

Microbial composition of tumorous and adjacent gastric tissue is associated with prognosis of gastric cancer

Konrad Lehr¹, Darja Nikitina², Ramiro Vilchez-Vargas¹, Ruta Steponaitiene ², Cosima Thon¹, Jurgita Skieceviciene², Denny Schanze³, Martin Zenker³, Peter Malfertheiner^{1,4}, Juozas Kupcinskas^{2,5}, Alexander Link¹

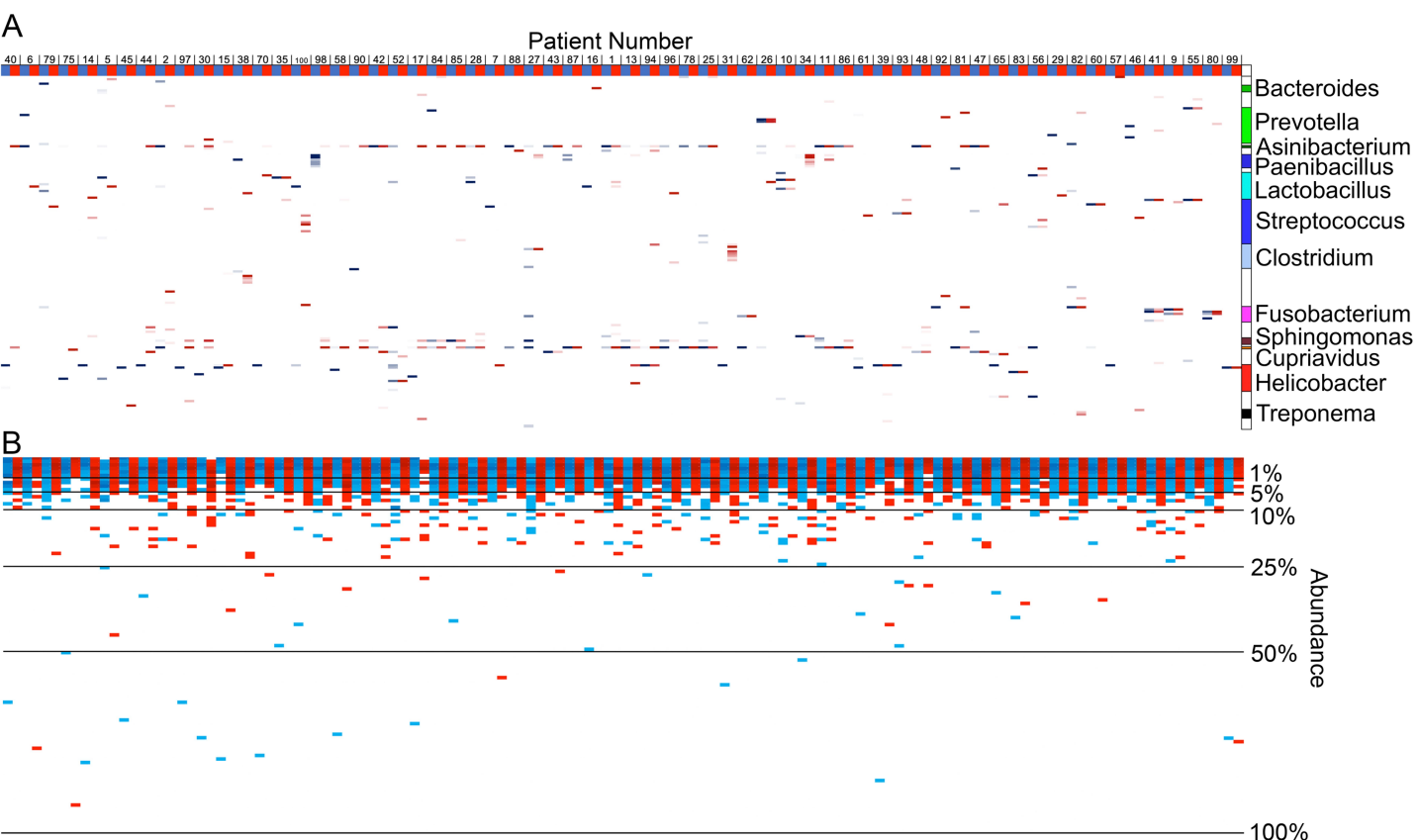
1 Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Magdeburg, Germany

