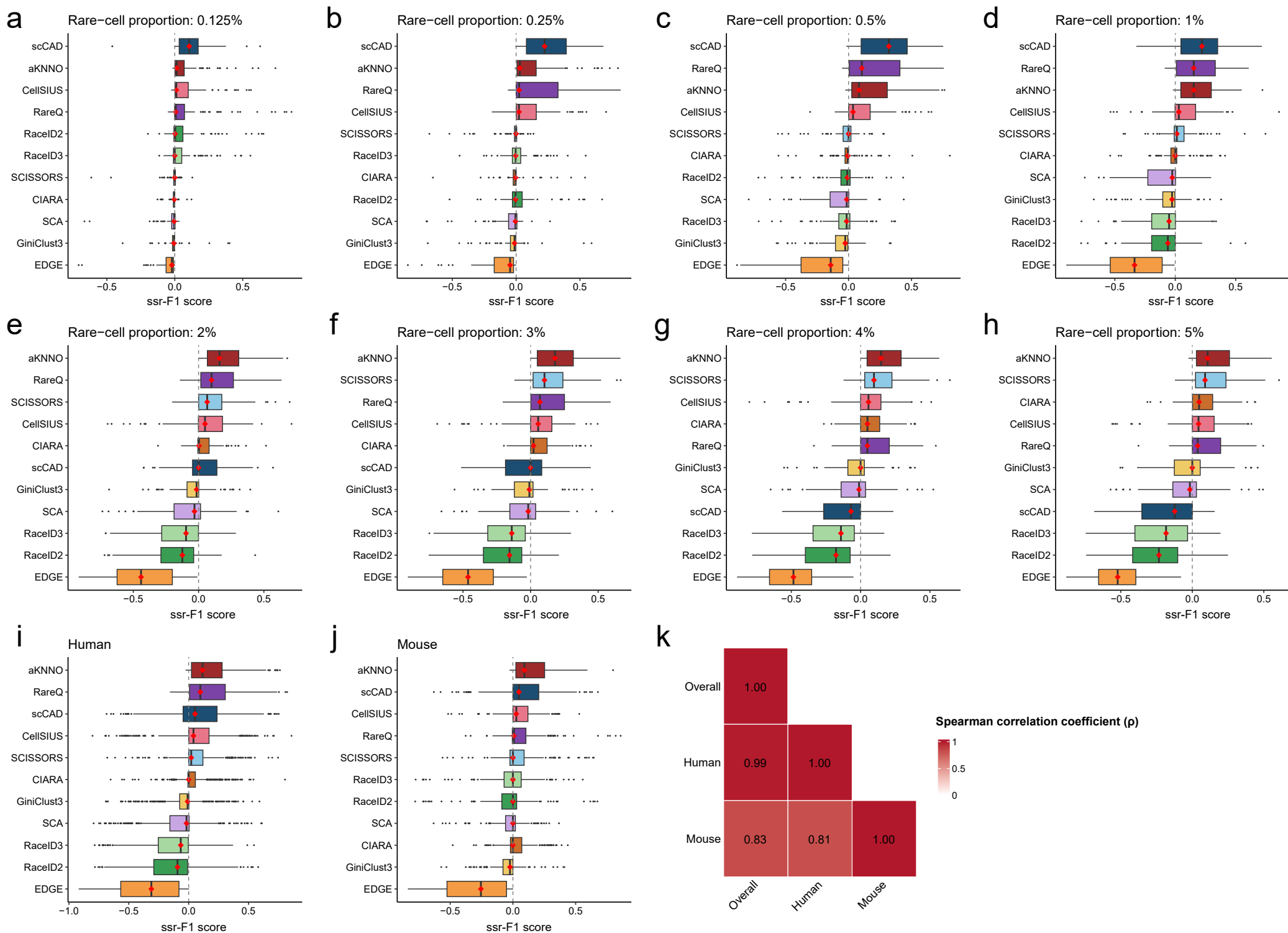


Supplementary Materials

Supplementary Figures



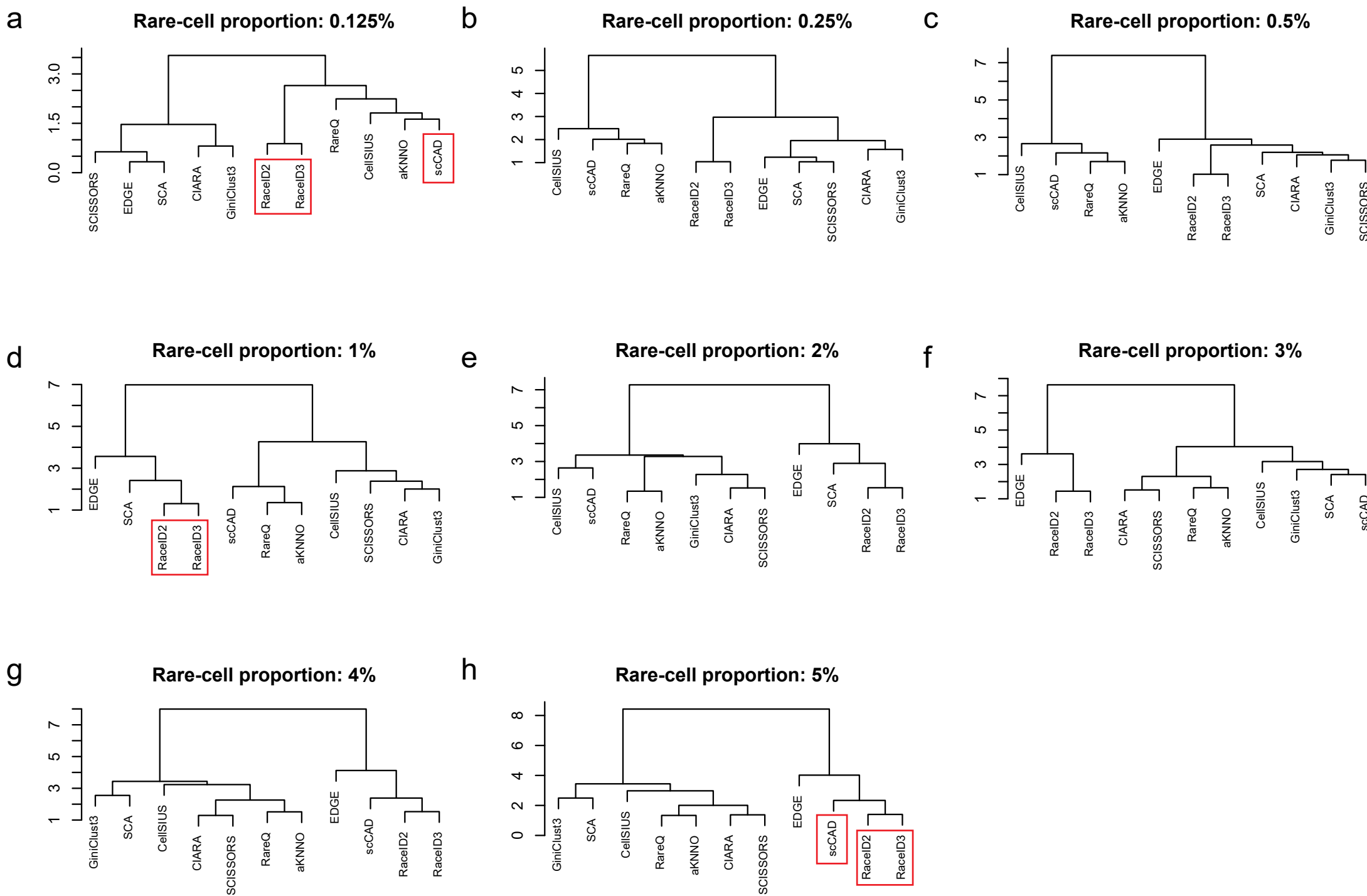
Supplementary Figure 1

Supplementary Figure 1

a–h, Distribution of scenario-specific relative F1 (ssr-F1) scores for each method at rare-cell proportions of **(a)** 0.125%, **(b)** 0.25%, **(c)** 0.5%, **(d)** 1%, **(e)** 2%, **(f)** 3%, **(g)** 4%, and **(h)** 5%.

i–j, Distribution of ssr-F1 scores for each method in benchmarking scenarios derived from **(i)** human and **(j)** mouse datasets.

