Munc18-2, but not Munc18-1 or Munc18-3, regulates platelet exocytosis, hemostasis, and thrombosis

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SUPPORTING INFORMATION

Supplemental Figures

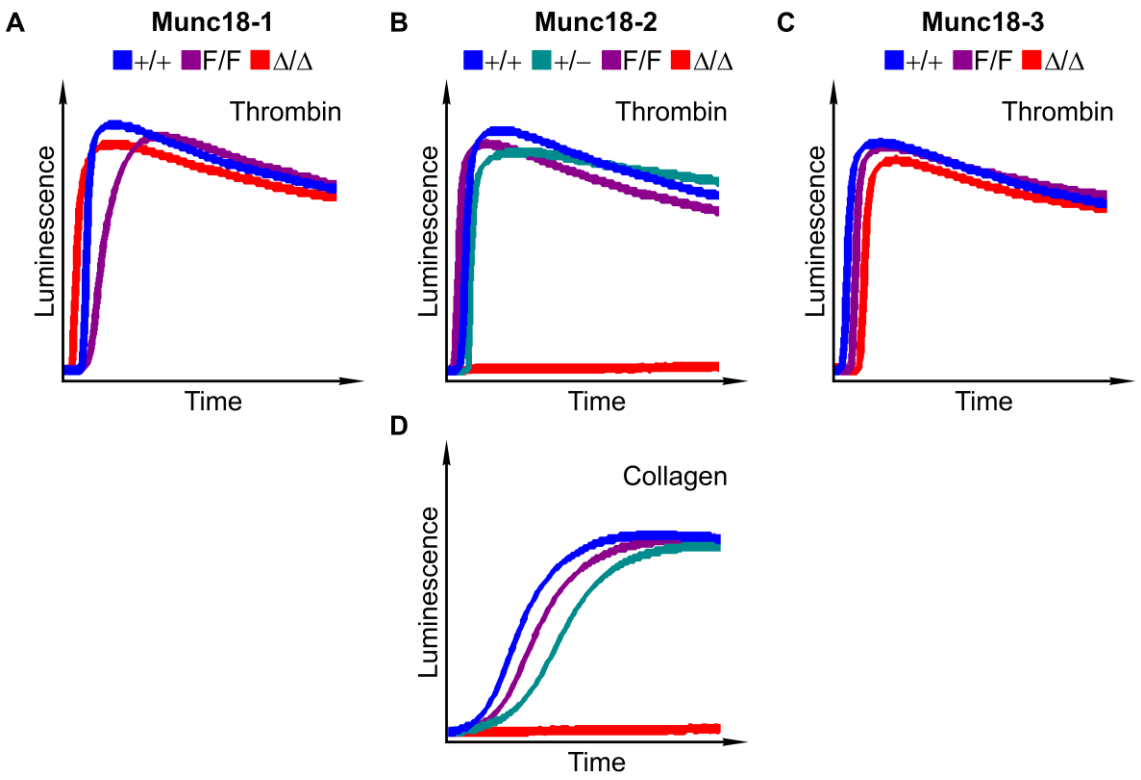

Figure S1. Deletion of Munc18-2 impairs dense granule release in platelets regardless of the agonist used. Samples from Munc18-1 (*A*), Munc18-2 (*B* and *D*), and Munc18-3 (*C*) mutant mice were stimulated with thrombin (1 U/ml) or collagen (10 μg/ml). *A*-*D*, representative tracings of ATP release (dense granules) measured by luminometry in whole blood.

