

Supplementary Notes

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# Comparison results

## S1.1 ARI

We use “adjustedRandIndex” in R (‘cidr’ package) to calculate Adjusted Rand Index (ARI). “OTHER” means “Non-reference”

**Supplementary Table 1 | ARI-Zeisel**

	pagoda2_inform	pagoda2_multilevel	SC3	CIDR	Seurat	tSNE	SAFE	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.235692663	0.590718097	0.424669823	0.367248095	0.180596042	0.862302873	0.718785282	0.4587997	0.4977975	0.4741055	0.5053194	0.5578852
Neuron merged	0.112323793	0.321984262	0.211593099	0.62575058	0.166885822	0.52733821	0.426741839	0.8210009	0.8626126	0.8495579	0.8624451	0.917321
Neuron removed	0.1739433	0.5899641	0.3259773	0.6563953	0.2497052	0.766662	0.4948344	0.6813724	0.8462789	0.8013861	0.8575611	0.9133551

**Supplementary Table 2 | ARI-Tasic**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.2063102	0.2139046	0.2195942	0.2178078	0.218299
Neuron merged + OTHER removed	0.9632103	0.987294	0.9990817	0.9911415	0.9956251
Neuron removed + OTHER removed	0.778748	0.8610543	0.9020829	0.8749216	0.9428578

**Supplementary Table 3 | ARI-Habib**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.8462835	0.9098513	0.8560124	0.9152363	0.9406015
OTHER removed	0.9830895	0.995678	0.9943954	0.9961101	0.9948532
Neuron removed + OTHER removed	0.8519236	0.9700085	0.8864094	0.97641	0.9435471

**Supplementary Table 4 | ARI-Hochgerner**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.1320347	0.1651151	0.1530798	0.1671729	0.1854772
Merged + OTHER removed	0.7888407	0.9600862	0.957464	0.950519	0.9086574
Merged + Neuron removed + OTHER removed	0.5974354	0.8854013	0.7476373	0.877548	0.8857942

## S1.2 NMI

We use “NMI” in R (‘NMI’ package) to calculate Normalized Mutual Information (NMI).

**Supplementary Table 5 | NMI-Zeisel**

	pagoda2_inform	pagoda2_multilevel	SC3	CIDR	Seurat	tSNE	SAFE	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.584084877	0.699891534	0.639689618	0.47436346	0.360502588	0.814605826	0.697543949	0.5415847	0.631015	0.6096761	0.63135	0.6895258
Neuron merged	0.445871248	0.547903315	0.478455719	0.579934692	0.4063472	0.647942782	0.546309753	0.6572838	0.7720847	0.7460005	0.7714603	0.8373372
Neuron removed	0.5951252	0.7446338	0.5087087	0.6114579	0.5191517	0.7116153	0.5653107	0.4995832	0.702085	0.6362314	0.7113909	0.7955889

**Supplementary Table 6 | NMI-Tasic**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
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Original	0.4535323	0.4800227	0.4972161	0.4853185	0.4996632
Neuron merged + OTHER removed	0.8964497	0.9317615	0.9693537	0.9412241	0.9706097
Neuron removed + OTHER removed	0.8024618	0.8468327	0.9035316	0.862485	0.9343078

**Supplementary Table 7 | NMI-Habib**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.6880635	0.8247804	0.7485723	0.8320144	0.8733894
OTHER Removed	0.9165942	0.9776861	0.9582753	0.9805898	0.9708615
Neuron removed + OTHER removed	0.8289337	0.9560601	0.8919929	0.9635546	0.9368758

**Supplementary Table 8 | NMI-Hochgerner**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Original	0.3528099	0.46532	0.4422228	0.4561949	0.499306
Merged + OTHER removed	0.6654204	0.8837054	0.8433564	0.8652991	0.8310937
Merged + Neuron removed + OTHER removed	0.5565025	0.8295062	0.6925935	0.8125795	0.8440822

### S1.3 Precision & Recall

For precision and recall, we only show the result of scRef::kendall and scRef::semi-supervised in Zeisel test data (**Supplementary Table 9**). For the precision and recall of other test data, readers can calculate them by using the results of scRef. Users can download the results of scRef from:

