

Human epidermal growth factor receptor 3 serves as a novel therapeutic target for acral melanoma

Running title: HER3 as a therapeutic target for acral melanoma

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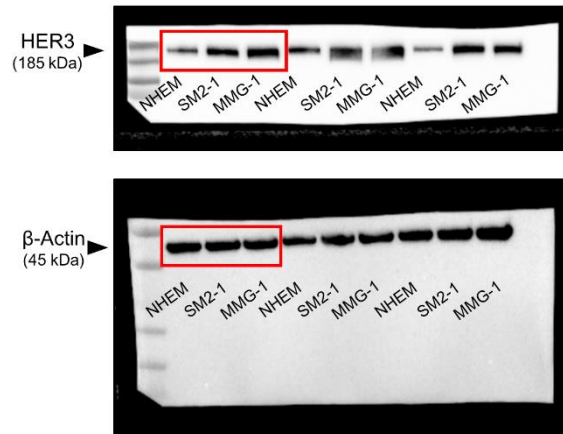
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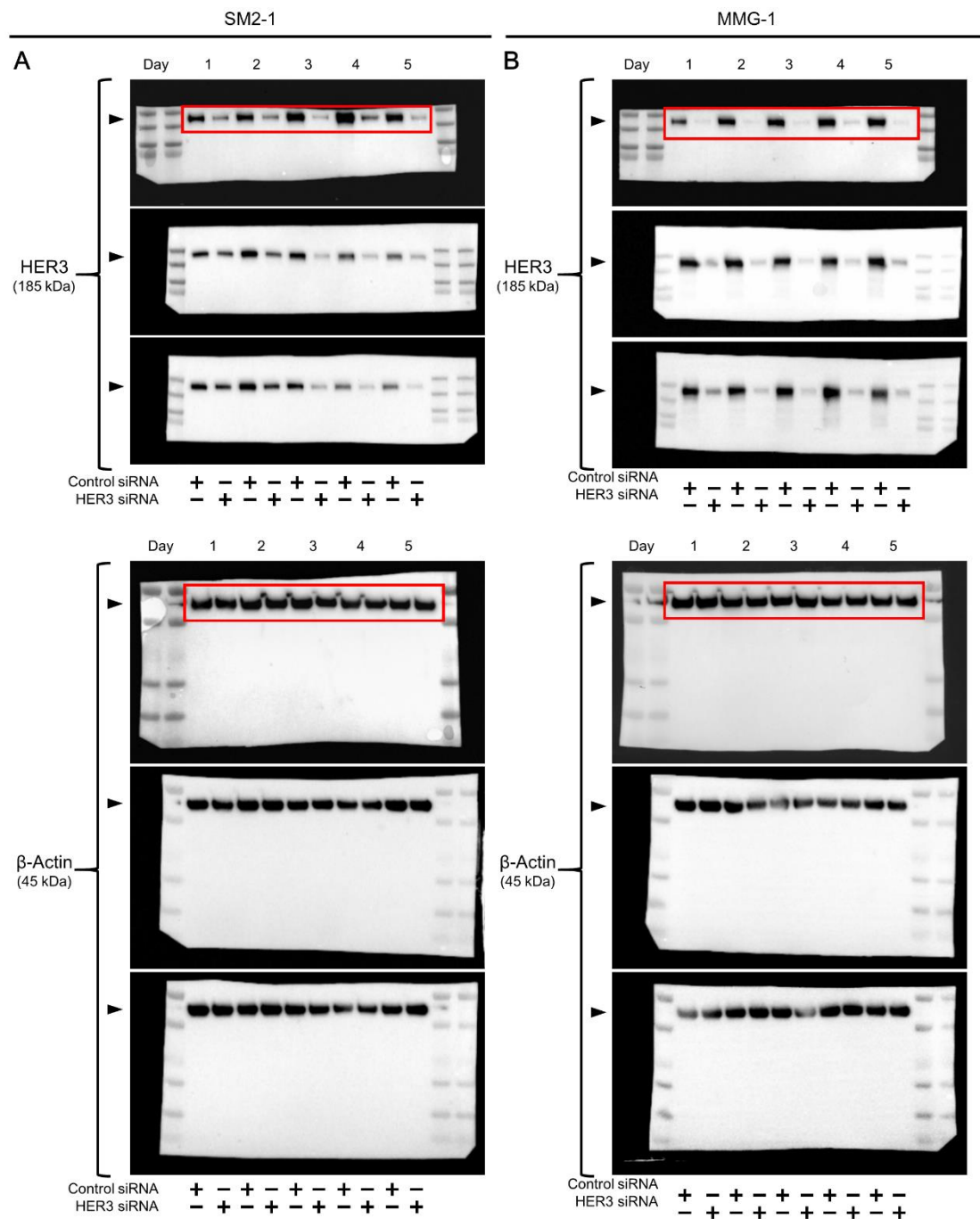
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Supplementary Figures

