

Global miRNA/Proteomic Analyses Identify miRNAs at 14q32 and 3p21, Which Contribute to Features of Chronic Iron-Exposed Fallopian Tube Epithelial Cells

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Supplementary Figure Legends

Figure S1. Protein targets of miR-138-5p predicted via IPA analysis. IPA analysis identified 28 upregulated proteins predicted to be downstream of miR-138-5p in 250 nM FAC-treated FT194 cells relative to Untreated. Of these, 7 (including ALDH1A2) were related to gynecological cancers. For detailed legend for all molecular processes in the protein network, refer to supplemental Fig. [S6](#).

Figure S2. Protein targets of miR-127-3p and miR-432, associated with malignant solid tumors, predicted via IPA analysis. IPA analysis identified 10 upregulated proteins predicted to be downstream of either miR-127-3p (5 proteins) or miR-432 (5 proteins) in FT194-OCV cells relative to FT194-CV and associated with malignant solid tumors. For detailed legend for all molecular processes in the protein network, refer to supplemental Fig. [S6](#).

Figure S3. Protein targets of miR-432, associated with binding of DNA, predicted via IPA analysis. IPA analysis identified 5 upregulated proteins predicted to be downstream of miR-432 in FT194-OCV cells relative to FT194-CV. Of these, three were associated with binding of DNA. For detailed legend for all molecular processes in the protein network, refer to supplemental Fig. [S6](#).

Figure S4. Protein targets of miR-127-3p predicted via IPA analysis. IPA analysis identified 5 upregulated proteins predicted to be downstream of miR-432 in FT194-OCV

cells relative to FT194-CV. For detailed legend for all molecular processes in the protein network, refer to supplemental Fig. S6.

Figure S5. Prediction of EVI1 binding with miR-138-5p, using Dialign Genomatrix software. The genomic sequence (5000bp upstream) of the promoter region of miR-138-5p-1 (located at 3p21.32) was obtained from the UCSC Genome Browser (www.genome.ucsc.edu, Human Dec. 2013 (GRCh38/hg38 Assembly)). The EVI1 N-terminal Zinc-finger binding, C-terminal Zinc-finger binding and consensus sequences were previously published³⁰⁻³². Prediction of EVI1 binding with miR-138-5p-1 promoter region was completed by aligning the sequences using Genomatix software suite (version 3.11, <http://www.genomatix.de/cgi-bin/dialign/dialign.pl>), and the aligned region is presented.

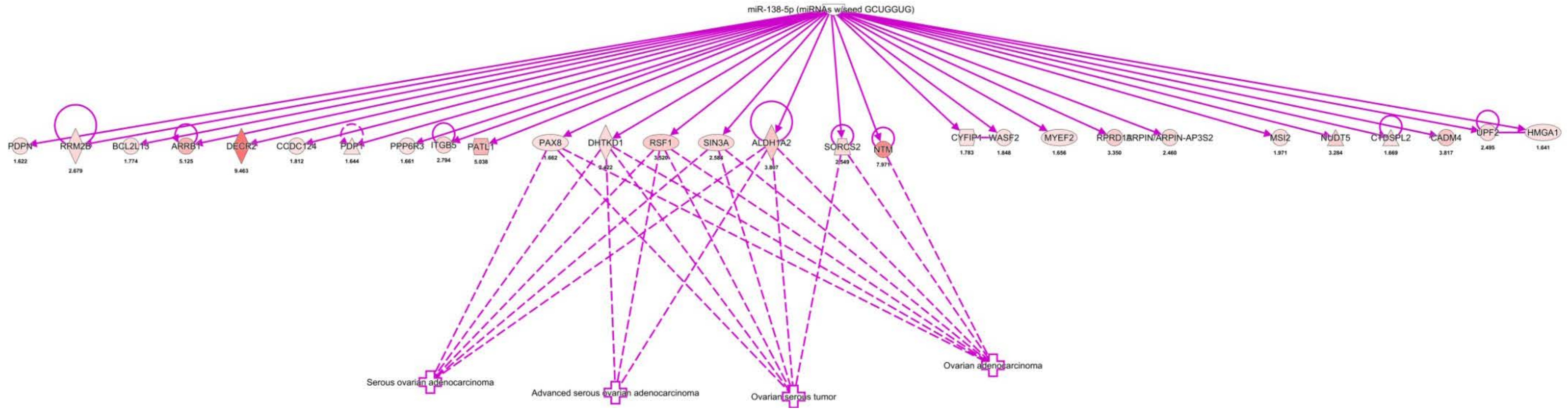
Figure S6. Detailed figure legend for Fig. 4D, Fig. 5A – 5D, and supplemental Fig. S1 - S4 provided by the Qiagen IPA Software Suite. The prediction legend (on left) and molecular shapes (on right) indicate the colors, patterns and shapes depicting protein measurements and alterations in molecular pathways associated with specific cellular functions. Upregulation and downregulation of targets is denoted by red and green, respectively, with the intensity of color demonstrating the level of change. Arrows indicated the prediction of activation (orange), inhibition (blue), inconsistent findings of downstream molecule (yellow) and no effect (black) while the different molecule shapes represent the type of protein that was expressed or predicted.

Supplementary Tables 1 and 2. Out of the total 4,402 proteins, 3,968 quantifiable proteins were identified after filtering. In OCV-infected compared to CV-infected FT194 cells, out of 4,691 total proteins, 4,148 quantifiable proteins were identified after filtering. Using Welch's t-test ($p < 0.05$) and z-score (z-score > 1), statistically significant targets were identified that had LFQ intensity ratio of ≥ 2 or ≤ 0.5 . Thus, 622 targets for OCV (relative to CV) and 243 targets for FAC-exposed FTSECs (relative to Untreated) were obtained. These tables show a raw list of total quantifiable as well as differentially expressed proteins.

