LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure S1: Characterisation of Sec23 depletion

(A) S2 cells that were (+) or not (-) treated with ds RNA for Sec23 were lysed and subjected to WB with an anti-sec23 antibody. Endogenous Sec23 was efficiently depleted. The asterisk indicates a background band that is not depleted.

(B) Effect of Sec23 depletion (+ds sec23) on cell growth rate.

(C) IF visualization of GFP-Sec23 in mock treated (-ds RNA) and Sec23 depleted (+ds sec23) GFP-Sec23 stably expressing S2 cells.

(D, D') EM of Sec23 depleted S2 cells showing ER proliferation and dilation (arrows).

Scale bars: 5 μm (C) and 200nm (D)

Supplementary Figure S2: Overexpression of Sar1[T34N] does not abolish dSec16 localisation to tER sites.

(A-D) IF localisation of dGMAP (A), d120kd (B), endogenous dSec16 (C), and Sec31 (D) in S2 cells transfected with Sar1[T34N]-GFP. Arrows point to transfected cells.

(D-E) IF localisation of endogenous dSec16 (red) in S2 cells transfected with wild type Sar1 (E) and with the GTP locked form of Sar1[H74G]-GFP (F). Surprisingly, the two fluorescent patterns seems to juxtapose rather than overlap, perhaps because, as COPII vesicles that are formed in the presence of the active Sar1 mutant do not uncoat, dSec16 is not accessible to its antibody.

(F) Double labeling of endogenous dSec16 (10nm) and GFP (15nm) on frozen sections of Sar1[H74G]-GFP transfected S2 cells shows that they both decorate the same tER sites. Scale bars: 5μm (A, B), 200nm (F).

Supplementary Figure S3: Inhibition of ER to Golgi transport does not change the localization of Sar1[H74G] and dSec16 to tER sites.

(A-A') Localisation of Sar1[H74G]-GFP to ER exit sites in mock (-BFA) and BFA treated S2 cells. Note that the Sar1[H74G]-GFP localization does not change (A, projection) and is still co-localised with dSec16 (A', confocal section).

(B) Localisation of Fringe-GFP in BFA treated cells. Note that, as expected, it is retained in the ER, showing that BFA was functional in blocking the ER to Golgi transport.

(C) IF localisation of endogenous dSec16 in cells mock treated or treated with BFA. Note that the pattern is identical.

Scale bars: 5μm

Supplementary Figure S4: Intracellular localisation of dSec16 truncations

(A-A') IF localisation (A) of ΔC-dSec16-V5 (green) and dGMAP (red) in S2 cells. ΔC-dSec16-V5 localisation by IEM (A').

