## **Supplementary Tables and Figures**

Table S1. Demographic information of Peranakan samples.

Characteristic	Sample size or mean±sd
Age	53.3±16.0
Self-reported sex	
Male	68
Female	107
Not available	2
Self-reported ancestry	
Peranakan Chinese	167
Peranakan Caucasian	1
Peranakan Eurasian	3
Peranakan Indian	1
Not available	5

Table S2. Genetic relatedness among Peranakan samples.

Relatedness	Criteria	No. of pairs
Parent-offspring	$2^{-5/2} < \varphi < 2^{-3/2}$ and $\pi_0 < 0.1$	23
Full sibling	$2^{-5/2} < \varphi < 2^{-3/2}$ and $\pi_0 > 0.1$	22
2 <sup>nd</sup> degree	$2^{-7/2} < \phi < 2^{-5/2}$	18
3 <sup>rd</sup> degree	$2^{-9/2} < \varphi < 2^{-7/2}$	33
Total		96

Notations:  $\varphi$ , kinship coefficient;  $\pi_0$ , the probability of zero identical-by-descent sharing.

Table S3. Global ancestry fractions of different Chinese groups inferred by the supervised ADMIXTURE method.

Chinese group	Chr	Chinese %	Malay %	Indian %	European %
Peranakan	1-22	93.9 (92.8-94.9)	5.14 (4.16-6.12)	0.76 (0.55-0.98)	0.22 (0.13-0.31)
	X	84.8 (80.4-89.2)	12.7 (8.55-16.8)	1.77 (0.90-2.63)	0.74 (0.41-1.07)
Singapore	1-22	97.7 (97.0-98.3)	2.22 (1.54-2.90)	0.07 (0.02-0.12)	0.06 (0.03-0.09)
	X	93.5 (91.3-95.6)	5.64 (3.51-7.78)	0.62 (0.25-1.00)	0.26 (0.10-0.43)
Southern	1-22	98.2 (97.7-98.8)	1.50 (0.96-2.05)	0.06 (0.02-0.09)	0.21 (0.14-0.27)
	X	96.1 (94.9-97.2)	2.54 (1.51-3.57)	1.02 (0.55-1.48)	0.38 (0.17-0.59)
Northern	1-22	98.1 (97.8-98.5)	0.32 (0.00-0.64)	0.37 (0.25-0.48)	1.19 (1.00-1.38)
	X	96.0 (94.6-97.3)	1.95 (0.73-3.17)	0.89 (0.52-1.26)	1.21 (0.80-1.61)

Mean and 95% CI (in parentheses) of ancestry fractions are presented. "Chr" stands for chromosome.

Table S4. Testing admixture with Malays in four Chinese groups by the  $f_3$  statistic.

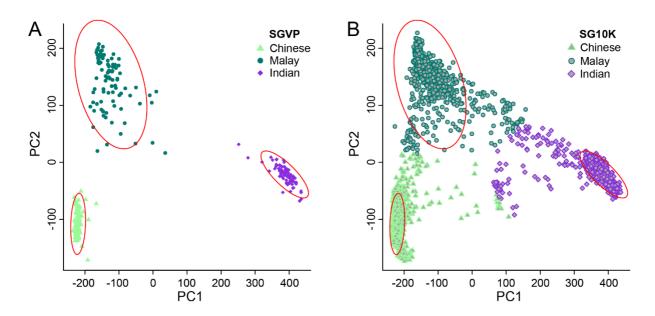
Tubic bar Testing au	mature with it	idiayo ili ibdi. Ci	intese groups by th	c j s statistic.
Target	Source 1	Source 2	$f_3$	Z
Peranakan Chinese	Chinese	Malay	-0.000715	-15.367
Singapore Chinese	Chinese	Malay	-0.000143	-2.756
Southern Chinese	Chinese	Malay	0.000586	8.222
Northern Chinese	Chinese	Malay	0.003058	33.264

Table S5. Mitochondrial haplogroup distribution in Chinese, Malays, Indians, and Peranakan Chinese.

