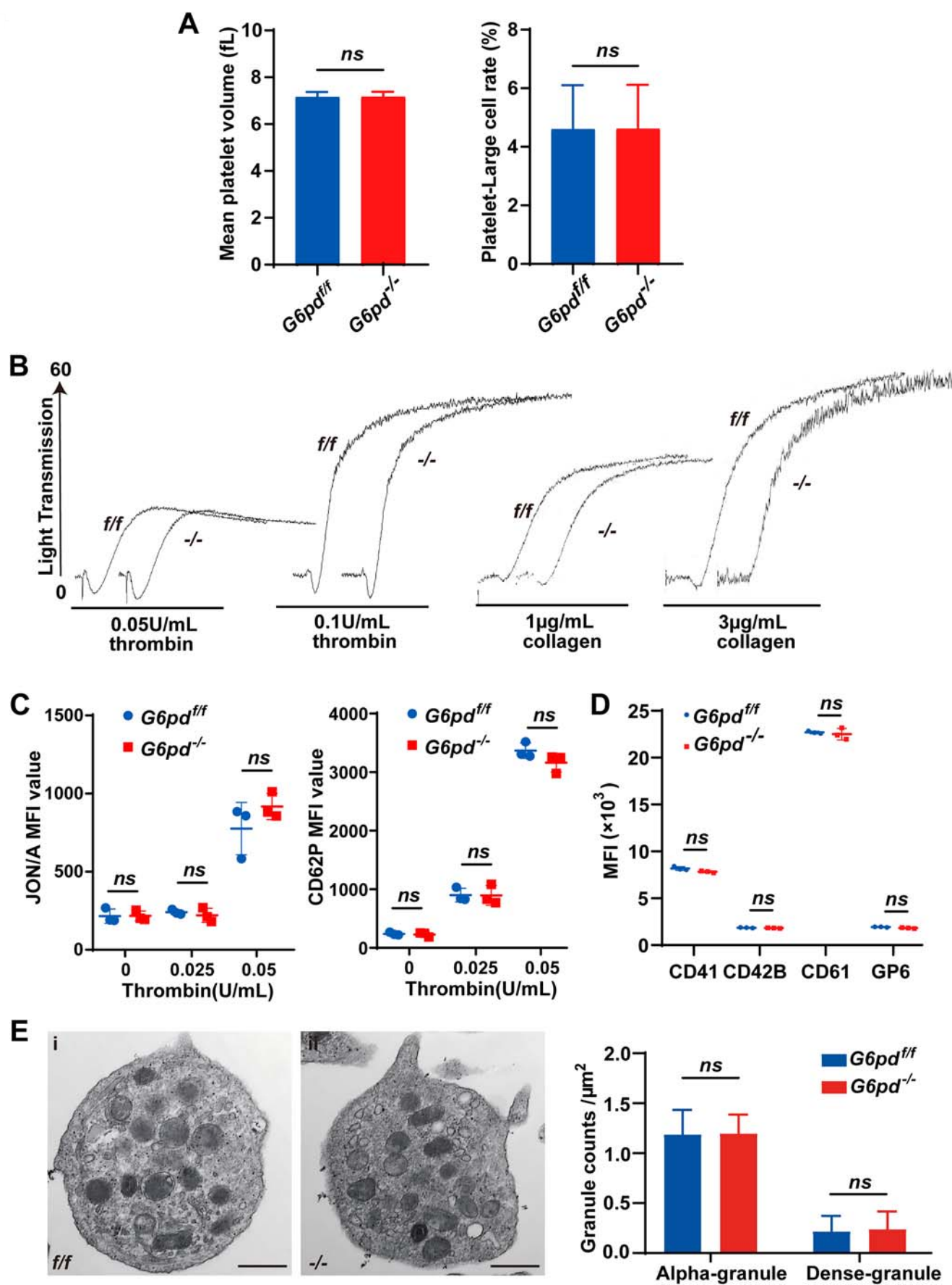


Expanded View Figures

Figure EV1. G6PD-deficient platelets displayed no abnormalities in size or in in vitro aggregation and secretion.

(A) Mean volume and large cell rate of platelet in *G6pd^{fl/fl}* and *G6pd^{-/-}* mice ($n = 7$). (B) Aggregation of washed *G6pd^{fl/fl}* and *G6pd^{-/-}* platelets in response to thrombin (0.05 U/mL, 0.1 U/mL) and collagen (1 μ g/mL, 3 μ g/mL). (C) JON/A and CD62P (P-selectin) exposure of washed *G6pd^{fl/fl}* and *G6pd^{-/-}* platelets stimulated with 0.025 and 0.05 U/mL thrombin ($n = 3$). (D) The expression of different surface glycoproteins of *G6pd^{fl/fl}* and *G6pd^{-/-}* platelets detected by flow cytometry ($n = 3$). (E) Representative ultrastructural images and granule analysis of washed *G6pd^{fl/fl}* and *G6pd^{-/-}* platelets visualized by electron microscopy ($n = 10$). Scale bars represented 0.5 μ m.