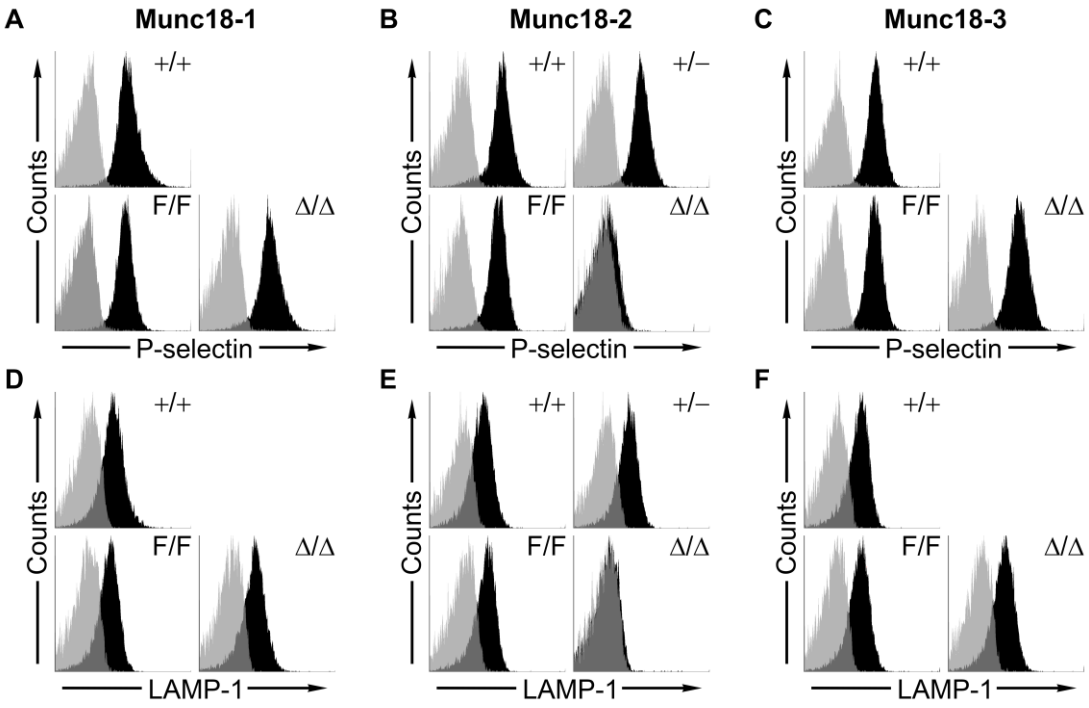

Figure S2. Deletion of Munc18-2 impairs alpha and lysosomal granule release in thrombin-stimulated platelets. Samples from Munc18-1 (*A* and *D*), Munc18-2 (*B* and *E*), and Munc18-3 (*C* and *F*) mutant mice were stimulated with thrombin (1 U/ml). *A*-*F*, representative tracings of P-selectin (*A*-*C*) and LAMP-1 (*D*-*F*) translocated to the surface of washed platelets measured by flow cytometry.

Table S1

Summary of results of secretion assays

Results are ratios of the means of experiments presented in Figs. 2 and 3. We include only values from platelets of Munc18-1$^{\Delta/\Delta}$, Munc18-2$^{\Delta/\Delta}$ and Munc18-3$^{\Delta/\Delta}$ mice, and from their respective F/F littermate controls.

Assay	**Agonist**	**Condition**	**Ratio: Δ/Δ platelets / F/F platelets**		
			Munc18-2	**Munc18-1**	**Munc18-3**
Dense granules (ATP)	Thrombin	Low	0.00 †	1.06	1.30
		High	0.02 *	0.94	1.11
	Collagen	Low	0.03 †		
		High	0.06 †		
Alpha granules (P-selectin)	Thrombin	Low	0.14 *	1.24	1.09
		High	0.16 *	1.03	0.93
		High + ADP	0.15 *		
Alpha granules (PF4)	Thrombin	High	0.26 *		
		High + ADP	0.33 *		
	Collagen	High	0.16 *		
		High + ADP	0.18 *		
Lysosomal granules (LAMP-1)	Thrombin	Low	0.08 *	0.79	1.39
		High	0.03 *	0.92	1.03
		High + ADP	0.10 †		

p values of comparing Δ/Δ to F/F: † = $p \leq 0.01$; * = $p \leq 0.001$

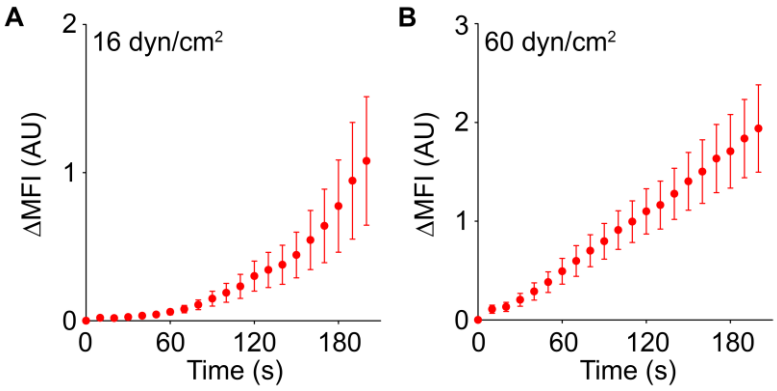

Figure S3. Platelets lacking Munc18-2 adhere to collagen but do not from thrombi under shear stress. Data from Munc18-2$^{\Delta/\Delta}$ platelets from Fig. 3, *C* and *E*, where we showed that in the absence of Munc18-2 platelets failed to from thrombi, is presented in a different scale. Whole blood was fluorescently-labeled and perfused over collagen-coated plates at low (*A*) or high (*B*) shear stress. The slight increase in fluorescence intensity above baseline (ΔMFI) over time represent adhesion of platelets to the collagen layer.