Current Biology, Volume *31*

Supplemental Information

Philippine Ayta possess the highest level of Denisovan ancestry in the world

Maximilian Larena, James McKenna, Federico Sanchez-Quinto, Carolina Bernhardsson, Carlo Ebeo, Rebecca Reyes, Ophelia Casel, Jin-Yuan Huang, Kim Pullupul Hagada, Dennis Guilay, Jennelyn Reyes, Fatima Pir Allian, Virgilio Mori, Lahaina Sue Azarcon, Alma Manera, Celito Terando, Lucio Jamero Jr., Gauden Sireg, Renefe Manginsay-Tremedal, Maria Shiela Labos, Richard Dian Vilar, Acram Latiph, Rodelio Linsahay Saway, Erwin Marte, Pablito Magbanua, Amor Morales, Ismael Java, Rudy Reveche, Becky Barrios, Erlinda Burton, Jesus Christopher Salon, Ma. Junaliah Tuazon Kels, Adrian Albano, Rose Beatrix Cruz-Angeles, Edison Molanida, Lena Granehäll, Mário Vicente, Hanna Edlund, Jun-Hun Loo, Jean Trejaut, Simon Y.W. Ho, Lawrence Reid, Kurt Lambeck, Helena Malmström, Carina Schlebusch, Phillip Endicott, and Mattias Jakobsson

Figure S1. Paleolithic archeological sites in the Philippines and the ISEA region, Related to Figure 1. Reconstructed map of Sundaland 21 kya and the location and chronology of paleolithic human remains discovered in the region (light red circular marker). Location of archeological sites within the Philippines associated with the presence of paleolithic artefacts (small grey circular marker), and location of present-day Negrito ethnic groups included in this study (small bright red circular marker). The arrows indicate the accessible corridors for the migration of populations during the period of maximum glaciation: the northern Palawan-Mindoro corridor and the southern Sulu-Zamboanga corridor.

