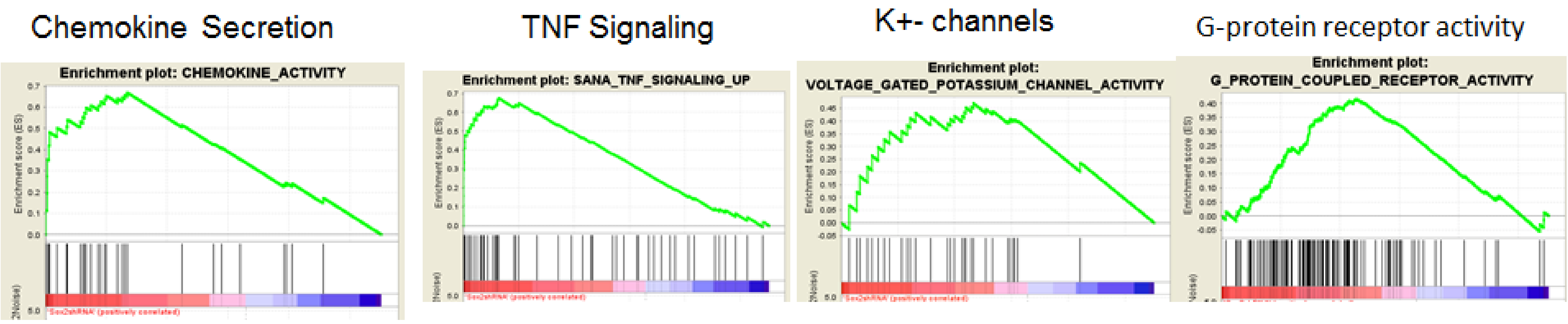
Supplementary Figure 1 GSEA enrichment plot showing significantly up and down regulated genesets in Sox2-deplted mOS-482 cells

Gene sets significantly UP upon Sox2 depletion in osteosarcoma 482 cells

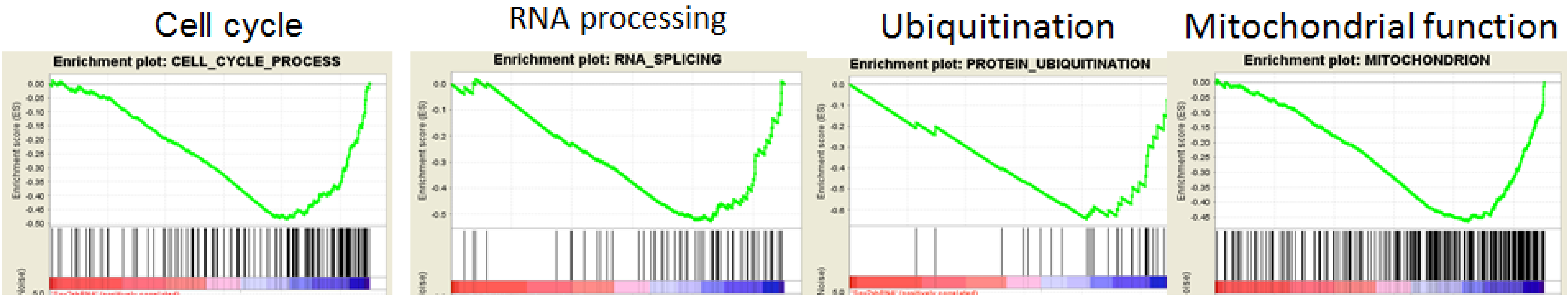

Chemokine Secretion

TNF Signaling

K+- channels

G-protein receptor activity

Gene sets significantly DOWN upon Sox2 depletion in osteosarcoma 482 cells


Cell cycle

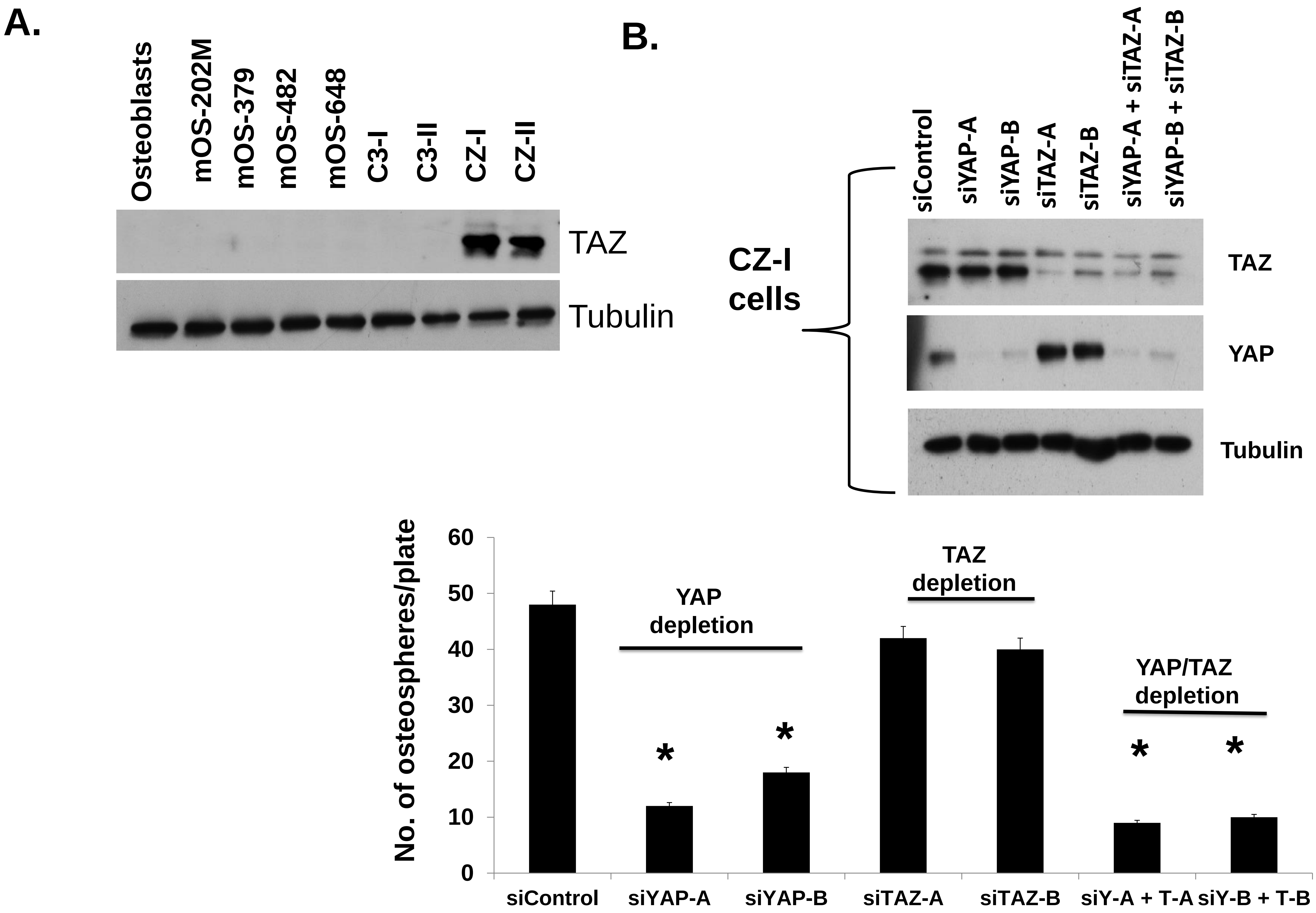
RNA processing

Ubiquitination

Mitochondrial function

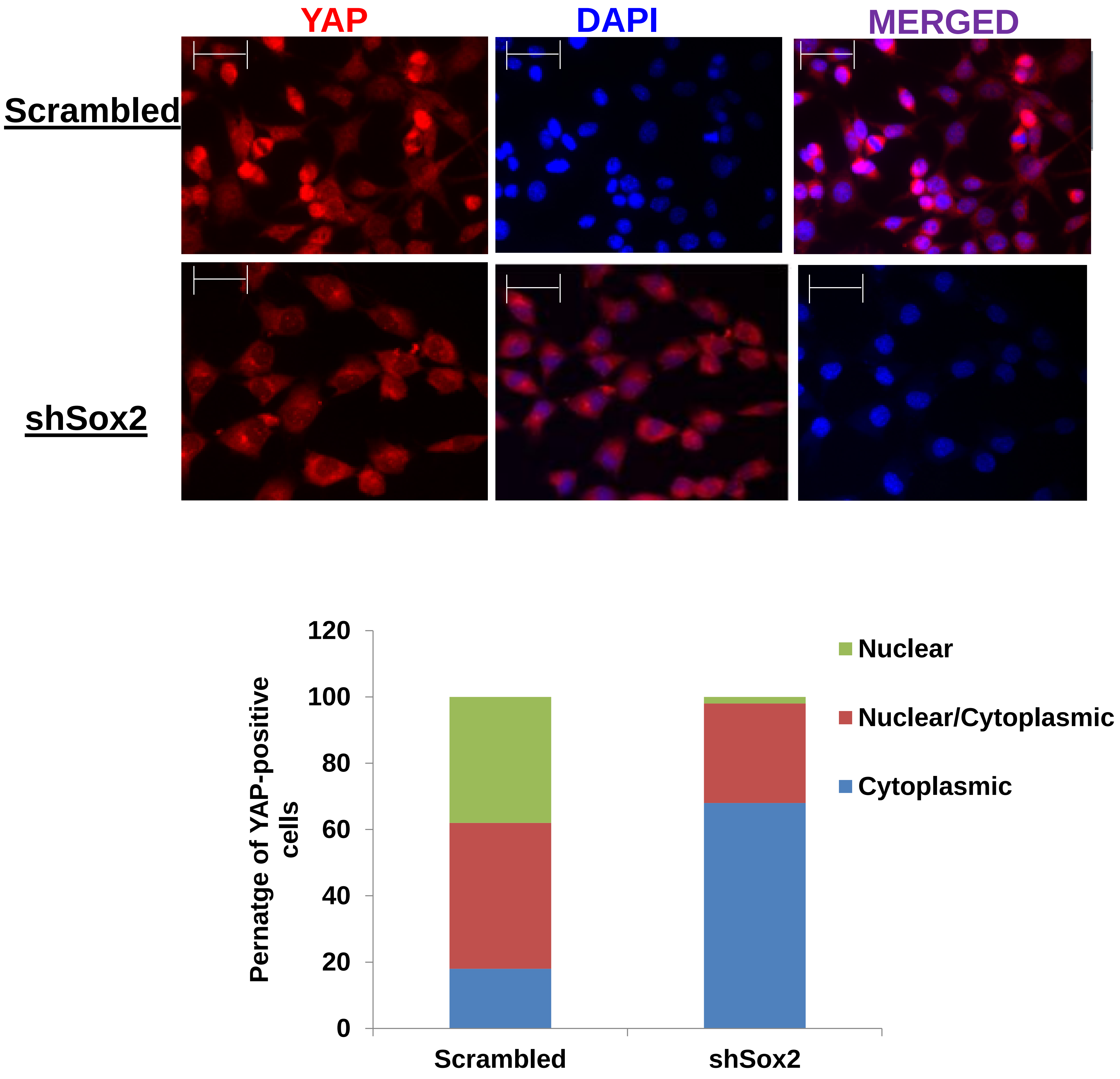
Supplementary Figure 1 GSEA enrichment plots showing expression enrichment of the indicated genesets.
A **positive enrichment** score (top) shows genesets enriched in Sox2 shRNA cells (red).
A **negative enrichment** score indicates enrichment in control cells expressing scrambled shRNA (blue). Bars represent individual genes in the geneset list.

Supplementary Figure 2 TAZ depletion does not affect sphere formation



Supplementary Figure 2. A**.** Western analysis of TAZ in murine osteosarcoma cells. Only CZ-I and CZ-II cells showed significant TAZ expression.
B. Depletion of YAP, but not TAZ, by siRNAs decreases sphere formation in CZ-I cells.
Upper panel – Western analysis of YAP and TAZ expression in CZ-I cells transfected with two independent siRNAs for YAP (siYAP-A and siYAP-B) and TAZ (siTAZ-A and siTAZ-B) either singly or together
Lower panel – Secondary sphere assay of CZ-I cells expressing indicated siRNAs singly or together (siY-A + T-A and siY-B + T-B), * = $p < 0.05$ by ANOVA. Error bars represent mean ± S.D.

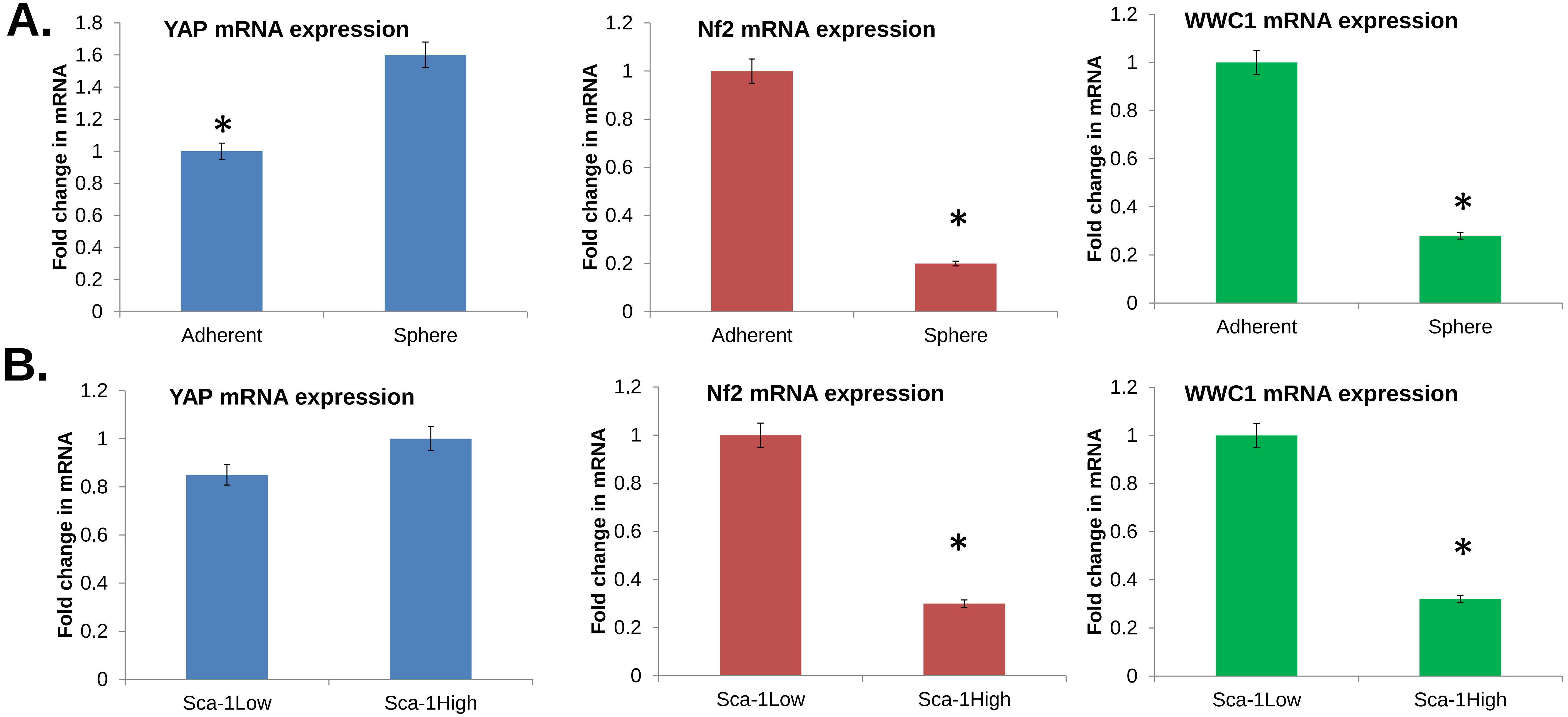
Supplementary Figure 3 Sox2 depletion leads to increased cytoplasmic YAP localization in mOS-482 cells.



Supplementary Figure 3. mOS-482 cells expressing scrambled or shSox2 retrovirus were grown for 48 hours, stained with anti-YAP antibody and imaged at 40X magnification using a Zeiss Axioplan 2 fluorescence microscope. Nuclei were stained with DAPI. Five representative fields were quantified for percentage of cells staining nuclear, cytoplasmic or both nuclear/cytoplasmic in each condition. Average counts are presented. Scale bar = 50 μM

Supplementary Figure 4

YAP, Nf2 and WWC1mRNA expression in spheres/adherent cells and Sca-1$^{High/Low}$ cells



Supplementary Figure 4A and B.

