Keenan, CM, Beckett, AJ, Sutherland, H, Ranganath, LR, Jarvis, JC, Prior, IA and Gallagher, JA

Concentric lamellae - novel microanatomical structures in the articular calcified cartilage of mice

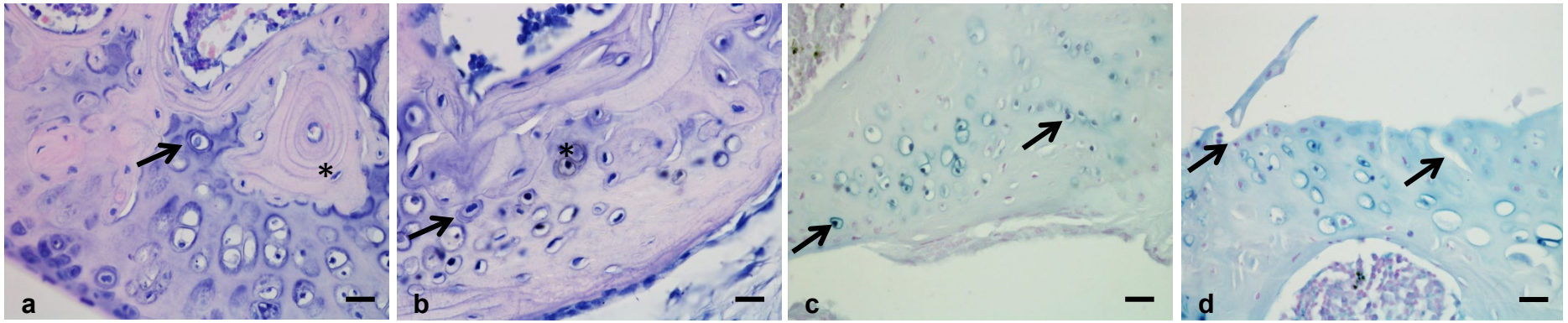

Fig. 1. Histological examination of BALB/c AKU mice. (a) H&E staining of a 31 week old BALB/c AKU mouse showed the appearance of a concentric ring like structure around a chondrocyte in the articular calcified cartilage (ACC) of the medial femoral condyle (arrowed). Remodelling of the subchondral bone (SCB) was also seen which is an indication of osteoarthritis (OA) (*). **(b)** H&E staining of a 60 week old BALB/c AKU mouse also showed the appearance of concentric ring structures around a chondrocyte in ACC of the lateral femoral condyle (LFC) (arrowed), along with protrusion of the SCB into ACC. **(c)** Schmorl's staining of a 60 week old BALB/c AKU mouse showed large numbers of pigmented chondrocytes, a hallmark of AKU, present throughout ACC of the LFC (arrowed). Pigmented chondrocytes were also visible in the H&E stained section (b) where they can be seen deep in ACC (*). **(d)** Analysis of a 49 week old BALB/c AKU mouse showed complete loss of the articular surface and vertical clefts running through the medial tibial plateau (arrowed), illustrating the severity of OA in these mice. Scale = 20µm.

