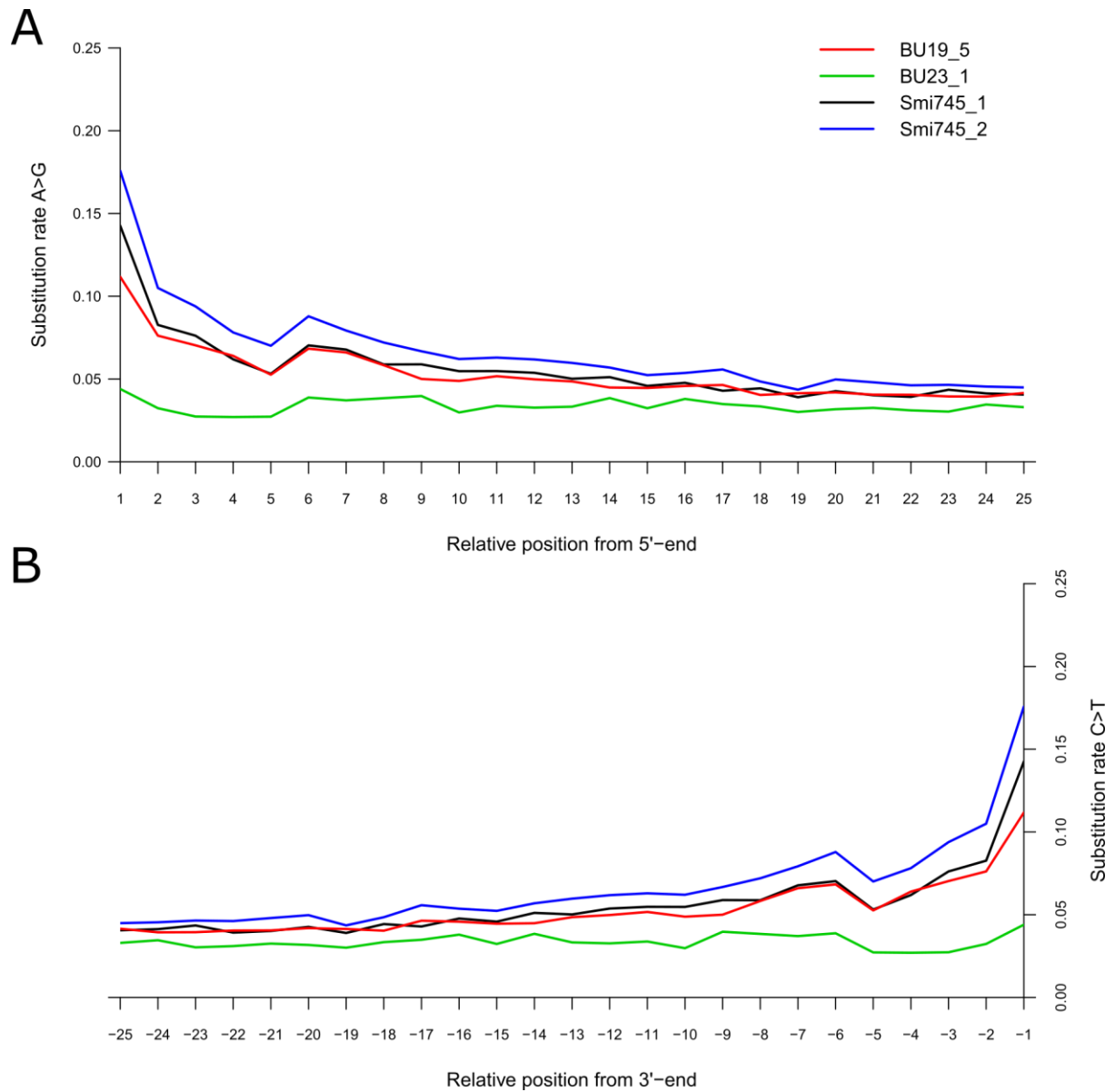
