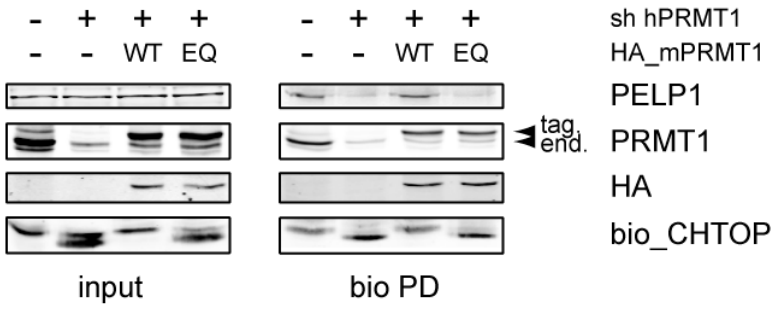
Supplementary Table 1. Oligonucleotides used in this study

Name	Sequence (5´-3´)
QPCR	
Rnh1_Fwd	TCCAGTGTGAGCAGCTGAG
Rnh1_Rev	TGCAGGCACTGACTGAAGCACCA
Senp3_Fwd	GAGAGGGTCTCCACCAGTGCC
Senp3_Rev	CTGCGCTCCCCTTCAGGACCA
Wdr18_Fwd	CTGGCCTGGAGCCTCTGCAGT
Wdr18_Rev	CCAATGAAGCGGTGGCCACCC
Las1L_Fwd	CGAGTGCCAGGGGTATCGTCG
Las1L_Rev	GCCAGGGGCAGTTCGTTGCCT
Tex10_Fwd	GCTGCTTGGAGTCTCTTGGAGG
Tex10_Rev	GCCAGCCAACGGGAGAGCACT
Pelp1_Fwd	GCTGGGCCTCAGACCAGAGTG
Pelp1_Rev	CTCGCAGGCCAGCTGTTGGAG
Dusp6_Fwd	GGCGAGTTCAAATACAAGCAA
Dusp6_Rev	ACCAGGACACCACAGTTTTTG
Tubb1_Fwd	TAAGAAGTATGTGCCGCGAG
Tubb1_Rev	AGTTGTTACCAGCACCAGAGTTA
Zbp89_Fwd	CATGAGGAGACAGTGAAAAATG
Zbp89_Rev	CTGTAAGTCTACTGGCTCTCTG
Atf5_Fwd	CTGGCTCGTAGACTATGGGAA
Atf5_Rev	TCCAATCAGAGAAGCCGTCA
ChIP	
Amylase_Fwd	CTCCTTGTACGGGTTGGT
Amylase_Rev	AATGATGTGCACAGCTGAA
Dusp6_Fwd	ACACACGATCTAAAGGAGGAC
Dusp6_Rev	CAATTAGCAAGCACAAAAGC
Tubb1_Fwd	GGATGTACAAGTGTCTCTGAGC
Tubb1_Rev	TATCTTTCCGCTCATTTCC
Zbp-89_Fwd	CTGGGAGGAGGAAGAGAAG
Zbp-89_Rev	GAGAGAACTTTTGCTGTGGC
Atf5_Fwd	GGTTCCTCACTTCGTCTCC
Atf5_Rev	TTCACTCTCCGCTCACACC
Osm_Fwd	CTGGGGAGACTTTGGTTTT
Osm_Rev	ACTGGGTCCTGGTACTCTGG
Gata-1 Upstream_Fwd	TGATGGCTTCTACTAGGCACACG
Gata-1 Upstream_Rev	GGCTTCACTCCCAGGAATGTAGG

