Supporting Information

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

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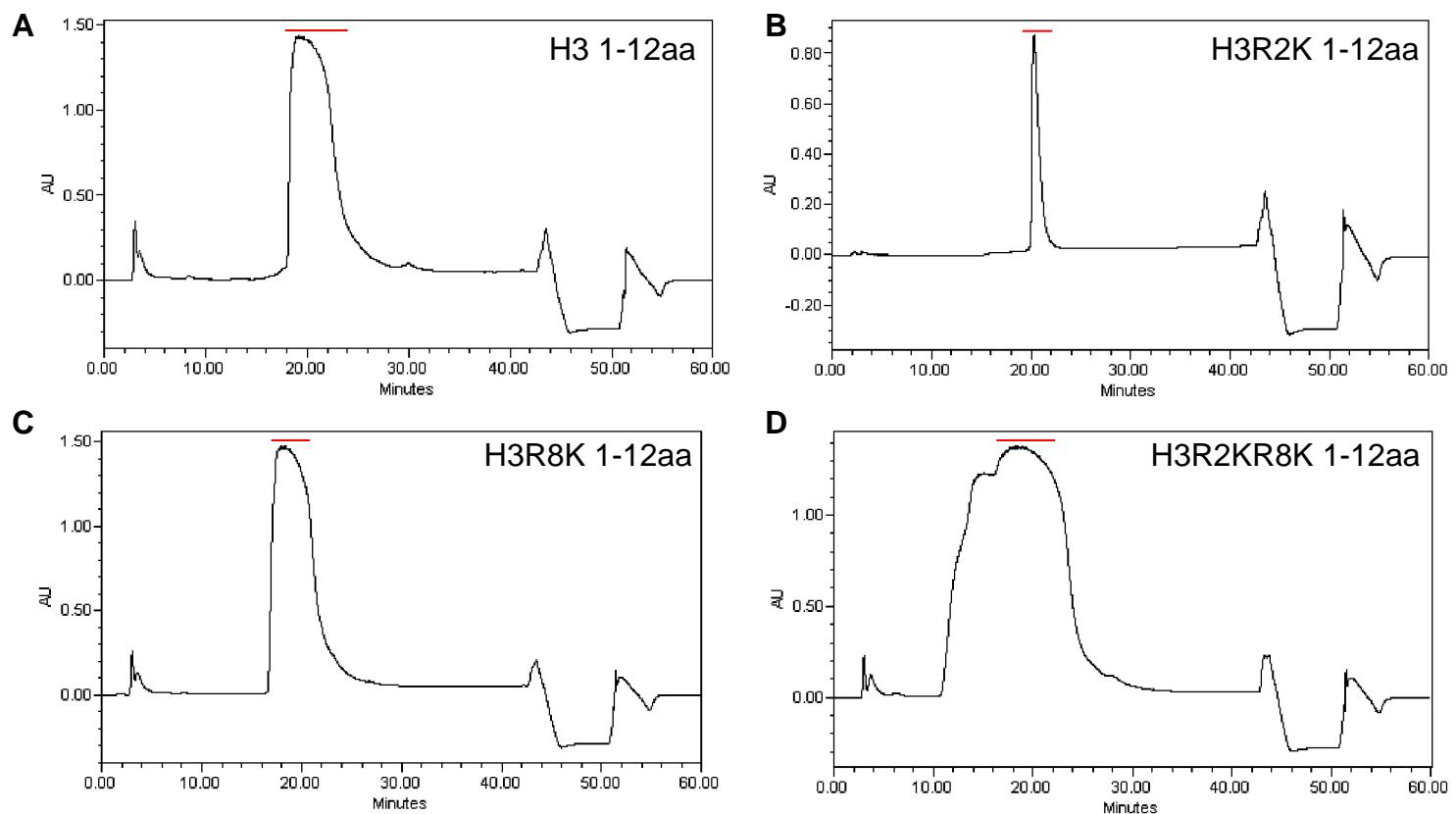
Figure S1. HPLC chromatograms at 205nm absorbance of H3 1-12aa peptides. Red bar indicates the collected fraction.**a.** H3 **b.** H3R2K **c.** H3R8K. **d.** H3R2KR8K
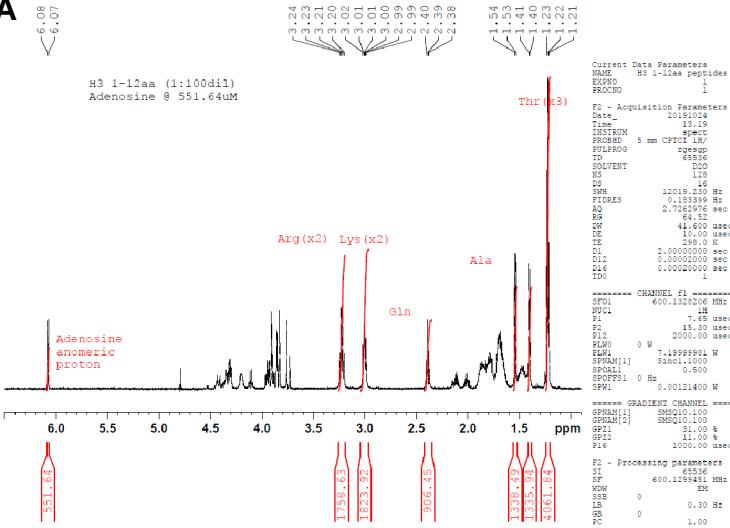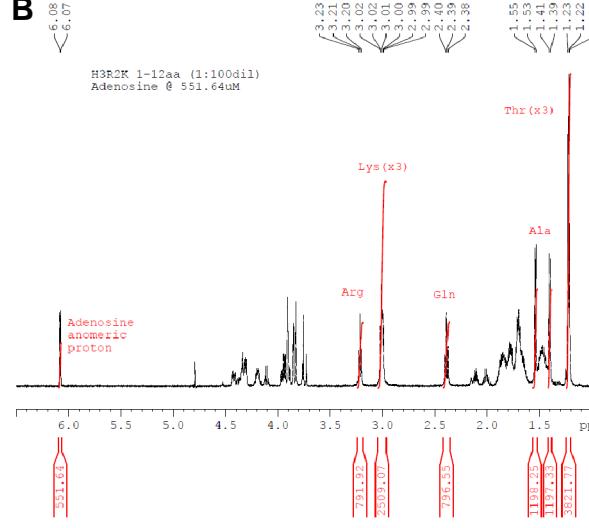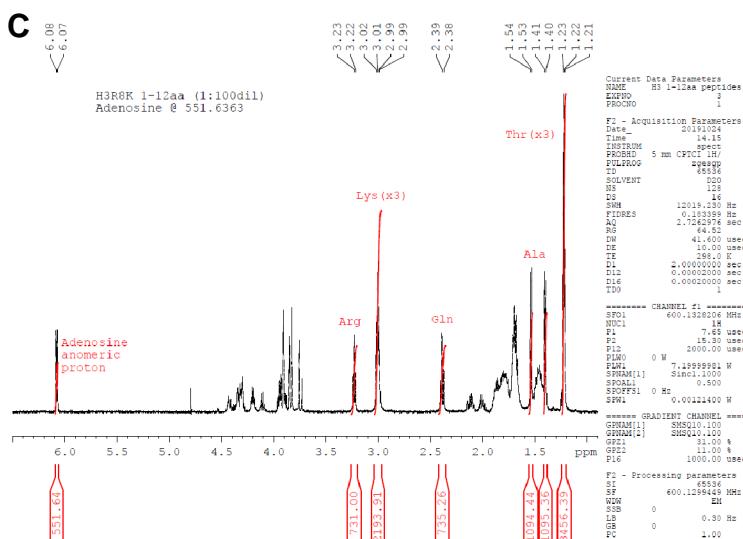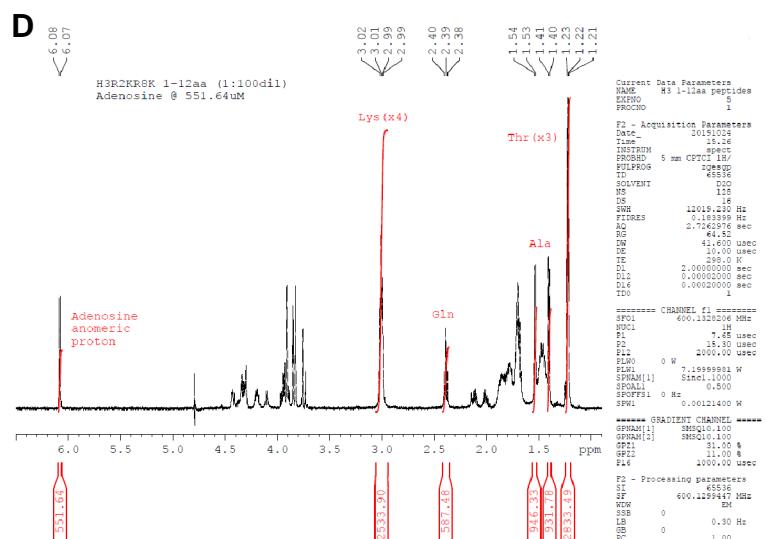
