Supplementary Methods

Histology.

Esophagi, tongue, forestomach, and ventral neck skin were removed from 1 month-old, 1.5 month-old, and 3 month-old, littermate control and *ED-L2-Cre/Rosa26-IKK2ca*^{SFL} mice, examined grossly, and then processed for histology. Briefly, tissue was fixed in 10% Buffered Formalin Phosphate (Fisher Scientific) or Zinc Formalin (Polysciences Inc, Warrington, PA) and embedded in paraffin, and 5-µm sections were applied to X-tra slides (Leica Biosystems, Buffalo Grove, IL). Slides were stained with hematoxylin and eosin, and images were captured on a Nikon Eclipse E600 microscope with a Photometrics CoolSNAP charge-coupled device camera (Roper Scientific, Tucson, AZ). The following numbers of matched littermate control and mutant mice were examined histologically: age 1 month, 6 controls and 6 mutants; age 1.5 months, 8 controls and 8 mutants; age 3 months, 5 controls and 5 mutants.

Cell Culture, Infection, and Transfection.

Cells were grown in keratinocyte serum-free medium (K-SFM, Invitrogen, Carlsbad, CA) supplemented with 40 µg/ml bovine pituitary extract (Life Technologies), 5 ng/ml epidermal growth factor (EGF) (Life Technologies), 100 units/ml penicillin, and 100 µg of streptomycin (Life Technologies). To express mouse and human *IKKβ*, cDNAs were subcloned into the pBabe-puro vector (Addgene plasmid # 1764) and the pFB-neo vector (Agilent Technologies, Wilmington, DE). For retroviral packaging, pBabe-puro and pFB-neo retroviral vectors were transfected into Phoenix-Ampho or Phoenix-Eco cells with Lipofectamine 2000 according to the manufacturer's instructions. Virus-containing medium was harvested 48 hours after transfection and filtered with 0.45µM Millex HV filters (EMD Millipore, Temecula, CA). Keratinocytes

were infected with culture supernatants from individual Phoenix-Ampho or Phoenix-Eco cells at a 1:3 dilution in KSFM. Cells selected after 48 hours with 1μg/ml puromycin (Sigma-Aldrich, St-Louis, MO) or 150 μg/ml G418 (Corning) for 10 days.

Immunofluorescence, Immunohistochemistry

Following microwave or proteinase K (for CD31 only) antigen retrieval, slides were incubated with one of the following primary antibodies: 1:500 rat anti-CD31 (BD Biosciences); 1:500 goat anti-GFP (Abcam, Cambridge, MA); 1:50 rat anti-CD45 (BD Biosciences); 1:500 mouse anti-desmin (Sigma-Aldrich), or 1:1,000 VWF (Dako, Carpinteria, CA). For CD31/Desmin co-staining, frozen esophageal sections fixed in cold acetone were used. Species-specific secondary antibodies were added, and detection was performed as previously described[1]. For fluorescent labeling, 1:600 Cy2 or 1:600 Cy3 (Jackson ImmunoResearch, West Grove, PA) was used.

Cell proliferation and apoptosis analyses.

We injected littermate control and *ED-L2-Cre/Rosa26-IKK2ca*^{SFL} mice with BrdU labeling reagent (Zymed, San Francisco, CA) 60 minutes before death. After death, esophagi were removed and processed as described earlier. Following microwave antigen retrieval, slides were incubated with rat anti-BrdU antibody (1: 1:15,000) (Accurate Chemicals and Scientific Corporation, Westbury, NY). The proliferative index was determined by counting the number of BrdU-labeled cells per 100 basal cells in a least 5 distinct regions of esophagi from at least 4 *ED-L2-Cre/Rosa26-IKK2ca*^{FL} mice and 4 littermate controls. Results were expressed as number of labeled cells ± standard error of the mean. TUNEL was performed using the In Situ Cell Death

Fluorescein Detection Kit (Sigma) according to the manufacturer's instructions.

Tissue microarray.

Tissue microarrays of human biopsies from esophageal squamous cell cancer (n =96), adjacent normal esophagus (n =38), adjacent inflamed esophagus (n=12) and normal esophagus (n=5) were stained using the following antibodies: p65 NFkappaB, GM-CSF and VWF. Staining intensity was scored on a scale of 0–4 (0 = none, 1= mild or weak, 2 = moderate, 3 = intense, 4 = very intense) by a pathologist (AJK-S) in a blinded fashion. Inflamed esophagus was defined as tissue with a CD45 score ≥ 2. Details on the numbers of specimens analyzed for each of the markers across patient diagnoses are illustrated in Supplementary Table 4.

Western blots

For each sample, 20 μg of total protein was separated on a NuPAGE 4–12% bis-tris acrylamide gel (Life Technologies) and transferred onto polyvinylidene difluoride membrane (Millipore). After blocking, membranes were incubated overnight at 4°C with 1:2,000 rabbit anti-IKKβ (Cell Signaling), 1:1,000 rabbit anti-IκBα antibody (Cell Signaling), and 1:10,000 mouse anti-β-actin (Sigma-Aldrich, St. Louis, MO). Membranes were then incubated with a 1:10,000 dilution of anti-rabbit/horseradish peroxidase or anti-mouse/horseradish peroxidase (GE Healthcare Bio-Sciences, Piscataway, NJ) and developed with Immobilon ECL (Millipore).

RNA analyses.

For quantitative real-time PCR, the TATA box binding protein or Glyceraldehyde-3-

phosphate dehydrogenase genes were used as the internal control. Reverse transcription was performed with the Maxima First-Strand cDNA Synthesis for RT-qPCR kit (Thermo Scientific) following the manufacturer's instructions. Primer sequences are available upon request. RT² Profiler PCR Array Mouse Cytokines & Chemokines (Qiagen) was utilized to examine cytokine expression for 3 pairs of 4 week-old mice.

Flow cytometry.

