# Supplementary Data

## Supplementary Materials and Methods

### *N-terminal sequence of TMEM98*

Human embryonic kidney (HEK) 293T cells were transfected with the pYD11-TMEM98-hFc plasmids using VigoFect reagent (Vigorous). The transiently transfected cells were cultured in serum-free media (Gibco) for 72 h before the culture medium was harvested and purified on a protein G column (GE Healthcare) according to the manufacturer's instructions. The purified transmembrane protein 98 (TMEM98)-Fc protein was dialyzed in phosphate-buffered saline at 4°C, then quantified and identified by SDS-PAGE and Western blot analysis with an anti-TMEM98 antibody. Purified TMEM98 protein was used to do the N-terminal sequence.

### *Semiquantitative reverse transcription–polymerase chain reaction and real-time polymerase chain reaction*

The cDNA panels for multiple human tissues (Human MTC™ Panels I and II) and the Human Immune System MTC Panel were purchased from Clontech. *TMEM98* mRNA level was assessed by semiquantitative reverse transcription–polymerase chain reaction (SqRT-PCR) with forward (5′-TTGCCACTTCCAGCAGCTTTAGC) and reverse (5′-CAGCCGTGGAGAACAACTAACTCTA) primers. DNA was denatured at 94°C for 5 min, followed by 35 cycles at 58°C and extension for 1 min at 72°C. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was amplified as before, 40 for 22 cycles. Five microliters of the polymerase chain reaction (PCR) product was analyzed on a 1.0% agarose gel.

Real-time PCR was performed for quantitative analyses in an ABI Prism 7000 Sequence Detection System (Applied Biosystems). Amplifications were carried out using Universal Probe Library (UPL) probes. The quantification data were analyzed with ABI Prism 7000 SDS software. The expression levels of the target genes were measured as fluorescence signal intensities and normalized to the internal standard gene *GAPDH*. The samples with unspecific amplification were regarded as zero. The real-time PCR was performed using the following primers:

TMEM98 forward primer, 5′-GTCAGCGACATCATTG TGGT-3′, and reverse primer, 5′-GGAGGGTACATCGAC TTCACA-3′.

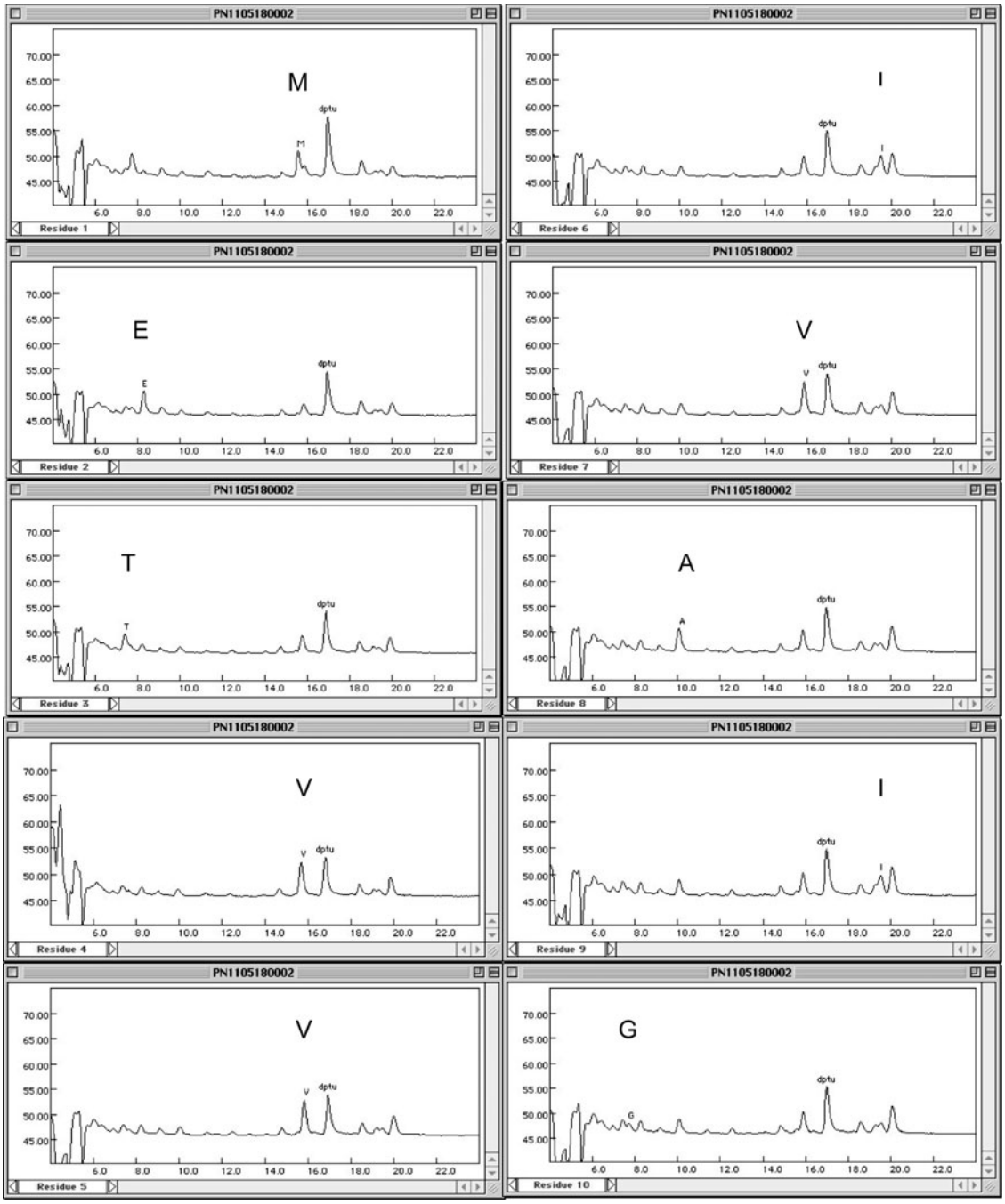
GAPDH forward primer, 5′-TCCACTGGCGTCTTCA CC-3′, and reverse primer, 5′-GGCAGAGATGATGACCC TTTT-3′.

