Ju et al., http://www.jcb.org/cgi/content/full/jcb.201105010/DC1

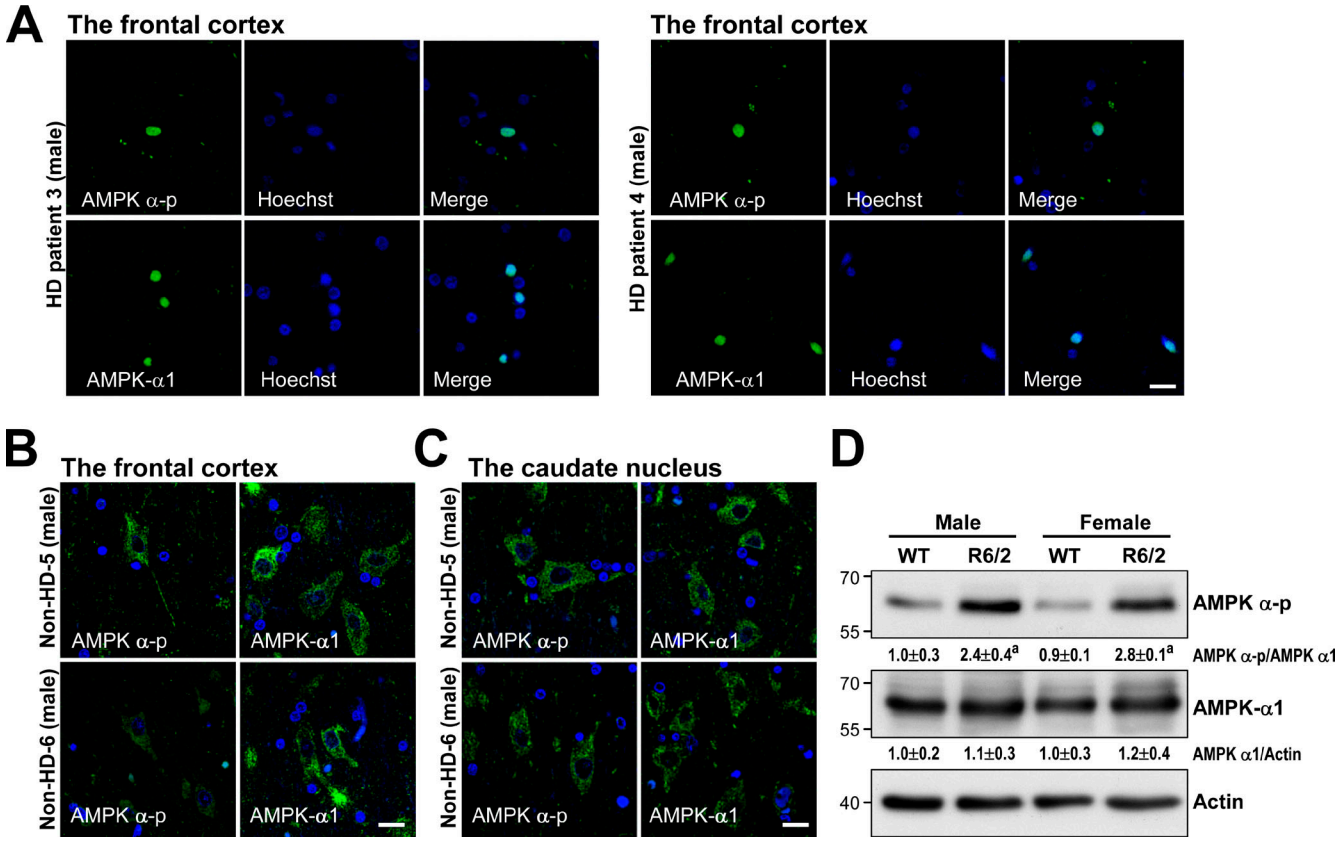

Figure S1. **Selective activation and nuclear enrichment of AMPK-α1 in brains of patients and mice with HD.** (A) The frontal cortex of two HD patients was analyzed (a 57-yr-old male on the left and 58-yr-old male on the right). (B and C) The frontal cortex (B) and caudate nucleus (C) of two non-HD and non-AD control subjects (a 74-yr-old male on the left and 83-yr-old male on the right) were assessed. Immunofluorescence staining of phosphorylated AMPK-α at Thr^{172} (AMPK-α-p [green]) or AMPK-α1 (green) was conducted. Nuclei were stained with Hoechst (blue). Bars, 20 µm. (D) Equal amounts of striatal lysates of 12-wk-old WT and