# Expanded View Figures

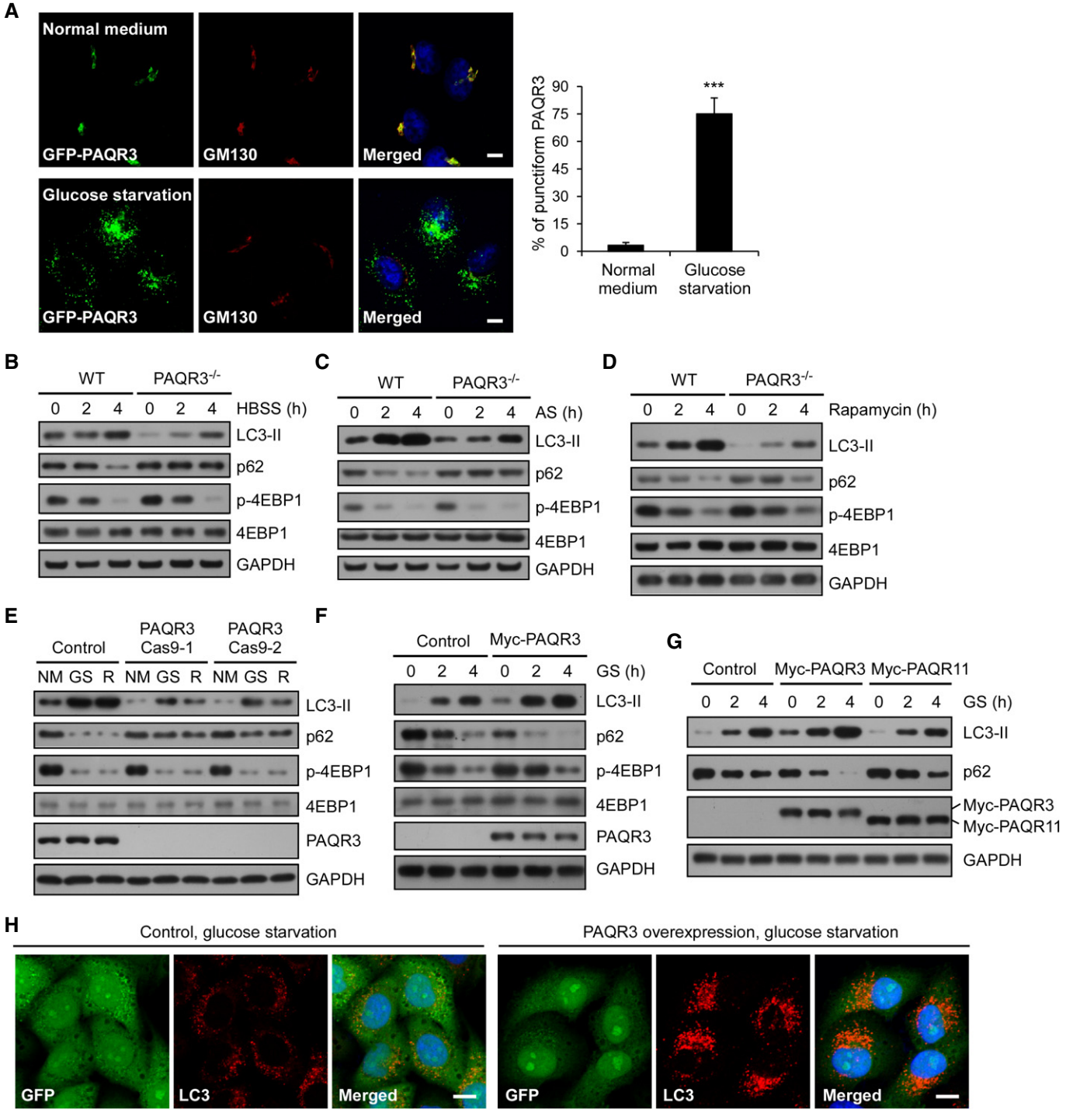


**Figure EV1.**

**Figure EV1. PAQR3 modulates autophagy induced by multiple stress conditions.**

A GFP-PAQR3-transfected HeLa cells were fixed for immunofluorescence staining with the indicated antibodies before or after glucose starvation (GS) for 4 h. At least 100 cells were counted per experiment and the data on the right represent the percentage of punctiform PAQR3 with or without GS, the results were obtained from three independent experiments. Values are presented as mean ± SD (***$P < 0.001$). Scale bar: 10 μm.