For flow cytometric analysis of *in vivo* experiments, spleen and esophagus were harvested at 6 weeks of age. Single-cell suspensions were prepared and red blood cells were lysed using ACK Lysis Buffer (Life Technologies). Live/dead cell discrimination was performed using Live/Dead Fixable Aqua Dead Cell Stain Kit (Life Technologies). Cell surface staining was done for 20–30 min. Intracellular staining was done using a fixation/permeabilization kit (eBioscience.) T helper cells were phenotyped as CD45+CD3^{+}CD4^{+}, cytotoxic T lymphocytes as CD45+CD3+Cd8+, T cells as CD45+CD3+, myeloid derived suppressor cells (MDSC) as CD45+CD11b^{+}Gr-1^{+}, dendritic cells as CD45+CD11c+, natural killer cells as CD45+, CD49b+, macrophages as CD45+F4/80+, and regulatory T cells (Treg cells) as CD45+CD3+CD4+CD44+FOXP3^{+}. All flow cytometric analyses were done using an LSR II (BD) and analysed using FlowJo software (TreeStar).

Antibodies for flow cytometry.

Antibodies for flow cytometry are provided in the table below.

Antibody	**Clone**	**Fluorophore**	**Company**
CD49b	DX5	FITC	BD Biosciences

CD4	RM4-5	V450	BD Biosciences
Live/Dead	N/A	AmCyan	Life Technologies
CD44	1M7	BV 785	Biolegend
CD11c	N418	QD605	Biolegend
FoxP3	Fjk-16s	APC	ebioscience
CD45.2	104	AF700	Biolegend
CD11b	M1/70	PE	BD Biosciences
F4/80	BM8	PE-Cy7	Biolegend
CD3	14S-2C11	PE-Cy5	Biolegend
CD8	53-6.7	PE-Texas Red	BD Biosciences

Statistical analyses.

For flow cytometry analyses, Student *t* tests were used to indicate the statistical difference between the groups using SAS, version 9.3 (SAS Inc. Cary, NC). As the cell counts were not normally distributed, these were log-transformed for statistical analyses. To allow us to combine all the data into a single model, adjusting for organ type and repeated measures within subject, a GEE (generalized estimating equations) model was run with the log transformed cell count as the outcome, stratified by cell type. Predictors were organ and mutant/control status. For mouse studies, the numbers of animals per group were chosen to allow us to detect differences in the proportion of mice having angiogenesis using 81% power and an alpha-level of 0.05. For human tissue microarray experiments, we included a small pilot patient sample in order to explore initial consistency of our findings in clinical populations. Since this patient population served as an exploratory sub-study, no formal power calculations were performed.

Descriptive statistics summarized patient tissue characteristics, both demographic/clinical and markers. A series of Spearman sample correlation coefficients and their respective 95% confidence intervals were used to compare associations between markers and clinical variables. In order to compare mean marker values across tissue types, we employed a series of linear mixed models with random patient effect (to account for within patient association if a patient contributed more than one tissue sample) and fixed tissue effects. Model estimated pairwise differences across tissue types were compared via least squares means with adjustment (Tukey-Kramer) for multiple pairwise comparisons. All statistical tests assumed a 5% level of significance. Figures for tissue microarray experiments were generated in SAS (version 9.4, copyright 2012, The SAS Institute Inc., Cary, NC).

References

1. Yang Y, Goldstein BG, Nakagawa H, et al. Kruppel-like factor 5 activates MEK/ERK signaling via EGFR in primary squamous epithelial cells. Faseb J 2007;21:543-50.

Supplemental Table 1: Differentially expressed cytokines in esophageal epithelia by quantitative PCR array			
Gene name	Gene Symbol	Fold change by qPCR array*	*P* value
Interleukin 10	IL-10	318.2572951	0.0384
Lymphotoxin A	Lta	317.4450426	0.0313
Tumor necrosis factor	TNF	199.9189105	0.0476
Interleukin 12B	IL-12b	190.0718692	0.0388
Chemokine (C-X-C motif) ligand 5	cxcl5	50.09473961	0.0378
Colony stimulating factor 2 (granulocyte-macrophage)	Csf2 or GM-CSF	5.062766526	0.0387
Thrombopoietin	Thpo	1.420995198	0.0380
Bone morphogenetic protein 2	Bmp2	0.647943946	0.0143
Bone morphogenetic protein 7	Bmp7	0.639018584	0.0369
Macrophage migration inhibitory factor	Mif	0.593148809	0.0417
Bone morphogenetic protein 4	Bmp4	0.525470363	0.0405
Cardiotrophin 1	ctf1	0.478189772	0.0268
Interleukin 12A	Il-12a	0.249996492	0.0406

