Supplementary Figure Legends

Supplementary Figure 1. Different methods to generate conditional KOs.

(a) Schematic drawing of the conventional strategy to generate conditional KOs utilizing CRISPR. Cas9 and gRNAs are used to flank the exon of one allele of the gene of interest with loxP sites. To achieve biallelic targeting the second allele needs to be generated via either a second round of recombination or breeding of mice. Orange triangles – loxP sites, DS – Drug selection gene.

(b) Conditionals by inversion (COIN) strategy to generate conditional KOs. A 'flippable' reporter gene combined with a drug selection cassette (DSC) is inserted into an exon or intron of the gene of interest via homologous recombination. The drug selection cassette is flanked by FRT sites and is removed prior to inversion of the reporter cassette. The reversing of orientation (of the reporter cassette) is mediated by Cre recombinase and converts the lox71 and lox66 sites to a lox72 and loxP site respectively. Following inversion the 3' splice site (3'SS) together with the polyA signal now in sense direction abrogate the transcription of the gene.

(c) Generation of conditional KOs in mouse zygotes. Co-injection of Cas9 mRNA, different gRNAs and DNA vectors/oligos in mouse zygotes allows generation of a conditional allele by flanking the exon of interest with loxP sites.
The gRNA sequence is red, capitalised and underlined, while the PAM is in green and the template oligos containing the loxP sites (light blue text with orange background) to be inserted are in light blue.

(d) Inducible Cas9 systems. In the first system (i), cells express the Tet-On 3G transactivator in an inactive form and Cas9 is not expressed. Addition and binding of doxycycline to the Tet-On 3G transactivator induces a conformational change allowing it to bind to the Tet responsive element 3G and initiate expression of the Cas9 protein. The constant transcription of the gRNA in combination with the induced expression of Cas9 allows gene editing. It is noteworthy that there is a similar system based on the same principle (although using M2rtTA rather than 3G) except that the gRNA is not constitutively expressed but needs to be provided through transfection. In the second system (ii) the Cas9 protein is split in two parts. Following translation the C-terminal part having two nuclear localization signals (NLS) is transported into the nucleus whereas the N-terminal part, having a nuclear export signal (NES) is kept in the cytoplasm. In addition the N- and C-terminal part of Cas9 are fused to FKBP (FK506 binding protein 12) and FRB (FKBP rapamycin binding) domains respectively. Thus, in the presence of rapamycin the FKBP and FRB domains will bring the Cas9 domains together, allowing their reassembly and import into the nucleus, due to the presence of the NLS sequences. Upon gRNA transfection the reassembled Cas9 can induce double stranded breaks.

Supplementary Figure 2. Step-wise Cre recombination and inversion of the FLIP cassette.

(a) Inversion (flipping) of the FLIP cassette.
Schematic showing the step wise recombination of loxP sites following Cre treatment. Following the first recombination the loxP sites represented by pink triangles (left) or the loxP sites represented by purple triangles (right) will be recombined. As the loxP sites are

facing each other the result is an inversion. During the second recombination the loxP sites, now aligned in the same direction recombine. The result is deletion of the PGK promoter and branch point 1 (BP1). SD – splice donor, SA – splice acceptor, pink and purple triangles – loxP site, BP – branching point.

(b) Genotyping strategy used to confirm clones targeted with the FLIP cassette.
The arrows represent primers, and the primer pairs are colour coded. The drawing shows the position of the primers in the genome and in the FLIP cassette. The blue and orange primers were used to confirm correct integration of the FLIP cassette. The allele not having integrated a FLIP cassette but potentially sustained indels due to NHEJ is genotyped and sequenced with the primer pair represented by the green arrows.

Supplementary Figure 3. Workflow

Representative image of the workflow including time estimate for generating bi-allelic conditional KOs using the CRISPR-FLIP technology.

Supplementary Figure 4. Validation of the FLIP cassette by insertion in the endogenous *Esrrb and Sox2* gene.

