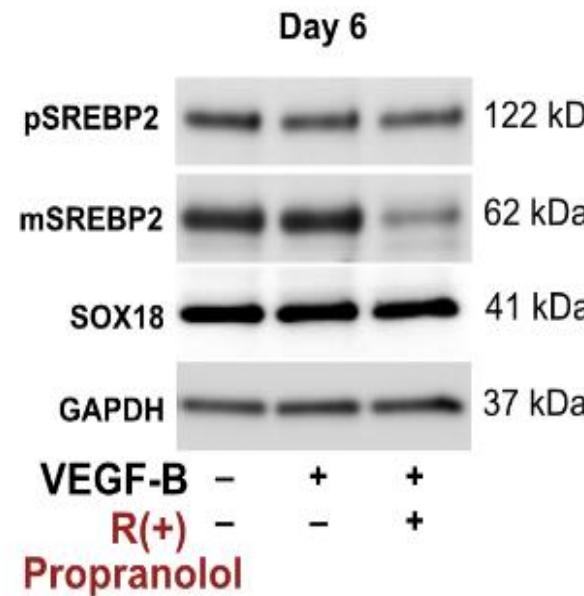
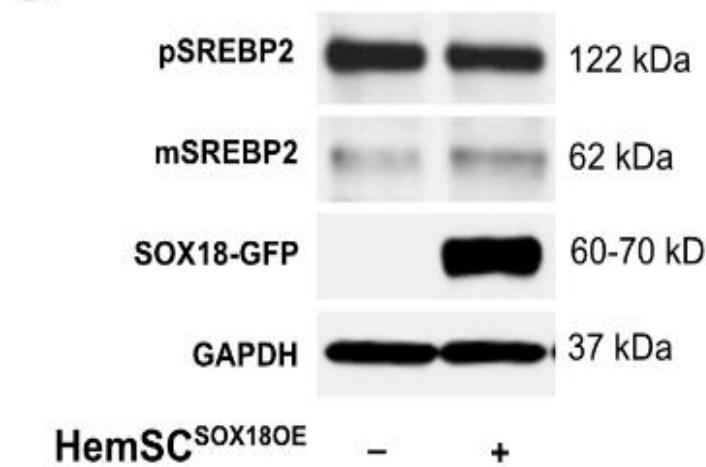


Figure 3 B, D, F Western Blots for full tiffs rev 11/26/24

B



D



F

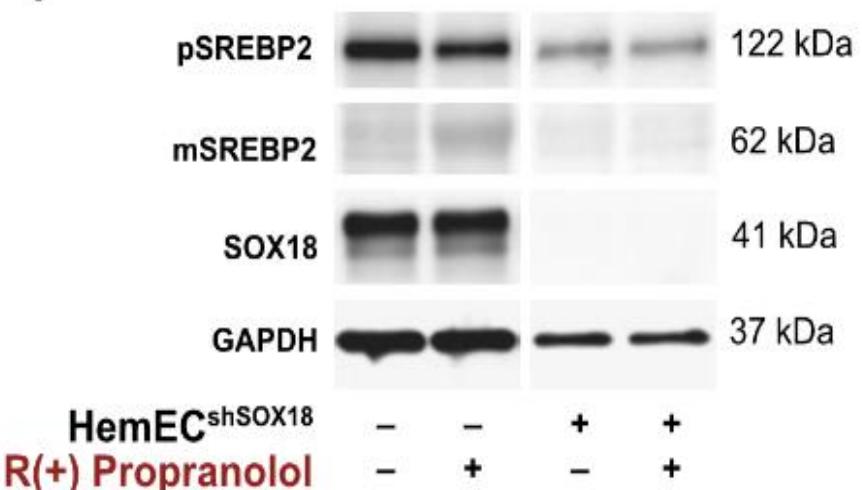


Figure 3 B Western Blots for full tiffs rev 11/26/24

B

Day 6

pSREBP2				122 kDa
mSREBP2				62 kDa
SOX18				41 kDa
GAPDH				37 kDa
VEGF-B	-	+	+	
R(+)	-	-	+	
Propranolol				



SREBP2

Tiff name: 042123 HemSC149 Differentiation 2hr R+ Treatments SREBP2 7m_PUB_600

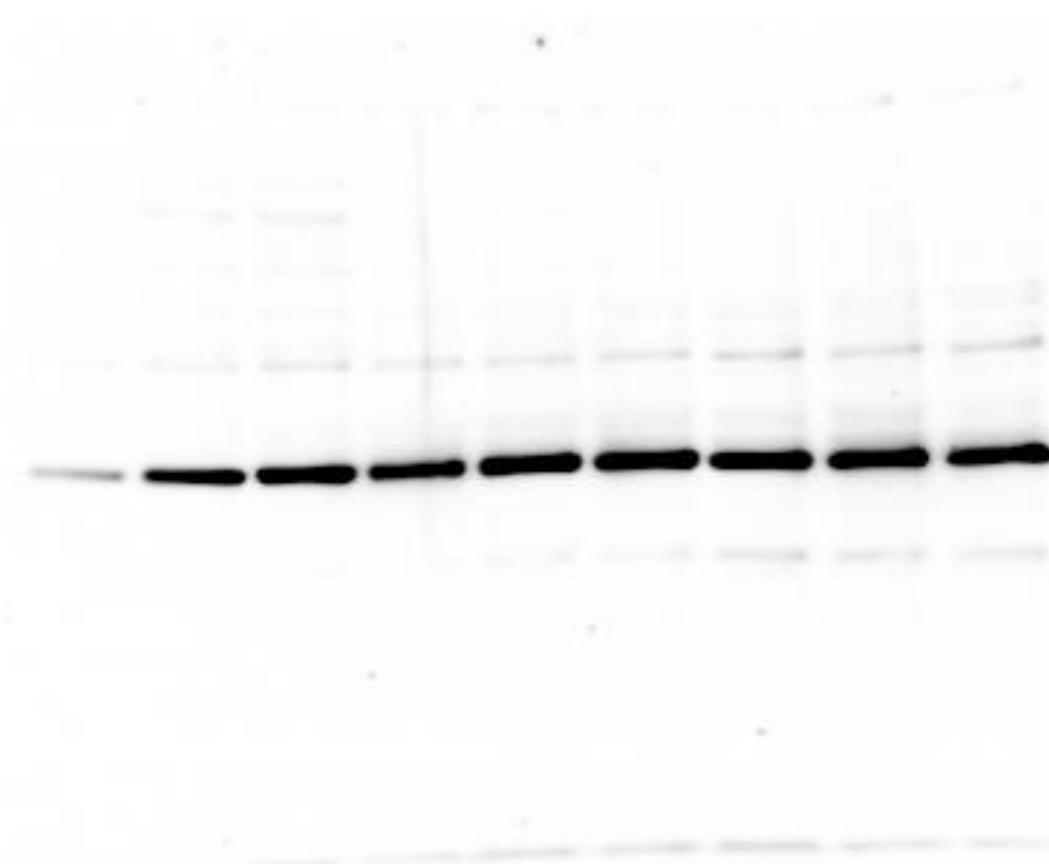
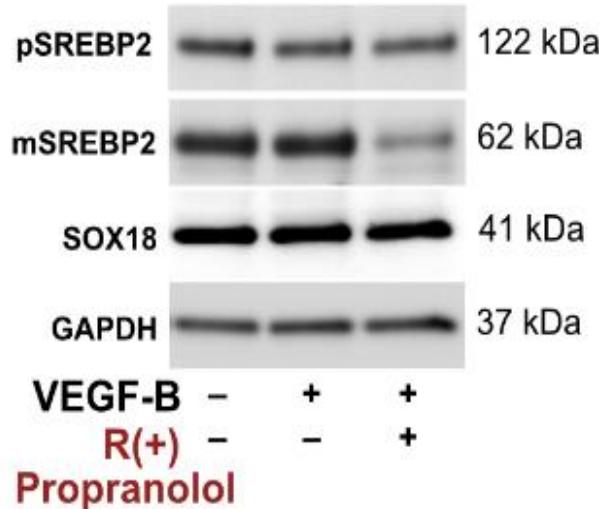
Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 B Luke's HemSC149 SREBP2 2 hrs R+ and original location:

G:\Shared drives\SR-Bischoff-Common\Luke Borgelt\Western Blot\042123 HemSC149 Differentiation 2hr R+ treated samples SREBP2 WB\042123 HemSC149 Differentiation 2hr R+ Treatments SREBP2 WB

Figure 3 B Western Blots for full tiffs rev 11/26/24

B

Day 6



Sox18

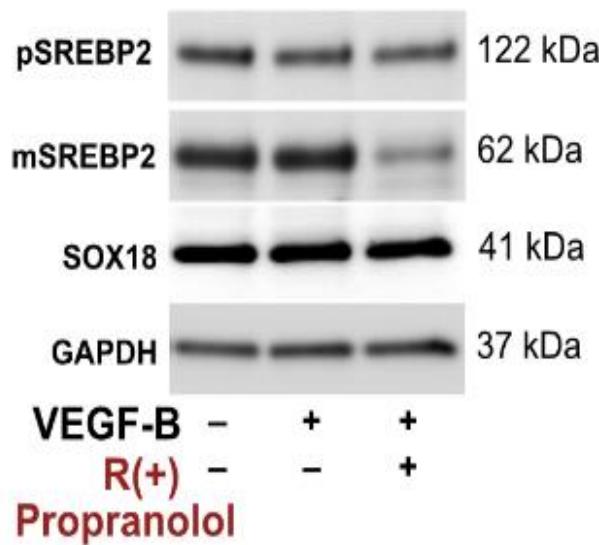
Tiff name: Sox18 cropped R+ 2 hrs 24.03.21_14.27.15_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 B Luke's HemSC149 SREBP2 2 hrs R+

Figure 3 B Western Blots for full tiffs rev 11/26/24

B

Day 6



GAPDH



Tiff name: 042123 HemSC149 Differentiation 2hr R+ Treatments SREBP2 GAPDH 10s_PUB_600

