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Supplementary Materials for

VSIG4 mediates transcriptional inhibition of *Nlrp3* and *Il-1*β in macrophages

Xiaoyong Huang, Zeqing Feng, Yuanzhong Jiang, Jialin Li, Qun Xiang, Sheng Guo, Chengying Yang, Lei Fei, Guoning Guo, Lixin Zheng, Yuzhang Wu*, Yongwen Chen*

*Corresponding author. Email: chenyongwen@tmmu.edu.cn (Y.C.); wuyuzhang@tmmu.edu.cn (Y.W.)

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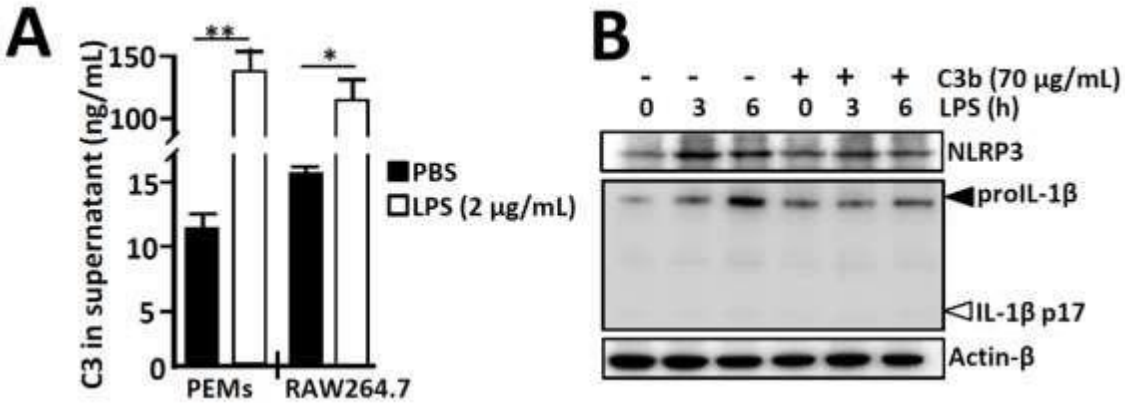

Fig. S1. Liganding VSIG4 by complement C3b results from decreasing NLRP3 and proIL-1β expression in macrophages. (**A**) PEMs and RAW264.7 cells were treatment with LPS (2 μg/mL) or PBS vehicle for 24h, ELISA analysis of C3 in supernatants. (**B**) The expression of VSIG4 in human THP-1 cells was induced by PMA, and cells were further treated with microbeads-C3b (70 μg/mL) in the presence of LPS (2 μg/mL), cell extracts were immunoblotted for NLRP3 and proIL-1β. Error bar, s.e.m. NS: not significant different, *p<0.05 and **p<0.01 (Student's t-test). Data represent one out of three biological replicates, with three technical replicates each at least.

