Supplementary Information

CD133 peptide-conjugated pyropheophorbide-a as a novel photosensitizer for targeted photodynamic therapy in colorectal cancer stem cells

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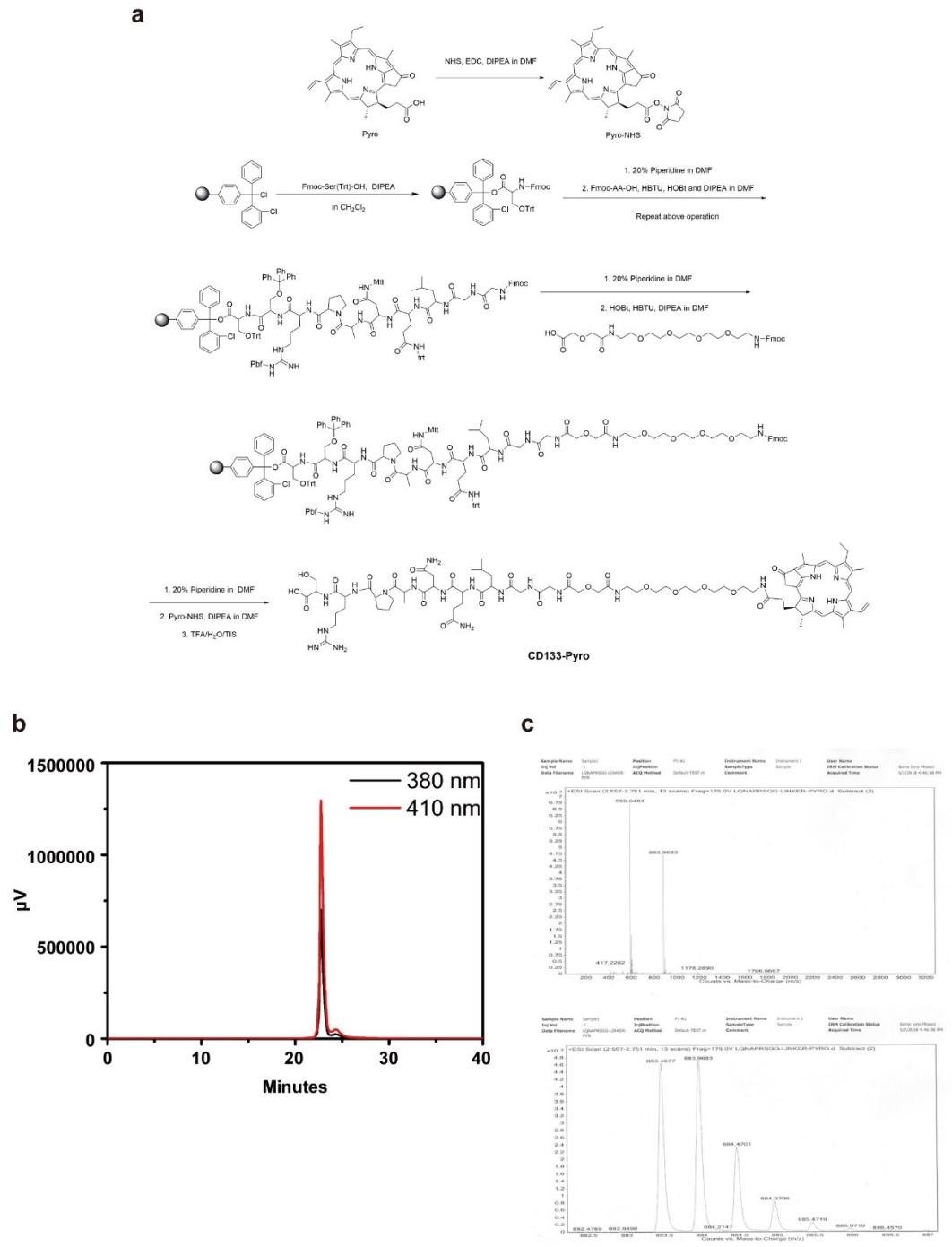
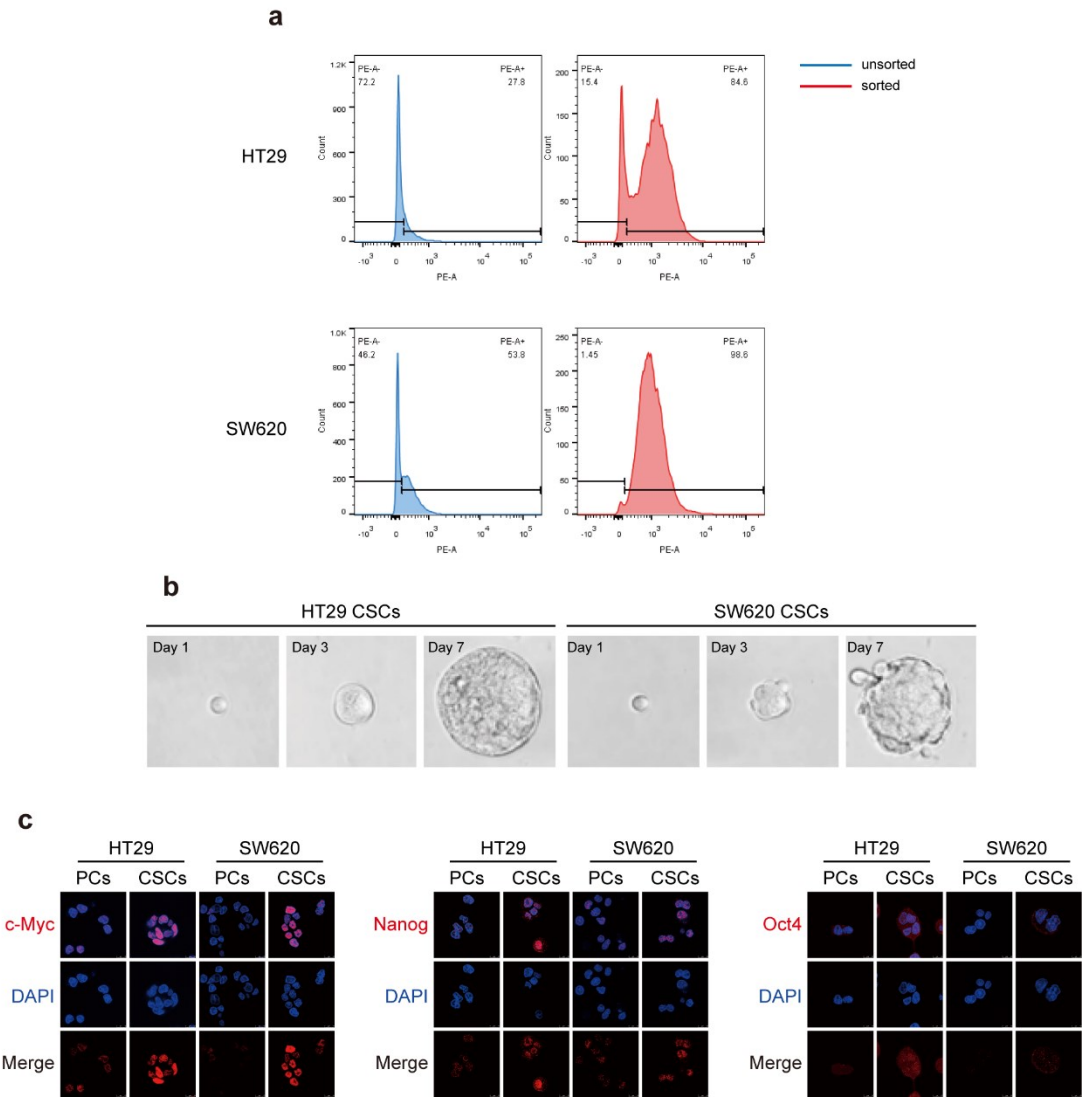
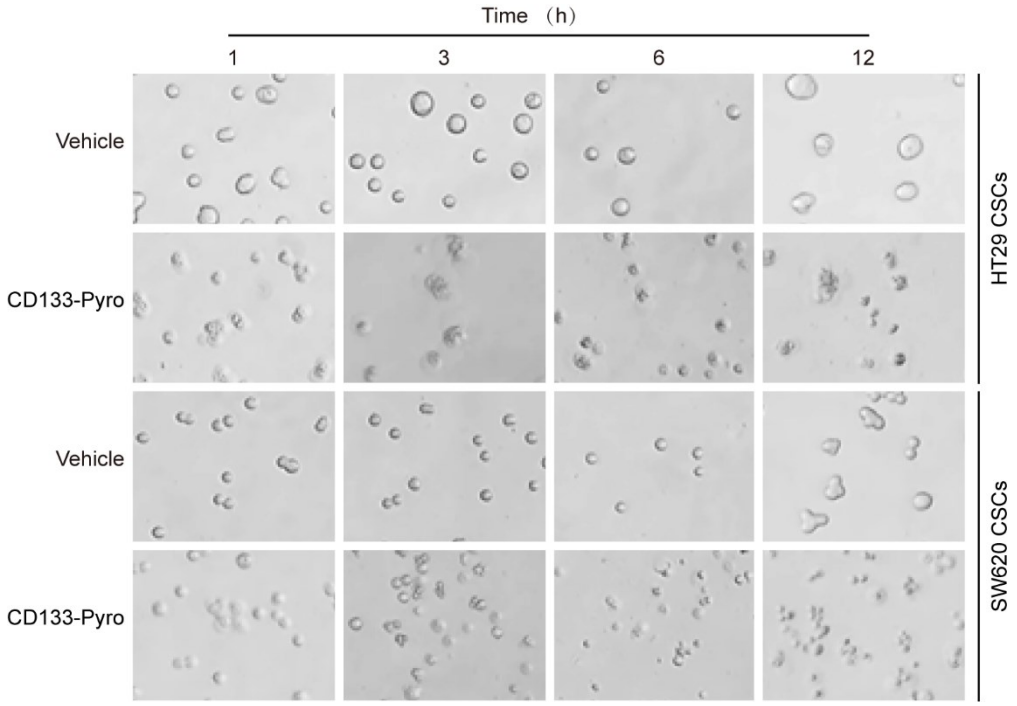
Supplementary Figures



