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# Supplemental Information

# *O*-GlcNAc transferase links glucose metabolism to MAVS-mediated antiviral innate immunity

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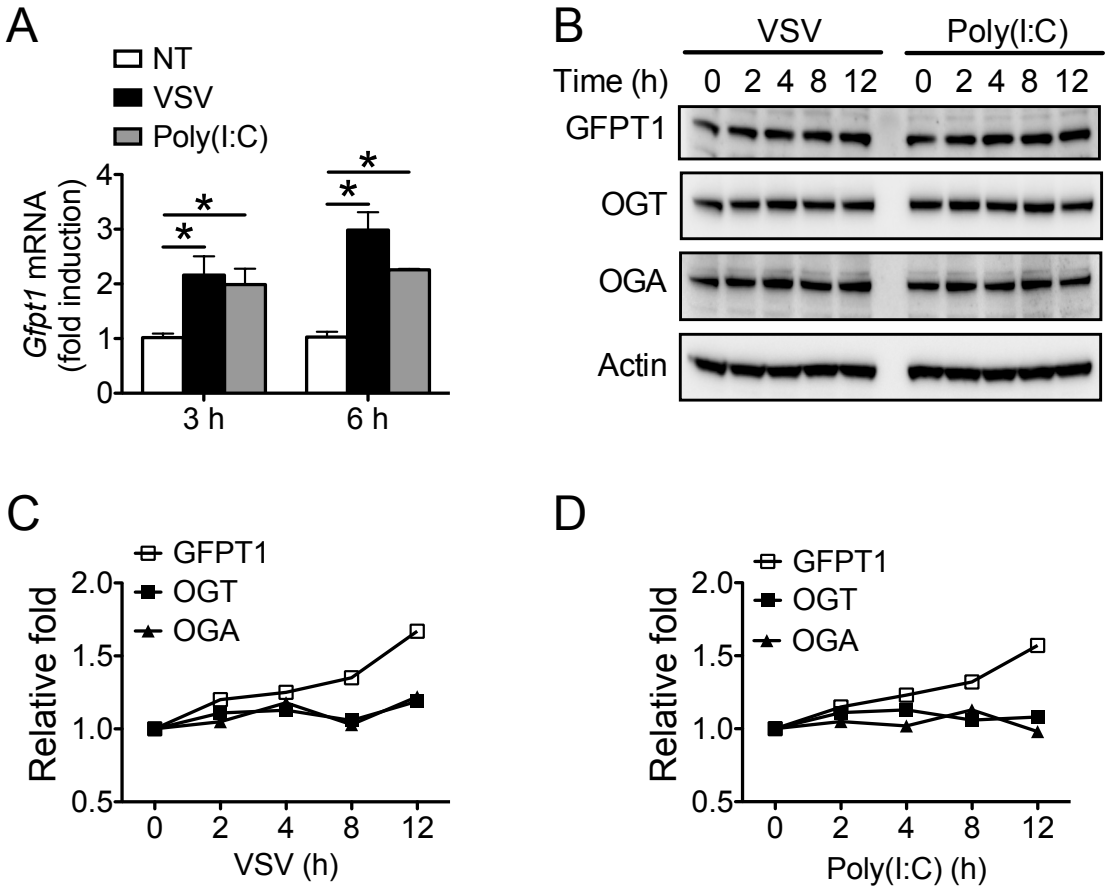


**Figure S1. Related to Figure 1.** Effect of RLR activation on expression levels of key HBP enzymes. (A-D) BMMs generated from C57BL/6 mice were left untreated, or stimulated with either vesicular stomatitis virus (VSV) (multiplicity of infection (MOI) = 1) or transfected poly(I:C) for indicated periods. Transcript level of glutamine fructose-6-phosphate transaminase (GFPT1) was assayed by RT-PCR (A). Immunoblotting (B) and densitometric analysis (C and D) to quantify ratio of protein level of GFPT1, OGT and OGA to actin in treated BMMs. * $P < 0.05$, versus controls (two-tailed Student's *t*-test (A)). Data are presented as mean values of biological triplicates ± s.d.

