**Supplementary materials and methods**

**Animal**

Female Sprague-Dawley rats at 11 weeks of age were purchased from the Charles River Laboratories (Raleigh, USA). All animals were housed under specific pathogen-free conditions in the animal care facility of MPI research. The procedures of animal experiments were performed in accordance with the regulations outlined in the United States Department of Agriculture (USDA) animal welfare act (9 CFR, parts 1-3).

**Preparation and treatment**

The codon-optimized human IL-7 gene (27) and granulocyte colony-stimulating factor (G-CSF) gene were individually fused with a hybrid Fc-fragment which contains the upper CH2 domain of IgD, and the last CH2 and CH3 domains of IgG4 (28). The schematic structure of Fc-fused IL-7 is presented in Supplementary Figure 1. Chinese hamster ovary (CHO) cells were stably transfected with a plasmid encoding IL-7-Fc or G-CSF-Fc, and proteins were prepared as previously described (29). Recombinant human IL-7 (rIL-7), which was purified from HEK 293E cells, was purchased from Biolegend (San Diego, USA). Mice were synchronized in a diestrus state with 3mg of medroxyprogesterone acetate (Depo-Provera, Pfizer) subcutaneous injection 4 days before treatment and received 10μg of cytokines IL-7, IL-7-Fc or G-CSF-Fc in PBS via intravaginal route by a micropipette after anesthetized with 100 mg/kg ketamine (Yuhan) and 10 mg/kg xylazine hydrochloride (Bayer) in PBS by intraperitoneal injection. To analyze the source of the genital tract T cells, mice were treated with 60μg of FTY720 (Cayman Chemical) in 0.1% BSA-containing PBS intraperitoneally from one day after IL-7-Fc treatment at 3 days intervals. HPV DNA vaccine (GX188) was prepared as previously described (7). For genital TetE749-57+ CD8 T cell analysis, mice were vaccinated with 50μg of electroporation-enhanced immunization with GX188 (GX188E) through intramuscular injection. Seven days after priming, mice were boosted with 10μg of GX188E, with or without intravaginal IL-7-Fc treatment. Electroporation was performed with field strength of 100 V/cm (constant), 6 pulses for 20ms each using an ECM 830 electroporation (BTX). For the in vivo anti-tumor experiment, tumor-bearing mice were vaccinated intramuscularly with 10μg of GX188E. After anesthesia, mice were intravaginally treated with 10μg of IL-7-Fc.

**Distribution and retention of fluorescence-conjugated IL-7-Fc in the genital tract**

IL-7-Fc was coupled with Cy-5.5 mono-reactive NHS ester according to the manufacturer’s instructions (GE Healthcare). Eluted proteins were desalted and concentrated by using centrifugal filter devices (Merck Millipore) and protein concentration of the dye-labeled IL-7-Fc was measured using an anti-human IL-7 ELISA set (Southern Biotech). Diestrus mice were intravaginally administered with an equivalent signal intensity of Cy-5.5-cojuganted IL-7-Fc (1 mg/kg) or unconjugated-Cy-5.5 in PBS under anesthesia. The vaginal lavage and selected organs were collected after euthanasia at 1 and 7 days after application, and processed for quantification of fluorescence signal intensity in an IVIS spectrum machine (Caliper Life Science). The signal intensities were measured quantitatively in the organs by measuring the photons per second per centimeter squared per steradian (p/s/cm2/sr).

**Quantification of serum IL-7**

To measure serum IL-7 concentration, blood sample were drawn before IL-7-Fc administration and at indicated timepoints up to 7 days post treatment and then measured by human IL-7 ELISA set (Southern biotech).