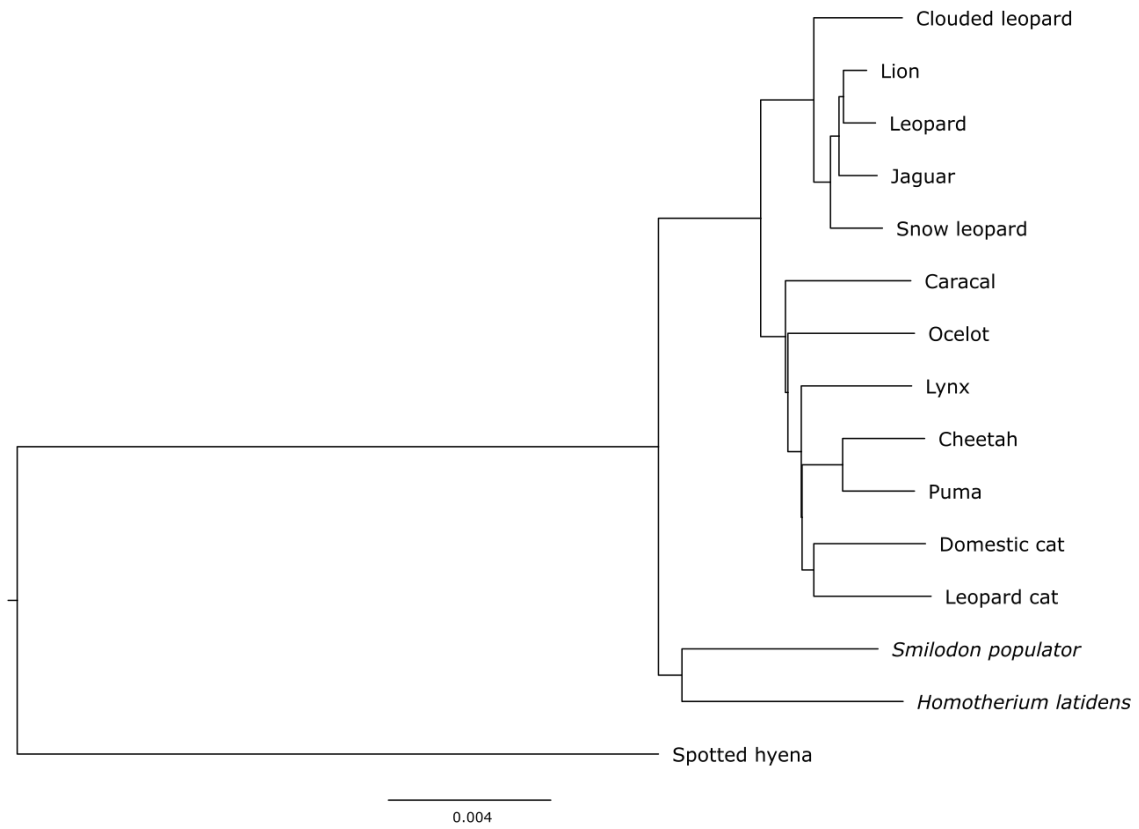