R6/2 mice were assessed by Western blot analyses for levels of phosphorylated AMPK-α at Thr^{172} and AMPK-α1. The data represent the mean ± SEM of three independent experiments. Molecular mass is indicated in kilodaltons. a, $P < 0.05$ versus WT.

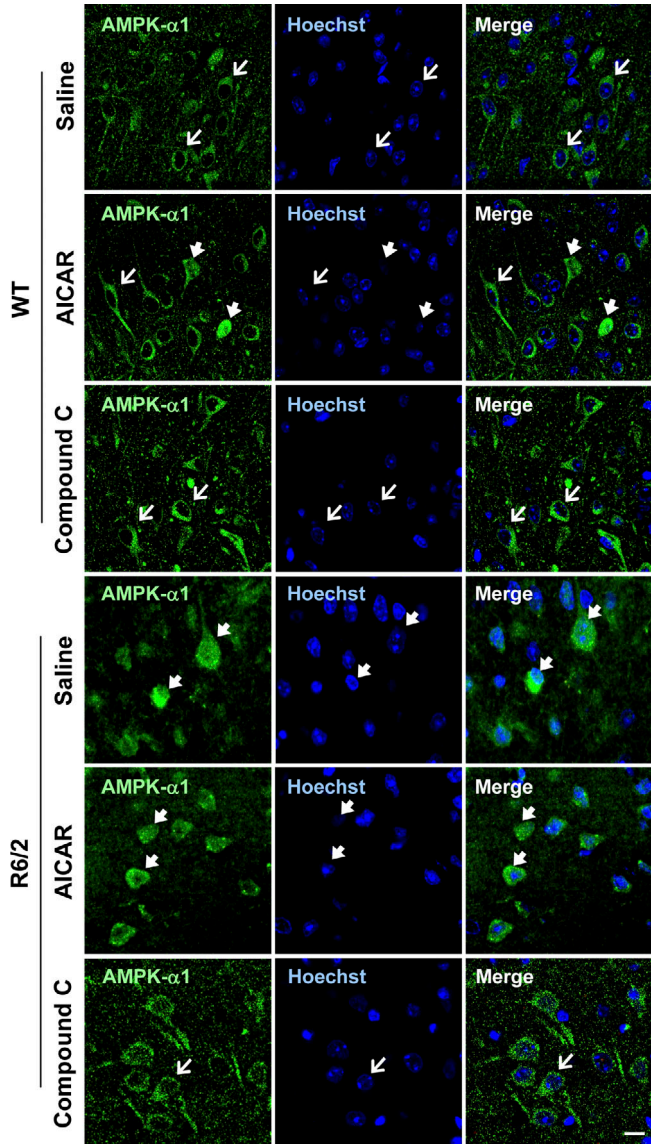

Figure S2. **An inhibitor of AMPK, CC, reduced the nuclear enrichment of AMPK-α1 in the striatum of R6/2 mice.** Mice were intrastriatally injected with 3 µg AICAR or 5 µg CC for 24 h. Immunofluorescence staining of AMPK-α1 (green) was conducted. Nuclei were stained with Hoechst (blue). Thin arrows mark cells containing AMPK-α1 that are located mostly in cytoplasmic regions. Thick arrows mark cells with nuclear enrichment of AMPK-α1. Bar, 20 µm.

