

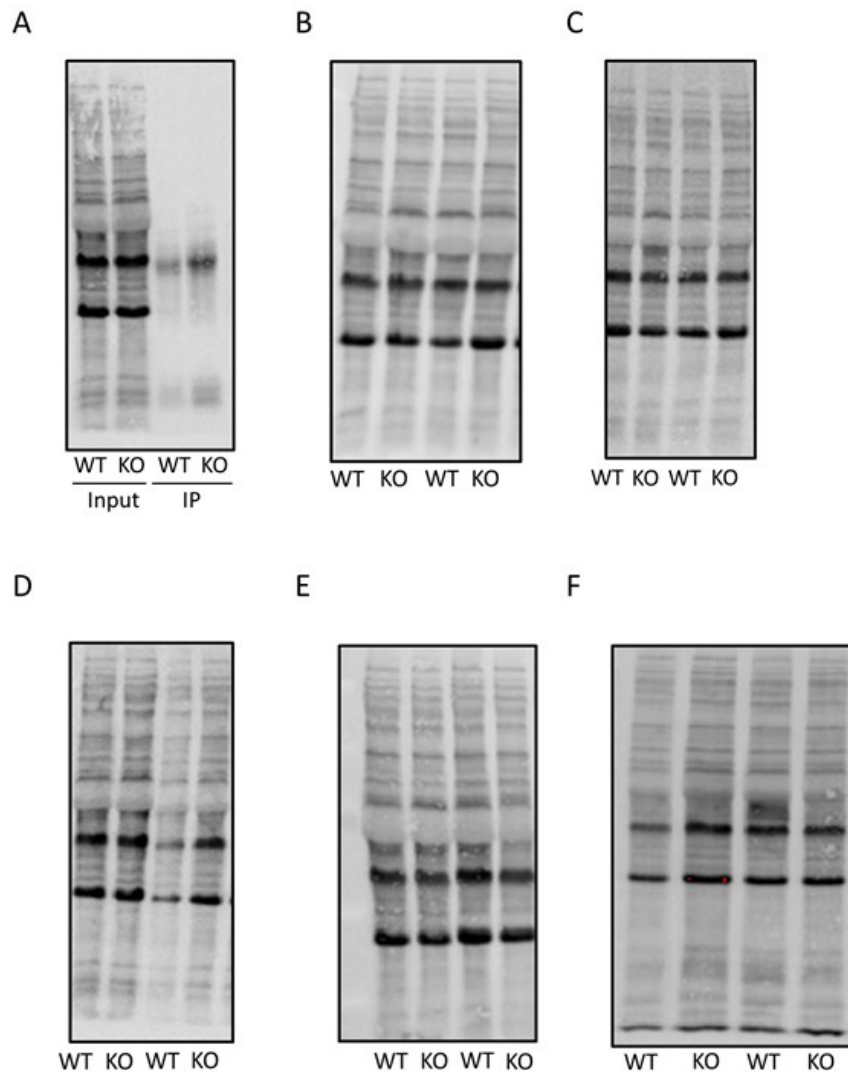
Supplementary Material

Figure S1. Total protein images of platelets from co-immunoprecipitation (A) and analysis of IP₃R1 (B), IP₃R2 (C), IP₃R3 (D), IRAG1 (E) and PKGI β (F) expression.

Total protein images from Western Blots for co-immunoprecipitation (Figure 2A) and analysis of expression of IP₃R1 (Figure 2B), IP₃R2 (Figure 2C), IP₃R3 (Figure 2D), IRAG1 (Figure 2E) and PKGI β (Figure 2F) in platelets from IRAG2-WT (WT) and IRAG2-KO (KO) mice. Input per lane: 70 μ g of protein for analysis of IRAG1, IP₃R1, IP₃R2 and IP₃R3 expression and 35 μ g of protein for PKGI β expression. For co-immunoprecipitation 1000 μ g of murine platelet lysate was used.