Detection of correctly targeted *Esrrb* clones **(a-d)**. The FLIP cassette containing a resistance gene was inserted into the 2nd exon of *Esrrb* (a). Detection of correctly targeted *Esrrb* clones. Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (b). The clones G11, B3 and H5 are correctly targeted. Sequencing results of the second allele of the *Esrrb* gene allow identification of insertions/deletions (c). Clone B3 has a 5 base pair (bp) deletion and clone H5 has a 34bp deletion. Loss of protein expression following Cre treatment was confirmed by western blot (d). Detection of correctly targeted *Sox2* clones (**e-i**). The FLIP cassette containing a resistance gene was inserted into the exon of *Sox2* (e). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (f). The clones A2, HOM are correctly targeted. Sequencing results of the second allele of the *Sox2* gene allow identification of insertions/deletions, this was used to confirm the FLIP/+ genotype of clone A2 (g). The lack of the exon band confirms the genotype of the HOM FLIP/FLIP clone. Loss of protein following Cre treatment (gene inactivation) was confirmed by immunofluorescence (h) and western blot (i). Please note that in this case a homozygous FLIP/FLIP clone was used to show the loss of protein expression and functionality of the FLIP cassette.

Supplementary Figure 5.

Detection of correctly targeted *Apc* clones **(a-c).** The FLIP cassette containing a resistance gene was inserted into the 16th exon of *Apc* (a). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (b). The clones A3, D5 are correctly targeted. Sequencing results of the second allele of the *Apc* gene allow identification of insertions/deletions (c). Clone D5 has a 10 bp deletion.

Detection of correctly targeted *Nfx1* clones (**d-f**). The FLIP cassette containing a resistance gene was inserted into the 2nd exon of *Nfx1* (d). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (e). The clones E1, F6 are correctly targeted. Sequencing results of the second allele of the *Nfx1* gene allow identification of insertions/deletions (f). Clone F6 has a 22 bp deletion.

Detection of correctly targeted *Tcf7l2* clones (**g-i**). The FLIP cassette containing a resistance gene was inserted into the 5th exon of *Tcf7l2* (g). Detection of correctly targeted *Tcf7l2* clones. Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (h). The clones C3, A6, A11 are correctly targeted. Sequencing results of the second allele of the *Tcf7l2* gene allow identification of insertions/deletions (i). Clone A6 has a 10 bp deletion and A11 has a 1 bp deletion.
Detection of correctly targeted *Trim13* clones (**j-l**). The FLIP cassette containing a resistance gene was inserted into the 3rd exon of *Trim13* (j). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (k). The clones H3, H4, G10 are correctly targeted. Sequencing results of the second allele of the *Trim* gene allow identification of insertions/deletions (bottom right and left). Clone H3 has a 2 bp insertion and G10 has a 1 bp deletion (l).
Detection of correctly targeted *Trim37* clones (**m-o**). The FLIP cassette containing a resistance gene was inserted into the 6th exon of *Trim37* (m). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (n). The clones E3, H5, F11 are correctly targeted. Sequencing results of the second allele of the *Trim37* gene allow identification of insertions/deletions (o). Clone H5 has a 13 bp deletion and F11 has a 4 bp deletion.
SD – splice donor, SA – splice acceptor, pink and purple triangles – loxP site, BP – branching point, pA- polyadenylation signal, gRNA and PAM recognition sequences are represented in blue and purple respectively.

Supplementary Figure 6.

