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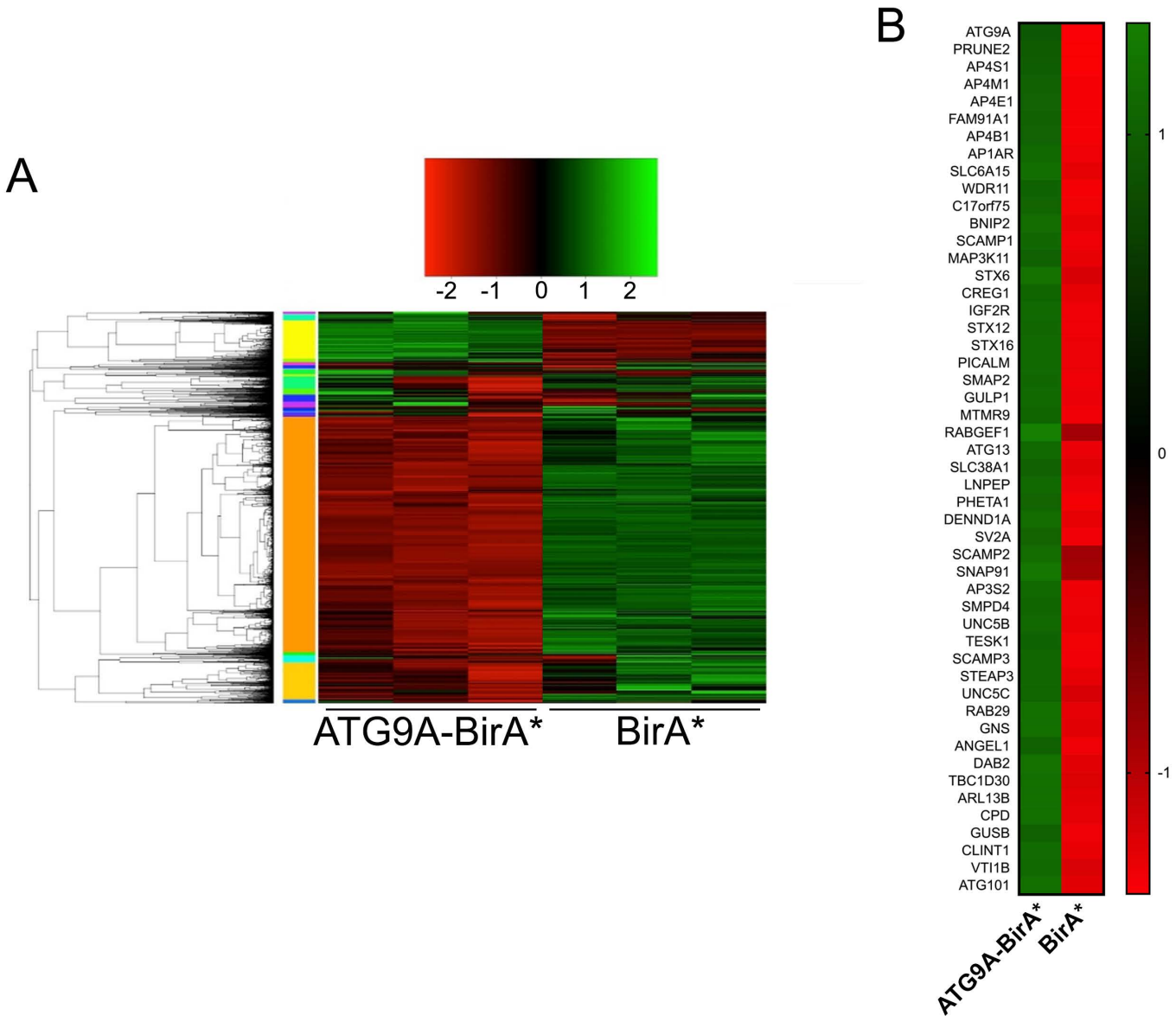
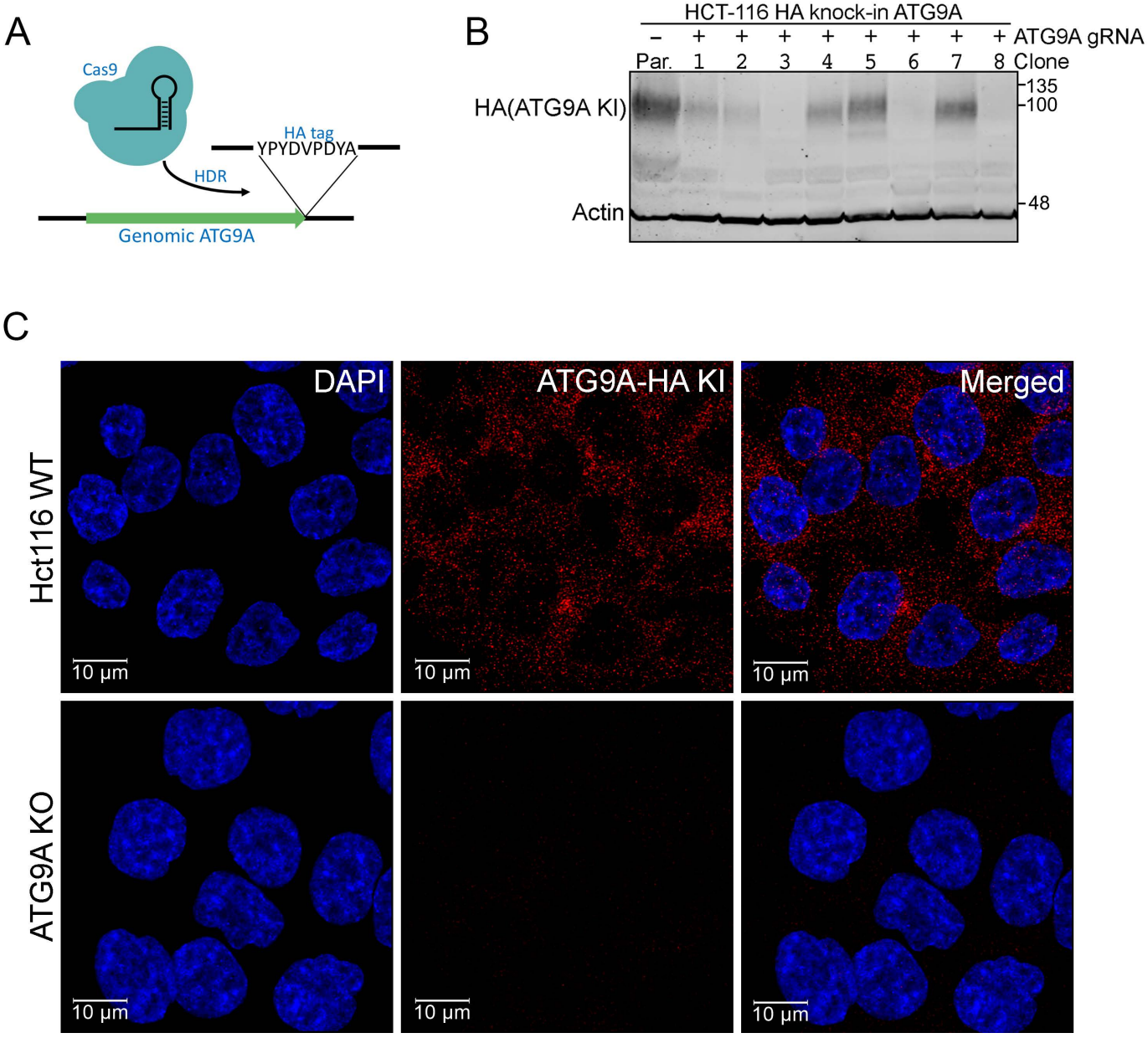
Appendix Figure S1



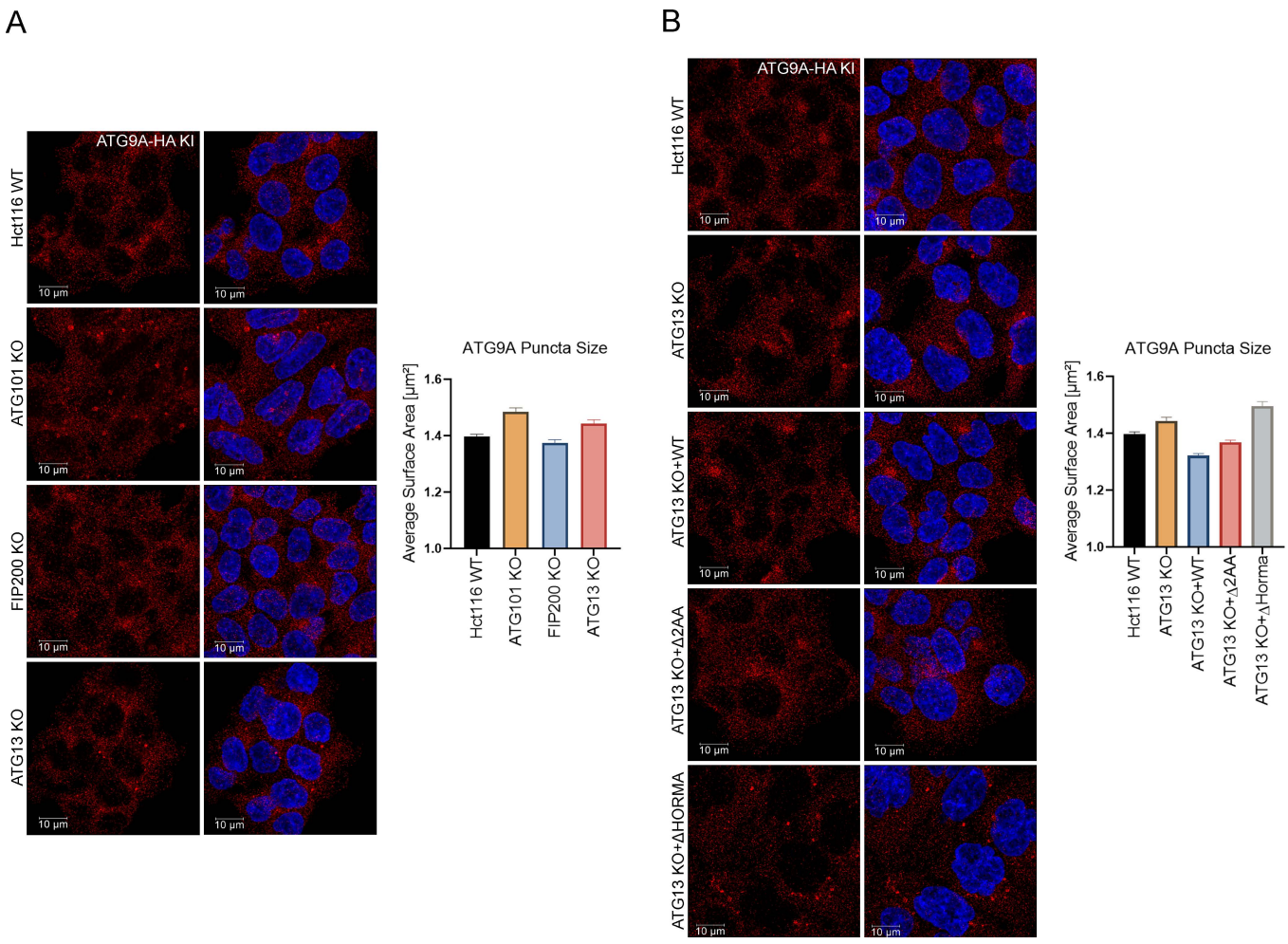
Appendix Figure S1: BioID identifies a network of proximity-based ATG9A interactions. (A) A heat map generated for all putative interactors from the BioID proteomics data for HA-BirA* vs HA-ATG9A BirA* streptavidin pull-downs. (B) A heat map of the top 50 putative interactors from the HA-ATG9A-BirA* BioID proteomics data with the highest p-value and fold-change. Normalized protein levels of HA-BirA* and HA-ATG9A-BirA* were utilized to calculate z-score.

