

Supplemental Information

Baf53a is involved in survival of mouse ES cells, which can be compensated by Baf53b

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Supplemental Table S1. List of primer sequences for RT-PCR

Gene Name		Sequences	
<i>For RT-PCR</i>			
Baf53a	F	TAGTTTCCAGGCCATTTTGG	
	R	GTGGTGTGGGTAGCTCCACT	
Nanog	F	AGGGTCTGCTACTGAGATGCTCTG	
	R	CAACCACTGGTTTTTCTGCCACCG	
p21	F	GAACGGTGGAACTTTGACTTC	
	R	AGAGTGCAAGACAGCGACAAG	
<i>For cloning</i>			
Baf53a full	F	CAATTGATGAGCGGCGGCGTGTACGGC	
	R	GCGGCCGCTCAAGGGCATTTTCTTTCTACA	
Baf53b full	F	GAATTCATGAGCGGGGCGTCTAC	
	R	GCGGCCGCTCAGGGGCACTTCCGCTC	
Baf53a M3	F	GACGGTGGCGGCAGCTTTCAGCT	
	R	AGCTGAAAGCTGCCGCCACCGTC	
Baf53a ^R	F	GGTTGACTTTCCTACCGCCATCGGTGTGGTGCTGGAGAGA	
	R	ACCGATGGCGGTAGGAAAGTCAACCTTAGGGCAGTCCTCG	

Legends for Supplemental Figures

Supplemental Figure 1. Baf53a knockdown in ES cells induces the expression of undifferentiated state marker genes and reduces ES cell proliferation.

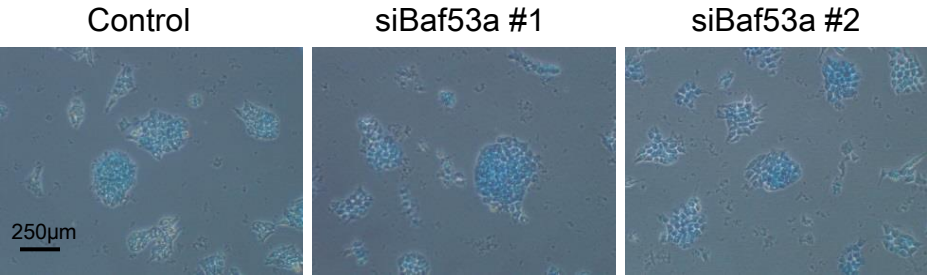
(A) Alkaline phosphatase stain in control and Baf53a knockdown ES cells. EGFP control siRNA (Control) or two independent Baf53a siRNAs (siBaf53a #1 and #2) were transfected into E14 ES cells. Alkaline phosphatase activity of these ES cells was detected by VECTOR Blue alkaline phosphatase substrate kit 48h after transfection and morphological features were observed. Scale bar is 250 μm . (B) Expression of undifferentiated state marker genes in Baf53a knockdown ES cells. Expression of Baf53a, Dax1, Oct3/4, Esrrb, and Nanog mRNA in control, siBaf53a #1, or siBaf53a #2 ES cells was examined by quantitative RT-PCR. (C) Expression of Oct3/4 protein in Baf53a knockdown ES cells. Protein expression of Baf53a and Oct3/4 in control, siBaf53a #1, or siBaf53a #2 ES cells was examined by Western blot. $\alpha\text{Tubulin}$ was used as a loading control. The result is representative of three independent experiments. (D) Proliferation of Baf53a knockdown ES cells. Control, siBaf53a #1, or siBaf53a #2 ES cells were seeded at 500, 1000, or 3000 cells per well in a 96-well dish, and subjected to WST-1 assay at 2 days after incubation. The result is representative of three independent experiments represents the means $\pm$ standard deviations of triplicate assays.

Supplemental Figure 2. (A) E14 ES cells were transfected with expression vectors for empty (ev), Baf53a WT, or Baf53a M3. Transfected ES cells were cultured in the presence of 1 $\mu\text{g/mL}$ puromycin; and these cells were subjected to the WST-1 assay. The data is representative of three independent experiments. (B) Expression of exogenous Myc-

