Legends for Supplementary Figures

Supplementary Figure 1 Expression of H3.3-YFP, H3K36me3, and STAT1-P in WT and *Whsc1*$^{-/-}$ cells. (**A**) Acid extracted histones from WT and *Whsc1*$^{-/-}$ cells expressing H3.1-YFP or H3.3-YFP were analyzed for total H3, H3K36me3, and H3-YFP by immunoblotting using respective antibodies. (**B**) Increasing amount of acid extracted histones from WT and *Whsc1*$^{-/-}$ cells (2, 4, and 8 μg) were tested for H3K36me3 as above. H3 was tested as a loading control. (**C**) WT and *Whsc1*$^{-/-}$ cells were stimulated with IFN for indicated times and nuclear extracts were tested for phosphorylated STAT1 (STAT1-P) by immunoblotting with specific antibody. β-ACTIN was used as a loading control.

Supplementary Figure 2 Levels of protein expressed in WT and *Whsc1*$^{-/-}$ cells upon IFN stimulation. WT and *Whsc1*$^{-/-}$ cells expressing H3.3-YFP were stimulated with IFN and whole cell extracts were tested for expression of indicated proteons at indicated times by immunoblotting using respective antibodies.

Supplementary Figure 3 Accumulation of total H3 on the ISGs is not affected by the absence of WHSC1. WT and *Whsc1*$^{-/-}$ cells were treated with IFN for indicated times and accumulation of total H3 in ISGs and *Gtf2b* was tested by ChIP using a specific antibody. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 4 Reduction of ISG mRNA induction in *Whsc1* knockdown cells. WT cells were transiently transfected with *Whsc1* siRNA or scrambled control siRNA, and treated with IFN for indicated times. Expression of transcripts for the ISGs in above cells was measured by qRT-PCR. *Gtf2b* transcripts were tested as a control. Values represent the average of two determinations +/- S.D.

Supplementary Figure 5 Recruitment of WHSC1 and H3.3 in ISGs. (**A**-**C**) WT and *Whsc1*$^{-/-}$ cells treated with IFN for indicated times were analyzed for the recruitment of WHSC1 (**A**), HIRA (**B**), and H3K36me3 (**C**) to the ISGs and *Gtf2b* by ChIP analyses. Values represent the average of duplicate determinations +/- S.D. (**D**) ChIP analysis was

performed for NIH3T3 cells expressing H3.3-YFP or H3.3K36R-YFP using anti-GFP antibody. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 6 IFN induced recruitment of H3K36me2 and SETD2. WT and *Whsc1*$^{-/-}$ cells were treated with IFN for indicated times and ChIP analyses were performed to detect recruitment of SETD2 (**A**) and H3K36me2 (**B**) to the ISGs and *Gtf2b*. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 7 Relative levels of H3.3-YFP and H3.3K36R-YFP. (**A**) Acid extracted histones from NIH3T3 cells expressing H3.1-YFP, H3.1K36R-YFP, H3.3-YFP, or H3.3K36R-YFP were analyzed for H3K36me3, H3-YFP, and H3 by immunoblotting using respective antibodies. (**B**) NIH3T3 cells expressing H3.3-YFP and H3.3K36R-YFP were stimulated with IFN for indicated times. Nuclear extracts were tested for expression of H3.3-YFP and H3.3K36R-YFP by immunoblotting with anti-GFP antibody. H3 was used as a loading control.

Supplementary Figure 8 IFN induced recruitment of Pol II to the ISGs. WT and *Whsc1*$^{-/-}$ cells were treated with IFN for indicated times and ChIP analyses were performed to detect recruitment of Pol II-2P (**A**), Pol II-HP (**B**), Pol II (**C**), and Pol II-5P (**D**) to the ISGs and *Gtf2b*. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 9 IFN induced recruitment of P-TEFb and BRD4 to the ISGs. WT and *Whsc1*$^{-/-}$ cells were treated with IFN for indicated times and ChIP analyses were performed to detect recruitment of CDK9 (**A**) and BRD4 (**B**) to the ISGs and *Gtf2b*. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 10 Reintroduction of WHSC1 deletions in *Whsc1*$^{-/-}$ cells. (**A**) *Whsc1*$^{-/-}$ cells transiently expressing WT WHSC1 or its deletion constructs were treated with IFN for indicated times and expression of *Whsc1* and ISGs mRNAs were tested by qRT-PCR. Values represent the average of two determinations +/- S.D. (**B**) Whole cell

extracts from the above cells were tested for WHSC1 protein expression by immunoblotting.

Supplementary Figure 11 Reduction of H3.3 deposition and ISG mRNA induction in *Hira* knockdown cells. (**A**) NIH3T3 cells transduced with a vector for *Hira* shRNA or scrambled control shRNA were treated with IFN for indicated times. mRNA levels of ISGs were measured by qRT-PCR. Values represent the average of two determinations +/- S.D. *Gtf2b* transcripts were tested as a control. (**B**) *Hira* and control knockdown cells expressing H3.3-YFP were analyzed for the incorporation of H3.3 in ISGs and *Gtf2b* by ChIP analysis using anti-GFP antibody. Values represent the average of duplicate determinations +/- S.D. (**C**) ChIP analysis was performed for *Hira* and control knockdown cells to detect recruitment of WHSC1. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 12 Reduction of H3.3 deposition and ISG mRNA induction in *Brd4* knockdown cells. (**A**) NIH3T3 cells transduced with a vector for *Brd4* shRNA or scrambled control shRNA were treated with IFN for indicated times. mRNA levels of ISGs were measured by qRT-PCR. Values represent the average of two determinations +/- S.D. *Gtf2b* transcripts were tested as a control. (**B** and **C**) *Brd4* and control knockdown cells expressing H3.3-YFP were analyzed for recruitment of BRD4 (**B**) and WHSC1 (**C**) by ChIP. Values represent the average of duplicate determinations +/- S.D. (**D**) *Brd4* and control knockdown cells expressing H3.3-YFP were analyzed for the incorporation of H3.3 in ISGs and *Gtf2b* by ChIP using anti-GFP antibody. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 13 Effect of the bromodomain inhibitor JQ1 on ISG mRNA induction and H3.3 deposition. (**A**-**C**) WT cells were treated with IFN for indicated times. JQ1 was added 1 h prior to IFN treatment. ChIP assays were performed to detect accumulation of BRD4 (**A**), WHSC1 (**B**), or H3.3-YFP (**C**) in ISGs and *Gtf2b* using respective antibodies. Values represent the average of duplicate determinations +/- S.D.