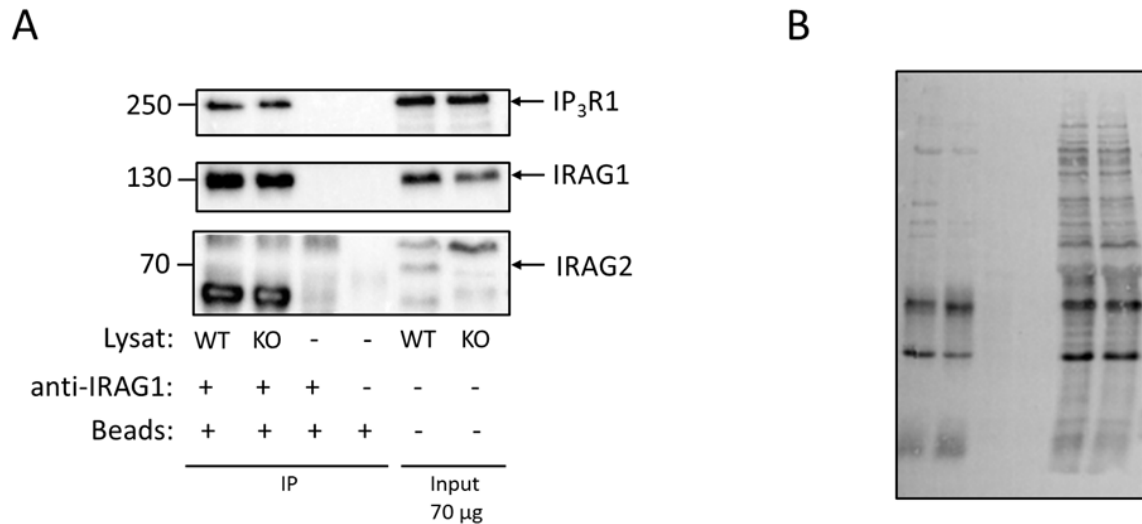


Figure S2. Immunoprecipitation of IRAG1 with IRAG1 specific antibody in platelets from IRAG2-WT (WT) and IRAG2-KO (KO) mice and Western Blot analysis of co-immunoprecipitated interaction partners. **(A)** Interaction of IRAG1 with IP₃R1 and PKG1 β in murine platelets was detected in IRAG2-WT (WT) and IRAG2-KO (KO) platelets. No direct interaction of IRAG1 and IRAG2 was detected neither in platelets from IRAG2-WT (WT) nor in IRAG2-KO (KO) platelets. **(B)** Total protein image from Western Blot for co-immunoprecipitation **(A)** is shown. As input probes for IRAG2-WT (WT) and IRAG2-KO (KO) platelets 70 µg of protein and for immunoprecipitation 1000 µg of platelet lysate was used.

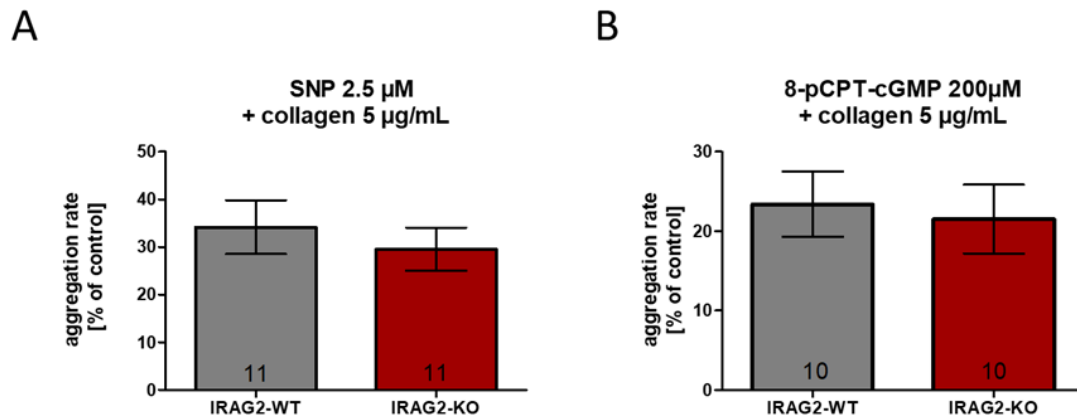


Figure S3: NO/cGMP-dependent inhibition of platelet aggregation in platelets from IRAG2-WT and IRAG2-KO mice using SNP 2.5 µM **(A)** or 8-pCPT-cGMP 200 µM **(B)** as an inhibitor and collagen 5 µg/mL to evoke platelet aggregation. Aggregation rate was calculated as maximal slope of the aggregation curve and aggregation rate under treatment with inhibitor is calculated as % of control (control: aggregation rate without inhibitor). Only a weak tendency for a reduced aggregation rate [% of control] was seen in IRAG2-KO platelets compared to IRAG2-WT platelets, which is not significant. For each aggregation 1.0×10^8 platelets/ml were used. The number of experiments is shown in the graphs and mean \pm SEM is shown by bars.