

Figure S1 – Trait analysis in surface, Pachón, surface-Pachón F1 and surface-Pachón F2 fish.

(A) 24-hour sleep profile depicting the amount slept at 1-hour intervals over a 24-hour time frame. Black = surface fish, Grey = F1 hybrids, White = Pachón cavefish. Shaded areas of the graph represent when lights were off. (B) Bout number (Kruskal-Wallis: $H_2=94.36$, $p<0.0001$; Dunn's multiple comparisons post hoc test: SF vs. F1: $z=3.243$, $p=0.0071$, Pa vs. F2: $p>0.9999$. All other: $p<0.0001$). (C) Bout duration (Kruskal-Wallis: $H_2=37.35$, $p<0.0001$; Dunn's multiple comparisons post hoc test: F1 vs. F2: $z=1.418$, $p=0.9372$, Pa vs. F1 and Pa vs. F2: $p>0.9999$. All other: $p<0.0001$). (D) Comparison of bout duration and about number in F2 hybrid crosses. (Spearman-rank correlation: $r=0.3604$, $p<0.0001$; linear regression is included as a descriptive: slope=0.1636.) (E) Image of 22dpf fish with adipose where Nile Red stain is pseudocolored green (scale=1mm). (F) Image of 22dpf fish without adipose (scale=1mm). Graphs (B) and (C) are representations of median \pm quartile.

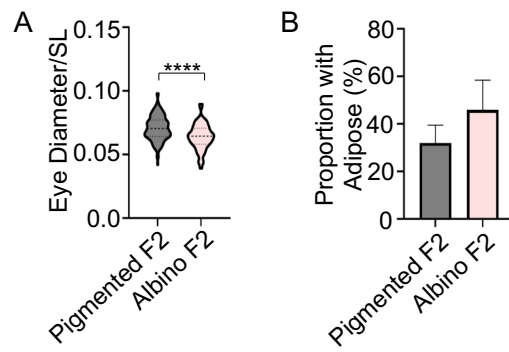


Figure S2 – Relationship between albinism and morphological traits in cave-surface F2 hybrid fish. (A) Eye diameter in albino vs. pigmented F2 hybrids, corrected for standard length (Mann-Whitney, $u=6211$, $p<0.0001$). This graph is a representation of median \pm quartile. (B) Proportion of pigmented and albino F2 hybrid individuals with adipose. Fisher's Exact Test. Error bars calculated using z^* -value of 1.96 and denote the margin of error of the sample proportion. (Fishers Exact tests: $p=0.0805$).

