

Learning from Whales: A Deep Dive in Marine Mammal Genetics

Background and Objective

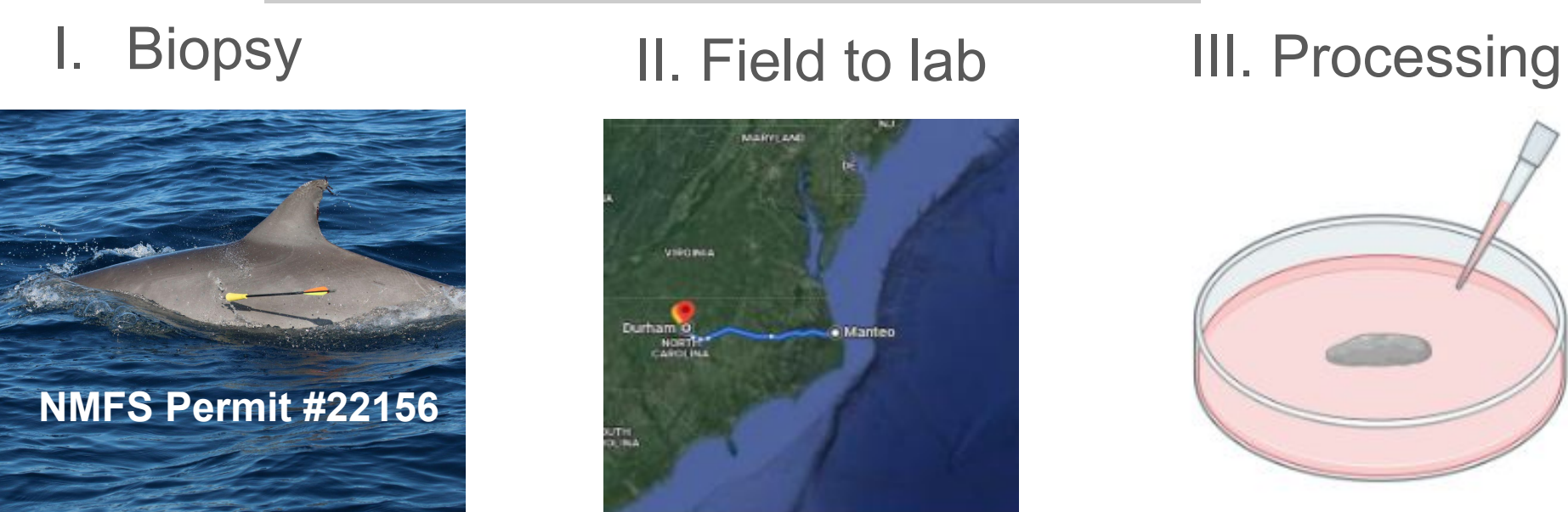
- Marine mammals exhibit physiological adaptations to low oxygen conditions (hypoxia) during prolonged breath holds
- Underlying cellular and molecular mechanisms remain largely unexplored
- Inshore and offshore dolphins have adapted into different ecotypes that can be differentiated through genetic analysis

