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Supplemental Information

Functional Specialization of Human Salivary Glands and Origins of Proteins Intrinsic to Human Saliva

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Supplemental Figures

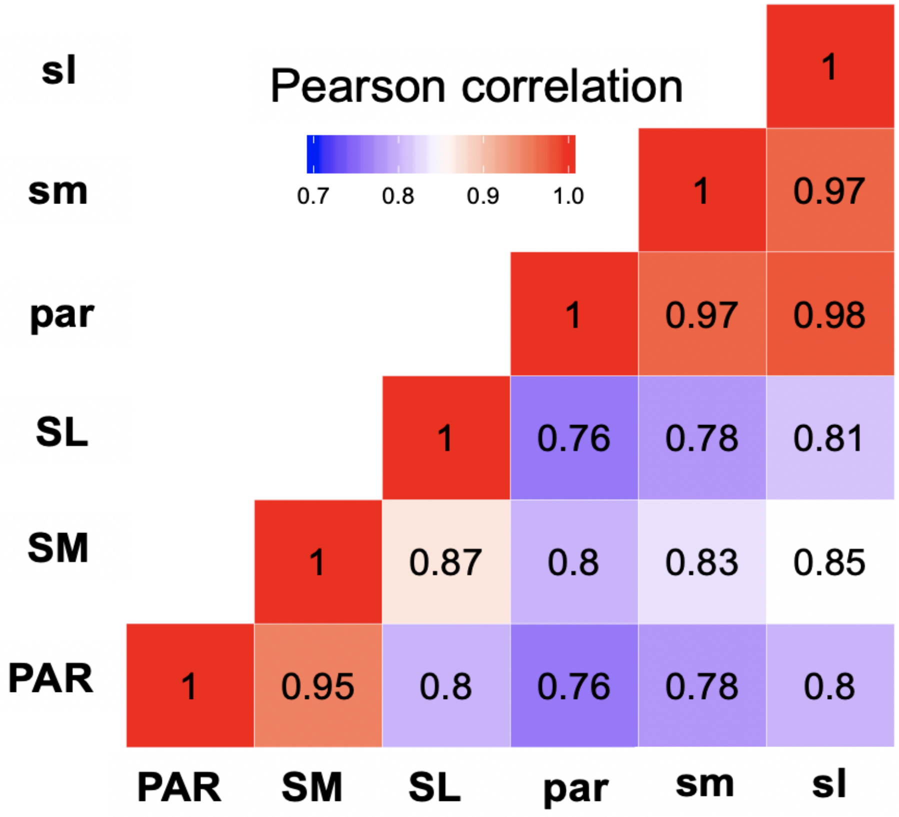

Figure S1. [Related to Figure 1] Correlation analysis of the genome-wide transcriptomes of all salivary gland samples included in this study. A heatmap was constructed by the Pearson correlation of gene expression of all salivary gland samples, with the number in each box representing the correlation coefficient among each pair compared (adult glands, upper case; fetal glands, lower case).

