**Supplementary Material and Methods**

*Patients and tissues.*

Clinicopathological information (n=294) and specimens (n=91) from patients treated for endometrial cancer between 2004-2012 at Kyoto University Hospital were obtained with written consent from each patient and used under protocols approved by the Kyoto University Institutional Review Board. Prognosis was determined using the survival rate and the *Kaplan-Meier* method.Ninety one adenocarcinomas (69 endometrioid and 22 SPECs) were obtained at the time of initial surgery, fixed in 10% buffered formalin, embedded in paraffin, and sectioned. RNA was extracted using the RNeasy Mini Kit (QIAGEN, Venlo, Netherlands) from tumor tissues of 63 of these patients (51 endometrioid and 12 SPECs) for gene expression analysis.

 Tissue microarrays obtained from the BC Cancer Agency and Vancouver General Hospital (Vancouver, Canada) included specimens of 460 endometrial cancers in five tissue microarrays (355 endometrioid and 105 SPECs) were also examined independently as an external validation. All patient samples were obtained after receiving written informed consent and research ethics approvals under the University of British Columbia and BC Cancer Agency.

*Cell lines and culture.*

Human endometrial cancer cell lines, HEC1A (a grade 2 endometrioid adenocarcinoma cell line, ATCC, Rockville, MD), HEC50B (a grade 3 endometrioid adenocarcinoma cell line, JCRB, Osaka, Japan), Ishikawa (a grade 1 endometrioid adenocarcinoma cell line, National Hospital Organization, Japan), SPAC-1L (a SPEC cell line, The Cancer Institute of the Japanese Foundation for Cancer Research, Japan) were maintained in RPMI1640 (Nacalai Tesque, Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (v/v; Biowest, France) and penicillin–streptomycin (100 IU/ml penicillin, 100 µg/ml streptomycin; Nacalai Tesque). All cells were seeded into Cellstars® tissue culture plates (Greiner, Frickenhausen, Germany) were regularly tested for mycoplasma contamination, and were authenticated by STR analysis.

These cells were used for further functional assays as described below.

*Immunohistochemistry.*

Immunohistochemical staining was done using the streptavidin-biotin peroxidase complex method as previously reported ([1](#_ENREF_1)). An endogenous peroxidase block was followed by nonspecific background blocking and incubation with a rabbit polyclonal anti-human STAT1 antibody (HPA000931; 1:250 dilution, Sigma Aldrich, Germany), a mouse monoclonal anti-human CD8+ antibody (Clone C8/144B; N1592, Dako, Carpinteria, CA, USA), a rabbit monoclonal anti-human ICAM1 (EPR4776) antibody (ab109361; 1:150 dilution, Abcam, Cambridge, UK) or a rabbit polyclonal anti-human B7-H1/PD-L1/CD274 antibody IHC-plus (LS-B480; 1:100 dilution, LifeSpan Biosciences, Seattle, WA, USA). Normal rabbit IgG antibody (sc-2027; 1:250 dilution, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was used as an isotype controls. The primary antibody was omitted for negative controls. The expression of STAT1, CD8+ T, ICAM1 and PD-L1 cells were independently evaluated by three examiners with integration of the intensity and area of the staining while blinded to the clinical history of the patients. The intensity was graded on a 0 to 4 scale: 0, negative; 1, weak positive (weak intensity and ≤25% area stained); 2, intermediate (weak intensity and 25-50% area stained); 3, positive (prominent intensity and 50-75% area stained); and 4, strong positive (prominent intensity and ≥75% area stained).

*STAT1 knockdown*

*STAT1*-specific short interfering RNAs (siRNA; FlexiTube siRNA QIAGEN catalogue no. SI02662884), *MYC*-specific siRNA (FlexiTube siRNA QIAGEN catalogue no. GS4609), and negative control siRNA (AllStars Negative Control siRNA; QIAGEN) were transfected into Ishikawa, HEC1A, HEC50B, and SPAC1L cells using HiPerFect Transfection Reagent (QIAGEN) as previously described ([2](#_ENREF_2)). Briefly, 105 cells per well were seeded in 24-well plate (Greiner, Frickenhausen, Germany), and incubated for 48 hours. The medium was aspirated and the cells were gently washed with RPMI1640 (Nacalai Tesque, Kyoto, Japan) followed by transfection with the siRNA.

