Supplementary Tables

Table S1: List of primers and probes used in quantitative real-time PCR

Primers and probes used in quantitative real-time PCR

AXL FL_forward	CCTCCCAGTACCCCTGGA	Probe 37
AXL FL_reverse	AGGCAGGAGTTGAAGGTTCC	Probe 37
AXL V_forward	TGTGTGGCAGCCTACACTG	Probe 1
AXL V_reverse	AAGGTTCCTTCACTGGGCG	Probe 1
GAPDH_forward	CTCTGCTCCTCCTGTTCGAC	Probe 60
GAPDH_reverse	AATCCGTTGACTCCGACCTT	Probe 60

Table S2: List of primers of sgRNAs used in cloning

Sequences of sgRNAs

hAxl-45F	**CACCgGTGCTTGGCGCTGTGCGGCT**
hAxl-45R	**AAACAGCCGCACAGCGCCAAGCACc**
hAxl-185F	**CACCgGAGAGCCCCCCGAGGTACAT**
hAxl-185R	**AAACATGTACCTCGGGGGGCTCTCc**
Ren-208F	**CACCgGTAGCGCGGTGTATTATACC**
Ren-208R	**AAACGGTATAATACACCGCGCTACc**

Supplementary Figures:

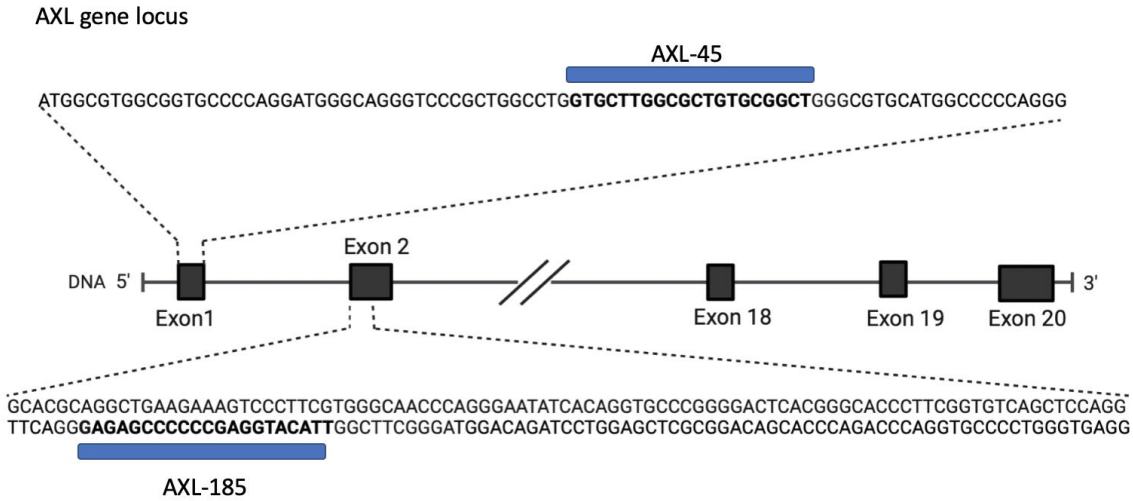
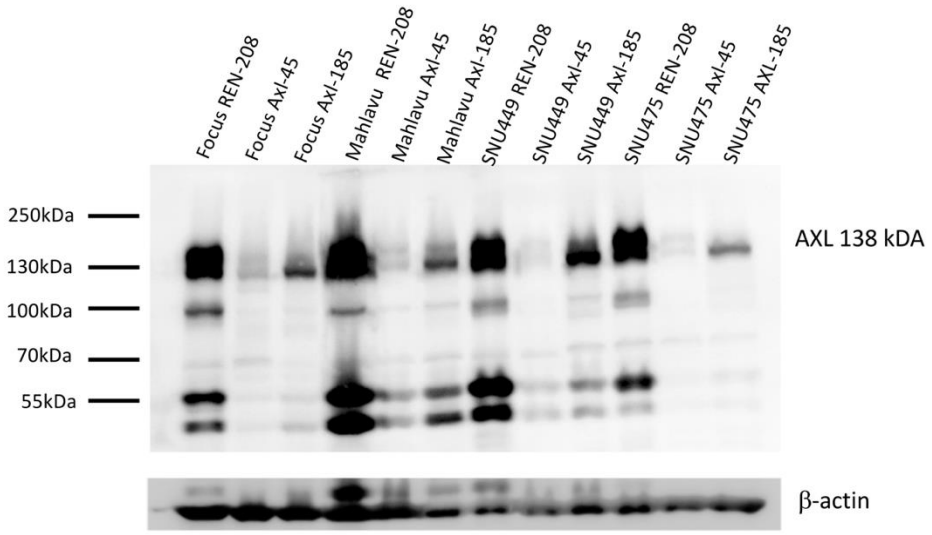


Fig S1: The schematic representation of the AXL gene target sequences for gRNAs used in CRISPR-Cas9-mediated gene knockout studies. AXL gene locus consisting of 20 exons (black) is shown in 5′ to 3′ orientation. The locations of gRNAs targeting the AXL gene locus are highlighted.