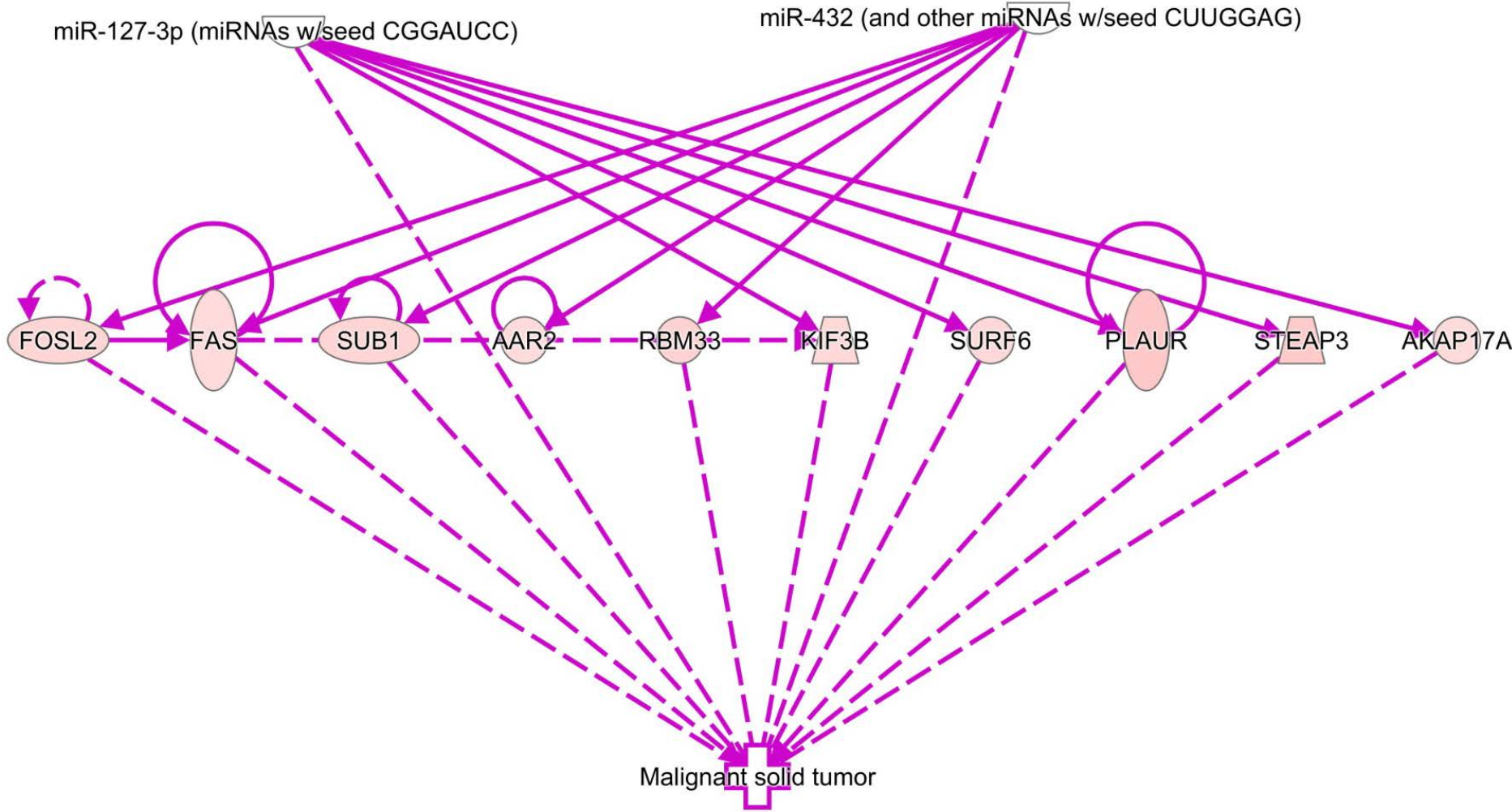
Additional Information for Western Blots

Uncropped images are displayed for all western blots presented in the manuscript including Fig. 4C, Fig. 6A, Fig 7B, Fig. 8B, and Fig. 8C. Cropped blots are presented in the main figure file; white space in-between each blot represents different antibody applications to the same blot.