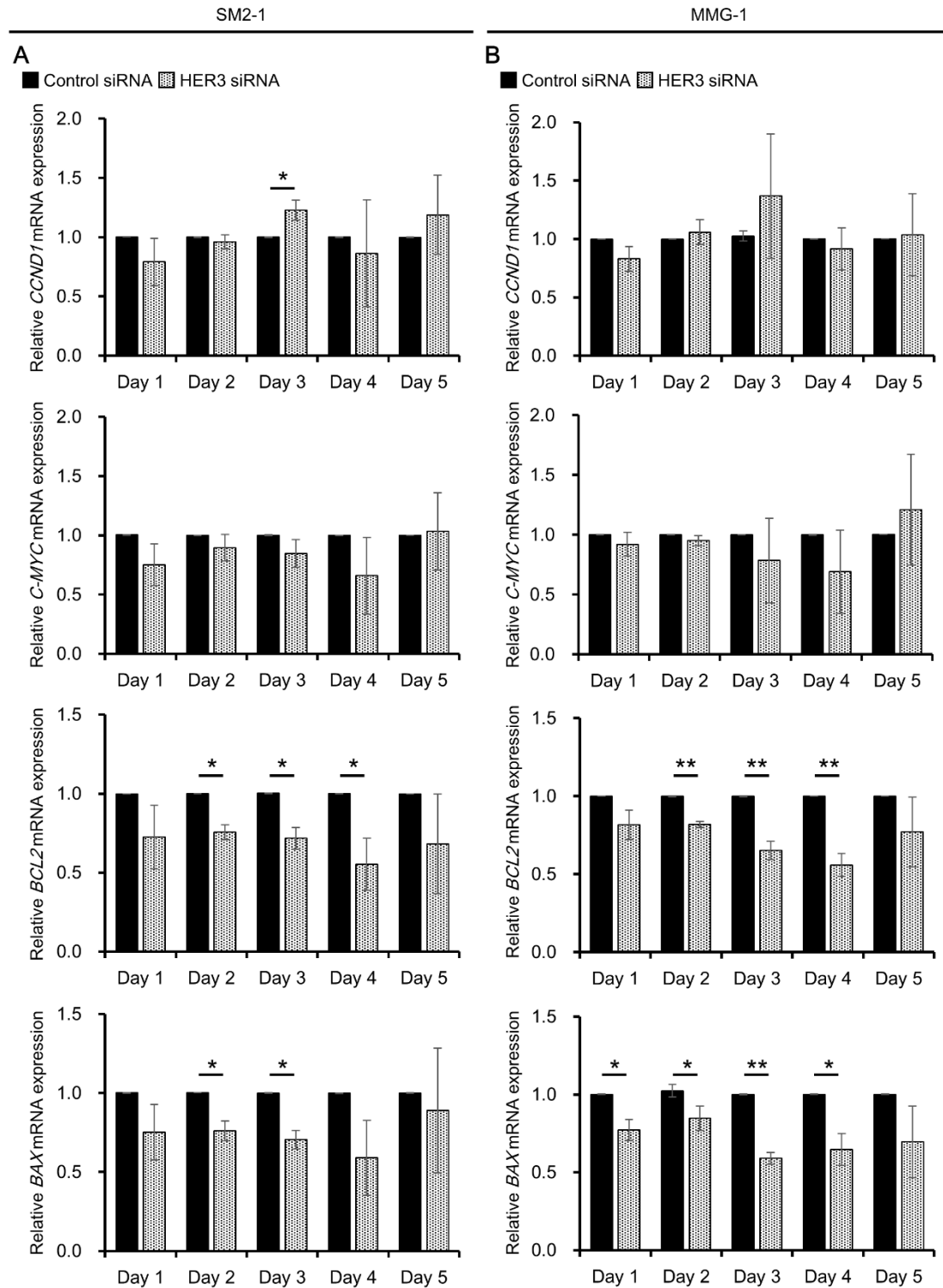
Supplementary Tables



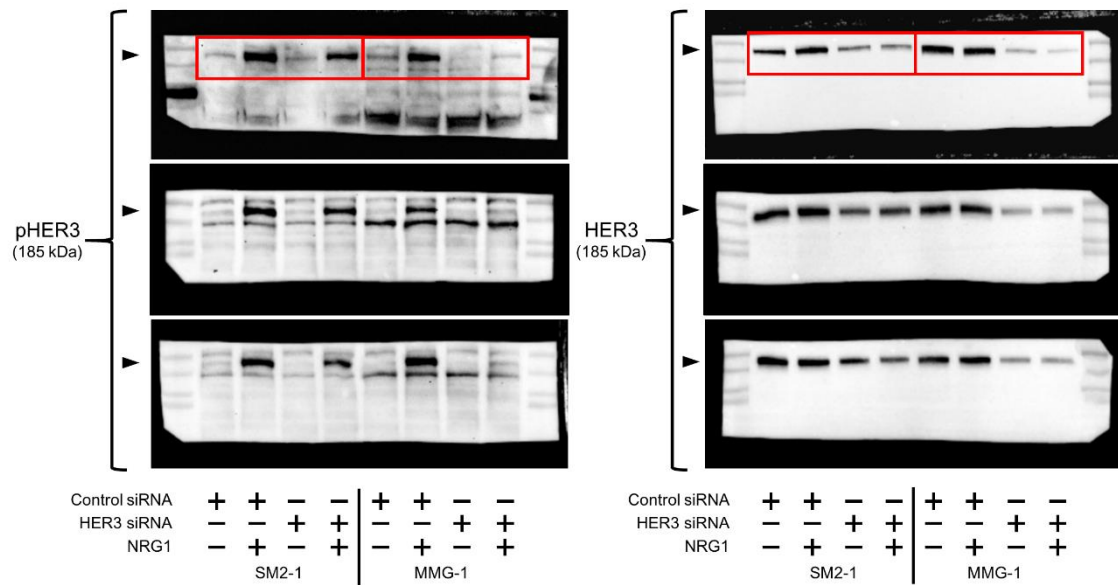
Supplementary Figure S1. Original full length western blot images presented in Fig. 2B. HER3 and β -actin protein expression in NHEM, SM2-1, and MMG-1 cells was determined by western blotting. Unedited original images of blots are shown. The samples were derived from three independent experiments. The red boxes indicate the cropped areas shown in Fig. 2B.



