

Supplementary Information

Table S1. Primer sequences for quantitative PCR

Gene	Sequence of forward (F) and reverse (R) primers	Start location of fox amplicon in CanFam3.1
<i>AKAP7</i>	F: CCTATCCATTCCGATCACCAAC R: AGTCGCTCATCTTGCTGGAG	chr1:69376373
<i>ANKRD6</i>	F: AACACGGCTCTACACGAAGC R: CTCTGGGAGTGGCTGTTCTG	chr12:48811400
<i>BRCA2</i>	F: GCAGAACTGGTAGGCTCTCC R: ATACCAGCAAGCAGGACGAG	chr25:7753591
<i>CDH4</i>	F: GACATCTGGATCCCCTGGC R: TCAGGACACTTCAGCATCCG	chr24:46055878
<i>ITGA1</i>	F: GGCCAATGAGACTGTCCCTG R: CAGCTTAAGTTCTGGCATCGG	chr4:62309206
<i>LAMA2</i>	F: ACCTCTGCTCGCTATATTCGC R:CCGACTGAAATATCCTTGACCG	chr1: 67735809
<i>NELL2</i>	F: CCTTCAGCGCACTCATCAATG R: GGCCGTTTGTAGCTGTCGAG	chr27: 9535623
<i>PDE7B</i>	F: CCCATTCATTGACTTCCGCC R: TCCACGAAGCAACCTCGATG	chr1: 28719580
<i>SDHA</i>	F: CCTGCGTCACAAAGCTCTTT R: TGTCATGTAGTGGATGGCGT	chr34:11957015
<i>SPOCK1</i>	F: GGGAGTCCTTGCAGATTGGC R: ACTGGTTTGGAGCCCTTCAC	chr11: 24748659
<i>THBS4</i>	F: TGCAAGTTGGTCAGTCTCAGG R: CACTGTGATGGGACGCTTAAAC	chr3: 26970860

Table S2. Reads from sequencing of tame and aggressive fox anterior pituitary RNA

Sample		Reads (unfiltered)	Reads (filtered)
Tame	1-601	34,577,964	32,479,541
	2-662	31,279,625	29,749,267
	3-663	33,576,358	31,677,842
	4-664	37,936,135	36,086,260
	5-665	34,731,107	31,347,816
	6-666	34,735,178	32,916,697
Aggressive	49-649	32,688,890	32,866,684
	50-710	33,570,511	32,694,392
	51-711	32,901,110	31,043,144
	52-712	33,126,696	31,429,811
	53-713	32,876,971	31,237,749
	54-714	34,760,977	31,328,261
Tame		Mean = 34,168,005 SD = 2,239,555	Mean = 32,376,237 SD = 2,121,953
Aggressive		Mean = 33,625,582 SD = 906,373	Mean = 31,766,674 SD = 797,449

Table S3. Alignment rate of reads from tame and aggressive fox anterior pituitary RNA

Sample		Alignment rate (%)
Tame	1-601	79.0
	2-662	78.9
	3-663	79.1
	4-664	79.5
	5-665	79.2
	6-666	79.3
Aggressive	49-649	78.5
	50-710	78.9
	51-711	78.8
	52-712	79.0
	53-713	79.0
	54-714	78.5

Table S4. Genes expressed in fox anterior pituitary tissue, with counts (normalized by DESeq2) averaged across all 12 samples. The table includes both tame and aggressive foxes.

fox-pituitary-expr.xlsx

Table S5. Significantly enriched GO terms in the 0.1% (180) most highly expressed genes in fox anterior pituitary tissue. The table includes GO identifier, name of enriched GO term, number of DE genes associated with the GO term, percent of genes associated with the GO term which are DE, p-value, corrected p-value (FDR), symbols of the DE genes in the GO term, and symbols of all the genes in the GO term.

fox-pituitary-topexpr-GO.xlsx

Table S6. Differentially expressed genes in tame versus aggressive fox anterior pituitary tissue.

