***Entamoeba gingivalis* exerts severe pathogenic effects on the oral mucosa**

Supplementary Methods

Cultivation of primary gingival epithelial and fibroblast cells from *ex vivo* biopsies

Ex vivo biopsies of the healthy oral masticatory mucosa were collected at the hard palate adjacent (<10 mm) to the fourth and fifth tooth by using a tissue puncher (3 mm diameter). Fresh gingival tissue was transferred into DMEM with 2× Penicillin-Streptomycin for 30 minutes to eliminate bacteria. The donors were orally and systemically healthy subjects. The Ethical Review Board of the Charité – Universitätsmedizin Berlin (EA4/088/15) approved this procedure. To separate the epithelial layer from the lamina propria, the biopsies were incubated in 10ml cell growth medium (DMEM, 1% Pen/Strep) with 5mg/ml Dispase II (Sigma Aldrich) on ice in a 4 ℃ refrigerator overnight. Primary epithelial cells were detached from the epithelial layer of the tissue by 5ml Trypsin/EDTA in a 37℃ 300 rpm shaker for 30min and neutralized by 10ml DMEM containing 10% FBS collected by 800 rpm 5min centrifugation. Primary gingival epithelial cells were cultured with Dermalife K keratinocyte Medium complete medium (LL-0007, LIFELINE), with 1× Penicillin-Streptomycin added. Primary gingival fibroblast cells (pGFs) were cultured in cell growth medium (DMEM, 1% Amphotericin B, 1% Pen/Strep, 1% non-essential Amino acids). Primary gingival cells were passaged when reaching 80% confluence. Primary gingival cells were only used before the fourth passage. 24 hours before E.g. infection, the pGFs (passage 3-6) and pECs (passage 3-4) were seeded in 6-well tissue culture plates (TPP Techno Plastic Products, Trasadingen, Switzerland) (1.8 x 105 cells per well).

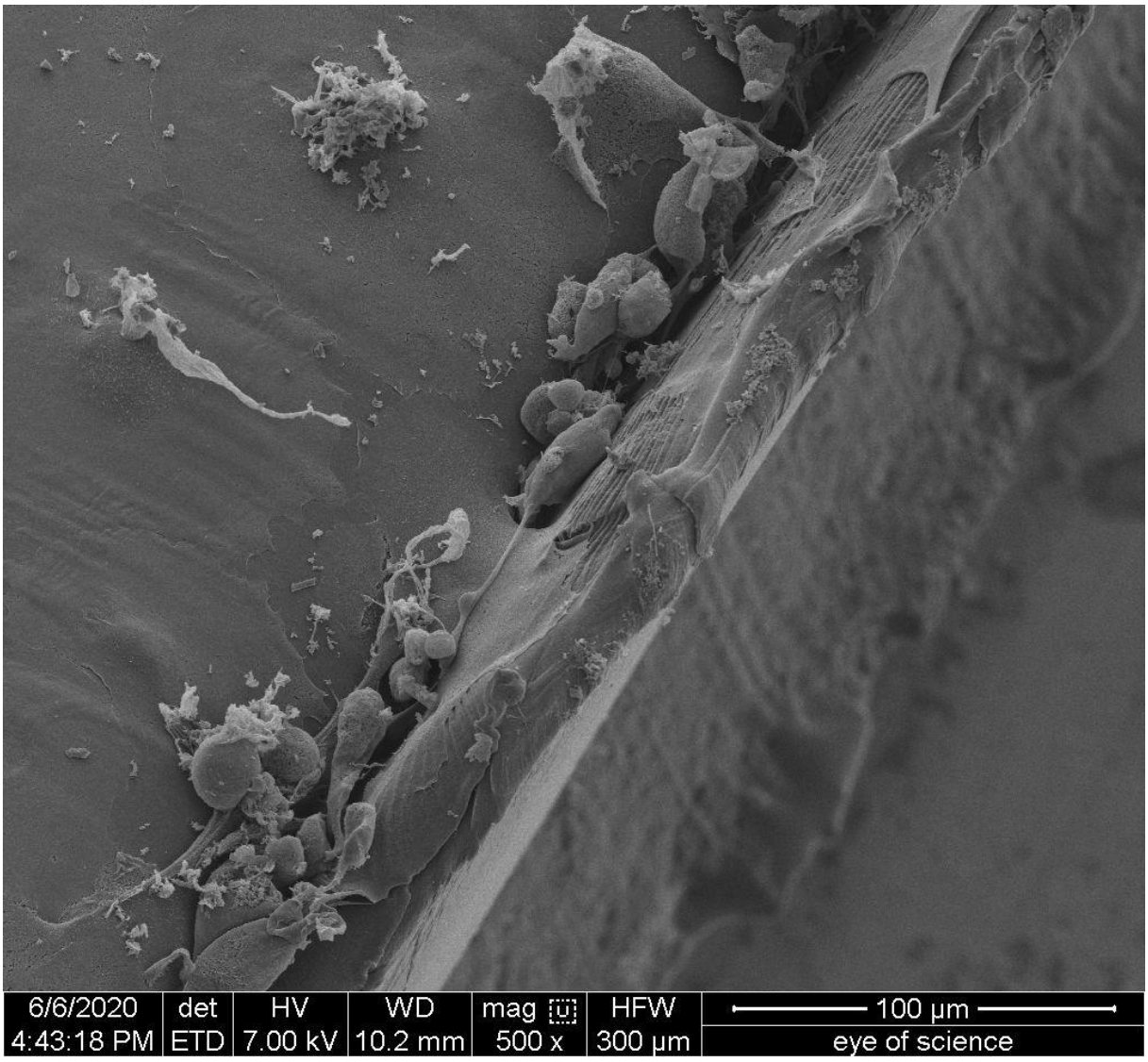
Infection of gingival cells with *E. gingivalis*

