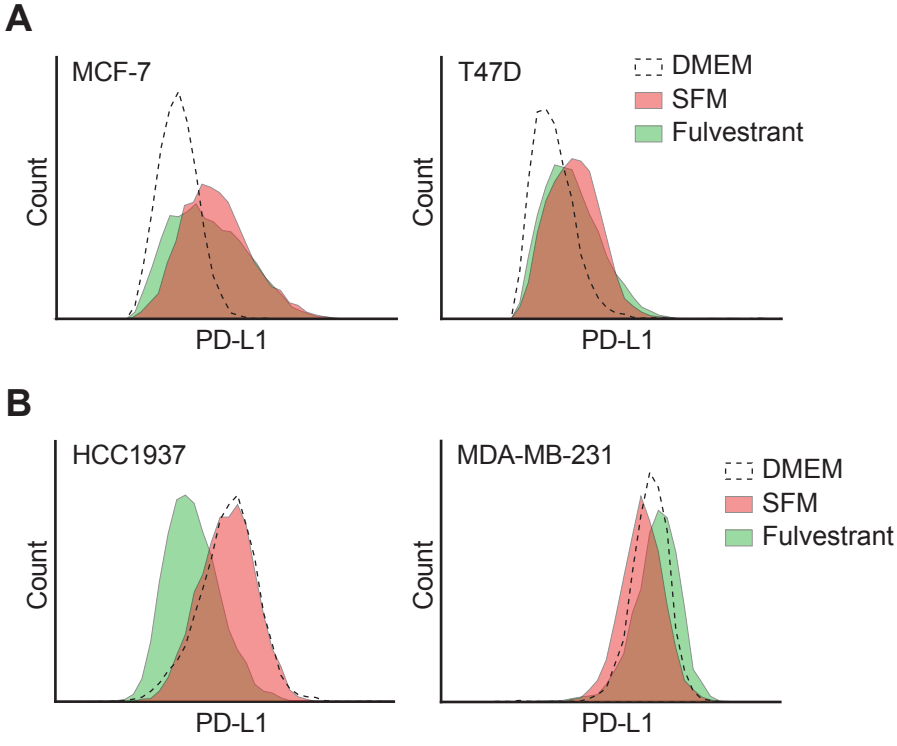
A
MCF-7
T47D
DMEM
SFM
Fulvestrant
Count
PD-L1
B
HCC1937
MDA-MB-231
DMEM
SFM
Fulvestrant
Count
PD-L1

Figure S1. **Estrogen-deprival induces PD-L1 expression in ER+ BC cells A,** Flow cytometry-mediated quantification of PD-L1 surface levels in ERα^+ BC cell lines (MCF7 and T47D), 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 ERα^- 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.