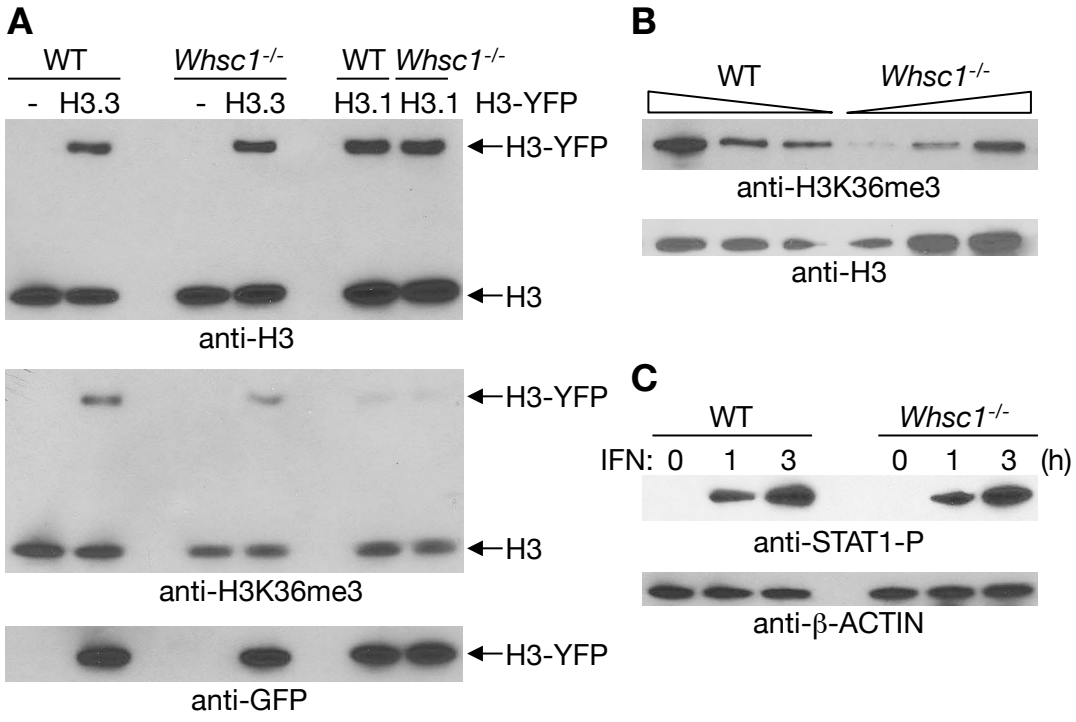
(**D**) Above cells were tested for ISGs and *Gtf2b* transcripts expression. Values represent the average of two determinations +/- S.D.

Supplementary Figure 14 Effect of P-TEFb inhibitor Flavopiridol in ISG mRNA induction and H3.3 deposition. (**A-C**) WT cells were treated with IFN for indicated times. Flavopiridol was added simultaneously with IFN. ChIP assays were performed to detect recruitment of CDK9 (**A**) or WHSC1 (**B**), or H3.3-YFP (**C**) in ISGs and *Gtf2b* using respective antibodies. (**C**) ChIP analysis was performed for WT cells expressing H3.3-YFP or using anti-GFP antibody. Values represent the average of duplicate determinations +/- S.D. (**D**) Above cells were tested for ISGs and *Gtf2b* transcripts expression. Values represent the average of two determinations +/- S.D.

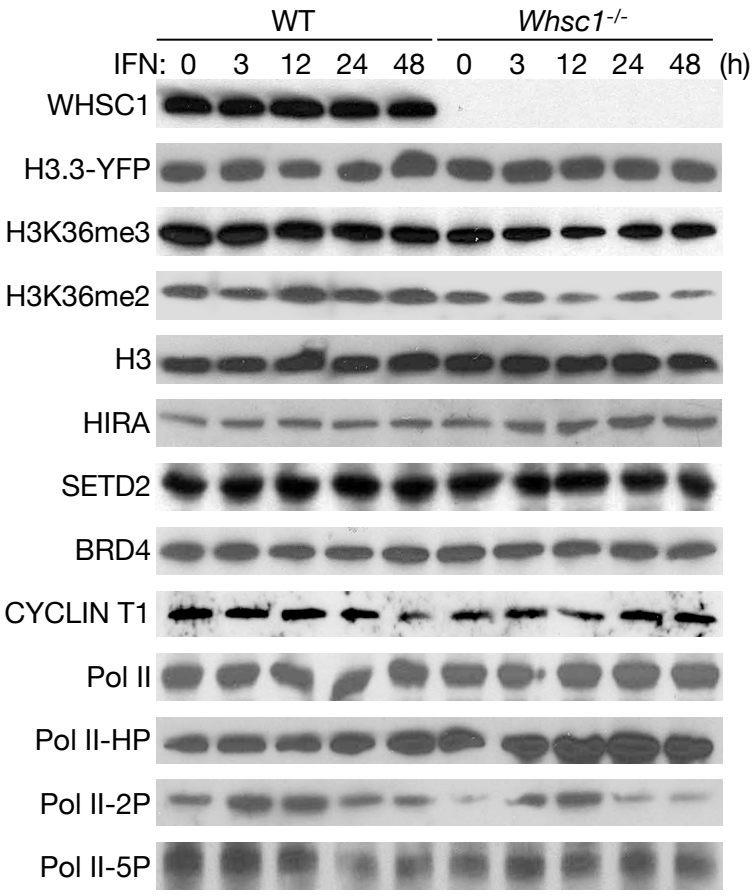
Supplementary Figure 15 Effects of Flavopiridol on IFN induced recruitment of BRD4 and HIRA. WT cells were treated with IFN for indicated times. Flavopiridol was added simultaneously with IFN. ChIP assays were performed to detect recruitment of BRD4 (**A**) or HIRA (**B**) in ISGs and *Gtf2b* using respective antibodies. Values represent the average of duplicate determinations +/- S.D.

Supplementary Figure 16 Expression of BRD4, P-TEFb, WHSC1, and H3.3-YFP in the presence of JQ1 and Flavopiridol, and after UV treatment. (**A**) WT cells expressing H3.3-YFP were stimulated with IFN at indicated times in the presence of JQ1 or the inactive steroisomer JQ1 (Ctrl). Whole cell extracts were tested for expression of BRD4, WHSC1, and H3.3-YFP by immunoblotting using respective antibodies. H3 was used as a loading control. (**B**) WT cells expressing H3.3-YFP were stimulated with IFN at indicated times in the presence of Flavopiridol or vehicle (Ctrl). Whole cell extracts were tested for expression of CYCLIN T1, WHSC1, H3.3-YFP, BRD4, and HIRA by immunoblotting using respective antibodies. H3 was used as a loading control. (**C**) WT and *Whsc1*$^{-/-}$ cells expressing H3.3-YFP were irradiated with UV-B (4 mJ/cm^2). Acid extracts prepared at indicated times were tested for expression of H3.3-YFP by immunoblotting with anti-GFP antibody. H3 was used as a loading control.

