**Supplementary Material**

|  |  |  |  |
| --- | --- | --- | --- |
| hsa-let-7a-5p | hsa-mir-150-5p | hsa-mir-204-5p | hsa-mir-301a-3p |
| hsa-let-7d-5p | hsa-mir-155-5p | hsa-mir-205-5p | hsa-mir-30d-5p |
| hsa-let-7g-5p | **hsa-mir-15a-5p** | **hsa-mir-20a-5p** | hsa-mir-31-3p |
| hsa-let-7i-5p | **hsa-mir-15b-5p** | hsa-mir-20b-5p | hsa-mir-31-5p |
| hsa-mir-100-5p | hsa-mir-16-5p | hsa-mir-210-3p | hsa-mir-338-3p |
| hsa-mir-101-3p | hsa-mir-17-5p | hsa-mir-211-5p | hsa-mir-33a-5p |
| hsa-mir-124-3p | hsa-mir-182-5p | hsa-mir-214-3p | hsa-mir-342-3p |
| hsa-mir-1246 | hsa-mir-185-5p | hsa-mir-218-5p | hsa-mir-34a-3p |
| hsa-mir-125b-5p | hsa-mir-192-5p | hsa-mir-21-5p | hsa-mir-34a-5p |
| hsa-mir-132-3p | hsa-mir-193b-3p | hsa-mir-221-3p | hsa-mir-34b-3p |
| hsa-mir-137 | hsa-mir-193b-5p | hsa-mir-222-3p | hsa-mir-425-5p |
| hsa-mir-142-3p | hsa-mir-197-3p | hsa-mir-23a-3p | hsa-mir-494-3p |
| hsa-mir-142-5p | hsa-mir-199a-3p | **hsa-mir-23b-3p** | hsa-mir-509-3p |
| hsa-mir-145-5p | hsa-mir-199a-5p | hsa-mir-29a-3p | hsa-mir-92b-3p |
| hsa-mir-146b-5p | hsa-mir-200c-3p | hsa-mir-29b-3p | hsa-mir-93-5p |
| hsa-mir-148a-3p | hsa-mir-203a-3p | **hsa-mir-29c-3p** |  |

