Electronic Supplementary Material (ESI) for Analyst.

SUPPLEMENTARY FIGURES

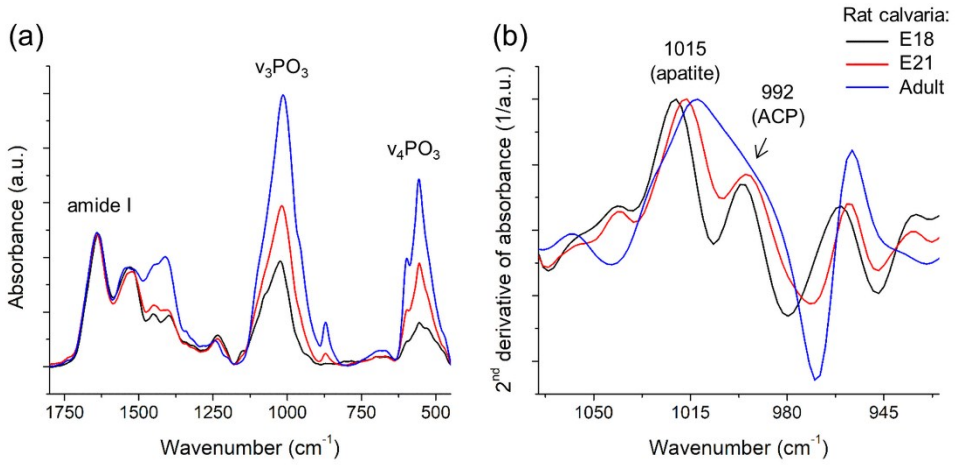

Figure S1: The amorphous calcium phosphate (ACP) component of bone is progressively lost during development. Calvaria bones from rats at progressive developmental stages were overlaid to illustrate changes in the ACP peak at ~992 cm^{-1} during development. **(a)** Fourier transform infrared (FTIR) spectra, showing the progressive growth of the PO_4 bands from mineral relative to the amide I band from protein. **(b)** Second derivative (inverted) of the v_3PO_4 band of the FTIR spectra. It is clear that the ACP peak at ~992 cm^{-1} becomes progressively less evident as bone matures.

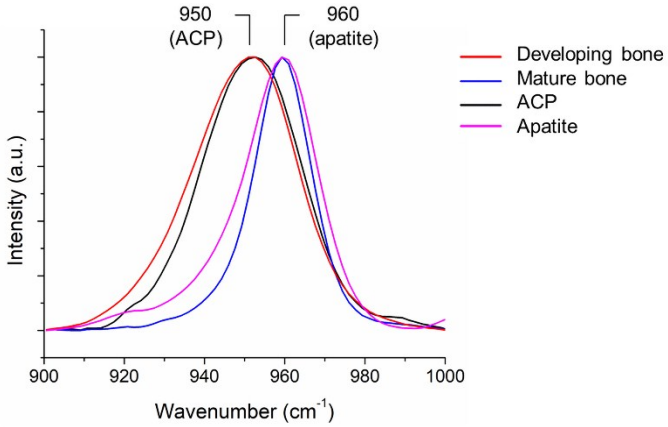

Figure S2: The presence of amorphous calcium phosphate (ACP) in developing bone is also observed with Raman spectroscopy. The results obtained using Fourier transform infrared (FTIR) were corroborated by the Raman spectra of developing (caudal fin) and mature (head) zebrafish bones. ACP and apatite standards were used to clarify the interpretation of the results. While the mature bones show the typical ν_1PO_4 band of apatite at ~960 cm^{-1}, the presence of ACP in the developing bones is clearly seen by the band at ~950 cm^{-1}.

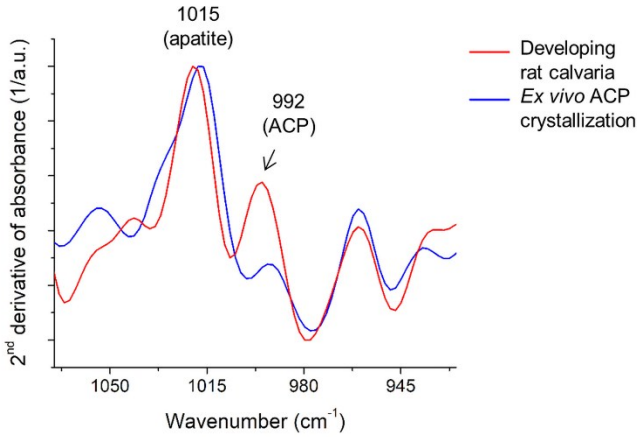

Figure S3: The amorphous calcium phosphate (ACP) component of developing bone acts as an apatite precursor when subjected to *ex vivo* crystallization conditions. The analysis described in Fig. 3 using tips of the developing zebrafish caudal fin bones were repeated using the calvaria from day 18 rats fetuses. The bones were analyzed and subjected to 24h in a buffer that favor the crystallization of ACP into apatite. Second derivative (inverted) of the ν_3PO_4 band of the Fourier transform infrared (FTIR) spectra confirmed the previous observations, showing a clear reduction of the ACP component peak at ~992 cm^{-1} after *ex vivo* crystallization.