<https://github.com/jumphone/scRef/tree/master/Benchmark>

**Supplementary Table 9 | Precision&Recall-Zeisel**

	scRef::kendall		scRef::semi-supervised	
	Precision	Recall	Precision	Recall
Astrocytes	94.44%	75.89%	96.41%	83.93%
Endothelial.Cells	88.18%	76.17%	89.18%	87.66%
Microglia	100.00%	66.33%	100.00%	71.43%
Oligodendrocytes	94.10%	97.32%	95.18%	98.78%
Neuron	94.32%	97.97%	97.91%	97.79%

### S1.4 Running time

For methods which can use multicores, we use 10 threads to run. The CPU of used server is Intel(R) Xeon(R) CPU E5-2650 v3 @ 2.30GHz. This result doesn't include the time cost of loading data.

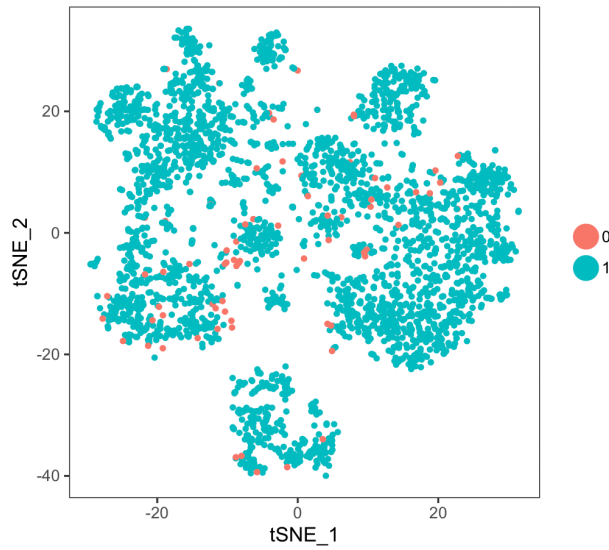
**Supplementary Table 10 | Running time-Zeisel**

	pagoda2_infomap	pagoda2_multilevel	SC3	CIDR	Seurat	tSNE	SAFE	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
Time (minute)	<1	<1	32	2	2	18	61	<1	<1	<1	<1	<1

### S1.5 Annotation error analysis

1 We have analyzed the annotation error in Zeisel's data: We define cells having wrong annotation in all  
2 five methods of scRef as "Error", and define cells having at least one correct annotation as "Correct".  
3 75 out of 3005 cells are Errors. tSNE plot shows that Errors are randomly distributed in different cell  
4 types (**Supplementary Figure 1**). In addition, we find that Errors occupy a large proportion in the  
5 cells having wrong labels of each measurements (see **Supplementary Table 11** and **Supplementary**  
6 **Figure 2**). Finally, by comparing the sequenced gene number between Errors and Corrects, we find  
7 that the wrong annotation of Errors may be caused by the low sequence depth of these cells  
8 (**Supplementary Figure 3**).

10 **Supplementary Figure 1 | "0" means Error; "1" means Correct.**



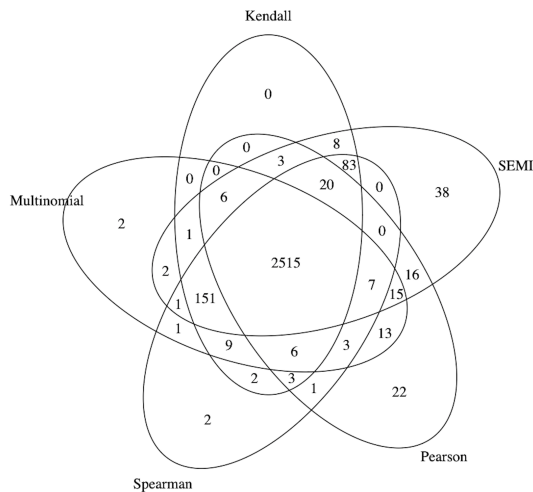
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12 **Supplementary Table 11 | Errors in five methods of scRef**

	scRef::pearson	scRef::spearman	scRef::multinomial	scRef::kendall	scRef::semi-supervised
#correct cells	2630	2804	2732	2807	2866
#wrong cells	375	201	273	198	139
#Errors / #wrong cells	20.00%	37.31%	27.47%	37.88%	53.96%