Because no axenic cultures of *E. gingivalis* exist to date, the petri dishes containing the amoebic cultures were placed on ice for 8 minutes to detach amoebae from the bottom. Subsequently, 500 µl of the medium was transferred to sterile 2 ml Eppendorf tubes and centrifuged for 10 minutes at 275g. The supernatant was discarded, the pellet was washed with 1.5 ml sterile 1×PBS and pre-warmed fresh culture medium was added. The pellet was dissolved by gentle pipetting and the washing was repeated 4× to eliminate bacteria from the amoeba. *E. gingivalis* at multiplicity of infection (MOI) 0.2 was added in 10µL PBS to primary gingival epithelial cells (GECs) and primary gingival fibroblasts (GFBs) and co-incubated for 2 hours. To generate

the mock-infection medium we used 10µl of the supernatant of the *E. gingivalis* growth medium of the last washing step.

E. gingivalis was cultured and counted as follows. Cultured *E. gingivalis* were detached from the petri-dish by placing it on ice for 8 min. *E. gingivalis* was re-suspended with a 1ml pipette in the petri-dish by pipetting 500µl gently up and down (3×). The suspension media were pooled from several petri-dishes and centrifuged 10 minutes at 275g. The supernatant was removed and the pellet was washed with 1×PBS (sterile). Centrifugation and washing was repeated 4×. 500ul of the supernatant of the last washing step was used for the mock infection. The *E. gingivalis* pellet was re-suspended in 100µl 1× PBS. For counting *E. gingivalis* numbers, 10µl of the *E. gingivalis* suspension was mixed with 10µl Typan blue solution. Subsequently, 10µl of the mixed solution was added to a cell counting Neubauer Chamber. The unstained *E. gingivalis* was counted under the light microscope (blue stained substances are cell debris of human and bacterial cells of the plaque). An average amount of the *E. gingivalis* counted from the four quadrants on the chamber equals × 10^3 *E. gingivalis*/100µl *E. gingivalis* suspension.

Supplementary Figure and Tables



Supplemental Figure 1. *Entamoeba gingivalis* accumulated in interstices of the culture plate

Supplemental Table 1. Results of the tmod enrichment analysis for contrast pGEC *E. gingivalis* stimulated vs control and pGF *E. gingivalis* stimulated vs control

