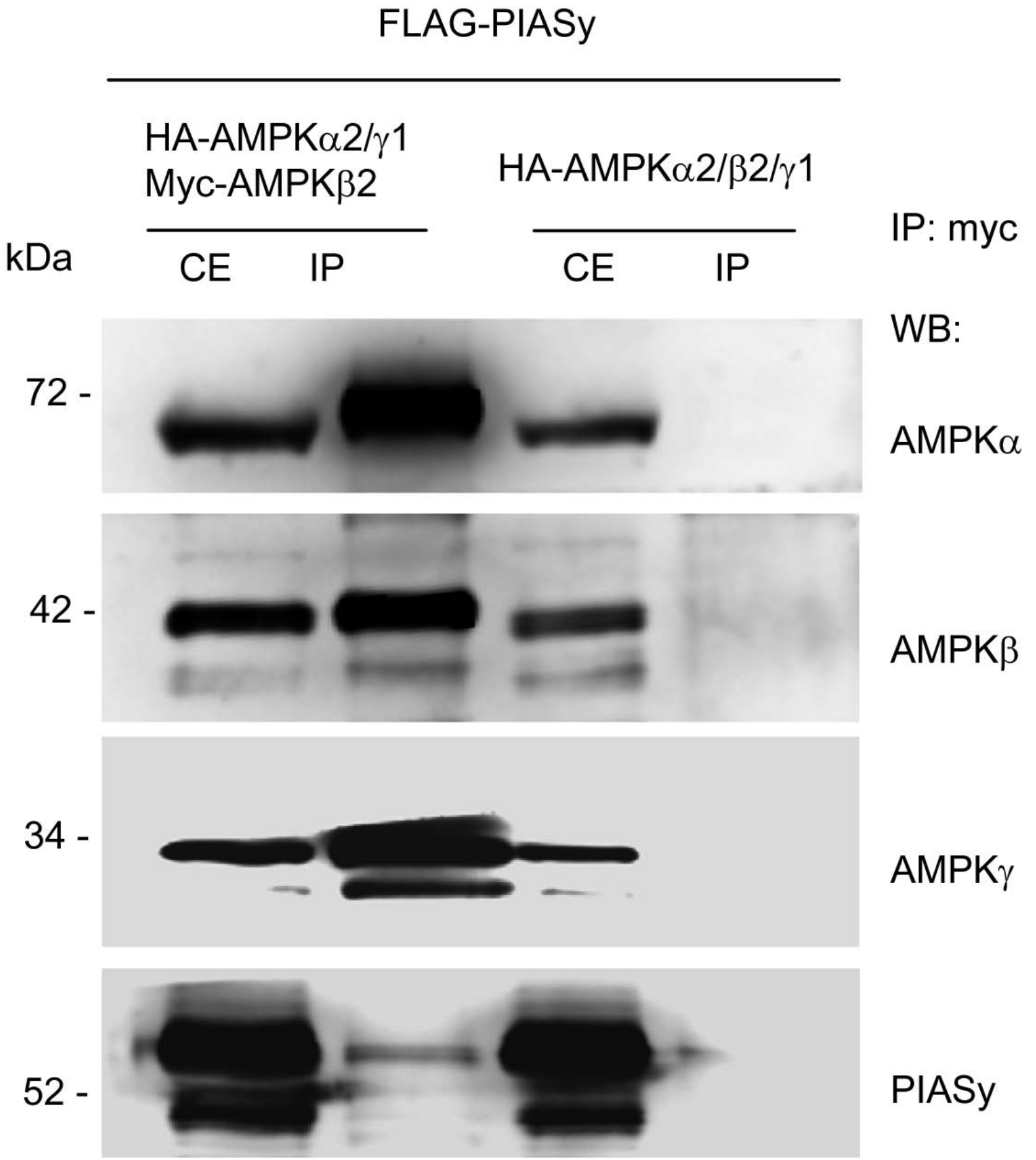
Supplementary Fig. S1. Co-immunoprecipitation of PIASy and AMPK subunits. Human embryonic kidney cells (Hek293) were transfected with plasmids pFLAG-PIASy, and a combination of plasmids pCMV-HA-AMPKα2, pCMV-**myc**-AMPKβ2 and pCMV-HA-AMPKγ1, or pCMV-HA-AMPKα2, pCMV-HA-AMPKβ2 and pCMV-HA-AMPKγ (as negative control of co-immunoprecipitation). Cell extracts were used in immunoprecipitation studies using anti-myc antibody (IP). The resulting immunoprecipitates were analyzed by Western blotting using anti-AMPKα, anti-AMPKβ, anti-AMPKγ and anti-PIASy antibodies.

