Supplementary Materials and Methods

Transfections

MDA-MB-231 cells were transfected with CMV-FLAG-ERβ expression vector or control vector using Lipofectamine transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. After 24 h, the transfected cells were collected and analyzed for ERβ expression by immunofluorescence.

Immunohistochemistry

Tissue samples were fixed in 4% paraformaldehyde over night, washed in 50% ethanol and embedded in paraffin for sectioning. Analysis of immunohistochemistry experiments were performed in a Fully Automated IHC and ISH Leica BOND-MAX (Leica Microsystems Inc, Buffalo Grove, IL, USA). Tissue sections were pretreated with citrate buffer pH 6 for 10 minutes. The primary antibody was incubated for 30 minutes. For the second step the Bond Polymer Refine Detection kit (Leica Microsystems Inc, Buffalo Grove, IL, USA) was used as recommended by the manufacturer. The nuclei were counterstained with hematoxylin. For immunohistochemistry, slides were mounted with Pertex mounting medium. Images were acquired using a Zeiss Axioplan2 microscope (Carl Zeiss AG, Germany) or a Nikon Eclipse E1000 microscope (Nikon Instruments, Inc., Melville, NY, USA).
Transfected with ERβ-expression vector

Transfected with empty (control) vector

Supplementary Figure 1.
Supplementary Figure 2.

Tumor volume, mm$^3$

- **Vehicle**
- KB099520

Days after tumor induction

Treatment starts

0 100 200 300 400 500 600 700 800 900 1000

Days after tumor induction

- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
Supplementary Figure 3.

Tumor volume, mm$^3$

- Vehicle
- DPN
- DPN + ICI

Days after tumor induction

Treatment starts
Supplementary Figure 4.

(a)和(b)图展示了不同治疗方案下肿瘤体积随时间的变化。横轴表示肿瘤诱导后的天数（6到12天），纵轴表示肿瘤体积（单位：mm³）。不同治疗方案的标记如下：
- 蓝色圆形：Vehicle
- 黑色方块：DPN
- 灰色菱形：DPN + E2 (a图)
- 黑色方形：Vehicle
- 灰色方块：E2
- 灰色菱形：PPT (b图)

所有图中都标示了“Treatment starts”（治疗开始）的标记。
Supplementary Figure 5.
Supplementary Figure 6.
Supplementary Figure 7.

(a) Relative mRNA expression for Vegf-d and Lyve-1.

(b) Relative mRNA expression for VEGF-C.

- **Vehicle**
- **DPN**
Supplementary Figure 8.
Supplementary Figure legends

Supplementary figure 1. ERβ expression in Granta-519 MCL cells and validation of the ERβ specificity of the anti-ERβ antibody. (a) ERβ expression was analyzed in Granta-519 MCL cells using the anti-ERβ PPG5/10 antibody. Strong ERβ staining was observed in the nuclei of MCL cells. To confirm the ERβ specificity of the anti-ERβ PPG5/10 antibody, ERβ-negative human breast cancer MDA-MB-231 cells were stained and showed to be negative (b), unless the cells were transfected with a human ERβ-expressing vector (c). Green color indicates ERβ staining. Magnification: 400×.

Supplementary figure 2. The highly selective ERβ-agonist KB099520 inhibits Granta-519 MCL growth in vivo. Male mice were transplanted with Granta-519 MCL cells as described in Methods and treated with 28 μmole/kg body weight of KB099520 (▲) or vehicle (●). The arrow indicates start of treatment. Similar to the structurally unrelated ERβ agonist DPN, this highly selective ERβ agonist significantly inhibited Granta-519 MCL growth in vivo.

Supplementary figure 3. The ER antagonist ICI 182.780 inhibits the tumor inhibiting effect of DPN. Male mice were transplanted with Granta-519 MCL cells as described in Methods and treated with Vehicle (●), DPN (12.5 μmole/kg/day) alone (■) or a combination of DPN (12.5 μmole/kg/day) and ICI 182.780 (1.65 μmole/kg/day) (▲). The ER antagonist ICI 182.780 blocked the tumor inhibiting effect of DPN. *P<0.05 or **P<0.01 for DPN vs. Vehicle for day 12 to 16 and ¶P<0.05 for DPN vs. DPN+ ICI 182.780 for day 15 to 16. The arrow indicates start of treatment.
Supplementary figure 4. Estradiol inhibits the tumor inhibiting effect of DPN. No effect of the ERα selective agonist PPT. Male mice were transplanted with Granta-519 MCL cells as described in Methods and treated with, vehicle (●), DPN (■, 12.5 μmole/kg/day), PPT (◇, 13 μmole/kg/day) or estradiol (◇, 11.5 μmole/kg/day) alone or in a combination of DPN and estradiol (◇, 12.5 μmole and 11.5 μmole/kg/day, respectively). PPT had no tumor inhibiting effect (b) in contrast to DPN (a) and estradiol impaired the tumor inhibiting effect of DPN (a). Note that estradiol is a weak partial agonist in this context (b) similar to what has seen for murine T cell lymphoma5. The arrow indicates start of treatment.

Supplementary figure 5. DPN inhibits the expression of VEGF-C in Granta-519 MCL. The expression of VEGF-C was analyzed by immunofluorescence. The number of VEGF-C-positive cells was decreased in DPN-treated Granta-519 MCL lymphomas compared to control. Red color indicates VEGF-C staining and blue indicates nuclear staining with DAPI. Magnification: 600×.

Supplementary figure 6. DPN inhibits lymphatic vessel formation in Granta-519 MCL. The expression of the lymphoendothelial marker LYVE-1 was analyzed by immunofluorescence. The number and size of LYVE-1-positive lymphatic vessels were decreased in DPN-treated Granta-519 MCL lymphomas compared to control. Red color indicates LYVE-1 staining and blue indicates nuclear staining with DAPI. Magnification: 200×.

Supplementary figure 7. DPN down-regulates VEGF and LYVE-1 expression in murine EG7 T cell lymphoma and human Raji BL tumors. Male mice were grafted with EG7 T cell lymphoma cells (a) or BL Raji (b) as described in Methods and treated with a daily
subcutaneous injection of DPN (12.5 μmole/kg/day) from start of palpable tumor until sacrifice (7-14 days). Expression of VEGF-C (Raji), Vegf-d and Lyve-1 (EG7) mRNA expression was quantified by qPCR. Gapdh was used as internal control when analyzing murine EG7 tumor tissue. Vegf-d, and not Vegf-c, was the major Vegf down-regulated by DPN in the murine EG7 lymphomas.

Supplementary figure 8. Selective ERβ agonist DPN inhibits Raji lymphoma dissemination in vivo. DPN treatment inhibited the dissemination of Raji lymphoma cells to the liver as demonstrated by a reduced number of lymphoma foci on the liver surface.
**Supplementary Table 1. Primers for qPCR.**

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<th>Name of the gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<td>CATCCCTCTTTGAACCTGGA</td>
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