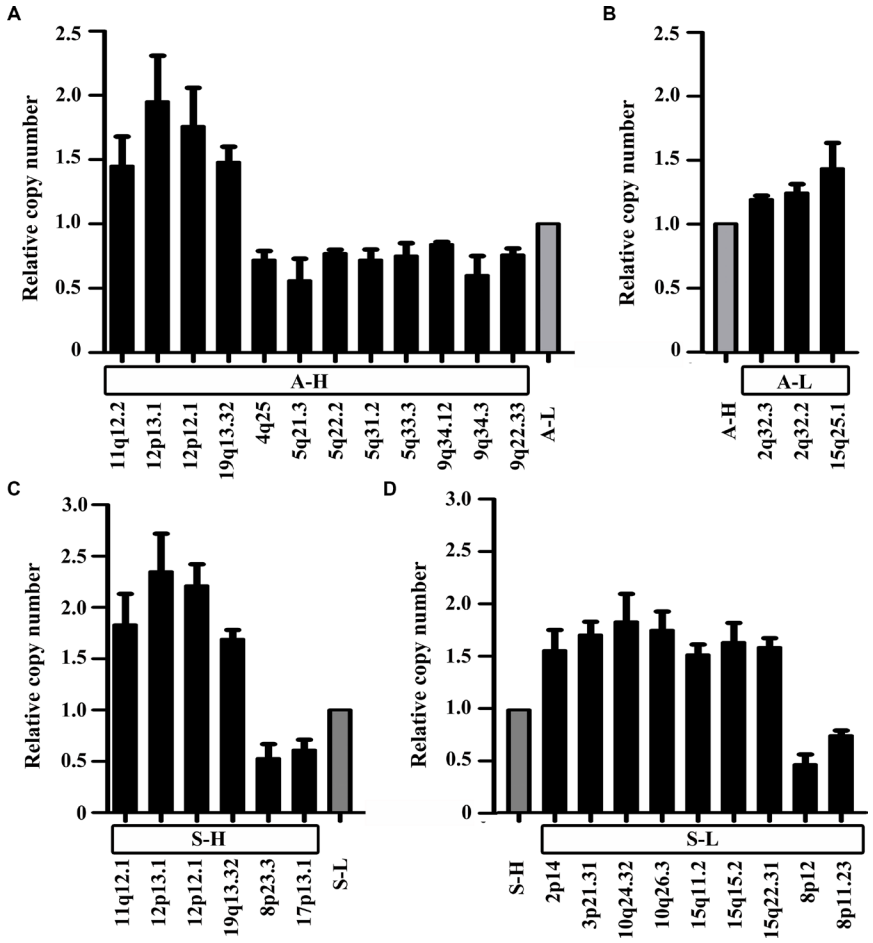
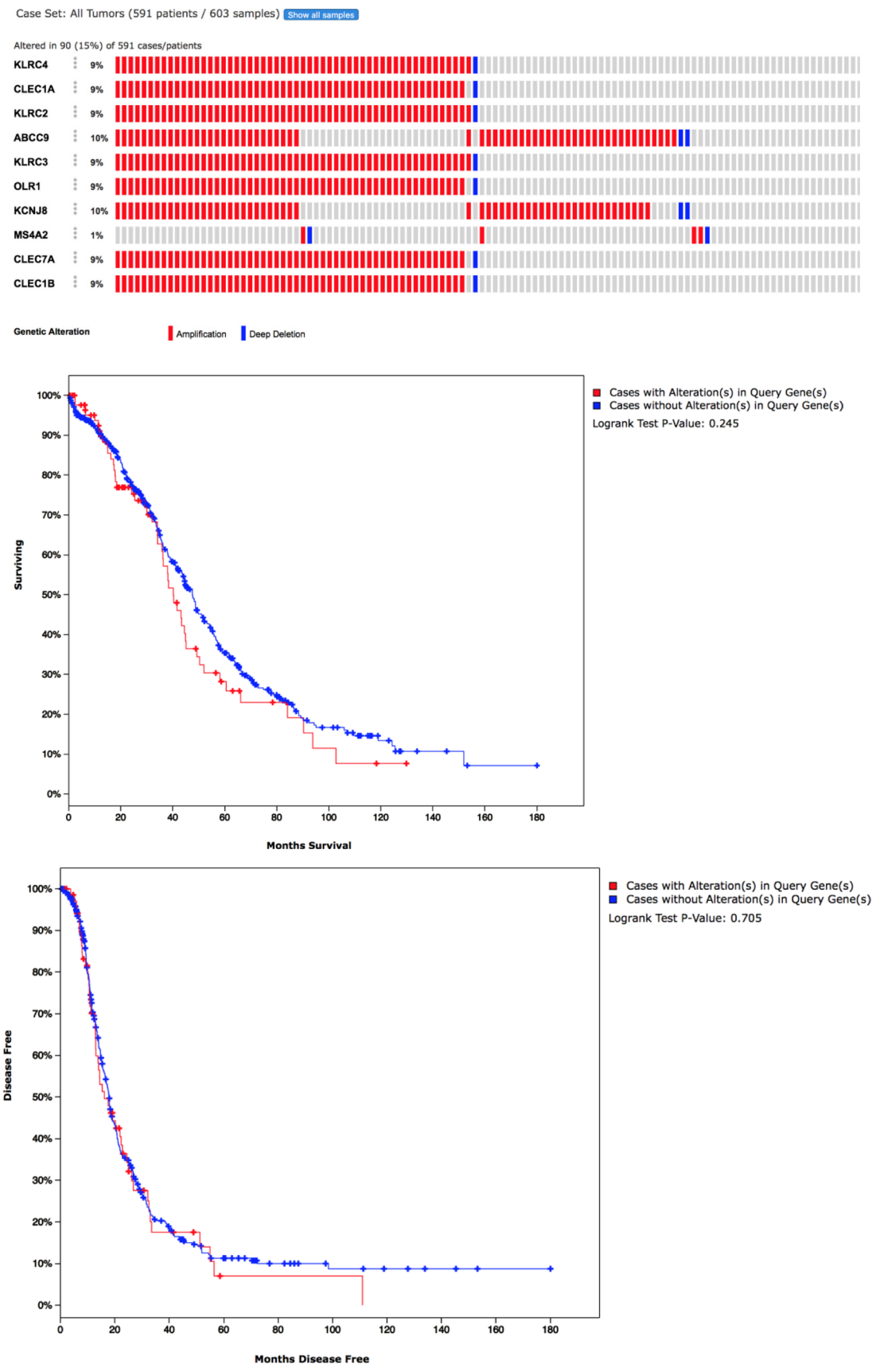
# Genome-wide DNA copy number analysis in clonally expanded human ovarian cancer cells with distinct invasive/migratory capacities

