

The PROTAC selectively degrading Bcl-x_L represents a novel Hedgehog pathway inhibitor with capacity of combating resistance to Smoothened inhibitors while sparing bone growth

Supplementary Figure 1

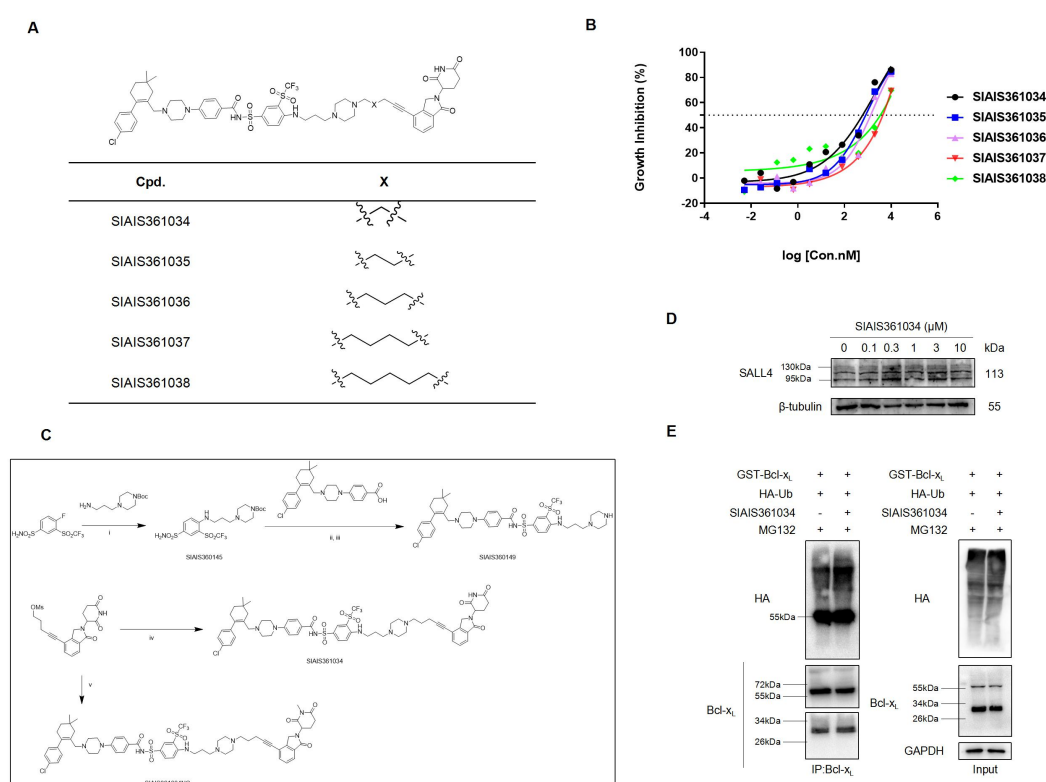


Figure S1. Optimization of linker length with linear aliphatic chains, synthetic schemes of SIAIS361034 and SIAIS361034NC. (A) Structures of Bcl-x_L degraders. **(B)** Cell viability of RS4;11 cells after treatment with increasing concentrations of Bcl-x_L degraders for 48 h. Data represent mean \pm SD (n = 3). **(C)** Reagents and conditions: i) DIPEA, DCM, rt; ii) EDCI, DMAP, DCM, rt; iii) TFA, DCM; iv) SIAIS360149, K₂CO₃, NaI, DMF, 80 °C; v) NaH, MeI, DMF, 0 °C to rt; then SIAIS360149, K₂CO₃, NaI, DMF, 80 °C; DIPEA = *N,N*-diisopropylethylamine; DCM = dichloromethane; EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

hydrochloride; DMAP = 4-dimethylaminopyridine; TFA = trifluoroacetic acid; DMF = *N,N*-dimethylformamide. **(D)** A representative western blot analysis of SALL4 in NIH-3T3 cells after being treated with SIAIS361034 as indicated for 24 h. **(E)** A representative western blot analysis of hemagglutinin (HA), Bcl-x_L, and GAPDH following GST-Bcl-x_L immunoprecipitation of protein extracts from 293T cells. Cells were cotransfected as indicated with GST-Bcl-x_L and HA-tagged ubiquitin (HA-Ub) and then were treated with or without SIAIS361034 (1 μM) and MG132 (10 μM) as indicated for 4 h.

