Article's title: Inhibition of interleukin 10 transcription through the SMAD2/3 signaling pathway by Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ activation in human T-cell lymphoma HuT-78 cells

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Supplementary Figure legends

Figure S1. Gene expression of Th1 and Th2 cytokines, CD25, Foxp3, LAG3, and EGR2 in HuT-78 cells and differential levels of IL-10 expression and secretion in HuT-78, Jurkat, Daudi, K562, and THP-1 cells. A: Quantitative real-time PCR assay for IFN-γ, IL-17A, IL-4, IL-5, and IL-13 in HuT-78 cells (n=4). Expression levels were shown as a ratio to ACTB. B: Real-time PCR assay for IL-10 in HuT-78, Jurkat, Daudi, K562, and THP-1 cells (n=4 for each). C: Quantitative detection of IL-10 by ELISA assays in HuT-78, Jurkat, Daudi, K562, and THP-1 cells (n=4 for each). Supernatant samples were collected 24 hr after cultivation. D: Real-time PCR assays for CD25, Foxp3, LAG3, and EGR2 in HuT-78 cells (n=4). Results are expressed as means ± S.D.

Figure S2. The SKA-31-induced hyperpolarization and current activation in HuT-78 cells in current- and voltage-clamp mode using whole-cell patch clamp recordings (at pCa 6.5). A: The time course of hyperpolarization responses induced by the application of 1 μM SKA-31. B: Summarized results of the changes in membrane potential was obtained from 'A' (n=5 for each). C: Effects of vehicle (black), 1 μM SKA-31 (red) and 1 μM SKA-31 + 1 μM TRAM-34 (blue) on the current density-voltage relationship for voltage ramp-induced currents from -120 to +60 mV in HuT-78 cells. D: Summarized results of 1 μM SKA-31-induced outward current density at +60 mV (n=7 for each). Results are expressed as means ± S.D. *,**: $P < 0.05$, 0.01 vs. vehicle control,

Figure S3. Effects of 30 mM K^+-containing solution and 1 nM thapsigargin on $K_{Ca}3.1$ activators-induced inhibition of IL-10 transcription in HuT-78 cells. A: The time course of depolarization responses induced by the application of 30 and 140 mM K^+-containing solution.

The fluorescent intensity of $DiBAC_4(3)$ before the application of the agents at 0 sec is expressed as 1.0. B: Disappearance of 1 μM SKA-31-induced hyperpolarization responses by the application of 30 mM K^+-containing solution. Cell numbers used for the experiments are shown in parentheses. C: Quantitative real-time PCR assay for IL-10 in HuT-78 cells treated with vehicle, DCEBIO (1 μM), DCEBIO + 30 mM K^+, SKA-31 (1 μM), and SKA-31 + 30 mM K^+ for 6 hr (n=4 for each). D: Real-time PCR assay for IL-10 in HuT-78 cells treated with vehicle, 1 nM thapsigargin (TG), and TG + 10 μM KN-62 for 6 hr (n=4 for each). Expression levels were expressed as a ratio to ACTB. Results are expressed as means ± S.D. **: $P < 0.01$ vs. vehicle control, $^{\#\#}$: $P < 0.01$ vs. DCEBIO-treated, $^{\$\$}$: $P < 0.01$ vs. SKA-31-treated, ¶¶: $P < 0.01$ vs. TG-treated.

