## **Supplemental Materials**

## Supplemental Data

## Supplemental Table 1

PEG-IGF-I exposure [ug/ml]									
Time (hrs)	0.3 mg/kg	1 mg/kg	3 mg/kg						
0	0.00	0.00	0.00						
6	0.76	2.94	8.87						
24	2.51	2.83	11.46						
48	1.00	3.80	9.16						

**Supplemental Table 1: Pharmacokinetics of PEG-IGF-I in** *Mecp2*<sup>*NULL/Y*</sup> **mice.** Mice (n=4 for each dose and time point) were injected I.P. with PEG-IGF-I at the dose listed. At the time listed, the animals were euthanized and serum collected for analysis of drug concentration.

Symbols	NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
key					
NULL 0.1			‡	0	+
NULL 0.3			+	<b>\$</b>	۸
NULL 1				•	#
NULL Veh					*

## Supplemental Table 2. Complete statistical summary for Figure 2.

	Statistical	· ·					
Age (wk)	Test	Comparison		5	tatistics		
	One-way	by genotype-					
	ANOVA	group		E(1 00)	-1 571 n-	100	
		group	20	1 (4, 80)	-1.371, p-	21	7
			20	NULL	15	NULL	1
4			NULL 0.1	0.3	NULL 1	Veh	WT veh
	Tukey HSD	NULL 0.1		1.000	0.292	0.871	0.980
		NULL 0.3			0.395	0.911	0.988
		NULL 1				0.718	0.271
		NULL Veh					0.720
	000 1101	by genotype-					
		treatment					
	ANOVA	group		F(4, 89)=	=8.334, p<.	0001	
		n	24	12	14	34	10
5				NULL		NULL	
	Tukey HSD		NULL 0.1	0.3	NULL 1	Ven	WI veh
		NULL 0.1		1.000	0.000	0.463	0.691
		NULL 0.3			0.001	0.713	0.759
		NULL 1				0.004	0.000
		NULL Ven					0.080
	One-way ANOVA	by genotype-					
		group	F(4, 88)=9.507, p<.0001				
		n	23	12	<u>14</u>	34	10
			20	NULL	17	NULL	10
6			NULL 0.1	0.3	NULL 1	Veh	WT veh
	<b>T</b> 1 1/00			~ ~ ~ ~	0 000	~	~
	Tukey HSD	NULL 0.1		0.973	0.000	0.738	0.143
	Tukey HSD	NULL 0.1 NULL 0.3		0.973	0.000	0.738 0.482	0.143 0.527
	Tukey HSD	NULL 0.1 NULL 0.3 NULL 1		0.973	0.000	0.738 0.482 0.005	0.143 0.527 0.000
	Tukey HSD	NULL 0.1 NULL 0.3 NULL 1 NULL Veh		0.973	0.000	0.738 0.482 0.005	0.143 0.527 0.000 0.009
	One-way	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype-		0.973	0.000	0.738 0.482 0.005	0.143 0.527 0.000 0.009
	One-way	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment		0.973	0.000	0.738 0.482 0.005	0.143 0.527 0.000 0.009
	One-way ANOVA	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group		0.973 F(4, 81)	0.000 0.000 =5.765, p=	0.738 0.482 0.005	0.143 0.527 0.000 0.009
	One-way ANOVA	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	23	0.973 F(4, 81) 12	0.000 0.000 =5.765, p= 11	0.738 0.482 0.005	0.143 0.527 0.000 0.009
7	One-way ANOVA	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	23	0.973 F(4, 81) 12 NULL 0.3	=5.765, p= 11	0.738 0.482 0.005	0.143 0.527 0.000 0.009 10
7	One-way ANOVA Tukey HSD	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	23 NULL 0.1	6.973 F(4, 81) 12 NULL 0.3	=5.765, p= 11 NULL 1	0.738 0.482 0.005 .002 .002 .002 .002 .002 .002	0.143 0.527 0.000 0.009 10 WT veh
7	One-way ANOVA Tukey HSD	NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1	23 NULL 0.1	0.973 F(4, 81) 12 NULL 0.3 0.902	=5.765, p= 11 NULL 1 0.461 0.184	0.738 0.482 0.005 .002 .002 .002 .002 .002 .002 .0	0.143 0.527 0.000 0.009 10 WT veh 0.033 0.341
