Germline polymorphisms in genes involved in the Hippo pathway as recurrence biomarkers in stages II/III colon cancer

The Hippo pathway regulates tissue growth and cell fate. In colon cancer, Hippo pathway deregulation promotes cellular quiescence and resistance to 5-Fluorouracil (5-Fu). In this study, 14 polymorphisms in 8 genes involved in the Hippo pathway (MST1, MST2, LATS1, LATS2, YAP, TAZ, FAT4 and RASSF1A) were evaluated as recurrence predictors in 194 patients with stages II/III colon cancer treated with 5-Fu-based adjuvant chemotherapy. Patients with a RASSF1A rs2236947 AA genotype had higher 3-year recurrence rate than patients with CA/CC genotypes (28 vs 33%, hazard ratio (HR): 1.87; P = 0.07). In left-sided tumors, this association was stronger (HR: 0.29; P = 0.0111) and a similar trend was found in an independent Japanese cohort. These promising results reveal polymorphisms in the Hippo pathway as biomarkers for stages II and III colon cancer.

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INTRODUCTION
Tumor recurrence following resection of stages II and III colon cancer occurs in approximately 25–40% of the patients.1 Adjuvant chemotherapy with 5-Fluorouracil (5-Fu) reduces the risk of recurrence,2 and the addition of oxaliplatin to 5-Fu can further decrease this risk in stage III colon cancer patients.3 However, in current practice the majority of patients does not benefit from adjuvant chemotherapy and will relapse despite treatment. The underlying mechanisms of tumor recurrence after curative treatment are not fully understood. Several processes have been proposed to influence tumor relapse and promote chemotherapy resistance such as the presence of cancer stem cells or the epithelial–mesenchymal transition (EMT) process.4,5 Disruption of the Salvador-Warts-Hippo pathway, commonly known as the Hippo pathway, is the newest contributor to these recurrence mechanisms. The Hippo pathway is a highly evolutionary conserved pathway, whose main physiological function is to control tissue growth and hence organ size.6,7 The core signaling consists of several kinases, STE20-like kinases 1 and 2 (MST1 and MST2), large tumor suppressors 1 and 2 (LATS1 and LATS2) and the adaptor proteins MOB kinase activator 1A and 1B (MOB1A and MOB1B). Together, these proteins facilitate the phosphorylation of homologous oncoproteins Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). Phosphorylation of YAP/TAZ leads to their accumulation in the cytoplasm and stimulates their proteosomal degradation.8 Inactivation of this cascade results in YAP/TAZ nuclear translocation. In the nucleus, YAP/TAZ exert their function by activating transcription factors such as SMAD1-3 and TEAD1-4 that induce the transcription of multiple target genes. Among others, these target genes include Axin2, Birc5, Myc, Ctgf and β2-integrin, which are involved in stem cell maintenance, EMT, metastasis development and regulation of microRNA biogenesis.9,10 The upstream regulation of the Hippo pathway remains poorly understood, however, several upstream branches have been described.11 One of them is the Ras-association domain 1 (RASSF1). RASSF1a is a putative tumor suppressor gene that is methylated in several tumor types including colorectal cancer.12 RASSF1a can activate Hippo signaling by protein–protein interaction by binding MST2 through its SARAH (Sav/Rassf/Hpo) domain.13 In colon cancer the Hippo effectors YAP/TAZ have been reported to contribute to 5-Fu resistance by inducing cellular quiescence,14 and their expression has been correlated with the patients’ prognosis.15–17 Furthermore, Hippo signaling is interconnected with several other pathways that are well-established major role players in the development and progression of colorectal cancer. Wnt/β-catenin pathway crosstalks with Hippo signaling through a mechanism scarcely understood. β-Catenin interacts with TAZ/YAP favoring their translocation to the nucleus, thus increasing the transcription of the Hippo targeted genes.18 Other colon cancer-associated pathways that engage in regulatory crosstalk with the Hippo signaling include among other transforming growth factor β, Hedgehog and Notch pathways.20

Based on the importance of Hippo signaling in processes possibly implicated in colon cancer recurrence, this work was designed to evaluate the potential role as prognostic biomarkers of single-nucleotide polymorphisms (SNPs) in genes involved in the Hippo pathway, in patients with resected stages II and III colon cancer.