Figure S2. 1D ^{1}H NMR spectra of H3 1-12aa peptides showing peak integrations of residues used to measure peptide concentration. **a.** H3 unmodified **b.** H3R2K **c.** H3R8K. **d.** H3R2KR8K
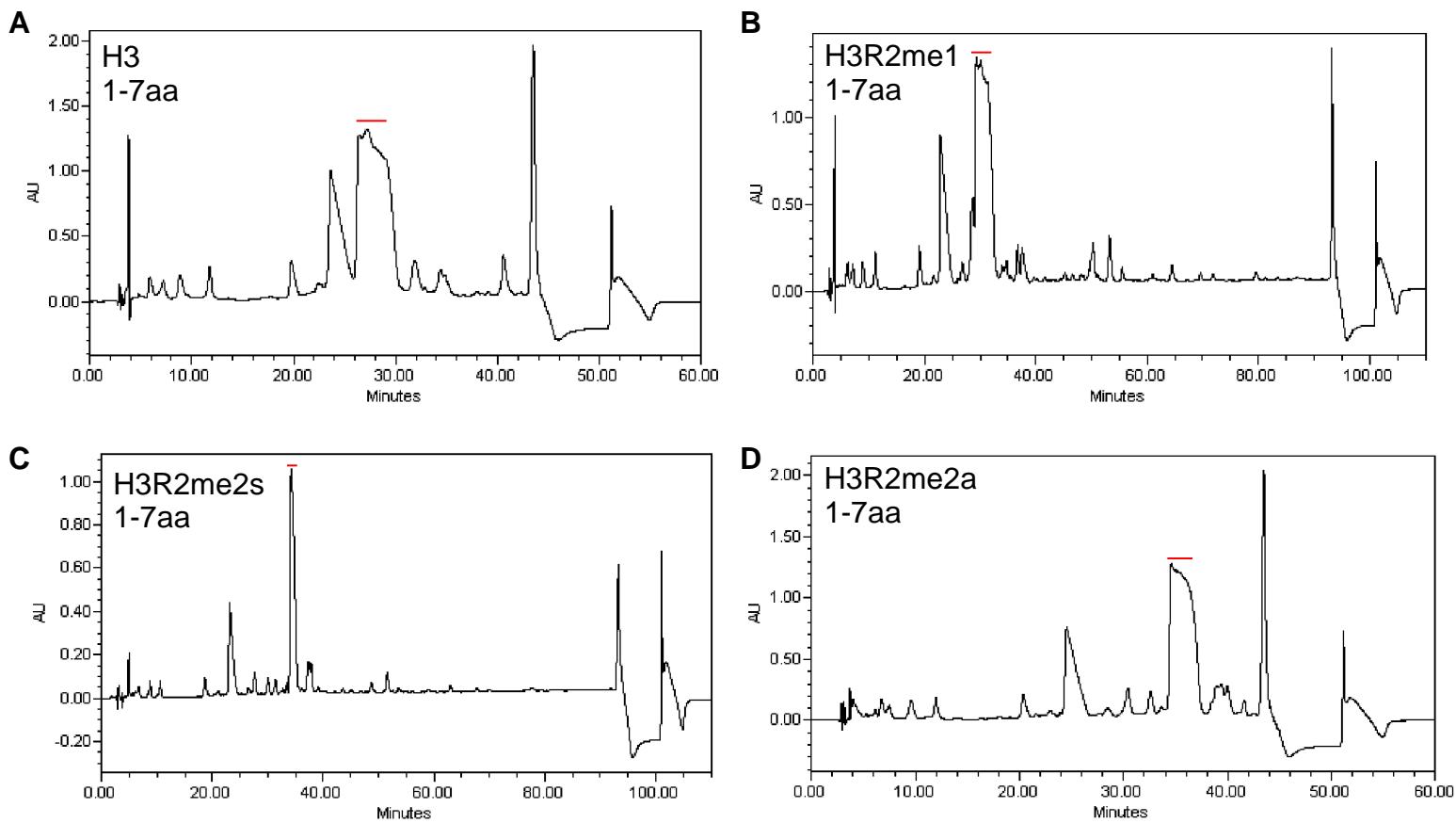
Figure S3. HPLC chromatograms at 205nm absorbance of H3 1-7aa peptides. Red bar indicates the collected fraction. **a.** H3 **b.** H3R2me1 **c.** H3R2me2s. **d.** H3R2me2a

