens.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Diagnostic Group** | **NS** | **CN** | **DN** | **MIS** | **PCM** |
| **Number of Samples** | 19 | 16 | 19 | 17 | 30 |
| Male | 9 | 9 | 6 | 7 | 21 |
| Female | 10 | 7 | 13 | 10 | 9 |
| **Anatomic Site** |  |  |  |  |  |
| Head & Neck | 3 | 2 | 1 | 3 | 9 |
| Upper Extremity | 2 | 1 | 0 | 4 | 4 |
| Trunk | 13 | 12 | 18 | 7 | 14 |
| Lower extremity | 1 | 1 | 0 | 3 | 3 |
| **Age at diagnosis (years)** |  |  |  |  |  |
| Mean | 58.6 | 36.5 | 43.9 | 53.2 | 54.9 |
| Min | 40 | 10 | 20 | 26 | 18 |
| Max | 94 | 68 | 77 | 79 | 94 |
| **Histology Subtype** |  |  |  |  |  |
| Superficial spreading |  |  |  | 8 | 22 |
| Lentigo maligna |  |  |  | 9 | 4 |
| Nodular |  |  |  | 0 | 3 |
| **Vertical growth phase** |  |  |  |  |  |
| Absent |  |  |  | 17 | 15 |
| Present |  |  |  | 0 | 14 |
| **Depth of invasion (mm)** |  |  |  |  |  |
| Mean |  |  |  |  | 1.29 |
| Min |  |  |  |  | 0.12 |
| Max |  |  |  |  | 11.5 |
| **Ulceration** |  |  |  |  |  |
| Absent |  |  |  |  | 29 |
| Present |  |  |  |  | 1 |
| **Mitotic index (/mm2)** |  |  |  |  |  |
| 0 |  |  |  |  | 17 |
| 1 |  |  |  |  | 4 |
| 2 |  |  |  |  | 5 |
| 4 |  |  |  |  | 2 |
| 6 |  |  |  |  | 1 |
| **Anatomic level of invasion (Clark’s)** |  |  |  |  |  |
| I |  |  |  |  | 1 |
| II |  |  |  |  | 13 |
| III |  |  |  |  | 6 |
| IV |  |  |  |  | 5 |
| V |  |  |  |  | 1 |
| Mean |  |  |  |  | 2.69 |
| **Tumor Inflammation** |  |  |  |  |  |
| Absent |  |  |  |  | 11 |
| Non-brisk |  |  |  |  | 14 |
| Brisk |  |  |  |  | 5 |
| **Regression** |  |  |  |  |  |
| Absent |  |  |  |  | 24 |
| Present |  |  |  |  | 5 |
| **Morphological Features** |  |  |  |  |  |
| *Scatter of IEM* |  |  |  |  |  |
| Absent |  |  |  | 7 | 11 |
| 1 |  |  |  | 3 | 6 |
| 2 |  |  |  | 3 | 3 |
| 3 |  |  |  | 4 | 8 |
| *Nesting of IEM* |  |  |  |  |  |
| Absent |  |  |  | 11 | 4 |
| 1 |  |  |  | 2 | 8 |
| 2 |  |  |  | 2 | 11 |
| 3 |  |  |  | 2 | 6 |
| *Cell Shapes* |  |  |  |  |  |
| Round |  |  |  | 4 | 7 |
| Ovoid |  |  |  | 11 | 13 |
| Elongated |  |  |  | 0 | 5 |
| Spindled |  |  |  | 2 | 4 |
| **Solar Elastosis** |  |  |  |  |  |
| Absent |  |  |  | 1 | 11 |
| Slight |  |  |  | 6 | 6 |
| Moderate |  |  |  | 5 | 4 |
| Severe |  |  |  | 5 | 7 |
| **Cytoplasmic Melanin** |  |  |  |  |  |
| Absent (amelanotic) |  |  |  | 2 | 2 |
| Faint |  |  |  | 6 | 11 |
| Moderate |  |  |  | 9 | 12 |
| Abundant |  |  |  | 2 | 4 |

NS, normal skin; CN, common nevus; MIS, melanoma in situ; PCM, primary cutaneous melanoma (invasive), IEM, intraepidermal melanocytes.

