

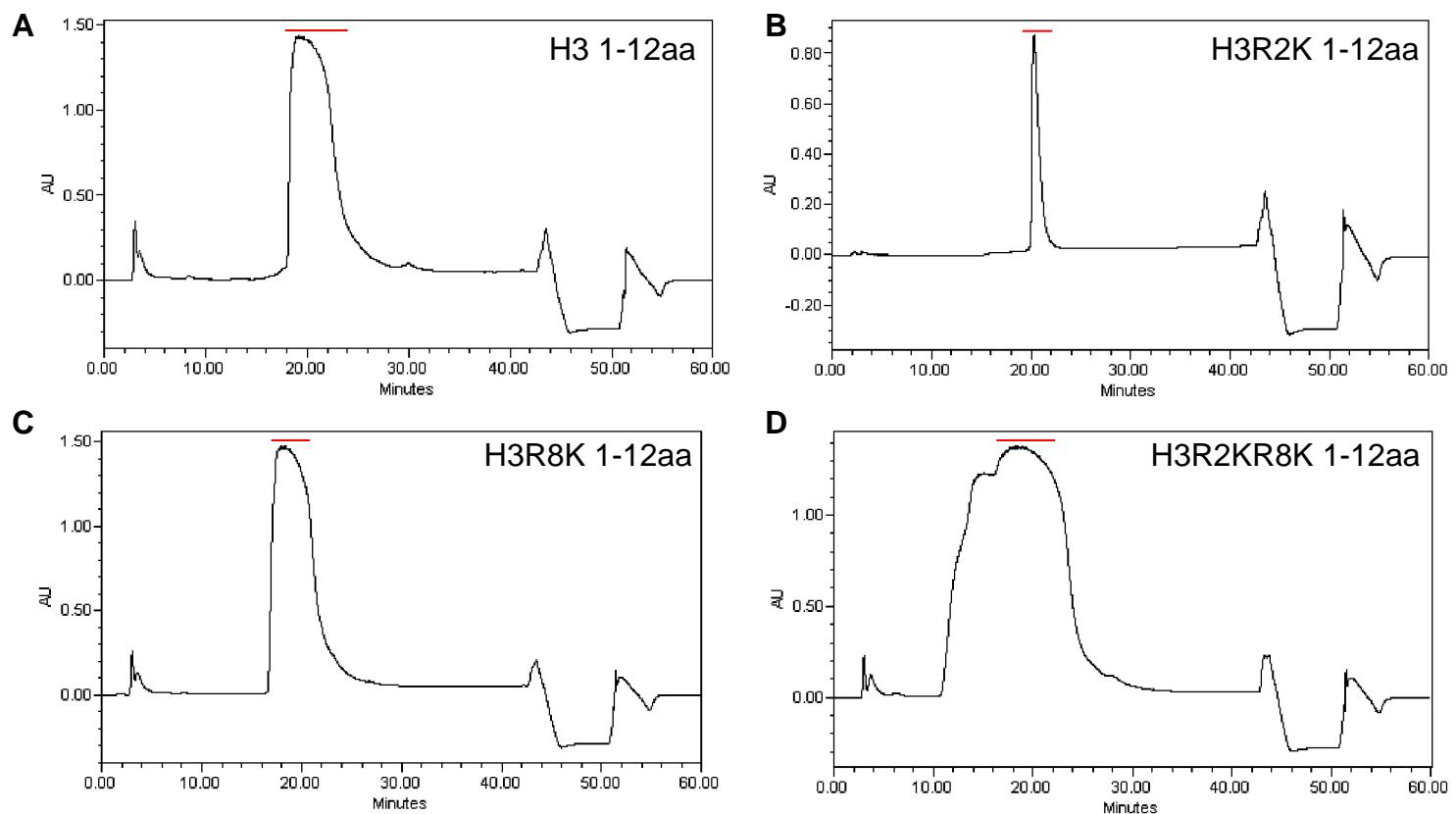
Supporting Information

## A binary arginine methylation switch on histone H3 Arginine 2 regulates its interaction with WDR5

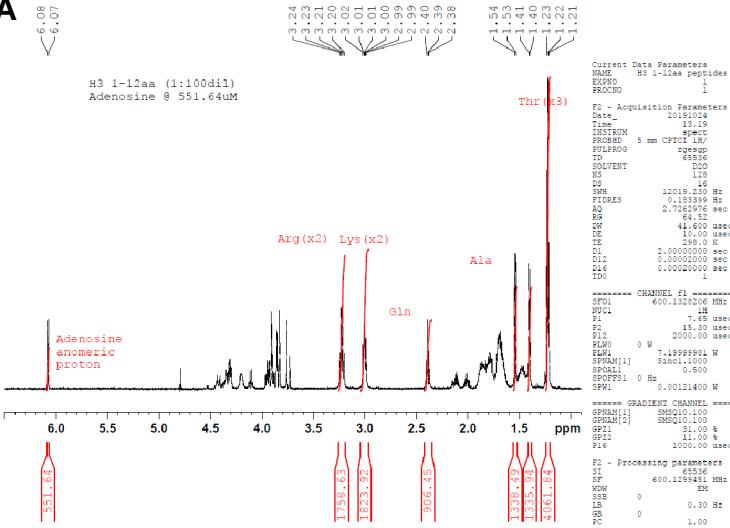
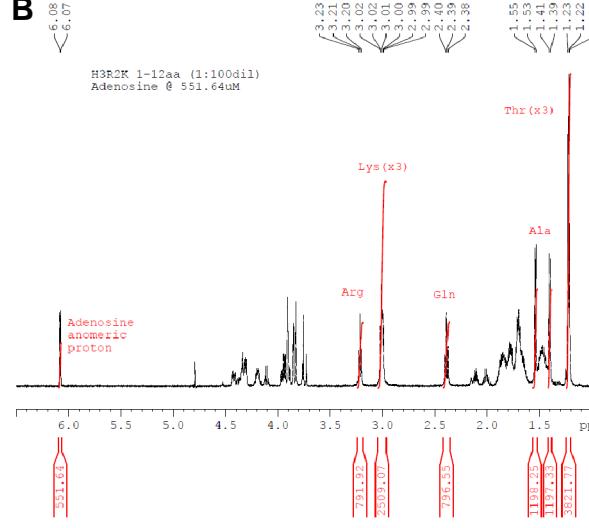
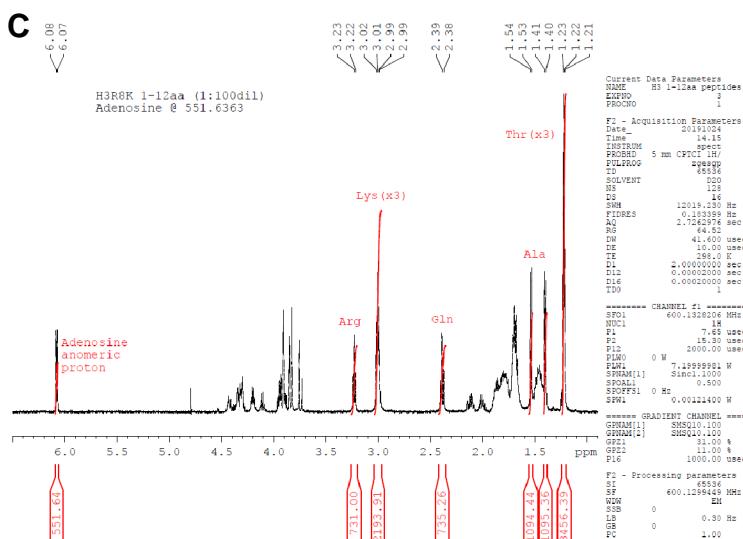
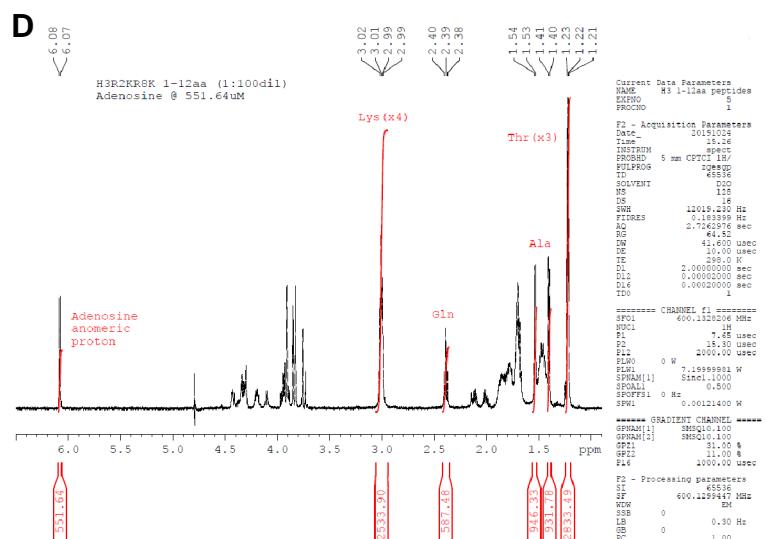
Benjamin M. Lorton, Rajesh K. Harijan, Emmanuel S. Burgos, Jeffrey B. Bonanno,  
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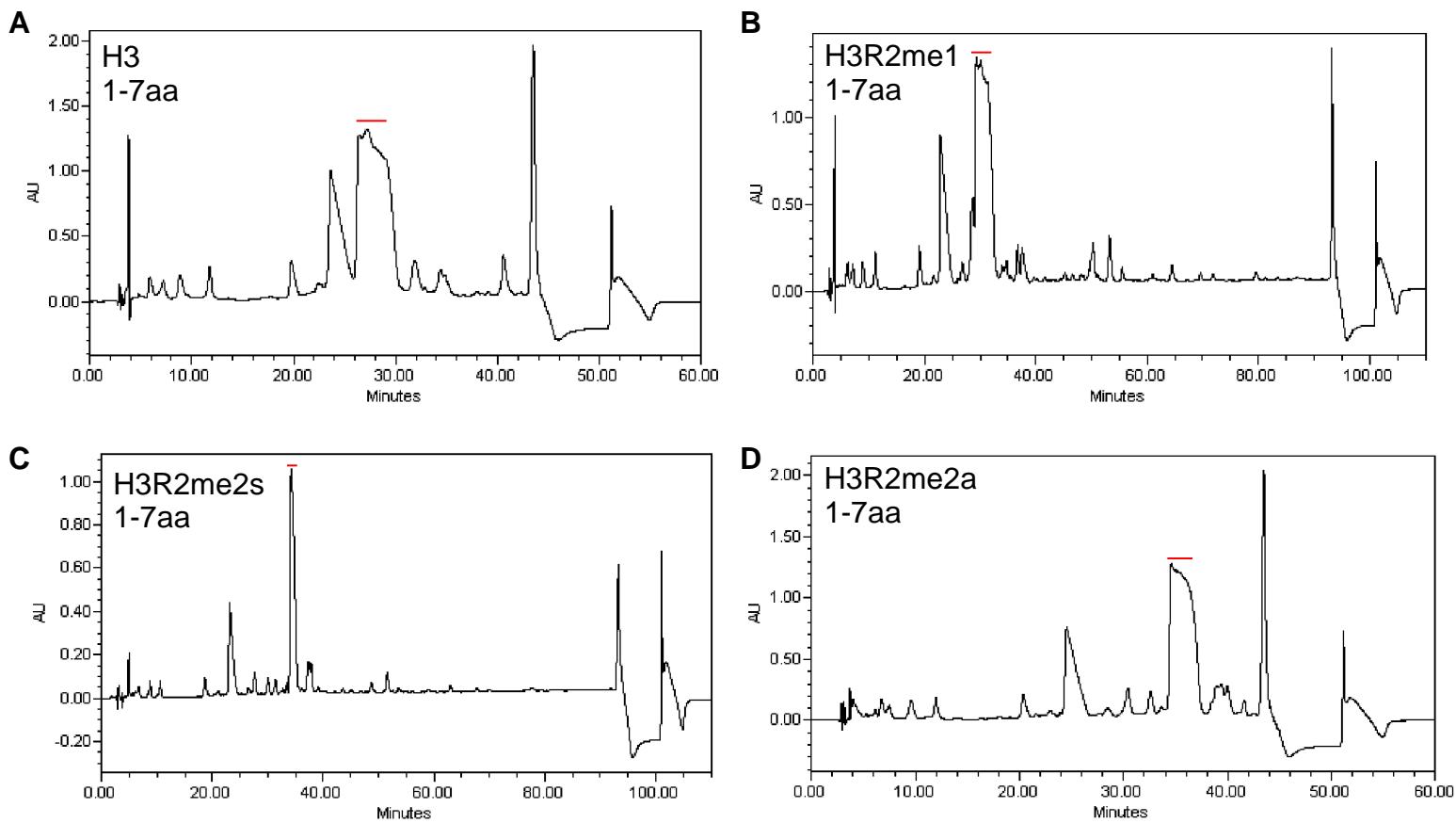