Supplementary Fig. S2. Leptomycin B prevents the nuclear export of AMPK complex. A) U2OS cells transfected with plasmids pFLAG-PIASy and pCMV-6xHis-SUMO2 were treated with 20 ng/ml leptomycin B for 20 min or left untreated. The subcellular localization of AMPKβ2 subunit was carried out as described in Materials and Methods by using anti-AMPKβ as a primary and anti-rabbit Alexa-Fluor 488 as a secondary antibody. The same samples were also immunodetected with anti-FLAG as a primary and anti-mouse Texas Red as a secondary antibody to determine the localization of PIASy. All the samples were treated with Topro3 to stain the nucleus and the three images were subjected to a merge analysis. Notice that leptomycin B treatment induces the nuclear accumulation of endogenous AMPKβ. B) U2OS cells were also transfected with pFLAG-PIASy, pCMV-6xHis-SUMO2 and a combination of plasmids pCMV-myc-AMPKα2, pCMV-myc-AMPKβ2 and pCMV-myc-AMPKγ1. Cells were treated or not with leptomycin B and analyzed as in part A.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Myc-AMPKβ2
HA-AMPKα2/β2/γ1
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CE
IP
CE
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WB:
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AMPKα
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AMPKβ
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AMPKγ
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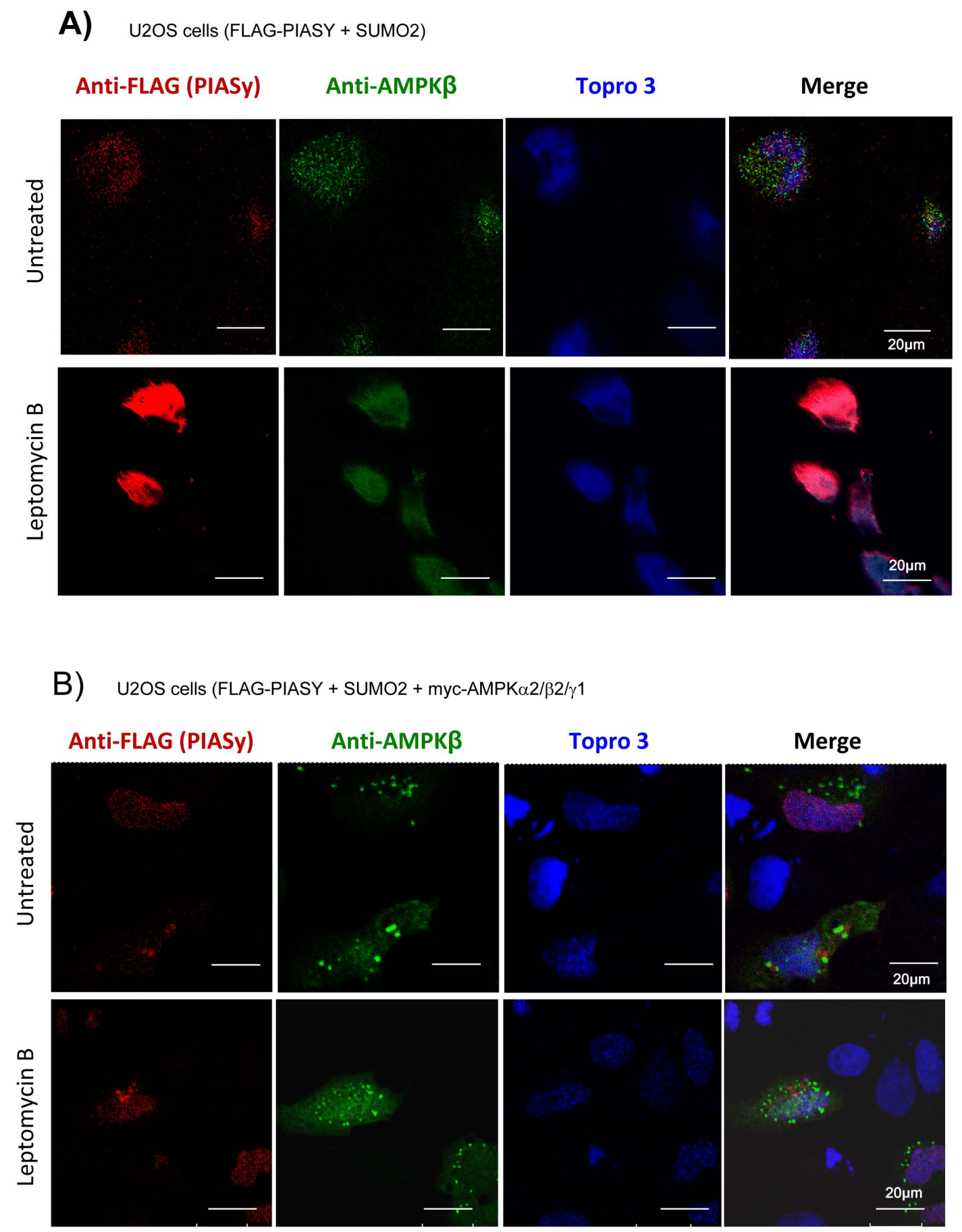
PIASy


A)
U2OS cells (FLAG-PIASY + SUMO2)
Anti-FLAG (PIASy)
Anti-AMPKβ
Topro 3
Merge
Untreated
Leptomycin B
20μm
20μm
B)
U2OS cells (FLAG-PIASY + SUMO2 + myc-AMPKα2/β2/γ1
Anti-FLAG (PIASy)
Anti-AMPKβ
Topro 3
Merge
Untreated
Leptomycin B
20μm
20μm