PrLZ increases prostate cancer docetaxel resistance by inhibiting LKB1/AMPK-mediated autophagy

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Supplementary Figure Legends

Supplementary Figure S1. PrLZ deficiency induces autophagosome formation in PCa. (**A**) The basal levels of LC3-Ⅰ, LC3-Ⅱ, the LC3-Ⅱ/LC3-Ⅰ ratio, LKB1, AMPK and PrLZ proteins were detected by western blotting in PC3 cells, C4-2 cells, or LNCaP cells. The expression of p62 was detected by western blotting in PC3-PrLZ vs. PC3-Vec cells and PrLZ knockdown (sh-PrLZ) C4-2 cells vs. control cells in the presence or absence of 10 mM NH_4CL (**B, C**), 10 nM BafA1 (**D, E**), and 50 μM CQ (**F, G**), and representative fluorescence photomicrographs (**H**) and quantification (**I**) of GFP-RFP-LC3 puncta in C4-2 cells with PrLZ shRNA (sh-PrLZ) or scramble. Yellow puncta denote autophagic vesicle structures. Scale bars: 10 μm. The results represent the mean ± S.D. of 3 independent experiments. **, $P < 0.01$.

Supplementary Figure S2. The protein level of androgen receptor (AR) is decreased in response to glucose starvation and docetaxel treatment. PCa C4-2 cells underwent glucose starvation (GS) (**A**) and docetaxel (DTX) treatment (**B**) for the indicated time (0, 6, 12, 24 h) and with the indicated concentration (0, 5, 10, 20 μM, for 24 h), respectively. The expression of AR was assayed by western blotting.

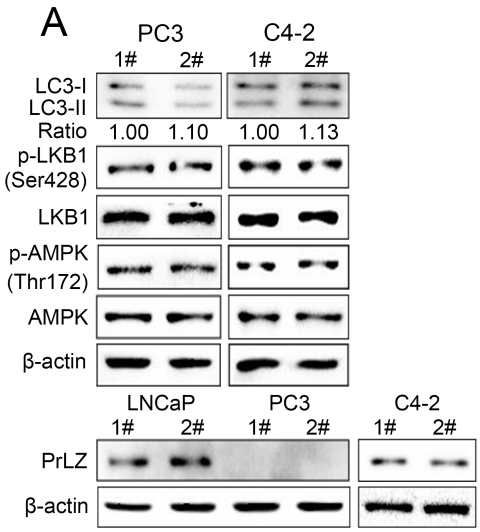
Supplementary Figure S3. **PrLZ-mediated autophagy is involved in docetaxel-induced apoptosis in PCa cells.** (**A**) The expressions of p-TAK1, TAK1 and CaMKKβ were detected by western blotting in PC3-PrLZ vs. PC3-Vec cells and PrLZ knockdown (sh-PrLZ) C4-2 vs. control cells. (**B**) C4-2 and PC3 cell viability was

assayed following treatment with the indicated concentrations of docetaxel (Ctrl, 2.5, 5, 10, 20, 30, 40 μM, for 24 h). (**C**) PC3 cells with PrLZ (PC3/PrLZ) and C4-2 cells with PrLZ shRNA (sh-PrLZ) or scramble (Sc) (**D**) underwent docetaxel (DTX) treatment (Ctrl, 5, 10, 20 μM) for 24 h. The cell viability was assayed by the MTT assay. The results represent the mean ± S.D. of 3 independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ and ****, $P < 0.0001$. (**E**) pcDNA3-Flag-PrLZ plasmid was transfected into C4-2 cells with PrLZ shRNA (sh-PrLZ) or scramble (Sc), and the cell viability was assayed by MTT following treatment with 20 μM docetaxel (DTX, 24 h). The results represent the mean ± S.D. of 3 independent experiments. ****, $P < 0.0001$. The expression levels of autophagy and apoptosis-related molecules were assayed by western blotting in LNCaP (**F**) and PC3 (**G**) cells exposed to 0, 5, 10, 20 μM docetaxel (DTX) for 24 h. (**H**) The expression levels of PrLZ, AR, p-LKB1 (Ser428), LKB1, p-AMPK (Thr172), AMPK, LC3-Ⅰ, LC3-Ⅱ and the LC3-Ⅱ / LC3-Ⅰ ratio were detected by western blotting in C4-2 parental or docetaxel-resistant (DTX-R) cells. (**I**) The PrLZ and AR mRNA levels were quantified by real-time quantitative PCR in C4-2 parental or docetaxel-resistant (DTX-R) cells. The results represent the mean ± S.D. of 3 independent experiments. **, $P < 0.01$.