\*For correspondence: david.shechter@einsteinmed.org



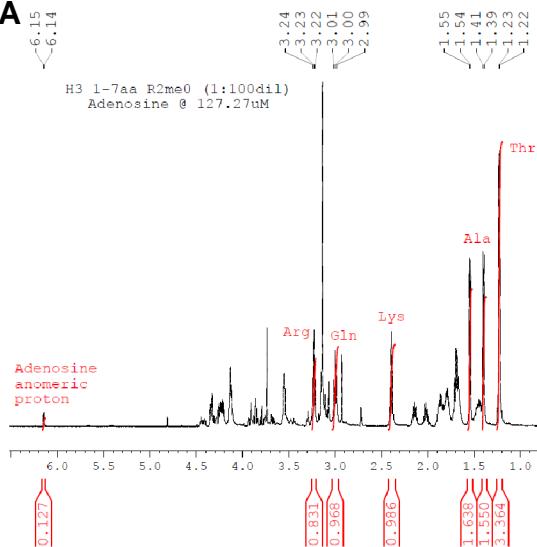
**Figure S1.** HPLC chromatograms at 205nm absorbance of H3 1-12aa peptides. Red bar indicates the collected fraction. **a.** H3 **b.** H3R2K **c.** H3R8K. **d.** H3R2KR8K

**A****B****C****D**

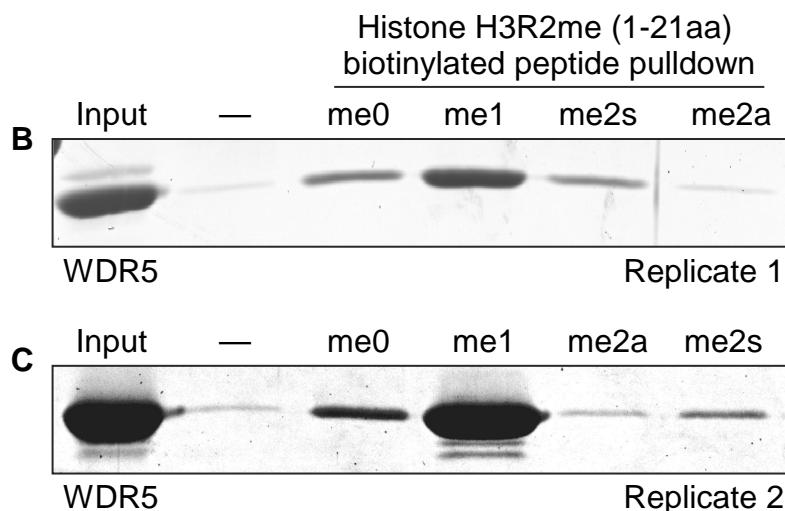
**Figure S2.** 1D  $^1\text{H}$  NMR spectra of H3 1-12aa peptides showing peak integrations of residues used to measure peptide concentration. **a.** H3 unmodified **b.