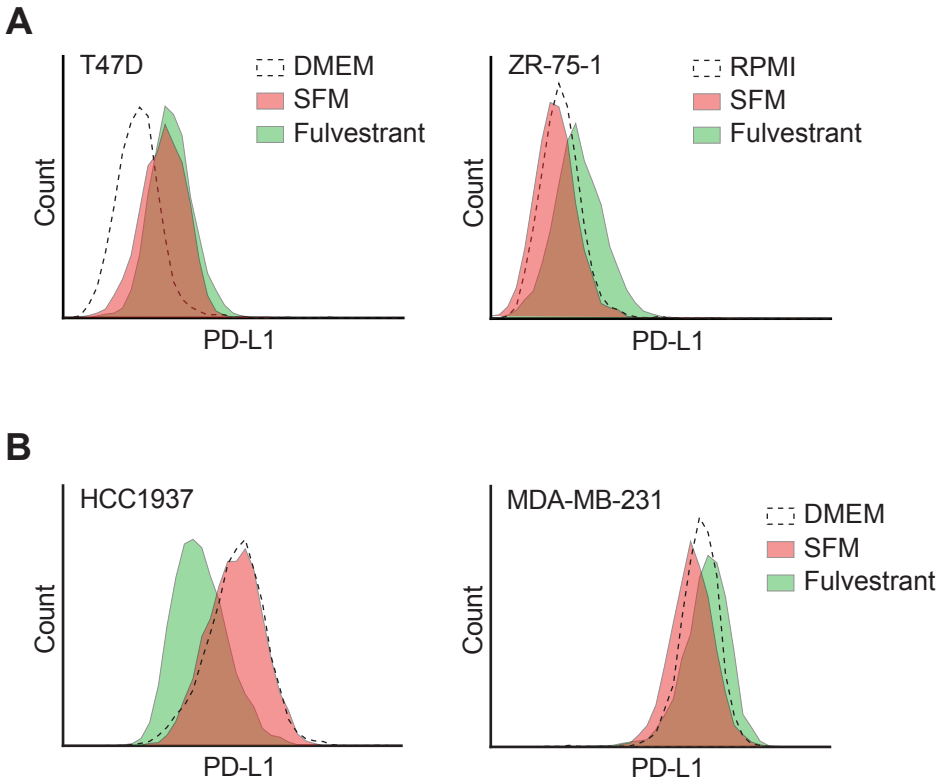
A
T47D
DMEM
SFM
Fulvestrant
Count
PD-L1
ZR-75-1
RPMI
SFM
Fulvestrant
Count
PD-L1
B
HCC1937
Count
PD-L1
MDA-MB-231
DMEM
SFM
Fulvestrant
Count
PD-L1

Figure S1. **Estrogen deprivation induces PD-L1 expression in ER+ BC cells.** (**A**) Flow cytometry-mediated quantification of PD-L1 surface levels in ER^+ BC cell lines, T47D and ZR-75-1, grown in DMEM, SFM or DMEM supplemented with fulvestrant (1 μM) for 18 days. (**B**) Flow cytometry-mediated quantification of PD-L1 surface levels in ER− BC cell lines (HCC1937 and MDA-MB-231), grown in normal medium (DMEM for MDA-MB-231 and RPMI 1640 for HCC1937), SFM or medium supplemented with fulvestrant (1 μM) for 18 days.