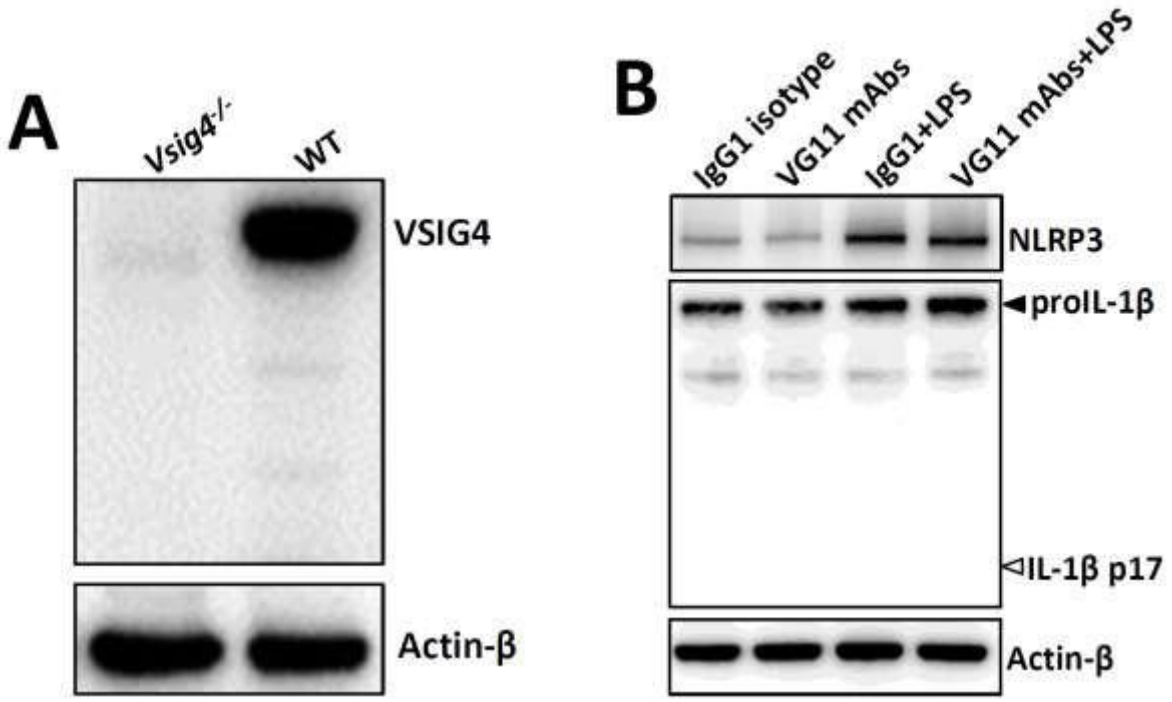

Fig. S2. The expression of NLRP3 and IL-1β in *Vsig4*$^{-/-}$ PEMs was not affected by VG11 mAbs. (**A**) Western-blot analysis of VSIG4 protein in the liver tissues isolated from C57BL/6 WT mice and *Vsig4*$^{-/-}$ littermates using the VG11 mAbs. (**B**) PEMs from *Vsig4*$^{-/-}$ mice were treatment with the VG11 mAbs (50 μg/mL) or isotype IgG1 (50 μg/mL) for 6h, cells were further activation without or with LPS (2 μg/mL) for 6h, cell extracts were immunoblotted for NLRP3 and proIL-1β. Black arrows on western blots denote proIL-1β (p31) and white arrows denote the processed p17 subunit (p17). Data represent one out of three biological replicates, with three technical replicates each at least.

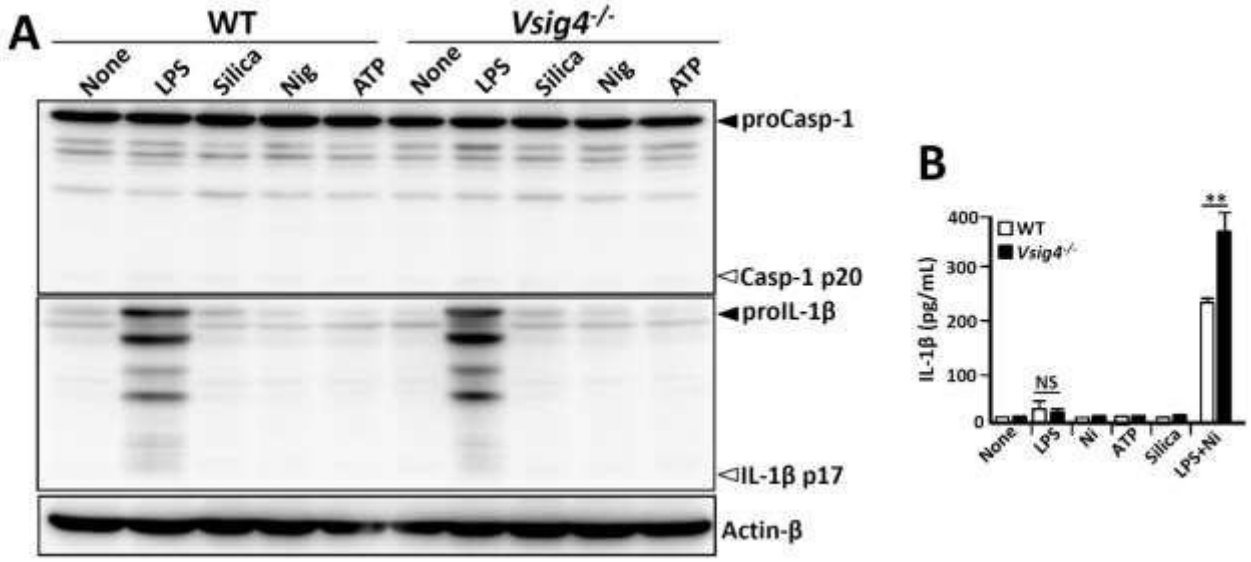

Fig. S3. *Vsig4* deficiency does not cause spontaneous NLRP3 inflammasome activation and IL-1β secretion. PEMs were isolated from C5BL/6 WT and *Vsig4*$^{-/-}$ mice, cells were stimulated with 2 μg/mL LPS for 3h, treated with 1.5 μM ATP or 5μM nigericin for 45 min, or added with SiO_2 (500 μg/mL) for 4h, respectively, (**A**) cell extracts were immunoblotted for caspase-1 and IL-1β, Black arrows on western blots denote proCasp-1 (p45) and proIL-1β, white arrows denote the processed Caspapse-1 p20 subunit (p20) and the matured IL-1β p17, respectively. (**B**) ELISA analysis of IL-1β in culture supernatants, PEMs were stimulated with 2 μg/mL LPS for 3h and then treated with 5 μM nigericin for 45 min used as positive controls. Error bar, s.e.m, $^{**}p<0.01$ (Student's *t*-test). One out of three biological replicates, with three technical replicates each.

