Supplementary Note 1 - Calculating Average Expression:

We average expression overall cells including those with zero reads for a particular gene which results in good fits for both Logistic and Michaelis-Menten models (**Figure 1, S3**). Whereas, in Peirson and Yau (2015)[1] average expression was calculated by averaging only over non-zero expression values. If there is a strong relationship between dropout rate and expression level then it implies that the presence of dropouts is indicative of low expression. Thus, excluding zeros when calculating average expression would lead to overestimation of the expression level for rarely detected genes. We also fit the three different models, Michaelis-Menten, Logistic, and double exponential, to the dropout rate vs average expression when calculated excluding zeros (**Figure S1**). Fitting quality was highly variable across datasets; for the UMI datasets only the logistic function fit well, whereas for Shalek, Pollen and Biase datasets the double exponential function was the best fit, followed closely by the Michaelis-Menten. In the Deng and Buettner datasets all three models fit reasonably well.

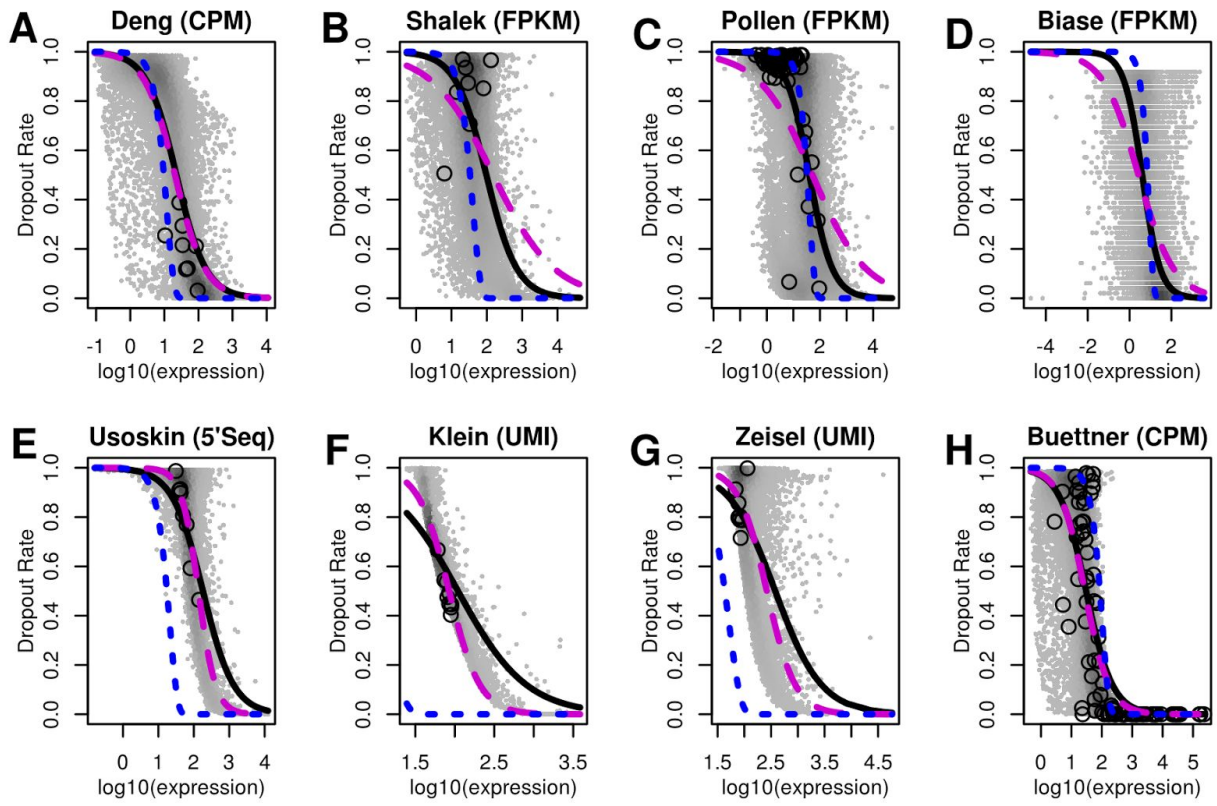


Figure S1 *The Michaelis-Menten (solid black), logistic (dashed purple), and double exponential (dotted blue) models fit to data when average expression is calculated using only expression values > 0. Black circles indicate spike-in RNAs.*

Supplementary Note 2 - Interpreting K_M

The fitted Michaelis constant (K_M) corresponds to the mean level of expression for a gene that is detected in 50% of cells. Thus, K_M is inversely related to the detection rate, and consequently samples with deeper sequencing have a lower K_M (Figure S2). In addition to being a function of the number reads, K_M is also related to the single-cell RNASeq method employed. Full transcript SMART-seq datasets had lower a K_M than the datasets which sequenced a single end of each transcript, while datasets employing UMIs had high K_M relative to the number of detected genes.

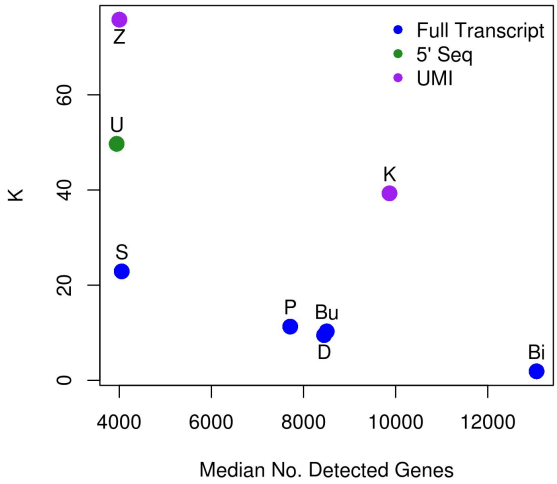


***Figure S2**: K_M is related to detection rate and sequencing method and a low value indicates fewer dropouts. Datasets are labelled by the first letter(s) of the author's name.*

Supplementary References

1. Pierson, E. & Yau, C. ZIFA: Dimensionality reduction for zero-inflated single-cell gene expression analysis. *Genome Biol.* **16,** 241 (2015).

