Supplementary information

Supplementary Table 1: Genome assembly metrics. The metrics of the different versions of the mountain lion genome. The final column represents the final assembly, PumCon1.0. We saw a marked improvement in the N50 as a result of scaffolding with HiRise. Gap filling with PB Jelly notably decreased the numbers of Ns and strings of Ns in the genome assembly. Due to gap filling and the creation of previously missing regions of the genomes, more Illumina reads mapped to the genome assembly. Due to the error frequency of ONT reads, the correction of the gap filled region sequence with iterative rounds of Pilon using the Illumina data also increased the number of reads that mapped to the genome. Asterisk denotes that the Meraculous assembly did not include the final versions of the X chromosome scaffolds.

	Meraculous	HiRise	PBJelly	Pilon iteration 1	Pilon iteration 2 (final)
Assembly step	Shotgun assembly	Scaffolding	Gap Filling	Error correcting	Error correcting
Input data	Illumina shotgun reads	Chicago & Hi-C libraries	ONT reads	Illumina shotgun reads	Illumina shotgun reads
Genome length (bp)	2,181,316,782	2,293,137,739	2,433,777,904	2,433,231,347	2,432,985,507
N50 scaffold	36.6kb	103.78 Mb	100.51 Mb	100.54 Mb	100.53 Mb
L50 scaffold	17,135	7	8	8	8
# of gaps	124,710	258,836	207,433	184,611	178,994
# of Ns	26,631,327	154,284,192	132,359,239	119,328,697	114,069,924
% of Ns in genome	1.22%	6.73%	5.44%	4.90%	4.69%
# Illumina shotgun reads mapping (samtools view -q 30 -c)	958,095,130*	979,540,843	982,339,501	985,819,801	987,305,346

Supplementary Table 2: Benchmarking Universal Single-Copy Orthologs (BUSCO) gene completeness score. The results of running BUSCO[1] on the PumCon1.0 genome using the human gene set (n=4104).

Complete BUSCOs	3832 (93.4%)
Complete and single-copy BUSCOs	3815 (93.0%)
Complete and duplicated BUSCOs	17 (0.4%)
Fragmented BUSCOs	141 (3.4%)
Missing BUSCOs	131 (3.2%)

Supplementary Table 3: Details for the panel of mountain lions used in this study. *Note: SMM13 was used solely for the X chromosome scaffold assembly, and thus further metrics (i.e. heterozygosity) were not calculated in this study.

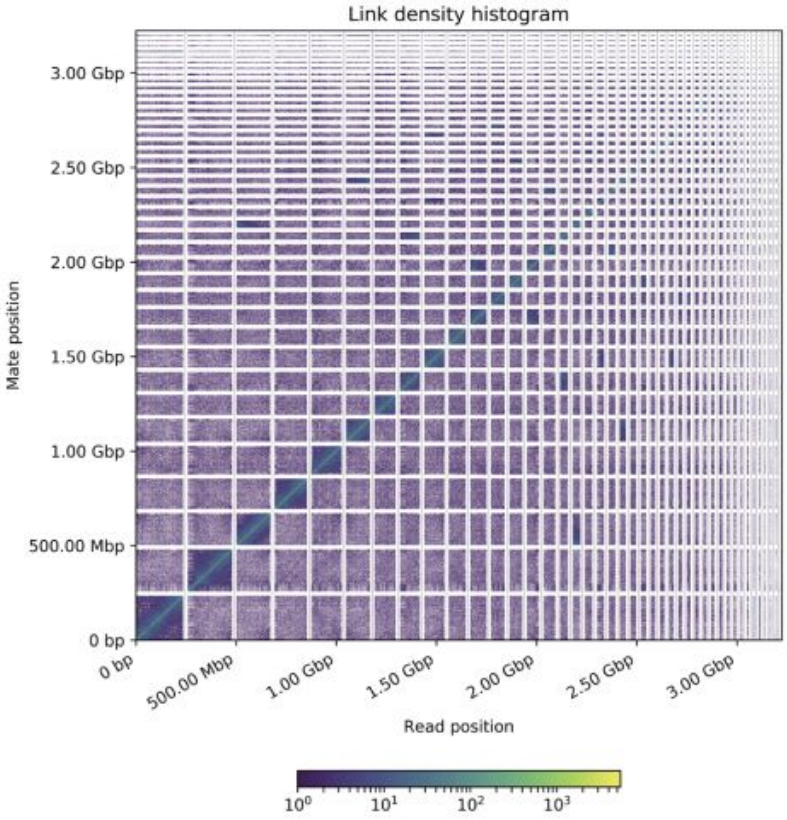
Mountain lion	Population	Gender	Coverage	Heterozygosity (pileup method)	Heterozygosity (PLINK 26 autosomal scaffolds)	Proportion of genome in an ROH	Alternate ID names	SRA accession IDs
BR338	Minas Gerais state, Brazil (BR)	male	48x	0.00155	0.00101	0.06736	bPco338	SRR7639695-6
BR406	São Paulo state, Brazil (BR)	male	27x	0.00166	0.00103	0.03970	D406	SRR7542886-8
EVG21	Everglades National Park (EVG)	female	51x	0.00121	0.00091	0.34221	FP021, Pco-0075	SRR7660678-9
CYP47	Big Cypress National Preserve (CYP)	male	43x	0.00033	0.00032	0.58485	FP047, Pco-0423	SRR7664677-8
CYP51	Big Cypress National Preserve (CYP)	male	55x	0.00034	0.00034	0.56168	FP051, Pco-0428	SRR7956993-4
YNP198	Yellowstone National Park (YNP)	male	40x	0.00090	0.00077	0.15873	M198	SRR7610940-1
SMM12	Santa Monica Mountains (SMM)	male	46x	0.00079	0.00073	0.18884	P12	SRR7661934-5
SMM13*	Santa Monica Mountains (SMM)	female	40x	NA	NA	NA	P13	SRR7690239-40
SMM22	Santa Monica Mountains (SMM)	male	34x	0.00059	0.00049	0.417637	P22	SRR7543017-8
SC29	Santa Cruz Mountains (SC)	female	35x	0.00062	0.00058	0.32707	29F	SRR7537344-5
SC36	Santa Cruz Mountains (SC)	male	47x	0.00049	0.00050	0.33987	36M	SRR7148342-54

