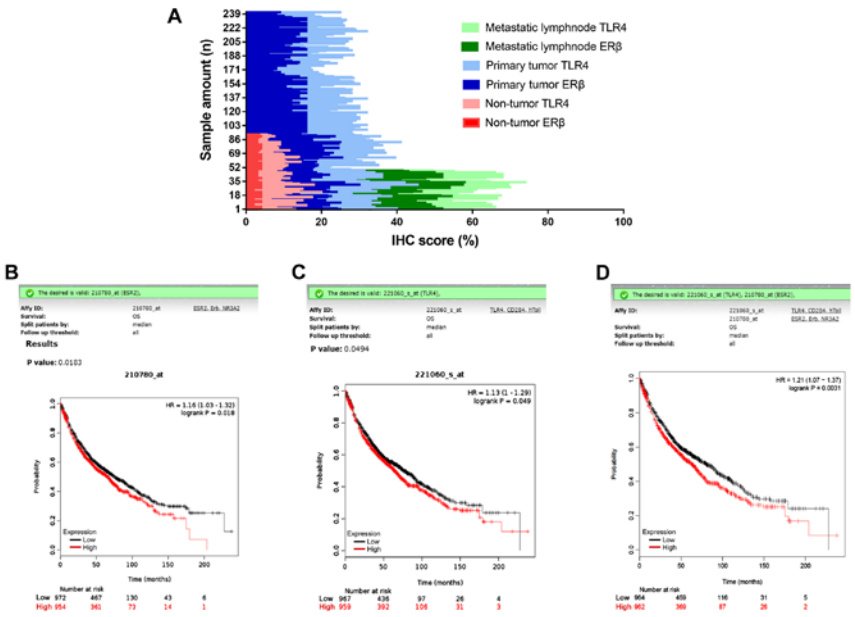

Figure S1. (A) Detailed immunohistochemical (IHC) scores for ERβ and TLR4 expression in adjacent non-tumor tissue, primary NSCLC tumors, and metastatic lymph nodes. (B) Kaplan-Meier overall survival (OS) curves (http://www.kmplot.com/lung) of 1,926 patients with lung cancer. The OS rate in patients with high ERβ expression was significantly lower than that in patients with no or low ERβ expression (log-rank P=0.018; HR=1.16 (1.03-1.32) (probe 210780_at). (C) Kaplan-Meier survival analysis using KMplotter revealed that high TLR4 expression was associated with shorter overall survival (log-rank P=0.049; HR=1.13 (1-1.29) (probe 221060_s_at). (D) Kaplan-Meier survival analysis using KMplotter revealed that co-expression of ERβ and TLR4 was associated with poor OS and higher hazard ratio (log-rank P=0.0031; HR=1.21 (1.07-1.37) (probe 221060_s_at). NSCLC, non-small cell lung cancer; ERβ, estrogen receptor β; TLR4, Toll-like receptor 4.