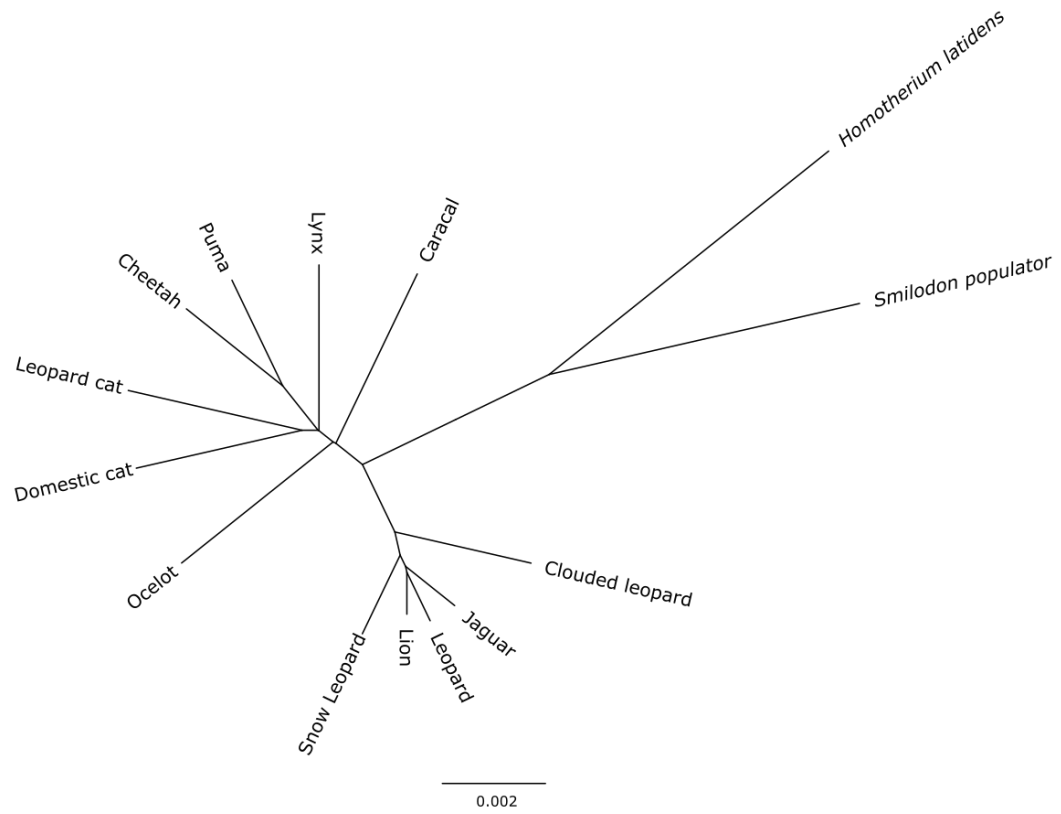
## Supplementary figures



**Supplementary figure S1:** Levels of ancient DNA damage in the four libraries used to construct the *Smilodon populator* draft genome. (A) Rate of A to G substitutions relative to the 5' end of the read. (B) Rate of C to T substitutions relative to the 3' end of the read.



**Supplementary figure S2:** Neighbour-joining (NJ) tree constructed using genome-wide transversion pairwise distances, rooted using the spotted hyena (*Crocota crocuta*). Scale bar represents the proportion of differences between sequences.



**Supplementary figure S3:** Neighbour-joining (NJ) tree constructed using genetic drift distances calculated using pairwise  $F_2$  -statistics. Scale bar represents the proportion of differences between sequences.

## Supplementary tables

| <b>H1</b>       | <b>H2</b>          | <b>H3</b>       | <b>D-statistic</b> | <b>Standard Error</b> | <b>Z-score</b> |
|-----------------|--------------------|-----------------|--------------------|-----------------------|----------------|
| <i>Smilodon</i> | <i>Homotherium</i> | Lion            | -0.2024            | 0.0030                | -67.69         |
| <i>Smilodon</i> | <i>Homotherium</i> | Leopard         | -0.1874            | 0.0030                | -62.39         |
| <i>Smilodon</i> | <i>Homotherium</i> | Jaguar          | -0.1853            | 0.0030                | -61.54         |
| <i>Smilodon</i> | <i>Homotherium</i> | Snow leopard    | -0.1780            | 0.0030                | -59.34         |
| <i>Smilodon</i> | <i>Homotherium</i> | Clouded leopard | -0.1629            | 0.0030                | -54.05         |
| <i>Smilodon</i> | <i>Homotherium</i> | Lynx            | -0.1230            | 0.0031                | -40.13         |
| <i>Smilodon</i> | <i>Homotherium</i> | Ocelot          | -0.1217            | 0.0031                | -39.42         |
| <i>Smilodon</i> | <i>Homotherium</i> | Cheetah         | -0.1216            | 0.0031                | -39.34         |
| <i>Smilodon</i> | <i>Homotherium</i> | Puma            | -0.1212            | 0.0031                | -39.45         |
| <i>Smilodon</i> | <i>Homotherium</i> | Caracal         | -0.1210            | 0.0031                | -39.20         |
| <i>Smilodon</i> | <i>Homotherium</i> | Cat             | -0.1208            | 0.0030                | -39.95         |
| <i>Smilodon</i> | <i>Homotherium</i> | Leopard cat     | -0.1207            | 0.0030                | -39.69         |