B–D Immunoblotting (IB) analysis of WT and PAQR3-deficient MEFs after HBSS treatment, amino acid starvation (AS), or rapamycin (50 nM) treatment for different times as indicated.

E WT and PAQR3-deficient HeLa (PAQR3 Cas9-1/2) cells were incubated with normal medium (NM), GS or treated with rapamycin (50 nM) for 4 h, followed by IB.

F HeLa cells stably transfected with PAQR3 were incubated in GS for different times as indicated, followed by IB.

G HeLa cells were infected with PAQR3 or PAQR11 overexpression of lentivirus, respectively. After GS for 2 or 4 h, the whole-cell lysates were harvested for IB.

H HeLa cells were infected by control or PAQR3-expressed lentivirus. After GS for 4 h, the cells were fixed for immunofluorescence staining with LC3 antibody (red). Scale bar: 10 μm.

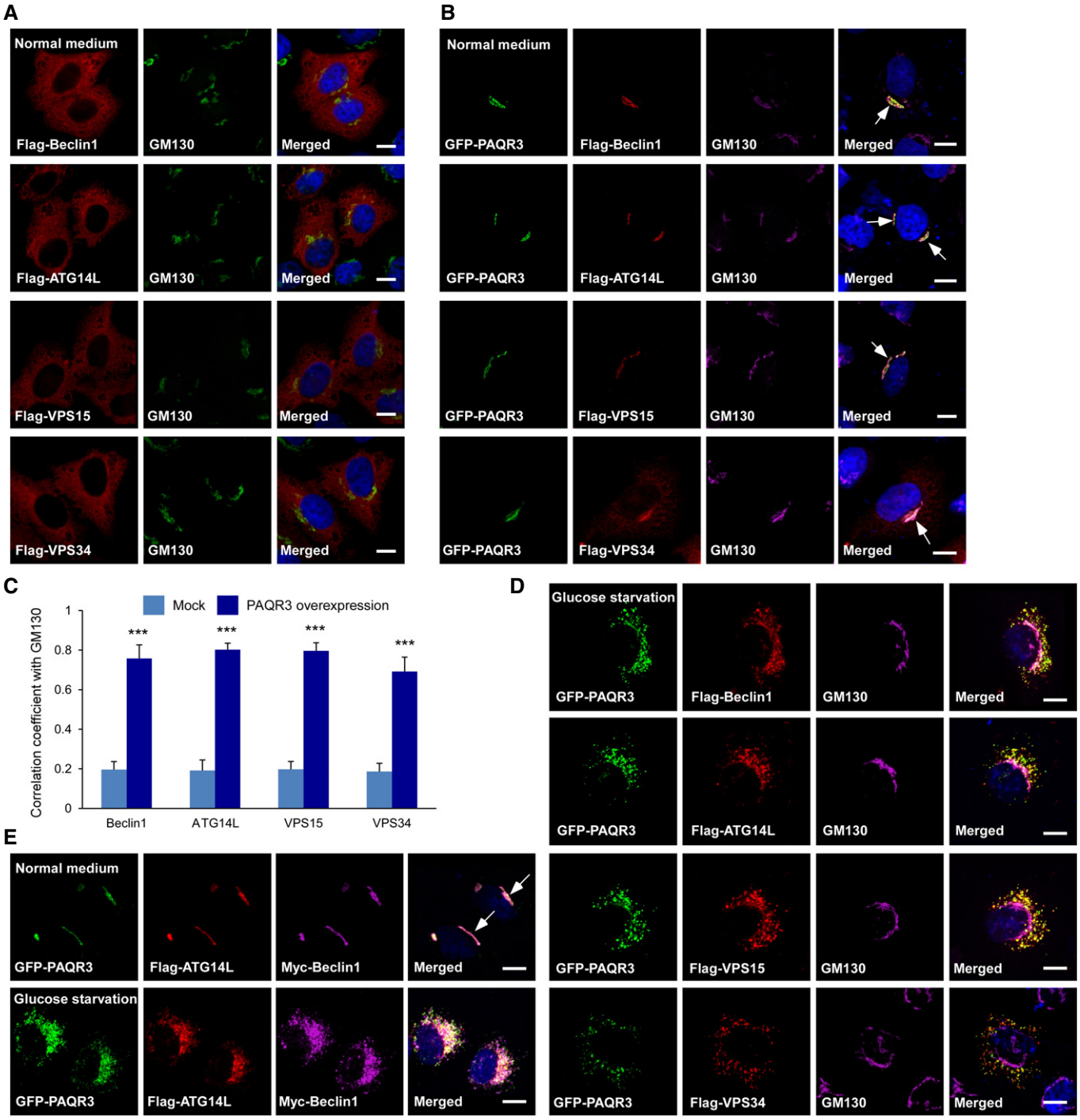


**Figure EV2. ATG14L-linked VPS34 complex coexists with PAQR3 in the Golgi apparatus.**

A, B HeLa cells were transfected with the plasmids as indicated, followed by immunofluorescence staining with the indicated antibodies. The arrow indicates co-localization of PAQR3 with the indicated proteins at the Golgi apparatus. Scale bar: 10 μm.

C Co-localization coefficient between the indicated proteins and GM130 in the presence or absence of PAQR3 overexpression. At least fifty cells were quantified from each independent experiment, which was repeated for three times with similar results. Values are presented as mean ± SD, ***$P < 0.001$.

D HeLa cells were transfected with the plasmids as indicated. After glucose starvation for 4 h, the cells were fixed and used in immunofluorescence staining and confocal analysis. Scale bar: 10 μm.

E HeLa cells were co-transfected with GFP-fused PAQR3, Flag-tagged ATG14L, and Myc-tagged Beclin1 simultaneously. At 24 h after transfection, the cells were fixed for immunofluorescence staining before or after glucose starvation as indicated. The arrow indicates apparent co-localization of ATG14L and Beclin1 with PAQR3. Scale bar: 10 μm.

