Novel long-lived esophageal progenitor cells contribute to homeostasis and regeneration
Giroux et al.

Supplemental Data

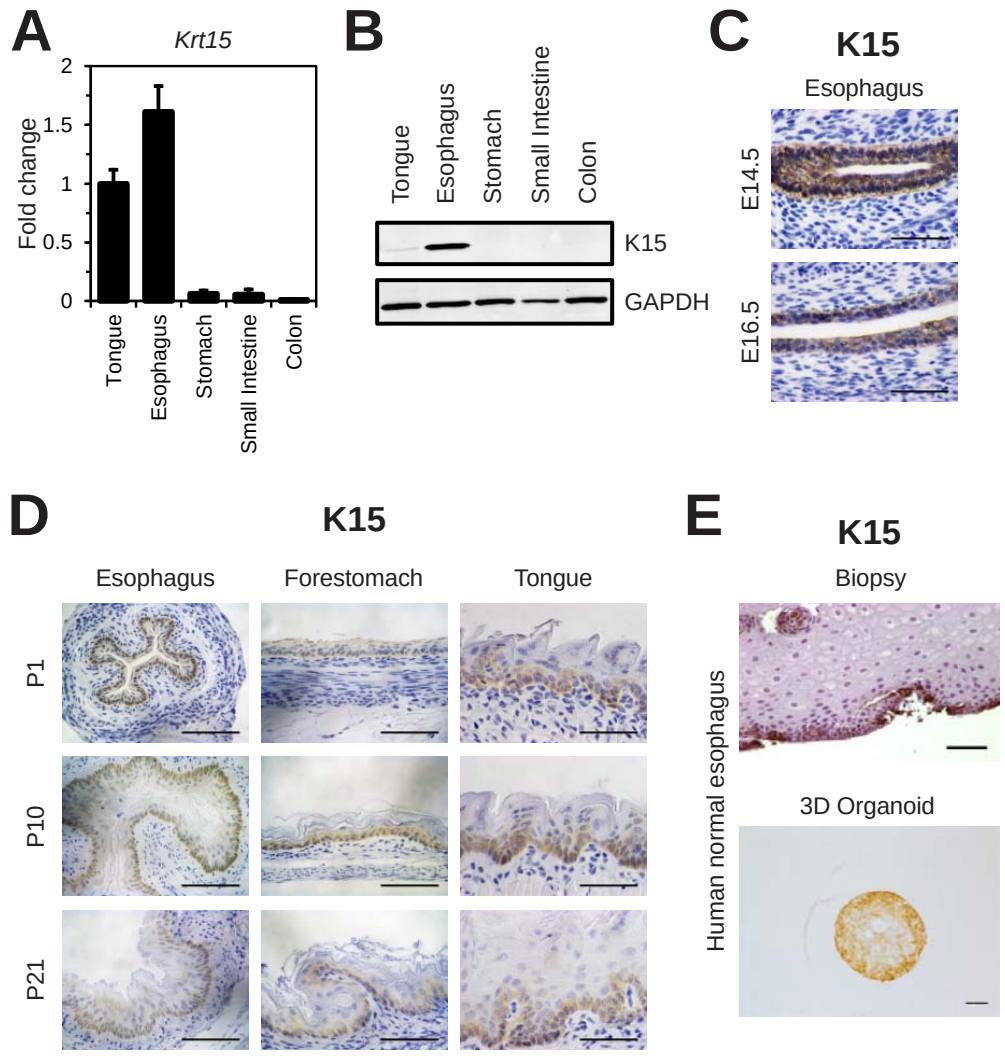

Figure S1: K15 is expressed in the esophagus of embryo and newborn mice
(A) qPCR for *Krt15* mRNA expression in several digestive tract tissues. Graph represents mean ± SEM (n=4). **(B)** Western Blot for K15 in mouse digestive tissues. **(C)** Immunohistochemistry for K15 in mouse embryonic esophagus (E14.5 and E16.5). **(D)** Immunohistochemistry for K15 in the esophagus, forestomach and tongue of newborn mice (P1, P10 and P21). **(E)** Immunohistochemistry for K15 in normal human esophagus and 3D organoids derived from normal human esophagus biopsies.
Scale bar = 50µm.

