iScience, Volume *25*

Supplemental information

Salivary ZG16B expression loss follows exocrine gland dysfunction related to oral chronic graft-versus-host disease

Ana Caroline Costa-da-Silva, Marit H. Aure, Joshua Dodge, Daniel Martin, Susan Dhamala, Monica Cho, Jeremy J. Rose, Carol W. Bassim, Kiran Ambatipudi, Frances T. Hakim, Steven Z. Pavletic, and Jacqueline W. Mays

SUPPLEMENTAL INFORMATION

Table S1: Clinical characteristics of patients and healthy volunteers, Related to all Figures

Demographics	Discovery			Validation				scRNAseq
				Cohort 1		Cohort 2		
	unaffected	affected	HV	unaffected	affected	unaffected	affected	HV
Number of cases	3	3	2	10	10	12	12	4
Age at sample, yr, median (range)	64 (25-68)	50(50-53)	56 (49-63)	46.5 (26-56)	57(27-67)	54 (19-68)	50 (20-68)	27 (24-34)
Sex (F/M)	0/3	0/3	0/2	5/5	3/7	6/6	6/6	2/2
Race (%)								
White, non-Hispanic	2 (66.6)	3 (100)	2 (20)	8 (80)	10 (100)	12 (100)	10 (83.3)	2 (50)
White, Hispanic	1 (33.3)			1 (10)			1 (8.3)	1(25)
African							1 (8.3)	1(25)
Asian				1 (10)				
Time post-HSCT, months, median (range)	9 (3-20)	12(2-20)	n/a	25.5(3-88)	11.5 (4-34)	36 (5-137)	18 (8-68)	n/a
Underlying Disease, n (%)								
Lymphoma (HD/NHL)	-	2 (66.6)	n/a	4 (40)	5 (50)	1 (8.3)	3 (25)	n/a
Acute leukemia (AML/ALL)	-	-	n/a	2 (20)	1 (10)	6 (50)	6 (50)	n/a
Chronic leukemia (CLL/CML)	3 (100)	1 (33.3)	n/a	2 (20)	4 (40)	2 (16.7)	1 (8.3)	n/a
MDS/myeloproliferative disorder (MDS, myelofibrosis, PV)	-	-	n/a	1 (10)	-	1 (8.3)	1 (8.3)	n/a
Other	-	-	n/a	1 (10)	-	2 (16.8)	1 (8.3)	n/a
Donor type, n (%)								
Related	-	-	n/a	3 (30)	-	5 (41.7)	5 (41.6)	n/a
Unrelated	3 (100)	3 (100)	n/a	7 (70)	10 (100)	7 (58.3)	7 (58.3)	n/a
HLA match, n (%)								
Matched	3 (100)	3 (100)	n/a	9 (90)	8 (80)	11 (91.7)	9 (75)	n/a
Mismatched	-	-	n/a	1 (10)	2 (20)	1 (8.3)	2 (16.7)	n/a
Haploidentical family donor	-	-	n/a	-	-	-	1 (8.3)	n/a
Sex match (donor -> recipient) , n (%)								
F → F	-	-	n/a	5 (50)	1 (10)	3 (25)	3 (25)	n/a
F → M	-	2 (66.7)	n/a	-	3 (30)	-	3 (25)	n/a
M → M	3 (100)	1 (33.3)	n/a	3 (30)	4 (40)	5 (41.7)	3 (25)	n/a
M → F	-	-	n/a	-	2 (20)	3 (25)	2 (16.7)	n/a
Not Available	-	-	n/a	2 (2)	-	1 (8.3)	1 (8.3)	n/a
Conditioning regimen , n (%)								
Myleoablative (%)	3 (100)	3 (100)	n/a	9 (90)	10 (100)	7 (58.3)	6 (50)	n/a
Nonmyeloablative			n/a				4	n/a
History of TBI, n(%)	3 (100)	3 (100)	n/a	9 (90)	10 (100)	7 (58.3)	6 (50)	n/a
Stem cell source , n (%)								
PBSC	2 (66.7)	3 (100)	n/a	10 (100)	10 (100)	11 (91.7)	9 (75)	n/a
Bone Marrow	1 (33.3)	-	n/a	-	-	1 (8.3)	3 (25)	n/a
History of aGVHD, yes, n (%)	1 (33.3)	1 (33.3)	n/a	4 (40)	6 (60)	8 (66.7)	8 (66.7)	n/a
Saliva production[1], g, mean (±SD)	2.13 (±0.99)	1.4 (±0.86)	4.32 (±0.86)	2.37(±1.98)	2.4 (±1.31)	2.12(±1.26)	2.27(±1.38)	3.78(±1.52)
Systemic immunosupressive treatment at time of saliva sample n (%)								
None		1 (33.3)	3 (100)	5 (50)			2 (16.7)	4 (100)
Steroid	1 (33.3)			1 (10)	2 (20)	5 (42)	1 (8.3)	
Calcineurin/mTOR inhibitor		2 (66.6)		3 (30)	4 (40)	5 (42)	3 (25)	
Calcineurin/mTOR inhibitor + Steroid				1 (10)	2 (20)	1 (8.3)	6 (50)	
Other	2 (66.6)				2 (20)	1 (8.3)		