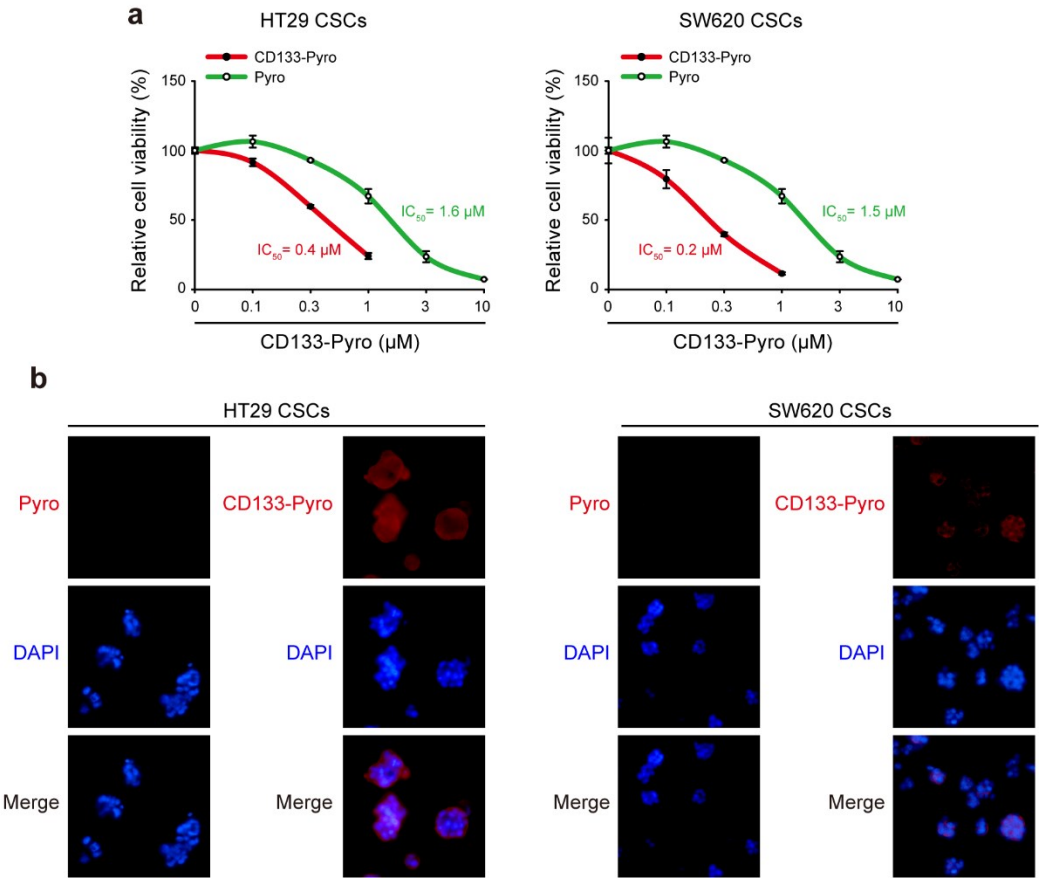
Supplementary Figure 1. Synthetic route and identification of CD133-Pyro conjugate. a Synthetic route and identification of CD133-Pyro conjugate. **b** HPLC analysis of CD133-Pyro conjugate. **c** Mass spectrometry analysis of CD133-Pyro conjugate.



