MOLECULAR ECOLOGY

Supplemental Information for:

Vascularization underlies differences in sexually selected skin coloration in a wild primate

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In addition to the 38 photos taken at the start of anesthetization (hereafter, "baseline"; **Fig. 2**), we took 16 photos from a subset of adult males (*n*=13) and females (*n*=3) after a heat pack was applied to one side of the chest for 1 minute (hereafter "heat application"). Although we were unable to make statistically meaningful comparisons due to a smaller sample size of photos from anesthetized adult females (*n*=3), females seem to follow a similar pattern to males for change in redness between baseline and application of a heat pack directly to the chest skin (**Fig. S1**).

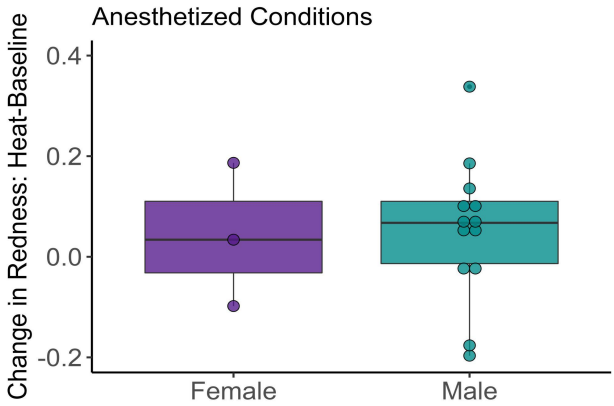

Figure S1. Adult males and females show a similar pattern in change in redness between baseline and application of a heat pack directly to the skin.

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Sample collection year (**Table S1**) is associated with the first and second principal components of gene expression which explained 78.2% and 15.5% of the variance, respectively (PC1: year β=0.39, *P*=0.02; PC2: year β=-0.22, *P*=0.003, **Fig. S2**). This variance could be a result of storage time, a batch effect of the RNAlater™ in which samples were stored, or temperature differences during shipment to the United States. This finding led us to include sample collection year in subsequent linear models of sex on the first and second principal component of gene expression.

Table S1. Sample collection by year, sex, and age category for the 36 samples used in analyses.

Year	Subadult female	Adult female	Subadult male	Adult male	Total
2017	2	6	1	8	17
2019	0	8	5	6	19

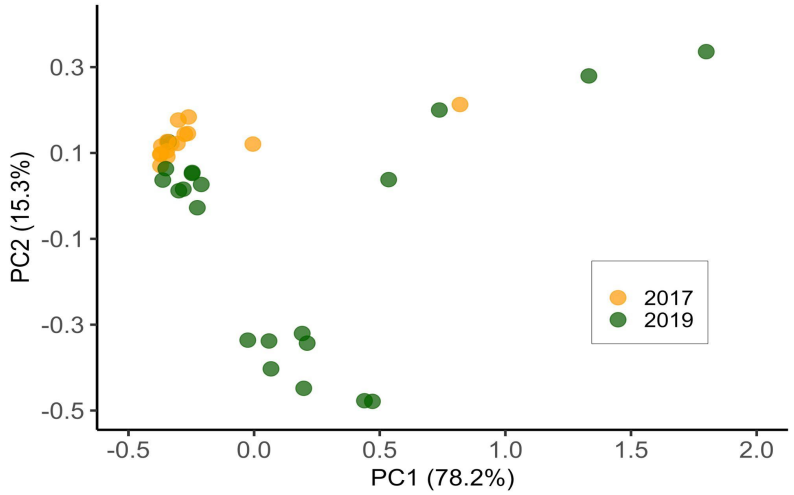

Figure S2. Sample collection year was significantly associated with the first and second principal component of gene expression.

When controlling for sample collection year, males and females differed along the first principal component of gene expression, which explained 78.2% of the overall variance in gene expression (**Fig. S3**). Year explained 12.2% of the variance and sex explained 13.1% of the variance in the first principal component of gene expression. We removed Y-linked genes from all analyses as they would be biased towards males. We did include X-linked genes in downstream analyses, but here we tested whether results of the sex difference in gene expression are influenced by X-linked genes. We found that the results of the principal component analysis did not change when we removed genes located on the X chromosome (**Fig. S4**).

