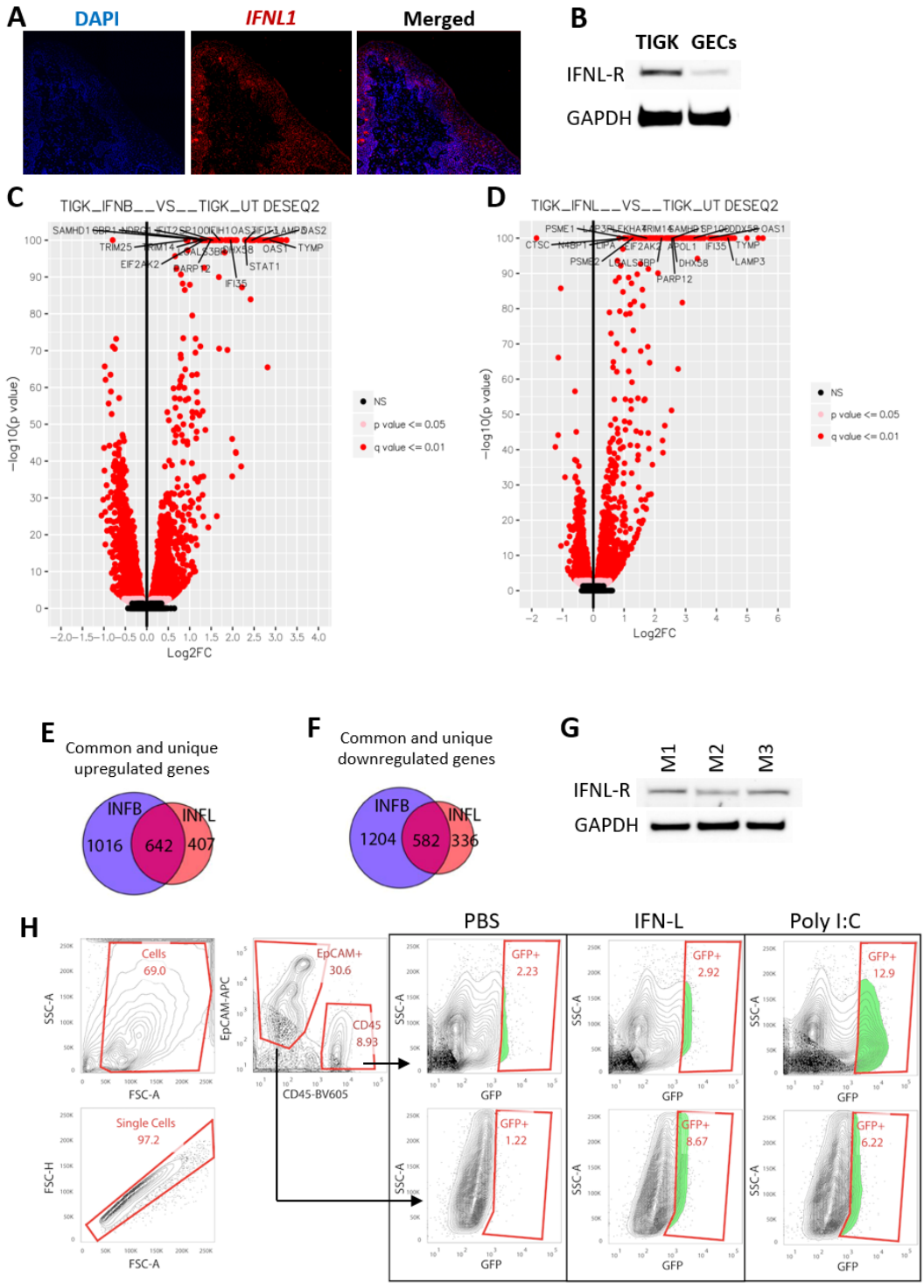
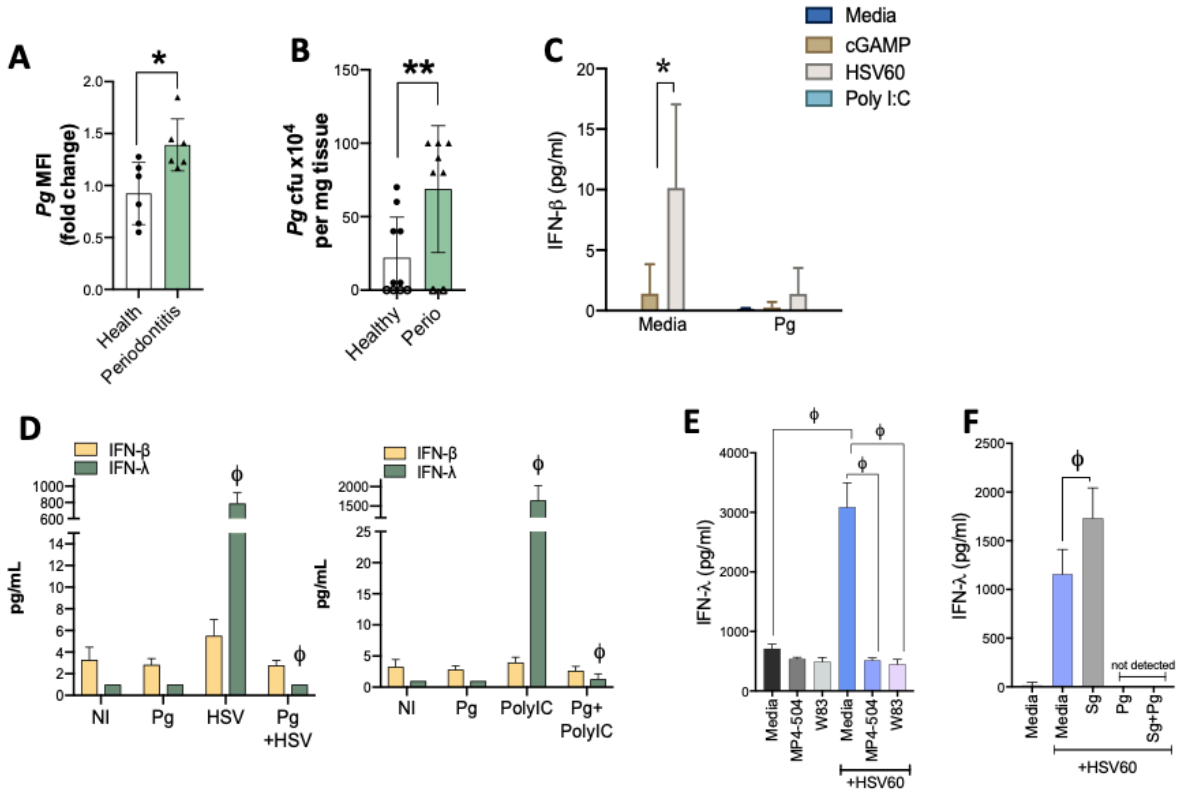
Supplementary Figures: Rodriguez-Hernandez et al

