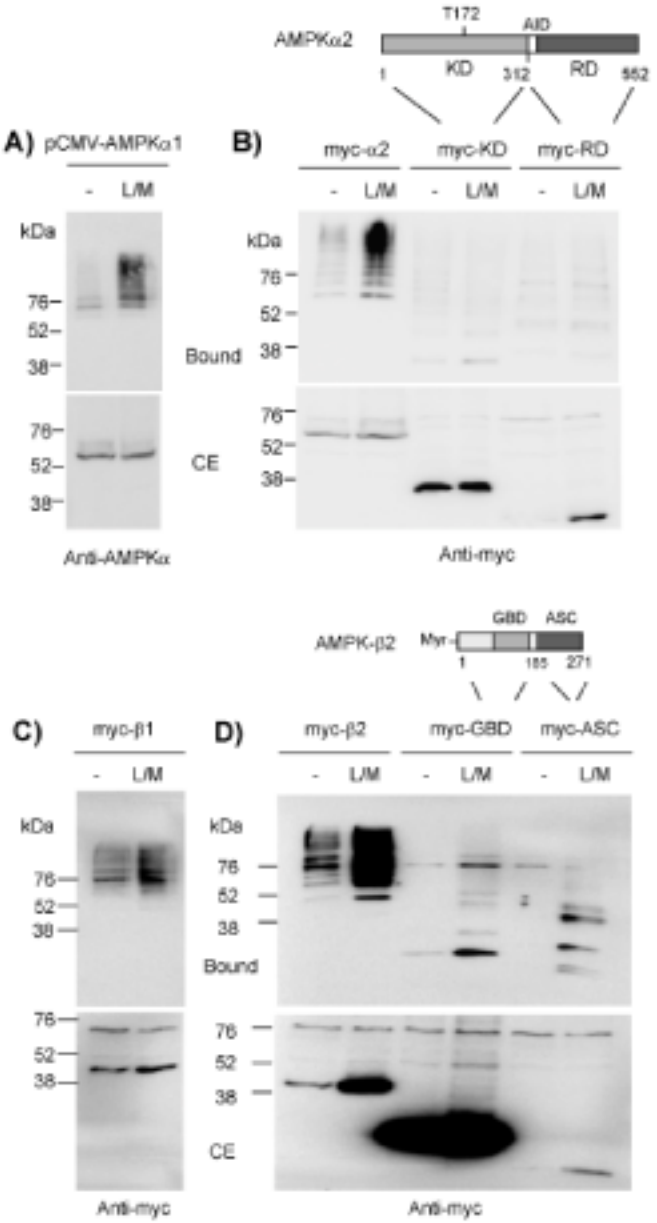
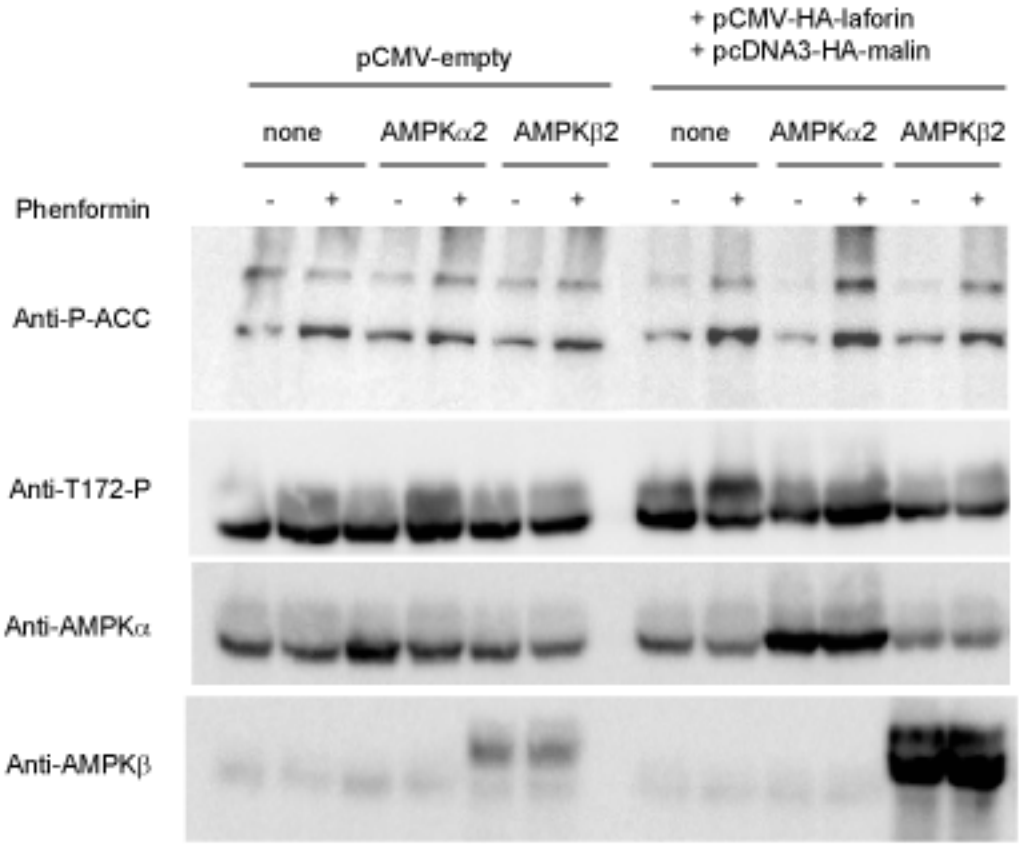
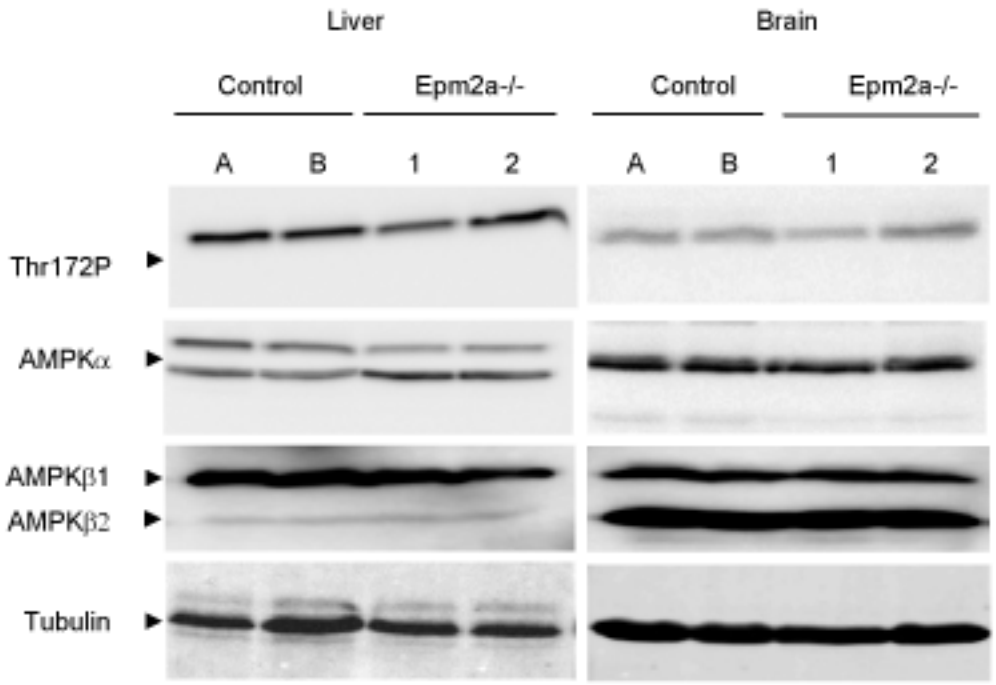


