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-$Flag_2$-tag.
Upper part:

Overlay histogram showing the intensity ditraibution for PGK-GFP (green) and PGK-$Flag_2$ (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.

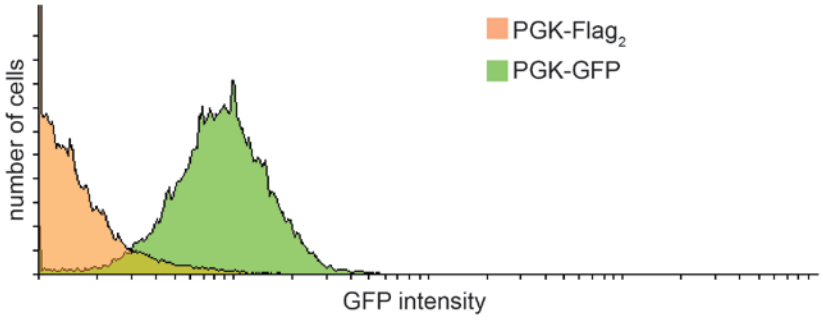
B) Comparison of targeting efficiency in a situation where the HR donor and the modified locus may be cleaved by the cas9 nuclease.

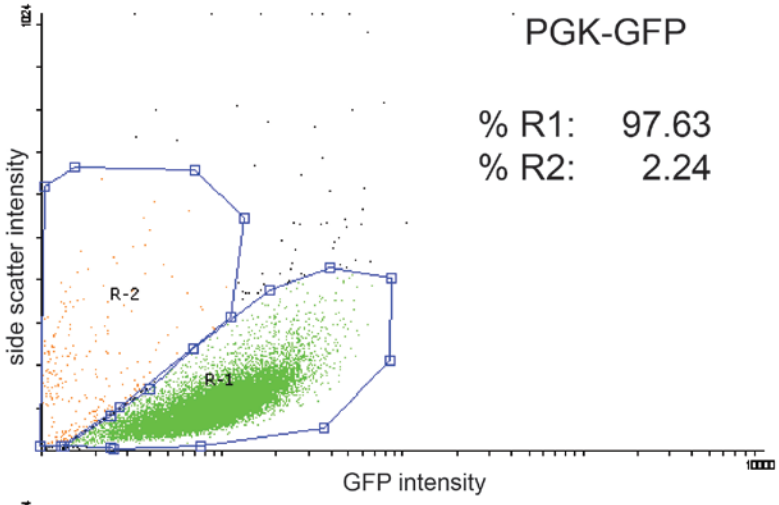
Upper part: Sequence diagram of the PGK locus as in A)

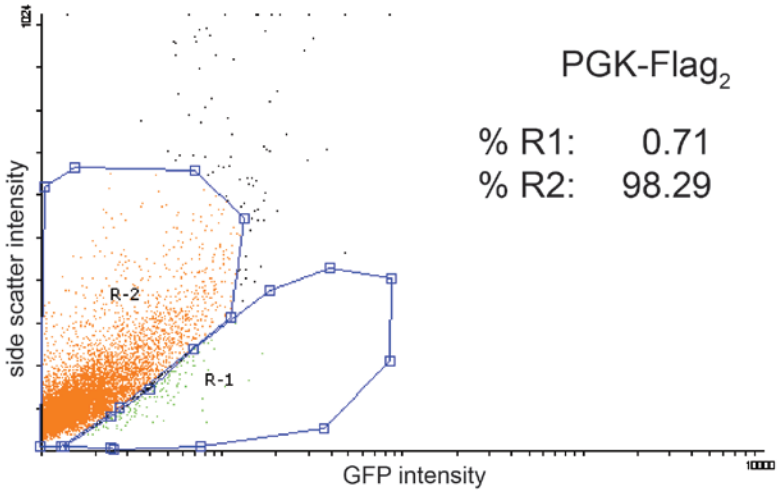
Lower part:

