

Supplementary Tables for:

**Low-dose Tacrolimus Promotes the Migration and Invasion and Nitric Oxide Production in the Human-derived First Trimester
Extravillous Trophoblast Cells *In Vitro***

By:

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Supplementary Table S1: Primary antibodies used in this study.

Antibody ID	Antibody Name	Target Antigen	Clonality	Host Organism	Dilution	Vendor	Catalogue Number	Specific Use
AB_2166687	Mouse Anti-PR Monoclonal Antibody, Unconjugated, Clone F-4	PGR human, mouse, rat	Monoclonal	Mouse	1:100	Santa Cruz Biotechnology	SC-166169	Western blot (WB) detection of the human PGR
AB_2864799	Mouse Anti-PR- Alexa Fluor® 647 Monoclonal Antibody, Conjugated, Clone F-4	PGR human, mouse, rat	Monoclonal	Mouse	1:100	Santa Cruz Biotechnology	sc-166169 AF647	Immunofluorescent (IF) detection of the human PGR
AB_325316	Phospho-Progesterone Receptor (Ser294) Monoclonal Antibody (608)	Phospho-Progesterone Receptor (Ser294) human	Monoclonal	Mouse	1:200	Thermo Fisher Scientific	MA1-414	Western blot (WB) detection of the human phosphorylated PGR (pPGR)
AB_628293	Stat3 (F-2) antibody	Recombinant protein corresponding to AA 50-240 mapping to amino acids of human origin human, mouse, rat	Monoclonal	Mouse	1:300	Santa Cruz Biotechnology	SC-8019	Western blot (WB) detection of the human STAT3
AB_2491009	Phospho-Stat3 (Tyr705) (D3A7) XP Rabbit mAb antibody	Phospho-Stat3 (Tyr705) human, mouse, rat	Monoclonal	Rabbit	1:200	Cell Signaling Technology	9145	Western blot (WB) detection of the human phosphorylated STAT3 (pSTAT3)

Supplementary Table S1: Primary antibodies used in this study (continued).

Antibody ID	Antibody Name	Target Antigen	Clonality	Host Organism	Dilution	Vendor	Catalogue Number	Specific Use
AB_2533121	Anti-eNOS Monoclonal Antibody, Unconjugated, Clone eNOS-9D10	eNOS bovine	Monoclonal	Mouse	1:300	ThermoFisher Scientific	33-4600	Western blot (WB) detection of the human eNOS
AB_2533285	Phospho-eNOS (Ser1179) Polyclonal Antibody	Phospho-eNOS (Ser1179) bovine	Polyclonal	Rabbit	1:200	ThermoFisher Scientific	36-9100	Western blot (WB) detection of the human phosphorylated eNOS ^{Ser1179} (p-eNOS ^{Ser1179})
AB_10707645	Phospho-NOS3 (p-NOS3) (pT495.33) antibody	p-NOS3 (pT495.33) human, mouse, rat	Monoclonal	Mouse	1:200	Santa Cruz Biotechnology	SC-136519	Western blot (WB) detection of the human phosphorylated eNOS ^{Thr495} (p-eNOS ^{Thr495})
AB_2293930	FKBP52 Affinity Purified Polyclonal antibody Human/Mouse/Rat	FKBP52 human, mouse, rat	Polyclonal	Goat	1:200	R and D Systems	AF4095	WB and IF detections of the human FKBP52 in
AB_626658	Akt1 (B-1) antibody	AKT1 human, mouse, rat	Monoclonal	Mouse	1:200	Santa Cruz Biotechnology	sc-5298	Wb detection of the protein kinase Akt in HTR-8/SVneo cells
AB_2861344	p-Akt1/2/3 Antibody (C-11)	p-Akt1/2/3 human, mouse, rat	Monoclonal	Mouse	1:200	Santa Cruz Biotechnology	sc-514032	Wb detection of the phosphorylated protein kinase Akt in HTR-8/SVneo cells

