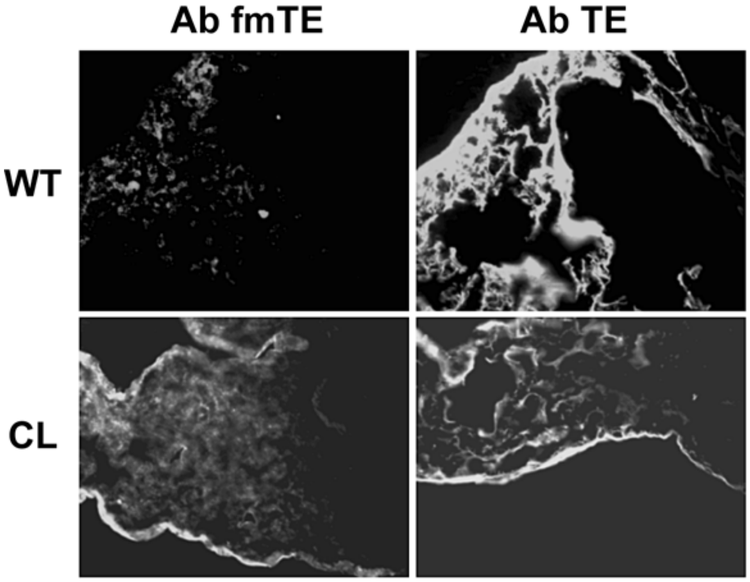
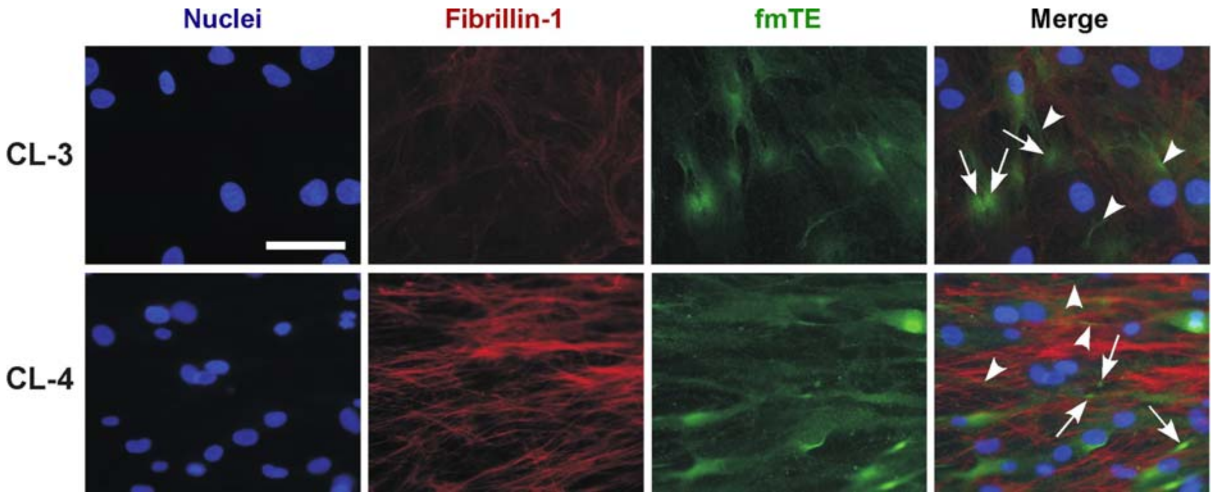