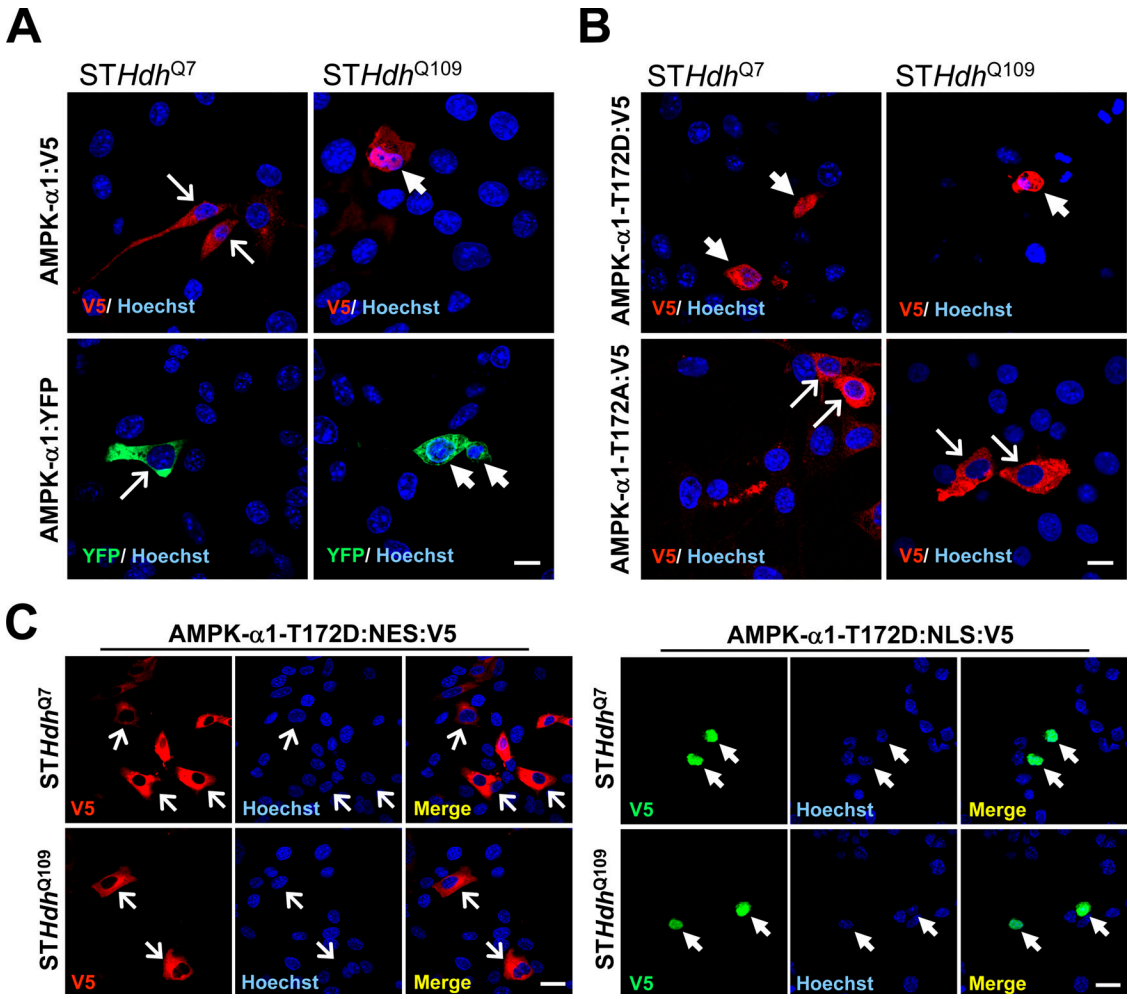

Figure S3. **Nuclear enrichment of AMPK-α1 in striatal cells expressing polyQ-expanded mHtt.** (A and B) Cells were transfected with the indicated construct for 48 h. Immunofluorescence staining of exogenously expressed AMPK-α1:V5 (A) and AMPK-α1-T172D:V5 or AMPK-α1-T172A:V5 (B) was conducted using an anti-V5 antibody as indicated. Nuclei were stained with Hoechst. (C) Immunofluorescence staining of AMPK-α1 variants was conducted as indicated (AMPK-α1-T172D:NES:V5 [red] or AMPK-α1-T172A:NLS:V5 [green]) using an anti-V5 antibody. Nuclei were stained with Hoechst. (A–C) Thin arrows mark cells that contain the AMPK-α1 variant and that are located mostly in cytoplasmic regions. Thick arrows mark cells with nuclei enriched with the indicated AMPK-α1 variant. Bars, 10 μm.

