**Supplementary Table S1. Correlation between Sema3d expression and clinicopathologic characteristics of HCC patients in training cohort and validation cohort.**

| Clinicopathologic variables | Training cohort | Validation cohort |
|-----------------------------|-----------------|-------------------|
|                             | Sema3d expression |                             |                             |
|                             | High    | Low   | P     | n    | Sema3d expression | High    | Low   | P     |
| Gender                      |         |       |       |      |                  |         |       |       |
| Female                      | 39      | 19    | 20    | 23   | 8                  | 15      |       |       |
| Male                        | 141     | 50    | 91    | 97   | 47                 | 50      |       |       |
| Age (years)                 |         |       |       |      |                  |         |       |       |
| ≤50                         | 116     | 49    | 67    | 87   | 36                 | 51      |       |       |
| >50                         | 64      | 20    | 44    | 33   | 19                 | 14      |       |       |
| AFP (ng/ml)                 |         |       |       |      |                  |         |       |       |
| <20                         | 29      | 14    | 15    | 22   | 12                 | 10      |       |       |
| ≥20                         | 151     | 55    | 96    | 98   | 43                 | 55      |       |       |
| Hepatitis B status          |         |       |       |      |                  |         |       |       |
| Negative                    | 29      | 15    | 14    | 27   | 15                 | 12      |       |       |
| Positive                    | 151     | 54    | 97    | 93   | 40                 | 53      |       |       |
| Liver cirrhosis             |         |       |       |      |                  |         |       |       |
| Absent                      | 64      | 29    | 35    | 48   | 25                 | 23      |       |       |
| Present                     | 116     | 40    | 76    | 72   | 31                 | 42      |       |       |
| Child-Pugh classification   |         |       |       |      |                  |         |       |       |
| A                           | 113     | 40    | 73    | 73   | 37                 | 36      |       |       |
| B                           | 67      | 29    | 38    | 47   | 18                 | 29      |       |       |
| Tumor size (cm)             |         |       |       |      |                  |         |       |       |
| ≤5                          | 74      | 35    | 38    | 46   | 29                 | 17      |       |       |
| >5                          | 106     | 34    | 72    | 74   | 26                 | 48      |       |       |
| Tumor nodule number         |         |       |       |      |                  |         |       |       |
| Solitary                    | 113     | 50    | 63    | 69   | 40                 | 29      |       |       |
| Multiple (≥ 2)              | 67      | 19    | 48    | 51   | 15                 | 36      |       |       |
| Capsulation formation       |         |       |       |      |                  |         |       |       |
| Presence                    | 91      | 39    | 52    | 50   | 27                 | 23      |       |       |
| Absence                     | 89      | 30    | 59    | 70   | 28                 | 42      |       |       |
| Edmondson-Steiner grade     |         |       |       |      |                  |         |       |       |
| I & II                      | 78      | 36    | 42    | 53   | 21                 | 32      |       |       |
| III & IV                    | 102     | 33    | 69    | 67   | 34                 | 33      |       |       |
| Microvascular invasion      |         |       |       |      |                  |         |       |       |
| Absence                     | 121     | 54    | 67    | 71   | 40                 | 31      |       |       |
| Presence                    | 59      | 15    | 44    | 49   | 15                 | 34      |       |       |
| Macrophascular invasion     |         |       |       |      |                  |         |       |       |
| Absence                     | 143     | 61    | 82    | 102  | 51                 | 51      |       |       |
| Presence                    | 37      | 8     | 29    | 18   | 4                  | 14      |       |       |
| BCLC stage                  |         |       |       |      |                  |         |       |       |
| 0 & A                       | 83      | 42    | 41    | 51   | 32                 | 19      |       |       |
| B & C                       | 97      | 27    | 70    | 69   | 23                 | 46      |       |       |
| TNM stage                   |         |       |       |      |                  |         |       |       |
| I                           | 73      | 36    | 37    | 53   | 31                 | 22      |       |       |
| II & III                    | 107     | 33    | 74    | 67   | 24                 | 43      |       |       |
| CNLC stage |     |     | <0.001 |     |     |
|------------|-----|-----|--------|-----|-----|
|            | I   |     |        | 48  | 29  | 19   |
|            | II & III | 94  | 24    | 70  | 72  | 26   | 46   |

