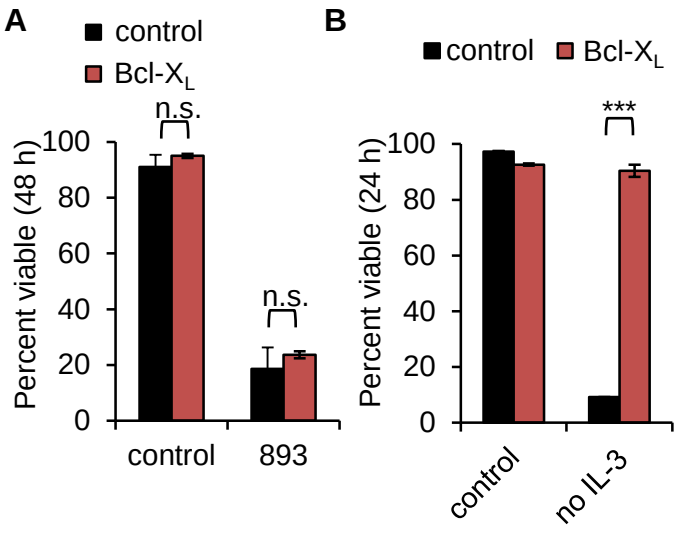
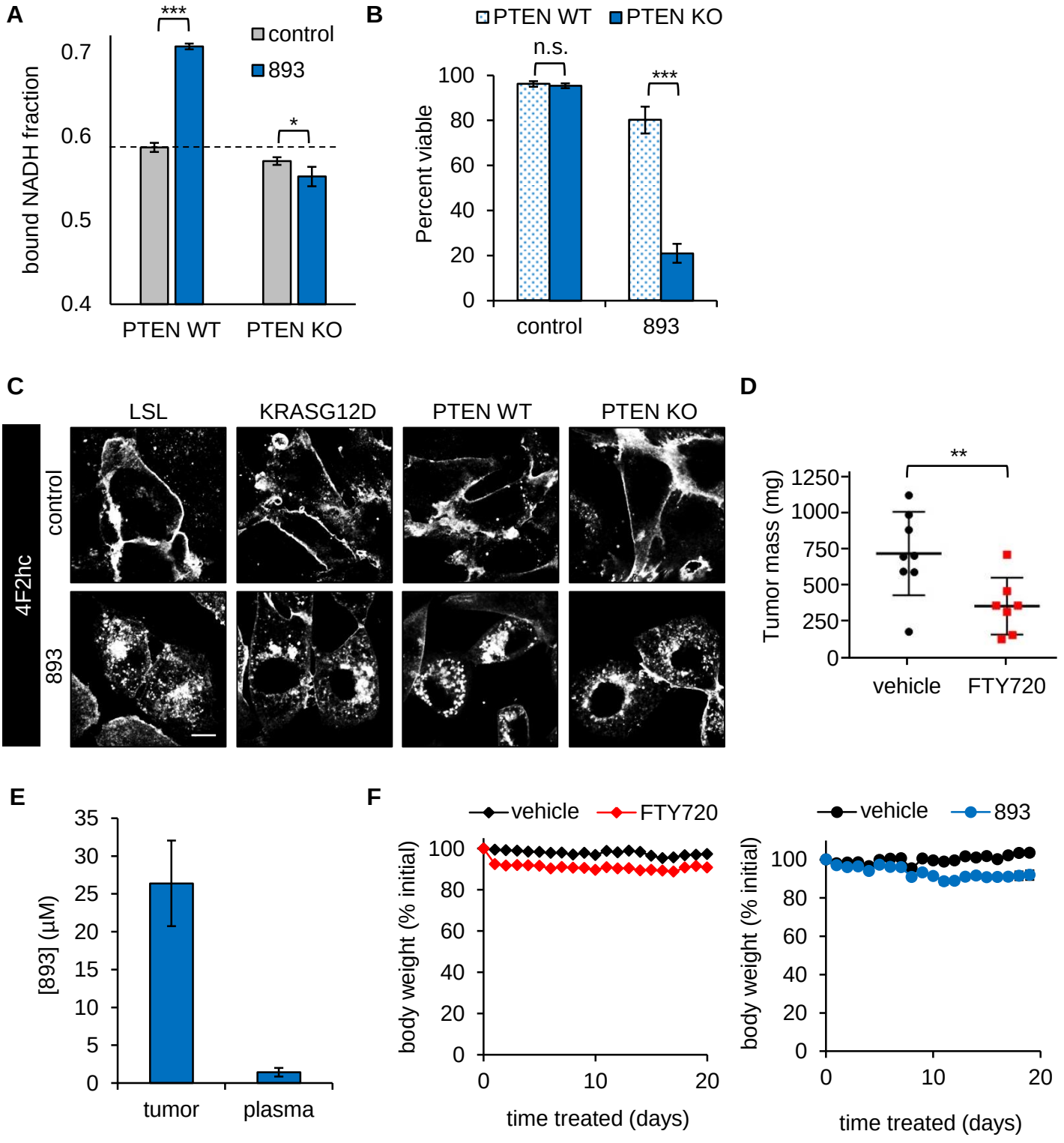
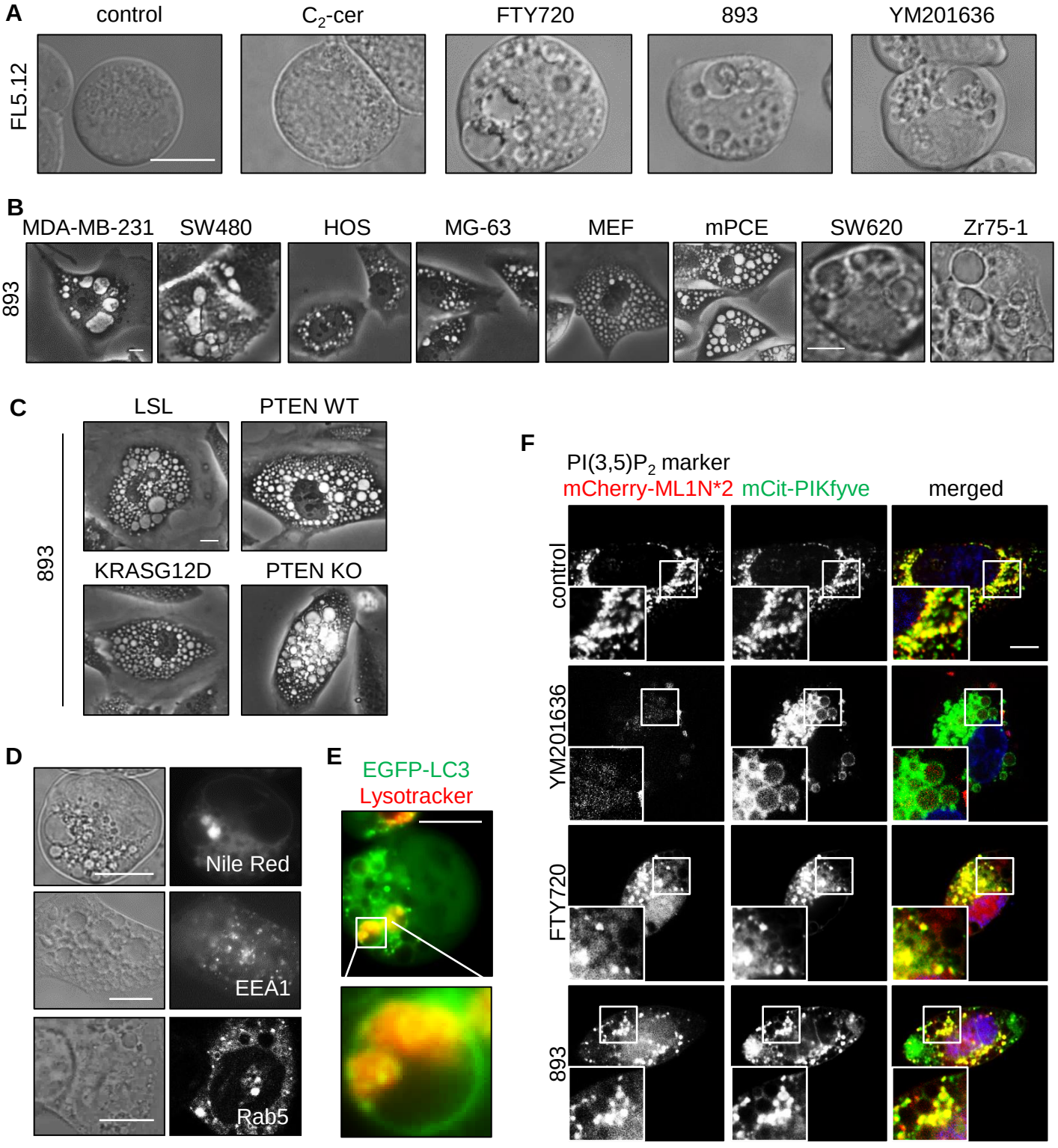


