Supplementary Materials for

Codelivery of CRISPR-Cas9 and chlorin e6 for spatially controlled tumor-specific gene editing with synergistic drug effects

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Figs. S1 to S9
Table S1
Synthesis of 6-benzylxcarbonylamino-2-(bis-tert-butoxycarbonylmethyl-amino)-hexanoic acid tert-butyl ester (tBuO₃-NTA-CBZ).

Tert-butyl bromoacetate (5.2, 26.6 mmol) and DIEA (6.4 mL, 37.5 mmol) were added sequentially to a solution of N⁵-carbonyloxyl-L-Lysine-tert-butylester hydrochloride (4.00 g, 10.7 mmol in 50 mL of DMF). After being stirred overnight at 70 °C, the reaction mixture was diluted with 300 mL of ethylacetate. The organic phase was washed with 2.5% citric acid, saturated NaHCO₃, saturated NaCl, and dried over MgSO₄. Organic solvent was removed under reduced pressure. The mixture was purified with silica gel chromatography (elution: Petroleum ether/ethylacetate, 3/1) to obtain tBuO₃-NTA-CBZ as a yellow liquid (4.5 g, 75% yield).¹-H-NMR (CDCl₃) resonance peaks (fig. S2A) at 1.42-1.48 ppm (s, 27H), 1.4-1.6 ppm (m, 6H), 3.2 ppm (t, 2H), 3.3 ppm (t, 1H), 3.5 ppm (dd, 4H), 5.1 ppm (s, 2H), 7.3-7.4 ppm (m, 5H). ESI-MS m/z: 565.2 [M+H]⁺.

Synthesis of 6-amino-2-(bis-tert-butoxycarbonylmethyl-amino)-hexanoic acid tert butyl ester (tBuO₃-NTA-NH₂)
tBuO₃-NTA-CBZ (2.8 g, 5 mmol) was dissolved in 50 mL of MeOH. After addition of 10% Pd/C catalyst (0.2 g), the solution was stirred under 1 atm of H₂ for 20 h. The catalyst was filtered off and the solvents were removed under reduced pressure to give tBuO₃-NTA-NH₂ (2.04 g; 95% yield).¹-H-NMR (CDCl₃) resonance peaks (fig. S2A) at 1.42-1.48 ppm (s, 27H), 1.4-1.6 ppm (m, 6H), 2.6 ppm (t, 2H), 3.3 ppm (t, 1H), 3.5 ppm (dd, 4H). ESI-MS m/z: 431.37 [M+H]⁺, 453 [M+Na]⁺.

Synthesis of 3,3'-dithio (succinimidyl propionate)
3,3'-Disulfanediylidipropionic acid (10g, 47.6 mmol), 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide hydrochloride (EDCI, 20 g, 105 mmol), N-hydroxysuccinimide (NHS, 12 g, 105 mmol) were dissolved in CH₂Cl₂ (200 mL). After stirred overnight at room temperature, the mixture reaction was washed with 1.0 M HCl twice, saturated NaHCO₃, and saturated NaCl, respectively. The Organic phase was dried over MgSO₄ and concentrated to 50 mL. The product was crystallized in ethanol (EtOH) as a white solid. After being filtered, the crystal was washed with absolute EtOH and dried under vacuum to give 3,3'-Dithio (succinimidyl propionate) (17.3 g, 90%).¹-H-NMR (CDCl₃) resonance peaks (fig. S2A) at 2.84 ppm (s, 8H), 3.00-3.04 ppm (m, 4H), 3.05-3.10 ppm (m, 4H).

Synthesis of 6-dithiobis (succinimidyl propionate)-2-(bis-tert-butoxycarbonyl methyl-amino)-hexanoic acid tert-butyl ester (tBuO₃-NTA-SS-SS-NHS)
A solution of tBuO₃-NTA-NH₂ (2.15 g, 5 mmol in 100 mL of CH₂Cl₂) was added dropwise to a solution of 3,3'-dithio (succinimidyl propionate) (10 g, 24.8 mmol in 200 mL of CH₂Cl₂). After being stirred overnight at room temperature, the mixture was concentrated to 20 mL under reduced pressure and precipitated with EtOH. Precipitate was filtered and concentrated to obtain a yellow paste. The resulting production was purified with silica gel chromatography (elution: ethylacetate/methanol/acetic acid, 100:20:1) to give tBuO₃-NTA-SS-SS-NHS as a yellow liquid (2.3 g; 65% yield).¹-H-NMR (CDCl₃) resonance peaks at 1.42-1.48 ppm (s, 27H), 1.4-1.6 ppm (m, 6H), 2.7 ppm (t, 2H), 2.9 ppm (s, 4H), 3.0-3.2 ppm (m, 8H), 3.3 ppm (t, 1H), 3.5 ppm (dd, 4H). ESI-MS m/z: 742.5 [M+Na]⁺.

Synthesis of APEG-PCL
APEG-PCL was synthesized by ring opening polymerization of e-caprolactone using stannous octoate as catalyst. APEG-OH (1.0g, 0.83 mmol, Mn=1.2 kDa) was dissolved in 100 mL of anhydrous toluene, and the solvent was distilled off to a final volume of 15 mL. After residual water was removed, e-caprolactone (4.5 g, 39.5 mmol) and a drop of stannous octoate were added under argon atmosphere. The mixture solution was stirred under argon at 120 °C for 24 h. The solution was precipitated with cold diethyl ether. The precipitate was filtered and vacuum-dried to obtain APEG1₃₋₅₋₅₆, as calculated based on ¹-H-NMR measurement. Yield: 93%.¹-H-NMR (fig. S2B) resonance peaks at 1.40 ppm (m, -COCH₂CH₂CH₂CH₂CH₂O-), 1.65 ppm (m, -COCH₂CH₂CH₂CH₂CH₂O-), 2.31 ppm (t, -COCH₂CH₂CH₂CH₂CH₂O-), 3.65ppm (m, -O(CH₂)₂O-), 4.05ppm (t, -COCH₂CH₂CH₂CH₂CH₂O-), 5.15-5.30 ppm (dd, CH₃=CH-), 5.80 ppm (m, CH₂ =CH-).

