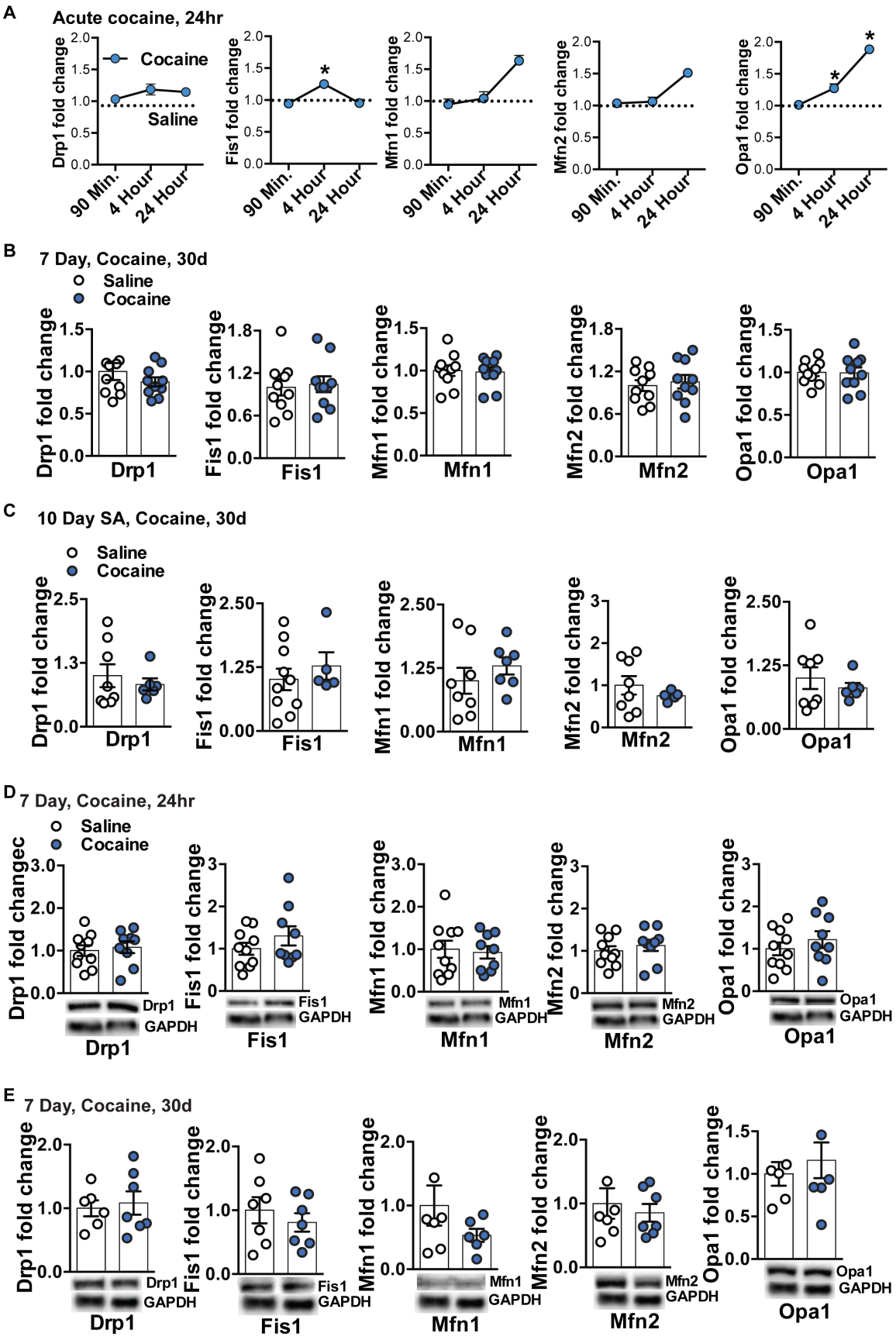
A
Acute cocaine, 24hr
Cocaine
Saline
Drp1 fold change
Fis1 fold change
Mfn1 fold change
Mfn2 fold change
Opa1 fold change
90 Min.
4 Hour
24 Hour
B
7 Day, Cocaine, 30d
Saline
Cocaine
Drp1
Fis1
Mfn1
Mfn2
Opa1
C
10 Day SA, Cocaine, 30d
D
7 Day, Cocaine, 24hr
Drp1 fold changec
GAPDH
E
7 Day, Cocaine, 30d

Figure S1. Related to Figure 1: Effect of short-term and long-term cocaine exposure on mitochondrial fission and fusion. (A) Drp1 mRNA levels are unaltered 90 min, 4 hours or 24 hours following an acute cocaine (20mg/kg, ip) injection: Student's t-test: t=0.657, df=7, p=0.53; t=1.742, df=8, p=0.11; t=1.317, df=9, p=0.22 respectively. Levels of Fis1 are increased 4 hours following an acute cocaine injection: Student's t-test: t=2.680, df=8, p=0.02. Mfn1 levels show no significant change following acute cocaine at any time point: Student's t-test: t=0.516, df=7, p=0.62; t=0.276, df=8, p=0.78; t=1.863, df=9, p=0.09 respectively. Similar pattern is observed for Mfn2: Student's t-test: t=0.845, df=7, p=0.42; t=0.692, df=8, p=0.50; t=1.187, df=9, p=0.26 respectively. Finally, Opa1 displayed increased mRNA levels 4 hours and 24 hours after acute cocaine: Student's t-test: t=2.584, df=8, p=0.03; t=2.630, df=9, p=0.02 respectively. Saline group: n=4, 5 and 5; cocaine group n=5, 5 and 6 respectively. **(B, C)** Mitochondrial fission and fusion mRNAs were unaltered in NAc 30 days after cocaine (7 days, 20mg/kg, ip) compared to saline controls. Student's t-test: t=1.069, df=18, p=0.29; t=0.267, df=18, p=0.79; t=0.178, df=18, p=0.86; t=0.448, df=18, p=0.65; t=0.077, df=18, p=0.93 respectively, n=10 in each group. Nor were they altered 30 days after cocaine self-administration (1mg/kg/infusion; n=7) compared to saline saline (n=8) for 10 days. Student's t-test: t=0.170, df=13, p=0.86; t=0.738, df=13, p=0.47; t=0.930, df=13, p=0.36; t=0.268, df=13, p=0.79; t=0.138, df=13, p=0.89 respectively. **(D, E)** Mitochondrial fission and fusion proteins were unaltered in NAc 24 hours (n=10 per group) or 30 days (n=7 per group) after cocaine (7 days, 20mg/kg, ip) compared to saline controls: Student's ttest (24 hours, in the order of the proteins displayed): t=1.035, df=18, p=0.31; t=1.575, df=18, p=0.13; t=0.232, df=18, p=0.81; t=1.273, df=18, p=0.21; t=1.331, df=18, p=0.19 respectively. Student's t-test (30 days, in the order of the proteins displayed): t=1.589, df=12, p=0.56; t=0.758, df=12, p=0.46; t=0.538, df=12, p=0.60; t=0.528, df=12, p=0.60; t=0.311, df=12, p=0.76. *p<0.05. Error bars, SEM.

