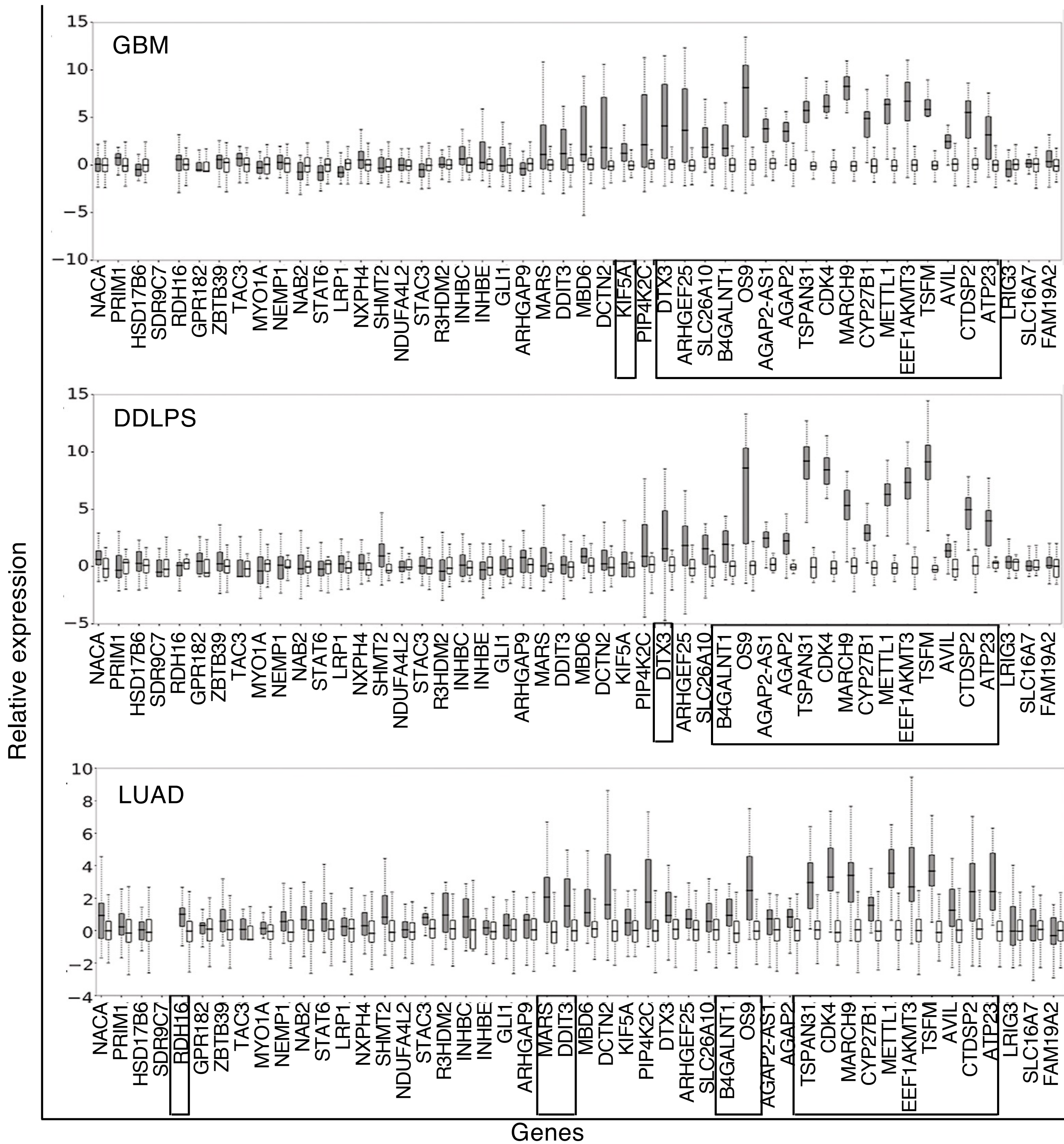
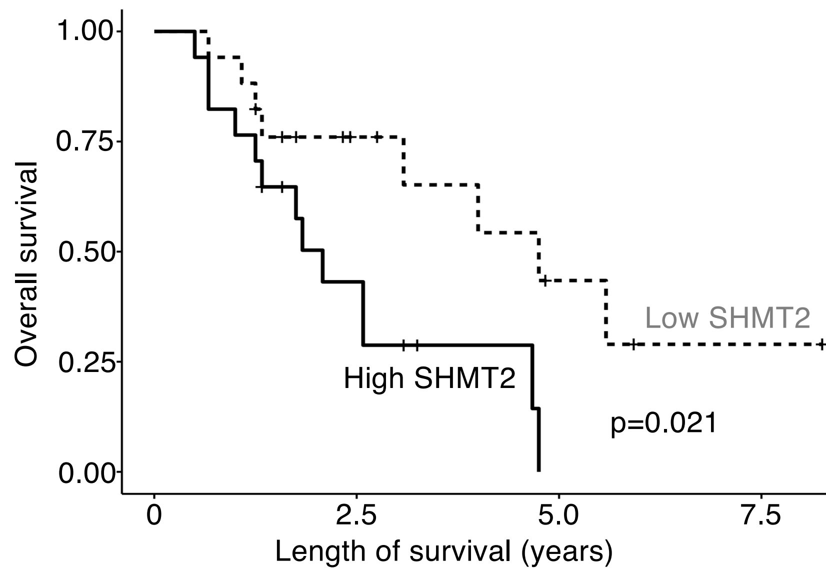
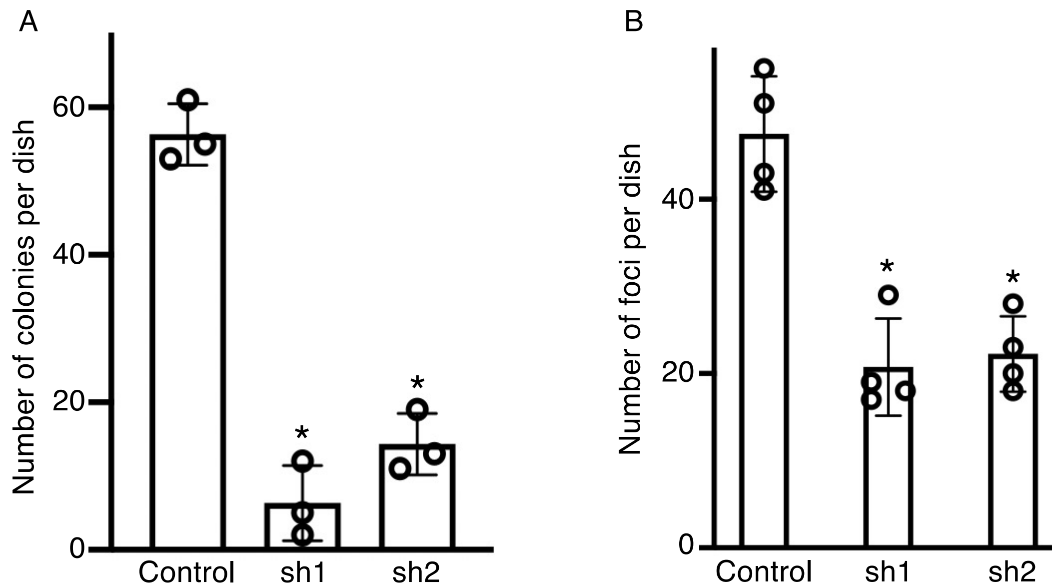


