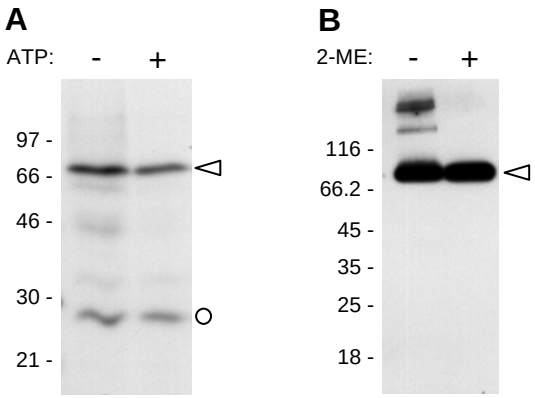

Supplemental Figure 1. The polypeptide with Mr lower than 30 kD is not released from BiP by ATP treatment.

A. Protoplasts from tobacco leaves were pulse-labeled with ^{35}S-Met and ^{35}S-Cys for 1 h in normal conditions and subjected to chase for 4 h in the presence of 20 mM 2-ME. Total protoplasts homogenates were immunoselected using anti-BiP antiserum. The immunoselected material bound to the Sepharose-protein A resin was split into two parts, which where incubated for 1 h with (+) or without (-) ATP, as described (Foresti et al., 2003). The material still bound to the resin after this treatment was analyzed by SDS-PAGE and fluorography. The positions of BiP (open arrowhead) and of the polypeptide with Mr lower than 30 kD (open circle) are indicated at right. Numbers at left indicate the positions of molecular mass markers in kD.

B. Protoplasts from tobacco leaves were incubated for 24 h in the presence (+) or absence (-) of 20 mM 2-ME. Equal amounts of total proplast homeogenated were analyzed by SDS-PAGE followed by protein blot with anti-BiP antiserum. The position of BiP is indicated at right (open arrowhead). Numbers at left indicate the positions of

molecular mass markers in kD. Notice that the component with Mr lower than 30 kD is not detected, strongly suggesting that it is not a BiP fragment.