Supplementary Figure S4. **LKB1-mediated autophagy is involved in docetaxel-induced apoptosis in PCa cells.** After transfected with siRNA against LKB1 (si-LKB1) for 48 h, Western blotting was used to detect the expressions of p-LKB1 (Ser428), caspase-3, cleaved caspase-3, PARP, and cleaved PARP in

docetaxel (DTX, 20 μM, for 24 h)-treated LNCaP (**A**) and PC3 (**B**) cells. LC3-Ⅰ, LC3-Ⅱ, and PrLZ expressions, and the LC3-Ⅱ/ LC3-Ⅰ ratio in PC3/PrLZ (**C**) cells were assayed by western blotting following transfection with LKB1 siRNA (si-LKB1) or control siRNA. (**D**) The expression levels of autophagy- and apoptosis-related proteins were determined by western blotting in PC3/PrLZ and PC3/Vec cells following transfection with LKB1 siRNA (si-LKB1). (**E**) Body weights of the nude mice in the 4 groups (PC3-Vec + Vehicle, PC3-PrLZ + Vehicle, PC3-Vec + DTX, PC3-PrLZ + DTX, 5 mice per group). The results represent the mean±S.D. of 3 independent experiments.

Supplementary Figure S5. **LKB1 and ATG5 are involved in docetaxel-induced autophagy in PCa cells.** (**A**) Representative fluorescence photomicrographs of GFP-RFP-LC3 puncta in docetaxel (DTX, 20 μM, for 24 h)-treated C4-2 cells transfected with LKB1 siRNA (si-LKB1) or control siRNA (si-NC). Scale bars: 10 μm. (**B**) Representative fluorescence photomicrographs of GFP-RFP-LC3 puncta in docetaxel (20 μM, for 24 h)-treated C4-2 cells transfected with ATG5 siRNA (si-ATG5) or negative control siRNA (si-NC). Scale bars: 10 μm.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PC3
C4-2
1#
2#
1#
2#
LC3-I
LC3-II
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1.10
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1.13
p-LKB1
(Ser428)
LKB1
p-AMPK
(Thr172)
AMPK
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2#
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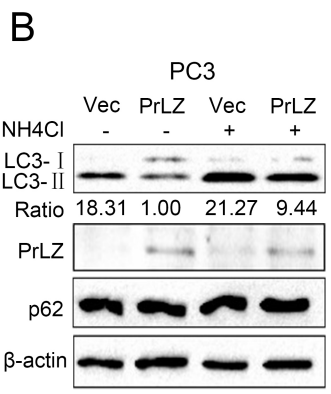
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Vec
PrLZ
NH4Cl
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-
+
+
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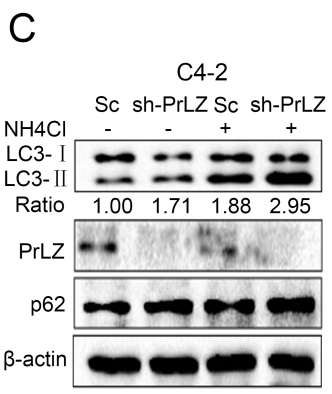
PrLZ
p62
β-actin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sh-PrLZ
Sc
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-
-
+
+
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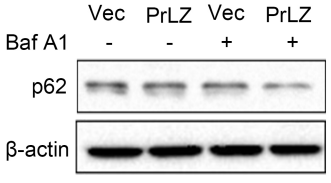
PrLZ
p62
β-actin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Vec
PrLZ
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-
-
+
+
p62
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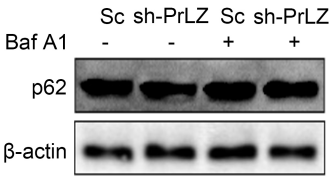
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sh-PrLZ
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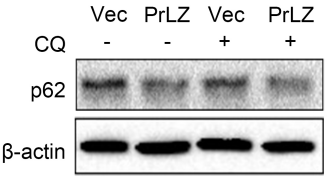
Baf A1
-
-
+
+
p62
β-actin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Vec
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CQ
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+
+
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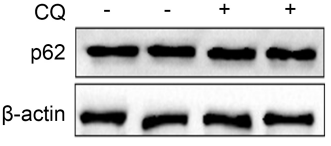
β-actin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CQ
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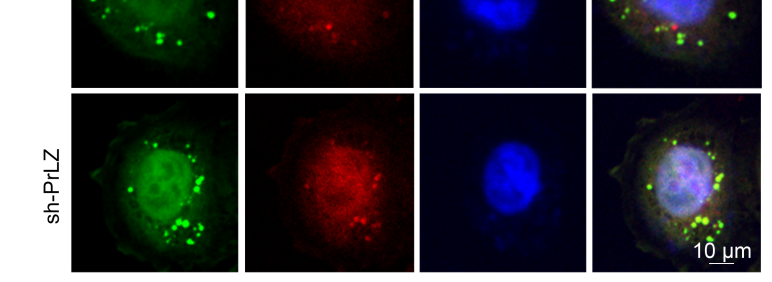
-
+
+
p62
β-actin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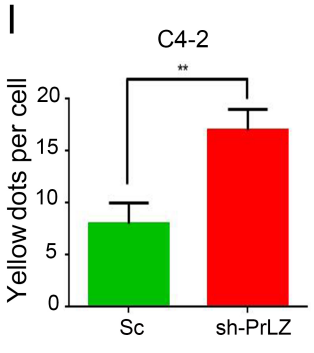
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C4-2
**
Yellow dots per cell
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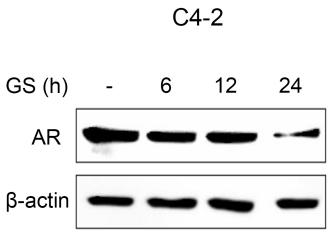
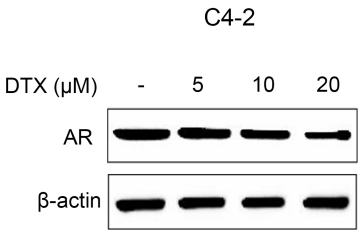
15
10
5
0
Sc
sh-PrLZ

