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*$^{NULL/Y}$ 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.

Supplemental Table 2. Complete statistical summary for Figure 2.

Symbols key	NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
NULL 0.1		□	‡	○	+
NULL 0.3			†	◊	^
NULL 1				●	#
NULL Veh					*

Panel	Age (wk)	Statistical Test	Comparison	Statistics				
Weight 2A	4	One-way ANOVA	by genotype-treatment group	F(4, 80)=1.571, p=.190				
		Tukey HSD	n	20	12	15	31	7
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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
	5	One-way ANOVA	by genotype-treatment group	F(4, 89)=8.334, p<.0001				
		Tukey HSD	n	24	12	14	34	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT 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 Veh					0.080
	6	One-way ANOVA	by genotype-treatment group	F(4, 88)=9.507, p<.0001				
		Tukey HSD	n	23	12	14	34	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.973	0.000	0.738	0.143
			NULL 0.3			0.000	0.482	0.527
			NULL 1				0.005	0.000
			NULL Veh					0.009
	7	One-way ANOVA	by genotype-treatment group	F(4, 81)=5.765, p=.002				
		Tukey HSD	n	23	12	11	30	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.902	0.461	0.939	0.033
			NULL 0.3			0.184	0.538	0.341
			NULL 1				0.791	0.002

			NULL Veh					0.004
	8	One-way ANOVA	by genotype-treatment group	F(4, 76)=4.386, p=.003				
		Tukey HSD	n	23	11	9	28	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.979	0.981	1.000	0.005
			NULL 0.3			0.877	0.951	0.074
			NULL 1				0.992	0.008
			NULL Veh					0.002
	9	One-way ANOVA	by genotype-treatment group	F(4, 66)=6.428, p<.0001				
		Tukey HSD	n	21	9	5	26	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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
	10	One-way ANOVA	by genotype-treatment group	F(4, 63)=5.813, p<.0001				
		Tukey HSD	n	20	9	5	24	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.663	0.132	1.000	0.017
			NULL 0.3			0.023	0.674	0.543
			NULL 1				0.110	0.000
			NULL Veh					0.015
Heart rate 2B	5	One-way ANOVA	by genotype-treatment group	F(4, 59)=.322, p=862				
		Tukey HSD	n	13	12	7	28	4
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		.876	.936	.994	1.000
			NULL 0.3			1.000	.956	.970
			NULL 1				.984	.981
			NULL Veh					.999
	6	One-way ANOVA	by genotype-treatment group	F(4, 49)=2.673, p=.043				
		Tukey HSD	n	9	12	7	20	6
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.166	0.066	0.589	0.999
			NULL 0.3			0.942	0.778	0.401

			NULL 1				0.398	0.183
			NULL Veh					0.854
	7	One-way ANOVA	by genotype-treatment group	F(4, 51)=5.721, p=.001				
		Tukey HSD	n	18	11	4	20	3
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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
	8	One-way ANOVA	by genotype-treatment group	F(3, 46)=3.399, p=.025				
		Tukey HSD	n	14	9		22	5
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.133		0.100	0.631
			NULL 0.3				0.984	0.037
			NULL 1					
			NULL Veh					0.038
	9	One-way ANOVA	by genotype-treatment group	F(3, 46)=5.546, p=.002				
		Tukey HSD	n	14	9		20	7
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.068		0.664	0.060
			NULL 0.3				0.335	0.000
			NULL 1					
			NULL Veh					0.005
	10	One-way ANOVA	by genotype-treatment group	F(3, 39)=8.276, p<.0001				
		Tukey HSD	n	11	8		16	8
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.030		0.556	0.030
			NULL 0.3				0.241	0.000
			NULL 1					
			NULL Veh					0.001
Glucose 2C	4	One-way ANOVA	by genotype-treatment group	F(4, 48)=.493, p=.741				
		Tukey HSD	n	5	12	7	24	5
				NULL 0.1	NULL 0.3	NULL 1	NULL 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				0.986	0.897
			NULL Veh					0.971
		One-way ANOVA	by genotype-treatment group	F(4, 61)=3.002, p=.025				
	5	Tukey HSD	n	10	12	5	29	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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 ANOVA	by genotype-treatment group	F(4, 61)=5.312, p=.001				
	6	Tukey HSD	n	9	12	7	28	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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 ANOVA	by genotype-treatment group	F(4, 62)=5.908, p<.0001				
	7	Tukey HSD	n	9	12	7	29	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.005	0.000	0.028	0.006
			NULL 0.3			0.719	0.696	1.000
			NULL 1				0.110	0.796
			NULL Veh					0.670
		One-way ANOVA	by genotype-treatment group	F(4, 54)=3.795, p=.009				
	8	Tukey HSD	n	9	11	4	25	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.043	0.512	0.214	0.005
			NULL 0.3			0.977	0.713	0.896
			NULL 1				0.999	0.728
			NULL Veh					0.169
		One-way ANOVA	by genotype-treatment group	F(3, 46)=6.645, p=.001				
	9	Tukey HSD	n	9	9		22	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.006		0.066	0.001
			NULL 0.3				0.387	0.950

