SUPPORTING MATERIAL AND METHODS

MAS mRNA expression in human cell lines

HEK293 and MCF7 cell lines were purchased from ATCC. AB116720FLCL1 immortalized human skeletal muscle cells derived from fascia lata muscle of healthy subject were obtained from the Institute of Myology (Paris). Total mRNA was extracted and purified using Trizol reagent (Life Technologies, France). RNAs were converted into cDNAs using Thermo Scientific Maxima H Minus Reverse Transcriptase before performing quantitative PCR using iTaq SybrGreen (Biorad, Marnes-la Coquette, France) and QuantiTect® human MAS1 primer assay (Qiagen, Hs_MAS1_1_SG). Relative MAS mRNA expression was quantified using Beta-2 microglobulin (β2M) housekeeping gene and 2^-∆∆CT method. MAS expression in AB1167 and MCF7 cells was calculated relatively to HEK293 MAS mRNA expression.

NO Measurement

Subconfluent MCF7 cells cultured in 96-well flat bottom black plates were loaded with 4µM DAF-FM in PBS for 30 minutes at 37°C in a humidified incubator under an atmosphere with 5% CO2. Then, the cells were washed with PBS and stimulated 30 minutes with 1, 5 or 10 µM 20E or Ang-(1-7) in the presence or absence of Mas receptor antagonist, A779 (10µM). The nitric oxide in the supernatant was measured by DAF-FM™ (4-amino-5-methylamino-2',7'-difluorescein; ThermoFisher Scientific). The fluorescence emission intensity was measured using a monochromator SpectraMax® ID3 plate reader (Molecular Devices). Endpoint fluorescence emission was measured at 525 nm after excitation at 485 nm. Each condition was studied in quadruplicates.