Table S1: List of genes co-regulated with PIMREG in the enriched pathways

Cell cycle:	E2F1, E2F2, DBF4, PKMYT1, TTK, CHEK1, PTTG1, CCNE2, CDC45, MCM7, CDKN2C, BUB1, ORC6, CCNA2, ORC1, CDC7, CDC6, CDK1, ESPL1, CDC20, MCM2, CDK4, CDC25C, CDC25A, CCNB1, CCNB2, MAD2L1, PLK1, PCNA, BUB1B, MAD2L2
DNA Replication:	RFC5, PRIM1, DNA2, RFC3, RFC4, MCM7, POLE2, PRIM2, PCNA, MCM2, POLA2, RNASEH2A
Fanconi anemia (FA):	RAD51C, FANCD2, FANCI, EME1, FANCE, BRIP1, FANCG, FANCA, RMI1, UBE2T, FANCB, RAD51
Pirimidine metabolism:	NT5C3B, DTYMK, CTPS1, POLA2, POLR2D, TK2, TK1, PRIM1, TYMS, POLE2, NME1, RRM2, PRIM2, DCTPP1
Oocyte meiosis:	CDK1, PKMYT1, CDC20, ESPL1, AURKA, PTTG1, CDC25C, ITPR1, CCNE2, MAD2L1, PLK1, BUB1, FBXO5, MAD2L2
Progesterone-mediated oocyte maturation:	CCNB1, CDK1, MAD2L1, CCNB2, PLK1, BUB1, PKMYT1, CDC25C, MAD2L2, CCNA2, CDC25A
P53 signaling pathway:	CCNB1, CCNE2, CDK1, PPM1D, CCNB2, RRM2, CHEK1, CDK4, GTSE1
Mismatch repair (MMR):	EXO1, RFC5, RFC3, RFC4, PCNA
Homologous recombination (HR):	RAD51C, EME1, RAD54B, RAD54L, RAD51
Nucleotide excision repair (NER):	RFC5, ERCC8, RFC3, RFC4, POLE2, PCNA

