Supplementary

Table S1 : Tabula Muris permuted datasets.

Tissue	Technology	Cells (filtered)	Closest cell-types	Permuted Genes	Best Fit Model*
Heart	Smartseq2	4098	Smooth muscle cell Endothelial cell	2912	ZINB: 99% ZILN: 0%
Kidney	Smartseq2	517	Fenestrated cell Endothelial cell	5728	ZINB: 77% ZILN: 22%
Pancreas	Smartseq2	1204	Pancreatic PP cell Pancreatic A cell	7300	ZINB: 95% ZILN: 0%
Trachea	Smartseq2	813	Leukocyte Epithelial cell	4156	ZINB: 97% ZILN: 3%
Lung	Smartseq2	1198	Stromal cell Endothelial cell	1862	ZINB: 91% ZILN: 1%
Bladder	Smartseq2	1287	Bladder cell Basal cell of urothelium	5460	ZINB: 97% ZILN: 1%
Muscle	Smartseq2	1831	Skeletal muscle stem cell Skeletal muscle cell	2665	ZINB: 99% ZILN: 0%
Tongue	Smartseq2	1394	Keratinocyte Basal cell of epidermis	3600	ZINB: 65% ZILN: 0%
Liver	Smartseq2	646	Kupffer cell Endothelial cell hepatic sinusoid	5288	ZINB: 80% ZILN: 20%
Mammary	Smartseq2	2255	Basal cell Luminal epithelial cell	2335	ZINB: 70% ZILN: 0%
Marrow	Smartseq2	4442	Neutrophil Granulocyte	1359	ZINB: 97% ZILN: 0%
Skin	Smartseq2	2218	Epidermal cell Basal cell of epidermis	4417	ZINB: 95% ZILN: 1%
Mammary	10X	4295	T cell B cell	4295	ZINB: 99% ZILN:0%

Bladder	10X	2375	Bladder cell Basal cell of urothelium	6239	ZINB: 90% ZILN:0%
Muscle	10X	4224	T cell B cell	3260	ZINB: 100% ZILN: 0%
Marrow	10X	3285	Hematopoietic stem cell B cell	2243	ZINB: 81% ZILN: 0%
Kidney	10X	2447	Macrophage Fenestrated cell	3630	ZINB: 99% ZILN: 0%
Lung	10X	4247	Stromal cell Endothelial cell	1936	ZINB: 90% ZILN: 0%
Tongue	10X	7501	Keratinocyte Basal epidermal cell	839	ZINB:43% ZILN: 0%

*Proportion of permuted (not DE) genes that were better fit by either a zero-inflated negative binomial distribution (ZINB), or a zero-inflated log-normal distribution (ZILN). Missing percentages are genes that didn’t fit either distribution.