Supplementary Tables

Table S1: Fitting the single (K_M) & double Michaelis-Menten (K_1, K_2)

	Buettner	Deng	Usoskin	Klein	Zeisel	Shalek	Pollen	Biase
K_M	10.3	9.5	49.7	39.3	75.8	22.9	11.3	1.9
K_1	9.7	7.3	48.5	39.4	72.4	18.7	11.3	2.3
K_2	$5x10^{-4}$	$9x10^{-4}$	$4x10^{-4}$	$9x10^{-4}$	1.09	$9x10^{-4}$	$3x10^{-4}$	$8x10^{-4}$

Table S2: *Groups used for pairwise differential expression analysis*

Buettner	**Shalek**	**Deng**	**Usoskin***	**Klein****	**Zeisel**	**Pollen**	**Biase**
G1	Unstimulated	2-4 cell	NP	Day 0	Neuron	Skin	zygote
S	4h LPS	8-16 cell	TH	Day 2	Glia	Blood	2 cell
G2/M		blast	NF	Day 4	Microglia	hiPSC	4 cell
			PEP	Day 7	Non-Neuro	Neuronal	ICM
							TE

**NP = nonpeptidergic nociceptors, TH = tyrosine hydroxylase expressing, NF = neurofilament expressing, PEP = peptidergic nociceptors*
***ESCs following LIF withdrawal*

Supplementary Figures

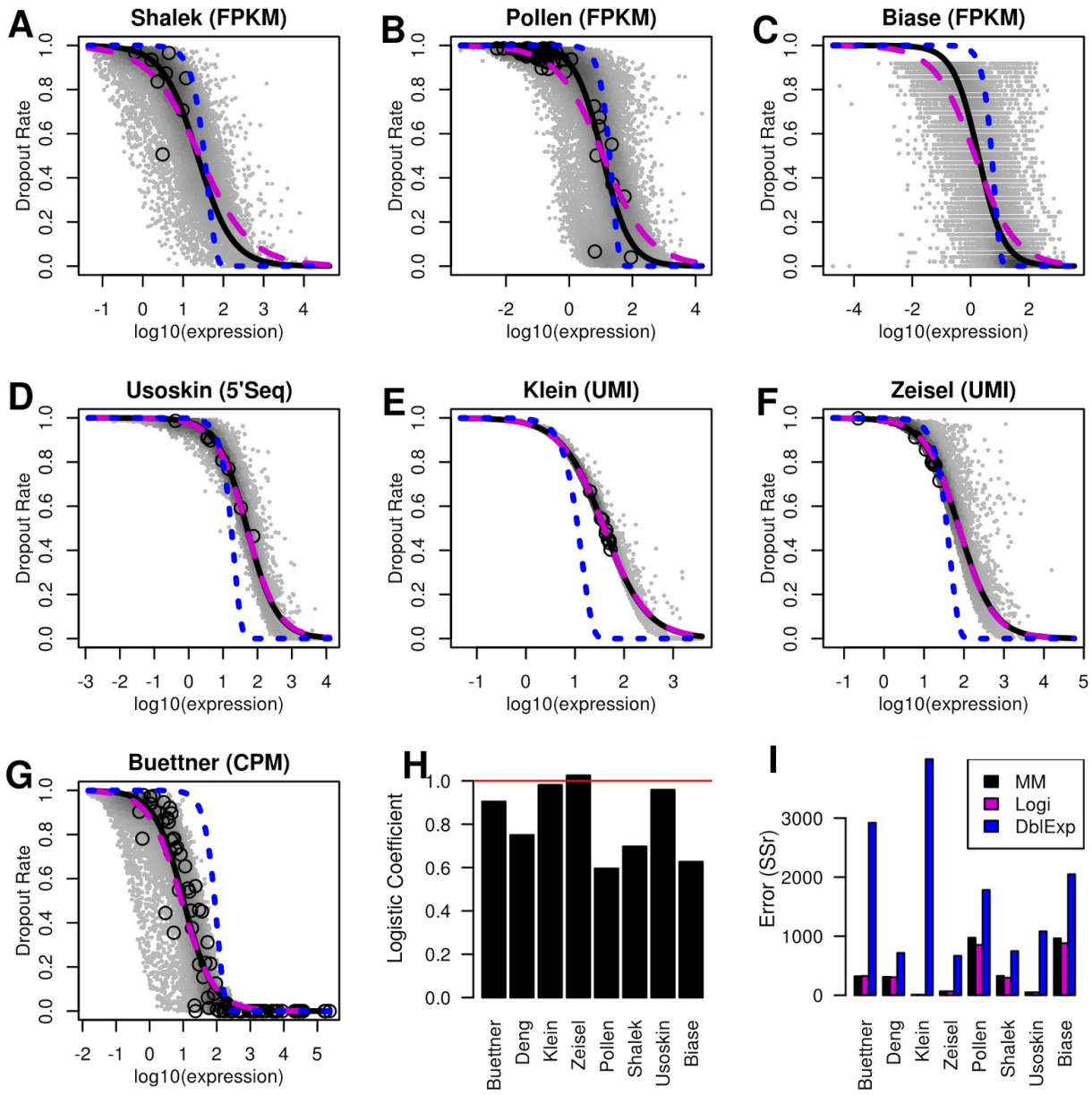


Figure S3 (A-G)The Michaelis-Menten (solid black), logistic (dashed purple), and double exponential (dotted blue) models are fit to the other five published datasets. Black circles indicate spike-in RNAs. (H) Due to noise in the data the logistic regression fits a coefficient < 1 for most datasets giving a flatter curve compared to Michaelis-Menten. (I) Logistic regression had the smallest sum of squared residuals (SSr) across all eight datasets considered.