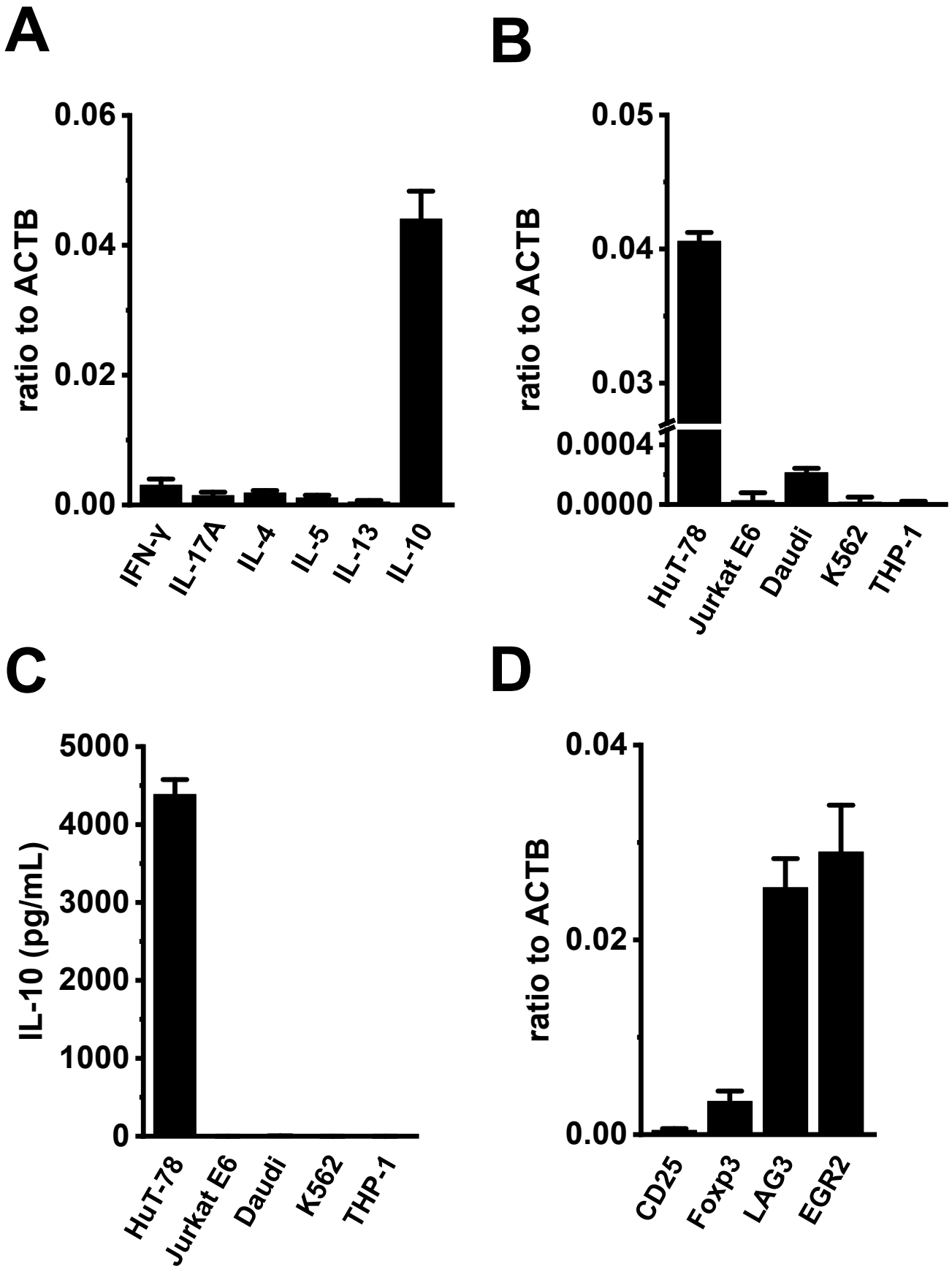
Figure S4. Time course of decreases in IL-10 transcripts by treatments with $K_{Ca}3.1$ activators in HuT-78 cells. A-C: Quantitative real-time PCR assay for IL-10 in HuT-78 cells treated with vehicle (A), DCEBIO (1 μM) (B), and SKA-31 (1 μM) (C) for 1, 3, 6, 12, and 24 hr (n=4 for each). Expression levels were expressed as a ratio to ACTB. Results are expressed as means ± S.D. **: $P < 0.01$ vs. at 0 hr.

Figure S5. Full images of cropped blots in Figure 4 (A) and Figure 7 (A)