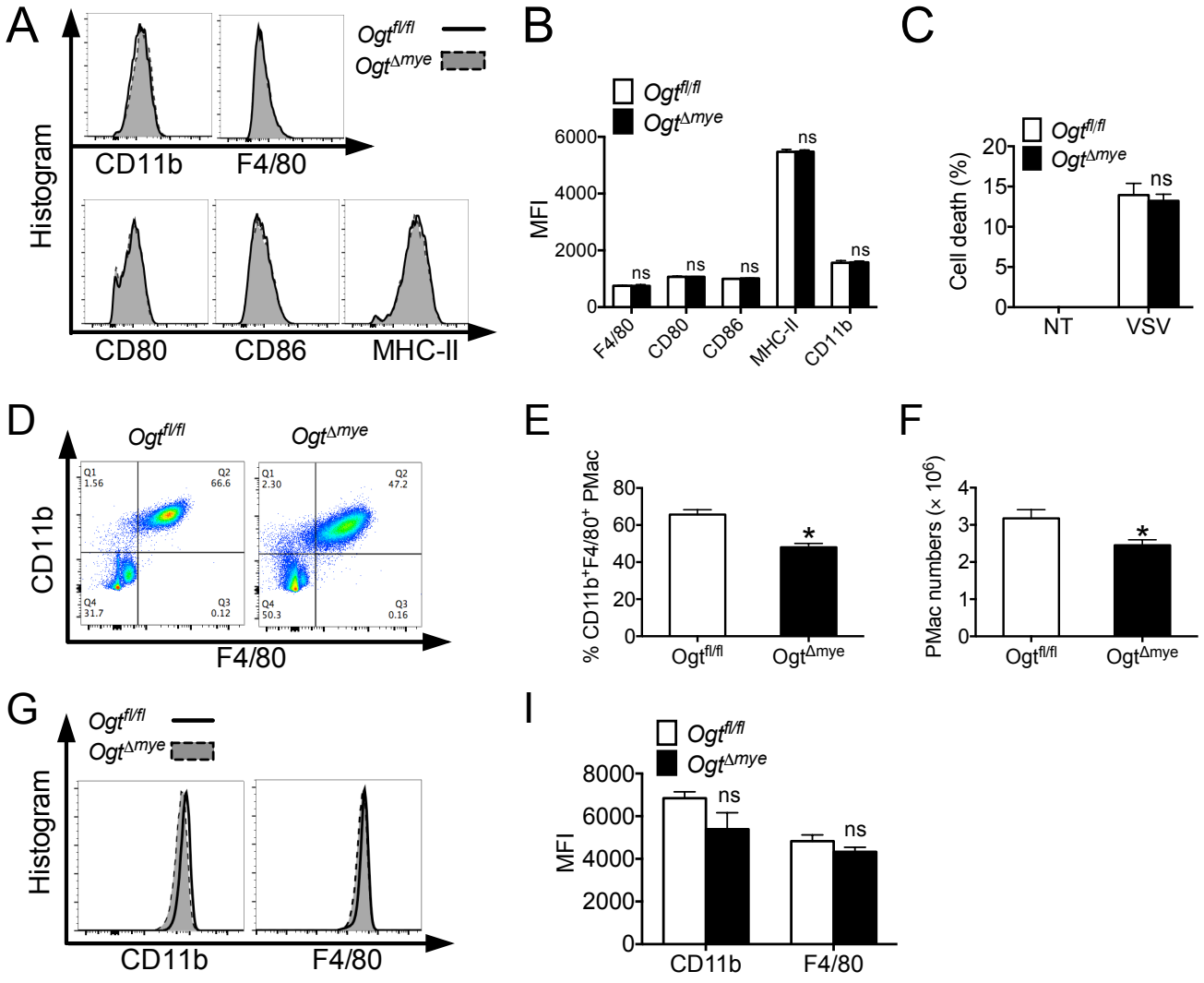


**Figure S2. Related to Figure 2. OGT deletion causes a moderate defect in differentiation of peritoneal macrophages (PMac), whereas does not affect BMM differentiation or cell viability after VSV stimulation.** (A-B) Macrophage differentiation markers CD11b and F4/80, and activation markers CD80, CD86 and MHCII were measured by flow cytometry assay in BMMs generated from *Ogt*$^{fl/fl}$ and *Ogt*$^{\Delta mye}$ mice. Histogram (A) and quantification of the mean fluorescence intensity (MFI) (B) were shown. (C) Cell viability was measured with the Cytotoxicity Detection Kit (LDH) in *Ogt*$^{fl/fl}$ and *Ogt*$^{\Delta mye}$ BMMs in the absence or presence of VSV stimulation (MOI = 1) for 6 h. (D-F) Peritoneal

macrophages ($CD11b^{+}F4/80^{+}$) in $Ogt^{fl/fl}$ and $Ogt^{\Delta mye}$ mice were measured by FACS analysis (D). The percent (E) and total number (F) of PMac were shown. (G-H) Histogram (G) and quantification of the MFI (H) of macrophage differentiation markers CD11b and F4/80 were measured by FACS assay in PMac isolated from $Ogt^{fl/fl}$ and $Ogt^{\Delta mye}$ mice. ns, not significant, versus controls (two-tailed Student's *t*-test (B and C)). Data are presented as mean values of biological triplicates ± s.d.

