Supplementary Methods:

LC-MS/MS analyses

Peptides from each immuno-affinity enrichment were analyzed by multidimensional RP-SAX-RP MS/MS (1) with the following conditions: peptides were serially eluted from the first and second dimension columns with the following buffers:

Acetonitrile (%)	Ammonium formate pH10 (mM)
12.5	250; 675
15	250; 675
20	250; 675
30	250; 675

The first and second dimension columns consisted of a 360 × 150 μm × 6 cm fused silica capillary packed with XBridge 5 μm beads and a 360 × 150 μm × 6 cm fused silica capillary packed with Poros 10HQ media, respectively. Eluting peptides were diluted online with a 10-fold excess of buffer A (0.2 M acetic acid in water) and automatically loaded onto a pre-column (360 × 150 μm × 6 cm fused silica capillary packed with Poros 10R2 media). Peptides were finally eluted with an organic gradient (NanoAcquity UPLC system, Waters, 5% to 35% B in 240 minutes, A = 0.2 M acetic acid in water, B = 0.2 M acetic acid in acetonitrile), resolved on an analytical column with an integrated 1 μm emitter tip (360 × 30 μm × 50 cm fused silica capillary packed with 5 μm Monitor C18) and electrosprayed into the mass spectrometer (Orbitrap Fusion; Thermo, ESI voltage = 2.8 kV). A digital PicoView platform (New Objective) was used to automatically position the emitter tip in spray or wash positions over the course of the experiment. The mass spectrometer was programmed to perform data-dependent MS/MS on the 15 most abundant precursors in each MS1 scan with the following parameters:

MS/MS acquisition parameters

	MS1	MS2	
Detector	Orbitrap	Ion Trap	Orbitrap
Dissociation	-	CAD	HCD
Acquisition type	Centroid	Centroid	Centroid
Resolution	120,000	-	15,000
Quadrupole isolation use	No	Yes	Yes
Isolation width (Da)	-	1.6	1.6
Excluded charge states	1, > 8, unknown	-	-
Precursor selection range	300-2000	-	-
Precursor selection threshold	-	5,000	5,000
Dynamic exclusion (sec.), after (count)	60, 1	-	-
Max injection time (ms)	50	25	50
Target number (ion counts)	500,000	5,000	50,000
Collision Energy (%)	-	35	30

Peptides in the supernatant from the last immuno-affinity purification were analyzed by multidimensional LC-MS/MS analysis as described above. The fractions consisted of the following buffers:

Acetonitrile (%)	Ammonium formate pH10 (mM)
10	100; 230; 675
12	100; 230; 675
14	100; 230; 675
16	100; 230; 675
18	100; 230; 675
20	100; 230; 675
25	675
40	400
90	*900
	* containing 10% acetic acid

The first and second dimension columns consisted of a 360 × 200 μm × 20 cm fused silica capillary packed with XBridge 5 μm beads and a 360 × 150 μm × 15 cm fused silica capillary packed with Poros 10HQ media, respectively. The analytical column and analytical chromatography gradient were as described above. Peptides were electrosprayed into the mass spectrometer (QExactive HF, Thermo ESI voltage = 3.8 kV). The mass spectrometer was programmed to perform data-dependent MS/MS on the 10 most abundant precursors in each MS1 scan with the following parameters:

MS/MS acquisition parameters

	MS1	MS2
Detector	Orbitrap	Orbitrap
Dissociation	-	HCD
Acquisition type	Profile	Profile
Resolution	240,000	15,000
Isolation width (Da)	-	1.5
Excluded charge states	1, > 8, unknown	-
Precursor selection range	300-2000	-
Underfill ratio (%)	-	5
Dynamic exclusion (sec.), after (count)	15, 1	-
Max injection time (ms)	100	50
Target number (ion counts)	1,000,000	100,000
Normalized collision energy	-	30
Peptide match	Preferred	-
Exclude isotopes	On	-
Lock mass (m/z, +/- 10 ppm)	445.12003	-

Peptides from enriched Eif4g1 were analyzed by multidimensional LC-MS/MS as described for the methylated peptides. Fractions consisted of the following buffers:

Acetonitrile (%)	Ammonium Formate pH10 (mM)
12.5	700
15	250; 700
20	250; 700
25	250; 700
40	250
90	*900
	* containing 10% acetic acid

The first and second dimension columns and the analytical column were exactly as described for the methylated peptides. Peptides were electrosprayed into the mass spectrometer (QExactive HF, Thermo, ESI voltage = 3.8 kV). The mass spectrometer was programmed to target the heavy isotope of the R4-monomethylated sequence: GPPRGGPGGELPR at m/z: 427.57.

Reference peptides were synthesized by the TUFTS University Core Facility. Synthetic peptides (1 pmole/µL in 50% acetonitrile, 0.1 M Acetic acid) were electrosprayed into the Orbitrap Fusion or the QExactive mass spectrometers at a voltage of 4 kV and a flow rate of 4 µL/min. MS2 spectra were recorded in the Orbitrap following fragmentation in the HCD cell.

Data processing and analysis

Spectra were converted into a Mascot generic file format (mgf) using multiplierz scripts (2). MS1 spectra acquired on the Orbitrap Fusion mass spectrometer were recalibrated using the background ion $(Si(CH_3)_2O)_6$ at m/z 445.12 ± 0.03. HCD and CAD (where applicable) spectra were searched separately using the Mascot search algorithm (search parameters are listed below).

Mascot search parameters

	Orbitrap Fusion	QExactive
Mascot version	2.4.1	
Precursor Search tolerance (ppm)	10	
Fragment ion tolerance (Da)	0.6 (CAD), 0.02 (HCD)	0.02
Databases (with reversed sequences)	Mouse RefSeq (2011/07/11), Lab contaminants	
Maximum missed cleavages	2	
Fixed modifications	Carbamidomethyl (C)	
Variable modifications	Oxidation (M), Methyl (R), Dimethyl (R)	Oxidation (M)
Quantitation modifications	SILAC K+6 R+10	

Peptide spectrum matches (psms) to reversed database sequences were used to limit psm-level false discovery rate to 1%. We used custom multiplierz/python scripts to calculate light and heavy isotope precursor intensities within overlapping chromatographic features. Averaged local noise intensities around calculated m/z values were used for psms with a missing SILAC

isotopic feature. Ratios for arginine-methylated sites were averaged across two independent biological replicates with permuted SILAC labeling scheme for the control and Prmt1 shRNAs conditions. These ratios were normalized to correct for changes in expression of protein substrates based on relative abundances measured in the supernatant from the last immuno-affinity purification. psms associated with a single SILAC channel were excluded from the protein ratio calculation if they were detected in the other biological replicate with the same single SILAC isotopic composition. Methylated peptides mapping to proteins not detected or not quantified in the supernatant are not reported. All methylated arginine sites were re-mapped to UniProt accessions during the quantification stage.

References

1. Wang LD, Ficarro SB, Hutchinson JN, Csepanyi-Komi R, Nguyen PT, Wisniewski E, et al. Phosphoproteomic profiling of mouse primary HSPCs reveals new regulators of HSPC mobilization. Blood. 2016;128:1465–74.

2. Askenazi M, Parikh JR, Marto JA. mzAPI: a new strategy for efficiently sharing mass spectrometry data. Nat Methods. 2009;6:240–1.