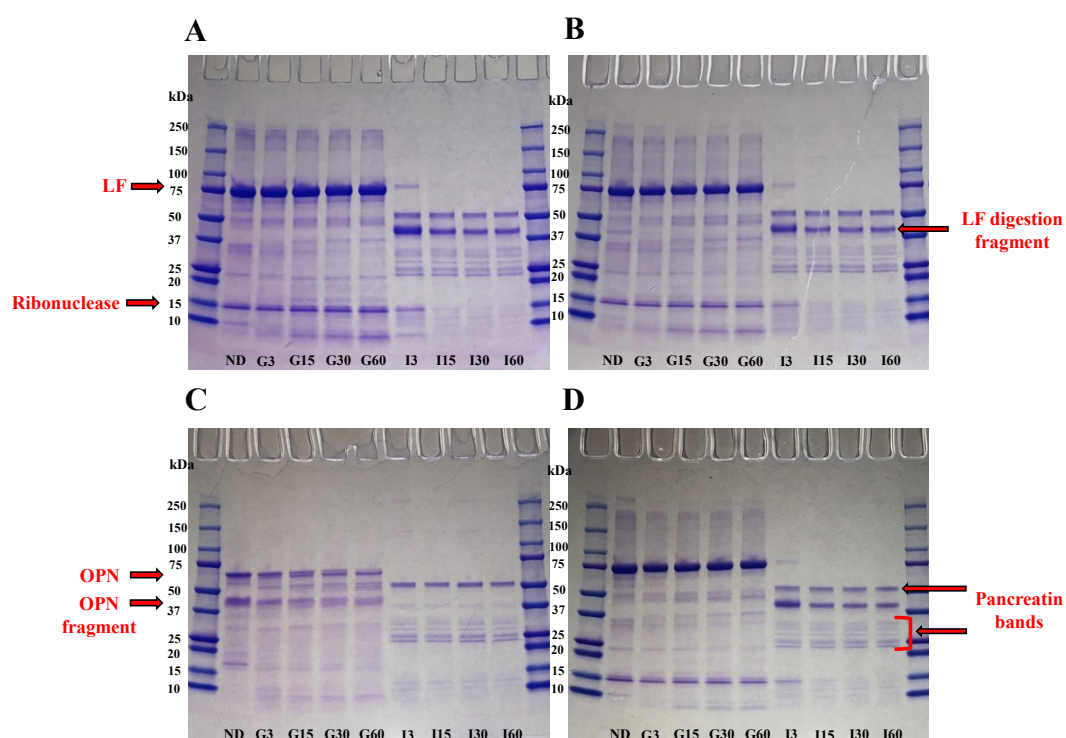


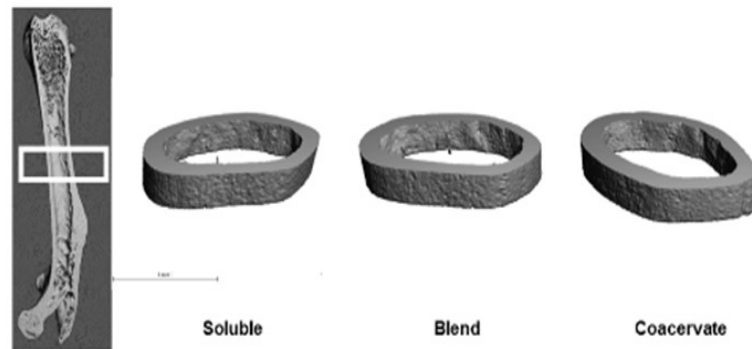
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Authors	Goulding, David A.;Bonnet, Nicolas;Horcajada, Marie-Noëlle;Baruchet, Michael;Bermont, Flavien;Hauser, Jonas;Macrì, Simone;Pisa, Edoardo;Nembrini, Chiara;Vidal, Karine;O'Brien, Nora M.;O'Mahony, James A.;O'Regan, Jonathan
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Supplementary information

The impact of complexation or complex coacervation of lactoferrin and osteopontin on simulated infant gastrointestinal digestion, intestinal inflammation, and in vivo bone development



Supplementary Figure 1. Proteolysis of uncomplexed and complexed lactoferrin (LF) and osteopontin (OPN) during simulated infant gastrointestinal digestion at pH 5.3 (gastric) and pH 6.6 (intestinal). Panels A-D show the apparent molecular weight profile of gastric and intestinal digestates of (A) LF, (B) LF-OPN soluble complexes (SC), (C) OPN, and (D) LF-OPN complex coacervates (CC), as analysed by reducing SDS-PAGE. Loading quantity was 6 µg protein/lane. Each lane contains a digestate collected at a specific timepoint (in minutes) during either the gastric (G) or intestinal (I) phases of simulated digestion, which is annotated at the bottom of each lane. ND is the non-digested sample.



Supplementary Figure 2. The impact of lactoferrin-osteopontin soluble complexes (SC), lactoferrin-osteopontin complex coacervates (CC), and a mixed blend of uncomplexed lactoferrin and osteopontin (LF & OPN mix) on cortical bone structure. Presented is a micro-CT image of bone cortical structures following supplementations. Data are shown as mean values $\pm$ standard error of triplicate measurements. ‘*’ represents statistically significant differences ($P < 0.05$) between treatments, other p-values compare CC to SC or to LF & OPN mix, as indicated on the figure.