

1 Distinct gene regulatory signatures of dominance rank and social bond 2 strength in wild baboons

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23 **Abstract:**

24 The social environment is a major determinant of morbidity, mortality, and Darwinian
25 fitness in social animals. Recent studies have begun to uncover the molecular processes
26 associated with these relationships, but the degree to which they vary across different
27 dimensions of the social environment remains unclear. Here, we draw on a long-term field study
28 of wild baboons to compare the signatures of affiliative and competitive aspects of the social
29 environment in white blood cell gene regulation, under both immune stimulated and non-
30 stimulated conditions. We find that the effects of dominance rank on gene expression are
31 directionally opposite in males versus females, such that high-ranking males resemble low-
32 ranking females, and vice-versa. Among females, rank and social bond strength are both
33 reflected in the activity of cellular metabolism and proliferation genes. However, pronounced
34 rank-related differences in baseline immune gene activity are near-absent for social bond
35 strength, while only bond strength predicts the fold-change response to immune
36 (lipopolysaccharide) stimulation. Together, our results indicate that the directionality and
37 magnitude of social effects on gene regulation depend on the aspect of the social environment
38 under study. This heterogeneity may help explain why social environmental effects on health
39 and longevity can also vary between measures.

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42 INTRODUCTION

43 Many animal species, including humans, live the majority of their lives as part of a larger
44 group of conspecifics. Social group living provides a number of benefits, including protection
45 from predators, improved territory and resource defense, and access to potential mates [1–4].
46 At the same time, it also generates competition for resources among group members. For many
47 group-living species, the outcome of competitive interactions is at least partially predictable,
48 giving rise to an observable social dominance hierarchy in which high status animals are
49 consistently able to displace lower status animals [5–7]. Due to correlated differences in
50 resource access, energy expenditure, and/or psychosocial stress, high-ranking and low-ranking
51 animals are frequently behaviorally and physiologically distinct. For example, across social
52 mammals, low status individuals often have elevated glucocorticoid levels or exhibit signs of
53 glucocorticoid resistance [8–12].

54 However, correlations between social status and physiological measures are highly
55 heterogeneous across species or between sexes, and sometimes even directionally
56 inconsistent [8,13–18]. This heterogeneity is likely explained in part by differences in how status
57 is attained and maintained. In some cases social status depends on individual characteristics,
58 such as the ability to physically dominate competitors (e.g., male bottlenose dolphins, male red
59 deer, female meerkats: [19–21]). In contrast, other types of social hierarchies are determined
60 via nepotism, and do not strongly covary with individual phenotype (e.g., female spotted hyenas,
61 some female cercopithecine primates: [22,23]). Hierarchies that are largely determined by
62 physical condition are often dynamic, whereas nepotistic hierarchies can remain highly stable
63 over time, and even extend across generations [24–26]. Consequently, while rank is an
64 important predictor of fitness in both types of hierarchies [27–31], its physiological signatures
65 may differ. For example, while high rank predicts lower glucocorticoid levels in female blue
66 monkeys, female baboons, and naked mole-rats of both sexes [10,13,32,33], glucocorticoid
67 levels tend to be higher in high rank female ring-tailed lemurs, female meerkats, and male
68 chimpanzees [18,34,35].

69 In addition to the competitive interactions that structure social hierarchies, group-living
70 animals can also form individually differentiated, affiliative social bonds. The affiliative behaviors
71 that give rise to social bonds (e.g., proximity or contact in cetaceans and ungulates, grooming
72 and proximity in primates) are often patterned, at least in part, by social status [36–42]. For
73 example, in cooperatively breeding meerkats, dominant males groom dominant females more
74 often than they groom subordinate females [43]. Similarly, attraction to high-ranking individuals
75 commonly structures grooming patterns in social primates [40]. However, rank is not the sole
76 determinant of affiliative behavior and social bond formation. In female yellow baboons, for
77 instance, a measure of female social connectedness to other females is better predicted by the
78 presence of maternal kin than by rank (although rank, not the presence of maternal kin, predicts
79 female social connectedness to males; [44]). Recent evidence also indicates that the fitness
80 effects of affiliative social relationships are also partially independent of rank. Stronger social
81 relationships predict natural lifespan in members of at least five mammalian orders, and this
82 relationship often persists after controlling for variation in rank or other measures of social status
83 [38,45–51]. Indeed, in yellow baboons, social relationships predict lifespan even when rank
84 does not [45].

85 Social status and social integration are therefore connected dimensions of the social
86 environment that nevertheless can have distinct fitness consequences. This observation
87 presents a puzzle about the mechanisms that make their consequences for health, physiology,
88 and survival distinct. To date, far more work has focused on the physiological and molecular
89 correlates of social status than of affiliative social bonds in natural animal populations. However,
90 four lines of evidence argue that differences in affiliative social interactions should also be
91 reflected in physiological or molecular variation. First, such changes are implied by cross-taxon
92 support for an association between lifespan and social integration [52], suggesting at least a
93 partial basis in physical condition. Second, studies in a small set of natural populations have
94 already identified links between affiliative relationships and biomarkers of stress, especially
95 glucocorticoid levels. For example, urinary glucocorticoids are lower in chimpanzees sampled
96 while interacting with closely bonded social partners than in those interacting with non-bonded
97 partners [53], and male rhesus macaques and female chacma baboons with stronger social
98 bonds show reduced glucocorticoid responses to environmental stressors [54–56]. Third, social
99 isolation and loneliness are associated with changes in human biology, including increased
100 proinflammatory activity [57–59], hypothalamic-pituitary-adrenal axis activation [60,61], and risk
101 for cardiovascular disease [62,63]. Finally, studies in captive rodents show that manipulation of
102 social integration and social support can causally alter glucocorticoid regulation and increase
103 the risk of cancer metastasis [64,65].

