Legends to supplementary material

Supplementary Table 1: List of siRNA sequences used in this study.

Supplementary Figure S1: Expression profiling of dynein subunits in HeLa, hTERT-RPE1, human dermal fibroblasts and HepG2 cells. Using reverse transcription PCR on total RNA from cells we were unable to detect expression of DYNC1I1, DYNLRB2, or DYNLL2 in any cell type tested. Human dermal fibroblasts express only very low levels of Roadblock 1 (DYNLRB1) and LC8 (DYNLL1). Samples are shown in duplicate with the second set containing two-fold more starting cDNA in each reaction.

Supplementary Figure S2: Effect of dynein subunit suppression on Golgi architecture and function. Cells were transfected with each siRNA as indicated and processed for immunofluorescence to label GalT (green in merges) and β'-COP (red in merges). Cells were counterstained with DAPI to label nuclei (blue). These data show entire fields of view from which the enlargements in Figure 2A are taken. Bar (all panels) = 20 μm.

Supplementary Figure S3: Dynein subunit suppression does not affect expression of GalT or other proteins. Cells were transfected with each siRNA as indicated, lysed, and separated by SDS-PAGE followed by immunoblotting with antibodies as indicated. No difference in expression level was observed in three independent experiments.

Supplementary Figure S4: Effect of dynein subunit suppression on Golgi (GM130) architecture. Cells were transfected with each siRNA as indicated and processed for immunofluorescence to label GM130 (green in merges). Cells were counterstained with DAPI to label nuclei (blue). These data show entire fields of view from which the enlargements in Figure 2B are taken. Bar (all panels) = 20 μm.

Supplementary Figure S5: Effect of dynein subunit suppression on ERES and ERGIC. Cells were transfected with each siRNA as indicated and processed for immunofluorescence to label ERGIC-53 (green in merges) and Sec24C (red in merges). Cells were counterstained with DAPI to label nuclei (blue). These data show entire fields of view from which the enlargements in Figure 3 are taken. Bar (all panels) = 20 μm.

Supplementary Figure S6: Effect of dynein subunit suppression on Golgi architecture and transferrin uptake in living cells. Cells stably expressing GRASP65-GFP (green) were depleted of targets as indicated and loaded with AlexaFluor®-568-transferrin (red) for 1 hour at 37°C followed by imaging also at 37°C. At this time AlexaFluor®-568-transferrin labels a significant intracellular pool with little peripheral labeling in controls; peripheral accumulation indicates a defect in centripetal translocation of transferrin-positive endosomes. These data show entire fields of view from which the enlargements in Figure 4 are taken. Bar (all panels) = 20 μm.

Supplementary Figure S7: Effect of dynein subunit suppression on the microtubule network. Cells were transfected with each siRNA as indicated and processed for immunofluorescence to label α-tubulin (green in merges). Cells were counterstained with DAPI to label nuclei (blue). These data show entire fields of view from which the enlargements in the right-hand column of Figure 5 are taken. Bar (all panels) = 20 μm.

