## Supplementary materials

Table S1  Characteristics of patients with esophageal squamous cell carcinomas

| Clinicopathological variables | Total cases ($n = 135$) |
|------------------------------|-------------------------|
| **Group**                    |                         |
| Normal tissue                | 90                      |
| Carcinoma tissue             | 135                     |
| **Age (years)**              |                         |
| ≤ 60                         | 55                      |
| > 60                         | 80                      |
| **Gender**                   |                         |
| Male                         | 84                      |
| Female                       | 51                      |
| **Differentiation**          |                         |
| Well                         | 45                      |
| Moderate                     | 68                      |
| Poor                         | 22                      |
| **T stage**                  |                         |
| 1                            | 12                      |
| 2                            | 46                      |
| 3                            | 77                      |
| **Lymph node metastasis**    |                         |
| N0 (No)                      | 98                      |
| N1 (Yes)                     | 37                      |
| **TNM stage**                |                         |
| I                            | 10                      |
| II                           | 96                      |
| III                          | 29                      |
Figure S1  Expression analysis of NEDD9 in sphere-forming cells compared to in parental KESE70 (A) and TE1 (B) cells. A total of 1,000 cells were seeded in 24-well Ultra Low Attachment Plates (Corning, Corning, NY, USA) in serum-free DMEM/F12 medium supplemented with heparin, B27, EGF, and bFGF. After culturing for 7 days, the number of spheres was counted using a microscope (Leica, Wetzlar, Germany). For secondary and tertiary sphere formation, the spheres were collected, dissociated into single cells, and seeded into 24-well Ultra Low Attachment Plates. Three independent experiments were conducted in parallel. 0: parental adherent cells; 1: first passage of spheres; 2: second passage of spheres; 3: third passage of spheres; 4: fourth passage of spheres. *P < 0.05; **P < 0.01; ***P < 0.001 compared to the parental adherent cells.

Figure S2  The mRNA and protein expressions of NEDD9 in human esophageal epithelial cell line, Het-1a, and esophageal squamous cell carcinoma cell lines were determined by real-time PCR and Western blot.