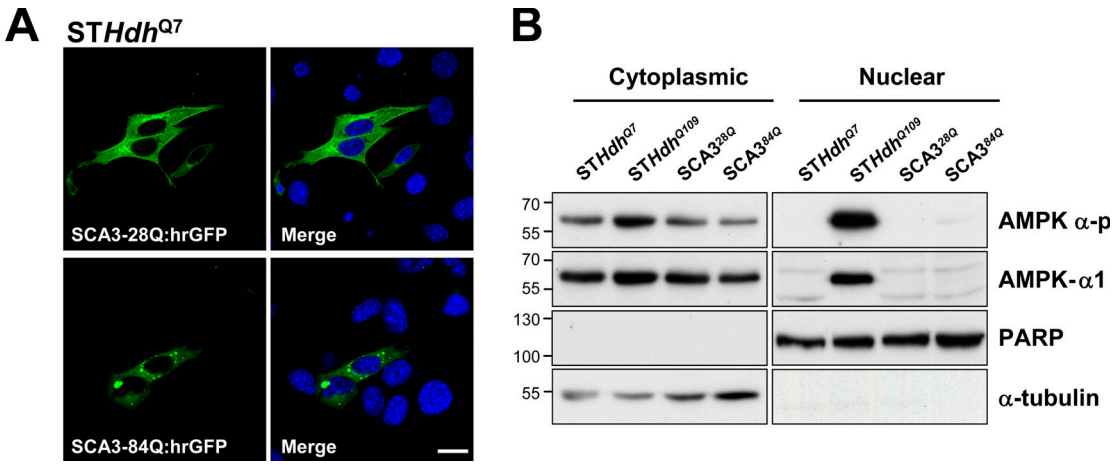

Figure S4. **PolyQ-expanded mHtt, but not SCA3, causes enrichment of AMPK-α1 in striatal cells.** (A) ST*Hdh*^{Q7} cells were transfected with the indicated construct (pcDNA3.1-SCA3-$[Q]_{28}$-hrGFP, SCA3-28Q:hrGFP; and pcDNA3.1-SCA3-$[Q]_{84}$-hrGFP, SCA3-84Q:hrGFP) for 48 h. Nuclei were stained with Hoechst (blue). Note that expression of SCA3-84Q:hrGFP caused aggregation in the perinuclear region. Bar, 20 µm. (B) Equal amounts (50 µg per lane) of the nuclear and cytosolic fractions were assessed by Western blot analyses for levels of phosphorylated AMPK-α at Thr^{172} (AMPK-α-p) and AMPK-α1 as indicated. PARP and α-tubulin were markers for the nuclear and cytoplasmic fractions, respectively. Molecular mass is indicated in kilodaltons.

