Statins reduce T cell inflammatory and pathogenic responses by inducing Kruppel-like factor 2 expression

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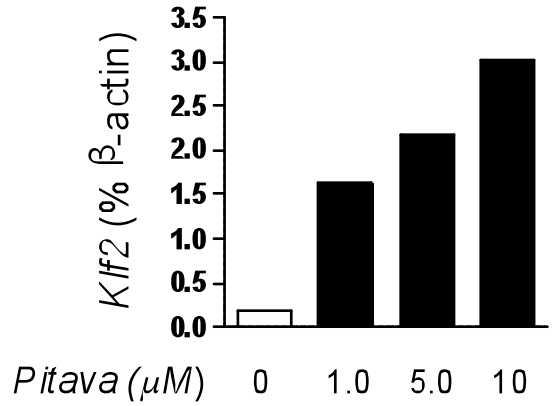
Nonstandard abbreviations used: KLF2, kruppel-like factor 2; S1PR1, sphingosine 1 phosphate receptor 1; PHA, phytohemagglutinin; IP-10, IFN-γ inducible protein 10;GGPP, geranygeranyl pyrophosphate;FPP, farnesyl pyrophosphate; GGTI, geranyl geranyl transferase inhibitor;FTI, farnesyl transferase inhibitor.

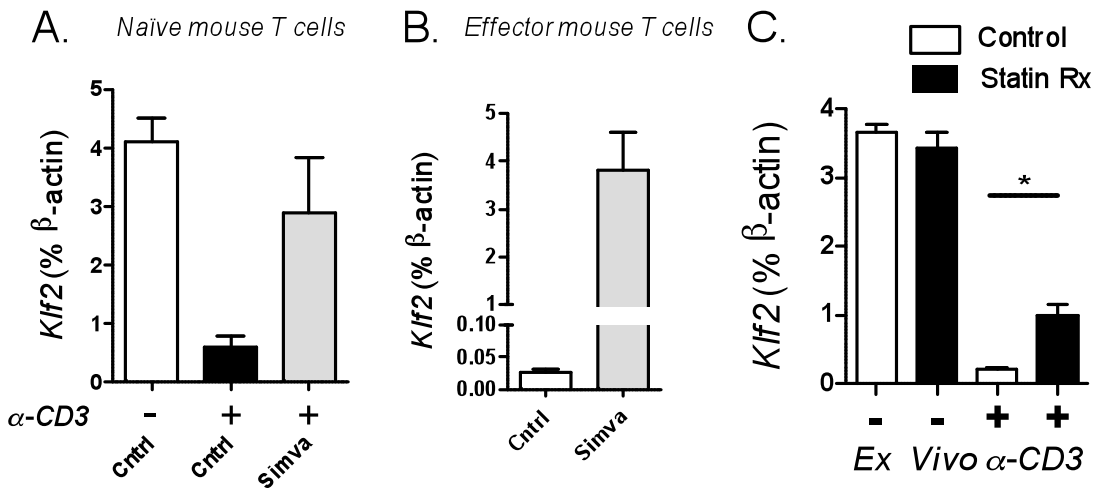
Conflict of interest: The authors have declared that no conflict of interest exists**.**