Supplemental Figure 1



Supplemental Figure 1: A) *IFNL1* transcript expression was determined in fixed human gingival tissues of healthy donors (n=3) by RNAscope. (B) IFNL-R expression in TIGKs and human primary GECs was determined by western blots. Volcano plot of differentially expressed transcripts between GECs incubated with (C) 20 ng/ml IFN-λ or (D) 0.2 ng/ml IFN-β compared to untreated cells. X-axis shows log-fold change between the two conditions with positive values showing upregulation and negative values showing downregulated genes. Y-axis denotes P values for corresponding genes. Significantly different genes are shown highlighted in red ($P<0.05$ as determined using the DESeq package in R). IFN-λ treatment resulted in differential expression of 1967 transcripts compared to 3444 after IFN-β treatment. Venn Diagrams of unique and differentially upregulated transcripts (E) and downregulated transcripts (F) between the two treatments are shown. (G) To confirm murine gingival tissues, express IFNL-R, gingival tissues were isolated from 3 $Mx1^{gfp}$ mice, and IFNL-R expression determined by immunoblots. (H) Gating strategy for determining GFP expression in gingival cells.

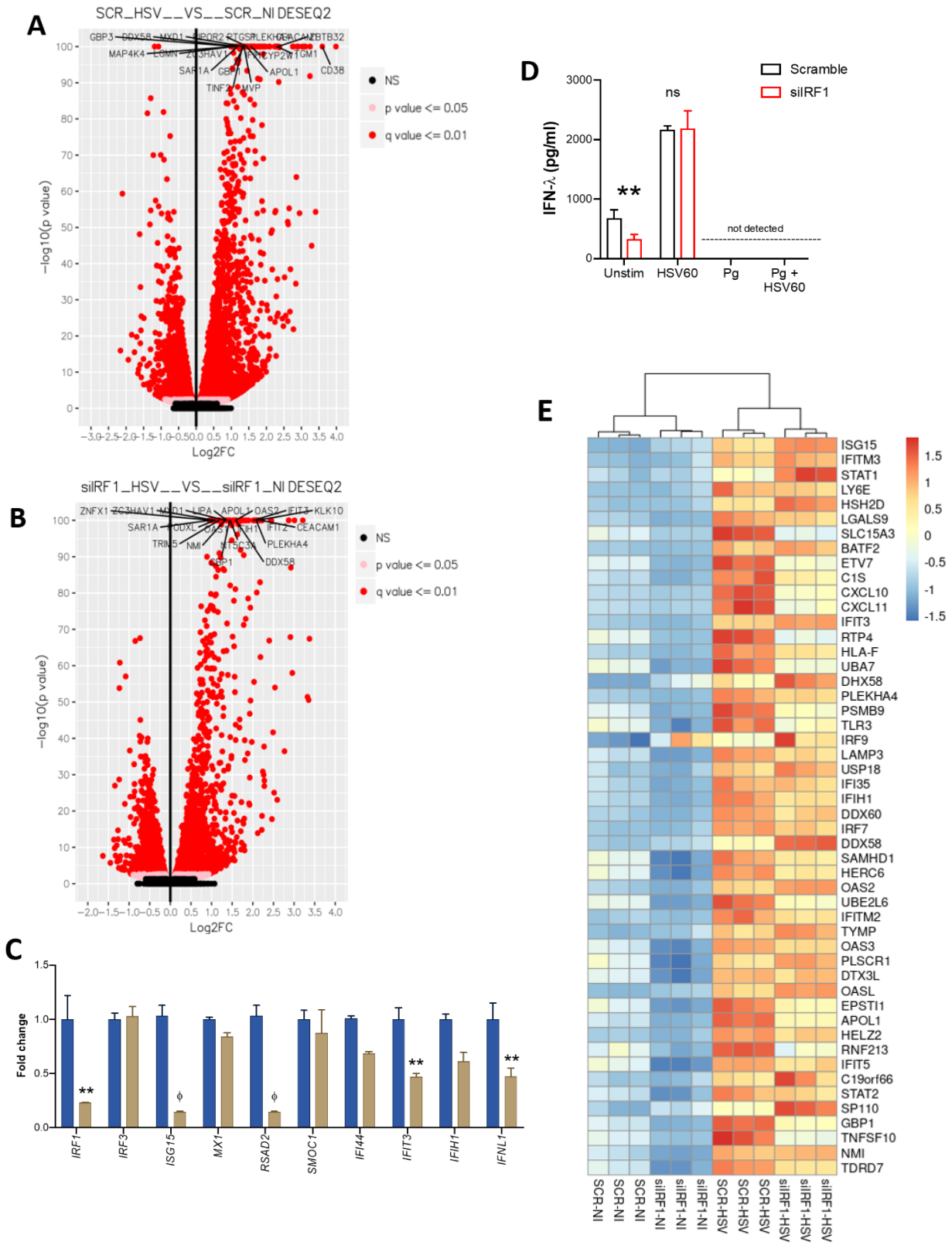
Supplemental Figure 2



