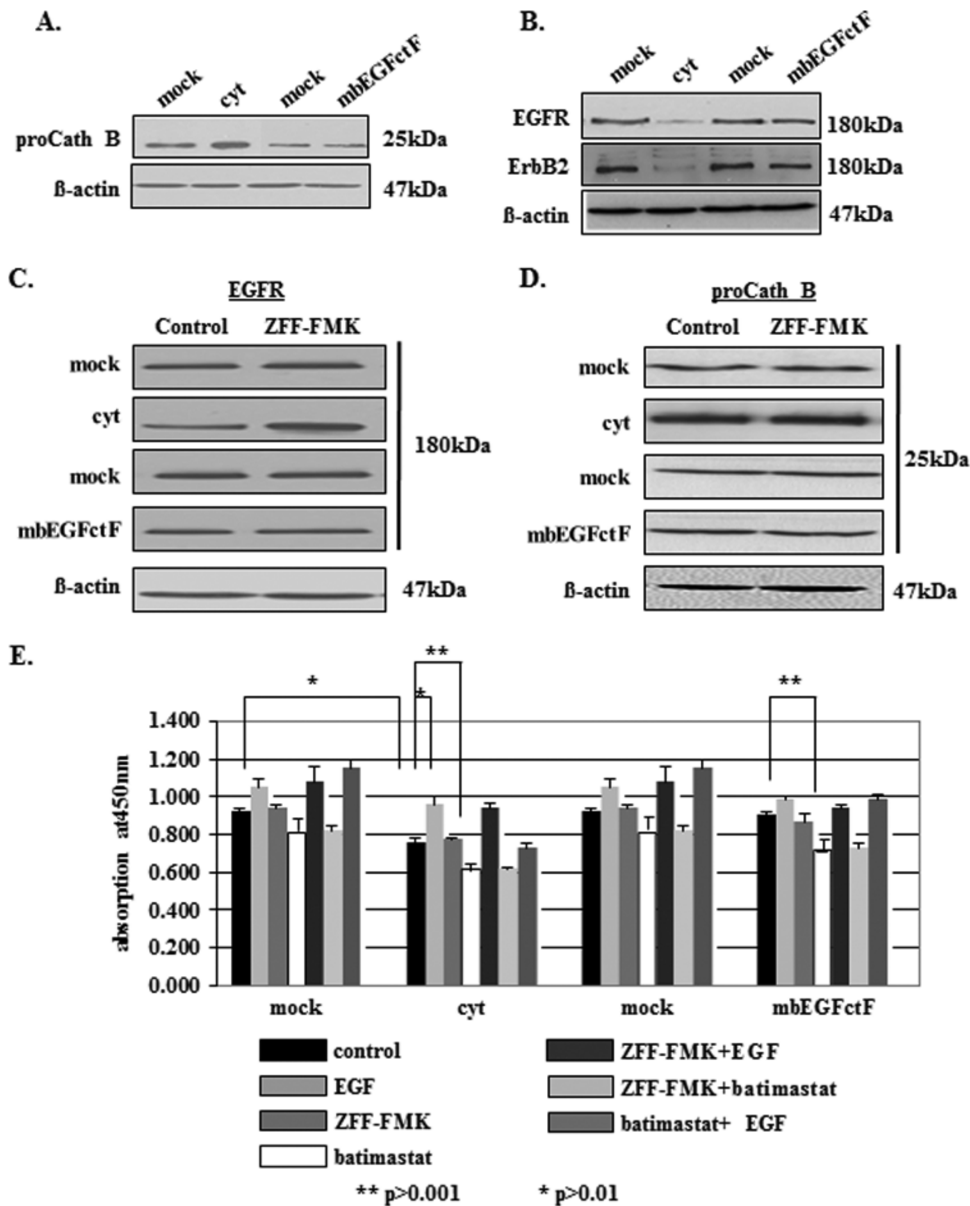
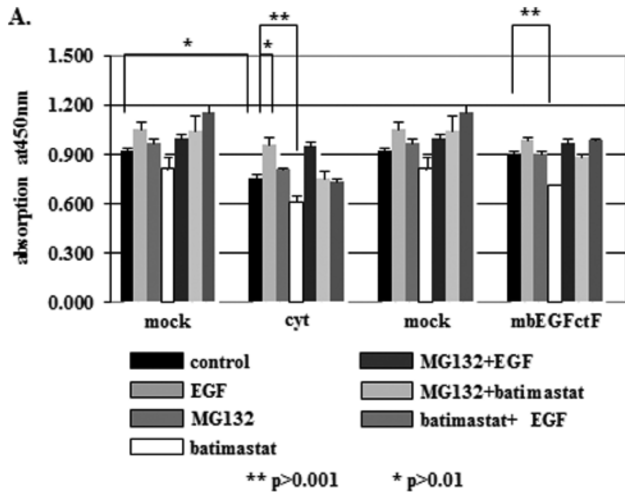
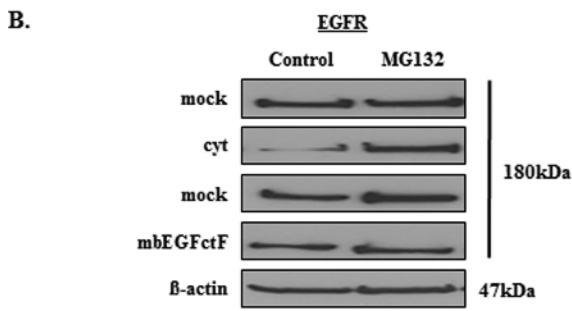


