**Table S1. Statistics for Golgi phenotypes following LIS1, NDE1, and NDEL1 overexpression**

For each set of experiments in which GFP fusion proteins were expressed in HeLaM cells (as indicated), a statistical analysis using multinomial logistic regression was performed. Data are not shown for the 'full scatter' phenotype, as these were not observed under any condition.

| GFP-LIS1* vs GFP expression | Compact | Ribbon | Broken ribbon | Partial scatter |
|---|---|---|---|---|
| Change in odds | 4.6 | (1.0) | 1.0 | 1.7 |
| 95% Confidence Interval | 2.9 – 7.1 | n/a | 0.7 – 1.4 | 0.8 – 3.7 |
| *P* value | <0.001 | n/a | 0.68 | 0.17 |

| GFP-NDE1* vs GFP expression | Compact | Ribbon | Broken ribbon | Partial scatter |
|---|---|---|---|---|
| Change in odds | 10.3 | (1.0) | 0.7 | 0.8 |
| 95% Confidence Interval | 6.7 – 15.7 | n/a | 0.5 – 1.1 | 0.3 – 2.2 |
| *P* value | <0.001 | n/a | 0.40 | 0.68 |

| GFP-NDEL1* vs GFP expression | Compact | Ribbon | Broken ribbon | Partial scatter |
|---|---|---|---|---|
| Change in odds | 6.9 | (1.0) | 0.9 | 1.5 |
| 95% Confidence Interval | 4.5 – 10.7 | n/a | 0.6 – 1.4 | 0.7 – 3.6 |
| *P* value | <0.001 | n/a | 0.77 | 0.26 |

**Table S2. Statistics for Golgi phenotypes in knockdown experiments**

For each set of knockdown experiments, as indicated, a statistical analysis using multinomial logistic regression was performed. Here, the odds of obtaining indicated Golgi phenotypes, relative to the most likely normal phenotype (i.e. a Golgi ribbon), was calculated in experimental samples (i.e. knockdown cells, or knockdown cells expressing GFP), compared to the odds in control cells (i.e. lamin knockdowns, or knockdown cells expressing GFP fusion proteins). This change in odds is shown, together with the 95% Confidence Interval and the *P* value that the change is significant.

| LIS1 kd vs Lamin kd | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | n.m. | (1.0) | 2.9 | 89.3 | 4088 |
| 95% Confidence Interval | n.m. | n/a | 2.1 - 4.0 | 57.7 - 138.2 | 1287 - 12982 |
| *P* value | n.m. | n/a | <0.001 | <0.001 | <0.001 |

| LIS1 kd; GFP vs GFP-LIS1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.1 | (1.0) | 2.3 | 8.7 | 154 |
| 95% Confidence Interval | 0.01 – 0.5 | n/a | 1.2 – 4.2 | 4.9 – 15.4 | 71 - 335 |
| *P* value | 0.07 | n/a | 0.05 | <0.001 | <0.001 |

| LIS1 kd; GFP vs GFP-NDE1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.2 | (1.0) | 1.0 | 1.7 | 8.3 |
| 95% Confidence Interval | 0.03 – 2.1 | n/a | 0.5 – 2.0 | 0.9 – 2.9 | 4.5 – 14.9 |
| *P* value | 0.19 | n/a | 0.90 | 0.11 | <0.001 |

| LIS1 kd; GFP vs GFP-NDEL1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.1 | (1.0) | 1.7 | 3.8 | 15.2 |
| 95% Confidence Interval | 0.01 – 1.0 | n/a | 0.9 – 3.2 | 2.1 – 6.7 | 8.7 – 26.5 |
| *P* value | 0.05 | n/a | 0.09 | <0.001 | <0.001 |

| NDE1/NDEL1 kd; GFP vs GFP-NDE1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.6 | (1.0) | 2.1 | 9.1 | 91 |
| 95% Confidence Interval | 0.1 – 2.1 | n/a | 1.0 – 4.4 | 4.7 – 17.4 | 43 - 190 |
| *P* value | 0.39 | n/a | 0.05 | <0.001 | <0.001 |

| NDE1/NDEL1 kd; GFP vs GFP-NDEL1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.5 | (1.0) | 2.4 | 9.5 | 387 |
| 95% Confidence Interval | 0.01 – 0.5 | n/a | 1.2 – 5.0 | 5.0 – 18.0 | 140 - 1067 |
| *P* value | 0.07 | n/a | 0.02 | <0.001 | <0.001 |

| NDE1/NDEL1 kd; GFP vs GFP-LIS1* expression | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.9 | (1.0) | 0.6 | 1.0 | 3.5 |
| 95% Confidence Interval | 0.2 – 4.7 | n/a | 0.3 to 1.4 | 0.5 to 2.1 | 1.6 to 7.7 |
| *P* value | 0.9 | n/a | 0.24 | 0.96 | 0.01 |

**Table S3. Microtubule phenotypes of RNAi-depleted cells**

RNAi-depleted cells scored for DAPI staining phenotypes (Table 2) were also stained with anti-tubulin antibody and scored for microtubule organization phenotypes as indicated. Examples of each phenotype are illustrated in Fig. S6.

| RNAi condition | Cell number | Broad central focus % | No focus % | Longitudinal % | Whorls % |
|---|---|---|---|---|---|
| Mock | 311 | 26.0 | 72.3 | 1.6 | 0 |
| Lamin | 242 | 23.6 | 70.7 | 5.8 | 0 |
| LIS1 | 257 | 8.6 | 77.8 | 6.6 | 7.0 |
| NDE1 | 268 | 23.1 | 65.3 | 4.1 | 7.5 |
| NDEL1 | 324 | 10.2 | 69.1 | 20.7 | 0 |
| NDE1+NDEL1 | 267 | 12.0 | 76.8 | 7.9 | 3.4 |

## Table S4. Statistics for Cdc42 expression

For each set of experiments in which Cdc42 mutants were expressed in HeLaM cells (as indicated), a statistical analysis using multinomial logistic regression was performed using cells expressing WT Cdc42 as a control.

| Cdc42(G12V) vs Cdc42 WT | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.7 | (1.0) | 1.8 | 3.4 | 2.4 |
| 95% Confidence Interval | 0.4 – 1.2 | n/a | 1.4 – 2.3 | 2.3 – 5.0 | 0.8 – 6.9 |
| $P$ value | 0.17 | n/a | <0.001 | <0.001 | 0.12 |

| Cdc42(T17N) vs Cdc42 WT | Compact | Ribbon | Broken ribbon | Partial scatter | Full scatter |
|---|---|---|---|---|---|
| Change in odds | 0.8 | (1.0) | 0.8 | 0.9 | 0.2 |
| 95% Confidence Interval | 0.5 – 1.3 | n/a | 0.7 – 1.1 | 0.6 – 1.4 | 0.02 – 1.4 |
| $P$ value | 0.38 | n/a | 0.20 | 0.6 | 0.11 |