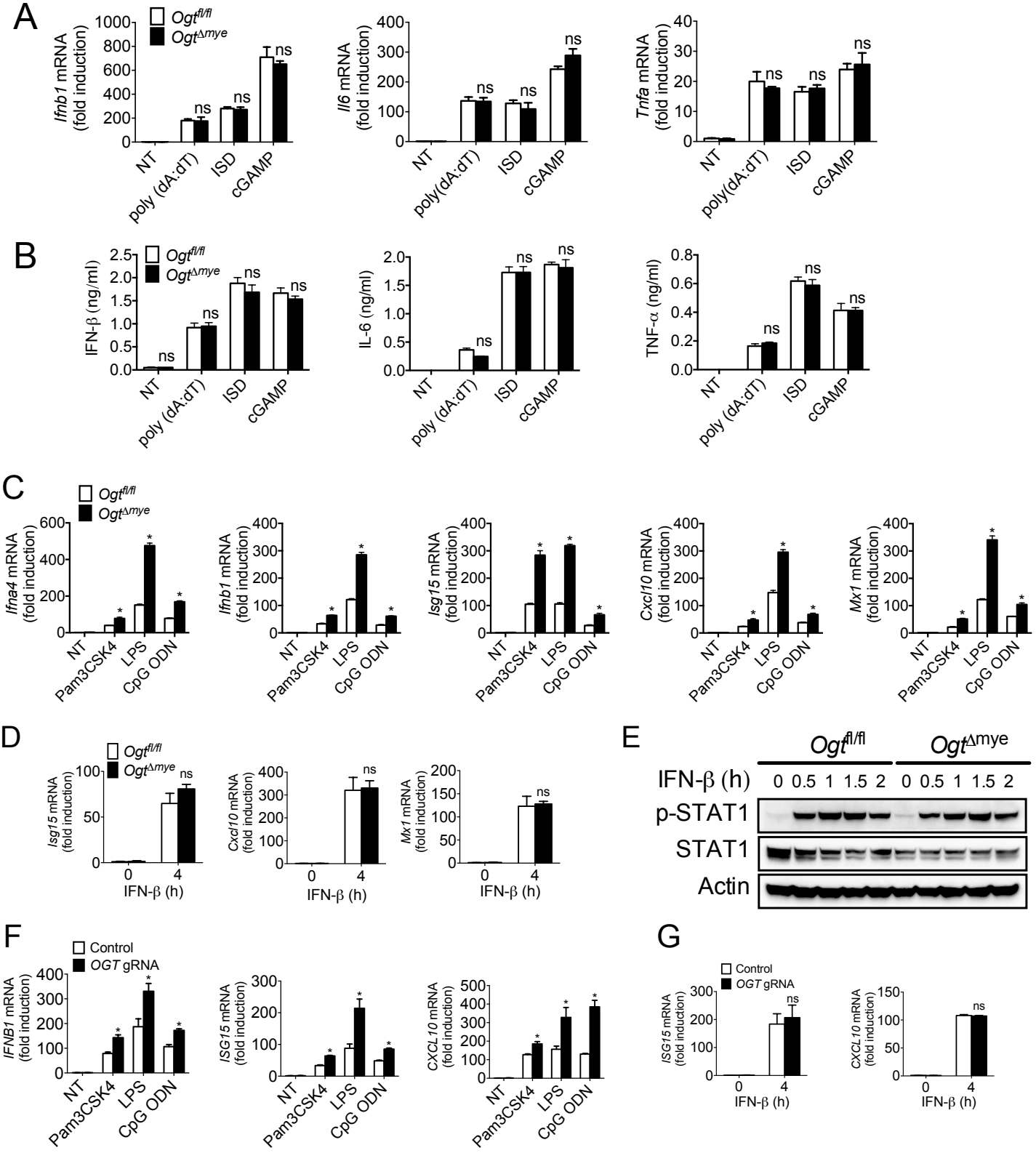
A
Ogtfl/fl
OgtΔmye
Ifnb1 mRNA (fold induction)
Il6 mRNA (fold induction)
Tnfa mRNA (fold induction)
ns
NT
poly (dA:dT)
ISD
cGAMP
B
IFN-β (ng/ml)
IL-6 (ng/ml)
TNF-α (ng/ml)
C
Ifna4 mRNA (fold induction)
Ifnb1 mRNA (fold induction)
Isg15 mRNA (fold induction)
Cxcl10 mRNA (fold induction)
Mx1 mRNA (fold induction)
Pam3CSK4
LPS
CpG ODN
D
Isg15 mRNA (fold induction)
Cxcl10 mRNA (fold induction)
Mx1 mRNA (fold induction)
IFN-β (h)
E
Ogtfl/fl
OgtΔmye
IFN-β (h) 0 0.5 1 1.5 2 0 0.5 1 1.5 2
p-STAT1
STAT1
Actin
F
Control
OGT gRNA
IFNB1 mRNA (fold induction)
ISG15 mRNA (fold induction)
CXCL10 mRNA (fold induction)
G
ISG15 mRNA (fold induction)
CXCL10 mRNA (fold induction)