Supplemental Figure 2: (A) Mean fluorescence intensity (fold change) for *P. gingivalis (Pg)* staining was estimated in gingival tissue sections from healthy (n=6) and periodontitis (n=6) samples. (B) *Pg* cfu per mg of tissue were determined by ELISA in gingival tissue homogenates of healthy (n=10) and periodontitis patients (n=8). Open symbols represent samples with values below detection limit). Statistical differences were calculated using students t-test (*P<0.05). (C) TIGKs were either left untreated or infected with *Pg* as described above with subsequent stimulation with 50 μg/ml Poly I:C (TLR3 agonist), 5 μg/ml ORN06 (TLR7 agonist), 5 μg/ml HSV60 or 25 μg/ml 2'3'-cGAMP (STING agonist) for 18 h. IFN-β levels in cell free supernatants are shown as mean ± SD. (D) OKF6 cells were infected with *Pg* for 5 h at MOI 100. Cells were washed once with PBS to remove non-internalized bacteria and immediately stimulated with 10 μg/ml HSV60 for 18h. IFN-β (white bars) and IFN-λ (green bars) levels (mean ± SD) in cell free supernatants were measured by ELISA. For C, D, statistical differences were determined by two-way ANOVA

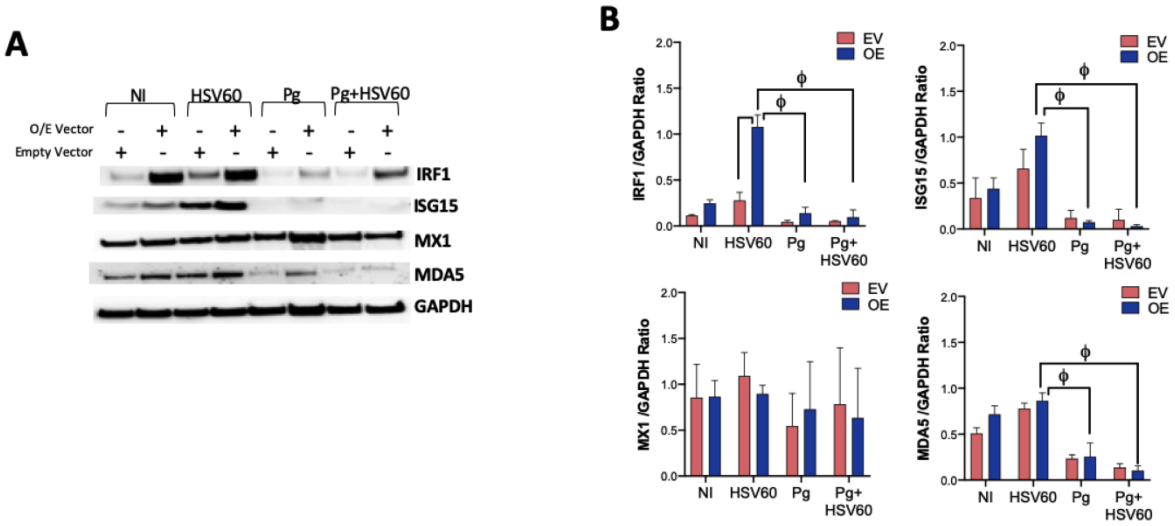
(**P<0.01; ϕP<0.001) with Holm-Sidak multiple comparison test. TIGKs were infected with *P. gingivalis* W83 or the clinical isolate MP4-504 for 5h at MOI 100 (E) or with a combination of (F) *Pg* 33277 (MOI 100) and *Sg* (MOI 10) with subsequent stimulation with 5 μg/ml HSV60 for 18 h. IFN-λ levels in cell free supernatants are shown as mean ± SD. Statistical differences were determined by one-way ANOVA with Tukey's multiple comparison test (ϕP<0.001)