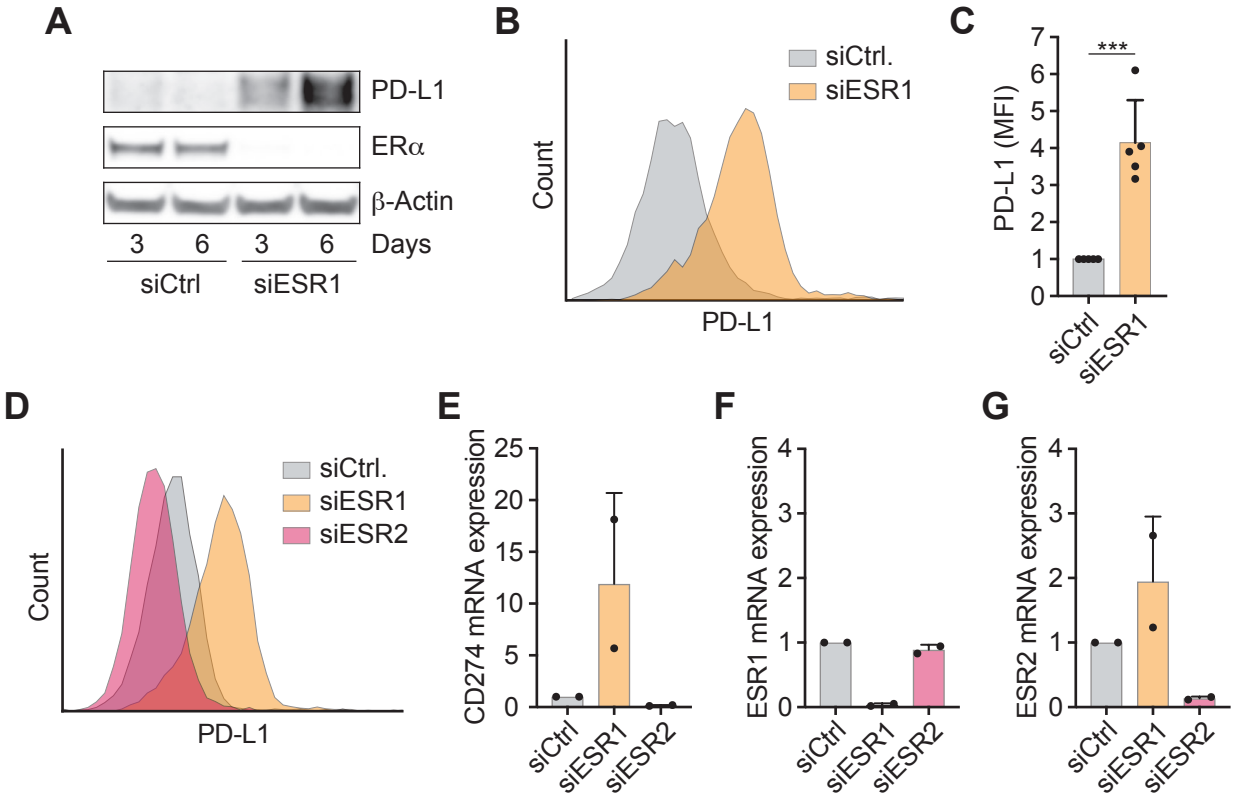
A
PD-L1
ERα
β-Actin
3
6
3
6
Days
siCtrl
siESR1
B
siCtrl.
siESR1
Count
PD-L1
C
***
PD-L1 (MFI)
siCtrl
siESR1
D
siCtrl.
siESR1
siESR2
Count
PD-L1
E
CD274 mRNA expression
siCtrl
siESR1
siESR2
F
ESR1 mRNA expression
siCtrl
siESR1
siESR2
G
ESR2 mRNA expression
siCtrl
siESR1
siESR2

Figure S2. **ERα, and not ERβ, suppresses PD-L1 expression in MCF7 cells.** (**A**) Western blot analysis of the indicated proteins in MCF7 cells treated with either 20 nM control or ERα-targeting (*ESR1*) siRNA for 3 or 6 days. β-Actin is shown as a loading control. (**B**) PD-L1 membrane levels analysed by flow cytometry after treatment of MCF7 cells with the indicated siRNAs for 6 days. (**C**) Quantification of 3 independent experiments as the one shown in (**B**). Data are presented as mean +SD (n=5). ***$p<0.001$. (**D**) PD-L1 membrane levels as analysed by flow cytometry in MCF7 cells transfected twice with 20nM of the indicated siRNAs for 6 days. (**E-G**) qRT-PCR analysis of *CD274* (**E**), *ESR1* (**F**) and *ESR2* (**G**) mRNA levels in MCF7 cells transfected as in (**D**). Levels of the *18S* rRNA served as an internal control.

