Supplementary

P. gingivalis peptidyl arginine deiminase can modulate neutrophil activity via infection of human dental stem cells (hDSCs)

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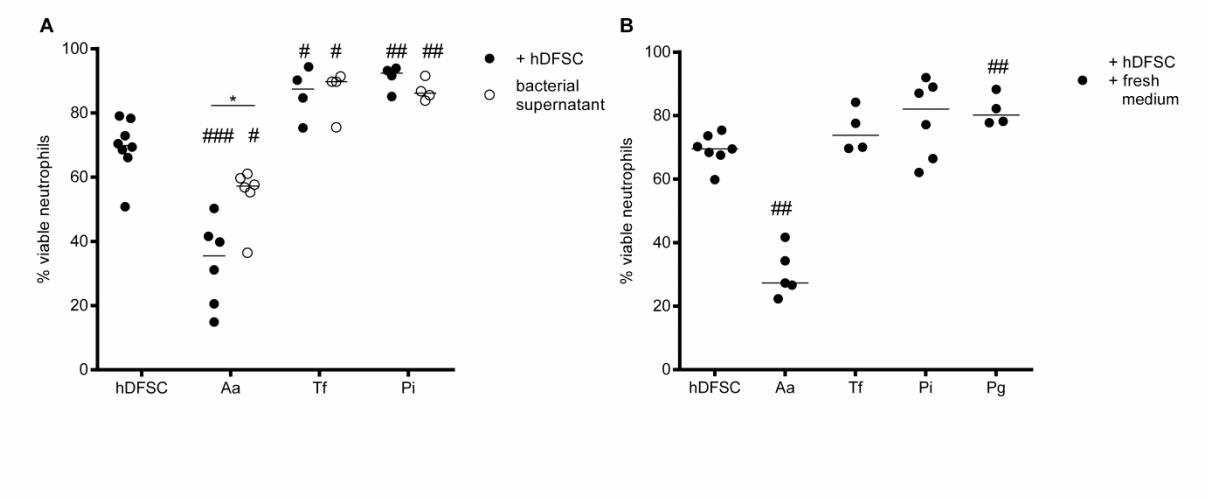
FIGURE S1 Influence of different oral bacterial pathobionts on the viability of neutrophils

