Deciphering Promoter Hypermethylation of Genes Encoding for RASSF/Hippo Pathway Reveals the Poor Prognostic Factor of RASSF2 Gene Silencing in Colon Cancers

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Table S1. List of primers used for MS-PCR for methylated (M) and non-methylated (U) genes.

| RASSF1A	Primer sequences	Tm(°C)
U	F: 5'-TTTGGTTGGAGTGTGTTAATGTG-3' R: 5'-CAAACCCCACAAACTAAAAACAA-3'	60
M	F: 5'-GTGTTAACGCGTTGCGTATC-3' R: 5'-AACCCCGCGAACTAAAAACGA-3'	62
RASSF2		
U	F: 5'-AGTTTGTTGTTGTTTTTTAGGTGG-3' R: 5'- AAAAAACCAACAACCCCCACA-3'	63
M	F: 5'-GTTCGTCGTCGTTTTTTAGGCG-3' R: 5'- AAAAACCAACGACCCCCGCG-3'	63
STK4		
U	F: 5'-TTTGTGGGGTGGGTTTAGGAGGTTTGT-3' R: 5'-AACCAATAACCCCTCACCAACACAACAA-3'	63
M	F: 5'-GCGGGGCGGGTTTAGGAGGTTC-3' R: 5'-CCAATAACCCCTCACCGACGC-3'	63
STK3		
U	F: 5'-TTTTAAGTGGGAGGGAGATTTGTTGTGG-3' R: 5'-AAAAACCAAAACACCAACCAACCAAACC-3'	61
M	F: 5'-CGGGAGGGAGATTCGTCGCG-3' R: 5'-AAACCGAAACACCGACCGACCG-3'	63
LATS1		
U	F: 5'-TGAATGATTAGAGTTGTGGGTGATGT-3' R: 5'-AAACATTTCCCAACATCACTTACACA-3'	60
M	F: 5'-GAACGATTAGAGTTGCGGGCGAC-3' R: 5'-AACATTTCCCGACGTCGCTTACG-3'	62
LATS2		
U	F: 5'-GGTGTTTTGTTTGGATTGGTATGTGGTT-3' R: 5'-CATCTTCCCAAAACACTCACACCACA-3'	60
M	F: 5'-TTCGTTCGGATTGGTATGCGGTC-3' R: 5'-CCATCTTCCCGAAACGCTCACG-3'	60
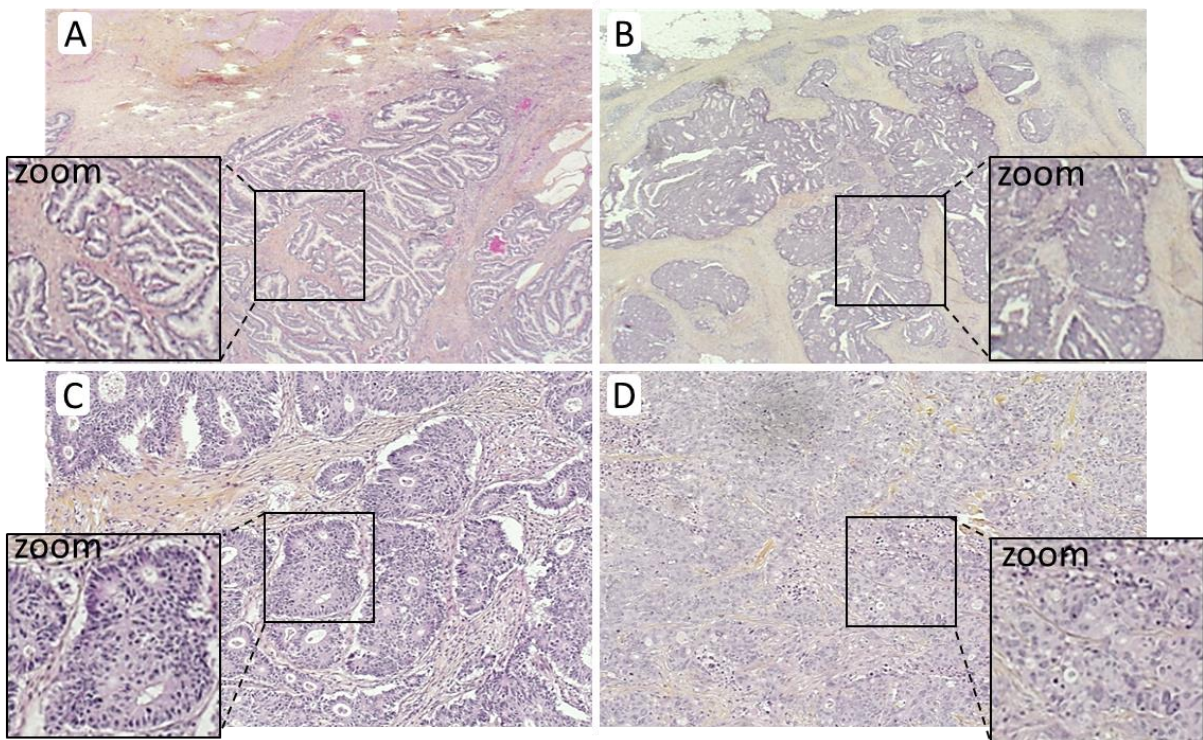
Figure S1. Illustrations of the different grades of differentiation of adenocarcinomas. (**A**) well differentiated adenocarcinoma: well individualized glandular structures, more or less branched (HES ×20). (**B** and **C**) Moderately differentiated adenocarcinoma: glandular structures of cribriform appearance, appearance of fused glands (HES ×20 and ×100). (**D**) Poorly differentiated adenocarcinoma: formation of clumps, with rare persistent glandular lumens (HES ×100).