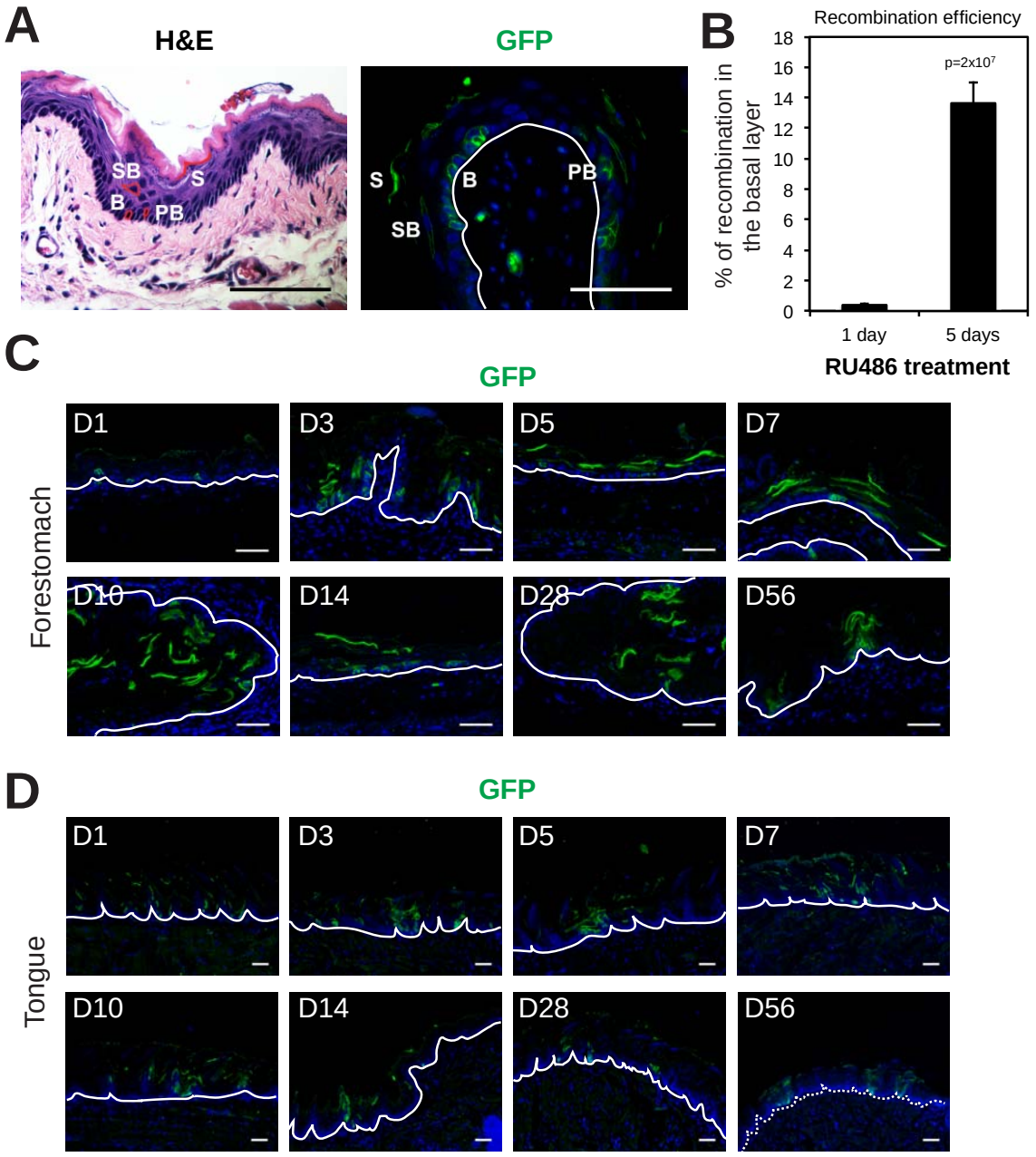

Figure S2: *Krt15* marks long-lived cells in the mouse forestomach and tongue

(A) Localization of basal [B], parabasal [PB], suprabasal [SB] and superficial [S] cells in normal esophagus as well as in an example of lineage-tracing experiment in *Krt15-CrePR1;R26*$^{mT/mG}$ mice. **(B)** *Krt15-CrePR1;R26*$^{mT/mG}$ mice were injected daily with 0.5mg RU486 to induce Cre recombination for 1 or 5 consecutive days and sacrificed one day following the last RU486 injection. Percentage of GFP+ cells in the basal layer was assessed. Graph represents mean ± SEM (n=4). **(C-D)** *Krt15-CrePR1;R26*$^{mT/mG}$ mice were injected daily with 0.5mg RU486 for 5 consecutive days and sacrificed at different time points. GFP (*Krt15*-derived cells) immunofluorescence in the forestomach **(C)** and the tongue **(D)**.
Dotted line marks the basement membrane. Scale bar = 50μm.