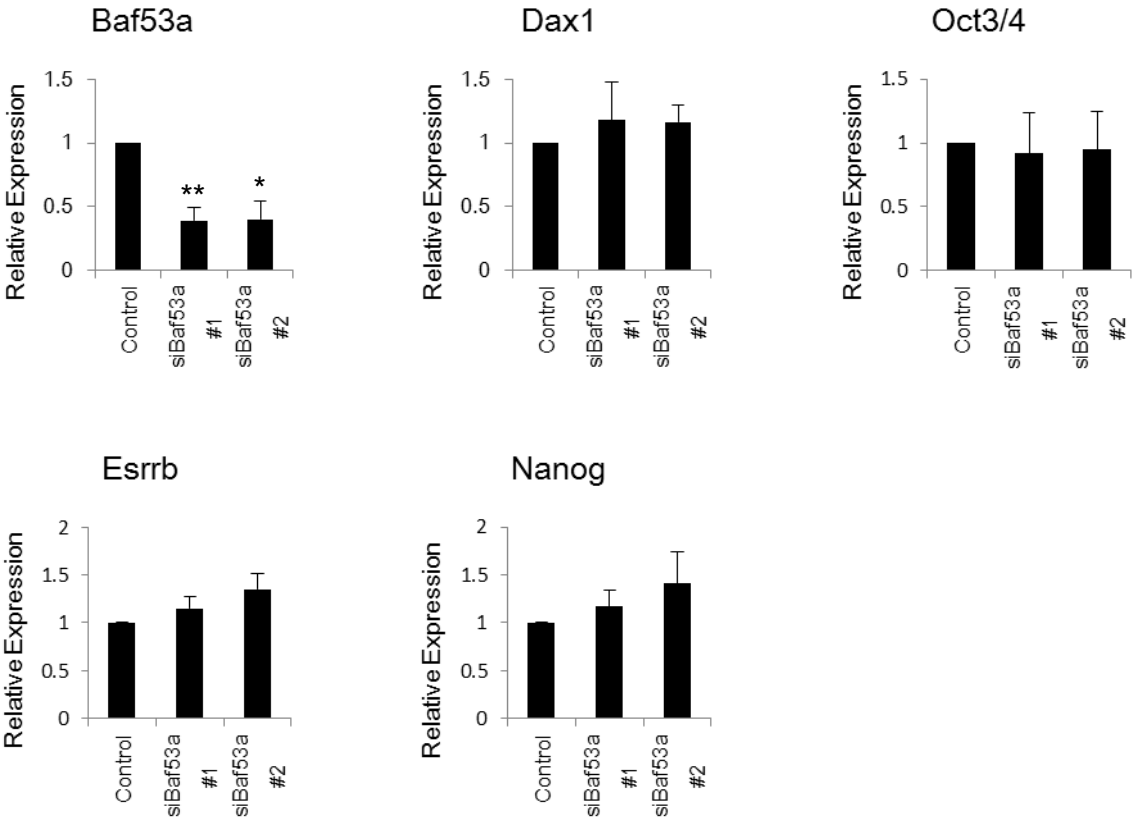
tagged Baf53a protein. Expression of either Myc-Baf53a WT or Myc-Baf53a M3 in the panel A was confirmed by Western blot analysis. α Tubulin was used as a loading control. The result is representative of three independent experiments. (C) Amino acid sequence alignment of Baf53a and Baf53b. Baf53a and Baf53b consist of 429 and 426 amino acid residues, respectively, and they exhibit 84% identity of these two amino acid sequences <<http://web.expasy.org/sim/>>. The three amino acids (ERR) of Baf53a to construct Baf53a M3 are shown as red letters.

Supplemental Figure 3. Original full blot images of Western blot analyses. (A) Original images of Figure 1C. (B) Original images of Figure 3A. (C) Original images of Figure 4D. (D) Original images of Figure 4F. (E) Original images of Figure 5E. (F) Original images of Figure 6D. (G) Original images of Supplemental Figure 1C. (H) Original images of Supplemental Figure 2B.

