

Wenderski et al. Supplementary File 3

This file contains:

- Raw Western blot scans
- Repeated experiments
- Genotyping

Western blot scans related to Figs. 2A and S4A, B

HEK293T cells transduced with wild type or ASD mutant ACTL6B (BAF53b) expressed from EF1a (constitutive) promoter. Cells were selected continuously with hygromycin over 10 days.

LI-COR

Image ID: 0004961_06
Acquire Time: Jul 3, 2018 2:27:24 PM

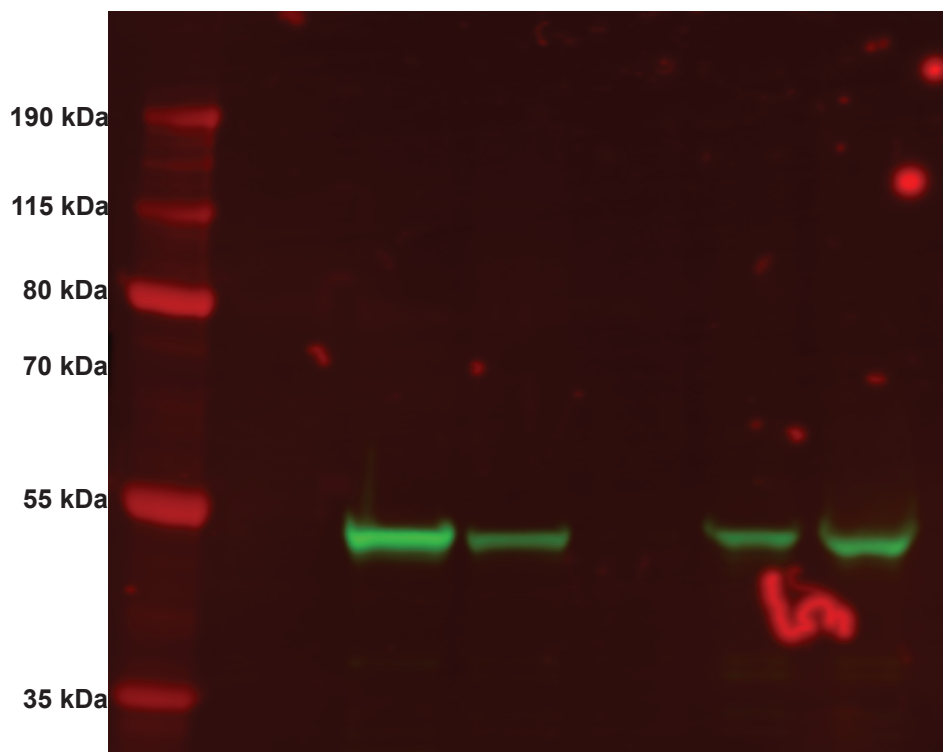
Page 1

Acquisition Information

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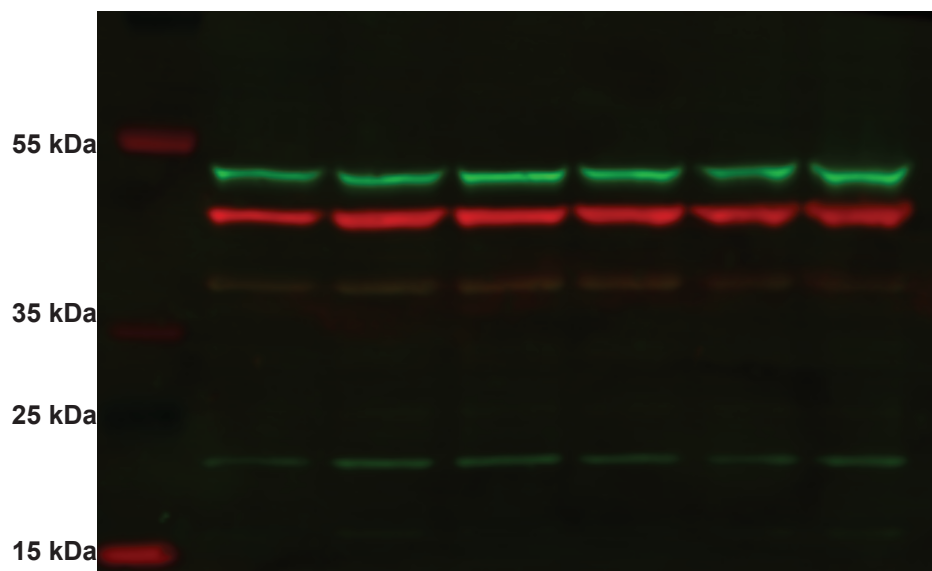
Image Display Values

Channel	Color	Minimum	Maximum	K
700	Red	0.191	294	1
800	Green	0.0561	68.5	0



Lanes:

1. Page Ruler Plus
2. N144 vector with RFP
3. N144 vector with BAF53b wild type
4. N144 vector with BAF53b L154F
5. N144 vector with BAF53b T175P
6. N144 vector with BAF53b G393R
7. N144 vector with BAF53b G417W



Western blot scans related to Figs. 2A and S4A, B (repeated experiment)

HEK293Ts transduced with pInducer20 containing wild type or ASD mutant *BAF53b*. Where indicated "+Dox", 1 ug/mL fresh doxycycline was applied daily for 7 days. Cytoplasmic fractions.



Image ID: 0003489_01
Acquire Time: Apr 14, 2017 1:17:58 PM

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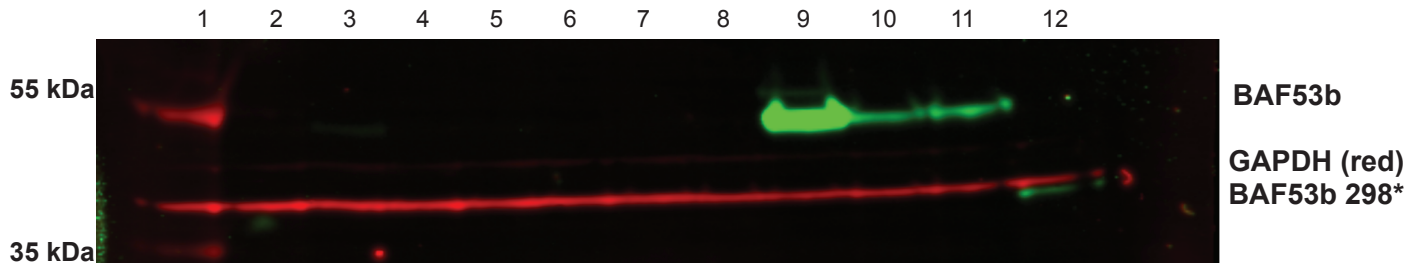
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Image Display Values

Channel	Color	Minimum	Maximum	K
700	Red	0.628	14.0	0.5
800	Green	0.400	2.95	0.37

1st scan:
immunoblot for Baf53b (rb) and Gapdh (ms)



Lanes:

- | | | |
|--------------------------|--------------------------|---|
| 1. Uninfected -dox* | 6. BAF53b R298* -dox | 11. BAF53b G393R +dox |
| 2. Empty vector- dox | 7. Uninfected +dox | 12. BAF53b R2988 +dox |
| 3. BAF53b wild type -dox | 8. Empty vector +dox | *NOTE: ladder run with Uninfected -dox sample |
| 4. BAF53b L154F -dox | 9. BAF53b wild type +dox | |
| 5. BAF53b G393R -dox | 10. BAF53b L154F +dox | |

Western blot scans related to Figs. 2C, D; S4E

Primary E18.5 cortical or striatal neurons infected with lentivirus containing plInducer20 with wild type or ASD mutant *BAF53b*.

