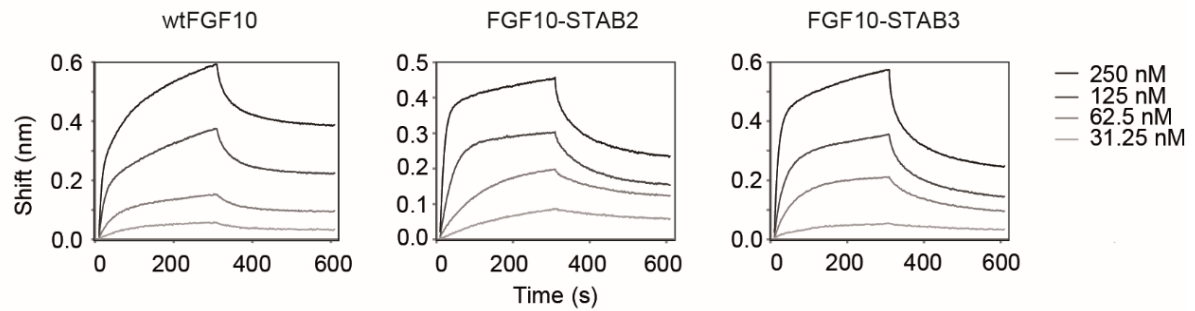


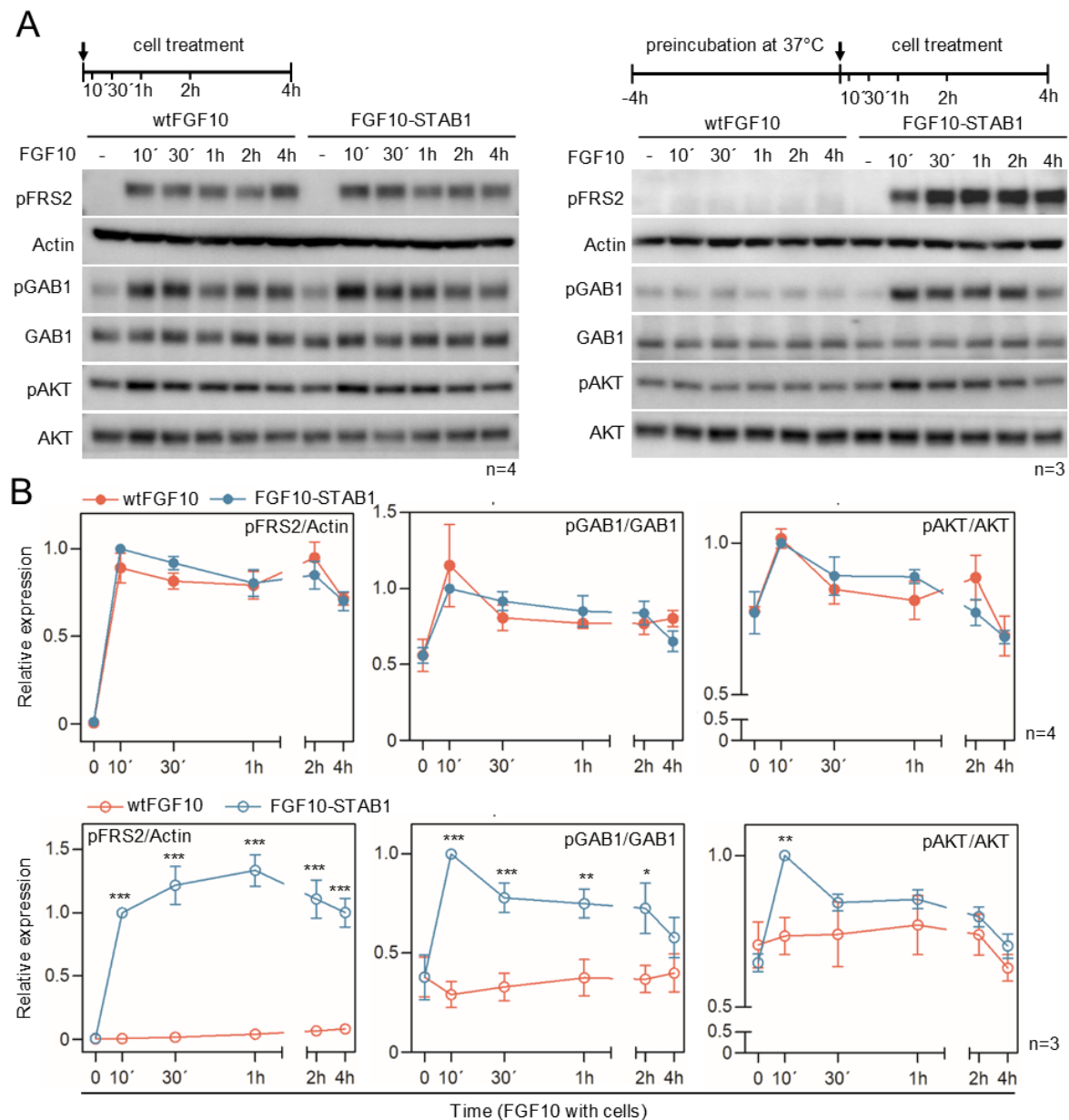
Supplementary information

Increased thermal stability of FGF10 leads to ectopic signaling during development



Protein	K_D [nM]
wtFGF10	764 ± 132
FGF10-STAB2	996 ± 380
FGF10-STAB3	913 ± 449

Figure S1 FGFR2b interaction kinetics of wildtype FGF10, FGF10-STAB2 and FGF10-STAB3 analyzed by biolayer interferometry. Recombinant extracellular domain of FGFR2b fused to the Fc fragment was immobilized on a Protein A biosensor. Dissociation constants were calculated from fitted saturation binding curves. Representative sensograms for three (n) independent experiments are shown.



