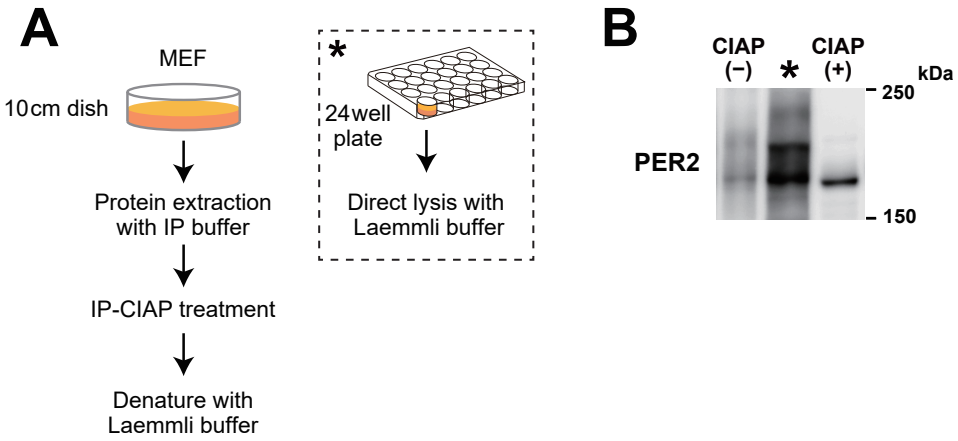
Supplemental Figure 2



Supplemental Figure 2. Immunoprecipitaton-phosphatase treatment on mPER2. (A) Schematic of the experimental design. Cells cultured in 10-cm dish were harvested at 4 h after DEX treatment. The endogenous mPER2 proteins were extracted into IP buffer and subjected to immunoprecipitation (IP)-calf-intestinal alkaline phosphatase (CIAP) treatment as described previously (Doi et al., 2004). Asterisk indicates the sampling protocol for the circadian time course experiments used in **Figure 1**. (B) After IP-CIAP treatment, nearly all mPER2 proteins migrate as a single lower molecular weight band, as was reported previously (Lee et al., 2001). For comparison, a lysate from **Figure 1** (*; a direct lysis by Laemmli buffer) was loaded in parallel, with the loading protein amount being adjusted to the IP-CIAP sample (~3 × 10^4 cells/load). Note that the high molecular weight forms of the endogenous mPER2 were better preserved in the direct lysis sample (*), which indicates the merit of the immediate lysis method that we employed for the analysis of the circadian profiles of mPER2.