Infected on DIV5, +dox on DIV6, collected DIV22.

Whole cell extract.

LI-COR®

Image ID: 0004995_01
Acquire Time: Jul 4, 2018 12:28:13 AM

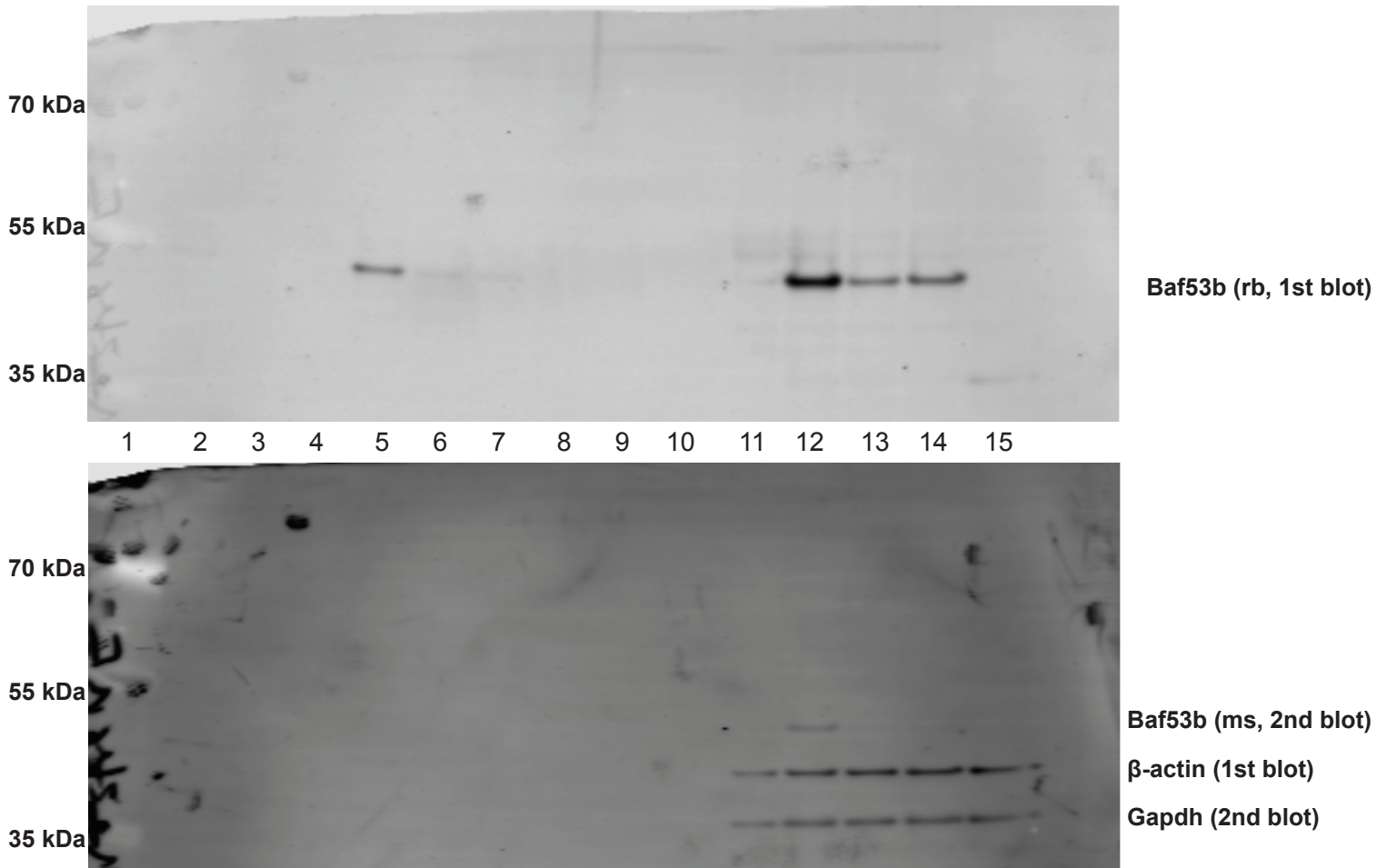
Page 1

Acquisition Information

#	Image ID	Acquire Time	Channels	Resolution	Intensities	Quality	Analysis	Image Name	Comment
1	0004995_01	Jul 4, 2018 12:28:13 AM	700 800	169um	Auto Auto	medium	Manual	0004995_01	

Image Display Values

Channel	Color	Minimum	Maximum	K
800	Gray Scale (Black on White)	0.482	15.0	1



Lanes:

1. Page Ruler Plus
2. Cortical neurons, *Baf53b*^{+/+} empty vector
3. Cortical neurons, *Baf53b*^{+/-} empty vector
4. Cortical neurons, *Baf53b*^{-/-} empty vector
5. Cortical neurons, *Baf53b*^{-/-} BAF53b wild type
6. Cortical neurons, *Baf53b*^{-/-} BAF53b L154F
7. Cortical neurons, *Baf53b*^{-/-} BAF53b G393R
8. Cortical neurons, *Baf53b*^{-/-} BAF53b R298*
9. empty
10. empty
11. Striatal neurons, *Baf53b*^{-/-} empty vector
12. Striatal neurons, *Baf53b*^{-/-} BAF53b wild type
13. Striatal neurons, *Baf53b*^{-/-} BAF53b L154F
14. Striatal neurons, *Baf53b*^{-/-} BAF53b G393R
15. Striatal neurons, *Baf53b*^{-/-} BAF53b R298*

Western blot scans related to Figs. 2C, D; S4E

Primary E18.5 cortical or striatal neurons infected with lentivirus containing plInducer20 with wild type or ASD mutant *BAF53b*.

Infected on DIV5, +dox on DIV6, collected DIV22.

Immunoprecipitation with anti-Brg1 (H-10) from nuclear extract (43 ug).

