Supplemental Information

Hypofunctional TrkA Accounts for the Absence of Pain Sensitization in the African Naked Mole-Rat

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Figure S1 related to Figure 2. Thr502 does not affect PKCε-mediated sensitization of NMR TRPV1. (A) In NMR TRPV1, a conserved serine is substituted by threonine at position 502 (numbering for rat TRPV1). (B) Rat TRPV1 expressed in CHO cells is potentiated by a 30 second pulse of PMA. (C) Generation of naked mole-rat immortalized fibroblasts, four clones were isolated and population doubling (PD) measured weekly. Clone 3 ceased to grow at a cumulative PD of 56, clones 1, 2 and 4 reached PDs of 87, 64 and 60 respectively. Note primary fibroblasts grow normally at 32°C, but form multi-nucleated syncytium at 37°C (D). Naked mole-rat TRPV1 expressed in NMRF cells (clone 1) is similarly potentiated by PMA as rat TRPV1. (E) Naked mole-rat TRPV1T502S expressed in NMRF cells is potentiated by PMA. Chi-squared test; **p < 0.01, ***p < 0.001. (F) pH5.0 solution elicits inward currents in Trpv1−/− mouse neurons transfected with naked mole-rat Trpv1, currents were inhibited by 10 µM ruthenium red (RR) and transfected Trpv1−/− DRG neurons displayed heat-activated currents with mean thresholds of 44.4 ± 0.7°C (n = 5), example heat activated current shown is from a Trpv1−/− DRG neuron transfected with naked mole-rat Trpv1.
Figure S2 related to Figure 3. Sequence alignment of naked mole-rat TrkA peptide sequence. Cloned and predicted naked mole-rat TrkA sequences (Kim et al., 2011) were aligned with sequences available from public databases. (A) Domain 5 of the extracellular region responsible for NGF binding shows less sequence conservation than the intracellular kinase domain (B). Black squares represent conserved naked mole-rat tyrosines; Tyr759 (human Tyr751) and Tyr793 (human Tyr785). Turquoise square indicates a residue solely found in the naked mole-rat TrkA sequence (see also Figure S3A).
Figure S3 related to Figure 3. Comparison of naked mole-rat TrkA kinase domain sequences those of five other African mole-rat species. (A) Alignment of the TrkA kinase domain sequence for mouse (M. musculus), naked mole-rat (H. glaber), Giant (Fukomys mechwii), Mashona (F. darlingi), Natal (Cryptomys hottentotus natalensis), Damaraland (F. damarensis), and Emin’s mole-rat (Heliophobius emini)(Faulkes et al., 2011). A purple square represents a bathyergid-specific amino acid variant while orange squares represent the residues defining unique naked mole-rat TrkA kinase domain sequence. (B) Unusual residues that are shared between the naked mole-rat and the designated species are highlighted in red. Numbering is based on NMR TrkA. (C) Phylogenetic relationships between African mole-rat species were calculated from 827 transcripts per species with Mus musculus and Tachyoryctes splendens (Tanzanian root rat) as outgroups. Sequences were aligned using Clustal Omega (version 1.2.0). A phylogenetic tree was calculated with a maximum likelihood approach implemented in the RAxML tool (version 8.2.3). The F. darlingi sequences were obtained from our RNAseq data after de novo transcriptome assembly, publicly available RNAseq data was used to assemble sequences from the other African mole-rat species (see Table S1 for details). Branch lengths are proportional to the average number of substitutions per site.
Figure S4 related to Figure 3. NGF-induced activation of rat and chimeric TrkA. Serum-depleted HEK 293 cells expressing either rat or chimeric TrkA were stimulated with NGF (50 or 100 ng/ml) for 1 and 5 minutes, or left untreated, and total cell lysates were subjected for Western blotting analysis. (A) Representative immunoblots against total TrkA and TrkA Tyr674/675. Due to overexpression, a substantial amount of total TrkA was present in its immature form, and only the bands representing mature TrkA (110 and 140 kDa), that can be activated by ligands (Wolf et al., 1998) were used for quantification. (B) Quantification of TrkA intensities from five independent experiments. In rat TrkA, phosphorylation levels were significantly increased after both 1 and 5 minutes of NGF treatment when compared to the basal state. In the cells expressing the chimeric TrkA, the phosphorylation levels after 1 and 5 minute NGF stimulation were not different to the control, non-stimulated receptor. (C) Immunoblotting against total TrkA and TrkA Tyr490. (D) Quantification of TrkA intensities from seven independent experiments. NGF triggered phosphorylation of the Tyr490 residue, where rat TrkA samples showed a significant increase in phosphorylation level after 1 minute NGF stimulation. NGF treatment did not have any effect on the phosphorylation level of the chimeric TrkA Tyr490 residue, where both 1 minute and 5 minute NGF-treated samples were not different to the constitutive phosphorylation level of this receptor. One-sample t-test was used, with the value of basal phosphorylation level set as 1, with *p < 0.05; **p < 0.01 and ***p < 0.001. Data are represented as mean ± SEM.
Figure S5 related to Figure 4. Over-represented motifs surrounding upregulated phosphoserine sites when signaling through NGF-stimulated cells expressing either rat or chimeric TrkA. Proline moieties in sequence motifs are common in Erk 1/2 kinase substrate motifs. (Amanchy et al., 2007).
Table S1

