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Supplementary Information for

Oocyte Age and Preconceptual Alcohol Use are Highly Correlated with Epigenetic Imprinting of a non-coding RNA (*nc886*)

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This PDF file includes:

Figures S1 to S6

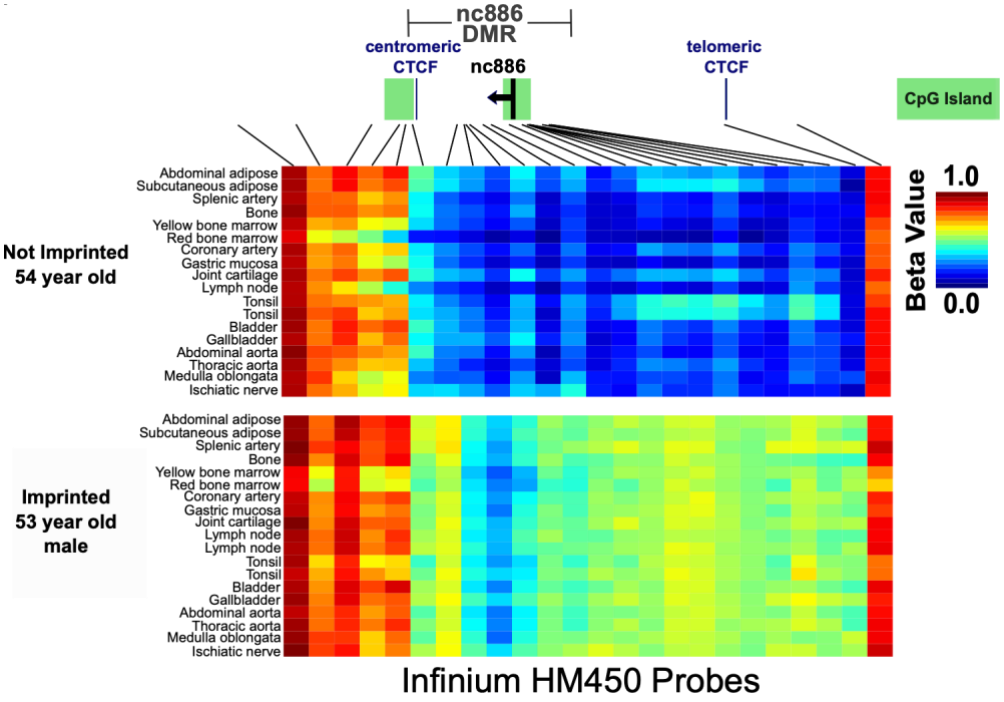

Fig. S1. Imprinting status across the *nc886* DMR is consistent across all tissue types in an individual. Beta values for DNA methylation were reanalyzed from Lokk et al. (17). Data are from Illumina Infinium 450K arrays across chr5:136075450−136084330 (hg38) and are plotted for two individuals from somatic tissues collected at autopsy. Data was obtained from GSE50192 Lokk et al. (17).

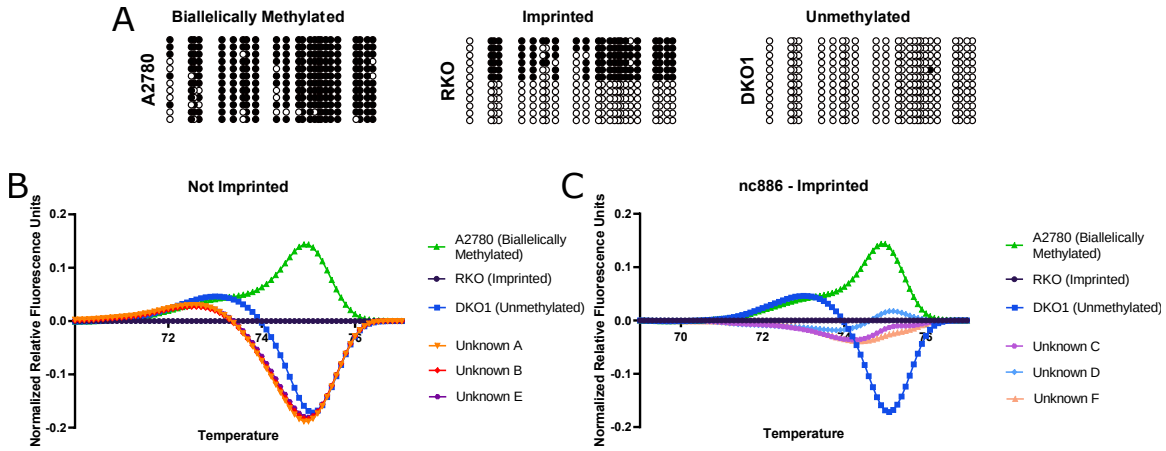

Fig. S2. High resolution melt curve analysis to determine imprinting of the *nc886* locus. A) Sodium bisulfite sequencing of control DNA from cancer cell lines where nc886 is biallelically methylated (A2780), imprinted (RKO), or unmethylated (DKO1). B) Melt curve analysis, normalized to RKO (imprinted), for control samples and three unknown samples determined to be “Not Imprinted”, C) Melt curve analysis, normalized to RKO (imprinted) for control samples and three unknown samples determined to be “Imprinted”.

