Figure S7

(A) Bright field / DAPI, LGR5 (Green) / PD-L1 (Red) / TDO2 (Blue)

(B) CRC-24M

(C) CRC-29M

(D) CRC-19

(E) CRC-24M

(F) CRC-20

(G) CRC-24M

(H) CRC-20

Figure S7
FIGURE S7 TDO2 is coexpressed with LGR5 and PD-L1 in colon tumors. A, In situ RNA hybridization of xenografted tumors derived from cancer spheroids. (Left column) Merged images of bright field microscopy and DAPI staining of the tumors derived from CRC-24M or CRC-20. Scale bar: 50 µm. (Right panels) Multiple fluorescence in situ RNA hybridization with LGR5 (green), PD-L1 (red) and TDO2 (blue) probes. Images acquired at a higher magnification are shown at right. B, Quantitation of the proportions of xenograft tumor cells with the indicated numbers of hybridizing dots for TDO2. Data from xenograft tumors derived from metastatic spheroids (CRC-24M and CRC-29M) and nonmetastatic spheroids (CRC-19 and CRC-20) are shown. C, D, Correlation of the average number of hybridizing dots for (C) LGR5 or (D) PD-L1 with the indicated number of dots for TDO2 in xenografted tumors (CRC-24M). The hybridization data shown in (A) were used for the counting (n=3). E, Representative coimmunostaining of xenograft tumors (CRC-24M and CRC-20) for PD-L1 and TDO2. Nuclei was counterstained with Hoechst 33342. Scale bar: 10 µm. F, Representative coimmunostaining of xenograft tumors (CRC-24M and CRC-20) for β-catenin and TDO2. Nuclei was counterstained with Hoechst 33342. Scale bar: 10 µm. G, Representative coimmunostaining of surgical specimens of primary colon cancer of patients with liver metastasis (patient-2). Nuclei was counterstained with Hoechst 33342. Scale bar: 10 µm. H, A proposed model for the TDO2-kynurenine pathway-mediated regulation of antitumor immunity and cancer stemness in the tumor microenvironment. The TDO2-kynurenine pathway activates AHR, and activated AHR induces PD-L1-mediated immune evasion and promotes cancer stemness, leading to the generation of immune-evasive CSCs, thereby facilitating liver metastasis. Pathways shown in red are presented in this paper and those in blue were previously documented. Values represent the mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001.