

**Fig. S1. The composition and diversities of gut microbiota in patients received ICI treatment for unresectable HCC and healthy controls**

Stacked bar plots of phylogenetic composition of common bacterial taxa (>0.1% abundance) at the (A) phylum and (B) family levels in fecal samples of healthy controls, HCC patients with objective response (OR) and progressive disease (PD) to ICI treatment. Alpha diversity indices of fecal bacteria by (C) Shannon index and (D) phylogenetic diversity whole tree index.

**Fig. S2. The composition and diversities of gut microbiota in patients received ICI monotherapy for unresectable HCC and healthy controls**

(A) Principle coordinates analysis of fecal microbiota by Bray-Curtis dissimilarity metrics. (B) Histograms and (C) cladogram of linear discriminant analysis (LDA) scores computed for differentially abundant taxa in the fecal microbiome. The length of each bar indicates the effect size associated with a taxon, which is significantly different when comparing to other groups.

OR, objective response; PD, progressive disease

**Fig. S3. The microbial composition and fecal bile acids according to the prior experience of tyrosine kinase inhibitor**

Stacked bar plots of phylogenetic composition of common bacterial taxa (>0.1% abundance) at the (A) phylum and (B) family levels as well as the (C) phylogenetic diversity whole tree index and (D) principle coordinates analysis by Bray-Curtis dissimilarity metrics in the fecal samples of HCC patients according to the prior experience of tyrosine kinase inhibitor (TKI) (TKI-naïve, n = 18; TKI-experienced, n = 23). (E) The relative abundance of *Prevotella 9* and *Lachnospirillum*, and (F) the

PC Lee, et al. 2

concentration of secondary bile acids in the feces of patients according to the experience of TKI.

DCA, deoxycholic acid; G, glycine conjugated species; LCA, lithocholic acid; MDCA, murideoxycholic acid (murocholic acid); nM, nanomolar; T, taurine conjugated species; UCA, ursocholic acid; UDCA, ursodeoxycholic acid.

**Fig. S4. Volcano plot analysis of fecal non-targeted metabolites altered in HCC patients with objective response to ICI treatment**

The non-target cations altered in the fecal samples with labeled substances.

41: oxypurinol; 45: nutriacholic acid; 60: methyl 3b, 24-dihydroxy-11, 13(18)-oleanadien-30-oate; 119: D-proline; 152: 6-methylquinoline; 185: 2-(Methoxycarbonyl)-5-methyl-2,4-bis(3-methyl-2-butenyl)-6-(2-methyl-1-oxopropyl)-5-(4-methyl-3-pentenyl)cyclohexanone; 203: (3beta,5alpha,6beta,22E,24R)-23-Methylergosta-7,22-diene-3,5,6-triol

The non-target anions altered in the fecal samples with labeled substances.

18: oxypurinol; 33: isohyodeoxycholic acid; 35: inosine; 48: D-glucuronic acid.