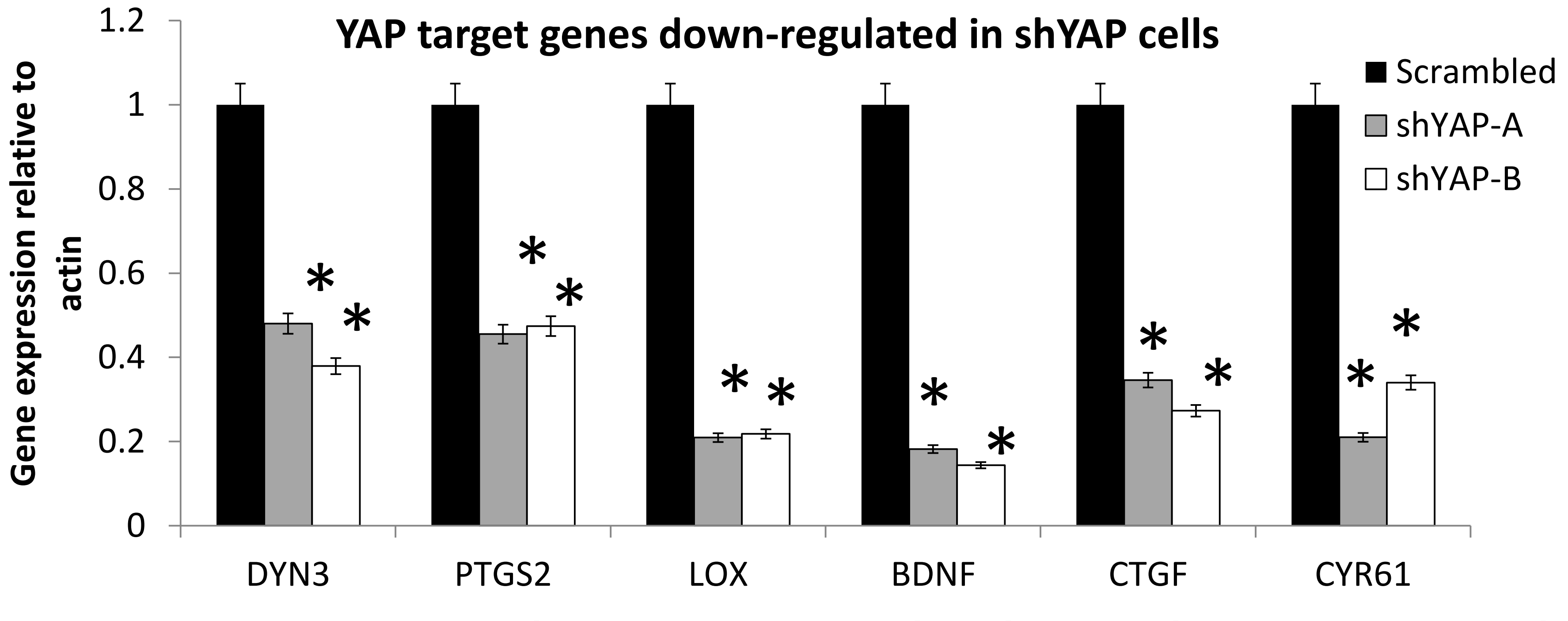
qRT-PCR analysis of YAP, Nf2 and WWC1 mRNA expression

A. Adherent mOS-482 cells and secondary spheres derived from mOS-482 cells

B. B. Sca1$^{High/Low}$ cells.

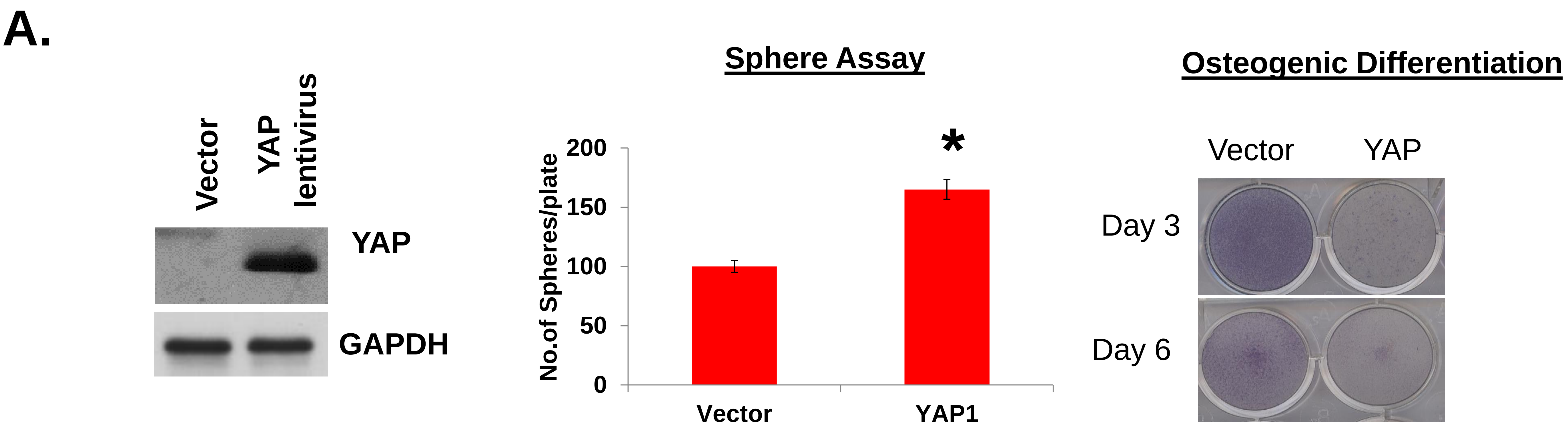
mRNA expression was normalized to actin. * = $p < 0.05$ by t-test. Error bars represent mean ± S.D.

C. mRNA expression of YAP target genes in mOS-482 cells expressing YAP shRNA

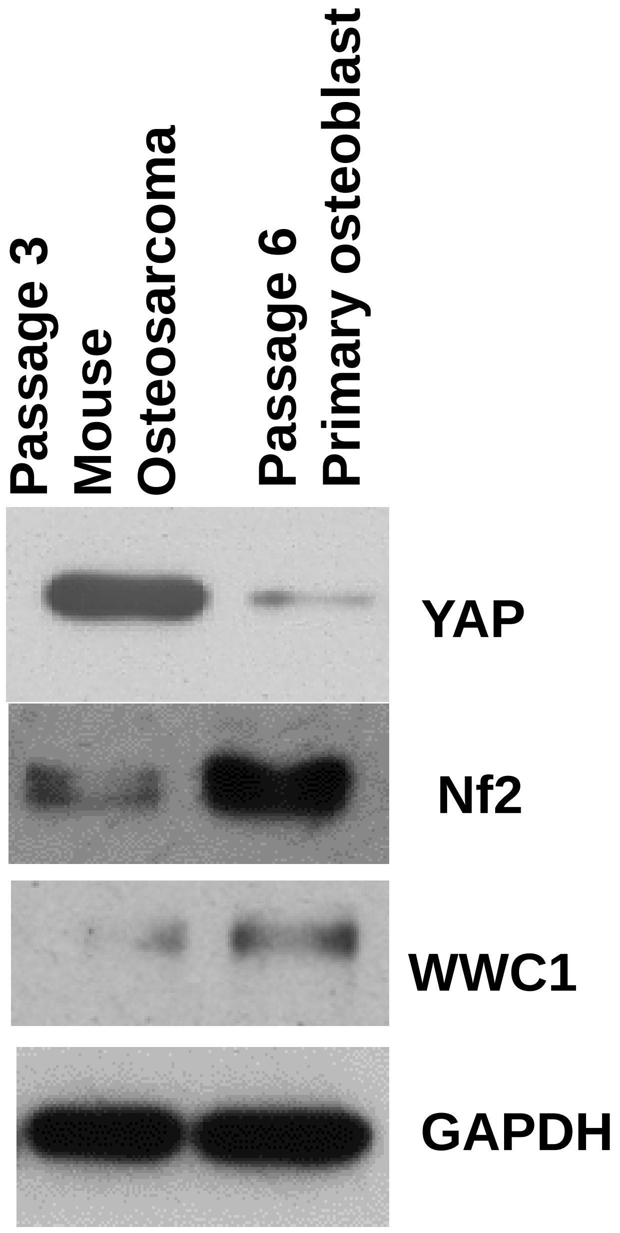


Supplementary Figure 4C mRNA expression was normalized to actin. * = $p < 0.05$ by ANOVA. Error bars represent mean ± S.D.

<u>Supplementary Figure 5</u> A. YAP overexpression in primary osteoblasts increases sphere formation and blocks osteogenic differentiation**.**



B. Increased YAP and low Nf2/WWC1 expression in low passage mOS cells.

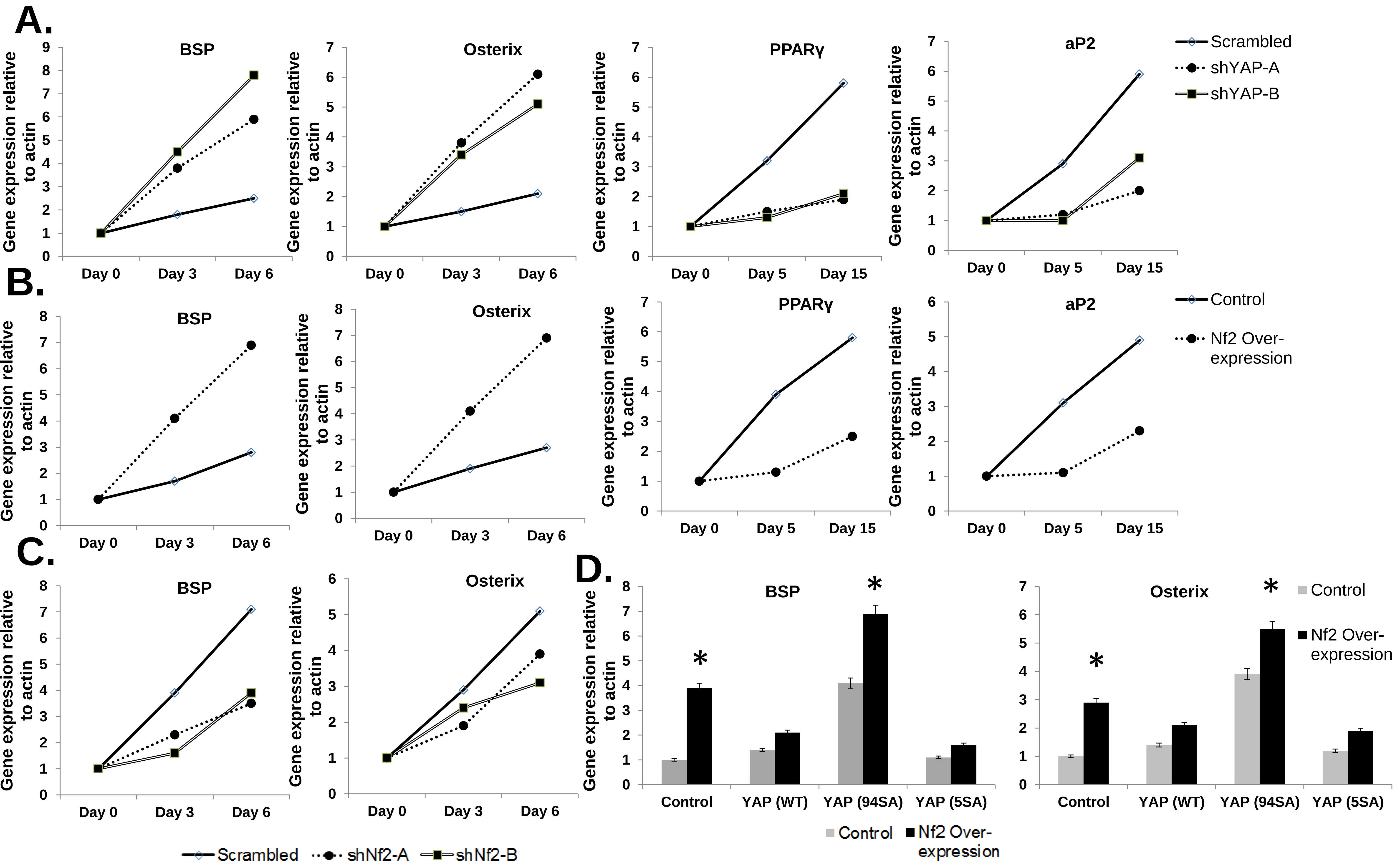


<u>Supplementary Figure 5</u>

A. YAP over expression increases sphere formation and decreases osteogenic differentiation of primary osteoblasts. Left panel – Western analysis of YAP expression in primary osteoblasts expressing control or YAP lentivirus. Center panel – osteosphere assay . Right panel – Osteogenic differentiation

B. Western analysis of YAP, Nf2 and WWC1 expression in mouse osteosarcoma cells (passage 3) and primary osteoblasts (passage 6). Low passage osteosarcoma cells also have high YAP and decreased Nf2 and WWC1 expression.

Supplementary Figure 6 Osteogenic and adipogenic differentiation marker genes by qRT-PCR analysis.



Supplementary Figure 6

mRNA expression is normalized to actin.

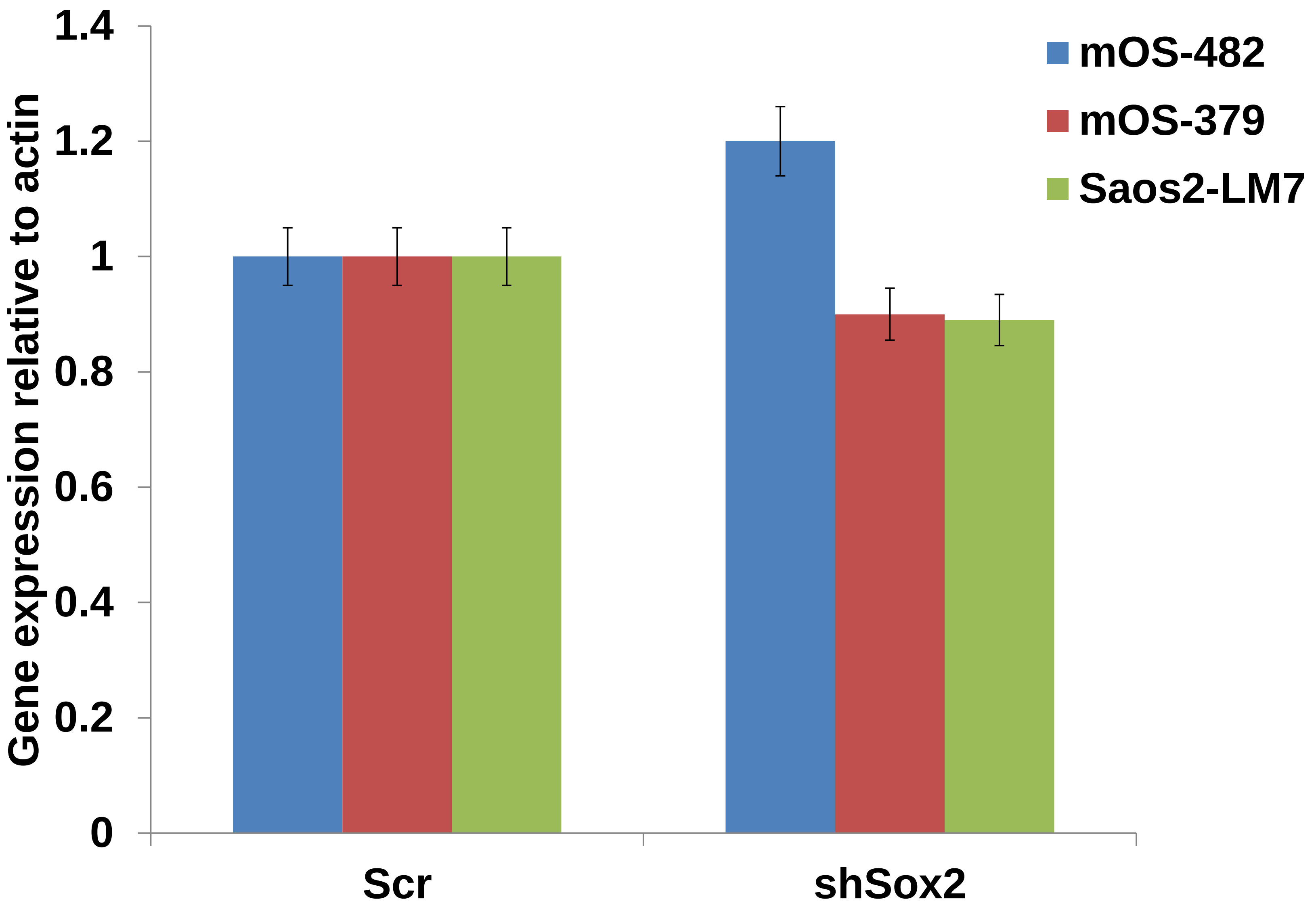
A. BSP and Osterix expression for osteogenic, and PPARγ and aP2 expression for adipogenic differentiation.shown in Figure 3D