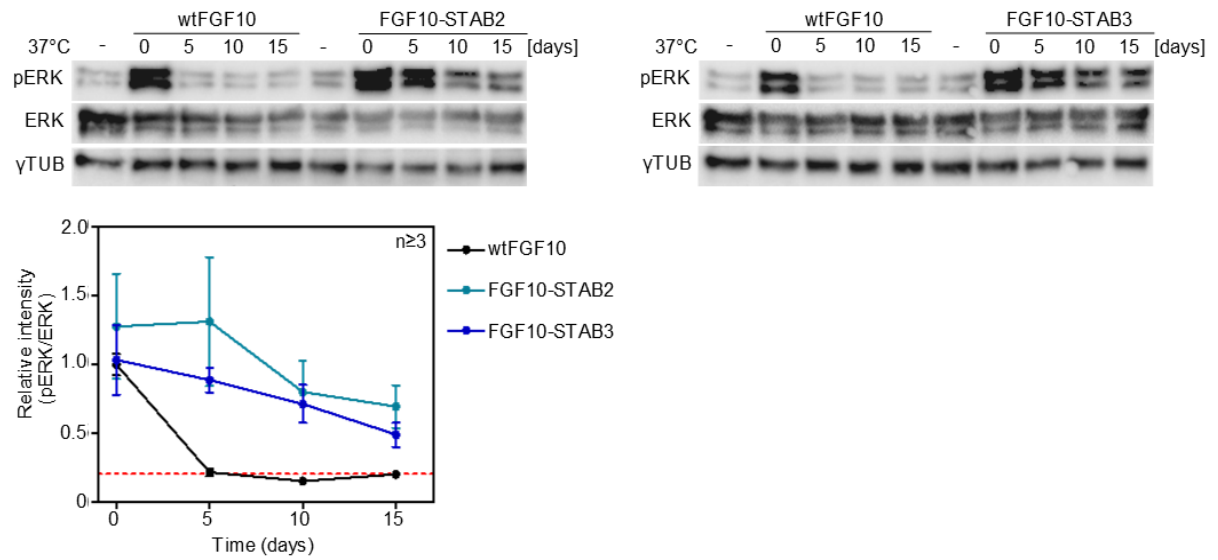


Figure S3 Recombinant FGF10 (0.5 μ g/ml) was incubated at 37°C in DMEM supplemented with 10% FBS for 5-15 days. MCF7 cells were stimulated with preincubated FGF10 (20 ng/ml) for 15 minutes and analyzed for activation of ERK pathway. Data are representative for at least three independent experiments (n). pERK signal was quantified and normalized to total ERK (mean $\pm$ SD for at least three independent experiments).

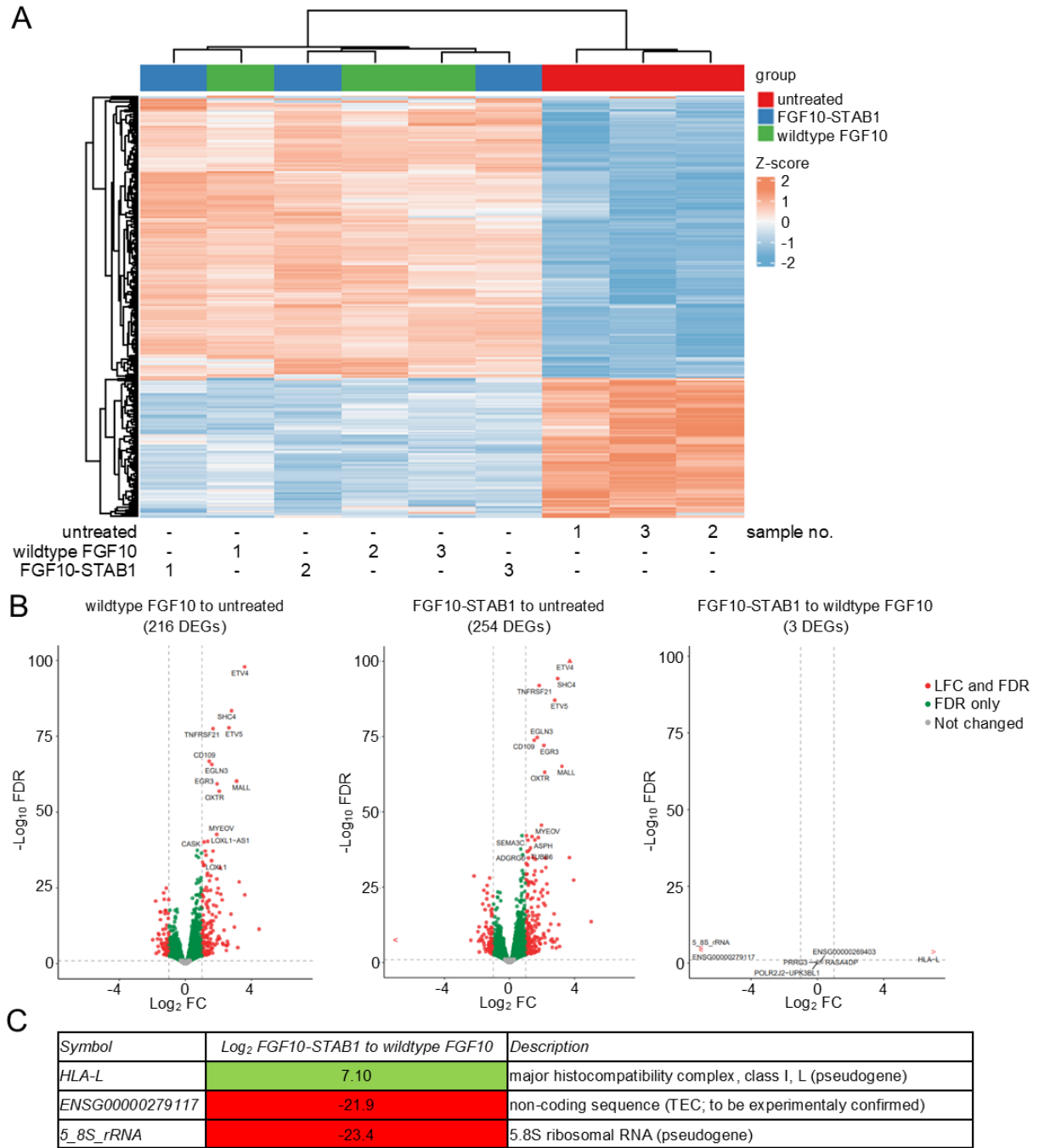


Figure S4 Differential expression analysis between MCF7 cells treated with wildtype FGF10 or FGF10-STAB1 and untreated cells. MCF7 cells were serum starved for two days, treated with wildtype FGF10 or FGF10-STAB1 (20 ng/ml) for 8 hours, and analyzed for changes in gene expression. Three independent cell samples were analyzed in each group. Compared to untreated controls, wildtype FGF10 regulated expression of 216 genes (175 protein-coding genes), while FGF10-STAB1 modulated the expression of 254 genes (201 protein-coding genes). (A) Expression heatmap of genes differentially expressed in at least one of the comparisons shows the minimal difference in induced gene expression changes between wildtype FGF10 and FGF10-STAB1. (B) Results of differential expression analysis for individual genes (points) showed as volcano plot. DEG, differentially expressed gene; LFC, $\log_2$ fold changes; FDR, false discovery rate. (C) Only three non-coding transcripts were found differentially expressed between FGF10-STAB1 and wildtype FGF10.

