

SUPPLEMENTARY DATA

Functional Consequences of C-Terminal Mutations in *RUNX2*

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	Arg131	Pro279	Asp287	Thr420	
	↓	↓	↓	↓	
<i>Homo sapiens</i>	SHWRCNK	PFNPQGQSQITDPRQ		HYHTYLP	NP_001019801.3
<i>Mus musculus</i>	SHWRCNK	PFNPQGQSQITDPRQ		---TVPA	XP_006523603.1
<i>Danio rerio</i>	SHWRCNK	SFNPQGQTQISDPRQ		HYHTYLP	NP_998023.1
<i>Rattus norvegicus</i>	SHWRCNK	PFNPQGQSQITDPRQ		HYHTYLP	XP_006244611.1
<i>Caenorhabditis elegans</i>	KHWRSNK	SF-----		IFIT---	NP_491679.1
<i>Xenopus tropicalis</i>	SHWRCNK	PFNPQGQSQIADPRQ		HYHTYLP	NP_001128588.1
<i>Drosophila melanogaster</i>	NHWRSNK	AGQP--AAMMPSPG		-----	NP_001285501.1

Fig S1. Amino acid conservations of p.Arg131, Pro279, Asp287, and Thr420 among several species.

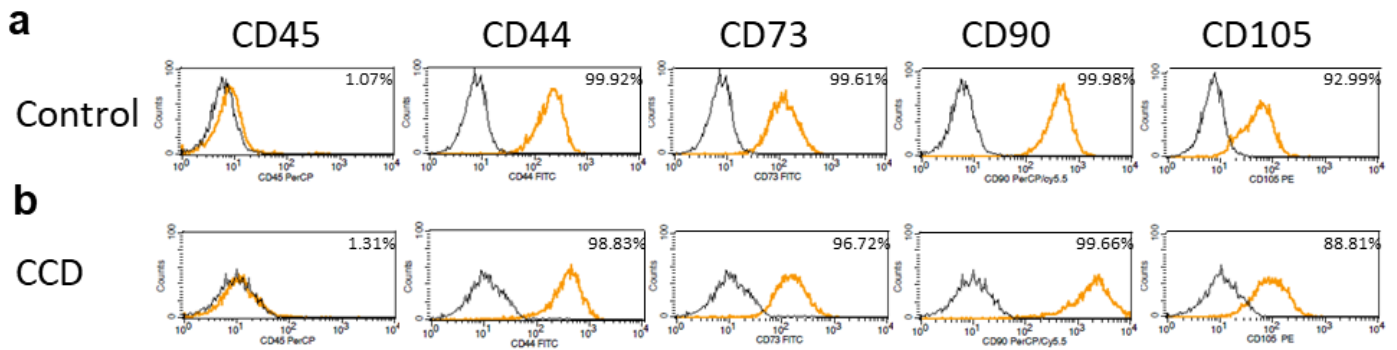


Fig S2. Flow cytometric analysis of aBMSCs marker expression. a, b. CCD and control cells were positive for CD44, CD73, CD90, and CD105, but negative for CD45.

