Supplementary Material to Madonna et al. “Omega-3 fatty acids attenuate constitutive and insulin-induced CD36 expression through a suppression of PPARα/γ activity in microvascular endothelial cells” (Thromb Haemost 2011; 106.3)

Supplement Figure 1. The effect of PI3-kinase and ERK1/2 kinase inhibitors on insulin-induced CD36 expression in HMVEC. HMVEC were preincubated for 30 min with the PI3-kinase inhibitor wortmannin (WT, 100 nmol/L), or the ERK1/2 inhibitor PD98059 (PD 50 µM), or the p38 mitogen activated protein kinase (MAPK) inhibitor SB202190, and then incubated with insulin (10⁻⁸ mol/L) for further 24 h. At the end of treatments cell surface expression of CD36 was measured by ELISA. Results are means ± SD of 3 separate experiments. *P< 0.05 vs. vehicle control; **, P< 0.05 vs. insulin.
Supplement Figure 2. The effect of insulin and DHA on proliferation and uptake of oxidized LDL in HMVEC. Representative photomicrographs of HMVEC expressing CD36, treated with insulin and/or DHA and then exposed to DiI-oxLDL for 6 h at 37°C, indicating the uptake of oxLDL in untreated vs treated cells with insulin or with insulin + DHA. Red fluorescence: cells positive for DiI-oxLDL uptake; green fluorescence: cells positive for CD36 expression.
Online Supplement Fig. 2

1. untreated
2. Insulin
3. DHA
4. DHA + Insulin
**Supplement Figure 3.** Expression and distribution of NF-κB subunits p50 and p65 in HMVEC treated with insulin ± TNF-α. Nuclear and cytosolic extracts derived from HMVEC subjected or not to treatment with insulin and TNF-α for short (45 min) or long incubation times (48 h) were analyzed by Western blotting, using rabbit anti-human p50 and p65 polyclonal antibodies. Lanes 1-4, samples subjected to no stimuli (control), short (45 min) TNF-α treatment, long (48 h) TNF-α treatment, treatment with insulin alone (10^{-8} mol/L) and treatment with TNF-α + insulin.
Cytosolic proteins

- + - - +  TNF-α 10 ng/mL, 45 min
- - + - -  insulin 10⁻⁸ mol/L, 45 min
- - - + +  insulin 10⁻⁸ mol/L, 48 h

1 2 3 4 5

Nuclear proteins

- + - - +  TNF-α 10 ng/mL, 45 min
- - + - -  insulin 10⁻⁸ mol/L, 45 min
- - - + +  insulin 10⁻⁸ mol/L, 48 h

1 2 3 4 5

Online Supplement Fig. 3

- p105 subunit (NF-κB precursor)
- p65 subunit
- p50 subunit

- p105 subunit (NF-κB precursor)
- p65 subunit
- p50 subunit