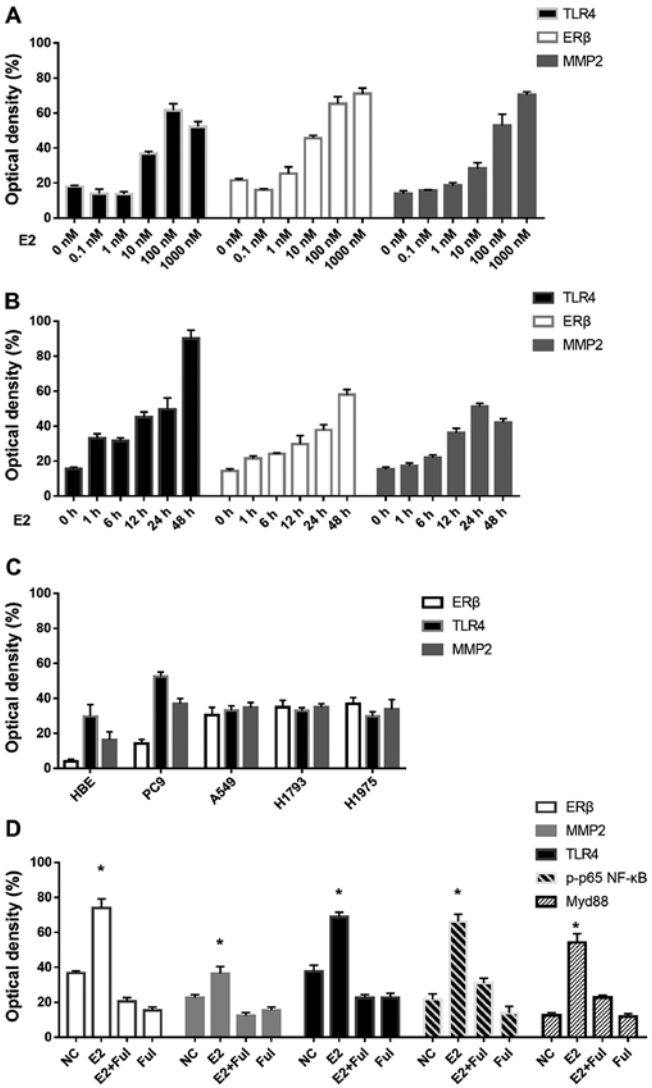

Figure S2. (A) Optical density analysis of the western blotting of the synchronized A549 cells treated with E2 at different concentrations (0, 0.1, 1, 10, 100 and 1,000 nM) for 48 h. (B) Optical density analysis of the western blotting of synchronized A549 cells treated with E2 at different time points (0, 1, 6, 12, 24 and 48 h) at concentrations of 100 nM. (C) Optical density analysis of the western blotting of ERβ, TLR4 and MMP2 expression in cultured NSCLC cell lines (PC9, A549, H1793, and H1975) and normal bronchial epithelial cell line (HBE). (D) Optical density analysis of the western blotting of ERβ, TLR4, MMP2, P65NF-κB, phosphorylated (p)-p65NF-κB, and myd88 protein levels at 48 h in A549 cells. *P<0.05, significant difference as compared with the negative control (NC) group. MMP2, matrix metalloprotease 2; ERβ, estrogen receptor β; TLR4, Toll-like receptor 4; myd88, myeloid differentiation primary response 88; NF-κB, nuclear factor-κB; Ful, fulvestrant (ER antagonist).

