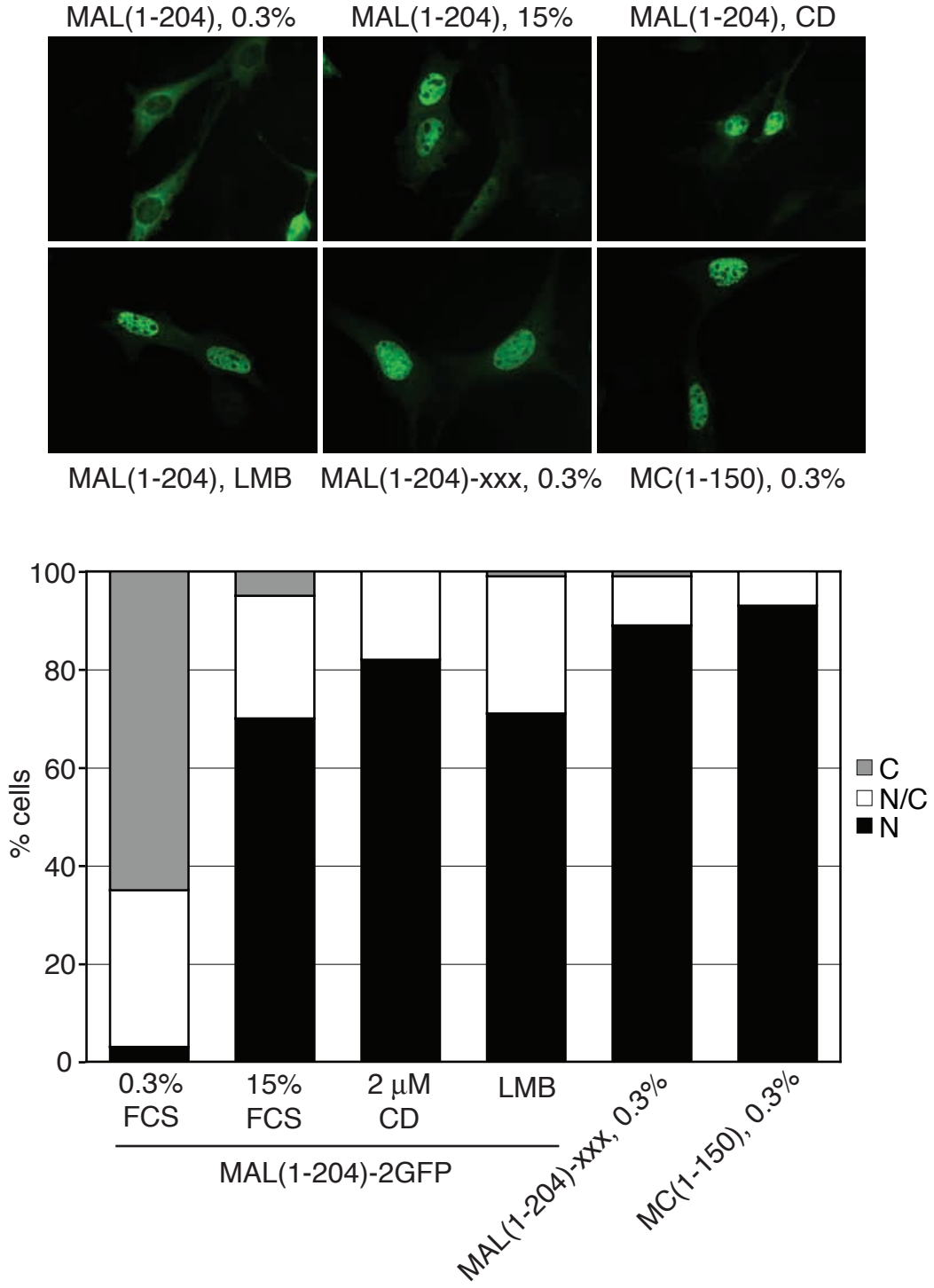