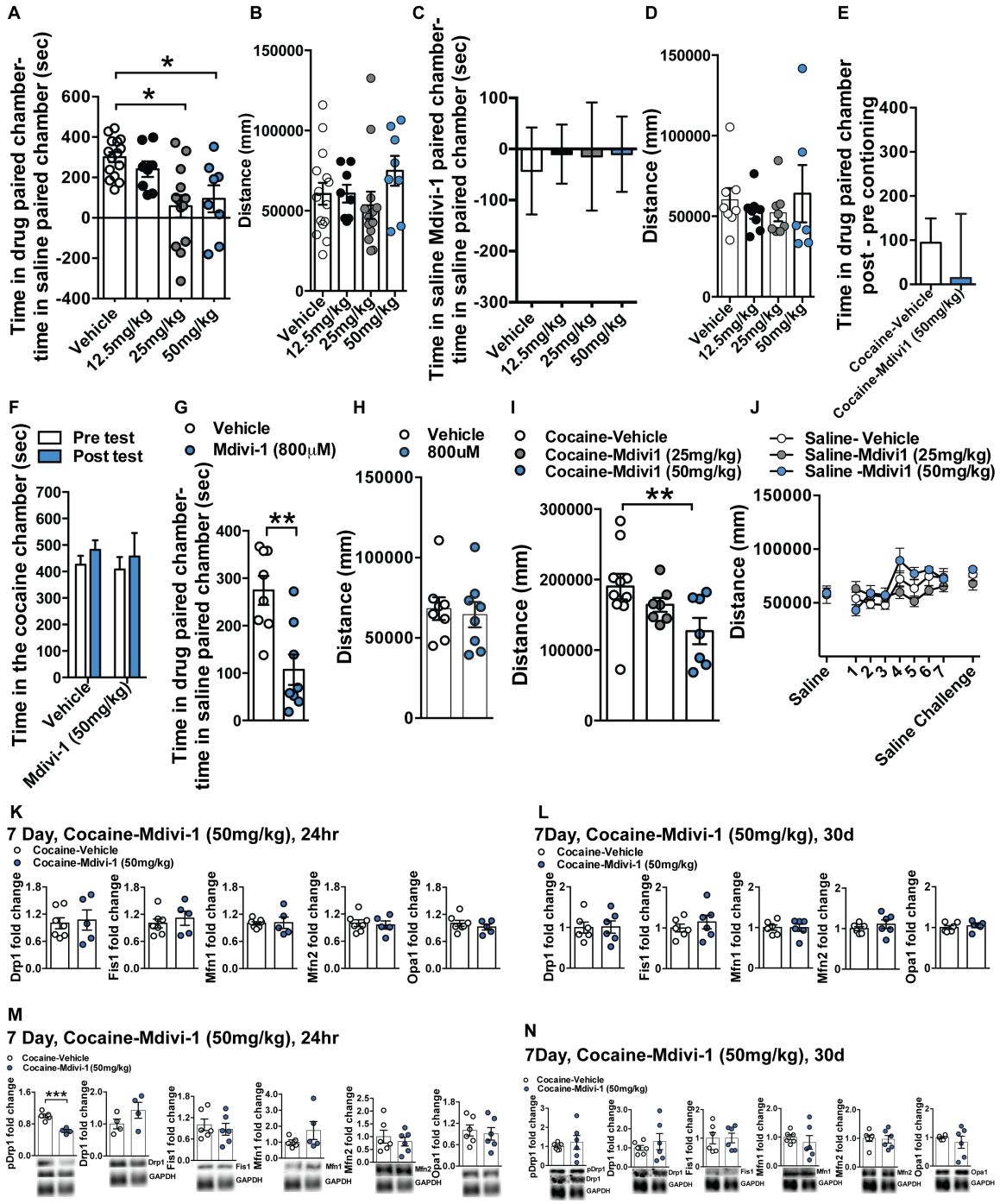

Figure S2. Related to Figure 2: Inhibiting mitochondrial fission with Mdivi-1 reduces cocaine behavioral outcomes without affecting overall locomotion. (A) 25mg/kg (n=13) and 50mg/kg (n=8) Mdivi-1 reduced cocaine (7.5mg/kg) conditioned place preference when compared to the vehicle-treated group (One-way ANOVA: $F(3,40)=4.278$, $p=0.0104$, Tukey post-hoc: $p<0.05$), while 12.5mg/kg (n=8) had no effect, compared to vehicle controls (n=15). **(B)** Mice did not exhibit differences in

