Figure S1. PU attenuates the reduced cell viability and increased oxidative stress in NRK-52E cells following exposure to Cd. The cells were treated with 20 μM Cd in the presence or absence of 100 μM PU for 12 h. (A) DCF fluorescence was measured to reflect ROS levels using flow cytometry. (B) Cell Counting Kit-8 reagent was employed to determine cell viability. (C) SOD levels were measured using a commercial kit. n=6. *P<0.05; **P<0.01. Cd, cadmium; PU, puerarin; DCF, dichlorofluorescein; ROS, reactive oxygen species; SOD, superoxide dismutase.

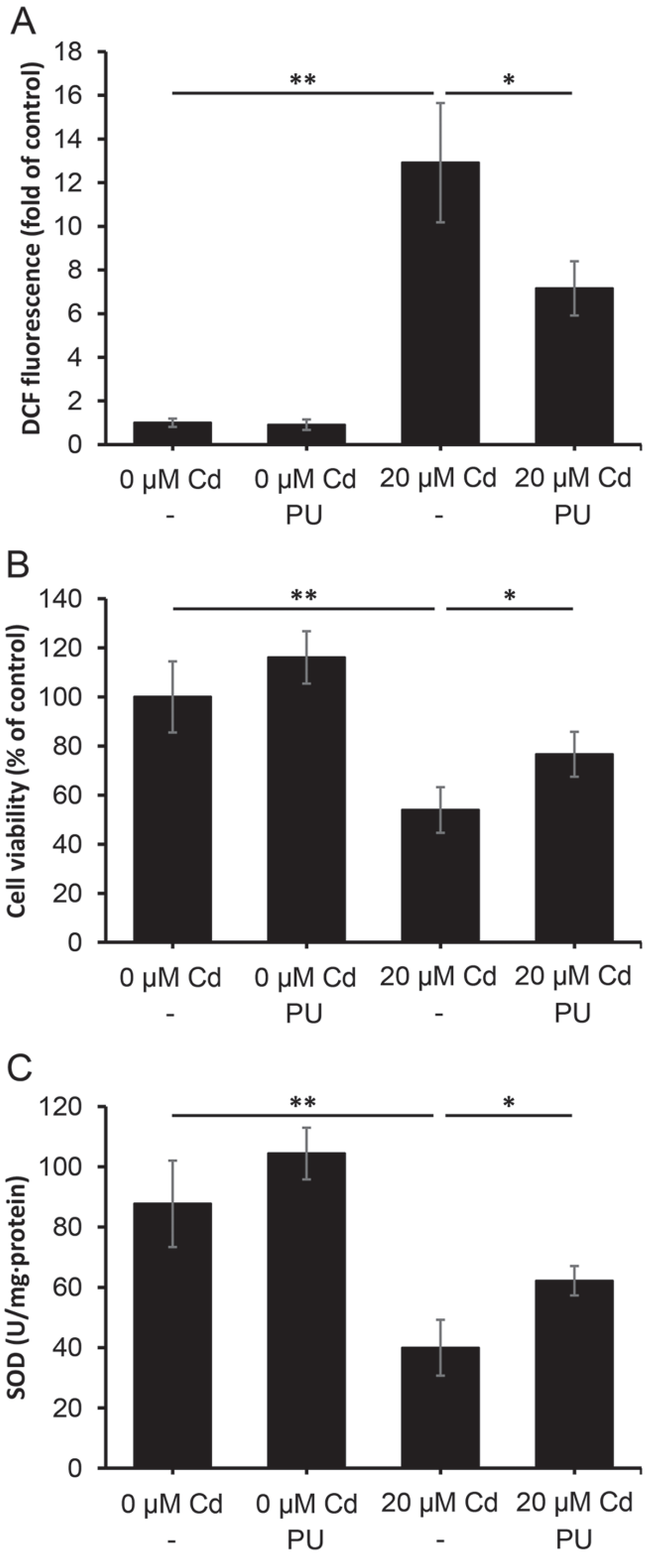

Figure S2. Efficiency of ATG7 knockdown in NRK-52E cells. The cells were transfected with shRNA ATG7, and they were subsequently collected and lysed, following treatment with 20 μM cadmium for 12 h. The protein level of ATG7 was quantified following western blotting. The upper panel depicts representative western blotting images and the lower panel indicates the quantitative analysis. n=3. **P<0.01. shRNA, short hairpin RNA; ATG7, autophagy-related protein 7.