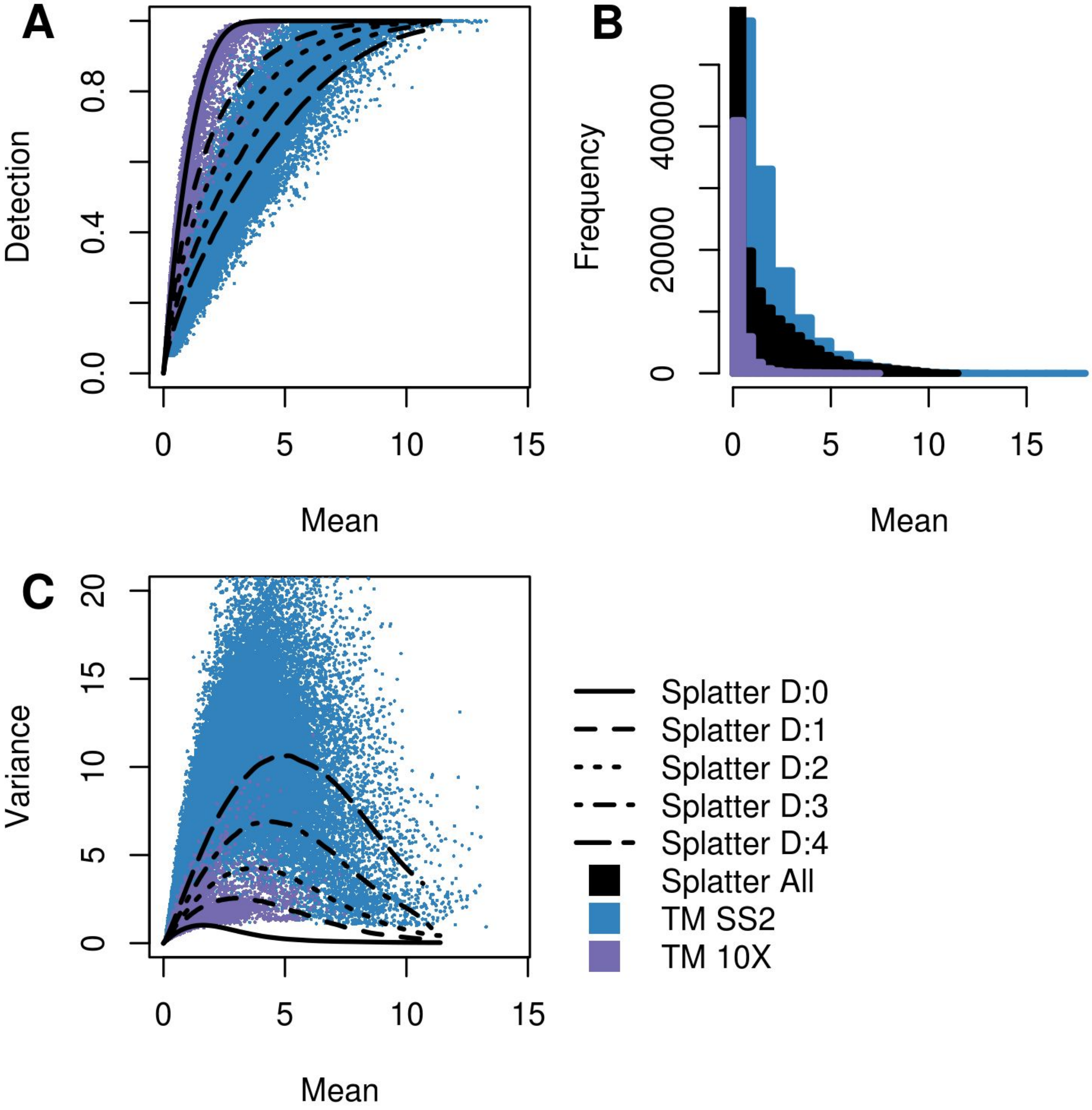


Figure S1 Comparison of Splatter simulations and real scRNA-seq data. Smartseq2 (blue) and 10X Chromium (purple) scRNA-seq data from the Tabula Muris dataset. Both were normalized and log2 transformed using scater. Results for splatter datasets with different values for the dropout-rate parameter: D, were spline-smoothed to generate curves. (A) Detection rate vs mean expression of genes across all cell-types in each tissue. (B) Distributions of mean expression levels. (C) Variance vs mean expression of genes.

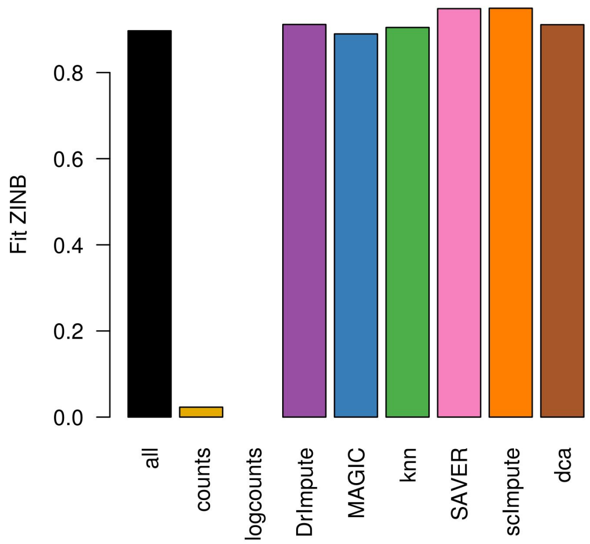


Figure S2 : **Imputed false differential expression is not due to genes violating a negative binomial.** Proportion of false-positive Tabula Muris markers that fit a zero-inflated negative binomial (ZINB) distribution better than a zero-inflated log-normal (ZILN) distribution. “All” indicates the proportion of all genes that were fit better by the ZINB distribution.