Questions

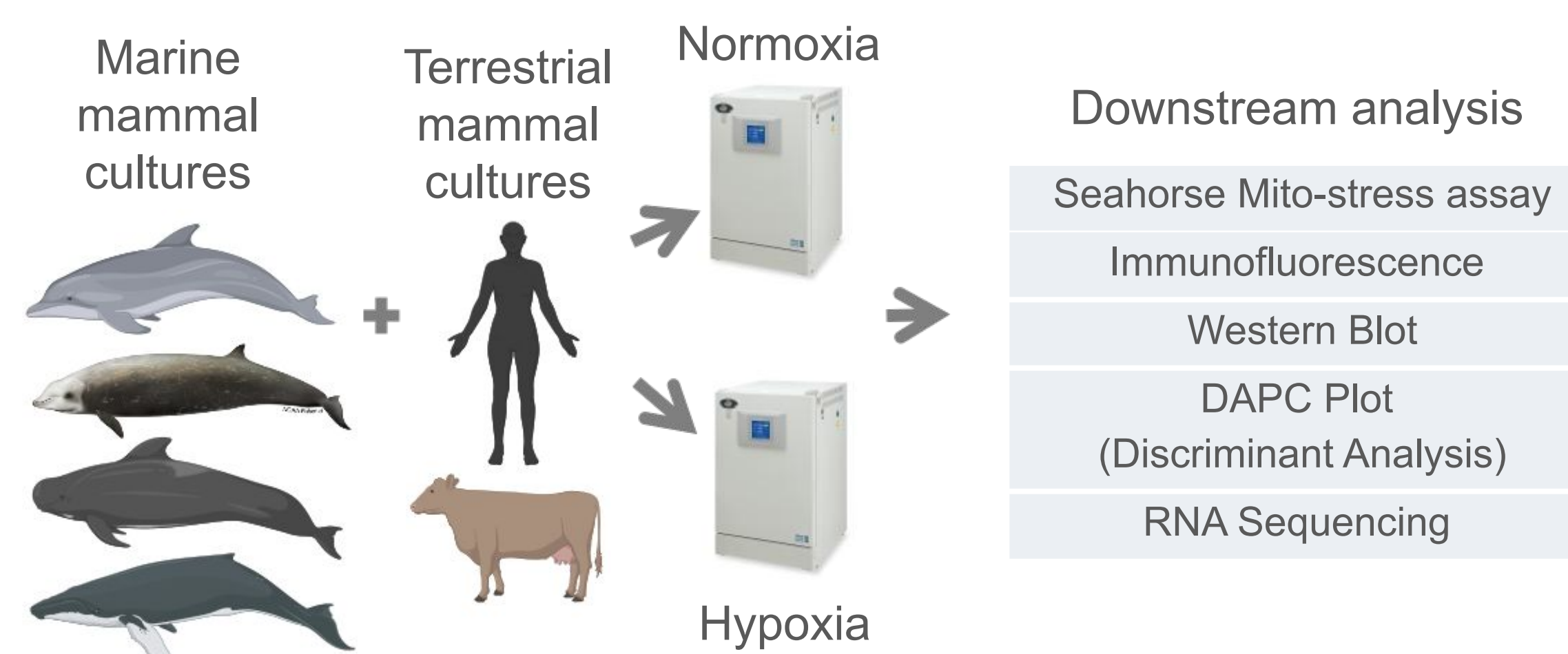
- What are the cellular and molecular mechanisms underlying marine mammal adaptations to hypoxia?
- How many genetically distinct groups exist within bottlenose dolphin populations in the Western North Atlantic?

