Supplemental material

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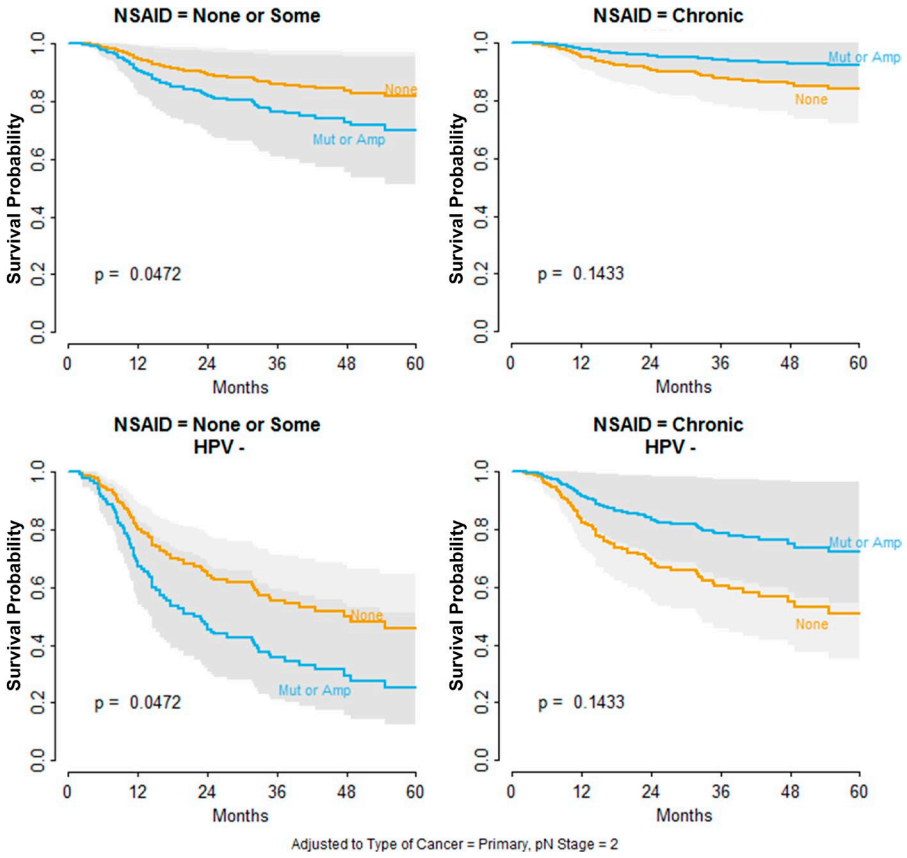

Figure S1. **DSS estimated from the proportional hazards regression model demonstrates benefit of NSAID irrespective of HPV status.** Plots are arrayed by HPV status (rows) and NSAID exposure (columns). Each plot shows model-predicted DSS probability by patients with *PIK3CA* mutation and/or copy number amplification (Mut or Amp, blue) versus patients with WT and nonamplified *PIK3CA* (None, orange). P values are based on Z statistics for testing contrasts derived from the design matrix of the selected regression model and are adjusted for age at surgery, type of cancer (primary versus recurrence), and pathological N stage. Plots reflect the interactions among NSAID status and mutation status.

