Supplemental Materials and Methods

Direct bisulfite sequencing of genomic DNA

Whole SMGs from 9 month-old female IL-14αTG and IL-14αTG x $P2Y_2R^{-/-}$ mice were utilized for genomic DNA isolation using a QIAamp Mini Kit (Qiagen), per manufacturer's protocol. Bisulfite conversion was performed on purified genomic DNA (500 ng) using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA), per manufacturer's protocol. Bisulfite-treated DNA was amplified by PCR using specific primers (forward: 5'-TGG GTT TGA TTT AGT TTT TTG TTA GTT TTT G-3'; reverse: 5'-ACC CAA AAA AAC AAA ATA AAA AAA ACT CAC CTA C-3') for a portion of the mouse *LTA* gene that includes a short region upstream of the transcription start site (TSS) and all of exon 1 (-46 bp to +189 bp from TSS). PCR amplification was performed using Platinum Hot Start PCR 2X Master Mix (ThermoFisher Scientific, Waltham, MA) on a MJ Research PTC-200 thermal cycler using the following protocol: 5 min at 95°C, 45 repeats at 95°C for 30 s, 60°C for 45 s, 72°C for 60 s and a final extension at 72°C for 7 min. PCR products were cleaned using ExoSAP-IT reagent (Affymetrix, Santa Clara, CA) before sequencing at the University of Missouri DNA Core Facility on an Applied Biosystems 3730xl DNA Analyzer. Using Chromas software (Technelysium Pty Ltd, South Brisbane, Australia) for analysis of the sequencing chromatogram, DNA methylation at each of the 14 cytosine-guanine dinucleotides (CGs) in the amplified *LTA* region was calculated using the equation [% cytosine methylation = (C/C+T) * 100], where C and T correspond to the maximum height of cytosine and thymine peaks, respectively, at each site (Parrish *et al*, 2012). CG sites within the *LTA* gene of a universal methylated mouse DNA standard (Zymo Research) served as a positive control, whereas unmethylated CC dinucleotides within the *LTA* gene served as a negative control.

Supplemental Results

Identification of 2 differentially methylated sites in the lymphotoxin-α gene in SMGs from IL-14αTG x $P2Y_2R^{-/-}$ mice, as compared to age-matched IL-14αTG mice

A portion of the mouse *LTA* gene (-46 bp to +189 bp from TSS; Supplemental Figure 3a) containing 14 CGs that are potential sites of DNA methylation was chosen for direct bisulfite sequencing analysis. This portion contains all of *LTA* exon 1 and has been previously shown to regulate *LTA* transcriptional activity (Worm *et al*, 1998, Yokley *et al*, 2013). Furthermore, previous studies have identified differentially methylated CGs upstream of the TSS and in exon 1 of the *LTA* gene in SS patients (Altorok *et al*, 2014, Braekke Norheim *et al*, 2016, Cole *et al*, 2016, Imgenberg-Kreuz *et al*, 2016, Miceli-Richard *et al*, 2016). As shown in Supplemental Figure 3b, 12 of the 14 potentially methylated CG sites in the mouse *LTA* gene showed no significant difference in methylation status between IL-14αTG and IL-14αTG x $P2Y_2R^{-/-}$ mice. Two of the CG sites (CG1 at -46 bp from TSS and CG11 at +162 bp from TSS) were slightly, but significantly, hypomethylated in SMGs from IL-14αTG x $P2Y_2R^{-/-}$ as compared to IL-14αTG mice. While these results may suggest that $P2Y_2$Rs influence the epigenetic regulation of *LTA* in IL-14αTG mice, decreased DNA methylation is generally associated with increased gene expression, whereas we observed decreased *LTA* expression following $P2Y_2$R knockout in IL-14αTG mice (manuscript Figure 5). These data suggest that $P2Y_2$Rs may regulate LTA expression through a different mechanism.

Supplemental References

Altorok N, Coit P, Hughes T, Koelsch KA, Stone DU, Rasmussen A, . . . Sawalha AH (2014). Genome-wide DNA methylation patterns in naive $CD4^+$ T cells from patients with primary Sjögren's syndrome. *Arthritis Rheumatol* **66**: 731-739.

Braekke Norheim K, Imgenberg-Kreuz J, Jonsdottir K, Janssen EA, Syvanen AC, Sandling JK, . . . Omdal R (2016). Epigenome-wide DNA methylation patterns associated with fatigue in primary Sjögren's syndrome. *Rheumatology* **55**: 1074-1082.

Cole MB, Quach H, Quach D, Baker A, Taylor KE, Barcellos LF and Criswell LA (2016). Epigenetic Signatures of Salivary Gland Inflammation in Sjögren's Syndrome. *Arthritis Rheumatol* **68**: 2936-2944.

Imgenberg-Kreuz J, Sandling JK, Almlof JC, Nordlund J, Signer L, Norheim KB, . . . Nordmark G (2016). Genome-wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. *Ann Rheum Dis* **75**: 2029-2036.