**Figure S3. Related to Figure 3. No defect in activation of immune signaling other than RLR pathway in OGT-KO cells.** (A to E) BMMs generated from *Ogt*$^{fl/fl}$ and *Ogt*$^{\Delta mye}$ mice were left untreated or transfected with poly(dA:dT), interferon stimulatory DNA (ISD), or cGAMP (all at 2 μg/ml) by lipofectamine 2000 for 6 h (A) or 16 h (B), various TLR agonists including Pam3Cys (500 ng/ml), LPS (100 ng/ml) or CpG oligonucleotide (ODN) 1826 (5 μg/ml) (C), or recombinant mouse IFN-β (20 ng/ml) (D and E). Cytokine gene transcripts in the cells (A, C and D) and cytokine proteins in the supernatants (B) were measured with RT-PCR and ELISA, respectively. (F and G) IFN-I gene transcripts in *OGT*-KO and WT control THP-1 cells left untreated or stimulated with LPS (F) or recombinant human IFN-β. * $P < 0.05$, ns, not significant, versus controls (two-tailed Student's *t*-test (A, B, C, D, F and G)). Data are presented as mean values of biological triplicates ± s.d.

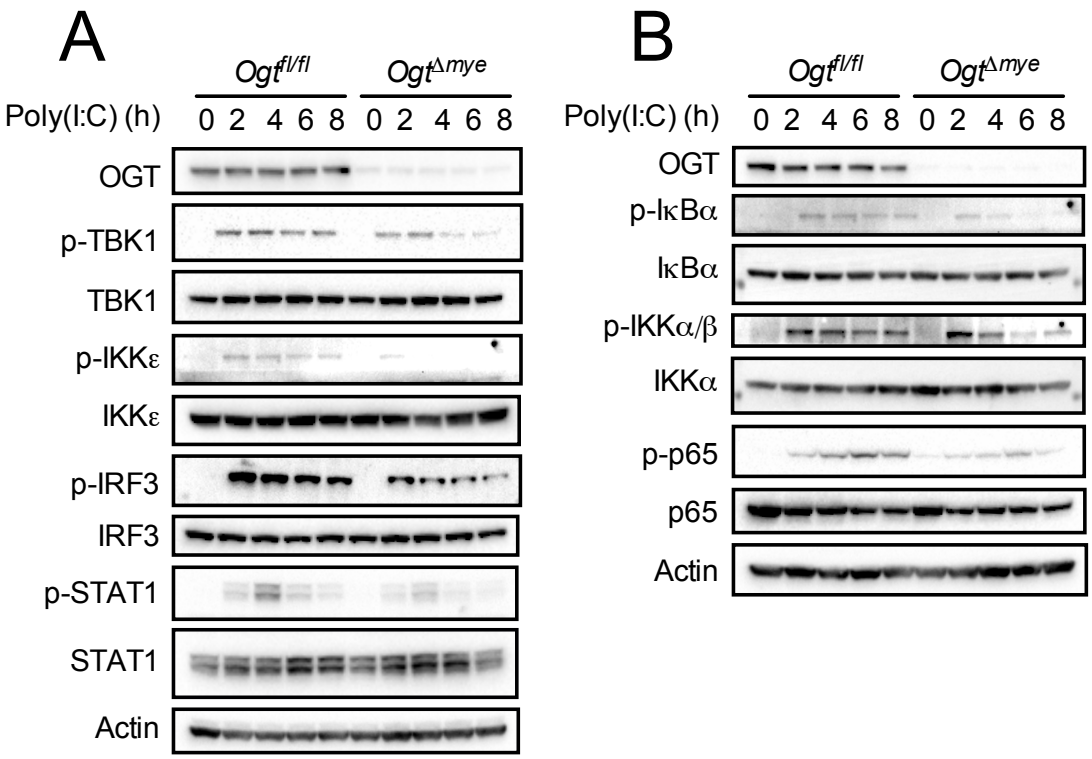


**Figure S4. Related to Figure 3. OGT is critical for the activation of antiviral immune signaling.**

(A and B) BMMs generated from *Ogt*$^{fl/fl}$ and *Ogt*$^{\Delta mye}$ mice were left untreated or transfected with 4 μg/ml poly(I:C) by lipofectamine 2000 for indicated periods. Phosphorylation of IFN-I signaling molecules including TBK1, IKKε, IRF3 and STAT1 (A) and NF-κB signaling molecules including IκBα, IKKα/β and p65 (B) was assessed by immunoblotting. Data are from one experiment representative of three experiments.