LI-COR

Image ID: 0003624_01
Acquire Time: May 5, 2017 5:49:30 PM

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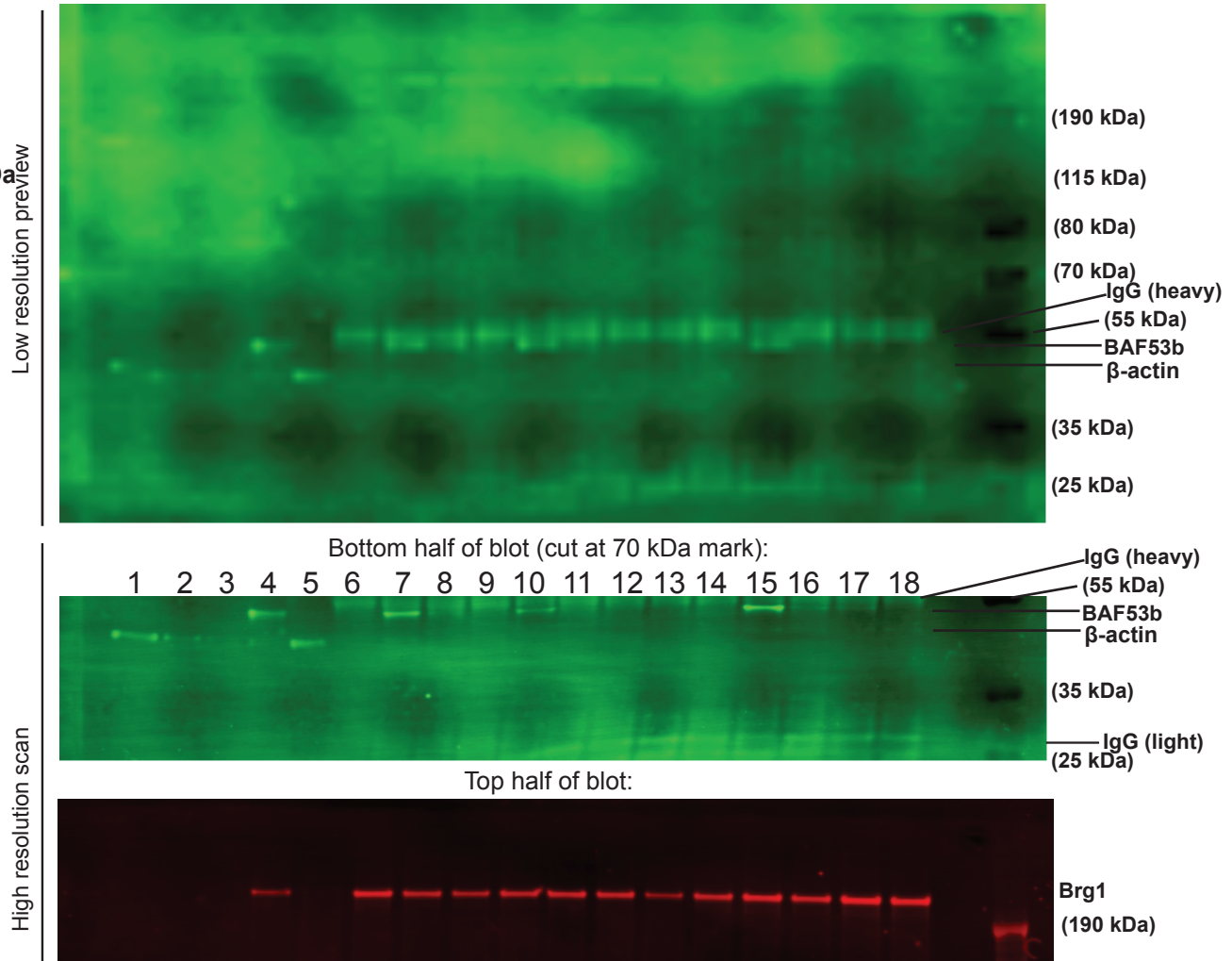
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1	0003624_01	May 5, 2017 5:49:30 PM	700 800	preview	Auto Auto	lowest	Manual	0003624_01	

Image Display Values

Channel	Color	Minimum	Maximum	K
800	Green	0.702	1.78	0

Immunoblot for Brg1 (G-7), Baf53b (rb), b-actin (rb)



Lanes:

- | | |
|--|--|
| 1. Cortical neurons, cytosol: <i>Baf53b</i> ^{+/+} empty vector | 11. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b L154F |
| 2. Cortical neurons, cytosol: <i>Baf53b</i> ^{+/-} empty vector | 12. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b G393R |
| 3. Cortical neurons, cytosol: <i>Baf53b</i> ^{-/-} empty vector | 13. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b R298* |
| 4. Cortical neurons, cytosol: <i>Baf53b</i> ^{-/-} BAF53b wild type | 14. Striatal neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} empty vector |
| 5. Cortical neurons, input: <i>Baf53b</i> ^{-/-} empty vector | 15. Striatal neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b wild type |
| 6. Cortical neurons, IgG IP: <i>Baf53b</i> ^{+/+} empty vector | 16. Striatal neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b L154F |
| 7. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{+/+} empty vector | 17. Striatal neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b G393R |
| 8. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{+/-} empty vector | 18. Striatal neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b R298* |
| 9. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} empty vector | 19. empty well |
| 10. Cortical neurons, Brg1 IP: <i>Baf53b</i> ^{-/-} BAF53b wild type | 20. Page Ruler Plus |

Western blot scans related to Figs. 2B and S4C

iPSC-derived neurons from 2 affected siblings ($BAF53b^{L154F/L154F}$) and unaffected father ($BAF53b^{+/L154F}$) from Family 2703, and unaffected control ($BAF53b^{+/+}$).

Whole cell protein extracts. 3 sets of samples collected at separate times after >30 days neural induction.



