Besirli et al. http://www.jcb.org/cgi/doi/10.1083/jcb.200501138

Supplemental materials and methods

Cerebellar granule neuron survival assays

To measure the percentage of viable granule cells, a fluorometric calcein-AM assay was used (Harris, C.A., M. Deshmukh, B. Tsui-Pierchala, A.C. Maroney, and E.M. Johnson, Jr. 2002. *J. Neurosci.* 22:103–113). Cultures were washed once with magnesium-free Locke's solution (154 mM sodium chloride, 5.6 mM potassium chloride, 3.6 mM sodium bicarbonate, 2.7 mM calcium chloride, 5.6 mM glucose, 2.5 mM Hepes, 2.5 mM sodium Hepes) and incubated for 30 min at 37ºC with Locke's solution containing 5 μM calcein AM (Molecular Probes). The cultures were lysed with Locke's solution supplemented with 0.1% Triton X-100; the fluorescence was measured on a TiterTek Fluoroskan II Plate Reader at emission 485 nm and excitation 538 nm.