Synthesis of NH₂-PEG-PCL
APEG-PCL (2 g, 0.304 mmol), 2-aminoethanethiol hydrochloride (0.7 g, 6.16 mmol), and 2,2-azobisisobutyronitrile (164 mg, 1 mmol) were mixed in 20 mL of DMF, the mixture was deoxygenated by N₂ bubble for 20 min and stirred at 65 °C for 24 h under N₂ atmosphere. The reaction mixture was dialyzed (MWCO: 3.5 kDa) against methanol for 2 d and precipitant was filtered out. The filtration was washed with diethyl ether and vacuum-dried to obtain a white powder. Yield: 85%.¹-H-NMR (fig. S2B) resonance peaks at 1.40 ppm (m, -COCH₂CH₂CH₂CH₂CH₂O-), 1.65 ppm (m, -COCH₂CH₂CH₂CH₂CH₂O-), 2.31 ppm (t, -COCH₂CH₂CH₂CH₂CH₂O-), 2.75 ppm (t, NH₂CH₂CH₂SCH₂-), 2.92 ppm (t, NH₂CH₂CH₂S-), 3.20 ppm (t, NH₂CH₂CH₂S-), 3.65 ppm (m, -O(CH₂)₂O-), 4.05 ppm (t, -COCH₂CH₂CH₂CH₂CH₂O-).

Synthesis of tBuO₃-NTA-SS-PEG-PCL
NH₂-PEG-PCL (0.5 g, 0.076 mmol) was dissolved in 5 mL of anhydrous CH₂Cl₂. After tBuO₃-NTA-SS-NHS (0.55 g, 0.76 mmol) and TEA (10.5 μL, 0.076 mmol) were added (fig. S1), the solution was stirred at room temperature
for 12 h. The solution was dialyzed (MWCO: 1 kDa) against methanol for 2 d, rotary-evaporated, and vacuum-dried to obtain the polymer tBuO-NTA-SS-PEG-PCL. (Mn=7.6 kDa, calculated from 1H-NMR spectrum; yield: 85%). 1H-NMR (fig. S2B) resonance peaks at 1.40 ppm (m, -COCH₂CH₂CH₂CH₂O₂), 1.48 ppm (m, (CH₂)₃-COOCH⁻), 1.65 ppm (m, -COCH₂CH₂CH₂CH₂O₂), 2.31 ppm (t, -COCH₂CH₂CH₂CH₂O₂), 2.60-3.05 ppm (m, -(CH₂)₃SS(CH₃)₂-), 3.35 ppm (t, -CH₂SSCH₂), 3.65 ppm (m, -(CH₂)₃SSCH₂), 3.65 ppm (m, -(CH₂)₃SSCH₂), 4.05 ppm (t, -COCH₂CH₂CH₂CH₂O₂).

Synthesis of NTA-SS-PEG-PCL

tBuO-NTA-SS-PEG-PCL (0.3 g, 0.008 mmol) was dissolved in 2 mL of TFA. After being stirred for 2 h at room temperature, the solution was evaporated to remove most of TFA, dialyzed (MWCO: 1 kDa) against methanol for 1 d, rotary-evaporated and vacuum-dried to obtain the finally polymer NTA-SS-PEG-PCL. (Mn=7.4 kDa, calculated from 1H-NMR spectrum; yield: 90%). 1H-NMR (fig. S2B) resonance peaks at 1.40 ppm (m, -COCH₂CH₂CH₂CH₂O₂), 1.65 ppm (m, -COCH₂CH₂CH₂CH₂O₂), 2.31 ppm (t, -COCH₂CH₂CH₂CH₂O₂), 2.60-3.05 ppm (m, -(CH₂)₃SS(CH₃)₂-), and -CH₂SSCH₂), 3.35 ppm (t, (-(CH₃)₂COOCCH₂)₂N-CH(CH₂COOC(CH₃)₃)-CH₂), 3.65 ppm (m, -(CH₂)₃SSCH₂), 4.05 ppm (t, -COCH₂CH₂CH₂CH₂O₂).

Synthesis of APEG-PBLA

APEG-PBLA was synthesized through ring-opening polymerization of N-carboxy anhydride of β-benzyl L-aspartate (BLA-NCA) using APEG-NH₂ as an initiator. In brief, 0.68 g of APEG-NH₂ (0.2 mmol, Mn=3.4 kDa) was vacuum-dried at 70 °C for 2 h in a 100 mL flask, and dissolved in 100 mL of anhydrous dichloromethane. Subsequently, 3.5 g of BLA-NCA (14 mmol) dissolved in 3 mL of anhydrous DMF was added into the above solution under argon atmosphere. The reaction was kept stirring for 72 h at 35 °C. The mixture was precipitated in excessive cool diethyl ether. The precipitant was washed with diethyl ether, and dried overnight under vacuum to obtain a white solid. The degree of polymerization for PBLA was 70, as calculated based on the 1H-NMR spectrum (fig. S3A). (APEG-PBLA: Mn=17.7 kDa, calculated from 1H-NMR spectrum; yield: 95%). 1H-NMR resonance peaks at 2.6-2.9 ppm (d, -(CH₂)₂COOCH₂), 3.56 ppm (s, -(CH₂)₃NHCH(CH₂)COO⁻), 5.0 ppm (s, -(CH₂)₂CH⁻), 5.1-5.4 ppm (m, CH₂=CH₂), 5.88 ppm (m, CH₂=CH₂), 7.30 ppm (m, C₆H₅CH₂⁻).

Synthesis of APEG-pAsp(DAB-boc)

First, APEG-PBLA was reacted with acetyl chloride to synthesize APEG-PBLA-Ac. Under N₂ atmosphere, 2 g of APEG-PBLA (0.11 mmol) was dissolved in 20 mL of anhydrous CH₂Cl₂ in a 50 mL schlenk flask and cooled in an ice water bath. 235 µL of acetyl chloride (3.3 mmol) was added. After 30 µL of anhydrous TEA (0.22 mmol) was added dropwise, the solution was stirred for 12 h at room temperature and dialyzed (MWCO: 3.5 kDa) against methanol for 1 d. Precipitant was filtered out. Filtration was vacuum-dried to obtain a white powder of APEG-PBLA-Ac. Next, 1.6 g of APEG-PBLA-Ac (0.09 mmol) and 6 g of N-Boc-1,4-butanediamine (DAB-boc) (31.7 mmol, about 5 eq.) were dissolved in 30 mL of anhydrous DMSO. The solution was stirred for 24 h at 35 °C and dialyzed (MWCO: 3.5 kDa) against methanol for 48 h to remove excess DAB-boc. The purified solution was rotary-evaporated to remove solvent and vacuum-dried to get polymer APEG-pAsp(DAB-boc) (Mn=23.4 kDa, calculated from 1H-NMR spectrum; yield: 89%). 1H-NMR (fig. S3A) resonance peaks at 1.30 ppm (s, -(CH₂)₂CH₃CH₂CH₂-), 1.38 ppm (s, (CH₃)₃COOCNH⁻), 3.0 ppm (s, -(CH₂)₂CONHCH₂-), 3.15 ppm (s, -(CH₂)₂CONHCH₂-), 3.56 ppm (s, -(CH₂)₂CONHCH₂-), 4.65 ppm (s, -(CH₂)₂CONHCH₂-), 5.1-5.4 ppm (m, CH₂=CH₂), 5.88 ppm (m, CH₂=CH₂), 6.8 ppm (s, (CH₃)₃COOCNH⁻).

