

Supplementary Figure S1: MicroRNA profiling and absolute miR-206 concentrations in plasma

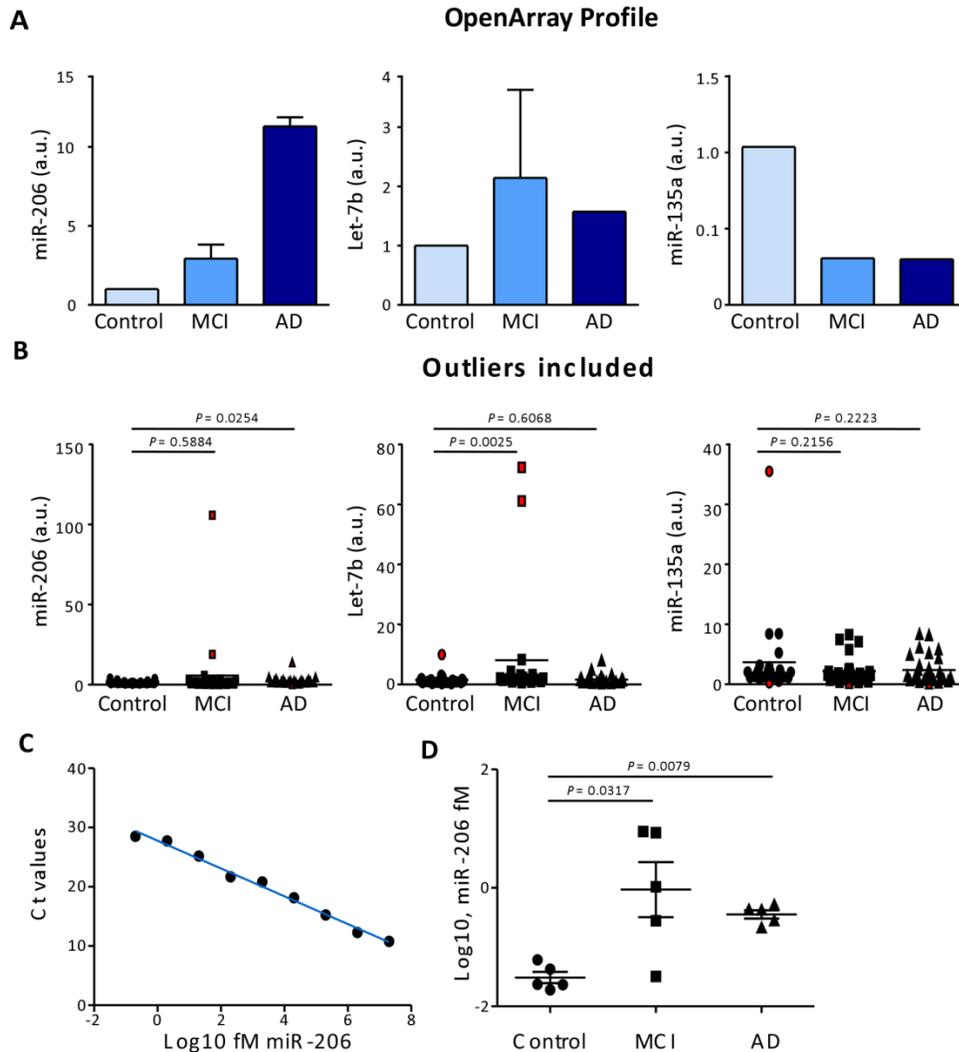


Figure S1. (A) Graphs showing increased miR-206 plasma concentrations in MCI and AD (left), increased Let-7b plasma concentrations in MCI (middle) and decreased miR-135a plasma concentrations in both MCI and AD (right) as detected by OpenArray (n = 2). (B) Individual RT-qPCR in validation cohort (with outliers included in red) shows increased miR-206 plasma levels in AD (left, MCI vs control $P = 0.5884$; AD vs control $P = 0.0254$; Mann-Whitney, n = 29 (control), n = 27 (MCI), 25 (AD)) and increased Let-7b plasma levels in MCI (middle, MCI vs control $P = 0.0025$; AD vs control, $P = 0.6068$, n = 24 (control), 22 (MCI), 23 (AD)). No

differences were observed for miR-135a between conditions (right, MCI vs control $P = 0.2156$; AD vs control, $P = 0.2223$, $n = 27$ (control), 26 (MCI), 23 (AD)). (C) Graph showing standard curve of miR-206 using 9 serial dilutions (1:10) of miR-206 mimics ranging from 20 nM - 0.2 fM analysed by RT-qPCR and plotted as Ct value against Log10 of femtomolar concentration. (D) Concentration of miR-206 (femtomolar) in small RNA extracts from plasma in control, MCI and AD subjects ($n = 5$ per condition). RT-qPCR was run at same time as standard curve. MiR-206 is increased in plasma of MCI ($P = 0.0317$, Mann-Whitney, $n = 5$) and in AD ($P = 0.0079$, Mann-Whitney, $n = 5$). * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure S2: MCI analysis with Last observation carried forward.

