

Table S1. Percentage of patients in the overall, localized and metastatic cohort with different intensities of Hepsin staining in each of the discrete clinical and histopathological categories recorded. The *p*-values of Pearson's Chi-square correlations performed between hepsin and these variables are shown. HPN: Hepsin; N (%): Number and percentage of patients; ECOG: Eastern Cooperative Oncology Group; TNM II: T-stage 3/4, N-stage 0, M-stage 0; TNM III: T-stage 1/2/3/4, N-stage 1/2, M-stage 0; TNM IV: T-stage 1/2/3/4, N-stage 0/1/2, M-stage 1; T-stage: size and extension of the primary tumor; N-stage: tumor involvement of nearby lymph nodes; *: significant *p*-value.

| Overall cohort | HPN staining intensity | | | p-value from Chi-square test |
|--|------------------------|-----------|-----------|------------------------------|
| Clinical/histopathological variables correlated with HPN | Low | Medium | High | |
| Sex | | | | 0.819 |
| Men: N (%) | 39 (20.9) | 66 (35.3) | 82 (43.9) | |
| Women: N (%) | 22 (22.0) | 38 (38.0) | 40 (40.0) | |
| ECOG at diagnosis | | | | 0.736 |
| 0: N (%) | 23 (20.0) | 48 (41.7) | 44 (38.3) | |
| 1: N (%) | 27 (22.1) | 40 (32.8) | 55 (45.1) | |
| 2: N (%) | 5 (15.2) | 11 (33.3) | 17 (51.5) | |
| ≥3: N (%) | 1 (25.0) | 1 (25.0) | 2 (50.0) | |
| Primary tumor site | | | | 0.551 |
| Ascending colon: N (%) | 19 (22.9) | 31 (37.3) | 33 (39.8) | |
| Descending colon: N (%) | 1 (7.1) | 5 (35.7) | 8 (57.1) | |
| Transverse colon: N (%) | 3 (20.0) | 8 (53.3) | 4 (26.7) | |
| Sigmoid colon: N (%) | 12 (18.5) | 24 (36.9) | 29 (44.6) | |
| Rectal: N (%) | 26 (26.3) | 30 (30.3) | 43 (43.4) | |
| Number of chemotherapy lines | | | | 0.231 |
| 1: N (%) | 11 (21.2) | 20 (38.5) | 21 (40.4) | |
| 2: N (%) | 11 (28.2) | 10 (25.6) | 18 (46.2) | |
| 3: N (%) | 5 (14.3) | 17 (48.6) | 13 (37.1) | |
| 4: N (%) | 3 (20.0) | 4 (26.7) | 8 (53.3) | |
| ≥5: N (%) | 0 (0.0) | 9 (52.9) | 8 (47.1) | |
| RAS mutations | | | | 0.574 |
| Yes: N (%) | 17 (17.3) | 38 (38.8) | 43 (43.9) | |
| No: N (%) | 15 (23.1) | 21 (32.3) | 29 (44.6) | |
| BRAF mutations | | | | 0.579 |
| Yes: N (%) | 2 (33.3) | 1 (16.7) | 3 (50.0) | |
| No: N (%) | 27 (20.5) | 47 (35.6) | 58 (43.9) | |
| Microsatellite instability | | | | 0.172 |
| Yes: N (%) | 2 (13.3) | 3 (20.0) | 10 (66.7) | |
| No: N (%) | 26 (21.8) | 44 (37.0) | 49 (41.2) | |
| Histologic grade | | | | <0.001* |
| Well differentiated: N (%) | 22 (17.3) | 55 (43.3) | 50 (39.4) | |
| Moderately differentiated: N (%) | 14 (16.5) | 26 (30.6) | 45 (52.9) | |