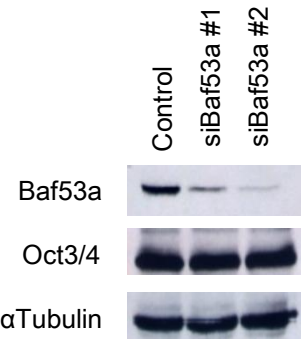
A



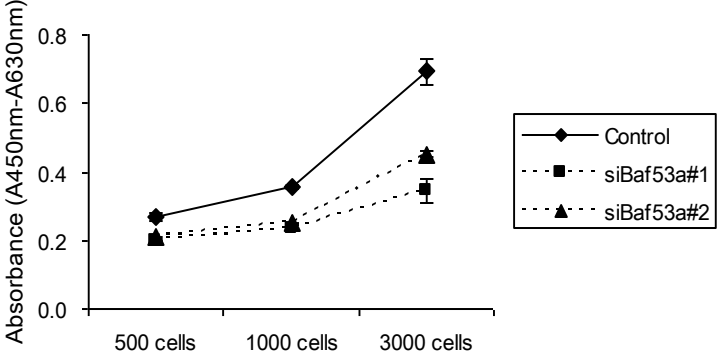
B



C

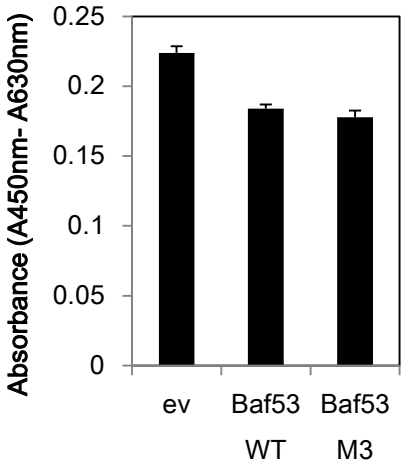


D

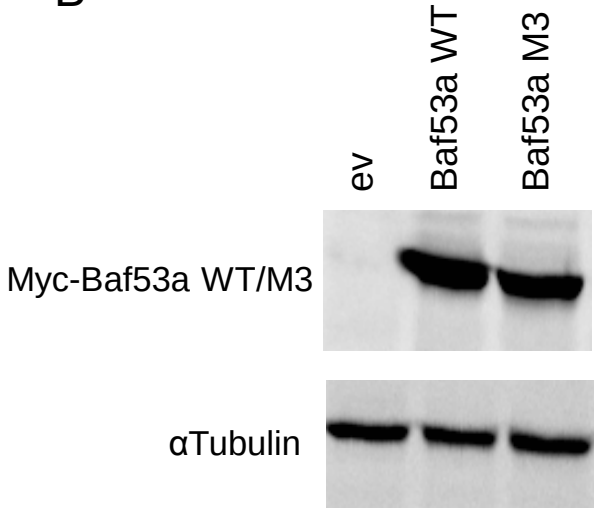


Supplemental Figure 1

A



B

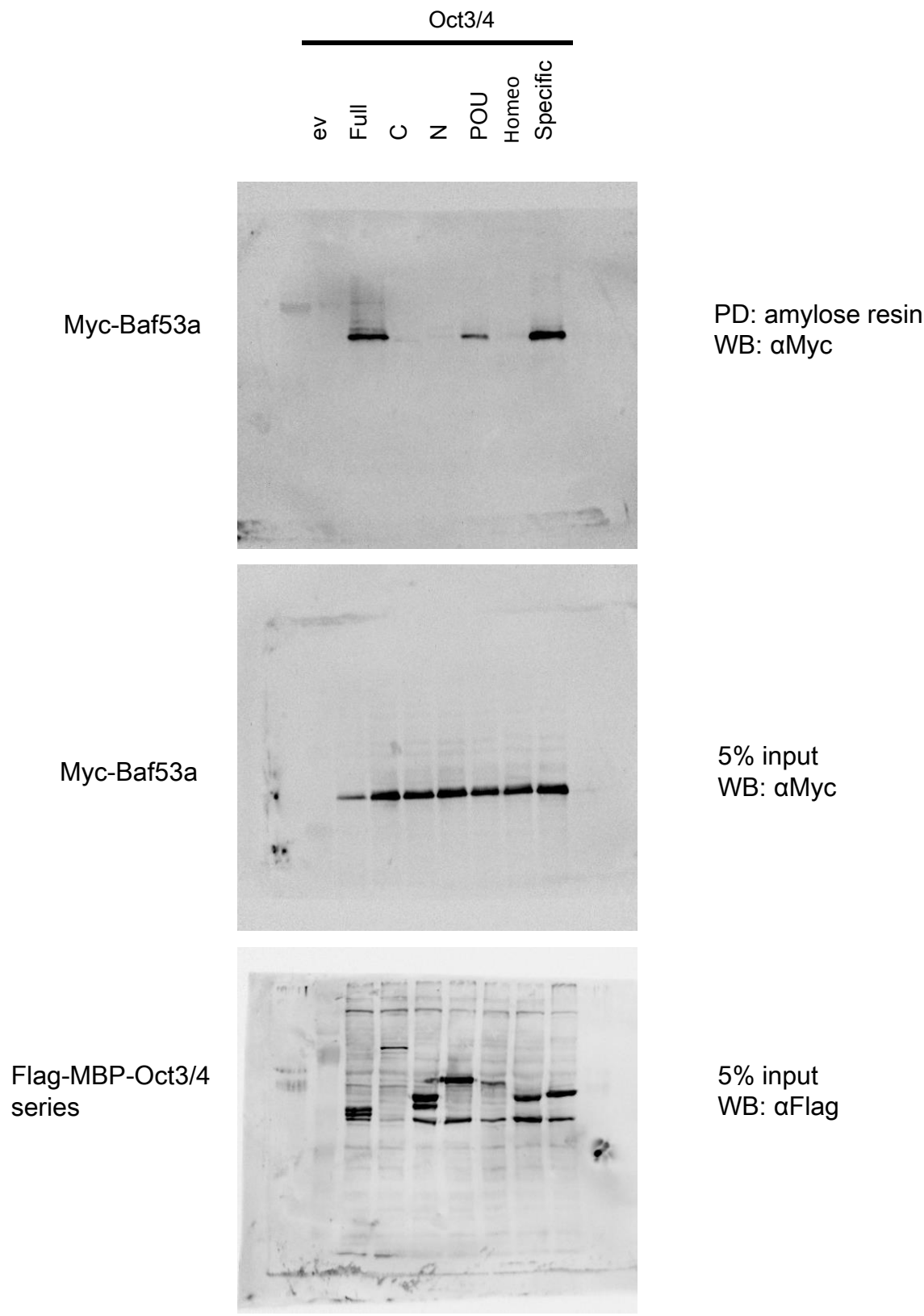


C

Baf53a	1	MSGGVYGGDEVGALVFDIGSYTVRAGYAGEDCPKVDFTAIGVVLERDDGSTMMEIDGDK
Baf53b	1	MSGGVYGGDEVGALVFDIGSFVRAGYAGEDCPKADFPTTVGLLAAEEGGG--LELEGEK

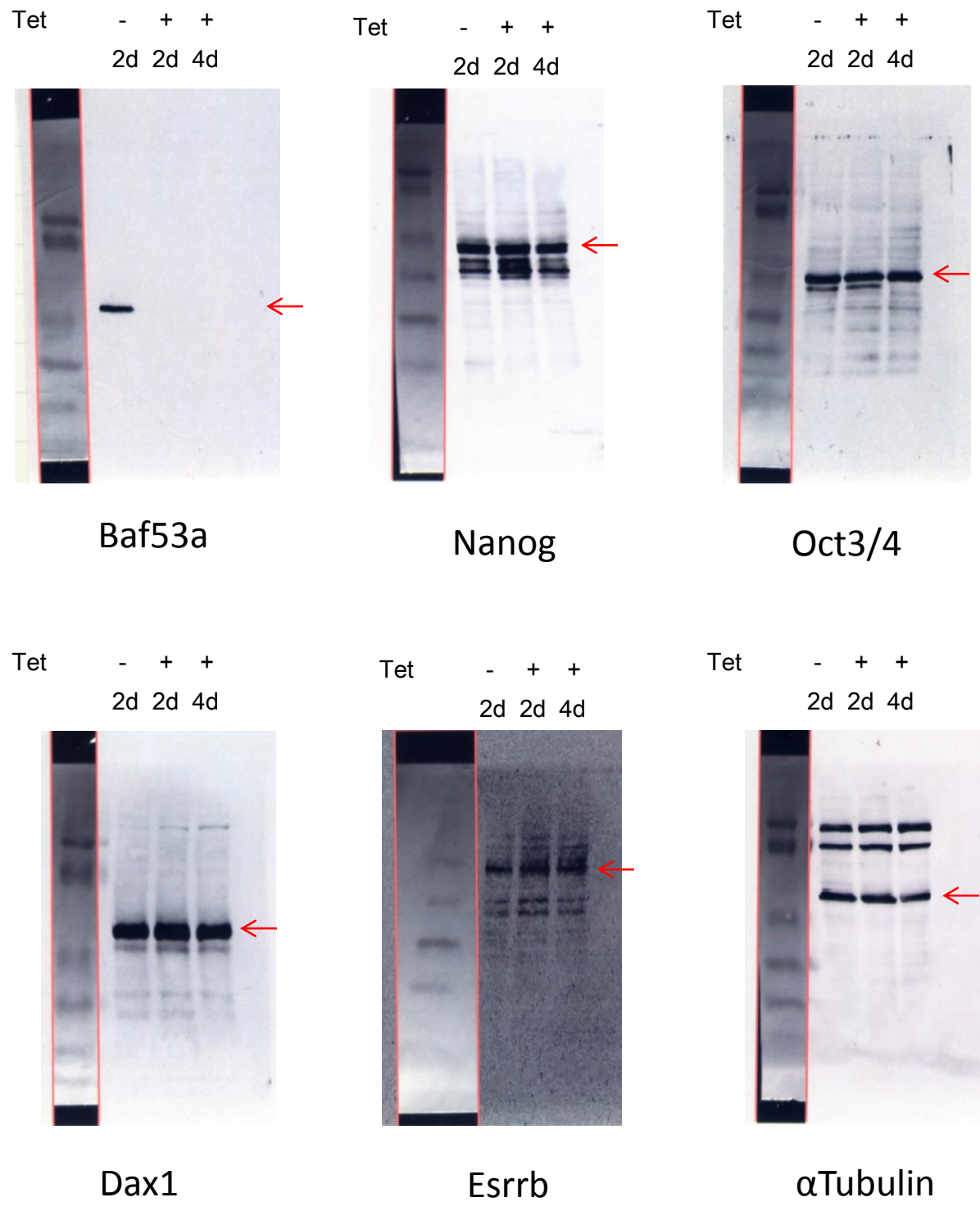