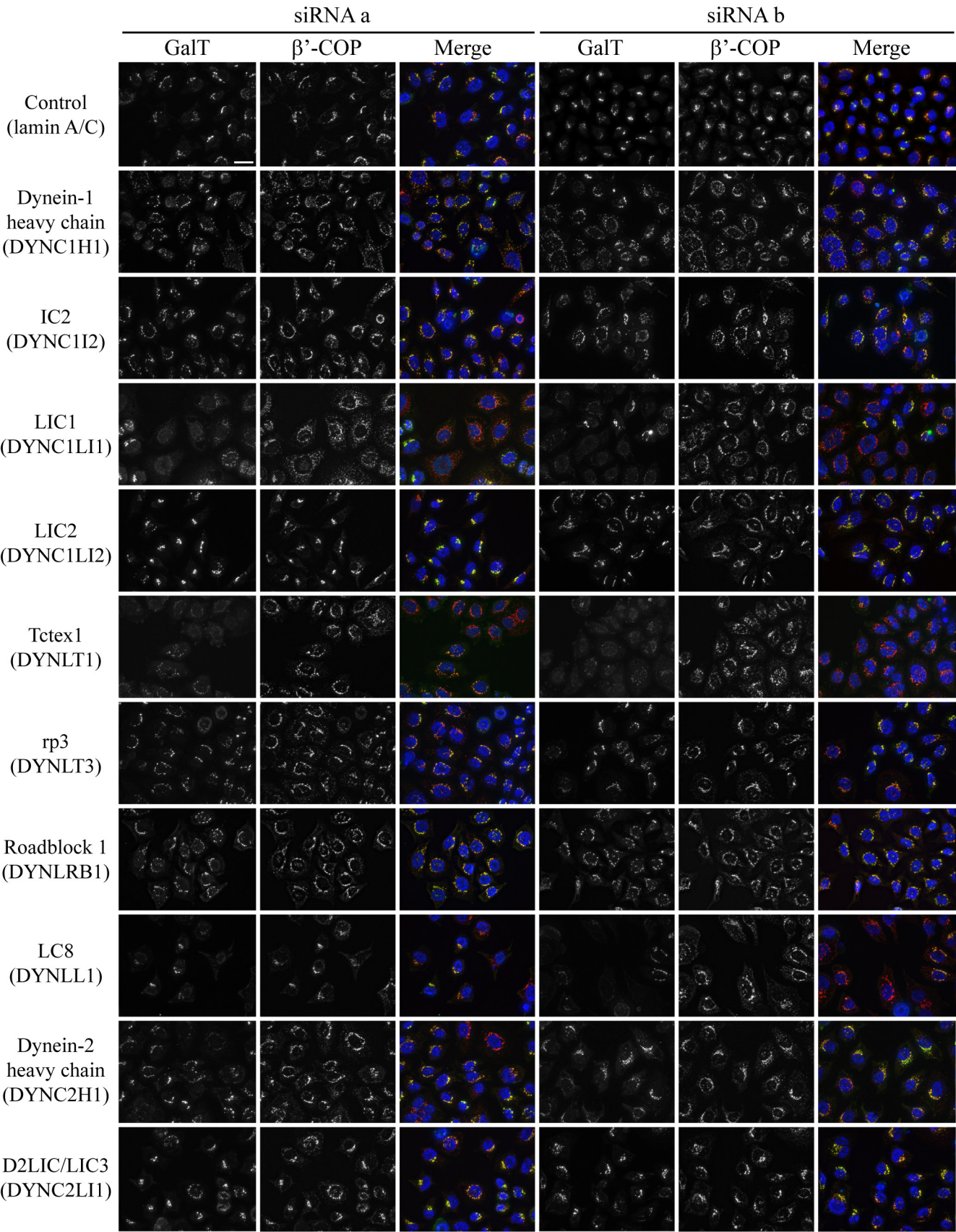
Supplementary Table 1: Sequences of siRNA duplexes used in this study.

Target	Sequence
DYNC1H1 a	ACA UCA ACA UAG ACA UUC A
DYNC1H1 b	CCA AGC AGA UAA GGC AAU A
DYNC1I1 a	CAG CAA GUG UGG CCA UUG A
DYNC1I1 b	ACA GCA GAU CCU UCA UUC A
DYNC1I2 a	UCA CUG GCA UCC AUU GUC A
DYNC1I2 b	GCA GUA GCU GUG ACA UCU A
DYNC1LI1 a	AGA UGA CAG UGU AGU UGU A
DYNC1LI1 b	GAA CAU GAC UAC AGA GAU G
DYNC1LI1 c	GCU GGA GUG CUU CUU CAG A
DYNC1LI1 d	UCA CUG AUC CUG UAA GUU G
DYNC1LI2 a	ACC UCG ACU UGU UGU AUA A
DYNC1LI2 b	GCC GGA AGA UGC AUA UGA A
DYNC1LI2 c	UAA UGC UGC UAG AUU GUU A
DYNC1LI2 d	GUC AGC AGU UAC AGA AUU A
DYNLT1 a	AUA CAU CGU GAC CUG UGU A
DYNLT1 b	GUG AAC CAG UGG ACC ACA A
DYNLT3 a	CCA GUG GAC UGC AAG CAU A
DYNLT3 b	UCC UUA ACA CAC CUG GUU A
DYNLRB1 a	UCA UGU CAG UGG ACU AGC A
DYNLRB1 b	UCA UCG UCG UGA ACA CAG A
DYNLRB2 a	UCA UGG UAG CUC CAG AUA A
DYNLRB2 b	GCA CAG UUC GUG AUA UUG A
DYNLL1 a	AUG CGG ACA UGU CGG AAG A
DYNLL1 b	AAC AAG GAC UGC AGC CUA A
DYNLL2 a	GGA CAU UGC UGC CUA UAU C
DYNLL2 b	GUU GCA AUC CUC CUC UUC A
DYNC2H1 a	GGA AUU GAA UAC UCU UCA A
DYNC2H1 b	ACA GGC UCU UCU CUC UGA A
DYNC2H1 c	GCA GUG CAC UUA UUC AAG A
DYNC2H1 d	GUC UGA AGA UAA CAU AUG A
DYNC2H1 e	UCA GUA GAA UCU AAU GAC A
DYNC2LI1 a	GAC UAC UAU UAU UCU AAG G
DYNC2LI1 b	UGG AAG UGA AGG UGA UGG A.

