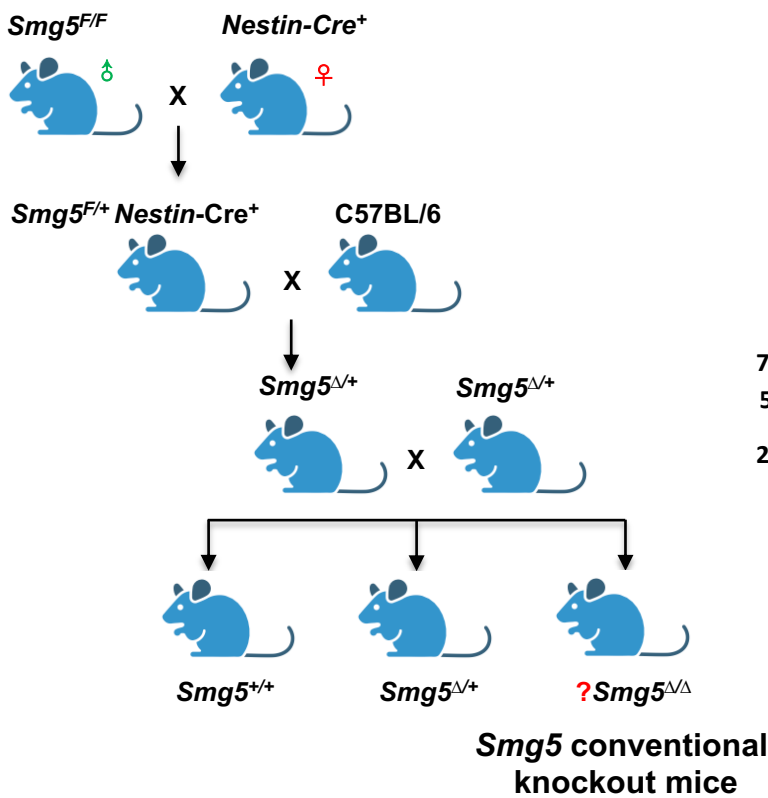


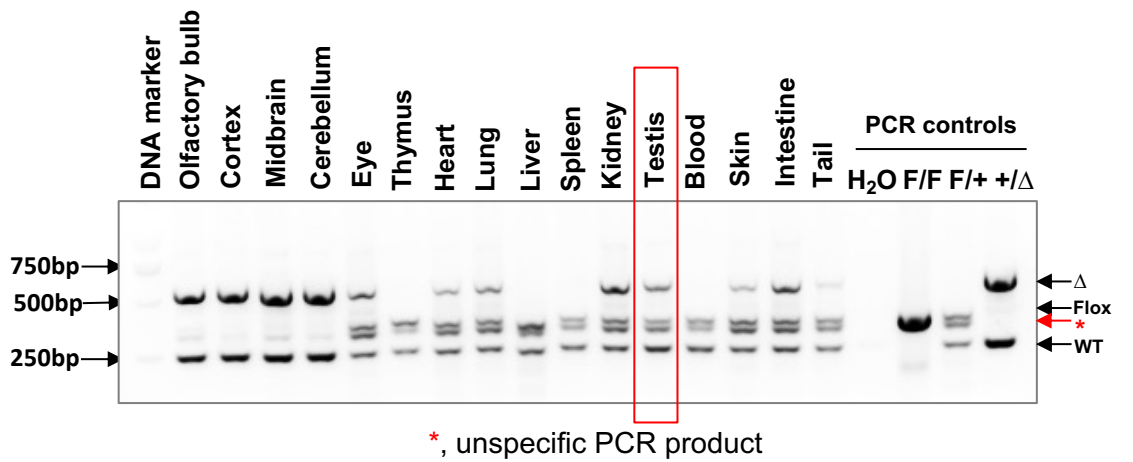
## **Supplementary Figures**

Supp Figure S1

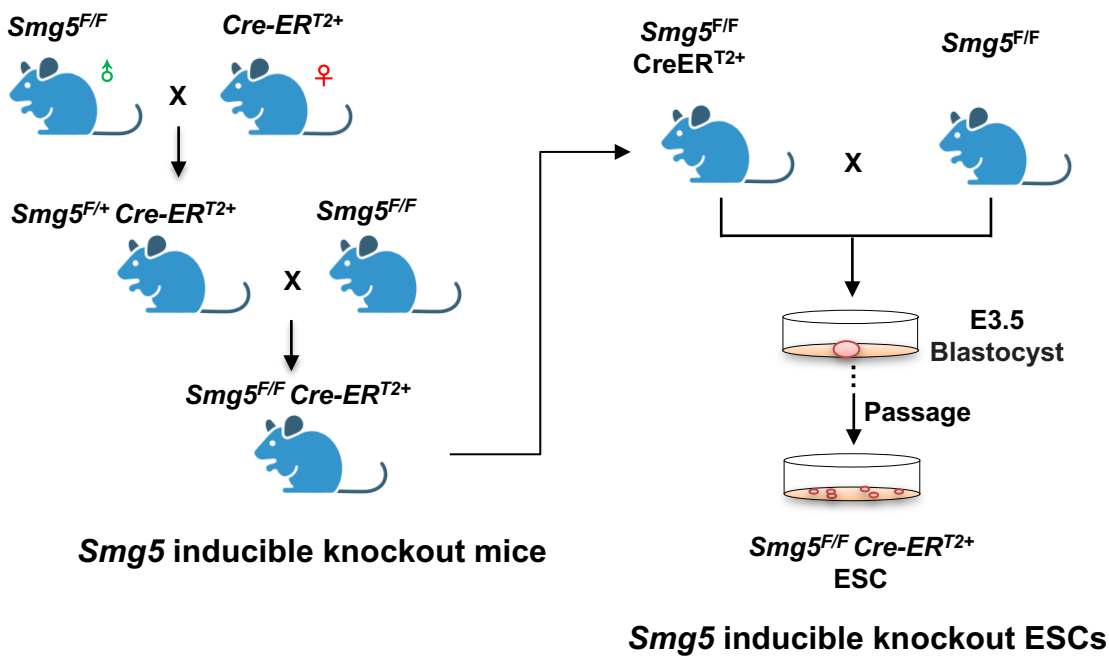
A



B



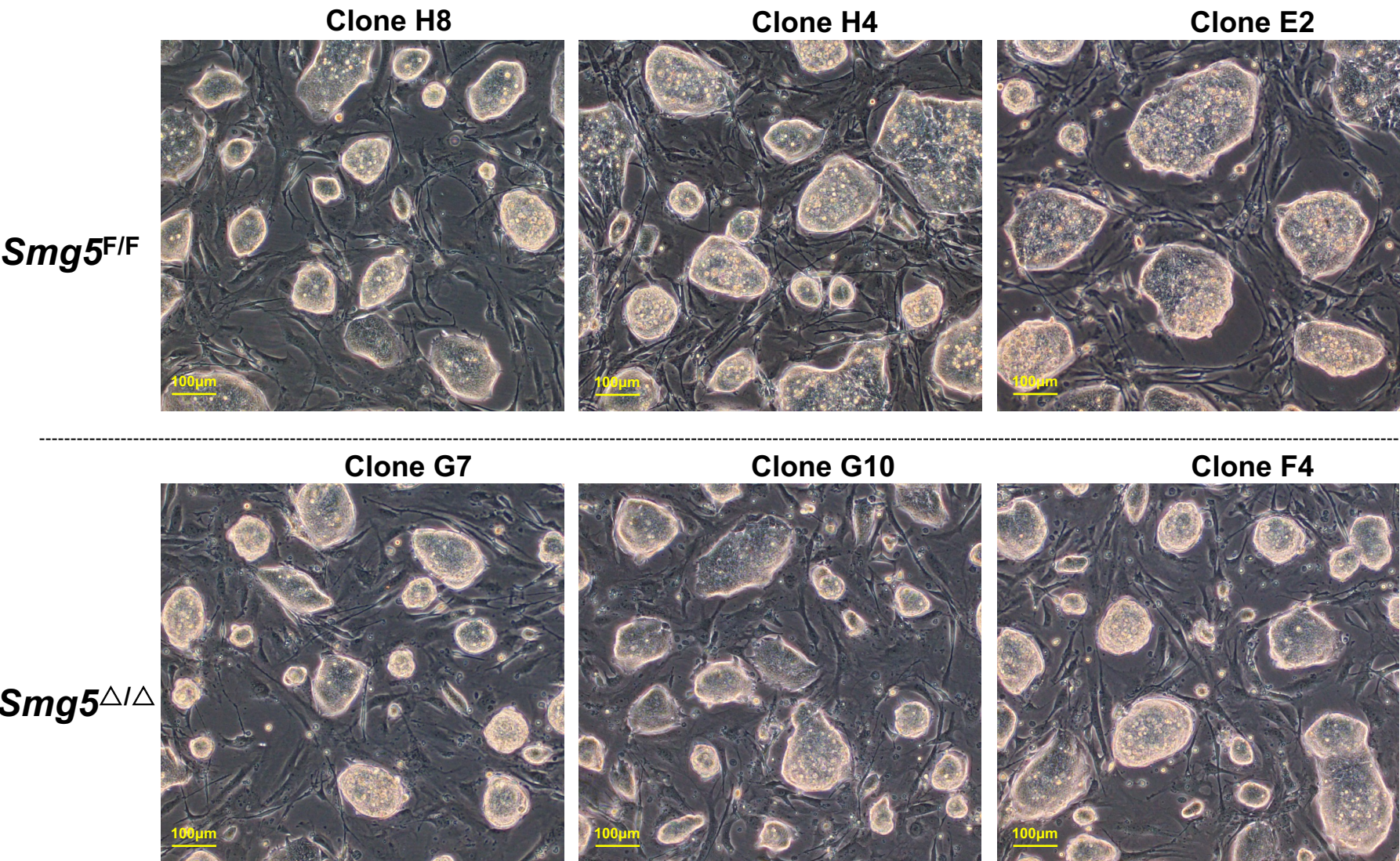
C



**Supp Figure S1. Generation of *Smg5*<sup>Δ/Δ</sup> mice and *Smg5* inducible knockout mESCs. A.** Breeding strategy to generate *Smg5*<sup>Δ/Δ</sup> mice. **B.** PCR analysis on *Smg5* deletion in various tissues from a male *Smg5*<sup>F/F</sup>*Nestin-Cre*<sup>+</sup> mouse. **C.** Strategy to establish *Smg5* inducible knockout mESCs (*Smg5*<sup>F/F</sup>*Cre-ERT2*<sup>+</sup> mESCs).

Supp Figure S2

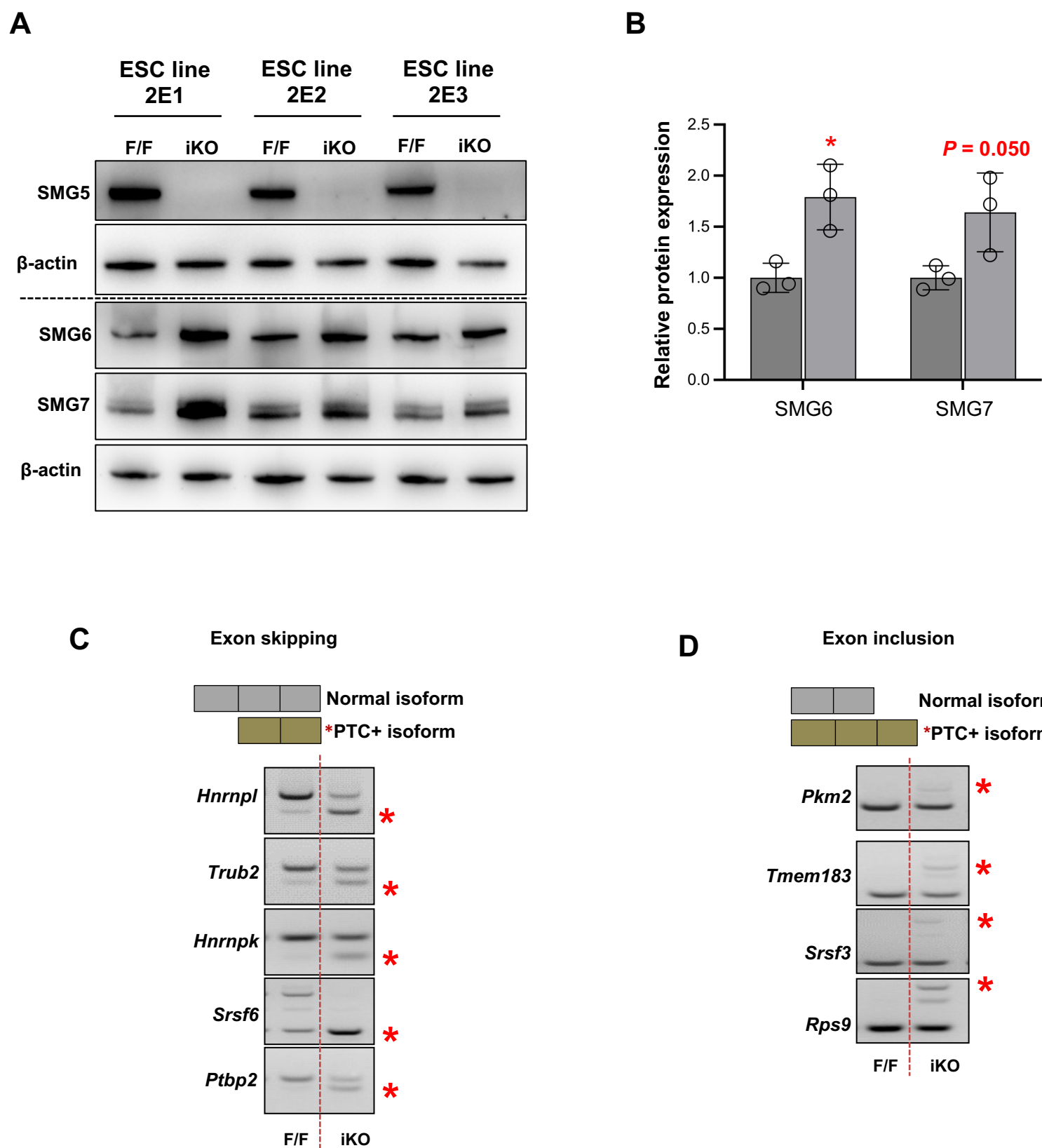
A



Supp Figure S2. Morphology of control (H8, H4, and E2) and *Smg5<sup>Δ/Δ</sup>* (G7, G10, F4) mESCs growing on feeders.