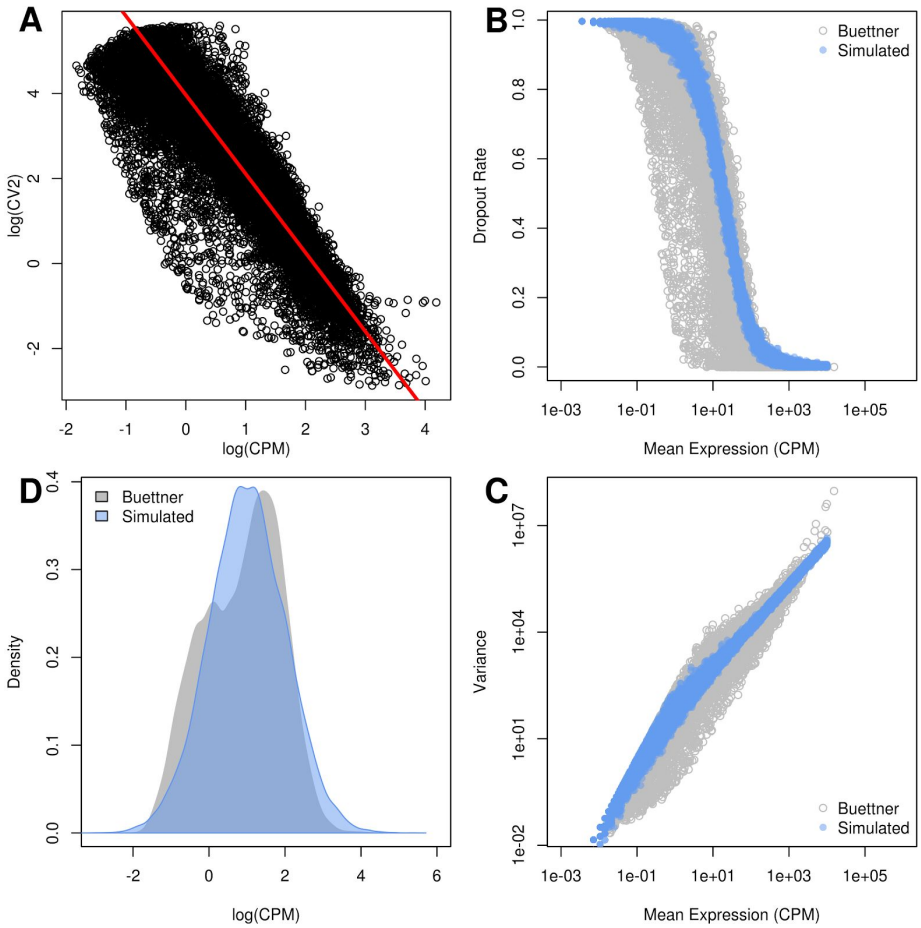


Figure S4: (A) relationship fit between dispersion (CV^2) and mean CPM-normalized expression. (B-C) Comparison of observed Buettner data with simulated single-cell data prior to addition of DE genes. (D) distribution of mean expression values observed in the Buettner data was approximate with a log-normal distribution (mu = 1, sd = 1).

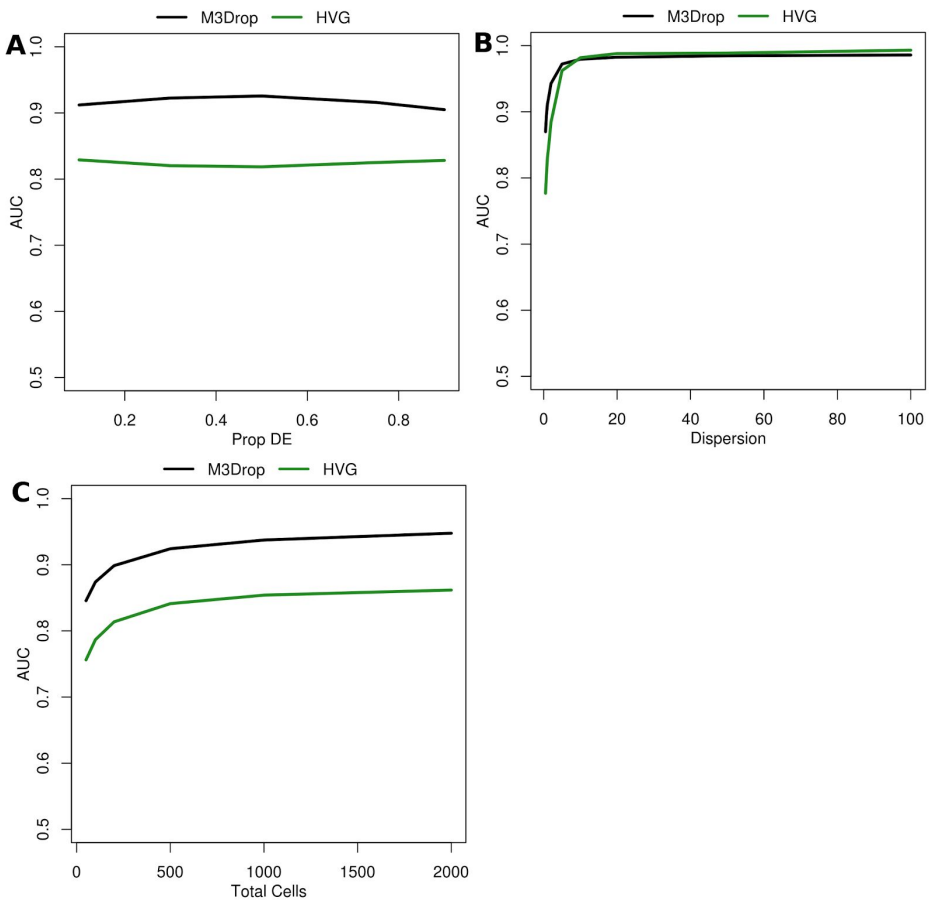
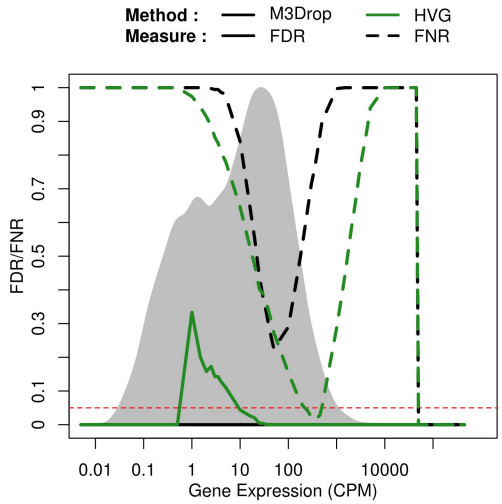