[1]Five-minute unstimulated whole saliva

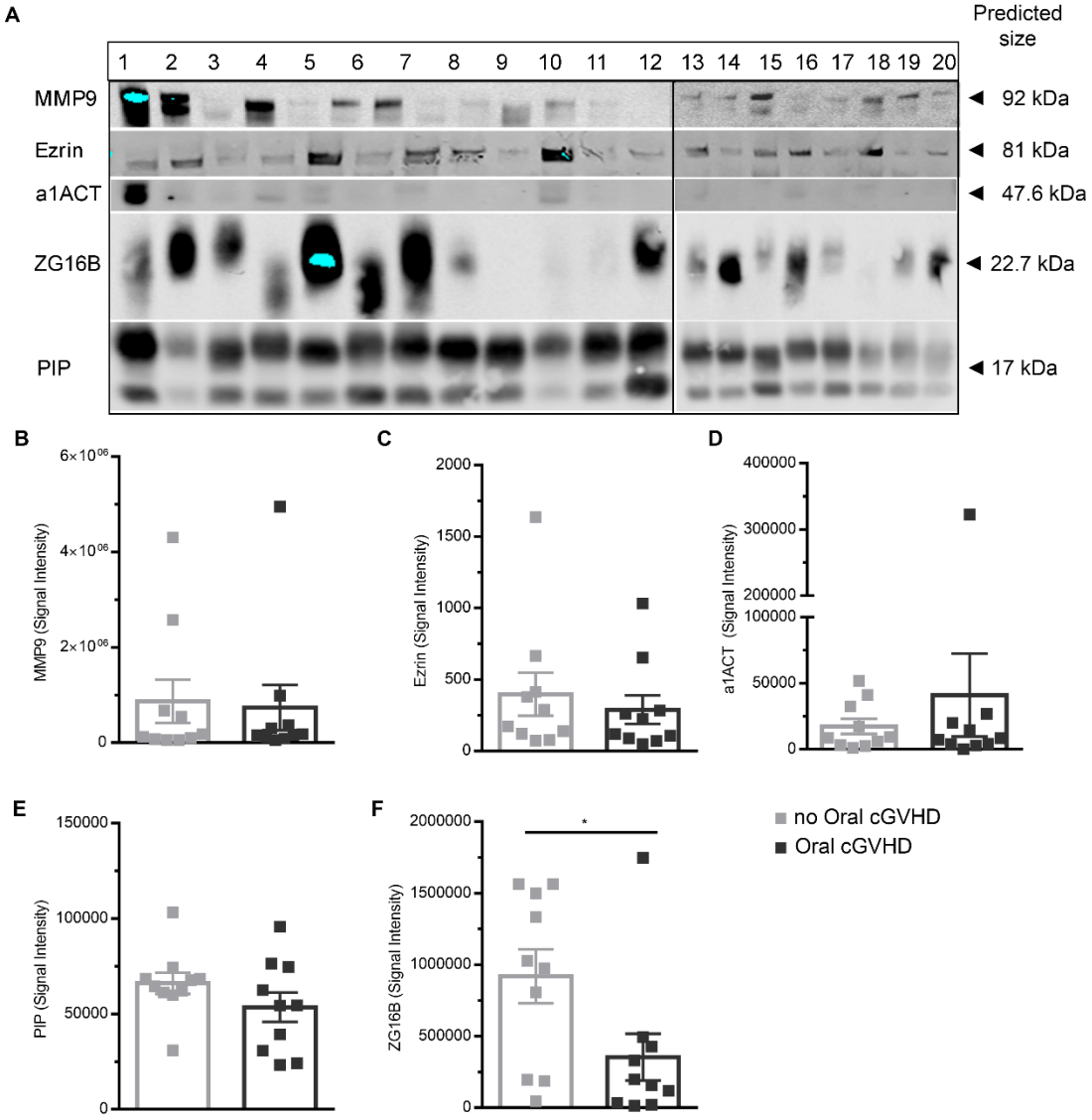

Figure S1: WB Validation of the differentially expressed proteins in the saliva samples of Cohort 2, Related to Figure 2 (A) Quantitative WB analysis of MMP9 (predicted MW of ~ 92 kDa), Ezrin(predicted MW of ~ 81 kDa), a1ACT (predicted MW of ~ 47.6 kDa), and both glycosylated and nonglycosylated forms of ZG16B (predicted MW of ~ 22.7 kDa) and PIP (predicted MW of ~ 17 kDa) in individual saliva samples from post-HSCT patients with oral cGVHD (odd numbers) compared with non-affected post-HSCT patients (even numbers) of Cohort 2 was undertaken. Equal amounts of protein (10 µg)

were loaded in each lane of the individual gels which are separated by the line (B) Densitometric analysis was performed using Image Studio Lite software (LiCor). Each dot represents 1 patient. Values are plotted as mean ± SEM. Differences between groups were calculated using unpaired student's *t*-test with $p \leq 0.05$ considered significant.