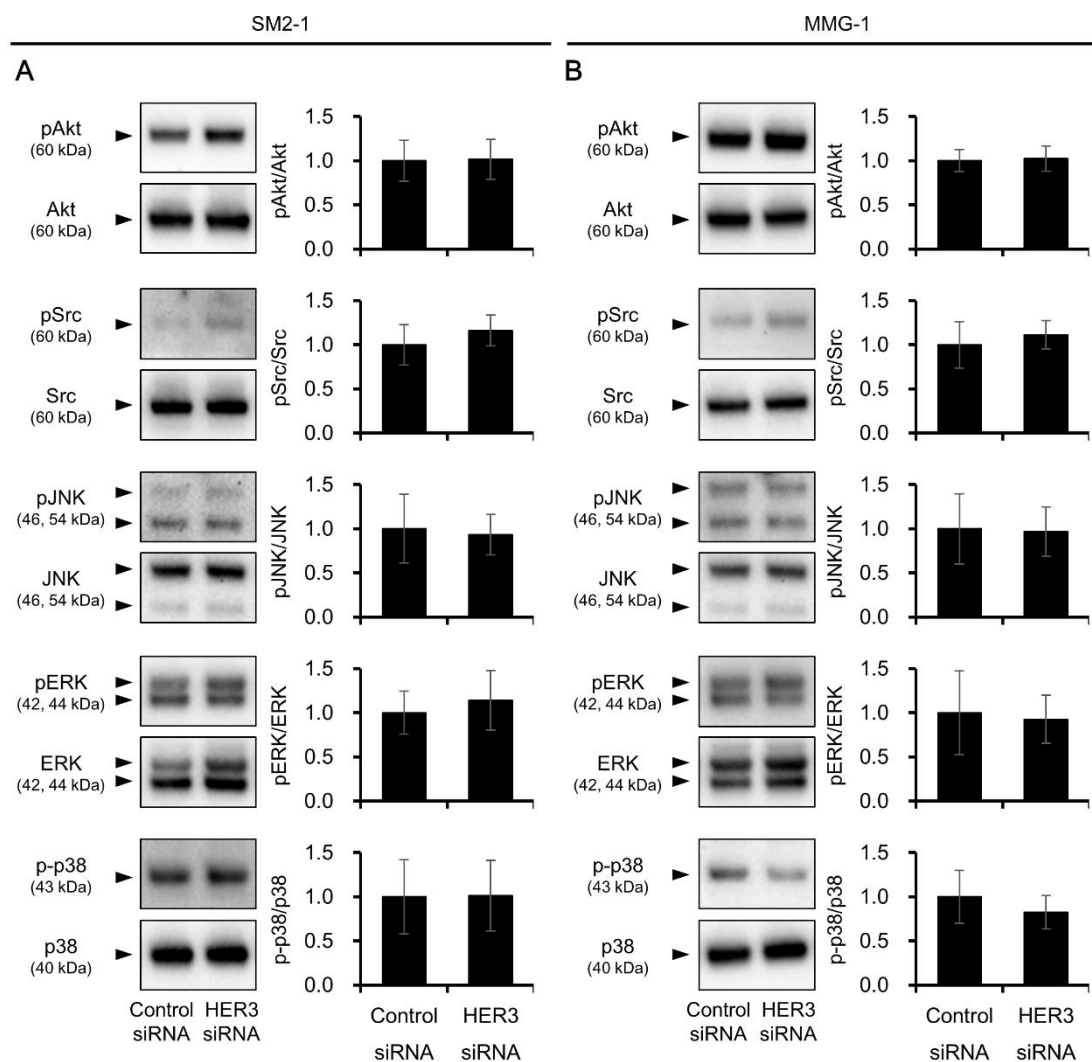
Supplementary Figure S2. Original full length western blot images presented in Fig. 3C and D. HER3 and β -actin protein expression in control or HER3 siRNA-transfected (A) SM2-1 and (B) MMG-1 cells was determined by western blotting. Unedited original images of blots are shown. The samples were derived from three independent experiments. The red boxes indicate the cropped areas shown in Fig. 3C and D.