104 Despite these findings, most studies consider either the physiological signature of social
105 status or of affiliative social relationships, not both. Further, those studies that incorporate both
106 dimensions often measure only a single outcome variable in one type of social status hierarchy
107 (i.e., physical competition-based or nepotistic). Because single measures vary along only one
108 dimension, they have limited ability to distinguish shared versus unique signatures of
109 competitive and affiliative interactions. Thus, it is possible that physiological changes in
110 response to the social environment converge on a generalized signature of stress and adversity,
111 in which low status and weak social bonds produce undifferentiable responses (e.g., the
112 “conserved transcriptional response to adversity”: [66]). Alternatively, different facets of the
113 social environment may be reflected in different biological pathways. If so, higher dimensional
114 measures of physiological or molecular state may be informative about multiple aspects of an
115 animal’s social experience, and help uncover why social status and social affiliation can be
116 related, yet have distinct effects on fitness.

117 Functional genomic analyses of gene regulation provide an opportunity to differentiate
118 these hypotheses. Importantly, previous work demonstrates the sensitivity of gene regulation to
119 the social environment. For example, competitive interactions to establish dominance rapidly
120 alter DNA methylation, histone marks, and gene expression across multiple vertebrate and
121 social insect species [67–73]. Affiliative interactions can also be reflected in altered gene
122 expression patterns. For example, experimental social isolation in piglets results in increased
123 plasma cortisol and altered glucocorticoid and mineralocorticoid receptor expression in stress-
124 related regions of the brain [74]. However, the species that have been central to understanding
125 the genomic signatures of social status and social competition (e.g., cichlid fish, mice) tend not
126 to be the same ones developed as models for social affiliation (e.g., voles, titi monkeys).
127 Additionally, few studies of social interactions and gene regulation have focused on species that

128 establish both clear social dominance hierarchies and long-term social bonds outside the mating
129 pair-bond.

130 To begin addressing this gap, this study draws on data and samples from a five decade-
131 long field study of wild baboons in Kenya, in which the fitness consequences of both social
132 status and social relationships have been extensively investigated in prior work [29,44,45,75–
133 78]. Gene regulatory signatures of the social environment have also been detected in this
134 population [15,79]. Most relevant to this work, high-ranking baboon males exhibit elevated
135 expression of inflammation-related genes both at baseline and upon stimulation with
136 lipopolysaccharide (LPS; a pathogen-associated molecular pattern associated with gram-
137 negative bacteria, and a strong driver of the innate inflammatory response: [15]). In contrast,
138 little signature of rank was detectable in females [15]. This result is consistent with findings that
139 high rank in males (but not females) predicts accelerated epigenetic aging, elevated
140 glucocorticoid and testosterone levels, and, to a lesser extent, higher mortality rates [14,45,80].
141 However, it contrasts with the hypothesis of a highly consistent gene regulatory response to the
142 social environment [66].

143 Together, these observations raise key questions about the extent to which the links
144 between social experience and gene regulation are sex- and/or context-specific. To address
145 them here, we expand on our previous white blood cell gene expression data sets by 64% (from
146 $n = 119$ to $n = 195$ samples, including paired baseline and LPS-stimulated samples from nearly
147 all individuals; Table S1). We also generated ATAC-seq data on chromatin accessibility in
148 baseline and LPS-stimulated samples to infer the transcription factor binding events that explain
149 social environment associations with gene expression. Our results indicate even more
150 substantial sex differences in the signature of dominance rank than was apparent in previous
151 work. We also identify, for the first time in any natural vertebrate population, a strong signal of
152 social bond strength on gene regulation. Although several of the major pathways associated
153 with rank and social bond strength overlap, their signatures are clearly distinct, and only social
154 bond strength predicts the gene expression *response* to pathogen stimulation (i.e., the
155 difference between baseline and LPS-stimulated cells from the same individual). Together, this
156 work emphasizes the strong relationship between the social environment and gene regulation in
157 the immune system in wild social mammals. It thus deepens our understanding of how fitness-
158 relevant social experiences “get under the skin” to affect health and fitness outcomes.

159 160 **RESULTS**

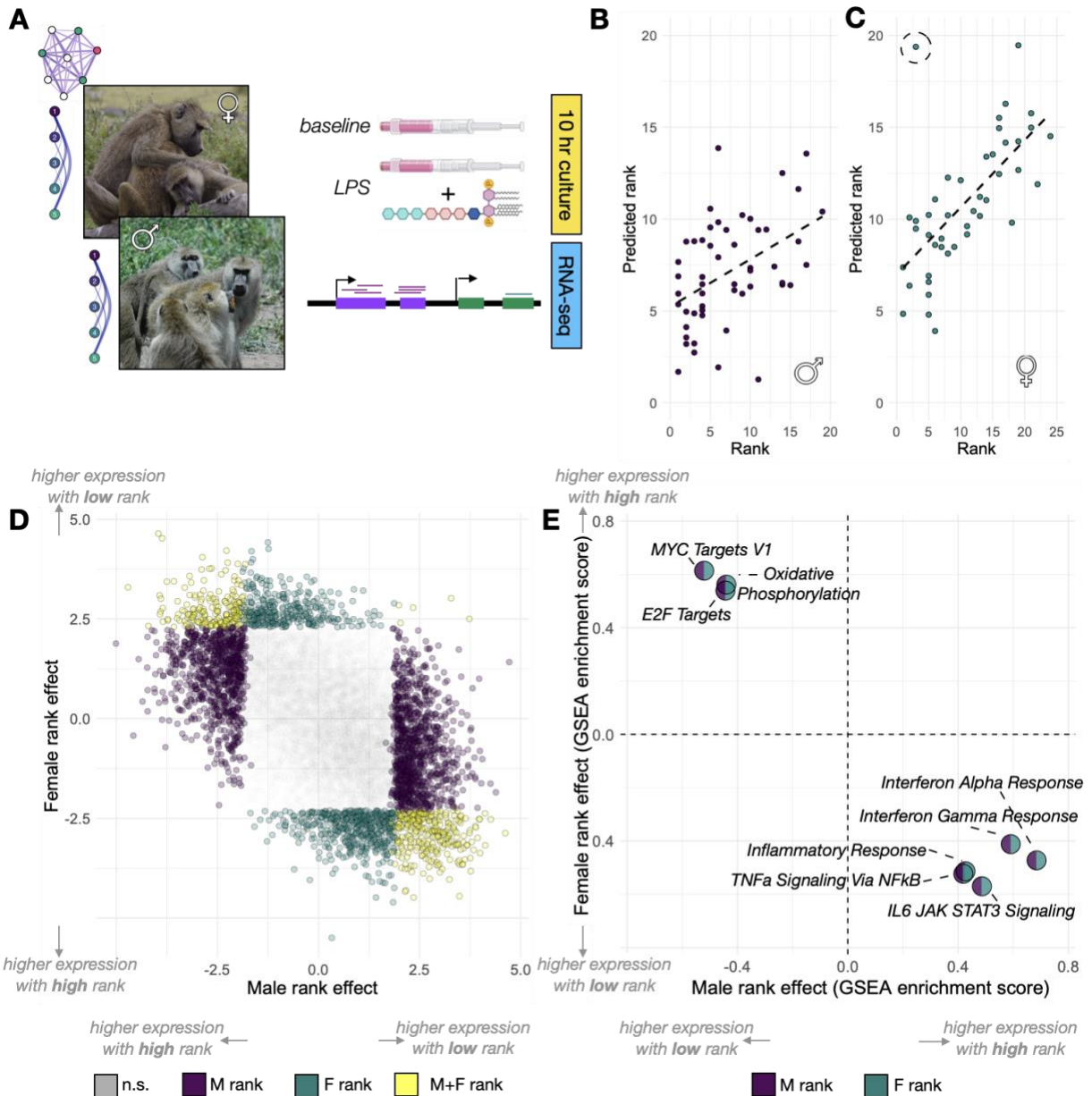
161 ***Directionally opposite gene expression signatures of dominance rank in male and female*** 162 ***baboons***

