Main Manuscript for: Modular genetic control of social status in a cichlid fish

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This PDF file includes:

- Supplementary Results
- Supplementary Figures 1-12
- Supplementary Tables 1-2

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Supplementary Information Appendix

Supplementary Results

Injuries to focal males

We found that 2 $AR\alpha^{d50/+}$ males and 1 $AR\alpha^{d50/d50}$ had damaged fins; 1 injured fish from each of these respective groups died on day 2 before the extractions. Injuries to these fish were likely caused by attacks from the stimulus male as these males fled at high levels from males compared to fish that were not injured (average flee from male±SEM= 74.4±43.29 and 12.16±5.72 for injured and uninjured fish, respectively) and fled from females at levels similar to uninjured fish (average flee from female±SEM= 0.83±0.60 and 1.41±0.26 for injured and uninjured fish, respectively. No $AR\alpha^{+/+}$ males exhibited injuries and all survived the assay. No injuries were observed in $AR\beta^{+/+}$, $AR\beta^{d5/+}$, or $AR\beta^{d5/d5}$ fish.

Supplementary Figures and Legends

A ARα

Gene	Amino acid sequence
ARα^{+}	(N$_{46}$)SSPPNNMNQSSSSAFAECDSTVADTS(N$_{620}$)
ARα^{d50}	(N$_{46}$)SSPPNNMNQSSSSAFAEC*

B ARβ

Gene	Amino acid sequence
ARβ^{+}	(N$_{388}$)FESSRE(N$_{123}$)GKQKYLCASKNDCTIDK(N$_{308}$)*
ARβ^{d5}	(N$_{388}$)FESRTH(N$_{44}$)AEIPVCQQE*

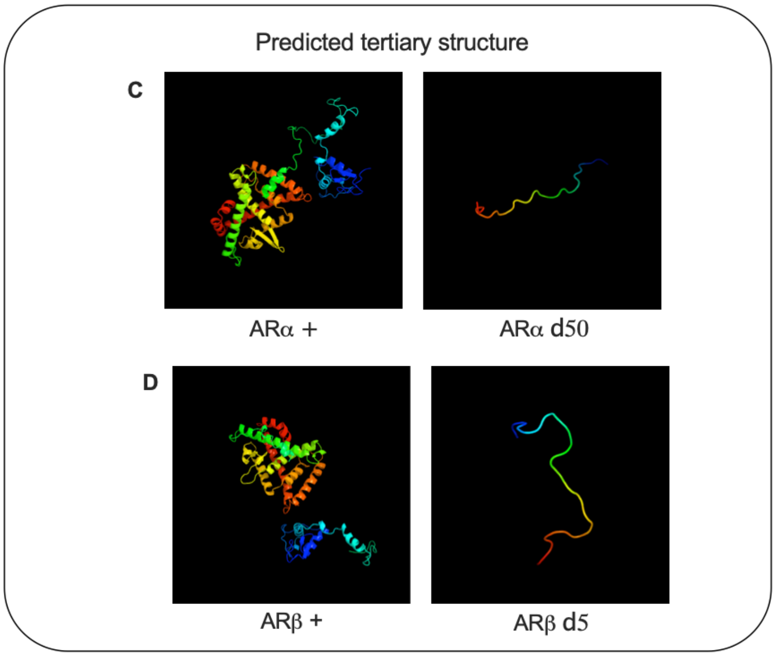

Fig. S1. Predicted amino acid sequence and tertiary structure of wild-type and mutant forms of *AR*α and *ARβ*. (A) Amino acid sequence for the wild-type version of *AR*α (*AR*α^{+}) and the mutant version of *AR*α (*AR*α^{d50}), with the latter containing a premature stop sequence (indicated by the red asterisk) induced by a frameshift mutation. **(B)** Amino acid sequence for the wild-type version of *ARβ* (*ARβ*$^{+}$) and the mutant version of *ARβ* (*ARβ*^{d5}), with the latter containing incorrect amino acids (indicated in red font) and a premature stop sequence (indicated by the red asterisk) induced by a frameshift mutation. **(C)** Computer modeling (Phyre2(17)) predicts from the ARα^{+} amino acid sequence extensive tertiary structure in the ARα^{+} protein that is absent from that predicted from the ARα^{d50} amino acid sequence. **(D)** Computer modeling (Phyre2(17)) predicts from the AR*β*$^{+}$ amino acid sequence extensive tertiary structure in the ARα^{+} protein that is absent from that predicted from the AR*β*^{d5} amino acid sequence. (N$_{\#}$) indicate the number of bases not shown in actual gene sequence for clarity. +=wild-type. d=deletion.