2 Institute for Digestive Research, Lithuanian University of Health Sciences Kaunas, Lithuania

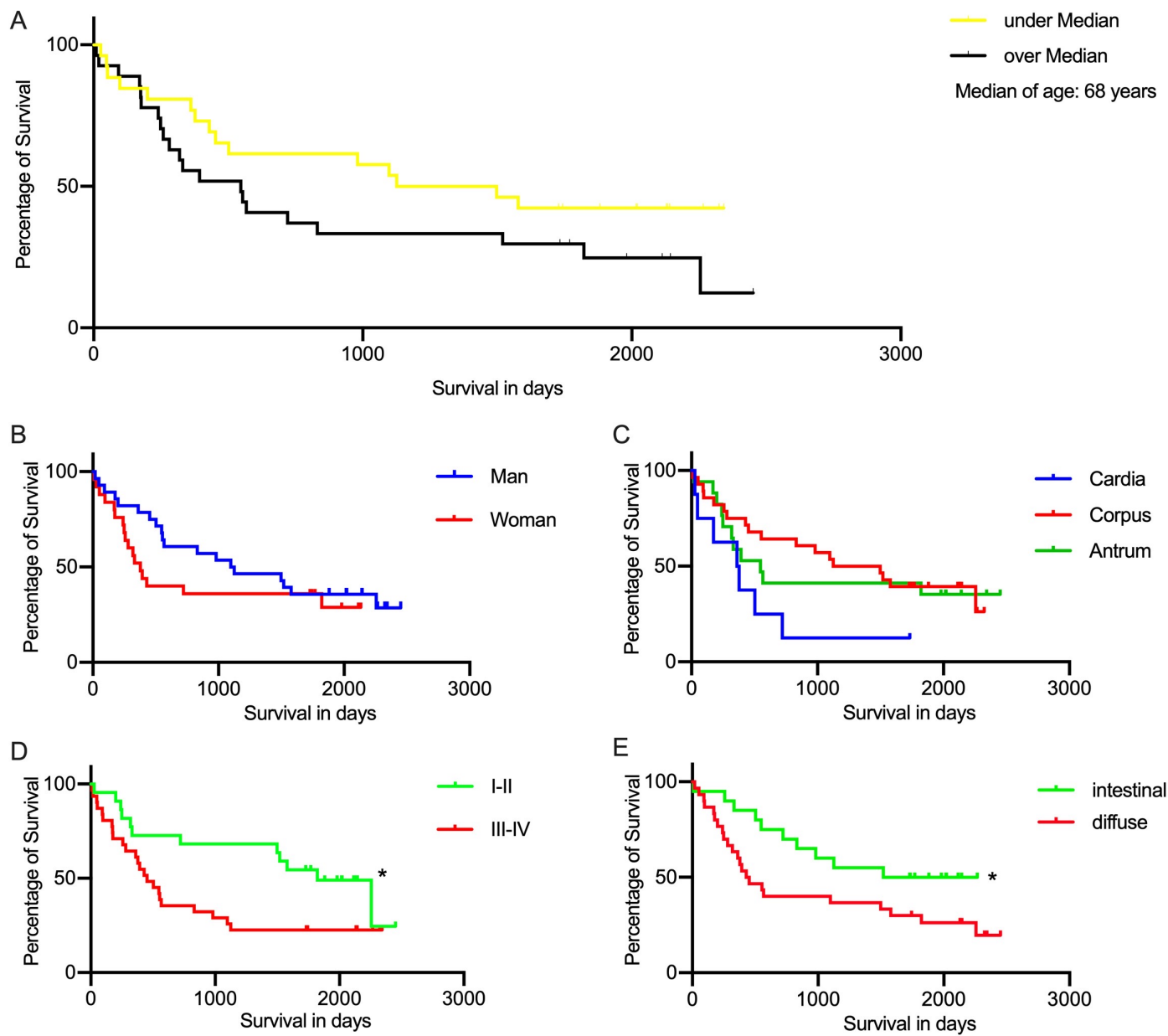
3 Institute of Human Genetics, Otto-von-Guericke University Magdeburg, Germany

4 Department of Internal Medicine 2, University Hospital, LMU Munich, Munich, Germany

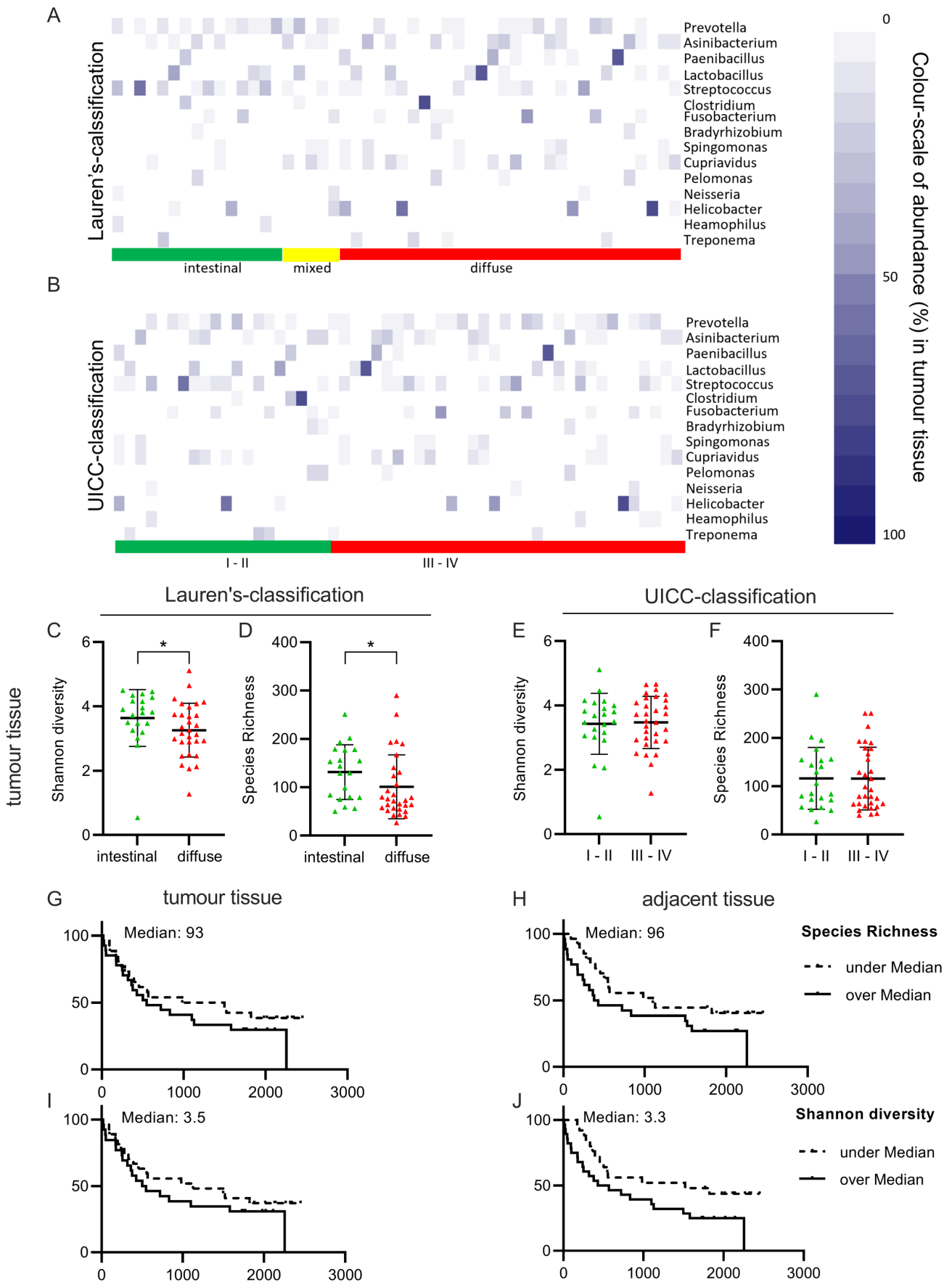
5 Department of Gastroenterology, Lithuanian University of Health Sciences Kaunas, Lithuania



