Supplemental Table 1. Differentially expressed genes in salivary glands between Sjögren's syndrome patients with high *BMP6* expression versus those with normal *BMP6* expression.

Gene symbol	Count per million	Log_2 fold change	*p* value	FDR
IGLVI-63	2.479239684	5.446	8.41E-06	0.028050458
UBD	3.416763525	5.205	4.89E-08	0.001686146
KRT4	2.809991105	5.191	1.08E-05	0.028050458
LOC105375724	3.809394049	3.578	2.19E-05	0.030829178
LTA	**2.001291919**	**3.365**	**0.00013041**	**0.049406587**
IGLV3-22	2.511103253	3.266	2.51E-05	0.031705253
CXCL13	**3.596567595**	**3.138**	**1.56E-05**	**0.028050458**
VPREB3	1.922862637	2.799	1.24E-05	0.028050458
CXCL9	**3.242140672**	**2.78**	**8.08E-06**	**0.028050458**
TCL1A	2.280466157	2.773	2.21E-05	0.030829178
TMEM163	1.919053457	2.675	9.61E-05	0.046670237
IGKV7-3	2.863533857	2.67	2.95E-05	0.033461111
NCR3	**1.758239343**	**2.658**	**4.63E-05**	**0.036352544**
CCL19	**4.877741778**	**2.613**	**9.43E-07**	**0.016252662**
IGKV1D-37	1.230044563	2.612	7.06E-05	0.041981695
LTA	**1.621659027**	**2.443**	**0.000128637**	**0.049276675**
MMP9	**2.405295351**	**2.361**	**8.68E-05**	**0.045060433**
CD52	5.74789247	2.356	2.01E-05	0.030829178
CD19	2.36792619	2.281	0.000107506	0.04691603
PLA2G2D	**3.920237621**	**2.169**	**0.000122961**	**0.049276675**
RP11-330H6.5	1.766939444	2.163	9.55E-05	0.046670237
PPP1R18	1.617340638	2.154	4.51E-05	0.036352544
IGKV1-8	4.652770241	2.144	8.64E-05	0.045060433
LTB	2.231687832	2.072	4.48E-05	0.036352544

Gene symbol	Count per million	Log_2 fold change	*p* value	FDR
IGKV2-10	1.707040236	2.034	1.43E-05	0.028050458
NKG7	4.076117313	2.013	7.70E-06	0.028050458
TIFAB	1.805860479	1.998	0.000104882	0.04691603
WDFY4	1.757147594	1.988	0.00010526	0.04691603
HCST	3.886703663	1.984	3.01E-05	0.033461111
BCL2A1	**2.379617337**	**1.981**	**5.10E-05**	**0.03700639**
CLECL1	2.216661299	1.972	0.00011509	0.047986684
ADAM19	3.888868174	1.943	3.49E-05	0.034783959
RBP5	2.090748562	1.924	1.17E-05	0.028050458
PTGDS	**5.927266531**	**1.893**	**3.55E-05**	**0.034783959**
IGKV1OR22-1	2.410195285	1.887	6.05E-05	0.039337364
HLA-DRB1	6.657364793	1.872	7.05E-06	0.028050458
CMPK2	**2.47488667**	**1.853**	**4.03E-06**	**0.028050458**
TMC8	2.052861999	1.851	9.45E-06	0.028050458
POU2F2	**3.306801345**	**1.834**	**5.15E-05**	**0.03700639**
GZMK	3.312458993	1.834	7.04E-05	0.041981695
TFEC	**2.385957817**	**1.822**	**9.75E-05**	**0.046670237**
LGALS2	3.304511646	1.797	1.48E-05	0.028050458
IGHV4-4	1.495989033	1.796	5.78E-05	0.038847864
GZMA	**2.88278428**	**1.78**	**1.48E-05**	**0.028050458**
CD3D	4.540737092	1.757	2.24E-05	0.030829178
SLC15A3	**1.95706469**	**1.729**	**0.000102935**	**0.04691603**
ITGAX	**4.663820874**	**1.723**	**2.67E-05**	**0.031705253**
SPIB	3.503693733	1.714	0.000112996	0.047986684
OASL	**3.118165737**	**1.704**	**8.65E-05**	**0.045060433**
P2RX5	1.696457884	1.675	0.000115527	0.047986684