### *Immunoprecipitation*

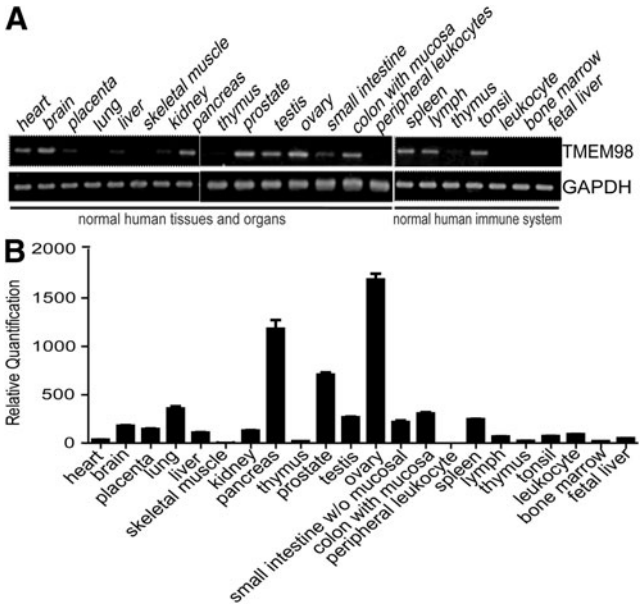
Supernatants (500 μL) collected from HEK293T cells transiently transfected with the TMEM98 plasmid or the pcDB vector were centrifuged (800 rpm, 10 min; 12,000 rpm, 10 min, 4°C) to eliminate the residual cells and debris. Then, they were incubated with the primary antibody (pAb1, 1 μg) against TMEM98 overnight at 4°C. Next, protein G beads (30 μL) were added, and the cultures were further incubated (overnight at 4°C). After washing, the pellet was resuspended with the loading buffer and microcentrifuged. The supernatants were collected as samples, and anti-myc was used for Western blot analysis.

