FIGURE LEGENDS

Fig. 1. Immunolocalisation of collagen XXVII in embryonic mouse tissues.

Collagen XXVII (brown staining) localises to the cartilaginous skeletal elements and in particular, the hypertrophic regions, shown here in the developing foot (A) and ribs (B), the inner limiting membrane of the retinal and developing corneal stroma (C) at E14.5 days. (Preimmune and no chromogen controls can be seen in supplementary material - Fig. S3) Strong staining for collagen XXVII is detected in the stellate reticulum of developing teeth at E18.5 days. p, prehypertrophic; h, hypertrophic; l, lung; r, rib; st, corneal stroma; b, non-specific staining of the lens (see Fig. S3); ilm, inner limiting membrane of retina; sr, stelate reticulum. Bar = 10 μM

Fig. 2. Expression of collagen XXVII is down-regulated in some tissues between E14.5 and E18.5 days of development.

Strong collagen XXVII is detected by both immunohistochemical (A, F) and ^{35}S *in situ* hybridisation (C, G) in the major arteries associated with the heart (A,C) and the dermis (F, G) of E14.5 day mouse embryos. However, at E18.5 days, immunolocalisation at these anatomical sites is no longer apparent (heart, D; dermis, I). Preimmune serum treated controls for heart at E14.5 (B) and E18.5 days (E). H is the brightfield image of G. a, aorta; d, dermis. Bar = 10 μM

Fig. 3. Localisation of collagen XXVII in new born and 6 week old mouse tibia.

Collagen XXVII (A, D, G), collagen II (B, E, H) and collagen X (C, F, I) immunolocalisation in tibia of newborn (A, B, C) and 6 week old mice (D-I). G, H & I are enlargements of the boxed areas in the adjacent micrographs. a, articular cartilage; r, p and h refer to the reserve proliferative and hypertrophic zones of the growth plate. Bar = 10 μM

Fig. 4. Collagen XXVII immunolocalises to 10 nm thick non-striated fibrils.

Fibril extracts of new born mouse rib growth plate were absorbed to grids, treated with the collagen XXVII antiserum, washed and bound antibody detected using protein A labelled with 10 nm gold particles. A. An area showing the localisation of 10 nm particles to thin fibrils. B. An adjacent area showing no localisation to the much thicker, cross-striated fibrils containing collagen II. Further micrographs are presented in supplementary material (Fig. S4). Bar = 100 nM

Supplementary Figure Legends.
Fig. S1. Production of antisera to a recombinant type XXVII variable domain.

A. Schematic representation of the 45 kDa fusion protein containing histidine tag (His) and the variable domain (VAR) of mouse collagen XXVII. Cultures of IPTG-induced expression clones were lysed and the supernatant (S) and insoluble pellet (P) analysed under reducing conditions on SDS/PAGE and western blotted. Blots were probed with an anti His-tag antibody. On preparative gels, the band corresponding to recombinant variable domain was excised and the gel slice homogenised. A sample of the homogenate was then re-analysed by SDS/PAGE and blotted (Gel slice). B. Control (M) and collagenase-treated M+C) ATDC5 cell conditioned medium was resolved under reducing conditions on SDS/PAGE, western blotted and probed with either the collagen XXVII antiserum (I) generated to the recombinant protein shown in (A) or pre-immune antiserum (PI). C. Control (M) and collagenase-treated (M+C) conditioned ATDC5 medium was resolved on SDS/PAGE and then coomassie blue stained. **

marks position that proα1(II) collagen band runs. * marks position that the bacterial collagenase enzyme runs.

Collagenase-sensitive bands of ~150 - 100 kDa are detected by the new collagen XXVII antiserum (Fig. S1B) which match the bands previously identified using an antiserum generated to two peptides derived from the variable domain (5). Coomassie blue stained lanes of the same conditioned media treated with and without highly purified bacterial collagenase reveal that the majority of protein bands are unaffected by treatment with highly purified bacterial collagenase (Fig. S1C). The collagenase-sensitive proα1(II) collagen band of 150 kDa (Fig. S1C, lane M labelled with **) co-migrates with the highest Mr type XXVII collagen band detected by western blotting (Fig. S1B).

Fig. S2. Immunolocalisation of collagen XXVII can first be detected at E12.5 days in mouse.

Serial sections of an E12.5 day embryo treated with collagen XXVII antiserum (A, B) or preimmune serum (C, D). B and D are enlargements of the boxed areas in A and C respectively. The first signs of collagen XXVII deposition become apparent in the cartilaginous condensations of the developing vertebrae (v). Background staining (b) is apparent in blood (see Fig. S3). Bar = 10 μM

Fig. S3 Type XXVII collagen expression is widespread at E114.5 days of development.

Sections fo E14.5 day mouse embryo's treated with collagen XXVII antiserum (A), preimmune serum (B), or photographed prior to the addition of the chromogen used for color development (C). Background staining in blood and the lens is apparent in the preimmune (B) and this chemical browning has developed prior to the addition of the DAB peroxidise substrate (C). Collagen XXVII deposition is apparent throughout the cartilaginous anlagen as well as in the eye (see Fig. 1C for enlargement) and in the developing dermis, particularly around the head (Fig. 2F for enlargement) and foot (A). Bar = 10 μM

Fig. S4. Collagen XXVII immunolocalises to 10 nm thick non-striated fibrils

Additional micrographs taken from the grids used to prepare Fig. 4. Fibril extracts of new born mouse rib growth plate were absorbed to grids, treated with the collagen XXVII antiserum, washed and bound antibody detected using protein A labelled with 10 nm gold particles. A-C. Areas showing the localisation of 10 nm particles to thin fibrils. D. An adjacent area showing no localisation to the much thicker, cross-striated fibrils containing collagen II. Bar = 100 nM