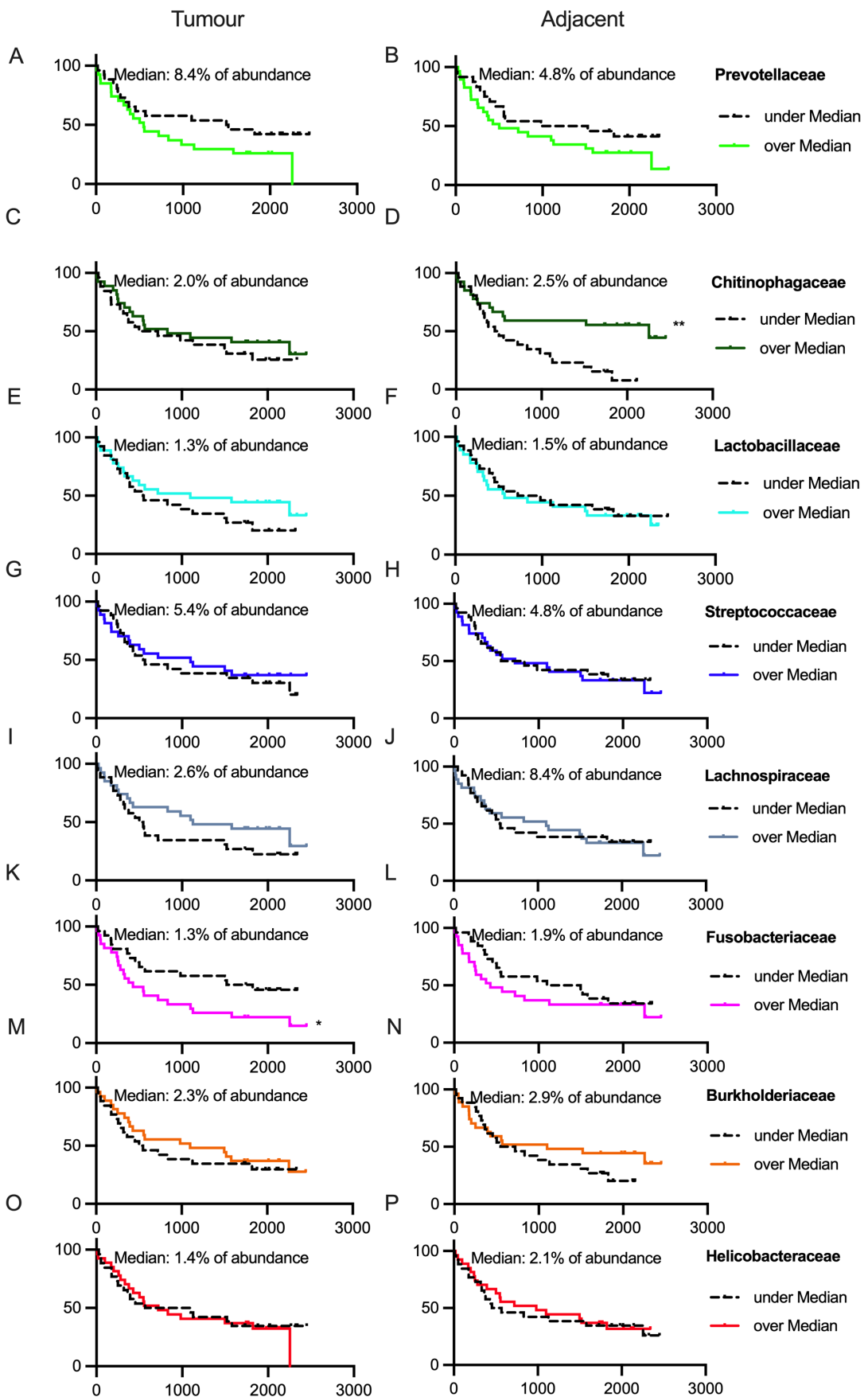
Supplementary Figure S1: Heatmap containing the phylotypes detected with $>5\%$ of abundance in tumour tissue (red) and adjacent tissue (blue). Samples are ordered in increasing % of Bray-Curtis similarity between paired samples of the same patient, and only the genera of the phylotypes found in more than 10 samples are displayed. Light red and light blue denote low abundance, while dark red and dark blue denote high abundances (A). Heatmap with the phylotype frequency per sample at a certain % of abundance (B). Samples are displayed in the same order as in A.