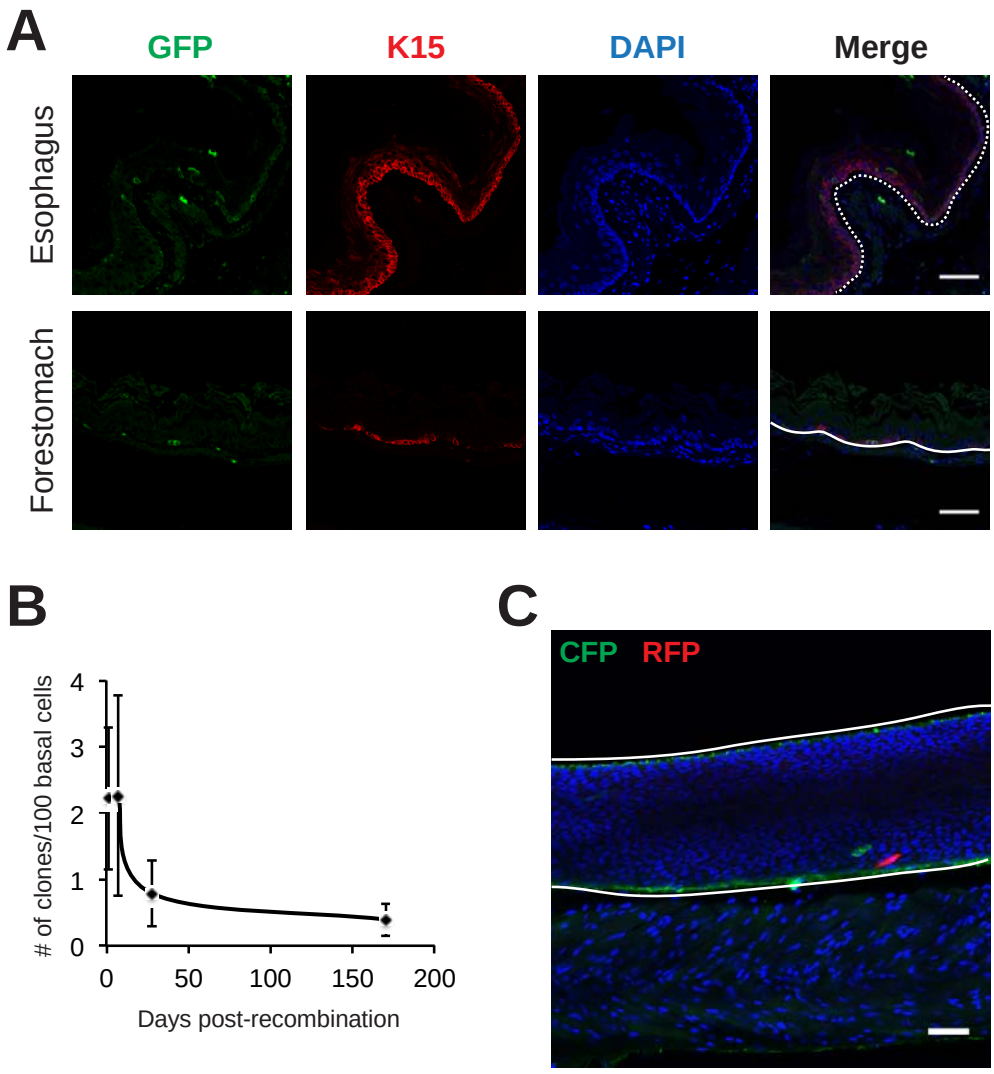

Figure S3: *Krt15*+ clones are maintained up to 6 months
(A) *Krt15-CrePR1;R26*$^{mT/mG}$ mice were injected daily with 0.5mg RU486 for five consecutive days and sacrificed one day after the last RU486 injection. Immunofluorescence for GFP (*Krt15*-derived cells) and K15 in esophagi. **(B)** *Krt15-CrePR1;R26*$^{mT/mG}$ mice were injected daily with 0.5mg RU486 for five consecutive days and sacrificed at different time points. Number of GFP clones was counted. Graph represents mean ± SD (n=4-5 mice/group, cross-sections of 4 different regions of the esophagus were analyzed for each mouse). Statistical significance was determined by the Wald chi-square test ($X^2(3)=106.28$, $p<0.0001$). **(C)** *Krt15-CrePR1;R26*^{Conf} mice were injected every 12 hours with 1mg of RU486 for 10 consecutive days and sacrificed 2 months later. Confocal microscopy of one CFP clone and one RFP clone.

Dotted line marks the basement membrane. Scale bar = 50μm.

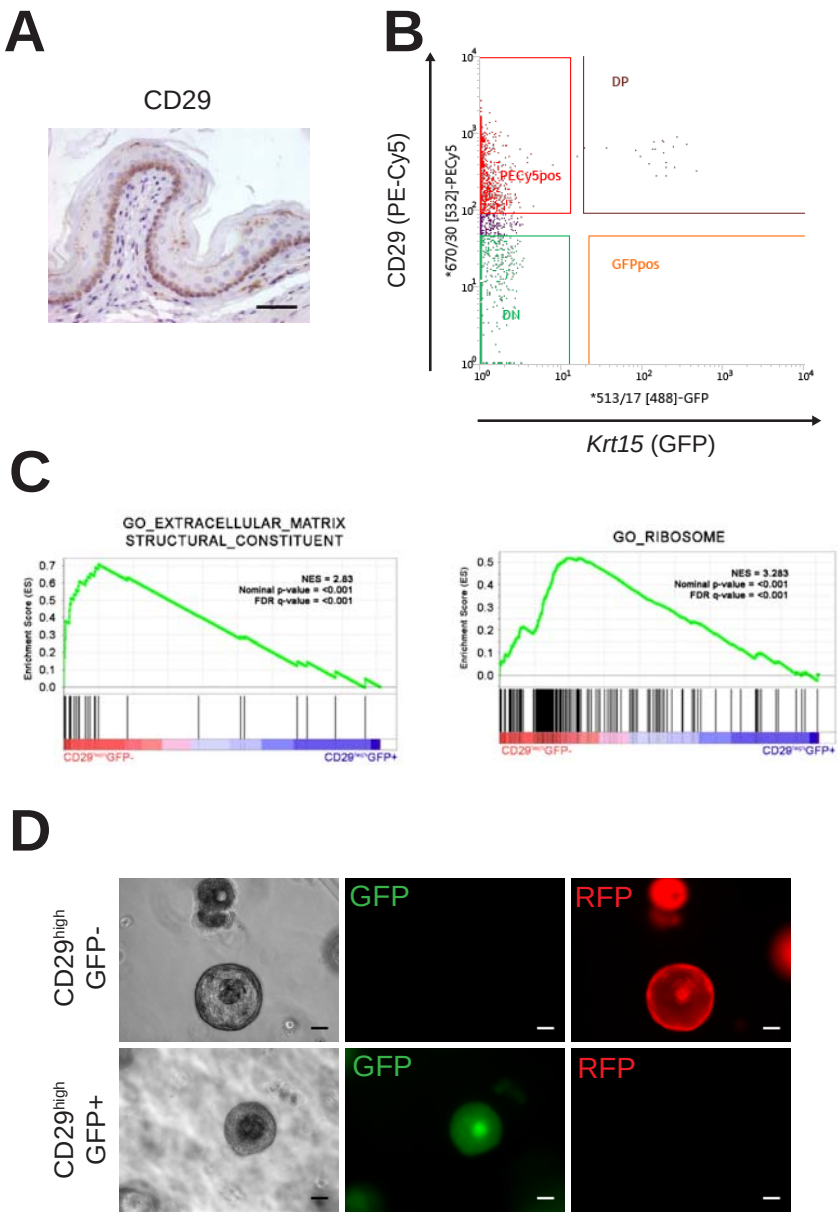

Figure S4: *Krt15*- cells are enriched for ECM constituents and ribosome gene sets
(A) CD29 immunohistochemistry in WT mouse esophagus. **(B-D)** *Krt15-CrePR1;R26mT/mG* mice were injected once with 0.5mg RU486 and sacrificed 24h later. CD29^{high}GFP+ (*Krt15*+ basal cells) and CD29^{high}GFP- (*Krt15*- basal cells) cells were sorted. **(C)** Transcriptional profiles of both cell populations were determined by RNA-seq. Plots for two of the gene sets enriched in *Krt15*- basal cells. **(D)** GFP and Tomato detection in CD29^{high}GFP- and CD29^{high}GFP+ cells-derived organoids.
Scale bar = 50µm.