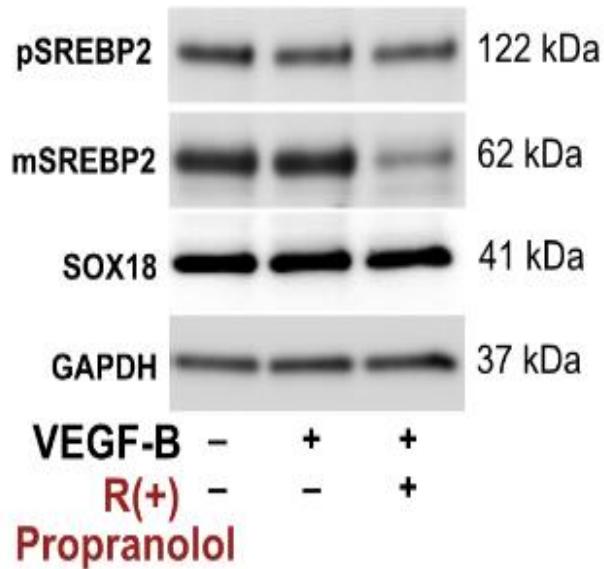
Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 B Luke's HemSC149 SREBP2 2 hrs R+ and original location:

G:\Shared drives\SR-Bischoff-Common\Luke Borgelt\Western Blot\042123 HemSC149 Differentiation 2hr R+ treated samples SREBP2 WB\042123 HemSC149 Differentiation 2hr R+ Treatments SREBP2 WB

Figure 3 B Western Blots for full tiffs rev 11/26/24

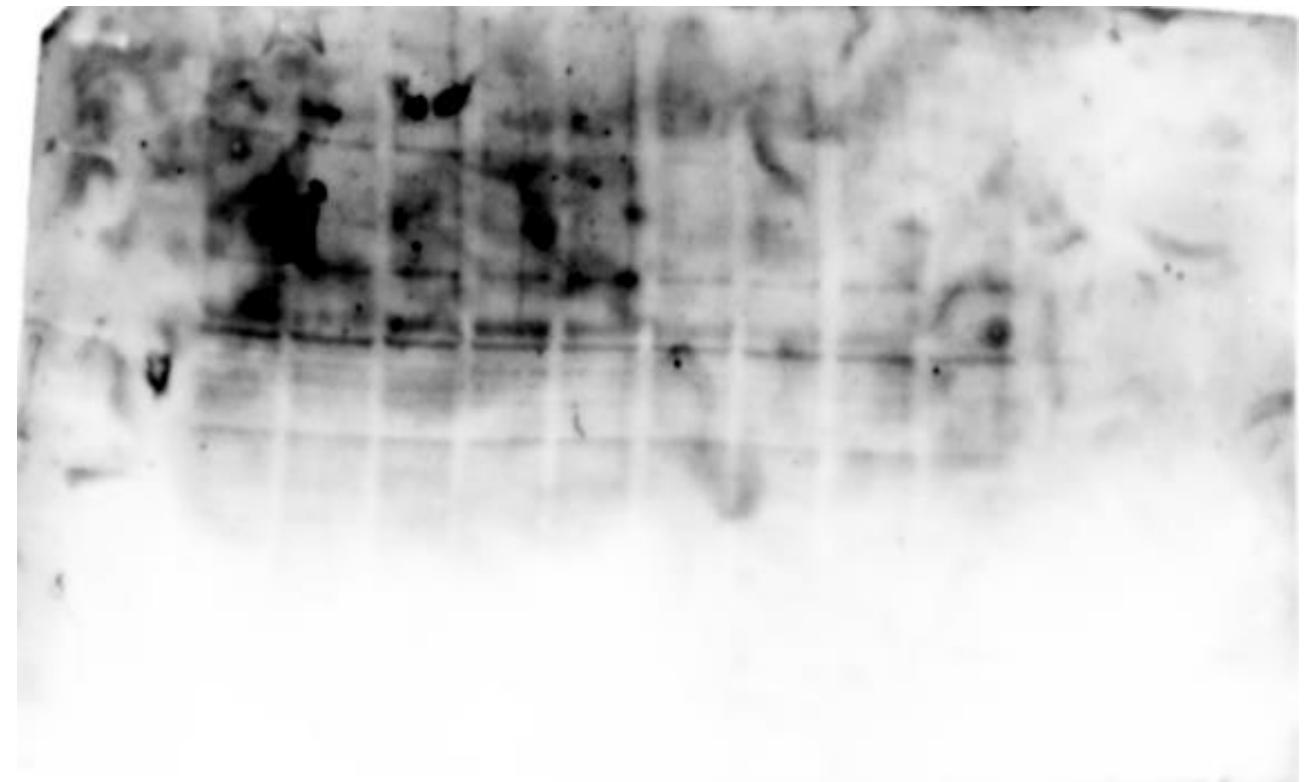
B

Day 6



**Tubulin
50 kDa**

Tubulin



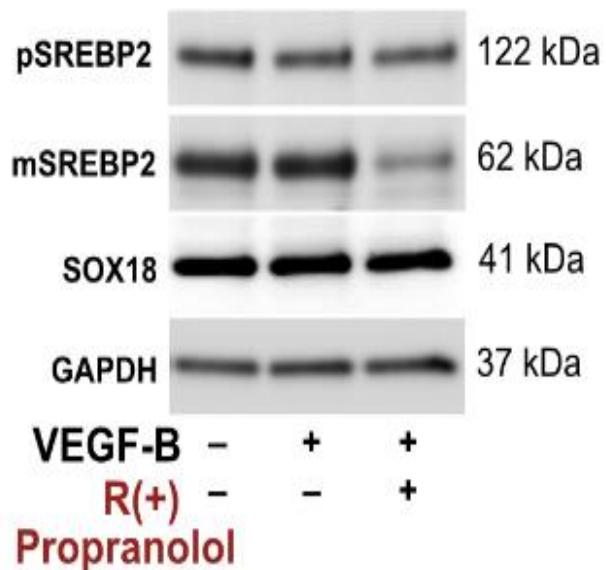
Tiff name: For Slide Tubulin B1 lighter crop 4m49s574ms 24.04.27_16.53.25_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 B Luke's HemSC149 SREBP2 2 hrs R+

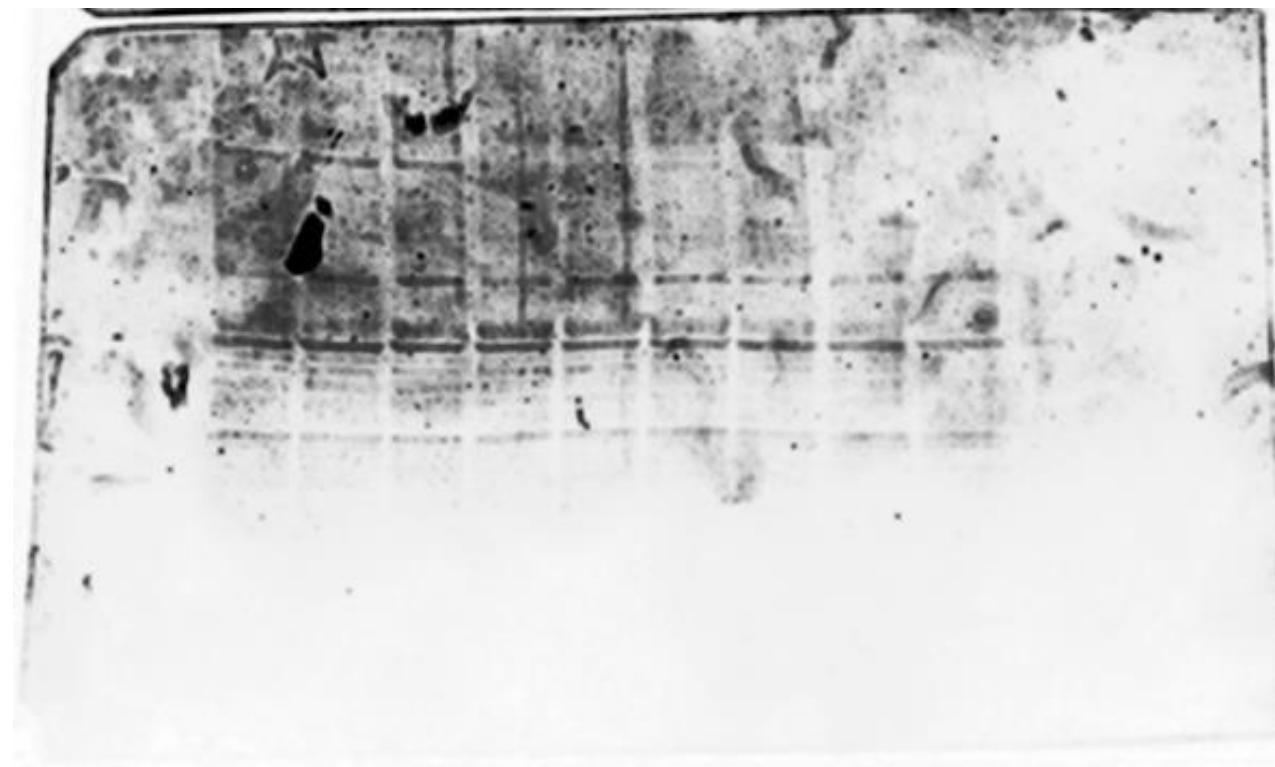
Figure 3 B Western Blots for full tiffs rev 11/26/24