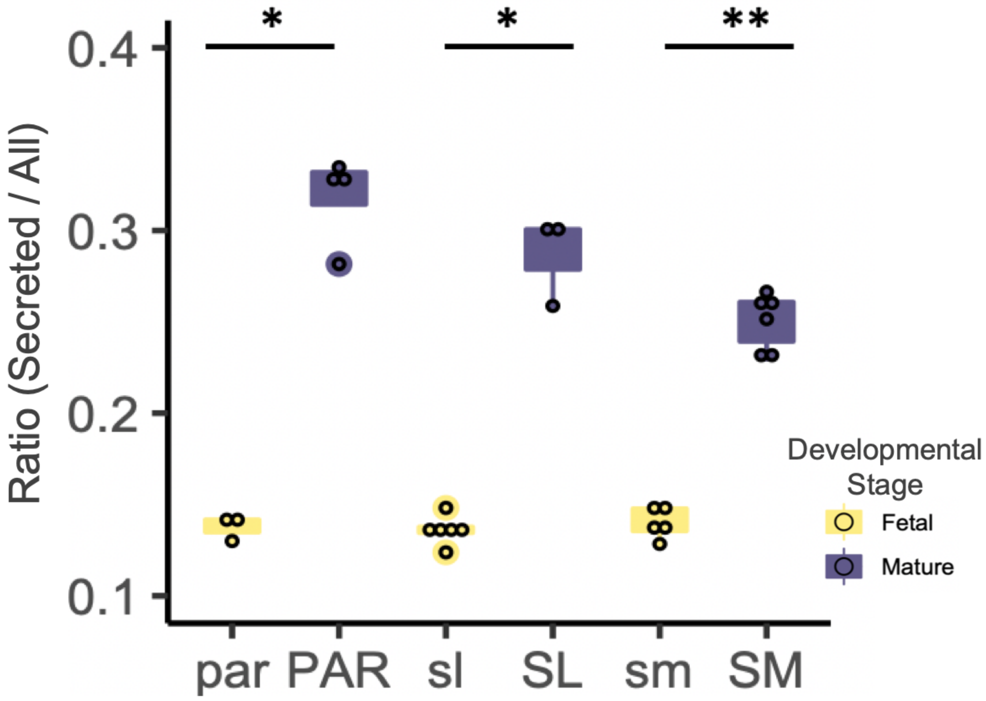

Figure S2. [Related to Figure 1] Bar plots showing the ratio of transcripts encoding for secreted proteins versus all proteins in adult and fetal salivary glands. Genes were classified based on whether they encode “secreted” proteins as per protein annotation obtained from the Human Protein Atlas (Uhlén et al., 2015). A ratio for each salivary gland was derived by dividing the sum total of all gene transcripts encoding secreted proteins by the sum total of all gene transcripts expressed. Each dot represents data from an individual salivary gland tissue sample. Mature glands show a significantly higher relative expression of genes that encode for secreted proteins than fetal glands (*$p < 0.05$, One-tailed Wilcoxon rank-sum test).

