Supplemental Data

Leucine-rich repeat and WD repeat-containing protein 1 is recruited to pericentric heterochromatin by trimethylated lysine 9 of histone H3 and maintains heterochromatin silencing

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Running title: Lrwd1 and heterochromatin silencing

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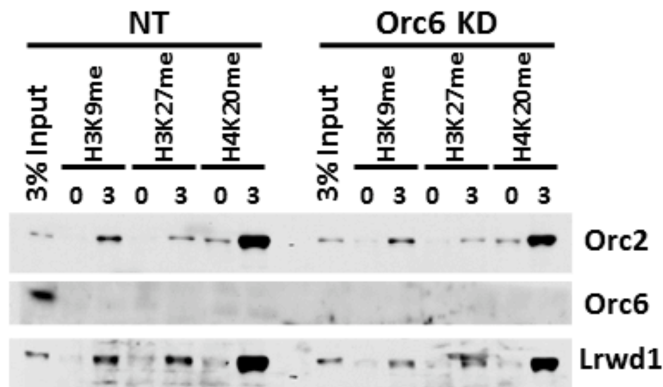
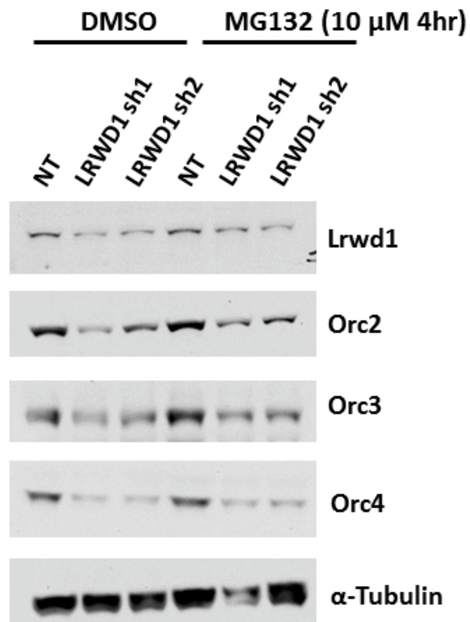
Fig. S1



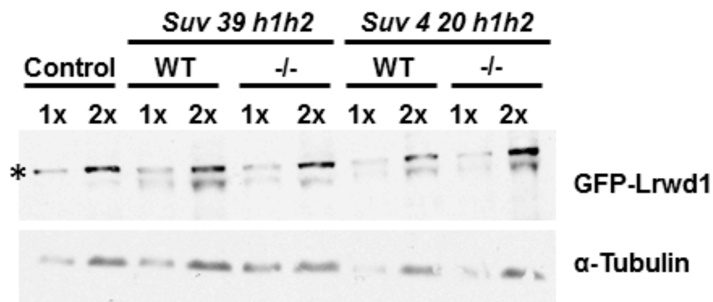
Supplemental Fig. S1. Orc6 is not required for Lrwd1 to bind histone peptides with repressive histone marks. Orc6 was depleted using shRNA, and nuclear extracts were prepared to perform the peptide pull down assay described in Fig. 1 using unmodified (0) and tri-methylated (3) H3K9, H4K20 and H3K27 peptides. As controls, nuclear extracts were prepared from cells infected with virus for non-targeting control (NT). Result shown is a representative of three independent experiments.

Fig. S2



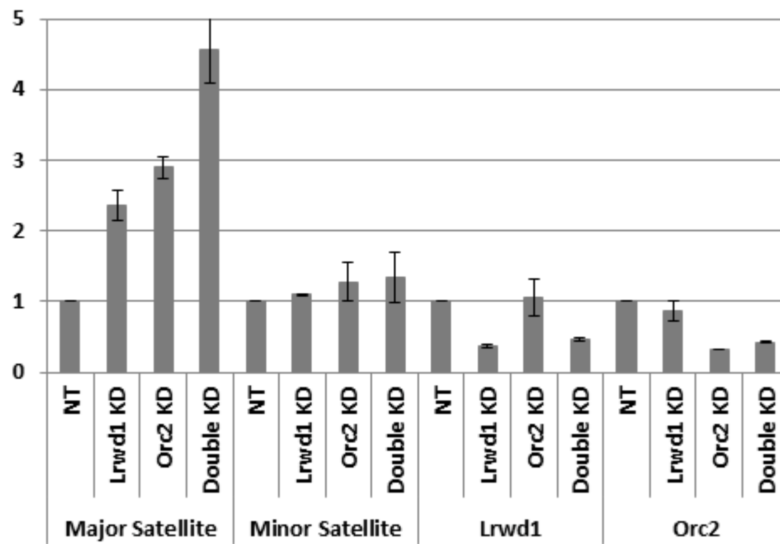
Supplemental Fig. S2. Proteasome inhibitor MG132 do not restore ORC protein levels in HeLa cells depleted with Lrwd1. HeLa cells were infected with viruses for non targeting control (NT) and two independent shRNAs against Lrwd1 (sh1 and sh2). After cells were cultured for 68 hours, DMSO or proteasome inhibitor MG132 was added to the culture medium for additional 4 hours. The cells were then collected and protein lysates prepared for Western Blot using antibodies against Lrwd1, Orc2, Orc3 and Orc4 proteins. α-Tubulin serves as a loading control.

Fig. S3



Supplemental Fig. S3. Expression of GFP-Lrwd1 in wild type, *Suv3 9 h1/h2*$^{-/-}$ and *Suv4 20 h1/h2*$^{-/-}$ MEF cells. The pEGFPC1-Lrwd1 construct was transfected into wild type, *Suv3 9 h1/h2*$^{-/-}$ and *Suv4 20 h1/h2*$^{-/-}$ MEF cells, and lysates were collected to detect the expression level of GFP-Lrwd1 by Western blot using antibodies against GFP. α-Tubulin serves as a loading control. (* non-specific band)

Fig. S4



Supplemental Fig. S4. Depletion of both Lrwd1 and Orc2 do not have a major effect on the expression of minor satellite DNA. RNA was isolated from cells infected with virus of non-targeting control (NT), Lrwd1 single knockdown (KD), Orc2 single knockdown and the double knockdown of Lrwd1 and Orc2 (double KD), reverse-transcribed. Expression levels of major satellite DNA, minor satellite DNA, Lrwd1 and Orc2 were examined by real-time PCR using primers described in experimental procedures as shown in Figure 7C. Results are the average of two independent experiments.