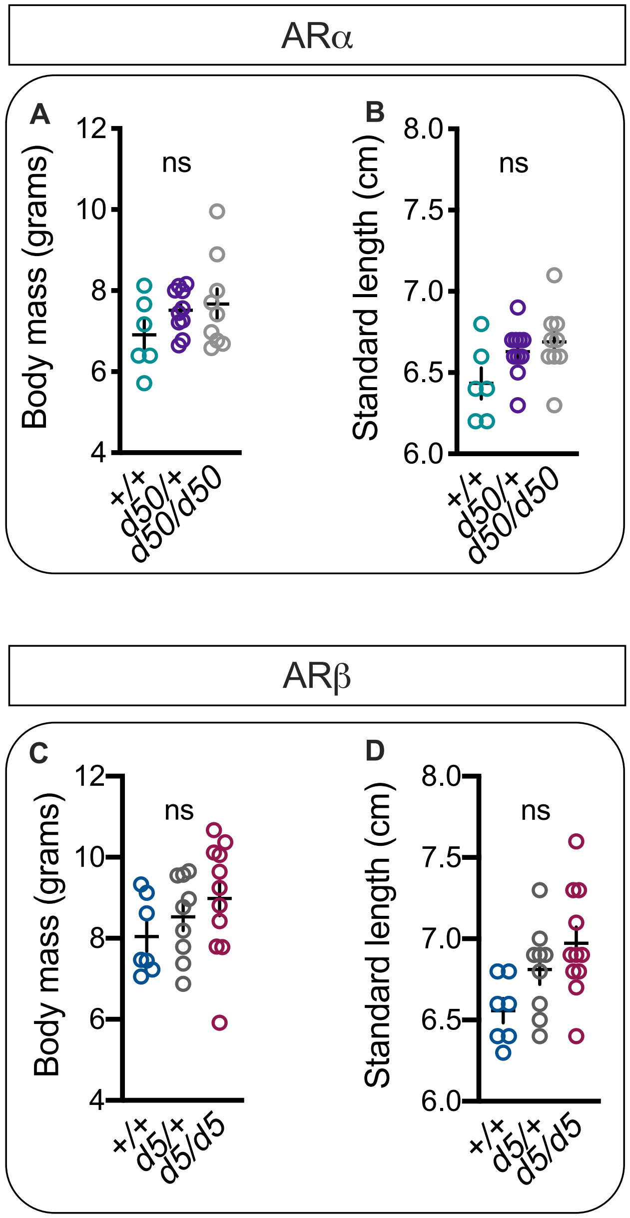

Fig. S2. No effects of AR genotype on body mass and standard length. There was no effect of *ARα* (**A** and **B**) or *ARβ* (**C** and **D**) genotype on body size or standard length. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. ns=not significant.

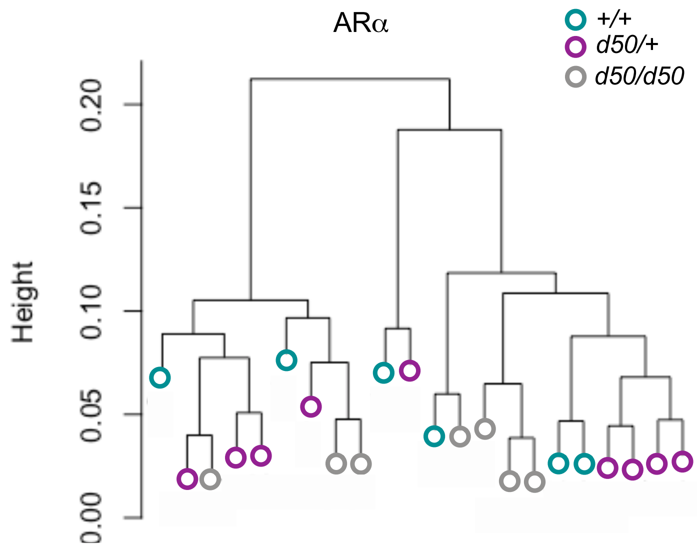

Fig. S3. Results of hierarchical clustering of *ARα* fish by color. Each branch with a distinct colored circle at the tip represents a fish from a given genotype. Fish form clusters that are unrelated to genotype, confirming visual qualitative observations that *ARα* mutant males do not look different than *ARα*$^{+/+}$ males.