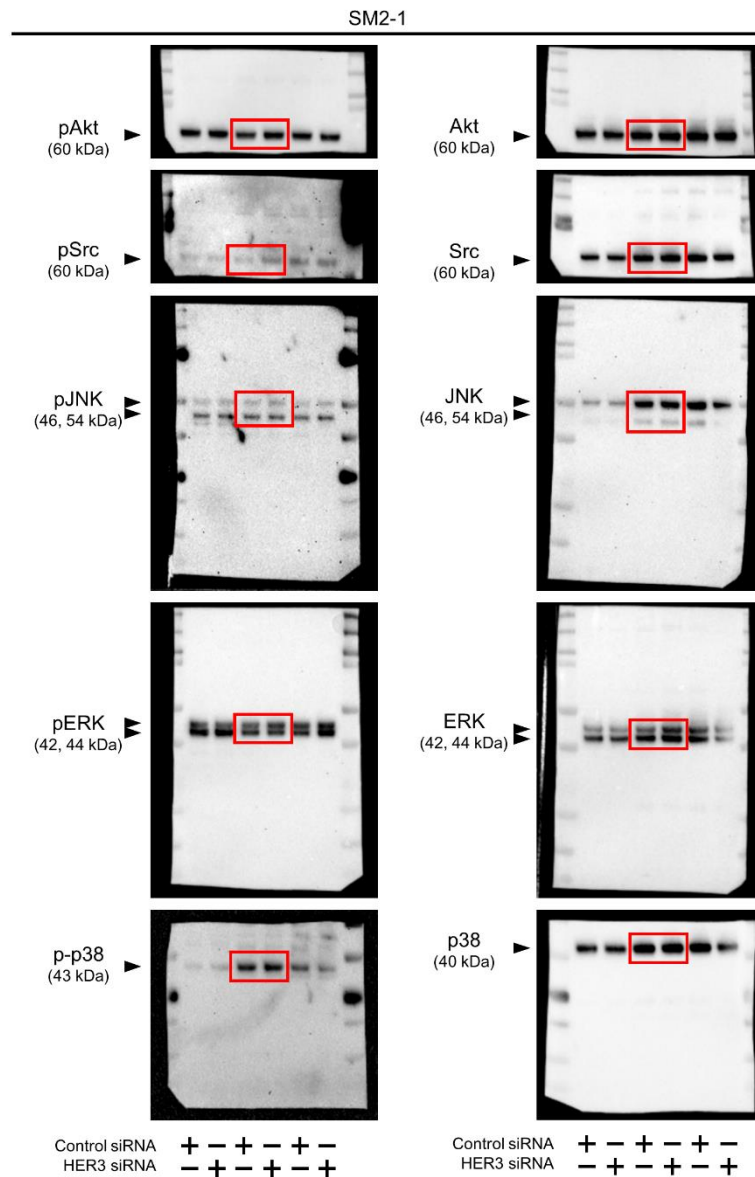
Supplementary Figure S3. Expression of *CCND1*, *C-MYC*, *BCL2*, and *BAX* in siRNA-transfected AM cells. Gene expression of *CCND1*, *C-MYC*, *BCL2*, and *BAX* was measured in control or HER3 siRNA-transfected (A) SM2-1 and (B) MMG-1 cells. Data are the mean $\pm$ SD of three independent experiments. * $p < 0.05$ and ** $p < 0.01$.