Supplementary Figure 2. Characteristics of CSCs isolated from CD133$^+$ cells of CRC. a CD133$^+$ cells were sorted from HT29 and SW620 PCs by MACS. The percentage of CD133$^+$ cells was analyzed by FlowJo. **b** The morphology showing tumorsphere formation of CD133$^+$ cells 1-7 d after cell sorting under serum-free suspension culture conditions. Original magnification at 200×. **c** Representative confocal images of protein levels of c-Myc, Nanog and Oct4 in HT29 and SW620 PCs and CSCs. Scale bars, 10 μm.



Supplementary Figure 3. Morphological change of CSCs upon treatment with CD133-Pyro PDT. Photomicrographic images of HT29 and SW620 CSCs obtained at indicated time points after treatment with CD133-Pyro PDT. Original magnification at 200×.



Supplementary Figure 4. ***In vitro*** **cytotoxic effect induced by Pyro and CD133-Pyro PDT in HT29 and SW620 CSCs. a** Relative cell viability of Pyro and CD133-Pyro-treated HT29 and SW620 CSCs under 670 nm light irradiation (5 J/cm^2). Data were shown as mean ± s.e.m. (n = 4) and the half-maximal inhibitory concentration (IC_{50}). **b** Representative fluorescence images showing the superior binding capacity of CD133-Pyro over Pyro to HT29 and SW620 CSCs after incubation with Pyro or CD133-Pyro (3 μM) for 10 min at ambient temperature. Original magnification at 400×.

Supplementary Tables

Supplementary Table 1. Tumor-initiating potential of SW620 PCs or CSCs in each treatment group.

Cell types	No. of cells inoculated	Tumor incidence	observation time (d)
SW620 PCs	5×10^4	0/4	30
	5×10^5	0/4	30
	5×10^6	4/4	30
SW620 CSCs	5×10^2	0/4	30
	5×10^3	0/4	30
	5×10^4	3/4	30

Different numbers of viable cells were injected subcutaneously into unilateral flank of nude mice. Tumor-initiating potential in the above groups was observed.

Supplementary Table 2. Reagents associated with this article.

Reagent Name	Company	Cat #
RPMI-1640	Hyclone	C11875500BT
DMEM/F-12	Gibco	C11330500BT
D-Hank's		14175095
B27	Invitrogen	17504-044
EGF	Prospec	Cyt-217-b
bFGF		Cyt-218-b
CellTiter-Blue® Cell Viability Assay	Promega	G808b
DCFH-DA	Sigma-Aldrich	D6883
NAC		A7250
CQ		C6628
Fetal bovine serum	BI	04-001-1A
Apoptosis Detection Kit	Miltenyi	130-092-052

BI: Biological industries; DCFH-DA: 2′-7′-dichlorofluorescein diacetate; NAC: N-acetyl-l-cysteine; CQ: chloroquine.

Supplementary Table 3 Antibodies associated with this article.

Antibody Name	Company	Cat #	Dilution (WB)	Dilution (IF)
CD133	CST	5860	/	1:100
c-Myc	CST	5605	/	1:100
Nanog	Abcam	ab109250	/	1:100
Oct4	Abcam	ab109183	/	1:100
Nrf2	CST	12721	1:1000	/
Keap1	CST	8047	1:1000	/
$mTOR^{Ser2448}$	CST	5636	1:1000	/
mTOR	CST	2983	1:1000	/
P62	CST	8025	1:1000	/
LC3-I/II	CST	12741	1:1000	1:100
Bcl-2	Abclonal	A0208	1:1000	/
BAD	Abclonal	A1593	1:1000	/
Bak	CST	12105	1:1000	/
GAPDH	Abclonal	AC002	1:10000	/

CST: Cell signaling technology; WB: Western blotting; IF: Immunofluorescence.

Supplementary Video. Uptake of CD133-Pyro in HT29 and SW620 CSCs were recorded by dynamic laser confocal 0-10 min post-treatment.