Baf53a	61	GKQGGPTYIIDTNALRVPRENMEAISPLKNGMVEDWDSFQAILDHTYKMHVKSEASLHPV
Baf53b	59	EKKG-KIFHIDTNALHVPRDGAEVMSPLKNGMIEDWECFRAILDHTYSKHKSEPNLHPV
		* * ***** *
Baf53a	121	LMSEAPWNTRAKREKLTLMFEHYSIPAFFLCKTAVLTAFANGRSTGLILDSGATHTTAI
Baf53b	118	LMSEAPWNTRAKREKLTLMFEQYNIPAFFLCKTAVLTAFANGRSTGLVLDGATHTTAI
		***** *
Baf53a	181	PVHDGYVLQGGIVKSPLAGDFITMQCRELFQEMNIELIPPYMIASKEAVREGSPANWKRK
Baf53b	178	PVHDGYVLQGGIVKSPLAGDFISMQCRELFQEMAIIDIPPYMIAAKEPVREGAPPNWKKK
		***** *
Baf53a	241	EKLPPQVTRSWHNYMCNCVIQDFQASVLQVSDSTYDEQVAAQMPTVHYEFPNGYNCDFGAE
Baf53b	238	EKLPPQVSKSWHNYMCNEVIQDFQASVLQVSDSPYDEQVAAQMPTVHYEMPNGYNTDYGAE
		***** *
Baf53a	301	RLKIPEGLFDPSNVKGLSGNTMLGVSHVVTTSVGMCDIDIRPGLYGSVIVAGGNTLIQSF
Baf53b	298	RLRIPEGLFDPSNVKGLSGNTMLGVGHVVTTSIGMCDIDIRPGLYGSVIVTGGNTLLQGF
		* * ***** *
Baf53a	361	TDRLNRELSQKTPPSMRLKLIANNTTVERRFSSWIGGSILASLGTFOQMWIISKQEYEEGG
Baf53b	358	TDRLNRELSQKTPPSMRLKLIASNSTMERKFSPWIGGSILASLGTFOQMWIISKQEYEEGG
		***** *
Baf53a	421	KQCVERKCP
Baf53b	418	KQCVERKCP