B

Day 6



**Tubulin
50 kDa**



Tubulin

Tiff name: For Slide 2hrs R+ Tubulin 4m wide dynamic crop 24.04.27_15

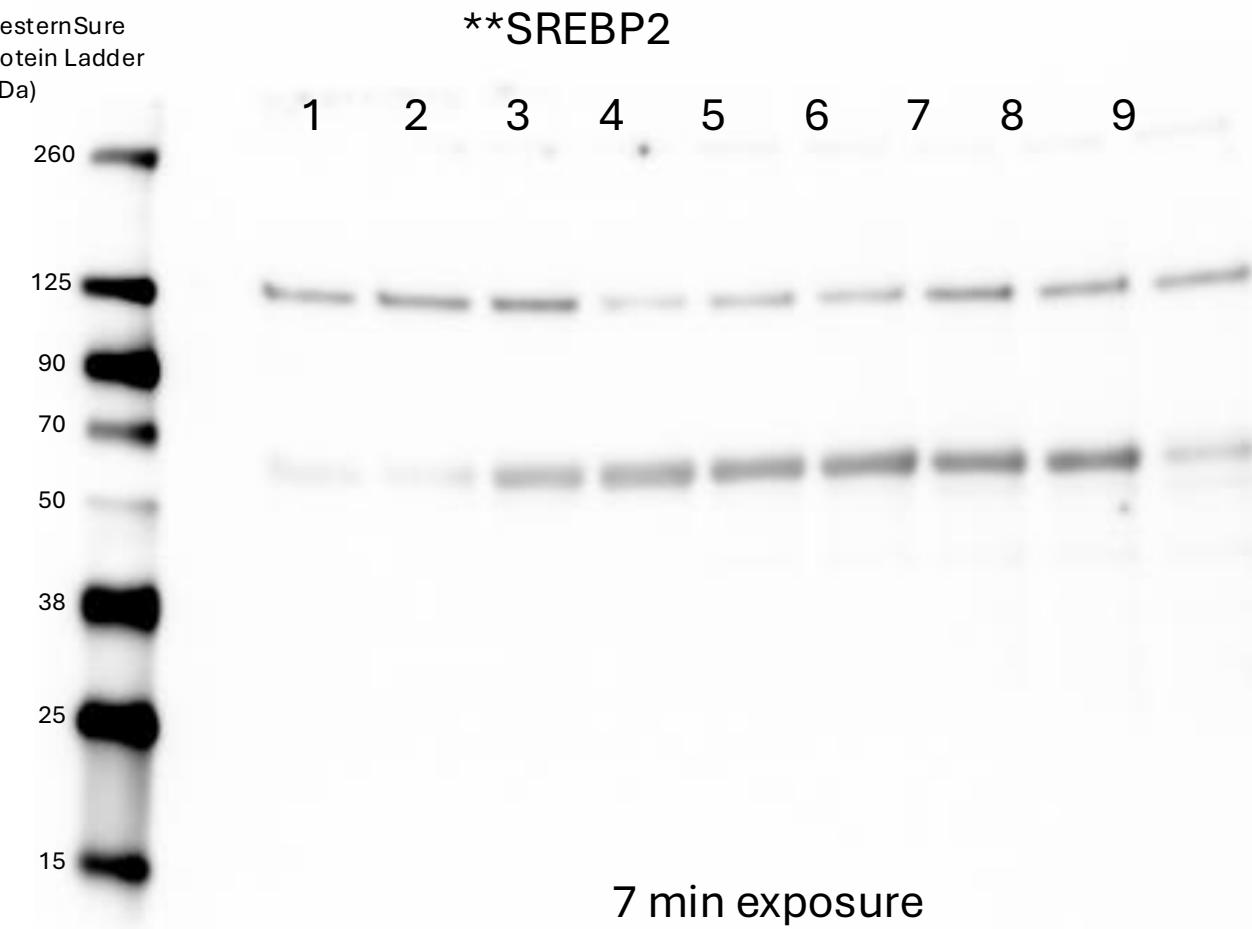
Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 B Luke's HemSC149 SREBP2 2 hrs R+

HemSC149 p8 Differentiation R+ 2hr treatments

Luke Borgelt

04/21/23

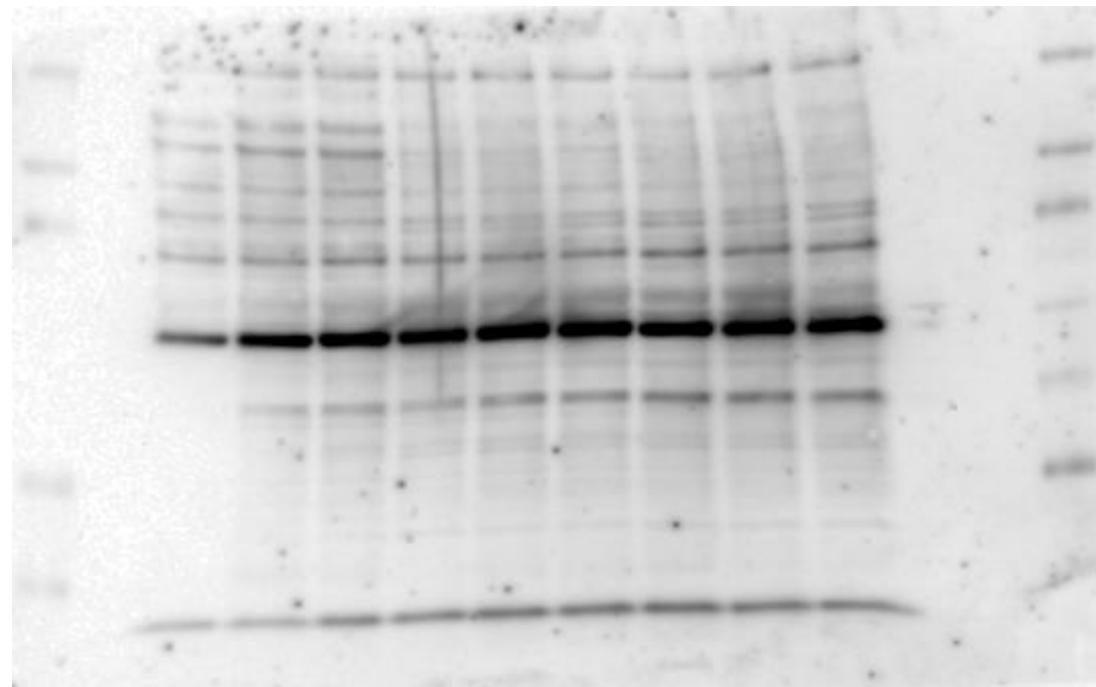
WesternSure
Protein Ladder
(kDa)



Sox18 Western Blot - HemSC149 p8 Differentiation R+ 2hr treatments (LB 4/21/23) reprobed 3/21/24 JWS

WesternSure
Protein Ladder
(kDa)

1. 2. 3. 4. 5. 6. 7. 8. 9.



Sox18
41-45 kDa

1 min Azure Imager

1. D2 Control
2. D2 VEGFB
3. D2 R+ (2hrs)
4. D4 Control
5. D4 VEGB
6. D4 R+ 2hrs
7. D6 Control
8. D6 VEGFB
9. D6 R+ 2hrs

30 ug protein lysate/well

Block 1 hour RT in 5% Milk/TBS-T

Mouse anti-human Sox18 (1:500)
Incubation overnight at 4C

2nd mouse HRP (1:2000)
Incubation 1 hour at room temperature (RT)

All antibodies in 5% Milk/TBS-T

WesternSure
Protein Ladder
(kDa)

260

125

90

70

50

38

25

15

**GAPDH

1 2 3 4 5 6 7 8 9

10s exposure

Lanes:

- 1- D2 -VEGFB, + 0.01% HCl (VEGFB vehicle)
- 2- D2 +VEGFB, 0.01% ddH₂O (R+ vehicle)
- 3- D2 +VEGFB, 20uM R+ (2hrs)
- 4- D4 -VEGFB, + 0.01% HCl (VEGFB vehicle)
- 5- D4 +VEGFB, 0.01% ddH₂O (R+ vehicle)
- 6- D4 +VEGFB, 20uM R+ (2hrs)
- 7- D6 -VEGFB, + 0.01% HCl (VEGFB vehicle)
- 8- D6 +VEGFB, 0.01% ddH₂O (R+ vehicle)
- 9- D6 +VEGFB, 20uM R+ (2hrs)

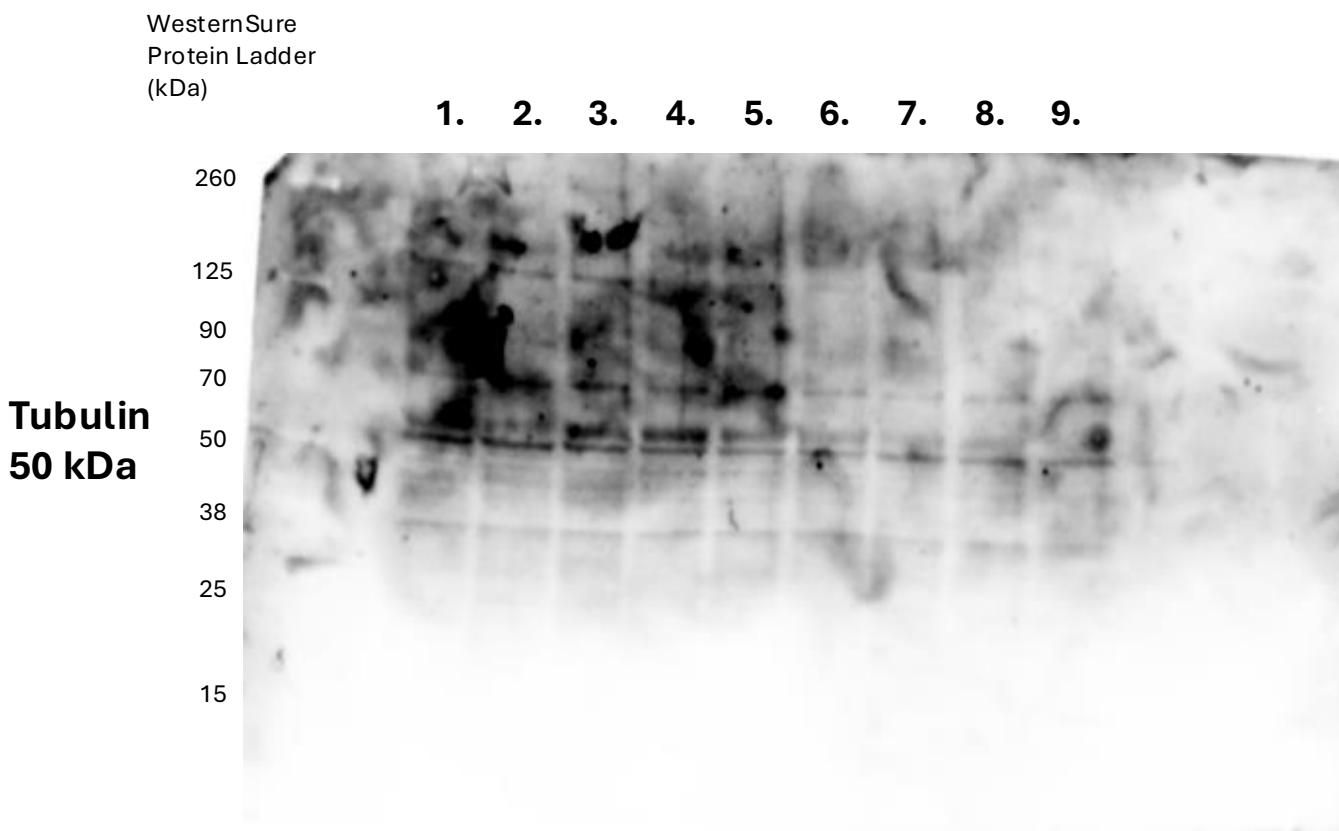
GAPDH
(37 kDa)

