Evaluation of relative quantification of alternatively spliced transcripts using droplet digital PCR

Supplementary information

Supplementary Data A

Primer specificity was tested on plasmids containing one single transcript of interest. Each assay was tested on all plasmids in the same manner as described for qPCR in the methods section. For all assays we observed a specificity of >99.90%, with amplification products of non-specific primer-plasmid combinations representing ≤0.08% of the specific primer-plasmid PCR product.

Supplementary Data B

Sample quality control was done as described in the methods section. Data shown here indicate that none of the cDNA samples are lagging by increased inhibition (SPUD assay). RT and DNase treatment were executed successfully on all samples. Original RNA samples depicted in grey shades (left), matched cDNA samples in yellow shades (right).

Supplementary Data C

Like in Figure 2 for all BRCA1 isoform-specific assays, linearity and dynamic range were investigated for the internal ddPCR standard RPP30. This assay shows a wide dynamic range (similar to that of BRCA1-ex5FL) and high level of linearity (R^2 >0.99). LOB was 0 for all NTCs and LOD and LOQ were also similar to those of BRCA1-ex5FL. Target-specific plasmids for RPP30 were unavailable.

Supplementary Data D

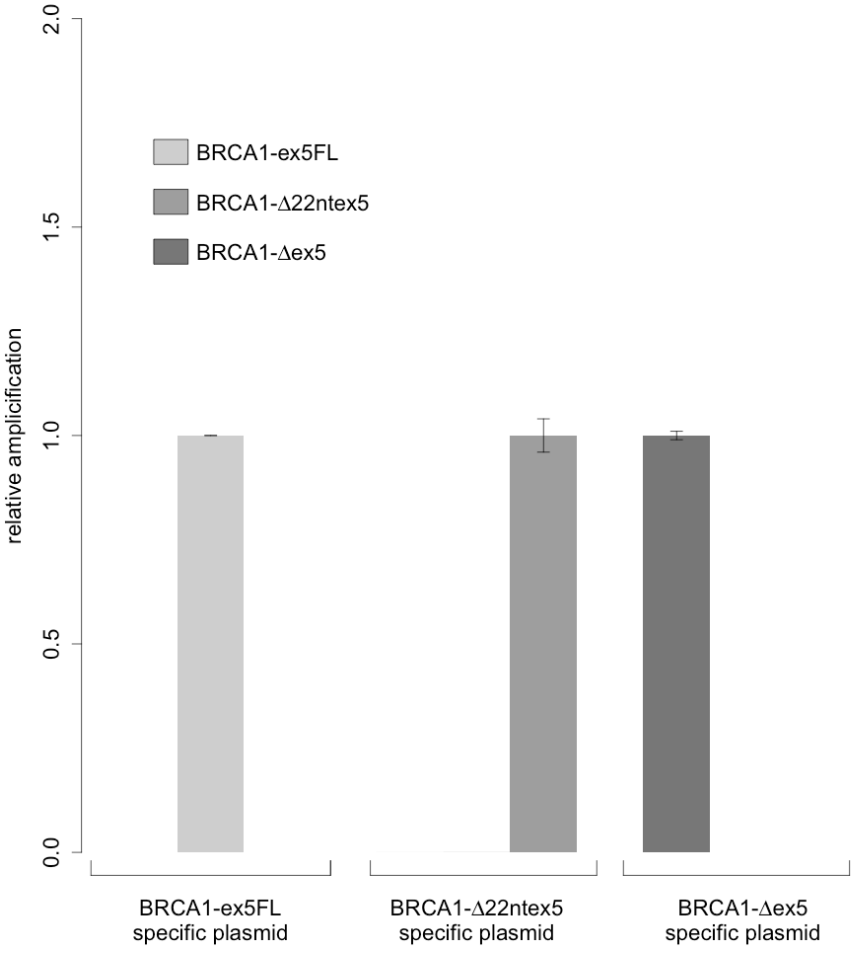
Correlation and Bland-Altman plot comparing relative abundance of relevant isoforms for both techniques. Data points of qPCR and ddPCR are located relatively close to the diagonal on a correlation plot (left side panel), but often allocate to one side of the

plot. The same holds true when presenting the data on a Bland-Altman plot (right side panel). This graph makes clear that most ddPCR measurements of BRCA1-Δ22ntex5 are larger than qPCR measurements and the opposite is true for BRCA1-ex5FL, although the same primers and samples were used with both methods. Control samples and BRCA1c.212+3A>G carrier samples are depicted in respectively black and grey.