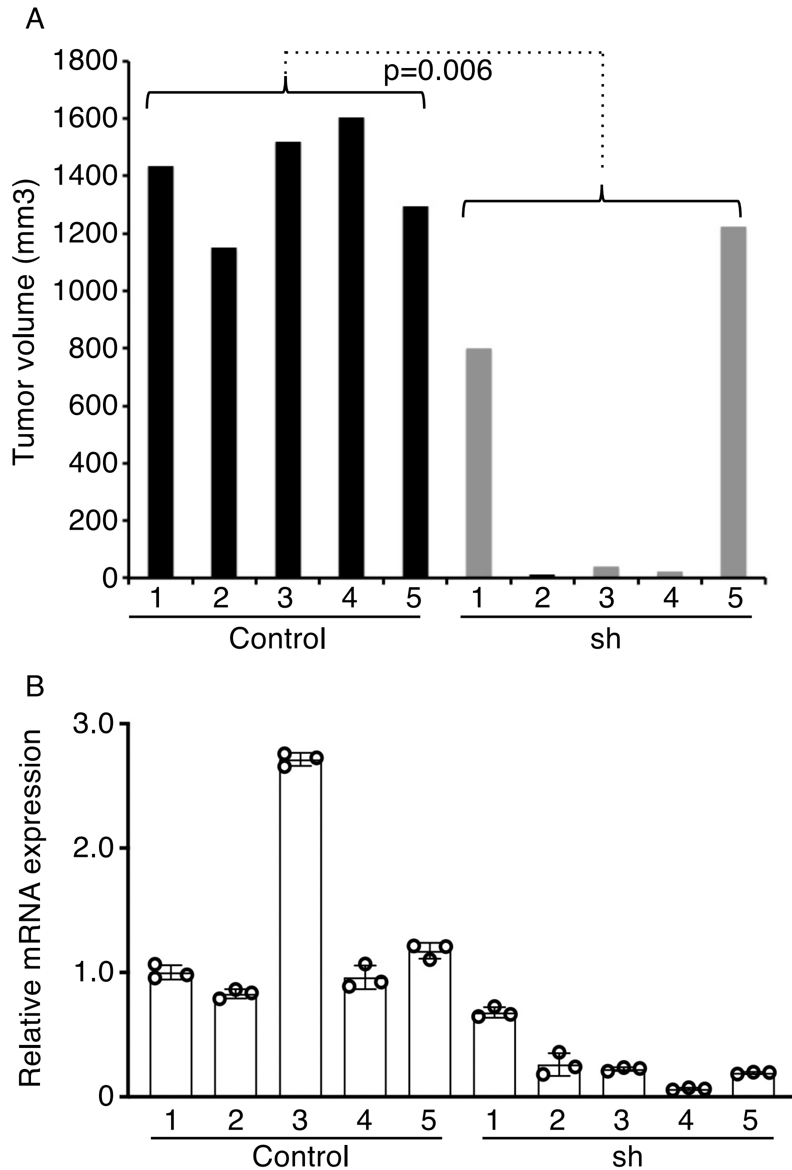
Supplemental Figure 1. Expression levels of genes in the 12q13-q14 region. Genes in and around the 12q13-q14 amplicon were analyzed for significant differences in gene expression between amplicon-positive (grey) and amplicon-negative (white) samples. Genes that had Bonferroni-corrected p-values ≤ 0.05 and fold changes ≥ 2.0 are indicated with black rectangles.