Figure W1. (A) Western blot analysis revealed the up-regulation of procathB in human glioma cell line LN-18 transiently transfected with EGFcyt. This effect was not observed with mbEGFctF construct. (B) Decreased EGFR and ErbB2 protein levels in LN-18 transiently transfected with EGFcyt. This effect was not observed with mbEGFctF construct. (C) Western blot analysis revealed an up-regulation of EGFR in the presence of ZFF-FMK (10 μM) in EGFcyt glioma transfectants. No change was observed in mbEGFctF transfectants and mock. (D) The same ZFF-FMK treatment did not alter cellular procathB expression in any of the LN-18 clones. (E) BrdU proliferation assays revealed decreased proliferation in LN-18 EGFcyt transfectants and treatment with the general MMP inhibitor batimastat caused a significant decrease in proliferation in EGFcyt and mbEGFctF clones suggestive of a possible weak EGFR activation through cleaved EGF-like ligand(s). In all cases, EGF treatment (10 ng/ml) caused a significant increase in proliferation in LN-18 EGFcyt clones. The procathB inhibitor ZFF-FMK (10 μM) did not affect cell growth in the presence or absence of exogenous EGF.





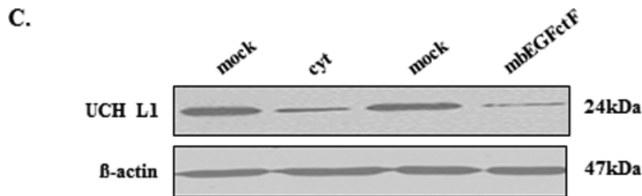


Figure W2. (A) BrdU proliferation assays revealed a 12% increase in proliferation in LN-18 EGFcyt transfectants on MG132 treatment, which was enhanced further in the presence of EGF. The general MMP inhibitor batimastat caused a small but significant decrease in proliferation in LN-18 EGFcyt and mbEGFctF clones suggestive of a possible weak EGFR activation through cleaved EGF-like ligand(s). (B) Western blot analysis revealed an exclusive up-regulation of EGFR in the presence of MG132 (10 μM) in LN-18 EGFcyt transfectants. No change was observed with mbEGFctF transfectants and mock. (C) Representative Western blot demonstrating that both EGFcyt and mbEGFctF glioma transfectants showed reduced levels of cellular UCH-L1. β-Actin served as a loading control.