	Age (wk) 4 5 6	Age (wk)Statistical TestAge (wk)One-way ANOVA4One-way Tukey HSD4One-way ANOVA5One-way ANOVA5Tukey HSD6One-way ANOVA	Age (wk)Statistical TestComparisonAge (wk)One-way ANOVAby genotype- treatment group4One-way ANOVANULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL Veh4One-way ANOVANULL 0.1 group5One-way ANOVAby genotype- treatment group5One-way ANOVAby genotype- treatment group5One-way ANOVANULL 0.1 NULL Veh5One-way ANOVANULL 0.1 by genotype- treatment group6One-way ANOVANULL 0.1 NULL 0.3 hull 1 hull Veh	Age (wk)Statistical TestComparisonAge (wk)Testby genotype- treatment groupANOVAby genotype- treatment groupANOVANULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL VehTukey HSDNULL 0.1 NULL VehOne-way ANOVAby genotype- treatment group5One-way ANOVANULL 0.1 NULL Veh5One-way ANOVANULL 0.1 NULL 0.1 NULL 0.3 NULL 0.1 NULL 0.3 NULL 1 NULL 0.4 So genotype- treatment group6NORe-way ANOVAn6NULL 0.1 NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL 0.3 NULL 1 NULL 0.3 NULL 1 NULL 0.3 NULL 0.1 NULL 0.3 NULL 1 NULL 0.3 NULL 0.1 NULL 0.1 NULL 0.3 NULL 0.1 NULL 0.3 NULL 1 NULL 0.3 NULL 0.1 NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL 0.3 NULL 1 NULL 0.1 NULL 0.1 	Age (wk)Statistical TestComparisonStatistical by genotype- treatment group $F(4, 80)$ 4One-way ANOVAn20127NULL0.1NULL0.37Tukey HSDNULL0.11.000NULLNULL0.3NULL1.000NULLNULL0.3NULL1.000NULLNULLNULL1.0001.000NULLNULLNULL1.0001.000NULLNULLNULL1.0001.000NULLNULLNULL1.0001.0005One-way ANOVANULL<0.1	Age (wk)         Statistical Test         Comparison         Statistics           One-way ANOVA         by genotype- treatment group         F(4, 80)=1.571, p= F(4, 80)=1.571, p= NULL           ANOVA         n         20         12         15 NULL           Tukey HSD         NULL 0.1         NULL 0.1         0.3         NULL 1           NULL 1         NULL Veh         1.000         0.292           NULL 1         NULL 0.3         0.395           NULL 1         NULL Veh         1.000         0.395           Streatment ANOVA         by genotype- treatment         1.000         0.395           NULL Veh         F(4, 89)=8.334, p<	Statistical Test         Comparison         Statistics           One-way ANOVA         by genotype- treatment group         F(4,80)=1.571, p=.190           ANOVA         n         20         12         31           NULL         NULL 0.1         NULL         0.3         NULL         Veh           Tukey HSD         NULL 0.1         NULL 0.1         1.000         0.292         0.871           NULL 1         NULL 0.1         NULL 0.1         1.000         0.292         0.871           NULL 1         NULL 0.1         NULL 0.1         1.000         0.292         0.871           NULL 1         NULL 0.3         NULL 1         0.395         0.911           NULL Veh         F(4,89)=8.334, p<001