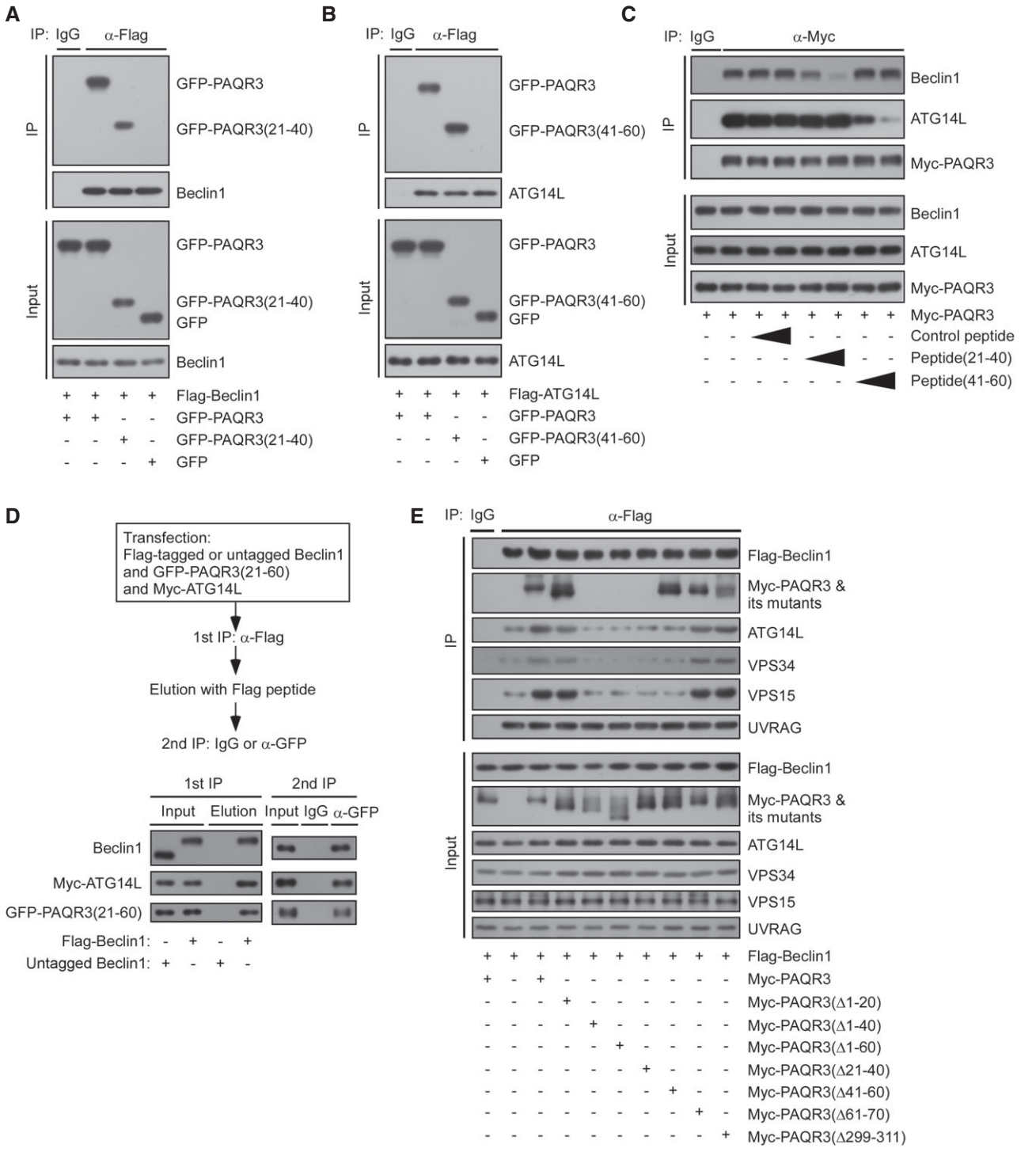


**Figure EV3. Beclin1 and ATG14L interact with the $NH_2$-terminal 21–60aa of PAQR3.**

A, B HEK293T cells were transfected with different plasmids as indicated. At 24 h after transfection, the cells were subjected to immunoprecipitation (IP) and immunoblotting (IB) analyses.

C HEK293T cells were transfected with Myc-tagged PAQR3. At 24 h after transfection, the cells were treated with P21–40 or P41–60 (4 or 20 ng/μl) for 12 h, respectively, and the cell lysates were used in IP and IB.

D A two-step co-immunoprecipitation assay to determine the complex formation among PAQR3 $NH_2$-terminal 21–60aa, Beclin1, and ATG14L. The procedures of the assay are outlined in the top panel. HEK293T cells were transfected with the plasmids as indicated. At 24 h after transfection, the cells were harvested for IP and IB.

E HeLa cells were transfected with the plasmids as indicated. The cell lysates were used for IP and IB.

