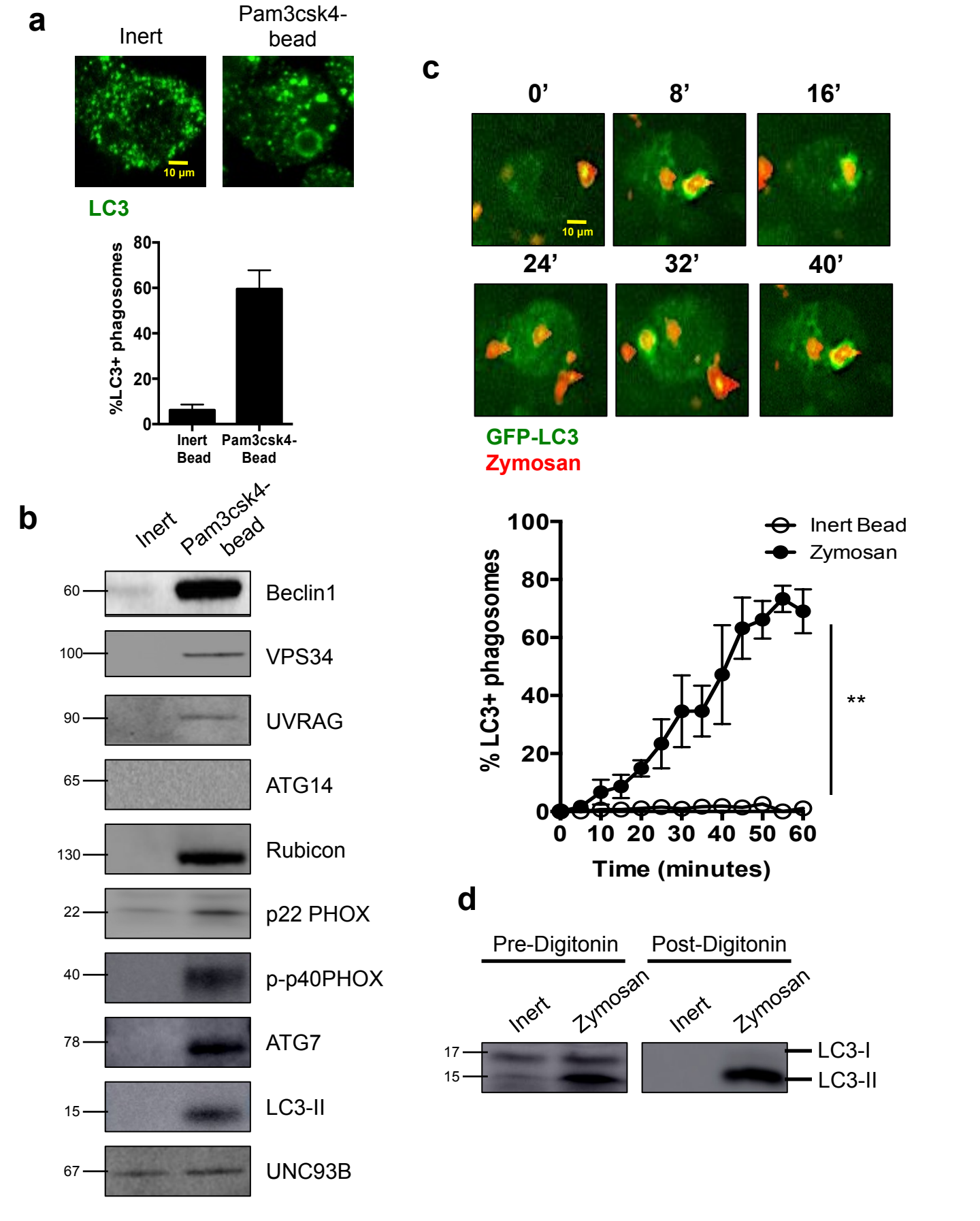
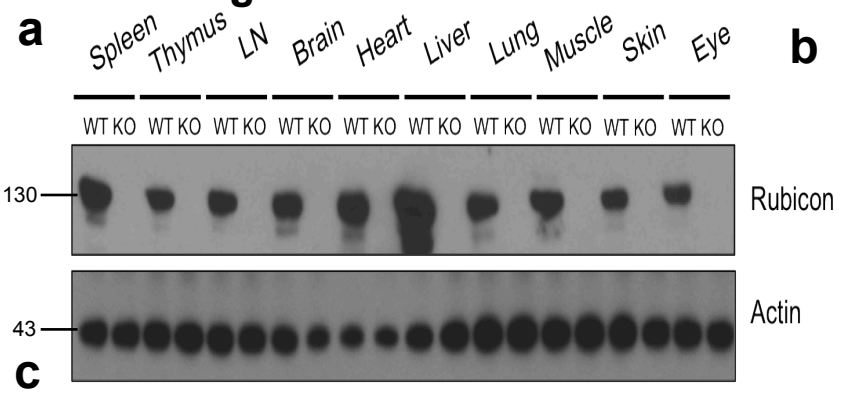
Supplementary Table 1: Proteins uniquely associated with the LAPosome

