# Input-specific regulation of hippocampal circuit maturation by non-muscle Myosin IIB

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**Supplementary Figure 1. The developmental expression of three non-muscle myosin isoforms**. Western blots from the hippocampus of PND8, PND14, PND21 and PND35 wild-type mice show protein expression of Myh9 (A), Myh10 (B) and Myh14 (C) proteins.

**Supplementary Figure 2. The compensation of non-muscle myosins in Myh10 ko slices**. Western blots from the hippocampus of PND14 wild type and *Myh10*$^{fl/fl}$ slices infected with Cre virus at PND1 show no compensation in Myh9 (A) and Myh14(B) protein levels. Student's t-test for Myh9 $t(7)=0.67$, $p=0.52$. Student's t-test for Myh14 $t(7)=1.77$, $p=0.12$.

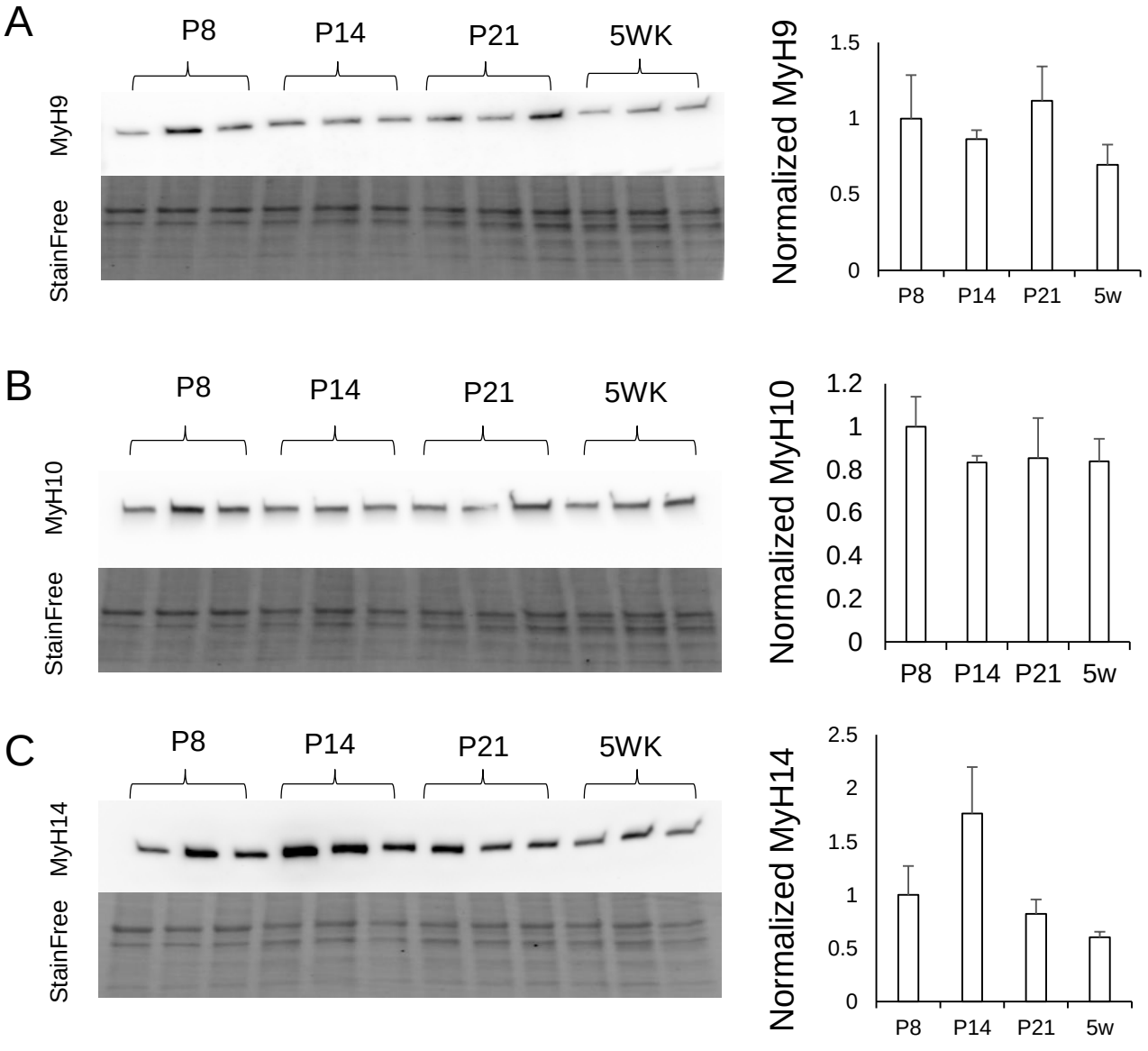
**Supplementary Figure 3. The effect of myosin II inhibition on basal synaptic transmission in adult hippocampal slices**. (A). 50 µM Blebbistatin did not affect magnitude of fEPSP slope in CA1 after 1 hour of incubation (RM ANOVA $F(1,13)=0.048$, $p=0.830$, n=9,control; n=6, Blebb). Black bar = bath application of drug. Traces: Averaged fEPSPs for pre(grey)- and post (black)-drug application. (B) Inhibition of myosin-II had no effect on pre-synaptic release probability as measured by paired pulse ratio in whole-cell voltage clamp recordings (RM ANOVA $F(1,14)=0.662$, $p=0.429$, n=9, control; n=7, blebb). All error bars represent SEM.