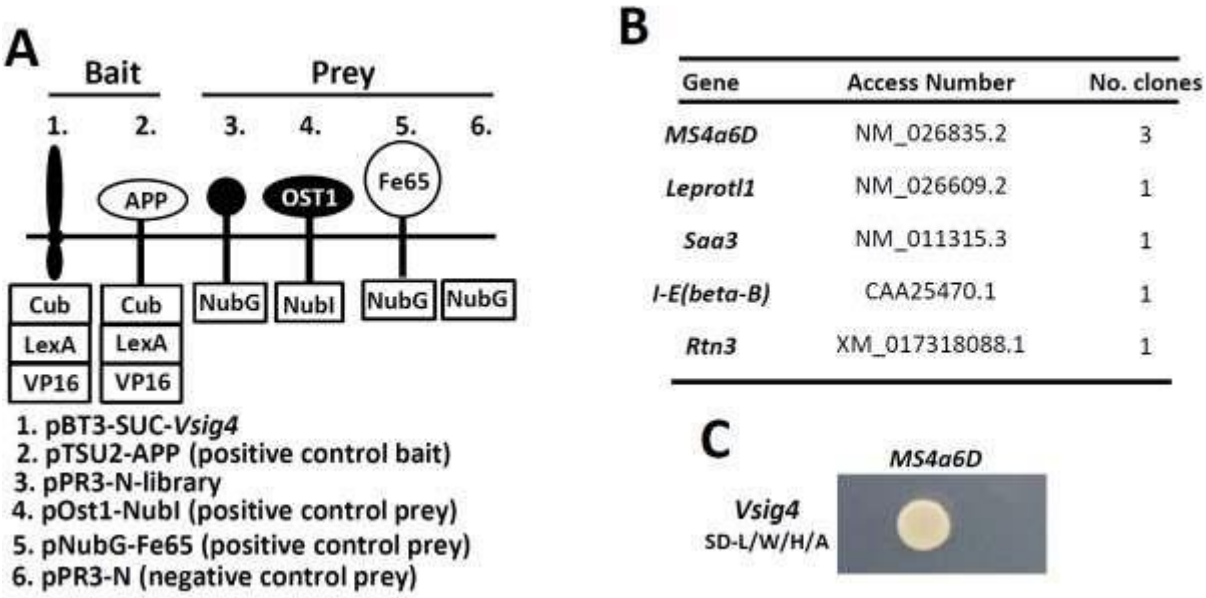

Fig. S4. MS4A6D interacts with VSIG4 by using a yeast split-ubiquitin screen. (**A**) Schematic illustrating topology of bait and prey constructs used in the split-ubiquitin screen. (**B**) Identification of bait-dependent *Vsig4* binding partners from the yeast split-ubiquitin screen. (**C**) Re-transformation experiments analysis of VSIG4 crosslinks with MS4A6D.

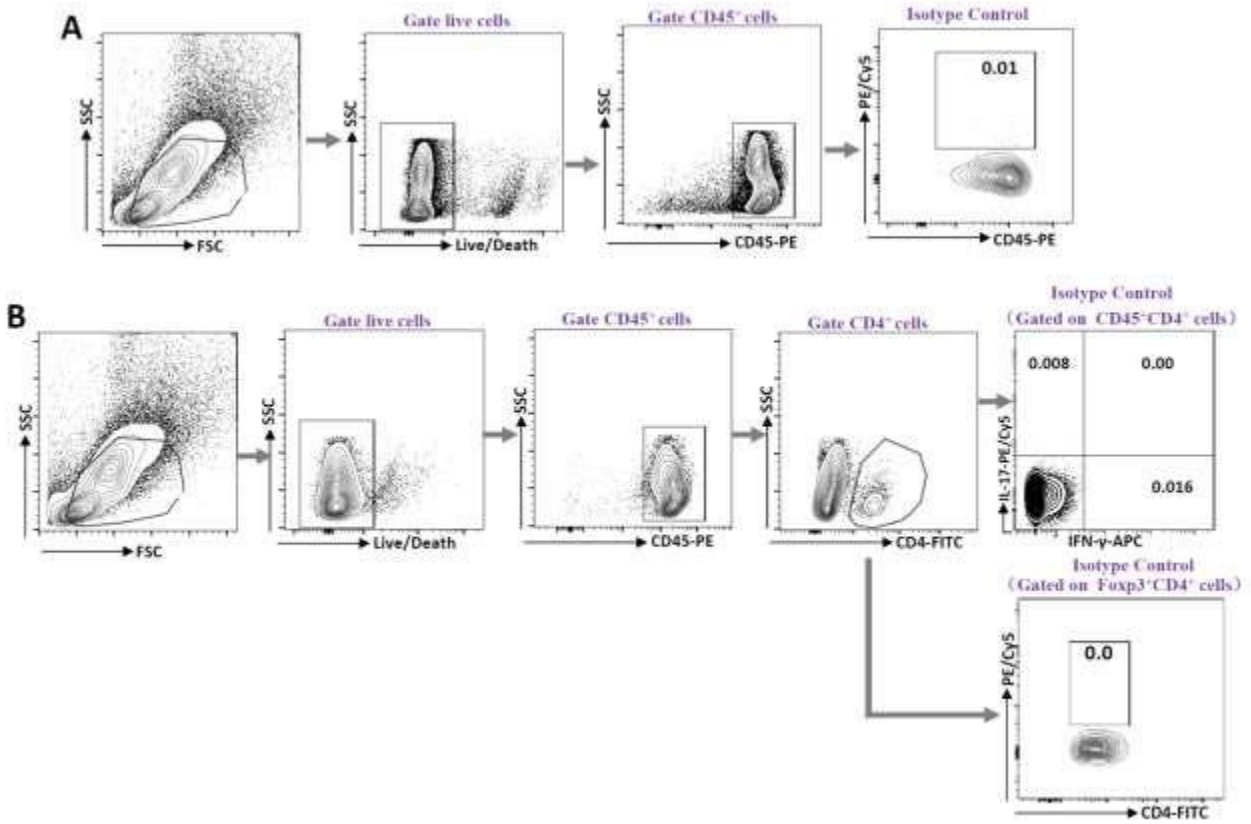

Fig. S5. Gating strategies of flow cytometry. (**A**) Flowcytometry gating strategy used in Fig. 4C and **H**; Fig. 5 **J** and **K**. (**B**) Flowcytometry gating strategy used in Fig. 4E, Fig. 5K and Fig. 6 E.