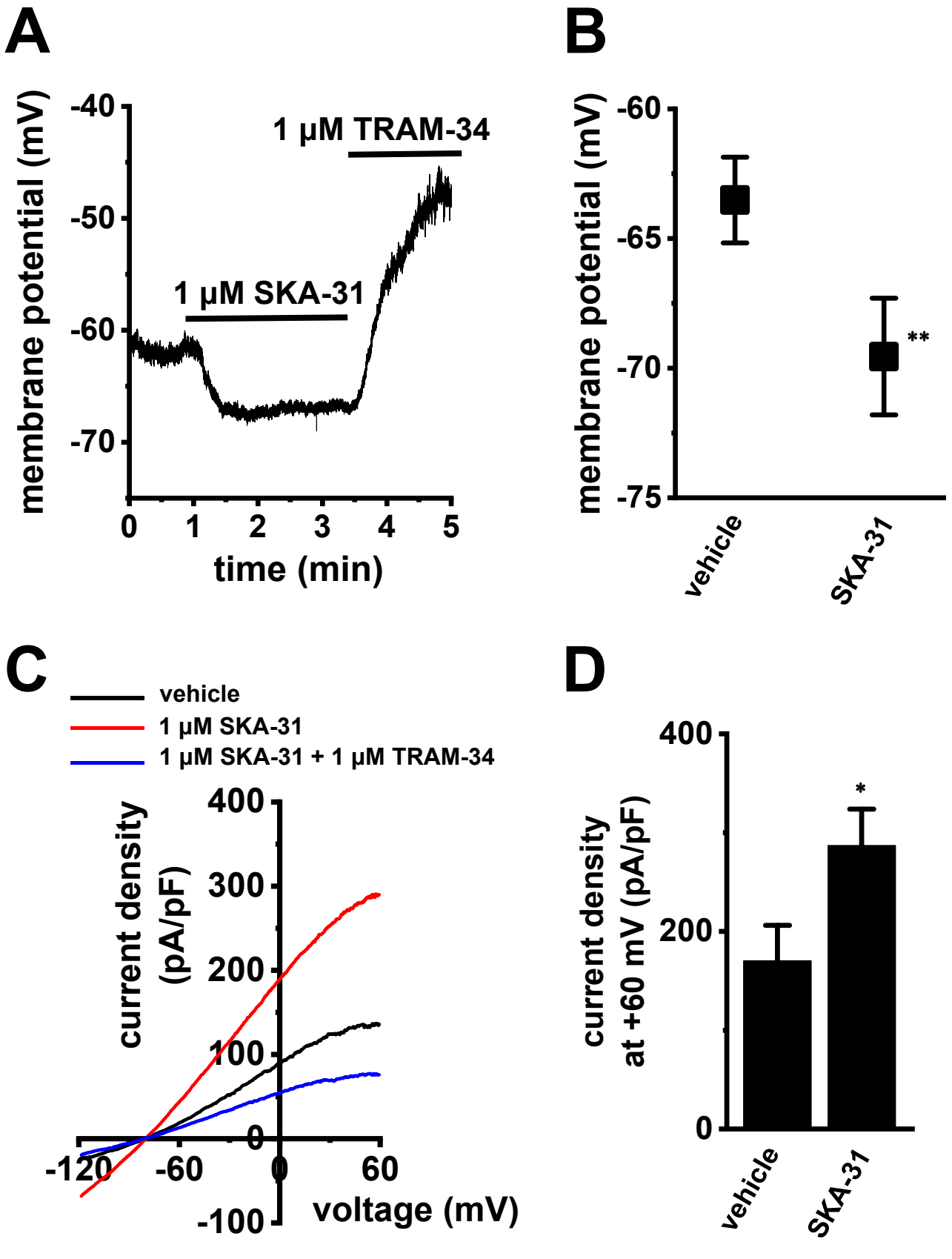
Figure S6. Effects of an ATP competitive inhibitor of the TGF-β1 receptor (LY364947), NF-κB inhibitor (BAY11-7082), mTOR inhibitor (everolimus), AKT inhibitor (AZD5363), and STAT3 inhibitor (5,15-DPP) on expression levels of IL-10 transcripts in HuT-78 cells. A-C: Quantitative real-time PCR assay for IL-10 in HuT-78 cells treated with vehicle, LY364947 (1 μM) (A), BAY11-7082 (1 μM) (B), everolimus (10 nM) (C), AZD5363 (1 μM) (C), and 5,15-

DPP (20 μM) (C) for 6 hr (n=4 for each). Expression levels were expressed as a ratio to ACTB. Results are expressed as means ± S.D. **: $P < 0.01$ vs. vehicle control.

