

Figure S1. HPLC chromatogram of an alkaloid extract from *C. roseus* leaves showing the major presence of vindoline (V), catharanthine (C) and α -3',4'-anhydrovinblastine (AVLB).

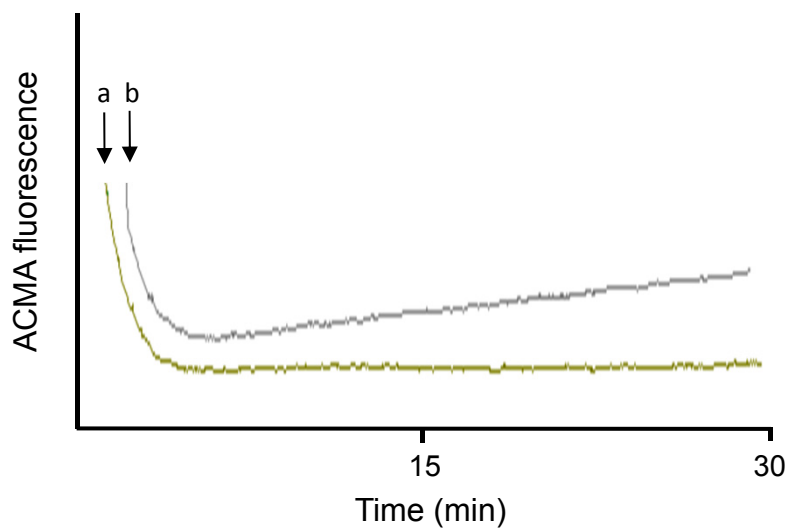


Figure S2. H⁺ pumping activity in tonoplast vesicles upon addition of 1 mM of ATP in the presence (a) or in the absence (b) of an ATP regenerating system (10 mM of creatine phosphate plus 10 $\mu\text{g mL}^{-1}$ of creatine kinase). In the absence of this regenerating system, after 1 min the system can not maintain the H⁺ gradient. The H⁺ pumping activity was measured by the fluorescence quenching of the pH-sensitive probe ACMA.

