Supplementary Figure Legends

Fig. S1: Total Protein Stains and Additional Blot for SBDP Following Mechanical Stretch Injury in Mixed-Sex WT Neurons. (A) Total protein stain of the PVDF membrane for densitometry as a loading control for sample set 1-3 on SBDPs (i.e., Fig. 1C). **(B)** Western shows SBDPs (n=3/group) for sample set 4-6. **(C)** Total protein stain for densitometry as a loading control for sample set 4-6 on SBDPs (Fig. S1B).

Fig. S2: **Additional Western Blots for Signaling Targets in Fig. 2. (A-F)** Western blots show target analysis results for sample set B on pERK, ERK total, pAKT, AKT Total, PERK, and GFAP (n=2/group). Results of sample set A and set B were combined to generate box plots in Fig. 2.

Fig. S3: Total Protein Stains for Western blots on Cell Signaling Targets in Fig. 2 Studies. (A-I) Total protein stains of PVDF membranes used for densitometry as a loading control in Fig. 2 and Fig. S2.

Fig. S4: Total Protein Stains and Additional SBDP Blot in Sex-Separated WT Neurons Subjected to Mechanical Stretch Injury. (A) Total protein stain of the PVDF membrane for densitometry as a loading control for sample set 1-4 on SBDPs (i.e., Fig. 3D). **(B)** Western shows SBDPs (n=4/group) for sample set 5-8. **(C)** Total protein stain for densitometry as a loading control for sample set 5-8 on SBDPs (Fig. S4B).

Fig. S5: Additional Western Blots for Signaling Targets in Fig. 4 and Fig. 5 on the Effect of RBM5 KO in Mechanical Stretch and OGD Injuries. (A) Western blots show the results of RBM5, SBDP, caspase-3, pAKt, AKT total, pCREB, and CREB total for sample set 5-8 (n=4/group) in the stretch injury model. Data were combined with results of sample set 1-4 in to generate graphs in Fig. 4. **(B)** Western blots show the results of RBM5, SBDP, caspase-3, pAKt,

AKT total, pCREB, and CREB total for sample set 5-8 (n=4/group) in the OGD model. Data were combined with results of sample set 1-4 to generate graphs in Fig. 5. The red asterisk indicates a single sample for caspse-3 in the stretch-injury group for which analysis was not possible due the lack of sample availability.

Fig. S6: Total Protein Stains for Cell Signaling Targets Measured in Fig. 4 and Fig. S5A in Stretch-Injury. (A-F) Total protein stain of PVDF membranes for densitometry as a protein loading control. Membranes correspond with cell signaling targets shown in Fig. 4D and in Fig. S5A.

Fig. S7: Total Protein Stains for Cell Signaling Targets Measured in Fig. 5 and Fig. S5B in OGD. (A-F) Total protein stain of PVDF membranes for densitometry as a protein loading control. Membranes correspond with cell signaling targets shown in Fig. 5E and in Fig. S5B.

Fig. S8: ERα Protein Levels in Sex-Separated EV-Control Versus RBM5 KO Neurons in the OGD Model. EV-control or CRE (RBM5 KO) neurons were pre-treated 3d with 1μM 17E or vehicle, injured with a 90min OGD, and returned to the incubator for an additional 24h with 1μM 17E or vehicle. **(A-B)** Western Blots for ERα and the corresponding total protein stains of PVDF membranes in male neurons. Panel A shows sample set 1-3, and panel B shows sample set 4-6. Total protein stain was collected after protein transfer and prior to incubation with a primary antibody. **(C-D)** Western Blots for ERα and the corresponding total protein stains of PVDF membranes in female neurons. Panel C shows sample set 1-3, and panel D shows sample set 4-6.

Fig. S9: GPR30 Protein Levels in Sex-Separated EV-Control Versus RBM5 KO Neurons and in the OGD Model. (A) Western blot for GPR30 and the corresponding total protein stain in uninjured male and female neurons of the control (EV) and KO (CRE) genotype. Sample set A (1-3) and set B (4-6) were run together on the same blot. No effect of KO was seen. **(B** and **C)** Western Blots for GPR30 and the corresponding total protein stains of PVDF membranes in male neurons in EV vs. KO, vehicle vs. 17E, uninjured and OGD-injured neurons. Panel B shows sample set 1-3, and panel C shows sample set 4-6. No effect KO or 17E was seen, but there was a robust downregulation of GPR30 at 24h post-injury.

Fig. S10: Total Protein Stains for Sample Set 1-3 on SBDP Blots in Fig. 6. (A) Total Protein stain of PVDF membrane corresponding to sample set 1-3 Western Blot on SBDPs in male neurons (Fig. 6D). **(B)** Total Protein stain of PVDF membrane corresponding to sample set 1-3 Western Blot on SBDPs in female neurons (Fig. 6E).

Fig. S11: Additional Blots on SBDP for Sample Set 4-6 in Support of Fig. 6 Studies. (A) Western blot on SBDPs in sample set 4-6 and the corresponding total protein stain of PVDF membrane for densitometry as a loading control in male neurons. **(B)** Western blot on SBDPs in sample set 4-6 and the corresponding total protein stain of PVDF membrane for densitometry as a loading control in female neurons.

Fig. S12: Volcano Plot on Differentially Expressed Genes Comparing Male vs. Female Uninjured EV-Control Neurons. (A) Volcano plot shows the fold-change and p-value of the proteins identified in TMT analysis comparing uninjured EV male neurons versus uninjured EV female neurons. **(B)** Heat map of the 4 DE proteins.

Fig. S13: Culture Purity (Sex Dichotomization) and Insult Severity in Cultures for TMT Analysis. (A) All 3 independent floxed cortical neuron cultures for TMT Analysis were confirmed by SRY genotyping of embryos to be 100% male or 100% female. **(B)** Box plots show 24h post-injury raw LDH values (OD450). There were no differences in insult severity comparing male vs. female EV injured neurons or male vs. female KO injured neurons.

Fig. S14: Uncropped Blots from Fig. 2. Images show the complete set of uncropped western blots to support Fig 2 studies.

Fig. S15: Uncropped Blots from Fig. 4 and Fig. 5. Images show the complete set of uncropped western blots used to support Fig. 4 and Fig. 5 studies. Some blots were physically cut in half after transfer to the PVDF membrane to permit the analysis of multiple targets at one time. This was necessary to conserve sample, which can only be obtained in limited quantities from 6-well plates.

Fig. S16: Uncropped Blots from Fig. 6. Images show the complete set of uncropped western blots to support Fig 6 studies.

Fig. S17: Validation of Lentivirus Dose (Multiplicity of Infection) in Floxed Neurons for KO Studies. **(Top)** Western blot shows RBM5 expression DIV6 cortical neurons in EV transduced vs. CRE transduced floxed neurons at 30, 60, and 100 MOI (n=3/group). **(Middle)** Western blot shows CRE Recombinase expression in EV transduced vs. CRE transduced floxed neurons at 30, 60, and 100 MOI (n=3/group). **(Bottom)** Representative total protein stain. 100 MOI was selected as the optimal dose due to maximal inhibition of RBM5 expression and confirmation of CRE expression. RBM5 expression was not inhibited by the EV control vector.