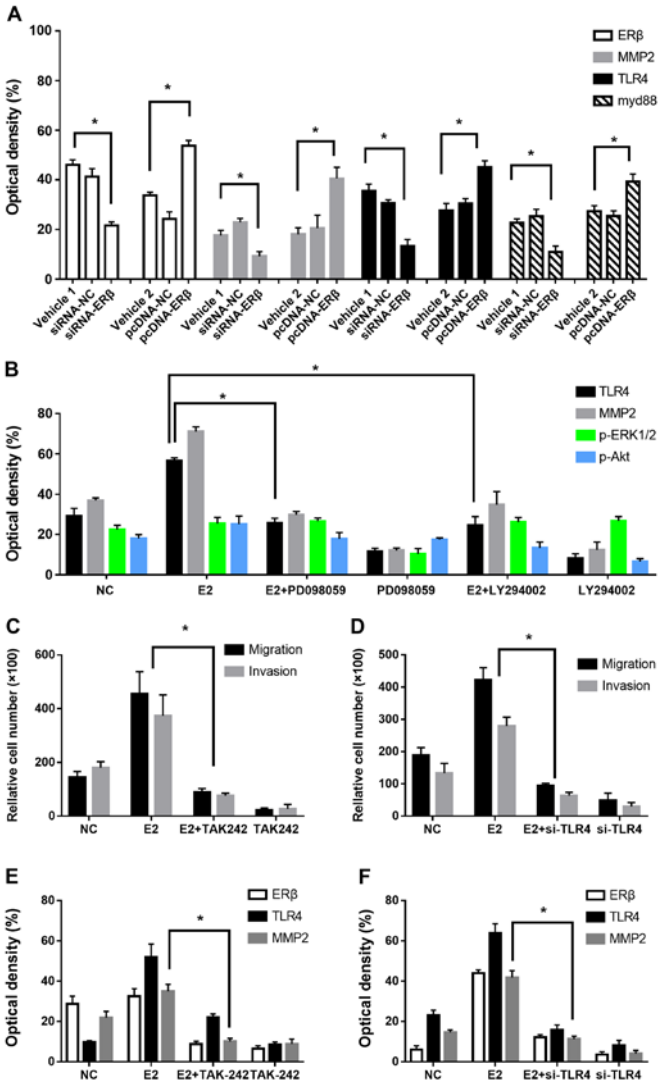

Figure S3. (A) Optical density analysis of the western blotting of ERβ, MMP2, TLR4 and myd88 in the synchronized A549 cells treated with siRNA-ERβ or pcDNA-ERβ. (B) Optical density analysis of the western blotting of TLR4, MMP2, p-ERK1/2 and p-AKT protein levels in A549 cells treated with E2, PD098059, and LY294002, in combination or alone. (C and D) Counting results of the relative Transwell cell numbers are shown (from the images in Fig. 3D and E). (E and F) Optical density analysis of the western blotting shown in Fig. 3F and 3G). *P<0.05, significant difference. MMP2, matrix metalloprotease 2; ERβ, estrogen receptor β; TLR4, Toll-like receptor 4; myd88, myeloid differentiation primary response 88.