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MATERIALS AND METHODS

Eligible patients
A total of 194 patients with high-risk stages II and III colon cancer were included. Patients with stage II were classified as high risk if they presented at a median overall survival of this study was time to recurrence (TTR) that was defined as a period from the date of diagnosis to the date of first documented tumor recurrence. TTR was censored at the time of last follow-up or death if patients remained recurrence free. With samples from 194 patients available (79 events) for genotyping the selected SNPs, this study had 80% power to detect a hazard ratio (HR) of 1.89 – 2.13 in a recessive model with a minor frequency of 0.25 – 0.45 using a two-sided log-rank test at a significance level of 0.05.

Deviations from the Hardy–Weinberg equilibrium were tested using χ² test. The association between the allelic distribution of the SNPs and their potential association with the baselines characteristics was examined using χ² or Fisher’s exact test. The true inheritance mode of the analyzed polymorphisms is unknown, therefore a co-dominant, dominant or recessive model was assumed wherever appropriate. The association of the SNPs and TTR was analyzed using Kaplan–Meier curves and log-rank test. In the multivariable Cox regression analysis, the model was adjusted by stage, type of adjuvant chemotherapy and stratified by race. Recursive partitioning (RP) analysis was conducted to explore patterns of recurrence by SNPs of Hippo pathway.

All calculations were performed using SAS statistical package version 9.4 (SAS Institute, Cary, NC, USA) and R package version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-sided at a significance level of 0.05.

RESULTS

The median follow-up of the USC cohort was 4.4 years (range 0.4–16.8 years) and the 3-year recurrence rate was 36% (±4% standard error, s.e.). The median overall survival for this cohort has not yet been reached.

The median follow-up of the Japanese cohort was 5 years (range 0.3–8.6) and the 3-year recurrence rate was 29% (±2% s.e.). The median overall survival of this series has not been reached.

| Table 1. Baseline characteristics and treatment of USC and Japanese cohorts |
|-----------------------------|-----------------------------|-----------------------------|
| USC (n = 194)               | Japanese (n = 350)          |
| Age, years                 |                              |
| 55–64                      | 70                          | 70                          | 20.0 0.001 |
| 65                         | 55                          | 28.4                        | 51.1 0.001 |
| Sex                        |                              |
| Female                     | 88                          | 175                         | 50.0 0.30  |
| Male                       | 106                         | 175                         | 50.0 0.30  |
| Stage                      |                              |
| II                         | 85                          | 43.8                        | 0       |
| III                        | 109                         | 56.2                        | 229     | 65.4 0.001 |
| IIIIC                      | 121                         |                              | 34.6    |
| N of resected lymph nodes  |                              |
| ≤ 12                       | 61                          | 31.4                        | 47      | 13.5 0.001 |
| > 12                       | 115                         | 59.3                        | 302     | 66.5 0.001 |
| Tumor side                 |                              |
| Right                      | 95                          | 49.0                        | 110     | 31.4  |
| Left                       | 92                          | 47.4                        | 238     | 68.0  |
| Left and right             | 2                            | 1.0                         | 2       | 0.6 0.001 |
| Missing                    | 5                            | 2.6                         |         |
| Adjuvant treatment         |                              |
| Fluoropyrimidines          | 129                         | 66.5                        | 206     | 58.9  |
| 5-FU/LV/Oxaliplatin        | 48                          | 24.7                        | 68      | 19.4  |
| 5-FU/LV/Irinotecan         | 17                          | 8.8                         | 0       | 0     |
| None                       | 76                          | 25.0                        |         | 21.7  |
| Ethnicity                  |                              |
| Asian                      | 27                          | 13.9                        | 350     | 100.0  |
| African American           | 13                          | 6.7                         | NA      |
| Caucasian                  | 108                         | 55.7                        |         | 0     |
| Hispanic                   | 46                          | 23.7                        |         |

