Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY APPENDIX

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METHODS

Study Plan

Drs Chan, and Ogino conceived and designed the study. Drs Liao, Lochhead, Nishihara, Morikawa, Kuchiba, Yamauchi, Imamura, Qian, Baba, Shima, Sun, Nosho, Meyerhardt, Giovannucci, Fuchs, Chan and Ogino gathered the data. Drs Liao, Lochhead, Nishihara, Morikawa, Kuchiba, Yamauchi, Imamura, Qian, Baba, Shima, Sun, Nosho, Meyerhardt, Giovannucci, Fuchs, Chan and Ogino analyzed the data. Drs Liao, Nishihara, Fuchs, Chan and Ogino vouch for the data and analysis. Dr Liao wrote the first draft of manuscript. All authors contributed to editing, and decided to publish the paper.

PTGS2 and Phospho-AKT Immunohistochemistry

Tissue microarray was constructed,[1] and immunohistochemistry analyses for PTGS2 (HGNC:9605; cyclooxygenase-2, COX-2) and phosphorylated AKT at Ser 473 (phospho-AKT)

were performed [anti-PTGS2 (1:300, mouse monoclonal; Cayman Chemical, Ann Arbor, MI) and Anti-phospho-AKT (1:100, rabbit monoclonal; Cell Signaling Technology, Boston, MA)].[2] Appropriate positive and negative controls were included in each run of assay. Immunostained tissues for each marker were scored by a pathologist (PTGS2 by S.O.; phospho-AKT by Y.B.) blinded to other data. A subset sample of over 100 cases was scored independently by a second pathologist (PTGS2 by T.M.; phospho-AKT by K.S.) unaware of other data. The concordance between the two observers ($P < 0.001$) was 0.85 ($\kappa = 0.69$, N=124) for PTGS2 and 0.81 ($\kappa = 0.59$, N=132) for phospho-AKT.

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.2; SAS Institute Inc, Cary, NC). All *P* values were two-sided. For our main hypothesis testing on the prognostic effect of aspirin use in strata of *PIK3CA* status, we interpreted results cautiously given the nature of subgroup analyses. To assess associations between categorical data, the chi-square test, or Fisher's exact test was performed. To compare mean age and mean LINE-1 methylation levels, a t-test or ANOVA (analysis of variance), assuming equal variances, was performed.

Our main hypothesis was the differential effects of post-diagnosis aspirin use on tumor behavior according to *PIK3CA* mutation status. We utilized all cases of colorectal cancers with available exposure information and tumor *PIK3CA* status, to maximize statistical power. All other analyses were sensitivity analyses or exploratory analyses on selected strata (such as stage I-III, stage II-III, stage III, pre-diagnosis aspirin use status), which were a part of the current study, but not our main analyses.

The Kaplan-Meier method and log-rank test were performed for survival analysis. In colorectal cancer-specific mortality analyses, deaths from causes other than colorectal cancer were censored. To control for confounding, we used Cox proportional hazards models to calculate mortality hazard ratio (HR) according to aspirin use status, or tumor *PIK3CA* mutation status. The multivariate model initially included age at diagnosis (continuous), sex, year of diagnosis (continuous), months from diagnosis to first measurement of post-diagnosis aspirin use (continuous), regular aspirin use before colorectal cancer diagnosis, body mass index (<30 kg/m^2 vs ≥ 30 kg/m^2), tumor location (proximal vs. distal colon vs. rectum), tumor differentiation (poor vs well-moderate), MSI (high vs. low/MSS), CIMP (high vs. low/0), LINE-1 methylation (continuous), *KRAS* mutation and *BRAF* mutation. To minimize residual confounding and overfitting, disease stage (I, II, III, IV, or unknown) was used as a stratifying variable using the "strata" option in the SAS "proc phreg" command. To avoid overfitting, variables in the final model were selected using backward stepwise elimination with a threshold of $P = 0.05$. Age at diagnosis, MSI and tumor differentiation remain in the final model after selection. Cases with missing data in any of the categorical variables [tumor location (<0.1% missing), tumor differentiation (<0.1%), CIMP (7.6%), MSI (5.8%), *BRAF* (4.5%), and *KRAS* (4.5%)] were included in the majority category for that variable. We confirmed that excluding cases with missing information in any of the covariates did not substantially change the results (data not shown). Significance of an interaction was assessed by the Wald test on the cross-product of the aspirin and *PIK3CA* variables in a Cox model. To assess the potential differential effect of aspirin use according to *PIK3CA* status, we performed a single Cox regression analysis, in which we could estimate the effect of aspirin use simultaneously in two strata of *PIK3CA* status, using a re-parameterization of the interaction term.[3-4] The proportionality of hazards assumption was