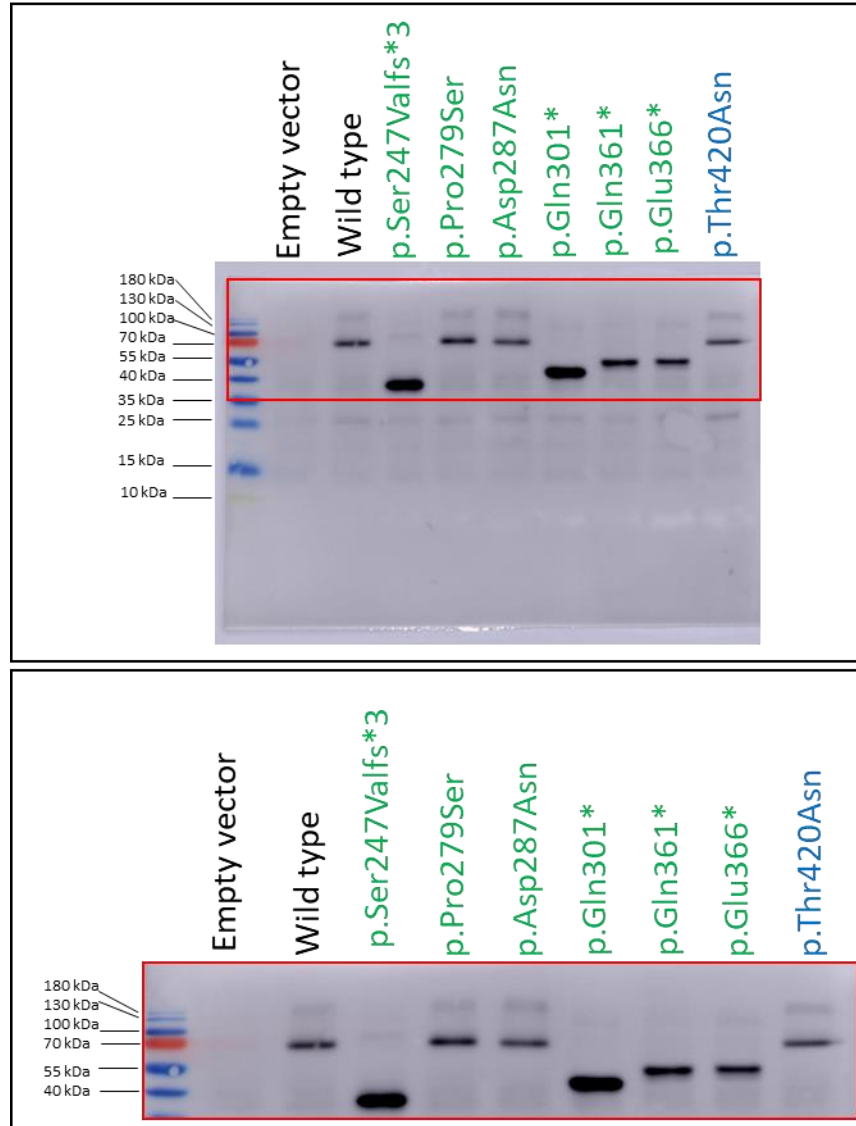


Fig S3. Full length blot of RUNX2 expression in empty, wildtype, and mutants (p.Ser247Valfs*3, p.Pro279Ser, p.Asp287Asn, p.Gln301*, p.Gln361*, p.Glu366*, and p.Thr420Asn) vectors transfected in HEK293 cells. Red boxes indicate the cropped blot shown in Figure 1c.