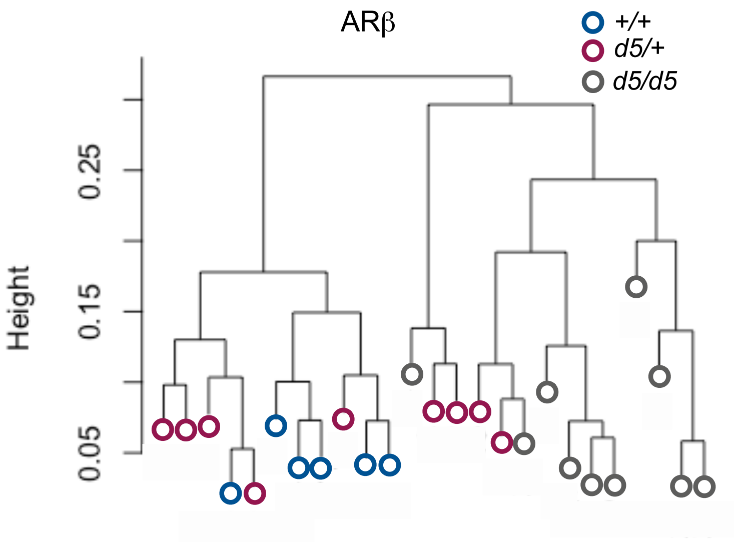

Fig. S4. Results of hierarchical clustering of *ARβ* fish by color. Each branch with a distinct colored circle at the tip represents a fish from a given genotype. $AR\beta^{d5/d5}$ and $AR\beta^{+/+}$ males fall into two distinct clusters, while $AR\beta^{d5/+}$ males are split nearly equally between those two clusters, confirming visual qualitative observations that $AR\beta^{d5/d5}$ males look different than $AR\beta^{+/+}$ males and reflecting an intermediate coloration pattern in $AR\beta^{d5/+}$ males.