| Species                          | SRA accession | # read pairs | # full-length transcripts | % TrkA sequence identified |
|---------------------------------|---------------|--------------|----------------------------|-----------------------------|
| *Fukomys darlingi*              | SRP066607*    | 107,855,116  | 9,798                      | 68                          |
| *Tachyoryctes splendens*        | SRR2141217    | 18,135,605   | 7,466                      | 35                          |
| *Heliophobius emini*            | SRR2141215    | 17,410,109   | 1,580                      | 0                           |
| *Bathyergus suillus*            | SRR2141210    | 18,175,296   | 5,849                      | 26                          |
| *Georychus capensis*            | SRR2141216    | 20,038,942   | 3,375                      | 0                           |
| *Cryptomys hottentotus mahali*  | SRR2141211    | 17,253,040   | 5,262                      | 0                           |
| *Cryptomys hottentotus*         | SRR2141212    | 17,290,951   | 4,973                      | 0                           |
| *Cryptomys hottentotus*         | SRR2141213    | 17,833,375   | 5,883                      | 0                           |
| *Fukomys damarensis*            | SRR2141214    | 18,220,998   | 6,109                      | 33                          |

Table S1 related to Figure 3. Summary of transcriptome assemblies from African mole-rat RNAseq data. Asterisk indicates data set deposited at NCBI accession code PRJNA303968.
Supplemental Experimental Procedures

**DRG neuron culture and transfection**
Animal housing, care and protocols for killing are registered with and approved by the appropriate German federal authorities (State of Berlin). DRG neurons were prepared from both naked mole-rat and mouse as described previously (Hu et al., 2010; Park et al., 2008) and plated onto glass coverslips plated with poly-L-lysine (200 μg/ml) and laminin (20 μg/ml). Neurons were maintained in DMEM (Life Technologies, U.S.A.) containing 10% heat-inactivated horse serum (Biochrom), 20 mM glutamine, 0.8% glucose, 100 U penicillin and 100 mg/ml streptomycin (Life Technologies) and incubated at 37°C in 5% CO₂. For transfection of naked mole-rat TRPV1/EGFP into DRG neurons from Trpv1<sup>Δ</sup> mice (Jax Mice, Bar Harbor, Maine), the Amaza Nucleofector system was used according to the manufacturer’s protocol (Amaza). All recordings were made within 36 hours of isolation. Note NGF was never added to the medium of cultured DRG neurons as this is known to lead to an up-regulation of heat-activated currents in sensory neurons (Stucky and Lewin, 1999).

