

Supplementary Table 1 Summary of patient characteristics

| Characteristic | Number of patients (%) |
|-----------------------------------|------------------------|
| Age (range) in years | 55.6 (23-79) |
| Gender | |
| Male | 35 (64.8) |
| Female | 19 (35.2) |
| Tumor size (largest diameter, cm) | 6.3 (3-12) |
| Tumor location | |
| Sacral vertebra | 42 (77.8) |
| Cervical vertebra | 6 (11.1) |
| Thoracic vertebra | 4 (7.4) |
| Lumbar vertebra | 2 (3.7) |
| Surrounding muscle invasion | |
| Yes | 35 (64.8) |
| No | 19 (35.2) |
| Preoperative recurrence | |
| Yes | 11 (20.4) |
| No | 43 (79.6) |
| Tumor grade | |
| High | 38 (70.4) |
| Low | 16 (29.6) |
| Tumor stage | |
| IA | 13 (24.1) |
| IB | 6 (11.1) |
| IIA | 4 (7.4) |
| IIB | 27 (50) |
| III | 4 (7.4) |
| Type of resection | |
| EI | 17 (31.5) |
| EA | 37 (68.5) |
| Tumor hemorrhage | |
| No | 10 (18.5) |
| Yes | 44 (81.5) |
| Tumor necrosis | |
| Absent | 13 (24.1) |
| Mild | 18 (33.3) |
| Moderate | 15 (27.8) |
| Severe | 8 (14.8) |
| Level of overall TILs | |
| Absent | 0 (0) |
| Rare/few | 23 (42.6) |

| | |
|---|---------------|
| Moderate | 15 (27.8) |
| Prominent | 16 (29.6) |
| Ki-67 staining index | |
| Low | 25 (46.3) |
| High | 29 (53.7) |
| Number of TILs subsets (cells/mm ²) | |
| PD-1 ⁺ TILs, mean ± SD | 151.8 ± 121.7 |
| PD-L1 ⁺ TILs, mean ± SD | 288.0 ± 182.5 |
| CD8 ⁺ TILs, mean ± SD | 311.0 ± 274.8 |
| Foxp3 ⁺ TILs, mean ± SD | 132.0 ± 115.0 |
| CD8 ⁺ /Foxp3 ⁺ ratio, mean ± SD | 3.3 ± 3.1 |
| Survival during follow-up | |
| Death | 24 (44.4%) |
| Alive | 30 (55.6%) |
| Relapse during follow-up | |
| Yes | 41 (75.9%) |
| No | 13 (24.1%) |

EI, Enneking inappropriate; EA, Enneking appropriate; TILs, tumor-infiltrating lymphocytes; PD-1, programmed cell death 1; PD-L1, programmed cell death-1 ligand 1; SD, standard deviation.

Supplementary Table 2 List of the specific primers that were used in this study

| Gene | Forward | Reverse |
|-------------|-----------------------------|----------------------------|
| miR-574-3p | 5'-GGCGCACGCTCATGCACACA-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| miR-4731-3p | 5'-GGCGCACACAAGTGGCCCCC-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| miR-4433-5p | 5'-GGCGCGTCCCACCCCCAC-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| miR-671-5p | 5'-GGCGAGGAAGCCCTGGAGGGG-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| miR-371b-5p | 5'-GGCGACTCAAAGATGGCGG-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| miR-1225-3p | 5'-GGCGTGAGCCCCTGTGCCGC-3' | 5'-GTGCAGGGTCCGAGGT-3' |
| U6 | 5'-CTCGCTTCGGCAGCACA-3' | 5'-AACGCTTCACGAATTTGCGT-3' |

Supplementary Table 3 Candidate miRNAs from microarray and bioinformatical analysis that could potentially regulate PD-L1 expression in spinal chordoma

| miRNAs | <i>P</i> -value | Fold change | FDR ^a | Expression | Sequence |
|-----------------|-----------------|-------------|------------------|------------|------------------|
| hsa-miR-574-3p | 2.72E-02 | 4.98 | 0.03210 | down | TGTGGGTGTGTGCATG |
| hsa-miR-4731-3p | 5.41E-03 | 10.81 | 0.00782 | down | AGTGTTGGGGGCCA |
| hsa-miR-4433-5p | 1.73E-02 | 3.82 | 0.02975 | down | ACAGGAGTGGGGGG |
| hsa-miR-671-5p | 6.43E-03 | 2.61 | 0.00684 | down | CTCCAGCCCCT |
| hsa-miR-371b-5p | 2.84E-02 | 2.30 | 0.00622 | down | AAAGTGCCGCCATCT |
| hsa-miR-1225-3p | 9.38E-03 | 12.08 | 0.02133 | down | CTGGGGGCGGC |

miRNA, microRNA; FDR, false discovery rate; ^aFDR < 0.05

Supplementary Table 4 Frequency of infiltration pattern according to TILs subsets in the chordoma microenvironment

| TILs subtypes | Frequency of infiltration pattern | | | |
|--------------------|-----------------------------------|----------|----------|-----------|
| | Absent | Rare/few | Moderate | Prominent |
| PD-1 ⁺ | 0 | 15 | 20 | 19 |
| PD-L1 ⁺ | 0 | 24 | 18 | 12 |
| Foxp3 ⁺ | 4 | 22 | 14 | 14 |
| CD8 ⁺ | 10 | 17 | 14 | 13 |

TILs, tumor-infiltrating lymphocytes; PD-1, programmed cell death 1; PD-L1, programmed cell death-1 ligand 1.