Synthesis of NH₂-PEG-pAsp(DAB-boc)

NH₂-PEG-pAsp (DAB-boc) polymer was synthesized by addition reaction of double bond with cysteamine hydrochloride using AIBN as initiator. In brief, 0.7 g of APEG-pAsp (DAB-boc)-Ac (0.03 mmol), 102 mg of cysteamine hydrochloride (0.9 mmol) and 24.6 mg of AIBN (0.15 mmol) were mixed in 5 mL of DMF. After being deoxygenated by N₂ bubble for 15 min, reaction mixture was stirred at 70 °C for 24 h under N₂ atmosphere, dialyzed (MWCO: 3.5 kDa) against methanol for 1 d, rotary-evaporated, and finally vacuum-dried to obtain the polymer NH₂-PEG-pAsp(DAB-boc). (Mn=23.5 kDa, calculated from 1H-NMR spectrum; yield: 92%). 1H-NMR (fig. S3A) resonance peaks at 1.30 ppm (s, -(CH₂)₂CH₃CH₂CH₂-), 1.38 ppm (s, (CH₃)₃COOCNH⁻), 3.0 ppm (s, -(CH₂)₂CONHCH₂-), 3.15 ppm (s, -(CH₂)₂CONHCH₂-), 3.56 ppm (s, -(CH₂)₂CONHCH₂-), 4.60 ppm (s, -(CH₂)₂CONHCH₂-), 6.8 ppm (s, (CH₃)₃COOCNH⁻).

Synthesis of Mal-PEG-pAsp (DAB-boc)

NH₂-PEG-pAsp (DAB-boc) (0.4 g, 0.017 mmol) was dissolved in 5 mL of anhydrous DMF. After 3-Maleimidopropionic acid N-hydroxysuccinimide ester (90 mg, 0.34 mmol) and TEA (2.3 µL, 0.017 mmol) were added, the solution was stirred at room temperature for 12 h, dialyzed (MWCO: 3.5 kDa) against methanol for 2 d, rotary-evaporated, and finally vacuum-dried to obtain the polymer Mal-PEG-pAsp (DAB-boc). (Mn=23.7 kDa, calculated from 1H-NMR spectrum; yield: 91%). 1H-NMR (fig. S3A) resonance peaks at 1.30 ppm (s, -(CH₂)₂CH₃CH₂CH₂-), 1.38 ppm (s, (CH₃)₃COOCNH⁻), 3.0 ppm (s, -(CH₂)₂CONHCH₂-), 3.15 ppm (s, -(CH₂)₂CONHCH₂-), 3.56 ppm (s, -(CH₂)₂CONHCH₂-), 4.60 ppm (s, -(CH₂)₂CONHCH₂-), 6.8 ppm (s, (CH₃)₃COOCNH⁻), 7.0 ppm (s, -(CH₂)₂CONHCH₂-).
Synthesis of iRGD-PEG-pAsp(DAB-boc)

After Mal-PEG-pAsp(DAB-boc) (237 mg, 0.01 mmol) were dissolved in 2 mL of DMF, the peptide iRGD-SH (109 mg, 0.1 mmol) dissolved in 1mL of DMF was added under argon atmosphere. After being stirred at room temperature for 24 h, the solution was dialyzed (MWCO: 7 kDa) against deionized water for 2 d, and then freeze-dried to obtain iRGD-PEG-pAsp(DAB-boc). (Mn=24.8 kDa, calculated from 1H-NMR spectrum; yield: 91%). 1H-NMR (fig. S3A) resonance peaks at 1.30 ppm (s, -CH_2CH_2CH_2), 1.38 ppm (s, (CH_3)COOCNH-), 3.0 ppm (s, -CH_2CONHC_2), 3.15 ppm (s, -CH_2CONHC_2), 3.56 ppm (s, -OCH_2CH_2O), 4.60 ppm (s, -NHCH(CH_2)CO-), 6.8 ppm (s, (CH_3)COOCNH-), new peaks at 1.92 and 4.21 ppm assigned to iRGD peptide.

Synthesis of iRGD-PEG-pAsp(DAB)

The Boc-protecting groups of iRGD-PEG-pAsp(DAB-boc) were removed by trifluoroacetic acid (TFA). In brief, iRGD-PEG-pAsp(DAB-boc) (200 mg, 0.008 mmol) was dissolved in 2 mL of TFA. After being stirred for 2 h at room temperature, the solution was evaporated to remove most of TFA, diluted with deionized water, dialyzed (MWCO: 3.5 kDa) against deionized water for 1 d, and freeze-dried to obtain the polymer. (Mn=17.8 kDa, calculated from 1H-NMR spectrum; yield: 93%). 1H-NMR (fig. S3A) resonance peaks at 1.30 ppm (s, -CH_2CH_2CH_2), 2.95 ppm (s, -CH_2CONHC_2), 3.10 ppm (s, -CH_2CONHC_2), 3.56 ppm (s, -OCH_2CH_2O), 4.60 ppm (s, -NHCH(CH_2)CO-), the peaks at 1.92 and 4.21 ppm assigned to iRGD peptide.

Synthesis of APEG-pAsp(DAB)

In brief, APEG-pAsp(DAB-boc) (234 mg, 0.1 mmol) was dissolved in 2 mL of TFA. After being stirred for 2h at room temperature, the solution was evaporated to remove most of TFA, diluted with deionized water, dialyzed (MWCO: 3.5 kDa) against deionized water for 1 d, and freeze-dried to obtain the aimed polymer. (Mn=16.4 kDa, calculated from 1H-NMR spectrum; yield: 91%).

Synthesis of (1S)-N-(5-Carbobenzyloxyaminoo-carboxypropyl) iminodiacetic Acid (N²-Cbz-NTA).

N²-Carbobenzyloxy-L-lysine (12 g, 42.8 mmol) was dissolved in 65 mL of 2 M NaOH, and the solution was added dropwise to a cooled solution (0 °C) of bromoacetic acid (11.9 g, 85.6 mmol, in 45 mL of 2 M NaOH). The mixture was stirred overnight at room temperature, heated at 65°C for 1 h, and cooled down. 130 mL of 1 M HCl was added to the solution. Precipitate was filtered off, washed with water, and dried to afford a crude white powder (14.6 g) in 86% yield. 1H-NMR (fig. S4B) resonance peaks at 7.42-7.13 ppm (m, 5H), 5.00 ppm (s, 2H), 3.61-3.39 ppm (m, 4H), 3.35 ppm (t, 1H, J = 7.2 Hz), 2.96 ppm (d, 2H, J = 5.9 Hz), 1.79-1.28 ppm (m, 6H).