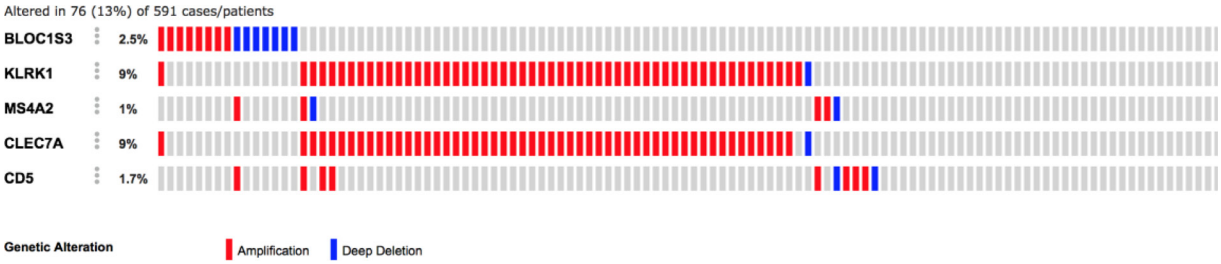
## Supplementary Materials

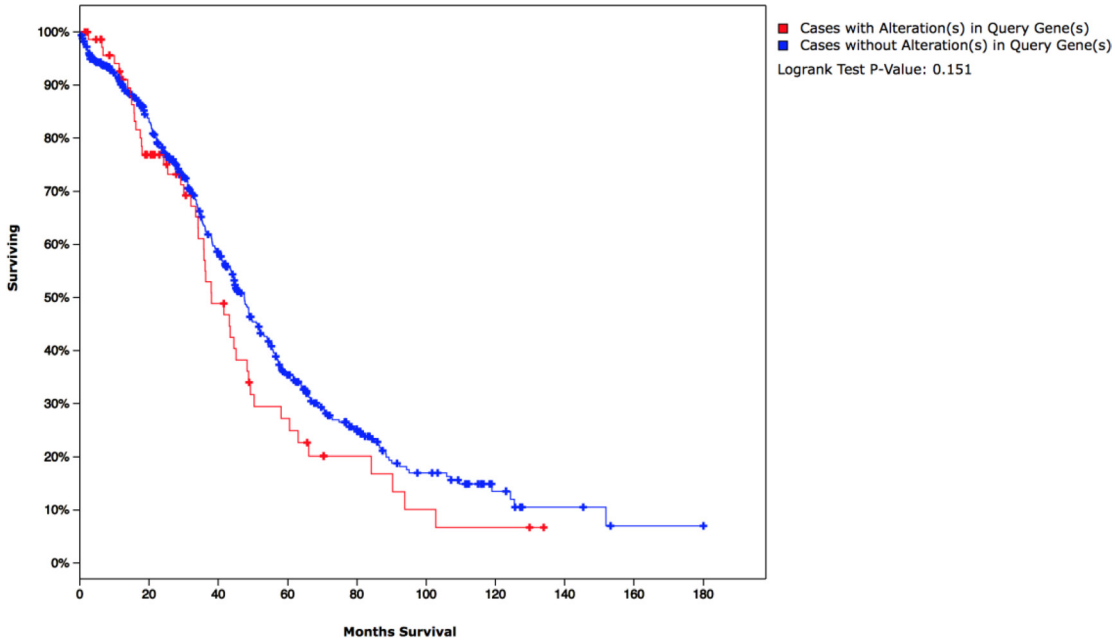


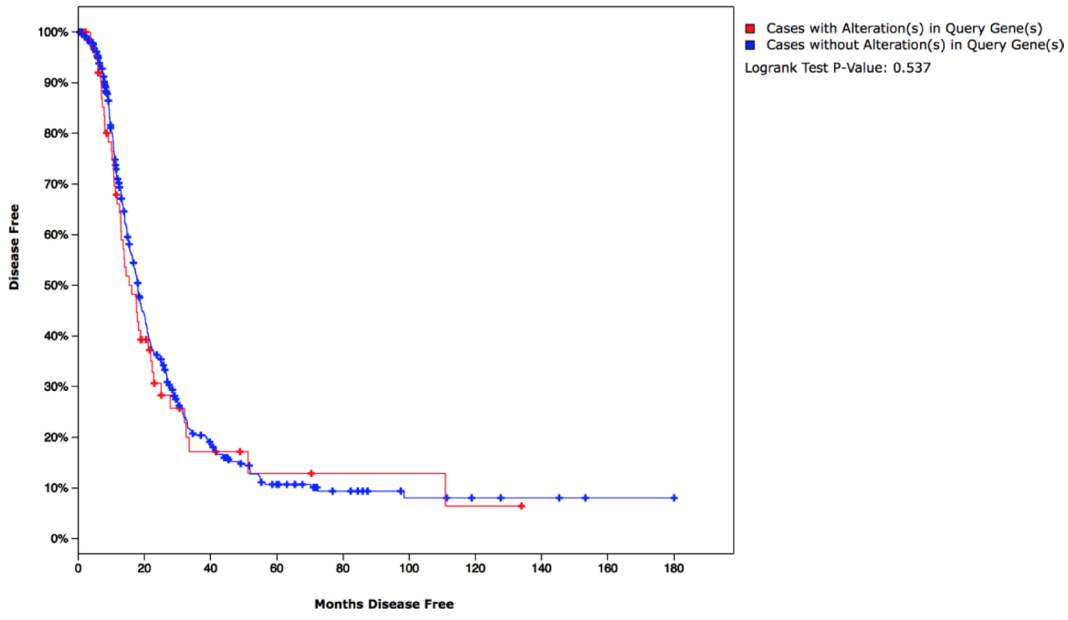

**Supplementary Figure 1: Validation of the CNV data.** Relative gene copy numbers in the selected regions were determined by RT-PCR with DNA from (**A**, **B**) A-H and A-L or (**C**, **D**) S-H and S-L cells. The data shown are the average values from three independent experiments ± SEM. For (A,C), the relative gene copy numbers of A-L/S-L cells were set as 1 and those of A-H/S-H cells were normalized to these values, whereas for (B,D), the relative gene copy numbers of A-H/S-H cells were set as 1 and those of A-L/S-L cells were normalized to these values (For each relative copy number of each selected region, $P < 0.05$; data not shown.) .



