



Supplemental Tables

Table S1. Primers used for the PCR studies.

Gene	Forward Primer	Reverse Primer
ER α 66	AATTCAGATAATCGACGCCAG	TTTCAACATTCTCCCTCCTC
ER α 36	CCAAGAATGTTCAACCACAACCT	GCACGGTTCATTAAACATCTTTCTG
GP α ER1	TGGTGGTGAACATCAGCTTC	TGAGCTTGTCCCTGAAGGTC
CXCL12	GGGCTCCTACTGTAAGGGTT	TTGACCCGAAGCTAAAGTGG
CXCR4	GGTGCTGAAATCAACCCAC	CGTGGAACGTTTTTCCTGTT
cMYC	CACCGAGTCGTAGTCGAGGT	TTTCGGGTAGTGGAACCA
TNF α	AGATGATCTGACTGCCTGGG	CTGCTGCACTTTGGAGTGAT
IL-6	GTCAGGGGTGGTTATTGCAT	AGTGAGGAACAAGCCAGAGC
PD-L1	TATGGTGGTGGCGACTACAA	TGGCTCCCAGAATTACCAAG
Cyclophilin A	ATGGTCAACCCACCGTGT	TTCTGCTGTCTTTGGAACCTTGTC

Table S2. Binding simulation studies of ER α and ER α 36 with GP α ER1 and NF κ B. X denotes either a non-measurable binding or a measurable yet non-expected binding. NR = Non-relevant information, either because the molecule did not participate in the complex or because of a non-identified molecular interaction.

MONOMERS					
No	Complex 1 (ligand)	Complex 2 (ligand)	ΔG (Kcal/mol)	NLS ER α 36	NLS NF- κ B
1	GP α ER1	ER α 36	−565.19	BLOCKED	NR
2	GP α ER1(Estradiol)	ER α 36	X	NR	NR
3	GP α ER1(Estradiol)	ER α 36(Estradiol)	−238.74 (X)	BLOCKED	NR
4	GP α ER1	ER α 36(Estradiol)	−296.07 (X)	FREE	NR
5	GP α ER1	G α iGDP	X	NR	NR
6	GP α ER1(Estradiol)	G α iGDP	−689.54	NR	NR
7	GP α ER1(Estradiol)-G α iGDP	ER α 36	X	NR	NR
8	GP α ER1(Estradiol)-G α iGDP	ER α 36(Estradiol)	−218.27 (X)	FREE	
9	GP α ER1	G α sGDP	X	NR	NR
10	GP α ER1(Estradiol)	G α sGDP	X	NR	NR
11	ER α 36	NF κ B	X	NR	NR
12	ER α 36(Estradiol)	NF κ B	−312.60 (X)	FREE	FREE
13	ER α 36(Estradiol)-NF κ B	GP α ER1	−676.75	FREE	FREE
14	ER α 36(Estradiol)-NF κ B	GP α ER1(Estradiol)	−815.93	FREE	BLOCKED
15	GP α ER1	NF κ B	−958.32	NR	FREE
16	GP α ER1(Estradiol)	NF κ B	−1015.87	NR	FREE
17	GP α ER1-NF κ B	ER α 36	X	NR	NR
18	GP α ER1-NF κ B	ER α 36(Estradiol)	−423.65	FREE	FREE
19	GP α ER1(Estradiol)-NF κ B	ER α 36	X	NR	NR
20	GP α ER1(Estradiol)-NF κ B	ER α 36(Estradiol)	−450.29	BLOCKED	FREE
21	GP α ER1-NF κ B	ER α 66	−797.14	FREE	FREE
22	GP α ER1-NF κ B	ER α 66(Estradiol)	−801.41	FREE	FREE
23	GP α ER1(Estradiol)-NF κ B	ER α 66	−845.62	FREE	FREE
24	GP α ER1(Estradiol)-NF κ B	ER α 66(Estradiol)	−681.13	BLOCKED	FREE
25	GP α ER1	ER α 66	−984.59	BLOCKED	NR