Methods

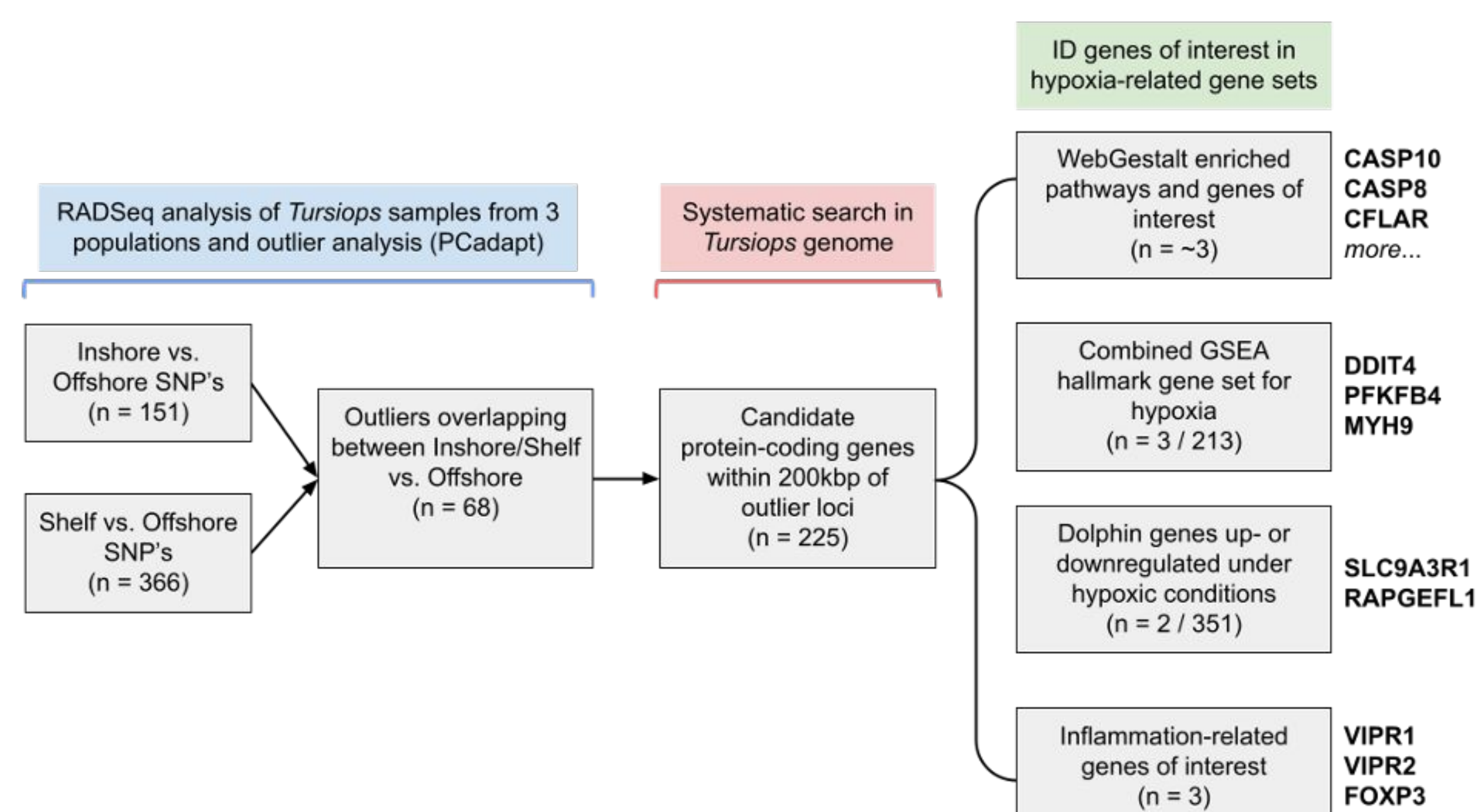
Cetacean Cell Cultures



Molecular Analysis



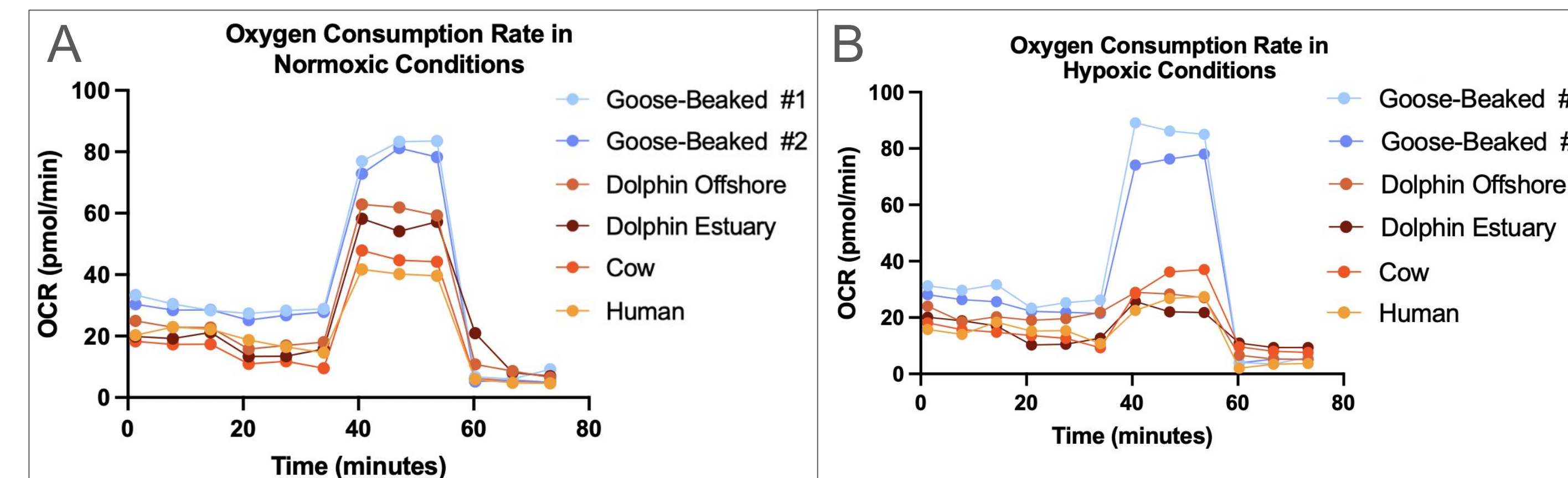
Identifying Hypoxia Candidate Genes



How do marine mammal genetics reflect the cellular adaptations of marine mammals to low oxygen conditions?

