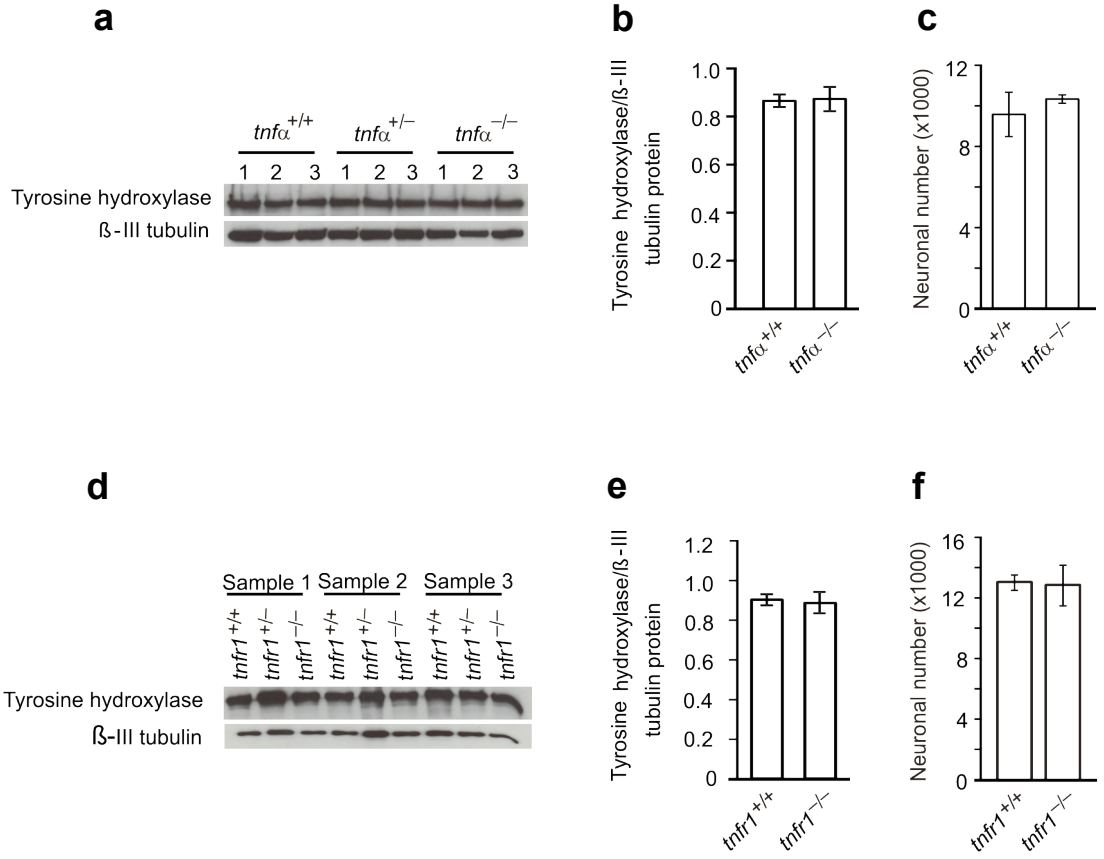
TNFα reverse signaling promotes sympathetic axon growth and target innervation

Lilian Kisiswa, Catarina Osório, Clara Erice, Thomas Vizard, Sean Wyatt and Alun Davies

Supplementary Figure 1: Deletion of either *tnfα* or *tnfr1* does not affect the levels of tyrosine hydroxylase and the number of neurons in the SCG.



(**a**) Western blots of lysates of SCG from three separate P10 *tnfα*$^{+/+}$, *tnfα*$^{+/–}$ and *tnfα*$^{–/–}$ mice probed for tyrosine hydroxylase and β-III tubulin.

(**b**) Levels of tyrosine hydroxylase protein relative to β-III tubulin in the SCG of P10 *tnfα*$^{+/+}$ and *tnfα*$^{–/–}$ littermates.

