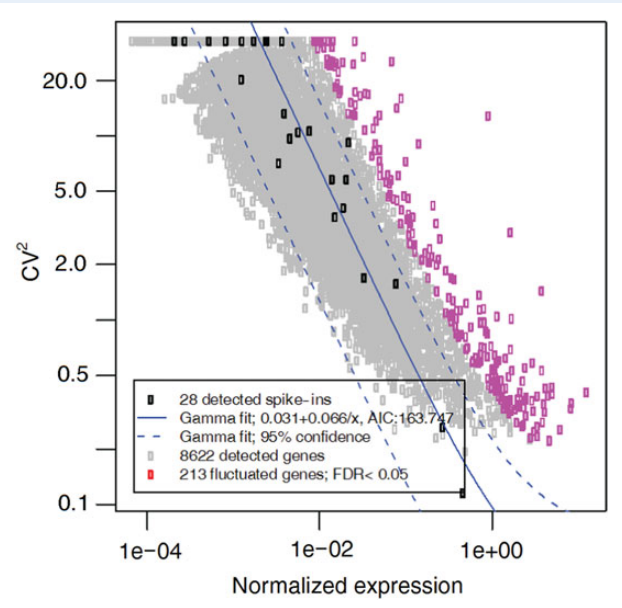
Supplementary Text S1 The concept of 'fluctuation'

SAMseq (Li and Tibshirani, 2013) and SAMstrt (Katayama *et al.*, 2013) estimate the significance of differential expression with respect for technical noise, which is the variation in sequencing depths and the sampling noise. SAMstrt uses only the total counts of the spike-in RNAs as the depths (d_i in Li *et al.* (Li and Tibshirani, 2013)) for the spike-in based normalization purpose, while SAMseq uses the total counts of all genes. This means that the noise component in the observed spike-in read counts affects the level of the normalized values. Therefore, to be of interest, the fold difference or the degree of differentially expressed genes, should be larger than the technical noise level.

'Fluctuation' is one solution to estimating the degree of variation. We used several spike-in RNAs, added to all samples at the same concentration, for estimation of the noise level. In detail, expectation of the noise is modelled by fitting against a Gamma distribution and identity link to $y = a_1/x + a_0$, where x is the average, and y is the squared coefficient of variation (CV^2), of the normalized spike-in levels (black points and blue lines in Supplementary Fig. S3). We can then find the significance of the degree of variation in each gene by comparison to the expected noise level (magenta points in Supplementary Fig. S3). For robustness against library biases, the noise modelling is performed by library, and the noise models and the observed CV^2 over all target libraries are integrated by summation and scaling properties on the Gamma distribution. Finally, the significance of the integrated variation in each gene is evaluated by comparison to the integrated noise model; the fluctuationScore in out/byGene/diffexp.xls is $CV^2_{observed}/CV^2_{expected}$, and fluctuation is the adjusted *P*-value.

Further thresholding of differentially expressed genes by the significant fluctuation reduces false positives from the technical noise in the spike-in read counts. The fluctuation estimation does not need a classification of the samples, while the differential expression test requires predefined classifications. You can therefore use this measure to select highly 'variable' genes within a cell population as (Brennecke *et al.*, 2013), or between the patients, and so on.



Supplementary Figure S3 Estimation of fluctuation among cultured cells.