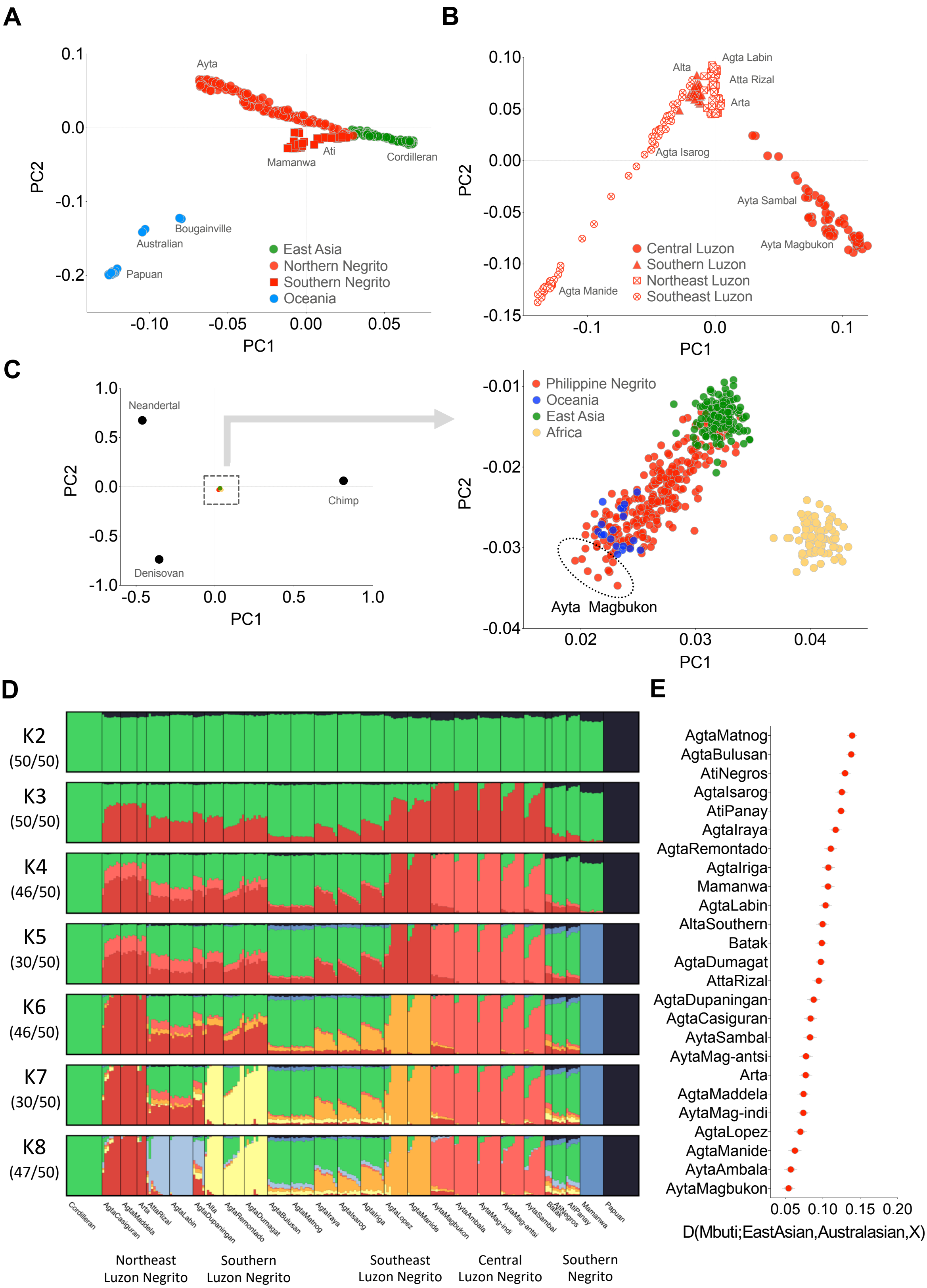

Figure S2. Genetic affinities and clusters of Philippine Negrito ethnic groups, Related to Figure 1. Principal Component analysis (PCA) restricted to Australasians and East Asians (**A**), and to Northern Negritos of the Philippines (**B**). PCA applying the least square equations projection (**C**); modern humans are projected onto the first two principal components defined by the Altai Denisovan, Altai Neandertal, and chimpanzee. All modern humans lie at the center of the plot, and an inset panel to the right shows Ayta Magbukon to lie at the edge defined by Denisovan ancestry. Admixture analysis of Philippine Negritos (**D**) together with the reference populations representing the least admixed East Asian or Australasian, Cordillerans and Papuans, respectively. The analysis was ran for 50 iterations, and for each K, the common modes of replicates were identified using CLUMPP. The major mode for each K was then plotted using Pong v1.4. Detection of East Asian-related ancestry in all Philippine Negritos using the test D(Mbuti;EastAsian,Australasian,X), with Cordilleran Balangao as the surrogate for the least admixed East Asian and Papuan as the surrogate for the least admixed Australasian (**E**).