163 We used RNA-seq to measure genome-wide gene expression levels in white blood cells
164 from 97 unique adult baboons (45 females, 52 males; Table S1). For each animal, we collected
165 RNA from paired baseline (unstimulated cells cultured in media) and LPS-stimulated samples
166 that were cultured in parallel for 10 hours (Fig 1A; following [15]). Following quality control, our
167 data set consisted of RNA-seq data from 195 samples (119 samples from previously published
168 work and an additional 76 samples that are newly reported here; 97 unique individuals total,
169 with 3 individuals represented by more than one blood draw; Table S1). Samples were
170 sequenced to a mean coverage of 17.4 million reads \pm 7.7 million s.d. (Table S1). After filtering

171 for genes that were detectably expressed in one or both conditions (median RPKM > 2 in either
172 condition), we retained 10,281 genes for downstream analysis.

173 We first investigated the signature of dominance rank separately in each sex. Consistent
174 with our earlier work [15], we found widespread associations between male ordinal dominance
175 rank and gene expression levels. 2,345 genes were significantly associated with male rank in
176 baseline samples and 2,996 in LPS-stimulated samples (22.8% and 29.1% of genes tested,
177 respectively; i.e., $\beta_{\text{rank}} \neq 0$ in a linear mixed effects model controlling for technical covariates,
178 age, and treatment effects, 10% FDR; Table S2. An elastic net model relating gene expression
179 to dominance rank thus predicted male rank with moderately high accuracy (Pearson's
180 $R=0.449$, $p=8.46 \times 10^{-4}$; Fig 1B; Fig S1). In females, gene expression data were also significant
181 predictors of dominance rank (Pearson's $R= 0.656$, $p= 1.31 \times 10^{-6}$; Fig 1C; Table S3). However,
182 the elastic net analysis for females revealed one female (AMB_2) who was high-ranking at the
183 time of sampling (ordinal rank 3) but was predicted to be very low-ranking in both baseline and
184 LPS samples (predicted ordinal rank 17.7 and 19.4, respectively; Fig S1). By substantially
185 increasing our sample size and excluding AMB_2, we were able to identify female dominance
186 rank-gene expression associations that were undetectable in previous work [15], including 1,285
187 rank-associated genes at baseline and 221 rank-associated genes after LPS stimulation (12.5%
188 and 2.1% of genes tested, respectively; 10% FDR; Table S2). Because AMB_2 was a clear
189 outlier in our sample, we report analyses excluding her in the remainder of our results; however,
190 our comparisons are qualitatively unchanged if AMB_2 is included (Fig S2).

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Figure 1. Strong, sex-specific signatures of dominance rank in white blood cell gene expression. (A) Study design: dominance rank (males and females) and social bond strength (females only) were evaluated for their relationship with white blood cell gene expression, generated from samples cultured for 10 hours in the absence (baseline) or presence of lipopolysaccharide (LPS). **(B-C)** A gene expression-based elastic net model accurately predicts dominance rank for male (B; Pearson's $R=0.449$, $p=8.46 \times 10^{-4}$) and female (C; Pearson's $R=0.656$, $p=1.31 \times 10^{-6}$) baboons. **(D)** The effect estimates for the rank-gene expression association are negatively correlated in males versus females (Pearson's $R=-0.544$, $p<10^{-50}$; colored dots are genes that pass a 10% false discovery rate threshold). **(E)** Gene sets enriched for higher expression in high-ranking males are enriched for lower expression in high-ranking females, and vice-versa. Enrichment in males is shown in purple; enrichment in females is shown in green. For all gene sets, enrichment score Bonferroni-corrected p -values are <0.005 . Photo credits in (A): Elizabeth Archie (females) and Courtney Fitzpatrick (males). Stock images of LPS and blood draw tubes courtesy of BioRender.com.

206 In both males and females, the second principal component (PC2) of the overall gene
207 expression data was correlated with rank (males: $p=1.68 \times 10^{-4}$; females: $p=0.013$; note that
208 PC1 splits baseline from LPS-stimulated cells). However, while high-ranking males tended to
209 project onto positive values of PC2, high-ranking females tended to project onto negative values
210 (Fig S3). Effect size estimates for individual genes were also anti-correlated by sex, such that
211 genes that were more highly expressed in high-ranking females tended to be more lowly
212 expressed in high-ranking males (Pearson's $R=-0.537$, $p<10^{-50}$; Fig 1D; Fig S4). As a result,
213 genes with increased activity in high-ranking males and low-ranking females were both enriched
214 for inflammatory and type I interferon pathways (all $p_{\text{adj}}<0.005$; Fig 1E; Table S4) [81].
215 Meanwhile, genes with increased activity in low-ranking males and high-ranking females were
216 both enriched for key metabolic and cell cycle-related pathways, including oxidative
217 phosphorylation and *myc* signaling (all $p_{\text{adj}}<0.005$; Table S4). Thus, the same genes and
218 pathways were sensitive to rank dynamics in males and females, but in opposing directions.
219 Indeed, when applying the predictive model trained for male rank to gene expression data from
220 females, the model predictions were negatively correlated with the observed female ranks
221 (Pearson's $R=-0.339$; $p=0.023$), and vice-versa (correlation between female-trained model
222 predictions and observed male rank: Pearson's $R=-0.339$, $p=0.014$). Similarly, accessible
223 binding sites for immune response-related transcription factors (e.g., ISL1, KLF3, defined based
224 on increased chromatin accessibility after LPS stimulation: see SI Methods; Tables S5-S6) were
225 over-represented near genes upregulated in high-ranking males and near genes upregulated in
226 low-ranking females (all $p < 0.05$; Fig S6; Table S7).