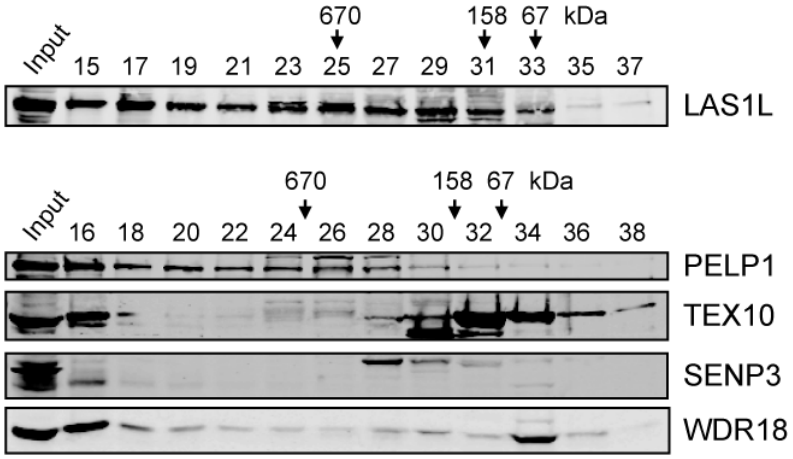
Supplemental Figure 1, Fanis et al.



Supplemental Figure 1. Methylation of Chtop is required for Pelp1 recruitment

293T cells were depleted for endogenous PRMT1 using an shRNA against PRMT1 (sh hPRMT1). PRMT1 depleted cells were transiently transfected with expression vectors encoding mouse wild type (WT) and enzymatic inactive (EQ) Prmt1 (HA_mPRMT1). Cell lysates were analyzed by streptavidin pull-down (bio PD) and Western blotting with the antibodies indicated. Arrows indicate endogenous (end.) and HA-tagged (tag.) Prmt1.

Supplemental Figure 2, Fanis et al.



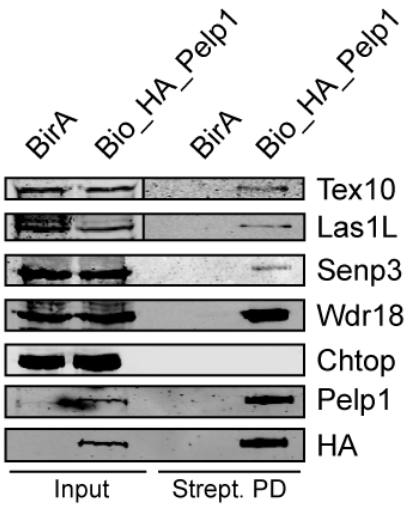
Supplemental Figure 2. 5FMC elution patterns in 293T cells

293T cell nuclear extracts were analyzed by sized-exclusion chromatography on a Superose 6 column. Proteins eluted from the indicated fractions were blotted with the indicated antibodies. Molecular mass markers are indicated at the top.

Supplemental Figure 3, Fanis et al.

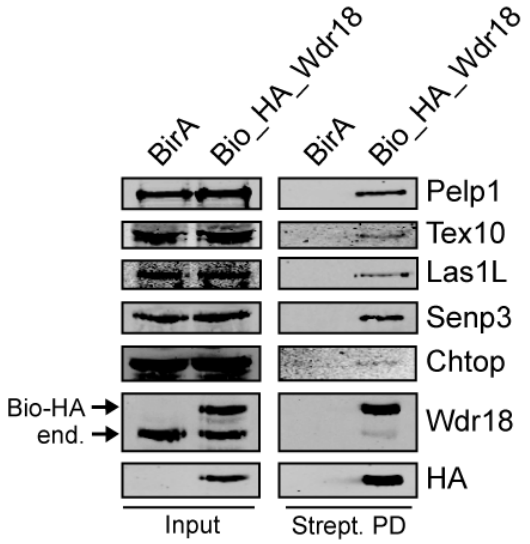
A

Strept. PD of Bio_HA_Pelp1		
Protein	uniq. pept.	coverage (%)
Las1L	19	26.3
Wdr18	14	39.7
Tex10	16	19.2
Pelp1	11	11.4
Senp3	8	16.9
Nol9	4	6.4