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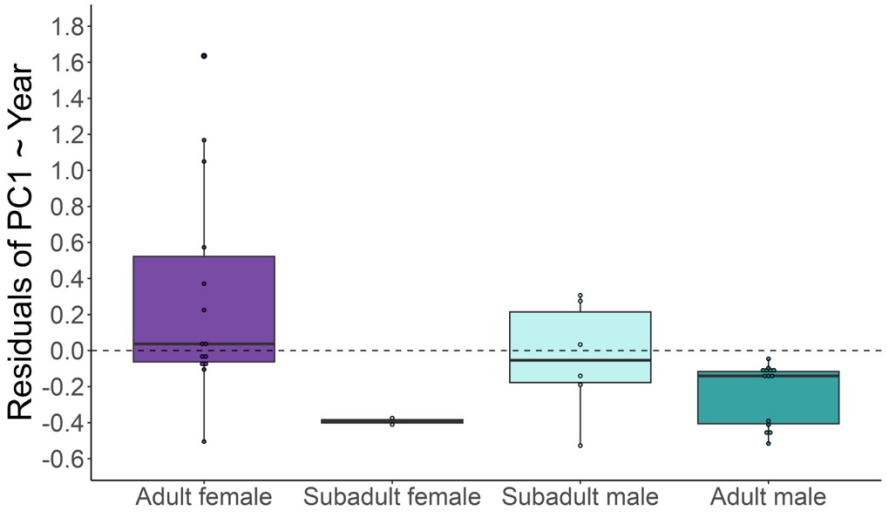

Figure S3. The first principal component of gene expression by age category while controlling for year of sample collection.

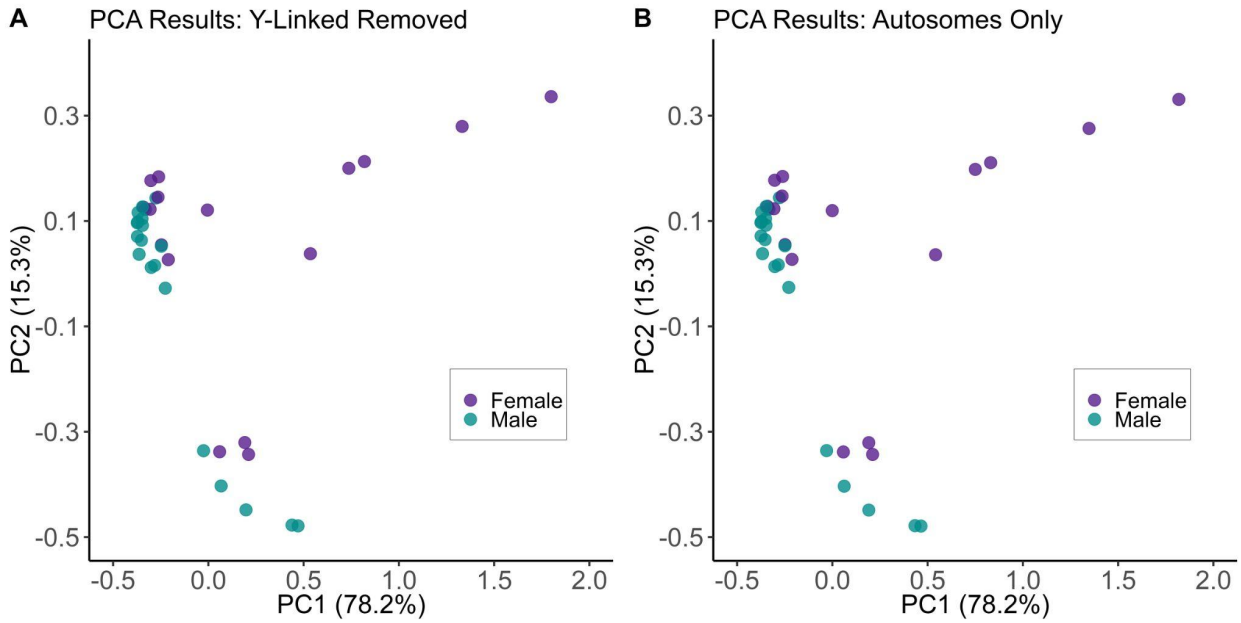

Figure S4. The results of the principal component analysis did not change between **A)** when we removed genes located on the Y chromosome and **B)** when we analyzed only those found on autosomes (both X-linked and Y-linked genes removed).