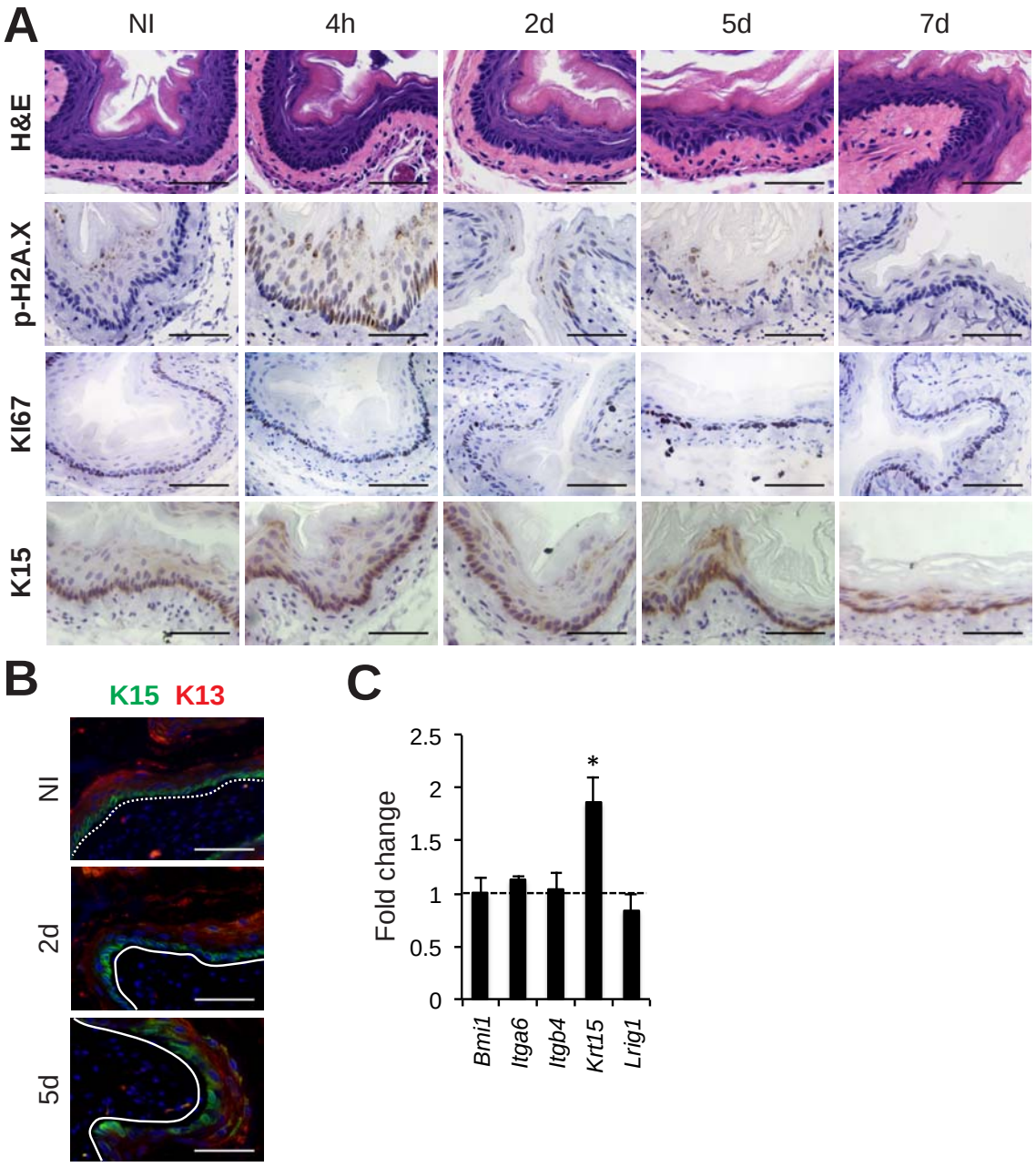

Figure S5: K15 is mislocalized following irradiation

(A-B) C57BL/J6 WT mice were subjected to 12 Gy whole-body irradiation and sacrificed 4h, 2d, 5d and 7d following irradiation **(A)** H&E and immunohistochemistry for p-H2A.X, KI67 and K15 in the esophagi of non-irradiated (NI) and irradiated mice. **(B)** Colocalization of K15 and K13 in the esophagi of non-irradiated (NI) and irradiated mice. **(C)** Organoids-derived from C57BL/J6 WT mice were submitted to 4 Gy irradiation 24h after seeding and harvested 7d later. Graph represents relative gene expression between irradiated and non-irradiated 3D organoids (mean ± SEM, n=3, *represents $p \leq 0.05$ *vs.* non-irradiated). Dotted line indicates the basement membrane. Scale bar = 50µm.