Appendix Figure S2



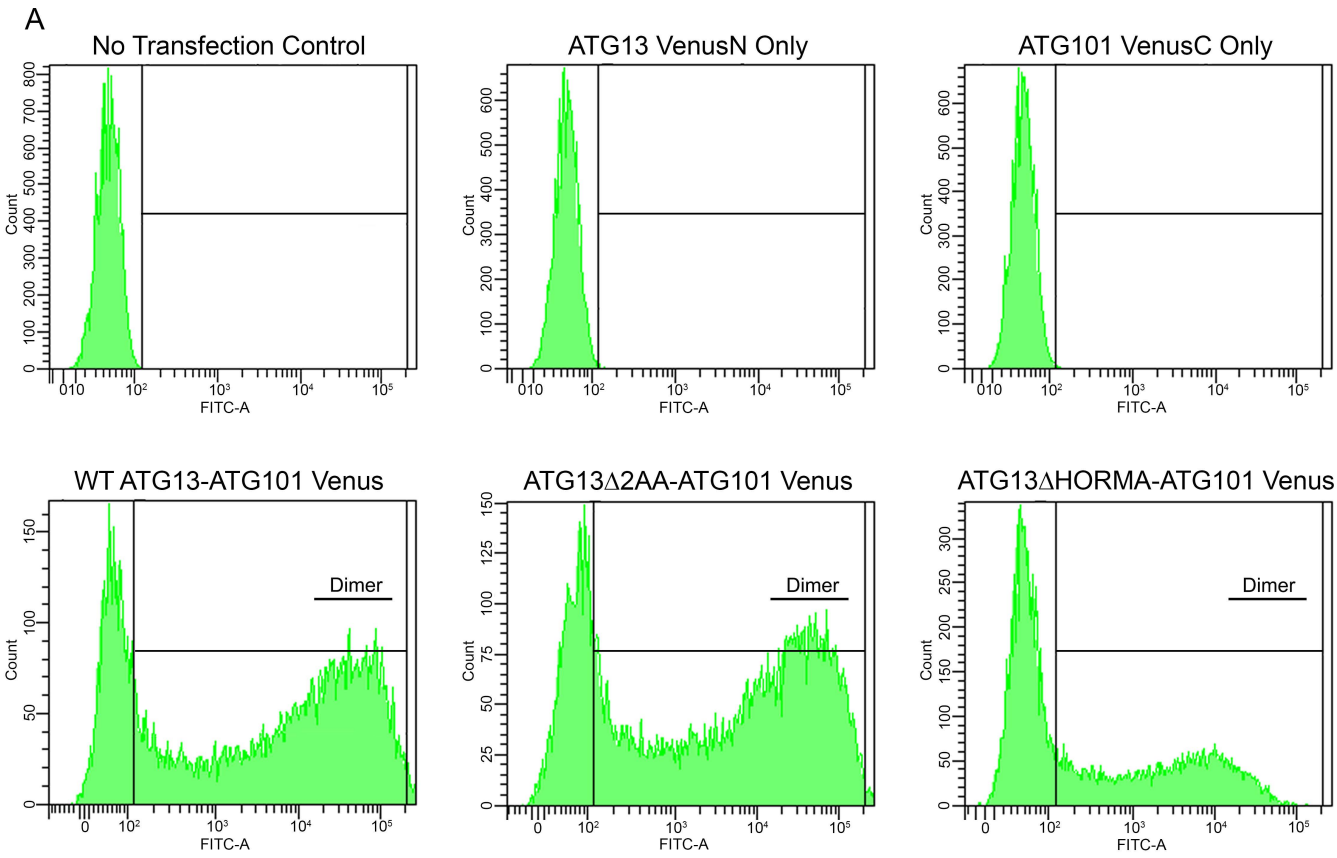
Appendix Figure S2: Validation of the HCT-116 ATG9A-HA KI cell line. (A) Schematic representation of the CRISPR/Cas9-mediated insertion of a 1x HA tag sequence at the C-terminal end of genomic *atg9a* (HCT-116 ATG9A-HA KI). Single cell-derived clones were validated by targeted deep sequencing. (B) The HCT-116 ATG9A-HA KI 2E6 clone was validated by knocking out the ATG9A locus with CRISPR/ Cas9 (clones 3, 6 and 8 were successful). Clones were validated by measuring the loss of HA signal by immunoblotting with indicated antibodies. (C) Confocal images of HCT-116 ATG9A-HA KI parental cell line or ATG9A KO cells generated in HCT-116 ATG9A-HI KI cell line by CRISPR/Cas9. Cells were grown in full DMEM media, fixed, labelled with HA and imaged (Scale bar, 10 µm).

