

Supplementary Materials:

Table S1. Summary of saline volume and synthetic adrenocorticotropic hormone (Synacthen® depot, Novartis, South Africa (Pty) Ltd.) administered to each individual during the ACTH challenge.

| ID | Sex | Dosage ($\mu\text{g}/100\text{g}$) | Mass (g) | Synacthen Volume (μL) | Saline Volume (μL) |
|----|--------|--------------------------------------|----------|------------------------------------|---------------------------------|
| F1 | Female | 20 | 45.1 | 36 | 164 |
| F2 | Female | 20 | 35.6 | 28 | 172 |
| F3 | Female | 20 | 38.2 | 31 | 169 |
| M1 | Male | 20 | 53.3 | 43 | 157 |
| M2 | Male | 20 | 40.5 | 33 | 167 |
| M3 | Male | 20 | 38.5 | 31 | 169 |
| F4 | Female | 60 | 43.0 | 103 | 97 |
| F5 | Female | 60 | 56.4 | 135 | 65 |
| F6 | Female | 60 | 50.1 | 120 | 80 |
| M4 | Male | 60 | 35.5 | 85 | 115 |
| M5 | Male | 60 | 33.2 | 80 | 120 |
| M6 | Male | 60 | 39.4 | 95 | 105 |

Table S2. Summary of EIA sensitivities and, intra-assay and inter-assay coefficients of variation of high and low value quality controls for all EIAs applied to measure immunoreactive urine glucocorticoid metabolite (uGCM) and fecal glucocorticoid metabolite in naked mole-rats.

| Enzyme immunoassay | Sensitivity | | | |
|---|---------------|-----------------------------|---------------------------|---------------------------|
| | Urine (pg/mL) | Feces (ng/g fecal dry mass) | Intra-assay variation (%) | Inter-assay variation (%) |
| Corticosterone | 80 | 2.4 | 4.57–5.74 | 10.60–13.49 |
| Cortisol | 25 | 0.75 | 4.42–6.24 | 14.11–14.89 |
| 11-oxoetiocholanolone I | 40 | 1.2 | 4.09–4.81 | 14.28–14.85 |
| 11-oxoetiocholanolone II | 25 | 0.75 | 4.94–6.84 | 10.91–12.74 |
| 5 α -pregnane-3 β ,11 β ,21-triol-20-one | 80 | 2.4 | 4.71–5.71 | 9.98–12.81 |