Image ID: 0005001_04
Acquire Time: Jul 4, 2018 12:52:10 AM

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Acquisition Information

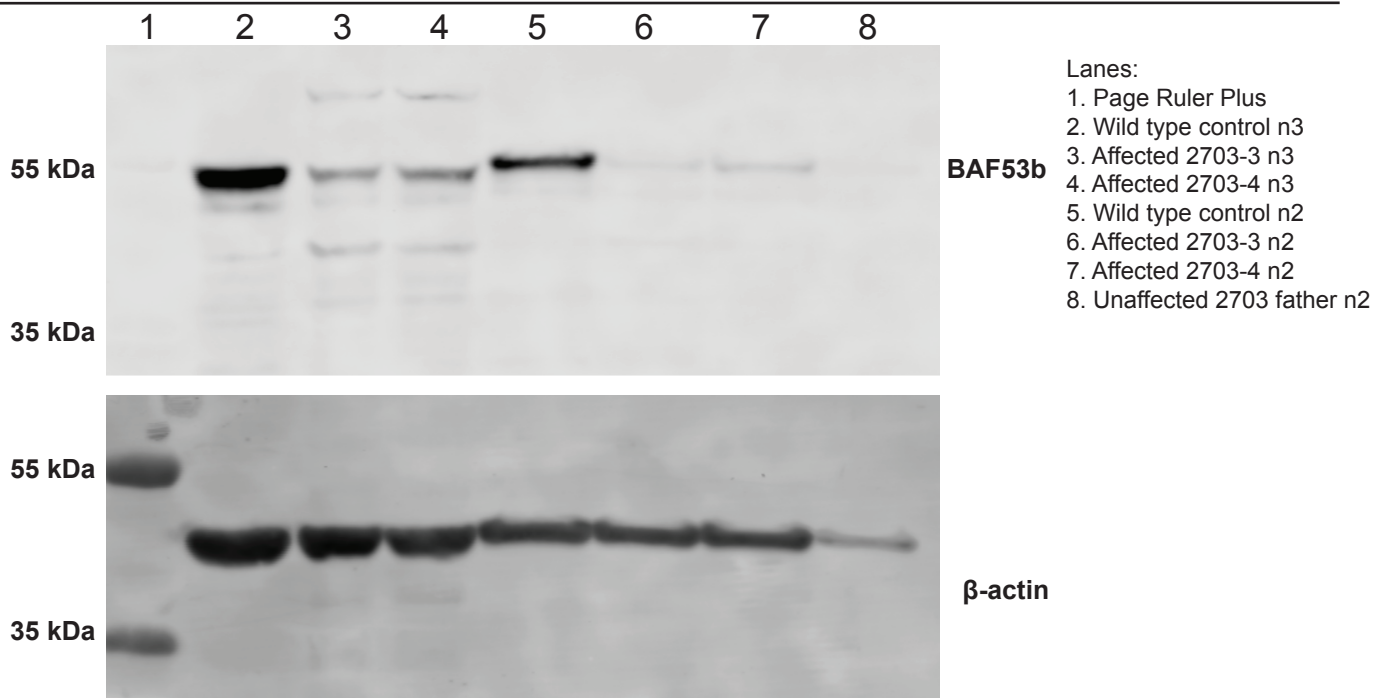
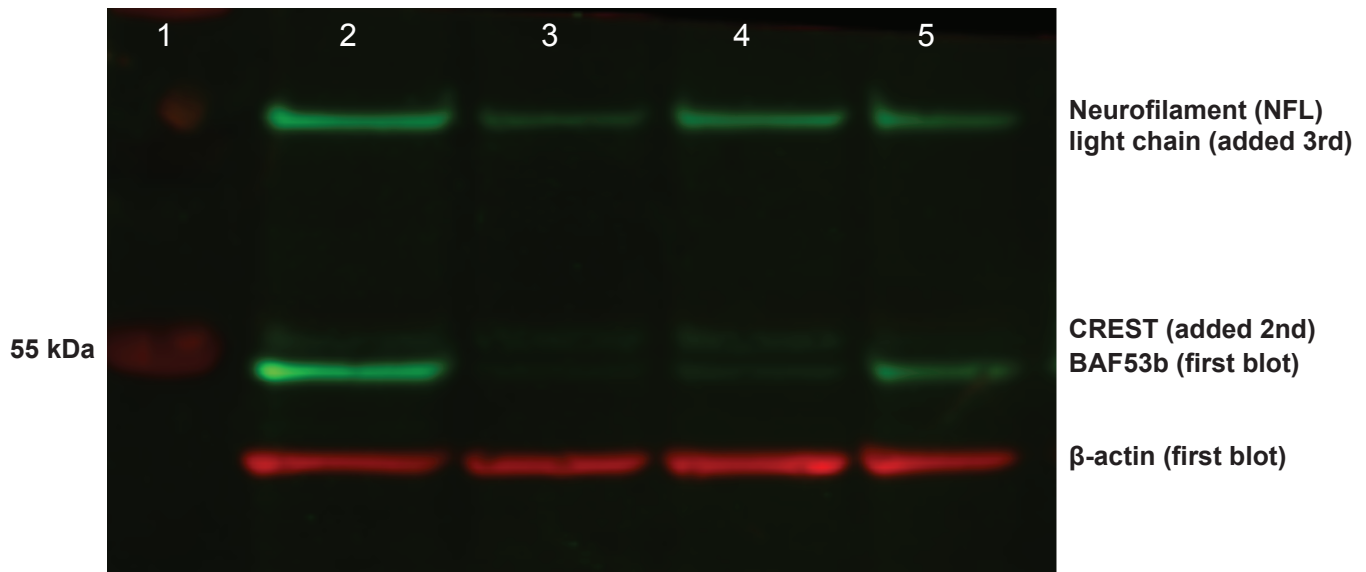
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Image Display Values

Channel	Color	Minimum	Maximum	K
700	Red	0.000119	223	0
800	Green	0.000119	30.7	0

Lanes:

1. Page Ruler Plus
2. Wild type control n1
3. Affected 2703-3 n1
4. Affected 2703-4 n1
5. Unaffected 2703 father n1



Western blot scans related to Figs. 2B and S4C

iPSC-derived neurons from 2 affected siblings (*BAF53b*^{L154F/L154F}) from Family 2703 and unaffected control (*BAF53b*^{+/+}). Immunoprecipitation with anti-Brg1 (H-10) from nuclear extract (25 ug).