A



Supplemental Figure 3A

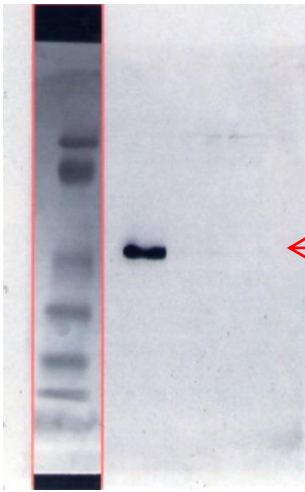
B



Supplemental Figure 3B

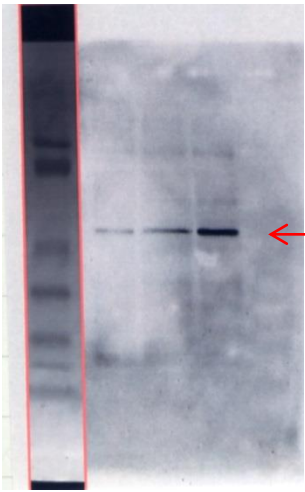
C

Tet - + +
 2d 2d 4d



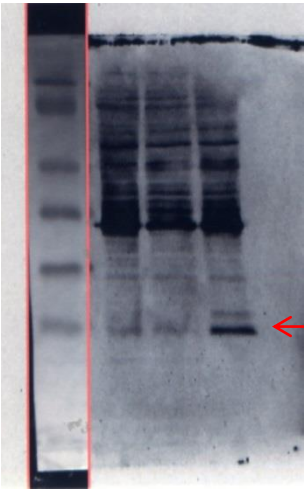
Baf53a

Tet - + +
 2d 2d 4d



p53

Tet - + +
 2d 2d 4d



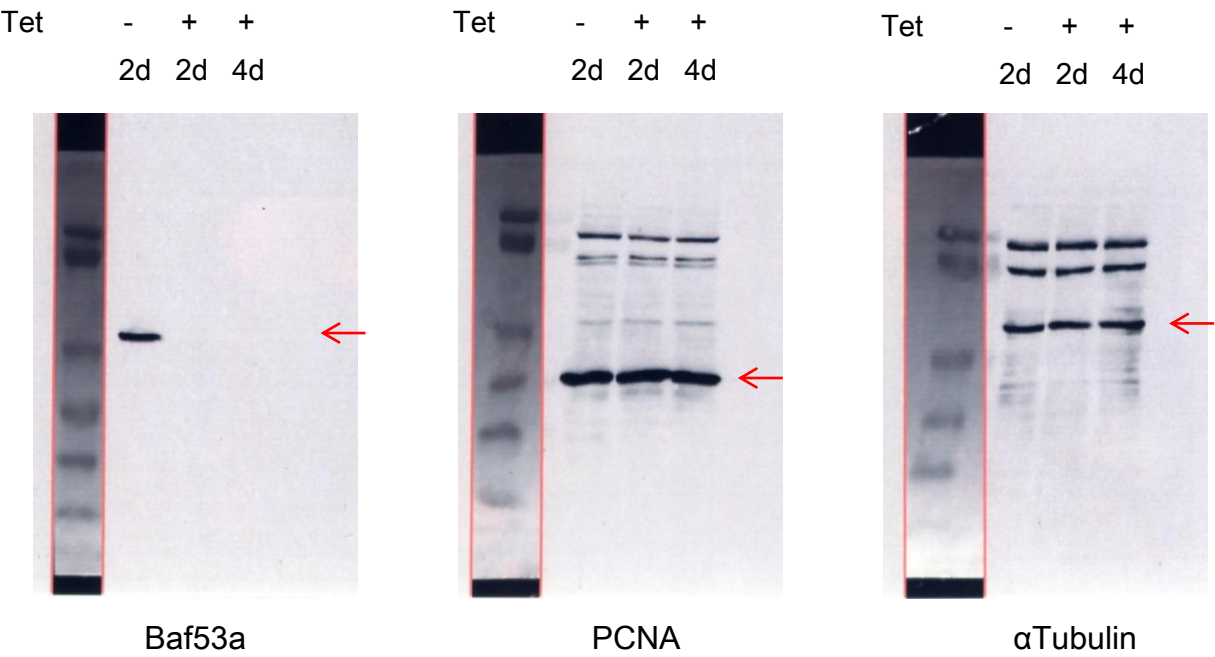
Cleaved Caspase3

Tet - + +
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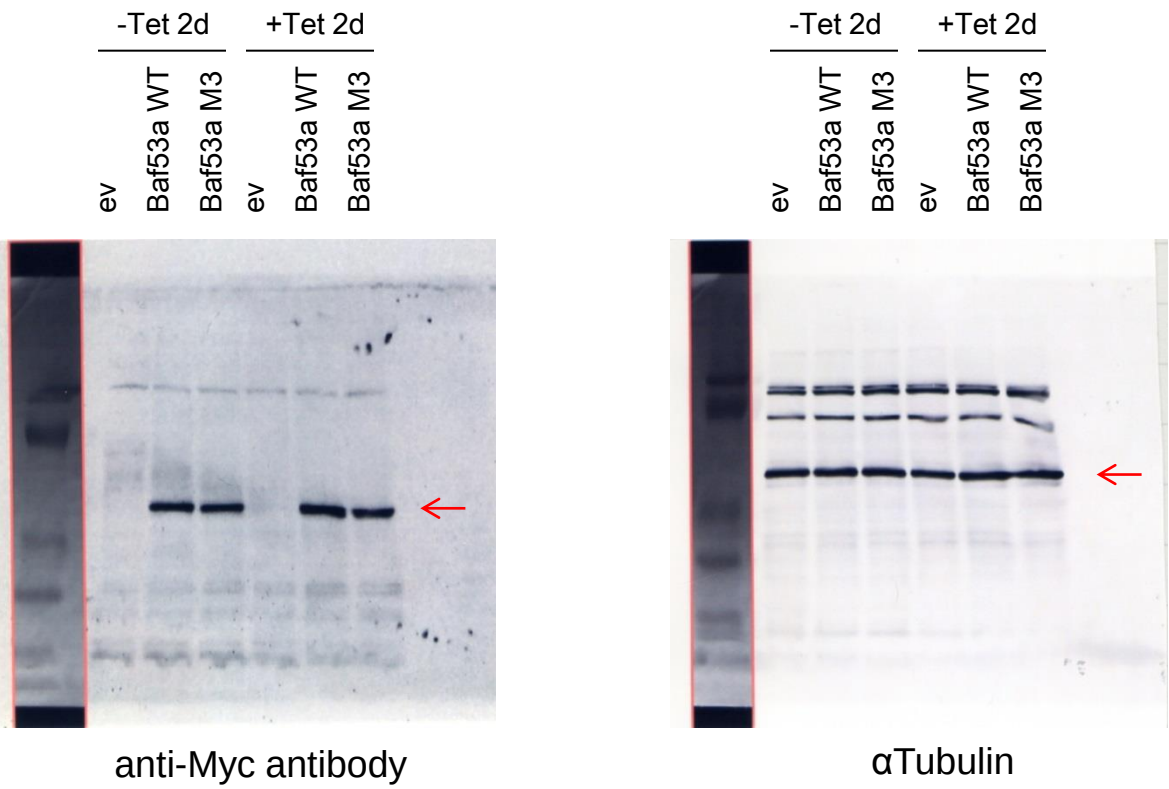