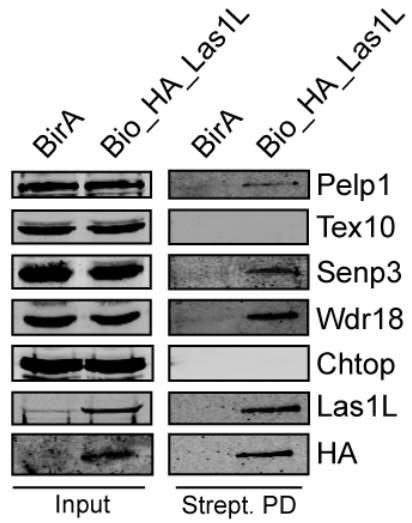
B

Strept. PD of Bio_HA_Wdr18		
Protein	uniq. pept.	coverage (%)
Las1L	20	31.3
Wdr18	15	41.3
Tex10	16	21
Pelp1	15	18.1
Senp3	15	30.1
Nol9	9	10.9



C

Strept. PD of Bio_HA_Las1L		
Protein	uniq. pept.	coverage (%)
Pelp1	9	9.7
Wdr18	9	23
Tex10	4	4.5
Las1L	4	5.9
Senp3	4	7.9



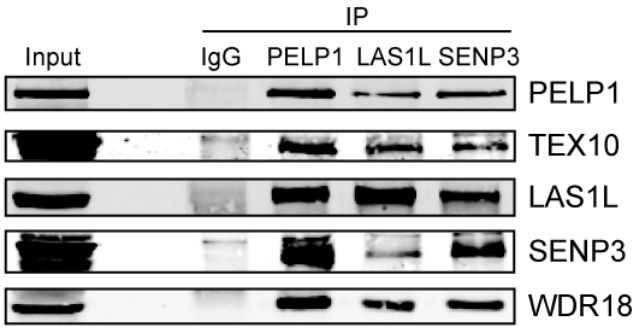
Supplemental Figure 3. 5FMC nuclear interactions

(A) Pelp1 interactions in MEL cells. Whole cell lysates (Input) and streptavidin pull downs (Strept. PD) from MEL_BirA (BirA) and MEL_BirA cells expressing biotinylated Pelp1 (Bio_HA_Pelp1) were analyzed by MS (table) and western blotting. Immunoblot probed with the antibodies indicated.

(B) Wdr18 interactions in MEL cells. Whole cell lysates (Input) and streptavidin pull downs (Strept. PD) from MEL_BirA (BirA) and MEL_BirA cells expressing biotinylated Wdr18 (Bio_HA_Wdr18) were analyzed by MS (table) and western blotting. Immunoblot probed with the antibodies indicated. Arrows indicate endogenous (end.) and biotinylated (Bio_HA) Wdr18.

(C) Las1L interactions in MEL cells. Whole cell lysates (Input) and streptavidin pull downs (Strept. PD) from MEL_BirA (BirA) and MEL_BirA cells expressing biotinylated Las1L (Bio_HA_Las1L) were analyzed by MS (table) and western blotting. Immunoblot probed with the antibodies indicated.