** H3R2K **c.** H3R8K. **d.** H3R2KR8K



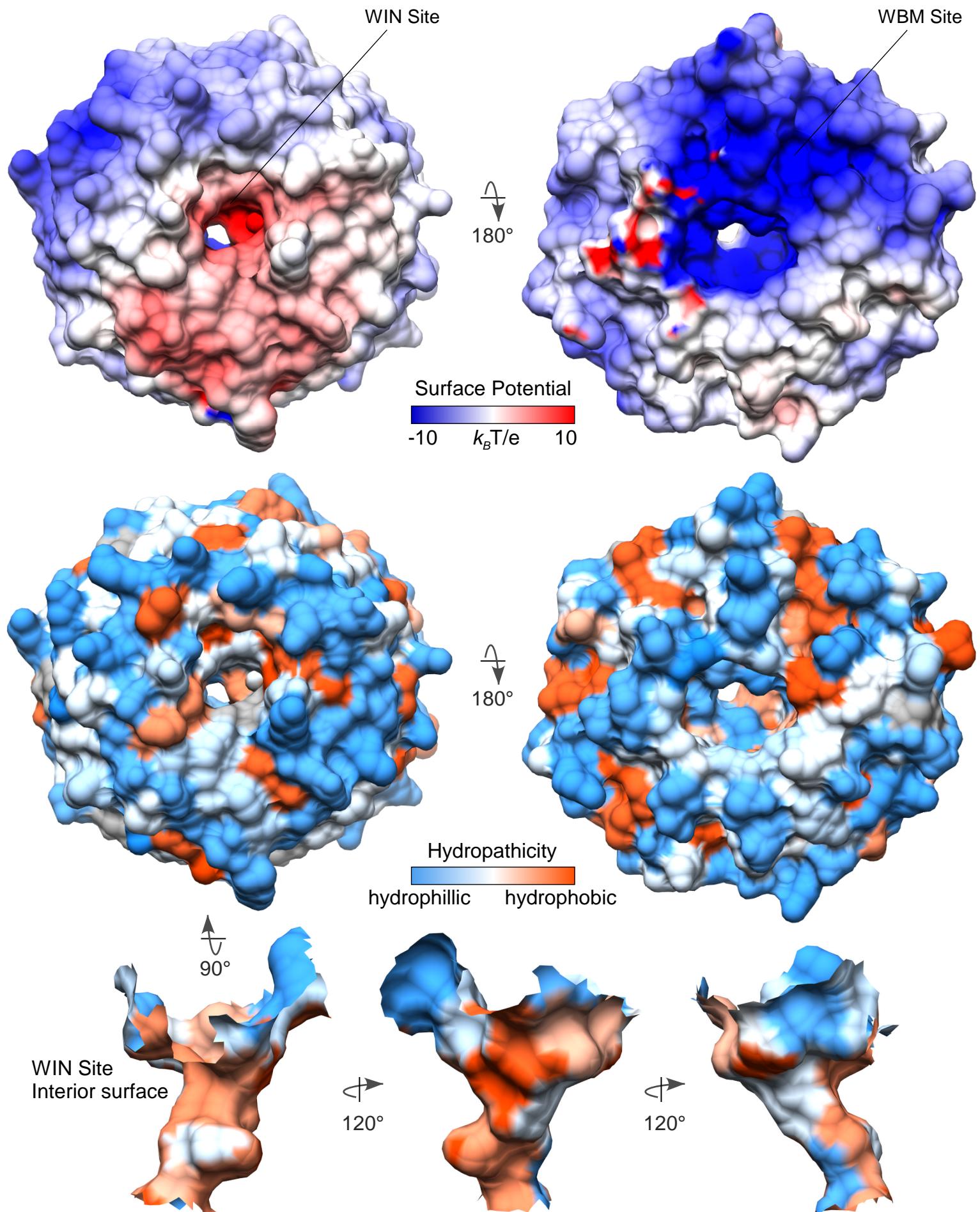
**Figure S3.** HPLC chromatograms at 205nm absorbance of H3 1-7aa peptides. Red bar indicates the collected fraction. **a.** H3 **b.** H3R2me1 **c.** H3R2me2s. **d.** H3R2me2a

**A**

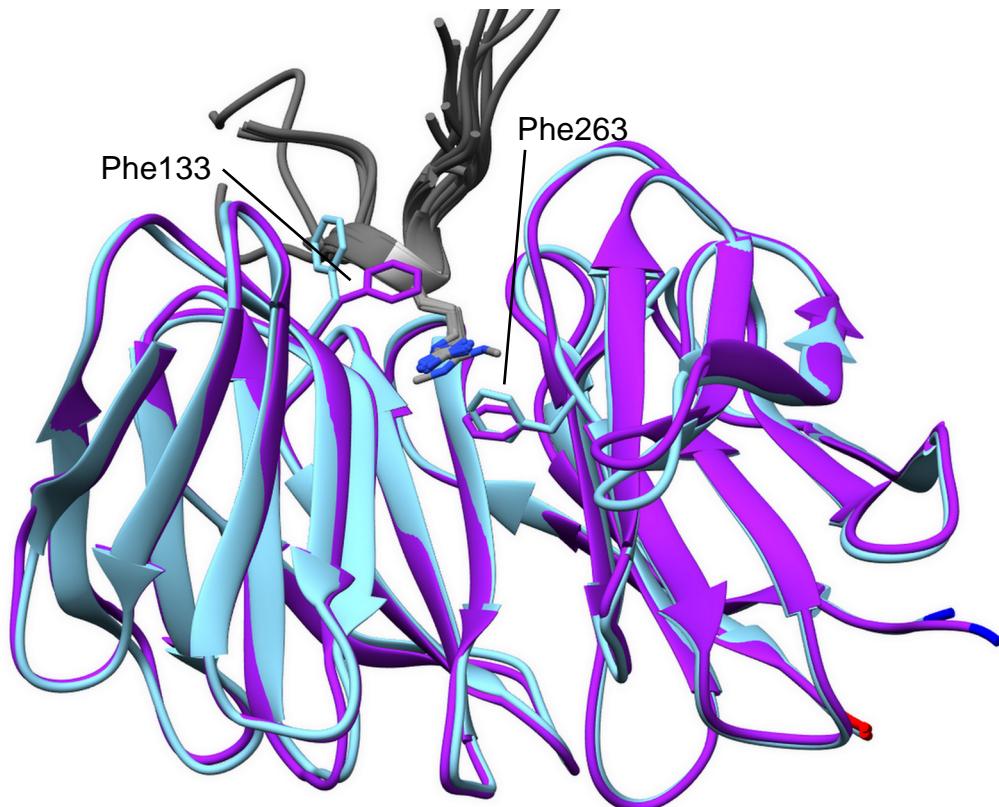
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S1293 16
S1294 16
S1295 16
S1296 16
S1297 16
S1298 16
S1299 16
S1300 16
S1301 16
S1302 16
S1303 16
S13
```

