Figure S1: Minimal contamination of neurons used for co-cultures with other cell types. Neurons were obtained from GFP (+) mice (A), and plated on astrocytes derived from C57Bl/6J mice. After maturing in culture until DIV9, the number of GFP(+) cell that were neurons was quantified. Over 96% of the GFP(+) cells were neurons as determined by identifying cells that were GFP(+) and expressed TJ1, a neuronal-specific marker (B) (Long Arrow-non-neuronal cell; Arrowhead- neuron).

Figure S2: Regardless of the presence of HIF-1 function, astrocytes do not die with exposure to hypoxia. Astrocyte cultures were exposed to hypoxia (0.5% O_2) for 14 or 72 hours. Cell death was assessed by live/dead assay (Molecular Probes) either immediately upon removal from hypoxia, or after 24 hours of reperfusion in normoxia. The 14 hours of hypoxia followed by 24 hours of normoxia was the same protocol as that utilized for the co-cultures, which provokes profound neuronal death. Astrocyte viability was not compromised by hypoxia regardless of the presence or absence of HIF-1α function in astrocytes.

Figure S3: Evaluation of HIF-1 loss in primary neuronal cultures and screening of co-cultures for loxP recombination in neurons. To demonstrate that loss of HIF-1α can be induced in primary neuronal cultures, neuronal cultures were prepared from HIF-$1\alpha^{f+/f+}$::ESRCre or HIF-$1\alpha^{f+/f+}$ embryos, treated with tamoxifen, and then exposed to cobalt (A). Loss of HIF-1α protein is achieved in neurons which contain EsrCre and are exposed to tamoxifen, but not in controls (B). (Specific HIF-1 band indicated by an arrow, while arrowhead marks the non-specific band) (HN33 cell lysates (+) or (-) cobalt

served as controls). In some cases, HIF-1$\alpha^{f+/f+}$::ESRCre or HIF-1$\alpha^{f+/f+}$ neurons were plated onto astrocytes derived from C57Bl/6J mice (C). To accomplish recombination of the floxed allele specifically in neurons, the cultures were exposed to tamoxifen whereas control cultures were not. To screen for loss of HIF-1 function in neurons, recombination of the floxed DNA segment was evaluated by PCR (D) and recombined (arrow) and non-recombined (arrowhead) products were identified. In this example (D), co-cultures were prepared with neurons obtained from 4 different embryos and plated onto C57Bl/6J embryos. Recombination is observed only in cultures derived from HIF-1$\alpha^{f+/f+}$::ESRCre mice treated with tamoxifen, not controls.