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After modeling the effect of sex on gene expression, we calculated a false discovery rate threshold (FDR) for each gene. We considered genes that passed a more permissive FDR threshold of 20% to be differentially expressed between the sexes as hypergeometric tests are (1) underpowered when few genes passing thresholds are included and (2) robust to false positives because tests focus on aggregate genes within pathways and incidences of false positives should be randomly distributed across pathways. We demonstrate below the set of genes that pass a FDR threshold of 5% (*n* genes=410), 10% (*n* genes=639), and 20% (*n* genes=1,068, **Fig. S5**).

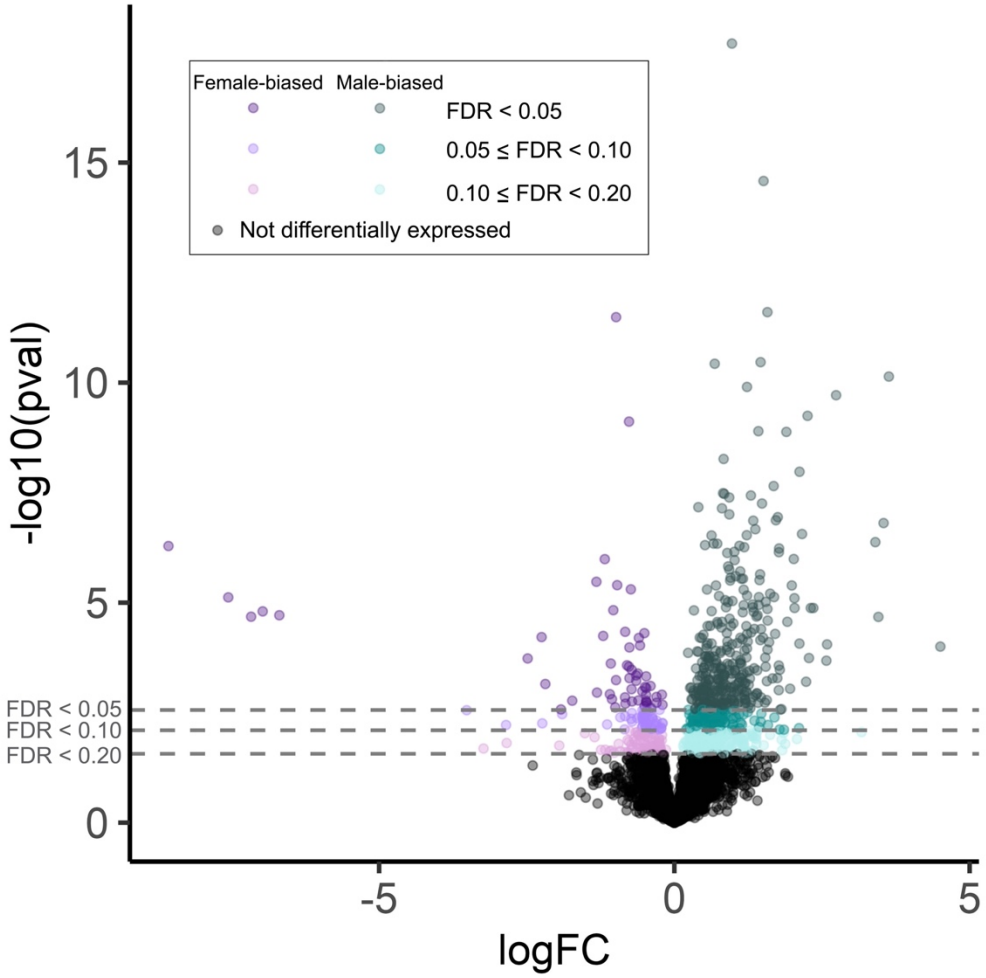

Figure S5. Of the 10,212 detectably expressed genes, 10.5% exhibited differential expression across males and females at a False Discovery Rate (FDR) threshold of 20% (*n* genes = 1,068). Volcano plot dots represent the *P* value versus the fold change (FC) for individual genes. The gray dotted lines represent the genes that pass a FDR threshold of 5%, 10%, and 20%. Genes with negative FC values are more highly expressed in females (female-biased) while genes with positive FC values are more highly expressed in males (male-biased).

