Supplement: Infusing mature megakaryocytes into mice yields functional platelets

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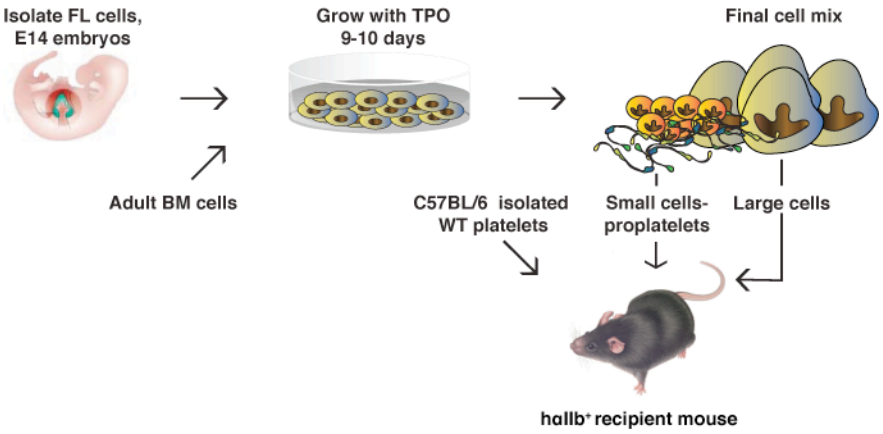

Figure 1supplement. Isolation of FL and BM derived megakaryocytes

The strategy followed in our study is presented by which the three different products for infusion were collected: isolated WT platelets, large and small cells were obtained from FL cells grown in the presence of TPO. Flow cytometric analysis of whole blood from h_IIb+ recipient mice before and at different time points after infusion of donor cells was performed. Blood was stained with labeled species-specific anti-_IIb antibodies. The % of platelets in the left upper quadrant represents the recipient's platelets, while the % in the right lower quadrant represents donor-derived platelets.

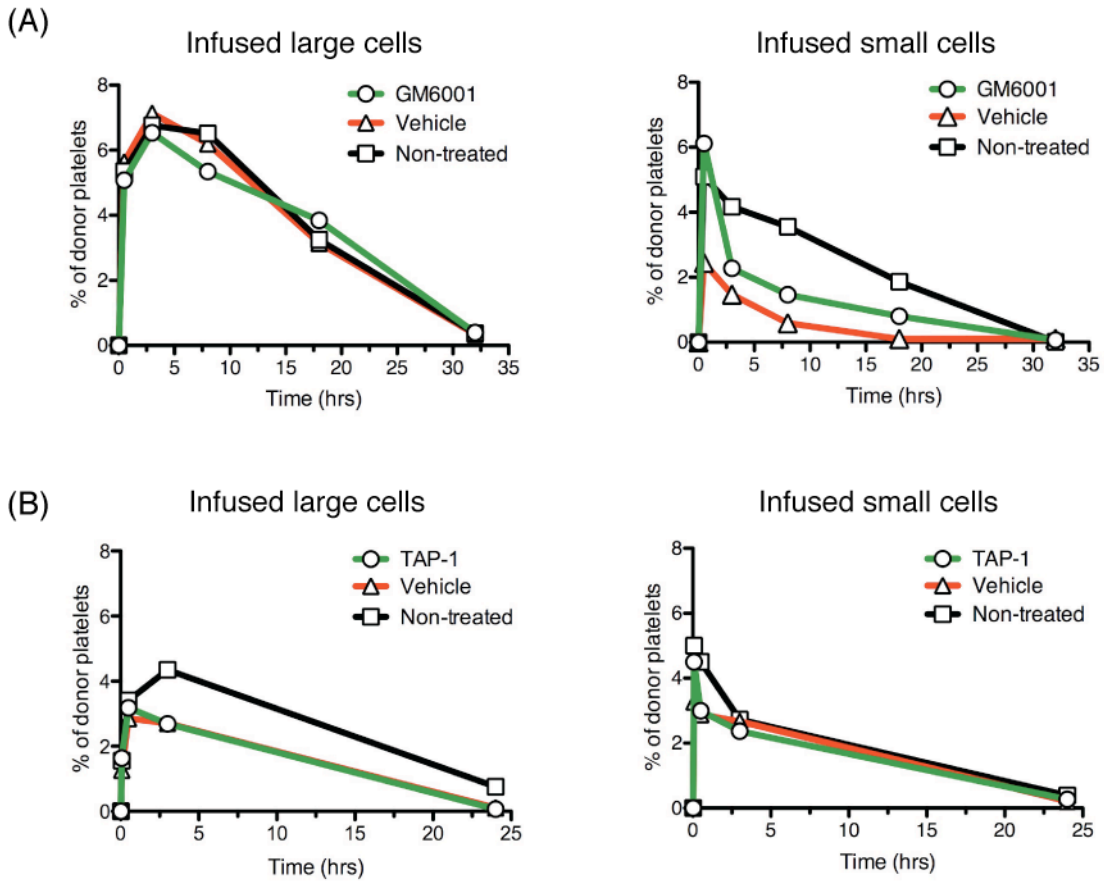

Figure 2supplement. Effect of metalloproteinase on thrombopoiesis from in vitro grown FL-derived cells.