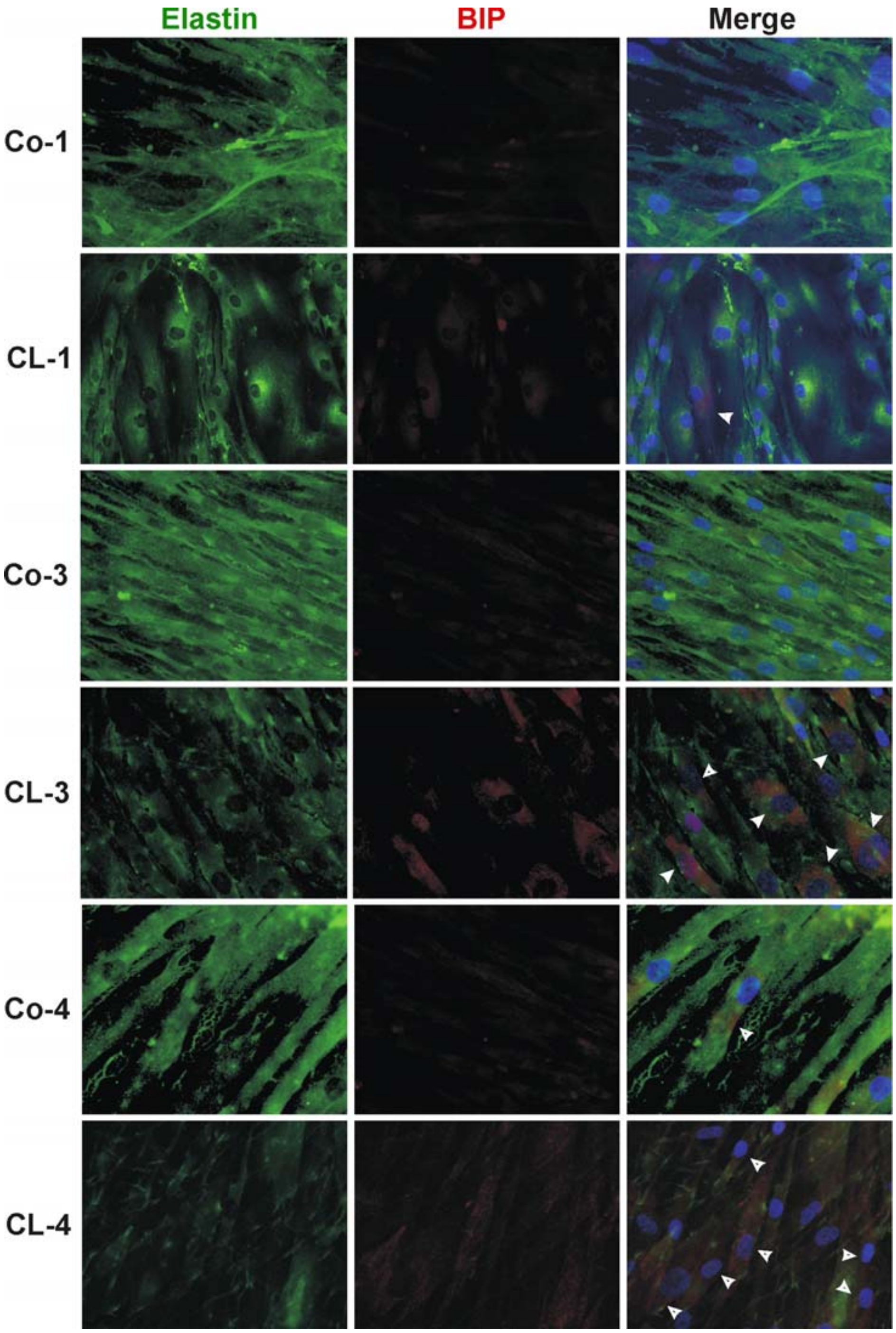