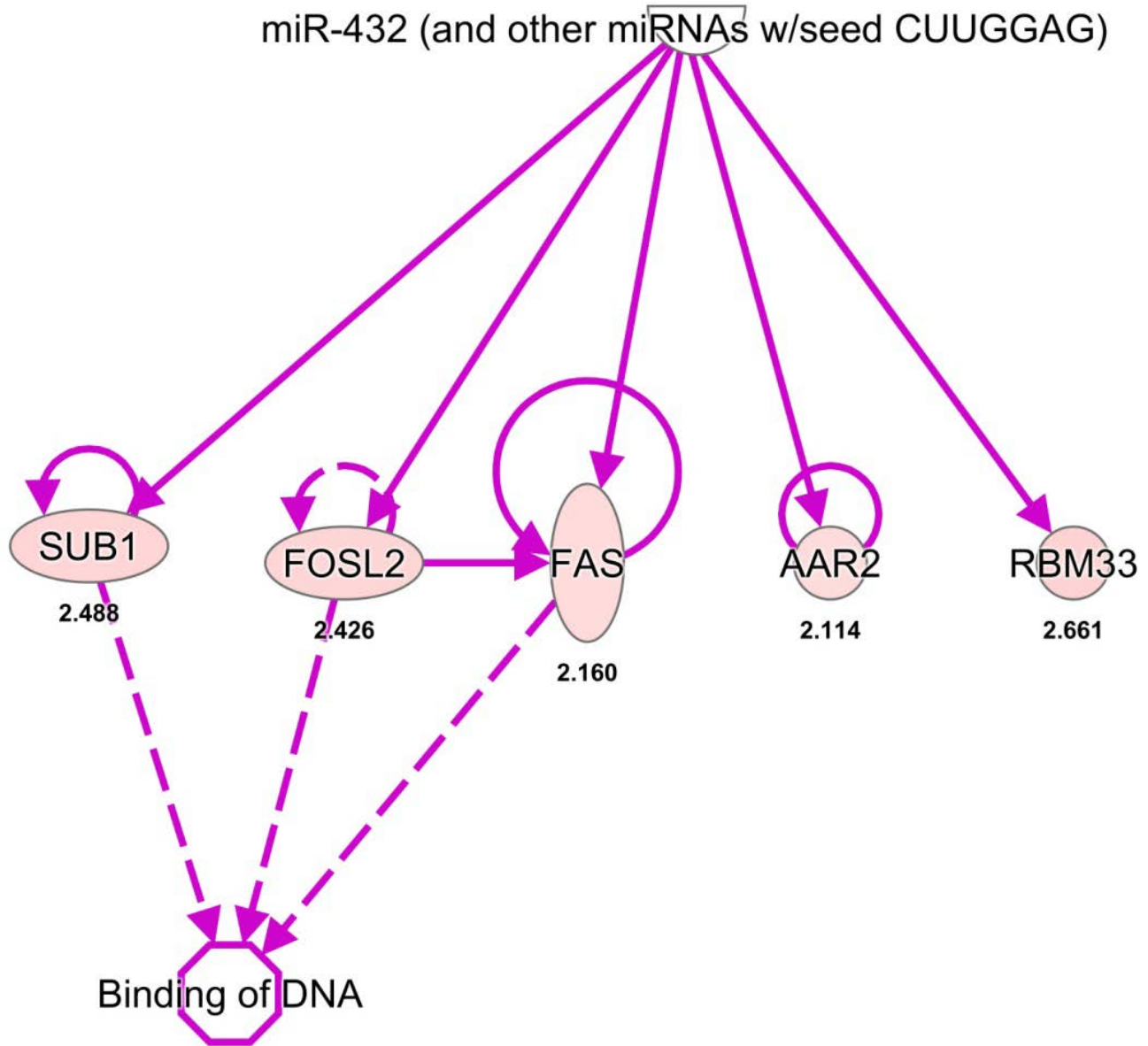
Supplemental Figure S1



Supplemental Figure S2

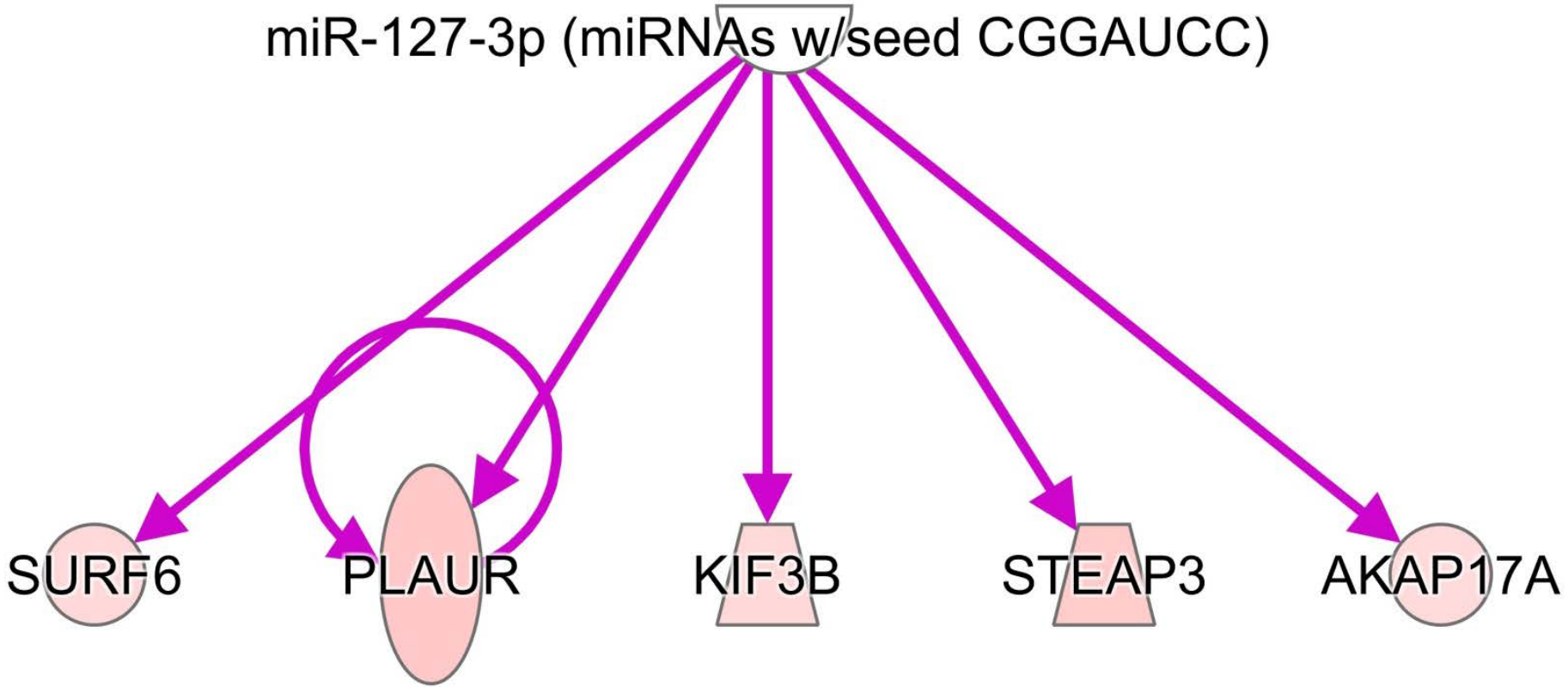


Supplemental Figure S3



Supplemental Figure S4

miR-127-3p (miRNAs w/seed CGGAUCC)

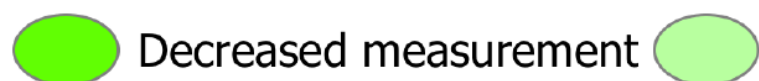


Supplemental Figure S5

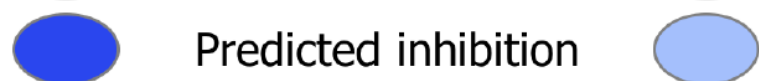
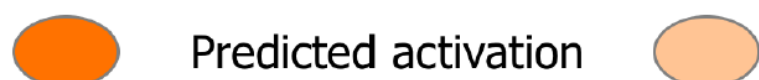
alignment position		2521.....	2531.....	2541.....	2551.....	2561.....	2571.....	2581.....
Promoter_miR-138	2521	tagcctagag	tcagGAAGAT	GAGttaagag	gtctgacagt	ctattgaagt	aaaattattc	attacagaat
EVI1_N-term_Zn_Finger_BD	4	-----	----AAGAT	A-----	-----	-----	-----	-----
EVI1_C-term_Zn_Finger_BD	1	-----	----GAAGAT	GAG-----	-----	-----	-----	-----
EVI1_Consensus	5	-----	----AAGAT	Aa-----	-----	-----	-----	-----

Prediction Legend

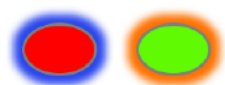
more extreme in dataset



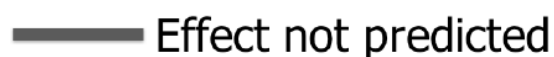
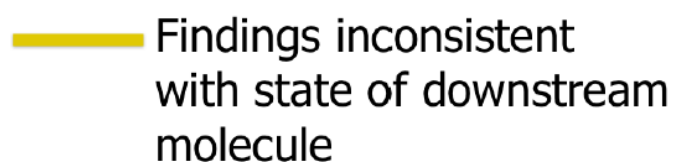
more confidence



Glow indicates activity when opposite of measurement

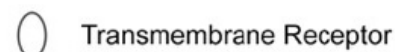
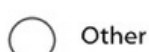


Predicted Relationships

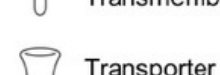
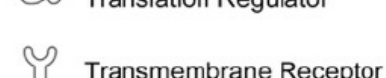
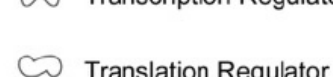
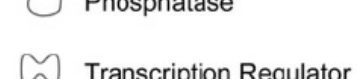
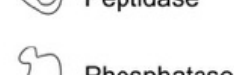
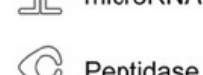
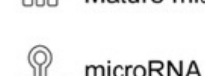
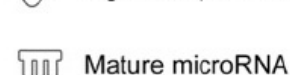
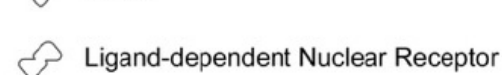
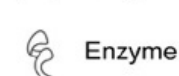