For establishing *STAT1* stably suppressed cells, *STAT1*-specific short hairpin RNA (shRNA) was introduced into SPAC1L cells using Turbofectin 8.0 Transfection Reagent (QIAGEN). STAT1-shRNA (HuSH 29mer shRNA pGFP-V-RS, Origene, Rockville, MD, USA) and negative control shRNA (scrambled shRNA cassette, Origene) were transfected, and stably transfected cells were selected with puromycin (0.5-1.0 µg/ml, Nacalai Tesque) as recommended by the manufacturer. A dominant-negative *STAT1* DNA plasmid (pBOS-STAT1-DN, Osaka University Graduate School of Medicine, Japan) ([3](#_ENREF_3)) was also transfected with Lipofectamine 2000 for obtaining *STAT1* dominant negative cells. Stably transfected cells were selected with Geneticin (800 µg/ml, Nacalai Tesque) as described previously ([4](#_ENREF_4)).

To confirm down-regulation of *STAT1* and *MYC* expression, quantitative RT-PCR and western blot were performed using cells collected 48 hours post-transfection as described below.

*Microarray analysis*

Gene expression microarray data was generated from 63 endometrial cancer samples using Affymetrix U133 Plus 2.0® gene chips (Affymetrix, Santa Clara, CA). The Significance Analysis of Microarrays (SAM) software (www.[http://statweb.stanford.edu/~tibs/ SAM/](http://statweb.stanford.edu/~tibs/%20SAM/)) was used to detect genes distinguishing type II cancers from type I cancers as described previously ([5](#_ENREF_5),[6](#_ENREF_6)). Supervised hierarchical clustering of these genes was performed and graphically viewed as a dendogram and heat map using Cluster 3.0 and Java TreeView (http://jtreeview. sourceforge.net/). The published microarray dataset TCGA UCEC\_ 2013 ([7](#_ENREF_7)) was also analyzed using this method, and the expression pattern for the group of genes commonly up- or down-regulated in type II cancers was designated as a type II signature. The cBioPortal for Cancer Genomics database (<http://www.cbioportal.org/public-portal/>) was used to analyze genetic oncoprints. Gene expression microarray data was also generated for SPAC-1L cells treated with or without STAT1-siRNAs. The Connectivity Map analysis software (Cmap; <http://www.broadinstitute.org/cmap/>) was used to mine potential therapeutic agents for SPECs based on genes whose expression is altered by STAT1 suppression. Using a Bayesian binary regression model as previously reported ([8](#_ENREF_8)), the MYC signature was generated and applied to our datasets *in vitro* and *in vivo* for assessing MYC pathway activity in endometrial cancers. Microarray data from 63 endometrial cancers and the SPAC-1L cells can be obtained at the Gene Expression Omnibus website (GSE56026, <http://www.ncbi>. nlm.nih.gov/geo/).

*Western Blot.*

Cells were lysed in radio-immunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific, Waltham, MA) with a protease inhibitor cocktail (EMD, Madison, WI) and a phosphatase inhibitor cocktail (Nacalai Tesque). Protein was quantified using the DC Protein Assay Kit (Bio-Rad, Hercules, CA). Twenty micrograms of sodium dodecyl sulfate (SDS)-treated protein was loaded onto a 10-20% Tris-Tricine Mini Protean® gel (Bio-Rad). Gels were electroblotted onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad). Nonspecific binding of the antibody was blocked by 1hr incubation at room temperature in Blocking One-P (Nacalai Tesque). The membranes were incubated overnight at 4oC with primary antibody (1:800, anti-STAT1 rabbit polyclonal Ab, Sigma Aldrich HPA000931; 1:1,000, anti-ICAM1 (EPR4776) rabbit monoclonal Ab, Abcam ab109361; 1:800, anti-human B7-H1/PD-L1/CD274 rabbit polyclonal Ab, LS-B480; 1:500, and anti-MYC (9E10) mouse monoclonal Ab, Abcam ab32). After washing in tris-buffered saline (TBS)-T, the blots were incubated with the appropriate peroxidase-coupled secondary antibody (1:6,000; Anti-rabbit HRP or anti-mouse HRP, GE Healthcare Life Sciences, Uppsala, Sweden). β-actin was used as an endogenous loading control, detected using an anti-human β-actin antibody (1:8,000; Rabbit mAb, Abcam, Cambidge, MA). Specific proteins were detected using ECL Plus Western Blotting Reagent (GE Healthcare Life Sciences). The bands were visualized using Molecular Imager® Gel DocTM XR+ and ChemiDocTM XRS+ Systems with Image Lab 2.0 software (Bio-Rad).