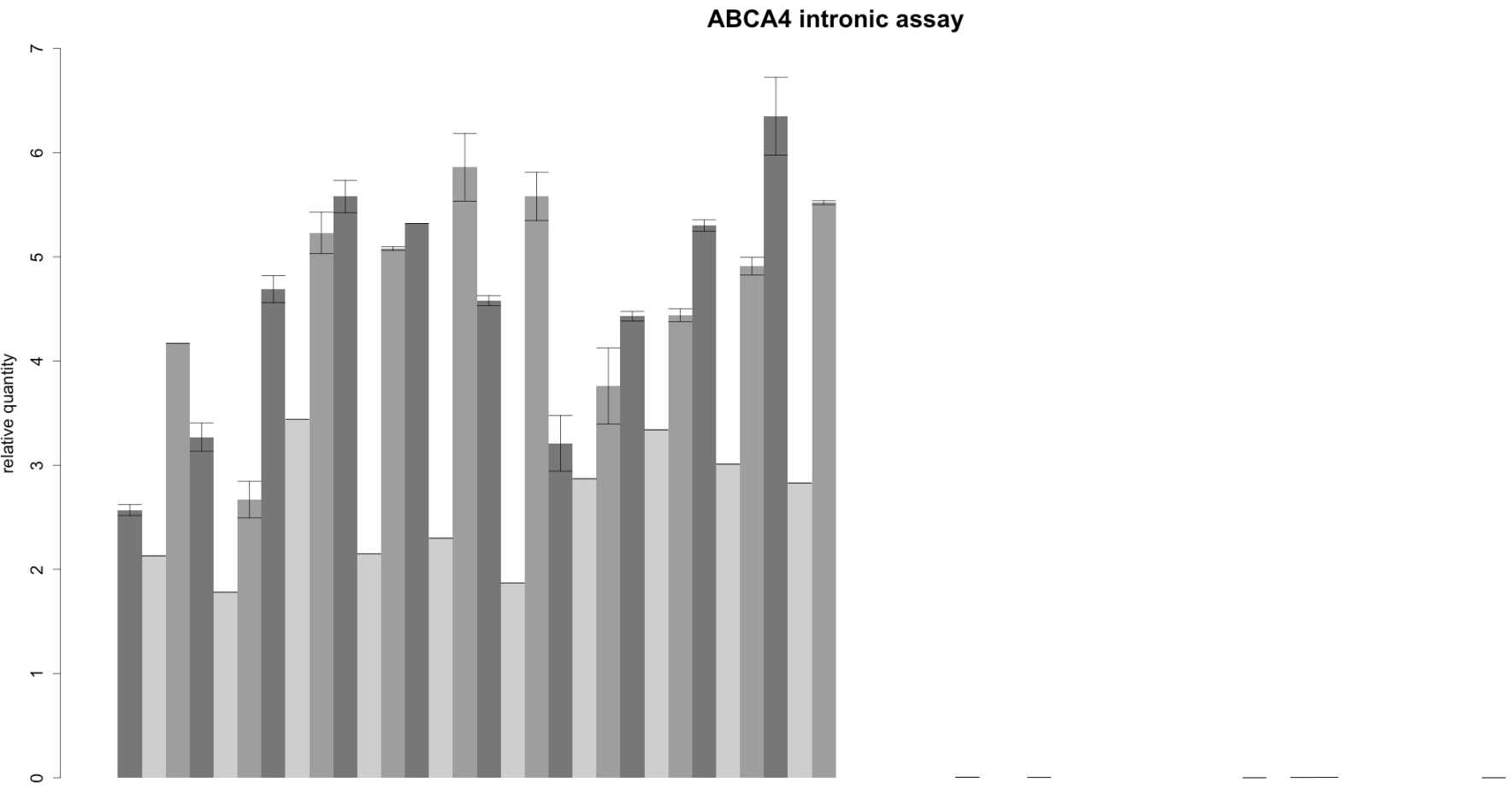
Supplementary Data E

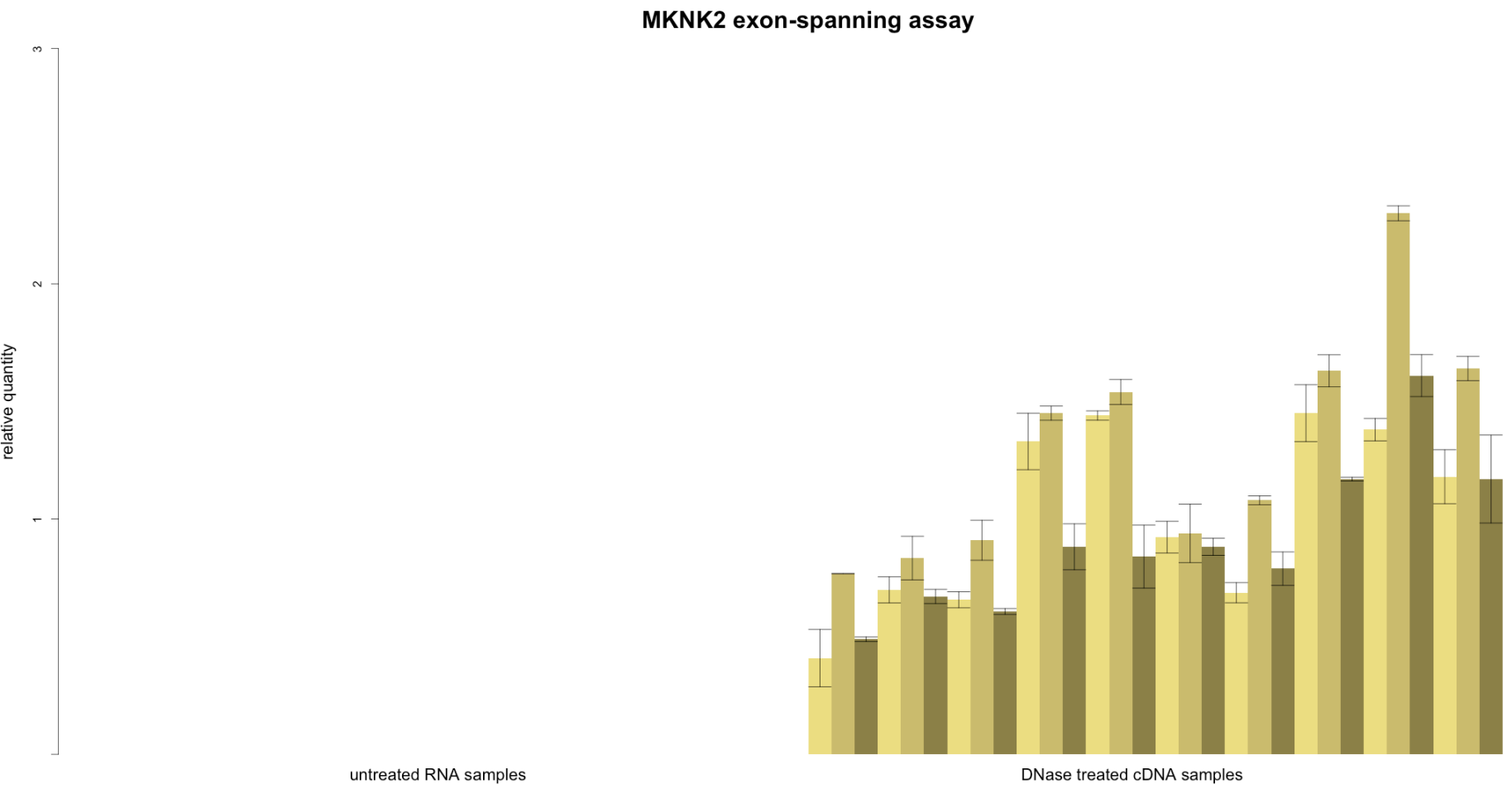
Correlation and Bland-Altman plot for qPCR versus ddPCR data. Positive droplet calling and concentration calculation was done using Quantasoft software (Bio-Rad). Comparing Supplementary data E to D shows that when calling positive droplets with Quantasoft there is a larger difference between qPCR and ddPCR measurements than when using ddpcRquant, as data shifts even more towards one side of the plot, indicating a true difference between measurements. Control samples and BRCA1c.212+3A>G carrier samples are depicted in respectively black and grey.

