



Figure 1 | Genetic affinities between USR1, present-day Native Americans and world-wide populations. a. f_3 statistics of the form $f_3(\text{San}; X, \text{USR1})$, for each population in the genotype panel. Warmer colours represent greater shared drift between a population (X) and USR1. **b.** D statistics of the form $D(\text{Native American, Aymara; USR1, Yoruba})$ (points). The Andean Aymara were used to represent SNA. *Native American populations with Asian admixture ($|Z|$ for $D(\text{H1, Aymara; Han, Yoruba}) > 3.3$) (Supplementary Fig. 5a). Error bars represent 1 and approximately 3.3 standard errors ($P \approx 0.001$). Native American populations were grouped by language family¹. **c.** Quantile-quantile plot comparing observed Z scores to the expected normal distribution under the null hypothesis (H_0), for all possible $D(\text{Native American, USR1; Siberian1,$

Siberian2). Colours correspond to the Z score obtained for $D(\text{H1, Aymara; Han, Yoruba})$. The expected normal distribution under the null hypothesis was computed for all groups jointly (Supplementary Information section 10.4). Thick and thin lines represent a Z score of approximately 3.3 ($P \approx 0.001$) and a Z score of approximately 4.91 ($P \approx 0.01$ after applying a Bonferroni correction for 11,322 tests). The bottom-right panel shows the expected tree under the null hypothesis. **d.** Admixture proportions estimated by ADMIXTURE²⁰ assuming $K = 20$ ancestral populations. Bars represent individuals, and colours represent admixture proportions from each ancestral component. Admixture proportions in ancient genomes (wider bars) were estimated using a genotype likelihood-based approach²¹. Nat. Am., Native American; Sib., Siberian.

Information section 6), using outgroup f_3 statistics¹⁹, model-based clustering^{20,21} and multidimensional scaling²² (Supplementary Information section 7–9). Outgroup f_3 statistics of the form $f_3(\text{San}; X, \text{USR1})$ revealed that USR1 is more closely related to present-day Native Americans than to any other tested population, followed by Siberian and East Asian populations^{1,2} (Fig. 1a). Pairwise comparisons of the f_3 statistics for USR1 and a set of ancient and contemporary Native American genomes^{2,7,14} (Supplementary Information section 6)

showed that all are similarly related to Eurasian, Australasian and African populations, although other Native American genomes (Aymara², Athabascan¹⁵, 939², Anzick¹⁷ and Kennewick¹⁴) have a higher affinity for contemporary Native Americans than does USR1 (Supplementary Information section 9). Multidimensional scaling and ADMIXTURE analysis showed that the USR1 genome did not cluster with any specific Native American group (Fig. 1d and Supplementary Fig. 3b). These results imply that USR1 belonged to a previously