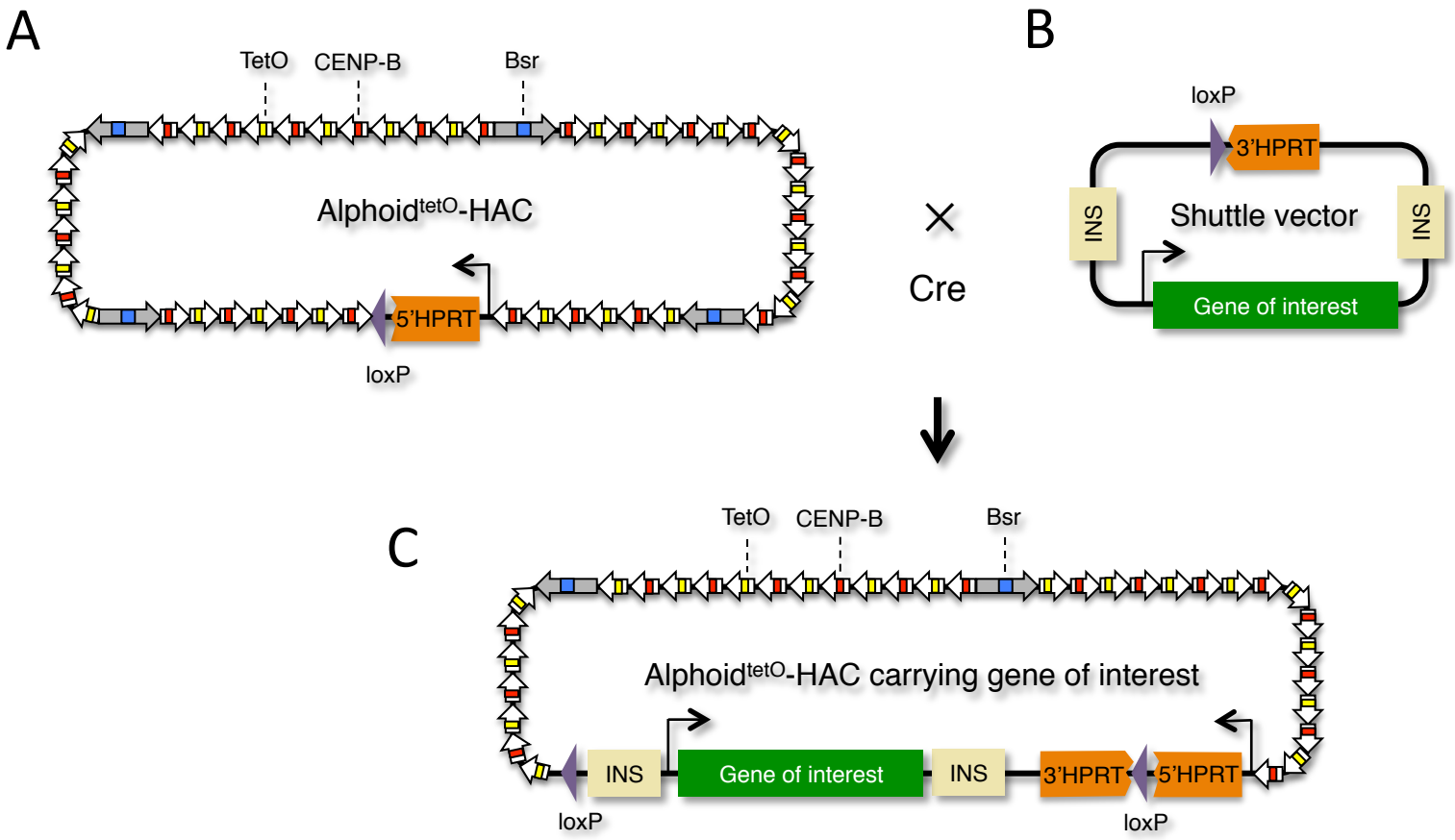

Figure S1: Scheme illustrating the uploading of gene of interest onto alphoid^{tetO}-HAC via Cre/loxP-mediated recombination. (**A**) "Empty" HAC consisting of multiply amplified alphoid-DNA (white block arrows) with either CENP-B box (red rectangles) or tetracycline operator sequence, tetO (yellow rectangles). The HAC also features clusters of blasticidine resistance genes (blue rectangles) within vector sequence (gray block arrows), a loxP site (purple arrowhead) and 5' portion of HPRT gene. (**B**) Basic shuttle-vector containing gene(s) of interest flanked by transcriptional insulators (INS), a loxP site, and 3' part of HPRT gene. (**C**) A result of Cre/loxP-mediated recombination; HPRT gene is restored after the successful recombination event in HPRT$^{-/-}$ CHO cells, allowing positive clone selection in HAT-containing media. The procedure is described in more details in ref. 13.