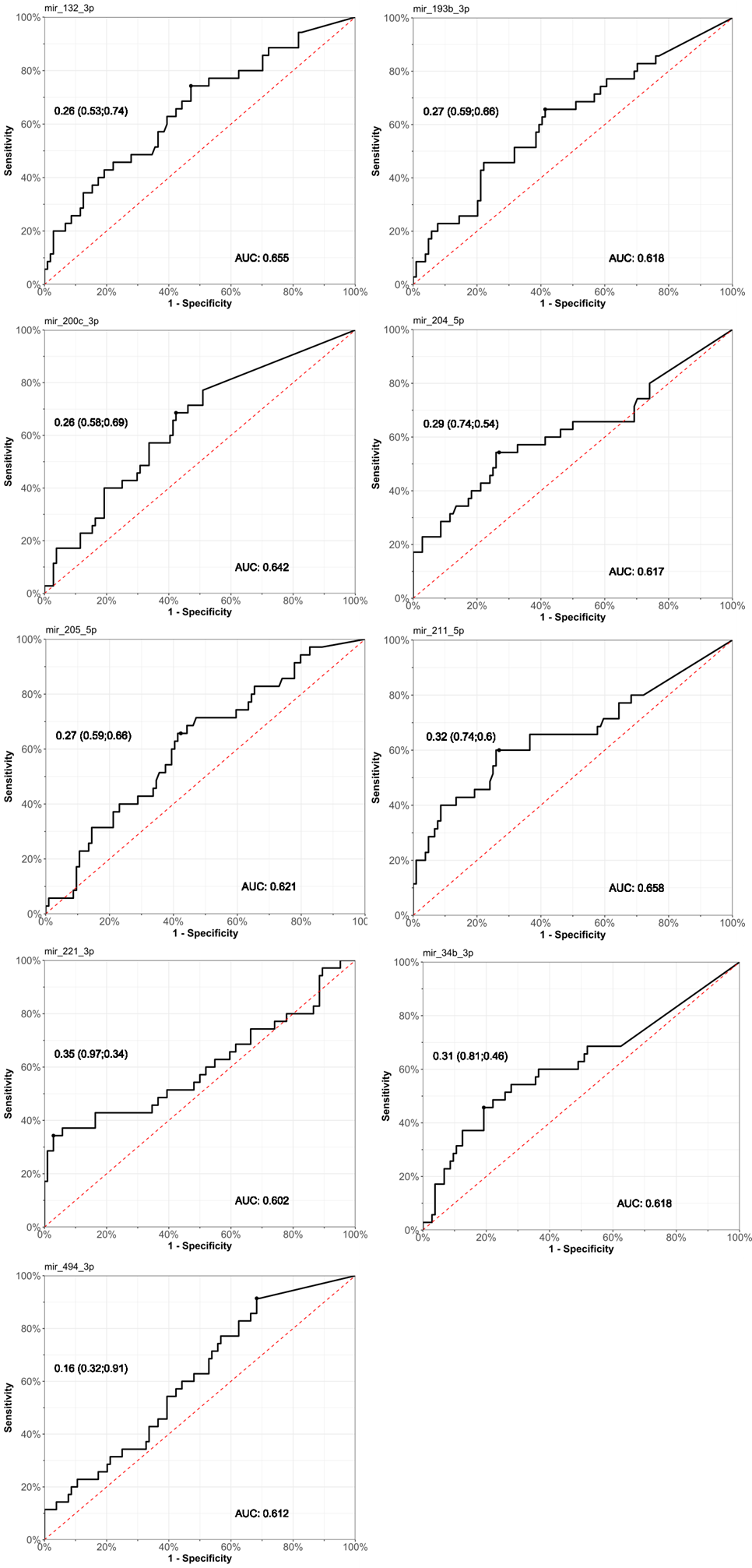
**Supplementary Table S1** Hypothesis-(literature research)driven list of miRNAs analyzed in this study using the Abcam FirePlex Assay. Expression levels of miRNAs in bold letters (most stable expressed miRNAs) were used for normalization.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **I** | **II** | **III** | **IV** | **Overall** |
| **(N=17)** | **(N=30)** | **(N=57)** | **(N=35)** | **(N=139)** |
| **Age** |  |  |  |  |  |
| Mean (SD) | 60.3 (13.0) | 65.0 (14.7) | 63.1 (14.8) | 62.2 (17.5) | 62.9 (15.2) |
| Median [Min, Max] | 66.0 [38.0, 78.0] | 66.0 [17.0, 82.0] | 63.0 [28.0, 86.0] | 67.0 [20.0, 96.0] | 66.0 [17.0, 96.0] |
| **Sex** |  |  |  |  |  |
| Male | 7 (41.2%) | 15 (50.0%) | 29 (50.9%) | 22 (62.9%) | 73 (52.5%) |
| Female | 10 (58.8%) | 15 (50.0%) | 28 (49.1%) | 13 (37.1%) | 66 (47.5%) |
| **BRAF** |  |  |  |  |  |
| Wildtype | 0 (0%) | 1 (3.3%) | 28 (49.1%) | 25 (71.4%) | 54 (38.8%) |
| Mutated | 0 (0%) | 2 (6.7%) | 21 (36.8%) | 10 (28.6%) | 33 (23.7%) |
| Unknown | 17 (100%) | 27 (90.0%) | 8 (14.0%) | 0 (0%) | 52 (37.4%) |
| **PD-L1 staining in tumor tissue** | |  |  |  |  |
| negative | 0 (0%) | 0 (0%) | 2 (3.5%) | 5 (14.3%) | 7 (5.0%) |
| low | 0 (0%) | 0 (0%) | 2 (3.5%) | 7 (20.0%) | 9 (6.5%) |
| high | 0 (0%) | 0 (0%) | 3 (5.3%) | 8 (22.9%) | 11 (7.9%) |
| Missing | 17 (100%) | 30 (100%) | 50 (87.7%) | 15 (42.9%) | 112 (80.6%) |
| **LDH (ULN = 225)** |  |  |  |  |  |
| Elevated | 2 (11.8%) | 1 (3.3%) | 10 (17.5%) | 22 (62.9%) | 35 (25.2%) |
| Normal | 2 (11.8%) | 13 (43.3%) | 36 (63.2%) | 11 (31.4%) | 62 (44.6%) |
| Missing | 13 (76.5%) | 16 (53.3%) | 11 (19.3%) | 2 (5.7%) | 42 (30.2%) |
| **Stage** |  |  |  |  |  |
| I | 17 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 17 (12.2%) |
| II | 0 (0%) | 30 (100%) | 0 (0%) | 0 (0%) | 30 (21.6%) |
| III | 0 (0%) | 0 (0%) | 57 (100%) | 0 (0%) | 57 (41.0%) |
| IV | 0 (0%) | 0 (0%) | 0 (0%) | 35 (100%) | 35 (25.2%) |
| **Number of metastatic sites** |  |  |  |  |  |
| 0 | 17 (100%) | 30 (100%) | 57 (100%) | 0 (0%) | 104 (74.8%) |
| 1 | 0 (0%) | 0 (0%) | 0 (0%) | 12 (34.3%) | 12 (8.6%) |
| 2 | 0 (0%) | 0 (0%) | 0 (0%) | 13 (37.1%) | 13 (9.4%) |
| ≥ 3 | 0 (0%) | 0 (0%) | 0 (0%) | 10 (28.6%) | 10 (7.2%) |

