

Supplementary Information

A user-friendly CRISPR/Cas9 system for mutagenesis of *Neurospora crassa*

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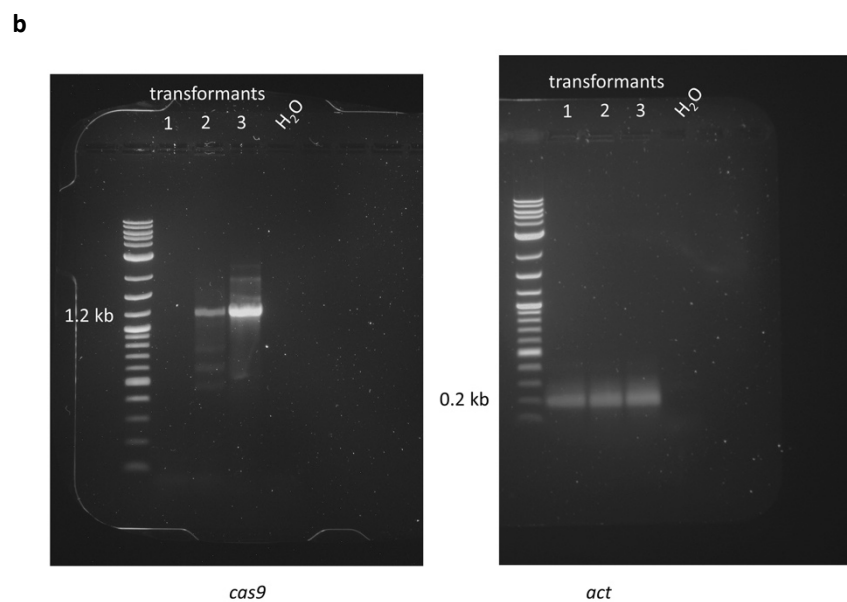
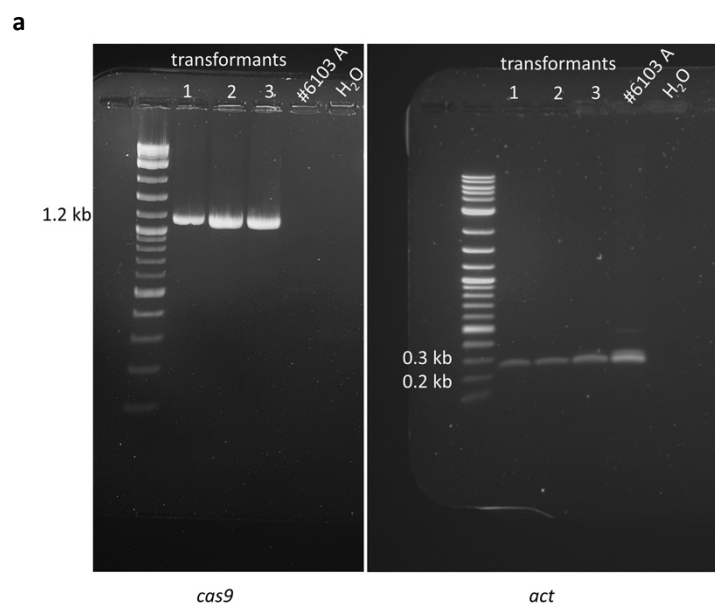
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Supplementary Figure 1. Full-length gels of DNA-gels shown in Figure 1c and d

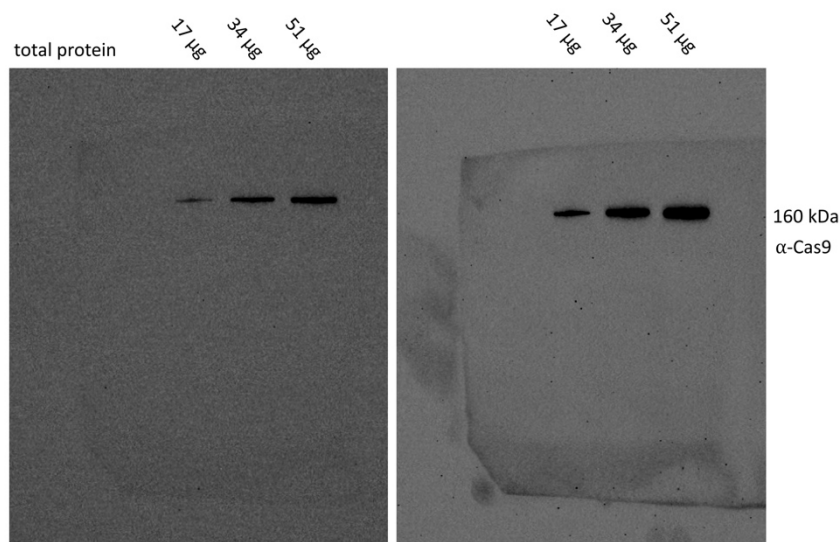
Supplementary Figure 2. Full-length blot of western blot shown in Figure 1e

Supplementary Figure 3. Southern blot to confirm the *cas9* integration into the genome

