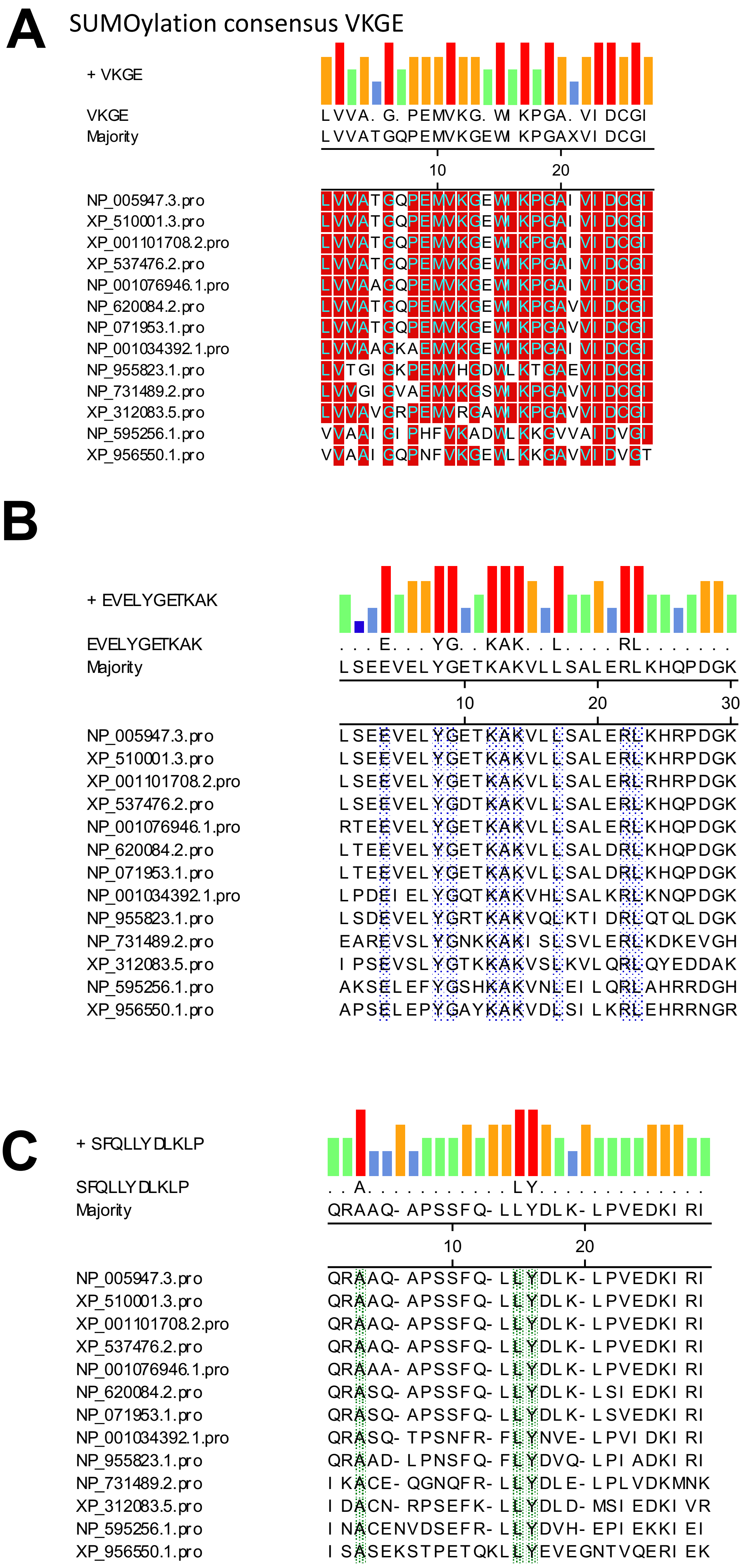


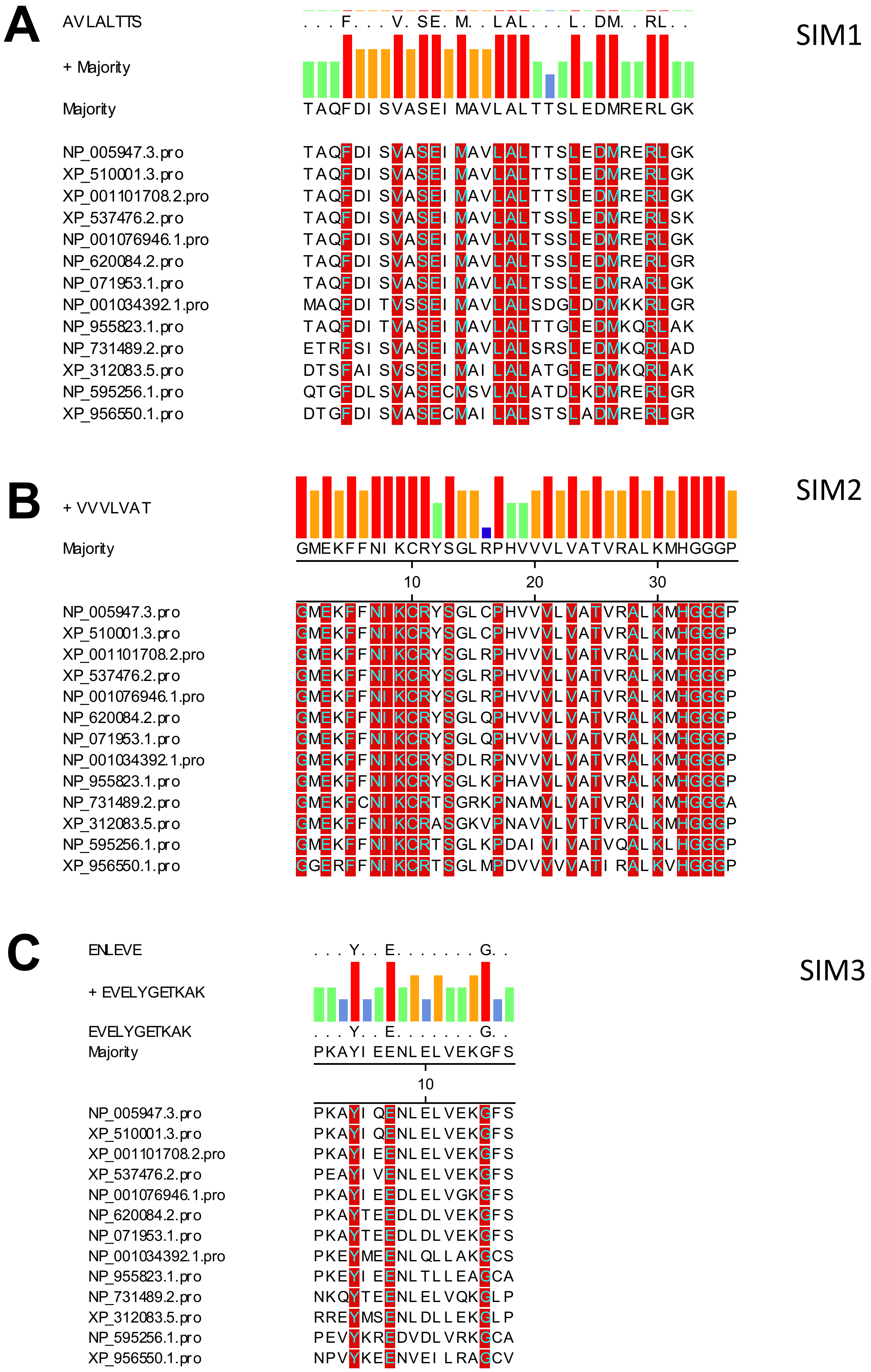
+ VKGE	
VKGE	LVVA. G. PEMVKG. W KPGA. VI DCGI
Majority	LVVATGQPEMVKGEW KPGAXVI DCGI
NP_005947.3.pro	LVVATGQPEMVKGEW KPGAI VI DCGI
XP_510001.3.pro	LVVATGQPEMVKGEW KPGAI VI DCGI
XP_001101708.2.pro	LVVATGQPEMVKGEW KPGAI VI DCGI
XP_537476.2.pro	LVVATGQPEMVKGEW KPGAI VI DCGI
NP_001076946.1.pro	LVVAAGQPEMVKGEW KPGAI VI DCGI
NP_620084.2.pro	LVVATGQPEMVKGEW KPGAVVI DCGI
NP_071953.1.pro	LVVATGQPEMVKGEW KPGAVVI DCGI
NP_001034392.1.pro	LVVAAGKAEMVKGEW KPGAI VI DCGI
NP_955823.1.pro	LVTGI GKPEMVHGDWLKTGAEVI DCGI
NP_731489.2.pro	LVVGI GVAEMVKGSW KPGAVVI DCGI
XP_312083.5.pro	LVVAVGRPEMVRGAW KPGAVVI DCGI
NP_595256.1.pro	VVAAI GI PHFVKADWLKKGVVAI DVGI
XP_956550.1.pro	VVAAI GQPNFVKGEWLKKGAVVI DVGT