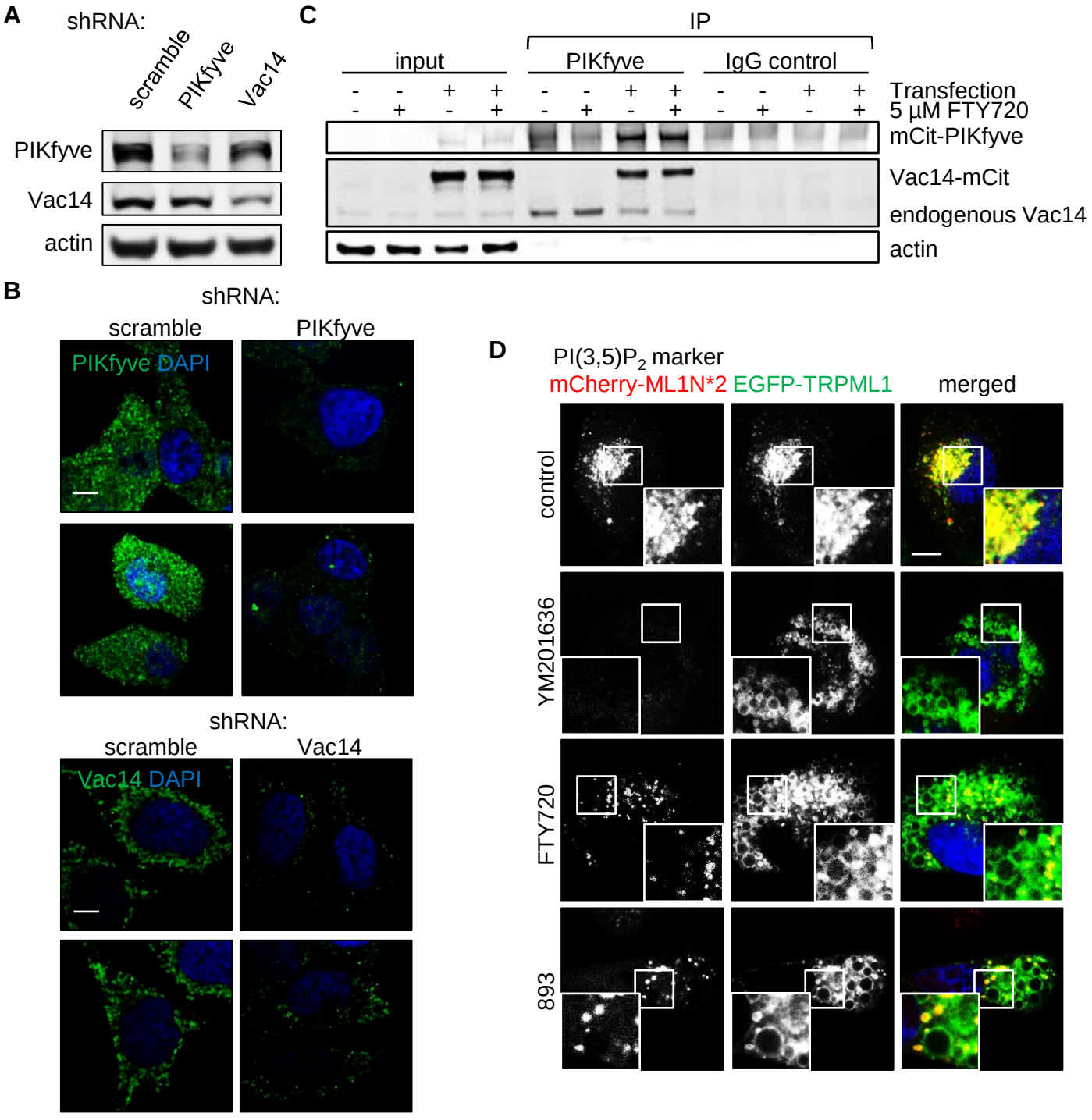
Supplemental Figure 1: Blocking apoptosis does not prevent SH-BC-893-induced cell death. (**A-B**) Viability of control or Bcl-X_L over-expressing FL5.12 cells 24 h after SH-BC-893 (893) treatment (**A**) or IL-3 withdrawal (**B**). Means +/- SEM, n ≥ 3. Using Student's unpaired, two-tailed t-test, ***, *P* <0.001; n.s., not significant (*P* >0.05).