Abbreviations: 5-FU, 5-Fluorouracil; LV, leucovorin; USC, University of Southern California. *Forty patients were excluded from the original cohort due to depletion of DNA specimen. **Based on χ² test and excluded patients with missing characteristics. Genotypes were achieved in at least 90% of the analyzed samples for each polymorphism. In failed cases, genotyping was not successful due to low quality of DNA or limited DNA quantity. All the analyzed SNPs but one (rs95522315) were within the probability limits of Hardy–Weinberg equilibrium.
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Based on tumor location, TAZ rs3811715 correlated strongly with the 3-year recurrence probability in patients with left-sided tumors. The genotype frequencies in this subgroup were CC = 55, CT = 28 and TT = 6. Patients harboring a CT or TT genotype had a 10% (±5% s.e.) 3-year recurrence probability whereas patients harboring a CC genotype had 48% (±7% s.e.) (HR: 0.25; 95% CI, 0.10–0.60; P = 0.001). Patients with a TT genotype (n = 6) had no recurrence. This association remained significant after adjusting for the relevant clinical parameters (HR: 0.29; 95% CI, 0.11–0.78; P = 0.011).

In the Japanese exploratory cohort in patients bearing left-sided tumors, patients carrying a TAZ rs3811715 TT genotype (TT = 14, CT = 78, CC = 129) had 7% (±7% s.e.) 3-year recurrence rate compared with 27% (±3% s.e.) for patients with at least a C genotype (HR: 0.21; 95% CI, 0.03–1.54; P = 0.091).

Additionally, in left-sided tumors a polymorphism located in MST1, rs17420378, was associated with the recurrence probability. Patients with a GA or AA genotypes had a higher recurrence probability than patients with a GG genotype (HR: 2.31; 95% CI, 1.21–4.43; P = 0.009). However, in multivariable analysis this association was not maintained (HR: 2.01; 95% CI, 0.98–4.10; P = 0.057). This polymorphism was not tested in the Japanese cohort, as the reported MAF is < 10%.

Based on gender, the association of TAZ rs3811715 with the 3-year recurrence rate was stronger in the female population (HR: 0.46; 95% CI, 0.22–0.96; P = 0.031), although this association did not retain significance in the multivariable analysis (P = 0.06) (Table 4).

RP analysis
RP analysis was applied to construct a decision tree as a model to classify patients according to their 3-year recurrence risk (Figure 1).

In the overall population, four terminal nodes arose showing significantly different 3-year recurrence probabilities ranging from 12% (±6% s.e.) for patients in node 1 to 56% (±9% s.e.) for patients allocated in node 4. The initial split was due to Rassf1a rs2236947, indicating that this SNP was the main contributor to the variation in the recurrence probability rate, followed by TAZ rs3811715 and FAT4 rs1039808 (Figure 1).

RP analysis also confirmed the influence of tumor location and revealed different patterns for patients bearing left- or right-sided tumors. For patients with right colon carcinomas, Rassf1a rs2236947 remained the most important polymorphism to predict recurrence probability followed by YAP rs8504 and LTS5 rs9552315, whereas for patients with left-sided tumors TAZ rs3811715 was responsible for the tree’s initial split (Figure 2).

DISCUSSION
The present study identifies polymorphisms within genes involved in the Hippo pathway as predictors of recurrence in patients with high-risk stage II and stage III colon cancer treated with adjuvant 5-Fu-based chemotherapy. Moreover, our data suggest that the value of these polymorphisms as biomarkers for localized colon cancer is influenced by tumor location and gender.

The Hippo signaling pathway has gained notoriety over the past few years. Despite this increasing interest, to our knowledge, polymorphisms located in genes involved in this pathway had never been evaluated as biomarkers for colon cancer. As an emerging cascade involved in cancer, in Hippo signaling neither the upstream regulators nor the downstream effectors are fully understood. One of the upstream regulators is Rassf1a, a tumor suppressor that is frequently methylated in colon cancer and that can activate Hippo signaling by binding to MST and ultimately promote apoptosis through p53. In this work, the Rassf1a rs2236947 polymorphism correlated with the recurrence