+ EVELYGETKAK	
EVELYGETKAK	. . . E. . . YG. . KAK. . L. . . . RL. . . . . . .
Majority	L SEEVELYGETKAKVLLSALERLKHQPDGK
NP_005947.3.pro	L SEEVELYGETKAKVLLSALERLKHRPDGK
XP_510001.3.pro	L SEEVELYGETKAKVLLSALERLKHRPDGK
XP_001101708.2.pro	L SEEVELYGETKAKVLLSALERLRHRPDGK
XP_537476.2.pro	L SEEVELYGDTKAKVLLSALERLKHQPDGK
NP_001076946.1.pro	RTEEVELYGETKAKVLLSALERLKHQPDGK
NP_620084.2.pro	L TEEVELYGETKAKVLLSALDRLKHQPDGK
NP_071953.1.pro	L TEEVELYGETKAKVLLSALDRLKHQPDGK
NP_001034392.1.pro	L PDEI ELYGQTKAKVHLSALKRLKNQPDGK
NP_955823.1.pro	L SDEVELYGRTKAKVQLKTI DRLQTQL DGK
NP_731489.2.pro	EAREVSLYGNKKAKI SLSVLERLKDKEVGH
XP_312083.5.pro	I PSEVSLYGTKKAKVSLKVLQRLQYEDDAK
NP_595256.1.pro	AKSELEFYGSHKAKVNLEI LQRLAHRRDGH
XP_956550.1.pro	APSELEPYGAYKAKVDLSI LKRLEHRRNGR

+ SFQLLYDLKLP	
SFQLLYDLKLP	. . A. . . . . LY. . . . .
Majority	QRAAQ- APSSFQ- LLYDLK- LPVEDKI RI
NP_005947.3.pro	QRAAQ- APSSFQ- LLYDLK- LPVEDKI RI
XP_510001.3.pro	QRAAQ- APSSFQ- LLYDLK- LPVEDKI RI
XP_001101708.2.pro	QRAAQ- APSSFQ- LLYDLK- LPVEDKI RI
XP_537476.2.pro	QRAAQ- APSSFQ- LLYDLK- LPVEDKI RI
NP_001076946.1.pro	QRAAA- APSSFQ- LLYDLK- LPVEDKI RI
NP_620084.2.pro	QRASQ- APSSFQ- LLYDLK- LSI EDKI RI
NP_071953.1.pro	QRASQ- APSSFQ- LLYDLK- LSVEDKI RI
NP_001034392.1.pro	QRASQ- TPSNFR- FLYNVE- LPVI DKI RI
NP_955823.1.pro	QRAAD- LPNSFQ- FLYDVQ- LPI ADKI RI
NP_731489.2.pro	I KACE- QGNQFR- LLYDLE- LPLVDKMNK
XP_312083.5.pro	I DACN- RPSEFK- LLYDLD- MSI EDKI VR
NP_595256.1.pro	I NACENVDSEFR- LLYDVH- EPI EKKI EI
XP_956550.1.pro	I SASEKSTPETQKLLYEVEGNTVQERI EK

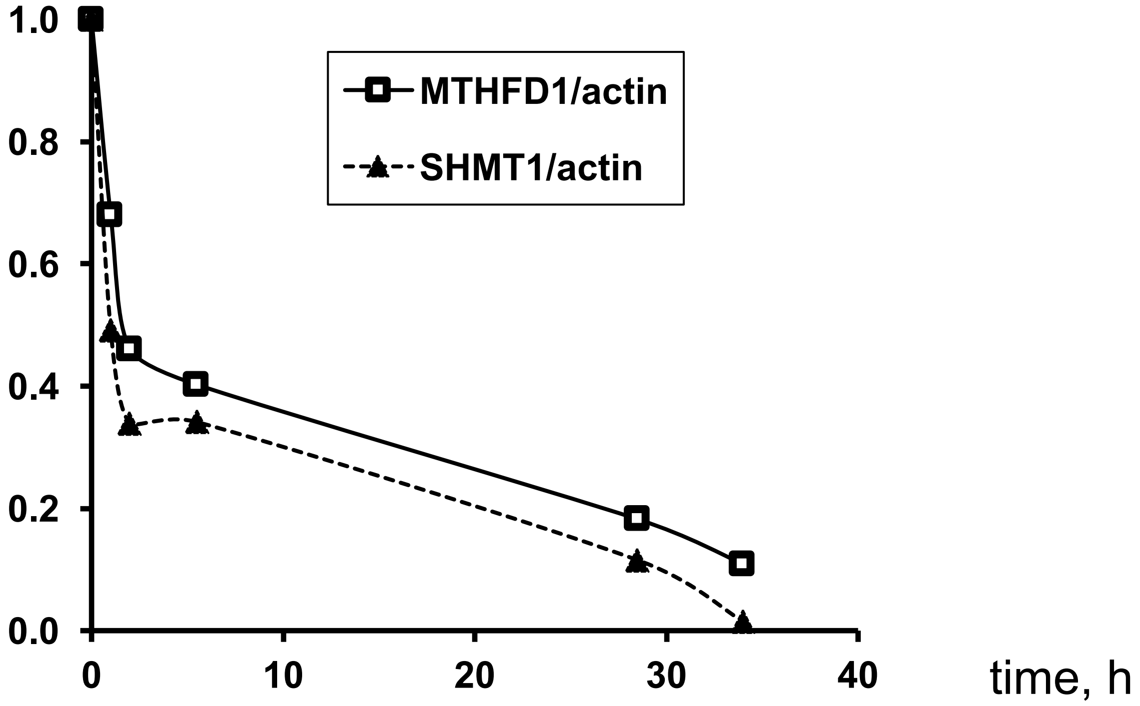
Supplementary Figure 1.