30 ug protein lysate/well

GAPDH

37 kDa

Tubulin* Western Blot - HemSC149 p8 Differentiation R+ 2hr treatments (LB 4/21/23) reprobed 4/26/24 JWS



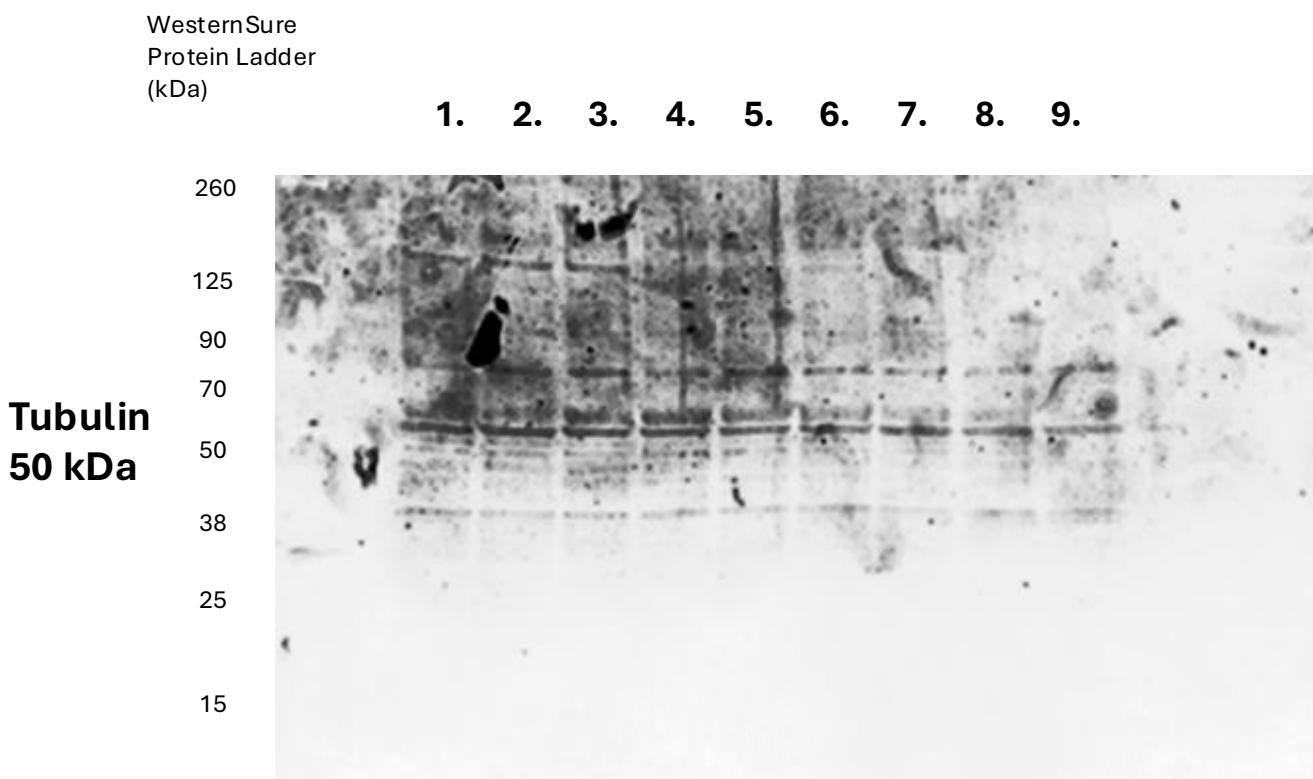
1 min Azure Imager

1. D2 Control
2. D2 VEGFB
3. D2 R+ (2hrs)
4. D4 Control
5. D4 VEGB
6. D4 R+ 2hrs
7. D6 Control
8. D6 VEGFB
9. D6 R+ 2 hrs

30 ug protein lysate/well
Block 1 hour RT in 5% Milk/TBS-T
Goat anti-mouse Tubulin (1:2000)
Incubation overnight at 4C
2nd goat HRP (1:2000)
Incubation 1 hour at room temperature (RT)
All antibodies in 5% Milk/TBS-T

*Recombinant Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - Goat IgG (Chimeric) (ab289875) reacts with mouse, rat, human
This goat monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (ab179484).
By necessity, some rabbit sequence is retained as part of the variable domain.
<https://www.abcam.com/products/primary-antibodies/alpha-tubulin-acetyl-k40-antibody-epr16772-goat-igg-chimeric-ab289875.html>

Tubulin* Western Blot - HemSC149 p8 Differentiation R+ 2hr treatments (LB 4/21/23) reprobed 4/26/24 JWS



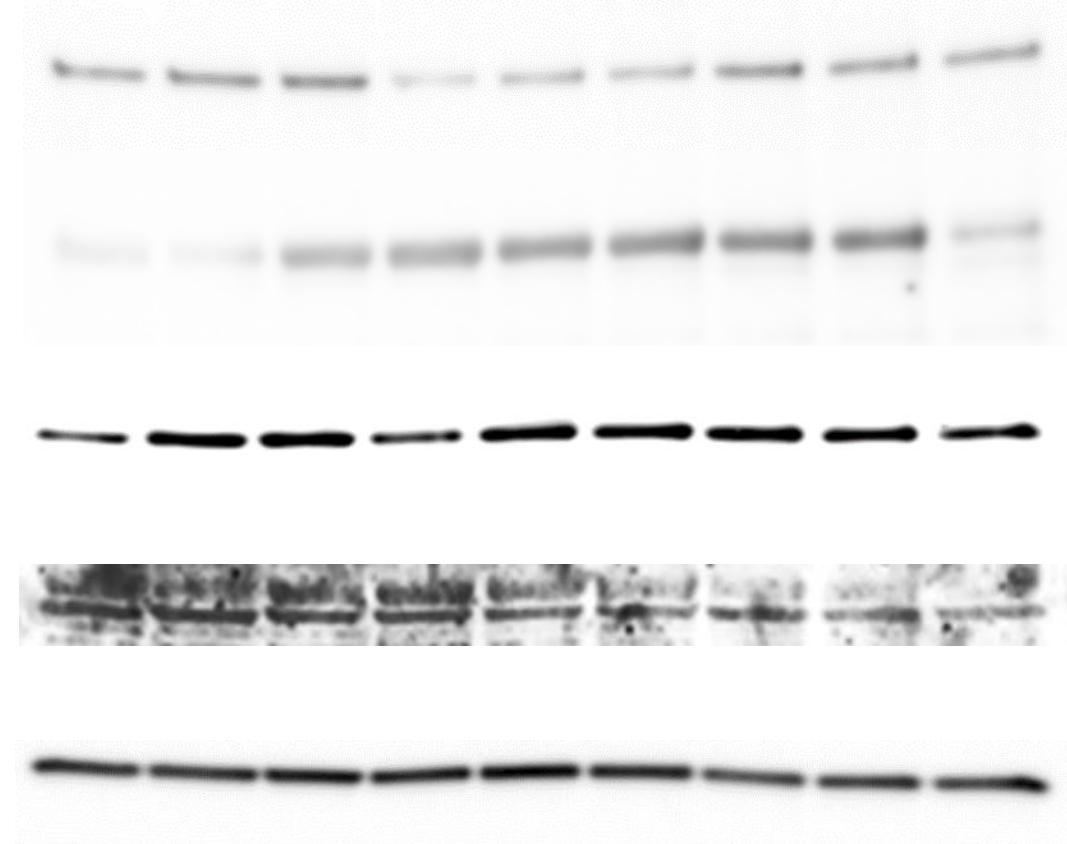
4 min less overall gamma Wide Dynamic Azure

1. D2 Control
2. D2 VEGFB
3. D2 R+ (2hrs)
4. D4 Control
5. D4 VEGB
6. D4 R+ 2hrs
7. D6 Control
8. D6 VEGFB
9. D6 R+ 2hrs

30 ug protein lysate/well
Block 1 hour RT in 5% Milk/TBS-T
Goat anti-mouse Tubulin (1:2000)
Incubation overnight at 4C
2nd goat HRP (1:2000)
Incubation 1 hour at room temperature (RT)
All antibodies in 5% Milk/TBS-T

*Recombinant Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - Goat IgG (Chimeric) (ab289875) reacts with mouse, rat, human
This goat monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (ab179484).
By necessity, some rabbit sequence is retained as part of the variable domain.
<https://www.abcam.com/products/primary-antibodies/alpha-tubulin-acetyl-k40-antibody-epr16772-goat-igg-chimeric-ab289875.html>

HemSC149 p8 EC Differentiation Assay, R+ Propranolol (20 uM) 2 hours, 04/21/23 LB, reprobe Sox18 03/21/24 JWS