Supplementary Table 4: ROH HMM parameters. Parameters used as input into the HMM ROH script for each mountain lion.

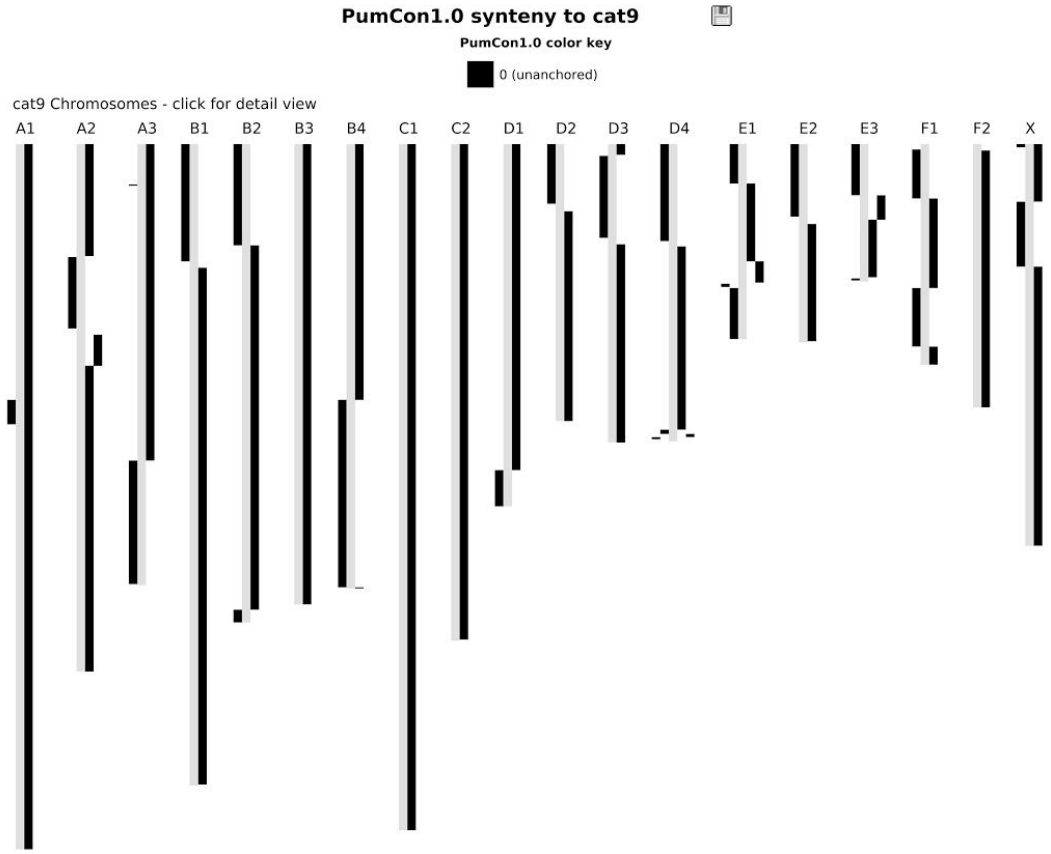
Sample	Genotyping error rate	Outbred heterozygosity
BR338	0.000146	0.0019
BR406	0.000054	0.0018
EVG21	0.000071	0.0019
CYP47	0.000078	0.0011
CYP51	0.000071	0.0010
SC29	0.000078	0.0012
SC36	0.000027	0.0010
SMM12	0.000075	0.0012
SMM22	0.000078	0.0012
YNP198	0.000087	0.0012

Supplementary Table 5: ROH pairwise IBD values. Percent of the genome in an IBD ROH between pairs of mountain lions as shown in Fig. 4D.