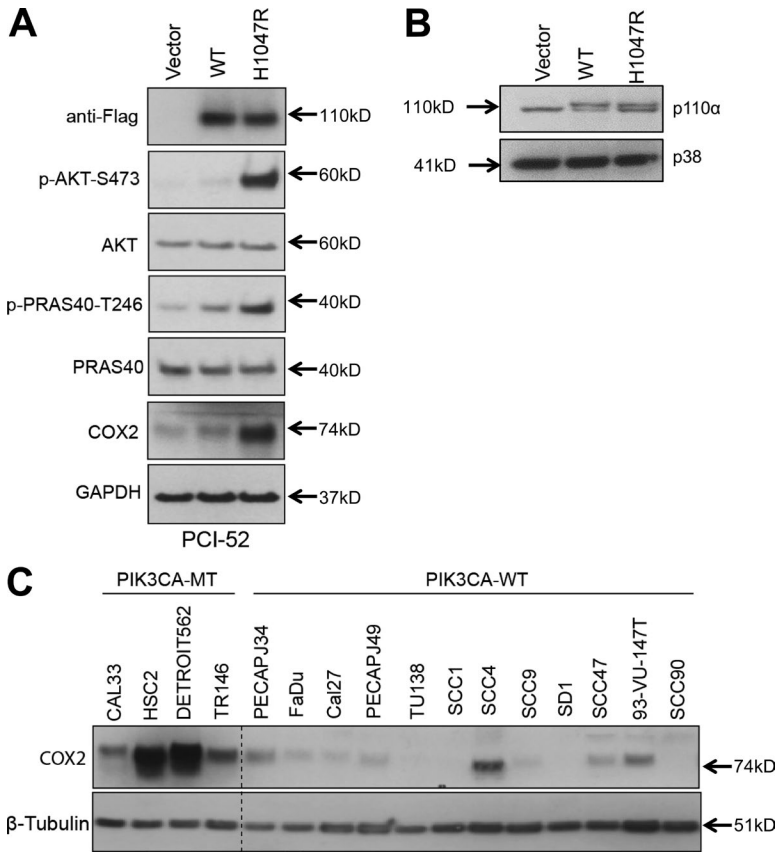

Figure S2. **Activation of PI3K signaling and COX2 expression in HNSCC cells overexpressing MT *PIK3CA* (H1047R). (A)** PCI-52 HNSCC cells engineered to express vector, WT *PIK3CA*, or MT *PIK3CA* (H1047R) were treated for 48 h with doxycycline (1 mg/ml) to induce expression of the Flag-tagged WT and MT proteins. Whole-cell lysates were then prepared and subjected to immunoblotting for the indicated proteins. GAPDH was used as a loading control. **(B)** Lysates from vector, *PIK3CA-WT*, or *PIK3CA-MT* expressing cells were probed with anti-p110α. P38 was used as a loading control. **(C)** HNSCC cell lines harboring MT *PIK3CA* or WT *PIK3CA* were subjected to immunoblotting for COX2. β-Tubulin was used as a loading control.

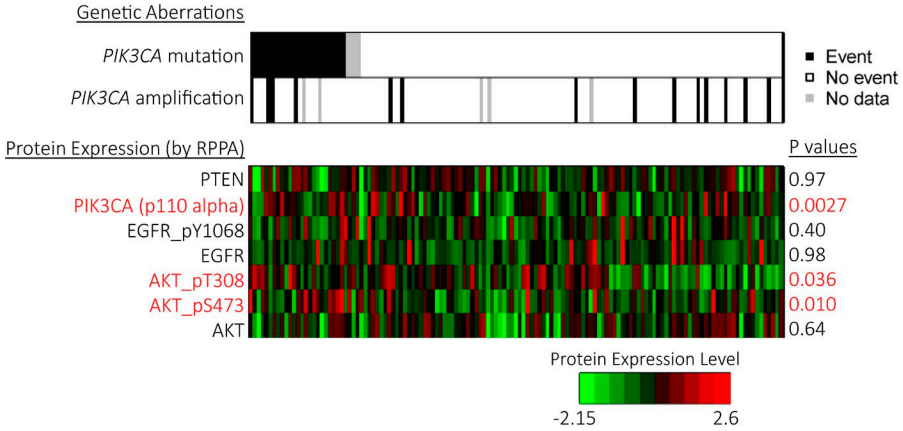

Figure S3. **Correlation of *PIK3CA* mutation or amplification with increased expression of p110α (P = 0.0027), pAKT(T308) (P = 0.036), and pAKT(S473) (P = 0.010) in 165 HNSCC tumors.** Expression of total and phosphorylated proteins was assessed by reverse phase protein array, amplification was determined by SNP array, and mutation was evaluated by whole-exome sequencing.