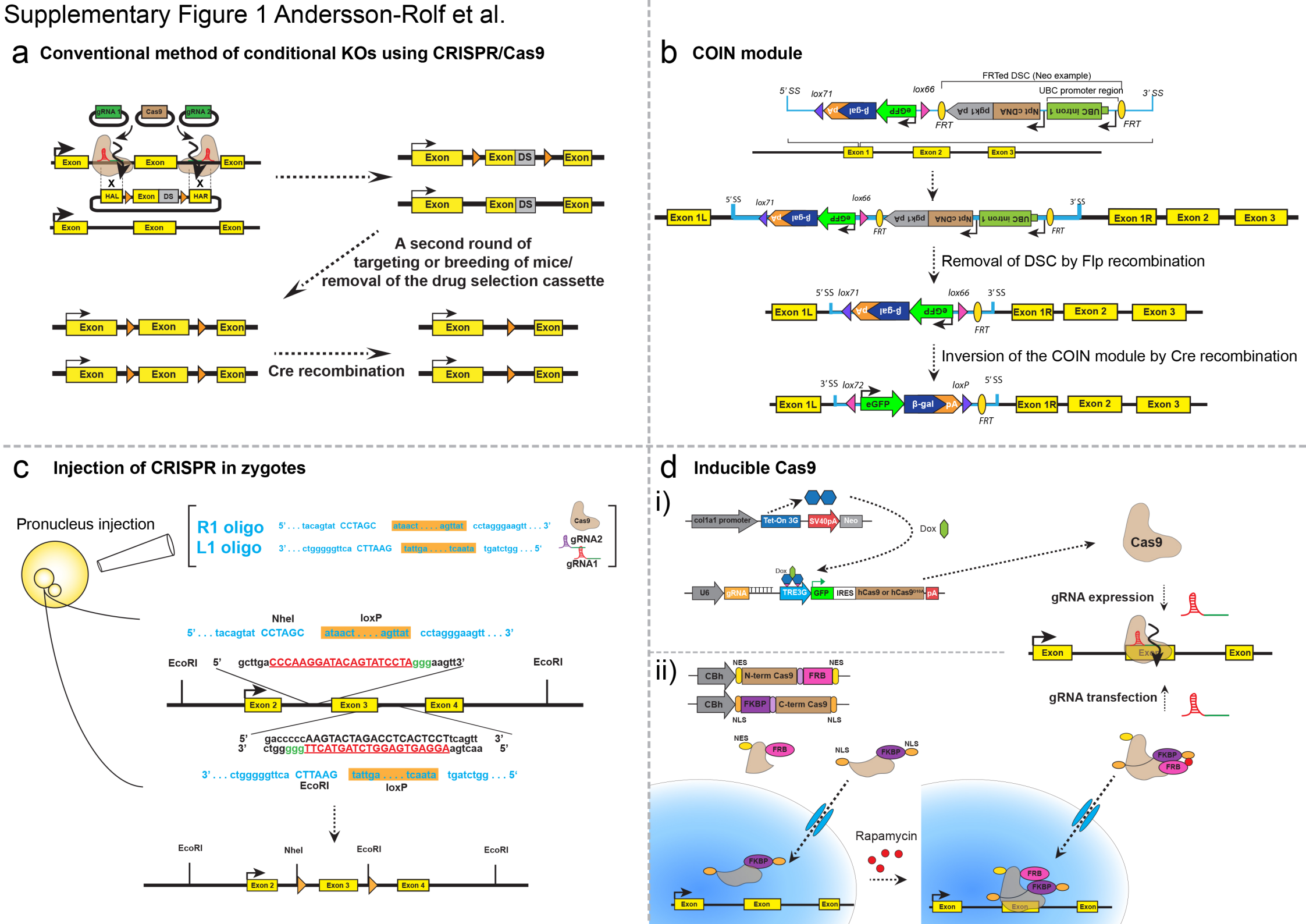
Detection of correctly targeted human *ARID1A (hARID1A)* in human embryonic kidney cells 293 (HEK293*)* clones **(a-c).** The FLIP cassette containing a resistance gene was inserted into the 3rd exon of *hARID1A* (a). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (b). The clones F1, F8, B8 are correctly targeted. Sequencing results of the second allele of the *hARID1A* gene allow identification of insertions/deletions (c). Clone F8 has a 5bp deletion and clone B8 has a 47bp deletion.

Detection of correctly targeted human *TP53 (hTP53)* in human embryonic kidney cells 293 (HEK293*)* clones **(d-f).** The FLIP cassette containing a resistance gene was inserted into the 4th exon of *hTP53* (d). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (e). The clones D1, E2, D6 are correctly targeted. Sequencing results of the second allele of the *hTP53* gene allow identification of insertions/deletions (f). Clone E2 has a 19bp deletion and clone D6 is homozygous for the FLIP cassette.

Supplementary Figure 7.