**Supplementary table S1:** D-statistics results when placing *Smilodon* and *Homotherium* monophyletically.

| <b>H1</b>          | <b>H2</b>       | <b>H3</b>          | <b>D-statistic</b> | <b>Standard Error</b> | <b>Z-score</b> |
|--------------------|-----------------|--------------------|--------------------|-----------------------|----------------|
| <i>Homotherium</i> | Lion            | <i>Smilodon</i>    | -0.5398            | 0.0019                | -286.56        |
| <i>Homotherium</i> | Leopard         | <i>Smilodon</i>    | -0.5425            | 0.0018                | -296.74        |
| <i>Homotherium</i> | Jaguar          | <i>Smilodon</i>    | -0.5428            | 0.0019                | -290.98        |
| <i>Homotherium</i> | Snow leopard    | <i>Smilodon</i>    | -0.5444            | 0.0018                | -297.45        |
| <i>Homotherium</i> | Clouded leopard | <i>Smilodon</i>    | -0.5490            | 0.0018                | -298.41        |
| <i>Homotherium</i> | Lynx            | <i>Smilodon</i>    | -0.5606            | 0.0019                | -300.92        |
| <i>Homotherium</i> | Ocelot          | <i>Smilodon</i>    | -0.5610            | 0.0019                | -301.52        |
| <i>Homotherium</i> | Cheetah         | <i>Smilodon</i>    | -0.5613            | 0.0019                | -302.34        |
| <i>Homotherium</i> | Puma            | <i>Smilodon</i>    | -0.5612            | 0.0019                | -302.26        |
| <i>Homotherium</i> | Caracal         | <i>Smilodon</i>    | -0.5604            | 0.0018                | -302.99        |
| <i>Homotherium</i> | Cat             | <i>Smilodon</i>    | -0.5617            | 0.0018                | -306.86        |
| <i>Homotherium</i> | Leopard cat     | <i>Smilodon</i>    | -0.5618            | 0.0018                | -303.95        |
| <i>Smilodon</i>    | Lion            | <i>Homotherium</i> | -0.6691            | 0.0015                | -442.49        |
| <i>Smilodon</i>    | Leopard         | <i>Homotherium</i> | -0.6626            | 0.0015                | -429.25        |
| <i>Smilodon</i>    | Jaguar          | <i>Homotherium</i> | -0.6615            | 0.0015                | -429.38        |
| <i>Smilodon</i>    | Snow leopard    | <i>Homotherium</i> | -0.6585            | 0.0016                | -420.54        |
| <i>Smilodon</i>    | Clouded leopard | <i>Homotherium</i> | -0.6535            | 0.0016                | -417.07        |
| <i>Smilodon</i>    | Lynx            | <i>Homotherium</i> | -0.6395            | 0.0016                | -397.97        |
| <i>Smilodon</i>    | Ocelot          | <i>Homotherium</i> | -0.6390            | 0.0016                | -395.76        |
| <i>Smilodon</i>    | Cheetah         | <i>Homotherium</i> | -0.6392            | 0.0016                | -395.61        |
| <i>Smilodon</i>    | Puma            | <i>Homotherium</i> | -0.6389            | 0.0016                | -390.09        |
| <i>Smilodon</i>    | Caracal         | <i>Homotherium</i> | -0.6381            | 0.0017                | -386.04        |
| <i>Smilodon</i>    | Cat             | <i>Homotherium</i> | -0.6391            | 0.0016                | -402.73        |
| <i>Smilodon</i>    | Leopard cat     | <i>Homotherium</i> | -0.6392            | 0.0016                | -398.00        |