Supplementary Table 5 Correlations between tumor PD-L1 expression, miR-574-3p level and microenvironmental TILs in spinal chordoma (n = 54)

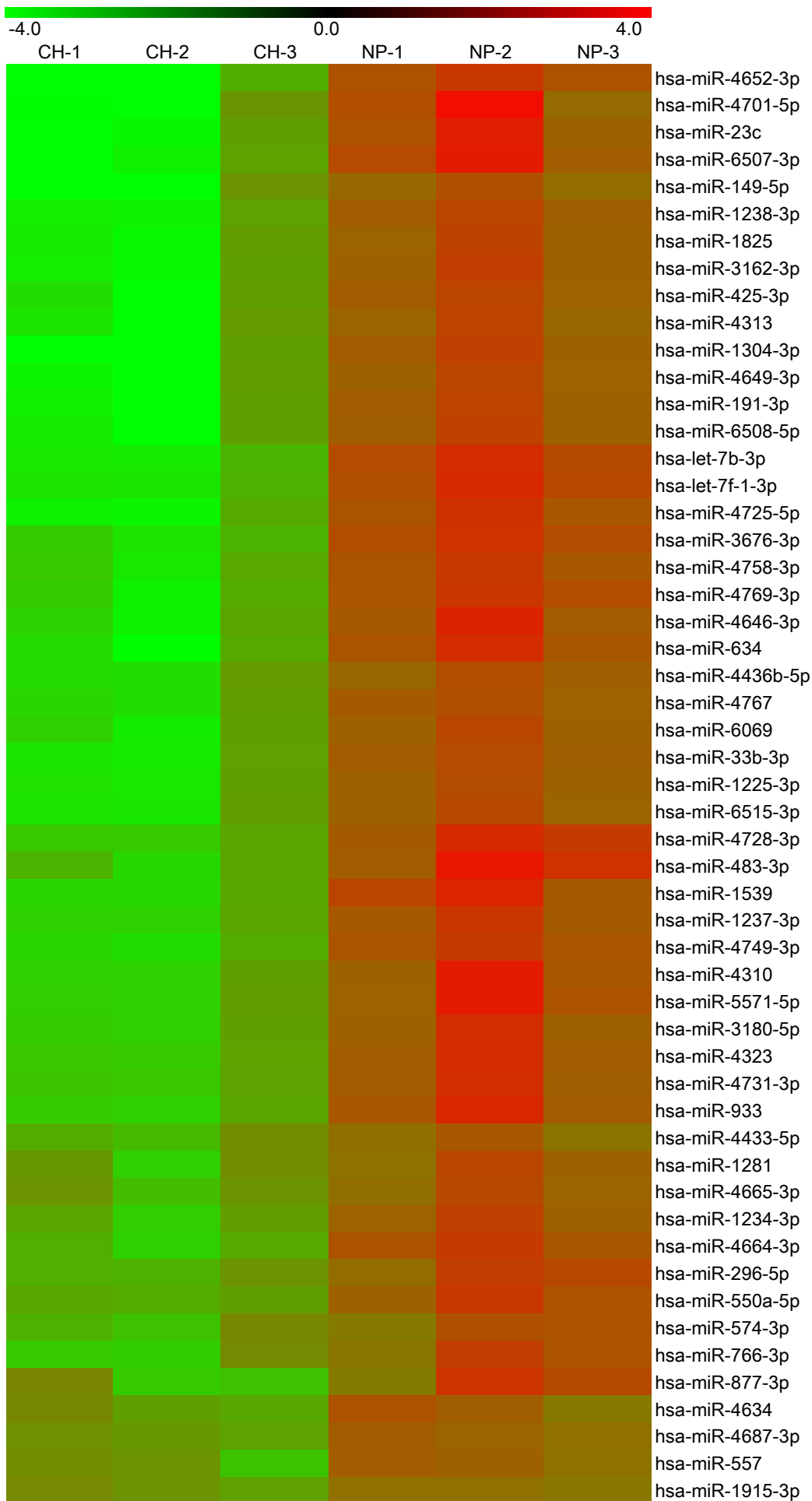
| | miR-574-3p expression | PD-L1 expression (positive vs negative) | Extent of overall TILs (positive vs negative) | PD-1 ⁺ TILs number | PD-L1 ⁺ TILs number | CD8 ⁺ TILs number | Foxp3 ⁺ TILs number | CD8 ⁺ /Foxp3 ⁺ ratio |
|---|-----------------------|---|---|-------------------------------|-----------------------------------|------------------------------|----------------------------------|--|
| miR-574-3p expression | | t = 3.435 P = 0.001 | t = 1.345 P = 0.185 | r = -0.102 P = 0.462 | r = -0.203 P = 0.140 | r = 0.156 P = 0.260 | r = -0.125 P = 0.367 | r = 0.503 P < 0.001 |
| PD-L1 expression (positive vs negative) | | | $\chi^2 = 7.953$ P = 0.005 | t = 2.718 P = 0.009 | t = 2.951 P = 0.005 | t = 1.005 P = 0.320 | t = 1.415 P = 0.163 | t = 2.764 P = 0.008 |
| Extent of overall TILs (positive vs negative) | | | | t = -0.555 P = 0.581 | t = 1.022 P = 0.312 | t = 0.054 P = 0.957 | t = -0.285 P = 0.777 | t = 0.084 P = 0.934 |
| PD-1 ⁺ TILs number | | | | | r = -0.521 P < 0.001 | r = -0.188 P = 0.174 | r = 0.031 P = 0.825 | r = -0.383 P = 0.006 |
| PD-L1 ⁺ TILs number | | | | | | r = 0.234 P = 0.089 | r = -0.013 P = 0.923 | r = 0.181 P = 0.208 |
| CD8 ⁺ TILs number | | | | | | | r = 0.595 P < 0.001 | r = 0.302 P = 0.033 |
| Foxp3 ⁺ TILs number | | | | | | | | r = -0.393 P = 0.005 |

