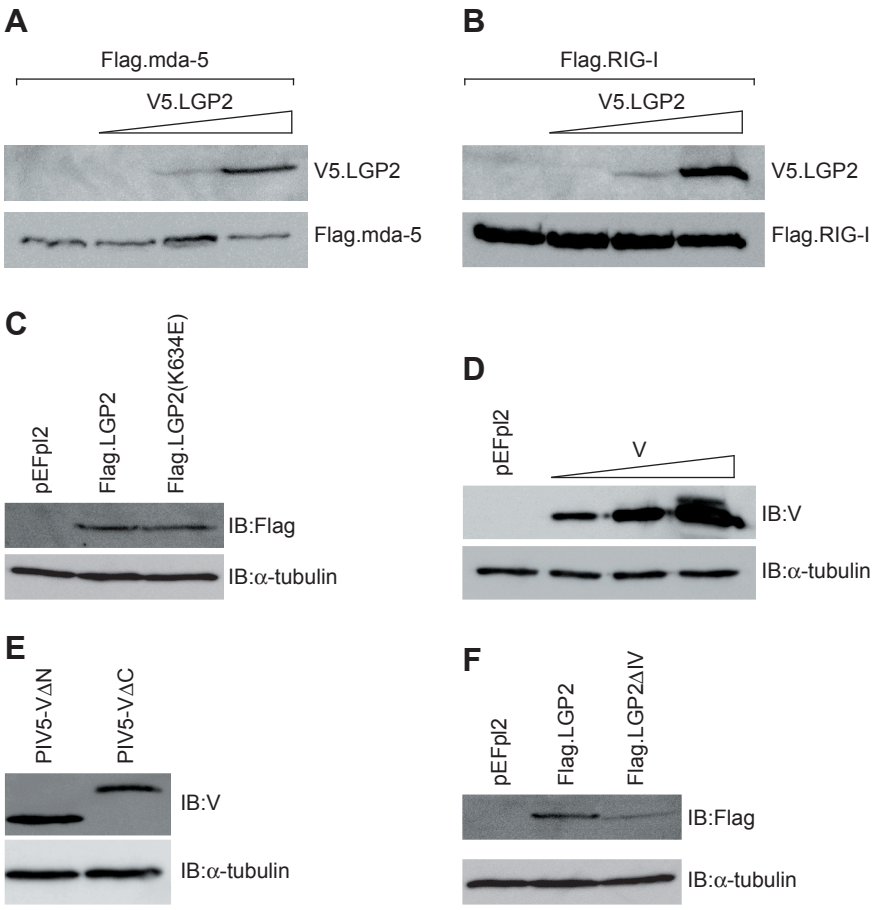
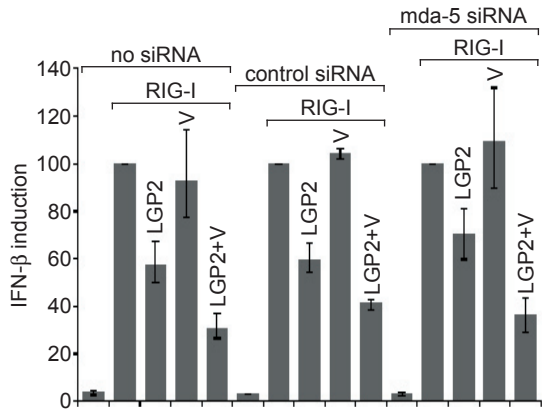
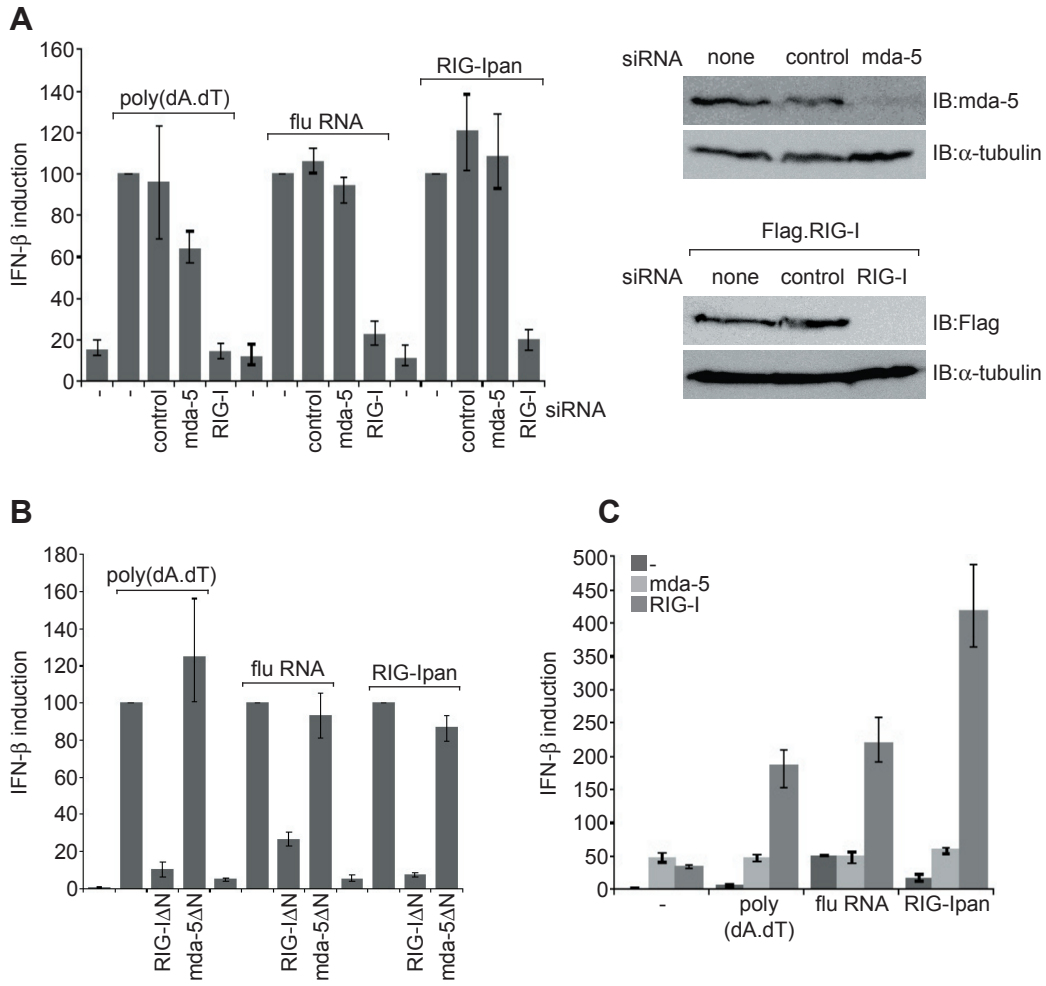