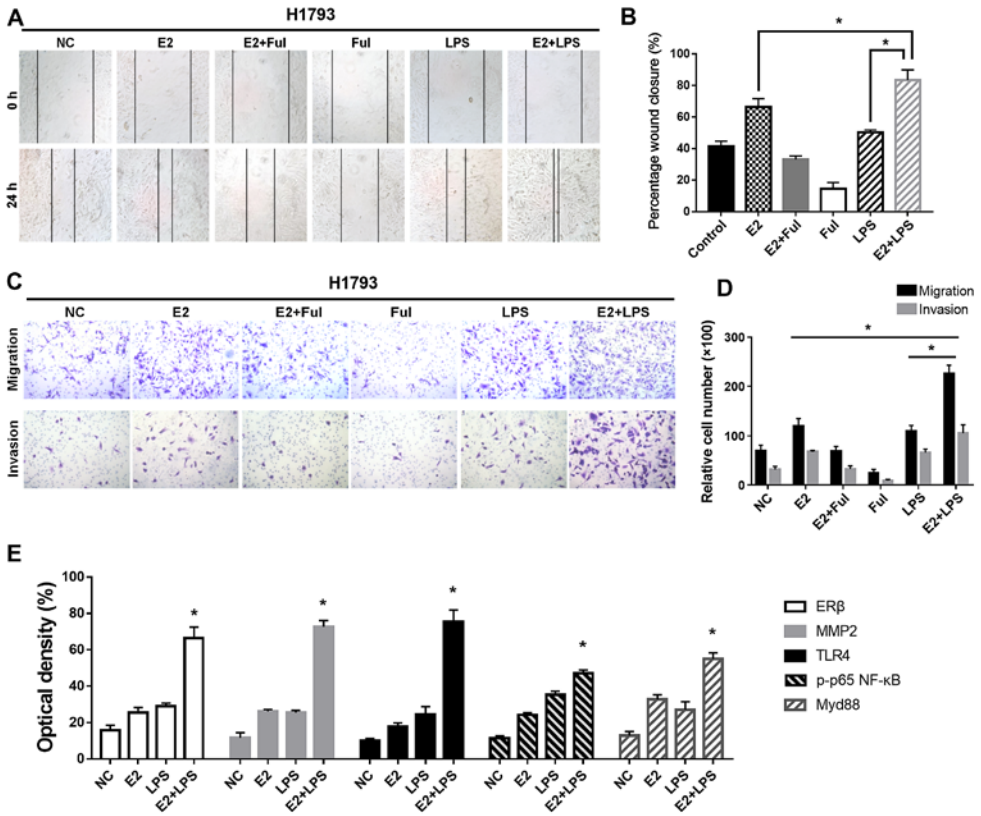

Figure S4. (A) Migration in a wound-healing assay of H1793 cells after treatment with DMSO (negative control, NC) or E2 (10 nM), Ful (1 μM), E2+Ful, LPS (10 μg/ml), and E2+LPS for 24 h (magnification x40). (B) Effect on wound closure (shown as a percentage) in H1793 cells. (C and D) Matrigel Transwell assay was performed to determine the invasiveness of H1793 cells in the different treatment groups. Exposure to E2 and LPS enhanced cell invasion, while the combination of E2 and LPS increased cell invasiveness more than did exposure to each agent alone. *P<0.05, significant difference. (E) Optical density analysis of the western blotting of ERβ, TLR4, MMP2, phosphorylated (p)-p65NF-κB, and myd88 protein levels at 48 h in A549 cells treated with E2, LPS, or the combination of both. *P<0.05, significant difference, compared to the NC group. MMP2, matrix metalloprotease 2; ERβ, estrogen receptor β; TLR4, Toll-like receptor 4; myd88, myeloid differentiation primary response 88, NF-κB, nuclear factor-κB.

