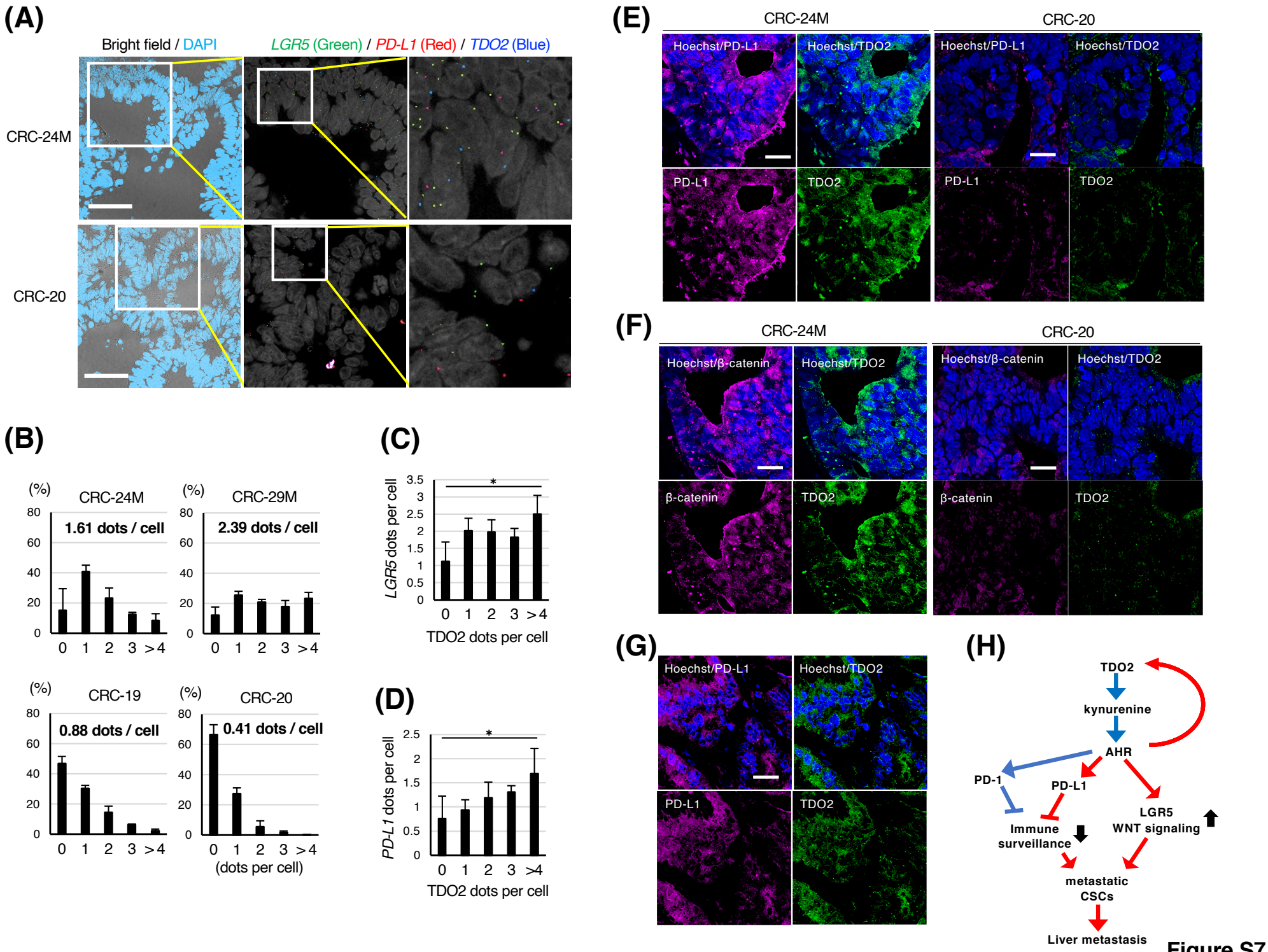
(A)
Bright field / DAPI
LGR5 (Green) / PD-L1 (Red) / TDO2 (Blue)
CRC-24M
CRC-20
(B)
(%)
CRC-24M
1.61 dots / cell
CRC-29M
2.39 dots / cell
CRC-19
0.88 dots / cell
CRC-20
0.41 dots / cell
0 1 2 3 >4
(dots per cell)
(C)
LGR5 dots per cell
TDO2 dots per cell
(D)
PD-L1 dots per cell
TDO2 dots per cell
(E)
CRC-24M
CRC-20
Hoechst/PD-L1
Hoechst/TDO2
PD-L1
TDO2
(F)
Hoechst/β-catenin
β-catenin
(G)
(H)
TDO2
kynurenine
AHR
PD-1
PD-L1
Immune surveillance
LGR5
WNT signaling
metastatic CSCs
Liver metastasis

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. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.