**Supplementary table S2:** D-statistics results when placing *Smilodon* and *Homotherium* parapytetically.

| <b>Genus 1</b>      | <b>Genus 2</b>      | <b>Divergence (Ma)</b> | <b>Recalculated divergence (Ma)</b> | <b>F2</b> | <b>Rate/million years</b> |
|---------------------|---------------------|------------------------|-------------------------------------|-----------|---------------------------|
| <i>Neofelis</i>     | <i>Panthera</i>     | 8.32                   | 6.82                                | 0.004161  | 0.000250                  |
| <i>Neofelis</i>     | <i>Felis</i>        | 14.08                  | 13.94                               | 0.008505  | 0.000302                  |
| <i>Neofelis</i>     | <i>Prionailurus</i> | 14.08                  | 13.84                               | 0.008443  | 0.000300                  |
| <i>Neofelis</i>     | <i>Caracal</i>      | 14.08                  | 12.92                               | 0.007884  | 0.000280                  |
| <i>Neofelis</i>     | <i>Acinonyx</i>     | 14.08                  | 13.65                               | 0.008328  | 0.000296                  |
| <i>Neofelis</i>     | <i>Lynx</i>         | 14.08                  | 13.22                               | 0.008065  | 0.000286                  |
| <i>Panthera</i>     | <i>Felis</i>        | 14.08                  | 12.68                               | 0.007732  | 0.000275                  |
| <i>Panthera</i>     | <i>Prionailurus</i> | 14.08                  | 12.58                               | 0.007675  | 0.000273                  |
| <i>Panthera</i>     | <i>Caracal</i>      | 14.08                  | 11.66                               | 0.007112  | 0.000253                  |
| <i>Panthera</i>     | <i>Acinonyx</i>     | 14.08                  | 12.38                               | 0.007554  | 0.000268                  |
| <i>Panthera</i>     | <i>Lynx</i>         | 14.08                  | 11.96                               | 0.007296  | 0.000259                  |
| <i>Felis</i>        | <i>Prionailurus</i> | 8.16                   | 10.21                               | 0.006230  | 0.000382                  |
| <i>Felis</i>        | <i>Caracal</i>      | 11.34                  | 11.76                               | 0.007174  | 0.000316                  |
| <i>Felis</i>        | <i>Acinonyx</i>     | 9.66                   | 11.10                               | 0.006774  | 0.000351                  |
| <i>Felis</i>        | <i>Lynx</i>         | 9.19                   | 10.64                               | 0.006493  | 0.000353                  |
| <i>Prionailurus</i> | <i>Caracal</i>      | 11.34                  | 11.72                               | 0.007148  | 0.000315                  |
| <i>Prionailurus</i> | <i>Acinonyx</i>     | 9.66                   | 11.06                               | 0.006746  | 0.000349                  |
| <i>Prionailurus</i> | <i>Lynx</i>         | 9.19                   | 10.60                               | 0.006465  | 0.000352                  |
| <i>Caracal</i>      | <i>Acinonyx</i>     | 11.34                  | 11.52                               | 0.007029  | 0.000310                  |
| <i>Caracal</i>      | <i>Lynx</i>         | 11.34                  | 11.11                               | 0.006777  | 0.000299                  |
| <i>Acinonyx</i>     | <i>Lynx</i>         | 9.66                   | 10.53                               | 0.006425  | 0.000333                  |
| <b>Average</b>      |                     |                        |                                     |           | 0.000305                  |

**Supplementary table S3:** Genus level pairwise F2 comparisons, divergence dates, and genome-wide rates of genetic drift per million years. “Average” indicates the mean value of all individual genome-wide rates of genetic drift. Divergence dates are those based on results from Barnett et al 2020 [1].

