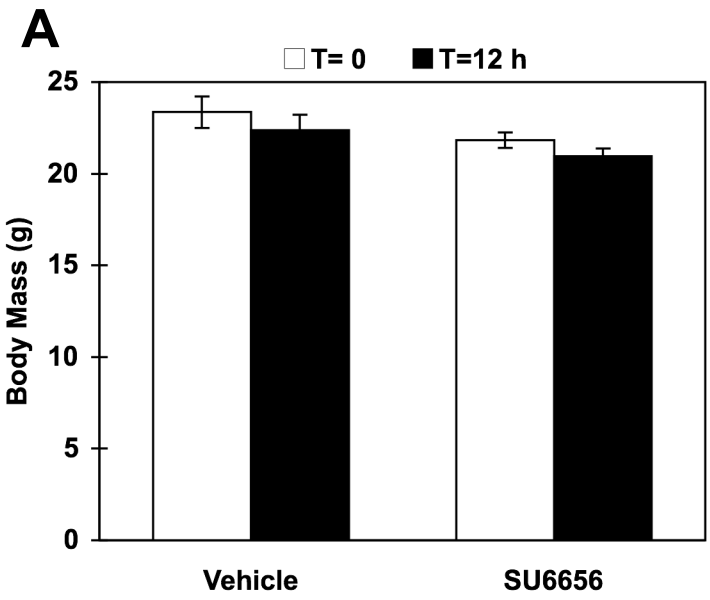

A
T= 0
T=12 h
Body Mass (g)
25
20
15
10
5
0
Vehicle