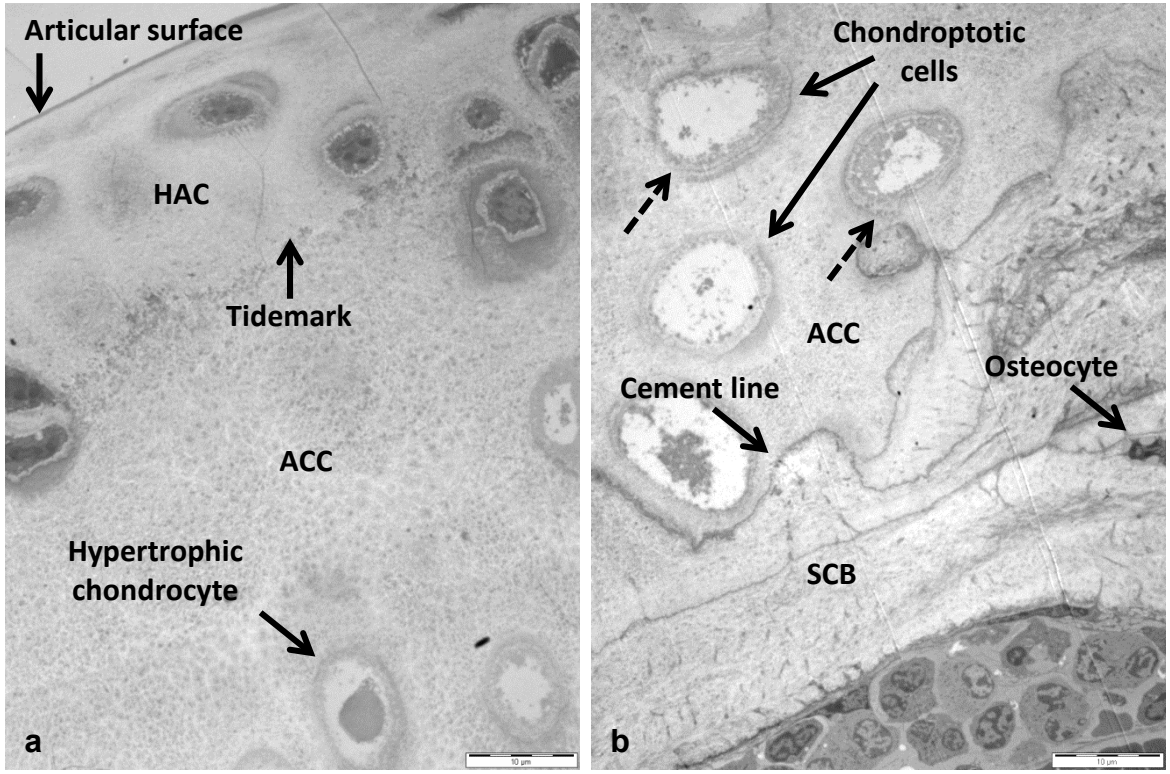

Fig. 2. TEM micrographs of the medial femoral condyle from a 53 week old BALB/c AKU mouse. (a) HAC and ACC with the tidemark, which separates the two types of articular cartilage, have been labelled. A hypertrophic chondrocyte can be seen deep in the ACC. **(b)** Chondrocytes undergoing chondroptosis were visible in the ACC. Concentric lamellae were also visible surrounding the cells (dashed lines). The cement line which separates the ACC from the underlying SCB is highlighted (x1250). Tissue fixed in glutaraldehyde. Scale = 10µm.