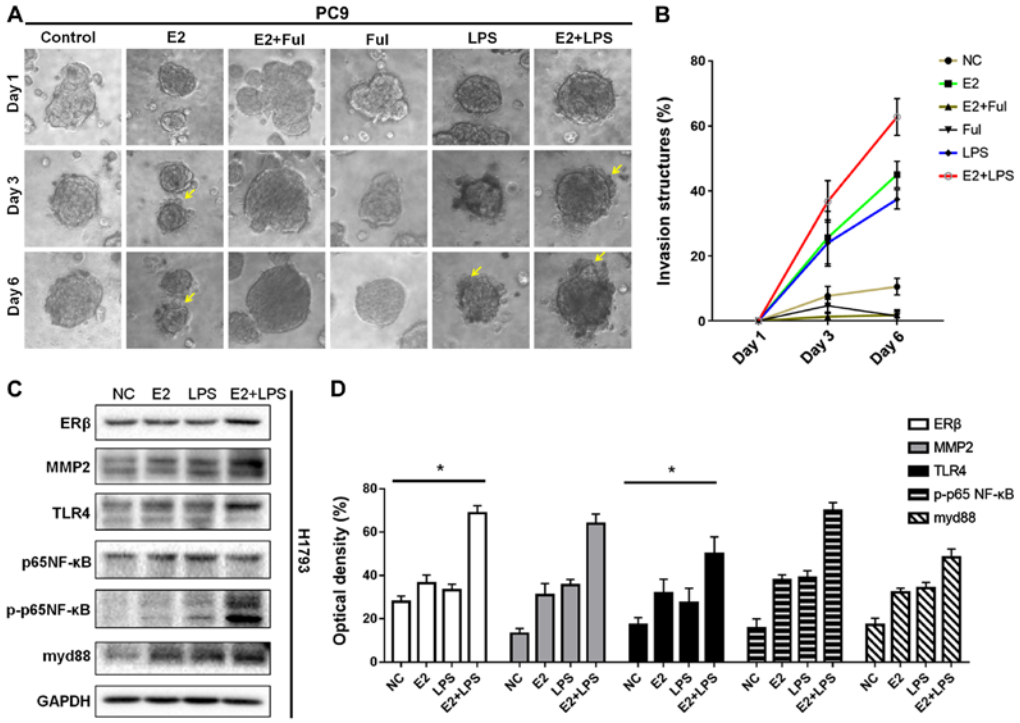

Figure S5. (A) 3D spheroid cell invasion assay of lung adenocarcinoma PC9 cells treated with DMSO (negative control, NC), E2 (10 nM), Ful (1 μM), E2+Ful, LPS (10 μg/ml), or E2+LPS. Representative images were acquired at day 1, 3 and 6 using a light microscope (x100). The combined treatment with E2 and LPS accelerated invadopodium formation (yellow arrow) compared with that of other treatment groups. (B) Quantification of 3D spheroid cell invasion assays. Quantification was carried out by measuring the distance between the invasive cell frontier and spheroid edge. (C) Western blot analysis of ERβ, TLR4, MMP2, p65NF-κB, phosphorylated (p)-p65NF-κB, and myd88 protein levels at 48 h in H1793 cells. Protein levels of ERβ, TLR4, myd88, MMP2, and p-p65NF-κB, induced by combined treatment with E2 and LPS were higher compared with groups treated with E2 or LPS alone. (D) Detection of optical density (*P<0.05 indicates statistical significance). Ful, fulvestrant (ER antagonist); LPS, lipopolysaccharide; MMP2, matrix metalloprotease 2; ERβ, estrogen receptor β; TLR4, Toll-like receptor 4; myd88, myeloid differentiation primary response 88, NF-κB, nuclear factor-κB.

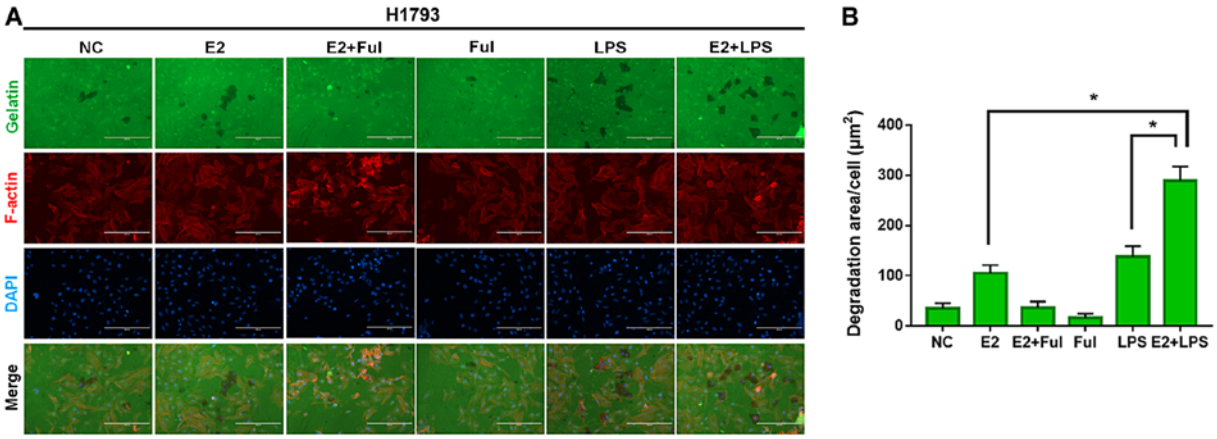

Figure S6. (A) Fluorescent-gelatin degradation assay of lung adenocarcinoma H1793 cells treated with DMSO (negative control, NC), E2 (10 nM), Ful (1 μM), E2+Ful, LPS (10 μg/ml), and E2+LPS at 48 h post-treatment. The dotted black areas show representative regions of gelatin degradation. Scale bar, 200 μm. (B) Quantification of degradation level in FITC-gelatin as determined by degradation assay (μm^2/cell). Combined treatment with E2+LPS significantly accelerated invadopodium formation in NSCLC cells compared with other treatments. *P<0.05 indicates statistical significance. Ful, fulvestrant (ER antagonist); LPS, lipopolysaccharide; NSCLC, non-small cell lung cancer.