APPENDIX

Receptor-mediated clustering of FIP200 bypasses the role of LC3 lipidation in autophagy

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Appendix Figure Legend:

1 Appendix Figure S1 – related to Fig 2.
2 (A) Gating strategy for tf-Reporter screens. Single cells were selected through the indicated forward scatter and back scatter gates. Singlets were then analyzed for red and green fluorescence, with the indicated gates sorted to enrich for cells with activated and inhibited autophagy.
3 (B) A rank-ordered list of autophagy modifiers based on average beta scores across all lipidation deficient cell lines (ATG7KO, ATG10KO and ATG3KO cells). Top modifiers of lipidation-deficient autophagy are indicated in blue. Data from Table EV2.
4 (C) Gene correlation plot of average beta scores for tf-NBR1 in wild-type cells (from Shoemaker et al, 2019)) and ATG7KO cells (this study). Highlighted in red are genes with a beta score > 0.5 across all three lipidation-deficient cell lines. Dashed lines, top 1% of beta scores.
5 Data from Table EV2.
Appendix Figure S2 – related to Fig 6.

(A, B) $ATG7^{K^O}/TAX1BP1^{K^O}$ K562 cells expressing tf-NBR1 were nucleofected with TagBFP-TAX1BP1 variants and analyzed for red:green ratio and BFP expression. (n = 10,000 cells for all samples in A, n>8,000 cells for all samples in B). Median values for each sample are identified by a black line within each violin. The red dotted line across all samples corresponds to the red:green ratio of rescued cells (i.e. $TAX1BP1^{WT}$). The red solid line across all samples corresponds to the ratio observed in non-transfected cells.

(C, D) $TAX1BP1^{K^O}$ K562 cells were nucleofected with tf-TAX1BP1 variants and analyzed for red:green ratio (n = 10,000 cells for all $TAX1BP1^{K^O}$ rescues. n>4,000 for $ATG9A^{K^O}$ control). Median values for each sample are identified by a black line within each violin. The red dotted line across all samples corresponds to the red:green ratio of tf-TAX1BP1$^{WT}$. The red solid line across all samples corresponds to the red:green ratio observed for tf-TAX1BP1$^{WT}$ in $ATG9A^{K^O}$ cells.

Source data are available online for this figure.
Aver age beta score $−$ tf−NBR1 (all KO cells compiled)

Rank−ordered gene list

B) Average beta score $−$ tf−NBR1 (all KO cells compiled)

Appendix Figure S1