26	GP1R(Estradiol)	ERa66	X	NR	NR
27	GP1R(Estradiol)	ERa66(Estradiol)	X	NR	NR
28	GP1R	ERa66(Estradiol)	X	NR	NR
29	GP1R-ERa36	NFkB	X	NR	NR
30	GP1R-ERa36	NFkB	X	NR	NR
31	GP1R(Estradiol)-GaiGDP	NFkB	-1028.28	NR	FREE
32	GP1R(Estradiol)-GaiGDP-ERa36(Estradiol)	NFkB	-446.71	FREE	FREE
33	GP1R(Estradiol)-GaiGDP-NFkB	ERa36	X	NR	NR
34	GP1R(Estradiol)-GaiGDP-NFkB	ERa36(Estradiol)	-564.81	BLOCKED	FREE
35	GP1R(Estradiol)-GaiGDP	ERa66	X	NR	NR
36	GP1R(Estradiol)-GaiGDP	ERa66(Estradiol)	-918.39	FREE	NR
37	GP1R(Estradiol)-GaiGDP-ERa66(Estradiol)	NFkB	-343.15 (X)	FREE	FREE
38	GP1R(Estradiol)-GaiGDP-NFkB	ERa66	X	NR	NR
39	GP1R(Estradiol)-GaiGDP-NFkB	ERa66(Estradiol)	-569.75	BLOCKED	FREE
DIMERS					
No	Receptor	Ligand	ΔG (Kcal/mol)	NLS ERa36	NLS NF-kB
1	ERa36(Estradiol)	ERa36(Estradiol)	-609.34	FREE	NR
2	GP1R(Estradiol)-GaiGDP	GP1R(Estradiol)-GaiGDP	-407.97	NR	NR
3	[GP1R(Estradiol)-GaiGDP] ₂	[ERa36(Estradiol)] ₂	X	NR	NR
4	GP1R(Estradiol)] ₂	[ERa36(Estradiol)] ₂	X	NR	NR
5	[GP1R(Estradiol)] ₂	NFkB	-508.50	NR	FREE
6	[GP1R(Estradiol)] ₂ -NFkB	NFkB	-1065.20	NR	FREE
7	[GP1R(Estradiol)-NFkB] ₂	[ERa36(Estradiol)] ₂	-664.51	FREE	FREE