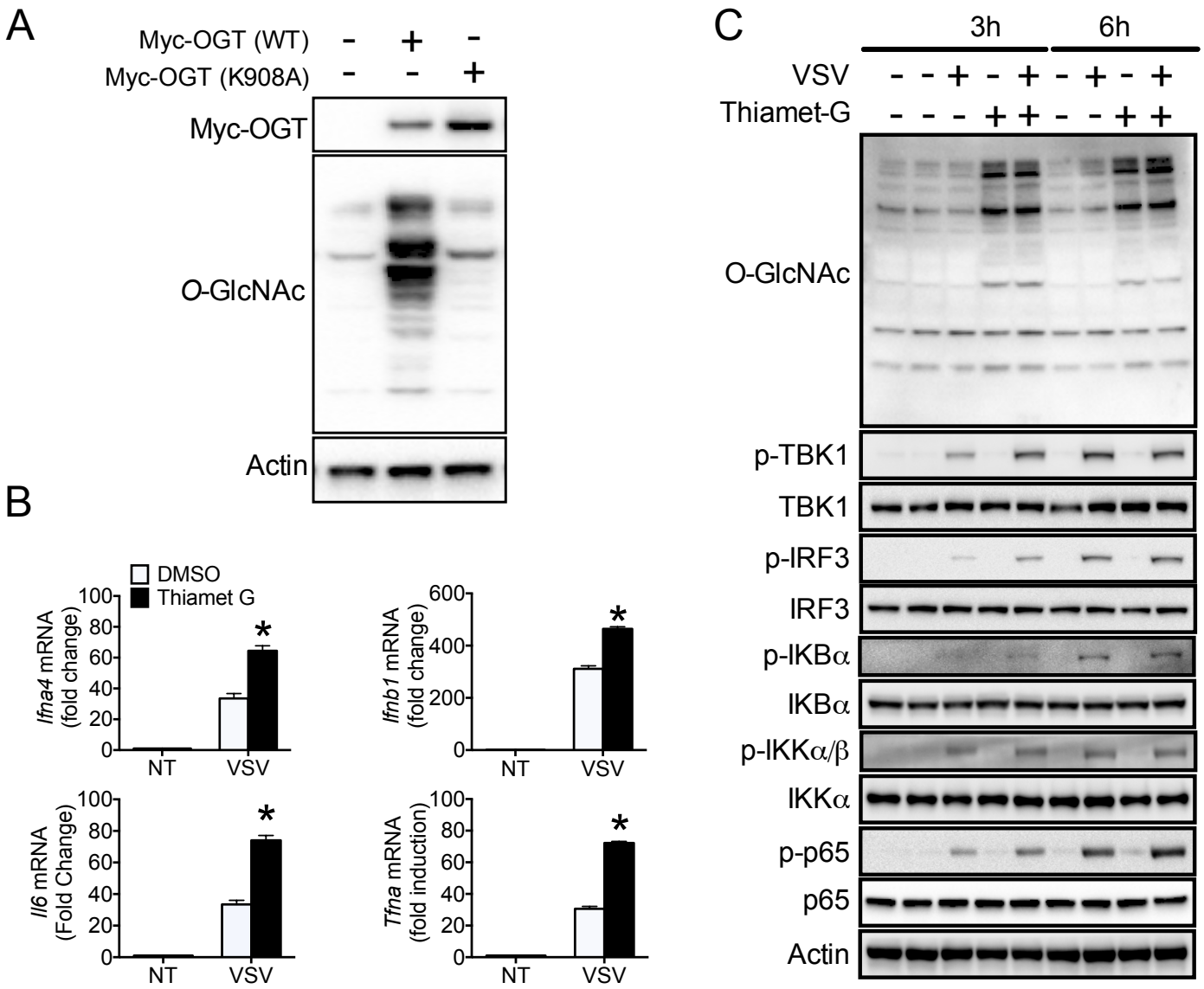


**Figure S5. Related to Figure 5. K908A mutation causes defective enzymatic activity of OGT, and pharmacological activation of the *O*-GlcNAc signaling promotes antiviral immune activation.** (A) 293T cells were transfected for 30 h with either Myc-tagged OGT WT (middle lane) or K908A mutant (right lane). Protein *O*-GlcNAcylation in total cell lysate was assessed with specific anti-*O*-GlcNAc antibody by immunoblotting. Data are from one experiment representative of six experiments. (B-C) BMMs generated from $Ogt^{fl/fl}$ and $Ogt^{\Delta mye}$ mice were pretreated with or without thiamet G (10 μM) for 2 h, followed by the challenge with VSV. Transcripts of *Ifna4*, *Ifnb1*, *Il6* and

*Tnfa* were measured with RT-PCR (A). Immunoblotting of total and phosphorylated TBK1, IRF3, IκBα, IKKα/β and p65 was performed (C). * $P < 0.05$, versus controls (two-tailed Student's *t*-test (B)). Data are presented as mean values of biological triplicates ± s.d.