Figure S1

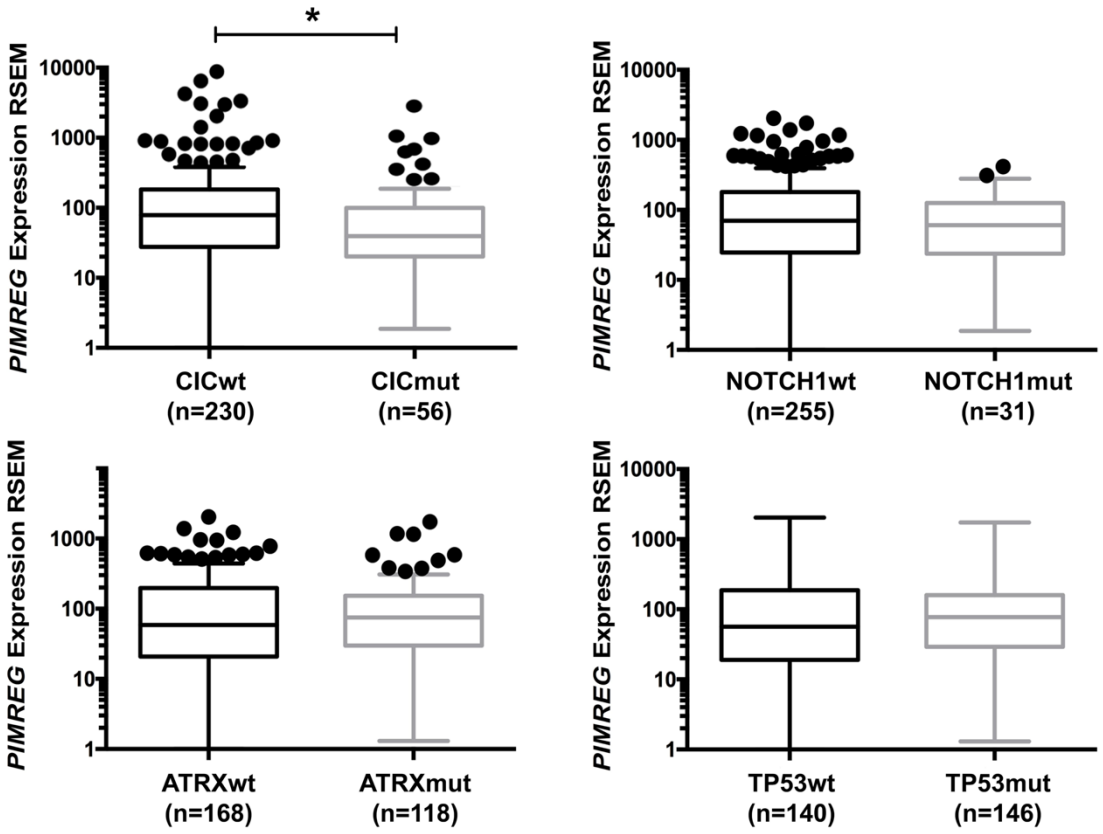

Fig. S1. *PIMREG* expression is significantly lower in patients carrying *CIC* mutations**.** *PIMREG* mRNA levels in TCGA LGG patients classified according to mutational status of recurrently affected genes: *CIC*, *ATRX*, *TRP53* and *NOTCH1*. CICwt: CIC wild type phenotype, CICmut: CIC mutated phenotype, ATRXwt: ATRX wild type phenotype, ATRXmut: ATRX mutated phenotype, TP53wt: TP53 wild type phenotype, TP53mut: TP53 mutated phenotype, NOTCH1wt: NOTCH1 wild type phenotype, NOTCH1mut: NOTCH1 mutated phenotype. (*) p-value = 0.0142, Mann-Whitney two-tailed test.