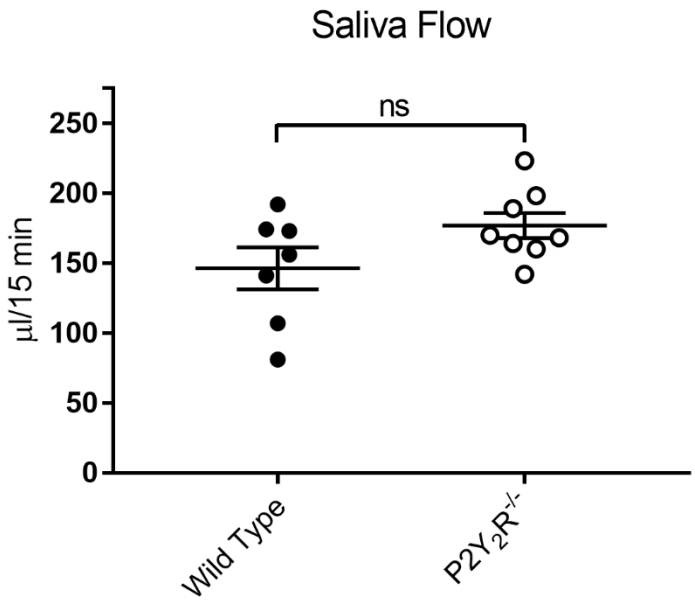
Miceli-Richard C, Wang-Renault SF, Boudaoud S, Busato F, Lallemand C, Bethune K, . . . Tost J (2016). Overlap between differentially methylated DNA regions in blood B lymphocytes and genetic at-risk loci in primary Sjögren's syndrome. *Ann Rheum Dis* **75**: 933-940.

Parrish RR, Day JJ and Lubin FD (2012). Direct bisulfite sequencing for examination of DNA methylation with gene and nucleotide resolution from brain tissues. *Current protocols in neuroscience* Chapter 7: Unit 7.24.

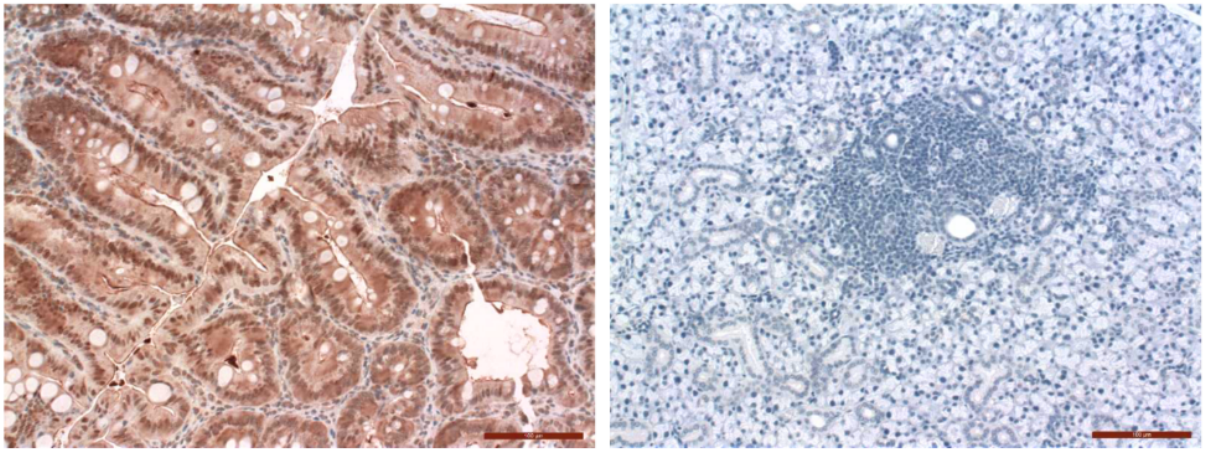
Worm MM, Tsytsykova A and Geha RS (1998). CD40 ligation and IL-4 use different mechanisms of transcriptional activation of the human lymphotoxin α promoter in B cells. *Eur J Immunol* **28**: 901-906.

Yokley BH, Selby ST and Posch PE (2013). A stimulation-dependent alternate core promoter links lymphotoxin α expression with TGF-β1 and fibroblast growth factor-7 signaling in primary human T cells. *J Immunol* **190**: 4573-4584.