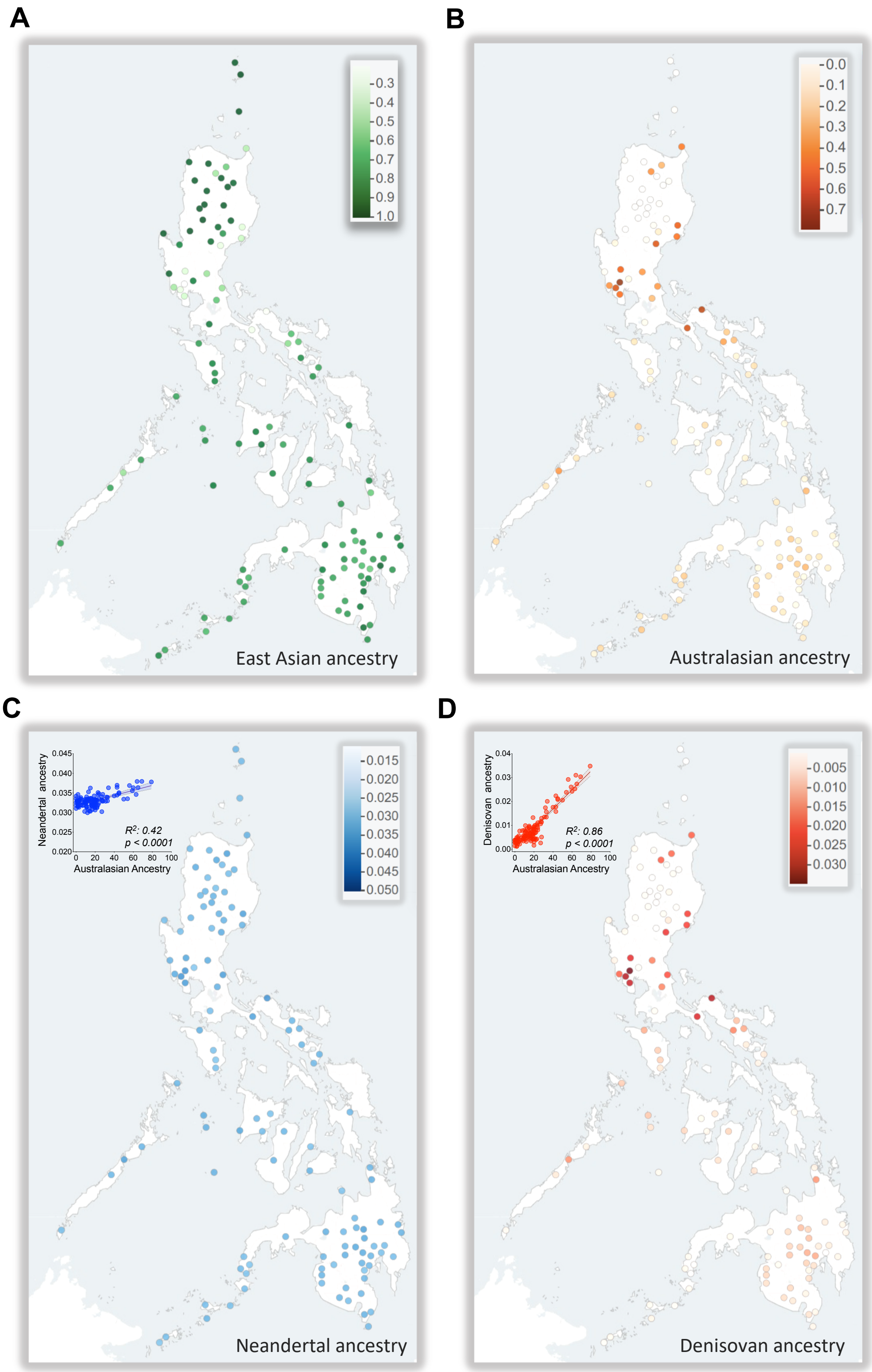

Figure S3. Levels of Neandertal, Denisovan, East Asian and Australasian-related ancestries among Philippine ethnic groups, Related to Figures 2 and 3. Map of the Philippines showing the location of the populations with circular markers indicating their levels of East Asian (**A**) and Australasian (**B**), Neandertal (**C**), and Denisovan (**D**) ancestry. Neandertal ancestry and Denisovan ancestries are estimated by *f4-ratio* statistics, while East Asian and Australasian-related ancestries are estimated by qpAdm (see STAR Methods). An overlap between the distribution pattern of Australasian-related (**B**) and Denisovan (**D**) ancestry is observed, indicating correlation. Inset scatter plots (**C**,**D**) display stronger positive correlation between Australasian-related and Denisovan ancestry than between Australasian-related and Neandertal ancestry (R2: 0.86 vs 0.42, respectively).

