

Supplementary figure 1



Supplementary figure 2



Supplementary figure 3

Supplementary figure legends

Supplementary Figure 1. Verification of the specificity of anti-erbB antibodies. (A) Specificity of anti-erbB antibodies used for immunohistochemistry (IHC) was assessed by performing immunoprecipitation (IPP) experiments. Protein extracts prepared from human fetal cortical astrocytes cultures were immunoprecipitated with the anti-erbB1 (Zymed), antierbB2 (DakoCytomation, Dako) or anti-erbB3 (Upstate Biotechnologies, Upstate) antibodies, and protein extracts prepared from human fetal hypothalamic astrocytes cultures were immunoprecipitated with the anti-erbB4 antibody (NeoMarkers, NeoM). Starting material before immunoprecipitation (before IPP), immunoprecipitated proteins (IPP erbB) and depleted fraction after IPP (IPP supernatant) were electrophorezed and immunoblotted (IB) with the anti-erbB antibodies used for western blot experiments (rabbit anti-erbB1 antibody from Upstate Biotechnologies, Upstate, and anti-erbB2, anti-erbB3 or anti-erbB4 antibodies from Santa Cruz, Sc). Each of the four anti-erbB antibodies used for IPP yielded a band at the expected molecular weight (MW) after immunoblotting with the anti-erbB antibodies used for western blot experiments. Note that erbB3 protein was readily detectable after IPP with anti-erbB3 antibody while its expression was below the detection threshold in starting material. All membranes were subsequently re-probed with the anti-Akt antibody to check for protein loading and IPP efficiency. Note that Akt protein was detected at equal amounts before IPP and after IPP in the depleted fraction, while no signal was seen in the immunoprecipitated fraction. The asterisk points to unbound immunoglobulins in the IPP fraction. (B) The anti-erbB1, anti-erbB2 and anti-erbB3 antibodies used for IHC experiments yielded a band at the expected MW after immunoblotting on protein lysates prepared from human cortical astrocytes cultures. (C) Protein lysates from human cortical astrocytes cultures (erbB1, erbB2 and erbB3 IB) or from fetal brain (erbB4 IB) were immunoblotted with the four anti-erbB antibodies used for western blot experiments in the absence or presence of a 10-fold molar excess of anti-erbB blocking peptides. Note that pre-incubation of each anti-erbB antibody with its respective blocking peptide abolished subsequent recognition of erbB receptors in protein extracts.

Supplementary Figure 2. Analysis of erbB1, erbB2 and erbB3 protein levels across the series of cerebro-cortical and glioblastoma tissues using a battery of distinct anti-erbB antibodies. Western blot analysis of erbB protein expression was made on three cortical samples (C1, C2 and C3) and on the nine glioblastoma samples (G1 to G9) as described in the legend of Figure 2A. Although distinct antibodies showed different detection sensibilities, all antibodies directed against the same erbB receptor yielded a similar expression profile across the samples series (compare the three anti-erbB1 antibodies, the two anti-erbB2 antibodies and compare all erbB antibodies to Fig. 2A). Note that the anti-erbB1 antibody from Zymed and the anti-erbB2 antibody from Dako yielded a readily detectable signal in the three cerebro-cortical samples despite absence of immunostaining when used in immunohistochemistry.

Supplementary Figure 3. Expression of GFAP and erbB1 in the human glioblastoma cell lines U251, U373 and T98G. Immunofluorescent labeling of GFAP (green, upper lane) and erbB1 (green, lower lane). While a strong GFAP immunolabeling was seen in most U251 and U373 cells, GFAP immunoreactivity was not detected in the T98G cell line. Note that the three cell lines expressed erbB1. Nuclei were counter-stained with Hoechst (blue). Scale bar = $20 \mu m$.