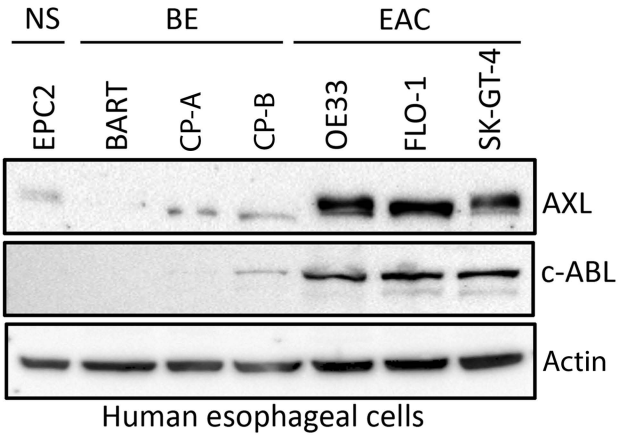
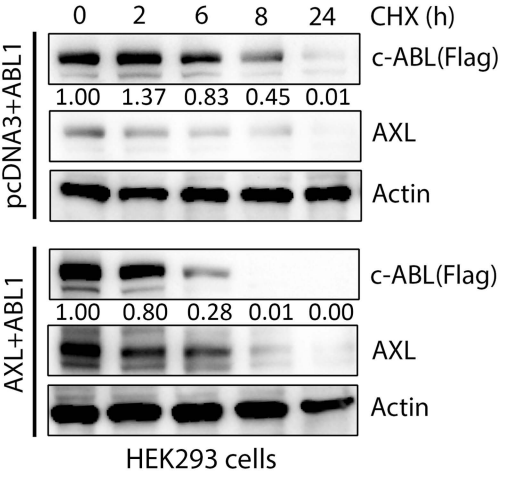


Supplemental Figure S1. Co-overexpression of AXL and c-ABL proteins in esophageal adenocarcinoma cell lines. Proteins from normal esophageal squamous epithelial cells (NS; EPC2), Barrett's cells (BE; BART, CP-A, and CP-B), and esophageal adenocarcinoma cells (EAC; OE33, FLO-1, and SK-GT-4) were subjected to Western blot analysis of AXL and c-ABL proteins.



S**upplemental Figure S2. AXL expression does not enhance c-ABL protein stability.** HEK293 cells were transiently co-transfected with ABL1 and pcDNA3 or AXL and treated with CHX for the indicated timepoints. Western blot analysis was used to assess c-ABL protein levels. The data showed that c-ABL protein stability was not increased by AXL expression.

Supplemental Table S1. Co-overexpression of AXL and c-ABL proteins in esophageal adenocarcinomas

	N	EAC $N = 53$	Normal $N = 11$	p-value
AXL index score	64			$<0.001^2$
0		13% (7)	0% (0)	
1		21% (11)	64% (7)	
2		11% (6)	36% (4)	
3		55% (29)	0% (0)	
c-ABL index score	64			$<0.001^2$
0		6% (3)	45% (5)	
2		8% (4)	27% (3)	
4		21% (11)	27% (3)	
8		26% (14)	0% (0)	
12		40% (21)	0% (0)	

N, the number of samples and numbers after proportions are frequencies. AXL (55%, IHC index score = 3) and c-ABL (66%, IHC index score ≥ 8) are frequently overexpressed in esophageal adenocarcinomas. 2, Pearson test was used to assess the differences in AXL and c-ABL index scores between normal and tumor tissue samples.

Supplemental Table S2: descriptive patient characteristics

	N	
Gender	53	
Female		11% (6)
Male		89% (47)
Age	53	56 63 70
Age categories	53	*a b c*
<60		36% (19)
≥60		64% (34)
Race/Ethnicity	44	
Latino		11% (5)
White		89% (39)
AXL index score	53	
0		13% (7)
1		21% (11)
2		11% (6)
3		55% (29)
AXL	53	
<2		34% (18)
≥2		66% (35)
c-ABL index score	53	
0		6% (3)
2		8% (4)
4		21% (11)
8		26% (14)
12		40% (21)
c-ABL	53	
<8		34% (18)
≥8		66% (35)
Location	53	
distal esophagus		4% (2)
Esophagus		51% (27)
G-E Junction		45% (24)
Tumor size (cm)	52	1.0 2.5 4.0
Tumor categories	52	
<3cm		48% (25)
≥3cm		52% (27)
Barrett's	27	
Yes		93% (25)
No		7% (2)
Surgery performed	49	
Yes		22% (11)
No		78% (38)

N, number of patients. Numbers after proportions are frequencies. *a b c* represent the lower quartile *a,* the median *b,* and the upper quartile *c* for continuos variables.