Supplemental Figure 2. Relationship of SHMT2 mRNA expression to overall survival in patients with FP RMS. Using expression microarray data (11), 34 FP-RMS tumors were stratified into low and high SHMT2 groups based on the median expression level of SHMT2 mRNA. The overall survival of patients with FP-RMS with high (n=17) versus low (n=17) SHMT2 expression was then compared. The p-value shown was calculated by the log-rank test.

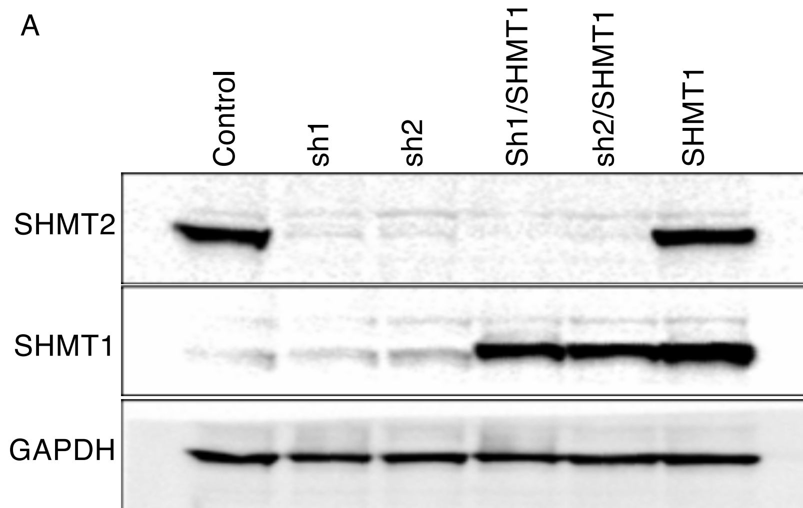


Supplemental Figure 3. Effect of SHMT2 knockdown on colony (A) and focus (B) formation of Rh30 cells. As described in Figure 6E-F, control or shRNA-expressing cells were cultured for 3 weeks with (A) or without (B) NIH 3T3 cells, and then fixed and stained with Giemsa. Counting in A (3 replicates) and B (4 replicates) was performed using ImageJ software (NIH) and displayed as the mean ± SD. Dunnett's multiple comparisons test was used for statistical analysis of differences between control and shRNA-expressing cells; an asterisk (*) indicates $p<0.001$.

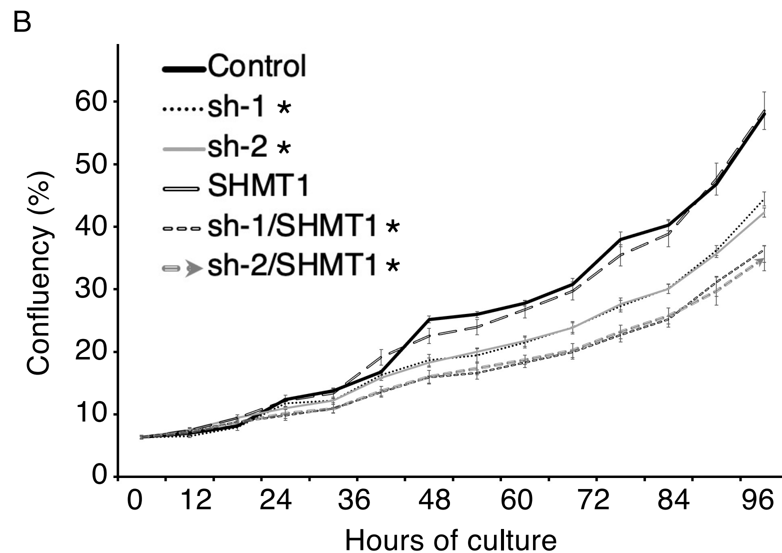


Supplemental Figure 4. Intramuscular xenograft tumor formation of control or SHMT2 shRNA-expressing Rh30 cells. (A). *Tumor volume.* Tumors (shown in Figure 6G) were excised and volumes were measured by the formula ($width^2$ x length)/2. Statistical analysis of differences in tumor volumes of control versus shRNA-expressing tumors was performed using the Student's *t*-test (2 tailed, type 2). (B) *Quantitation of SHMT2 mRNA expression in excised tumors*. The relative SHMT2 mRNA expression in each control or SHMT2 shRNA-expressing tumor was determined by qRT-PCR, using GAPDH for normalization. Data represent mean ± SD of 3 replicates. The tumor numbers in parts A and B refer to the tumors shown in Figure 6G.