*Real Time Quantitative PCR.*

Total RNA was extracted from cell lines using the RNeasy® Mini Kit (QIAGEN). To monitor gene expression, quantitative reverse transcriptase (RT)-PCR amplification of target genes and *GAPDH* mRNAs was done using the Light Cycler 480-II (Roche, Basel, Switzerland) and a Dual Color Hydrolysis Universal Probe System (Roche). Supplementary Table 1 lists the primers used, which were obtained from the Universal Probe Library Assay Design Center (Roche). Cycling parameters were 95oC for 10 seconds followed by 40 cycles of 95oC for 5 seconds and 60oC for 30 seconds, followed by a dissociation cycle of 95oC for 15 seconds, 60oC for 20 seconds, and 95oC for 15 seconds. The relative expression of target genes was estimated by dividing the threshold cycle (CT) value of target genes by the *GAPDH* CT values.

*Cell Proliferation Assay.*

Cells were seeded into 96-well tissue culture plates at 2×103 cells per well. The cell culture medium was replaced with fresh medium and incubated for 24hr. The number of viable cells in each well was examined using the WST-1 assay kit (Premix WST-1®, Takara, Otsu, Japan) following the manufacturer’s instructions, and was compared between groups. The experiments were carried out in sextuplicate.

*Soft Agar Colony Formation Assay.*

Soft agar assays were performed in 6-well plates. The base layer of each well consisted of a 1 ml volume with final concentrations of 1x media (RPMI-1640 plus 10% inactivated FBS) and 0.6% low melting point agarose. Plates were chilled at room temperature until the agarose was solidified, at which point a 1 ml volume of a growth agar layer was added, consisting of 1 x 104 cells suspended in 1x media and 0.3% low melting point agarose. Plates were again chilled at room temperature until the growth layer congealed. A further 1 ml of 1x media without agarose was added on top of the growth layer on day 0 and again on day 14 of growth. Cells were allowed to grow at 37°C for 4 weeks and total colonies were counted.

*Adhesion assay*

Human umbilical vein endothelial cells (HUVEC) were obtained fresh and were cultured until just confluent. Background signaling was minimized by serum starvation for 12 hours followed by rinsing with sterile PBS. HUVEC mono-layer cells (95% confluent) and 105 cancer cells were co-cultured and labeled using the Vybrant® CFDA SE Cell Tracer Kit according to the manufacturer’s protocol for four hours, then the remaining floating cancer cells were removed with culture media. Fresh media was added and relative fluorescence units were measured by spectrophotometer at excitation 492 nm and emission 517 nm. Adhered cells were visualized using a fluorescent microscope equipped with a digital camera (IX71/AP71, Olympus) and analyzed using Metamorph software (Molecular Devices, LLC., Sunnyvale, California, USA).

*Motility tracking assay*

Cells were placed into 6 cm dishes and cultured until they were 90-100% confluent. Experimental wounds were introduced by dragging a sterile 200µl plastic pipette tip across the cell monolayer. After wounding, the cultured cells were rinsed with PBS and media was added with or without 250 IU/ml recombinant human interferon gamma (R&D Systems, Minneapolis, MN, USA). The cells were then incubated for 24 hours to track cell motility. A total of six wounds were sampled for each specimen. Cell migration was evaluated by measuring the gap between the cells most closely spaced on each leading edge at 0, 6, 12, 18, and 24 hours post-wounding and expressed as the percentage of the distance filled.

*Invasion assay*

Invasive potential into a reconstituted basement membrane was assayed on 24-well companion plates (Becton Dickinson Labware, Massachusetts, USA) with an 8-mm pore polycarbonate filter coated with Matrigel (Becton Dickinson Labware, USA) as described previously ([1](#_ENREF_1)). The lower chamber contained 0.8 ml medium with 10% FBS as a chemo- attractant. In the upper compartment, 0.8 – 1 x 10­6 cells in 0.2 ml medium were seeded and incubated with or without treatment at 37oC for 24 hours to allow for migration to the lower chamber. Student’s t-tests or One way ANOVA were used to analyze the differences in invasion between groups.

*In Vivo Experiments*

Female NOD-SCID mice were purchased from Nihon Clea (Kyoto, Japan). Animal care and experimental procedures under pathogen-free conditions were performed in accordance with the guidelines of the Institute of Laboratory Animals Graduate School of Medicine, Kyoto University. Subcutaneous xenografts were established in the flanks by inoculating 5×106 SPAC-1L cells with and without STAT1 alteration by dominant negative and shRNA transduction methods. Tumor growth in inoculated mice was sequentially monitored twice a week for 8 weeks by measuring the volume of tumors.

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