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| Poorly differentiated: N (%) | 13 (46.4) | 4 (14.3) | 11 (39.3) | 0.656 |
| Lymphovascular invasion | | | | |
| No: N (%) | 21 (21.9) | 35 (36.5) | 40 (41.7) | |
| Yes: N (%) | 21 (17.1) | 46 (37.4) | 56 (45.5) | 0.633 |
| Perineural invasion | | | | |
| No: N (%) | 31 (19.4) | 56 (35.0) | 73 (45.6) | |
| Yes: N (%) | 10 (17.5) | 24 (42.1) | 23 (40.4) | 0.197 |
| Presence of tumor deposits distant to the primary tumor | | | | |
| No: N (%) | 36 (20.6) | 62 (35.4) | 77 (44.0) | |
| Yes: N (%) | 5 (12.5) | 20 (50.0) | 15 (37.5) | 0.727 |
| Tumor stages: TNM II vs III vs IV | | | | |
| II: N (%) | 12 (21.8) | 19 (34.5) | 24 (43.6) | |
| III: N (%) | 14 (15.6) | 36 (40.0) | 40 (44.4) | |
| IV: N (%) | 27 (22.9) | 40 (33.9) | 51 (43.2) | 0.750 |
| Tumor stages: TNM II-III vs IV | | | | |
| II-III: N (%) | 34 (20.1) | 64 (37.9) | 71 (42.0) | |
| IV: N (%) | 27 (22.9) | 40 (33.9) | 51 (43.2) | 0.110 |
| T-stage | | | | |
| T1: N (%) | 2 (25.0) | 2 (25.0) | 4 (50.0) | |
| T2: N (%) | 3 (17.6) | 3 (17.6) | 11 (64.7) | |
| T3: N (%) | 23 (16.1) | 53 (37.1) | 67 (46.9) | |
| T4: N (%) | 12 (25.0) | 23 (47.9) | 13 (27.1) | 0.611 |
| N-stage | | | | |
| N0: N (%) | 16 (21.3) | 27 (36.0) | 32 (42.7) | |
| N1: N (%) | 16 (19.5) | 33 (40.2) | 33 (40.2) | |
| N2: N (%) | 7 (12.3) | 21 (36.8) | 29 (50.9) | |
| | | | | |
| Localized cohort | HPN staining intensity | | | p-value from Chi-square test |
| Clinical/histopathological variables correlated with HPN | Low | Medium | High | |
| Sex | | | | 0.902 |
| Men: N (%) | 21 (19.3) | 41 (37.6) | 47 (43.1) | |
| Women: N (%) | 13 (21.7) | 23 (38.3) | 24 (40.0) | |
| ECOG at diagnosis | | | | 0.334 |
| 0: N (%) | 15 (17.6) | 38 (44.7) | 32 (37.6) | |
| 1: N (%) | 15 (22.4) | 21 (31.3) | 31 (46.3) | |
| 2: N (%) | 2 (18.2) | 2 (18.2) | 7 (63.6) | |
| ≥3: N (%) | 0 (0.0) | 1 (100.0) | 0 (0.0) | 0.513 |
| Primary tumor site | | | | |
| Ascending colon: N (%) | 12 (25.0) | 16 (33.3) | 20 (41.7) | |
| Descending colon: N (%) | 1 (11.1) | 3 (33.3) | 5 (55.6) | |
| Transverse colon: N (%) | 1 (10.0) | 6 (60.0) | 3 (30.0) | |
| Sigmoid colon: N (%) | 4 (11.4) | 16 (45.7) | 15 (42.9) | |
| Rectal: N (%) | 16 (25.0) | 21 (32.8) | 27 (42.2) | 0.275 |
| Number of chemotherapy lines | | | | |
| 1: N (%) | 5 (22.7) | 6 (27.3) | 11 (50.0) | |
| 2: N (%) | 0 (0.0) | 5 (50.0) | 5 (50.0) | |
| 3: N (%) | 0 (0.0) | 6 (50.0) | 6 (50.0) | |
| 4: N (%) | 0 (0.0) | 1 (33.3) | 2 (66.6) | |
| ≥5: N (%) | 1 (25.0) | 3 (75.0) | 0 (0.0) | |

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| RAS mutations | | | | 0.381 |