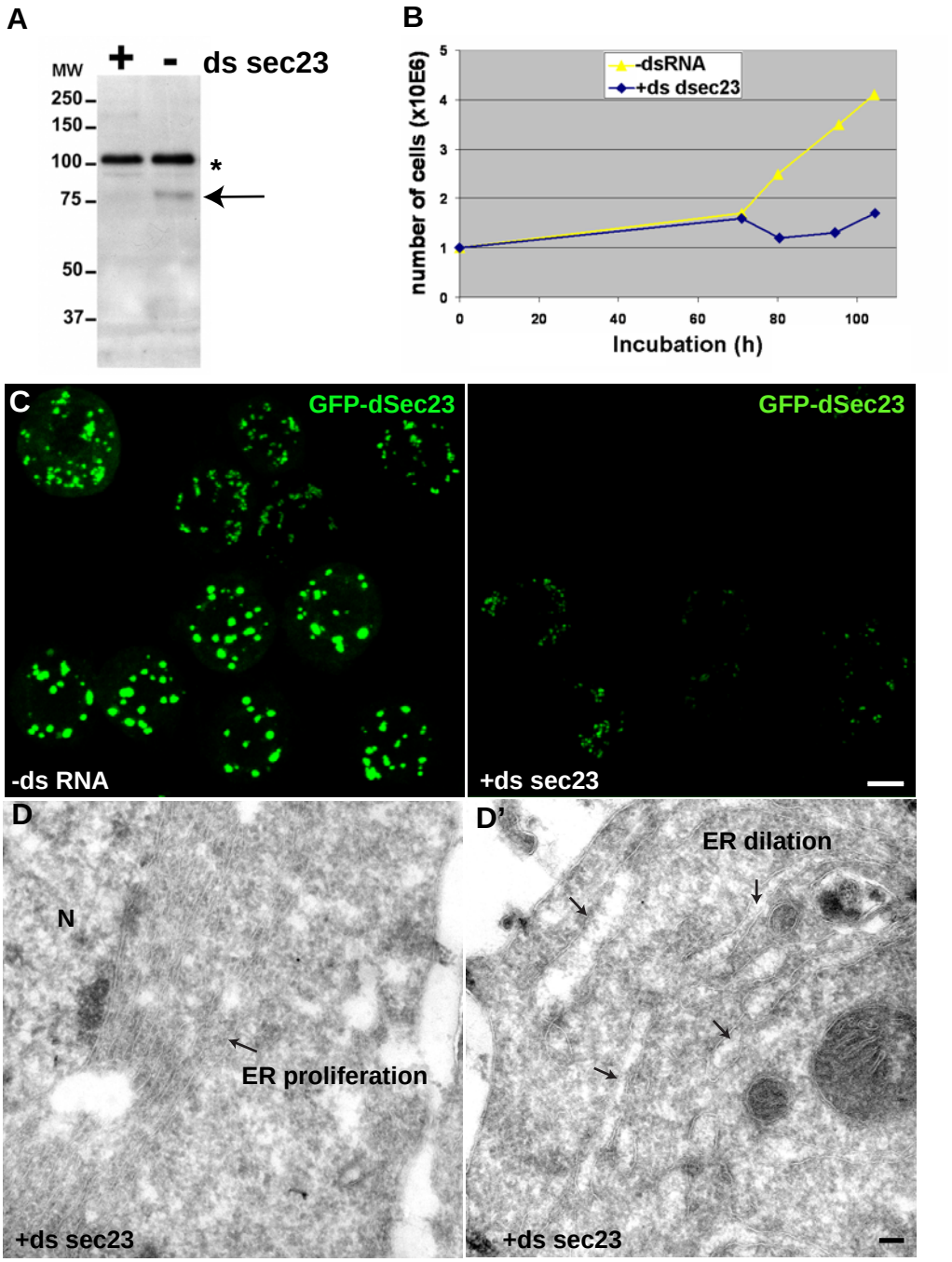
(B-E) IF localisation of V5 tagged dSec16 truncated mutants (green) and dGMAP (red). Note that NC3.4-CCD and NC2-CCD show partial colocalisation with dGMAP whereas NC2-CCD and CCD alone do not.

(F-G) IF localisation of ΔC-dSec16-V5 (green, F) and NC2.3-CCD-V5 (green, G) and dGMAP (red) in dSec16 depleted S2 cell). ΔC-dSec16-V5 localisation by IEM in the depleted cells (F'). Note that it still localises to ER cup in the absence of endogenous dSec16.