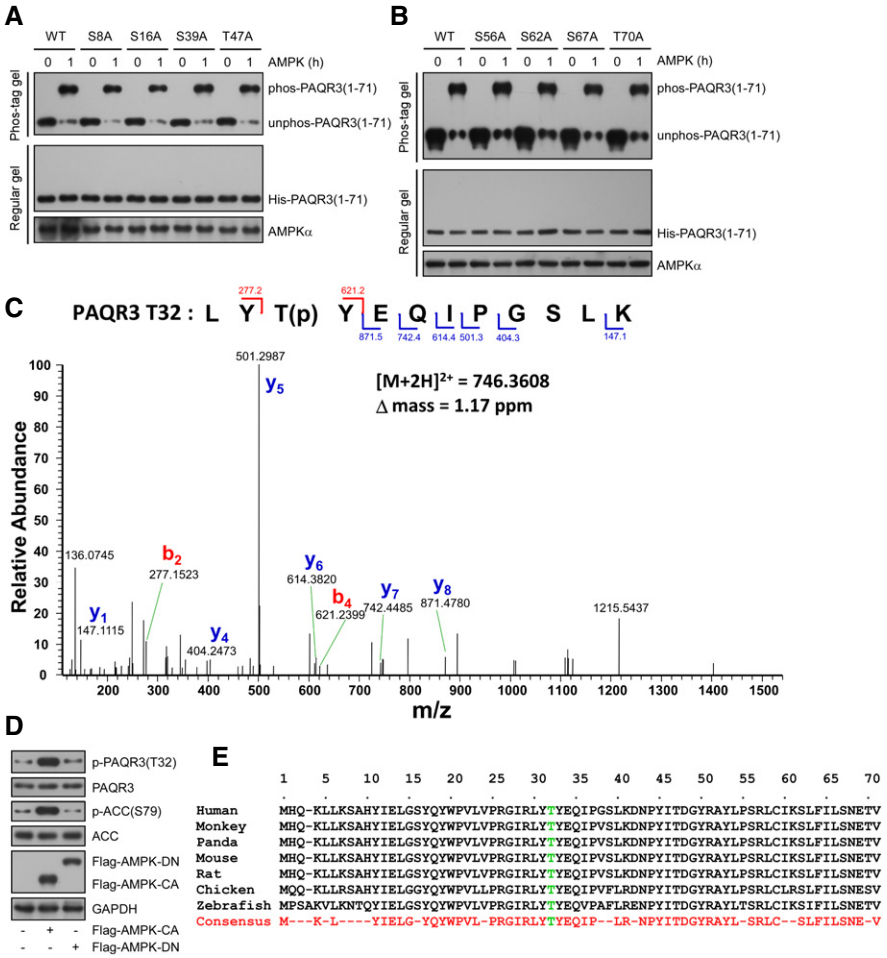


**Figure EV4. PAQR3 T32 is directly phosphorylated by AMPK.**

A, B Bacterial purified His-tagged WT or mutant $NH_2$-terminal 71aa of PAQR3 was subjected to AMPK kinase assay for the indicated time, followed by Phos-tag gel or regular SDS–PAGE analysis.

C Bacterial purified His-tagged $NH_2$-terminal 71aa of PAQR3 was subjected to AMPK kinase assay, followed by mass spectrometry analysis. The MS/MS spectrum of a precursor ion with $[M + 2H]^{2+}$ = 746.3608 was identified as PAQR3 T32-phosphorylated tryptic peptide LYT(p)YEQIPGSLK. The labeled mass peaks indicate b and y ion series that matched the phosphopeptide.

D HeLa cells were transfected with constitutively activated AMPK (AMPK-CA) or AMPK (AMPK-DN). At 24 h after transfection, the cell lysates were harvested for immunoblotting.