**Supplemental Table 4. Pairwise statistical comparisons of miRNA levels among diagnostic groups by Tukey and non-parametric methods.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **miRNA** | **NS** | **CN** | **DN** | **MIS** | **PCM** |
| **miR-451at, n** | \* |  |  |  |  |
|  | \* |  |  |  |  |
|  | \* |  |  |  |  |
|  | \* |  |  |  |  |
|  | | | | | |
| **miR-451a.1t, n** | \* |  |  |  |  |
|  | \* |  |  |  |  |
|  | \* |  |  |  |  |
|  | \* |  |  |  |  |

The pairwise comparisons of the two statistically significant disease groups are shown in the same color. \*The group with higher miRNA levels. tTukey and nNon-parametric methods.

**Supplemental Table 5. Correlation between melanoma clinicopathological characteristics and miR-211, miR-451a and miR-451a.1 expression**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **PCM (n=30)** | | **MIS (n=17)** | |
|  | **miRNA** | **Parameter with higher value** | **miRNA** | **Parameter with higher value** |
| **Gender** | miR-211m | Male | NSD |  |
| miR-451am, t |
| miR-451a.1m, t |
| **Age at diagnosis** | NSD |  | NSD |  |
| **Histological subtype** | miR-451am | SSM | NSD |  |
| **Cytoplasmic melanin** | miR-451a.1m, t | Absent to faint | miR-451am, t  miR-451a.1m | Absent to moderate |
| **Upward scatter of IEM** | NSD |  | miR-451am | Absent |
| **Nesting of IEM** | NSD |  | NSD |  |
| **Solar elastosis** | NSD |  | NSD |  |
| **Cell shape** | NSD |  | NSD |  |
| **Vertical growth phase** | NSD |  |  |  |
| **Depth of invasion (mm)** | NSD |  |  |  |
| **Clark’s level** | NSD |  |  |  |
| **Mitotic index (/mm2)** | NSD |  |  |  |
| **Tumor inflammation** | miR-211m, t  miR-451am, t | Present, non-brisk |  |  |

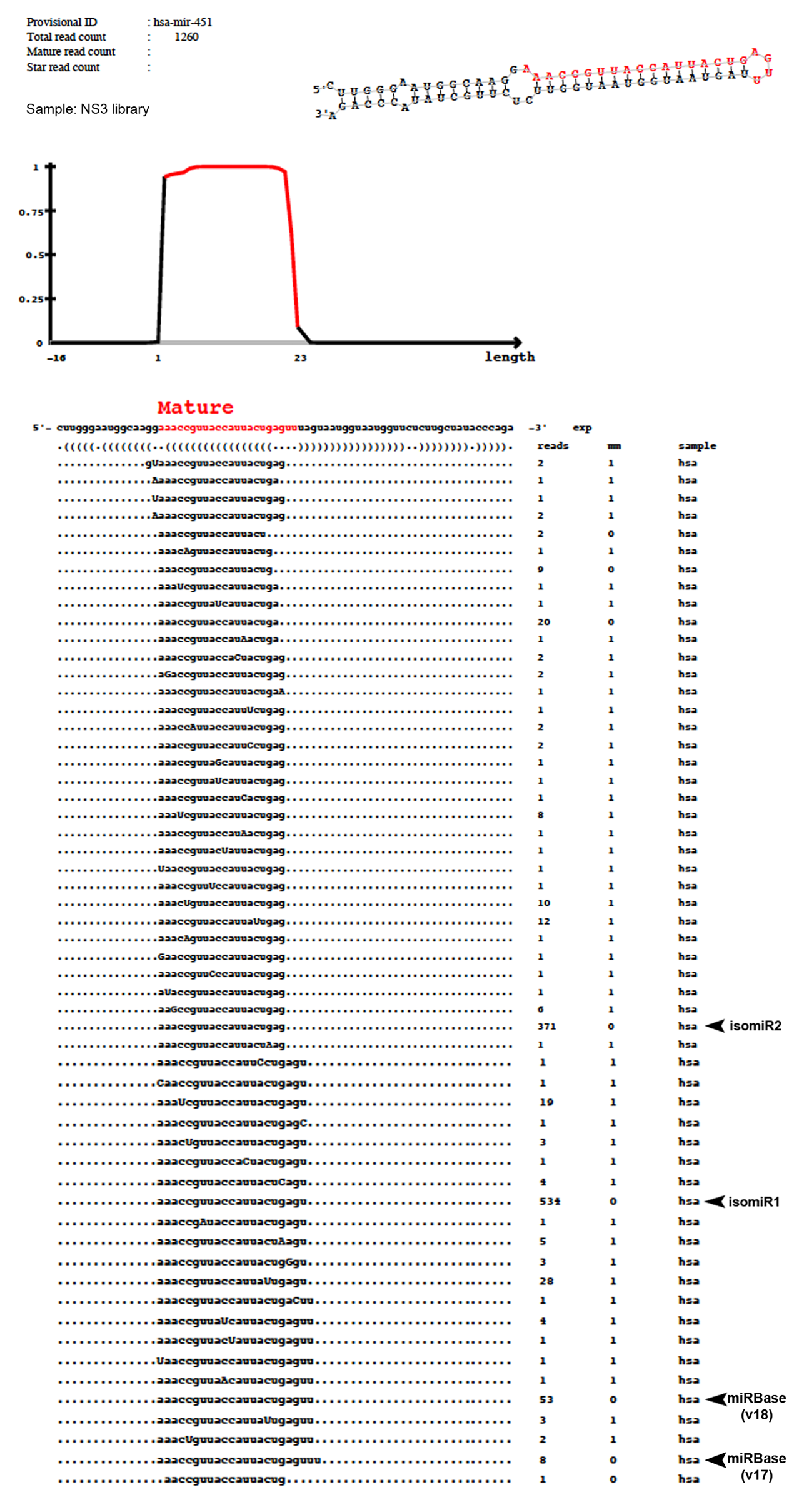
PCM, primary cutaneous melanoma (invasive); MIS, melanoma in situ; NSD, no significant difference for any miRNA. mStatistically significant by Wilcoxon-Mann-Whitney test (alpha =0.05); tStatistically significant by t-test (alpha =0.05). IEM, intraepidermal melanocytes; SSM, superficial spreading melanoma.