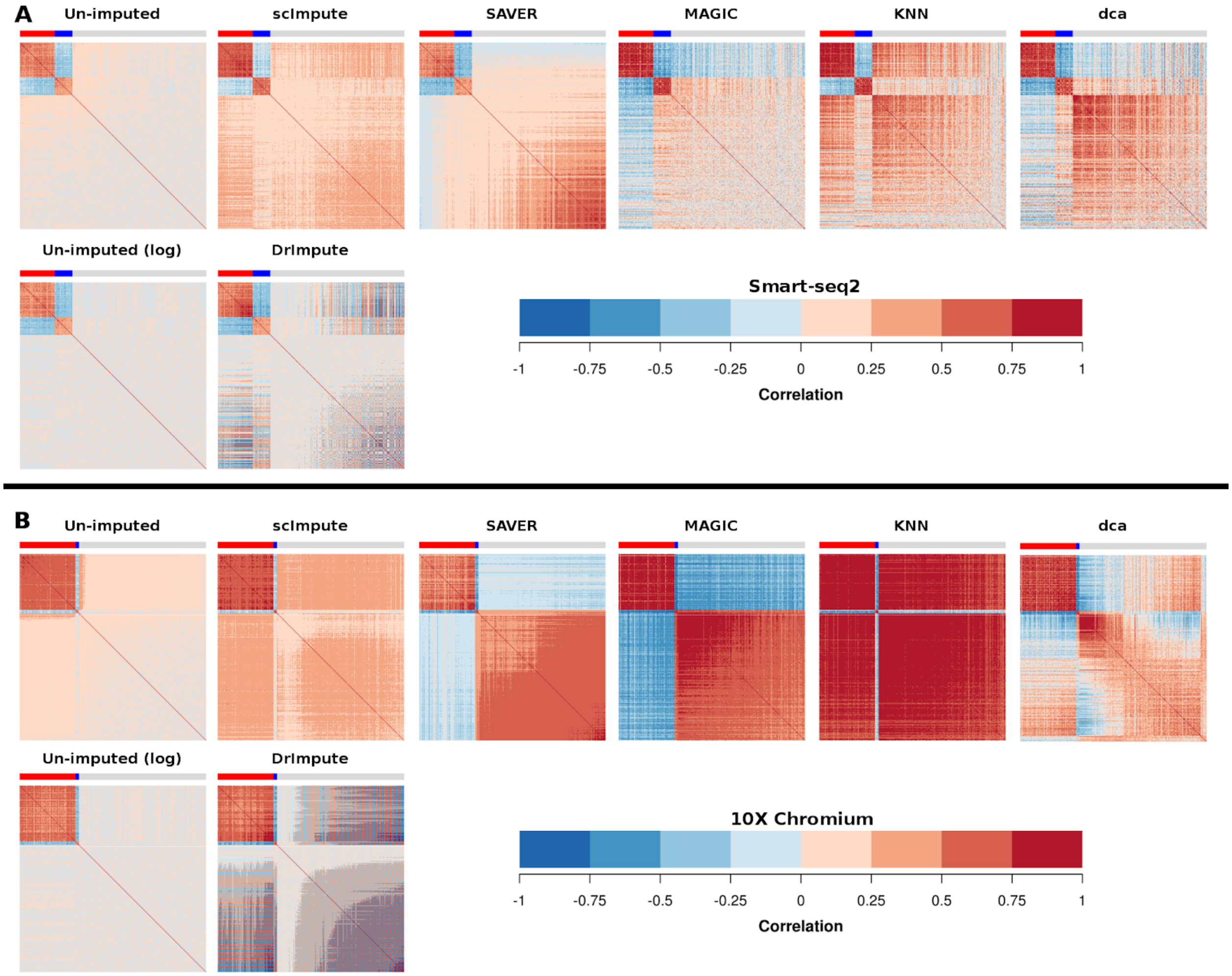


Figure S3: False gene-gene correlations induced by single-cell imputation methods. Gene-gene Spearman correlations between genes in permuted mouse tongue before and after imputation using suggested parameter values. Coloured bars indicate marker genes (AUC > 0.75, FDR 5%) for the two different cell-types (red & blue) or permuted genes (grey). Genes are ordered left to right by DE direction then by expression level (high to low). For visualization, 500 genes were randomly subset from Smart-seq2 (A) and 10X Chromium (B) ensuring at least 50% were the permuted genes.