Molecule Shapes

Network Shapes



Path Designer Shapes



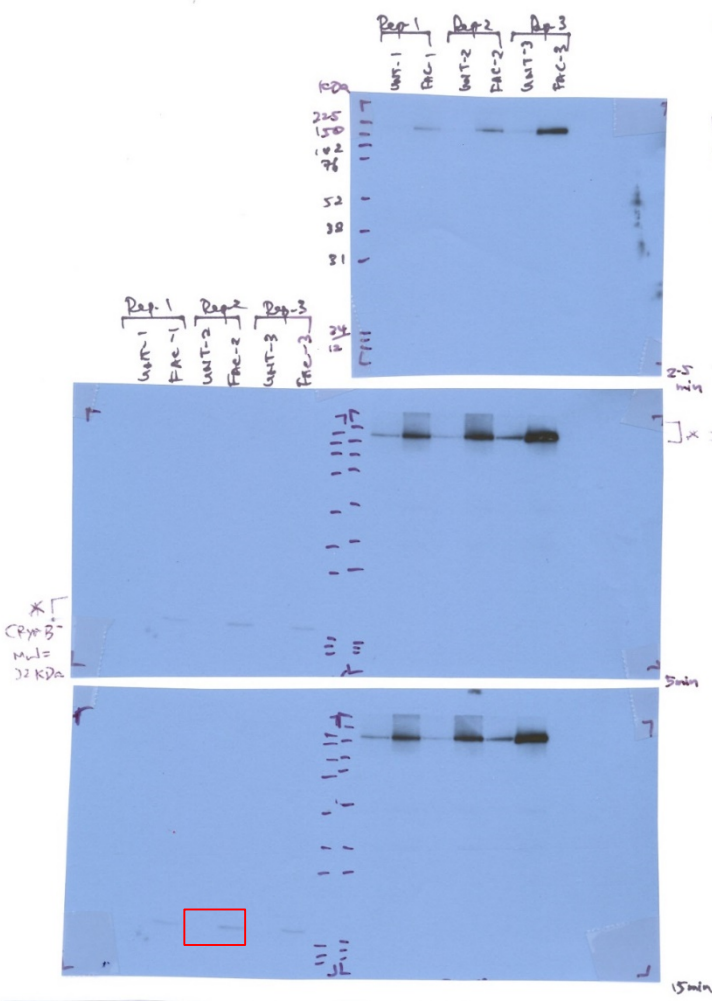
Additional Information for Western Blots

**Uncropped -
Fig. 4C, Fig. 6A, Fig, 7B, Fig. 8B, and Fig. 8C**

Figure 4C: Replicate #2 (Left Side)

CRYAB

10% FTIR4: UNT/FAC (@p=5i) Rep1/2/3 (New Samples)



Ab: Left Side -
CRYAB @
1:1000 @ Rb
2x

Made: 11/25/26
Next → ITGA2

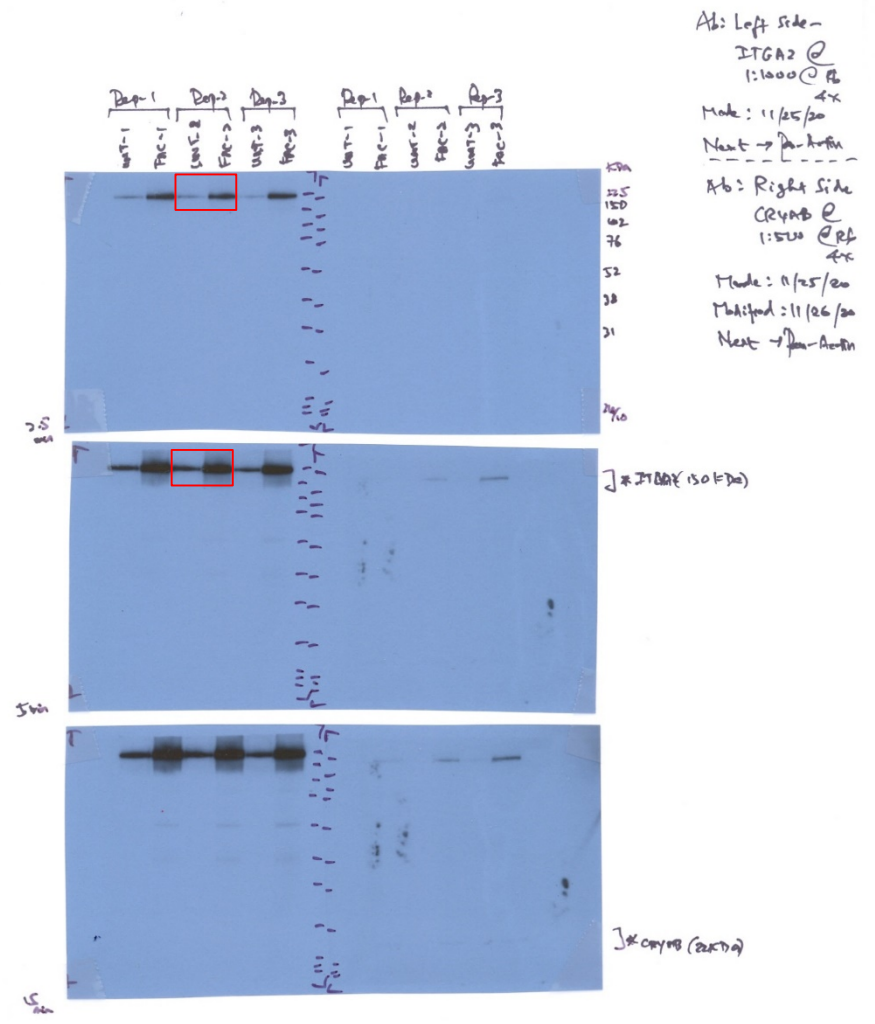
Ab: Right Side -
ITGA2 @
1:1000 @ Rb
4x

Made: 11/25/26
Next → CRYAB

* ITGA2 MW ~ 150 kDa

ITGA2

10% FTIR4: UNT/FAC (@p=5i) Rep1/2/3 (New Samples)



Ab: Left Side -
ITGA2 @
1:1000 @ Rb
4x

Made: 11/25/26
Next → Pan-Ac-tin

Ab: Right Side -
CRYAB @
1:500 @ Rb
4x

Made: 11/25/26
Next → Pan-Ac-tin

Figure 4C: Replicate #2 (Left Side)

Pan-Actin

10% FT194: CNT (FAC @ p=50) Rep(1/2/3) (New Samples)