satisfied by evaluating time-dependent variables, which were the cross products of the post-diagnosis aspirin variable and survival time (P = 0.23 for colorectal cancer-specific mortality; P = 0.75 for overall mortality) and the cross products of the *PIK3CA* variable and survival time (P = 0.44 for colorectal cancer-specific mortality; P = 0.95 for overall mortality).

Table S1. Post hoc subgroup analyses that were performed and are reported in the current study.

Exposure ("A")	Primary subgroups (classified by "B" variable)	Secondary strata (or subsets)	Primary study endpoint	Secondary study endpoint	Method to assess heterogeneity of exposure effects	Table
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type		Colorectal cancer-specific mortality	Overall mortality	Interaction ("A" x "B"). Assessment of effect estimates in 2 strata. Fisher's exact test	Table 2, Table 3 (for Fisher's exact test), Table S2 (for all variables in the final model)
Post-diagnosis aspirin use (2 levels of regular user; >5 tablets / week, and ≤5 tablets / week vs. non-user)	*PIK3CA* mutant vs. wild-type		Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimates in 2 strata.	Data not shown
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Pre-diagnosis aspirin use (regular user vs. not)	Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimates in 4 strata	Table 4
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Stage I-III	Colorectal cancer-specific mortality	Overall mortality	Interaction ("A" x "B"). Assessment of effect estimates in 2 strata.	Table S3
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Stage II-III	Colorectal cancer-specific mortality	Overall mortality	Interaction ("A" x "B"). Assessment of effect estimates in 2 strata.	Table S4
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Stage III	Colorectal cancer-specific mortality	Overall mortality	Interaction ("A" x "B"). Assessment of effect estimates in 2 strata.	Table S5
Pre-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant	Post-diagnosis non-user	Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimate.	Table S6 (top rows)

Pre-diagnosis aspirin non-user/post-diagnosis user vs. post-diagnosis non-user	*PIK3CA* mutant		Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimate.	Table S6 (bottom rows)
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	PTGS positive vs. negative	Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimates in 4 strata	Table S7
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	MSS	Colorectal cancer-specific mortality	Overall mortality	Interaction (“A” x “B”). Assessment of effect estimates in 2 strata.	Table S8
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Phospho-AKT positive vs. negative	Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimates in 4 strata	Table S9
Post-diagnosis aspirin use (regular user vs. not)	*PIK3CA* mutant vs. wild-type	Tumor location (proximal vs. distal)	Colorectal cancer-specific mortality	Overall mortality	Assessment of effect estimates in 4 strata	Table S10
Post-diagnosis NSAID use (regular user vs. not)	*PIK3CA* mutant vs. wild-type		Colorectal cancer-specific mortality	Overall mortality	Interaction (“A” x “B”). Assessment of effect estimates in 2 strata	Table S11

MSS, microsatellite stable

Table S2. Colorectal cancer patient survival in multivariate analysis according to the variables remained in the final model of Table 2.

	Colorectal cancer-specific mortality	Overall mortality
	Multivariate stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
PIK3CA wild-type		
Aspirin non-user	1 (referent)	1 (referent)
Aspirin user	0.96 (0.69-1.32)	0.94 (0.75-1.17)
PIK3CA mutant		
Aspirin non-user	1 (referent)	1 (referent)
Aspirin user	0.18 (0.06-0.61)	0.54 (0.31-0.94)
Age at diagnosis (for 10-year increment)	1.33 (1.12-1.59)	2.06 (1.78-2.39)
Time from diagnosis to first measurement of post-diagnosis aspirin use (for 1-month increment)	0.98 (0.96-0.99)	0.99 (0.98-1.00)
MSI		Did not remain in this model
MSI-low/MSS	1 (referent)	
MSI-high	0.18 (0.09-0.39)	
Tumor differentiation		Did not remain in this model
Well-moderate	1 (referent)	
Poor	2.14 (1.34-3.43)	
Year of diagnosis (for 1-year increment)	Did not remain in this model	0.96 (0.94-0.98)

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), regular aspirin use before diagnosis, tumor location, tumor differentiation, body mass index, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.

