Hypoxic conditions were examined as it was recently shown that hypoxic signaling plays a role in the progression of VC during CKD.

Methods HASMCs (P4-5; PromoCell) were grown in 6- or 12-well cell culture plates until about 70% confluence in regular commercial smooth muscle cell growth media (PromoCell). The cells were then: 1) Cultured for 21 days in DMEM mineralisation media (10nM dexamethasone and 10mM sodium β-glycerophosphate) or control media, 2) Cultured for 21 days in commercial mineralisation media or control media (PromoCell), 3) Cultured for 4, 6, and 8 days in high phosphate media (PromoCell; 2.5mM PO4) or control media in hypoxic conditions (1% O2), 4) Cultured for 6 days in commercial mineralisation media (PromoCell) or control media in hypoxic conditions, or 5) Cultured for 8 and 12 days in DMEM mineralisation media or control media in hypoxic conditions. Alizarin red staining was used to detect calcification at the end of the experiments, cell morphology was examined, and alkaline phosphatase (ALP) activity and total protein content were measured.

Results Alizarin red staining showed that DMEM mineralisation media cultured cells were completely calcified after 21 days and very faint staining was visually seen in DMEM control media cultured cells (protocol 1; figure 1). PromoCell mineralisation media cultured cells were moderately calcified (protocol 2) and control cells in PromoCell regular media maintained the characteristic morphology of HASMCs, while mineralised cells were characteristic of transdifferentiated, calcifying HASMCs (protocol 2, figure 1). DMEM mineralisation media (protocol 1) significantly decreased cell protein after 21 days compared to DMEM control media cultured cells, while this did not occur in PromoCell media treated cells (protocol 2; figure 2). PromoCell mineralisation media and DMEM mineralisation media both significantly increased ALP activity in cell lysates compared to respective controls (figure 2). No calcification was detected in cells subjected to the hypoxic conditions (protocols 3–5).

Conclusion Culturing cells in PromoCell mineralisation media for 21 days in normoxic conditions was evaluated to be the most suitable method for inducing calcification in HASMCs. Hypoxia failed to induce calcification in HASMCs in any of the tested media conditions.

Conflict of interest None