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Supplemental Information

Methylation and Transcripts Expression at the Imprinted *GNAS* Locus in Human Embryonic and Induced Pluripotent Stem Cells and Their Derivatives

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Supplemental Information

Legends for supplemental figures

Figure S1 : Relative quantitative real-time expression of the five *GNAS* transcripts, Related to Figure 2. A color code is attributed to each fibroblasts (triangle), PSC (circles) and progeny (squares), in order to distinguish expression changes for a given Fibroblast/PSC/Progeny pair at each transcript.Fb: fibroblasts ; PSC: pluripotent stem cells ; Pro: progenies.

Figure S2 : Methylation quantification at the DMRs of *GNAS* comparing PSC, Related to Figure 3. Methylation at each of the four DMRs was similar. Cell cultures, quantification of methylation and statistical analysis were performed as described in the methods section. This figure shows the same PSC data than that presented figure 2, but showing in different columns data for hESC (❍) and hiPSC (●), in order to facilitate the comparison between the two types of PSC. The median for each group is indicated by the horizontal line.

Figure S3 : Methylation quantification at the four DMRs of *GNAS* in PSC compared to progenies, Related to Figure 4. This figure shows the same data than that presented figure 4. However a color code is attributed to each PSC (circles) and progeny (squares), in order to distinguish methylation changes for a given PSC/Progeny pair at each DMR. PSC: pluripotent stem cells; Pro: progenies.

Figure S4 : Analysis of mono- or bi-allelic expression of *GNAS* transcripts quantified by pyrosequencing, Related to Methods and Figure 5. Pyrograms of the TACAT C/T CTG sequence covering the rs7121 coding polymorphism (T393C) analyzed in PCR products obtained from gDNA (A) and cDNA (B and C) are shown for iPSC90_c17. (A) Heterozygosity at the gDNA level, indicated by 44.7 % and 55.3 % T and C respectively; (B) Biallelic expression of Gsα transcript indicated by 57.5 and 42.5 T and C respectively; (C) Monoallelic expression of *NESP55* transcript indicated by 98.3% and 1.7% T and C respectively. The rs7121polymorphism is indicated in bold letters (C/T). Heterozygosity at the *GNAS* polymorphism rs7121 (T393C) and transcript allelic expression analysis were analyzed as described in the methods section.

Figure S5 : Allelic transcript expression in parental fibroblasts, hiPSC and progenies, Related to Figure 5. Panels A and B : Results from parental fibroblasts 4603 and i90 respectively. This figure shows the same data than that presented figure 5. However, results from the two fibroblast lines are presented in different panels in order to distinguish DMR methylation changes between the two cell lines. Fb: fibroblasts ; Pro: progenies.

Table S1 : primer sequences, CpGs positions and PCR conditions for *GNAS* DMRs amplification and methylation analysis, Related to Methods

Table S2 : primer sequences and PCR conditions to analyze the *GNAS* polymorphism rs7121 on genomic DNA and transcript cDNA, Related to Methods

