Supplemental Figures

Figure S1

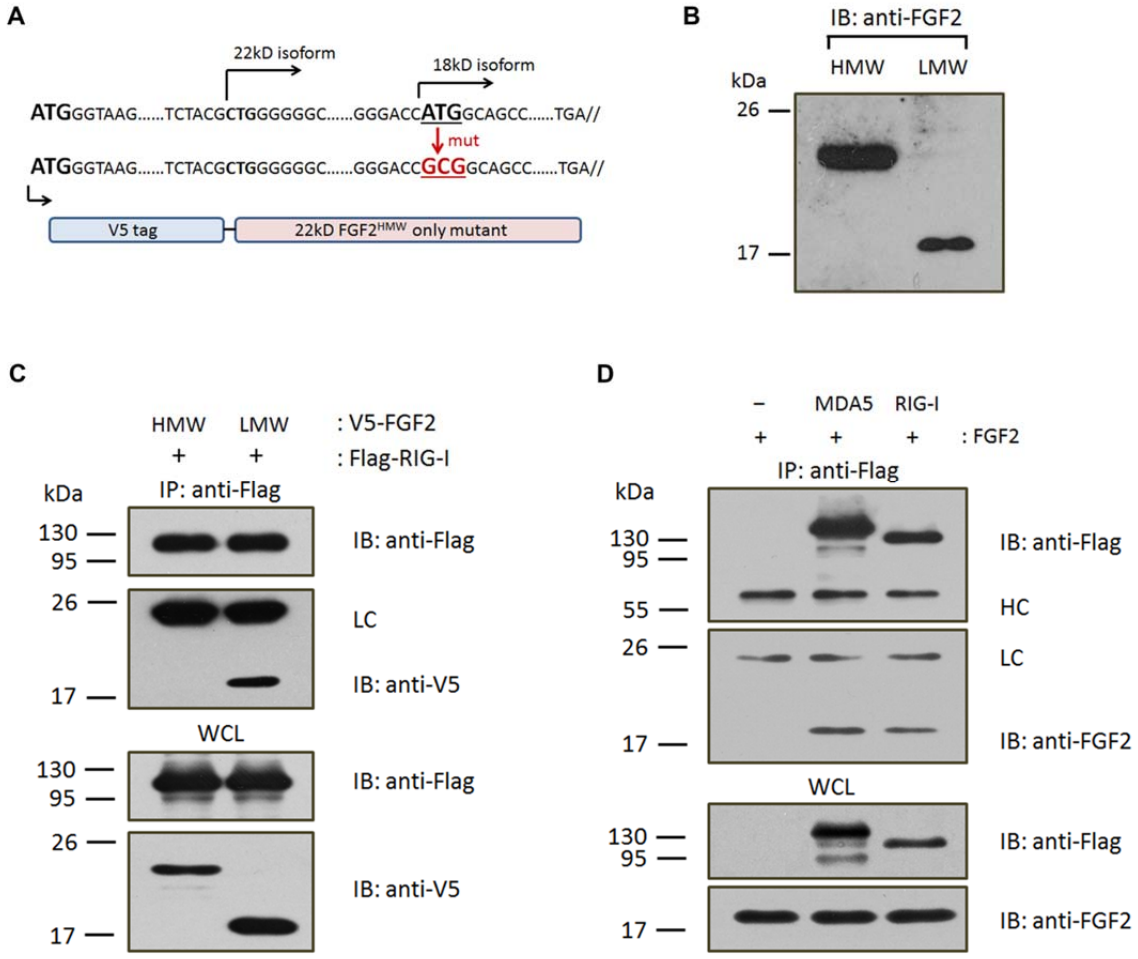

Figure S1. The specificity of the interaction between LMW or HMW FGF2 and RIG-I or MDA5 was detected. (A) V5-tagged HMW FGF2 (22 kDa) plasmid was constructed by substitution of GCG for LMW FGF2 initiation codon ATG. **(B)** 293T cells were transfected with V5-tagged HMW FGF2 or LMW FGF2, and 48 h later, the expression of HMW and LMW FGF2 was determined by immunoblotting using an anti-FGF2 antibody. **(C)** 293T cells were transfected with plasmids expressing Flag-RIG-I and V5-tagged HMW or LMW FGF2. After a 48-h transfection, WCLs of 293T cells were immunoprecipitated with an anti-Flag antibody, followed by IB assay with anti-Flag and anti-V5 antibodies. **(D)** 293T cells were transfected with plasmids expressing LMW FGF2 and Flag-RIG-I or MDA5. WCLs of 293T cells were immunoprecipitated with an anti-Flag antibody, followed by IB assay with anti-Flag and anti-FGF2 antibodies.

