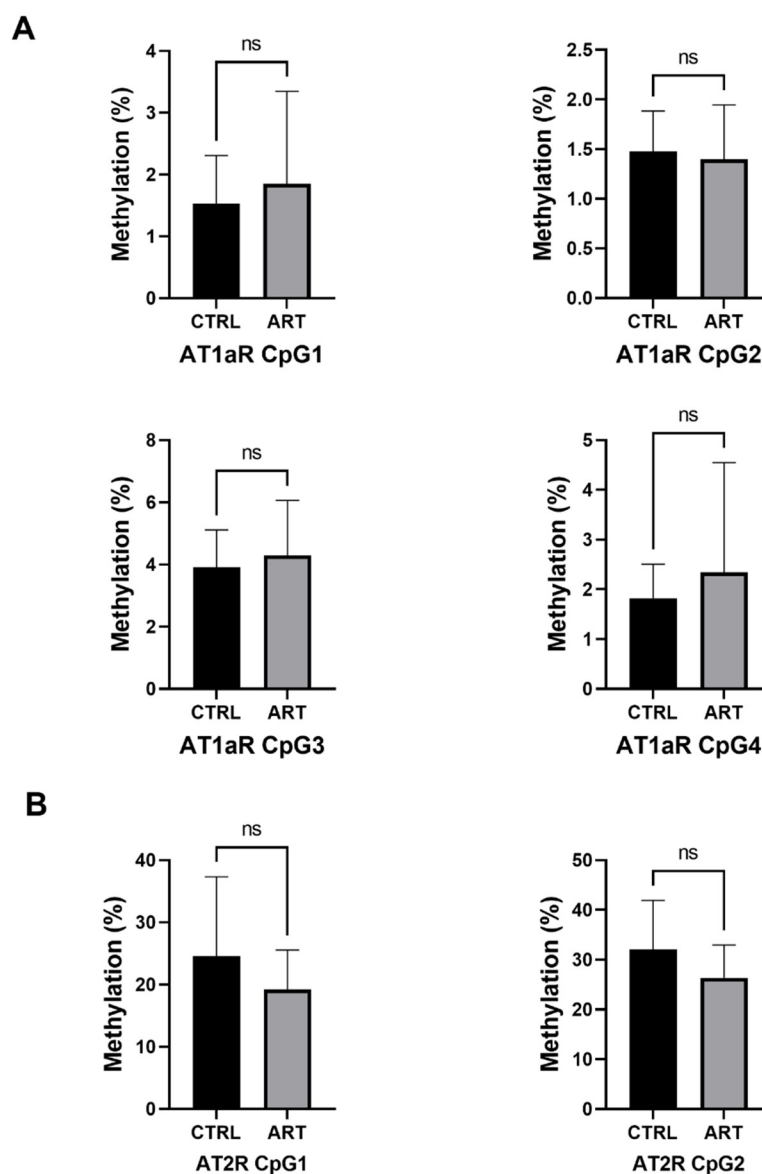


# Supplementary Materials



**Figure S1.** *At1aR* and *At2R* genes promoter DNA methylation level determined by bisulfite pyrosequencing ( $n \geq 10/\text{group}$ ). Values are mean  $\pm$  SD. **A)** *At1aR*; CpG1  $1.9 \pm 1.5$  vs.  $1.5 \pm 0.78$  [% of Methylation] ART vs. Ctrl;  $P = 0.5$ . CpG2  $1.4 \pm 0.55$  vs.  $1.5 \pm 0.41$  [% of Methylation] ART vs. Ctrl;  $P = 0.7$ . CpG3  $4.3 \pm 1.8$  vs.  $3.9 \pm 1.2$  [% of Methylation] ART vs. Ctrl;  $P = 0.5$ . CpG4  $2.3 \pm 2.2$  vs.  $1.8 \pm 0.68$  [% of Methylation] ART vs. Ctrl;  $P = 0.4$ . **B)** *At2R*; CpG1  $19 \pm 6.3$  vs.  $25 \pm 13$  [% of Methylation] ART vs. Ctrl;  $P = 0.2$ . CpG2  $26 \pm 6.6$  vs.  $32 \pm 9.9$  [% of Methylation] ART vs. Ctrl;  $P = 0.08$ .

## Supplementary material, promoter methylation in *At1aR*, *At1bR* and *At2R*

DNA methylation measured using bisulfite pyrosequencing in the promoters of *At1aR*, *At1bR* and *At2R* genes. Sequences uploaded from Mouse mm10 version. Primer designed using MethPrimer and assay designed using PyroMark q24 Advanced version 3.0.0.

