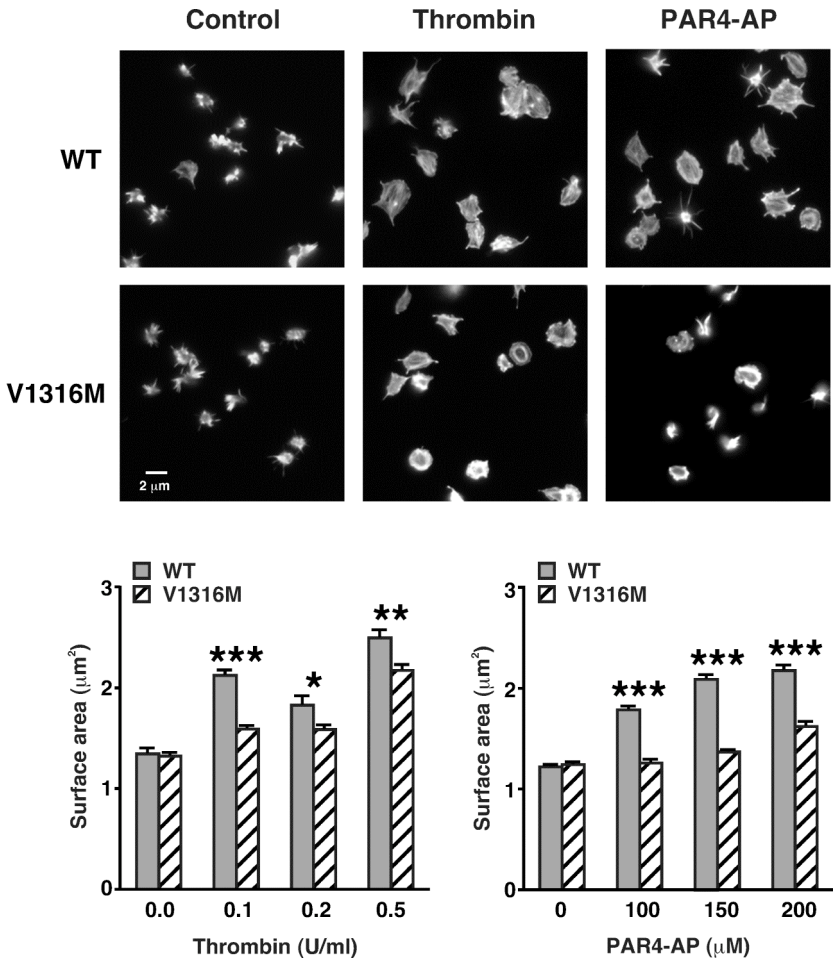

Figure S1: Platelet spreading in mVWF/V1316M

Platelet suspensions from mVWF/V1316M or WT were stimulated with either thrombin (0.1 to 0.5 U/ml) or PAR4-AP (100 to 200 μM) and immediately plated for 30 min at room temperature onto coverslips precoated with fibrinogen (100 μg/ml). Platelets were stained with Alexa Fluor 488-labeled phalloidin. The surface area was quantified as described under Material and Methods. Data are expressed as the means ± SEM of at least 20 determinations and are representative of 2 independent experiments; **P< 0.01; ***P< 0.001 (unpaired Student's *t* test).