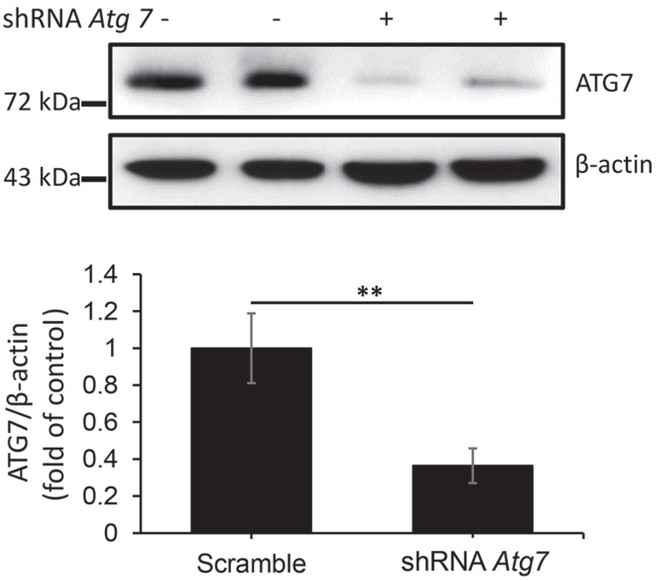

Figure S3. ATG7 knockdown augments the decrease in cell viability and the increase in oxidative stress induced by Cd in NRK-52E cells. NRK-52E cells were transfected with shRNA ATG7 and were subsequently treated with 20 μM Cd for 12 h. (A) DCF fluorescence was measured to reflect ROS levels using flow cytometry. (B) SOD levels were measured using a commercial kit. (C) Cell Counting Kit-8 reagent was used to determine cell viability. n=6. *P<0.05; **P<0.01. Cd, cadmium; DCF, dichlorofluorescein; ROS, reactive oxygen species; SOD, superoxide dismutase; shRNA, short hairpin RNA; ATG7, autophagy-related protein 7.