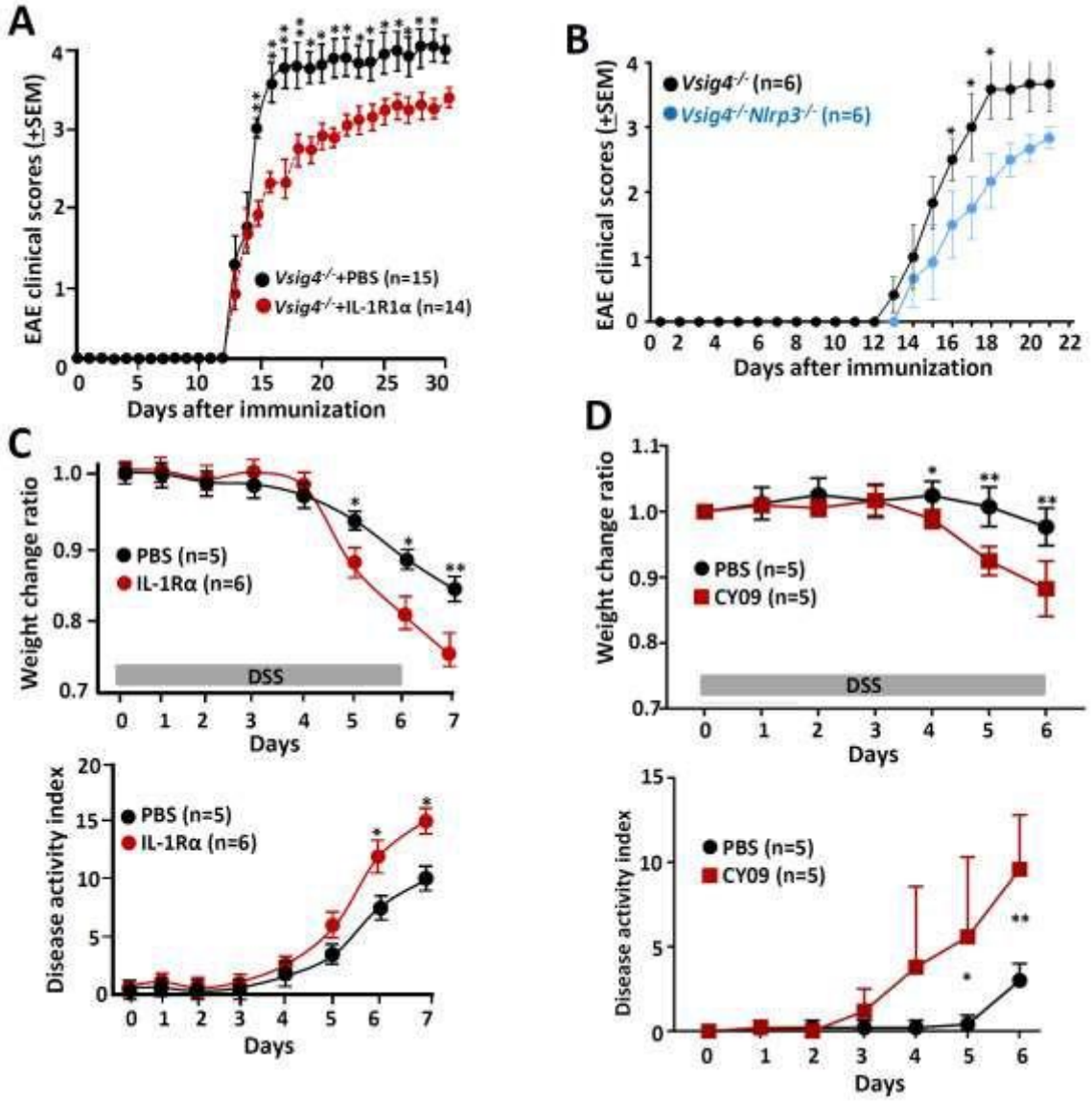

Fig. S6. Inhibition of NLRP3 activity or blocking IL-1β signaling controls the progress of EAE and colitis. (**A**) The *Vsig4*$^{-/-}$ mice were induced to develop EAE by treated with $MOG_{35\sim55}$ peptides in the presence of IL-1Rα (200 ng/mouse/3 days) or PBS vehicle, the clinical scores were assessed and compared daily. Data are merged of two independent experiments with similar results. (**B**) The *Vsig4*$^{-/-}$ and *Nlrp3*$^{-/-}$*Vsig4*$^{-/-}$ DKO mice were induced to develop EAE by treated with $MOG_{35\sim55}$ peptides, the clinical scores were assessed and compared daily. One of three independent experiments with similar results was shown. *Vsig4*$^{-/-}$ mice were given 3.5% DSS in their drinking water for 6 days to induce colitis, mice were also treated with (**C**) IL-1Rα (100 ng/mouse/day) or (**D**) CY09 (50 μg/mouse/day) or PBS- vehicle for continuous 6 days, comparative analysis of body weight change ratio and disease activity index daily. One of three independent experiments with similar results was shown. Error bar, s.e.m. *p<0.05 and **p < 0.01 (Student's t-test).