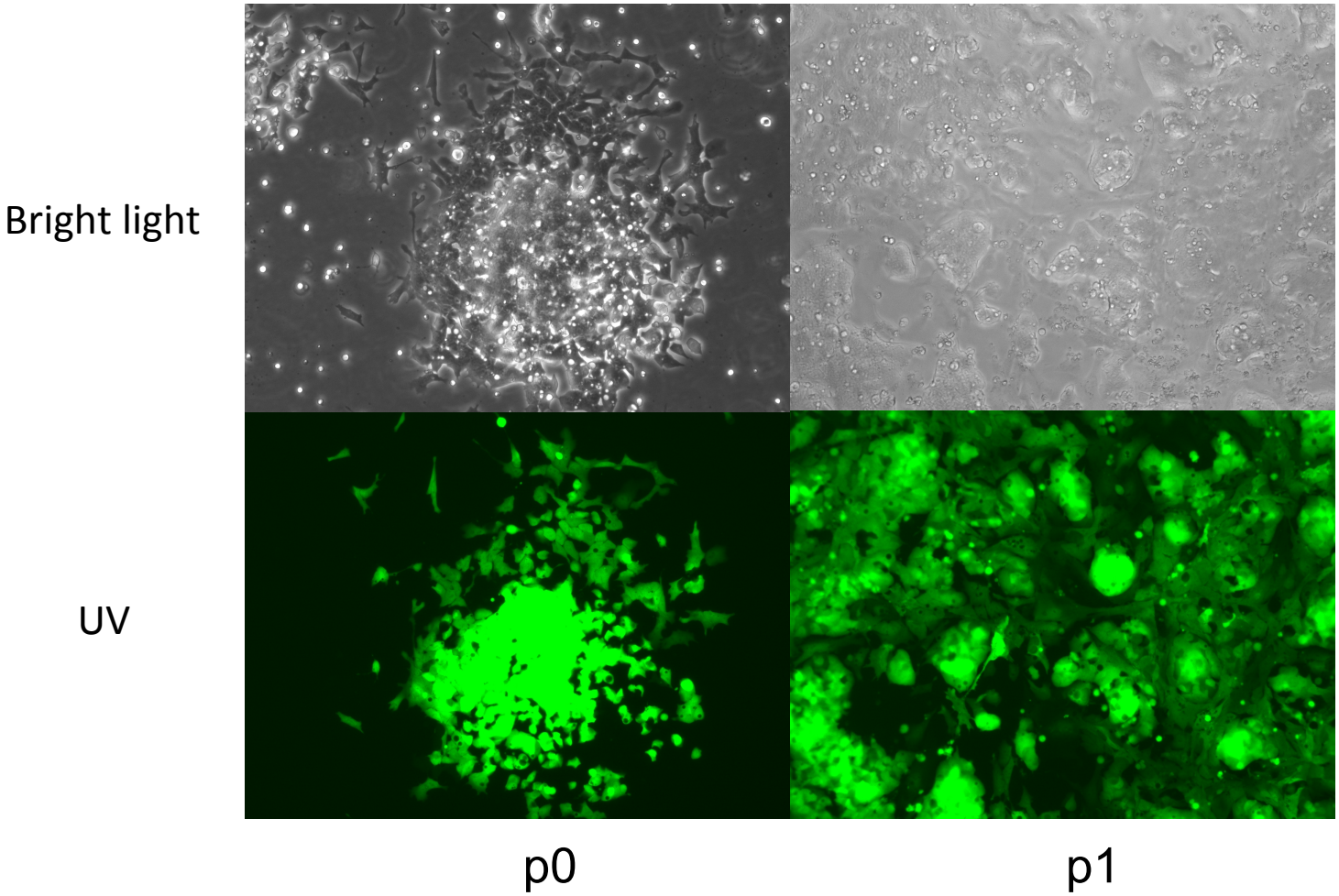

Figure S2: Primary clones of mouse ES cells, carrying HAC-GFP after MMCT procedure. Left panel – primary clone of mouse ES cells, carrying HAC-GFP during Blasticidin selection. Right panel – the same clone after first passage in culture.