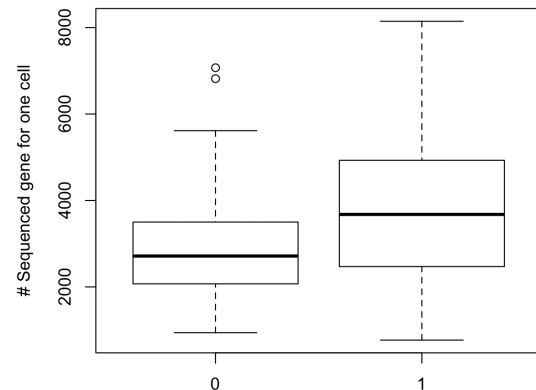
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14 **Supplementary Figure 2 | Venn plot of correct cells**



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## Supplementary Figure 3 | Number of sequenced genes



0: Wrong in all methods. 1: Correct in at least one method

## S1.6 Annotation with most variable genes

“Ref-var” and “Sc-var” means that scRef only uses top 20% variable genes in reference and single-cell data, respectively. We use “var” function in R to calculate the variance of each gene.

## Supplementary Table 12 | Variable gene performance-Zeisel

	Zeisel	scRef::semi (All genes)	scRef::semi (Ref-var)	scRef::semi (Sc-var)
ARI	Original	0.5578852	0.5604749	0.5618369
	Merged + OTHER removed	0.917321	0.9280562	0.8955228
	Merged + Neron removed + OTHER removed	0.9133551	0.9191855	0.9163205
NMI	Original	0.6895258	0.7024494	0.6874412
	Merged + OTHER removed	0.8373372	0.853632	0.8315773
	Merged + Neron removed + OTHER removed	0.7955889	0.8072065	0.8171042

## Supplementary Table 13 | Variable gene performance-Tasic

	Tasic	scRef::semi (All genes)	scRef::semi (Ref-var)	scRef::semi (Sc-var)
ARI	Original	0.218299	0.2183459	0.2196549
	Merged + OTHER removed	0.9956251	0.9957325	0.9959257
	Merged + Neron removed + OTHER removed	0.9428578	0.9543684	0.9748919
NMI	Original	0.4996632	0.5024584	0.5047601
	Merged + OTHER removed	0.9706097	0.976027	0.9822003
	Merged + Neron removed + OTHER removed	0.9343078	0.9513827	0.9707728

## Supplementary Table 14 | Variable gene performance-Habib

	Habib	scRef::semi (All genes)	scRef::semi (Ref-var)	scRef::semi (Sc-var)
ARI	Original	0.9406015	0.8800211	0.9503378
	Merged + OTHER removed	0.9948532	0.9959139	0.9950213
	Merged + Neron removed + OTHER removed	0.9435471	0.9809136	0.9187328

<b>NMI</b>	<b>Original</b>	0.8733894	0.7894564	0.8682525
	<b>Merged + OTHER removed</b>	0.9708615	0.9818982	0.9673631
	<b>Merged + Neron removed + OTHER removed</b>	0.9368758	0.9665326	0.9225051

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2 **Supplementary Table 15 | Variable gene performance-Hochgerner**

	Hochgerner	scRef::semi (All genes)	scRef::semi (Ref-var)	scRef::semi (Sc-var)
<b>ARI</b>	<b>Original</b>	0.1854772	0.1826365	0.1944175
	<b>Merged + OTHER removed</b>	0.9086574	0.9211051	0.8647135
	<b>Merged + Neron removed + OTHER removed</b>	0.8857942	0.8320005	0.8801124
<b>NMI</b>	<b>Original</b>	0.499306	0.5045318	0.5019407
	<b>Merged + OTHER removed</b>	0.8310937	0.8422553	0.8051473
	<b>Merged + Neron removed + OTHER removed</b>	0.8440822	0.8076233	0.8167469

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