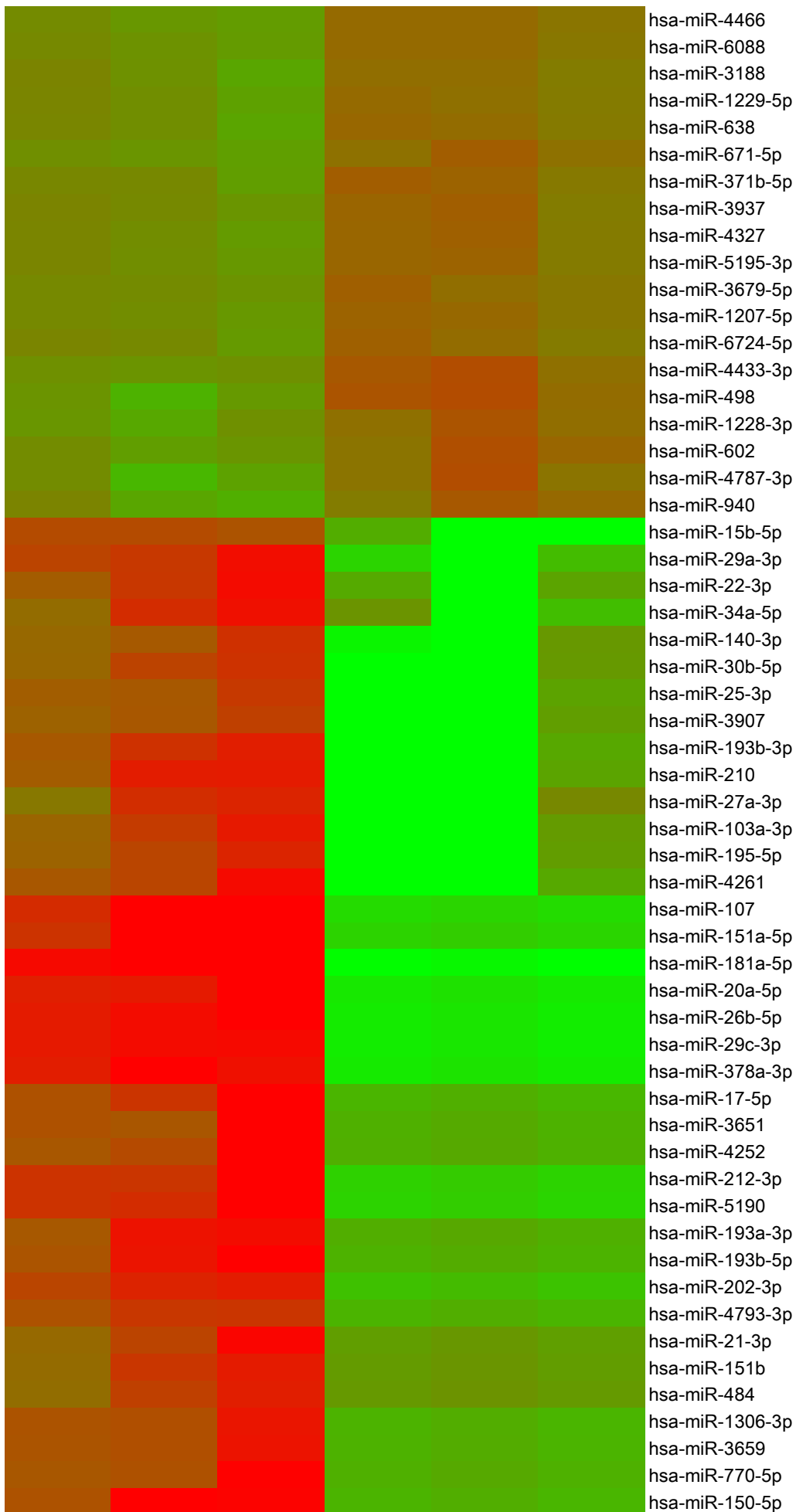
TILs, tumor-infiltrating lymphocytes; PD-1, programmed cell death 1; PD-L1, programmed cell death-1 ligand