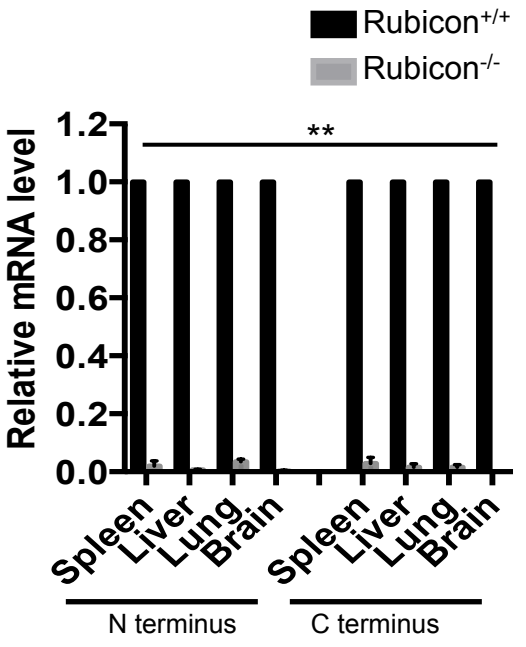
Protein	Description	Log2 (IgG-bead / Inert-bead)	Log2 (Pam3csk4-bead / Inert-bead)	Log2 (Avg)
Rubicon	Run domain Beclin-1 interacting and cysteine-rich containing protein	1.21	1.35	1.28
Prkcd	Protein kinase C δ	1.91	2.63	2.23
Lgals3	Galectin-3	3.21	2.93	3.07
Ifitm3	Interferon-induced transmembrane protein 2	2.36	1.66	2.01
Srrm2	Serine/arginine repetitive matrix protein 2	2.19	1.85	2.02
Myof	Myoferlin	3.13	2.68	2.89
2-Sep	Septin-2	1.55	1.92	1.995
Ap2s1	AP-2 complex subunit ς 1	2.63	2.25	2.44
Trim33	E3 ubiquitin-protein ligase	2.58	1.69	2.135
Xrcc5	X-ray repair cross-complementing protein 5	1.03	5.98	3.505
Lalba	Lactalbumin-α	5.23	1.39	3.31
Saa3	Serum amyloid A-3	3.43	2.54	2.985
Hck	Tyrosine-protein kinase HCK	2.28	1.81	2.045
Tmem206	Transmembrane protein 206	1.71	2.44	2.075
Snx20	Sorting nexin-20	2.37	2.83	2.6
Atp8b3	ATPase, class I, type 8B, member 3	0.96	0.81	0.89
Reps1	RalBP1-associated Eps domain-containing protein 1	4.87	1.29	3.08
Gabarap	Gamma-aminobutyric acid receptor-associated protein	2.04	2.33	2.185
Gabarapl 1	Gamma-aminobutyric acid receptor-associated protein-like 1	1.17	1.77	1.47
Map1lc3b	Microtubule-associated proteins 1A/1B light chain 3B	0.91	0.71	0.81
Pik3c3	Phosphatidylinositol 3-kinase catalytic subunit type 3	1.09	1.17	1.13
Atg3	Ubiquitin-like-conjugating enzyme ATG3	N/A	0.72	0.72
Atg9	Autophagy 9-like 1 protein	0.89	0.53	0.71

Supplementary Figure 1: LAP is a pathway distinct from canonical autophagy.