* *L2/IKKβca* mice compared with littermate controls

Supplemental Table 2. Immune cell counts by organ							
		Raw			**Log-transformed**		
Organ	**Cell type**	**CONTROL**	**MUTANT**	**p**	**LN_CONTROL**	**LN_MUTANT**	**p**
Esophagus	CD45+	8.31 (4.13)	18.88 (13.74)	0.271	2.16 (0.46)	2.69 (1.10)	0.488
Spleen	CD45+	67.60 (3.97)	63.50 (19.83)	0.743	4.23 (0.06)	4.13 (0.34)	0.654
Esophagus	T cells (CD45+CD3+)	21.29 (17.14)	18.43 (3.45)	0.791	2.73 (1.23)	2.96 (0.18)	0.769
Spleen	T cells (CD45+CD3+)	24.47 (13.29)	17.53 (5.95)	0.456	3.16 (0.48)	2.89 (0.31)	0.462
Esophagus	Th (CD45+CD3+CD4+)	31.27 (21.77)	46.73 (10.54)	0.330	3.34 (0.62)	3.85 (0.21)	0.245
Spleen	Th (CD45+CD3+CD4+)	55.83 (14.13)	48.70 (24.01)	0.680	4.02 (0.27)	3.80 (0.61)	0.600
Esophagus	CD44+ Th	45.43 (18.13)	26.97 (6.07)	0.170	3.78 (0.45)	3.31 (0.23)	0.192
Spleen	CD44+ Th	28.80 (5.46)	28.03 (1.36)	0.825	3.38 (0.18)	3.37 (0.05)	0.894
Esophagus	Tregs (CD45+CD3+CD4+CD44+FoxP3+)	2.34 (4.05)	1.43 (1.72)	0.737	0.69 (1.20)	0.71 (0.73)	0.984
Spleen	Tregs (CD45+CD3+CD4+CD44+FoxP3+)	6.43 (2.11)	3.00 (2.42)	0.138	1.97 (0.32)	1.27 (0.58)	0.139
Esophagus	Tregs (CD45+CD3+CD4+FoxP3+)	12.43 (12.50)	4.37 (5.12)	0.359	1.95 (1.72)	1.27 (1.21)	0.605
Spleen	Tregs (CD45+CD3+CD4+FoxP3+)	12.67 (2.14)	10.86 (7.77)	0.718	2.61 (0.15)	2.32 (0.68)	0.520
Esophagus	CTL (CD45+CD3+CD8+)	26.40 (18.36)	27.20 (4.59)	0.945	3.18 (0.61)	3.33 (0.16)	0.694
Spleen	CTL (CD45+CD3+CD8+)	29.03 (5.14)	36.90 (14.38)	0.423	3.39 (0.17)	3.59 (0.36)	0.432
Esophagus	CD44+CTL	46.33 (27.57)	34.00 (9.44)	0.504	3.73 (0.65)	3.53 (0.30)	0.652
Spleen	CD44+CTL	35.53 (10.42)	37.27 (3.07)	0.796	3.57 (0.32)	3.64 (0.08)	0.710
Esophagus	MDSC (CD45+Gr1+CD11b+)	2.42 (1.76)	14.76 (12.20)	0.158	1.10 (0.67)	2.57 (0.72)	0.061
Spleen	MDSC (CD45+Gr1+CD11b+)	2.68 (2.50)	24.43 (23.27)	0.183	1.13 (0.76)	2.93 (0.97)	0.065
Esophagus	DC (CD45+CD11c+)	4.73 (2.14)	4.19 (1.24)	0.726	1.70 (0.38)	1.63 (0.23)	0.800
Spleen	DC (CD45+CD11c+)	2.49 (0.55)	3.17 (0.25)	0.118	1.24 (0.16)	1.43 (0.06)	0.133
Esophagus	NK (CD45+CD49b+)	3.23 (0.97)	3.60 (0.98)	0.666	1.42 (0.25)	1.51 (0.22)	0.673
Spleen	NK (CD45+CD49b+)	2.64 (0.80)	1.74 (0.75)	0.229	1.27 (0.23)	0.98 (0.30)	0.251
Esophagus	Macrophages (CD45+F4/80+)	4.26 (2.66)	4.89 (1.59)	0.743	1.54 (0.65)	1.75 (0.30)	0.644
Spleen	Macrophages (CD45+F4/80+)	2.68 (0.57)	5.03 (2.98)	0.249	1.29 (0.15)	1.72 (0.45)	0.194

Model based type III p-value for mutant / control log transformed immune cell counts

Cell type	**Pr > ChiSq**
CD45+	0.442
T cells (CD45+CD3+)	0.911
Th (CD45+CD3+CD4+)	0.582
CD44+ Th	0.194
Tregs (CD45+CD3+CD4+CD44+FoxP3+)	0.391
Tregs (CD45+CD3+CD4+FoxP3+)	0.423
CTL (CD45+CD3+CD8+)	0.279
CD44+CTL	0.793
MDSC (CD45+Gr1+CD11b+)	0.036
DC (CD45+CD11c+)	0.659
NK (CD45+CD49b+)	0.547
Macrophages (CD45+F4/80+)	0.227

Supplementary Table 3. Clinicopathological characteristics of patients with esophageal squamous cell carcinoma.

Characteristic	No. of patients (n=96)	Percent
Sex		
Male	85	88.54
Female	11	11.46
Age		
≤ 60	26	27.08
> 60	70	72.9
Histological grade		
Well differentiated	38	39.58
Moderately differentiated	40	41.67
Poorly differentiated	18	18.75
Tumor Stage		
T1	5	5.21
T2A	19	19.79
T2B	9	9.38
T3	27	28.13
T4	36	37.50

Supplementary Table 4. Number of patients used for the scoring of each marker across pathology diagnosis

Pathology diagnosis	Marker	N
Normal esophagus	GM-CSF	5
	MVD	5
	p65	5
	TNF	5
	CD45	5
Adjacent normal	GM-CSF	38
	MVD	12
	p65	38
	TNF	38
	CD45	38
Adjacent inflammation	GM-CSF	12
	MVD	4
	p65	12
	TNF	12
	CD45	12
ESCC	GM-CSF	96
	MVD	23
	p65	96
	TNF	96
	CD45	96

Supplementary Table 5. Comparison of markers across pathologic diagnoses.

		(A) Normal esophagus	*(B) Adjacent Normal*	*(C) Adjacent Inflammation*	*(D) ESCC*
GM-CSF	*Mean*	0.00	0.89	1.33	2.36
	Std	0.00	0.83	0.89	0.90
	Min	0.00	0.00	0.00	0.00
	Q1	0.00	0.00	1.00	2.00
	Median	0.00	1.00	1.00	2.50
	Q3	0.00	1.50	2.00	3.00
	Max	0.00	2.50	3.00	4.00
MVD	*Mean*	21.50	36.79	52.03	79.42
	Std	6.17	5.03	9.18	14.43
	Min	14.50	30.70	41.60	43.00
	Q1	18.00	32.15	44.65	72.20
	Median	19.00	36.50	52.10	78.20
	Q3	27.50	40.45	59.40	85.20
	Max	28.50	45.00	62.30	113.60
p65	*Mean*	0.00	0.75	1.58	2.62
	Std	0.00	0.85	0.90	0.82
	Min	0.00	0.00	0.00	0.50
	Q1	0.00	0.00	1.00	2.00
	Median	0.00	0.75	2.00	2.50
	Q3	0.00	1.00	2.00	3.00
	Max	0.00	3.00	3.00	4.00

		(A) Normal esophagus	*(B) Adjacent Normal*	*(C) Adjacent Inflammation*	*(D) ESCC*
TNF	*Mean*	0.00	0.24	1.08	1.79
	Std	0.00	0.43	0.67	0.83
	Min	0.00	0.00	0.00	0.00
	Q1	0.00	0.00	1.00	1.00
	Median	0.00	0.00	1.00	1.75
	Q3	0.00	0.00	1.50	2.50
	Max	0.00	1.00	2.00	4.00
CD45	*Mean*	0.60	0.70	2.17	2.13
	Std	0.55	0.51	0.39	0.84
	Min	0.00	0.00	2.00	0.00
	Q1	0.00	0.00	2.00	1.50
	Median	1.00	1.00	2.00	2.00
	Q3	1.00	1.00	2.00	2.50
	Max	1.00	2.00	3.00	4.00

Supplementary Table 6. Correlations between markers in patients with esophageal squamous cell cancer.