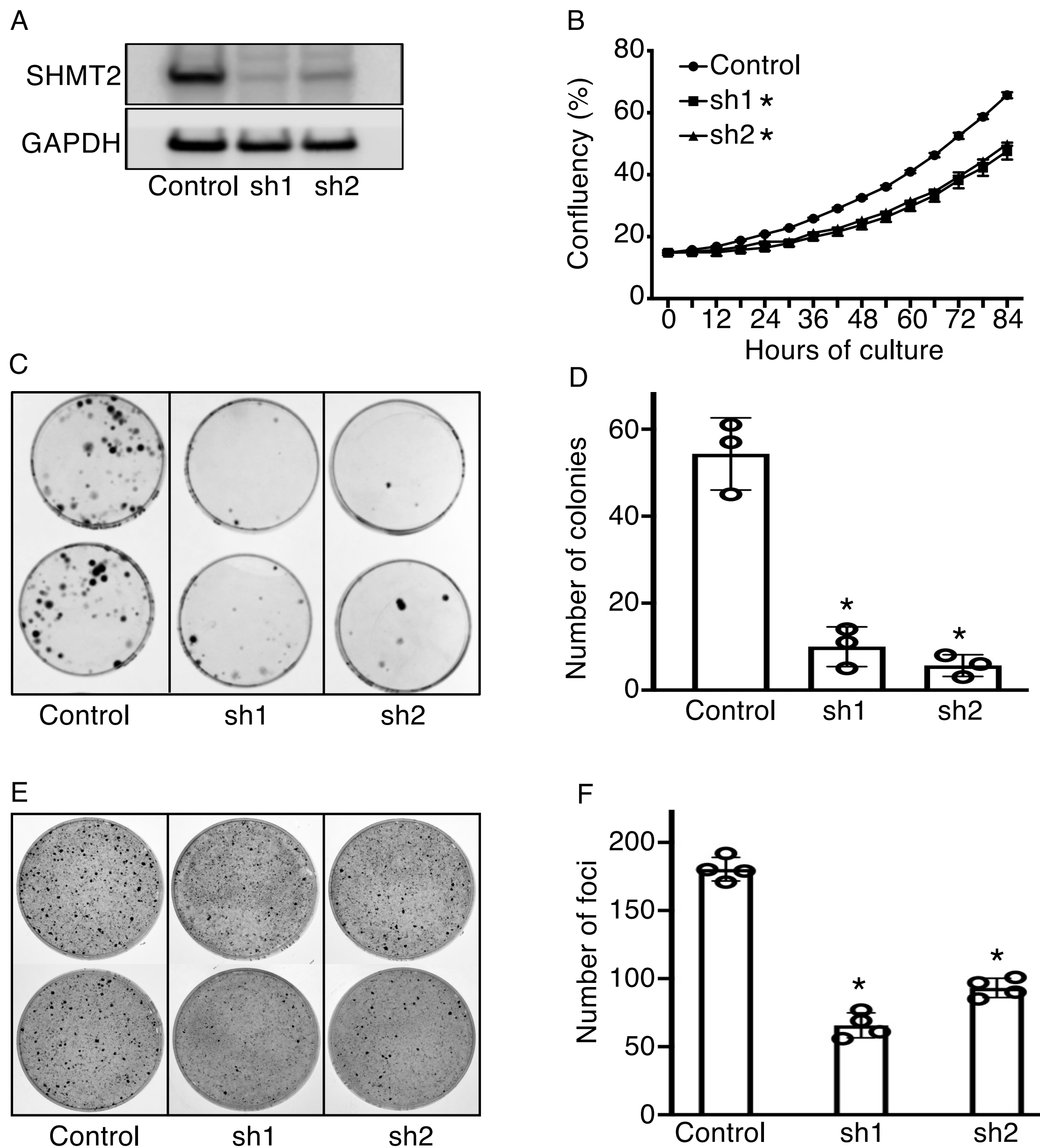
A



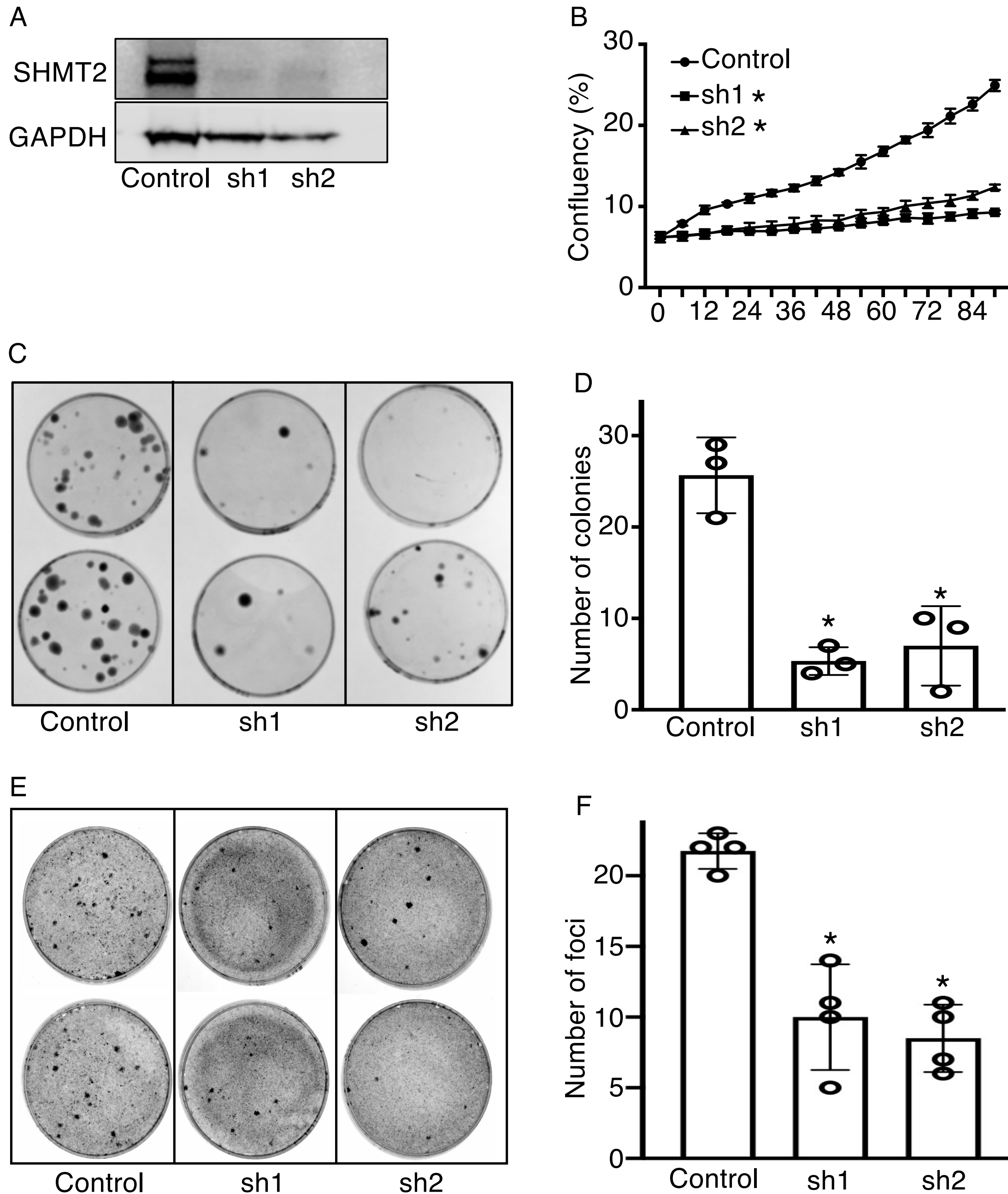
B



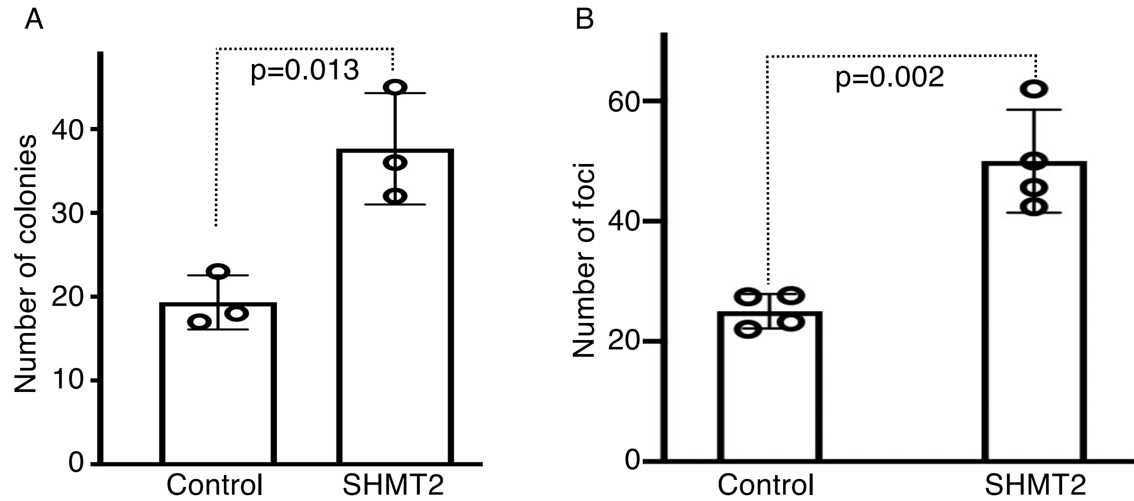
Supplemental Figure 5. Effect of SHMT1 on Rh30 cell growth. (A) *Western blot analysis of SHMT1 and SHMT2 expression*. Immunoblots of of Rh30 cells expressing the indicated genetic elements were probed with specific antibodies against SHMT1 and SHMT2. GAPDH was used as a loading control. (B). *IncuCyte assay*. Data indicate mean confluence ± SE of 4 different wells. ANOVA tests with correction for multiple comparisons using the Sidak-Bonferroni method were performed for the last 6 time points. An asterisk (*) indicates $p<0.05$ when compared with Rh30-control cells. Experiments were repeated twice, and representative data are shown.