**Supplementary Table S2** Demographics and patient characteristics for melanoma patients in cohort 1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Rank** | **Category** | **Subcategory** | **p-value** |
| 1 | KEGG (miRPathDB) | MicroRNAs in cancer | 1.55e-19 |
| 2 | KEGG (miRPathDB) | Olfactory transduction | 8.05e-15 |
| 3 | KEGG (miRPathDB) | Proteoglycans in cancer | 2.02e-12 |
| 4 | KEGG (miRPathDB) | Pathways in cancer | 2.59e-11 |
| 5 | KEGG (miRPathDB) | Cell cycle | 3.11e-11 |
| 6 | KEGG (miRPathDB) | Prostate cancer | 1.57e-09 |
| 7 | KEGG (miRPathDB) | Focal adhesion | 2.50e-09 |
| 8 | KEGG (miRPathDB) | Hepatitis B | 3.74e-09 |
| 9 | KEGG (miRPathDB) | Endocrine resistance | 4.06e-09 |
| 10 | KEGG (miRPathDB) | Colorectal cancer | 4.54e-09 |
| 11 | KEGG (miRPathDB) | Cellular senescence | 1.19e-08 |
| 12 | KEGG (miRPathDB) | Human T-cell leukemia virus 1 infection | 3.69e-08 |
| 13 | KEGG (miRPathDB) | AGE-RAGE signaling pathway in diabetic complications | 3.81e-08 |
| 14 | KEGG (miRPathDB) | Neurotrophin signaling pathway | 3.98e-08 |
| 15 | KEGG (miRPathDB) | Breast cancer | 7.19e-08 |
| 16 | KEGG (miRPathDB) | Hepatocellular carcinoma | 7.19e-08 |
| 17 | KEGG (miRPathDB) | Pancreatic cancer | 1.21e-07 |
| 18 | KEGG (miRPathDB) | Gastric cancer | 1.57e-07 |
| 19 | KEGG (miRPathDB) | Hippo signaling pathway | 1.75e-07 |
| 20 | KEGG (miRPathDB) | Yersinia infection | 2.34e-07 |

**Supplementary Table S3** Top 20 KEGG pathways enriched in the 9-miRNA signature examined in Fig.1 for discriminating lower-stage melanoma patients and actively metastasizing stage IV melanoma patients.