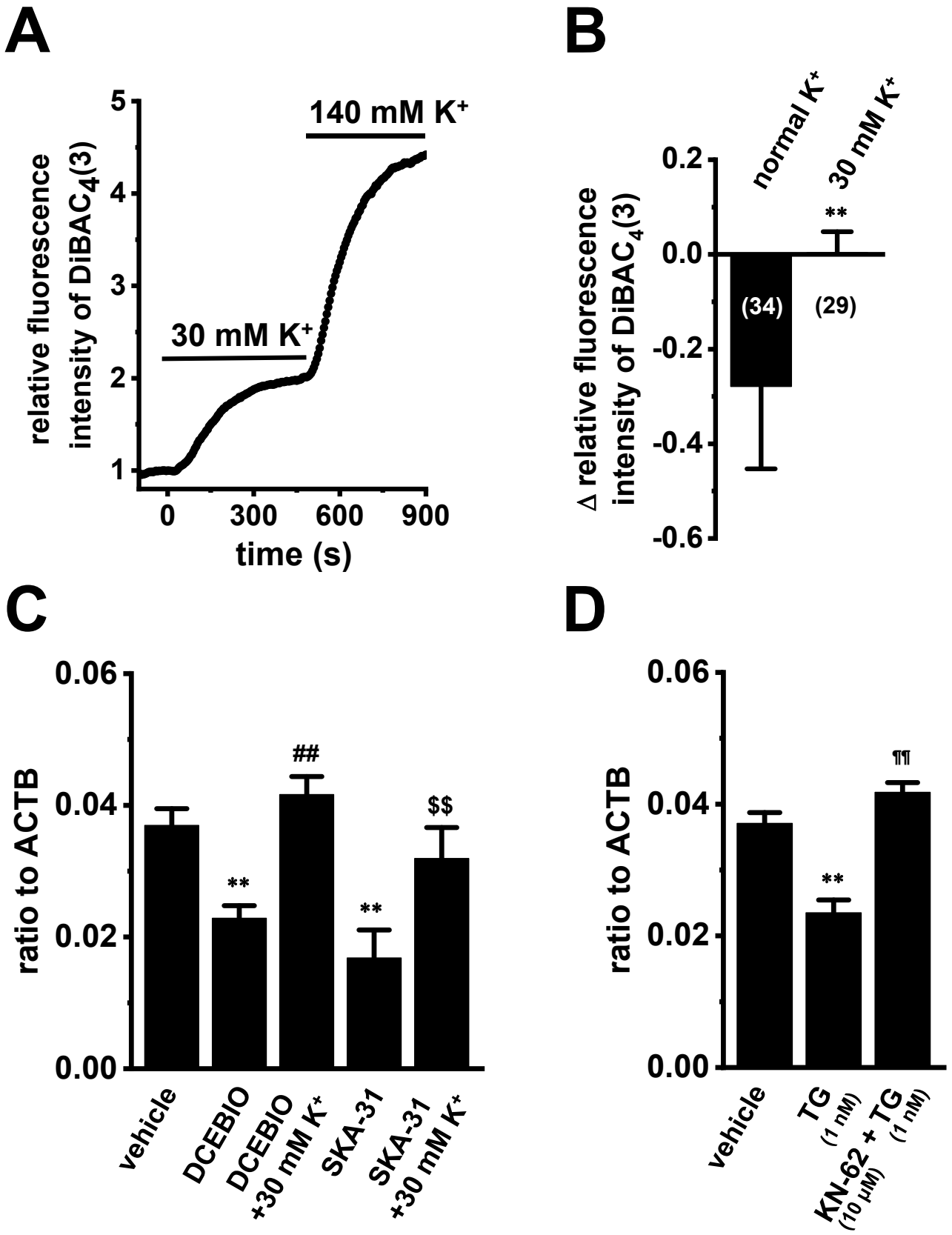
supplementary Fig. S1



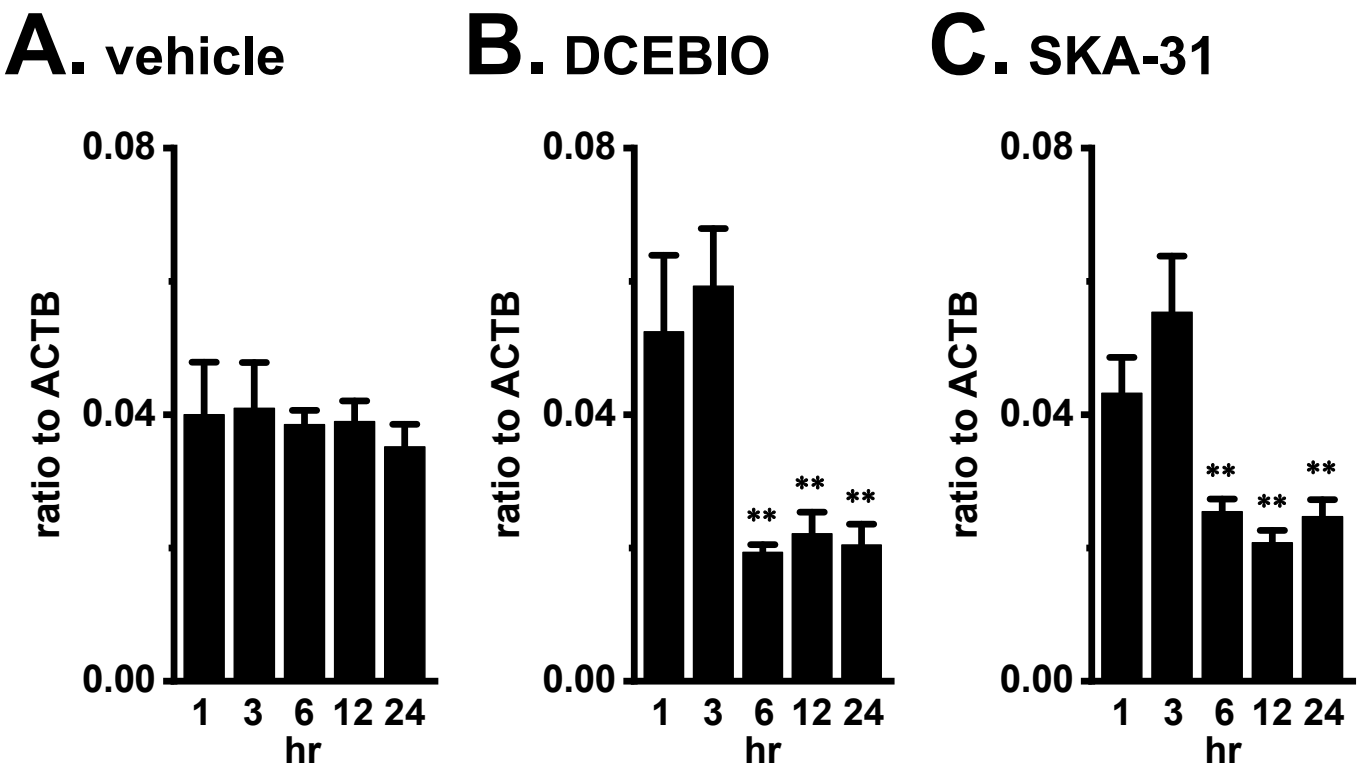
supplementary Fig. S2



supplementary Fig. S3

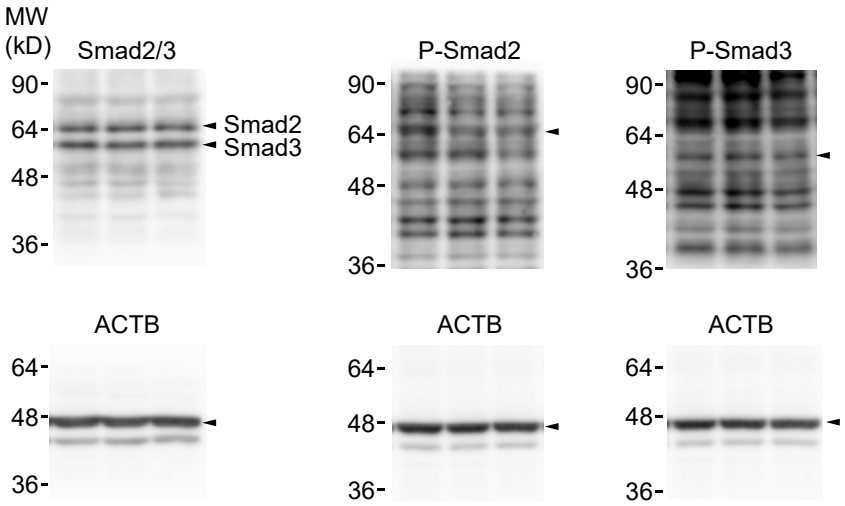