**CHO cell culture and transfection**
CHO cells were cultured in F12-Ham medium (Life Technologies) and incubated at 37°C in 5% CO₂. For electrophysiology experiments cells were plated onto PLL-coated plastic dishes and the following day transfected with Lipofectamine (Invitrogen). The plasmid of interest was transfected at a 4:1 ratio with EGFP. For calcium imaging experiments cells were plated onto PLL-coated glass coverslips and transfected with Fugene (Promega). xTRPV1 and rat TrkA (kind gift of P.A. McNaughton, Kings College London) were transfected at a 5:5:1 ratio with EGFP.

**Electrophysiology**
Recordings took place after a 10 – 20 min incubation with either IB4-Alexa Fluor®-488 or IB4-Alexa Fluor®-568 (Invitrogen) to allow IB4-positive and -negative neurons to be discerned from one another. Whole-cell recordings were made from DRG neurons using pipettes (3 – 6 MΩ resistance) pulled with a Flaming-Brown puller (Sutter Instruments, USA). Extracellular solution contained (mM): NaCl 140, MgCl<sub>2</sub> 1, CaCl<sub>2</sub> 2, KCl 4, glucose 4, HEPES 10, pH7.4 with NaOH. Electrodes were filled with (mM): KCl 110, NaCl 10, MgCl<sub>2</sub> 1, EGTA 1 and HEPES 10, pH7.3. Solutions were applied and heated using a gravity driven multi-barrel perfusion system (WAS-02) (Dittert et al., 2006). All recordings were made using an EPC-10 amplifier in combination with Patchmaster® and Fitmaster® software (HEKA). Pipette and membrane capacitance were compensated using the auto function of Patchmaster and series resistance was compensated by ~70% to minimize voltage errors. IB4-positive of GFP-positive cells were observed using a Polychromator V (Visitron) and MetaFluor (Visitron).

**Molecular biology and cloning strategies**
Cloning of naked mole-rat Trpv1 was described before (Smith et al., 2011). QuickChange II XL kit (Stratagene) was used to introduce point mutations. A plasmid coding for rat TrkA was a kind gift from P. A. McNaughton, Cambridge, UK. In order to clone NMR TrkA, total RNA was isolated from naked mole-rat DRGs with TRIzol (Life Technologies) and dissolved in 30 μl of RNase-free water. 1 – 3 μg of total RNA, and oligo(dT) and random hexamers (BioteZ, Berlin) were used for cDNA synthesis using SuperScript III Reverse Transcriptase (Life Technologies). Given that no genomic data was available for naked mole-rat at the time when TrkA was cloned, cloning primers were designed in silico by aligning the nucleotide sequence of a TrkA coding sequence (CDS) from mouse, rat, human, orangutan and cow. Primers were designed from regions of high sequence conservation. This allowed us to clone and sequence a 2092 bp long transcript, aligning to the 3’ of the TrkA CDS. Sequence was proofread by cloning the respective exons from NMR genomic DNA. The remaining 305 bp, aligning to the 5’ end of the transcript, were devised from Kim et al., 2011. The final 2397 bp long transcript was synthesized and cloned into pUC57 by GenScript. Chimeric TrkA was cloned by overlap extension PCR, by using rat and NMR TrkA constructs. The overlapping primers used were 5’TMRmnr CAG TGG AGA AGA GAG ACG ACA CGC CT and 3’TMRrat AGG CGT GTC GTC TCT CTT CAC TGG.
In order to sequence the CDS for the TrkA intracellular kinase domain of other African mole-rat species, primers specific for NMR TrkA were used to amplify exons 12 – 17 from species’ genomic DNA. Five mole-rats representative of the Bathyergidae family were used: Giant (Fukomys mechowii), Damaraland (F. damarensis), Mashona (F. darlingi), Natal (Cryptomys hottentotus natalensis) and Emin’s (Heliophobius emini) mole-rats. The constructs used for Xenopus oocyte recordings (rat TRPV1, rat TrkA, NMR TrkA, chimeric TrkA) were subcloned into a modified pCI vector (Promega), linearized with ClaI and cRNA was synthesized by using the mMESSAGE mMACHINE T7 Transcription Kit (Life Technologies) according to the manufacturer’s protocol. The quality and integrity of the cRNA constructs was verified by RNA electrophoresis. The constructs used for activation of TrkA in HEK 293 cells (rat TrkA, chimeric TrkA) were subcloned into pEXPR-IBA105. All
Xenopus laevis oocyte recordings
cRNA was diluted to 0.55 μg/μl (TRPV1: TrkA = 2.15 : 1) in ultra-pure water. X. laevis defolliculated oocytes (stage V or VI) were purchased from EcoCyte Bioscience and delivered in Barth solution complemented with Pen/Strep. Glass capillaries were pulled on DMZ-Universal Puller (Zeitz, Germany) and each oocyte was injected in Barth solution using the Nanoject II Auto-Nanoliter Injector (Drummond, USA) with 32.2 nl of cRNA mix and kept at 16°C in Barth solution complemented with 10% horse serum and 1% Pen/Strep. Two-electrode voltage-clamp recordings were performed at RT 3 – 5 days after injection using a GeneClamp500B Amplifier, Digidata 1322A and pClamp 8.0 Software (Axon Instruments). Borosilicate glass electrodes (0.5 – 1 MΩ) were filled with 3 M KCl. Solutions were gravity fed with a flow rate of ~5 ml/min using a Bath Perfusion System valve controller (ALA-VM8, Ala Scientific Instruments, USA). Membrane potential was clamped to -40 mV and only those oocytes with leak currents < 500 nA were used for analysis. Between every acid pulse, oocytes were allowed to recover for 2 min with constant oocyte Ringer perfusion. For NGF sensitization, two or three acid pulses (pH5.8) were administered before NGF perfusion (1 – 1000 ng/ml for 5 min) followed by two acid pulses. Current responses right before and after NGF application were used for analysis. Currents were analyzed with pCLAMP9 Software (Axon instruments) after digital filtering at 1 kHz.