**Supplementary Figure 4. Normal pharmacologically isolated NMDA currents in Myh10 ko neurons**. (A) Representative NMDA EPSCs (in the presence of NBQX and picrotoxin) from proximal and distal stimulation locations in *Myh10*$^{fl/fl}$ animals injected with Cre. Recordings from neighboring Cre negative and Cre positive neurons (*Myh10* KO) show (B) normal proximal (paired $t(14)=0.98$,$p=0.34$) and (C) normal distal EPSCs (paired $t(14)=0.33$, $p=0.74$) at proximal stimulation locations (n=15 pairs from n=5 mice).

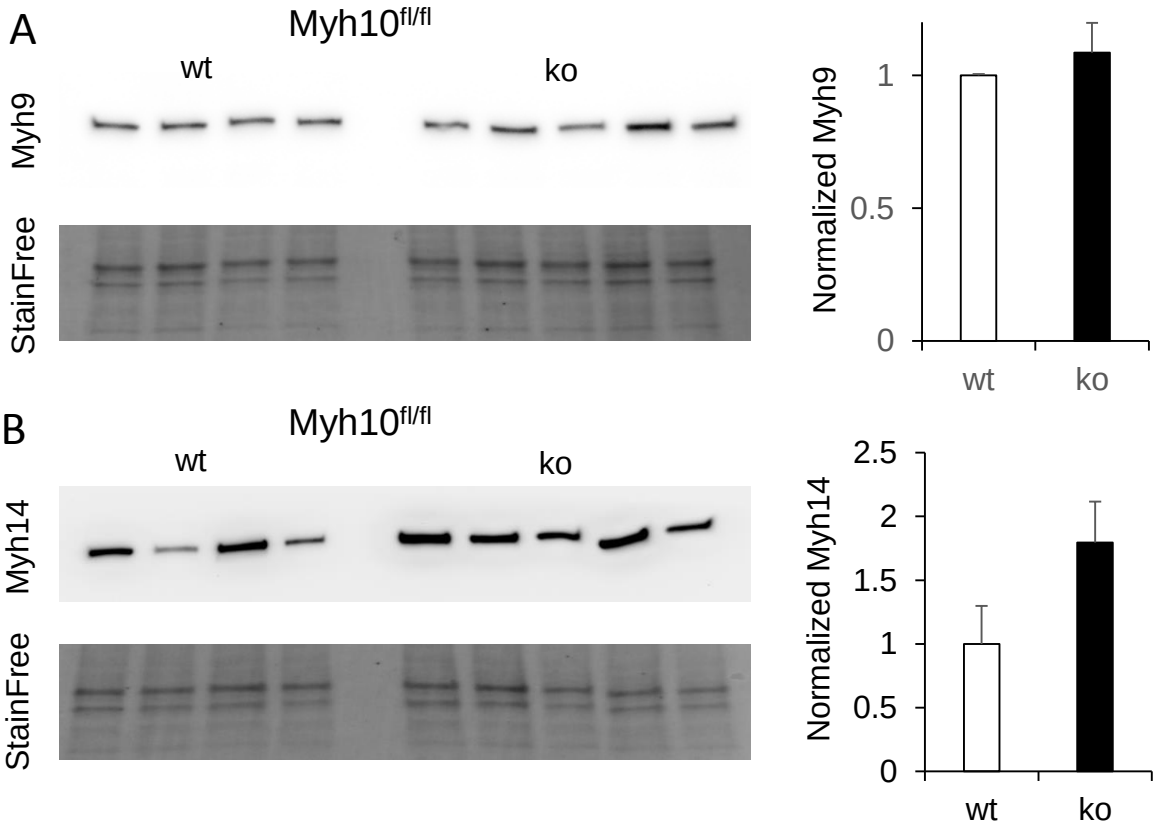
**Supplementary Figure 5. The compensation of non-muscle myosins in Myh9 ko and double ko slices**. Western blots from the hippocampus of PND14 *Myh9*$^{fl/fl}$ or *Myh10*$^{fl/fl}$*; Myh9*$^{fl/fl}$ slices infected with Cre virus at PND1 show no compensation in Myh10 (A) and Myh14(B) protein levels in Myh9 ko slices, and no compensation in Myh14 levels (C) in double ko slices. Student's t-test for Myh10 $t(6)=0.96$, $p=0.37$. Student's t-test for Myh14 in Myh9 ko $t(6)=0.98$, $p=0.36$. Student's t-test for Myh14 in double ko $t(8)=1.78$, $p=0.11$.