Supplemental Figures

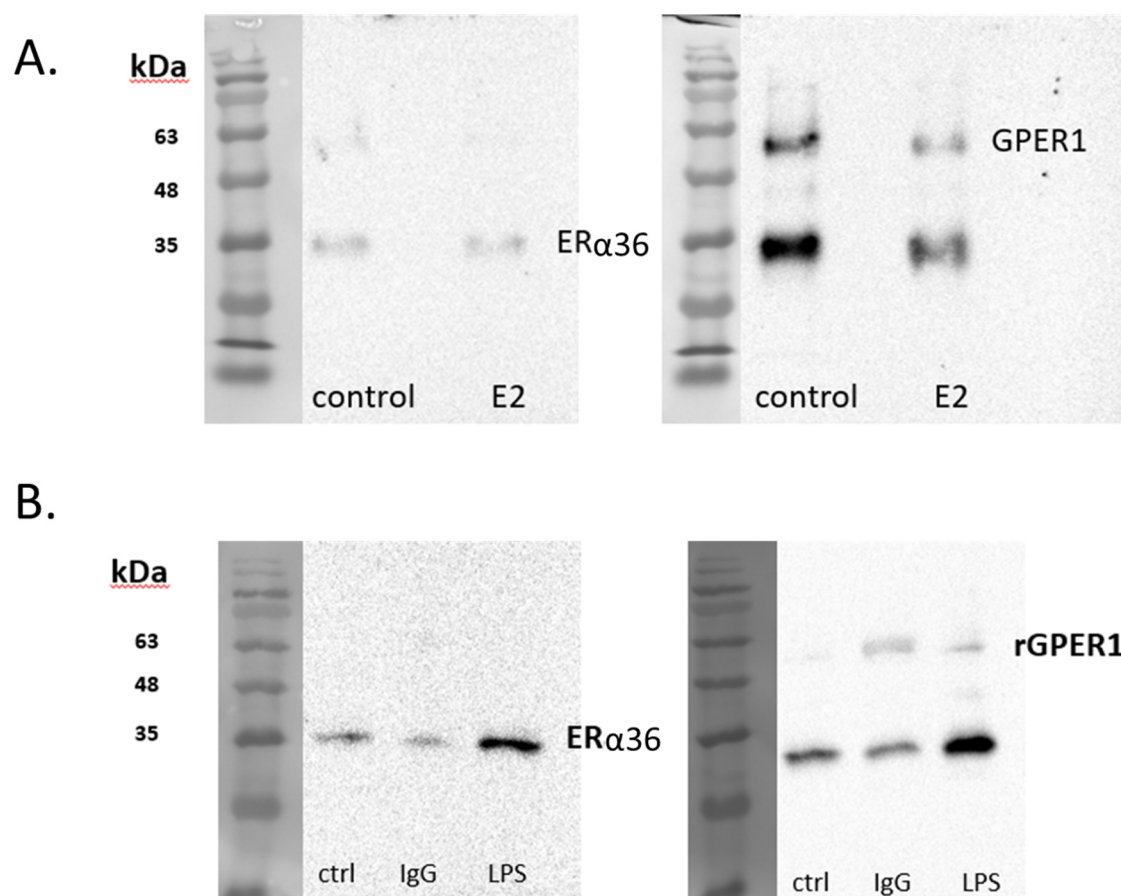


Figure S1. Co-immunoprecipitation studies in SKBR3 cells. A. E2 (10^{-6} M) treatment of SKBR3 cells did not lead to increased precipitation of ERα36 when an anti-GPER1 was used for the initial protein precipitation. B. However, when cells were treated with LPS, an increased amount of ERα36 could be found in the precipitate. All experiments were repeated three times.