Figure S4. 1D ^{1}H NMR spectra of H3 1-7aa peptides showing peak integrations of residues used to measure peptide concentration. **a.** H3 unmodified **b.** H3R2me1 **c.** H3R2me2s. **d.** H3R2me2a

A H3: NH$_2$-A$\underline{R}$TKQTARKSTGGKAPRKQLA(GGK-biotin)

Histone H3R2me (1-21aa)
biotinylated peptide pulldown

Input	—	me0	me1	me2s	me2a

B

WDR5   Replicate 1

Input	—	me0	me1	me2a	me2s

C
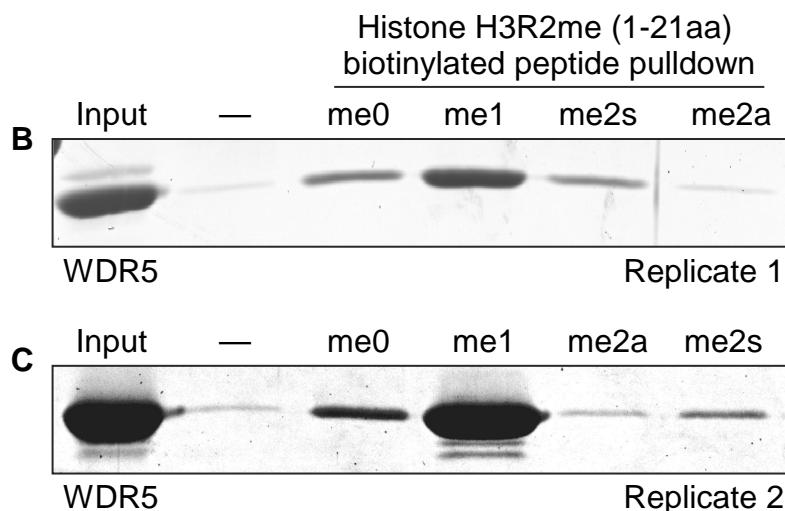
WDR5   Replicate 2

