

**Insertion of a knockout-first cassette in *Ampd1* gene leads to neonatal death by
disruption of neighboring genes expression**

Yongcheng Pan^{1,2#}, Lusi Zhang^{1,3#}, Qiong Liu¹, Ying Li¹, Hui Guo¹, Yu Peng¹,
Hexiang Peng¹, Beisha Tang^{1,2,4}, Zhengmao Hu¹, Jingping Zhao⁵, Kun Xia^{1, 6, 7*},
Jia-Da Li^{1,7*}

Supplementary Information

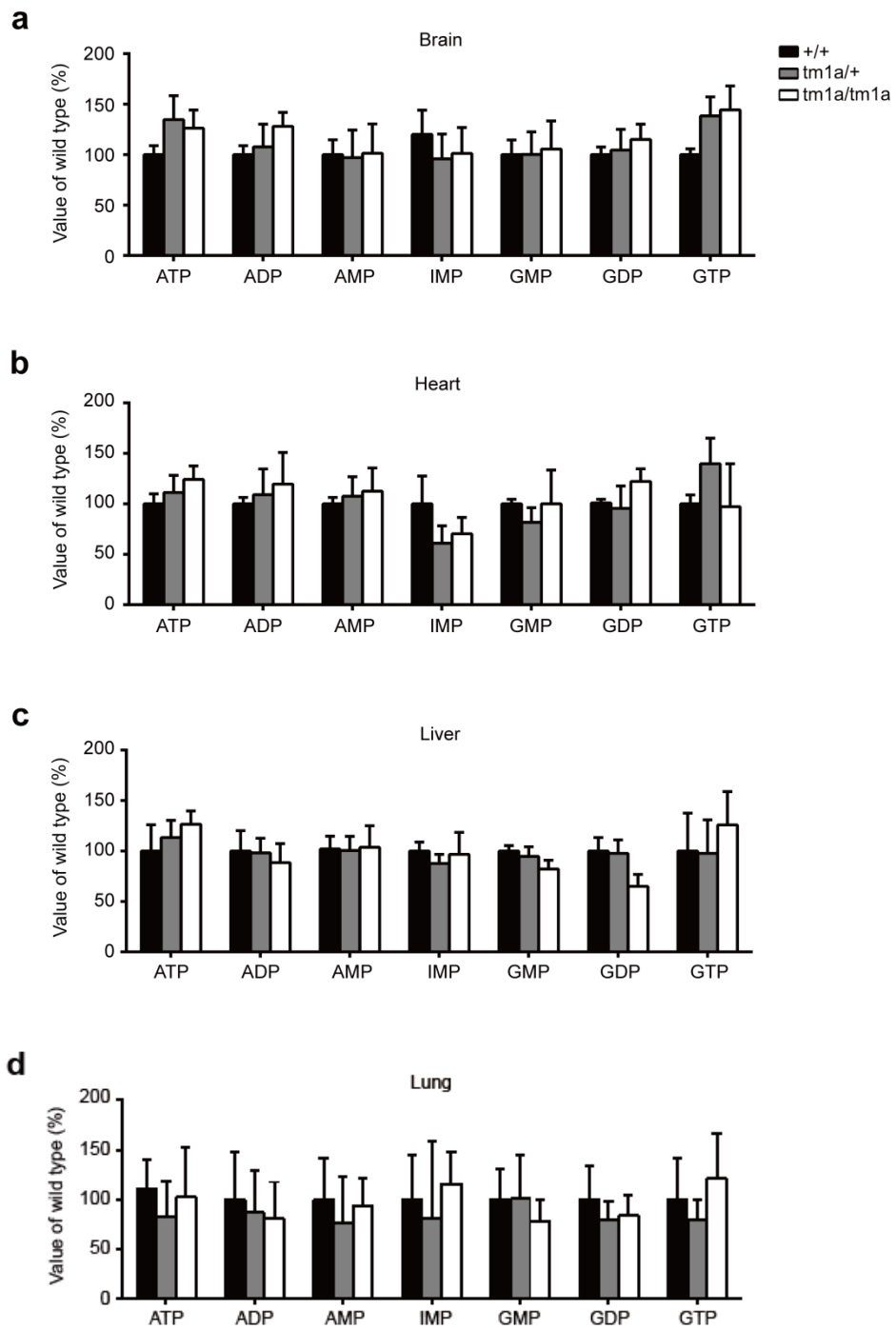
Supplementary Table 1: Differentially expressed genes identified by RNA sequencing and RT-PCR verification.