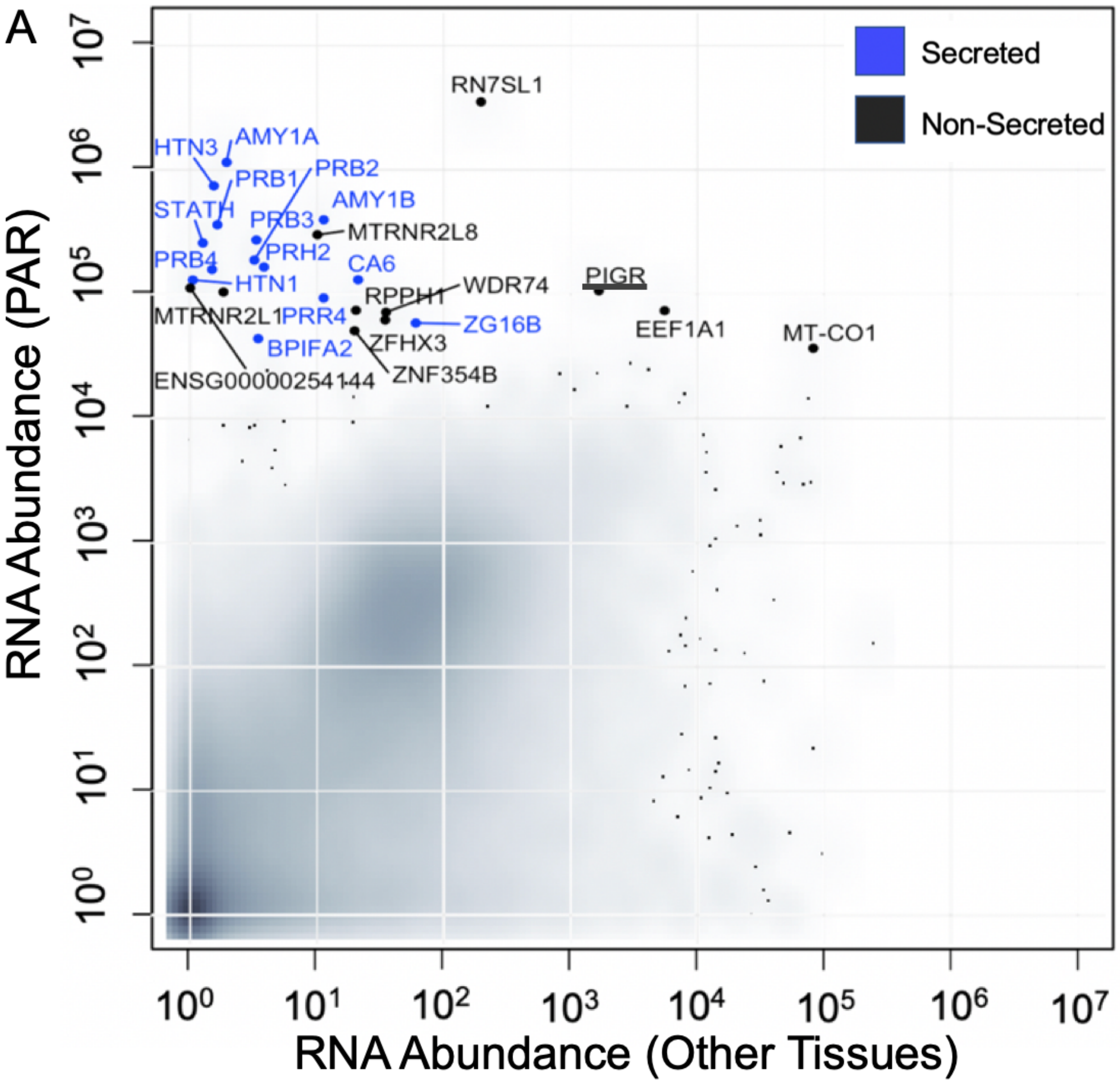

Figure S3. [Related to Figure 3] Salivary gland-specific gene expression (PAR) compared to other tissues. The y-axis shows the transcript levels of each gene ($\log_{10}$ (1+normalized read counts)) for each adult major salivary gland type. For comparative purposes, the x-axis shows the maximum gene expression levels through GTEx Portal. Genes coding for secreted proteins are highlighted in blue. Note that PIGR, IGHA1, and MUC19 (underlined) can occur as both membrane-bound and secreted proteins, although they were not classified as “secreted protein” in the Human Protein Atlas.

