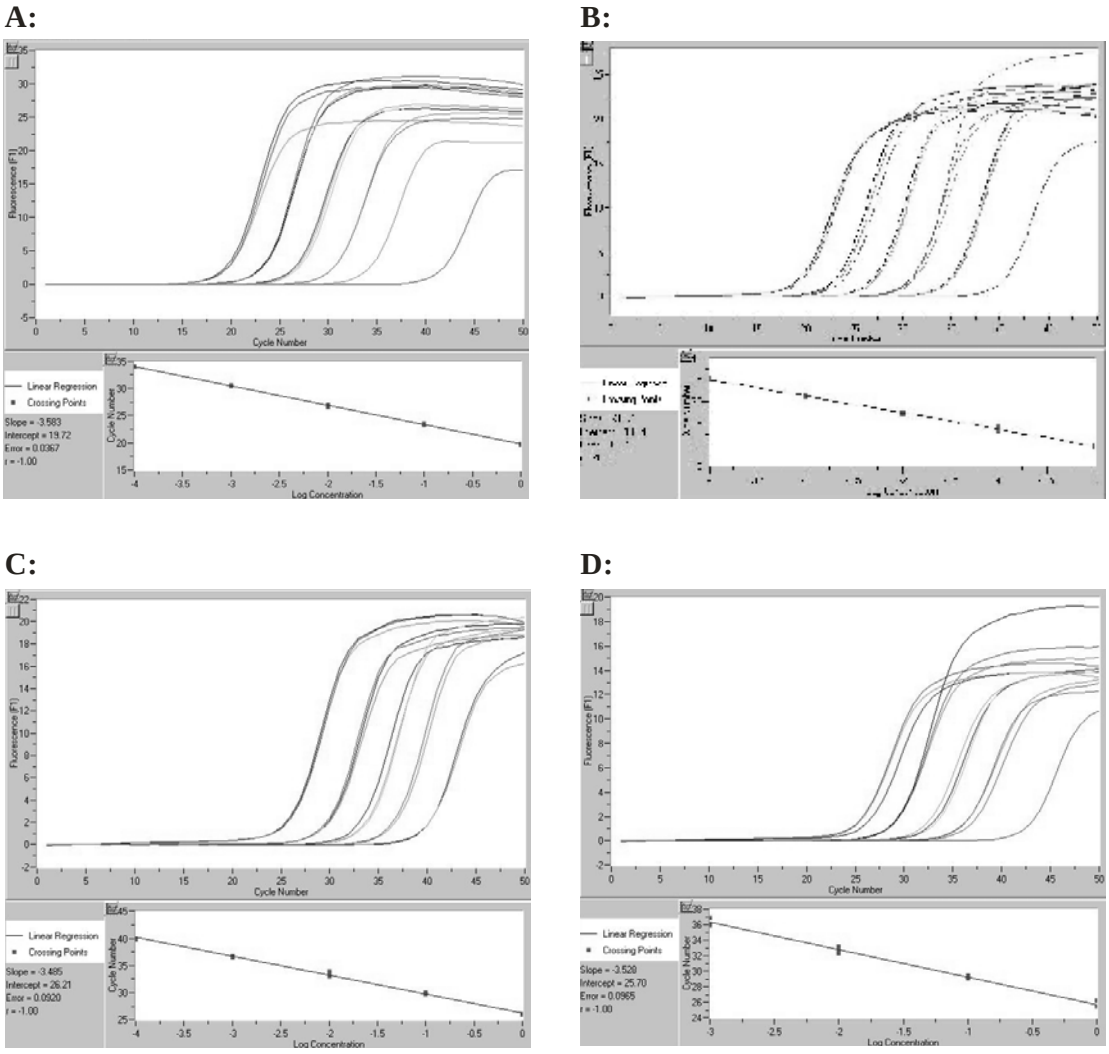
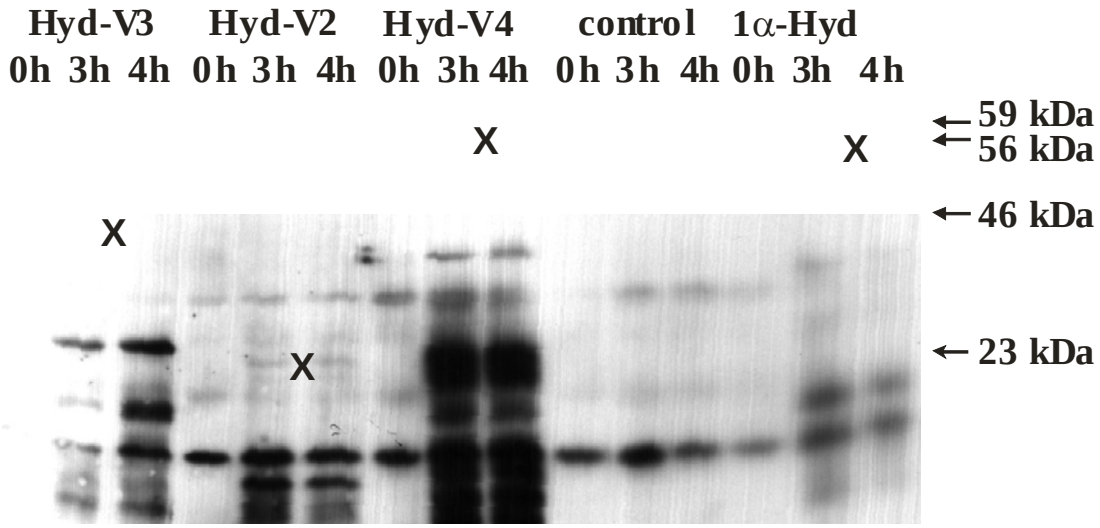
SUPPLEMENTAL FIGURES