Figure S5: Effect of changing the proportion of genes which are DE (A), overall dispersion (B) and total number of cells (C) on the performance of M3Drop (black) and HVG (green).



Figures S6: Quality of calls for M3Drop and HVG for genes 3-fold more variable in all 300 cells across a range of expression values. Grey indicates distribution of observed mean expression

values in Buettner dataset. Red dashed line indicates FDR used in multiple-testing correction. Note that the solid black line is at 0 for all expression levels.

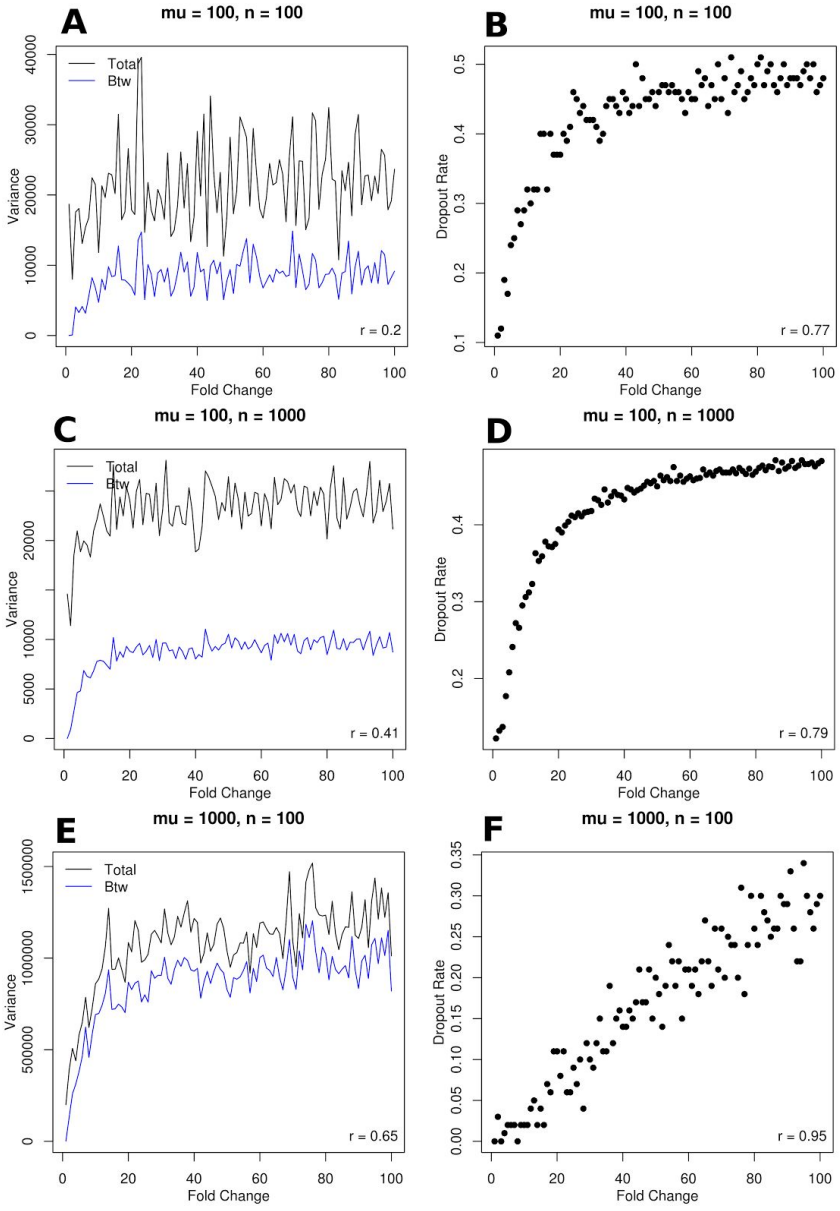


Figure S7: Relationship between fold-change in mean-expression and observed sample variance (left column) or dropout rate (right column) for a sample of “n” cells with 50% high expression, 50% low expression and an overall mean of “mu”. Btw is the amount of variance between the two subpopulations. The relationship between fold change and dropout rate/variance in each panel can be summarized by the Pearson correlation coefficient and it is reported in **Figure S8**.

