SUPPORTING INFORMATION

NMR investigation of the role of osteocalcin and osteopontin at the organic-inorganic interface in bone

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Bone mineral

We have attempted to distinguish bone mineral of OC-OPN$^{-/-;-/-}$ bone from that of WT control in terms of chemical shift and peak widths:

1/ The difference in ^{31}P chemical shifts Δδ between the individual phosphorous environments extracted from the HETCOR spectra was defined (see Figure S1 A for OH…**P**O$_4^{3-}$ *vs* H-**P**O$_4^{2-}$) and plotted as function of CP contact time (Figure S1 B for OH…**P**O$_4^{3-}$ *vs* H-**P**O$_4^{2-}$).

2/ The ^{31}P peak widths of each individual ^{31}P environment were evaluated as a function of CP time (Figure S2). Although these ^{31}P peak widths appear to drift slightly, the difference between WT and OC-OPN$^{-/-;-/-}$ at any given CP time (see Table S1 for 1.5 ms) was small.

3/ The ^{1}H peak widths, obtained from deconvolution of the projection spectrum into the ^{1}H signals corresponding to the three ^{31}P environments, were examined. (Table S1) Note that the OH signal was deconvoluted into two contributions (Figure S3).

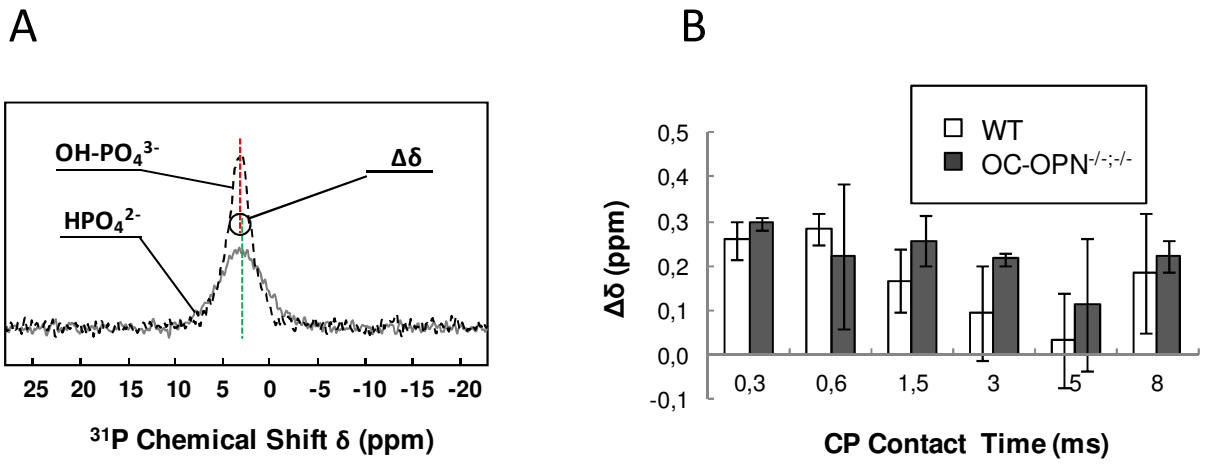

Figure S1 A/ ^{31}P chemical shift difference Δδ between OH-**P**O$_4^{3-}$ and H**P**O$_4^{2-}$ resonances (i.e. difference in ppm between the red and green lines, which cross through the maxima of the OH-**P**O$_4^{3-}$ and H**P**O$_4^{2-}$ resonances, respectively).

B/ Changes in the measured difference Δδ with CP contact time.

Table S1 Relative ^{31}P chemical shift Δδ (as defined in Figure S1 A), and ^{1}H and ^{31}P peak widths for the three ^{31}P environments distinguished by ^{1}H-^{31}P HETCOR for bone specimens from OC-OPN$^{-/-;-/-}$ mutants and their WT littermates.

