

Target	Mutation	Organism	H3K79 methylation	In vivo/in vitro	Citation
H2B-Ub	H2B K123R	Yeast	abolished	<i>In vivo</i>	(Briggs et al., 2002; Ng et al., 2002c)
	Δ Rad6	Yeast	abolished	<i>in vivo</i>	(Briggs et al., 2002; Ng et al., 2002c)
	Rnf20 (knockdown)	Human	reduced	<i>in vivo</i>	(Wang et al., 2013)
	Rnf20 (overexpression)	Human	increased	<i>in vivo</i>	(Zhu et al., 2005)
Ubiquitin	L71A, L73A	Human	decreased	<i>in vitro</i>	(Holt et al., 2015)
	L8,I44A	Human	no effect	<i>in vitro</i>	(McGinty et al., 2009)
Acidic patch	H2A E64A	Human	no effect	<i>in vitro</i>	(McGinty et al., 2009)
	H2A E68A	Human	no effect	<i>in vitro</i>	(McGinty et al., 2009)
H4 tail	R19A, R17A	Human	Severe reduction	<i>in vitro</i>	(McGinty et al., 2009)
	R19A, R17A	Yeast	abolished	<i>in vivo/in vitro</i>	(Altaf et al., 2007; Fingerman et al., 2007)
	R19E, R17E	Yeast	abolished	<i>in vivo</i>	(Fingerman et al., 2007)
	R17A	Yeast	Abolished di- and tri- methyl	<i>in vivo</i>	(Fingerman et al., 2007)
	R17E	Yeast	abolished	<i>in vivo</i>	(Fingerman et al., 2007)
	R17K	Yeast	no effect	<i>in vivo</i>	(Fingerman et al., 2007)
	R19A	Yeast	Abolished di- and tri- methyl	<i>in vivo</i>	(Fingerman et al., 2007)
	R19E	Yeast	Abolished di- and tri- methyl	<i>in vivo</i>	(Fingerman et al., 2007)
	R19K	Yeast	no effect	<i>in vivo</i>	(Fingerman et al., 2007)
	Δ 17-20	Yeast	abolished	<i>in vivo/in vitro</i>	(Fingerman et al., 2007)
Dot1L	H4 R17 binding pocket E374A, E374Q	Yeast	abolished	<i>in vitro</i>	(Sawada et al., 2004)
	H4 R17 binding pocket Y350F	Yeast	severe reduction	<i>in vitro</i>	(Sawada et al., 2004)
	Lysine tunnel, W543A	Yeast	abolished	<i>in vitro</i>	(Sawada et al., 2004)
	Lysine tunnel, W543F	Yeast	abolished	<i>in vitro</i>	(Sawada et al., 2004)
	Lysine Tunnel N241D, N241D	Human	abolished	<i>in vitro</i>	(Min et al., 2003)
	Y312A	Human	abolished	<i>in vitro</i>	(Min et al., 2003)
	C-term Ub-binding helix (Δ EDVDE)	Yeast	abolished	<i>in vivo/in vitro</i>	(Fingerman et al., 2007)
	SAM binding site G163R/S164C/G165R	human	abolished	<i>in vivo/in vitro</i>	(Feng et al., 2002; Kim et al., 2012)
	SAM binding site E422D	Yeast	abolished	<i>in Vitro</i>	(Sawada et al., 2004)
	DNA binding deletion Dot1L(1-351), Dot1L(1-370), Dot1L(1-385)	Human	abolished	<i>In vitro</i>	(Feng et al., 2002; Min et al., 2003)
siRNA	human	reduced	<i>In vivo</i>	(Kim et al., 2012)	

Table S1: Published mutants and their effects on H3K79 methylation by Dot1L, Related to Figures 4-7