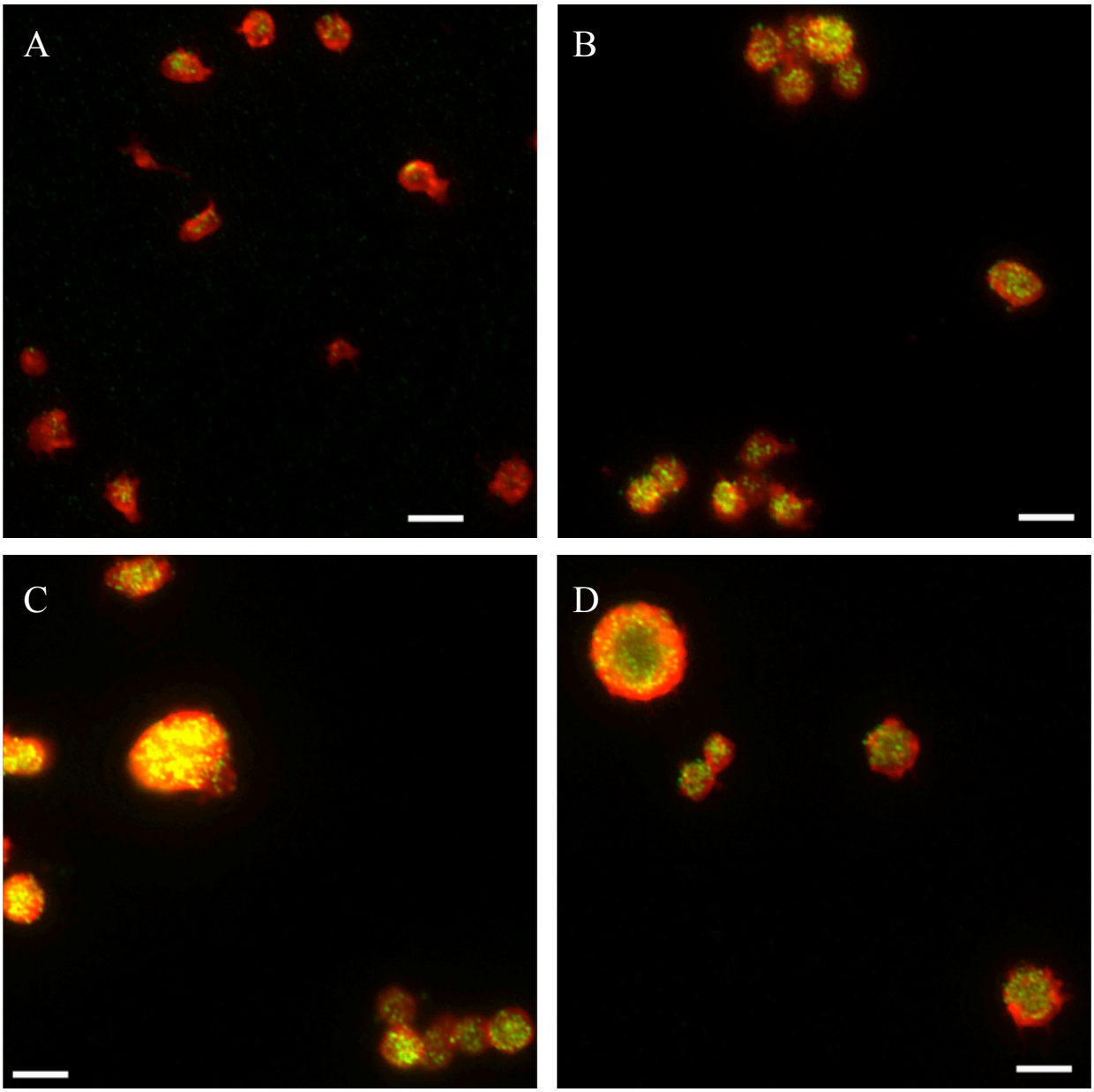

Figure S2: Binding of VWF/pV1316M to patient's platelets
Control and p.V1316M PRP were isolated as described in Material and Methods and smears were prepared with 5µl of this preparation. Unpermeabilized platelets were detected by staining for the αIIb subunit of integrin αIIbβ3 (CD41, red) (Monoclonal Mouse anti-Human CD41 followed by AlexaFluor 546-Goat anti-Mouse secondary antibody). VWF (green) was revealed by staining with a Rabbit anti-Human VWF followed by AlexaFluor 488-Goat anti-Rabbit secondary antibody. (A) In control platelets, numerous regular platelets with barely detectable VWF staining were found. (B-D) In the patient carrying the p.V1316M/VWF mutation, the majority of the platelets were enlarged and small platelets aggregates were also detectable. Note the strong labeling for VWF in panels B-D. Original magnification was 100x. Scale bar is 5µm.