Abbreviations: AFP, alpha-fetoprotein; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; CNLC, China Clinic Liver Cancer.
Supplementary Table S2. Univariate and multivariate analyses of risk factors associated with overall survival and disease-free survival of HCC patients in training cohort.

| Clinicopathologic variables | OS | | | DFS | | |
|-----------------------------|--|--|---|--|--|---|
|                            | P  | HR(95% CI) | P  | HR(95% CI) | P  | HR(95% CI) |
| **Gender** | 0.940 | NA | 0.271 | NA |  |
| Female | 1 |  | 1 |
| Male | 1.017(0.661-1.565) | 1.224(0.854-1.754) |  |
| **Age (years)** | 0.389 | NA | 0.100 | NA |  |
| ≤50 | 1 |  | 1 |
| >50 | 1.83(0.807-1.733) | 1.304(0.951-1.790) |  |
| **AFP (ng/ml)** | 0.033 | 0.077 | <0.001 | 0.057 |  |
| <20 | 1 | 1 | 1 |
| ≥20 | 1.600(1.039-2.464) | 1.573(0.953-2.597) | 1.993(1.417-2.804) | 1.487(0.989-2.238) |
| **Hepatitis B status** | 0.606 | NA | 0.152 | NA |  |
| Negative | 1 |  | 1 |
| Positive | 1.116(0.736-1.692) | 1.292(0.910-1.835) |  |
| **Liver cirrhosis** | 0.138 | NA | 0.064 | NA |  |
| Absent | 1 |  | 1 |
| Present | 1.345(0.909-1.989) | 1.351(0.983-1.858) |  |
| **Child-Pugh classification** | 0.145 | NA | 0.104 | NA |  |
| A | 1 |  | 1 |
| B | 1.318(0.909-1.909) | 1.320(0.945-1.845) |  |
| **Tumor size (cm)** | 0.002 | 0.337 | 0.013 | 0.529 |  |
| ≤5 | 1 | 1 | 1 |
| >5 | 1.774(1.228-2.563) | 1.236(0.802-1.907) | 1.514(1.091-2.100) | 1.127(0.777-1.634) |
| **Tumor nodule number** | <0.001 | 0.052 | 0.018 | 0.063 |  |
| Solitary | 1 | 1 | 1 |
|  |  |  |  |  |  |
|                          | Multiple (≥ 2) | 1.500(0.996-2.413) | 1.476(1.068-2.038) | 1.472(0.979-2.212) |
|--------------------------|---------------|---------------------|---------------------|---------------------|
| **Capsulation formation**|               |                     |                     |                     |
| Presence                 |               |                     |                     |                     |
| Absence                  | 1.472(1.020-2.124) | 1.388(0.674-1.708) | 1.536(1.113-2.121) | 1.392(0.978-1.981) |
| **Edmondson-Steiner grade** |               |                     |                     |                     |
| I & II                   | 0.008         | 0.140               | 0.007               | 0.190               |
| III&IV                   | 1.643(1.139-2.371) | 1.364(0.903-2.059) | 1.571(1.134-2.177) | 1.297(0.879-1.915) |
| **Microvascular invasion** |               |                     |                     |                     |
| Absence                  | 0.006         | 0.005               | 0.003               | 0.017               |
| Presence                 | 1.707(1.169-2.492) | 1.996(1.231-3.236) | 1.686(1.199-2.370) | 1.541(1.080-2.197) |
| **Macrovascular invasion** |               |                     |                     |                     |
| Absence                  | <0.001        | 0.002               | <0.001              | <0.001              |
| Presence                 | 2.206(1.362-3.012) | 1.971(1.289-3.013) | 2.047(1.394-3.003) | 2.078(1.399-3.086) |
| **BCLC stage**           | 0.018         | 0.025               | 0.003               | 0.034               |
| 0&A                      | 1.628(1.086-2.441) | 1.731(1.071-2.797) | 1.665(1.191-2.328) | 1.531(1.033-2.268) |
| B&C                      |               |                     |                     |                     |
| **TNM stage**            | 0.014         | 0.017               | 0.001               | <0.001              |
| I                        |               |                     |                     |                     |
| II &III                  | 1.635(1.105-2.421) | 1.783(1.107-2.873) | 1.821(1.294-2.563) | 2.124(1.476-3.076) |
| **CNLC stage**           |               |                     |                     |                     |
| Absence                  | <0.001        | 0.007               | 0.003               | 0.004               |
| Presence                 | 1.971(1.366-2.844) | 1.777(1.168-2.702) | 1.616(1.171-2.230) | 1.801(1.213-2.673) |
| **Sema3d expression**    |               |                     |                     |                     |
| Low                      | 0.013         | 0.019               | 0.001               | 0.022               |
| High                     | 1.579(1.103-2.262) | 1.502(1.069-2.110) | 1.840(1.276-2.653) | 1.484(1.058-2.081) |