locomotion during the probe test: One-way ANOVA: $F(3,40)=1.163$, $p=0.335$. **(C)** Control mice, pre-treated with Mdivi-1 (or vehicle) and receiving saline, did not display preference for any chamber: One-way ANOVA: $F(3,26)=0.036$, $p=0.99$, and **(D)** did not exhibit differences in locomotion during the probe test due to Mdivi-1 treatment: One-way ANOVA: $F(3,26)=0.415$, $p=0.743$; n=8, 8, 8 and 6 respectively. **(E, F)** Mdivi-1 (50mg/kg, ip) pre-treatment did not alter conditioned place preference for a low (4mg/kg, ip) dose of cocaine with or without Mdivi-1 treatment: Student's t-test: $t=0.513$, $df=10$, $p=0.6$ and Two-way Repeated Measure ANOVA: Time: $F(1,10)=1.018$, $p=0.336$, respectively; n=6 in each group. **(G)** NAc infusion of Mdivi-1 (800μM; 0.5 μL/side) reduced cocaine (7.5mg/kg) conditioned place preference when compared to vehicle-treated group. Student's t-test, $t=3.791$, $df=14$, $p=0.002$. n=8 in each group. **(H)** Mdivi-1 infusion (800μM/0.5μL/side) in the NAc did not alter the overall locomotion during the probe test: Student's t-test: $t=0.351$, $df=14$, $p=0.73$. **(I)** Contrary to vehicle treated or 25mg/kg dose, 50mg/kg Mdivi-1 (ip) pre-treatment reduced cocaine-induced locomotor sensitization when tested with a challenge cocaine injection (10mg/kg, ip) 7 days after withdrawal (detail from Figure 2D 'Challenge'): Two-way Repeated Measure ANOVA: Interaction: $F(16,176)=1.79$, $p=0.0356$, Tukey post-hoc: $p<0.01$. n=10, 7 and 7 respectively. **(J)** Mice pre-treated with Mdivi-1 (25mg/kg, ip, n=5) or (50mg/kg, ip, n=6) showed similar locomotion over 7 days of saline treatment as well as saline challenge after 7 days compared to vehicle treated mice (n=6): Two-way Repeated Measure ANOVA: Treatment: $F(2,14)=1.010$, $p=0.389$. **(K)** Mice pre-treated with Mdivi-1 (50mg/kg, ip) during 7 days of cocaine (20mg/kg, ip) injections regiment show no change in mRNA levels for our target genes 24h after the last injection: Student's t-test (in the order of the genes displayed): $t=0.29$, $df=10$, $p=0.77$; $t=0.65$, $df=10$, $p=0.52$; $t=0.10$, $df=10$, $p=0.91$; $t=0.34$, $df=10$, $p=0.73$; $t=0.86$, $df=10$, $p=0.40$. Cocaine-vehicle: n= 7, cocaine-Midivi: n=5. **(L)** Similar results were found when mRNA levels were assessed 30 days following the last injection: Student's t-test (in the order of the genes displayed): $t=0.06$, $df=10$, $p=0.94$; $t=0.76$, $df=10$, $p=0.46$; $t=0.04$, $df=10$, $p=0.96$; $t=0.94$, $df=10$, $p=0.36$; $t=0.61$, $df=10$, $p=0.54$. Cocaine-vehicle: n= 6, cocaine-Midivi: n=6. **(M)** Mdivi-1 (50mg/kg, ip) reduced phosphorylation of Drp1 induced by 7 days of cocaine injections (20mg/kg, ip) when measured 24h after the last injection:

Student's t-test: t=6.023, df=7, p=0.005. However, protein levels for all other targets were unchanged Student's t-test (in the order of the proteins displayed): t=0.09, df=7, p=0.92; t=0.54, df=10, p=0.59; t=0.26, df=9, p=0.79; t=0.62, df=10, p=0.54; t=0.40, df=10, p=0.69; vehicle group n=4-6, Mdivi-1 group n=4-6. **(N)** Protein measured 30 days after the last injection showed no change between both groups: Student's t-test (in the order of the proteins displayed): t=0.57, df=10, p=0.57; t=0.74, df=10, p=0.47; t=0.02, df=10, p=0.97; t=0.72, df=10, p=0.48; t=0.18, df=10, p=0.85; t=0.78, df=10, p=0.45; n=6 in each group. *p<0.05, **p<0.01, ***p<0.001. Error bars, SEM.

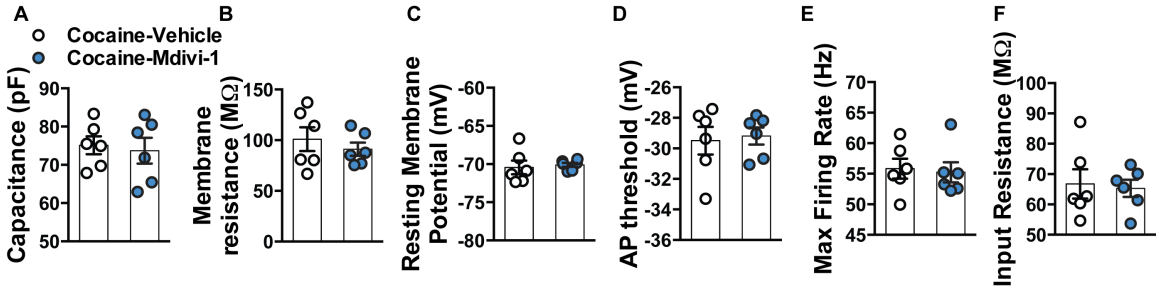

Figure S3. Related to Figure 3: Inhibiting mitochondrial fission with Mdivi-1 does not affect basic electrophysiological properties of D1-MSNs. (A) Mdivi-1 treatment (50mg/kg, ip) had no effect on general membrane properties of D1-MSNs including capacitance: Student's t-test: t=0.344, df=10, p=0.73, **(B)** membrane resistance: t=0.736, df=10, p=0.47, **(C)** resting membrane potential: t=0.362, df=10, p=0.72, **(D)** action potential threshold: t=0.285, df=10, p=0.78, **(E)** maximum firing rate: t=0.255, df=10, p=0.80, **(F)** or input resistance: t=0.262, df=10, p=0.79. Data were averaged by animal, n=6 mice in each group. Error bars, SEM.