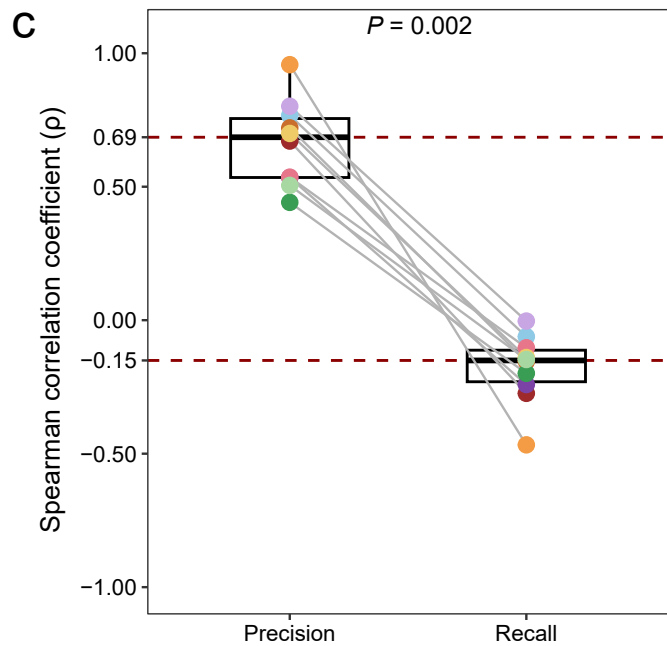
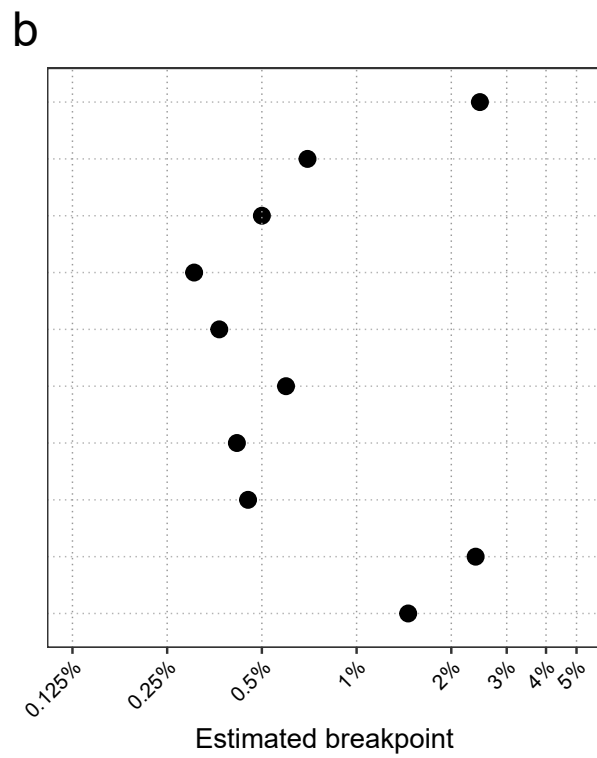
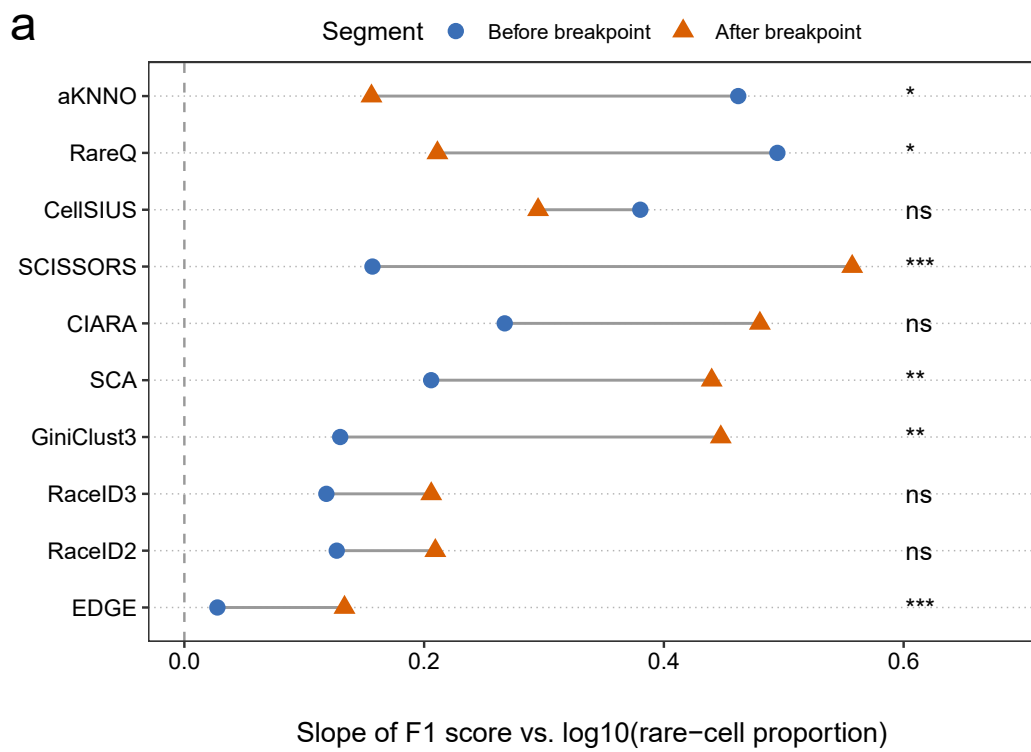
k, Spearman correlation matrix comparing method rankings derived from the overall benchmark, human datasets, and mouse datasets.