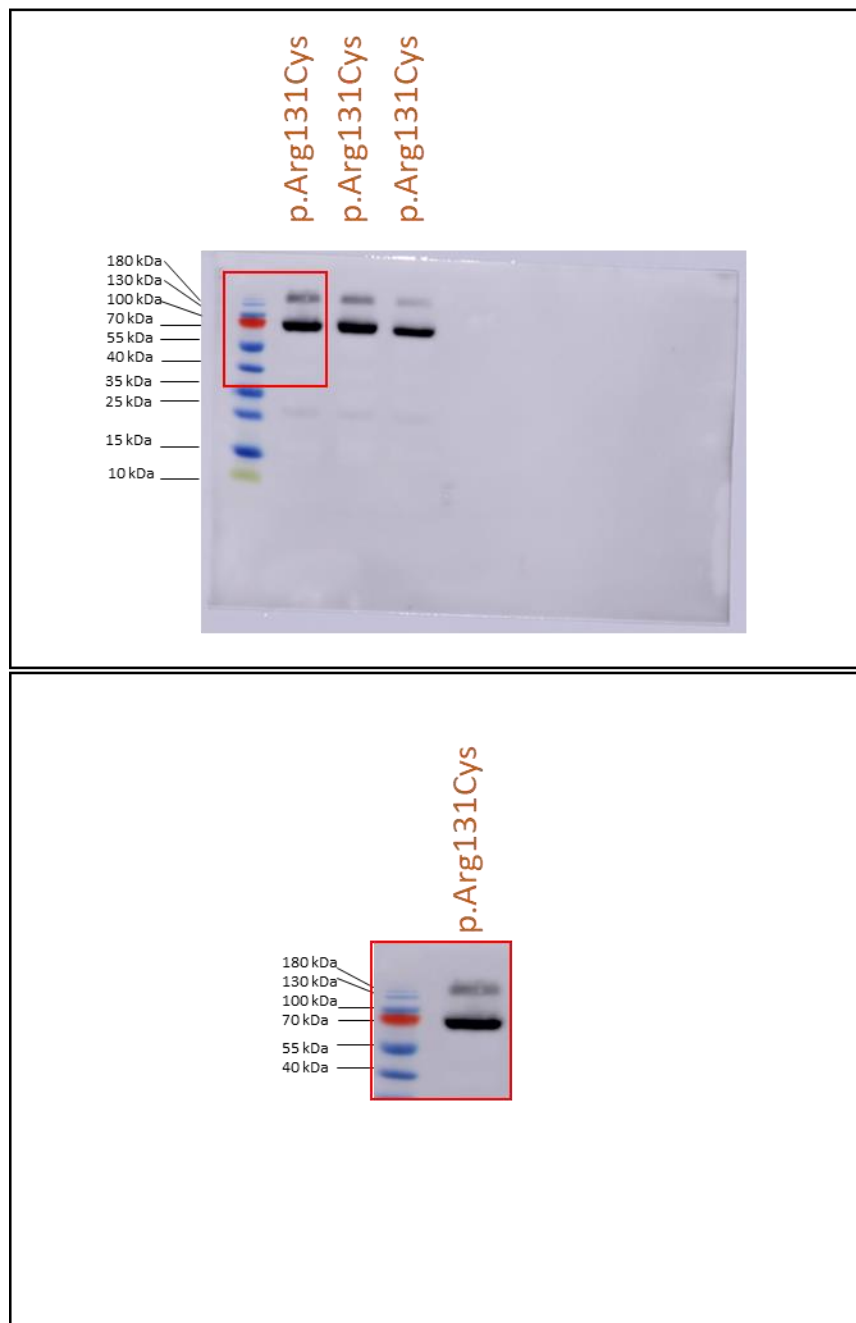


Fig S4. Full length blot of RUNX2 expression in mutants (p.Arg131Cys) vectors transfected in HEK293 cells. Red boxes indicate the cropped blot shown in Figure 1c.

