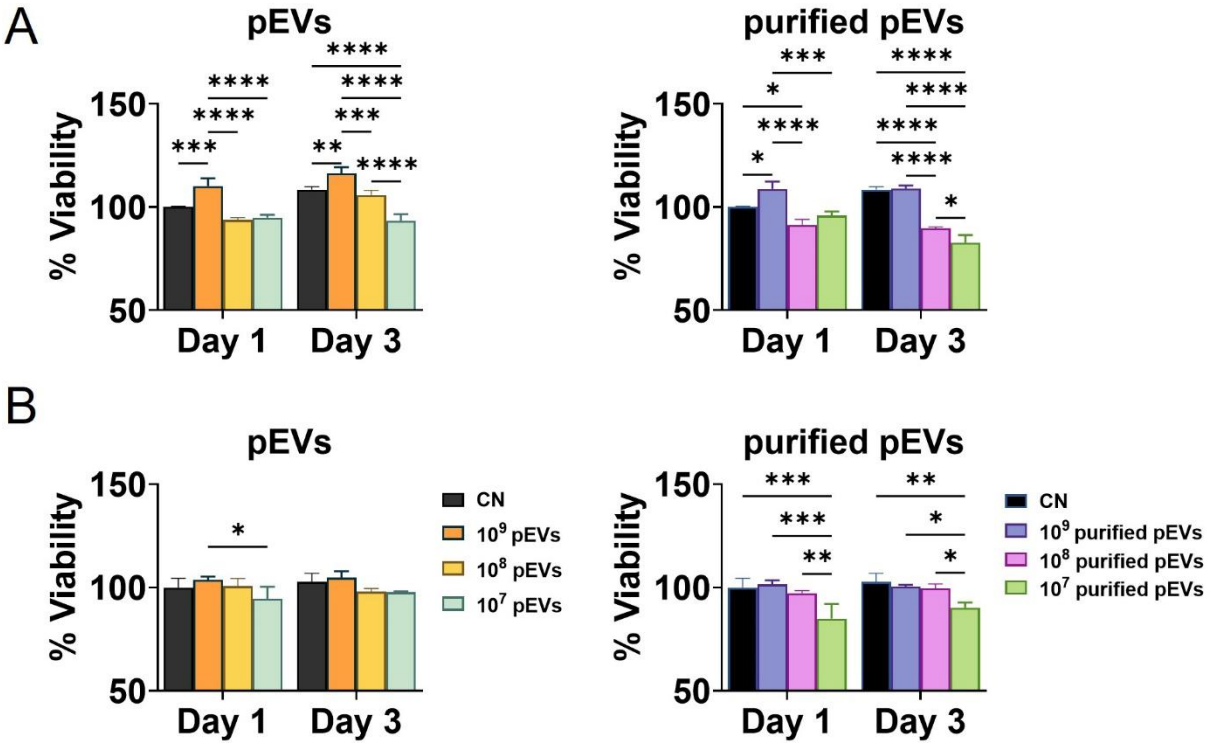
Supplementary Materials

Purified plasma vesicles modulate inflammation and mineralisation

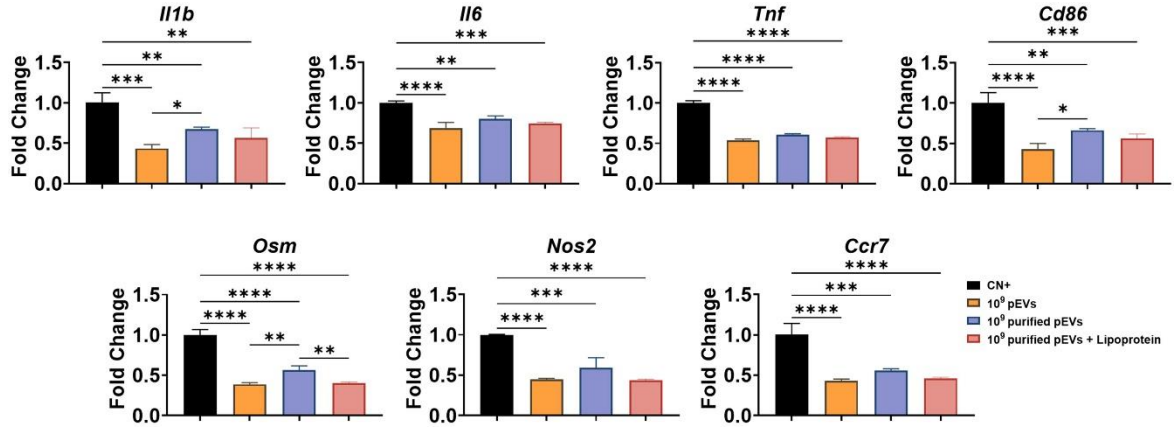
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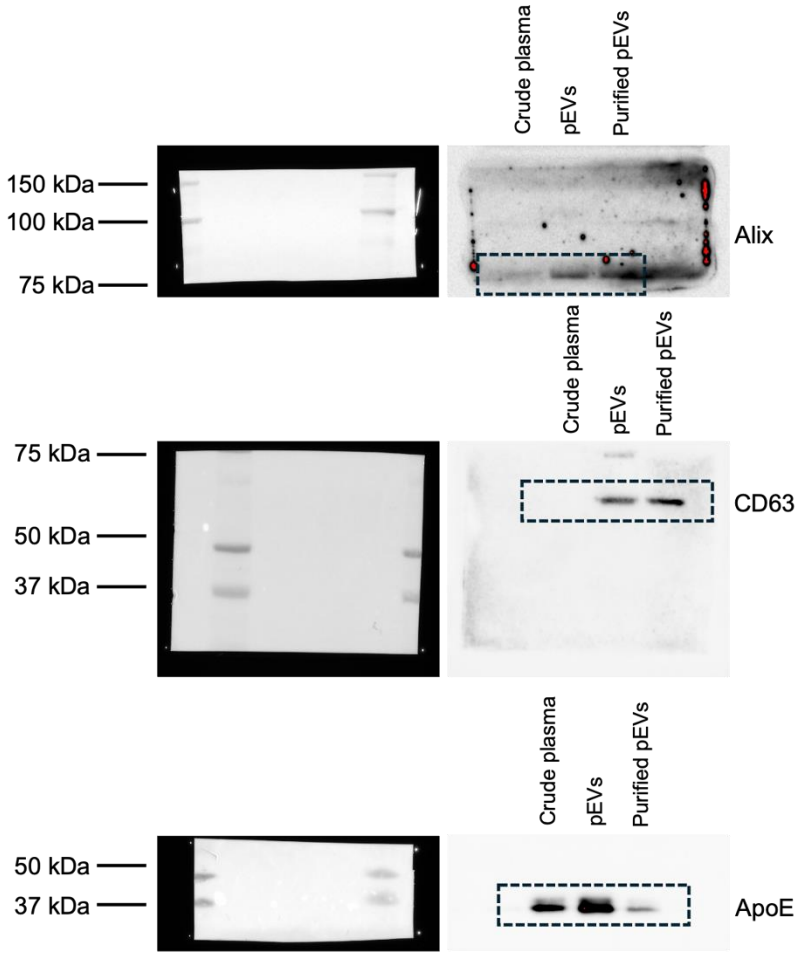
Correspondence to: Prof. Sašo Ivanovski, Dr. Yinghong Zhou, School of Dentistry, Centre for Orofacial Regeneration, Reconstruction and Rehabilitation (COR3), The University of Queensland, Brisbane 4072, Australia. E-mail: s.ivanovski@uq.edu.au; yinghong.zhou@uq.edu.au; Prof. Joy Wolfram, School of Chemical Engineering, The University of Queensland, Brisbane 4072, Australia. E-mail: j.wolfram@uq.edu.au