fox-pituitary-DEG.xlsx

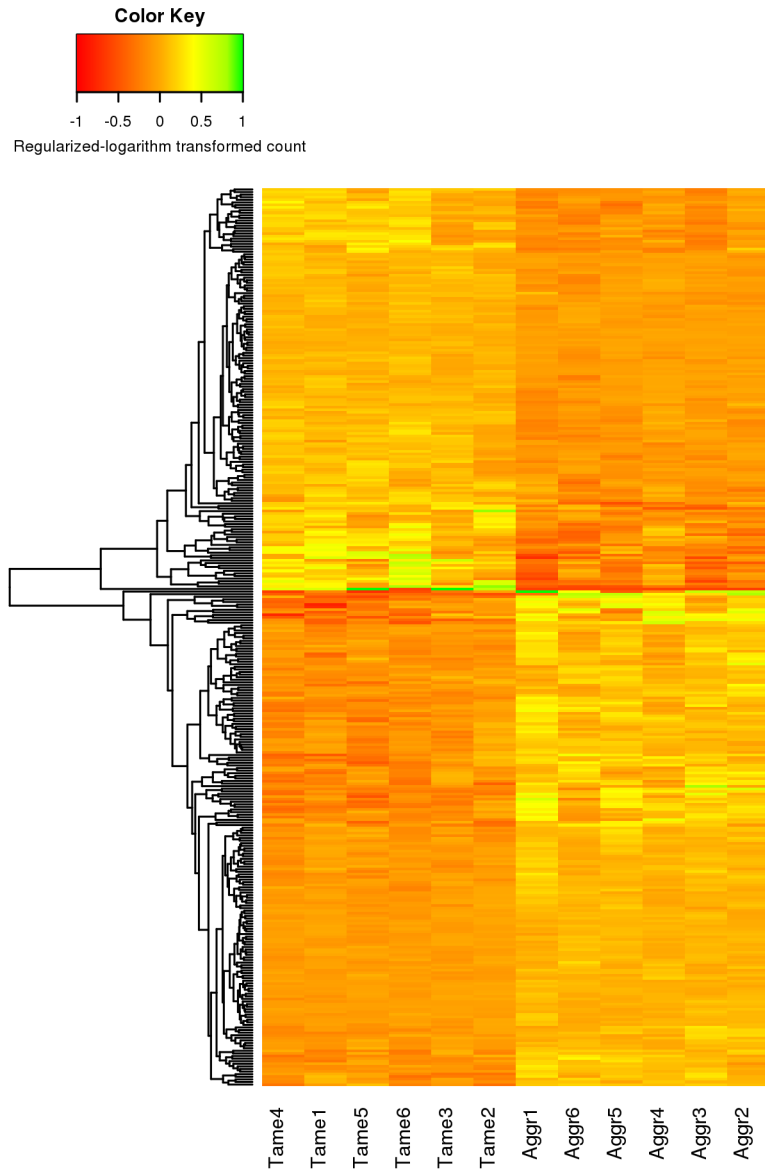


Figure S1. Heat map of differentially expressed genes (346) in tame versus aggressive fox pituitary tissue (FDR < 0.05).

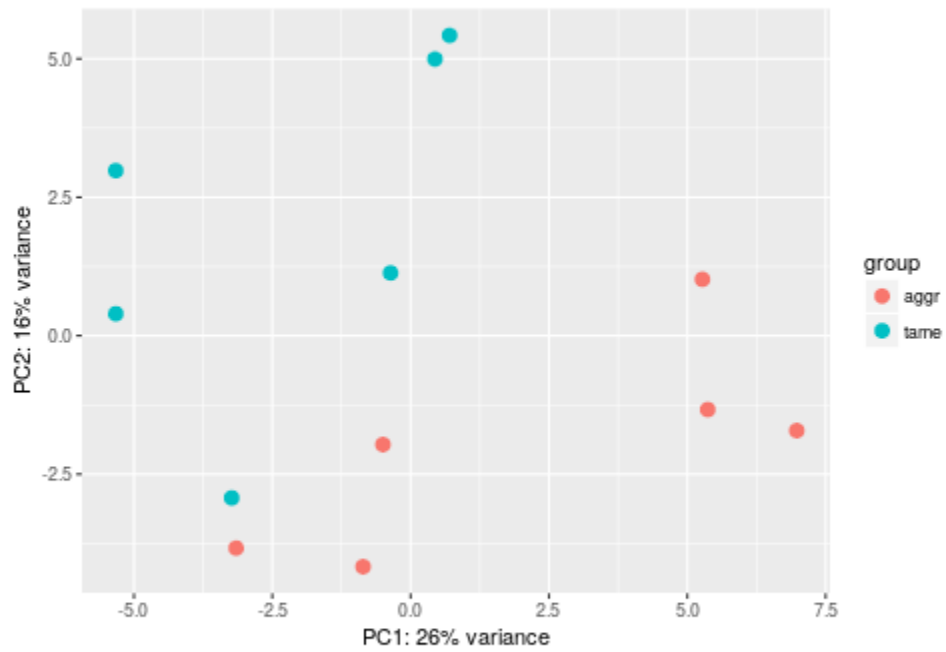


Figure S2. Principal components analysis of tame versus aggressive fox pituitary samples by gene expression, comparing PC1 versus PC2. Note clustering of tame (blue) versus aggressive (red) samples.

