Results

We investigated if human platelets shed GPIbα and GPV in similar fashion to mouse platelets. Refrigeration for 24 or 48 h led to a progressive loss of GPIbα and GPV, but not GPIX or β3, from the platelets, as determined by flow cytometry using specific antibodies (Fig. 1A). The metalloprotease inhibitor GM6001 inhibits the shedding of GPIbα (Fig. 1A) and GPV (not shown). We confirmed that glycocalicin is released into the media by immunoblotting of refrigerated platelets versus the storage plasma using the specific anti GPIbα antibody SZ2 (Fig. 1B).

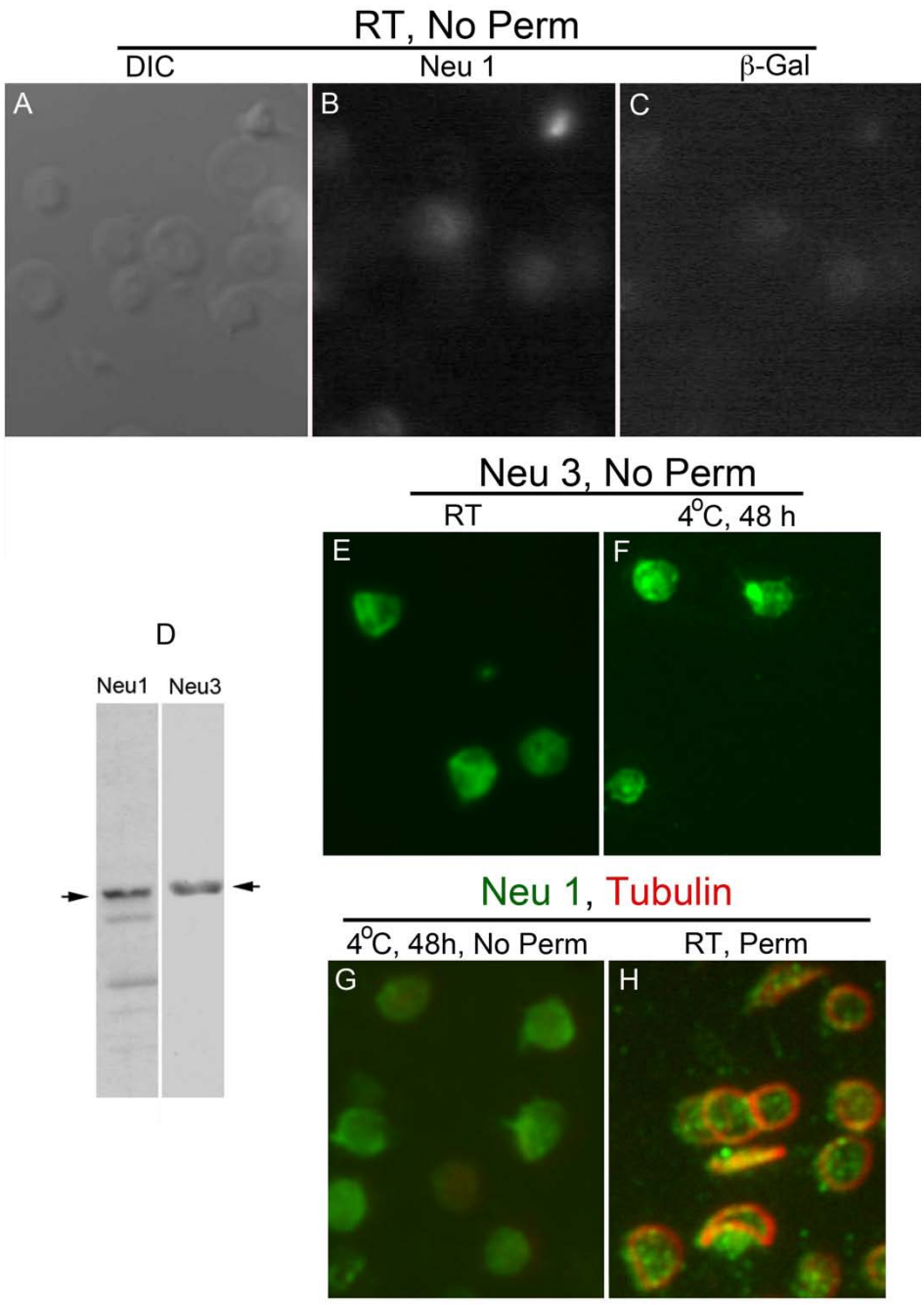

Figure S1. Resting human platelet do not label with anti-Neu1 or anti–β-Gal antibodies in the absence of detergent treatment

(A) DIC image, (B) anti-Neu1 labeling (C) anti–β-gal labelling. (D) Immunoblot for Neu1 and Neu 3 in lysates of normal human platelets. (E, F) Neu 3 is found on the surface for both resting and refrigerated platelets. (G, H) Refrigeration does not permeabilize platelets. (G) Fixed refrigerated platelets were stained for Neu1 (green) and β1-tubulin (red). Note the absence of tubulin staining in the refrigerated platelets. (H) Fixed resting platelets stained for Neu1 (green) and β1-tubulin (red) after detergent permeabilization.

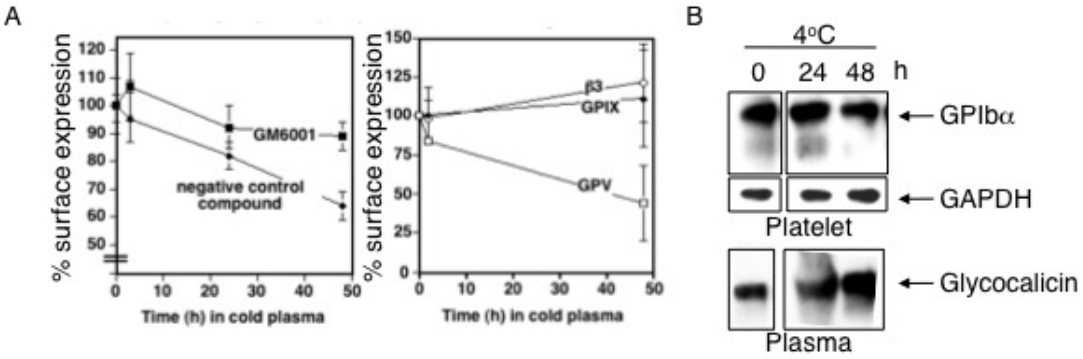

Figure S2. (A) GPIbα, GPV, GPIX, and β3 surface expression was assessed by flow cytometry. Human platelets were stored in plasma in the presence of absence of 100 μM GM6001 for the indicated time points. GPIbα expression is the average derived from labeling platelets with 6 different anti-GPIbα mAbs (WM23, AN51, 6D1, VM16d, SZ2, and HIP1) n = 4 (left panel). GPV, GPIX, and β3 surface expression were also measured by flow cytometry (right panel). Expression at time 0 was set as 100%. Results are expressed relative to the amount of GPIbα on fresh platelets (mean % relative to time 0 ± s.e.m.), n=5. (B) Immunoblot for GPIbα in lysates of human platelets refrigerated for 0, 24 and 48 h and glycocalicin in released into plasma (lower panel). GADPH is used as a loading control (upper panel).