|                     |                 |                   | Subsample            |         | High coverage        |         |                       |
|---------------------|-----------------|-------------------|----------------------|---------|----------------------|---------|-----------------------|
| Genus 1             | Genus 2         | Actual divergence | Estimated divergence | F2      | Estimated divergence | F2      | Divergence difference |
| <i>Caracal</i>      | <i>Acinonyx</i> | 11.34             | 11.36                | 0.00693 | 11.52                | 0.00703 | 0.16                  |
|                     | <i>Lynx</i>     | 11.34             | 10.88                | 0.00663 | 11.11                | 0.00678 | 0.23                  |
|                     | <i>Felis</i>    | 11.34             | 11.40                | 0.00695 | 11.76                | 0.00717 | 0.36                  |
| <i>Prionailurus</i> | <i>Felis</i>    | 8.16              | 10.05                | 0.00613 | 10.21                | 0.00623 | 0.16                  |

**Supplementary table S4:** Comparison of divergence date estimates to their next closest relatives using the full high coverage data and subsampled 0.7x data from the *Caracal* and *Prionailurus* using an average drift rate of 0.000305. Divergence dates are shown in millions of years. “Actual divergence” is the mean divergence from Barnett et al 2020 [1].

| Genus 1            | Genus 2            |           | Divergence (Ma) | F2      |
|--------------------|--------------------|-----------|-----------------|---------|
| <i>Smilodon</i>    | <i>Homotherium</i> | Mean      | 20.65           | 0.01260 |
| <i>Smilodon</i>    | <i>Homotherium</i> | Upper 95% | 26.06           | 0.01590 |
| <i>Smilodon</i>    | <i>Homotherium</i> | Lower 95% | 15.25           | 0.00930 |
| <i>Homotherium</i> | <i>Felinae</i>     |           | 22.75           | 0.01388 |
| <i>Smilodon</i>    | <i>Felinae</i>     |           | 21.44           | 0.01308 |
| Machairodontinae   | <i>Felinae</i>     |           | 22.10           | 0.01348 |

**Supplementary table S5:** Divergence times within Machairodontinae and between Machairodontinae and Felinae based on F2 values and an average genome-wide genetic drift rate of 0.000305. Upper and lower 95% intervals are taken from 1.96x the standard deviation (0.001485) of the F2 calculated between *Smilodon* and *Homotherium*.

| <b>A</b>        | <b>B</b>           | <b>C</b>        | <b>F3</b> | <b>SE</b> | <b>Z-score</b> |
|-----------------|--------------------|-----------------|-----------|-----------|----------------|
| <i>Smilodon</i> | <i>Homotherium</i> | Felinae         | 0.00434   | 0.00009   | 48.29          |
| <i>Smilodon</i> | <i>Homotherium</i> | <i>Panthera</i> | 0.00595   | 0.00012   | 49.77          |
| <i>Smilodon</i> | <i>Homotherium</i> | Jaguar          | 0.00682   | 0.00014   | 47.18          |
| <i>Smilodon</i> | <i>Homotherium</i> | Ocelot          | 0.00782   | 0.00017   | 46.36          |
| <i>Smilodon</i> | <i>Homotherium</i> | Puma            | 0.00788   | 0.00017   | 47.36          |

**Supplementary table S6:** F3 statistics results based on the topology [[A,B],C]. SE shows the standard error and the Z-score shows the number of SE the F3 is away from 0. A negative F3 indicates gene flow while positive shows inconclusive results.