Supplementary Figures

Fig. S1

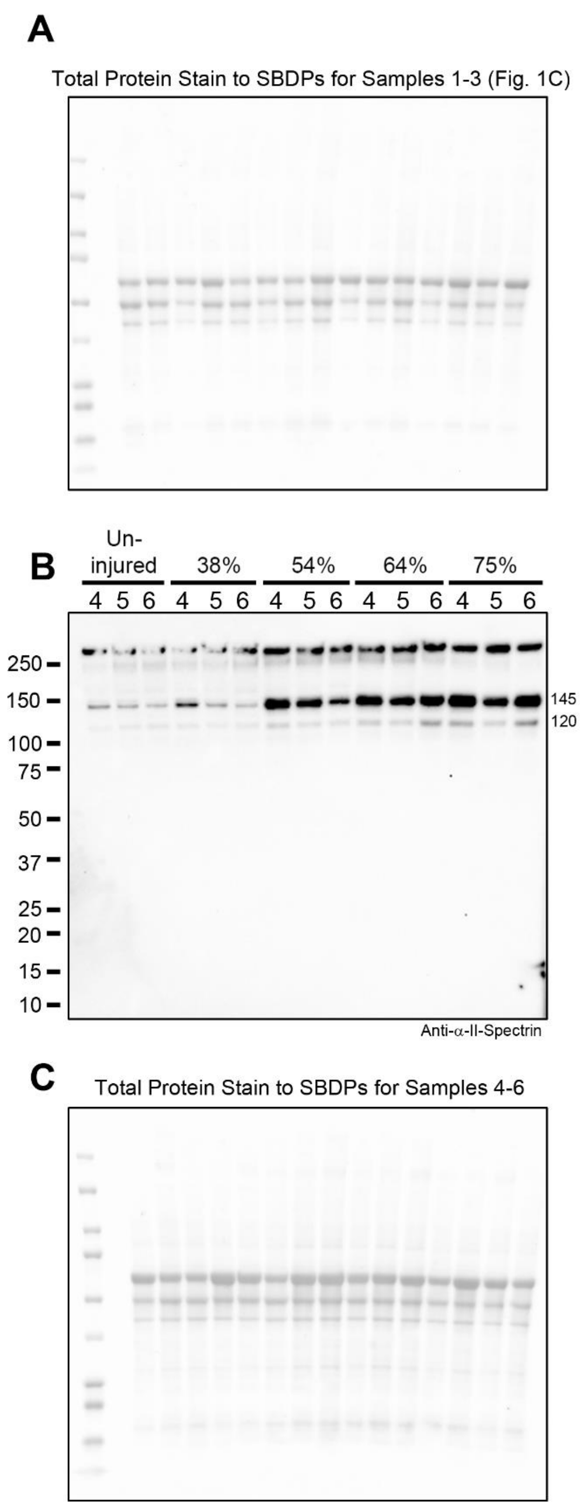


Fig. S2

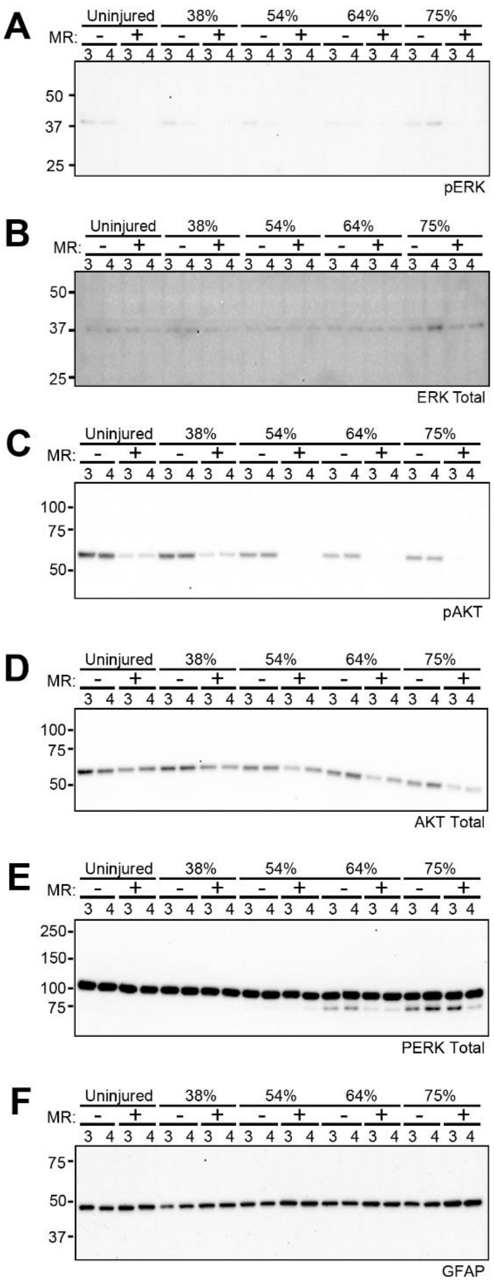

A
Uninjured 38% 54% 64% 75%
MR: - + - + - + - + - +
3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4
50
37
25
pERK
B
ERK Total
C
100
75
50
pAKT
D
AKT Total
E
250
150
100
75
PERK Total
F
75
50
37
GFAP

Fig. S3

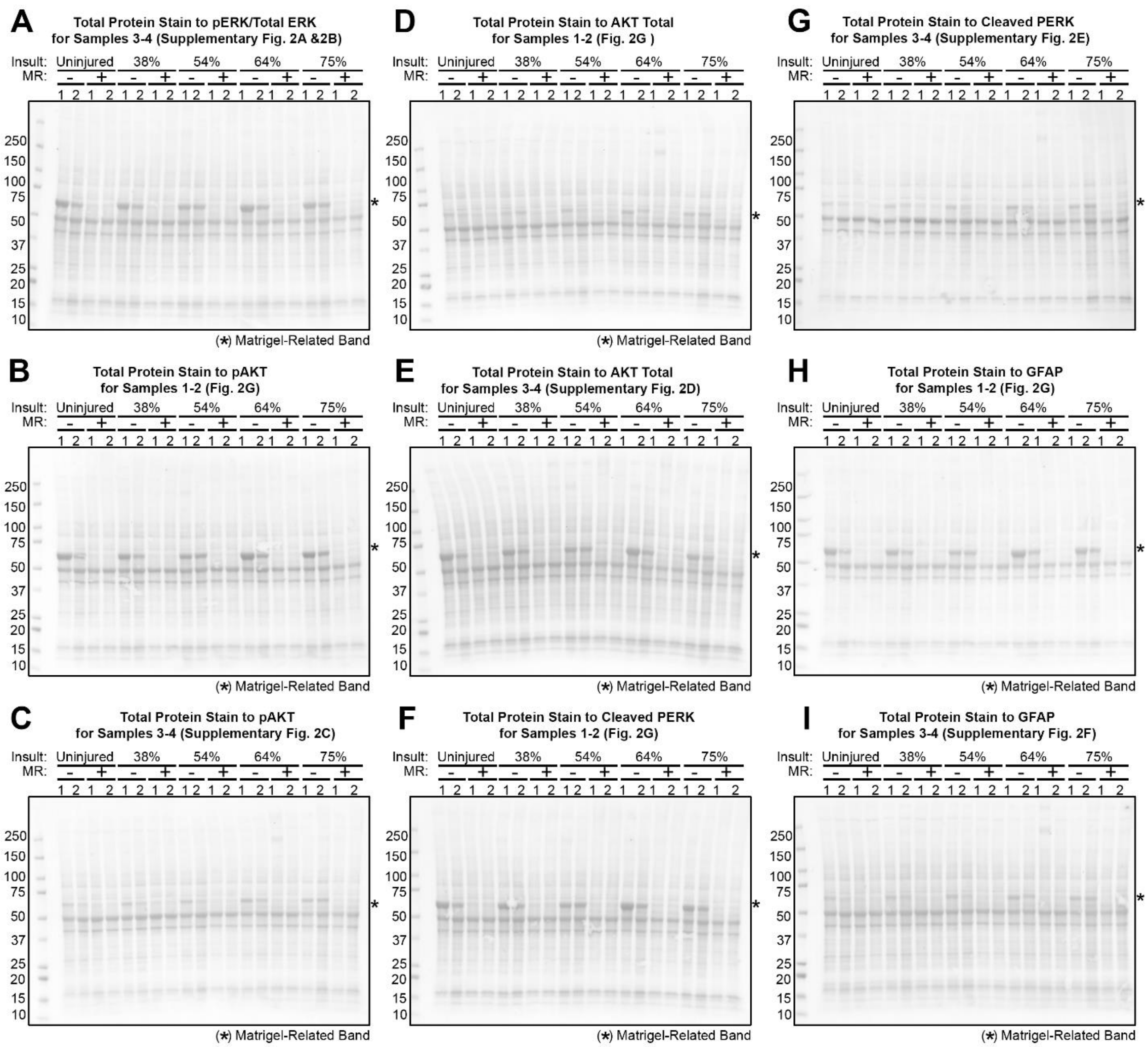
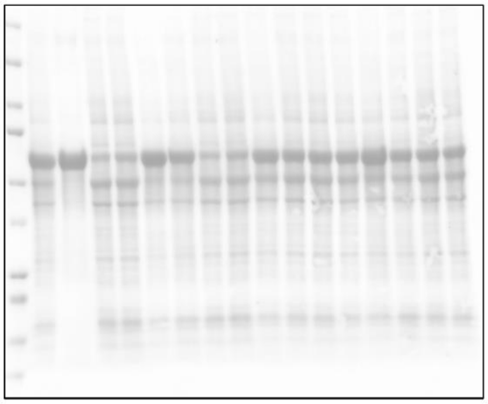

A
Total Protein Stain to pERK/Total ERK
for Samples 3-4 (Supplementary Fig. 2A &2B)
D
Total Protein Stain to AKT Total
for Samples 1-2 (Fig. 2G)
G
Total Protein Stain to Cleaved PERK
for Samples 3-4 (Supplementary Fig. 2E)
B
Total Protein Stain to pAKT
for Samples 1-2 (Fig. 2G)
E
Total Protein Stain to AKT Total
for Samples 3-4 (Supplementary Fig. 2D)
H
Total Protein Stain to GFAP
for Samples 1-2 (Fig. 2G)
C
Total Protein Stain to pAKT
for Samples 3-4 (Supplementary Fig. 2C)
F
Total Protein Stain to Cleaved PERK
for Samples 1-2 (Fig. 2G)
I
Total Protein Stain to GFAP
for Samples 3-4 (Supplementary Fig. 2F)
Insult: Uninjured 38% 54% 64% 75%
MR: - + - + - + - + - +
250
150
100
75
50
37
25
20
15
10
*
(*) Matrigel-Related Band

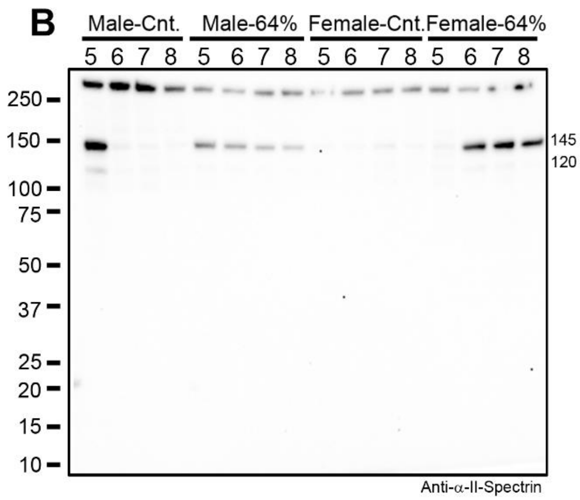
Fig. S4

A

Total Protein Stain to SBDPs for Samples 1-4 (Fig. 3D)