### *Flow cytometry*

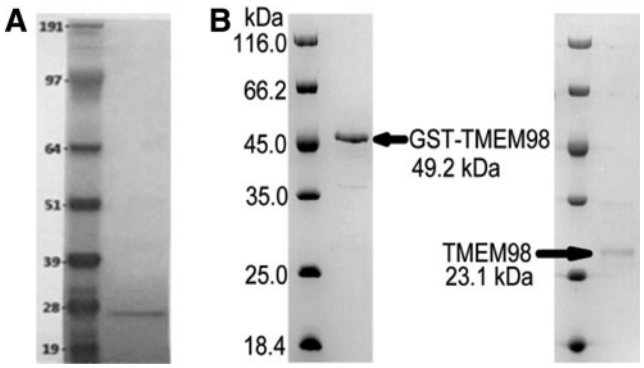
For pSTAT1 (Y701) and pSTAT4 (pY693) staining, cells were fixed and permeabilized with the Foxp3 Fix/Perm Buffer Set (BioLegend) and stained with corresponding Abs (Cell Signaling Technology). Labeled cells were used for flow cytometric analysis. Data were analyzed using FlowJo software (Tree Star) by gating on live cells based on their forward versus side scatter profiles.



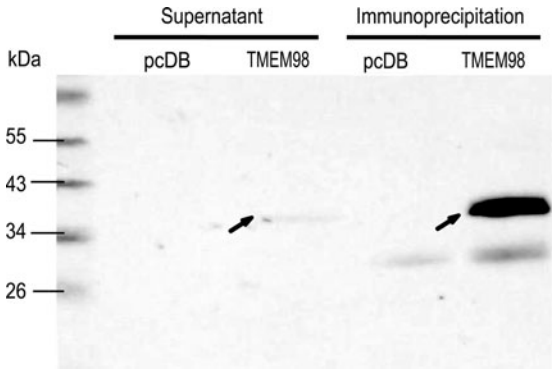

**SUPPLEMENTARY FIG. S1.** Protein preparation and N-terminal sequence of TMEM98. Purified TMEM98 protein was subjected to the N-terminal sequence. The figure shows the maps of the detected amino acids. They are METVVIVAIG, respectively, from the N terminus. TMEM98, transmembrane protein 98.



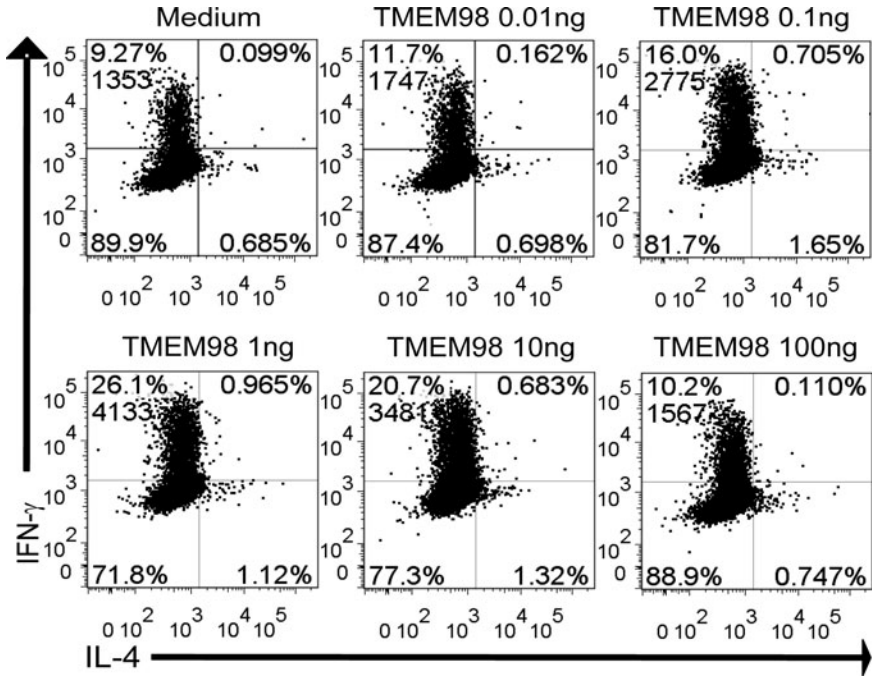

**SUPPLEMENTARY FIG. S2.** Expression profile of *TMEM98* in normal human tissues and immune organs was detected by SqRT-PCR and real-time PCR. **(A)** *TMEM98* was amplified for 35 cycles, and *GAPDH* was amplified for 22 cycles. **(B)** As for real-time PCR, values were normalized to the internal standard gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The expression level of *TMEM98* in peripheral leukocyte was set as 1. PCR, polymerase chain reaction; SqRT-PCR, semiquantitative reverse transcription–polymerase chain reaction.