	Peak[a]		WT	OC-OPN$^{-/-;-/-}$
$\Delta\delta\ (^{31}P) = (\delta_X - \delta_{OH\text{-}PO4})$ [b] (ppm)	**#1**	OH-**P**O_4^{3-}	/	/
	#2	H_2O-**P**O_4^{3-}	0.0 ± 0.1	0.1 ± 0.1
	#3	H**P**O_4^{2-}	0.2 ± 0.1	0.2 ± 0.1
^{31}P peak width [c] (ppm)	**#1**	OH-**P**O_4^{3-}	3.2 ± 0.1	3.2 ± 0.1
	#2	H_2O-**P**O_4^{3-}	5.5 ± 0.1	5.4 ± 0.1
	#3	H**P**O_4^{2-}	5.8 ± 0.1	5.8 ± 0.1
^{1}H peak width [c] (ppm)	**#1a**	O**H**-PO_4^{3-}	1.2 ± 0.2	1.1 ± 0.2
	#1b	O**H**-PO_4^{3-}	2.0 ± 0.4	1.9 ± 0.4
	#2	**H**$_2$O-PO_4^{3-}	2.0 ± 0.3	2.1 ± 0.3
	#3	**H**PO_4^{2-}	9.8 [d]	9.8 [d]

[a] Peak numbering is defined in Figures 2A (#1, #2 and #3) and S3 (#1a and #1b).
[b] Average for contact times from 0.3 to 8 ms.
[c] Width at half maximum measured for HETCOR spectra recorded at 1.5 ms contact time.
[d] Larger error on the peakwidth (very broad, low intensity signal).

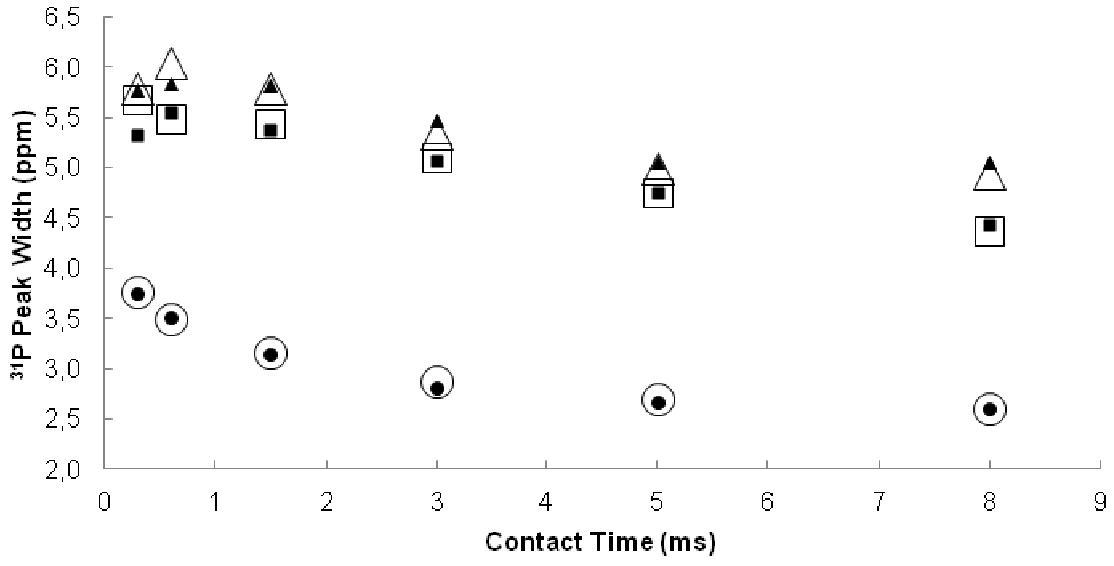

Figure S2 ^{31}P peak widths extracted from the 2D ^{1}H-^{31}P HETCOR spectra of WT (hollow markers) and OC-OPN$^{-/-;-/-}$ (filled markers) bone specimens. The widths of OH-**P**O_4^{3-}, H_2O-**P**O_4^{3-} and H**P**O_4^{2-} environments are shown as circle, rectangle and triangle, respectively. Errors are in the order of size of the hollow markers.

