

**Table S1.** Proposed hydrogen bonds between methyltransferase and cofactor based on the crystal structure of each methyltransferase with bound AdoHcy

| Enzyme              | PDB  | Substrate                 | <sup>a</sup> Amino acid CO <sub>2</sub> H       |                 |                                   | <sup>a</sup> Amino acid NH <sub>2</sub> |   |                                   | Nucleoside OH |  |                                   |                  |                  |
|---------------------|------|---------------------------|---|-----------------|-----------------------------------|---|---|-----------------------------------|---------------|--|-----------------------------------|------------------|------------------|
|                     |      |                           | L1  | L4              | X                                 | L1                                      | L4  | X                                 |               | N6   | N1                                | N3               |                  |
| HhaI                | 1MHT | DNA C (C5)                | L <sub>21</sub> G <sub>23</sub>                 |                 | S <sub>305</sub>                  |   |   |                                   |               | E <sub>40</sub>  | D <sub>60</sub>                   | I <sub>61</sub>  | W <sub>41</sub>  |
| DNMT2               | 1G55 | DNA C (C5)                | V <sub>13</sub> G <sub>15</sub>                 |                 | S <sub>376</sub>                  |   | S <sub>76</sub>                                 |                                   |               | D <sub>34</sub>  | E <sub>58</sub>                   | I <sub>57</sub>  | Y <sub>10</sub>  |
| PvuII               | 1BOO | DNA C (N4)                | G <sub>273</sub> S <sub>276</sub>               |                 |                                   |   |   | F <sub>250</sub>                  |               | H <sub>246</sub> E <sub>294</sub>                                  | D <sub>34</sub>                   | S <sub>33</sub>  |                  |
| TaqI                | 1AQI | DNA A (N6)                |   |                 | T <sub>23</sub>                   |   | E <sub>45</sub> A <sub>47</sub> C <sub>48</sub> |                                   |               | E <sub>71</sub>  | D <sub>89</sub>                   | F <sub>90</sub>  | I <sub>72</sub>  |
| <sup>b</sup> DpnM   | 2DPM | DNA A (N6)                | E <sub>41</sub> G <sub>46</sub> A <sub>48</sub> |                 | K <sub>21</sub>                   |   | E <sub>41</sub> F <sub>43</sub>                 | D <sub>194</sub>                  |               | W <sub>17</sub> D <sub>62</sub>                                    | D <sub>177</sub>                  | F <sub>178</sub> | F <sub>63</sub>  |
| T4dam               | 1Q0T | DNA A (N6)                | S <sub>37</sub>                                 |                 | K <sub>11</sub>                   |   |   | D <sub>171</sub>                  |               | Y <sub>7</sub> D <sub>50</sub> Q <sub>52</sub>                     | F <sub>184</sub>                  | F <sub>157</sub> |                  |
| <sup>b,d</sup> RsrI | 1NW5 | DNA A (N6)                |   |                 | K <sub>227</sub> S <sub>253</sub> |   |   | D <sub>65</sub>                   |               | H <sub>223</sub> D <sub>271</sub>                                  | D <sub>46</sub>                   | C <sub>47</sub>  | A <sub>272</sub> |
| <sup>b</sup> RsrI   | 1NW7 | DNA A (N6)                |   |                 |                                   |   |   | D <sub>65</sub> P <sub>66</sub>   |               | H <sub>223</sub> D <sub>271</sub>                                  | D <sub>46</sub>                   | C <sub>47</sub>  | A <sub>272</sub> |
| <sup>b</sup> MboII  | 1G60 | DNA A (N6)                | T <sub>225</sub> S <sub>223</sub>               |                 | K <sub>197</sub>                  |   |   | D <sub>30</sub>                   |               | D <sub>241</sub>   | N <sub>11</sub>                   | C <sub>12</sub>  | M <sub>242</sub> |
| <sup>b</sup> FtsJ   | 1EIZ | RNA                       | G <sub>63</sub> G <sub>64</sub> G <sub>65</sub> |                 | S <sub>33</sub>                   |   | D <sub>57</sub> G <sub>59</sub>                 | D <sub>124</sub>                  |               | A <sub>1</sub> D <sub>83</sub>                                     | D <sub>99</sub>                   | F <sub>100</sub> |                  |
