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Figure S1 Morphology and growth of BDOs from non-cancer patients.

(a, b) Microscopic images of the two lines of BDOs from non-cancer patients. Both of the BDOs showed a regular cystic shape with a thin cyst wall. Bars in (a) and (b) are 100 μ m.



Figure S2 Microscopic images of all BDOs established in this study.

All of the 60 successfully established BDOs exhibited a diverse range of morphology, such as a regular cystic shape, a solid mass, a multi-cystic form and an irregular cyst with a markedly thick cyst wall, compared with BDOs from non-cancer patients. Bar is 100 µm.



Figure S3 Microscopic images of XDOs.

XDOs showed a more solid and compact morphology than the corresponding pre-transplant BDOs in Figure S2. Bar is 100 μ m.

B35 Organoid



Figure S4 Increased Ki67- and p53-positive cells in XDOs for B35.

Related to Figure 4(b, c). Immunohistochemistry of XDOs in B35 showed increased Ki67-positive (left panel) and TP53-positive cells (right panel) compared with those of the pre-transplant BDOs. The percentages of Ki67- and TP53-positive cells are shown as mean values \pm SD of triplicates. The differences were analyzed statistically using Student's t test (n = 3). *p <0.01, **p <0.001. Bars are 50 µm.

R273C



Figure S5 Enrichment of TP53-mutated cancer cells in B35 BDOs by Nutlin-3a treatment.

Related to Figure 5(b-d). In another BDO, B35, including a minor population of cancer cells with TP53 mutation (R273C), the effectiveness of Nutlin-3a treatment for enriching the TP53-mutated cancer cells in organoids was confirmed by sequencing analysis and immunohistochemistry. The percentages of TP53-positive cells demonstrated by immunohistochemistry are shown as mean values \pm SD of triplicates. The differences were analyzed statistically using Student's t test (n = 3). Red arrows and red underbars indicate the mutation sites and the associated changes in codons, respectively. ***p <0.0001. Bars are 50 µm.