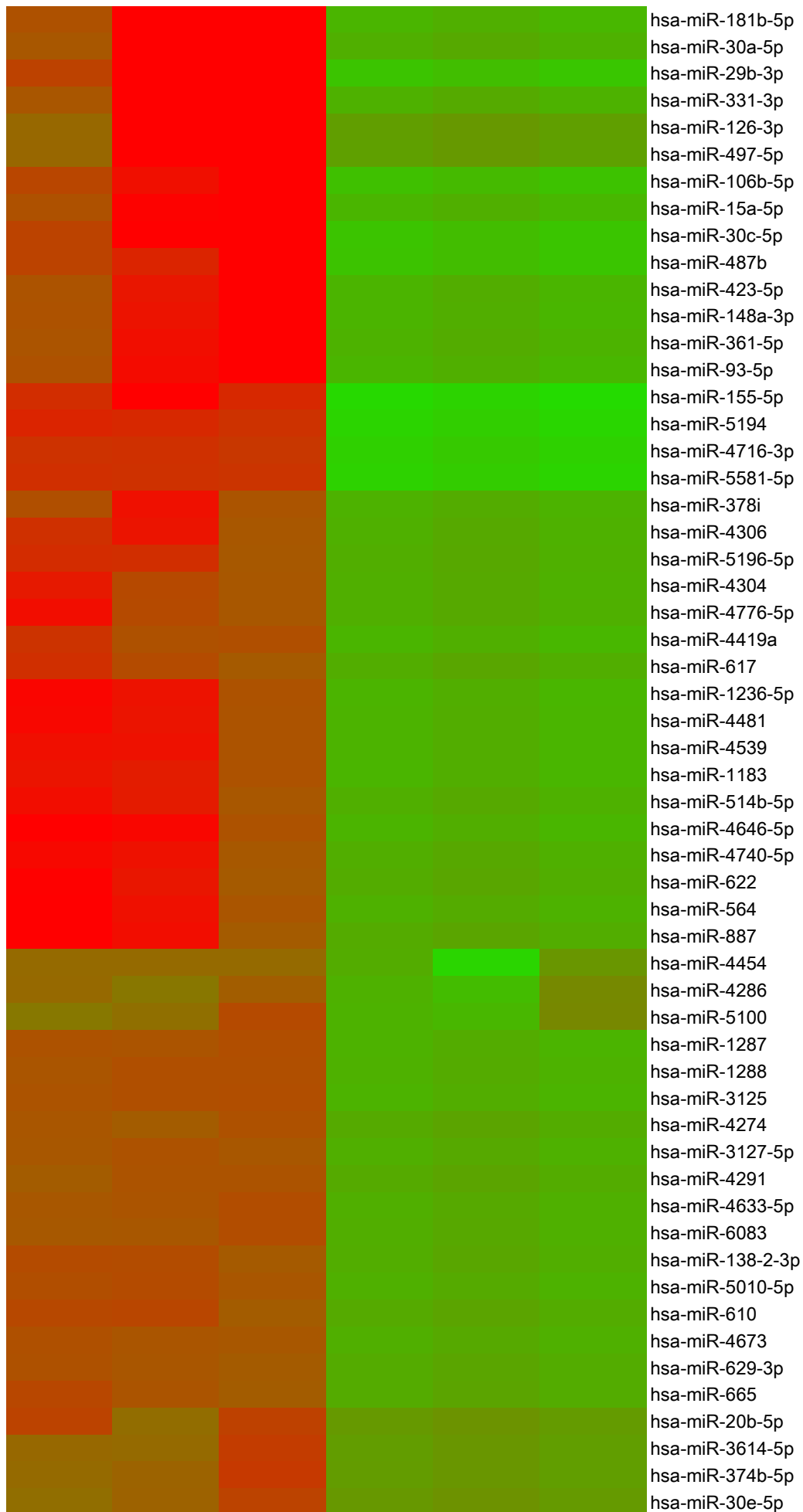
Supplementary Table 6 List of the cut-off points for miRNA-574-3p and TILs in prognosis analysis of LRFS and OS