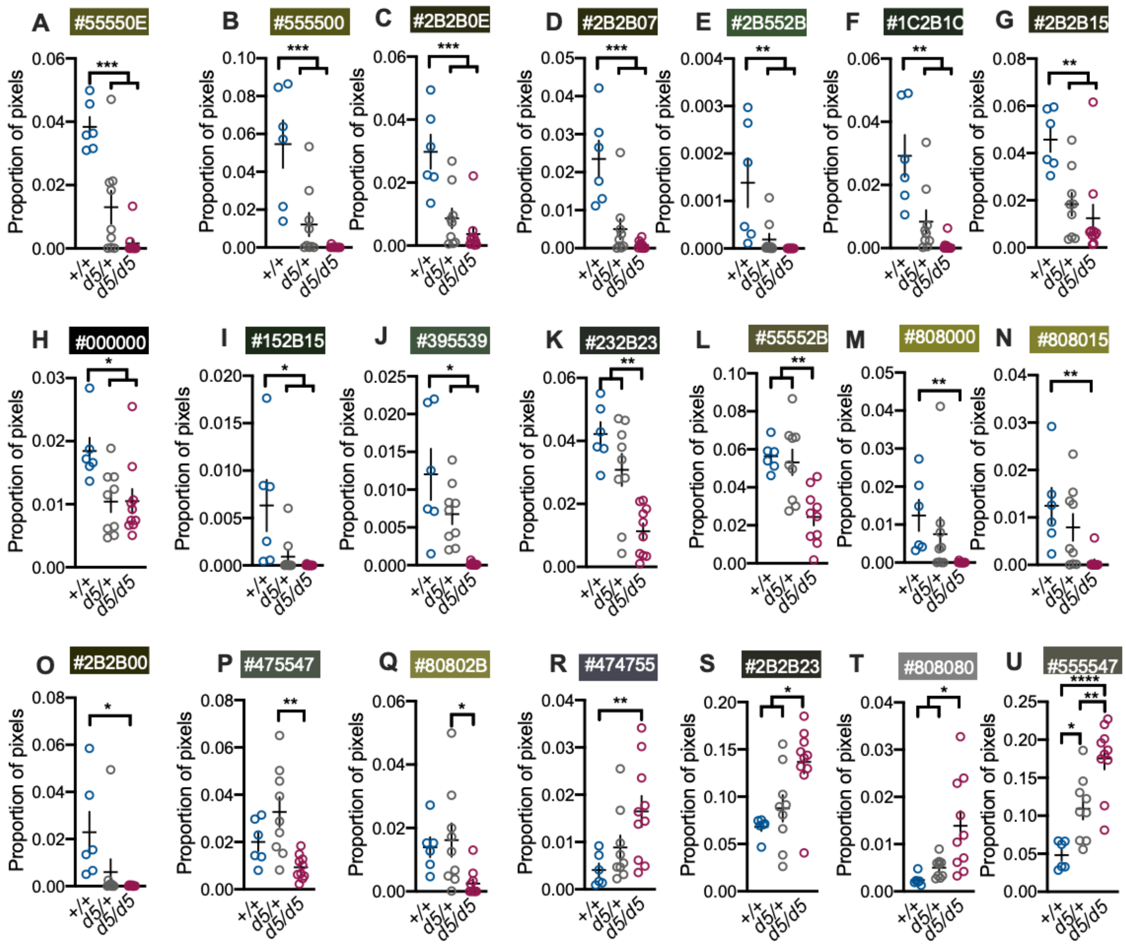

Fig. S5. *ARβ* is necessary for the normal expression of numerous colors. (**A** to **J**) $AR\beta^{+/+}$ males exhibit a larger proportion of pixels for several colors compared to *ARβ* mutant males. (**K** and **L**) *ARβ* mutant males show a greater proportion of pixels for two colors compared to $AR\beta^{d5/d5}$ males. **(M** to **O**) $AR\beta^{+/+}$ males show a greater proportion of pixels for three colors compared to $AR\beta^{d5/d5}$ males but $AR\beta^{d5/+}$ males were not different from either group for these colors. (**P** and **Q**) For two colors, $AR\beta^{d5/+}$males had a greater proportion of pixels than $AR\beta^{d5/d5}$ males but not $AR\beta^{+/+}$ males. **(R)** For one color, $AR\beta^{d5/d5}$ males had more pixels than $AR\beta^{+/+}$ males, **(S** to **T)** while for two colors $AR\beta^{d5/d5}$ males had a greater proportion of pixels compared to both groups. **(V)** $AR\beta^{d5/+}$ males had more pixels for one color. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. ****P<0.0001; ***P<0.001; **P < 0.01; *P < 0.05.