Lanes:

1. D2 (-)VEGFB, + 0.01% HCl (VEGFB vehicle)
2. D2 +VEGFB, 0.01% ddH₂O (R+ vehicle)
3. D2 +VEGFB, 20uM R+ (2hrs)
4. D4 (-)VEGFB, + 0.01% HCl (VEGFB vehicle)
5. D4 +VEGFB, 0.01% ddH₂O (R+ vehicle)
6. D4 +VEGFB, 20uM R+ (2hrs)
7. D6 (-)VEGFB, + 0.01% HCl (VEGFB vehicle)
8. D6 +VEGFB, 0.01% ddH₂O (R+ vehicle)
9. D6 +VEGFB, 20uM R+ (2hrs)

SREBP2 (122 kDa band)

SREBP2 (55-65 kDa band)

Sox18 (41-45 kDa band)

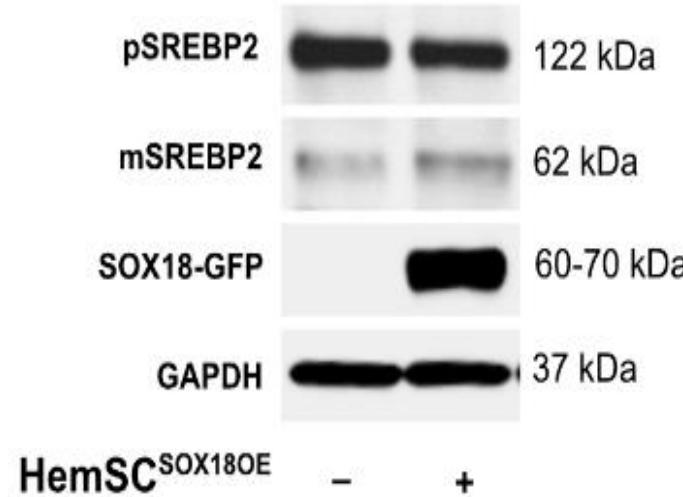
(Remnant SREBP2 (60 kDa)
above)

Tubulin (50 kDa)

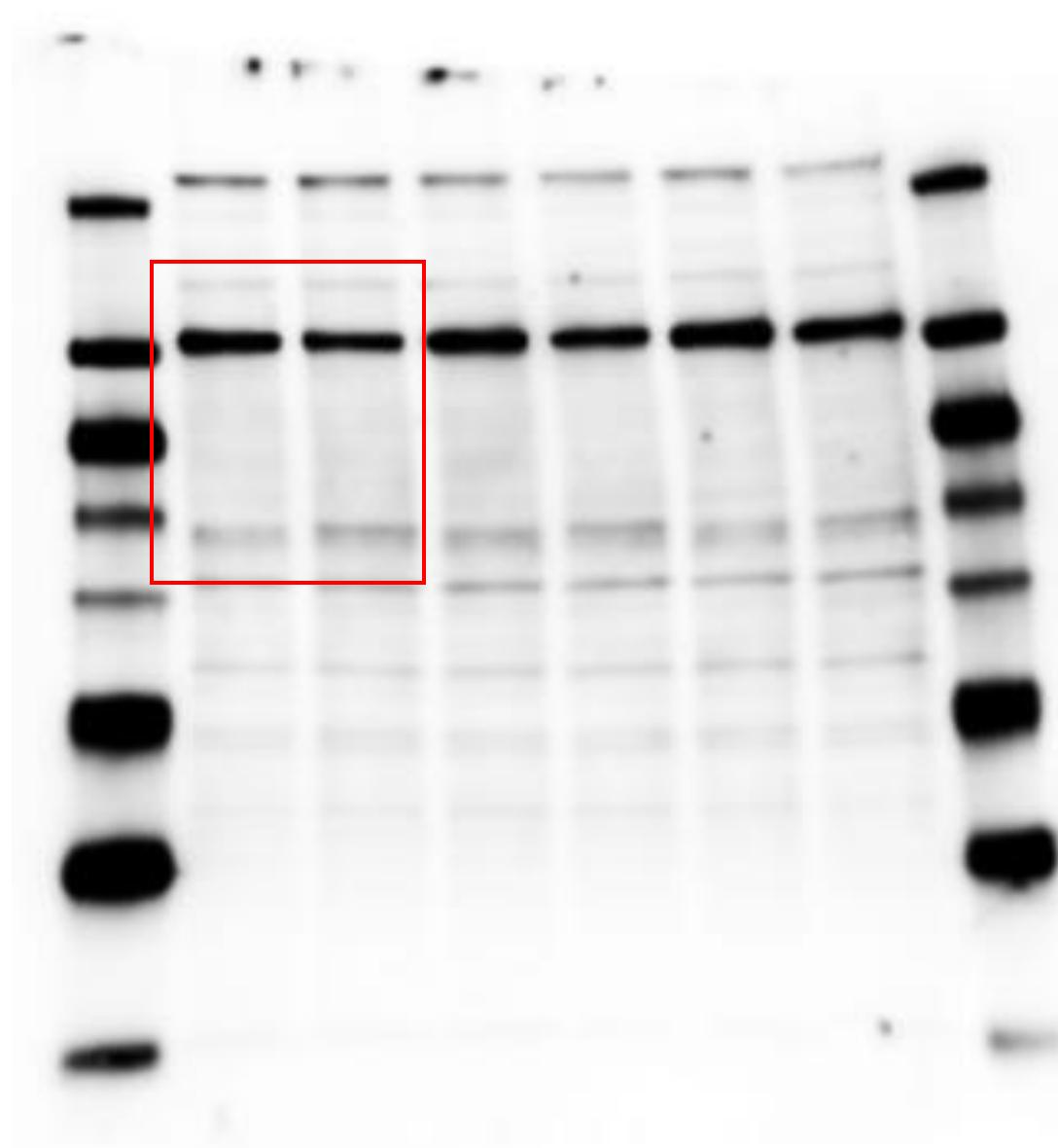
GAPDH (37 kDa band)

Figure 3 D Western Blots for full tiffs rev 11/26/24

D



SREBP2

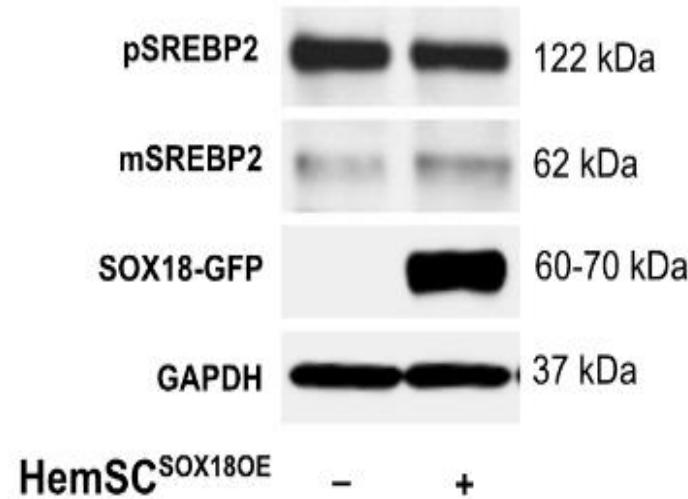


Tiff name: 8 bit SREBP2 3 min sharp auto straight crop 24.09.01_15.14.57_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 D Sox18 OE HemSC SREBP2 Sox18GFP GAPDH

Figure 3 D Western Blots for full tiffs rev 11/26/24

D



Sox18, GAPDH

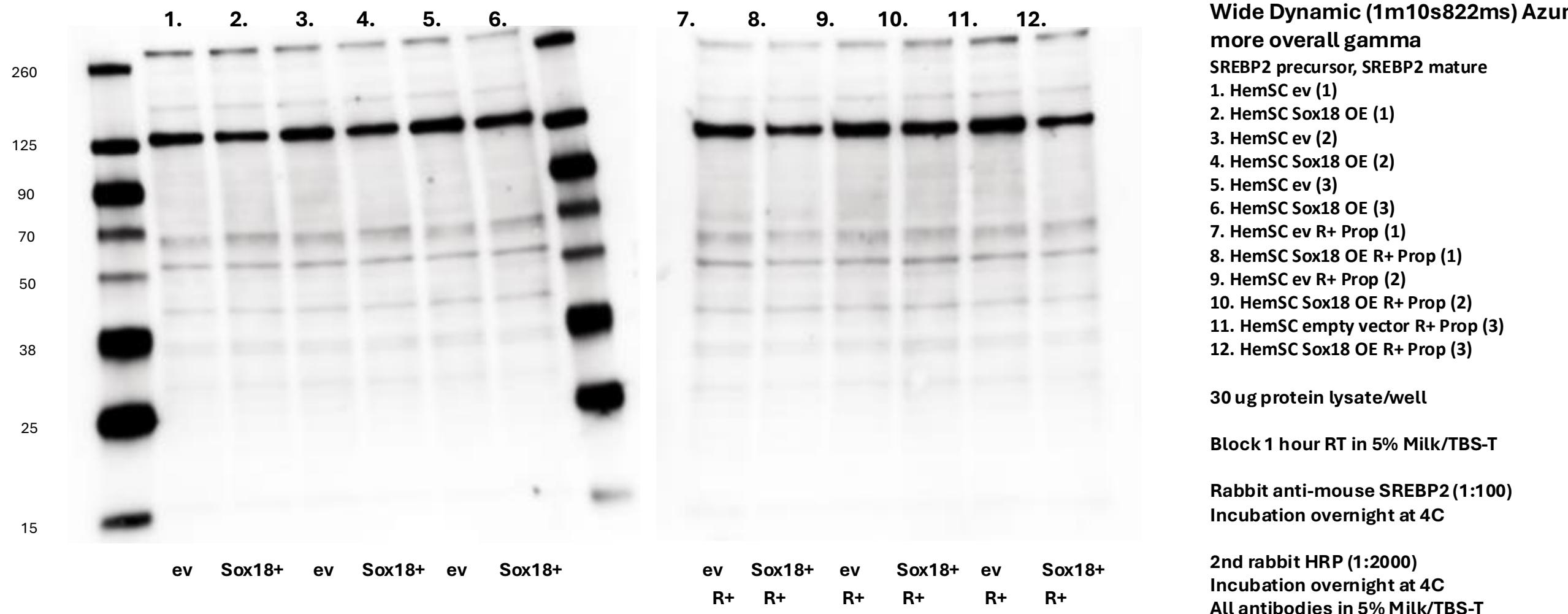


Tiff name: 420ms autoexposure more gamma 24.08.10_15.16.11_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 D Sox18 OE HemSC SREBP2 Sox18GFP GAPDH

SREBP2 precursor, mature forms – 2 Days, HemSC171 ev, Sox18 Overexpression (triplicates) in EGM-2 Complete media 8/9/24

5uM ALLN in RIPA proteinase inhibitors cocktail AND 2 hours 100 uM ALLN pre-treatment (following Luke's 4/21/23 protocol) - triplicates



**** Protein ladder lanes, very narrow and blurred into multiple lanes, did not show top 260 kDa band at all, which led to incorrect blot cut at top.**

Old SREBP2 antibody in 5% Milk/TBS-T/0.02% Na Azide with SREBP2 Lot# 4120677 (MilliporeSigma Cat# MABS1988)

Previous lots not available.