Supplemental Table S2: ***(continued)***

	N	
Tumor core histology	51	
Diffuse		8% (4)
Intestinal		73% (37)
mixed		4% (2)
Papillary		2% (1)
poorly diff		4% (2)
Signet-Ring		8% (4)
well diff		2% (1)
Differentiation	49	
intestinal		6% (3)
Moderately-differentiated		49% (24)
Mod-poorly differentiated		18% (9)
Poorly differentiated		18% (9)
Well-differentiated		8% (4)
Lymph Nodes	53	
Negative		38% (20)
Positive		62% (33)
Stage	53	
0		9% (5)
1		30% (16)
2		38% (20)
3		21% (11)
4		2% (1)
N Classification	53	
N0		49% (26)
N1		19% (10)
N2		32% (17)
T Classification	53	
T1		51% (27)
T2		26% (14)
T3		17% (9)
T4		6% (3)
Number Positive Nodes	51	
0		47% (24)
1		24% (12)
2		10% (5)
3		6% (3)
4		4% (2)
5		2% (1)
6		4% (2)
7		2% (1)

N, number of patients. Numbers after proportions are frequencies. *a b c* represent the lower quartile *a,* the median *b,* and the upper quartile *c* for continuos variables.

Supplemental Table S2: *(continued)*

	N	
8		2% (1)
Number Total Nodes	50	5 8 14
		a b c
Cancer reccurence	53	
Yes		96% (51)
No		4% (2)
Recurrence after chemo	53	
Yes		100% (53)
No		0% (0)
Recurrence Free Survival	53	114 845 1890
		a b c
location.of.tumor.recurrence	27	
anastamosis		4% (1)
Bone		4% (1)
Brain		7% (2)
Esophagus		4% (1)
Femur		4% (1)
Liver		11% (3)
Lung		7% (2)
Lymph node (supraclavicular)		4% (1)
Mediastinum		7% (2)
NA		19% (5)
Neck		4% (1)
Perirenal		4% (1)
Pleura		15% (4)
Ribs		4% (1)
Stomach		4% (1)
Died of cancer	53	
Yes		100% (53)
No		0% (0)
Follow-up period	53	45 450 1740
Recurrence survival days	53	114 845 1890
		a b c
Post Op CT	34	
Yes		41% (14)
No		59% (20)

N, number of patients. Numbers after proportions are frequencies. *a b c* represent the lower quartile *a,* the median *b,* and the upper quartile *c* for continuos variables.

Supplemental Table S3. List of quantitative real-time PCR primers sequences

HPRT1 (forward)	5'-ACCCTTTCCAAATCCTCAGC-3'
HPRT1 (reverse)	5'-GTTATGGCGACCCGCAG-3'
AXL (forward)	5'-GAAGGTACCATGACAACCCAGGCAAAGTG-3'
AXL (reverse)	5'-GAACTCGAGACGCCATGGGTGCCAAAC-3'
ABL1 (forward)	5'-AAGCCGCTCGTTGGAACTC-3'
ABL1 (reverse)	5'-AGACCCGGAGCTTTTCACCT-3'
EIF4E1 (forward)	5'-GAAACCACCCCTACTCCTAATCC-3'
EIF4E1 (reverse)	5'-AGAGTGCCCATCTGTTCTGTA-3'
EIF4E2 (forward)	5'- ACAACAAGTTCGACGCTTTGA-3'
EIF4E2 (reverse)	5'- TCTCTTGCTACTGCTCTGATTCT-3'
EIF4E3 (forward)	5'- GAGTTAGTGTCAGTGTTCGGG-3'
EIF4E3 (reverse)	5'- TGTGGGGCAGAAGTTCATAGA-3'

Cell culture and reagents

The human EAC cancer cell line, SK-GT-4, was purchased from Sigma-Aldrich (St. Louis, MO, USA). SK-GT-4 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (FBS, Invitrogen Life technologies, Carlsbad, CA, USA) and 1% penicillin/streptomycin (GIBCO). CP-A and CP-B cells were obtained from ATCC (Manassas, VA, USA) and BAR-T cells [1] were cultured in DMEM/F12 medium supplemented with 0.4 µg/ml hydrocortisone, 20 ng/ml recombinant human epidermal growth factor, 20 mg/ml adenine, 140 µg/ml bovine pituitary extract, 0.1% ITS Supplement (Sigma-Aldrich; I1884), 4 mM glutamine, and 5% FBS. EPC2 cells were kindly provided by Dr. Hiroshi Nakagawa (University of Pennsylvania, Philadelphia, PA, USA) and cultured in keratinocyte serum-free medium supplemented with 40 mg/ml bovine pituitary extract and 1 ng/ml EGF (Invitrogen). HEK-293 were obtained from ATCC and maintained in F12 (HAM) medium (GIBCO) supplemented with 10% FBS and 1% penicillin/streptomycin. All cell lines were authenticated using short tandem repeat (STR) profiling (Genetica DNA Laboratories, Burlington, NC, USA). Cycloheximide was purchased from Sigma-Aldrich.

c-ABL protein stability assay

HEK-293 cells were transiently co-transfected with Flag-ABL1 in combination with AXL-Myc-His or pcDNA3 plasmids and cultured for 24 h. The cells were then treated with 80 µg/ml of cycloheximide (CHX) and harvested at 0-, 2-, 6-, 8-, and 24-hour time points. Proteins from each time point were prepared and analyzed by Western blotting to evaluate c-ABL protein

stability. The mean intensity of protein bands was analyzed by densitometry using IMAGEJ (NIH Image) and normalized to β-actin.

References

1 Jaiswal KR, Morales CP, Feagins LA, Gandia KG, Zhang X, Zhang HY *et al*. Characterization of telomerase-immortalized, non-neoplastic, human Barrett's cell line (BAR-T). *Dis Esophagus* 2007; 20: 256-264.