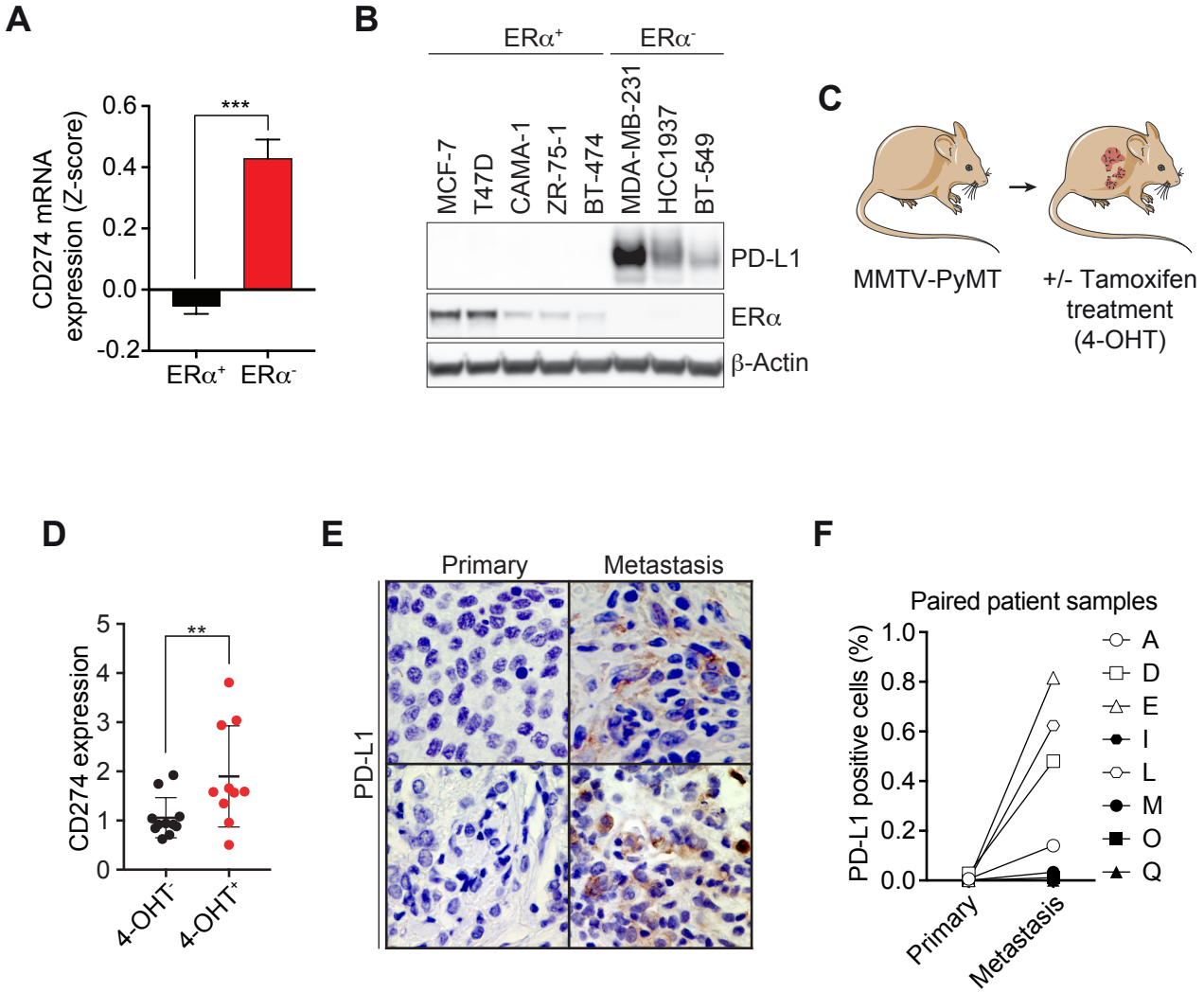
A
CD274 mRNA expression (Z-score)
0.6
0.4
0.2
0.0
-0.2
***
ERα+
ERα-
B
ERα+
ERα-
MCF-7
T47D
CAMA-1
ZR-75-1
BT-474
MDA-MB-231
HCC1937
BT-549
PD-L1
ERα
β-Actin
C
MMTV-PyMT
+/- Tamoxifen treatment (4-OHT)
D
CD274 expression
5
4
3
2
1
0
**
4-OHT-
4-OHT+
E
Primary
Metastasis
PD-L1
F
Paired patient samples
PD-L1 positive cells (%)
1.0
0.8
0.6
0.4
0.2
0.0
A
D
E
I
L
M
O
Q
Primary
Metastasis

Figure S3. **Inverse correlation between ERα and PD-L1 in BC.** (**A**) *CD274* mRNA expression levels in ERα$^+$ (n=1459) and ERα$^-$ (n=445) patient samples. Normalised Z-scores were extracted from the METABRIC breast cancer dataset. A factor was added to both populations in order to set the ERα+ group to 0. Data are presented as mean +SEM and two-tailed Student's t-test was used to calculate the statistical significance, ***p<0.001. (**B**) Western blot analysis of PD-L1 and ERα protein in a panel of ERα$^+$ and ERα$^-$ BC cell lines. β-Actin served as a loading control. (**C**) Schematics of the experimental procedure in mice. MMTV-PyVT mice that spontaneously develop mammary tumors were