Upon RNA virus recognition RIG-I activates Card9 and inflammasome signaling to facilitate interleukin 1β production

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Supplementary Figures 1 - 4

Supplementary Table 1
Supplementary Figure 1. Distinct 3pRNAs induce comparable caspase-1 activation

BMDCs of WT mice were stimulated with two different 3pRNAs for 6h. Supernatants were analyzed for processing of caspase-1 by immunoblot (a) and for IL-1β production by ELISA (b). All data presented in (b) are means +/- s.e.m. All results shown are representative of at least two independent experiments.
Supplementary Figure 2. Trim25 is required for full IL-1β production upon RNA virus infection

(a) Left Panel: BMDCs of WT and Trim25-deficient (Trim25-KO) mice were stimulated as indicated and cell culture supernatants were analysed for IL-1β production by ELISA. Right panel: BMDCs of WT and Trim25-deficient mice were stimulated as in (a) and intracellular pro-IL-1β concentrations were determined by ELISA after cell lysis with repeated freeze-thaw cycles. (b) Cell culture supernatants of WT and Trim25-deficient cells stimulated as indicated were analyzed for processing of caspase-1 by immunoblot. All data presented in (a) are means +/- s.e.m. All results shown are representative of at least two independent experiments.
Supplementary Figure 3. Card9 deletion or IKK inhibition abrogates RIG-I-induced pro-IL-1β mRNA induction and IL-1β secretion

(a, b) BMDCs from WT or Card9-deficient (Card9-KO) mice were pretreated with 3μM IKK inhibitor Bay11-7082 or only DMSO for 1 h as indicated and subsequently stimulated for 6 h with VSV where indicated. 

(a) Relative amounts of pro-IL-1β mRNA were determined by quantitative RT-PCR analysis.

(b) Supernatants were analyzed for IL-1β concentrations by ELISA.

All data are means +/- s.e.m.. All results shown are representative of at least two independent experiments.
Supplementary Figure 4. Scheme of RIG-I-induced inflammasome activation

Pathway leading to IFN production and NF-κB-inflammasome activation

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Supplementary Table 1. Primers used for quantitative real-time (RT) PCR

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<th>Species</th>
<th>Gene Symbol</th>
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<th>Reverse</th>
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<td><em>Il1b</em></td>
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<td>5’-tcttttgggtattgcttg-3’</td>
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1Mm = mus musculus