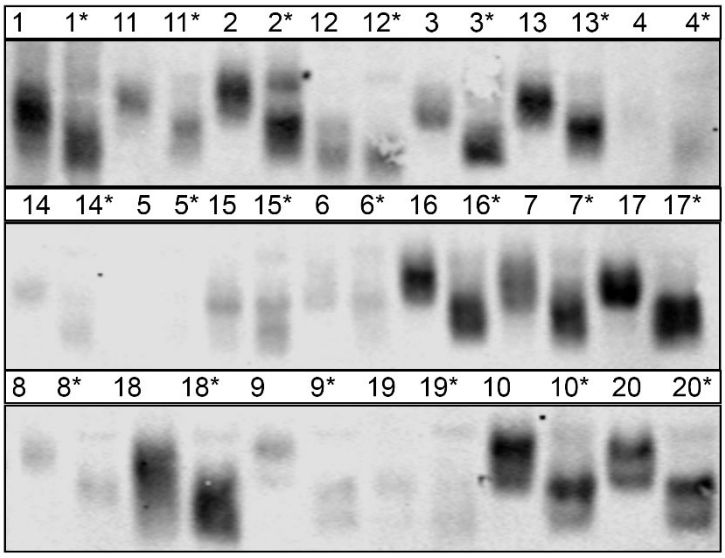

Figure S2: WB analysis of PNGase F-treated (*) and untreated saliva from post-HSCT patients with oral cGVHD (# 1-10) compared with non-affected post-HSCT patients (# 11-20) of cohort 2, Related to Figure 3. A 5 µg aliquot of ethanol-precipitated saliva, treated with or without PNGase F digestion, was loaded per lane and detected by anti-ZG16B WB. Untreated samples migrate differently at, typically, a higher apparent molecular weight. After PNGase F treatment, all samples migrate at the predicted molecular weight of ~22,7 kDa [UniProtKB – Q96DA0]. The asterisked numbers denote samples with PNGase treatment.

Table S2: Summary of HV information and sequencing statistics, Related to Figure 4

Samples	Age/Sex	Nº of cells	Mean Reads/cell	Median genes/cell	Nº of cells post filtering
HV1	28/M	7707	14403	912	6747
HV2	26/M	7000	18387	577	6488
HV3	34/F	4124	32805	1305	3579
HV4	24/F	6978	20852	756	5600