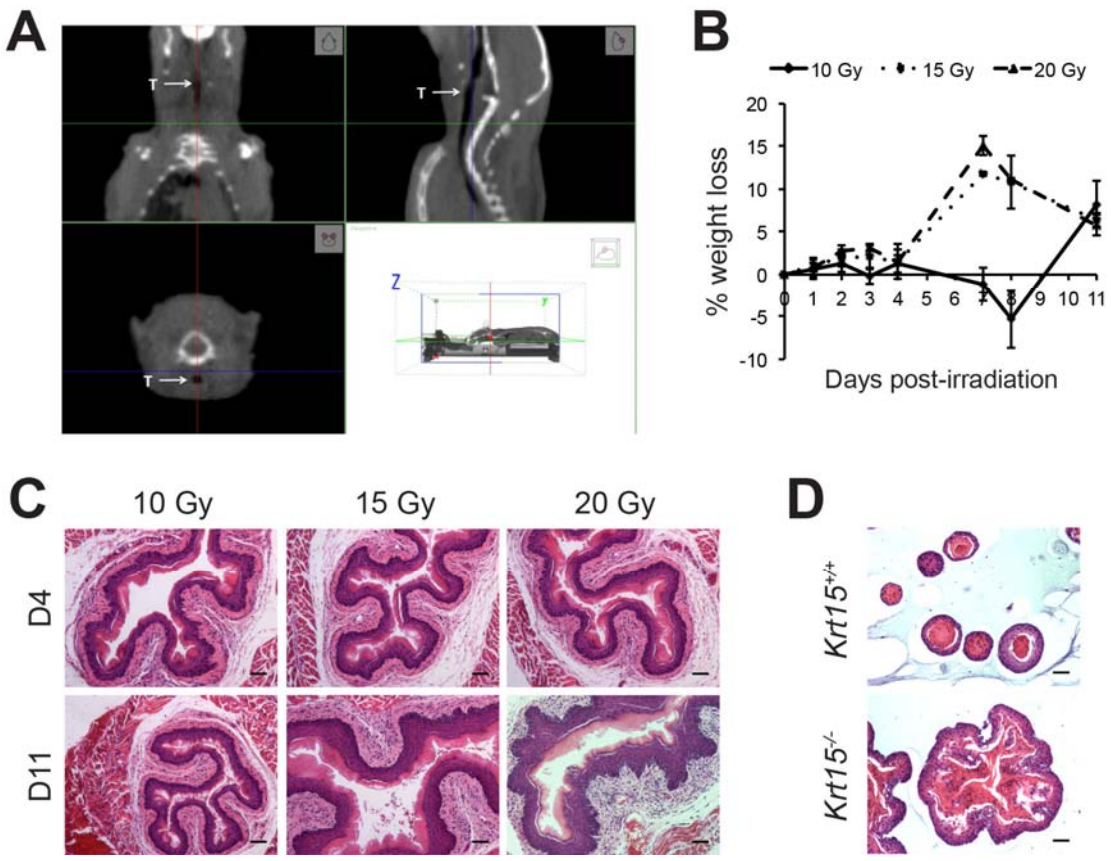

Figure S5: Esophageal-targeted irradiation

(A) Esophagi were locally irradiated in mice. Following CT scanning, isocenter was determined using MuriSlice Software. Esophagi of the mice were then irradiated at the isocenter using a 3x12mm collimator at a 90° angle. T indicates the trachea. **(B-C)** Esophagi of C57BL/J6 WT mice were locally irradiated with 10, 15 or 20 Gy and mice were sacrificed 4 or 11 days following irradiation. **(B)** Weight loss was assessed. Graph represents mean ± SEM (n=4 mice/dose). **(C)** H&E staining of irradiated mice with 10, 15 or 20 Gy of esophagus-targeted radiation and sacrificed at D4 or D11 following radiation. **(D)** *Krt15*$^{+/+}$ and *Krt15*$^{-/-}$ mice were subjected to esophagus-targeted 20 Gy irradiation and sacrificed 15 days following radiation. H&E staining of 3D organoids grown from esophageal epithelial cells isolated from those mice.
Scale bar = 50µm.

Table S1: Up-regulated genes in *Krt15+* basal cells vs. *Krt15-* basal cells

Gene ID	Gene Full Name	Fold Change (log2)	p-value (adj)
Msh2	MutS Homolog of, 2	9.23	6.3E-07
Slc12a2	Solute Carrier Family 12 (Sodium/Potassium/Chloride Transporter), Member 2	8.63	1.4E-05
Ascl2	Achaete-Scute Complex, Drosophila, Homolog of, 2	8.55	5.8E-06
Sh3bp4	SH3 domain-binding protein 4	8.39	3.2E-05
Sectm1b	Secreted and Transmembrane 1	8.01	0.0002
Zcchc14	Zinc Finger CCHC Domain-containing protein 14	7.97	0.0001
Btbd11	BTB/POZ domain-containing Protein 11	7.97	0.0002
Orc5	Origin Recognition Complex, Subunit 5, Sc. Cerevisiae, Homolog of	7.83	0.00009
Zfp868	Zinc Finger Protein 868	7.77	0.0004
Rad51b	Rad51, S. Cerevisiae, Homolog of, B	7.77	0.0004
Nup133	Nucleoporin, 133-KD	7.75	0.0004
Zufsp	Zinc Finger With UFM1 Specific Peptidase Domain	7.74	0.0004
Ubr3	Ubiquitin Protein Ligase E3 Component N-Recognin 3	7.65	0.0002
Pds5a	PDS5 Cohesion Associated Factor A	7.62	0.0006
Epha2	Ephrin Receptor EphA2	7.61	0.0001
Slc30a4	Solute Carrier Family 30 Member 4	7.59	0.0006
Gripap1	GRIP1-associated protein 1	7.59	0.0003
A830080D01 Rik		7.56	0.0007
Cnksr3	Connector Enhancer Of KSR 3	7.43	0.001
Amer1	APC Membrane Recruitment Protein 1	7.36	0.001
Sgcb	Sarcoglycan Beta	7.32	0.001
Foxj3	Forkhead Box J3	7.26	0.002
Dgkd	Diacylglycerol Kinase Delta	7.24	0.0007
E230016M11 Rik		7.23	0.002
Parg	Poly(ADP-Ribose) Glycohydrolase	7.17	0.0001
Acer2	Alkaline Ceramidase 2	7.13	0.003
Srfbp1	Serum Response Factor Binding Protein 1	7.10	0.003
2810001G20R ik		7.09	0.004
Zfp422	Zinc Finger Protein 22	7.03	0.003
D730001G18 Rik		7.00	0.004
Pwp2	PWP2 Periodic Tryptophan Protein Homolog	6.98	0.002
Trappc9	Trafficking Protein Particle Complex 9	6.97	0.004
Rdh10	Retinol Dehydrogenase 10	6.96	0.005
Zfp746	Zinc Finger Protein 746	6.95	0.005
Slc19a2	Solute Carrier Family 19 Member 2	6.95	0.006

Tnc	Tenascin C	6.94	0.004
Zbtb40	Zinc Finger And BTB Domain Containing 40	6.85	0.006
MARCH8	Membrane Associated Ring-CH-Type Finger 8	6.78	0.007
Hmgxb4	HMG-Box Containing 4	6.76	0.007
Epb4.1l5	Erythrocyte Membrane Protein Band 4.1 Like 5	6.76	0.002
Pla2g7	Phospholipase A2 Group VII	6.73	0.007
Ust	Uronyl 2-Sulfotransferase	6.68	0.01
Lrrc8d	Leucine Rich Repeat Containing 8 Family Member D	6.62	0.01
Atmin	ATM Interactor	6.61	0.003
1700019D03Rik		6.61	0.01
Arhgef19	Rho Guanine Nucleotide Exchange Factor 19	6.57	0.01
Aplf	Aprataxin And PNKP Like Factor	6.57	0.01
Zer1	Zyg-11 Related Cell Cycle Regulator	6.53	0.01
Katnal1	Katanin Catalytic Subunit A1 Like 1	6.51	0.005
B4galt7	Beta-1,4-Galactosyltransferase 7	6.48	0.01
Ermard	ER Membrane Associated RNA Degradation	6.46	0.01
Mxd1	Max Dimerization Protein 1	6.44	0.01
Slc25a27	Solute Carrier Family 25 Member 27	6.42	0.01
F2r	Coagulation Factor II Receptor	6.41	0.02
Cep350	Centrosomal Protein 350	6.41	0.02
Mboat2	Membrane Bound O-Acyltransferase Domain Containing 2	6.41	0.02
Tmem8	Transmembrane 8	6.41	0.02
Ythdc2	YTH domain-containing protein 2	6.40	0.02
Gtf2ird2	GTF2I Repeat Domain Containing 2	6.40	0.02
Serac1	Serine Active Site Containing 1	6.39	0.02
6030458C11Rik		6.37	0.02
Casd1	CAS1 Domain Containing 1	6.37	0.01
Zfp52	Zinc Finger Protein 52	6.36	0.02
Efcab1	EF-Hand Calcium Binding Domain 1	6.35	0.02
Ccdc125	Coiled-Coil Domain Containing 125	6.34	0.02
Mdm4	Mouse Double Minute 4 Homolog	6.34	0.01
Tecpr2	Tectonin Beta-Propeller Repeat Containing 2	6.33	0.02
Rbm48	RNA Binding Motif Protein 48	6.32	0.02
Fbxo42	F-Box only protein 42	6.26	0.01
2410131K14Rik		6.26	0.01
Stk3	Serine/Threonine Kinase 3	6.23	0.02
Myh10	Myosin Heavy Chain 10	6.22	0.00
Rlf	Rearranged L-Myc Fusion	6.19	0.01
Wdr91	WD Repeat Domain 91	6.19	0.03
Ttc21b	Tetratricopeptide Repeat Domain 21B	6.19	0.02