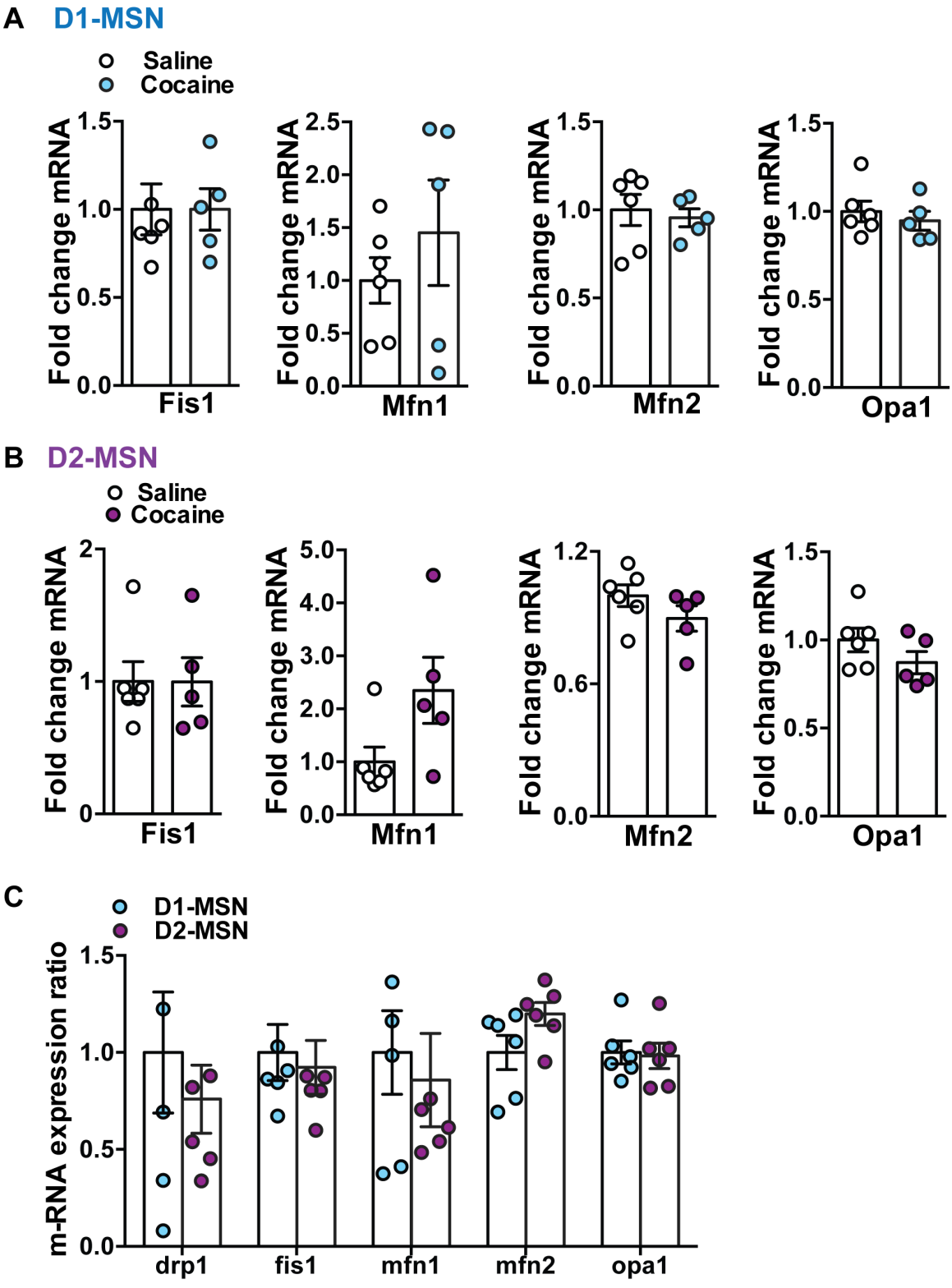

Figure S4. Related to Figure 4: Mitochondrial fission and fusion gene mRNAs are unaltered in MSN subtypes after repeated cocaine and are not differentially expressed in MSN subtypes. (A) Cell type specific mRNA of Fis1, Mfn1, Mfn2 and

Opa1 fold changes after repeated cocaine (20mg/kg, 7 days) in D1-Cre-RT (Student's t-test: t=0.001, df=9, p=0.99; t=0.887, df=9, p=0.39; t=0.414, df=9, p=0.68; t=0.65, df=9, p=0.53, in the order of the genes displayed) and **(B)** D2-Cre-RT mice (Student's t-test (in the order of the genes displayed): t=0.01, df=9, p=0.99; t=2.097, df=9, p=0.07; t=1.365, df=9, p=0.21; t=1.379, df=9, p=0.20). There was no change in mRNAs for these genes in MSN subtypes in the cocaine condition compared to saline controls. n=6 saline and 5 cocaine for both D1- and D2-Cre-RT mice. **(C)** Drp1, Fis1, Mfn1, Mfn2, and Opa1 mRNAs are not differentially expressed in NAc D1-MSNs vs. D2-MSNs: Student's t-test (in the order of the genes displayed): t=0.672, df=10, p=0.52; t=0.382, df=10, p=0.71; t=0.442, df=10, p=0.67; t=1.863, df=10, p=0.09; t=0.197, df=10, p=0.84. n=6 in each group. Error bars, SEM.

