Connectivity Map analysis of NMD+ *BMPR2* related HPAH provides insights into disease penetrance

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ONLINE DATA SUPPLEMENT

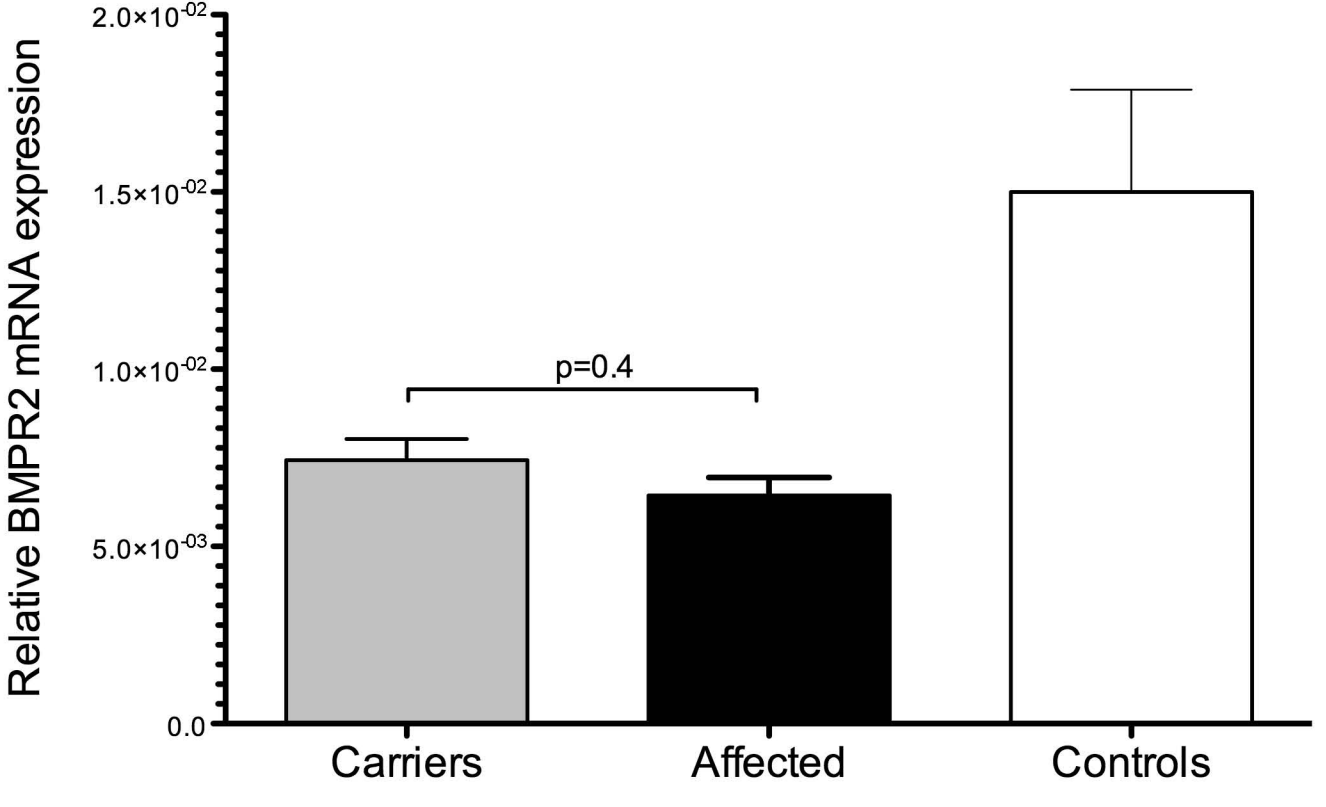
Supplementary figure 1: Real time PCR analysis of total *BMPR2* mRNA expression in the 20 samples analyzed in the array study. Data presented in an aggregate fashion for each group. Real-time PCR analysis was carried out using Taqman Universal Master Mix and a 7500 Real-Time PCR system according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).[11] *HPRT* was used as the endogenous housekeeping control. The error bars represent SEM. P values were two tailed and were calculated using students t test. Statistical analysis was done using the Prism V software package for Mac.