Brms1l	Breast Cancer Metastasis-Suppressor 1-Like	6.18	0.02
Dsg2	Desmoglein 2	6.18	0.01
Ttc26	Tetratricopeptide Repeat Domain 26	6.14	0.03
Zfp407	Zinc Finger Protein 407	6.09	0.03
Nek1	NIMA Related Kinase 1	6.09	0.01
9230114K14Rik		6.05	0.03
Ch25h	Cholesterol 25-Hydroxylase	6.04	0.04
Aox4		6.04	0.04
Dapp1	Dual Adaptor Of Phosphotyrosine And 3-Phosphoinositides 1	6.03	0.02
Asap3	ArfGAP With SH3 Domain, Ankyrin Repeat And PH Domain 3	5.99	0.04
Fzd8	Frizzled 8	5.90	0.04
AI607873		5.90	0.05
Flrt2	Fibronectin Leucine Rich Transmembrane Protein 2	5.89	0.03
Sirpa	Signal Regulatory Protein Alpha	5.88	0.05
Tbc1d16	TBC1 Domain Family Member 16	5.86	0.05
D930016D06Rik		5.86	0.02
Apcdd1	Apc, downregulated by, 1	5.83	0.05
Arrdc2	Arrestin Domain Containing 2	5.82	0.04
Atp7a	ATPase Copper Transporting Alpha	5.80	0.02
Slc22a4	Solute Carrier Family 22 Member 4	5.80	0.02
Gadd45a	Growth arrest- and DNA damage-inducible Gene, 45 alpha	5.79	0.01
Ptbp2	Polypyrimidine Tract Binding Protein 2	5.77	0.04
Ovol2	Ovo-like 2	5.72	0.01
Mkl1	Megakaryoblastic Leukemia (Translocation) 1	5.51	0.04
Synj1	Synaptojanin 1	5.45	0.04
Dhx38	DEAH-Box Helicase 38	5.44	0.03
Wdr11	WD Repeat Domain 11	5.42	0.02
Ranbp9	RAN Binding Protein 9	5.30	0.01
Ltn1	Listerin E3 Ubiquitin Protein Ligase 1	5.29	0.02
Ephb4	EPH Receptor B4	5.29	0.04
Zbtb21	Zinc Finger And BTB Domain Containing 21	5.22	0.04
Srrt	Serrate, RNA Effector Molecule	5.14	0.02
Akap11	A-Kinase Anchoring Protein 11	5.03	0.05
Smim1	Small Integral Membrane Protein 1	5.01	0.01
Eps15l1	Epidermal Growth Factor Receptor Pathway Substrate 15 Like 1	5.00	0.04
Polr2a	RNA Polymerase II Subunit A	4.87	0.04
Nf2	Neurofibromin 2	4.86	0.047
Nfat5	Nuclear Factor Of Activated T-Cells 5	4.72	0.047
Arhgap12	Rho GTPase Activating Protein 12	4.53	0.047

Prpf8	Pre-MRNA Processing Factor 8	4.51	0.049
Cnot1	CCR4-NOT Transcription Complex Subunit 1	4.24	0.047

Table S2: Down-regulated genes in *Krt15+* basal cells vs. *Krt15-* basal cells

Gene ID	Gene Full Name	Fold Change (log2)	p-value (adj)
Col3a1	Collagen Type III Alpha 1 Chain	-10.09	1.77E-10
Fxyd1	FXYD Domain Containing Ion Transport Regulator 1	-10.09	2.74E-08
Mfap5	Microfibrillar Associated Protein 5	-10.04	3.54E-10
Dpt	Dermatopontin	-9.60	1.46E-08
Cd302	CD302 Molecule	-9.31	1.14E-07
Col1a1	Collagen Type I Alpha 1 Chain	-9.08	5.41E-06
Saa3	Serum Amyloid A3 Pseudogene	-9.03	7.85E-06
Col1a2	Collagen Type I Alpha 2 Chain	-9.03	5.80E-06
Ctla2a	Cytotoxic T lymphocyte-associated protein 2 complex	-9.00	1.46E-05
Bgn	Biglycan	-8.65	4.42E-05
Sparcl1	SPARC Like 1	-8.21	0.0002
Ccl7	C-C Motif Chemokine Ligand 7	-8.04	0.0004
Rgs1	Regulator Of G-Protein Signaling 1	-7.90	0.0004
Serpinf1	Serpin Family F Member 1	-7.75	0.0004
Inmt	Indolethylamine N-Methyltransferase	-7.66	0.001
Pi16	Peptidase Inhibitor 16	-7.47	0.0001
Slit3	Slit Guidance Ligand 3	-7.46	0.001
Lims2	LIM Zinc Finger Domain Containing 2	-7.44	0.001
Bcl2l11	BCL2 Like 11	-7.36	0.002
Aspn	Asporin	-7.33	0.001
Nid1	Nidogen1	-7.32	0.001
Tnxb	Tenascin XB	-7.31	0.001
Col5a1	Collagen Type V Alpha 1 Chain	-7.27	0.002
Fbln2	Fibulin2	-7.25	0.0004
Lum	Lumican	-7.23	0.003
Cd37	CD37 Molecule	-6.96	0.003
Olfml2b	Olfactomedin Like 2B	-6.90	0.007
Cd34	CD34 Molecule	-6.89	0.007
Jam3	Junctional Adhesion Molecule 3	-6.87	0.005
Abca8a	ATP binding cassette Subfamily A Member 10	-6.86	0.006
Scara5	Scavenger Receptor Class A Member 5	-6.69	0.01
Clec3b	C-Type Lectin Domain Family 3 Member B	-6.63	0.01
Lrrc17	Leucine Rich Repeat Containing 17	-6.52	0.01
Ace2	Angiotensin I Converting Enzyme 2	-6.42	0.02
Rhoj	Ras Homolog Family Member J	-6.41	0.02
Dmtn	Dematin Actin Binding Protein	-6.37	0.02
Gpr133	Adhesion G Protein-Coupled Receptor D1	-6.17	0.03
Fbn1	Fibrillin1	-6.15	0.01
Sod3	Superoxide Dismutase 3, extracellular	-6.14	0.03