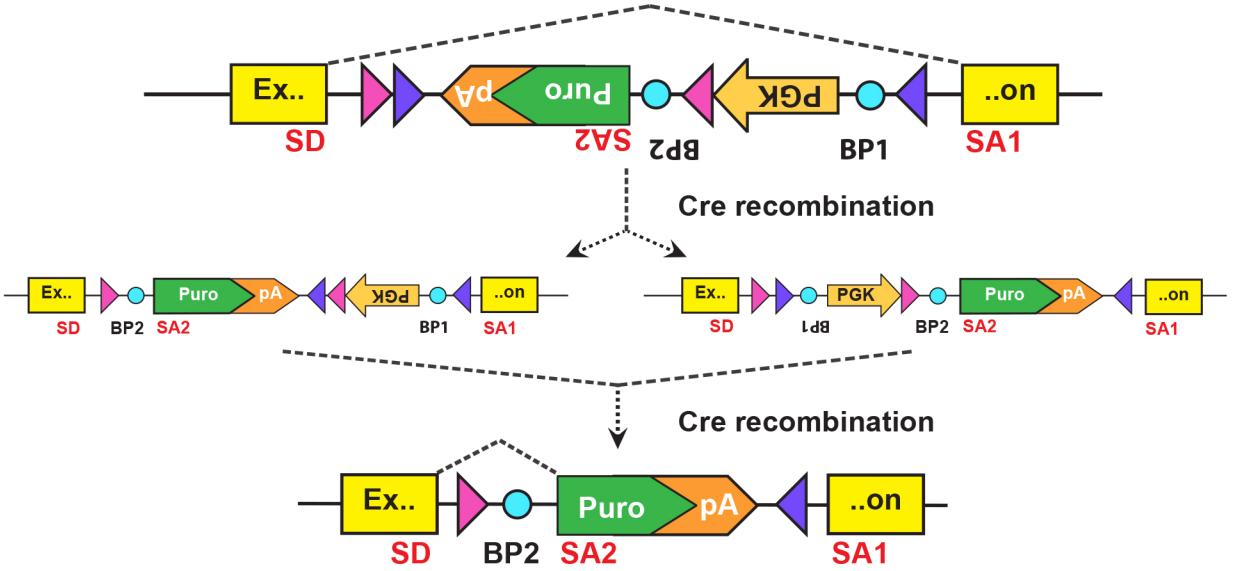
Detection of correctly targeted human *TP53 (hTP53)* in human induced pluripotent stem cell (hiPSC) clones **(a-c).** The FLIP cassette containing a resistance gene was inserted into the 4th exon of *hTP53* (a). Detection of correctly integrated 5'arm and 3'arms by PCR in ESC clones targeted with the FLIP cassette (b). The clones H4, C4, F4 are correctly targeted. Sequencing results of the second allele of the *hTP53* gene allow identification of insertions/deletions (c). Clone C4 has an 11bp deletion and clone F4 has a 13bp insertion.

Supplementary Figure 1 Andersson-Rolf et al.



Supplementary Figure 2 Andersson-Rolf et al.

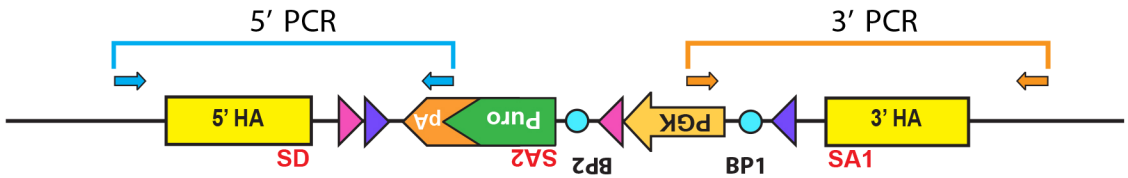
a Stepwise Cre recombination of the FLIP cassette



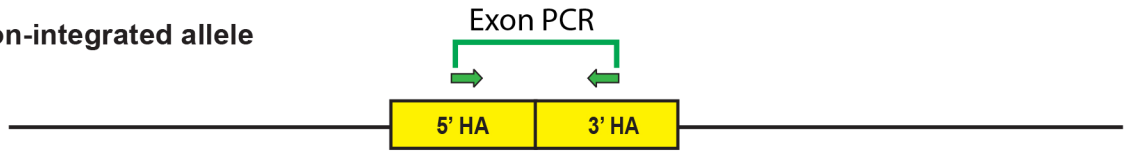
1. Disruption of the original splice site (BP1 removed)
2. Polyadenylation signal (pA) to terminate transcription
3. New splice acceptor (BP2-SA2)

