**Quantitative RT-PCR analysis**

Total RNA was extracted with the TRIZOL Reagent (Invitrogen), according to the manufacturer’s instructions. To degrade any contamination of DNA, RNA was treated with DNase (BioLabs). 5 µg of RNA was converted into first-stranded DNA by a Superscript First-Strand Synthesis System for RT-PCR (Invitrogen). Real time quantitative RT-PCR was performed on an 7500 Real-Time PCR System (Applied Biosystems,). The cDNA generated from the reverse transcription reactions was amplified by PCR with the Sensimix SYBR Kit (Bioline) in a total volume of 25 µl according to the manufacturer’s instructions. The primers used were as follows: TPC1, 5'-AGC CGC CTC TAC TTC ATG ACC TTT-3’ and 5'-ACC ATC AAC TTC CGA GTC CTG GTT-3’; TPC2, 5'-AAC CTG GTG TCC ATT TGC GTG TTC -3’ and 5'-AGA CCT TGA GCA GCA TCT CCA ACA-3’; HPRT1, 5'-TGG AGT CCT ATT GAC ATC GCC AGT -3’ and 5'-AAC AAC AAT CCG CCC AAA GGG AAC-3’. The level of messenger analyzed was expressed as relative fold change vs. the HPRT1 messenger RNA and analyzed by means of the 7500 System (Applied Biosystems). For the absolute quantitation of TPC1 and TPC2 transcripts, specific standard curves were obtained by running five 5-fold serial dilution of in vitro transcribed RNA (range 100 to 0,16 ng). Equal volumes of standard and sample cDNA were used in Real Time PCR assay. Quantitation of TPC1 and TPC2 and linear regression analyses were performed using 7500 v.2.0.5 software (Applied Biosystems).
Figure S1. NAADP levels in response to thrombin. NAADP levels measured over time in HUVEC-derived EA.hy926 endothelial cells stimulated with 2U/ml thrombin. The kinetics of the variable but well apparent increase in NAADP synthesis, at variance with that triggered by H1-receptor stimulation, consists in a delayed and transient peak of NAADP synthesis reverting to basal level within 2 min. Error bars represent SEM; n = 3 independent experiments; *student’s t-test p < 0.05.