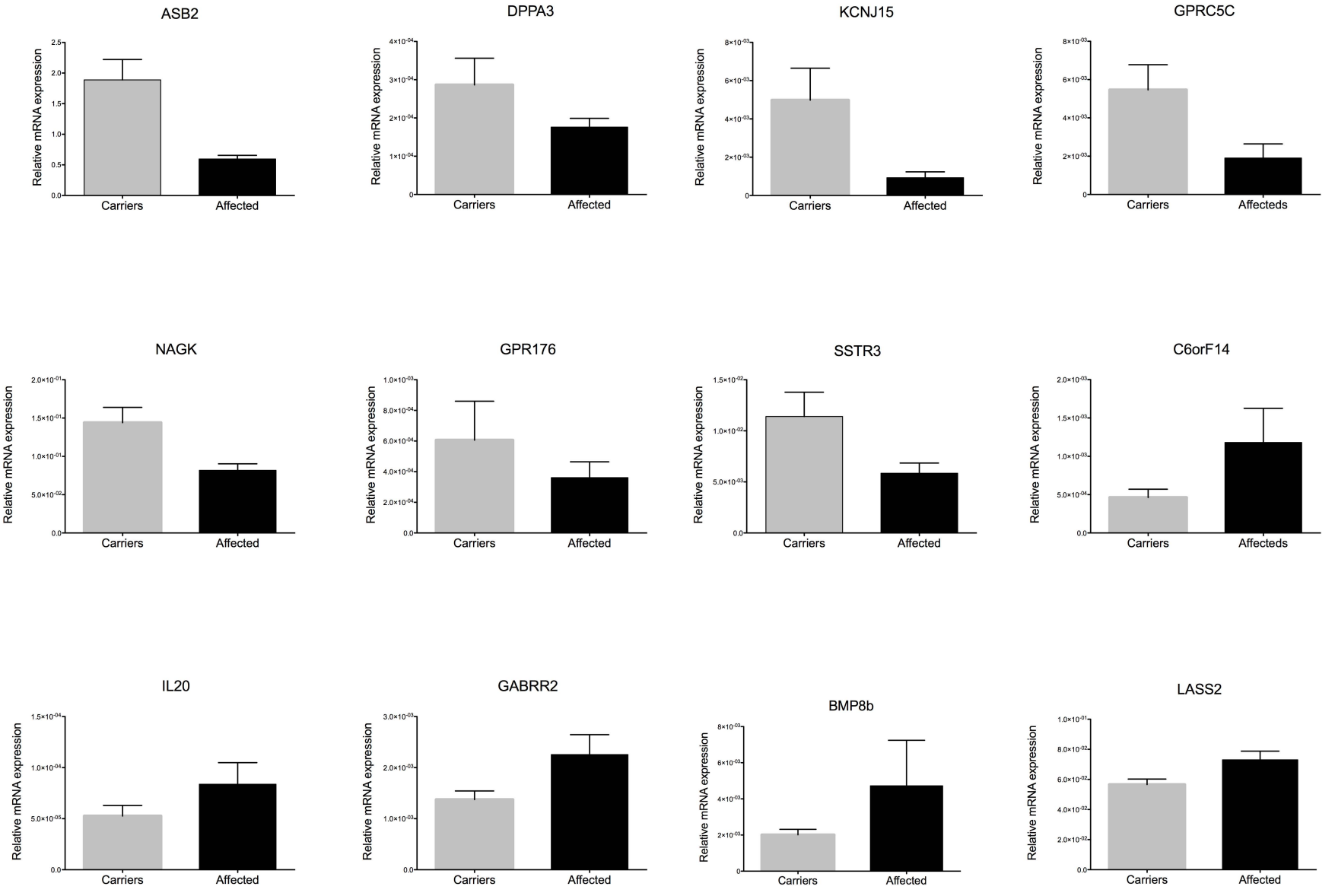
Supplementary figure 2: Real time PCR validation of the expression signature. Data are presented in aggregate fashion. The analysis was done as previously reported using Taqman predesigned assays[11] (Applied Biosystems, Foster City, CA). Error bars represent SEM. *HPRT* was used as the endogenous housekeeping control.