**Supplementary Figure 6. The input specific effects of NMIIA and NMIIB deletion on evoked EPSCs**. Cre positive neurons from *Myh10*$^{fl/fl}$*; Myh9*$^{fl/fl}$ slices have lower ratio of proximal to distal

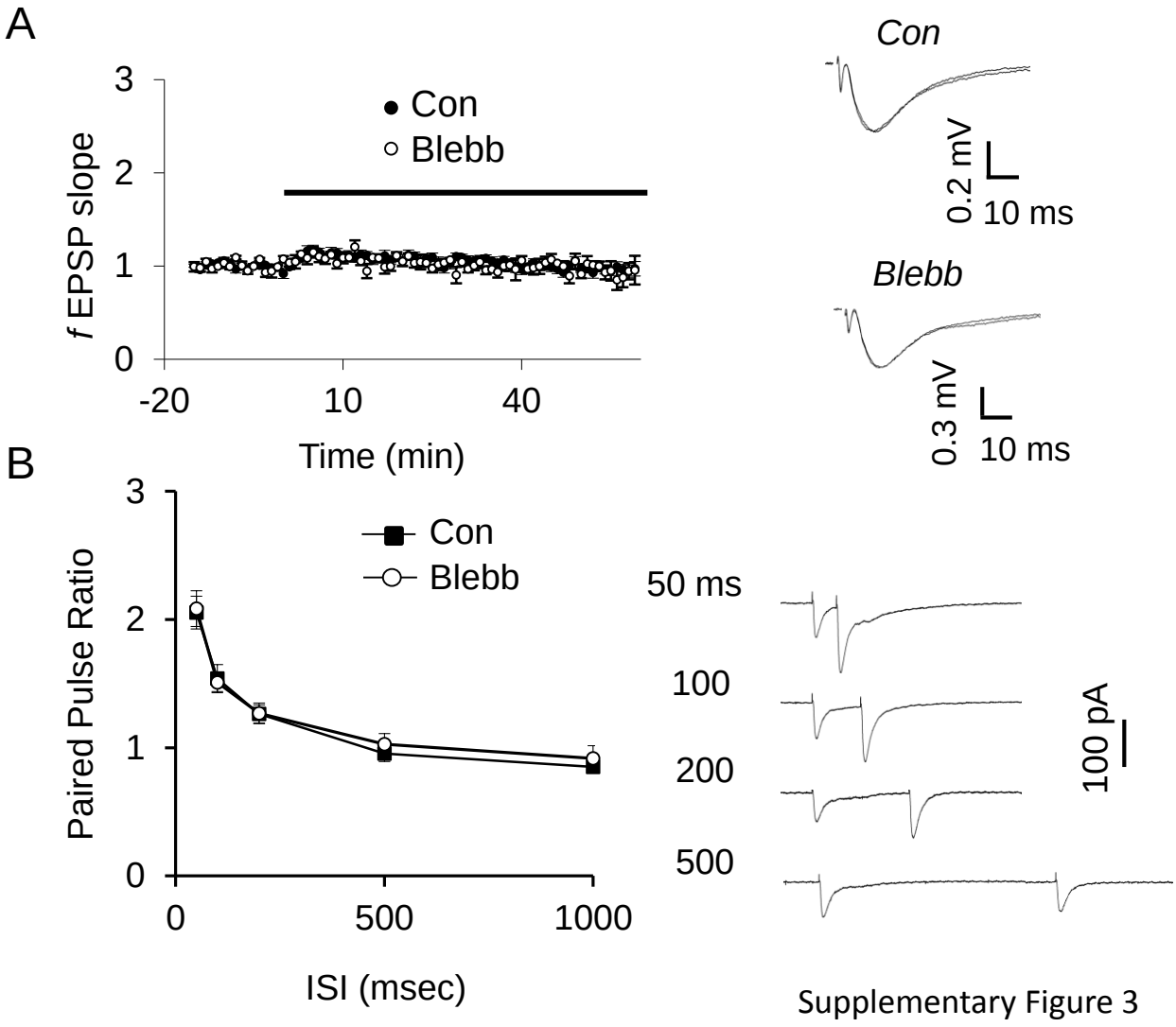
EPSC amplitude compared to Cre negative neurons from the same slice. $t(13)=2.34$, $p=0.034$. Dashed black line is identity line. Filled circle is the sample mean.