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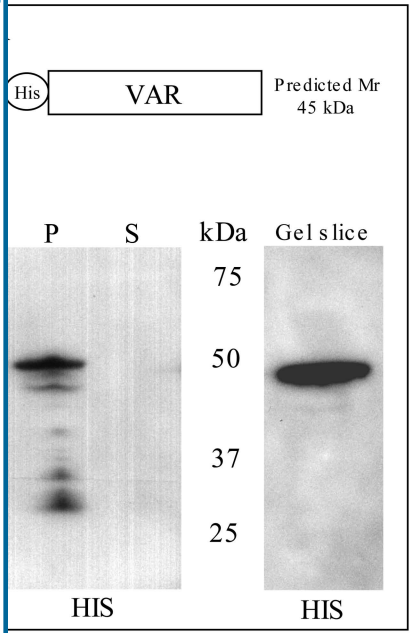
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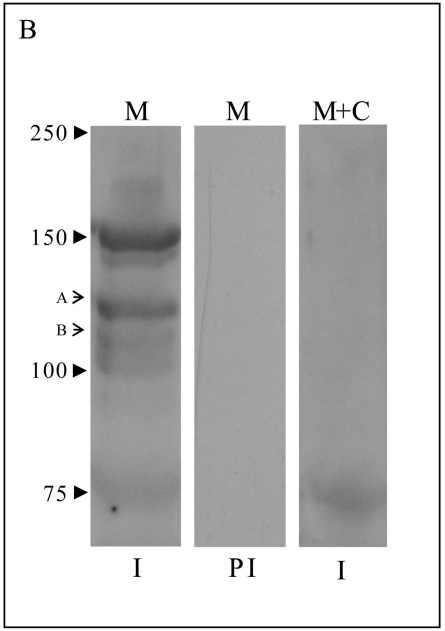
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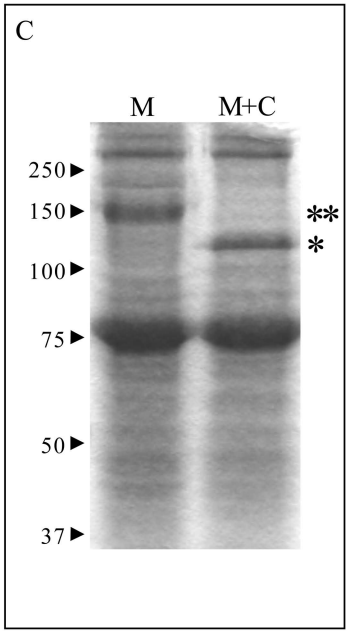
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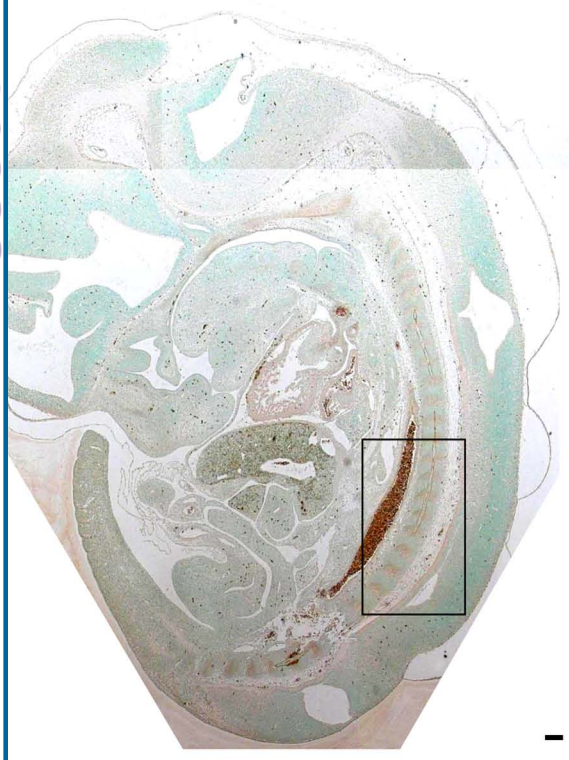

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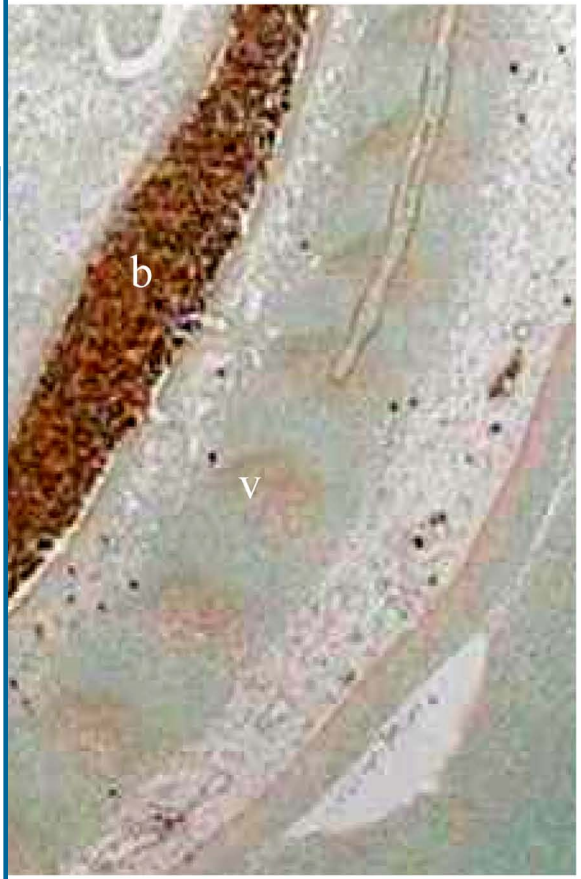

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