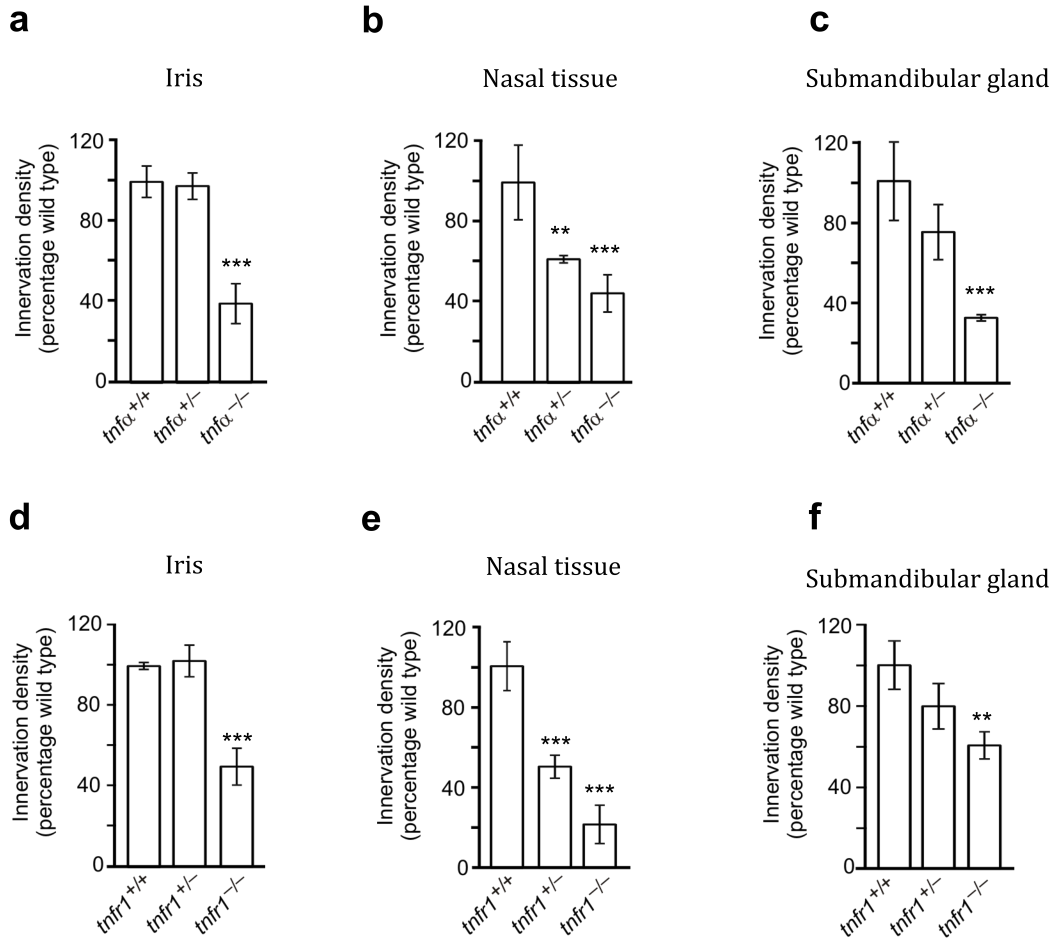
(**c**) Estimates of the number of neurons in the SCG of P10 *tnfα*$^{+/+}$ and *tnfα*$^{–/–}$ littermates. Mean ± s.e.m of data from three animals of each genotype are shown.

(**d**) Western blots of lysates of SCG from three separate P10 *tnfr1*$^{+/+}$, *tnfr1*$^{+/–}$ and *tnfr1*$^{–/–}$ mice probed for tyrosine hydroxylase and β-III tubulin.

(**e**) Levels of tyrosine hydroxylase protein relative to β-III tubulin in the SCG of P10 *tnfr1*$^{+/+}$and *tnfr1*$^{–/–}$ littermates.