## Supp Figure S3

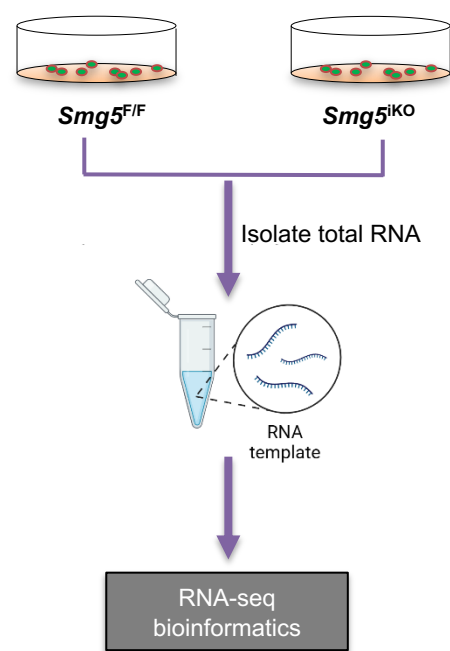


**Supp Figure S3. SMG5 loss inhibits NMD activity in mESCs.** **A.** Western blotting analysis of SMG5, SMG6 and SMG7 in control and *Smg5*<sup>iKO</sup> mESCs. β-actin is used as a loading control. Note: 2E1, 2E2 and 2E3 are three parental *Smg5* inducible knockout mESC lines. **B.** Quantification of the protein levels of SMG6 and SMG7 in control and *Smg5*<sup>iKO</sup> mESCs. **C.** Accumulation of PTC<sup>+</sup> isoforms generated with exon skipping events in *Smg5*<sup>iKO</sup> mESCs. **D.** Accumulation of the PTC<sup>+</sup> isoforms generated with exon inclusion in *Smg5*<sup>iKO</sup> mESCs. Note: Unpaired Student's *t*-test was carried out for statistical analysis. \*,  $p < 0.05$ .