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 ocurring at Arg2, underlined. **b.** and **c.** Replicate pulldowns 1 and 2
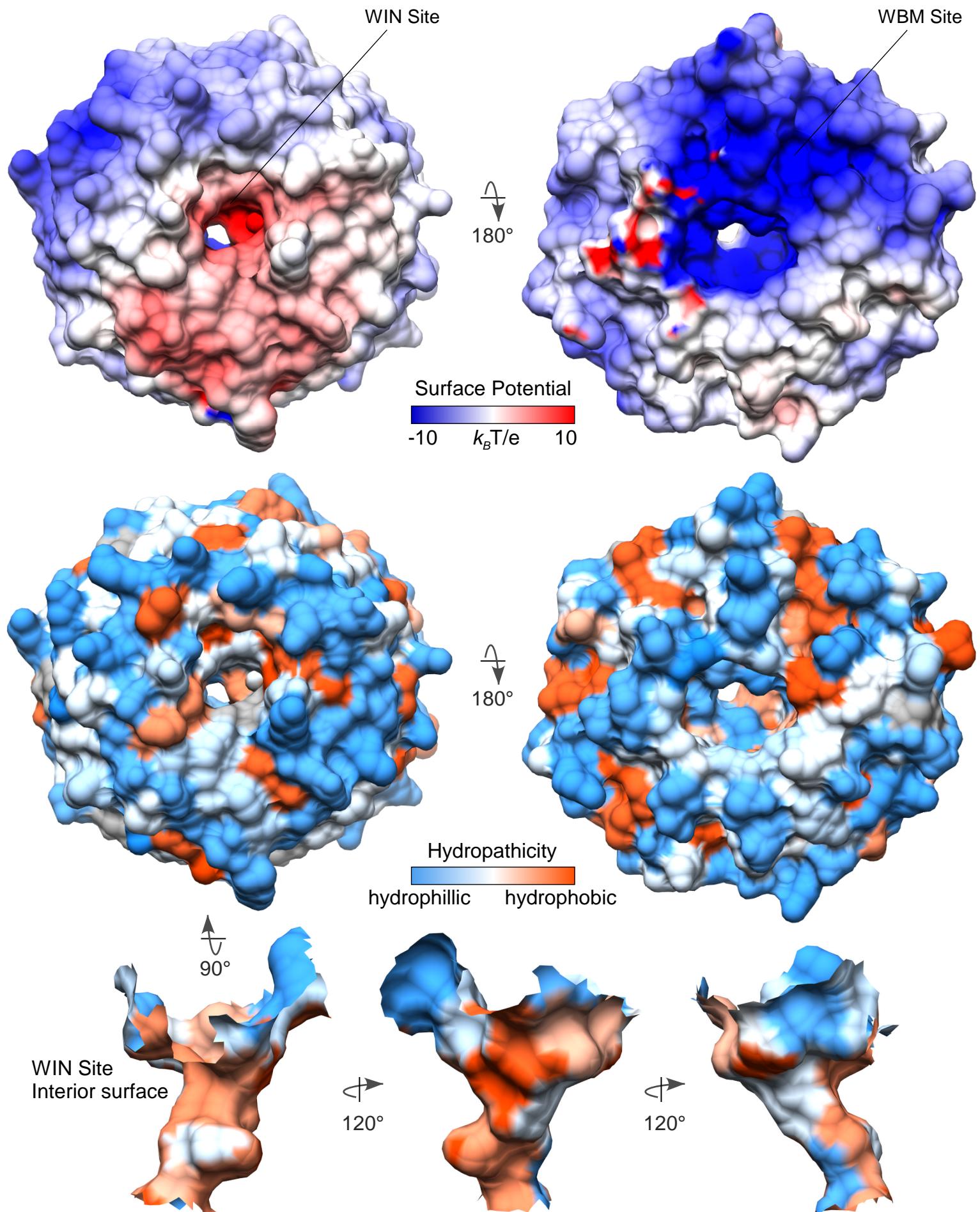
Figure S6. Electrostatic potential (top) and hydropathicity (bottom) surface representations of the WIN site
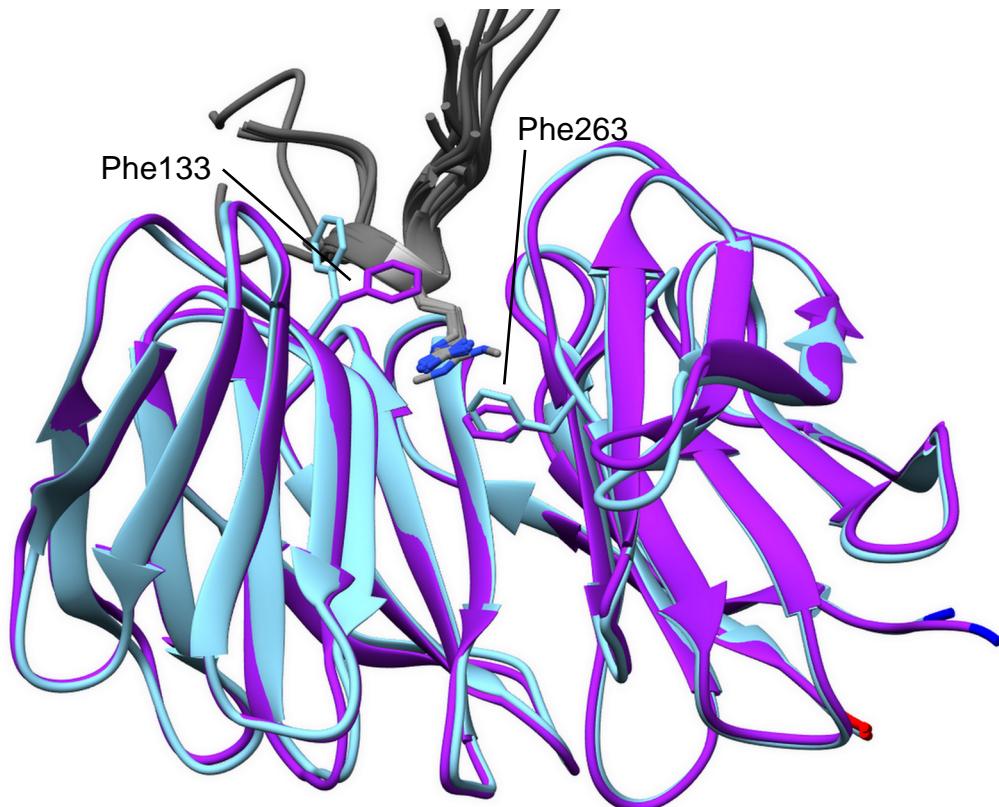
|	
H3	**ARTK**TKQTA
MLL1	GS**ARAE**AEVHL
MLL2	GC**ARSE**SEPKI
MLL3	GS**ARAE**AEPKM
MLL4	GA**ARAE**AEVYL
SET1A	GS**ARSE**SEGGY
SET1B	GC**ARSE**SEGFY
SETD1A	GS**ARSE**SEGGY
SETD1B	GC**ARSE**SEGFY
KANSL1	VA**ARTR**TRPVL
NS1	KM**ARTA**TARSK

