Supplementary Information

Engineered mesenchymal stem cell-derived exosomes with high CXCR4 levels for targeted siRNA gene therapy against cancer

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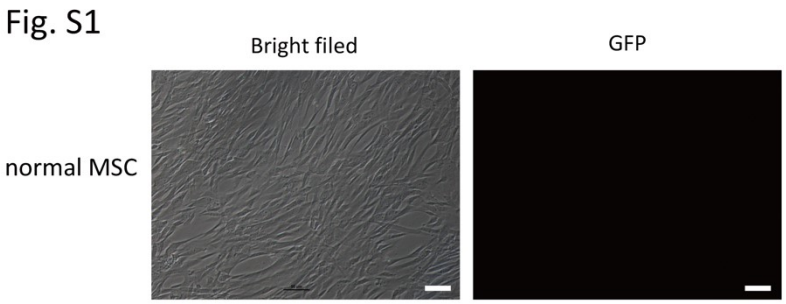

Fig. S1. Morphological characteristics of normal MSCs. Confocal microscopy images of normal MSCs. Scale bar: 50 μm.

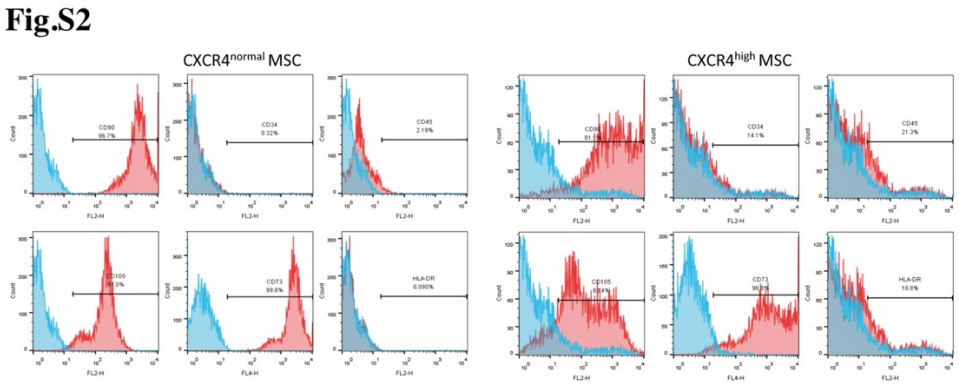

Fig. S2. Characterization of normal MSCs. Expression of signature proteins (CD90, CD34, CD45, CD105, CD73, HLA-DR) on the surface of MSCs surface measured by flow cytometry.

Fig. S3

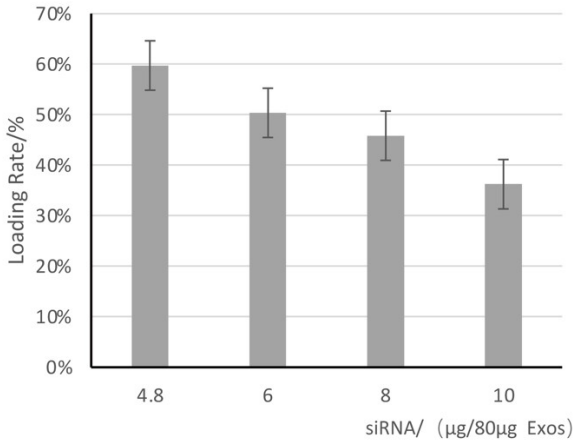

Fig. S3. The efficiency of loading siRNA into exosomes by electrotransfer. The post-electroporation exosomes were treated with RNase A, gel electrophoresis experiments were performed, and band brightness was analyzed via ImageJ.

Fig. S4

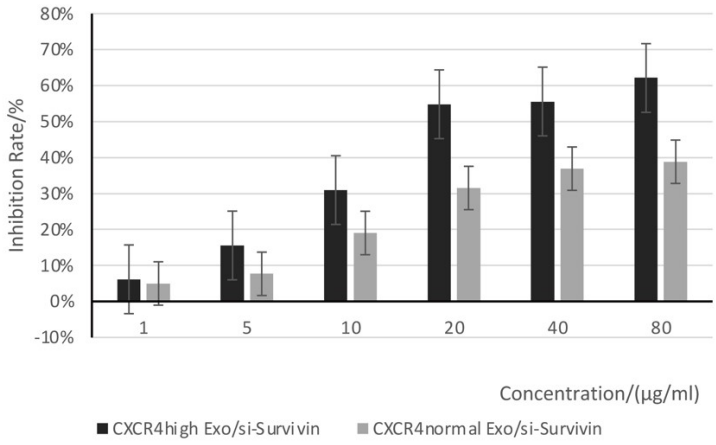

Fig. S4. Test of the best drug concentration of MGC803 cells. MGC803cells were cocultured with different concentrations of $CXCR4^{high}$ Exo/si-Survivin and $CXCR4^{normal}$ Exo/si-Survivin. The absorbance was measured after 24 hours and statistics were analyzed.