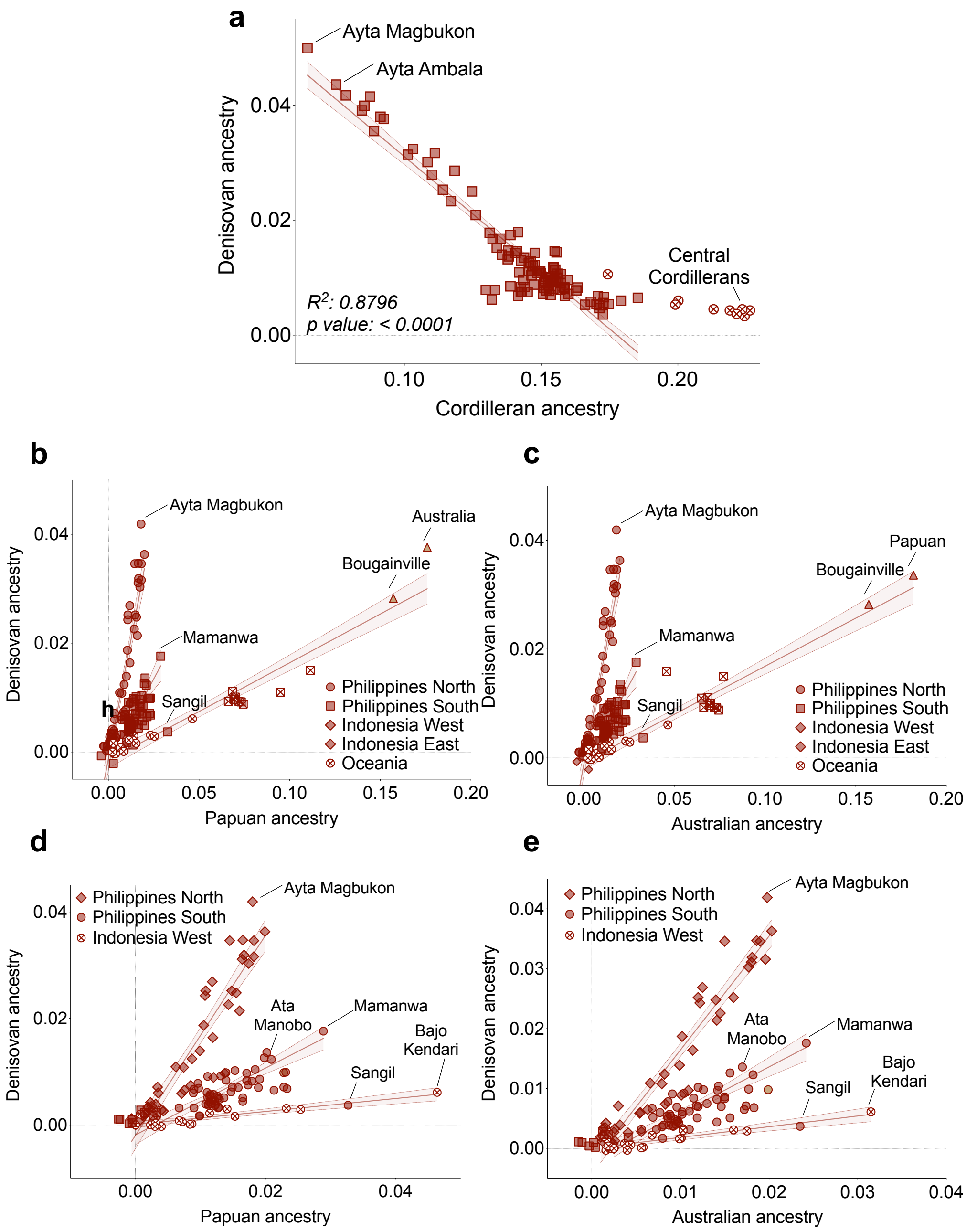

Figure S4. Correlation between Denisovan and Australasian-related ancestry, Related to Figure 3. Inverse correlation between Denisovan ancestry and East Asian/Cordilleran-related ancestry (**A**). Correlation between Denisovan ancestry and Papuan-related ancestry (**B**) or Australian-related ancestry (**C**) restricted to Philippine and western Indonesian populations, showing distinct slopes for Northern Negritos, Southern Negritos, and populations with Papuan-related ancestry. Correlation between Denisovan ancestry and Papuan-related ancestry (**D**) or Australian-related ancestry (**E**) of ISEA and Oceanian populations, showing distinct slopes for Northern Negritos, Southern Negritos, and populations with Papuan-related ancestry such as Sangil, eastern Indonesians, and Oceanians. Cordilleran ancestry is estimated by D(Mbuti;Balangao,Papuan,X), Denisovan ancestry is estimated by D(Mbuti;Denisovan,Balangao,X), and Papuan or Australian-related ancestries are estimated by D(Mbuti;Australian/Papuan,Balangao,X).