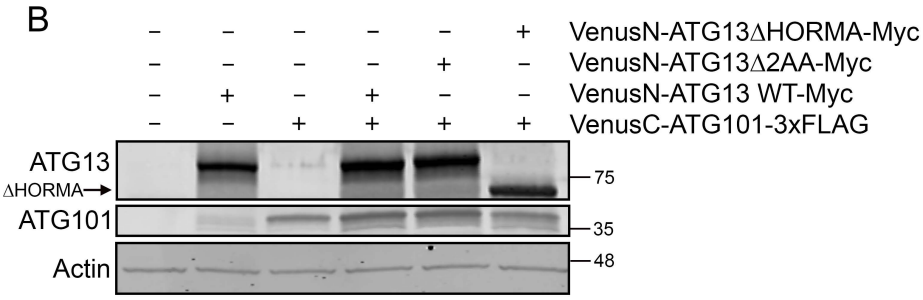
Appendix Figure S3

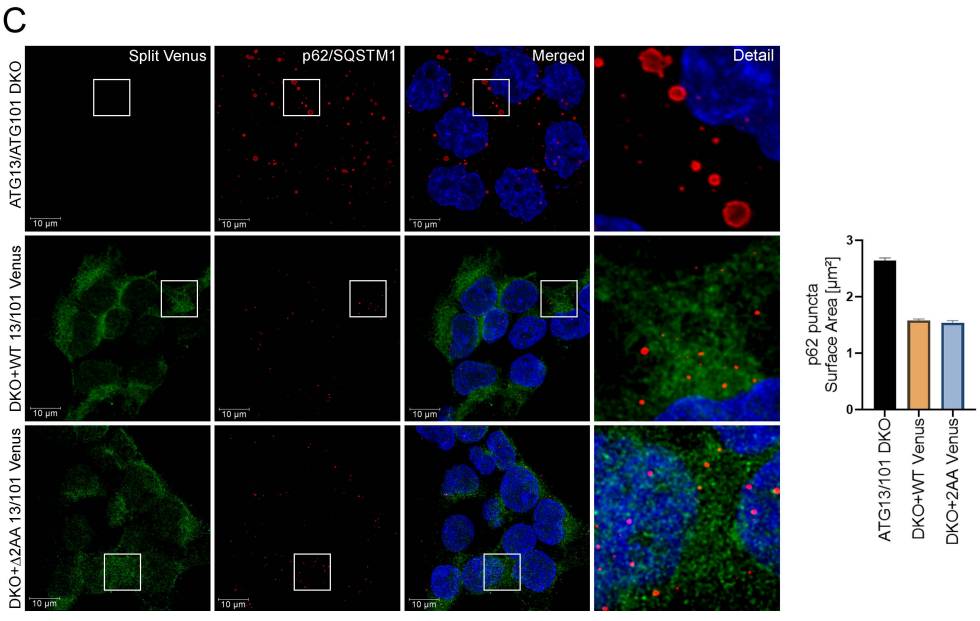


Appendix Figure S3: Loss of ATG13 and ATG101, but not FIP200, lead to the accumulation of ATG9A in large puncta. (A) Confocal images of ATG9A puncta in HCT-116 ATG9A-HA KI-ATG13 WT, ATG101 KO, FIP200 KO and ATG13 KO cells. Cells were grown in full DMEM media, fixed, labelled with HA and imaged (Scale bar=10 µm) (left). Quantification of average ATG9A puncta surface area. Mean ± SEM, n=1 independent experiments with 30 technical replicates. (B) Confocal images of ATG9A puncta in HCT-116 ATG9A-HA KI-ATG13 WT, ATG13 KO or ATG13 KO cells reconstitutes with ATG13 Δ2AA and ATG13 ΔHORMA. Cells were grown in full DMEM media, fixed, labelled with HA and imaged (Scale bar=10 µm) (left). Quantification of average ATG9A puncta surface area. Mean ± SEM, n=1 independent experiment with 30 technical replicates. Confocal images for A and B were obtained from the same single independent experiment as Figures EV4 and EV5.

Appendix Figure S4







Appendix Figure S4: Evaluation of a split mVenus system to capture the ATG13-ATG101 dimer. (A) HEK293T cells were overexpressed with VenusC-ATG101-3X FLAG only, VenusN-ATG13-Myc only, VenusN-ATG13 Δ2AA only, VenusN-ATG13 ΔHORMA or both Venus N and C halves together as indicated. Cells were grown in full DMEM media, trypsinized, separated in a cell strainer, and analyzed by flow cytometry without fixation. Gates, laser power, and detector were kept constant between samples. 10,000 cells displayed per histogram. (B) HEK29T cells in A were also collected and analyzed by western blot to show overall expression. (C) Confocal images of HCT116 ATG9A-HA KI ATG13-ATG101 double KO cells with or without stable reconstitution with VenusC-ATG101-3X FLAG and VenusN-ATG13-Myc or VenusN-ATG13 Δ2AA. Cells were grown in full DMEM media, fixed, and stained with p62/SQSTM1 antibody as indicated (Scale bar=10 μm). Quantification of average ATG9A puncta surface area. Mean ± SEM, n=1 independent experiments with 30 technical replicates.