Abbreviations: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; CNLC, China Clinic Liver Cancer.
Supplementary Table S3. Univariate and multivariate analyses of risk factors associated with overall survival and disease-free survival of HCC patients in validation cohort.

| Clinicopathologic variables | OS   | DFS   |
|-----------------------------|------|-------|
|                             | Univariate Analysis | Multivariate Analysis | Univariate Analysis | Multivariate Analysis |
|                             | P     | HR(95% CI) | P     | HR(95% CI) | P     | HR(95% CI) | P     | HR(95% CI) |
| Gender                      | 0.489 | NA      | 0.496 | NA      |
| Female                      | 1     | 1       |
| Male                        | 1.213(0.703-2.093) | 1.195(0.715-1.998) |
| Age (years)                 | 0.510 | NA      | 0.889 | NA      |
| ≤50                         | 1     | 1       |
| >50                         | 1.173(0.729-1.887) | 1.033(0.654-1.633) |
| AFP (ng/ml)                 | 0.039 | 0.317   | 0.013 | 0.132   |
| <20                         | 1     | 1       | 1     | 1       |
| ≥20                         | 1.702(1.028-2.818) | 1.320(0.766-2.277) | 1.819(1.135-2.914) | 1.478(0.889-2.458) |
| Hepatitis B status          | 0.215 | NA      | 0.175 | NA      |
| Negative                    | 1     | 1       |
| Positive                    | 1.413(0.818-2.440) | 1.418(0.857-2.347) |
| Liver cirrhosis             | 0.131 | NA      | 0.142 | NA      |
| Absent                      | 1     | 1       |
| Present                     | 1.417(0.902-2.227) | 1.368(0.900-2.078) |
| Child-Pugh classification   | 0.162 | NA      | 0.053 | NA      |
| A                           | 1     | 1       |
| B                           | 1.365(0.883-2.109) | 1.497(0.994-2.252) |
| Tumor size (cm)             | 0.064 | NA      | 0.233 | NA      |
| ≤5                          | 1     | 1       |
| >5                          | 1.545(0.976-2.448) | 1.289(0.849-1.957) |
| Tumor nodule number         | 0.017 | 0.002   | 0.001 | 0.031   |
| Solitary                    | 1     | 1       | 1     | 1       |
### Table

|                          | Multiple (≥ 2)                | 2.242(1.358-3.701) | 1.995(1.327-3.000) | 1.643(1.048-2.578) |
|--------------------------|------------------------------|---------------------|-------------------|-------------------|
| **Capsulation formation**| Presence                     | 0.023               | 0.371             | 0.032             | 0.243             |
|                          | Absence                      | 1.682(1.074-2.636)  | 1.362(0.692-2.678) | 1.576(1.040-2.388) | 1.460(0.773-2.757) |
| **Edmondson-Steiner grade** | I & II                      | 0.019               | 0.028             | 0.007             | 0.011             |
|                          | III & IV                     | 1.692(1.091-2.625)  | 1.691(1.057-2.705) | 1.750(1.161-2.638) | 1.802(1.145-2.835) |
| **Microvascular invasion** | Presence                    | 1.801(1.168-2.775)  | 1.713(1.058-2.773) | 1.778(1.187-2.665) | 1.870(1.150-3.042) |
|                          | Absence                      | 0.006               | 0.002             | 0.002             | 0.027             |
| **Macrovascular invasion** | Presence                    | 1.944(1.212-3.118)  | 2.242(1.358-3.701) | 2.049(1.300-3.231) | 1.795(1.067-3.019) |
| **BCLC stage**           | 0&A                          | 0.040               | 0.038             | 0.019             | 0.009             |
|                          | B&C                          | 1.774(1.028-3.064)  | 1.850(1.035-3.308) | 1.832(1.105-3.034) | 2.406(1.192-3.513) |
| **TNM stage**            | I                            | 0.002               | 0.018             | <0.001            | 0.013             |
|                          | II & III                     | 2.078(1.320-3.272)  | 1.913(1.120-3.269) | 2.154(1.409-3.292) | 1.880(1.141-3.097) |
| **CNLC stage**           | I                            | 0.012               | 0.019             | 0.006             | 0.026             |
|                          | II & III                     | 1.807(1.140-2.865)  | 1.782(1.099-2.890) | 1.828(1.191-2.805) | 1.842(1.077-3.152) |
| **Sema3d expression**    | High                         | 0.002               | 0.031             | 0.004             | 0.021             |
|                          | Low                          | 1.865 (1.260-2.761) | 1.567(1.043-2.355) | 1.788(1.207-2.647) | 1.486(1.061-2.083) |