supplementary Fig. S4

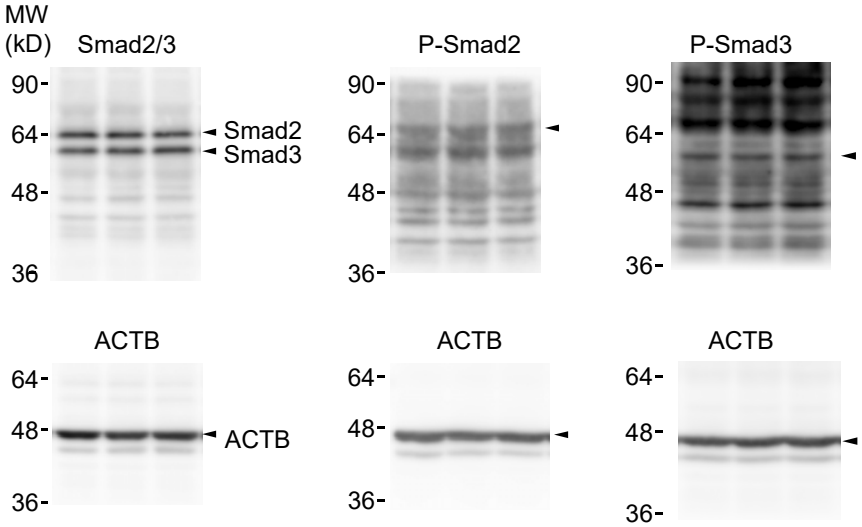


supplementary Fig. S5

A. full images of cropped blots shown in Figure 4



B. full images of cropped blots shown in Figure 7



supplementary Fig. S6