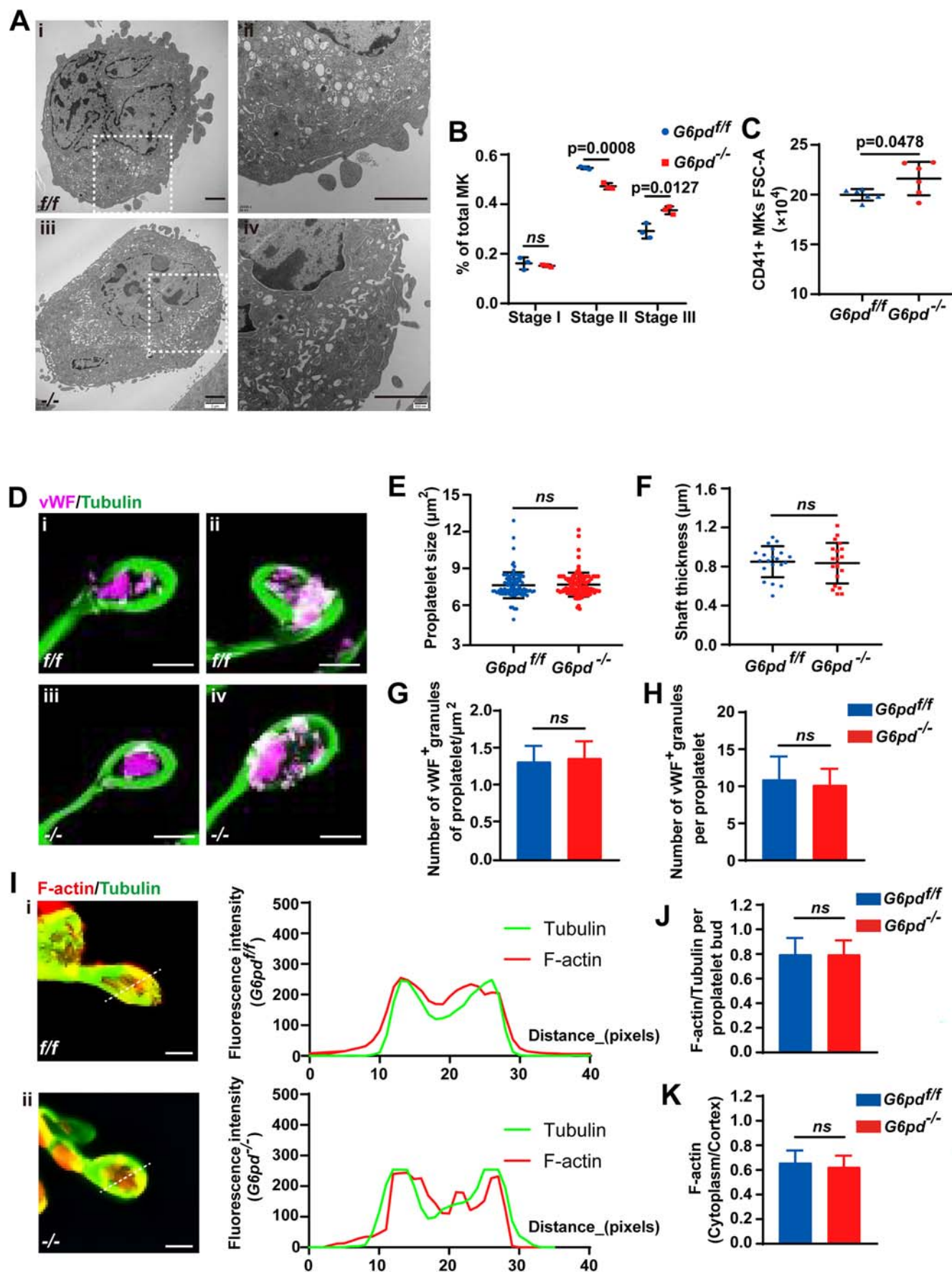


Figure EV2. G6PD deficiency influenced demarcation membrane systems (DMS) formation of megakaryocyte.

(A) $G6pd^{+/l}$ and $G6pd^{-/-}$ MKs derived from induced fetal liver cells were visualized by electron microscopy. Representative ultrastructural images (i and ii for $G6pd^{+/l}$ MKs, iii and iv for $G6pd^{-/-}$ MKs) were presented. Scale bars represented 2 μm . (B) Megakaryocytes were categorized into three maturation stages based on ultrastructural features under the electron microscopy. Percentage of different stages of MKs from three groups $G6pd^{+/l}$ and $G6pd^{-/-}$ fetal livers were analyzed ($n = 3$, $*P < 0.05$, $***P < 0.001$). Stage I corresponded to cells approximately 10 μm in diameter with a large nucleus; Stage II to cells 10 to 20 μm in diameter containing platelet-specific granules; and Stage III to mature MKs with well-developed DMS, distinct platelet territories, and a peripheral zone. (C) MK size analysis by flow cytometry using the forward scatter (FSC) value of CD41⁺ cells (MKs) derived from induced fetal liver cells. (D) Representative high magnification images of vWF (purple) and tubulin (green) fluorescence in $G6pd^{+/l}$ and $G6pd^{-/-}$ proplatelets. Scale bars represented 2 μm . (E) Sizes of proplatelet buds produced by cultured MKs ($n = 100$). (F) Thickness of proplatelet shaft connected with buds ($n = 20$). (G, H) The density and number of vWF⁺ granules in proplatelets ($n = 20$). (I) High magnification images and representative line scans of tubulin (green) and F-actin (red) fluorescence in $G6pd^{+/l}$ and $G6pd^{-/-}$ proplatelets. Scale bars represented 2 μm . (J) Relative level of F-actin vs tubulin per proplatelet bud, calculated from fluorescence intensity ratios with Image J ($n = 20$). (K) F-actin intensity of cytoplasm vs cortex in proplatelet bud ($n = 20$), which were determined from intensities within $\sim 0.75 \mu\text{m}$ diameter circles placed over the edge or middle of the proplatelet, using Image J software.