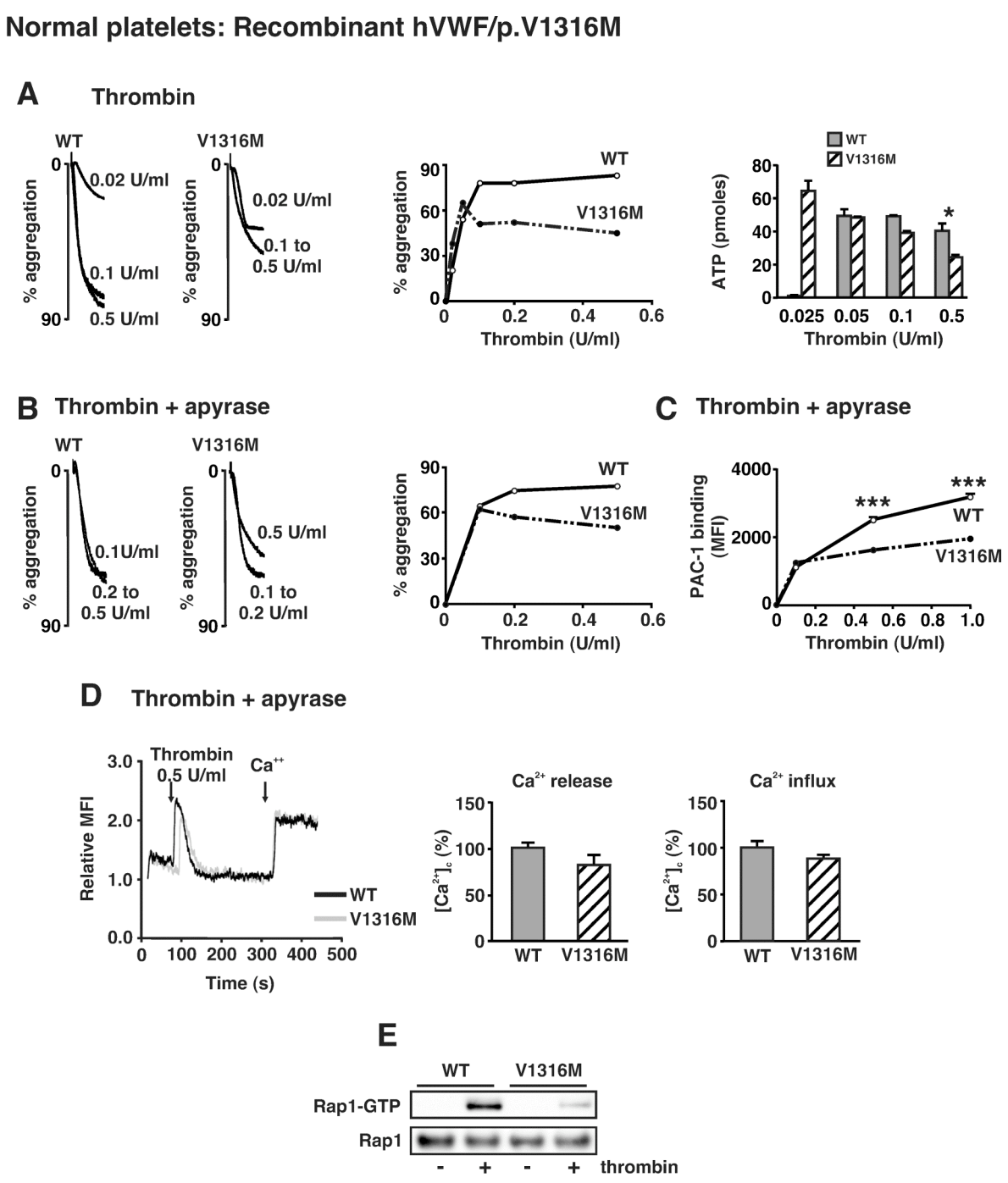

Figure S3: Thrombin-induced activation of human control platelets pretreated with recombinant hVWF/p.V1316M