**Supplemental Table 6. Association between histopathological phenotype and miR-451a isomiRs in melanoma (n=47)**

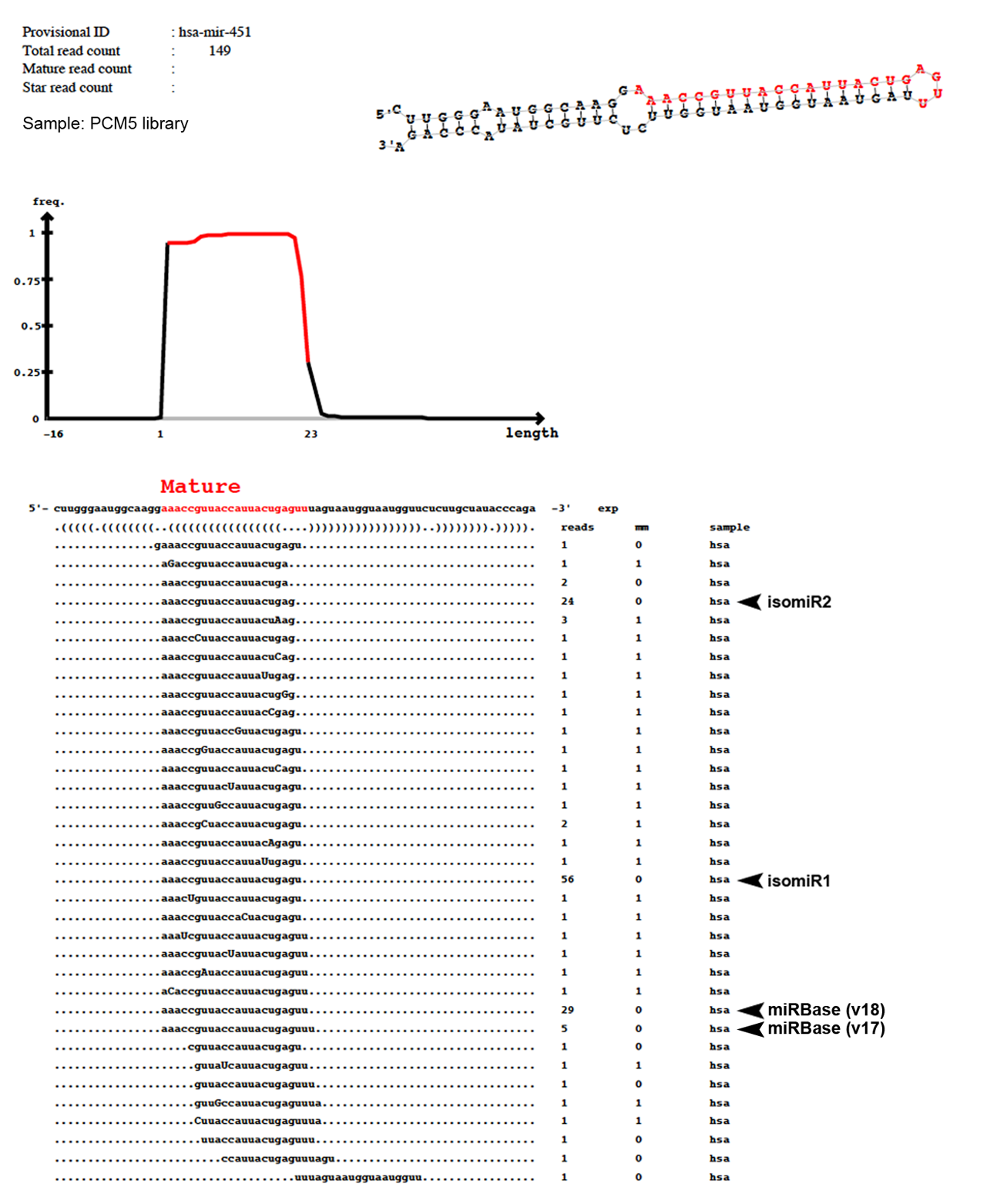
|  |  |  |
| --- | --- | --- |
| **Melanoma phenotype** | **miR-451a** | **miR-451a.1** |
| Melanin | Melanotic | Amelanotic |
| Pagetoid scatter of melanocytes | Absent |  |
| Histological subtype | Superficial spreading |  |
| Tumor inflammation | Present |  |