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
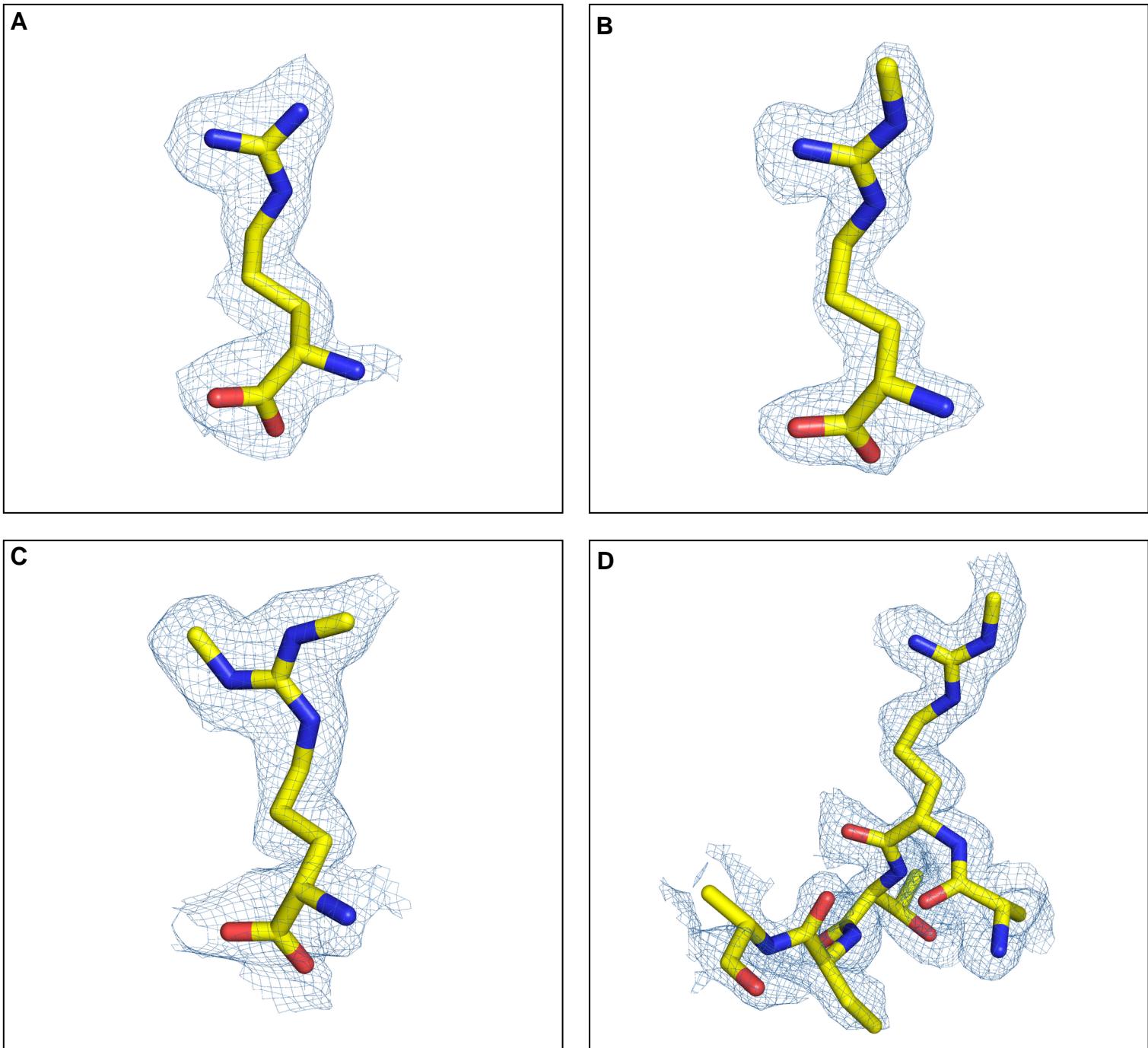
Figure S8. Ligand density maps of **a.** L-Arg **b.** me1-L-Arg **c.** me2s-L-Arg and **d.** H3R2me1 peptide in complex with WDR5
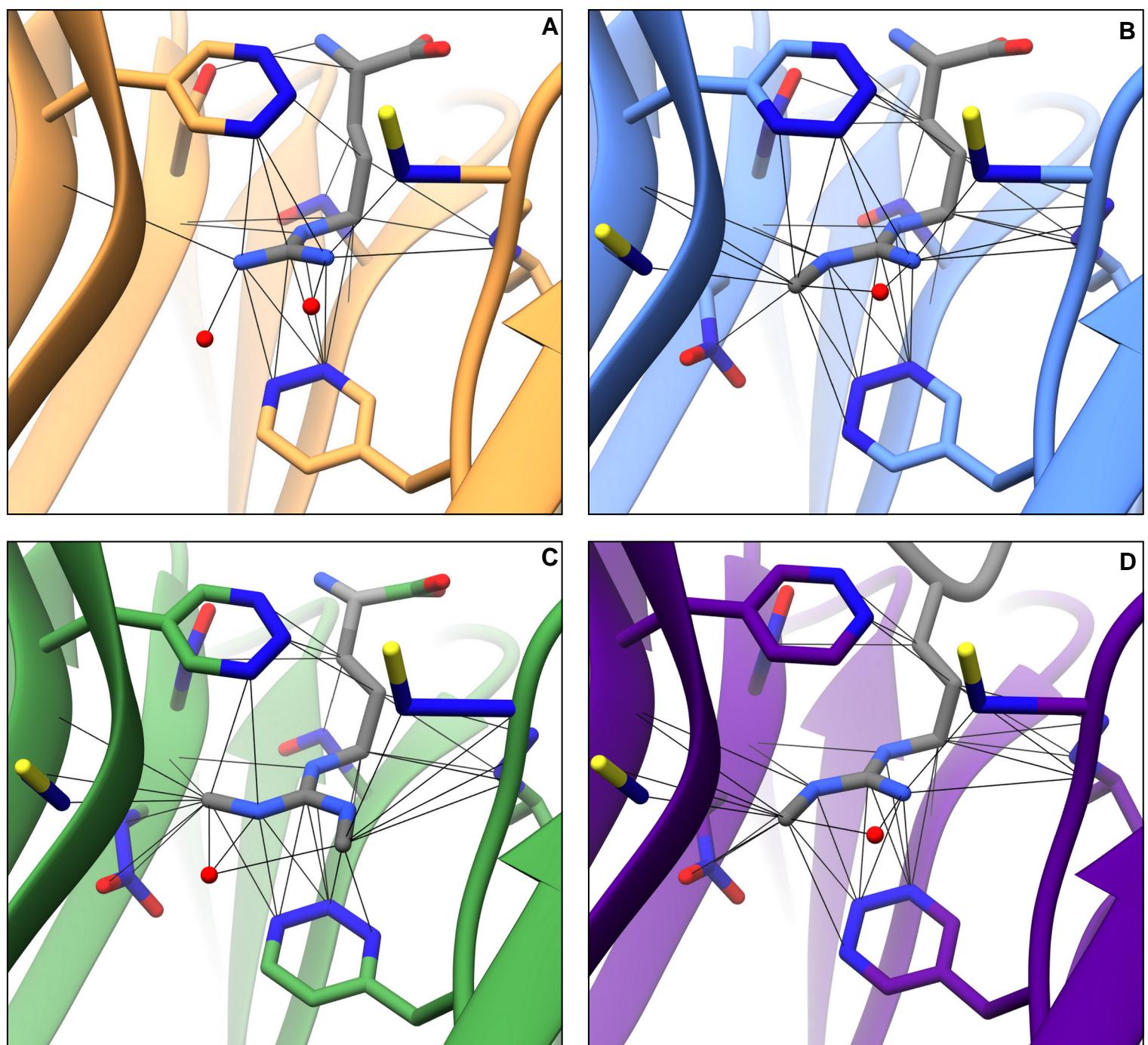
**Figure S9.** Contacts within VDW distances depicted for **a.** L-Arg, **b.** L-Arg me1, **c.** L-Arg me2s, and **d.** H3R2me1 peptide ligands. Backbone contacts uncolored; contacts with WDR5 residue sidechain atoms are colored dark blue. VDW contacts between L-Arg me2s ωme' and S218 and between L-Arg me2s ωme' and F219 sidechain omitted for clarity. Difference in contacts summarized in Tables 2, S3. H2O (red sphere).