B. BSP and Osterix expression for osteogenic, and PPARγ and aP2 expression adipogenic differentiation shown in Figure 4A

C. BSP and Osterix expression for osteogenic differentiation shown in Figure 4B

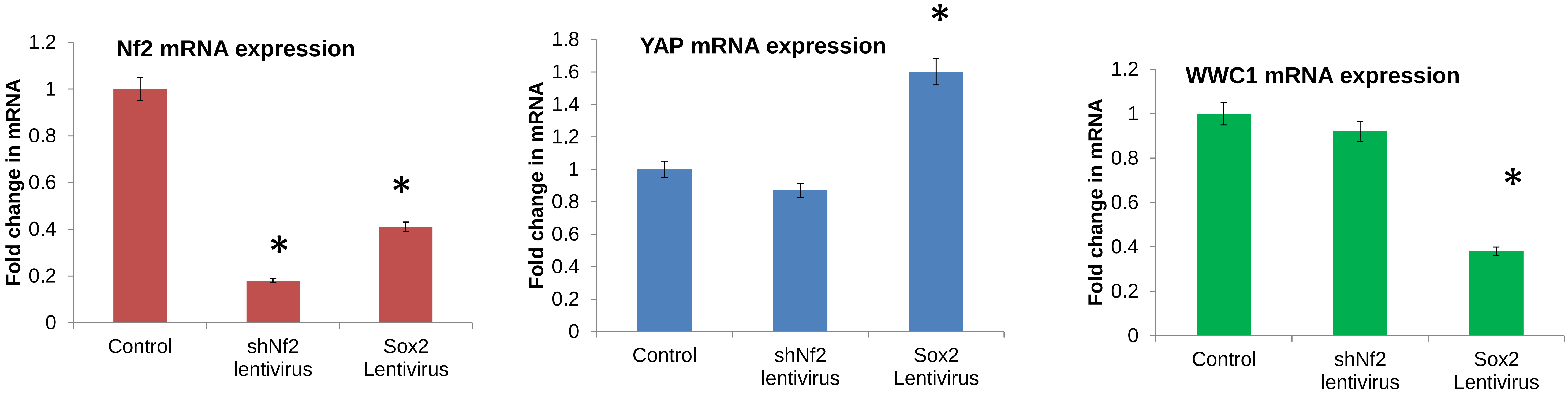
D. BSP and Osterix expression for osteogenic differentiation (day 6) shown in Figure 4D. * = p <0.05 by t-test. rror bars represent mean ± S.D.

Supplementary Figure 7 YAP mRNA expression is not significantly altered in Sox2-depleted murine and human OS lines



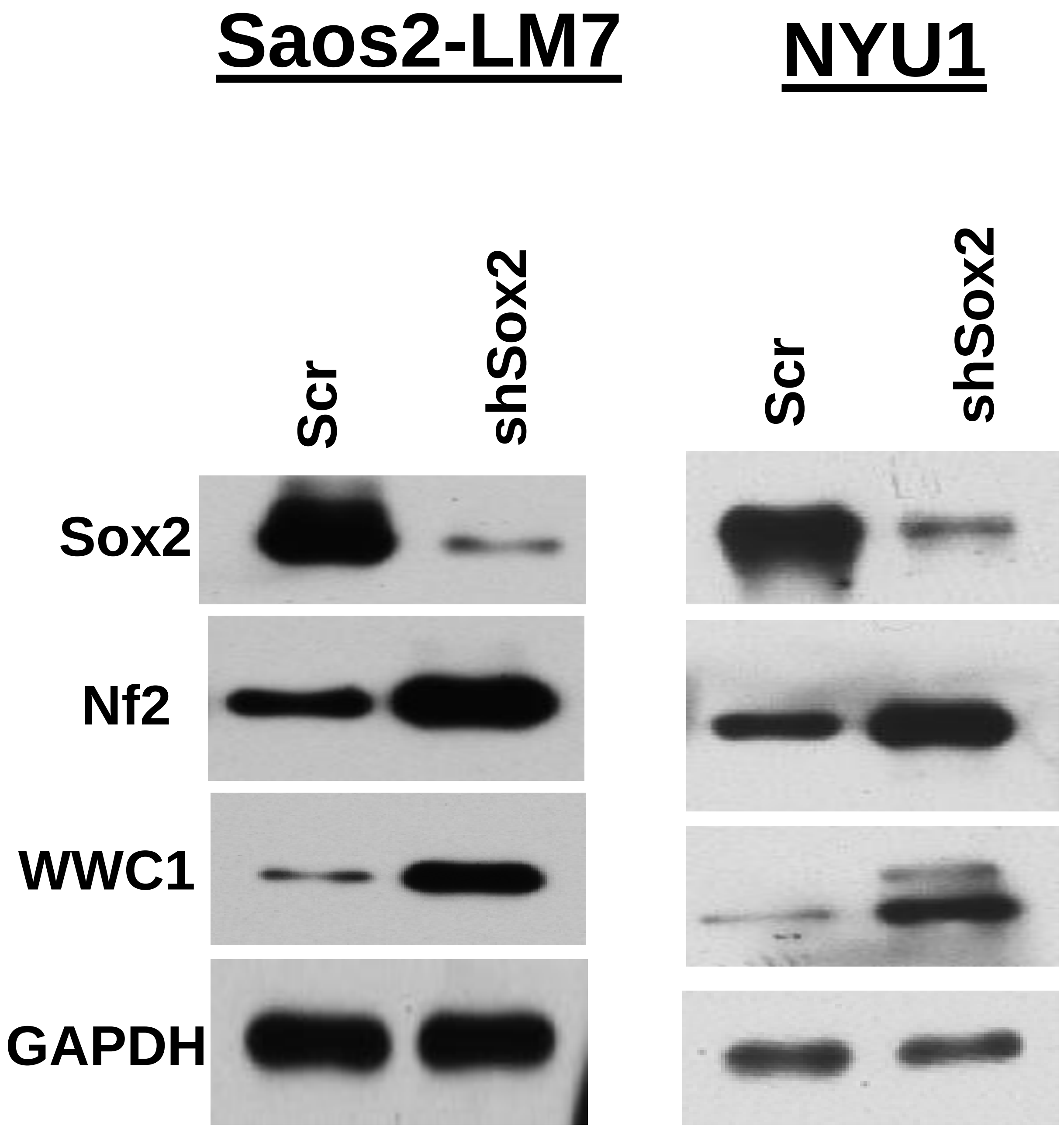
Supplementary Figure 7 mOS-482 cells (murine), mOS-379 cells (murine) and Saos2-LM7 (human) cells were transduced with a control (scr) or Sox2 shRNA lentivirus. qRT-PCR of YAP mRNA expression normalized to actin. * = $p < 0.05$. Error bars represent mean ± S.D.

Supplementary Figure 8 mRNA expression of Nf2, YAP and WWC1 in NIH-3T3 cells expressing control, shNf2 lentivirus or Sox2 lentivirus (associated with Figure 8A)



Supplementary Figure 8. qRT-PCR analysis mRNA expression normalized to actin. * = $p < 0.05$ by t-test. Error bars represent mean ± S.D.

Supplementary Figure 9 Nf2 and WWC1 expression are increased in human osteosarcoma cell lines depleted of Sox2



Supplementary Figure 9. Saos-2-LM7 or primary human osteosarcoma cells from fresh biopsy (NYU1) expressing either Scrambled (Scr) or human Sox2 shRNA (shSox2) were analyzed for Sox2, Nf2 and WWC1 expression by Western analysis. GAPDH was used as a loading control.

Supplementary Figure 10 Uncropped Western Blot Images

Figure 1C

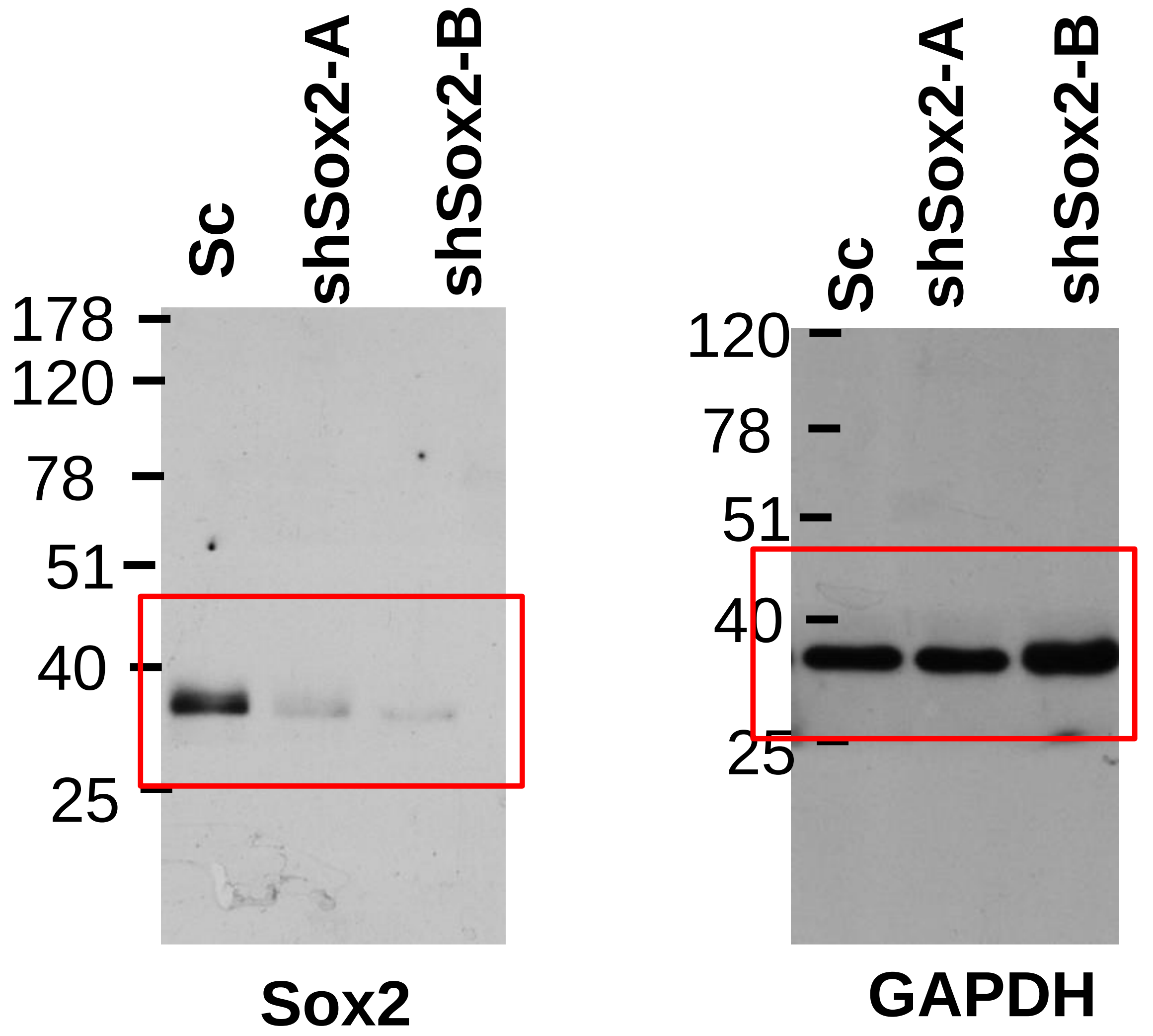


Figure 2E

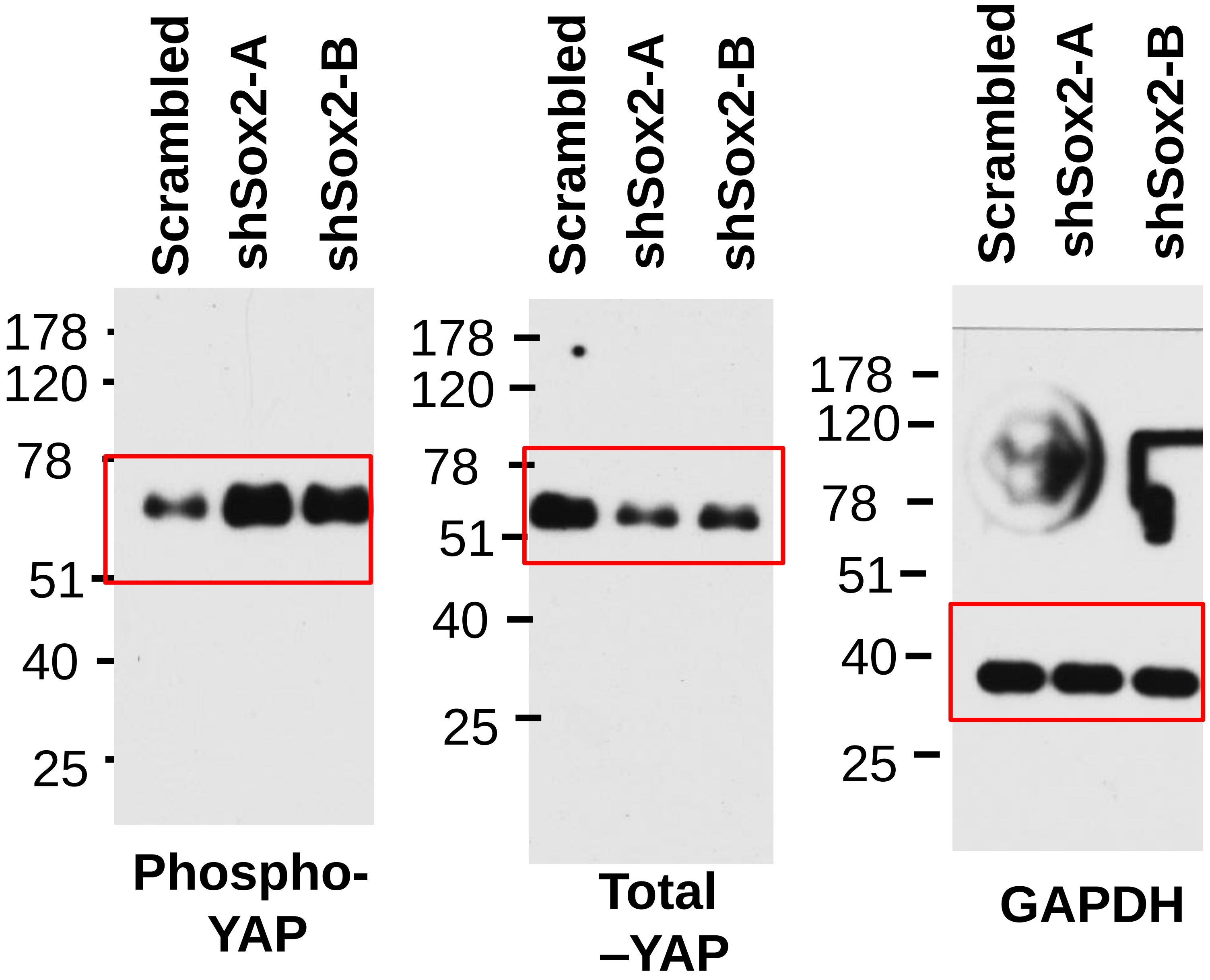
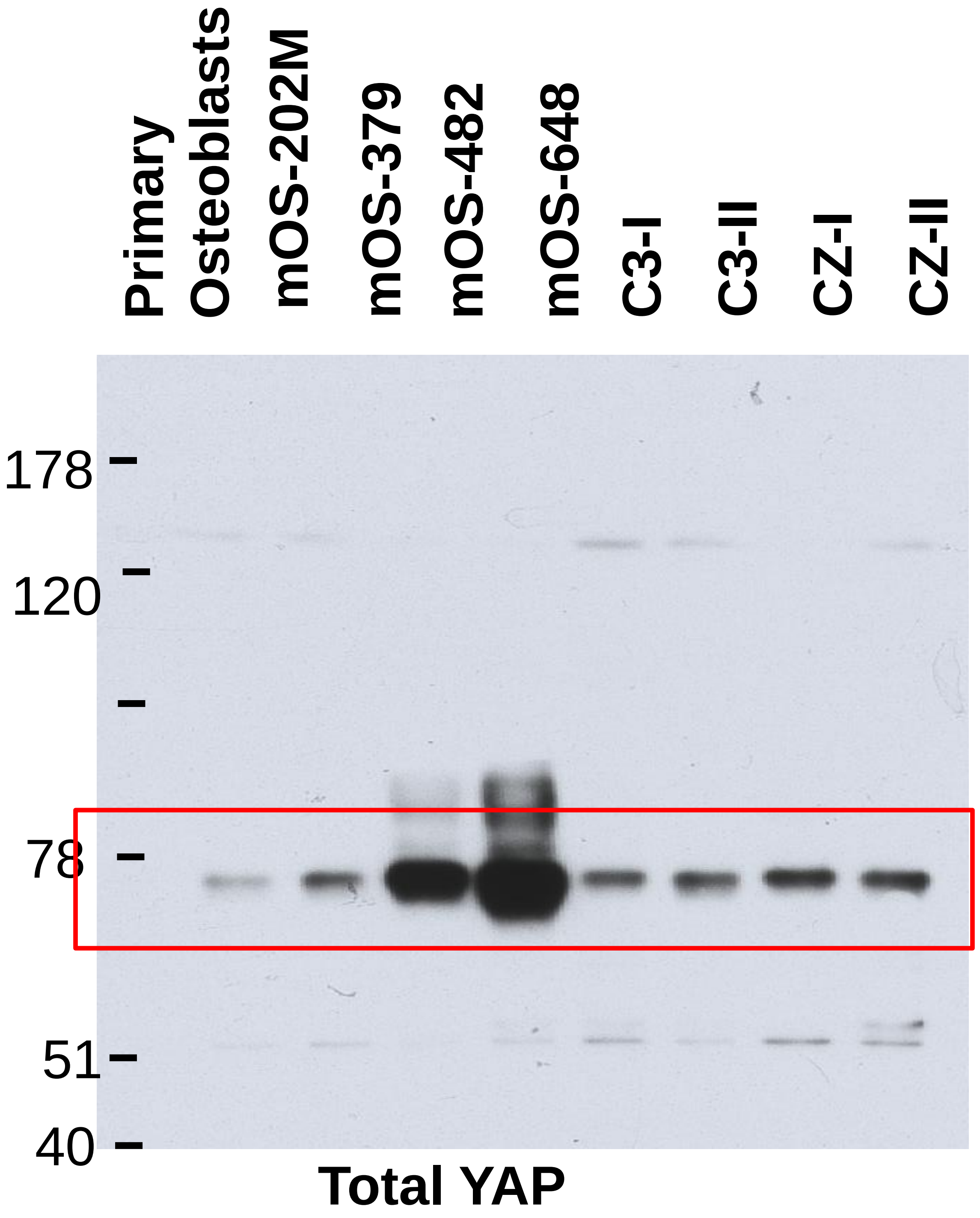