			NULL Veh					0.004
		One-way	by genotype-					
		ANOVA	treatment					
			group		F(4, 76)	=4.386, p=	.003	
			n	23	11	9	28	10
	8			NULL 0.1	NULL 0.3	NULL 1	Veh	WT veh
		Tukey HSD	NULL 0.1		0.979	0.981	1.000	0.005
		rancy nob	NULL 0.3			0.877	0.951	0.074
			NULL 1				0.992	0.008
			NULL Veh					0.002
		One-wav	by genotype-					
		ANOVA	treatment			C 120	0004	
			group	01	F(4, 66)=	=6.428, p<.	0001	10
			n	21	9 NULL	5	26 NULL	10
	9			NULL 0.1	0.3	NULL 1	Veh	WT veh
		Tukey HSD	NULL 0.1		0.682	0.195	1.000	0.004
			NULL 0.3			0.040	0.733	0.298
			NULL 1				0.147	0.000
			NULL Veh					0.004
		One-way	by genotype-					
		ANOVA	group		F(4 63)=	=5 813 n<	0001	
	10		n	20	9	<u>5.015, p &lt;.</u> 5	24	10
					NULL	· ·	NULL	
	10			NULL 0.1	0.3	NULL 1	Veh	WT veh
		Tukey HSD	NULL 0.1		0.663	0.132	1.000	0.017
			NULL 0.3			0.023	0.674	0.543
			NULL I				0.110	0.000
			by genotype-					0.015
		One-way	treatment					
		ANOVA	group		F(4, 59	)=.322, p=	862	
			n	13	12	7	28	4
	5			NULL 0.1	NULL	NULL 1	NULL	WT veh
	-	Tulanduco			0.3	026	Ven	1 000
		тикеу нър			.870	.930	.994	1.000
Heart rate			NULL 1			1.000	984	981
2B			NULL Veh				1501	.999
			by genotype-					
		One-way	treatment					
		ANOVA	group		F(4, 49)	=2.673, p=	.043	
	6		n	9		7	20	6
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
		TUKEY HSD	NULL 0.1		0.166	0.066	0.589	0.999
			NULL 0.3			0.942	0.778	0.401

			NULL 1 NULL Veh				0.398	0.183 0.854
		One-way ANOVA	by genotype- treatment		F(A 51)	-5 721 n-	001	
			n	18	1(4, 51)	-3.721, p-	20	3
	7			NULL 0.1	NULL 0.3	۲ NULL 1	NULL Veh	WT veh
		Tukey HSD	NULL 0.1		0.012	0.005	0.599	0.970
			NULL 0.3			0.724	0.203	0.089
			NULL 1				0.050	0.022
			NULL Veh					0.634
		One-way ANOVA	by genotype- treatment group		F(3, 46)	=3.399, p=	.025	
			n	14	9	•	22	5
	8			NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
		Tukey HSD	NULL 0.1		0.133		0.100	0.631
			NULL 0.3				0.984	0.037
			NULL 1					
			NULL Veh					0.038
		One-way	by genotype-					
		ANOVA	group		F(3, 46)	=5.546. n=	002	
			n	14	9	0.0.076	20	7
	9			NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
		Tukey HSD	NULL 0.1		0.068		0.664	0.060
			NULL 0.3				0.335	0.000
			NULL 1					
			NULL Veh					0.005
		One-way ANOVA	by genotype- treatment					
			group		F(3, 39)	=8.276, p<.	0001	
			n	11	8		16	8
	10			NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
		Tukey HSD	NULL 0.1		0.030		0.556	0.030
			NULL 0.3				0.241	0.000
			NULL 1					0.004
			hy genotype-					0.001
		One-way ANOVA	treatment		F(4. 48	)=.493. p=.	741	
Glucose		<b></b>	n	5	12	<u>, , , , , , , , , , , , , , , , , , , </u>	24	5
2C	4				NULL	-	NULL	-
		Tukey HSD		NULL 0.1	0.3	NULL 1	Veh	WT veh
			NULL 0.1		0.820	0.995	0.892	0.757
			NULL 0.3			0.951	0.997	0.997

