

## **A congenital pain insensitivity mutation in the nerve growth factor gene uncouples nociception from affective pain in heterozygous humans and mice**

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**Supplementary Figure 1. Molecular strategy for the generation of wild type and R100W knock-in mice.** **a**, *Up*, endogenous mouse NGF locus with 5' and 3' Southern blot probes and expected sizes of wild type Southern blot bands; *middle*, targeting vector for site-specific recombination; *down*, targeted NGF locus with 5' and 3' Southern blot probes and expected sizes of recombinant Southern Blot bands. Color codes are: pink, mouse NGF coding sequence; yellow, human NGF coding sequence; brown, NeoR positive selection cassette; orange, PGK promoter; blue, loxP sites; light green, left and right homology arms; dark green, Diphtheria Toxin A (DTA) negative selection marker. **b**, Representative image of ES cells Southern Blot. **c**, PCR genotyping of NGF<sup>R100W/m</sup>, NGF<sup>m/m</sup> and NGF<sup>R100W/R100W</sup> mice; wild-type band: 400bp, mutant band: 200bp.

**Supplementary Figure 2. Rescue of early lethality of homozygous R100W mice by NGF.** Homozygous R100W mice (NGF<sup>R100W/R100W</sup>) die within the first month of life. Treatment of pregnant dams with subcutaneous NGF (1 µg/kg) until 10 days after delivery, followed by subcutaneous and intranasal NGF (480 ng/kg) to pups until two months of age, rescued lethality in NGF<sup>R100W/R100W</sup> mice.

**Supplementary Figure 3. Decreased warm and hot sensitivity.** **a**, increased latency in NGF<sup>R100W/m</sup> mice to respond to high temperatures. **b**, Higher temperature threshold for eliciting licking reaction in NGF<sup>R100W/m</sup> mice.

**Supplementary Figure 4. Age-dependent reduction in hairy skin innervation, but unaffected tape removal performance in HSAN V mice.** **a**, NGF<sup>R100W/m</sup> mice display a normal number of attempts in the tape removal test in both juveniles and adults despite a longer response latency at 6 months of age. **b**, Representative images and quantification of PGP9.5 expression show normal innervation at 2 months and a significant reduction at 6 months of age (scale bars, 50  $\mu$ m).

**Supplementary Figure 5. Neurotrophic effect of hNGF<sup>R100W</sup> in DRG cultures.** Comparable number of NeuN-immunoreactive cells after treatment with hNGF<sup>WT</sup> and hNGF<sup>R100W</sup>.

**Supplementary Figure 6. NGF bioavailability in R100W condition.** **a**, Impaired secretion of human NGF<sup>R100W</sup> in HEK293 cells transfected with the corresponding plasmid, similarly to what reported in Larsson et al.2009. The co-transfection of HEK293 cells with human NGF<sup>WT</sup> and human NGF<sup>R100W</sup>, to mimic heterozygous condition, shows that the latter induces a decrease in the secretion of human NGF<sup>WT</sup>. **b**, Consistently, the amount of NGF in brain extracts from NGF<sup>R100W/m</sup> mice was significantly diminished.

**Supplementary Figure 7. Normal performance in Y maze.** NGF<sup>R100W/m</sup> mice do not show difference in spatial memory compared to NGF<sup>m/m</sup>.

**Supplementary Figure 8. Age-related effect of R100W mutation on anxiety behaviour.** **a**, In the elevated plus maze, 6-month-old NGF<sup>R100W/m</sup> mice spent more time in the open arms than NGF<sup>m/m</sup> mice. **b**, The amount of marbles buried by NGF<sup>R100W/m</sup> and NGF<sup>m/m</sup> is similar at 2 months of age but is significantly reduced in NGF<sup>R100W/m</sup> at 6 months of age. **c**, Nesting ability is normal in juvenile but impaired in adult HSAN V mice. Data are presented as mean  $\pm$  SEM.

**Supplementary Figure 9. Normal social interaction in HSAN V mice assessed with 3-chamber test.** 6-month-old HSAN V mice perform similarly to NGF<sup>h/m</sup> mice in the sociability phase.

**Supplementary Figure 10. Decreased plasmatic levels of oxytocin in HSAN V mice.**