Supplementary Table 1: Human fibroblasts used in organotypic 3D culture

#	Name	Description/genotype	References
1	FSF2447	fetal skin fibroblasts	18
2	FSF2463	fetal skin fibroblasts	
3	FEF3	fetal esophageal fibroblasts	
4	FEF9003	fetal esophageal fibroblasts	
5	FEF3303	fetal esophageal fibroblasts	
6	FEF2008	fetal esophageal fibroblasts	
7	FEF4736	fetal esophageal fibroblasts	
8	FEF4736-puro	fetal esophageal fibroblasts (empty vector control)	
9	FEF4736	fetal esophageal fibroblasts stably expressing HGF	
10	TEF1947F1	ESCC-derived cancer-associated fibroblasts	

Supplementary Table 2: EPC2 cell derivatives used extensively in organotypic culture

#	Genotype (Selection markers), description	References
1	hTERT (H), immortalized	14,30
2	hTERT (HN), immortalized, control for #3, #4, and #5	31
3	hTERT;myrAKTΔ4-129-ER (HN), control for #6, inducible inactive form of AKT	
4	hTERT;myrAKT4-129-ER (HN), Tamoxifen-inducible constitutively active AKT	
5	hTERT;EGFR (HN)	
6	hTERT (HNP), immortalized, control for #10	19, 32
7	hTERT;EGFR (HNP), control for #10	

8	hTERT;p53^{R175H} (HNP), control for #10	
9	hTERT;p53^{R175H} (HNZ), control for #11	
10	hTERT;EGFR;p53^{R175H} (HNP)	
11	hTERT;EGFR;p53^{R175H} (HNZ)	
12	hTERT;EGFR;p53^{R175H} (HNZP), control for #17	17
13	hTERT;EGFR;p53^{R175H};POSTN (HNZP)	
14	hTERT;EGFR;p53^{R175H};non-silencing scramble control shRNA (HNZP), control for #19 and #20	
15	hTERT;EGFR;p53^{R175H};shRNA α-POSTN (#1)(HNZP)	
16	hTERT;EGFR;p53^{R175H};shRNA α-POSTN (#2)(HNZP)	
17	hTERT;p53^{R175H} (HNZP), control for #22	14
18	hTERT;p53^{R175H};TRP-Met (HNZP)	
19	hTERT;EGFR;p53^{R175H} (HNPB), control for #24 and #25	33
20	hTERT;EGFR;p53^{R175H};IGFBP3 (HNPB)	
21	hTERT;EGFR;p53^{R175H};IGFBP3^{GGG} (HNPB)	
22	hTERT;EGFR;p53^{R175H};non-silencing scramble control shRNA (HNPG), control for #27 and #28	
23	hTERT;EGFR;p53^{R175H};shRNA α-IGFBP3 (#1)(HNPG)	
24	hTERT;EGFR;p53^{R175H};shRNA α-IGFBP3 (#2)(HNPG)	
25	hTERT;EGFR;p53^{R175H};non-silencing scramble control shRNA (HNPGB), control for #30, #31 and #32	
26	hTERT;EGFR;p53^{R175H};shRNA α-IGFBP3 (#2)(HNPGB)	
27	hTERT;EGFR;p53^{R175H};shRNA α-IGFBP3 (#2);IGFBP3 (HNPGB)	
28	hTERT;EGFR;p53^{R175H};shRNA α-IGFBP3 (#2);IGFBP3^{GGG} (HNPGB)	

29	hTERT;Myc (HNG), control for #34	21
30	hTERT;Myc;CDX1 (HNG)	
31	hTERT;scrabmle shRNA (HPG), control for #36 and #37	22
32	hTERT;shRNA α-NOTCH3 (#1)(HPG)	
33	hTERT;shRNA α-NOTCH3 (#2)(HPG)	
34	hTERT;GFP (HG), control for #39	
35	hTERT;DNMAML1-GFP (HG)	
36	hTERT;DNMAML1-GFP$^{Tet\text{-}Off}$ (HPG)	

Selection markers: H, Hygromycin B; P, Puromycin; N, Neomycin (Geneticin/G418); Z, Zeocin; B, Blasticidin S; G, Green Fluorescent Protein. Primary human esophageal keratinocytes EPC2 and telomerase-immortalized derivatives[2,10] were stably transduced with retrovirus or lentivirus to express indicated genes or short hairpin RNA sequences, either alone or in combination, and selected using antibiotics resistance genes or green fluorescent protein as described in the references cited.