		NULL 1 NULL Veh				0.986	0.897 0.971
	One-way ANOVA	by genotype- treatment group		F(4, 61)	=3.002, p=	.025	
		n	10	12	5	29	10
5			NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
	Tukey HSD	NULL 0.1		0.116	0.806	0.990	0.161
		NULL 0.3			0.917	0.091	1.000
		NULL 1				0.899	0.937
		NULL Veh					0.149
	One-way	by genotype-					
	ANOVA	treatment			F 212	001	
		group		F(4, 61)	=5.312, p=	.001	10
		n	9	1Z NULL	1	28 NHLI I	10
6			NULL 0.1	0.3	NULL 1	Veh	WT veh
	Tukey HSD	NULL 0.1		0.001	0.027	0.265	0.026
	/ -	NULL 0.3			0.987	0.040	0.908
		NULL 1				0.382	0.999
		NULL Veh					0.437
	One-way	by genotype-					
	ANOVA	treatment		- (			
		group		F(4, 62)=	=5.908, p<.	0001	
			-		_		
		n	9	12 NUULI	7	29	10
7		n	9 NULL 0.1	12 NULL 0.3	7 NULL 1	29 NULL Veh	10 WT veh
7	Tukev HSD	n NULL 0.1	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000	29 NULL Veh 0.028	10 WT veh <u>0.006</u>
7	Tukey HSD	n NULL 0.1 NULL 0.3	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000 0.719	29 NULL Veh 0.028 0.696	10 WT veh <u>0.006</u> 1.000
7	Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000 0.719	29 NULL Veh 0.028 0.696 0.110	10 WT veh 0.006 1.000 0.796
7	Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000 0.719	29 NULL Veh 0.028 0.696 0.110	10 WT veh 0.006 1.000 0.796 0.670
7	Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype-	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000 0.719	29 NULL Veh 0.028 0.696 0.110	10 WT veh 0.006 1.000 0.796 0.670
7	Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment	9 NULL 0.1	12 NULL 0.3 0.005	7 NULL 1 0.000 0.719	29 NULL Veh 0.028 0.696 0.110	10 WT veh 0.006 1.000 0.796 0.670
7	Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group	9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54)	7 NULL 1 0.000 0.719 =3.795, p=	29 NULL Veh 0.696 0.110	10 WT veh 0.006 1.000 0.796 0.670
7	Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54)	7 NULL 1 0.000 0.719 =3.795, p= 4	29 NULL Veh 0.696 0.110	10 WT veh 0.006 1.000 0.796 0.670 10
7	Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1	29 NULL Veh 0.696 0.110 .009 25 NULL Veh	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh
7	Tukey HSD One-way ANOVA Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005
8	Tukey HSD One-way ANOVA Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.3	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896
7	Tukey HSD One-way ANOVA Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.3 NULL 1	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728
7 8	Tukey HSD One-way ANOVA Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.3 NULL 1 NULL Veh	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 .009 25 NULL Veh 0.214 0.713 0.999	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169
7 8	Tukey HSD One-way ANOVA Tukey HSD One-way	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype-	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169
7 8	Tukey HSD One-way ANOVA Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 .009 25 NULL Veh 0.214 0.713 0.999	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169
8	Tukey HSD One-way ANOVA Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043 F(3, 46)	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 .009 25 NULL Veh 0.214 0.713 0.999 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169
7 8	Tukey HSD One-way ANOVA Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043 F(3, 46) 9	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977	29 NULL Veh 0.028 0.696 0.110 .009 25 NULL Veh 0.214 0.713 0.999 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169
7 8 9	Tukey HSD One-way ANOVA Tukey HSD One-way ANOVA	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	9 NULL 0.1 9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043 F(3, 46) 9 NULL 0.3	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977 =6.645, p= NULL 1	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169 10 WT veh
7 8	Tukey HSD One-way ANOVA Tukey HSD One-way ANOVA Tukey HSD	n NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n NULL 0.1 NULL 0.1 NULL 0.3 NULL 1 NULL Veh by genotype- treatment group n	9 NULL 0.1 9 NULL 0.1 9 NULL 0.1	12 NULL 0.3 0.005 F(4, 54) 11 NULL 0.3 0.043 F(3, 46) 9 NULL 0.3 0.006	7 NULL 1 0.000 0.719 =3.795, p= 4 NULL 1 0.512 0.977 =6.645, p= NULL 1	29 NULL Veh 0.028 0.696 0.110 	10 WT veh 0.006 1.000 0.796 0.670 10 WT veh 0.005 0.896 0.728 0.169 10 WT veh 10 WT veh 0.001