SREBP2 Precursor 122 kDa

Mature form 55, 62 kDa

Sox18, GAPDH – 2 Days, HemSC171 ev, Sox18 Overexpression (triplicates) in EGM-2 Complete media 8/10/24

5uM ALLN in RIPA proteinase inhibitors cocktail AND 2 hours 100 uM ALLN pre-treatment (following Luke's 4/21/23 protocol) - triplicates

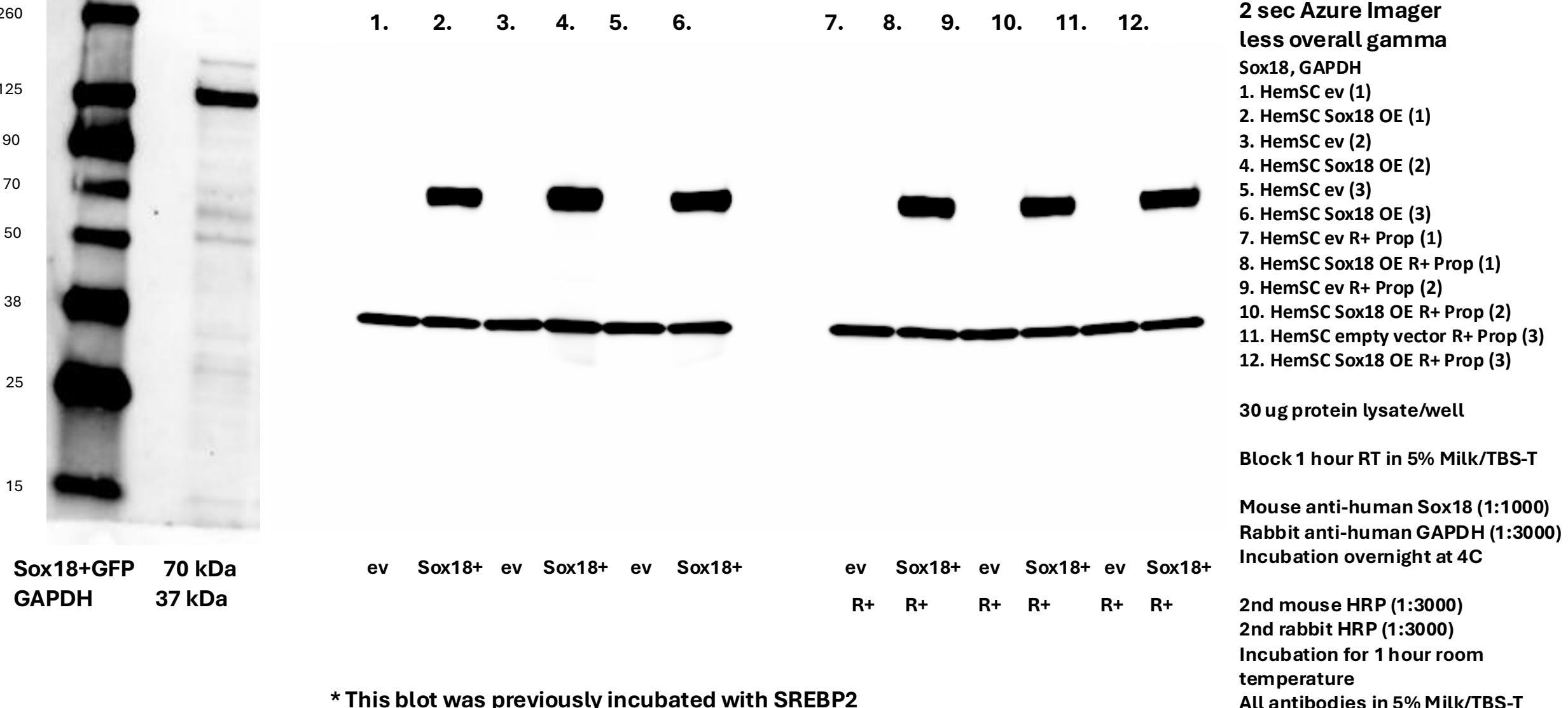
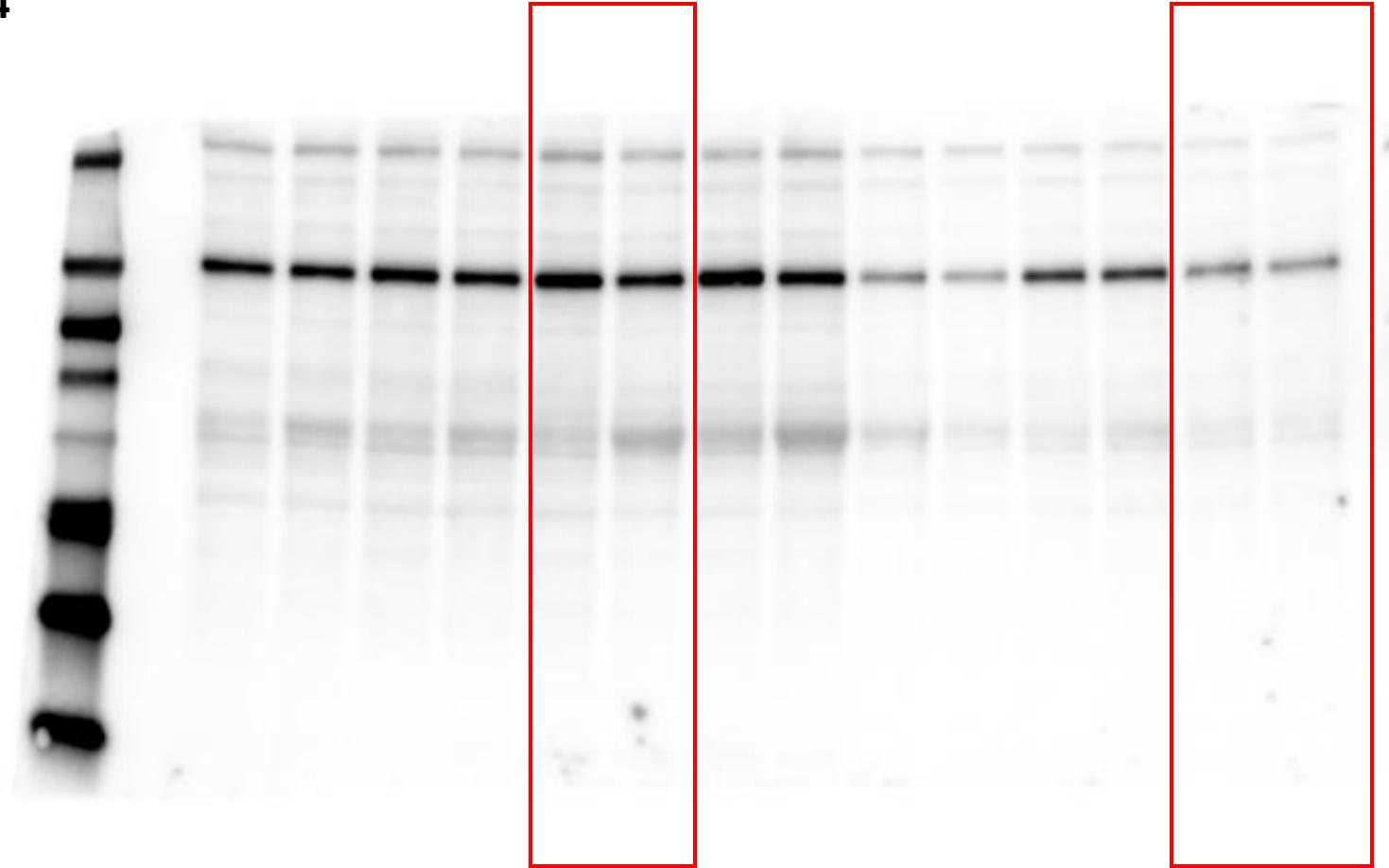


Figure 3 F Western Blots for full tiffs rev 11/26/24

F

pSREBP2					122 kDa
mSREBP2					62 kDa
SOX18					41 kDa
GAPDH					37 kDa
HemEC	-	-	+	+	
R(+) Propranolol	-	+	-	+	