B



C

Total Protein Stain to SBDPs for Samples 5-8

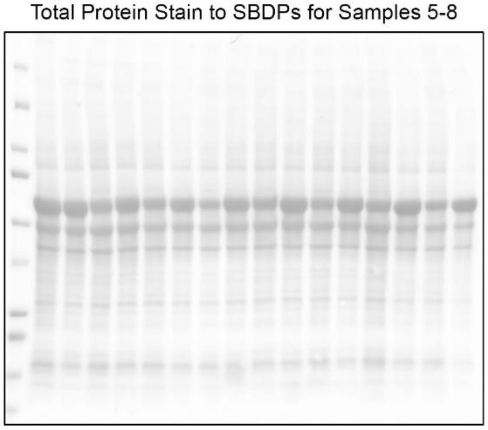
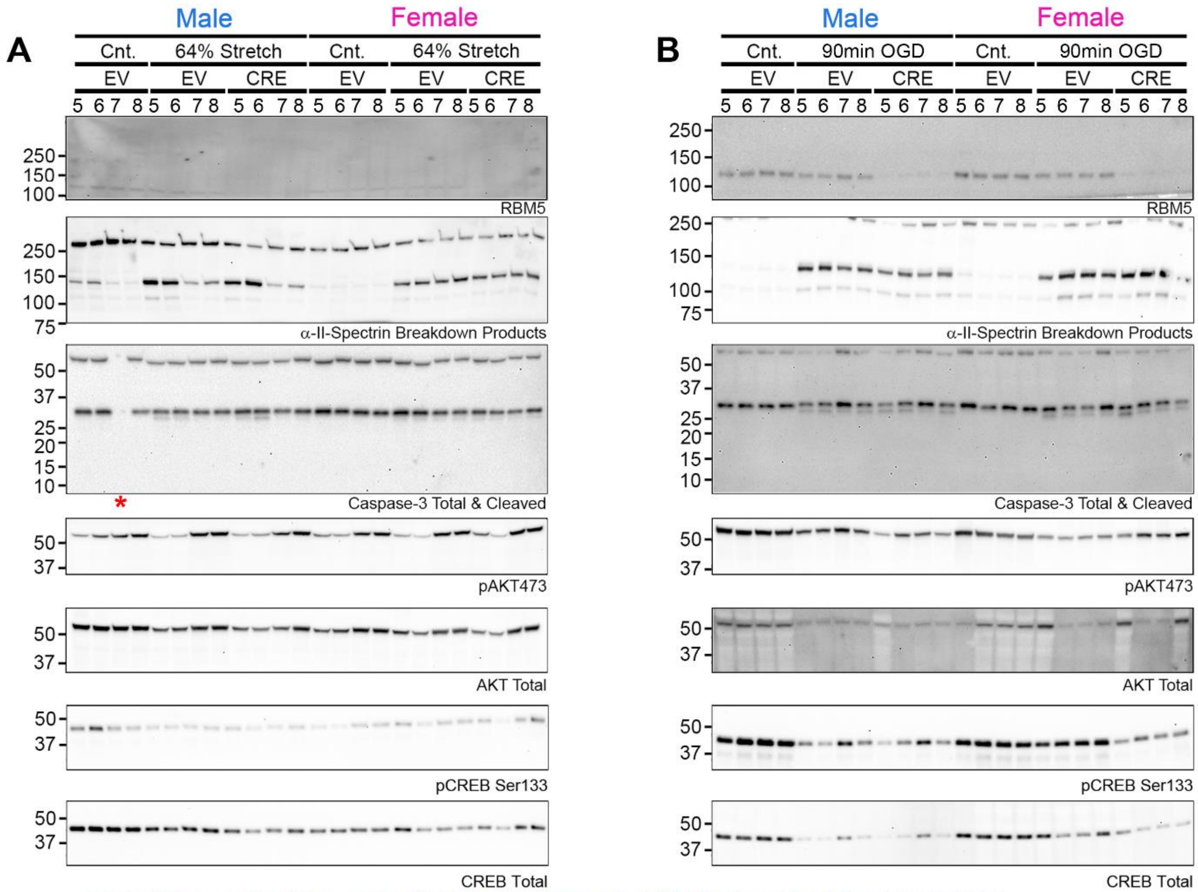


Fig. S5



* = Empty Lane for Caspase-3-Total Sample **Male-Cnt.-EV-#7** (Due to Sample Unavailability)

Fig. S6

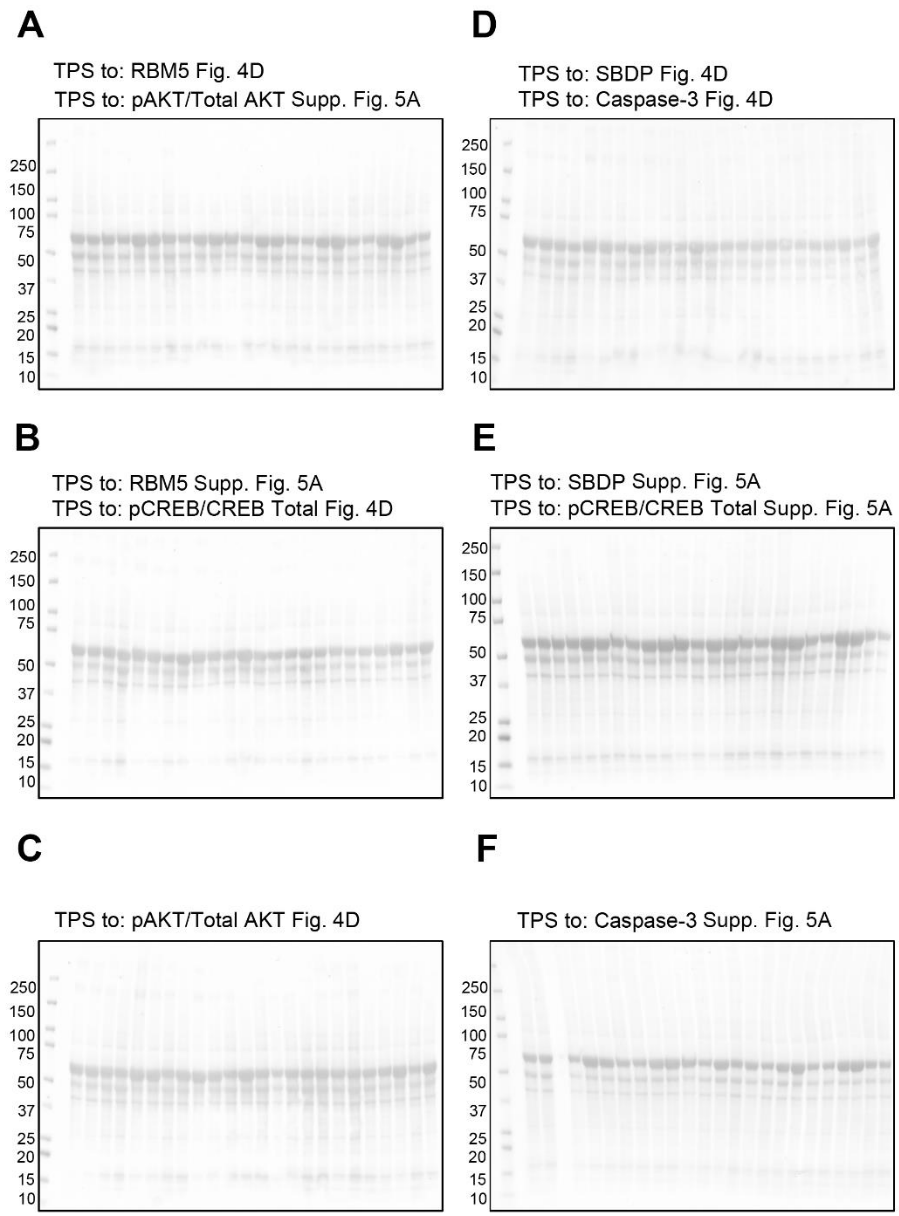

A
TPS to: RBM5 Fig. 4D
TPS to: pAKT/Total AKT Supp. Fig. 5A
250
150
100
75
50
37
25
20
15
10
D
TPS to: SBDP Fig. 4D
TPS to: Caspase-3 Fig. 4D
250
150
100
75
50
37
25
20
15
10
B
TPS to: RBM5 Supp. Fig. 5A
TPS to: pCREB/CREB Total Fig. 4D
250
150
100
75
50
37
25
20
15
10
E
TPS to: SBDP Supp. Fig. 5A
TPS to: pCREB/CREB Total Supp. Fig. 5A
250
150
100
75
50
37
25
20
15
10
C
TPS to: pAKT/Total AKT Fig. 4D
250
150
100
75
50
37
25
20
15
10
F
TPS to: Caspase-3 Supp. Fig. 5A
250
150
100
75
50
37
25
20
15
10