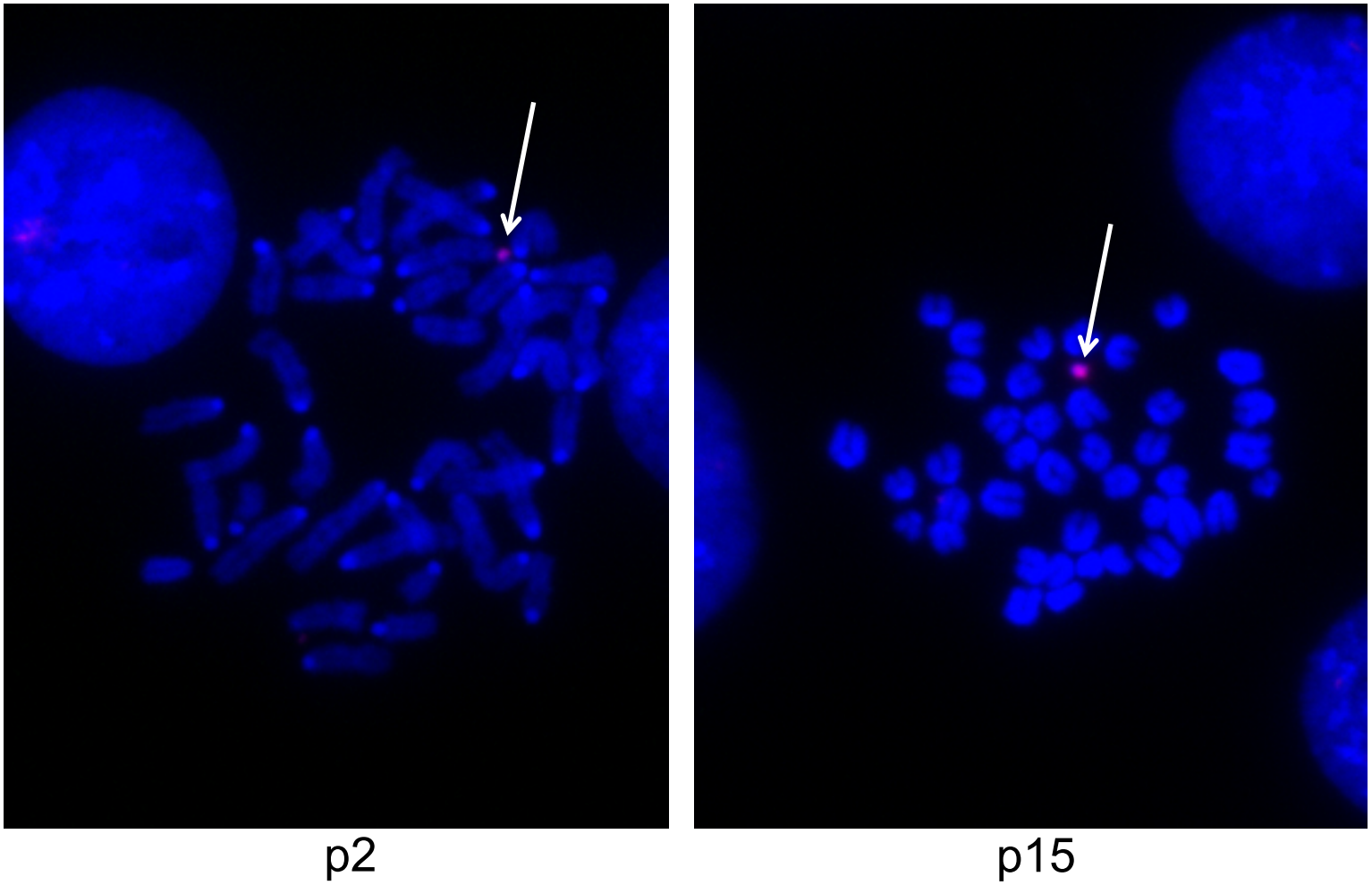

Figure S3: Mitotical stability of HAC in mouse ES cells during culturing. Left panel – FISH analysis of metaphase spread prepared from mouse ES cells, carrying HAC-GFP at passage 2 (1st week of culturing). Right panel – FISH analysis of metaphase spread prepared from mouse ES cells, carrying HAC-GFP at passage 10 (4th week of culturing). Red signal – probe against the alphoid-tetO array labeled with Alexa546. White arrows indicate HAC.