treated daily from week 5 until with 4-hydroxytamoxifen (4-OHT) or vehicle. (**D**) *Cd274* expression in tumors isolated from MMTV-PyVT mice treated with 4-OHT or vehicle as determined by RT-qPCR. Data were normalized to β-*Actin* mRNA levels. Graph represents the mean ±SD and two-tailed Student's t-test was used to determine statistical significance, **p<0.01. (**E**) Immunohistochemical staining of PD-L1 (brown) in paired samples of primary and metastatic human ER+ BC from two different patients. Sections were stained with Hematoxylin-Eosin to aid in the pathological analysis. (**F**) Quantification of the percentage of PD-L1 positive cells in paired primary tumor and metastasis samples from 8 patients of ERα-positive BC.

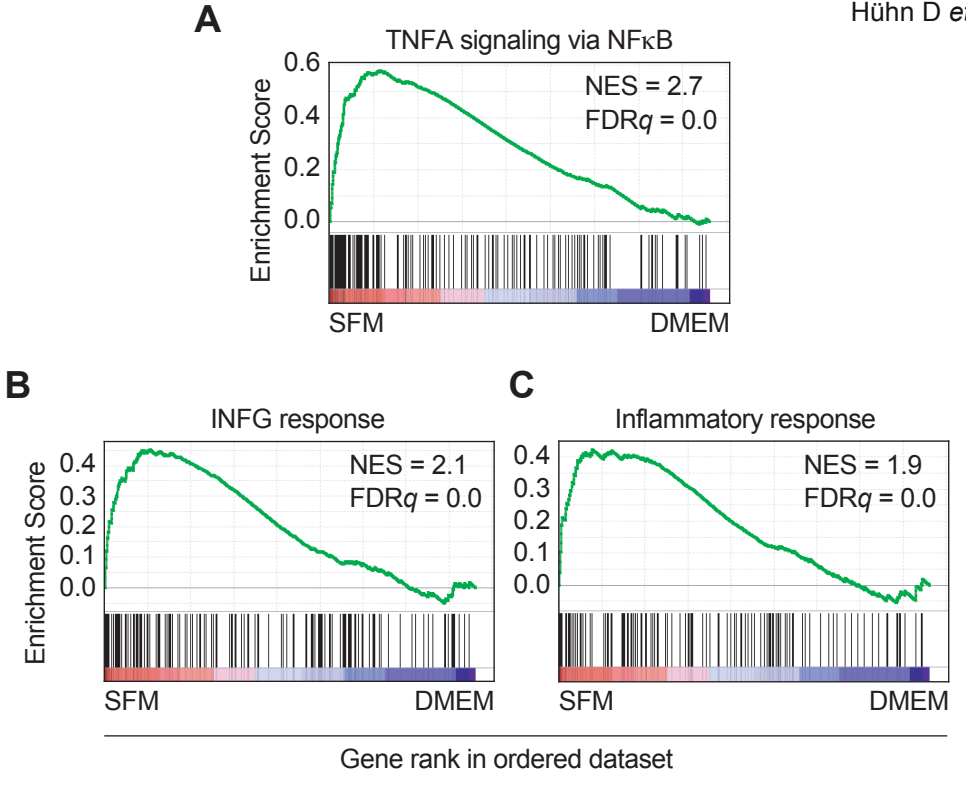
A
TNFA signaling via NFκB
NES = 2.7
FDRq = 0.0
Enrichment Score
0.6
0.4
0.2
0.0
SFM
DMEM
B
INFG response
NES = 2.1
FDRq = 0.0
Enrichment Score
0.4
0.3
0.2
0.1
0.0
SFM
DMEM
C
Inflammatory response
NES = 1.9
FDRq = 0.0
0.4
0.3
0.2
0.1
0.0
SFM
DMEM
Gene rank in ordered dataset