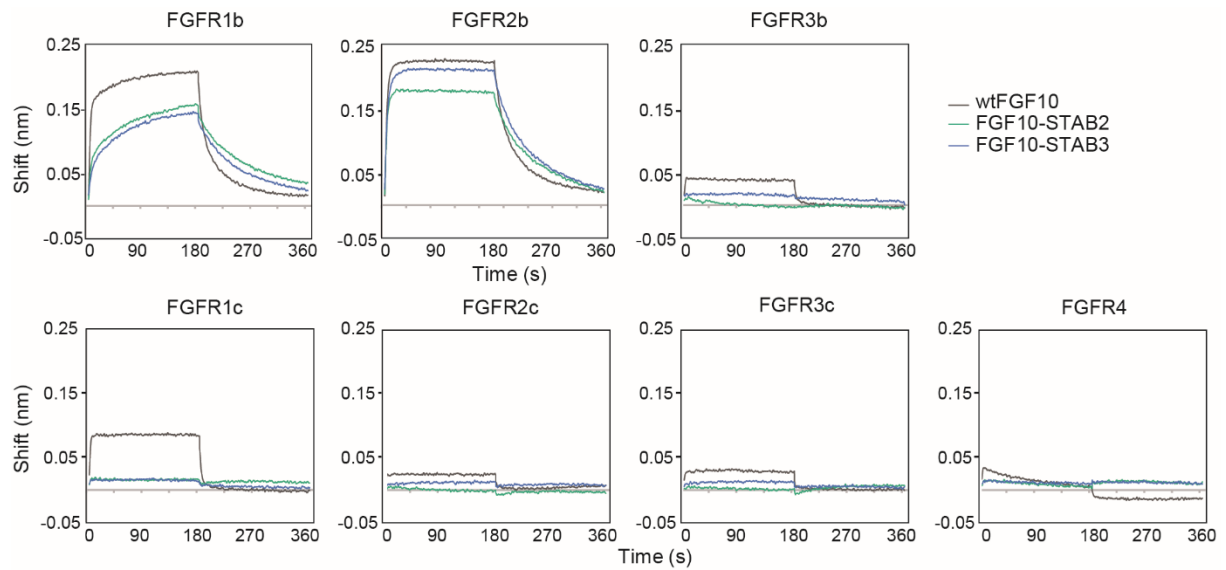


Figure S5 FGFR specificity analysis of wild type of FGF10, FGF10-STAB2 and FGF10-STAB3 variants designed by semi-rational approach assessed by biolayer interferometry (BLI) technique. BLI experiments were performed on an Octet K2 instrument (Sartorius AG) using Protein A biosensors. Recombinant extracellular domains of individual FGFRs (isoforms IIIb and IIIc) fused to the Fc fragment were immobilized on a Protein A biosensor. FGFR coupled to the sensor was then used to capture the analyzed FGF10 variants at a concentration of 250 nM. Association and dissociation processes were monitored for 180 seconds each. ForteBio's Data Analysis 11.0 software was used to analyze the binding curves. Representative sensograms are shown (n=3).

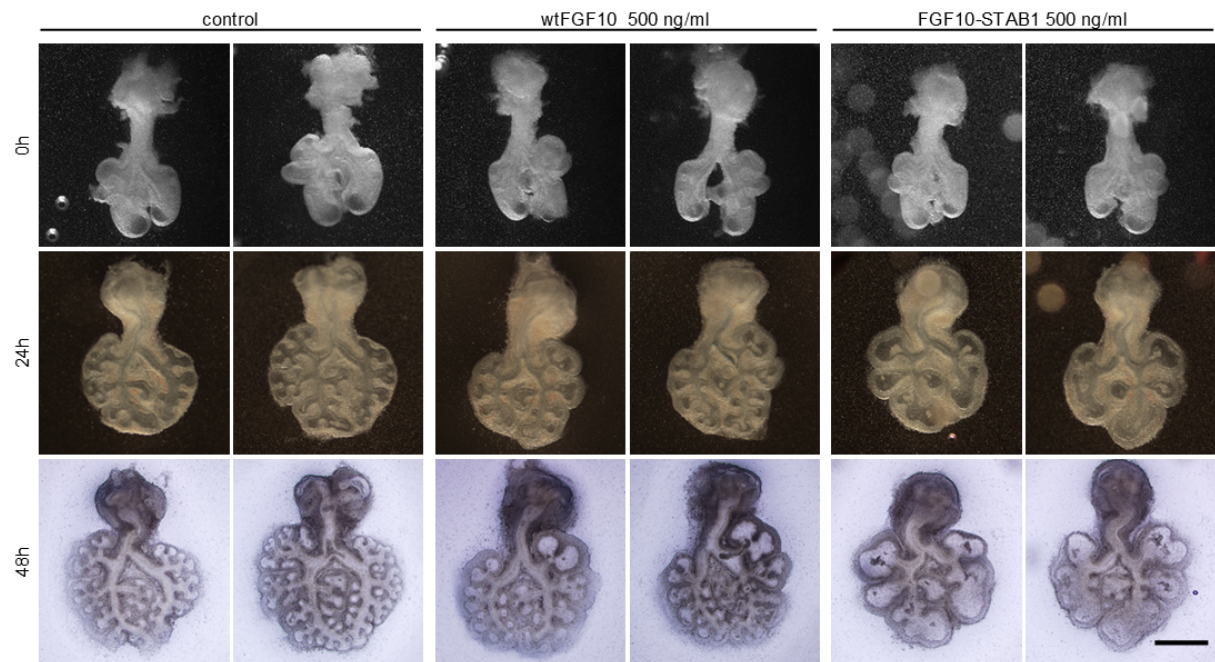


Figure S6 FGF10-STAB1 alters cell differentiation in embryonic lung explants. E11.25 mouse lungs were explanted and incubated for up to 48 hours with 500 ng/ml FGF10 added every 12-24 hours. Overall morphology of lung explants reveals significant suppression of branching by FGF10-STAB1, while only small effect was found with 500 ng/ml of wildtype FGF10 (scale bar, 0.5 mm). Two explants are shown for each treatment.