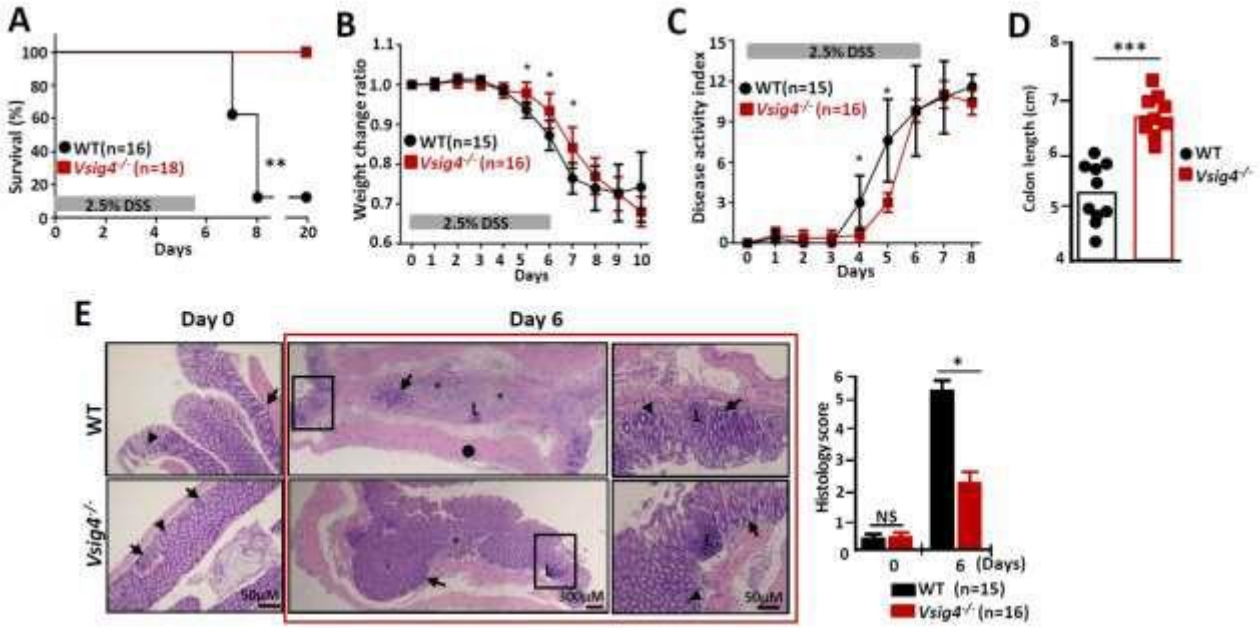

Fig. S7. *Vsig4*$^{-/-}$ mice are resistant to 2.5% DSS-induced colitis. Mice were given 2.5% DSS in their drinking water for 6 days to induce colitis, (**A**) the survival rate was assessed daily for a total of 20 days. Data are merged of five independent experiments with similar results. Comparative analysis of (**B**) body weight and (**C**) disease activity index, data are merged of three independent experiments with similar results. The colonic tissues were collected at day 6 after DSS fed, (**D**) colon length was compared, each circle represents an individual mouse. (**E**) H&E staining analysis of histopathological changes (left), and semi-quantitative scoring of histopathology (right). The circle indicated swelling vessels, asterisk indicated severe edema/inflammation with large lymphoid nodules (L), arrows indicated retention/regeneration of crypts, and arrowheads showed the normal epithelium. Scale bar=20 μm. Error bar, s.e.m. *p<0.05, **p < 0.01 and ***p < 0.001. (A) was analyzed by a log-rank test, others were compared by Student's *t*-test.