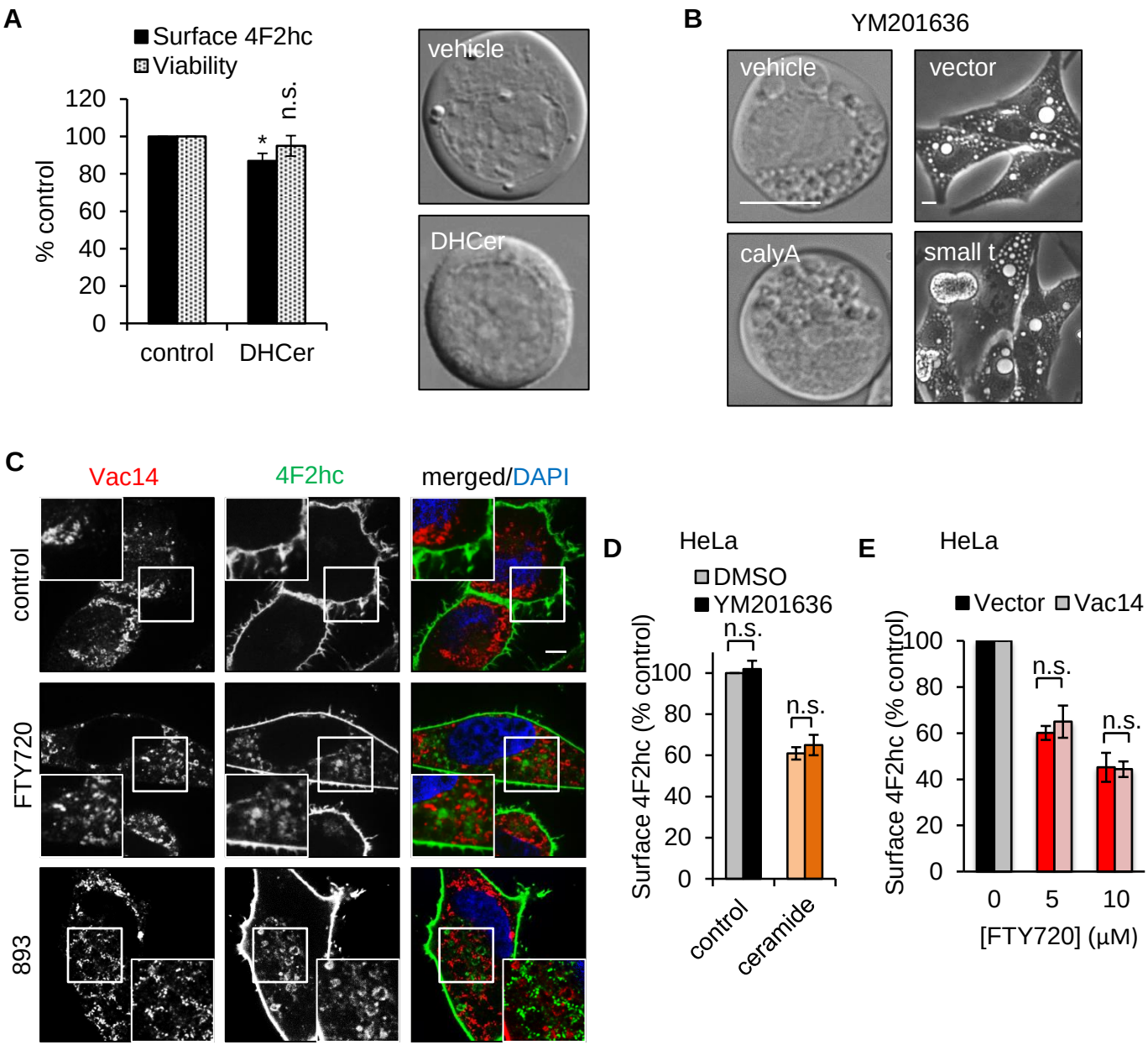
Supplemental Figure 2: FTY720 and SH-BC-893 inhibit tumor growth in vivo. (**A**) Bound NADH fraction in PTEN WT or PTEN KO MEFs treated with 7.5 μM SH-BC-893. (**B**) Viability of p53$^{-/-}$ MEFs that are wildtype (WT) or knocked-out (KO) for PTEN. (**C**) SH-BC-893-treated control (LSL), K-RasG12D-expressing, PTEN WT, or PTEN KO MEFs stained for 4F2hc. Scale bar, 20 μm. (**D**) SW620 tumor weight upon excision in mice treated with 10 mg/kg FTY720. Means +/- S.D., n ≥ 7. (**E**) SH-BC-893 concentration in SW620 tumors and blood plasma 24 h after 11 d of 20 mg/kg i.p. treatment. Means +/- SEM except for (**D**), n ≥ 3. Using Student's unpaired, two-tailed t-test, *, P <0.05; **, P <0.01; ***, P <0.001; n.s., not significant (P >0.05). (**F**) Percent change in body weight of NSG mice from SW620 xenograft study shown in Figure 2G-I or (**D**).



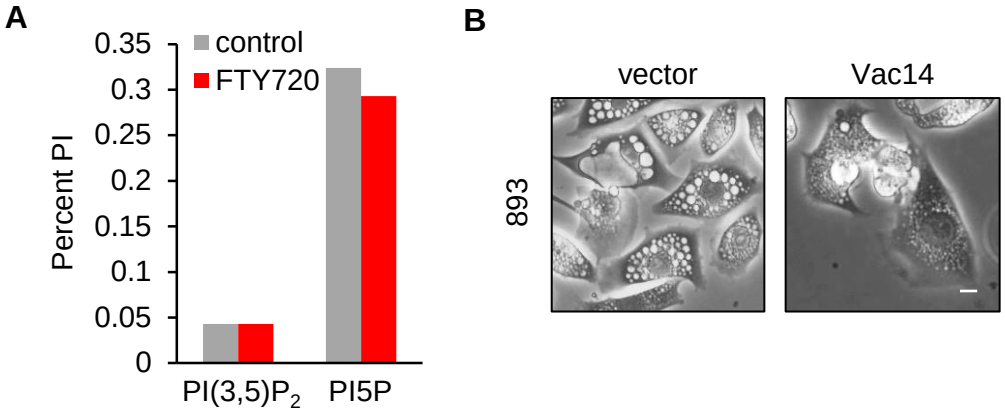
Supplemental Figure 3: Characterization of FTY720- and SH-BC-893-induced vacuolation. (**A,B**) FL5.12 cells (**A**) or the indicated cell lines (**B**) were treated with drugs for 2 h or 6 h, respectively (50 μM C_2-ceramide, 5 μM FTY720, 5 μM SH-BC-893, 800 nM YM201636). (**C**) Control (LSL), K-RasG12D-expressing, PTEN WT, or PTEN KO MEFs treated with SH-BC-893 for 6 h. (**D**) FL5.12, HeLa, or MEFs treated with FTY720 and stained with Nile Red, probed with EEA1 antibodies, or expressing GFP-Rab5, respectively. (**E**) GFP-LC3-expressing FL5.12 treated with 2.5 μM FTY720 for 2 h and stained with Lysotracker Red. (**F**) HeLa cells expressing the PI(3,5)P_2 marker mCherry-ML1N*2 and mCit-PIKfyve were treated with 800 nM YM201636, 5 μM FTY720, or 5 μM SH-BC-893 for 6 h. Scale bar, 10 μm.