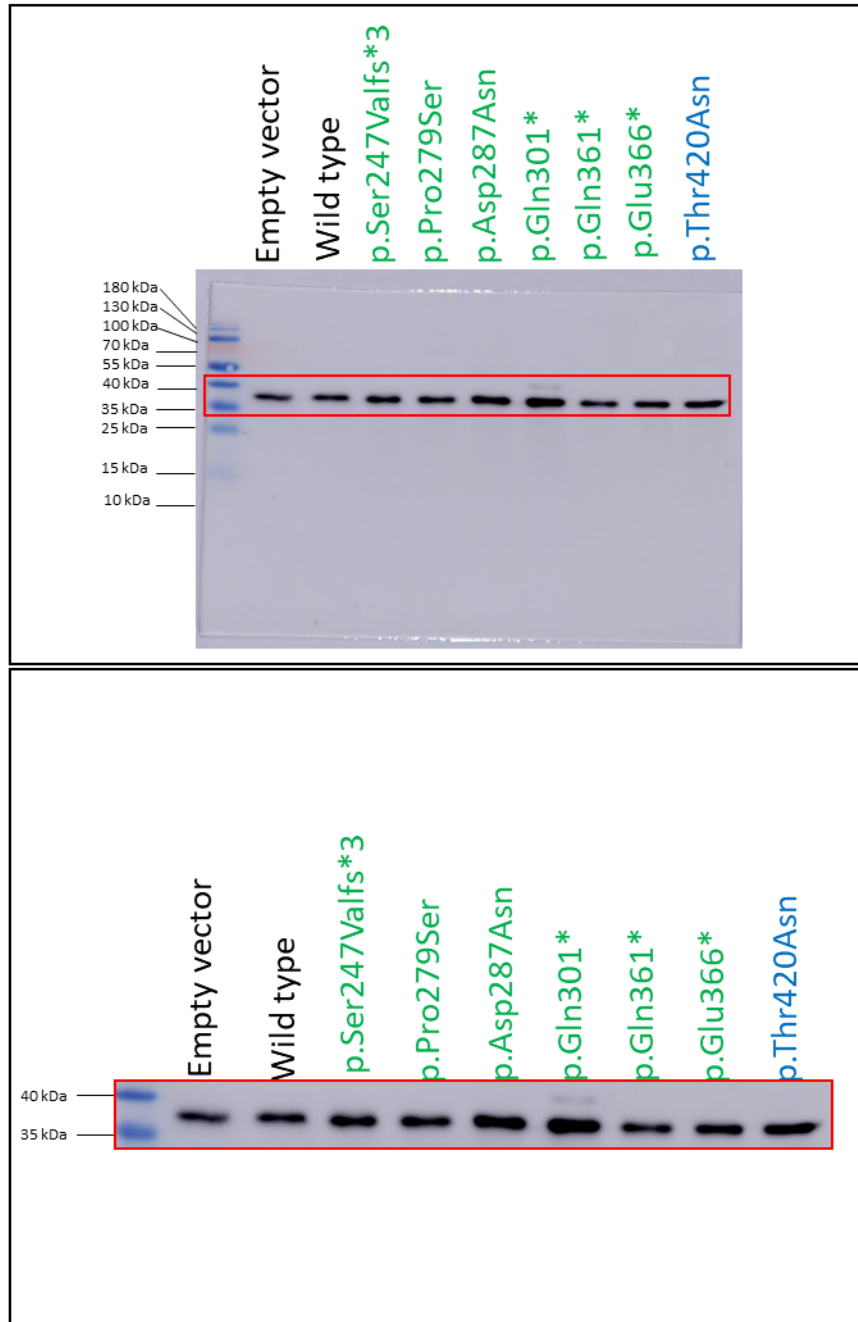


Fig S5. Full length blot of GAPDH expression in empty, wildtype, and mutants (p.Ser247Valfs*3, p.Pro279Ser, p.Asp287Asn, p.Gln301*, p.Gln361*, p.Glu366*, and p.Thr420Asn) vectors transfected in HEK293 cells. Red boxes indicate the cropped blot shown in Figure 1c.