Ab: Pan Actin
(Left & Right)
@ 1:5000
Ab 2x
Moe: 2/5/20
Next -> Binder

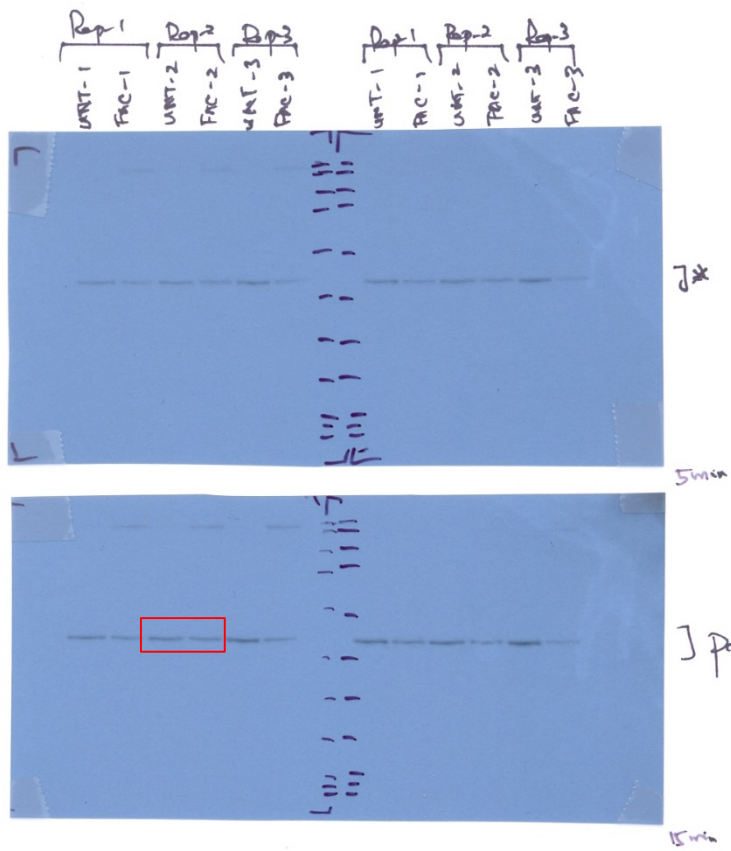
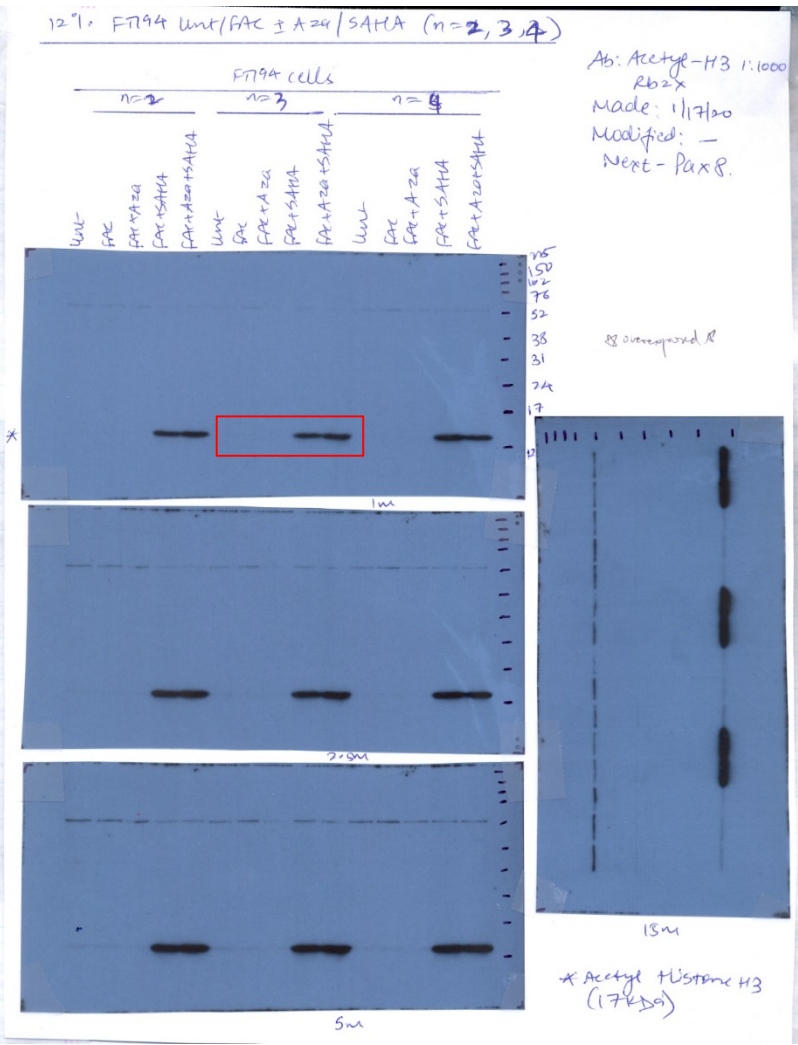


