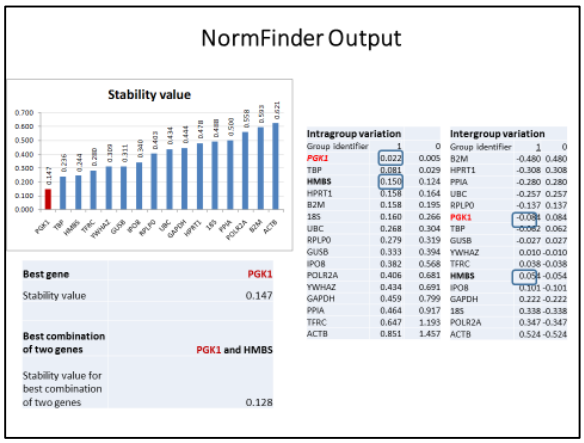
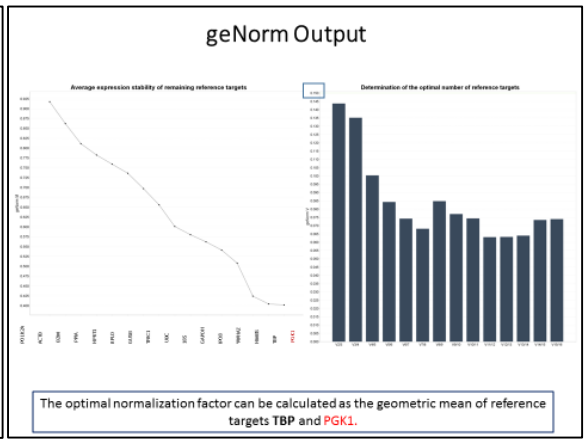
Additional file 13. Selection of reliable reference genes for peripheral blood gene expression analyses. TaqMan® array human endogenous control cards (Applied Biosystems, Foster City, CA, USA), which are 384-well microfluidic cards containing 16 human TaqMan Gene Expression Assays. They were used to evaluate the endogenous controls specific for peripheral blood that exhibit minimal differential expression. Peripheral blood samples from 8 PD and 8 HC gender- and age-matched subjects were processed following the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The expression stability was determined and compared by two commonly used algorithms (geNorm and NormFinder). By comparing the output of these two methods and by accepting gene expression levels of qPCR at Ct values≤ 29, we obtained a list of the most stable reference genes in human peripheral blood. See the following Figures reporting output files of the analyses. As the best reference genes, PGK1, UBC and GAPDH were selected according to the following practical considerations: PGK1 is the best reference gene according to geNorm and NormFinder analyses; UBC presents similar stability strength to PGK1 and a different threshold Ct value; GAPDH is one of the most widely used reference genes in peripheral blood expression studies. The gene expression analyses of the first experimental data sets (data not shown) reveled that GAPDH had a higher variability compared to PGK1 and UBC; therefore, we decided to evaluate the relative gene expression by normalizing the data to the geometric mean of PGK1 and UBC.





Reference Genes: Mean CT values

Gene ID	Average Ct
18S	13.85
B2M	20.55
RPLPO	24.54
PPIA	25.15
UBC	25.69
PGK1	26.03
ACTB	26.12
GAPDH	27.07
YWHAZ	29.63
HPRT1	29.67
GUSB	29.7
TFRC	30.23
IPO8	30.25
POLR2A	30.64
TBP	31.56
HMBS	32.02