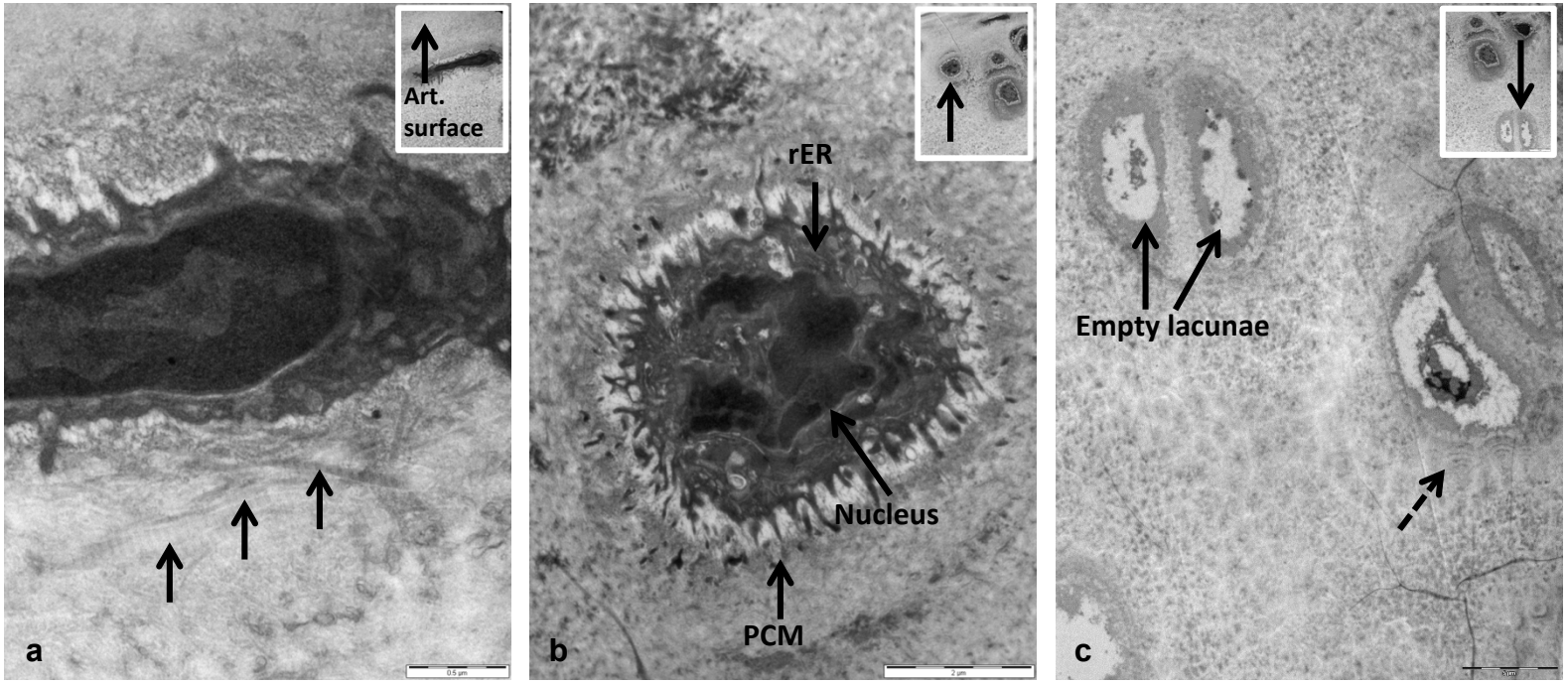

Fig. 3. Ultrastructural examination of chondrocytes from different zones of cartilage in a 53 week old BALB/c AKU mouse. (a) TEM micrograph of a flattened chondrocyte in the superficial zone of the HAC. Individual collagen fibres, located in the pericellular matrix (PCM), lie parallel to the articular surface (arrowed) (x26,500). Inset: Location of the chondrocyte in HAC (x8250). **(b)** TEM micrograph of a chondrocyte in the deep zone of the HAC. Specific structures within the cell have been labelled (x9900). **(c)** TEM micrograph of hypertrophic chondrons in the ACC. Both sets of chondrocytes appeared chondroptotic with chromatin condensation, cellular disintegration and the final stage of chondroptosis, empty lacunae, all present. Concentric lamellae were also visible surrounding the cells (dashed lines). Inset: Location of the chondrocyte in the HAC (arrowed) (x2500). Inset: Location of the cells in the ACC (x2500). Tissue fixed in glutaraldehyde. Scale = (a) 0.5µm, (b) 2µm, (c) 5µm.