SU6656

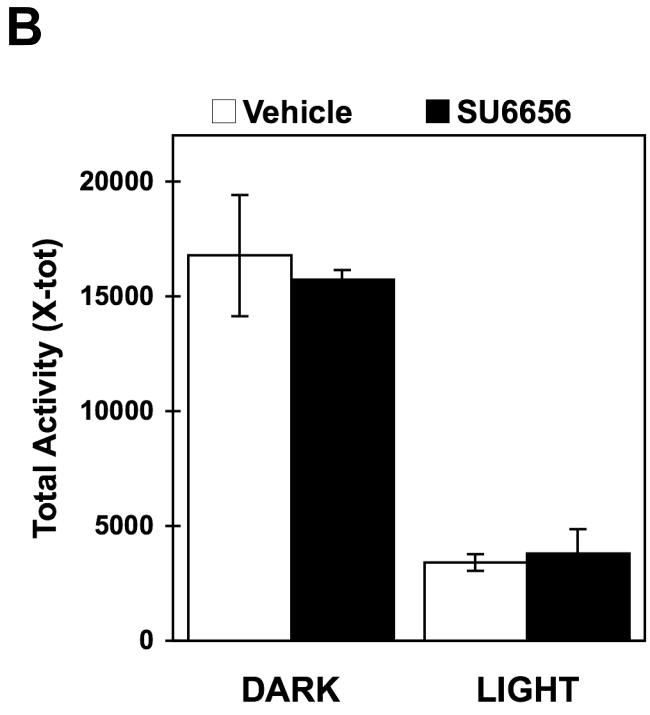

B
Vehicle
SU6656
Total Activity (X-tot)
20000