Variable	*With Variable*	*N*	*Correlation Estimate*	*95% Confidence Limits*		*p Value for H0:Rho=0*
GM-CSF	MVD	23	0.33392	-0.090779	0.655848	0.1124
GM-CSF	p65	96	0.53097	0.369860	0.661085	<.0001
GM-CSF	TNF	96	0.36414	0.176539	0.526208	0.0002
GM-CSF	CD45	96	0.11539	-0.087107	0.308737	0.2611
MVD	p65	23	0.08161	-0.342102	0.477740	0.7082
MVD	TNF	23	0.01558	-0.399189	0.425050	0.9432
MVD	CD45	23	0.10477	-0.321309	0.495569	0.6304
p65	TNF	96	0.36480	0.177275	0.526756	0.0002
p65	CD45	96	0.00270	-0.197896	0.203073	0.9791
TNF	CD45	96	0.20332	0.002959	0.387995	0.0456

Supplementary Table 7. Correlations between markers and clinical variables in patients with esophageal squamous cell cancer.

Variable	*With Variable*	*N*	*Correlation Estimate*	*95% Confidence Limits*		*p Value for H0:Rho=0*
GM-CSF	months	96	0.10475	-0.097790	0.298957	0.3081
GM-CSF	age	96	0.07427	-0.128124	0.270725	0.4707
GM-CSF	stage	96	-0.11580	-0.309108	0.086700	0.2595
GM-CSF	grade	96	-0.10970	-0.303510	0.092828	0.2856
MVD	months	23	0.40290	-0.011156	0.699012	0.0510
MVD	age	23	-0.35999	-0.672416	0.061306	0.0849
MVD	stage	23	-0.06610	-0.465613	0.355799	0.7620
MVD	grade	23	-0.18718	-0.556448	0.243836	0.3862
p65	months	96	0.04780	-0.154165	0.245928	0.6428
p65	age	96	0.05873	-0.143443	0.256201	0.5687
p65	stage	96	-0.05357	-0.251358	0.148509	0.6032
p65	grade	96	-0.15431	-0.344150	0.047648	0.1316
TNF	months	96	0.13724	-0.065032	0.328685	0.1806
TNF	age	96	0.17821	-0.023103	0.365631	0.0808
TNF	stage	96	-0.18959	-0.375791	0.011328	0.0628
TNF	grade	96	-0.09196	-0.287153	0.110563	0.3713
CD45	months	96	0.00420	-0.196451	0.204514	0.9675
CD45	age	96	0.05322	-0.148855	0.251027	0.6056
CD45	stage	96	-0.13497	-0.326617	0.067339	0.1880
CD45	grade	96	0.03515	-0.166505	0.233991	0.7331

Supplementary Figure legends

Supplementary Figure 1. Activated IKKβ was expressed in esophageal epithelia of *L2/IKKβca* mice (**A**) Targeting strategy for the generation of *ED-L2-Cre/Rosa26-IKK2ca*^{SFL} mice. (**B**) Western blot of epithelial scrapings from 1.5 month-old mice revealed activation of IKKβ/NFκB signaling in *L2/IKKβca* mice (Mut), demonstrated by increased IKKβ expression and reduced IκB levels compared to *IKKβca*^{SFL} controls (Con). β-actin was a loading control. **(C- D)** GFP (green) was not expressed in *IKKβca*^{SF} mice **(C)** but was observed in esophageal epithelia of *L2/IKKβca* mice **(D)**, indicating Cre-mediated recombination in these cells. DAPI was used as a nuclear stain. Scale bars, 10μm.

Supplementary Figure 2. *L2/IKKβca* mutant mice had increased proliferation and apoptosis in esophageal epithelia. **(A)** Quantitation of BrdU-labeled cells revealed a statistically significant increase in cell proliferation in *L2/IKKβca* mutant mice ($P<0.05$) at 4 weeks, 6 weeks, and 3 months of age. **(B-C)** Compared with littermate controls **(B)**, *L2/IKKβca* mutant mice **(C)** showed increased numbers of apoptotic cells in the superficial layers of esophageal epithelia at 3 months of age by TUNEL staining. DAPI was used as a nuclear stain. Scale bars, 50μm.

Supplementary Figure 3. *L2/IKKβca* mutant mice had squamous epithelial hypertrophy of the tongue and the ventral neck skin but not the forestomach. Control tongue (A), forestomach (C), and skin (E), from 1.5 month-old mice were examined, as well as tongue (B), forestomach (D), and skin (F), of matched *L2/IKKβca* mice. Scale bars, 50μm (A-D), 100μm (E-F).

Supplementary Figure 4. Activation of epithelial IKKβ signaling leads to increased GM-CSF expression in esophageal epithelia of mice. **(A-B)** At 4 weeks of age, *L2-IKKβca* mice *(***B)** had

increased GM-CSF, indicated by brown staining within epithelial cells, compared to littermate controls **(A)**. Scale bars, 50μm.

Supplementary Figure 5. Intraepithelial leukocytes were present in *L2/IKKβca* mice treated with an IgG2a control antibody **(A)** or with a blocking antibody against GM-CSF **(B)**, as indicted by staining for CD45. Scale bars: 25μm.