b Genotyping stratgey

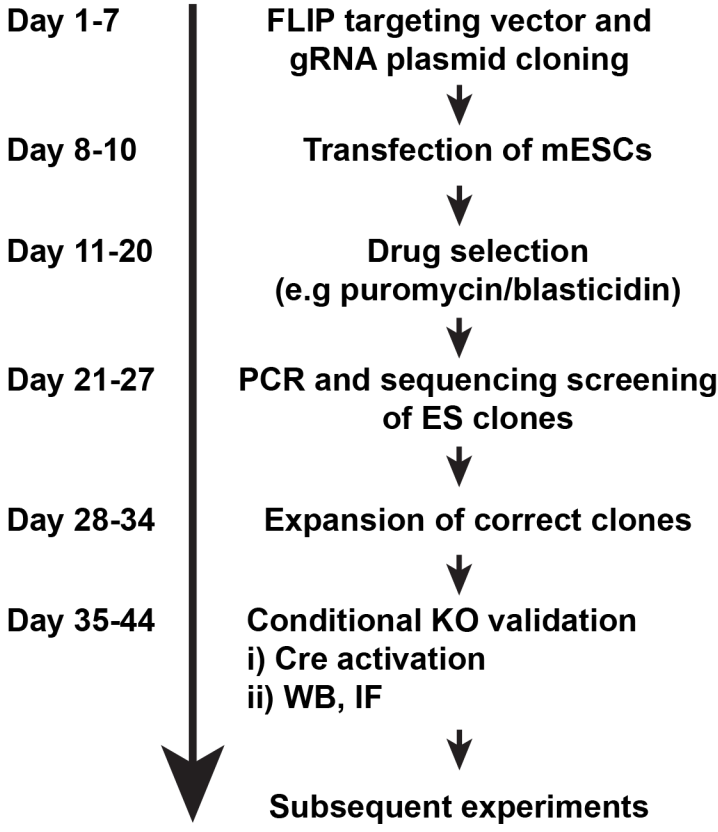
Cassette integrated allele



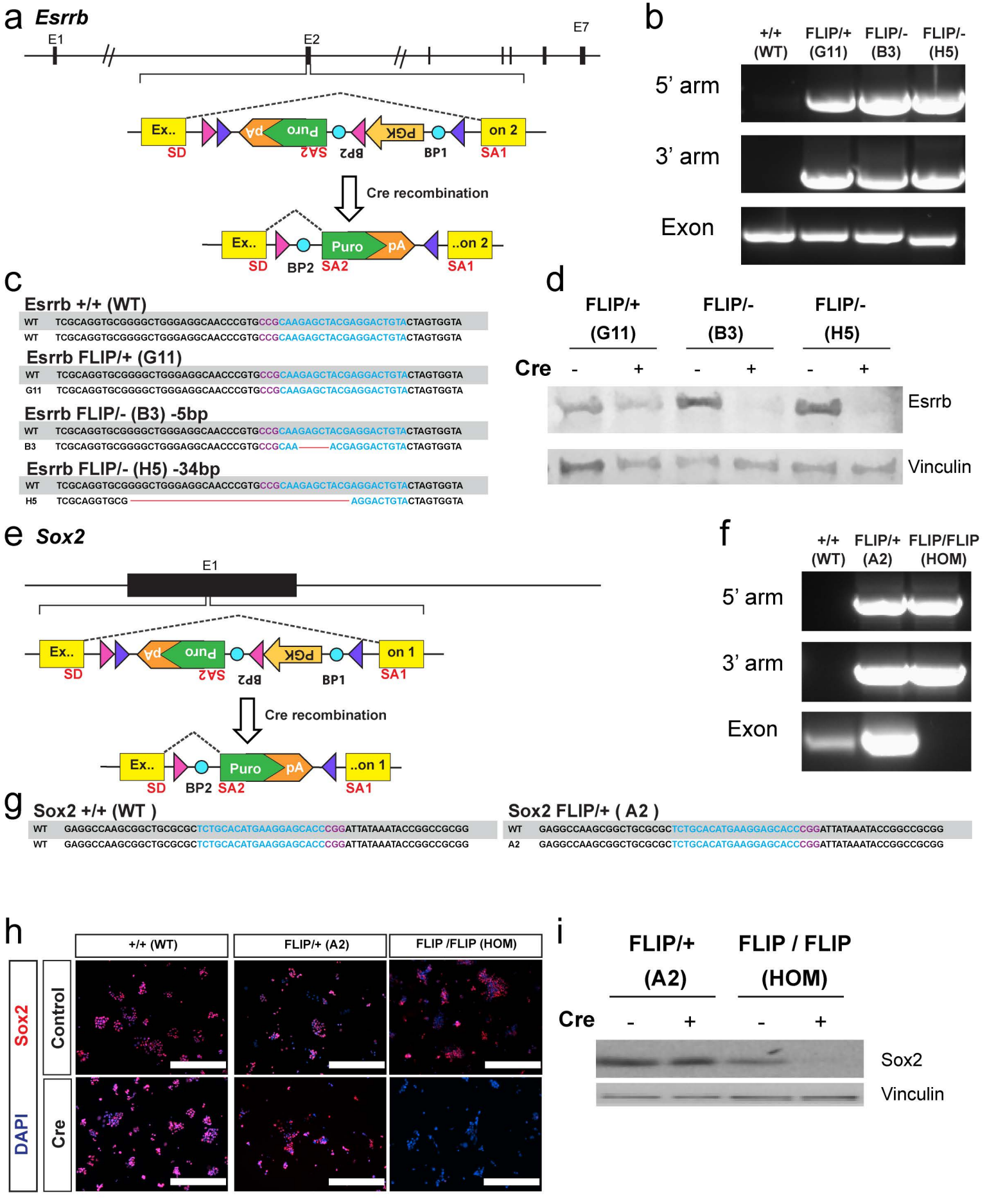
