

Figure S1. Characterization of AAVS1 CAGs.TK and LV CAGs.TK-IPSC. (a) PCR-based analysis of targeted integration into the AAVS1 locus. WT=Wild type; 5'TG= 5' targeted integration; 3'TG= 3' targeted integration; 5'off= 5' off-target integration; 3'off= 3' off-target integration; bi= biallelic-targeted control; mono= mono allelic-targeted control. (b) Vector copy number of transduced cells as determined by qPCR detecting wPRE. Technical replicates N=3. (c) RT-qPCR expression data of TK.007-modified cells for *NANOG* and *OCT4*. Expression levels are relative to H9 embryonic stem cells (ES). Technical replicates N=3. (d) Flow cytometry analysis of TRA-1-60 and Δ CD19 expression. Shown is the percentage of positive cells within the population of viable cells among $0.5-1 \times 10^5$ analyzed cells in total. (e) Flow cytometry analysis of Kat2S and Δ CD19 expression intensity in pluripotent TK.007-modified iPSC based on the analysis of a total of $0.5-1 \times 10^5$ cells.

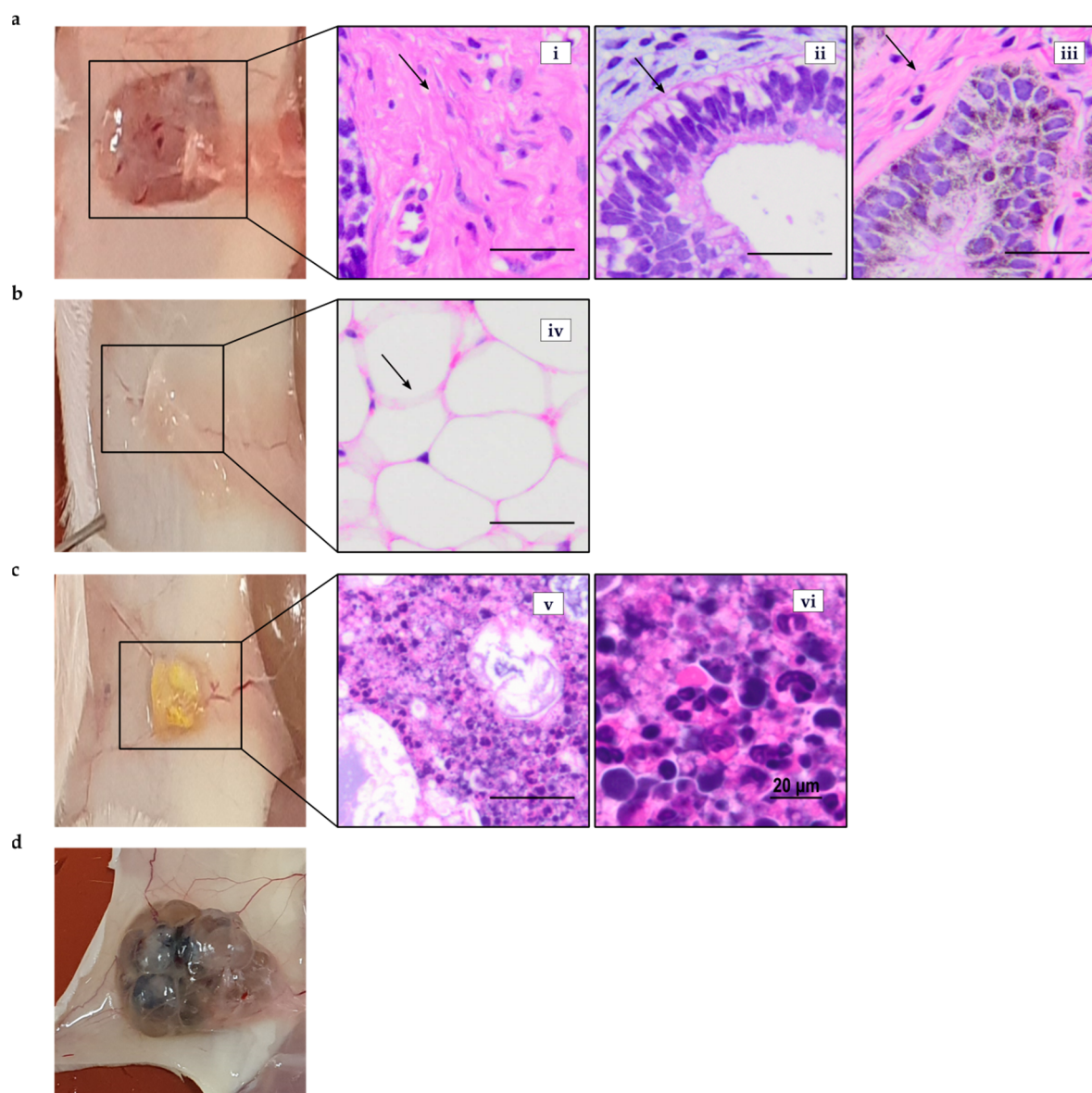
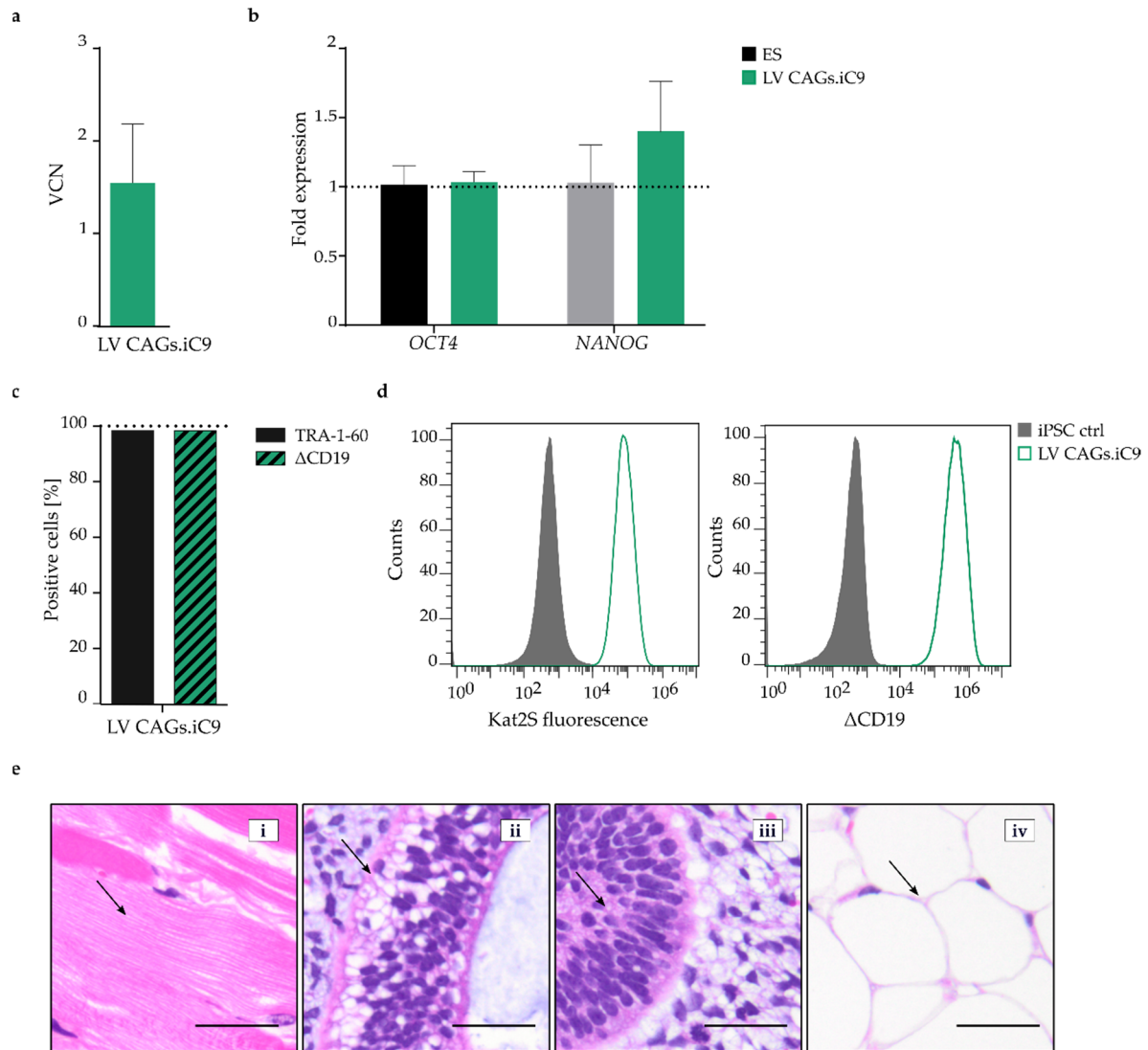
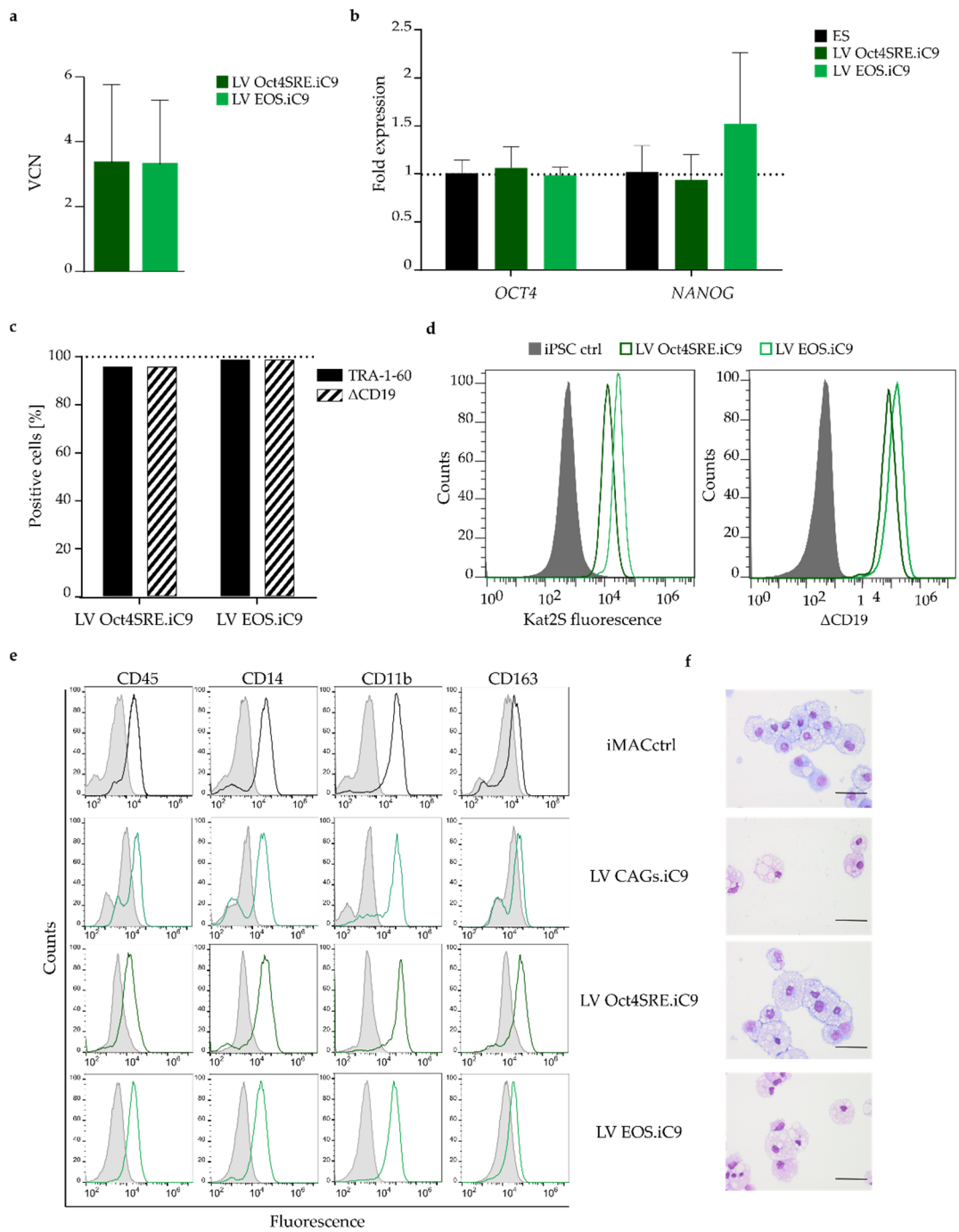


Figure S2. Teratoma sections from AAVS1 CAGs.TK-iPSC and LV CAGs.TK-iPSC injected mice. (a) Vehicle-treated teratomas, established from AAVS1 CAGs.TK and LV CAGs.TK iPSC are composed of all three germ layers (indicated by the arrows), mesoderm (muscular structure; i), endoderm (mucinous glandular epithelium; ii) and ectoderm (pigment epithelium; iii). (b) Animals that were immediately treated with GCV upon iPSC-injection demonstrated no palpable or visible teratomas. The tissue present at the injection site was adipose tissue (iv). (c) Animals treated with GCV upon tumor