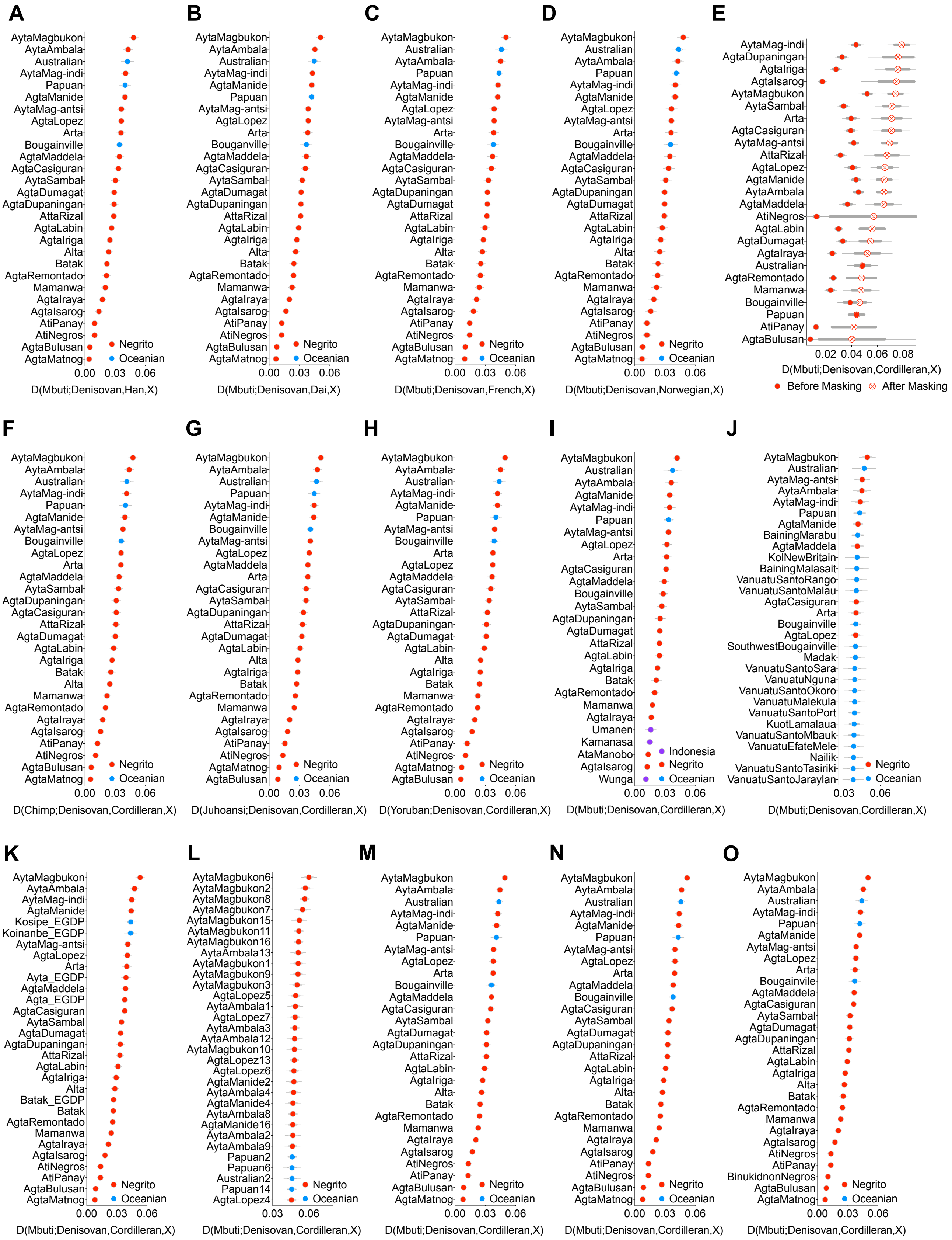

Figure S5. Ayta Magbukon exhibit the highest detectable level of Denisovan ancestry, Related to Figures 2 and 3. Levels of Denisovan affinity among Asia-Pacific populations relative to (**A**) Han, (**B**) Dai, (**C**) French, (**D**) or Norwegian. Levels of Denisovan affinity accounting for admixture with East Asians (**E**). Levels of Denisovan affinity, using Phil_1KGP_SGDP_1.92M dataset, among Asia-Pacific populations with (**F**) Chimp, (**G**) Juhoansi, or (**H**) Yoruba as an outgroup. Levels of Denisovan affinity using (**I**) Phil_AsiaPacific_315K, (**J**) Phil_HO_201K, or (**K**) Phil_EGVP datasets. Using Phil_1KGP_SGDP_1.92M dataset, levels of Denisovan affinity for individuals instead of populations (**L**), or for a restricted SNP panel that is ascertained for (**M**) European, (**N**) African populations, or (**O**) H3Africa SNP panel. Thick and thin error bars represent 1 and 1.96 standard error of the estimate, respectively.