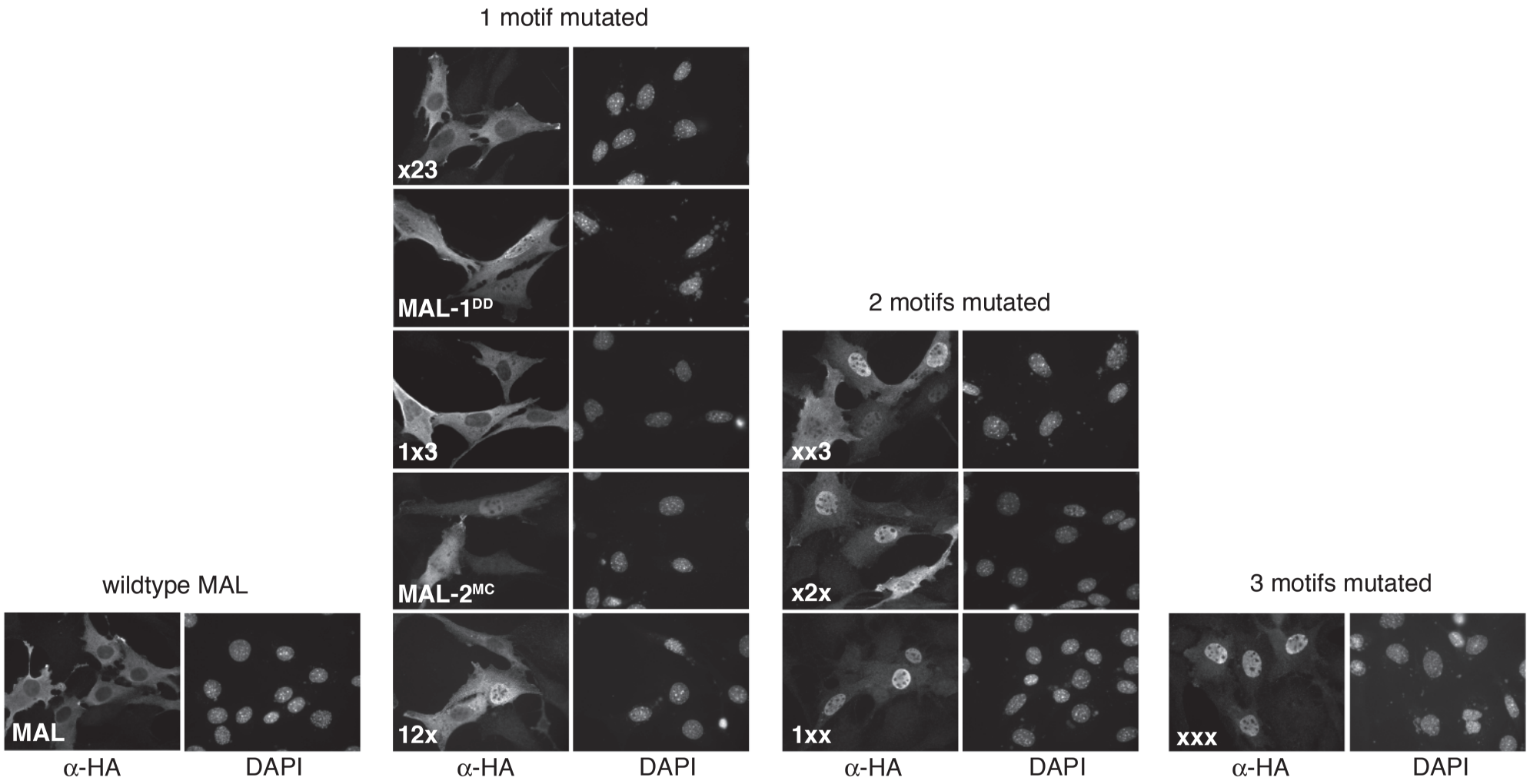