Supp. Figure S1. Specific detection of mutant elastin by the fmTE antibody. Frozen lung sections of transgenic mice expressing fmTE from a bacterial artificial chromosome transgene (CL) and control wildtype (WT) mice were stained with antibodies against fmTE and against normal tropoelastin (TE). The fmTE antibody reacted only the mutant transgenic tissue (CL) whereas the TE antibody reacted with both mutant and wild type tissue.



Supp. Figure S2. Colocalization immunofluorescent staining for fibrillin-1 (red) and fmTE (green) with a polyclonal antibody against a C-terminal epitope of -1 frameshift mutant elastin in permeabilized fibroblast cultures from both patients CL-3 and CL-4 showed more incorporation of mutant elastin into globules and less deposition into elastic fibers than TE. Arrows: clumps of elastin; arrowhead: elastic fiber. Nuclei were stained blue. Scale bar: 25 µm.



Supp. Figure S3. Immunofluorescent staining for elastin (green) and BiP (red) of permeabilized fibroblast cultures of patients (CL-1, CL-3, and CL-4) and matching controls (Co-1, Co-3, Co-4). Arrowheads show double positive cells in merged images. Nuclei were counterstained blue.

Supp. Table S1. Primers and PCR conditions for gDNA sequencing of Elastin exon 28-34

Primers ELN ex. 28-34 (gDNA)		**PCR method**
ELN_Ex28_F	gacccatcgttcagaaatgg	TD 62 - 50°C
ELN_Ex28_R	agggaggcactctgtactgg	TD 62 - 50°C
ELN_Ex29_F	cagagtgcctccctgaactc	TD 62 - 50°C
ELN_Ex29_R	gtcagaagctcctcccacac	TD 62 - 50°C
ELN_Ex30_F	tcagtcccacctttctgacc	TD 62 - 50°C
ELN_Ex30_R	ctcgcatacacacaccacac	TD 62 - 50°C
ELN_Ex31_F	attcctgagccgtcatgtg	TD 62 - 50°C
ELN_Ex31_R	cactggcaggaaggcatc	TD 62 - 50°C
ELN_Ex32_F	tcttatcctgaccccacctg	TD 62 - 50°C
ELN_Ex32_R	ggagtccccactgctagatg	TD 62 - 50°C
ELN_Ex33_F	tgcaggcagaaagtgatgag	TD 62 - 50°C
ELN_Ex33_R	gagatggcacaggagaggag	TD 62 - 50°C
ELN_Ex34_F	attcgagtgggtcagagcag	TD 62 - 50°C
ELN_Ex34_R	cgttgatgaggtcgtgagtc	TD 62 - 50°C

TD: touch-down PCR.
Each PCR mix contains 14 µl H2O, 2µl of 10 µM forward primer, 2µl of 10 µM reverse primer, 2 µl of 10x supermix (Invitrogen), and 0.05 µl of platinum Taq polymerase (Invitrogen).

Supp. Table S2. Antibodies used in this study

Antigen	Host (type)	Label	Conc.	Provider	Cat. #
TE/elastin	Rabbit (polyclonal)	-	1:200	Dr. Mecham*	
fmTE	Rabbit (polyclonal)	-	1:200	Dr. Mecham	
Fibrillin-1	Mouse (monoclonal)	-	1:500	Chemicon, Billerica, MA	MAB1919
BiP	Mouse (monoclonal)	-	1:1000	BD Bioscience, Franklin Lakes, NJ	610978
peIF2α	Rabbit (polyclonal)	-	1:500	Cell Signaling Technology, Danvers, MA	3597
Caspase-3	Rabbit (monoclonal)	-	1:200	Cell Signaling Technology	9664
pSMAD2	Rabbit (polyclonal)	-	1:500	Cell Signaling Technology	3101S
rabbit IgG	Donkey (monoclonal)	AlexaFluor 488 (red)	1:500	Invitrogen, Carlsbad, CA	A21203
Mouse IgG	Donkey (monoclonal)	AlexaFluor 594 (green)	1:500	Invitrogen, Carlsbad, CA	A21206

Conc.: concentration
*: Washington University School of Medicine, St-Louis, MO
Cat. #: catalog number

Supp. Table S3. Relative abundance of *ELN* transcript species

	Without CHX					**With CHX**				
	Exon 32 skip		Full length		Total (%)	Exon 32 skip		Full length		Total (%)
Patient	WT (%)	MT (%)	WT (%)	MT (%)		WT (%)	MT (%)	WT (%)	MT (%)	
Control	**69.3**	**-**	**30.7**	**-**	**100**	**62.9**	**-**	**37.1**	**-**	**100**
(SD)	(8.2)		(8.2)			(6.7)		(6.7)		
CL-3	**22.4**	**63.3**	**7.2**	**7.1**	**100**	**27**	**51**	**11.2**	**10.9**	**100**
(SD)	(2.1)	(4.0)	(1.1)	(1.4)		(3.0)	(5.7)	(1.6)	(1.3)	
CL-4	**36.1**	**-**	**9.6**	**54.3**	**100**	**40.5**	**-**	**11.3**	**48.2**	**100**
(SD)	(3.1)		(0.8)	(3.7)		(1.1)		(0.5)	(0.8)	
CL-1	**17.6**	**49.8**	**6.8**	**25.8**	**100**	**23.6**	**43.7**	**9.2**	**23.5**	**100**
(SD)	(0.8)	(2.6)	(0.3)	(1.6)		(1.7)	(2.9)	(0.7)	(0.7)	

CHX: cycloheximide
WT: wild type allele
MT: mutant allele
SD: standard deviation of the mean

Supp. Table S4. Aortic involvement in all published ADCL patients to date with an N-terminal *ELN* mutation

	ARD	**No ARD**	**All patients**
Ex. 32 mutations	0	4	4
Mutations outside ex. 32	9	1	10
All mutations	9	5	14

ARD: Aortic root dilatation.
Fisher's exact test: $p = 0.005$
3 patients with unknown clinical data and 3 patients with a chromosomal rearrangement were excluded from the analysis.