Supplementary Data

Cell Transfection

siRNA *LOX* and their corresponding negative control, siControl (Life Technologies, Thermo Fisher Scientific, Rockford, IL), were transiently transfected into 2×10^5 cells on six-well plates using the transfection reagent RNAiMAX (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's protocol. THJ-16T and HEK-293 were used to generate inducible stable cell lines overexpressing $BRAF^{V600E}$ or $BRAF^{E586K}$. The vectors were purchased from GeneCopoeia (GeneCopoeia, Rockville, MD).

Invasion and Migration Assay

A cell migration assay using polycarbonate filters with a pore size of 8 μm (BD Biosciences, Bedford, MA) was performed according to the manufacturer's protocol. Briefly, thyroid cell lines were incubated with doxycycline on six-well plates for 24 hours. Then, the cells were trypsinized and seeded in the upper chamber of the insert (2.5×10^5 cells) in serum-free medium with doxycycline. In the lower chamber, medium supplemented with 10% fetal bovine serum was added. Following incubation for 22 hours, the inserts were removed and stained according to the manufacturer's protocol.

Protein Extraction and Western Blot Assay

Cells were lysed in a buffer containing 10 mM of Tris and 1% sodium dodecyl sulfate (SDS), protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO), and PhosphoSTOP phosphatase inhibitors (Roche, Indianapolis, IN). The lysates were quantified for protein concentrations using a Pierce BCA Protein Assay kit (Life Technologies). Cell lysates were analyzed in SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with 5% bovine serum albumin in Tris-buffered saline–Tween buffer and then incubated with primary antibodies overnight at 4°C. The membranes were then incubated with the horseradish peroxidase–conjugated secondary antibodies. Protein bands were analyzed using Pierce enhanced chemiluminescence reagent (Thermo Fisher Scientific). The band densitometry was performed using the Image Lab software (Bio-Rad Laboratories).

Supplementary Table S1. Comparison of Clinical and Pathologic Characteristics and *LOX* and *BRAF* Status in TCGA Data Set of Patients with Thyroid Cancer

	BRAF *status*			BRAF-*positive*		
Clinical characteristics	BRAF-*negative* % (n)	*BRAF-positive* % (n)	p	*LOX low* % (n)	*LOX high* % (n)	p
Age, years						
≥45	53 (141)	56.6 (136)	0.4	56 (83)	57.6 (53)	0.89
<45	47 (125)	43.3 (104)		43.9 (65)	42.4 (39)	
Sex						
Female	74 (197)	72 (173)	0.61	71.6 (106)	72.8 (67)	0.88
Male	26 (69)	28 (67)		28.4 (42)	27.2 (25)	
Overall stage						
Stage I–II	71 (188)	62.3 (149)	0.05	69.4(102)	51 (47)	0.006
Stage III–IV	29 (77)	37.6 (90)		30.6 (45)	49 (45)	
Extrathyroidal extension						
No	76.3 (193)	61 (143)	0.0001	72.2 (104)	42.8 (39)	<0.0001
Yes	33.6 (60)	39 (92)		27.8 (40)	57.1 (52)	
T stage						
T1–T2	55.3 (176)	45.6 (135)	0.03	68.7 (101)	36.9 (34)	<0.0001
T2–T3	44.7 (89)	54.3 (104)		31.3 (46)	63 (58)	
Lymph nodes						
No	55.3 (131)	45.6 (100)		54 (73)	32.1 (27)	
Yes	44.7 (106)	54.3 (119)	0.05	46 (62)	67.8 (57)	0.002
Recurrence						
No	92.7 (241)	87.9 (204)		91.7 (133)	81.6 (71)	
Yes	7.3 (19)	12.1 (28)	0.09	8.3 (12)	18.4 (16)	0.035