A



B

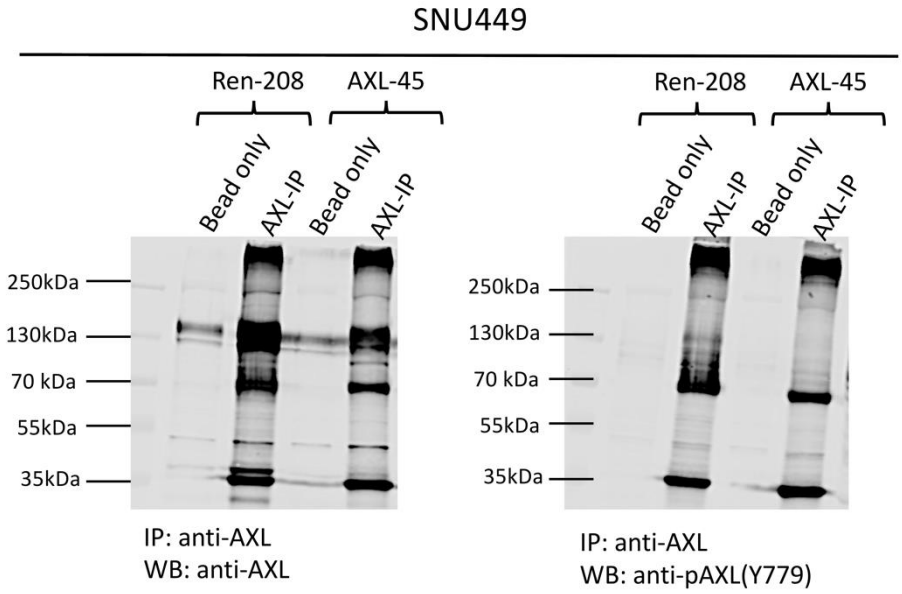
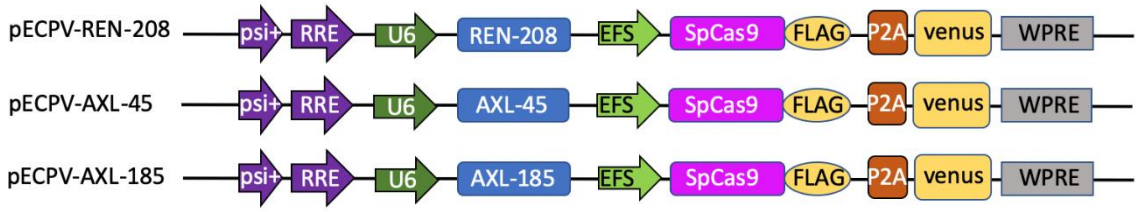


Figure S2: AXL expression in CRISPR-Cas9-mediated knock-out cells. A. AXL gene was targeted in 4 different HCC cell lines Focus, Mahlavu,SNU449 and SNU475 using gRNAs REN-208,AXL-45 and AXL-185. B. Anti-AXL blot and anti-pAXL(Y779) blot of anti AXL immunoprecipitated SNU449 cells targeted by Ren-208 and AXL-45.

