Supplementary Figure Legends

Supplementary Table S1 Target sequences of shRNAs against Notch2, CBF1.

Supplementary Table S2 Sequences of the primers specific for Notch pathway components.

Supplementary Figure S1

Notch pathway components are expressed in uveal melanoma cell lines. A,B, mRNA levels of Notch1, 2, 3 receptors (A) and Jag1 and Jag2 ligands (B) were determined by qPCR in five uveal melanoma cell lines.

Supplementary Figure S2

Treatment with MRK003 inhibits Hes1 and Hey1 mRNA expression. A,B, Hes1 mRNA levels were determined by qPCR in OCM3 (A) and OCM8 (B) cells treated with MRK003 at 2, 5, 10 μM for 48 h. **C,D,** mRNA levels of Hey1 were determined by qPCR in OCM1 (C) and OCM3 (D) cells after 48 h treatment with 2, 5, or 10 μM MRK003. **E,** Luciferase activity was determined in OCM3 cells transfected with CBF1 responsive element (CBF-RE) or CBF1 binding mutant (CBF-BM) luciferase constructs and treated for 48 h with MRK003. βgalactosidase plasmid was co-transfected as internal control. The data are the mean value from triplicate experiments. **F,** Clonogenic growth in soft agar was significantly reduced by 2 and 5 μM MRK003 in OCM8 cells; ND: not detected (*** p=0.0005).

Supplementary Figure S3

Notch pathway inhibition reduces cellular invasion. A, CBF1 and Hey1 mRNA levels were determined by qPCR in OCM3 and OCM1 cells infected with shCBF1 or scrambled shRNA (** p=0.009, *** p<0.0006). **B,** Annexin V positive cells were detected 72 h after infecting OCM3 and OCM1 cells with shCBF1 or scrambled shRNA. **C,** Notch2 and Hey1 mRNA levels were reduced in OCM1 cells infected with shNotch2 compared to scrambled shRNA or PLKO.1 empty vector controls, whereas no effects were observed on Notch1 mRNA levels.

Supplementary Figure S4

Constitutive Notch activation rescues Notch pathway suppression induced by MRK003.

A, MTS assay was performed in OCM1 cells infected with CLE, CLEN1 or CLEN2 vectors, after treatment for 5 or 7 days with MRK003 at the indicated doses. Photographs were taken before performing the MTS assay after 7 days of culture (** p=0.03, *** p<0.0001). **B,** Invasion assay was carried out in OCM3 cells expressing CLE, CLEN1 or CLEN2 vectors, after 24 hour treatment with MRK003 at the indicated doses (** p=0.008, *** p<0.0001). Photographs show the migrated cells. **C,** Hey1 mRNA levels were increased in Mel290 cells infected with CLEN1 or CLEN2 vectors compared to CLE control, as determined by qPCR (* p=0.04, *** p<0.0001).

Supplementary Figure S5

Notch pathway inhibition reduces activation of Erk, Akt and STAT3 A, Phosphorylation levels of Akt^{Ser473}, Erk1-2$^{Thr202/Tyr204}$ and $STAT3^{Tyr705}$ were measured by immunoblotting in OCM1 cells treated with DMSO or MRK003 for 48 h at 2 and 5 μM. **B,** Phosphorylation levels of Erk1-2$^{Thr202/Tyr204}$ and $STAT3^{Tyr705}$were determined in OCM3 cells treated with DMSO or MRK003 for 48 h or infected with sh-CBF1.