227 228 ***Distinct signatures of dominance rank and social bond strength in female baboons***

229 To investigate whether genes that are sensitive to dominance rank (regardless of effect
230 direction) also carry a signature of other aspects of the social environment, we next assessed
231 the relationship between social bond strength and gene expression in the same sample. We
232 focused exclusively on females ($n=88$ samples from $n=44$ unique individuals), using the “dyadic
233 sociality index” (DSI), a strong predictor of lifespan in our study population that captures an
234 annual measure of the strength of a female's bonds with her top three female partners [45].
235 Female-to-female DSI is uncorrelated with dominance rank in this data set (Pearson's $R = 0.11$,
236 $p = 0.468$), allowing us to assess the overlap between DSI and rank associations with gene
237 expression independently of a correlation between the predictor variables themselves. While
238 male social bonds to females also predict male survival [45], our DSI data set for males ($n=30$
239 unique individuals) was too small to support a parallel analysis.

240 Controlling for dominance rank and other biological and technical sources of variance,
241 we identified 529 DSI-associated genes (5.1% of genes tested) in female Amboseli baboons
242 ($\beta_{\text{DSI}} \neq 0$ in a linear mixed effects model; 10% FDR; Table S2). The vast majority of cases (522
243 genes, 98.6%) were specifically identified in the LPS-stimulated condition, although gene-level
244 DSI effect sizes are correlated between conditions (Pearson's $R=0.524$, $p<10^{-50}$). Surprisingly,
245 genes that were more highly expressed in females with strong social bonds (high DSI) also
246 tended to be more highly expressed in low-ranking females, and vice versa, resulting in a
247 positive correlation between the parameter estimates for rank and DSI (at baseline: Pearson's
248 $R=0.516$; in LPS-stimulated samples: $R= 0.351$; Fig S5; note that the positive correlation arises

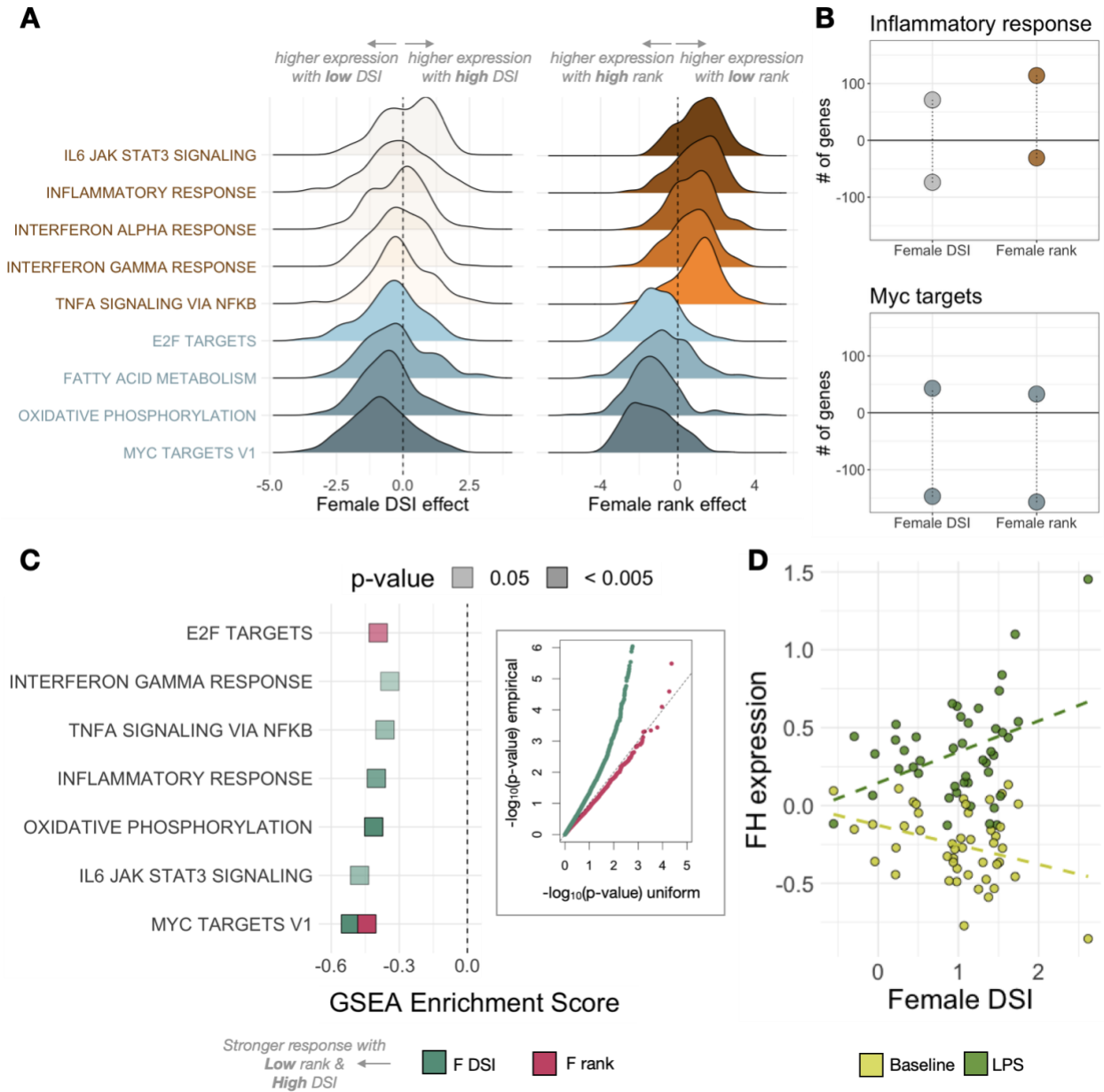
249 because low ordinal rank values reflect high rank: i.e., the top-ranked female has an ordinal
250 rank of 1 and lower ranked females have ranks >1).