Supplementary Figure 1



Supplementary Figure 2



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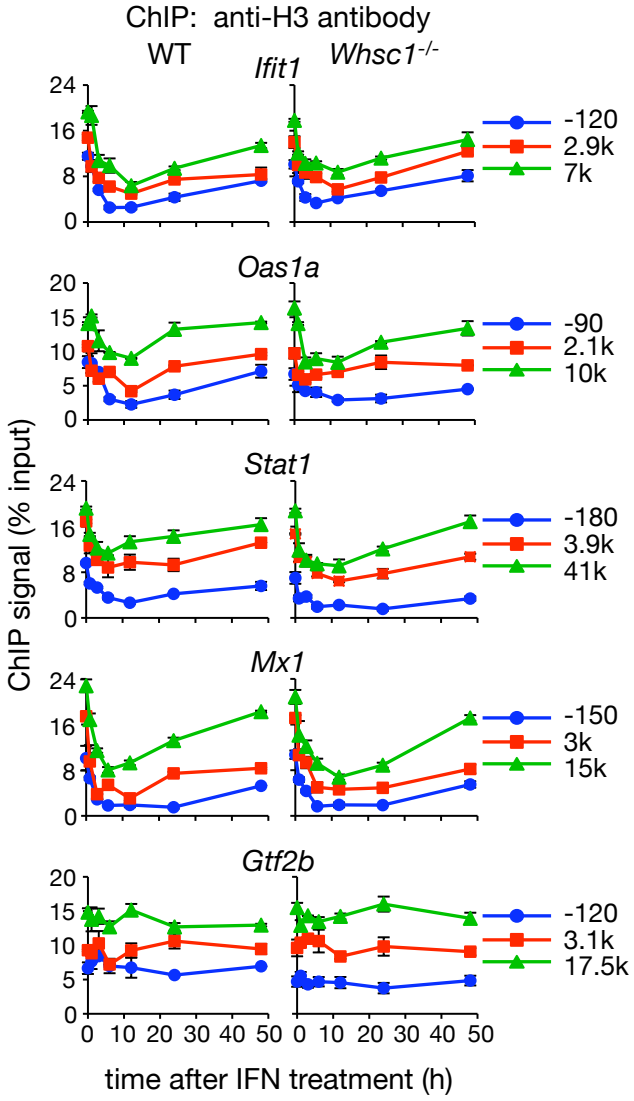

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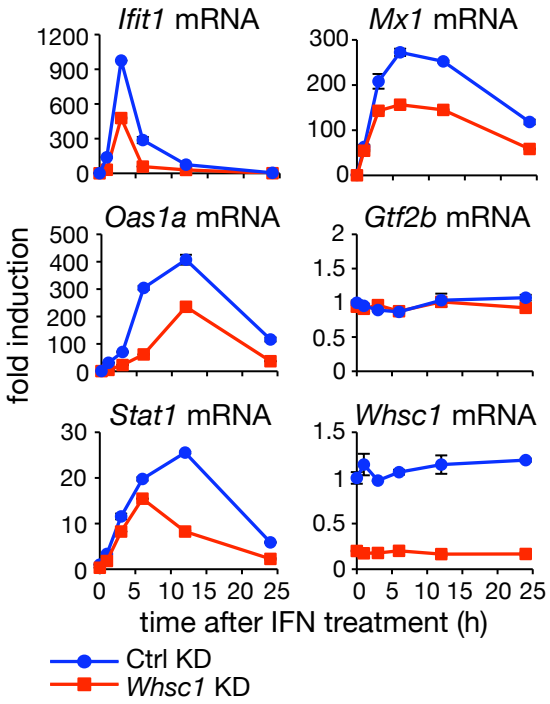

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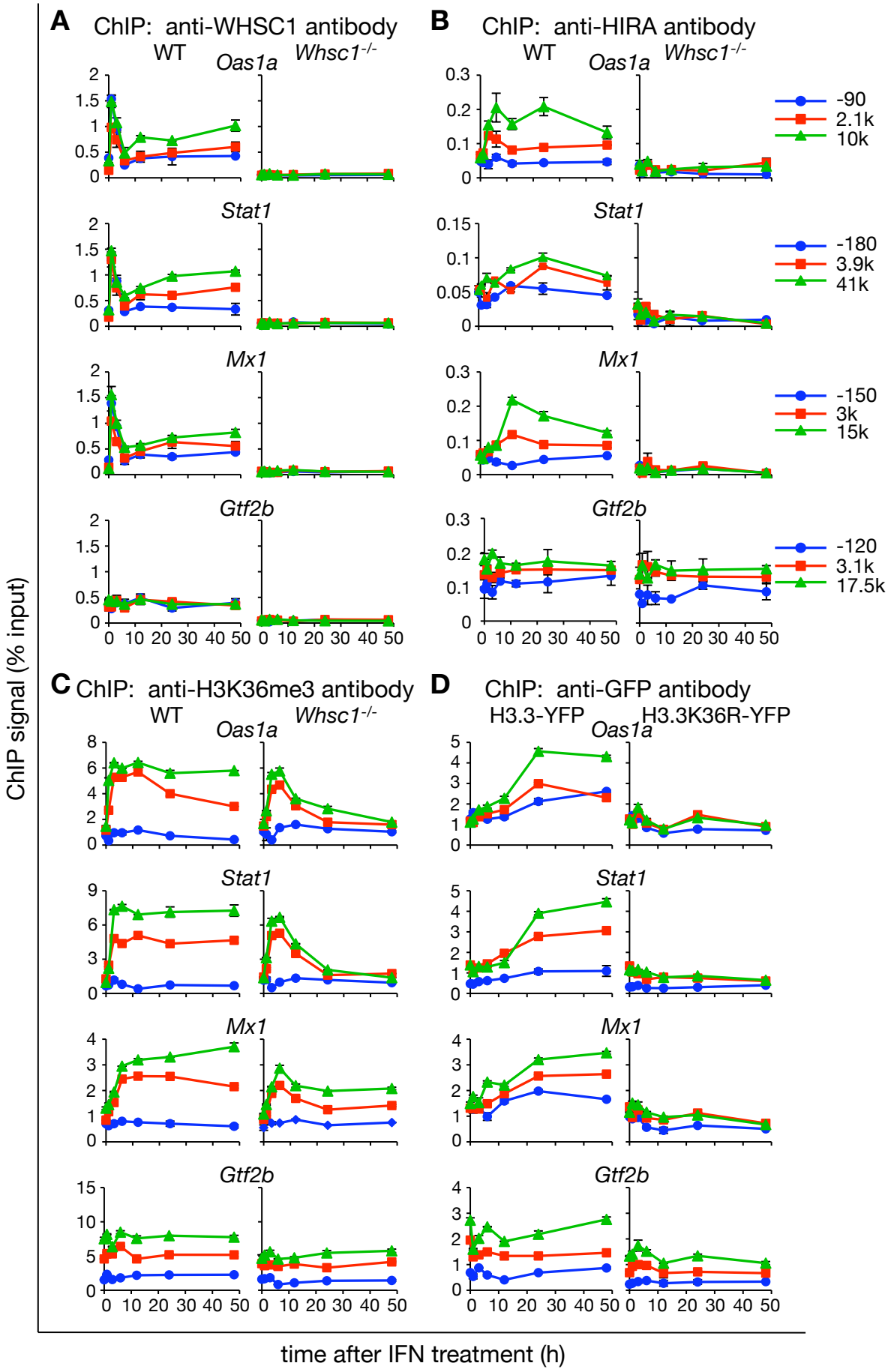

