Supplementary Figures and Legends for Böttcher et al.

Supplementary Figure 1:

FACS analysis of a clonally derived cell line harboring a PGK-GFP tag and, as controls, a PGK-Flag₂-tag.

Upper part:

Overlay histogram showing the intensity distribution for PGK-GFP (green) and PGK-Flag₂ (red). The two curves overlap slightly, therefore quantification is performed on two-dimensional dot plots.

Lower part:

Two-dimensional dot-plot for GFP fluorescence intensity (abscissa) and side scatter intensity (ordinate). In this diagram, the two cell populations can be clearly separated.

Supplementary Figure 2:

A) Details on the targeting design for the PGK locus (top) and the Tub56D locus (bottom). The CRISPR target site (boxed) and a small portion of the homologous sequence included in the primers to generate the HR donor PCR product are shown. PAM: protospacer associated motif; magenta triangles: predicted sites of DNA cleavage by the cas9 nuclease programmed to target the indicated sequence.

B) Comparison of targeting efficiency in a situation where the HR donor and the modified locus may be cleaved by the cas9 nuclease.

Upper part: Sequence diagram of the PGK locus as in A)

Lower part:

Western Blot analysis of tagging efficiency at the Blanks locus using wild type (left) and point mutant (right) HR donors. Three technical replicates (= parallel transfection and selection) are shown for each condition. Tubulin served as loading control. The tag was detected with the anti-Flag M2 monoclonal antibody (Sigma).
Supplementary Figure 2

A

HR primer sense
5’ ... GCTGCAATGACCGACGCG ...−3’

PGK locus
... GCTGCAATGACCGACGCGCTAAAaacgtacatataataggt ...
... CGACGGAACGCTGCTGGGATGcgcgtatatatgc ...

PAM △ CRISPR target
3’−... CGCATATATATGGCA ... 5’
HR primer antisense

HR primer sense
5’ ... GAGGTCGACGAGGAAC ...−3’

CRISPR target

E V D E N * △ PAM

Tub56D locus
... GAGGTCGACGAGGAACCTAAacctgagttaaatc ...
... CTCCAGCTCGTCGTAGTTtaagctgcttttagtt ...

3’−... TAAGGTTAGCCTTCTAGT ... 5’
HR primer antisense

B

HR primer sense
5’ ... ACGGACTATCCGACAAAAA ...−3’

blanks locus
... ACGGACTATCCGACAAAAATACcaacgggaactttatatgattttaaa ...
... TTGCTGATAGCCTTTTATTatgtttgtactagctagagccttggcactaaatattt ...

PAM △ CRISPR target
3’−... TGCTTTTAGTAGCCTAGACCCCTGGG ... 5’
3’−... TGCTTTTAGTAGCCTAGACCCCTGGG ... 5’
HR primer antisense point mutations

<table>
<thead>
<tr>
<th>no mutation</th>
<th>with double point mutation</th>
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Blanks-Flag
Tubulin