Supplementary information, Data S14 Population substructure in wolves between the eastern and western parts of Eurasia

In our structure and PCA analysis, we did not observe any population substructure among the gray wolves that we collected. However, in the SNP array dataset, we see differences among wolves sampled from different locations. In addition, a previous study using wolves from the Middle East (Israel), Europe (Croatia) as well as China found genetic differentiation among these wolf populations[9]. When we overlaid these three individuals onto the large PCA plot, we found that, wolves from western Eurasia do not group together with the wolves we collected, and are genetically closer to dogs (Supplementary information, Figure S14).

One possible explanation for the genetic differentiation is admixture with local dog populations. In order to test this hypothesis, we employed the D test, which can explicitly test for potential gene flow between a focal population and a target group of populations. To be more precise, we setup a D test in the form D((outgroup, focal_wolf),(dogY, dogZ)), where we used the dhole (Supplementary information, Table S1) as the outgroup. For each focal wolf population and dogY, we tried all combinations of dogZ from our sample. For each combination, a negative Z score will indicate gene flow between the dogY and the focal wolf population. We report the number of significant combinations where the D test gives a significant negative Z score.

Using the three wolves (one Israeli wolf, one Croatian wolf and one Chinese wolf) from the Freedman *et al.*'s study[9] together with our own dataset, we extracted all possible combinations that indicate admixture between wolves and dogY. As shown in Supplementary information, Table S8, the Israeli wolf has a wide range of detected admixture/gene flow with dogs from the Middle East as well as Europe. The amount of admixture/gene flow found in Croatian and Chinese wolves from Freedman *et al*. and wolves from this study are slightly less. This indicates that, gene flow between wolves and local dog populations does occur, but the amounts are not high (Supplementary information, Data S8). In general, the D test is quite sensitive at detecting low levels of migration between populations[23].