Supplemental Figure 3



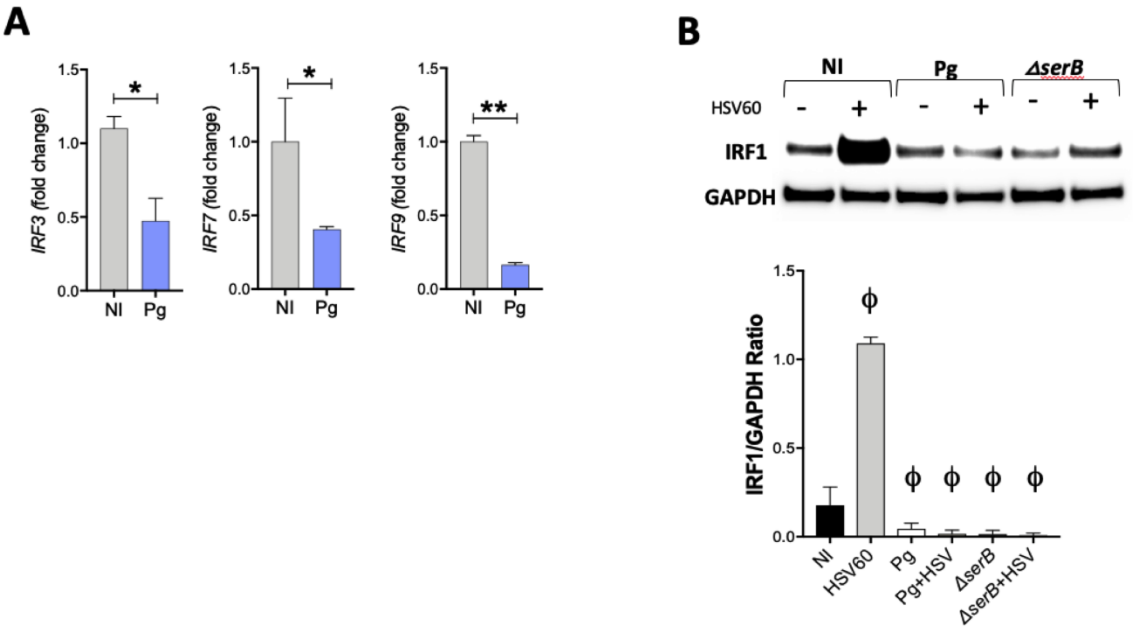
Supplemental Figure 3: TIGKs were transfected with siIRF1 or scrambled control siRNA for 24 h. Cells were either left non-infected (NI) or stimulated with 5 μg/ml HSV60 for 18h. Volcano plots of differentially expressed transcripts between (A) NI-siIRF1 Vs NI-Scrambled control and (B) HSV-siIRF1 Vs HSV-scrambled are shown. X-axis shows log-fold change between the two conditions with positive values showing upregulation and negative values showing downregulated genes. Y-axis denotes P values for corresponding genes. Significantly different genes are shown highlighted in red ($P<0.05$ as determined using the DESeq package in R). (C) Changes in transcript abundance of select genes from RNA-seq datasets in A, B were confirmed by qRT-PCR, normalized to GAPDH ($2^{-\Delta\Delta CT}$). Data are shown as mean ± SD and statistical differences were determined by two-way ANOVA with Holm-Sidak multiple comparison test (**$P<0.01$; ϕ $P<0.001$). (D) TIGKs were transfected with siIRF1 or scrambled control siRNA for 24 h and then stimulated with 5 μg/ml HSV60 with or without *P. gingivalis* infection for 18 h. IFN-λ levels in cell free supernatants are shown as mean ± SD. Statistical differences were determined by two-way ANOVA with Holm-Sidak multiple comparison test (**$P<0.01$). (E) Hierarchical clustering heatmap (based on log (RPKM) values) of the top 50 IFN stimulated genes (ISGs) under all conditions (siIRF1 Vs scrambled control) are shown. Color intensity denotes level of gene expression.