Figure S2

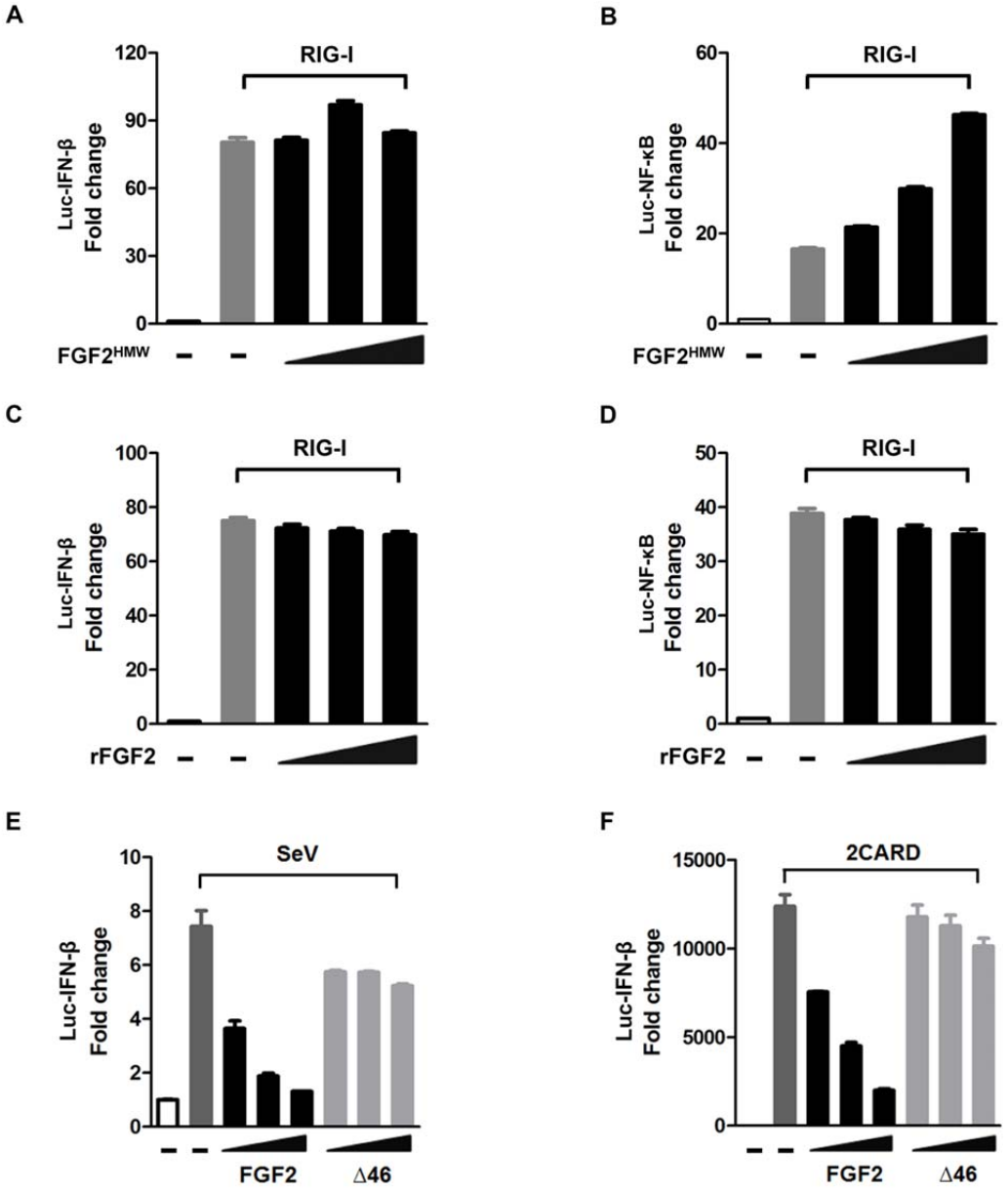

Figure S2. Intracellular LMW FGF2 specifically antagonizes RIG-I-mediated antiviral signaling. (A-D) 293T cells were transfected with plasmids expressing full-length RIG-I, and luc-IFN-β (A, C) or luc-NF-κB (B, D), and various amounts of HMW FGF2 (0, 20, 50, and 100 ng) or recombinant human LMW FGF2 proteins (rFGF2; 10, 25, and 50 ng/ml). After a 48-h transfection, cells lysates were prepared for luciferase activity detection. **(E, F)** 293T cells were transfected with various amounts of LMW FGF2 (20, 50, and 100 ng) or LMW FGF2 Δ46 (20, 50, and 100 ng) and with SeV (50 HA unit/ml) infection after 24 h of transfection (E) or co-transfected with 2CARD (F). Cells were harvested after 48 h of treatment. The IFN-β promoter activity was assessed by dual luciferase assay.