Vitamin D_3 Metabolism in Human *Glioblastoma Multiforme*: Functionality of CYP27B1 Splice Variants, Metabolism of Calcidiol and Effect of Calcitriol

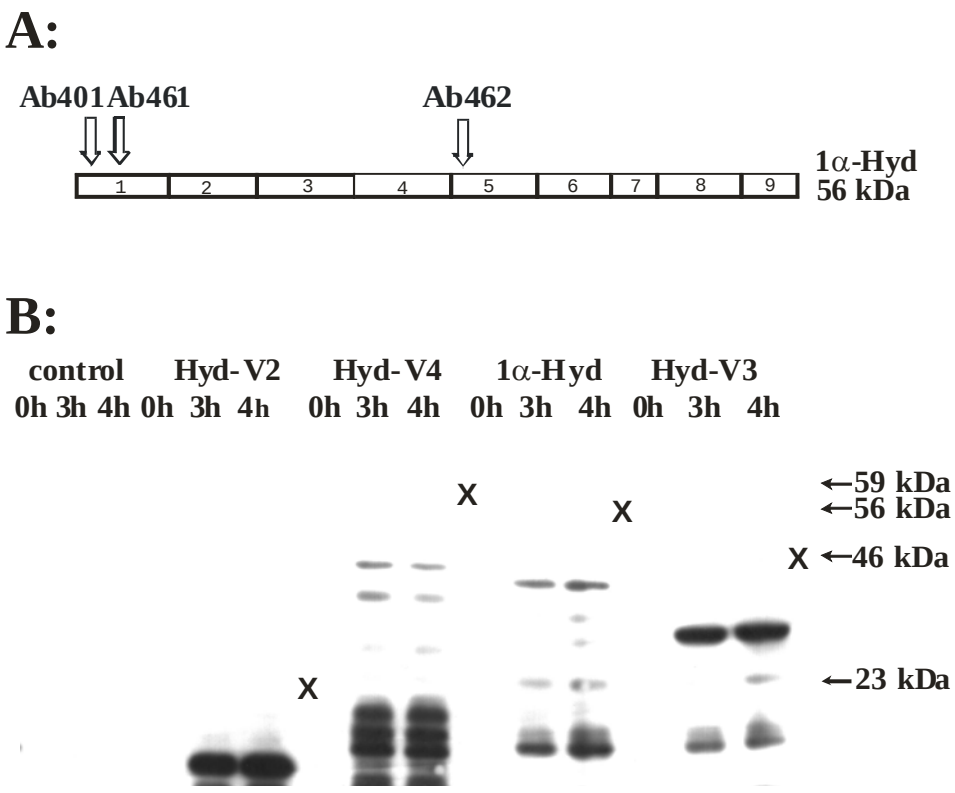
Diesel, Radermacher, Bureik, Bernhardt, Seifert, Reichrath, Fischer and Meese