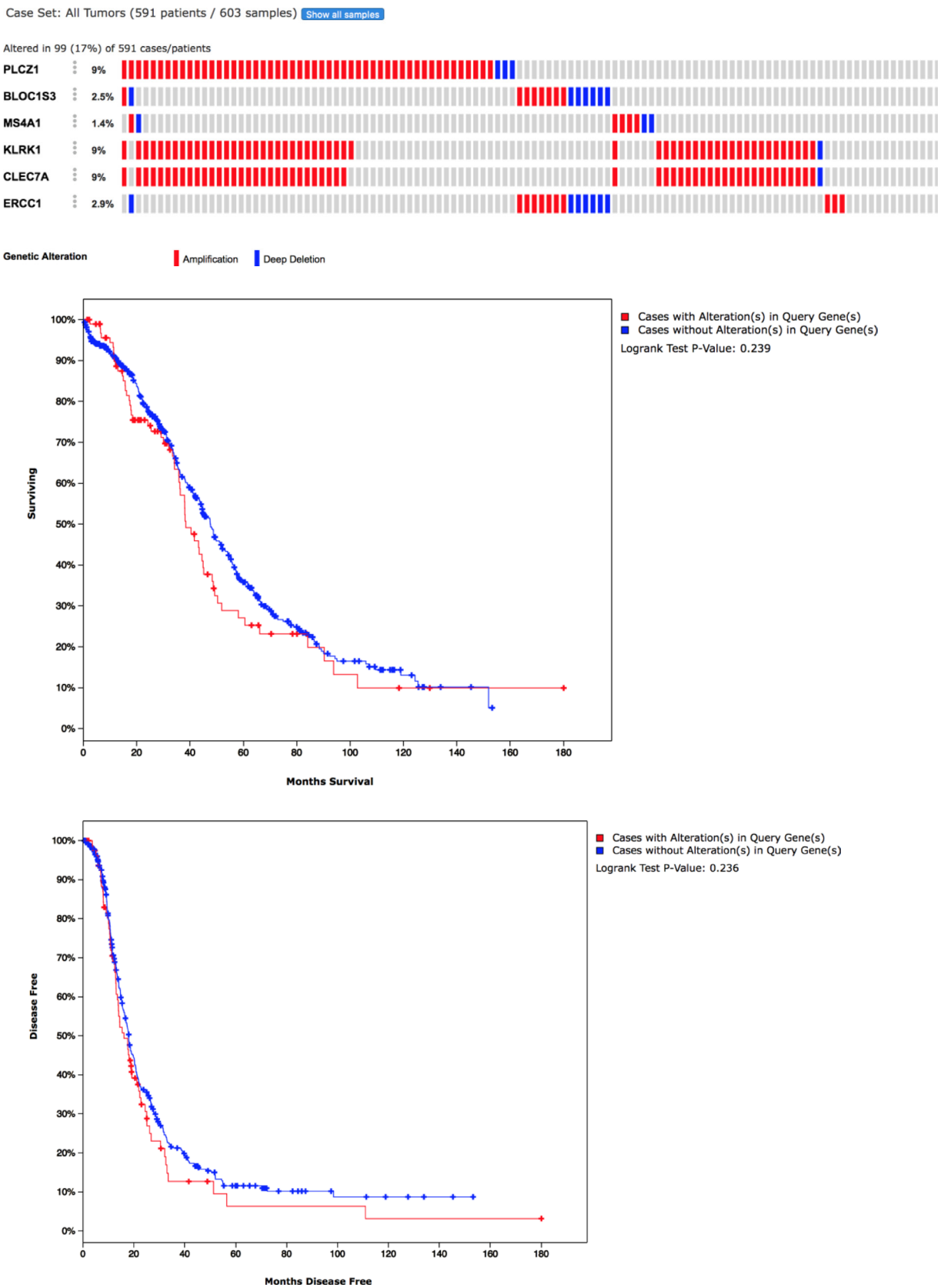

**Supplementary Figure 2: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.



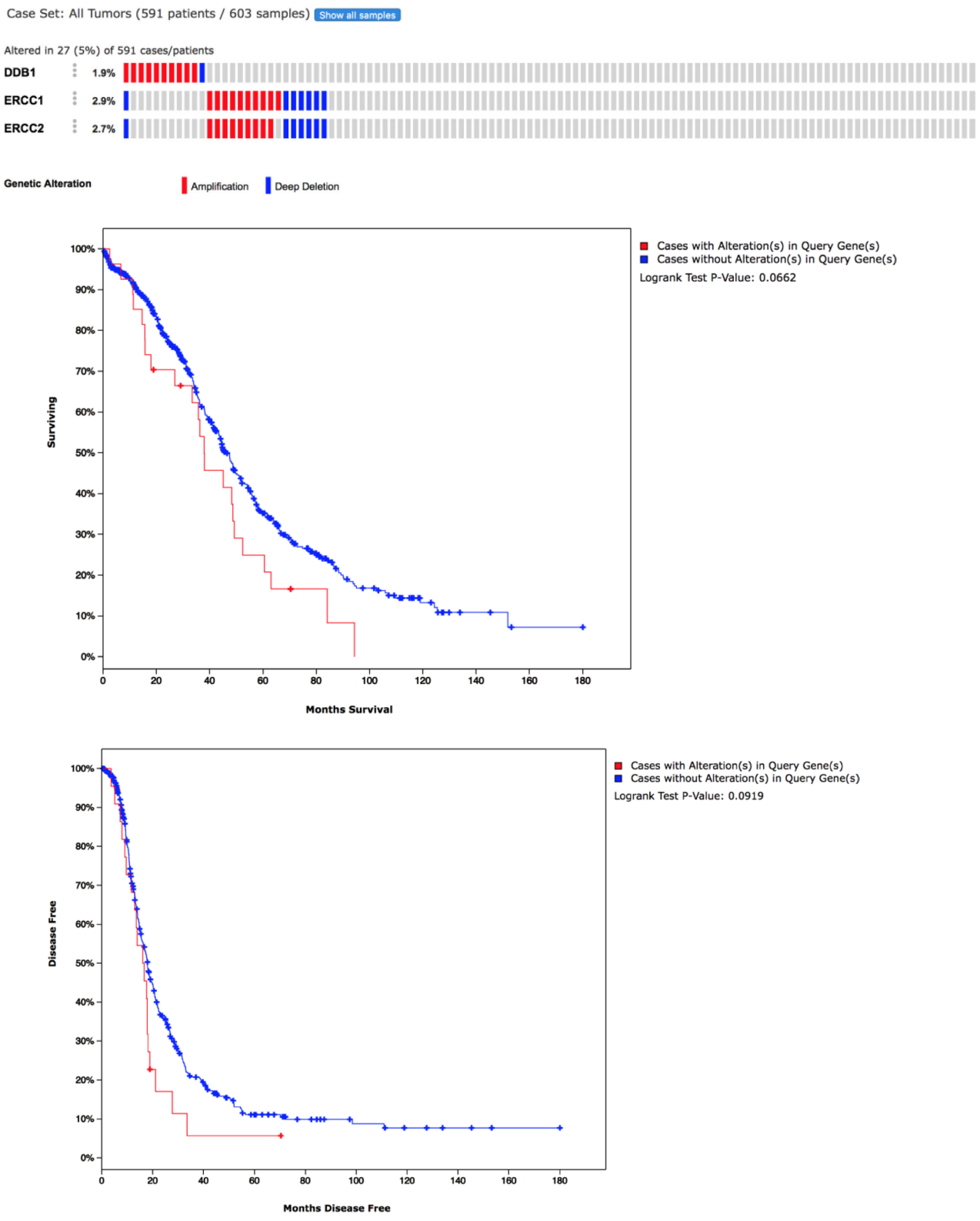



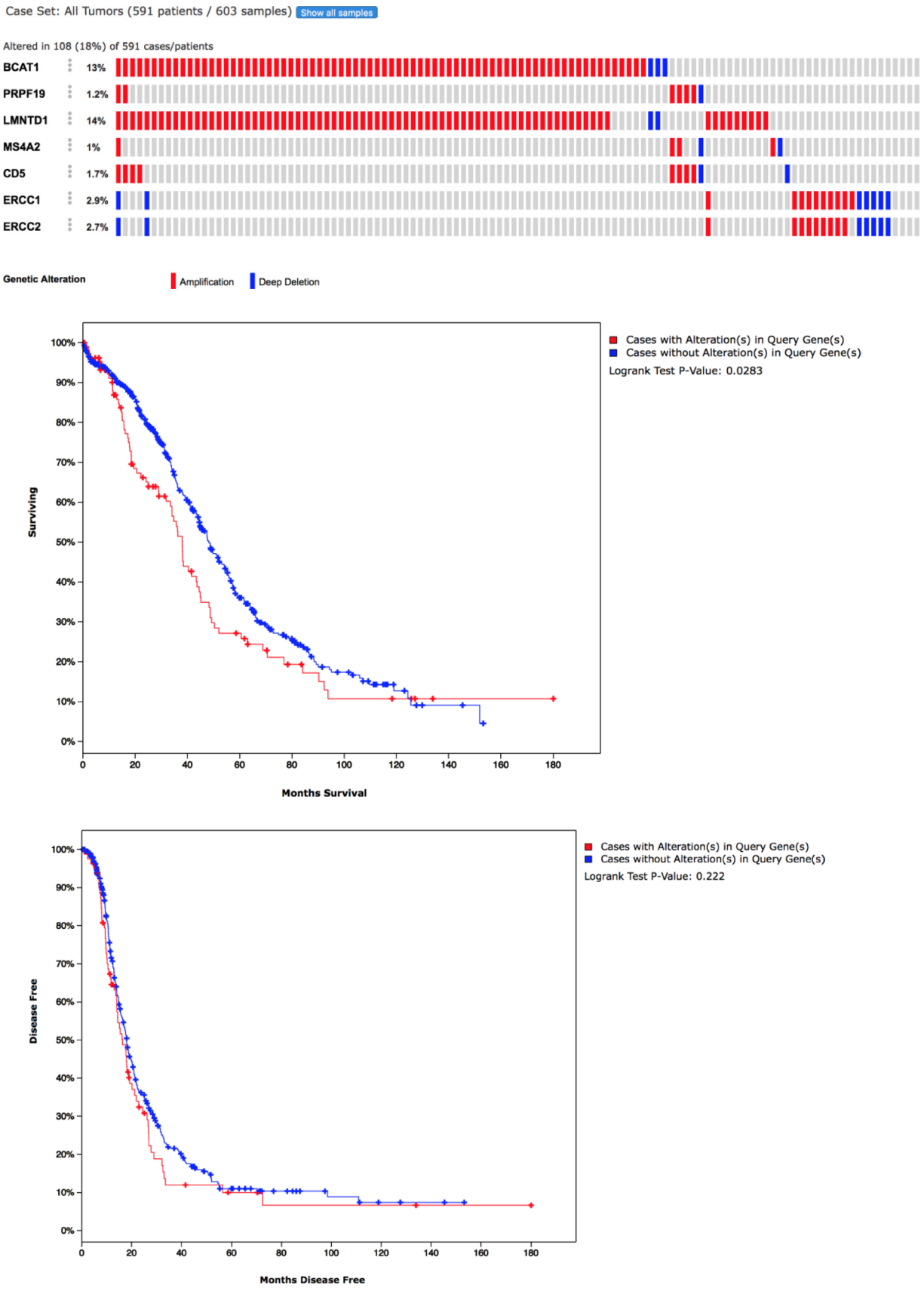

**Supplementary Figure 3: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.



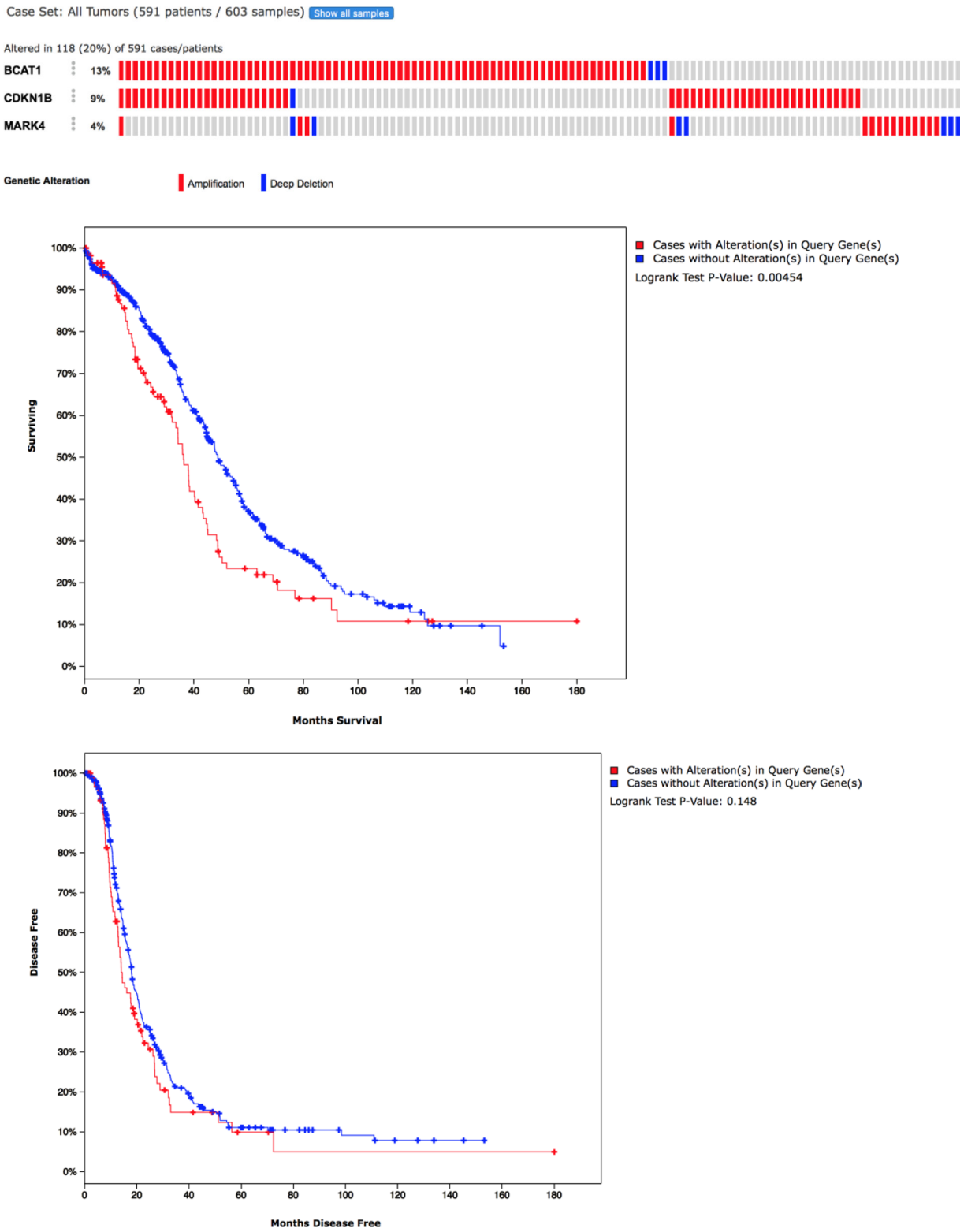

**Supplementary Figure 4: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.




**Supplementary Figure 5: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.



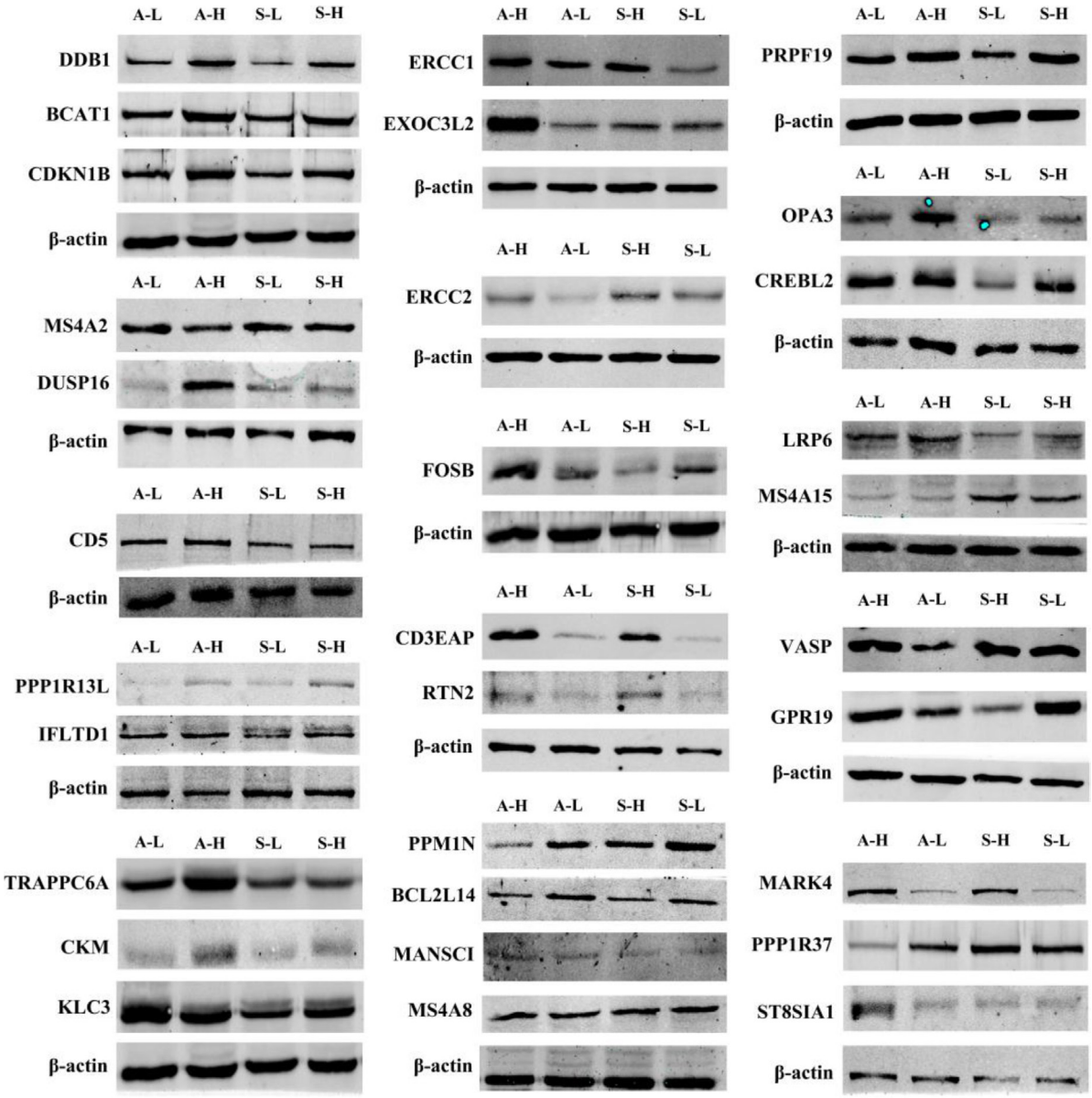

**Supplementary Figure 6: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.




**Supplementary Figure 7: Clinical outcomes associated with the functional gene groups amplified in both A-H- and S-H-specific gains from the TCGA database.