| Ermc                | 1QAN | RNA A (N6)                |   | I <sub>13</sub> | N <sub>101</sub>                  |   | G <sub>38</sub>                                 |                                   |               | E <sub>59</sub>  | D <sub>84</sub>                   | I <sub>85</sub>  |                  |
| Vp39                | 1AV6 | RNA OH                    | G <sub>72</sub> H <sub>74</sub>                 |                 | Q <sub>39</sub>                   |   | D <sub>68</sub>                                 | G <sub>138</sub>                  |               | D <sub>95</sub> R <sub>97</sub>                                    | V <sub>116</sub>                  |                  |                  |
| CheR                | 1AF7 | Protein E (O)             |   |                 | T <sub>94</sub> R <sub>98</sub>   |   | A <sub>123</sub> E <sub>129</sub>               | R <sub>230</sub>                  |               | D <sub>154</sub>   | A <sub>38</sub> N <sub>212</sub>  |                  |                  |
| Prmt1               | 1ORI | Protein R (N)             |   |                 | R <sub>54</sub>                   |   |   |                                   |               | H <sub>45</sub> E <sub>100</sub>                                   | E <sub>129</sub>                  | V <sub>128</sub> |                  |
| Prmt3               | 1F3L | Protein R (N)             |   |                 | R <sub>236</sub>                  |   | D <sub>258</sub>                                |                                   |               | D <sub>282</sub>   | E <sub>311</sub>                  | I <sub>310</sub> |                  |
| PIMT                | 1DL5 | Protein D-NH <sub>2</sub> | G <sub>85</sub> G <sub>87</sub>                 |                 |                                   |   | G <sub>83</sub>                                 |                                   |               | E <sub>107</sub>   | D <sub>134</sub>                  | G <sub>135</sub> | V <sub>106</sub> |
| <sup>c</sup> HPIMT  | 1I1N | Protein D-NH <sub>2</sub> | S <sub>88</sub>                                 |                 | S <sub>59</sub> H <sub>64</sub>   |   |   |                                   |               | D <sub>109</sub>   | D <sub>141</sub> T <sub>216</sub> | G <sub>142</sub> |                  |
| <sup>c</sup> PIMT   | 1JG1 | Protein D-NH <sub>2</sub> | S <sub>102</sub> N <sub>105</sub>               |                 |                                   |   | E <sub>97</sub> G <sub>99</sub>                 |                                   |               | E <sub>121</sub>   | D <sub>148</sub> L <sub>221</sub> |                  |                  |
| <sup>b</sup> DOT1L  | 1NW3 | Protein K (N)             | Q <sub>168</sub>                                |                 | T <sub>139</sub>                  |   | D <sub>161</sub> G <sub>163</sub>               |                                   |               | E <sub>186</sub>   | D <sub>222</sub>                  | F <sub>223</sub> |                  |
| <sup>b</sup> HemK   | 1NV8 | Protein Q (N)             | G <sub>129</sub>                                |                 | N <sub>197</sub>                  |   | D <sub>127</sub>                                |                                   |               | D <sub>151</sub>   |                                   |                  |                  |
| CmaAI               | 1KPG | Lipid C=C                 |   |                 | Y <sub>33</sub> S <sub>34</sub>   |   | G <sub>72</sub>                                 | I <sub>136</sub>                  |               | G <sub>74</sub> T <sub>90</sub> L <sub>94</sub> Q <sub>95</sub>    | E <sub>124</sub>                  |                  |                  |
| CmaA2               | 1KPI | Lipid C=C                 |   |                 | Y <sub>41</sub> S <sub>42</sub>   |   | G <sub>80</sub>                                 | L <sub>144</sub>                  |               | G <sub>82</sub> T <sub>102</sub> L <sub>103</sub> Q <sub>107</sub> | E <sub>132</sub>                  | W <sub>131</sub> |                  |