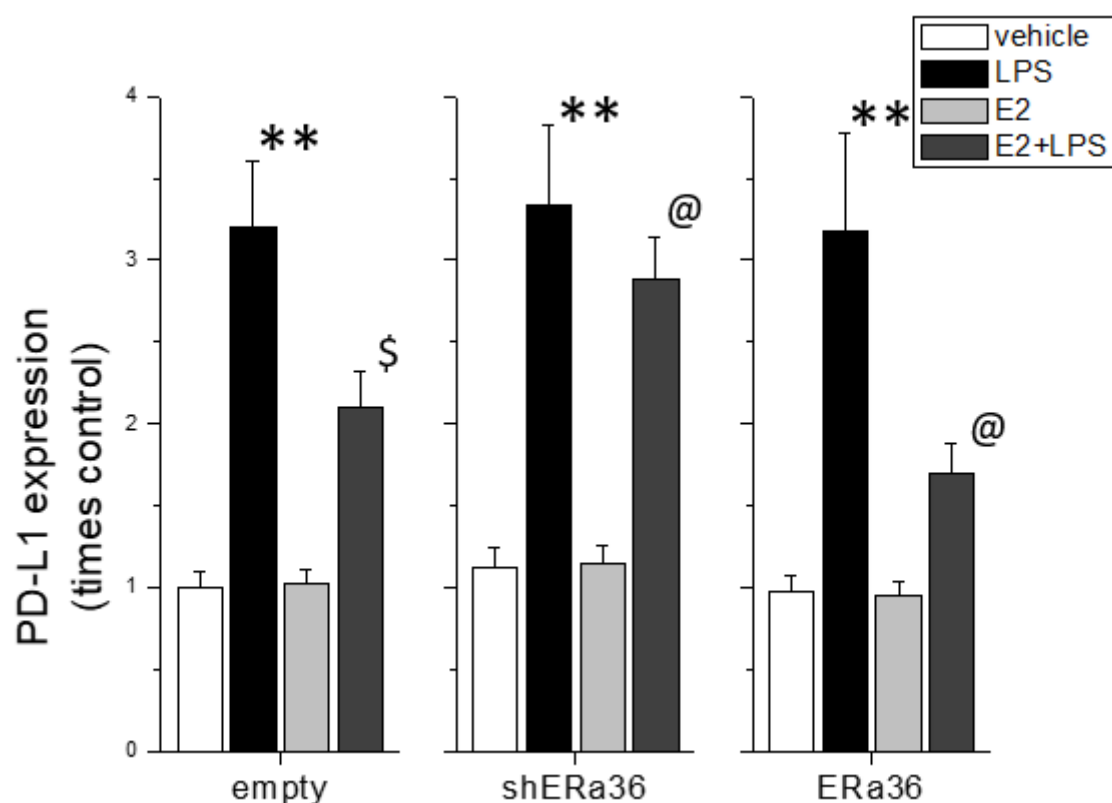


Figure S2. qPCR analysis of PD-L1 expression in SKBR3 cells under conditions of ERα36 knock-down or overexpression. All experiments were repeated three times in triplicates. * $p < 0.05$ and ** $p < 0.01$ vs. vehicle, \$ $p < 0.05$ vs. LPS, @ $p < 0.05$ and @@ $p < 0.01$ vs. E2 + LPS in empty, one-way ANOVA with Dunnet's test for multiple comparisons.

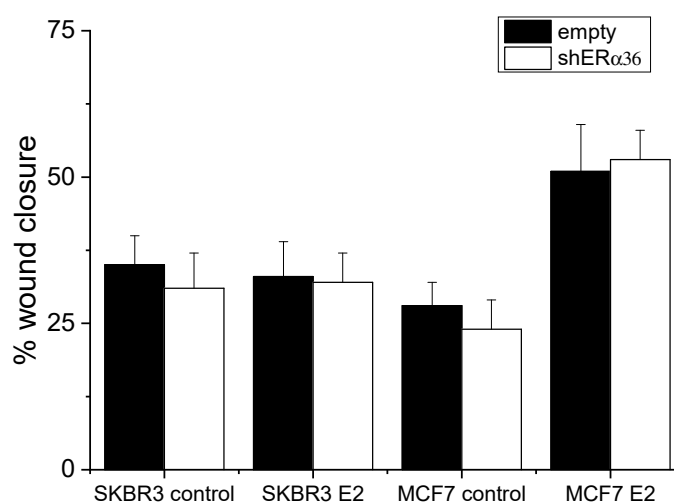


Figure S3. Wound healing assay in SKBR3 and MCF7 cells in the presence and the absence of estrogen. Knock-down of ERα36 expression did not affect the wound healing capacity of the studied cell lines, $n = 3$.

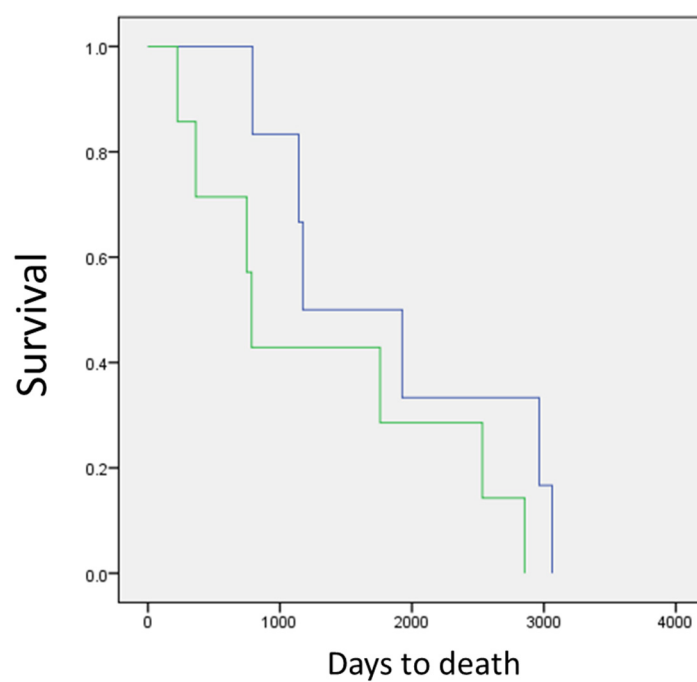


Figure S4. Kaplan–Mayer survival curves of Caucasian breast cancer patients that displayed an expression of ERα36 above (blue) or below (green) the median of the whole TCGA breast cancer cohort. The difference between the two groups was not statistically significant, probably due to the small number of patients with reported cancer-related death in the cohort.