Gene symbol	Count per million	Log_2 fold change	*p* value	FDR
CHST11	1.79255394	1.668	8.00E-05	0.045060433
RASGRP1	1.850078463	1.65	9.08E-05	0.045993801
CD72	2.128759224	1.644	2.53E-05	0.031705253
HLA-DRB3	3.192818499	1.639	6.53E-05	0.04096311
ITGAL	**3.416188199**	**1.628**	**3.37E-05**	**0.034783959**
ARHGAP9	2.758677331	1.609	3.74E-05	0.034836115
CD69	**1.943597729**	**1.598**	**1.12E-05**	**0.028050458**
TBC1D10C	2.316705466	1.57	5.86E-05	0.038847864
CSF2RB	**3.037698016**	**1.568**	**1.63E-05**	**0.028050458**
BMP6	1.62140251	1.518	0.000125796	0.049276675
LIMD2	2.747846772	1.515	6.68E-05	0.041103297
ANXA2R	2.705991112	1.486	8.57E-05	0.045060433
CD8A	**2.874337505**	**1.48**	**3.98E-05**	**0.036088675**
GPR65	2.336738777	1.464	4.42E-05	0.036352544
FCRL5	3.432757457	1.438	9.21E-05	0.045993801
CD37	4.247881191	1.433	6.70E-06	0.028050458
TRAF1	**2.193123799**	**1.432**	**0.000127498**	**0.049276675**
GVINP1	2.151973077	1.388	7.54E-05	0.04404202
PTPN7	**2.729516376**	**1.379**	**5.43E-05**	**0.037423639**
CD48	**3.875190034**	**1.379**	**0.000107124**	**0.04691603**
KIF20B	2.307512186	1.34	8.15E-05	0.045060433
SNX20	3.170497354	1.322	0.000101553	0.04691603
SERPINB9	**5.728634271**	**1.321**	**1.50E-05**	**0.028050458**
CERKL	2.198168143	1.312	4.79E-05	0.036686171
LY86	2.834416189	1.303	0.000127154	0.049276675
EPSTI1	3.513596227	1.298	8.23E-05	0.045060433

Gene symbol	Count per million	Log_2 fold change	*p* value	FDR
PYHIN1	2.216694521	1.292	0.000124394	0.049276675
IL32	**5.263001527**	**1.266**	**8.76E-05**	**0.045060433**
SFMBT2	2.048830941	1.246	4.57E-05	0.036352544
PCED1B	2.876793685	1.234	6.37E-05	0.040669271
BCL2	2.579611406	1.205	0.000102361	0.04691603
APOBEC3D	4.706302587	1.181	1.81E-05	0.029672261
SEMA4D	4.13171056	1.174	4.64E-05	0.036352544
GRK6	2.368965859	1.143	2.62E-05	0.031705253
GLCCI1	3.598950935	1.106	5.12E-05	0.03700639
IRF7	**2.585094745**	**1.08**	**5.26E-05**	**0.03700639**
MEX3C	3.180689227	0.932	0.000111219	0.047929645
KRT38	1.578279786	-3.886	0.000123737	0.049276675
RPL3P4	3.677699188	-4.622	3.63E-05	0.034783959
HNRNPA1P40	4.294464998	-5.5	8.46E-06	0.028050458
BPIFA1	3.613842413	-5.681	3.20E-05	0.034458527

Genes shown in bold were reported to be regulated by LPS.

Supplemental Table 2. Literature searching strategy and result.