Supplementary Fig. S1: Analysis of the ubiquitination of AMPKα and β subunits by the laforin-malin complex. AMPKα1 (A) and AMPKβ1 (C) are also substrates for laforin-malin dependent ubiquitination. HEK293 cells were transfected with plasmids pCMV-His6xUbiq, pCMV-AMPKα1or pCMV-AMPKβ1 with or without plasmids pCMV-HA-laforin and pcDNA3-HA-malin (L/M). Then, cell extracts were analyzed as in Fig. 1 using anti-AMPKα total (A) or anti-myc (C) antibodies. Mapping of the domains of AMPKα2 (B) and AMPKβ2 (D) involved in the laforin-malin dependent ubiquitination. HEK293 cells were transfected with plasmid pCMV-His6xUbiq and the indicated combination of plasmids (myc-α2: pCMVmyc-AMPKα2; myc-KD: pCMVmyc-AMPKα2 KD; myc-RD: pCMVmyc-AMPKα2 RD; myc-β2: pCMVmyc-AMPKβ2; myc-GBD: pCMVmyc-AMPKβ2 GBD; myc-ASC: pCMVmyc-AMPKβ2 ASC; L/M: pCMV-HA-laforin/pcDNA3-HA-malin). Cell extracts were analyzed as in Fig 1 using anti-myc antibodies.



Supplementary Fig. S2: The expression of laforin and malin does not affect the activity of AMPK complex. HEK293 cells were co-transfected with plasmid pCMVmyc-AMPKα2, pCMVmyc-AMPKβ2 or pCMVmyc (none) and a combination or not of pCMV-HA-laforin and pcDNA3-HA-malin. After 24 hours of transfection, cells were treated or not with 5 mM phenformin for 1 hour. Cell extracts (25 μg) were then analyzed by SDS-PAGE and Western blotting using the indicated antibodies. A representative Western blot is presented. Intensity of the P-ACC bands was measured and no statistically significant differences were found when comparing the cells transfected with the laforin/malin plasmids with the cells treated with an empty vector (n: 3).



Supplementary Fig. S3: Laforin deletion does not affect expression of endogenous AMPKα and AMPKβ subunits or the regulation of AMPKα-Thr172 phosphorylation. Cell extracts (30 μg) from liver and brain biopsies from two control (A and B) and two Epm2a-/- mice lacking laforin (1 and 2), were obtained as in *Vernia et al. 2009; PLoS ONE e5907* and analyzed by Western blotting using anti-Thr172P, anti-AMPKα, anti-AMPKβ and anti-Tubulin antibodies.