### Supplementary Material and Methods Section

#### Table S1. Forward primers to produce the sgRNA in vitro transcription template. Each primer contains the T7 promoter sequence, the spacer sequence (different for each sgRNA) and the scaffold template-annealing sequence.

| sgRNA name | T7 promoter sequence | Spacer sequence | Guide-it Scaffold Template-annealing sequence |
|------------|----------------------|----------------|---------------------------------------------|
| G3         | GCGGCCTCTAATACGACTCAGCACTATAGGG | AGTGCATGGGCGCTGGGCGG | GTTTTAGATGAGCTGAAATAGCA                     |
| G4         | GCGGCCTCTAATACGACTCAGCACACACAGG | GGCCAGTCTGAGCCAGACGCGC | GTTTTAGATGAGCTGAAATAGCA                     |
| G5         | GCGGCCTCTAATACGACTCAGCACTATAGGG | GTCATGCAATACGCGACCTC | GTTTTAGAGCTGAAATAGCA                       |
| G6         | GCGGCCTCTAATACGACTCAGCACTATAGGG | GCCGGTGTTCGAGCGGAAC | GTTTTAGAGCTGAAATAGCA                       |
| G7         | GCGGCCTCTAATACGACTCAGCACTATAGGG | TGGGAAGAGGCGCGCGGTC | GTTTTAGAGCTGAAATAGCA                       |
| G8         | GCGGCCTCTAATACGACTCAGCACTATAGGG | CGCGCTATTGCTGATGAGAC | GTTTTAGAGCTGAAATAGCA                       |
| G9         | GCGGCCTCTAATACGACTCAGCACTATAGGG | AGGCACGCCGCCCCTACCGGC | GTTTTAGAGCTGAAATAGCA                       |
| G10        | GCGGCCTCTAATACGACTCAGCACTATAGGG | GCCGGTGTACGAGCGGTCGCT | GTTTTAGAGCTGAAATAGCA                       |
| G11        | GCGGCCTCTAATACGACTCAGCACTATAGGG | CACGCTTGTGTCCCGCGTC | GTTTTAGAGCTGAAATAGCA                       |
| G12        | GCGGCCTCTAATACGACTCAGCACTATAGGG | TGATGACGGGTTGGCGGTTT | GTTTTAGAGCTGAAATAGCA                       |

#### Table S2. Primers for PCR reactions. For each primer are reported the nucleotide sequence, the Melting temperature and what reaction it was used for.

| Name                  | Sequence            | Tm  | Use                          |
|-----------------------|---------------------|-----|------------------------------|
| TempForward           | GAATGCCTGCTTCCCAAGAA | 57,8| Cutting template production  |
| TempReverse           | AGTTTGGAAACGGCAATCA | 55,5|                             |
| BCOR-NGS-Forw         | GGAGGACTGGAACCCCT   | 55  | Amplicon NGS off-target      |
| BCOR-NGS-Rev          | TTCGCGGAAACTACCTACAC| 57  |                             |
| INNP5A-NGS-Forw       | TATGAAGGACTGCAAGGC  | 54  |                             |
| INNP5A-NGS-Rev        | CGCTTGTTGAGGAGGCTGTCTTA | 57  |
| ZNRF1-NGS-Forw        | CCTGCAGGAATGTGAAATGG | 58  |                             |
| ZNRF1-NGS-Rev         | CACTGCTACTAAGAGGTGA | 58  |                             |
| G8_COL3A1-Forw        | TTGTAGGGTAAACCAGGACC | 56,1|                             |
| G8_COL3A1-Rev         | CCTTGCTATTTACTCCTGAGCAC | 55,2|
| KCNQ1-NGS-Forw        | TTGTGTGGAGGGCTGTAGAG | 56  |                             |
| KCNQ1-NGS-Rev         | GGACCGGCTTCTCCTCA   | 55  |                             |
| MEIS1-NGS-Forw        | GAAGGACCCAGCTGTAGAG | 57  |                             |
| MEIS1-NGS-Rev         | CACATGGCGTTGTTAGAG  | 56  |                             |
**Table S3. Primers for first step PCR reactions for libraries production.** For each primer are reported the adaptor, spacer and locus sequences.