Supplemental Figure 4



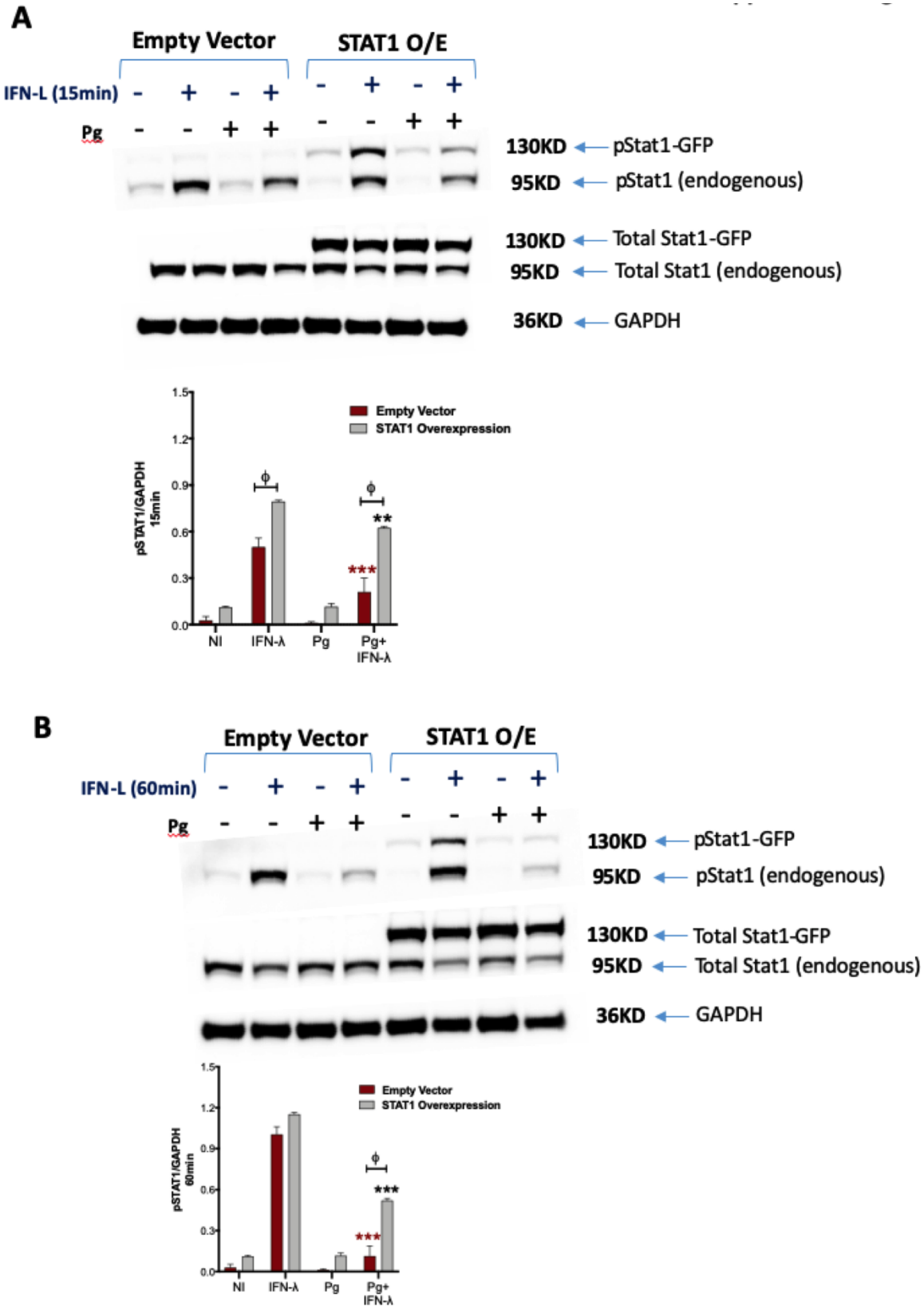
Supplemental Figure 4: (A) TIGKs were transfected with pCMV-IRF1 overexpression vector (OE) or control empty pCMV vector (EV) and immunoblotted for IRF1, ISG15, MX1, MDA5 and GAPDH after treatment with HSV60 +/- *P. gingivalis*. (B) Band intensities of immunoblots were determined, and ratios of IRF1, ISG15, MX1 and MDA5 to GAPDH from 3 different blots are shown (mean ± SD). Statistical differences were determined by two-way ANOVA with Holm-Sidak multiple comparison test (ϕ $P<0.001$).