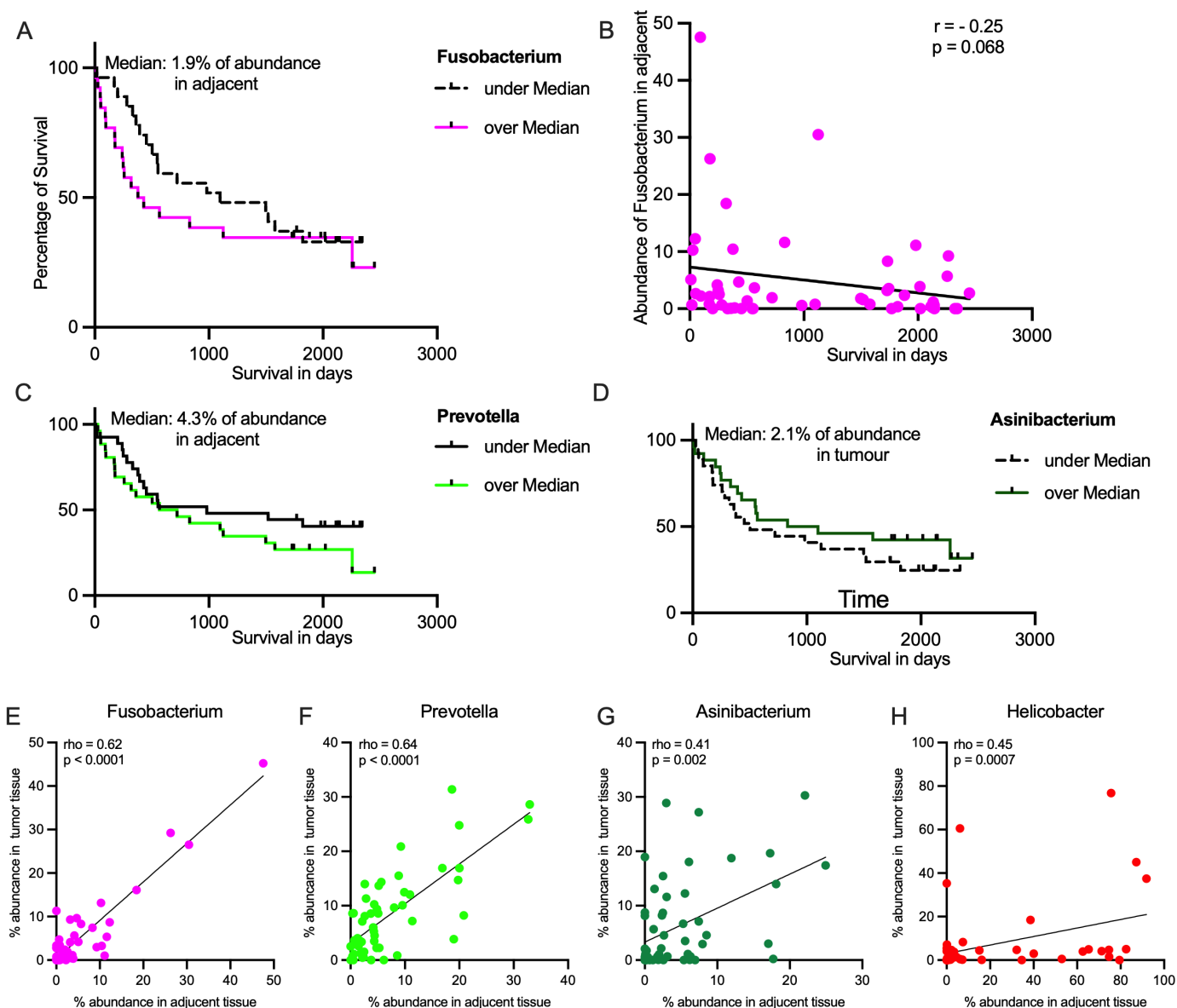
Supplementary Figure S2: Kaplan-Meier-Curves for the age (A), the gender (B), the location of tumour (C), the UICC-classification (D), and the Lauren classification (E). Significant differences in OS are indicated by * if $q < 0.05$.