Supplementary Figure 2: Generation of the Rubicon-deficient mouse model using the CRISPR/Cas9 method.

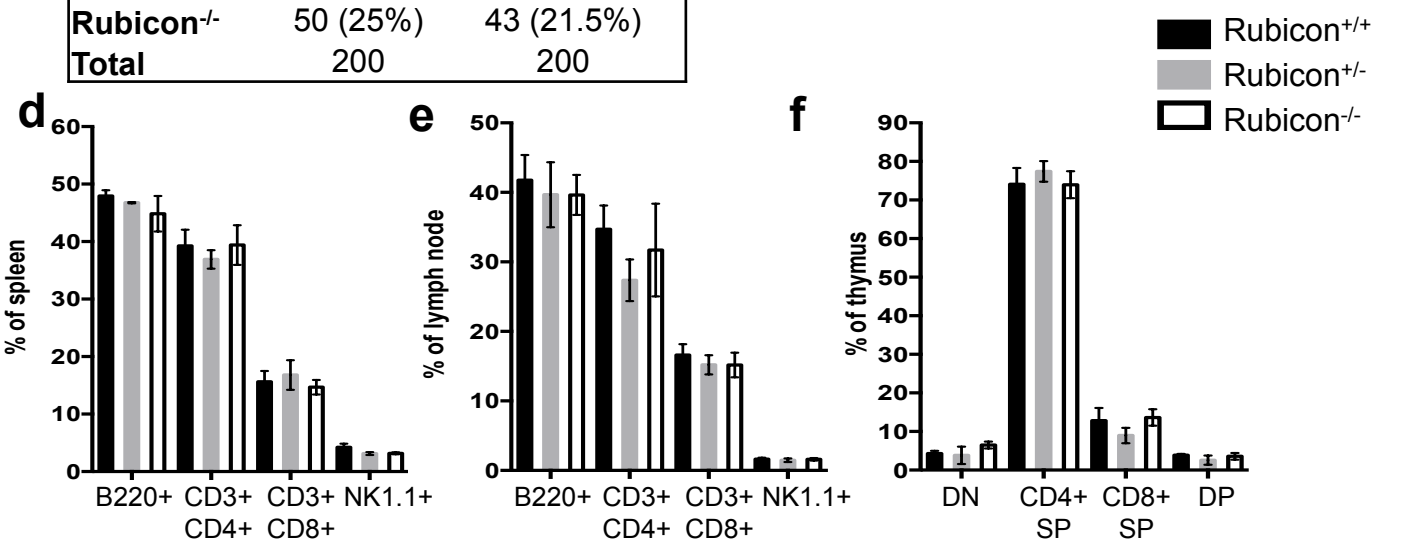


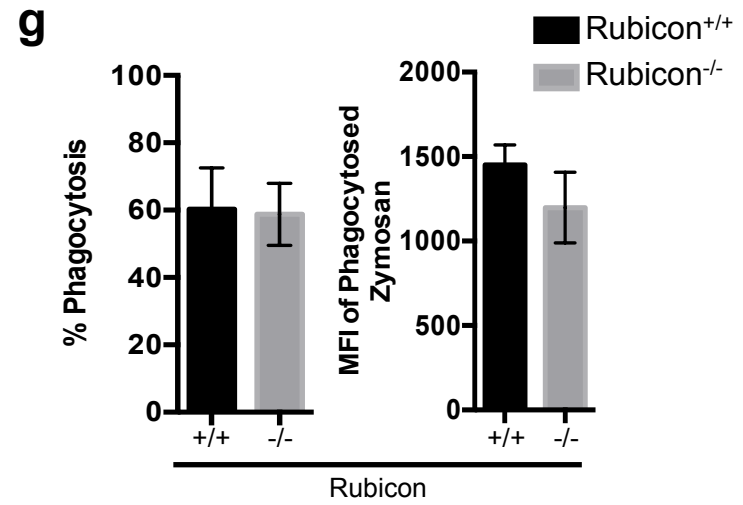


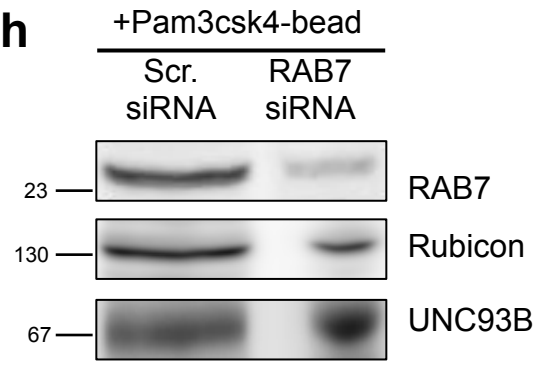