| PcaA                | 1L1E | Lipid C=C                 |   |                 | Y <sub>33</sub> S <sub>34</sub>   |   | G <sub>72</sub>                                 | I <sub>136</sub>                  |               | G <sub>74</sub> T <sub>94</sub> L <sub>95</sub> Q <sub>99</sub>    | W <sub>123</sub> E <sub>124</sub> |                  |                  |
| ChOMT               | 1FP1 | Small                     |   |                 | K <sub>274</sub>                  |   | D <sub>213</sub>                                |                                   |               | D <sub>240</sub>   | D <sub>260</sub>                  |                  |                  |
| IOMT                | 1FP2 | Small                     |   |                 | K <sub>253</sub>                  |   | D <sub>194</sub>                                |                                   |               | D <sub>219</sub>   | D <sub>293</sub>                  |                  |                  |
| <sup>b</sup> COMT   | 1VID | Small                     |   |                 | V <sub>42</sub>                   |   | G <sub>66</sub> S <sub>72</sub>                 | D <sub>141</sub>                  |               | E <sub>90</sub>  | S <sub>119</sub>                  | S <sub>119</sub> |                  |
| Gamt                | 1KHH | Small                     | M <sub>70</sub> I <sub>72</sub> A <sub>73</sub> |                 |                                   |   |   | D <sub>134</sub>                  |               | G <sub>60</sub> E <sub>89</sub> C <sub>90</sub>                    | E <sub>117</sub>                  | W <sub>116</sub> |                  |
| Pnmt                | 1HNN | Small                     | Y <sub>85</sub>                                 |                 | Y <sub>35</sub> Y <sub>40</sub>   |   | G <sub>79</sub> S <sub>80</sub>                 | A <sub>181</sub>                  |               | D <sub>81</sub> G <sub>101</sub>                                   | D <sub>158</sub>                  | V <sub>159</sub> |                  |
| <sup>b</sup> COMT   | 1KYZ | Small                     |   |                 | K <sub>265</sub>                  |   | D <sub>206</sub>                                |                                   |               | D <sub>231</sub>   | D <sub>251</sub>                  |                  |                  |
| Gnmt                | 1NBH | Small                     |   |                 | W <sub>30</sub> R <sub>40</sub>   |   | A <sub>64</sub>                                 | L <sub>136</sub>                  |               | D <sub>85</sub>  | N <sub>116</sub>                  | W <sub>117</sub> |                  |
| Hnmt                | 1JQD | Small                     | E <sub>65</sub>                                 |                 | E <sub>28</sub> H <sub>29</sub>   |   | G <sub>60</sub> D <sub>67</sub>                 | H <sub>140</sub> I <sub>142</sub> |               | E <sub>89</sub> Q <sub>94</sub>                                    | S <sub>120</sub> S <sub>121</sub> | S <sub>120</sub> |                  |
| <sup>b</sup> SAMT   | 1M6E | Small                     | N <sub>65</sub>                                 |                 |                                   |   | D <sub>57</sub>                                 |                                   |               | D <sub>98</sub>  | S <sub>129</sub>                  |                  |                  |

<sup>a</sup> The residues contacting the methionine moiety are grouped according to their position in the type I AdoMet-dependent methyltransferase fold: L1 indicates the loop following strand β<sub>1</sub>, L4 the loop following β<sub>4</sub> and X indicates residues outside the conserved domain. Residues forming side chain interactions are written in black, main chain interactions in blue and water mediated interactions in magenta. Conserved residues are highlighted in yellow.

<sup>b</sup> 2DPM, 1NW5, 1G60, 1EIZ, 1NW3, 1NV8, 1VID, 1KYZ and 1M6E were solved with bound AdoMet and 1IM8 was solved with AdoHcy.

<sup>c</sup> Hydrogen bonds with adenine N<sup>7</sup> were predicted: made by the main chain of Thr 216 in 1I1N and Ile 221 in 1JG1.

<sup>d</sup> AdoMet and AdoHcy have different conformations and different interactions with M.RsrI in 1NW5 and 1NW7, therefore both structures are included.