Search term		Hit	Match
"Sjogren syndrome"	+ "**Proteomics**"	183	**17**
"Sjogren syndrome"	+ "**HMGB1**"	10	**3**
	+ "**Heat shock protein**"	60	**2**
	+ "**Hyaluronan**"	36	**1**
	+ "**Fibronectin**"	30	**1**
	+ "Histone"	88	0
	+ "Fibrinogen"	25	0
	+ "S100"	9	0
	+ "Defensin"	9	0
	+ "Syndecans"	7	0
	+ "Tenascin C"	4	0
	+ "Decorin"	2	0
	+ "Heparan sulfate"	2	0
	+ "Biglycan"	1	0
	+ "Granulysin"	0	0
	+ "Glypicans"	0	0
	+ "HMGN1"	0	0

HMGB1, high mobility group box 1; HMGN1, high mobility group nucleosome binding domain 1.

Supplemental Table 3. Potential TLR4 ligands reported to be increased in Sjögren's syndrome (SS) patients.

Reference		Sample	Potential TLR4 ligands increased in SS
Human Salivary Proteome Wiki *		**Saliva**	**HSP70, S100, Histones**
Sembler-Møller ML (1)	**2020**	**Saliva**	**HMGB1**
Wei P (2)	**2020**	**Saliva**	
Cecchettini A (3)	**2019**	**Saliva**	**HSP70, S100, Histones**
Aqrawi LA (4)	**2019**	**Saliva**	
Hall SC (5)	**2017**	**Saliva**	**Histones**
Aqrawi LA (6)	**2017**	**Saliva**	**HSP70**
Deutsch O (7)	**2015**	**Saliva**	**HSP70, S100, Histones, Fibrinogen**
Ambatipudi KS (8)	**2012**	**Saliva**	**HSP70, S100, Histones, Fibrinogen**
Baldini C (9)	**2011**	**Saliva**	**S100**
Fleissig Y (10)	**2009**	**Saliva**	**S100, Fibrinogen**
Silvestre FJ (11)	2009	Saliva	Fibronectin
Peluso G (12)	**2007**	**Saliva**	**Defensin**
Hu S (13)	**2007**	**Saliva**	
Giusti L (14)	**2007**	**Saliva**	
Ryu OH (15)	**2006**	**Saliva**	
Tishler M (16)	1998	Saliva	Hyaluronan
Hjelmervik TO (17)	**2009**	**MSG**	**HSP60, HSP70, HSP90, Decorin**
Ek M (18)	2006	MSG	HMGB1
Bodewes ILA (19)	**2019**	**Serum**	
Kanne AM (20)	2018	Serum	HMGB1
Nishikawa A (21)	**2016**	**Serum**	
Bårdsen K (22)	2016	Plasma	HSP90
Dupire G (23)	2012	Serum	HMGB1
Aragona P (24)	1999	Serum	HSP60

Proteomic studies are indicated as bold. HSP, heat shock protein; HMGB1, high mobility group box 1; MSG, minor salivary gland. *https://salivaryproteome.nidcr.nih.gov.

Supplemental Table 4. Demographic and clinical characteristics between Sjögren's syndrome patients with high *BMP6* expression (n = 20) versus those with normal *BMP6* expression (n = 23).

Characteristics	High *BMP6*	Normal *BMP6*	*p* value*
Age, years	55 ± 12	50 ± 16	0.230
Female, *n (%)*	20 (100)	23 (100)	-
Low salivary flow (<0.1 mL/min), *n (%)*	14 (70)	16 (70)	0.975
Positive Schirmer's test (≤5mm/5min), *n (%)*	10 (50)	9 (39)	0.474
Anti-SSA Ab positive, *n (%)*	14 (70)	16 (70)	0.975
Anti-SSB Ab positive, *n (%)*	11 (55)	11 (48)	0.639
Focus score	1.2 ± 1.3	1.6 ± 1.1	0.264
Prior medications during 3 months before recruitment			
Glucocorticoids, *n (%)*	9 (45)	7 (30)	0.361
Immunosuppressants†, *n (%)*	9 (45)	6 (26)	0.219
Hydroxychloroquine, *n (%)*	3 (15)	1 (4)	0.323