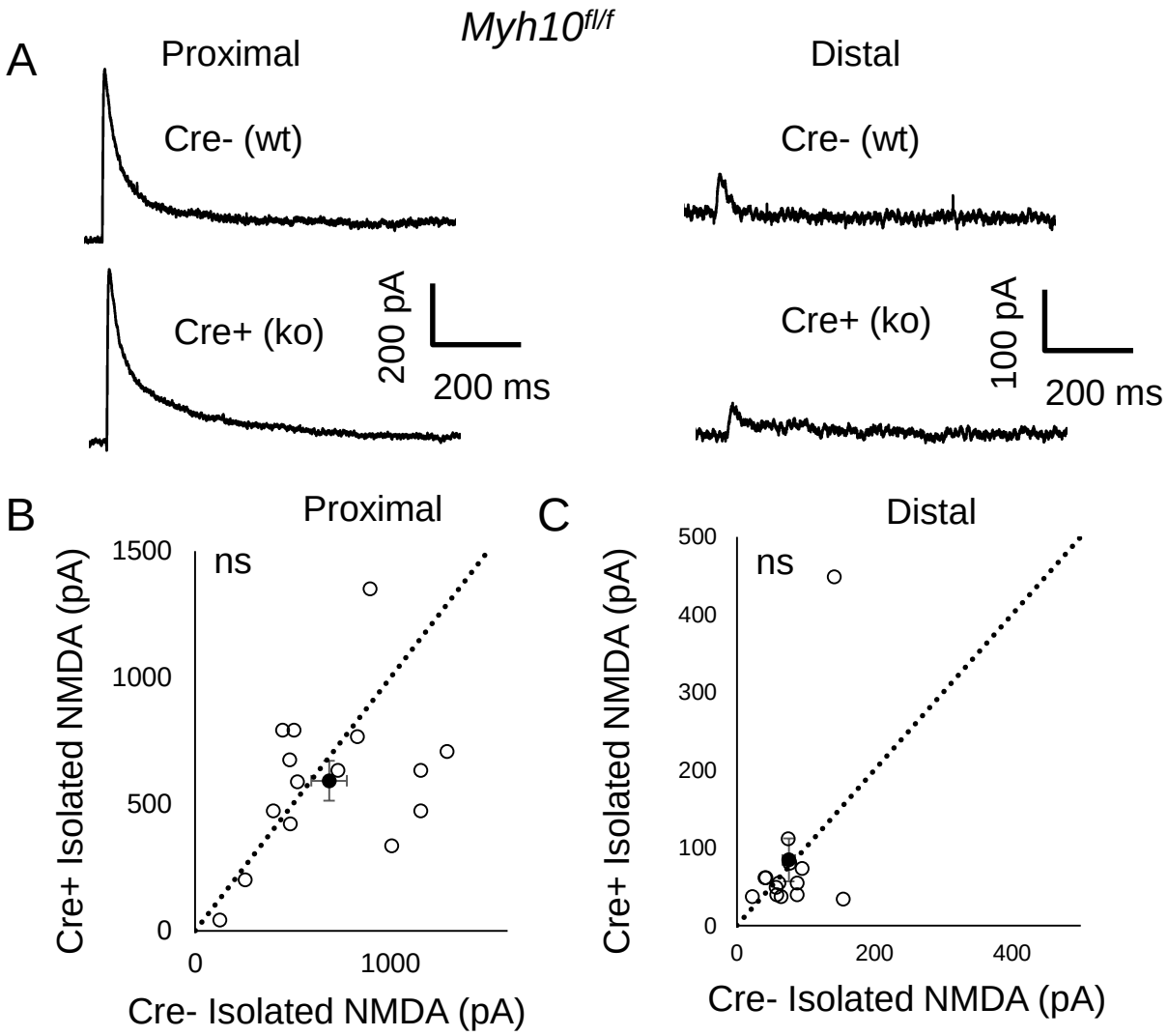

Supplementary Figure 1



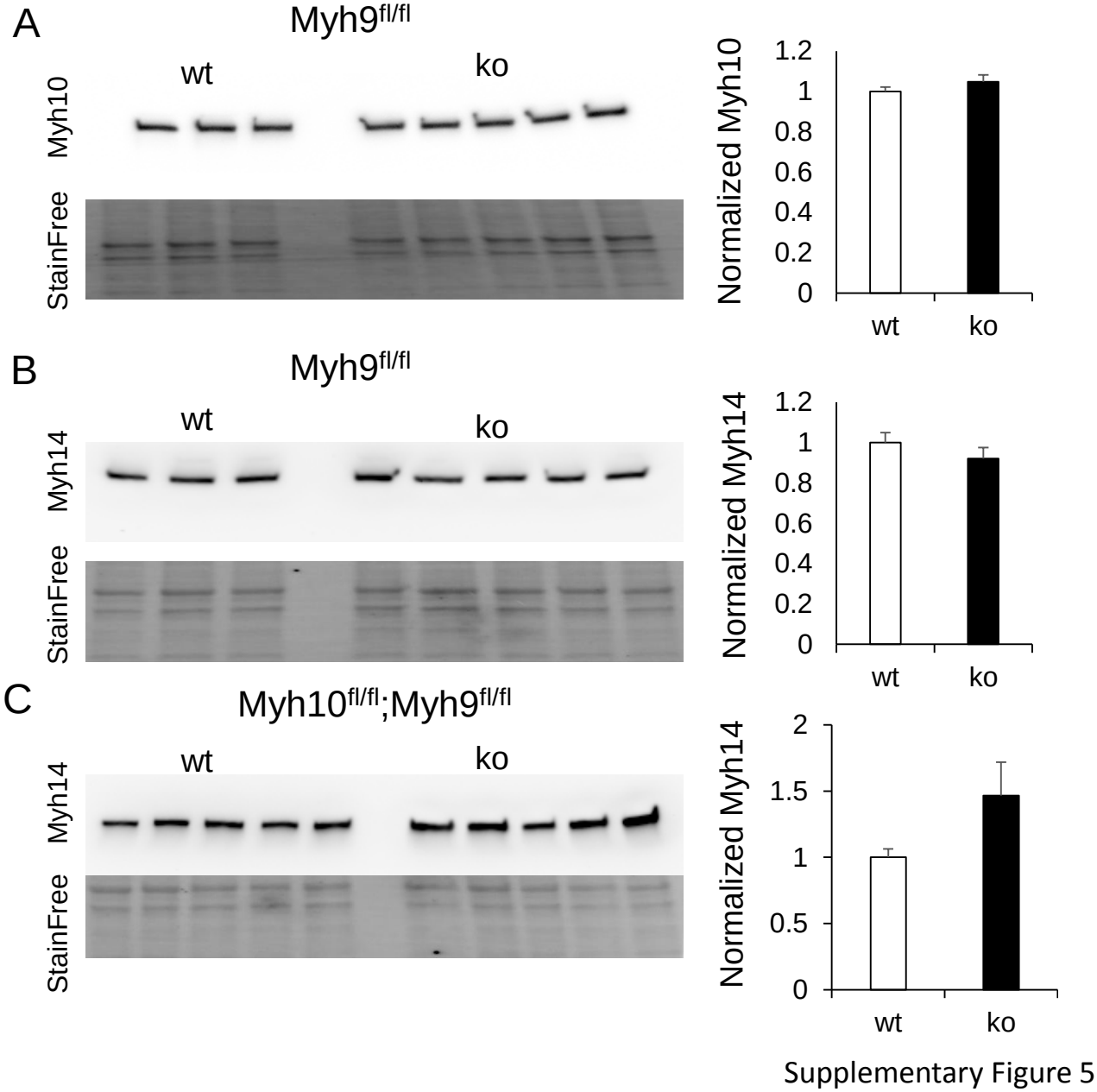

