Supplemental Materials and Methods

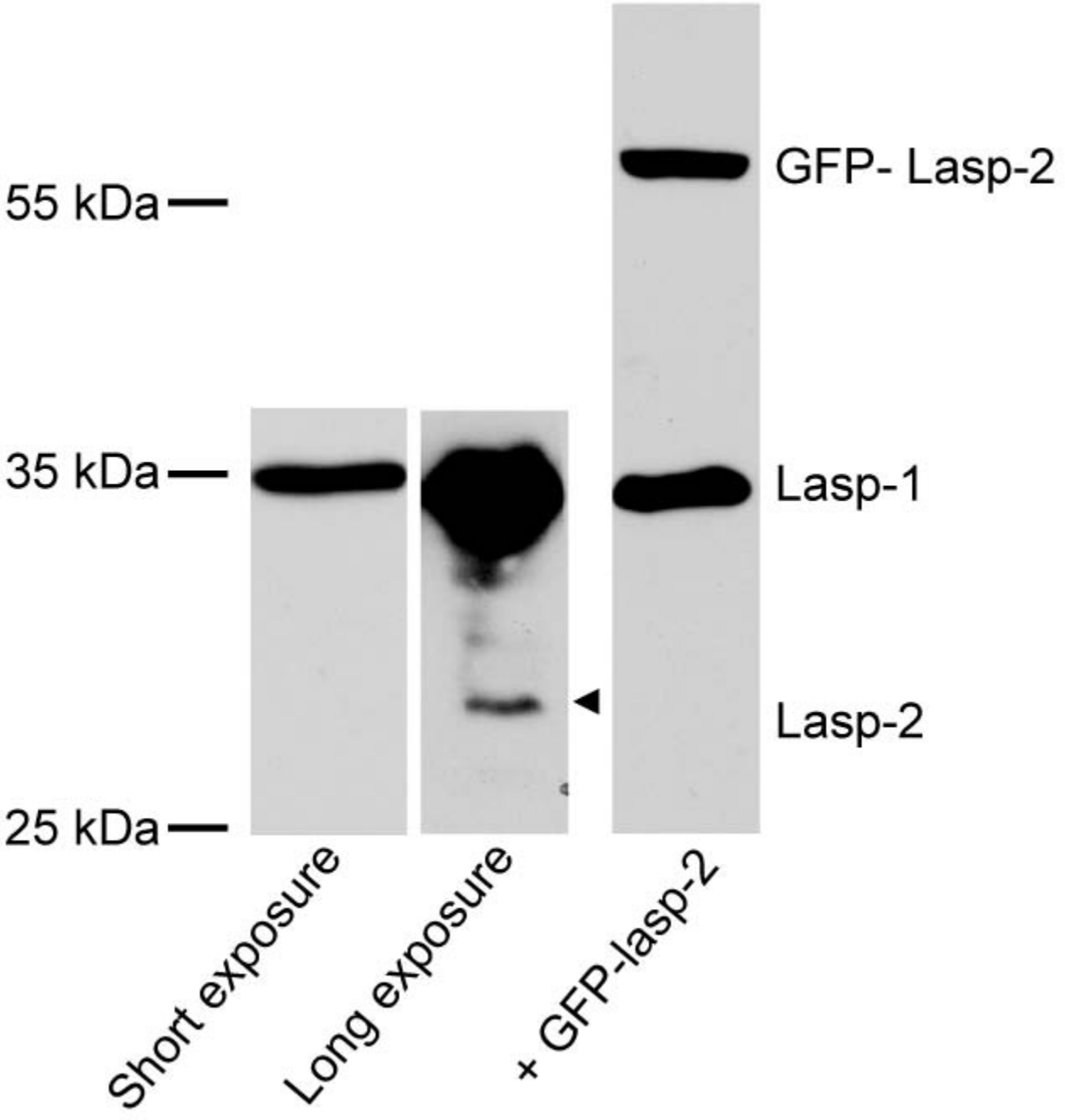
Determination of vinculin and paxillin solubility in co-IP buffer

