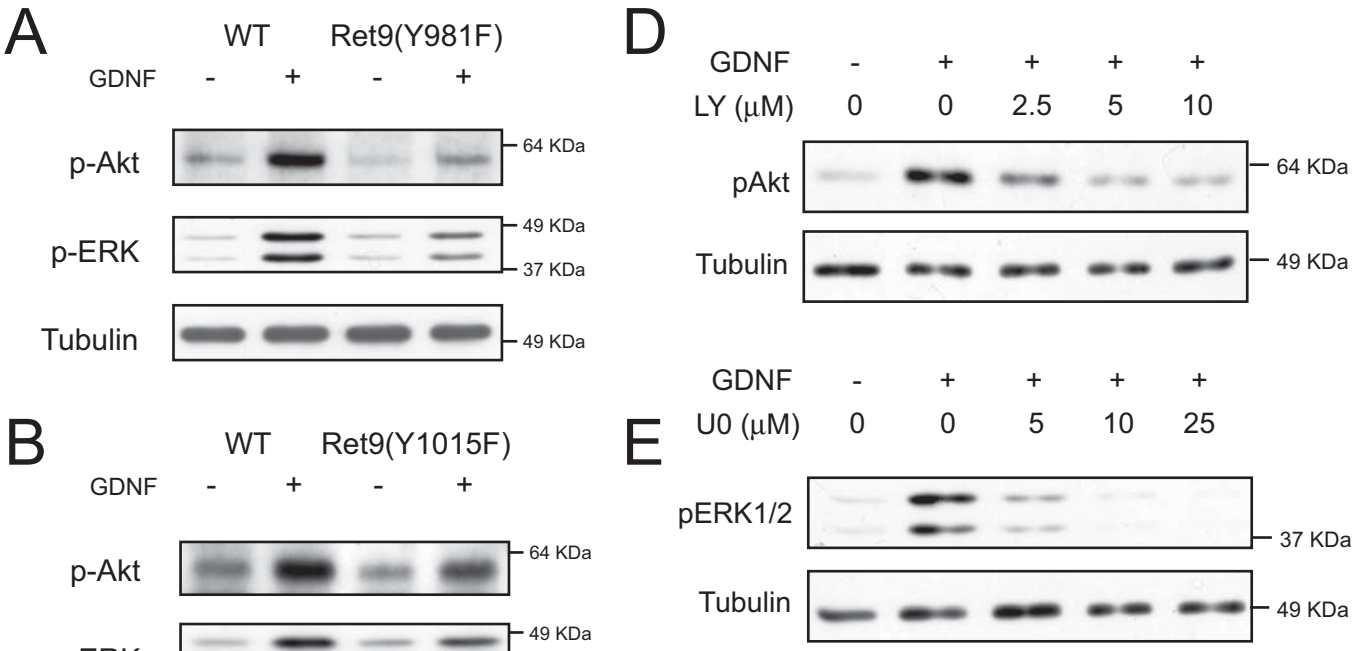
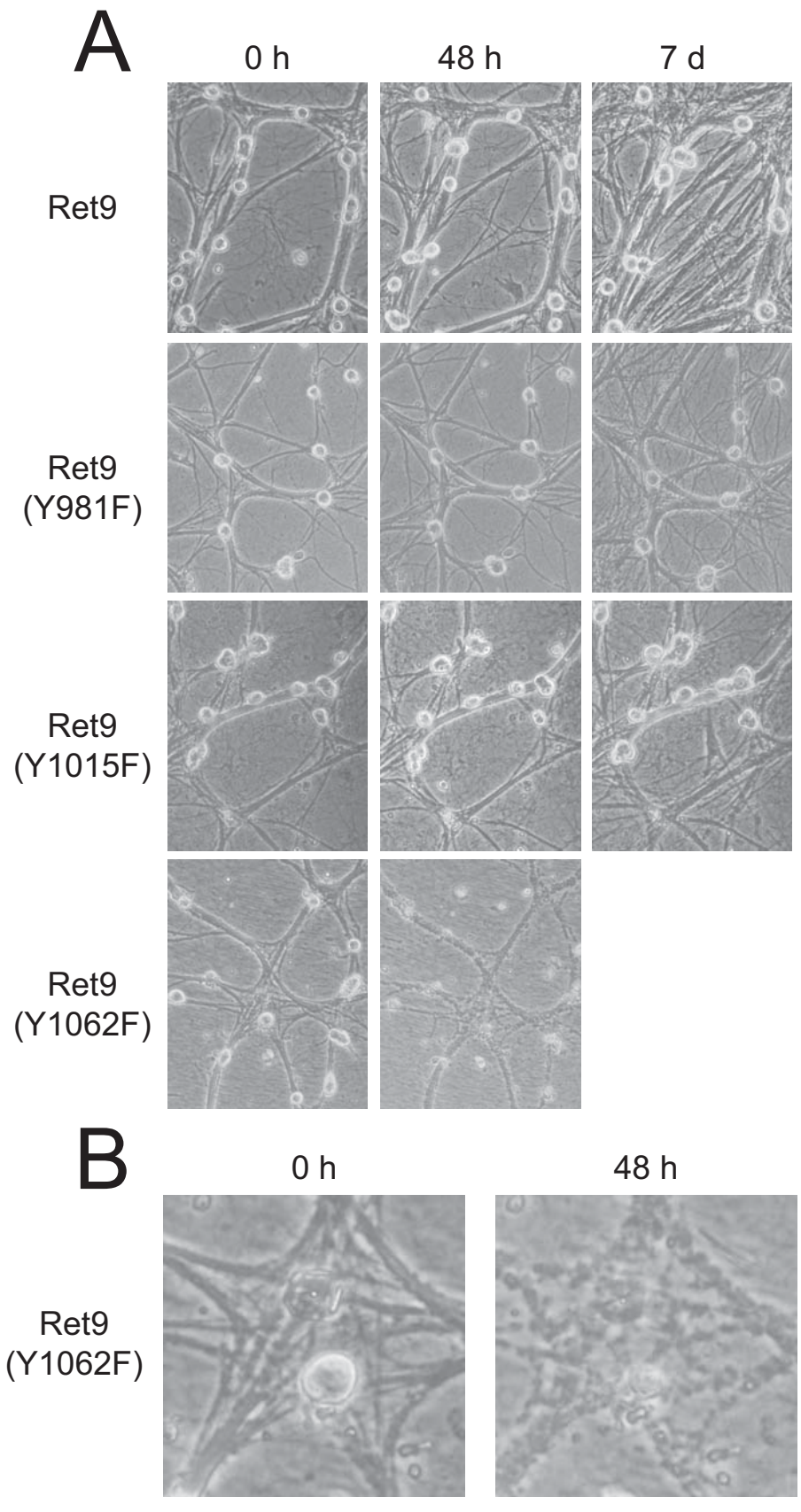