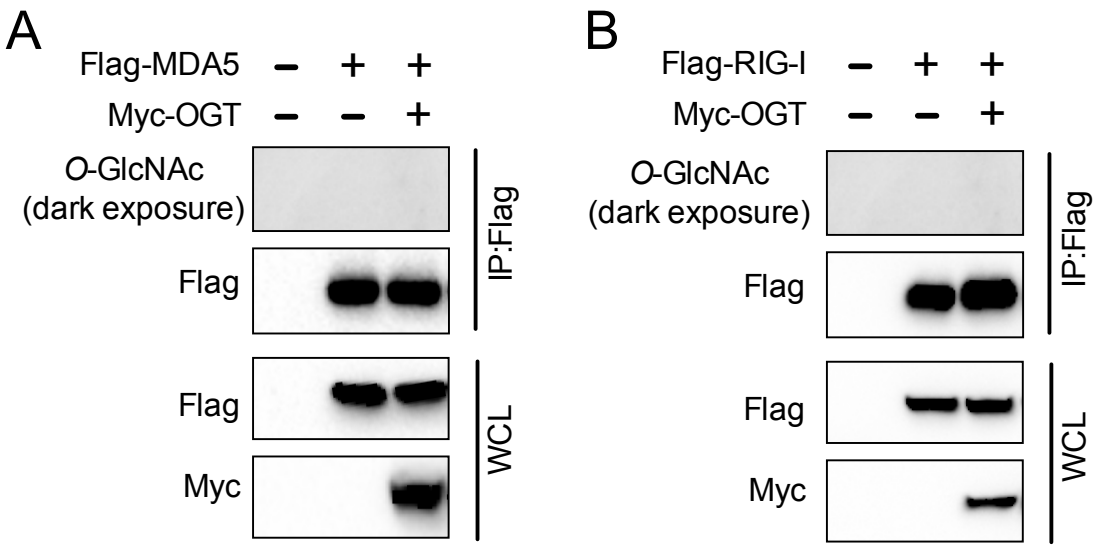


**Figure S6. Related to Figure 6. Neither RIG-I nor MDA5 is targeted for *O*-GlcNAcylation.** (A and B) 293T cells were transfected for 30 h with the expression vector for either Flag-tagged MDA5 (A) or Flag-tagged RIG-I (B) in the presence or absence or Myc-tagged OGT. Total MDA5 (A) or RIG-I (B) was immunoprecipitated with anti-Flag agarose beads and then assessed for *O*-GlcNAcylation with specific anti-*O*-GlcNAc antibody by immunoblotting. Data are from one experiment representative of two independent experiments.

