Nitric oxide (NO) inhibits platelet activation and aggregation by mechanisms that are not completely defined. Platelet activation involves exocytosis of platelet granules, releasing mediators that regulate interactions between platelets, leukocytes, and endothelial cells. We hypothesized that NO inhibits platelet granule exocytosis by inhibiting N-ethylmaleimide Sensitive Factor (NSF), an ATPase that regulates exocytosis. We now demonstrate that exogenous and endogenous NO inhibits exocytosis of dense, lysosomal, and -granules from human platelets. NO inhibits platelet granule exocytosis by pathways independent of guanylate cyclase, but dependent upon S-nitrosylation. NO inhibits NSF regulation of platelet granule exocytosis by increasing the affinity of NSF for Soluble NSF Attachment Receptor (SNARE) molecules that mediate exocytosis, and by inhibiting the ability of NSF to disassemble SNARE complexes. Inhibition of NO synthase (NOS) increases the thrombus burden in a murine model of pulmonary thromboembolism. Addition of NSF, but not NSF pre-incubated with NO, to platelets treated with a NO donor restores the exocytosis capacity of the platelets. Regulation of NSF is a novel mechanism by which NO regulates thrombosis.