Table 2. Primary information on the analyzed polymorphisms

| Gene | SNP | Location | SNP function/association | F-SNP score |
|------|-----|----------|--------------------------|-------------|
| MST1 | rs17420378 | Exon 11 | Missense | 0.533 |
|     | rs6073629 | 3’UTR | Transcriptional regulation | 0.5 |
| MST2 | rs10955176 | 3’UTR | NA | NFI |
| LATS1 | rs12174349 | 5’UTR | NA | NFI |
| LATS2 | rs558614 | Exon 4 | Missense | 0.156 |
| LATS2 | rs9552315 | 3’UTR | Transcriptional regulation | 0.5 |
| YAP | rs8504 | 3’UTR | NA | NFI |
| TAZ | rs3811715 | Intron | Splice donor | 0.242 |
| TAZ | rs6783790 | Intron | Splice donor | 0.389 |
| FAT4 | rs1014867 | Exon 17 | Missense | 0.59 |
|     | rs1039808 | Exon 1 | Missense | NFI |
|     | rs2073498 | Exon 3 | Missense | 0.5 |
| RASSF1 | rs2236947 | Intron | Transcriptional regulation | 0.268 |

Abbreviations: FAT4, atypical cadherin 4; LATS1, large tumor suppressor 1; LATS2, large tumor suppressor 2; NA, not analyzed; NFI, no functional information; NSCLC, non-small cell lung cancer; MST1, STE20-like kinase 1; MST2, STE20-like kinase 2; RASSF1, Ras-association domain 1; SNP, single-nucleotide polymorphism; TAZ, transcriptional co-activator with PDZ-binding motif; YAP1, yes associated protein.

There were significant differences in some polymorphisms in the allele frequencies across races in the USC cohort (Supplementary Table 1).

Genetic determinants and outcome
Located in the Rassf1a gene, the rs2236947 polymorphism was associated with the 3-year recurrence probability; patients homozygous for the variant A allele had a 56% (±10% s.e.) 3-year recurrence probability compared with 33% (±4%) for patients with a CC or CA genotype (HR: 1.87; 95% confidence interval (CI), 1.10–3.17; P = 0.017). In multivariable analysis, this association remained significant (HR: 1.78; 95% CI, 1.03–3.06; P = 0.039) (Table 3).

In the TAZ gene, the variant allele of the rs3811715 polymorphism was associated with a lower 3-year recurrence rate. Patients with a CT or TT genotype had a 28% (±5% s.e.) 3-year recurrence probability compared with 40% (±5% s.e.) for patients with a homozygous wild type CC genotype, although this association did not reach statistical significance (HR: 0.66; 95% CI, 0.41–1.05; P = 0.077).

No association was found in the overall population in the Japanese cohort for these two SNPs. The MAF for these polymorphisms in the Japanese cohort was 25% for both SNPs. In the Asian population included in the USC cohort, the MAF for these SNPs was 27 and 34% for Rassf1a rs2236947 and TAZ rs3811715, respectively.

Subgroup analysis by gender and tumor location
Differences were detected for the association of the analyzed SNPs and the 3-year recurrence probability based on gender and tumor location.
probability in this cohort of patients. Although no functionality is known for this SNP, in silico analysis revealed that this SNP could affect transcriptional regulation (http://compbio.cs.queensu.ca/F-SNP/).

At the center of the Hippo signaling cascade, the highly homologous YAP and TAZ are the main effectors of the pathway. When phosphorylated YAP/TAZ remain in the cytoplasm, Hippo signaling acts as a tumor suppressor pathway. In the cytoplasm, YAP/TAZ interact with β-catenin, which can lead to inhibition of Wnt signaling. Moreover, YAP/TAZ form cytoplasmic complexes with junctional proteins like Scribble or α-catenin maintaining cell polarity. Disruption of the pathway leads to increased YAP/TAZ translocation into the nucleus, which promotes tissue growth, cell viability and stem cell maintenance by regulation of different transcription factors. 12,22 Even more, loss of cell polarity due to lack of TAZ regulation has been implicated in the EMT. 23 In this work, the TAZ rs3811715 polymorphism correlated with the recurrence probability. This SNP is located intronic and prediction tools revealed that affects a splicing site leading to a frameshift coding change. 24 In our work, patients with at least a
Table 4. Subgroup analyses of Hippo SNPs and time to recurrence in patients with stage II or III colon cancer at USC