Supplemental Figure 5



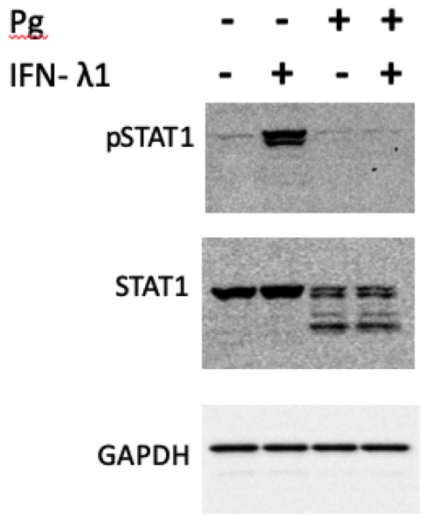
Supplemental Figure 5: (A) Transcript levels of *IRF3, IRF7 and IRF9* were determined in unstimulated or *Pg* challenged TIGKs by qRT-PCR, normalized to GADPH ($2^{-\Delta\Delta CT}$) and are shown as mean ± SD. Statistical differences were determined by student's t-test (ϕ P<0.001). (B) TIGKs were infected with *P. gingivalis* WT (Pg) or SerB deficient isogenic mutant (Δ*serB*) for 5 h, followed by 5 μg/ml HSV DNA for additional 18 h. IRF1 and GAPDH levels were detected by immunoblotting, band intensities were determined and ratios of IRF1 to GAPDH from 3 different blots are shown (mean ± SD) in the lower panel. Statistical differences were determined by one-way ANOVA and Tukey's multiple comparison test (ϕ P<0.001).