(**f**) Estimates of the number of neurons in the SCG of P10 *tnfr1*$^{+/+}$ and *tnfr1*$^{–/–}$ littermates. Mean ± s.e.m of data from three animals of each genotype are shown.

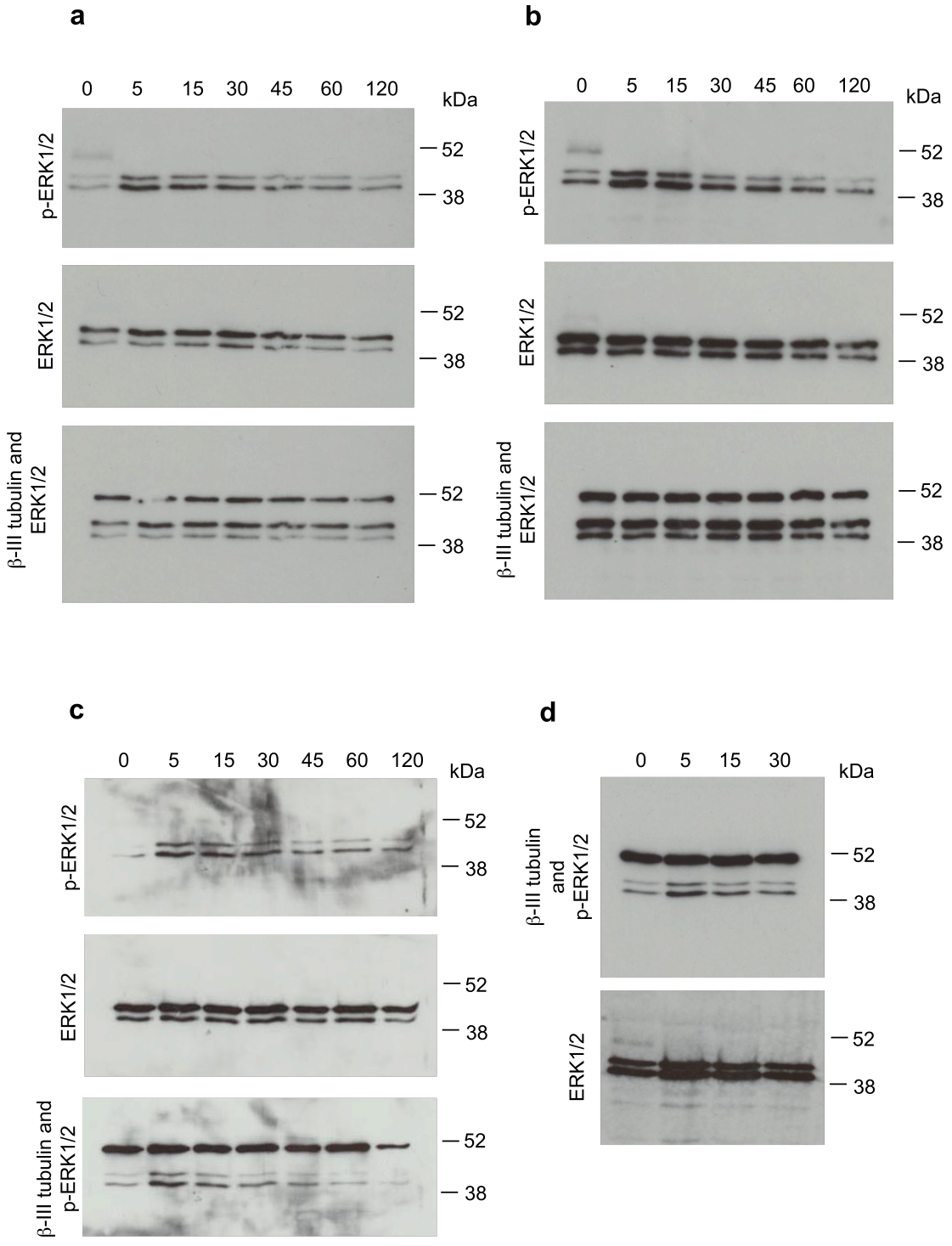
Supplementary Figure 2: Reduced sympathetic innervation density in *tnfα*$^{-/-}$ and *tnfr1*$^{-/-}$ mice quantified by DBH immunohistochemistry.