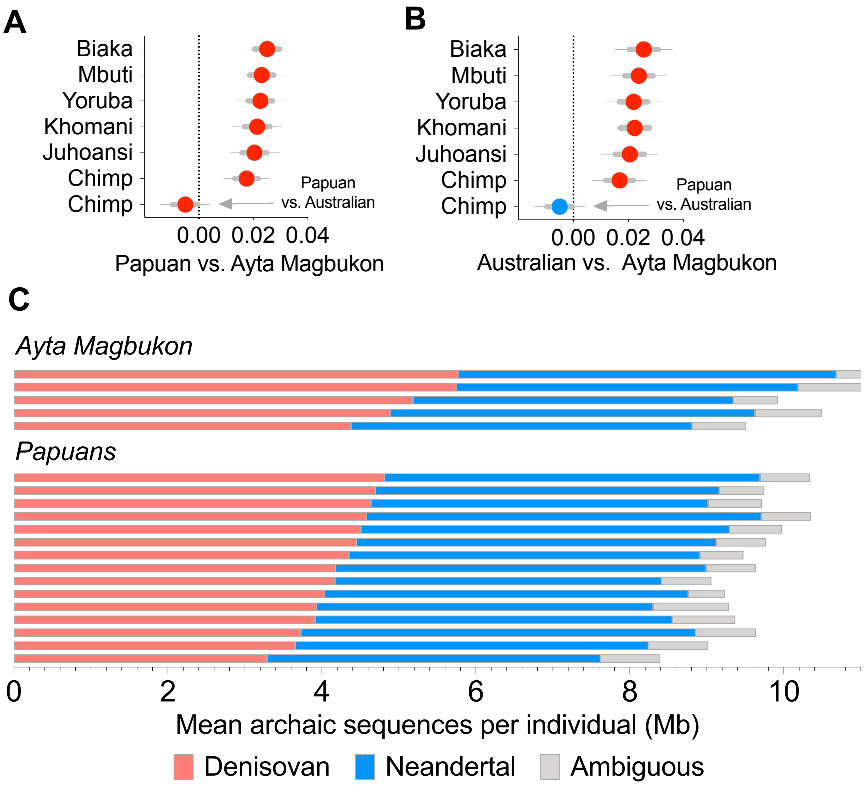

Figure S6. Analysis of sequence data confirm higher levels of Denisovan ancestry in Ayta Magbukon, Related to Figures 4 and 5. Direct comparison of Denisovan ancestry between AustraloPapuans and Ayta Magbukon, using the statistic D(Outgroup;Denisovan,X,AytaMagbukon), with Chimp or various African groups used as an outgroup (**A**,**B**) (all comparisons between Papuans or Australians vs. Ayta Magbukon have a Z score > 3). Average amounts of introgressed Denisovan, Neandertal, or ambiguous sequences detected per individual by the S' approach (**C**) (see STAR Methods).