αTubulin

D

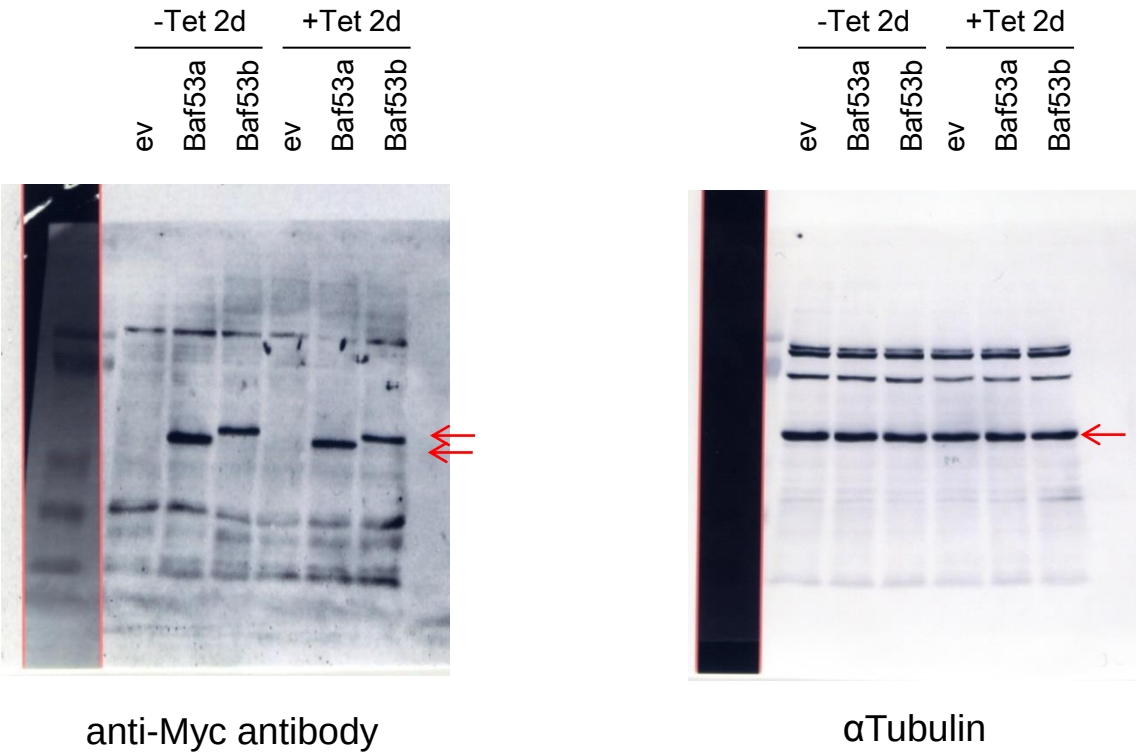


E

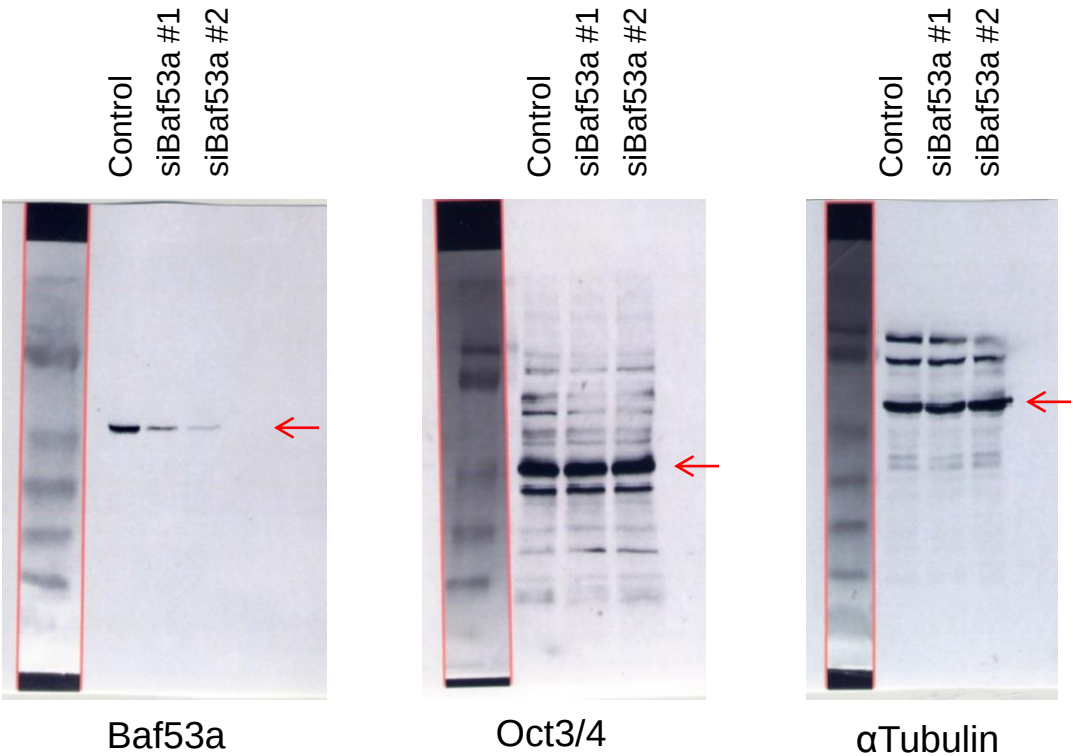


Supplemental Figure 3DE