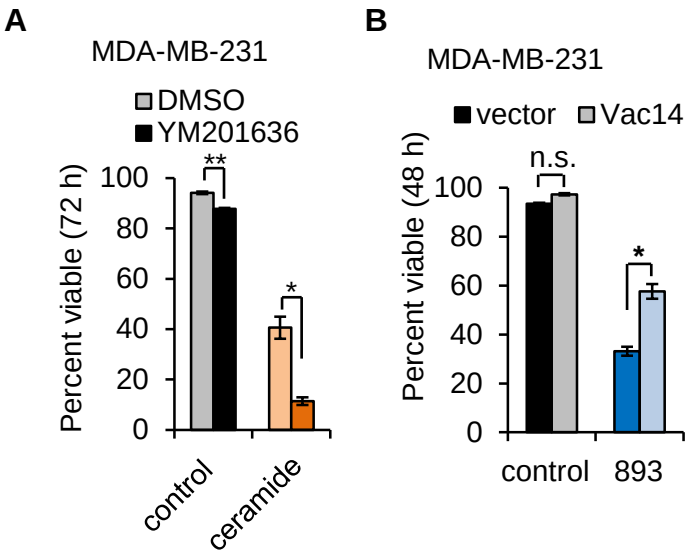
Supplemental Figure 4: FTY720 and SH-BC-893 mislocalizes PIKfyve. (**A,B**) HeLa cells transfected with shRNA scramble, PIKfyve, or Vac14 were evaluated by western blotting (**A**) or by confocal immunofluorescence microscopy (**B**). (**C**) PIKfyve immunoprecipitation from mCitrine-PIKfyve- and Vac14-mCitrine-expressing HeLa cells treated with FTY720. (**D**) HeLa cells expressing PI(3,5)P_2 marker mCherry-ML1N*2 and EGFP-TRPML1 were treated with 800 nM YM201636, 5 μM FTY720, or 5 μM SH-BC-893 for 6 h. Scale bar, 10 μm.



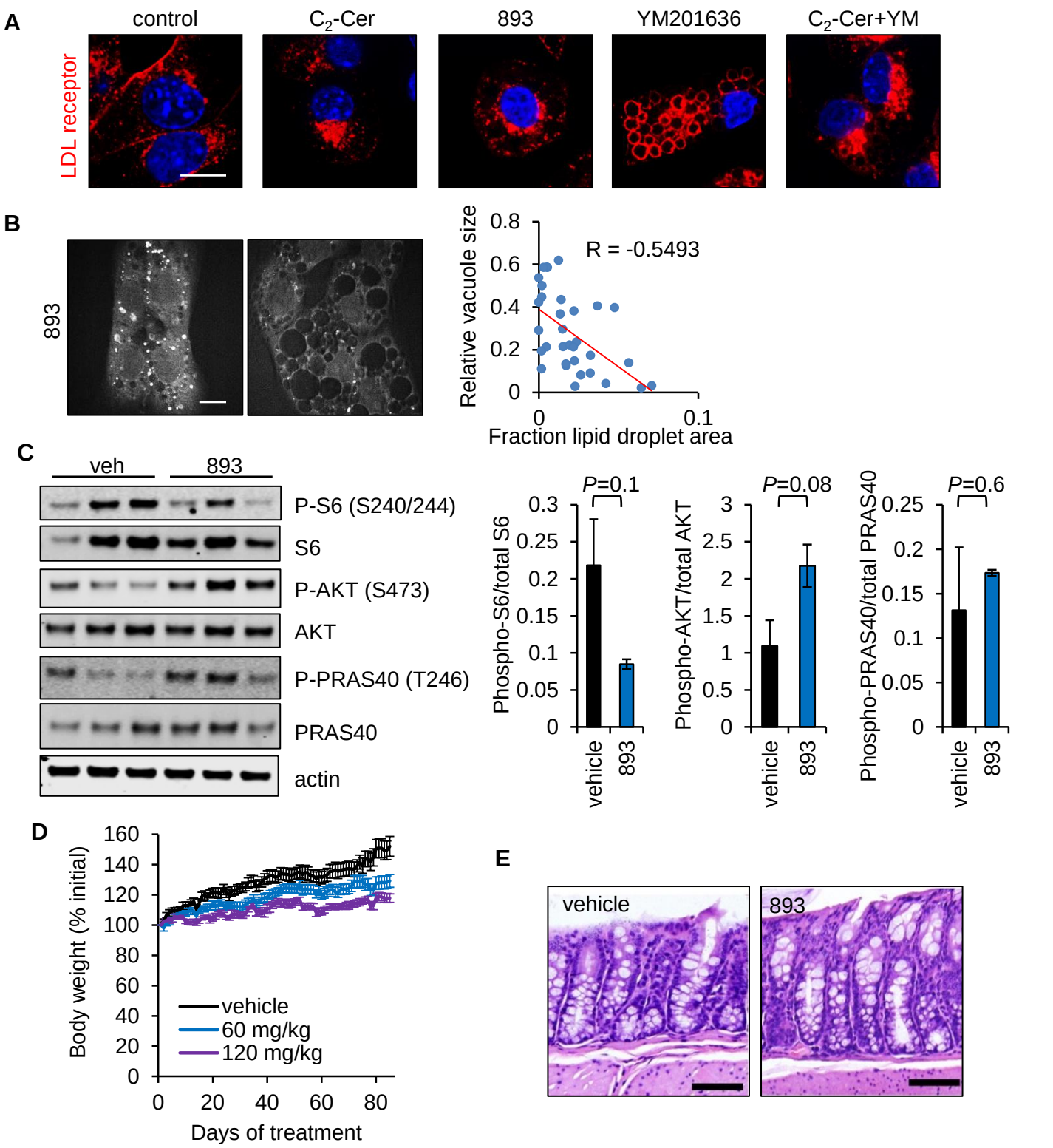
Supplemental Figure 5: FTY720 and SH-BC-893 induce surface nutrient transporter loss and vacuolation via two distinct PP2A-dependent mechanisms. (**A**) Surface 4F2hc levels and viability (left) and vacuolation (right) of FL5.12 cells treated with 50 μM dihydro-C_2-ceramide (DHCer). Statistics comparing to respective controls. (**B**) FL5.12 cells (left) treated with 800 nM YM201636 +/- 5 nM calyculinA (calyA) or HeLa cells (right) expressing SV40 small t antigen treated with YM201636. (**C**) HeLa cells were treated with 5 μM FTY720 or SH-BC-893, stained as indicated, and evaluated by confocal microscopy. (**D**) Surface 4F2hc levels in HeLa cells treated with DMSO, 800 nM YM201636, 25 μM C_2-ceramide, or with YM201636 + C_2-ceramide. (**E**) Surface 4F2hc levels in vector or Vac14 over-expressing HeLa cells treated with FTY720 for 6 h. Scale bar, 10 μm. Means +/- SEM n ≥ 3. Using Student's unpaired, two-tailed t-test, *, P <0.05; n.s., not significant (P >0.05).