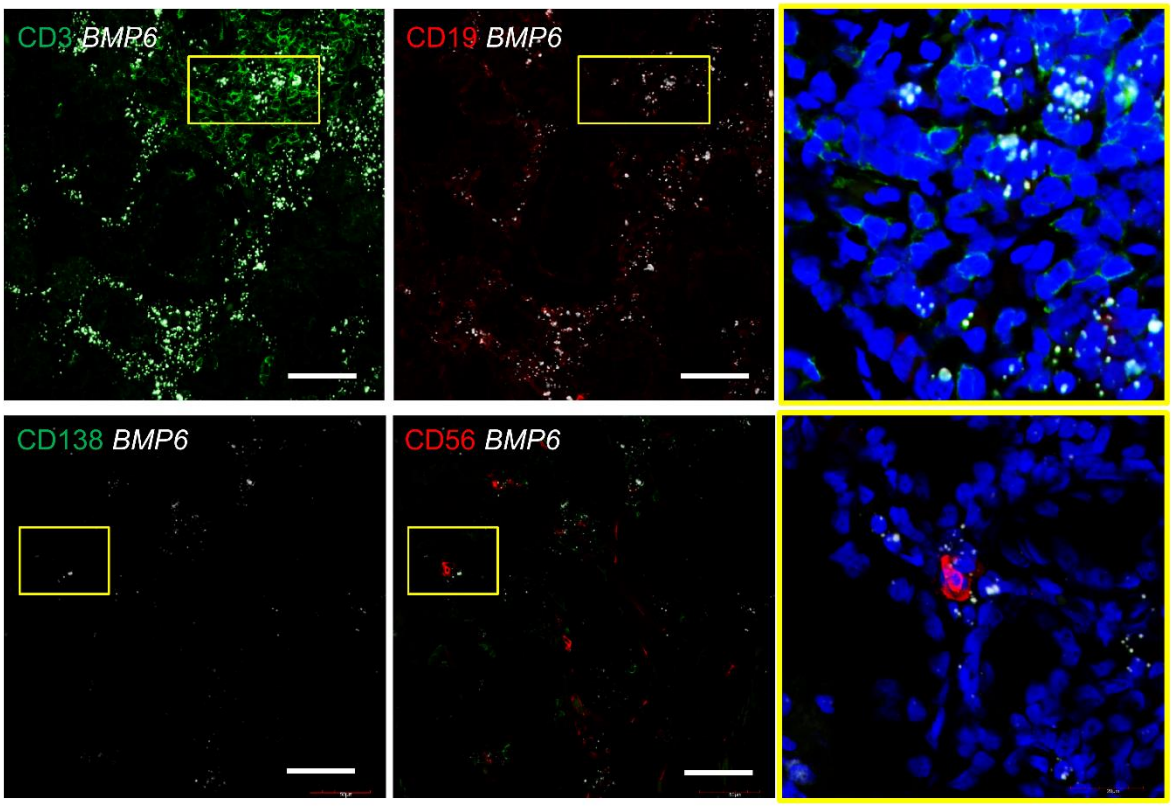
†Immunosuppressants include methotrexate, azathioprine, cyclosporine and leflunomide.

*Fisher's exact test for categorical variables and *t*-test for continuous variables.