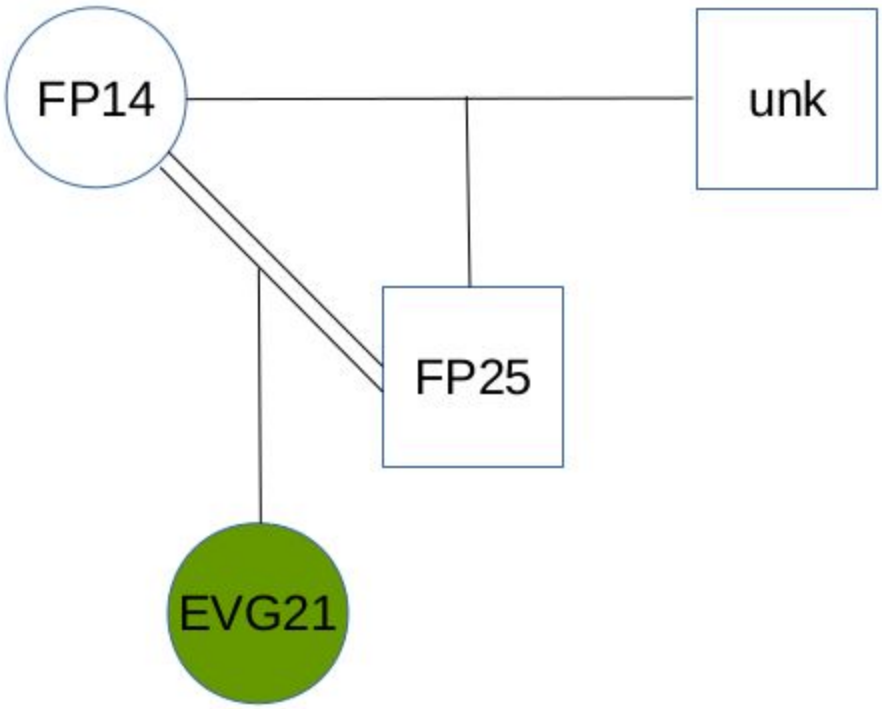
	BR338	BR406	EVG21	CYP47	CYP51	SC29	SC36	SMM12	SMM22	YNP198
BR338	6.74%									
BR406	0.00%	3.97%								
EVG21	0.00%	0.00%	34.22%							
CYP47	0.00%	0.00%	8.72%	58.49%						
CYP51	0.00%	0.00%	6.85%	35.87%	56.17%					
SC29	0.00%	0.00%	0.19%	1.17%	1.07%	32.71%				
SC36	0.00%	0.00%	0.09%	0.70%	0.70%	11.55%	33.99%			
SMM12	0.00%	0.00%	0.00%	0.60%	0.48%	2.96%	2.72%	18.88%		
SMM22	0.00%	0.00%	0.09%	0.92%	0.76%	5.10%	4.16%	4.39%	41.76%	
YNP198	0.00%	0.00%	0.18%	0.30%	0.22%	0.56%	0.16%	0.67%	0.56%	15.87%



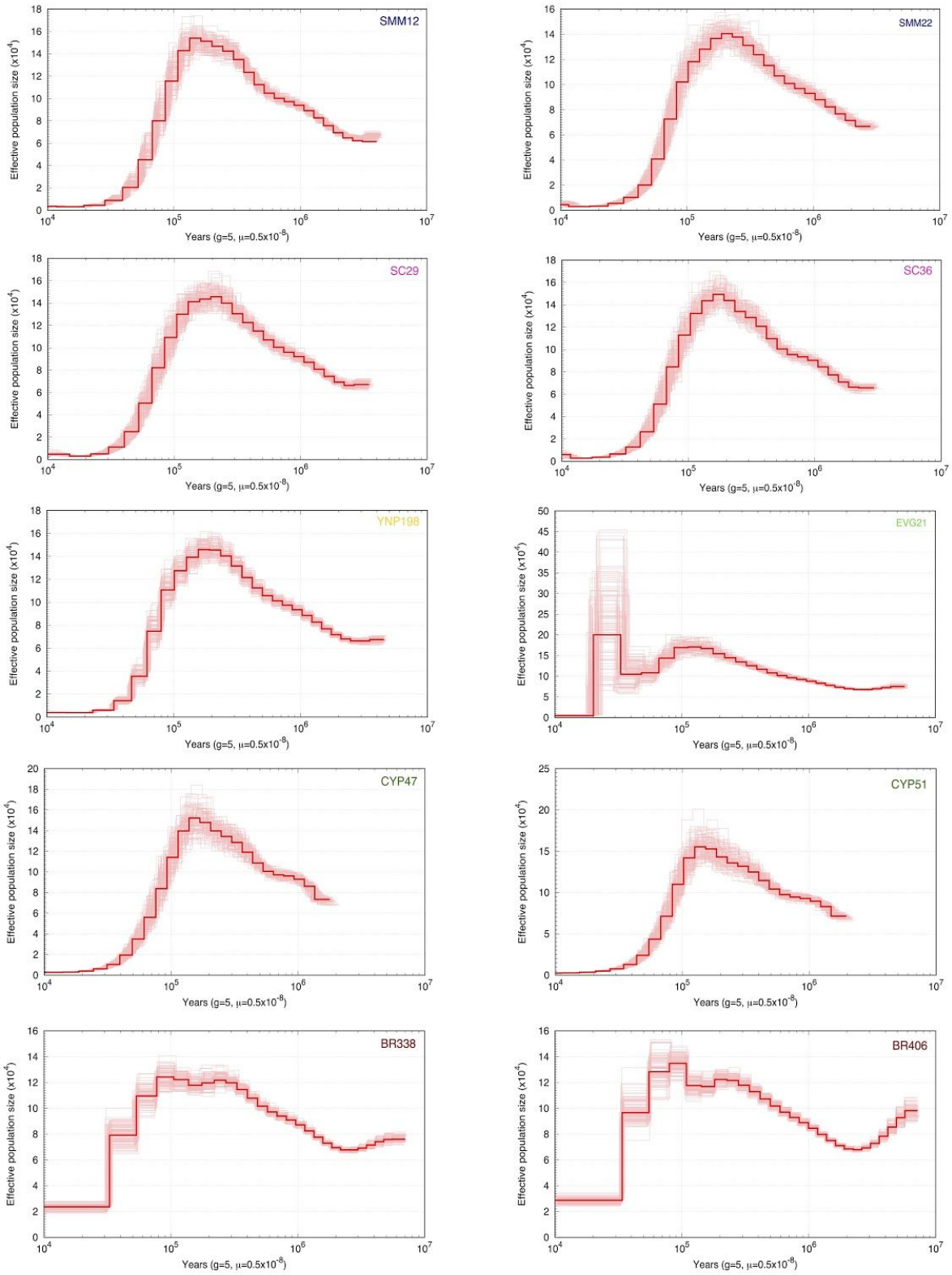
Supplementary Figure 1: Linkage map of HiRise genome assembly for SC36. The x and y axes mark the mapping positions of the first and second read in a read pair of the Hi-C library respectively, grouped into bins which represent scaffolds. Each square is colored according to the number of read pairs within the bin. Scaffolds less than 1 Mb in length are not shown.