| Name        | Adaptor                          | Spacer   | Locus               |
|-------------|----------------------------------|----------|---------------------|
| G3Nv2_1F    | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | TGCCACAGGGCTGGACTACT |
| G3Nv2_2F    | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | TGCCACAGGGCTGGACTACT |
| G3Nv2_3F    | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | TGCCACAGGGCTGGACTACT |
| G8_1R       | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | GCT      | GCTCCAGCTTCTGTGGT   |
| G8_2R       | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | TGCT     | GCTCCAGCTTCTGTGGT   |
| G8_3R       | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | CAGTACTG | GCTCCAGCTTCTGTGGT   |
| G3_1F       | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | AATACAGTGGAACCTATGGCCACG |
| G3_2F       | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | AATACAGTGGAACCTATGGCCACG |
| G3_3F       | GTCTCGGTGGGTCTCGGAGATGTGTATAAGAGACAG |          | AATACAGTGGAACCTATGGCCACG |
| G8B_1R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | TCGTGA   | TCTCTCCATCTACGGTCCA |
| G8B_2R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | CTA      | TCTCTCCATCTACGGTCCA |
| G8B_3R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | AGCGAGTAC | CTCTCCATCTACGGTCCA |
| G8N_1F      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | ATCTCGGTCATCTACGGTCCA |
| G8N_2F      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | GCTC     | ATCTCGGTCATCTACGGTCCA |
| G8N_3F      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | TGGACT   | ATCTCGGTCATCTACGGTCCA |
| G8N_1R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | G        | CTGGTGATTCCGGTTTCTCG |
| G8N_2R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | AAC      | CTGGTGATTCCGGTTTCTCG |
| G8N_3R      | TCCTCGGGAGGCTCAATGTGATAAGAGACAG     | TGGACAAAG | CTGGTGATTCCGGTTTCTCG |

**Lipofection**

The day before the lipofection, 5x10^5 cells were plated in 60 mm-diameter plates, in the specific medium for fibroblasts. The cells were then incubated at 37°C to reach a confluence of 70-80%. The next day two test tubes were prepared for each sample to be transfected. In the first tube the plasmid vector pcDNA3 or pcDNA3-EGFP was diluted in 250 µL of Opti-MEM medium and mixed gently; in the second one each selected transfection agent was added to 250 µL of Opti-MEM medium depending on volumes reported in table S4. This last step was carried out using polystyrene tubes, a material capable of preventing the adhesion of the transfecting agent to the surface of the tube itself, to facilitate its dilution with the medium and, subsequently, the formation of complexes with DNA. Subsequently the two solutions were combined and mixed and then incubated at room temperature from 15 to 30 minutes depending on the transfecting agent used, to promote the formation of complexes between cationic lipids and DNA. After removing the spent medium from the cells and replacing it with fresh serum-free medium, it was possible to add the cationic lipid-DNA complexes previously prepared to each well, favouring their mixing through circular and cross movements. In the wells corresponding to the negative controls only the medium and the liposomal agents were added, without the plasmid DNA. Finally, the plates were incubated at 37°C and, after 4 hours, the medium was replaced with complete medium, to limit toxic effects due to the permanence of the transfecting agents in culture. Transfected cells were incubated at 37°C in an 80% humidified atmosphere, containing 5% CO₂.
### Table S4. Transfection agents used for lipofection tests.
The table summarizes the various transfecting agents used in the fibroblast lipofection tests, their volumes, the amount of DNA used and the relationship between DNA and transfecting agent.