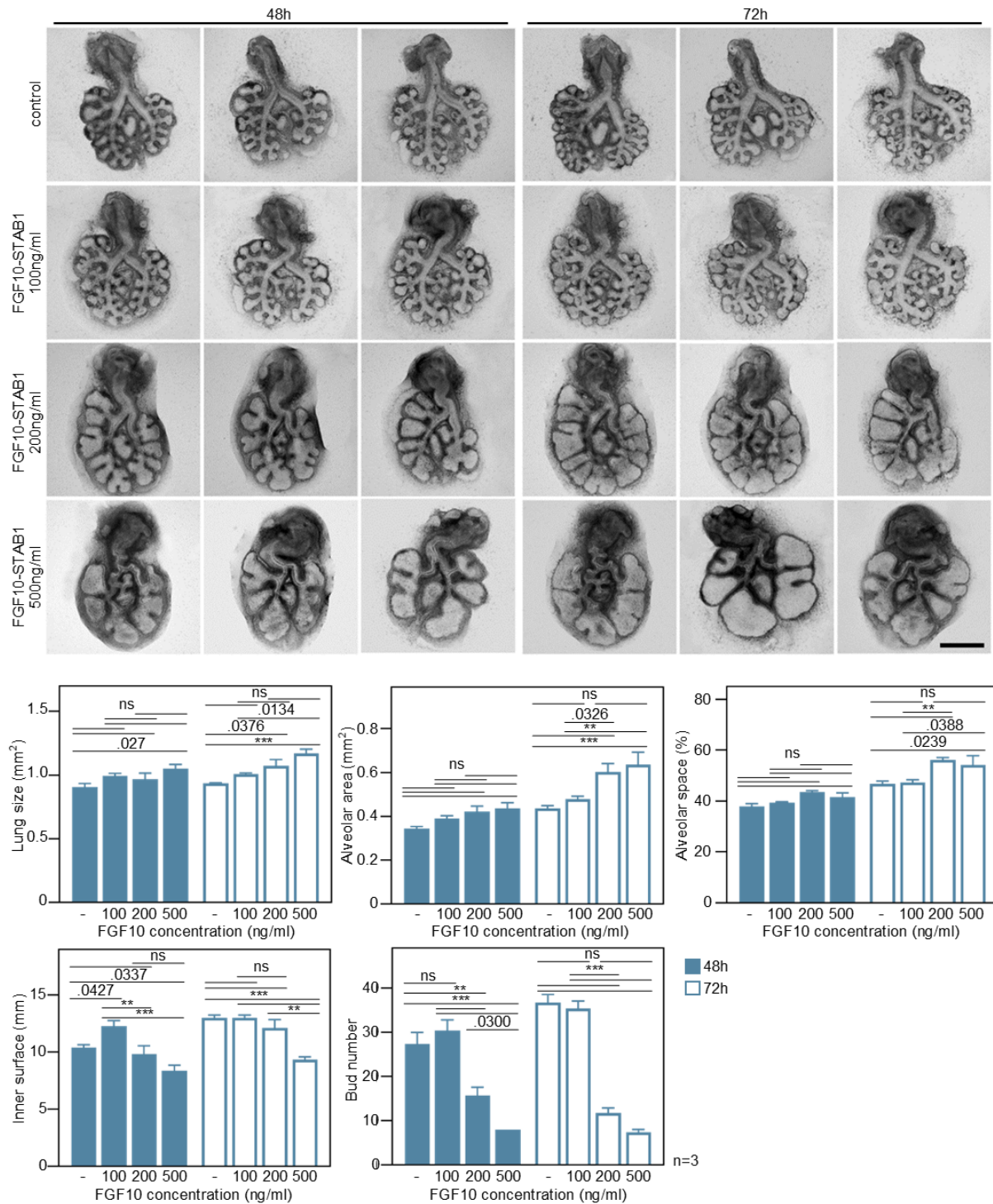


Figure S7 FGF10-STAB1 alters cell differentiation in embryonic lung explants. E11.25 mouse lungs were explanted and incubated for up to 48 and 72 hours with FGF10 added every 12-24 hours. The overall morphology of lung explants reveals significant suppression of branching by FGF10-STAB1, apparent at 200 and 500 ng/ml (scale bar, 0.5 mm). Three explants are shown for each treatment (mean±SEM, Two-Way ANOVA, ***p<0.001, **p<0.01).

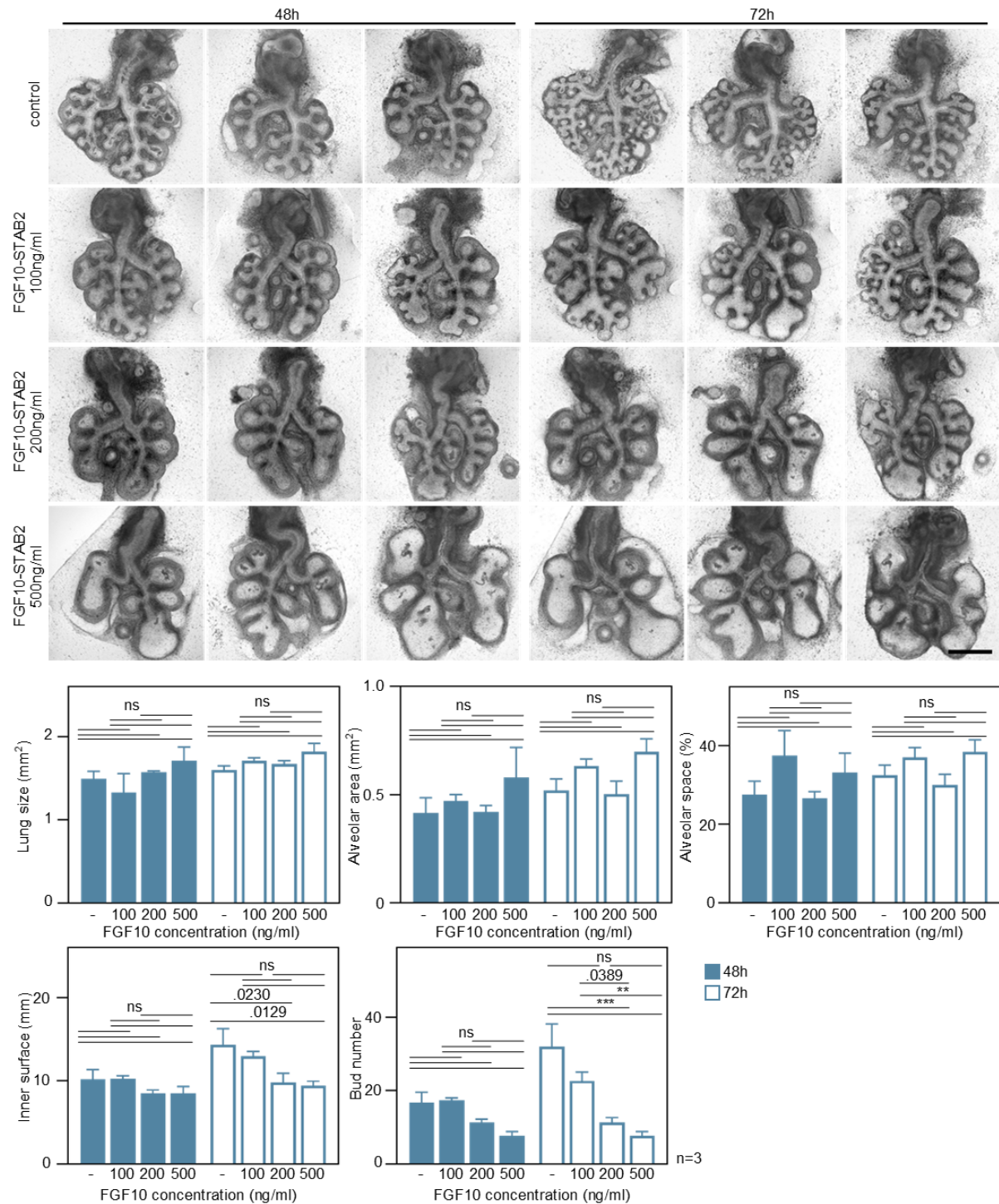


Figure S8 FGF10-STAB2 alters cell differentiation in embryonic lung explants. E11.25 mouse lungs were explanted and incubated for up to 48 and 72 hours with FGF10 added every 12-24 hours. The overall morphology of lung explants reveals significant suppression of branching by FGF10-STAB2, apparent at 100, 200 and 500 ng/ml (scale bar, 0.5 mm). Three explants are shown for each treatment treatment (mean±SEM, Two-Way ANOVA, ***p<0.001, **p<0.01).