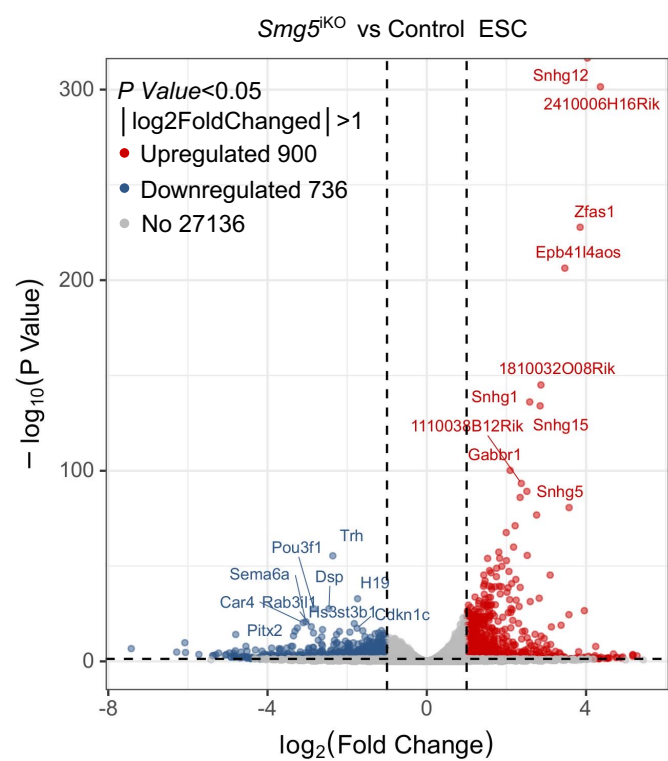


# Supp Figure S4

A



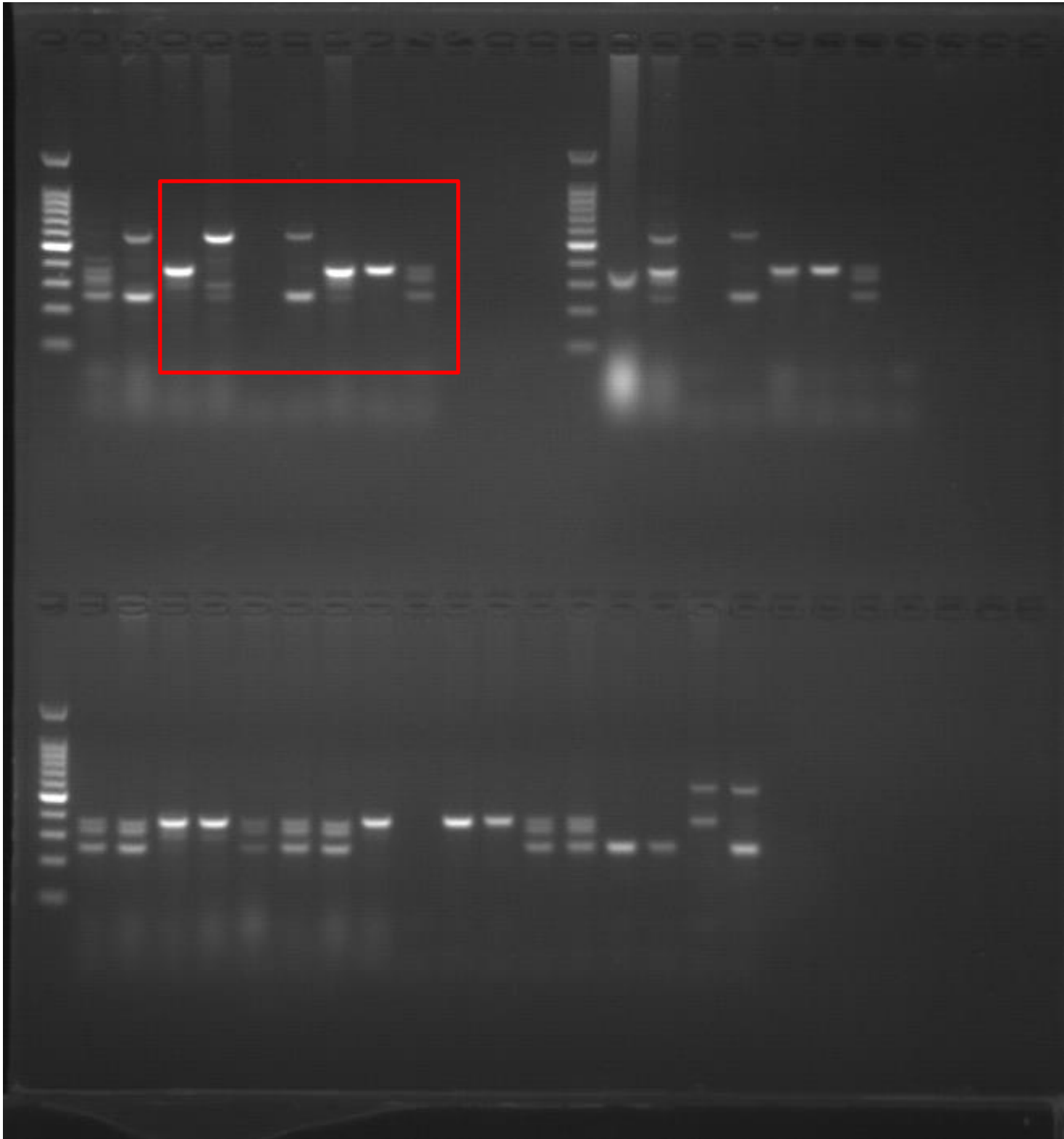
B



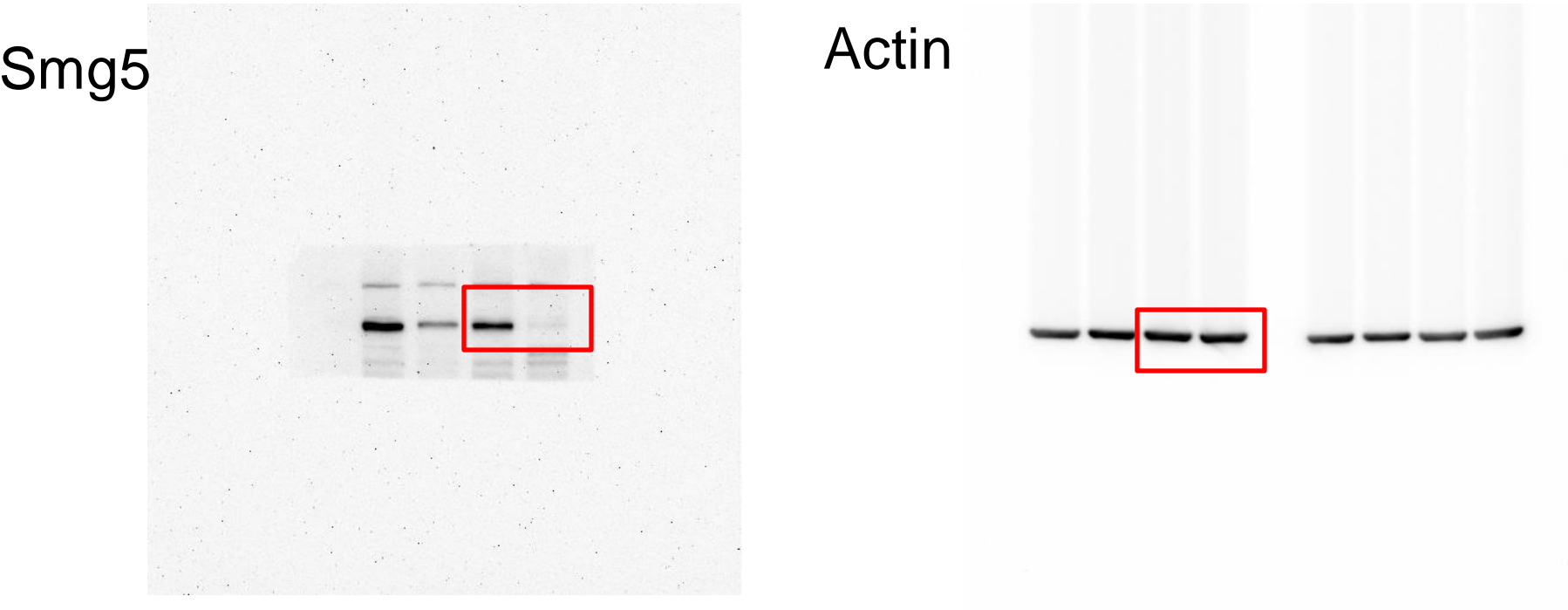
**Supp Figure S4. Transcriptome analysis between control and *Smg5<sup>IKO</sup>* mESCs.**  
**A.** Schematic diagram of mESCs transcriptome sequencing. **B.** Volcano map displayed differentially expressed genes in control and *Smg5<sup>IKO</sup>* mESCs. A total of 1636 differentially expressed genes were identified. Of these DEGs, 900 genes were upregulated, and 736 genes were downregulated ( $|\log_2\text{FoldChanged}| > 1$ ,  $P \text{ Value} < 0.05$ ).