15000
10000
5000
0
DARK
LIGHT

Figure S1

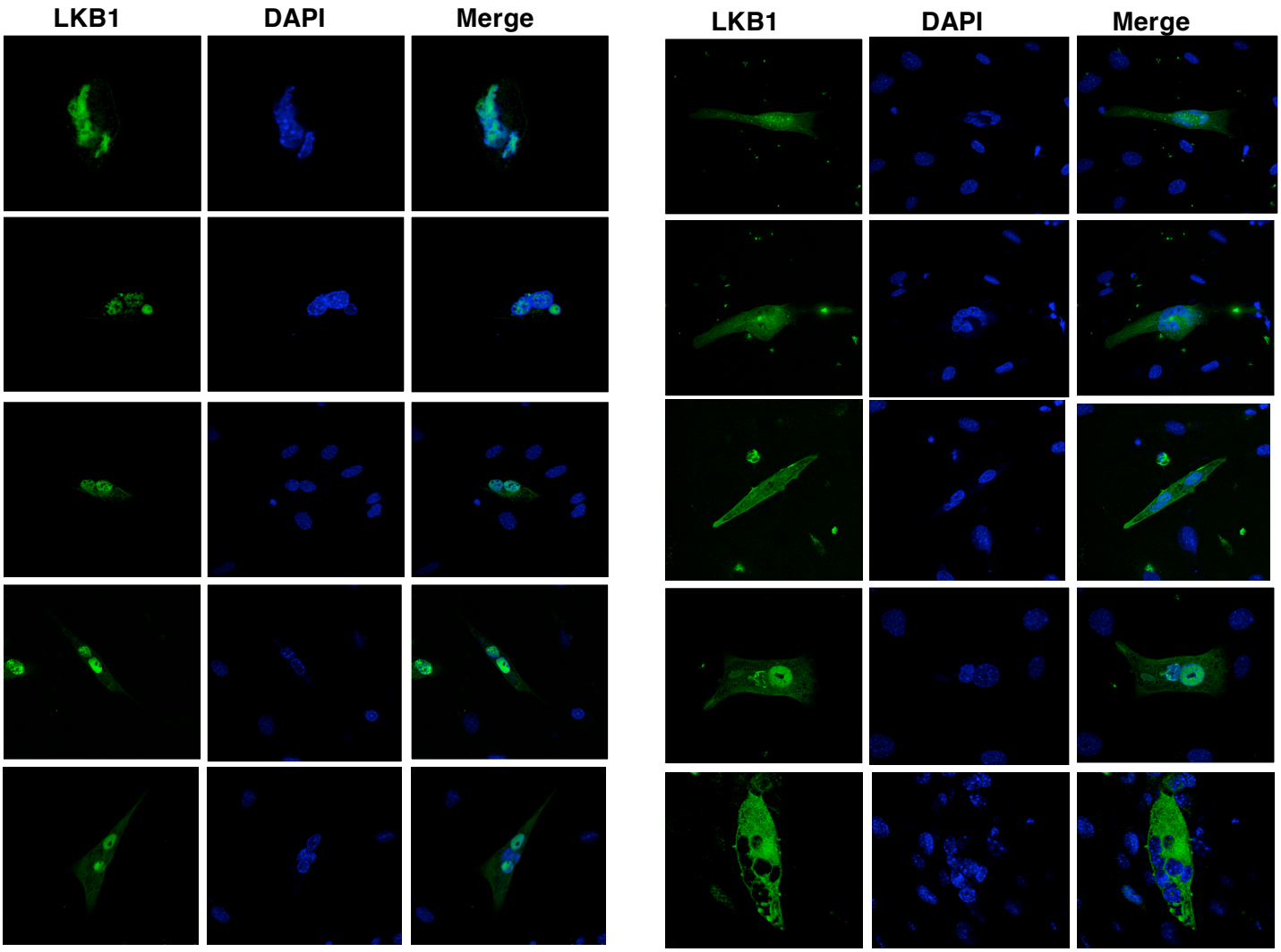


Figure S2