TrkA activation in HEK cells
HEK 293 cells (grown in DMEM (Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin, 4 mM L-glutamine, 10% fetal calf serum (FCS)) were transfected on Day 0 with either rat or chimeric TrkA construct with the PEI reagent. In some experiments, to control for equal level of transfection, cells were co-transfected with a plasmid coding for EGFP protein (DNA ratio TrkA : EGFP = 5 : 1). The GFP signal was visualized on the Day 1, prior to serum depletion. Recombinant human NGF (Sigma, cat. number N1408) was reconstituted in water (1 μg/ml stock) under sterile conditions and stored at -20°C. Prior to NGF stimulation, cells were serum depleted for 2 hours. 30 min prior to NGF stimulation, 0.5 mM sodium orthovanadate (Sigma) was added in order to block the phosphatases. This step was introduced in order to reduce dephosphorylation by phosphatases in stimulated cells, given that pilot experiments showed low relative increase in TrkA phosphorylation upon NGF stimulation. NGF working solutions (50 and 100 ng/ml) were prepared freshly in DMEM. Cells were incubated with NGF for 1 and 5 min to allow for TrkA receptor activation. Cells were washed 2x in ice cold DPBS and collected by scraping in 1x RIPA buffer (New England Biolabs) supplemented with cOmplete, Mini and PhosSTOP (Roche). Cell lysates were homogenized by passing them 10x and 5x through a 20 G and 23 G needle, respectively. Lysates were incubated for 10 min on ice and cell debris was pelleted at 13,000 rpm at 4°C. The supernatant was subjected to Western blotting. Proteins were transferred to a nitrocellulose membrane (pore size 0.2 μm, GE Healthcare). Membranes were blocked under agitation in blocking solution (5% BSA in TBS, 0.1 % Tween20) for 30 min at RT. Primary (anti-TrkA (kind gift from L.F. Reichardt, UCSF), and TrkA and TrkB Antibody Sampler Kit (CST) and secondary (anti-rabbit and anti-mouse HRP conjugated) antibodies were diluted in 5% BSA in TBST. The chemiluminescent signal was detected by the photo imager device FusionSolo (Vilber Lourmat) using Super Signal West Dura Chemiluminescent Substrate (Thermo Scientific). For quantification purpose, in order to account for loading and transfer errors, each membrane was immunoblotted twice. Phosphorylation status of a specific residue was assayed first, followed by membrane stripping and immunoblotting for the total TrkA. Membranes were stripped by agitation (75 rpm, 55°C, 25 min) in 25 ml prewarmed stripping solution (2% SDS, 0.8% B-ME, 80 mM Tris HCl pH6.8).