CI, confidence interval; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable.

Table S3. Post-diagnosis aspirin use and stage I-III colorectal cancer mortality in strata of tumor *PIK3CA* mutation status.

			Colorectal cancer-specific mortality				Overall mortality			
PIK3CA status	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
Wild-type	Non-user	399	63	1 (referent)	1 (referent)	1 (referent)	152	1 (referent)	1 (referent)	1 (referent)
	User	288	44	1.01 (0.68-1.48)	0.99 (0.67-1.46)	0.92 (0.62-1.37)	110	1.06 (0.83-1.35)	1.05 (0.82-1.35)	0.92 (0.72-1.19)
Mutant	Non-user	78	15	1 (referent)	1 (referent)	1 (referent)	30	1 (referent)	1 (referent)	1 (referent)
	User	60	3	0.23 (0.07-0.80)	0.26 (0.07-0.89)	0.26 (0.08-0.90)	16	0.60 (0.33-1.11)	0.64 (0.35-1.18)	0.65 (0.36-1.20)
P for interaction (aspirin use and *PIK3CA* status)				0.027	0.043	0.045		0.096	0.14	0.30

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), regular aspirin use before diagnosis, tumor location, tumor differentiation, body mass index, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.

CI, confidence interval; HR, hazard ratio.

Table S4. Post-diagnosis aspirin use and stage II-III colorectal cancer mortality in strata of tumor *PIK3CA* mutation status.

			Colorectal cancer-specific mortality				Overall mortality			
PIK3CA status	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
Wild-type	Non-user	287	54	1 (referent)	1 (referent)	1 (referent)	120	1 (referent)	1 (referent)	1 (referent)
	User	186	35	1.04 (0.68-1.59)	0.95 (0.62-1.45)	0.91 (0.59-1.41)	77	1.01 (0.76-1.35)	0.99 (0.74-1.31)	0.89 (0.67-1.20)
Mutant	Non-user	59	14	1 (referent)	1 (referent)	1 (referent)	25	1 (referent)	1 (referent)	1 (referent)
	User	33	3	0.34 (0.10-1.17)	0.32 (0.09-1.11)	0.28 (0.08-1.00)	9	0.55 (0.26-1.18)	0.54 (0.25-1.16)	0.55 (0.26-1.18)
P for interaction (aspirin use and *PIK3CA* status)				0.094	0.11	0.084		0.14	0.15	0.24

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), regular aspirin use before diagnosis, tumor location, tumor differentiation, body mass index, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.
* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.
CI, confidence interval; HR, hazard ratio.

Table S5. Post-diagnosis aspirin use and stage III colorectal cancer mortality in strata of tumor *PIK3CA* mutation status.

			Colorectal cancer-specific mortality			Overall mortality		
PIK3CA status	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)	No. of events	Univariate HR (95% CI)	Multivariate HR (95% CI)
Wild-type	Non-user	128	38	1 (referent)	1 (referent)	59	1 (referent)	1 (referent)
	User	99	25	0.86 (0.52-1.43)	0.88 (0.53-1.45)	47	1.06 (0.72-1.55)	0.90 (0.61-1.34)
Mutant	Non-user	23	8	1 (referent)	1 (referent)	11	1 (referent)	1 (referent)
	User	14	2	0.35 (0.07-1.64)	0.29 (0.06-1.37)	3	0.38 (0.10-1.35)	0.34 (0.09-1.22)
P for interaction (aspirin use and *PIK3CA* status)				0.27	0.18		0.13	0.15

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), regular aspirin use before diagnosis, tumor location, tumor differentiation, body mass index, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.

CI, confidence interval; HR, hazard ratio.

Table S6. Selected comparisons of colorectal cancer mortality among patients with *PIK3CA*-mutated tumors.