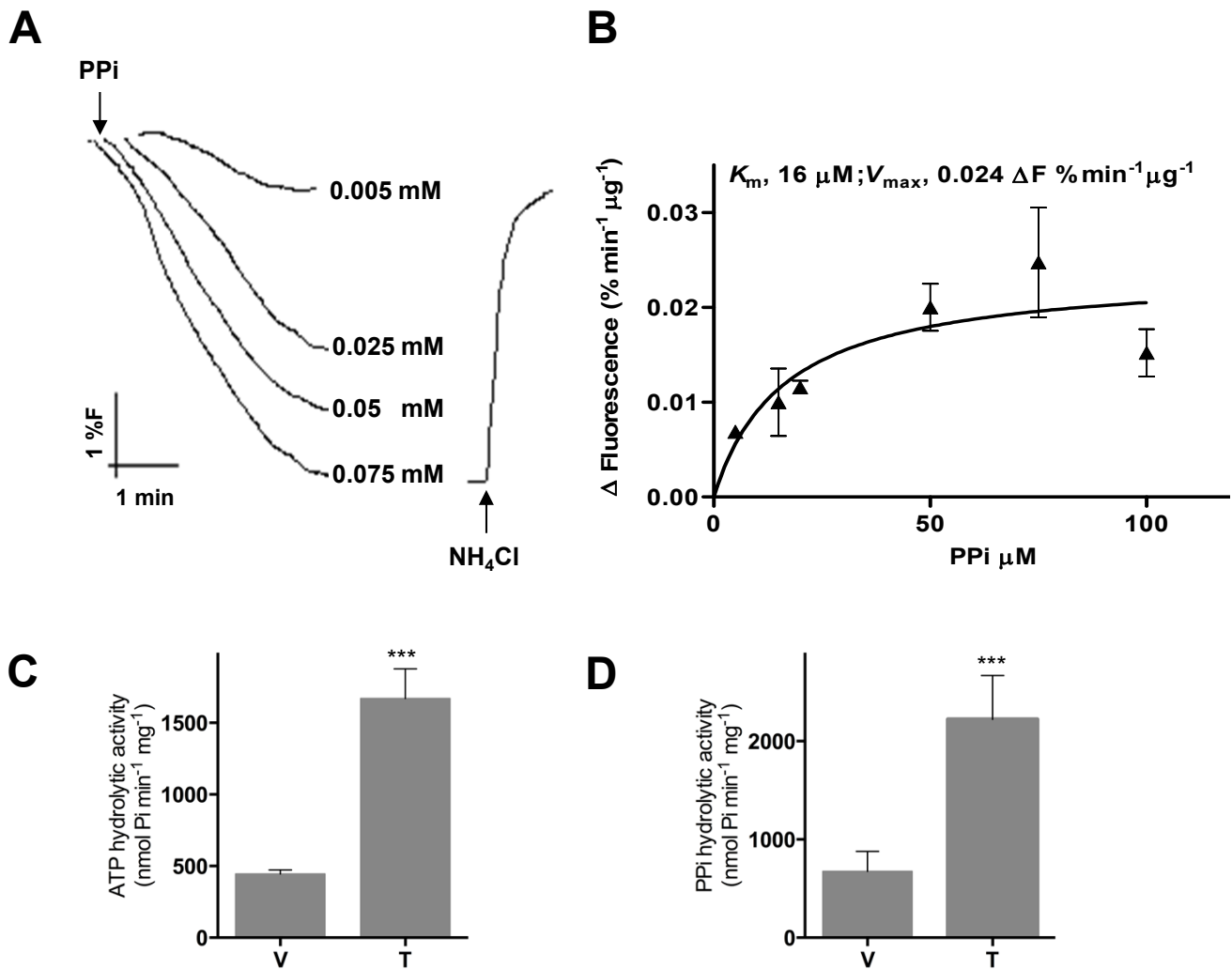


Figure S3. Pumping activities of V-H⁺-PPase in tonoplast vesicles and ATP and PPI hydrolytic activities in vacuoles and tonoplast vesicles isolated from *C. roseus* leaves. A, H⁺ pumping activity in tonoplast vesicles upon addition of PPI, measured by the fluorescence quenching of the pH-sensitive probe ACMA. B, Michaelis-Menten plot of the initial rates of proton pumping in A. Error bars indicate SD (n = 3). C, ATP hydrolytic activity of intact vacuoles (V) and tonoplast vesicles (T), in the presence of the P-H⁺-ATPase inhibitor vanadate (100 μM) and the F-H⁺-ATPase inhibitor azide (5 mM), to detect only the V-H⁺-ATPase activity. D, PPI hydrolytic activity of intact vacuoles (V) and tonoplast vesicles (T). Statistical significance was evaluated using Student's *t* test for pairwise comparison (***) P < 0.001). Data significantly different are indicated. Error bars indicate SD (n = 3).

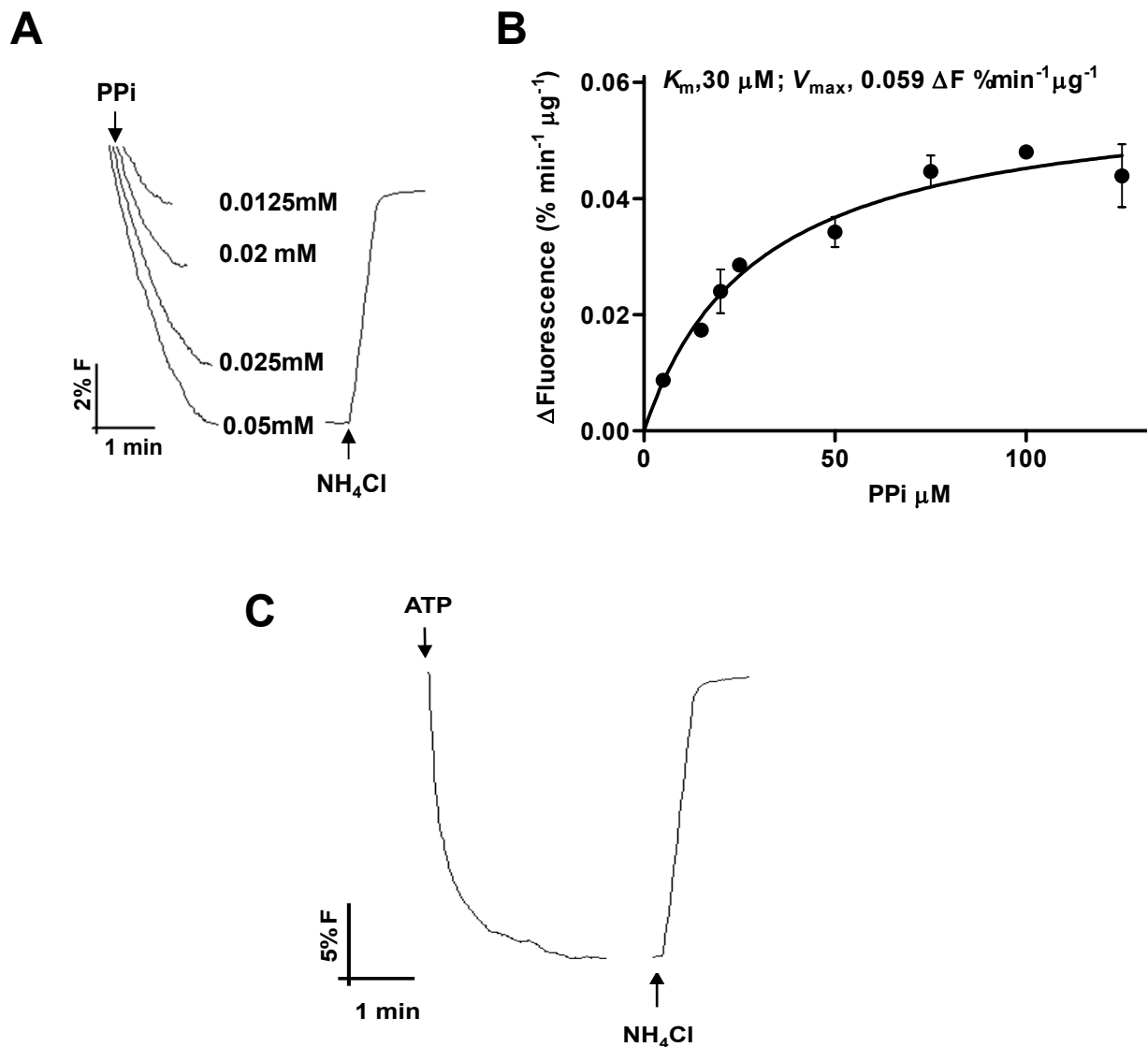


Figure S4. Pumping activity of V-H⁺-PPase and V-H⁺-ATPase in intact vacuoles from *C.roseus* leaves. A, H⁺ pumping activity in intact vacuoles upon addition of PPI, measured by the fluorescence quenching of the pH-sensitive probe ACMA. B Michaelis-Menten plot of the initial rates of proton pumping in A. C, H⁺ pumping activity in intact vacuoles upon addition of ATP, measured by the fluorescence quenching of the pH-sensitive probe ACMA. Error bars indicate SD (n = 3).