(A) SUMOylation consensus VKGE (corresponding to human MTHFD1 aa 222-227) is highly conserved in MTHFD1 proteins. Accession IDs for the species aligned are: H.sapiens NP_005947.3, P.troglodytes XP_510001.3, M.mulatta XP_001101708.2, C.lupus XP_537476.2, B.Taurus NP_001076946.1, M.musculus NP_620084.2, R.norvegicus NP_071953.1, G.gallus NP_001034392.1, D.rerio NP_955823.1, D.melanogaster NP_731489.2, A.gambiae XP_312083.5, S.pombe NP_595256.1, N.crassa XP_956550.1. Megalign (DNAStar, Lasergene) package was used to align sequences using Clustal W method with default settings. (B) Reverse SUMOylation consensus EVELYGETKAK demonstrated a lower degree of conservation across species. C. Reverse SUMOylation consensus SRQLLYDLKLP is not conserved in 6 out of 13 (46%) MTHFD1 proteins.



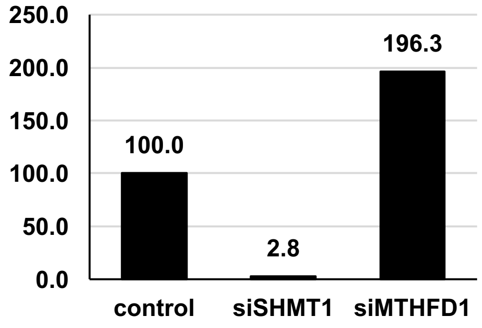
Supplementary Figure 2.
A. SUMO-interacting motif (SIM) AVLALTTS (corresponding to human MTHFD1 aa 571-578) is conserved in MTHFD1 proteins. Accession IDs for the species aligned are: H.sapiens NP_005947.3, P.troglodytes XP_510001.3, M.mulatta XP_001101708.2, C.lupus XP_537476.2, B.Taurus NP_001076946.1, M.musculus NP_620084.2, R.norvegicus NP_071953.1, G.gallus NP_001034392.1, D.rerio NP_955823.1, D.melanogaster NP_731489.2, A.gambiae XP_312083.5, S.pombe NP_595256.1, N.crassa XP_956550.1. B. SUMO-interacting motif VVVLVAT (human MTHFD1 694-700) demonstrated a high degree of conservation across species. C. SUMO-interacting motif ENLELVE (human MTHFD1 726-732) is not conserved across species.

A. Densitometry data for Fig. 4A

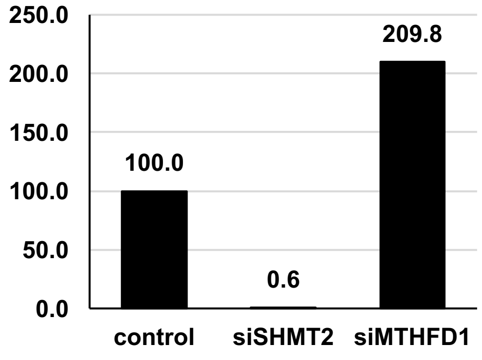


B. Densitometry data for Fig. 5C

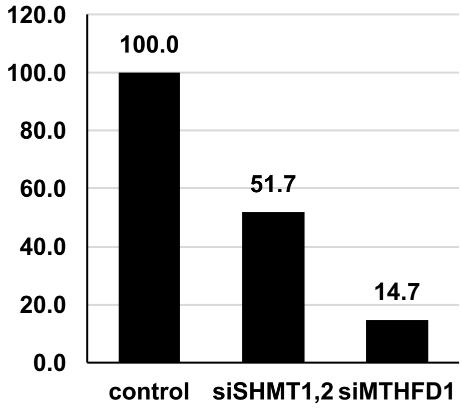
SHMT1/actin levels



SHMT2/actin levels



MTHFD1/actin levels



Supplementary Figure 3.
(A) Densitometry data for Fig. 4A
(B) Densitometry data for Fig. 5C