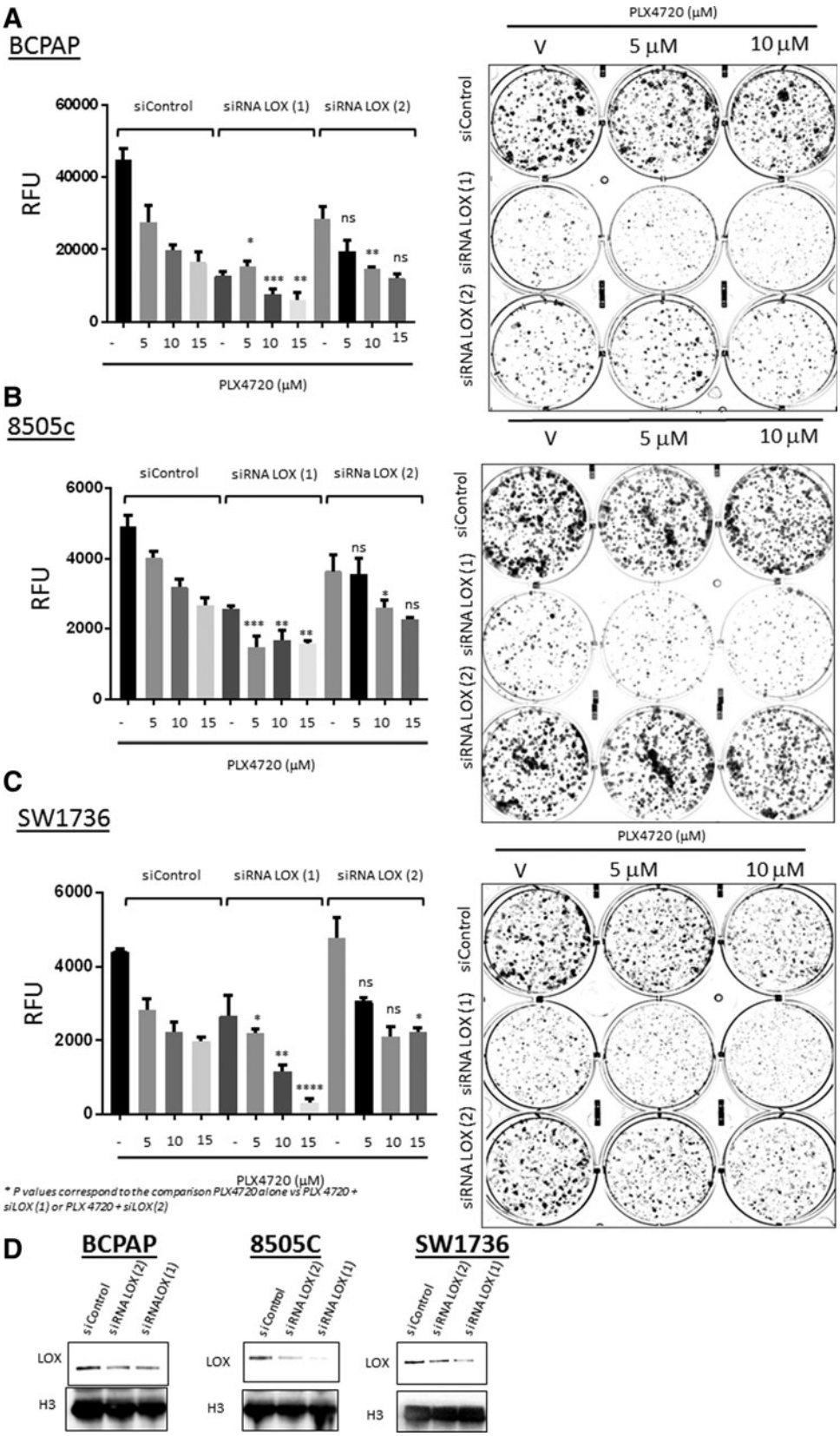
Supplementary Table S2. Clinical Characteristics of the Cohort from the Authors' Institution