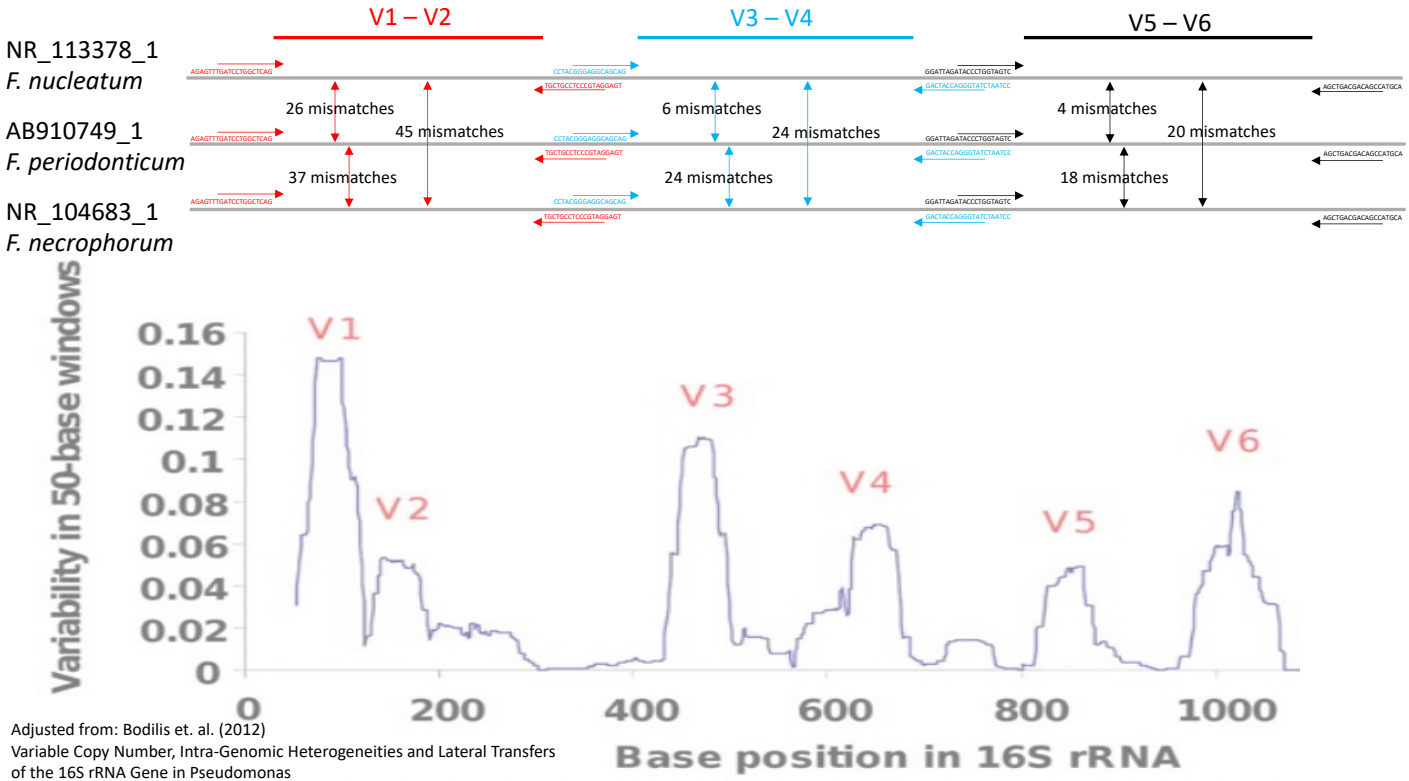
Supplementary Figure S3: Abundances of the genera grouped according to Lauren's classification (A) and UICC classification (B) of 53 GC patients. Only the genera with more abundance than 10% were considered. Shannon diversity index (C, E) and species richness (D, F) in samples with different tumour stages, according to Lauren's classification and UICC classification. Significant differences between groups are indicated by * if $q < 0.05$. Kaplan-Meier-Curves for Shannon Index and species richness in tumour and adjacent tissues samples (G-J).



Supplementary Figure S4: Kaplan-Meier-Curves for Prevotellaceae (A-B), Chitinophagaceae (C-D), Lactobacillaceae (E-F), Streptococcaceae (G-H), Lachnospiraceae (I-J), Fusobacteriaceae (K-L), Burkholderiaceae (M-N), and Helicobacteraceae (O-P) in tumour or adjacent tissues. X-axes shows the OS in days and y-axes the percentage of OS.