Supplementary Table S2: Secondary antibodies used in this study

Antibody ID	Antibody Name	Target Antigen	Clonality	Host Organism	Dilution	Vendor	Catalogue Number	Specific Use
AB_628489	Mouse anti-goat IgG-FITC	IgG goat	Monoclonal	Mouse	1:3000	Santa Cruz Biotechnology	33-4600	Secondary Ab used in the IF detection of the human FKBP52
AB_631746	Goat anti-rabbit IgG-HRP antibody	IgG rabbit	Polyclonal	Rabbit	1:5000	Santa Cruz Biotechnology	SC-2004	Secondary Ab used in WB
AB_2536527	Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP	IgG (H+L) mouse	Polyclonal	Goat	1:1000	ThermoFisher Scientific	G21040	Secondary Ab used in WB

Supplementary Figures for:

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Supplementary Figure Legends

Supplementary Figure S1:

Detection of PGR mRNA in HTR8/SVneo cells treated with DMSO only (Control), tacrolimus (TAC), L-NAME, TAC+L-NAME, Mifepristone and TAC+ Mifepristone, respectively. Tacrolimus significantly ($p < 0.05$) induced the expression of the PGR mRNA in the HTR-8/SVneo cells irrespective of the presence or absence of the eNOS and PGR inhibitors; L-NAME and Mifepristone, respectively. mRNA copies were calculated using the comparative threshold cycle ($\Delta\Delta CT$) method with normalization to GAPDH. R values were measured as the negative values of $\Delta\Delta CT$ as exponent of 2 according to the equation: $R = 2^{-\Delta\Delta CT}$ where $\Delta\Delta CT = \Delta CT (\text{Target}) - \Delta CT (\text{Endogenous Control})$. GAPDH primers were utilized as positive controls. Negative controls without RNA and without reverse transcriptase were also performed.

Supplementary Figure S2:

Effects of low-dose tacrolimus on IL6 secretion in HTR8/SVneo cells. A highly sensitive human IL6 ELISA kit (Catalog number: EH2IL6, Invitrogen) was used to detect alterations in concentrations of this cytokine in HTR8/SVneo cells in conditioned media after 24 hours of treatment with low-dose tacrolimus (10ng/ml) alone or in a combination formulation with L-NAME or mifepristone. Compared to the DMSO-free control, a significant trend ($p = 0.06$) increase in IL6 production was observed among all treatment conditions. However, compared to DMSO-only treated controls, none of the treatment conditions used in this study was able to influence the release of IL6 in HTR8/SVneo cells. Data are presented as mean \pm SD.