Fig. S7

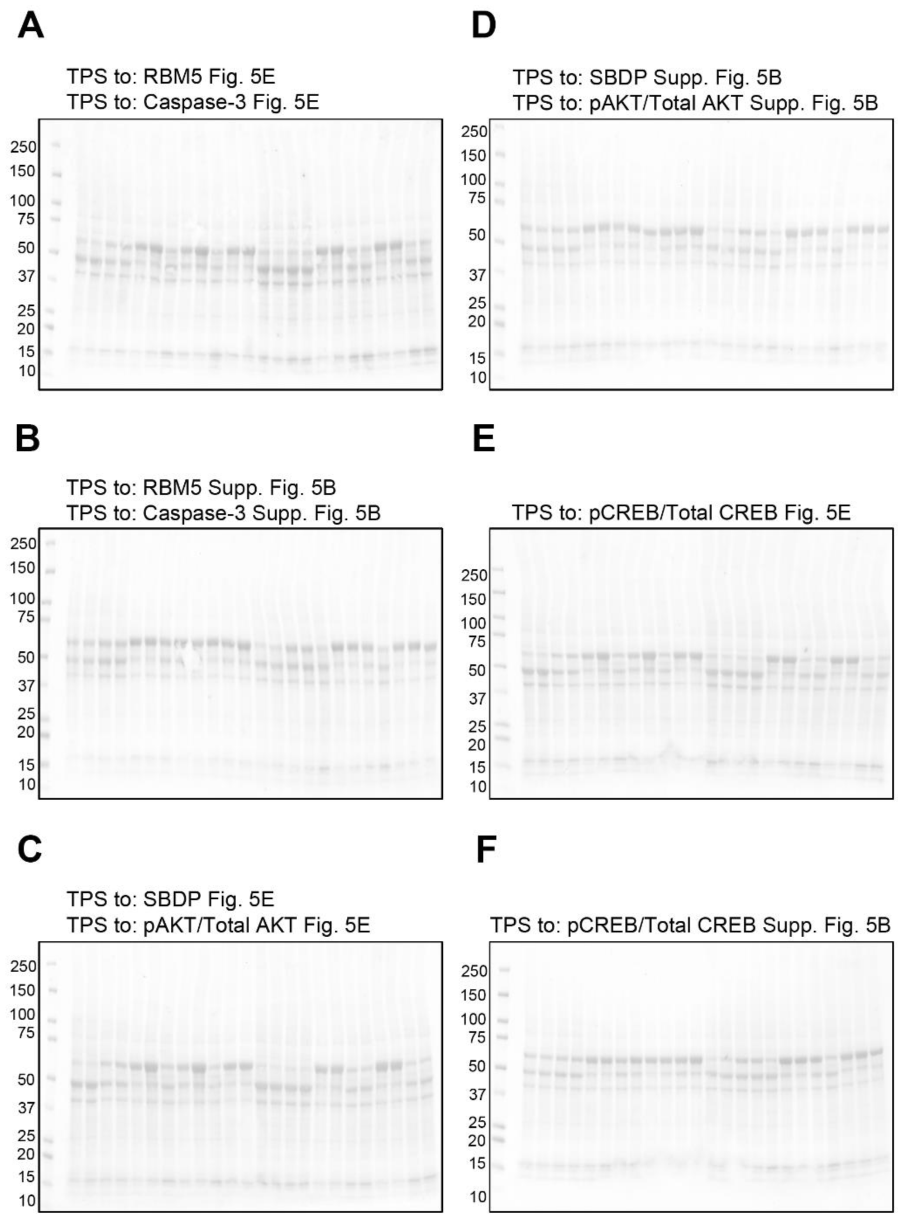

A
TPS to: RBM5 Fig. 5E
TPS to: Caspase-3 Fig. 5E
D
TPS to: SBDP Supp. Fig. 5B
TPS to: pAKT/Total AKT Supp. Fig. 5B
B
TPS to: RBM5 Supp. Fig. 5B
TPS to: Caspase-3 Supp. Fig. 5B
E
TPS to: pCREB/Total CREB Fig. 5E
C
TPS to: SBDP Fig. 5E
TPS to: pAKT/Total AKT Fig. 5E
F
TPS to: pCREB/Total CREB Supp. Fig. 5B
250
150
100
75
50
37
25
20
15
10

Fig. S8

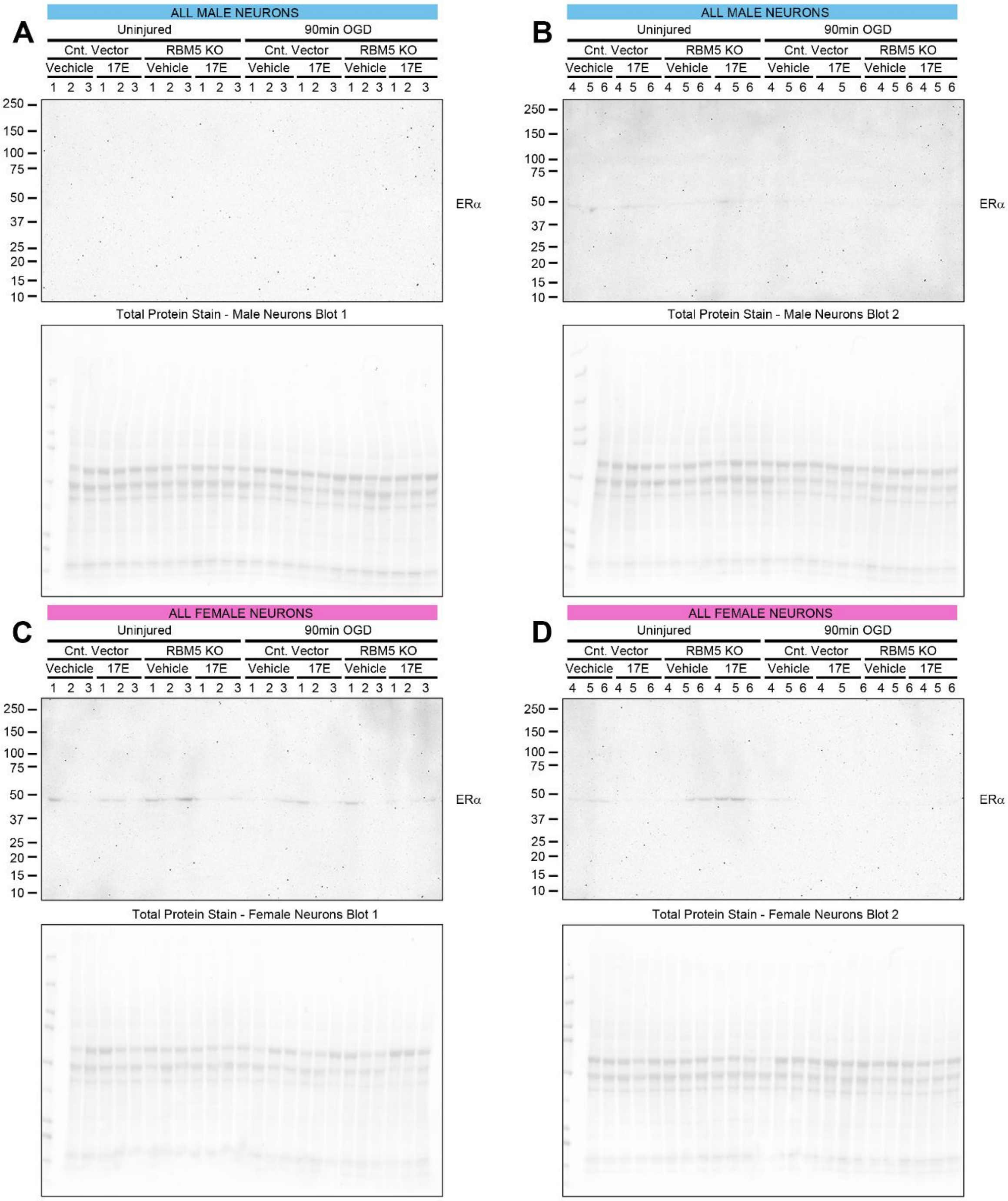

A
ALL MALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
250
150
100
75
50
37
25
20
15
10
ERα
Total Protein Stain - Male Neurons Blot 1
B
ALL MALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6
250
150
100
75
50
37
25
20
15
10
ERα
Total Protein Stain - Male Neurons Blot 2
C
ALL FEMALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
250
150
100
75
50
37
25
20
15
10
ERα
Total Protein Stain - Female Neurons Blot 1
D
ALL FEMALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6
250
150
100
75
50
37
25
20
15
10
ERα
Total Protein Stain - Female Neurons Blot 2