c

Rubicon$^{+/-}$ x Rubicon$^{+/-}$

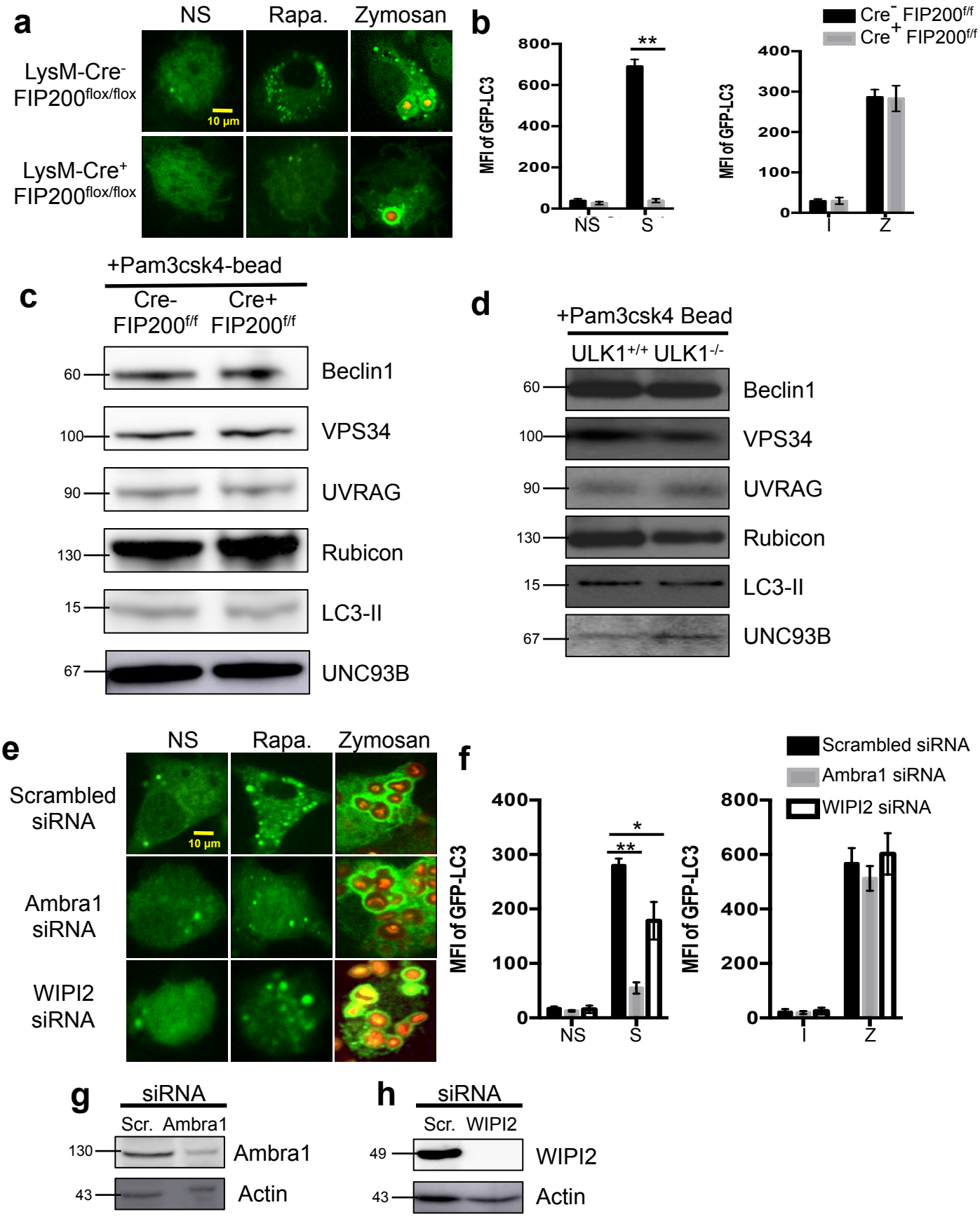
	Expected	Observed
Rubicon$^{+/+}$	50 (25%)	58 (29%)
Rubicon$^{+/-}$	100 (50%)	99 (49.5%)
Rubicon$^{-/-}$	50 (25%)	43 (21.5%)
Total	200	200