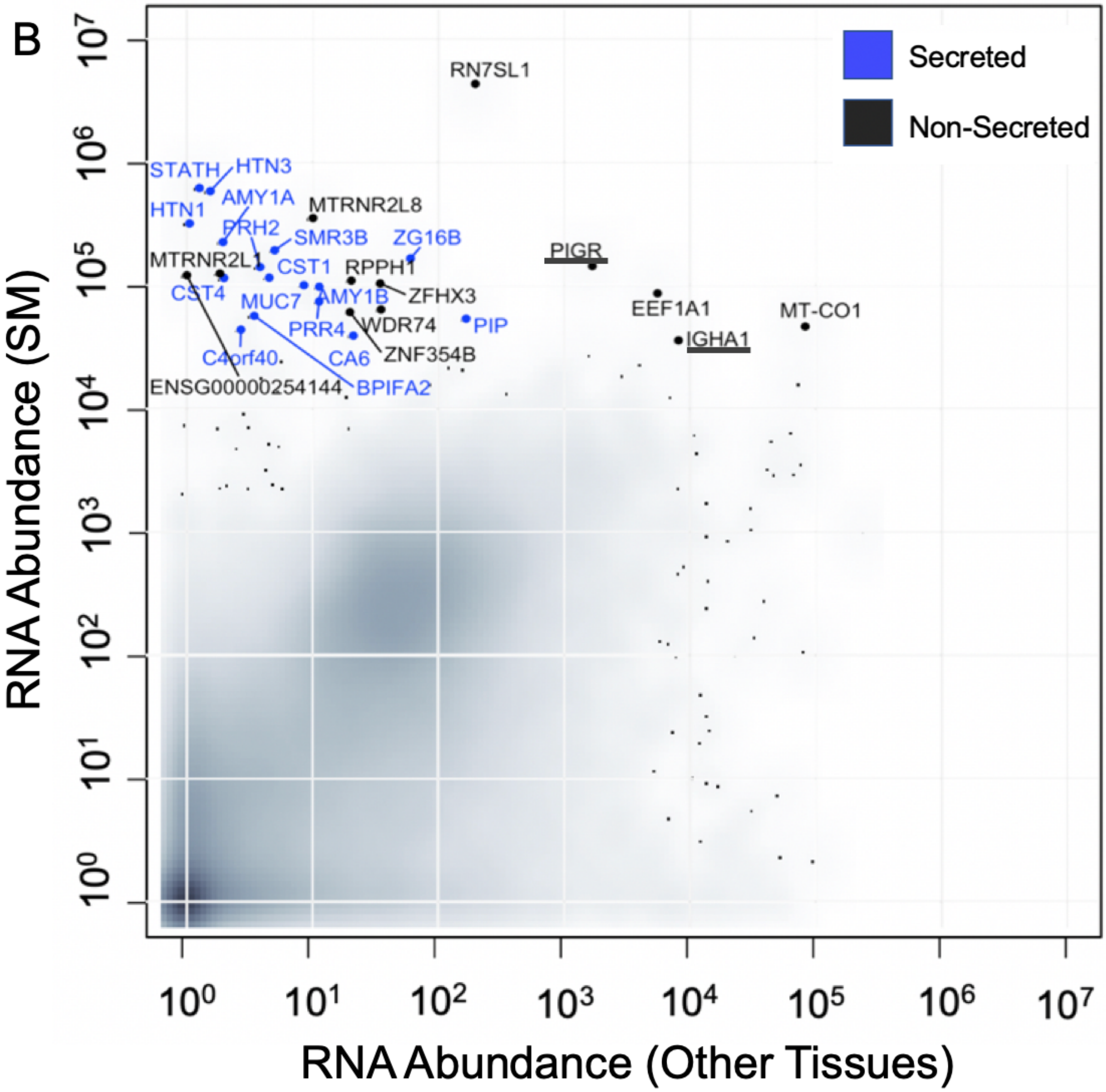

Figure S4. [Related to Figure 3] Salivary gland-specific gene expression (SM) compared to other tissues. The y-axis shows the transcript levels of each gene ($\log_{10}$ (1+normalized read counts)) for each adult major salivary gland type. For comparative purposes, the x-axis shows the maximum gene expression levels through GTEx Portal. Genes coding for secreted proteins are highlighted in blue. Note that PIGR, IGHA1, and MUC19 (underlined) can occur as both membrane-bound and secreted proteins, although they were not classified as "secreted protein" in the Human Protein Atlas.