Figure S4. **Estrogen deprivation triggers an inflammatory transcriptional program in MCF7 cells.** (**A**-**C**) Pre-ranked GSEA on the genes from the hallmarks "TNFA signaling via NF-κB" (**A**), "IFNG response" (**B**) and "Inflammatory response" (**C**) obtained from RNAseq analysis comparing the transcriptome of MCF7 cells grown in DMEM or SFM for 3 weeks. The heatmap representation illustrates the overall upregulation of these pathways in SFM-grown MCF7 cells.

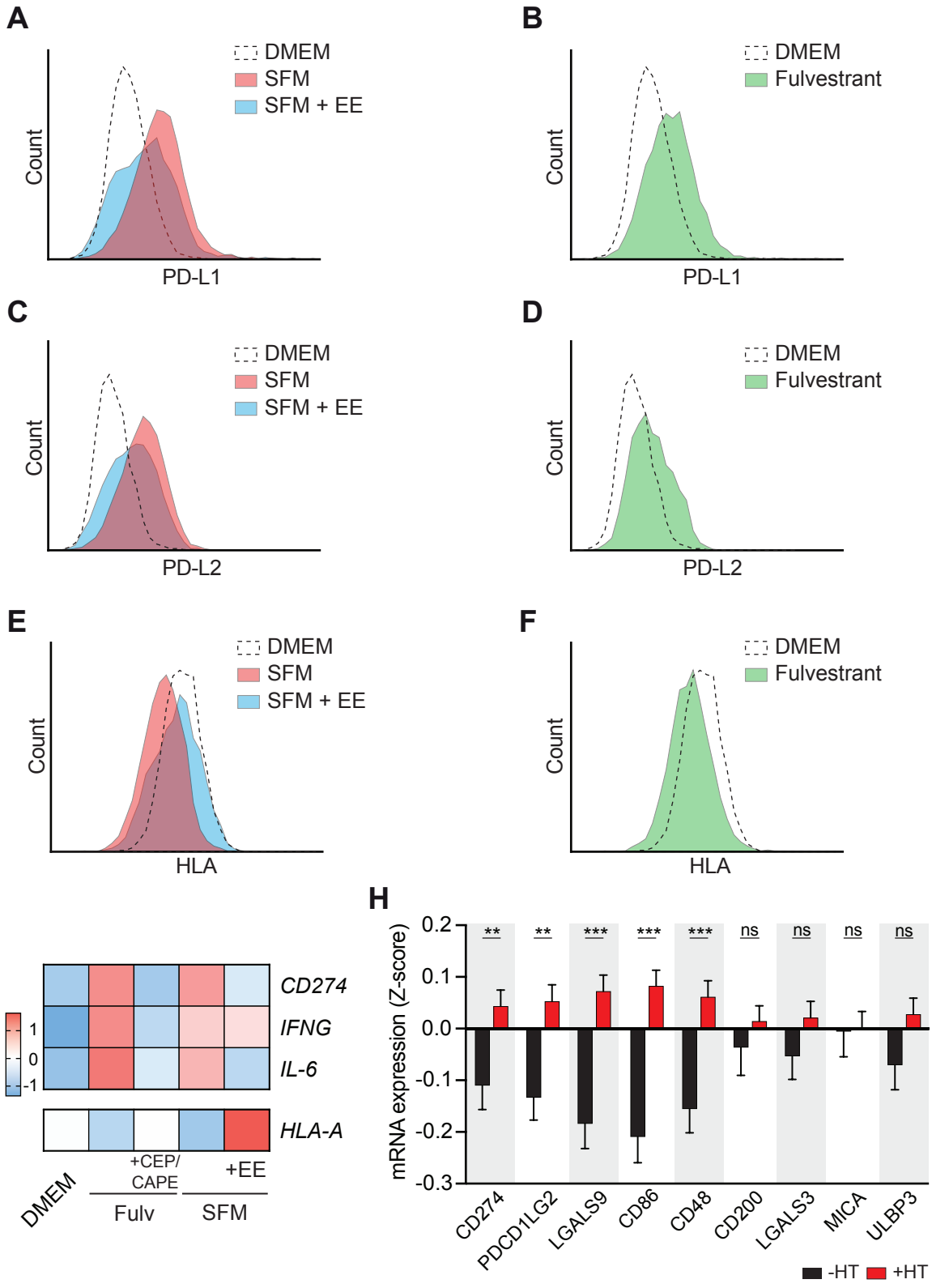
A
DMEM
SFM
SFM + EE
Count
PD-L1
B
DMEM
Fulvestrant
Count
PD-L1
C
DMEM
SFM
SFM + EE
Count
PD-L2
D
DMEM
Fulvestrant
Count
PD-L2
E
DMEM
SFM
SFM + EE
Count
HLA
F
DMEM
Fulvestrant
Count
HLA
G
mRNA expression (Z-score)
1
0
-1
CD274
IFNG
IL-6
HLA-A
DMEM
+CEP/
CAPE
+EE
Fulv
SFM
H
mRNA expression (Z-score)
0.2
0.1
0.0
-0.1
-0.2
-0.3
**
**
***
***
***
ns
ns
ns
ns
CD274
PDCD1LG2
LGALS9
CD86
CD48
CD200
LGALS3
MICA
ULBP3
-HT
+HT

Figure S5. **Estrogen deprivation induces the expression of immune checkpoints and the downregulation of HLA in T47D cells.