| SNP          | N   | 3-Year recurrence probability ± s.e.* | HR (95%CI)*  | P-valueb | HR (95%CI)* multivariate | P-valueb |
|--------------|-----|---------------------------------------|--------------|----------|--------------------------|----------|
| **Left-sided colon cancer** |     |                                       |              |          |                          |          |
| LATS1rs12174349 | 21  | 0.44 ± 0.11                           | 1 (reference)| 0.32     | 1 (reference)            | 0.68     |
| LATS2rs558614  | 24  | 0.41 ± 0.11                           | 1 (reference)| 0.49     | 1 (reference)            | 0.78     |
| MST1rs6073629  | 69  | 0.38 ± 0.06                           | 1 (reference)| 0.25     | 1 (reference)            | 0.35     |
| MST1rs17420378 | 19  | 0.28 ± 0.11                           | 0.62 (0.27,1.43) | 0.009   | 0.67 (0.29,1.56)        | 0.057    |
| RASSF1rs2073498 | 24  | 0.44 ± 0.11                           | 1 (reference)| 0.93     | 1 (reference)            | 0.95     |
| C/T          | 49  | 0.35 ± 0.07                           | 0.83 (0.41,1.69) | 0.26     | 1 (reference)            | 0.92     |
| C/T          | 16  | 0.30 ± 0.13                           | 0.60 (0.21,1.70) | 0.001   | 1.04 (0.43,2.57)        | 0.011    |
| T/T          | 76  | 0.34 ± 0.06                           | 1 (reference) |          |                          |          |
| A/A          | 13  | 0.54 ± 0.15                           | 1.59 (0.70,3.63) | 0.031    | 1 (reference)            | 0.072    |
| C/T or C/T   | 55  | 0.48 ± 0.07                           | 1 (reference) | 0.26     | 0.29 (0.11,0.76)        | 0.16     |
| T/T          | 34  | 0.10 ± 0.05                           | 0.25 (0.10,0.60) | 0.031    | 1 (reference)            | 0.072    |
| **Female population** |     |                                       |              |          |                          |          |
| LATS1rs104867 | 81  | 0.35 ± 0.06                           | 1 (reference) | 0.55     | 1 (reference)            | 0.37     |
| C/T          | 10  | 0.44 ± 0.17                           | 1.33 (0.52,3.42) | 0.93     | 1.51 (0.58,4.28)        | 0.73     |
| LATS1rs1039008 | 36  | 0.42 ± 0.09                           | 1 (reference) | 0.49     | 1 (reference)            | 0.68     |
| C/T          | 35  | 0.32 ± 0.08                           | 0.87 (0.42,1.79) | 0.70     | 0.31 (0.13,0.71)        | 0.32     |
| T/T          | 0.27 ± 0.12                           | 0.95 (0.39,2.30) | 0.32     | 1 (reference)            | 0.78     |
| LATS2rs558614 | 44  | 0.31 ± 0.07                           | 0.57 (0.27,1.20) | 0.49     | 0.87 (0.36,2.10)        | 0.78     |
| A/T          | 24  | 0.41 ± 0.11                           | 0.73 (0.32,1.67) | 0.83     | 0.64 (0.18,2.33)        | 0.55     |
| C/T          | 56  | 0.41 ± 0.07                           | 1 (reference) | 0.25     | 1 (reference)            | 0.35     |
| T/T          | 6   | 0.50 ± 0.25                           | 0.75 (0.18,3.16) | 0.009   | 2.01 (0.98,4.40)        | 0.057    |
| RASSF1rs2073498 | 24  | 0.44 ± 0.11                           | 1 (reference) | 0.93     | 1 (reference)            | 0.95     |
| C/A or A/A   | 49  | 0.35 ± 0.07                           | 0.83 (0.41,1.69) | 0.26     | 1 (reference)            | 0.92     |
| C/A or C/A   | 16  | 0.30 ± 0.13                           | 0.60 (0.21,1.70) | 0.001   | 1.04 (0.43,2.57)        | 0.011    |
| C/C          | 72  | 0.38 ± 0.06                           | 1 (reference) | 0.26     | 1 (reference)            | 0.77     |
| C/A or A/A   | 19  | 0.29 ± 0.11                           | 0.62 (0.27,1.43) | 0.031    | 1 (reference)            | 0.072    |
| C/C or C/A   | 76  | 0.34 ± 0.06                           | 1 (reference) | 0.65     | 1 (reference)            | 0.072    |
| A/C or A/C   | 13  | 0.54 ± 0.15                           | 1.59 (0.70,3.63) | 0.031    | 1 (reference)            | 0.072    |
| A/C or A/C   | 42  | 0.37 ± 0.08                           | 1.10 (0.52,2.34) | 0.65     | 1 (reference)            | 0.072    |
| C/C or C/C   | 14  | 0.36 ± 0.13                           | 1.50 (0.60,3.72) | 0.66     | 1.32 (0.49,3.57)        | 0.74     |
| A/C or A/C   | 82  | 0.35 ± 0.06                           | 1 (reference) | 0.66     | 1 (reference)            | 0.67     |
| C/T          | 4   | 0.25 ± 0.22                           | 0.64 (0.09,4.72) | 0.30     | 0.64 (0.09,4.85)        | 0.08     |
| LATS1rs12174349 | 46  | 0.33 ± 0.07                           | 1 (reference) | 0.95     | 1 (reference)            | 0.95     |
| C/T          | 32  | 0.35 ± 0.09                           | 1.03 (0.48,2.18) | 0.33     | 1 (reference)            | 0.33     |
| T/T          | 10  | 0.42 ± 0.16                           | 2.09 (0.77,5.71) | 0.49     | 1 (reference)            | 0.50     |
| RASSF1rs2073498 | 21  | 0.30 ± 0.10                           | 1 (reference) | 0.047    | 1 (reference)            | 0.14     |
| G/A or C/A   | 35  | 0.33 ± 0.08                           | 0.81 (0.33,1.99) | 0.047    | 1 (reference)            | 0.14     |
| A/A or A/A   | 29  | 0.42 ± 0.11                           | 1.31 (0.54,3.17) | 0.65     | 1.00 (0.43,2.85)        | 0.74     |
| LATS2rs558614 | 38  | 0.39 ± 0.08                           | 1 (reference) | 0.66     | 1 (reference)            | 0.67     |
| C/T          | 40  | 0.35 ± 0.08                           | 0.84 (0.42,1.70) | 0.33     | 1 (reference)            | 0.33     |
| G/A or C/A   | 10  | 0.21 ± 0.13                           | 0.43 (0.10,1.85) | 0.44     | 1 (reference)            | 0.44     |
| LATS1rs1039008 | 58  | 0.42 ± 0.07                           | 1 (reference) | 0.047    | 1 (reference)            | 0.14     |
### Table 4. (Continued)