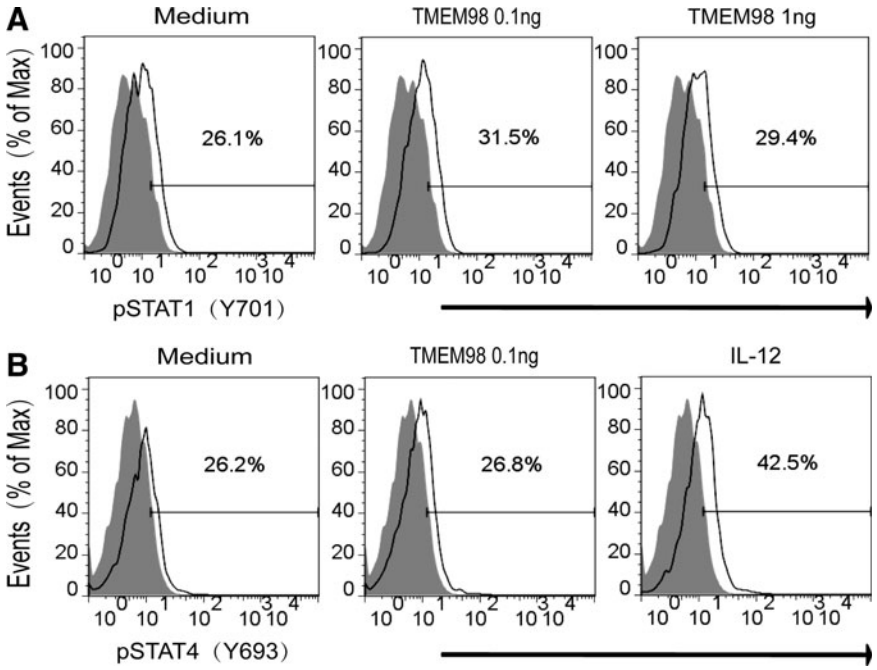

**SUPPLEMENTARY FIG. S4.** Purification of the eukaryotic and prokaryotic proteins of TMEM98. **(A)** The eukaryotic TMEM98-myc-DDK protein purification. Purity was >90% as determined by SDS-PAGE and Coomassie blue staining. **(B)** The prokaryotic purification of TMEM98 (27AA–226AA) (*left*). The GST tag was removed using the thrombin (*right*). The *arrows* show the position and the molecular weight of the target protein.




**SUPPLEMENTARY FIG. S3.** Immunoprecipitation of the secreted TMEM98 using pAb1. Supernatants (500 μL) collected from the HEK293T cells transiently transfected with pcDB-TMEM98 or the pcDB vector were immunoprecipitated using pAb1. These samples and the supernatant (50 μL) (*arrows*) were then analyzed by Western blot using anti-myc. HEK, human embryonic kidney; pAb, primary antibody.




**SUPPLEMENTARY FIG. S5.** Prokaryotic protein of TMEM98 promotes the differentiation of Th1 cells *in vitro*. Naïve $CD4^+$ T cells ($1 \times 10^6$/mL) were cultured under Th1 conditions, and various concentrations of prokaryotic rTMEM98 were added. On day 3, the cells were stimulated by PMA and ionomycin for 5 h with GolgiStop, followed by intracellular staining. Data are representative of 3 independent experiments. Live cells were gated for flow cytometry analysis. rTMEM98, recombinant TMEM98; Th1, T helper 1.




**SUPPLEMENTARY FIG. S6.** TMEM98 can slightly increase the phosphorylation of STAT1, but not STAT4. **(A)** After being stimulated with immobilized anti-CD3e and anti-CD28 Abs for 48 h, naïve $CD4^+$ T cells ($2 \times 10^6$/mL) were washed and restimulated with anti-CD3e and anti-CD28 in serum-free conditions for 24 h. The cells were then treated with eukaryotic rTMEM98 for 20 min. The level of phosphorylated STAT1 was determined by flow cytometry. **(B)** Naïve $CD4^+$ T cells were prepared as described above and were then treated with IL-12 (100 ng) or eukaryotic rTMEM98 for 10 min. The level of phosphorylated STAT4 was determined by flow cytometry. The shading represents staining with the isotype control. This experiment was repeated at least thrice.