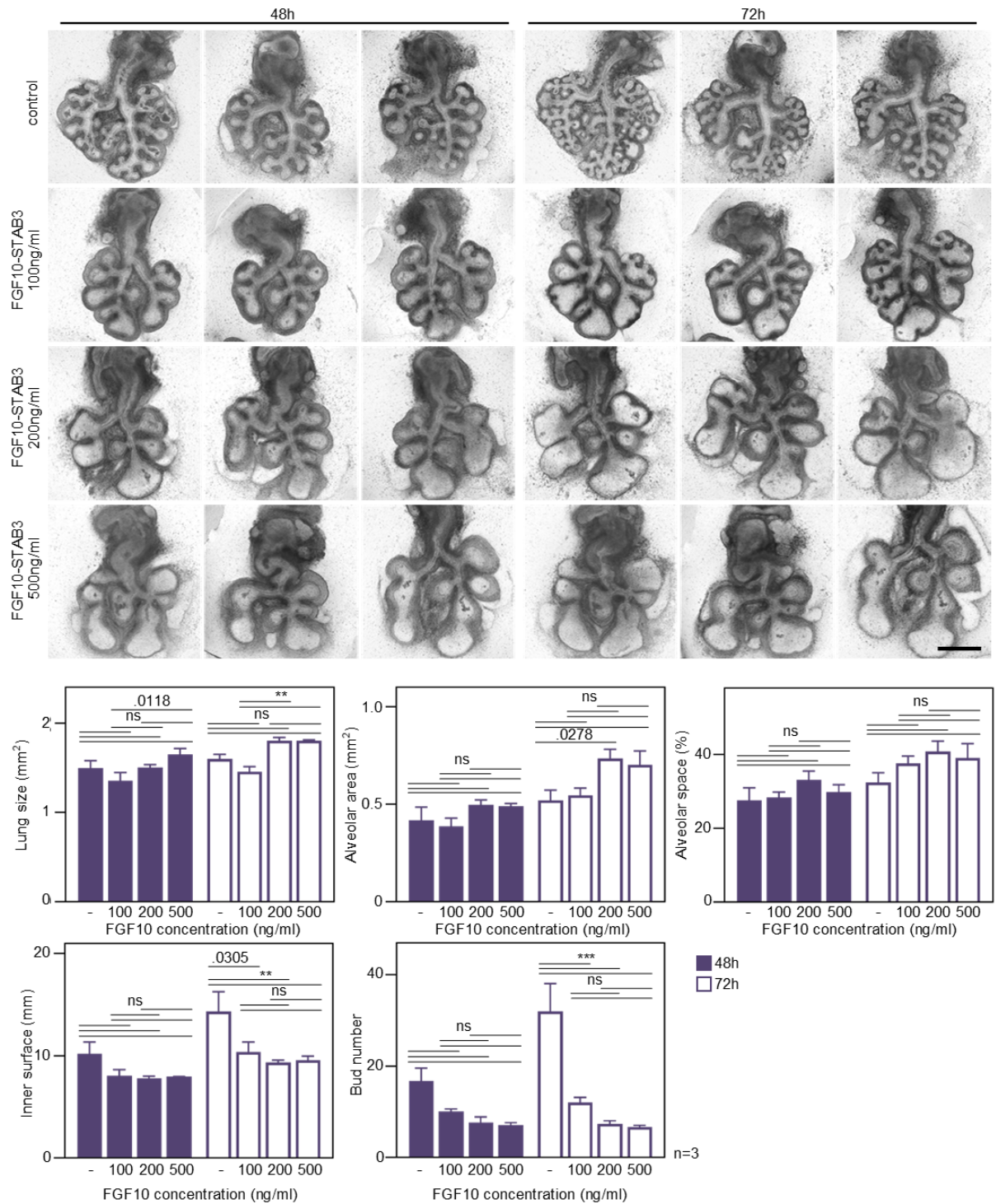


Figure S9 FGF10-STAB3 alters cell differentiation in embryonic lung explants. E11.25 mouse lungs were explanted and incubated for up to 48 and 72 hours with FGF10 added every 12-24 hours. The overall morphology of lung explants reveals significant suppression of branching by FGF10-STAB3, apparent at 100, 200 and 500 ng/ml (scale bar, 0.5 mm). Three explants are shown for each treatment treatment (mean±SEM, Two-Way ANOVA, *** $p < 0.001$, ** $p < 0.01$).

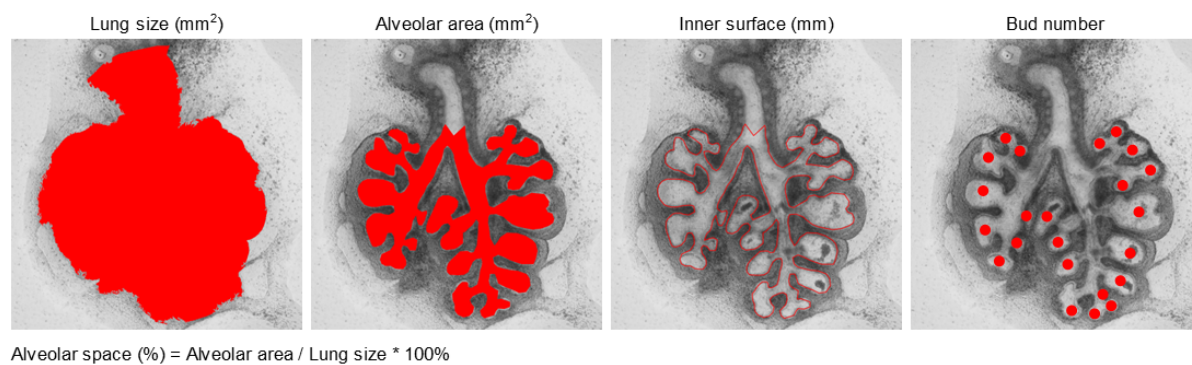


Figure S10 Scheme showing the measurement of embryonic lung explants stimulated with FGF10 variants.