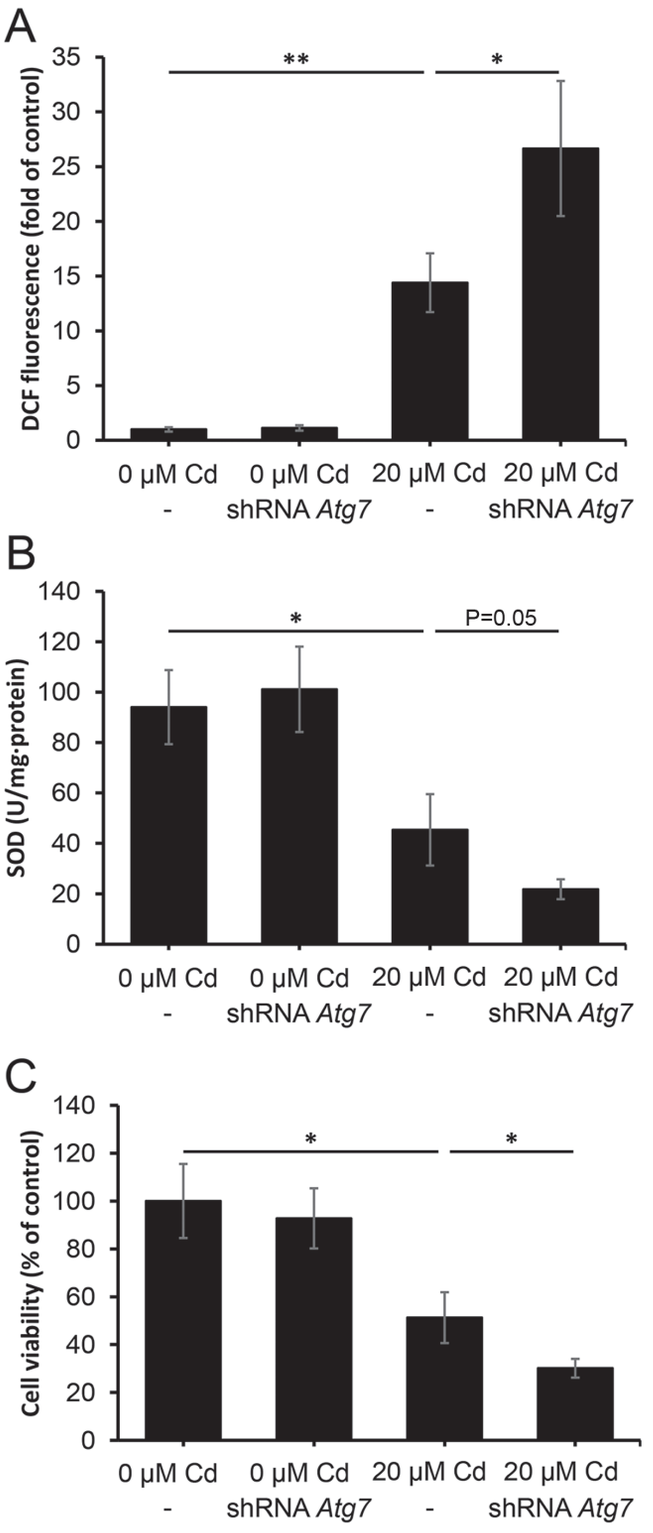

Figure S4. Efficiency of RAB7 overexpression in NRK-52E cells. Cells were transfected with ORF RAB7 followed by treatment with 20 μM cadmium for 12 h, and were subsequently collected and lysed. The protein level of RAB7 was quantified following western blotting. The upper panels depicts representative western blotting images and the lower panel indicates the quantitative analysis. n=3. **P<0.01. ORF, open reading frame; RAB7, Ras-related protein Rab-7.

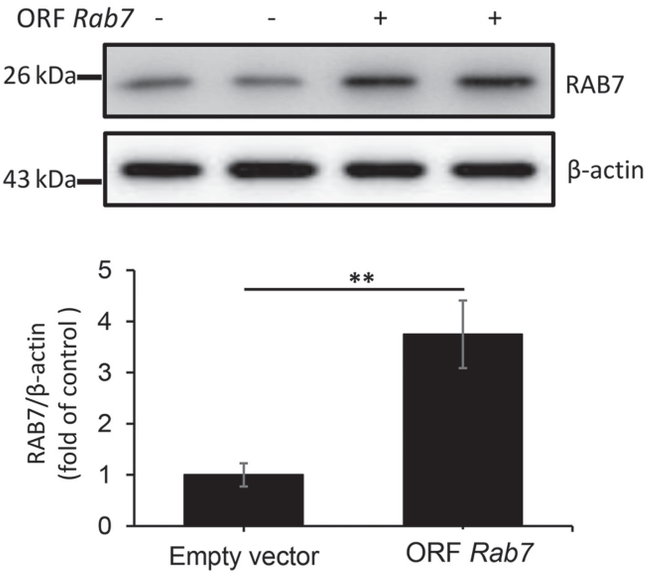

Figure S5. RAB7 overexpression restores the autophagic flux in Cd-treated NRK-52E cells. The cells were transfected with ORF RAB7 followed by treatment with 20 μM Cd for 12 h. In the representative confocal images (magnification, x630), puncta with both green and red fluorescence (indicated as yellow) illustrate the autophagic flux blockade, while the red puncta depict a normal autophagic flux. Cd, cadmium; ORF, open reading frame; RAB7, Ras-related protein Rab-7; RFP, red fluorescent protein; GFP, green fluorescent protein.