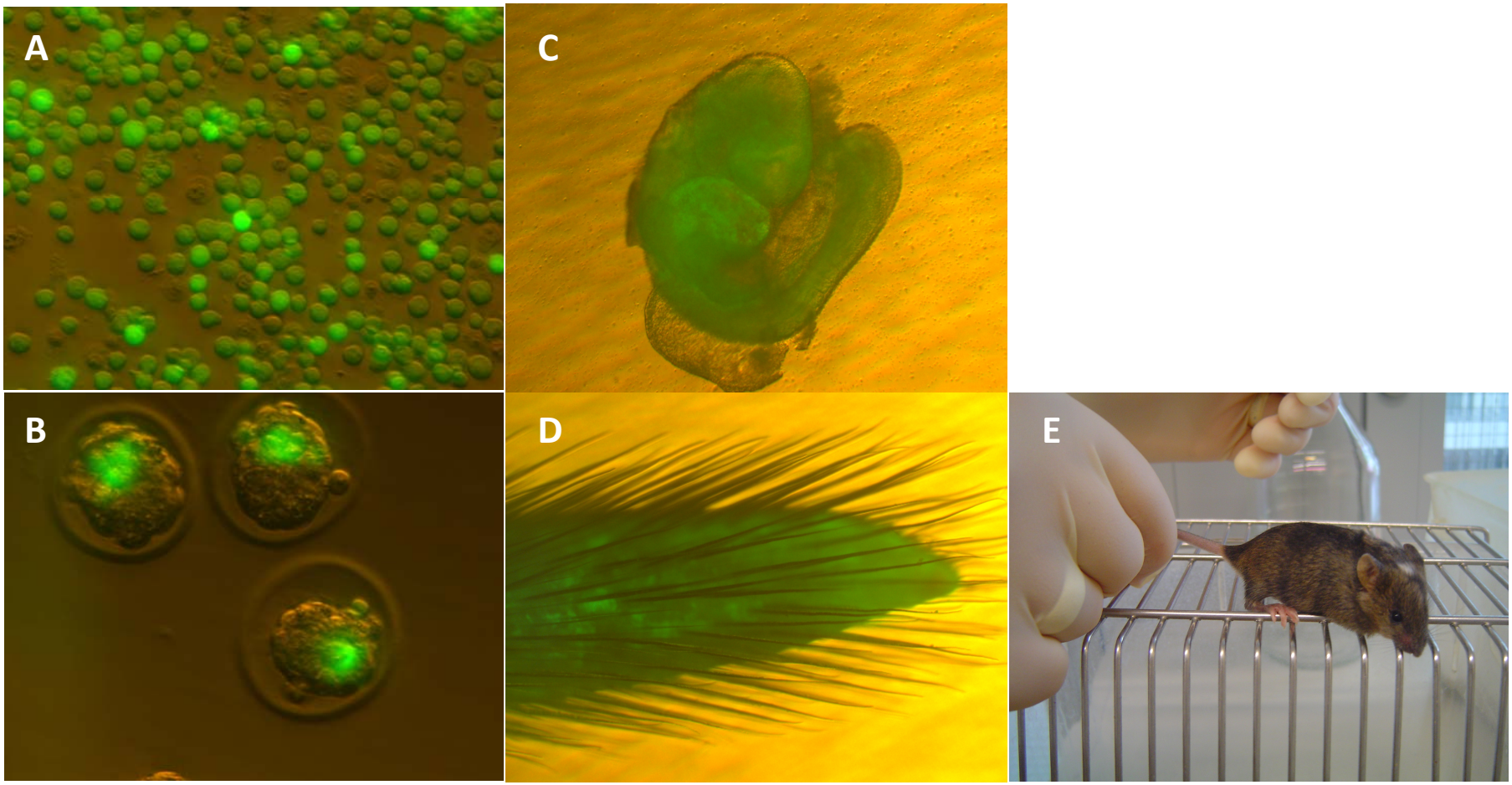

Figure S4: Whole cycle of chimera producing. Mouse ES cells, carrying HAC-GFP (A), preimplantated donor blastocysts immediately after injection (B), chimeric fetus (C), tail of adult chimeric mouse (D), adult chimeric mouse (E).