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**Supplementary Figure S1** ROC analysis of the single nine miRNAs to distinguish stage IV melanoma patients from melanoma patients with lower stages. Cutoff-value is the Youden-Index. Specificity and Sensitivity at cutoff are shown in parentheses. The red line indicates a random classifier. AUC, area under the curve. Data from Cohort 1.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Baseline** | **Progress** | **Overall** |
| **(N=35)** | **(N=14)** | **(N=49)** |
| **Age** |  |  |  |
| Mean (SD) | 62.2 (17.5) | 57.4 (12.0) | 60.9 (16.2) |
| Median [Min, Max] | 67.0 [20.0, 96.0] | 57.5 [35.0, 76.0] | 61.0 [20.0, 96.0] |
| **Sex** |  |  |  |
| Male | 22 (62.9%) | 10 (71.4%) | 32 (65.3%) |
| Female | 13 (37.1%) | 4 (28.6%) | 17 (34.7%) |
| **BRAF** |  |  |  |
| Wildtype | 25 (71.4%) | 5 (35.7%) | 30 (61.2%) |
| Mutated | 10 (28.6%) | 9 (64.3%) | 19 (38.8%) |
| **PD-L1 staining in tumor tissue** |  |  |  |
| negative | 5 (14.3%) | 3 (21.4%) | 8 (16.3%) |
| low | 7 (20.0%) | 0 (0%) | 7 (14.3%) |
| high | 8 (22.9%) | 0 (0%) | 8 (16.3%) |
| Missing | 15 (42.9%) | 11 (78.6%) | 26 (53.1%) |
| **LDH (ULN = 225)** |  |  |  |
| Elevated | 22 (62.9%) | 5 (35.7%) | 27 (55.1%) |
| Normal | 11 (31.4%) | 8 (57.1%) | 19 (38.8%) |
| Missing | 2 (5.7%) | 1 (7.1%) | 3 (6.1%) |
| **AJCC Stage** |  |  |  |
| Stage IV M1a | 4 (11.4%) | 0 (0%) | 4 (8.2%) |
| Stage IV M1b | 11 (31.4%) | 4 (28.6%) | 15 (30.6%) |
| Stage IV M1c | 14 (40.0%) | 5 (35.7%) | 19 (38.8%) |
| Stage IV M1d | 6 (17.1%) | 5 (35.7%) | 11 (22.4%) |
| **Number of metastatic sites** |  |  |  |
| 0 | 0 (0%) | 0 (0%) | 0 (0%) |
| 1 | 12 (34.3%) | 5 (35.7%) | 17 (34.7%) |
| 2 | 13 (37.1%) | 6 (42.9%) | 19 (38.8%) |
| ≥ 3 | 10 (28.6%) | 3 (21.4%) | 13 (26.5%) |

**Supplementary Table S4** Demographics and patient characteristics for melanoma patients in cohort 2 (actively metastasizing stage IV melanoma patients at therapy-start (baseline) and stage IV melanoma patients with *the* first occurrence of progressive disease after *the* start of immune therapy (progress).