**A H3: NH<sub>2</sub>-ARTKQTARKSTGGKAPRKQLA(GGK-biotin)**

**Figure S5.** Replicate peptide pulldown assays showing WDR5 interacts with H3R2me0, me1, and me2s but not H3R2me2a. (-) negative control: no peptide, resin only. **a.** Histone H3 1-21aa peptide sequence with methylarginine isoforms occurring at Arg2, underlined. **b.** and **c.** Replicate pulldowns 1 and 2

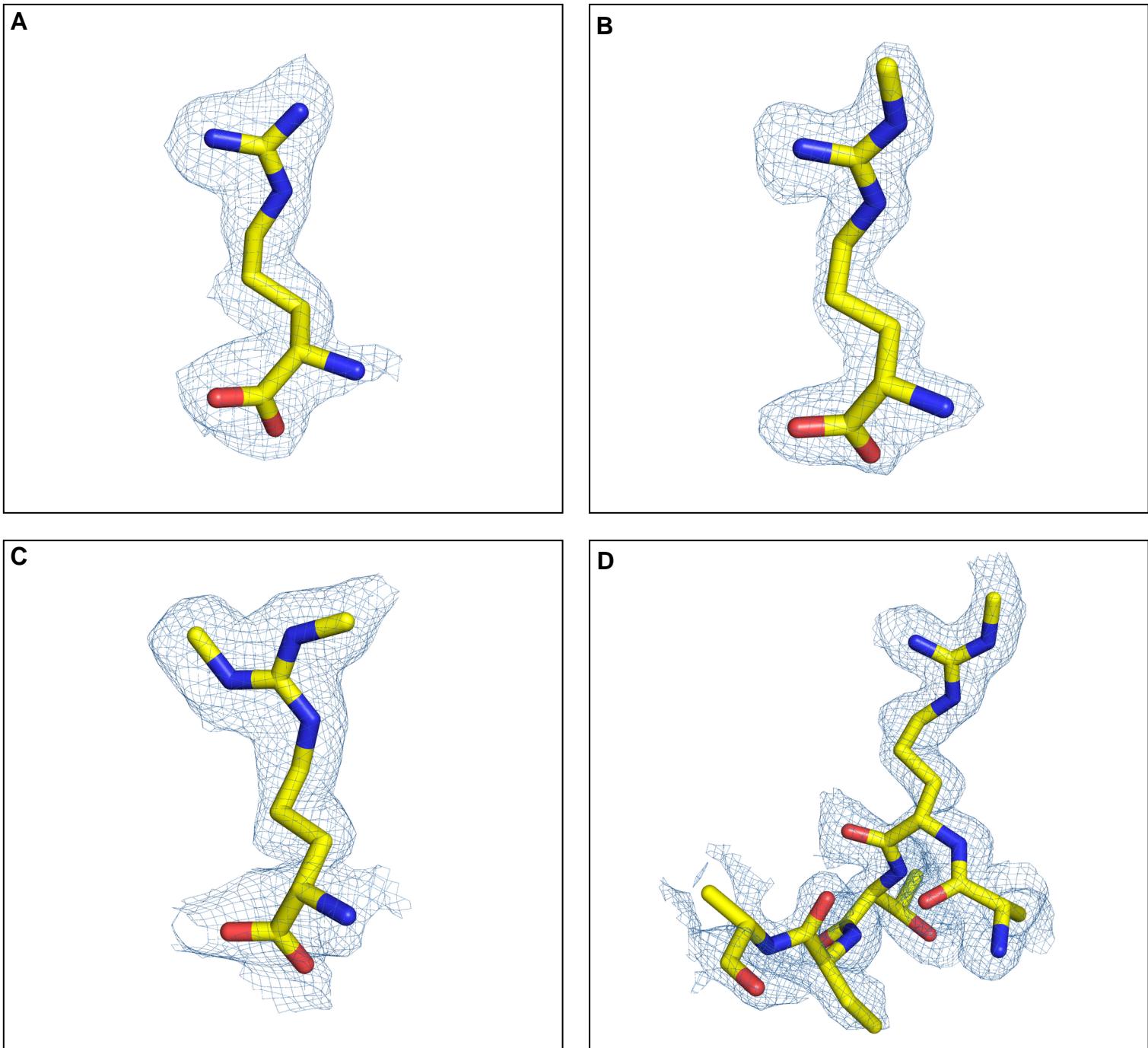


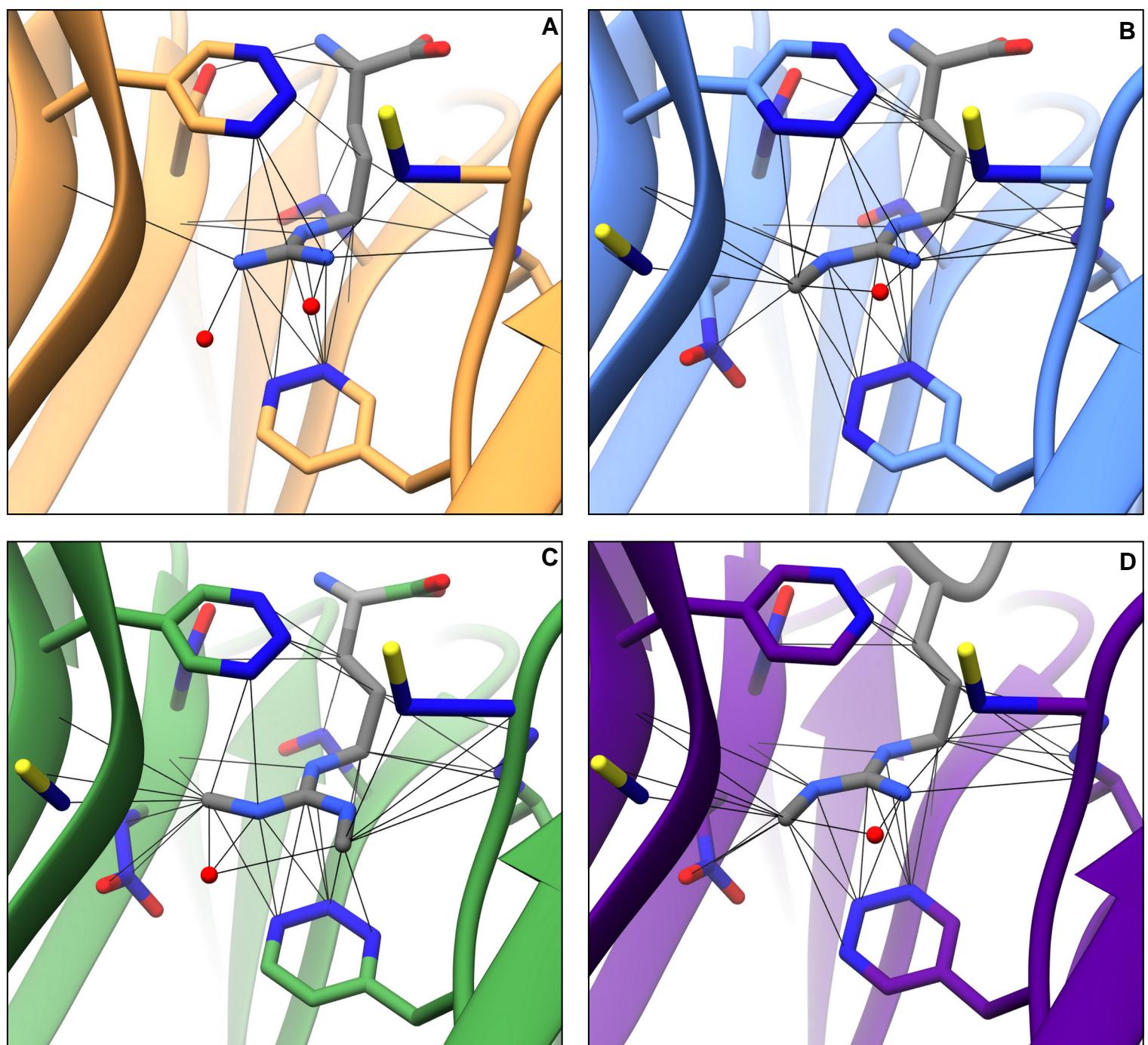
**Figure S6.** Electrostatic potential (top) and hydropathicity (bottom) surface representations of the WIN site



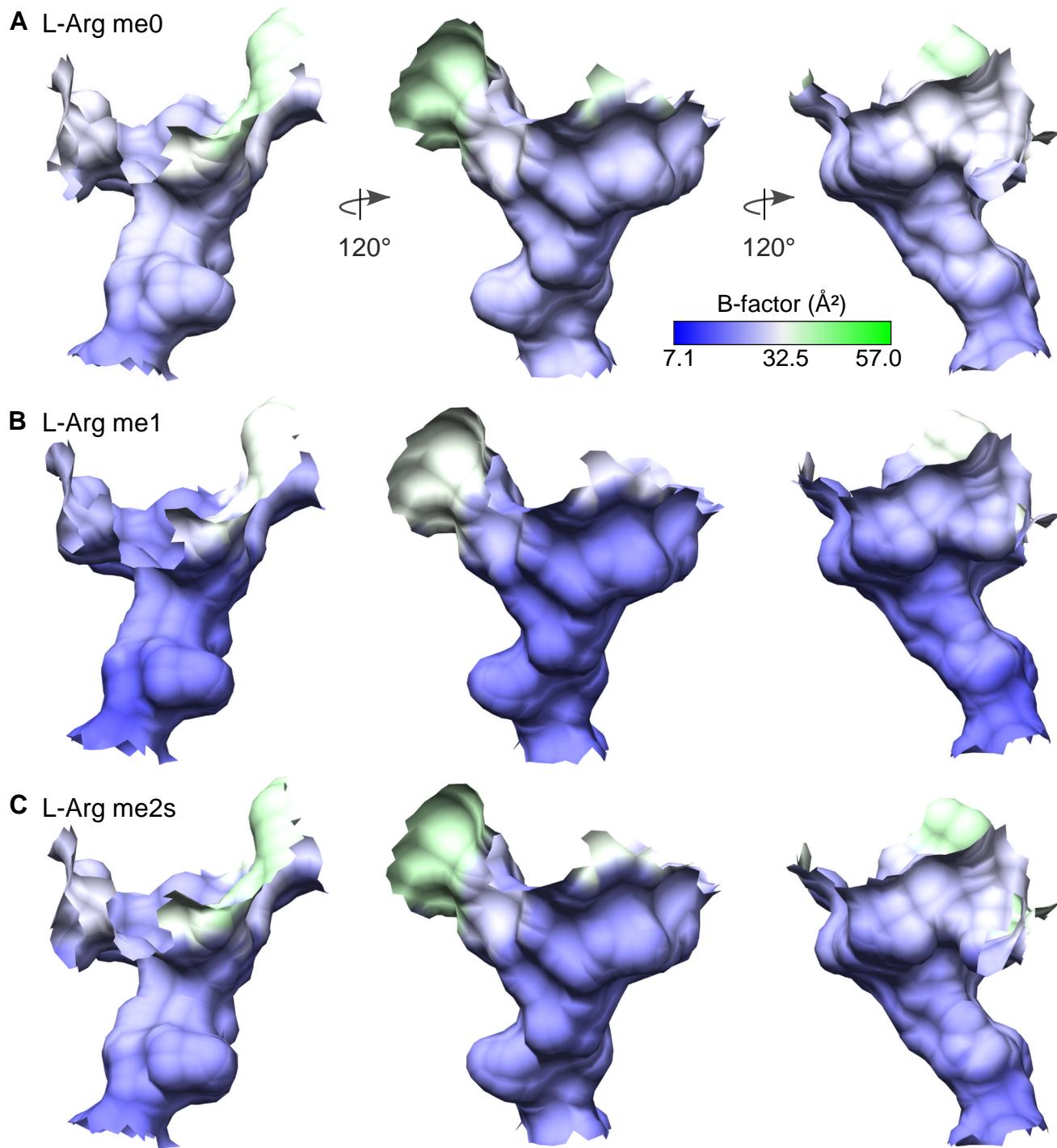