| Yes: N (%) | 2 (5.7) | 16 (45.7) | 17 (48.6) | |
| No: N (%) | 4 (16.7) | 9 (37.5) | 11 (45.8) | |
| BRAF mutations | | | | 0.168 |
| Yes: N (%) | 1 (50.0) | 0 (0.0) | 1 (50.0) | |
| No: N (%) | 5 (9.8) | 21 (41.2) | 25 (49.0) | |
| Microsatellite instability | | | | 0.218 |
| Yes: N (%) | 2 (15.4) | 2 (15.4) | 9 (69.2) | |
| No: N (%) | 13 (17.1) | 29 (38.2) | 34 (44.7) | |
| Histologic grade | | | | 0.057 |
| Well differentiated: N (%) | 17 (18.9) | 33 (36.7) | 40 (44.4) | |
| Moderately differentiated: N (%) | 10 (18.9) | 23 (43.4) | 20 (37.7) | |
| Poorly differentiated: N (%) | 5 (50.0) | 0 (0.0) | 5 (50.0) | |
| Lymphovascular invasion | | | | 0.626 |
| No: N (%) | 16 (21.3) | 28 (37.3) | 31 (41.3) | |
| Yes: N (%) | 12 (15.8) | 28 (36.8) | 36 (47.4) | |
| Perineural invasion | | | | 0.653 |
| No: N (%) | 23 (19.7) | 44 (37.6) | 50 (42.7) | |
| Yes: N (%) | 5 (15.2) | 11 (33.3) | 17 (51.5) | |
| Presence of tumor deposits distant to the primary tumor | | | | 0.156 |
| No: N (%) | 26 (20.5) | 46 (36.2) | 55 (43.3) | |
| Yes: N (%) | 2 (10.0) | 9 (45.0) | 9 (45.0) | |
| Tumor stages: TNM II vs III | | | | 0.601 |
| II: N (%) | 12 (21.8) | 19 (34.5) | 24 (43.6) | |
| III: N (%) | 14 (15.6) | 36 (40.0) | 40 (44.4) | |
| T-stage | | | | 0.256 |
| T1: N (%) | 2 (40.0) | 1 (20.0) | 2 (40.0) | |
| T2: N (%) | 3 (20.0) | 3 (20.0) | 9 (60.0) | |
| T3: N (%) | 17 (16.0) | 40 (37.7) | 49 (46.2) | |
| T4: N (%) | 5 (23.8) | 11 (52.4) | 5 (23.8) | |
| N-stage | | | | 0.357 |
| N0: N (%) | 12 (21.8) | 19 (34.5) | 24 (43.6) | |
| N1: N (%) | 12 (20.3) | 24 (40.7) | 23 (39.0) | |
| N2: N (%) | 2 (6.5) | 12 (38.7) | 17 (54.8) | |
| | | | | |
| Metastatic cohort | HPN staining intensity | | | p-value from Chi-square test |
| Clinical/histopathological variables correlated with HPN | Low | Medium | High | |
| Sex | | | | 0.827 |
| Men: N (%) | 18 (23.1) | 25 (32.1) | 35 (44.9) | |
| Women: N (%) | 9 (22.5) | 15 (37.5) | 16 (40.0) | |
| ECOG at diagnosis | | | | 0.814 |
| 0: N (%) | 8 (26.7) | 10 (33.3) | 12 (40.0) | |
| 1: N (%) | 12 (21.8) | 19 (34.5) | 24 (43.6) | |
| 2: N (%) | 3 (13.6) | 9 (40.9) | 10 (45.5) | |
| ≥3: N (%) | 1 (33.3) | 0 (0.0) | 2 (66.7) | 0.645 |
| Primary tumor site | | | | |
| Ascending colon: N (%) | 7 (20.0) | 15 (42.9) | 13 (37.1) | |
| Descending colon: N (%) | 0 (0.0) | 2 (40.0) | 3 (60.0) | |
| Transverse colon: N (%) | 2 (40.0) | 2 (40.0) | 1 (20.0) | |

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| Sigmoid colon: N (%) | 8 (26.7) | 8 (26.7) | 14 (46.7) | |