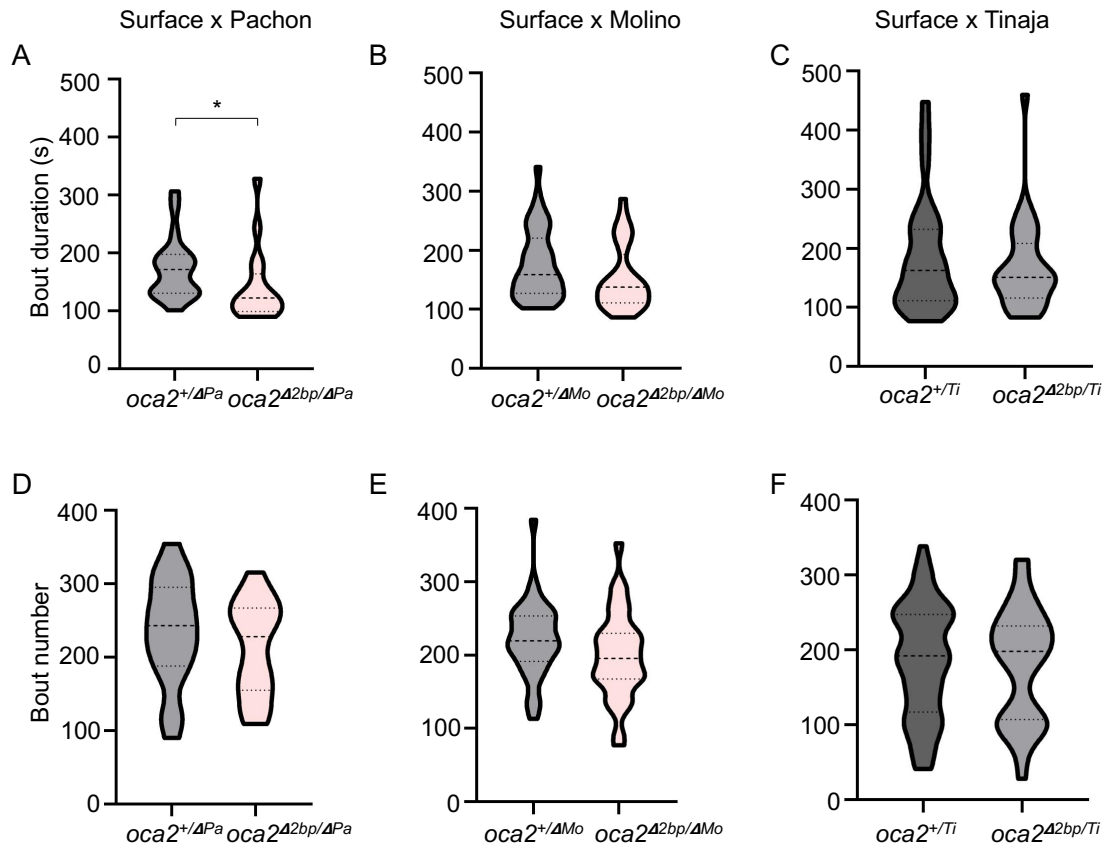


Figure S3 – Sleep architecture in surface-cave hybrid fish harboring an engineered *oca2* mutation. Average bout duration (A-C) and total number of bouts (D-F) were assessed in Pachón-surface F1 hybrids (A, D), Molino-surface F1 hybrid (B,E) and Tinaja-surface F1 hybrid fish (C,F). Average bout duration in (A) *oca2*^{+/ΔPA} (n=21) compared to *oca2*^{Δ2bp/ΔPA} (n=17) siblings (Mann-Whitney, U=93, p=0.0114). (B) *oca2*^{+/ΔMo} (n=38) compared to *oca2*^{Δ2bp/ΔMo} (n=32) siblings (Mann-Whitney, U=447, p=0.0581). (C) *oca2*^{+/Ti} (n=56) compared to *oca2*^{Δ2bp/Ti} (n=33) siblings (Mann-Whitney, U=884, p=0.7386). Number of bouts in (D) *oca2*^{+/ΔPA} (n=21) compared to *oca2*^{Δ2bp/ΔPA} (n=17) siblings (t-test, t=0.9381, p=0.3544). (E) *oca2*^{+/ΔMo} (n=38) compared to *oca2*^{Δ2bp/ΔMo} (n=32) siblings (t-test, t=1.631, p=0.1075). (F) *oca2*^{+/Ti} (n=56) compared to *oca2*^{Δ2bp/Ti} (n=33) siblings (t-test, t=0.2707, p=0.7873).

