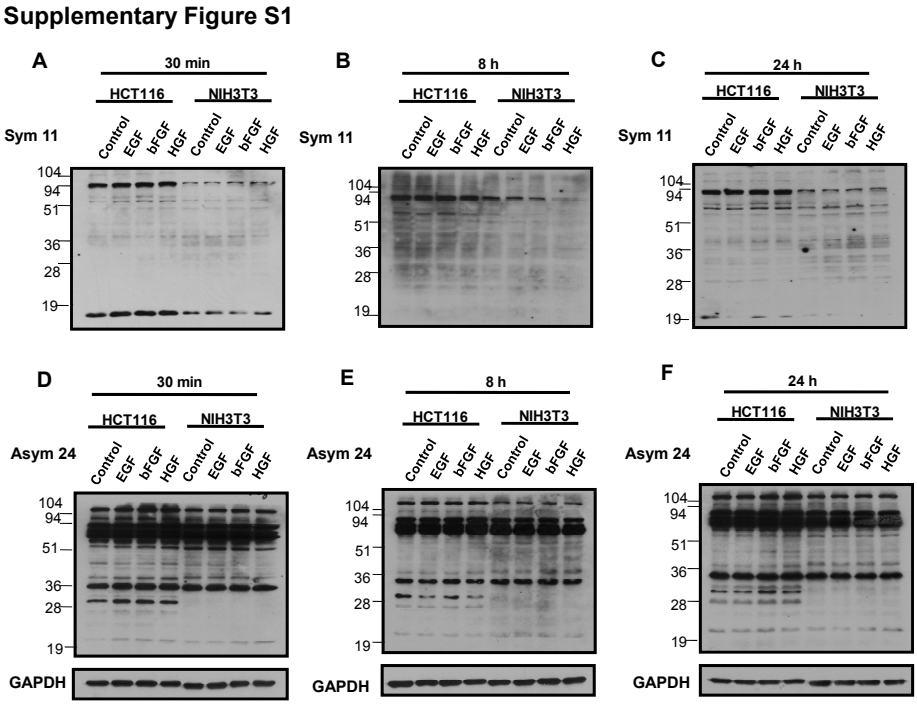
Supplemental data

Jung *et al*., Methylation of eukaryotic elongation factor 2 induced by basic fibroblast growth factor via MAPK

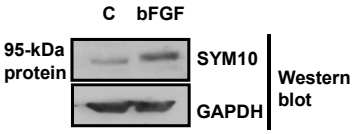


Supplementary Figure S1. No significant change in protein arginine methylation patterns as detected by SYM11 and ASYM24 was induced by growth factors such as EGF, bFGF and HGF in NIH3T3 and HCT116 cells.

Symmetrically (A-C) and asymmetrically (D-F) dimethylated arginines on proteins in cell lysates obtained 30 min (A, D), 8 h (B, E), 24 h (C, F) after the administration of growth factors were detected by Western blots using SYM11 for symmetric dimethylation of arginine or ASYM24 for asymmetric dimethylation of arginine. Cells were cultured in the absence or presence of EGF, bFGF, or HGF and cell lysates were prepared at indicated time points. Western blot analysis was performed with SYM11 and ASYM24, respectively.

GAPDH was used as a loading control. Protein bands were visualized using enhanced chemiluminescence.

Supplementary Table S1
Identification of the 95-kDa protein by mass spectromethy in which symmetric dimethylation of arginine was induced by bFGF as detected by SYM10 .



Sample	Protein name	NCBI accession number	kDa/pI	Z score	MS analysis % coverage
Control 95-kDa protein	EE eukaryotic translation elongation factor 2	gi|26328763|dbj|BAC28120.1	95.24/6.5	1.40	23
bFGF-treated 95-kDa protein	EE eukaryotic translation elongation factor	gi|33859482|ref|NP_031933.1	95.30/6.4	1.56	29

Supplementary Table S2
Sequence coverage of peptides identified by mass spectrometry of eEF2