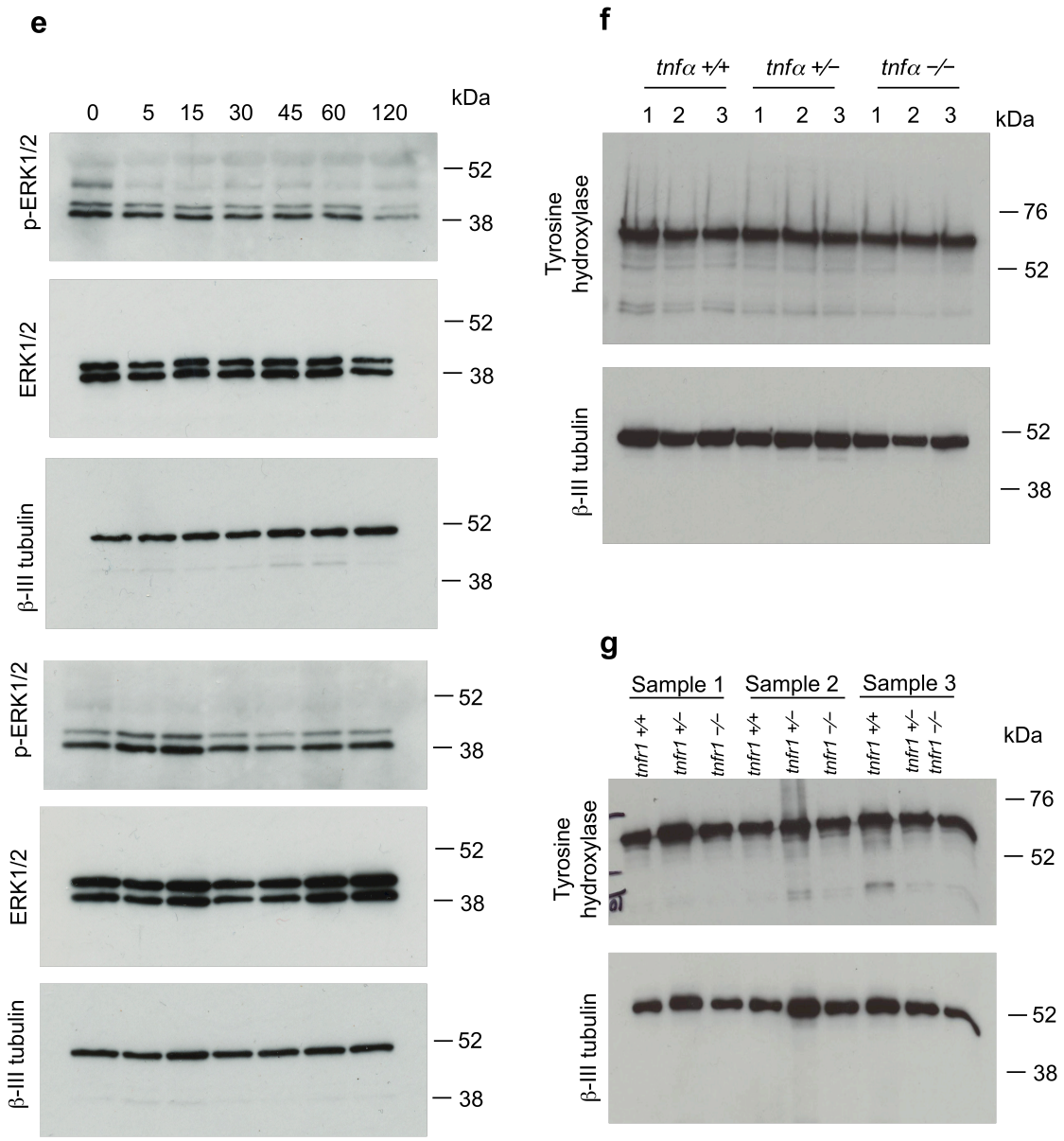


(a) Relative levels of DBH immunofluorescence in the iris, nasal turbinate tissue and submandibular gland of P10 *tnfα*$^{+/+}$, *tnfα*$^{+/-}$ and *tnfα*$^{-/-}$ mice expressed as a percentage of the mean level in *tnfα*$^{+/+}$ mice. Mean ± s.e.m of data from three animals of each genotype (** indicates P<0.01 and *** indicates P<0.001, statistical comparison with control).

(b) Relative levels of DBH immunofluorescence in the iris, nasal turbinate tissue and submandibular gland of P10 *tnfr1*$^{+/+}$, *tnfr1*$^{+/-}$ and *tnfr1*$^{-/-}$ mice expressed as a percentage of the mean level in P10 *tnfr1*$^{+/+}$ mice. Mean ± s.e.m of data from three animals of each genotype (** indicates P<0.01 and *** indicates P<0.001, statistical comparison with control).

Supplementary Figure 3: Full-length Western blots.




e
0 5 15 30 45 60 120
kDa
p-ERK1/2
ERK1/2
β-III tubulin
52
38
f
tnfα +/+
tnfα +/–
tnfα –/–
1 2 3 1 2 3 1 2 3
kDa
Tyrosine hydroxylase
β-III tubulin
76
52
38
g
Sample 1
Sample 2
Sample 3
tnfr1 +/+
tnfr1 +/–
tnfr1 –/–
kDa
Tyrosine hydroxylase
β-III tubulin
76
52
38

(a) Full-length Western blots of Fig. 6a. Top panel shows blot of membrane probed for phospho ERK1/2, middle blots is the blot probed with antibody against total ERK1/2 and the bottom blots was probed with antibody against β-III tubulin (top band) without total ERK1/2 (bottom two bands) being stripped off. All three antibodies were stained using the same membrane after stripping off previous antibody.

(b) Full-length Western blots of Fig. 6b. Top panel shows the blot of membrane probed for phospho ERK1/2, middle blots is the blot probed with antibody against total ERK1/2 and the bottom blots was probed with antibody against β-III tubulin (top band) without total ERK1/2 (bottom two bands) being stripped off. All three antibodies were stained using the same membrane after stripping off previous antibody.