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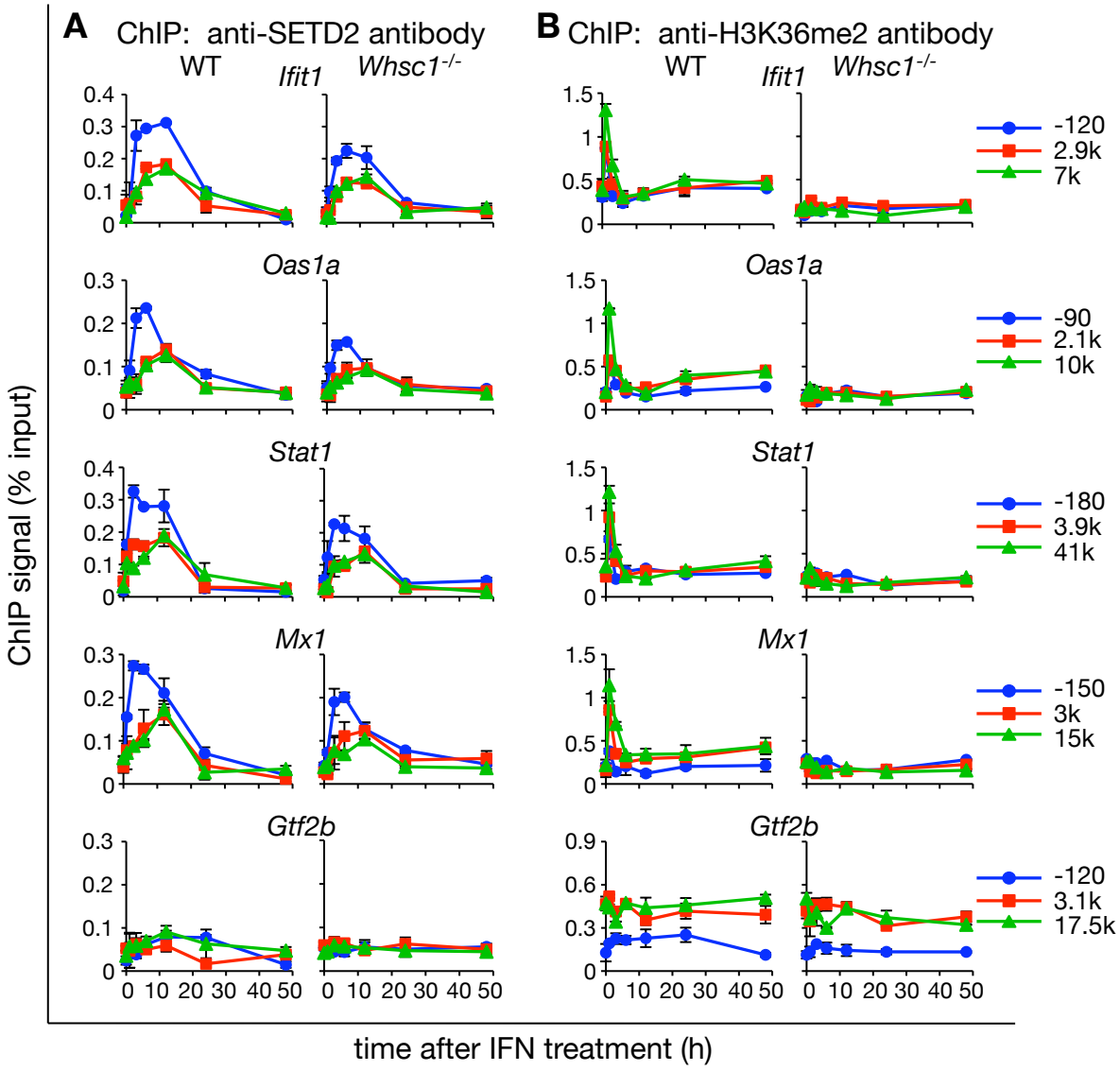

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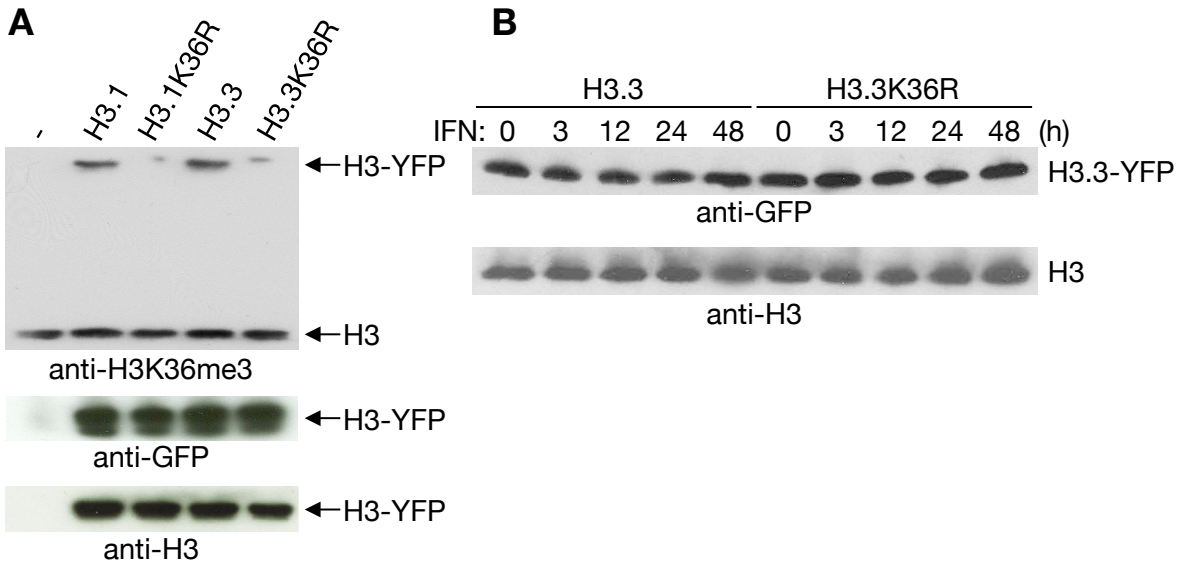