HEK 293 cells were plated on 10 cm tissue culture dishes and transfected with plasmids encoding GFP, GFP-lasp-2, Cherry-lasp-1 or Cherry-lasp-1 plus GFP-lasp-2. 48h after transfection, lysate was harvested in ice-cold immunoprecipitation buffer (137 mM NaCl, 1% NP-40, 20 mM Tris-HCl pH 8.0, 2 mM EDTA, 10% glycerol) with protease inhibitors. Lysates were then sonicated and centrifuged for 15 min at 16,000 x g to remove insoluble debris. The resulting pellet was resuspended in a more “stringent” buffer containing 25 mM HEPES pH 7.4, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% Na-deoxycholate, 1% Triton X-100, 0.1% SDS, 10% glycerol with protease inhibitors, sonicated and spun for 15 min at 16,000 x g. The protein levels in the final lysates were measured using a BCA (Thermo Fisher Scientific, Waltham, MA) assay and the lysates were each adjusted to 1 mg/ml. Equal amounts of protein were loaded onto SDS-PAGE gels and transferred onto nitrocellulose membranes (Whatman, Kent, UK) for Western blot analysis.

Supplemental Figure S1. Anti-lasp-1 antibodies detect both lasp-1 and lasp-2 proteins. Protein lysates were taken from HEK 293 cells and analyzed by Western blot analysis. Using a commercially available anti-lasp-1 monoclonal antibody, short exposures reveal a band corresponding to lasp-1 at ~35 kDa. A band corresponding to the migration of GFP-lasp-2 (~60 kDa) also is easily detected in lysates expressing GFP-lasp-2. Longer exposures reveal a band (~33 kDa) corresponding to the migration of endogenous lasp-2 in the cell lysates (arrowhead).

Supplemental Figure S2. The solubilization conditions of the co-IP buffer (containing 1% NP-40) is sufficient to solubilize the majority (>80%) of vinculin and paxillin protein in HEK 293 cells. Cell lysates from HEK 293 cells expressing either GFP, GFP-lasp-2, Cherry-lasp-1 or GFP-lasp-2 plus Cherry-lasp-1 were harvested using the 1% NP-40 co-IP buffer. After sonication and centrifugation, the resulting insoluble pellet was solubilized using a more "stringent" buffer condition including 1% Na-deoxycholate, 1% TritionX-100 and 0.1% SDS. Equal amounts of protein were loaded from each preparation. Western blots show that the majority (>80%) of vinculin and paxillin protein is present in the 1% NP-40 fraction and the presence of GFP-lasp-2 or Cherry-lasp-1 does not change the solubility of vinculin or paxillin. Asterisk (*) indicates some degradation of Cherry-lasp-1.