251 This result was counterintuitive to us because strong social bonds predict longer lifespan
252 in Amboseli baboon females [45], but the inflammation-related pathways associated with low
253 female rank in this population are commonly thought to be costly to health [82,83]. We therefore
254 investigated the pathways that account for the correlation in rank and DSI effect sizes at
255 baseline. We found that this correlation is not, in fact, driven by immune process and
256 inflammation-related genes: social environmental effects on these genes are specific to rank,
257 and absent for social bond strength (Fig 2A). Specifically, genes involved in the inflammatory
258 response are highly enriched for upregulated expression in low-ranking females at baseline
259 ($p_{\text{adj}} < 0.005$) and the majority of genes in this set exhibit a positive effect size (i.e., increased
260 expression with lower rank: binomial test $p = 2.35 \times 10^{-12}$). In contrast, there is no enrichment of
261 inflammation-related genes for DSI ($p_{\text{adj}} > .05$), nor any bias in the sign of the DSI effect ($p =$
262 0.868). Consistent with these observations, accessible binding sites for TFs active in the
263 immune response (e.g., STAT5, Smad3, STAT3) are not enriched in or near DSI-upregulated
264 genes (all $p > 0.5$; Table S7). Instead, the overall correlation in rank and DSI effect sizes is
265 driven by genes involved in cellular metabolism and cell cycle control, particularly targets of the
266 transcription factor *myc* and genes that function in fatty acid metabolism and oxidative
267 phosphorylation (both $p_{\text{adj}} < 0.005$; Fig 2A-B).

268 Notably, while genes involved in immune defense are not associated with DSI at
269 baseline, a number of immune-related gene sets are significantly enriched for large DSI effects
270 in the LPS-stimulated condition. After LPS stimulation, high social bond strength predicts higher
271 expression of genes involved in the inflammatory response ($p_{\text{adj}} = 2.0 \times 10^{-3}$). Because these
272 genes are not detectably associated with DSI in baseline samples, this observation suggests a
273 potential interaction between social bond strength and the cellular environment after bacterial
274 exposure. In support of this possibility, DSI predicts the magnitude of the *response* to LPS (i.e.,
275 the foldchange difference between LPS and baseline samples, within females) for 200 genes
276 (10% FDR; Fig 2C; Table S8). Females with strong social bonds nearly always exhibit a more
277 dynamic response to LPS than those with weaker social bonds (binomial test for LPS-
278 upregulated genes: $p = 1.55 \times 10^{-10}$; binomial test for LPS-downregulated genes: $p = 3.12 \times 10^{-$
279 12). In contrast, because dominance rank effects are highly consistent between baseline and
280 LPS conditions, rank does not predict the magnitude of the response to LPS (1 rank-associated
281 gene; 10% FDR; Table S8). While many of the associations between DSI and the LPS response
282 occur in immune pathways (Fig. 2C), females with stronger social bonds also exhibit markedly
283 stronger responses to LPS in key cellular metabolism genes, including a key enzyme that
284 catalyzes transitions through the Krebs cycle (*FH*: $q = 0.024$; Fig 2D).

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290 **Figure 2. Social status and social bond strength predict distinct patterns of gene expression.** (A) Distribution

291 of DSI effects (left) and rank effects (right) on gene expression at baseline for genes within the MolSigDB Hallmark

292 gene sets labeled at left. Genes within immune-related pathways (red/orange) are polarized towards higher

293 expression in low-ranking females (positive effect sizes, because low rank is represented by high ordinal rank

294 values). In contrast, genes in the same pathways show no pattern for association with DSI (small effect sizes

295 centered on zero). Cellular metabolism and cell cycle-related gene sets (blue) tended to be more highly expressed

296 with high rank (negative effect sizes) and low DSI (negative effect sizes). Translucent density plots indicate no

297 significant bias in the direction of effects (binomial test $p > 0.05$). (B) Effect size bias for genes in the Hallmark

298 inflammatory response and myc (v1) target gene sets, for DSI and rank respectively. (C) Gene set enrichment

299 analysis results for female DSI (green) and rank (pink) effects on the foldchange response to LPS stimulation. Inset:

300 QQ-plot of the $-\log_{10}(p\text{-value})$ for DSI and rank effects on the LPS response, relative to a uniform null distribution. We