Figure 2A – Left Panel



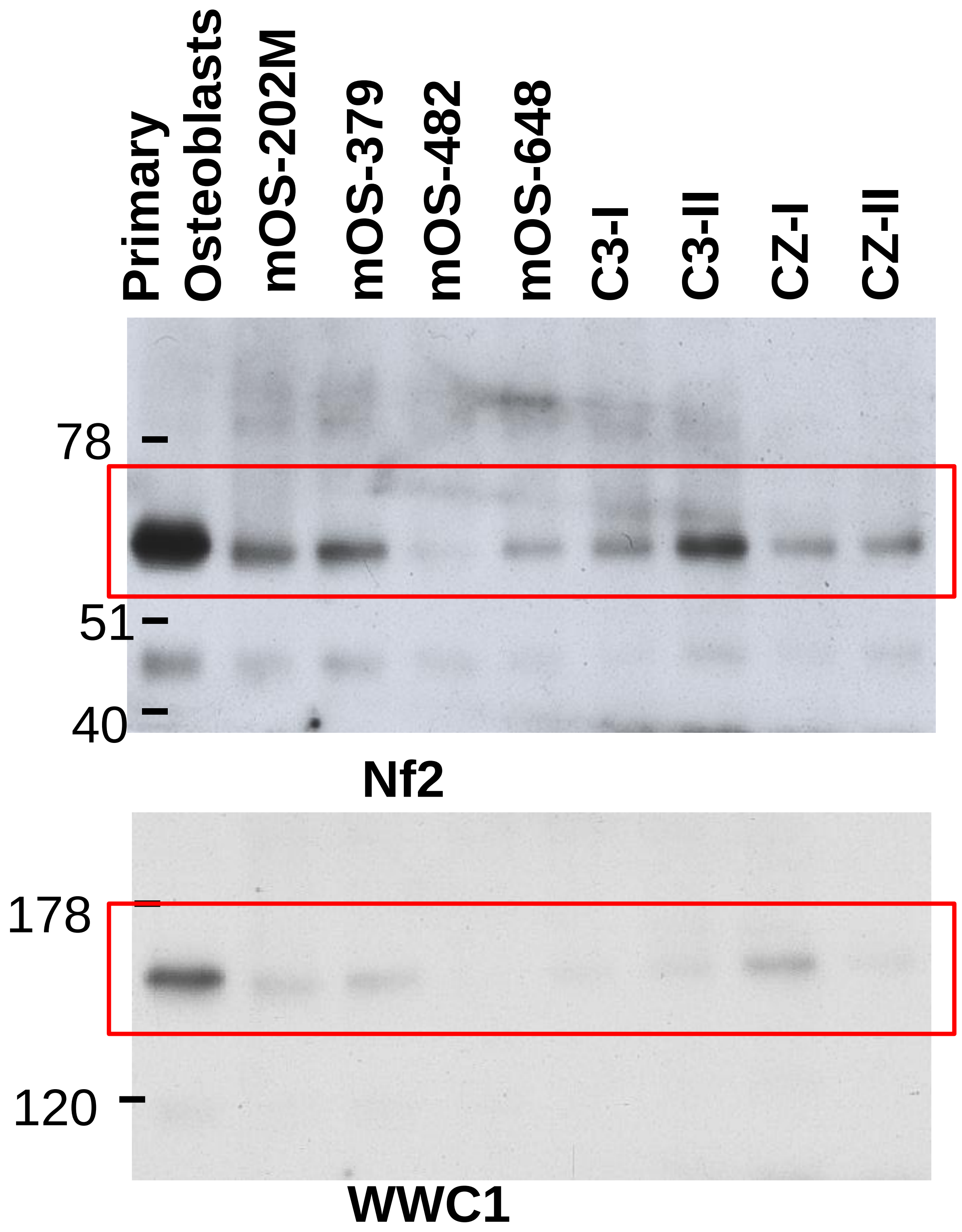


Figure 2A – Right Panel

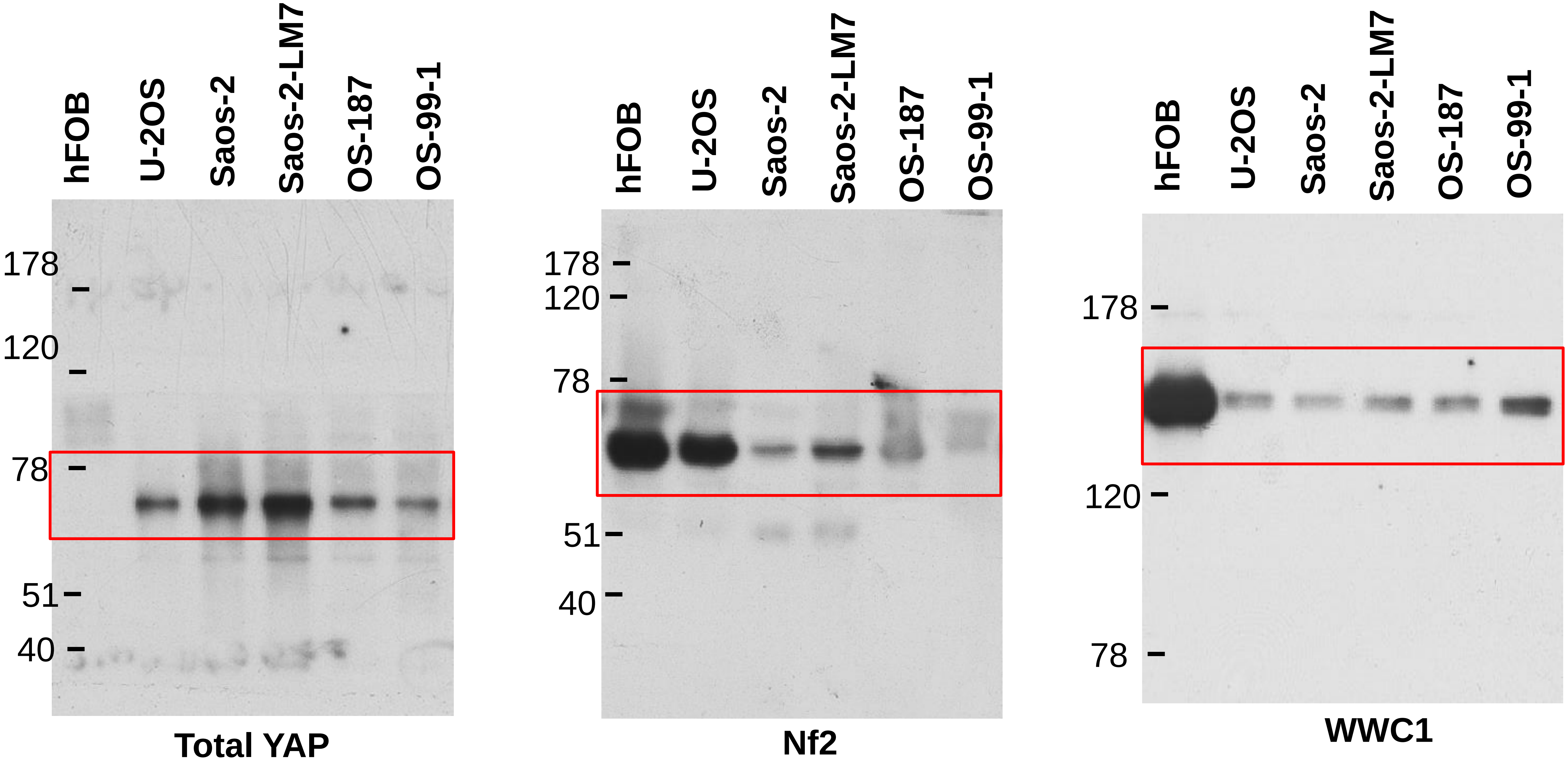


Figure 3A –Adherent and Sphere

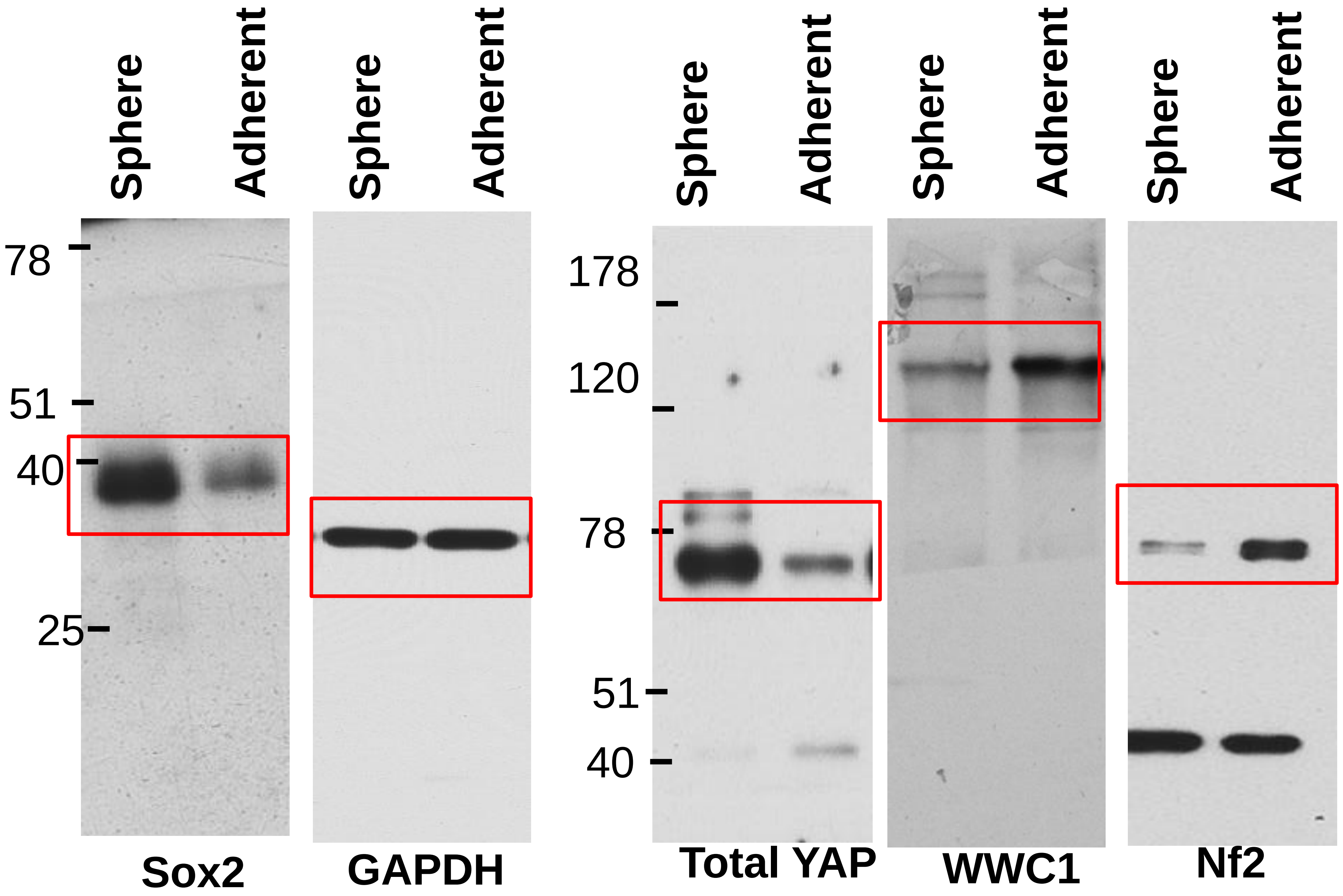


Figure 3A –Sca1^{High}Sox2^{High} and Sca1^{Low}Sox2^{Low}

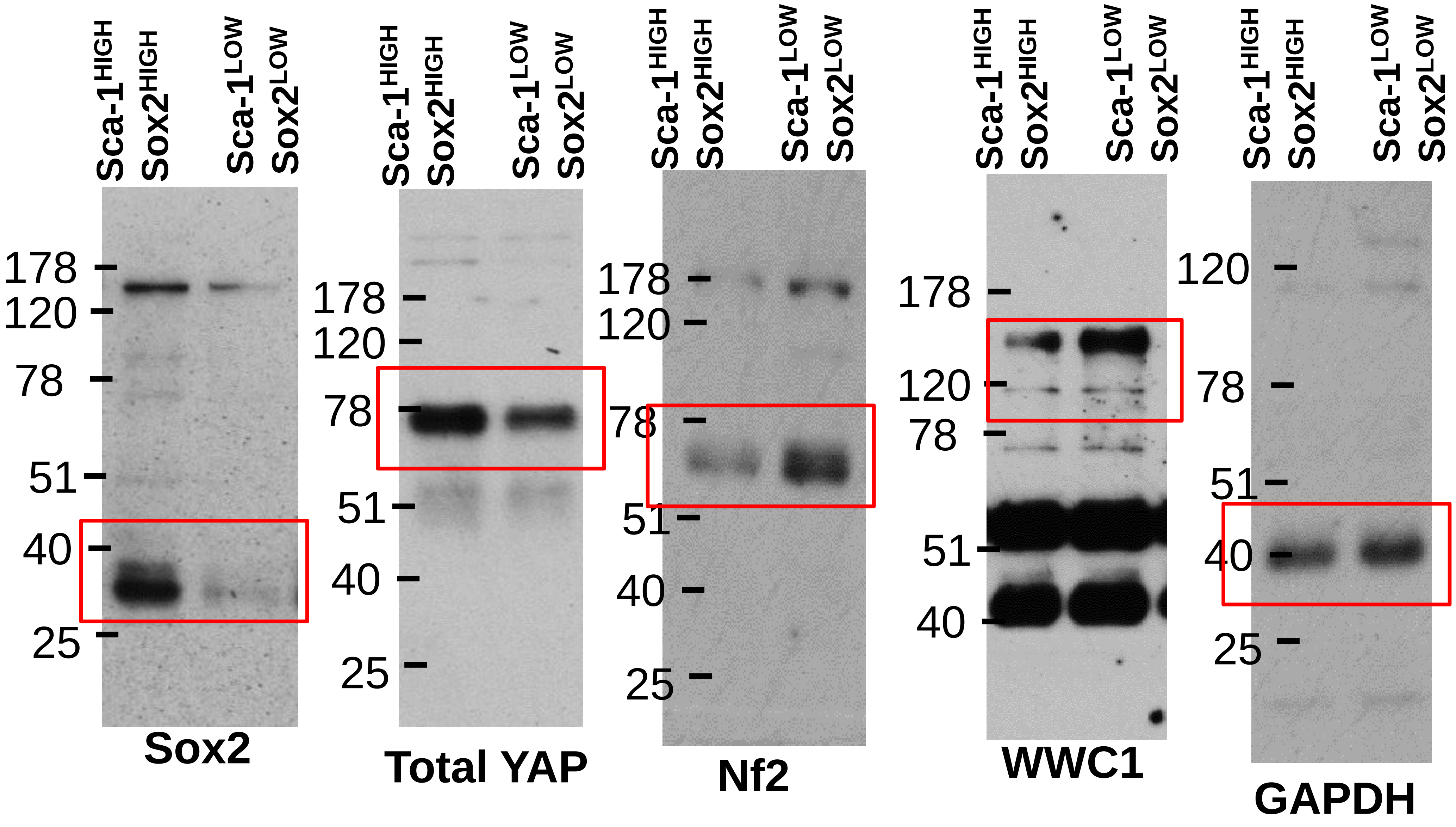


Figure 3B

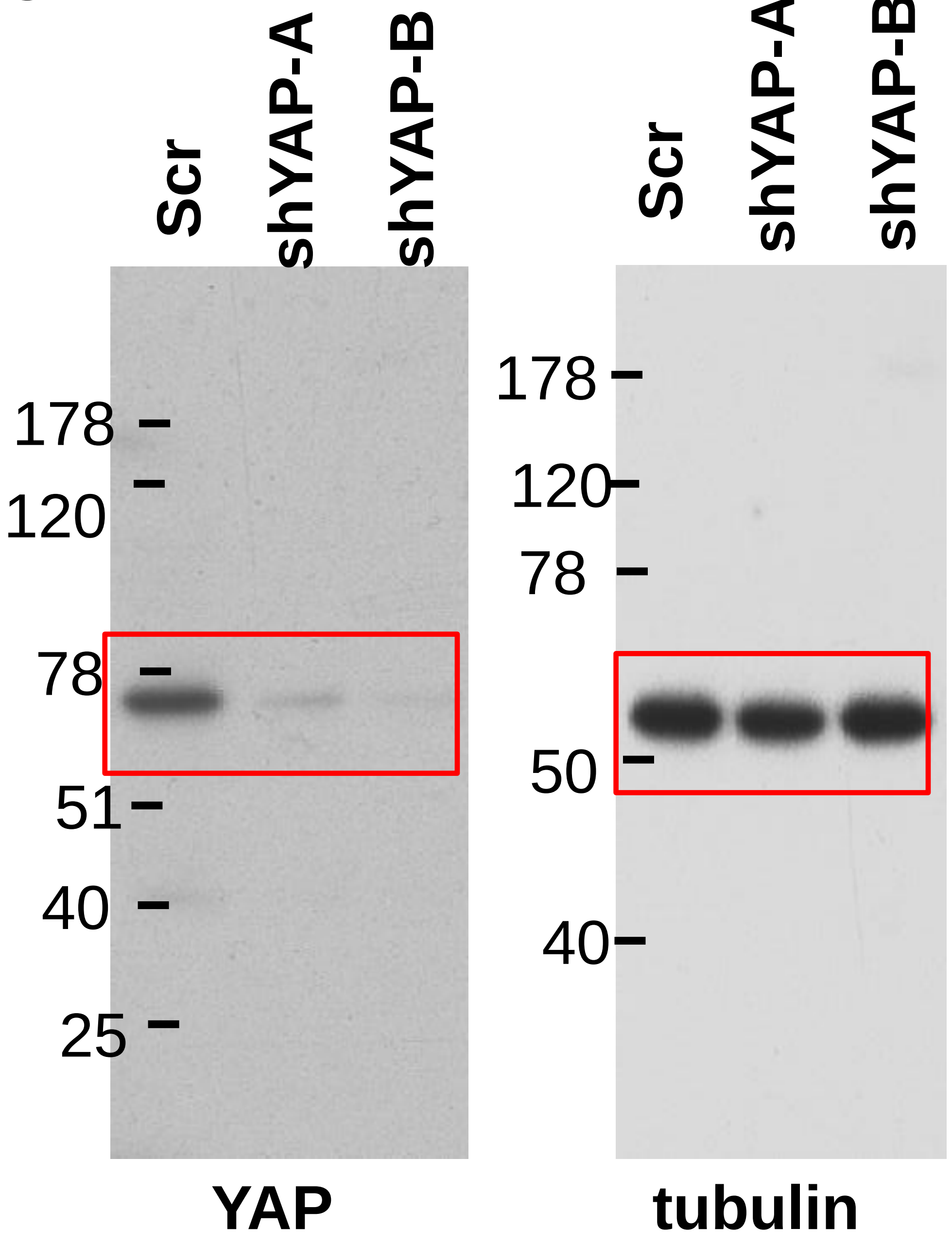


Figure 4A

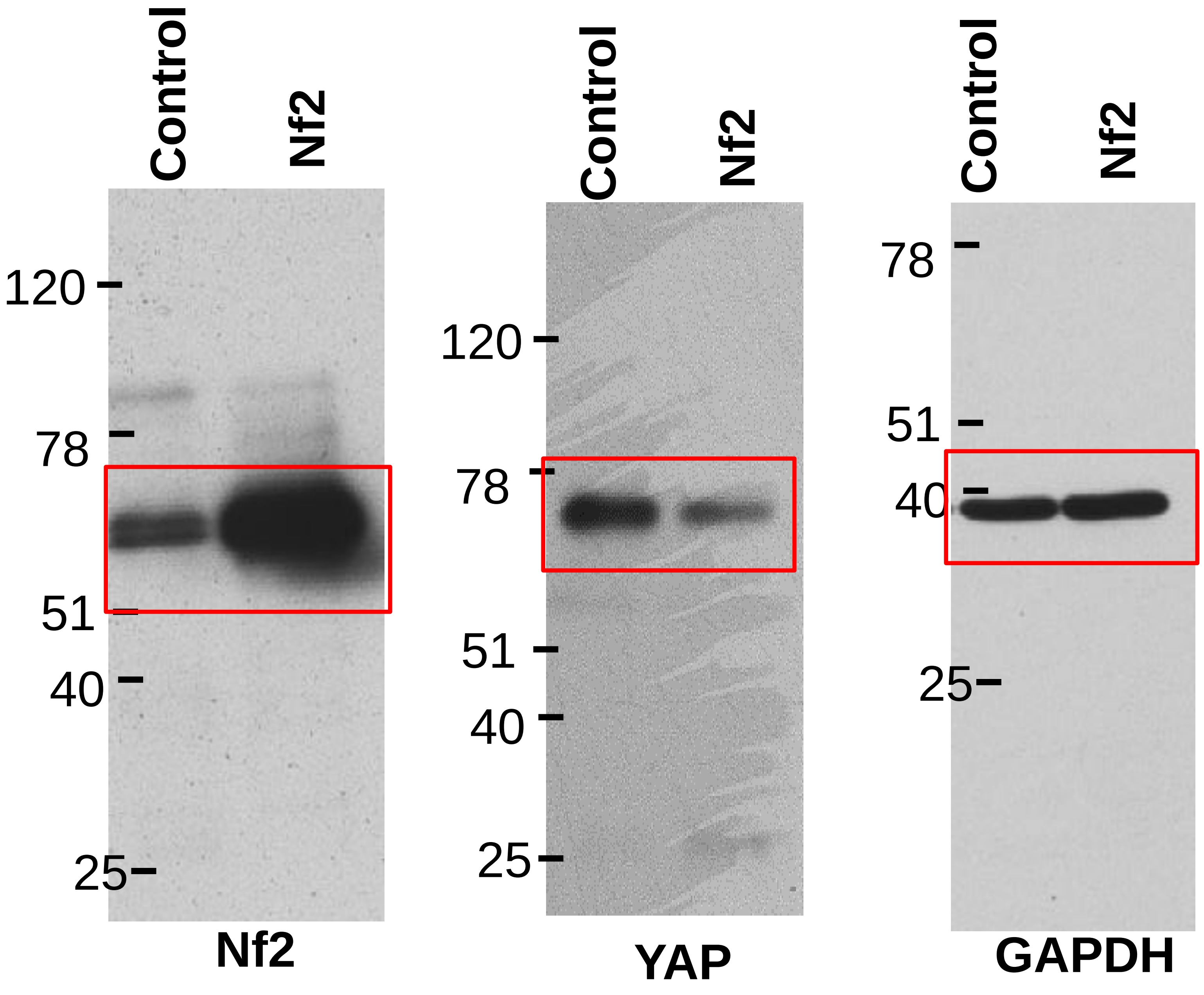


Figure 4B

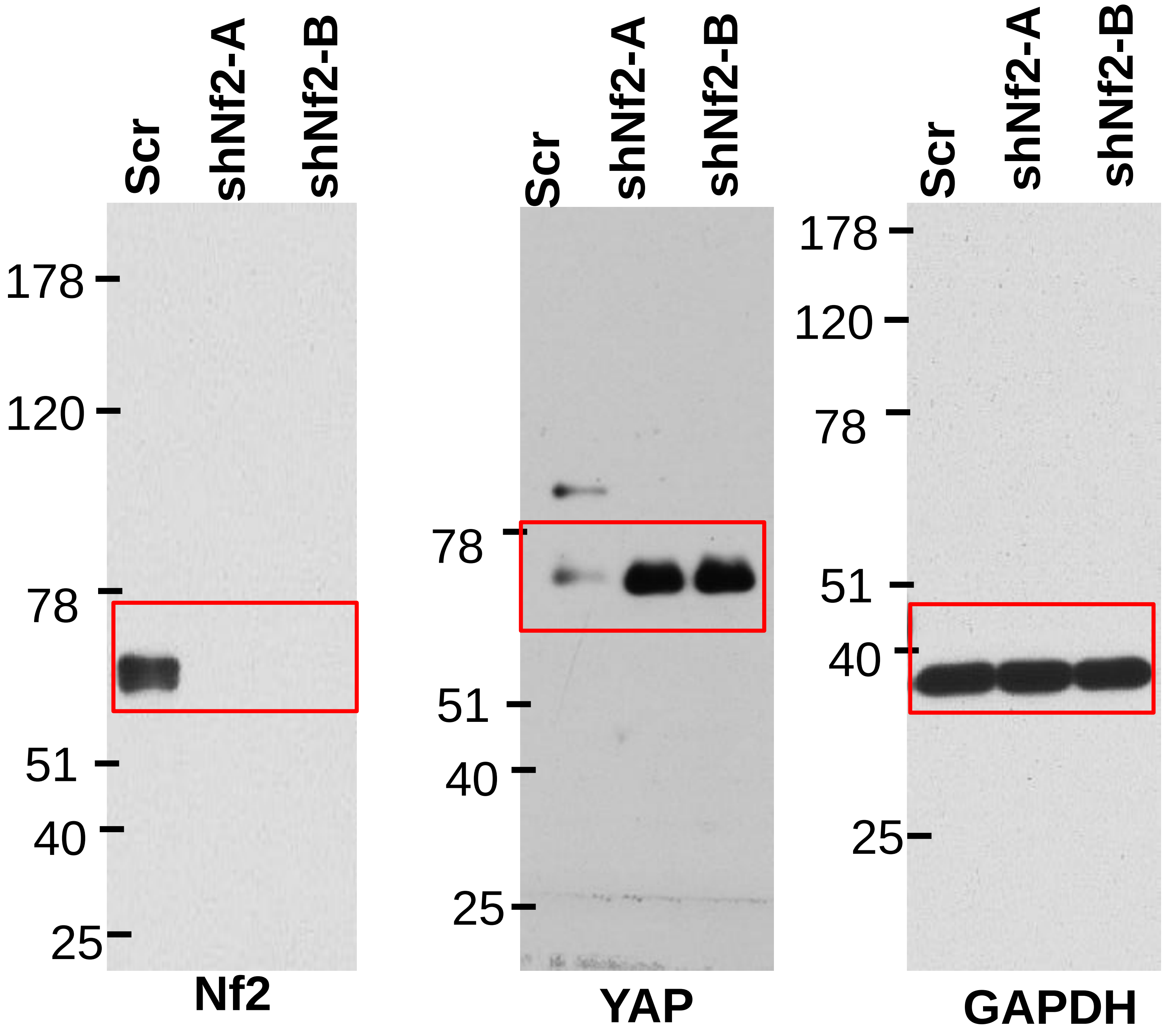


Figure 4C

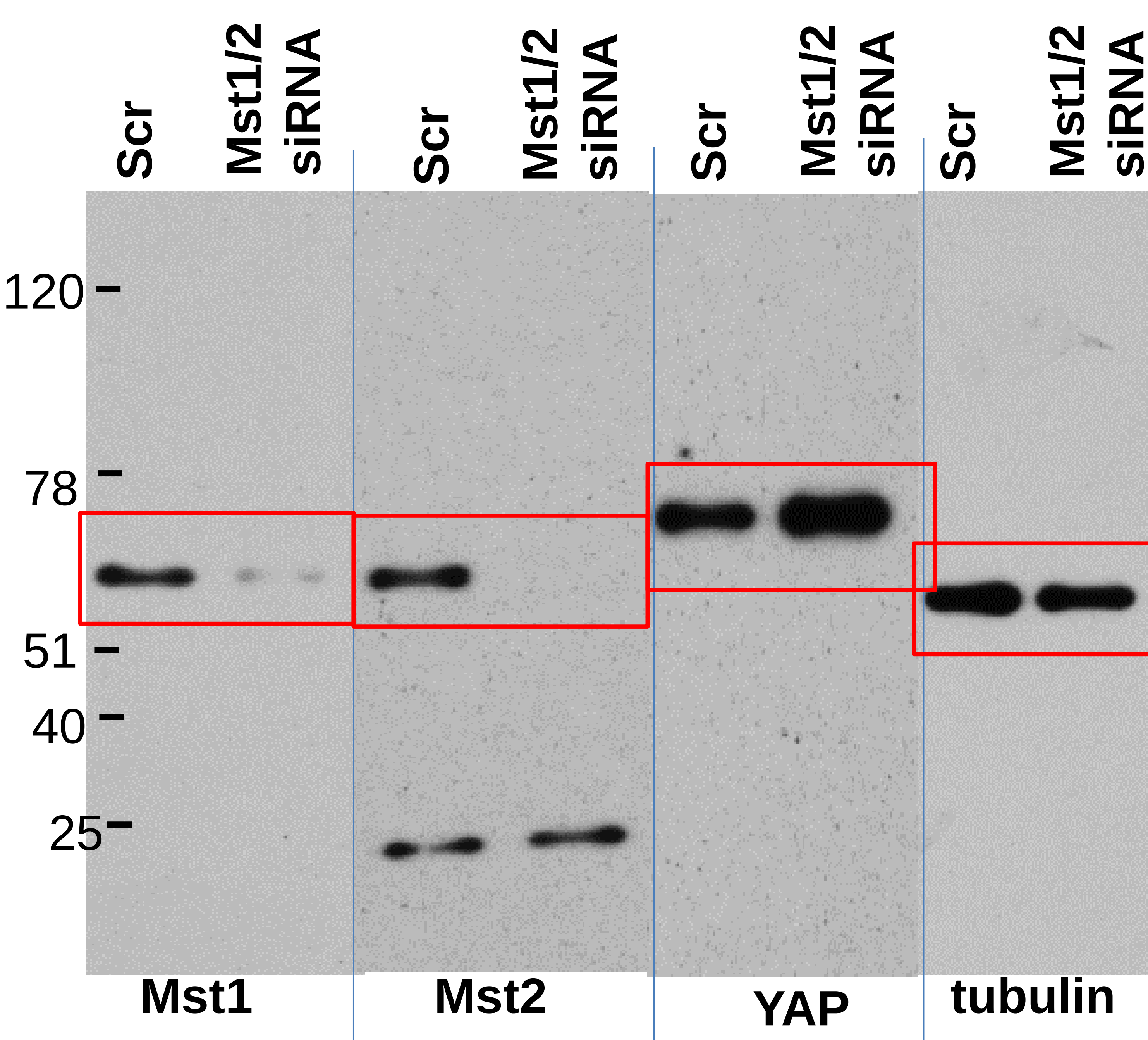


Figure 4C-Continued

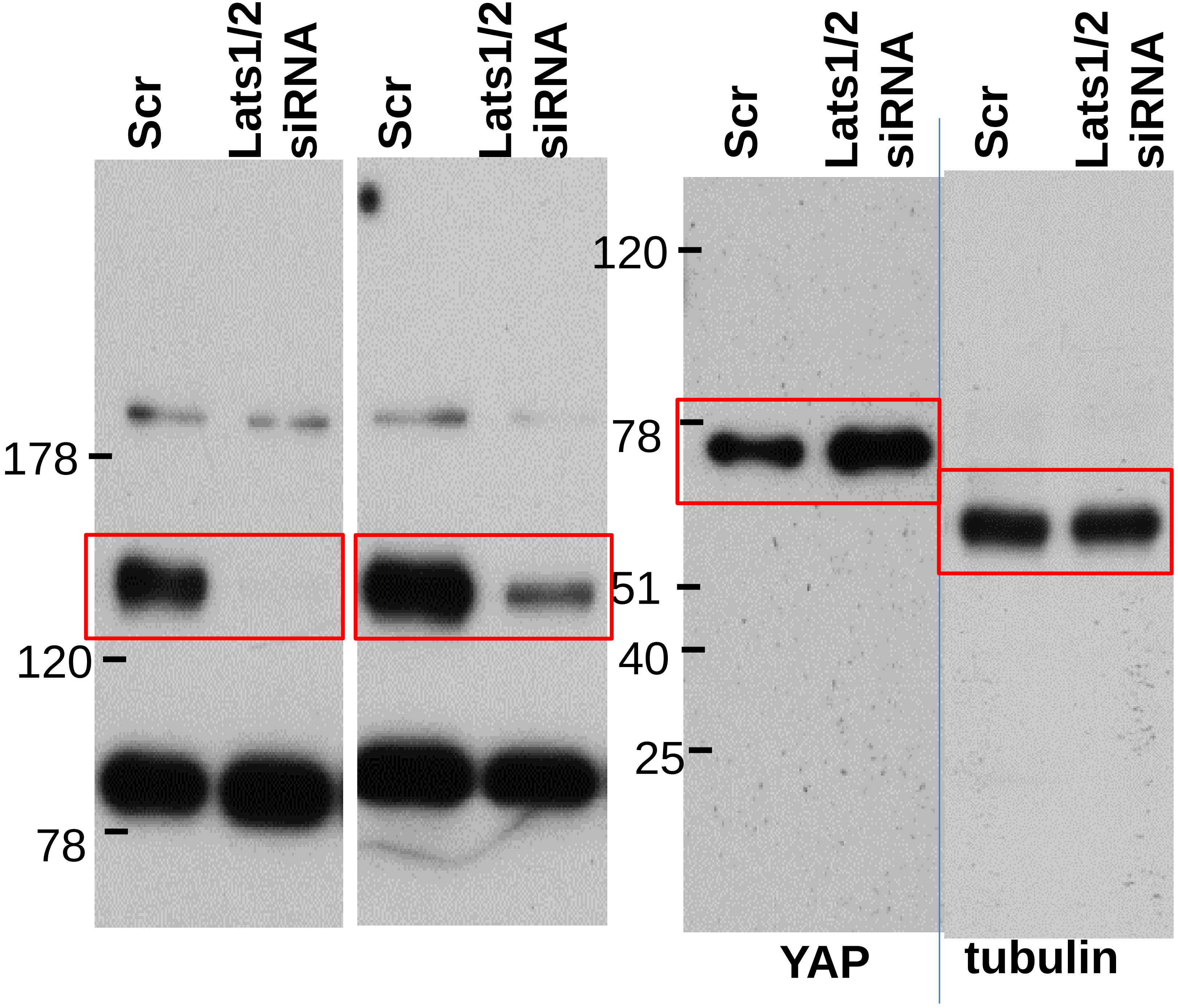


Figure 4D

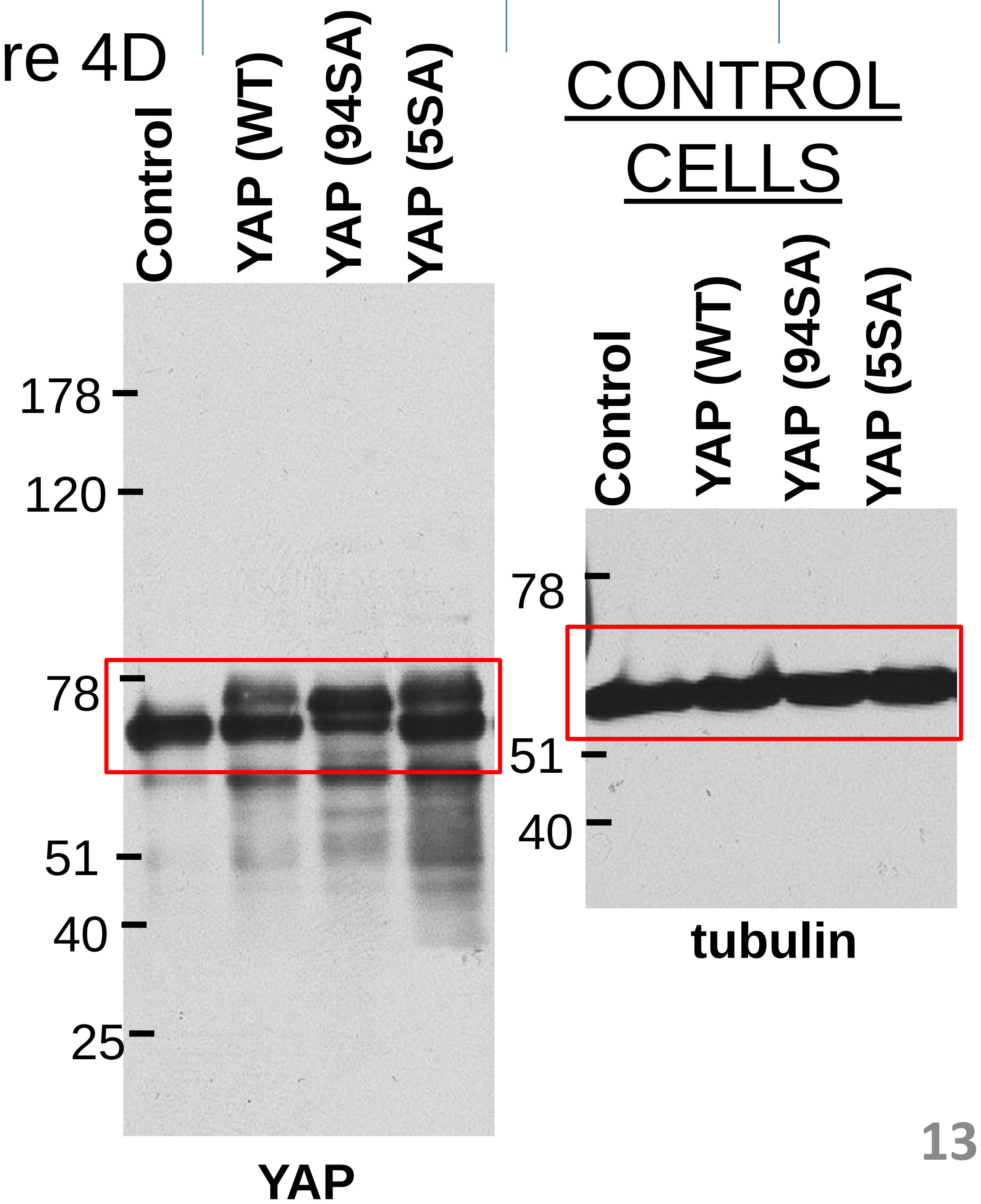


Figure 4D- Continued

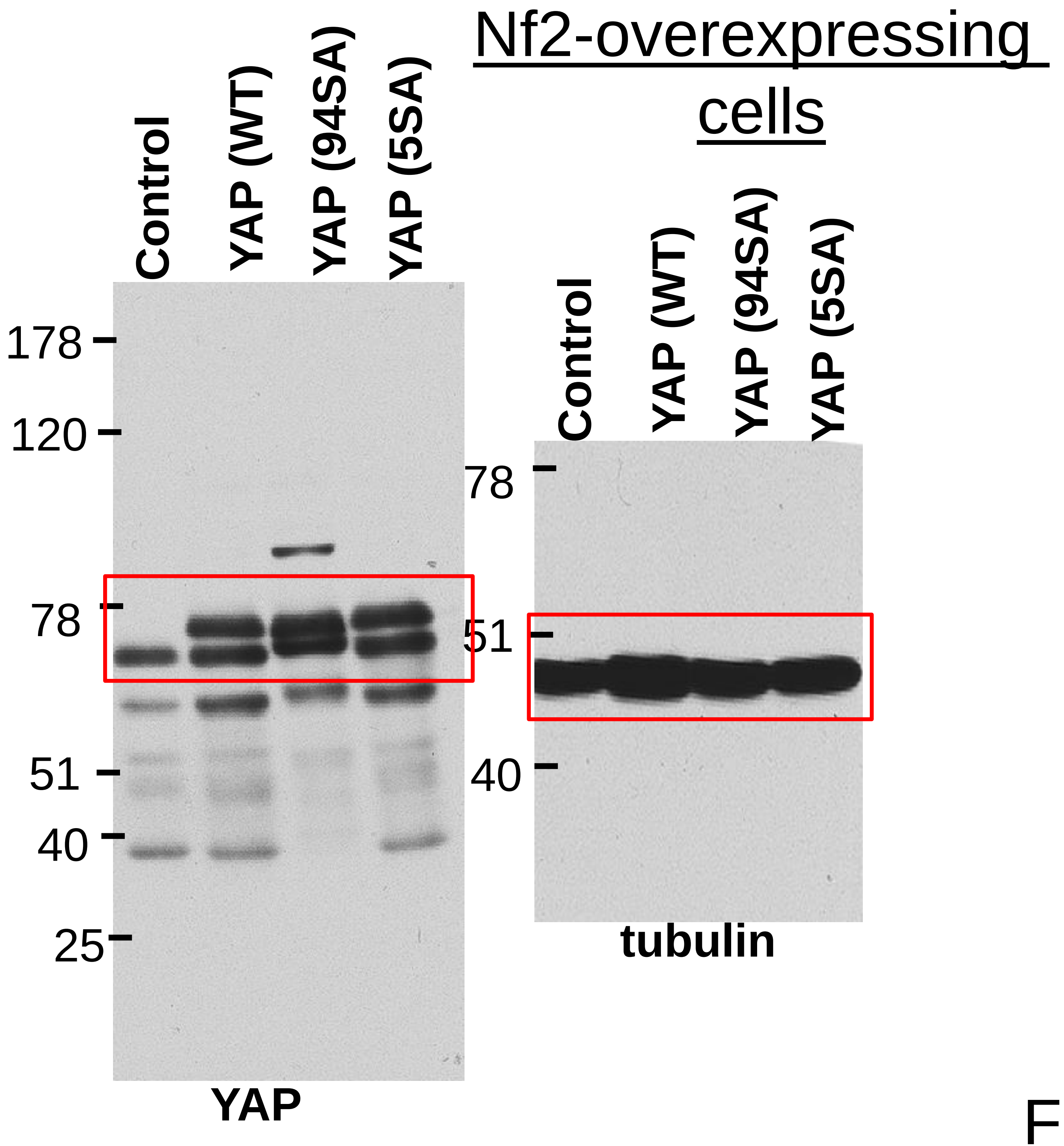


Figure 5A

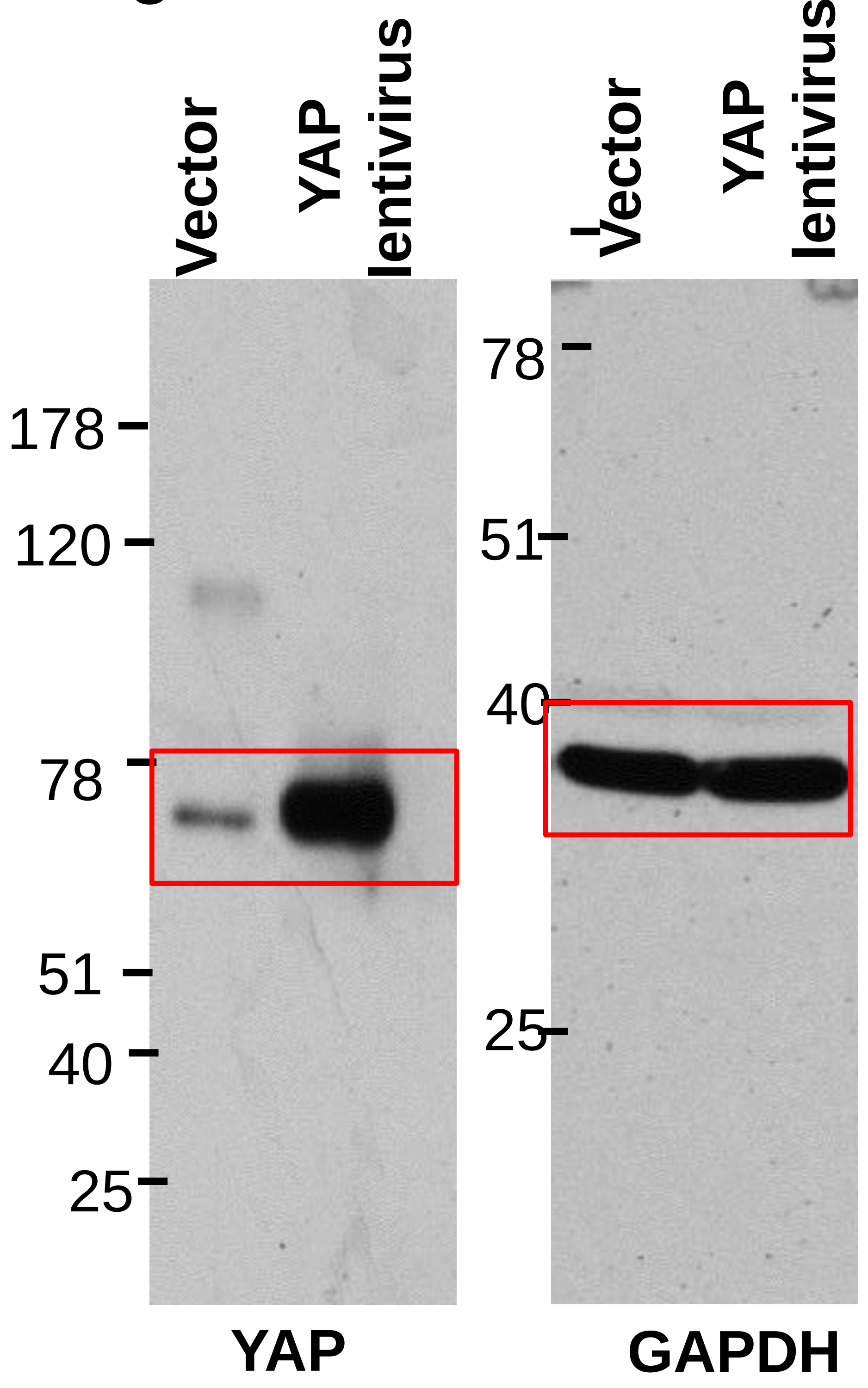


Figure 5B

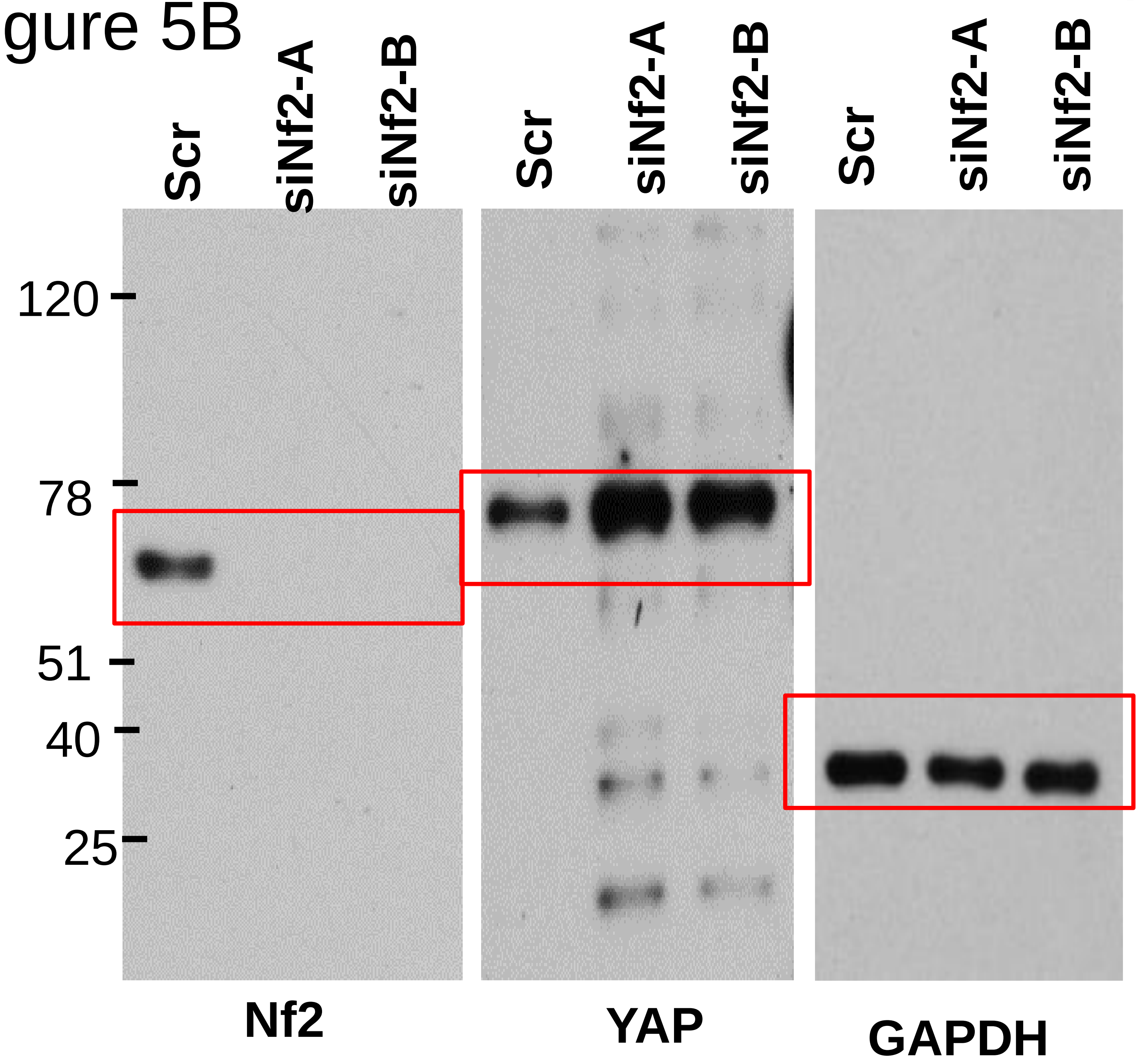


Figure 5C

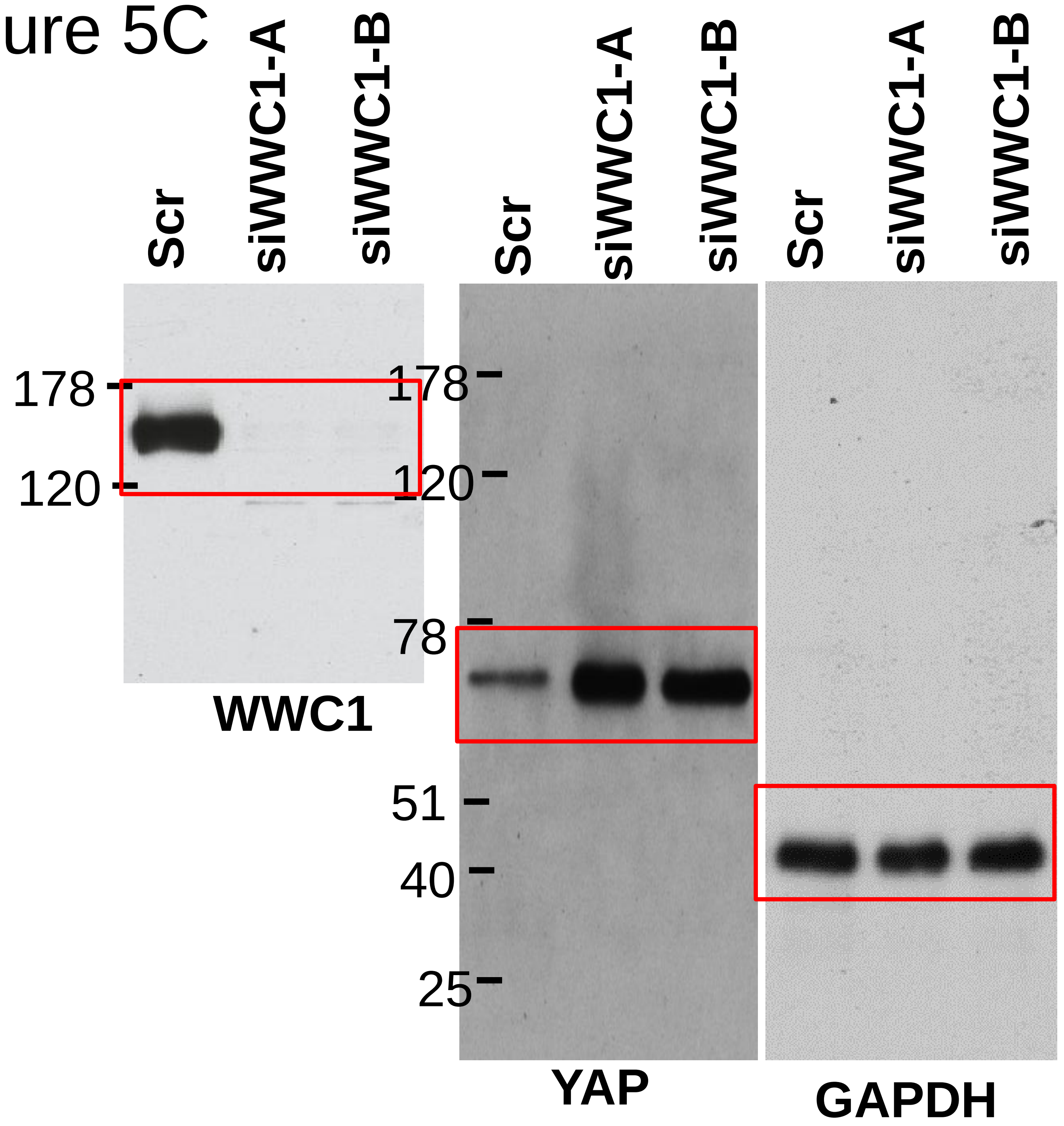


Figure 6B