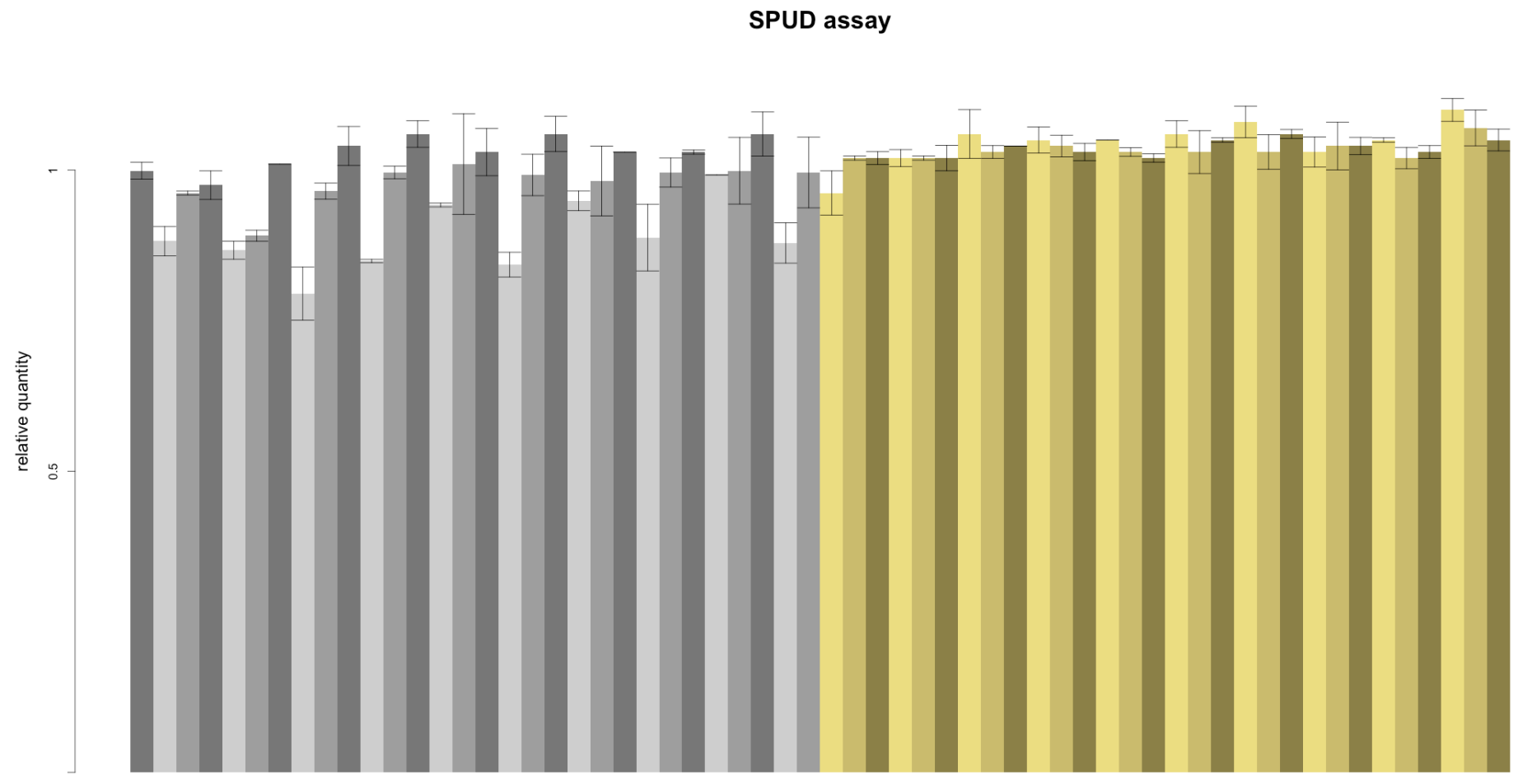
Supplementary Data A



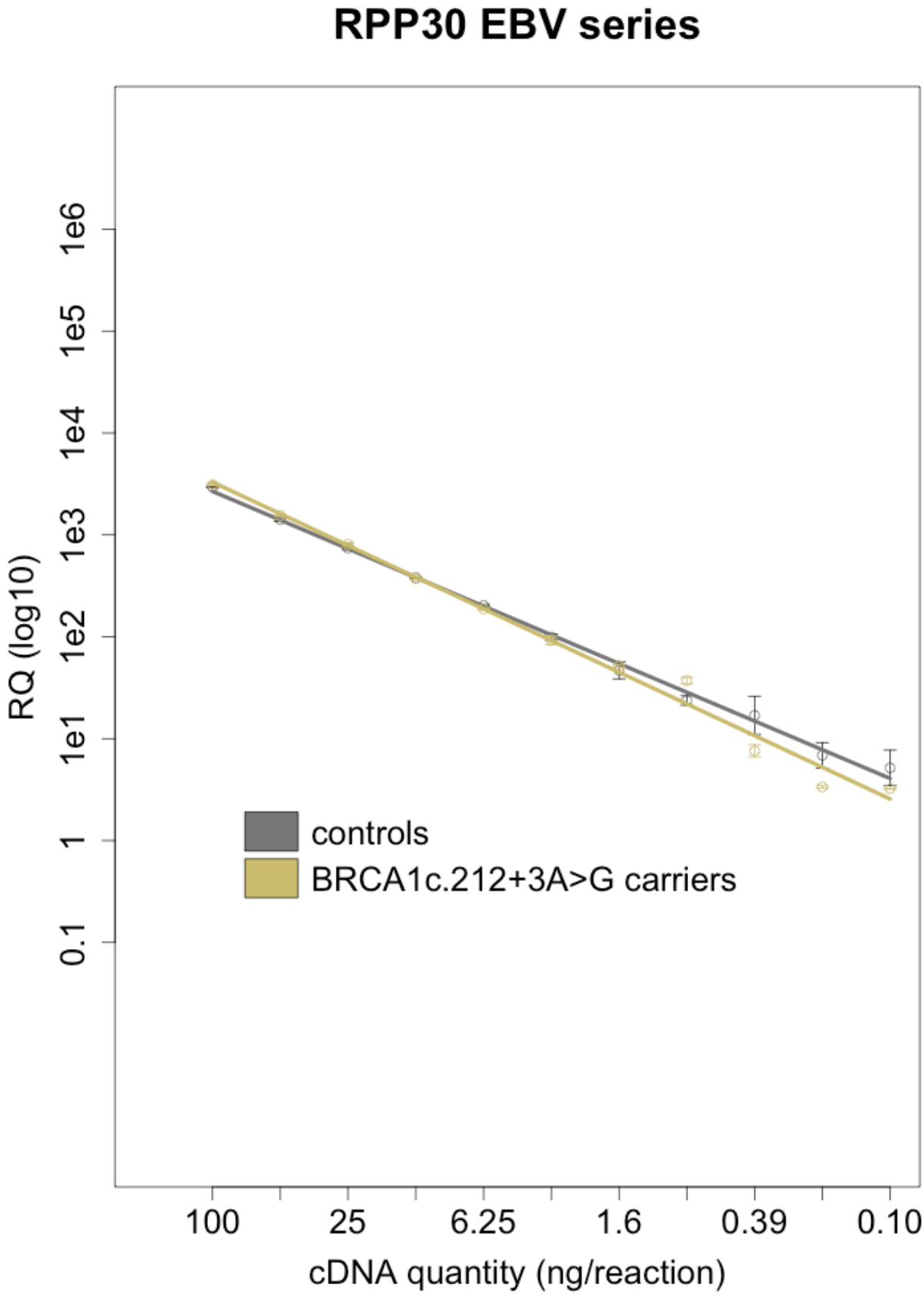
