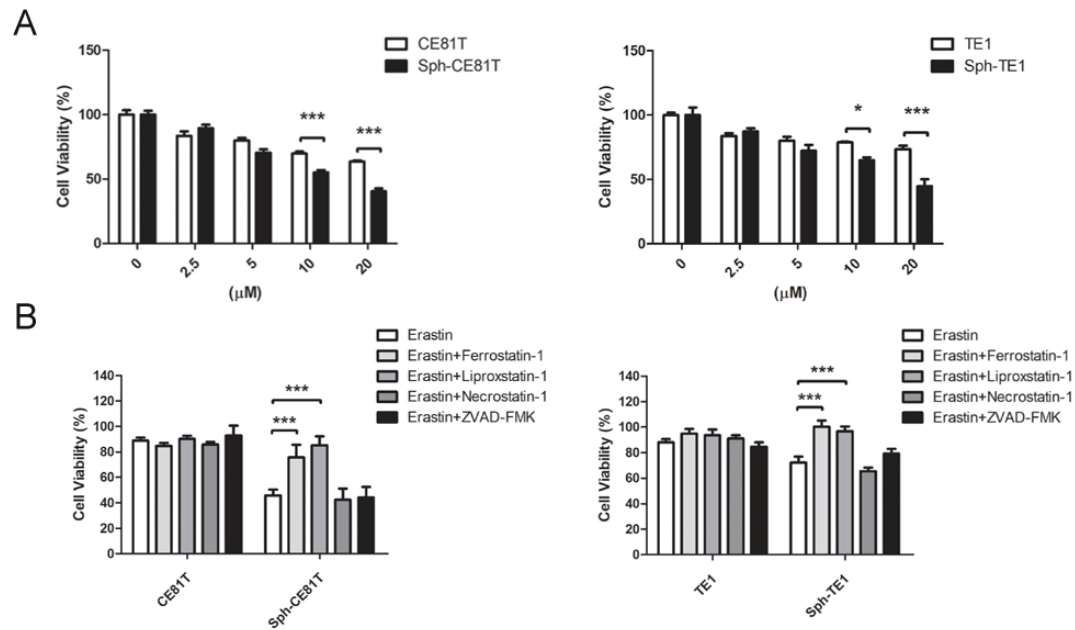


**Supplementary Figure S1.** Real-time PCR of Nanog, Sox2, and Oct4 of CE81T and TE1 cells in spheroid (Sph) and adherent cultures.



**Supplementary Figure S2. Erastin induced more ferroptosis in esophageal cancer cells in spheroid culture.**

(A) Cell viability of TE1 and CE81T cells cultured in spheroid (Sph-TE1, Sph-CE81T) or adherent system and treated with erastin in different concentration to induce ferroptosis. (B) Viability of cells in different cultures with erastin (20  $\mu\text{M}$ ) were treated with ferrostatin-1 (1  $\mu\text{M}$ ), liproxstatin-1 (1  $\mu\text{M}$ ), ZVAD-FMK (50  $\mu\text{M}$ ), or Necrostatin-1 (50  $\mu\text{M}$ ) for 24 h.

**Supplementary Table S1. Demographic data of 70 patients who underwent surgery for esophageal cancer.**

| Variables (n=70)                     | Number(%)        |
|--------------------------------------|------------------|
| Age(year)                            | 38-91(69.4±10.5) |
| Sex                                  |                  |
| Male                                 | 67(95.7)         |
| Female                               | 3(4.3)           |
| Histological type                    |                  |
| Epidermoid carcinoma                 | 16(22.9)         |
| Squamous cell carcinoma              | 52(74.3)         |
| Adenosquamous carcinoma              | 1(1.4)           |
| Epidermoid carcinoma, adenocarcinoma | 1(1.4)           |
| T status                             |                  |
| T1                                   | 4(5.7)           |
| T2                                   | 13(18.6)         |
| T3                                   | 45(64.3)         |
| T4                                   | 8(11.4)          |
| N status                             |                  |
| N0                                   | 31(44.3)         |
| N1                                   | 37(52.9)         |
| N2                                   | 2(2.9)           |
| M status                             |                  |
| M0                                   | 62(88.6)         |
| M1                                   | 8(11.4)          |
| pHsp27 expression                    |                  |
| Increased expression (+)             | 16(22.9)         |
| Decreased expression (-)             | 54(77.1)         |
| GPX4 expression                      |                  |
| Increased expression (+)             | 55(78.6)         |
| Decreased expression (-)             | 15(21.4)         |
| pHsp27 and GPX4 coexpression         |                  |
| pHsp27 (+)/GPX4 (+)                  | 14(20)           |
| pHsp27 (+)/GPX4 (-)                  | 2(2.9)           |
| pHsp27 (-)/GPX4 (+)                  | 41(58.6)         |
| pHsp27 (-)/GPX4 (-)                  | 13(18.6)         |