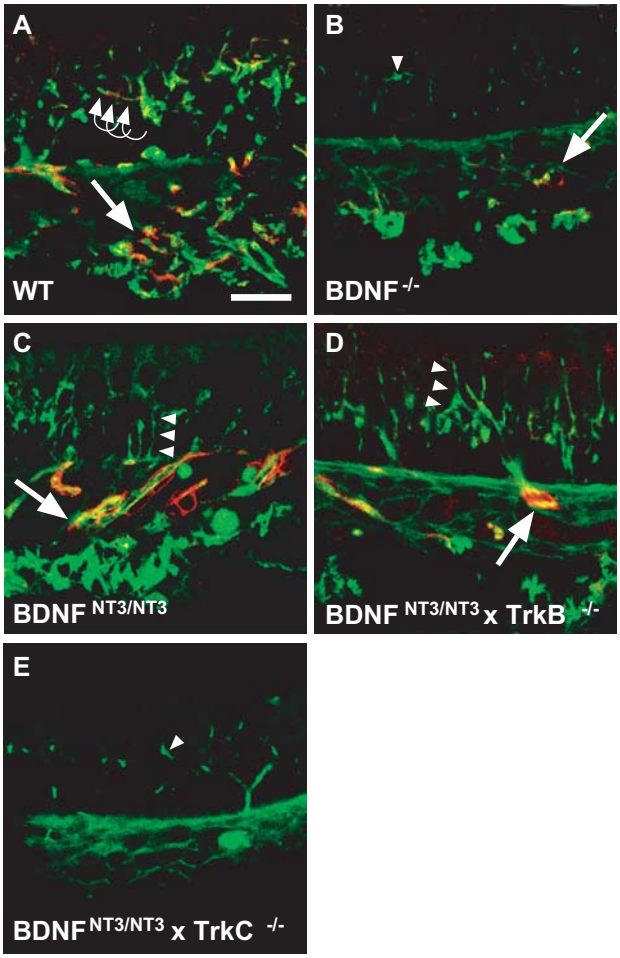
SUPPLEMENTARY FIGURE



Supplementary fig 1 Differences in rescue of afferent and efferent innervation of the vestibular sensory epithelium. (A-E) High power micrographs of $p75^{NTR}$ (red) and βIII tubulin (green) double staining to discriminate between afferent and efferent innervation in the utricular maculae at E18 in mice of indicated genotypes. Double stained nerve fibers (i.e. afferents) are indicated by curved arrows in the epithelial layer and one arrow in the subepithelial layer. βIII tubulin stained fibers (i.e. efferents) are indicated by arrowheads in the epithelium. Note the difference in rescue of afferents and efferents between the genotypes. Scale bar = 20 μm.

SUPPLEMENTARY MATERIALS

Quantitative Real-Time PCR

Total RNA from inner ears of E18 mice was extracted using the Absolutely RNA™ Nanoprep kit (Stratagene, LaJolla, USA) following the manufacturer's instructions. Reverse Transcription (RT) was carried out for 10 minutes at 65°C followed by 1 hour at 42°C and 15 minutes at 70°C in a 20 µl reaction containing 1µg of total RNA, 4 µl of 5x first strand buffer, 0.5 mM of each dNTP, 10 mM DTT, 0.5 µg oligod$(T)_{15}$ (Promega) and 200 U of Super Script II RT (Invitrogen, Life Technologies).

Primers amplifying the extracellular domains (all) and the intracellular tyrosine kinase domains (FL) of either TrkB or TrkC were designed in different exons to avoid amplification of eventual DNA contamination. PCR products were purified, subcloned in pCR II-TOPO vector (Invitrogen) and verified by sequencing. These subcloned PCR products allowed us to generate the standard curves used for quantification of the Real-Time PCR assay.

The primer sequences were as follows:

HPRT forward 5'-GAATCTGCAAATACGAGGAGTCCT-3'
HPRT reverse 5'-CTTTACTAGGCAGATGGCCACA-3'
TrkB-all forward 5'-TGTGACCCTTTCCTGCAGTGT-3'
TrkB-all reverse 5'-CCCTGTGTGTGGCTTGTTTCA-3'
TrkB-FL forward 5'-GTGCTGATGGCAGAGGGTA-3'
TrkB-FL reverse 5'-ATTTTCACCAGCAGGTTCTCT-3'
TrkC-all forward 5'-AGTAACCGGCTCACCACACTC-3'
TrkC-all reverse 5'-AGCGGATGTCACAGCTGCAGT-3'
TrkC-FL forward 5'-TGATCCTCGTGGATGGACAG-3'
TrkC-FL reverse 5'-CTTCACTAGTAGATTGGCTCC-3'

Real-Time PCR was conducted in a 25 µl reaction containing 5% RT product, 12.5 µl of SYBR Green PCR Master Mix (Applied Biosystems) and 5 pmol of each primer (MWG-Biotech AG). cDNA was denatured 10 minutes at 95°C and amplified in 45 cycles in a 2 step program as follows: 30 seconds 95°C, 30 seconds annealing temperature (58°C for TrkB-FL and TrkC-FL, 69°C for TrkB-all and 60°C for TrkC-all and HPRT).