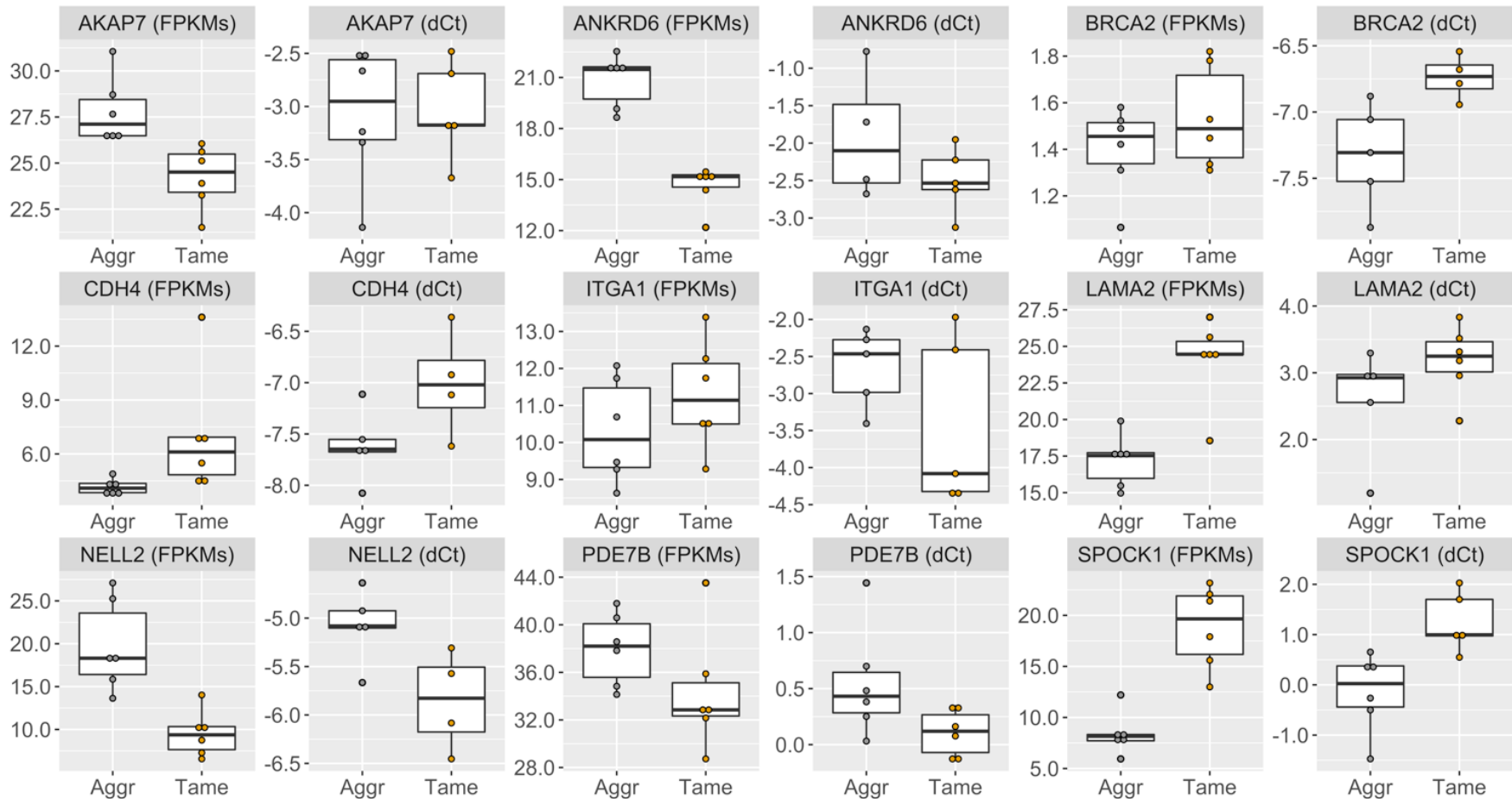


Figure S3. Side-by-side comparison of FPKM counts (RNA-seq gene expression results) and dCt (qPCR gene expression results) for nine genes. All FPKM counts include six tame and six aggressive samples; dCt results have fewer samples for some genes due to failed qPCR reactions. All dCt values are relative to the *SDHA* reference gene, and therefore some values may be negative.