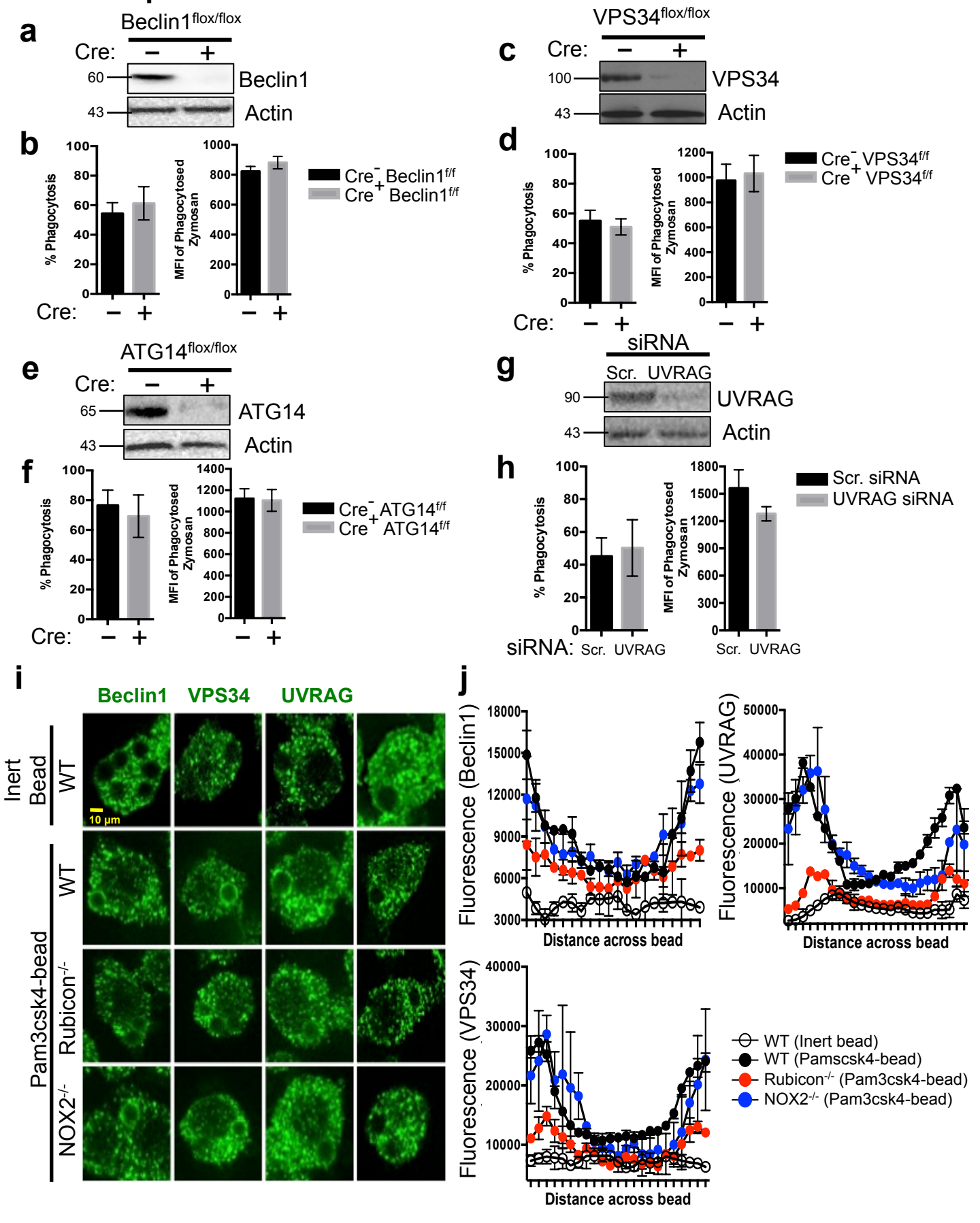




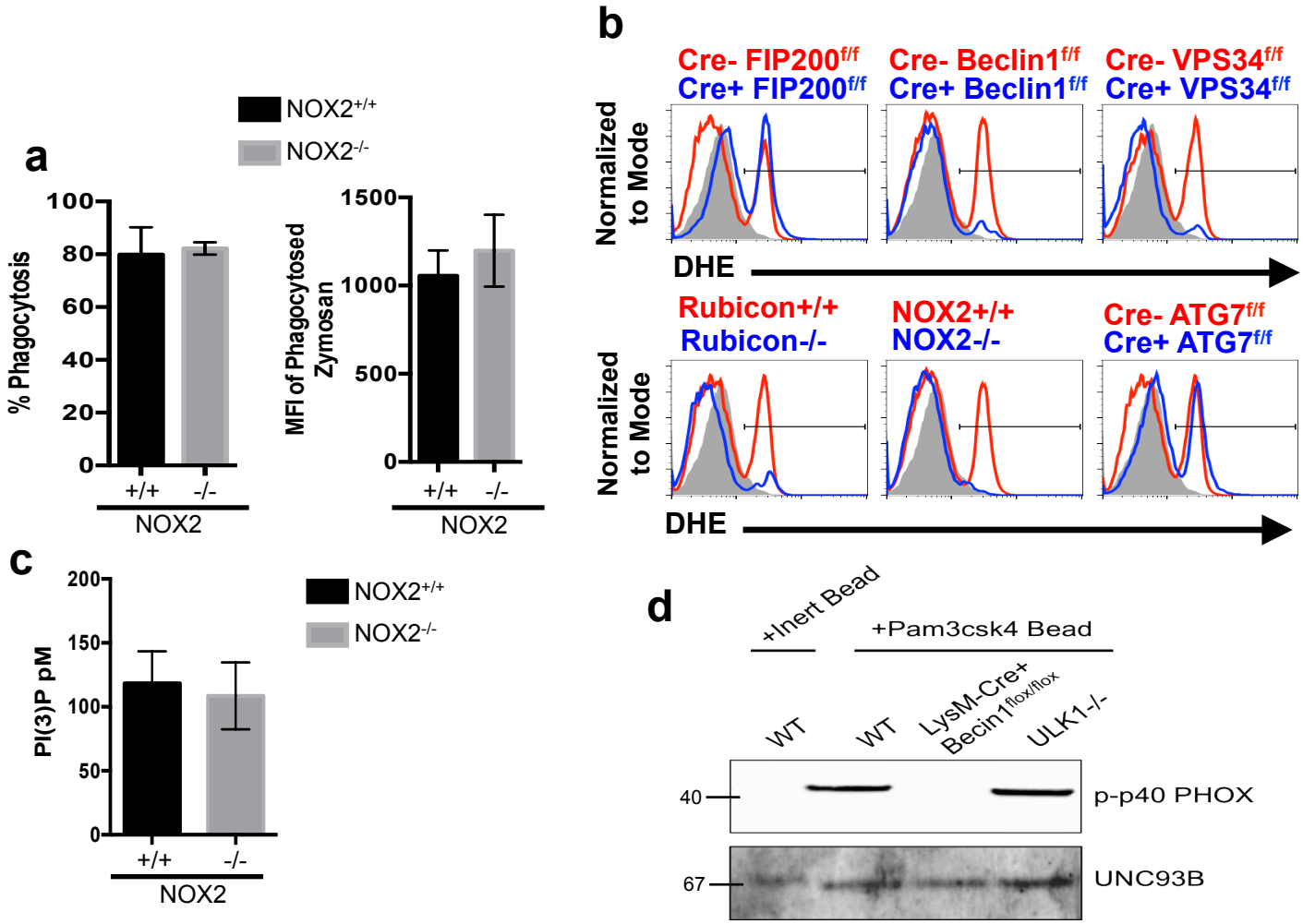
Supplementary Figure 3: LAP occurs independently of the pre-initiation complex.