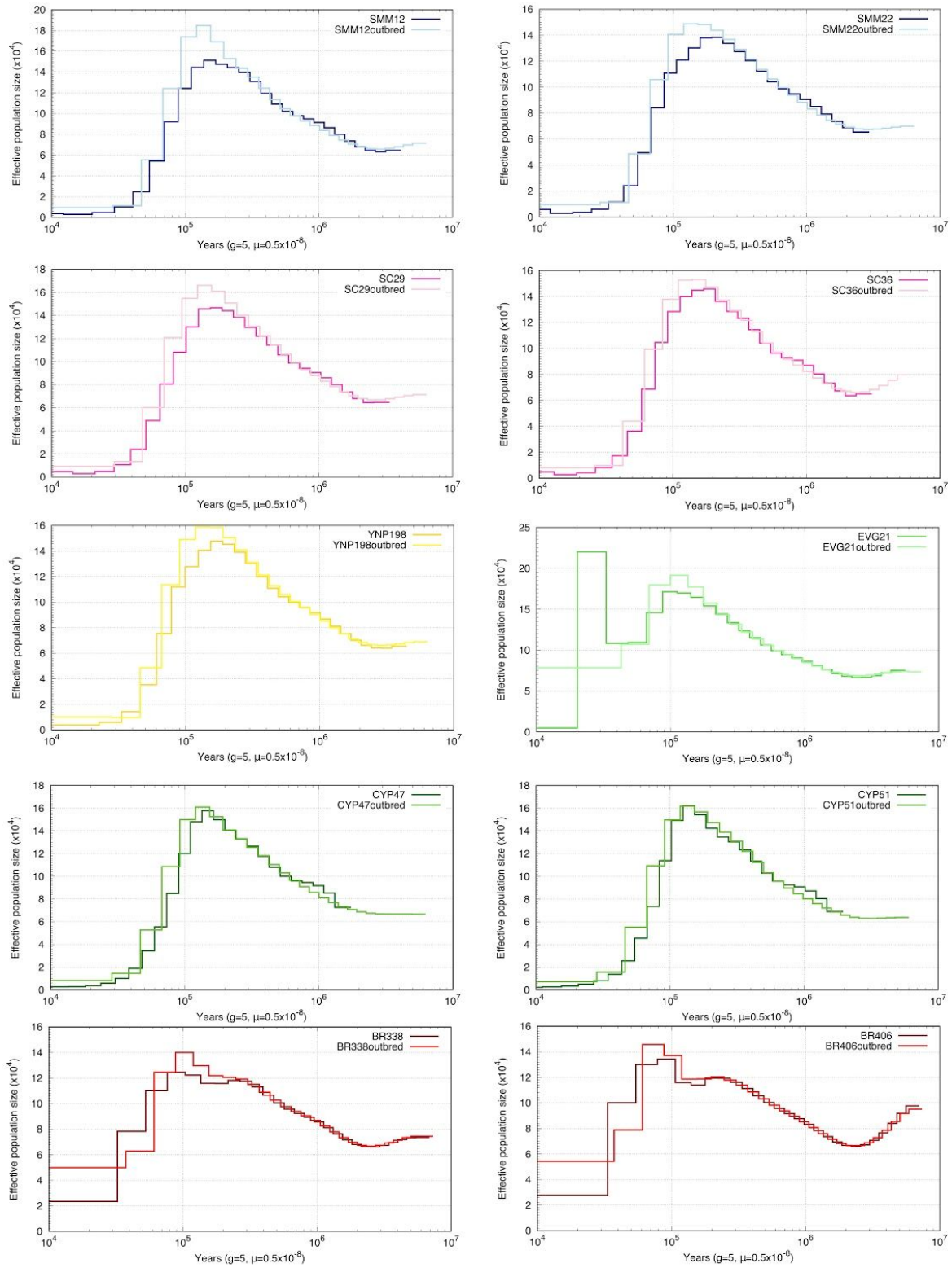
Supplementary Figure 2: Synteny between PumCon1.0 and Felis catus 9.0 genome.
Alignment of syntenic regions of the Felis catus 9.0 (domestic cat, gray) genome assembly with our PumCon1.0 mountain lion assembly (black) using SyMap2[2]. Four of the scaffolds in our assembly mapped to the entirety of a chromosome in the cat genome.