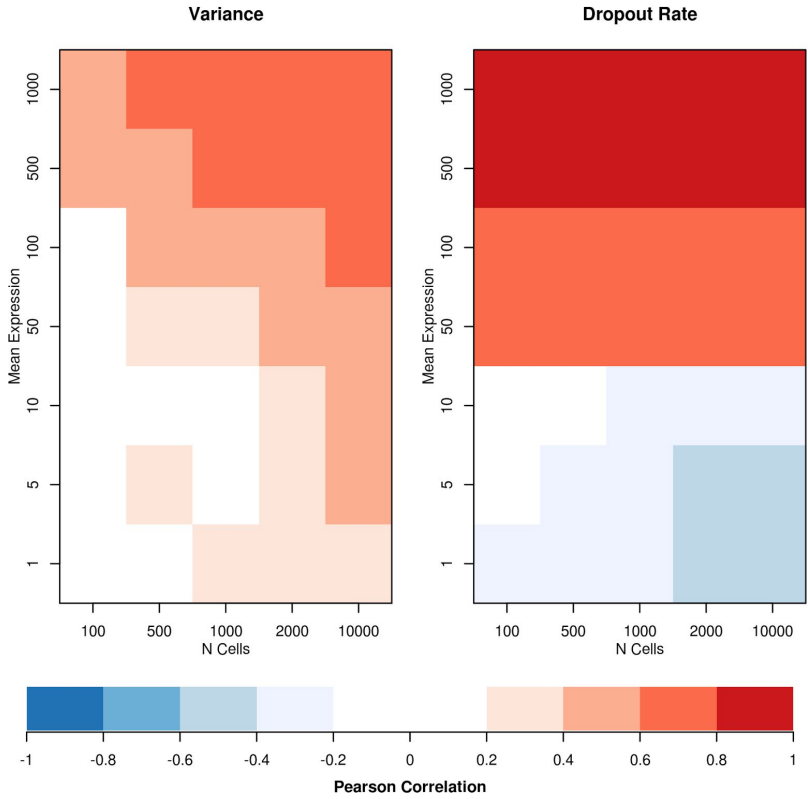


Figure S8: Pearson correlation between fold change in mean-expression and variance (left) or dropout rate (right) for different combinations of “n” (number of cells) and “mu” (mean expression).

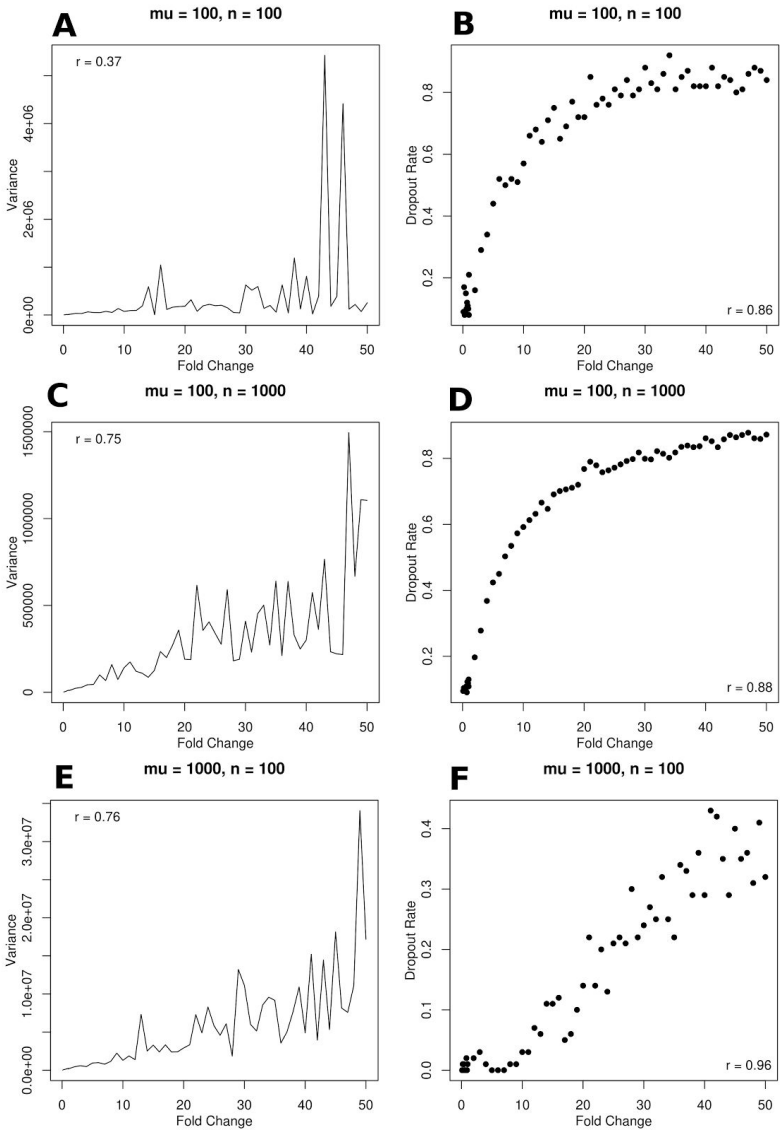


Figure S9: Relationship between fold change in overall variability relative to the fitted relationship (Figure S6) and observed sample variance (left column) or dropout rate (right column) for different combinations of “n” (number of cells) and “mu” (mean expression). The relationship between fold change of the variance and dropout rate/variance in each panel can be summarized by the Pearson correlation coefficient and it is reported in **Figure S10**.