All sequences are shown 5'-3'

Suffixes "a", "b" etc are used throughout to denote the two different duplexes used.

Marker
Negative control
HeLa
hTERT-RPE1
HDF
HepG2
HeLa
hTERT-RPE1
HDF
HepG2
IC1 (DYNC1I1)
IC2 (DYNC1I2)
D2LIC/LIC3 (DYNC2LI1)
Tctex1 (DYNLT1)
Roadblock 1 (DYNLRB1)
LC8 (DYNLLL1)
rp3 (DYNLT3)
Roadblock 2 (DYNLRB2)
8 light chain 2 (DYNLLL2)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siRNA b
GalT
β'-COP
Merge
GalT
β'-COP
Merge
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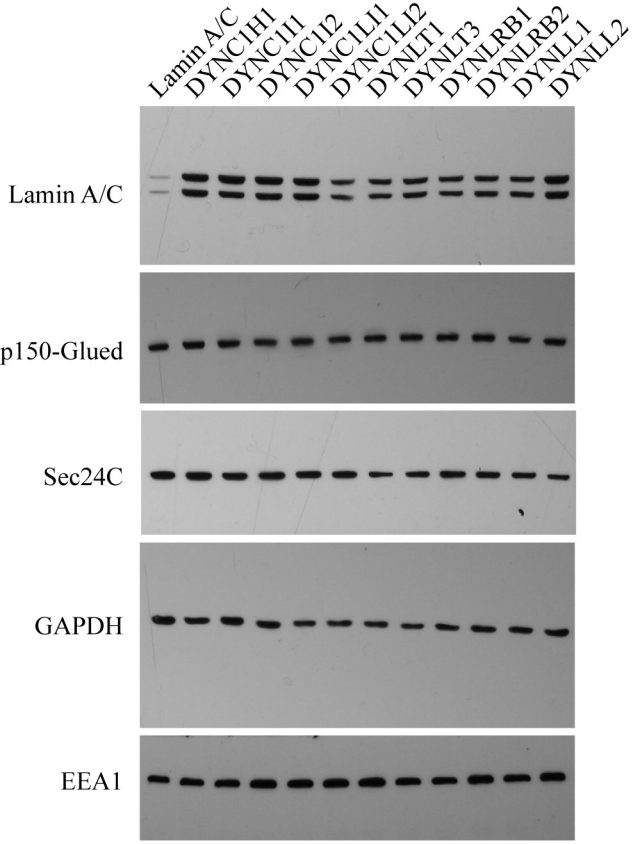
IC2 (DYNC1I2)
LIC1 (DYNC1LI1)
LIC2 (DYNC1LI2)
Tctex1 (DYNLT1)
rp3 (DYNLT3)
Roadblock 1 (DYNLRB1)
LC8 (DYNLL1)
Dynein-2 heavy chain (DYNC2H1)
D2LIC/LIC3 (DYNC2LI1)