Supplementary Figure 6. Representative IHC panels from normal esophagus, cancer adjacent area (normal), cancer adjacent (inflammation) and ESCC from human patients. Expression of GM-CSF, VWF, p65 NFκB, TNF and CD45 increased progressively in cancer adjacent area with inflammation and ESCC, compared to normal esophagus and cancer adjacent area (normal). Scale bars: 50μm.

Supplementary Figure 7. p65 NFκB expression increases from normal to inflamed tissues and from inflamed tissues to ESCC. **(A)** Box plots indicate the scoring of p65 NFκB expression by pathologic diagnosis for human patients with normal esophagus, esophageal cancer adjacent area (normal), esophageal cancer adjacent area (inflammation), and esophageal squamous cell cancer (ESCC, tumor). *Dots* represent outliners and *diamonds* represents the mean values. The number of patients analyzed per pathologic diagnosis is detailed in Supplementary Table 4. (**B-C**) Overall (**B**) and pairwise (**C**) significance of the data represented in (**A**). All statistical tests assumed a 5% level of significance.

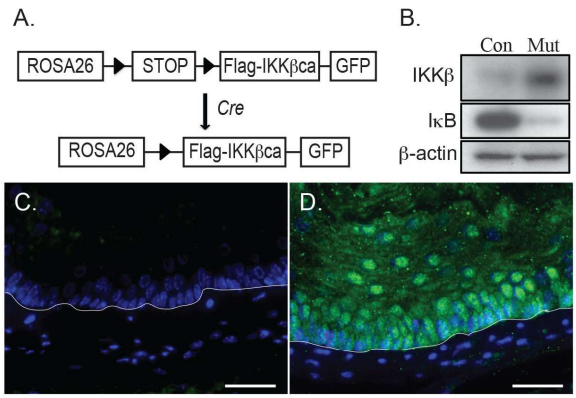
Supplementary Figure 8. Increasing angiogenesis is seen during progression from normal to inflamed tissues and from inflamed tissues to ESCC. **(A)** Box plots indicate the scoring of microvessel density (MVD) by pathologic diagnosis for human patients with normal esophagus, esophageal cancer adjacent area (normal), esophageal cancer adjacent area (inflammation), and

esophageal squamous cell cancer (ESCC, tumor). *Dots* represent outliners and *diamonds* represents the mean values. The number of patients analyzed per pathologic diagnosis is detailed in Supplementary Table 4. (**B-C**) Overall (**B**) and pairwise (**C**) significance of the data represented in (**A**). All statistical tests assumed a 5% level of significance.

Supplementary Figure 9. GM-CSF expression increases from normal to inflamed tissues and from inflamed tissues to ESCC. **(A)** Box plots indicate the scoring of GM-CSF levels by pathologic diagnosis for human patients with normal esophagus, esophageal cancer adjacent area (normal), esophageal cancer adjacent area (inflammation), and esophageal squamous cell cancer (ESCC, tumor). *Dots* represent outliners and *diamonds* represents the mean values. The number of patients analyzed per pathologic diagnosis is detailed in Supplementary Table 4. (**B-C**) Overall (**B**) and pairwise (**C**) significance of the data represented in (**A**). All statistical tests assumed a 5% level of significance.

Supplementary Figure 10. TNF expression increases from normal to inflamed tissues and from inflamed tissues to ESCC. **(A)** Box plots indicate the scoring of TNF expression by pathologic diagnosis of human patients with normal esophagus, esophageal cancer adjacent area (normal), esophageal cancer adjacent area (inflammation), and esophageal squamous cell cancer (ESCC, tumor). *Dots* represent outliners and *diamonds* represents the mean values. The number of patients analyzed per pathologic diagnosis is detailed in Supplementary Table 4. (**B-C**) Overall (**B**) and pairwise (**C**) significance of the data represented in (**A**). All statistical tests assumed a 5% level of significance.