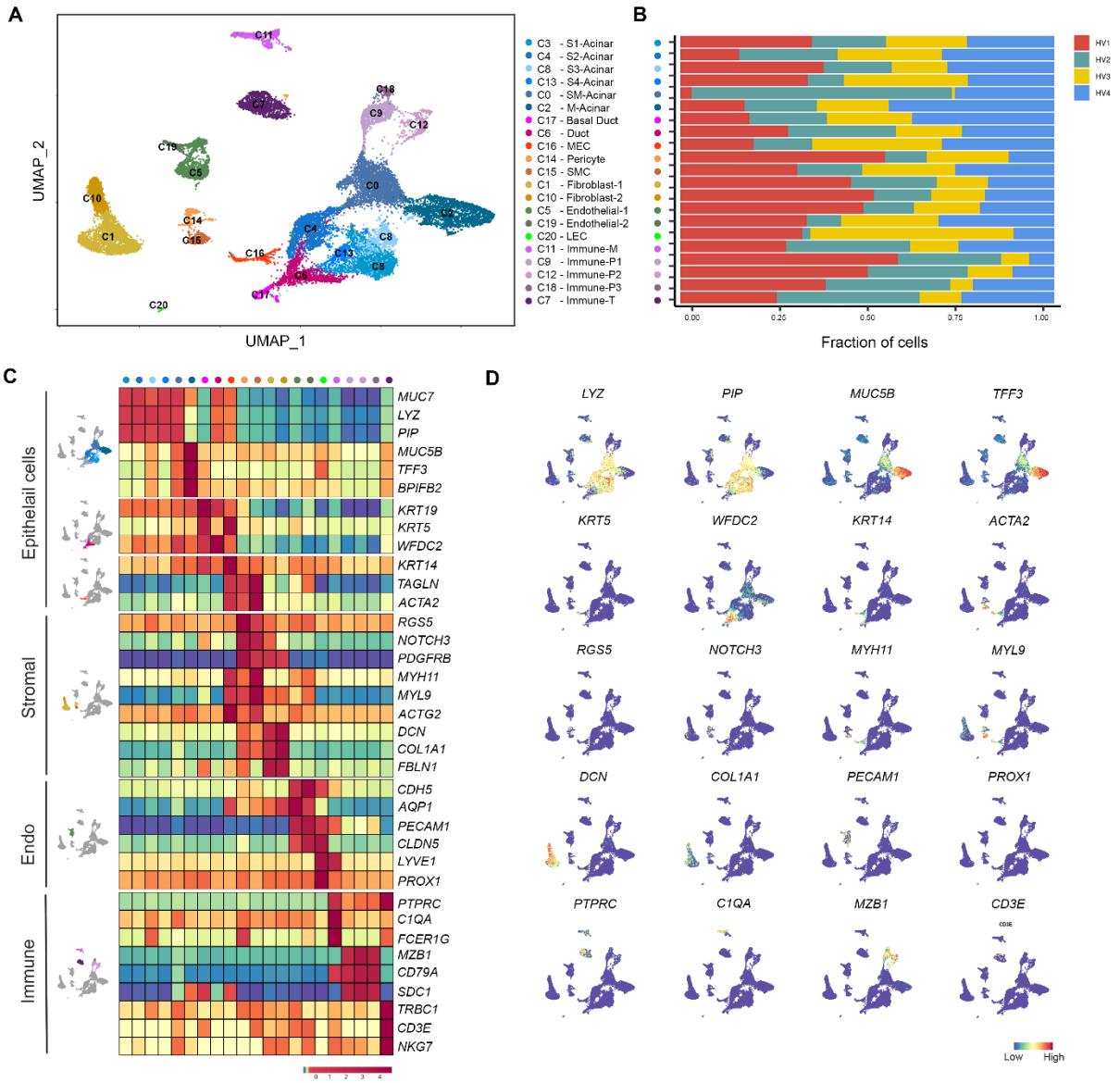

Figure S3: Single-cell transcriptomic sequencing and clustering of healthy volunteer minor salivary gland cells, Related to Figure 4. (A) UMAP embedding of data integrated from 4 HV MSG that were clustered into 20 populations and annotated according to expression of known marker genes. **(B)** Bar chart showing the fraction of cells from each sample in each of the 20 identified clusters. **(C)** UMAP projection plots colored by cell populations along with heatmaps of normalized gene expression of known markers used for their identification. **(D)** Feature plots illustrating the expression pattern

of selected makers genes of each cluster. Blue denotes minimal expression and red, high. MEC - Myoepithelial Cells; LEC – Lymphatic Endothelial Cells; SMC- Smooth Muscle Cells.