** For each gene group, copy number alterations of the member genes are listed. Kaplan-Meier analysis of OS and PFS was performed with the copy number of the gene group as a categorical variable, so that the groups with unaltered and altered copy numbers could be compared. The results of a Cox proportional hazards test, with residual disease as a copredictor, are shown as *P* values. The diagram depicts the detailed number of patients and survival months with unaltered and altered copy numbers.

| Genes | Differential expression | | *P* value | |
|---|---|---|---|---|
| | A-H VS A-L | S-H VS S-L | A-H VS A-L | S-H VS S-L |
| *MS4A2* | -- | -- | ns | ns |
| *CD5* | -- | -- | ns | ns |
| *ERCC1* | ↗ | ↗ | ** | * |
| *DDB1* | ↗ | ↗ | ** | * |
| *ERCC2* | ↗ | ↗ | * | ** |
| *BCAT1* | ↗ | ↗ | * | ** |
| *PRPF19* | ↗ | ↗ | * | * |
| *IFLTD1* | -- | -- | ns | ns |
| *CDKN1B* | ↗ | ↗ | ** | * |
| *MARK4* | ↗ | ↗ | ** | * |
| *TRAPPC6A* | ↗ | -- | ** | ns |
| *EXOC3L2* | ↗ | -- | *** | ns |
| *CKM* | ↗ | ↗ | * | * |
| *KLC3* | ↘ | ↗ | ns | ns |
| *PPP1R13L* | ↗ | ↗ | * | * |
| *CD3EAP* | ↗ | ↗ | ** | ** |
| *FOSB* | ↗ | ↘ | * | * |
| *RTN2* | ↗ | ↗ | * | * |
| *PPM1N* | ↘ | ↘ | * | ns |
| *VASP* | ↗ | -- | * | ns |
| *OPA3* | ↗ | ↗ | * | * |
| *BCL2L14* | ↘ | -- | ns | ns |
| *LRP6* | ↗ | ↗ | * | * |
| *MANSC1* | ↗ | -- | ns | ns |
| *DUSP16* | ↗ | ↗ | ** | ns |
| *CREBL2* | -- | ↗ | ns | * |
| *GPR19* | ↗ | ↘ | * | ** |
| *ST8SIA1* | ↗ | -- | * | ns |
| *PPP1R37* | ↘ | -- | * | ns |
| *MS4A8* | -- | -- | ns | ns |
| *MS4A15* | -- | -- | ns | ns |

Note: ↗: increase; ↘: decrease; --: no change; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.