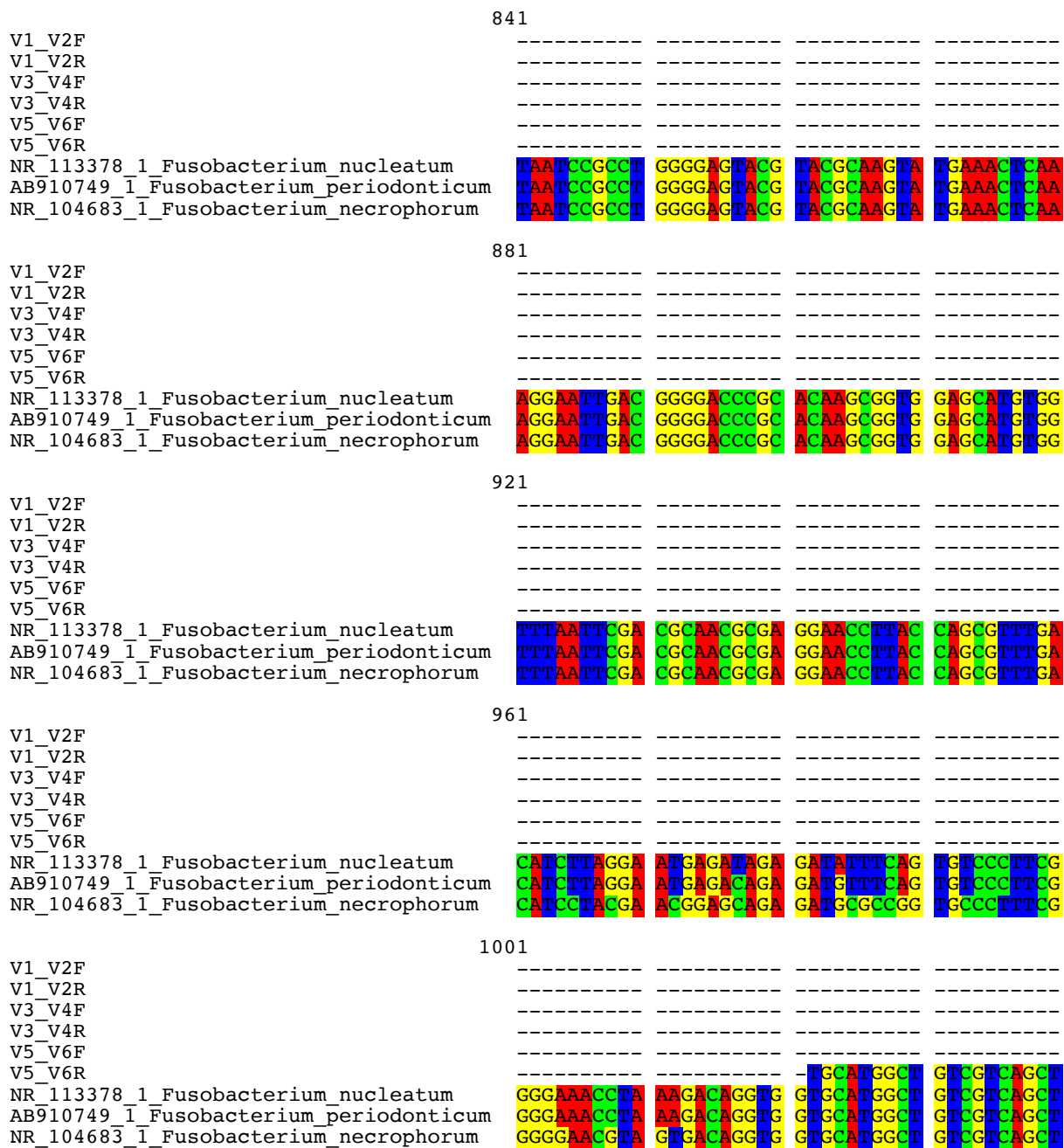
Supplementary Figure S5: Kaplan-Meier-Curves of the abundances of *Fusobacterium* in adjacent tissue (A). Correlation of OS data (in days) with the abundance of *Fusobacterium* in adjacent tissue, with linear regression and Spearman rank coefficient (B). Kaplan-Meier-Curves of the abundances of *Prevotella* in adjacent tissue (C) and the abundances of *Asinibacterium* in tumour tissue (D). X-axes show survival data in days. Significant differences in survival are indicated by * if $q < 0.05$ and ** if $q < 0.01$. Correlation of the abundance of *Fusobacterium* (E), *Prevotella* (F), *Asinibacterium* (G), *Helicobacter* (H) in tumour and adjacent tissue.