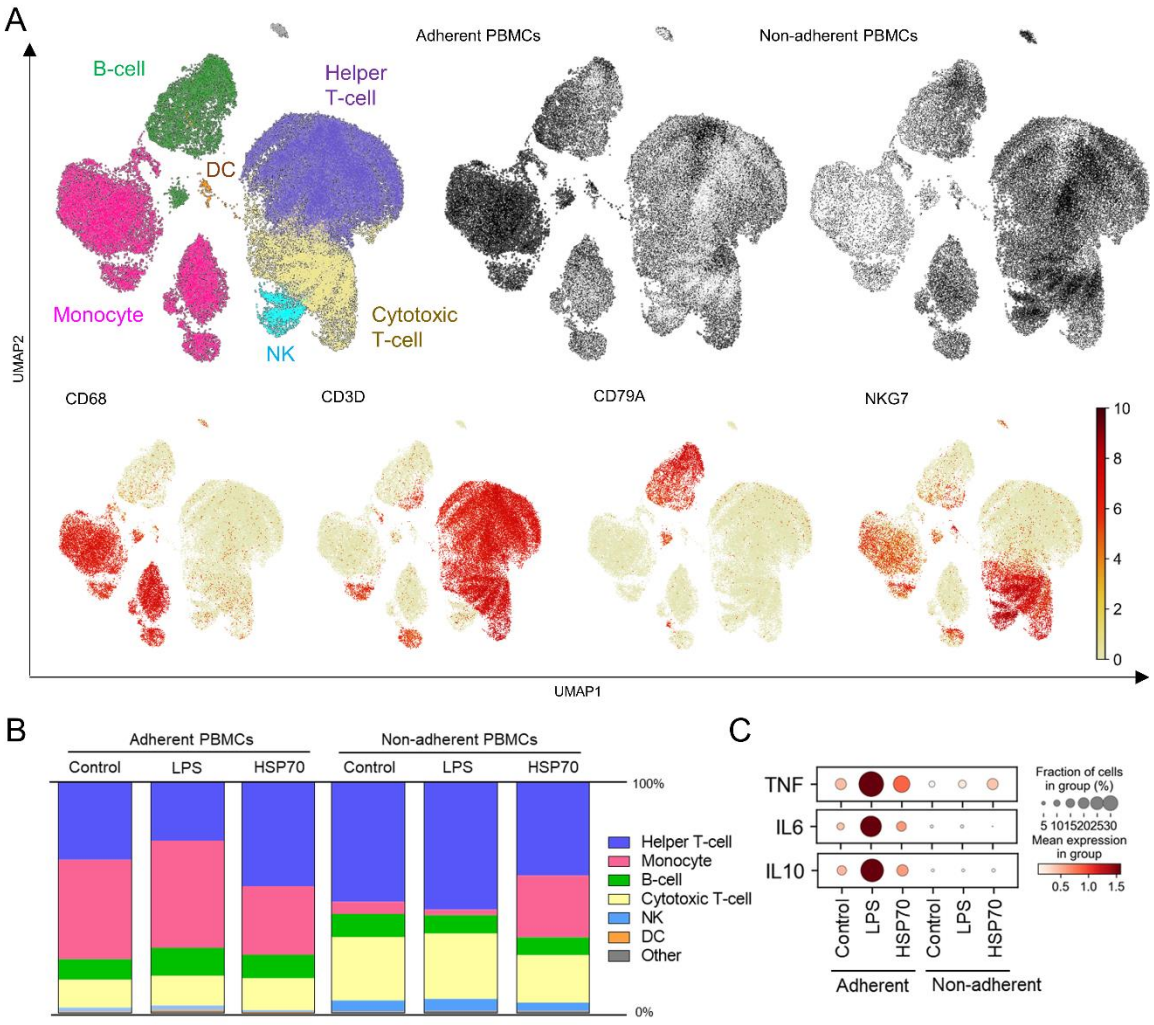
Supplemental Table 5. Quality assessment summary of PBMCs samples.

	Adherent			Non-adherent		
	Sham	LPS	HSP70	Sham	LPS	HSP70
Number of cells	9272	11844	32415	12005	9547	11674
Mean reads per cell	8011	25671	32590	10577	15112	37946
Median reads per cell	901	1715	1506	1052	1315	1424
Number of reads	74×10^6	304×10^6	1056×10^6	127×10^6	144×10^6	443×10^6
Valid Barcodes	96.5%	97.0%	96.6%	97.0%	96.9%	96.1%
Valid UMIs	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%
Sequencing Saturation	32.0%	53.1%	59.2%	44.9%	50.3%	74.7%
Reads Mapped to Genome	97.6%	97.6%	97.5%	97.3%	97.2%	97.4%
Reads Mapped to Transcriptome	58.5%	60.8%	57.8%	56.6%	55.5%	56.7%
Fraction Reads in Cells	94.7%	94.7%	86.0%	93.2%	93.0%	94.5%
Total Genes Detected	19619	21756	23764	20530	20476	21875
Median UMI Counts per Cell	2048	4949	4782	2537	3293	4188