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To test our prediction that males would exhibit increased expression of genes associated with blood flow, we searched for relevant terms (including the phrase "blood") and removed those not involved in blood pressure regulation and blood vessel maintenance (i.e. "establishment of blood-retinal barrier"). We compared the standardized effect of sex for these 149 blood pressure regulation and blood vessel maintenance genes to the standardized effect of sex for all other 10,066 detectably expressed genes with a Kolmogorov-Smirnov's test. Genes more highly expressed in males were enriched for biological processes associated with blood pressure regulation and blood vessel maintenance (K-S Test: D=0.19, P=2.59x10^{-5}). Enriched genes in the blood pressure regulation group included functions such as regulation of systemic arterial blood pressure while genes in the blood vessel maintenance group included regulation of blood vessel diameter, branching, remodeling, and development (**Fig. S6**).

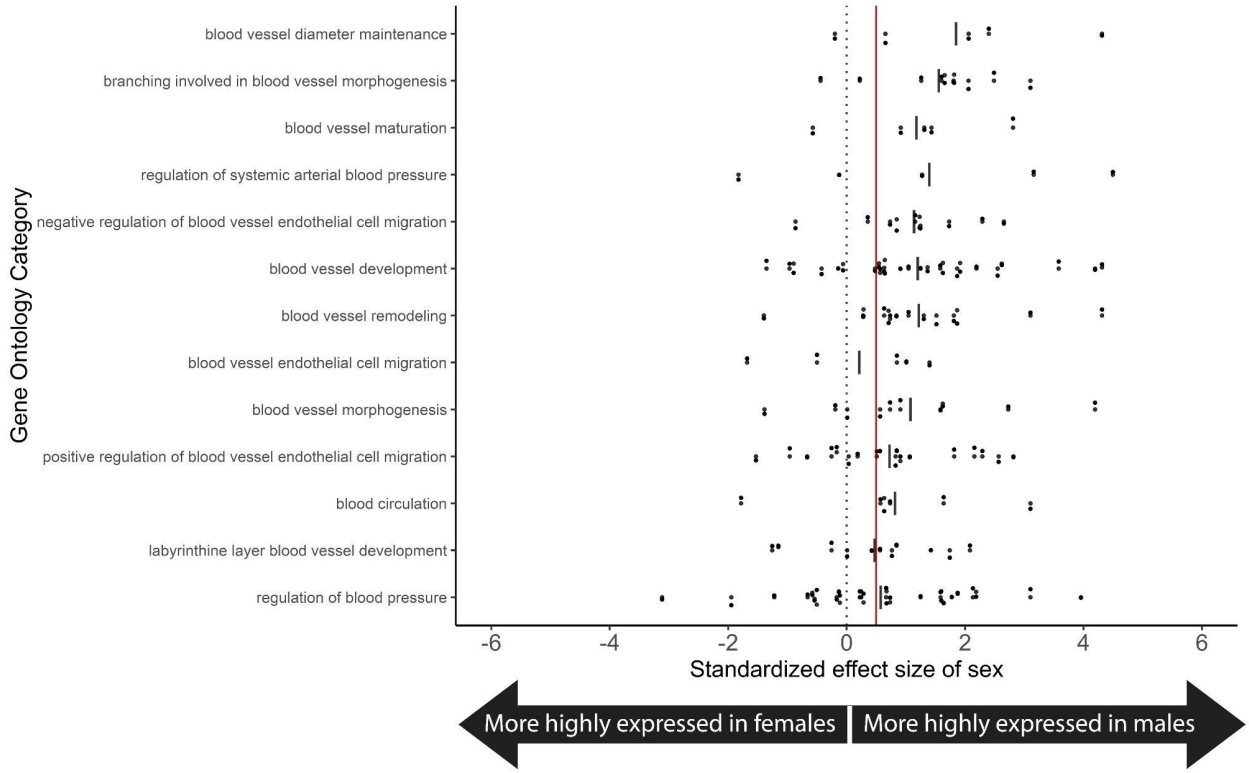

Figure S6. Genes more highly expressed in males were enriched for biological processes associated with blood pressure regulation and blood vessel maintenance. Beeswarm dots represent individual genes within the top 13 most represented subcategories of the blood pressure and blood vessel maintenance gene ontology category. The solid vertical lines reflect the average standardized effect of sex for genes for each subcategory. The solid red line represents the median standardized effect size of sex for all genes not in blood pressure regulation or blood vessel maintenance associated categories.