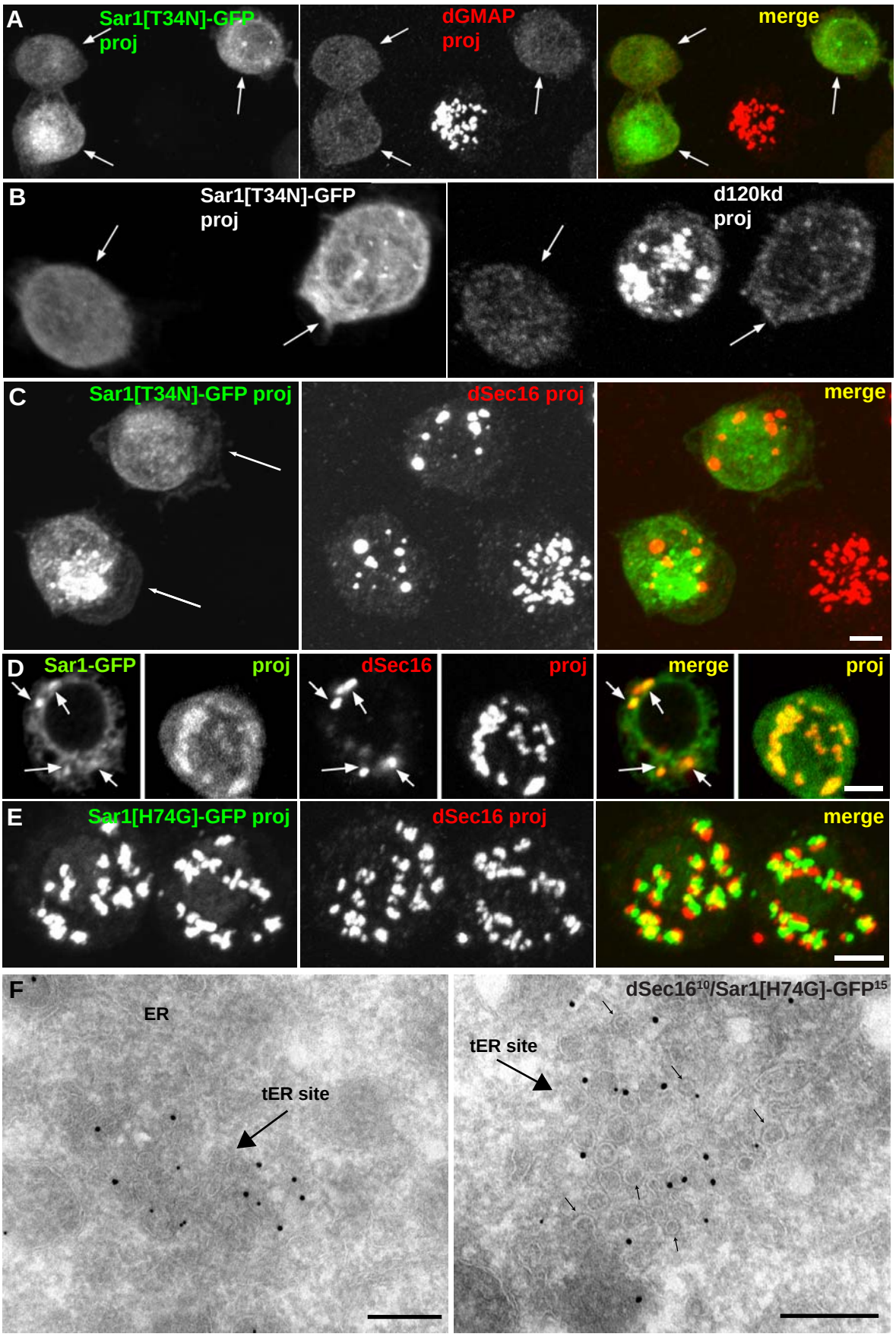
Scale bars: 5μm (A-G) and 200nm (A', F').