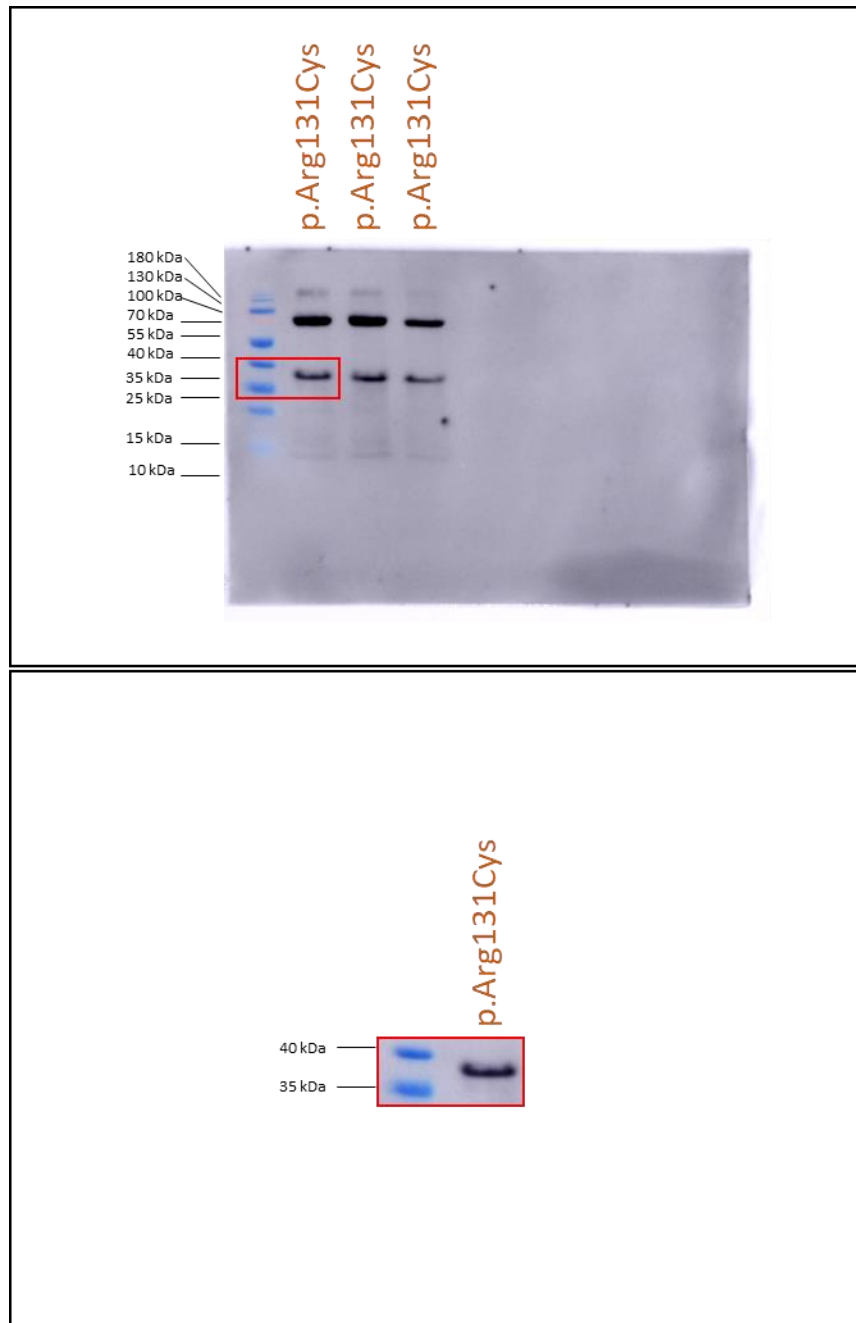


Fig S6. Full length blot of GAPDH expression in mutants (p.Arg131Cys) vectors transfected in HEK293 cells. Red boxes indicate the cropped blot shown in Figure 1c.

Table S1. Primers used for site-directed mutagenesis.

Variants	Primers	Sequence 5'> 3'	Annealing temperature [°C]
c.A739del p.Ser247Valfs*3	Forward Reward	TCTCTGACCGCCTCGTGATTTAGGGC GC GAGGCGGTCAGAGAACAA	60.6
c.835C>T p.Pro279Ser	Forward Reward	TCCTTTTAATTCACAAGGACAGAG CTTGGTGCAGAGTTCAGG	53.0
c.859G>A p.Asp287Asn	Forward Reward	TCAGATTACAAACCCAGGCA CTCTGTCCTTGTGGATTAAGG	54.9
c.901C>T p.Gln301*	Forward Reward	GTCCTATGACTAGTCTTACCCCTCC CACGGCGGGGAAGACTGT	58.5
c.1081C>T p.Gln361*	Forward Reward	TAAGAAGAGCTAGGCAGGTGC CTGAGAGTGGAAGGCCAG	56.0
c.1096 G>T p.Glu366*	Forward Reward	AGGTGCTTCATAACTGGGCCC GCCTGGCTCTTCTTACTG	55.5
c.1259C>A p.Thr420Asn	Forward Reward	CACTACCACAACTACCTGCCA AGTGGTGGCGGACATACC	57.0

Table S2. Primers used for Sanger sequencing to validate the mutagenesis.

Primer	Sequence 5'> 3'	Annealing temperature [°C]
Forward Reward	GCGCATTCTCATCCCAGTA GGCTCAGGTAGGAGGGGTAA	60

Table S3. Primers used for Sanger sequencing of the alveolar bone mesenchymal stem cells (aBMSC) obtained from the CCD patient harboring the p.Ser247Valfs*3 mutation.

Primer	Sequence 5'> 3'	Annealing temperature [°C]
Forward	TGGCCACCAGATACCGCTTA	60
Reward	ATAAGCCGCTTCACAGCTCC	

Table S4. Primers used for real-time PCR.

Genes	Primers	Primer Sequence (5' > 3')	Annealing temperature [°C]
<i>ALP</i>	Forward	CGA GAT ACA AGC ACT CCC ACT TC	60
	Reverse	CTG TTC AGC TCG TAC TGC ATG TC	
<i>COL1A1</i>	Forward	CTG GCA AAG AAG GCG GCA AA	60
	Reverse	CTC ACC ACG ATC ACC ACT CT	
<i>OCN</i>	Forward	CTT TGT GTC CAA GCA GGA GG	60
	Reverse	CTG AAA GCC GAT GTG GTC AG	
<i>RUNX2</i>	Forward	ATG ATG ACA CTG CCA CCT CTG A	60
	Reverse	ATG GAG GGC GGA TTG GAA A	
<i>β-actin</i>	Forward	TGG AAC GGT GAA GGT GAC AG	60
	Reverse	AAC AAC GCA TCT CAT ATT TGG AA	