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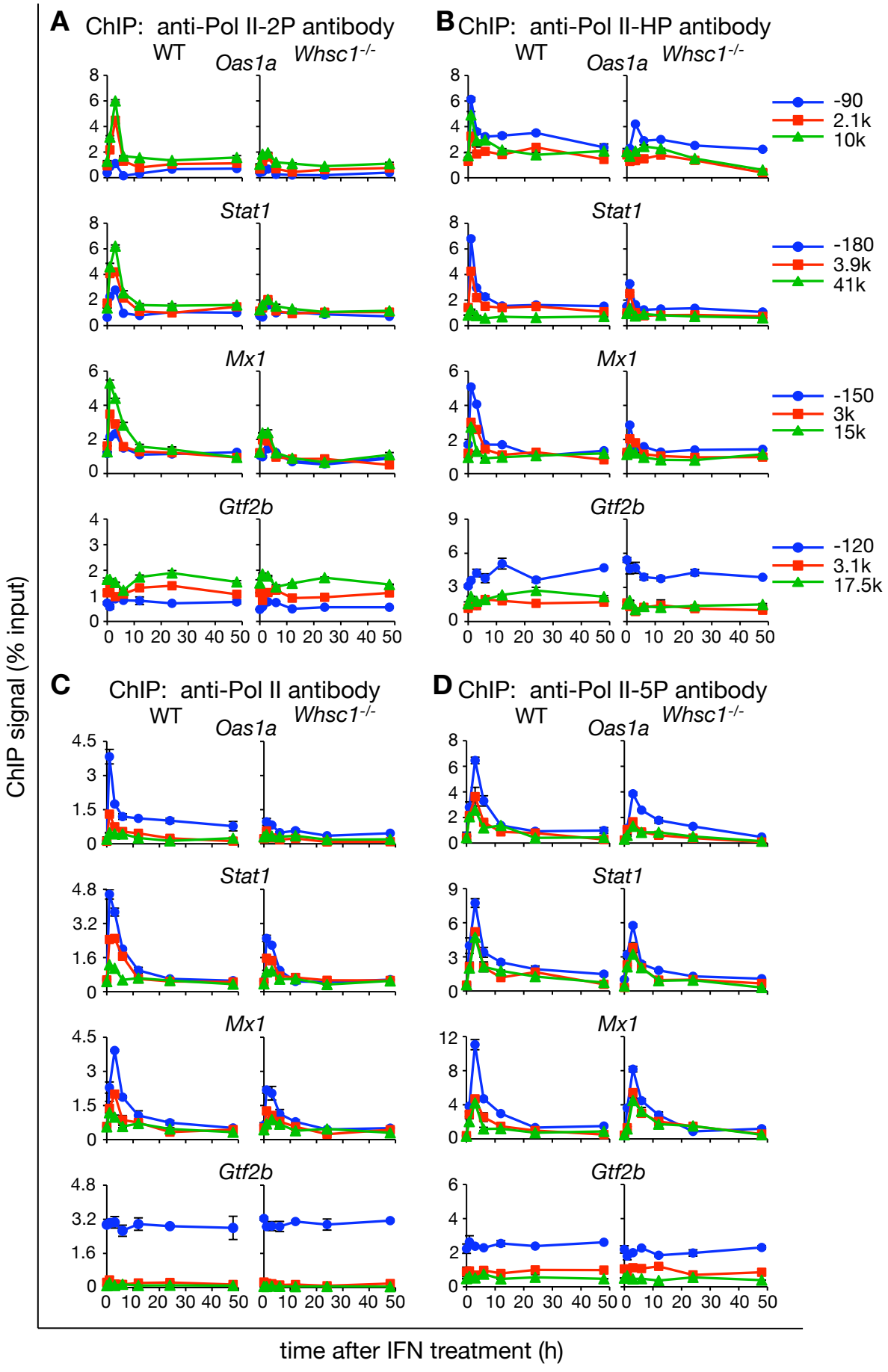

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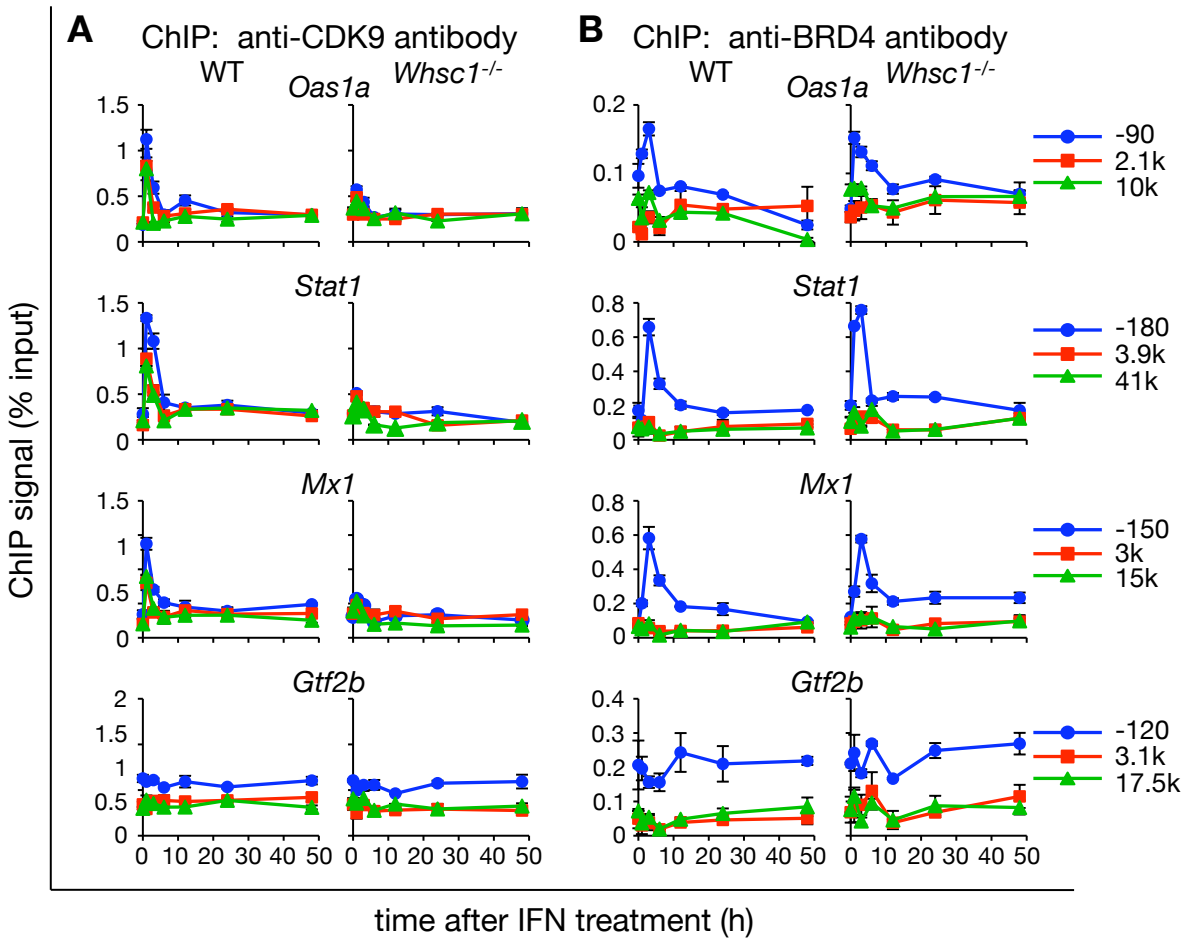