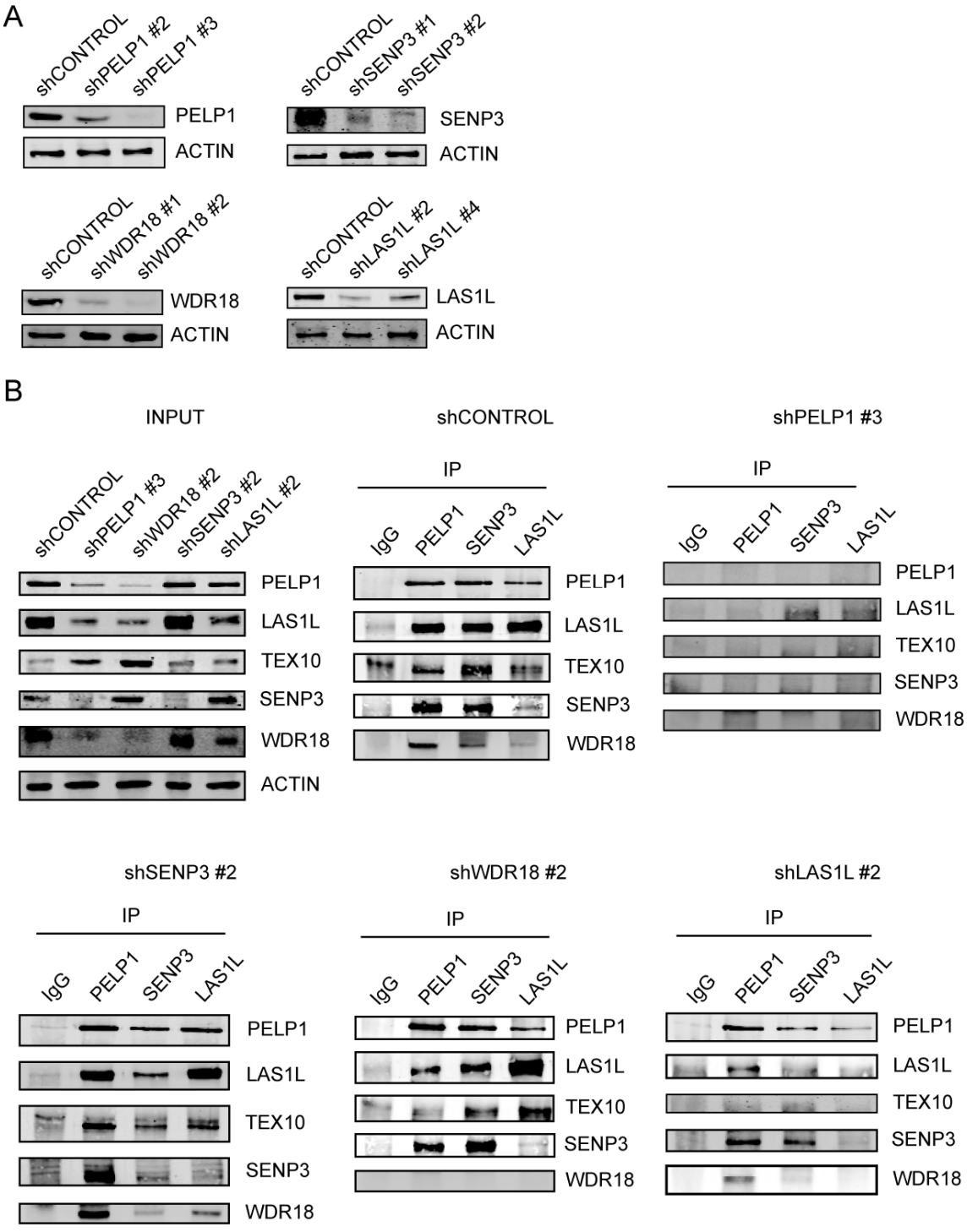
Supplemental Figure 4, Fanis et al.



Supplemental Figure 4. 5FMC is a nuclear complex

Endogenous association between the 5FMC components. 293T cell nuclear lysates were analyzed by immunoprecipitation (IP) and western blotting with the antibodies indicated.

Supplemental Figure 5, Fanis et al.