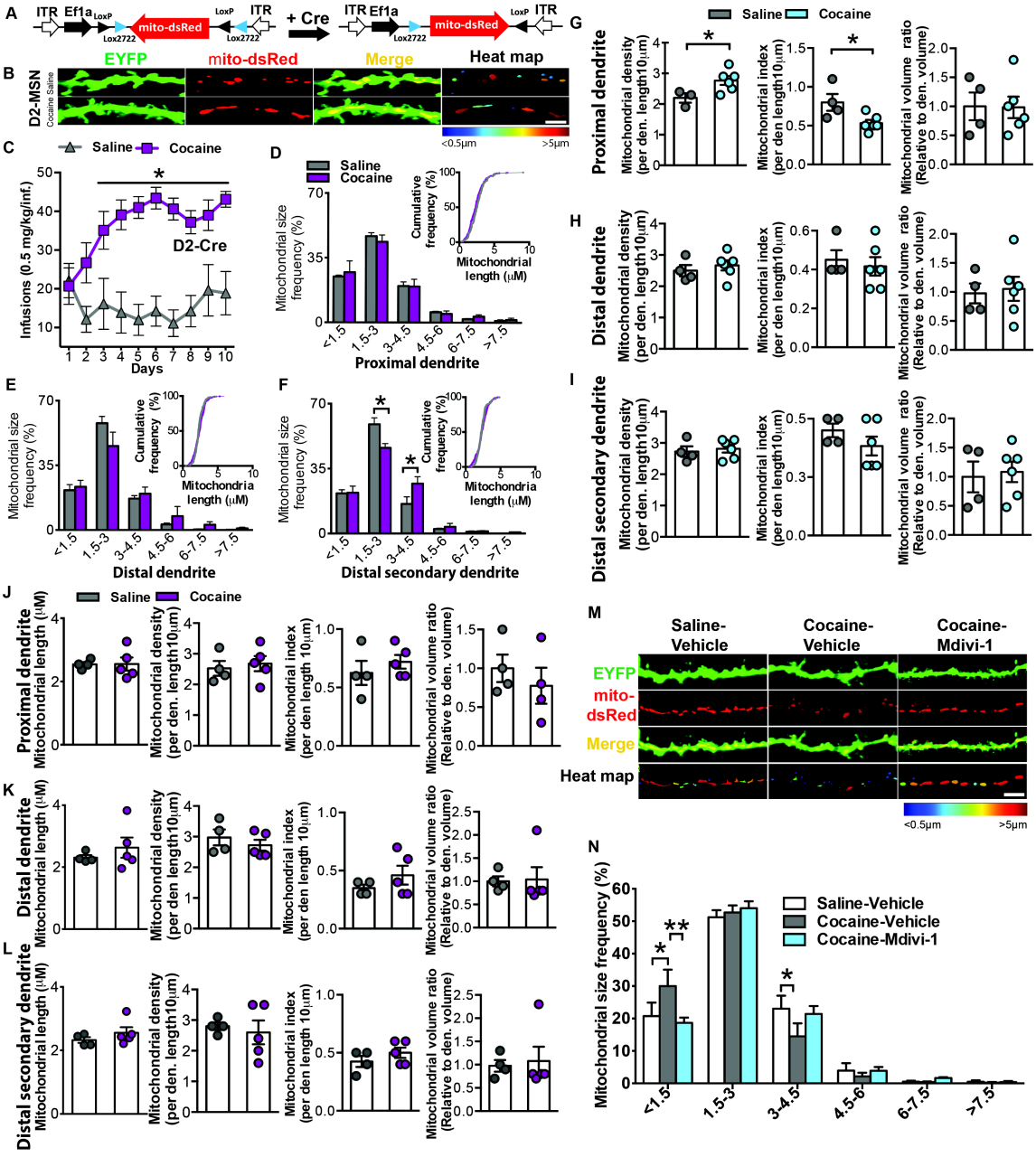

Figure S5. Related to Figure 5: Limited alterations in mitochondrial size of D2-MSNs following cocaine self-administration. (A) Schematic of the double-floxed, inverted, open reading frame Cre-dependent AAV vector expressing mitodsRed. **(B)** Representative confocal images of a D2-MSN co-labeled with EYFP (green) and mito-dsRed (red). (**C**) D2-Cre mice developed stable cocaine intake (0.5mg/kg/infusion, n=10) from day 3 onward compared to saline controls (n=8): Two-way Repeated Measures ANOVA: Interaction: $F(9,144)=5.847$, $p<0.0001$, Tukey post-hoc: $p<0.05$. **(D)** 3D reconstruction of dendrites and mitochondria showed no

difference in mitochondria size in proximal dendrites (Two-way ANOVA: Interaction: $F(5,42)=0.217$, $p=0.95$) and, **(E)** distal dendrites (Two-way ANOVA: Interaction: $F(5,42)=1.403$, $p=0.24$) and **(F)** only showed significant reduction of smaller mitochondria and increased medium-sized mitochondria in distal secondary dendrites: Two-way ANOVA: Interaction: $F(5,42)=4.888$, $p=0.001$, Bonferroni posthoc $p<0.05$). The insets display cumulative frequency distribution plots. Kolmogorov-Smirnov test did not showed differences in D2-MSNs for these 3 parameters. **(G)** Mitochondria density was increased and mitochondria index was significantly reduced in D1-MSN dendrites following cocaine self-administration: Student's t-test: $t=2.443$, $df=7$, $p=0.04$ and $t=2.657$, $df=8$, $p=0.02$, whereas mitochondria volume ratio was unchanged $t=0.05$, $df=8$, $p=0.95$. n=4 and 6 respectively. **(H)** In distal dendrites $t=0.67$, $df=8$, $p=0.51$; $t=0.46$, $df=8$, $p=0.65$; $t=0.25$, $df=8$, $p=0.80$ and **(I)** distal secondary dendrites: $t=0.44$, $df=8$, $p=0.66$; $t=1.20$, $df=8$, $p=0.26$; $t=0.28$, $df=8$, $p=0.78$ of D1-MSNs, none of these parameters was changed. **(J)** Similarly, none of these parameters was changed in D2-MSN mitochondria for proximal dendrites: $t=0.03$, $df=7$, $p=0.97$; $t=0.44$, $df=7$, $p=0.67$; $t=0.84$, $df=7$, $p=0.42$; $t=0.76$, $df=7$, $p=0.47$, **(K)** Distal dendrites: $t=0.85$, $df=7$, $p=0.42$; $t=0.83$, $df=7$, $p=0.42$; $t=1.15$, $df=7$, $p=0.28$; $t=0.12$, $df=7$, $p=0.90$; and **(L)** distal secondary dendrites: $t=1.09$, $df=7$, $p=0.30$; $t=0.44$, $df=7$, $p=0.67$; $t=1.13$, $df=7$, $p=0.29$; $t=0.28$, $df=7$, $p=0.78$. **(M)** Representative confocal images of D2-MSN dendrites (green) with labeled mitochondria (red) after cocaine or saline self-administration (left and middle panels). The right panel displays a heat map of mitochondrial length in MSN dendrites in cocaine and saline conditions. Scale bar 5µm. **(N)** Mdivi-1 pre-treatment (50mk/kg, ip) prevented the cocaine-induced increase in smaller size mitochondria (<1.5µm) and prevented the decrease in longer size mitochondria (3-4.5µm): Two-way ANOVA: Interaction: $F(10,66)=2.175$, $p<0.03$, Tukey post-hoc: $p<0.01$, $p<0.05$. n=4, 5 and 5 respectively. *$p<0.05$, **$p<0.01$. Error bars, SEM.