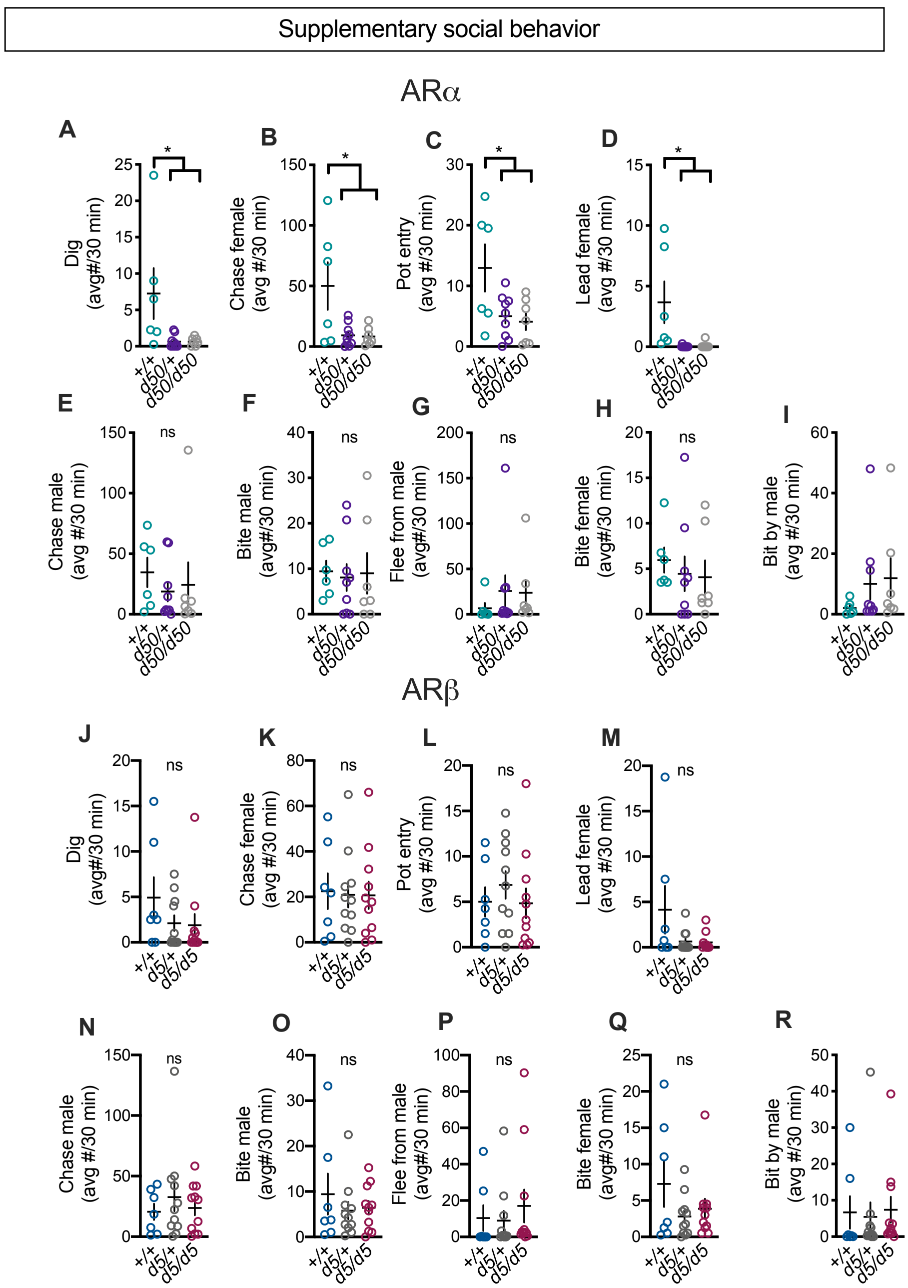

Fig. S6. Effects of AR genotype on different social behaviors. (**A** to **D**) *AR*α is required for the performance of numerous reproductive behaviors. **(E** and **F)** *AR*α is not required for attacking the stimulus male. **(G)** *AR*α genotype does not affect the number of times males flee from the stimulus male. **(H and I)** *AR*α genotype does not affect the number of times males bite females or are bit by the stimulus male. (**J** to **M**) *ARβ* is not required for reproductive behaviors or (**N** and **O**) attacking the stimulus male. (**P**) *ARβ* genotype does not affect the number of times males flee from the stimulus male. (**Q** and **R**) *ARβ* genotype does not affect the number of times males bite females or are bit by the stimulus male. wt=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. ns=not significant. *$P < 0.05$.

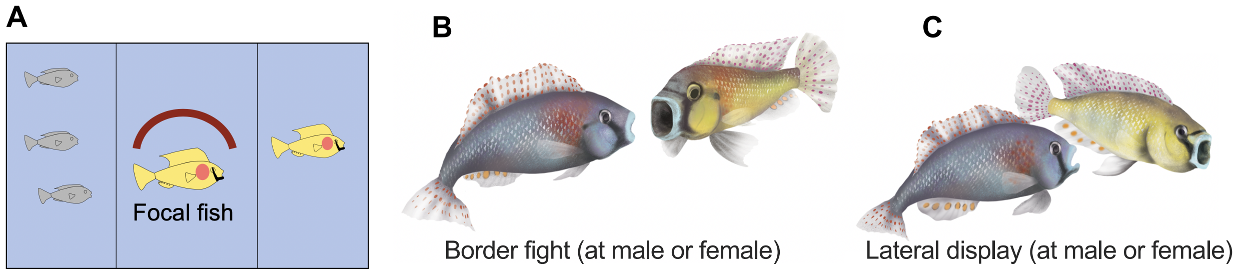

Fig. S7. Scoring behavior during modified dyad assays. (A) A modified dyad assay was used to assess aggression. The focal fish was separated physically from the stimulus male and females by a transparent, perforated, acrylic barrier. (**B** and **C**) *A. burtoni* will perform border fights at either sex and perform lateral displays at either sex during modified dyad assays.