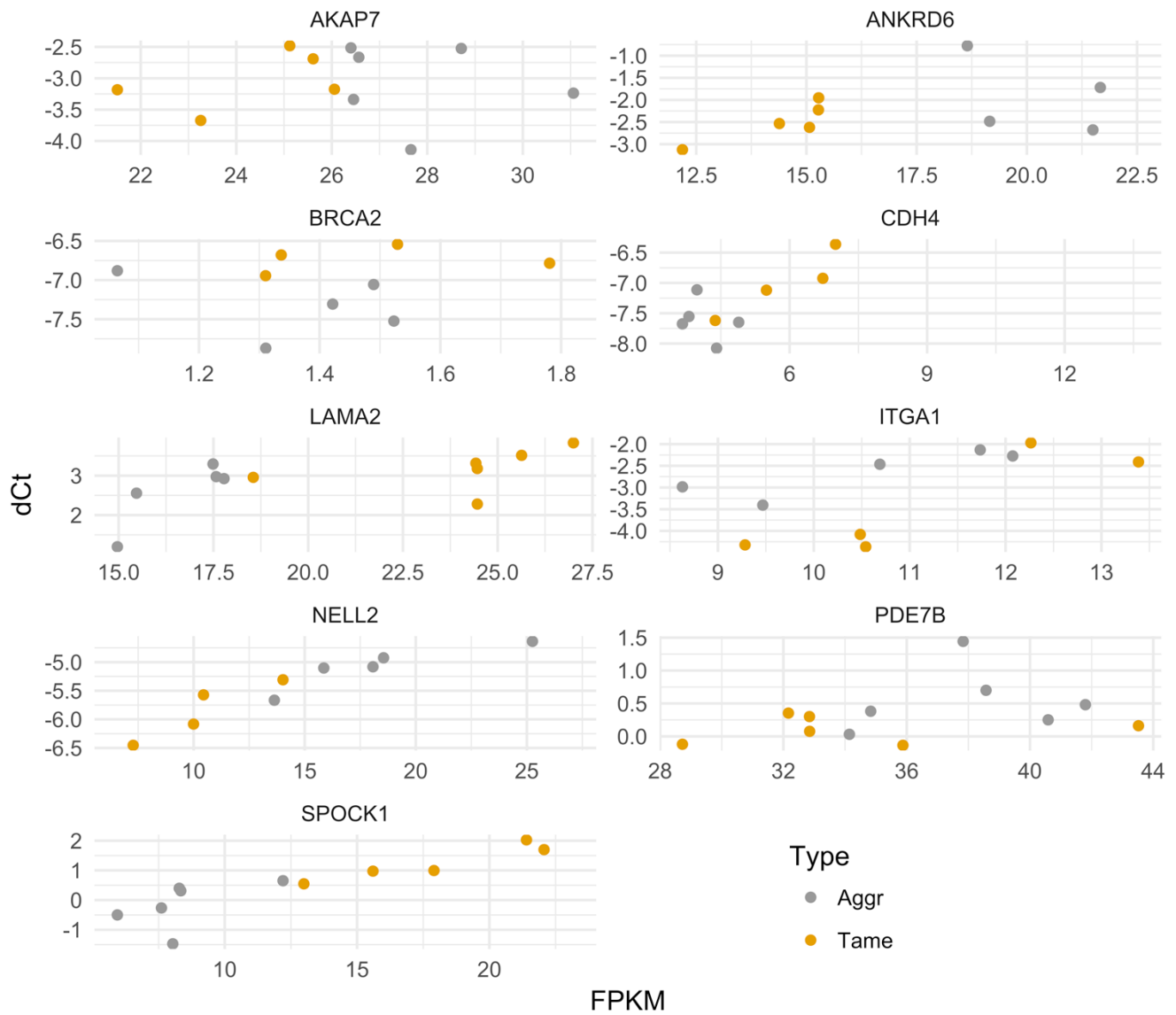


Figure S4. Scatterplot of RT-qPCR (dCt, y-axis) and RNA sequencing (FPKM, x-axis) results in tame and aggressive fox anterior pituitary tissue for nine genes.