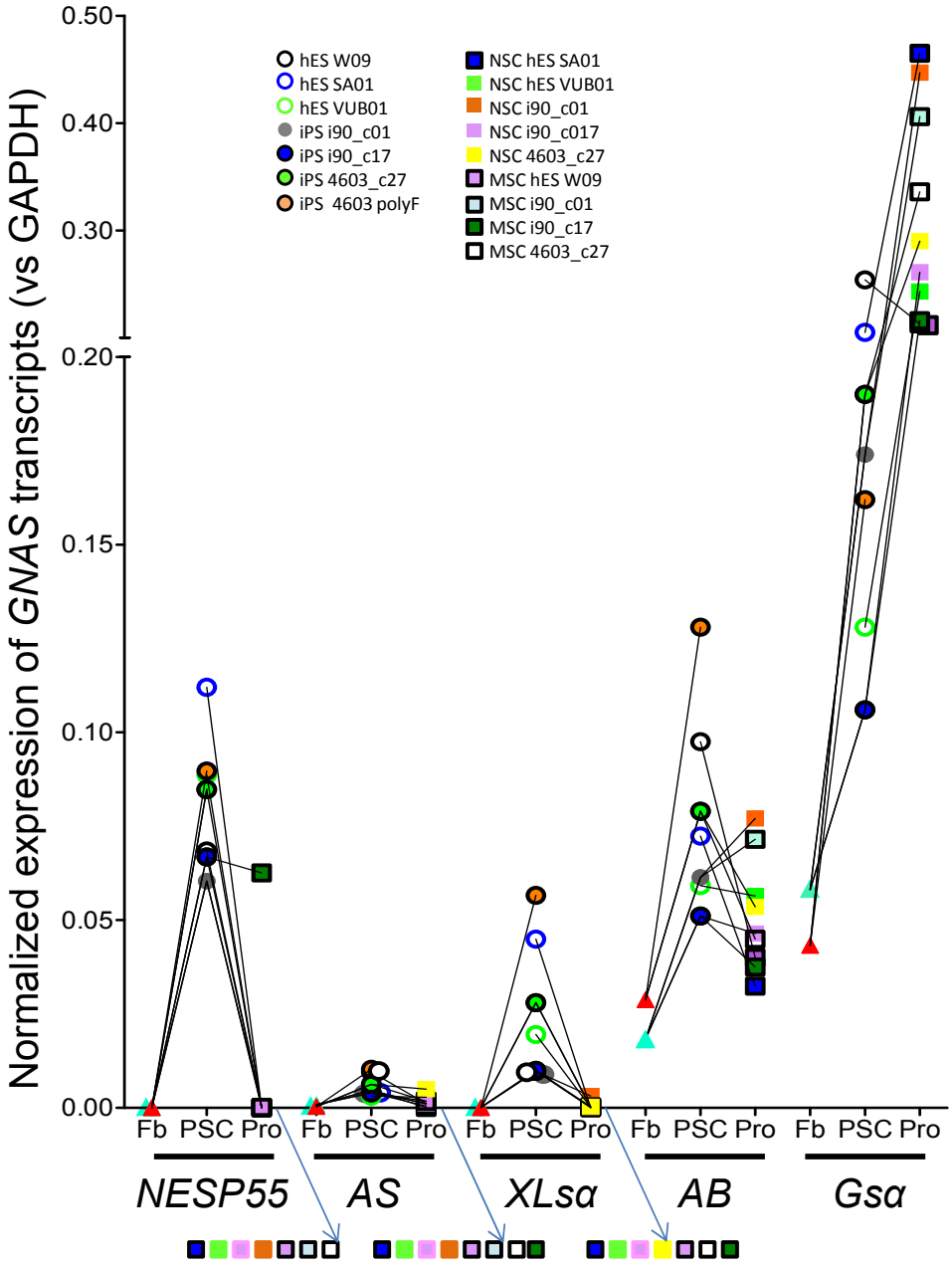

Figure S1, Related to Figure2

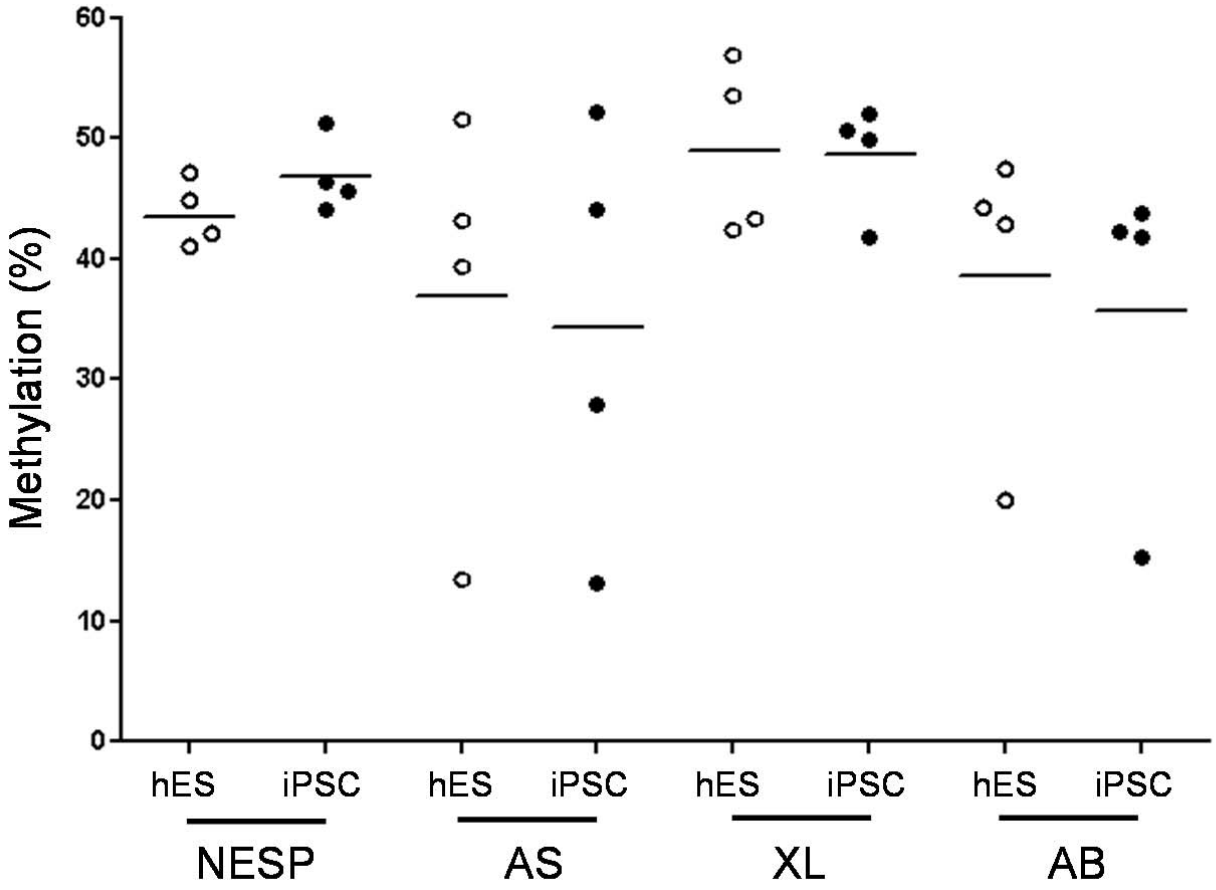