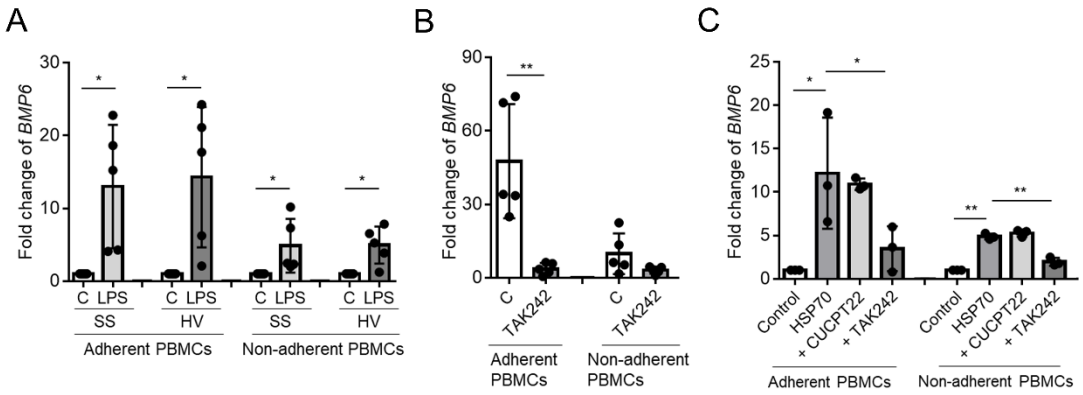
UMI, unique molecular identifier.



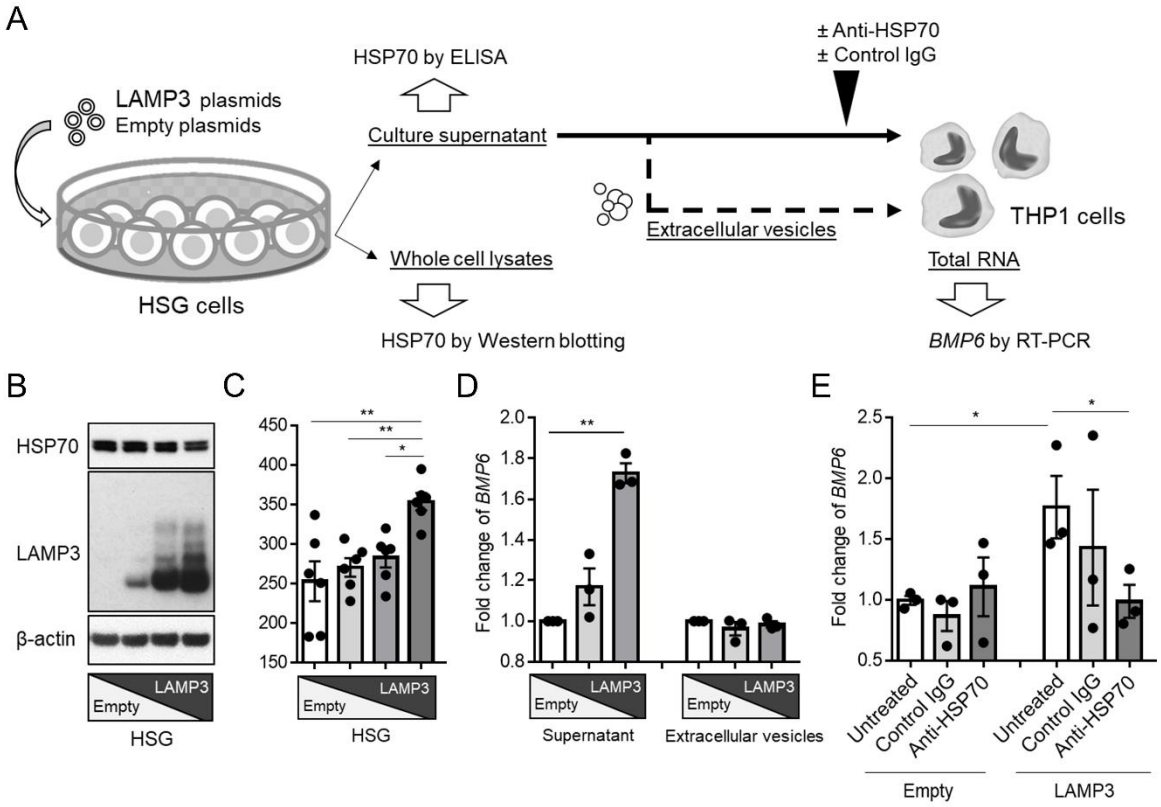
Supplemental Figure 1. *BMP6* mRNA expressing cells in salivary glands of patients with Sjögren's syndrome. Representative images of dual *in situ* hybridization for *BMP6* (white) and immunofluorescence for CD3 (green), CD19 (red), CD138 (green) or CD56 (red) on labial minor salivary gland sections. Scale bars = 50 µm.



