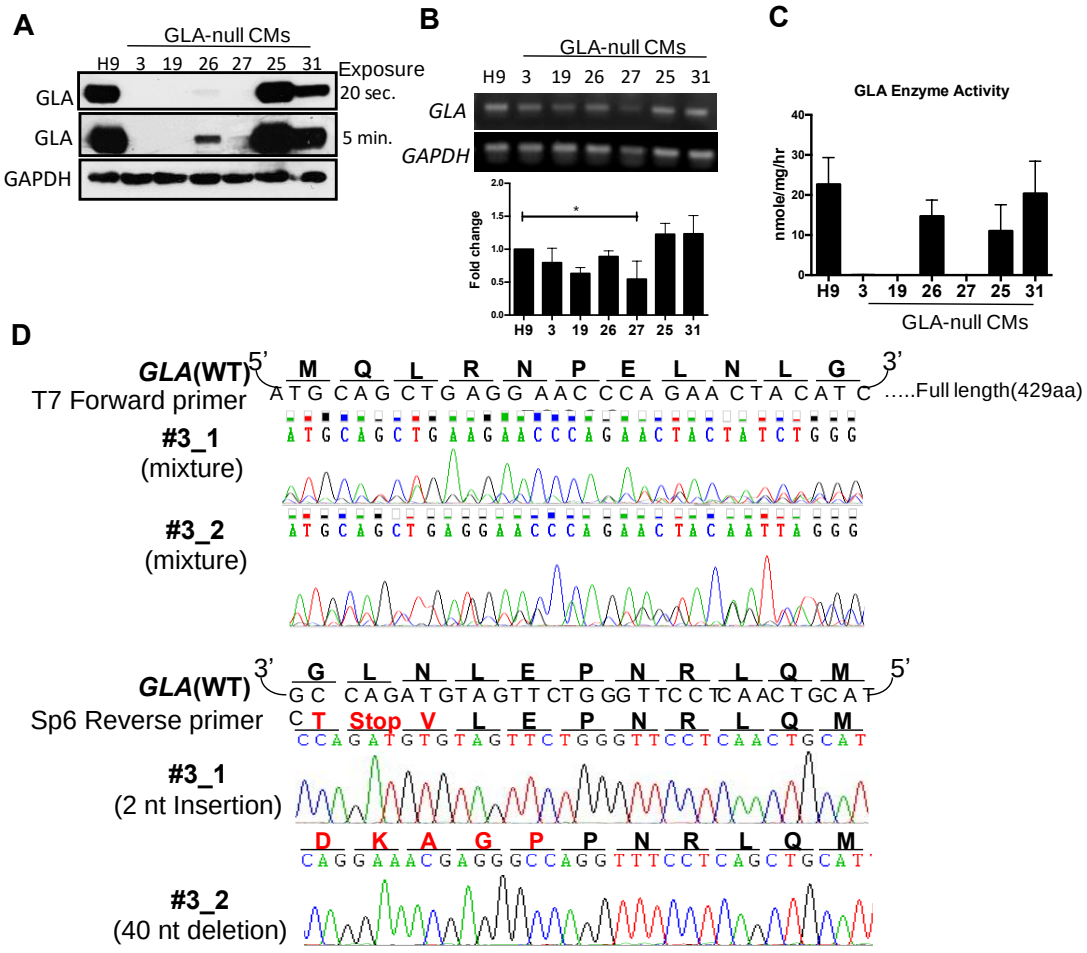
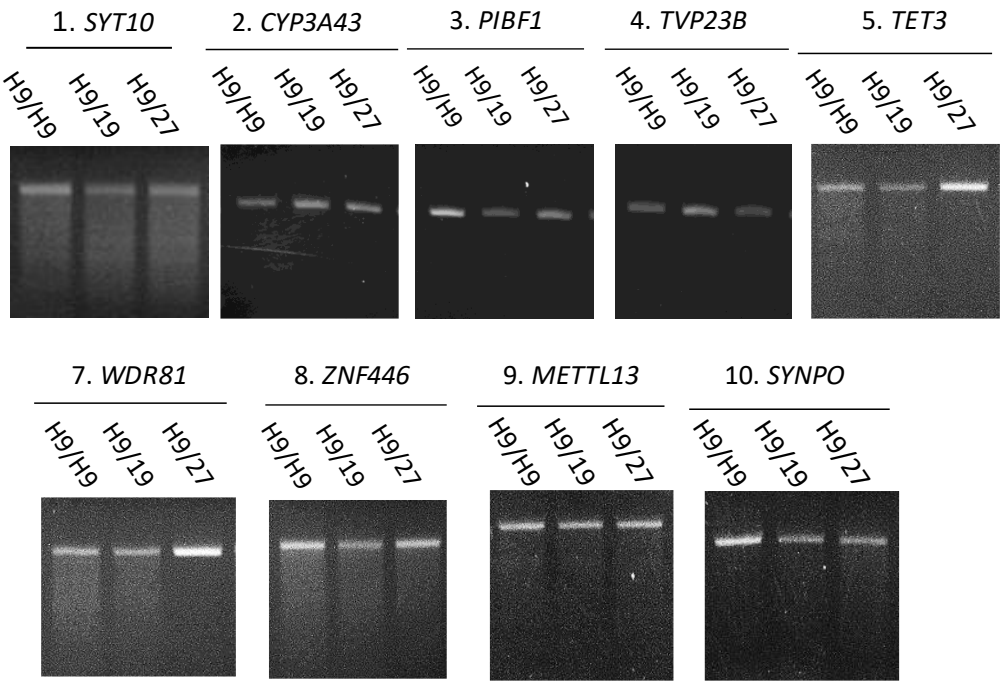