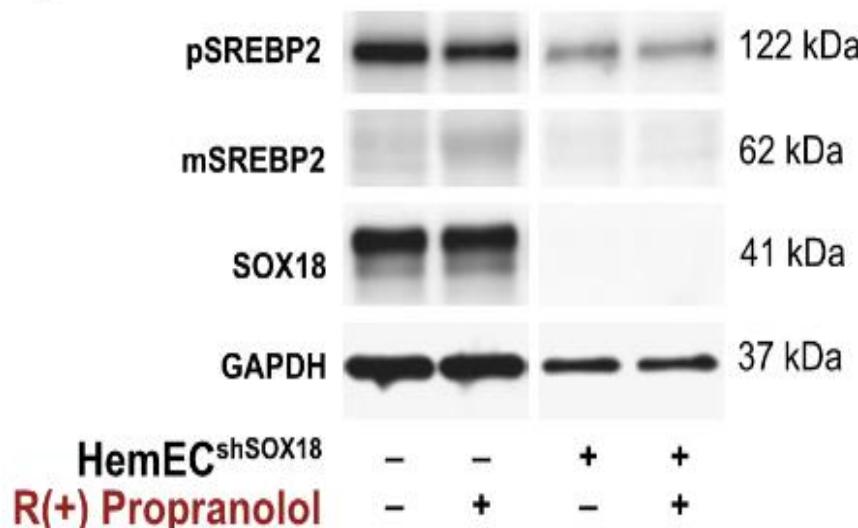
SREBP2

Tiff name: SREP2 straightened 23.12.13_12.56.57_PUB_600

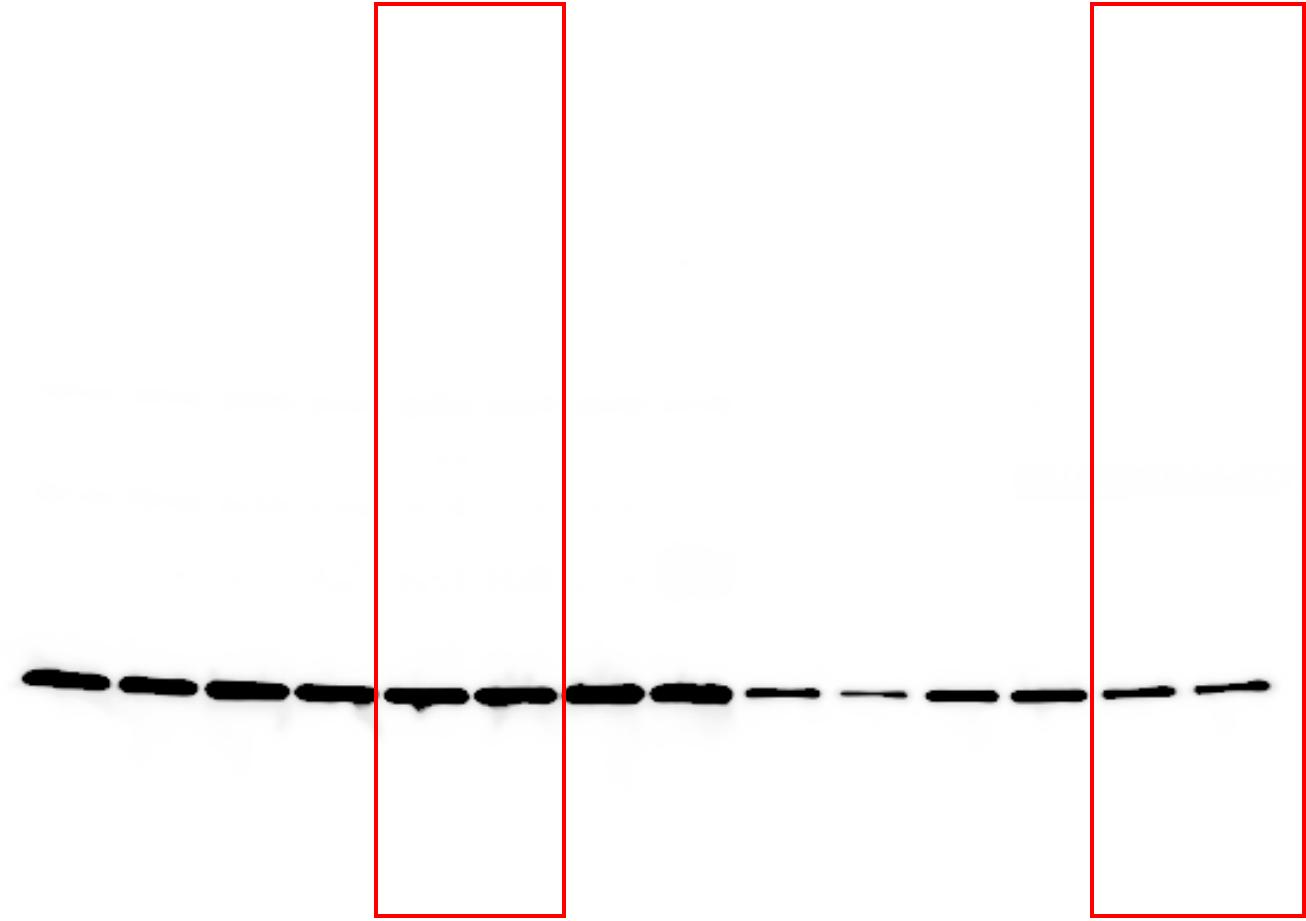
Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 F Sox18 ctrl kdn HemEC SREBP2 Sox18 GAPDH R+

Figure 3 F Western Blots for full tiffs rev 11/26/24

F



GAPDH

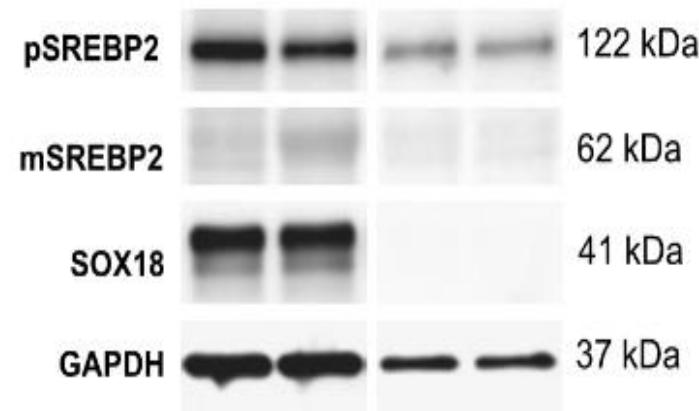


Tiff name: GAPDH threshold affinity autoexposure 23.12.14_16.33.04_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 F Sox18 ctrl kdn HemEC SREBP2 Sox18 GAPDH R+

Figure 3 F Western Blots for full tiffs rev 11/26/24

F



Sox18

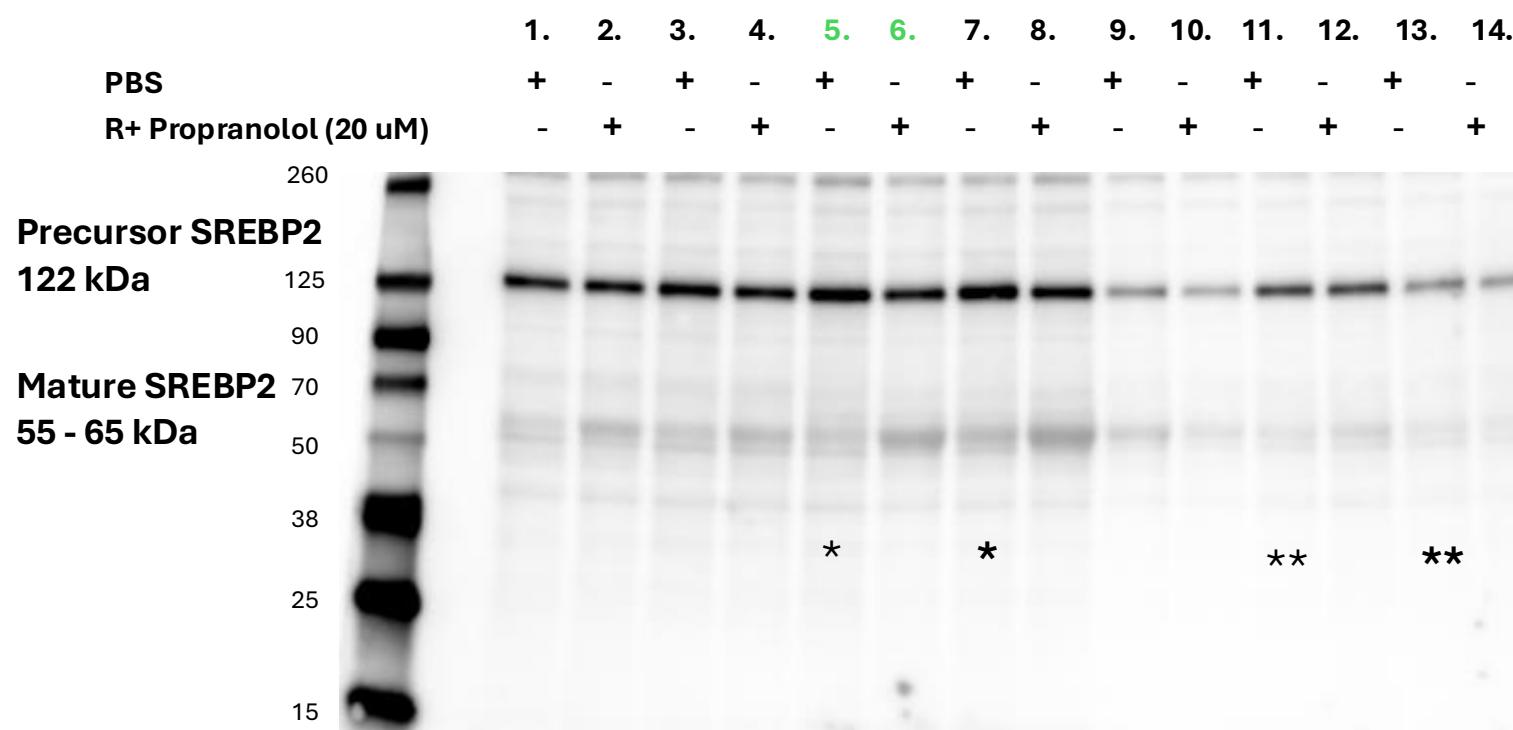
Tiff name: Sox18 GAPDH less gamma 23.12.12_13.30.44_PUB_600

Location: G:\Shared drives\SR-Bischoff-Common\Jill Wylie-Sears\For Anne\Figure 3 Find full tiffs 11262024\Figure 3 F Sox18 ctrl kdn HemEC SREBP2 Sox18 GAPDH R+