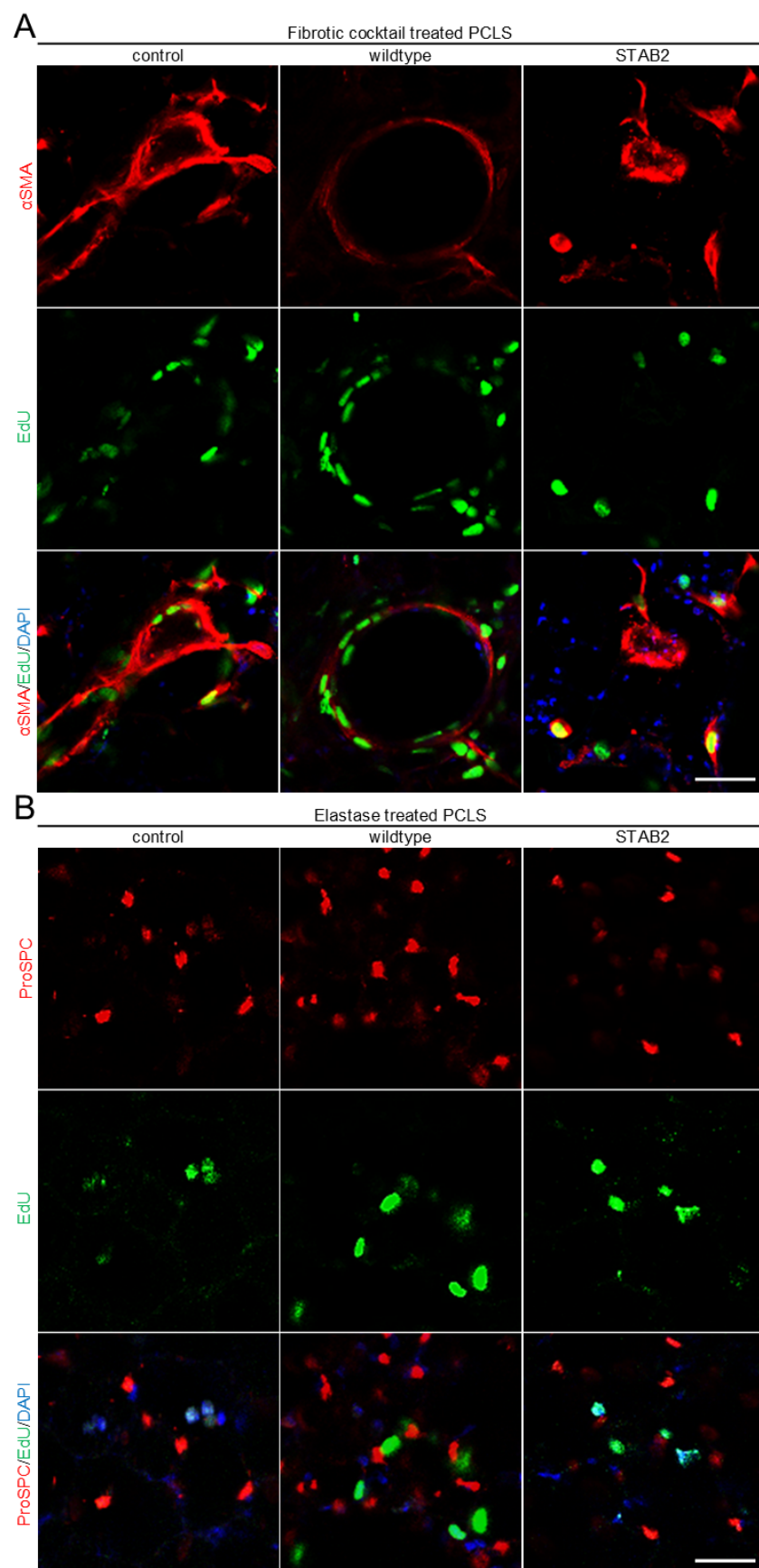


Figure S11 (A) Representative confocal images of PCLS fibrosis. PCLS pretreated with

fibrotic cocktail upon 72 hours stimulation with vehicle (control), wildtype FGF10 and FGF-STAB2 (250 ng/ml) were stained with EdU (green), α SMA (red) and DAPI (blue). **(B)** Representative confocal images of PCLS regeneration. PCLS injury was induced by preincubation in elastase for 24 hours before treatment with vehicle, wildtype FGF10 and STAB2 (250 ng/ml) and stained with EdU (green), ProSPC (red) and DAPI (blue). Scale bar 50 μ m.

Table S1 Antibodies used in the study. WB, western blot; IHC, immunohistochemistry; 2nd, secondary antibody.

<i>Protein</i>	<i>Source</i>	<i>Cat. No.</i>	<i>Method</i>
Actin	Cell Signaling	3700	WB
AKT	Cell Signaling	4691	WB
pAKT ^{S473}	Cell Signaling	4060	WB
ERK	Cell Signaling	9102	WB
pERK ^{T202/Y204}	Cell Signaling	9101	WB
pERK ^{T202/Y204}	Cell Signaling	4376	WB
FGF10	Santa Cruz	sc-7917	WB
pFRS2 ^{Y196}	Cell Signaling	3864	WB
pFRS2 ^{Y436}	Cell Signaling	3861	WB
Gab1	Cell Signaling	3232	WB
pGab1 ^{Y627}	Cell Signaling	3231	WB
γ Tubulin	Sigma Aldrich	T6557	WB
V5	Invitrogen	46-0705	WB
mouse IgG	Sigma Aldrich	A6782	2 nd , WB
rabbit IgG	Sigma Aldrich	A6667	2 nd , WB
mouse IgG	Jackson ImmunoResearch	115-035-003	2 nd , WB
rabbit IgG	Jackson ImmunoResearch	111-035-144	2 nd , WB
α SMA	Abcam	ab5694	IHC
α SMA	Merck Millipore	C6198	IHC
DRAQ5	Thermo Fisher	62251	IHC
E-cadherin	eBioscience	53-3249-82	IHC
Pro-SPC	Abcam	ab270521	IHC
Sox2	Cell Signaling	2748S	IHC
Sox9	Abcam	ab185230	IHC
Sox9	Sigma Aldrich	HPA001758	IHC
rabbit IgG	Invitrogen	A-31573	2 nd , IHC
rat IgG	Invitrogen	A-21208	2 nd , IHC

Table S2 Stabilizing mutations identified in FGF10 by energy-based and evolution-based predictions. Mutations identified by evolution-based approach are highlighted in bold. $\Delta\Delta G$, predicted change in Gibbs free energy; MSA, multiple sequence alignment.

<i>Mutation</i>	<i>$\Delta\Delta G$ (kcal/mol)</i>	<i>Conservation</i>	<i>Consensus</i>	<i>Amino acids in MSA</i>
N71E	-2.331	5	-	D, G, E, K, Y, N, F, S, P, V, H
T86F	-1.783	7	-	H, N, P, T, Q
I126Y	-3.167	7	-	I, F, M, L, V
N127Y	-2.637	3	-	G, A, E, Y, K, N, F, Q, T, S, H
S143P	-2.089	7	-	K, V, A, E, T, S
H171Y	-1.996	5	-	Q, R, K, H, N, P, E
R174Y	-3.650	6	-	G, E, H, K, R, T, Q
Q175E	-2.102	6	-	Q, A, E, P, H, R
N181D	-2.100	7	-	N, R, G, D, M, S
V205P	-1.377	2	-	P, V, F, I, Q, M, T, A, E, K, N, L
I118V	0.008	8	0.69	M, T, Q, I, E, V
V123I	-1.590	8	0.83	L, I, V
L152F	0.395	9	0.62	L, F
Y177F	-0.100	8	0.83	Y, F

Table S3 *In silico* prediction of stabilizing effect of multiple-point FGF10 mutants. $\Delta\Delta G$, predicted change in Gibbs free energy.