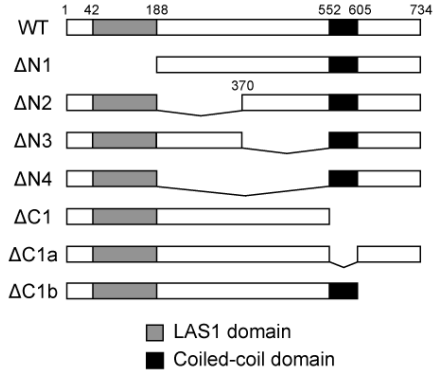
Supplemental Figure 5. PELP1 is the key stabilizing component of 5FMC complex

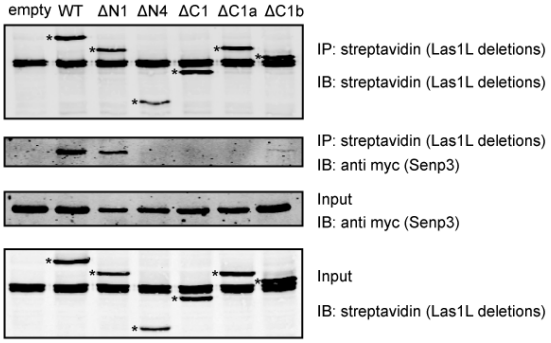
(A-B) 293T cells were transfected with the indicated shRNAs; whole cell lysates were analyzed by Western blotting using the indicated antibodies. Actin served as a loading control.

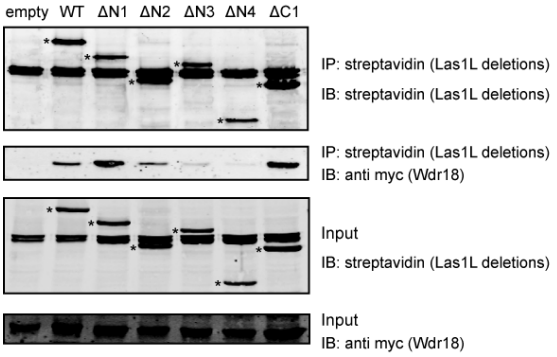
(B) Whole cell lysates (Input) and immunoprecipitations (IP) from 293T cells were analyzed by Western blotting to study the stability and interactions of 5FMC components after depletion of individual 5FMC members. Immunoblots were probed with the indicated antibodies; actin serves as a loading control. Immunoprecipitation results are shown only for the indicated shRNA. Similar results were obtained using the second shRNA construct.

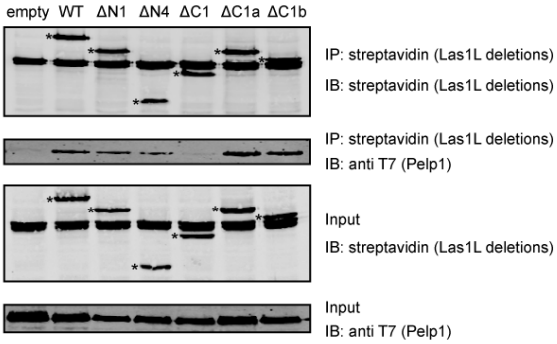
Supplemental Figure 6, Fanis et al.