Gene	Forward Primer	Reverse Primer
MOUSE		
β–actin	5’-TCC TTC GTT GCC GGT CCA-3’	5’-ACC AGC GCA GCG ATA TCG TC-3’
Klf2	5’-ACA GAC TGC TAT TTA TTG GAC CTT AG-3’	5’-CAG AAC TGG TGG CAG AGT CAT TT-3’
Ccr7	5’-CCA GAC CGT GGC CAA TTT CAA CAT-3’	5’-ACA AGA AAG GGT TGA CAC AGC AGC-3’
Ccr5	5’-ACT GCT GCC TAA ACC CTG TCA TCT-3’	5’-TTC ATG TTC TCC TGT GGA TCG GGT-3’
Sell (Cd62l)	5’-CAT TCC TGT AGC CGT CAT GG-3’	5’-AGG AGG AGC TGT TGG TCA TG-3’
Ifng	5’-AAC GCT ACA CAC TGC ATC TTG G-3’	5’-GCC GTG GCA GTA ACA GCC-3’
S1pr1	5’-GTG TAG ACC CAG AGT CCT GCG-3’	5’-AGC TTT TCC TTG GCT GGA GAG-3’
Vcam1	5’-CCA AAT CCA CGC TTG TGT TGA-3’	5’-GGA ATG AGT AGA CCT CCA CCT-3’
Cxcl10	5’-GCC GTC ATT TTC TGC CTC A-3’	5’-CGT CCT TGC GAG AGG GAT C-3’
Ccl5	5’-CAA GTG CTC CAA TCT TGC AGT C-3’	5’-TTC TCT GGG TTG GCA CAC AC-3’
HUMAN		
β–actin	5’-GAG CTA CGA GCT GCC TGA CG-3’	5’-GTA GTT TCG TGG ATG CCA CAG GAC T-3’
KLF2	5’-CTT TCG CCA GCC CGT GCC GCG-3’	5’-AAG TCC AGC ACG CTG TTG AGG-3’
IFNG	5'-ATA TTT TAA TGC AGG TCA TTC AGA TGT AG-3'	5'-TGA AGT AAA AGG AGA CAA TTT GGC T-3'
CD59	5’-ATG CGT GTC TCA TTA C-3’	5’-TTC TCT GAT AAG GAT GTC-3’

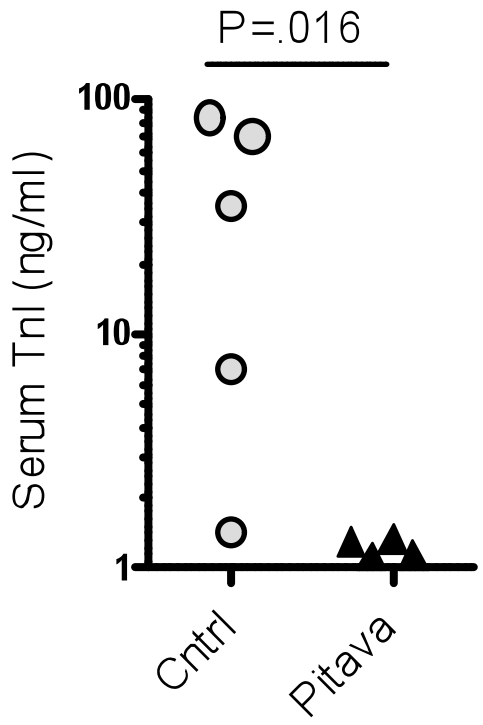
Supplemental Table 1: Oligonucleotide primers used for qRT-PCR



Supplemental Figure 1. Dose response of pitavastatin induction of *Klf2* mRNA in effector mouse T cells. *In vitro*-generated effector OT-1 cells were treated with the indicated concentrations of pitavastatin for 18h before RNA isolation and qRT-PCR analysis of *Klf2*.



Supplemental Figure 2. Statins increase mouse CD4$^+$ T cell *Klf2* mRNA expression. Naïve OT-1I (CD4$^+$) T cells were treated with the vehicle only (control), or 10 μM simvastatin (Simva) for 18h, then stimulated with αCD3 for 6h before RNA isolation and qRT-PCR analysis of *Klf2* (A). *In vitro*-generated effector OT-II cells were treated with the vehicle (control) or simvastatin for 18h, before RNA isolation and qRT-PCR analysis of *Klf2*. The data are the mean ± s.d. of two experiments. C57BL/6 mice were injected i.p. with 20mg/kg lovastatin or DMSO vehicle control for 3 consecutive days. Splenic CD4$^+$ T cells were purified and cultured with or without α-CD3 for 6h, RNA was isolated and qRT-PCR analysis was performed for *Klf2* expression (C), Data in C are the mean ± S.E.M. from 3 experiments.



Supplemental Figure 3. ***In vivo*** **statin treatment reduces pathogenicity of T cells.** cMy-mOva mice were fed pitavastatin (30 mg/kg) or vehicle (control) by gavage twice per day for 8 days consecutive days. On the third day of statin treatment, 5 x 10^4 effector OT-1 cells were adoptively transferred into the mice. The day following the last pitavastatin treatment, the mice were sacrificed, and serum was sampled for determination of troponin I levels. Data are the mean ± S.E.M. of samples from 4 or 5 mice in each group.