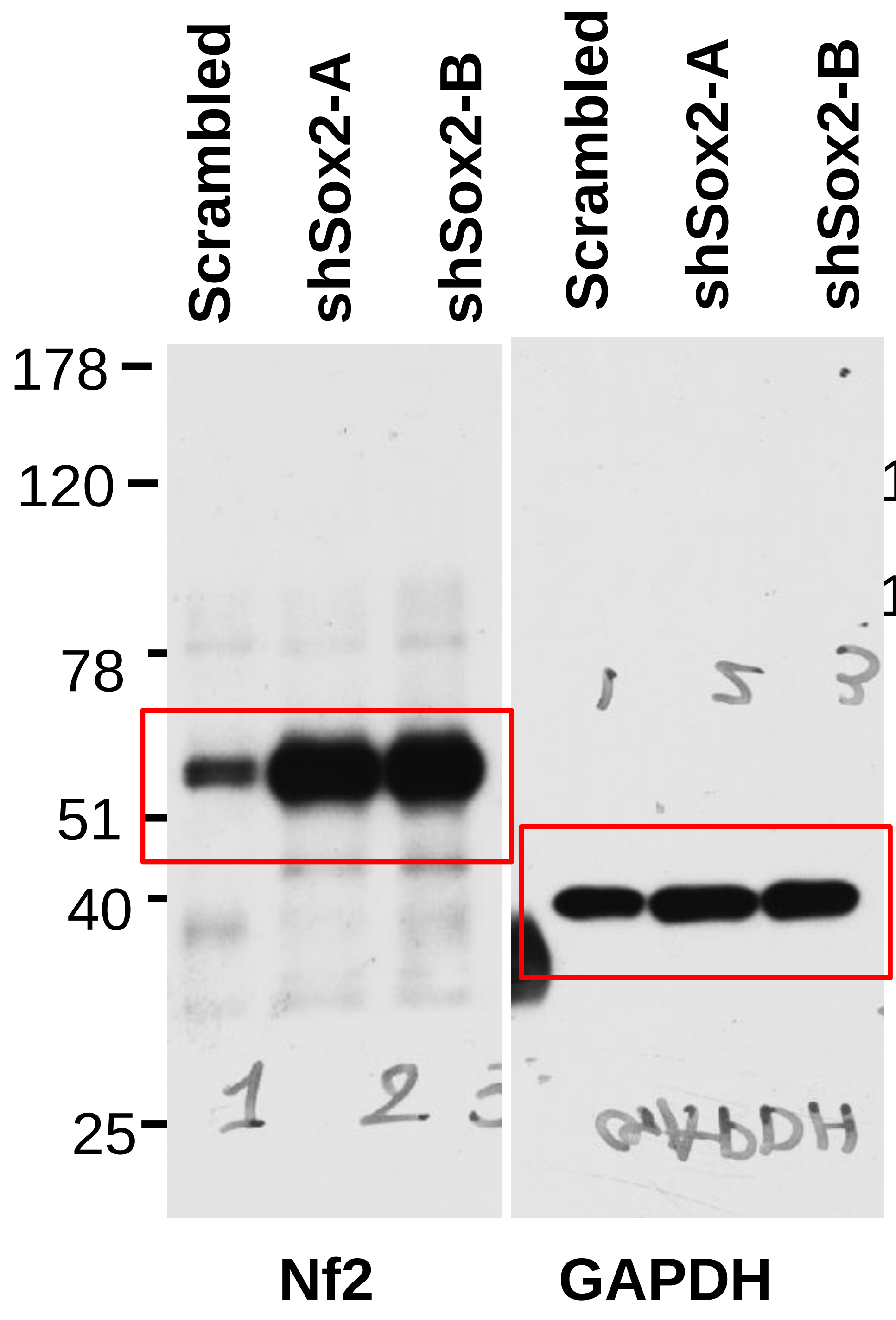


Figure 6C

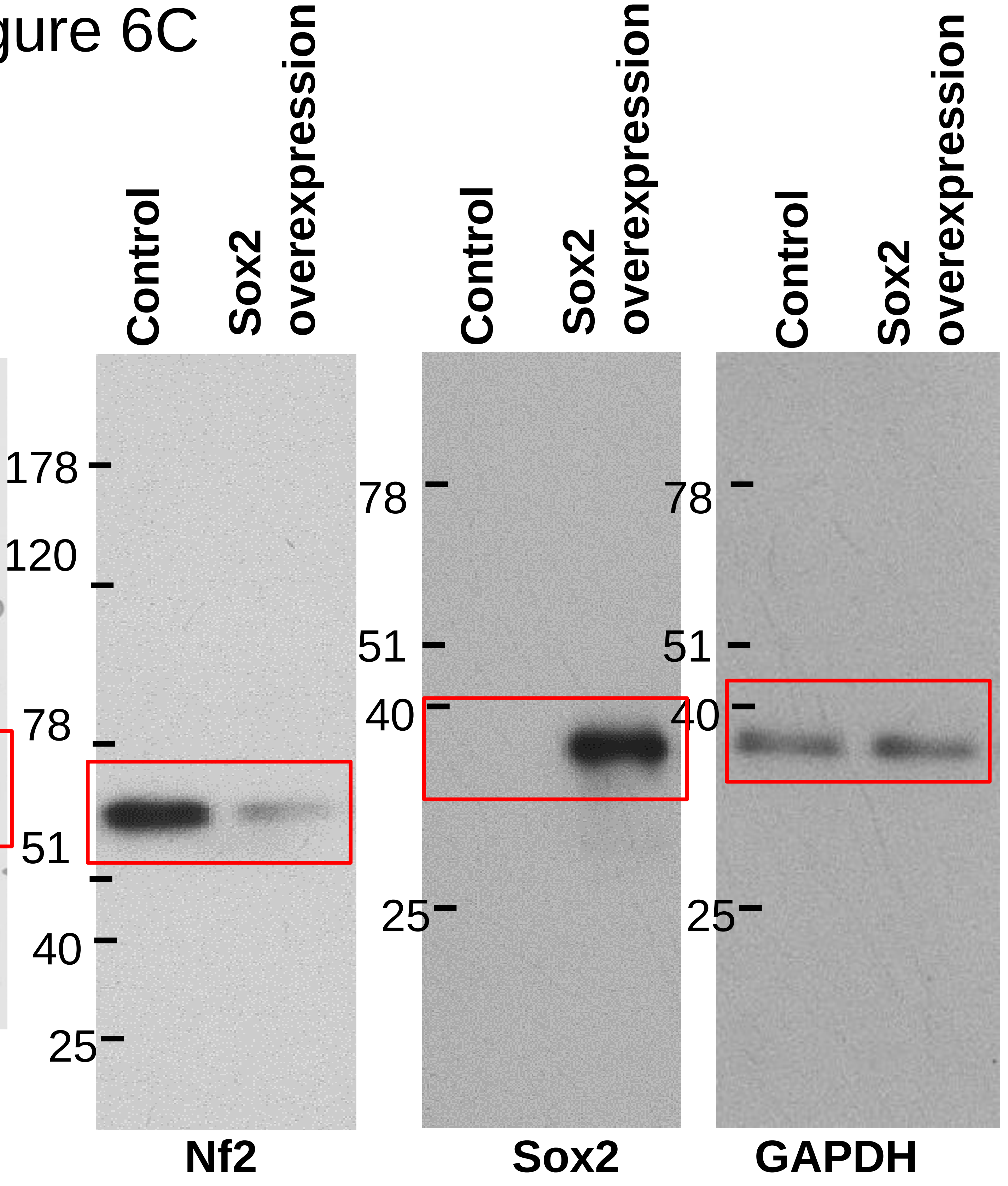


Figure 7B

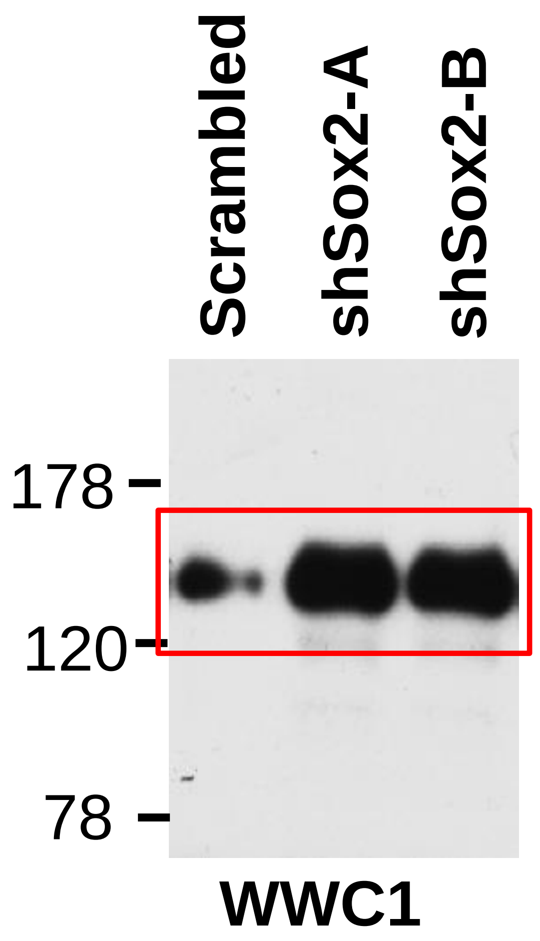


Figure 7C

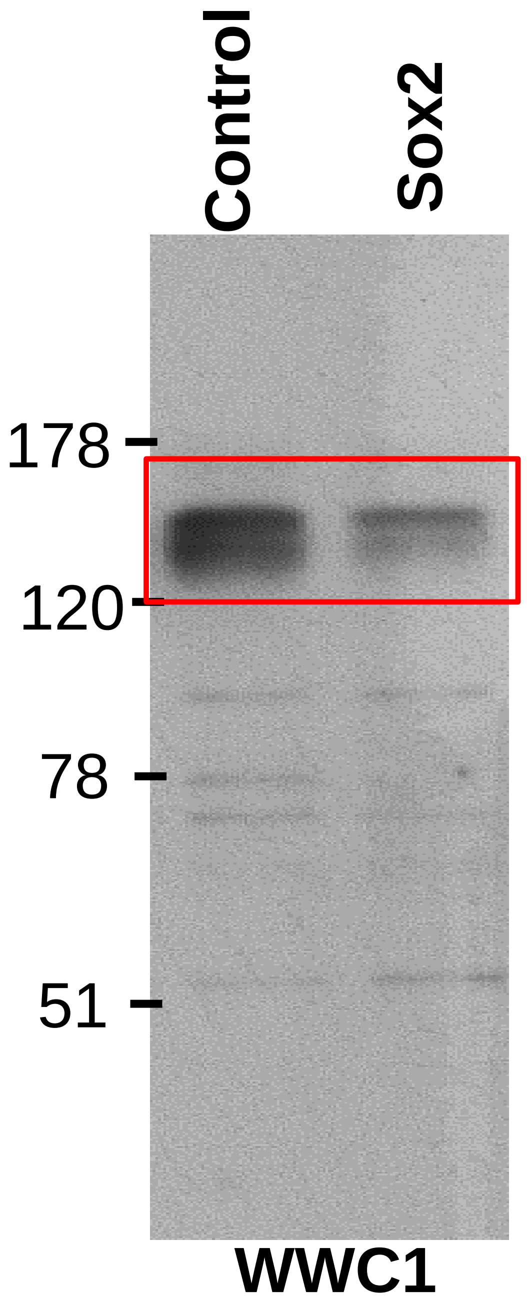


Figure 8A

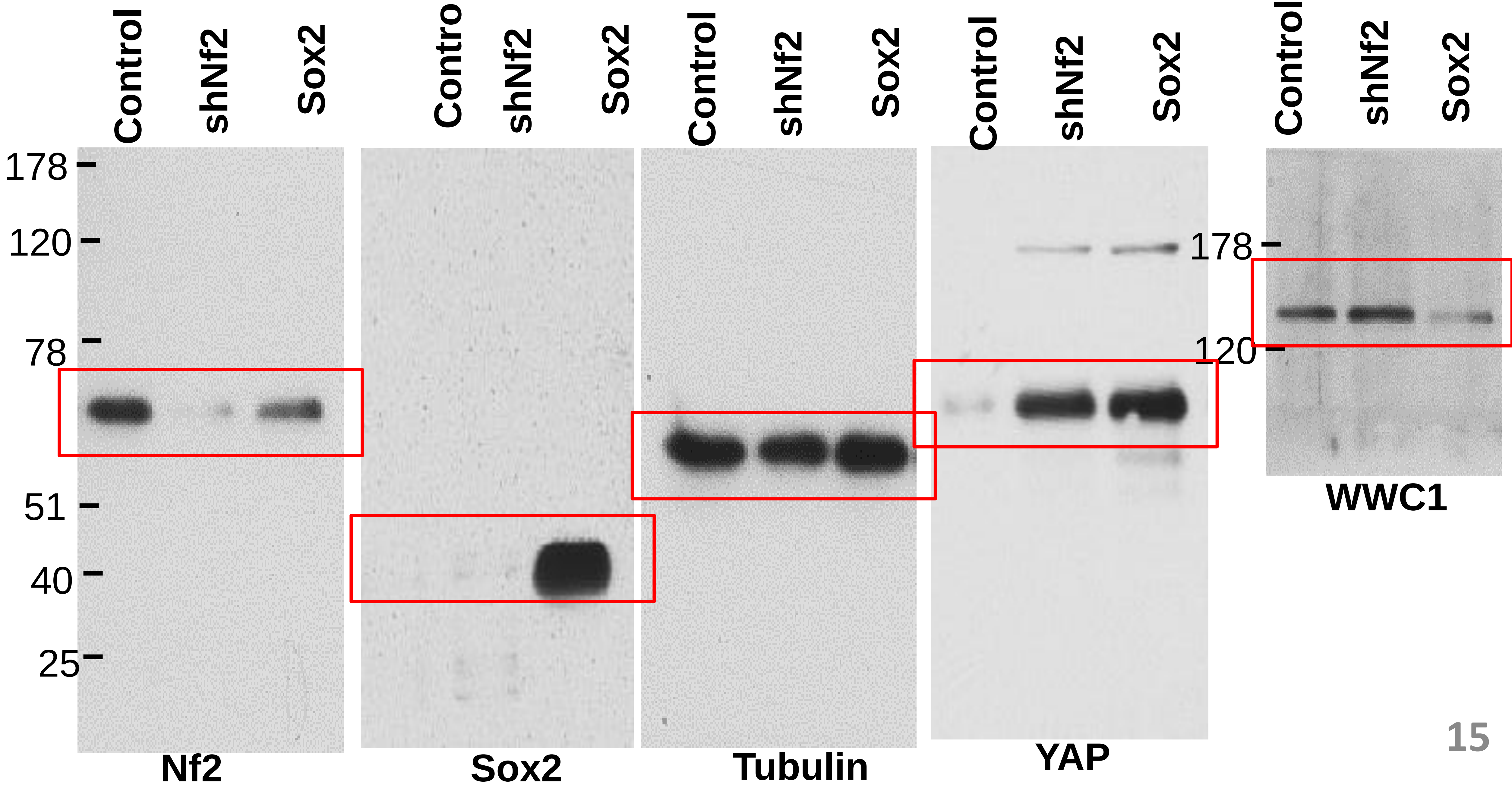


Figure 8B

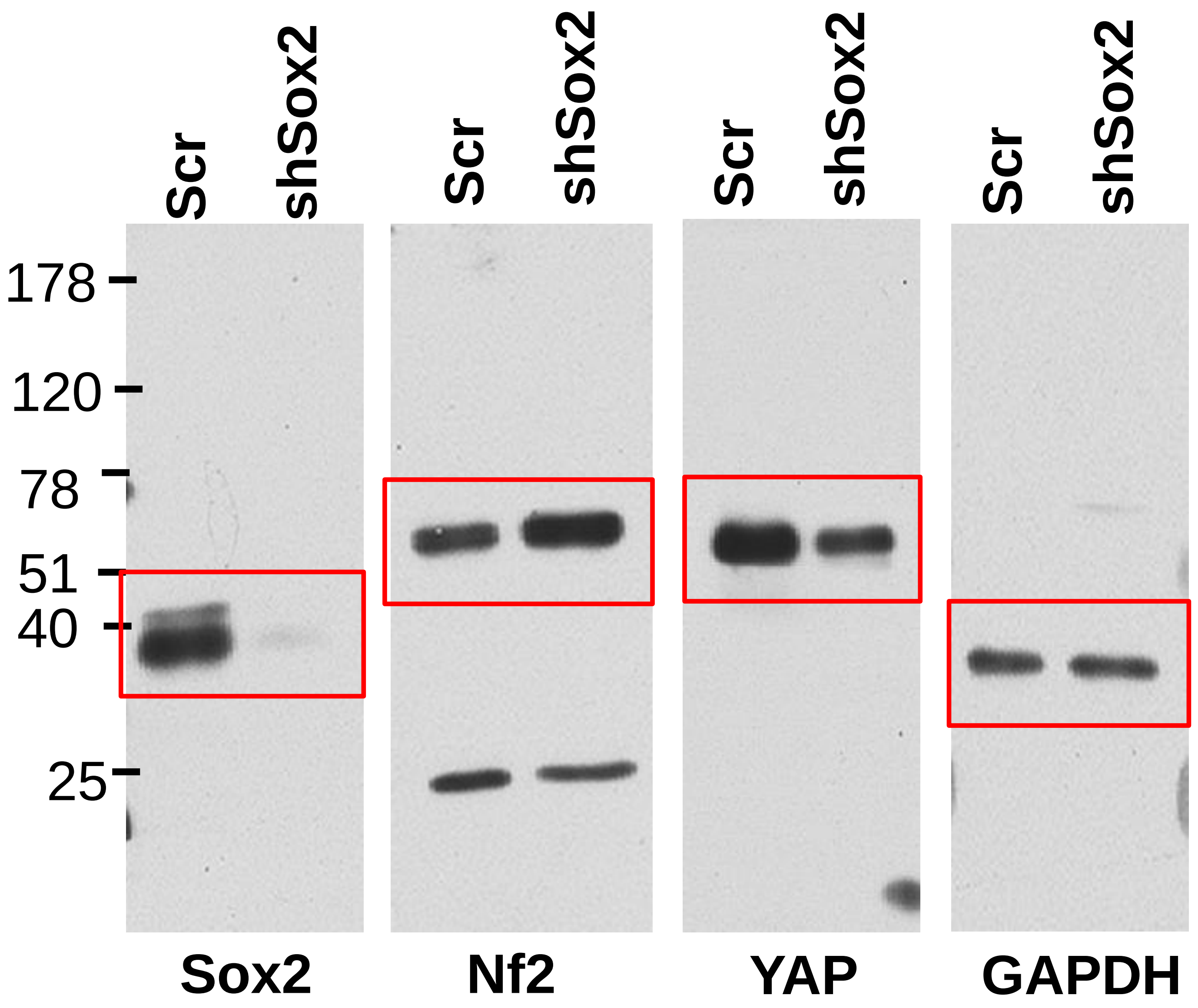
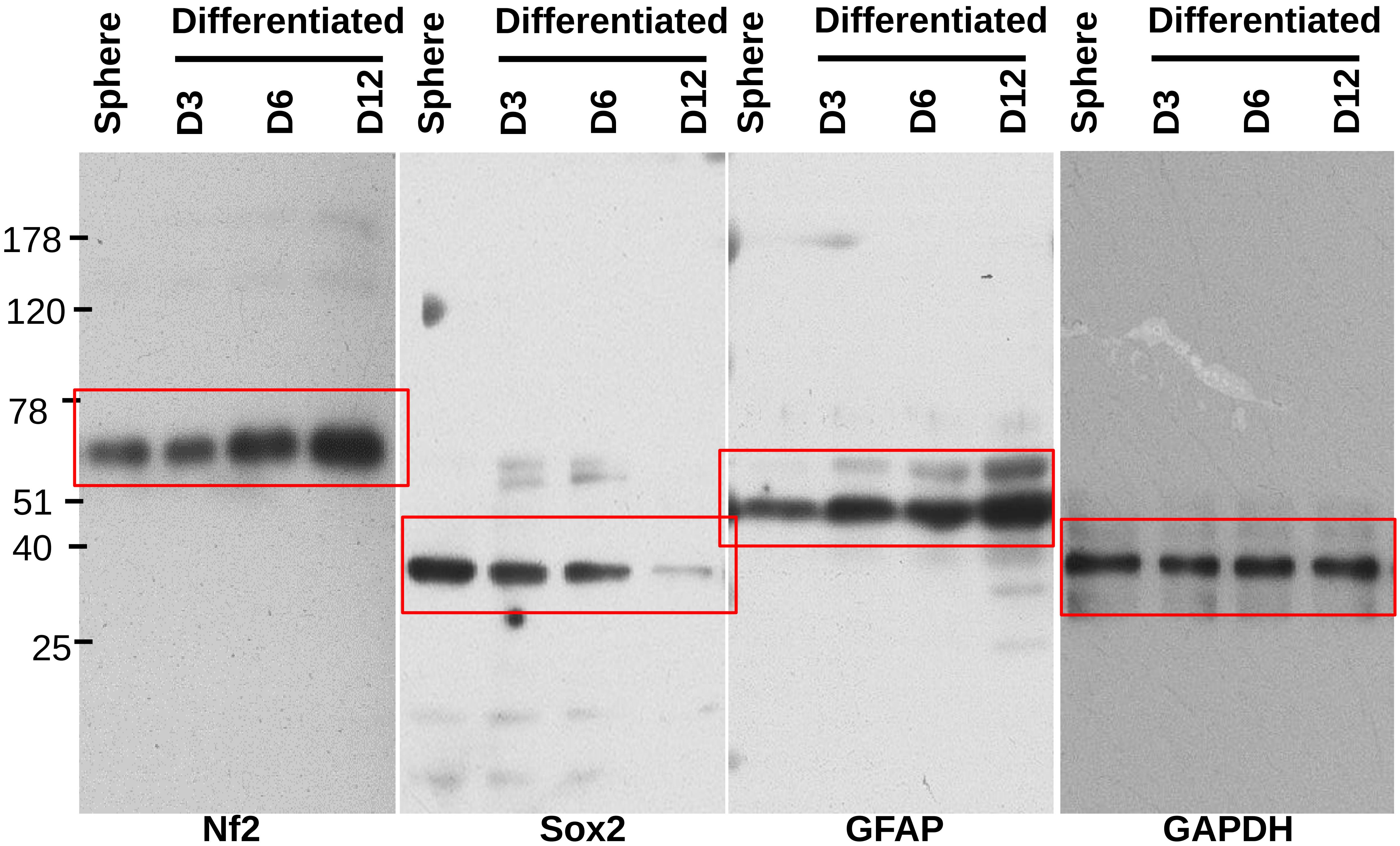


Figure 8C



Supplementary Table 1- Antibodies used in the study

Western Blotting

Protein	**Manufacturer**	**Catalog Number**	**Dilution**
Sox2	Cell Signaling	2748	1:500
YAP	Cell Signaling	4912	1:1000
Phospho-YAP	Cell Signaling	4911	1:1000
Nf2	Cell Signaling	6995	1:500
WWC1	Cell Signaling	8774	1:500
Mst1	Cell Signaling	3682	1:1000
Mst2	Cell Signaling	3952	1:1000
Lats1	Cell Signaling	3477	1:1000
Lats2	Cell Signaling	5888	1:1000
GFAP	Dako	M076101-2	1:500
GAPDH	SCBT	sc-32233	1:1000
Tubulin	Sigma	T6074	1:2000

Immunofluorescence