			NULL 1					
			NULL Veh					0.111
	10	One-way ANOVA	by genotype-treatment group	F(4, 45)=3.707, p=.011				
		Tukey HSD	n	7	9	4	20	10
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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
Glucose tolerance test 2D	6	One-way ANOVA	by genotype-treatment group	F(4, 25)=1.123, p=.368				
		Tukey HSD	n	6	4	2	14	4
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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
	7-8	One-way ANOVA	by genotype-treatment group	F(4, 30)=4.778, p=.004				
		Tukey HSD	n	6	8	3	14	4
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			NULL 0.1		0.846	0.995	0.994	0.019
			NULL 0.3			0.740	0.454	0.095
			NULL 1				1.000	0.027
			NULL Veh					0.002
	9-11	One-way ANOVA	by genotype-treatment group	F(4, 25)=3.782, p=.023				
		Tukey HSD	n	5	8		12	4
				NULL 0.1	NULL 0.3	NULL 1	NULL Veh	WT veh
			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		‡	o	+
NULL VEH			^	*
WT INS				#

Panel	Age (wk)	Statistical Test	Comparison	Statistics			
Weight 4A	4	One-way ANOVA	by genotype-treatment group	F(3, 28)=.871, p=.468			
		Tukey HSD	n	8	8	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.581	0.632	0.996
			NULL VEH			1.000	0.715
			WT INS				0.762
	5	One-way ANOVA	by genotype-treatment group	F(3, 28)=3.651, p=.024			
		Tukey HSD	n	8	8	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.511	0.016	0.178
			NULL VEH			0.280	0.896
			WT INS				0.675
	6	One-way ANOVA	by genotype-treatment group	F(3, 28)=10.698, p<.0001			
		Tukey HSD	n	8	8	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.522	0.000	0.003
			NULL VEH			0.005	0.073
			WT INS				0.681
	7	One-way ANOVA	by genotype-treatment group	F(3, 27)=20.032, p<.0001			
		Tukey HSD	n	7	8	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.976	0.000	0.000
			NULL VEH			0.000	0.000

			WT INS				0.742
	8	One-way ANOVA	by genotype-treatment group	F(3, 26)=17.896, p<.0001			
		Tukey HSD	n	7	8	7	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.999	0.000	0.000
			NULL VEH			0.000	0.000
			WT INS				0.970
	9	One-way ANOVA	by genotype-treatment group	F(3, 25)=64.667, p<.0001			
		Tukey HSD	n	6	8	7	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.543	0.000	0.000
			NULL VEH			0.000	0.000
			WT INS				0.972
	10	One-way ANOVA	by genotype-treatment group	F(3, 25)=63.209, p<.0001			
		Tukey HSD	n	6	8	7	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.263	0.000	0.000
			NULL VEH			0.000	0.000
			WT INS				0.996
Heart rate 4B	5	One-way ANOVA	by genotype-treatment group	F(3, 11)=.998, p=.430			
		Tukey HSD	n	4	3	4	4
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.884	0.429	0.539
			NULL VEH			0.882	0.946
			WT INS				0.997
	6	One-way ANOVA	by genotype-treatment group	F(3, 28)=1.401, p=.263			
		Tukey HSD	n	8	8	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.369	0.844	0.286
			NULL VEH			0.841	0.998

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

			NULL VEH			0.710	0.082
			WT INS				0.493
	6-7	One-way ANOVA	by genotype-treatment group	F(3, 35)=11.718, p<.0001			
		Tukey HSD	n	9	10	10	10
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.053	0.000	0.000
			NULL VEH			0.262	0.027
			WT INS				0.693
	>8	One-way ANOVA	by genotype-treatment group	F(3, 22)=18.557, p<.0001			
		Tukey HSD	n	3	7	8	8
				NULL INS	NULL VEH	WT INS	WT VEH
			NULL INS		0.512	0.000	0.000
			NULL VEH			0.000	0.000
			WT INS				0.976

