



Figure S3

FIGURE S3 The Induction of TDO2-Kynurenine-AHR Pathway Transactivated Pd-I1 and Compromised immunological Responses in Metastasized Liver. A, Effects of Tdo2 expression upon liver metastasis in CT26 colon cancer cells in *Balb/c* nude mice. (Left) GFP images of liver after splenic injection of CT26 cells introduced with GFP and the indicated vectors. (Right) Relative number of the liver-metastasized GFP-positive cells. B, Macrophage infiltration into metastatic foci was not significantly affected in Tdo2-expressing tumors. (Left) Immunohistochemistry analyses with anti-macrophage antibody (F4/80⁺). Images with higher magnification are also shown. Scale bar: 100 μm. (Right) Relative number of F4/80⁺ infiltrating macrophages in the indicated tumors was determined by counting the numbers of positive cells at three representative areas. C, D, Percentage fractions of the indicated subsets of T lymphocytes (CD3⁺, CD4⁺, CD8⁺) in CD45⁺ cells, Treg cells in CD4⁺ T cells, and IFN-γ⁺ cells in CD8⁺ T cells in the metastasized liver (C) and metastasized lung (D). The lymphocytes were extracted from the liver and cell numbers were evaluated by flow cytometry. E, F, Western blot analyses of Pd-I1 in the indicated CT26-derived cells treated with the indicated concentrations of the inhibitor of (E) TDO2 or (F) AHR. G, Alignment of consensus AHR-binding sequences of the indicated genes. Consensus sequences are shown in red. Values represent the mean ± s.d. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.