Supplementary Figure 11. CD45 expression is increased in ESCC compared to normal esophagus. **(A)** Box plots indicate the scoring of CD45 staining by pathologic diagnosis of human patients with normal esophagus, esophageal cancer adjacent area (normal), esophageal cancer adjacent area (inflammation), and esophageal squamous cell cancer (ESCC, tumor). *Dots* represent outliners and *diamonds* represents the mean values. The number of patients analyzed per pathologic diagnosis is detailed in Supplementary Table 4. (**B-C**) Overall (**B**) and pairwise (**C**) significance of the data represented in (**A**). All statistical tests assumed a 5% level of significance.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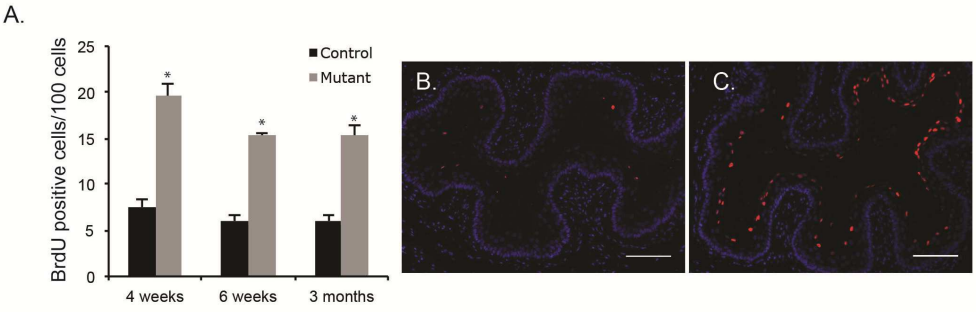
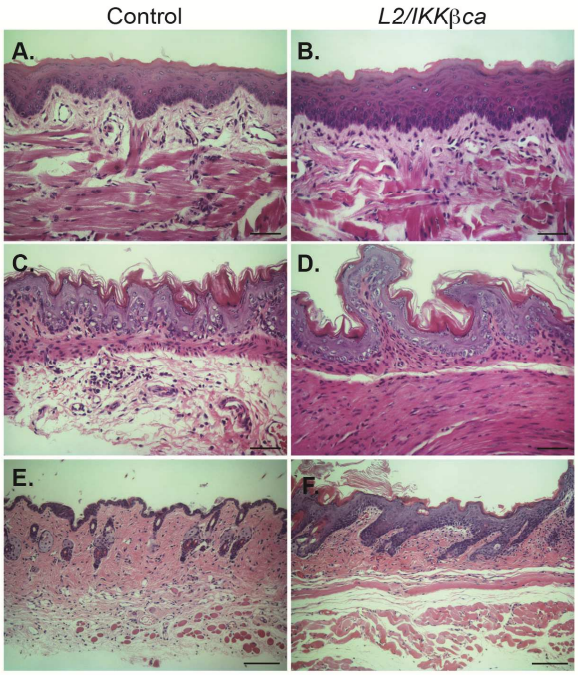
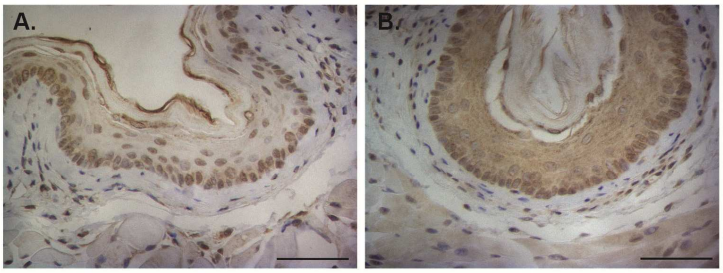
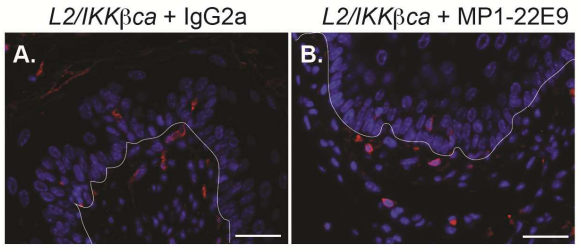
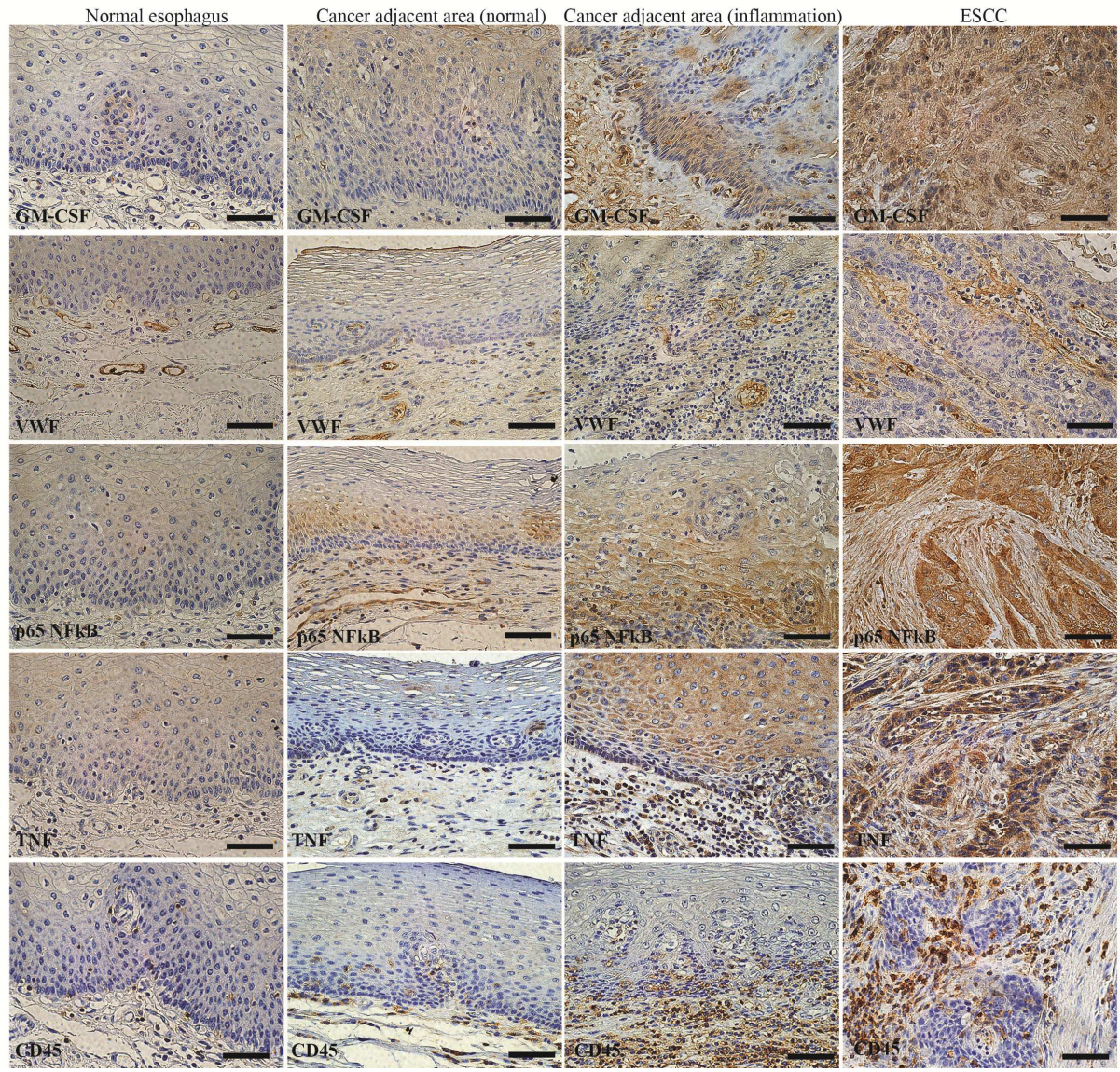
IκB
β-actin
C.
D.