Protein	**Manufacturer**	**Catalog Number**	**Dilution**
YAP	SCBT	sc-271134	1:200

Immunohistochemistry

Protein	**Manufacturer**	**Catalog Number**	**Dilution**
Sox2	Millipore	AB5603	1:200
YAP	SCBT	sc-271134	1:200

Supplementary Table 2 – Primers/siRNA/shRNA used in this study

ChIP PCR Primer

Gene	Direction	Sequence
Nf2	Forward	CGC TCC TTC TCA ACC AGT CT
	Reverse	GGC CTA CTC CAC CAA TTG AA
WWC1	Forward	GCG CTC TTG TTG GTT TGA AG
	Reverse	GCC ACT TAA TGC TGC TTT AGT TC

qRT-PCR Primer

Gene	Direction	Sequence
Nf2	Forward	CTA GTT CAA GAG ATC ACG CAA CA
	Reverse	GGC AGT AGA CCT TTT CAT CCA AA
WWC1	Forward	TGC TGA GGG AAA CCA AAG CC
	Reverse	CTG GAC CAT AGG TCG GAG TG
YAP	Forward	ACC CTC GTT TTG CCA TGA AC
	Reverse	TGT GCT GGG ATT GAT ATT CCG TA
Actin	Forward	GGC TGT ATT CCC CTC CAT CG
	Reverse	CCA GTT GGT AAC AAT GCC ATG T
aP2	Forward	AAG GTG AAG AGC ATC ATA ACC CT
	Reverse	TCA CGC CTT TCA TAA CAC ATT CC
