

Table SI. Patient characteristics of the validation dataset.

Characteristics	ARMS	ERMS
Sex		
Male	3	6
Female	7	4
Age (years)		
Mean	12.3	3.6
SD	(± 3.4)	(± 2.8)
Survival (years)		
Mean	3.6	4.7
SD	(± 0.9)	(± 3.6)
Event-free survival(years)		
Mean	1.5	3.8
SD	(± 1.2)	(± 3.9)
Tumor size		
≤ 5 cm	1	2
> 5 cm	8	8
Not known	1	

RMS,rhabdomyosarcoma; ARMS, alveolar RMS; ERMS, embryonal RMS.

Table SII. Antibodies.

Antibody	Dilution	Application
Primary antibodies for western blotting		
mAb rabbit anti- β -Actin, Cell Signaling Technology, Inc.(cat. no. 4970)	1:10,000	Western blotting
mAb rabbit anti-non-phospho- β -Catenin (Ser33/37/Thr41), Cell Signaling Technology, Inc. (cat. no. 8814)	1:1,000	Western blotting
mAb rabbit anti-Frizzled5, Cell Signaling Technology, Inc. (cat. no. 5266)	1:1,000	Western blotting
pAb rabbit anti-ROR1, Cell Signaling Technology, Inc. (cat. no. 4102)	1:1,000	Western blotting
pAb rabbit anti-SP1, Cell Signaling Technology, Inc. (cat. no. 5931)	1:1,000	Western blotting
mAb rabbit anti-Wnt5a, Cell Signaling Technology, Inc.(cat. no. 2530)	1:1,000	Western blotting
mAb mouse anti-Wnt5a, Thermo Fisher Scientific, Inc. (cat. no. MA5-15511)	1:500	Immunohistochemical staining
mAb mouse anti-GAPDH, Cell Signaling Technology, Inc. (cat. no. 97166)	1:1,000	Western blotting
pAb rabbit anti-Myogenin, Thermo Fisher Scientific, Inc. (cat. no. PA5-110007)	1:1,000	Western blotting
mAb rabbit anti-MyoD1, Abcam (cat. no. ab133627)	1:1,000	Western blot
mAb mouse anti-Desmin, Leica Biosystems (cat. no. DES-DERII)	1:1,000	Western blotting
pAb rabbit anti-Frizzled2, Thermo Fisher Scientific, Inc. (cat. no. 38-4700)	1:1,000	Western blotting
pAb rabbit anti-ROR2, Thermo Fisher Scientific, Inc. (cat. no. PA5-14727)	1:1,000	Western blotting
Secondary antibodies for Western blotting ^a		
pAb goat anti-rabbit IgG/HRP, Jackson ImmunoResearch Laboratories, Inc. (cat. no. 111-035-045) **	1:10,000	Western blotting
pAb rabbit anti-mouse IgG/HRP, Jackson ImmunoResearch Laboratories, Inc. (cat. no. 315-035-003) **	1:10,000	Western blotting
pAb goat anti-rabbit IgG/HRP, Cell Signaling Technology, Inc. (cat. no. 7074) **	1:1,000	Western blotting
pAb horse anti-mouse IgG/HRP, Cell Signaling Technology, Inc. (cat. no. 7076) **	1:1,000	Western blotting

^aSignals were visualized using the ECL plus detection system (Cytiva).HRP, horseradish peroxidase;mAb, monoclonal antibody; pAb, polyclonal antibody.

Table SIII. Primers and short hairpin RNA.

Name	Orientation	Sequence
AXIN2	Forward Reverse	GCCAACGACAGTGAGATATCC CTCGAGATCAGCTCAGCTGCA
B2M	Forward Reverse	ACTCTCTCTTTCTGGCCTGG GACAAGTCTGAATGCTCCACT
CD133	Forward Reverse	GCTGACCCTCATCGTGATTT TTCCACATTTGCACCAAAGA
cMYC	Forward Reverse	GTAGTGGAAAACCAGCAGCC CCTCCTCGTCGCAGTAGAAA
CTNNB1	Forward Reverse	GAAACGGCTTTTCAGTTGAGC CTGGCCATATCCACCAGAGT
FZD2	Forward Reverse	CATCATGCCCAACCTTCT GGCTTCGCAGCCCT
FZD4	Forward Reverse	CGAGCTGCAGCTGACAACTT TGGTCGTTCTGTGGTGGGAA
FZD5	Forward Reverse	CCCAGCGTCAAGTCCAT TTGATGACGCTGCGGAT
GAPDH	Forward Reverse	TGCACCACCAACTGCTTAGC GGCATGGACTGTGGTCATGAG
ROR1	Forward Reverse	AATGATGCTCCTGTGGTCCAG GCACATGCAATCCCTCTGTAT
ROR2	Forward Reverse	AACCGGACCATTTATGTGGA ACACGAAGTGGCAGAAGGAT
RPL13A	Forward Reverse	CCTGGAGGAGAAGAGGAAAGAGA TTGAGGACCTCTGTGTATTTGTCAA
TBP	Forward Reverse	AGTTCTGGGATTGTACCGCA TGTGCACACCATTTTCCCAG
SOX2	Forward Reverse	AGCTCGCAGACCTACATGAA TGGAGTGGGAGGAAGAGGTA
WNT5A	Forward Reverse	TGGCTTTGGCCATATTTTTC CCGATGTACTGCATGTGGTC
WNT5A-specific short hairpin RNA		GGACGTTAAGAGATATTCAA

Table SIV. Differences of *WNT5A* and *WNT5A* receptors (and *CTNNB1*) expression levels in ARMS and ERMS from two publically available microarray datasets [dataset 1(1) and dataset 2(2)]. In the validation dataset, gene expression levels were investigated by reverse transcription-quantitative PCR with primers listed in Table S3. Significant P-values are in bold.

	dataset 1			dataset 2			Validation dataset		
	ARMS (n=37)	ERMS (n=66)	P-value	ARMS (n=34)	ERMS (n=36)	P-value	ARMS (n=10)	ERMS (n=10)	P-value
<i>WNT5A</i>	2.30	2.35	0.0068	2.20	2.68	<0.0001	1.69	3.40	0.0185
<i>FZD2</i>	2.49	2.59	0.0007	2.43	2.66	0.0001	0.69	2.95	0.0002
<i>FZD4</i>	2.78	2.96	0.0001	2.26	2.48	<0.0001	0.28	8.68	0.0068
<i>FZD5</i>	2.31	2.32	0.1046	1.67	1.69	0.3171	1.25	1.24	0.9705
<i>ROR1</i>	2.40	2.41	0.0173	1.63	1.80	<0.0001	2.21	5.94	0.0630
<i>ROR2</i>	2.31	2.32	0.0093	1.50	1.49	0.2878	274.4	79.94	0.0630
<i>CTNNB1</i>	3.18	3.18	0.8254	2.48	2.360	0.0593	61.39	74.12	0.3150

References

1. Davicioni E, Anderson MJ, Finckenstein FG, et al.: Molecular classification of rhabdomyosarcoma--genotypic and phenotypic determinants of diagnosis: a report from the Children's Oncology Group. *The American journal of pathology* 174: 550-564, 2009.
2. Williamson D, Missiaglia E, de Reynies A, et al.: Fusion gene-negative alveolar rhabdomyosarcoma is clinically and molecularly indistinguishable from embryonal rhabdomyosarcoma. *J Clin Oncol* 28: 2151-2158, 2010.