Supplementary methods

Grading of elastin degradation

The grading of elastic fiber degradation was carried out based on the extent of elastic fiber fragmentation and branching, according to a reported method [1] with the following modifications. One point was given to one incident of elastic fiber fragmentation or branching; the total points were summated to calculate “score” for each section from each aorta. Grade 1 = none (score 0-7); grade 2 = minimal (8-15); grade 3 = moderate (16-23); grade 4 = severe (>23) (adapted from Deckert et al [1]).

PCNA immunostaining and TUNEL assay

To assess VSMC proliferation in the aorta, paraffin-embedded aorta sections were immunostained for PCNA (Biovision, 1:400). The sections were also subjected to a TUNEL assay using an in situ apoptosis detection kit (Takara) according to the manufacturer’s instruction.

Reference