A

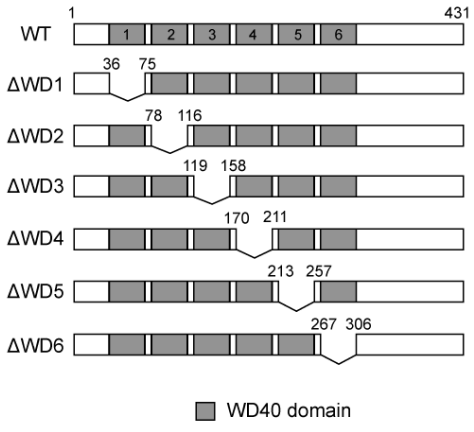


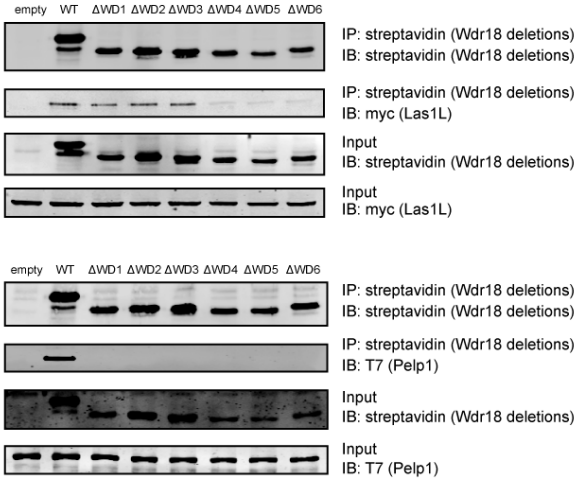






B



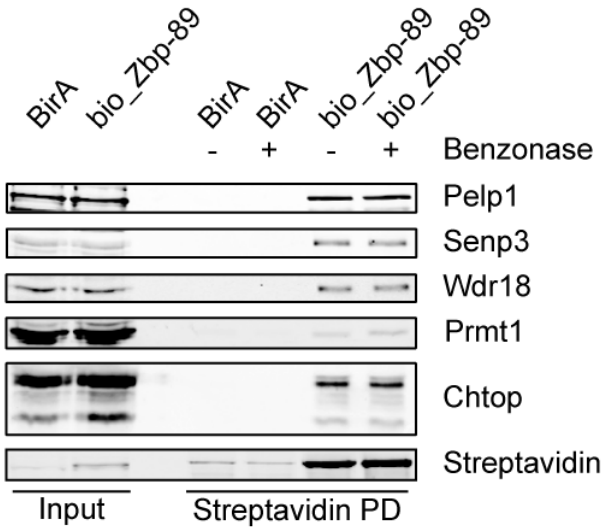


Supplemental Figure 6. Mapping the interaction regions of Las1L and Wdr18

(A) Bio_HA-tagged wild-type Las1L and its deletion mutants were expressed in 293T cells. Cell lysates were immunoprecipitated (IP) and blotted (IB) with the indicated antibodies. Whole cell lysates (Input) were blotted and ectopically expressed wild-type Wdr18 and its deletion mutants were visualized using a streptavidin antibody. Asterisks indicate wild-type Las1L and its deletion mutants.

(B) Bio_HA-tagged wild-type Wdr18 and its deletion mutants were expressed in 293T cells. Cell lysates were immunoprecipitated (IP) and blotted (IB) with the antibodies indicated. Whole cell lysates (Input) were blotted and ectopically expressed wild-type Wdr18 and its deletion mutants were visualized using a streptavidin antibody.