Supplementary Figure 1. Expression of proteins used in transfection experiments
(A) and (B) HEK293 cells were transfected with pEF.Flag.mda-5 (A) or pEF.Flag.RIG-I (B) and either 0, 2, 20 or 200ng pEF.V5.LGP2. Cell extracts were made 24h after transfection and subjected to immunoblotting with anti-V5 or anti-Flag antibodies. (C) HEK293 cells were transfected with pEFpl2, pEF.Flag.LGP2 or pEF.Flag.LGP2(K634E). Cell extracts were made 24h after transfection and subjected to immunoblotting with anti-Flag or anti-α-tubulin antibodies. (D) HEK293 cells were transfected with pEFpl2, or either 2, 20 or 200ng pEF.PIV5-V. Cell extracts were made 24h after transfection and subjected to immunoblotting with anti-V5 or anti-α-tubulin antibodies. (E) HEK293 cells were transfected with pEF.V5.PIV5-VΔN or pEF.PIV5-VΔC. Cell extracts were made 24h after transfection and subjected to immunoblotting with anti-V5 or anti-α-tubulin antibodies. (F) HEK293 cells were transfected with pEFpl2, pEF.Flag.LGP2 or pEF.Flag.LGP2ΔIV. Cell extracts were made 24h after transfection and subjected to immunoblotting with anti-Flag or anti-α-tubulin antibodies.



Supplementary Figure 2. Inhibition of RIG-I by LGP2 and PIV5-V is independent of mda-5

HEK293 cells were transfected with pIFΔ(-116)lucter, pJatlacZ, pEFplink2 or plasmids expressing RIG-I, LGP2 or PIV5-V as indicated, in the presence of no siRNA (lanes 1-5), a control siRNA (lanes 6-10) or the mda-5 siRNA (lanes 11-15).



Supplementary Figure 3. Poly(dA.dT), RNA from influenza virus-infected cells and RIG-Ipan induce IFN through RIG-I and not mda-5.

(A) HEK293 cells were transfected with pIFΔ(-116)lucter, pJatlacZ and either no siRNA n(-), a control siRNA, an mda-5 siRNA or a RIG-I siRNA. 30h after transfection cells were induced with either poly(dA.dT), RNA from influenza-virus infected cells or RIG-Ipan. To confirm the effectiveness of the mda-5 siRNA, HEK-293 cells transfected with either no siRNA, the control siRNA or the mda-5 siRNA were treated with IFN overnight to induce the expression of mda-5. Extracts from these cells were subjected to immunoblotting with an anti-mda-5 antibody and an anti-α-tubulin antibody to confirm equal loading. To confirm the effectiveness of the RIG-I siRNA HEK-293 cells were transfected with pEF.Flag.RIG-I and either no siRNA, the control siRNA or the RIG-I siRNA. Extracts from these cells were subjected to immunoblotting with anti-Flag and anti-α-tubulin antibodies. (B) HEK293 cells were transfected with pIFΔ(-116)lucter, pJatlacZ and either pEFpl2, pEF.RIG-IΔN or pEF.mda-5ΔN. 30h after transfection cells were induced with either poly(dA.dT), RNA from influenza-virus infected cells or RIG-Ipan. (C) HEK293 cells were transfected with pIFΔ(-116)lucter, pJatlacZ and either pEFpl2 (dark grey bars), pEF.mda-5 (light grey bars) or pEF.RIG-I (mid-grey bars). 30h after transfection cells were uninduced (-) or induced with either poly(dA.dT), RNA from influenza-virus infected cells or RIG-Ipan.