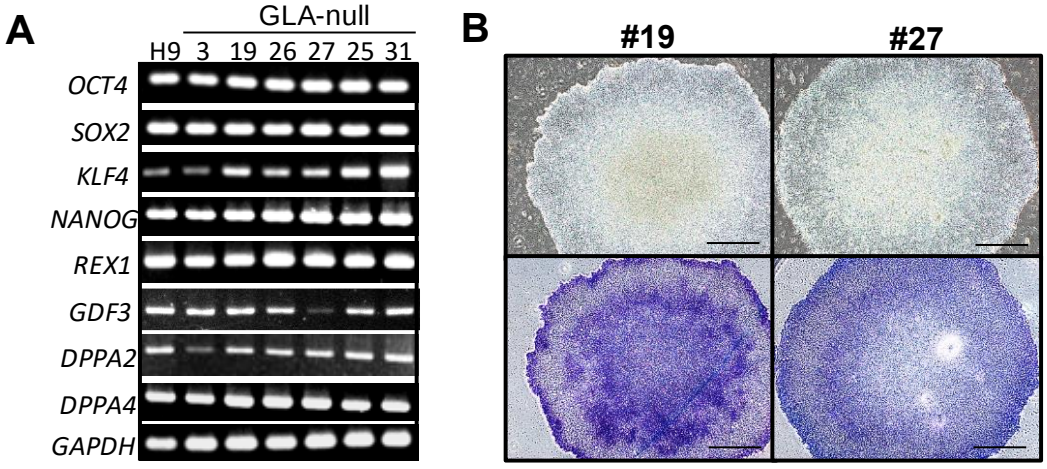