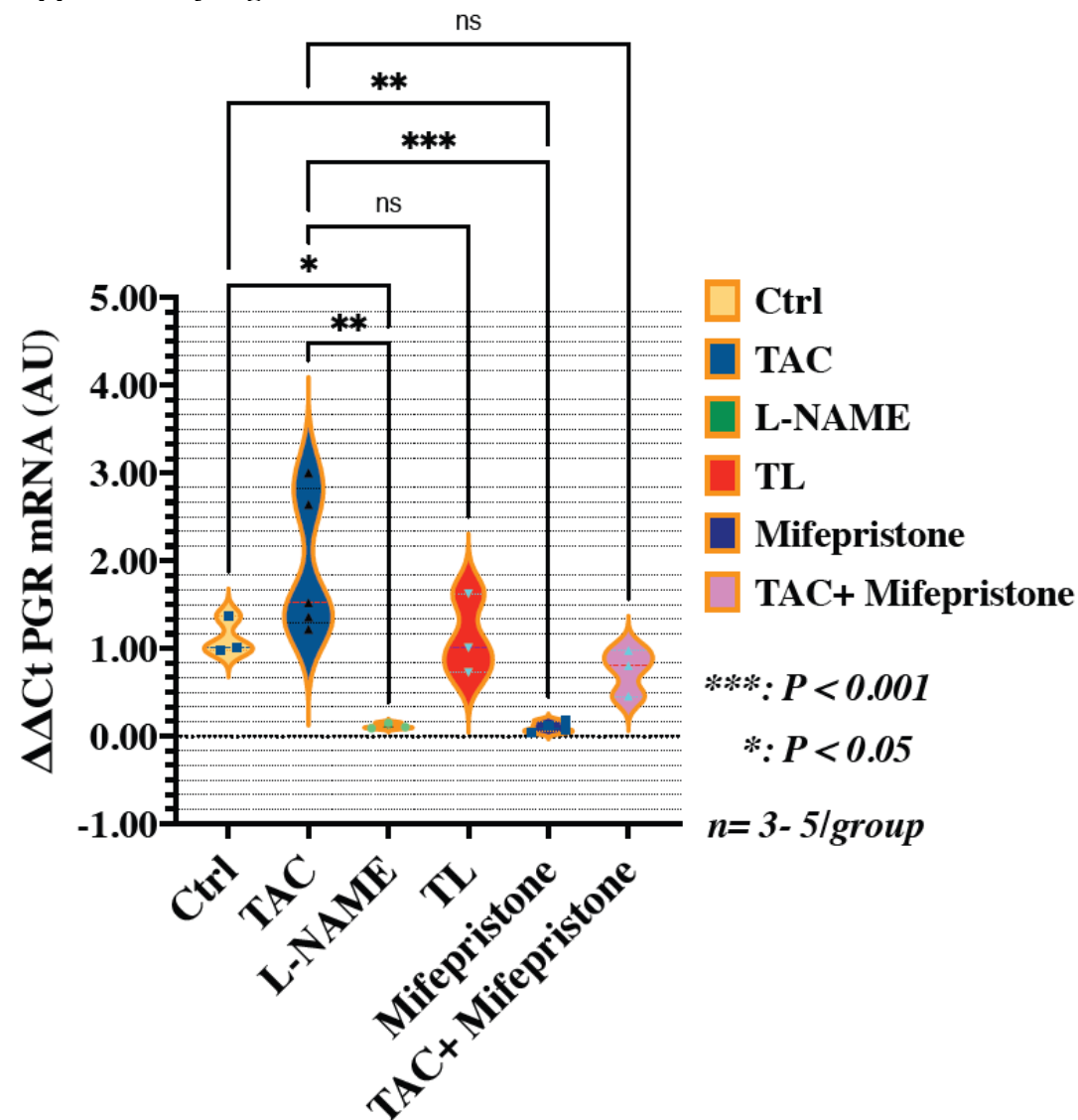
Supplementary Figure S3:

Effects of low-dose tacrolimus on TNF α secretion in HTR8/SVneo cells. A highly sensitive human TNF α ELISA kit (Catalog number: BMS223HS, Invitrogen) was used to detect alterations in concentrations of this cytokine in HTR8/SVneo cells in conditioned media after 24 hours of treatment with low-dose tacrolimus (10ng/ml) alone or in a combination formulation with L-NAME or mifepristone. Compared to DMSO-only treated controls, Low-dose tacrolimus and L-NAME significantly increased the concentrations of TNF α in the condition medium of HTR8/SVneo cells. Data are presented as mean \pm SD.

Supplementary Figure S4:

A: Representative Western blot detection of protein expression and phosphorylation of Akt in HTR8/SVneo cells treated with DMSO-only (Control: Ctrl), tacrolimus (TAC), mifepristone (Mife), and TAC+ mifepristone (TAC+ Mife), respectively. B: Histogram analysis of the relative expression of pAkt/Akt in HTR8/SVneo cells demonstrating a significant time-dependent increase in protein expression and phosphorylation of Akt in the TAC- and TAC+ Mife treated cells over 72 hours of incubation. MWM: Molecular Weight Markers.