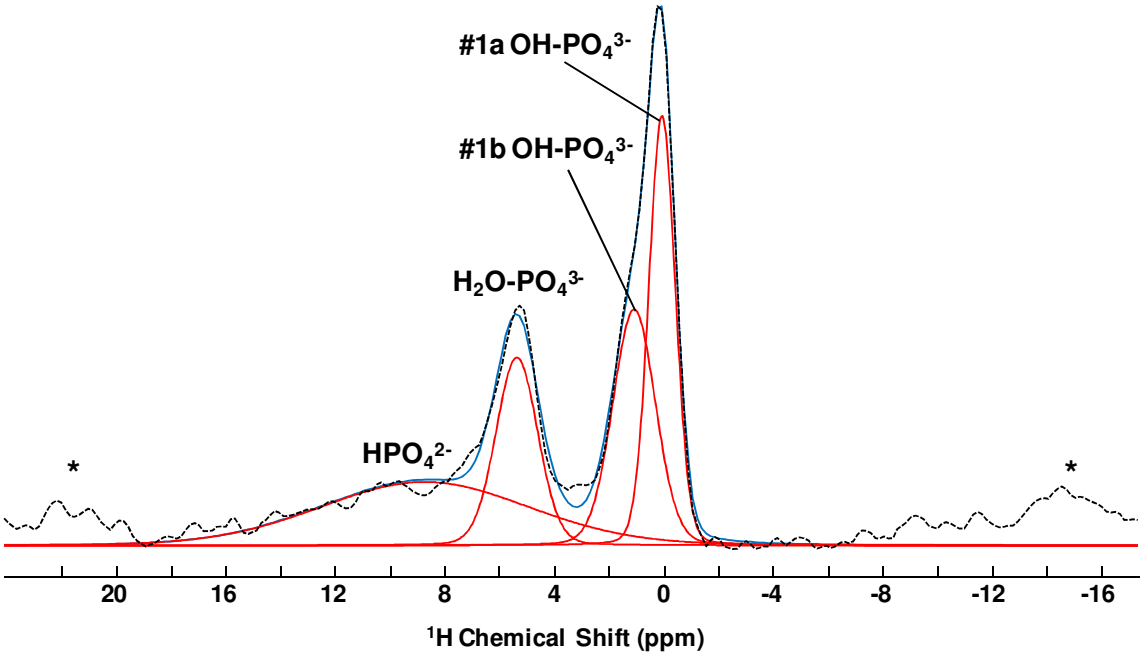

Figure S3 Deconvolution of the F1 projection of one of the $^{1}H \rightarrow ^{31}P$ HETCOR spectra recorded. Two environments for the O**H**-PO_4^{3-} signals appear to be present (**#1a** and **#1b**). Spinning sidebands are marked by the asterisk.

Organic matrix

The intensity of individual ^{13}C signals of organic matrix were deconvoluted and compared between WT and OC-OPN$^{-/-;-/-}$ (Table S2).

Table S2 Parameters extracted from the deconvolution of the ^{1}H-^{13}C CPMAS NMR spectra of WT and OC-OPN$^{-/-;-/-}$ bone specimens: signal intensity and peak width of individual amino acids ^{13}C signals.

Peak Number (Figure 4)				**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Partial Assignment		C=O	GAG/Cit	Gly C-2 Arg C-5	Pro C-2 Hyp C-2 Phe C-2	Pro C-3 Arg C-3 Lys C-3	Pro C-4 Glu C-3 Leu C-4	Pro C-5	Ala C-2	Ala C-3	Hyp C-3 Lys C-6 Asp C-3 Leu C-3 Ile C-3 Phe C-3	Hyp C-4
Signal Intensity (%)	WT	14.3 ± 0.4	1.0 ± 0.1	9.7 ± 0.1	8.7 ± 0.2	5.6 ± 0.9	5.5 ± 0.3	3.1 ± 0.1	3.9 ± 0.3	3.5 ± 1.0	5.8 ± 0.2	3.0 ± 0.1
	OC-OPN$^{-/-;-/-}$	13.5 ± 0.3	0.9 ± 0.1	9.5 ± 0.1	8.7 ± 0.2	5.6 ± 0.6	5.6 ± 0.3	2.9 ± 0.2	3.9 ± 0.4	3.1 ± 1.2	5.9 ± 0.2	3.1 ± 0.1
Peak Width (ppm)	WT	5.0 ± 0.1	5.7 ± 0.5	3.0 ± 0.1	3.7 ± 0.1	3.8 ± 0.5	2.8 ± 0.1	2.2 ± 0.1	3.1 ± 0.3	3.4 ± 1.0	5.0 ± 0.3	2.3 ± 0.1
	OC-OPN$^{-/-;-/-}$	4.6 ± 0.1	4.8 ± 0.2	2.9 ± 0.1	3.6 ± 0.1	3.8 ± 0.3	2.8 ± 0.1	2.1 ± 0.1	3.1 ± 0.3	3.1 ± 1.1	5.0 ± 0.3	2.2 ± 0.1