** (**A, C, E**) Flow cytometry-mediated assessment of surface PD-L1 (**A**), PD-L2 (**C**) or HLA-A, -B, -C levels (**E**) in T47D cells grown in DMEM or SFM for 21 days. Where indicated EE (10nM) was added for the final 4 days. (**B, D, F**) Flow cytometry-mediated assessment of surface PD-L1 (**A**), PD-L2 (**C**) or HLA-A, -B, -C levels (**E**) in T47D cells grown in DMEM or DMEM supplemented with 1uM Fulvestrant for 21 days. (**G**) Heatmap of mRNA expression levels (Z-score) of the indicated genes as analyzed by qRT-PCR in T47D cells grown in DMEM or SFM for 21 days, alone or in combination with the NFkB inhibitor CAPE (10μM) or JAK2 inhibitor CEP-33779 (10μ) for the last 4 days. Where indicated EE (10nM) was added for the final 4 days. *18S* RNA served as an internal control. (**H**) mRNA expression levels of the indicated genes related to immune checkpoints in ERα$^+$ patients receiving (+HT; 1047) or not receiving (-HT; 412) hormone therapy. Normalised Z-scores were extracted from the METABRIC breast cancer dataset. A factor was added to both populations in order to set the ERα+ group to 0. Data are presented as mean +SEM and two-tailed Student's t-test was used to calculate the statistical significance, **p<0,01;***p<0.001;ns: not significant.

