

## **SUPPLEMENT**

# **Trimethylamine N-Oxide, Circulating Endothelial Progenitor Cells, and Endothelial Function in Patients with Stable Angina**

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## **Methods**

### **Human endothelial progenitor cell (EPC) culture**

Peripheral blood samples for EPC culture were obtained from healthy young volunteers. Total mononuclear cells (MNCs) were isolated by density gradient centrifugation with Histopaque-1077 (1.077 g/mL, Sigma) from the peripheral blood of the healthy young volunteers. Briefly,  $5 \times 10^6$  MNCs were seeded in EGM-2MV medium (Cambrex, East Rutherford, NJ, USA), supplemented with hydrocortisone, human epidermal growth factor, R3-insulin-like growth factor 1, human fibroblast growth factor, vascular endothelial growth factor, gentamicin, amphotericin B, vitamin C, and 20% fetal bovine serum, on 0.1% fibronectin-coated plates. The medium was changed after every 4 days of culture, and EPCs appeared within 7–15 days after the start of the MNC culture. EPC passage numbers 3–7 were used. Additionally, we identified the EPCs by the antibodies CD34, CD133, KDR, CD31 (Santa Cruz), and vWF (Neomarkers) using immunofluorescence assays. The fluorescent images were obtained by a laser scanning confocal microscope.