E T32 of PAQR3 is conserved in different species. Sequence alignment of PAQR3 T32 and surrounding amino acids in a few representative species are depicted.

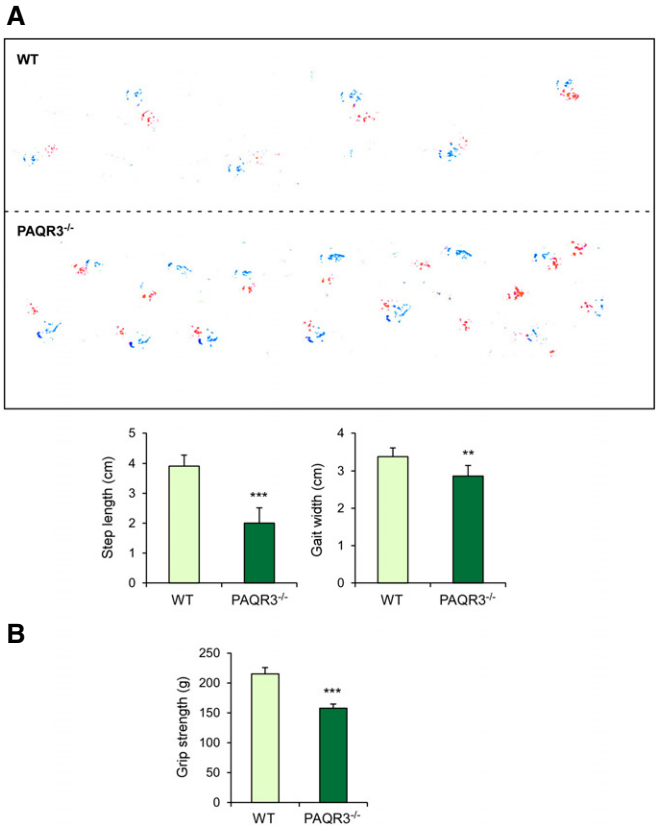


**Figure EV5. PAQR3-deleted mice exhibit ataxic walking pattern and ameliorated grip strength.**

A Gait abnormalities in the PAQR3 knockout mice were evaluated by footprint analysis during walking (red, forelimbs; blue, hindlimbs). Quantification analyses of stride length and gait width of paws print were shown in the bottom panels.

B Grip strength analysis of all limbs in PAQR3 knockout mice and their WT littermates.

Data information: Values are presented as mean ± SD ($n = 7$; $^{**}P < 0.01$; $^{***}P < 0.001$).