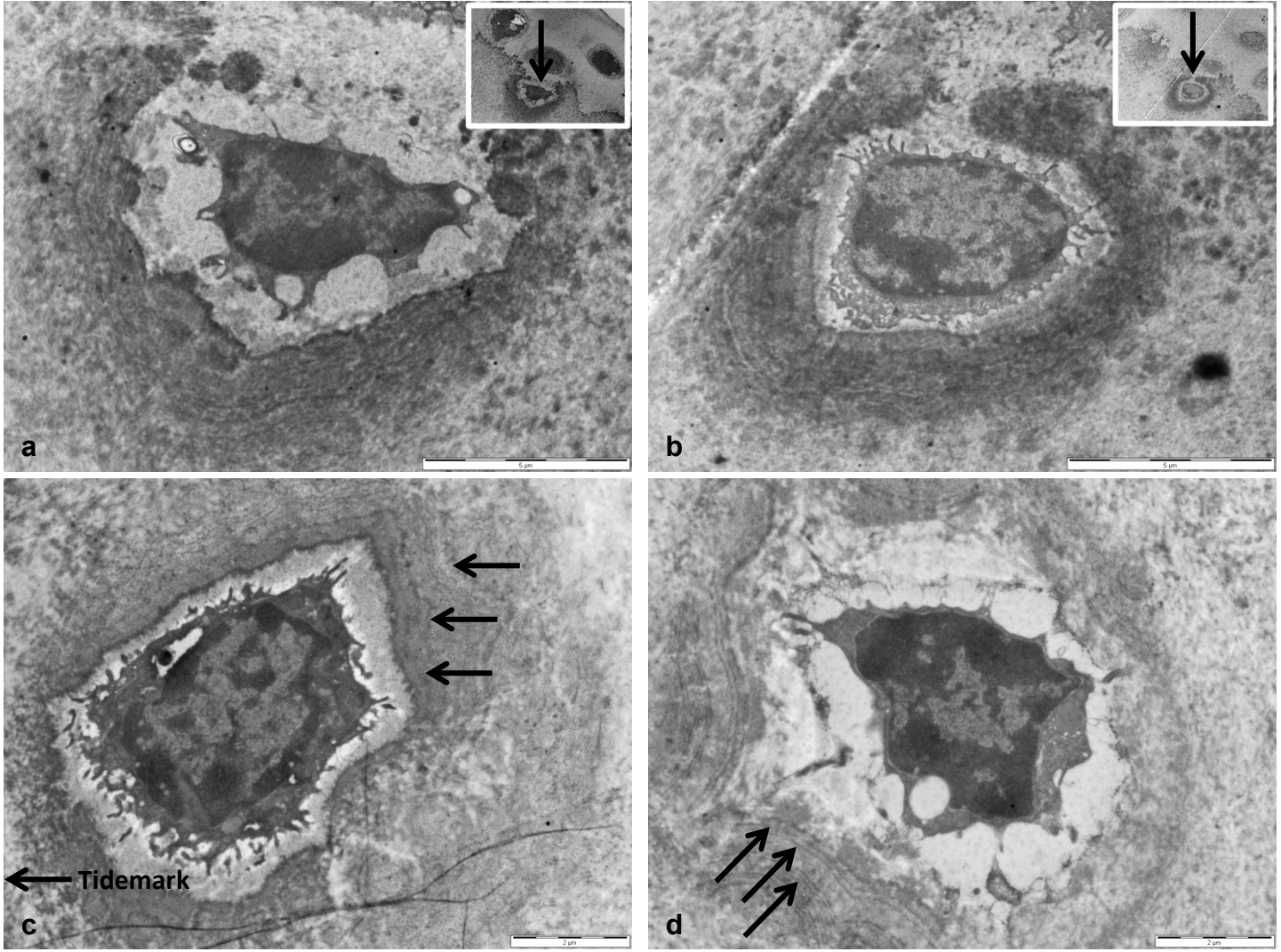

Fig. 4. The appearance of concentric lamellae around chondrocytes in the ACC of aged BALB/c AKU mice. (a) A chondrocyte partially surrounded by concentric lamella, yet not completely enclosed in the ACC (x6000). Inset: Location of chondrocyte in the ACC, showing apparent 'opening' of the tidemark (arrowed) resulting in the cell becoming engulfed by the ACC (x2500). **(b)** A chondrocyte almost completely surrounded by lamellae, progressing deeper into the ACC (x6000). Inset: Location of chondrocyte in the ACC, showing apparent 'closing' of the tidemark (arrowed) resulting in the cell becoming completely embedded in the ACC (x2500). **(c)** A chondrocyte surrounded by numerous concentric lamellae (arrowed) in a periodic-like manner (x8200). **(d)** Concentric lamellae surrounding a chondrocyte deep in the ACC, in a periodic manner (arrowed) identical to what was seen in (c) (x8200). Tissues fixed in (a,b) PBFS, (c,d) glutaraldehyde. Ages = (a,b) 60 wks, (c,d) 54.4 wks. Scale = (a,b) 5μm, (c,d) 2μm.

