Study sheds light on a mitochondrial disease

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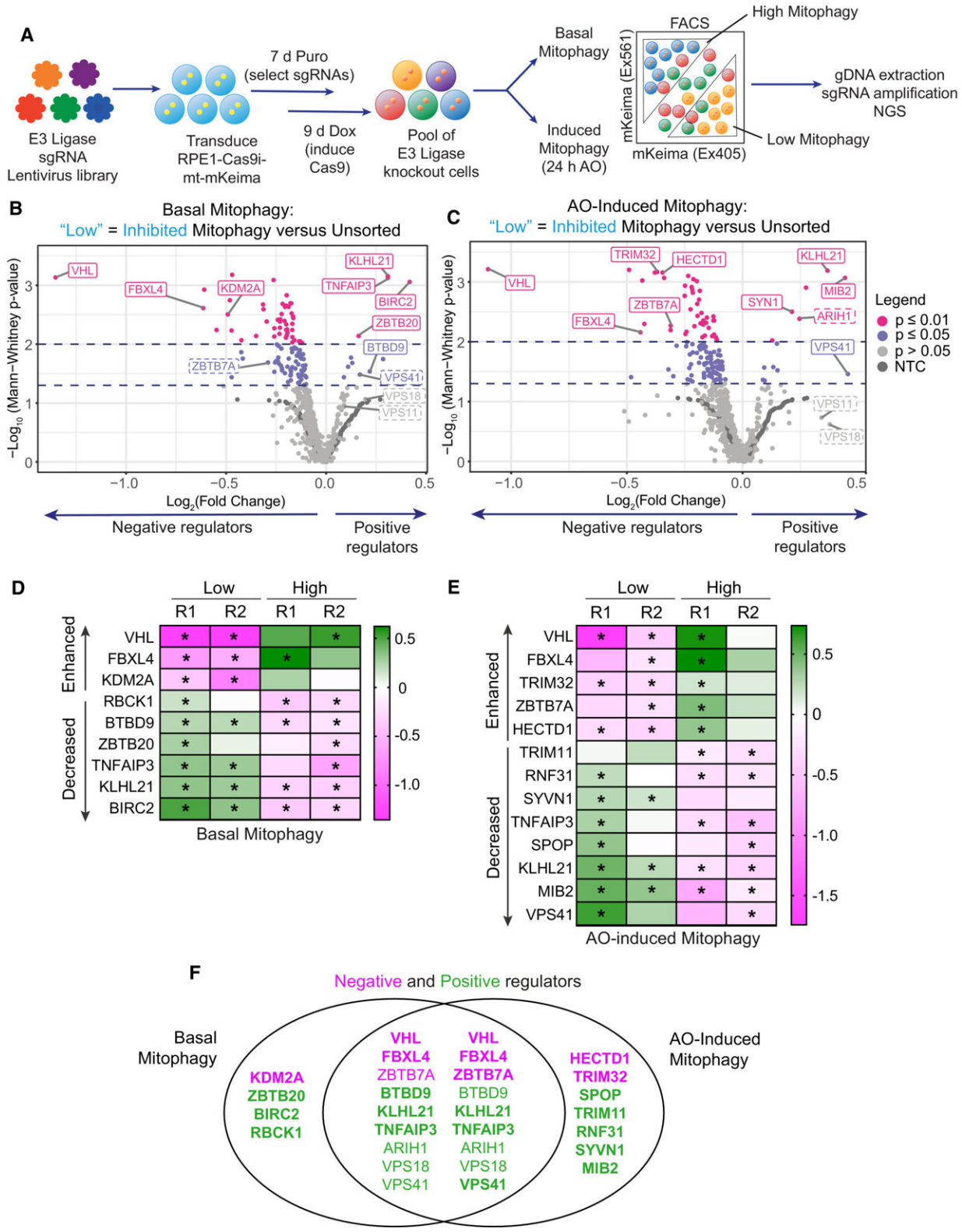

CRISPR screen targeting E3 ligases identifies major regulators of Parkin-

independent mitophagy A. Schematic depicting the CRISPR screening strategy. RPE1-Cas9i-mt-mKeima cells were transduced with a lentiviral CRISPR sgRNA library targeting 606 E3 ligases. sgRNA-expressing cells were selected for 7 days with puromycin (Puro) and Cas9 expression induced with doxycycline (Dox) for 9 days. Fourteen days post-transduction, half the cells were treated with antimycin and oligomycin (AO; 1 and 10 μM respectively) for 24 h, then sorted alongside untreated cells (basal mitophagy) by FACS into "high" and "low mitophagy" populations based on mt-mKeima fluorescence. Genomic DNA was extracted from sorted and unsorted reference cells, sgRNAs amplified by barcoded PCRs and samples analyzed by next-generation sequencing (NGS). B, C. Volcano plot showing the average $\log_2$ fold change and $-\text{Log}_{10}$ *P*-value of genes in low mitophagy versus unsorted cells in the AO-induced and basal mitophagy screen for two independent biological replicates. Statistical thresholds of 2 and 3 standard deviations from the mean are indicated by dashed lines and color coding. Indicated are high-confidence (unbroken line) and lower-confidence (dashed line) candidates shown in (D–F). D, E. High-confidence candidate list of positive (decreased) and negative (enhanced) regulators of Parkin-independent-induced mitophagy. Heatmap showing the Log_2 fold change of genes in low/high mitophagy versus unsorted cells in the induced and basal mitophagy screen for each of two independent biological replicates. Genes with *P*-values