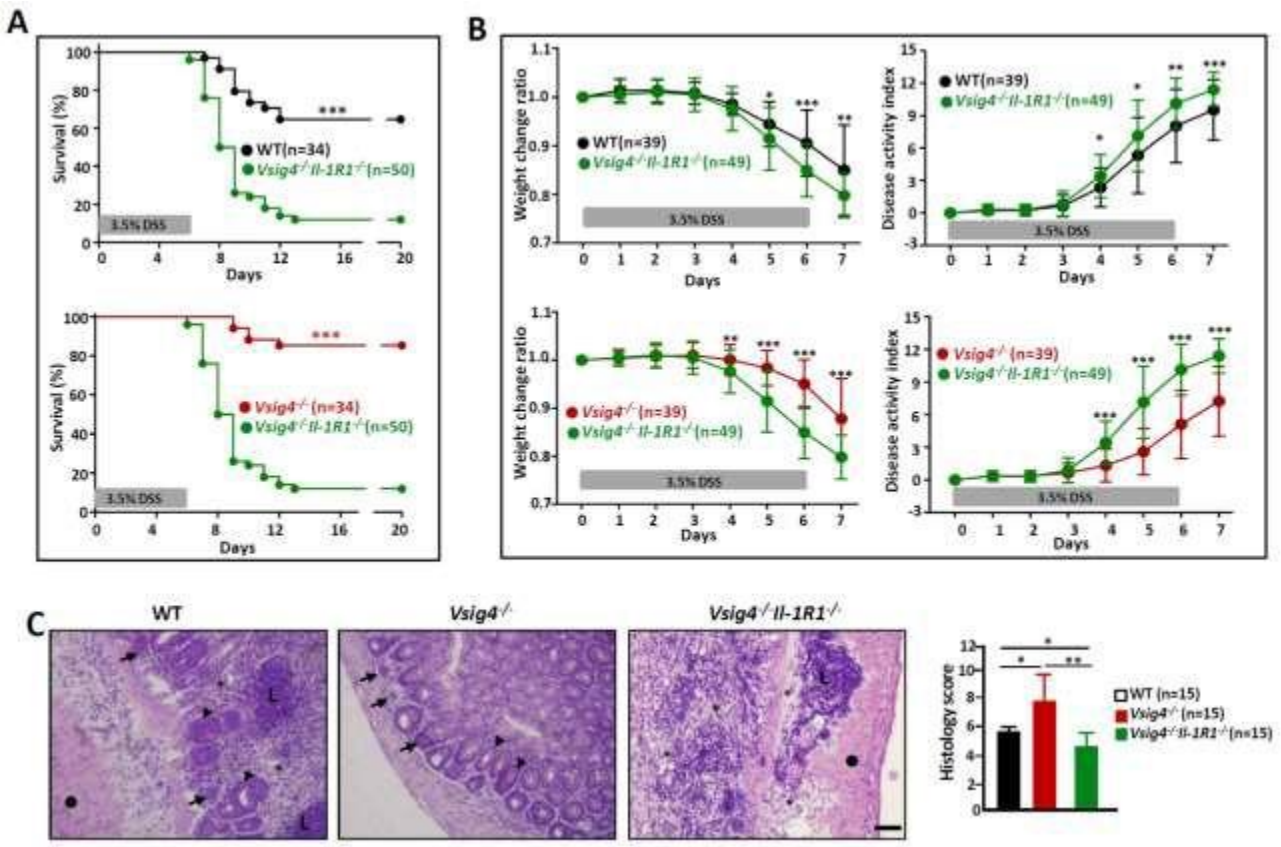

Fig. S8. *Vsig4*$^{-/-}$*Il-1R1*$^{-/-}$ mice deteriorated DSS-induced colonic damage. Mice were given 3.5% DSS in their drinking water for 6 days to induce colitis, (**A**) the survival rate was assessed daily for a total of 20 days. Data are merged of six independent experiments with similar results. (**B**) Comparative analysis of body weight and disease activity index. Data are merged of six independent experiments with similar results. (**C**) The colonic tissues were collected at day 6 after DSS fed, H&E staining analysis of histopathological changes (left), and semi-quantitative scoring of histopathology (right). The circle indicated swelling vessels, asterisk indicated severe edema/inflammation with large lymphoid nodules (L), arrows indicated retention/regeneration of crypts, and arrowheads showed the normal epithelium. Scale bar=20 μm. Error bar, s.e.m. **p*<0.05, ***p* < 0.01 and ****p* < 0.001. (A) was analyzed by a log-rank test; (B) and (C) were compared by Student's *t*-test.

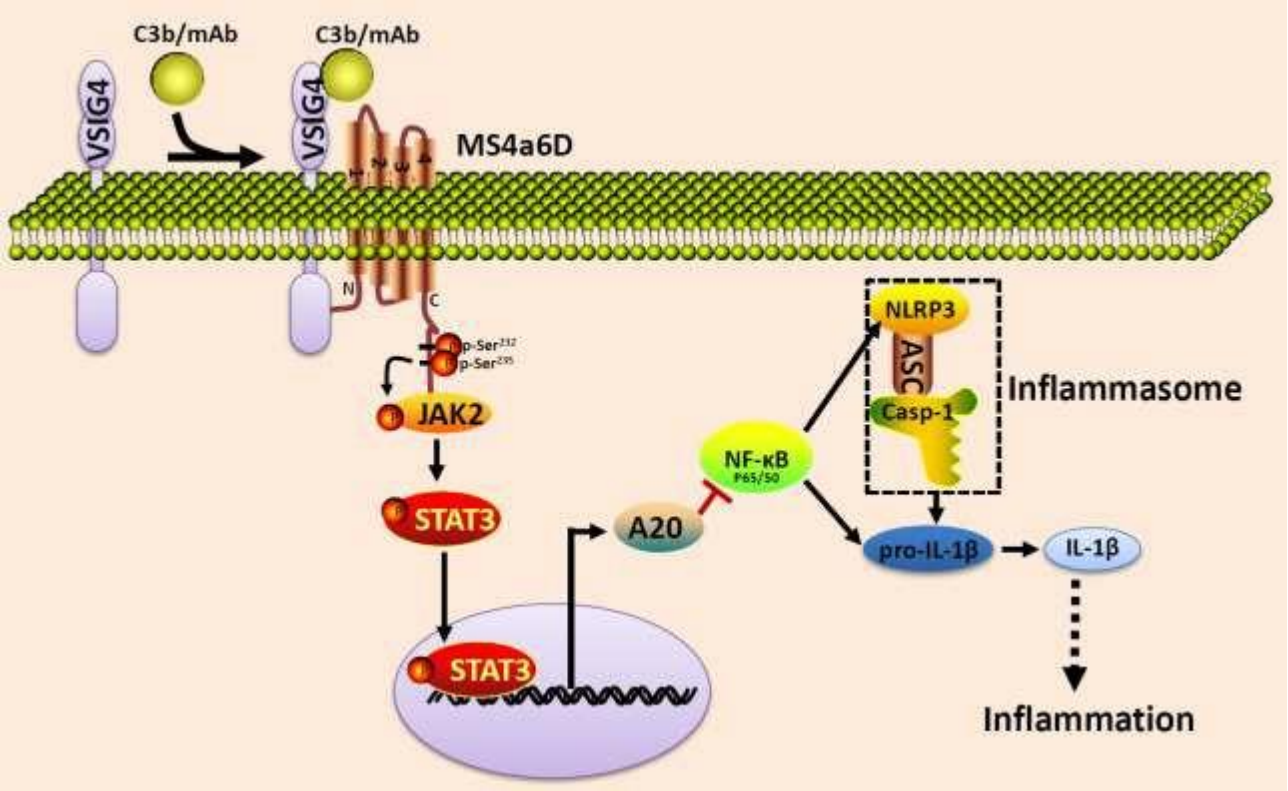

Fig. S9. Model of VSIG4/MS4A6D signaling complex attenuates NLRP3 inflammasome activation. Liganding VSIG4 with complement C3b or the agonist VG11 mAbs results from intracellular Ser^{273}/Ser^{276} phosphorylation of VSIG4, by thus recruits VSIG4/MS4A6D interaction and form a surface inhibitory signaling complex (SISC), Both Ser^{232} and Ser^{235} phosphorylation at C-terminal of MS4A6D further triggers JAK2-STAT3-A20 cascades to inactivate NF-kB. Finally, VSIG4 prevents *Nlrp3* and *Il-1β* gene transcription, in turn controls the progress of NLRP3-mediated pathogenesis.