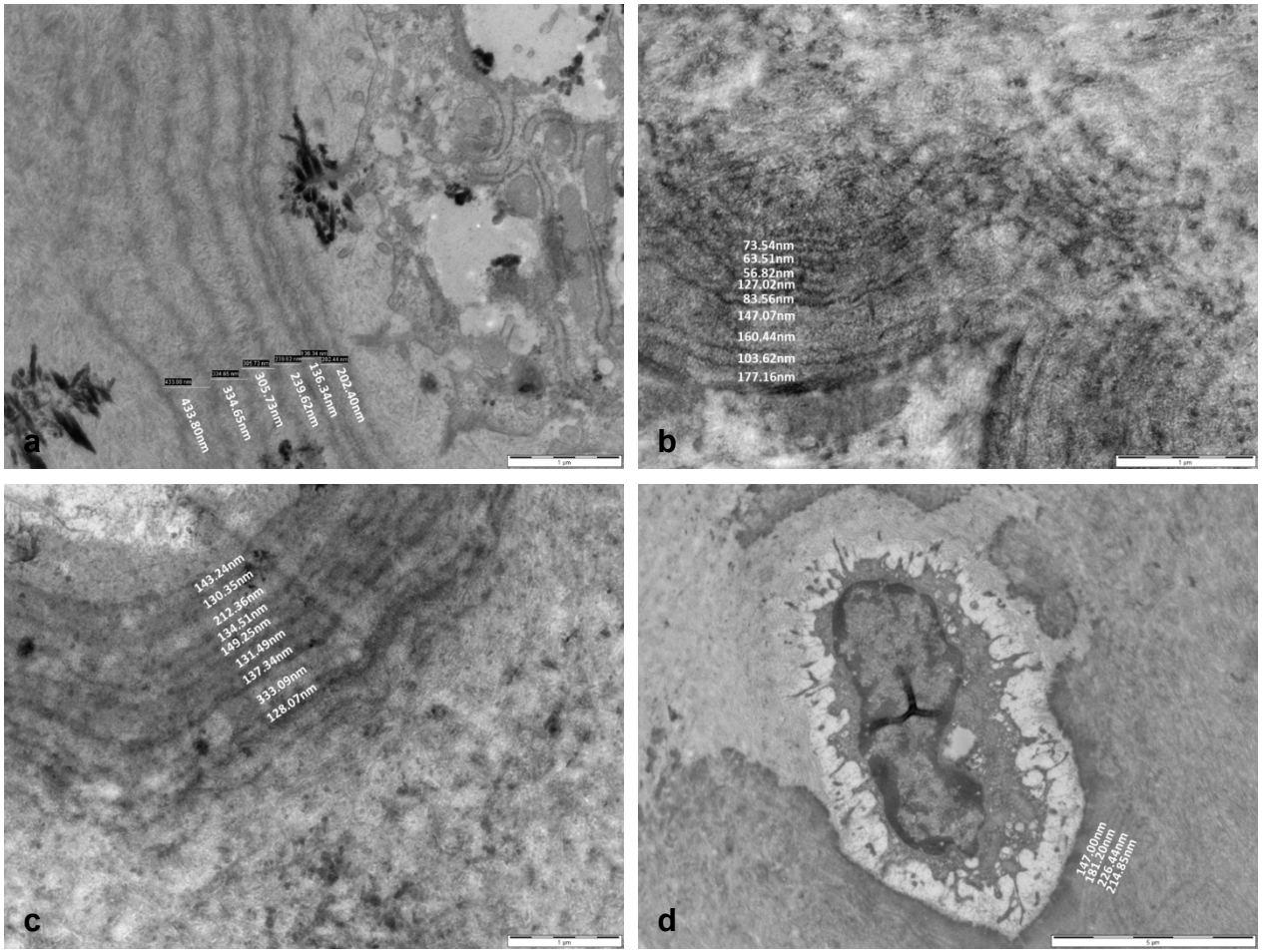

Fig. 5. Measurements of concentric lamellae in BALB/c AKU and WT mice. (a) Quantification of the lamella in a 7.8 week old AKU mouse showed a general increase in width as they progressed further away from the chondrocyte (x16,500). **(b,c)** The number of lamellae surrounding chondrocytes in aged AKU mice (53 + 61 weeks old respectively) increased in comparison to young AKU mice (a), however the widths of the lamellae were significantly narrower (x26,500). **(d)** Quantification of the lamellae in an aged WT mouse (69 weeks) revealed the number of lamellae was comparable to that seen in young AKU mice (a), whilst the width was comparable to that seen in aged AKU mice (b,c) (x4200). Tissues fixed in (a,b) glutaraldehyde (c,d) PBFS. Scale = (a,b,c) 1µm, (d) 5µm.