Lamin A/C
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DYNC1I1
DYNC1I2
DYNC1LI1
DYNC1LI2
DYNLT1
DYNLT3
DYNLRB1
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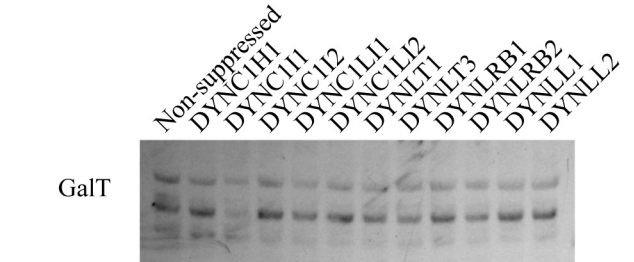
Lamin A/C
p150-Glued
Sec24C
GAPDH
EEA1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DYNC1LI2
DYNLT1
DYNLT3
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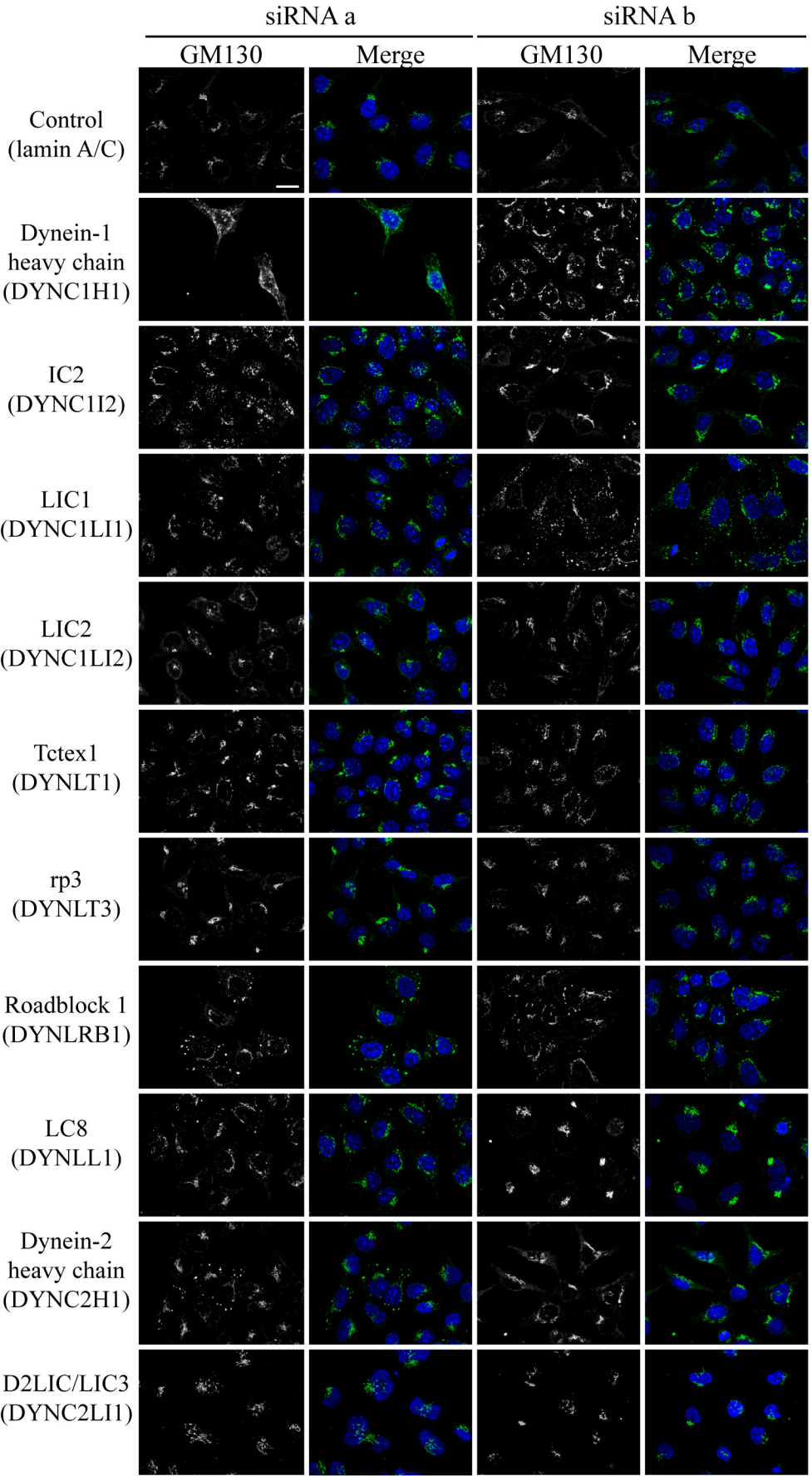
DYNLRB2
DYNLL1
DYNLL2
