Supplemental Materials

Molecular Biology of the Cell

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Supplemental Data: Mass spectrometry analysis for TurboID-zyxin (excel file). The analysis is based on triplicate samples with following conditions:

B1-B3: Biotinylated proteins identified from cells without biotin for 30 min
C1-C3: Biotinylated proteins isolated from cells exposed to biotin for 30 min
S1-S3: Biotinylated proteins isolated from cells exposed to biotin and 60% stretch for 30 min

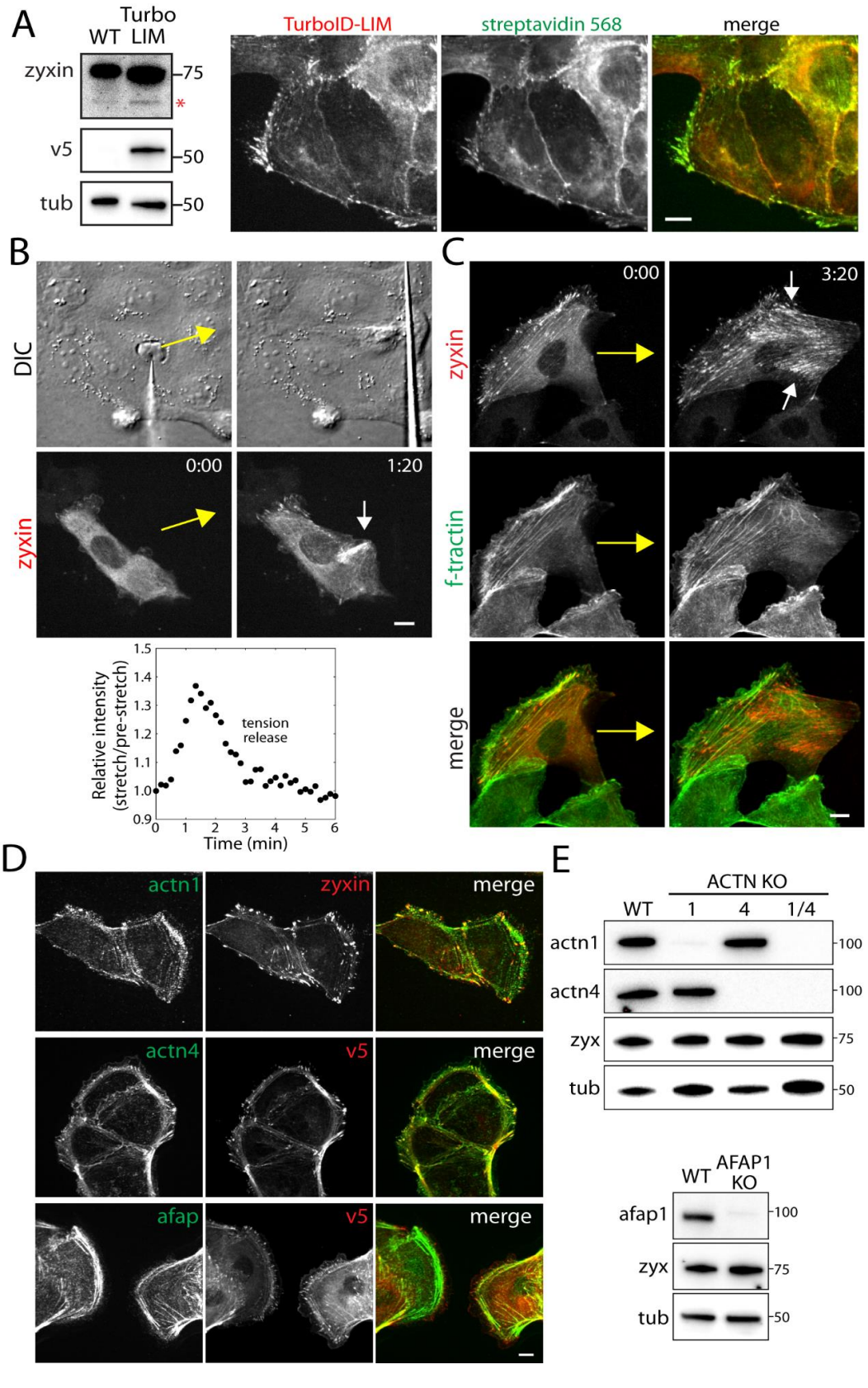
The first tab ("raw data") contains the original list of proteins. The second tab ("ratio") contains data sorted based on the ratio of relative abundance of biotinylation in stretch and control conditions, while the third tab ("S only") lists the proteins identified only in all stretch samples but not in any of control samples (see highlighted proteins at the top).