| Variables | Cut-off values |
|--|-----------------------------|
| Outcome parameter: LRFS | |
| miRNA-574-3p | 8.425 |
| PD-1 ⁺ TILs | 88.5 cells/mm ² |
| PD-L1 ⁺ TILs | 450 cells/mm ² |
| Foxp3 ⁺ TILs | 162.5 cells/mm ² |
| CD8 ⁺ TILs | 5 cells/mm ² |
| CD8 ⁺ /Foxp3 ⁺ ratio | 1.090 |
| Outcome parameter: OS | |
| miRNA-574-3p | 18.41 |
| PD-1 ⁺ TILs | 80 cells/mm ² |
| PD-L1 ⁺ TILs | 446.5 cells/mm ² |
| Foxp3 ⁺ TILs | 196.5 cells/mm ² |
| CD8 ⁺ TILs | 402.5 cells/mm ² |
| CD8 ⁺ /Foxp3 ⁺ ratio | 4.585 |

TILs, tumor-infiltrating lymphocytes; PD-1, programmed cell death 1; PD-L1, programmed cell death-1 ligand 1; LRFS, local recurrence-free survival; OS, overall survival.

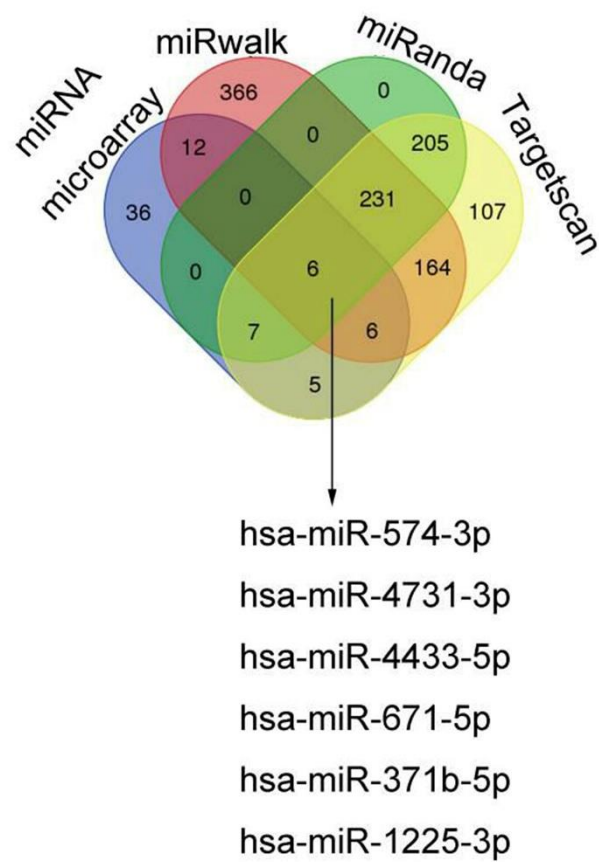




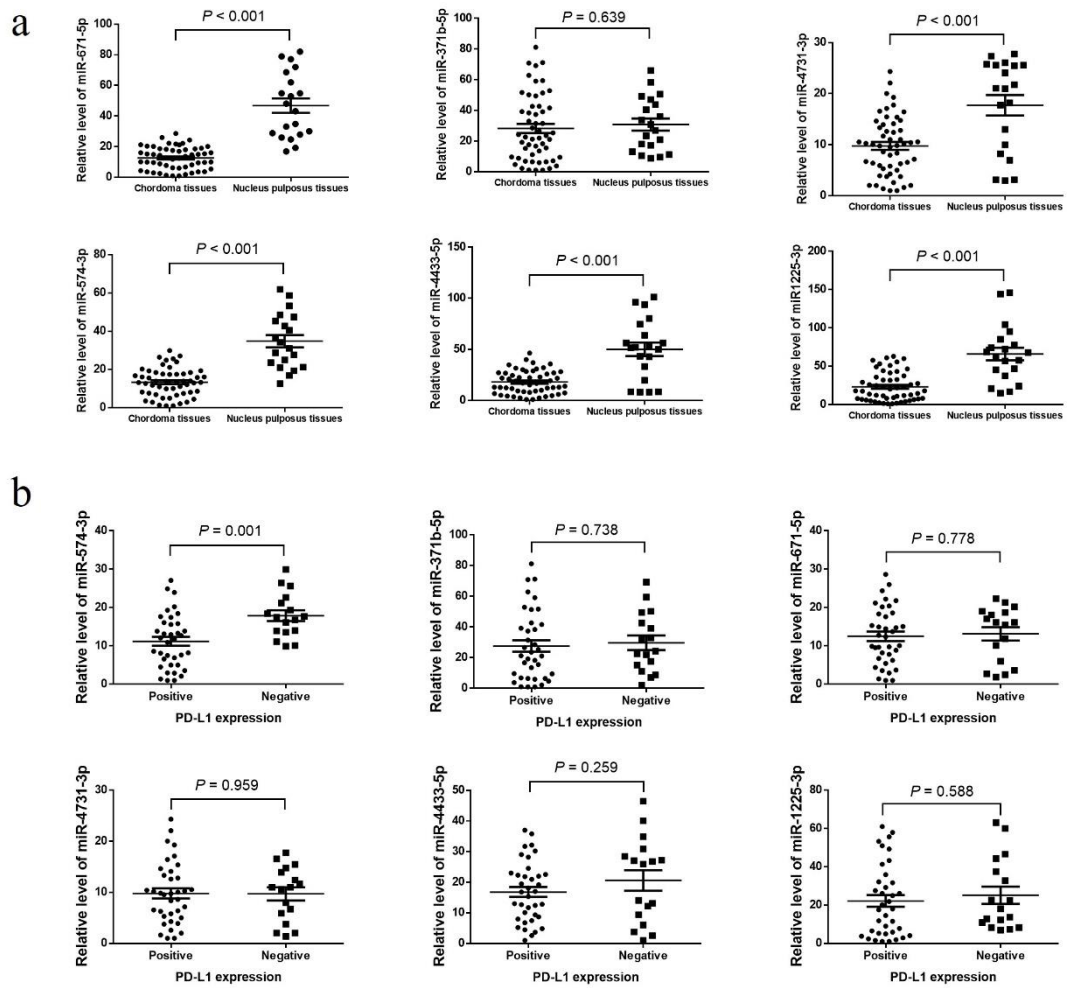




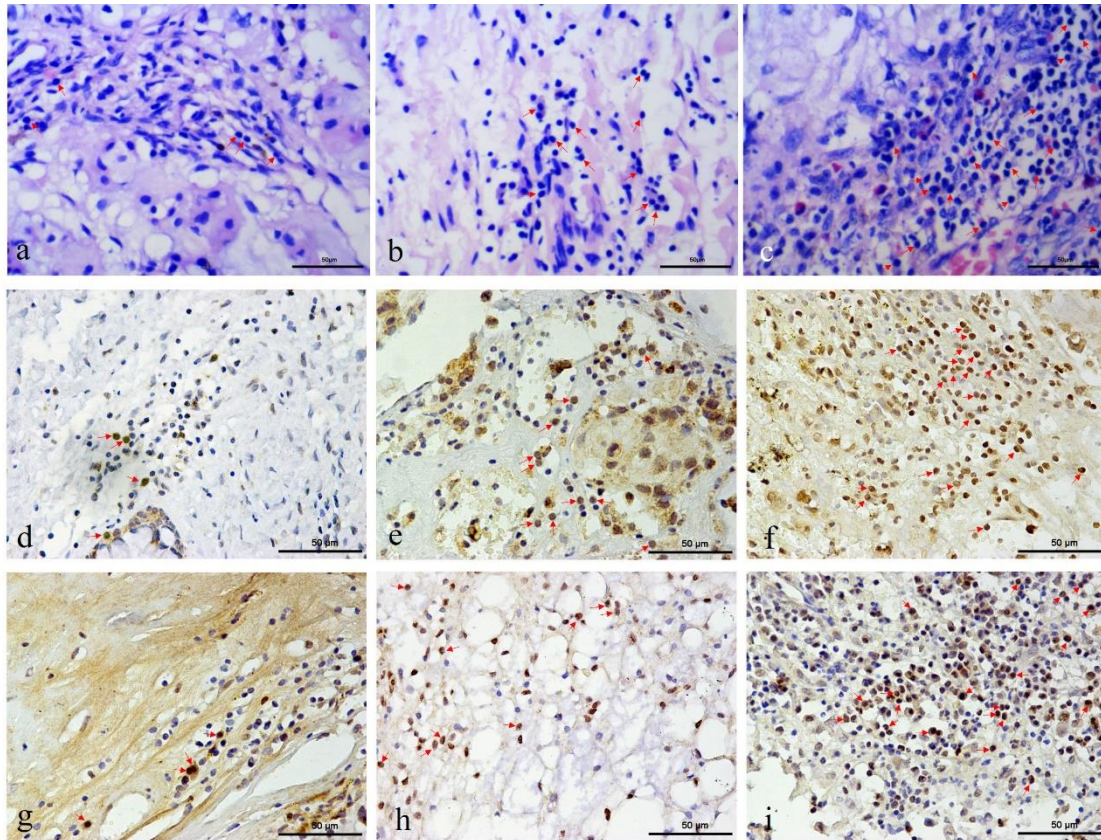
Supplementary Figure 1 A heat-map representation of the differentially expressed miRNAs patterns in chordoma (CH) versus nucleus pulposus tissues (NP). Relative expression is presented as a colorgram (*red*: high expression).