WT FL-cells were grown in culture in the presence of either 100 µM GM6001 (A) or 10 µM TAP-1 (B) and then separated into small and large cells as in Fig. 1A and infused into h_IIb+ recipient mice to see if the metalloproteinase inhibitors improved the shorten half-life observed with non-treated derived platelets. N=2, performed in duplicates.

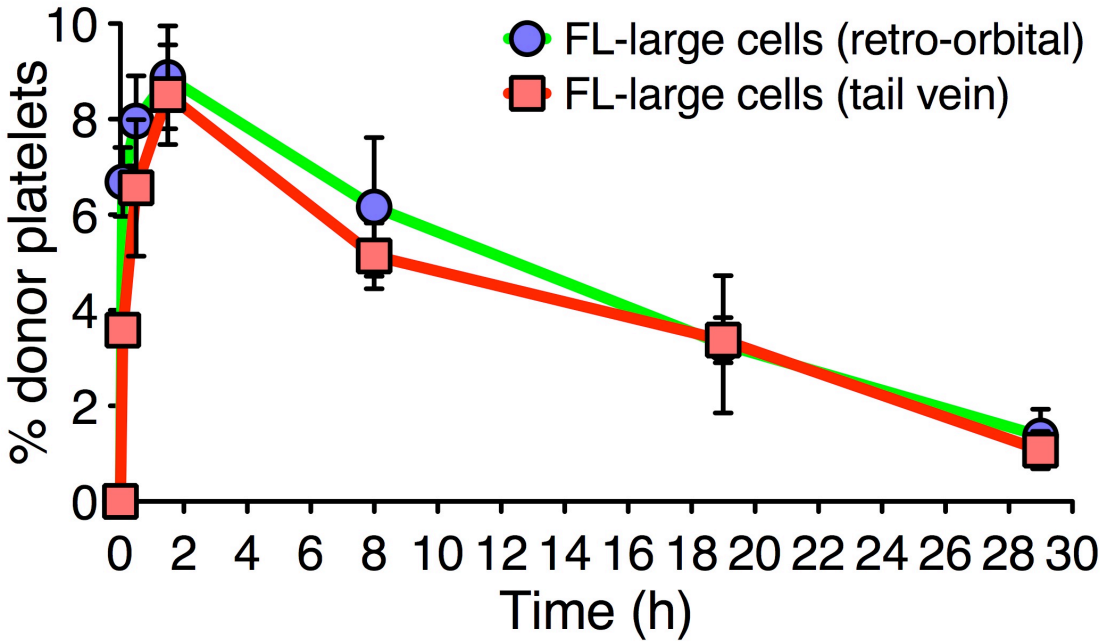

Figure 3supplement. Comparison of retro-orbital Vs tail vein infusions of FL-large cells

Flow cytometric percentage of infused ~10^6 FL-large cells in recipient animals. N=5 study per arm, Mean ± 1 standard deviation (SD) are shown.

Table 1supplement. Calculation of number of alveoli capillaries blocked by infused megakaryocytes.

Mice platelet content: 1-2 X 10^9 platelets (1)
Mice platelet half-life: 37 hrs (2)
Megakaryocyte sheds 10^2 platelets (determined in this study).
Calculate: 2-4 X 10^6 megakaryocytes each day traveling to the lungs to maintain the animal's platelet count.
Mice alveoli: 2.3 X 10^6 in both lungs (3)
Capillaries per alveoli: 25-100; based on human alveolar structure, corrected for the difference in volume between human and murine alveoli) (4)
Calculate: Infusion of = 10^6 megakaryocytes over 10 mins into the mice will block 0.4-1.7% of the entire capillary bed for a 24 hr period.

References:

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2. Baker, G.R., Sullam, P.M., and Levin, J. 1997. A simple, fluorescent method to internally label platelets suitable for physiological measurements. *Am J Hematol* 56:17-25.
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Supplemental movie legend

Video 1. Incorporation of donor platelets in arteriole thrombi of h_IIb^{+} recipient mouse after infusion of FL- small cells, following laser injury. (MOV 3.1 MB)

As in Fig. 2C with an injury created near the center of the video and blood flow from top to bottom on the screen. The platelets depicted in green were detected with an anti-mouse CD41 antibody labeled with Alexa^{488}. Incorporation of platelets from small cells into the thrombi was seen with a distinct population of CD41^{+} cells re-circulating and rarely incorporated into the growing thrombus.

Video 2. Incorporation of donor platelets in arteriole thrombi of h_IIb^{+} recipient mouse after infusion of FL-large cells, following laser injury. (MOV 3.1 MB)

As in Fig. 2B with an injury created near the center of the video and blood flow from top to bottom on the screen. The platelets depicted in green were detected with an anti-mouse CD41 antibody labeled with Alexa^{488}. Incorporation of platelets from the large cells into the growing thrombi was detected similar to infusion of WT platelets.

Video 3. Incorporation of WT donor platelets in arteriole thrombi of h_IIb^{+} recipient mouse following laser injury. (MOV 3.1 MB)

As in Fig. 2A with an injury created near the center of the video and blood flow from top to bottom on the screen. The platelets depicted in green were detected with an anti-mouse CD41 antibody labeled with Alexa^{488}. Incorporation of the WT infused platelets into the growing thrombi was clearly detected.