Figure S2

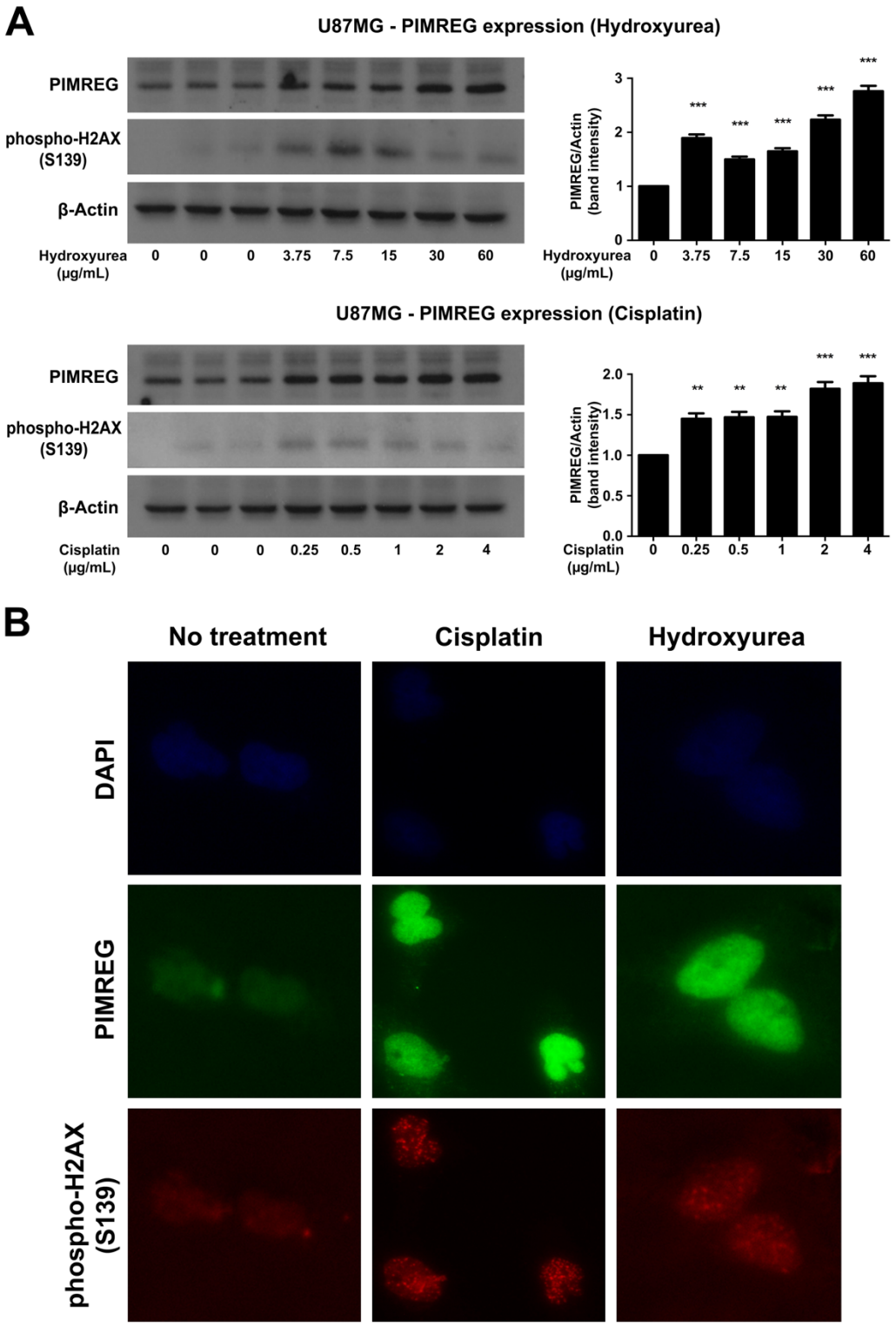

Fig. S2. PIMREG expression is induced upon hydroxyurea and cisplatin treatment of GBM cells. **(A)** Western blot analysis of protein extracts from U87MG cells treated for 24h with the indicated concentration of hydroxyurea or cisplatin. Membranes were blotted with

PIMREG, phospho-H2AX(S139) and β-Actin antibodies. Representative blots are shown (left panel). The bar graphs represent densitometric measurement of the bands normalized by β-Actin and relative to untreated control cells set as 1 (right panels). Results are shown as mean ± SD of three independent experiments. (**) p-value < 0.001, and (***) p-value < 0.0001, One-way ANOVA test**. (B)** Nuclear expression of PIMREG and phospho-H2AX(S139) in U87MG cells treated with hydroxyurea (15 μg/ml) or cisplatin (1 μg/ml) for 24h and analyzed by immunofluorescence microscopy. 63X magnification.