Non-integrated allele



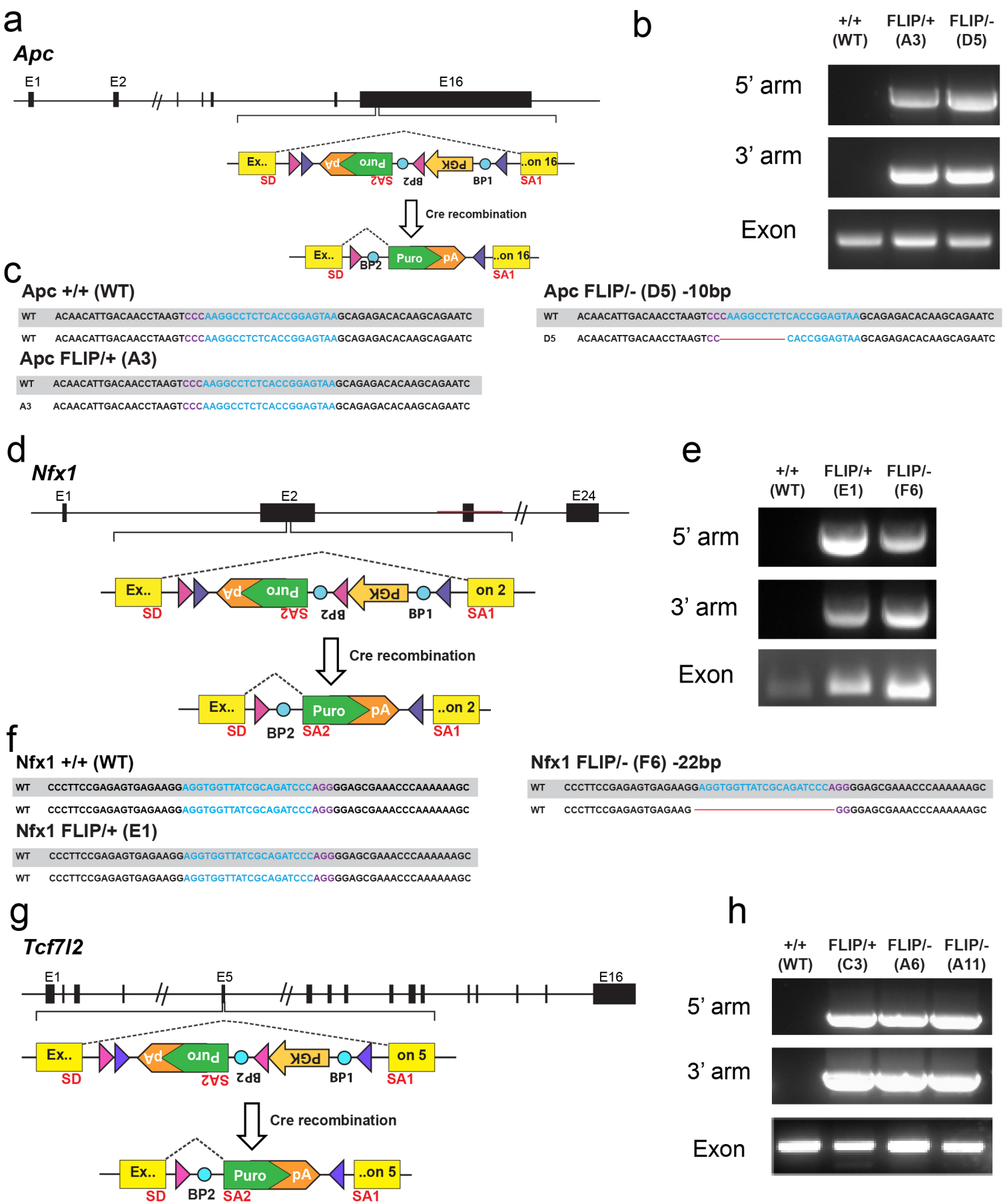
Supplementary figure 3 Andersson-Rolf et al.