Although we did not find evidence for increased expression of genes associated with estrogen or androgen regulation in males, we further investigated whether the average standardized expression of these genes correlated with chest redness at baseline while under anesthesia within females or within males. For the subset of individuals for which we had matched skin biopsies and chest redness data at baseline while under anesthesia (*n*=10 males, *n*=8 females), we Z-transformed expression values for each set of genes associated with PPI networks for ERα, ERβ, and AR across these 18 individuals and averaged Z-transformed expression levels of these genes per individual to obtain a composite ERα, ERβ, and AR gene expression score for each individual. We then ran three linear regression models within females and three linear regression models within males with expression score as the outcome variable and chest redness as the predictor variable. We did not find a correlation between average standardized sex-bias gene expression level of ERα, ERβ, or AR and chest redness within females (ERα: β=3.75, *P*=0.28; ERβ: β=3.47, *P*=0.29; AR: β=2.89, *P*=0.38) or within males (ERα: β=0.48, *P*=0.21; ERβ: β=0.45, *P*=0.26; AR: β=0.47, *P*=0.31; **Fig. S7**). The small sample sizes and the impact of anesthesia on chest redness may have influenced these results.

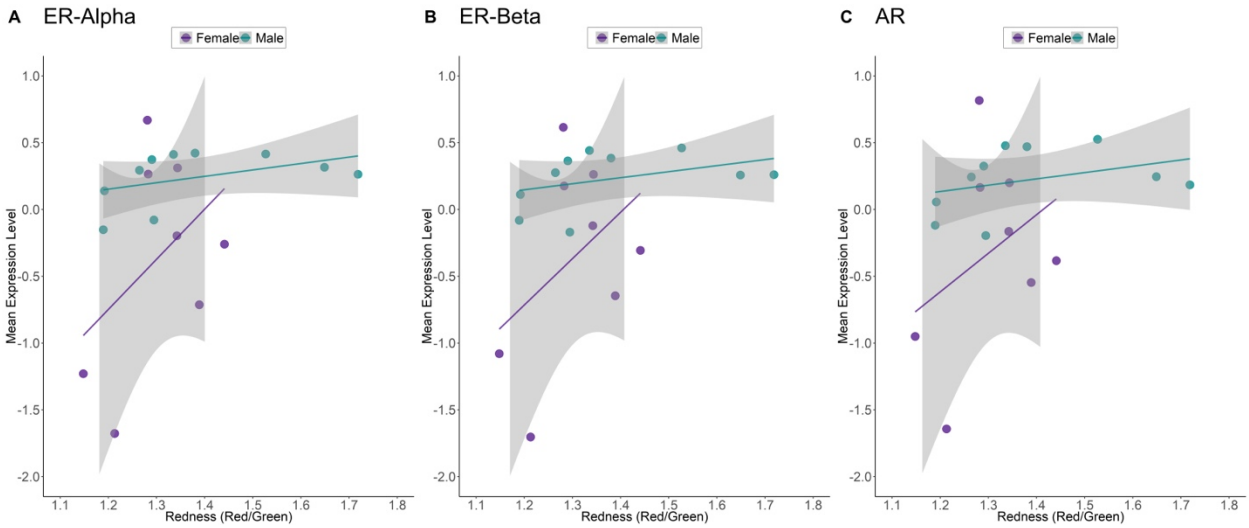

Figure S7. Expression of genes associated with ERα, ERβ, or AR did not correlate with chest redness at baseline while under anesthesia within males or within females.