A.
BrdU positive cells/100 cells
25
20
15
10
5
0
Control
Mutant
*
*
*
4 weeks
6 weeks
3 months
B.
C.

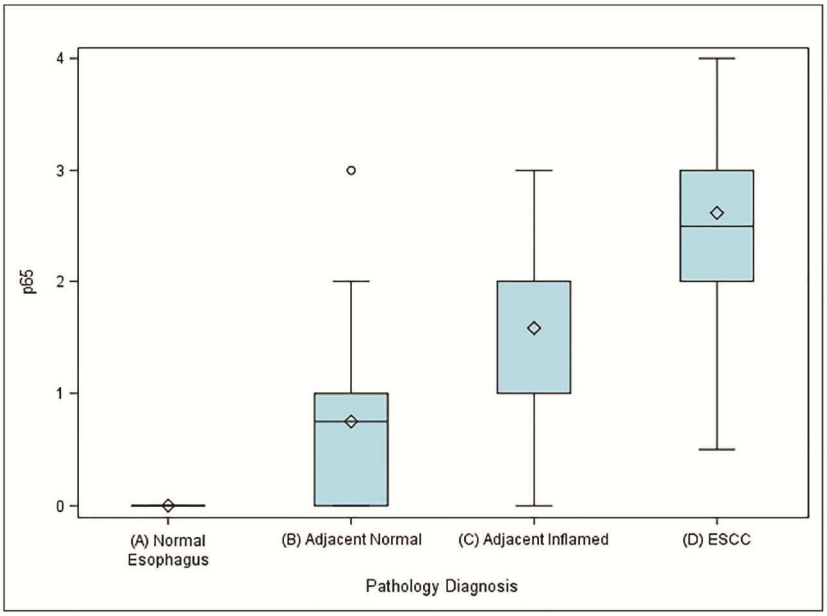

Control
L2/IKKβca
A.
B.
C.
D.
E.
F.


A.
B.


L2/IKKβca + IgG2a
L2/IKKβca + MP1-22E9
A.
B.


Normal esophagus
Cancer adjacent area (normal)
Cancer adjacent area (inflammation)
ESCC
GM-CSF
VWF
p65 NFkB
TNF
CD45

A.



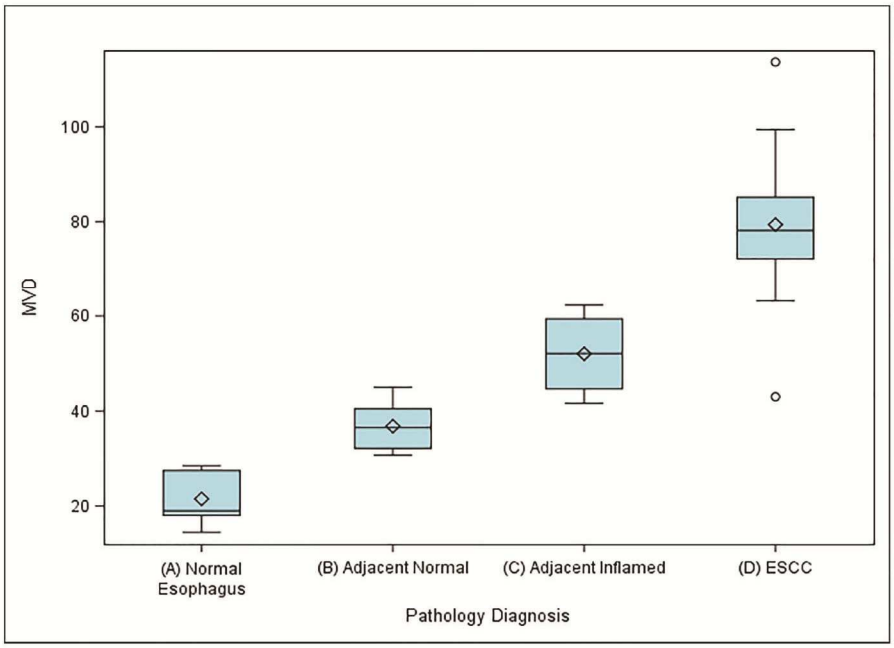
B.

Pathology diagnosis	Pr > |t|
Normal esophagus	1.0000
Adjacent normal	<.0001
Adjacent inflammation	<.0001
ESCC	<.0001

C.

Pathology diagnosis	Pathology diagnosis	Adjusted P value
Adjacent normal	Normal esophagus	0.2440
Adjacent Inflammation	Normal esophagus	0.0032
ESCC	Normal esophagus	<.0001
Adjacent normal	Adjacent inflammation	0.0065
Adjacent normal	ESCC	<.0001
ESCC	Adjacent inflammation	0.0004

A.



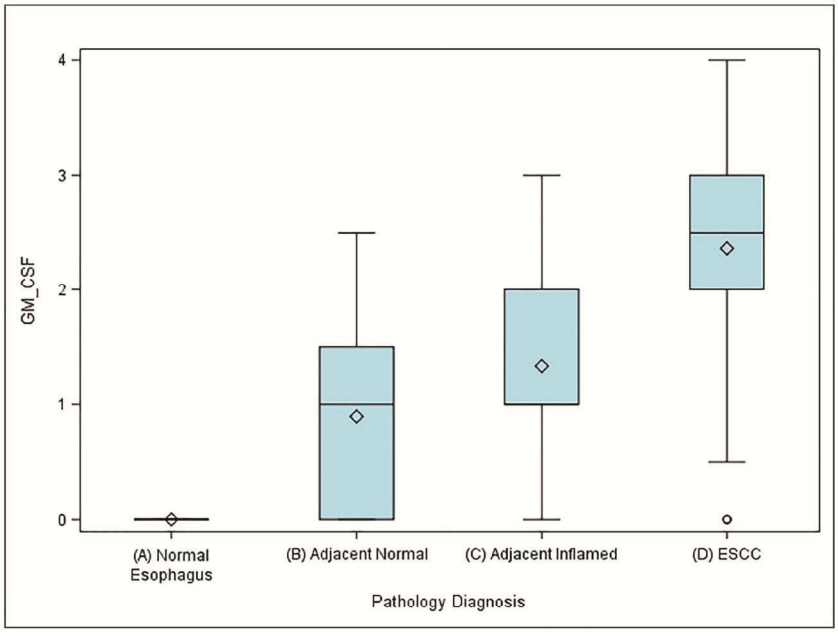
B.