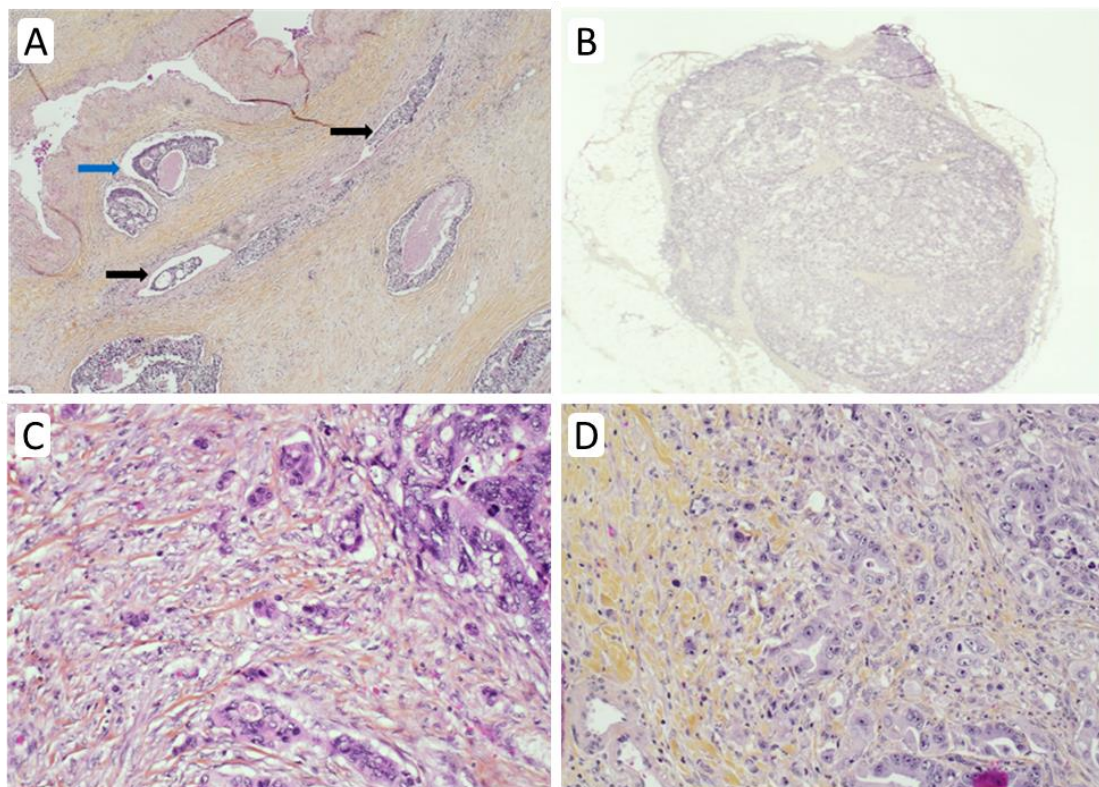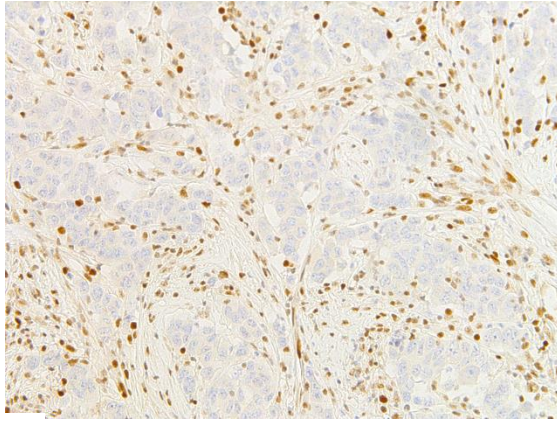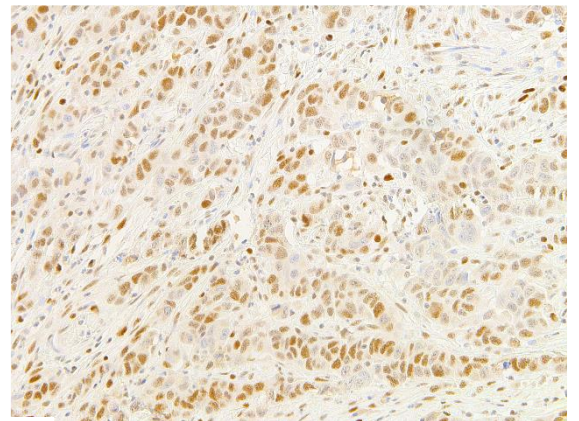
Figure S2. Illustration of the histological criteria of the cohort. (**A**) Tumor venous and lymphatic emboli. Tumor cell clusters are located in lymphatics (blue arrow), as well as in venous (black arrows) (HES ×50). (**B**) Illustration of a tumor deposit located in the mesocolon without identifiable lymph node parenchyma (HES ×20). (**C**) Illustration of grade 2 budding: a few isolated cells or clusters of less than 5 cells are seen detaching from the tumor glands (HES ×200). (**D**) Grade 3 budding: many single cells or clusters of less than 5 cells are detached from the tumor glands (HES ×200).

A

B

Figure S3. Illustration of deficient MMR (dMMR) status. (**A**) Example of a loss of MLH1 expression in favor of a dMMR-IHC (×20). (**B**) The same specimen with expression of MSH2 (×20).
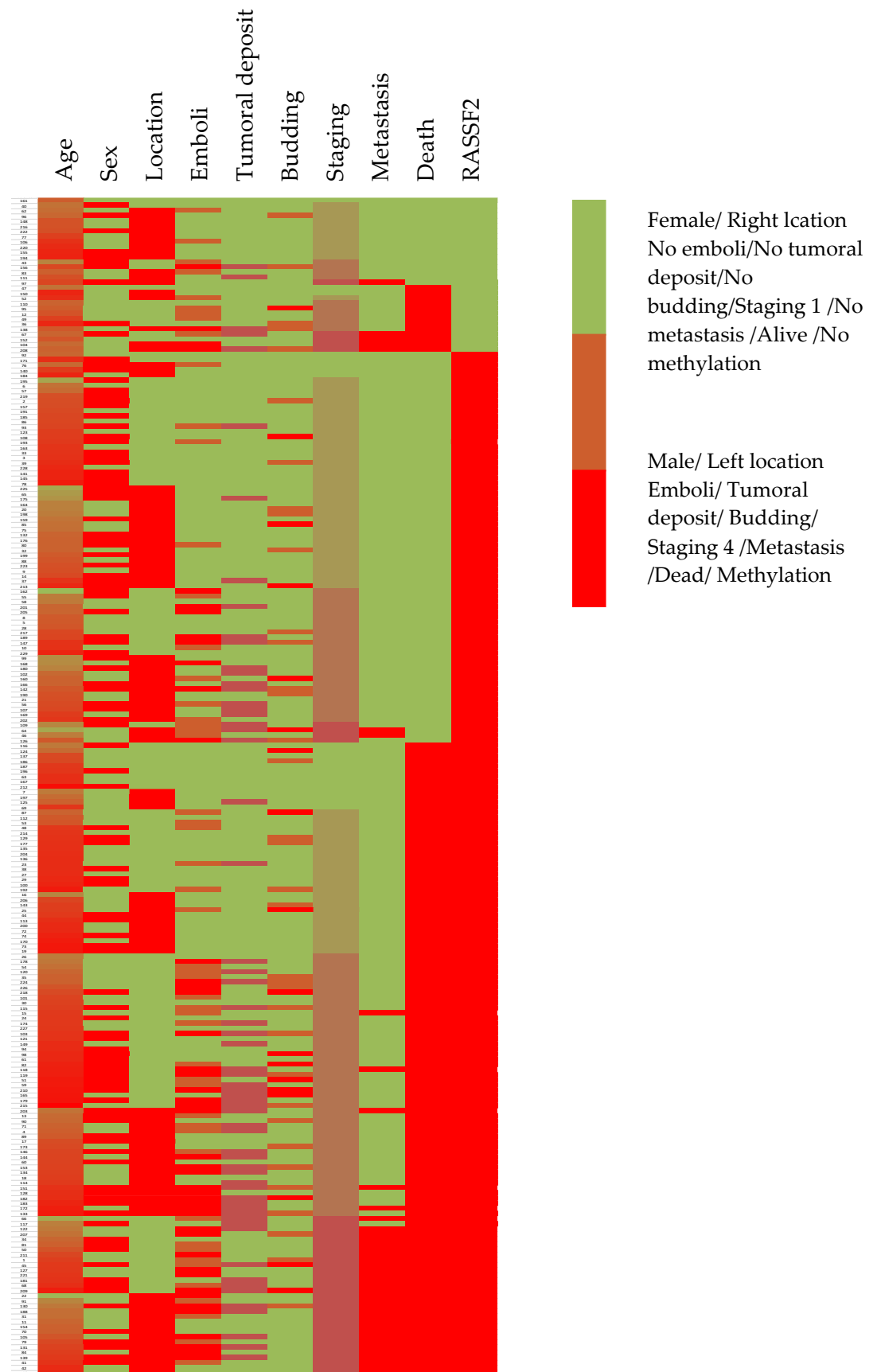