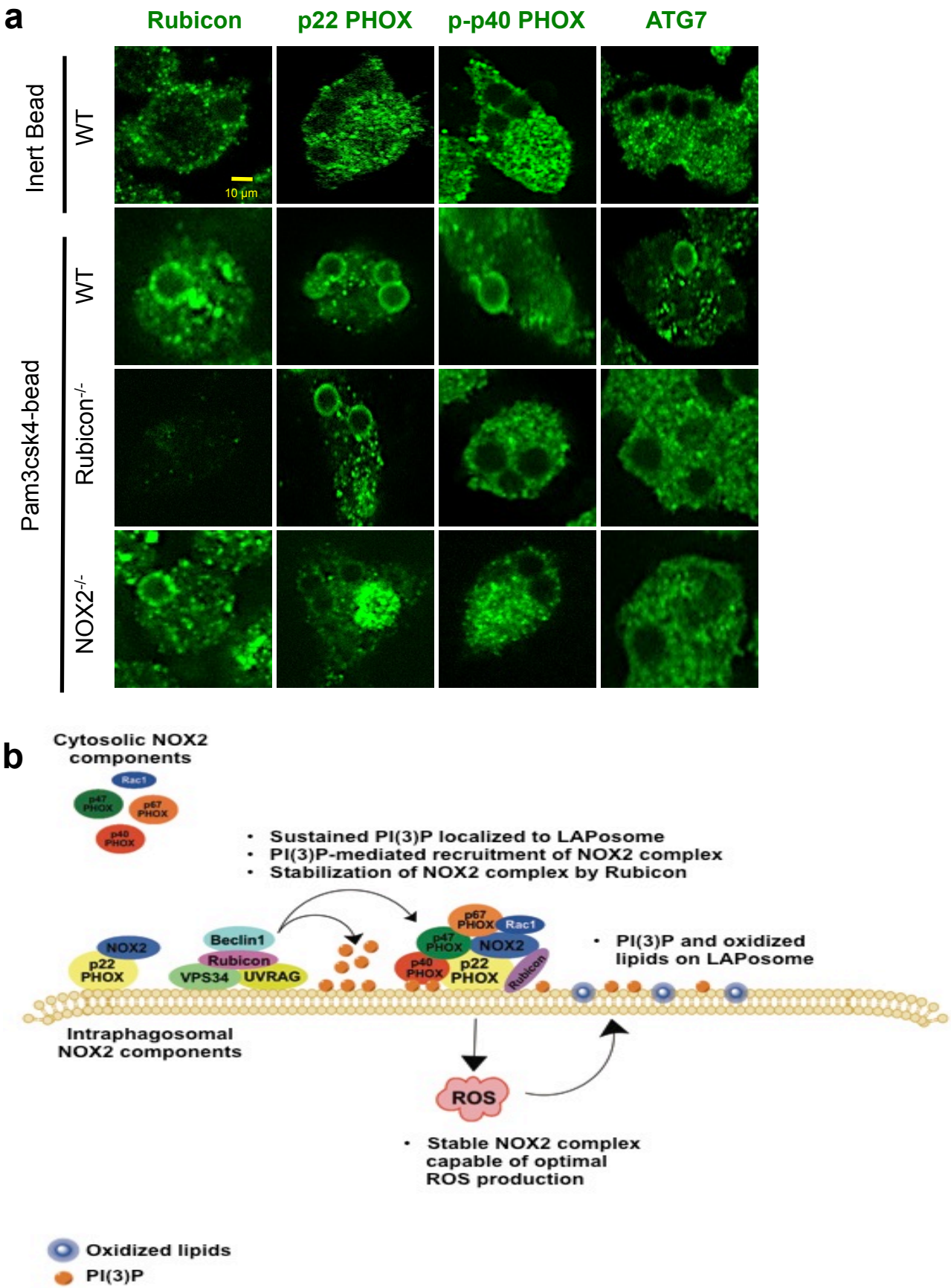
Supplementary Figure 4: LAP utilizes a UVRAG-containing Class III PI3K complex.