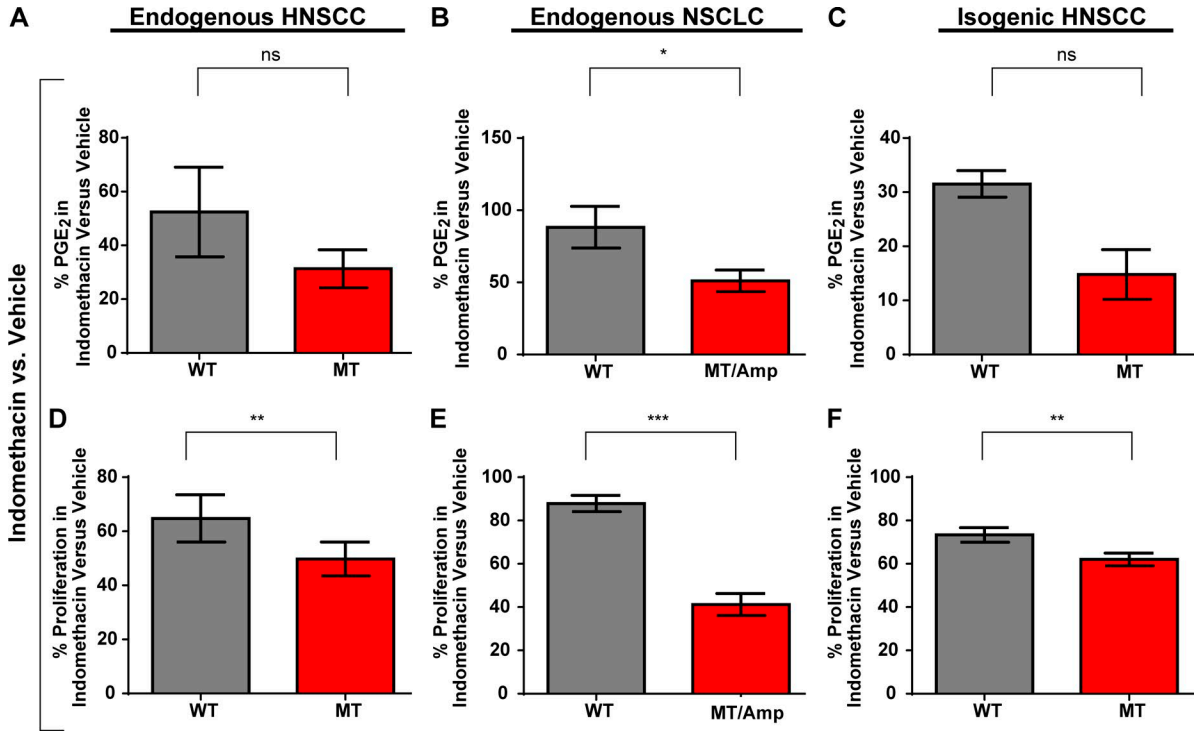

Figure S4. **Cancer cell lines with *PIK3CA* alteration are more sensitive to indomethacin treatment. (A–C)** Indomethacin treatment (48 h) leads to greater reduction of PGE_2 secretion in (A) HNSCC (P = 0.065) cells harboring endogenous *PIK3CA* mutations (Cal33, Detroit562, and HSC-2) or (B) NSCLC (P = 0.030) cells harboring endogenous *PIK3CA* mutation or amplification (H460, *PIK3CA* MT; H3255, *PIK3CA* amplification), and in (C) isogenic HNSCC cells overexpressing MT *PIK3CA* (P = 0.12). **(D–F)** Indomethacin treatment (48 h) more potently inhibits cell proliferation in (D) HNSCC (P = 0.0027) or (E) NSCLC (P < 0.0001) cells harboring endogenous *PIK3CA* mutations/amplification, and in (F) isogenic HNSCC cells overexpressing MT *PIK3CA* (P = 0.0025). All panels are depicted as mean ± SD (error bars). All experiments were performed in triplicate and repeated once. Data were analyzed by two-way analysis of variance with interaction in which cell type (mutated versus WT) was crossed with treatment (vehicle, indomethacin). Specific hypotheses were evaluated by a *t* test with pooled estimates of SE. *, P ≤ 0.05; **, P ≤ 0.005; and ***, P ≤ 0.001.