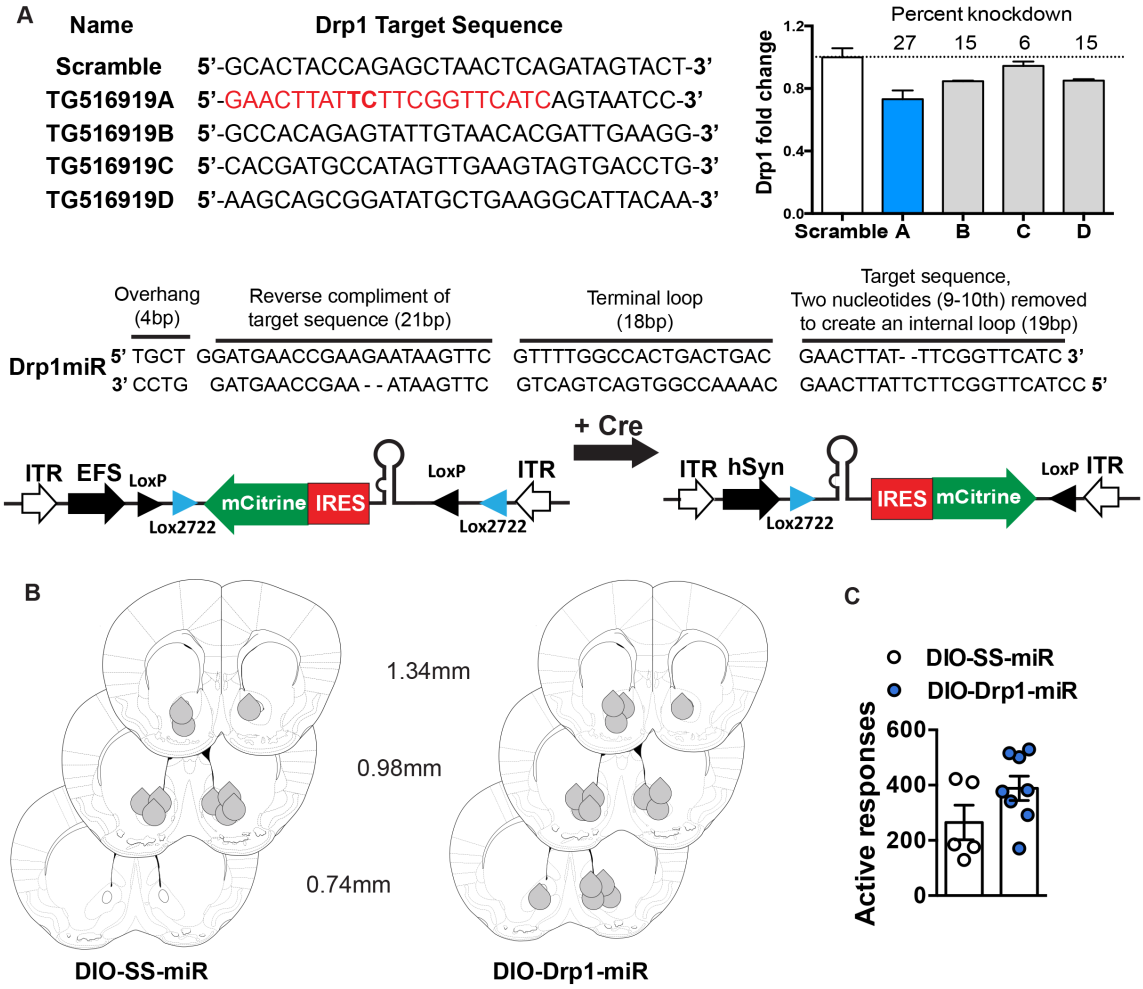

Figure S6. Related to Figure 6: Drp1 knockdown has no effect on cocaine seeking after withdrawal. (A) Mouse Drp1 shRNA sequences and their targeting of Drp1 (left). shRNAs reduced Drp1 mRNA levels in Neuro2a cells. Blue bar indicates that sequence A was selected for miR engineering (right). Illustration of the miRNA engineering sequence: the first 21 bp of the Drp1 target sequence (red) of shRNA was used for miRNA engineering (middle). Schematic of the double-floxed, inverted, open reading frame Cre-dependent AAV vector expressing Drp1-miR (bottom). **(B)** Viral placement of AAV-DIO-SS-miR-mCitrine and AAV-DIO-Drp1-miR-mCitrine in the nucleus accumbens of mice tested for cocaine seeking 24h after the last FR1 session. Adapted from Paxinos and Franklin, 2001. **(C)** Active responses (average) during the last day of water training. Mice infused with AAV-DIO-Drp1-miRmCitrine showed similar response for a natural reward when compared to controls: Student's t-test: t=1.669, df=11, p=0.123. n=5 SS-miR and n=8 Drp1-miR. Error bars, SEM.