LI-COR

Image ID: 0004346_01
Acquire Time: Oct 4, 2017 5:25:32 PM

Page 1

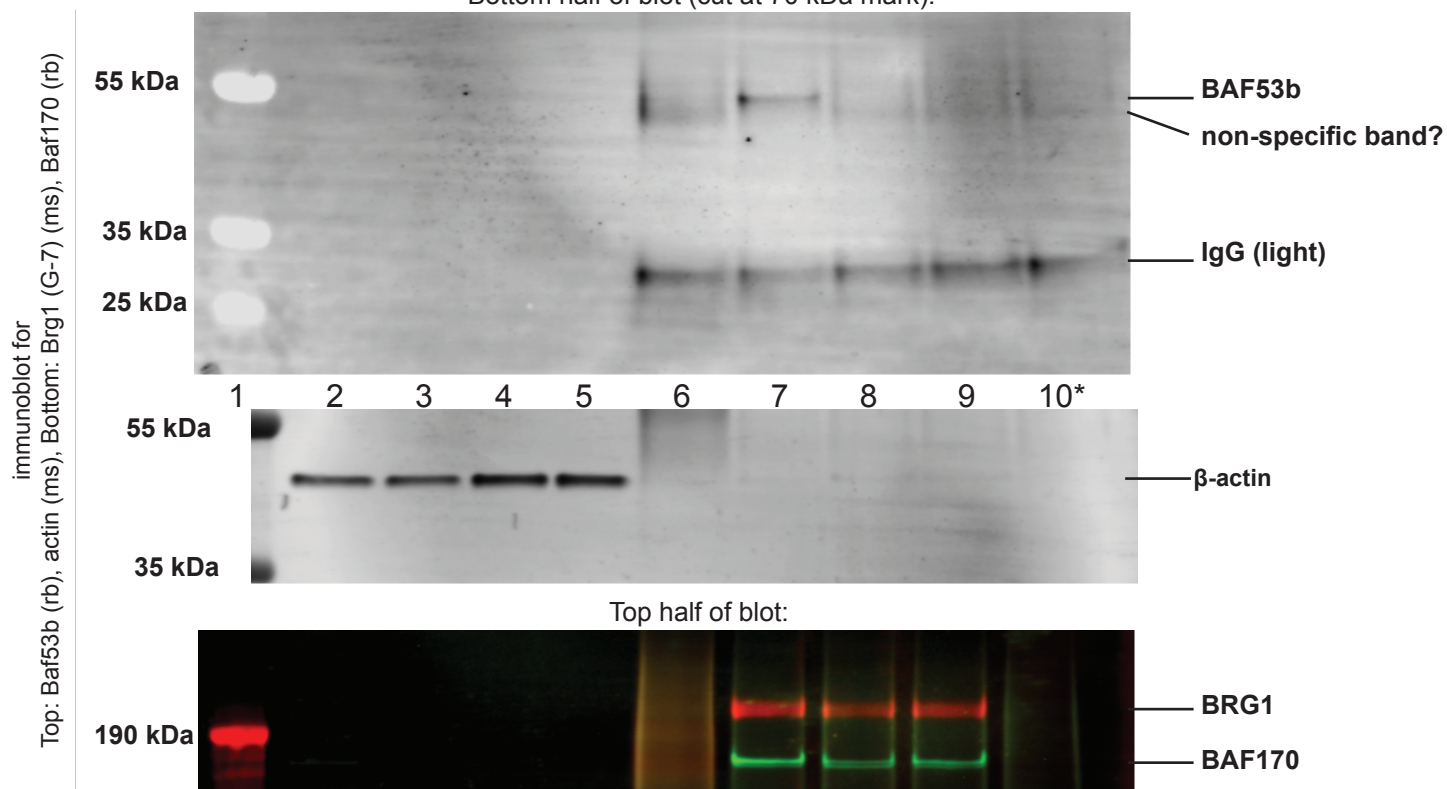
Acquisition Information

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1	0004346_01	Oct 4, 2017 5:25:32 PM	700 800	169um	Auto Auto	high	Manual	0004346_01	

Image Display Values

Channel	Color	Minimum	Maximum	K
800	Gray Scale (Black on White)	3.43	8.39	0

Bottom half of blot (cut at 70 kDa mark):



Lanes:

1. Page Ruler Plus
2. Flow-through: Unaffected
3. Input: Unaffected
4. Input: Affected 1
5. Input: Affected 2
6. IgG IP: Unaffected
7. Brg1 IP: Unaffected
8. Brg1 IP: Affected 1
9. Brg1 IP: Affected 2
- 10*: Brg1 IP: Unaffected relative with *BAF53b*^{+/L154F} did not have enough starting sample for IP but tried it anyway

Western blot scans related to Fig. S4A

HEK293Ts transduced with pInducer20 containing wild type or ASD mutant *BAF53b*. Where indicated "+Dox", 1 ug/mL fresh doxycycline was applied daily for 7 days. Immunoprecipitation with anti-Brg1 (H-10) from nuclear extract (250 ug).



Image ID: 0003424_01
Acquire Time: Mar 31, 2017 8:33:55 PM

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1st scan:
immunoblot for Baf53b (rb)

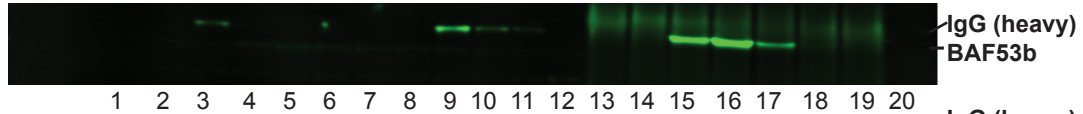
Acquisition Information

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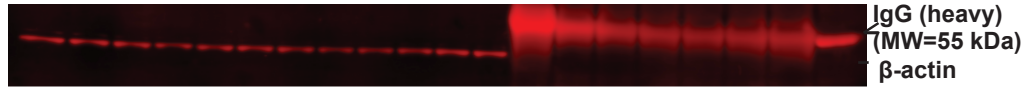
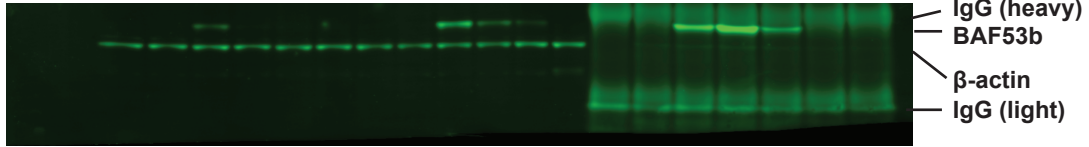
Image Display Values

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800	Green	0.456	4.46	0

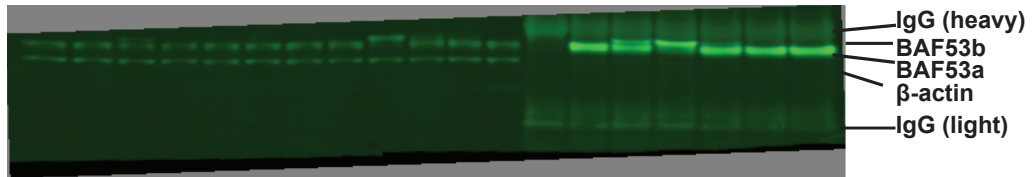
Bottom half of blot (cut at 70 kDa mark):



2nd scan:
reprobe with
b-actin (rb), b-actin (ms)

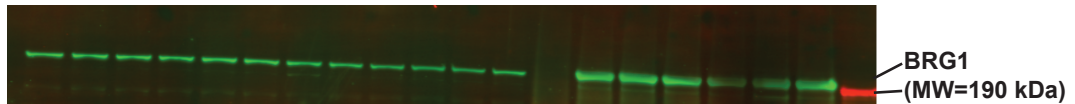


3rd scan:
reprobe with
Baf53a (rb)

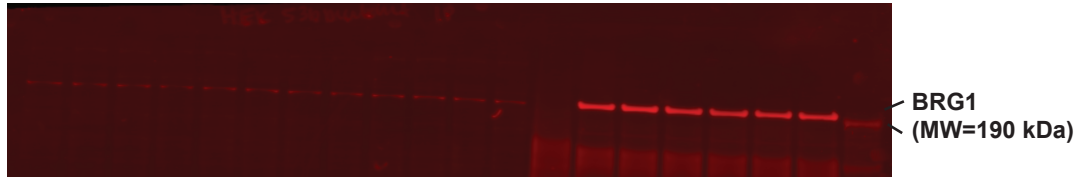
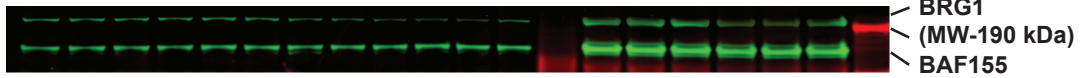


Top half of blot:

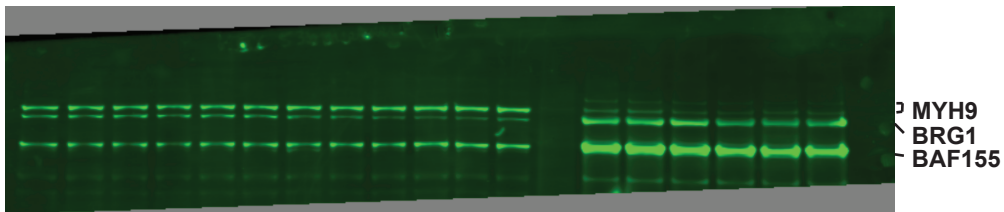
1st scan:
Brg1 H-88 (rb), Kat6a (ms)



2nd scan:
reprobe with
Baf155 (rb), Brg1 G-7 (ms)



3rd scan:
reprobe with
MYH9 (rb)



- Lanes:
- | | | | |
|---------------------------------|---------------------------------|------------------------------------|------------------------------------|
| 1. Input: uninfected -dox | 6. Input: BAF53b R298* -dox | 11. Input: BAF53b G393R +dox | 16. Brg1 IP: BAF53b wild type +dox |
| 2. Input: empty vector- dox | 7. Input: uninfected +dox | 12. Input: BAF53b R2988 +dox | 17. Brg1 IP: BAF53b L154F +dox |
| 3. Input: BAF53b wild type -dox | 8. Input: empty vector +dox | 13. IgG IP: uninfected +dox | 18. Brg1 IP: BAF53b G393R+dox |
| 4. Input: BAF53b L154F -dox | 9. Input: BAF53b wild type +dox | 14. Brg1 IP: empty vector +dox | 19. Brg1 IP: BAF53b R298* +dox |
| 5. Input: BAF53b G393R -dox | 10. Input: BAF53b L154F +dox | 15. Brg1 IP: BAF53b wild type -dox | 20. Page Ruler Plus Ladder |

Western blot scans related to Fig. S4A (repeated experiment)

HEK293Ts transduced with pInducer20 containing wild type or ASD mutant *BAF53b*. Where indicated "+Dox", 1 ug/mL fresh doxycycline was applied daily for 7 days. Immunoprecipitation with anti-Brg1 (H-10) from nuclear extract (250 ug).



Image ID: 0004700_07
Acquire Time: May 3, 2018 3:49:01 PM

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Acquisition Information

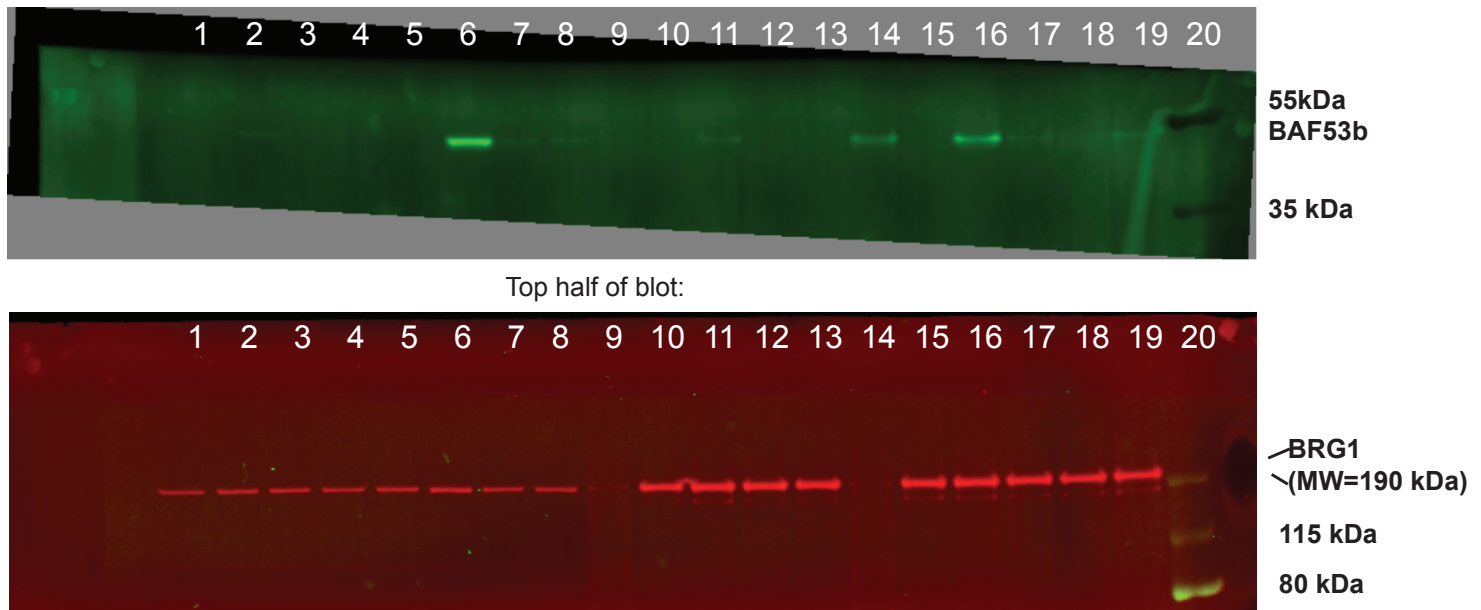
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Image Display Values

Channel	Color	Minimum	Maximum	K
800	Green	0.0869	11.6	0

Bottom half of blot (cut at 70 kDa mark):

1st scan:
Immunoblot for Baf53b (rb), Brg1 (G-7) (ms)

