

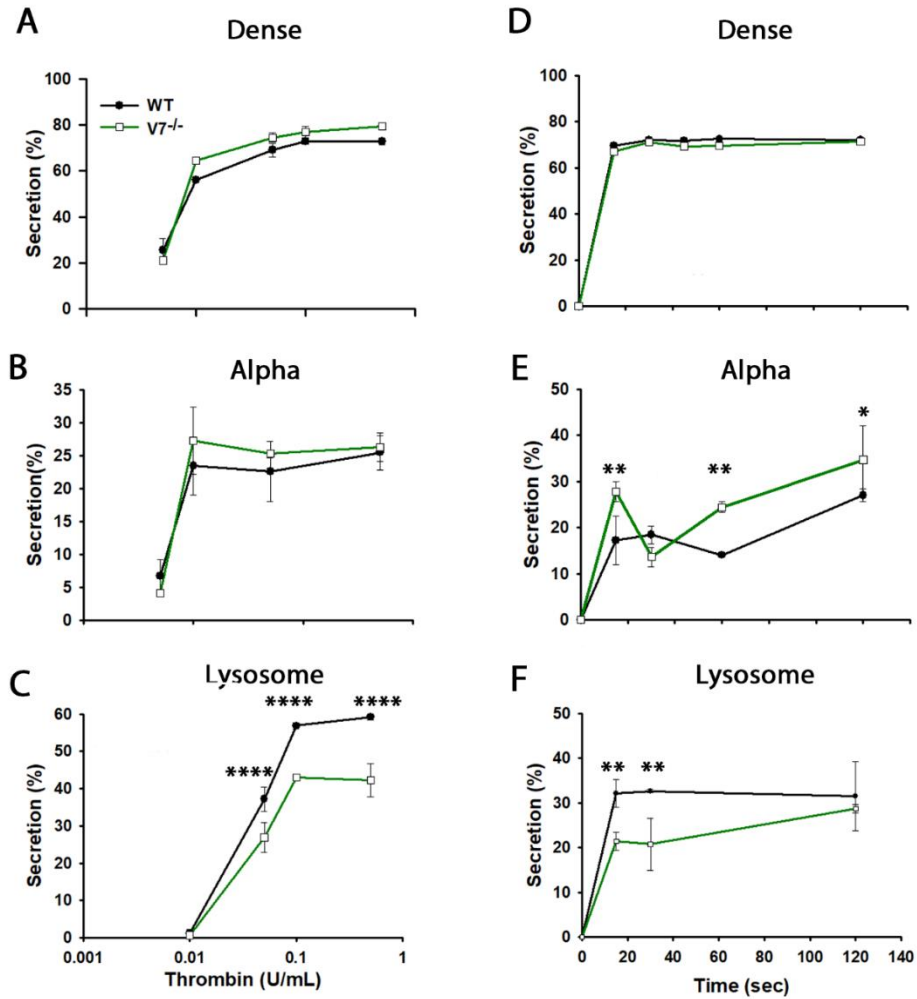
SUPPLEMENTAL MATERIAL FOR:

The complementary roles of VAMP-2, -3, and -7 in platelet secretion and function

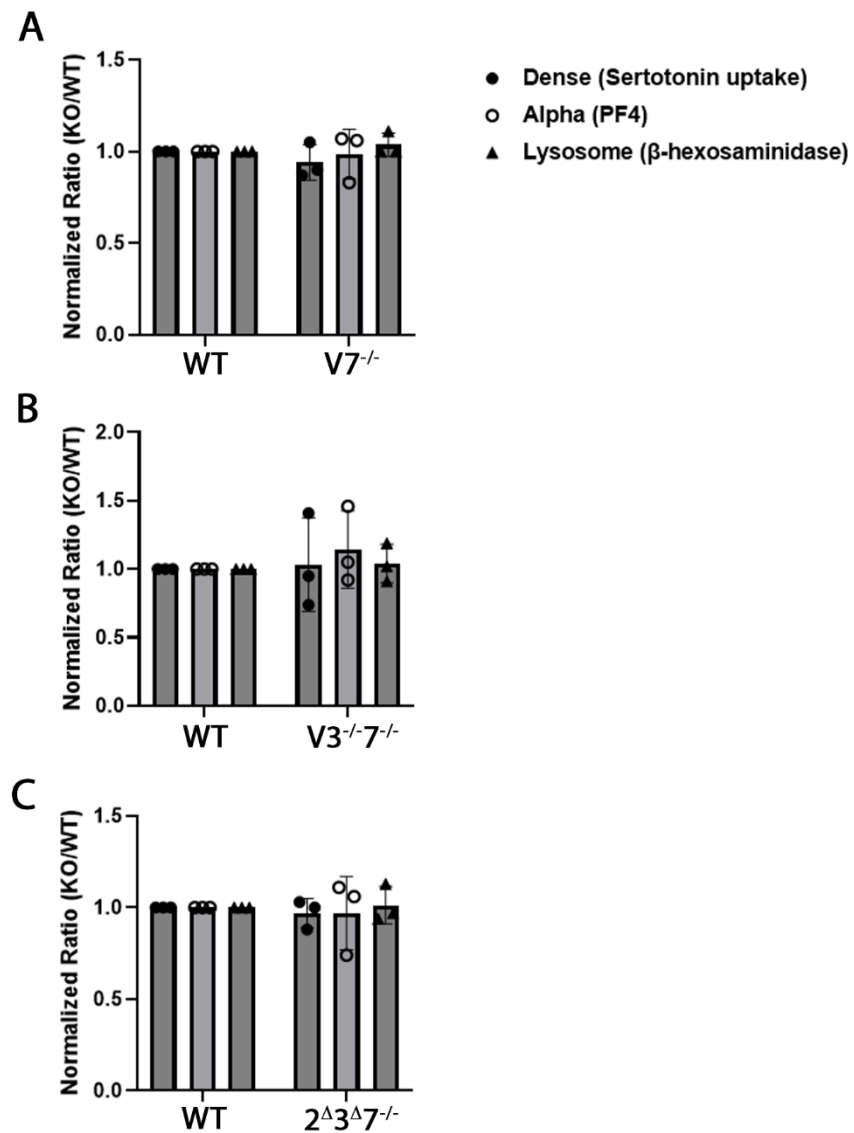
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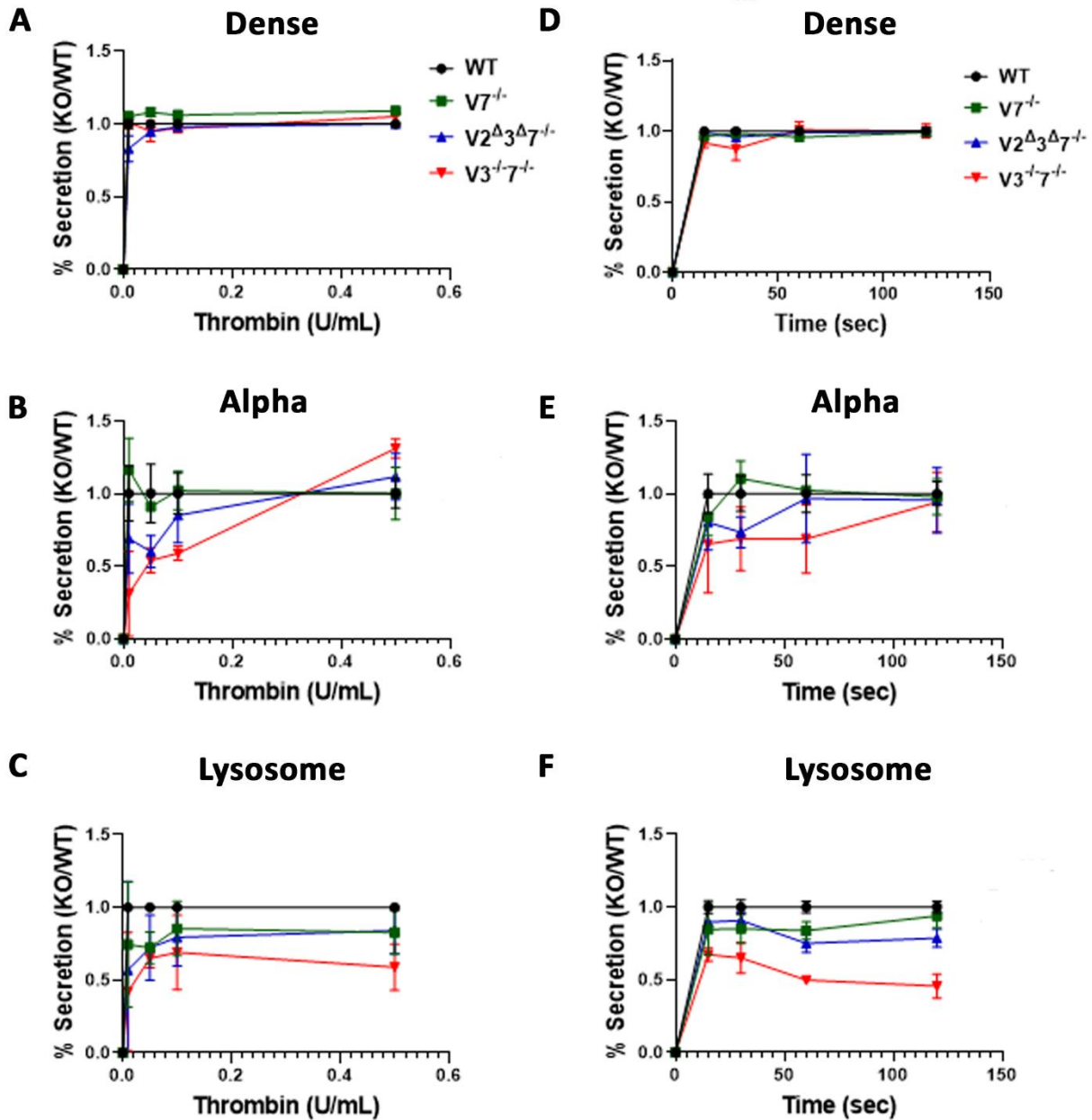
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Supplemental Figure 1: Loss of V7 affects the kinetics and the extent of lysosomal secretion. [³H]-5-[HT] (serotonin)-labeled platelets from wild type (WT), V7^{-/-} mice were prepared as in Methods. The release of [³H]-5-[HT] from dense granules (**A, D**), PF4 from α granules (**B, E**), and β -hexosaminidase from lysosomes (**C, F**) was measured, and percentage secretion was calculated. (**A-C**) For the thrombin dose-response experiment, platelets were stimulated for 2 min with the indicated concentrations of thrombin. (**D-F**) For the time-course experiments, platelets were stimulated with 0.05 U/mL thrombin for the indicated times. Data are mean \pm standard error of the mean of triplicate measurements and is representative of ≥ 3 independent experiments. Statistical significance was calculated using a two-way ANOVA. ns not significant, * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$



