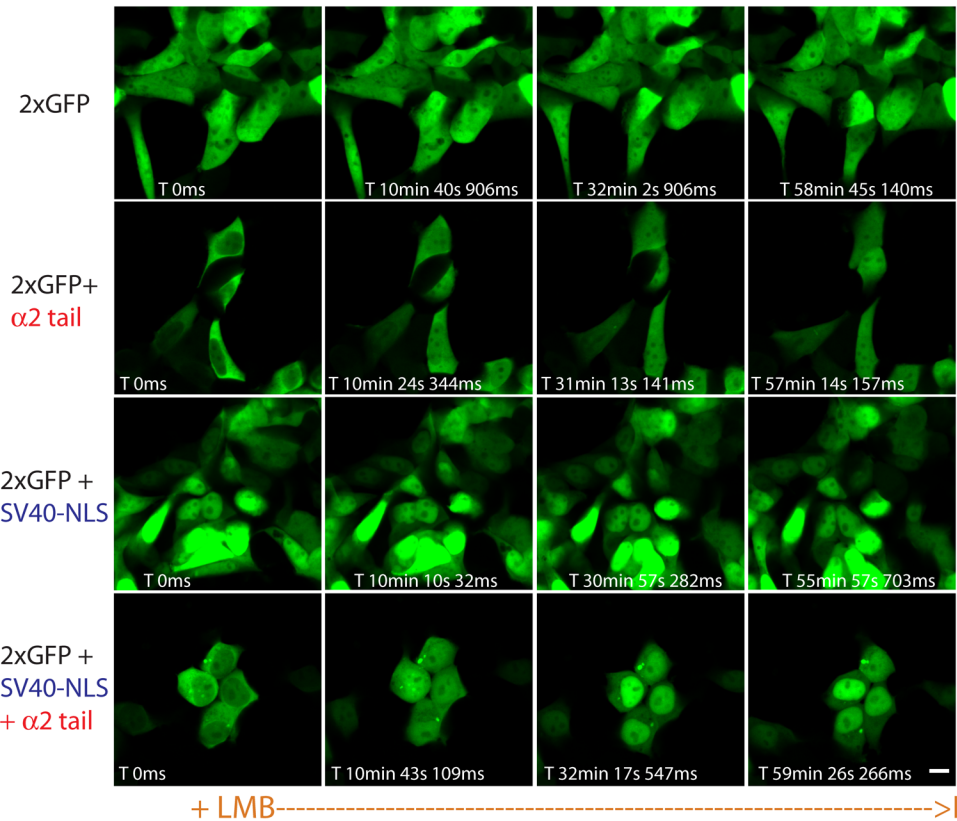


Supplementary Figure 1. Leptomycin B (LMB) treatment, a specific inhibitor of CRM1-mediated nuclear export, induces accumulation of AMPK α carboxy tail-containing proteins in the nucleus in a time-dependent manner. HEK 293 cells transiently transfected with either 2xGFP or 2xGFP fusions containing the AMPK α 2 C-terminal tail, the SV40 NLS, or both tail and NLS, imaged before and at indicated time points after treatment with Leptomycin-B (LMB). Bar, 10 μ m.

Supplementary Figure 2. Truncated AMPK α lacking the carboxy-terminal 23 amino acids (AMPK $\alpha\Delta$ C) associates with β/γ subunits in transfected cells by anti-myc immunoprecipitation. Western blot of anti-myc immunoprecipitations from lysates of HEK 293 cells co-transfected with HA-tagged AMPK β , FLAG-tagged AMPK γ (all three lanes), and either myc-tagged full-length AMPK α 2 (lane 1) or C-terminally truncated AMPK $\alpha\Delta$ C (lane 3). Cells with no myc-AMPK α were used as a negative control (lane 2).

Supplementary Figure 3. Total phospho-AMPK α levels *in vivo* are highly regulated. Transgenic expression of wild type (mCherry-AMPK α) but not nuclear relatively unphosphorylated AMPK α (mCherry-AMPK $\alpha\Delta$ C) causes increased endogenous phospho-AMPK α (pAMPK α). Western blot of total lysates from transgenic *Drosophila* adults expressing either Gal4 alone (control), or the respective mCherry-tagged AMPK α transgene probed for phospho-AMPK α and Lamin C (as loading control).



Immunoprecipitation anti-Myc

Myc-
AMPK α 2

NT
 α 2

Myc-
AMPK $\alpha\Delta$ C

Myc-alpha2



HA-beta1



FLAG-gamma1



Western

Gal4 mCherry mCherry-
only -AMPK α AMPK α Δ C



◀ transgenic pAMPK α



◀ endogenous pAMPK α



◀ Lamin C