1 MVNFTVDQIR AIMDKKANIR NMSVIAHVDH GKSTLTDSLV CKAGIIASAR
51 AGETRFTDTR KDEQERCITI KSTAISLFYE LSENDLNFIK QSKDGSGFLI
101 NLIDSPGHVD FSSEVTAALR VTDGALVVVD CVSGVCVQTE TVLRQAIAER
151 IKPVLMMNKM DRALLELQLE PEELYQTFQR IVENVNVIIS TYGEGESGPM
201 GNIMIDPVLG TVGFGSGLHG WAFTLKQFAE MYVAKFAAKG EGQLSAAERA
251 KKVEDMMKKL WGDRYFDPAN GKFSKSANSP DGKKLPRTFC QLILDPIFKV
301 FDAIMNFRKE ETAKLIEKLD IKLDSEDKDK EGKPLLKAVM RRWLPAGDAL
351 LQMITIHLPS PVTAQKYRCE LLYEGPPDDE AAMGIKSCDP KGPLMMYISK
401 MVPTSDKGRF YAFGRVFSGV VSTGLKVRIM GPNYTPGKKE DLYLKPIQRT
451 ILMMGRYVEP IEDVPCGNIV GLVGVDQFLV KTGTITTFEH AHNMRVMKFS
501 VSPVVRVAVE AKNPADLPKL VEGLKRLAKS DPMVQCIIEE SGEHIIAGAG
551 ELHLEICLKD LEEDHACIPI KKSDPVVSYR ETVSEESNVL CLSKSPNKHN
601 RLYMKARPFP DGLAEDIDKG EVSARQELKA RARYLAEKYE WDVAEARKIW
651 CFGPDGTGPN ILTDITKGVQ YLNEIKDSVV AGFQWATKEG ALCEENMRGV
701 RFDVHDVTLH ADAIHRGGGQ IIPTARRCLY ASVLTAQPRL MEPIYLVEIQ
751 CPEQVVGGIY GVLNRKRGHV FEESQVAGTP MFVVKAYLPV NESFGFTADL
801 RSNTGGQAFP QCVFDHWQIL PGDPFDNSSR PSQVVAETRK RKGLKEGIPA
851 LDNFLDKL

1. Sequence Coverage: **81%**
2. Matched peptides shown in **Bold Red**

Supplementary Table S3
Validation of siRNA inhibition of PRMT5 and PRMT7 mRNA expression in NIH3T3 cells by real-time quantitative PCR

siRNA transfected	primer	Reduction(%)
PRMT5 siRNA	PRMT5	71
PRMT7 siRNA	PRMT7	46
PRMT5+PRMT7 siRNA	PRMT5	35
	PRMT7	34

* Actin was used for normalization

Materials and methods

MS analysis

Analysis of arginine methylation was performed as previously described (Kirino *et al*., 2010). A 95-kDa arginine-methylated protein gel band was excised from SDS-PAGE gel. The protein band was subjected to reduction in 10 mM dithiothreitol for 45 min at 37℃ and alkylation in 55 mM iodoacetamide for 15 min at room temperature in the dark and was digested overnight at 37℃ with 12.5 ng/μl trypsin (Promega, Madison, WI, USA). The digested peptides were acidified to 0.1% trifluoroacetic acid and desalted onto C18 Ziptips (Millipore, Billerica, MA, USA) according to the manufacturer′s instruction. The desalted peptides were analyzed in an LCQ Deca mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled online to an Agilent 1100 HPLC system. An in-house pulled 0.075 x 130 mm emitter column was prepared by packing with

Zorbax 300SB-C18, 5 μm (Agilent, Santa Clara, CA, USA) using a slurry packer (Alltech, Lexington, Ky, USA). Peptides were separated with a linear acetonitrile gradient: 0 % solvent A (5% acetonitrile, 0.1% formic acid) to 40% solvent B (95% acetonitrile, 0.1% formic) over 80 min and then 90% solvent B in 15 min at a flow rate of 0.2 μL/min. For tandem mass spectrometry, MS and MS/MS data were obtained in a data-dependent mode. The full mass scan range mode was m/z = 250 − 2000 Da for the selection of a precursor ion. An exclusion dynamic mode was applied to exclude the most intense ion from further selection over a 2-min period. The subsequent MS/MS data were acquired using a 2 m/z unit ion isolation window in the automated gain control (AGC) mode where AGC values of 5.00e+05 and 1.00e+04 were set for full MS and MS/MS, respectively. The normalized CID was set at 35.0.

Reference

Kirino Y, Vourekas A, Kim N, de Lima Alves F, Rappsilber J, Klein PS, Jongens TA and Mourelatos Z. Arginine methylation of vasa protein is conserved across phyla. J Biol Chem 2010;285:8148-54