			Colorectal cancer-specific mortality				Overall mortality			
Pre-diagnosis regular aspirin use*	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
Non-user	Non-user	71	20	1 (referent)	1 (referent)	1 (referent)	34	1 (referent)	1 (referent)	1 (referent)
User		24	6	0.86 (0.34-2.2)	0.64 (0.25-1.67)	0.54 (0.20-1.43)	10	0.80 (0.39-1.62)	0.68 (0.32-1.43)	0.68 (0.32-1.47)
P value				0.75	0.36	0.21		0.53	0.31	0.33
User or non-user (any status)	Non-user	95	26	1 (referent)	1 (referent)	1 (referent)	44	1 (referent)	1 (referent)	1 (referent)
Non-user	User	20	1	0.16 (0.02-1.20)	0.22 (0.03-1.68)	0.22 (0.03-1.68)	6	0.61 (0.26-1.44)	0.78 (0.32-1.88)	0.71 (0.29-1.73)
P value				0.075	0.15	0.15		0.26	0.58	0.45

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), tumor location, tumor differentiation, body mass index, microsatellite instability, *KRAS* mutation, *BRAF* mutation, CpG island methylator phenotype, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.

CI, confidence interval; HR, hazard ratio.

Table S7. Post-diagnosis aspirin use and colorectal cancer mortality in strata of combined *PIK3CA* mutation and PTGS2 (cyclooxygenase-2) expression status.

PIK3CA status	PTGS2 (cyclooxygenase-2) expression status	Post-diagnosis regular aspirin use*	Total No.	Colorectal cancer-specific mortality			Overall mortality		
				No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)
Wild-type	Negative	Non-user	149	28	1 (referent)	1 (referent)	61	1 (referent)	1 (referent)
		User	104	21	1.11 (0.63-1.96)	0.96 (0.54-1.70)	42	1.08 (0.73-1.61)	1.03 (0.69-1.53)
	Positive	Non-user	246	55	1 (referent)	1 (referent)	116	1 (referent)	1 (referent)
		User	172	36	0.95 (0.62-1.45)	0.92 (0.60-1.40)	78	0.97 (0.73-1.30)	0.97 (0.73-1.29)
Mutant	Negative	Non-user	37	6	1 (referent)	1 (referent)	14	1 (referent)	1 (referent)
		User	25	2	0.47 (0.09-2.32)	0.94 (0.19-4.72)	7	0.72 (0.29-1.78)	0.99 (0.40-2.46)
	Positive	Non-user	42	16	-	-	25	1 (referent)	1 (referent)
		User	23	0	-	-	6	0.29 (0.12-0.71)	0.34 (0.14-0.82)

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.
CI, confidence interval; HR, hazard ratio.

Table S8. Post-diagnosis regular aspirin use and colorectal cancer mortality in strata of tumor *PIK3CA* mutation status in MSS patients.

			Colorectal cancer-specific mortality				Overall mortality			
PIK3CA status	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
Wild-type	Non-user	382	90	1 (referent)	1 (referent)	1 (referent)	163	1 (referent)	1 (referent)	1 (referent)
	User	283	63	0.97 (0.70-1.33)	0.98 (0.71-1.36)	0.92 (0.67-1.28)	121	1.05 (0.83-1.33)	1.07 (0.84-1.35)	0.95 (0.75-1.21)
Mutant	Non-user	81	25	1 (referent)	1 (referent)	1 (referent)	38	1 (referent)	1 (referent)	1 (referent)
	User	54	3	0.16 (0.05-0.51)	0.20 (0.06-0.68)	0.19 (0.06-0.64)	17	0.57 (0.32-1.01)	0.66 (0.37-1.18)	0.64 (0.36-1.14)
P for interaction (aspirin use and *PIK3CA* status)				0.004	0.013	0.013		0.052	0.14	0.17

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, time from diagnosis to first measurement of post-diagnosis aspirin use (months), regular aspirin use before diagnosis, tumor location, tumor differentiation, body mass index, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.

CI, confidence interval; HR, hazard ratio; MSS, microsatellite stable.

Table S9. Post-diagnosis aspirin use and colorectal cancer mortality in strata of combined *PIK3CA* mutation and tumor phospho-AKT expression status.

				Colorectal cancer-specific mortality			Overall mortality		
PIK3CA status	Phospho-AKT status	Post-diagnosis regular aspirin use*	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)
Wild-type	Negative	Non-user	102	21	1 (referent)	1 (referent)	42	1 (referent)	1 (referent)
		User	73	21	1.51 (0.83-2.77)	1.09 (0.59-2.02)	38	1.37 (0.89-2.13)	1.16 (0.74-1.81)
	Positive	Non-user	164	36	1 (referent)	1 (referent)	73	1 (referent)	1 (referent)
		User	127	23	0.83 (0.49-1.41)	0.87 (0.51-1.47)	55	1.00 (0.71-1.43)	1.03 (0.72-1.47)
Mutant	Negative	Non-user	11	1	1 (referent)	1 (referent)	2	1 (referent)	1 (referent)
		User	9	0	-	-	3	1.71 (0.29-10.27)	1.81 (0.30-10.93)
	Positive	Non-user	47	16	1 (referent)	1 (referent)	25	1 (referent)	1 (referent)
		User	29	0	-	-	8	0.39 (0.18-0.86)	0.57 (0.25-1.28)

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.
CI, confidence interval; HR, hazard ratio.