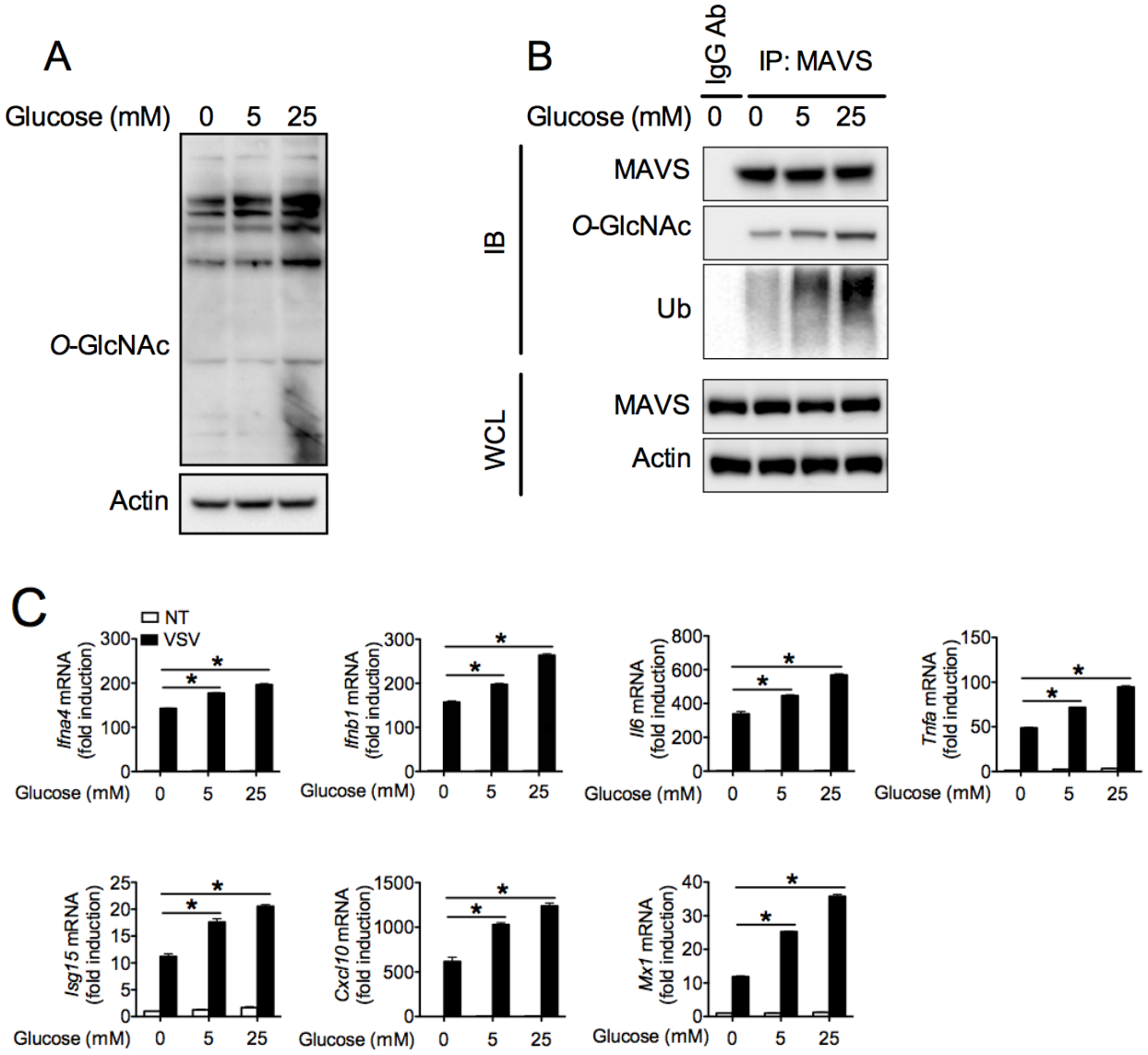


**Figure S7. Related to Figure 7. Glucose availability determines MAVS *O*-GlcNAcylation and ubiquitination.** (A) Total protein *O*-GlcNAcylation in BMMs generated from B57BL/6 mice placed in culture medium with 0, 5 or 25 mM glucose for 16 h. (B and C) BMMs generated from B57BL/6 mice were placed in culture medium with 0, 5 or 25 mM glucose for 16 h, followed by VSV stimulation for 4 h. Endogenous MAVS was immunoprecipitated, followed by immunoblotting with either anti-ubiquitination or anti-*O*-GlcNAcylation antibodies (B). Gene transcripts including *Ifna4*, *Ifnb1*, *Il6*, *Tnfa*,

*Isg15*, *Cxcl10* and *Mx1* in the cells were measured with RT-PCR (C). * $P < 0.05$, versus controls (two-tailed Student's *t*-test (C)). Data are presented as mean values of biological triplicates ± s.d.

**Table S2. Primer sequences for RT-PCR. Related to Figure 2, 3 and 4.**

| Genes | Forward | Reverse |
|---|---|---|
| Mouse *Ifna4* | CCTGTGTGATGCAGGAACC | TCACCTCCCAGGCACAGA |
| Mouse *Ifnb1* | ATGAGTGGTGGTTGCAGGC | TGACCTTTCAAATGCAGTAGATTCA |
| Mouse *Il6* | AGCTGGAGTCACAGAAGGAG | AGGCATAACGCACTAGGTTT |
| Mouse *Tnfa* | GTCAGGTTGCCTCTGTCTCA | TCAGGGAAGAGTCTGGAAAG |
| Mouse *Isg15* | TGGAAAGGGTAAGACCGTCCT | GGTGTCCGTGACTAACTCCAT |
| Mouse *Cxcl10* | CCTGCCCACGTGTTGAGAT | TGATGGTCTTAGATTCCGGATTC |
| Mouse *Mx1* | GGGGAGGAAATAGAGAAAATGAT | GTTTACAAAGGGCTTGCTTGCT |
| Mouse *Actb* | AGGGCTATGCTCTCCCTCAC | CTCTCAGCTGTGGTGGTGAA |
| Human *IFNB1* | CATTACCTGAAGGCCAAGGA | CAATTGTCCAGTCCCAGAGG |
| Human *IL6* | CGGGAACGAAAGAGAAGCTCTA | GGCGCTTGTGGAGAAGGAG |
| Human *TNFA* | GAGGCCAAGCCCTGGTATG | CGGGCCGATTGATCTCAGC |
| Human *ISG15* | CTGAGAGGCAGCGAACTCAT | AGCATCTTCACCGTCAGGTC |
| Human *CXCL10* | CTCCAGTCTCAGCACCATGA | GCTCCCCTCTGGTTTTAAGG |
| Human *GADPH* | ATGACATCAAGAAGGTGGTG | CATACCAGGAAATGAGCTTG |
| VSV L-domain | TGATACAGTACAATTATTTTGGGAC | GAGACTTTCTGTTACGGGATCTGG |