Supplementary Table 1: Cloning of the dSec16 constructs

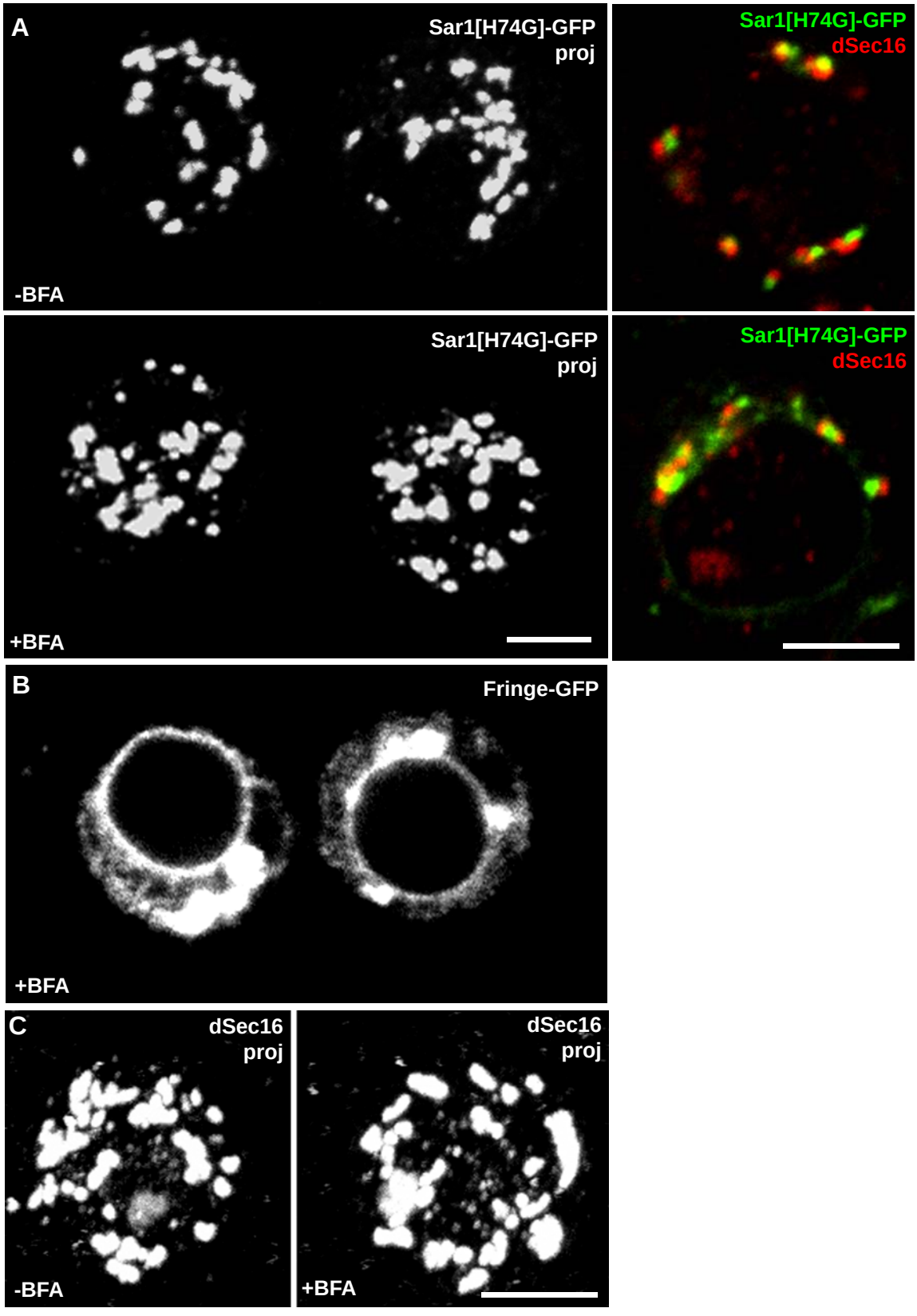
Construct	Amino acids	restriction sites
dSec16	1-1953	SpeI/XbaI
ΔC-dSec16	1-1532	SpeI/XbaI
CCD	1062-1535	EcoRI/XhoI
NC2.3.4-CCD	690-1535	NotI/XhoI
NC3.4-CCD	890-1535	NotI/XhoI
NC4-CCD	990-1535	NotI/XhoI
NC2	690-900	NotI/XhoI
NC3.4	890-1080	NotI/XhoI
NC2.3.4	690-1080	NotI/XhoI
NC1.2.3.4	1-1080	NotI/XhoI
NC2-CCD	690-900V1062-1535	NotI/XhoI
NC2.3-CCD	690-1000V1062-1535	NotI/XhoI
ΔNC23	1-689V990-1953	NotI/XbaI
ΔArg	1-859V925-1953	NotI/XbaI