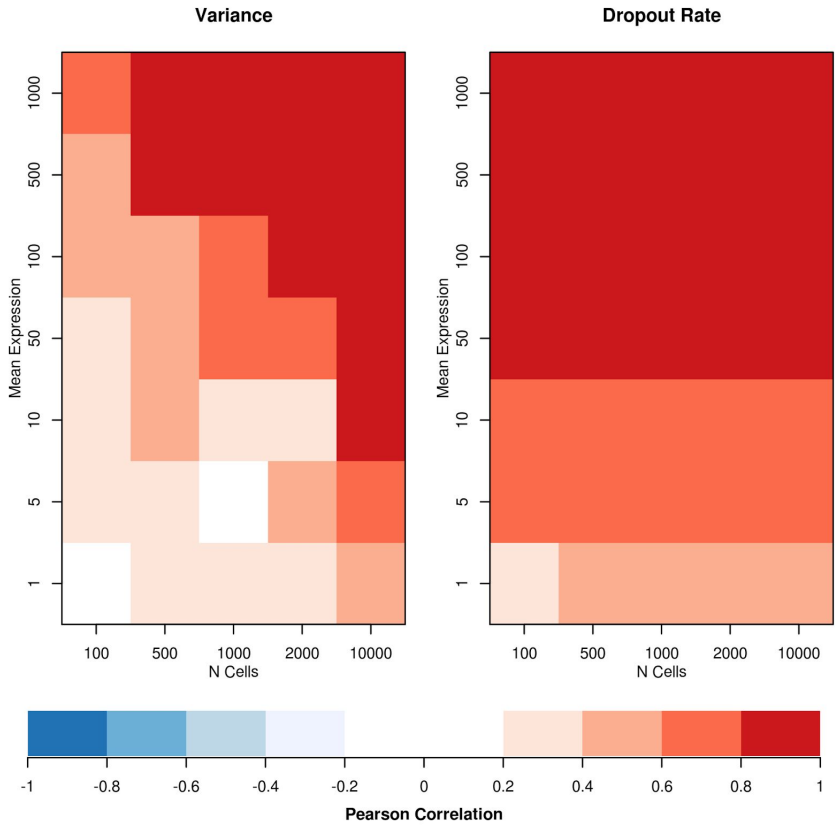


Figure S10: Pearson correlation between fold change in overall variability relative to the fitted relationship (Figure S5A) and variance (left) or dropout rate (right) for different combinations of “n” (number of cells) and “mu” (mean expression).

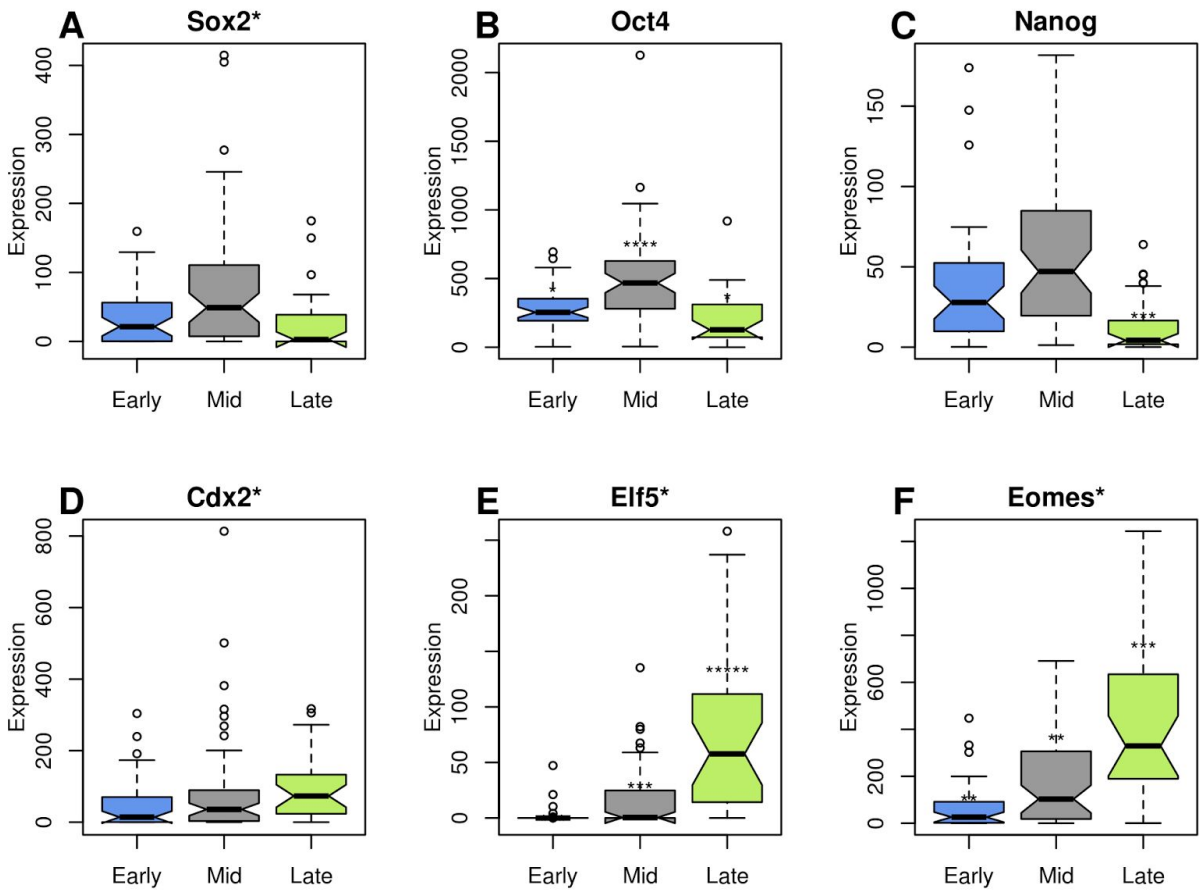


Figure S11 (A-C) Expression of markers of the inner cell mass (ICM) for the three blastocyst timepoints. (D-F) Expression of markers of the trophectoderm (TE), Only the late blastocyst timepoint is significantly different from the other timepoints for multiple markers. This is due to a higher proportion of TE cells (**Figure S12**). Stars indicate order of magnitude of significant differences from other groups from a Wilcox rank-sum test (one star indication $p < 0.01$).