A L-Arg me0

120°

120°

B-factor (Å²)

7.1   32.5   57.0

B L-Arg me1
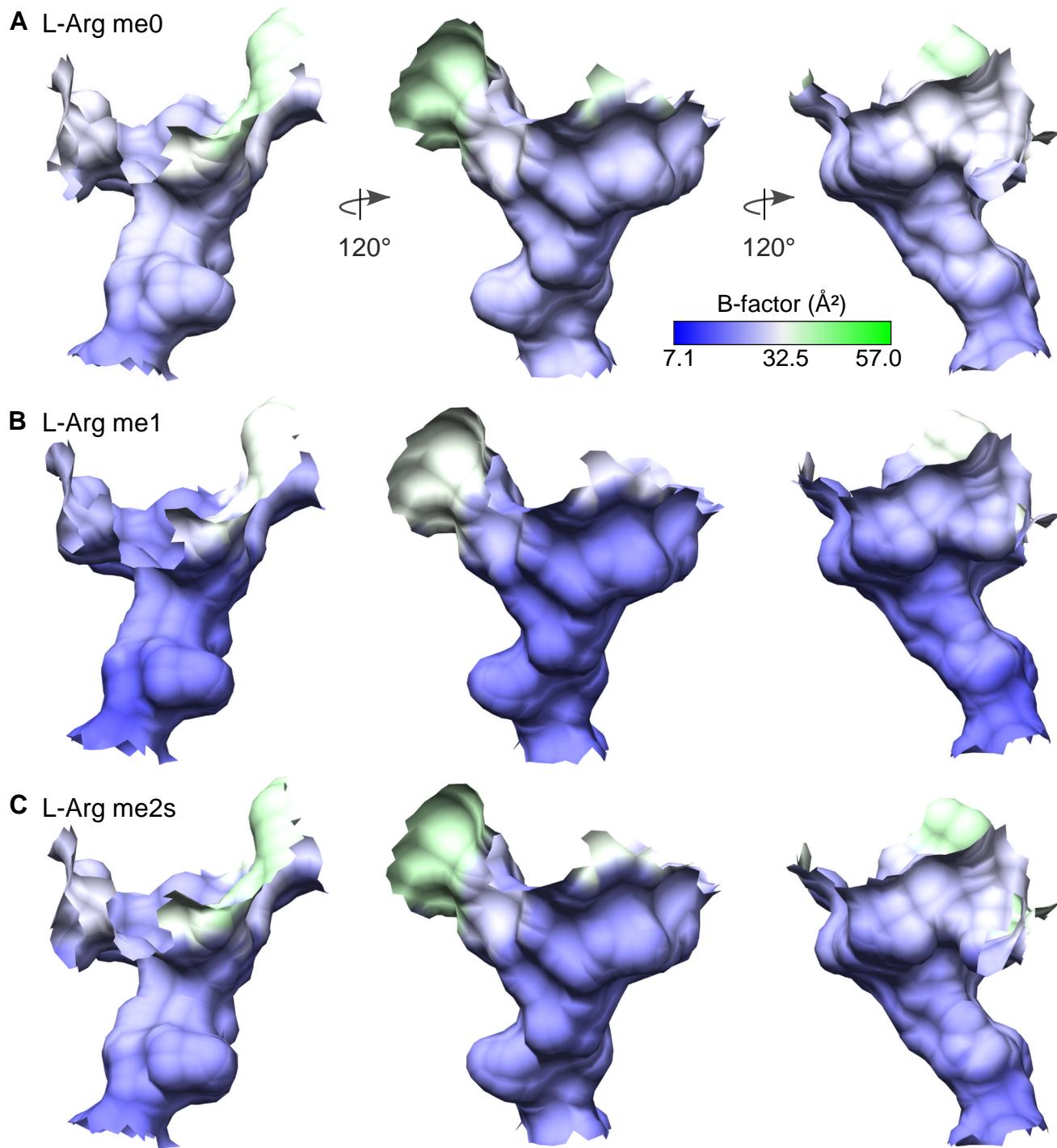
C L-Arg me2s

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.

A

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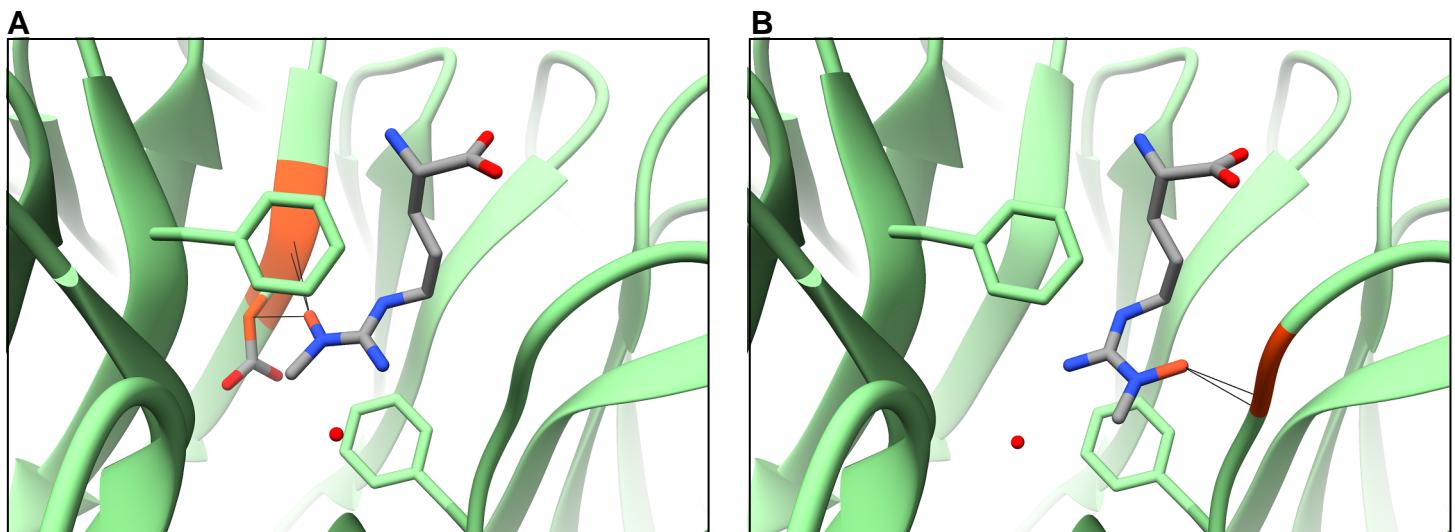
Figure S11. Rme2a modeled in the WIN site depicting clashes that potentially inhibit binding. **a.** Rme2a modeled from Rme1 structure. Clashes with Ser91 backbone and Asp92 sidechain higlighed in orange. **b.** Rme2a modeled from Rme2s structure. Clashes with Cys261 backbone highlighted in orange.

