Supplementary material

**Immune competence and spleen size scale with colony status in the naked mole-rat**

Valérie Bégay1\*, Branko Cirovic2, Alison J. Barker1, Robert Klopfleisch3, Daniel W. Hart4, Nigel C. Bennett4 and Gary R. Lewin1\*

1Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Laboratory of Molecular Physiology of Somatic sensation, Robert-Rössle Straße 10, D-13125 Berlin, Germany

2German Cancer Research Center, Division of Cellular Immunology, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

3Free University of Berlin, Robert von Ostertag strasse 15, Institute of Veterinary Pathology, 14163 Berlin, Germany

4Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, Republic of South Africa

\*Correspondence to Valérie Bégay [vbegay@mdc-berlin.de](mailto:vbegay@mdc-berlin.de) or Gary R Lewin [glewin@mdc-berlin.de](mailto:glewin@mdc-berlin.de)

**References:**

1. Emmrich S, Mariotti M, Takasugi M, Straight ME, Trapp A, Gladyshev VN, Seluanov A, Gorbunova V. 2019 The hematopoietic landscape at single-cell resolution reveals unexpected stem cell features in naked mole-rats. *BioRxiv*

2. Hilton HG *et al.* 2019 Single-cell transcriptomics of the naked mole-rat reveals unexpected features of mammalian immunity. *Plos Biol.* **17**, e3000528. (doi:10.1371/journal.pbio.3000528)

3. Eigenbrod O *et al.* 2019 Rapid molecular evolution of pain insensitivity in multiple African rodents. *Science* **364**, 852-859. (doi:10.1126/science.aau0236)

**Table legends:**

**Table S1: Differential gene expression analysis of NM-R and MM spleen transcriptomes**

DESeq2 output tables for differential gene expression analysis comparing spleen samples from lsNM-R versus MM ("lsNM-R\_vs\_MM"), ssNM-R versus MM ("ssNM-R\_vs\_MM") and ssNM-R versus lsNM-R ("ssNM-R\_vs\_lsNM-R"), respectively. baseMean, average of the normalised count values; lfcSE, standard error estimate for log2FoldChange; stat, Wald statistic; padj, adjusted p value. For more details, see methods section.

**Table S2: Gene set enrichment analysis**

GSEA results comparing ssNM-R versus MM spleen transcriptomic data sets. padj, adjusted p value; ES, enrichment score; NES, normalised enrichment score; nMoreExtreme, number of more significant random gene pathways. Pathway size: number of tested genes in the pathway. For more details, see methods section.

**Table S3: Hallmark gene set enrichment analysis**

GSEA results comparing ssNM-R versus MM spleen transcriptomic data sets focusing on hallmark pathways. padj, adjusted p value; ES, enrichment score; NES, normalised enrichment score; nMoreExtreme, number of more significant random gene pathways. For more details, see methods section.

**Table S4: Enrichment analysis of NM-R immune cell gene signatures**

GSEA results with custom pathway signature defined by the immune cell signatures from single cell transcriptomic data as described in [2]. Transcriptome data from spleens of ssNM-R versus MM were compared. NES, normalised enrichment score; pval, p value; padj, adjusted p value. For more details, see methods section.