**Original scans for main figures and supp figures**

Related to Figure 2B



Related to Figure 2D





# Related to Fig 2

Fig 2F

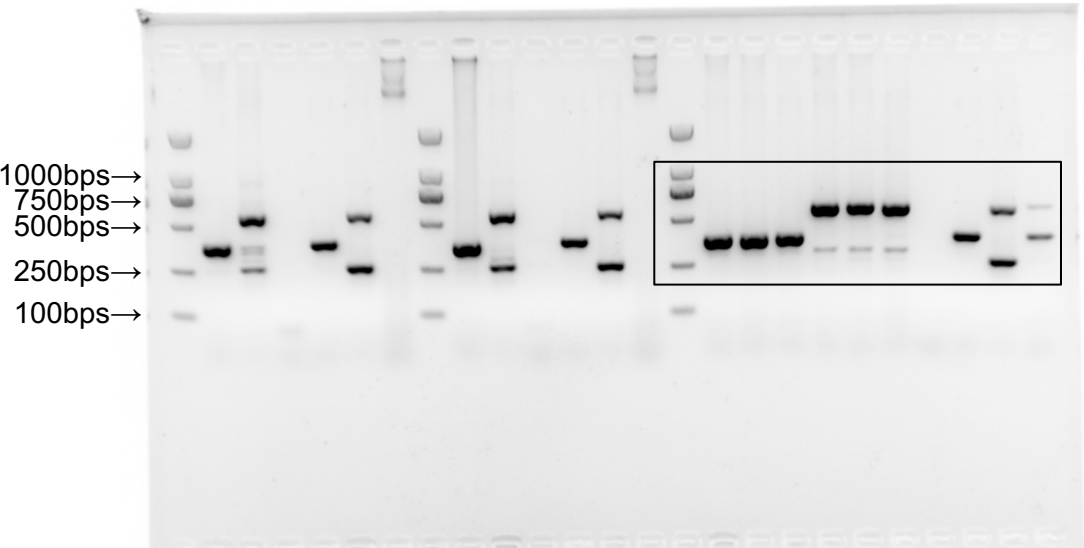
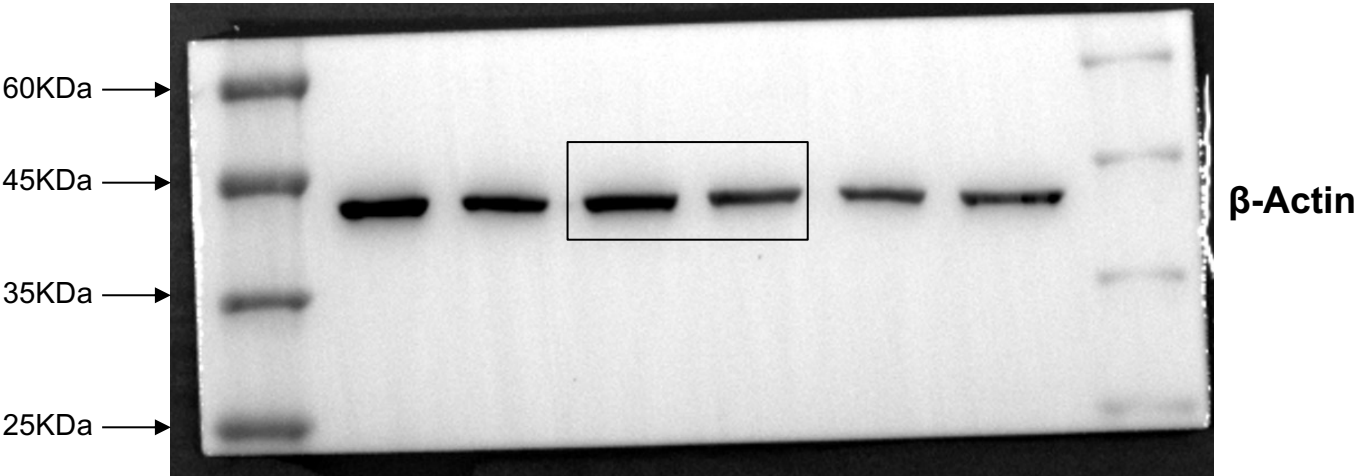