Table S10. Post-diagnosis aspirin use and colorectal cancer mortality in strata of combined *PIK3CA* mutation and tumor location.

PIK3CA status	Tumor location	Post-diagnosis regular aspirin use*	Total No.	Colorectal cancer-specific mortality			Overall mortality		
				No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)
Wild-type	Proximal colon	Non-user	199	39	1 (referent)	1 (referent)	89	1 (referent)	1 (referent)
		User	151	24	0.81 (0.49-1.35)	0.77 (0.46-1.29)	62	0.95 (0.69-1.31)	0.93 (0.67-1.29)
	Rectum / distal colon	Non-user	266	57	1 (referent)	1 (referent)	107	1 (referent)	1 (referent)
		User	184	40	1.04 (0.69-1.56)	1.04 (0.70-1.57)	74	1.04 (0.77-1.40)	1.05 (0.78-1.42)
Mutant	Proximal colon	Non-user	52	10	1 (referent)	1 (referent)	20	1 (referent)	1 (referent)
		User	34	0	-	-	8	0.52 (0.23-1.17)	0.60 (0.26-1.37)
	Rectum / distal colon	Non-user	43	16	1 (referent)	1 (referent)	24	1 (referent)	1 (referent)
		User	32	3	0.22 (0.06-0.75)	0.25 (0.07-0.86)	10	0.47 (0.22-0.97)	0.52 (0.25-1.11)

* Aspirin use was defined as regular use of aspirin in most weeks, whereas aspirin nonuse was defined as no regular use of aspirin during most weeks.
CI, confidence interval; HR, hazard ratio.

Table S11. Colorectal cancer patient mortality according to post-diagnosis regular NSAID use in strata of *PIK3CA* mutation status.

		Colorectal cancer-specific mortality				Overall mortality			
	Total No.	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)	No. of events	Univariate HR (95% CI)	Stage-stratified HR (95% CI)	Multivariate stage-stratified HR (95% CI)
All cases									
NSAID non-user	760	152	1 (referent)	1 (referent)	1 (referent)	320	1 (referent)	1 (referent)	1 (referent)
Regular NSAID user*	162	28	0.85 (0.57-1.27)	1.00 (0.66-1.49)	1.09 (0.72-1.64)	58	0.84 (0.64-1.12)	0.91 (0.69-1.21)	1.09 (0.82-1.45)
PIK3CA wild-type									
NSAID non-user	633	128	1 (referent)	1 (referent)	1 (referent)	267	1 (referent)	1 (referent)	1 (referent)
Regular NSAID user*	136	24	0.87 (0.56-1.34)	0.95 (0.61-1.48)	1.02 (0.66-1.59)	51	0.89 (0.66-1.20)	0.94 (0.69-1.27)	1.11 (0.82-1.50)
PIK3CA mutant									
NSAID non-user	127	24	1 (referent)	1 (referent)	1 (referent)	53	1 (referent)	1 (referent)	1 (referent)
Regular NSAID user*	26	4	0.78 (0.27-2.24)	1.25 (0.43-3.65)	1.53 (0.52-4.50)	7	0.61 (0.28-1.33)	0.76 (0.34-1.67)	0.94 (0.42-2.09)
P for interaction (NSAID use and *PIK3CA* mutation status)			0.85	0.64	0.50		0.37	0.62	0.71

The multivariate, stage-stratified Cox regression model initially included age, sex, year of diagnosis, regular aspirin use after diagnosis, tumor location, tumor grade, body mass index, microsatellite instability, CpG island methylator phenotype, *KRAS* mutation, *BRAF* mutation, LINE-1 methylation and PTGS2 expression. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

* Regular NSAID user was defined as those who regularly used NSAID two or more times per week, whereas NSAID non-user was defined as those who used NSAID less frequently, or never used NSAID.

CI, confidence interval; HR, hazard ratio; NSAID, non-steroidal anti-inflammatory drug.

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