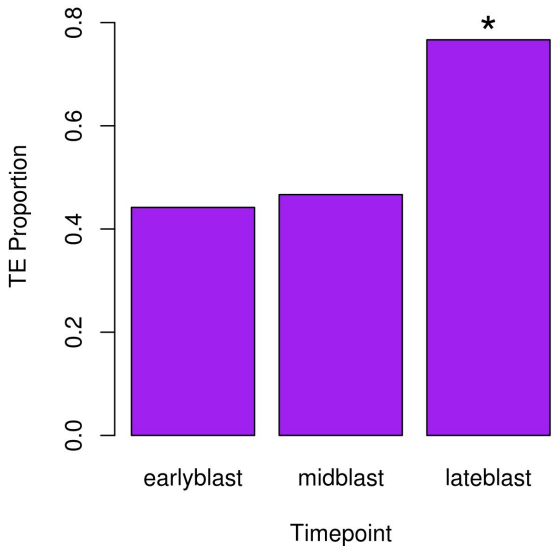


Figure S12 Late blastocysts were significantly enriched in TE (Group 4) cells compared to early and mid stage blastocysts. Star indicates $p < 0.01$ using Fisher's exact test.

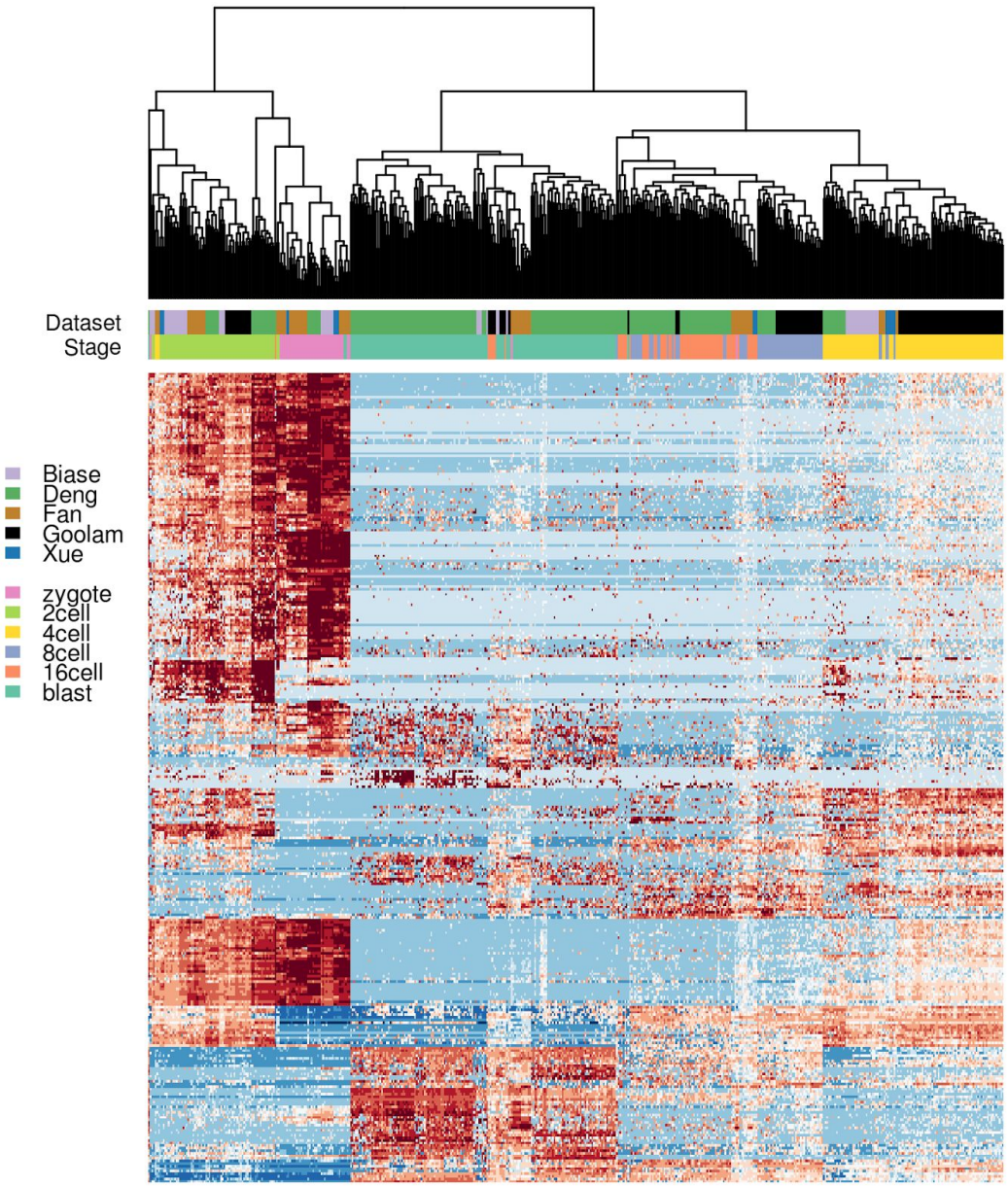


Figure S13: M3Drop marker genes makes it possible to merge datasets from different experiments. Expression of 316 differentially expressed using M3Drop genes seen in both Deng and Biase datasets across five mouse preimplantation datasets.