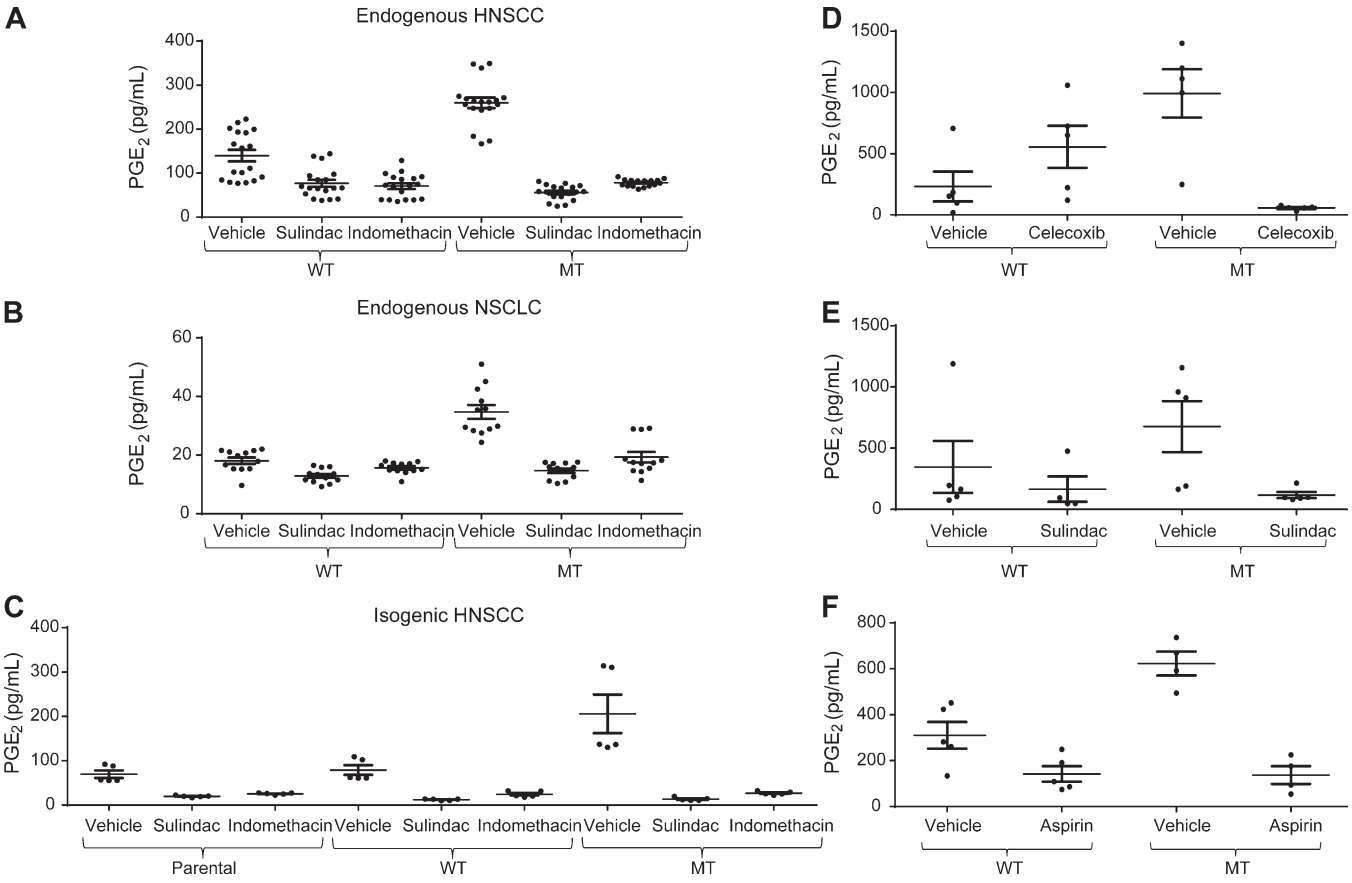

Figure S5. ***PIK3CA* alteration is associated with greater reduction in PGE2 in vitro and lower circulating PGE2 in vivo following NSAID treatment. (A–F)** Cancer cell lines with *PIK3CA* alteration exhibit a greater reduction in PGE_2 upon sulindac or indomethacin treatment (A–C) and tumor-bearing mice with *PIK3CA* MT tumors have lower circulating PGE_2 than do mice with WT tumors following NSAID treatment (D–F). Cells were treated as described in Fig. 3 followed by measurement of PGE_2 by ELISA. Data are depicted as mean ± SEM. Mice were treated as described (see Materials and methods) with celecoxib (D), sulindac (E), or aspirin (F) followed by collection of blood and measurement of circulating PGE_2 by ELISA. Data are depicted as mean ± SEM.