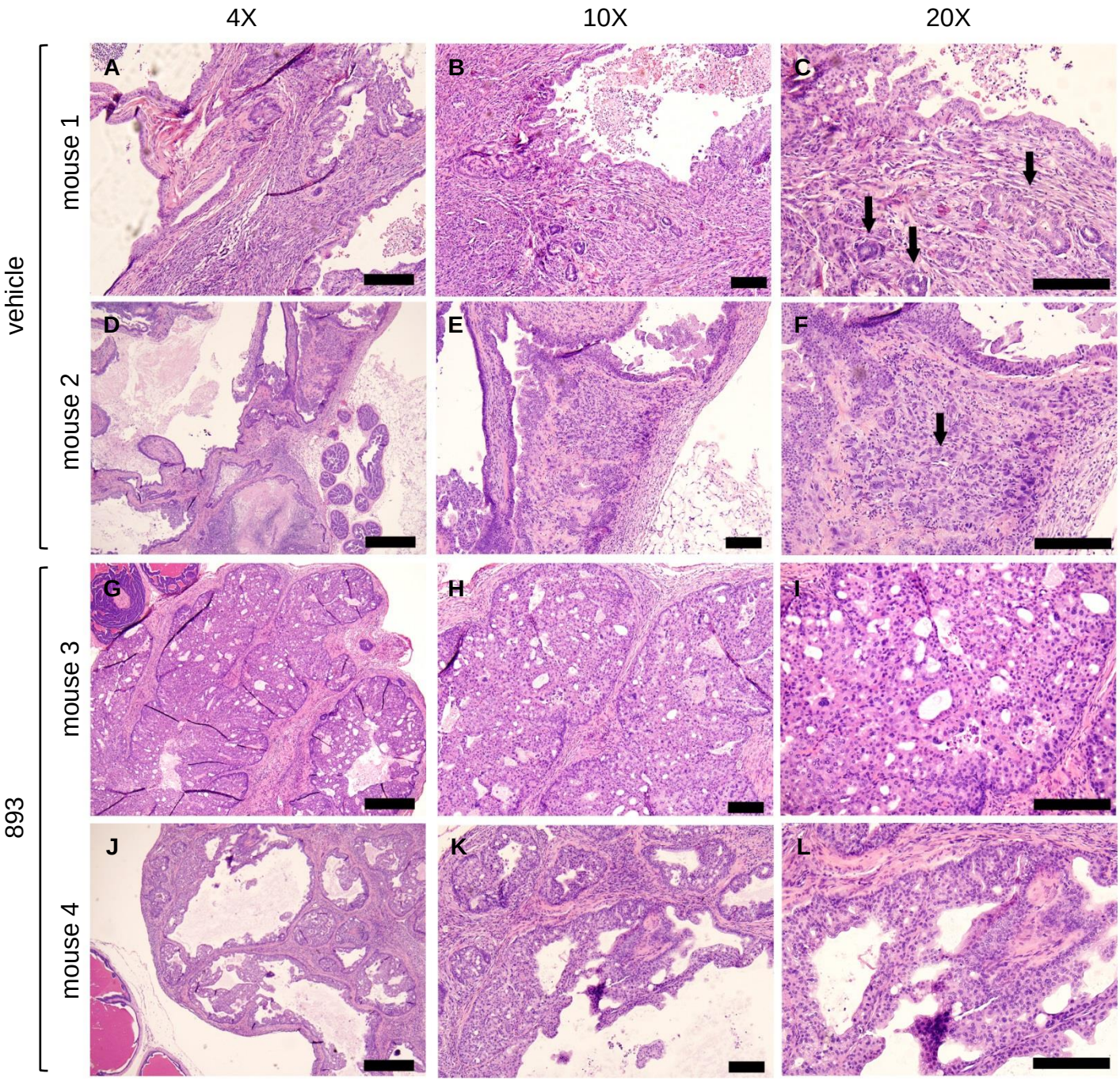
Supplemental Figure 6: SH-BC-893 blocks autophagic flux. (**A**) $PI(3,5)P_2$ and PI5P levels in MEF cells treated with FTY720 expressed as a percentage of total phosphatidylinositol. Average from two independent experiments. (**B**) Mouse prostate cancer epithelial cells expressing vector or Vac14 treated with 5 µM SH-BC-893. Scale bar, 10 µm.



Supplemental Figure 7: Vacuolation enhances cell death. (**A**) Viability of MDA-MB-231 cells treated with DMSO, 800 nM YM201636, 25 µM C_2-ceramide, or with YM201636 + C_2-ceramide. (**B**) Viability of vector or Vac14 over-expressing MDA-MB-231 cells treated with SH-BC-893. Means +/- SEM, n ≥ 3. Using Student's unpaired, two-tailed t-test, *, $P < 0.05$; **, $P < 0.01$; n.s., not significant ($P > 0.05$).