Figure S2, Related to Figure 3

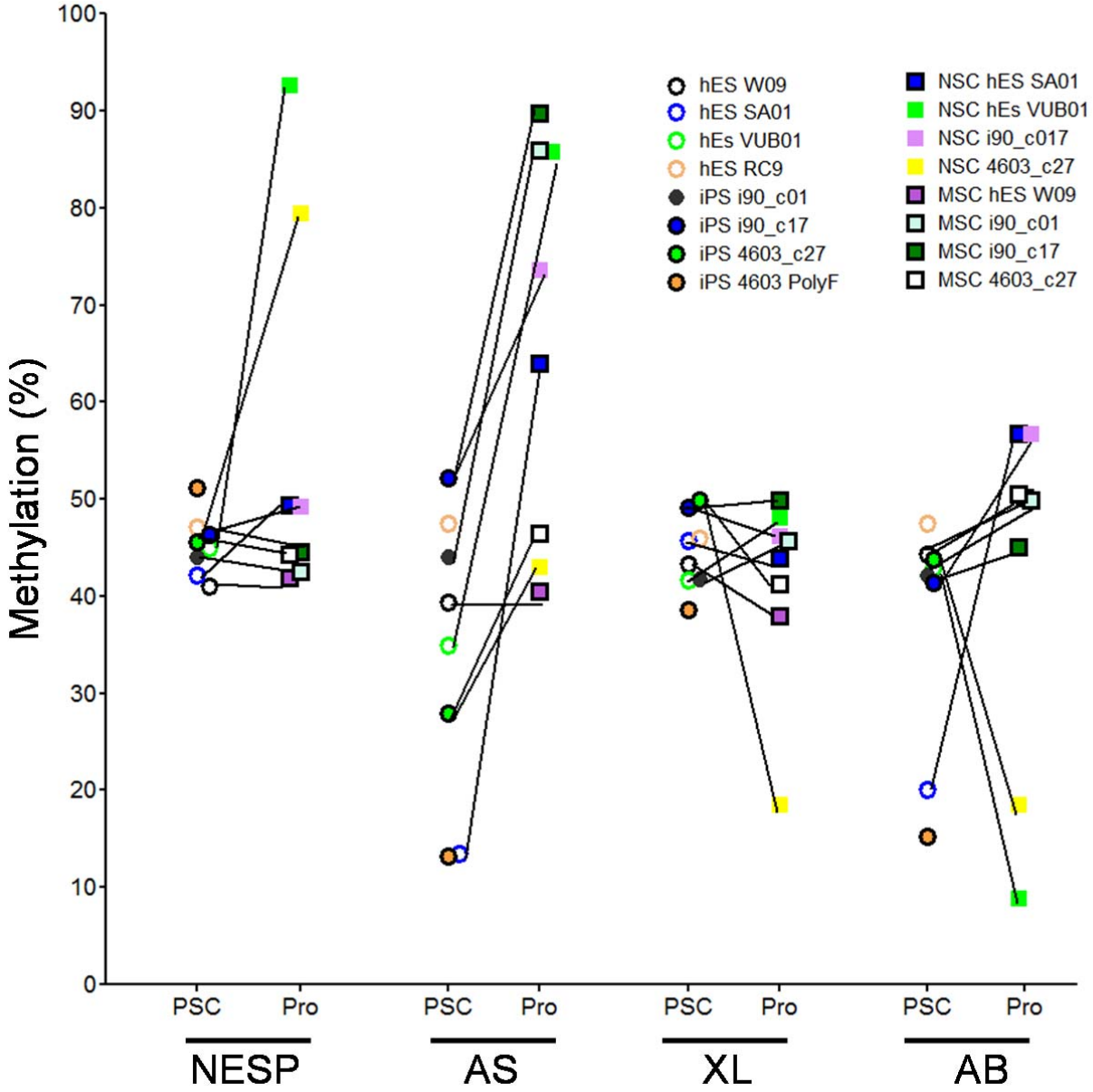

Figure S3, Related to Figure 4

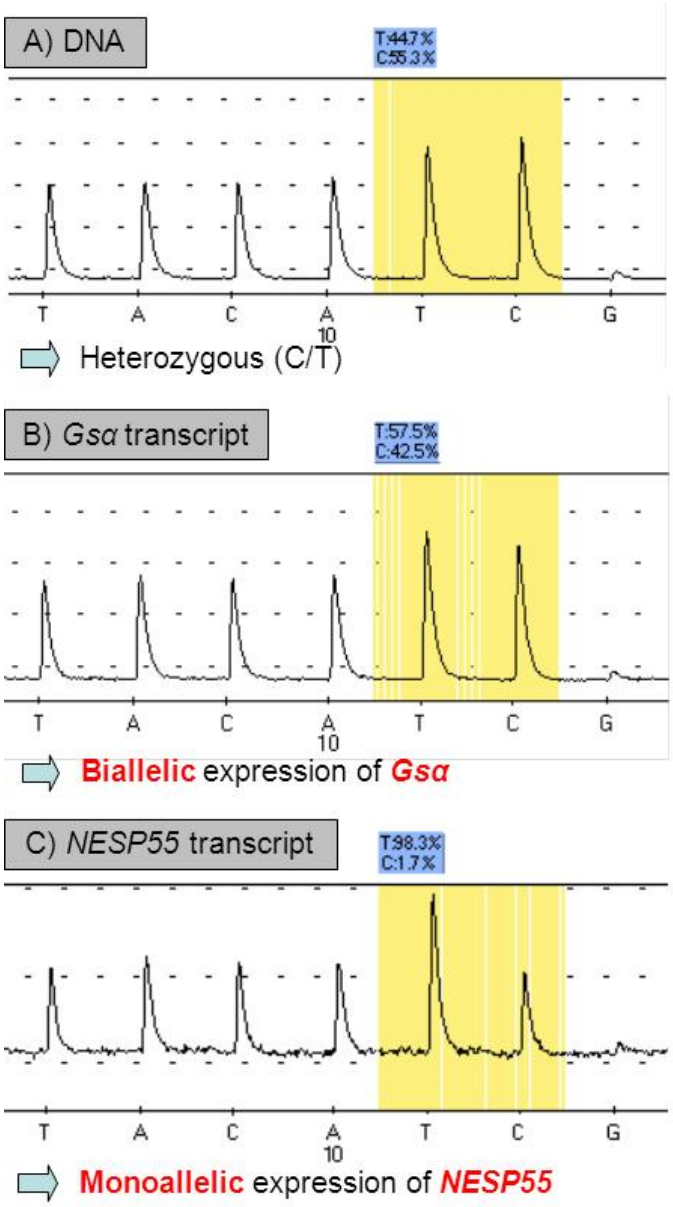