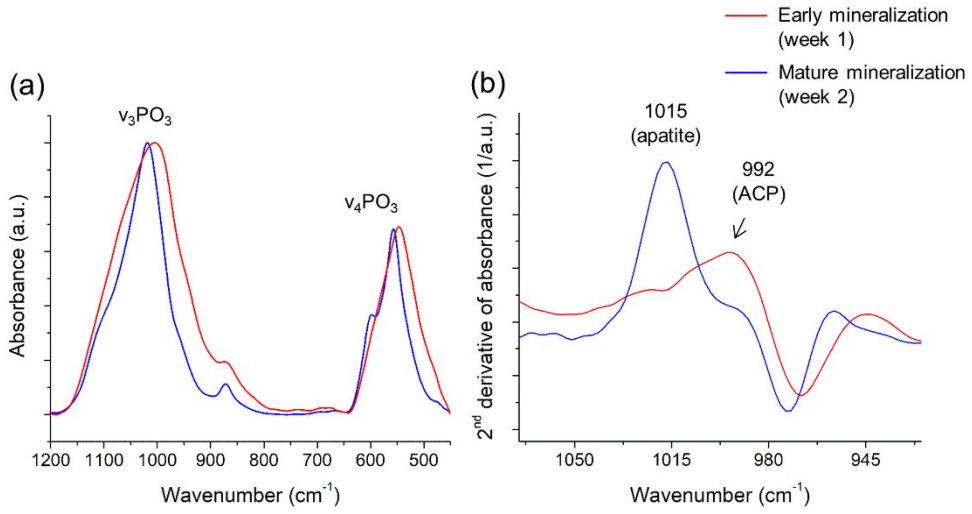

Figure S4: Amorphous calcium phosphate (ACP) is a major transient component of early mineralization in cell culture, preceding the formation of apatite in the mature matrix. The analysis described on Fig. 4 using 7F2 osteoblasts were repeated using hFOB osteoblasts. The cells were seeded as micromasses and cultured in osteogenic medium for 1 and 2 weeks. **(a)** Fourier transform infrared (FTIR) spectra. **(b)** Second derivative (inverted) of the v_3PO_4 band of the FTIR spectra. Early mineralization shows PO_4 bands typical of ACP, as well as the prominent ACP peak at ~992 cm^{-1}, whereas mature mineralization shows primarily apatite features, with a clear reduction of the ACP component peak — thus confirming the previous observations.

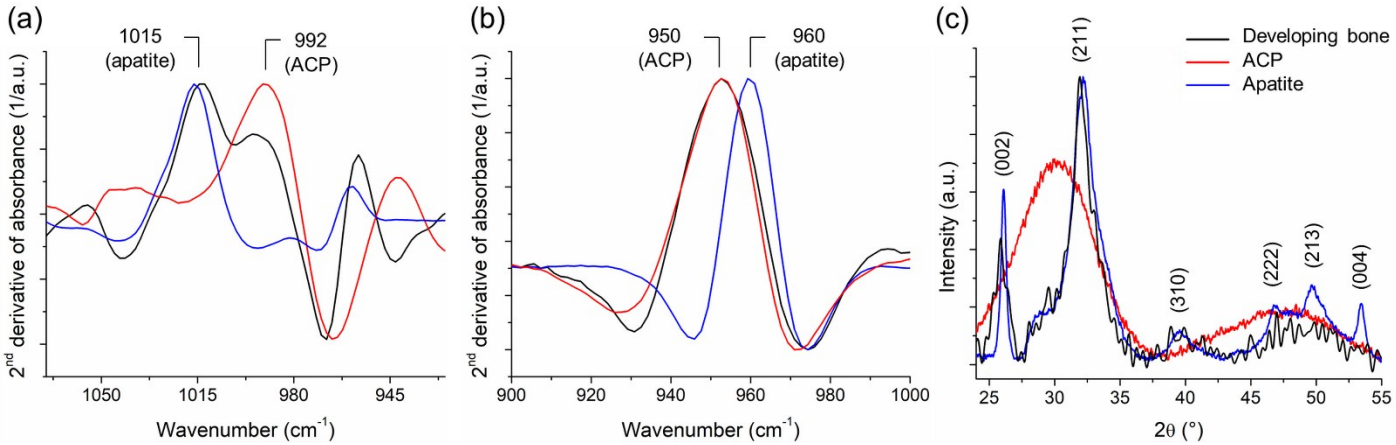

Figure S5: Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy and X-ray diffraction (XRD) are sensitive to distinct components of bone mineral. We analyzed developing bones from zebrafish (caudal fin) and standards of ACP and apatite to illustrate differences among the analytical approaches. **(a)** Second derivative (inverted) of the ν_3PO_4 band of the FTIR spectra. **(b)** Second derivative (inverted) of the ν_1 PO_4 band of the Raman spectra. **(c)** XRD patterns. An advantage of the FTIR approach we used in this work was that it was able to identify specific markers of both ACP and apatite in the developing bones, while Raman showed only the amorphous component and XRD, the crystalline.