Fig. S5

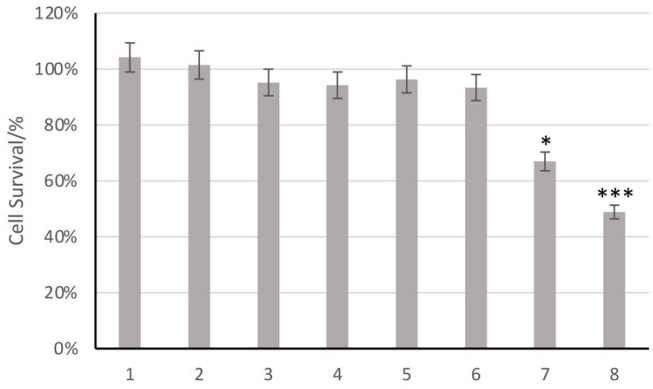

Fig. S5. *In vitro* therapeutic effect of exosomes. CKK-8 assay of MGC803 cells treated with Exos+siRNA or Exos/siRNA after 24h. Data are presented as the mean ± SEM, 0.01<*p < 0.05, **p < 0.01, ***p < 0.001. (n = 6 per group) 1: PBS; 2: naked si-Survivin; 3: $CXCR4^{normal}$ Exo/si-NC; 4: $CXCR4^{high}$ Exo/si-NC; 5: $CXCR4^{normal}$ Exo+si-Survivin; 6: $CXCR4^{high}$ Exo+si-Survivin; 7: $CXCR4^{normal}$ Exo/si-Survivin; 8: $CXCR4^{high}$ Exo/si-Survivin.

Fig. S6

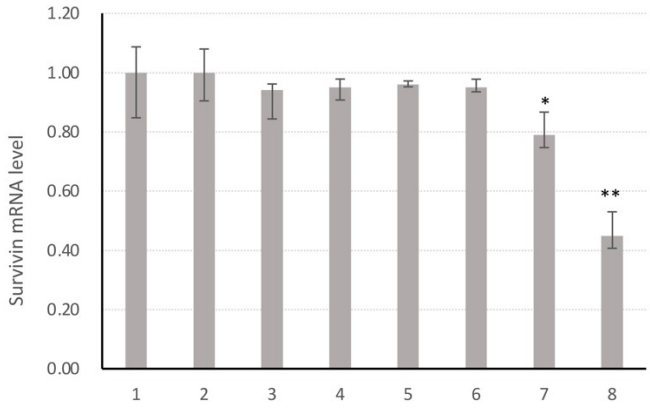

Fig. S6. Survivin expression in MGC803 cells. RT-PCR analysis of the expression of Survivin mRNA in MGC803 cells after transfection with different agents for 24 h. The expression level was relative to that in the PBS group. Data are presented as the mean ± SEM, 0.01<*p < 0.05, **p < 0.01, ***p < 0.001. (n = 6 per group). 1: PBS; 2:

naked si-Survivin; 3: CXCR4^{normal} Exo/si-NC; 4: CXCR4^{high} Exo/si-NC; 5: CXCR4^{normal} Exo+si-Survivin; 6: CXCR4^{high} Exo +si-Survivin; 7: CXCR4^{normal} Exo/si-Survivin; 8: CXCR4^{high} Exo/si-Survivin.

Fig. S7

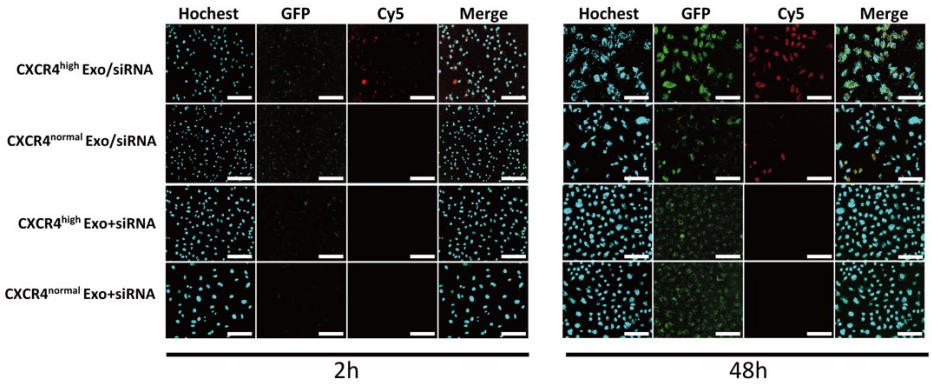

Fig. S7. Cellular uptake of exosomes. Confocal microscopy images of A549 cells co-cultured with CXCR4^{high} Exo/si-Survivin, CXCR4^{normal} Exo/si-Survivin, CXCR4^{high} Exo and CXCR4^{normal} Exo for several hours separately. Exosomes were labeled with GFP (green), and siRNA was conjugated with Cy5 (Red). Scale bar: 100 μm.