Fig. S9

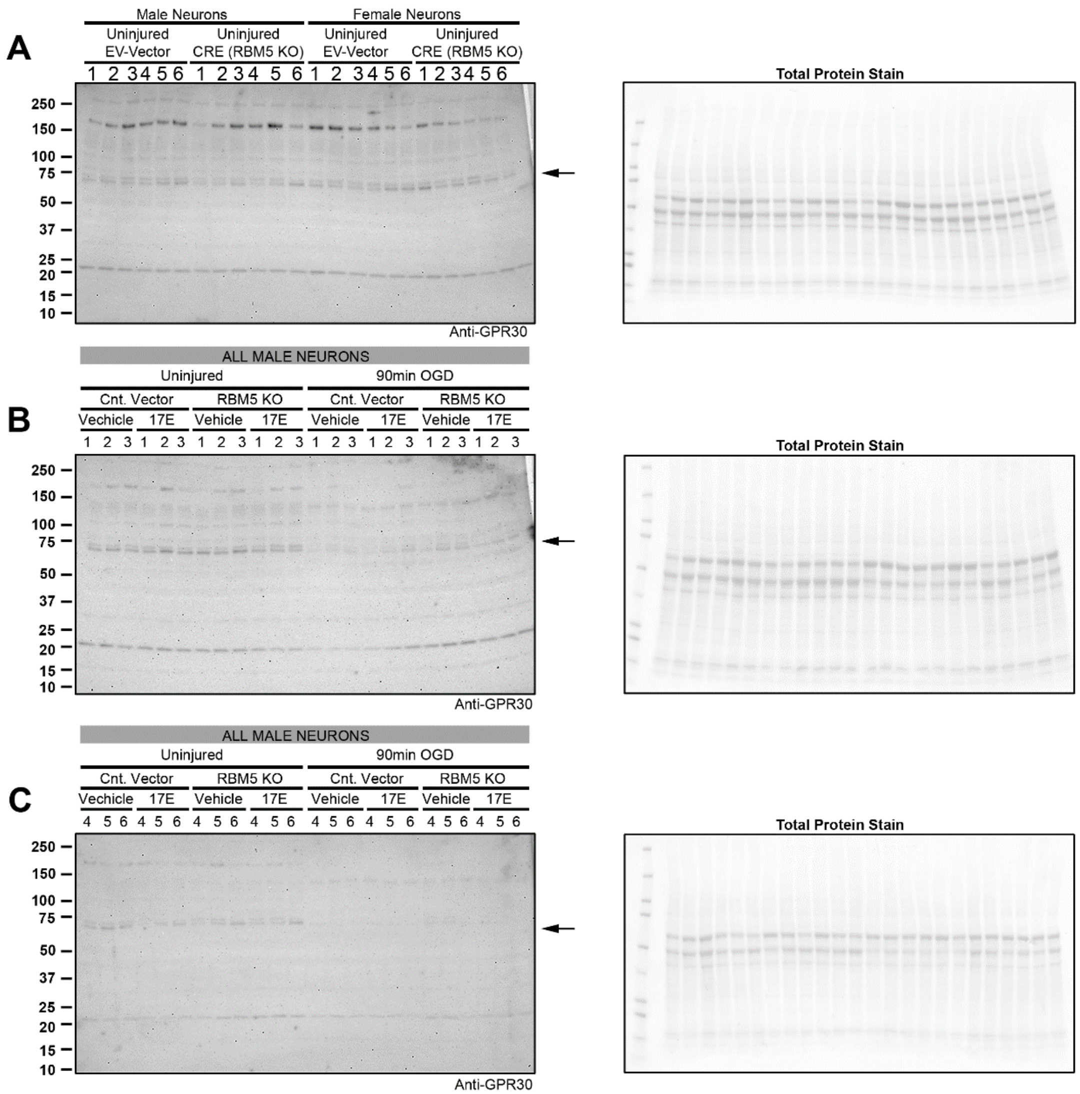
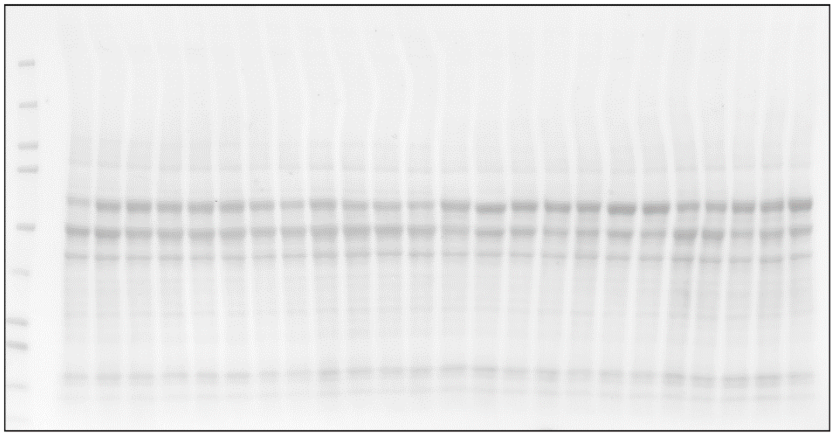

A
Male Neurons
Female Neurons
Uninjured EV-Vector
Uninjured CRE (RBM5 KO)
Uninjured EV-Vector
Uninjured CRE (RBM5 KO)
250
150
100
75
50
37
25
20
15
10
Anti-GPR30
Total Protein Stain
B
ALL MALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
Anti-GPR30
Total Protein Stain
C
ALL MALE NEURONS
Uninjured
90min OGD
Cnt. Vector
RBM5 KO
Cnt. Vector
RBM5 KO
Vechicle
17E
Vehicle
17E
Vehicle
17E
Vehicle
17E
Anti-GPR30
Total Protein Stain

Fig. S10

A

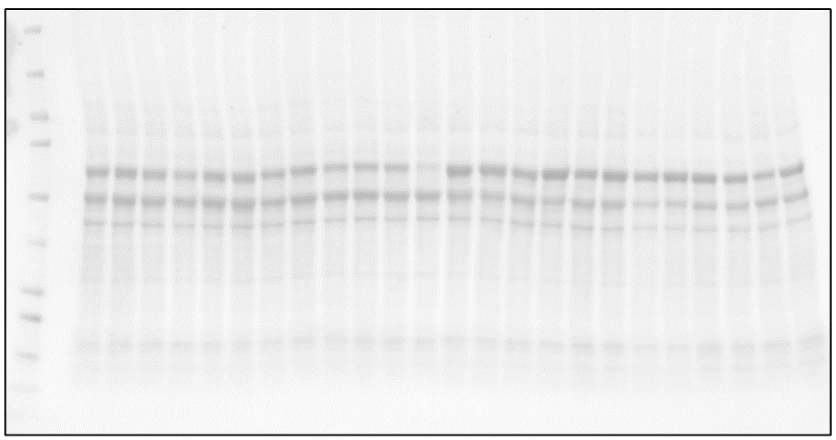
Total Protein Stain to SBDP Blot in Fig 6D



(All Male Samples)

B

Total Protein Stain to SBDP Blot in Fig. 6E

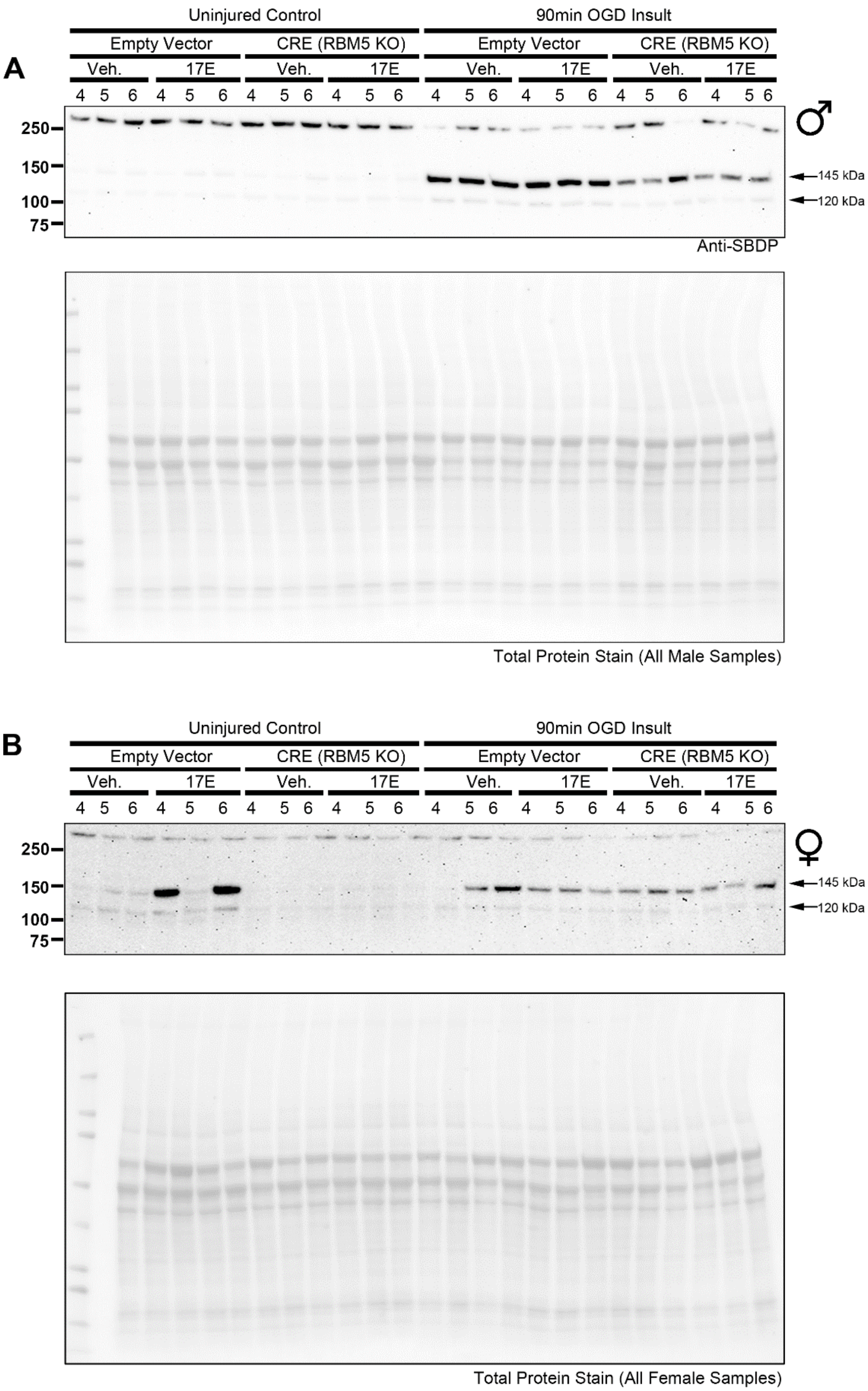


(All Female Samples)