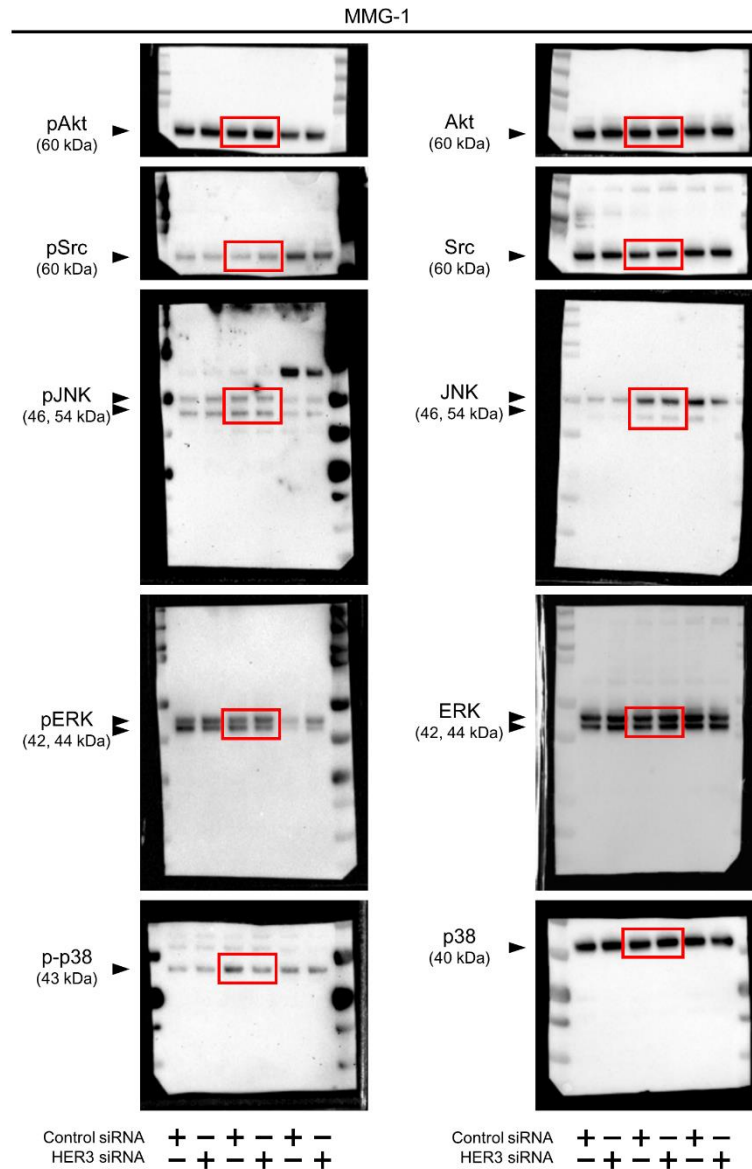
Supplementary Figure S4. Original full length western blot images presented in Fig. 4A and B. Phospho-HER3 (pHER3) and HER3 protein expression in control or HER3 siRNA-transfected SM2-1 and MMG-1 cells treated with vehicle control or NRG1 (10 ng/mL) was determined by western blotting. Unedited original images of blots are shown. The samples were derived from three independent experiments. The red boxes indicate the cropped areas shown in Fig. 4A and B.