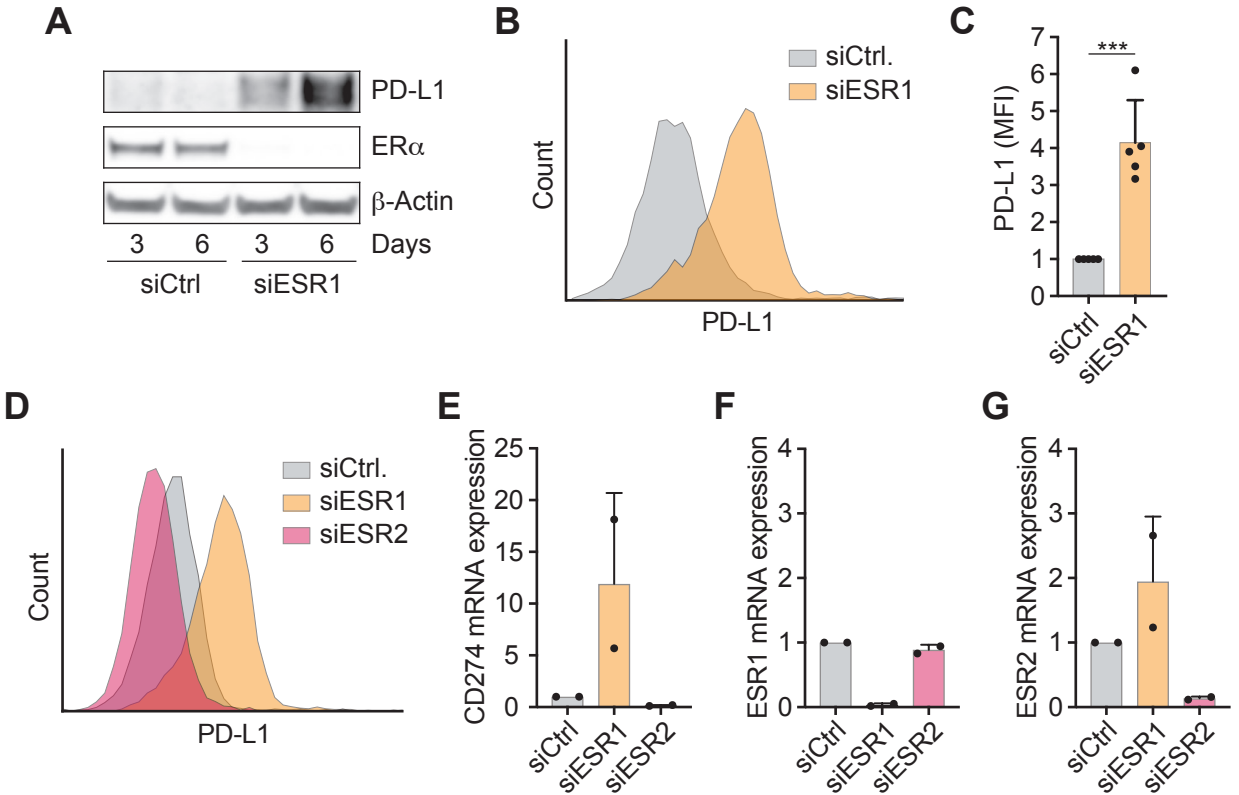
A
PD-L1
ERα
β-Actin
3 6 3 6 Days
siCtrl siESR1
B
siCtrl.
siESR1
Count
PD-L1
C
***
PD-L1 (MFI)
siCtrl
siESR1
D
siCtrl.
siESR1
siESR2
Count
PD-L1
E
CD274 mRNA expression
siCtrl
siESR1
siESR2
F
ESR1 mRNA expression
siCtrl
siESR1
siESR2
G
ESR2 mRNA expression
siCtrl
siESR1
siESR2

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.

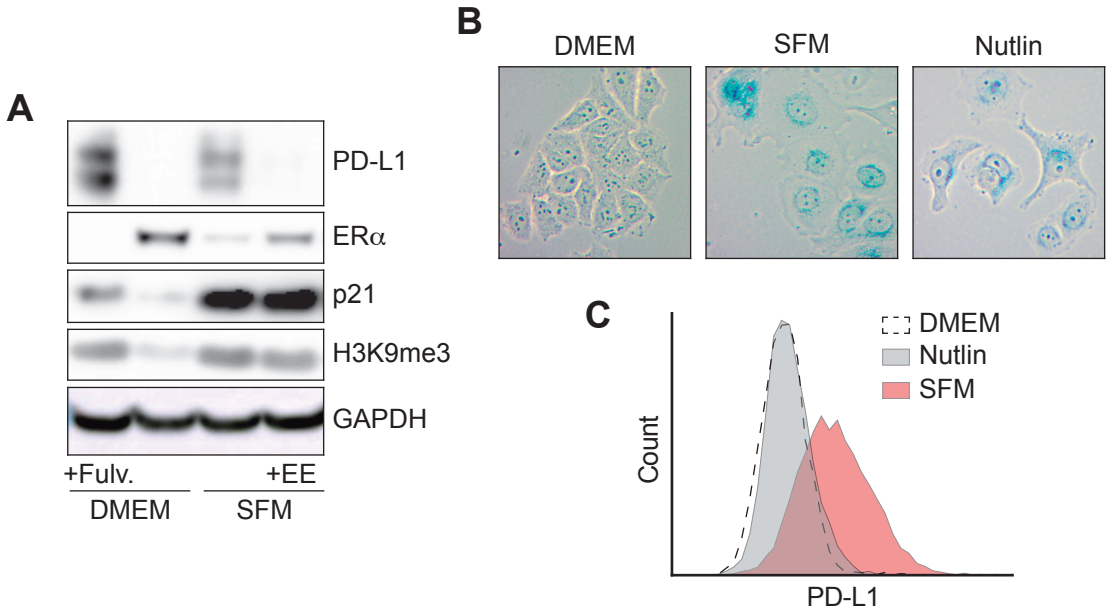
A
PD-L1
ERα
p21
H3K9me3
GAPDH
+Fulv.
+EE
DMEM
SFM
B
DMEM
SFM
Nutlin
C
DMEM
Nutlin
SFM
Count
PD-L1

Figure S3. **Senescence fails to upregulate 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**).