**Table S3. Primer sequences for molecular cloning. Related to Figure 5, 6 and 7.**

| | | |
|---|---|---|
| pWPXLd-OGT | Forward | GCGTTTAAACATGGCGTCTTCCGTGGGCAAC |
| | Reverse | GCGGATCCCGTGCTGACTCAGTGACTTCAAC |
| pWPXLd-MAVS | Forward | GCGTTTAAACATGCCGTTTGCTGAAGACAAG |
| | Reverse | GCACGCGTAAGTGCAGACGCCGCCGGTACAG |
| MAVS N-terminus | Forward | GCCTCGAGATGCCGTTTGCTGAAGACAAG |
| | Reverse | GCTCTAGACTATTTACCCTCTGCAGCCCCTG |
| MAVS C-terminus | Forward | GCCTCGAGCAGGGTGCAGAGAGTGACCAG |
| | Reverse | GCTCTAGACTAGTGCAGACGCCGCCGGTAC |

**Table S4. Primer sequences for site-directed mutagenesis. Related to Figure 4, 6 and 7.**

| | | |
|---|---|---|
| MAVS (S284/285A) | Forward | CAGGCCGAGCCTATCATCTGCGCCGCTGGGGCAGAGGCACCTGCCAAC |
| | Reverse | GTTGGCAGGTGCCTCTGCCCCAGCGGCGCAGATGATAGGCTCGGCCTG |
| MAVS (T300/301A) | Forward | CTCTGCCCTCCAAAGTGCCTGCCGCCTTGATGCCTGTGAACACAGTG |
| | Reverse | CACTGTGTTCACAGGCATCAAGGCGGCAGGCACTTTGGAGGGCAGAG |
| MAVS (T328/S329/S330A) | | |
| | Forward | CAGTGCCCTCCAAGTTGCCAGCTGCCGCAAAGCCCCCTGGTGCAGTGCC |
| | Reverse | GGCACTGCACCAGGGGGCTTTGCGGCAGCTGGCAACTTGGAGGGCACTG |
| MAVS (T342A) | Forward | CAGTGCCTTCTAATGCGCTCGCCAATCCAGCACCATCCAAATTG |
| | Reverse | CAATTTGGATGGTGCTGGATTGGCGAGCGCATTAGAAGGCACTG |
| MAVS (S353/T354A) | Forward | CATCCAAATTGCCCATCAACGCAGCCCGTGCTGGCATGGTGCCATC |
| | Reverse | GATGGCACCATGCCAGCACGGGCTGCGTTGATGGGCAATTTGGATG |
| MAVS (S361A) | Forward | CCCGTGCTGGCATGGTGCCAGCCAAAGTGCCTACTAGCATGG |
| | Reverse | CCATGCTAGTAGGCACTTTGGCTGGCACCATGCCAGCACGGG |
| MAVS (T365/S366A) | Forward | CATGGTGCCATCCAAAGTGCCTGCTGCAATGGTGCTCACCAAGGTGTC |
| | Reverse | GACACCTTGGTGAGCACCATTGCAGCAGGCACTTTGGATGGCACCATG |
| MAVS (T365A) | Forward | GGTGCCATCCAAAGTGCCTGCTAGCATGGTGCTCACCAAGGTG |
| | Reverse | CACCTTGGTGAGCACCATGCTAGCAGGCACTTTGGATGGCACC |
| MAVS (S366A) | Forward | GCCATCCAAAGTGCCTACTGCAATGGTGCTCACCAAGGTGTC |
| | Reverse | GACACCTTGGTGAGCACCATTGCAGTAGGCACTTTGGATGGC |
| MAVS (T370A) | Forward | GTGCCTACTAGCATGGTGCTCGCCAAGGTGTCTGCCAGCACAG |
| | Reverse | CTGTGCTGGCAGACACCTTGGCGAGCACCATGCTAGTAGGCAC |
| OGT (K908A) | Forward | TGCTCCTGCAGAGGAACACG |
| | Reverse | GTTCCTCTGCAGGAGCAACAG |
| OGT with gRNA non-recognizable synonymous mutation | | |
| | Forward | GCCCTAAGTTTGAGTCCAAACCATGCTGTCGTCCATGGCAACCTGGCTTGTGTATAC |
| | Reverse | GTATACACAAGCCAGGTTGCCATGGACGACAGCATGGTTTGGACTCAAACTTAGGGC |