|  |  |  |  |
| --- | --- | --- | --- |
| **Rank** | **Category** | **Subcategory** | **p-value** |
| 1 | KEGG (miRPathDB) | Pathways in cancer | 3.12335e-10 |
| 2 | KEGG (miRPathDB) | Proteoglycans in cancer | 3.12335e-10 |
| 3 | KEGG (miRPathDB) | MicroRNAs in cancer | 2.68618e-09 |
| 4 | KEGG (miRPathDB) | Pancreatic cancer | 2.4139e-08 |
| 5 | KEGG (miRPathDB) | Cellular senescence | 5.90468e-08 |
| 6 | KEGG (miRPathDB) | Human T-cell leukemia virus 1 infection | 2.49835e-07 |
| 7 | KEGG (miRPathDB) | Hepatitis B | 5.91831e-07 |
| 8 | KEGG (miRPathDB) | Hepatocellular carcinoma | 8.30673e-07 |
| 9 | KEGG (miRPathDB) | Human papillomavirus infection | 8.30673e-07 |
| 10 | KEGG (miRPathDB) | Prostate cancer | 8.30673e-07 |
| 11 | KEGG (miRPathDB) | MAPK signaling pathway | 1.47004e-06 |
| 12 | KEGG (miRPathDB) | Chronic myeloid leukemia | 3.48845e-06 |
| 13 | KEGG (miRPathDB) | Thyroid hormone signaling pathway | 4.20469e-06 |
| 14 | KEGG (miRPathDB) | Gastric cancer | 4.40756e-06 |
| 15 | KEGG (miRPathDB) | Longevity regulating pathway | 6.5572e-06 |
| 16 | KEGG (miRPathDB) | AMPK signaling pathway | 1.14065e-05 |
| 17 | KEGG (miRPathDB) | Central carbon metabolism in cancer | 1.14065e-05 |
| 18 | KEGG (miRPathDB) | Measles | 1.14065e-05 |
| 19 | KEGG (miRPathDB) | Adherens junction | 1.43192e-05 |
| 20 | KEGG (miRPathDB) | Cell cycle | 1.70659e-05 |

**Supplementary Table S5**Top 20 pathways enriched in the five-miRNA signature examined in Fig. 3 for discriminating therapy naïve stage IV melanoma patients (baseline) and stage IV melanoma patients at progress during therapy (progress).

|  |  |
| --- | --- |
|  | **Overall** |
| **(N=20)** |
| **Age** |  |
| Mean (SD) | 61.2 (20.9) |
| Median [Min, Max] | 63.5 [20.0, 96.0] |
| **Sex** |  |
| Male | 12 (60.0%) |
| Female | 8 (40.0%) |
| **BRAF** |  |
| Wildtype | 14 (70.0%) |
| Mutated | 6 (30.0%) |
| **AJCC Stage** |  |
| Stage IV M1a | 3 (15.0%) |
| Stage IV M1b | 8 (40.0%) |
| Stage IV M1c | 6 (30.0%) |
| Stage IV M1d | 3 (15.0%) |
| **PD-L1 staining in tumor tissue** | |
| Negative (<1%) | 5 (25.0%) |
| Low (1-20%) | 7 (35.0%) |
| High (>20%) | 8 (40.0%) |
| **LDH (ULN = 225)** |  |
| Elevated | 16 (80.0%) |
| Normal | 4 (20.0%) |
| **Number of metastatic sites** |  |
| 0 | 0 (0%) |
| 1 | 8 (40.0%) |
| 2 | 9 (45.0%) |
| ≥ 3 | 3 (15.0%) |

**Supplementary Table S6** Demographics and patient characteristics in cohort 3 (for treatment-naïve, actively metastasizing stage IV melanoma patients with negative, compared to low and high PD-L1 immunostaining of melanoma tissue).