Variables	% (n)
Median age at diagnosis	
Male	48 ± 13.23
Female	43.5 ± 13.69
Sex	
Male	23.9 (26)
Female	76.1 (83)
Stage	
I–II	63.3 (69)
III–IV	14.7 (16)
N/A	22 (24)
Histological type	
cPTC	71.5 (78)
Other[a]	28.4 (31)
Recurrence[b]	
Yes	9.2 (10)
No	84.4 (92)
N/A	6.4 (7)
Mortality	
Alive	100 (109)
Dead	0 (0)
BRAF mutation status	
$BRAF^{V600E}$	47.7 (52)
No $BRAF^{V600E}$	52.3 (57)

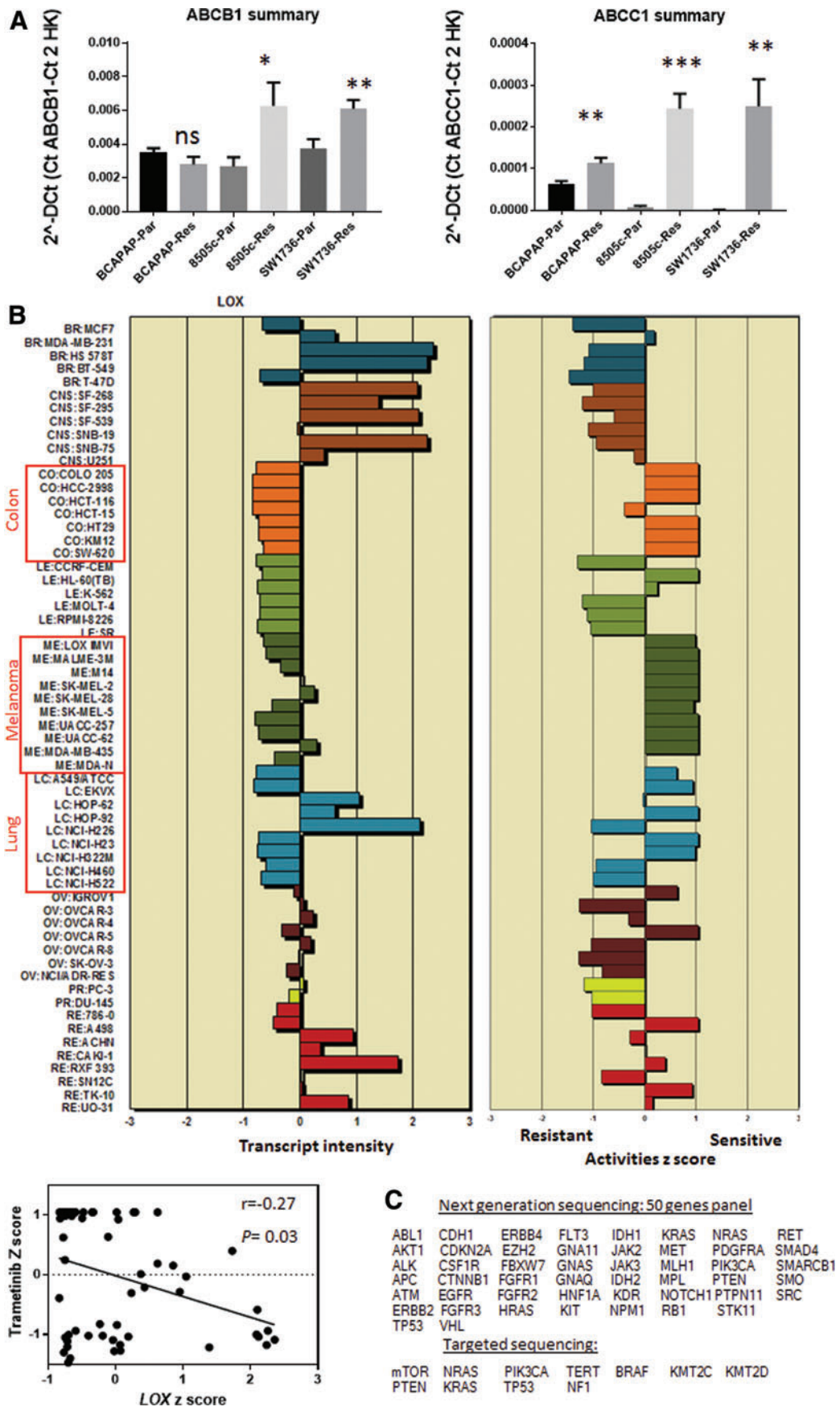
[a]Tall-cell variant of papillary thyroid cancer ($n = 5$), follicular variant of papillary thyroid cancer ($n = 17$), and poorly differentiated thyroid cancer ($n = 1$).