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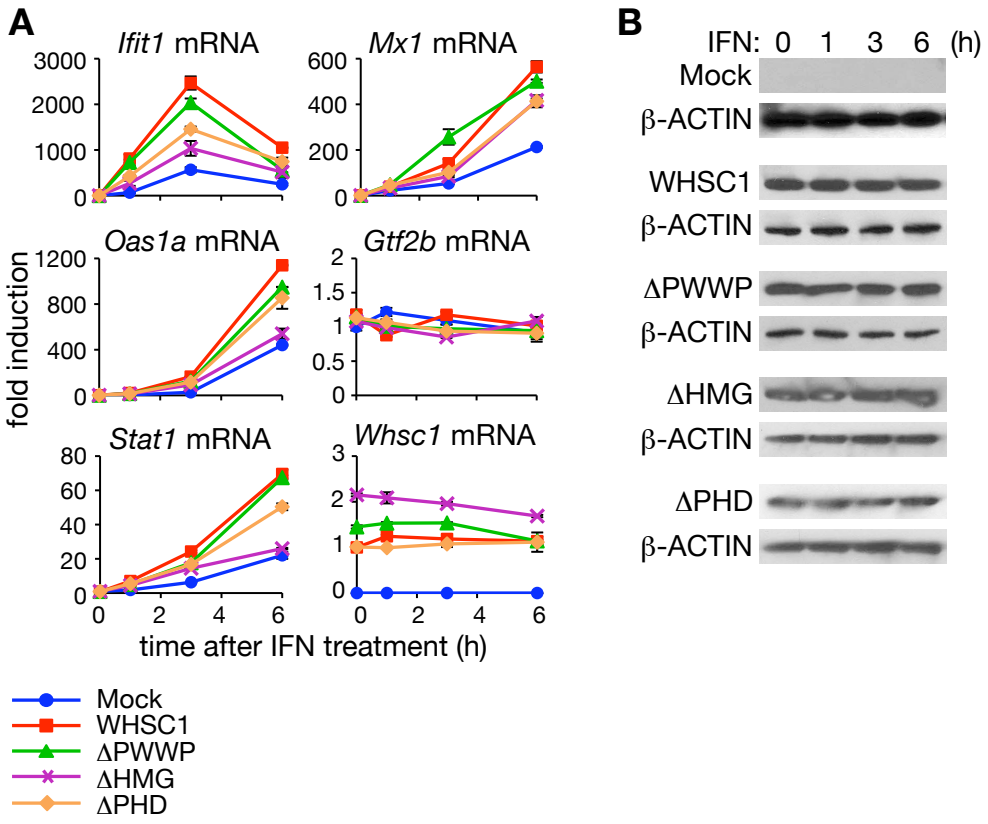

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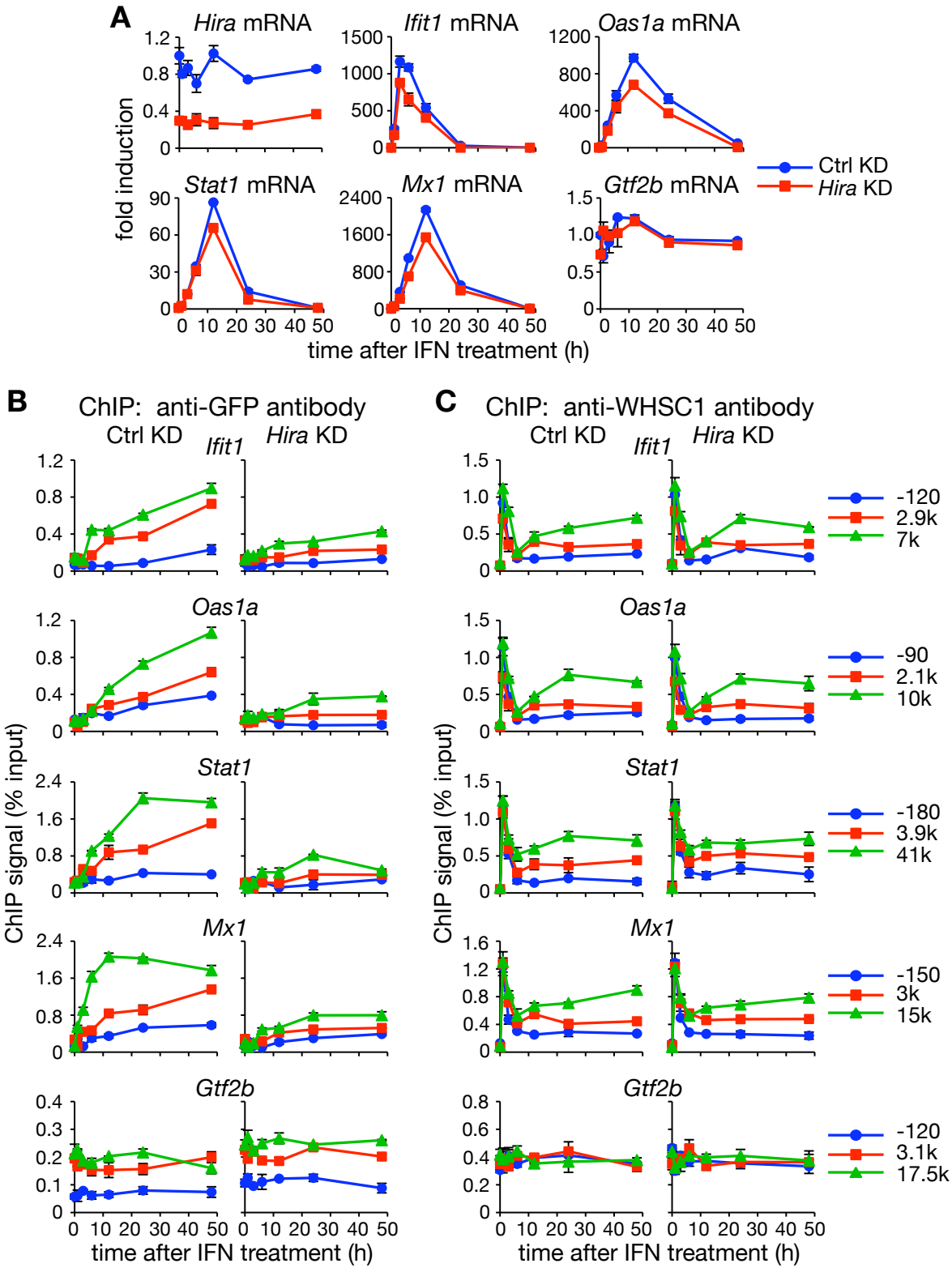