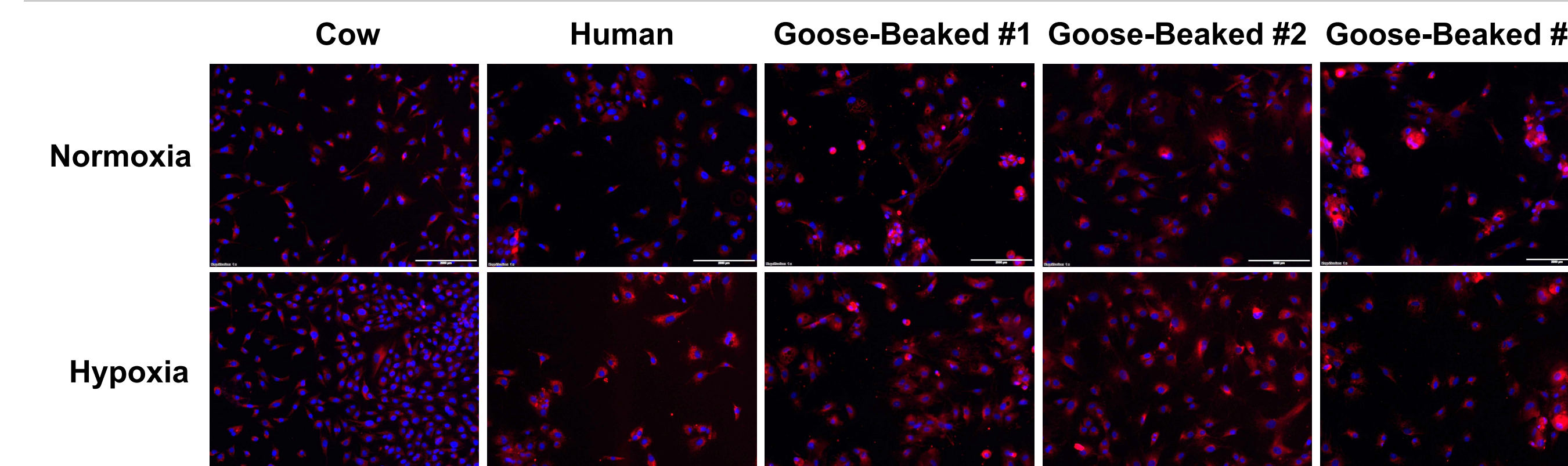
Results

Figure 1. Deep diving whales maintain high oxygen consumption rates under hypoxic conditions.



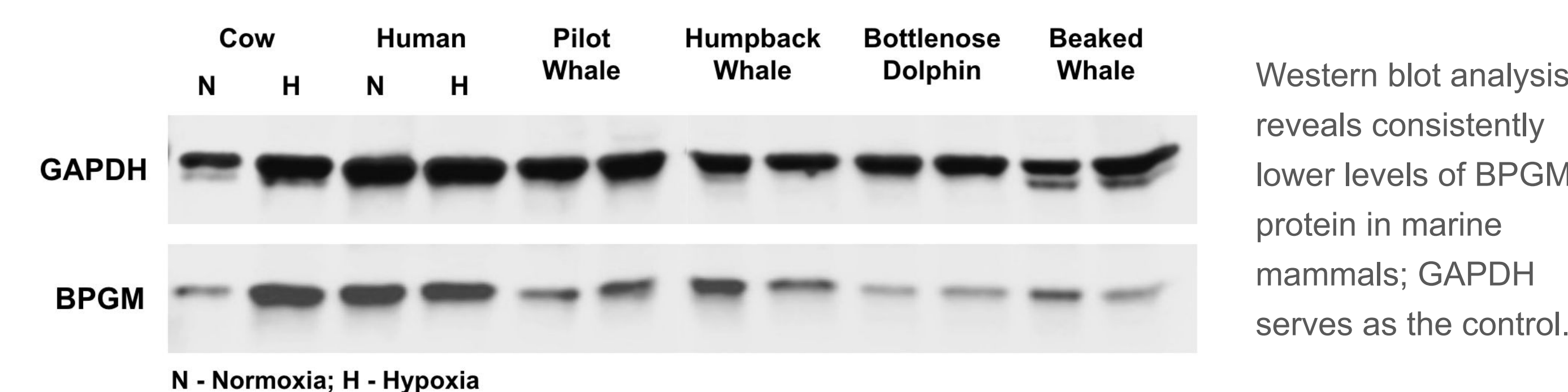
A. Seahorse Mito-Stress assay analysis showing higher oxygen consumption rates in Goose-beaked whale fibroblasts than terrestrial mammal and common bottlenose dolphin fibroblasts in normoxic conditions.
B. Seahorse Mito-Stress assay analysis showing maintained high consumption rate in Goose-beaked whale fibroblasts under hypoxic conditions while other species' fibroblasts lowered their oxygen consumption rate.