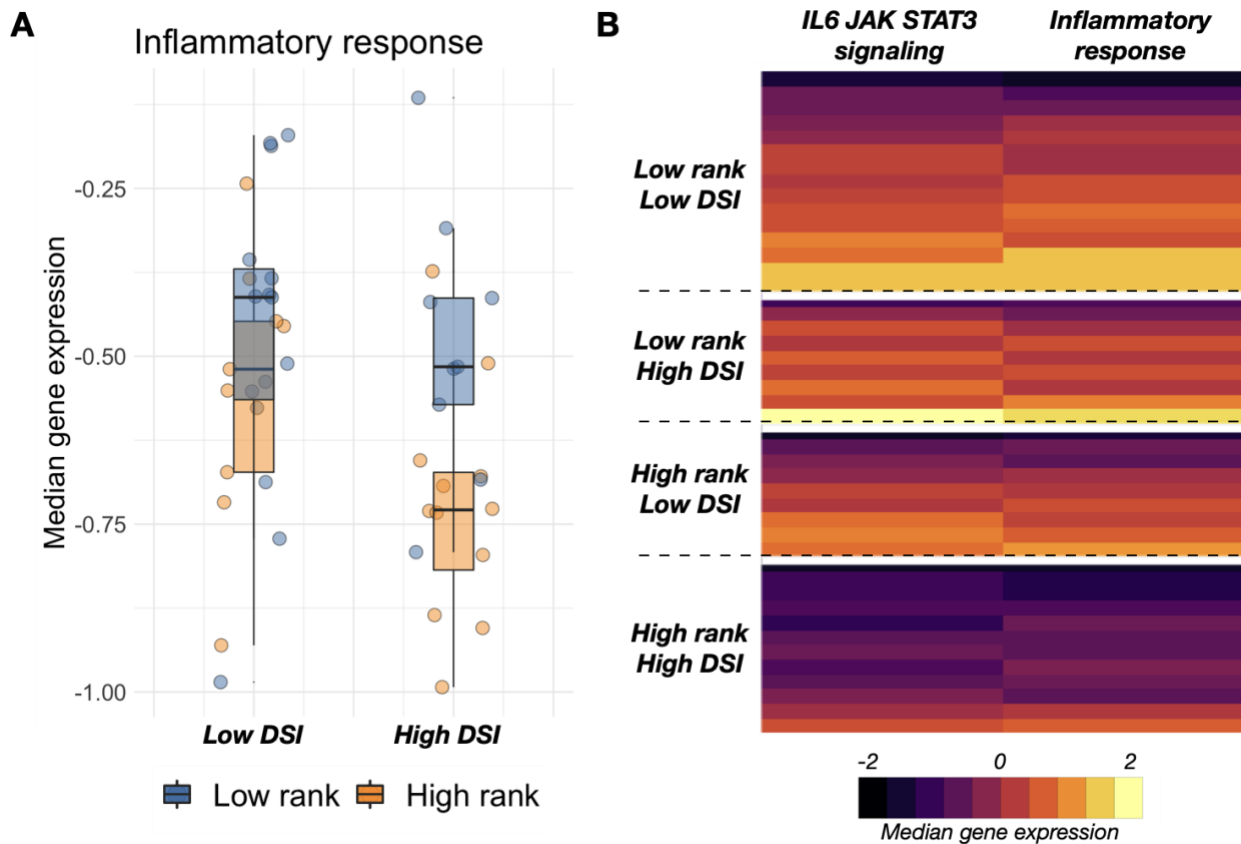
301 observe strong evidence for associations between DSI and the LPS response, but not for rank. (D) Example of FH, a

key enzyme in the Krebs cycle that responds more strongly to LPS in high DSI females than low DSI females.

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Gene expression patterns and multidimensional social advantage

To investigate the combined signatures of social status and social bond strength, we asked whether females that were relatively advantaged in both respects—and who therefore experienced advantages to both fertility and survival [44,45,75,84] — appeared physiologically distinct from other females. To do so, we binned females into four categories, corresponding to high rank/high DSI, high rank/low DSI, low rank/high DSI, and low rank/low DSI (stratified based on median rank and median DSI values in our sample). This classification reveals that, at baseline, high rank/high DSI females exhibited the lowest median expression values of genes in the Hallmark inflammatory response and IL6 signaling via JAK/STAT3 gene sets ($p < 0.05$ for Wilcoxon summed ranks test of high rank/high DSI females against all three other categories, for both gene sets; Fig 3). Thus, females with social capital in both dimensions—status and affiliation—present a distinct, potentially advantageous gene regulatory profile as well.



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Figure 3. The gene expression signature of multidimensional social advantage. (A) Median gene expression for genes in the inflammatory response gene set illustrates that high ranking animals exhibit lower inflammation-related gene expression regardless of social bond strength (main effect of rank = -0.15, $p = 0.019$). There is no main effect of DSI ($p = 0.166$), but the difference between high- and low-ranking females is greater when high-ranking females also have strong social bonds. (B) Median, rescaled gene expression per individual in the Hallmark IL6 signaling via JAK/STAT3 and the inflammatory response gene sets. Each row represents a different female, with rows stratified by median dominance rank and median DSI.

326 DISCUSSION

327 Social interactions, both affiliative and competitive, determine much about the daily
328 experience of group-living animals. Over the life course, these experiences compound to
329 powerfully predict health, survival, and reproductive success. Our findings reinforce that the
330 signature of the social environment is not only observable at the whole-organism level, but also
331 in widespread differences in gene expression. They therefore contribute to a modest but
332 expanding body of work linking gene expression variation to social experience in natural animal
333 populations, in both the brain and the periphery [15,70]. Together, this work generalizes
334 extensive research on social interactions and gene regulation in laboratory models [67,85–87] to
335 freely interacting animals in the wild. It also argues that correlations between gene expression,
336 social status, and social integration in humans capture a broader pattern of molecular sensitivity
337 to the social environment that predates the evolution of our own lineage [66,88].

338 Our findings converge with much of the previous work in humans and captive primates
339 to indicate that innate immune defense and cellular metabolism-related pathways are closely
340 entwined with social experience [15,57,89–92]. However, the signature of social bond strength
341 is much more apparent after immune stimulation than at baseline, and the signature of
342 dominance rank is substantially stronger in male versus female baboons. Thus, the functional
343 genomic signatures of different aspects of the social environment are themselves distinct. Our
344 results are consistent with observations that the fertility and survival consequences of male
345 rank, female rank, and female social bond strength also differ in this population [29,44,45,75–
346 78]. They thus call the hypothesis of a strongly conserved signature of social disadvantage into
347 question [66]. Tests for such a signature have particularly emphasized social disadvantage-
348 linked increases in the expression of inflammation and interferon signaling-related genes. This
349 prediction is supported for low rank in females but not for low social bond strength—and
350 strikingly, is directionally reversed in male baboons.

351 Consequently, only female social status-related differences in gene expression
352 recapitulate the pattern reported in studies of socioeconomic status, loneliness, and social
353 integration in humans and experimental studies of dominance rank in captive female rhesus
354 macaques [57,89,91,93–97]. Our results suggest that low social status in female baboons may
355 therefore be a better model for social disadvantage in humans than low social status in male
356 baboons—perhaps because social status in male baboons is driven almost entirely by fighting
357 ability, which is not the primary determinant of social status in modern human societies. Indeed,
358 social environment-associated gene expression signatures in humans are often interpreted
359 through the lens of chronic psychosocial stress [88,96,98]. While the importance of chronic
360 stress in natural animal populations remains an open question [11], low ranking females in both
361 this study population, wild blue monkeys, and captive rhesus macaques do exhibit higher
362 glucocorticoid levels and/or a blunted diurnal rhythm [12,13,32,99]. Psychosocial stress may
363 therefore be the common explanatory factor underlying conserved signatures of social
364 adversity, when they are observed. In contrast, high rank in baboon males imposes energetic
365 stress due to competition with other males and the demands of mate-guarding [29,100],
366 although males may experience forms of psychosocial stress as well. And while the stability of
367 social hierarchies and experimental work in captive primates suggests that rank precedes the
368 gene expression patterns we observe in females, males that achieve high rank may already be
369 physiologically distinct [15].