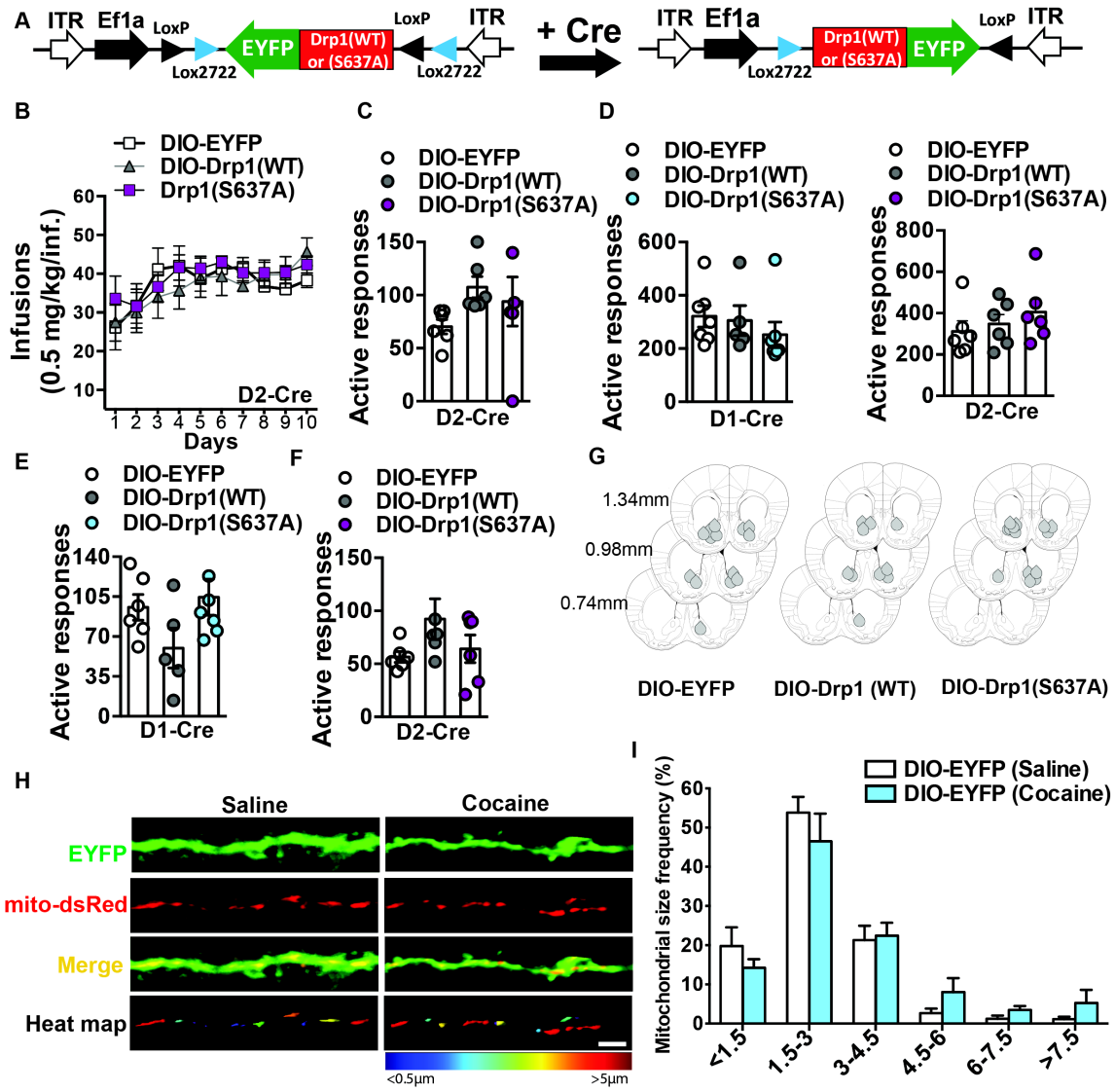

Figure S7. Related to Figure 7: Enhancing fission promoting Drp1 in D2-MSNs has no effect on cocaine self-administration and seeking. (A) Schematic of the double-floxed, inverted, open reading frame Cre-dependent AAV vector expressing Drp1(WT) and Drp1(S637A). **(B)** All virus groups in the D2-Cre mice displayed similar cocaine intake (FR1, 0.5mg/kg/inf): Two-way Repeated Measures ANOVA: Interaction: $F(18,135)=0.628$, $p=0.72$ and, **(C)** drug seeking after 30 days withdrawal: One-way ANOVA $F(2,15)=1.889$: $p=0.18$. n= 6 in each group. **(D)** Active responses (average) during the last day of water training. All groups showed similar responding for a natural reward in both D1-Cre (left) and D2-Cre (right) mice: One-way ANOVA $F(2,16)=0.638$: $p=0.54$ (n=7, 5 and 7 respectively) and One-way ANOVA $F(2,15)=0.775$:

p=0.47 (n=6 in each group). **(E)** Drp1 manipulation had no effect on cocaine seeking 24h after the last FR1 session in either D1-Cre mice (One-way ANOVA $F(2,15)=2.292$: $p=0.135$) or, **(F)** D2-Cre mice: One-way ANOVA $F(2,15)=1.558$: $p=0.242$. **(G)** Viral placement of AAV-DIO-EYFP, AAV-DIO-Drp1(WT)-EYFP and AAV-DIO-Drp1(S637A)-EYFP in the nucleus accumbens of D1-Cre mice tested for cocaine seeking after 30 days of withdrawal. Adapted from Paxinos and Franklin, 2001. **(H)** Representative confocal images of D1-MSN dendrites (green) with labeled mitochondria (red) after cocaine (top) or saline (bottom) self-administration and 30 days withdrawal (left and middle panels). The right panel displays a heat map of mitochondrial length in MSN dendrites in cocaine and saline conditions. Scale bar 5μm. **(I)** After 30 days of withdrawal, mice that self-administered cocaine (0.5mg/kg/inf; n=5) display no difference in mitochondria length in NAc D1-MSNs compared to saline controls (n=5): Two-way ANOVA: Interaction: $F(5,48)=1.120$, $p=0.362$. Error bars, SEM.