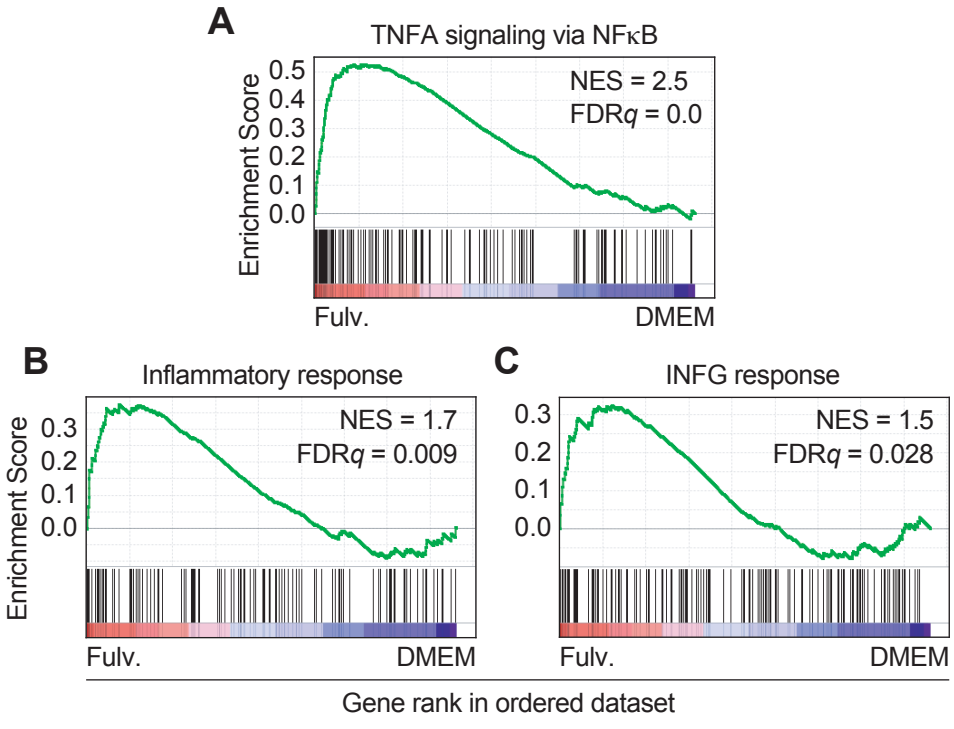
A
TNFA signaling via NFκB
NES = 2.5
FDRq = 0.0
Enrichment Score
0.5
0.4
0.3
0.2
0.1
0.0
Fulv.
DMEM
B
Inflammatory response
NES = 1.7
FDRq = 0.009
Enrichment Score
0.3
0.2
0.1
0.0
Fulv.
DMEM
C
INFG response
NES = 1.5
FDRq = 0.028
0.3
0.2
0.1
0.0
Fulv.
DMEM
Gene rank in ordered dataset

Figure S4. **Fulvestrant 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**), "Inflammatory response" (**B**) and "IFNG response" (**C**) obtained from RNAseq analysis comparing the transcriptome of MCF7 cells grown in DMEM or fulvestrant (1 μM) for 3 weeks. The heatmap representation illustrates the overall upregulation of these pathways in fulvestrant-treated MCF7 cells.