Figure 6A: Replicate #3

Acetyl-H3



DNMT1

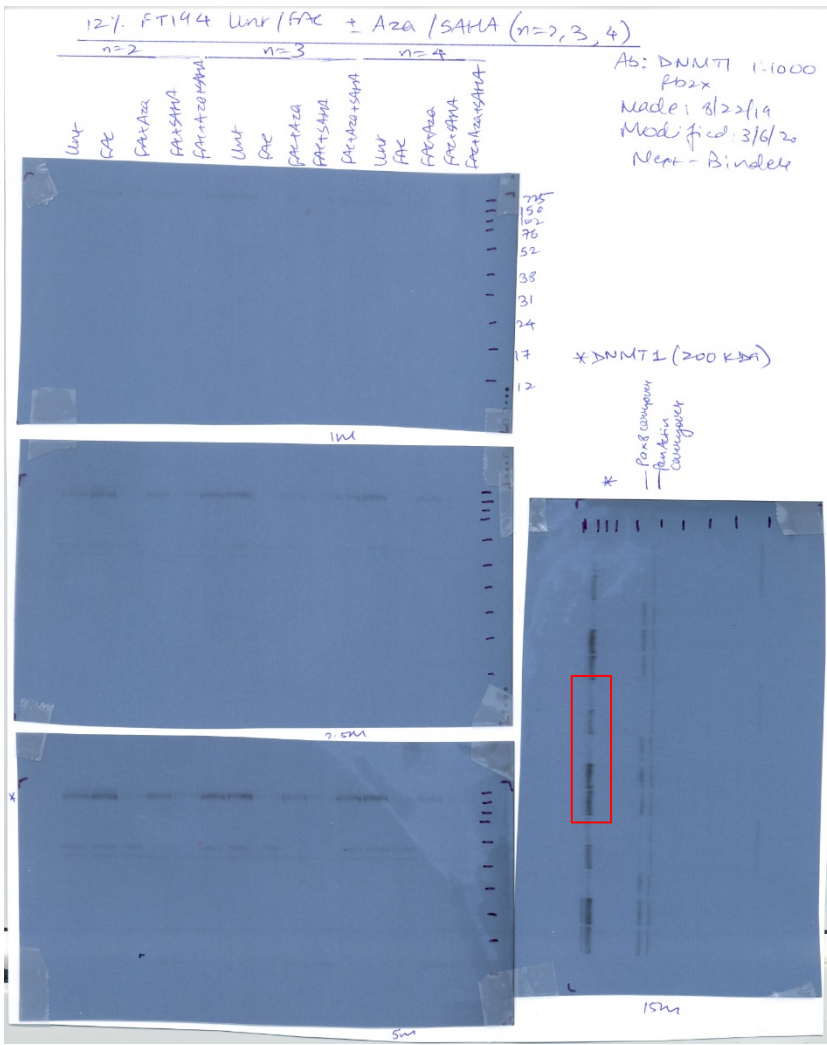
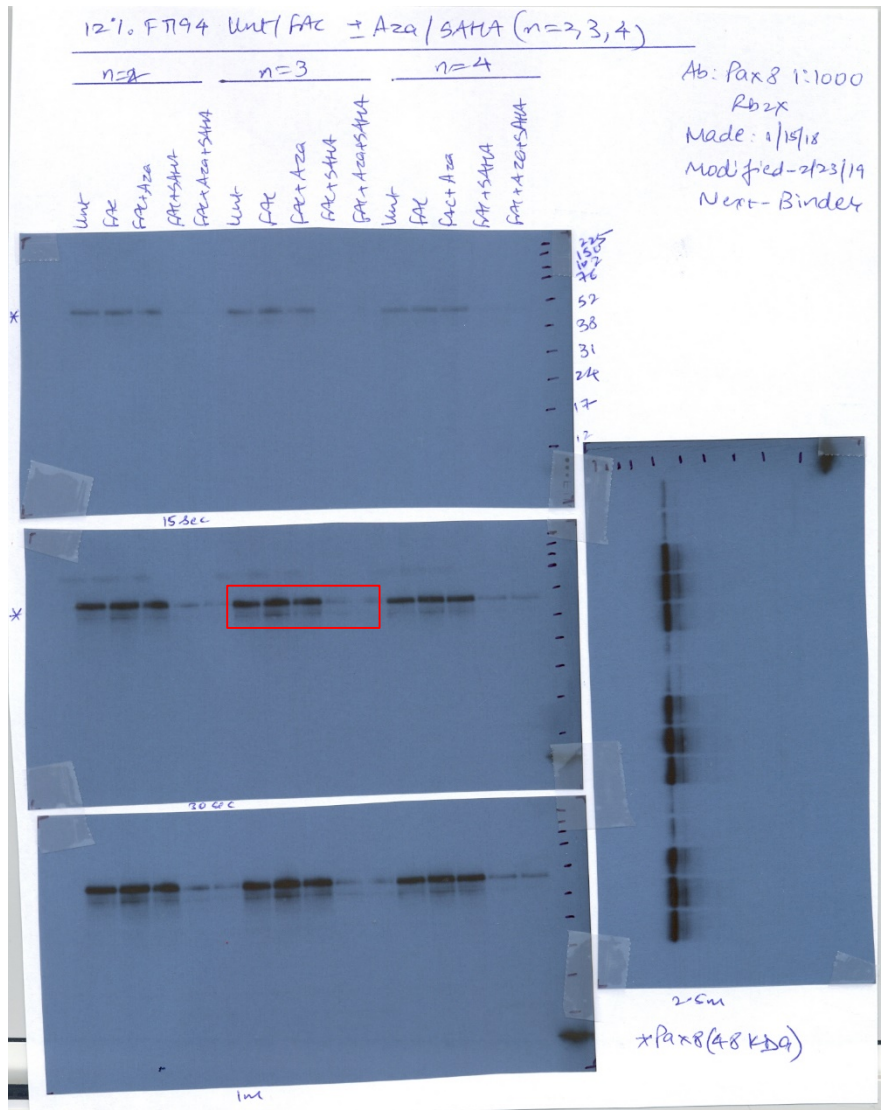