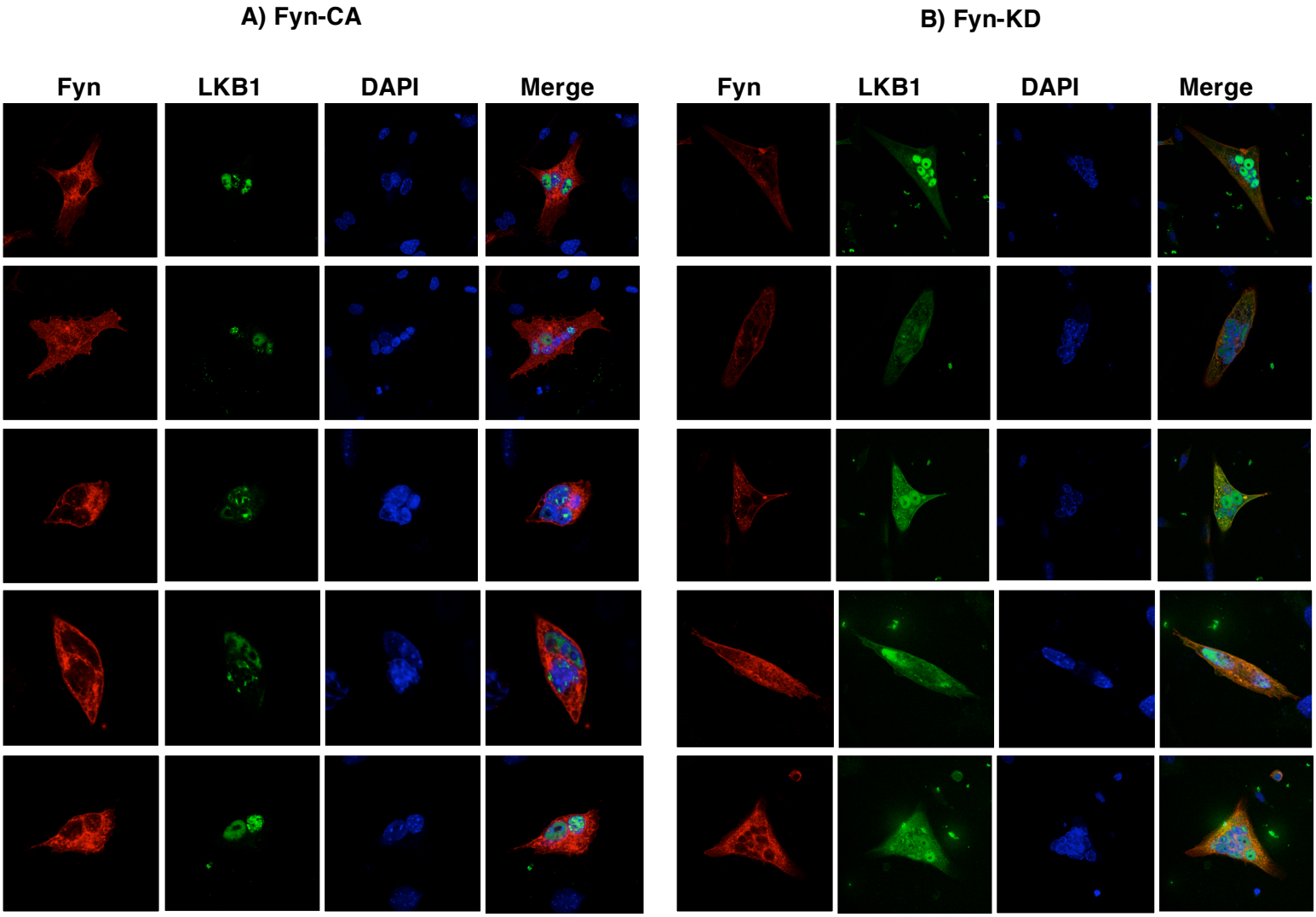


Figure S3

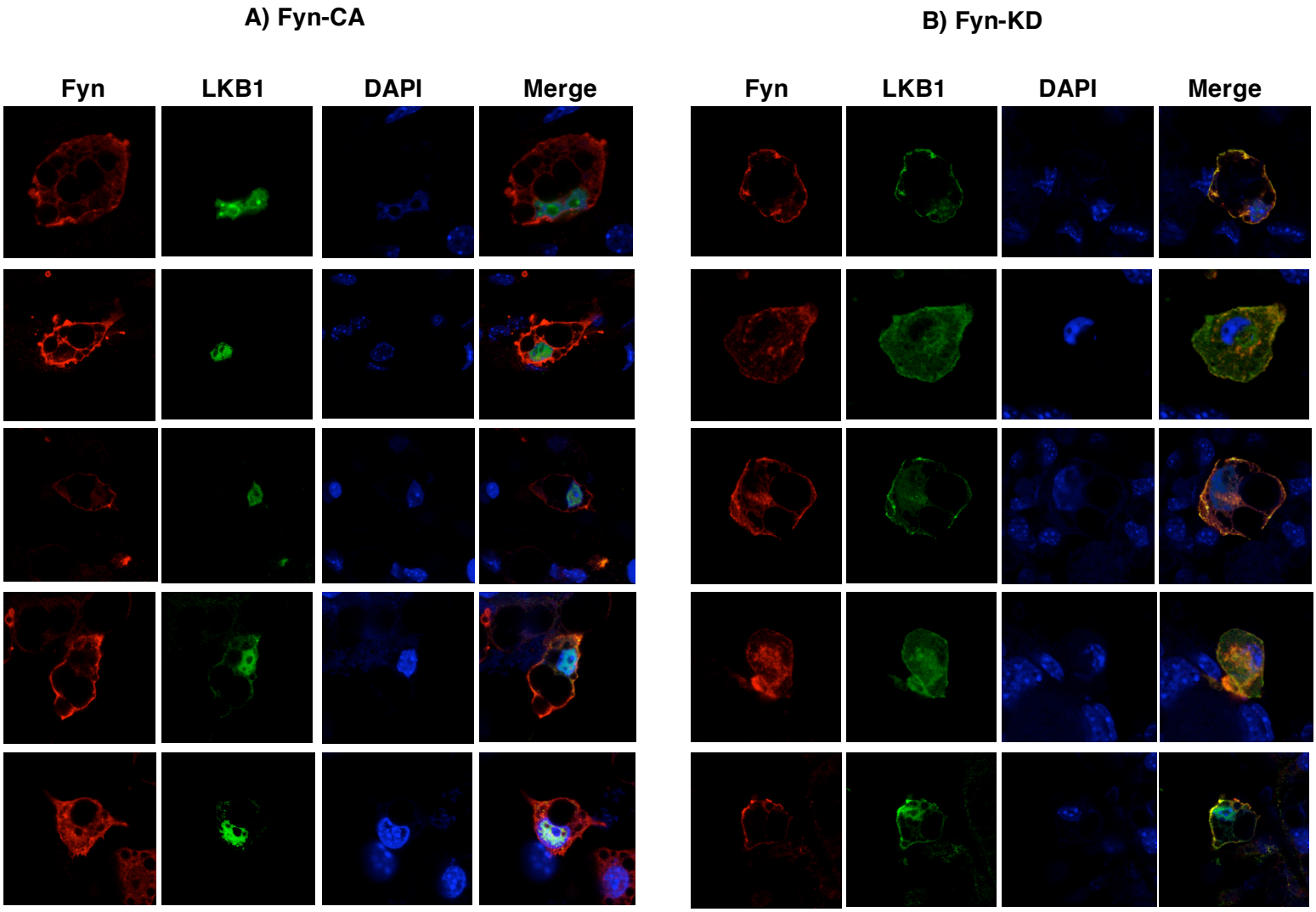