Supplemental Figure 6

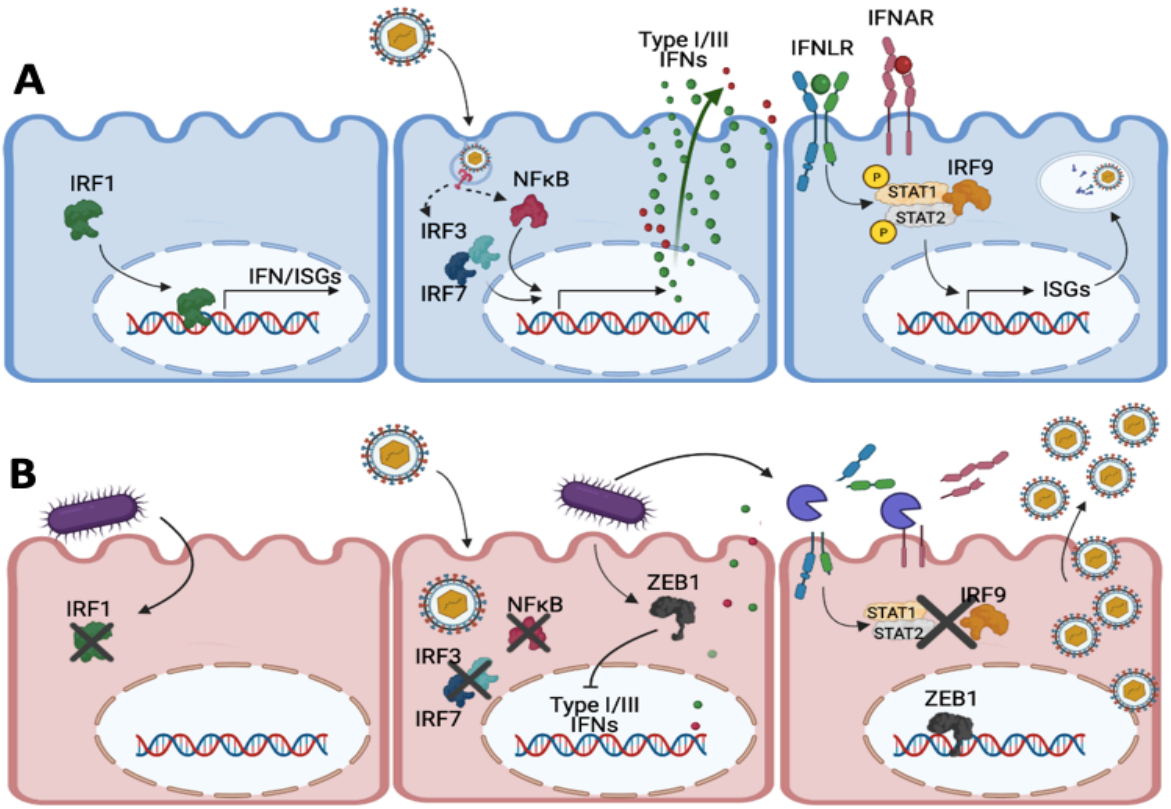


Supplemental Figure 6: GECs were transfected with STAT1-GFP overexpression plasmid or vector control 24h prior to infection with *P. gingivalis* WT (*Pg*). After 1 h of infection, cells were stimulated with 20 ng/ml IFN-λ for 15 min (A) and 60 min (B). (A) phospho-STAT1 (pSTAT1) and total STAT1 expression was determined by western blotting. GAPDH was used as the loading control. (B) Band intensities were determined and ratios of pSTAT1 to GAPDH from 3 different blots are shown (mean ± SD). Statistical differences were determined at each timepoint by two-way ANOVA (**P<0.05; ***P<0.01; ϕ P<0.001) ANOVA with Holm-Sidak multiple comparison test. Red and black symbols depict statistical comparisons to the empty vector control and STAT1 overexpression (NI) groups respectively.

Supplemental Figure 7



Supplemental Figure 7: A) GECs were infected with *P. gingivalis* (Pg) MOI 100 for 5 h and stimulated with IFN-λ (20 ng/ml) for 5h. Phospho-STAT1 (pSTAT1, total STAT1 and GAPDH expression was determined by western blotting.



Supplemental Figure 8: (A) IRF1 signaling maintains a basal antiviral state in gingival epithelial cells (GECs) via constitutive expression of multiple ISGs. Viral infection in GECs activates several pattern recognition receptors resulting in the preferential induction of Type III IFN that signals via IFN-L receptors to augment ISG expression and antiviral defenses. (B) *P. gingivalis* infection compromises both, constitutive and inducible IFN responses in GECs by degrading or inactivating IFN-inducing transcription factors and cleaving IFN receptors making cells refractory to exogenous IFNs, and inducing a state of broad IFN paralysis. Thus, *P. gingivalis* infection severely compromises anti-viral immunity, providing an early replicative niche for oral viruses.

Supplemental Table 1: Antiviral restriction factors downregulated by *P. gingivalis*

ENSEMBL GENE	GENE SYMBOL	Log2 Fold Change	q_value
ENSG00000183486	***MX2***	-8.41016	0.001087
ENSG00000137959	***IFI44L***	-6.85783	0.001087
ENSG00000134321	***RSAD2***	-6.63744	0.001087
ENSG00000187608	***ISG15***	-5.87784	0.001087
ENSG00000185885	***IFITM1***	-4.99342	0.001087
ENSG00000185745	***IFIT1***	-4.97384	0.001087
ENSG00000138135	***CH25H***	-4.77437	0.001087
ENSG00000157601	***MX1***	-4.63737	0.001087
ENSG00000126709	***IFI6***	-4.57048	0.001087
ENSG00000137965	***IFI44***	-4.56653	0.001087
ENSG00000119917	***IFIT3***	-4.06795	0.001087
ENSG00000165949	***IFI27***	-4.06526	0.001087
ENSG00000185201	***IFITM2***	-3.94771	0.024228
ENSG00000068079	***IFI35***	-3.86895	0.001087
ENSG00000111335	***OAS2***	-3.74836	0.001087
ENSG00000135114	***OASL***	-3.66909	0.001087
ENSG00000119922	***IFIT2***	-3.64383	0.002842