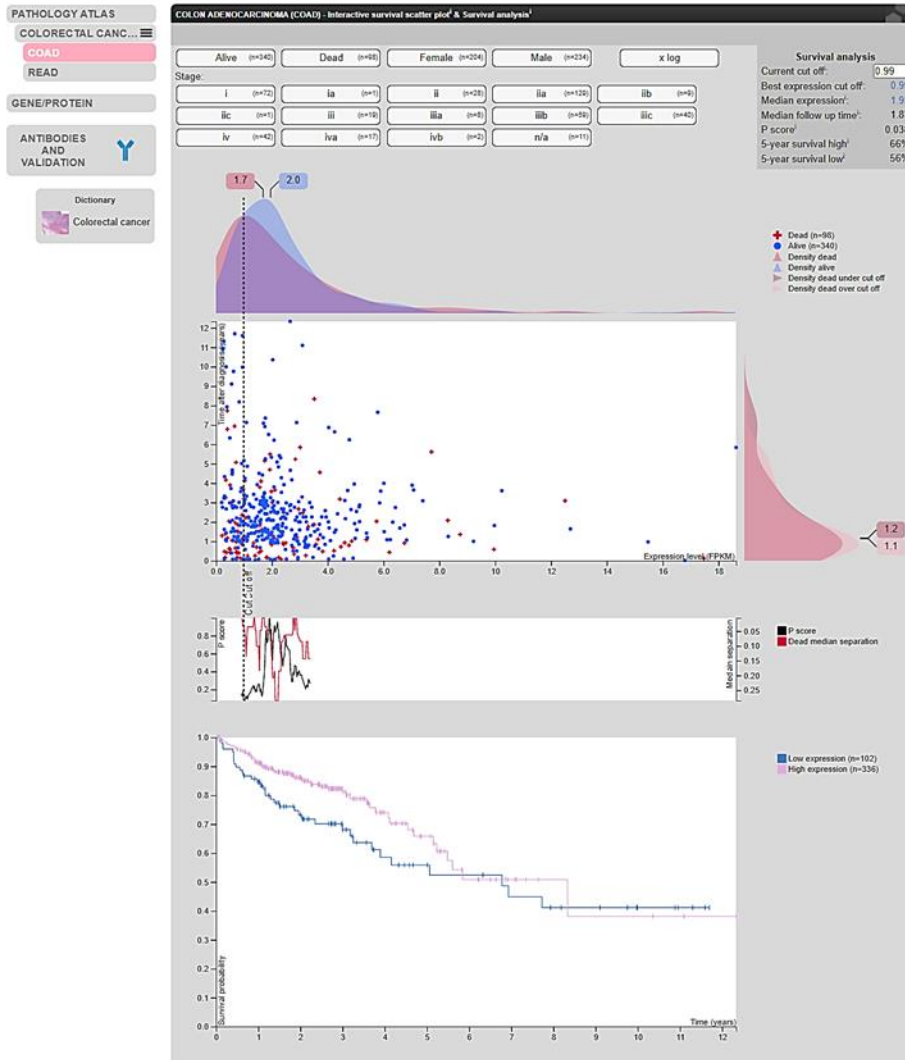**Figure S4.**: Heat map of clinical and histological data of the cohort.

Figure S5: Low RASSF2 mRNA expression predicts significant worse overall survival in 438 patients with colon cancer from the Table 336. or weakly (*n* = 102) expresses the RASSF2 messenger. Survival analysis reveals that the low RASSF2 mRNA expression predicts significant worse overall survival in 438 patients with colon cancer (P score: 0.038, Expression of RASSF2 in colorectal cancer—The Human Protein Atlas).