Supplemental Figure 8: SH-BC-893 is selectively toxic to cancer cells. (**A**) mPCE cells treated with indicated compounds and stained for the LDL receptor. Scale bar, 20 μm. (**B**) CARS images of lipid droplets in mPCE cells treated with SH-BC-893. Linear regression plot representing correlation between vacuole size and lipid droplet amount in mPCE cells treated with SH-BC-893. R, Pearson Coefficient. Scale bar, 20 μm. (**C**) Western blotting of tumors from vehicle or SH-BC-893 treated mice in Fig. 8J. On the right, ratio of phosphorylated over total S6, AKT, and PRAS40 from tumors excised from pDKO mice treated with vehicle or 120 mg/kg SH-BC-893. Means +/- SEM, n ≥ 3, *P*-values using Student's unpaired, two-tailed t-test. (**D**) Percent change in body weight of pDKO mice treated with vehicle, 60 mg/kg, or 120 mg/kg SH-BC-893. (**E**) Histology of intestinal crypts in mice treated with 120 mg/kg SH-BC-893 for 11 weeks. Scale bar, 100 μm.



Supplemental Figure 9: SH-BC-893 inhibits prostate cancer progression. (**A**-**L**) H&E staining of p53$^{-/-}$ PTEN$^{-/-}$ prostates excised from mice treated with vehicle (**A**-**F**) or 120 mg/kg SH-BC-893 (**G**-**L**) for 11 weeks. At high power, arrows indicate invasive glandular structures in a reactive stroma (**C,F**). Prostates in SH-BC-893-treated mice exhibited exclusively prostatic intraepithelial neoplasia (**G**-**L**) rather than the locally invasive adenocarcinomas seen in vehicle controls (**A**-**F**) indicating that SH-BC-893 slowed disease progression. Although prostates of treated mice still developed prostatic intraepithelial neoplasia, no invasive component was identified in SH-BC-893-treated pDKO mice. The intraepithelial proliferation present in treated mice showed much lower degrees of cellular pleomorphism, hyperchromasia, and nuclear atypia. Scale bars = 500 mm (4X), 100 mm (10X & 20X).

Supplemental Table 1: Blood chemistry of vehicle or SH-BC-893-treated pDKO mice at sacrifice. Means +/- SEM are shown, n=4. AP: alkaline phosphatase; SGPT (ALT): serum glutamic-pyruvic transaminase (alanine aminotransferase); SGOT (AST): serum glutamic oxaloacetic transaminase (aspartate aminotransferase); CPK: creatine phosphokinase; BUN: blood urea nitrogen; CR: creatinine; CHOL: cholesterol; Ca^{2+}: Calcium; P: phosphorus; $HCO3^{-}$: bicarbonate.

Blood Chemistry Panel

	AP	SGPT (ALT)	SGOT (AST)	CPK	ALBUMIN	TOTAL PROTEIN	GLOBULIN
vehicle	93 ± 5.4	65 ± 10.4	809 ± 200	5,017 ± 1199	3 ± 0.2	6 ± 0.3	3 ± 0.13
60 mg/kg 893	77 ± 7.8	78 ± 25	640 ± 136	5,340 ± 2366	3 ± 0.2	5 ± 0.4	2 ± 0.18
120 mg/kg 893	83 ± 6.2	91 ± 17.2	907 ± 171	10,441 ± 3014	3 ± 0.1	6 ± 0.2	2 ± 0.03

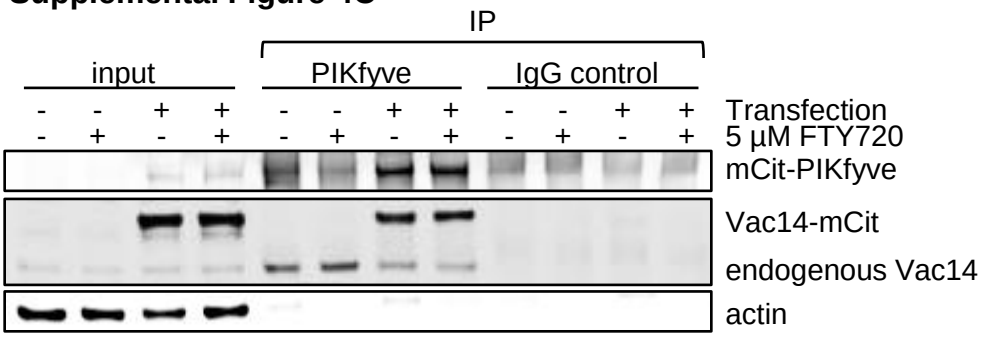
	TOTAL BILIRUBIN	BUN	CR	CHOL	GLUCOSE	Ca^{2+}	P	$HCO3^{-}$
vehicle	0.2 ± 0.03	29 ± 3.1	<0.2	187 ± 8.8	264 ± 43	11 ± 0.8	13 ± 0.6	19 ± 5.8
60 mg/kg 893	0.1 ± 0	19 ± 1.1	<0.2	134 ± 15.8	282 ± 24	10 ± 0.7	11 ± 1.0	20 ± 3.2
120 mg/kg 893	0.2 ± 0.03	23 ± 1.5	<0.2	107 ± 7.5	307 ± 28	10 ± 0.1	13 ± 0.1	17 ± 2.8

Supplemental Table 2: Complete blood count of vehicle or SH-BC-893-treated pDKO mice at sacrifice. Means +/- SEM are shown, n=4. WBC: white blood cells; RBC: red blood cells; HCT: hematocrit