Postn	Periostin	-6.14	0.03
Lrrc32	Leurine Rich Repeat Containing 32	-6.13	0.03
Flt3l	Fms Related Tyrosine Kinase 3 Ligand	-6.09	0.03
Ptgis	Prostaglandin I2 (Prostacyclin) Synthase	-6.01	0.04
Ldlrad4	Low Density Lipoprotein Receptor Class A Domain Containing 4	-5.98	0.04
Igfbp6	Insulin Like Growth Factor Binding Protein 6	-5.85	0.004
Trappc12	Trafficking Protein Particle Complex 12	-5.21	0.03
Rarres2	Retinoic Acid Receptor Responder 2	-5.02	0.03
Ttc39c	Tetratricopeptide Repeat Domain 39C	-4.54	0.04

Supplemental Methods

Animal studies

Krt15-CrePR1 mice were bred with *R26*$^{mT/mG}$ mice and 6-week old progeny mice were used for lineage tracing experiments. Cre recombination was induced in *Krt15-CrePR1;R26*$^{mT/mG}$ mice by intraperitoneal (IP) injections of 0.5 mg of RU486 (Sigma-Aldrich). RU486 was dissolved in ethanol and then diluted in peanut oil. RU486 was administered daily for five continuous days except if mentioned otherwise and mice were then sacrificed at different time points (n=4-7 for each time point).

Krt15-CrePR1 mice were bred with *R26*^{Conf} mice and 6-week old progeny mice were used for lineage tracing experiments. Cre recombination was induced in *Krt15-CrePR1;R26*^{Conf} mice by IP injections of 1 mg of RU486 every 12 hours for ten continuous days and mice were then sacrificed at different time points (n=6-8 for each time point). Tissues were fixed in 4% PFA for 1 hr. and then incubated overnight in 30% sucrose before embedding in OCT compound.

Krt15-CrePR1 mice were bred with *R26*^{iDTR} and 6-week old progeny mice were used for *Krt15*+ cell ablation experiments. Cre recombination was induced in *Krt15-CrePR1;R26*^{iDTR} mice by IP injection of 0.5 mg of RU486 and DTR activation was induced by IP injection of 1ug *Diphteria Toxin* (DT, Sigma-Aldrich) diluted in PBS. Mice were administered RU486 daily for five continuous days followed by a single DT injection the following day. Mice were then sacrificed 12 days following DT injection (n=5 mice/group).

When experimental mice were sacrificed, they were generally injected with BrdU IP (0.1mg/g body weight) 1.5 hrs. prior to sacrifice to mark proliferative cells. Except if mentioned otherwise, esophagus, forestomach and tongue were removed and fixed in Zinc Formalin.

For each mouse, esophagi were cut in 4 pieces and embedded such that upon slide sectioning, we would obtain transverse planes of 4 different regions of the esophagi to control for regional differences.

Vibratome sectioning and Confocal Imaging

Tissue was embedded in 4% low melt agarose and 100μm thick cross sections were cut with a vibratome (Leica VT1000S). Sections were stained with anti-GFP (Abcam) and/or anti-RFP (Abcam) diluted 1:200 in PBS 5% BSA 0.1% Triton overnight. Sections were then incubated with secondary antibodies and DAPI. Confocal imaging was performed on a Leica SP8 confocal microscope with a 20X dry or 40X water objective. Application Suit (Leica) and Imaris software were used for image processing.

Esophageal epithelial cell isolation

Primary esophageal epithelial cells were cultured and maintained as described previously (Kalabis et al. 2012). Briefly, the esophagus was digested in Dispase I (BD Biosciences). Epithelial sheets were peeled and dissociated in 0.05% Trypsin-EDTA. Trypsinization was stopped using soybean trypsin inhibitor and epithelial cells were then filtered through a 40-μm cell strainer. Primary esophageal epithelial cells were expanded in Keratinocyte-serum free medium (KSFM) without $CaCl_2$ and supplemented with 0.018mM $CaCl_2$, 50ug/ml bovine pituitary extract, 5 ng/ml human recombinant EGF, 5 μg/ml gentamicin sulfate, 0.2% Fungizone® Antimycotic and penicillin-streptomycin.

EdU detection by Flow cytometry

Krt15-CrePR1;R26$^{mT/mG}$ mice were injected with 0.5mg of RU486 and sacrificed 24 or 48 hrs. following Cre recombination (n=4 mice/group). Before sacrifice, mice were injected IP with EdU (20mg/kg body weight). Esophagi were harvested and epithelial cells were isolated was described above. Cells were stained for EdU using Click-iT® Plus EdU Alexa Fluor® 647 Flow Cytometry Assay Kit (ThermoFisher Scientific) according to manufacturer's instructions. Cells were also stained with CD29-PE-Cy5 as described above. Percentage of EdU+ cells in *Krt15*+ and *Krt15*- basal cell populations was determined by flow cytometry.