Supplementary Figure 2

Supplementary Figure 2

a–h, Hierarchical clustering of methods based on their ssr-F1 score profiles across benchmarking scenarios at rare-cell proportions of **(a)** 0.125%, **(b)** 0.25%, **(c)** 0.5%, **(d)** 1%, **(e)** 2%, **(f)** 3%, **(g)** 4%, and **(h)** 5%. Red boxes highlight selected clustering patterns, including the close clustering of RaceID2 and RaceID3 and the distinct position of scCAD at the lowest and highest rare-cell proportions.

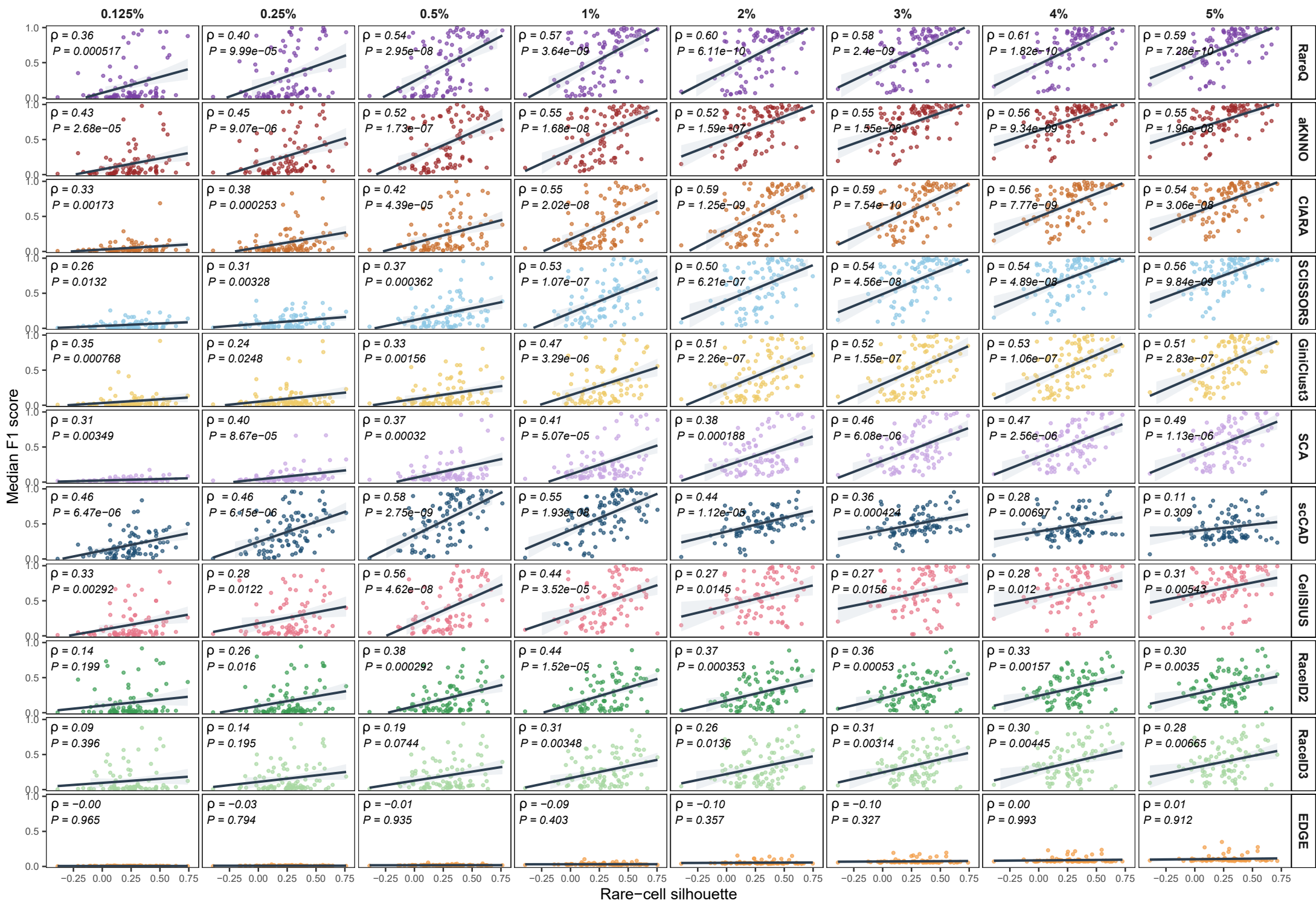


Supplementary Figure 3

a, Estimated slopes of F1 score against log10-transformed rare-cell proportion before and after the breakpoint for the ten methods showing overall monotonic performance trends. The P values were calculated using the Davies test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

b, Estimated breakpoints for the ten methods showing overall monotonic performance trends.