Supplementary figure 3: False positive and false negative curves with the varying size of the gene signature. The point (n=35) was selected where both false positive and false negative determinations reach their lowest point.

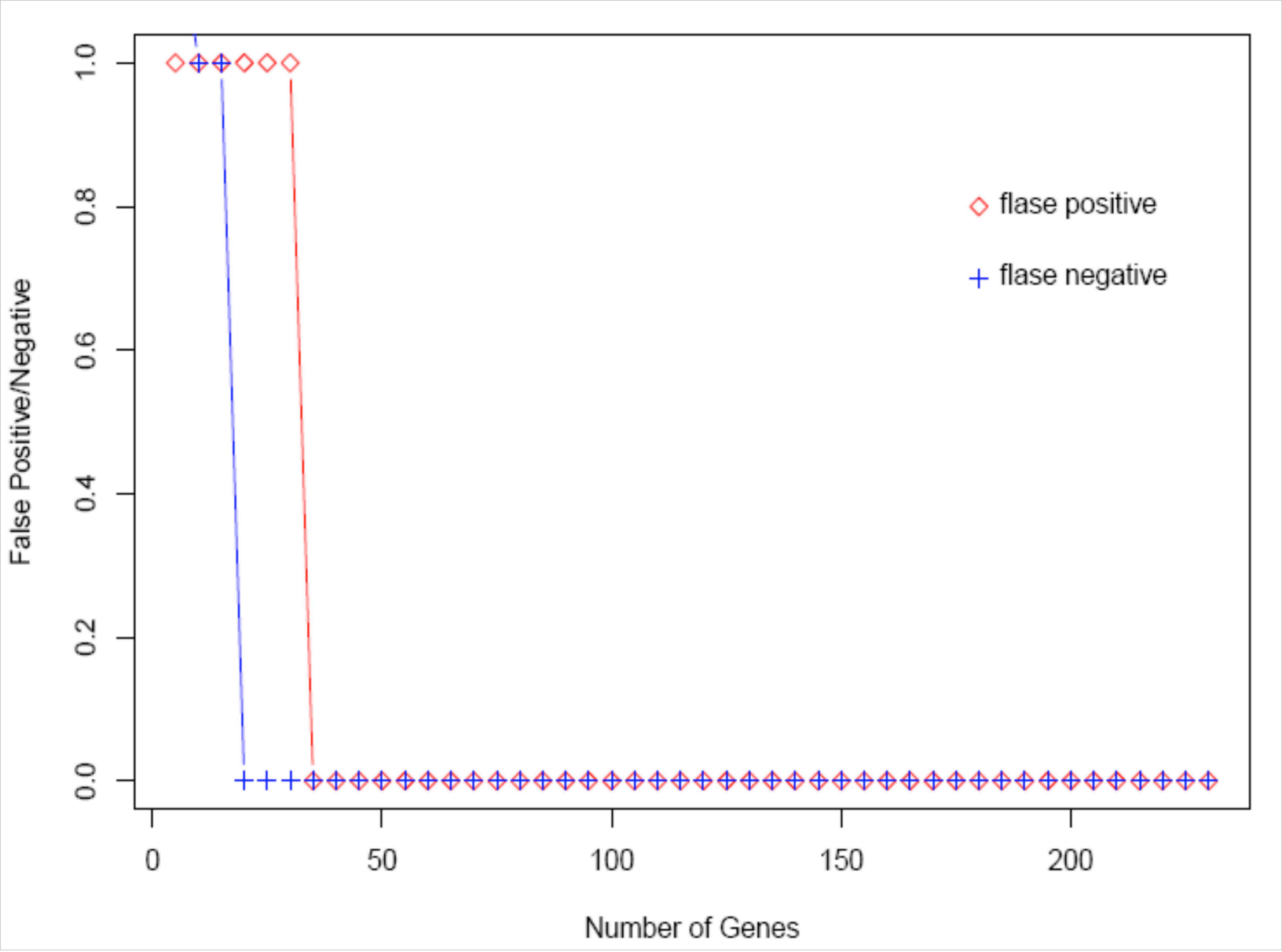
Supplementary figure 4: ROS measurement using the ROS-sensitive dye DCFDA. Aggregate data from CLs derived from 5 affected and 5 carrier individuals is presented. Each individual sample was measured in replicates of 8. The experiment was repeated 3 times. Results of one representative experiment are shown. The error bars represent