Figure S4

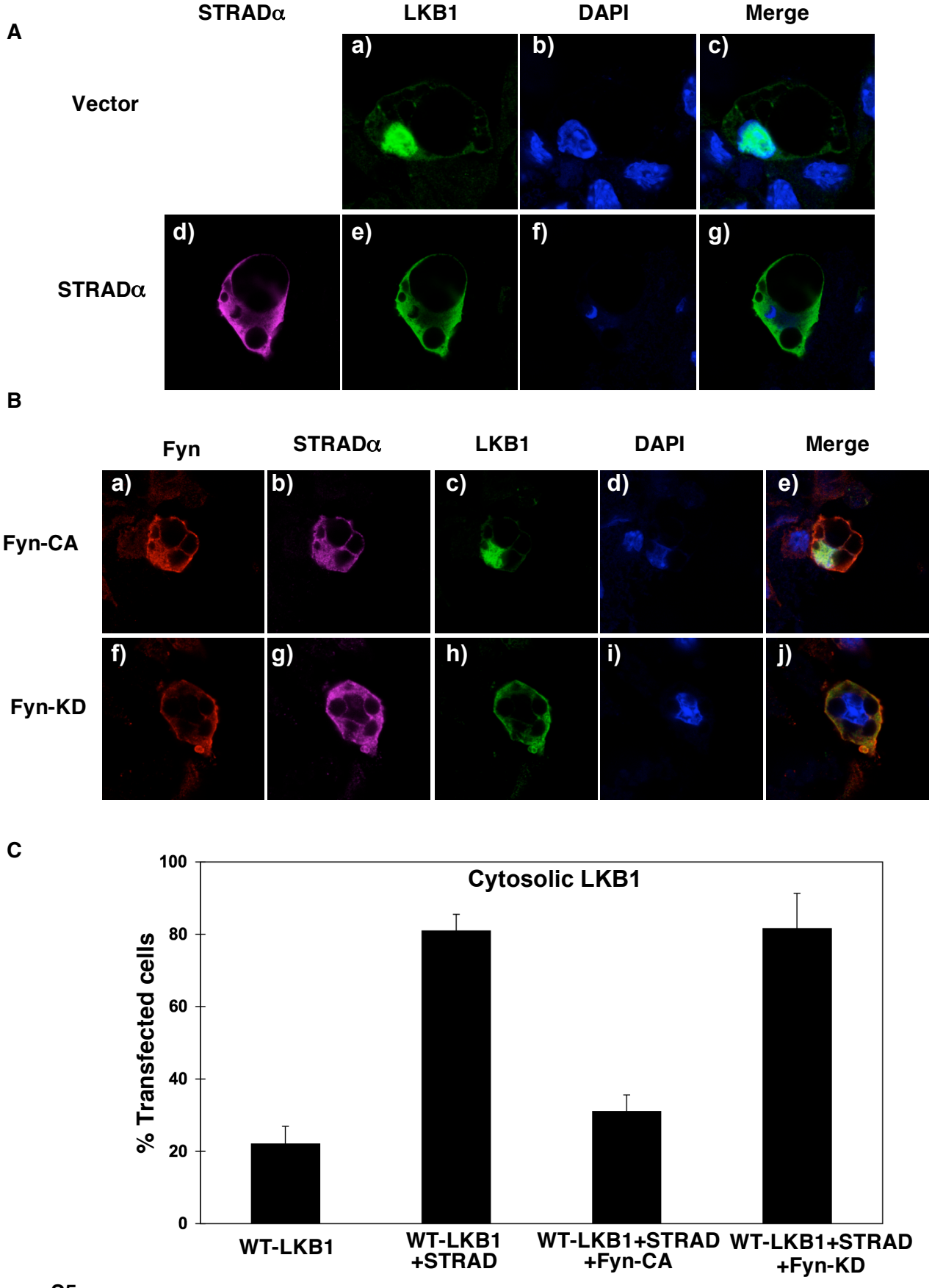
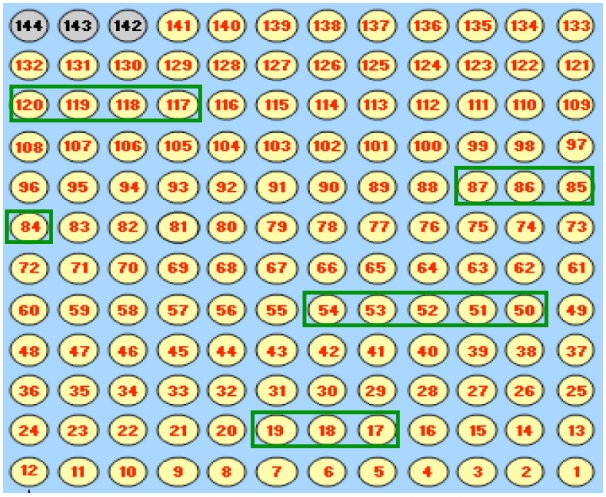