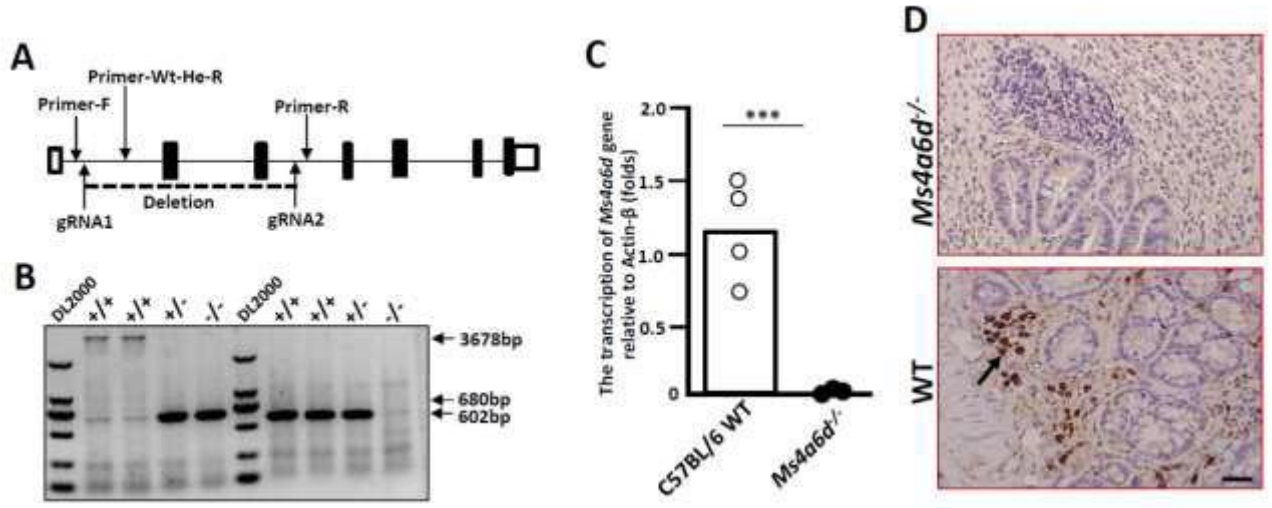

Fig. S10. Creating *Ms4a6d*$^{-/-}$ mice by CRISPR/Cas9-mediated genome engineering. (**A**) Seven exons have been identified, with the ATG start codon in exon 2 and TAA stop codon in exon 7. Exon 2 and exon 3 were selected as target sites. (**B**) Screening of *Ms4a6d*$^{-/-}$ mice by PCR, the PCR products for WT: 3678bp and 602bp, Heterozygous: 680bp and 602bp; KO: only 680bp. (**C**) The PEMs were isolated from C57BL/6 WT mice and their *Ms4a6d*$^{-/-}$ littermates, and qRT-PCR analysis of *Ms4a6d* gene transcription, each circle represents one mouse. (**D**) Immunohistochemistry analysis of the expression of MS4A6D in colonic tissues isolated from WT and *Ms4a6d*$^{-/-}$ mice. Arrow indicated positive cells, scale bar= 40μm.

Table S1. The PCR primers used in this study.

Item	gene	Sense	Anti-sense
Primers for qRT-PCR	*IL-1β*	5' -CAGGCAGGCAGTATCACTCAT TG-3`	5'-CGTCACACACCAGCAGGTTATC-3`
	IL-18	5' -TGAAGTAAGAGGACTGGCTG TGA-3`	5'-ATCTTGTTGTGTCCTGGAACACG-3`
	Nlrp3	5' -CCTGACCCAAACCCACCAGT-3`	5'-TTCTTTCGGATGAGGCTGCTTA-3`
	Caspase-1	5' -AAGAACAGAACAAAGAAGA TGGA-3`	5'-ACCCTCGGAGAAAGATGTTGAAA-3`
	Vsig4	5' -GGATCCCACCCCACCCTAAAAACA-3`	5' -CTCGAGTCAGCAGGCAGGAATAGA-3`
	Actin-β	5'-CACTATCGGCAATGAGCGGTTCC-3`	5'-CAGCACTGTGTTGGCATAGAGGTC-3`
	Ms4a6d	5'-TGTGGTGGAAACAGAGCTCC-3`	5'-TTCTGCTATATAACCTCTAC-3`
Primers for shRNA	*Ms4a6d#1*	5'-aattaatctggctacccatttgtagctcgagctacaaatgggtagccagatttttttttat-3'	5'-aaaaaaaaatctggctacccatttgtagctcgagctacaaatgggtagccagatt-3'
	Ms4a6d#2	5'-aattcagcaatgtaagctggctttcctcgaggaaagccagcttacattgctgttttttttat-3'	5'-aaaaaaacagcaatgtaagctggctttcctcgaggaaagccagcttacattgctg-3'
	Ms4a6d#3	5'-aattgagccattaaacagctgcttcctcgaggaagcagctgtttaatggctctttttttat-3'	5'-aaaaaaagagccattaaacagctgcttcctcgaggaagcagctgtttaatggctc-3'
	Stat3 #1	5'-ccggcagcacaaccttcgaatcctcgaggattcttcgaaggtttggctgtttttg-3'	5'-aaggcaaaaacagcacaaccttcgaagaatcctcgaggattcttcgaaggttgtgctg-3'
	Stat3 #2	5'-ccggcagcacaaccttcgaagaatcctcgaggattcttcgaaggttgtgctgttttg-3'	5'-aattcaaaaacagcacaaccttcgaagaatcctcgaggattcttcgaaggttgtgctg -3'
	Stat3#3	5'-aattaagcgtaatctccaggataacctcgaggttatcctggagattacgctttttttttat-3'	5'-aaaaaaaaagcgtaatctccaggataacctcgaggttatcctggagattacgctt-3'
	A20	5'-aattgctatcactcatggatataaactcgagtttatatccatgagtgatagctttttttat-3'	5'-aaaaaaagctatcactcatggatataaactcgagtttatatccatgagtgatagc-3'
Primers for ChIP-qPCR	STAT3 binding motif in *A20* promoter (660-670)	5'-GAAGCACATGTCTCTAGTTC-3'	5'-TGTGAGTGTGTATCTCTAGG-3'
	STAT3 binding motif in *A20* promoter (486-496)	5'-CATCAGGTCACTCCGCAGAC-3'	5'-AGACCTCAAGGGTCAGCCAC-3'
Primers for *A20* gene promoter luciferase	*P2000*	5'-GGGGTACCCCTGGGTTCGTTGAGGAACTGTC-3'	5'-GGCTAGCCGTGCCAACAGGGGGATTTCCGAT-3'
	P1300	5'-GGGGTACCCCTGGCACTGAGTATTTCTATCTG-3'	5'-CGGCTAGCCGTGCCAACAGGGGGATTTCCGAT-3'
	P1000	5'-GGGGTACCCCTCTGCTCAGCC	5'-CGGCTAGCCGTGCCAACAGG