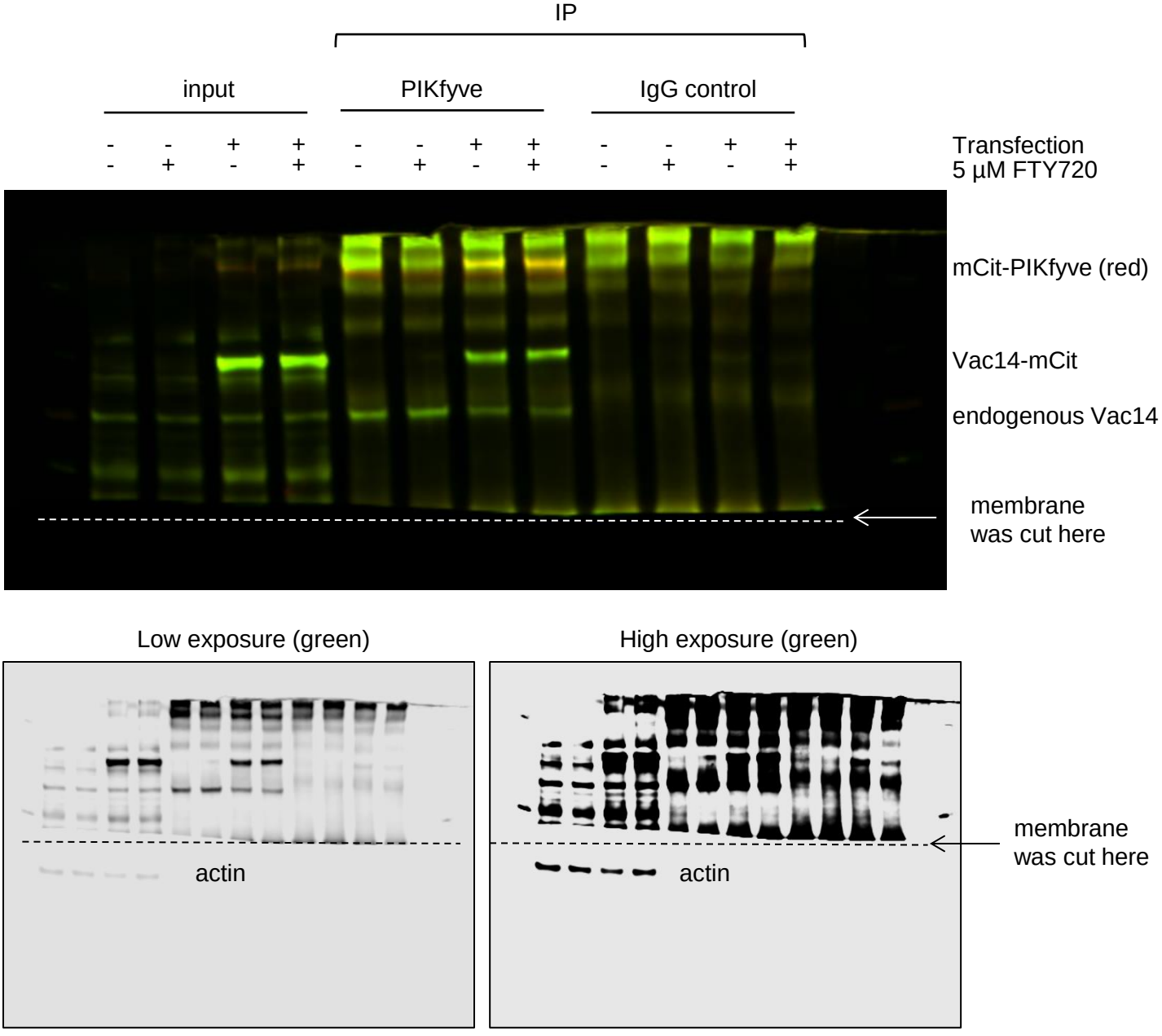
Complete Blood Count

	WBC [10^3 cells/µL]	% neutrophil	% lymphocyte	RBC [M/µL]	HCT
vehicle	5 ± 1	16 ± 5	76 ± 7	9 ± 0	38 ± 3
60 mg/kg 893	5 ± 1	14 ± 3	77 ± 4	8 ± 1	32 ± 5
120 mg/kg 893	8 ± 1	19 ± 5	73 ± 5	9 ± 1	41 ± 3

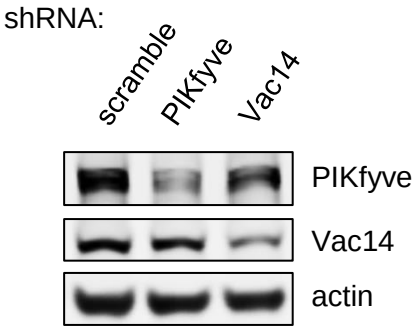
Supplemental Figure 4C



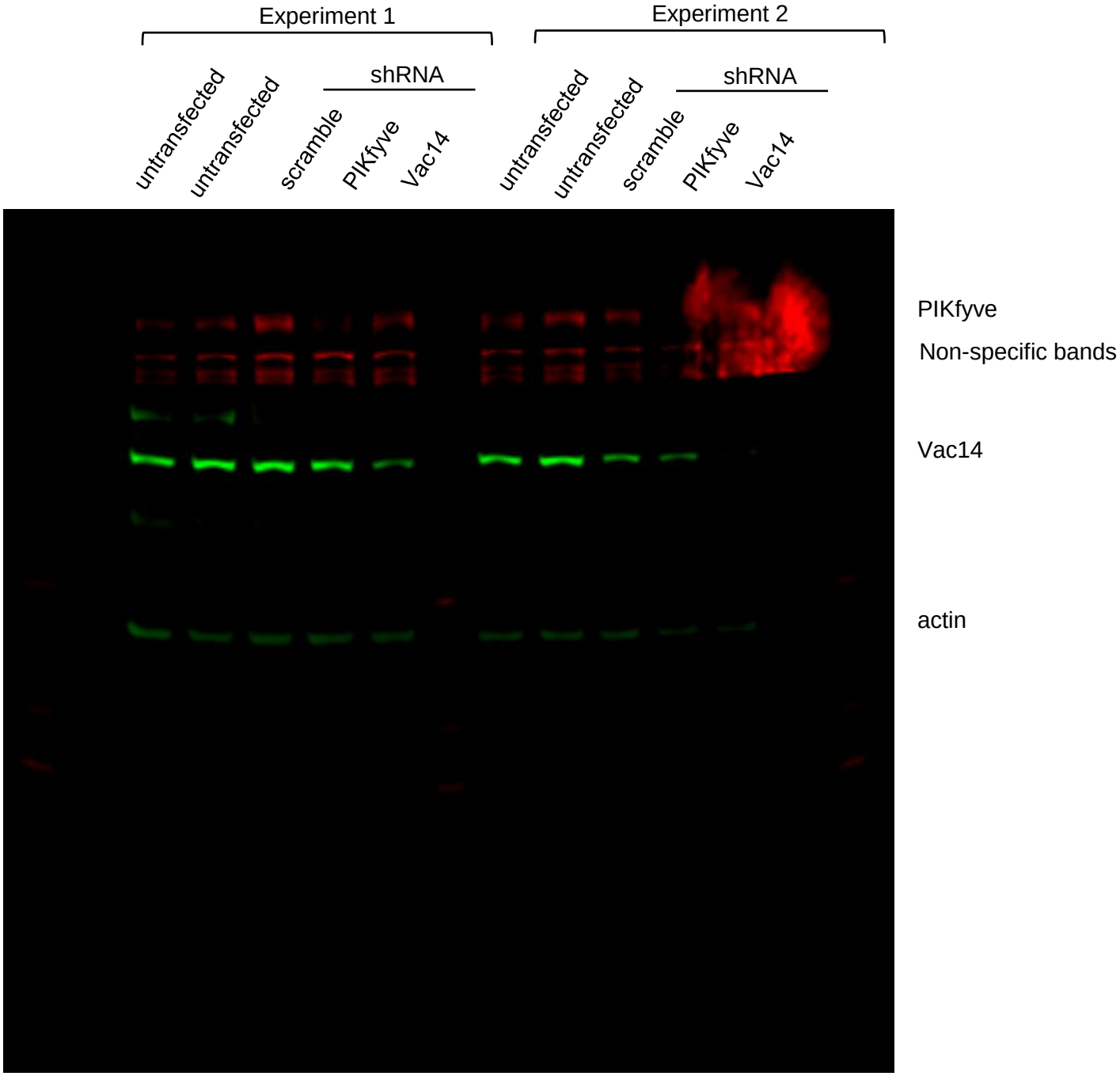
Full unedited gel for Supplemental Figure 4C