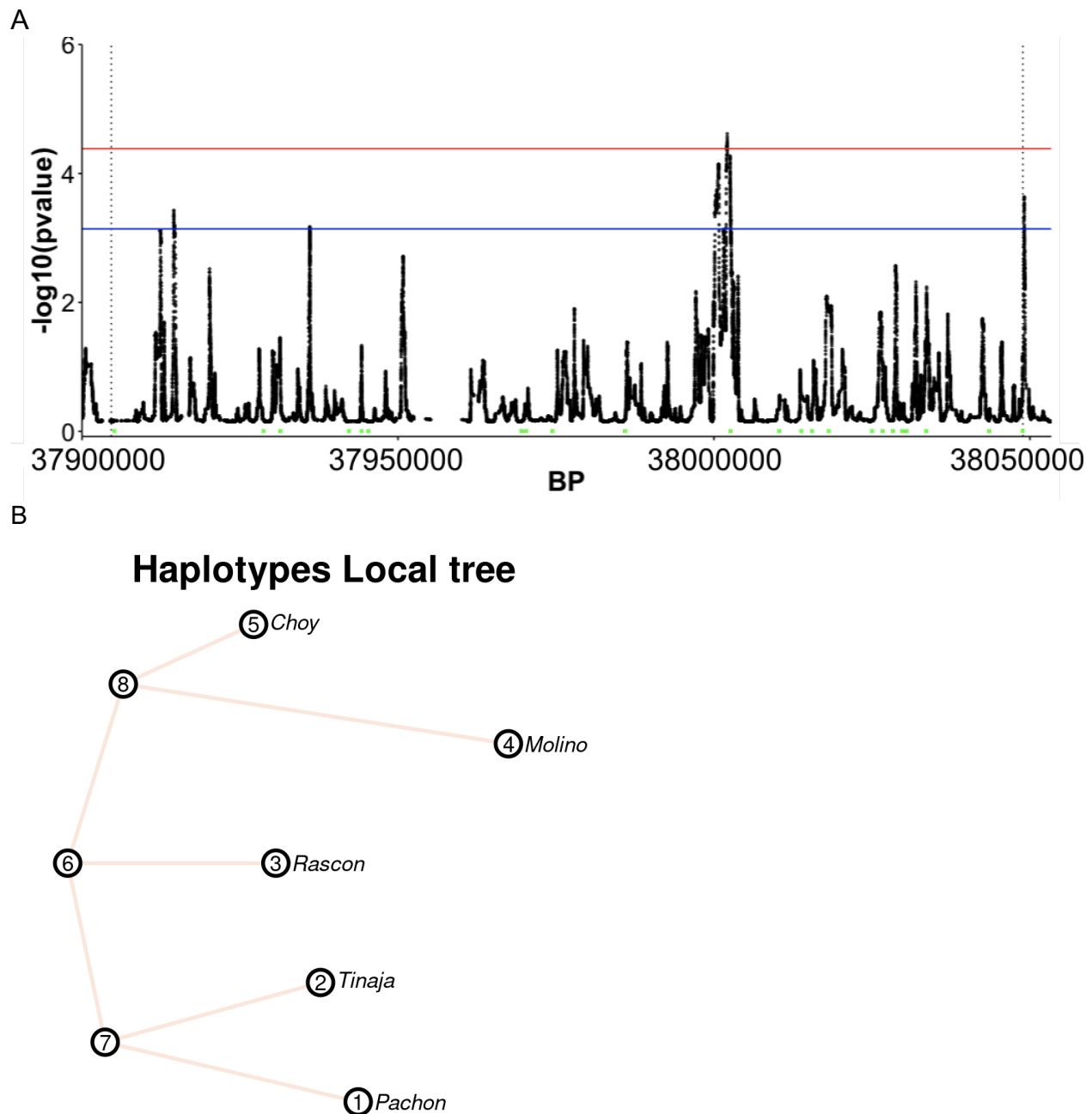


Figure S4 - Population genetics analysis at the *oca2* locus. (A) hapFLK p-values across *oca2* (region within dotted lines). Red line = 1% FDR cutoff. Blue line = 5% FDR cutoff. The 24 exons are shown in green boxes at the bottom of the plot. P-values were plotted along the antisense strand, so exon 24 is on the left end of the plot, near 37,900,000 bp, and exon 1 is on the right end of the plot near 38,050,000 bp. Peaks above the 1% FDR cutoff are present at exon 14 and a peak above the 5% FDR cutoff is present at exon 1.

(B) Local population tree for the *oca2* region of chromosome 13 (37,904,635-38,048,888 bp) using Reynolds distances based on haplotype frequencies.

Table S1 - P-values for branches within the haplotype-based population tree. Branch segments occur between nodes 1-8, corresponding to those shown in Supplementary Fig 4.

Branch	Std. Error	t-value	Pr(> t)	P-value
8<->4	-0.388	0.01322	-29.36	0.000087
8<->5	-0.118	0.01322	-8.96	0.002900
7<->1	-0.312	0.01322	-23.63	0.000170
7<->2	-0.266	0.01322	-20.09	0.000270
6<->7	-0.009	0.01536	-0.57	0.610000
6<->3	-0.215	0.01145	-18.8	0.000330
6<->8	-0.05	0.01536	-3.25	0.048000