Synthesis of (1S)-N-(5-Amino-carboxypropyl) iminodiacetic Acid (NTA)

N²-Cbz-NTA (6 g, 15.1 mmol) was dissolved in 100 mL MeOH/H_2O (95: 5). After addition of 10% Pd/C catalyst (0.6 g), the solution was stirred under 1 atm of H_2 for 20 h. After addition of 100 mL of water to the solution, MeOH was removed in vacuo. The catalyst and water were removed from the filtrate to give a colorless paste. The colorless paste was precipitated from EtOH. The resulting precipitate was dissolved in 5 mL of H_2O and recrystallized from EtOH at -20 °C. The white crystals were filtered off and dried to get NTA (2.7g,10.3 mmol) in 68% yield. 1NMR (fig. S4B) resonance peaks at 3.77 ppm (m, 5H), 2.95 ppm (t, 2H), 1.85-1.40 ppm (m, 6H).

Synthesis of (1S)-N-[(4-Mercaptobutanoyl) amino]-1-carboxypropyl] iminodiacetic Acid (NTA-SH)

NTA (1.5 g, 5.7 mmol), NaHCO_3 (1.5 g, 17.8 mmol) and γ-butyrolactone (0.9 g, 8.8 mmol) were dissolved in 10 mL of H_2O. After being heated for 15 h at 72 °C, solution was cooled, acidified to pH 3 with 1 mL of AcOH, and concentrated under reduced pressure to give NTA-SH as a yellow paste. A yellow solid was crystallized from EtOH. The resulting solid was filtered, washed with EtOH, and dried under vacuum to give NTA-SH (1.4 g, 3.8 mmol) in 66.7% yield. 1NMR (fig. S4B) resonance peaks at 3.72 ppm (m, 5H), 3.12 ppm (t, 2H), 2.48 ppm (t, 2H), 2.29 ppm (t, 2H), 1.90-1.35 ppm (m, 8H).

Synthesis of NTA-PEG-PCL

After APEG-PCL (0.5 g, 0.076 mmol) and AIBN (41 mg, 0.25 mmol) were dissolved in 10 mL of DMF, NTA-SH (356 mg, 0.98 mmol) dissolved in 2 mL of water was added under argon atmosphere. After being stirred for 24 h at 70 °C, solution was dialyzed (MWCO: 3.5 kDa) against methanol for 1 d and deionized water for 2 d, and then freeze-dried to obtain NTA-PEG-PCL. (Mn = 6.9 kDa, calculated from 1H-NMR spectrum; yield: 88%).

Expression and purification of Cas9 protein

pET-NLS-Cas9-6xHis-tag containing NLS-Cas9-6xHis-tag fusion sequence under T7 promotor was an acquisition from David Liu (Addgene plasmid # 62934; http://n2t.net/addgene: 62934). The plasmid was transformed Escherichia coli BL21 Star (DE3) (Takara, Japan). The resultant bacteria were pre-grown for 16 h in Luria-Bertani (LB) broth containing 50 μg/mL of ampicillin at 37 °C. This pre-culture products were diluted 1:100 in the same growth medium and grown to an OD₆₀₀ of 0.6 at 37 °C. Cas9 expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.5 mM following growth at 20 °C overnight. Cells were
harvested by centrifugation and resuspended in wash buffer (20 mM Tris-HCl pH 8.0, 20 mM imidazole, 50 mM NaCl, 1 mM PMSF). Cells were lysed three times with an ultra-high cell crusher (JN-3000 PLUS, Guangzhou, China). The cellular debris were cleared by centrifugation for 30 min at 12,000 g. Column purification was performed according to the manufacturer’s protocol. Cell lysate was injected into a HisTrap Ni Crude column (GE Healthcare) attached to an AKTA Start System (GE Healthcare). The column containing Cas9 was washed with 10 column volumes of wash buffer. Cas9 proteins were eluted using an elution buffer (20 mM Tris-HCl, 300 mM imidazole, 50 mM NaCl, 1 mM PMSF, pH 8.0). Next, the eluent containing Cas9 was purified with a HiTrap SP HP column (GE Healthcare). Cas9 was eluted with a purification buffer (20 mM HEPES, pH 7.4) containing a linear NaCl gradient from 0.1 M to 1 M over five column volumes. The eluted fractions containing Cas9 was assessed using colloidal Coomassie-stained SDS-PAGE. Cas9 yield was quantified with a BCA protein assay kit (Sangon Biotech Co. Ltd., Shanghai, China). Then, Cas9 was changed to desalting buffer (20 mM HEPES, 150 mM KCl, 50% glyceride, pH 7.4) using a desalination column (GE Healthcare). Finally, Cas9 was concentrated to 1 mg/mL in desalting buffer, frozen in liquid nitrogen, and stored at -80 °C until use.

**In vitro transcription of sgRNAs**

20-bp sgRNA sequences were designed using the Zhang Lab Guide Design Resources (https://www.synthego.com). DNA sequences encoding sgRNA were inserted into pSpCas9(BB)-2A-GFP (PX458) plasmid vector under T7 promoter (Addgene plasmid # 48138). Recombinant plasmids were transformed DH5α Competent Cells (Tokara, Japan) to amplify plasmid. Plasmids were purified from lysate of DH5α cells and linearized as template for sgRNA transcription. sgRNAs were transcribed using *in vitro* GeneArt™ Precision gRNA Synthesis Kit (Invitrogen, USA) and were purified using GeneArt™ gRNA Clean-up Kit (Invitrogen, USA). The resultant sgRNAs were diluted to 1 mg/mL in RNase-free water and stored at -80 °C until use.

**Blood circulation and in vivo biodistribution of nanoparticles**

Nude mice were randomly divided into three groups (n = 3): control group for data normalization, T-CC-NPs group, and NT-CC-NPs group. After T-CC-NPs and NT-CC-NPs were injected intravenously into mice, orbital blood of 50 μL were collected at different time points (1 h, 3 h, 6 h, 12 h, 24 h and 48 h) after injection. The sera and blood cells were separated by centrifugation. The fluorescence intensity of Ce6 (Ex/Em: 410nm/670 nm) and Cas9-eGFP (Ex/Em: 488nm/510 nm) in 30-fold diluted sera were detected using a fluorescence spectrophotometer. The deceasing of Ce6 and Cas9-eGFP fluorescence intensities represented the clearance rates of nanoparticle in blood. The fluorescence of Ce6 and Cas9-eGFP in blood cells was analyzed using flow cytometry.