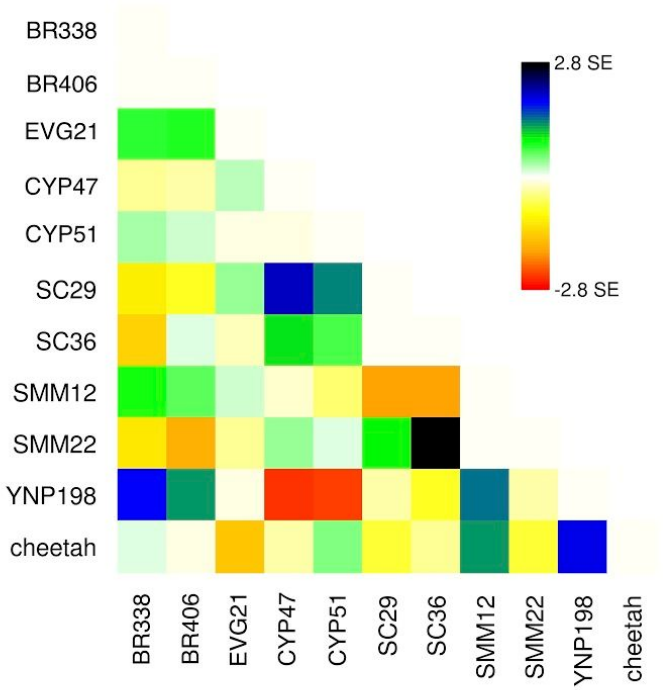
Supplementary Figure 3: Pedigree of EVG21. Pedigree of the inbred and admixed Florida panther sequenced from Everglades National Park[3]. All panthers in the pedigree are of Everglades ancestry. The Central American admixture into this population occurred approximately 6-9 generations prior to these individuals[3]. Note: EVG21 is referred to as FP021 in the original dataset[3].



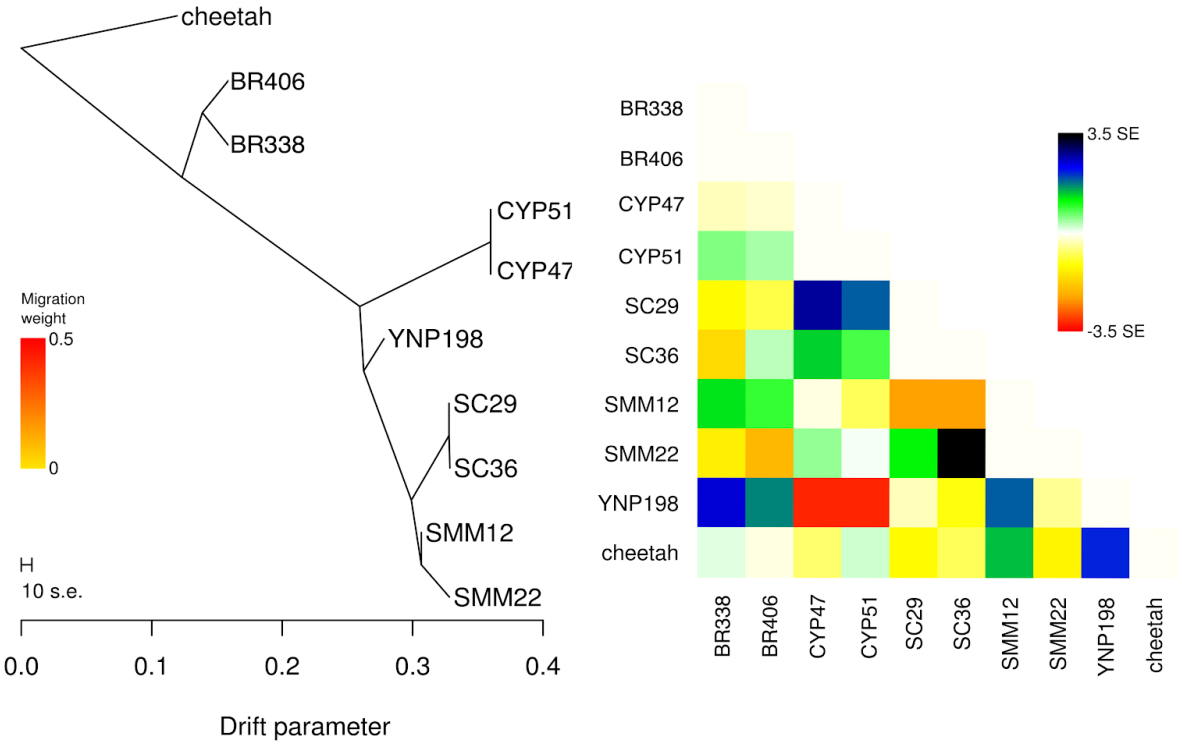
Supplementary Figure 4: Bootstrap replicate PSMC plots for ten mountain lions. We ran one hundred bootstrap replicates for each of the mountain lions using the PSMC model[4]. Generation time is 5 years, and per generation mutation rate is 0.5e-8[5].