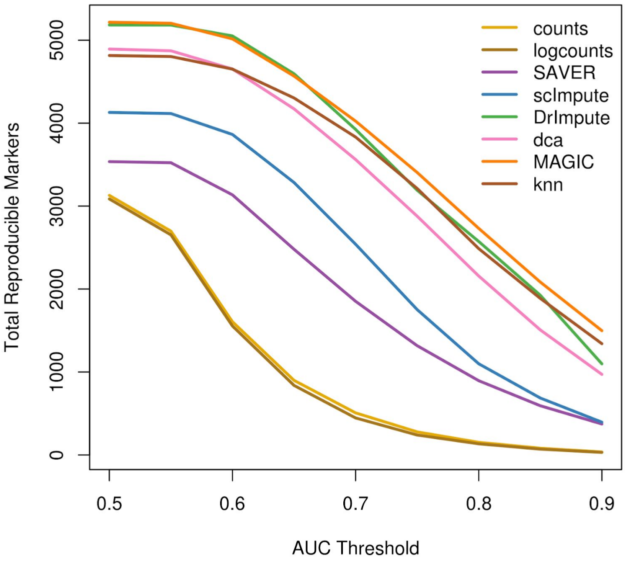
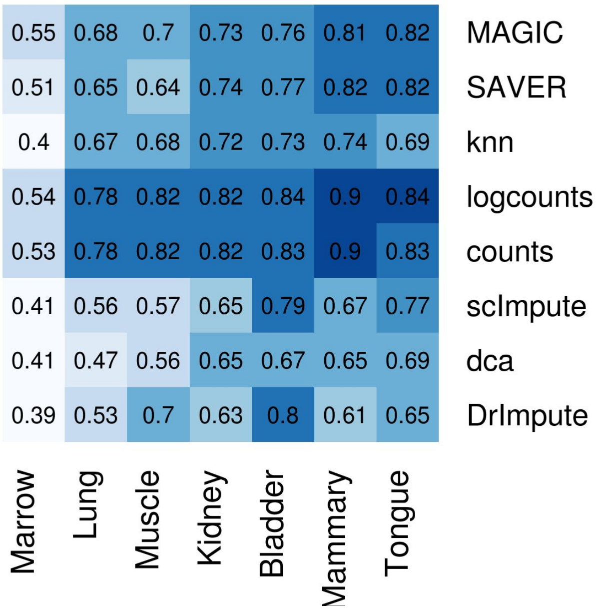
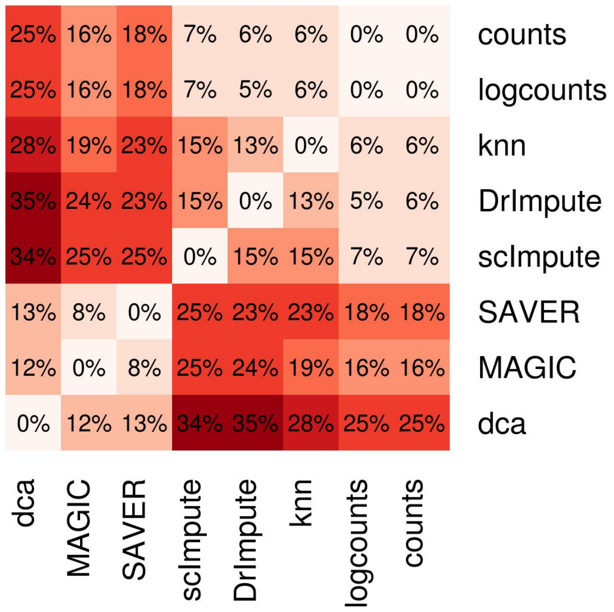


Figure S4: Absolute number of reproducible markers before and after imputation averaged across all Tabula Muris datasets. Markers were identified using a Mann-Whitney-U test, FDR 5%, and requiring an AUC score for classifying cells into the respective cell-type above a particular threshold. Genes identified as markers of the same cell-type in both Smart-seq2 and 10X datasets were considered reproducible.



Marrow	Lung	Muscle	Kidney	Bladder	Mammary	Tongue	
0.55	0.68	0.7	0.73	0.76	0.81	0.82	MAGIC
0.51	0.65	0.64	0.74	0.77	0.82	0.82	SAVER
0.4	0.67	0.68	0.72	0.73	0.74	0.69	knn
0.54	0.78	0.82	0.82	0.84	0.9	0.84	logcounts
0.53	0.78	0.82	0.82	0.83	0.9	0.83	counts
0.41	0.56	0.57	0.65	0.79	0.67	0.77	scImpute
0.41	0.47	0.56	0.65	0.67	0.65	0.69	dca
0.39	0.53	0.7	0.63	0.8	0.61	0.65	DrImpute

Figure S5: Concordance of markers test results across Tabula Muris datasets. Spearman correlations between marker p-values in matching 10X Chromium and Smart-seq2 datasets.



	dca	MAGIC	SAVER	scImpute	DrImpute	knn	logcounts	counts
counts	25%	16%	18%	7%	6%	6%	0%	0%
logcounts	25%	16%	18%	7%	5%	6%	0%	0%
knn	28%	19%	23%	15%	13%	0%	6%	6%
DrImpute	35%	24%	23%	15%	0%	13%	5%	6%
scImpute	34%	25%	25%	0%	15%	15%	7%	7%
SAVER	13%	8%	0%	25%	23%	23%	18%	18%
MAGIC	12%	0%	8%	25%	24%	19%	16%	16%
dca	0%	12%	13%	34%	35%	28%	25%	25%

Figure S6: Contradictory marker directions between imputation methods. Proportion of significant markers (FDR 5%) that were assigned to different cell-types by different imputation methods in the same Tabula Muris dataset. Results were averaged across all Tabula Muris datasets.