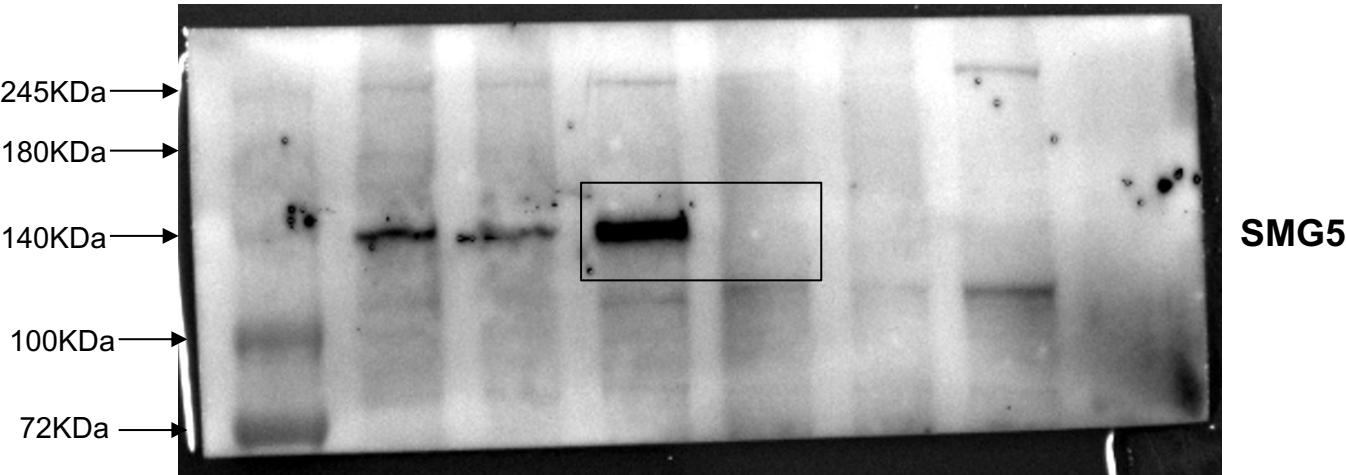
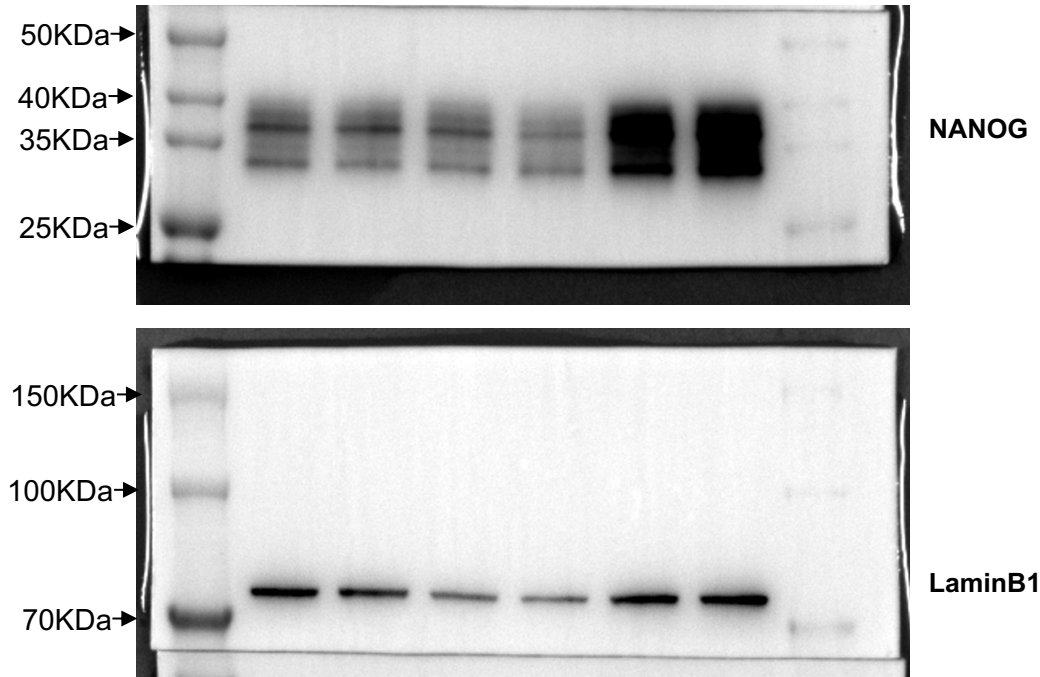
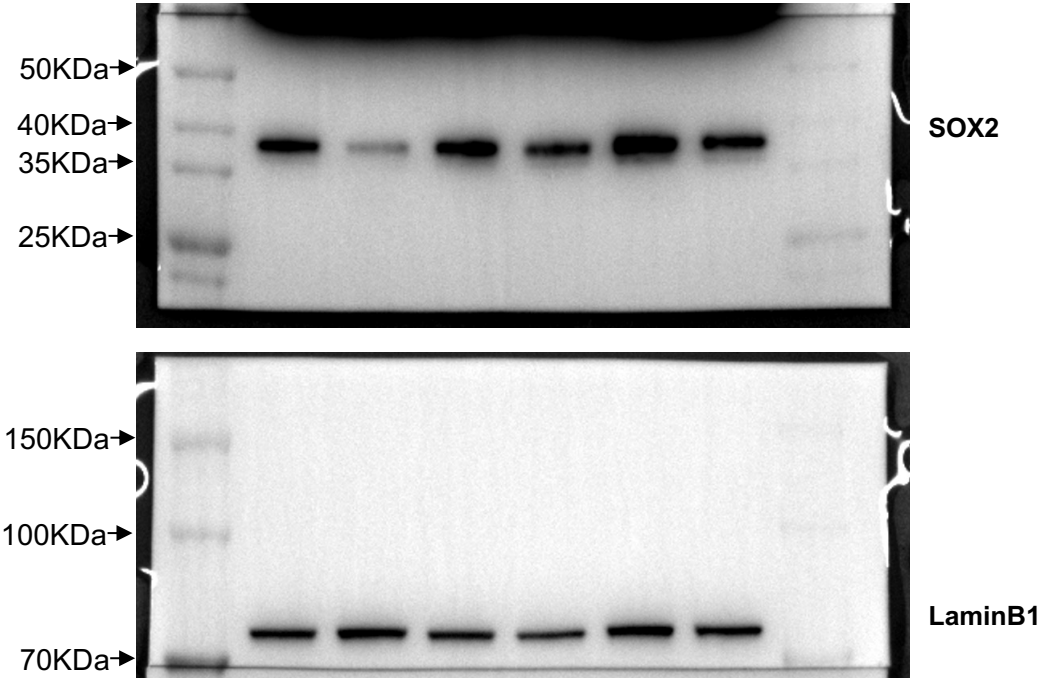
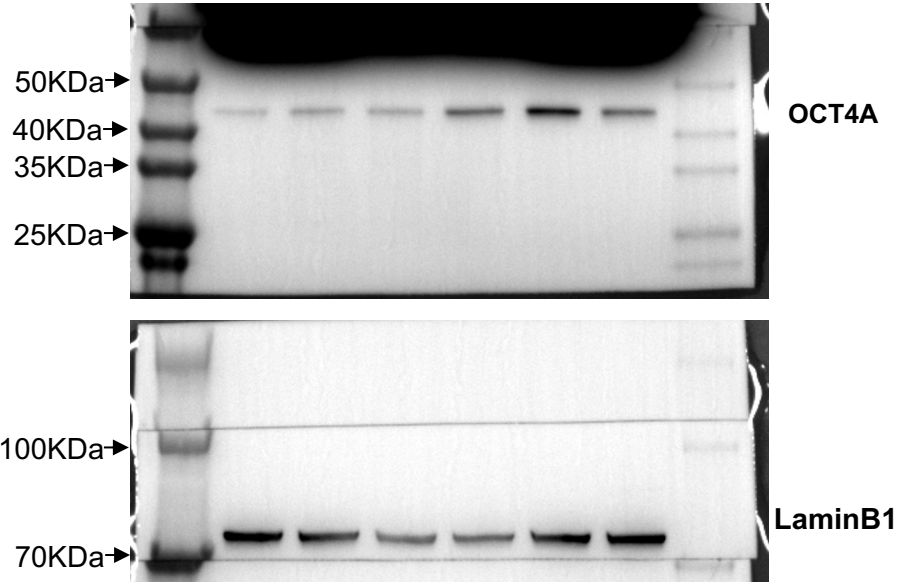
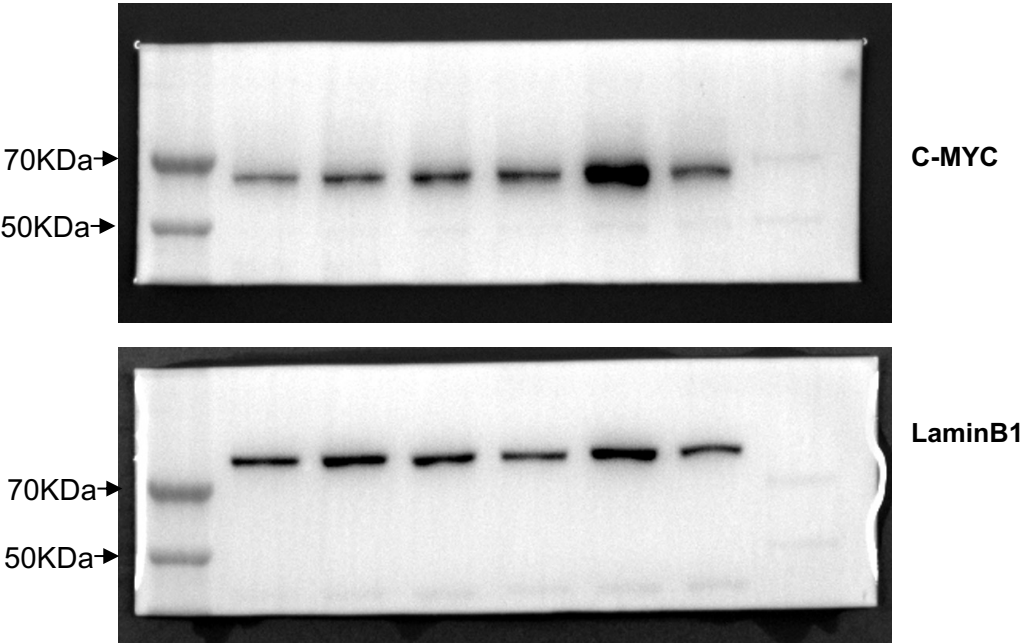
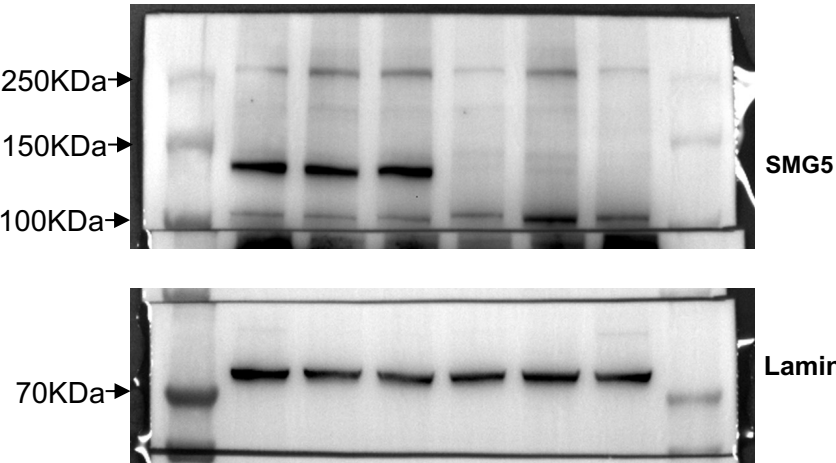