**Immunohistochemistry staining and quantification of fluorescence intensity or the number of T cells**

Tissues were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek), frozen in liquid nitrogen, and stored at -80oC. Frozen blocks were cut into 7µm thick sections, fixed in acetone, and nonspecific binding was blocked for 1 hr with a solution of 5% bovine serum albumin in PBS. To label CD31 and VCAM-1, sections were stained with anti-CD31 (MEC13.3, Biolegend), and biotin-conjugated VCAM-1 (429 (MVCAM.A), Biolegend). Alexa Fluor 594-conjugated streptavidin (Biolegend) was used for secondary staining of VCAM-1. Sections were rinsed for 10 min in PBS and then preserved in mounting medium with DAPI (Sigma). For the quantification of fluorescence intensity, a previously described method was employed (30). To label CD4 and CD8, and collagen IV, sections were stained with anti-CD4 (RM4-5, BD) and anti-CD8 (53-6.7, Biolegend), and rabbit anti-collagen IV (Abcam). Donkey anti-rabbit Alexa Fluor 647 (Invitrogen) was used for secondary staining of collagen IV. For calculating T cell numbers in cervicovaginal tissue (epithelial and lamina propria), the number of T cells in each region was quantitated by normalization against the area of the sections (mm2) from mice after 7 days after IL-7-Fc administration. Images were acquired with an LSM510Meta (Zeiss) confocal microscope and analysis of longitudinal sections was performed using the ZEN 2 software.

**Repeated dose toxicity study**

For the microscopic histopathology analysis after topical IL-7-Fc treatment, rats were anesthetized to receive either a 0.8, 3, 8 mg/kg/dose of IL-7-Fc via intravaginal administration once weekly for 4 weeks (5 total doses). Dose volumes were adjusted based on assigned dose level and animal weight on the day of dosing. The cervix and vaginal tissue were excised from all animals and were fixed in 10% neutral-buffered formalin. Fixed tissues were embedded in paraffin, sectioned at a thickness of 4-6 µm, and stained with hematoxylin and eosin (H&E, Sigma-Aldrich). Microscopic examination was performed by a certified veterinary pathologist. To observe the dose-dependent extent of vaginal irritation, rats were individually observed 4 and 24 hours post each dose administration and weekly using the scoring scale as follows: 0 = no erythema, 1 = very slight erythema (barely perceptible), 2 = well-defined erythema, 3 = moderate erythema, 4 = severe erythema (redness) to eschar formation.

**Flow cytometry analysis**

Cells were incubated with CD16/32 (2.4G2) to block non-specific binding of immunoglobulin to Fc receptors and stained with the appropriate combination of the following monoclonal antibodies: CD4 (RM4-5), CD8 (53-6.7), CD44 (IM7), CD62L (MEL-14), CD11 b (M1/70), CD11c (N418), and MHCII (M5/114.15.2), from eBioscience; CD3e (145-2C1), and TCRGL3), from BD; CXCR3 (CXCR3-173), from Biolegend; Live/Dead (Life technologies). For the TetE749-57 (TCMetrix, Switzerland) and L1165-173 (TCMetrix, Switzerland)H-2Db-restricted CD8 T cell tetramer staining, splenocytes and cervicovaginal cells were stained with tetramer and incubated for 30 min at 25oC. Samples were also stained with CD3e, CD4, CD8, CXCR3, Live/Dead for 30 min at 4oC. All samples were analyzed using an LSR Fortessa (BD) and FlowJo software (Tree Star)

**In vivo tumor experiments**

The TC-1/luc cell line, TC-1 co-transformed with the HPV16 E6, E7 gene and transfected with a luciferase gene, was kindly provided by Professor T.-C. Wu (Johns Hopkins Medical Institutions, Baltimore, MD) (31). Each diestrus mice was intravaginally administered a 50µl dose composed of 10µl of 20% nonoxynol-9 (USP) mixed with 40µl of 3% carboxymethyl cellulose (CMC) (Sigma-Aldrich) under anesthesia. After 6 hours, the genital tract was washed with phosphate buffered saline (PBS) and inoculated with 1ⅹ105 TC-1/luc cells mixed with 20µl of 3% CMC using a micropipette. Genital tumor growth was monitored through bioluminescence 10 min after 3mg of D-luciferin (Goldbio) intraperitoneal injection in an IVIS machine. The signal intensities were measured quantitatively in the tumor regions. The mice were euthanized for ethical reasons (under prolonged exposure to stress, body weight loss is greater than 20%). For T cell depletion, antibody against mouse CD8 (2.43) and isotype (rat-IgG2b) were purchased from Bioxcell (USA). After two days of the therapeutic treatment regimen in tumor-bearing mice, mice were intraperitoneally injected with 200μg of depleting antibody at 3 days intervals for 22 days after tumor challenge to maintain close to 100% cell depletion in the spleen, PBMC, and CV tissues as confirmed using CD3, CD8 antibody detection with flow cytometry in pilot experiments.