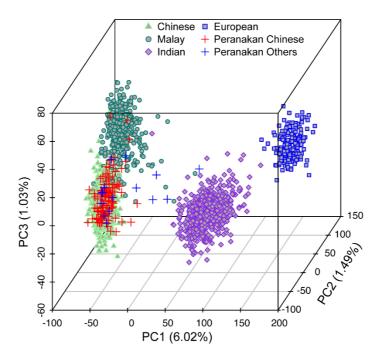
Macro-	acro- Chinese		Malay		Indian		Peranakan Chinese	
haplogroup	Counts	Percentage	Counts	Percentage	Counts	Percentage	Counts	Percentage
A	30	3.0	2	0.5	0	0.0	0	0.0
В	185	18.6	90	22.6	0	0.0	21	18.3
C	25	2.5	0	0.0	0	0.0	3	2.6
D	160	16.1	6	1.5	1	0.2	9	7.8
E	5	0.5	50	12.5	0	0.0	12	10.4
F	193	19.4	68	17.0	3	0.5	22	19.1
G	26	2.6	1	0.3	2	0.3	0	0.0
Н	0	0.0	0	0.0	28	4.5	0	0.0
I	0	0.0	0	0.0	2	0.3	0	0.0
J	1	0.1	0	0.0	7	1.1	0	0.0
K	0	0.0	0	0.0	1	0.2	1	0.9
M	270	27.1	125	31.3	373	59.3	35	30.4
N	47	4.7	23	5.8	12	1.9	5	4.3
P	0	0.0	1	0.3	1	0.2	0	0.0
R	34	3.4	26	6.5	91	14.5	5	4.3
T	0	0.0	0	0.0	7	1.1	0	0.0
U	0	0.0	0	0.0	90	14.3	0	0.0
$\mathbf{W}$	0	0.0	0	0.0	9	1.4	0	0.0
X	0	0.0	0	0.0	1	0.2	0	0.0
Y	2	0.2	5	1.3	0	0.0	1	0.9
Z	18	1.8	2	0.5	1	0.2	1	0.9
Total	996	100	399	100	629	100	115	100

Table S6. Y haplogroup distribution in Chinese, Malays, Indians, and Peranakan Chinese.

Macro-	Chinese		Malay		Indian		Peranakan Chinese	
haplogroup	Counts	Percentage	Counts	Percentage	Counts	Percentage	Counts	Percentage
С	14	4.3	11	7.3	6	2.7	3	6.7
D	6	1.8	0	0.0	0	0.0	0	0.0
E	0	0.0	0	0.0	1	0.4	0	0.0
F	0	0.0	2	1.3	0	0.0	0	0.0
G	0	0.0	1	0.7	5	2.2	0	0.0
Н	0	0.0	3	2.0	75	33.6	0	0.0
J	0	0.0	4	2.6	31	13.9	0	0.0
K	0	0.0	2	1.3	0	0.0	0	0.0
L	0	0.0	0	0.0	48	21.5	0	0.0
M	0	0.0	1	0.7	0	0.0	0	0.0
N	15	4.6	0	0.0	0	0.0	2	4.4
O1	95	29.0	93	61.6	5	2.2	9	20.0
O2	194	59.1	23	15.2	0	0.0	31	68.9
P	0	0.0	1	0.7	0	0.0	0	0.0
Q	1	0.3	0	0.0	4	1.8	0	0.0
R	3	0.9	7	4.6	48	21.5	0	0.0
S	0	0.0	3	2.0	0	0.0	0	0.0
Total	328	100	151	100	223	100	45	100



**Figure S1.** Choice of reference individuals based on PC coordinates. (A) PC coordinates of SGVP samples and the corresponding 95% concentration ellipses (solid red lines). (B) SG10K samples projected on the ancestry space created by SGVP samples (not shown) and SGVP confidence ellipses.



**Figure S2. Top 3 PCs of Peranakan samples based on 113,037 X SNPs.** Peranakan Chinese and Peranakan Others were defined based on autosomal SNPs as shown in **Figure 1**.

