**Supplementary information**

***Experimental Section***

**DNA preparation**

To demonstrate the feasibility of the assay, a 300 bp DNA fragment model system over the NPY gene was PCR amplified using standard procedures with forward primer TGGGATGATCGCGCTCCAC and reverse primer GGGCTCTAGAGCTCCAGGG (Integrated DNA Technologies, Australia). Amplicons were then purified using QIAquick Gel extraction kit (Qiagen, Australia). To generate methylated sequences, amplicons were treated with SssI methyltransferase (New England Biolabs, USA) overnight as recommended by the manufacturer and purified using the Agentcourt AMPure XP kit (Beckman Coulter, Australia). Finally, the amplicons were treated with 5 μM biotin-14-dUTP (Thermo Fisher, Australia) and terminal transferase (TdT, New England Biolabs, USA) in a 25 μL reaction as recommended by the manufacturer. Reactions were then diluted 10 fold in water to 0.2 ng/μL prior to detection.

WGA DNA was generated using the REPLI-g UltraFast Mini kit (Qiagen, Australia) and purified using the DNeasy Blood and Tissue kit (Qiagen, Australia). An aliquot of WGA DNA was then treated with SssI methyltransferase overnight and purified to generate highly methylated genomic DNA. Genomic DNA from Jurkat cells representing before and after 5-Aza treatment were purchased from New England Biolabs.

To generate DNA fragments compatible for the assay, 50 ng of genomic DNA (both WGA and cell line derived) was enzymatically digested with the endonucleases DpnII and MseI (7.5 units each, New England Biolabs) at 37 ºC in a 20 μL reaction supplemented with the NEB Buffer 3.1 system to generate DNA fragments. Since both DpnII and MseI recognize 4-base sequences, the resulting fragments have average lengths of 128bp with 5' overhangs. This fragment lengths allow for efficient oxygen channelling while the 5' overhangs allow for "fill-in" reaction by a DNA polymerase to biotinylate the fragments. After 30 minutes, the reaction was supplemented to a final volume of 25 μL with 5 units of Klenow fragment (3'→5' exo-) DNA polymerase (New England Biolabs, USA) and 5 μM of biotin-14-dUTP, dATP, dGTP and dCTP and incubated at 37 ºC for another 30 minutes to "fill-in" the overhangs and biotinylate the fragmented DNA. Reactions were then heat inactivated at 75ºC for 20 minutes. Reactions were then diluted 10 fold in water to 0.2 ng/μL prior to detection.

**Oxygen channelling assay**

The commercial oxygen channelling chemistry, AlphaScreen® General Protein A Detection Kit (Perkin Elmer, Australia), was used in this study. Briefly, for each reaction, 50 ng of MBD2-Fc protein (New England Biolabs, USA) was conjugated to 1250 ng of Protein A Acceptor beads in 20 μL of 1x MBD binding buffer (New England Biolabs, USA) for 20 minutes at room temperature and then supplemented with 400 mM NaCl. 2 μL of diluted DNA (approximately 0.4 ng per reaction) was added immediately to the MBD2-Acceptor Beads, pipette mixed rigorously 20 times and left to incubate for 20 minutes at room temperature. 2500 ng of streptavidin donor beads in 5 μL of 1x MBD binding buffer was then added to each reaction and mixed by pippetting. Luminescence mediated by oxygen channelling chemistry[9] was immediately measured over a 30 min period at room temperature on the EnSpire® plate reader (Perkin Elmer, Australia).

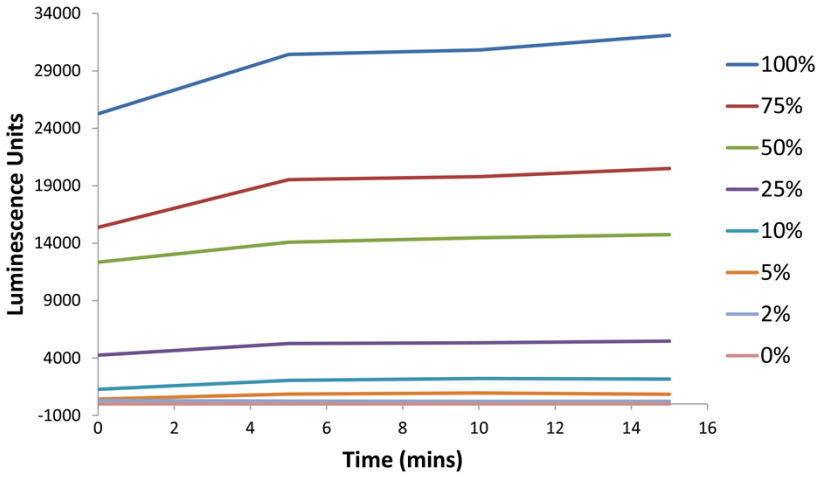


**Fig S1.** Representative time course of oxygen channelling mediated luminescence at various levels of % methylation.

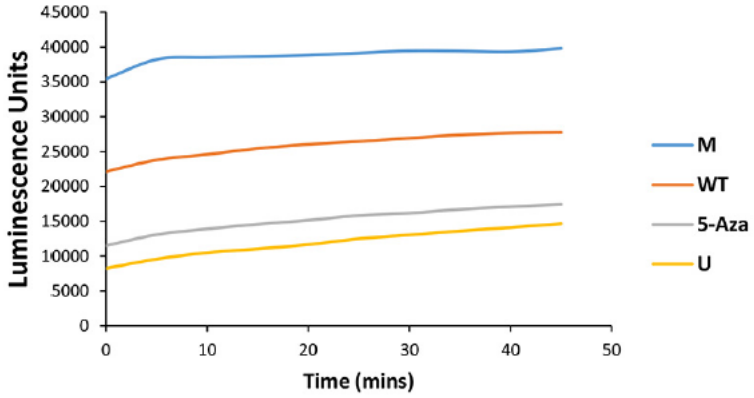


**Fig S2.** Representative time course of oxygen channelling mediated luminescence for (M) methylated WGA DNA control, (U) unmethylated WGA DNA control, Jurkat cells before (WT) and after (5-Aza) demethylating therapy.