Structure	Human chromodomain Y-linked 1, chromodomain, in complex with H3K9me3 peptide
PDB Code	6V41
Entry clone accession	
Entry clone source	
SGC clone accession	JMC095B09
Tag	N-terminal tag: MHHHHHHSSGRENLYFQG
Construct sequence	MHHHHHHSSGRENLYFQG ASQEFEVEA IVDKRQDKNG NTQYLVRWKG YDKQDDTWEP EQHLMNCEKC VHDFNRRQTE KQK
Vector	pET28-MHL
Expression host	BL21 (DE3) Codon plus RIL (Stratagene)
Growth method	The protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 ºC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.2 mM, and incubated overnight at 16 ºC. Cell pellets collected by centrifugation and frozen at -80 ºC.
Extraction buffers	**Lysis buffer**: **20 mM Tris-HCl pH 7.5, 400 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol**
Extraction procedure	Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at 15000rpm for 1h.
Purification buffers	**Wash buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 25 mM imidazole; **Elution buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 300 mM imidazole; **Gel filtration buffer**: 20 mM Tris-HCl pH 7.5, 100 mM NaCl and 1 mM DTT.

Purification procedure	**The fusion proteins were purified by Ni-NTA agarose column. The His tag was cleaved by His-tagged TEV protease (purified in-house) with an approximate molar ratio of 1 : 20 in the dialysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl and 5 mM beta-mercaptoethanol) at 4 °C overnight. The protein was diluted and applied onto HiTrap Q chromatography column (GE Healthcare) equilibrated with 20 mM Tris-HCl pH 7.5, 25mM NaCl and 1mM DTT. The proteins were eluted with a linear gradient of 0-50% elution buffer (20 mM Tris-HCl pH 7.5, 1M NaCl and 1 mM DTT). The proteins were further purified by gel filtration Superdex 200 10/300 (GE Healthcare). The gel filtration buffer contains 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM DTT.**
Protein stock concentration	**The purified protein was concentrated to 18 mg mL-1.**
Crystallization	Protein was incubated with H3K9me3 peptide at a molar ratio of 1:1.5 for 1 h on ice before setting up the crystallization trial. The crystals were obtained in 1.4 Na Cit, 0.1 Na Hepes, 7.5. The crystals were cryo-protected in the reservoir solution supplemented with 20% (v/v) glycerol and flash-frozen in liquid nitrogen.