Figure S23. Description of the quantile system of classification

(A) The left figure depicts a theoretical waterfall distribution of pathogenic and neutral missense mutations, as in Fig 1A. (1) Variant classification according to the experimental best cut-off. This cut-off (horizontal black line), that maximizes the experimental sensitivity and specificity in the waterfall distribution, is obtained by ROC curve analysis, as in S2 Fig. In the case of the Colony Size assay, mutations above the best cut-off are classified as pathogenic and mutations below are classified as neutral. (2) Bootstrap analysis provides a fluctuation of the best cut-off, depending on the values of the mutations and the WT BRCA1 reference randomly chosen. The fluctuating best cut-off values form a distribution, as depicted in the schematic. (3) Quantile system of variant classification according to the fluctuation of the best cut-off, depending on the values of the mutations and the WT BRCA1 reference randomly chosen. The fluctuating best cut-off values delimit the probability of the presence of this variable. As an example, the quantile Q0.99 is the value that separates the 99% lowest values from the 1% highest values in a distribution. This means that the probability to have the best cut-off above the quantile Q0.99 is 1%. Thus, in the Colony Size assay using the standard method, a mutation with the median above the quantile Q0.99 can be considered as pathogenic with a 1% probability of error. Indeed, this mutation could be neutral, but only if the best cut-off is above the median, which has a 1% probability, or less, to occur. This reasoning allows separation of the best cut-off distribution into 5 intervals, based on the five-class nomenclature proposed by Plon et al, with each interval defining the probability of the best cut-off presence within the waterfall distribution.

(B) Quantiles that delimit the 5 intervals of classification according to the assay and the method used. CS, Colony Size; LM, Liquid Medium; SF, Spot Formation; YL, Yeast Localization assay. Note that the quantiles differ, depending on whether the pathogenic mutations are above or below the best cut-off. For instance, in the standard method, the quantiles of the Colony Size assay are Q0.99, Q0.95, Q0.05 and Q0.001 (pathogenic mutants above the best cut-off), while quantiles are Q0.01, Q0.05, Q0.95 and Q0.999 in the Spot Formation assay (pathogenic mutants below the best cut-off). However, these two cases generate the same intervals (e.g., probability 1% for the class 5, see C and D). Cut-off values corresponding to these quantiles are listed in S5 Table for each assay and for each method.

(C) Interval limits in the case of the Colony Size assay, using the standard or the standard with reference method. P(X > Q0.99) = 1% is the probability to obtain the best cut-off variable X strictly over the quantile Q0.99, shown here as 26,222 cells per colony for the standard method, and 2,416 x 11,200 (BRCA1 median of the experimental data) = 27,062 cells per colony for the standard with reference method.

(D) Interval limits in the case of the Colony Size assay, using the MWW method.