**Supplemental Table 7. List of used abbreviations**

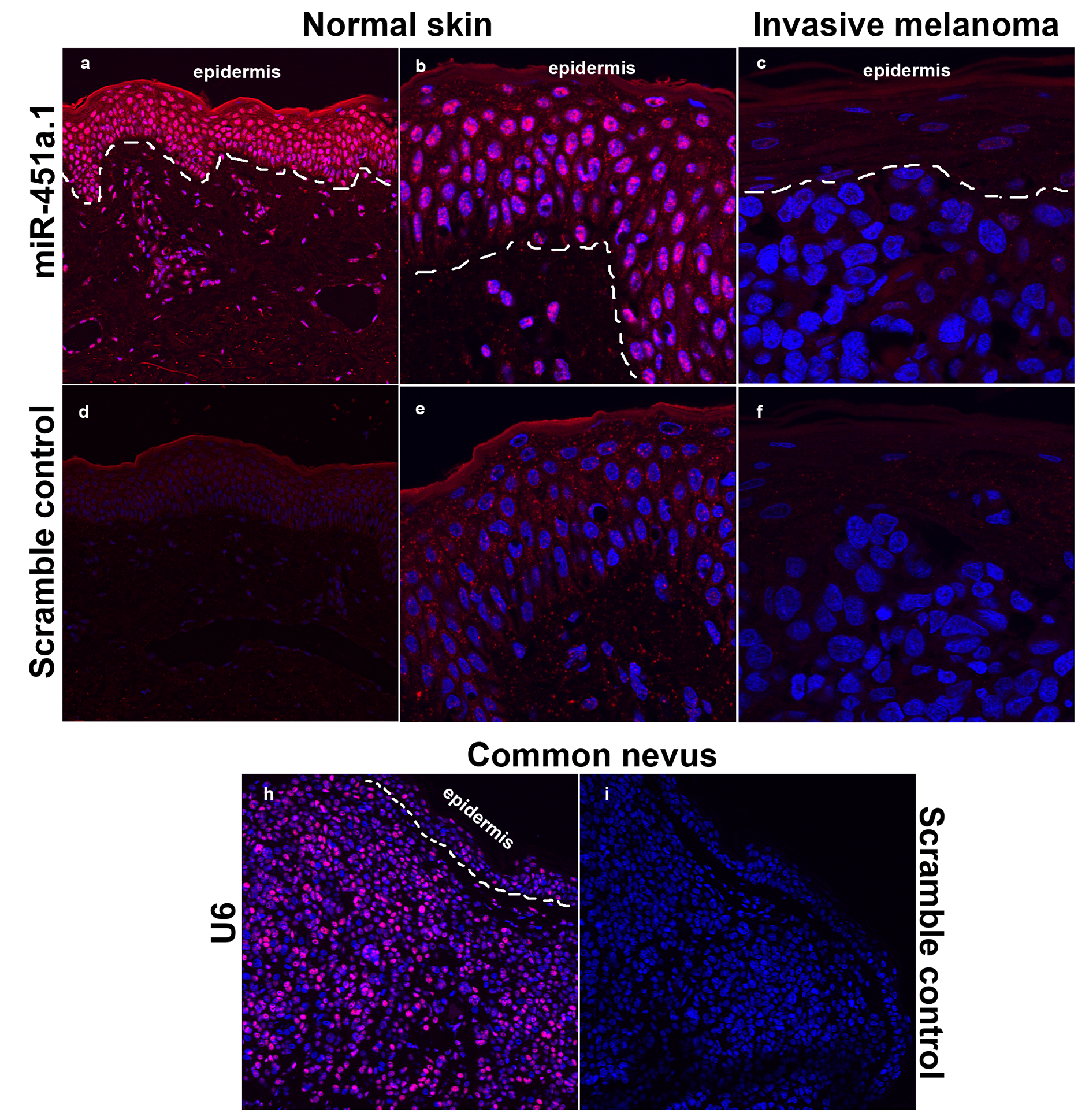
|  |  |
| --- | --- |
| **Abbreviations** | **Description** |
| NS | Normal skin |
| CN | Common (melanocytic) nevus |
| DN | Dysplastic nevus |
| PCM | Primary cutaneous melanoma |
| qRT-PCR | Quantitative real time polymerase chain reaction |
| NGS | Next generation sequencing |
| FFPE | Formalin-fixed paraffin-embedded |
| CMEL | Cultured primary melanocytes |
| CPM | Cultured primary melanoma cell lines |
| CMM | Cultured metastatic melanoma cell lines |
| isomiR | Isomeric microRNAs |
| miRNA | microRNA |
| LKB1 | Liver kinase B1 |
| AMPK | 5' adenosine monophosphate-activated protein |