Figure 6A: Replicate #3

PAX8



Pan-Actin

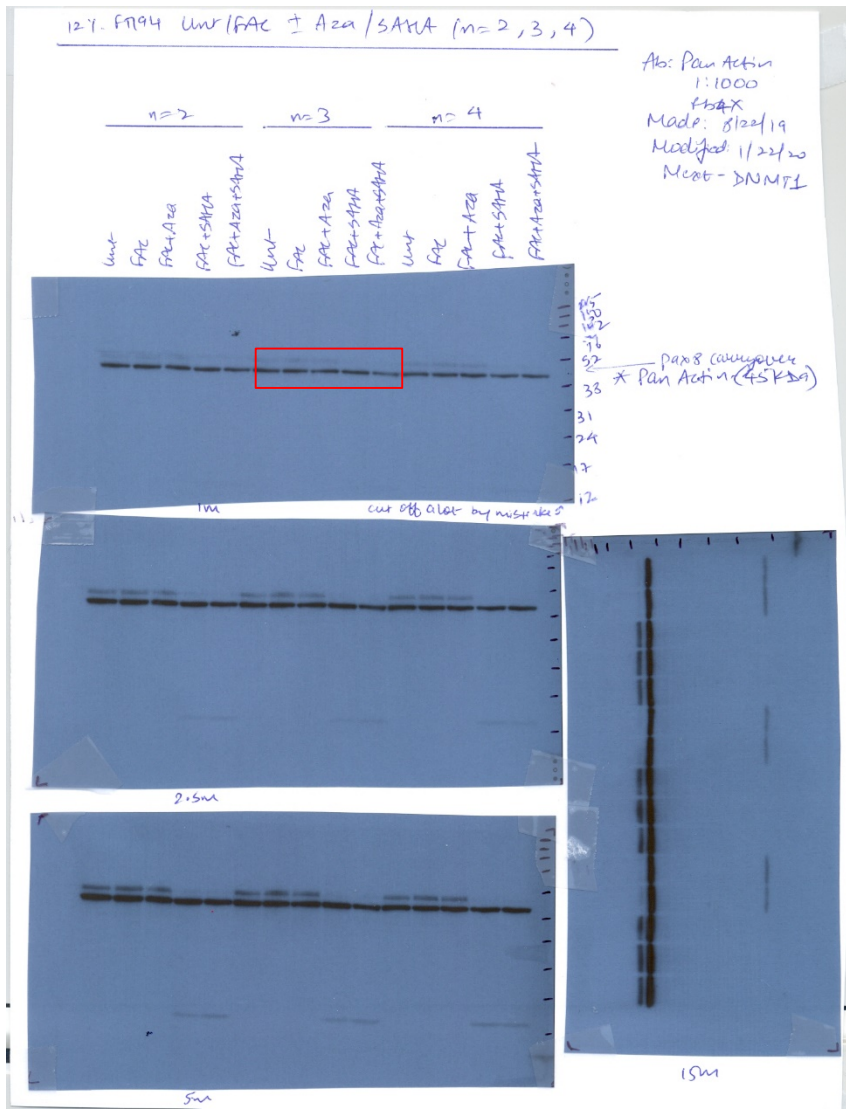


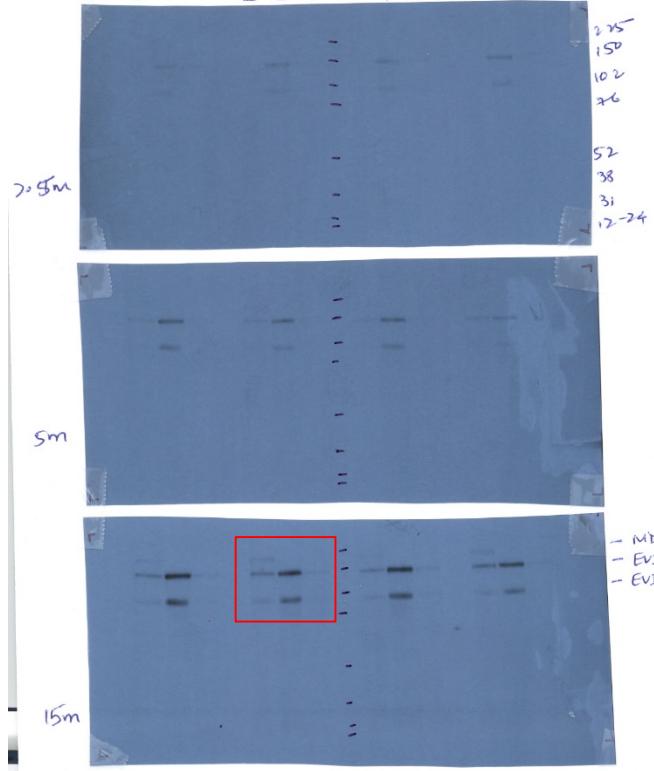
Figure 7B: Replicate #3 (Left Side)

EVI1

87. FT94 Unt/FAC siB n=2,3 (samples run twice)

FT94							
n=2			n=3		n=2		n=3
Unt	FAC	siB	Unt	FAC	siB	Unt	FAC
CTsi	CTsi	siB	CTsi	CTsi	siB	CTsi	CTsi
siB	siB		siB	siB		siB	siB

Ab: EVI1 1:500
 Sec: Rb4x
 Made: 6/18/18
 Modified: 10/22/19
 Next - Pan Actin



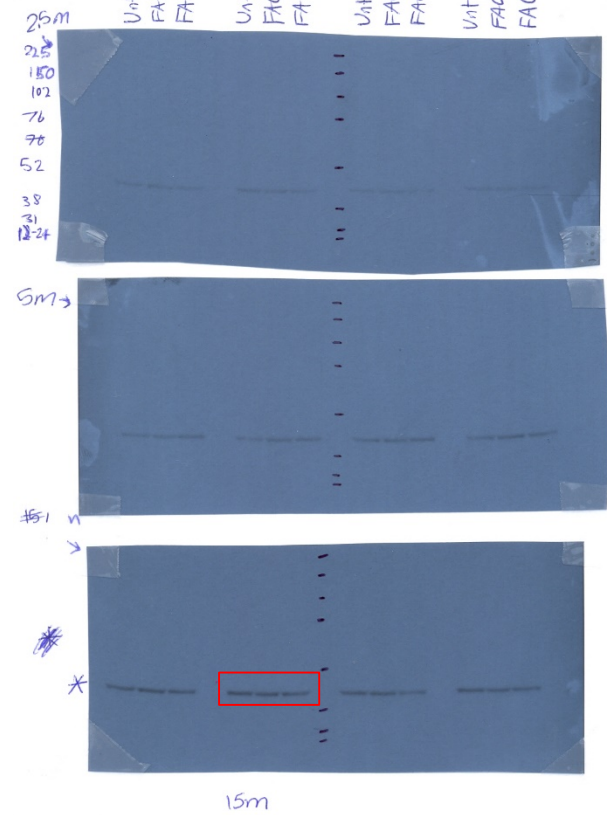
- MDS1/EVI1 (200kDa)
 - EVI1 wildtype (105kDa)
 - EVI1 del 190-55 (105kDa)

Pan-Actin

87. FT94 Unt/FAC siB n=2,3 (samples run twice)

FT94			FT94		
Unt	FAC	siB	Unt	FAC	siB
CTsi	CTsi	siB	CTsi	CTsi	siB
siB	siB		siB	siB	

Ab: Pan-Actin
 1:1000 Rb2x
 Made: 8/22/19
 Next - Bindley



* - Pan-actin (45 kDa)