Washed human control platelet pretreated for 10 minutes with recombinant hVWF/p.V1316M or WT were stimulated with thrombin in the (A) absence or (B,C, D) presence of apyrase (2U/ml) or (E) in the presence of the antagonists of ADP receptors (AR-C69931MX: 10 μM and MRS 2179: 10 μM). (A) Aggregation and secretion of washed platelets were initiated by adding various concentrations of thrombin (0.02U/ml to 0.5U/ml). Dense granule secretion was assessed by measuring the amount of ATP release (pmoles). (C) Integrin αIIbβ3 activation induced by thrombin (0.1 U/ml to 1.0 U/ml) was assessed by flow cytometry using integrin αIIbβ3 mAb (PAC1) specific for the activated conformation of the human integrin. The level of activated integrin is indicated by the mean fluorescence intensities (MFI). (D) Thrombin induced Ca^{2+} signaling was monitored by flow cytometry using the Oregon Green-488 BAPTA1-AM. Histograms represent the area under the curve of both the Ca^{2+} store release and Ca^{2+} influx. (E) Rap1 activity was measured by pull-down assay after 30 s of stimulation with thrombin(0.5 U/ml) in the absence of stirring. Data from one experiment done in triplicate are presented as mean ± SEM; *P<0. 05, ***P<0.001 (unpaired Student's *t* test). Results are representative of 3 independent experiments.

