



Supplementary Figure 1. Gene targeting. **(A)** In the targeting vector, the longer arm of homology contained a 2.06 segment flanked by *loxP* sites (orange triangles) for conditional removal with Cre recombinase. This segment contained *Dicer1* terminal exons 26, 26 and 27, the translation termination codon (TGA) and the putative polyadenylation signal (pAs) as shown for the wild-type allele (*Dicer1*⁺). The positive selection cassette (*neo*) was the mouse phosphoglycerate kinase 1 (*Pgk1*) promoter-*neo* coding sequence (cgs)-bovine growth hormone polyadenylation signal sequence (bpA), and was flanked by *frt* recombination sites (blue triangles) for excision with FLPe recombinase. The negative selection cassette was *Pgk1* promoter-diphtheria toxin A chain (*dtA*) cgs-bpA, or, *Pgk1* promoter-equine herpes virus 4 thymidine kinase (*EHV4tk*) cgs-bpA (Loubiere *et al.* 1999). The EHV4tk cgs was derived from pORF-EHV4-tk (InvivoGen, San Diego, California, USA). Direction of transcription of the cassettes and *Dicer1* (arrows). Two probes (Probe a, green bar; Probe b, red bar) were used to assay for homologous recombination in Southern blots, with respective band sizes for the *Dicer1* wild-type allele given underneath the bars. Restriction enzymes used in Southern blots were *Hind* III (H3) and *Kpn* I (K). **(B)** Targeting was initially identified by PCR, with the position of the two primers used indicated (half-arrows). Limits of the targeting vector (TV end). The deduced change in band sizes expected in Southern blots (Probe a, green bar; Probe b, red bar). **(C)** Southern blots indicating conservative recombination in five of five clones identified by PCR. Wild-type genomic DNA of strain C57BL/6J (B6) and wild-type genomic DNA of strain 129S1/SvImJ (129). Clones 13 and 14 were derived from the *dtA* vector (2 of 96 assayed), while clones 58, 65 and 82 were derived from the *EHV4tk* vector (3 of 96 assayed). **(D)** Conditional allele after removal of the *neo* cassette with FLPe (*Dicer1*^c). Position of primers used for identification (half-arrows). **(E)** Mutant allele produced by Cre mediated excision of floxed sequence (*Dicer1*⁻). Position of primers used for identification (half-arrows). **(F)** Region of DICER1 protein removed in the present conditional allele (purple bar).

Supplementary reference

Loubiere L, Tiraby M, Cazaux C, Brisson E, Grisoni M, Zhao-Emonet J, Tiraby G, Klatzmann D 1999 The equine herpes virus 4 thymidine kinase is a better suicide gene than the human herpes virus 1 thymidine kinase. *Gene Therapy* **6** 1638–1642.