Supplemental Table: CRISPR/Cas9 gRNA sequences and resulting mutations

Gene		*Sequence*	*Note*
ACTN1	gRNA	**GCAGATCGAGAACATCGAGG**	
		GCAGATCGAGAACAT--AGG	2 bp deletion (4 out of 4 sequenced)
ACTN4	gRNA	**GGTACGACTGGTTCGCCGCG**	
		GGTACGACTGGTTCGCCGGCG	1 bp insertion (3 out of 3 sequenced)
ACTN1/4	gRNA	**GCAGAAGCTGGAGGACTTCC**	
	(ACTN1)	GCAGAAGCTGGAGGACTTTCC	1 bp insertion (3 out of 4 sequenced)
		GCAGAAGCTGGAGGACTTTTCC	2 bp insertion (1 out of 4 sequenced)
	(ACTN4)	GCAGAAGCTGGAGGACTTTCC	1 bp insertion (4 out of 5 sequenced)
		GCAGAAGCTGGAG--------	8 bp deletion (1 out of 5 sequenced)
AFAP1	gRNA	**GTCACGTTGCAGCCCTGCAG**	
		GTCACGTTGCAGCCCTG----	4 bp deletion (3 out of 4 sequenced)
		GTCACGTTGCAGCCCTGTCAG	1 bp insertion (1 out of 4 sequenced)
ZYX	gRNA	**GAACGGAGTGGTGACTGGTG**	
		GAACGGAGTGGTGAC--GTG	2 bp deletion (1 out of 1 sequenced)
ARHGAP42	gRNA	**GATTTCCAGTTTGAATGTAT**	Amplicon sequencing:
		GATTTCCAGT----------	47.08% of 32843 reads
		GATTTCCAGTTTGAA-----	46.43% of 32383 reads

Supplemental Movie:
Movie 1: Stretch-induced accumulation of zyxin using a microneedle. See also Figure 1A.
Movie 2: Single cell wound healing of zyxin-GFP (left) and GFP-ARHGAP42 (right) expressing cells. See also Figure 4B.

Supplemental Figure 1. Analysis of stable cell lines and force-induced dynamics of zyxin. (A) Characterization of v5-TurboID-tagged LIM domain of zyxin. v5-TurboID-LIM was detected by v5 tag in Western blot at a low level (left). Star denotes the band for v5-TurboID-LIM. Anti-v5 antibody and streptavidin labeling co-localizes at focal adhesions and along cell-cell contacts. (B) The application of force by pulling on neighboring, non-expressing cell using a microneedle. Yellow arrow points to the direction of microneedle movement and white arrow indicates tandem dimer (td) DsRed-tagged zyxin accumulation. The intensity of tdDsRed-tagged zyxin increases as tension is applied by a microneedle, and decays as tension is released. (C) Relative dynamics of zyxin and F-tractin under tension applied by a microneedle. In all images, time in min:sec. Yellow arrows indicate the movement of microneedle and white arrows indicate the zyxin accumulation. (D) Relative localization of zyxin and α-actinin 1, α-actinin 4, and AFAP1. Localization of zyxin was detected using anti-zyxin (zyxin) or anti-v5 (v5) antibody in v5-TurboID-zyxin expressing cells. (E) Western blot analysis of α-actinin and AFAP1 knockout cell lines. All scale bars are 10 μm.


A
Turbo
WT LIM
zyxin
75
*
v5
50
tub
50
TurboID-LIM
streptavidin 568
merge
B
DIC
zyxin
0:00
1:20
Relative intensity
(stretch/pre-stretch)
tension
release
Time (min)
C
zyxin
0:00
3:20
f-tractin
merge
D
actn1
zyxin
merge
actn4
v5
merge
afap
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E
ACTN KO
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afap1
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