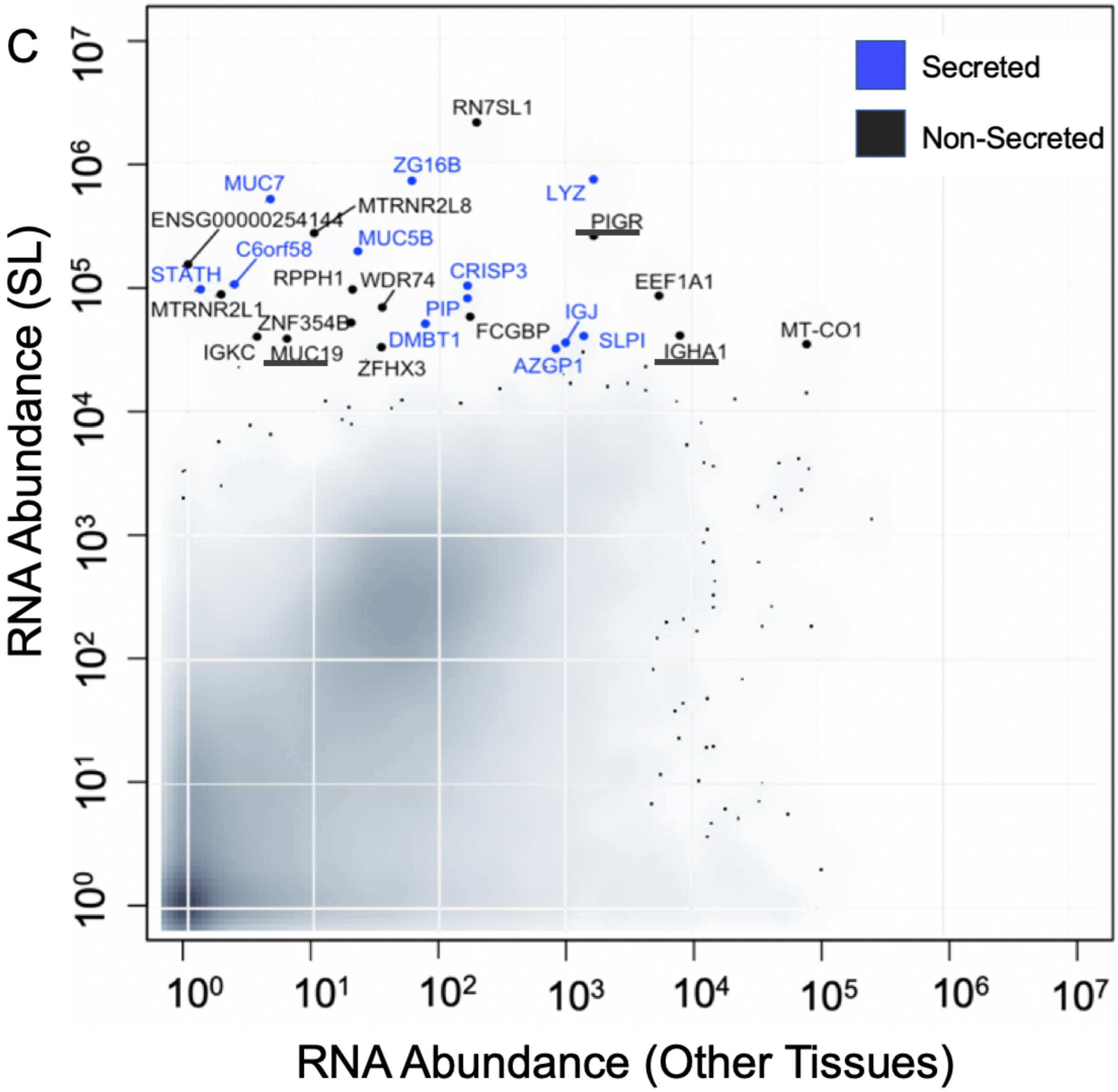

Figure S5. [Related to Figure 3] Salivary gland-specific gene expression (SL) compared to other tissues. The y-axis shows the transcript levels of each gene ($\log_{10}$ (1+normalized read counts)) for each adult major salivary gland type. For comparative purposes, the x-axis shows the maximum gene expression levels through GTEx Portal. Genes coding for secreted proteins are highlighted in blue. Note that PIGR, IGHA1, and MUC19 (underlined) can occur as both membrane-bound and secreted proteins, although they were not classified as "secreted protein" in the Human Protein Atlas.