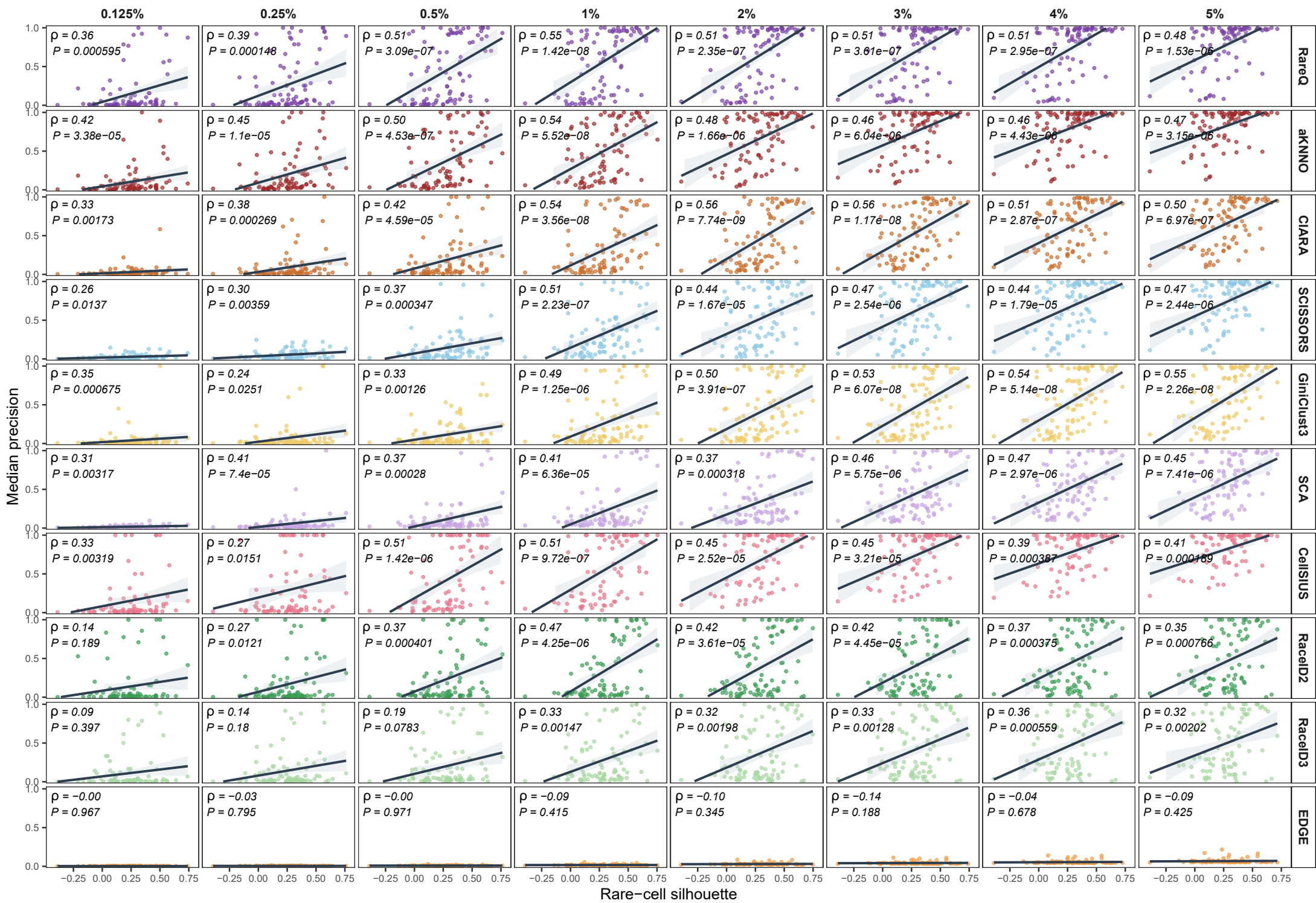
c, Spearman correlation coefficients between rare-cell proportion and precision or recall across the ten methods showing overall monotonic performance trends. Each point represents one method, with precision and recall values for the same method connected by gray lines. Dashed horizontal lines indicate the median correlation coefficients for precision ($\rho = 0.69$) and recall ($\rho = -0.15$). The two distributions were compared using a paired Wilcoxon signed-rank test.