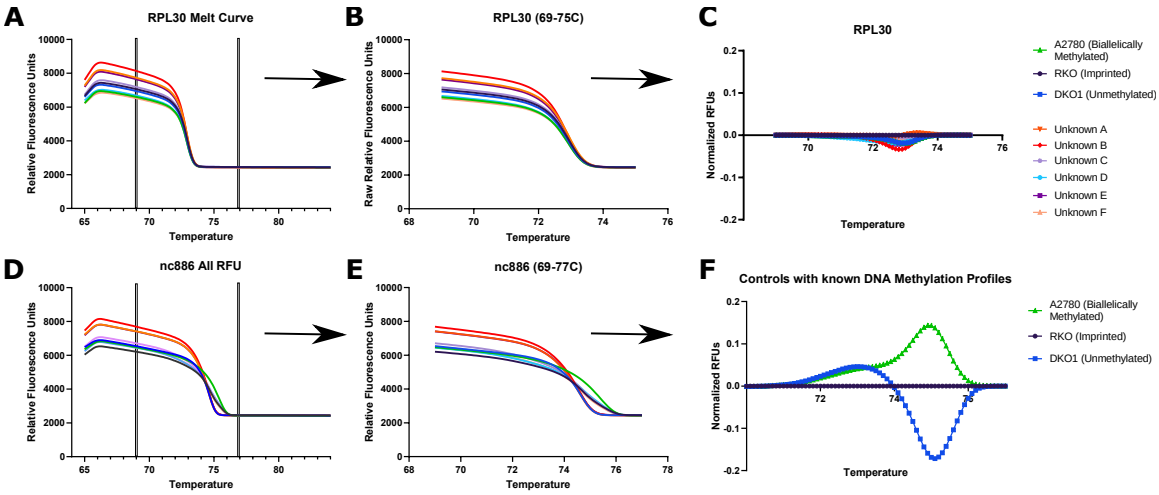

Fig. S3. Melt curve analysis for DNA methylation at *nc886*. Melt curves as measured in raw relative fluorescence units at each 0.1^{o}C decrease in temperature for *RPL30* (A) and *nc886* (D). For *RPL30*, melt curves were analyzed from 69^{o}C-75^{o}C (B). For *nc886*, melt curves were analyzed from 69^{o}C-77^{o}C (E). Melt curves were normalized to DNA from the imprinted cell line RKO (C, F). Melt curves are shown for three control and six unknown samples in triplicate for *RPL30* (C). Melt curves are shown for control samples with known DNA methylation profiles in triplicate for *nc886* (F).

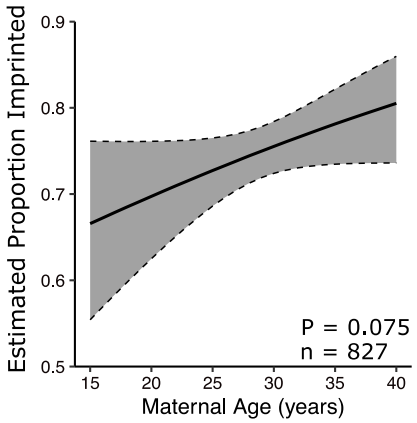

Fig. S4. Maternal age is positively correlated with imprinting of the *nc886* DMR in children at birth in Norway. Plot of proportion of individuals with imprinting at *nc886* with increasing maternal age, estimated using standard logistic regression with maternal age as the only covariate. Data from Markunas et al. (20), was analyzed in Carpenter et al. (10). Here, we reanalyzed Markunas et al. (20), as a continuous variable. Estimates were calculated. The shaded grey area is the 95% confidence interval ribbon.