Supplementary Data B



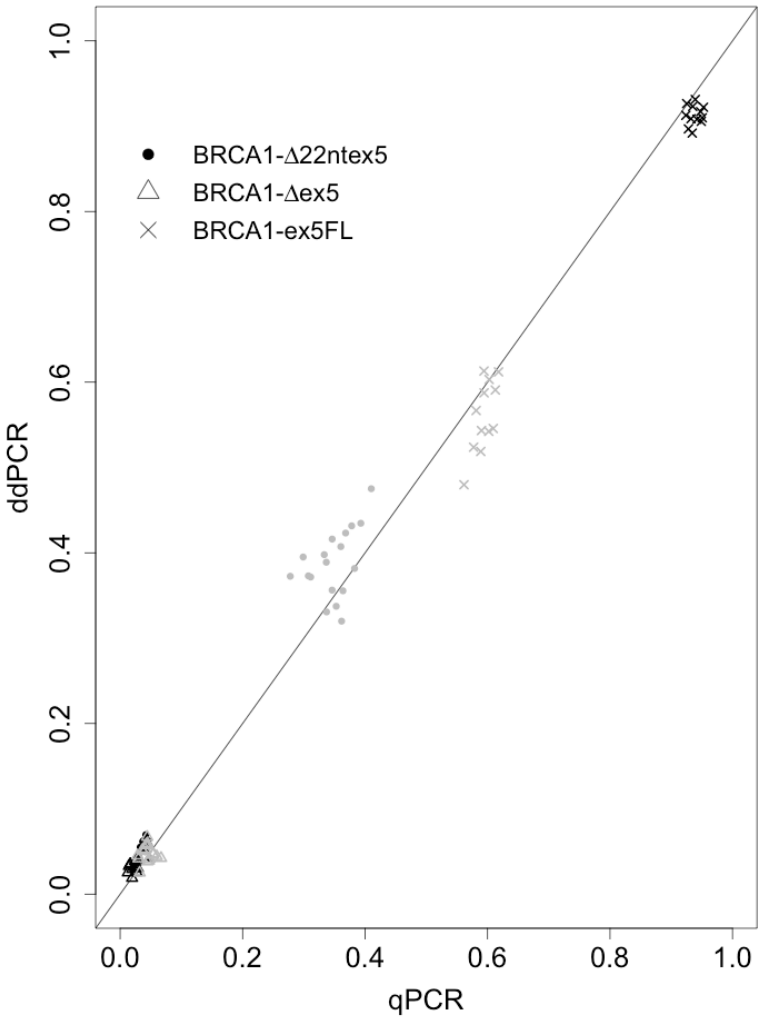