Calcium imaging
Calcium imaging was conducted as described previously (Milenkovic et al., 2007). Standard Fura-2 ratiometric calcium imaging was conducted to measure responses to capsaicin in CHO and NMRF cells transfected with rat TRPV1and NMR TRPV1 respectively, with rTrkA co-transfected in certain experiments. An inverted microscope (Zeiss Observer A1) equipped with MetaFluor photons imaging system, including Polychromator V or DG4 (Sutter Instruments), a CoolSNAP ES camera (Visitrion) was used for cell imaging. Paired images (340 and 380 nm excitation, 510 nm emission) were collected every 1.7 s. Capsaicin (1μM), NGF (100ng/ml) and PMA (1μM) were diluted in the same extracellular buffer as described for electrophysiology experiments previously. A 5 second capsaicin pulse was followed by 10 minutes perfusion with NGF or buffer lacking NGF before a second capsaicin response was applied. After the first capsaicin pulse only cells whose baseline recovered to at least 50% of the initial value before the second capsaicin application were analyzed. A 10 minute perfusion time was chosen because more cells recovered to within this 50% limit. Sensitization was scored if a cell’s percentage change was > mean percentage change + 2 s. d. in controls with percentage change calculated as: (100*(peak 2 – peak 1)/peak 1). For experiments with PMA, normal extracellular buffer was superperfused for 9.5 minutes followed by 30 seconds PMA treatment and then capsaicin. DRG neurons plated on a 5 mm
glass coverslip were placed in a recording chamber of 300 μl volume (Harvard Apparatus) and were continuously perfused with extracellular solution at a rate of 2 ml/min. Cells were loaded with Cal-520 (5 μM, AAT Bioquest) for 1 hour at 37 °C in the presence of Pluronic acid 0.02% dissolved in Ringer solution \([\text{mM}]: 140 \text{ NaCl}, 5 \text{ KCl}, 2 \text{ CaCl}_2, 2 \text{ MgCl}_2, 10 \text{ HEPES and 10 glucose, adjusted to pH 7.4}]\). Capsaicin (100 nM, Tocris) was dissolved in extracellular solution from a stock concentration of 10 mM in ethanol. Capsaicin was perfused for 30 s at 5 minutes interval. A solution contained 100mM KCl and 40mM NaCl was applied at the end of each experiment in order to select only viable and neuronal cells.

**Statistical analysis**
All statistical analyses were performed with GraphPad Prism 5. For electrophysiological analysis and for calcium imaging in DRG neurons, the Mann-Whitney U test was used to detect differences between neurons subjected to NGF and those not. For calcium imaging in CHO and NMRF cells, Fishers exact test was used. For oocyte recordings, One-way ANOVA with Bonferroni’s Multiple Comparison Test was used. For Western blotting analysis, one-sample t-test was used, with the basal state phosphorylation of the non-stimulated samples set to 1. All data are represented as mean ± SEM, unless otherwise stated. *p < 0.05; **p < 0.01; ***p < 0.001 and ****p < 0.0001.

