## Robust differential expression testing for single-cell CRISPR screens

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## Typical differential expression analysis for single cell CRISPR screens Group cells based on target Infect cells with targeting (T) and non-targeting (NT) perturbations 3 Differential expression analyses for perturbation-gene pairs Multiple testing correction to obtain significant associations Gene 1 expr. Gene 2 expr. Observed p-value Significant NT3 p = 0.005= 0.6 -TЗ Non- ↓ significant NT3 10 p = 0.05p = 0.02p = 0.008= 0.2. $10^{-1}$ $10^{-2}$ $\widetilde{\mathsf{T3}}$ Expected null p-value b Undercover differential expression analysis for calibration assessment Differential expression analyses Multiple testing correction to for UC perturbation-gene pairs btain significant associations Label NT perturbations as Group cells based Label NT perturbations as Group cells based undercover (UC), one at a time on target (NT vs UC) Gene 1 expr. Gene 2 expr. NT1 Undercover T3 p = 0.005UC1 UC1 false positives NT2 Undercover Observed p-value Significant TЗ Non- ↓ significant 10 p = 0.08 - $10^{-2}$ $10^{-1}$ NT3 Undercover Expected null p-value $\widetilde{\mathsf{T3}}$ ŨC3 p = 0.25p = 0.2UC3 Frangieh (IFN- $\gamma$ ) data Papalexi data **d** 8000 C е 1000 Observed p-value Observed p-value 1000 False positives False positives 1e-06 1e-06 100 100 10 1e-03 1e-03 10 MIMOSCA MAST

Method

1e+00

1e-03

Expected null p-value

1e-05

Method

Seurat De KS test

1e-04

1e-02

Expected null p-value

1e+00

Figure 1: Insert caption here.