Supplemental Figure 1

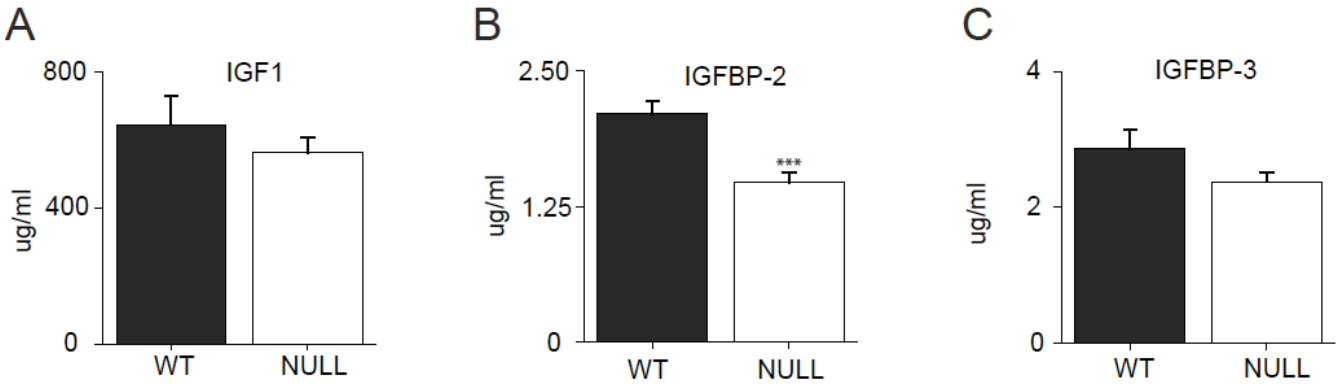

Figure S1. NULL mice have a metabolic syndrome. (A) At 9-14 weeks age, NULL mice (n=10) had unchanged IGF-I compared to WT mice (n=14). **(B)** In contrast, IGFBP-2 was decreased in NULL animals compared to WT animals. **(C)** IGFBP-3 levels were comparable between NULL and WT mice. ***p<0.001. Error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