Supplementary Figure 2



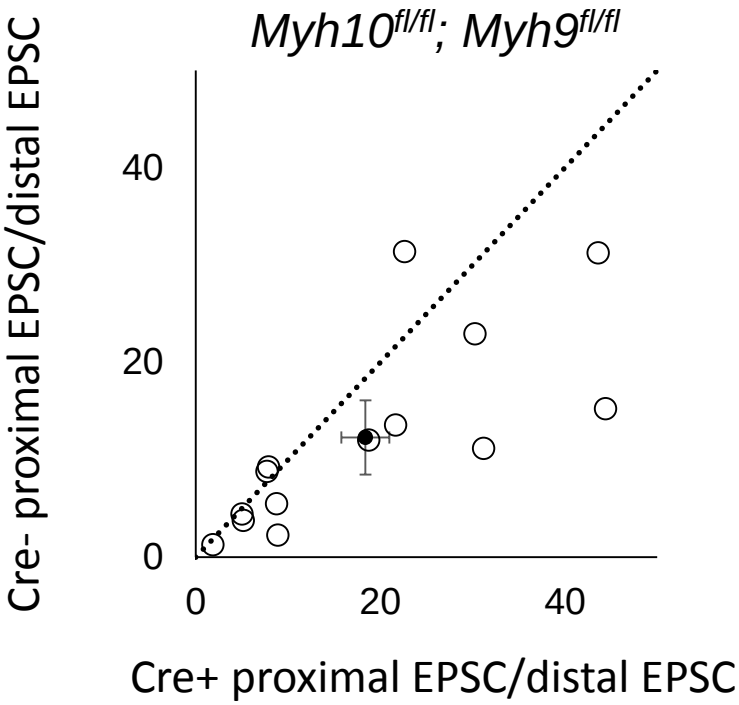

Supplementary Figure 3




Supplementary Figure 4




Supplementary Figure 5




Supplementary Fig 6