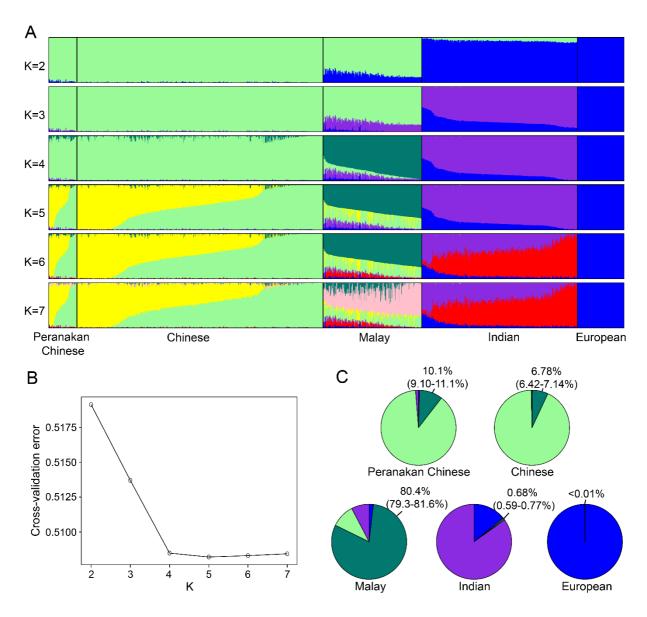
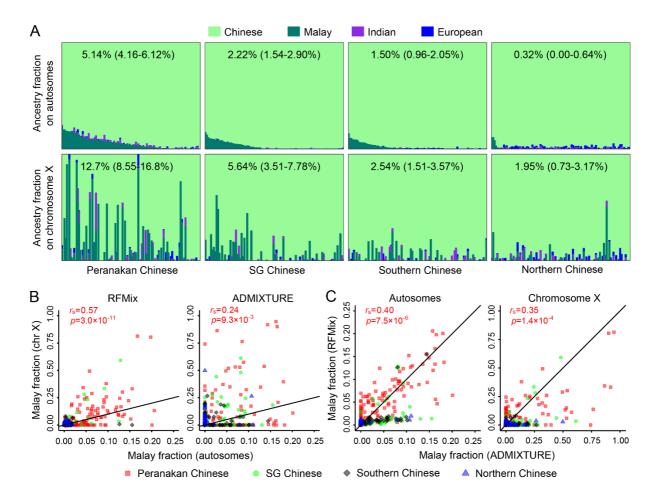
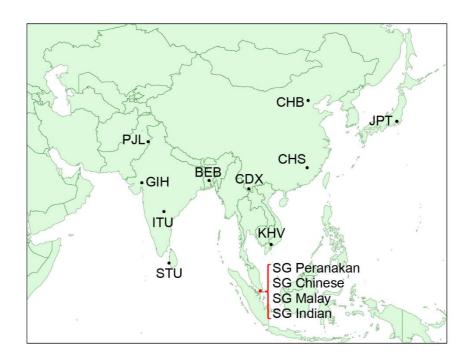


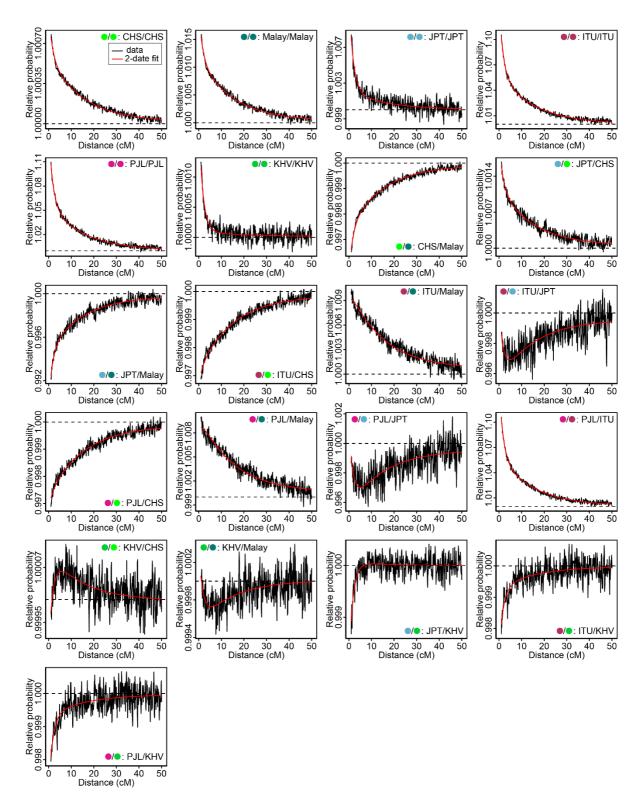
Figure S3. Unsupervised ADMIXTURE analysis of Peranakan Chinese and four reference populations. Unsupervised ADMIXTURE analyses were performed on biallelic autosomal SNPs with MAF>0.05 and at least 2 kb apart from each other. The number of hypothetical ancestral components, K, varied from 2 to 7. (A) Estimated ancestry fractions with different values of K. (B) Five-fold cross-validation errors for different K. While the minimum cross-validation error was achieved at K=5, there is only small difference compared to K=4, at which the ancestral components align well with the reference populations. (C) Pie charts of mean ancestry for each population when K=4. Mean and 95% CI of the Malay-like ancestral fraction (dark green) are indicated for each population.