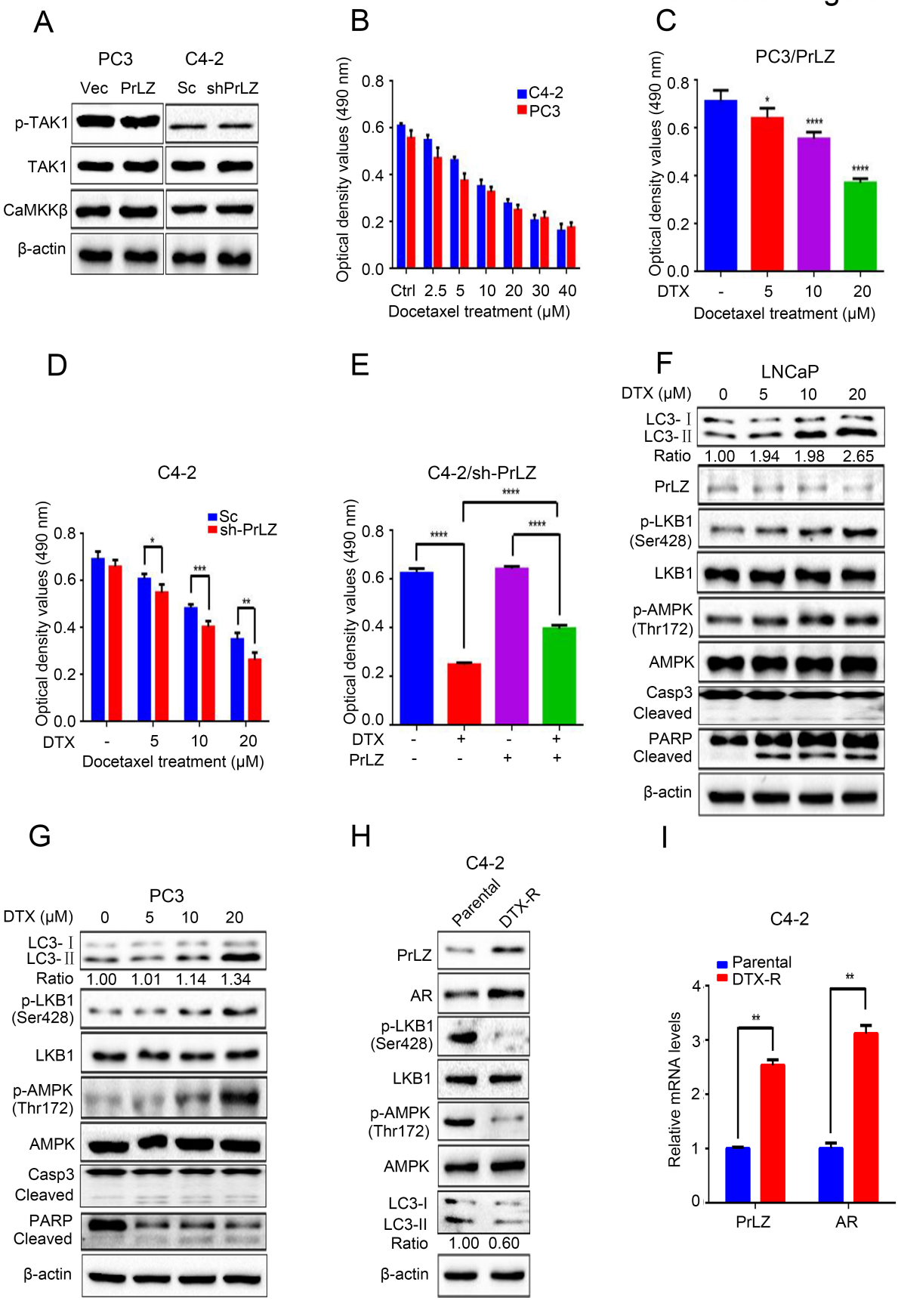
A



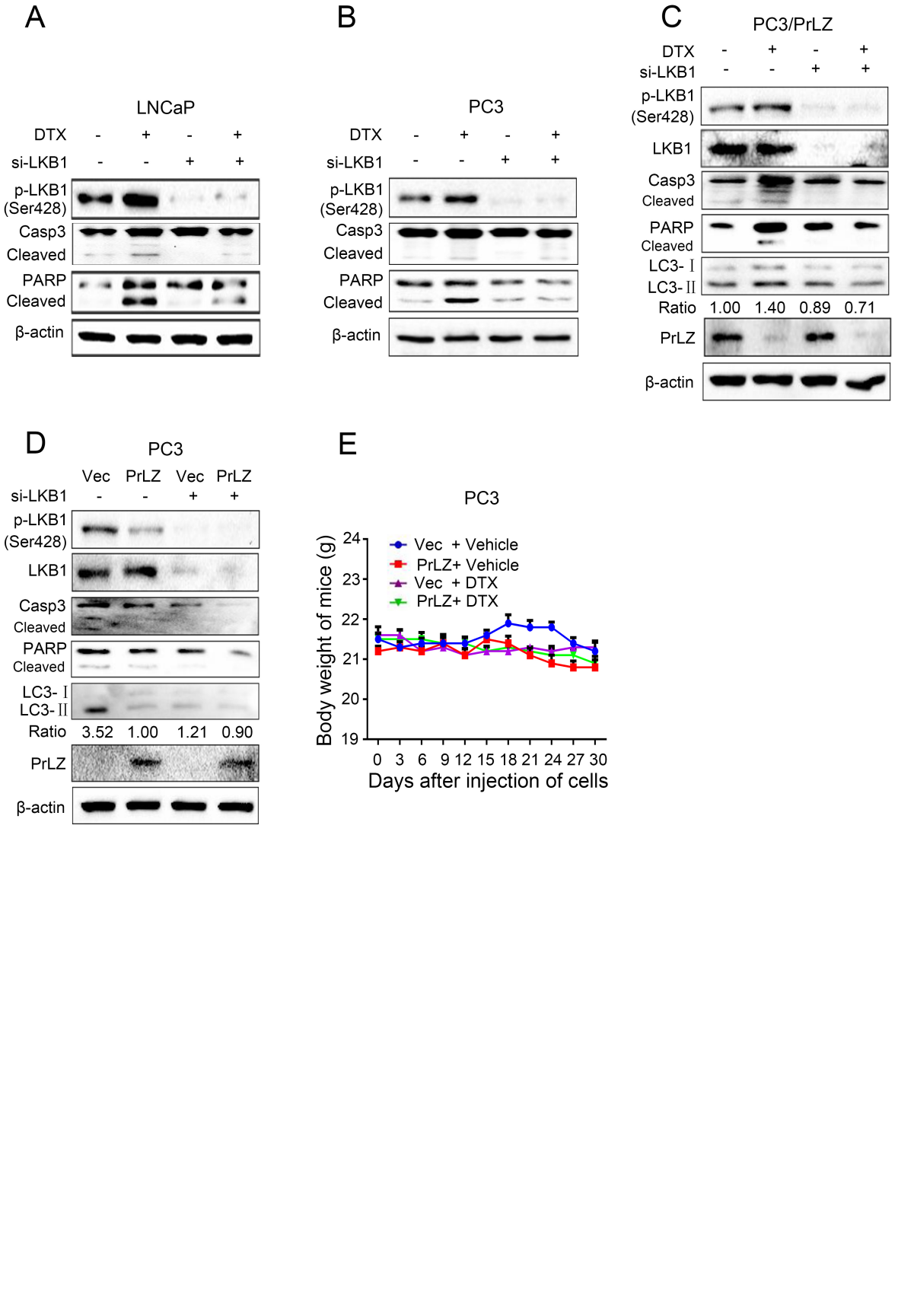
B