Fig. S11

A

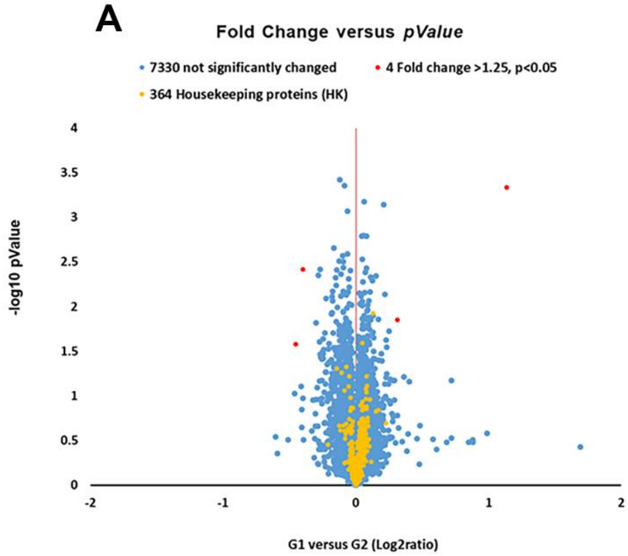
B



Uninjured Control
90min OGD Insult
Empty Vector
CRE (RBM5 KO)
Empty Vector
CRE (RBM5 KO)
Veh.
17E
Veh.
17E
Veh.
17E
Veh.
17E
4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6
250
150
100
75
♂
145 kDa
120 kDa
Anti-SBDP
Total Protein Stain (All Male Samples)
Uninjured Control
90min OGD Insult
Empty Vector
CRE (RBM5 KO)
Empty Vector
CRE (RBM5 KO)
Veh.
17E
Veh.
17E
Veh.
17E
Veh.
17E
4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6 4 5 6
250
150
100
75
♀
145 kDa
120 kDa
Total Protein Stain (All Female Samples)

Fig. S12

A


Fold Change versus pValue
7330 not significantly changed
4 Fold change >1.25, p<0.05
364 Housekeeping proteins (HK)
-log10 pValue
4
3.5
3
2.5
2
1.5
1
0.5
0
-2
-1
0
1
2
G1 versus G2 (Log2ratio)

B

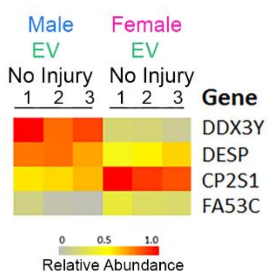

Male
EV
No Injury
1 2 3
Female
EV
No Injury
1 2 3
Gene
DDX3Y
DESP
CP2S1
FA53C
0
0.5
1.0
Relative Abundance

Fig. S13

TMT Analysis Neuron Cultures

A

SRY-Confirmed

Isolation 1:
Male Purity 100%
Female Purity 100%
Isolation 2:
Male Purity 100%
Female Purity 100%
Isolation 3:
Male Purity 100%
Female Purity 100%

B

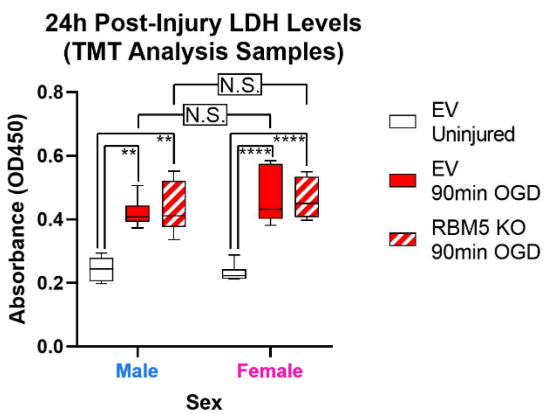


Fig. S14

Uncropped Blots (Fig. 2)

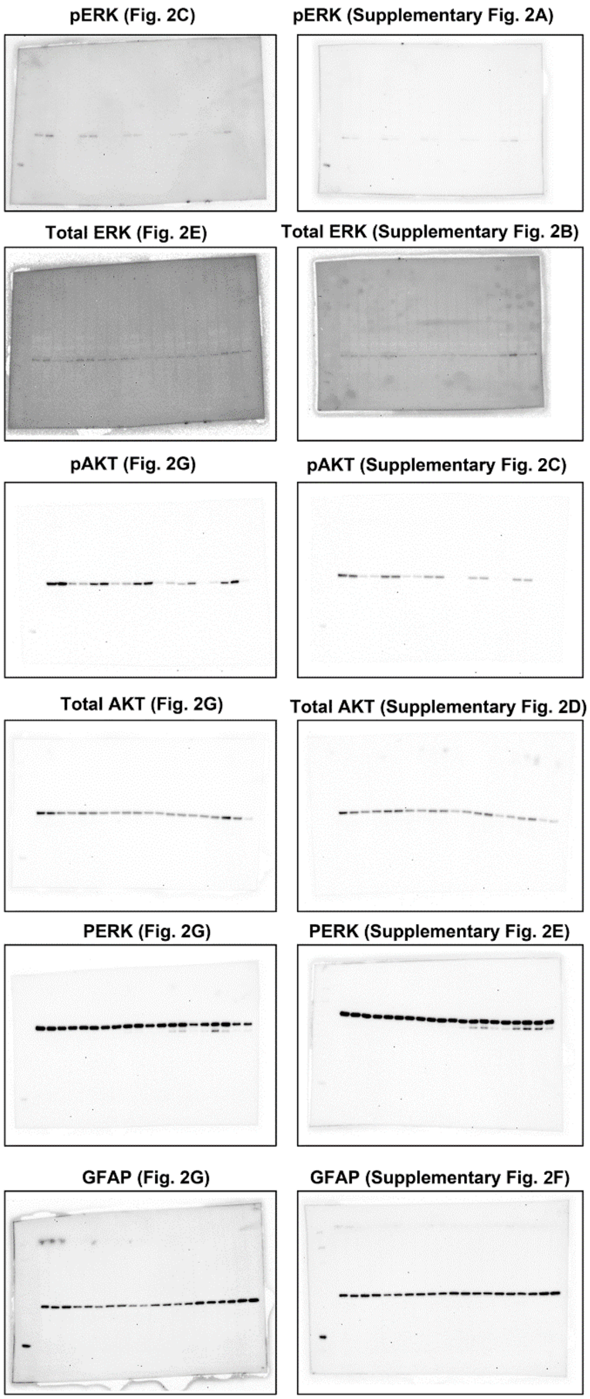
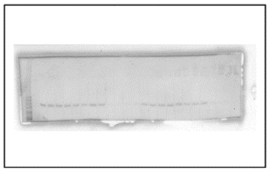


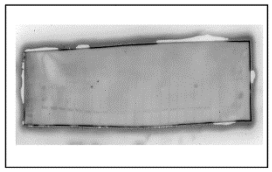
Fig. S15

Uncropped Blots (Fig. 4 and Fig. 5)

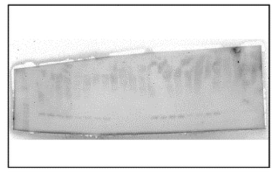
RBM5 (Fig. 4D)



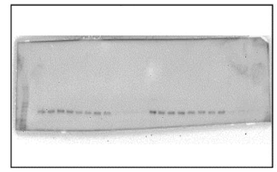
RBM5 (Supplementary Fig. 5A)



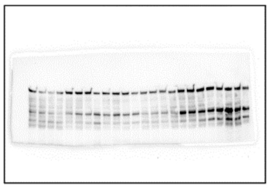
RBM5 (Fig. 5E)



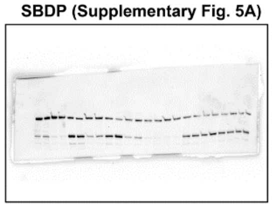
RBM5 (Supplementary Fig. 5B)



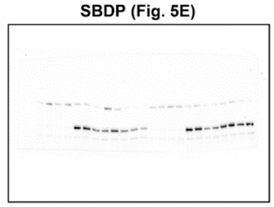
SBDP (Fig. 4D)



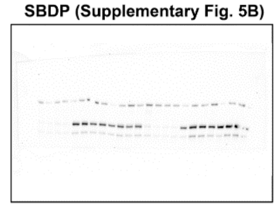
SBDP (Supplementary Fig. 5A)



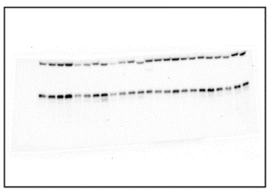
SBDP (Fig. 5E)



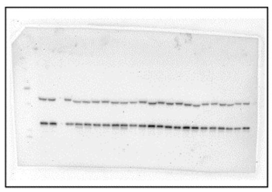
SBDP (Supplementary Fig. 5B)



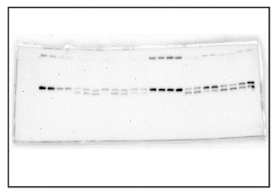
Caspase-3 (Fig. 4D)