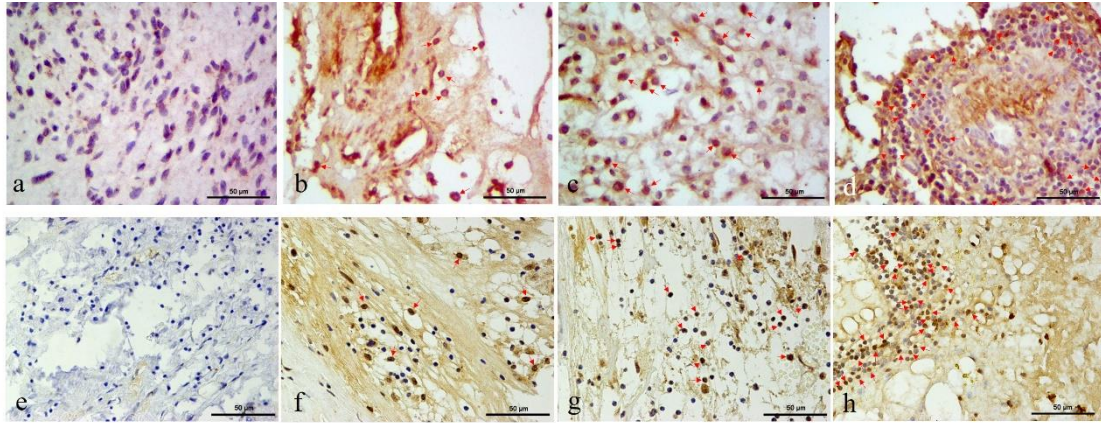
Supplementary Figure 2 Identification of six candidate miRNAs that could potentially regulate PD-L1 expression in chordoma tissues by microarray and bioinformatical analysis.



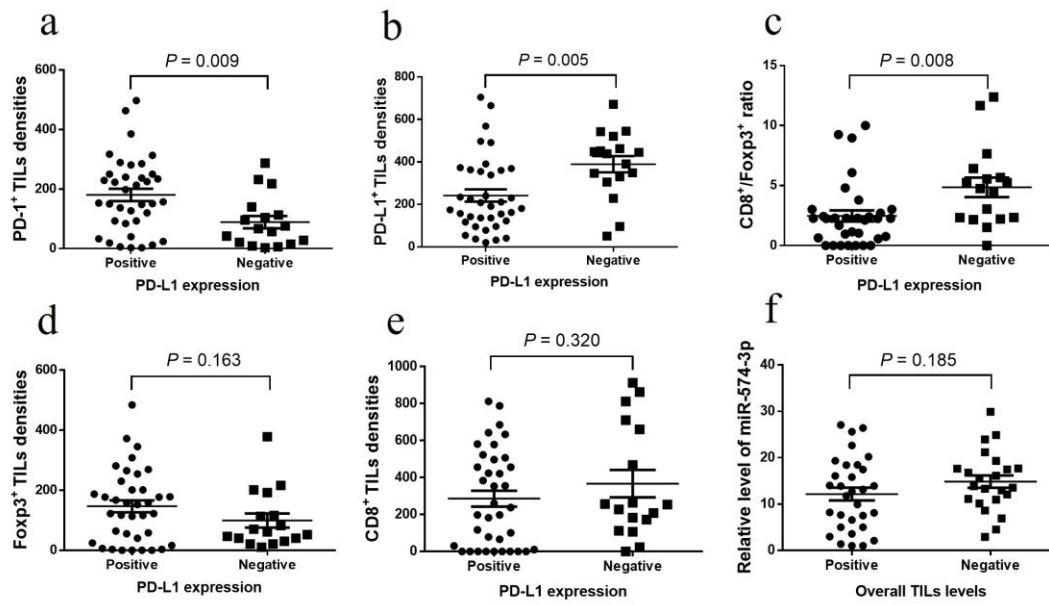
Supplementary Figure 3 (a) Comparison of six candidate miRNAs expression by qRT-PCR in 54 chordoma and 20 nucleus pulposus tissues; (b) Association of candidate miRNAs expression and PD-L1 level in chordoma tissues.