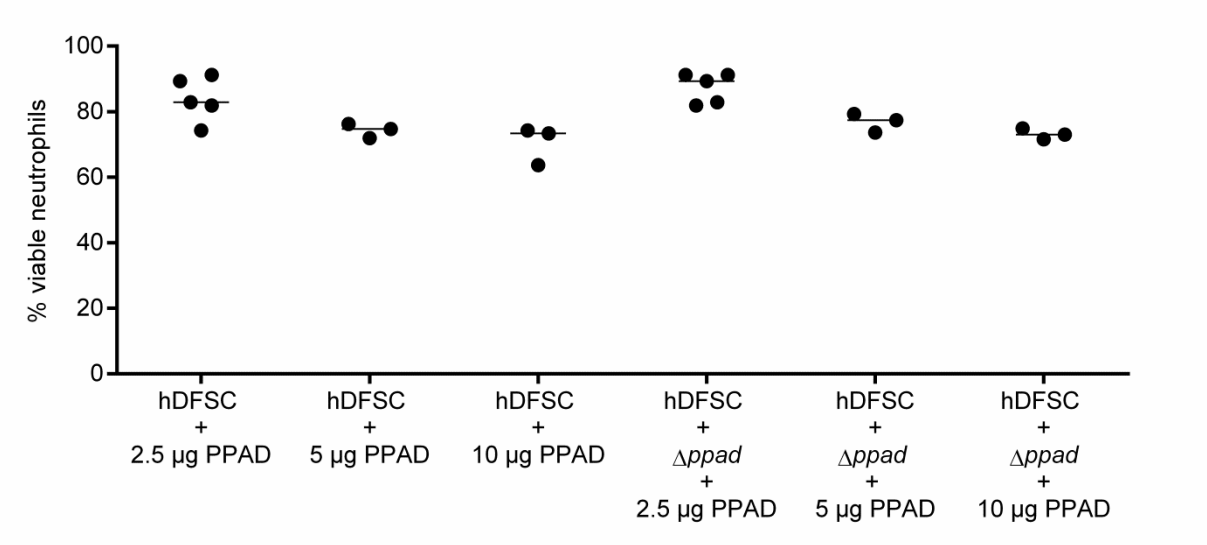


(A) Viability of neutrophils cultivated with primed stem cells or in bacterial supernatant HDFSCs were co-cultivated with *A. actinomycetemcomitans* (Aa), *T. forsythia* (Tf) or *P. intermedia* (Pi) for 24 h under anaerobic conditions, unprimed hDFSCs and bacteria only were used as control. After sterile filtration of the DMEM supernatants and antibiotic treatment, neutrophils were co-cultivated with primed hDFSCs for another 24 h in anaerobic conditions. (B) Viability of the neutrophils cultivated with primed stem cells and fresh medium. HDFSCs were co-cultivated with *A. actinomycetemcomitans* (Aa), *T. forsythia* (Tf), *P. intermedia* (Pi) and *P. gingivalis* W83 (Pg) for 24 h under anaerobic conditions. After antibiotic treatment, fresh medium was added, and neutrophils were co-cultivated for another 24 h in anaerobic conditions.

The apoptosis of neutrophils was analyzed via Annexin V/7-AAD staining and flow cytometry analysis. The percentage of viable cells without signs of apoptosis is plotted in the diagram. The results are displayed as the median. Significance of the unprimed stem cell control to the co-culture conditions: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p <$

0.001, n ≥ 4 in all the experiments (each data point represents and independent biological experiment).

FIGURE S2 Recombinant PPAD shows no dose dependent effect on the apoptosis of neutrophils.



Viability of neutrophils cultivated with *P. gingivalis* strains and recombinant PPAD. HDFSCs were incubated with recombinant PPAD or a combination of recombinant PPAD and *P. gingivalis* Δ*ppad* as described in Figure 2. Increasing amounts of recombinant PPAD (2.5 µg, 5 µg and 10 µg) were analyzed in the co-culture. No dose dependent effect was detected. Thus, 2.5 µg of recombinant PPAD was used in further experiments. The results are displayed as the median, n ≥ 3 (each data point represents and independent biological experiment).

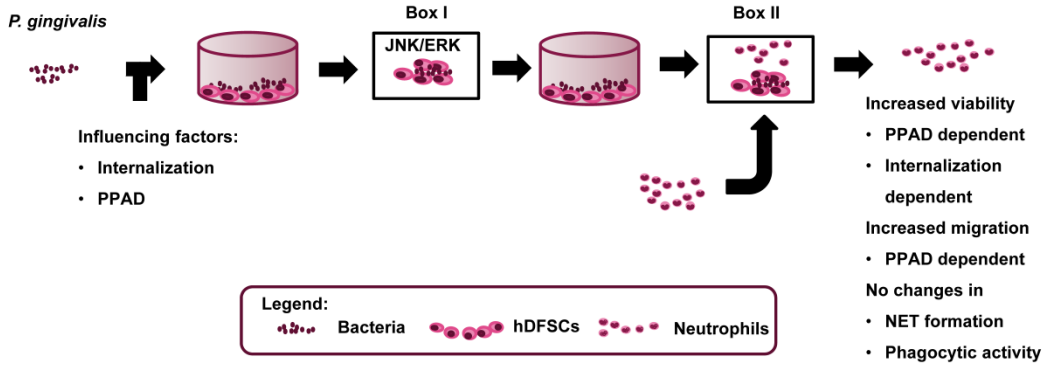


FIGURE S3 Summary of the experiments investigating the interaction of *P. gingivalis* with human dental follicle stem cells and the resulting effects on neutrophils in the established triple culture system.

Summary of the results reported in this study. Direct interaction of living *P. gingivalis* and hDFSCs is required for downstream effects on the neutrophils.