| Phenotype       | N  | 3-Year recurrence probability ± s.e. | HR (95%CI) | P-value | HR (95%CI) | P-value |
|-----------------|----|-------------------------------------|------------|---------|------------|---------|
| MST2rs10955176  |    |                                     | 0.34       | 0.67    |             |         |
| C/C             | 20 | 0.36 ± 0.11                         | 1 (reference) |        | 1 (reference) |         |
| C/T             | 49 | 0.40 ± 0.08                         | 1.04 (0.47,2.27) |        | 1.18 (0.51,2.71) |         |
| T/T             | 18 | 0.24 ± 0.11                         | 0.48 (0.15,1.56) |        | 0.73 (0.21,2.53) |         |
| RASSF1rs2073498 |    |                                     | 0.96       | 0.79    |             |         |
| C/C             | 71 | 0.33 ± 0.06                         | 1 (reference) |        | 1 (reference) |         |
| C/A or A/A      | 13 | 0.41 ± 0.14                         | 1.03 (0.39,2.68) |        | 1.15 (0.41,3.22) |         |
| RASSF1rs2236947 |    |                                     | 0.17       | 0.26    |             |         |
| C/C or C/A      | 73 | 0.32 ± 0.06                         | 1 (reference) |        | 1 (reference) |         |
| A/A             | 13 | 0.58 ± 0.14                         | 1.77 (0.76,4.11) |        | 1.66 (0.69,4.00) |         |
| TAZrs3811715    |    |                                     | 0.031      | 0.060   |             |         |
| C/C             | 48 | 0.43 ± 0.08                         | 1 (reference) |        | 1 (reference) |         |
| C/T or T/T      | 39 | 0.23 ± 0.07                         | 0.46 (0.22,0.96) |        | 0.47 (0.21,1.03) |         |
| YAP1rs18504     |    |                                     | 0.85       | 0.75    |             |         |
| C/C             | 30 | 0.32 ± 0.09                         | 1 (reference) |        | 1 (reference) |         |
| C/T             | 42 | 0.40 ± 0.08                         | 0.91 (0.44,1.90) |        | 1.21 (0.56,2.61) |         |
| T/T             | 13 | 0.23 ± 0.12                         | 0.72 (0.23,2.22) |        | 1.67 (0.41,6.84) |         |
| YAP1rs1820453   |    |                                     | 0.94       | 0.79    |             |         |
| A/A             | 36 | 0.33 ± 0.08                         | 1 (reference) |        | 1 (reference) |         |
| G/G             | 35 | 0.38 ± 0.09                         | 0.88 (0.41,1.87) |        | 0.77 (0.35,1.71) |         |
| G/A             | 16 | 0.29 ± 0.12                         | 0.99 (0.38,2.58) |        | 1.00 (0.37,2.72) |         |
| A/A or A/C      | 27 | 0.30 ± 0.09                         | 1 (reference) |        | 1 (reference) |         |
| A/C             | 46 | 0.33 ± 0.08                         | 0.98 (0.44,2.20) |        | 0.90 (0.38,2.12) |         |
| C/C             | 8  | 0.38 ± 0.17                         | 1.56 (0.53,4.64) |        | 1.68 (0.54,5.23) |         |