A-L A-H S-L S-H
DDB1
BCAT1
CDKN1B
β-actin
A-L A-H S-L S-H
MS4A2
DUSP16
β-actin
A-L A-H S-L S-H
CD5
β-actin
A-L A-H S-L S-H
PPP1R13L
IFLTD1
β-actin
A-L A-H S-L S-H
TRAPPC6A
CKM
KLC3
β-actin
A-H A-L S-H S-L
ERCC1
EXOC3L2
β-actin
A-H A-L S-H S-L
ERCC2
β-actin
A-H A-L S-H S-L
FOSB
β-actin
A-H A-L S-H S-L
CD3EAP
RTN2
β-actin
A-H A-L S-H S-L
PPM1N
BCL2L14
MANSCI
MS4A8
β-actin
A-L A-H S-L S-H
PRPF19
β-actin
A-L A-H S-L S-H
OPA3
CREBL2
β-actin
A-L A-H S-L S-H
LRP6
MS4A15
β-actin
A-H A-L S-H S-L
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GPR19
β-actin
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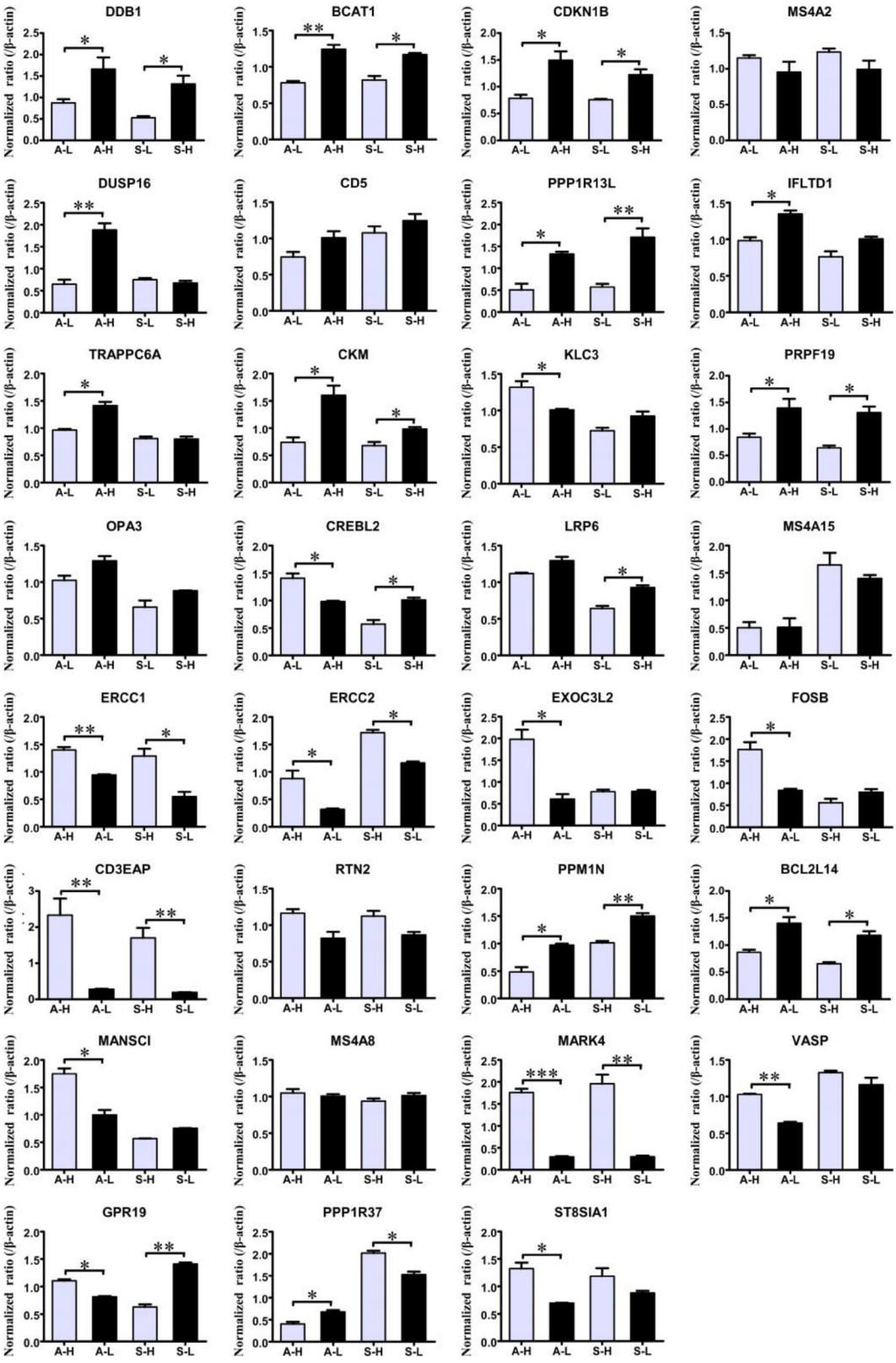
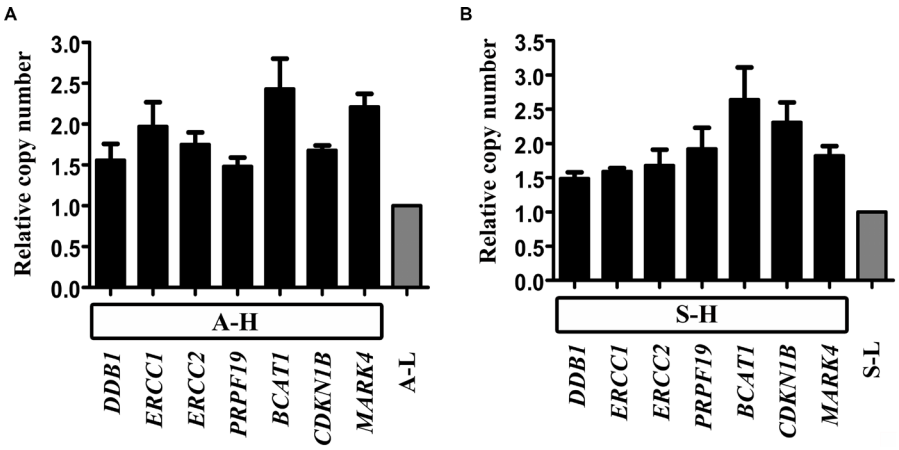
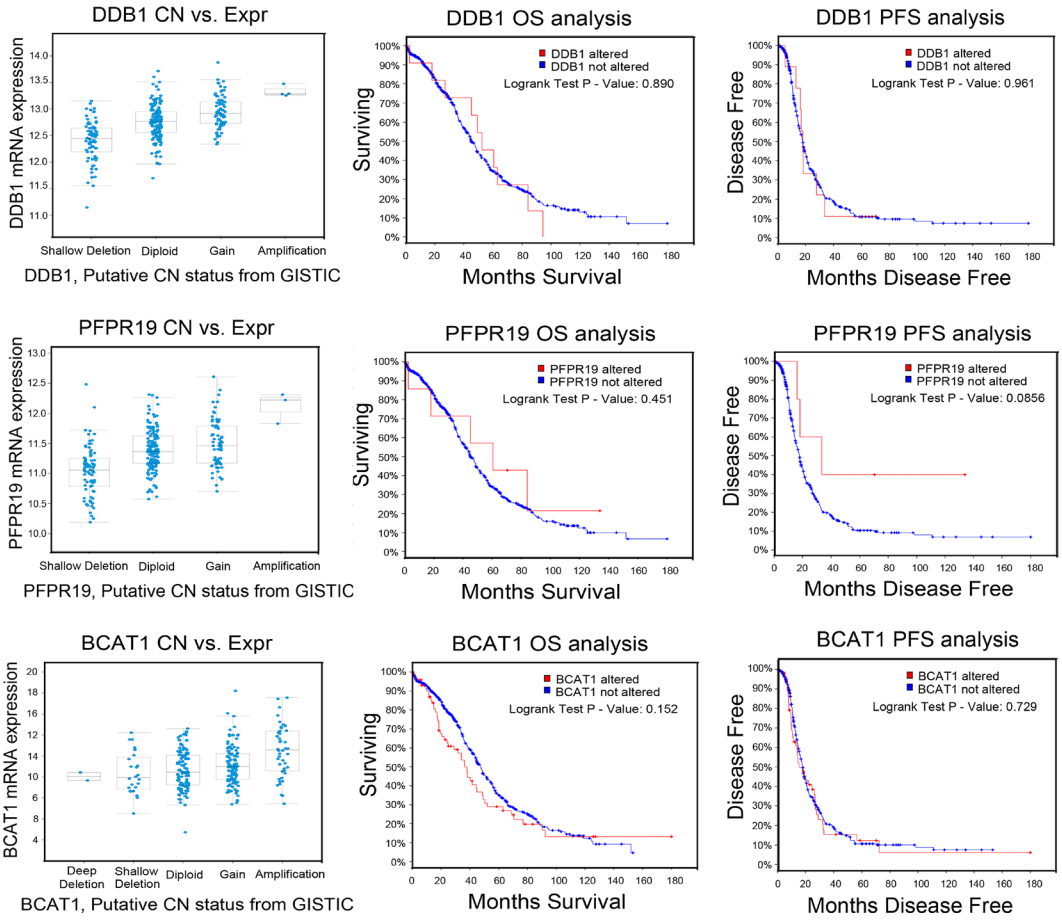
MARK4
PPP1R37
ST8SIA1
β-actin




**Supplementary Figure S8: Expression levels of all the selected genes in the distinct invasive/migratory subclones.** (**A**) RT-PCR analysis of the mRNA expression of the selected genes. (**B**) Western blot analysis displayed the protein levels of all the selected genes in A-H vs. A-L and S-H vs. S-L. (**C**) The data in the bar graphs were normalized to β-actin. Error bars represent the SEM, $n = 3$ (*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$).