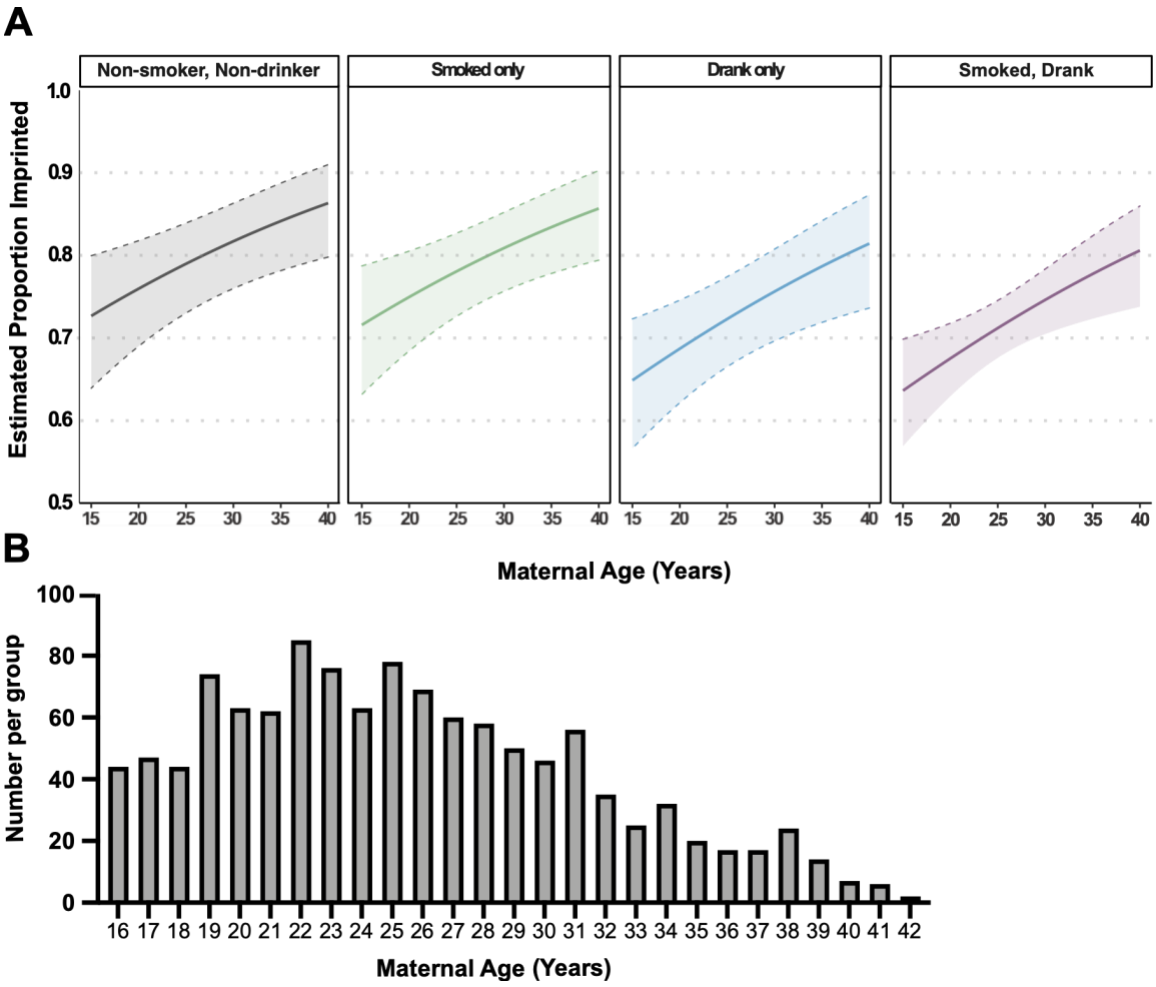

Fig. S5. Maternal age and drinking the year prior to pregnancy independently alter the probability that *nc886* will be imprinted. A) Plot of the estimated proportion of individuals with imprinting at *nc886* as a function of maternal years, separated by drinking and smoking behavior. Dotted lines represent 95% confidence interval ribbons. Estimates were calculated using standard logistic regression with drinking status (yes/no the year prior to pregnancy), smoking status (yes/no the year prior to pregnancy), and maternal age as covariates. Sample sizes from left to right: 120, 197, 203, 612. B) Histogram plotting the number of mothers at each age by year from the South African cohort.

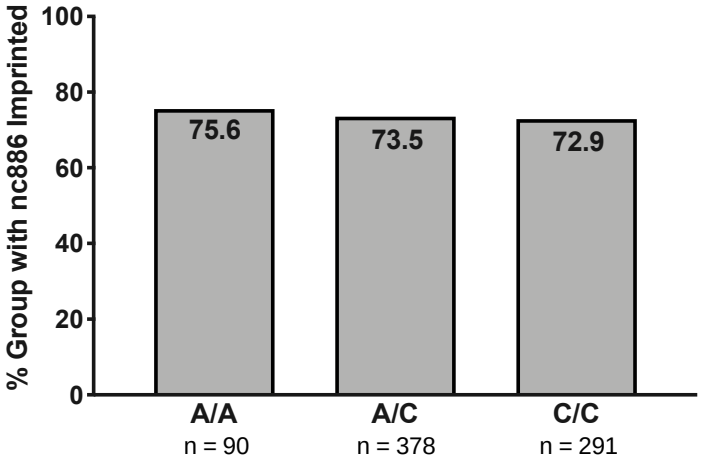

Fig. S6. SNP status at rs2346018, located in the centromeric CTCF site, does not alter the likelihood that *nc886* will be imprinted in children born in South Africa.
Histogram representing the percentage of each group, separated by genotype at rs2346018, that demonstrates imprinting at *nc886*.
P = 0.9688 (A/A - A/C), 0.9208 (A/A - C/C), 0.9682 (A/C - C/C).