PPARγ	Forward	TTT TCA AGG GTG CCA GTT TC
	Reverse	AAT CCT TGG CCC TCT GAG AT
Osterix	Forward	GGA AAG GAG GCA CAA AGA AGC
	Reverse	CCC CTT AGG CAC TAG GAG C
BSP	Forward	GGA GGG GGC TTC ACT GAT
	Reverse	AAC AAT CCG TGC CAC CA

Gene	**Direction**	**Sequence**
CTGF	Forward	GGC CTC TTC TGC GAT TTC G
	Reverse	GCA GCT TGA CCC TTC TCG G
Cyr61	Forward	CTC CAG AAT CTA CCA AAA CGG G
	Reverse	CGT CCA GGG AGT CCT TAA TGC
DYN3	Forward	GAC GGC CTC TTG TAC TCC AG
	Reverse	CTG TTA CGC GGT CTG TTT CTG
PTGS2	Forward	TGC ACT ATG GTT ACA AAA GCT GG
	Reverse	TCA GGA AGC TCC TTA TTT CCC TT
LOX	Forward	TCT TCT GCT GCG TGA CAA CC
	Reverse	GAG AAA CCA GCT TGG AAC CAG
BDNF	Forward	TTA CCT GGA TGC CGC AAA CAT
	Reverse	TGA CCC ACT CGC TAA TAC TGT C

Primers for site-directed mutagenesis (Figure 6D)

Gene	**Direction**	**Sequence**
Nf2 promoter (Sox2-binding site)	Forward	GGT CAC TCC GAG CGC TTT CGA TCC TAA AGT ATA CTC CTA C