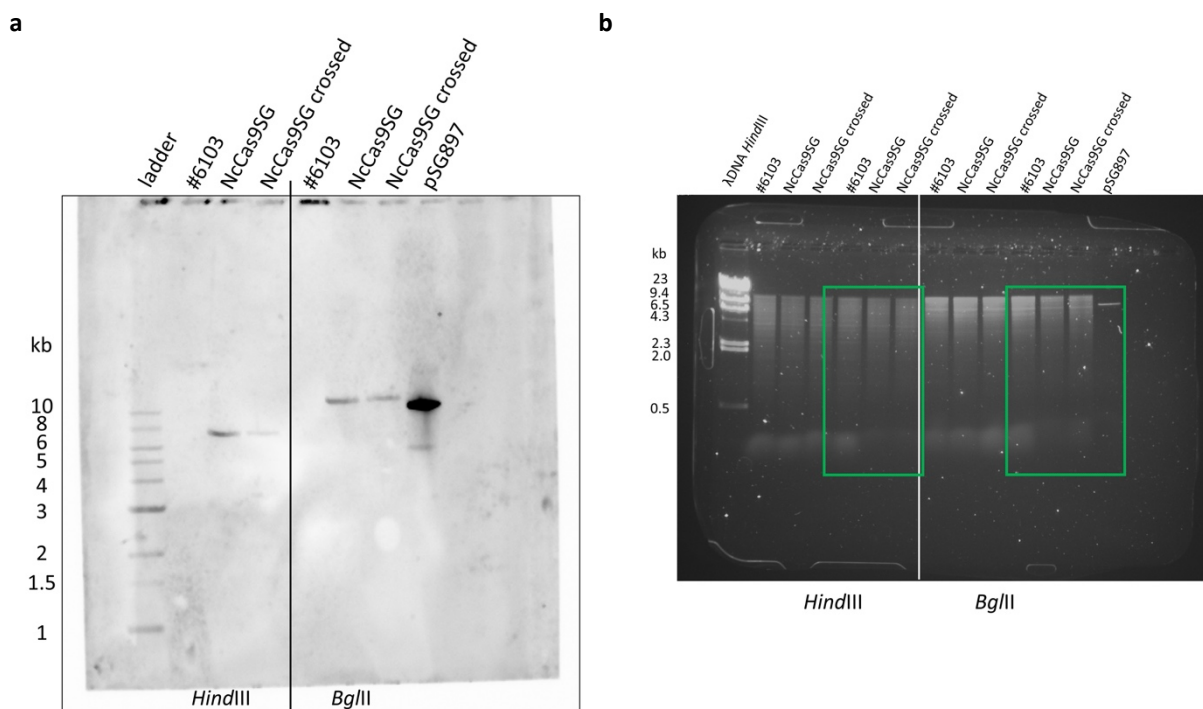
Supplementary Figure 4. *N. crassa* colonies on CsA containing VMM+SGF plates after transformation with different gRNA-c2 amounts



Supplementary Figure 1. Full-length gels of DNA-gels shown in Figure 1c and d. Gels for PCR (a) and RT-PCR (b)

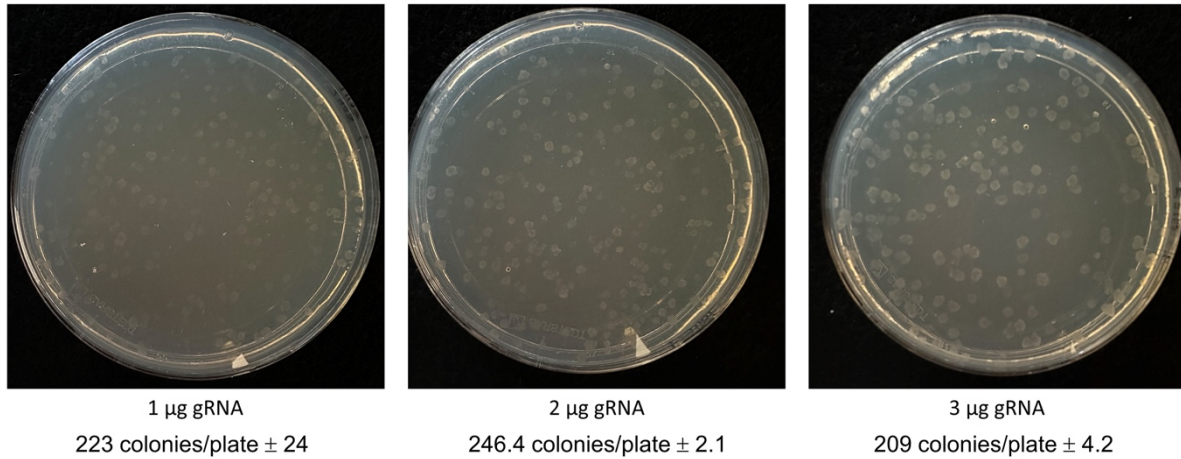


Supplementary Figure 2. Full-length blot of western blot shown in Figure 1e. Shown are two different exposures to make the membrane edges visible. The right side of the membrane was cut before antibody incubation, since the rest of the membrane belonged to another experiment.



Supplementary Figure 3. Southern blot to confirm the *cas9* integration into the genome. Genomic DNA from #6103 the *Cas9* transformant *NcCas9SG* used for all experiments and *NcCas9SG* crossed with #9716 were hydrolyzed with *HindIII* and *BglII*, respectively. A *cas9* probe targeting the transformation cassette was DIG-labeled and hybridized to the membrane. The bound probe was detected via chemiluminescence (a). Expected signals seen for #6103: none (hydrolyzed with *HindIII* and *BglII*). Expected signals seen for *NcCas9SG* and crossed strains: 7,355 bp (hydrolyzed with *HindIII*) and 11,399 bp

(hydrolyzed with *Bgl*II). Gel electrophoresis as a control for the hydrolyzation of the DNA used for the southern blot (green rectangle) (b).



Supplementary Figure 4. *N. crassa* colonies on CsA containing VMM+SGF plates after transformation with different gRNA-c2 amounts