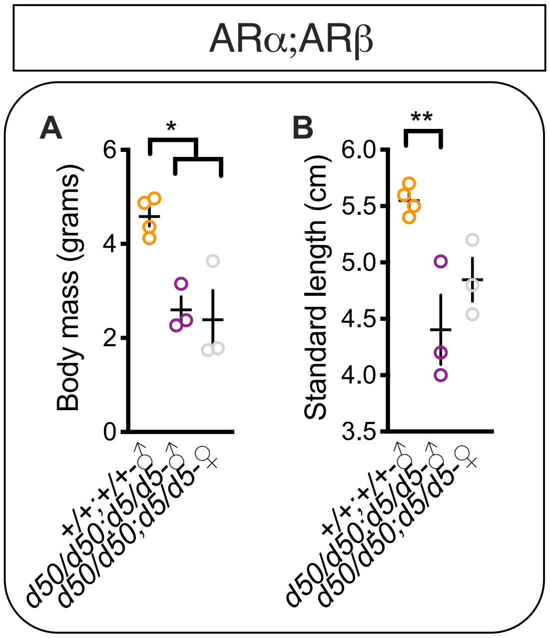

Fig. S8. *ARα;ARβ* genotype affects body mass and standard length. (A) *ARα*$^{+/+}$;*ARβ*$^{+/+}$ males weighed more than *ARα*$^{d50/d50}$;*ARβ*$^{d5/d5}$ males and females **(B)** and were greater in standard length than *ARα*$^{d50/d50}$;*ARβ*$^{d5/d5}$ males. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. $^{**}P < 0.01$; $^{*}P < 0.05$.

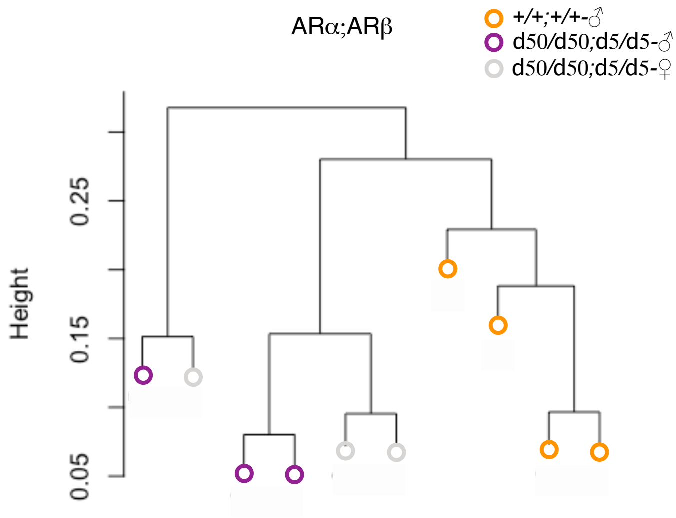

Fig. S9. Results of hierarchical clustering of *ARα;ARβ* fish by color. Each branch with a distinct colored circle at the tip represents a fish from a given genotype. $AR\alpha^{+/+};AR\beta^{+/+}$ males form a cluster that is distinct from $AR\alpha^{d50/d50};AR\beta^{d5/d5}$ males and females, confirming visual qualitative observations that $AR\alpha^{+/+};AR\beta^{+/+}$ males look different from $AR\alpha^{d50/d50};AR\beta^{d5/d5}$ males and females.