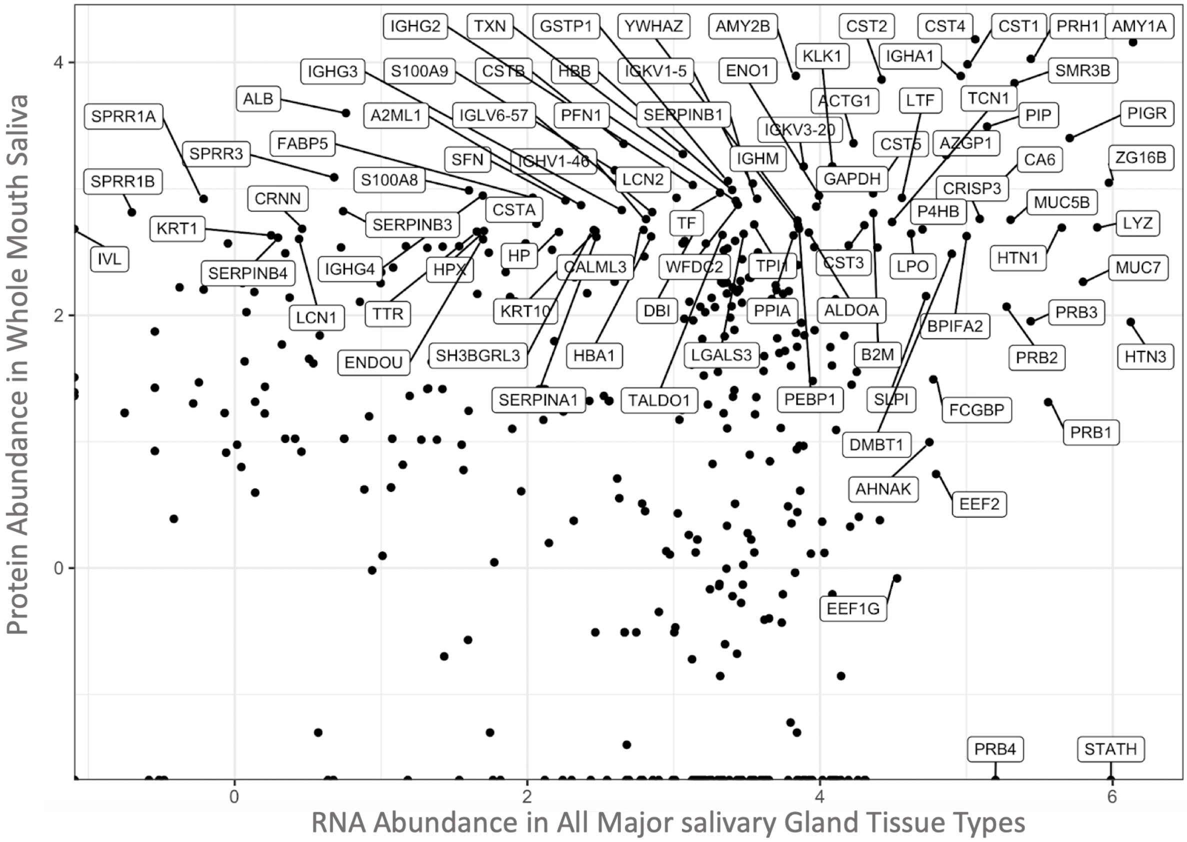

Figure S6. [Related to Figure 4] Secreted proteins in whole saliva identified by RNAseq analysis of major salivary gland tissues. The total sum of transcripts encoding for secretory proteins in all the three types of adult glands (x-axis, log$_{10}$) was plotted against the secreted protein abundance in whole mouth saliva (x-axis, log$_{10}$) (source: Human Salivary Proteome Wiki https://salivaryproteome.nidcr.nih.gov/). Well-known secreted proteins (*e.g.*, PIGR, AMY1A, CST4) were found highly abundant at both the mRNA and protein levels, indicating that these proteins are likely derived from the salivary glands. In contrast, non-secreted proteins that are abundant in whole saliva, such as ALB, KRT1 and SERPINB4, showed negligible transcript levels (<10 reads) in salivary gland tissue, suggesting that they originate from other tissues or organs.

Table S1. [Related to Figure 1] Summary information on the salivary glands used in the study. The tissue of origin, developmental stage (mature or fetal), sex, age, and the sample size for each category.

	Number of glands and sex distribution		
Gland type	Total number	Male	Female
Adult parotid	4	2	2
Adult submandibular	6	3	3
Adult sublingual	3	0	3
Fetal parotid	3	2	1
Fetal submandibular	3	4	1
Fetal sublingual	6	4	2