| Abbreviation | Transfection agent | Amount of DNA (µg) | Volume of the transfection agent (µL) | Ratio DNA:transfection agent | Note |
|--------------|--------------------|--------------------|----------------------------------------|-----------------------------|------|
| L-NT         | Lipofectamine 2000 | -                  | 20 µL                                  | -                           |      |
| L1           | Lipofectamine 2000 | 3 µg               | 20 µL                                  | -                           |      |
| L2           | Lipofectamine 2000 + Plus Reagent | 3 µg | 20 µL | - | Addition of Plus Reagent (60 µL) |
| T-NT         | TransIT-X2         | -                  | 7,5 µL                                 | -                           |      |
| T1           | TransIT-X2         | 2,5 µg             | 7,5 µL                                 | 1:3                         |      |
| T2           | TransIT-X2         | 2,5 µg             | 10 µL                                  | 1:4                         |      |
| E-NT         | Effectene          | -                  | 10 µL                                  | -                           |      |
| E1           | Effectene          | 0,4 µg             | 10 µL                                  | 1:25                        |      |
| E2           | Effectene          | 0,4 µg             | 20 µL                                  | 1:50                        |      |
| J-NT         | JetPRIME           | -                  | 4 µL                                   | -                           |      |
| J1           | JetPRIME           | 2 µg               | 4 µL                                   | 1:2                         |      |
| J2           | JetPRIME           | 2 µg               | 8 µL                                   | 1:4                         |      |
| X-NT         | X-TremeGENE        | -                  | 3 µL                                   | -                           |      |
| X1           | X-TremeGENE        | 1,00 µg            | 3 µL                                   | 1:3                         |      |
| X2           | X-TremeGENE        | 1,00 µg            | 6 µL                                   | 1:6                         |      |
| N-NT         | Nano-Juice         | -                  | 1,25 µL                                | -                           |      |
| N1           | Nano-Juice         | 1,25 µg            | 1,25 µL                                | 1:1                         | Addition of Transfection Booster (1,25 µL) |
| N2           | Nano-Juice         | 1,25 µg            | 2,5 µL                                 | 1:2                         | Addition of Transfection Booster (1,25 µL) |
| N3           | Nano-Juice         | 1,25 µg            | 1,25 µL                                | 1:1                         | Addition of Transfection Booster (2,5 µL) |
| N4           | Nano-Juice         | 1,25 µg            | 2,5 µL                                 | 1:2                         | Addition of Transfection Booster (2,5 µL) |
| F-NT         | Fail-Safe          | -                  | 8 µL                                   | -                           |      |
| F1           | Fail-Safe          | 2 µg               | 8 µL                                   | 1:4                         |      |
| F2           | Fail-Safe          | 4 µg               | 16 µL                                  | 1:4                         |      |
Table S5. Barcodes for NGS libraries. For each barcode are reported the nucleotide sequence and orientation.

| Name  | Sequence                                                                 | Orientation |
|-------|--------------------------------------------------------------------------|-------------|
| N701  | CAAGCAGAAAGCACGGGATACCGACTTCGCTTGCGGCTCGG                               | Forward     |
| N702  | CAAGCAGAAGACGGGATACCTAGACCGGTCCTTGCGGCTCGG                               |             |
| N703  | CAAGCAGAAAGACGGGATTTTCTGCTGCTGCTGCTCAGGCTCGG                           |             |
| N704  | CAAGCAGAAAGACGGGATACAGTGCAGGATCTGCTGCTGCTGCTGCTGCTGCTCGG               |             |
| N705  | CAAGCAGAAAGACGGGATATGCCTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| N706  | CAAGCAGAAAGACGGGATAGCTAGGAGGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| N707  | CAAGCAGAAAGACGGGATAGCTAGGAGGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| N708  | CAAGCAGAAAGACGGGATAGCTAGGAGGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| N709  | CAAGCAGAAAGACGGGATAGCTAGGAGGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| N710  | CAAGCAGAAAGACGGGATACCGACTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S501  | AATGATAGCGGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG  | Reverse     |
| S503  | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S505  | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S506  | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S507  | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S508  | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S5010 | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S5011 | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |
| S5013 | AATGATACGCGGAGACACAGGTACCTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG |             |