| <b>A</b>        | <b>B</b>           | <b>C</b>        | <b>D3</b> | <b>SD</b> | <b>p-value</b> |
|-----------------|--------------------|-----------------|-----------|-----------|----------------|
| <i>Smilodon</i> | <i>Homotherium</i> | Felinae         | -0.00004  | 0.00677   | 0.49           |
| <i>Smilodon</i> | <i>Homotherium</i> | <i>Panthera</i> | 0.00544   | 0.00740   | 0.07           |
| <i>Smilodon</i> | <i>Homotherium</i> | Jaguar          | 0.00491   | 0.00769   | 0.10           |
| <i>Smilodon</i> | <i>Homotherium</i> | Ocelot          | -0.00413  | 0.00894   | 0.18           |
| <i>Smilodon</i> | <i>Homotherium</i> | Puma            | -0.00358  | 0.00759   | 0.17           |

**Supplementary table S7:** D3 statistics results based on the topology [[A,B],C]. D3 shows the average D3 value taken from a non-overlapping sliding window size of 1Mb. SD is the standard deviation and significance from 0 is estimated by calculating a p-value assuming a normal distribution. A p-value less than 0.05 is considered significant. A significantly negative D3 value shows gene flow between B and C while a significantly positive D3 shows admixture between A and C.



| <b>A</b>           | <b>B</b>        | <b>C</b>        | <b>Date of gene flow</b> | <b>D3</b> | <b>SD</b> | <b>p-value</b> |
|--------------------|-----------------|-----------------|--------------------------|-----------|-----------|----------------|
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 20Ma                     | -0.0002   | 0.0044    | 0.467046       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 18Ma                     | -0.0026   | 0.0045    | 0.127785       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 17Ma                     | -0.0035   | 0.0047    | 0.063969       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 16Ma                     | -0.0048   | 0.0049    | 0.023450       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 15Ma                     | -0.0057   | 0.0050    | 0.010962       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 10Ma                     | -0.0114   | 0.0065    | 0.000248       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 5Ma                      | -0.0171   | 0.0085    | 0.000029       |
| <i>Homotherium</i> | <i>Smilodon</i> | Felinae species | 50kya                    | -0.0227   | 0.0105    | 0.000007       |

**Supplementary table S8:** D3 statistics results based on simulations using the topology [[A,B],C] with predefined gene flow between B and C at different time periods after divergence. The labels given to the taxon A,B, and C are based on the results of the current study but could represent any arbitrary species with the same divergence times, mutation rates, and recombination rates. D3 shows the average D3 value taken from a non-overlapping sliding window size of 1Mb. SD is the standard deviation and significance from 0 is estimated by calculating a p-value assuming a normal distribution. A p-value less than 0.05 is considered significant. A significantly negative D3 value shows gene flow between B and C while a significantly positive D3 shows admixture between A and C.

| <b>Species name:</b>            | <b>Source:</b>                            | <b>Accession code:</b>                                |
|---------------------------------|---|---|
| <i>Felis catus</i>              | 99 Lives Cat Genome Sequencing Initiative | NCBI SRA accession code: SRR2224864                   |
| <i>Prionailurus bengalensis</i> | [1]                                       | NCBI Bioproject accession code: PRJNA649572           |
| <i>Lynx pardinus</i>            | [2]                                       | European nucleotide archive accession code: ERA562804 |
| <i>Acinonyx jubatus</i>         | [3]                                       | NCBI SRA accession code: SRS1123638                   |
| <i>Caracal caracal</i>          | [1]                                       | NCBI SRA sample accession code: SAMN15096300          |
| <i>Neofelis nebulosa</i>        | [4]                                       | NCBI SRA sample accession code: SAMN14352199          |
| <i>Panthera uncia</i>           | [5]                                       | NCBI SRA accession code: SRR836372                    |
| <i>Panthera onca</i>            | [6]                                       | NCBI SRA sample accession code: SAMN05907657          |
| <i>Panthera pardus</i>          | [7]                                       | NCBI SRA accession code: SRR3041424                   |
| <i>Panthera leo</i>             | [5]                                       | NCBI SRA accession code: SRR836361                    |
| <i>Crocuta crocuta</i>          | [8]                                       | NCBI Bioproject accession code: PRJNA554753           |
| <i>Homotherium latidens</i>     | [1]                                       | NCBI Bioproject accession code: PRJNA649760           |
| <i>Puma concolor</i>            | [9]                                       | NCBI SRA accession code: SRR6467080                   |
| <i>Leopardus pardalis</i>       | [10]                                      | NCBI SRA accession code: SRR6071643                   |

**Supplementary table S9:** Genbank accession codes and the corresponding sources of the extant cat short read genomic data used in this study.