Supplemental Figure 2: No significant changes in granule cargo levels in V7^{-/-}, V3^{-/-}7^{-/-}, and V2^Δ3^Δ7^{-/-} platelets. Serotonin uptake, PF4 and β-hexosaminidase levels were measured based on secretion assays (see methods for details). The data are shown from the V7^{-/-} (A), V3^{-/-}7^{-/-} (B), and V2^Δ3^Δ7^{-/-} (C) platelets. The data are represented from at least 3 different biological samples. Statistical analysis was performed using unpaired Students t-test.



Supplemental Figure 3: Loss of V7 affects lysosomal secretion while combined loss of V3 and V7 or V2, V3 and V7 affects both alpha and lysosomal secretion. Serotonin uptake (A and D), PF4 (B and E) and β -hexosaminidase levels (C and F) were measured based on secretion assays (see methods for details). Average platelet secretion (%) from WT platelets were calculated and normalized by dividing the percent secretion for each data point by the average of all the data points, to get a ratio of 1. The ratio of KO/WT percent secretion was calculated by dividing corresponding KO percent secretion values with average percent secretion from WT platelets. The data are plotted as line graphs. The data are shown from the WT (black line), V7^{-/-} (green), V3^{-/-}7^{-/-} (red), and V2^{Δ3Δ7}^{-/-} (blue) platelets. The data are calculated from at least 2 different biological samples.

SUPPLEMENTAL TABLE 1

Blood Type or Metric [unit]	Genotype	Sex	Value	p value
RBC [M/uL]	WT	M	9.0±0.55	
		F	8.61±0.42	
	V3 ^{-/-} 7 ^{-/-}	M	9.07±0.24	0.81
		F	9.04±0.37	0.16
	V2 ^{Δ3} Δ7 ^{-/-}	M	8.79±0.69	0.54
		F	7.99±0.39	0.06
WBC [K/uL]	WT	M	9.96±2.05	
		F	8.52±2.09	
	V3 ^{-/-} 7 ^{-/-}	M	10.42±3.19	0.72
		F	11.10±1.56	0.08
	V2 ^{Δ3} Δ7 ^{-/-}	M	9.02±2.37	0.45
		F	9.5±2.15	0.51
Platelets [K/uL]	WT	M	823.08±101.92	
		F	703±58.71	
	V3 ^{-/-} 7 ^{-/-}	M	825.2±239.29	0.98
		F	810.5±314	0.47
	V2 ^{Δ3} Δ7 ^{-/-}	M	1232.75±78.95	2.489E-06
		F	928.5±223.63	0.064
Mean Platelet Volume [fL]	WT	M	6.08±0.44	
		F	6.32±0.95	
	V3 ^{-/-} 7 ^{-/-}	M	6.96±1.21	0.03
		F	6.92±1.29	0.44
	V2 ^{Δ3} Δ7 ^{-/-}	M	8.3±0.2	7.941E-08
		F	8.37±0.15	0.003

Supplemental Table 1- Hematological parameters for V3^{-/-}7^{-/-} and V2^{Δ3}Δ7^{-/-} animals. Whole blood was analyzed using an IDEXX analyzer - WT (n=18, M=13, F=5) V3^{-/-}7^{-/-} (n=7, M=4, F=3), V2^{Δ3}Δ7^{-/-} (n=6, M=3, F=3). Statistical analysis was done using Student t-test, p values are as shown.