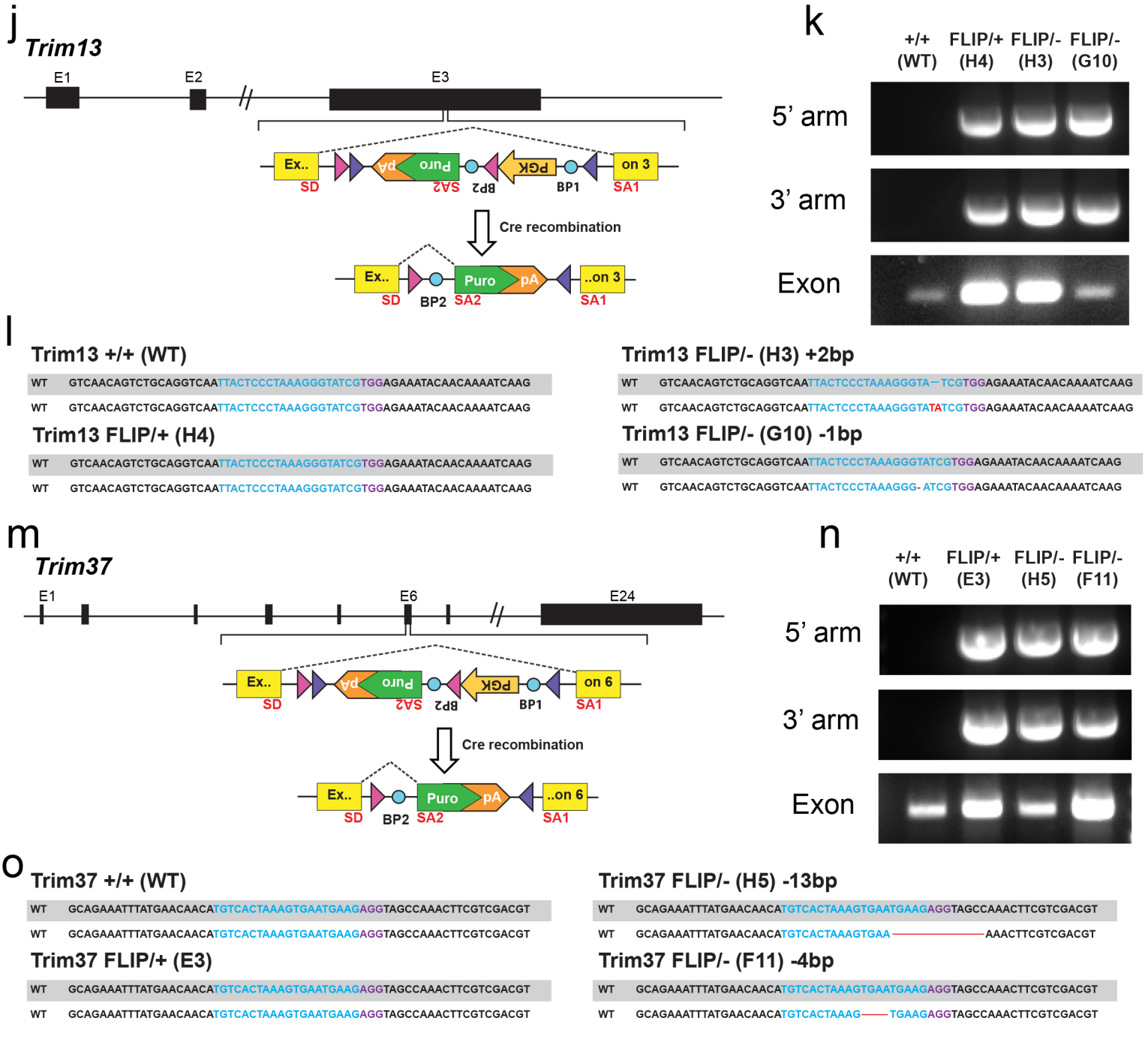
Supplementary Figure 4 Andersson-Rolf et al.