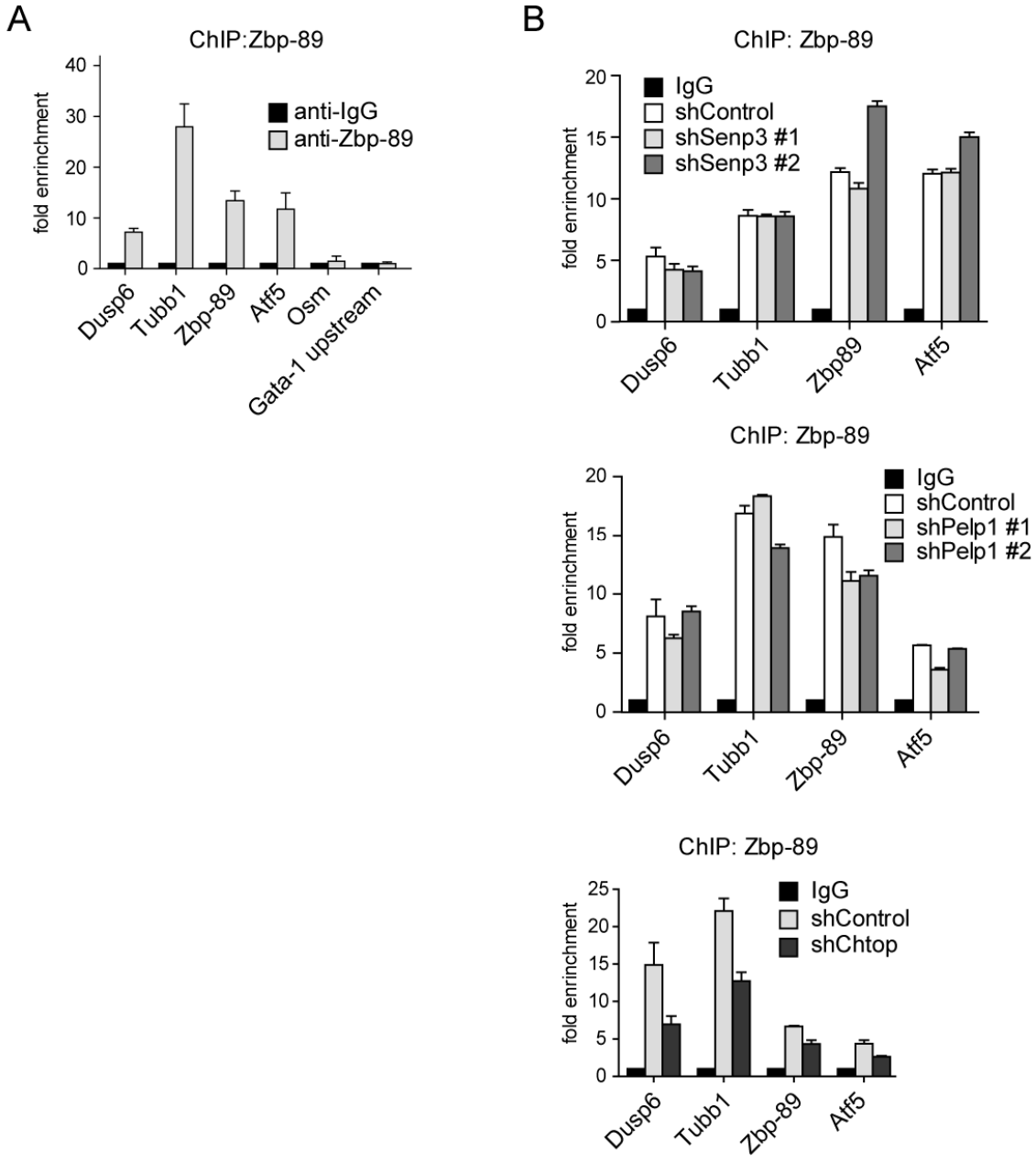
Supplemental Figure 7, Fanis et al.



Supplemental Figure 7. Chtop, Prmt1 and 5FMC complex are associated with Zbp-89

Streptavidin pull downs (Streptavidin PD) from MEL_BirA (BirA) and MEL_BirA cells expressing biotinylated Zbp-89 (Bio_Zbp-89) were treated with Benzonase. Whole cell lysates (Input) and streptavidin pull downs were analyzed by western blotting. Immunoblot probed with the antibodies indicated.

Supplemental Figure 8, Fanis et al.



Supplemental Figure 8. Recruitment of Zbp-89 to its target genes does not depend on 5FMC

(A) Zbp-89 is recruited to the promoter regions of *Dusp6*, *Zbp-89*, *Atf5* and the coding region of *Tubb1*. MEL_BirA cells analysed by ChIP using Zbp89 antibody for the indicated gene promoter or coding regions. The promoter region of the *Osm* gene and the region upstream of *Gata-1* promoter (*Gata-1* upstream) were used as negative controls.

(B) Pelp1, Senp3 and Chtop knockdowns do not affect Zbp-89 binding at the promoter or coding regions of *Dusp6*, *Tubb1*, *Zbp-89* and *Atf5*. MEL_BirA cells were treated as in (Fig. 6B). ChIP analysis at the indicated regions was performed using Zbp-89 antibody. Error bars: SD of triplicate experiment.