## Supplementary references:

1. Barnett, R., Westbury, M.V., Sandoval-Velasco, M., Vieira, F.G., Jeon, S., Zazula, G., Martin, M.D., Ho, S.Y.W., Mather, N., Gopalakrishnan, S., *et al.* (2020). Genomic Adaptations and Evolutionary History of the Extinct Scimitar-Toothed Cat, *Homotherium latidens*. *Curr. Biol.* *30*, 1–8.
2. Abascal, F., Corvelo, A., Cruz, F., Villanueva-Cañas, J.L., Vlasova, A., Marcet-Houben, M., Martínez-Cruz, B., Cheng, J.Y., Prieto, P., Quesada, V., *et al.* (2016). Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. *Genome Biol.* *17*, 251.
3. Dobrynin, P., Liu, S., Tamazian, G., Xiong, Z., Yurchenko, A.A., Krasheninnikova, K., Kliver, S., Schmidt-Küntzel, A., Koepfli, K.-P., Johnson, W., *et al.* (2015). Genomic legacy of the African cheetah, *Acinonyx jubatus*. *Genome Biol.* *16*, 277.
4. de Manuel, M., Barnett, R., Sandoval-Velasco, M., Yamaguchi, N., Garrett Vieira, F., Zepeda Mendoza, M.L., Liu, S., Martin, M.D., Sinding, M.-H.S., Mak, S.S.T., *et al.* (2020). The evolutionary history of extinct and living lions. *Proc. Natl. Acad. Sci. U. S. A.* *117*, 10927–10934.
5. Cho, Y.S., Hu, L., Hou, H., Lee, H., Xu, J., Kwon, S., Oh, S., Kim, H.-M., Jho, S., Kim, S., *et al.* (2013). The tiger genome and comparative analysis with lion and snow leopard genomes. *Nat. Commun.* *4*, 2433.
6. Figueiró, H.V., Li, G., Trindade, F.J., Assis, J., Pais, F., Fernandes, G., Santos, S.H.D., Hughes, G.M., Komissarov, A., Antunes, A., *et al.* (2017). Genome-wide signatures of complex introgression and adaptive evolution in the big cats. *Sci Adv* *3*, e1700299.
7. Kim, S., Cho, Y.S., Kim, H.-M., Chung, O., Kim, H., Jho, S., Seomun, H., Kim, J., Bang, W.Y., Kim, C., *et al.* (2016). Comparison of carnivore, omnivore, and herbivore mammalian genomes with a new leopard assembly. *Genome Biol.* *17*, 211.
8. Westbury, M.V., Hartmann, S., Barlow, A., Preick, M., Ridush, B., Nagel, D., Rathgeber, T., Ziegler, R., Baryshnikov, G., Sheng, G., *et al.* (2020). Hyena paleogenomes reveal a complex evolutionary history of cross-continental gene flow between spotted and cave hyena. *Science Advances* *6*, eaay0456.
9. Saremi, N.F., Supple, M.A., Byrne, A., Cahill, J.A., Coutinho, L.L., Dalén, L., Figueiró, H.V., Johnson, W.E., Milne, H.J., O'Brien, S.J., *et al.* (2019). Puma genomes from North and South America provide insights into the genomic consequences of inbreeding. *Nat. Commun.* *10*, 4769.
10. Li, G., Figueiró, H.V., Eizirik, E., and Murphy, W.J. (2019). Recombination-Aware Phylogenomics Reveals the Structured Genomic Landscape of Hybridizing Cat Species. *Mol. Biol. Evol.* *36*, 2111–2126.