Supplementary Figure 2

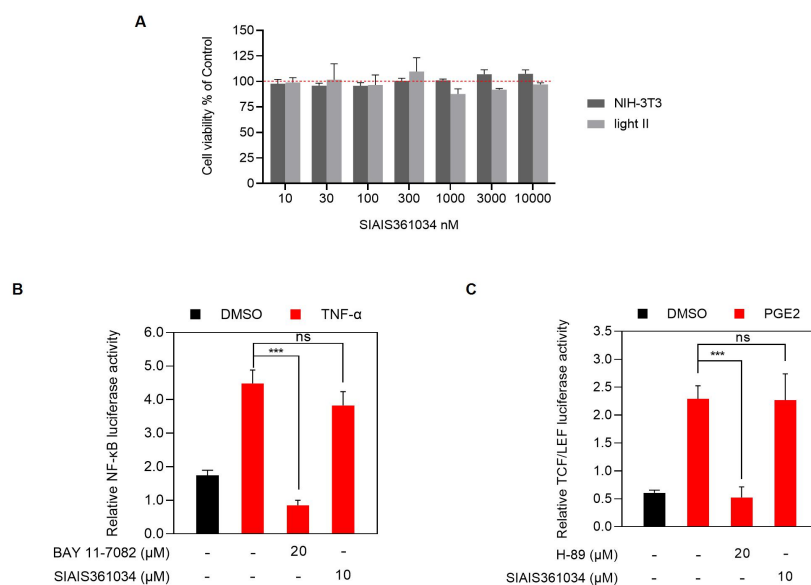


Figure S2. (A) Viability of NIH-3T3 cells and light II cells was determined by MTT cytotoxicity assays after incubation with various concentrations of SIAIS361034 as indicated for 72 h. Data represent mean \pm SD ($n = 3$). **(B)** NF- κ B luciferase analysis of the effect of SIAIS361034 on NF- κ B signaling pathway in 293T cells. The 293T cells were transfected with NF- κ B luciferase reporter and *Renilla*-TK plasmids and then exposed to TNF- α (10 ng/mL) with BAY 11-872 (20 μ M) and SIAIS361034 (10 μ M) for 6 h. Data represent mean \pm SD ($n = 3$). **(C)** TCF/LEF luciferase analysis of the effect of SIAIS361034 on Wnt signaling pathway in LS174T cells. The LS174T cells were transfected with TCF/LEF luciferase reporter and *Renilla*-TK plasmids and then exposed to PGE2 (1 μ M) with H-89 (20 μ M) and SIAIS361034 (10 μ M) for 24 h. Data represent mean \pm SD ($n = 3$).

Supplementary Figure 3

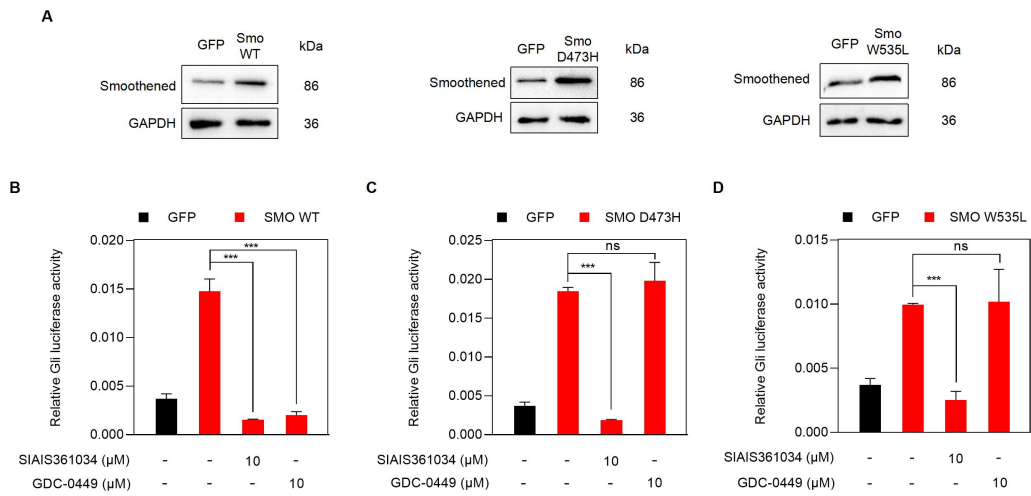


Figure S3. (A) A representative western blot analysis of Smo in light II cells after transfected with Smo-WT, Smo-D473H, Smo-W535L, and GFP plasmids. GAPDH was used as a protein loading control in western blot analysis. **(B-D)** The effect of SIAIS361034 (10 μ M) and GDC-0449 (10 μ M) on the Gli-luciferase activity initiated by ectopic expression of Smo-WT, Smo-D473H, and Smo-W535L plasmids, respectively. Data represent mean \pm SD (n = 3).