			NULL 1					0 111
		One-way ANOVA	by genotype- treatment					0.111
			group		F(4, 45)	=3.707, p=	.011	
	10		n	7	9 NULL	4	20 NULL	10
	10			NULL 0.1	0.3	NULL 1	Veh	WT veh
		Tukey HSD	NULL 0.1		0.010	0.722	0.301	0.035
			NULL 0.3			0.500	0.193	0.973
			NULL 1				1.000	0.774
			NULL Veh					0.524
		One-way	by genotype-					
		ANOVA	treatment					
			group		F(4, 25)	=1.123, p=	.368	
			n	6	4	2	14	4
	6			NULL 0.1	NULL 0.3	NULL 1	Veh	WT veh
		Tukey HSD	NULL 0.1		0.524	0.918	0.931	0.408
			NULL 0.3			0.995	0.797	1.000
			NULL 1				0.995	0.982
			NULL Veh					0.669
		One-way	by genotype-					
			treatment					
			group		F(4, 30)	=4.778, p=	.004	
Glucose			n	6	8	3		4
tolerance	7-8				NULL 0.3	NUUL 1	NULL Veb	W/T veb
test 2D		Tukau UCD		NOLL 0.1	0.0	0.005		
		тикеу нър			0.040	0.995	0.994	0.015
						0.740	1 000	0.000
			NULL Veh				1.000	0.027
			by genotype-					0.002
		One-way	treatment					
		ANOVA	group		F(4, 25)	=3.782, p=	.023	
			n	5	8		12	4
	9-11				NULL		NULL	
	5 11			NULL 0.1	0.3	NULL 1	Veh	WIveh
		Tukey HSD	NULL 0.1		0.115		1.000	0.305
			NULL 0.3				0.040	0.996
			NULL 1					
			NULL Veh					0.209

# Supplemental Table 3. Complete statistical summary for Figure 4.

Symbols key

	NULL INS	NULL VEH	WT INS	WT VEH
NULL INS		‡	0	+
NULL VEH			۸	*
WT INS				#

Panel	Age (wk)	Statistical Test	Comparison		S	statistics		
		One-way ANOVA	by genotype- treatment		F(3, 28	)= 871 n=	468	
	4		n	8	8 NULL VEH		8 WT VEH	
		Tukey HSD	NULL INS NULL VEH WT INS		0.581	0.632	0.996 0.715 0.762	
		One-way ANOVA	by genotype- treatment group		F(3, 28)	=3.651, p=	.024	
	5		n	8 NULL INS	8 NULL VEH	8 WT INS	8 WT VEH	
Weight 4A		Tukey HSD	NULL INS NULL VEH WT INS		0.511	0.016 0.280	0.178 0.896 0.675	
		One-way ANOVA 6	by genotype- treatment group		F(3, 28)=	10.698, p<	.0001	
	6		n	8 NULL INS	8 NULL VEH	8 WT INS	8 WT VEH	
		Tukey HSD	NULL INS NULL VEH WT INS		0.522	0.000 0.005	0.003 0.073 0.681	
		One-way ANOVA	by genotype- treatment group		F(3, 27)=	20.032, p<	.0001	
	7	Tukey HSD	n	7 NULL INS	8 NULL VEH	8 WT INS	8 WT VEH	
		•	NULL INS NULL VEH		0.976	0.000 0.000	0.000 0.000	

			WT INS				0.742	
		One-way ANOVA	by genotype- treatment		F(3, 26)=	17.896 n<	0001	
			n	7	8	<u>- 7</u>	8	
	8			NULL INS	NULL VEH	WT INS	WT VEH	
		Tukey HSD	NULL INS NULL VEH WT INS		0.999	0.000 0.000	0.000 0.000 0.970	
		One-way ANOVA	by genotype- treatment group		F(3, 25)=	64.667, p<	.0001	
			n	6	8	7	8	
	9			NULL INS	NULL VEH	WT INS	WT VEH	
		Tukey HSD	NULL INS NULL VEH		0.543	0.000 0.000	0.000 0.000	
			WT INS				0.972	
		One-way ANOVA	by genotype- treatment		F(3-25)-	63 200 nc	0001	
			n	6	8	<u>03.203, p</u> 7	.0001 8	
	10			NULL INS	NULL VEH	, WT INS	WT VEH	
		Tukey HSD	NULL INS NULL VEH		0.263	0.000	0.000 0.000	
			WT INS				0.996	
		One-way ANOVA	by genotype- treatment		F(3 11	)= 998 n=	430	
			n	4	3	<u></u>	4	
	5			NULL INS	NULL VEH	WT INS	WT VEH	
		Tukey HSD	NULL INS		0.884	0.429	0.539	
			NULL VEH			0.882	0.946	
Heart rate 4B			WTINS				0.997	
		One-way ANOVA	by genotype- treatment		F(2, 20)	1 401 -	262	
			group	0	r(3, 28) °	=1.401, р= о	.203	
	6	Tukev HSD		NULL INS	NULL VEH	o WT INS	WT VEH	
			NULL INS		0.369	0.844	0.286	
			NULL VEH			0.841	0.998	