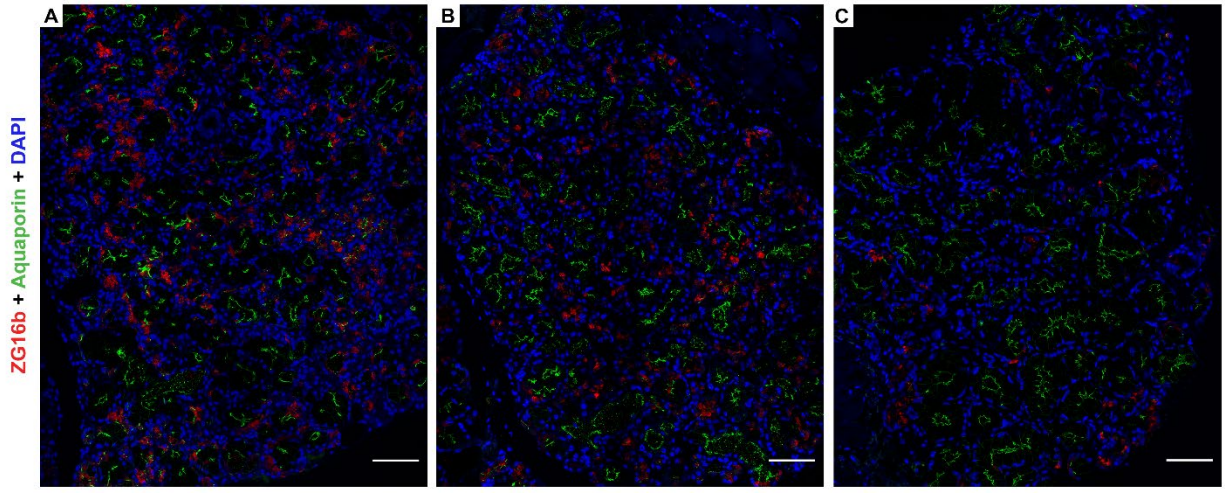

Figure S4: Expanded images of ZG16B in the specimens shown within Figure 5A-C. IHC staining highlights ZG16B (red) in the labial MSG of (A) a healthy volunteer, (B) a post-HSCT patient without oral cGVHD and (C) a post-HSCT patient with oral cGVHD. DAPI (blue) indicates nucleated cells and aquaporin 5 (green) labels the apical membrane of acinar cells. Magnification 400x, scale bars=200 μm.

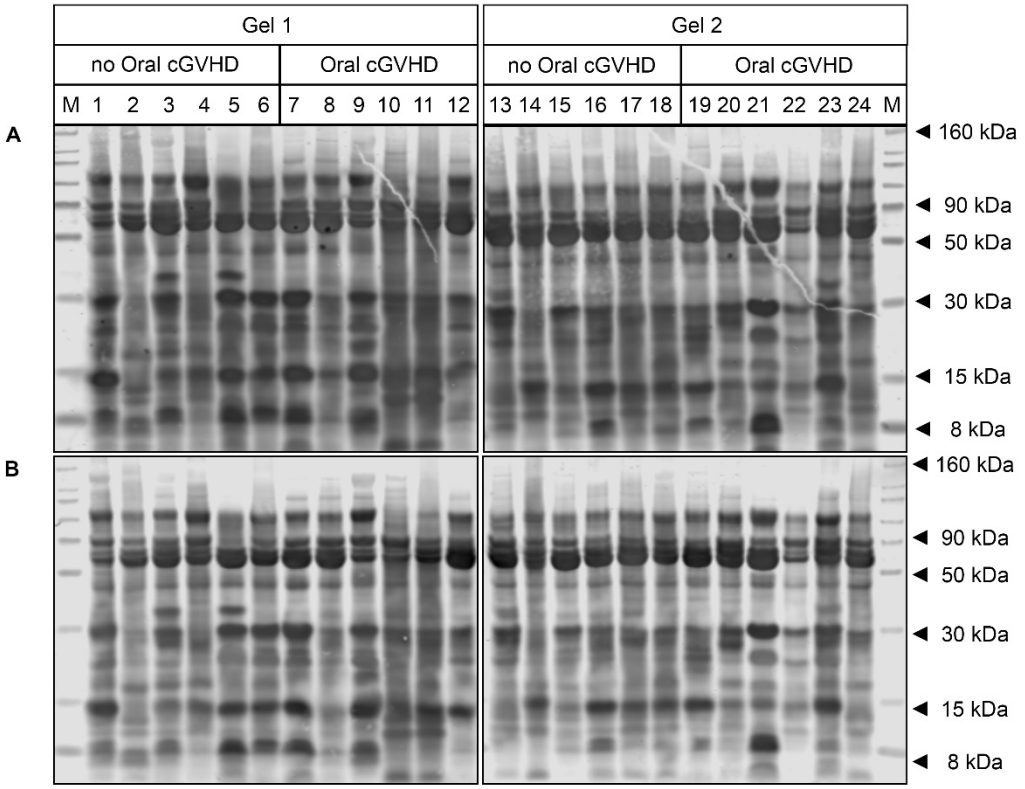

Figure S5: Loading Controls for Immunoblots, Related to Figures 2 and 3. Nitrocellulose membranes stained with Revert® Total protein stain prior to western blotting to verify consistent protein loading and electrotransfer. (A) Membrane Figure 2A. (B) Membrane Figure 3.