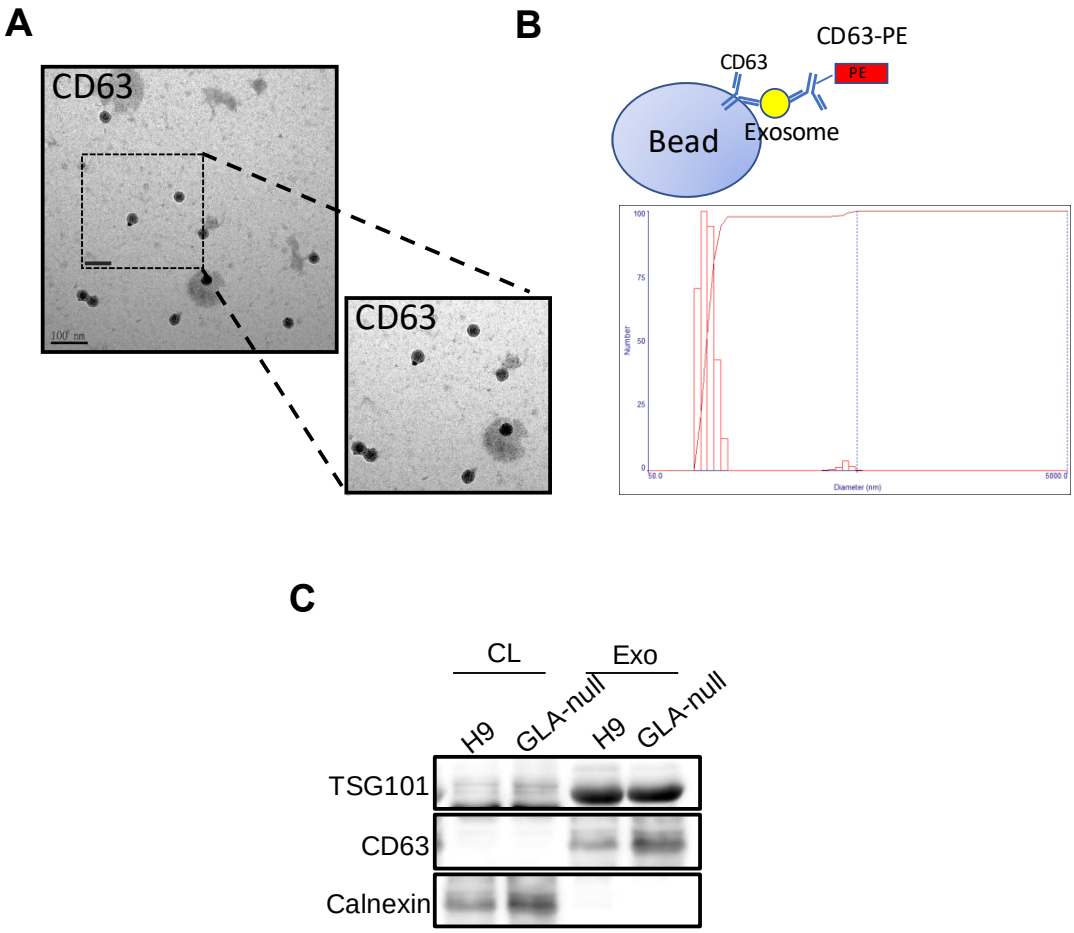

Supplementary Figure 1. (A) GLA protein expression level in GLA-null clones #3, 19, 26, 27, 25, and 31 derived CMs Exposure in short time:20 sec and longer time:5 mins. (B) mRNA level in GLA-null clones #3, 19, 26, 27, 25, and 31 derived CMs. (C) GLA enzyme activity in GLA-null clones #3, 19, 26, 27, 25, and 31 derived CMs. (D) Sanger sequencing analysis revealed heterogeneous population in clone #3.




Supplementary Figure 2. T7E1 digestion assay validating the absence of CRSIPR/Cas9-induced mutations in the predicted off-target genes. T7E1 cleavage assay showing the absence of mismatch cleavage products.




Supplementary Figure 3. Characterization of CRISPR/Cas9-edited GLA-null hESC clones. (A) RT-PCR analysis of expression of pluripotency-associated genes in CRISPR/Cas9-transfected hESC clones, including GLA-null clones #19 and #27. Untransfected parental hESCs (H9) served as a positive control. (B) Morphology and alkaline phosphatase activity of GLA-null hESC clones #19 and #27.




Supplementary Figure 4. Characterization of CM-derived exosomes. (A) Representative electron microscopy images of isolated exosomes. Scale bar: 100 uM. (B) CD63 PE-conjugated dynabeads isolated and identified distribution of CMs derived exosome sizes though Nanosight tracking system with a diameter range of 50–100 nm. (C) Western blot showing expression of exosomal markers TSG101 and CD63 in cell lysate (CL) and exosome-containing culture medium (Exo) of H9 and GLA-null CMs. Calnexin used as CL positive control.

*Supplementary Table 1.* List of primers used to amplify the predicted off-target gene loci.

| | Gene No. | Gene Name | Chromosome Site | Primer Sequence | Product Size |
|---|---|---|---|---|---|
| **1** | NM_198992 | *SYT10* | chr12:-33559846 | TAGCATGGGCACAGAACCTG<br>TTCAGCATTAGGTGCCTGAATTA | 684 |
| **2** | NR_103869 | *CYP3A43* | chr7:+99454520 | CCAGGAAGTTGTGTCCAAAGG<br>CAGTGTGTCTCCTGATTGGATG | 699 |
| **3** | NM_006346 | *PIBF1* | chr13:+73467918 | AGCAAGCTGGGAAACAATGC<br>CCCTCCCAGAAACATGGTGT | 867 |
| **4** | NM_016078 | *TVP23B* | chr17:+18702311 | TCCTTGATCATTGGTAGGAAAGGT<br>TTGTTAGGCAGCAATAGGTTACCA | 739 |
| **5** | NM_144993 | *TET3* | chr2:-74328546 | GTCCCCCAAGAGGACTAACG<br>GGGCACACTCGATGAGGAT | 674 |
| **7** | NM_152348 | *WDR81* | chr17:-1639089 | GCGGTGAGTTGGGGGATTAG<br>AGCACCATGAAGCCTGAGGA | 660 |
| **8** | NM_017908 | *ZNF446* | chr19:-58992296 | ACCGCAAGAGCCACACAG<br>AACTGCCTATTTCCCGACCA | 679 |
| **9** | NM_014955 | *METTL13* | chr1:+171759733 | GCACACTGCTGCCAGTAACC<br>CAGGGACTTCAGGTGAAAACG | 736 |
| **10** | NM_001166208 | *SYNPO* | chr5:-150031531 | CCTGGATTCTAACAGACCAACTGC<br>GAGCAGGCCCACTCCACTT | 699 |