A
STRADα
LKB1
DAPI
Merge
Vector
STRADα
a)
b)
c)
d)
e)
f)
g)
B
Fyn
STRADα
LKB1
DAPI
Merge
Fyn-CA
Fyn-KD
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
C
Cytosolic LKB1
% Transfected cells
100
80
60
40
20
0
WT-LKB1
WT-LKB1 +STRAD
WT-LKB1+STRAD +Fyn-CA
WT-LKB1+STRAD +Fyn-KD

Figure S5

A

1	MEVVDPQQLGMFT	31	GEANVKKEIQLLR	61	NLLLTTGGTLKIS	91	SYAIPGDCGPPLS	121	IYTQDFTVPGQVP
2	VDPQQLGMFTEGE	32	NVKKEIQLLRRLR	62	LTTGGTLKISDLG	92	IPGDCGPPLSDLL	122	QDFTVPGQVPEEE
3	QQLGMFTEGELMS	33	KEIQLLRRLRHKN	63	GGTLKISDLGVAE	93	DCGPPLSDLLKGM	123	TVPGQVPEEEAS H
4	GMFTEGELMSVGM	34	QLLRRLRHKNVIQ	64	LKISDLGVAEALH	94	PPLSDLLKGMLEY	124	GQVPEEEASHNGQ
5	TEGELMSVGMDTF	35	RRLRHKNVIQLVD	65	SDLGVAEALHPFA	95	SDLLKGMLEYEPA	125	PEEEASHNGQRRG
6	ELMSVGMDTFIHR	36	RHKNVIQLVDVLY	66	GVAEALHPFAADD	96	LKGMLEYEPAKRF	126	EASHNGQRRGLPK
7	SVGMDTFIHRIDS	37	NVIQLVDVLYNEE	67	EALHPFAADDTCR	97	MLEYEPAKRFSIR	127	HNGQRRGLPKAVC
8	MDTFIHRIDSTEV	38	QLVDVLYNEEKQK	68	HPFAADDTCRTSQ	98	YEPAKRFSIRQIR	128	QRRGLPKAVCMNG
9	FIHRIDSTEVIYQ	39	DVLYNEEKQKMYM	69	AADDTCRTSQGSP	99	AKRFSIRQIRQHS	129	GLPKAVCMNGTEA
10	RIDSTEVIYQPRR	40	YNEEKQKMYMVME	70	DTCRTSQGSPAFQ	100	FSIRQIRQHSWFR	130	KAVCMNGTEAAQL
11	STEVIYQPRRKRA	41	EKQKMYMVMEYCV	71	RTSQGSPAFQPPE	101	RQIRQHSWFRKKH	131	CMNGTEAAQLSTK
12	VIYQPRRKRAKLI	42	KMYMVMEYCVCGM	72	QGSPAFQPPEIAN	102	RQHSWFRKKHPPA	132	GTEAAQLSTKSRA
13	QPRRKRAKLIGKY	43	MVMEYCVCGMQEM	73	PAFQPPEIANGLD	103	SWFRKKHPPAEA P	133	AAQLSTKSRAE G R
14	RKRAKLIGKYLM G	44	EYCVCGMQEMLDS	74	QPPEIANGLDTFS	104	RKKHPPAEAPVPI	134	LSTKSRAEGRAP N
15	AKLIGKYLMGDLL	45	VCGMQEMLDSVPE	75	EIANGLDTFSGFK	105	HPPAEAPVPIPPS	135	KSRAEGRAPNPA R
16	IGKYLMGDLLGEG	46	MQEMLDSVPEKRF	76	NGLDTFSGFKVDI	106	AEAPVPIPPSPDT	136	AEGRAPNPARKA C
17	**YLMGDLLGEGSYG**	47	MLDSVPEKRFPVC	77	DTFSGFKVDIWSA	107	PVPIPPSPDTKDR	137	RAPNPARKACSAS
18	**GDLLGEGSYGKVK**	48	SVPEKRFPVCQAH	78	SGFKVDIWSAGVT	108	IPPSPDTKDRWR S	138	NPARKACSASSK I
19	**LGEGSYGKVKEVL**	49	**EKRFPVCQAHGYF**	79	KVDIWSAGVTLYN	109	SPDTKDRWRSMTV	139	RKACSASSKIRRL
20	GSYGKVKEVLDSE	50	**FPVCQAHGYFCQL**	80	IWSAGVTLYNITT	110	TKDRWRSMTVVP Y	140	CSASSKIRRLSAC
21	GKVKEVLDSETLC	51	**CQAHGYFCQLIDG**	81	AGVTLYNITTGLY	111	RWRSMTVVPYLED	141	SSKIRRLSACKQQ
22	KEVLDSETLCRRA	52	**HGYFCQLIDGLEY**	82	TLYNITTGLYPFE	112	SMTVVPYLEDLHG		
23	LDSETLCRRAVKI	53	**FCQLIDGLEYLHS**	83	NITTGLYPFEGDN	113	VVPYLEDLHGADE		
24	ETLCRRAVKILKK	54	**LIDGLEYLHSQGI**	84	**TGLYPFEGDNIYK**	114	YLEDLHGADEDED		
25	CRRAVKILKKKKL	55	GLEYLHSQGIVHK	85	**YPFEGDNIYKLFE**	115	DLHGADEDEDLFD		
26	AVKILKKKKLRRI	56	YLHSQGIVHKDIK	86	**EGDNIYKLFENIG**	116	GADEDEDLFDIED		
27	ILKKKKLRRIPNG	57	SQGIVHKDIKPGN	87	**NIYKLFENIGKGS**	117	**EDEDLFDIEDDII**		
28	KKKLRRIPNGEA N	58	IVHKDIKPGNLLL	88	KLFENIGKGSYAI	118	**DLFDIEDDIIYTQ**		
29	LRRIPNGEANVKK	59	KDIKPGNLLLTTG	89	ENIGKGSYAIPGD	119	**DIEDDIIYTQDFT**		
30	IPNGEANVKKEIQ	60	KPGNLLLTTGGTL	90	GKGSYAIPGDCGP	120	**DDIIYTQDFTVPG**		