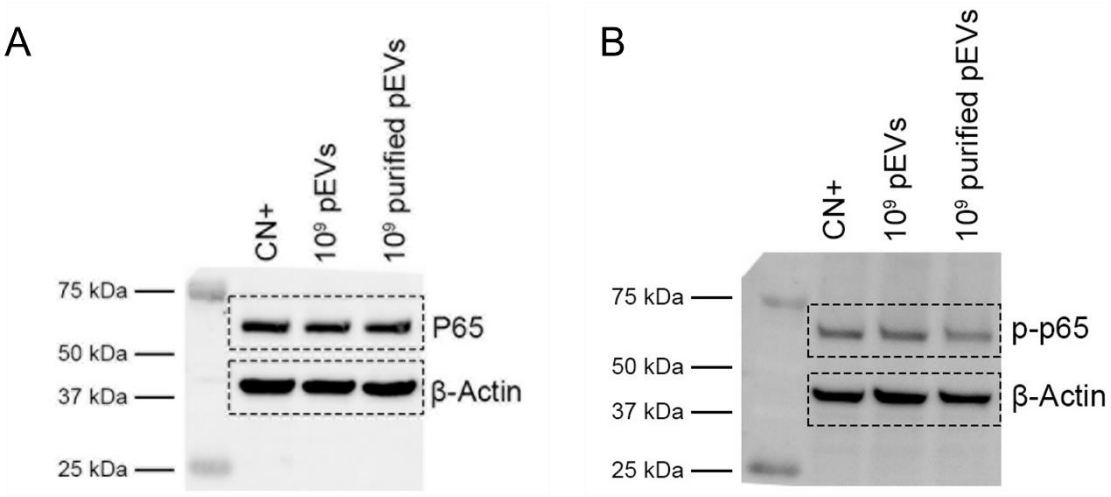
Supplementary Figure 1. Effects of pEVs and purified pEVs at 1×10^7, 1×10^8 and 1×10^9 particles/mL on the viability of RAW cells and hPDLCs. Alamar Blue assay showing cell viability of (A) RAW cells and (B) hPDLCs on days 1 and 3 with the treatment of pEVs or purified pEVs. Data are presented as mean ± SD from three independent experiments, each using paired pEV and purified pEV preparations from a different plasma donor (n = 3). Statistical significance is indicated as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. CN: Control (cells cultured in normal growth medium without EVs); EVs: extracellular vesicles; hPDLCs: human periodontal ligament cells; pEVs: plasma-derived extracellular vesicles; RAW: RAW264.7 macrophages; SD: standard deviation.



Supplementary Figure 2. Immunoregulatory effects of pEVs, purified pEVs and purified pEVs with reconstituted lipoproteins on RAW cells. Fold change in pro-inflammatory marker expression in RAW cells primed with *E. coli* LPS (1 µg/mL for 2 h), washed three times with PBS and subsequently cultured with 100 ng/mL LPS and the indicated EV preparations (1×10^9 particles/mL) for 24 h. Data are presented as mean ± SD from three independent experiments, each using paired pEV and purified pEV preparations from a different plasma donor ($n = 3$). Statistical significance is indicated as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. CN^+: Positive control (RAW cells treated with LPS only); EVs: extracellular vesicles; LPS: lipopolysaccharide; PBS: phosphate-buffered saline; pEVs: plasma-derived extracellular vesicles; SD: standard deviation.



Supplementary Figure 3. Original, uncropped Western blot images of Alix, CD63 and ApoE corresponding to Figure 1C. Alix: ALG-2-interacting protein X; ApoE: apolipoprotein E; CD63: cluster of differentiation 63.



Supplementary Figure 4. Original, uncropped Western blot images of (A) total NF-κB p65 and β-actin and (B) phosphorylated p65 (p-p65) and β-actin corresponding to Figure 5B. NF-κB: Nuclear factor-κB; p65: nuclear factor-κB p65; p-p65: phosphorylated p65.