Immunohistochemistry and immunofluorescence

Immunohistochemical (IHC) staining was performed as described previously (Long et al. 2015). Briefly, zinc formalin-fixed paraffin-embedded tissues were sectioned (6μm). Antigen retrieval was performed by

pressure cooking using citric acid pH6.0 buffer. For IHC, peroxidases were quenched with peroxide prior to blocking with avidin, biotin and blocking buffer at room temperature. Primary antibodies were incubated overnight at 4°C. Slides were then incubated with biotinylated antibodies for 30 min at 37°C and then with ABC Reagent (Vector Laboratories) for an additional 30 min at 37°C. Finally, slides were treated with DAB substrate and counterstained with hematoxylin. For immunofluorescence (IF), sections were blocked using blocking solution (PBS 1% BSA 0.3% Triton 5% serum) for 1h at room temperature and incubated with primary antibodies overnight at 4°C. Sections were then incubated with secondary antibodies for 1 hr. at room temperature and counterstained with DAPI. For GFP immunofluorescence, TSA® Amplification signal kit (Perkin-Elmer) was used according to the manufacturer's instructions. Antibodies used are as follows:

Table : Antibodies for IHC and IF

PROTEIN	SPECIES	DILUTION	CATALOG #	SUPPLIER
BrdU	Mouse	1:100	5292	Cell Signaling
E-CADHERIN	Rabbit	1:200	3195	Cell Signaling
GFP	Goat	1:200	ab6673	Abcam
Phospho-H2A.X	Rabbit	1:200	9718	Cell Signaling
KI67	Rabbit	1:200	ab16667	Abcam
K13	Mouse	1:200	sc-58721	Santa Cruz
K15	Rabbit	1:400	ab52816	Abcam
K15	Mouse	1:100	VP-C411	Vector Laboratories
p63	Mouse	1:200	sc-8431	Santa Cruz

RNA Extraction and qPCR

Total RNA was extracted from cells or tissues using the GeneJET™ RNA Purification Kit (Thermo Scientific) or from sorted cells using RNAqueous®-Micro Kit (Ambion) following the manufacturer's recommendations. RNA was then reverse transcribed with oligo-dT primers using the Taqman® Reverse Transcription Kit (Invitrogen) following the manufacturer's recommendations. QPCR was performed with Power SYBR® Green PCR Master Mix (Applied Biosystems) using the StepOnePlus™ Real-Time PCR System (Applied Biosystems). Primer sequences are listed in Supplemental Table S2.

Table : QPCR Primer Sequences

GENE	SPECIES	FORWARD	REVERSE
Bmi1	Mouse	CCAGACCACTCCTGAACATAAG	CTTCTCCTCGGTCTTCATTGG
Cd34	Mouse	TGGGCACCACTGGTTATTT	GAGGAGAGCACAAAGGAAGTAG
Cd71	Mouse	CTCGGCAAGTAGATGGAGATAAC	CGCTTACAATAGCCCAGGTAG
Cd73	Mouse	GACACTCCAACACCTTTCTCTAC	CATCATCTGCGGTGACTATGAA
Gapdh	Mouse	GGGTGTGAACCACGAGAAATA	AGTGATGGCATGGACTGTG
Itga6	Mouse	GCTTCCTCGTTTGGCTATGA	TCGAGTCTTTGGTCCCATTTAG
Itgb1	Mouse	GACAGTGTGTGTGTAGGAAGAG	ACTGCCAGTGTAATTGGGATAG
Itgb4	Mouse	TGGAGAGCAGCCTTGAAATC	GTCAGACATGGAGTTGGAGAAG
Krt15	Mouse	GCTGGTATTGGTGTCAGAGAAG	CCTGCACCAGACACTTAGATTT
Lrig1	Mouse	GAACACCTGAACCTTGGAGAG	CTGCAGCATCCTACCCATTAG
Prom2	Mouse	CACCATCCATGAACCTATCCTATC	CAGGTGCTTATCCAGGTCATAC
Smoc2	Mouse	TCGGCAGAACAAGACCAATAA	CTAGCACACACCAGCAGTATC

Western Blot

Protein lysis was performed using RIPA buffer supplemented with protease and phosphatase inhibitors. Proteins were separated by SDS-PAGE electrophoresis and transferred to PVDF membrane. Membrane was blocked using Odyssey® Blocking buffer (LI-COR). Primary antibodies (K15 1:1000 (ab52816, Abcam), GAPDH 1:10000 (MAB374, EMD Millipore) were incubated overnight at 4°C and secondary antibodies 1h at room temperature. Western blots were revealed using the Odyssey® Infrared Imager (LI-COR).

3D organoid culture

Epithelial cells were isolated from the mouse esophagus and 10,000 cells were seeded in 50µl Matrigel in 24-well plates. When sorted cells were used, 1,000 cells were seeded in 50µl Matrigel and passaged until a sufficient number of 3D organoids was obtained (n=4). After solidification, media (DMEM/F12, 1X Glutamax, 1X HEPES, 1X N2 Supplement, 1X B27 Supplement, 0.1mM N-Acetylcysteine, 50 ng/ml recombinant EGF, Noggin/R-Spondin conditioned media, 10µM Y27632) was added and replenished every 2-4 days. For human 3D organoids, cells were isolated from esophageal biopsies and 20,000 cells were seeded in 50µl Matrigel in 24-well plates. Media was as described before supplemented with 500nM A83-01, 10µM SB202190, 10nM Gastrin, 10mM Nicotiamide, 100ng/ml recombinant Wnt3a. For IHC or IF, 3D organoids were recovered from Matrigel with dispase digestion, fixed overnight in 4% paraformaldehyde (PFA) and embedded in 2% Bacto-Agar:2.5% gelatin. 3D organoids were grown for 7 to 10 days.

Human Samples

Normal de-identified human esophageal biopsies were obtained under an approved Institutional Review Board protocol.

Statistics

Values were presented as mean ± SEM, except mentioned otherwise. Two-tailed Student's t-test was used to determine the statistical significance except mentioned otherwise. A value of $p \leq 0.05$ was considered significant (*). To compare KI67 positivity in *Krt15*+ (GFP+) cells (Figure 3D), we performed a logistic regression. Odds Ratio/z-test was used to determine statistical significance and a value of $p \leq 0.05$ was considered significant (*). Wald chi-square test was also performed. To compare probability of having a KI67+ cells in the *Krt15*+ basal cells vs. the neighboring cells (Figure 3F), a repeated measures logistic regression was performed. Odds Ratios and z-test were performed following the regression. Also, chi-square test was performed to test the differences between each position. Clone counting was analyzed using binomial regression and chi-square test (Wald).

Study approval

Animal studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC), Philadelphia, USA. Human study was approved by the University of Pennsylvania Institutional Review Board (IRB), Philadelphia, USA. All subjects provided informed consent prior to their participation in the study.

Supplemental References

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