B



C

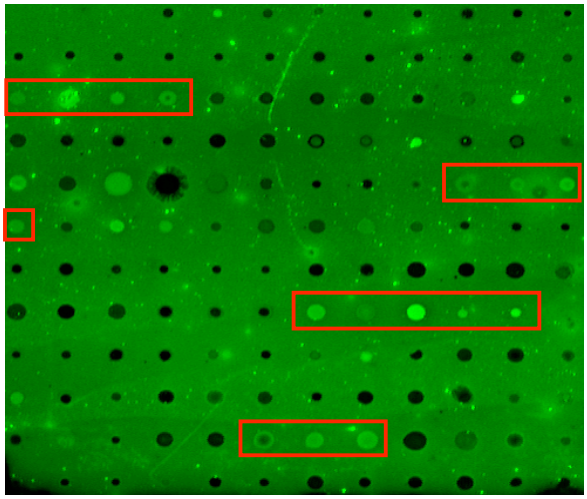


Figure S6

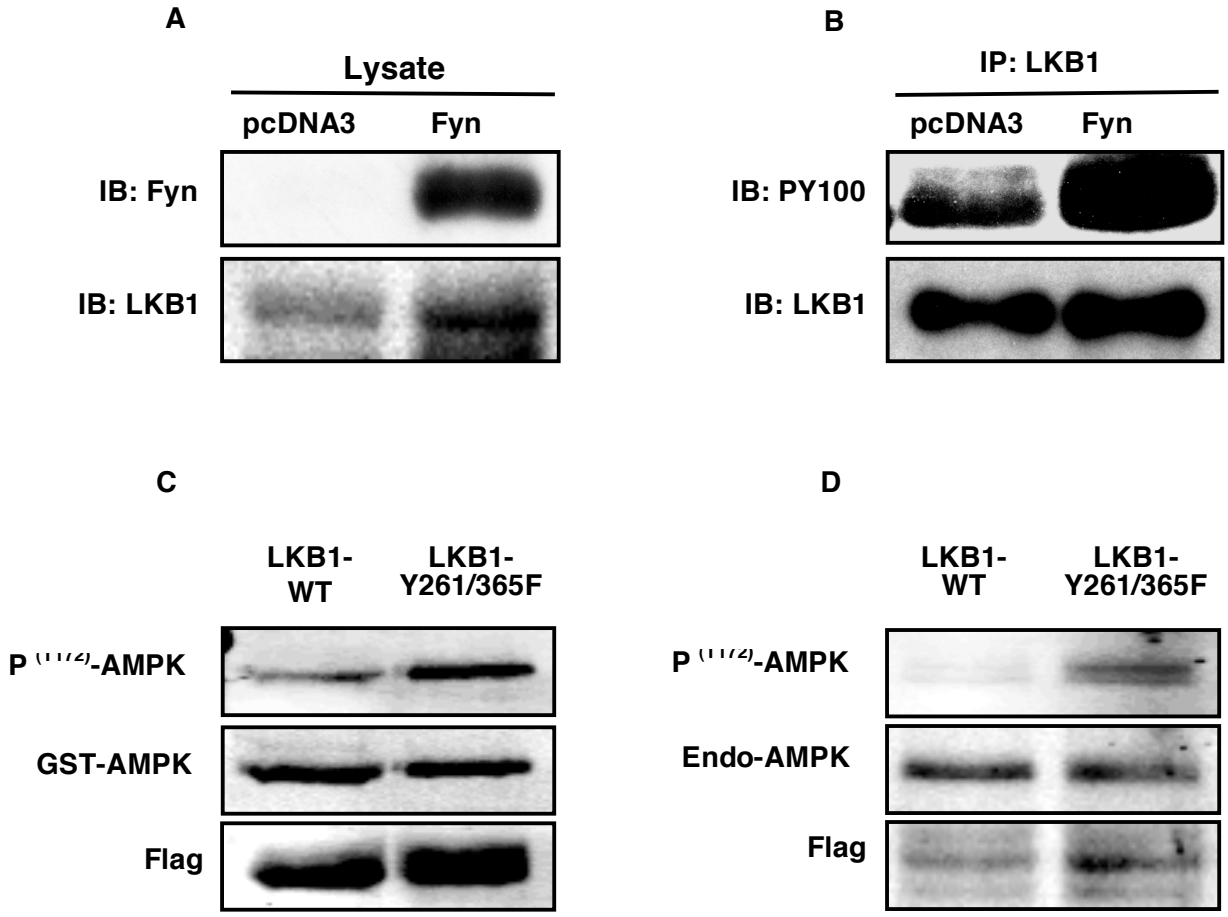

A
Lysate
pcDNA3
Fyn
IB: Fyn
IB: LKB1
B
IP: LKB1
pcDNA3
Fyn
IB: PY100
IB: LKB1
C
LKB1-WT
LKB1-Y261/365F
P (172)-AMPK
GST-AMPK
Flag
D
LKB1-WT
LKB1-Y261/365F
P (172)-AMPK
Endo-AMPK
Flag

Figure S7

Inventory

There are no supplemental experimental procedures or additional references.

Figure 1S: Effects of the SU6656 on body mass and physical activity in the Fyn null mice. Related to Figure 2 that shows the effects of SU6656 in wild type mice.

Figure 2S: Requested by the editor. Composite image for significant representation of C2C12 cells treated with vehicle or SU6656. Related to Figure 4A.

Figure 3S: Requested by the editor. Composite image for significant representation of C2C12 cells transfected with Fyn-CA or Fyn-KD. Related to Figure 4C.

Figure 4S: Requested by the editor. Composite image for significant representation of 3T3L1 cells transfected with Fyn-CA or Fyn-KD. Related to Figure 4E.