<i>Mutations</i>	<i>Sum of single-point mutant contribution $\Delta\Delta G$ (kcal/mol)</i>	<i>Calculated values for multiple-point mutants $\Delta\Delta G$ (kcal/mol)</i>
V123I_L152F	-1.2	-4.3
V123I_Q175E	-3.7	-3.3
V123I_N181D	-3.7	-1.8
L152F_Q175E	-1.7	-2.2
L152F_N181D	-1.7	-0.8
Q175E_N181D	-4.2	-0.5
V123I_L152F_Q175E	-3.3	-5.2
V123I_L152F_N181D	-3.3	-3.3
V123I_Q175E_N181D	-5.8	-2.7
L152F_Q175E_N181D	-3.8	-3.7
V123I_L152F_Q175E_N181D	-5.4	-5.0

Table S4 Thermodynamic parameters of wildtype FGF10 and its single mutants designed by semi-rational approach, determined from thermal denaturation monitored by measuring ellipticity at 228 nm. Data presented are mean values from three independent experiments +/- SD. Variants with higher stability than the wildtype FGF10 are in bold. The mutations used in STAB1 were italicized. In green are variants designed with a homology strategy. In blue is variant designed to lower affinity for heparin.

<i>Protein</i>	ΔT_{den}	T_{den}	ΔH_{den}
wildtype FGF10	-	53.8±0.8	137.7±23.0
F89H	-10.5	43.3±1.0	107.6±22.7
C150S	-9.0	44.8±1.1	123.2±5.4
M176W	-8.2	45.6±1.7	144.2±27.1
G160Y	-7.5	46.3±0.1	167.8±2.4
E93L	-7.3	46.5±0.7	148.5±7.9
C106S	-6.6	47.2±0.2	144.3±0.5
S99D	-5.8	47.9±0.2	153.0±2.5
K91Q	-3.8	50±0.4	154.6±0.9
N148P	-3.6	50.2±4.8	139.6±30.7
K151V	-2.2	51.6±0.6	149.9±9.6
F167R	-2.1	51.7±0.5	144.8±7.5
E93H	-1.5	52.2±0.3	145.0±6.1
K87N	-1.5	52.3±0.0	148.8±6.3
N196G	-1.4	52.4±0.3	152.3±5.5
Y108F	-0.8	53.0±0.2	156.4±3.1
C106D	-0.7	53.1±0.1	158.6±4.4
A185T	-0.4	53.4±0.0	159.3±1.3
N196Q	-0.2	53.6±0.0	159.5±2.7
W169Y	-0.1	53.7±0.1	146.9±1.9
T114I	0.0	53.8±0.3	155.3±10.3
I126V	0.0	53.8±0.6	105.9±14.0
K103E	0.3	54.1±0.3	160.3±11.6
Q170S	0.4	54.2±1.0	159.4±1.4
N148S	0.6	54.4±0.6	151.5±7.1
A185R	0.6	54.4±1.2	154.1±9.9
K191A	0.7	54.5±0.1	162.4±5.8
N135D	1.1	54.9±0.2	150.7±7.7
N148D	1.5	55.3±0.1	155.0±11.5
T86N	1.9	55.7±0.8	123.8±5.1
<i>V123I</i>	2.6	56.4±0.3	161.1±1.6
<i>L152F</i>	6.6	60.4±0.7	102.5±15.6

Table S5 Thermodynamic parameters of wildtype FGF10 and its multiple mutants designed by semi-rational approach, determined from thermal denaturation monitored by measuring ellipticity at 228 nm (mean from at least three independent experiments $\pm$ SD). The mutations used in STAB1 were italicized.

<i>Protein</i>	ΔT_{den}	T_{den}	H_{den}
wildtype FGF10	-	53.8 $\pm$ 0.8	137.7 $\pm$ 23.0
W79I K87G N129G Q170R N181P R187V	9.7	63.5 $\pm$ 0.3	125.1 $\pm$ 4.7
W79I F89Y E93N <i>L152F</i>	11.1	64.9 $\pm$ 1.1	166.1 $\pm$ 15.1
W79I E93N N129G <i>L152F</i>	12.9	66.7 $\pm$ 1.1	172.0 $\pm$ 10.8
W79I L111F <i>V123I</i> N129G <i>L152F</i>	13.5	67.3 $\pm$ 4.5	172.7 $\pm$ 22.6
W79I E93N <i>V123I</i> N129G <i>L152F</i>	15.1	68.9 $\pm$ 0.8	148.0 $\pm$ 27.3
W79I F89Y <i>V123I</i> N129G <i>L152F</i>	15.5	69.3 $\pm$ 1.2	168.3 $\pm$ 26.2
W79I <i>V123I</i> N129G <i>L152F</i> F167A	16.0	69.8 $\pm$ 0.3	117.6 $\pm$ 22.5
W79I <i>V123I</i> N129G <i>L152F</i>	16.7	70.5 $\pm$ 0.0	182.3 $\pm$ 0.4
W79I K87G N129G Q170R R187E	16.9	70.7 $\pm$ 0.4	131.6 $\pm$ 14.4
W79I K87G F89Y <i>V123I</i> N129G R187V	17.5	71.3 $\pm$ 0.6	125.6 $\pm$ 35.9
W79I K87G E93N <i>V123I</i> N129G R187V	18.3	72.1 $\pm$ 0.2	137.2 $\pm$ 24.8
W79I K87G N129G Q170R R187V	18.5	72.3 $\pm$ 0.6	138.2 $\pm$ 18.8
W79I F89Y E93N K94P <i>V123I</i> N129G <i>L152F</i> F167A Q170T	18.6	72.4 $\pm$ 2.4	123.3 $\pm$ 30.1
W79I K87G N129G Q170R R187V R188A	18.8	72 $\pm$ 1.3	143.3 $\pm$ 15.6
W79I K87G N129G Q170R R187V R188E	19.5	73.3 $\pm$ 0.8	150.2 $\pm$ 15.6
W79I K94P <i>V123I</i> N129G <i>L152F</i>	19.8	73.6 $\pm$ 0.3	162.5 $\pm$ 9.5
W79I K87G K94P N129G Q170R R187E	20.4	74.2 $\pm$ 0.1	142.3 $\pm$ 9.3
W79I E93N K94P <i>V123I</i> N129G <i>L152F</i> F167A Q170T	20.5	74.3 $\pm$ 0.4	149.4 $\pm$ 6.7
W79I K87G <i>V123I</i> N129G <i>L152F</i>	20.7	74.5 $\pm$ 0.1	179.8 $\pm$ 4.5
W79I K87G E93D K94P N129G Q170R R187E	20.9	74.7 $\pm$ 1.0	118.0 $\pm$ 19.5
W79I K87G K94P N129G Q170R R187V	20.9	74.7 $\pm$ 0.6	132.8 $\pm$ 5.8

Table S6 The affinity of wildtype FGF10, FGF10-STAB2 and FGF10-STAB3 for heparin. Proteins were eluted from a Heparin-Sepharose column by 0.15-1.5M NaCl gradient. Data shown are mean values of the salt concentration at which proteins were eluted, from at least three independent experiments $\pm$ SD.