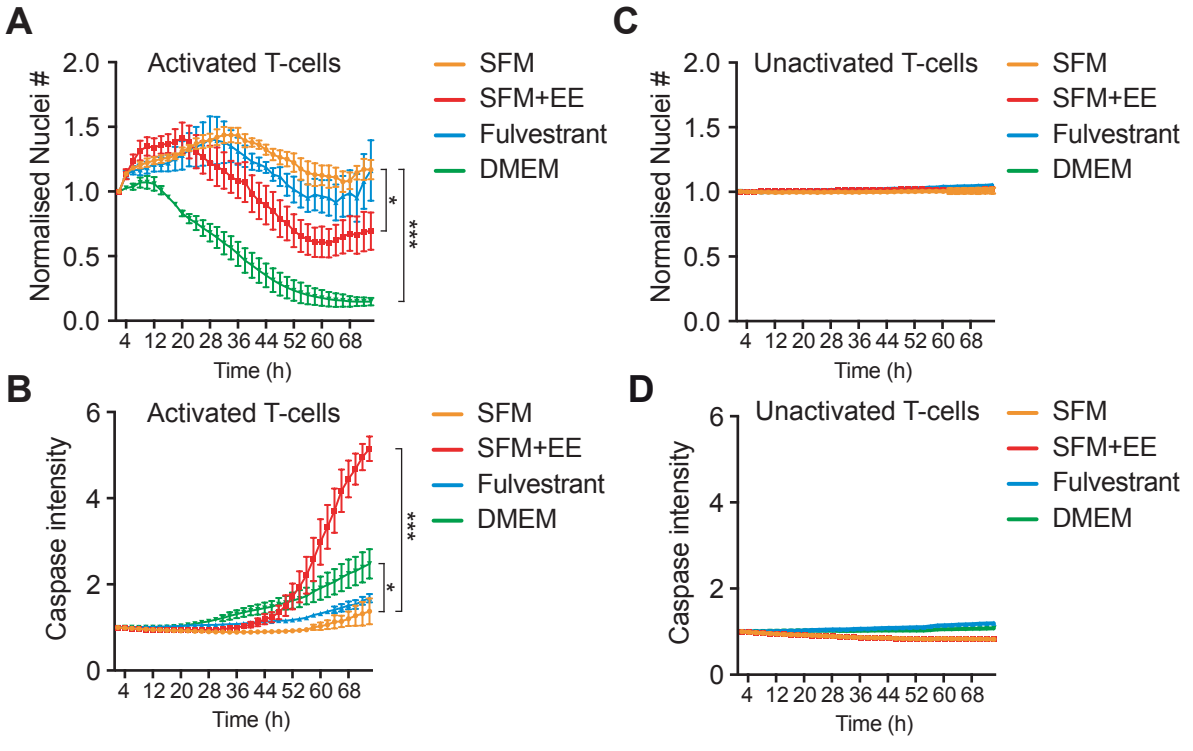
A
Activated T-cells
Normalised Nuclei #
2.0
1.5
1.0
0.5
0.0
4 12 20 28 36 44 52 60 68
Time (h)
SFM
SFM+EE
Fulvestrant
DMEM
*
***
B
Activated T-cells
Caspase intensity
6
4
2
0
4 12 20 28 36 44 52 60 68
Time (h)
SFM
SFM+EE
Fulvestrant
DMEM
***
*
C
Unactivated T-cells
Normalised Nuclei #
2.0
1.5
1.0
0.5
0.0
4 12 20 28 36 44 52 60 68
Time (h)
SFM
SFM+EE
Fulvestrant
DMEM
D
Unactivated T-cells
Caspase intensity
6
4
2
0
4 12 20 28 36 44 52 60 68
Time (h)
SFM
SFM+EE
Fulvestrant
DMEM

Figure S5. **Estrogen-deprival limits T-cell mediated killing of MCF7 cells A,** Live cell imaging of MCF7 nuclei count after addition of activated primary T lymphocytes isolated from human peripheral blood. Nuclei counts were normalized to the value at T = 0 hrs for each condition and then each timepoint was subsequently normalized to the value of the control to which no T-cells were added. Values represent the mean ± SEM. **B,** Time-lapse microscopy of the intensity of a fluorescently labelled caspase-3/7 substrate in MCF7 cells treated with activated primary T-cells. Values represent the mean ± SEM. **C,D** Equivalent analyses as the ones shown in (**A**) and (**B**), but where non-activated T-cells were used, illustrating that activation of the T-cells is a requisite for their cytotoxic activity against MCF7 cells.