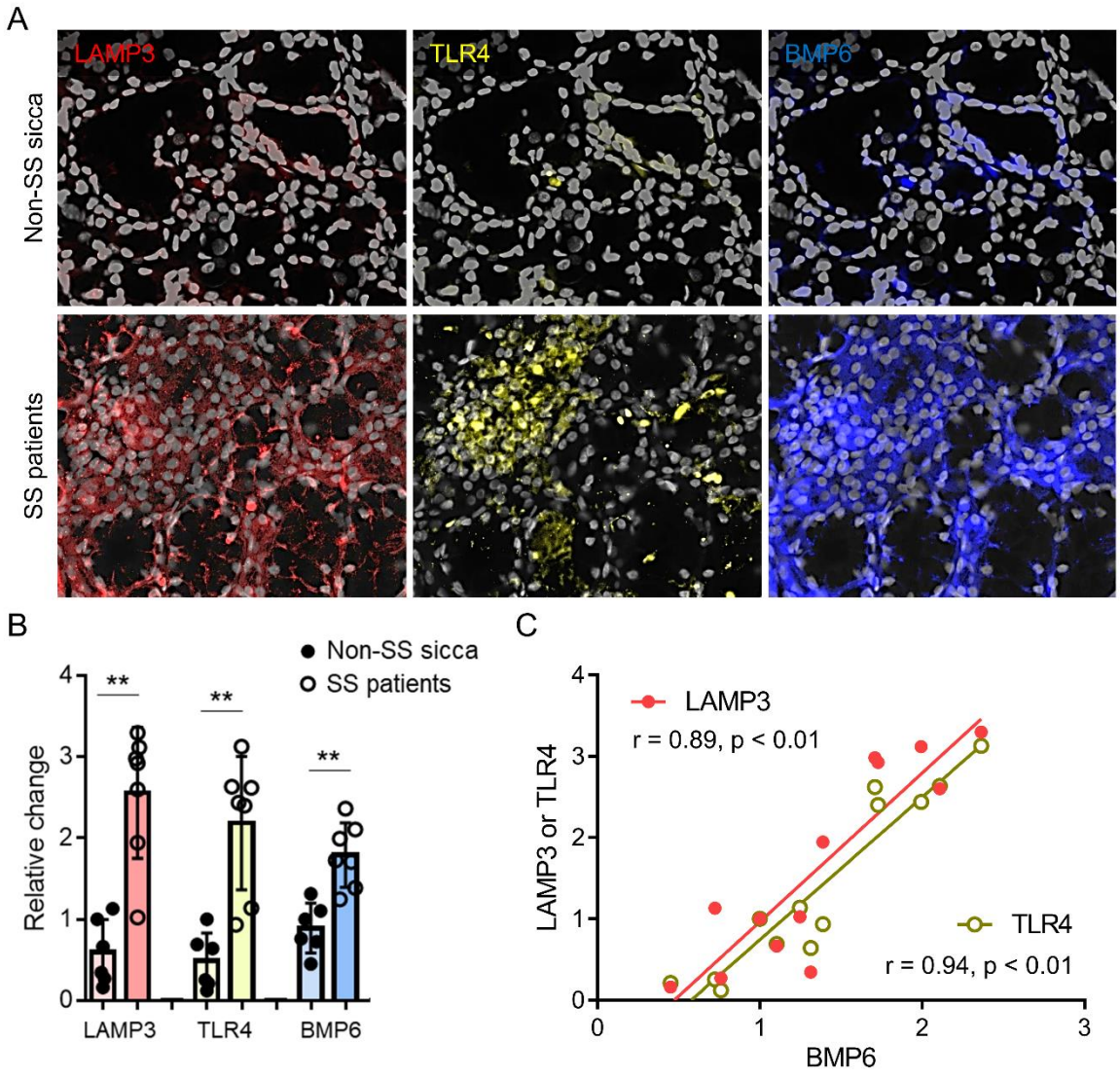
Supplemental Figure 2. Single-cell RNA sequencing libraries of human PBMCs. PBMCs were captured 20 hours after treatment with sham control, LPS or HSP70. The adherent and non-adherent populations were processed separately. (**A**) Annotated PBMCs are displayed in Uniform Manifold Approximation and Projection (UMAP) format with distribution of cells expressing the representative cell markers. (**B**) Proportion of each cell type among adherent and non-adherent groups. (**C**) Proportion and relative expression of indicted genes among adherent and non-adherent groups.