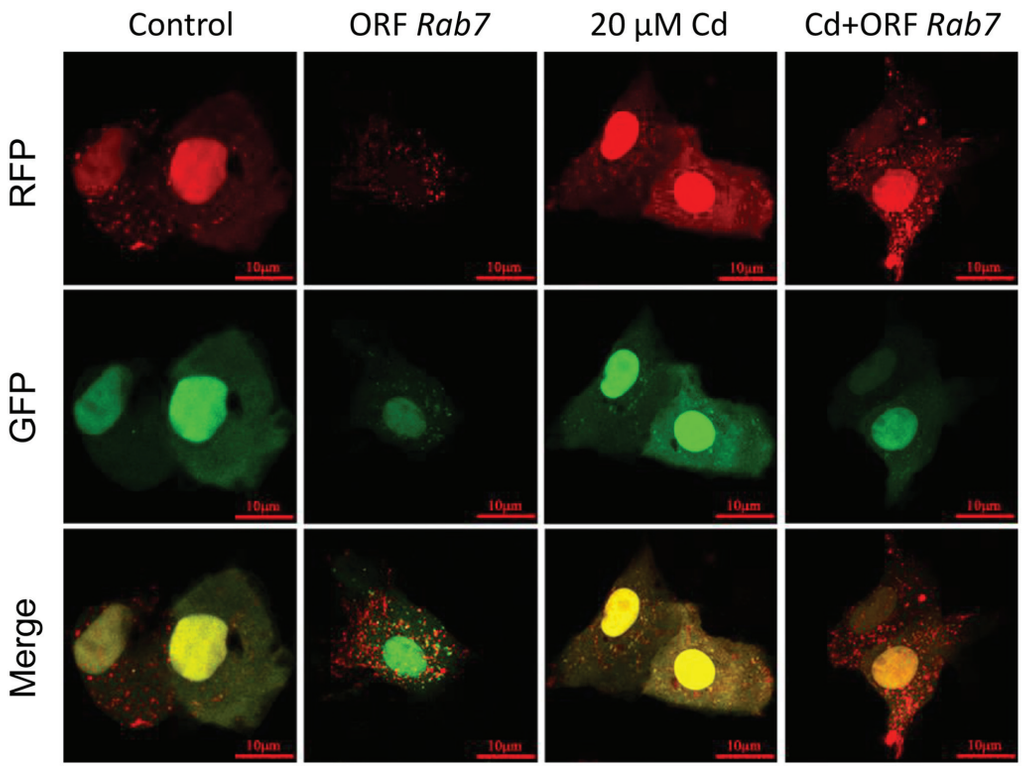

Figure S6. RAB7 overexpression alleviates the decrease in cell viability and the increase in oxidative stress induced by Cd in NRK-52E cells. Cells were transfected with ORF RAB7 followed by treatment with 20 μM Cd for 12 h. (A) DCF fluorescence was measured to reflect ROS levels using flow cytometry. (B) SOD levels were measured using a commercial kit. (C) Cell Counting Kit-8 reagent was employed to determine cell viability. n=6. *P<0.05; **P<0.01. Cd, cadmium; DCF, dichlorofluorescein; ROS, reactive oxygen species; SOD, superoxide dismutase; ORF, open reading frame; RAB7, Ras-related protein Rab-7.

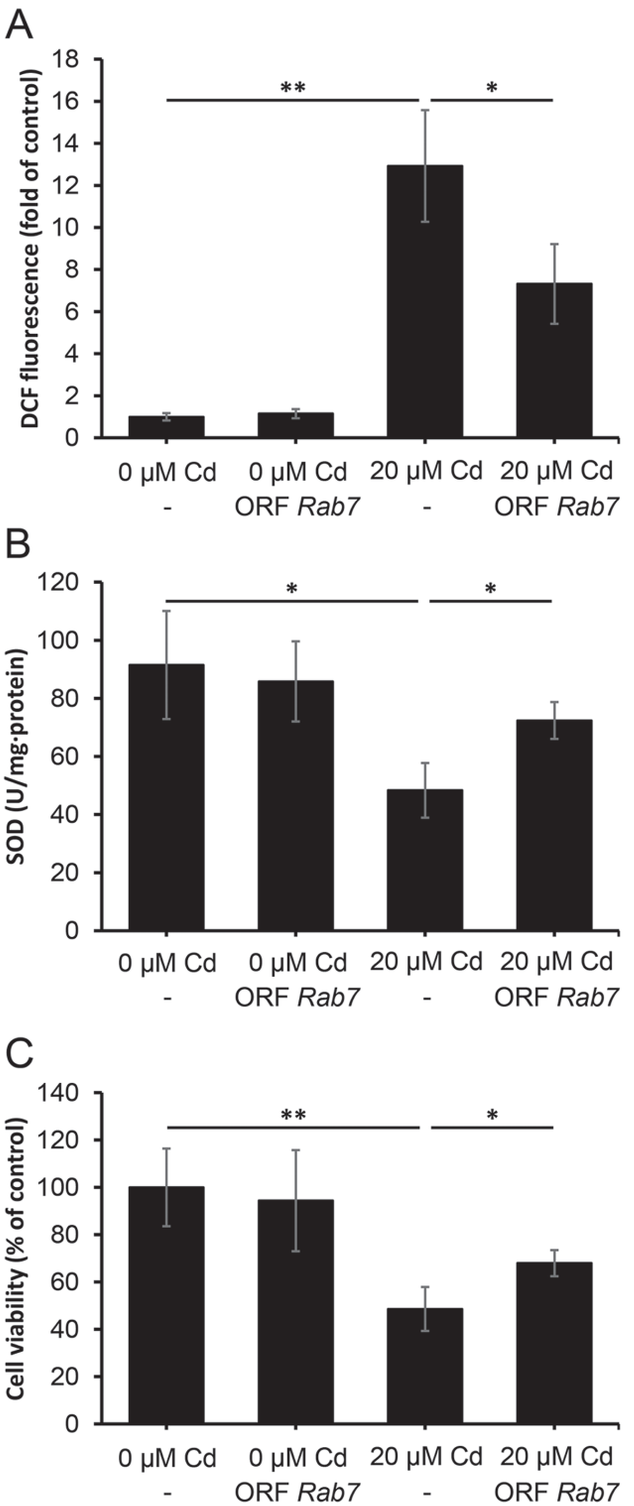

Figure S7. PU inhibits JNK and p38 activation and alleviates ERK inhibition induced by Cd in NRK-52E cells. The cells were treated with 20 μM Cd in the presence or absence of 100 μM PU for 12 h, and they were subsequently collected and lysed. Protein levels were presented as representative western blotting images. (A) p-p38 and p38, (B) p-JNK and JNK and (C) p-ERK and ERK. Cd, cadmium; PU, puerarin; p, phosphorylated.