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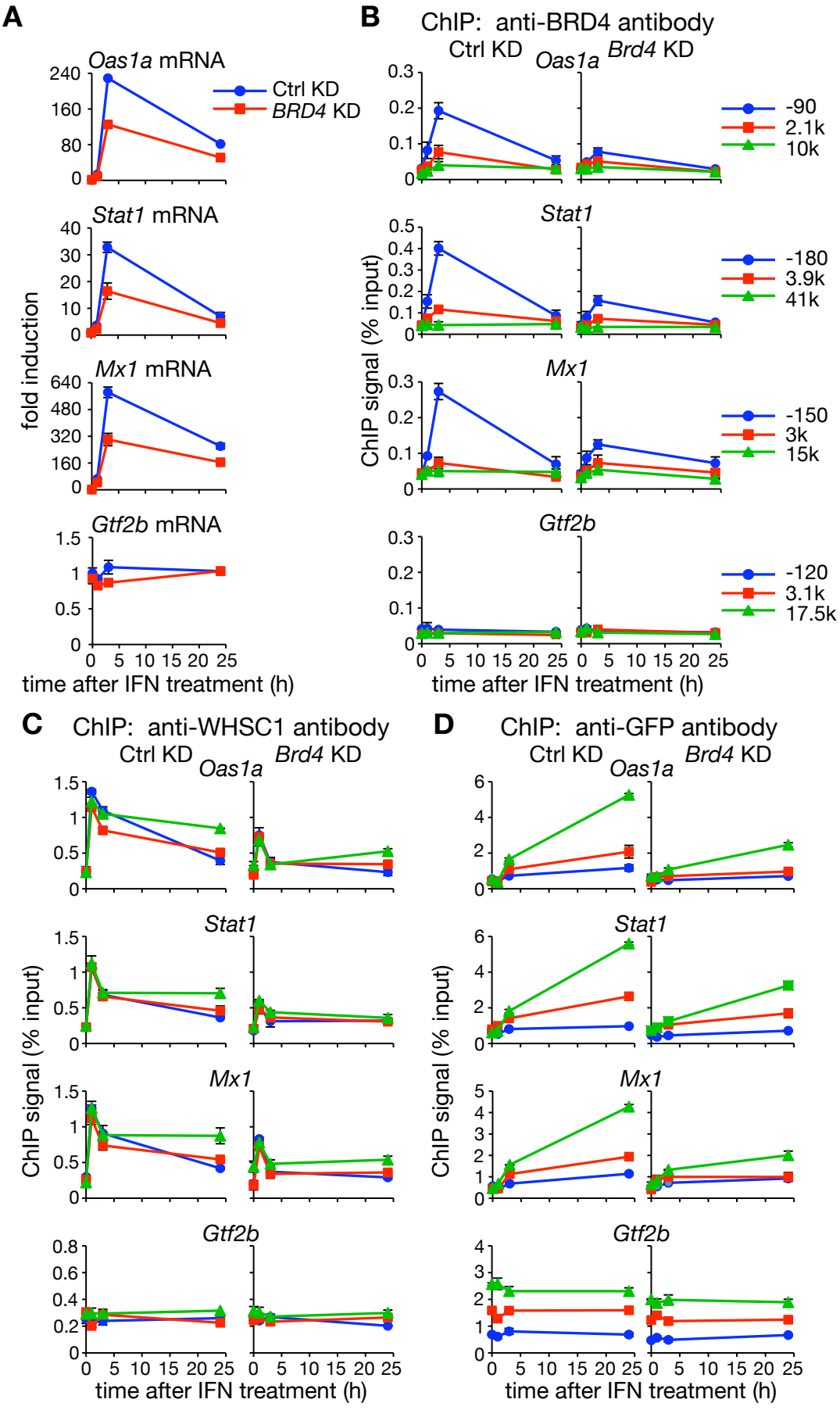

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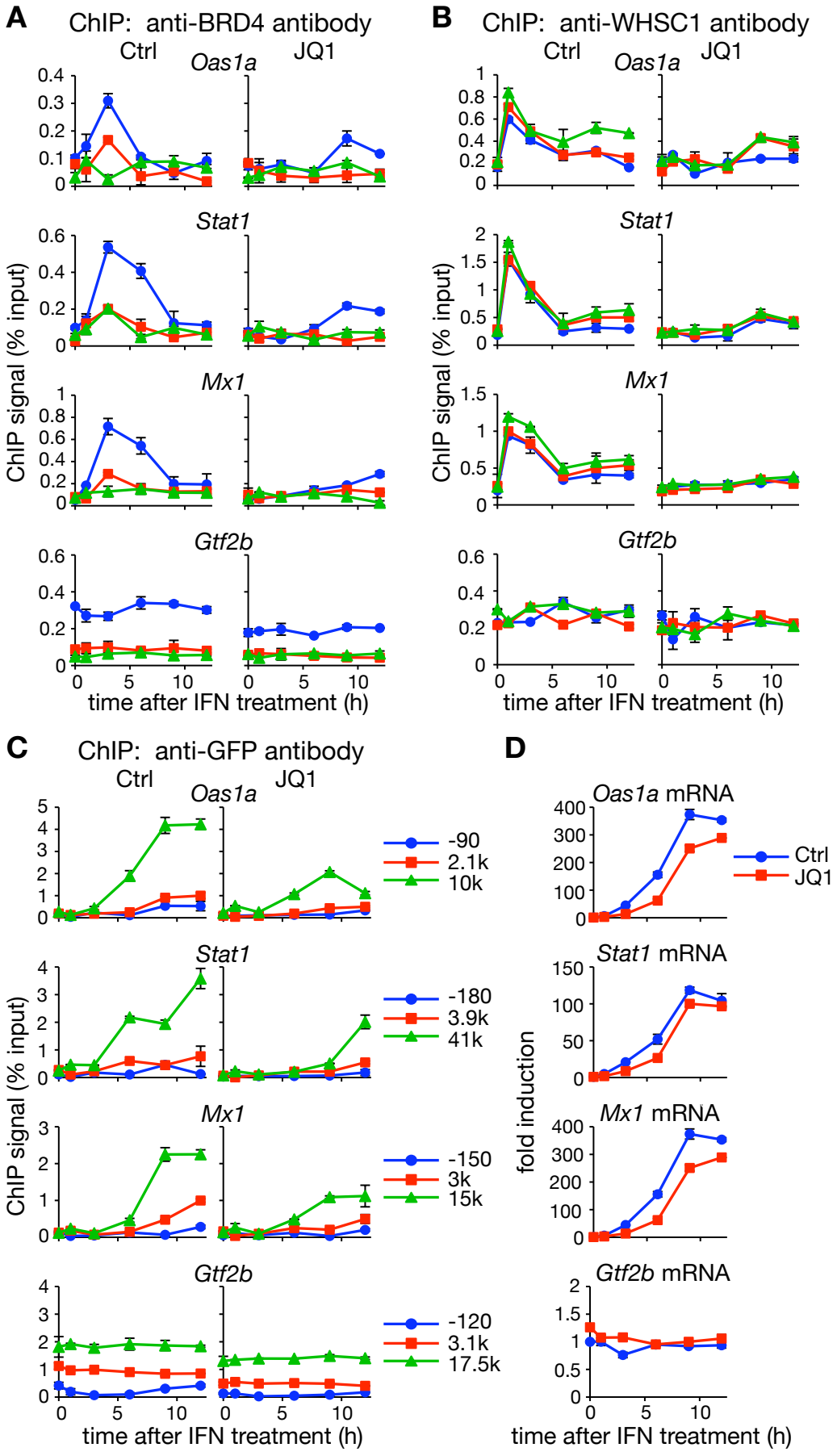