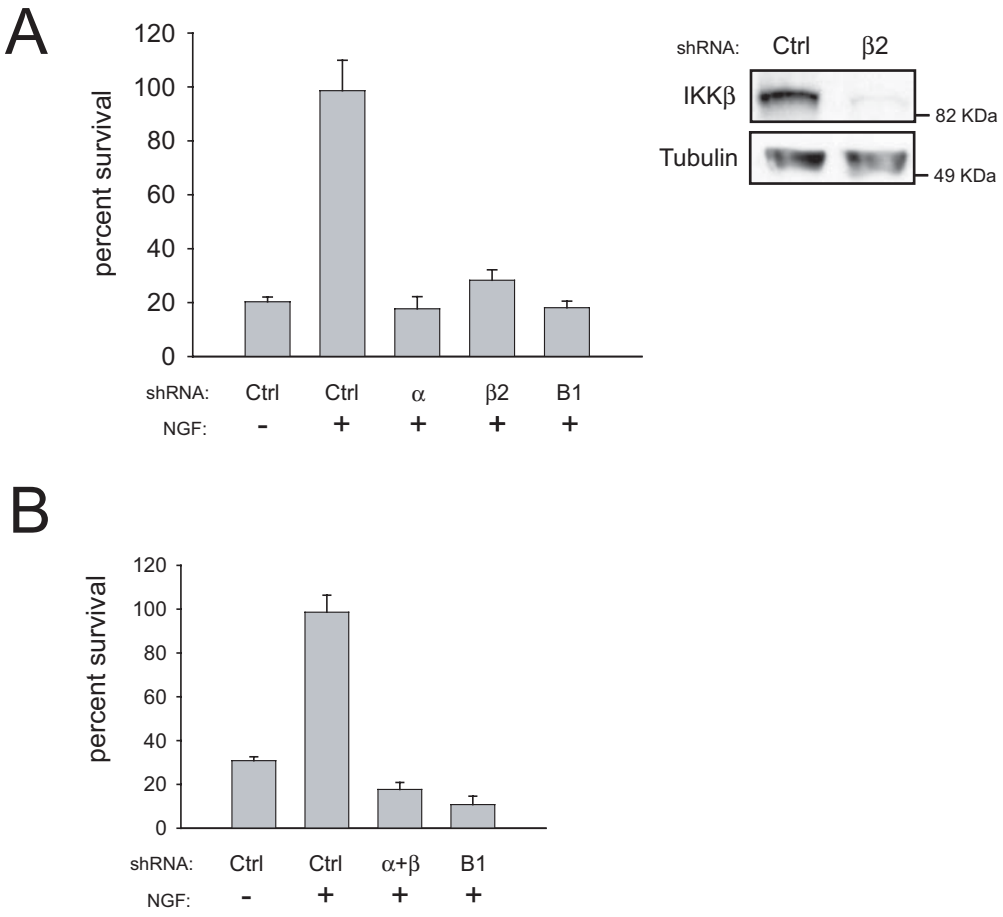
# SUPPLEMENTAL FIGURE 1, Encinas et al.