GalT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siRNA b
GM130
Merge
GM130
Merge
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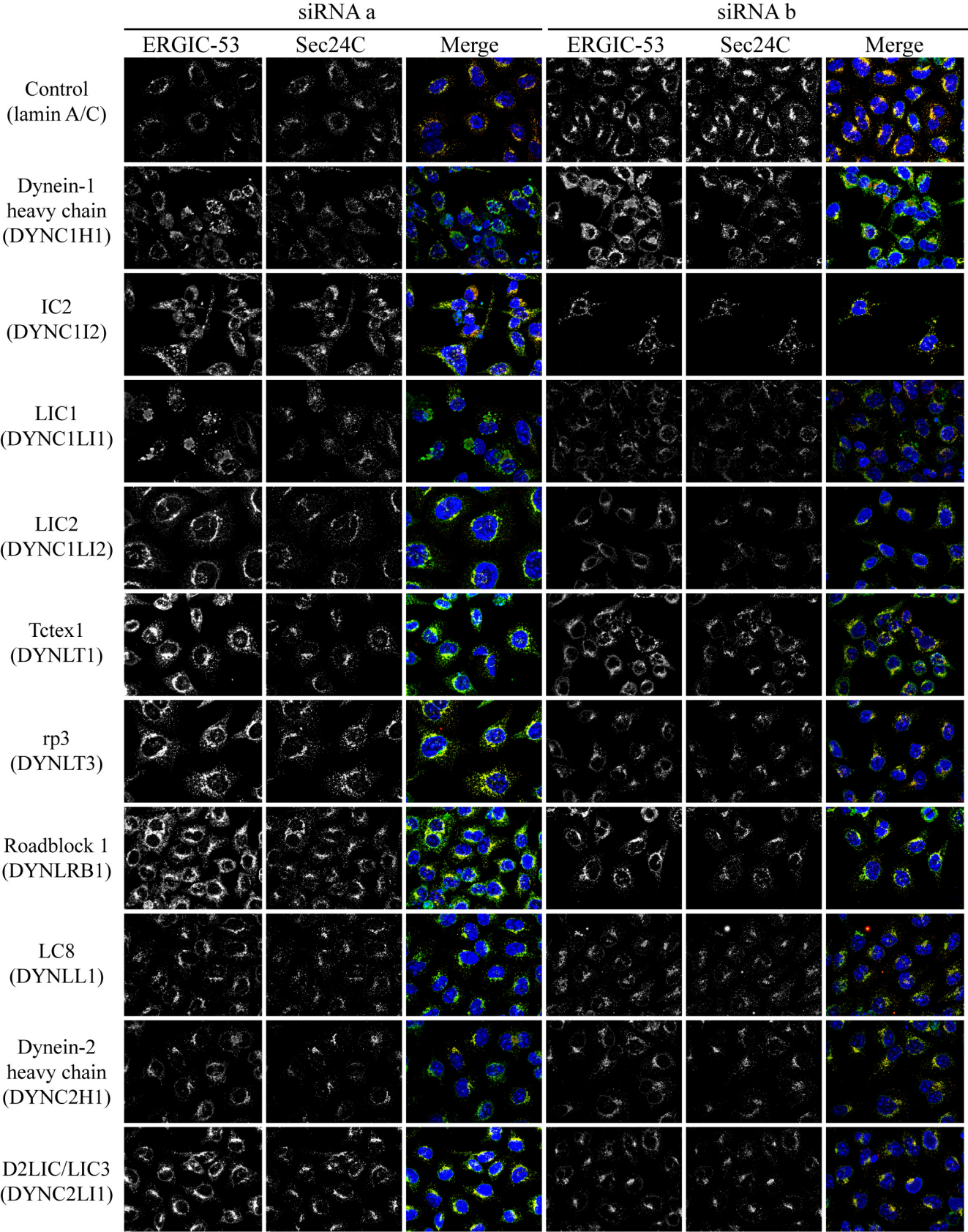
IC2 (DYNC1I2)
LIC1 (DYNC1LI1)
LIC2 (DYNC1LI2)
Tctex1 (DYNLT1)
rp3 (DYNLT3)
Roadblock 1 (DYNLRB1)
LC8 (DYNLL1)
Dynein-2 heavy chain (DYNC2H1)
D2LIC/LIC3 (DYNC2LI1)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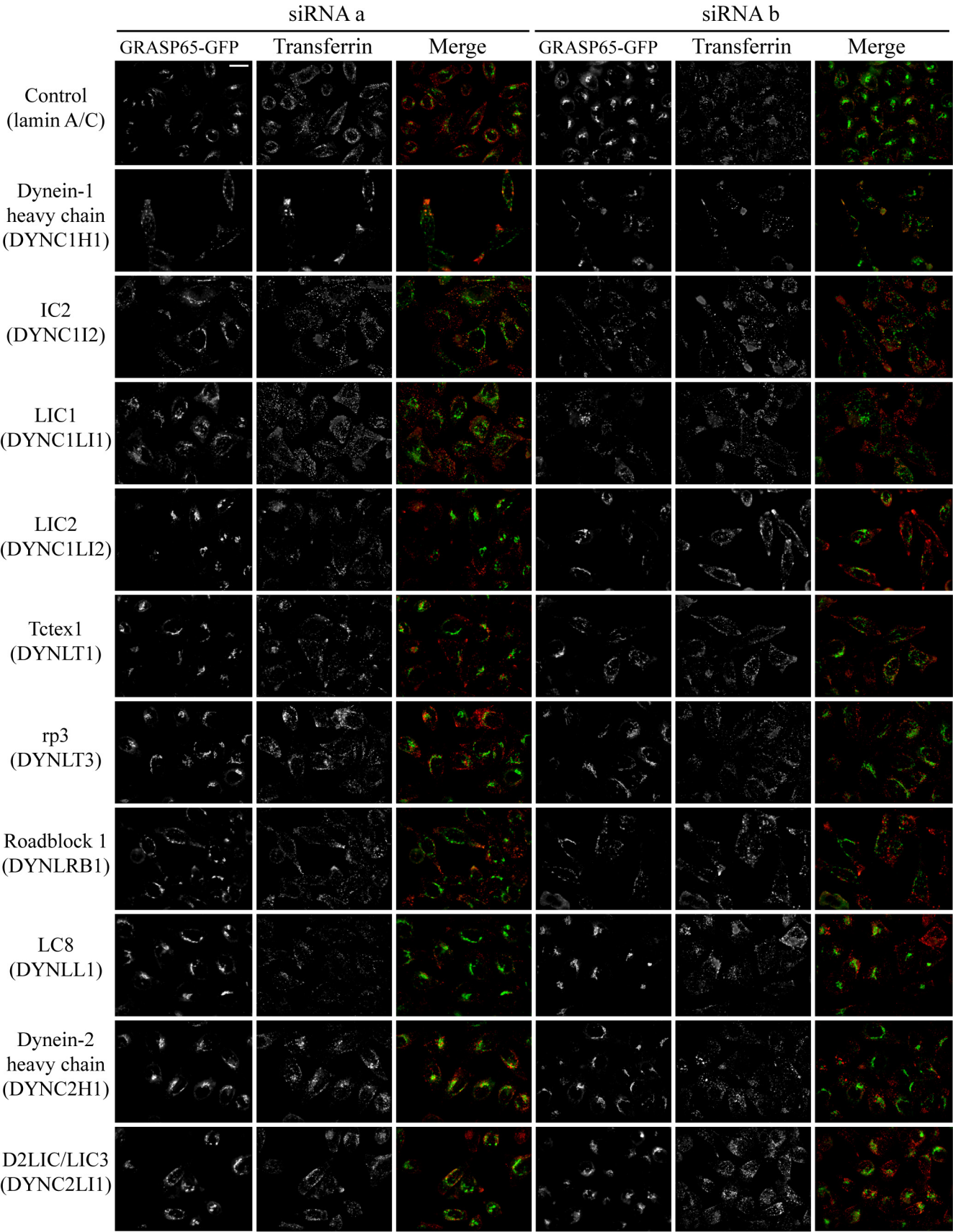
siRNA b
ERGIC-53