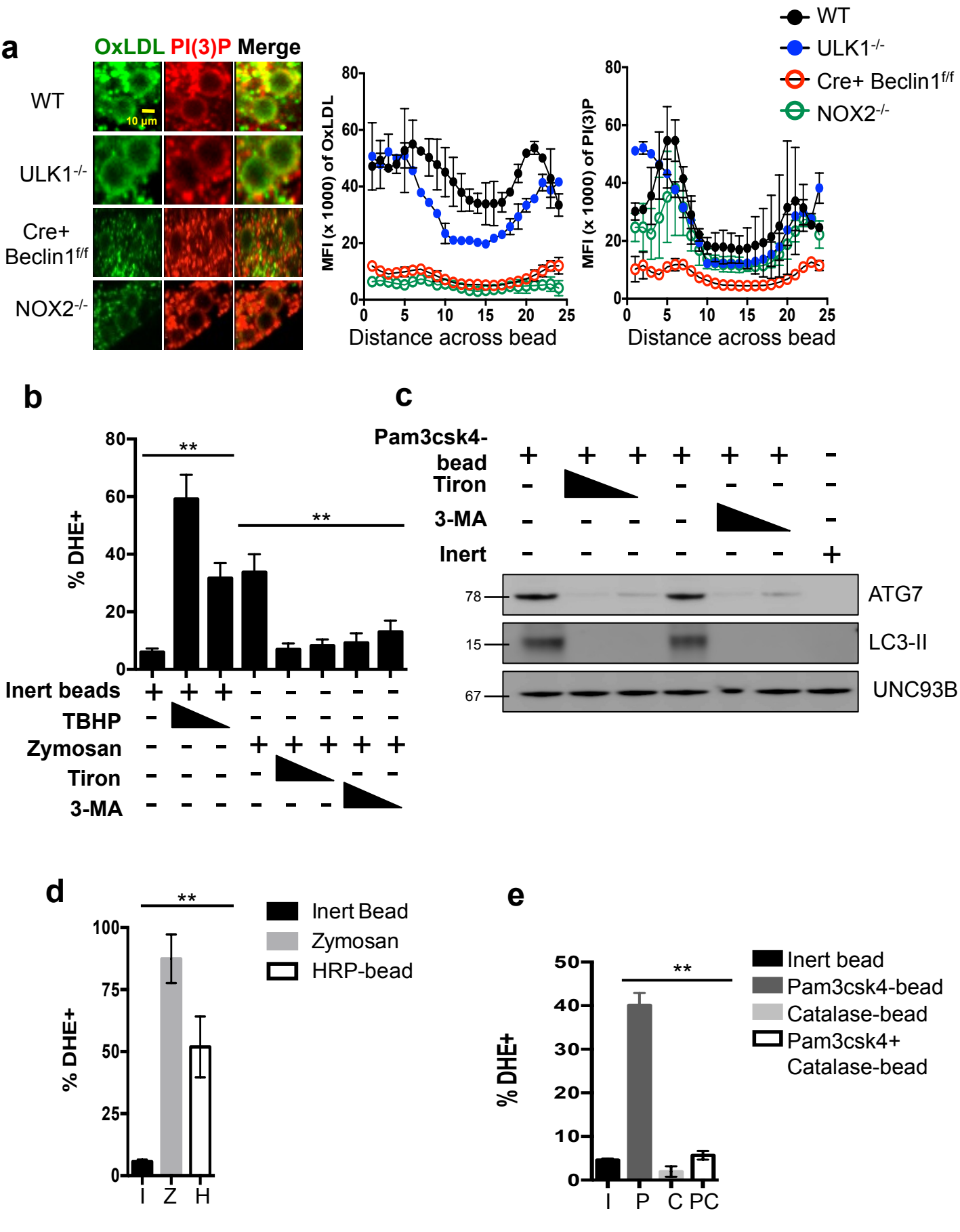
Supplementary Figure 5: NOX2 is downstream of the Class III PI3K complex and required for LAP



Supplementary Figure 6: The activity of Rubicon and NOX2 are required for translocation of the downstream conjugation systems to the LAPosome.



Supplementary Figure 7: PI(3)P and ROS are both required for LAP



Supplemental 8: Clearance of *Aspergillus fumigatus* requires LAP.