Figure 8B: Replicate #2

ALDH1A2

PAX8

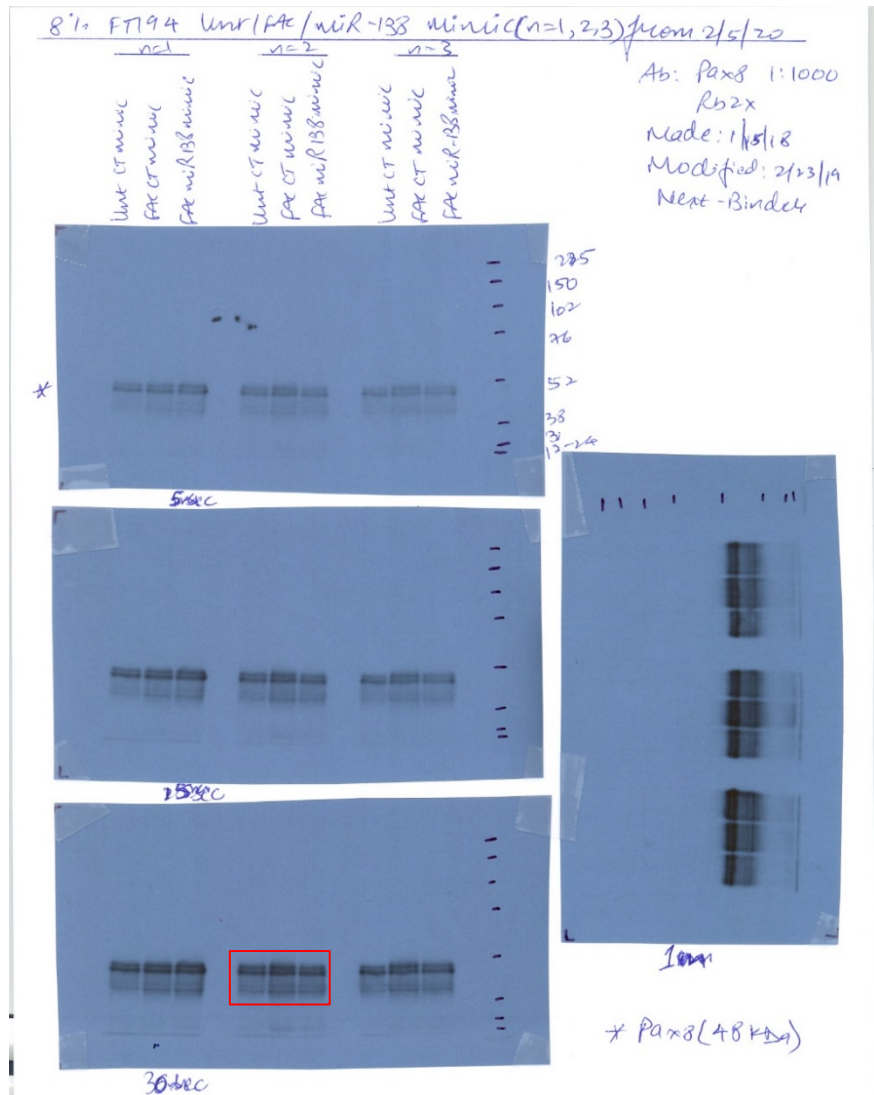
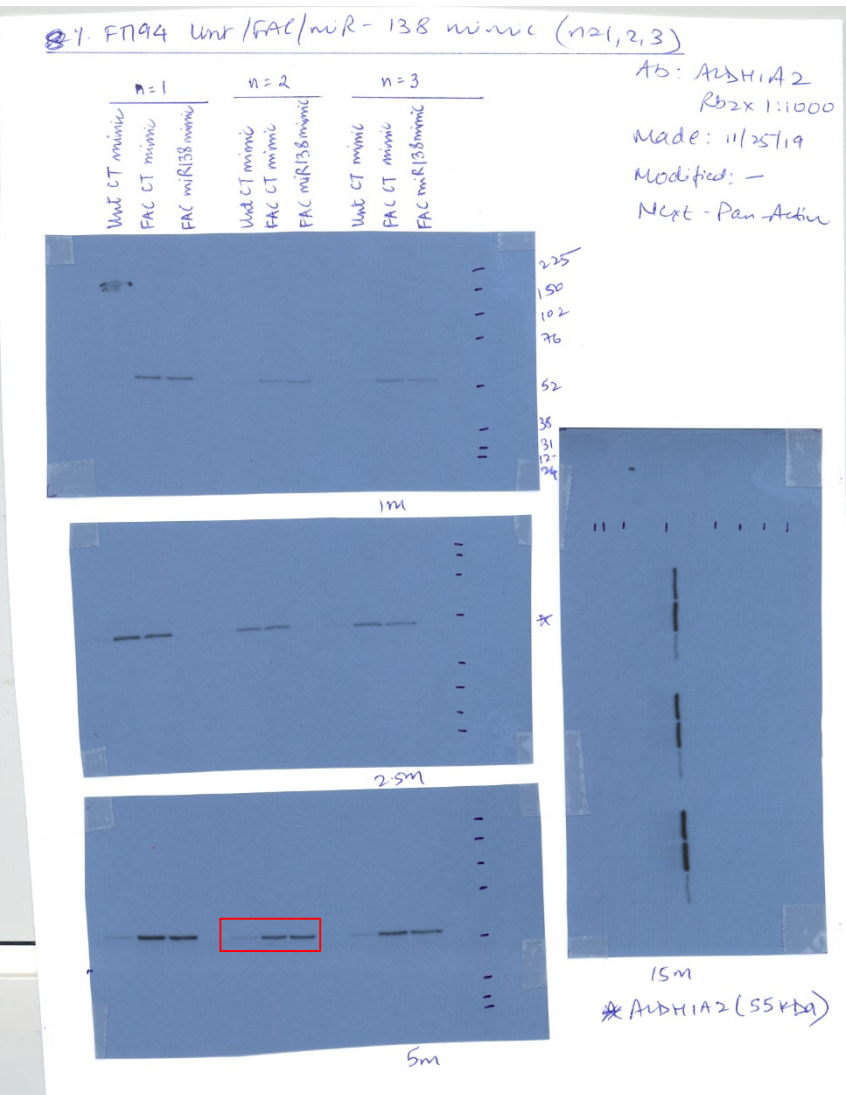


Figure 8B: Replicate #2

Pan-Actin

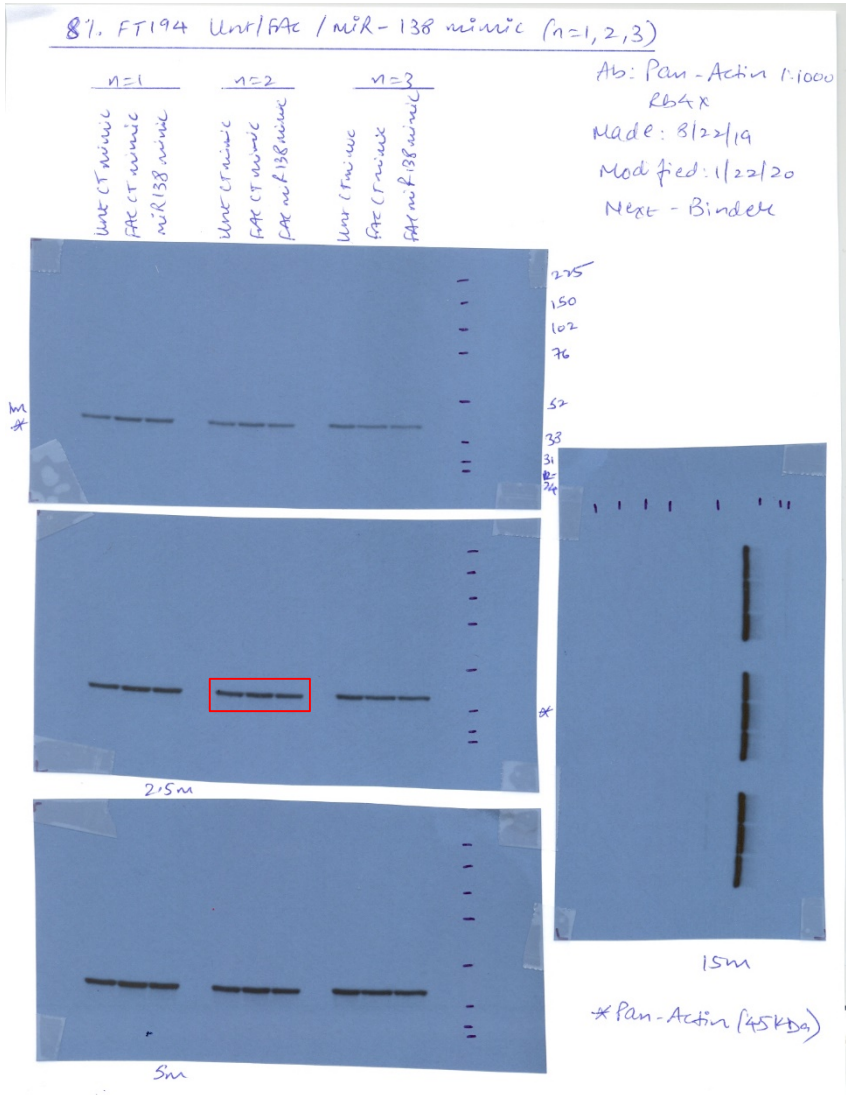


Figure 8C: Replicate #2

PAX8

Pan-Actin

10% FTH94: UNT & FTH94: FAC +/- miR-432 or miR-127 (Rep-1, 2-3)

10% FTH94: UNT & FTH94: FAC +/- miR-432 or miR-127 (Rep-1, 2, 3)