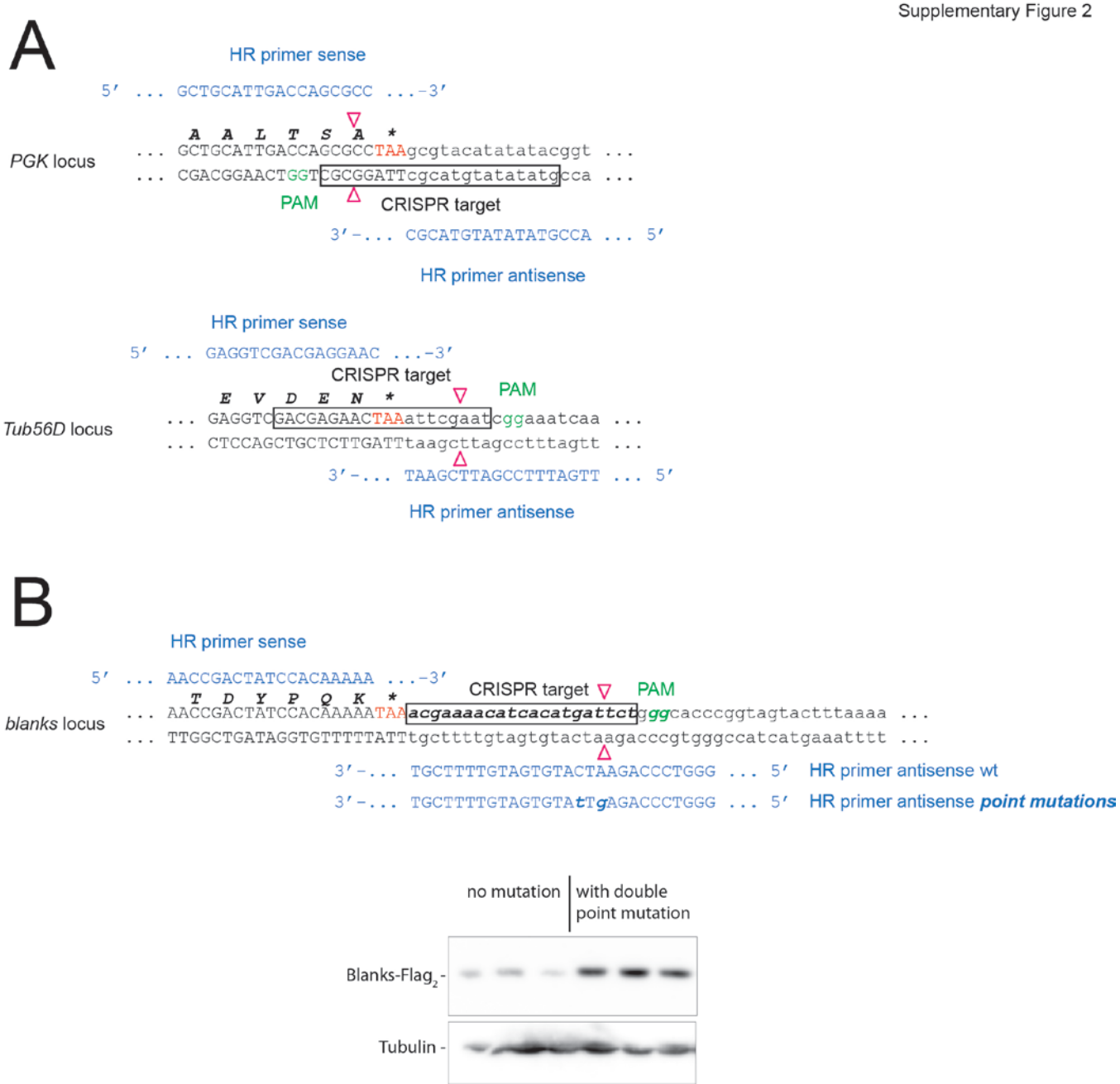
Western Blot analysis of tagging efficiency at the Blanks locus using wild type (left) and point mutant (right) HR donors. Three technical replicates (= parallel transfection and selection) are shown for each condition. Tubulin served as loading control. The tag was detected with the anti-Flag M2 monoclonal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

Supplementary Figure 1








A
HR primer sense
5′ ... GCTGCATTGACCAGCGCC ...-3′
A A L T S A *
PGK locus
... GCTGCATTGACCAGCGCCTAAgcgtacatatatacggt ...
... CGACGGAACTGGTCGCGGATTcgcatgtatatatgcca ...
PAM
CRISPR target
3′-... CGCATGTATATATGCCA ... 5′
HR primer antisense
HR primer sense
5′ ... GAGGTCGACGAGGAAC ...-3′
CRISPR target
E V D E N *
PAM
Tub56D locus
... GAGGTCGACGAGAACTAAattcgaatcggaaatcaa ...
... CTCCAGCTGCTCTTGATTtaagcttagcctttagtt ...
3′-... TAAGCTTAGCCTTTAGTT ... 5′
HR primer antisense
B
HR primer sense
5′ ... AACCGACTATCCACAAAAA ...-3′
T D Y P Q K *
CRISPR target
PAM
blanks locus
... AACCGACTATCCACAAAAATAAacgaaaacatcacatgattctgggcacccggtagtactttaaaa ...
... TTGGCTGATAGGTGTTTTTATTtgcttttgtagtgtactaagacccgtgggccatcatgaaatttt ...
3′-... TGCTTTTGTAGTGTACTAAGACCCTGGG ... 5′ HR primer antisense wt
3′-... TGCTTTTGTAGTGTAtTgAGACCCTGGG ... 5′ HR primer antisense point mutations
no mutation
with double point mutation
Blanks-Flag2 -
Tubulin -