Supplemental Figure 3. *BMP6* expression is upregulated via TLR4 pathway in human PBMCs. (**A**) Human PBMCs isolated from patients with Sjögren's syndrome (SS, $n = 5$) or healthy volunteers (HVs, $n = 5$) were treated with LPS (100 ng/mL) for 20 hours. (**B**) PBMCs from HVs ($n = 5$) were treated with LPS ± TAK242 (40 µM) for 20 hours. (**C**) PBMCs from HVs ($n = 3$) were treated with recombinant HSP70 (1 µg/mL) ± CUCPT22 (20 µM) or TAK242 (40 µM) for 20 hours. *BMP6* transcript was quantified using $\Delta\Delta$Ct method relative to *ACTB* in adherent and non-adherent population. Values shown are mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *t*-test with multiple testing correction using Dunnett's method.



Supplemental Figure 4. *BMP6* expression is not stimulated by extracellular vesicles from LAMP3-overexpressing HSG cells (**A**) Schematic methods of the following *in vitro* assays. (**B**) Representative Western blot with indicated antibodies using lysate of HSG cells 72 hours after transfection with empty and/or *LAMP3* expression plasmids. (**C**) HSP70 concentration in culture supernatant collected 96 hours after transfection. (**D**) Culture supernatant of HSG cells was collected 96 hours after transfection with empty and/or *LAMP3* expression plasmids and extracellular vesicles were isolated from supernatant. THP1 cells were treated with unfractionated supernatant or isolated EVs for 20 hours. (**E**) THP1 cells were treated with culture supernatant of HSG cells ± HSP70 neutralizing antibody (1 µg/mL) or control IgG (1 µg/mL). *BMP6* transcript was quantified using *ΔΔ*Ct method relative to *ACTB.* Values shown are mean ± SEM from three or five independent experiments. **p* < 0.05, ***p* < 0.01, *t*-test with multiple testing correction using Tukey's method.



Supplemental Figure 5. LAMP3, TLR4 and BMP6 expression in salivary glands. (**A**) Representative images of immunofluorescence for LAMP3 (red, left panel), TLR4 (yellow, center panel) or BMP6 (blue, right panel) and nucleus (DAPI, gray) on labial minor salivary gland sections from patients with Sjögren's syndrome (SS, n = 7) or non-SS sicca. (n = 6). Original magnification: 40x. (**B**) Bar chart showing mean (± SD) relative change of each protein expression area. $^{**}p < 0.01$, t-test. (**C**) Correlation between BMP6 and LAMP3 or TLR4 expression in labial minor salivary glands.

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