A



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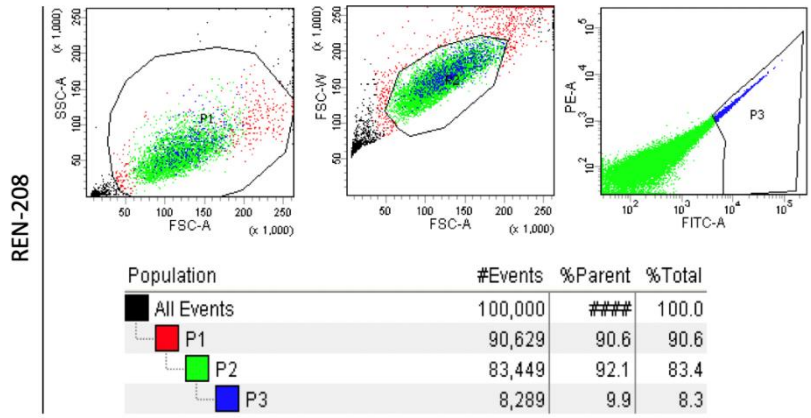
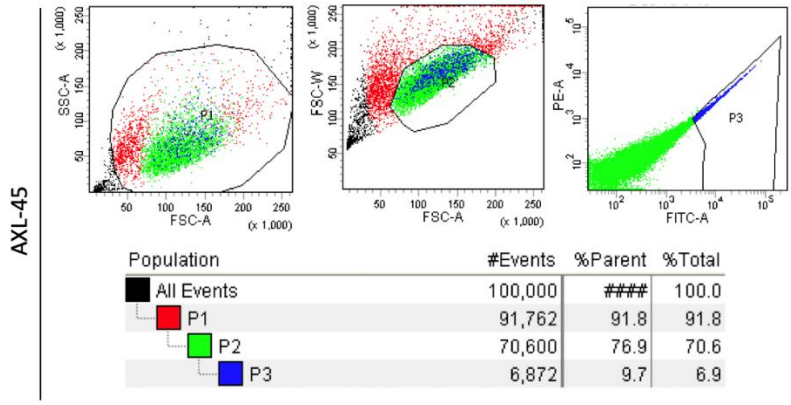


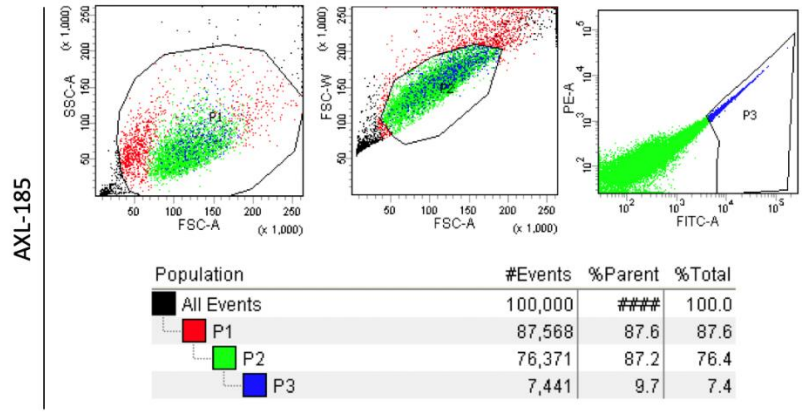
Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
P1	90,629	90.6	90.6
P2	83,449	92.1	83.4
P3	8,289	9.9	8.3

C



Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
P1	91,762	91.8	91.8
P2	70,600	76.9	70.6
P3	6,872	9.7	6.9

D



Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
P1	87,568	87.6	87.6
P2	76,371	87.2	76.4
P3	7,441	9.7	7.4

Fig S3: The schematic representation of vector maps carrying Renilla and AXL-specific gRNAs. (A) Backbone plasmid harbors psi+, RRE virus packaging elements; U6promoter and

gRNA expression cassette; EFS promoter and, SpCas9 (streptococcus pyogenes Cas9) coding sequence; P2A self-cleaving peptide, YFP (yellow fluorescent protein) variant Venus gene expression cassette; and WPRE post-transcriptional regulatory element. (B,C,D) Cell sorting gates for Venus-positive cells are depicted for REN-208 (B), AXL-45 (C), and AXL-185 (D)