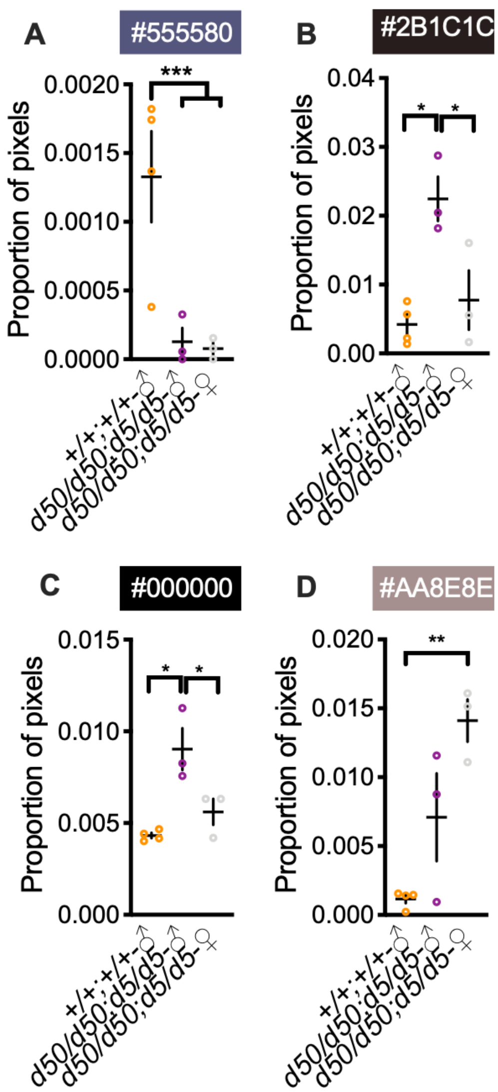

Fig. S10. *ARα;ARβ* genotype affects whole-body coloration. (A) *ARα*$^{+/+}$*;ARβ*$^{+/+}$ males had a greater proportion of pixels for one color compared to than *ARα*$^{d50/d50}$*;ARβ*$^{d5/d5}$ males and females. **(B** and **C)** *ARα*$^{d50/d50}$*;ARβ*$^{d5/d5}$ males expressed more pixels for two colors than *ARα*$^{+/+}$*;ARβ*$^{+/+}$ males and *ARα*$^{d50/d50}$*;ARβ*$^{d5/d5}$ females. **(D)** *ARα*$^{d50/d50}$*;ARβ*$^{d5/d5}$ females expressed more pixels for one color compared to *ARα*$^{+/+}$*;ARβ*$^{+/+}$ males. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. ***P<0.001; **P < 0.01; *P < 0.05.

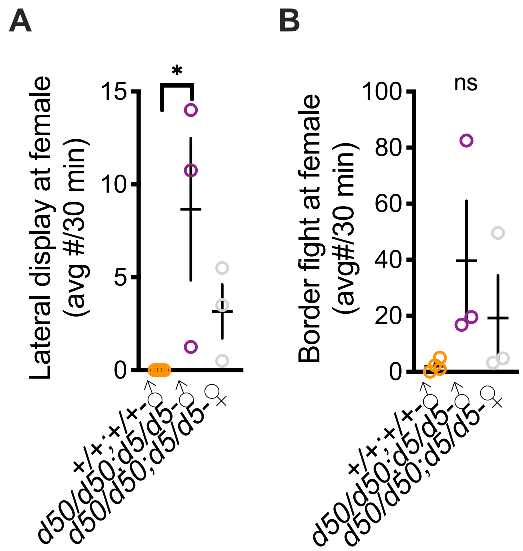

Fig. S11. Effects on female-directed aggression. (A) *ARα*$^{d50/d50}$*;ARβ*$^{d5/d5}$ males performed more lateral displays directed towards females than *ARα*$^{+/+}$*;ARβ*$^{+/+}$ males. **(B)** There was a statistical trend (omnibus ANOVA P=0.06) for an effect of *ARα;ARβ* genotype on attacks directed towards females. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. *P < 0.05.