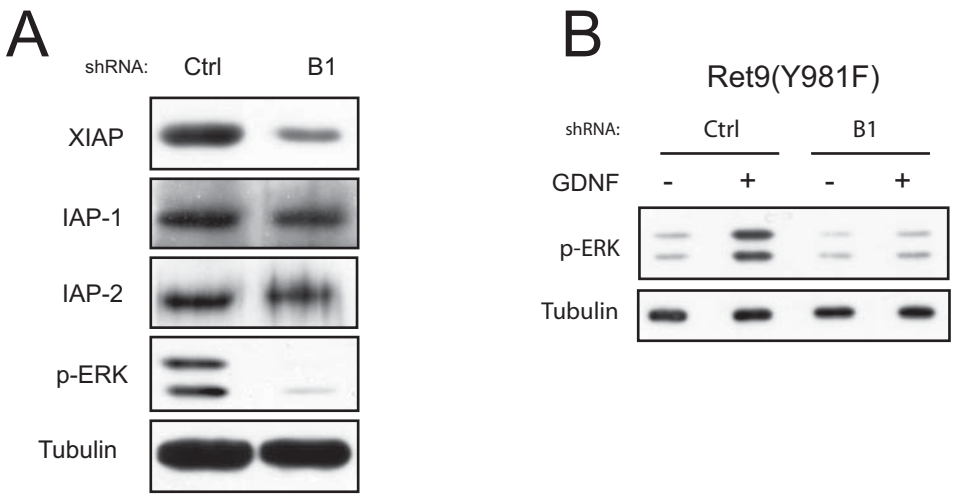
SUPPLEMENTAL FIGURE 2, Encinas et al.