**Supplementary Figure 9: Validation of the CNV data of the candidate genes.** Relative gene copy numbers of the 7 candidate genes were determined by RT-PCR with DNA from (**A**) A-H and A-L or (**B**) S-H and S-L cells. The data shown are the average values from three independent experiments ± SEM. The relative gene copy numbers of A-L/S-L cells were set as 1 and those of A-H/S-H cells were normalized to these values (For each relative copy number of each gene, $P < 0.05$; data not shown).




**Supplementary Figure 10: Reproduction of data from the TCGA database with the cBioPortal tool.** The graphs indicated the association of amplifications of candidate genes *DDB1*, *PFPR19* and *BCAT1* with gene expression and patient outcomes.

**Supplementary Table 1: Primers used for qPCR validation of CNVs**

| Primer name | Forward | Reverse | Amplicon/bp |
|---|---|---|---|
| RNase P-2 | TTTTACTCCCACAGCACCTCC | GGAATTTGGAGTGACCAAAGG | 105 |
| **A-H-specific gain** | | | |
| 11q12.2 | AAAAGCGCTCCATCTCCCAA | CTGGGTTGGGACAGAGTCAA | 140 |
| 12p13.1 | CAGCTTGCCCGAGTTCTACT | TGTCCTCAGAGTTAGCCGGA | 137 |
| 12p12.1 | CACAAAGAAAGCCCTCCCCA | CTGTGTTTCTCCCTTCTCAGGAT | 150 |
| 19q13.32 | GTCGCCAGAAATGGATGTGC | CTCTTCTTGGTGGTGGACGG | 147 |
| **A-H-specific loss** | | | |
| 4q25 | TATGGAACCTTCGCCTTCCTC | ATCAGCTTGTCCTGGGAATCA | 150 |
| 5q21.3 | GAACATGTATCGATTCGAGGGC | AAGCACCTACCTTAGGAATAGGATT | 134 |
| 5q22.2 | TCCCTGGAGTAAAACTGCGG | ACCCATAGGAACAGGACTGC | 104 |
| 5q31.2 | GAGGAATGCTGGCAATGAACA | TCCCCAAATCATGACTGTACCTG | 117 |
| 5q33.3 | GGAATTGGGGCAGAAATGAGC | TCACCATGGGAAAACGTCACT | 117 |
| 9q34.12 | GGAGATCTGCCTGAAGCTGG | CTACTCAGCCCAACCCGTG | 107 |
| 9q34.3 | AGTGCCTCTGACCACACTTC | TTCCCCAAGGGACCCTAGAA | 145 |
| 9q22.33 | TGGCTGTTAGGCTATTGGCTT | TGGCACACTACACACAGCAT | 131 |
| **A-L-specific gain** | | | |
| 2q32.3 | TGCCTTCCCACAGAGTTAAAAGA | TTATGTTTCACCCACCTGAGCC | 147 |
| 2q32.2 | ACACCATTGGTCTCGTGTTCT | ACCTGTCACTAGGCAGCATT | 129 |
| 15q25.1 | TGTTCTGGCAGTGTCTACGG | AGGTCCAAGCAAAACGTCCA | 124 |
| **S-H-specific gain** | | | |
| 11q12.1 | GCGAATACCATGTGACTC | ATCGGGCTTCTATCTACC | 165 |
| 12p13.1 | CAGCTTGCCCGAGTTCTACT | TGTCCTCAGAGTTAGCCGGA | 137 |
| 12p12.1 | CACAAAGAAAGCCCTCCCCA | CTGTGTTTCTCCCTTCTCAGGAT | 150 |
| 19q13.32 | GTCTCCAGAGTATCCCTGTCA | AGAGCAAGACTCCGTTCC | 179 |
| **S-H-specific loss** | | | |
| 8p23.3 | ACCTTTCTGCTGCGTGAC | GGACCTGAGTTTGATTTG | 158 |
| 17p13.1 | ATCTTATAGTTGCCTGTGGG | CTTCAATCTTAATTTGGGTG | 199 |
| **S-L-Specific gain** | | | |
| 2p14 | GTATCCCTTCTCCTCGTTTG | TCAGGACTGCTGTCACCC | 155 |
| 3p21.31 | TACAGGAAACCCATAGAA | ATAACCGTCTGAAACTCA | 116 |
| 10q24.32 | AGGTGTAAGGGCAAGAGTG | GAGTCAGCGAAGGCGATA | 117 |
| 10q26.3 | CCCTGATGCTTCCTGTTC | CACAAGTTTCCTCGATTACTG | 118 |
| 15q11.2 | AGTGAGCCTAGATTACACC | TATACTTGGCACCTTTGT | 182 |
| 15q15.2 | AGGGAAGCCACGACTCTG | TTCGGTCTTCACAACAATCC | 216 |
| 15q22.31 | GAGTGGCGGCTGATTGTT | TGGTGCTGGACTTCGTTC | 168 |
| **S-L-Specific loss** | | | |
| 8p12 | GCGGTGCGGTAAGTTCCT | CAGTGGCAAAGCCTAAGACAA | 212 |
| 8p11.23 | CTCGGCTGCCATCCTCCT | CCACCACCACCTTCCACA | 242 |
| **Candidate genes** | | | |
| *DDB1* | ACAGGGACAGCAGGTGGT | CTGCCTCTTCAGGATACACC | 246 |
| *ERCC1* | AAGGCACTGTCCATTCATC | GGAGGGTAGCAGCAGATT | 102 |
| *ERCC2* | GGTTTGAAGAGTGGTTGG | CTCATAGAATCGGCAGTG | 177 |
| *PRPF19* | CCAAGGGCTGACTCCACT | GAGACGGGCAATGACACG | 185 |
| *BCAT1* | CACTTCCAGCTTTCCCTT | GTACCAGAGCCAAACATC | 234 |
| *CDKN1B* | GAATAAGGAAGCGACCTG | AATCAGAATACGCCGAAA | 122 |
| *MARK4* | CCAGAAGGTCAAGCCACT | AACGCTCAGAAGAACAAA | 197 |