Supplementary Figure S1



IL6 (pg/ml)

Legend:

- DMSO-Free Ctrl
- DMSO
- TAC
- L-MAME
- TAC+L-NAME
- Mifepristone
- TAC+ Mifepristone

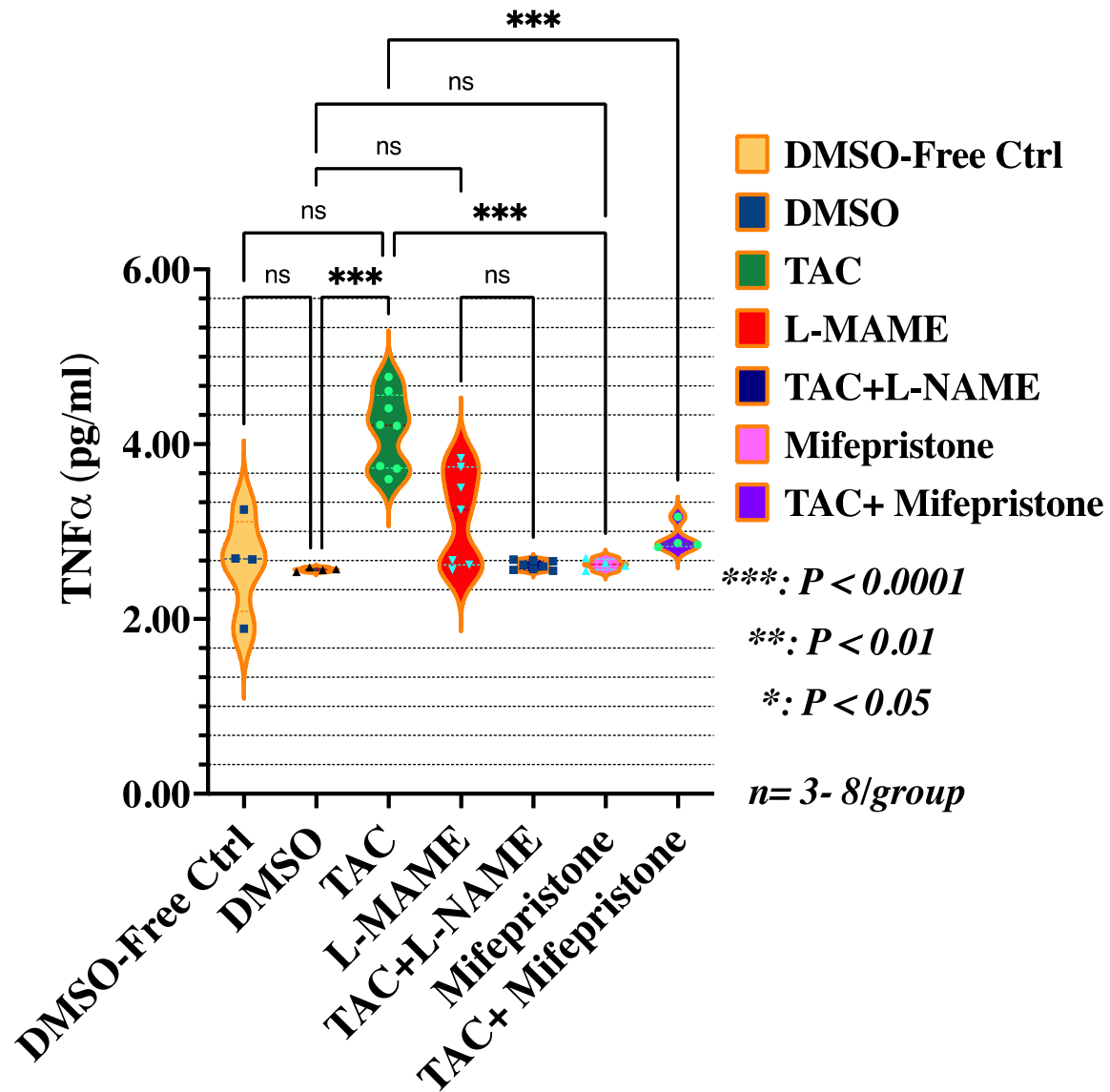
Statistical Significance:

- *: $P < 0.05$
- ns: $P \geq 0.05$

Approximate IL6 (pg/ml) values:

Group	Approximate IL6 (pg/ml)
DMSO-Free Ctrl	1.2
DMSO	2.1
TAC	2.5
L-MAME	2.1
TAC+L-NAME	2.8
Mifepristone	2.1
TAC+ Mifepristone	2.1

Supplementary Figure S3



Supplemental Figure S4