SUPPLEMENTAL FIGURE 3, Encinas et al.



SUPPLEMENTAL FIGURE 4, Encinas et al.



Supplemental Figure 1

Phosphorylation patterns of Akt and ERK1/2 after 24 hours of stimulation with GDNF. (A, B, C) Sympathetic neurons from mice of the indicated genotypes were cultured in the presence of NGF for five days, washed three times with media, and either deprived or stimulated for 24 hours in the presence of GDNF. Lysates were probed with the indicated antibodies.
(D, E) Sympathetic neurons from wild type mice were cultured for five days in the presence of NGF, washed three times and either deprived or incubated with GDNF alone or in the presence of the indicated inhibitors for 24 hours. Lysates were probed with the indicated antibodies.

Supplemental Figure 2

(A) Only neurons from Ret9(Y1062F) mice fail to survive in presence of GDNF. Pictures from the same field were taken before switching cells to GDNF (0h), and 48h and 7 days afterwards. The majority of neurons survived up to one week (the last time point analyzed) in neurons from all genotypes analyzed except those from mice lacking tyrosine 1062 which had died within 48h in culture. (B) Higher magnification of the centre of the field of neurons from Ret9(Y1062F) mice allows visualization of disintegrating cell bodies and neurites.

Supplemental Figure 3

(A) Silencing of IKKs or B-Raf prevents NGF-mediated survival of rat sympathetic neurons. SCG neurons from newborn rats were cultured exactly as described for mouse sympathetic neurons. The day after plating cells were infected with lentiviruses carrying shRNAs to IKKα ("α"), IKKβ ("β2") or B-Raf ("B1"). Four days later cells were switched to the indicated treatments for two additional days, after which survival was assessed. Shown is a representative graph of three independent experiments. Note that the shRNA used for rat neurons ("β2") is different from that used for mouse neurons. Its efficacy was tested by western blot on the right panel. The sequence of β2 is as follows: 5'-GCTCTTAGATACCTTCATGAA-3'.

(B) Silencing of IKKs or B-Raf prevents NGF-mediated survival of mouse trigeminal neurons. Trigeminal ganglia from newborn mice were dissected and cells dissociated by sequential treatment with collagenase and trypsin. Cells were and plated onto collagen-coated 24-well dishes (one tenth of a ganglion/well) and cultured in AM0 medium supplemented with 3.33 μg/ml aphidicolin (AG Scientific, San Diego, CA) to remove mitotic cells. The day after plating neurons were infected with lentiviruses bearing the indicated shRNAs to IKKα and IKKβ ("α+β") or B-Raf ("B1"). On the fifth day after plating cells were switched to anti-NGF- or NGF-containing media as indicated. Survival was assessed three days after switch. Shown is a representative graph of three independent experiments.

Supplemental Figure 4

(A) Knockdown of B-Raf results in diminished levels of XIAP and phospho-ERK1/2, but not c-IAP-1 or c-IAP-2. Sympathetic neurons were infected one day after plating with the indicated shRNA to B-Raf ("B1"). Five days after plating cells were switched to GDNF in the presence of 50mM of the pan-caspase inhibitor BAF, and kept in culture for two additional days. Levels of the indicated proteins or phospho-proteins were assessed by immunoblot with the indicated antibodies. Antibodies to c-IAP-1 and c-IAP-2 were from Santa Cruz Biotechnology (Santa Cruz, CA).

(B) B-Raf is activated by GDNF in sympathetic neurons from $Ret^{RET9(Y981F)}$ mice. Neurons were infected one day after plating with scrambled ("Ctrl") or B-Raf ("B1") shRNAs. On the fifth day in vitro, cells were stimulated for 10 minutes with GDNF and lysates probed with anti-phospho-ERK1/2. Knockdown of B-Raf abolished ERK phosphorylation, indicating that the main, if not the only MEK kinase activated by Ret9 (Y981F) mutant is B-Raf.