| Rectal: N (%) | 10 (28.6) | 9 (25.7) | 16 (45.7) | |
| Liver metastasis at diagnosis | | | | 0.810 |
| Yes: N (%) | 20 (23.8) | 27 (32.1) | 37 (44.0) | |
| No: N (%) | 7 (20.6) | 13 (38.2) | 14 (41.2) | |
| Peritoneal metastasis at diagnosis | | | | 0.110 |
| Yes: N (%) | 11 (34.4) | 7 (21.9) | 14 (43.8) | |
| No: N (%) | 16 (18.6) | 33 (38.4) | 37 (43.0) | |
| Lung metastasis at diagnosis | | | | 0.245 |
| Yes: N (%) | 10 (32.3) | 11 (35.5) | 10 (32.3) | |
| No: N (%) | 17 (19.5) | 29 (33.3) | 41 (47.1) | |
| >1 metastasis site at diagnosis | | | | 0.023* |
| Yes: N (%) | 16 (34.8) | 16 (34.8) | 14 (30.4) | |
| No: N (%) | 11 (15.3) | 24 (33.3) | 37 (51.4) | |
| Number of chemotherapy lines | | | | 0.106 |
| 1: N (%) | 6 (20.0) | 14 (46.7) | 10 (33.3) | |
| 2: N (%) | 11 (37.9) | 5 (17.2) | 13 (44.8) | |
| 3: N (%) | 5 (21.7) | 11 (47.8) | 7 (30.4) | |
| 4: N (%) | 3 (25.0) | 3 (25.0) | 6 (50.0) | |
| ≥5: N (%) | 0 (0.0) | 6 (46.2) | 7 (53.8) | |
| RAS mutations | | | | 0.829 |
| Yes: N (%) | 15 (23.8) | 22 (34.9) | 26 (41.3) | |
| No: N (%) | 11 (26.8) | 12 (29.3) | 18 (43.9) | |
| BRAF mutations | | | | 0.929 |
| Yes: N (%) | 1 (25.0) | 1 (25.0) | 2 (50.0) | |
| No: N (%) | 22 (27.2) | 26 (32.1) | 33 (40.7) | |
| Microsatellite instability | | | | 0.654 |
| Yes: N (%) | 0 (0.0) | 1 (50.0) | 1 (50.0) | |
| No: N (%) | 13 (30.2) | 15 (34.9) | 15 (34.9) | |
| Histologic grade | | | | <0.001* |
| Well differentiated: N (%) | 5 (13.5) | 22 (59.5) | 10 (27.0) | |
| Moderately differentiated: N (%) | 4 (12.5) | 3 (9.4) | 25 (78.1) | |
| Poorly differentiated: N (%) | 8 (44.4) | 4 (22.2) | 6 (33.3) | |
| Lymphovascular invasion | | | | 0.626 |
| No: N (%) | 16 (21.3) | 28 (37.3) | 31 (41.3) | |
| Yes: N (%) | 12 (15.8) | 28 (36.8) | 36 (47.4) | |
| Perineural invasion | | | | 0.653 |
| No: N (%) | 23 (19.7) | 44 (37.6) | 50 (42.7) | |
| Yes: N (%) | 5 (15.2) | 11 (33.3) | 17 (51.5) | |
| Presence of tumor deposits distant to the primary tumor | | | | 0.156 |
| No: N (%) | 26 (20.5) | 46 (36.2) | 55 (43.3) | |
| Yes: N (%) | 2 (10.0) | 9 (45.0) | 9 (45.0) | |
| T-stage | | | | 0.399 |
| T1: N (%) | 0 (0.0) | 1 (33.3) | 2 (66.6) | |
| T2: N (%) | 0 (0.0) | 0 (0.0) | 2 (100.0) | |
| T3: N (%) | 6 (16.2) | 13 (35.1) | 18 (48.6) | |
| T4: N (%) | 7 (25.9) | 12 (44.4) | 8 (29.6) | |
| N-stage | | | | 0.993 |
| N0: N (%) | 4 (20.0) | 8 (40.0) | 8 (40.0) | |
| N1: N (%) | 4 (17.4) | 9 (39.1) | 10 (43.5) | |

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| N2: N (%) | 5 (19.2) | 9 (34.6) | 12 (46.2) | |
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