assay		CCTTGATGTAT-3'	GGGATTTCCGAT-3'
Primers for whole gene expression of *Vsig4*, *Vsig4* truncation mutants and *Vsig4* site-directed mutagenesis	*Vsig4*$_{\text{whole}}$ (human)	5'-CCGGAATTCATGGGGATCTTACTGGGCCTG-3'	5'-AAGGAAAAAAGCGGCCGCACAGACACTTTTGCCCTCAGT-3'
	Vsig4$_{\text{whole}}$ (mouse)	5'-AGTCGCTAGCCACCATGGACTACAAGGACGACGATGACAAGCTAATAGTGCTCACCTATGG-3'	5'-AGTCGGATCCCTCTTGTTGGAATGTCCTGCA- 3'.
	Vsig4$_{\Delta 119\text{-}280}$	5'-TAGTCTAGCTAGCATGCGCTGGGTCCGGGC-3'	5'-AGTCGGATCCCTGGCTGAGGCAAGGATCATCA- 3'
	Ms4a6d$_{whole}$	5'-CTAGCTAGCATGATTCCACAAGTAGTGAC-3'	5'-AAGGAAAAAAGCGGCCGCGCGTAATCTGGAACATCGTATGGGTATGAAGTCAATATGTTTTCAT-3'
	Ms4a6d$_{\Delta 210\sim 247}$	5'-CTAGCTAGCATGATTCCACAAGTAGTGAC-3'	5'-AAGGAAAAAAGCGGCCGCAGCGTAATCTGGAACATCGTATGGGTAgacagccaggccaagctcca-3'
	Ms4a6d$_{\Delta 221\sim 247}$	5'-CTAGCTAGCATGATTCCACAAGTAGTGAC-3'	5'-AAGGAAAAAAGCGGCCGCAGCGTAATCTGGAACATCGTATGGGTAgctctgtttccaccacagtg
	Ms4a6d$_{\Delta 231\sim 247}$	5'-CTAGCTAGCATGATTCCACAAGTAGTGAC-3'	5'-AAGGAAAAAAGCGGCCGCAGCGTAATCTGGAACATCGTATGGGTAgaaaatcacattcccagaga-3'
	Ms4a6d$_{\Delta 241\sim 247}$	5'-CTAGCTAGCATGATTCCACAAGTAGTGAC-3'	5'-AAGGAAAAAAGCGGCCGCAGCGTAATCTGGAACATCGTATGGGTAggaaaacactgaggtaaagtg-3'
	Ms4a6d $_{230S\ to\ A}$	5'-AAAGAATAAATCCGCTGTATCTTCAGAGTCAC-3'	5'-ACTCTGAAGATACA**GC**GGATTTATTCTTTGAG-3'
	Ms4a6d $_{232S\ to\ A}$	5'-TAAATCCAGTGTA**G**CTTCAGAGTCACTTTG-3'	5'-CAAAGTGACTCTGAAGCTACACTGGATTTATT-3'
	Ms4a6d $_{233S\ to\ A}$	5'-AATCCAGTGTATCTGCAGAGTCACTTTGTAACC-3'	5'-GTTACAAAGTGACTCTG**C**AGATACACTGGATTTA-3'
	Ms4a6d $_{235S\ to\ A}$	5'-AGTGTATCTTCAGAG**G**CACTTTGTAACCCTAC-3'	5'-AGGGTTACAAAGTG**C**CTCTGAAGATACACTGG-3'
	Ms4a6d $_{241Y\ to\ A}$	5'-TTTGTAACCCTACA**GC**TGAAAACATATTGACTT-3'	5'-GTCAATATGTTTTCA**GC**TGTAGGGTTACAAAGTG-3'