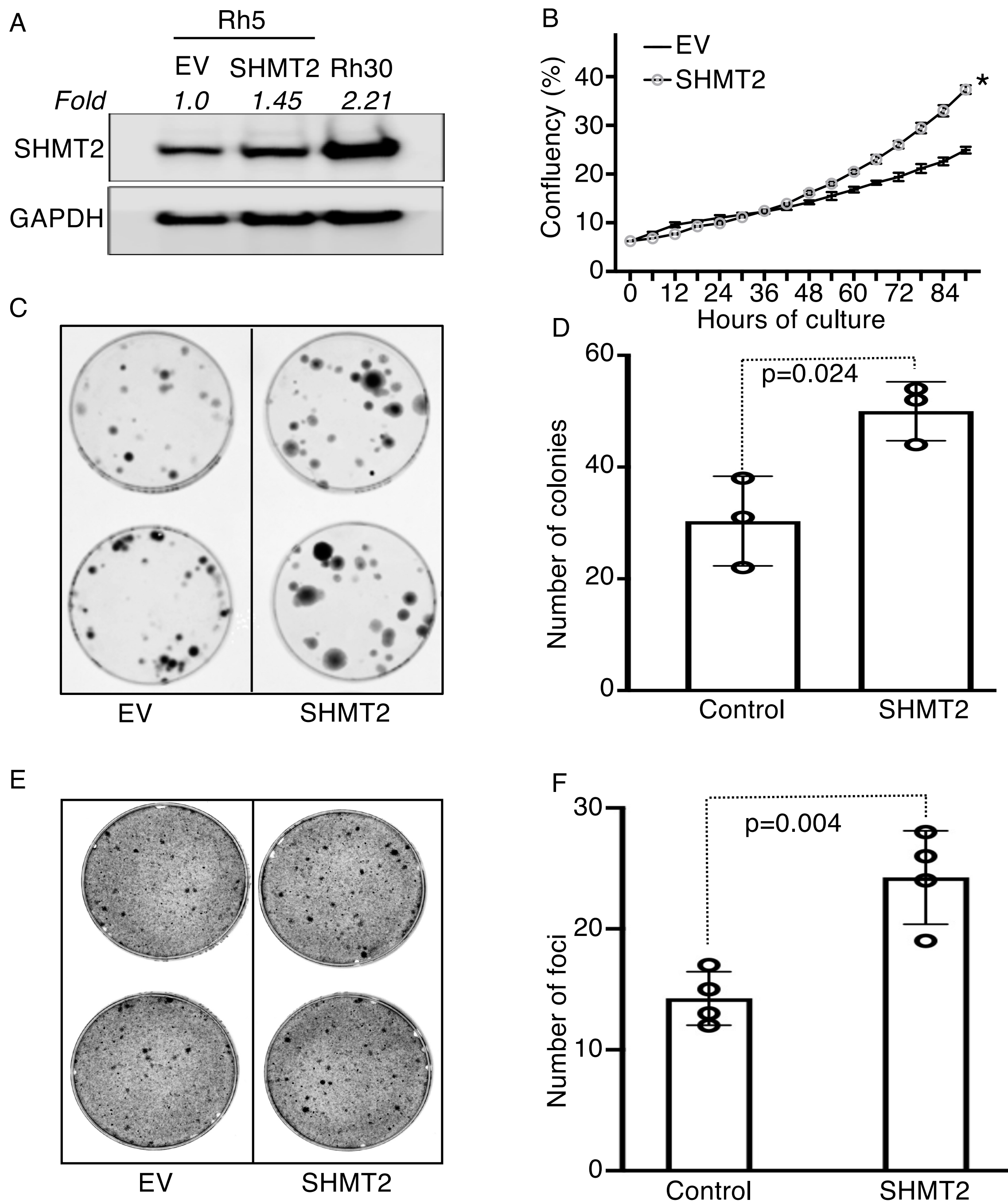
Supplemental Figure 6. Effect of SHMT2 knockdown in Rh41 cells. (A) *Western blot analysis of SHMT2 expression of control and shRNA-expressing cells*. GAPDH was used as a loading control. (B) *Growth assay.* Data indicate mean confluence values ± SE of 4 different wells. ANOVA tests with correction for multiple comparisons using the Sidak-Bonferroni method were performed for the last 6 time points. An asterisk (*) indicates $p<0.01$. (C) *Clonogenic assay and* (D) *colony counting*. 360 cells were seeded in 6-cm dishes, grown for 3 weeks, and then fixed and stained with Giemsa. (E) *Focus formation assay and* (F) *counting*. 500 cells were co-cultured with 2×10^5 NIH 3T3 fibroblasts for 4 weeks, and then fixed and stained with Giemsa. Counting in D (3 replicates) and F (4 replicates) was performed using the ImageJ software (NIH). Experiments were repeated at least twice, and representative data are shown. In D and F, Dunnett's multiple comparisons test was used for statistical analysis of differences between control and shRNA-expressing cells; an asterisk (*) indicates $p<0.001$.