palpation demonstrated necrotic tissue remnants with infiltrating leukocytes at the former injection site (v, vi). (d) Exemplary picture of a cystic fluid-filled teratoma excluded from the analysis. Scale bar 100 μ m (if not indicated otherwise).





8

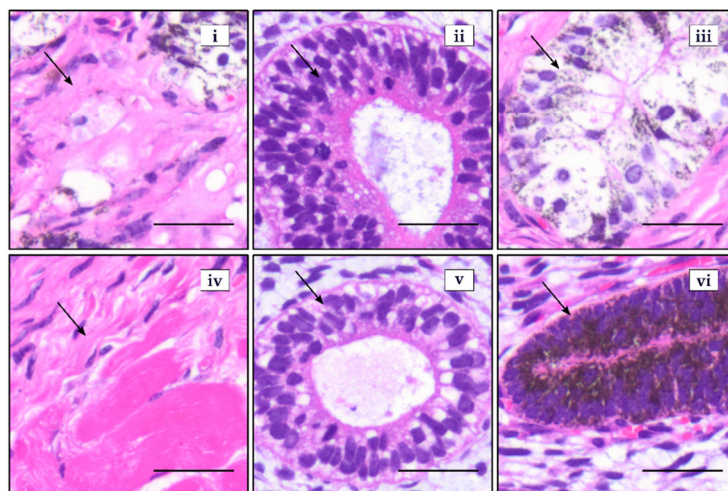


Figure S4. Characterization of LV Oct4SRE.iC9 and LV EOS.iC9 cells. (a) Vector copy number as determined by qPCR for wPRE. Technical replicates, N=3. (b) RT-qPCR analysis of *OCT4* and *NANOG* expression relative to embryonic stem cells (ES). Technical replicates N=3. (c) TRA-1-60 and Δ CD19 antibody staining analyzed by flow cytometry. Shown is the percentage of positive cells within the viable population among $0.5-1 \times 10^5$ cells analyzed in total. (d) Kat2S and Δ CD19 expression intensity analyzed by flow cytometry using a total of $0.5-1 \times 10^5$ cells. (e) Flow cytometry analysis of differentiated iMAC for expression of surface markers CD45, CD14, CD11b and CD163. Gray histograms indicate isotype control. Colored lines indicate stained cells. (f) Cytospin analysis of differentiated iMAC. Scale bar 50 μ M. (g) Upper part: sections of vehicle-treated LV Oct4SRE.iC9 iPSC-derived teratomas, representing cells of all three germ layers (indicated by the arrows), i.e. mesoderm (muscular structures; i), endoderm (mucinous glandular epithelium; ii), ectoderm (pigment epithelium; iii); lower part: sections of AP20187-treated LV-Oct4SRE.iC9 iPSC-derived teratomas representing cells of all three germ layers (indicated by the arrows), i.e. mesoderm (muscular structures; iv), endoderm (mucinous glandular epithelium; v) and ectoderm (pigment epithelium; vi). Scale bar 100 μ m.