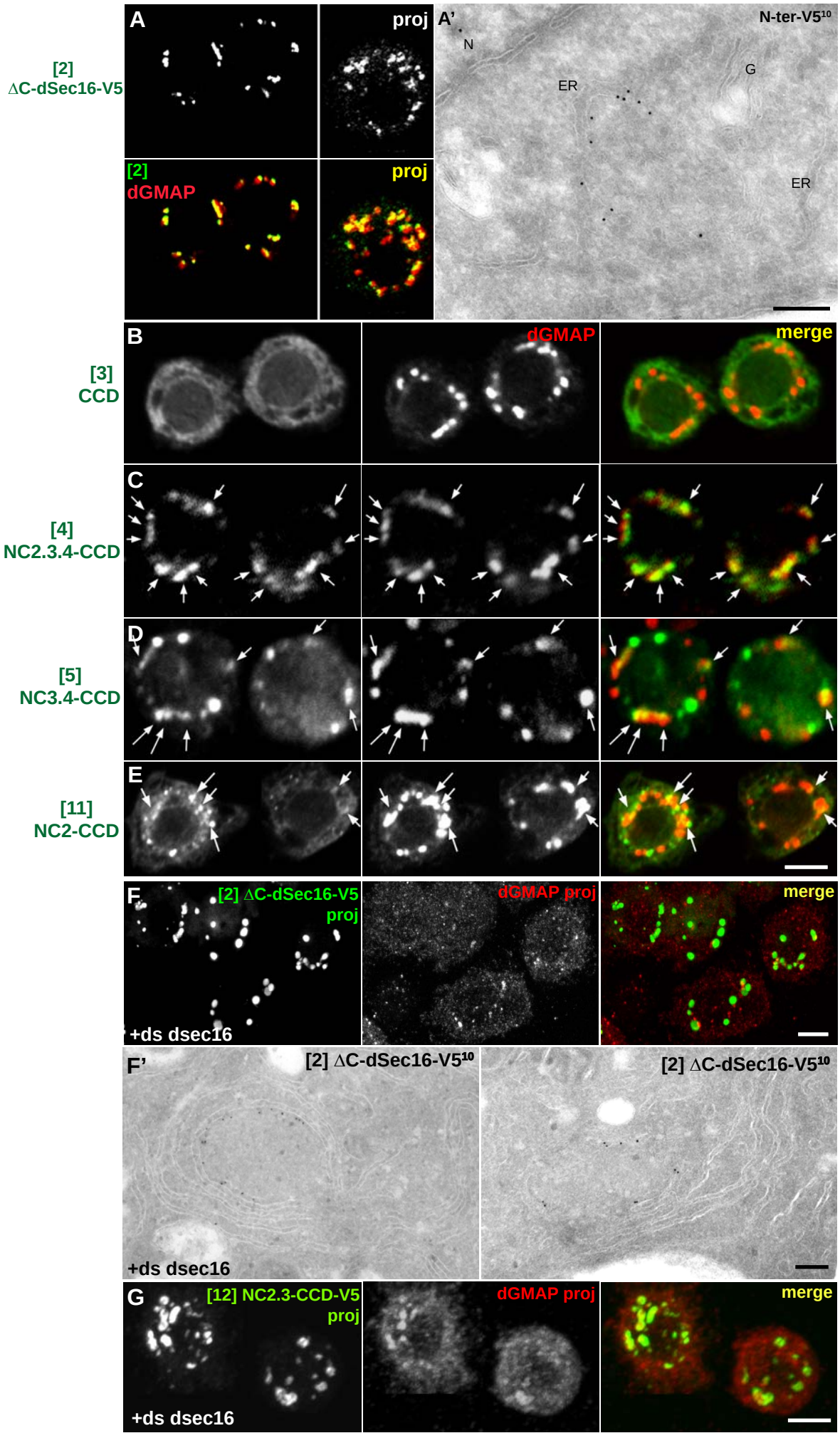
Supplementary Figure S1. Ivan et al, 2008.



Supplementary Figure S2. Ivan et al, 2008.



Supplementary Figure S3. Ivan et al, 2008.



Supplementary Figure S4. Ivan et al, 2008.