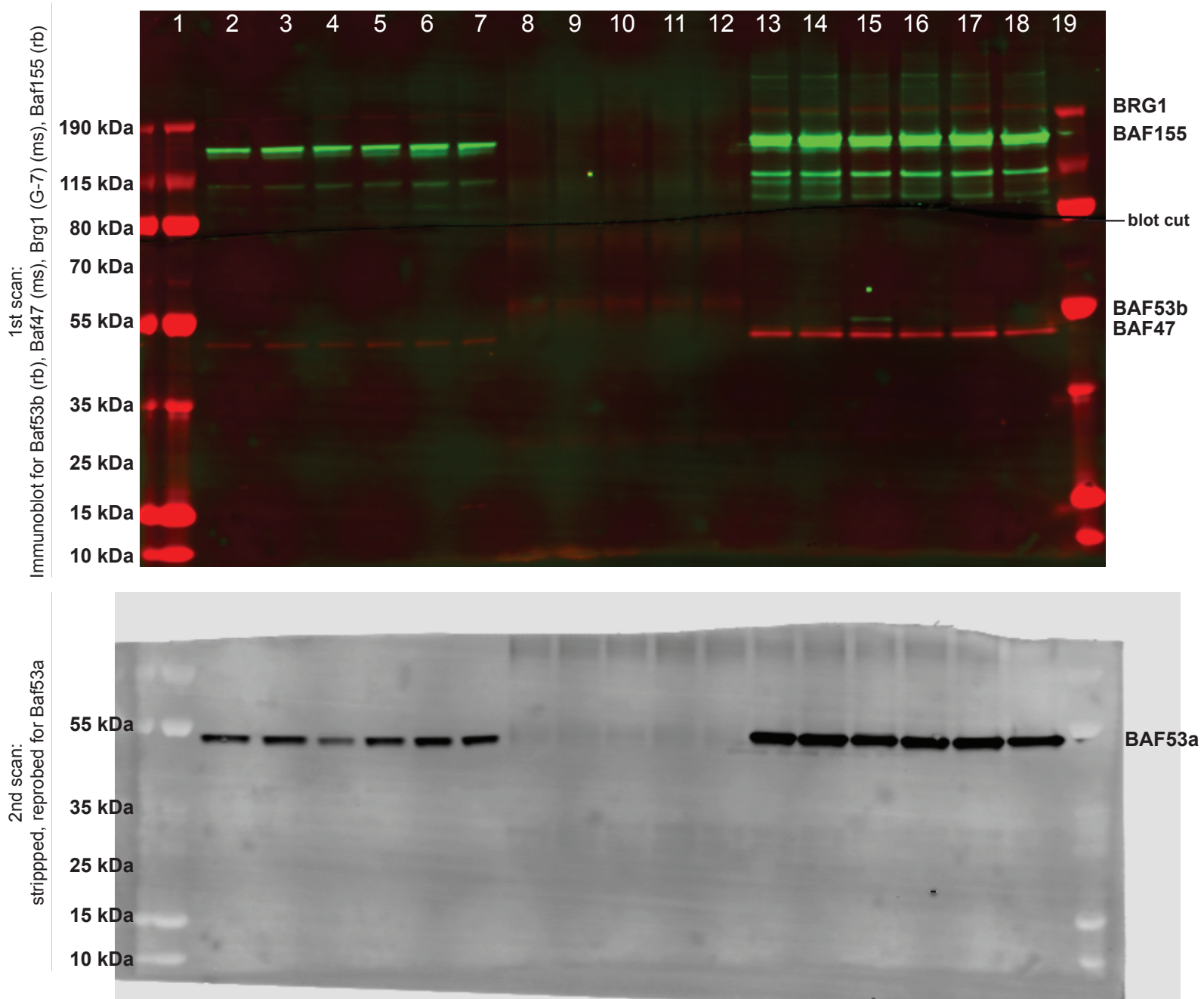


Lanes:

- | | | | |
|---------------------------------|----------------------------------|------------------------------------|--|
| 1. Input: empty vector -dox | 6. Input: BAF53b wild type +dox | 11. Brg1 IP: BAF53b wild type -dox | 16. Brg1 IP: BAF53b wild type +dox |
| 2. Input: BAF53b wild type -dox | 7. Input: BAF53b L154F +dox | 12. Brg1 IP: BAF53b L154F -dox | 17. Brg1 IP: BAF53b L154F +dox |
| 3. Input: BAF53b L154F -dox | 8. Input: BAF53b G393R +dox | 13. Brg1 IP: BAF53b G393R -dox | 18. Brg1 IP: BAF53b G393R+dox |
| 4. Input: BAF53b G393R -dox | 9. IgG IP: BAF53b wild type -dox | 14. IgG IP: BAF53b wild type +dox | 19. Brg1 IP: BAF53b wild type -dox n=2 |
| 5. Input: empty vector +dox | 10. Brg1 IP: empty vector -dox | 15. Brg1 IP: empty vector +dox | 20. Page Ruler Plus Ladder |

Western blot scans related to Fig. S4D

H9 hESCs infected with lentivirus containing plInducer20 with wild type or ASD mutant *BAF53b*. Immunoprecipitation with anti-Brg1 (G-7) from nuclear extract (90 ug).



Lanes:

- | | | | |
|---------------------------------|----------------------------------|------------------------------------|--------------------------------|
| 1. Page Ruler Plus Ladder | 6. Input: BAF53b G393R +dox | 11. IgG IP: BAF53b G393R +dox | 16. Brg1 IP: BAF53b L154F +dox |
| 2. Input: empty vector -dox | 7. Input: BAF53b R298* +dox | 12. IgG IP: BAF53b R298* +dox | 17. Brg1 IP: BAF53b G393R +dox |
| 3. Input: BAF53b wild type -dox | 8. Input: BAF53b G393R +dox | 13. Brg1 IP: empty vector -dox | 18. Brg1 IP: BAF53b R298*+dox |
| 4. Input: BAF53b wild type +dox | 9. IgG IP: BAF53b wild type +dox | 14. Brg1 IP: BAF53b wild type -dox | 19. Page Ruler Plus Ladder |
| 5. Input: BAF53b L154F +dox | 10. IgG IP: BAF53b L154F +dox | 15. Brg1 IP: BAF53b wild type +dox | |

Western blot scans related to Fig. S6E
P0 cerebellar tissue, whole cell lysate.



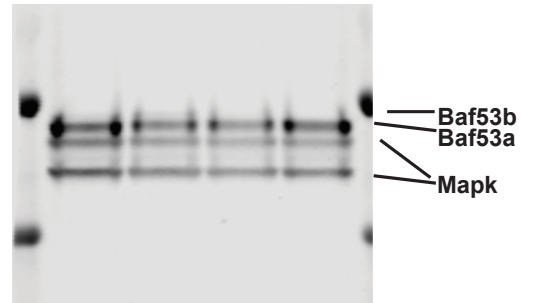
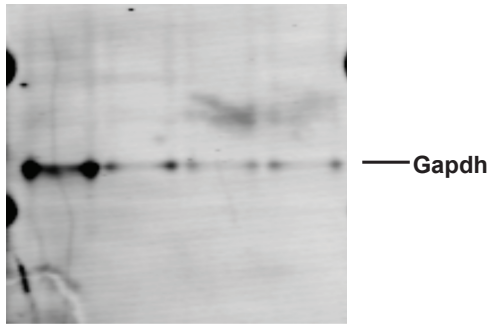
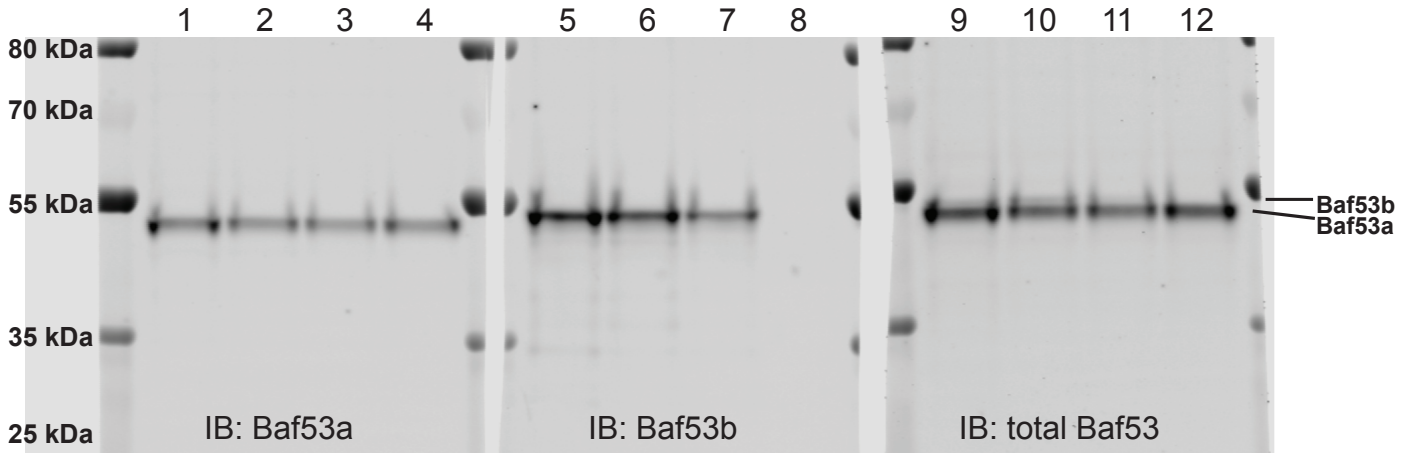
Image ID: 0005631_01
Acquire Time: Jan 11, 2019 11:06:47 PM