[b]Patients with excellent response were assessed for recurrence by serum thyroglobulin and ultrasound for recurrent disease. Recurrence is defined as patients with structurally incomplete response.

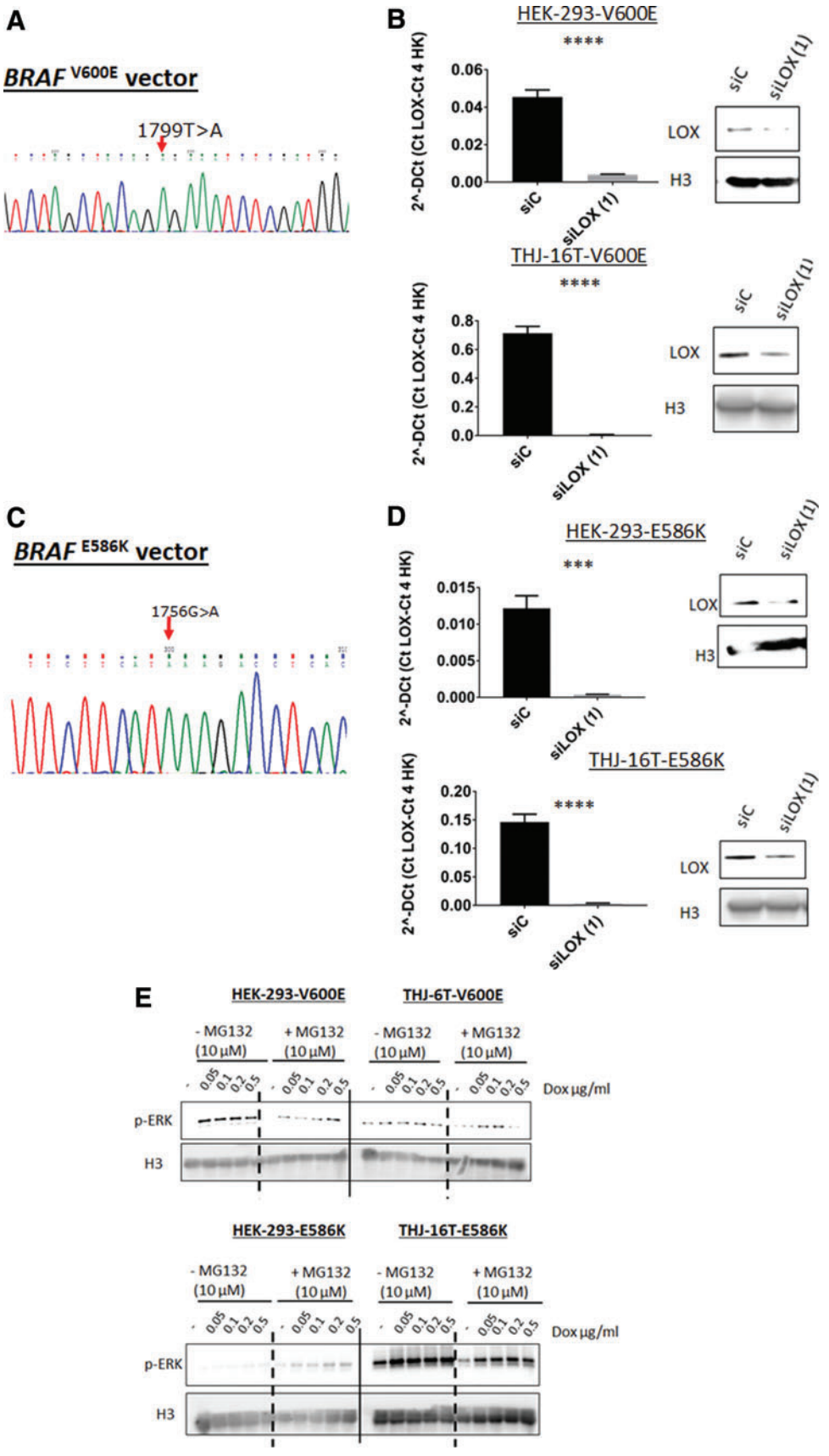
cPTC, classical papillary thyroid cancer.