Figure S3

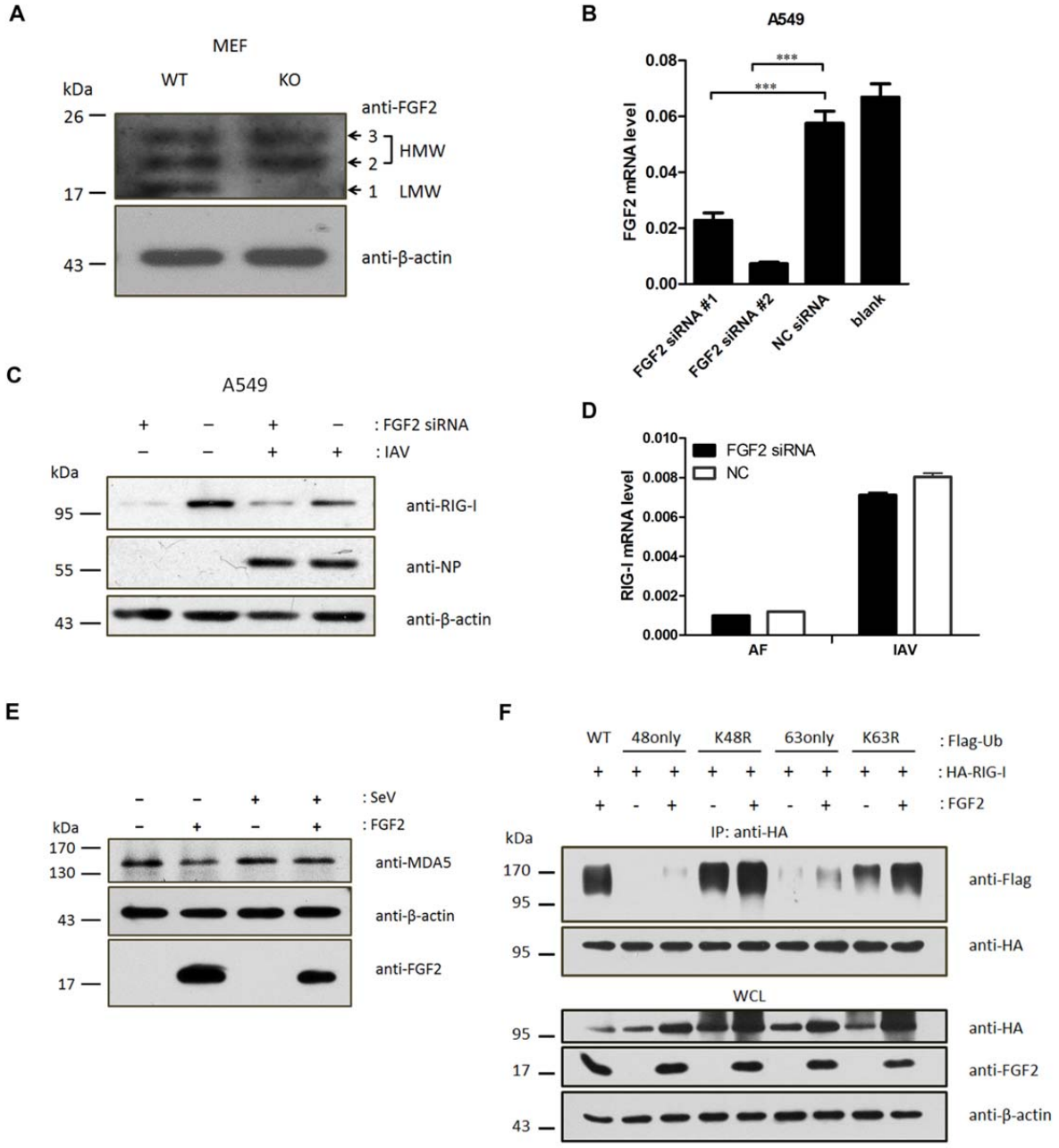

Figure S3. LMW FGF2 inhibits Lys 48-linked ubiquitination -mediated RIG-I degradation. **(A)** Alternative translation of FGF2 in lung tissues of the $Fgf2^{lmw+/+}$ (WT) and the $Fgf2^{lmw-/-}$ (KO) mice was detected by immunoblotting using an anti-FGF2 antibody. β-actin was used as an internal control. The arrows indicated three FGF2 isoforms of 18, 21, and 22 kDa individually. **(B)** A549 cells were transfected with FGF2 siRNA#1 and siRNA#2 against two different regions of FGF2 gene, and negative control (NC) siRNA or not (blank). After 48 h of transduction, the FGF2 knockdown efficiency was tested using real-time PCR. **(C, D)** A549

cells were transfected with FGF2 siRNA#2 for the endogenous FGF2 knockdown. After 48 h of transduction, A549 cells were infected with IAV or mock infected. Cell lysates were harvested at 12 hpi and then subjected to IB assay using anti-RIG-I, anti-influenza A virus nuclear protein (NP) and anti-β-actin antibodies. The RIG-I mRNA level was assessed by real time-PCR. **(E)** 293T cells were transfected with LMW FGF2 or vector. After 48 h transfection, 293T cells were infected with SeV. WCLs of 293T cells were immunoblotted with anti-MDA5, anti-FGF2 and anti-β-actin antibodies. **(F)** 293T cells were transfected with HA-RIG-I, Flag-Ub (WT, 48only, K48R mutant, 63only, or K63R mutant) together with LMW FGF2 or vector control. Forty-eight hours later, WCLs of 293T cells were immunoprecipitated with an anti-HA antibody, followed by IB assay with anti-Flag, anti-HA, anti-FGF2 and anti-β-actin antibodies.