Figure 2. Goose-beaked whales maintain Tom20 protein levels under hypoxic conditions, while cows and humans experience an increase in these levels.



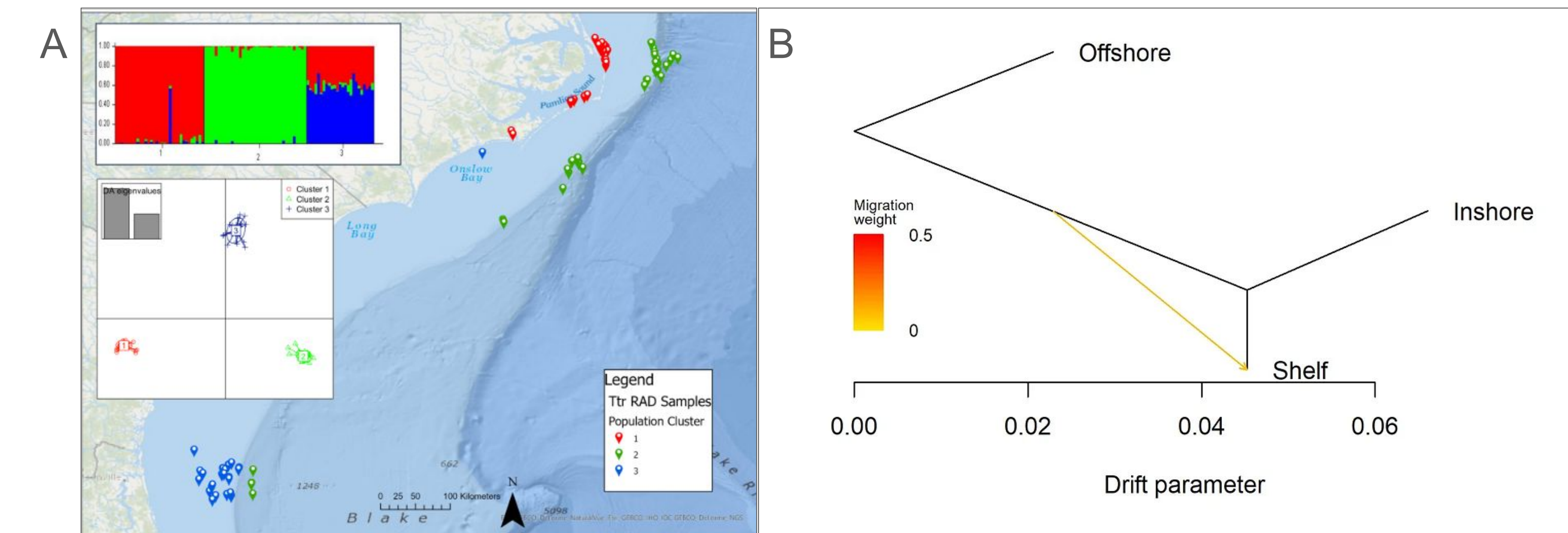
Immunofluorescence analysis demonstrates that Tom20 protein levels increase in cows and humans under hypoxic conditions when compared to normoxic conditions. Goose-beaked whales maintain consistent levels of Tom20 protein under these conditions. Tom20 protein is shown as a red stain and nuclei are shown as the blue stain.

Figure 3. Marine mammals have lower levels of BPGM protein (related to hemoglobin's oxygen affinity) than terrestrial mammals.