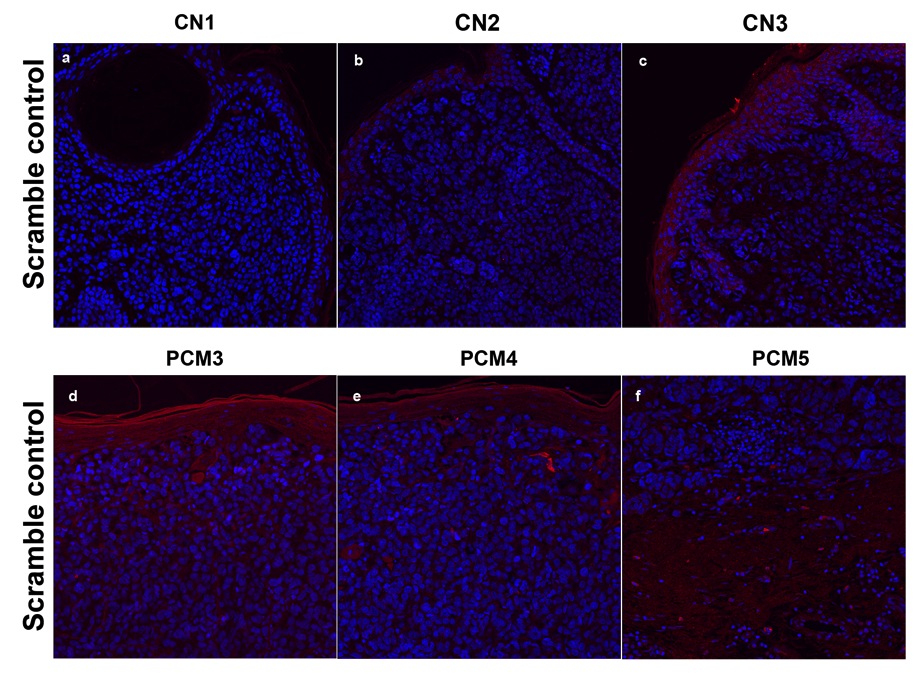
Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.