ID	Title	AUC	cES	adj.P.Val	Significant genes
pGEC					
LI.M35.0	signaling in T cells (I)	0.97	5.15	6.0E-09	*JUNB; FOS; FOSB; EGR1; JUN; TNF; ATF3; TNFRSF9*
LI.M86.0	chemokines and inflammatory molecules in myeloid cells	0.90	5.15	3.1E-19	*CXCL1; TRAF1; CXCL2; NFKBIA; BCL2A1; GCH1; G0S2; TNFAIP3; TNFAIP6; TNF; HS3ST3B1; NFKB1; CCL20; IL1B; DUSP1; PDE4B*
LI.M31	cell cycle and growth arrest	0.94	5.03	2.0E-10	*RARA; GADD45B; JUNB; TNFAIP3; THBS1; PPP1R15A; IL1A; IL1B; DUSP1*
LI.M29	proinflammatory cytokines and chemokines	0.80	5.02	1.2E-08	*PTX3; IL6; TNF; IL1A; CCL20; IL1B*
LI.M94	growth factor induced, enriched in nuclear receptor subfamily 4	0.99	4.67	3.4E-10	*PPP1R15A; PLK2; NR4A2; EGR3; CDKN1A; NR4A1; EPHA2; ID1; IL6; NR4A3; DUSP1*
LI.M89.0	putative targets of PAX3	0.93	4.24	1.9E-11	*PLK2; G0S2; CXCL2; EGR2; EGR3; CD83; EGR1; NR4A1; HBEGF; NR4A2; MAFF; CDKN1A; GEM; DUSP2*
LI.M20	AP-1 transcription factor network	0.94	4.22	3.6E-10	*MMP9; JUNB; FOS; FOSB; EGR1; JUN; PLAU; IL6; FOSL1; FOSL2; ATF3; MMP1*
LI.M89.1	putative targets of PAX3	0.94	4.16	3.4E-07	*EGR2; EGR3; CD83; NR4A1; INHBA; NR4A2; DUSP1; ATF3; DUSP2*
LI.M115	cytokines - recepters cluster	0.88	3.96	5.6E-06	*LIF; IL23A; IL15; CSF2; CSF3; IL6; IL15RA*
LI.M43.0	myeloid, dendritic cell activation via NFkB (I)	0.86	3.78	2.7E-07	*MAP3K8; IL23A; CCL5; CD83; NFKBID; TNF; ICAM1; RELB; NFKB2; BCL3*
LI.M172	enriched for TF motif TTCNRGNNNNTTC	0.88	3.49	6.8E-06	*CXCL3; EGR3; FOSB; GJB2; PLAU; NRIP3; NR4A3; EDN1; PHLDA2*
LI.M160	leukocyte differentiation	0.82	3.41	1.7E-08	*RARA; LIF; BTG2; EGR2; CD83; JUNB; ADAM8; ID1; KLF6; NR4A2; IL1A; NFKB2; BCL3*
LI.M43.1	myeloid, dendritic cell activation via NFkB (II)	0.87	3.40	4.9E-06	*IL1RN; IL23A; CCL5; NFKBIA; BIRC3; PRKCD; ICAM1; RELB; NFKB2; BCL3*
LI.M68	RIG-1 like receptor signaling	0.81	3.27	1.0E-04	*DDX58; TRIM25; NFKBIA; IFIH1; TNF; ISG15; CXCL10*
LI.M78	myeloid cell cytokines,	0.86	2.92	3.2E-04	*IL1RN; IL23A; IL6; LAMC2; MMP9; EDN1; SFN; LAMB3*

	metallopeptidases and laminins				
LI.M17.2	Hox cluster III	0.91	2.44	2.4E-01	*MEIS1; MEIS2*
pGF					
LI.M31	cell cycle and growth arrest	0.91	4.55	4.8E-09	*RARA; SIK1; GADD45B; TNFAIP3; THBS1; PPP1R15A; IL1A; IL1B; DUSP1*
LI.M89.0	putative targets of PAX3	0.90	3.95	2.8E-10	*PLK2; G0S2; CXCL2; SIK1; EGR2; EGR3; EGR1; HBEGF; NR4A2; MAFF; CDKN1A; GEM; DUSP2*
LI.M35.0	signaling in T cells (I)	0.88	4.08	4.0E-06	*FOS; FOSB; EGR1; JUN; TNF; ATF3; TNFRSF9*
LI.M89.1	putative targets of PAX3	0.87	3.77	5.2E-06	*EGR2; EGR3; INHBA; NR4A2; DUSP1; ATF3; DUSP2*
LI.M4.14	Rho GTPase cycle	0.86	2.39	1.0E-02	*ECT2; RACGAP1; CEP55; DEPDC1; ARHGAP11A; DEPDC1B; PRC1; ARHGAP19*
LI.M241	TBA	0.86	2.53	1.8E-01	*PAN2; CCNL2*
LI.M86.0	chemokines and inflammatory molecules in myeloid cells	0.86	4.43	3.4E-15	*CXCL1; TRAF1; CXCL2; NFKBIA; BCL2A1; G0S2; TNFAIP3; TNFAIP6; TNF; HS3ST3B1; NFKB1; CCL20; IL1B; DUSP1; PDE4B*
LI.M4.7	mitotic cell cycle	0.85	2.19	6.2E-04	*CCNB2; CCNB1; CCNA2; SHCBP1; PLK1; CDC25C; CDC25A; EXO1; CDT1; PKMYT1; E2F8; TUBG1; CDK1; CEP76; FOXM1; NEK2; PLK4; CKAP5*
LI.M4.2	PLK1 signaling events	0.85	2.37	2.0E-07	*BUB1B; PLK1; CEP55; KIF14; PRC1; MYBL2; NDC80; TPX2; HJURP; ECT2; RAD51AP1; ESPL1; WEE1; ASF1B; BUB1; CDC25C; CENPE; NUSAP1; TRIP13; GINS2; KIF23; CDC20; TUBG1; NCAPG; CENPU; CKAP2L; SPC25; KIF20A*
LI.M160	leukocyte differentiation	0.84	2.75	1.8E-05	*KLF13; RARA; LIF; NAA15; BTG2; EGR2; ID1; KLF6; NR4A2; IL1A; NFKB2; BCL3*
LI.M115	cytokines - recepters cluster	0.84	3.42	1.4E-04	*IL7R; LIF; IL23A; CSF2; CSF3; IL6*
DC.M3.3	Cell Cycle	0.84	2.27	3.3E-09	*TYMS; CDC20; BUB1; CCNB2; CDK1; UHRF1; TOP2A; CDCA5; GINS2; DTL; CCNA2; PRC1; ELL2; TPX2;*