370 This explanation does not, however, account for why social bond strength does not
371 follow the same pattern as female dominance rank. Weak social bond strength in Amboseli
372 baboon females is also correlated with elevated glucocorticoid levels, although this effect is
373 modest in comparison to other predictors (e.g., early life adversity [101]). If glucocorticoids are a
374 major determinant of social environment-associated variation in immune pathway gene
375 expression, these observations may account for why (unlike rank) we did not observe a strong
376 signature of social bond strength in immune genes at baseline. Instead, social bond strength is
377 most consistently linked to oxidative phosphorylation and *myc* signaling (a key regulator of cell
378 growth, metabolism, and apoptosis). Intriguingly, *myc* activity has also been implicated in social
379 regulation of brain gene expression in mice and as a mediator of social isolation-induced cancer
380 susceptibility in mice and rats [64,102]. These observations suggest that social bond strength
381 may be involved in altered energy metabolism and energetics in the baboons, as suggested in
382 other studies of chronic and/or psychosocial stress [103].

383 Together, our findings emphasize substantial complexity in how the social environment
384 is reflected at the molecular level. If we had focused only on an *a priori* subset of genes in the
385 genome, we could have concluded that social interactions do not predict gene expression levels
386 at all; that social status, but not social affiliation, predicts gene expression; or that social status
387 and social affiliation generate highly similar gene expression signatures. Similarly, if we had
388 focused only on one sex, we would have missed the shared sensitivity, but reversed
389 directionality, of status-related pathways in males versus females. Finally, if we had only
390 measured gene expression levels at baseline, we would have inferred that social bond strength
391 has little relevance to immune gene regulation, when in fact it is a much better predictor of
392 variation in the response to immune stimulation than dominance rank. While this complexity
393 presents a challenge—additional dimensions we did not explore, including developmental,
394 tissue, and cell type differences, are also likely to be important—it also illustrates the potential
395 for high-dimensional genomic data to capture heterogeneity in the signature of social
396 relationships that is impossible to infer from single measures. Indeed, our results suggest that,
397 even in the blood, social regulation of gene expression must be the consequence of multiple
398 upstream signaling pathways. Future studies thus have the opportunity both to test existing
399 hypotheses about the role of glucocorticoids in social environment-associated gene regulation,
400 and to identify alternative pathways that may also play an important role.

401

402 **Methods:**

403 *Study subjects and samples*

404 Study subjects were 97 adult baboons (52 males; 45 females) sampled from an
405 intensively monitored population of hybrid yellow baboons (*Papio cynocephalus*) and anubis
406 baboons (*Papio anubis*) in the Amboseli ecosystem of southern Kenya [104,105]. Genome-wide
407 gene expression measures were generated from blood samples collected during opportunistic
408 dartings from 2013 – 2018. Data from samples collected in 2013 – 2016 were previously
409 reported in [15], while the remaining 76 samples are newly reported here (Table S1). For all
410 sampling efforts, subjects were anesthetized using Telazol-loaded darts and safely removed
411 from their social groups for sample collection (as in [15,106,107]). Darted individuals were
412 allowed to recover from anesthesia and released to their social group the same day.

413 For each study subject, we drew 1 mL of blood directly into a sterile TruCulture tube
414 (Myriad RBM) containing cell media only (the baseline sample), and another 1 mL of blood into
415 a second TruCulture tube containing cell media plus 0.1 ug/mL lipopolysaccharide (LPS; Fig
416 1A). Samples were incubated for 10 hours at 37 C. Following incubation, white blood cells were
417 extracted and stored in RNALater at -20 C until further processing. To control for cell type
418 composition, we also measured peripheral blood mononuclear cell type proportions for five
419 major cell types, for each individual. To do so, we purified peripheral blood mononuclear cells
420 (PBMCs) from blood drawn into Cell Preparation Tubes (CPT tubes; BD Biosciences) and
421 stained the PBMCs using fluorophore-conjugated antibodies to the cell surface markers CD3,
422 CD14, CD16, CD8, and CD20, which together differentiate classical monocytes (CD3⁻/CD14⁺/
423 CD16⁻), natural killer cells (CD3⁻/CD14⁻/CD16⁺), B-cells (CD3⁻/CD20⁺), helper T-cells
424 (CD3⁺/CD4⁺/CD8⁻), and cytotoxic T-cells (CD3⁺/CD4⁻/CD8⁺) [15]. PBMC composition was then
425 profiled on a BD FACS Calibur flow cytometer and analyzed in FlowJo 10.7.1 (Table S1 with
426 additional cell type discrimination based on cell size and granularity).

427 To measure chromatin accessibility, 50 mL of blood was drawn from three male anubis
428 baboons housed at Texas Biomedical Research Institute's Southwest National Primate
429 Research Center into CPT tubes (BD Biosciences), spun for 30 minutes at 1800 rcf, and
430 shipped to Duke University for PBMC isolation. 50,000 PBMCs from each individual were
431 incubated for 10 hours at 37C and 5% CO₂ in either the presence or absence of LPS (0.1
432 ug/mL, Invivogen ultrapure LPS from *E. coli* strain 055:B5). We then generated ATAC-seq
433 libraries from 50,000 cells per sample (n=6 baseline and LPS-stimulated samples total from the
434 3 baboons; see SI Methods; [108]).