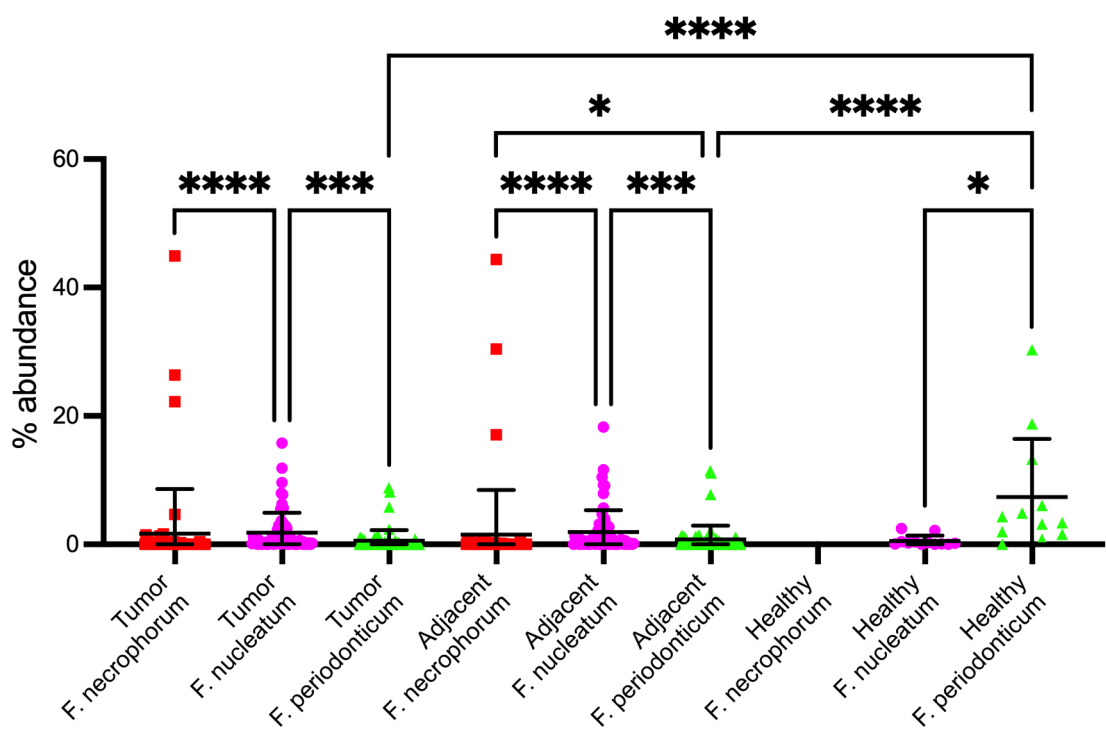
	1			
V1_V2F	AGAGTTTGAT	CCTGGCTCAG	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	AGAGTTTGAT	CCTGGCTCAG	GATGAACGCT	GACAGAATGC
AB910749_1_Fusobacterium_periodonticum	AGAGTTTGAT	CCTGGCTCAG	GATGAACGCT	GACAGAATGC
NR_104683_1_Fusobacterium_necrophorum	AGAGTTTGAT	CCTGGCTCAG	GATGAACGCT	GACAGAATGC
	41			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	TTAACACATG	CAAGTCAACT	TGAATTTGGG	TTTTTAACTT
AB910749_1_Fusobacterium_periodonticum	TTAACACATG	CAAGTCTACT	TGAACT	-----
NR_104683_1_Fusobacterium_necrophorum	TTAACACATG	CAAGTCGACT	CGAGTCT	-----
	81			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	AGATTTTGGGT	GGCGGACGGG	TGAGTAACGC	GTAAGAAGCT
AB910749_1_Fusobacterium_periodonticum	CGGTTTGGGT	GGCGGACGGG	TGAGTAACGC	GTAAGAAGCT
NR_104683_1_Fusobacterium_necrophorum	GGACTTTGGGT	GGCGCACGGG	TGAGTAACGC	GTAAGAAGCT
	121			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	TGCCCTCACAG	TTAGGGACAA	CATTTAGAAA	TGAATGCTAA
AB910749_1_Fusobacterium_periodonticum	TGCCCTCACAG	TTAGGGACAA	CATTTAGAAA	CGAATGCTAA
NR_104683_1_Fusobacterium_necrophorum	TGCCCTCTTAG	ACCGGGACAA	CATCTGAAA	CGGATGCTAA
	161			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	TACCTGATAT	TATGATTTTA	AGGCATCTTA	GAATTATGAA
AB910749_1_Fusobacterium_periodonticum	TACCTGATAT	TATGATTTTA	GGGCATCCTA	AGATTATGAA
NR_104683_1_Fusobacterium_necrophorum	TACCGGATAT	TATGGTTTTT	TCGCATGGAG	GAATCATGAA
	201			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	AGCTATAAGC	ACTGTGAGAG	AGCTTTGCGT	CCCATTAGCT
AB910749_1_Fusobacterium_periodonticum	AGCTATATGC	GCTGTGAGAG	AGCTTTGCGT	CCCATTAGCT
NR_104683_1_Fusobacterium_necrophorum	AGCTAGATGC	GCTAAGAGAG	AGCTTTGCGT	CCCATTAGCT
	241			
V1_V2F	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	AGTTGGAGAG	GTAACAGCTC	ACCAAGGCGA	TGATGGGTAG
AB910749_1_Fusobacterium_periodonticum	AGTTGGAGAG	GTAACGGCTC	ACCAAGGCGA	TGATGGGTAG
NR_104683_1_Fusobacterium_necrophorum	AGTTGGTTAG	GTAACGGCCC	ACCAAGGCAA	TGATGGGTAG

	281				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	CCGGCCTGAG	AGGGTGAACG	GCCACAAGGG	GACTGAGACA	
AB910749_1_Fusobacterium_periodonticum	CCGGCCTGAG	AGGGTGAACG	GCCACAAGGG	GACTGAGACA	
NR_104683_1_Fusobacterium_necrophorum	CCGGCCTGAG	AGGGTGAACG	GCCACAAGGG	GACTGAGACA	
	321				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----AC	TCCTACGGGA	GGCAGCA	-----	-----
V3_V4F	-----	CCTACGGGA	GGCAGCAG	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	CGGCCCTTAC	TCCTACGGGA	GGCAGCAGTG	GGGAATATTG	
AB910749_1_Fusobacterium_periodonticum	CGGCCCTTAC	TCCTACGGGA	GGCAGCAGTG	GGGAATATTG	
NR_104683_1_Fusobacterium_necrophorum	CGGCCCTTAC	TCCTACGGGA	GGCAGCAGTG	GGGAATATTG	
	361				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	GACAAATGGAC	CGAGAGTCTG	ATCCAGCAAT	TCTGTGTGCA	
AB910749_1_Fusobacterium_periodonticum	GACAAATGGAC	CAAGAGTCTG	ATCCAGCAAT	TCTGTGTGCA	
NR_104683_1_Fusobacterium_necrophorum	GACAAATGGAC	CACAAGTCTG	ATCCAGCAAT	TCTGTGTGCA	
	401				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	CGATGAAGTT	TTTCGGAATG	TAAAGTGCTT	TCAGTTGGGA	
AB910749_1_Fusobacterium_periodonticum	CGATGACGTT	TTTCGGAATG	TAAAGTGCTT	TCAGTTGGGA	
NR_104683_1_Fusobacterium_necrophorum	CGATGACGTT	TTTCGGAATG	TAAAGTGCTT	TCAGTTGGGA	
	441				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	AGAAAGAAAT	GACGGTACCA	ACAGAGAAG	TGACGGCTAA	
AB910749_1_Fusobacterium_periodonticum	AGAAAAAAAT	GACGGTACCA	ACAGAGAAG	TGACGGCTAA	
NR_104683_1_Fusobacterium_necrophorum	AGAAGTCAGT	GACGGTACCG	ACAGAGAAG	CGACGGCTAA	
	481				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	ATACGTGCCA	GCAGCCGCGG	TAATACGTAT	GTCACGAGCG	
AB910749_1_Fusobacterium_periodonticum	ATACGTGCCA	GCAGCCGCGG	TAATACGTAT	GTCACGAGCG	
NR_104683_1_Fusobacterium_necrophorum	ATACGTGCCA	GCAGCCGCGG	TAATACGTAT	GTCGCAAGCG	
	521				
V1_V2F	-----	-----	-----	-----	-----
V1_V2R	-----	-----	-----	-----	-----
V3_V4F	-----	-----	-----	-----	-----
V3_V4R	-----	-----	-----	-----	-----
V5_V6F	-----	-----	-----	-----	-----
V5_V6R	-----	-----	-----	-----	-----
NR_113378_1_Fusobacterium_nucleatum	TTATCCGGAT	TTATTGGGCG	TAAAGCGCGT	CTAGGTGGTT	
AB910749_1_Fusobacterium_periodonticum	TTATCCGGAT	TTATTGGGCG	TAAAGCGCGT	CTAGGTGGTT	
NR_104683_1_Fusobacterium_necrophorum	TTATCCGGAT	TTATTGGGCG	TAAAGCGCGT	CTAGGCGGCA	