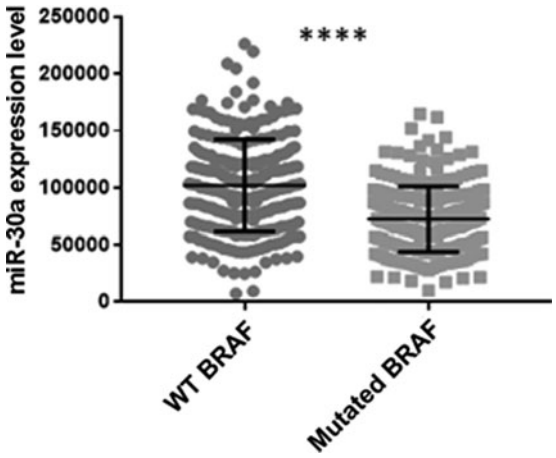
SUPPLEMENTARY FIG. S1. Knockdown of *LOX* sensitizes cancer cells to $BRAF^{V600E}$ inhibitor PLX4720. Analysis of cell proliferation and colony formation after treatment with different concentrations (0, 5, 10, and 15 μM) of PLX4720 alone or in combination with siRNA LOX(1) or siRNA LOX(2) in (**A**) BCPAP, (**B**) 8505C, or (**C**) SW1736 cell lines. The *p*-values correspond to the comparison drug alone versus drug with siLOX(1) or siLOX(2). (**D**) Western blot analysis showing *LOX* knockdown efficiency in the three cancer cell lines 48 hours post transfection. Ns, not significant. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. Error bars correspond to standard deviations.



SUPPLEMENTARY FIG. S2. (**A**) PLX4720-resistant cells. RT-PCR validation of the expression of ABCB1 and ABCC1 in the parental and resistant cells. (**B**) A mean-centered graph representing *LOX* expression and sensitivity to MEK inhibitor Trametinib in the NCI-60 screening. The red squares highlight the human cancers with activation of the MAPK pathway. Spearman correlation and *p*-value between *LOX* expression (z-score transcript) and response to the drug (z-score activities). (**C**) The next-generation sequencing of a 50-gene panel and genes analyzed by targeted sequencing to evaluate the additional mutations. Error bars correspond to standard deviations. Ns, not significant. $*p<0.05$; $**p<0.01$; $***p<0.001$.



SUPPLEMENTARY FIG. S3. Induction of $BRAF^{V600E}$ or $BRAF^{E586K}$ (**A**) Sanger sequencing of the lentiviral vector with $BRAF^{V600E}$ mutation. (**B**) *LOX* knockdown efficiency by Western blot and RT-PCR in $BRAF^{V600E}$ cells. (**C**) Sanger sequencing of the lentiviral vector with $BRAF^{E586K}$ mutations. (**D**) LOX knockdown efficiency by Western blot and RT-PCR in $BRAF^{E586K}$ cells. Error bars correspond to standard deviations. (**E**) Western blot of p-ERK expression in the $BRAF^{V600E}$ and $BRAF^{E586K}$ cell lines induced with doxycycline, with and without MG-132. ***$p < 0.001$; ****$p < 0.0001$.



SUPPLEMENTARY FIG. S4. MiR-30a is downregulated in *BRAF*-mutated tumors. miR-30a is significantly downregulated in *BRAF*-mutated tumors in TCGA cohort of thyroid cancer patients. ****$p < 0.0001$.