Supplementary Figure legends

Supplementary Fig. 1 The role of tumor-derived G-CSF in the induction of MDSC in ovarian cancer. a. Establishment of G-CSF-producing ovarian cancer cell lines assessed by RT-PCR analysis. **b-d**. Effects of tumor-derived G-CSF on the induction of MDSC in mice models of ovarian cancer. Nude mice were subcutaneously inoculated with A2780-Control or A2780-GCSF cells. Three weeks after inoculation, the spleen, bone marrow, blood and tumors were collected for analyses (five mice per group). **b**. G-CSF expression in A2780-Control cell- or A2780-GCSF cell-derived tumors. Scale bar, 50 μm. **c**. WBC/granulocyte counts of A2780-Control-derived tumor-bearing mice and A2780-GCSF-derived tumor-bearing mice. Bars, mean SD. **, $P< 0.01$, according to Welch's *t* test. **d**. MDSC in the peripheral blood, spleen, bone marrow and tumor of A2780-Control cell- or A2780-GCSF cell-inoculated mice. $CD11b^+Ly6G^+$ cell populations detected in the peripheral blood, spleen, bone marrow and tumor by flow cytometry. (i) Representative dot plots. (ii) and (iii) Graphs depicting the proportion of $CD11b^+Ly6G^+$ cells. Bars, mean SD. *, $P< 0.05$, **, $P< 0.01$, according to Welch's *t* test.

Supplementary Fig. 2 Cancer stem-like properties of ALDH-high ovarian cancer cells. **a**. *In vitro* tumorigenic and self-renewal activity of ovarian cancer cells according to ALDH-activity. A2780 cells that had been labeled with an Aldefluor kit were sorted using a flow cytometer. Then, ALDH-high and -low A2780 cells (1.5×10^3 cells) were separately plated on 60-mm ultra-low attachment surface dishes and

cultured for 2 weeks in the serum-free medium. The tumorigenic capacity and self-renewal activity of the cells were assessed by sphere formation assays. Photographs; representative photos of the spheres formed by the ALDH-high and -low cells (phase-contrast microscopy). Bars, 50 μm. Graph; the number of spheres counted (n = 3). Bars, SD. **, $p<0.01$, according to two-sided Student's t test. **b**. *In vitro* tumorigenic capacity of A2780 cells according to ALDH-activity. ALDH-high and -low A2780 cells (1×10^2 cells) were separately cultured in 60-mm dishes in the presence of 10% FBS for 2 weeks. Then, the colonies were stained with 0.5% crystal violet and the numbers of colonies were counted. Photographs; representative photos of the colonies formed by the ALDH-high and -low cells. Graph; the numbers of colonies counted (n = 3). Bars, SD. **, $p<0.01$, according to two-sided Student's t test. **c**. Differentiation capacity of A2780 cells according to ALDH-activity. ALDH-high and -low A2780 cells were separately cultured in the presence of 10% FBS for 3 days. Then, they were assessed using an Aldefluor assay (n = 5). Bars SD. **, $p<0.01$, according to two-sided Student's t test. Figures; Representative dot plots are shown. Graph; Population of ALDH-high cells. **d**. The CSC-related gene expression in A2780 cells according to ALDH-activity. The mRNA expression levels of SOX2, Nanog and OCT4 in ALDH-high and ALDH-low A2780 cells were measured by real-time quantitative RT-PCR (n = 4). GAPDH was used as internal reference for each sample. Bars SD. *, $p<0.05$, according to two-sided Student's *t* test.

Supplementary Fig. 3 The mechanism responsible for the increased stemness by MDSC. **a**. Effects of

MDSC on the induction of CSC *in vitro*. A2780 cells (3×10^5 cells /well) were cultured with MDSC or splenocytes (excluding MDSC) (3×10^4 cells /well) in the presence of 0.1% FBS for 18 hours in 6-well dishes. The mouse $EpCAM^+$ $CD45^-$ cells were gated using flow cytometry and then the percentages of ALDH-high cells were assessed using the Aldefluor assay (n = 5). Bars, SD. **, p <0.01, according to two-sided Student's *t* test. **b**. PGE2 expression in A2780-Control cell- or A2780-GCSF cell-derived tumors. Scale bar, 50 μm. **c**. Expression levels of EP2 and EP4 receptors in A2780 cells. EP2 and EP4 receptors and GAPDH mRNA levels in A2780 cells assessed by RT-PCR. **d**. Effects of PGE2 on the induction of CSC *in vitro*. A2780 cells were treated with PGE2 (50 ng/mL) with or without EP2 antagonist (20 nM) and EP4 antagonist (200 nM) in the presence of 0.1% FBS for 18 hours. The frequencies of ALDH-high A2780 cells were assessed using an Aldefluor assay (n = 5) Bars, SD. **, p<0.01, according to two-sided Student's *t* test. **e.** Effect of PGE2-inhibition on the MDSC-mediated CSC induction. A2780 cells (3×10^5 cells /well) and MDSC (3×10^4 cells /well) were co-cultured in 6-well dishes and treated either with celecoxib (20 μM), EP2 antagonist (20nM), or EP4 antagonist (200 nM) in the presence of 0.1% FBS for 18 hours *in vitro*. The mouse $EpCAM^+$ $CD45^-$ cells were gated using flow cytometry and then the percentages of ALDH-high cells were assessed using the Aldefluor assay (n = 5). Bars, SD. ** p <0.01, according to two-sided Student's *t* test.