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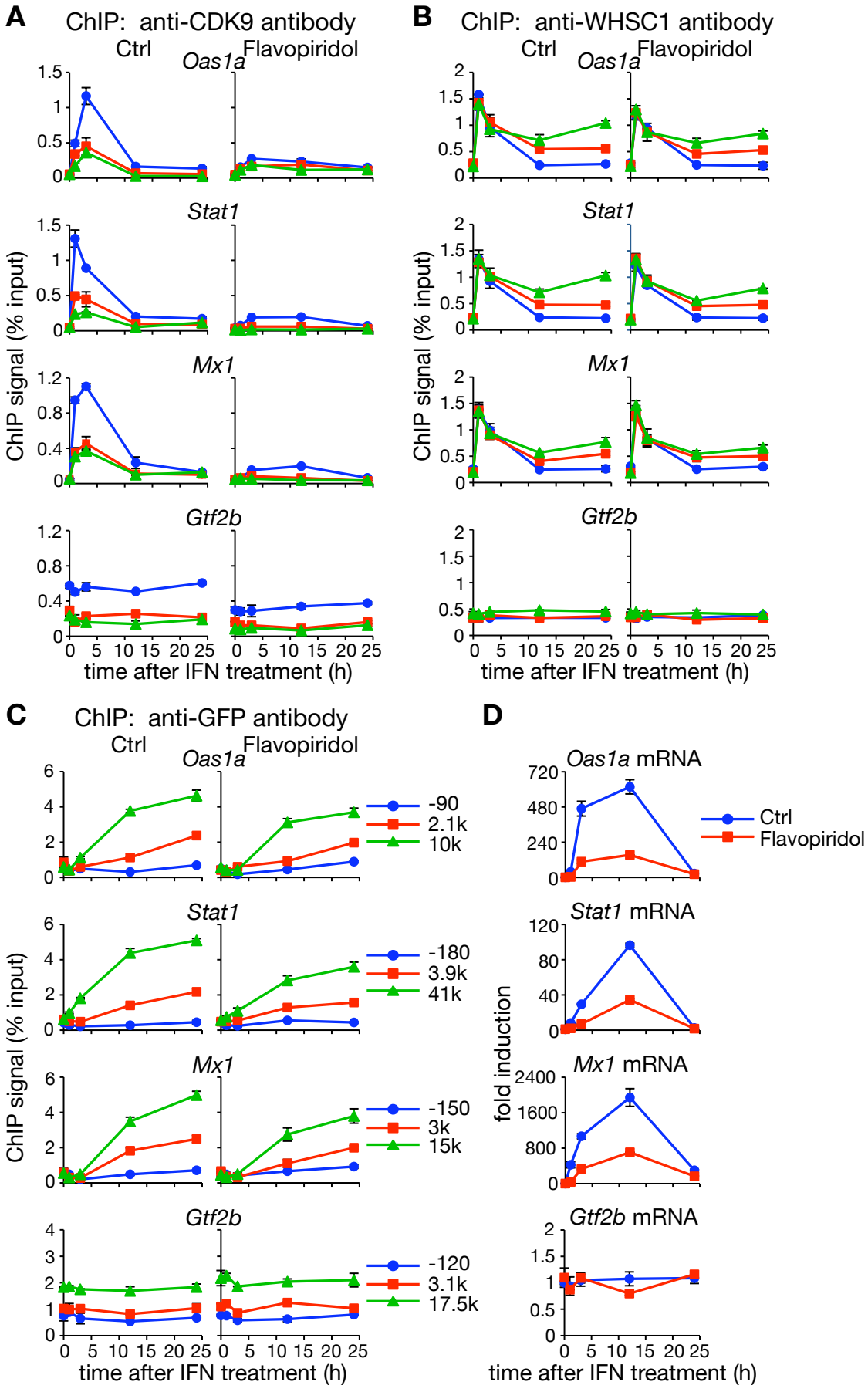

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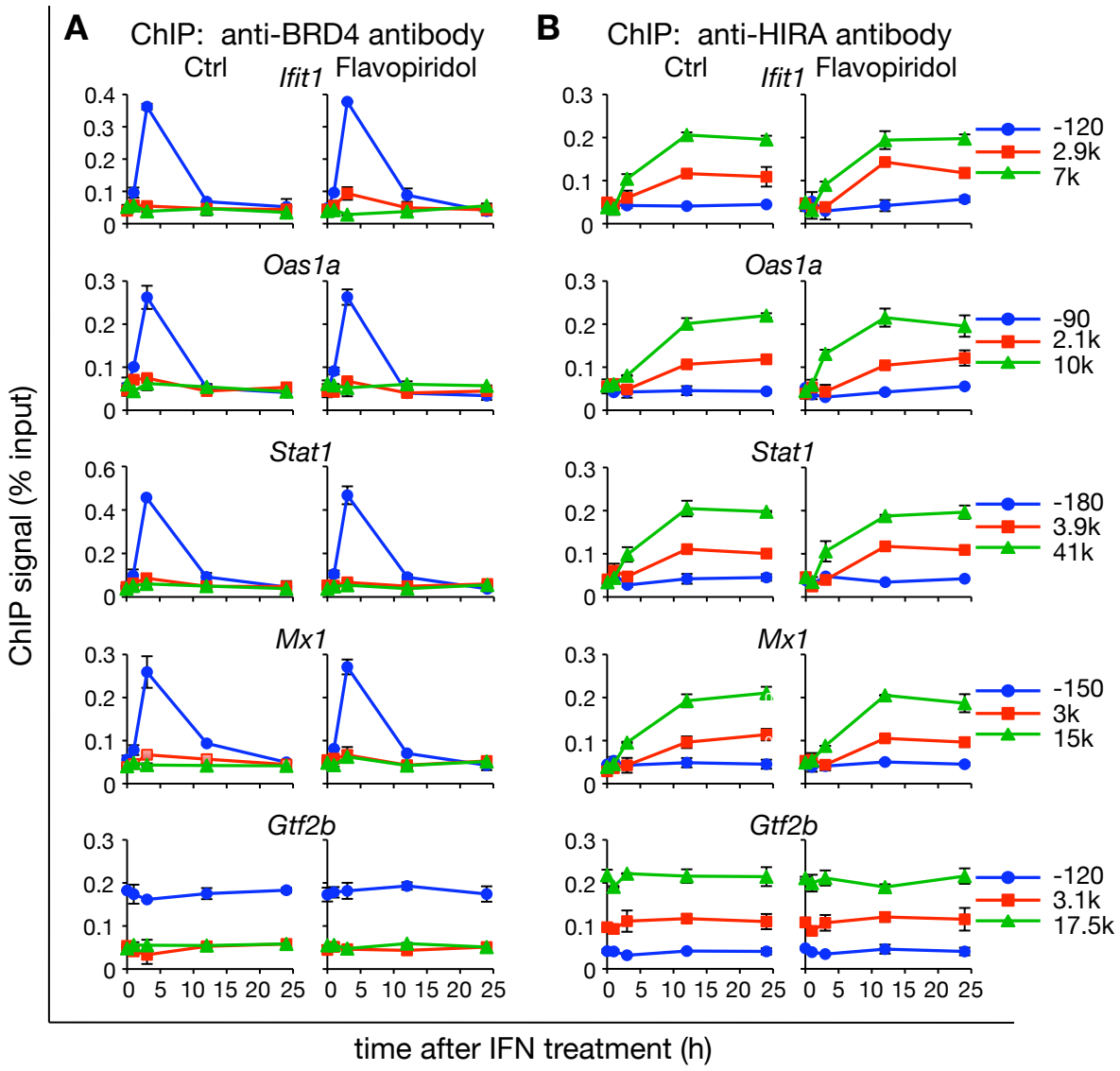

Supplementary Figure 16