Table S7. Comparison of RT-qPCR and RNA sequencing results in tame and aggressive fox anterior pituitary tissue.

Gene	RNA-seq		qPCR	
	Log ₂ fold change	FDR	ddCt	Wilcoxon
<i>AKAP7</i>	-0.23	0.003	-0.32	W=4, p=0.63
<i>ANKRD6</i>	-0.51	7.98e-10	-0.15	W=9, p=0.40
<i>BRCA2</i>	0.54	8.46e-5	0.33	W=0, p=0.10
<i>CDH4</i>	0.40	0.049	0.83	W=1, p=0.11
<i>ITGA1</i>	0.21	0.029	-0.89	W=7, p=0.85
<i>LAMA2</i>	0.46	4.66e-6	0.58	W=1, p=0.11
<i>NELL2</i>	-0.61	0.0001	-0.64	W=11, p=0.11
<i>PDE7B</i>	-0.25	0.024	-0.35	W=12, 0.34
<i>SPOCK1</i>	0.78	3.58e-8	1.19	W=1, 0.06

ddCt values for qPCR assays were calculated using the Applied Biosystems StepOnePlus Real Time PCR System software (Applied Biosystems, Foster City, CA) for all tame versus all aggressive samples for each gene. Significant differences in gene ddCt values were calculated by comparing individual ddCt values from tame and aggressive samples for each gene using the Wilcoxon rank-sum test.

Table S8. Significantly enriched GO terms in differentially expressed genes in tame versus aggressive fox anterior pituitary tissue. The table includes GO identifier, name of enriched GO term, number of DE genes associated with the GO term, percent of genes associated with the GO term which are DE, p-value, corrected p-value (FDR), symbols of the DE genes in the GO term, and symbols of all the genes in the GO term.

fox-pituitary-GO.xlsx

Table S9. Significantly enriched GO terms in genes significantly up-regulated in aggressive fox anterior pituitary tissue. The table includes GO identifier, name of enriched GO term, number of DE genes associated with the GO term, percent of genes associated with the GO term which are DE, p-value, corrected p-value (FDR), symbols of the DE genes in the GO term, and symbols of all the genes in the GO term.

fox-pituitary-GO-aggr.xlsx

Table S10. Significantly enriched GO terms in genes significantly up-regulated in tame fox anterior pituitary tissue. The table includes GO identifier, name of enriched GO term, number of DE genes associated with the GO term, percent of genes associated with the GO term which are DE, p-value, corrected p-value (FDR), symbols of the DE genes in the GO term, and symbols of all the genes in the GO term.

fox-pituitary-GO-tame.xlsx

Table S11. Antique White 4, Pale Turquoise, and Brown WGCNA modules. The table includes gene symbol and module membership (e.g., the correlation of its gene expression profile with the module eigengene), and whether individual genes are differentially expressed in tame versus aggressive fox anterior pituitary.

fox-pituitary-WGCNA.xlsx

Table S12. All WGCNA modules identified in fox anterior pituitary. The table includes genes in each module and the module membership of each gene (e.g., the correlation of its gene expression profile with the module eigengene).

fox-pituitary-WGCNA-membership.xlsx

Table S13. Enriched GO terms in 13 WGCNA modules in tame and aggressive fox pituitary. The table includes GO identifier, name of enriched GO term, p-value, corrected p-value (FDR), percent of

genes in the GO term which are associated with the module, number of genes in the GO term which are associated with the module, and the list of gene symbols in the GO term which are associated with the module. Modules were selected from those with eigengenes that significantly differed between tame and aggressive samples before correction with Benjamini-Hochberg.

fox-pituitary-WGCNA-GO.xlsx

Table S14. Exons differentially skipped in tame versus aggressive fox anterior pituitary tissue, as identified by rMATS. The table includes Ensembl ID, gene symbol, p value, FDR, percent of reads covering skipped exon in tame samples (mean), and percent of reads covering skipped exon in aggressive samples (mean).

fox-pituitary-skippedexons.xlsx