Supplementary Figure 4

Supplementary Figure 4

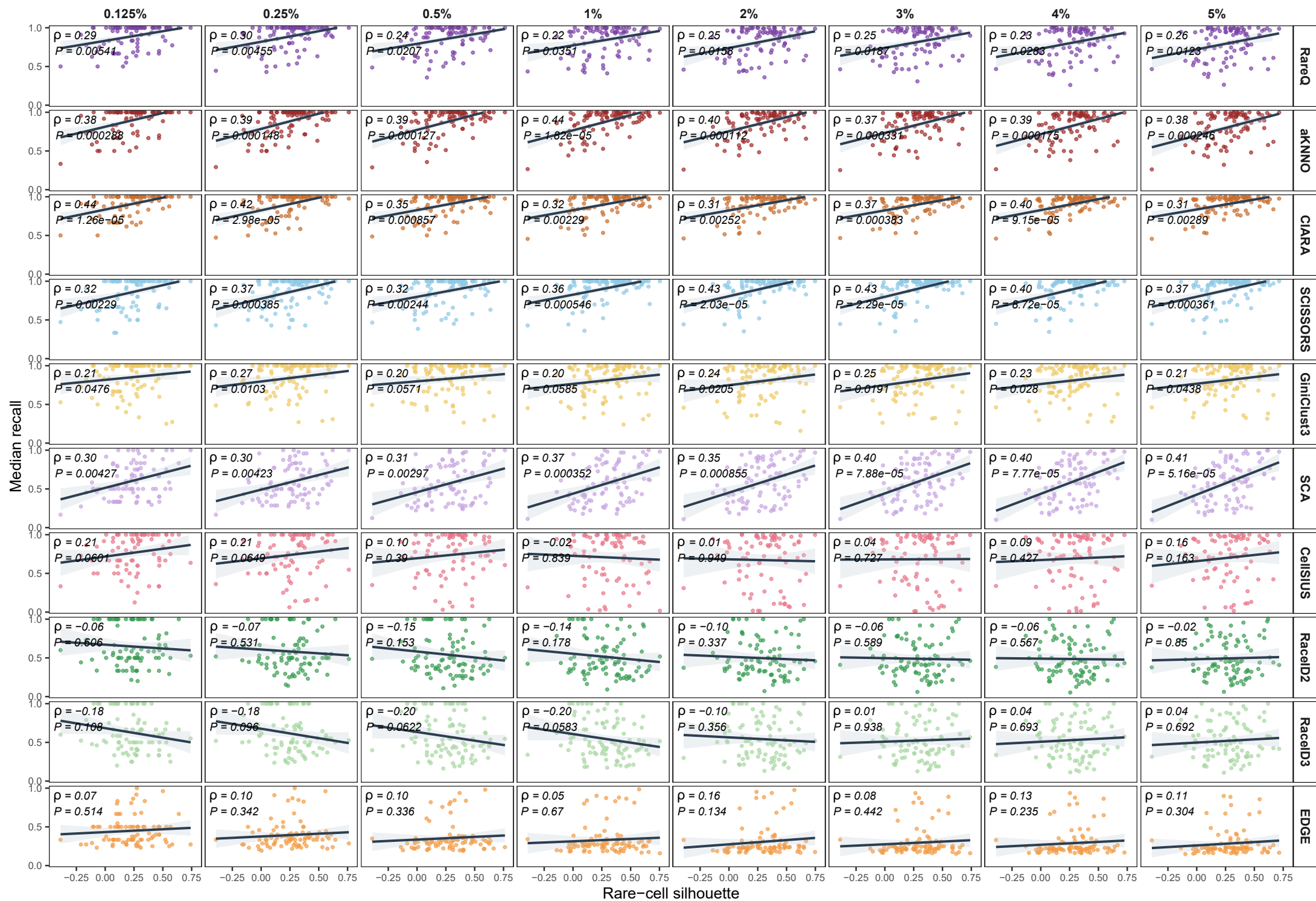
Scatterplots showing the relationship between rare-cell silhouette score and F1 score for each method at each rare-cell proportion. Each point represents a benchmarking scenario, and lines indicate fitted linear trends with 95% confidence intervals. Spearman correlation coefficients (ρ) and corresponding P values are shown in each panel.



Supplementary Figure 5

Supplementary Figure 5

Scatterplots showing the relationship between rare-cell silhouette score and precision for each method excluding scCAD at each rare-cell proportion. Each point represents a benchmarking scenario, and lines indicate fitted linear trends with 95% confidence intervals. Spearman correlation coefficients (ρ) and corresponding P values are shown in each panel.