**Immunohistochemistry and immunocytochemistry**
Standard immunohistochemistry and immunocytochemistry protocols on NMR and mouse DRGs were used (Wetzel et al., 2007) using an anti-TrkA antibody (kind gift from L.F. Reichardt, UCSF) and IB4-488. Immunofluorescent images were examined with a Leica DM 5000B microscope and MetaVue software (Visitron).

**Generation of NMRF cell line**
Fibroblast isolation and immortalization was conducted along similar lines to that described previously (Silva et al., 1995). Both kidneys were removed from 1 naked mole-rat and washed 3× in DMEM with 100 U penicillin, 100 mg/ml streptomycin. Tissue was chopped into small pieces and the subsequent sludge was shaken at ~37°C for 10 min in 0.05% trypsin (Sigma) in PBS. A 5 ml aliquot was taken and placed in a 50 ml tube with 5 ml DMEM (with 10% FCS, 100 U penicillin and 100 mg/ml streptomycin). 5 ml 0.05% trypsin was added to the original tissue suspension, shaken again for 10 min and 5 ml were taken again. This procedure was repeated for another 40 min. The trypsin/DMEM suspension was then spun down, cells resuspended in 20 ml supplemented DMEM and plated onto two 10 cm dishes. After initially noticing growth problems at 37°C (as has been noted previously (Salmon et al., 2008), cells from further isolation procedures were incubated at 32°C in 5% CO₂ and split when confluent. A pMSPE plasmid encoding SV40LT (kind gift from J. Fuhrmann, MDC) and G418 (800 μg/ml) were used for immortalization. Clones were selected passaged weekly calculating population doubling (PD). The cells frozen and stored in liquid N₂ could be defrosted and grown at the same rate as the same clone still in culture. Furthermore, after successful transformations cells could grow at 37°C with no major change in weekly PD (tested for 4 passages). However, cells used for experiments were incubated at 32°C to maintain the environment preferred by primary fibroblasts. Clone 1 was used in the experiments described here.

**Cell culture for SILAC and sample preparation for MS based protein quantification**
HEK293 cells were grown and labelled in SILAC media for at least seven population doublings. SILAC media were prepared as described before (Paul et al., 2011). Briefly, DMEM lacking pyruvate, glutamine, arginine and lysine (GIBCO) were supplemented with 10% dialyzed fetal bovine serum (dFBS, Gibco), pyruvate (1x, GIBCO), Glutamax (1x, GIBCO) and L-proline (20 mg/ml, Sigma). “Heavy” (H) SILAC media contained 28 mg/l \(^{13}\)C\(_6\) \(^{15}\)N\(_4\) L-arginine (Arg-10) and 49 mg/l \(^{13}\)C\(_6\) \(^{15}\)N\(_2\) L-lysine (Lys-8; all labeled amino acids from Sigma Isotec). “Light” (L) SILAC medium was prepared by adding the corresponding non-labeled amino acids (Arg-0 and Lys-0; Sigma). TrkA plasmids were co-transfected with pEGFP plasmid (5:1) to ensure equal transfection, and linear polyethyleneimine (Sigma) was used as a carrier. Cells were grown on 10 cm plates to reach 80%-90% confluence on the day of NGF stimulation. Twenty-four hours after transfection cells were washed once in warm DPBS and incubated for four hours in corresponding media lacking dFBS for serum starvation. Cells were stimulated for 10 min with 100 ng/ml NGF (murine 2.5S, Promega) or left untreated, and washed once in ice-cold DPBS before lysis. For total and upregulated phosphopeptide analysis, a biological duplicate was performed where H-labeled cells were stimulated with NGF, and L-labeled cells were left untreated (PMID 27136326). For assessment of MAPK phosphorylation levels a biological duplicate (label swap) was performed where in the forward set-up, H-cells were stimulated with NGF and L-cells left untreated, and in the reverse set-up L-cells were stimulated with NGF and H-cells left untreated. Cells were collected and lysed in 200 μl lysis buffer (8M urea, 100 mM Tris-HCl pH8.0 and benzonase (Merck)) and sonicated in ice-cold water for 5 min.
Protein concentration was measured by DC assay (Bio-Rad) and 250 µg of protein from each SILAC pair were mixed together. Protein mixture was reduced with 10 mM DTT in 50 mM ammonium bicarbonate, alkylated in 50 mM ammonium bicarbonate, 55 mM iodoacetamide, pre-digested with Lysyl endopeptidase (LysC, Wako, Osaka, Japan), and subjected to trypsin digestion (Promega) overnight. Digestion was stopped by trifluoroacetic acid (TFA). Peptides were purified from stop-and-go extraction (STAGE) tips (Rappsilber et al., 2003) containing C18 Empore disks (3M, Minneapolis, USA) pre-loaded with 10 mg of Reprosil-Gold 120 C18, 3 µm beads (Dr Maisch, Germany). Peptides were eluted from the C18 material with 400 µl TiO2 loading buffer (80% acetonitrile (ACN), 6% TFA). Phosphopeptide enrichment was performed on 0.5 mg TiO2 beads loaded into small stage tip containing a C8 Empore disk (3M, Minneapolis, USA). The TiO2 tips were washed once with TiO2 loading buffer and once with washing buffer (50% ACN, 0.1% TFA). The first elution was performed with 5% NH4OH and the second elution was performed with 5% piperidine. Enriched phosphopeptides were purified and eluted from the C18 Empore disks (Rappsilber et al., 2003).