Pathology diagnosis	Pr > |t|
Normal esophagus	0.0015
Adjacent normal	<.0001
Adjacent inflammation	<.0001
ESCC	<.0001

C.

Pathology diagnosis	Pathology diagnosis	Adjusted P value
Adjacent normal	Normal esophagus	0.1014
Adjacent Inflammation	Normal esophagus	0.0126
ESCC	Normal esophagus	<.0001
Adjacent normal	Adjacent inflammation	0.1014
Adjacent normal	ESCC	<.0001
ESCC	Adjacent inflammation	0.0016

A.



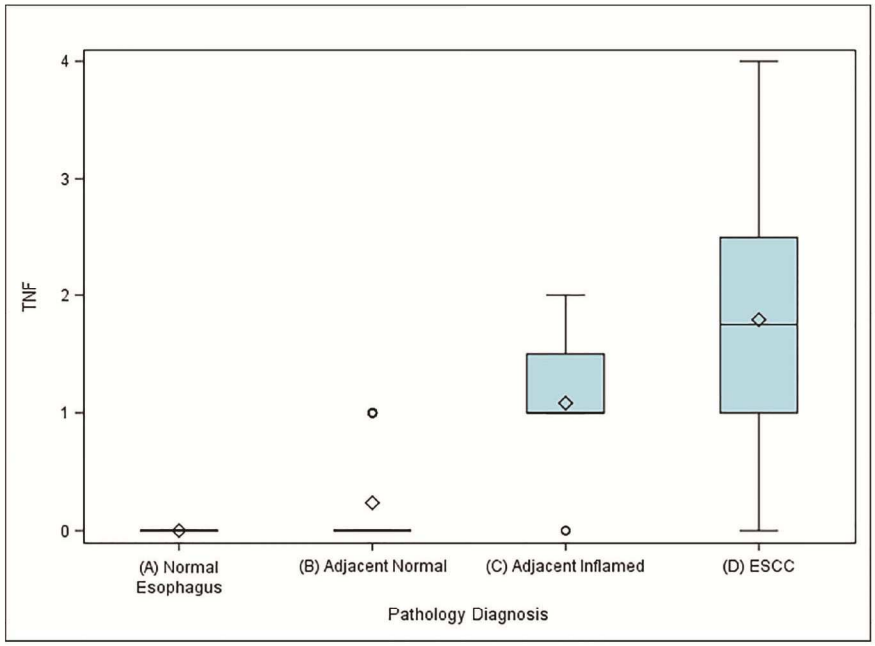
B.

Pathology diagnosis	Pr > |t|
Normal esophagus	1.0000
Adjacent normal	<.0001
Adjacent inflammation	<.0001
ESCC	<.0001

C.

Pathology diagnosis	Pathology diagnosis	Adjusted P value
Adjacent normal	Normal esophagus	0.1443
Adjacent Inflammation	Normal esophagus	0.0179
ESCC	Normal esophagus	<.0001
Adjacent normal	Adjacent inflammation	0.2260
Adjacent normal	ESCC	<.0001
ESCC	Adjacent inflammation	0.0015

A.



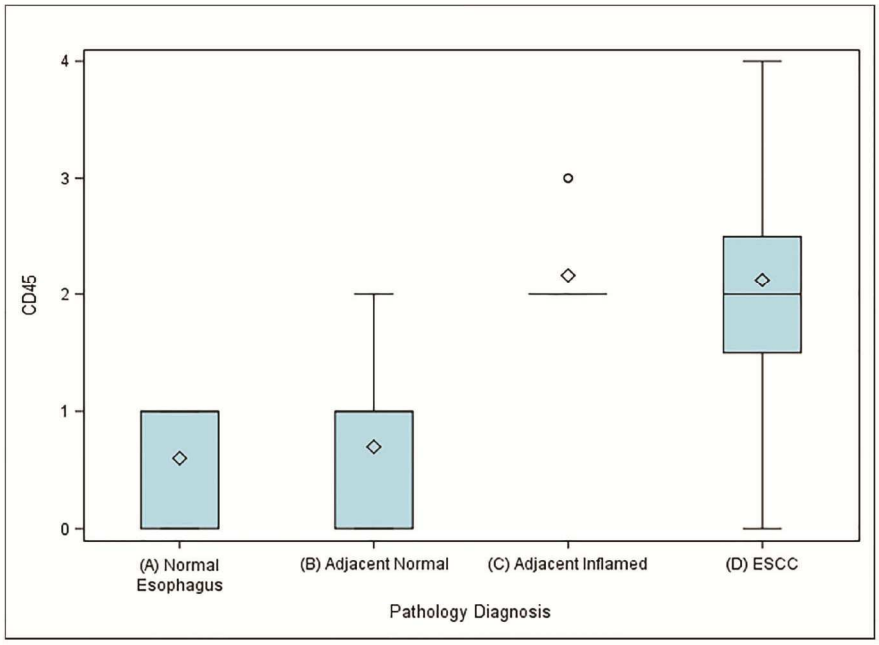
B.

Pathology diagnosis	Pr > |t|
Normal esophagus	1.0000
Adjacent normal	0.0349
Adjacent inflammation	<.0001
ESCC	<.0001

C.

Pathology diagnosis	Pathology diagnosis	Adjusted P value
Adjacent normal	Normal esophagus	0.8869
Adjacent Inflammation	Normal esophagus	0.0234
ESCC	Normal esophagus	<.0001
Adjacent normal	Adjacent inflammation	0.0018
Adjacent normal	ESCC	<.0001
ESCC	Adjacent inflammation	0.0117

A.



B.

Pathology diagnosis	Pr > |t|
Normal esophagus	0.0748
Adjacent normal	<.0001
Adjacent inflammation	<.0001
ESCC	<.0001

C.

Pathology diagnosis	Pathology diagnosis	Adjusted P value
Adjacent normal	Normal esophagus	0.9921
Adjacent Inflammation	Normal esophagus	0.0017
ESCC	Normal esophagus	0.0004
Adjacent normal	Adjacent inflammation	<.0001
Adjacent normal	ESCC	<.0001
ESCC	Adjacent inflammation	0.9976