					UBE2C; MELK; CKS2; CDT1; SPAG5; NCAPG; STIL; TTK; CHEK1; RRM2; CEP55; TK1; MCM4; APOBEC3B; NUSAP1; HMMR; KIF11; PTTG1; PLK4; ANLN; TRIP13; DLGAP5
LI.M29	proinflammatory cytokines and chemokines	0.83	3.50	9.1E-05	*PTX3; IL6; TNF; IL1A; C3; CCL20; IL1B*
LI.M73	enriched in monocytes (III)	0.83	2.74	5.9E-04	*CEBPB; DUSP6; FOS; IER3; RIN2; BCL6; PLXDC2*
LI.M4.9	mitotic cell cycle in stimulated CD4 T cells	0.81	2.18	2.4E-03	*SPDL1; CENPF; CENPE; MASTL; PLK4; KIF14; EZH2; RAD51; NCAPH; TRIP13; RRM1; CDC6*
LI.M85	platelet activation and degranulation	0.81	2.12	1.2E-02	*F3; TIMP1; FN1; THBS2; THBS1; SPARC; COL1A1; CLU; VWF*
LI.M20	AP-1 transcription factor network	0.80	3.61	1.1E-07	*FOS; FOSB; EGR1; JUN; PLAU; IL6; FOSL1; FOSL2; ATF3*

Significantly enriched genesets are shown with adj. P.Val < 0.01 and AUC ≥ 0.8. P-values from enrichment tests were corrected for multiple testing with the Benjamini-Hochberg method.

Supplemental Table 2. Results of the tmod enrichment analysis for database microRNA targets from the Molecular Signatures DB (MSigDB) for contrast EG stimulated pGEC (upper panel) and pGF (lower panel) vs controls.

miR ID	**miR standard name**	**AUC**	**cES**	**adj.P.Val**	**Significant predicted gene targets**
M32768	MIR3677-3P	0,92	3,40	0,008	*HAS3; HAUS6; SF3B6*
M32780	MIR196B-3P	0,91	2,39	0,054	*LRRC1; MCTP2; PPFIBP1*
M32749	MIR518A-3P, MIR518B, MIR518C-3P, MIR518D-3P, MIR518F-3P, MIR526A-3P	0,86	2,70	0,001	*ATXN7L3; EGR1; FBXO3; NDUFA4; TEAD3; ZNF281 ; ZNF282*
M32656	MIR8088	0,83	1,76	0,153	*CXXC5; DMTF1; DOCK7*
M32671	MIR4787-5P	0,82	2,19	0,025	*ARID2; CEMIP2; INF2; PDLIM5; ZNF385A*
M32723	MIR10392-3P	0,82	2,13	0,060	*CISH; GRAMD1A; PTPN14*
miR ID	**miR standard name**	**AUC**	**cES**	**adj.P.Val**	**Significant predicted gene targets**
M32683	MIR136-3P	0,87	2,52	0,013	*SHROOM2; SNTB2; TAB2; TOR1AIP2; VASN*
M32724	MIR8053	0,80	1,67	0,148	*CAPRIN1, DEDD, RMDN1, TPST2, UBQLN1*