	Reverse	GTA GGA GTA TAC TTT AGG ATC GAA AGC GCT CGG AGT GAC C

shRNAs used in this study (obtained from Sigma unless otherwise stated)

Gene	Sample	Catalog Number
Nf2	shRNA-A	SHCLNG-NM_010898 _TRCN0000042519
	shRNA-B	SHCLNG-NM_010898 _TRCN0000042520
Sox2	shRNA-A	Described in BasuRoy et al, Cell Death and Differentiation, 2010
	shRNA-B	Described in BasuRoy et al, Cell Death and Differentiation, 2010
YAP	shRNA-A	SHCLNG-NM_009534_TRCN0000238436
	shRNA-B	SHCLNG-NM_009534_TRCN0000238432

siRNAs used in this study (obtained from Ambion/Life Technologie)

Gene	Sample	Catalog Number
Nf2	siRNA-A	4390771-s70500
	siRNA-B	4390771-s70501
WWC1	siRNA-A	4390771- s102485
	siRNA-B	4390771-s102486
YAP	siRNA-A	4390771-s76160
	siRNA-B	4390771-s202423
TAZ	siRNA-A	4390771-s97145
	siRNA-B	4390771-s97146
Lats1		4390771-s201588
Lats2		4390771-s78350
Mst1		AM16708-62215
Mst2		AM16708 - 73946