Figure S3

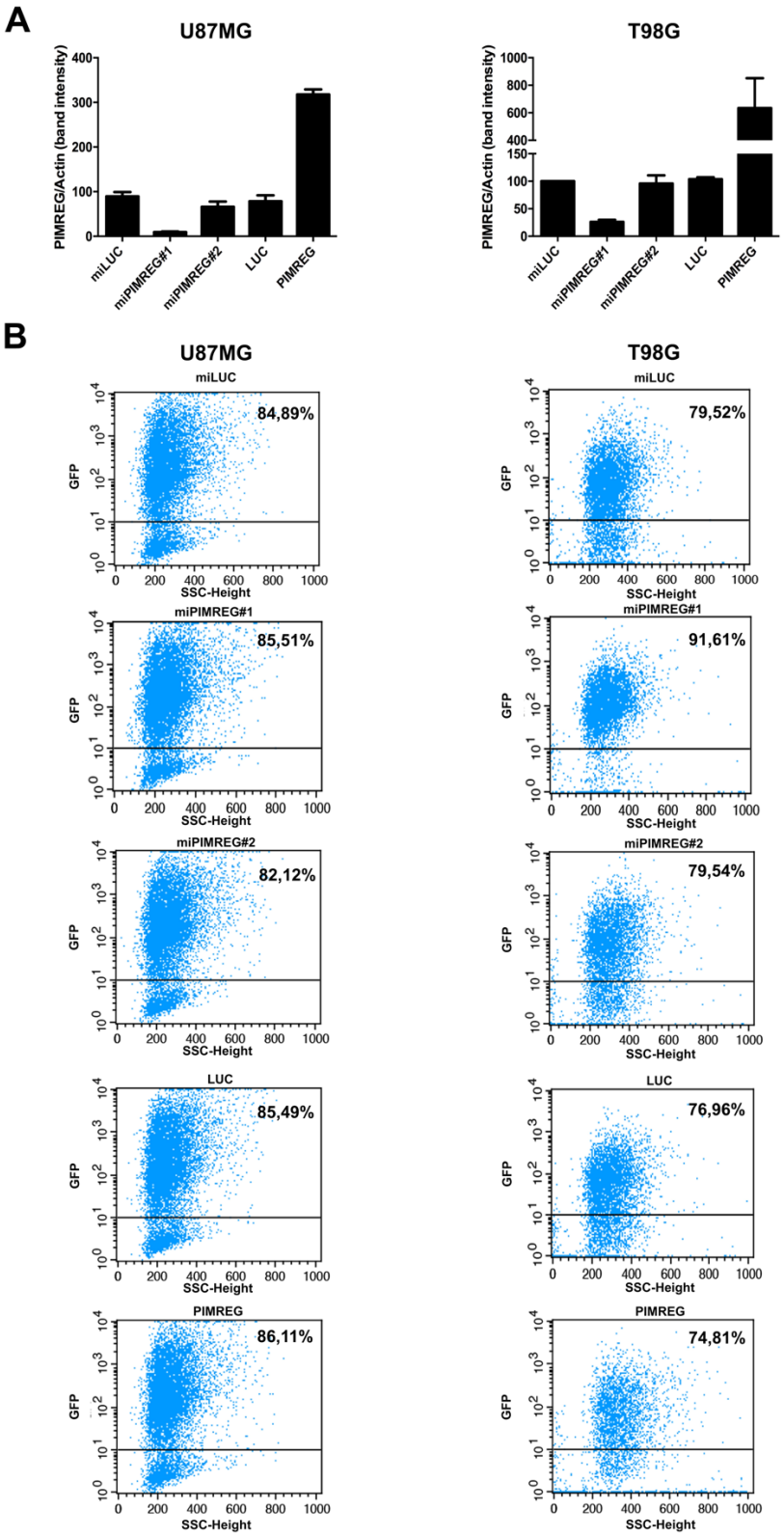

Fig. S3. PIMREG knockdown and overexpression in GBM cells. **(A)** PIMREG expression was evaluated by western blot on protein extracts of U87MG and T98G cells carrying the

pRTS-miLUC, pRTS-miPIMREG#1, pRTS-miPIMREG#2, pRTS-LUC or pRTS-PIMREG constructs. The bar graphs represent densitometric analysis of the bands normalized by β-Actin and relative to miLUC control cells set as 1. The results shown are mean ± SD of three independent experiments (including the representative blots shown in Fig. 4A). Note that PIMREG targeting sequence miPIMREG#2 was not effective in silencing the gene. **(B)** Percentage of the cells carrying the constructs within each cell population determined by GFP expression and FACS analysis.