|  |  |  |  |
| --- | --- | --- | --- |
| **Rank** | **Category** | **Subcategory** | **p-value** |
| 1 | KEGG (miRPathDB) | Irritable Bowel Syndrome | 2.22e-08 |
| 2 | KEGG (miRPathDB) | Cardiac Myocyte Injury | 9.32e-08 |
| 3 | KEGG (miRPathDB) | Aplastic Anemia | 1.33e-07 |
| 4 | KEGG (miRPathDB) | mir-199 family | 5.84e-06 |
| 5 | KEGG (miRPathDB) | Stroke, Ischemic | 6.64e-06 |
| 6 | KEGG (miRPathDB) | Autoimmune Diseases [unspecific] | 7.93e-06 |
| 7 | KEGG (miRPathDB) | Osteoblast\_differentiated | 1.17e-05 |
| 8 | KEGG (miRPathDB) | Endometriosis | 1.18e-05 |
| 9 | KEGG (miRPathDB) | Cardiomegaly | 1.94e-05 |
| 10 | KEGG (miRPathDB) | Cardiomyopathy, Hypertrophic | 2.91e-05 |
| 11 | KEGG (miRPathDB) | Atrial Fibrillation | 3.80e-05 |
| 12 | KEGG (miRPathDB) | Endocytosis | 5.44e-05 |
| 13 | KEGG (miRPathDB) | TWIST1 | 8.73e-05 |
| 14 | KEGG (miRPathDB) | Cell Proliferation | 9.12e-05 |
| 15 | KEGG (miRPathDB) | Chronic Hepatitis | 1.07e-04 |
| 16 | KEGG (miRPathDB) | Immune Response | 1.39e-04 |
| 17 | KEGG (miRPathDB) | Keloid | 1.51e-04 |
| 18 | KEGG (miRPathDB) | Coronary Atherosclerosis | 1.76e-04 |
| 19 | KEGG (miRPathDB) | Polycystic Kidney Disease | 1.76e-04 |
| 20 | KEGG (miRPathDB) | Cystic Fibrosis | 2.32e-04 |

**Supplementary Table S7** Top 20 pathways enriched in the two-miRNA signature examined in Fig. 4 for discriminating miRNA expression depending on PD-L1 expression in tumor tissue.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **miR-150-5p Expression ≤ Median** | **miR-150-5p Expression > Median** | **P-value** | **Overall** |
| **(N=76)** | **(N=76)** |  | **(N=152)** |
| **Age** |  |  |  |  |
| Mean (SD) | 63.6 (14.0) | 61.4 (15.9) | 0.368 | 62.5 (15.0) |
| Median [Min, Max] | 64.0 [28.0, 86.0] | 64.5 [17.0, 96.0] |  | 64.5 [17.0, 96.0] |
| **Sex** |  |  |  |  |
| Male | 46 (60.5%) | 37 (48.7%) | 0.192 | 83 (54.6%) |
| Female | 30 (39.5%) | 39 (51.3%) |  | 69 (45.4%) |
| **Stage** |  |  |  |  |
| <IV | 46 (60.5%) | 58 (76.3%) | 0.0758 | 104 (68.4%) |
| I | 7 (9.2%) | 10 (13.2%) |  | 17 (11.2%) |
| II | 10 (13.2%) | 20 (26.3%) | 30 (19.7%) |
| III | 29 (38.2%) | 28 (36.8%) | 57 (37.5%) |
| IV | 30 (39.5%) | 18 (23.7%) |  | 48 (31.6%) |
| **BRAF** |  |  |  |  |
| Wildtype (-) | 32 (42.1%) | 27 (35.5%) | 0.228 | 59 (38.8%) |
| Mutated (+) | 23 (30.3%) | 18 (23.7%) |  | 41 (27.0%) |
| Unknown | 21 (27.6%) | 31 (40.8%) |  | 52 (34.2%) |
| **Melanoma subtype** |  |  |  |  |
| Cutaneous | 70 (92.1%) | 68 (89.5%) | 0.779 | 138 (90.8%) |
| CUP | 6 (7.9%) | 8 (10.5%) |  | 14 (9.2%) |
| **LDH (ULN = 225)** |  |  |  |  |
| Normal | 40 (52.6%) | 29 (38.2%) | 0.0555 | 69 (45.4%) |
| Elevated | 21 (27.6%) | 19 (25.0%) |  | 40 (26.3%) |
| Unknown | 15 (19.7%) | 28 (36.8%) |  | 43 (28.3%) |

**Supplementary Table S8** Baseline differences in patients with lower or higher miR-150-5p expression than the median, respectively. Statistical differences were tested with the chi² test for categorical data and ANOVA or Welch’s t-test for numerical data.