H3	<b>ARTK</b> TKQTA
MLL1	GS <b>ARAE</b> AEVHL
MLL2	GC <b>ARSE</b> SEP <span style="color: red;">KI</span>
MLL3	GS <b>ARAE</b> AEPKM
MLL4	GA <b>ARAE</b> AEVYL
SET1A	GS <b>ARSE</b> SEGGY
SET1B	GC <b>ARSE</b> SEGFY
SETD1A	GS <b>ARSE</b> SEGGY
SETD1B	GC <b>ARSE</b> SEGFY
KANSL1	VA <b>ARTR</b> TRPVL
NS1	KM <b>ARTA</b> TARSK

**Figure S7.** Superposition of apo-WDR5 (cyan) and WDR5 in complex (purple) with structurally characterized WIN site ligands (grey) showing common binding mode with the arginine sidechain guanidino group stacking between WDR5 residues Phe133 and Phe263. Ligand WIN motifs (bolded) are aligned.

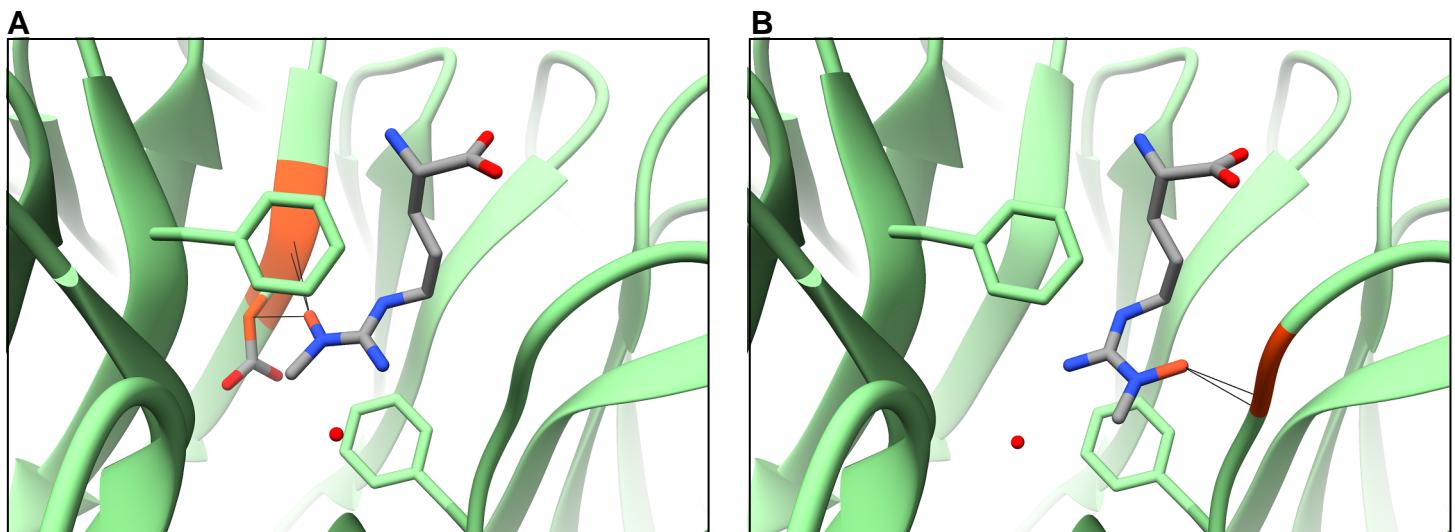
**Figure S6:** Ligand electron density**Figure S8.** Ligand density maps of **a.** L-Arg **b.** me1-L-Arg **c.** me2s-L-Arg and **d.** H3R2me1 peptide in complex with WDR5



**Figure S9.** Contacts within VDW distances depicted for **a.** L-Arg, **b.** L-Arg me1, **c.** L-Arg me2s, and **d.** H3R2me1 peptide ligands. Backbone contacts uncolored; contacts with WDR5 residue sidechain atoms are colored dark blue. VDW contacts between L-Arg me2s ωme' and S218 and between L-Arg me2s ωme' and F219 sidechain omitted for clarity. Difference in contacts summarized in Tables 2, S3. H<sub>2</sub>O (red sphere).



**Figure S10.** Surface representation of WIN site colored by B-factor showing no major differences when liganded to **a.** L-Arg, **b.** L-Arg me1, and **c.** L-Arg me2s.