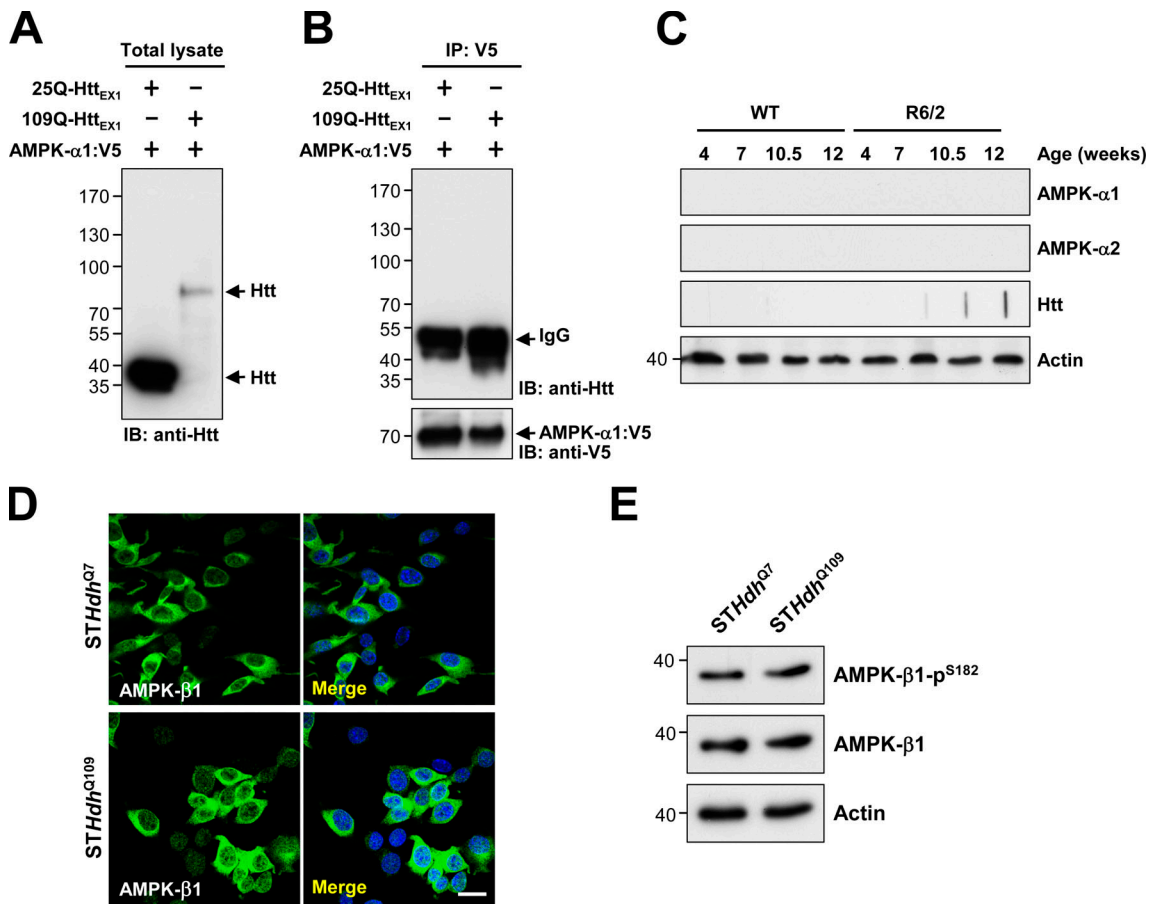

Figure S5. **AMPK-α1 does not interact with the N-terminal fragment of Htt.** (A and B) ST14 cells were transfected with the indicated expression construct of Htt (pcDNA3.1-Htt$_{EX1}$-[Q]$_{25}$-hrGFP, 25Q-Htt$_{EX1}$; or pcDNA3.1-Htt$_{EX1}$-[Q]$_{109}$-hrGFP, 109Q-Htt$_{EX1}$) plus pcDNA3.1-AMPK-α1:V5 (AMPK-α1:V5) at a 1:1 molar ratio for 48 h. IB, immunoblotted. (A) Equal amounts of the total lysate were assessed by Western blot analyses for protein levels of Htt$_{EX1}$-(Q)$_{25}$-hrGFP and Htt$_{EX1}$-(Q)$_{109}$-hrGFP using an anti-Htt antibody (EM48). (B) Total lysate was immunoprecipitated (IP) using an anti-V5 antibody and was subjected to Western blot analyses using an anti-Htt antibody (EM48) and V5 antibody as indicated. (C) The amount of Htt aggregates in the striatum of R6/2 mice and WT mice at the indicated age was determined by a filter retardation assay. Insoluble aggregates retained on the filters were detected using anti-Htt (EM48), anti–AMPK-α1, and anti–AMPK-α2 antibodies as indicated. (D) Localization of AMPK-β1 (green) in ST*Hdh*^{Q7} and ST*Hdh*^{Q109} cells was analyzed by immunofluorescence staining. Nuclei were stained with Hoechst (blue). Bar, 20 µm. (E) Equal amounts of total lysates were assessed by Western blot analyses for levels of phosphorylated AMPK-β1 at Ser^{182} (AMPK-β1-p^{S182}) and AMPK-β1 as indicated. Molecular mass is indicated in kilodaltons.