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eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/ An Endogenous Nanomineral Chaperones Luminal Antigen and Peptidoglycan to Intestinal Immune Cells.

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Supplementary Fig. 1 | Endogenous nanomineral of the murine intestinal lumen

Typical examples of the luminal endogenous nanomineral, in both discrete and agglomerated forms, derived from murine luminal contents, of the distal small bowel and visualised by transmission electron microscopy (TEM). Inset are the elemental compositions for Mg, P and Ca by standardless X-ray microanalysis.



Supplementary Fig. 2 | Endogenous nanomineral of the human Peyer's patch subepithelial dome

Monochromatic light microscopy image of the sub-epithelial dome (SED) after modified Von Kossa staining: regions of mineralised phosphate are shown as dense black dots as indicated by the red arrows.



Supplementary Fig. 3 | Nanomineral formation persists despite dietary deficiency of calcium and phosphorus.

a-b – Peyer's patch (PP) sections from adult BALB/c mice maintained for 3 weeks on **a**) a low Ca/P diet (< 0.3 g for both elements/kg diet) or **b**) a high Ca/P diet (> 3.8 g for both elements/kg diet) were stained with propidium iodide for nuclei (shown grey) and calcein for endogenous nanomineral (green). Under both dietary conditions mineralised calcium was stained in the sub-epithelial dome (SED). Scale bar, 50 μ m. Relative elemental (i.e. magnesium, phosphorus and calcium) composition of the nanomineral of the murine SED, after feeding **c**) a low Ca and P diet or **d**) a high Ca and P diet as determined by standardless X-ray microanalysis. Data are mean + SD of n = 19 (low Ca/P diet) and n = 26 (high Ca/P diet) separate regions.



Supplementary Fig. 4 | Calcein staining of the colon in wild type mice.

a – Colonic lymphoid follicles and **b** – regular mucosa do not demonstrate little calcein staining of AMCP nanomineral (green) within the tissue. Instead discrete calcein staining is often observed above the epithelium (white arrows) suggesting that the nanoparticles may agglomerate in and adhere to the tissue-associated mucus layer. **c** – The caecal patch, on the other hand, displays evident calcein staining (green) in the sub-epithelial area indicating that, in addition to Peyer's patches, immune-active lymphoid patches of the appendix also take up the endogenous nanomineral. Nuclei are shown in grey; Scale bars 50 μ m.



Supplementary Movie 1 | Localisation of the endogenous nanomineral to lysosomes of sub-epithelial dome antigen presenting cells.

3D reconstruction, imaged from a human Peyer's patch, showing typical localisation (orange) of the endogenous nanomineral (green-but not separately visible) to lysozyme-stained lysosomes (red).

Supplementary Movie 2 | 3D reconstruction of the endogenous nanomineral

Tomographic reconstruction of a large cluster of intra-cellular nanomineral imaged from the sub-epithelial dome of mouse Peyer's patch (as shown in Fig. 3e).