Supporting Information

Pseudopterosin Inhibits Proliferation and 3D Invasion in Triple Negative Breast Cancer by Agonizing Glucocorticoid Receptor Alpha

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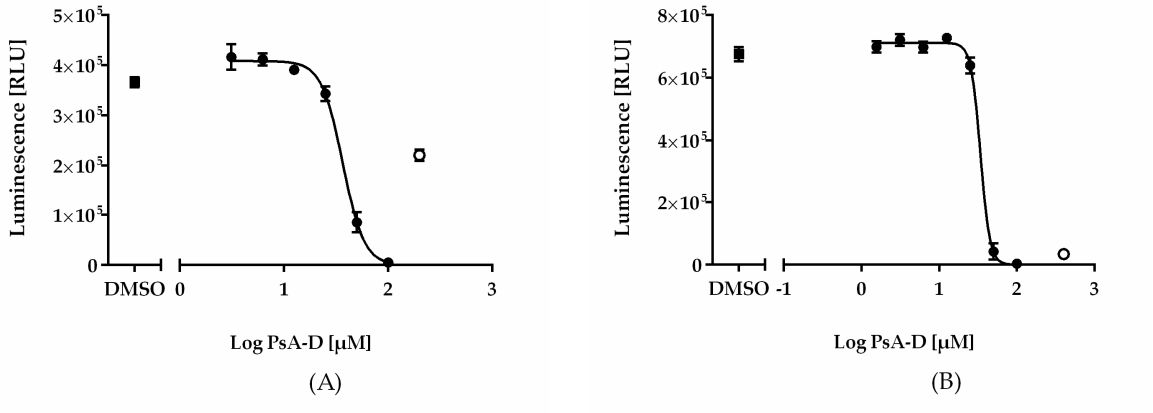
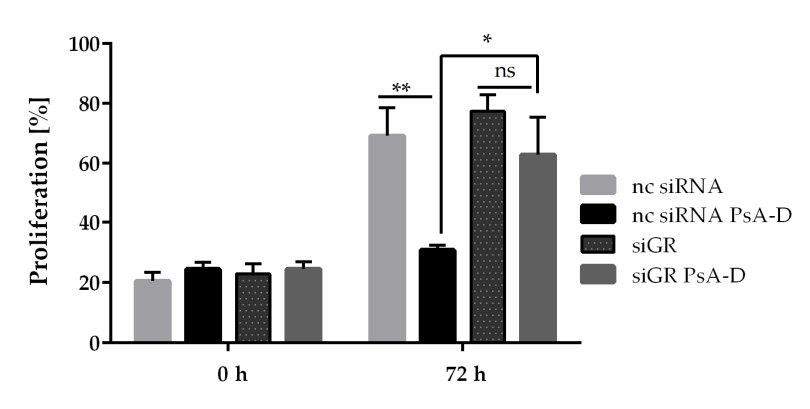
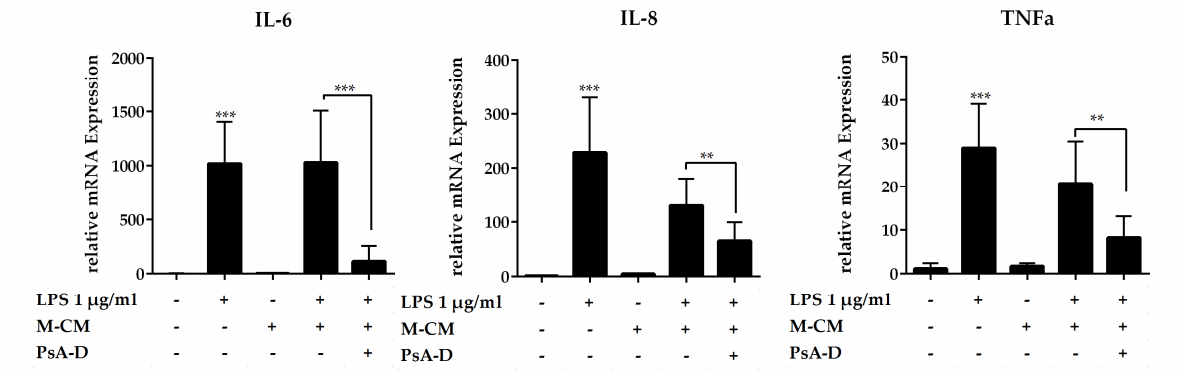


S1. Cell Viability of MDA-MB-231 cells after pseudopterosin treatment. Increasing amounts of PsA-D were incubated for either 24 hours showing an IC_{50} value of 31.4 µM (**A**) or for 48 hours leading to an IC_{50} value of 32.16 µM (**B**). Staurosporine (white circles) serves as positive control and DMSO as negative control. Error bars were calculated using ±SEM. Graphs represent exemplary data. Means of IC_{50} values were calculated of three independent experiments.



S2. Pseudopterosin failed to inhibit breast cancer cell proliferation after knockdown of the glucocorticoid receptor alpha (GRα) after 72 hours. Knockdown of GRα was done with the Lonza Nucleofector 2b device. The cells were seeded and proliferating cells were imaged with the IncuCyte® ZOOM every hour. Confluency of cells was determined with IncuCyte® software indicated in proliferation in percent. Cells were treated with a concentration of 15 µM of PsA-D. The bar diagram shows the proliferation rate at time points 0 and 72 hours. The data represent means of three independent experiments. Error bars were calculated using ±SEM. Two stars represent a significance of $p<0.01$ and one star of $p<0.05$.



S3. Pseudopterosin inhibited bidirectional communication between triple negative breast cancer (TNBC) and peripheral blood mononuclear cells (PBMC). Tumor-conditioned medium of MDA-MB-231 cells was produced using 1 µg/ml LPS and $1*10^6$ cells per ml. After 24 hours of incubation, tumor conditioned medium (MDA-MB-231-conditioned medium, M-CM) was harvested, centrifuged and sterile filtered. Afterwards, M-CM was added at 25 volume percentages to $1*10^6$ cells per ml of PBMC. After 5 hours of incubation, cells were harvested, RNA isolated and analyzed with qPCR.

Supplementary Method 1: Measurement of cell viability: MDA-MB-231 cells were seeded at a density of $2.8*10^5$ cells per ml in 384 well plates (Greiner Bio-One, Kremsmuenster, Austria) with the CyBio pipetting robot (Analytic Jena AG, Jena, Germany). The cells were incubated for 24 hours at 37°C before treatment. Compounds were added at different concentrations and incubated for 24 or 48 hours, respectively. Measurement of cell viability was performed with CellTiterGlo® from Promega (Darmstadt, Germany) according to the manufacturer's instructions.

Supplementary Method 2: Production of conditioned medium (CM) from MDA-MB-231 cells: MDA-MB-231 cells were seeded at a density of 1×10^6 cells into a 25 cm^2 flask. Cells were either stimulated with 1 µg/mL LPS or without, serving as a negative control. MDA-MB-231 conditioned media (M-CM) was collected after 24 hours, centrifuged and sterile filtered. Peripheral blood mononuclear cells (PBMC) were freshly thawed and seeded at 1×10^6 cells per ml. PsA-D was added at a concentration of 30 µM for 20 minutes followed by addition of 25 volume percentage of M-CM for 5 hours. Cells were then harvested and RNA isolated with RNase Mini Kit (Qiagen, Hilden, Germany) for further quantitative realtime PCR analysis.