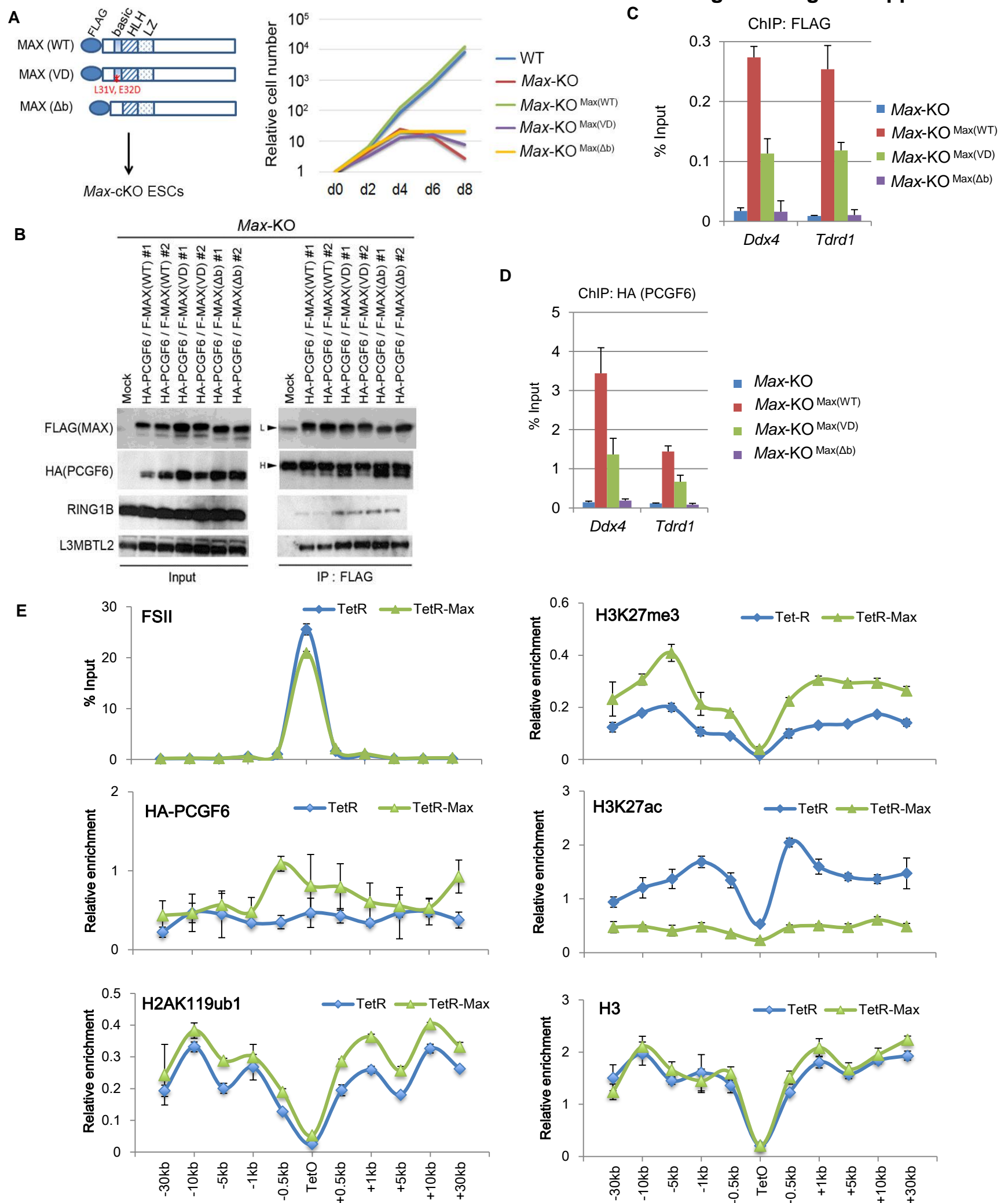


Figure 4–Figure Supplement 2

**Figure 4–Figure Supplement 2. Sequence recognition by MAX/MGA is critical for recruiting PCGF6-PRC1 to its target genes.**

(A) MAX-dependent proliferation of ESCs. Schematic representation of FLAG-tagged wild type (WT) and mutant [L31V and E32D substitution (VD) and basic region deletion (Db)] MAX proteins (left). Failure to rescue growth defects of *Max*-KO ESCs by either mutant MAX. Mock-transfected *Max* conditional KO ESCs (WT) stopped growing 4 days after doxycycline treatment (KO) (right). Stable expression of FLAG-tagged WT [KO+MAX(WT)] rescued the growth defect but VD or Db mutants [KO+MAX(VD) and KO+MAX(Db)] did not. (B) Association of mutant MAX proteins with other components of the PCGF6-PRC1 complex. Immunoprecipitation-immunoblot (IP-IB) analysis revealed the association of FLAG-tagged MAX WT, VD or Db with HA-tagged PCGF6, RING1B and L3MBTL2. *Max*-KO ESCs that expressed HA-tagged PCGF6 and FLAG-tagged wild type or mutant MAX were subjected to IP with anti-FLAG antibody. Resulting precipitates (IP) and lysates (Input) were immunoblotted with antibodies against FLAG, HA, RING1B or L3MBTL2. (C) Binding of FLAG-tagged WT or mutant MAX to target of ChIPed DNA is depicted as a percentage of input DNA. Error bars represent standard deviation determined from at least three independent experiments. (D) Binding of HA-tagged genes in *Max*-KO ESCs. Local levels of FLAG-tagged WT or mutant MAX at *Ddx4* or *Tdrd1* promoter regions were determined by ChIP-qPCR. The relative amount PCGF6 to target genes in *Max*-KO ESCs that express WT or mutant MAX. Local levels of HA-tagged PCGF6 at *Ddx4* or *Tdrd1* promoter regions were determined by ChIP-qPCR. (E) Forced tethering of MAX to a TetO array induced activation of PCGF6-PRC1 recruitment. ChIP analysis for TetR, HA-tag (PCGF6), H2AK119ub1, H3K27me3, H3K27ac and H3 across the TetO-containing locus in ESCs revealed TetR-MAX-mediated local activation of the PCGF6-PRC1 pathway. ChIP experiments were performed at least in biological duplicate with error bars showing SEM.