Table S1. **Baseline characteristics**

Characteristic	All patients (*n* = 266)	WT/nonamplified *PIK3CA*		MT/amplified *PIK3CA*		Test of equality P value[a]
		None/some NSAID use (*n* = 117)	Regular NSAID use (*n* = 74)	None/some NSAID use (*n* = 50)	Regular NSAID use (*n* = 25)	
Sex						
Male	195 (73)	85 (73)	57 (77)	38 (76)	15 (60)	0.3921
Female	71 (27)	32 (27)	17 (23)	12 (24)	10 (40)	
Age at surgery (yr)						
Median	60	58	63	59	62	0.0071
Year of surgery						
Prior to 2011	100 (38)	47 (40)	27 (36)	14 (28)	12 (48)	0.3227
2011 or later	166 (62)	70 (60)	47 (64)	36 (72)	13 (52)	
Tumor site						
Oral cavity	113 (42)	52 (44)	32 (43)	20 (40)	9 (36)	0.9301
Oropharynx	66 (25)	31 (26)	18 (24)	11 (22)	6 (24)	
Hypopharynx	16 (6)	5 (6)	6 (8)	4 (8)	1 (4)	
Larynx	71 (27)	29 (27)	18 (24)	15 (30)	9 (36)	
Pathological T stage[b]						
1	50 (19)	22 (20)	15 (21)	12 (24)	1 (4)	0.1629
2	70 (27)	35 (32)	22 (31)	8 (16)	5 (22)	
3	56 (22)	23 (21)	15 (21)	13 (27)	5 (22)	
4	64 (24)	27 (25)	17 (24)	12 (24)	8 (35)	
X	14 (5)	3 (3)	3 (4)	4 (8)	4 (17)	
Pathological N stage						
0	83 (31)	30 (26)	24 (32)	20 (40)	9 (36)	0.5131
1	34 (13)	20 (17)	10 (14)	3 (6)	1 (4)	
2	124 (47)	57 (49)	33 (45)	22 (44)	12 (48)	
X	25 (9)	10 (9)	7 (9)	5 (10)	3 (12)	
HPV status[c]						
Positive	60 (25)	26 (24)	19 (29)	11 (24)	4 (19)	0.8133
Negative	180 (75)	82 (76)	47 (71)	34 (76)	17 (81)	
Cancer type						
Primary	238 (89)	110 (94)	65 (88)	44 (88)	19 (76)	0.0508
Recurrence	28 (11)	7 (6)	9 (12)	6 (12)	6 (24)	

Data indicate *n* (%).
[a]Tests of equality across the four columns: χ^2 test for categorical data (either asymptotic form or permutation), Kruskal–Wallis test for continuous variables.
[b]Pathological T stage was missing for 12 patients.
[c]HPV status was missing for 26 patients.

Table S2. **Adjuvant therapies used in this cohort**

Adjuvant chemotherapy	*n*	%
None	139	52
Cisplatin	56	21
Cetuximab	16	6
Cisplatin + panitumumab	14	5
Carboplatin	11	4
Unknown	6	2
Carboplatin	5	2
Cetuximab + pemetrexed	4	2
Unspecified chemotherapy	3	1
Cisplatin + cetuximab	2	1
Cisplatin then carboplatin + taxol	2	1
Erlotinib	2	1
Carboplatin + cetuximab	1	0.4
Carboplatin + taxol	1	0.4
Cisplatin then carboplatin	1	0.4
Cisplatin + docetaxel	1	0.4
Cetuximab + bevacizumab	1	0.4
Panitumumab + cisplatin + carboplatin	1	0.4

Table S3. **Specific NSAID use in cohort among 99 regular users**

NSAID	*n*	%
Aspirin	74	74.7
Ibuprofen	24	24.2
Naproxen	7	7.1
Celecoxib	3	3.0
Rofecoxib	2	2.0
Diclofenac	1	1.0
Meloxicam	1	1.0
Indomethacin	1	1.0
Oxaprozen	1	1.0

Table S4. **HRs for DSS by *PIK3CA* status and regular NSAID exposure**

PIK3CA	Covariate	Univariate			Adjusted[a]		
		n at risk/*n* events	HR	95% CI	*n* at risk/*n* events	HR	95% CI
MT/A	Regular NSAID use	75/30	0.42	0.18–1.00	66/27	0.24	0.09–0.62
WT/NA	Regular NSAID use	191/53	0.86	0.49–1.50	174/50	0.86	0.48–1.54

MT/A, *PIK3CA* mutation or amplification; WT/NA, WT and not amplified.
[a]Adjusted for N stage, HPV status, and tumor type (primary or recurrence).

Table S5. **HRs for OS by *PIK3CA* status and regular NSAID exposure**

PIK3CA	Covariate	*n*	Univariate		*n*	Adjusted[a]	
			HR	95% CI		HR	95% CI
M/A	Regular NSAID use	75	0.53	0.26–1.07	66	0.31	0.14–0.69
WT/NA	Regular NSAID use	191	0.97	0.62–1.53	174	0.98	0.60–1.62

M/A, *PIK3CA* mutation or amplification; WT/NA, WT and not amplified.
[a]Adjusted for age at surgery, N stage, HPV status, and tumor type (primary or recurrence).

Table S6. ***PIK3CA* mutations in HNSCC PDX models**

PDX	PI3K mutation
MT1	E542K, F744L
MT2	R38C
MT3	M1043V