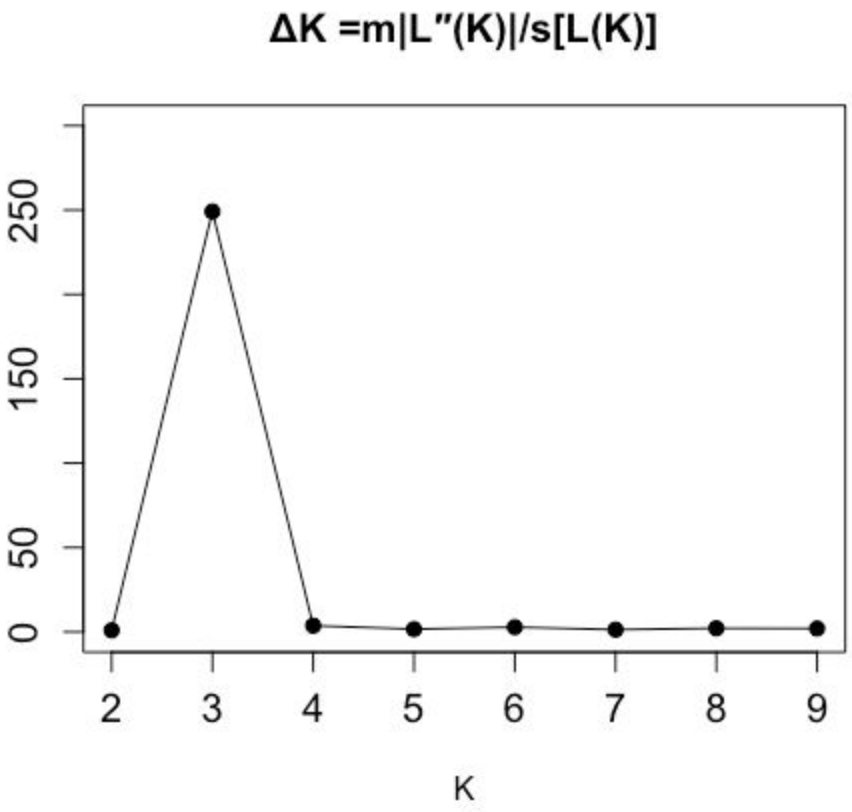
Supplementary Figure 5: PSMC plots for outbred (non-ROH) and entire genomes of the ten mountain lions. We saw no significant difference between the two models[4]. Generation time is 5 years, and per generation mutation rate is 0.5e-8[5].



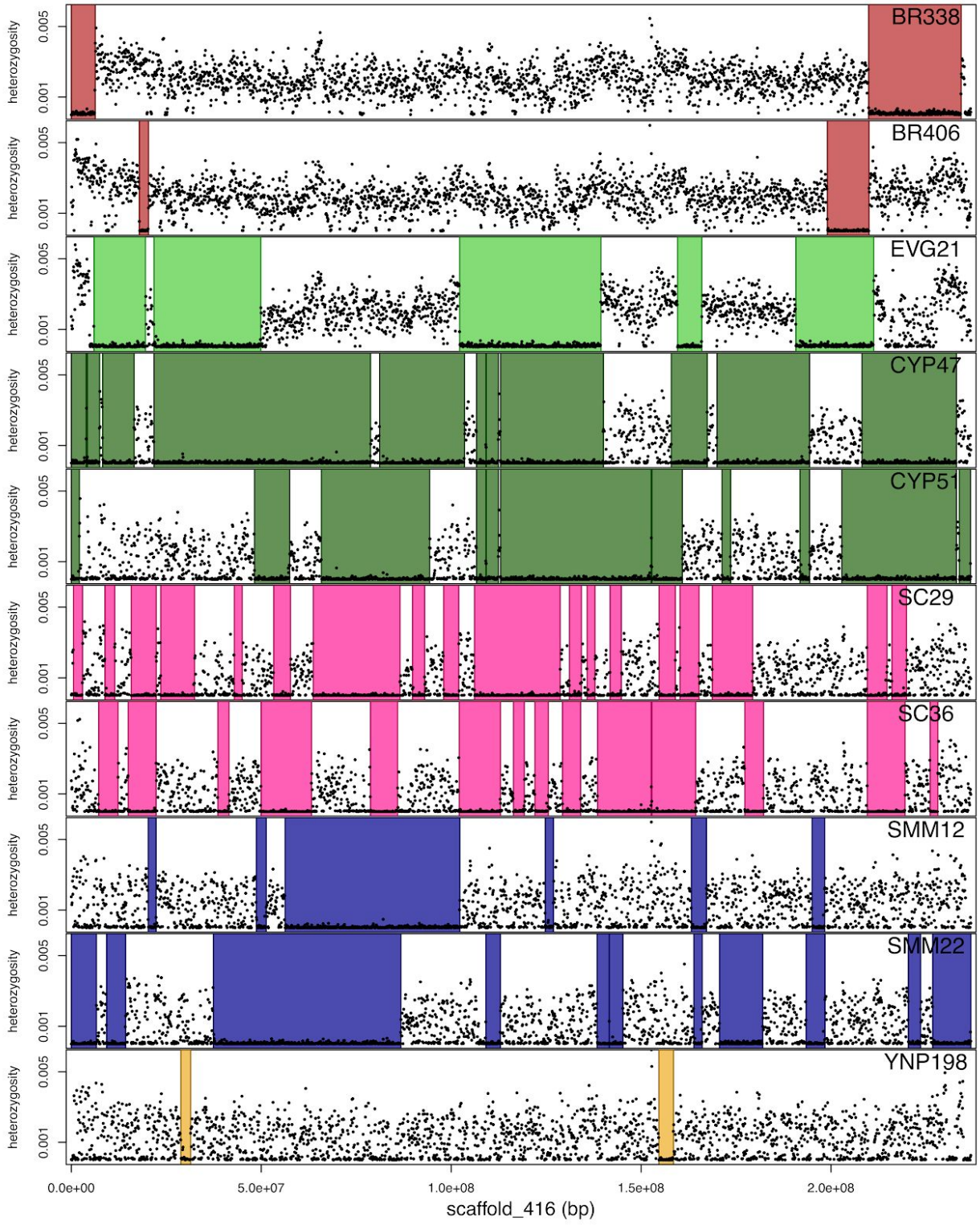
Supplementary Figure 6: TreeMix residual fit of the model. TreeMix[6] run on LD filtered variant file using ten mountain lions and African cheetah, 1 migration, and k =5000, where 99.91% of the variation is explained.