**Supplementary Table 2: Primers used for mRNA expression of the selected genes**

| Gene | Forward | Reverse | Amplicon/bp |
|---|---|---|---|
| MS4A2 | GGCCTATATCCACATCCACAGT | TACCAAGTCCCAGAATGGTGA | 116 |
| CD5 | TGACCTGCTTAGAACCCCAGA | GCTGCCGCTGTAGAACTCC | 152 |
| ERCC1 | CTACGCCGAATATGCCATCTC | GTACGGGATTGCCCCTCTG | 161 |
| DDB1 | ACCGGACACTTTACTTCGGC | TCGGCGGTGACCACATAGA | 83 |
| ERCC2 | AGAAGGTGATTGAAGAGCTTCG | ACCTCAGGGTGAATACACAAGT | 121 |
| BCAT1 | AGCCCTGCTCTTTGTACTCTT | CCAGGCTCTTACATACTTGGGA | 103 |
| PRPF19 | CGAGAAGTACATTGCGGAGAAT | GCTGGTACAGAGCGTGTGAC | 239 |
| IFLTD1 | TCCGAAAGCGTGTGTTTCAGT | CTTGAGCTTGTTCCACCTGAT | 108 |
| CDKN1B | TAATTGGGGCTCCGGCTAACT | TGCAGGTCGCTTCCTTATTCC | 116 |
| MARK4 | TGAAGGGCCTAAACCACCC | CCAGCACTTGCGTACTCCA | 94 |
| TRAPPC6A | CTGGAGGGTATGGGGTTCC | TTGTCTTGCAGGACGTAGGTC | 185 |
| EXOC3L2 | CGTGTGGAGCGATTCCATGA | GGCGATGGTCTTGCTGATATAG | 75 |
| CKM | ATGCCATTCGGTAACACCCAC | GCTTCTTGTAGAGTTCAAGGGTC | 124 |
| KLC3 | GCCACGCTCAACAACTTGG | CGCTCCACGTCCTCAAACTT | 188 |
| PPP1R13L | TGCAACGACACAGTCATCTG | GCCCCATACTCTGCTCGAC | 163 |
| CD3EAP | AGATACGGAGCTGTGGCTTAT | CCCATTGAAGCATTCTGGGG | 61 |
| FOSB | GCTGCAAGATCCCCTACGAAG | ACGAAGAAGTGTACGAAGGGTT | 249 |
| RTN2 | TCAGGGTTTACCGCAAAGTG | CTTGAGGGAATCCACGAGGTC | 200 |
| PPM1N | CGAGCGTTGGGCGACTTTA | CAGGAGCATGAACTCGTCCTC | 123 |
| VASP | ATGGCAACAAGCGATGGCT | CGATGGCACAGTTGATGACCA | 147 |
| OPA3 | CCTATGGCGAAGCTGCTATAC | GGCGGCCTCCTTAATACGG | 75 |
| BCL2L14 | CAAAATCCTCGCCTACTACACC | GACTCATTTGCTGAACAATTCCC | 126 |
| LRP6 | TGTCAGCGAAGAAGCCATTAAA | TCTAAGGCAATAGCTCTGGGT | 231 |
| MANSC1 | CACTAAGGCTGTCTGCTAGTCA | TCGAGTGTCGAAGATCATCAAGT | 203 |
| DUSP16 | CCTGACTTTATCCCCGAGTCT | GAGATCCCAGCTAAACAGTGC | 155 |
| CREBL2 | AGTAAAGAAGCCCGGTAAACG | CGACTGGATACCAACTCTTCCAA | 144 |
| GPR19 | ATCTTCGGCAATTCCCTGGTT | GCAACGCTGATGAGAAGGTC | 113 |
| ST8SIA1 | GTCCTCTGTTGGCTCTACATCT | CCCCGTCATACCACATGCTC | 214 |
| PPP1R37 | CTCGACTGTCTGGACCTGAAA | GCCGAGGCACCATCTTCATC | 134 |
| MS4A8 | GGCTTGAACATCGTCAGT | GCGTAAGGATAATAGTCG | 107 |
| MS4A15 | TGTGGACAGGGGCTATCTGG | GAAGTGCATGGCAATGACCG | 79 |
| GAPDH | TGTTGCCATCAATGACCCCTT | CTCCACGACGTACTCAGCG | 102 |

**Supplementary Table 3: Details about primary antibodies used for Western blotting**

| Genes | Antibodies | Catalog No. | Supplier |
|---|---|---|---|
| MS4A2 | Rabbit polyclonal to MS4A2 | ab203747 | ABCAM, Inc. UK |
| CD5 | Anti-CD5 (26-40) antibody produced in rabbit | SAB1104201 | Sigma-Aldrich, USA |
| ERCC1 | Anti-ERCC1 antibody produced in rabbit | SAB4500795 | Sigma-Aldrich, USA |
| DDB1 | Anti-DDB1 antibody produced in mouse | SAB1405702 | Sigma-Aldrich, USA |
| ERCC2 | Monoclonal Anti-ERCC2 antibody produced in mouse | WH0002068M1 | Sigma-Aldrich, USA |
| BCAT1 | rabbit BCAT1 Antibody | 12822 | CST, USA |
| PRPF19 | Anti-PRPF19 antibody produced in rabbit | SAB4501215 | Sigma-Aldrich, USA |
| IFLTD1 | ANTI-IFLTD1 (C-TERM) antibody produced in rabbit | SAB1303298 | Sigma-Aldrich, USA |
| CDKN1B | p27 Kip1 (D69C12) XP® Rabbit mAb | 3686 | CST, USA |
| MARK4 | rabbit MARK4 Antibody | 4834 | CST, USA |
| TRAPPC6A | Anti-TRAPPC6A antibody produced in rabbit | HPA043043 | Sigma-Aldrich, USA |
| EXOC3L2 | ANTI-EXOC3L2 (N-TERM) antibody produced in rabbit | SAB1302947 | Sigma-Aldrich, USA |
| CKM | Anti-CKM (166-180) antibody produced in rabbit | C1869 | Sigma-Aldrich, USA |
| KLC3 | Anti-KLC3 antibody | ab168242 | ABCAM, Inc. UK |
| PPP1R13L | Anti-iASPP antibody | ab34898 | ABCAM, Inc. UK |
| CD3EAP | Anti-PAF49 antibody | ab76933 | ABCAM, Inc. UK |
| FOSB | FosB (5G4) Rabbit mAb | 2251 | CST, USA |
| RTN2 | Anti-Reticulon 2 antibody | ab103170 | ABCAM, Inc. UK |
| PPM1N | Anti-PPM1N antibody | ab168300 | ABCAM, Inc. UK |
| VASP | VASP (9A2) Rabbit mAb | 3132 | CST, USA |
| OPA3 | Anti-OPA3 antibody | ab69163 | ABCAM, Inc. UK |
| BCL2L14 | Anti-Bcl G antibody | ab115467 | ABCAM, Inc. UK |
| LRP6 | LRP6 (C5C7) Rabbit mAb | 2560 | CST, USA |
| MANSC1 | Anti-MANSC1 antibody produced in rabbit | HPA007955 | Sigma-Aldrich, USA |
| DUSP16 | Anti-DUSP16, C-Terminal antibody produced in rabbit | SAB4503215 | Sigma-Aldrich, USA |
| CREBL2 | Anti-CREBL2 (N-term K27) antibody produced in rabbit | SAB1300866 | Sigma-Aldrich, USA |
| GPR19 | Anti-GPR19 antibody produced in rabbit | SAB2900709 | Sigma-Aldrich, USA |
| ST8SIA1 | Anti-ST8SIA1 antibody | ab37806 | ABCAM, Inc. UK |
| PPP1R37 | Anti-PPP1R37 antibody produced in rabbit | HPA041500 | Sigma-Aldrich, USA |
| MS4A8 | Anti-MS4A8 antibody produced in rabbit | HPA007318 | Sigma-Aldrich, USA |
| MS4A15 | Mouse polyclonal to MS4A15 | ab72697 | ABCAM, Inc. UK |

**Supplementary Table 4: Shared and specific CNV regions for A-H and A-L.**
See Supplementary_Table_4

**Supplementary Table 5: Functional enrichment of genes in A-H- or A-L-specific CNV regions.**
See Supplementary_Table_5

**Supplementary Table 6: Shared and specific CNV regions for S-H and S-L .**
See Supplementary_Table_6

**Supplementary Table 7: Functional enrichment of genes in S-H- or S-L-specific CNV regions.**
See Supplementary_Table_7

**Supplementary Table 8: Analysis of genes identified in CNV regions shared by both highly and minimally invasive/migratory subclones and further amplified in one subclone.**
See Supplementary_Table_8

**Supplementary Table 9: Functional enrichment of genes identified in CNV regions in both highly or both minimally invasive/migratory subclones.** See Supplementary_Table_9

**Supplementary Table 10: Analysis of genes amplified in both highly invasive/migratory subclones in the TCGA ovarian cancer database (TCGA, Provisional) with the cBioPortal tool.**
See Supplementary_Table_10