	561				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	-----	
V5_V6F	-----	-----	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	ATGTAAGTCT	GATGTGAAAA	TGCAGGGCTC	AACCTCTGTAT	
AB910749_1_Fusobacterium_periodonticum	ATGTAAGTCT	GATGTGAAAA	TGCAGGGCTC	AACCTCTGTAT	
NR_104683_1_Fusobacterium_necrophorum	AGGAAAGTCT	GATGTGAAAA	TGCGGAGCTC	AACCTCGTAT	
	601				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	-----	
V5_V6F	-----	-----	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	TGCGTTGGAA	ACTGTATAAC	TAGAGTACTG	GAGAGGTAAG	
AB910749_1_Fusobacterium_periodonticum	TGCGTTGGAA	ACTGCATGAC	TAGAGTACTG	GAGAGGTAAG	
NR_104683_1_Fusobacterium_necrophorum	GGCGTTGGAA	ACTGCCCTAC	TAGAGTACTG	GAGAGGTAAG	
	641				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	-----	
V5_V6F	-----	-----	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	CGGAAC TACA	AGTGTAGAGG	TGAAATTCGT	AGATATTTGT	
AB910749_1_Fusobacterium_periodonticum	CGGAAC TACA	AGTGTAGAGG	TGAAATTCGT	AGATATTTGT	
NR_104683_1_Fusobacterium_necrophorum	CGGAAC TACA	AGTGTAGAGG	TGAAATTCGT	AGATATTTGT	
	681				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	-----	
V5_V6F	-----	-----	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	AGGAATGCCG	ATGGGGAAGC	CAGCCTACTG	GACAGATACT	
AB910749_1_Fusobacterium_periodonticum	AGGAATGCCG	ATGGGGAAGC	CAGCCTACTG	GACAGATACT	
NR_104683_1_Fusobacterium_necrophorum	AGGAATGCCG	ATGGGGAAGC	CAGCCTACTG	GACAGATACT	
	721				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	GGATTAG	
V5_V6F	-----	-----	-----	GGATTAG	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	GACGCTAAAG	CGCGAAAGCG	TGGGTAGCAA	ACAGGATTAG	
AB910749_1_Fusobacterium_periodonticum	GACGCTAAAG	CGCGAAAGCG	TGGGTAGCAA	ACAGGATTAG	
NR_104683_1_Fusobacterium_necrophorum	GACGCTAAAG	CGCGAAAGCG	TGGGTAGCAA	ACAGGATTAG	
	761				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	ATACCTGGT	AGTC	-----	-----	
V5_V6F	ATACCTGGT	AGTC	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	ATACCTGGT	AGTCCACGCT	GTAACGATG	ATTACTAGGT	
AB910749_1_Fusobacterium_periodonticum	ATACCTGGT	AGTCCACGCC	GTAACGATG	ATTACTAGGT	
NR_104683_1_Fusobacterium_necrophorum	ATACCTGGT	AGTCCACGCT	GTAACGATG	ATTACTAGGT	
	801				
V1_V2F	-----	-----	-----	-----	
V1_V2R	-----	-----	-----	-----	
V3_V4F	-----	-----	-----	-----	
V3_V4R	-----	-----	-----	-----	
V5_V6F	-----	-----	-----	-----	
V5_V6R	-----	-----	-----	-----	
NR_113378_1_Fusobacterium_nucleatum	GTTGGGGGTC	GAACCTCAGC	GCCCCAAGCAA	ACGCGATAAG	
AB910749_1_Fusobacterium_periodonticum	GTTGGGGGTC	GAACCTCAGC	GCCCCAAGCTA	ACGCGATAAG	
NR_104683_1_Fusobacterium_necrophorum	GTTGGGGGTC	AAACCTCAGC	GCCCCAAGCTA	ACGCGATAAG	



Supplementary Figure S6: Analysis of the species resolution in *Fusobacterium* using different regions of the 16S rRNA gene.



Supplementary Figure S7: Abundance of *Fusobacterium* species in tumour, adjacent and healthy tissue.