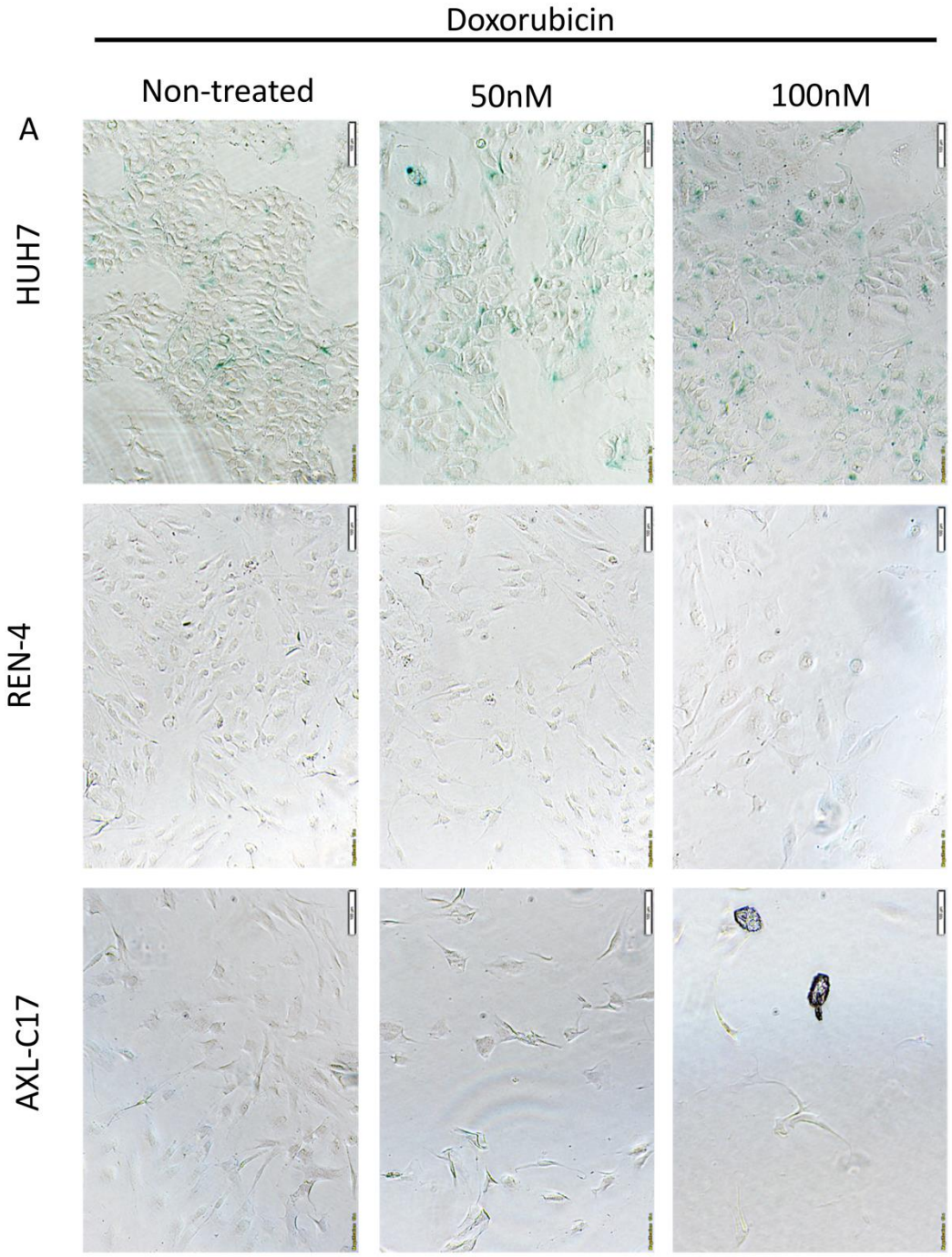


Figure S4: SA-β -Galactosidase Assay of AXL-knockout cells. Huh7 parental was used as a positive control. Representative images non-treated, and 50 and 100 nM of Doxorubicin treated cells are shown.