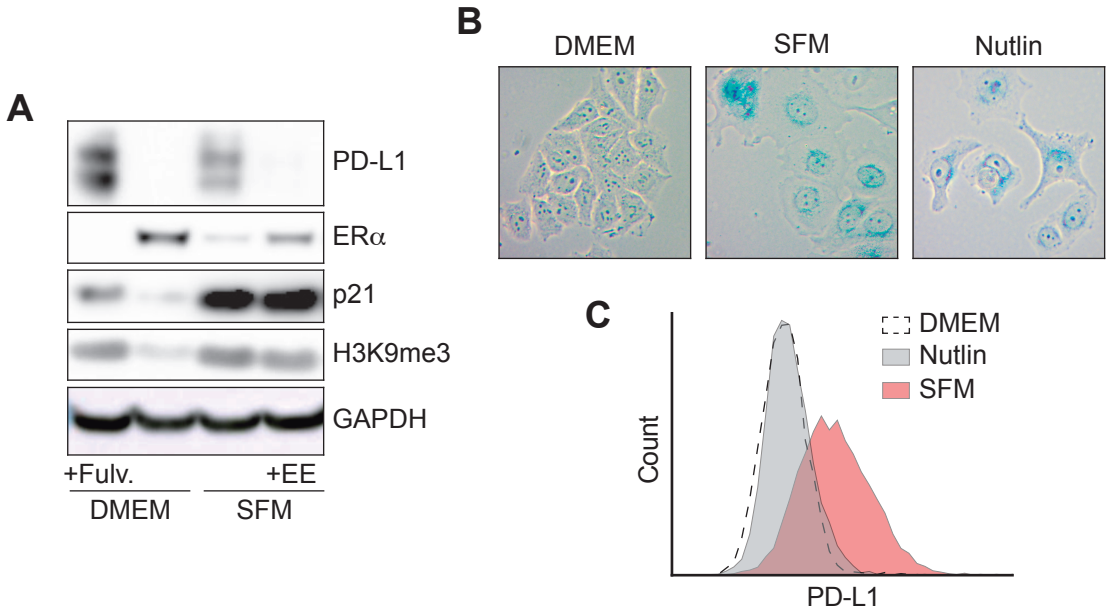
A
PD-L1
ERα
p21
H3K9me3
GAPDH
+Fulv.
+EE
DMEM
SFM
B
DMEM
SFM
Nutlin
C
DMEM
Nutlin
SFM
Count
PD-L1

Figure S6. **Induction of senescence is not sufficient to trigger PD-L1 expression in MCF7 cells.** (**A**) MCF7 cells were grown in DMEM, SFM or DMEM with fulvestrant (1 μM) for 17 days. Where indicated, EE (10 nM) was added for the last 3 days. Whole-cell extracts were subjected to western blot analysis of the indicated proteins. (**B**) SA-β-Galactosidase assay of MCF7 cells cultured in DMEM, SFM (19 days) or Nutlin-3 (5 μM, 5 days). Representative images for each condition are shown. (**C**) FACS analysis of PD-L1 membrane levels in MCF7 cells cultivated as in (**B**).