Figure S4, Related to methods and Figure 5

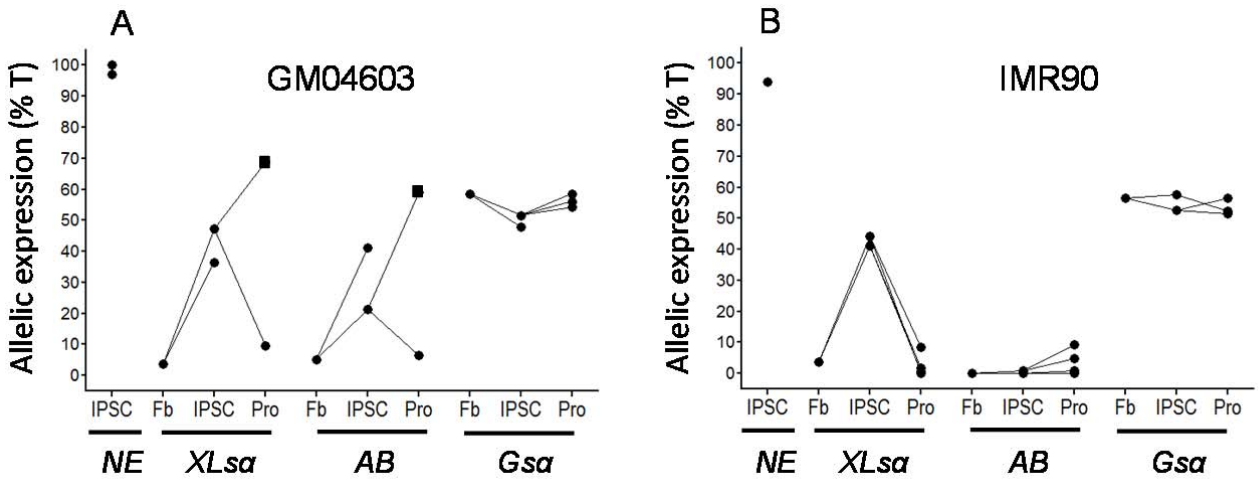

Figure S5, Related to Figure 5

Table S1: Primer sequences, CpGs position and PCR conditions for *GNAS* DMRs amplification and methylation analysis, Related to methods