Supplementary information and cell culture protocol for EPC2-hTERT and its derivatives

While several ESCC cell lines as well as primary and immortalized esophageal keratinocytes have been used for organotypic 3D culture in our laboratory, EPC2 and its derivatives have been most extensively characterized and used in this system, thus serving as good positive controls. EPC2 derives from a 55-year-old male, who underwent esophagectomy for Barrett's esophagus with severe dysplasia. There is no history of preoperative radiotherapy or chemotherapy. Specimen was harvested from morphologically normal proximal esophagus (upper 1/3). H&E showed normal mucosa, no inflammation, no dysplasia, and no cancer. EPC2-hTERT is immortalized, but not transformed, with functionally intact RB and p53 pathways [2], key tumor suppressors in esophageal carcinogenesis. Concomitant expression of EGFR and mutant p53 resulted in malignant transformation. Resulting EPC2-hTERT-EGFR-p53^{R175H} cells display invasive growth in organotypic 3D culture and form tumors in nude mice [5].

Reagents: See the main text for medium (h-KSFM) preparation, Trypsin-EDTA Soybean Trypsin Inhibitor (STI) and FBS. DMSO (Fisher Scientific) is used to prepare freezing medium.

To thaw cells

1) Thaw a cryogenic vial by incubating at 37ºC in a water bath for about 2 min.
2) Transfer the content into a 15-ml conical tube containing about 10 ml of full h-KSFM.
3) Pellet the cells by centrifugation for 3 min at 1,000 rpm at 4ºC.
4) Resuspend the cells with 15 ml of h-KSFM.
5) Seed the cells in a 75-cm^2 flask.

To subculture cells

6) Grow cells to 80% confluency. Do not allow them to grow over 90-100% confluency as post-confluent cells undergo terminal differentiation.
7) Add 5 ml of Trypsin-EDTA and rock the flask gently to distribute well. There is no need to rinse with DPBS prior to trypsinization
8) Remove Trypsin-EDTA by suction.
9) Incubate the dish at 37ºC for 2-3 min.
10) Add ~12 ml of STI into the flask to suspend the cells. **Critical:** EPC2 cells are very sensitive to trypsin, and therefore it is very important to block trypsin activity with soybean trypsin inhibitor.
11) Pellet the cells by centrifugation for 3 min at 1,000 rpm at 4ºC.
12) Resuspend the cells with 200-300 µl of h-KSFM medium, count cell number, and seed them ($0.5x10^6$ cells/75-cm^2 flask) into a new flask.

To freeze cells

13) Make freezing medium by add 1 ml of DMSO into 9 ml of fetal bovine serum. Keep on ice prior to use. The freezing medium can be stored at -20°C.
14) Start from the above step #12.
15) Add pre-chilled freezing medium and mix (~$1x10^6$ cells/1 ml).
16) Dispense into cryogenic vials (1 ml/vial).
17) Put the cryogenic vials into isopropanol-filled freezing container. (Alternatively, wrap them with a blue-pad/paper towel and put in a closed polystyrene box).
18) Transfer the box to –80ºC and keep 5 hours-overnight.
19) Transfer the cryogenic vials into a liquid nitrogen tank for long-term storage.

Supplementary References (References 14, 17, 19, 21, 22 and 30 are in main text)

31. Oyama, K.*, et al.* AKT induces senescence in primary esophageal epithelial cells but is permissive for differentiation as revealed in organotypic culture. *Oncogene* **26**, 2353-2364 (2007).

32. Ohashi, S.*, et al.* Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res* **70**, 4174-4184 (2010).

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