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	**ARTKQT**ARKSTGGKA	2CO0	2.25	[3]
H3	**ARTKQ**TARKS	2H13	1.58	[2]
H3	**ARTKQT**ARKST	2H9M	1.90	[1]
(ac)H3	**(ac)ARTK**Q	3PSL	1.70	[4]
H3R2me2s	M**ARTKQTA**RKSTGKA	4A7J	1.90	[5]
H3K4me1	**ARTKQTARK**	2H6K	1.89	[3]
H3K4me2	**ARTKQTAR**K	2H6N	1.50	[3]
H3K4me2	**ARTKQ**	2CNX	2.10	[3]
H3K4me2	**ARTKQT**ARKST	2O9K	1.90	[1]
H3K4me2	**ARTKQ**TARKS	2G99	1.90	—
H3K4me2	**ARTKQ**TARKS	2G9A	2.70	—
H3K4me3	**ARTK**QTARKST	2H9P	1.91	[1]
H3K4me3	**ARTKQTA**RK	2H6Q	1.87	[3]
MLL1	**GSARAEVHL**RKS	3EG6	1.72	[6]
MLL1	**ARAEVH**LRKSAFD	3EMH	1.37	[7]
MLL1	**EPPLNPHGSARAEVHL**R	4ESG	1.70	[8]
MLL2	G**CARSEPKILT**	3UVK	1.40	[9]
MLL2	**INPTGCARSEPKI**L	4ERQ	1.91	[8]
*rn*MLL3	G**SARAEPK**MSA	3UVL	2.20	[9]
MLL3	**VNPTGCARSEPKMS**	4ERY	1.30	[8]
MLL4	G**AARAEVYLR**	3UVM	1.57	[9]
MLL4	**LNPHGAARAEVY**LR	4ERZ	1.75	[8]
SET1A	**GSARSEGYY**PI	3UVN	1.79	[9]
SET1B	G**CARSEGFYT**I	3UVO	2.20	[9]
SETd1a	EH**QTGSARSEGY**YP	4EWR	1.50	[8]
SETd1b	EHVT**GCARSEGFYT**	4ES0	1.82	[8]
KANSL1	D**GTCVAARTRPVLSY**	4CY1	1.50	[10]
NS1	PKQKRKM**ARTAR**SKV	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

WDR5 crystals	Crystallization conditions	Soaking/Cryoprotectant Solution	Space group	PDB ID
WDR5-apo	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	$P2_12_12$	6OFZ
WDR5-Rme0	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	$P2_12_12$	6OI0
WDR5-Rme1	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	$P2_12_12$	6OI1
WDR5-Rme2s	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	$P2_12_12$	6OI2
WDR5-H3R2me1	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	$C222_1$	6OI3

Supplemental References

[1] Schuetz, A., Allali-Hassani, A., Martin, F., Loppnau, P., Vedadi, M., Bochkarev, A., Plotnikov, A. N., Arrowsmith, C. H., and Min, J. (2006) Structural basis for molecular recognition and presentation of histone H3 by WDR5, *The EMBO journal 25*, 4245-4252.

[2] Couture, J. F., Collazo, E., and Trievel, R. C. (2006) Molecular recognition of histone H3 by the WD40 protein WDR5, *Nature structural & molecular biology 13*, 698-703.

[3] Ruthenburg, A. J., Wang, W., Graybosch, D. M., Li, H., Allis, C. D., Patel, D. J., and Verdine, G. L. (2006) Histone H3 recognition and presentation by the WDR5 module of the MLL1 complex, *Nature structural & molecular biology 13*, 704-712.

[4] Avdic, V., Zhang, P., Lanouette, S., Voronova, A., Skerjanc, I., and Couture, J. F. (2011) Fine-tuning the stimulation of MLL1 methyltransferase activity by a histone H3-based peptide mimetic, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology 25*, 960-967.

[5] Migliori, V., Muller, J., Phalke, S., Low, D., Bezzi, M., Mok, W. C., Sahu, S. K., Gunaratne, J., Capasso, P., Bassi, C., Cecatiello, V., De Marco, A., Blackstock, W., Kuznetsov, V., Amati, B., Mapelli, M., and Guccione, E. (2012) Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance, *Nature structural & molecular biology 19*, 136-144.

[6] Patel, A., Dharmarajan, V., and Cosgrove, M. S. (2008) Structure of WDR5 bound to mixed lineage leukemia protein-1 peptide, *The Journal of biological chemistry 283*, 32158-32161.

[7] Song, J. J., and Kingston, R. E. (2008) WDR5 interacts with mixed lineage leukemia (MLL) protein via the histone H3-binding pocket, *The Journal of biological chemistry 283*, 35258-35264.

[8] Dharmarajan, V., Lee, J. H., Patel, A., Skalnik, D. G., and Cosgrove, M. S. (2012) Structural basis for WDR5 interaction (Win) motif recognition in human SET1 family histone methyltransferases, *The Journal of biological chemistry 287*, 27275-27289.

[9] Zhang, P., Lee, H., Brunzelle, J. S., and Couture, J. F. (2012) The plasticity of WDR5 peptide-binding cleft enables the binding of the SET1 family of histone methyltransferases, *Nucleic acids research 40*, 4237-4246.

[10] Dias, J., Van Nguyen, N., Georgiev, P., Gaub, A., Brettschneider, J., Cusack, S., Kadlec, J., and Akhtar, A. (2014) Structural analysis of the KANSL1/WDR5/KANSL2 complex reveals that WDR5 is required for efficient assembly and chromatin targeting of the NSL complex, *Genes & development 28*, 929-942.

[11] Qin, S., Liu, Y., Tempel, W., Eram, M. S., Bian, C., Liu, K., Senisterra, G., Crombet, L., Vedadi, M., and Min, J. (2014) Structural basis for histone mimicry and hijacking of host proteins by influenza virus protein NS1, *Nature communications 5*, 3952.