*Supplementary Table 2. Sequences of the primers used to analyze stemness markers byRT-PCR*

| **Name** | **Sequence** | **Predicted size** |
|---|---|---|
| *OCT4* | F_CTTCAGGCACTGTGTTCATTG<br>R_TTTGGCTGAACACCTTCCCA | 672 bp |
| *SOX2* | F_GCCCTGCAGTACAACTCCAT<br>R_TTCCTGCAAAGCTCCTACCG | 735 bp |
| *KLF4* | F_AGTTTCCCGACCAGAGAGA<br>R_ACGCGAACGTGGAGAAAGAT | 667 bp |
| *NANOG* | F_GAAGACAAGGTCCCGGTCAA<br>R_GGATTCAGCCAGTGTCCAGA | 709 bp |
| *REX1* | F_GTGGGCCTTATGTGATGGCT<br>R_TGCGTTAGGATGTGGGCTTT | 759 bp |
| *GDF3* | F_ GTTTGTGTTGCGGTCAGTCC<br>R_ CTTGGGGGCAATGATCCACT | 361 bp |
| *DPPA2* | F_CCGTCCCCGCAATCTCCTTCCATC<br>R_ATGATGCCAACATGGCTCCCGGTG | 606 bp |
| *DPPA4* | F_TAGCACAGCAAAAGAGGCCA<br>R_TGCATGGCCCATAAACAGGT | 635 bp |
| *GAPDH* | F_AGAAGGCTGGGGCTCATTTG<br>R_AGGGGCCATCCACAGTCTTC | 258 bp |

*Supplementary Table 3. Antibodies used in this study*

| **Target** | **Source** | **Catalog number** |
|---|---|---|
| GLA | GeneTex | GTX101178 |
| NANOG | Cell Signaling Technology | #4903 |

| | | |
|---|---|---|
| OCT4 | Cell Signaling Technology | #2750 |
| TRA-1-81 | Abcam | Ab16289 |
| TRA-1-60 | Abcam | Ab16288 |
| Nestin | Cell Signaling Technology | #4760 |
| alpha-SMA | Cell Signaling Technology | #19245 |
| AFP | Cell Signaling Technology | #4448 |

*Supplementary Table 4.* List of the differentially downregulated proteins involved in regulated exosome release.

| Uniprot | Protein Name | Gene Name |
|---|---|---|
| Q13636 | Ras-related protein Rab-11 | RAB11 |
| Q86VN1 | Vacuolar protein-sorting-associated protein 36 | VPS36 |
| Q13017 | Rho GTPase-activating protein 5 | ARHGAP5 |
| P52566 | Rho GDP-dissociation inhibitor 2 | ARHGDIB |
| Q9NP61 | ADP-ribosylation factor GTPase-activating protein 3 | ARFGAP3 |
| P35475 | Alpha-L-iduronidase | IDUA |
| Q96AJ9 | Vesicle transport through interaction with t-SNAREs homolog 1A | VTI1A |
| Q14894 | Ketimine reductase mu-crystallin | CRYM |
| P07996 | Thrombospondin-1 | THBS1 |
| Q02952 | A-kinase anchor protein 12 | AKAP12 |
| Q9UJA5 | tRNA-methyltransferase non-catalytic subunit TRM6 | TRMT6 |
| Q96EY4 | Translation machinery-associated protein 16 | TMA16 |
| Q86SZ2 | Trafficking protein particle complex subunit 6B | TRAPPC6B |

*Supplemental Reference*

1. Labuhn, M., et al., Refined sgRNA efficacy prediction improves large- and small-scale CRISPR-Cas9 applications. Nucleic Acids Res, 2018. 46(3): p. 1375-1385.