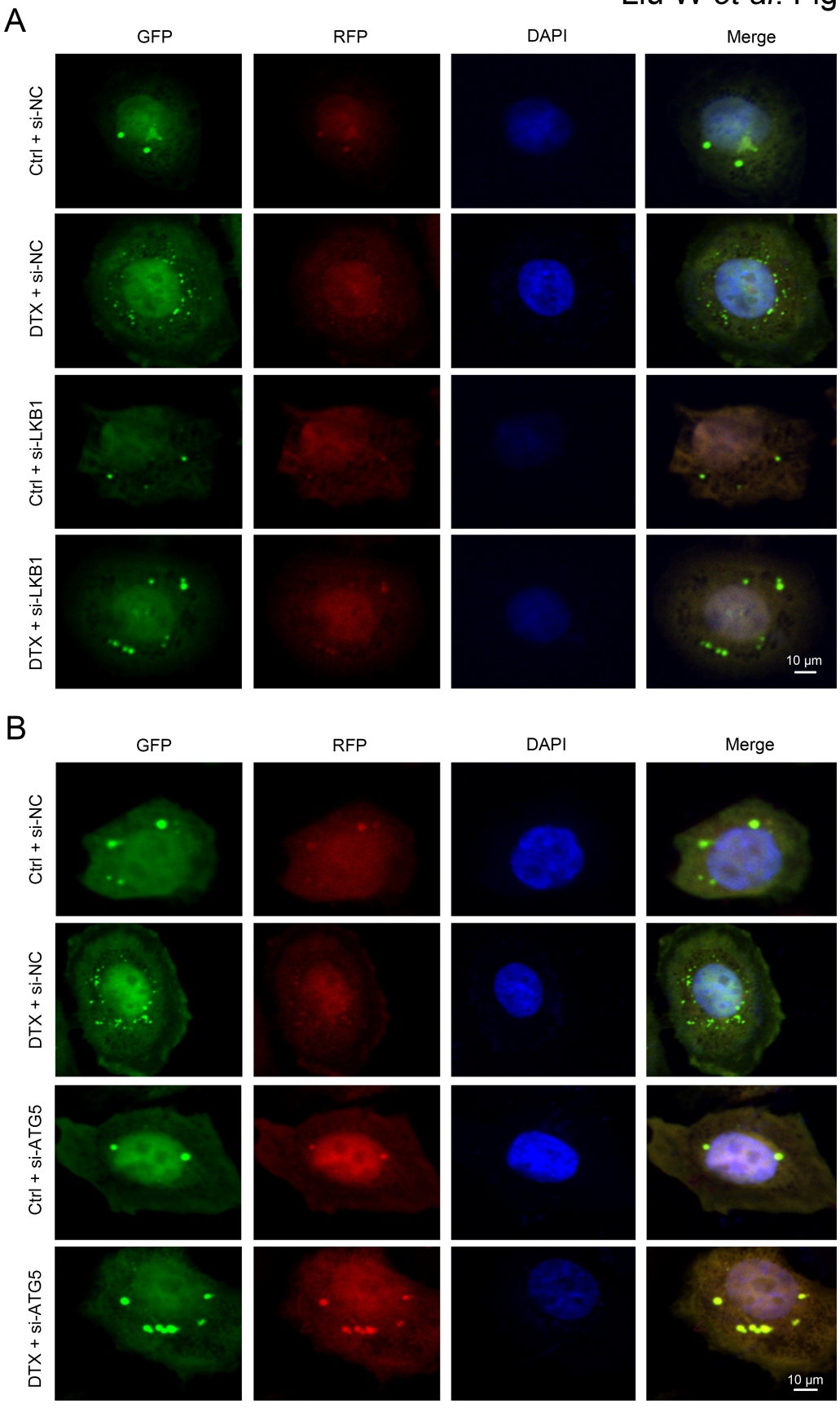


Liu W *et al*. Figure S3



Liu W *et al*. Figure S4

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RFP
DAPI
Merge
Ctrl + si-NC
DTX + si-NC
Ctrl + si-ATG5
DTX + si-ATG5
10 μm