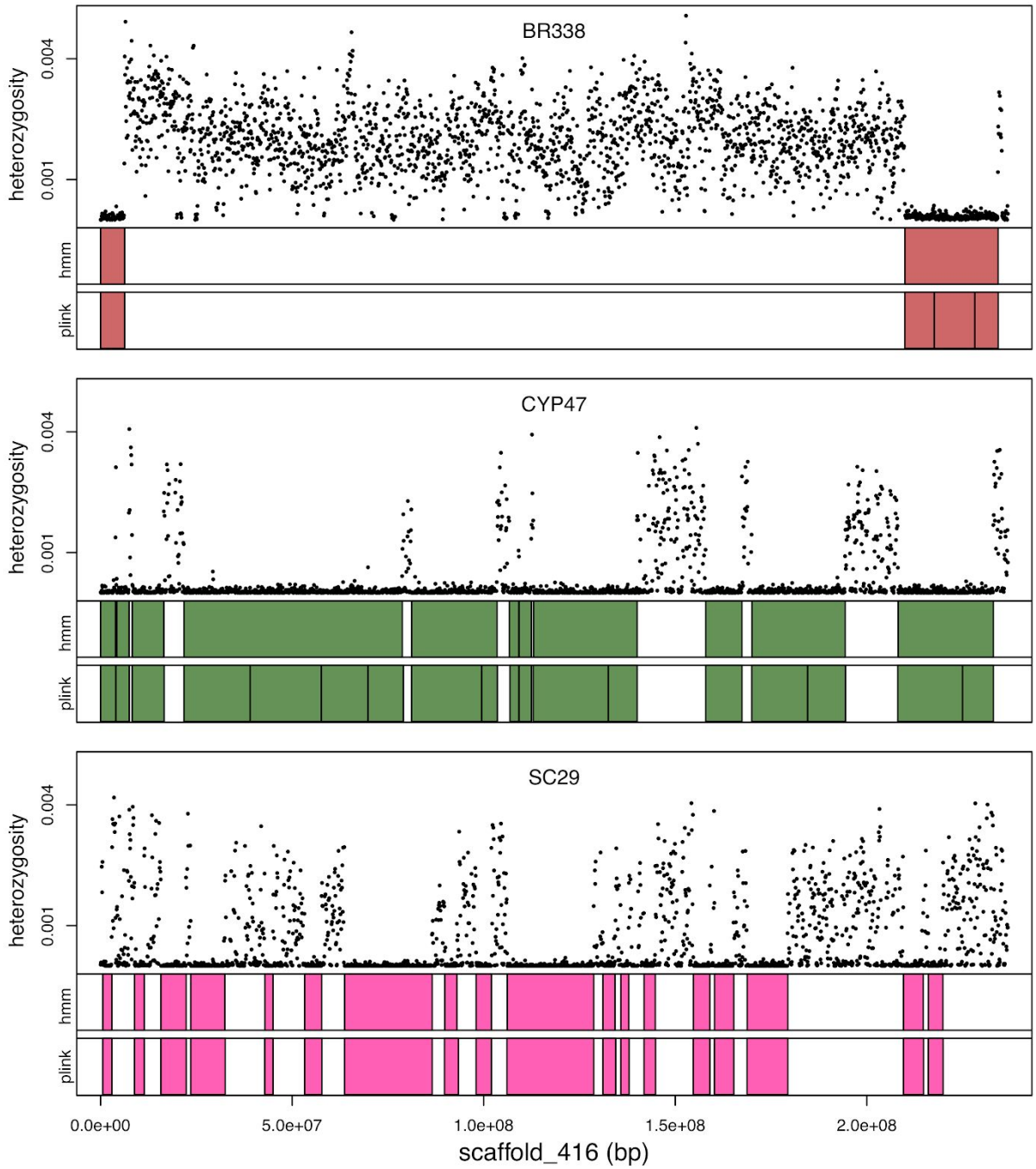
Supplementary Figure 7: TreeMix without EVG21. Best result of TreeMix[6] run on LD filtered variant file using nine mountain lions and African cheetah, no migrations, and k= 5000, where 99.91% of the variation is explained.