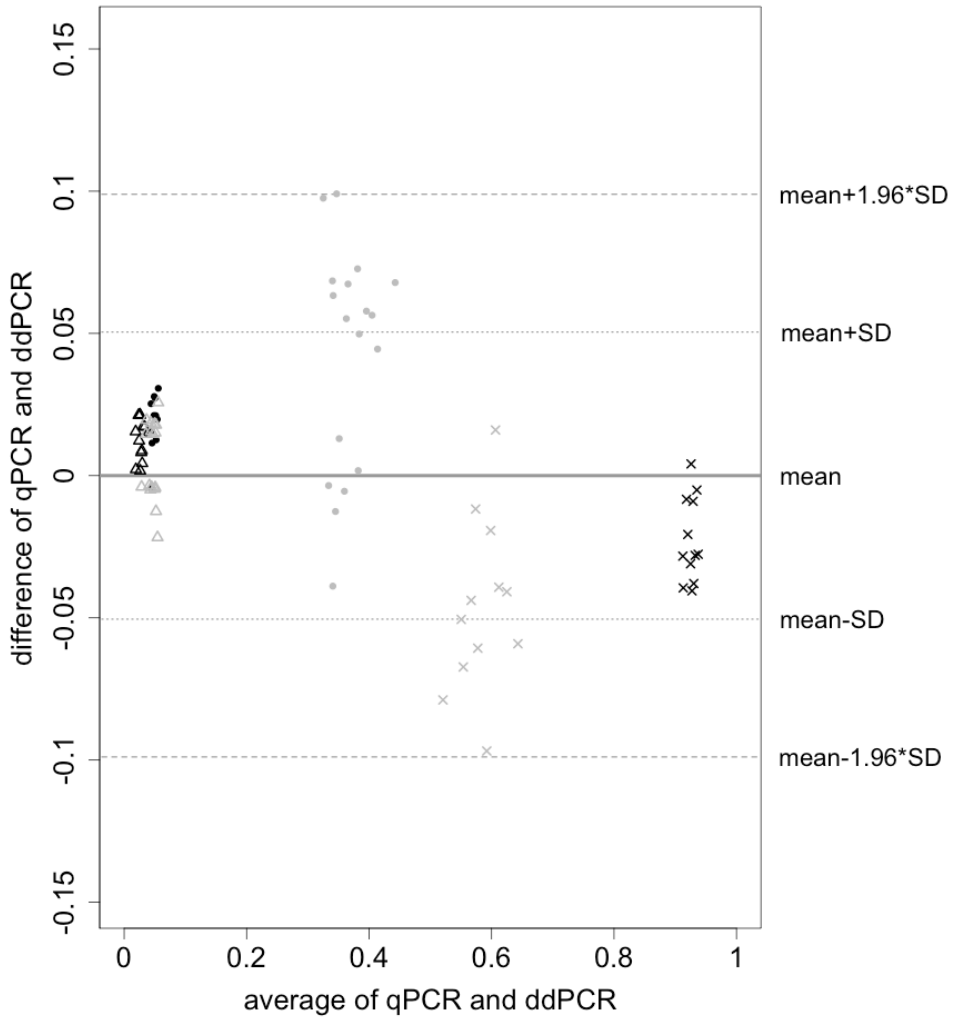


Supplementary Data C



Supplementary Data D





Supplementary Data E