**Table S1.** primers.

| gene  | Primer           | Sequence (5' to 3')             |
|-------|------------------|---------------------------------|
| AT1aR | AT1aR_TATA_F     | GTGTAAAGTTTTAGGGTTGGAATTT       |
| AT1aR | AT1aR_TATA_R_bio | Bio-ACCAAACAACATCATCCAATC       |
| AT1bR | AT1bR_F          | TAGAATTTGTTAAGGGAGGGGTTA        |
| AT1bR | AT1bR_R_bio      | Bio-TCCCTACTACCTAAAATCCAAACTAC  |
| AT2R  | AT2R_F           | GTTGAAGGTTTTTTAGTGGATAGAG       |
| AT2R  | AT2R_R_bio       | Bio-CAAAATACAATTTAAAATACAAAAAAA |

Bio- : biotin incorporated in the 5' end of the primer.

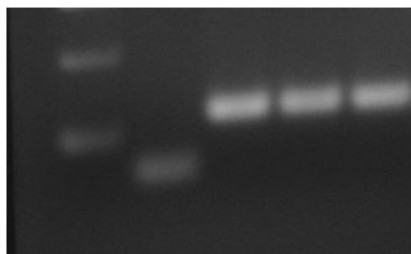
**Table S2.** Resume of pyrosequencing assays.

| gene  | original sequences  | sequence to analyse   | mm10 coordinates                | strand | amplicon size |
|-------|---|---|---------------------------------|--------|---------------|
| AT1aR | <u>GTGCAAAGCCTTAGGGTTGGAAC-</u><br><u>CTG(CG)<sup>1</sup>GAGTAGCAA(CG)<sup>2</sup>TCCCCCTAGGC-</u><br><b>TATAAA-</b><br>TATGGAAGTGCCT(CG) <sup>3</sup> CTGCTGAT(CG) <sup>4</sup> CCA<br>GGCAGTTGGGAGGGACTG-<br><u>GATGATGCTGTCTGGC</u>  | GYGGAGTAG-<br>TAAYGTTTTTTTAGGTTATAAA-<br>TATGGAAGTGTTTYGTTGTT-<br>GATYGTTAGGTAGTTGGGAGGGATTGG<br>ATGATGTTGTTTGGT  | chr13:30,336,361-<br>30,336,478 | +      | 118           |
| AT1bR | <u>CAGAATCTGTCAAGGGAGGGGTCAG-</u><br>GAGGGTCTTTAAGG(CG) <sup>1</sup> GGG(CG) <sup>2</sup> AGAAGG<br>GAAGGCAAGCCCAGACT(CG) <sup>3</sup> GATCCCAA-<br>GAAACAGCAA(CG) <sup>4</sup> CCCCTTTGCTG-<br><b>TATAAATT</b> GTAGCTGTCTC(CG) <sup>5</sup> CTAGGAT-<br>TACAGCTGCAGGCAGCCTGGATCCCAGGCAG-<br><u>CAGGGA</u>  | GGAGGGTTTTTAAGGYGGGGYGA-<br>GAAGGGAAGGTAAGTTTAGATTYG-<br>GATTTTAAGAAA-<br>TAGTAAYGTTTTTTTGTGTATAAATTG<br>TAGTTGTTTTYGTAGGATTATAGTT-<br>GTAGGTAGTTTGGATTTTAGGTAG-<br>TAGGGA  | chr3:20,367,144-<br>20,367,310  | -      | 167           |
| AT2R  | <u>GTTGAAGGCTCCCCAGTG-</u><br><u>GACAGAG(CG)<sup>1</sup>AATATATAAGAAGGAAAC-</u><br>CAGAGATCTGGTGCAG-<br>TTACATCTCAGAGGCTGG(CG) <sup>2</sup> ATGGAGGGAG<br>CT(CG) <sup>3</sup> GAACTGAAAGCTTACTTCAGCCTG-<br>CATTTTAAGGTAAGGCAGAACAATTTACACAT<br>GCTTGACTAGGGAAGGGGAACATACAGCAG-<br>TAAAAATGTTTCTGTGTGTG(CG) <sup>4</sup> TGTATGAAT<br><u>GTTTTCTTTTCTGTATTTTAACTGCATTTTG</u> | YGAATATATAAGAAGGAAATTAGA-<br>GATTTGGTGTAGTTATATTTTA-<br>GAGGTTGGYG-<br>ATGGAGGGAGTTYGGAATTGAAAGTTT<br>ATTTTAGTTT-<br>GTATTTTAAGGTAAGGTAGAA-<br>TAATTTATATATGTTT-<br>GATTAGGGAAGGG-<br>GAATATATAGTAGTAAAAATGTTTTTG<br>TGTGTGYGTG-<br>TATGAATGTTTTTTTTTTTTT-<br>GTATTTTAATTGTATTTTG | chrX:21,484,567-21,484,811      | +      | 245           |

Predicted TATA boxes in bold. Primers positions underlined. Transcripts based in italic. CG sites numerated. "Y" corresponds to the C of the CpG analysed for methylation.