Supplementary Fig. 4 The correlation among tumor-derived G-CSF, MDSC and ALDH activity *in vivo*.

a. Induction of CSC by tumor-derived G-CSF. Nude mice were inoculated with A2780-GCSF cells or A2780-Control cells (five mice per group). Three weeks after inoculation, subcutaneous tumors were collected and assessed. The human $EpCAM^+$ mouse $CD45^-$ cells in the tumors were gated using flow cytometry and then the percentages of ALDH-high cells were assessed using the Aldefluor assay. Bars, SD. **, $p < 0.01$, using two-sided Student's *t*-test. **b-d**. *In vivo* effects of PGE2-inhibition on the induction of MDSC and CSC. Nude mice were inoculated with A2780-GCSF cells. Nude mice bearing A2780-GCSF-derived tumors were randomly assigned into treatment groups: 5 mg/kg of daily celecoxib or PBS starting 1 day after inoculation (five mice per group). Three weeks after inoculation, their subcutaneous tumors were collected for evaluation. **b**. PGE2 expression in A2780-GCSF cell-derived tumors treated with celecoxib or PBS. Scale bar, 50 μm. **c**. Effects of celecoxib on the induction of MDSC in tumors. $CD11b^+Ly6G^+$ cell populations were detected in tumors by flow cytometry. Bars, SD. *, $p<0.05$, according to two-sided Student's t test. **d**. Effects of PGE2-inhibition using celecoxib on the induction of CSC in tumors. ALDH-high cells assessed using an Aldefluor assay. Bars, SD. *, $p<0.05$, according to two-sided Student's t test. **e-f**. Effects of an anti-Ly6G neutralizing antibody on MDSC recruitment and CSC induction in A2780-GCSF-derived tumors. Nude mice bearing A2780-GCSF-derived tumors were randomly assigned to treatment groups: anti-Ly6G neutralizing antibody (200 μg/mouse) or isotype control every 2 days starting 1 day after inoculation (five mice per group). Three weeks after inoculation, their subcutaneous tumors were collected for evaluation. **e**. $CD11b^+ Ly6G^+$ cell populations in tumor cells assessed using flow

cytometry. *, p <0.05, according to two-sided Student's *t*-test. **f**. ALDH-high cells assessed using an Aldefluor assay. Bars, SD. *, p <0.05, according to two-sided Student's *t*-test.

Supplementary Fig. 5 PGE2 produced by MDSC increased tumor PD-L1 expression via the mammalian target of rapamycin (mTOR) pathway in ovarian cancer cells. **a**. The role of mTOR signaling in MDSC-mediated PD-L1 expression. A2780 cells were seeded in the bottom chamber and MDSC were seeded in the top chamber of a 6-well plate (5:1 ratio of A2780: MDSC) in the presence of 0.1% FBS for 6 hours. Then, total protein of the bottom chamber was extracted, and the expression of P70S6 kinase, P-P70S6 kinase, AKT, P-AKT and PD-L1 was examined by Western blotting. Splenocytes (excluding MDSC) were also used for comparison. **b.** The effects of mTOR inhibition using rapamycin on MDSC-mediated increase in PD-L1 expression. A2780 cells were seeded in the bottom chamber and MDSC were seeded in the top chamber of a 6-well plate (5:1 ratio of HM-1: MDSC) with or without 50 nM rapamycin in the presence of 0.1% FBS for 6 hours. Then, total protein of the bottom chamber was extracted, and the expression of PD-L1 was examined by Western blotting. **c**. The role of PGE2 in the mTOR-dependent PD-L1 expression in A2780 cells. A2780 cells were treated with 0.25 μM PGE2 in the presence of 0.1% FBS in a time-dependent manner. Total protein was extracted, and the expression of P70S6 kinase, P-P70S6 kinase, AKT, P-AKT and PD-L1 was examined by Western blotting. **d**. The effects of mTOR inhibition using rapamycin on the PGE2-mediated increase in PD-L1 expression. A2780 cells were treated with 0.25 μM PGE2 with or

without 50 nM rapamycin in the presence of 0.1% FBS for 6 hours. Total protein of the bottom chamber was extracted, and the expression of P70S6 kinase, P-P70S6 kinase, AKT, P-AKT and PD-L1 was examined by Western blotting.