Supplemental Figure 2

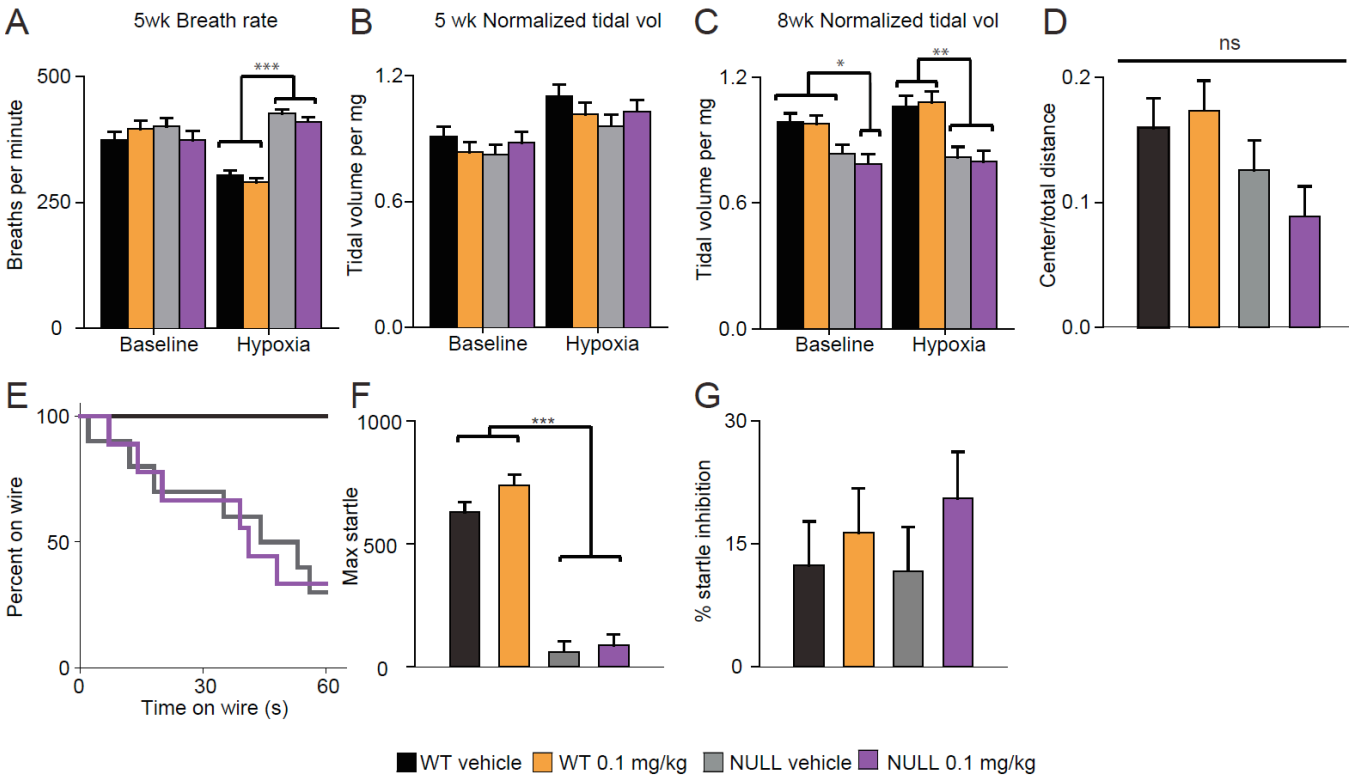

Figure S2. Treatment of NULL animals with 0.1mg/kg PEG-IGF-I. (A) Treatment with 0.1mg/kg PEG-IGF-I did not correct the abnormal breathing response seen in NULL animals at 5 weeks age. **(B, C)** Additionally, treatment had no effect on normalized tidal volume at 5 weeks or 8 weeks of age, either at 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.

Supplemental Figure 3

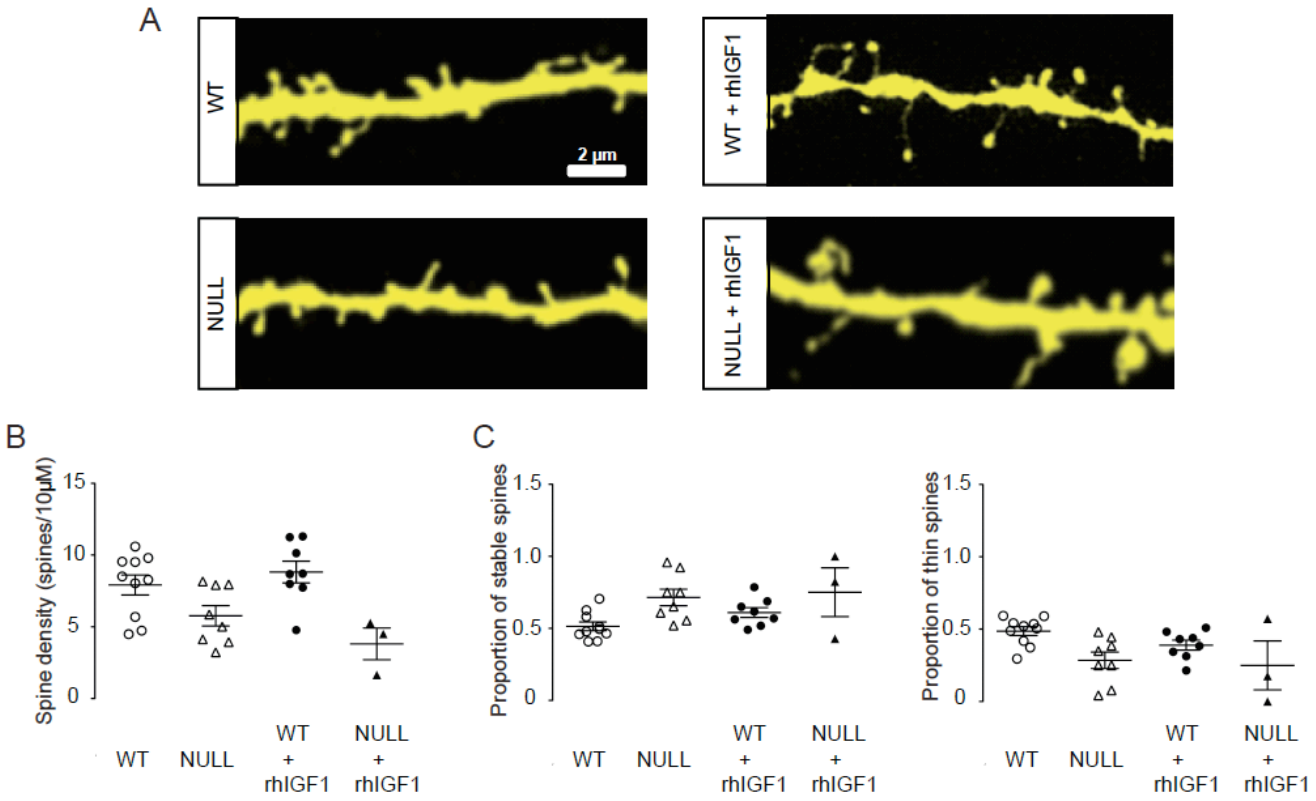

Figure S3. Treatment with rhIGF-I does not improve spine density in hippocampal neurons from NULL mice. (A) *Mecp2*$^{NULL/Y}$ mice show lower density of dendritic spines in pyramidal neurons from several brain regions (1), including the hippocampus (2). Intriguingly, treatment with the N-terminal tripeptide 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*$^{NULL/Y}$ 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*$^{NULL/Y}$ 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/10µm, n=8) nor in *Mecp2*$^{NULL/Y}$ ones (3.81±1.10 spines/10µm, 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 ($p>0.05$).