Supplemental Figure S3. The SH3 domain of lasp-2 is sufficient to bind to vinculin and paxillin. (A) Endogenous vinculin was immunoprecipitated from HEK 293 cell lysates expressing either GFP, GFP-lasp-2, GFP-161-273 (linker/SH3) or GFP-1-214 (ΔSH3). Full-length GFP-lasp-2 and GFP-161-273 co-immunoprecipitated with vinculin. GFP-1-124, however, was nearly undetectable, indicating that the SH3 domain of lasp-2 is sufficient to bind to vinculin. (B) Endogenous paxillin was immunoprecipitated from HEK 293 cell lysates expressing either GFP, GFP-lasp-2, GFP-161-273 (linker/SH3) or GFP-1-214 (ΔSH3). Full-length GFP-lasp-2 and GFP-161-273 co-immunoprecipitated with paxillin. GFP-1-124, however, was nearly undetectable, indicating that the SH3 domain of lasp-2 is sufficient to bind to paxillin.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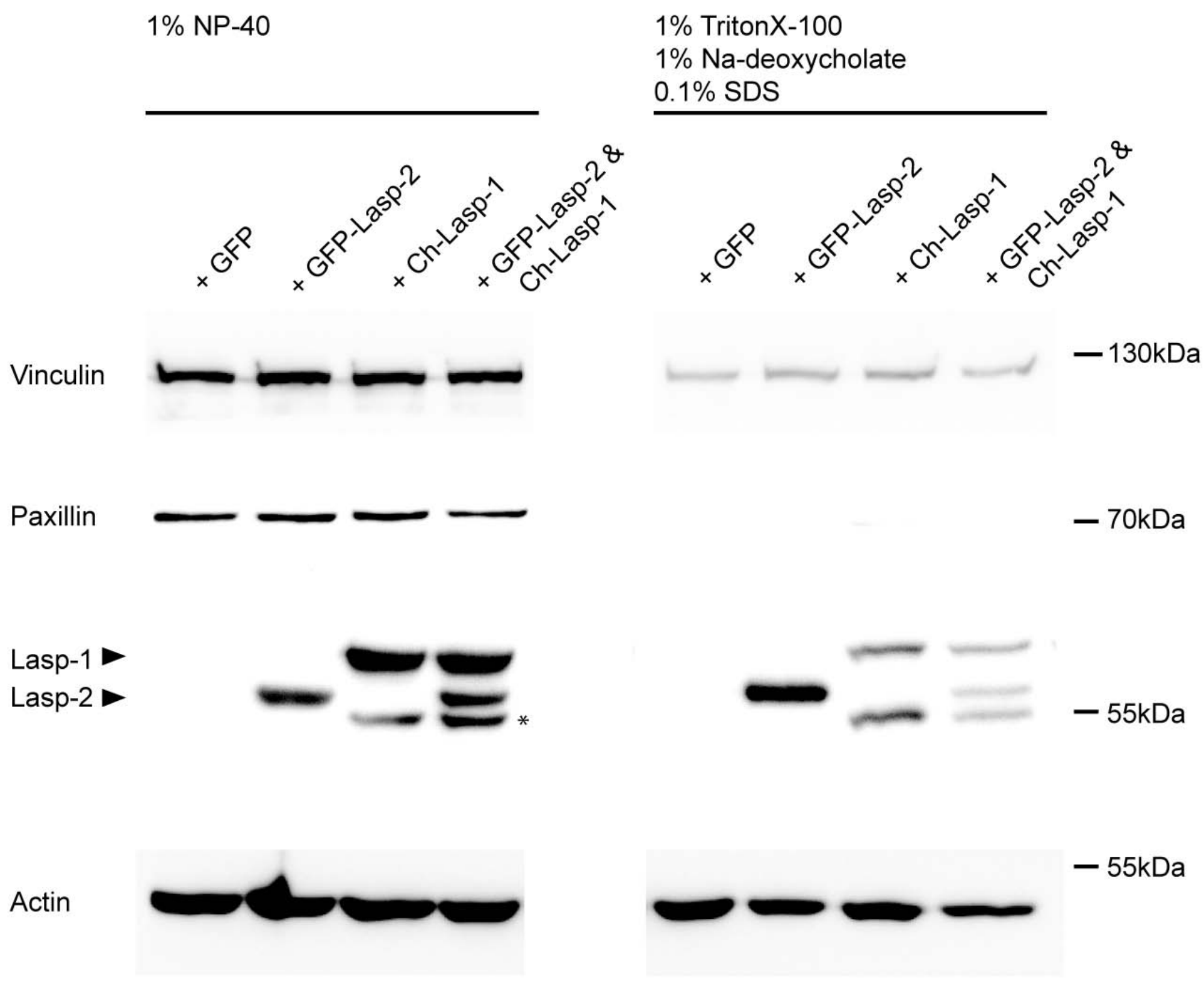