PCR	**NESP1**	**NESP2**	**AS1**	**AS2**	**XL1**	**XL2**	**AB**
Forward primer	5'AGAGTTTTAGGGAAGGGGAGGA3'	5'GAAGGAGTTTAAGGAGGAGAAGTAG3'	5'GTAGTGGGGTTAAAGGAG3'	5'GTGGGTATTTATTTTTGGTTAGT3'	5'AGTTTATTTTAAGAGGTTGTTAGAT3'	5'GGGTAGTAGTTTTTGGATGGAGAT3'	5'GGGATATTTGAGAGTTTTGAAAGAA3'
Reverse primer[1]	5'ACTAACCTAAATCCATAAAAACAAA3'	5CCATAAAAACAAAAAAAATCTAAAC3'	5'CTAAACCCACAAATAAATACCAT3'	5'CTAAACCCACAAATAAATACCAT3'	5'ACTCCTTCCATCTCTACTACT3'	5'CATCTCTACTACTTCCTCCTCAACTAAA3'	5'AAAAATACAAAACCTCCCCTACTC3'
PCR conditions	5 min, 95°C; 55 cycles of (20s, 95°C; 20s, 56°C; 20s, 72°C); 7 min, 72°C	5 min, 95°C; 55 cycles of (30s, 95°C; 30s, 60°C; 30s, 72°C); 7 min, 72°C	5 min, 95°C; 55 cycles of (15s, 95°C; 15s, 56°C; 15s, 72°C); 7 min, 72°C	5 min, 95°C; 55 cycles of (30s, 95°C; 30s, 56°C; 30s, 72°C); 7 min, 72°C	5 min, 95°C; 55 cycles of (30s, 95°C; 30s, 54°C; 30s, 72°C); 7 min, 72°C	15 min, 95°C; 55 cycles of (30s, 95°C; 30s, 60°C; 30s, 72°C); 7 min, 72°C	5 min, 95°C; 55 cycles of (20s, 95°C; 20s, 56°C; 20s, 72°C); 7 min, 72°C
Sequencing primer 1	5'GTTGTAAGTTAAAGAAGTT3'		5'GGGGTTAAAGGAGTTG3'		5'GTTAATATGGATAGTTTT3'		5'AGGGTGTTTAGGTAAG3'

Sequencing primer 2				5'AGGGTGTTTAGGTAAG3'
CpGs position (Hg19)	57,415,837 57,415,840 57,415,845 57,415,847 57,415,853	57,426,926 57,426,931 57,426,935 57,426,942 57,426,950 57,426,957 57,426,970	57,429,310 57,429,312 57,429,319 57,429,324 57,429,340	57,463,629 57,463,653 57,463,658 57,463,665 57,463,679 57,463,686 57,463,693 57,463,700

The HotStarTaq DNA Polymerase (Qiagen) was used for the amplification of XL-PCR2 and the Platinum Taq DNA Polymerase (Life Technologies) was used for the others PCR

1: For DMR amplification, the reverse primers were biotinylated

Table S2 : Primer sequences and PCR conditions to analyze the *GNAS* polymorphism rs7121 on genomic DNA and transcript cDNA, Related to methods

PCR	**gPCR**	**RT-PCR_NESP**	**RT-PCR_XL**	**RT-PCR_AB**	**RT-PCR_Gs**
Forward primer[1]	5'TGAAAGCAGTACTCCTAACTGACA3'	5'CTTCCAAAAAGGGACCCATC3'	5'CGCAGTAAGCTCATCGACAA3'	5'CCTTGCGTGTGAGTGCACCT3'	5'CGTGAGGCCAACAAAAAGAT3'
Reverse primer[1]	5'TGTTCCTATATGGACACTGTGCT3'	5'ATGGCAGTCACATCGTTGAA3'			
PCR conditions	5min, 95°C; 35 cycles of (30s, 95°C; 30s, 60°C; 30s, 72°C); 7 min, 72°C ; hold 4°C	5min, 95°C; 40 cycles of (30s, 95°C; 30s, 58°C; 30s, 72°C); 7 min, 72°C ; hold 4°C			
Forward Sequencing primer[2]	5'GAGAACCAGTTCAGAGT 3'				
Reverse Sequencing primer[2]	5'GTCAGGCACGTTCATCACACTC 3'				

1. Amplifications were performed for each product with either the forward or reverse primer being biotinylated

2. As a function of the biotinylated primer used for cDNA amplification, either the forward or reverse sequencing primer was biotinylated