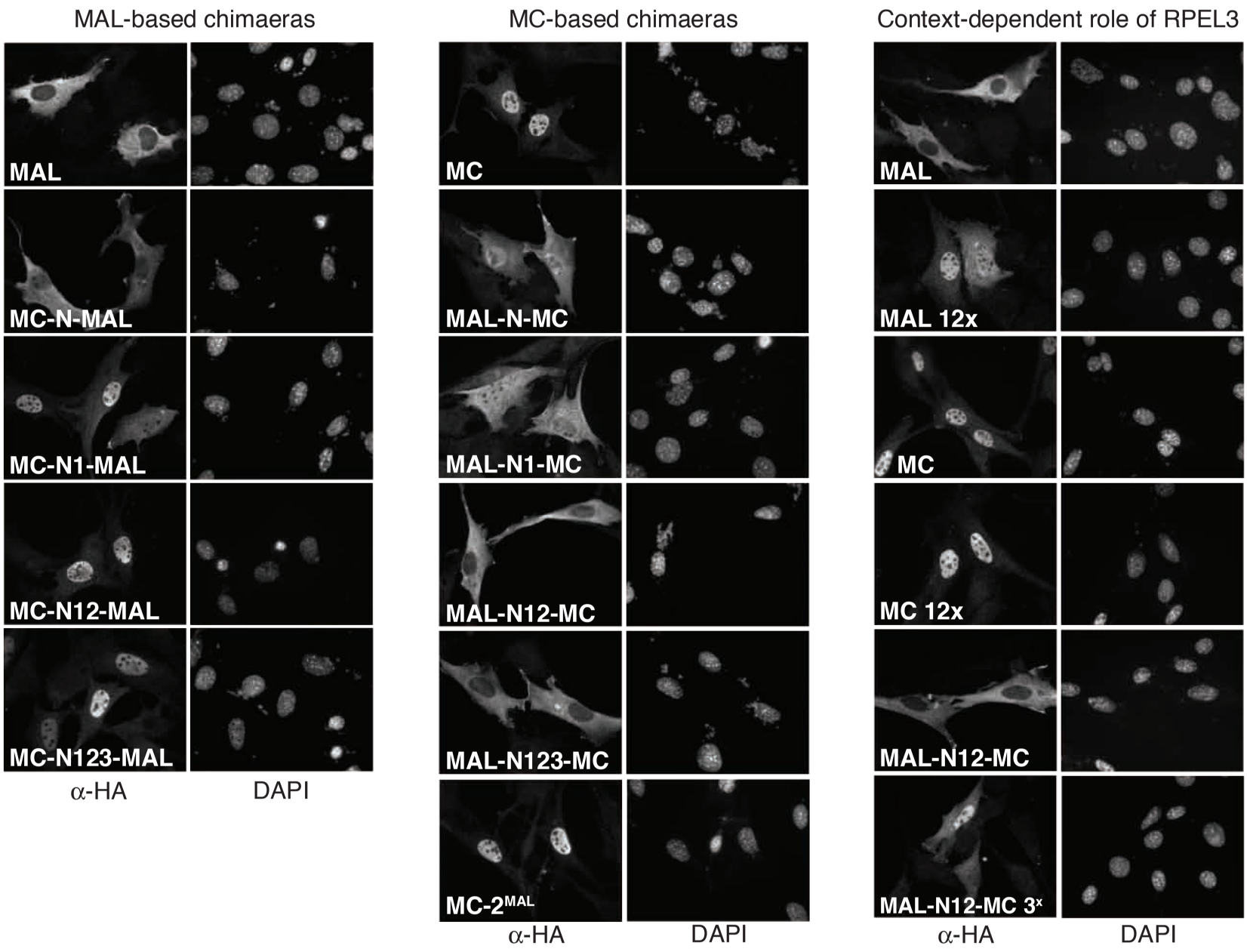
Supplemental Figure S1: Localisation of MAL(1-204)-2GFP and MC(1-150)-2GFP.