**Antibody treatment**

Antibody against mouse IL-7Rα (A7R34) and isotype (rat-IgG2a) were purchased from Bioxcell (USA). Four days prior to IL-7-Fc treatment, mice were intraperitoneally injected with 1mg of blocking antibody at 2 day-intervals until 6 days post topical administration of IL-7-Fc.

**Supplementary Table 1. Tolerable toxicity after repeated intravaginal administration of IL-7-Fc.**

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SD rats were intravaginally treated with indicated dose of IL-7-Fc at day 1, 8, 15, 22, and 29 for a total of five doses. (A) Sections of the genital tract were microscopically examined at 33 days post initial treatment. (B) Vaginal irritation scores were recorded prior to dosing and at 4 and 24 hours post each dose treatment using the scoring scale as explained at below.

**Supplementary figure legend**

**Supplementary Figure 1. Schematic diagram of Fc-fused IL-7**

The numbers in the boxes indicate the amino acid numbers from the CH1 region of immunoglobulin.

**Supplementary Figure 2. FcRn-mediated transcytosis and transport of Fc-fused IL-7 into serum after intravaginal treatment**

Mice (n=7/group) were intravaginally administered with PBS, rIL-7, or IL-7-Fc. Serum levels of IL-7 were analyzed by human IL-7 ELISA. Data, shown as means ± SEMs, are representative of two independent experiments. \* *p* < 0.05 (rIL-7 vs IL-7-Fc) by student’s *t*-test.

**Supplementary Figure 3. Distinct localization patterns of CD4 and CD8 T cells after intravaginal administration of IL-7-Fc**

Mice (n=4) were intravaginally administered with IL-7-Fc and sacrificed for CV harvest at day 7. Sections of CV were stained with antibodies against CD4, CD8, collagen IV and DAPI. (A) Microscopic images of CV tissue with CD4 (red), CD8 (green), collagen IV (white), and DAPI (blue) labeling are shown. E, Epithelial layer LP, lamina propria L, lumen. Scale bars indicate 100µm (left and middle) and 50µm (right). (B) Quantification of the number of CD4 (left) or CD8 (right) T cells in the E or LP from 10 sections. Data, shown as means ± SEMs, are representative of two independent experiments. \*\*, *p* < 0.01 by student’s *t*-test.

**Supplementary Figure 4. Comparison of Fc-fragment and IL-7-Fc on accumulation of T cells in cervicovaginal tissue**

Mice (n=5/group) were intravaginally treated with indicated dose of IL-7-Fc or Fc-fragment. After 7 days, CD4 and CD8 T cells in the CV tissue were analyzed by flow cytometry. (A) Representative dot plots of T cells in CV tissue. (B) Absolute number of CD4 or CD8 T cell counts. Data, shown as means ± SEMs, are representative of two independent experiments. \*\*, *p* < 0.01 by student’s *t*-test.

**Supplementary Figure 5. Abrogation of T cell accumulation after IL-7Rα blocking**

Mice (n=5) were received with either isotype (Rat-IgG2a) or anti-IL-7Rα antibodies at 2-day intervals beginning on four days prior to intravaginal IL-7-Fc treatment. After 7 days, CD4 and CD8 T cells in the CV tissue were analyzed by flow cytometry. (A) Representative dot plots of T cells in CV tissue. (B) Absolute number of CD4 or CD8 T cell counts. Data, shown as means ± SEMs, are representative of two independent experiments. \*\*, *p* < 0.01 by student’s *t*-test.

**Supplementary Figure 6. Route-dependency of IL-7-Fc administration on recruitment of T cells in cervicovaginal tissue**

Mice (n=5/group) were treated with IL-7-Fc via the subcutaneous (SC) or intravaginal (IVAG) route. (A) Serum levels of IL-7 analyzed by human IL-7 ELISA. \*\*, *p* < 0.01 (IL-7-Fc via SC vs IL-7-Fc via IVAG) by student’s *t*-test. (B-C) After 7 days, CD4 and CD8 T cells in the CV tissue were analyzed by flow cytometry. (B) Representative dot plots of T cells in CV tissue. (C) Absolute number of CD4 or CD8 T cell counts. Data, shown as means ± SEMs, are representative of two independent experiments. \*\*, *p* < 0.01 by student’s *t*-test.