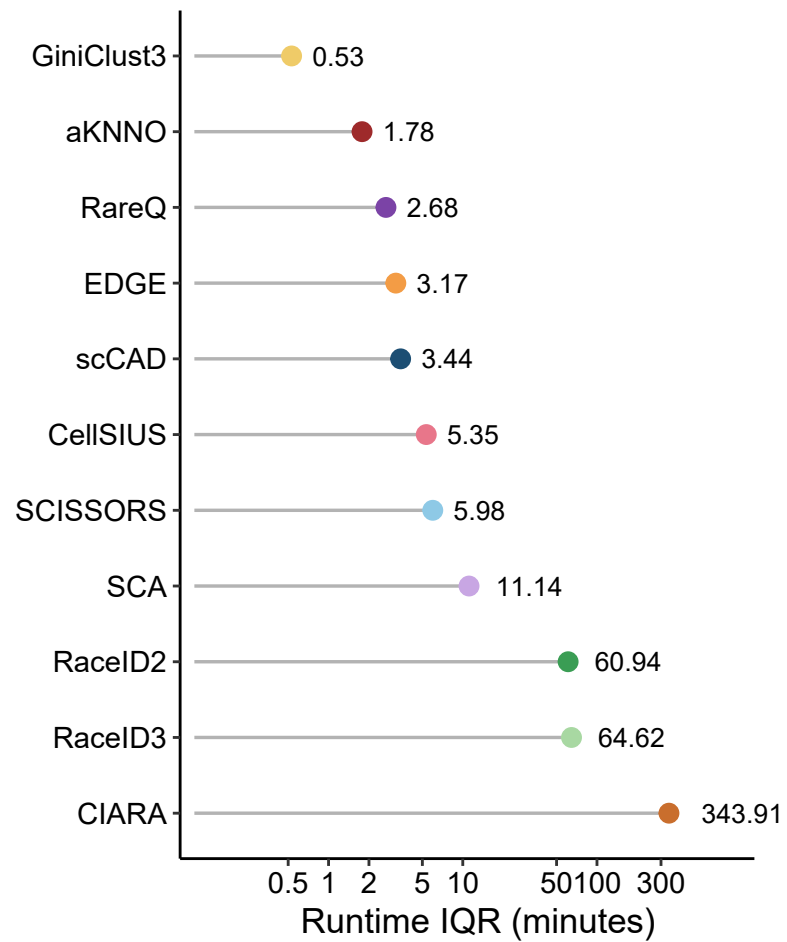


Supplementary Figure 6

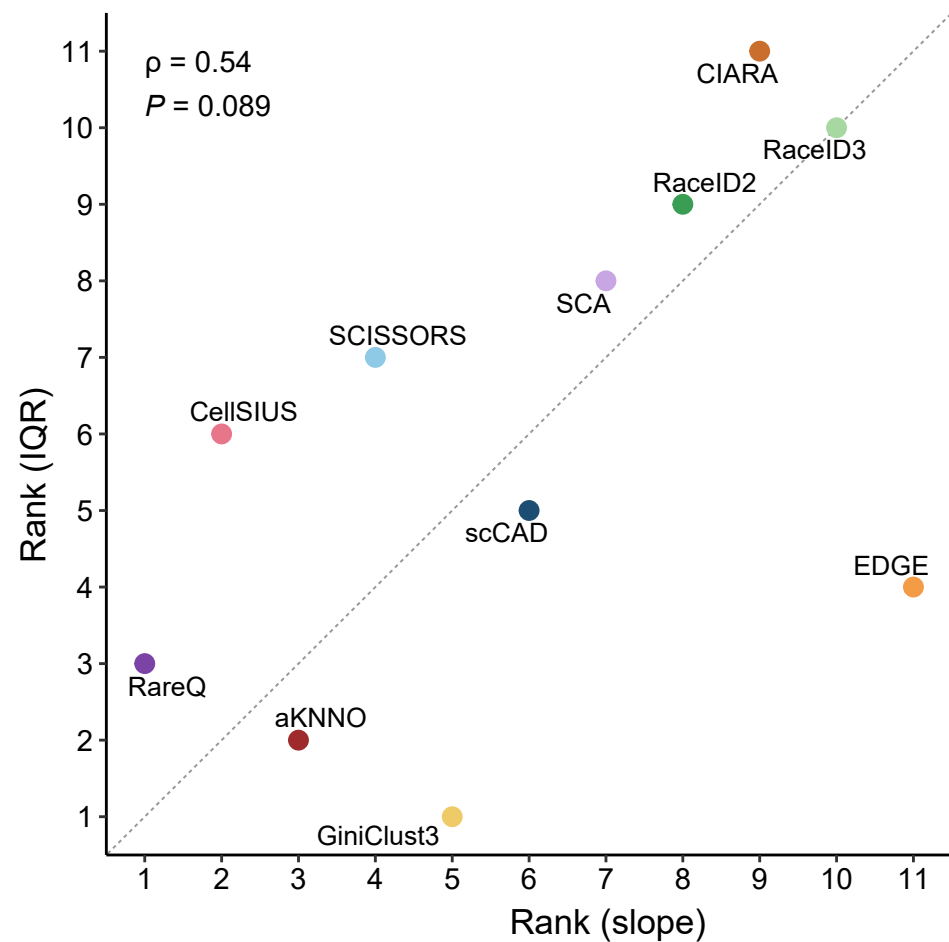
Supplementary Figure 6

Scatterplots showing the relationship between rare-cell silhouette score and recall for each method excluding scCAD at each rare-cell proportion. Each point represents a benchmarking scenario, and lines indicate fitted linear trends with 95% confidence intervals. Spearman correlation coefficients (ρ) and corresponding P values are shown in each panel.