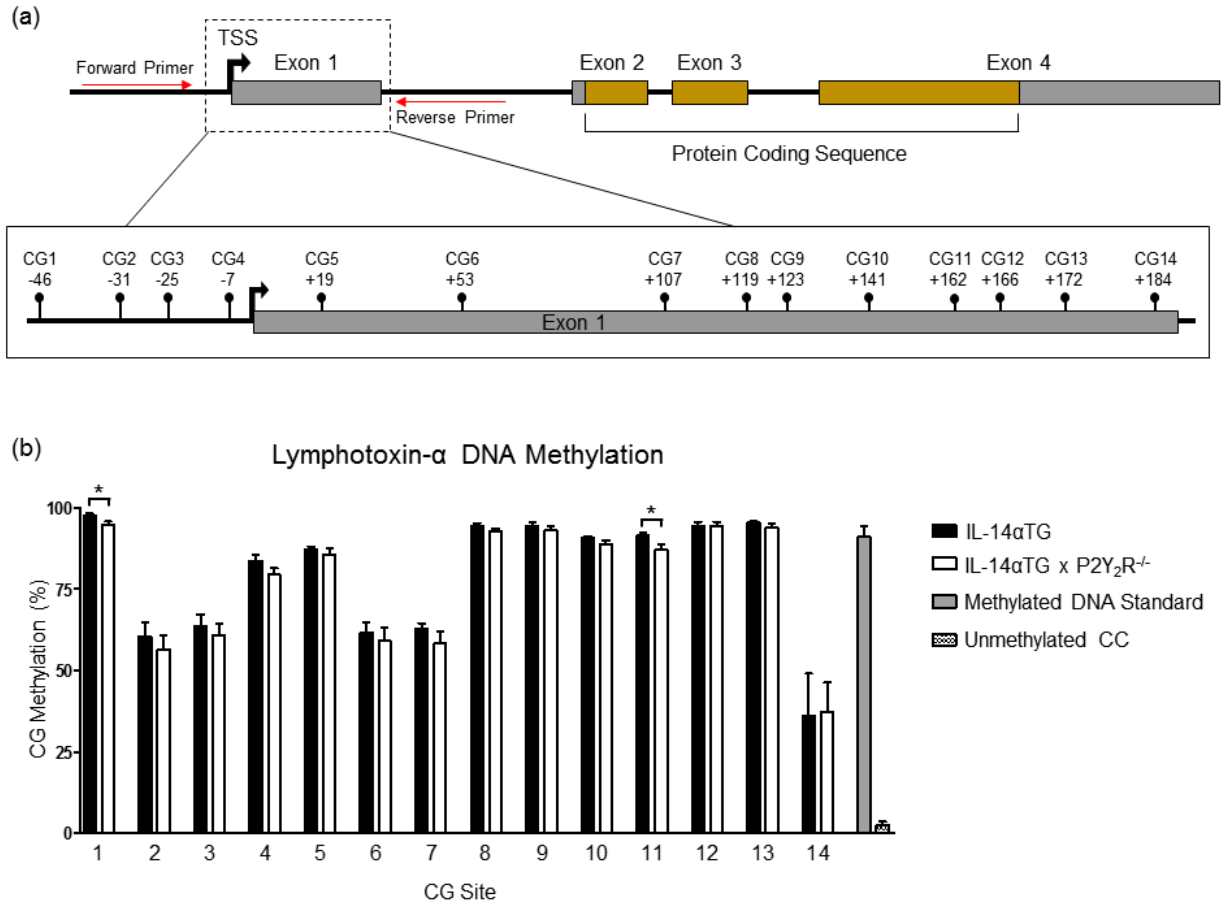
Supplemental Figures



Supplemental Figure 1. Analysis of saliva secretion. Following intraperitoneal injection of carbachol (0.25 mg/kg mouse weight), whole saliva was collected from the oral cavity of 12 week-old wild type C57BL/6 and $P2Y_2R^{-/-}$ mice. Data are means ± S.E.M. for wild type (n = 7) and $P2Y_2R^{-/-}$ (n = 8) mice, where ns indicates no significance.



Supplemental Figure 2. Immunohistochemical analysis of lymphotoxin-α expression in control tissues. Anti-lymphotoxin-α antibody was utilized for immunohistochemical analysis of formalin-fixed paraffin-embedded mouse colon adenocarcinoma (left; positive control), as described in the Materials and Methods section of the manuscript. Alternatively, SMG sections from 9 month-old IL-14αTG mice (right; negative control) were subjected to immunohistochemical analysis where the primary anti-lymphotoxin-α antibody was omitted and only the secondary horseradish peroxidase-conjugated antibody was used. Scale bar = 100 μm.



Supplemental Figure 3. *Lymphotoxin-α* gene DNA methylation in submandibular glands from IL-14αTG and IL-14αTG x $P2Y_2R^{-/-}$ mice. Bisulfite-converted genomic DNA from SMGs of 9 month-old IL-14αTG and IL-14αTG x $P2Y_2R^{-/-}$ mice was utilized for amplification and sequencing of (a) a fragment of the *lymphotoxin-α* (*LTA*) gene containing 14 CG dinucleotide methylation sites. (b) CG methylation % was determined at each CG site by the equation [% cytosine methylation = (C/C+T) * 100], where C and T correspond to the maximum height of cytosine and thymine peaks, respectively, at each CG site in the *LTA* DNA sequencing chromatogram. CG sites within the *LTA* gene of a universal methylated mouse DNA standard served as a positive control, whereas unmethylated CC dinucleotides within the *LTA* gene served as a negative control. Data are presented as means ± S.E.M. for IL-14αTG (n = 6) and IL-14αTG

x $P2Y_2R^{-/-}$ (n = 6) mice, where $P < 0.05$ indicates a significant difference, as determined by Student's t test.