**SUPPLEMENTARY MATERIAL**

**Novel start codon variant in the 5’UTR of *LDLR* associated with Familial Hypercholesterolaemia.**

Martin Bird1, Chris Jyun-Peng Tung2, Alan Pittman1, Elijah R Behr1, Axel Nohturfft2, and Marta Futema1,3

1Cardiovascular and Genomics Research Institute, School of Health & Medical Sciences, City St George's, University of London, London, UK

2Neuroscience and Cell Biology Research Institute, School of Health & Medical Sciences, City St George's, University of London, London, UK

3Institute of Cardiovascular Science, Faculty of Population Health, University College London, London, UK

Corresponding author: mbird@sgul.ac.uk and mfutema@sgul.ac.uk

**SUPPLEMENTARY METHODS**

**S1 Reagents**

Reagents were sourced as follows: DMEM/F12 medium (Gibco brand) and oligonucleotides from Life Technologies (Paisley, UK); antibiotics, DL-mevalonolactone (Sigma brand, M4667), fetal bovine serum (Sigma brand), ortho-nitrophenyl-β-galactoside (ONPG) and single-stranded oligonucleotides from Merck (Darmstadt, Germany); 25-hydroxycholesterol from Cambridge Bioscience (Bar Hill, UK); fetal, bovine lipoprotein-deficient serum (LPDS; AlphaDiagnostics brand) and lovastatin from CliniSciences Ltd (Slough, UK); and double-stranded oligonucleotides from Life Technologies. To prepare a stock solution of mevalonic acid, mevalonolactone was dissolved in water, hydrolysed by adding an equal volume of 1N KOH, incubated at 37˚C for 1 hour and neutralized with 1M HCl; the final concentrations was then adjusted to 50 mM mevalonate and 1x phosphate-buffered saline.

**S2 Oligonucleotides replacing BmgBI-NcoI fragment of pLDLR-Luc2p corresponding to -97 to -2**

**Oligos (wild-type):**

5’- GTGGGCCCCGAGTGCAATCGCGGGAAGCCAGGGTTTCCAGCTAGGACACAGCAGGTCGTGATCCGGGTCGGGACACTGCCTGGCAGAGGCTGCGAG -3’

3’- CACCCGGGGCTCACGTTAGCGCCCTTCGGTCCCAAAGGTCGATCCTGTGTCGTCCAGCACTAGGCCCAGCCCTGTGACGGACCGTCTCCGACGCTCGTAC -5’

Lower strand 5’to3’:

5’-CATGCTCGCAGCCTCTGCCAGGCAGTGTCCCGACCCGGATCACGACCTGCTGTGTCCTAGCTGGAAACCCTGGCTTCCCGCGATTGCACTCGGGGCCCAC-3’

**Oligos (c.-35C>G):**

5’- GTGGGCCCCGAGTGCAATCGCGGGAAGCCAGGGTTTCCAGCTAGGACACAGCAGGTCGTGATGCGGGTCGGGACACTGCCTGGCAGAGGCTGCGAG -3’

3’- CACCCGGGGCTCACGTTAGCGCCCTTCGGTCCCAAAGGTCGATCCTGTGTCGTCCAGCACTACGCCCAGCCCTGTGACGGACCGTCTCCGACGCTCGTAC -5’

Lower strand 5’to3’:

5’-CATGCTCGCAGCCTCTGCCAGGCAGTGTCCCGACCCGCATCACGACCTGCTGTGTCCTAGCTGGAAACCCTGGCTTCCCGCGATTGCACTCGGGGCCCAC-3’

**Oligos (c.-22del):**

5’- GTGGGCCCCGAGTGCAATCGCGGGAAGCCAGGGTTTCCAGCTAGGACACAGCAGGTCGTGATCCGGGTCGGGACATGCCTGGCAGAGGCTGCGAG -3’

3’- CACCCGGGGCTCACGTTAGCGCCCTTCGGTCCCAAAGGTCGATCCTGTGTCGTCCAGCACTAGGCCCAGCCCTGTACGGACCGTCTCCGACGCTCGTAC -5’

Lower strand 5’to3’:

5’-CATGCTCGCAGCCTCTGCCAGGCATGTCCCGACCCGGATCACGACCTGCTGTGTCCTAGCTGGAAACCCTGGCTTCCCGCGATTGCACTCGGGGCCCAC-3’