			WT INS				0.752	
		One-way ANOVA	by genotype- treatment group		F(3, 26)=	=12.621. p<	<.000	
			n	7	8	7	8	
	7			NULL INS	NULL VEH	WT INS	WT VEH	
		Tukey HSD	NULL INS NULL VEH WT INS		0.068	0.000 0.013	0.001 0.204 0.523	
		One-way ANOVA	by genotype- treatment group		F(2, 8)=	=2.516, p=.	142	
	8		n NULL INS	NULL INS	4 NULL VEH	3 WT INS	4 WT VEH	
		Tukey HSD	NULL VEH WT INS			0.546	0.123 0.605	
		One-way ANOVA	by genotype- treatment group		F(3, 25)=	21.470, p<	.0001	
	9	Tukey HSD	n NULL INS NULL VEH WT INS	6 NULL INS	8 NULL VEH 0.481	7 WT INS 0.000 0.000	8 WT VEH 0.000 0.001 0.590	
		One-way ANOVA	by genotype- treatment group		F(3, 25)=	=17.839, p∢	<.000	
	10	Tukey HSD	n NULL INS NULL VEH	6 NULL INS	8 NULL VEH 0.059	7 WT INS 0.000 0.002	8 WT VEH 0.000 0.010	
			WT INS				0.901	
Glucose		One-way ANOVA	treatment group		F(3, 28)	=2.804, p=	.058	
4C	4-5	Tukey HSD	n	8 NULL INS	8 NULL VEH	8 WT INS	8 WT VEH	
			NULLINS		1.000	0.710	0.082	

		NULL VEH WT INS			0.710	0.082	
						01100	
	One-way ANOVA	by genotype- treatment		F(3, 35)=	11 718 n<	0001	
		n	9	10	10	10	I
				NULL	10	10	
6-7			NULL INS	VEH	WT INS	WT VEH	
	Tukey HSD	NULL INS		0.053	0.000	0.000	
		NULL VEH			0.262	0.027	
		WT INS				0.693	
	One-way	by genotype-					
	ANOVA	treatment		E(2 22)-	10 EE7 n/	0001	
		n	3	- ( <i>3, 22)</i> - 7	20.557, p 8	.0001	
		11	5	, NULL	0	0	
>8			NULL INS	VEH	WT INS	WT VEH	
	Tukey HSD	NULL INS		0.512	0.000	0.000	
	-	NULL VEH			0.000	0.000	
		WT INS				0.976	



**Figure S1. NULL mice have a metabolic syndrome. (A)** At 9-14 weeks age, NULL mice (n=10) had unchanged IGF-I compared to WT mice (n=14). **(B)** In contrast, IGFBP-2 was decreased in NULL animals compared to WT animals. **(C)** IGFBP-3 levels were comparable between NULL and WT mice. \*\*\*p<0.001. Error bars are SEM.



WT vehicle - WT 0.1 mg/kg NULL vehicle NULL 0.1 mg/kg

**Figure S2. Treatment of NULL animals with 0.1mg/kg PEG-IGF-I. (A)** Treatment with 0.1mg/kg PEG-IGF-I did not correct the abnormal breathing response seen in NULL animals at 5 weeks age. **(B, C)** Additionally, treatment had no effect on normalized tidal volume at 5 weeks or 8 weeks of age, either at baseline or when exposed to hypoxia. **(D)** Center/total distance traveled ratio in the open field was unaffected by treatment. **(E)** NULL mice perform poorly on the wire hanging task, and this was unaffected by treatment. **(F, G)** Neither acoustic startle nor prepulse inhibition was affected by treatment. NULL 0.1mg/kg n=9, for all others n=10. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars are SEM.