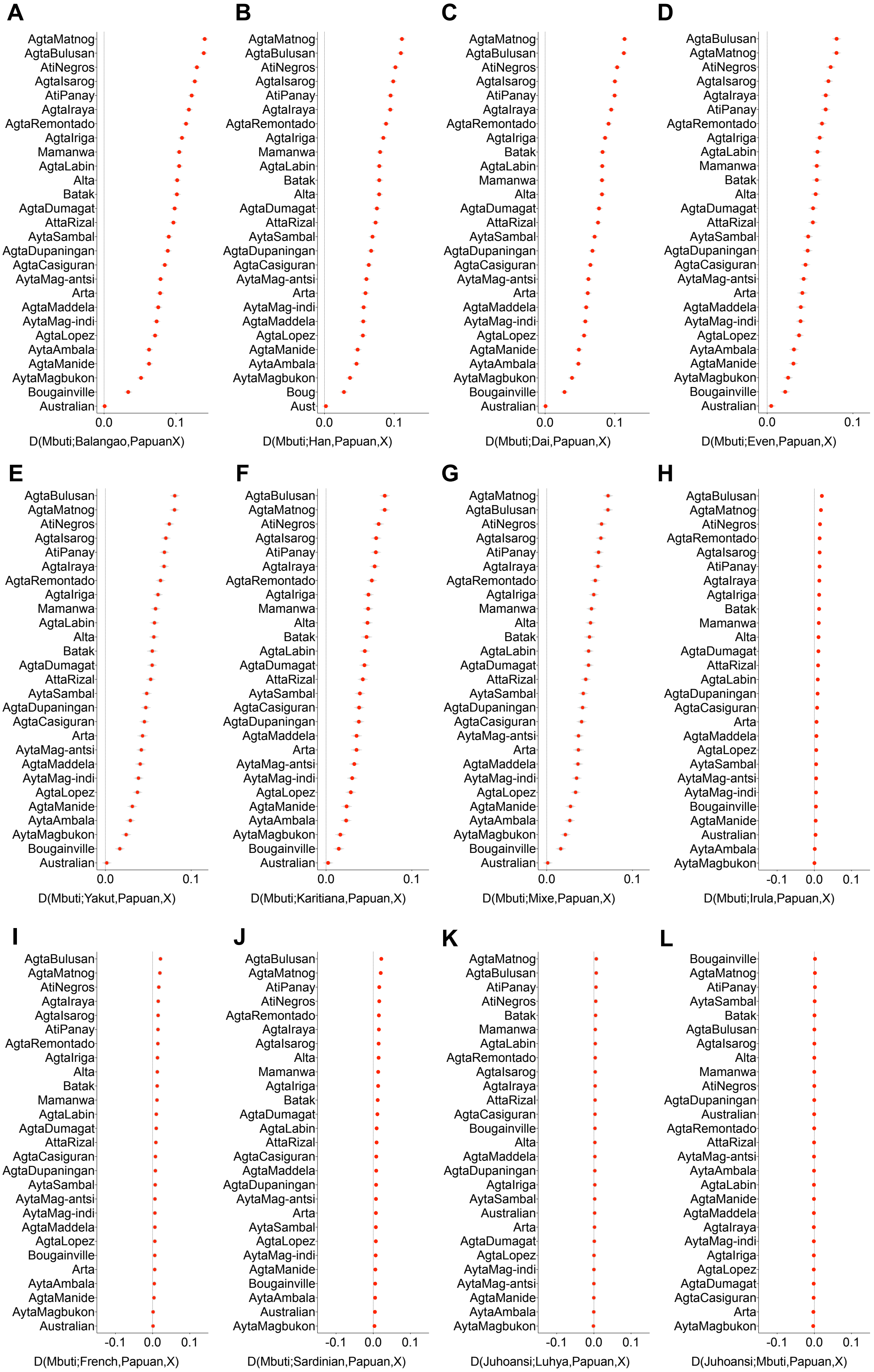

Figure S7. Presence of East Asian gene flow into Philippine Negritos, Related to Figures 4 and 7. Direct comparisons between Papuans and Philippine Negritos to assess the presence of gene flow from an East Asian (**A-C**), Siberian (**F,E**), Native American (**F,G**), South Asian (**H**), European (**I,J**), or African (**K,L**) source population. Thick and thin error bars represent 1 and 1.96 standard error of the estimate, respectively.
]]>

Parameters (all models)	
Length simulated sequence	100M
Samples per population	10
Mutation rate	$1.25\ e^{-8}$
Recombination rate	$1.25\ e^{-8}$
Generation time	25 years
Papuan - Ayta split	46 kya
East Asian -Ayta split	50 kya
European - Ayta split	55 kya
African – non-African split	70 kya
Denisovan 1 – Denisovan 0 split	200 kya
Denisovan 2 – Denisovan 0 split	200 kya
Denisovan 0 – Denisovan (Altai) split	300 kya
Neanderthal – Denisovan (Altai) split	400 kya
Denisovan (Altai) – African split	600 kya
African – Chimp split	4 mya
Time of sampling (Neanderthal)	60 kya
Time of sampling (Denisovan (Altai))	40 kya
Time of sampling (all other pops)	Present
Pop size Denisovan 0/1/2/Altai	1500
Pop size Neanderthal	2000
Pop size African	15000
Pop size non-African (initial)	5000
Pop size non-Africans (bottleneck 60kya-70kya)	2000
Pop size East Asian	3500
Pop size Papuan	3500
Pop size Ayta	3500
Pop size Chimp	30000
Admixture time / prop Neanderthal - non-African	68 kya / 0.02
Admixture time / prop East Asian - Ayta	2500 / 0.1

Table S1. Fixed parameters for all simulation models, Related to Figure 7.

	Models				
Parameters	**NULL**	**ALT1**	**ALT2**	**ALT3**	**ALT4**
Admix time / proportion Denisovan 1 – ancestor Ayta/Papuan	48 kya / 0.04	48 kya / 0.04	-	-	-
Admix time / proportion Denisovan 2 – Ayta	-	40 kya / 0.02	35 kya / 0.06	35 kya / 0.06	25 kya / 0.06
Admix time / proportion Denisovan 1 – Papuan	-	-	45 kya / 0.04	25 kya / 0.04	25 kya / 0.04

Table S2. Variable parameters for simulation models, related to Figure 7.