Supplemental Figure 4

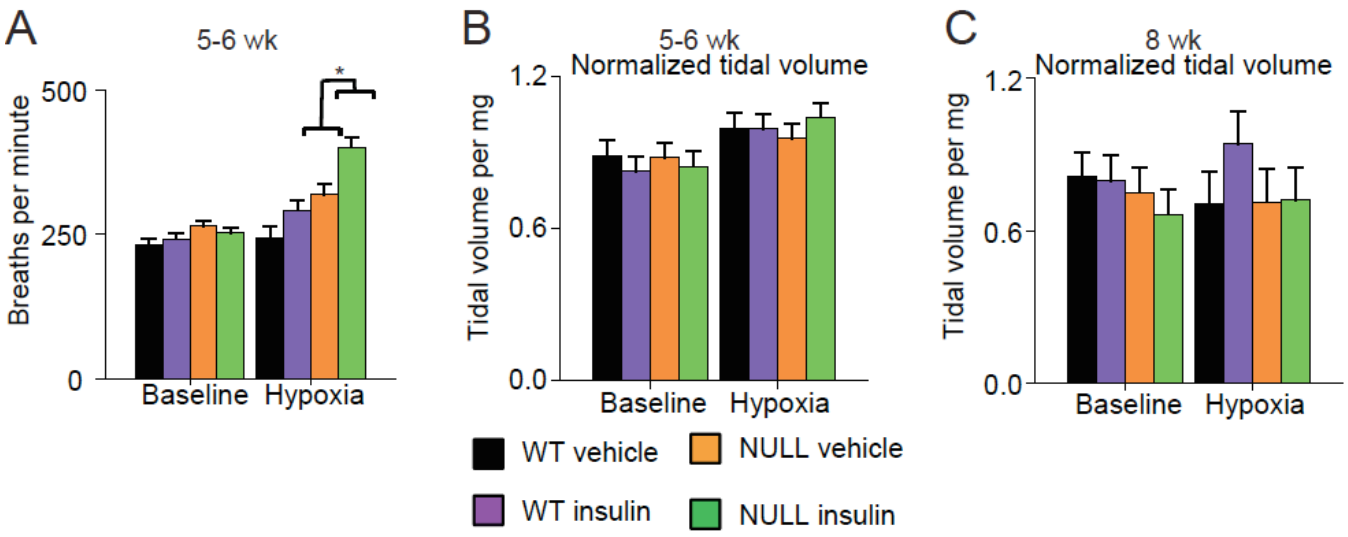

Figure S4. Treatment of NULL animals with insulin. (A) Surprisingly, treatment with insulin increased the breathing response to hypoxia in both WT as well as NULL animals, but had no effect on breathing rates at baseline in either genotype. **(B, C)** Insulin treatment had no effect on tidal volume at 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*$^{NULL/Y}$ 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 semi-automatically 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 post-hoc.

Supplemental References

1 Belichenko, P. (2009) Widespread changes in dendritic and axonal morphology in Mecp2-mutant mouse models of Rett syndrome: evidence for disruption of neuronal networks. *J. Comp. Neurol.*, **514**, 240-258.

2 Chapleau, C.A., Boggio, E.M., Calfa, G., Percy, A.K., Giustetto, M. and Pozzo-Miller, L. (2012) Hippocampal CA1 Pyramidal Neurons of Mecp2 Mutant Mice Show a Dendritic Spine Phenotype Only in the Presymptomatic Stage. *Neural plasticity*, **2012**, 976164.

3 Tropea, D. (2009) Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. *PNAS*, **106**, 2029-2034.

4 Chapleau, C. (2008) The actions of BDNF on dendritic spine density and morphology in organotypic slice culture depend on the presence of serum in culture media. *J. Neurosci. Methods*, **169**, 182-190.

5 Rodriguez, A. (2008) Automated three-dimensional detection and shape classification of dendritic spines from fluorescence microscopy images. *PloS one*, **3**, e1997.