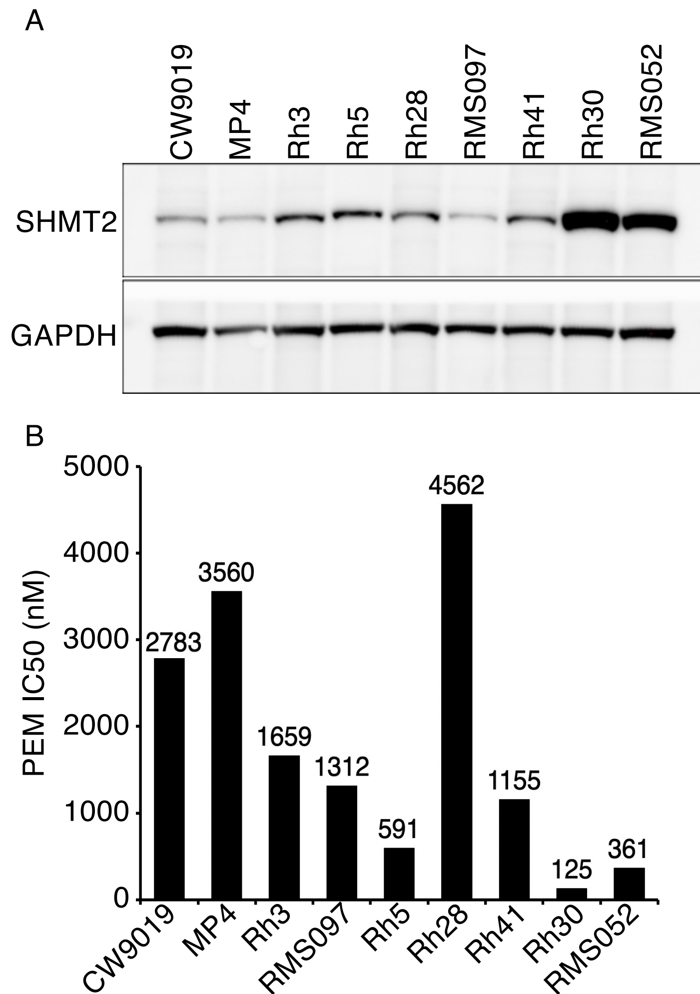
Supplemental Figure 7. Effect of SHMT2 knockdown in Rh5 cells. (A) *Western blot analysis of SHMT2 expression in Rh5 cells expressing control or SHMT2 shRNAs*. GAPDH was used as a loading control. (B) *IncuCyte assay.* Data indicate mean confluence values ± SE of 4 different wells. ANOVA tests (corrected for multiple comparisons using the Sidak-Bonferroni method) were performed for the last 6 time points. An asterisk (*) indicates an adjusted $p<0.01$. (C) *Clonogenic assay and* (D) *colony counting*. 500 cells were seeded in 6-cm dishes, grown for 3 weeks, and then fixed and stained with Giemsa. (E) *Focus formation assay and* (F) *focus counting*. 10^3 cells expressing control or shRNAs were co-cultured in 6-cm dish with 2 x 10^5 NIH 3T3 fibroblasts for 5 weeks, and then fixed and stained with Giemsa. Counting in D (3 replicates) and F (4 replicates) was performed using the ImageJ software (NIH). Experiments were repeated at least twice, and representative data are shown. In D and F, Dunnett's multiple comparisons test was used for statistical analysis of differences between control and shRNA-expressing cells; an asterisk (*) indicates $p<0.01$.