a



b



Supplementary Figure 7

a, Runtime interquartile range (IQR) for each rare cell detection method across benchmarking scenarios. Points indicate the runtime IQR for each method.

b, Comparison of method rankings based on runtime IQR and runtime slope. Each point represents a method. Spearman correlation coefficient (ρ) and corresponding P value are shown.

Supplementary Figure 8

a–c, Boxplots of the performance of multiple ensemble strategies. For reference purposes, the performance of each individual method is also shown. **(a)** Recall using a 1% threshold to define candidate rare-cell clusters; **(b)** precision and **(c)** recall using a 5% threshold. In each box, the central line represents the median value and the bounds represent the 25th and 75th percentiles (interquartile range). The whiskers encompass 1.5 times the interquartile range. The P values were calculated using paired Wilcoxon signed-rank tests. **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

d, Violin plots showing the distribution of marker gene expression levels in originally annotated rare cell types, corresponding clusters, and non-target cells in sample GSM8217713. In each embedded box, the central line represents the median value and the bounds represent the 25th and 75th percentiles (interquartile range). The P values were calculated using Wilcoxon rank-sum tests.

Supplementary Tables

Supplementary Table 1. Rare cell detection methods evaluated in this study.

Method	Language	Source code	Publication year	Ref
aKNNO	R	https://github.com/liuqivandy/aKNNO	2024	1
CellSIUS	R	https://github.com/Novartis/CellSIUS/	2019	2
CIARA	R/Python	https://github.com/ScialdoneLab/CIARA/	2023	3
EDGE	R	https://github.com/shawnstat/EDGE	2020	4
GiniClust3	Python	https://github.com/rdong08/GiniClust3/	2020	5
RaceID2	R	https://github.com/dgrun/StemID	2016	6
RaceID3	R	https://github.com/dgrun/RaceID3_StemID2_package	2018	7
RareQ	R	https://github.com/fabotao/RareQ	2026	8
SCA	Python	https://github.com/bendemeo/shannonca	2023	9
scCAD	Python	https://github.com/xuyp-csu/scCAD	2024	10
SCISSORS	R	https://github.com/jr-leary7/SCISSORS	2023	11

Supplementary Table 2. Overview of the scRNA-seq datasets used in benchmarking analyses.

Dataset	Sample ID	#Cells	#Genes	# Abundant cell types	Description	Protocol	Ref
Kumar et al.	P15	5,367	33,234	4	human breast	10x 3'	12
Travaglini et al.	P1	9,744	26,485	6	human lung	10x 3'	13
Guilliams et al.	H23	10,891	30,933	4	human liver	10x 3'	14
Lake et al.	PRE019	2,971	27,305	8	human kidney	10x 3'	15
Litviňuková et al.	D1	15,397	33,234	4	human heart	10x 3'	16
Elmentaite et al.	A26 (386C)	10,120	33,234	6	human intestine	10x 5'	17
Ulrich et al.	donor_1	10,457	36,020	6	human fallopian tube	10x 3'	18
PBMC3k	pbmc3k	2,638	13,714	7	human peripheral blood mononuclear cells	10x 3'	19
Tabula Sapiens	TSP14	10,935	58,482	7	human spleen	10x 3'	20
Tabula Sapiens	TSP14	3,066	58,482	5	human bladder	10x 3'	20
Tabula Sapiens	TSP14	3,282	58,482	5	human prostate	10x 3'	20
Tabula Sapiens	TSP4	7,124	58,482	4	human uterus	10x 3'	20
Rausell et al.	GSM3376172	3,010	16,653	4	mouse kidney	sNucDrop	21
Joseph et al.	musAd003	9,830	20,807	4	mouse prostate	10x 3'	22
Isola et al.	mouse_3	2,563	20,186	4	mouse ovary	10x 3'	23
Tabula Muris	30-M-4	3,392	17,984	3	mouse spleen	10x 3'	24
Tabula Muris	30-M-3	1,638	17,984	4	mouse lung	10x 3'	24
Tabula Muris	30-M-2	4,124	17,984	5	mouse heart	10x 3'	24

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