(c) Full-length Western blots of Fig. 6c. Top panel shows the blot of membrane probed for phospho ERK1/2,middle blots is the blot probed with antibody against total ERK1/2 and the bottom blots was probed with antibody against β-III tubulin (top band) without phospho ERK1/2 (bottom two bands) being stripped off. All three antibodies were stained using the same membrane after stripping off previous antibody.

(d) Full-length Western blots of Fig. 6d. Top blot if of membrane probed with antibody against β-III tubulin (top band) without phospho ERK1/2 (bottom two bands) being stripped off. The bottom blot shows total ERK1/2. All three antibodies were stained using the same membrane after stripping off previous antibody.

(e) Full-length Western blots of Fig. 6i. The first three panels shows blots of membrane probed with antibodies against phospho ERK1/2 (top panel), total ERK 1/2 (second panel) and β-III tubulin (third panel) of figure 6i top western blot. The bottom three blots are of membrane probed with antibodies against phospho ERK1/2 (forth panel), total ERK1/2 (fifth panel) and β-III tubulin (last panel) of figure 6i bottom western blot.

(f) Full-length Western blots of Suppl. Fig.1a. Blot of membrane probed with antibody against tyrosine hydroxylase (top panel). The membrane was then stripped off and re-probed with antibody against β-III tubulin (bottom panel)

(g) Full-length Western blots of Suppl. Fig.1d. Top panel show blot of membrane probed with antibody against tyrosine hydroxylase. The membrane was then stripped off and re-probed with antibody against β-III tubulin (bottom panel)