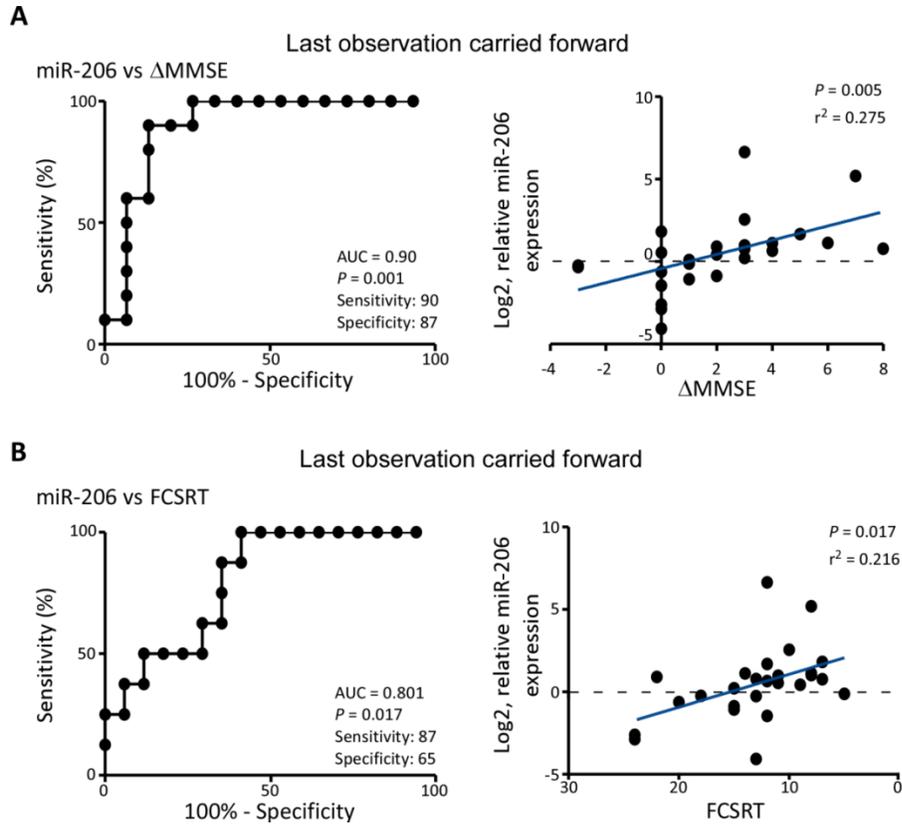


Figure S2. (A) MCI subjects with the inclusion of omitted subjects by Last observation carried forward (LOCF) method were grouped by changes in MMSE over 4 years. Relative miR-206 expression was significantly higher in cognitively declining group compared to stable MCI ($P = 0.001$; Mann-Whitney, $n = 15$ (MCI stable) and 10 (MCI decliners)) and plotting miR-206 against Δ MMSE showed a strong correlation ($P = 0.005$, $n = 25$). (B) MCI subjects including omitted subjects by LOCF method were grouped based on age-adjusted Free-cued-selective-reminding-test (FCSRT). Relative miR-206 expression was significantly higher in subjects below the FCSRT-free recall cut off ($P = 0.017$; Mann-Whitney, $n = 17$ (above cut-off) and 8 (below cut-off)) while relative expression of miR-206 showed a strong correlation with FCSRT-free scores ($P = 0.017$, $n = 25$).

Supplementary Figure S3: Development of a fast electrochemical detection method for miR-206 in plasma.

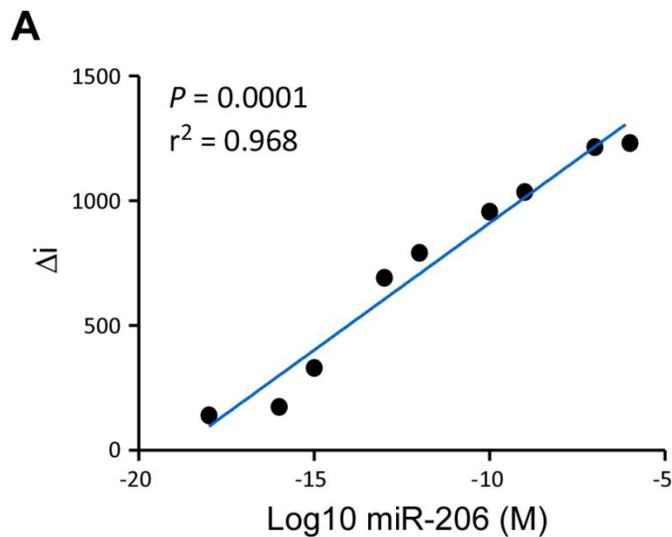


Figure S3: (A), Calibration curve for difference in current (Δi) before and after injection of hydrogen peroxide against known concentration of miR-206 oligonucleotides (linear between 100 nM – 100 μ M).