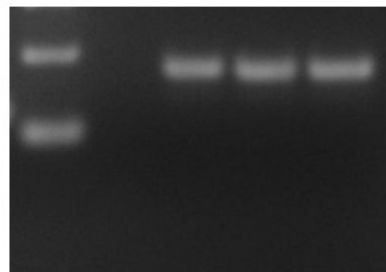
## Resume of amplification

### PCR 22mars2016 AT1aR



100 bp MM / BS DNA from mouse :  
0ng / 50ng / 100ng / 150ng /  
Assay AT1aR (118 bp) TM = 54°C

### PCR 22mars2016 AT1bR



100 bp MM / BS DNA from mouse :  
0ng / 50ng / 100ng / 150ng /  
Assay AT1bR (167 bp) TM = 56°C

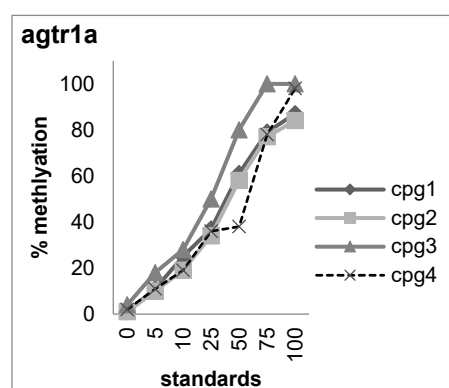
### PCR 23 mars 2016 AT2R



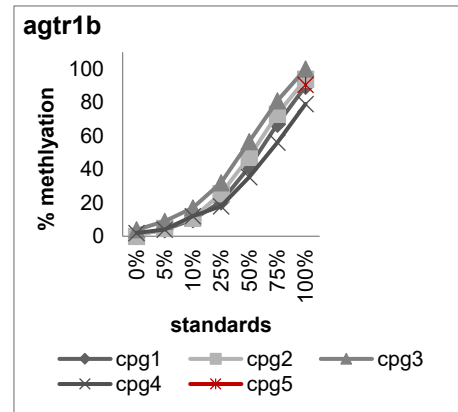
100 bp MM      0 ng      50      100      150      MM  
TM = 54 °C Assay **AT2R (245 bp)**

Validation of assay using methylated standards varying between 0 and 100 % methylation.

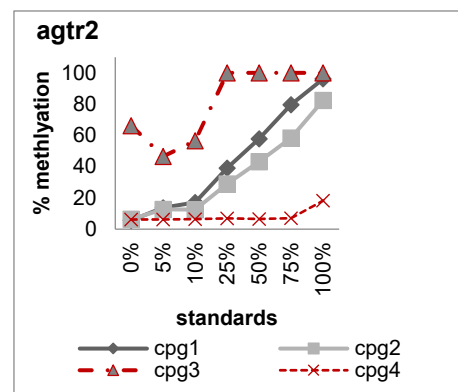
| AT1aR (all sites validated) |      |      |      |      |
|-----------------------------|------|------|------|------|
| standard                    | cpg1 | cpg2 | cpg3 | cpg4 |
| 0%                          | 1    | 1    | 4    | 2    |
| 5%                          | 11   | 10   | 18   | 11   |
| 10%                         | 25   | 19   | 28   | 19   |
| 25%                         | 37   | 34   | 50   | 36   |
| 50%                         | 61   | 58   | 80   | 38   |
| 75%                         | 79   | 77   | 100  | 78   |
| 100%                        | 87   | 84   | 100  | 98   |



| AT1bR (all sites validated) |      |      |      |      |      |
|-----------------------------|------|------|------|------|------|
| standard                    | cpg1 | cpg2 | cpg3 | cpg4 | cpg5 |
| 0%                          | 1    | 0    | 4    | 2    | ND   |
| 5%                          | 4    | 5    | 9    | 4    | ND   |
| 10%                         | 10   | 11   | 17   | 12   | ND   |
| 25%                         | 21   | 26   | 32   | 18   | ND   |
| 50%                         | 41   | 47   | 57   | 35   | ND   |
| 75%                         | 67   | 73   | 81   | 56   | ND   |
| 100%                        | 90   | 94   | 100  | 79   | 91   |