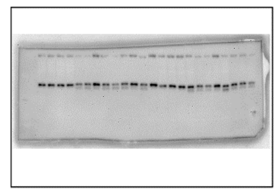
Caspase-3 (Supplementary Fig. 5A)



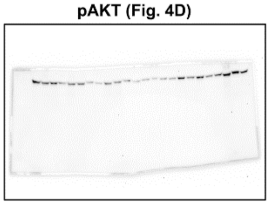
Caspase-3 (Fig. 5E)



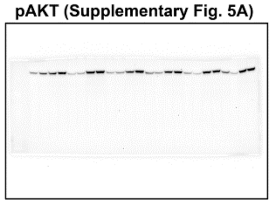
Caspase-3 (Supplementary Fig. 5B)



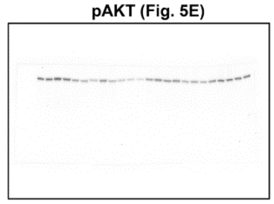
pAKT (Fig. 4D)



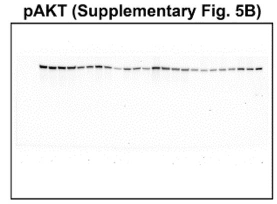
pAKT (Supplementary Fig. 5A)



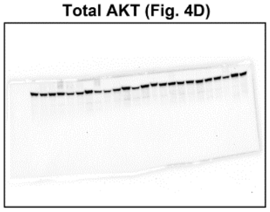
pAKT (Fig. 5E)



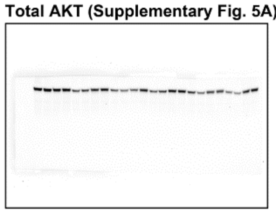
pAKT (Supplementary Fig. 5B)



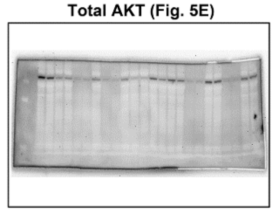
Total AKT (Fig. 4D)



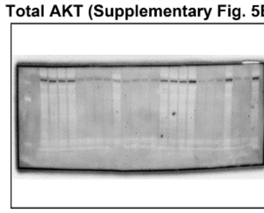
Total AKT (Supplementary Fig. 5A)



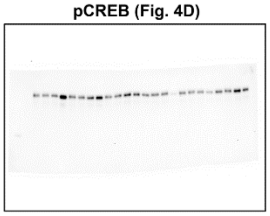
Total AKT (Fig. 5E)



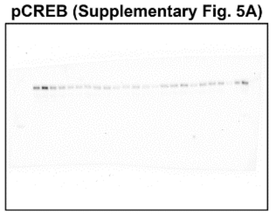
Total AKT (Supplementary Fig. 5B)



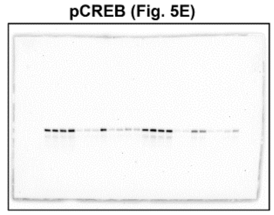
pCREB (Fig. 4D)



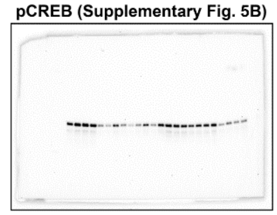
pCREB (Supplementary Fig. 5A)



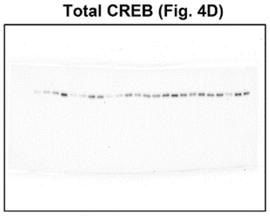
pCREB (Fig. 5E)



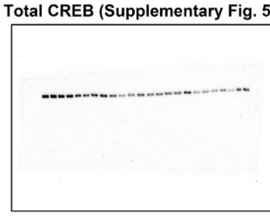
pCREB (Supplementary Fig. 5B)



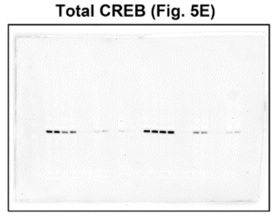
Total CREB (Fig. 4D)



Total CREB (Supplementary Fig. 5A)



Total CREB (Fig. 5E)



Total CREB (Supplementary Fig. 5B)

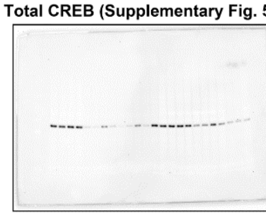


Fig. S16

Uncropped Blots (Fig. 6)

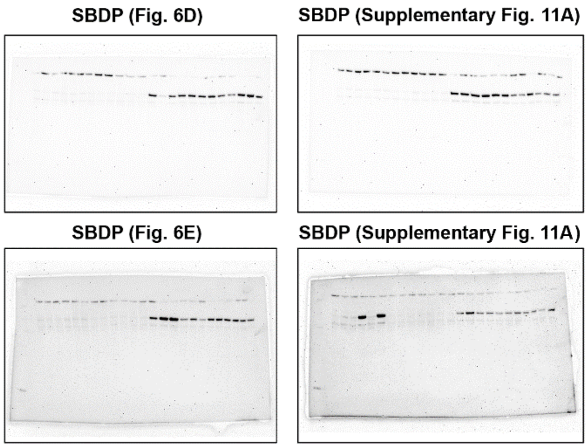
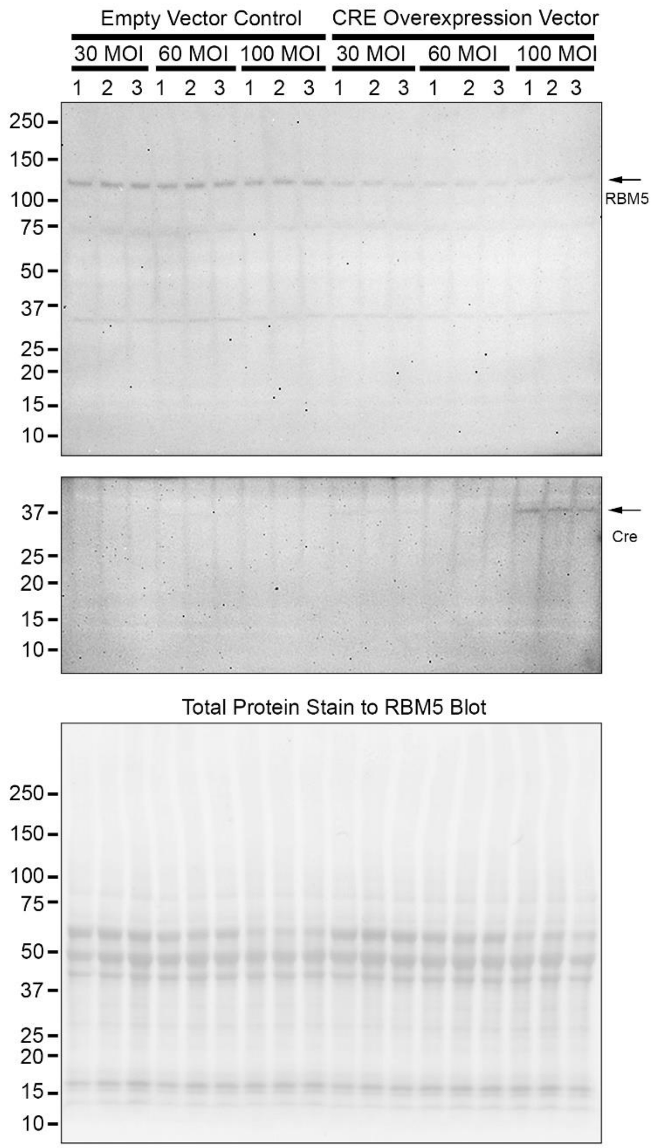


Fig. S17


Empty Vector Control
CRE Overexpression Vector
30 MOI
60 MOI
100 MOI
30 MOI
60 MOI
100 MOI
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
250
150
100
75
50
37
25
20
15
10
RBM5
37
25
20
15
10
Cre
Total Protein Stain to RBM5 Blot
250
150
100
75
50
37
25
20
15
10

Supplementary Tables 1-10

Statistical Tests & Outcomes

Table 1 Statistical Results: Matrigel Removal Protocol Protein Targets (2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Matrigel Removal	Factor 2: Insult Severity	Interaction Term
Fig. 2 Panel D	Phospho-ERK	NO **(p=0.0001)**	NO	YES	**p<0.0001**	**p=0.0002**	**p<0.0001**
Fig. 2 Panel F	Total ERK	YES (p=0.0510)	YES	NO	p=0.2162	**p=0.0002**	p=0.6792
Fig. 2 Panel H	Phospho-AKT	YES (p=0.1000)	YES	NO	**p<0.0001**	**p<0.0001**	**p<0.0001**
Fig. 2 Panel I	Total AKT	YES (p=0.1000)	YES	NO	**p<0.0001**	**p=0.0143**	p=0.2594
Fig. 2 Panel J	Cleaved PERK	NO **(p<0.0001)**	NO	YES	**p<0.0001**	**p<0.0001**	**p=0.0014**
Fig. 2 Panel K	Total GFAP	NO **(p=0.0475)**	NO	YES	**p=0.0018**	**p=0.0019**	p=0.3957

Table 2 Statistical Results: Mechanical Stretch WT Neurons Peak Pressure, LDH, & Protein Targets (Unpaired t-test and 2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Sex	Interaction Term
Fig. 3 Panel A	Well Peak Pressure (Injured)	NO **(p=0.0013)**	NO	YES	p=0.2331	**N/A**	**N/A**
Fig. 3 Panel C	LDH Levels	NO **(p=0.0149)**	NO	YES	**p<0.0001**	p=0.0924	p=0.7451
Fig. 3 Panel E	SBDP 145 Levels	NO **(p=0.0035)**	NO	YES	**p<0.0007**	p=0.1265	p=0.0804