Tissues including tumor, liver, spleen, kidney, lung, and hear, excised from mice receiving various treatments were grinded into tissue homogenates. Cells isolated from these homogenates were subjected to flow cytometry analysis.

**Preparation of T-Au-NPs**

Preparation of His-tagged Au-NPs was described briefly as follow: a solution of his-tag-SH peptide (HHHHHHGGC, 24 mg, 0.022 mmol in 1.3 mL of water) was mixed with a solution of GSH (20 mg, 0.066 mmol in 3.4 mL of water). The mixed solution was added to a solution of hydrogen tetrachloroaurate (HAuCl₄, 5 mg, 0.015 mmol in 0.5 mL of water) and pH of solution was adjusted to 6.0-6.5 with 1 M NaOH. A solution of sodium borohydride (5.6 mg, 0.15 mmol in 2.8 mL of water) was added and vigorously stirred for 10 min at room temperature. The mixture was allowed to react overnight. His-tagged Au-NPs were purified by gel filtration chromatography (GH-25, water) and concentrated with ultrafiltration (MWCO 30kDa, Millipore Amico Ultra). The solution of His-tagged Au-NPs (30 μg) was mixed with the solution of NTA-NPs (240 μg) to prepare Au nanoparticles conjugated to NTA-NPs (Au-NPs). The mixture was centrifuged three times using ultrafiltration (Sartorius Vivaspin 500, MWCO 300 kDa) to remove free His-tagged Au-NPs, and then was mixed with 15 μg of cationic iRGD-PD to form iRGD-PD-coated nanoparticles (T-Au-NPs). Finally, a solution of T-Au-NPs was centrifuged three times using ultrafiltration to remove free iRGD-PD.

**Quantitative determination of disulfide bond in NTA-SS-PEG-PCL**

2 mg of reducing-agent TCEP was added to 5 mL of NTA-NPs (1mg/mL) and incubated for 2 h. The excess TCEP was removed by ultrafiltration to obtain reduced NTA-NPs referred as SH-NPs. Solutions of NTA-NPs (2 mL, 1 mg/mL) and SH-NPs (2 mL, 1 mg/mL) were mixed with 1 mL of DTNB reagent (0.5 mg/mL) and incubated for 30 min at room temperature. The absorbance at 410 nm were measured using a UV-Vis spectrometer (PerkinElmer UV750, USA). Cysteine standards were used to calculate the number of thiol groups in SH-NPs which is equal to the number of disulfide bonds in NTA-SS-PEG-PCL.

**Cellular internalization and intracellular transportation pathways of nanoparticles**

CNE-2 cells were pretreated at 4 °C for 30 mins and treated with T-CC-NPs and NT-CC-NPs for 2h at 4 °C, wherein energy-dependent endocytosis was inhibited. Cells were pre-incubated with 50 μmol/L iRGD, 25 μg/mL nystatin, 10 μg/mL chlorpromazine (CPZ), 6.5 μg/mL dynasore, 12.5 μg/mL amiloride, 10 μg/mL β-cyclodextrin (CD), 3 μg/mL nocodazole, 0.5 mg/mL NaNO₃ for 1 h at 37 °C, respectively. Then, cells were incubated with T-CC-NPs and NT-CC-NPs for 4 h at 37 °C, respectively. Cell culture medium was replaced with fresh medium and cells
were irradiated with a 671 nm of NIR at a power of 800 mW/cm² for 1 min. Intracellular Cas9-eGFP fluorescence was detected by a flow cytometer. To analyze intracellular transportation of Cas9, cells were stained with Lysotracker Red (Invitrogen, USA), Golgi-Tracker (Invitrogen, USA), and Hoechst 33342, respectively, and observed with CLSM.

**Determination intracellular concentration of GSH**

CNE-2 cells were inoculated at a density of 5×10⁵ cells/well in 12-well plates and cultured overnight. Cells were incubated with T-Ce6-NPs for 4 h. After cell culture mediums were replaced with fresh mediums, cells were irradiated with a 671 nm NIR at a power of 800 mW/cm² for 1 min. At 0.5 h, 1 h, 2 h, and 3 h post-NIR irradiation, cells were collected and lysed to detect the intracellular GSH concentration using a Reduced Glutathione (GSH) Assay Kit (Solarbio, Beijing).

**Live/dead cell staining analysis**

CNE-2 cells were seeded into 12-well plates (1 × 10⁵ per well) for overnight growth at 37 °C and 5% CO₂. The cells were transfected with T-Ce6-NPs, T-Cas9-NPs, NT-CC-NPs and T-CC-NPs, respectively. If required, NIR laser irradiation was performed. The concentration of Ce6 and Cas9/sgRNA were 0.2 μg/mL and 2.5 μg/mL, respectively. Cells were stained using 500 μL of culture medium with 1 μM Calcein-AM (KeyGEN BioTECH, China) and 1 μM propidium iodide (KeyGEN BioTECH, China) for 15 min. Cells were washed with PBS three times and observed using CLSM.

**Real-time PCR quantitative analysis**

1 × 10⁵ CNE-2 cells per well were plated into 12-well plates and cultured overnight. After cells were treated with different formulations for 48 h, total RNAs were collected to perform quantitative real-time PCR (qRT-PCR) assays. Briefly, total RNAs were extracted using the TRIzol reagent (Invitrogen, USA) as the manufacturer’s protocol. mRNA was reverse-transcribed to cDNA using PrimeScript™ RT reagent Kit (Takara, Japan). mRNA expressions of Nrf2, HIF-1α, VEGF-A, VCAM were quantified with a StepOne Plus real-time PCR System (ABI, USA) using a FastStart Universal SYBR Green Master (ROX) Kit (Roche, Switzerland). Human β-actin was used as an internal normalization standard. The forward and reverse sequences of each gene primer were listed in **Table S1**.

**Western blot analysis**

CNE-2 cells (5 × 10⁵ per well) were seeded into 6-well plates and cultured overnight. Cells were treated with T-Ce6-NPs, T-Cas9/NIR, T-Ce6/NIR, NT-CC/NIR, and T-CC/NIR, respectively. After subsequent incubation for 48 h, cells were washed three times with PBS, and lysed by using RIPA lysis buffer (Abcam, Cambridge, UK) with protease inhibitors. Protein content was determined by BCA protein assay kit (Sangon Biotech Co. Ltd., Shanghai, China), 40 μg of proteins from each treatment was electrophoresed in 10% SDS-PAGE and transferred onto a PVDF membrane (Millipore Corp., USA). The membranes were blocked with 5% non-fat milk for 2 h at room temperature and incubated with 1000×dilutions of mouse monoclonal anti-Cas9 antibody (Cell Signaling Technology, Danvers, MA, USA), rabbit monoclonal anti-Nrf2 antibody (Abcam, Cambridge, UK), mouse monoclonal anti-HIF-1 alpha antibody (Abcam, Cambridge, UK), rabbit monoclonal anti-VEGFα antibody (Abcam, Cambridge, UK) or rabbit monoclonal anti-VCAM1 antibody (Abcam, Cambridge, UK) overnight at 4 °C. The membranes were immersed into solution with secondary antibody (Abcam, Cambridge, UK) for 1 h. The protein bands were imaged in a chemiluminescence imaging system (GE ImageQuant LAS 500, USA).