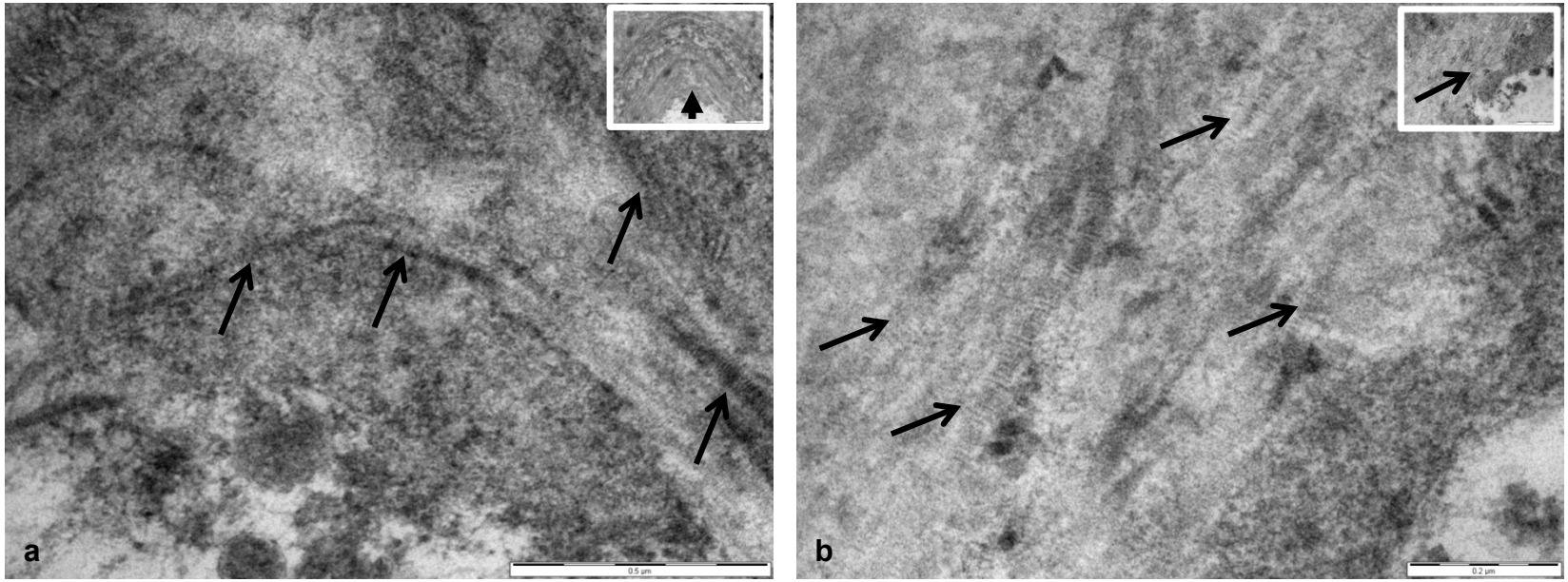

Fig. 6. Identification of collagen fibres in aged BALB/c AKU mice. (a) Collagen fibres were identified in the lamellae of a 56 week old AKU mouse (arrowed). Periodic banding can be seen along the fibres which is distinctive of collagen (x60,000). Inset: Low power image highlighting the location of the collagen fibres in the lamellae (x16,500). Tissue fixed in glutaraldehyde. **(b)** Collagen fibres were identified in the lamella of a chondron deep in the ACC of a 60 week old AKU mouse. Again, periodic banding can be seen along the fibres which is distinctive of collagen (x87,000). Inset: Low power image highlighting the location of the collagen fibres in the lamella (x43,000). Tissue fixed in PBFS. Scale = (a) 0.5µm, (b) 0.2µm.