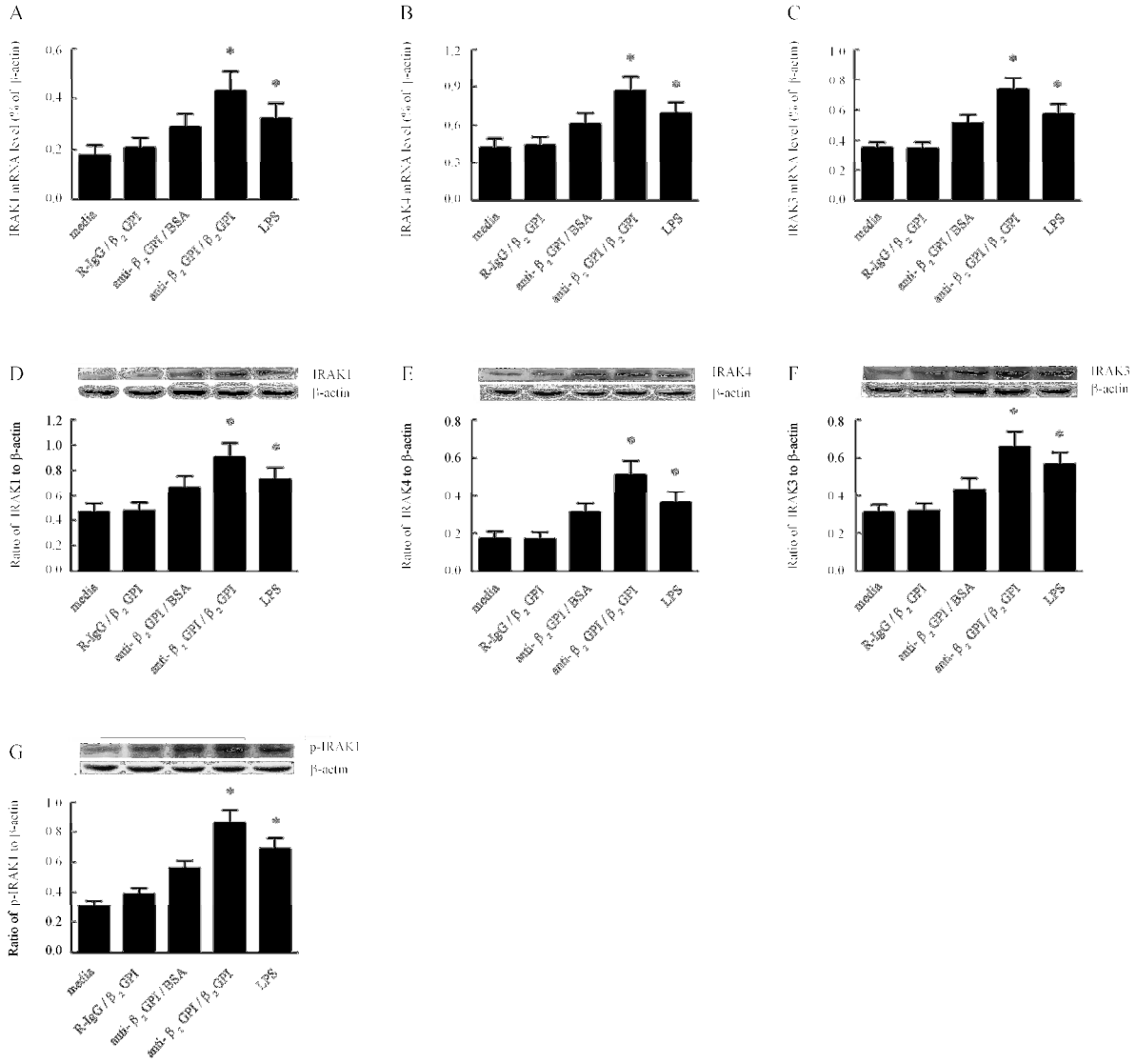
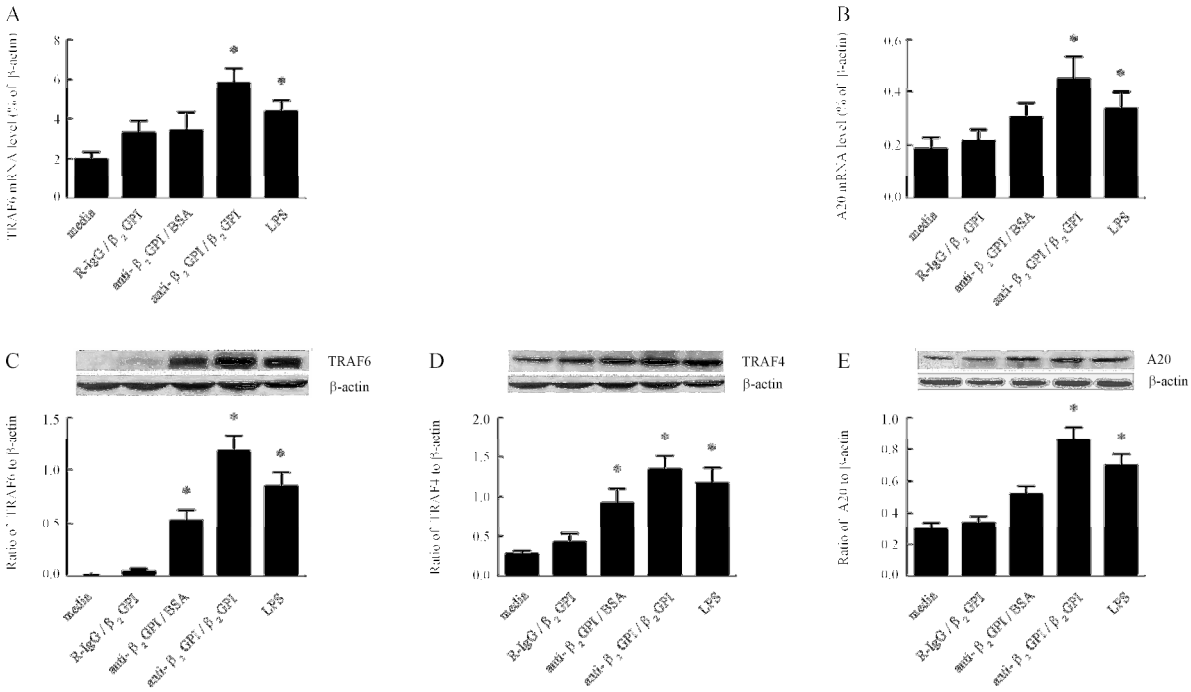
Supplementary Material to Xu et al. "Involvement of IRAKs and TRAFs in anti-β_2GPI/β_2GPI-induced tissue factor expression in THP-1 cells" (Thromb Haemost 2011; 106.5)

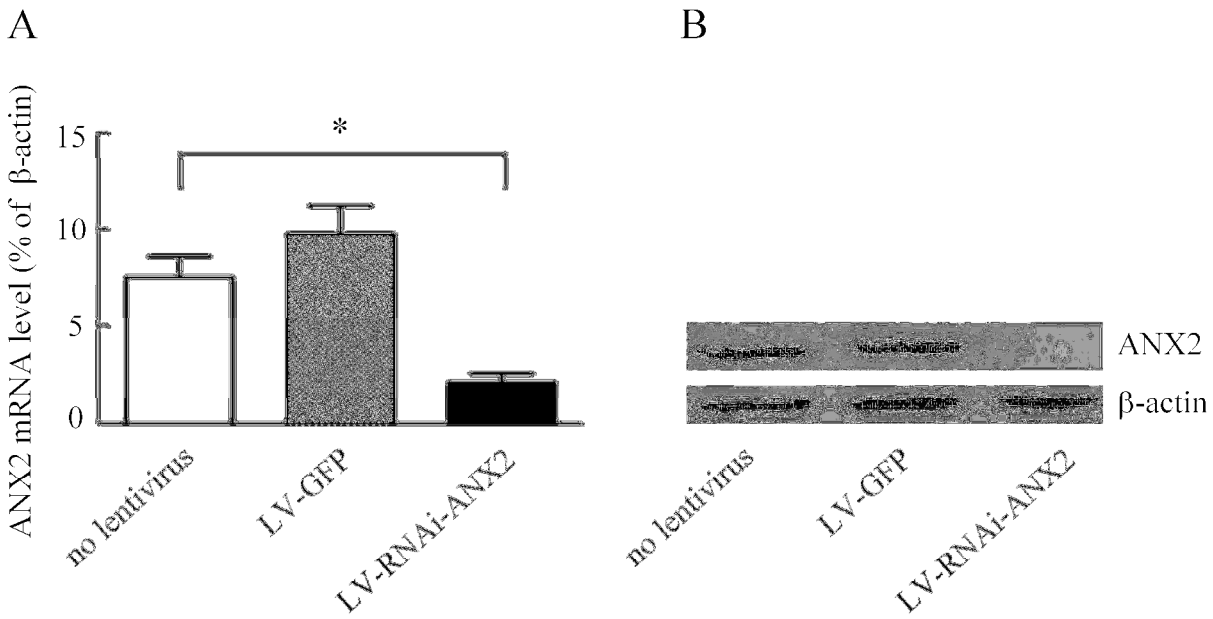


Supplemental Figure 1: The stimulatory effects of anti-β_2GPI/β_2GPI on IRAKs expression at mRNA and protein levels in THP-1 cells The THP-1 cells (2 x 10^6) were treated with anti-β_2GPI (10 μg/ml) / β_2GPI (100 μg/ml) complex, isotype control rabbit immunoglobulin G (R-IgG) (10 μg/ml) / β_2GPI (100 μg/ml), control of anti-β_2GPI (10 μg/ml) / bovine serum albumin (BSA, 100μg/ml), LPS (500 ng/ml) for different times. Total RNAs were extracted and mRNA levels of IRAK1 (A), IRAK4 (B) and IRAK3 (C) were measured by real-time quantitative PCR. Cells were lysed and proteins were subjected to