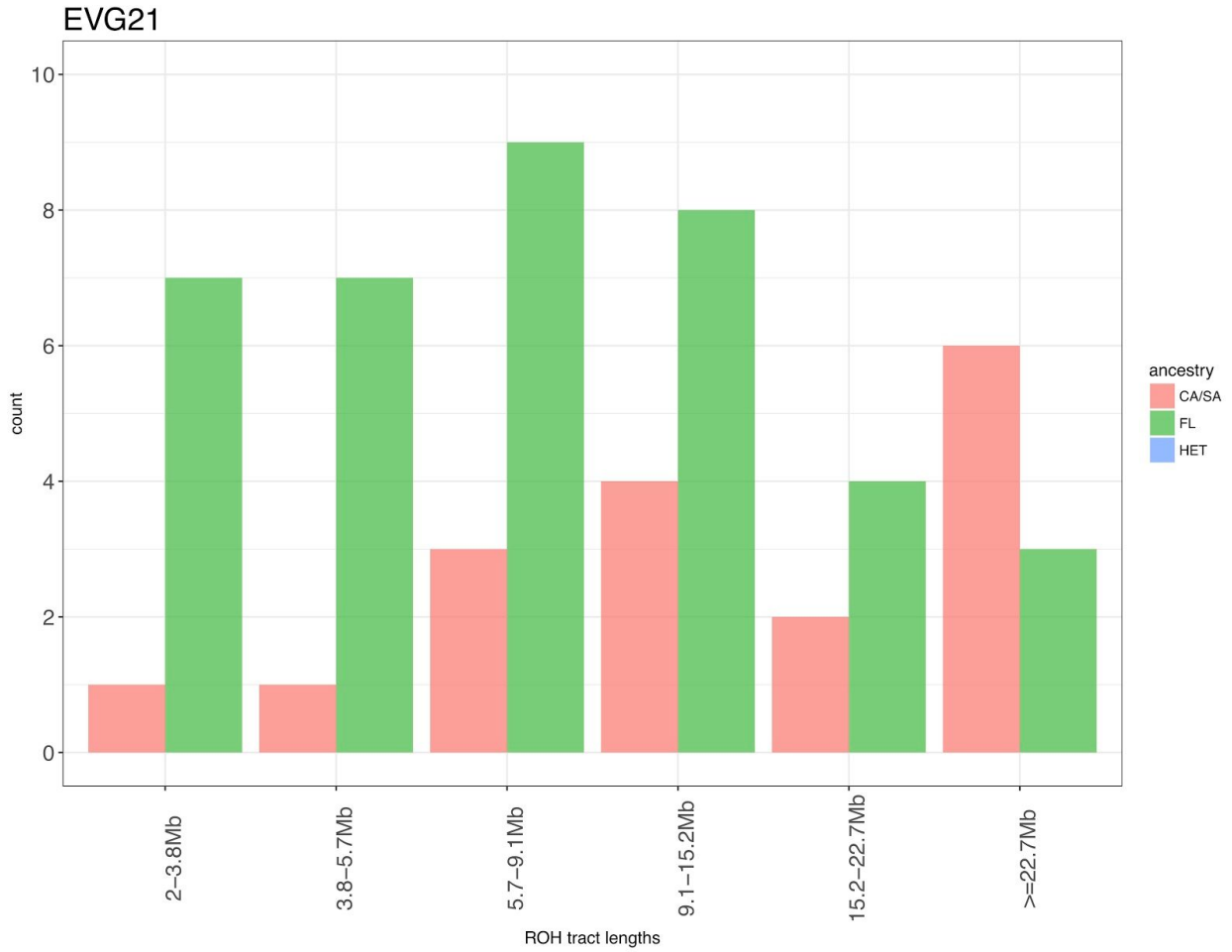
Supplementary Figure 8: Selection of best K in STRUCTURE. Using delta K[7], the rate of change in the log probability of data between successive K values, we identified three as the best K for our panel.



Supplementary Figure 9: ROH for all ten mountain lions as called by our ROH HMM. Black dots represent average heterozygosity in 100 kb windows; colored regions represent blocks called as ROH. Scaffold_416 represents the largest scaffold in the genome assembly at 236.8 Mb.



Supplementary Figure 10: ROH calls using two different methods. Top panel for each sample shows heterozygosity in 100 kbp windows. Bottom panels for each sample shows colored boxes indicating ROH called using our ROH HMM (top), and PLINK (bottom). PLINK tended to break up long tracts of ROH. Given that we were interested in the distribution of ROH lengths, we decided to use our HMM for ROH calls for further analyses.



Supplementary Figure 11: Ancestry of ROH in EVG21 genome. We assigned ancestry to the haploid fasta sequence of EVG21 using an ancestry HMM, subdividing the ancestry into three types: homozygous Central/South American ancestry, heterozygous ancestry, or homozygous Floridian ancestry. We identified which ancestry type each ROH > 2 Mb. We observed no ROH greater than 2 Mb in length in heterozygous ancestry regions for EVG21. Admixture effectively rescued the long tracts of homozygosity. We still saw ROH of greater than 2 Mb for both homozygous Central/South American ancestry and homozygous Floridian ancestry, both at a significant occurrence relative to the occurrence of heterozygous ancestry ROH.

References

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7. Evanno, G., Regnaut, S. & Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14,** 2611–2620 (2005).