SEM. P values were two tailed and were calculated using students t test. Statistical analysis was done using the Prism V software package for Mac.

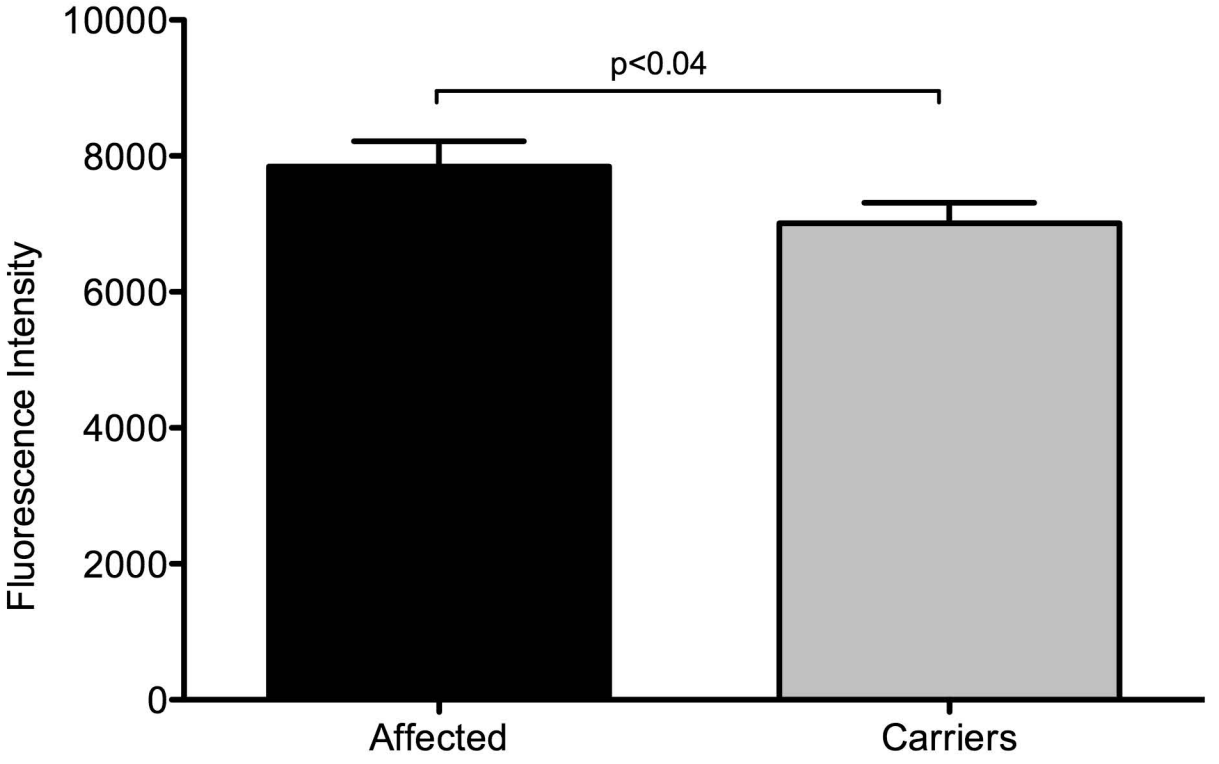

Relative BMPR2 mRNA expression
2.0×10-02
1.5×10-02
1.0×10-02
5.0×10-03
0.0
p=0.4
Carriers
Affected
Controls



Supplementary Figure 2



Supplementary Figure 3



Supplementary figure 4