Supplementary Figures to Amadio, Tarantino, et al. “Prostaglandin-endoperoxide synthase-2 deletion affects the natural trafficking of Annexin A2 in monocytes and favours venous thrombosis in mice” (https://doi.org/10.1160/TH16-12-0968)

Suppl. Figure 1: Effect of depletion of monocyte activation on venous thrombus size. Gadolinium chloride was administrated in WT (open squares) and PTGS2−/− (gray squares) mice 24 hours before IVC ligation and venous thrombi were collected 48 hours after surgery. Data are mean ± SEM. Significant P-values (2way-ANOVA for main effects of genotype and treatment, followed by a Bonferroni post hoc analysis).

** P<0.01 PTGS2−/− control versus WT control; °° P<0.01 PTGS2−/− gadolinium control versus PTGS2−/− control. n=6/group.
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Suppl. Figure 2: Effect of PTGS2 deletion on vessel wall. Representative image of quantification of Annexin A2 (ANXA2) expression in (A) total lysed, and in (B) membrane and nucleus normalized to tubulin or Lamin A/C from vessel of WT (open bars) and PTGS2<sup>−/−</sup> (KO; grey bars) mice. Data are mean ± SEM. Significant P-values (Wilcoxon rank-sum test) are shown. ns= no significant. n=4/group.
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Suppl. Figure 3: Representative immunofluorescence image and distribution of Annexin A2 (ANXA2, red) expression and DAPI (Blue) nuclear staining in circulating monocytes of WT and PTGS2^-/- mice. Scale bar = 10 μm. n = 4/group.
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Suppl. Figure 4: Effect of PTGS2 depletion on TF and ANXA2 in RAW 264.7 cells. Cells were transfected with PTGS2-directed siRNA or nonspecific siRNA as indicated, and (A) TF procoagulant activity, and ANXA2 expression (B) by western blotting and (C) immunohistochemistry were determined (mean value ± SEM of 5 independent experiments). Significant P-values (Wilcoxon rank-sum test) are shown. **P<0.01.
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**Suppl. Figure 5:** Effect of ANXA2 depletion on TF activity in RAW 264.7 cells. Cells were transfected with ANXA2-directed siRNA or nonspecific siRNA as indicated, and TF procoagulant activity was determined (mean value ± SEM of 5 independent experiments). Significant P-values (Wilcoxon rank-sum test) are shown. *P<0.05.
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Suppl. Figure 6: Effect of carbaprostacyclin on platelet activation. Mice were treated with carbaprostacyclin or vehicle 24 hours and 1 hour before blood collection. Percentage of (A) monocyte/platelet and (B) neutrophil/platelet aggregates analysed by flow cytometry. Platelet aggregation in response to (C) collagen 2 μg/ml and (D) ADP 4 μM. n=4-5/group (mean value + SEM). Significant P-values (Anova two-way with repeated measures, followed by a Bonferroni post hoc analysis) are shown.

*P<0.05, **P<0.01.
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**Suppl. Figure 7:** Effect of Carbaprostacyclin on venous thrombus cells. Mice were treated with Carbaprostacyclin or vehicle 24 hours before IVC ligation and venous thrombi were collected 48 hours after surgery. Representative image of MabF4/80 (monocyte/macrophages), TF and Annexin A2 (ANXA2) by immunoperoxidase staining from section thrombi.