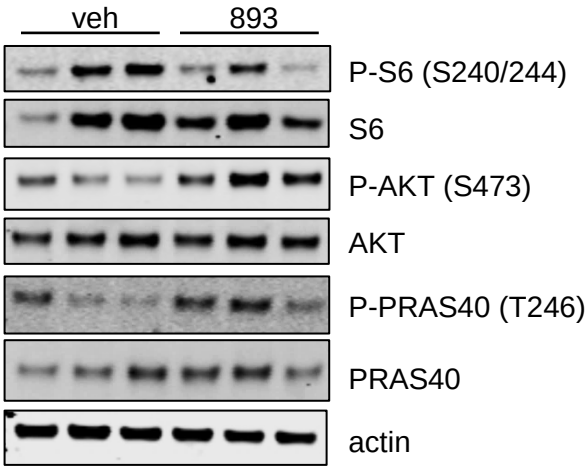
Supplemental Figure 4A



Full unedited gel for Supplemental Figure 4A
(Experiment 1 is shown on Supplemental Figure 4A)



Supplemental Figure 8C



Full unedited gel for Supplemental Figure 8C

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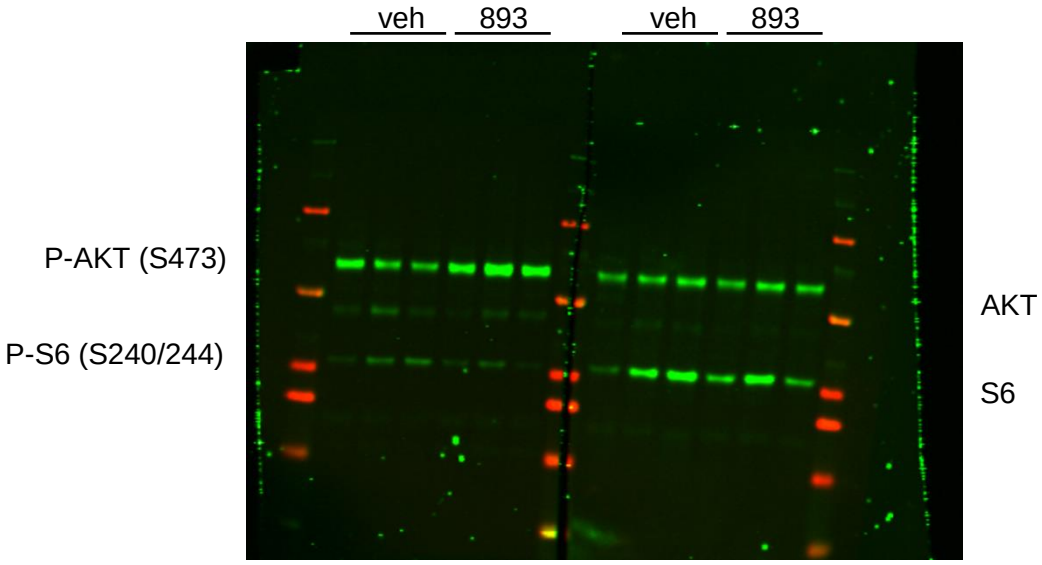
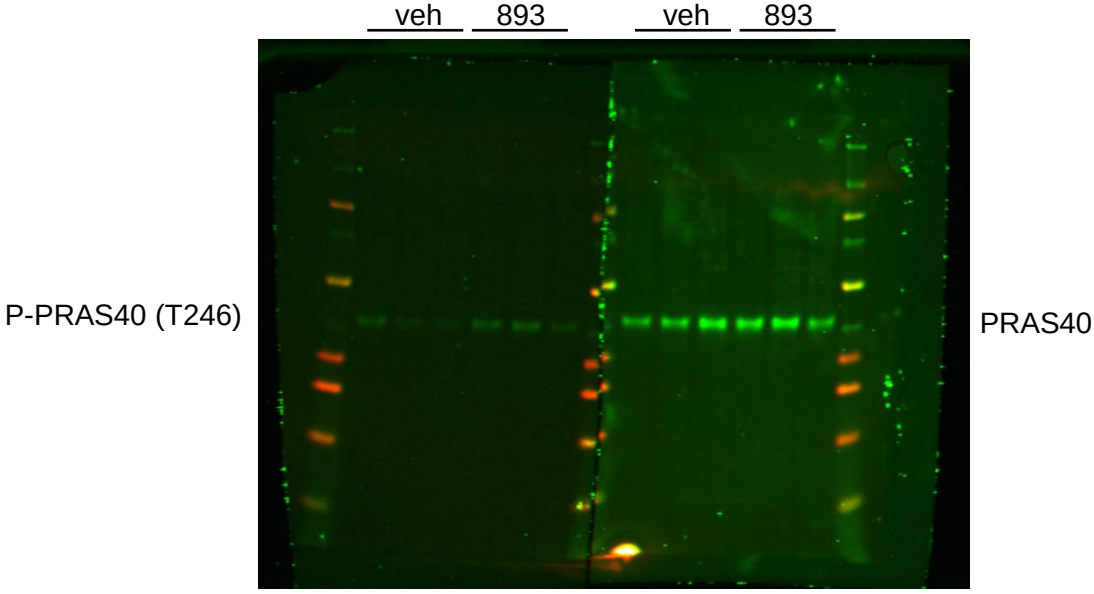


Gel 2



Full unedited gel for Supplemental Figure 8C continued

Gel 3