Figure S4

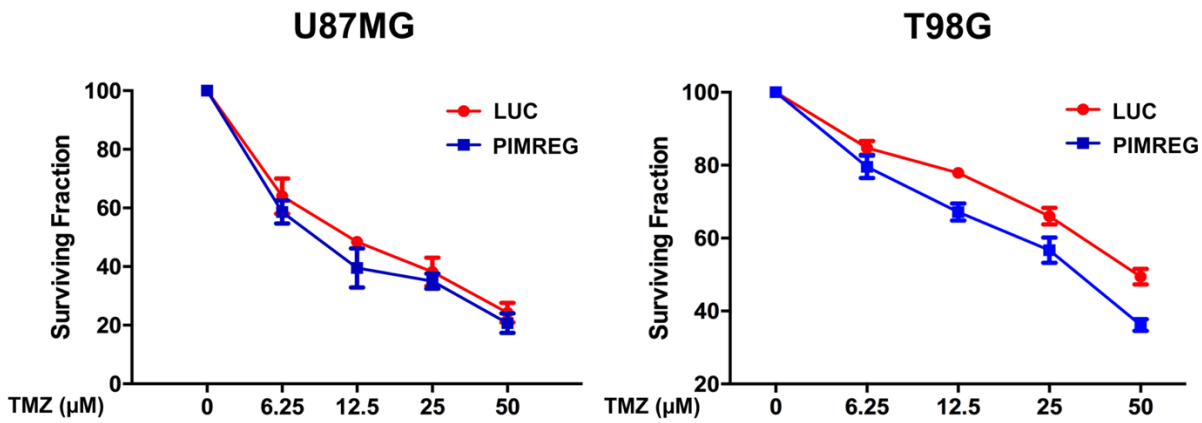

Fig. S4. PIMREG overexpression did not alter TMZ sensitivity of U87MG and T98G cells. PIMREG overexpressing (PIMREG) and luciferase control (LUC) cells were treated with the indicated concentrations of TMZ for 24h and assessed for clonogenic survival after 9 days. TMZ: temozolomide.

Figure S5

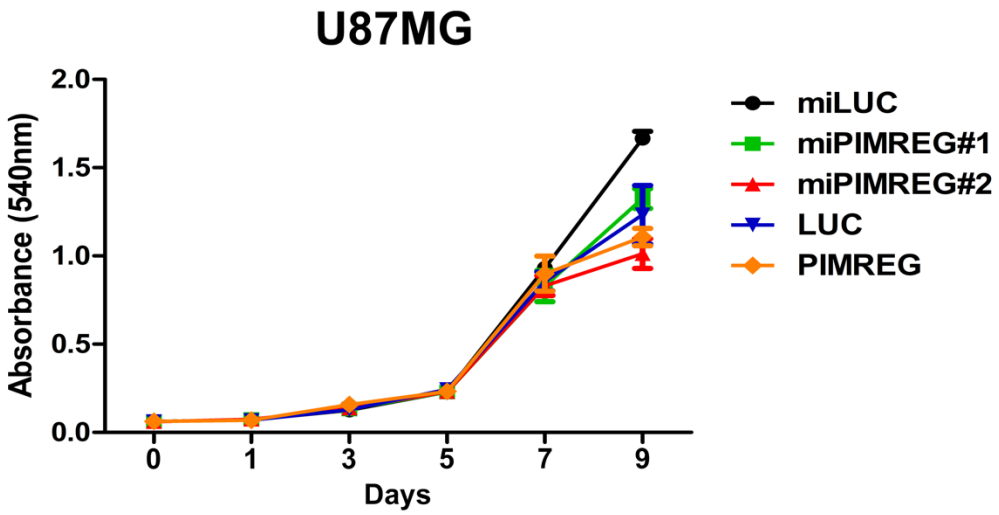

Fig. S5. PIMREG modulation did not alter proliferation of U87MG cells. U87 cells were cultured in serum depleted medium were assessed for growth on day 0, 1, 3, 5, 7 and 9 of culture. Growth curve of U87MG cells carrying the pRTS-miPIMREG#1, pRTS-miPIMREG#2, pRTS-miLUC constructs (knockdown and control, respectively) and PIMREG overexpressing (PIMREG) and luciferase control (LUC) cells.