Table 3 Statistical Results: Mechanical Stretch Floxed Neurons Peak Pressure & LDH (3-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Sex	Factor 3 Genotype
Fig. 4 Panel A	Well Peak Pressure	NO **(p<0.0001)**	NO	YES	**p<0.0001**	**p<0.0001**	p=0.0805
Fig. 4 Panel C	LDH Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	p=0.9112	p=0.2148
Cont'd	**Interaction Term 1 Injury x Sex**	**Interaction Term 2 Injury x Genotype**	**Interaction Term 3 Sex x Genotype**	**Interaction Term 4 Genotype x Sex x Injury**			
Fig. 4 Panel A	**p<0.0001**	p=0.0805	**p=0.0080**	**p=0.0080**			
Fig. 4 Panel C	p=0.9752	p=0.1525	p=0.4924	p=0.4338			

Table 4 Statistical Results: Mechanical Stretch Floxed Neurons Protein Targets (2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Vector/Injury	Factor 2: Sex	Interaction Term
Fig. 4 Panel E	RBM5 Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	**p=0.0004**	**p<0.0195**
Fig. 4 Panel F	SBDP 145 Levels	NO **(p=0.0128)**	NO	YES	**p<0.0001**	**p=0.0019**	**p=0.0015**
Fig. 4 Panel G	pAKT473 Levels	NO **(p=0.0072)**	NO	YES	p=0.2503	p=0.8461	p=0.1482
Fig. 4 Panel H	pCREB133 Levels	YES (p=0.1000)	YES	NO	p=0.2395	p=0.5732	**p=0.0456**

Table 5 Statistical Results: OGD LDH WT Neurons (2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Sex	Interaction Term
Fig. 5 Panel A	LDH Levels	NO **(p=0.0124)**	NO	YES	**p<0.0001**	p=0.1323	**p=0.0043**

Table 6 Statistical Results: OGD LDH RBM5 Floxed Neurons (3-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Sex	Factor 3 Genotype
Fig. 5 Panel C	LDH Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	**p=0.0170**	p=0.6416
Cont'd	**Interaction Term 1 Injury x Sex**	**Interaction Term 2 Injury x Genotype**	**Interaction Term 3 Sex x Genotype**	**Interaction Term 4 Genotype x Sex x Injury**			
Fig. 5 Panel C	p=0.8277	p=0.1087	p=0.2146	p=0.8339			

Table 7 Statistical Results: OGD Protein Targets Floxed Neurons (2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Vector/Injury	Factor 2: Sex	Interaction Term
Fig. 5 Panel F	RBM5 Levels	NO **(p=0.0017)**	NO	YES	**p<0.0001**	p=0.6510	**p=0.0013**
Fig. 5 Panel G	SBDP 145 Levels	NO **(p=0.0015)**	NO	YES	**p<0.0001**	**p=0.0004**	**p=0.0018**
Fig. 5 Panel H	SBDP 120 Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	**p=0.0078**	p=0.3917
Fig. 5 Panel I	Caspase 3 Fragment	NO **(p=0.0209)**	NO	YES	**p<0.0001**	**p=0.0135**	**p=0.0072**
Fig. 5 Panel J	pAKT473 Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	p=0.2200	**p<0.0001**
Fig. 5 Panel K	pCREB133 Levels	YES (p=0.1000)	YES	NO	**p<0.0001**	p=0.1194	p=0.1803

Table 8 Statistical Results: OGD Floxed Neurons & Estradiol Treatment LDH (3-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Drug	Factor 3 Genotype
Fig. 6 Panel A	LDH Levels	NO **(p<0.0209)**	NO	YES	**p<0.0001**	p=0.1700	p=0.1990
Fig. 6 Panel B	LDH Levels	NO **(p<0.0440)**	NO	YES	**p<0.0001**	**p=0.0090**	p=0.1297
Cont'd	**Interaction Term 1 Injury x Drug**	**Interaction Term 2 Injury x Genotype**	**Interaction Term 3 Drug x Genotype**	**Interaction Term 4 Genotype x Drug x Injury**			
Fig. 6 Panel A	p=0.2480	p=0.0568	p=0.9849	p=0.8944			
Fig. 6 Panel B	**p=0.0033**	p=0.0842	p=0.9534	p=0.4760			

Table 9 Statistical Results: OGD Floxed Neurons & Estradiol Treatment SBDP Targets (3-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Injury	Factor 2: Drug	Factor 3 Genotype
Fig. 6 Panel F	SBDP Levels 145kDa	NO **(p<0.0001)**	NO	YES	**p<0.0001**	**p=0.0537**	**p=0.0025**
Fig. 6 Panel G	SBDP Levels 145kDa	NO **(p=0.0029)**	NO	YES	**p<0.0001**	p=0.4425	p=0.5196
Fig. 6 Panel H	SBDP Levels 120kDa	YES (p=0.1000)	YES	NO	**p<0.0001**	p=0.0669	p=0.9995
Fig. 6 Panel I	SBDP Levels 120kDa	YES (p=0.1000)	YES	NO	p=0.3085	p=0.6086	p=0.1120
Cont'd	**Interaction Term 1 Injury x Drug**	**Interaction Term 2 Injury x Genotype**	**Interaction Term 3 Drug x Genotype**	**Interaction Term 4 Genotype x Drug x Injury**			
Fig. 6 Panel F	p=0.0593	**p=0.0029**	p=0.1900	p=0.1257			
Fig. 6 Panel G	p=0.0225	p=0.1762	p=0.2485	p=0.0190			
Fig. 6 Panel H	p=0.2414	p=0.6696	p=0.3096	p=0.2025			
Fig. 6 Panel I	p=0.3705	**p=0.0314**	**p=0.0372**	**p=0.0019**			

Table 10 Statistical Results: OGD Floxed Neurons LDH for TMT Study (2-WAY-ANOVA)

Figure Panel	Target	Passed Normality Test	Gaussian Distribution	Aligned Rank Transformation Of Data (ARTool)	Factor 1: Vector/Injury	Factor 2: Sex	Interaction Term
Supplementary Figure	LDH Levels	NO (p=0.0055)	NO	YES	**p<0.0001**	p=0.2547	p=0.4646

Supplementary Table 11

Embryo Number	Sex Determination Made Visually	Actual Sex (SRY Genotyping)	Incorrect Assignment (**X**)	Embryo Culture Isolation Date
1	Female	Female		4.2.21
2	Female	Female		
3	Male	Male		
4	Female	Female		
5	Male	Male		
6	Female	Female		
7	Male	Male		
8	Male	Male		
9	Male	Male		
10	Male	Male		
11	Female	Female		
12	Male	Female	**X**	
13	Male	Male		
14	Male	Male		
15	Male	Male		
16	Male	Male		
17	Female	Female		
18	Female	Female		
19	Female	Female		
20	Female	Female		
21	Female	Female		
22	Male	Male		
23	Male	Male		
24	Male	Male		
25	Male	Male		4.30.21
26	Female	Male	**X**	Out of Protocol
27	Male	Male		(Below 80%)
28	Male	Male		
29	Female	Female		
30	Male	Male		
31	Male	Male		
32	Male	Male		
33	Male	Male		
34	Male	Female	**X**	
35	Male	Female	**X**	
36	Female	Female		
37	Male	Female	**X**	
38	Female	Female		
39	Female	Female		
40	Male	Male		
41	Female	Female		5.3.21
42	Female	Male	**X**	

43	Male	Male		
44	Female	Female		
45	Male	Male		
46	Male	Male		
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55	Female	Female		
56	Male	Male		5.7.21
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61	Female	Female		
62	Female	Male	**X**	
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65	Male	Male		
66	Female	Female		
67	Female	Male	**X**	5.21.21
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71	Female	Female		
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74	Male	Male		
75	Female	Female		
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88	Female	Female		4.21.22

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391	Male	Male		
392	Male	Male		
393	Female	Female		
394	Male	Male		
395	Female	Female		
396	Female	Female		
397	Male	Male		
398	Male			12.7.22
399	Female			
400	Female			
401	Female			
402	Male			
403	Male			
404	Male			
405	Female			
406	Female			
407	Female			
408	Male			

409	Male			
410	Male			
411	Male			
412	Male			
413	Male			
414	Male			
415	Male			
416	Female			
417	Female			3.14.23
418	Female			**Male Dishes Lost**
419	Female			**(Technical Issue)**
420	Female			
421	Female			
422	Female			
423	Female			
424	Female			
425	Female			
426	Female			
427	Female			3.21.23
428	Female			
429	Male			
430	Female			
431	Female			
432	Female			
433	Female			
434	Male			
435	Male			
436	Male			
437	Male			
438	Male			
439	Female			
440	Female			
441	Male			
442	Male			
443	Female			
444	Female			
445	Male			
446	Male			
447	Male			
448	Female			
449	Male			
450	Male			
451	Female			
452	Female			
453	Male			
454	Male			

456	Male			
457	Female			
458	Female			
459	Male			
460	Female			
461	Female			
462	Male			
463	Female			
464	Male			
465	Female			
466	Female			
467	Male			

12 Embryos Incorrectly Assigned out of 467 Total Embryos = **97.4% Success Rate to Appropriately Assign Sex**