Supplementary Figure 4

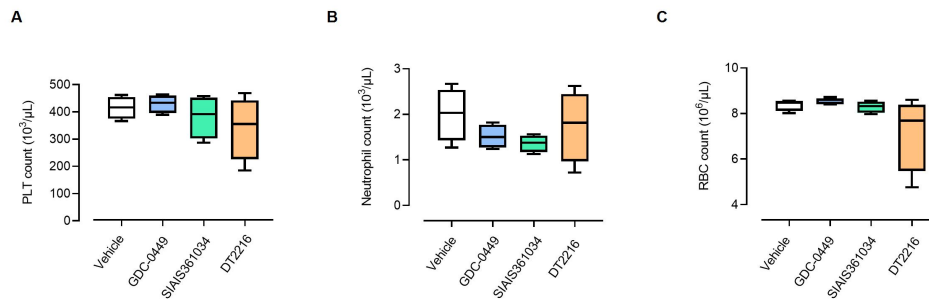


Figure S4. Numeration of platelets (A), neutrophils (B), and red blood cells (C) of 6-week-old mice after various treatments from P12 to P16. Data represent mean \pm SD (n = 4).

Supplementary Table 1. Mouse PK parameters following IV/IP/PO Administration with SIAIS361034.

IV	Dose Level	T _{1/2}	T _{max}	C _{max}	AUC _(0-t)	AUC _(0-∞)	MRT _(0-t)	MRT _(0-∞)	C ₀	V _{ss}	V _z	Cl	
Administration Route	mg/kg	h	h	ng/mL	h*ng/mL	h*ng/mL	h	h	ng/mL	mL/kg	mL/kg	mL/h/kg	
Animal No.													
Mouse 1	IV	2.00	1.79	0.083	10300	3193	3205	0.22	0.26	34215	163	1609	624
Mouse 2	IV	2.00	6.38	0.083	17900	6203	6222	0.30	0.40	79513	130	2957	321
Mouse 3	IV	2.00	2.29	0.083	13200	4263	4305	0.31	0.42	43287	193	1535	465
n		3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mean		2.00	3.49	0.083	13800	4553	4577	0.28	0.36	52339	162	2034	470
SD		0.000	2.52	0.00	3835	1526	1527	0.05	0.09	23967	32	801	151

IP	Administration Route	Dose Level	T _{1/2}	T _{max}	C _{max}	AUC _(0-t)	AUC _(0-∞)	MRT _(0-t)	MRT _(0-∞)	F
Animal No.		mg/kg	h	h	ng/mL	h*ng/mL	h*ng/mL	h	h	%
Mouse 1	IP	10	5.94	1.00	7630	15533	16333	3.84	5.25	71.4
Mouse 2	IP	10	6.44	0.50	9700	20871	22061	5.16	6.68	96.4
Mouse 3	IP	10	5.23	0.50	4920	15558	16141	4.24	5.22	70.5
n		3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mean		10.0	5.87	0.67	7417	17321	18178	4.41	5.72	79.4
SD		0.00	0.61	0.29	2397	3075	3364	0.68	0.83	14.7

PO	Administration Route	Dose Level	T _{1/2}	T _{max}	C _{max}	AUC _(0-t)	AUC _(0-∞)	MRT _(0-t)	MRT _(0-∞)	F
Animal No.		mg/kg	h	h	ng/mL	h*ng/mL	h*ng/mL	h	h	%
Mouse 1	PO	10	8.98	1.00	50.9	265	305	6.29	10.30	1.33
Mouse 2	PO	10	NA	2.00	54.9	213	NA	2.88	NA	NA
Mouse 3	PO	10	2.39	1.00	49.1	101	111	2.01	2.82	0.484
n		3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mean		10.0	5.68	1.33	51.6	193	208	3.73	6.56	NA
SD		0.00	4.66	0.58	2.97	83.7	137	2.27	5.28	0.60