**LC-MS/MS and MS data processing**

Phosphopeptides were separated on a monolithic column (100 µm i.d. x 2,000 mm, MonoCap C18 High Resolution 2000 [GL Sciences] kindly provided by Dr. Yasushi Ishihama [Kyoto University]) using 6 hour gradient of increasing ACN concentration at a flow rate of 300 nl/min. The Q Exactive instrument (Thermo Fisher Scientific) was operated in the data dependent mode with a full scan in the Orbitrap followed by top 10 MS/MS scans using higher-energy collision dissociation (HCD). MaxQuant software (v1.5.1.2)(Cox and Mann, 2008) was used to identify and quantify proteins. False discovery rate was set to 1% at both peptide and protein level. Carbamidomethylation of cysteine was selected as a fixed modification, and oxidation of methionine, acetylation of the protein N terminus and phosphorylation of serine, threonine and tyrosine (Phospho (STY)) were used as variable modifications. MS/MS spectra were searched using the Andromeda search engine (Cox et al., 2011) against a UniProt human database (release 2014–10) with an additional 248 common contaminants, and a separate search was performed for rat and chimeric TrkA sequences. All protein sequences were also reversed to generate a target-decoy database. Peptides were scored as up-regulated if log2(NGF+/NGF-) > 0.3 in at least one replicate. UniProt database was used to assess protein phosphorylation sites of serine, threonine and tyrosine.

**Electron microscopy and quantification of peripheral nerves**

The general procedure followed for quantification of peripheral nerves was described in (St John Smith et al., 2012). Three NMR pups (postnatal day 3) were intracardially perfused and the nerves of interest were dissected from both legs. Three ultra thin sections were taken from at least two nerves, usually three (nerve loss or damage sometimes occurred during either dissection or the embedding procedure), and on each ultra thin section five images (9422.22 x 7233.52 nm) were taken. Myelinated and unmymelinated axons were counted in these areas in iTEM software (Olympus Soft Imaging Solutions, Münster, Germany) and normalized to the whole nerve. For calculating C:A-fiber ratios (C-fiber count/A-fiber count), an average was taken for each ultra thin section per nerve.

**RNA sequencing and de novo transcriptome annotation and assembly**

RNAseq data for *Fukomys darlingi* were generated from three brain samples using paired-end, strand-specific (dUTP) libraries that were sequenced on an Illumina HiSeq2000 platform. Multiplexed libraries were sequenced for 2x101 cycles. Quality clipping of the raw reads was performed with Trimmomatic 0.32 (Bolger et al., 2014). Adapters were clipped off using 1 seed mismatch, a palindromic score threshold of 30 and a simple clip threshold of 15. Minimum quality for trailing bases was set to 20. Leading 10 bases were clipped off any read due to a sequence bias introduced by random hexamer priming. Read pairs with at least one read shorter than 30 bases after quality clipping were discarded.