**Immunofluorescence and immunohistochemistry**

Immunofluorescence analyses were conducted on CNE-2 cells and frozen tissue sections from tumor and major organs, respectively. Briefly, samples were washed with PBS three times and blocked with 5% bovine serum albumin (BSA) for 30 min. Cells and tissue sections were incubated with primary antibodies (i.e., anti-Cas9, anti-Nrf2, anti-VEGF-A, anti-CD31, anti-VCAM and anti-HIF-1 α antibodies) at 4 °C overnight, and treated with fluorescence-labeled secondary antibody. Nuclei were stained with DAPI or Hoechst. The fluorescence of cells and tissue sections were imaged with CLSM. For immunohistochemical (IHC) analysis, tissue sections were treated with 3% H₂O₂ and MeOH solution to inactivate endogenous peroxidase, and followed by treatment with citrate buffer (10 mM, pH 6.0) to retrieve antigen. After being blocked with 5% bovine serum albumin (BSA), tissue sections were incubated with anti-firefly luciferase antibody, anti-Cas9 antibody and anti-Nrf2 antibody for 2 h at 37 °C, and treated with biotin-labeled goat anti-mouse IgG secondary antibody. Then, streptavidin-biotin complex (SABC) was used to magnify immunohistochemical signal by cascade amplification between streptavidin and biotin. Immunoreactivity was visualized using DAB as substrate in a Vectra Polaris (PerkinElmer, USA).

**Preparation of recombinant CNE-2 cells constitutively expressing luciferase and red fluorescence proteins (CEN-2-Luc-RFP cells)**
A lentiviral vector containing luciferase (Luc) and red fluorescence protein (RFP) gene was constructed and packaged in HEK293T cells using packaging plasmids. Viral supernatant was collected to infect CNE-2 cells. The CNE-2 cells expressing Luc and RFP (CNE-2-Luc-RFP cells) were screened out under puromycin (2.5 μg/mL) for 3 weeks. The infection efficiency was analyzed using flow cytometry and fluorescent microscopy assays.

**Targeted DNA deep sequencing**

Genomic DNA of CNE-2 cells treated with T-CC/NIR was extracted using the Genomic DNA Isolation Kit (Tiangen Biotech CO., LTD, Beijing, China). 150 ng of genomic DNA was used as template to perform PCR using primers designed against on-target and off-target sites (Table S1), respectively. A second round of PCR was performed using primers with flanking HTS sequence on the end of specific primers. For in vivo analyses, ~30 mg of mouse tumor tissues or liver tissues was isolated from anesthetized mouse and genomic DNA was extracted as described above. Relative amounts of crude PCR products were quantified by gel electrophoresis and purified with the QIAquick PCR Purification Kit (Qiagen, USA). Purified DNA was amplified by PCR with primers containing sequencing adapters, and then sequenced and analyzed by Sangon Biotechnology Company (Shanghai, China) to detect indels around target sites.

**Hematoxylin and eosin (H&E) and TUNEL staining**

Tumor tissues and organs were embedded in paraffin and sectioned into samples of 2 μm thick. Sections were subjected to H&E staining. The FragEL™ DNA Fragmentation Detection Kit (Merck, Germany) was applied to detect apoptotic tumor cells. Briefly, after permeabilization using proteinase K and inactivation of endogenous peroxidase with 3% H₂O₂/MeOH, the samples were treated with terminal deoxynucleotidyl transferase (TdT) for 1.5 h at 37 °C to label the exposed 3'-OH ends of DNA fragment in apoptotic cells. The positive signals of apoptotic cells were visualized by staining with diaminobenzidine (DAB) in a Vectra Polaris (PerkinElmer, USA).
Fig. S1. Synthetic routes of (A) NTA-SS-PEG-PCL copolymer, (B) iRGD-PEG-pAsp(DAB) and APEG-pAsp(DAB) copolymers.
Fig. S2. Characterizations of NTA-SS-PEG-PCL copolymers. (A) $^1$H-NMR and MS analyses of tBuO$_2$-NTA-CBZ, tBuO$_2$-NTA-NH$_2$ and tBuO$_2$-NTA-SS-NHS. (B) $^1$H-NMR analyses of APEG-PCL, NH$_2$-PEG-PCL, tBuO$_2$-NTA-SS-PEG-PCL and NTA-SS-PEG-PCL. Analyses were performed in CDCl$_3$. (C) GPC curve of NTA-SS-PEG-PCL (Mw/Mn=1.18), in DMF containing LiBr (1 g/L) at a flow rate of 1.0 mL/min. Copolymer NTA-SS-PEG-PCL showed a unimodal molecular weight distribution in chromatograms and a higher molecular weight than that of macro-initiator (APEG$_1$-2k-OH). (D) The UV-Vis spectra of NTA-NPs and SH-NPs in Ellman’s reagent. NTA-NPs were self-assembled with NTA-SS-PEG-PCL polymers, and SH-NPs was prepared by reducing NTA-NPs with reducing-agent TCEP. (E)
Raman spectrum of NTA-SS-PEG-PCL. The characteristic peak at 509 cm\(^{-1}\) of disulfide bonds indicated that NTA was conjugated to PEG-PCL via disulfide bond.