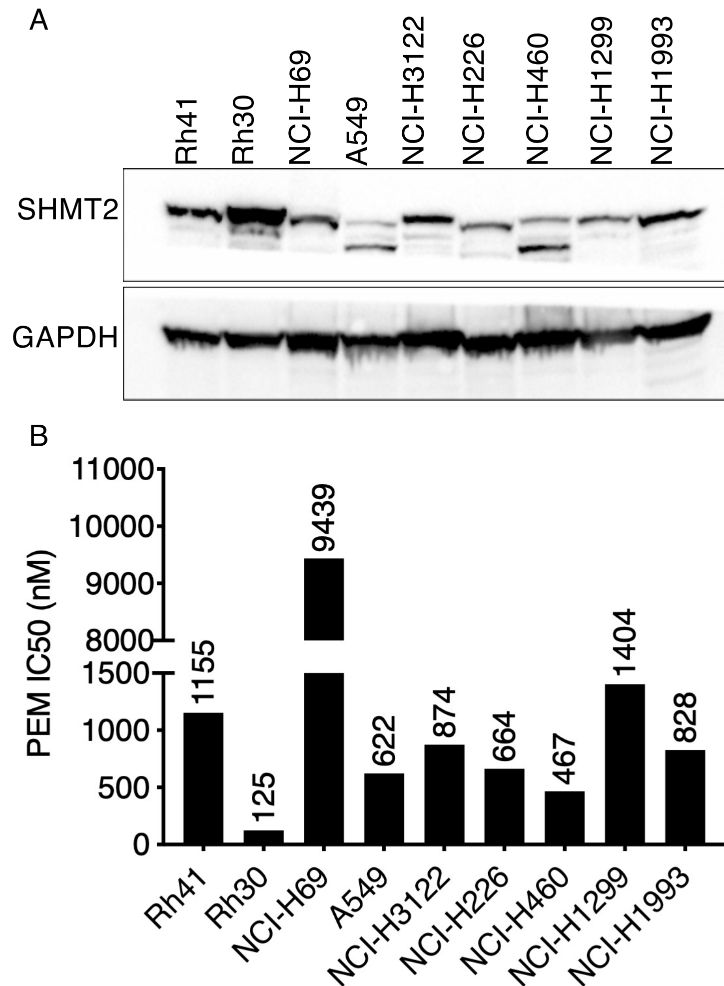
Supplemental Figure 8. Effect of SHMT2 expression on colony (A) and focus (B) formation in Rh41 cells. As previously described in Figure 7D-E, control (empty vector) or SHMT2-expressing cells were cultured for 3 weeks (colony) or 4 weeks (focus), and then fixed and stained with Giemsa. Colony and focus counting were performed using the ImageJ software (NIH). Data represent 3 replicates in A and 4 replicates in B ± SD. In B, the 4 replicates consisted of 2 dishes with 500 and 2 dishes with 1,000 tested cells, and counting results were normalized for 500 input cells. Student's *t*-test was used for statistical analysis.



Supplemental Figure 9. Effect of SHMT2 overexpression on Rh5 cell growth and transformation. (A) *Western blot analysis of SHMT2 protein expression in Rh5 cells*. Immunoblots show SHMT2 expression in control or SHMT2-expressing Rh5 cells. Rh30 cells were used for comparison and GAPDH was used as a loading control. Expression was quantified as described in Figure 5. (B) *Growth assay.* Data indicate mean confluence values ± SE of 4 different wells. Multiple t tests (correct for multiple comparisons using the Sidak-Bonferroni method) were performed for the last 6 time points to assess the significance in growth differences and asterisk (*) indicates an adjusted $p<0.05$. (C) *Clonogenic assay and* (D) *counting*. 500 cells were seeded in 6-cm dishes, grown for 3 weeks, and then fixed with methanol and stained with Giemsa. (E) *Focus formation assay* and (F) *counting*. 1000 cells were co-cultured with 2×10^5 NIH 3T3 fibroblasts in 6-cm dishes, and then fixed and stained after 4 weeks. Counting in D (3 replicates) and F (4 replicates) was performed using the ImageJ software (NIH). Experiments were repeated twice, and representative data are shown.



Supplemental Figure 10. Comparison of the FP RMS line RMS052 with other FP RMS cell lines. (A) Expression of SHMT2 FP RMS cell lines. The immunoblot was probed with SHMT2 antibody, stripped and reprobed with GAPDH antibody for loading control. (B) *IC50 of PEM in RMS cell lines*. The assay was performed as described in Figure 8 with PEM concentrations ranging from 50 to 6400 nM.



Supplemental Figure 11. Expression of SHMT2 and PEM IC50 in RMS and lung cancer cell lines. (A) *Protein expression of SHMT2*. The immunoblot was probed with SHMT2 antibody, stripped and reprobed with GAPDH antibody for loading control. (B) *IC50 of PEM*. The assay was performed as described in Figure 8 with PEM concentrations ranging from 100 to 6400 nM. All cell lines were assayed under the same experimental conditions.