Figure S6

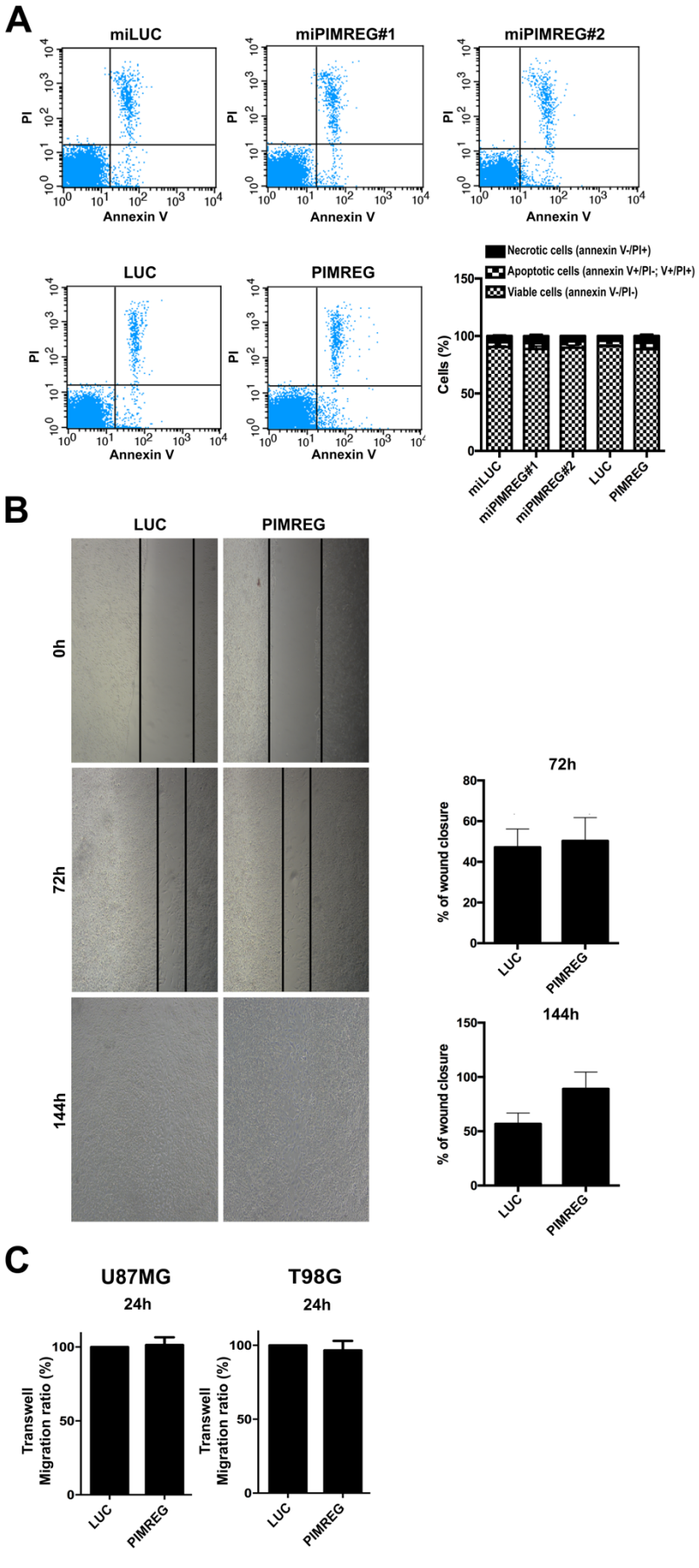
A
miLUC
miPIMREG#1
miPIMREG#2
LUC
PIMREG
PI
Annexin V
Necrotic cells (annexin V-/PI+)
Apoptotic cells (annexin V+/PI-; V+/PI+)
Viable cells (annexin V-/PI-)
Cells (%)
B
LUC
PIMREG
0h
72h
144h
72h
% of wound closure
144h
C
U87MG
T98G
24h
Transwell Migration ratio (%)

Fig. S6. PIMREG overexpression did not alter migration of GBM cells. **(A)** Percentage of viable cells in the T98G populations submitted to wound healing assay. PIMREG knockdown (miPIMREG#1, miPIMREG#2) and control (miLUC) cells and PIMREG overexpressing (PIMREG) and LUC control cells were assessed for annexin /PI staining at timepoint 144h. FACS plots show the percentage of viable (annexin V^-/PI^-), apoptotic (annexin V^+/PI^-; V^+/PI^+), and necrotic cells (annexin V^-/PI^+), determined by flow cytometry. The bars on the graph represent the mean values ± SD of three independent experiments. **(B)** Wound healing assay. PIMREG overexpressing (PIMREG) and luciferase control (LUC) T98G cells were grown to confluence and wounded. The pictures were taken in the indicated time points (0h, 72h and 144h). Representative image is shown. Bar graphs represent the percentage of closure, determined by reduction of the distance between the wound edges from time 0h set as 100%. **(C)** Transwell migration assay. Migration of PIMREG overexpressing (PIMREG and LUC, used as control) U87MG and T98G cells was evaluated by counting the number of cells that passed through the 8 μm pore-sized transwell after 24h exposure towards fetal bovine serum-containing media. Results are shown as mean ± SD of three independent experiments.