Fig 2H



# Related to Fig 5

Fig 5D



# Related to Fig 6

Fig 6C

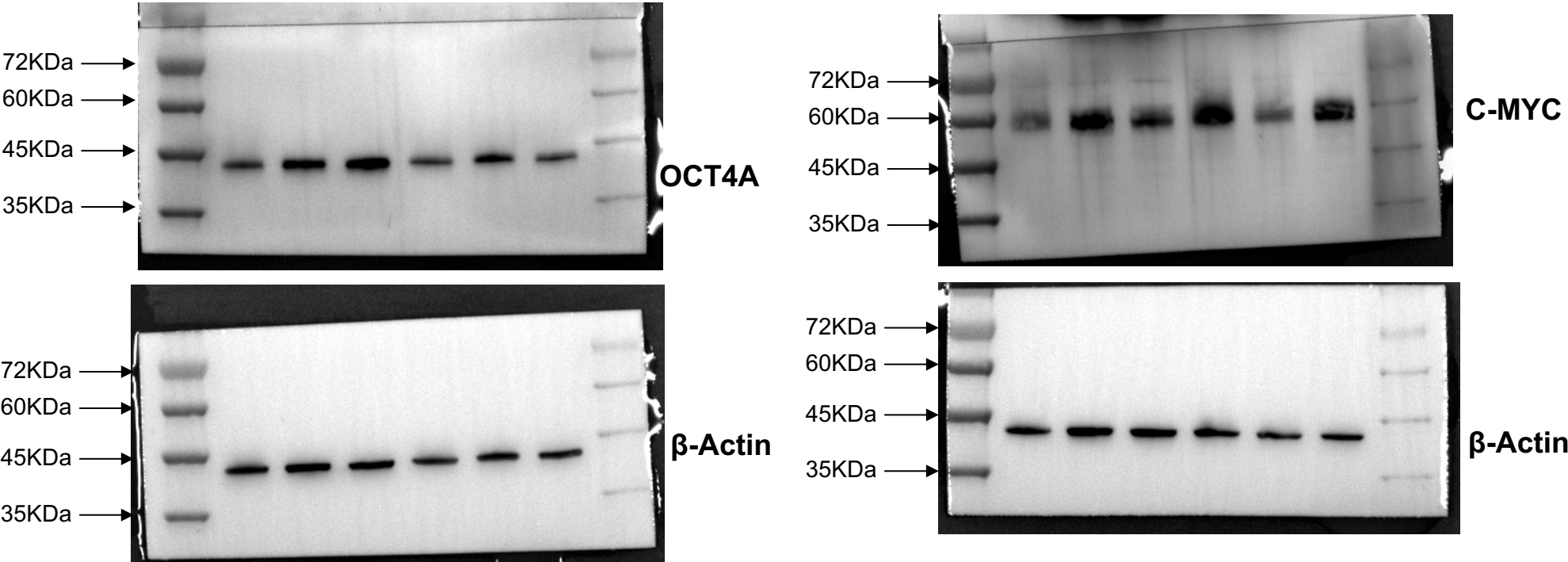
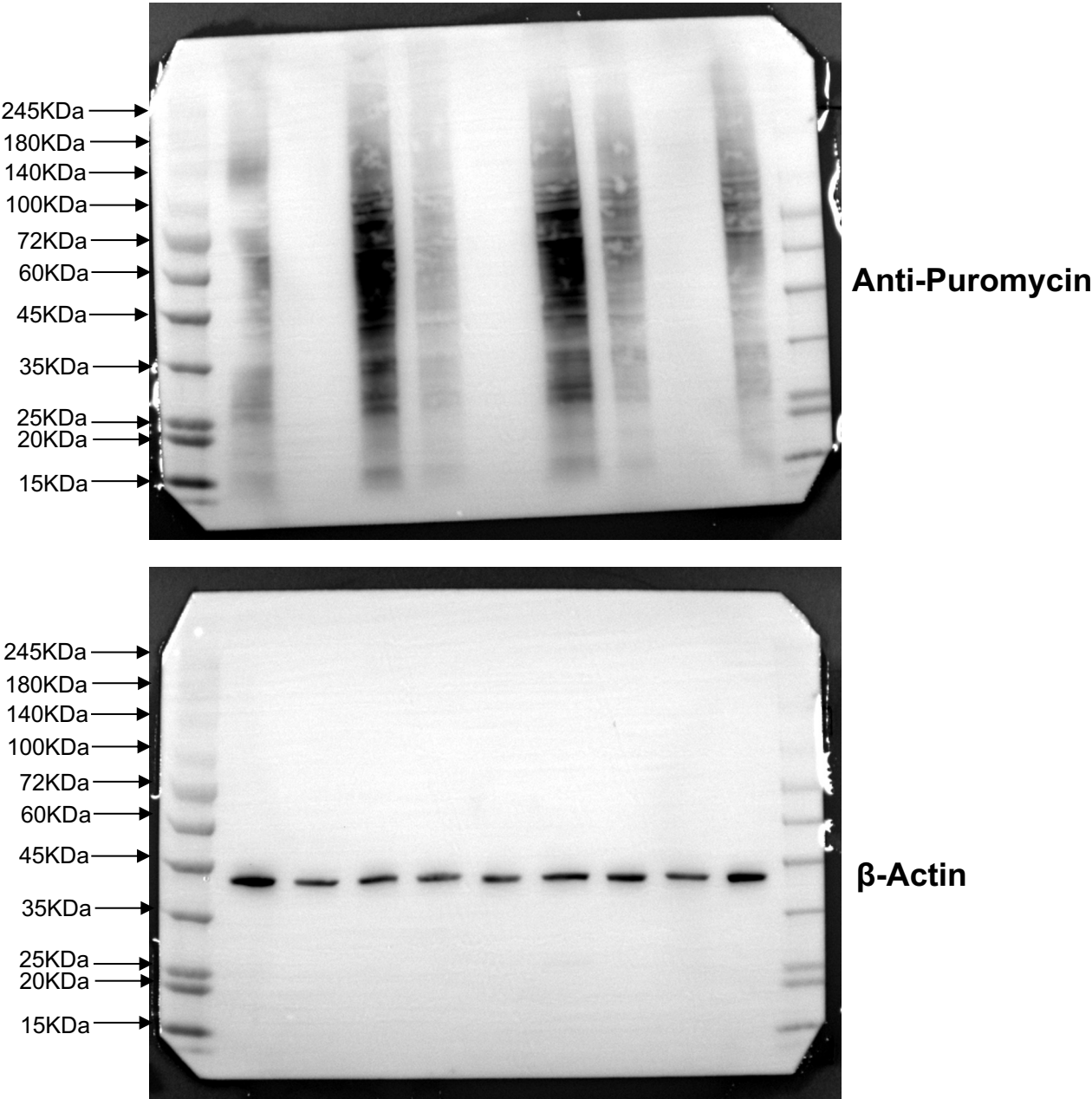
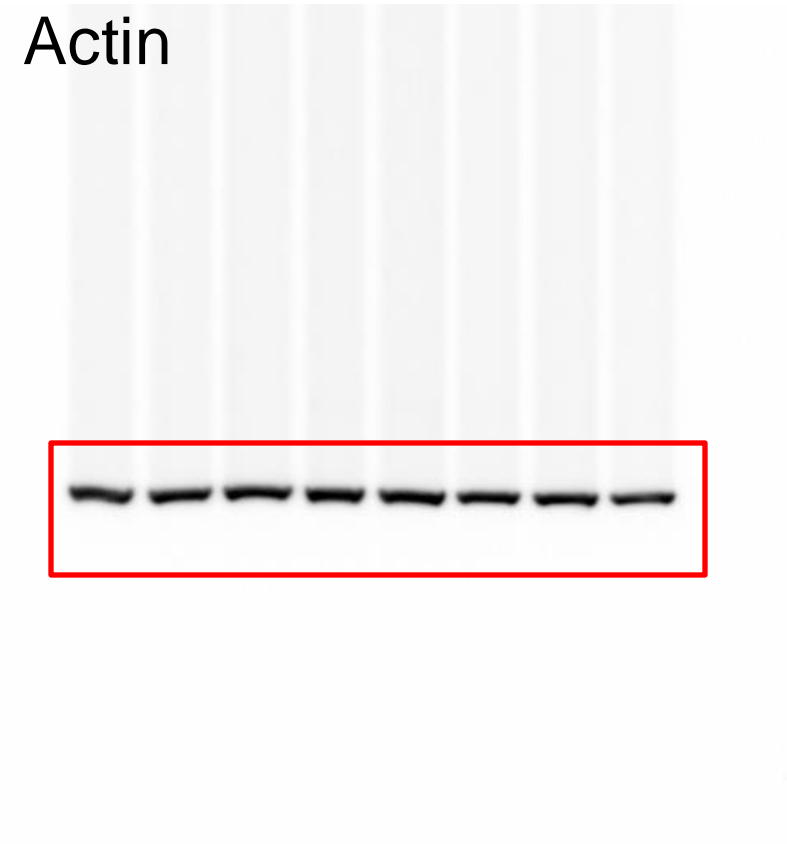
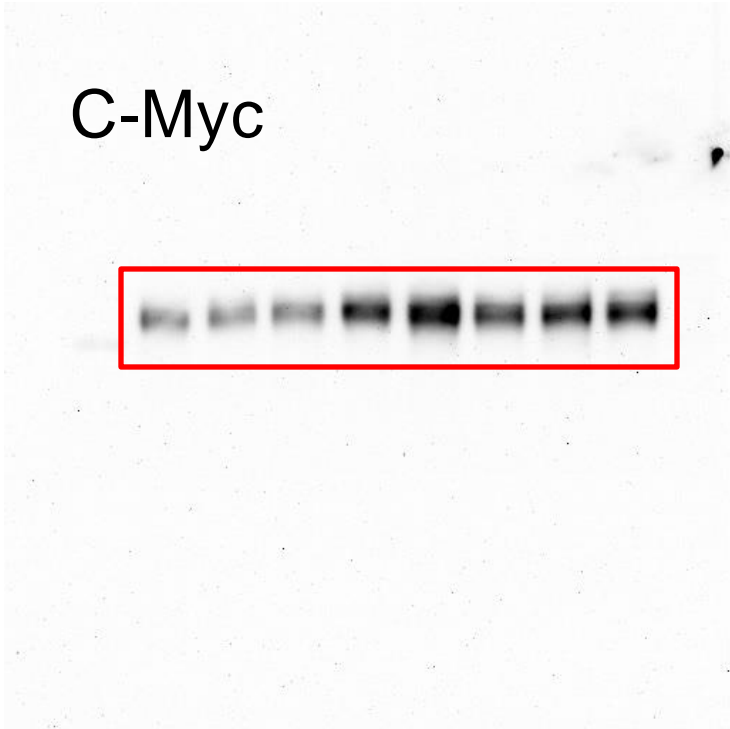