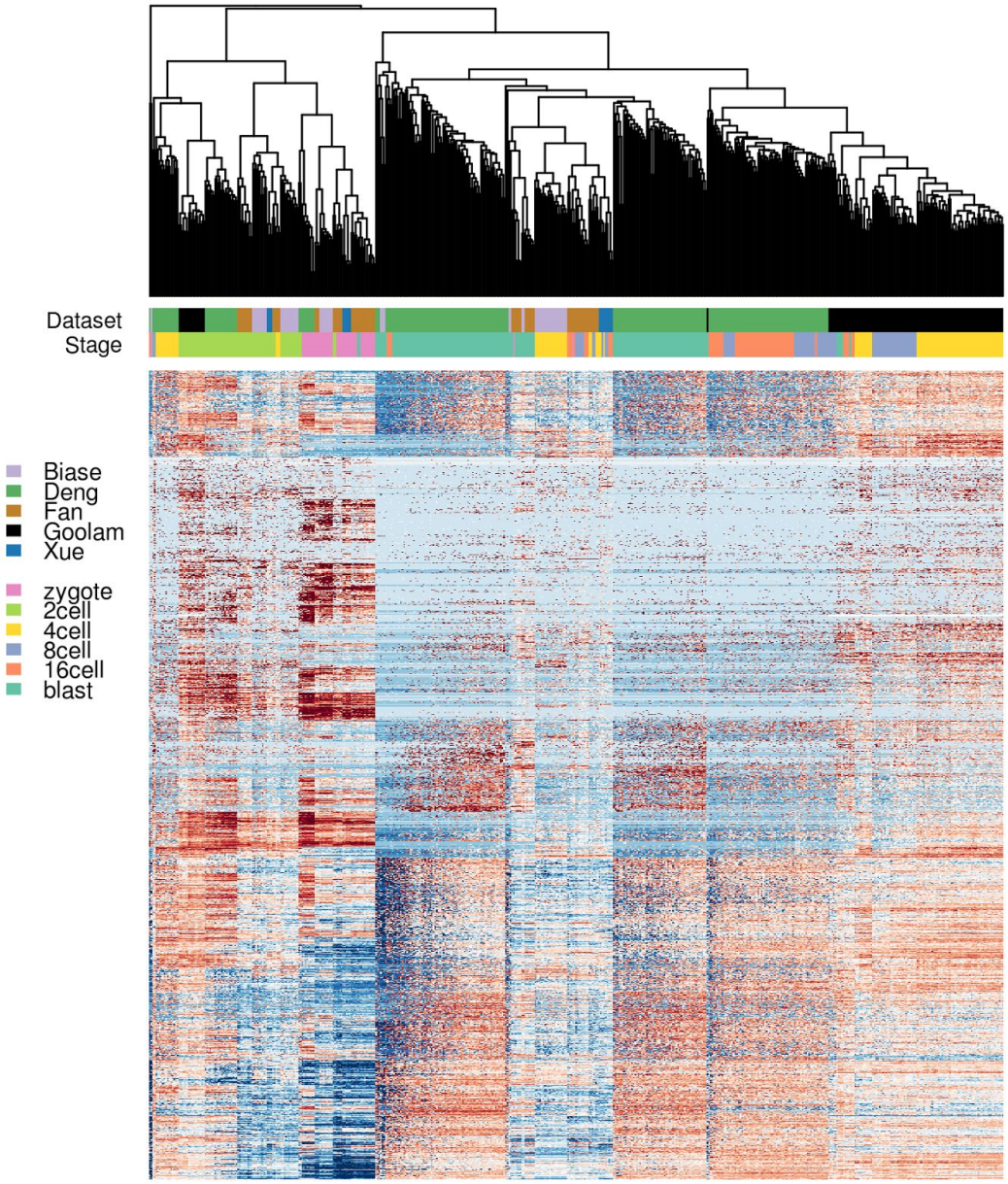


Figure S15: Expression of all 11,440 genes detected in all datasets across five mouse preimplantation datasets.

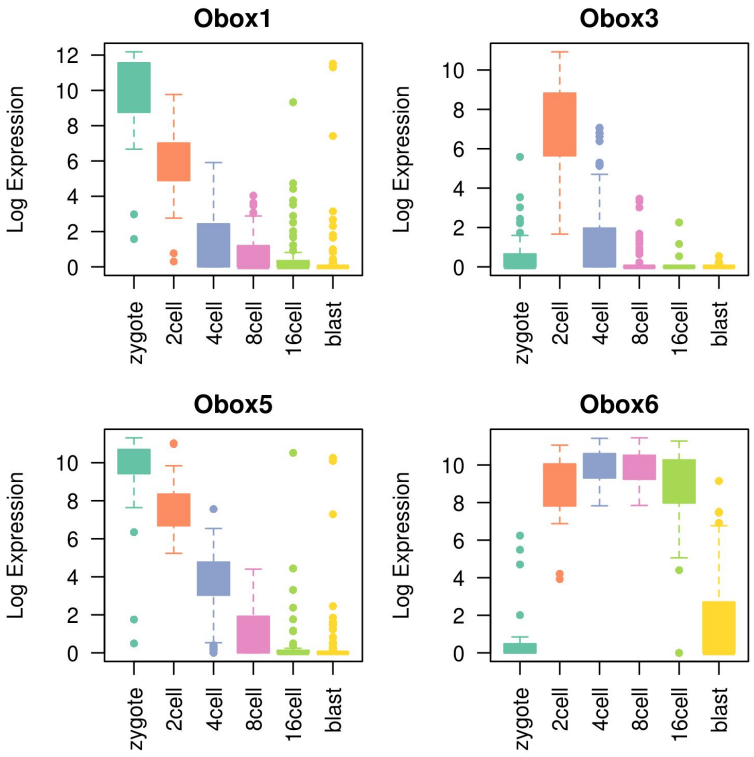


Figure S16: Expression of Obox genes across developmental stages for five mouse preimplantation datasets.