SN #	Cocaine Dep.	Brain ID	Cause Death	Age	Gender	Weight (g)	PH	Refrig Delay (hrs)	Additional Comorbidity
1	Cocaine dependent	7	Accident	51	Male	1428	6.73	1.50	Major Depression
2	Cocaine dependent	22	Natural	39	Male	1140	6.86	27.50	Depressive NOS
3	Cocaine dependent	26	Suicide	36	Male	1548	6.54	12.00	BiPolar Disorder
4	Cocaine dependent	33	Suicide	45	Male	1430	6.57	2.75	Major Depression
5	Cocaine dependent	34	Suicide	35	Male	1425	6.81	3.50	
6	Cocaine dependent	112	Suicide	24	Male	1460	6.89	2.50	
7	Cocaine dependent	120	Suicide	48	Male	1460	6.56	1.75	Major Depression
8	Cocaine dependent	121	Suicide	33	Male	1580	6.75	9.00	
9	Cocaine dependent	140	Suicide	43	Male	1445	6.78	5.25	Psychotic Disorder NOS
10	Cocaine dependent	147	Accident	24	Male	1480	6.33	7.00	
11	Cocaine dependent	156	Suicide	39	Male	1552	6.70	4.25	Major Depression
12	Cocaine dependent	193	Suicide	38	Male	1511.4	6.50	16.00	Depressive NOS
13	Cocaine dependent	194	Suicide	53	Male	1366.1	6.50	28.00	
14	Cocaine dependent	176	Suicide	50	Female	1255.5	6.30	7.00	
15	No cocaine dependent	128	Suicide	46	Male	1600	6.83	12.00	
16	No cocaine dependent	15	Natural	30	Male	1517	6.37	11.00	
17	No cocaine dependent	17	Natural	41	Male	1376	6.00	3.00	
18	No cocaine dependent	14	Natural	47	Male	1412	6.49	3.50	
19	No cocaine dependent	20	Accident	32	Male	1516	6.67	4.00	
20	No cocaine dependent	36	Natural	27	Male	1595	6.55	3.00	
21	No cocaine dependent	94	Accident	15	Male	1420	6.72	16.75	
22	No cocaine dependent	133	Accident	42	Male	1470	6.75	2.50	
23	No cocaine dependent	135	Natural	18	Male	1470	6.87	2.00	
24	No cocaine dependent	16	Accident	28	Male	1565	6.32	2.25	
25	No cocaine dependent	197	Suicide	41	Female	1355.2	6.50	3.50	
26	No cocaine dependent	173	Accident	20	Male	1533	6.30	12.00	

Table S1: Complete demographics for human samples. Related to STAR methods.

Mouse Primers	
Drp1-Forward	GGGCACTTAAATTGGGCTCC
Drp1-Reverse	TGTATTCTGTTGGCGTGGAAC
Fis1-Forward	GGCTGTCTCCAAGTCCAAATC
Fis1-Reverse	GGAGAAAAGGGAAGGCGATG
Opa1-Forward	TCACCTCTGCGTTTATTTGAAGA
Opa1-Reverse	GGGTAGAACGGGAGGAAAGG
Mfn1-Forward	TATCGATGCCTTGCGGAGAT
Mfn1-Reverse	GGCGAATCACAACACTTCCA
Mfn2-Forward	GGAGACCAACAAGGACTGGA
Mfn2-Reverse	TGCACAGTGACTTTCAACCG
Gapdh-Forward	AGGTCGGTGTGAACGGATTTG
Gapdh-Reverse	TGTAGACCATGTAGTTGAGGTCA
Rat Primers	
Drp1-Forward	GCAGCCGTAGTCCTCAAAGA
Drp1-Reverse	CTCCACCTTTTGAAGCCAGG
Fis1-Forward	CTGTTACAGACTGAGCCCCA
Fis1-Reverse	TGAGGCCTGTCACCTTTCTT
Opa1-Forward	CCGTGTGAGCAGAAGAACAC
Opa1-Reverse	AGCCTCAAGGCCAACTATGT
Mfn1-Forward	CAACTTGAACGTGAGCTGGA
Mfn1-Reverse	ACAGTGCCTCTGTTGTTAGGA
Mfn2-Forward	AGTCGGTTGGAAGTCACTGT
Mfn2-Reverse	TGTACTCGGGCTGAAAGGAG
Gapdh-Forward	AACGACCCCTTCATTGAC
Gapdh-Reverse	TCCACGACATACTCAGCAC
Human Primers	
Drp1-Forward	CAAAGCAGTTTGCCTGTGGA
Drp1-Reverse	TCTTGGAGGACTATGGCAGC
Fis1-Forward	CCAAATCCTGAAGGAGACGC
Fis1-Reverse	GCTGAAGGCCACAGAGGATA
Opa1-Forward	ACGTCTTTTGTCCAGCCTCT
Opa1-Reverse	GGTTAAAGCGCCCGTAACAT
Mfn1-Forward	CCTGGCATCCAGGAGTTAGA
Mfn1-Reverse	TGGTTCCAGCAATGCGATTT
Mfn2-Forward	TGCAGGTGTAAGGGACGATT
Mfn2-Reverse	GAGGCTCTGCAAATGGGATG
Gapdh-Forward	GAGAGAGACCCTCACTGCTG
Gapdh-Reverse	TCCCCTCTTCAAGGGGTCTA

Table S2: qPCR primers sequences. Related to STAR methods.