Fig 6E

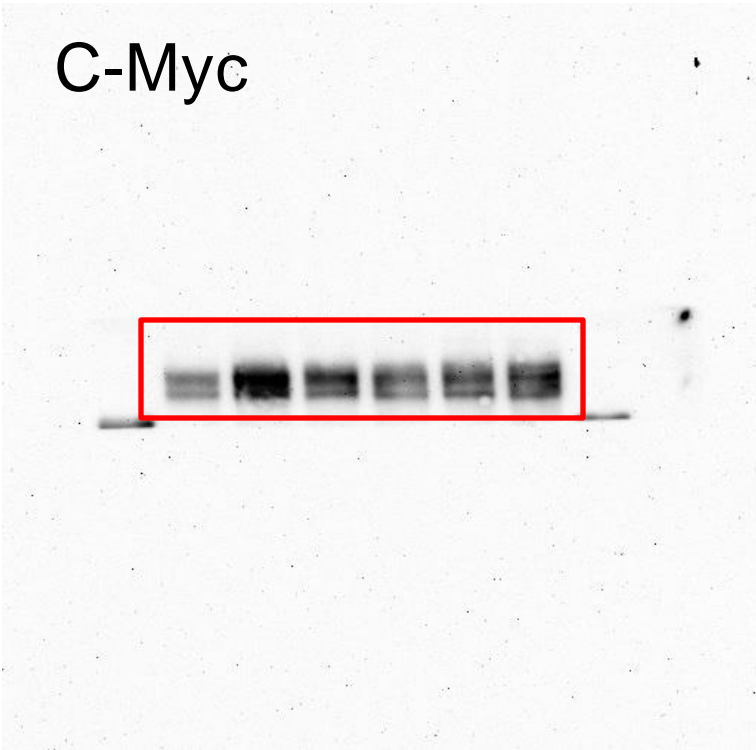




Related to Figure 6B

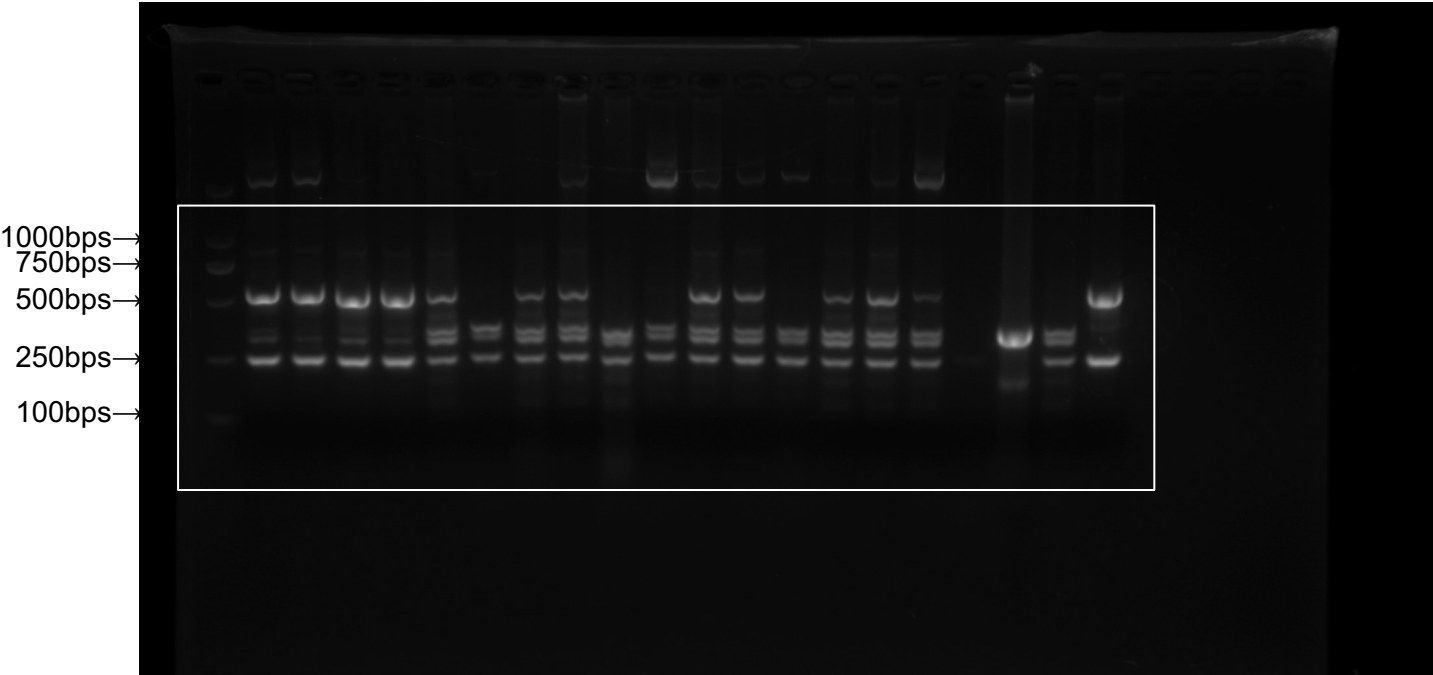


Related to Figure 6D



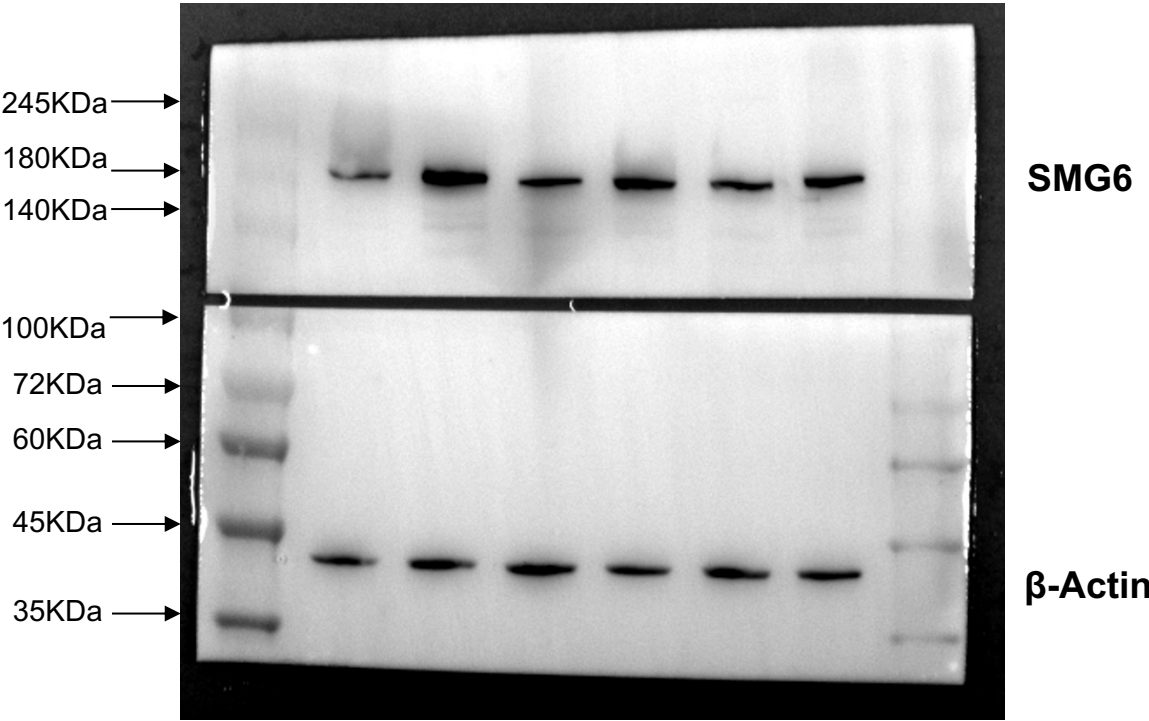