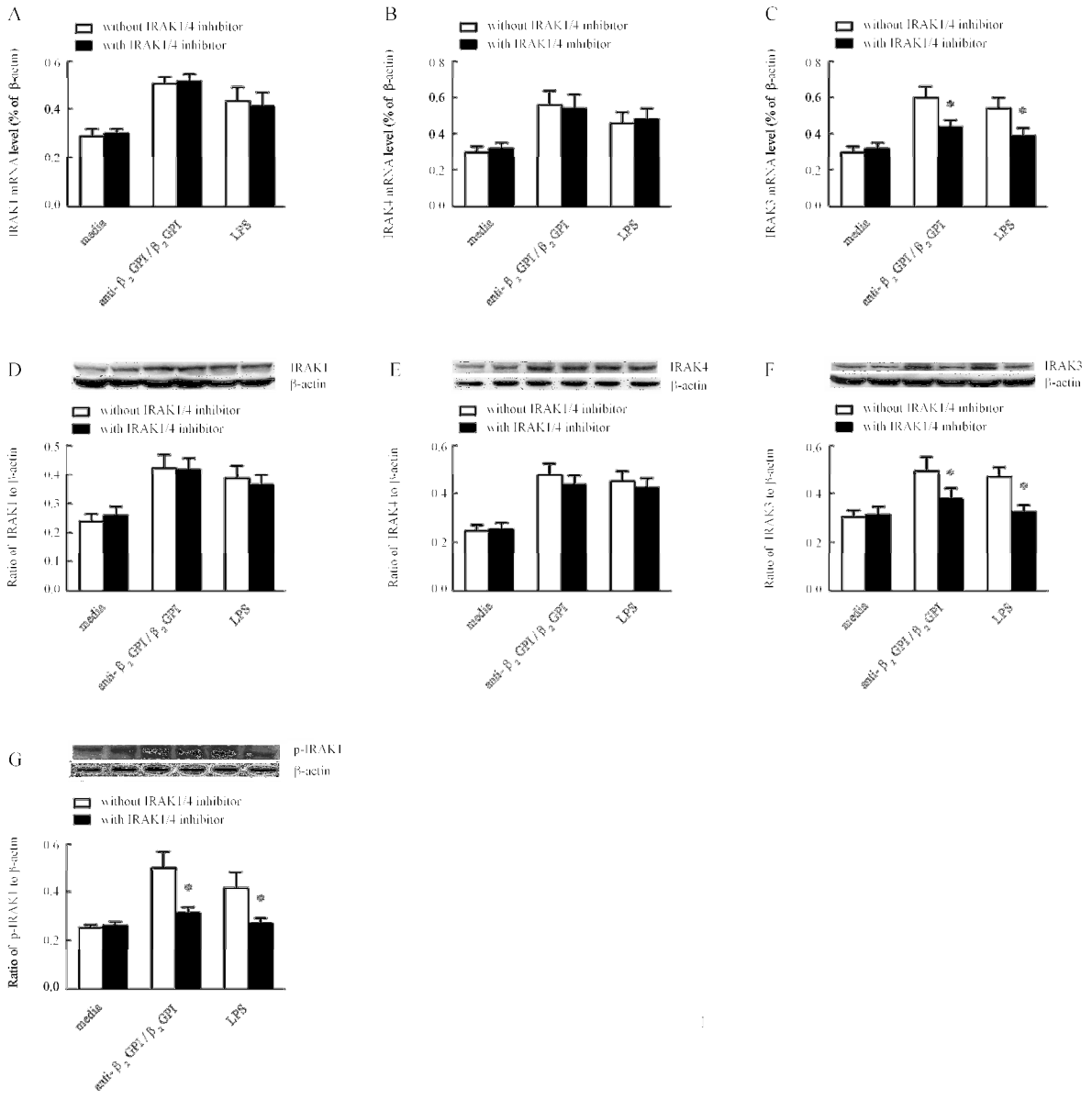
Western blot using IRAK1 (D), p-IRAK1 (G), IRAK4 (E), IRAK3 (F) and β-actin antibodies, respectively. Shown are representative blot and pooled data from three independent experiments. * $p < 0.05$ vs control of media.



Supplemental Figure 2: The stimulatory effects of anti-β_2GPI/β_2GPI on TRAFs expression at mRNA and protein levels in THP-1 cells The THP-1 cells (2 x 10^6) were treated with anti-β_2GPI (10 μg/ml) / β_2GPI (100 μg/ml) complex, isotype control rabbit immunoglobulin G (R-IgG) (10 μg/ml) / β_2GPI (100 μg/ml), control of anti-β_2GPI (10 μg/ml) / bovine serum albumin (BSA, 100μg/ml), LPS (500 ng/ml) for