Figure S3. Treatment with rhIGF-I does not improve spine density in hippocampal neurons from **NULL mice. (A)** *Mecp2*<sup>*NULL/Y*</sup> mice show lower density of dendritic spines in pyramidal neurons from several brain regions (1), including the hippocampus (2). Intriguingly, treatment with the N-terminal tripeptide of IGF1 ameliorates this spine phenotype in pyramidal neurons of the visual cortex (3). To determine if full length recombinant human IGF1 is also effective at reversing this phenotype in *Mecp2*<sup>NULL/Y</sup> neurons, we treated hippocampal slice cultures containing eYFP-expressing CA1 and CA3 pyramidal neurons with rh-IGF1. Using confocal microscopy, dendritic spines were identified as small protrusions that extended from the parent dendrite. Pyramidal neurons in slices from *Mecp2<sup>NULL/Y</sup>* mice have a lower spine density than that of wild type littermates (5.77±0.71 spines per 10µm of dendrite, n=8 cells vs. 7.92±0.69 spines/10µm, n=10; p=0.0038, ANOVA, Newman-Keuls). In addition, the proportion of morphological types of spines in  $Mecp2^{NULL/Y}$  slices is significantly biased towards the more stable spine types (stubby and mushroom) in detriment of more motile thin spines compared to wild-type slices (p<0.05). (B) 48h exposure to 50µg/mL rh-IGF-1 did not affect spine density neither in wild-type neurons (8.81±0.76 spines/10 $\mu$ m, n=8) nor in  $Mecp2^{NULL/Y}$  ones (3.81±1.10 spines/10 $\mu$ m, n=3; p>0.05, compared to saline-treated controls). (C) Similarly, rh-IGF-1 did not affect the proportions of dendritic spine types in neither of the genotypes (p>0.05).



**Figure S4. Treatment of NULL animals with insulin.** (A) Surprisingly, treatment with insulin increased the breathing response to hypoxia in both WT as well as NULL animals, but had no effect on breathing rates at baseline in either genotype. (B, C) Insulin treatment had no effect on tidal volume at 5-6 weeks or 8 weeks age in either genotype in either baseline breathing or during exposure to hypoxia. At 5-6 weeks age n=10 for each group in each segment. At 8 weeks age NULL 0.1mg/kg n=9, all others n=10. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001. Error bars are SEM.

#### **Supplemental Materials and Methods**

Quantitative confocal microscopy of dendritic spines. Organotypic cultures of hippocampal slices were prepared from postnatal day 5-8 *Mecp2*<sup>*NULL/Y*</sup> mice and wild-type littermates following standard procedures (4). At 4 days in vitro (DIV) media serum was titrated to serum-free conditions using Neurobasal-A with B-27 and L-glutamine supplementation. After 7 DIV, cDNA plasmids encoding enhanced Yellow Fluorescent Protein (eYFP, Clontech) were introduced by biolistic gene transfer using a Helios handheld gene gun (Bio-Rad). After 48 hours of expression, slice cultures were randomly assigned to treatment with 50µg/ml rhIGF-I or vehicle (0.9% NaCl). After 48 hours, slice cultures were fixed by immersion in 4% paraformaldehyde and mounted in glass slides with Vectashield (Vector labs). eYFP-expressing CA1 and CA3 pyramidal neurons were imaged in a Fluoview FV-300 laser-scanning confocal microscope (Olympus) using an oil immersion 63x (NA 1.45) objective lens (PlanApo). eYFP was excited with the 488nm line of an Ar laser and its fluorescence emission detected using standard FITC filters (Semrock). Individual secondary or tertiary branches of apical dendrites were imaged using a 3X digital zoom, and optical sections were acquired at 0.1µm intervals in the Z-axis using pinhole aperture 1. The x-y resolution is 0.09µm, measured with fluorescence microspheres. Dendritic spines were identified as small protrusions that extended from the parent dendrite, and detected semiautomatically in maximum-intensity projections of the confocal z-stacks using NeuronStudio (5). Spine density was calculated by quantifying the number of spines per dendritic segment, and normalized to 10μm of dendritic length. Data were analyzed using ANOVA followed by Newman-Keuls t-test posthoc.

## **Supplemental References**

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