Fig. S3. Characterizations of copolymer iRGD-PEG-pAsp(DAB). (A) \(^1\)H-NMR analyses of APEG-PBLA, APEG-pAsp(DAB-boc), NH\(_2\)-PEG-pAsp (DAB-boc), Mal-PEG-pAsp (DAB-boc), iRGD-PEG-pAsp(DAB-boc), and iRGD-PEG-pAsp(DAB). Analyses were performed in DMSO-\(d_6\). (B) FTIR spectra of iRGD-PEG-pAsp(DAB), iRGD-PEG-pAsp(DAB-boc), APEG-pAsp(DAB-boc), and APEG-PBLA. After aminolysis reaction, the characteristic peak at 1740 cm\(^{-1}\) of ester (s, \(\nu\)C=O, ester) and peaks at 745 and 700 cm\(^{-1}\) of benzene (s, \(\gamma\)C-H, benzene) disappeared. The characteristic peak at 1650 cm\(^{-1}\) of amide (s, \(\nu\)C=O, amide) was intensified. After deprotection reaction of N-Boc amines, the characteristic peak at 1250 cm\(^{-1}\) of Boc group (s, \(\delta\)C-O-C, amide) and the characteristic peak at 715 cm\(^{-1}\) of Primary amine (s, \(\delta\)N-H, amine) appeared. (C) MS analysis of the iRGD-SH peptide. (D) GPC curve of iRGD-PEG-pAsp(DAB) (Mw/Mn=1.20), in DMF containing LiBr (1 g/L) at a flow rate of 1.0 mL/min. iRGD-PEG-pAsp(DAB) Copolymer showed a unimodal molecular weight distribution in chromatograms and a higher molecular weight than that of macro-initiator (APEG\(_{3.4K}\)-NH\(_2\)).
Fig. S4. Synthetic route and characterizations of reducing agent-insensitive polymer NTA-PEG-PCL. (A) Synthetic route of reducing agent-insensitive polymer NTA-PEG-PCL. (B) $^1$H-NMR analyses of N$^\epsilon$-Cbz-NTA in DMSO-$d_6$, NTA and NTA-SH in D$_2$O. (C) $^1$H-NMR analyses of APEG-PCL and NTA-PEG-PCL in CDCl$_3$. 
### Table

| Concentration (µg/mL) | Tumor | Liver | Spleen | Kidney |
|-----------------------|-------|-------|--------|--------|
| 25                    | 99.94%| 13.07%| 99.36% | 99.44% |
| 50                    | 95.57%| 27.74%| 95.65% | 99.62% |
| 100                   | 96.16%| 2.74% | 96.72% | 99.56% |
| 200                   | 99.99%| 0.02% | 99.57% | 95.57% |
| 300                   | 99.99%| 0.02% | 99.56% | 95.57% |

**Notes:**
- **T-CC-NPs in PBS**
- **T-CC-NPs in mouse serum**

### Graphs

**A**
- Cell viability (%) vs. Concentration of nanoparticles (µg/mL)

**B**
- Size (nm) vs. Time (h)

**C**
- Fluorescence intensity (%)

**D**
- NIR imaging of T-CC-NPs in different tissues

**E**
- Relative fluorescence intensity (%)

**F**
- Flow cytometry analysis of Cas9-eGFP expression

**G**
- Flow cytometry analysis of Cas9-eGFP expression in different organs
Fig. S5. Cytotoxicity, colloidal stability, optimization of transfection, \textit{in vivo} biodistribution of nanoparticles, \textit{in vitro} ROS generation, and intracellular degradation of Cas9-eGFP analyses. (A) Cytotoxicity of blank nanoparticle (iRGD-PD coated NTA-NPs without Ce6 and Cas9 RNP, T-B-NPs). Size and zeta potential of nanoparticles were 95.3 nm and -3.8 mV, respectively. Data are shown as the mean ± SD (n = 3). (B) Colloidal stability of T-CC-NPs in PBS solution and mouse serum by dynamic light scattering (DLS). After incubation with serum, the serum was exchanged into PBS by ultrafiltration to avoid disturbance of serum proteins. Data are shown as the mean ± SD (n = 3). (C) Cellular uptake of T-CC-NPs under various conditions. Scale bar, 25 μm. (D) Fluorescence signals of tumor and major organs from mice receiving T-CD-NPs and NT-CD-NPs at 48 h after intravenous injection of nanoparticles. T-CD-NPs and NT-CD-NPs were prepared according to the preparation procedure of T-CC-NPs and NT-CC-NPs but using DiR instead of Ce6. Dose of DiR was 500 μg/kg body weight. (E) Relative fluorescence intensity of Ce6 and Cas9-eGFP in sera at various time points post-injection. Flow cytometry analyses of Ce6- and Cas9-eGFP-positive cells in (F) blood and (G) major organs from mice receiving various nanoparticles. (H) Fluorescence profiles of SOSG (λex = 488 nm) in T-CC-NPs solution under NIR (671 nm) irradiation for various times. (I) Degradation of Cas9-eGFP in the lysosome without NIR irradiation. Scale bar, 50 μm.
Fig. S6. Internalization pathway, intercellular transport, and cytotoxicity of nanoparticles analyses. (A) Quantification of ROS and GSH levels at various time points after cells were treated with T-Ce6/NIR. Scale bar, 75 μm. (B) CLSM and (C) flow cytometry analyses of nanoparticles internalization. After being preincubated with various...
inhibitors or free iRGD, cells were treated with nanoparticles plus NIR irradiation. T and NT indicated T-CC-NPs and NT-CC-NPs, respectively. The chlorpromazine, nystatin, dynasore, amiloride, nocodazole, and β-cyclodextrin were abbreviated to CPZ, Nystai, Dynas, Amilor, Nocod, and CD, respectively. The Golgi apparatus and nuclei were stained as red and blue fluorescence using Golgi-Tracker (Invitrogen, USA) and Hoechst 33342. Cytotoxicity of various nanoparticles to CEN-2 cells without NIR irradiation (D) and with NIR irradiation (E) (mean ± SD, n = 3). (F) Live/dead staining analysis of cells treated with various treatments. Scale bar, 50 μm. If applied, the concentrations of Cas9 and sgRNA were 2 and 0.5 μg/mL, respectively.
Fig. S7. Antitumor activity and histological outcome of the combined photodynamic therapy and Nrf2 gene editing. (A) Body weights of mice treated with various nanoparticles. The T-Cas9-NPs, T-Ce6-NPs, T-CC-NPs, and
NT-CC-NPs under NIR irradiation are abbreviated to T-Cas9/NIR, T-Ce6/NIR, T-CC/NIR, and NT-CC/NIR, respectively. Data are shown as the mean ± SD (n = 6). (B) Tumor weights and (E) excised tumor tissues in CEN-2 xenograft tumor-bearing mice after tail vein injection of various nanoparticles with or without NIR irradiation. Data are mean ± SD (n = 6). ** P < 0.01, *** P < 0.001. (D) H&E and immunohistochemistry analyses of Cas9 and Nrf2 proteins and TUNEL analyses in tumor tissue sections from CNE-2 xenograft mice receiving various nanoparticles. (E) H&E analyses in heart, liver, spleen, lung, and kidney tissues section. Doses of Ce6 and Cas9/sgRNA were 1 and 1.5 mg/kg, respectively. Cas9/sgRNA complex was prepared at a molar ratio of 1. Scale bar, 50 μm. Photo credit: Xiaoxia Li, Sun Yat-sen University.
Fig. S8. Mechanism of the combined Ce6 photodynamic therapy and CRISPR-Cas9-mediated gene editing. (A) Flow cytometry and (B) CLSM image of ROS levels in CNE-2 cells receiving various treatments. Data are shown as
the mean ± SD (n = 3). Scale bar, 50 μm in panel B. (C) Immunofluorescence analyses of Cas9 and Nrf2 proteins in CNE-2 cells receiving various nanoparticles. (D) Immunofluorescence analyses of Nrf2 and Cas9 proteins in tumor tissues sections from CNE-2 xenograft mice receiving various treatments. (E) Deep sequencing analysis of indel percentage in CNE-2 cells receiving T-CC/NIR treatment. (F) Relative mRNA expression level of Nrf2, HIFα, VEGF, and VCAM in CNE-2 cells receiving different treatments. Data are shown as the mean ± SD (n = 3). Scale bar, 100 μm.
Fig. S9. Immunofluorescence and immunohistochemistry in tumor sections from CNE-2 xenograft mice receiving various treatments. (A) Immunofluorescence analyses of proliferative biomarker Ki67, (B) apoptotic protein caspase 3, (C) HIF1α, (D) VCAM, (E) VEGF-A and CD31 in tumor tissues section. Scale bar, 100 μm.
Table S1 Molecular weight of copolymer, drug loading efficiency, drug loading content, and sequences of DNA oligos.