<i>Protein</i>	<i>NaCl concentration [M]</i>
wildtype FGF10	1.057 $\pm$ 0.04
FGF10-STAB2	0.855 $\pm$ 0.04
FGF10-STAB3	0.785 $\pm$ 0.04

Table S7 Genes differentially expressed between wildtype FGF10 or FGF10-STAB1 and untreated MCF7 cells. Only protein-coding genes exhibiting absolute log2 fold change value higher or equal to 1.5 in at least one of the comparisons are shown.

<i>Symbol</i>	<i>Log₂ fold change</i>		<i>Description</i>
	<i>wildtype to untreated</i>	<i>STAB1 to untreated</i>	
<i>B3GNT6</i>	4.46	5.01	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6
<i>MUC2</i>	3.6	3.94	mucin 2, oligomeric mucus/gel-forming
<i>ETV4</i>	3.59	3.7	ETS variant transcription factor 4
<i>SPRY4</i>	3.26	3.67	sprouty RTK signaling antagonist 4
<i>LGALS7B</i>	3.17	2.87	galectin 7B
<i>MALL</i>	3.1	3.22	mal, T cell differentiation protein like
<i>A2M</i>	2.97	3.02	alpha-2-macroglobulin
<i>SHC4</i>	2.79	2.96	SHC adaptor protein 4
<i>ETV5</i>	2.64	2.78	ETS variant transcription factor 5
<i>KRT20</i>	2.59	2.92	keratin 20
<i>GPR3</i>	2.54	2.54	G protein-coupled receptor 3
<i>C10orf67</i>	2.5	2.77	chromosome 10 open reading frame 67
<i>RAB7B</i>	2.48	3.07	RAB7B, member RAS oncogene family
<i>AXL</i>	2.47	2.66	AXL receptor tyrosine kinase
<i>IL2RB</i>	2.34	2.53	interleukin 2 receptor subunit beta
<i>RARRES2</i>	2.26	2.19	retinoic acid receptor responder 2
<i>AREG</i>	2.14	2.29	amphiregulin
<i>FN1</i>	2.1	2.21	fibronectin 1
<i>ZNF365</i>	2.07	2.19	zinc finger protein 365
<i>OXTR</i>	2.05	2.16	oxytocin receptor
<i>DUSP6</i>	1.99	2.24	dual specificity phosphatase 6
<i>CATSPER1</i>	1.99	1.96	cation channel sperm associated 1
<i>KCNF1</i>	1.93	1.92	potassium voltage-gated channel modifier subfamily F member 1
<i>EGR3</i>	1.92	2.11	early growth response 3
<i>PTGER2</i>	1.9	1.98	prostaglandin E receptor 2
<i>MYEOV</i>	1.9	1.96	myeloma overexpressed
<i>RASGEF1A</i>	1.88	2.02	RasGEF domain family member 1A
<i>TNIK</i>	1.85	2.1	TRAF2 and NCK interacting kinase
<i>SYT13</i>	1.79	1.63	synaptotagmin 13
<i>SDR16C5</i>	1.69	1.79	short chain dehydrogenase/reductase family 16C member 5
<i>TNFRSF21</i>	1.68	1.82	TNF receptor superfamily member 21
<i>DUSP5</i>	1.68	1.77	dual specificity phosphatase 5
<i>TMPRSS11E</i>	1.67	1.84	transmembrane serine protease 11E
<i>KRT23</i>	1.67	1.81	keratin 23
<i>ACOX2</i>	1.64	1.87	acyl-CoA oxidase 2
<i>GPR50</i>	1.64	1.6	G protein-coupled receptor 50
<i>TNFRSF11B</i>	1.63	1.75	TNF receptor superfamily member 11b
<i>EGLN3</i>	1.6	1.7	egl-9 family hypoxia inducible factor 3

<i>KRT7</i>	1.59	1.56	keratin 7
<i>LOXL1</i>	1.58	1.59	lysyl oxidase like 1
<i>UPP1</i>	1.56	1.76	uridine phosphorylase 1
<i>EMP1</i>	1.55	1.72	epithelial membrane protein 1
<i>MUC5AC</i>	1.54	2.03	mucin 5AC, oligomeric mucus/gel-forming
<i>TMT1B</i>	1.54	1.57	thiol methyltransferase 1B
<i>LONRF3</i>	1.54	1.54	LON peptidase N-terminal domain and ring finger 3
<i>NIPAL1</i>	1.53	1.62	NIPA like domain containing 1
<i>LOXL2</i>	1.53	1.48	lysyl oxidase like 2
<i>CDC42EP3</i>	1.52	1.62	CDC42 effector protein 3
<i>KRT86</i>	1.49	1.64	keratin 86
<i>SEC14L2</i>	1.49	1.51	SEC14 like lipid binding 2
<i>SERPINA3</i>	1.46	1.51	serpin family A member 3
<i>CAPN8</i>	1.45	1.81	calpain 8
<i>CD109</i>	1.45	1.52	CD109 molecule
<i>DMP1</i>	1.44	1.53	dentin matrix acidic phosphoprotein 1
<i>RNF183</i>	1.42	1.59	ring finger protein 183
<i>ALOXE3</i>	1.39	1.72	arachidonate lipoxygenase 3
<i>SPNS2</i>	1.38	1.55	SPNS lysolipid transporter 2, sphingosine-1-phosphate
<i>LIF</i>	1.37	1.62	LIF interleukin 6 family cytokine
<i>LONRF2</i>	1.37	1.55	LON peptidase N-terminal domain and ring finger 2
<i>GAL</i>	1.35	1.56	galanin and GMAP prepropeptide
<i>ERRF1</i>	1.34	1.51	ERBB receptor feedback inhibitor 1
<i>EGR1</i>	1.32	1.56	early growth response 1
<i>JCAD</i>	1.3	1.59	junctional cadherin 5 associated
<i>APOBR</i>	-1.08	-1.74	apolipoprotein B receptor
<i>TPPP3</i>	-1.1	-2.37	tubulin polymerization promoting protein family member 3
<i>DBP</i>	-1.13	-1.58	D-box binding PAR bZIP transcription factor
<i>SYNDIG1</i>	-1.28	-1.8	synapse differentiation inducing 1
<i>SMIM39</i>	-1.29	-1.86	small integral membrane protein 39
<i>TMT1A</i>	-1.39	-1.66	thiol methyltransferase 1A
<i>PGM5</i>	-1.48	-1.5	phosphoglucomutase 5
<i>GPR68</i>	-1.48	-1.87	G protein-coupled receptor 68
<i>KIT</i>	-1.51	-1.42	KIT proto-oncogene, receptor tyrosine kinase
<i>MMP16</i>	-1.53	-1.65	matrix metalloproteinase 16
<i>SOX6</i>	-1.63	-1.58	SRY-box transcription factor 6
<i>ABCC9</i>	-1.64	-1.9	ATP binding cassette subfamily C member 9
<i>SEC14L5</i>	-1.68	-1.94	SEC14 like lipid binding 5
<i>MAP2K6</i>	-1.8	-2.18	mitogen-activated protein kinase kinase 6