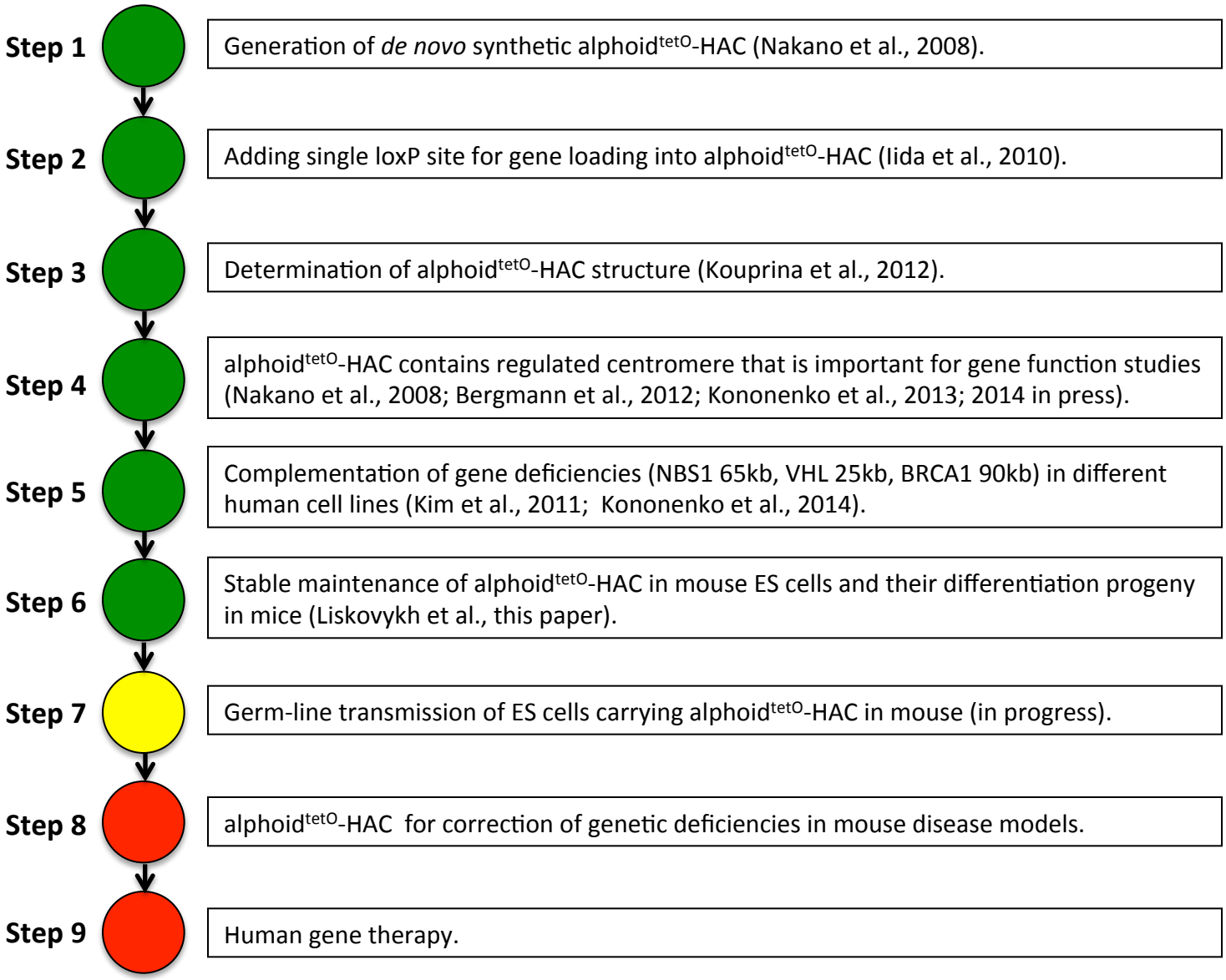

Figure S5: Evolution of de novo assembled alphoid^{tetO}-HAC technology toward gene therapy.