**Figure S11.** Rme2a modeled in the WIN site depicting clashes that potentially inhibit binding. **a.** Rme2a modeled from Rme1 structure. Clashes with Ser91 backbone and Asp92 sidechain highlighted in orange. **b.** Rme2a modeled from Rme2s structure. Clashes with Cys261 backbone highlighted in orange.

**Supplementary Table 1.** Alignment of WIN site ligands in solved WDR5 structures

WIN Ligand	Amino Acid Sequence	PDB	Resolution (Å)	Citation
apo	—	2GNQ	1.80	[1]
apo	—	2H14	1.48	[2]
apo	—	2H68	1.79	[3]
H3	<b><u>ARTKQT</u></b> ARKSTGGKA	2CO0	2.25	[3]
H3	<b><u>ARTKQT</u></b> ARKS	2H13	1.58	[2]
H3	<b><u>ARTKQT</u></b> ARKST	2H9M	1.90	[1]
(ac)H3	<b>(ac)<u>ARTKQ</u></b>	3PSL	1.70	[4]
H3R2me2s	<b><u>MARTKQT</u></b> ARKSTGKA	4A7J	1.90	[5]
H3K4me1	<b><u>ARTKQT</u></b> TARK	2H6K	1.89	[3]
H3K4me2	<b><u>ARTKQT</u></b> TARK	2H6N	1.50	[3]
H3K4me2	<b><u>ARTKQ</u></b>	2CNX	2.10	[3]
H3K4me2	<b><u>ARTKQT</u></b> ARKST	2O9K	1.90	[1]
H3K4me2	<b><u>ARTKQT</u></b> ARKS	2G99	1.90	—
H3K4me2	<b><u>ARTKQT</u></b> ARKS	2G9A	2.70	—
H3K4me3	<b><u>ARTKQT</u></b> ARKST	2H9P	1.91	[1]
H3K4me3	<b><u>ARTKQT</u></b> TARK	2H6Q	1.87	[3]
MLL1	<b><u>GSARAEVH</u></b> LRKS	3EG6	1.72	[6]
MLL1	<b><u>ARAEVH</u></b> LRKSAFD	3EMH	1.37	[7]
MLL1	<b><u>EPPLNPHG</u></b> SARA <u>EVHLR</u>	4ESG	1.70	[8]
MLL2	<b><u>GCARSEP</u></b> KILT	3UVK	1.40	[9]
MLL2	<b><u>INPTGCARSEP</u></b> KIL	4ERQ	1.91	[8]
<i>rn</i> MLL3	<b><u>GSARAEP</u></b> KMSA	3UVL	2.20	[9]
MLL3	<b><u>VNPTGCARSEP</u></b> KMS	4ERY	1.30	[8]
MLL4	<b><u>GAARAEVY</u></b> LR	3UVM	1.57	[9]
MLL4	<b><u>LNP</u></b> GAARA <u>EVY</u> LR	4ERZ	1.75	[8]
SET1A	<b><u>GSARSEGYY</u></b> PI	3UVN	1.79	[9]
SET1B	<b><u>GCARSEGFY</u></b> TI	3UVO	2.20	[9]
SETd1a	<b><u>EHQTGSARSEGYY</u></b> YP	4EWR	1.50	[8]
SETd1b	<b><u>EHVTGCARSEGFY</u></b> T	4ES0	1.82	[8]
KANSL1	<b><u>DGT</u></b> CVAA <u>RT</u> RPVLSY	4CY1	1.50	[10]
NS1	PKQKRKM <b><u>ART</u></b> ARSKV	4O45	1.87	[11]

Bolded residues resolved in structural data with the interacting arginine residue underlined.  
Grey bar highlights WIN motif consensus sequence

**Supplementary Table 2.** WDR5 crystallization and crystal handling

<b>WDR5 crystals</b>	<b>Crystallization conditions</b>	<b>Soaking/Cryoprotectant Solution</b>	<b>Space group</b>	<b>PDB ID</b>
<b>WDR5-apo</b>	100 mM Bis-Tris pH5.9 32% PEG3350 54.6 mM Ammonium Sulfate	100 mM Bis-Tris pH5.9 32.5% PEG3350 54.6 mM Ammonium Sulfate 20% Glycerol	P2 <sub>1</sub> 2 <sub>1</sub> 2	6OFZ
<b>WDR5-Rme0</b>	100 mM Bis-Tris pH5.9 32% PEG3350 54.6 mM Ammonium Sulfate	100 mM Bis-Tris pH5.9 32.5% PEG3350 54.6 mM Ammonium Sulfate 20% Glycerol 2mM L-Arg	P2 <sub>1</sub> 2 <sub>1</sub> 2	6OI0
<b>WDR5-Rme1</b>	100 mM Bis-Tris pH5.9 32% PEG3350 54.6 mM Ammonium Sulfate	100 mM Bis-Tris pH5.9 32.5% PEG3350 54.6 mM Ammonium Sulfate 20% Glycerol 2mM L-Arg_me1	P2 <sub>1</sub> 2 <sub>1</sub> 2	6OI1
<b>WDR5-Rme2s</b>	100 mM Bis-Tris pH5.9 32% PEG3350 54.6 mM Ammonium Sulfate	100 mM Bis-Tris pH5.9 32.5% PEG3350 54.6 mM Ammonium Sulfate 20% Glycerol 2mM L-Arg_me2s	P2 <sub>1</sub> 2 <sub>1</sub> 2	6OI2
<b>WDR5-H3R2me1</b>	100 mM Bis-Tris pH5.9 32% PEG3350 54.6 mM Ammonium Sulfate	100 mM Bis-Tris pH5.9 32.5% PEG3350 54.6 mM Ammonium Sulfate 20% Glycerol 2mM H3R2me1 1-21aa peptide	C222 <sub>1</sub>	6OI3

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