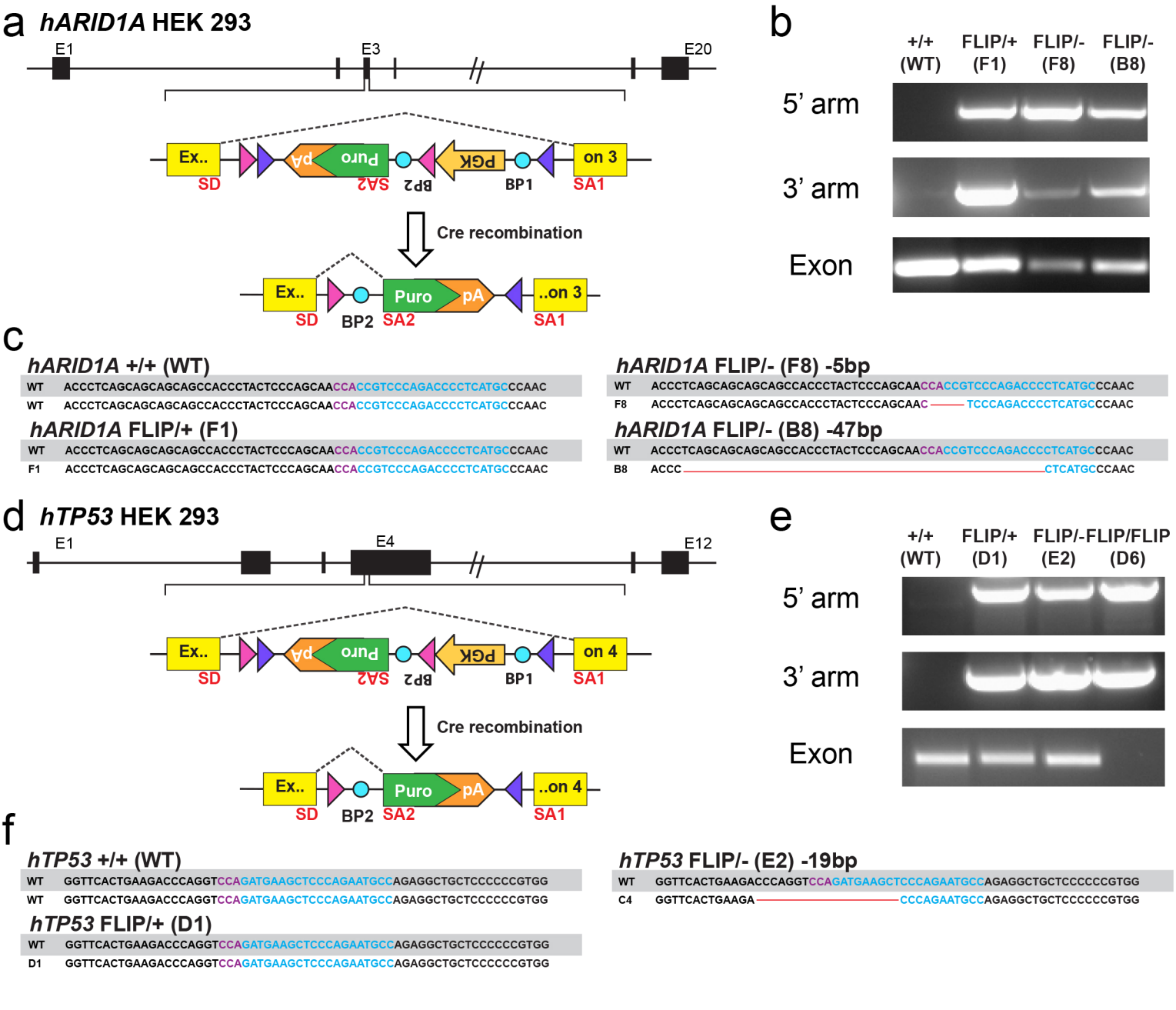
Supplementary Figure 5 Andersson-Rolf et al.



Supplementary Figure 5 Andersson-Rolf et al.



Supplementary Figure 6 Rolf et al.



Supplementary Figure 7 Rolf et al.