F

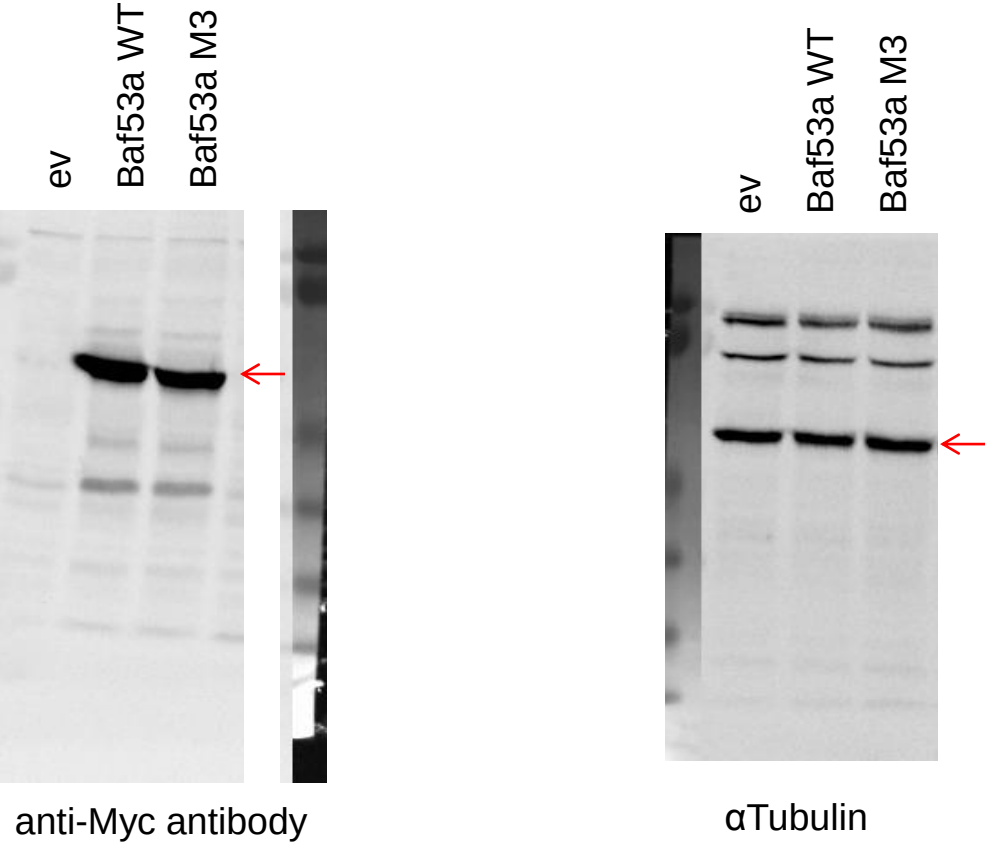


G



Supplemental Figure 3FG

H



Supplemental Figure 3H