**Abbreviations:** AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; CNLC, China Clinic Liver Cancer.
### Supplementary Table S4. Clinicopathological characteristics of HCC patients in training cohort and validation cohort.

| Clinicopathologic variables | Counts | P       |
|-----------------------------|--------|---------|
|                            | Training cohort | Validation cohort |
| Gender                      |         |         |
| Female                      | 39(21.67%) | 23(19.17%) | 0.600 |
| Male                        | 141(78.33%) | 97(80.83%)  |
| Age (years)                 |         |         |
| ≤60                         | 116(64.44%) | 87(72.50%) | 0.144 |
| >60                         | 64(35.56%) | 33(27.50%)  |
| AFP (ng/ml)                 |         |         |
| <20                         | 31(17.22%) | 22(18.33%) | 0.804 |
| ≥20                         | 149(82.78%) | 98(81.67%)  |
| Hepatitis B status          |         |         |
| Negative                    | 29(16.11%) | 27(18.33%) | 0.615 |
| Positive                    | 151(83.89%) | 93(81.67%)  |
| Liver cirrhosis             |         |         |
| Absent                      | 64(35.56%) | 48(40.00%) | 0.436 |
| Present                     | 116(64.44%) | 72(60.00%)  |
| Child-Pugh classification   |         |         |
| A                           | 113(62.78%) | 73(60.83%) | 0.734 |
| B                           | 67(37.22%) | 47(39.17%)  |
| Tumor size (cm)             |         |         |
| ≤5                          | 74(41.11%) | 46(38.33%) | 0.630 |
| >5                          | 106(58.89%) | 74(61.67%)  |
| Tumor nodule number         |         |         |
| Solitary                    | 113(62.78%) | 69(57.50%) | 0.359 |
| Multiple (≥ 2)              | 67(37.22%) | 51(42.50%)  |
| Capsulation formation       |         |         |
| Presence                    | 91(50.56%) | 50(41.67%) | 0.131 |
|                       | Absence | Presence |
|-----------------------|---------|----------|
| Absence               | 89(49.44%) | 70(58.33%) |
| **Edmondson-Steiner grade** | 0.886   |          |
| I & II                | 78(43.33%) | 53(44.17%) |
| III&IV                | 102 (56.67%) | 67(55.83%) |
| **Microvascular invasion** | 0.154 |          |
| Absence               | 121(67.22%) | 71(59.17%) |
| Presence              | 59(32.78%) | 49 (40.83%) |
| **Macrovascular invasion** | 0.223 |          |
| Absence               | 143(79.44%) | 102(85.00%) |
| Presence              | 37(20.56%) | 18(15.00%) |
| **BCLC stage**        | 0.538   |          |
| 0&A                   | 83(46.11%) | 51(42.50%) |
| B&C                   | 97 (53.89%) | 69(65.38%) |
| **TNM stage**         | 0.535   |          |
| I                     | 73(40.56%) | 53(44.17%) |
| II &III               | 107(59.44%) | 67(55.83%) |
| **CNLC stage**        | 0.184   |          |
| I                     | 86(44.78%) | 48(40.00%) |
| II &III               | 94(52.22%) | 72(60.00%) |