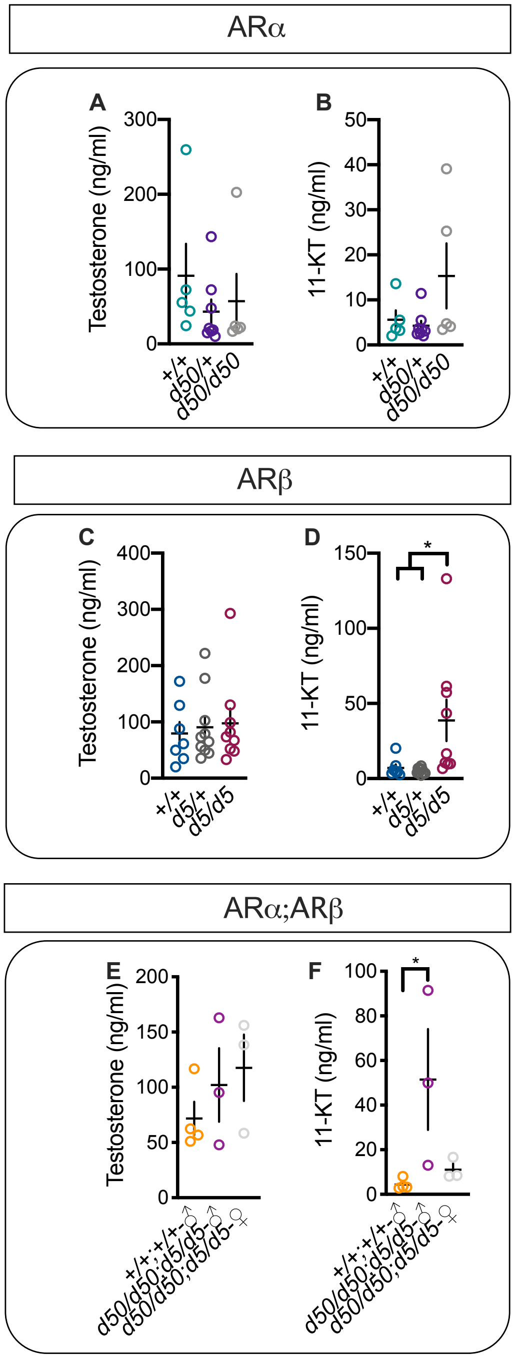

Fig. S12. Effects on AR genotype on testosterone and 11-ketotestosterone levels. (A and **B)** *ARα* genotype has no effect on testosterone or 11-ketotestosterone (11-KT) levels. (**C**) *ARβ* genotype has no effect on testosterone levels, **(D)** but $AR\beta^{d5/d5}$ males have higher levels of 11-KT than $AR\beta^{d5/+}$ and $AR\beta^{+/+}$ males. **(E)** *ARα;ARβ* genotype had no effect on testosterone levels, **(F)** but $AR\alpha^{d50/d50}$;$AR\beta^{d5/d5}$ had higher levels of 11-KT than $AR\alpha^{+/+}$;$AR\beta^{+/+}$ males. +=wild-type. d=deletion. Circles represent data points for individual fish. Crosses represent Mean±SEM. $^{*}P < 0.05$.

Supplementary Tables and Legends

Gene Name/Alias	Description	Alias	Location
ar	androgen receptor	ARα	NW_005179415
LOC102296288	androgen receptor-like	ARβ	NW_005179497

Table S1. Gene information. Information was gathered from NCBI (https://www.ncbi.nlm.nih.gov/gene/?term=androgen+receptor+burtoni) on *A. burtoni* ARs.

sgRNA	Oligo-1	Oligo-2
gARα-A	TTAATACGACTCACTATAGGACTGTGGCGGATACTTCTCGGTTTTAGAGCTAGAAATAG	AAAAGCACCGACTCGGTGCCACTT TTTCAAGTTGATAAC GGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
gARα-B	TTAATACGACTCACTATAGGGGTGCGCAAACTGTGACGCGGTTTTAGAGCTAGAAATAG	AAAAGCACCGACTCGGTGCCACTT TTTCAAGTTGATAAC GGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
gARβ-A	TTAATACGACTCACTATAGGGGGAAACATGTGTTCTCTACGTTTTAGAGCTAGAAATAG	AAAAGCACCGACTCGGTGCCACTT TTTCAAGTTGATAAC GGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
gARβ-B	TTAATACGACTCACTATAGGGGGGGAAAGAAGAACTCCATGTTTTAGAGCTAGAAATAG	AAAAGCACCGACTCGGTGCCACTT TTTCAAGTTGATAAC GGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

Table S2. Oligonucleotides used to synthesize single-guide RNA (sgRNA). We synthesized sgRNA by annealing oligo-1, which was specific to each target site, to oligo-2, a generic oligo that allows for amplification of the sgRNAs. g=guide. AR=androgen receptor.