Abbreviations: CI, confidence interval; HR, hazard ratio; SNP, single-nucleotide polymorphism; USC, University of Southern California. *Greenwood s.e. Based on log-rank test in the univariable analysis and based on Wald test within multivariable Cox proportional hazards model adjusting for stage and type of adjuvant therapy and stratified by race. In the dominant model.

**Figure 1.** Recursive partitioning analysis and estimated recurrence-free probability for all patients.

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variant allele at this locus had lower recurrence probability than patients with a homozygous wild-type genotype, suggesting that the variant allele could reduce TAZ’s nuclear ability to promote cell proliferation, survival and EMT. The presence of a variant allele for TAZ rs3811715 and the correlation with a lower recurrence probability was stronger in patients bearing left-sided tumors. Increasing data have underlined the fact that right- and left-sided tumors are different entities. Particularly in the adjuvant setting, these molecular differences might influence, in part, the response and the benefit from 5-Fu-based adjuvant treatment. Interestingly, Hippo signaling has been implicated in resistance to 5-Fu in CRC cell lines as YAP overexpression has been shown to lead to cellular quiescence and chemoresistance. However, the potential differences in the Hippo signaling activity depending on the tumor location have not been studied. In an exploratory analysis performed in an independent Japanese cohort, a similar trend was found for TAZ rs3811715 in patients bearing a left-sided tumor. However, this association was found in a different genetic model, and did not reach statistical significance. Many reasons could account for this fact such as the differences in MAFs between the two cohorts. The American cohort comprises different races including Caucasian, African-American, Hispanic as well as Asian, and MAFs among these groups differ greatly. We also believe that the clear differences in the baseline characteristics of the patients in these two cohorts have clearly influenced these results. These differences include the percentage of stages II and III (the Japanese cohort comprises only stage III patients), the number of resected lymph nodes or the tumor location as it shown in Table 1. Surprisingly, despite of being all stage III patients, the Japanese cohort had a lower recurrence rate than the American cohort (36 vs 29%). This fact could be explained by the higher rate of optimal lymphadenectomy in the Japanese cohort.

Overall, this work represents the first approach to the evaluation of polymorphisms within genes involved in the Hippo pathway as prognostic factors. This hypothesis generating study lacks correction for multiple testing and a more similar validation cohort; therefore, these results should be interpreted with caution. Nonetheless, the critical implications of the Hippo signaling in several recurrence mechanisms like stem cell maintenance, EMT and resistance to 5-Fu make this pathway a highly interesting target for colon cancer treatment. Therefore, further genetic studies are warranted.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Figure 2. Recursive partitioning analyses based on tumor location.
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