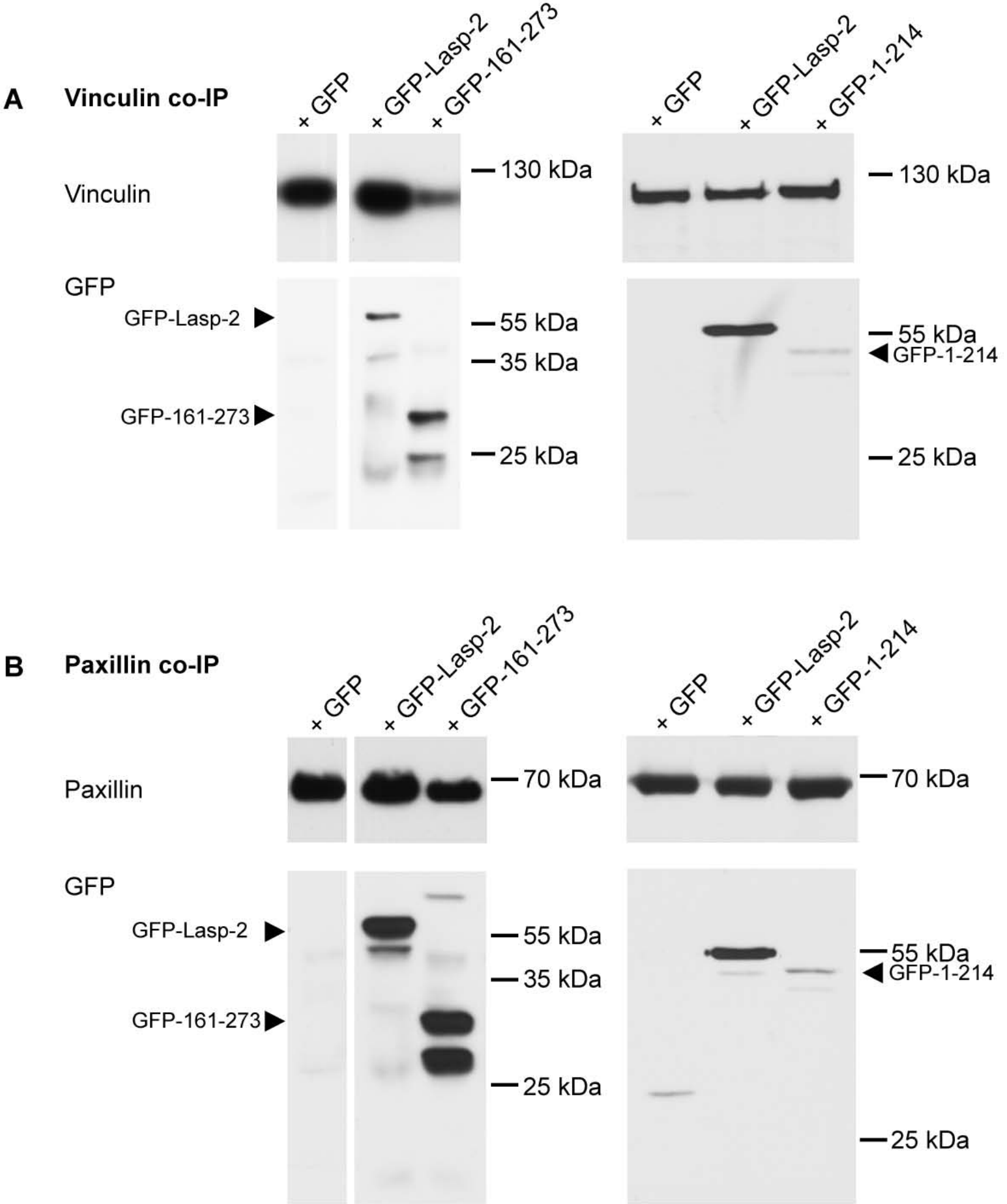
35 kDa
25 kDa
GFP- Lasp-2
Lasp-1
Lasp-2
Short exposure
Long exposure
+ GFP-lasp-2


1% NP-40
1% TritonX-100
1% Na-deoxycholate
0.1% SDS
+ GFP
+ GFP-Lasp-2
+ Ch-Lasp-1
+ GFP-Lasp-2 & Ch-Lasp-1
+ GFP
+ GFP-Lasp-2
+ Ch-Lasp-1
+ GFP-Lasp-2 & Ch-Lasp-1
Vinculin
Paxillin
Lasp-1 ►
Lasp-2 ►
*
Actin
130kDa
70kDa
55kDa
55kDa


A
Vinculin co-IP
+ GFP
+ GFP-Lasp-2
+ GFP-161-273
+ GFP
+ GFP-Lasp-2
+ GFP-1-214
Vinculin
130 kDa
130 kDa
GFP
GFP-Lasp-2
55 kDa
35 kDa
GFP-161-273
25 kDa
55 kDa
GFP-1-214
25 kDa
B
Paxillin co-IP
+ GFP
+ GFP-Lasp-2
+ GFP-161-273
+ GFP
+ GFP-Lasp-2
+ GFP-1-214
Paxillin
70 kDa
70 kDa
GFP
GFP-Lasp-2
55 kDa
35 kDa
GFP-161-273
25 kDa
55 kDa
GFP-1-214
25 kDa