different times. Total RNAs were extracted and used to detect mRNA levels of TRAF6 (A) and zinc finger protein A20 (B) by real-time quantitative PCR. Cells were lysed and proteins were subjected to Western blot using TRAF6 (C), TRAF4 (D), A20 (E) and β-actin antibodies, respectively. Shown are representative blot and pooled data from three independent experiments. * $p <$ 0.05 vs control of media.



Supplemental Figure 3: ANX2 mRNA (A) and protein (B) levels in lentivirus-infected THP-1 cells The empty lentivirus (LV-GFP) and ANX2 siRNA (LV-RNAi-ANX2) were transferred into target THP-1 cells at MOI equal to 100 with ENi.S and 5 μg / mL polybrene. After 72 h, the ANX2 mRNA (A) and its protein (B) levels on the cells were detected by real time quantitative PCR or Western blot. * $p < 0.01$ vs no lentivirus cells. Data shown with three separate experiments.



Supplemental Figure 4: The effects of IRAK1/4 inhibitor on IRAKs expression in THP-1 cells THP-1 cells (2×10^6) were pre-incubation with of IRAK1/4 inhibitor (5 μM)) for 2 h and then stimulated by anti-β_2GPI (10 μg/mL)/β_2GPI (100 μg/mL) complex or LPS (500 ng/ml) for additional different times. Total RNAs were extracted and mRNA levels of IRAK1 (A), IRAK4 (B) and IRAK3 (C) were measured by real-time quantitative PCR. Cells were lysed and proteins were subjected to Western blot using IRAK1 (D), p-IRAK1 (G), IRAK4 (E), IRAK3 (F) and β-actin antibodies, respectively. Shown are representative blot and pooled data from three independent experiments. * $p<0.05$ vs control of media.