Gene	Chr/Strand	Start/End	RNA seq		RT-PCR	
			Value of WT	p-adjust	Value of WT	p-value
<i>Man1a2</i>	3/-	100562208/100685503	18.07% ±2.30%	9.070E-53	24.37% ±2.19%	0.0001
<i>Ptgfrn</i>	3/-	101040232/101110278	100.33% ±5.80%	1	107.37% ±23.93%	0.7776
<i>Igsf3</i>	3/+	101377125/101463060	101.78% ±4.13%	1	103.57% ±19.44%	0.8723
<i>Atp1a1</i>	3/-	101576219/101604603	113.65% ±0.79%	1	115.33% ±14.01%	0.4038
<i>Casq2</i>	3/+	102086415/102146514	84.78% ±7.41%	1	80.17% ±16.35%	0.4416
<i>Csde1</i>	3/+	103020546/103058189	101.64% ±7.75%	1	86.48% ±10.34%	0.1547
<i>Nras</i>	3/+	103058339/103067914	63.63% ±5.43%	3.314E-04	50.85% ±5.53%	0.0037
<i>Ampd1</i>	3/+	103074014/103099714	0.16% ±0.04%	6.089E-38	0.11% ±0.16%	0.0003

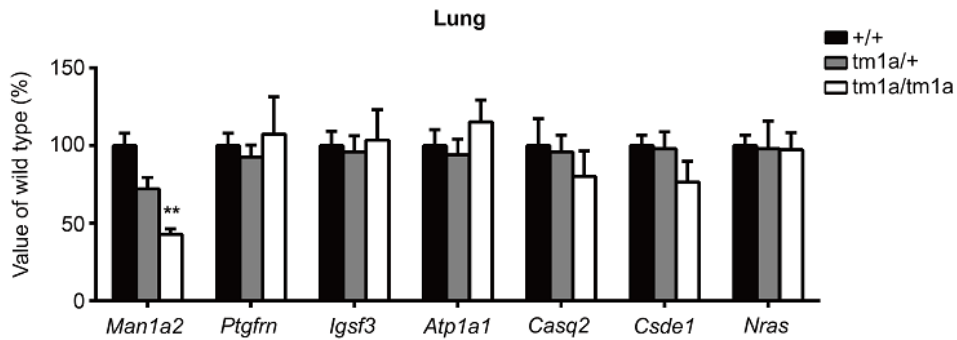
In RNA sequencing, muscle tissue come from E18.5 WT mice (n=3) and *Ampd1^{tmla/tmla}* mice (n=3). The P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Another group of E18.5 WT mice (n=5) and *Ampd1^{tmla/tmla}* mice (n=5) muscle were used for RT-PCR. Two-tailed Student's *t* test was used to determine the significance of differences, mean ± SEM.

Supplementary Table 2: Genotyping primers and PCR products size of different *Ampd1* mice.