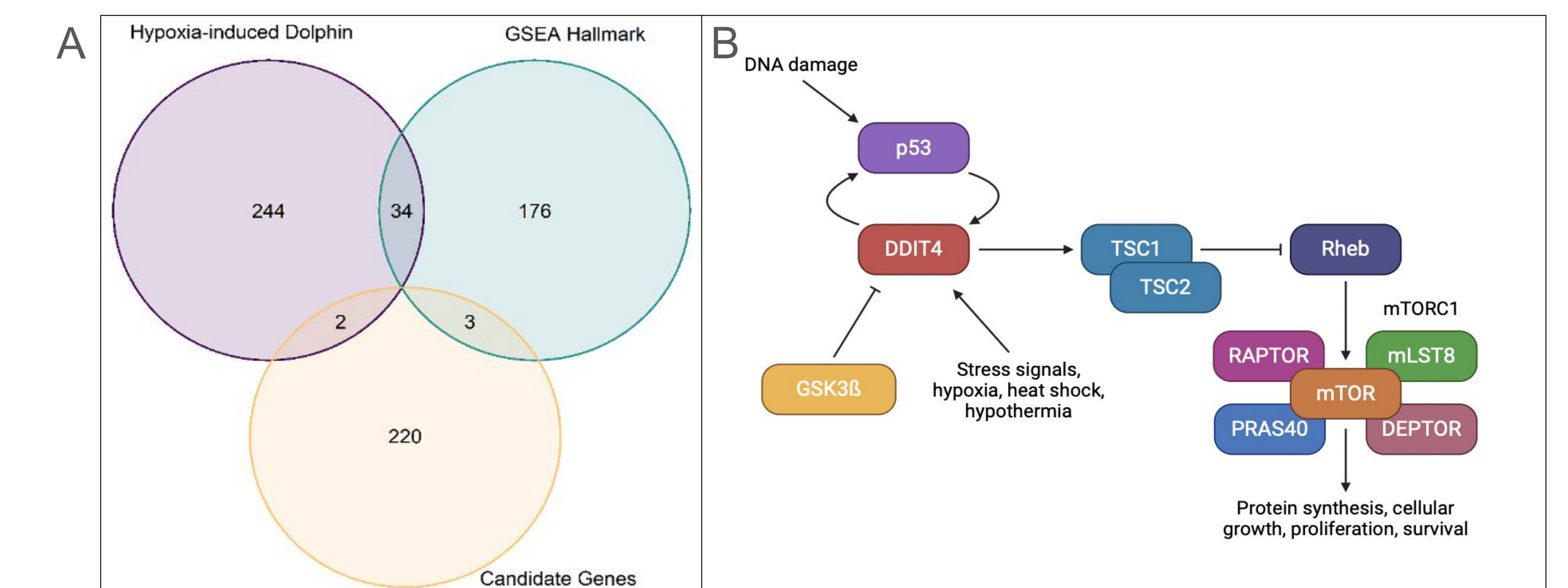
Western blot analysis reveals consistently lower levels of BPGM protein in marine mammals; GAPDH serves as the control.

Figure 4. There are 3 genetically distinct clusters of bottlenose dolphins in the Western North Atlantic.



A. Map, DAPC plot, and Structure plot showing the three genetically distinct clusters of *Tursiops truncatus/Tursiops erebennus* that were identified in the Western North Atlantic. Inshore bottlenose dolphins are shown in red, offshore are shown in green, and the coastal group living on primarily on the continental shelf is shown in blue. The points on the map identify where the biopsy sample of each individual was obtained.
B. Evolutionary tree generated by TreeMix that shows the relationships between the three genetically distinct clusters of bottlenose dolphins. The inshore (red) population mixed with the shelf (blue) population at some point in the past. The arrow indicates the direction of the mixture.

Figure 5. Genome-wide scan revealed candidate genes associated with hypoxia responses, including DDIT4.



A. Overlap of candidate gene set and hypoxia-related gene sets from dolphin RNASeq data and from GSEA hallmark gene set.
B. DDIT4 plays a crucial role in both p53-mediated and non-mediated pathways of mTOR inhibition, subsequently regulating cell growth and proliferation.

Summary and Future Directions

- There are significant cellular differences between marine and terrestrial mammals' responses to hypoxic conditions, suggesting important adaptive differences in respiration, oxygen affinity, and mitochondrial volume.
- Genetically distinct groups can be identified within bottlenose dolphin data by analyzing Single Nucleotide Polymorphisms (SNPs) across the genome, which can help inform management units and better understand these populations.
- These cellular analyses will be compared to similar respiration, oxygen affinity, and mitochondrial measures on cultured muscle and blood plasma cell lines from marine mammals.
- The bottlenose dolphin sample dataset will be expanded to include 52 stranded samples, then genetically analyzed to identify population structure within the inshore subpopulations