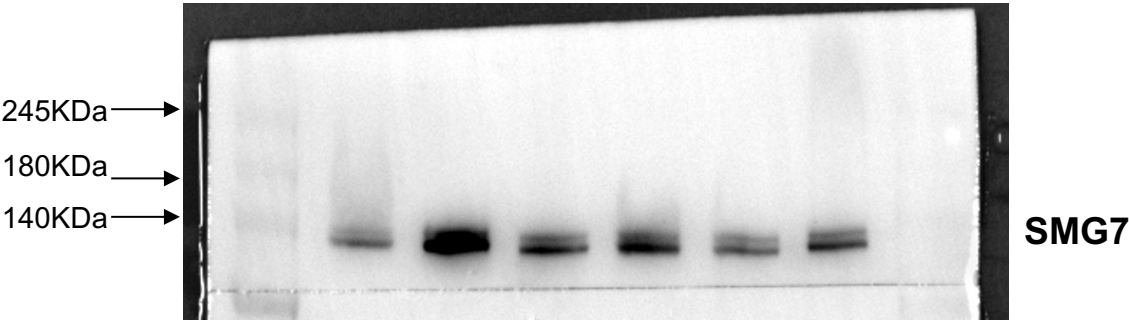
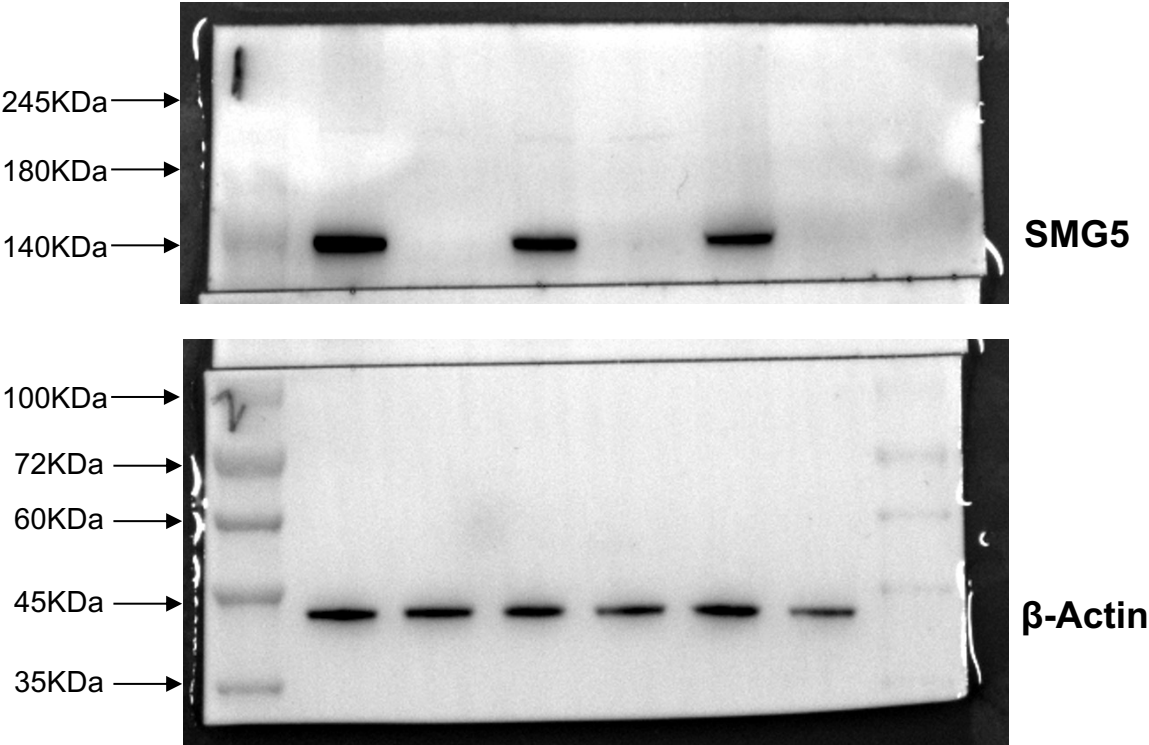
# Related to Supp Figure1

Supp Figure 1B



# Related to Supp Figure3

Supp Figure 3A





Related to Supp Figure 3C-D