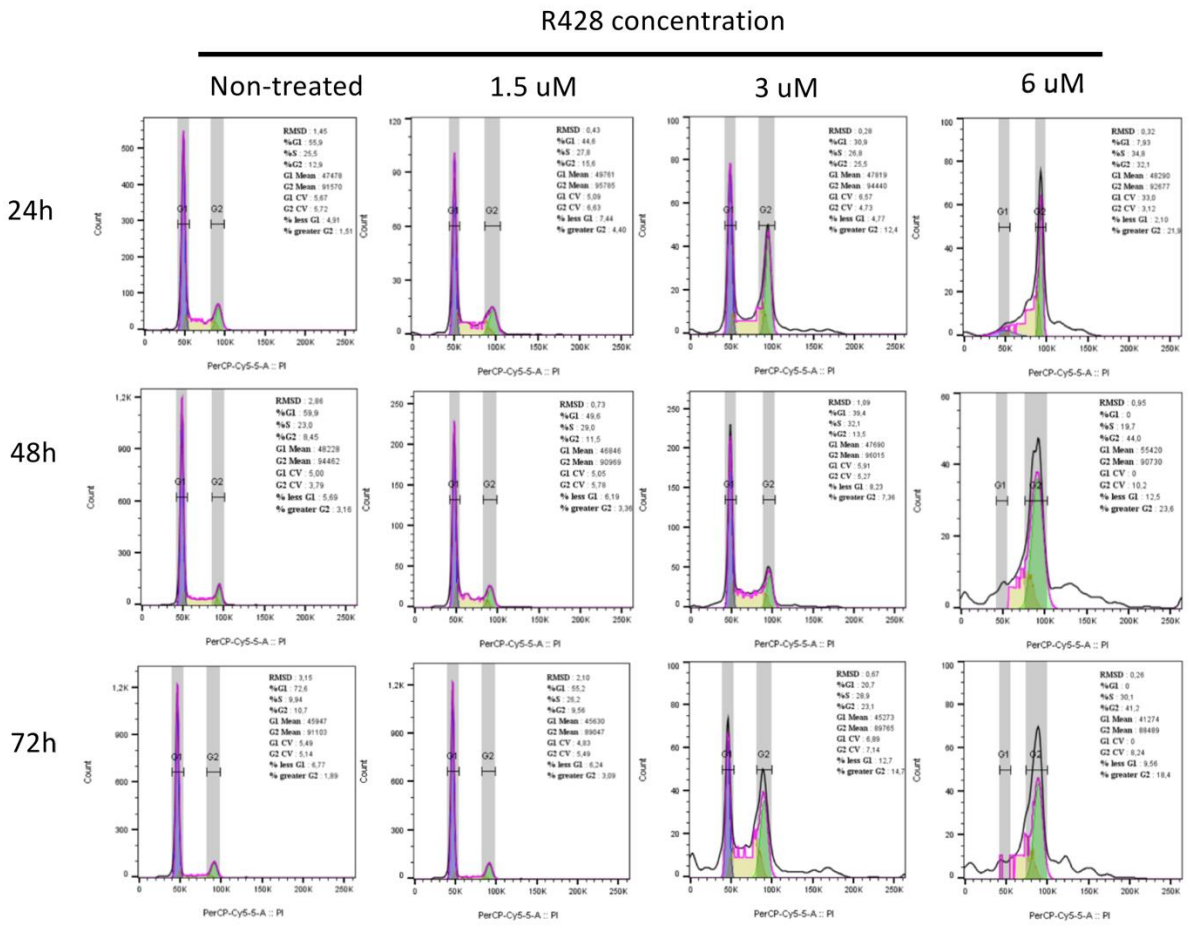


Figure S5: Cell cycle analysis of the parental SNU475 cell line treated with 0,1.5, 3 and 6μM R428 for 24, 48 and 72 hours.

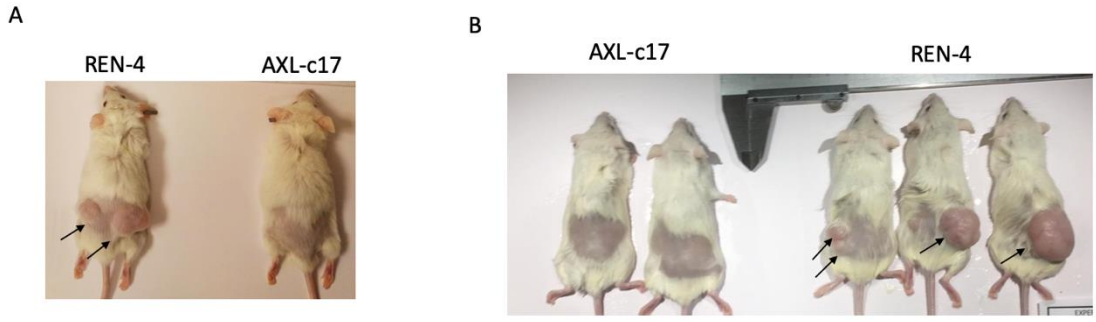


Figure S6: Tumorgenicity of SNU475-derived REN-4 and AXL-c17 clones. (A,B)Examples of subcutaneous tumors formed by REN-4 as compared to the absence of tumors by AXL knock-out AXL-c17 cells in immunodeficient NOD-SCIDγ mice. Tumors of REN-4 cells were observed as a result of the two-sided injection, $1x10^6$ cells on each side (A) and the one-sided injection of $2x10^5$ cells (B). Animals were shaved at cell injection sites to better visualize tumor formation.

Supplementary Methods:

SA-β-Galactosidase Assay

SA-β-Galactosidase Assay was performed as previously described Briefly, non-treated controls and cells treated with 50 and 100 nM doxorubicin (Sigma, D15151) for 72 hours were fixed in 3% formaldehyde at room temperature. Cells were washed twice with 1xPBS. X-gal staining solution (40mM citric acid, 140 mM Na_2HPO_4, 60 mM NaH_2PO_4, 5 mM potassium ferricyanide and 5 mM potassium ferrocyanide 150 mM NaCl, 2 mM MgCl2, and 1mg/ml X-gal at ph=6.0) was added. Cells were incubated at 37°C for 16 hours. 10x Bright Field images were obtained using Olympus IX71.