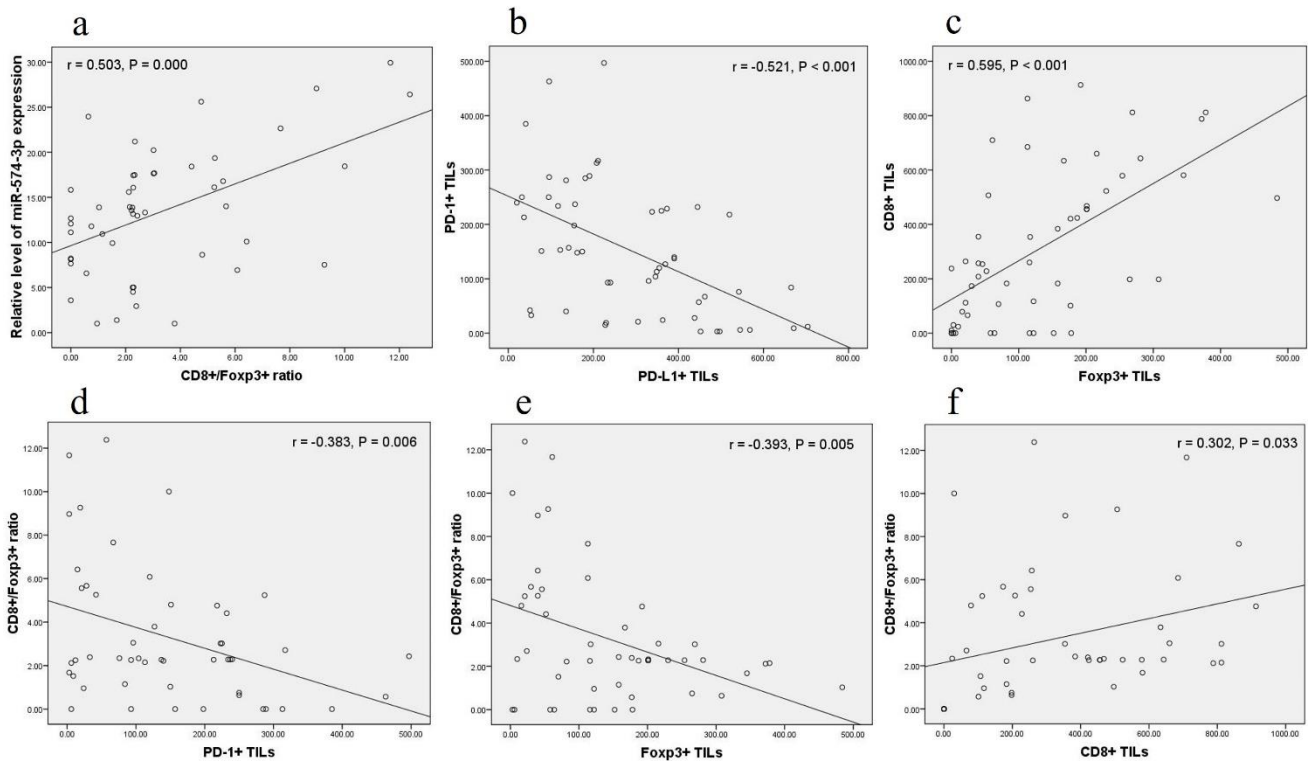
Supplementary Figure 4 The pattern of overall TILs, PD-1⁺ TILs and PD-L1⁺ TILs in chordoma tissues (*red arrow*). **(a)** Rare/few TILs (HE × 400); **(b)** Moderate TILs (HE × 400); **(c)** Prominent TILs (HE × 400); **(d)** Rare/few TILs that expressed PD-1; **(e)** Moderate TILs that expressed PD-1; **(f)** Prominent TILs that expressed PD-1; **(g)** Rare/few TILs expressing PD-L1; **(h)** Moderate TILs expressing PD-L1; **(i)** Prominent TILs expressing PD-L1 (×400).



Supplementary Figure 5 The pattern of CD8⁺ TILs and Foxp3⁺ TILs in chordoma tissues (*red arrow*). (a) Lack of TILs that expressed CD8 in chordoma tissues; (b) Rare/few TILs with CD8 expression; (c) Moderate TILs with CD8 expression; (d) Prominent TILs with CD8 expression; (e) Lack of TILs that expressed Foxp3 in chordoma tissues; (f) Rare/few TILs that expressed Foxp3; (g) Moderate TILs that expressed Foxp3; (h) Prominent TILs expressing Foxp3 ($\times 400$).



Supplementary Figure 6 Association of PD-L1 expression and PD-1⁺ TILs density (a), PD-L1⁺ TILs density (b), CD8⁺/Foxp3⁺ ratio (c), Foxp3⁺ TILs density (d) and CD8⁺ TILs density (e). Association of miR-574-3p expression and overall TILs level (f).



Supplementary Figure 7 (a) Association of miR-574-3p expression and CD8⁺/Foxp3⁺ ratio via Pearson correlation test; (b) Association of PD-1⁺ TILs and PD-L1⁺ TILs densities; (c) Association of CD8⁺ TILs and Foxp3⁺ TILs densities. Association of CD8⁺/Foxp3⁺ ratio and PD-1⁺ TILs density (d), Foxp3⁺ TILs density (e) and CD8⁺ TILs density (f).