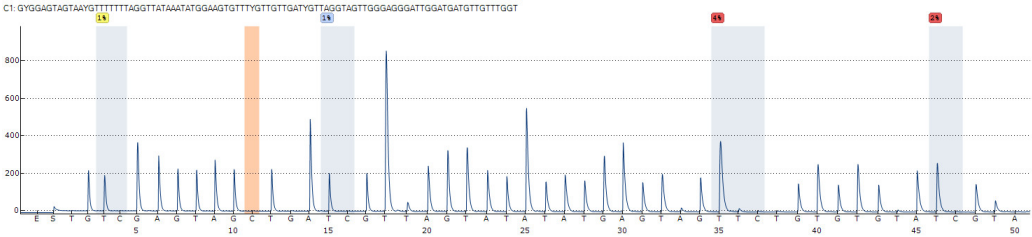


| AT2R assay, CpG1 and CpG2 validated |      |      |      |      |
|-------------------------------------|------|------|------|------|
| standard                            | cpg1 | cpg2 | cpg3 | cpg4 |
| 0%                                  | 6    | 6    | 66   | 6    |
| 5%                                  | 14   | 13   | 47   | 6    |
| 10%                                 | 17   | 12   | 57   | 6    |
| 25%                                 | 39   | 29   | 100  | 7    |
| 50%                                 | 58   | 43   | 100  | 6    |
| 75%                                 | 80   | 58   | 100  | 7    |
| 100%                                | 96   | 82   | 100  | 18   |

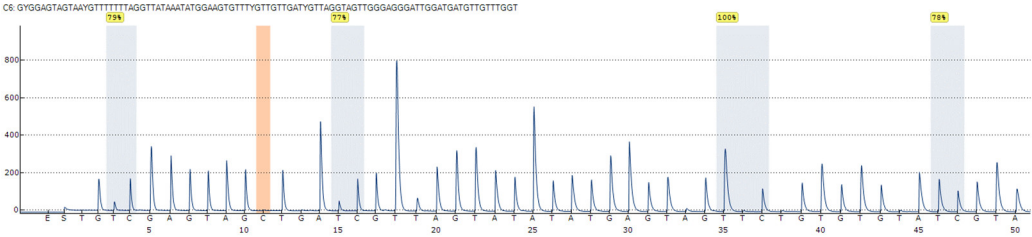


Illustrative pyro-histograms

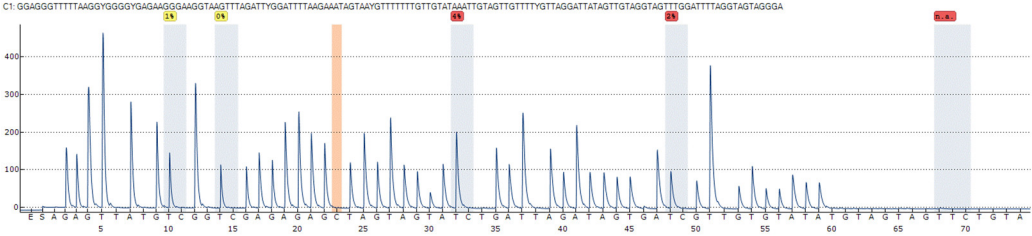
AT1AR - 0% methylated



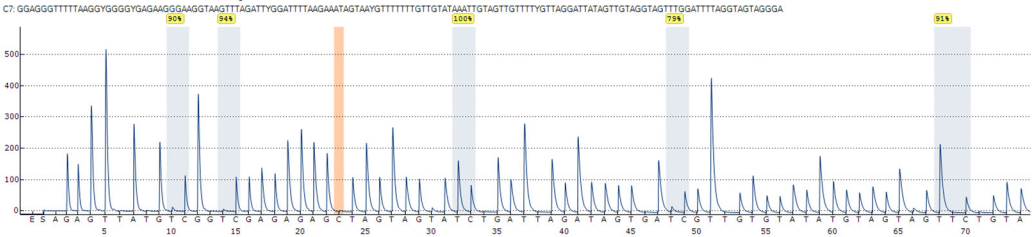
AT1AR - 75% methylated



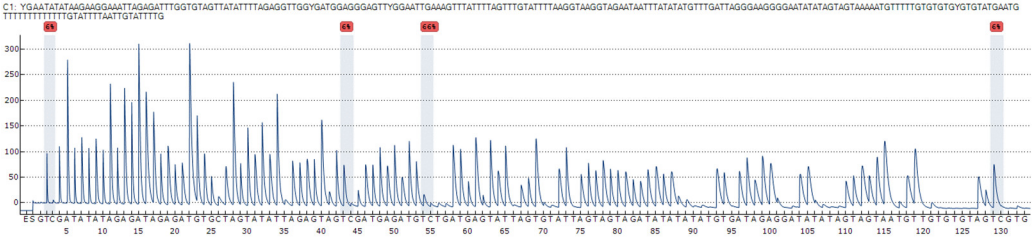
AT1BR - 0% methylated



AT1BR - 100% methylated



AT2R - 0% methylated



AT2R - 100% methylated