Fig. S8

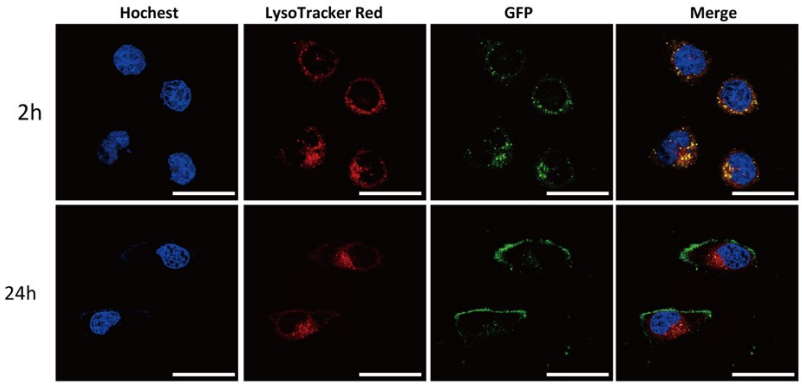

Fig. S8. Escape of exosomes in MGC803 cells. Localization of lysosome (LysoTracker, Red) and exosomes (GFP, Green) at different time points. Scale bar: 50 μm.

Fig. S9

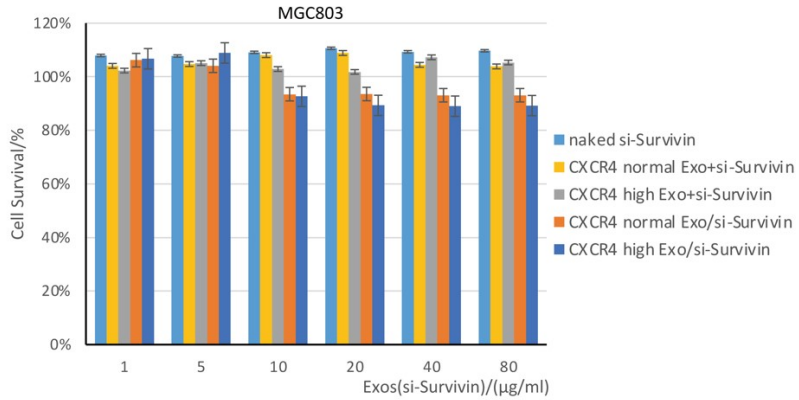

Fig. S9. Cytotoxicity testing of exosomes. MGC803 cells were cocultured with different types of exosomes, the cytotoxicity of which was detected by CCK8.

Fig. S10

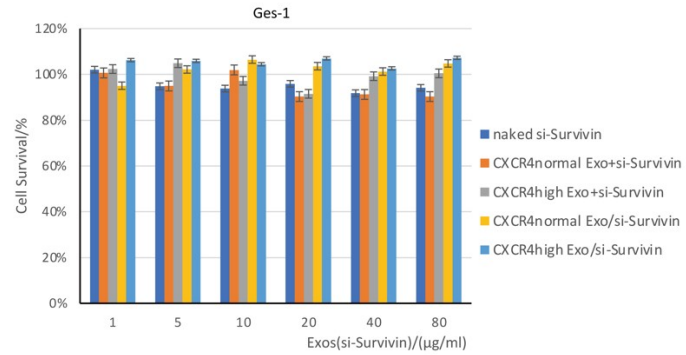

Fig. S10. Cytotoxicity testing of exosomes. Ges-1 cells were cocultured with different types of exosomes, the cytotoxicity of which was detected by CCK8.

Fig. S11

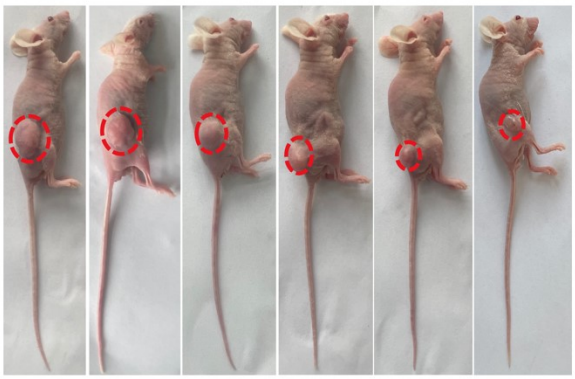

Fig. S11 **Treatment effect of exosomes.** Photographs of nude mice in different experimental groups after 21 days of treatment.