Acquisition Information

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Image Display Values

Channel	Color	Minimum	Maximum	K
700	Gray Scale (Black on White)	2.96	79.4	0
800	Gray Scale (Black on White)	0.239	12.8	0.41



Lanes:
1,5,9: Baf53b+/- #1 (2x concentrated)
2,6,10: Wild type (1x conc)
3,7,11: Baf53b+/- #2 (1x conc)
4,8,12: Baf53b-/- (1x conc)

Data related to Fig. S6F and G

Baf53b heterozygous cross P0 cortex (nBAF), hippocampus (nBAF) or cerebellum (npBAF + nBAF)

-Mouse littermate genotyping

-Input nuclear extracts

-Western blot scans for nBAF immunoprecipitation with anti-Crest from brain tissue nuclear extract (45 ug/cerebellar IP, 65 ug/cortical IP)

Genotypes:

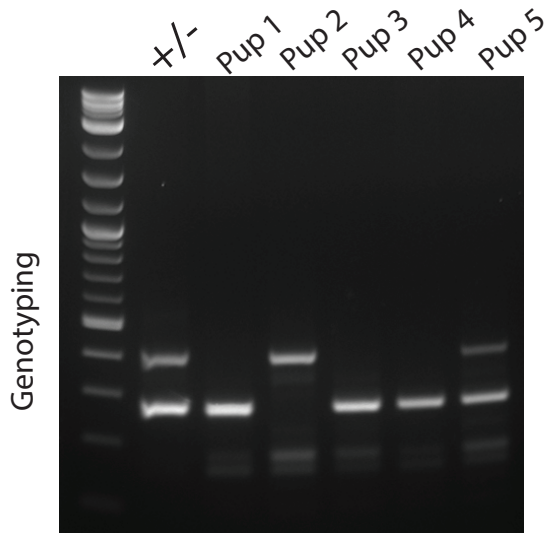
Pup 1: dead, blood on brain, -/-

Pup 2: +/+

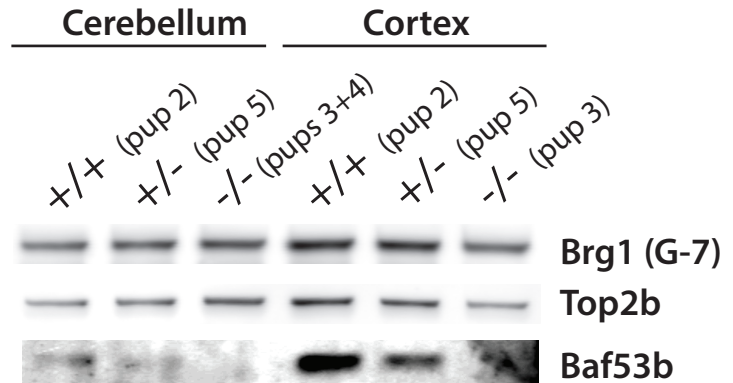
Pup 3: -/-

Pup 4: -/-

Pup 5: +/-



Input nuclear extracts:



LI-COR

Image ID: 0002805_01
Acquire Time: Oct 28, 2016 9:56:28 AM

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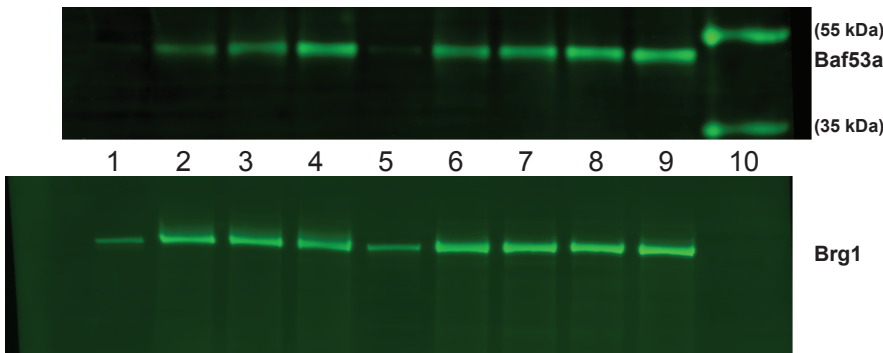
Acquisition Information

#	Image ID	Acquire Time	Channels	Resolution	Intensities	Quality	Analysis	Image Name	Comment
1	0002805_01	Oct 28, 2016 9:56:28 AM	700	84um	Auto	high	Manual	0002805_01	

Image Display Values

Channel	Color	Minimum	Maximum	K
700	Green	1.46	37.6	1

immunoblot for
Top: Baf53b (rb), Brg1 (H-88) (rb)



Lanes:

1. P0 cerebellum, input: *Baf53b*^{+/+}
2. P0 cerebellum, CREST IP: *Baf53b*^{+/+}
3. P0 cerebellum, CREST IP: *Baf53b*^{+/-}
4. P0 cerebellum, CREST IP: *Baf53b*^{-/-}
5. P0 cortex, input: *Baf53b*^{+/+}
6. P0 cortex, CREST IP: *Baf53b*^{+/+}
7. P0 cortex, CREST IP: *Baf53b*^{+/-}
8. P0 cortex, CREST IP: *Baf53b*^{-/-} n=1
9. P0 cortex, CREST IP: *Baf53b*^{-/-} n=2
10. Page Ruler Plus

Replicate experiment shown in Fig. S6J

E18/DIV7 cortical neuronal nuclei were lysed and exposed to increasing concentrations of salt. The chromatin and soluble fractions were separated by differential centrifugation. Full Western blot images were no longer available.