The Trinity tool (Grabherr et al., 2011) (version 20140717) and the Bridger software (Chang et al., 2015) (version 2014-12-01) were used with default parameters to assemble the raw transcriptomes from *Fukomys darlingi* and other African mole-rat species (SRA accession: SRP061925). After the primary assembly, both assemblies were combined using CAP3 (Huang and Madan, 1999) and all merged and non-merged sequences were used for downstream analyses. To identify sequencing library contamination and exclude ribosomal RNA and mitochondrial DNA sequences, the assembly was aligned using BLASTn (Altschul and Lipman, 1990) against mouse and human mRNA sequences from RefSeq (Pruitt et al., 2014), sequences of bacterial genomes often found in laboratory samples (Salter et al., 2014) as well as rRNA sequences from mouse, rat and human and mitochondrial sequences from mouse, rat, human and naked mole-rats. All assembled transcripts with a BLAST hit with an e-value <1e-20 against rRNA or bacterial or mitochondrial DNA that covers at least 10% of the transcript were discarded. Transcripts putatively originating from humans or mice were discarded if they
showed an e-value <1e-20 and sequence identity of >99% and the BLAST hit covered at least 70% of the respective transcript.

To identify protein-coding transcripts in the assembly, we performed a reciprocal best hit (RBH) strategy using UniProt consortium (2014) data sets from four organisms. The UniProt data were downloaded on February 2nd, 2015 and included sequence information from human, mouse, rat and guinea pig. Those species were chosen to be able to annotate transcript sequences that are well conserved among the mammalian kingdom. As proteins are annotated on isoform level in the UniProt data base, only the longest isoform per protein was considered for annotation. After removal of putatively contaminating sequences from the assembly, remaining transcript sequences were mapped to the four protein data sets using BLASTx (Altschul and Lipman, 1990). All hits with an e-value <1e-20 in both forward and reverse direction were considered for further analyses. The same stringency level was used for the alignment of protein sequences against the transcript sequences using tBLASTn (Altschul and Lipman, 1990). Transcripts were discarded as putatively chimeric if there was more than one protein with a best hit to the respective transcript (“collapse factor” >1 (O’Neil and Emrich, 2013). RBHs were identified per species and a full-length annotation was assigned if the BLASTx hit covered at least 70% of the protein. To integrate information on transcript annotation across species and to increase the specificity level, a transcript was only annotated as being coding for a specific protein if there existed a full-length annotation in at least two of the four species used for transcript annotation. Sequencing data and annotated transcripts from the Mashona mole-rat can be found under the accession number PRJNA303968 at the NCBI database.

Phylogenetic tree reconstruction:
Eight hundred and twenty-seven (827) transcripts were used per species to reconstruct a phylogenetic tree of the African mole-rats with Mus musculus and Tachyoryctes splendens as outgroups. De novo assembled transcriptomes were used for eight African mole-rat species and Tachyoryctes splendens, while naked mole-rat and mouse sequences were obtained from the RefSeq data base using the longest transcript isoform as a gene representative. In all species 827 transcripts were found and aligned using Clustal Omega (Sievers et al., 2011) (version 1.2.0). Their intersecting regions were compared to compute the phylogeny to avoid biases due to falsely assembled 3'- or 5'-UTRs. A phylogenetic tree was calculated with a maximum likelihood approach implemented in the RAxML tool (version 8.2.3). The general time reversible (GTR) model (Tavaré, 1986) was used to account for variable base frequencies and symmetrical substitution rates. A gamma distribution was assumed to underlie the rate heterogeneity over the sites. One hundred rapid bootstrap searches were performed in addition to 20 ML searches, the best ML tree was reported.

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