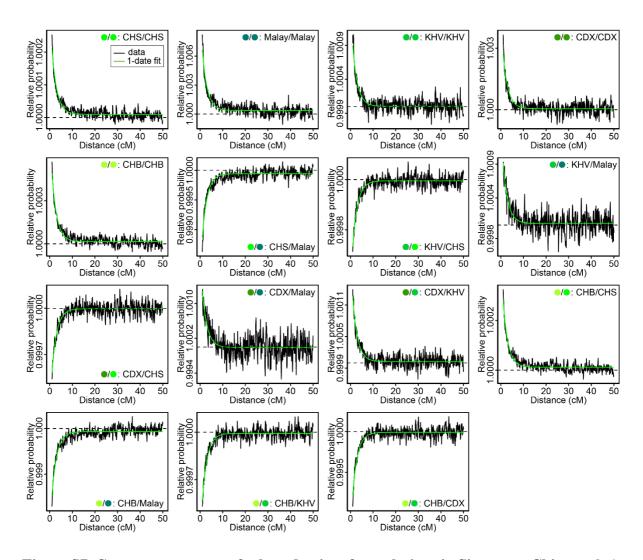
**Figure S4. Global ancestry fractions of Peranakan Chinese estimated by the supervised ADMIXTURE method.** (A) Ancestry fractions on autosomes and the X chromosome for Peranakan Chinese, SG Chinese, southern Chinese, and northern Chinese. Each bar represents the ancestry composition of one individual. Individuals on the top and bottom panels are in the same order. The mean and 95% CI of Malay ancestry for each population are labelled within each panel. (B) Comparison of the Malay ancestry fraction inferred using SNPs on chromosome X and autosomes by RFMix (left) or ADMIXTURE (right). Spearman's correlations and corresponding *p*-values for Peranakan Chinese are indicated in red text. (C) Comparison of the Malay ancestry fraction inferred by RFMix and ADMIXTURE using SNPs on autosomes (left) or chromosome X (right). Spearman's correlations and corresponding *p*-values for Peranakan Chinese are indicated in red text. The black line indicates the diagonal of y=x in panels B and C.



**Figure S5.** Geographic distribution of Asian populations included in the analysis. Abbreviations of 1KGP populations: BEB, Bengali; GIH, Gujarati; ITU, Telugu; PJL, Punjabi; STU, Sri Lankan Tamil; CDX, Chinese Dai; CHB, Han Chinese in Beijing; CHS, Southern Han Chinese; JPT, Japanese; KHV, Kinh in Vietnam.



**Figure S6. Co-ancestry curves of selected pairs of populations in Peranakan Chinese.** Only populations with more than 2% contribution to an ancestral source in either of the two admixture events were included. The solid black curves were observed co-ancestry curves, and the solid red curves were 2-date fit estimated by GLOBETROTTER. A horizontal black dashed line at y=1.0 was drawn in every plot for visual aid.



**Figure S7. Co-ancestry curves of selected pairs of populations in Singapore Chinese.** Only populations with more than 2% contribution to an ancestral source in the admixture event were included. The solid black curves were observed co-ancestry curves, and the solid green curves were 1-date fit estimated by GLOBETROTTER. A horizontal black dashed line at y=1.0 was drawn in every plot for visual aid.

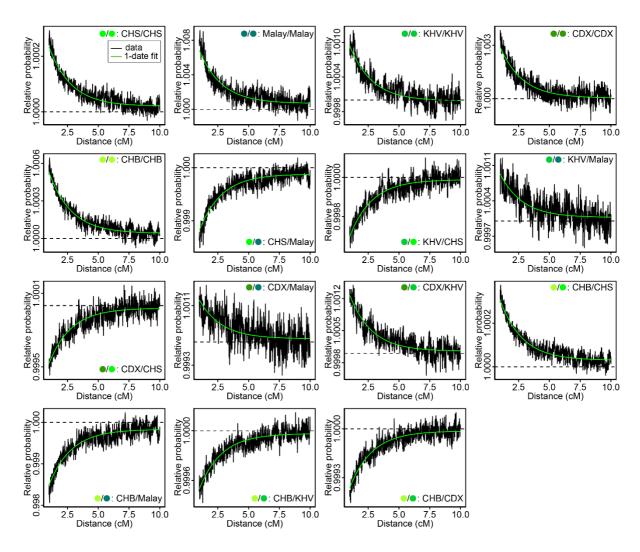


Figure S8. Co-ancestry curves of selected pairs of populations in Singapore Chinese at a grid range of 1 to 10cM. Only populations with more than 2% contribution to an ancestral source in the admixture event were included. The solid black curves were observed co-ancestry curves, and the solid green curves were 1-date fit estimated by GLOBETROTTER. A horizontal black dashed line at y=1.0 was drawn in every plot for visual aid.

## **Supplementary Note**

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