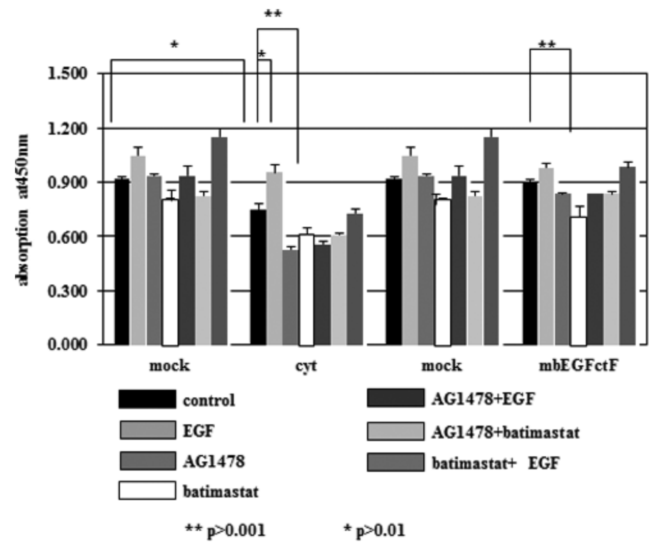
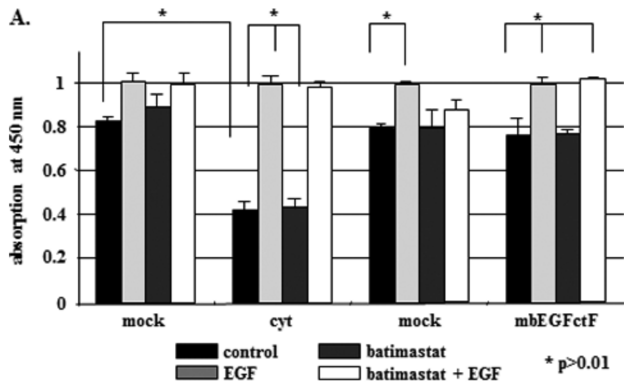
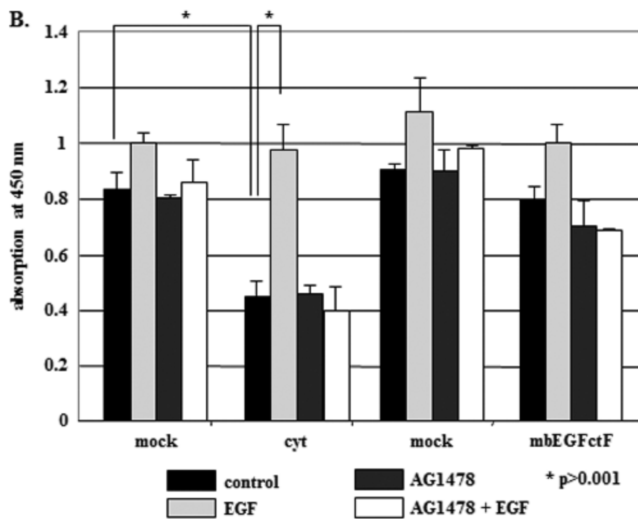
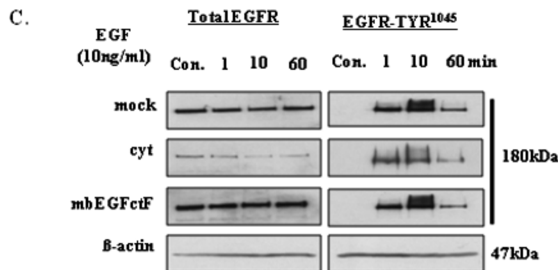


Figure W3. BrdU proliferation assays showing the increased proliferation in EGFcyt glioma transfectants and mock on EGF treatment (10 ng/ml). The EGF-induced proliferation was blocked by the EGFR inhibitor AG1478 (10 μM). AG1478 alone or in combination with the MMP inhibitor batimastat failed to significantly affect glioma cell growth.







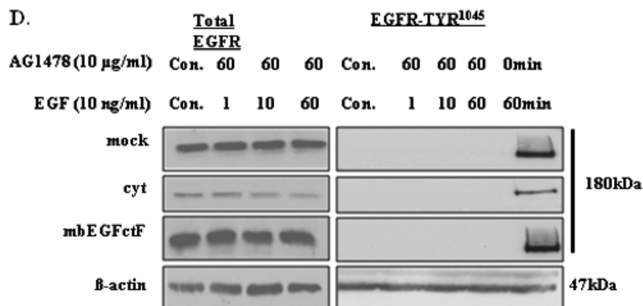


Figure W4. (A) Treatment of EGF-responsive EGFcyt and mbEGFctF thyroid transfectants with the general MMP inhibitor batimastat and subsequent BrdU assays. The increased proliferation of the mbEGFctF compared with EGFcyt transfectants was not the result of EGFR activation due to the proteolytic release of the extracellular bioactive EGF domain of the mbEGFctF construct. Batimastat failed to cause a change in proliferation in any of the transfectants tested. (B) The EGF-induced (10 ng/ml) increase in proliferation of EGFcyt, mbEGFctF, and corresponding mock thyroid transfectants was blocked by the EGFR inhibitor AG1478 (10 μM) as determined by BrdU proliferation assays. AG1478 itself did not affect cell growth. EGF treatment caused EGFcyt transfectants to significantly increase their proliferation rate to reach growth rates similar to mock. (C) Western blot detection of total EGFR and EGFR phosphorylated at residue Tyr^{1045} (EGFR-Tyr^{1045}) in thyroid EGFcyt, mbEGFctF, and mock transfectants. EGF (10 ng/ml) caused the specific Tyr1045 phosphorylation of EGFR in all transfectants investigated. (D) EGFR phosphorylation at residue Tyr^{1045} was completely blocked in the presence of the specific EGFR inhibitor AG1478.