### Molecular weight and molecular weight distribution of copolymer

| Polymer          | \(M_n\) (PEG) | \(M_n\) | \(M_w/M_n\) |
|------------------|---------------|---------|-------------|
| NTA-SS-PEG-PCL   | 1200          | 7400    | 1.18        |
| iRGD-PD          | 3400          | 17800   | 1.20        |

\(a\) calculated by \(^1\)H-NMR; \(b\) calculated by GPC.

### Loading efficiency and loading content of Cas9 and Ce6

|          | LE (Cas9) | LC (Cas9) | LE (Ce6) | LC (Ce6) |
|----------|-----------|-----------|----------|----------|
| CC-NPs   | 91.2 ± 1.6 % | 9.9 ± 1.3 % | 82.4 ± 2.1 % | 8.6 ± 2.7 % |
| T-CC-NPs | 91.2 ± 1.6 % | 9.5 ± 1.2 % | 82.4 ± 2.1 % | 8.2 ± 2.6 % |

LE (Cas9), Loading efficiency of Cas9; LC (Cas9), Loading content of Cas9; LC (Ce6), Loading content of Ce6

### Sequences of DNA oligos.

| Gene names                  | Sequences                                      | Notes               |
|-----------------------------|------------------------------------------------|---------------------|
| **Target Sequences**        |                                                |                     |
| EGFP/GFP                    | GTGAACCGCATCGAGCTGAagg                        | PAM                 |
| Luciferase                  | ATAAAAACGCAGCCCAACACcgg                       |                     |
| Humo Nrf2                   | TGGAGGCAAGATATAGCTTagg                       |                     |
| Mus Nrf2                    | AGAATTCCTCCCAATTCACGcgg                      |                     |
| **Off-Target Sequences**    |                                                |                     |
| H-Nrf2 Off-Target 1         | TGGAGGCTGATATAGAC CTagg                     | PAM                 |
| H-Nrf2 Off-Target 2         | TTAGGGCAAAATATAGATATTagg                    | Unmatched Sites     |
| M-Nrf2 Off-Target 1         | AGAGATCTGCAATTCAGCagg                       |                     |
| M-Nrf2 Off-Target 2         | ACAATTATCCAAATTTCACGcgg                     |                     |
| **Primers for sgRNA**       |                                                |                     |
| Transcription Template      |                                                |                     |
| EGFP/GFP sgRNA-F            | GCTAATACGACTCATAATAGGAGTGAACCCATCGAGCTGAA    | T7                   |
| Luciferase-sgRNA-F          | GCTAATACGACTCATAATAGGAGTGAACCCATCGAGCTGAA    | promoter            |
| H-Nrf2-sgRNA-F              | GCTAATACGACTCATAATAGGAGTGAACCCATCGAGCTGAA    | Target Sequence     |
| M-Nrf2-sgRNA-F              | GCTAATACGACTCATAATAGGAGTGAACCCATCGAGCTGAA    |                     |
| SgRNA Scaffold              | AAAAGCACCAGACTCGGTCCACT                       | Common              |
| **Primers for GCD**         |                                                |                     |
| Assay                       |                                                |                     |
| EGFP/GFP On-Target-F        | GACGTAAACCGCCACAGGTTT                       |                     |
| EGFP/GFP On-Target-R        | GCGGATCTTGAAGTTACCT                         |                     |
| H-Nrf2 On-Target1-F         | CCCACTCCCACCATCAACA                         |                     |
| H-Nrf2 On-Target1-R         | ACCTGCCATAACTTCCACAGA                      |                     |
| H-Nrf2 On-Target1-F         | CACTCTTTCCCTACAGGACGCTTCCGT                 |                     |
| H-Nrf2 On-Target1-R         | GAGGTTTCAGAGTGCTTCCCGATC                    |                     |
| H-Nrf2 Off-Target1-F        | CACTCTTTCCCTACAGGACGCTTCCGT                 |                     |
| H-Nrf2 Off-Target1-R        | GACGCTTTCCCTACAGGACGCTTCCGT                 |                     |

Notes:
- PAM: Protospacer Adjacent Motif
- T7: T7 promoter
- Common: Common sequence
### Primers for high-throughput sequencing

|                   | HTS-Target Sequence               |
|-------------------|----------------------------------|
| H-Nrf2 Off-Target1-F | GAGAATCCGCCTCTTGAGTAATGTAAGTACAGTGC |
| H-Nrf2 Off-Target1-R | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| H-Nrf2 Off-Target2-F | CACTCTTTTCTACAGACGCTCTTCCGATC   |
| H-Nrf2 Off-Target2-R | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| M-Nrf2 On-Target1-F | GAGAATCCGCCTCTTGAGTAATGTAAGTACAGTGC |
| M-Nrf2 On-Target1-R | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| M-Nrf2 Off-Target1-F | CACTCTTTTCTACAGACGCTCTTCCGATC   |
| M-Nrf2 Off-Target1-R | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| M-Nrf2 Off-Target2-F | CACTCTTTTCTACAGACGCTCTTCCGATC   |
| M-Nrf2 Off-Target2-R | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |

### Real-Time PCR Primers

|                        |                                |
|------------------------|--------------------------------|
| Nrf2-F                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| Nrf2-R                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| HIF-1α-F               | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| HIF-1α-R               | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| VEGF-F                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| VEGF-R                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| VCAM-F                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |
| VCAM-R                 | TCTCCACCAGGGAATGTAATGTAAGTACAGTGC |