Sec24C
Merge
ERGIC-53
Sec24C
Merge
Control (lamin A/C)
Dynein-1 heavy chain (DYNC1H1)
IC2 (DYNC1I2)
LIC1 (DYNC1LI1)
LIC2 (DYNC1LI2)
Tctex1 (DYNLT1)
rp3 (DYNLT3)
Roadblock 1 (DYNLRB1)
LC8 (DYNLL1)
Dynein-2 heavy chain (DYNC2H1)
D2LIC/LIC3 (DYNC2LI1)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Transferrin
Merge
GRASP65-GFP
Transferrin
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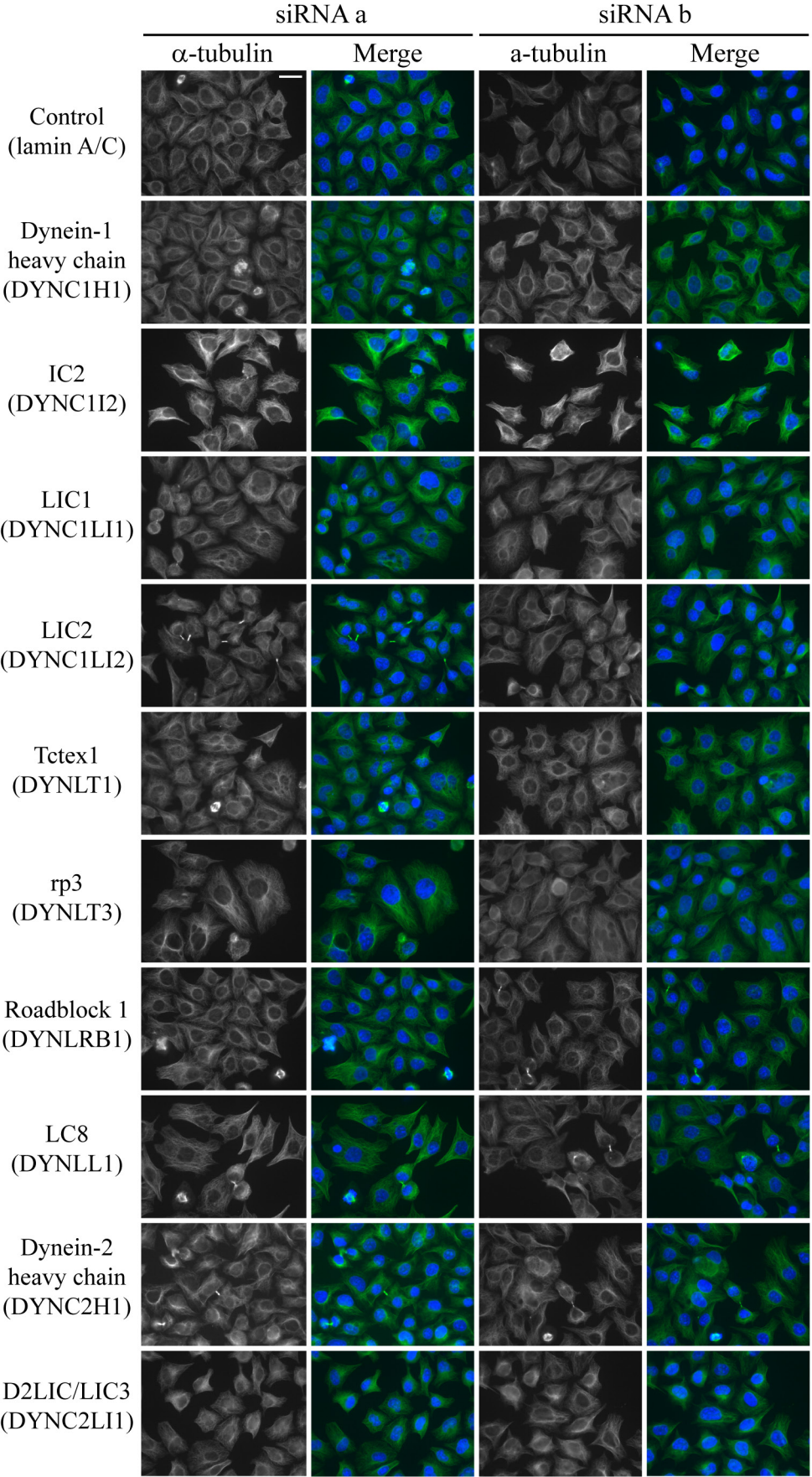
IC2 (DYNC1I2)
LIC1 (DYNC1LI1)
LIC2 (DYNC1LI2)
Tctex1 (DYNLT1)
rp3 (DYNLT3)
Roadblock 1 (DYNLRB1)
LC8 (DYNLL1)
Dynein-2 heavy chain (DYNC2H1)
D2LIC/LIC3 (DYNC2LI1)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siRNA b
α-tubulin
Merge
a-tubulin
Merge
Control (lamin A/C)
Dynein-1 heavy chain (DYNC1H1)
IC2 (DYNC1I2)
LIC1 (DYNC1LI1)
LIC2 (DYNC1LI2)
Tctex1 (DYNLT1)
rp3 (DYNLT3)
Roadblock 1 (DYNLRB1)
LC8 (DYNLL1)
Dynein-2 heavy chain (DYNC2H1)
D2LIC/LIC3 (DYNC2LI1)