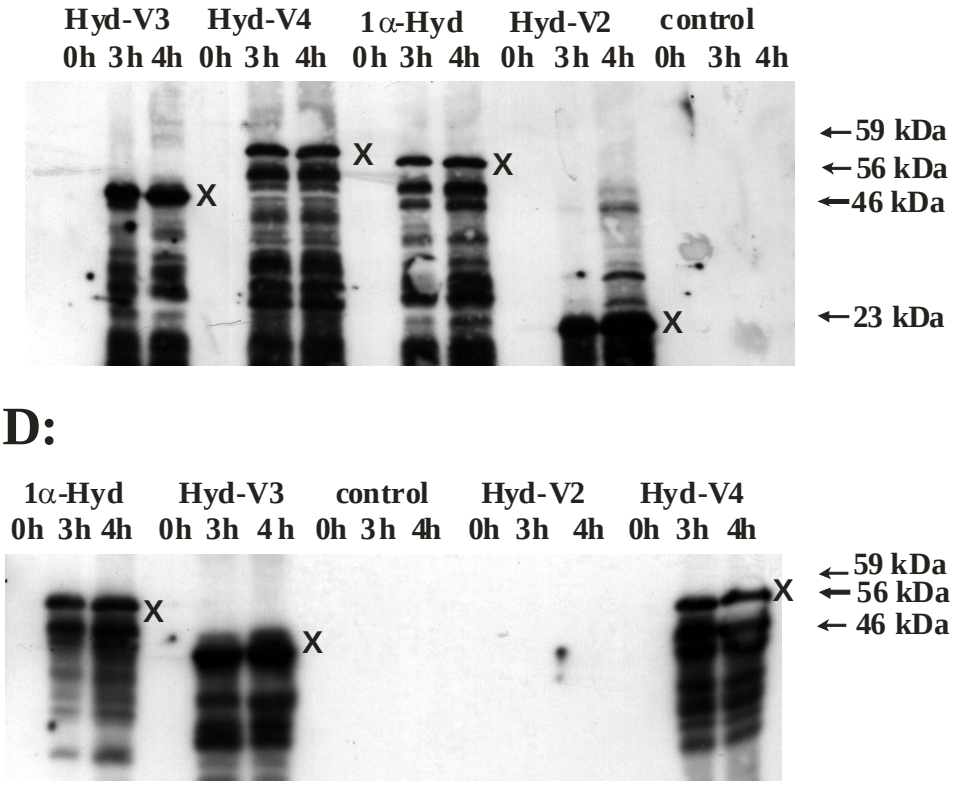


Supplemental Figure 1 Relative standard curve of CYP27B1 (**A**), β2-microglobulin (**B**), CYP24 (**C**) and VDR (**D**) generated by the LightCycler software. Different dilutions of cDNA from a CYP27B1 expressing cell line were analysed in triplicates (curves from left to right: 1:2, 1:20, 1:200, 1:2000, 1:20000). Efficiency of standard curves was 95 % for CYP27B1 and 91 % for β2-microglobulin.

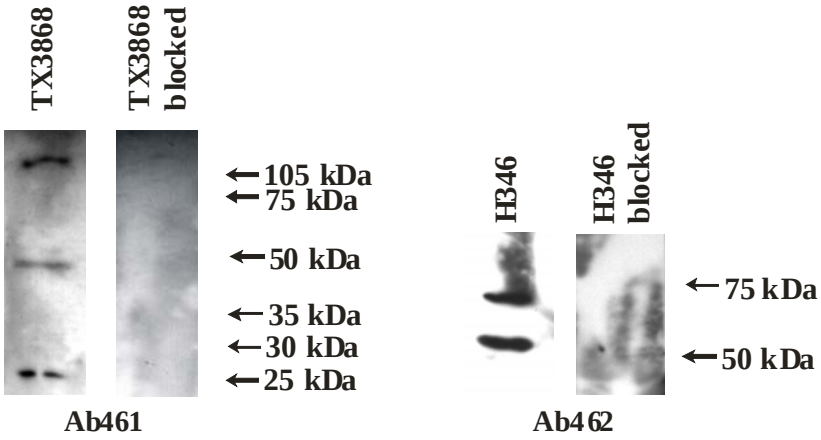


Supplemental Figure 2 Expression of CYP27B1 and splice variants in *E. coli*. Western Blot of recombinantly expressed CYP27B1 and splice variants Hyd-V2, Hyd-V3, Hyd-V4. Detection of C-terminal 6x His-tag using Ni-NTA-conjugate. Protein expression was determined before induction (0h) and 3h or 4h after induction. Negative control: vector without insert. Signals of intact proteins are marked (**X**).





Supplemental Figure 3 Specifity of antibodies against CYP27B1. **A:** Localisation of peptides in the normal CYP27B1 sequence used for the immunization of rabbits. Ab401, Ab461 specific for exon 1, Ab462 specific for exon 5. Ab: antibody. **B, C, D:** Western blot of recombinantly expressed CYP27B1 and splice variants Hyd-V2, Hyd-V3, Hyd-V4. Detection using purified polyclonal rabbit antibodies Ab401 (B), Ab461 (C), Ab462 (D) specific for CYP27B1. Protein expression was determined before induction (0h) and 3h or 4h after induction. Negative control: vector lacking insert.



Supplemental Figure 4 Western blot of total proteins isolated from GBM cell line TX3868 and GBM biopsy H346, using purified polyclonal rabbit antibodies Ab461 and Ab462. Antibody Ab461 identified proteins at 23 kDa, 56 kDa, and 140 kDa for TX3868. Antibody Ab462 detected proteins at 56 kDa and 58 kDa for H346. The specific bands disappeared when the antibodies were blocked with a 100 fold excess of immunizing peptide.