Figure S4

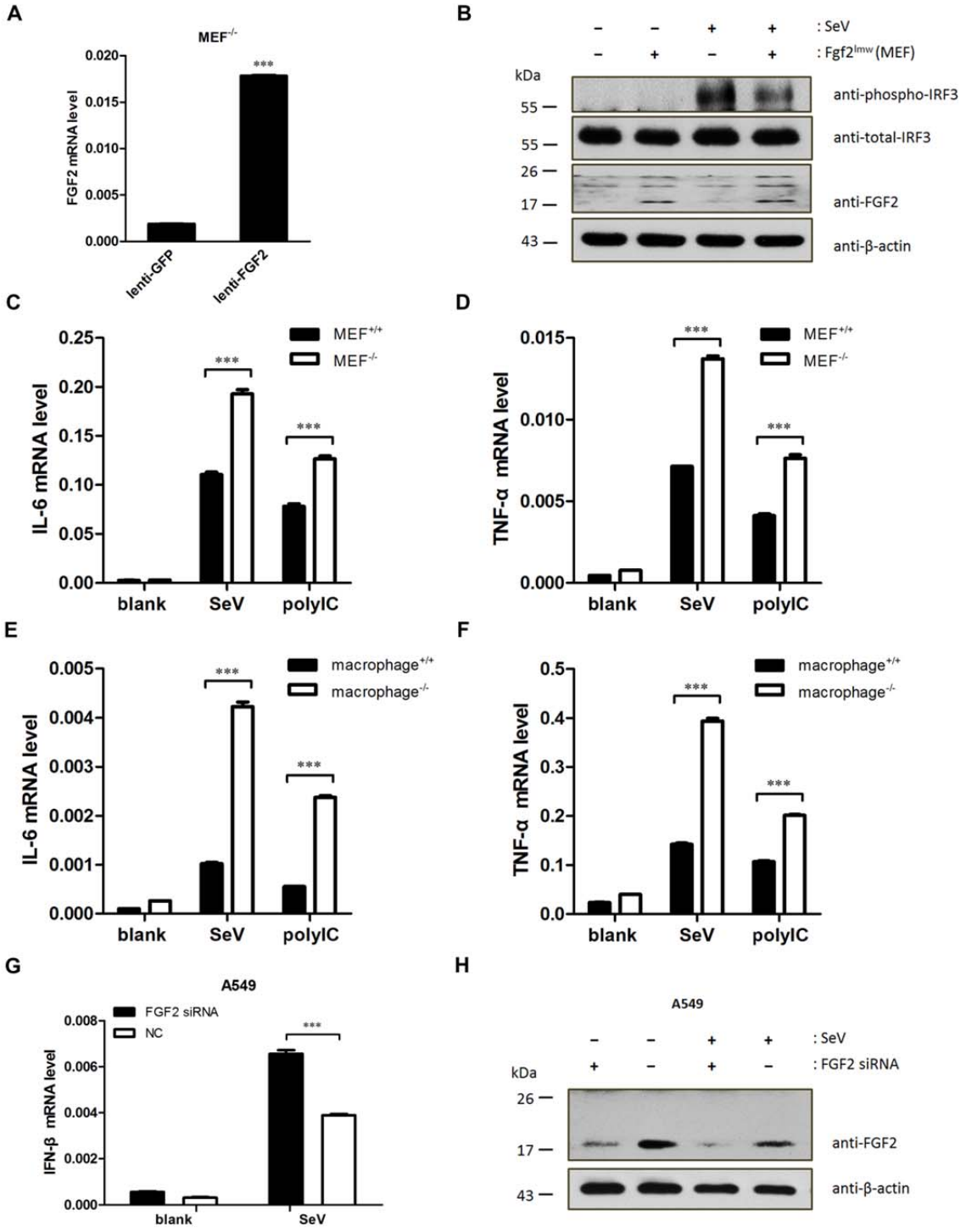

Figure S4. Cytosolic LMW FGF2 suppresses antiviral innate immune response.

(A) Primary Fgf2$^{lmw-/-}$ MEF cells were infected with recombinant lentivirus for LMW FGF2 expression, and lentivirus-GFP was used as control. Forty-hours later, the effective exogenous expression of LMW FGF2 was detected by real-time PCR assay. **(B)** The phosphorylation of IRF3 activation in SeV- or mock-infected Fgf2$^{lmw-/-}$ and Fgf2$^{lmw+/+}$ MEF cells was detected by

immunoblotting with anti-phospho-IRF3, anti-total-IRF3, anti-FGF2 and anti-β-actin antibodies. **(C-F)** The $Fgf2^{lmw+/+}$ and $Fgf2^{lmw-/-}$ MEFs (C, D) and peritoneal macrophages (E, F) were treated with or without polyIC or SeV. After 12 h of treatment, IL-6 and TNF-α mRNA expression levels were detected by real-time PCR assays. **(G, H)** A549 cells were transfected with FGF2 siRNA for the endogenous FGF2 knockdown. After 48 h of transfection, A549 cells were infected with SeV or mock infected. (G) IFN-β mRNA expression in A549 cells at 12 hpi were detected by real time-PCR. (H) LMW FGF2 protein levels were detected by immunoblotting with anti-FGF2 and anti-β-actin antibodies.