

Figure S1 Optimization of the organoid culture system. Different morphologies of organoids grown in Collagen I and Matrigel can be observed under light microscopy. (A) Matrigel increased the number of NPCOs compared to collagen I. Left, representative images of NPCOs using a microscope; right, number of NPCOs from 3 independent experiments. (B) Analysis of the optimal concentrations of cytokines in the NPCO culture medium. Organoids were cultured in different concentrations of the indicated cytokines for 5 days. The optimal cytokine concentrations were determined according to the resulting organoid numbers. (C) Number of NPCOs or NMOs in standard organoid medium (SOM) or conditional organoid media with the removal of Wnt3a, Rspo-1, Y27632, EGF, Noggin, or FGF2. NMO, nasal mucosa organoid; NPCO, nasopharyngeal carcinoma organoid.

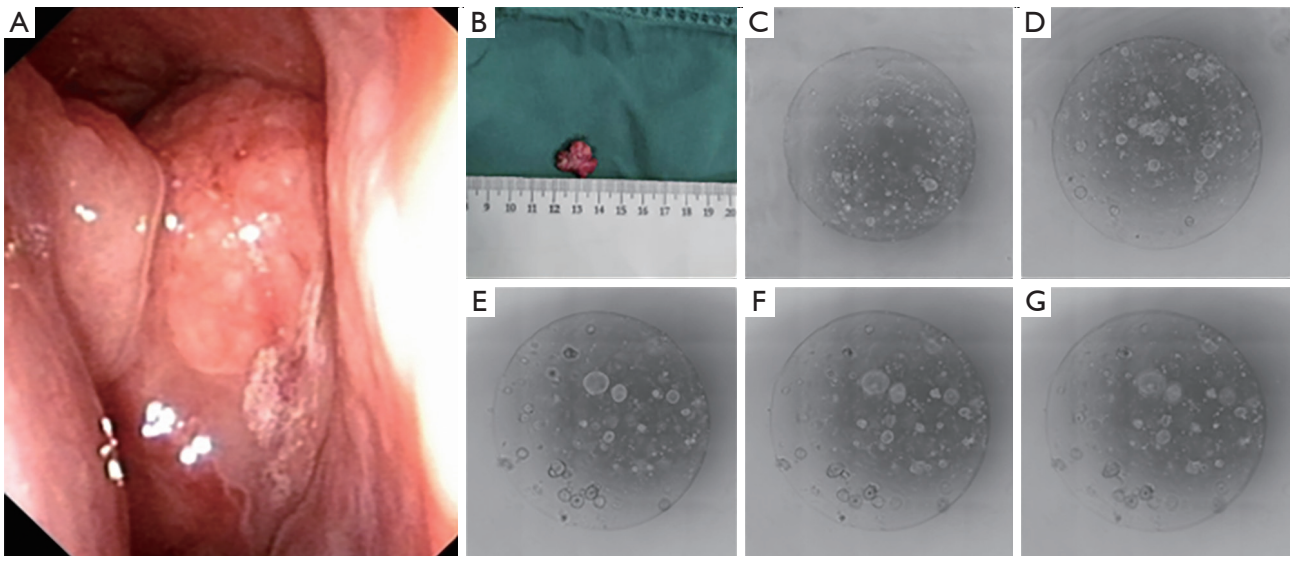


Figure S2 Establishment of NPCOs. (A) An NPC biopsy sample obtained during nasopharyngoscopy. (B) Macroscopic NPC tissues were used for 3-dimensional primary culture. (C-G) Phase-contrast microscopy showing the process of expansion on day 1 (C), day 3 (D), day 5 (E), day 7 (F), and day 9 (G) after seeding. 20× magnification used for organoid models. NPC, nasopharyngeal carcinoma; NPCO, nasopharyngeal carcinoma organoid.