Supplementary Figure S5. Phosphorylation status of signaling molecules in siRNA-transfected AM cells. Protein expression of signaling molecules and their phosphorylated forms in control or HER3 siRNA-transfected (A) SM2-1 and (B) MMG-1 cells was detected by western blotting. The signal of each molecule was analyzed using ImageJ software. The samples were derived from three independent experiments. Full-length blot images are shown in Supplementary Figs. S6 and S7.



Supplementary Figure S6. Original full length western blot images presented in Supplementary Fig. S5A. Protein expression of signaling molecules and their phosphorylated forms in control or HER3 siRNA-transfected SM2-1 cells was detected by western blotting. Unedited original images of blots are shown. The samples were derived from three independent experiments. The red boxes indicate the cropped areas shown in Supplementary Fig. S5A.



Supplementary Figure S7. Original full length western blot images presented in Supplementary Fig. S5A. Protein expression of signaling molecules and their phosphorylated forms in control or HER3 siRNA-transfected MMG-1 cells was detected by western blotting. Unedited original images of blots are shown. The samples were derived from three independent experiments. The red boxes indicate the cropped areas shown in Supplementary Fig. S5B.

Supplementary Table S1. HER3 positivity and clinicopathological factors

	Positive (n = 23)	Negative (n = 49)	<i>p</i> -value
Age (year, mean $\pm$ SD)	66.7 $\pm$ 16.1	64.8 $\pm$ 15.8	0.649
Sex (n)			
Male	11	23	>0.999
Female	12	26	
Tumor site (n)			
Hand	4	6	0.463
Foot	12	33	
Nail apparatus	7	10	
Breslow thickness (mm, mean $\pm$ SD)	4.26 $\pm$ 3.25	2.38 $\pm$ 3.42	0.029
AJCC stage			
I, II	13	39	0.052
III, IV	10	10	

AJCC, American Joint Committee on Cancer; SD, standard deviation

Supplementary Table S2. Primer sequences for qRT-PCR

Gene symbol	Primer sequence
<i>ACTB</i>	Sense: 5'-ATTGCCGACAGGATGCAGA-3' Antisense: 5'-GAGTACTTGCGCTCAGGAGGA-3'
<i>BAX</i>	Sense: 5'-GGACGAACTGGACAGTAACATGG-3' Antisense: 5'-GCAAAGTAGAAAAGGGCGACAAC-3'
<i>BCL2</i>	Sense: 5'-ATCGCCCTGTGGATGACTGAG-3' Antisense: 5'-CAGCCAGGAGAAATCAAACAGAGG-3'
<i>CCND1</i>	Sense: 5'-GCTGCGAAGTGGAACCATC-3' Antisense: 5'-CCTCCTTCTGCACACATTTGAA-3'
<i>C-MYC</i>	Sense: 5'-GAGGCGAACACACAACGTCTT-3' Antisense: 5'-CGCAACAAGTCCTCTTCAGAAA-3'
<i>HER3</i>	Sense: 5'-CTATGAGGCGATACTTGGAACGG-3' Antisense: 5'-GCACAGTTCCAAAGACACCCGA-3'

Supplementary Table S3. Primary antibodies used for western blotting

Antibody	Host	Manufacturer	Catalog no.	Dilution
Anti-human β -actin	Rabbit	Cell Signaling Technologies	#4970	1:2,000
Anti-Akt	Rabbit	Cell Signaling Technologies	#9272	1:1,000
Anti-pAkt	Rabbit	Cell Signaling Technologies	#4969	1:2,000
Anti-ERK	Rabbit	Cell Signaling Technologies	#9102	1:1,000
Anti-pERK	Rabbit	Cell Signaling Technologies	#4370	1:2,000
Anti-HER3/ErbB3	Rabbit	Cell Signaling Technologies	#12708	1:1,000
Anti-pHER3(Tyr1197)	Rabbit	Cell Signaling Technologies	#4561	1:1,000
Anti-JNK	Rabbit	Cell Signaling Technologies	#9258	1:1,000
Anti-pJNK	Rabbit	Cell Signaling Technologies	#4668	1:1,000
Anti-p38 MAPK	Rabbit	Cell Signaling Technologies	#8690	1:1,000
Anti-p-p38 MAPK	Rabbit	Cell Signaling Technologies	#4511	1:1,000
Anti-Src	Rabbit	Cell Signaling Technologies	#2123	1:1,000
Anti-pSrc	Rabbit	Cell Signaling Technologies	#2101	1:1,000