SREPB2 Western Blot HemEC naive and shSOX18 +/- R+ Propranolol 12/13/23

5uM ALLN in RIPA proteinase inhibitors cocktail, No ALLN 2 hrs pre-treatment (following Luke's 4/21/23 protocol)

Overexposure setting Azure Imager



Hemangioma Endothelial Cells

HemEC 171

* HemEC 171
Naïve (PBS)

HemEC Sox18 Knockdowns

Knockdown
HemEC 171

** Sox Kdn
HemEC 171
(PBS)

1. HemEC133 PBS

2. HemEC133 R+

3. HemEC150 PBS

4. HemEC150 R+

5. HemEC171 #1 PBS

6. HemEC171 #1 R+

7. HemEC171 #2 PBS

8. HemEC171 #2 R+

9. HemEC133.4 shSOX18 PBS

10. HemEC133.4 shSOX18 R+

11. HemEC150.4 shSOX18 PBS

12. HemEC150.4 shSOX18 R+

13. HemEC171.2 shSOX18 PBS

14. HemEC171.2 shSOX18 R+

All in EGM-2 Complete medium

30 ug protein lysate/well

High MW setting Turbo Transfer

Block 1 hour RT in 5% Milk/TBS-T

Rabbit anti-human SREBP2 (1:125)

Incubation overnight for 23 hours at 4C

2nd rabbit HRP (1:2000)

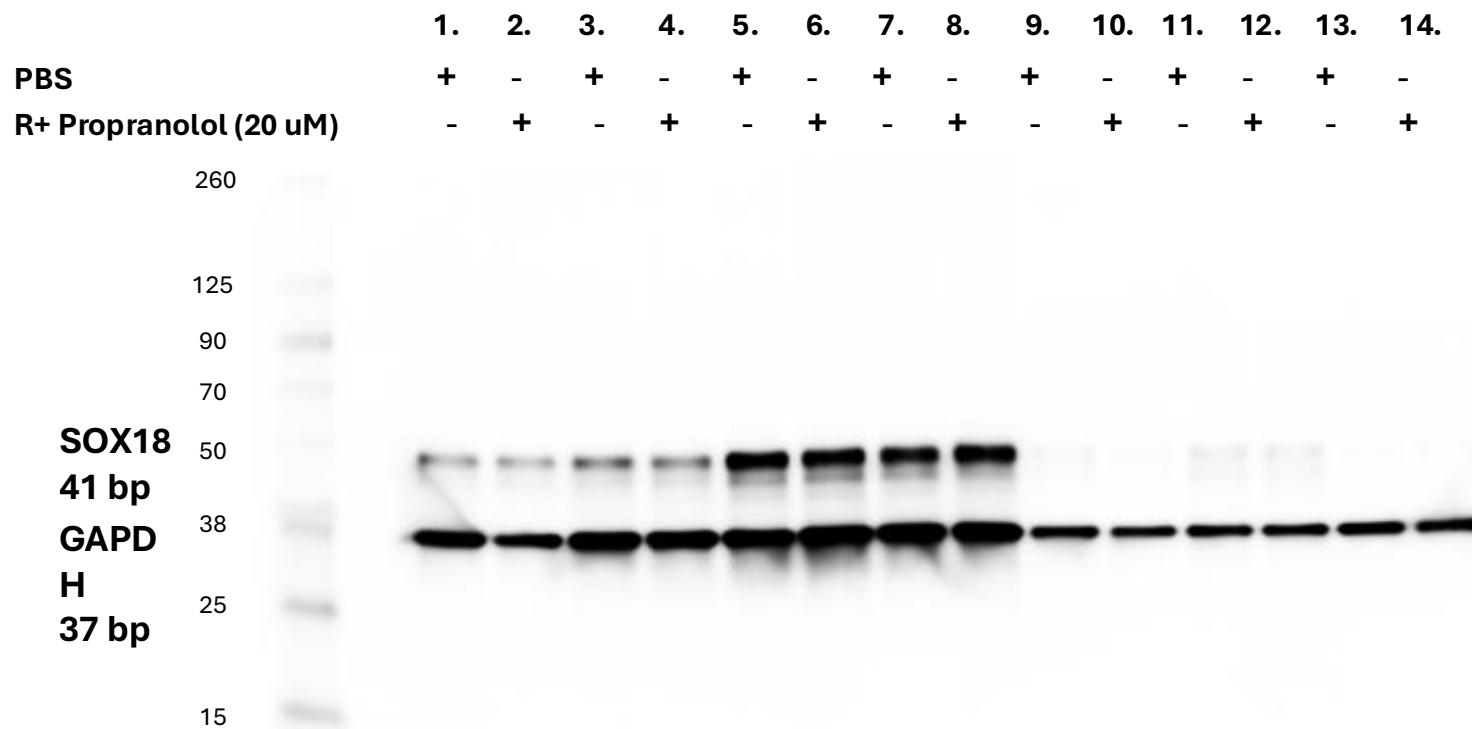
Incubation overnight (18 hrs) at 4C

All antibodies in 5% Milk/TBS-T

4 ml Clarity Max ECL developer per blot

Sox18, GAPDH Western Blot HemEC naive and shSOX18 +/- R+ Propranolol 12/13/23

30 sec setting Azure Imager



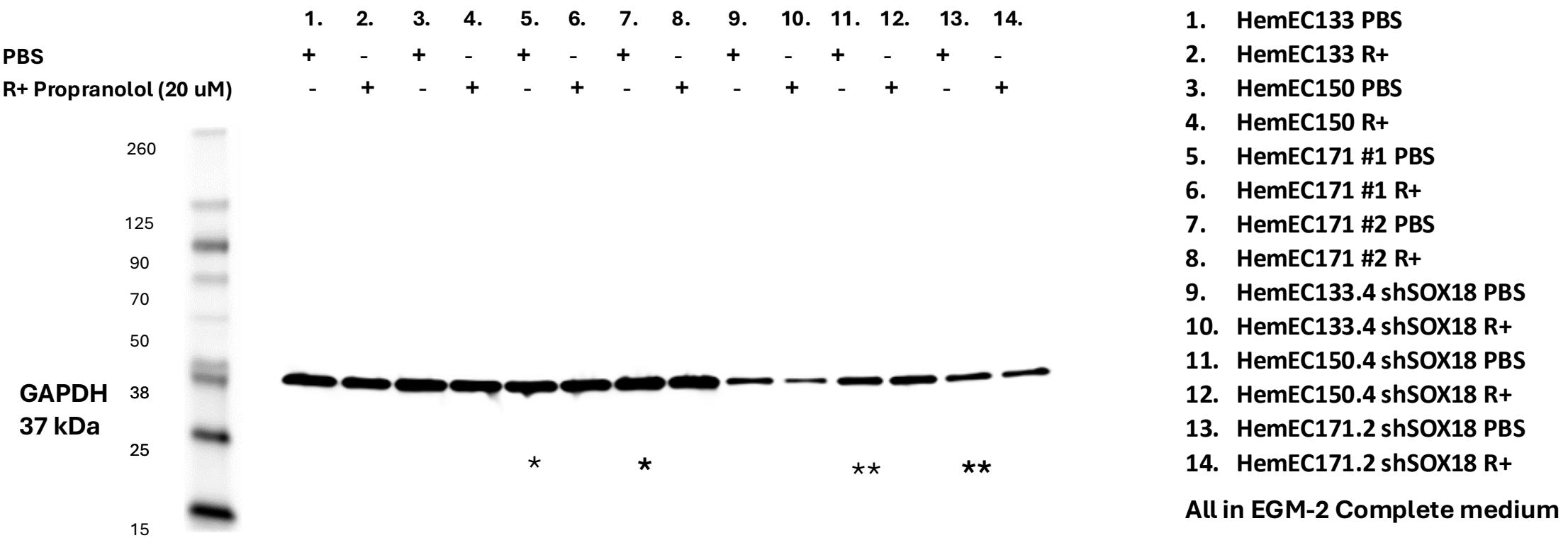
1. HemEC133 naïve PBS
2. HemEC133 naïve R+
3. HemEC150 naïve PBS
4. HemEC150 naïve R+
5. HemEC171 naïve #1 PBS
6. HemEC171 naïve #1 R+
7. HemEC171 naïve #2 PBS
8. HemEC171 naïve #2 R+
9. HemEC133.4 shSOX18 PBS
10. HemEC133.4 shSOX18 R+
11. HemEC150.4 shSOX18 PBS
12. HemEC150.4 shSOX18 R+
13. HemEC171.2 shSOX18 PBS
14. HemEC171.2 shSOX18 R+

15 ug protein lysate/well
Mixed MW setting Turbo Transfer
Block 1 hour RT in 5% Milk/TBS-T
Mouse anti-human Sox18 (1:500)
Rabbit anti-human GAPDH (1:2500)
Incubation overnight at 4C
2nd mouse HRP (1:2000)
2nd rabbit HRP (1:6000)
Incubation 1 hour at room temperature
All antibodies in 5% Milk/TBS-T
3 ml Clarity Max ECL developer per blot

GAPDH following SREPB2 Western Blot HemEC naive and shSOX18 +/- R+ Propranolol 12/13/23

5uM ALLN in RIPA proteinase inhibitors cocktail, No ALLN 2 hrs pre-treatment (following Luke's 4/21/23 protocol)

Autoexposure Azure Imager



Hemangioma Endothelial Cells

HemEC 171

* HemEC 171
Naïve (PBS)

HemEC Sox18 Knockdowns

Knockdown
HemEC 171

** Sox Kdn
HemEC 171
(PBS)

30 ug protein lysate/well
High MW setting Turbo Transfer
Block 1 hour RT in 5% Milk/TBS-T
Rabbit anti-human SREBP2 (1:125)
Incubation overnight for 23 hours at 4C
2nd rabbit HRP (1:2000)
Incubation overnight (18 hrs) at 4C
All antibodies in 5% Milk/TBS-T
4 ml Clarity Max ECL developer per blot