NIH3T3 cells transfected with 20 ng of 2GFP fusion constructs were serum-starved (0.3% FCS) and treated for 30 min with 15% FCS, 2 μM cytochalasin D (CD) or 20 nM leptomycin B (LMB) as indicated.
Upper panel shows representative micrographs; lower panel shows quantitation of 2GFP fusion protein localisation from 100 cells. See Figure 3 for dynamic localisation and FLIP analysis.



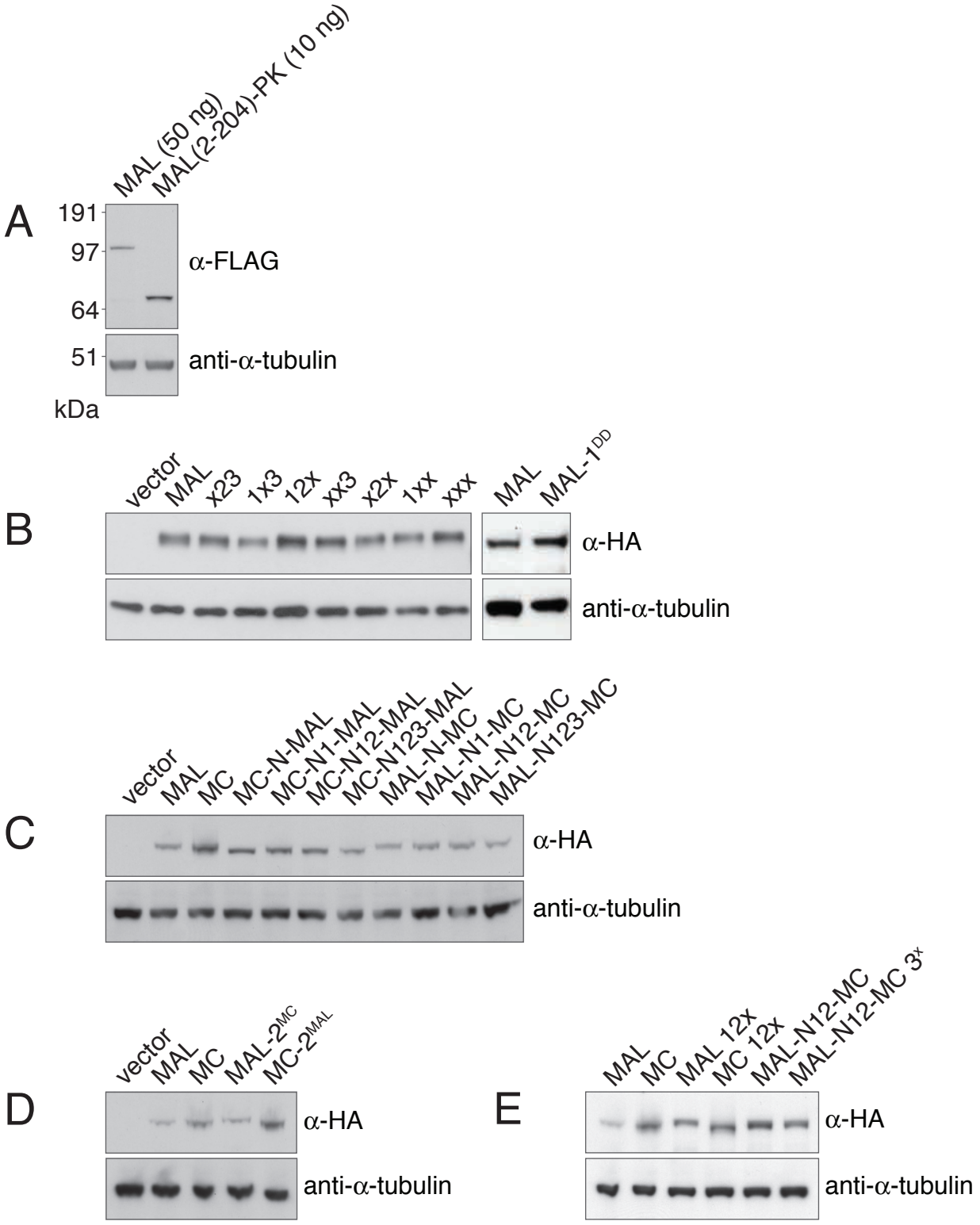
Supplemental Figure S2: RPEL motifs cooperate in MAL regulation.

NIH3T3 cells in a 6-well dish were transfected with 100 ng of the indicated C-terminally HA-tagged MAL constructs and maintained in 0.5% serum. Cells were stained against the double HA tag for immunofluorescence. DAPI was used to counterstain cell nuclei. See Figure 5B and 5D for quantitation.



Supplemental Figure S3: An intact unit of MAL RPEL motifs 1 and 2 is required for MAL regulation.

NIH3T3 cells in a 6-well dish were transfected with 100 ng of the indicated C-terminally HA-tagged constructs and maintained in 0.5% serum. Cells were stained against the double HA tag for immunofluorescence. DAPI was used to counterstain cell nuclei. See Figure 6B, 6E and 6G for quantitation.



Supplemental Figure S4: Expression of the used constructs.

NIH3T3 cells in a 24-well dish were transfected with the indicated constructs. Relative amounts of DNA were identical to those used for immunofluorescence microscopy and luciferase reporter assays. Expression levels were assessed by Western blotting against the FLAG or double HA tags as indicated. α-tubulin was detected as loading control.
(A) FLAG-MAL and FLAG-MAL(2-204)-PK. (**B**) RPEL motif mutants. See Figure 5A for nomenclature. (**C**) MAL-MC chimaeras. See Figure 6A for nomenclature. (**D**) Reciprocal RPEL2 exchange. See Figures 5A and 6A for nomenclature. (**E**) RPEL3 mutation in different contexts.