435 436 *Dominance rank and social bond strength*

437 Sex-specific dominance ranks are assigned each month for each social group in the
438 study population based on the outcomes of dyadic agonistic interactions observed on a near-
439 daily basis [104,109]. Dominance rank assignments produce a hierarchy structure that
440 minimizes the number of cases in which higher ranking individuals lose interactions to lower
441 ranking ones [110]. To investigate rank-gene expression associations, we extracted ordinal
442 dominance rank values concurrent with blood sample collection, which represent rank as integer
443 values where rank 1 denotes the top-ranking individual, rank 2 denotes the second highest-
444 ranking individual, and so on. We note that previous analyses in this and other social mammals
445 show that alternative rank metrics sometimes confer improved predictive power [110,111]. In the
446 Amboseli baboon population, this is especially observable in females, where proportional rank
447 (i.e., ordinal rank scaled by group size) is more closely associated with fecal glucocorticoid
448 levels and injury risk than ordinal rank [110]. In this data set, substituting ordinal rank for
449 proportional rank produces highly concordant effect size estimates (R^2 for baseline male, LPS
450 male, baseline female, and LPS female rank effects = 0.75, 0.79, 0.88, 0.85, respectively), so
451 we reported the results for ordinal rank for both sexes.

452 To measure social bond strength, we used the dyadic sociality index (DSI, as in
453 [45,80,101]). The DSI calculates the mean grooming-based bond strength between a focal
454 female and her top three grooming partners in the year prior to sample collection, controlling for
455 observer effort and dyad co-residency times (see details in the Supplementary Methods). High

456 DSI values thus correspond to strong social bonds, and low DSI values correspond to weak
457 social bonds.

458

459 *Genomic data generation*

460 For gene expression measurements, RNA was extracted from each sample (n=195 from
461 n=97 unique baboons) using the Qiagen RNeasy kit, following manufacturer's instructions
462 (mean RIN=9.19 in a random subset of n=21 samples). We constructed indexed RNA-seq
463 libraries using the NEBNext Ultra I or II library prep kits, followed by paired-end sequencing on
464 an Illumina HiSeq 2500 (for samples collected from 2013 – 2016) or single-end on a HiSeq
465 4000 (for samples collected after 2016) to a mean depth of 17.4 million reads (± 7.7 million SD;
466 Table S1). Trimmed reads were mapped to the *Panubis 1.0* genome (GCA_008728515.1) using
467 the STAR 2-pass aligner [112,113]. Finally, we generated gene-level counts using *HTSeq* and
468 the *Panubis1.0* annotation (GCF_008728515.1) [114]. We retained genes with median RPKM >
469 2 in the baseline samples, LPS samples, or both for downstream analysis (n=10,281 genes).

470 For chromatin accessibility estimates, ATAC-seq libraries were sequenced on a HiSeq
471 2500 to a mean depth of 40.0 million paired-end reads (± 13.7 million SD; Table S5). Trimmed
472 reads were mapped to the *Panubis 1.0* genome using *BWA* [115]. We then combined mapped
473 reads across samples in the same condition (baseline or LPS) and called chromatin
474 accessibility peaks for each condition separately using *MACS2* (see Supplementary Methods;
475 [116]).

476

477 *Gene expression analysis*

478 To identify social environment associations with gene expression, we first normalized the
479 gene expression data set using *voom* [117] and regressed out year of sampling (the primary
480 source of batch effects in our data set), sequencing depth, and the first three principal
481 components summarizing cell type composition using *limma* [118]. For each gene, we then
482 modeled the resulting residuals as the response variable in a sex-specific linear mixed model
483 including the fixed effects of treatment (LPS or baseline), dominance rank, DSI (for females
484 only), age, and a random effect that controls for kinship and population structure [119]. We
485 nested age, rank, and DSI within treatment condition to evaluate condition-specific versus
486 shared effects. To estimate genetic covariance between individuals, which is required for the
487 random effect estimates, we genotyped samples from the RNA-seq data using the Genome
488 Analysis Toolkit (see Supplementary Methods; [120]). To control for multiple hypothesis testing,
489 we calculated false discovery rates using the R package *qvalue* after verifying the empirical null
490 was uniformly distributed [121].

491 To investigate how social interactions influence the *response* to LPS treatment, we
492 calculated an equivalent to the fold-change in residual gene expression between paired LPS
493 and baseline samples in the 44 females with both samples available. We then modeled this
494 response using a mixed effects model, with fixed effects of age, dominance rank, and DSI, and
495 a random effect to control for genetic relatedness/population structure. To test for enrichment of
496 specific gene sets among rank- or DSI-associated genes, we used Gene Set Enrichment
497 Analysis (GSEA; [122]), across the 50 Hallmark gene sets in the Molecular Signatures
498 Database (MolSigDB; [81]). We assessed the significance of pathway enrichment scores via

499 comparison to 10,000 random permutations of gene labels across pathways, and controlled for
500 multiple hypothesis testing using a Bonferroni correction.

501 All statistical analyses in this section and below were performed in R (R version 3.6.1;
502 [123]).

503

504 *Elastic net rank predictions*

505 To generate predictive models for rank, we used the elastic net approach implemented
506 in the R package *glmnet* [124]. For within-sex predictions, samples from the same treatment
507 condition (baseline or LPS) were iteratively removed from the training set. An elastic net model
508 was then trained using N-fold internal cross-validation on the remaining samples, and rank was
509 predicted from the normalized gene expression data for the left-out test sample (see
510 Supplementary Methods). To predict across sex, we trained a single model on all samples from
511 a single treatment-sex combination, and used the model to predict rank for all samples from
512 animals of the other sex, collected in the same treatment condition.

513

514 *Transcription factor binding motif enrichment*

515 To investigate transcription factor binding motif (TFBM) enrichment, we focused on the 5
516 kb sequence upstream of rank or DSI-associated genes. We intersected these regions with
517 areas of open chromatin called from the ATAC-seq samples, merged within treatment (e.g. the
518 combined baseline or combined LPS samples). We then performed TFBM enrichment analysis
519 in these regions for rank- or DSI-associated genes relative to the background set of all
520 expressed genes using *Homer* (see Supplementary Methods) [125].

521

522 *Data accessibility*

523 The sequencing data analyzed here have been deposited in the NCBI Short Read
524 Archive under BioProject (PRJNA480672) for previously published data, PRJNA731520 for
525 newly generated RNA-seq data, and PRJNA731674 for baboon PBMC ATAC-seq data. Data
526 analysis and figure code is deposited at

527 https://github.com/janderson94/Anderson_et_al_distinct_social_signatures.

528

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557

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