Abbreviations: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; CNLC, China Clinic Liver Cancer.
### Supplementary Table S5. High expressed pathways in the Sema3d_Low group of Gene set enrichment analysis (GSEA)

| NAME                                                                 | NES     | NOM p-val  |
|----------------------------------------------------------------------|---------|------------|
| HALLMARK_PI3K_AKT_MTOR_SIGNALING                                     | 1.724282| 0.005714   |
| HALLMARK_KRAS_SIGNALING_UP                                           | 1.597425| 0.006085   |
| HALLMARK_INFLAMMATORY_RESPONSE                                       | 1.586428| 0.018256   |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION                           | 1.563879| 0.027944   |
| HALLMARK_APICAL_JUNCTION                                             | 1.554019| 0.034205   |
| HALLMARK_ALLOGRAFT_REJECTION                                         | 1.538907| 0.046122   |
| HALLMARK_APOPTOSIS                                                   | 1.521574| 0.047423   |
| HALLMARK_IL2_STAT5_SIGNALING                                         | 1.513966| 0.029598   |
| HALLMARK_IL6_JAK_STAT3_SIGNALING                                     | 1.512123| 0.036511   |
| HALLMARK_ANGIogenesis                                                | 1.488971| 0.049180   |

### Supplementary Table S6. Low expressed pathways in the Sema3D_Low group of Gene set enrichment analysis (GSEA)

| NAME                                                                 | NES     | NOM p-val  |
|----------------------------------------------------------------------|---------|------------|
| HALLMARK_OXIDATIVE_PHOSPHORYLATION                                   | -1.80412| 0.040698   |
| HALLMARK_FATTY_ACID_METABOLISM                                       | -1.08988| 0.369388   |
| HALLMARK_PEROXISOME                                                 | -1.04845| 0.411157   |
| HALLMARK_DNA_REPAIR                                                 | -0.95115| 0.522088   |
| HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY                             | -0.87475| 0.614801   |
| HALLMARK_ADIPOGENESIS                                                | -0.84374| 0.640657   |
| HALLMARK_BILE_ACID_METABOLISM                                        | -0.73125| 0.748441   |
| HALLMARK_XENOBIOTIC_METABOLISM                                       | -0.65223| 0.860082   |
| HALLMARK_MYC_TARGETS_V1                                              | -0.63144| 0.757085   |
| HALLMARK_MYC_TARGETS_V2                                              | -0.51806| 0.928425   |
### Supplementary Table S7. Result of co-IP combined LC-MS/MS

| Entry name   | Coverage [%] | # PSMs | Score Sequest HT: Sequest HT |
|--------------|--------------|--------|-----------------------------|
| K2C1_HUMAN   | 59           | 74     | 221.85                      |
| K1C9_HUMAN   | 69           | 49     | 169.45                      |
| K22E_HUMAN   | 67           | 50     | 161.64                      |
| K1C10_HUMAN  | 52           | 35     | 117.44                      |
| **FLNA_HUMAN** | **12**     | **30** | **85.13**                  |
| HSP7C_HUMAN  | 32           | 28     | 78.14                       |
| ALBU_HUMAN   | 31           | 26     | 70.7                        |
| ACTB_HUMAN   | 43           | 22     | 68.15                       |
| TBB4B_HUMAN  | 42           | 25     | 67.21                       |
| TBB5_HUMAN   | 44           | 23     | 63.66                       |
| HS90A_HUMAN  | 20           | 23     | 57.81                       |
| K2C6A_HUMAN  | 29           | 19     | 50.6                        |
| TBA1B_HUMAN  | 24           | 15     | 43.82                       |
| TBA1A_HUMAN  | 24           | 15     | 43.56                       |
| HS90B_HUMAN  | 21           | 18     | 43.17                       |
| G3P_HUMAN    | 50           | 14     | 41.06                       |
| BIP_HUMAN    | 19           | 11     | 39.18                       |
| H2A2C_HUMAN  | 30           | 14     | 38.35                       |
| K1C16_HUMAN  | 31           | 12     | 35.97                       |
| CH60_HUMAN   | 18           | 10     | 35.46                       |