Figure 5S: Requested by the editor and the reviewers. Representation of 3T3L1 cells transfected with STRADa, Fyn-KD or Fyn-CA and signal quantification. Related to Figure 4.

Figure 6S: Represents the JPT peptide technology. Related to Figure 5.

Figure 7S: Represents the effects of LKB1-Y261/365F double mutant in vivo. Related to Figure 7.

Supplemental Figure Legends

Figure S1: SU6656 does not induced weight loss or alter locomoter activity in the Fyn null mice. A) Body Mass before (T= 0, open bar) and after (T= 12 h (dark bar) vehicle or SU6656 injection to the Fyn null mice. **B)** Locomotor activity.

Figure S2: The selective Src family kinase inhibitor SU6656 alters LKB1 subcellular distribution in C2C12 myotubes. Five representative images of C2C12 myotubes transfected with pEGFP-LKB1 and incubated with vehicle **(A)** or SU6656 **(B).** Cells were imaged as described in Figure 4.

Figure S3: Expression of kinase-defective Fyn in C2C12 myotubes results in nuclear export of LKB1. Five representative images of C2C12 myotubes co-transfected with pEGFP-LKB1 and pcDNA3-Fyn-CA **(A)** or pcDNA3-Fyn-KD **(B).** Cells were imaged as described in Figure 4.

Figure S4: Expression of kinase-defective Fyn in 3T3L1 adipocytes results in nuclear export of LKB1. Five representative images of differentiated 3T3L1 adipocytes co-transfected with pcDNA3-LKB1 and pcDNA3-Fyn-CA **(A)** or pcDNA3-Fyn-KD **(B).** Cells were imaged as described in Figure 4.

Figure S5: STRADα does not affect Fyn-induced LKB1 translocation into the nucleus. A) Fully differentiated 3T3L1 adipocytes were transfected with pcDNA3-Flag-LKB1 (panels a-c) or with pcDNA3-Flag-LKB1 and Omni-STRADα (panels d-g). Immunofluorescence was performed using a mouse Flag

monoclonal antibody and rabbit Omni monoclonal antibody followed by Alexa Fluor 488 anti-mouse IgG (green) and Alexa Fluor 647 anti-rabbit IgG (purple). Nuclei were visualized with DAPI (blue). **B)** Fully differentiated 3T3L adipocytes were co-transfected with pcDNA3-Flag-LKB1, Omni-STRADα and pcDNA3-V5-Fyn-CA (panels a-e) or pcDNA3-V5-Fyn-KD (panels f-j). Immunofluorescence was performed using a mouse Flag monoclonal antibody, a chicken V5 polyclonal antibody and rabbit Omni monoclonal antibody followed by Alexa Fluor 488 anti-mouse IgG (green), Alexa Fluor 594 anti-chicken (red) and Alexa Fluor 647 Anti-rabbit IgG (purple) secondary antibodies. Nuclei were visualized with DAPI (blue). **C)** Quantification of the LKB1 signal in the cytoplasm of 3T3L1 cell. Data are representative of n = 3 experiments.

Figure S6: A) Sequence of the 141 LKB1 overlapping peptides used as targets for the identification of tyrosine acceptors for Fyn kinase phosphorylation. **B)** Scheme of the glass slide where the 141 overlapping peptides corresponding to LKB1 sequence was immobilized. **C)** Potential tyrosine site phosphorylated by Fyn.

Figure S7: Expression of the LKB1-Y261/365F double mutant increases T^{172}-AMPK phosphorylation. A) Tibialis anterior muscle was transfected with V5-Fyn-CA and muscle extracts were immunoblotted for Fyn and LKB1. **B)** Muscle extracts were immunoprecipitated with the LKB1 antibody and immunoblotted with the PY100 phosphotyrosine and LKB1 antibody. **C)** HeLa cells were transfected with the GST-AMPK α subunit cDNA and Flag-LKB1-WT or Flag-LKB1-Y261/365F double mutant cDNAs. Levels of LKB1 and AMPK α subunit expression were assessed by immunoblotting with a Flag or AMPK α subunit antibody, respectively. AMPK α subunit phosphorylation were determined using the phospho T172 specific antibody. **D)** Tibialis anterior muscle was transfected with the Flag-LKB1-WT or Flag-LKB1-Y261/365F double mutant cDNAs. Levels of LKB1 expression and endogenous AMPK α subunit levels were assessed by immunoblotting with a Flag or AMPK α subunit antibody, respectively. Endogenous AMPK α subunit phosphorylation were determined using the phospho T172 specific antibody.