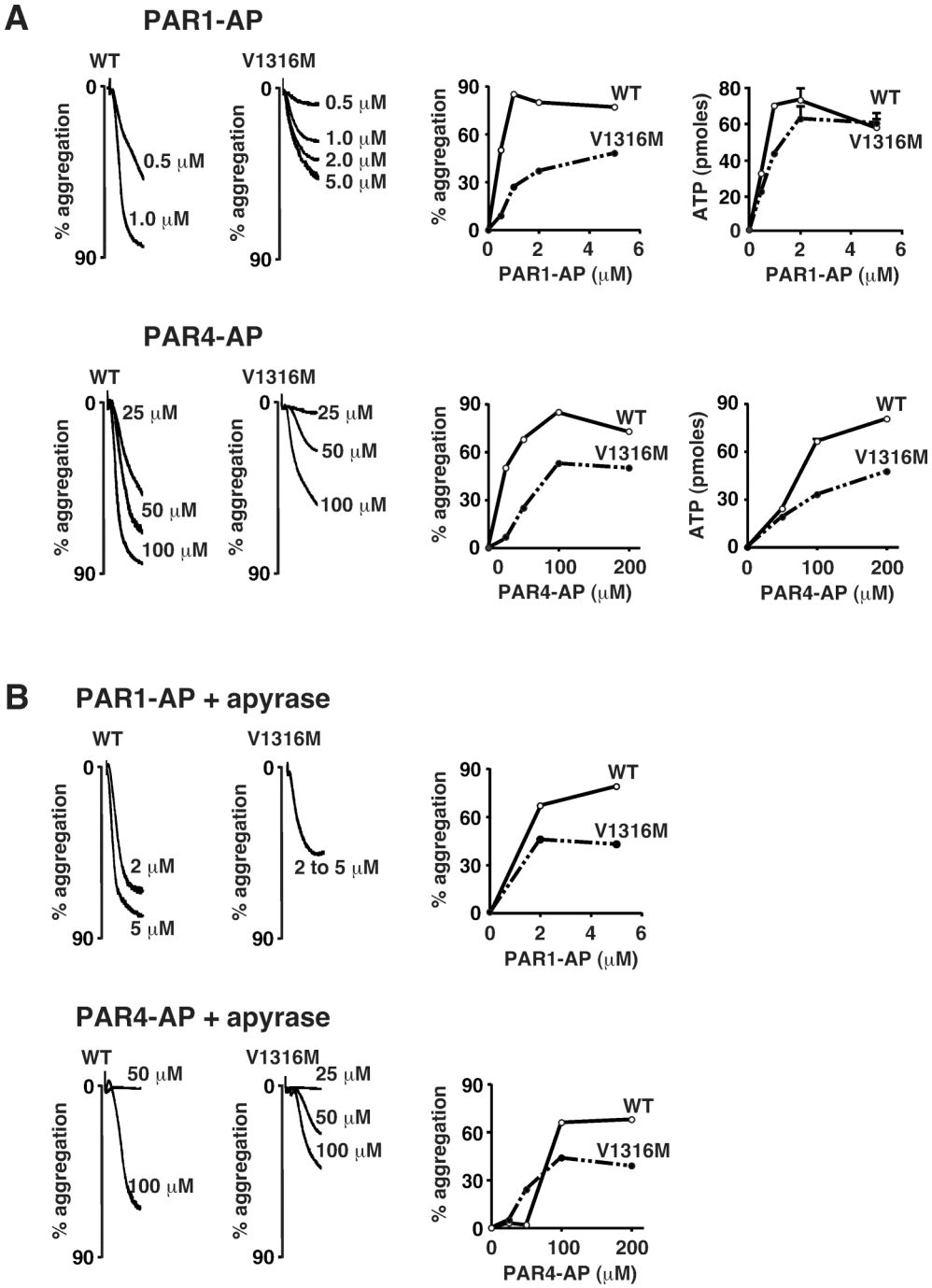

Figure S4: Aggregation and secretion of human control platelets induced by PAR1-AP and PAR4-AP after pretreatement with recombinant hVWF/p.V1316M.

Human control platelet suspensions were pretreated for 10 minutes with recombinant hVWF/p.V1316M or WT in the (A) absence or (B) presence of apyrase (2U/ml). Aggregation and secretion of washed platelets were initiated by adding various concentrations of PAR1-AP (0.5 μM to 5 μM) and PAR4-AP (25 μM to 200 μM). Dense granule secretion was assessed by measuring ATP release. Results are representative of 3 independent experiments.

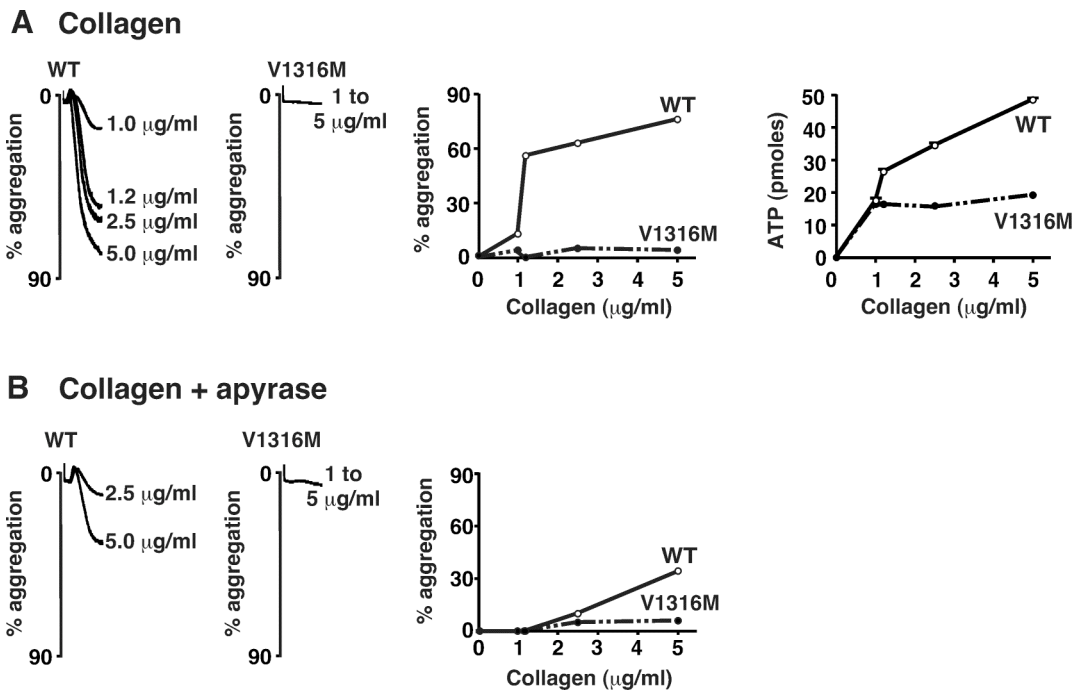

Figure S5: Aggregation and secretion of human control platelets induced by collagen after pretreatement with recombinant hVWF/p.V1316M.

Human control platelet suspensions were pretreated for 10 minutes with recombinant hVWF/p.V1316M or WT in the (A) absence or (B) presence of apyrase (2U/ml). Aggregation and secretion of washed platelets were initiated by adding various concentrations of collagen (1 μg/ml to 5 μg/ml). Dense granule secretion was assessed by measuring ATP release. Results are representative of 3 independent experiments.