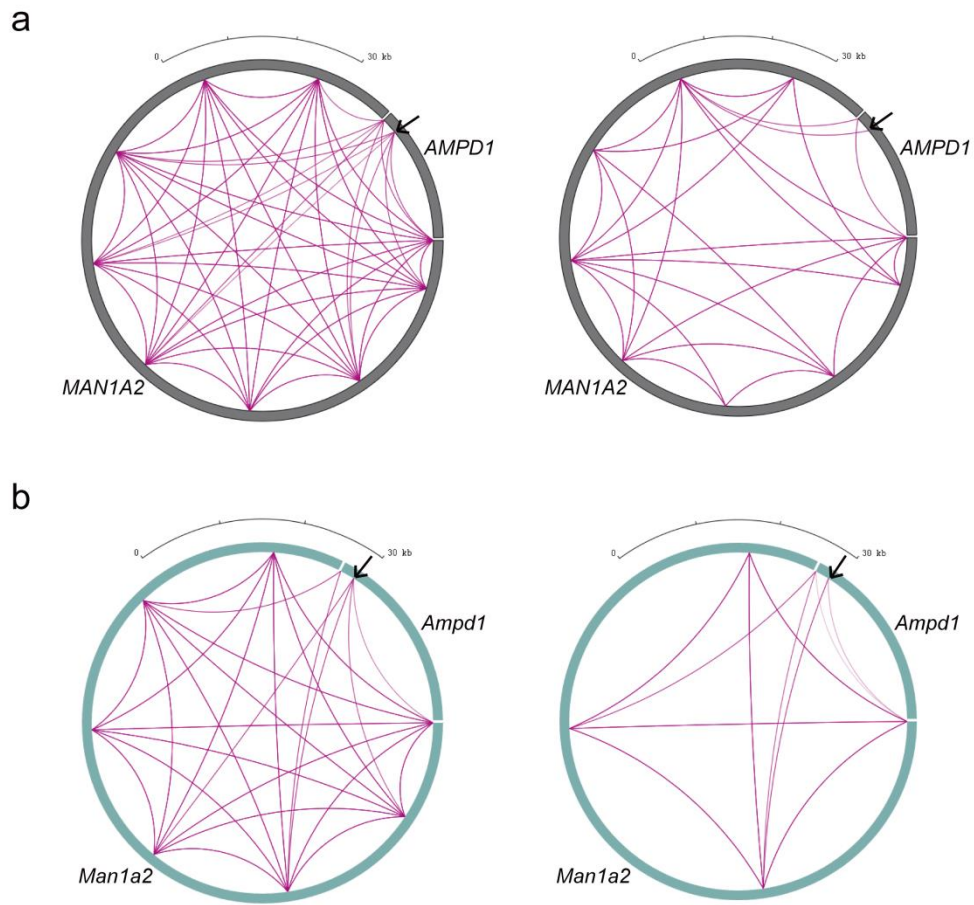
Allele	Primer	Sequence(5'-3')	Product size(bp)
<i>Ampd1</i> ^{tm1a}	WT-F	tgggctgacagctgcagttagca	WT:354bp tm1a/+:354bp & 410bp tm1a/tm1a:410bp
	loxP-F	gagatggcgcaacgcaattaat	
	Tm1a-R	ccaagaaaaagacagcacaggagacc	
<i>Ampd1</i> ^{tm1c}	Tm1c-F:	ggcaactccctttaccaca	WT:352bp tm1c/+:516bp & 352bp tm1c/tm1c:516bp
	Tm1c-R	tttcctgatctccaaccag	
<i>Ampd1</i> ^{tm1d}	Tm1d-F	ggcaactccctttaccaca	WT:1090bp tm1d/+:1090bp & 400bp tm1d/tm1d:400bp
	Tm1d-R	ggtaacacgcatgacaggtg	



Supplementary Figure 1: Nucleotide levels in the brain, the heart and the liver of E18.5 mice. There is no genotypic difference in the nucleotide levels of the brain (a), the heart (b), the liver (c) and the lung (d) in E18.5 *Ampd1*^{tm1a/tm1a} mice. n=5/each genotype, mean ± SEM.



Supplementary Figure 2: RNA level of different genes, which located between *Ampd1* and *Man1a2*, in the lung of *Ampd1^{tm1a/tm1a}* mice. *Man1a2* expression level was significantly decreased to 42.69% \pm 3.52% in lung of *Ampd1^{tm1a/tm1a}* mice, but the expression of genes located between *Man1a2* and *Ampd1* (*Ptgfrn*, *Igsf3*, *Atp1a2*, *Casq2*, *Csde1* and *Nras*) did not change in lung (n=5/each genotype, mean \pm SEM. As compared with WT, **P<0.01, two-way ANNOVA followed with Bonferroni's multiple comparisons test).



Supplementary Figure 3: Potential long range interactions between *Ampd1* and *Man1a2* gene. Hi-C results hosted in WashU Epigenome Browser indicated probable long range interaction between *Ampd1* and *Man1a2* in (a) human H1 embryonic stem cell or (b) mouse embryonic stem cell. Purple arc represented Long range interaction.