Supplemental Information

Supplemental Methods

The following overlapping single stranded oligonucleotides were inserted into the miR^{GFP} or miR^{DsRed} plasmids to generate expression constructs with gene-specific synthetic miRs embedded in the 3'UTR of the GFP or DsRed mRNA:

Primer name	Primer sequence (5'-3')
lacZ miR Top	TGCTGAAATCGCTGATTTGTGTAGTCGTTTTGGCCACTG ACTGACGACTACACATCAGCGATTT
lacZ miR Bottom	CCTGAAATCGCTGATGTGTAGTCGTCAGTCAGTGGCCA AAACGACTACACAAATCAGCGATTTC
OAZ miR Top	TGCTGAAACTTCAAGAAATACACCGCGTTTTGGCCACT GACTGACGCGGTGTATCTTGAAGTTT
OAZ miR Bottom	CCTGAAACTTCAAGATACACCGCGTCAGTCAGTGGCCA AAACGCGGTGTATTTCTTGAAGTTTC
AZI miR Top	TGCTGTTTGCTATGATATTAACTGCGGTTTTGGCCACTG ACTGACCGCAGTTAATCATAGCAAA
AZI miR Bottom	CCTGTTTGCTATGATTAACTGCGGTCAGTCAGTGGCCA AAACCGCAGTTAATATCATAGCAAAC

Supplemental Figure Legends

Supplemental Figure 1. Antizyme (OAZ) and its inhibitor (AZI) localize to centrosomes throughout the cell cycle in HeLa.

HeLa cells were grown in culture for 24 hrs and immunofluorescence was performed using antibodies against γ-tubulin (green) and either OAZ (A) or AZI (B) (red). Shown are representative interphase and mitotic cells (as determined by DNA staining, blue), with centrosomes indicated by arrows; bars = 5μm.

Supplemental Figure 2. Expression and localization of fluorescently tagged versions of AZI and OAZ.

(A) HeLa cells were analyzed by dual-color immunoblot with a mouse antibody against GFP (red) and a rabbit antibody agsinst AZI (green) 24 hrs after transfection with GFP alone or GFP-AZI. The sizes of molecular weight markers (MW), which are visible in both channels using the LI-COR Odyssey scanner, are indicated in KDa (the 72 KDa marker is only visible in the green channel). Similar results were obtained using DsRed-AZI (not shown). (B) Identical U2OS cell lysates were analyzed on separate immunoblots with rabbit antibodies against DsRed or OAZ, as indicated, 24 hrs after transfection with DsRed alone or DsRed-OAZ. The sizes of molecular weight markers (MW) are indicated in KDa. Similar results were obtained using GFP and GFP-OAZ (not shown). (C) GFP-AZI and GFP-OAZ localize to centrosomes. HeLa cells were analyzed

by IIF with an antibody against γ-tubulin 24 hrs after transfection with GFP, GFP-AZI, or GFP-OAZ; shown are representative cells expressing GFP, GFP-AZI, or GFP-OAZ, as indicated (bar = 5 μm) above digitally magnified images of the box surrounding the centrosome (bar = 1 μm); GFP, green; γ-tubulin, red; DNA, blue.

Supplemental Figure 3. miR-dependent depletion of OAZ and AZI.
(A and B) miR constructs reduce the AZI protein and the OAZ message by roughly 50%. Cells transfected with lacZ miR^{GFP}, AZI miR^{GFP}, or OAZ miR^{GFP} were treated with 5 μg/ml blasticidin for three days to kill non-transfected cells. (A) The level of AZI protein was determined in pooled drug resistant cells as described in Figure 1. Numbers below the tubulin loading control indicate the relative normalized AZI levels. (B) The level of the OAZ and GAPDH messages were determined in pooled drug resistant cells by RT-PCR as described in Figure 1. Numbers below the GAPDH loading control indicate the relative normalized levels of the OAZ message. (C) OAZ miR^{GFP} only partially depletes centrosomal OAZ. HeLa cells transfected with lacZ miR^{GFP} or OAZ miR^{GFP} were arrested in S-phase for 48h and analyzed by IIF with antibodies against OAZ (red) and γ-tubulin (green). Shown at left is a representative pair of OAZ miR^{GFP}-expressing (GFP positive) and GFP negative cells showing GFP (green), OAZ (red), DNA (blue) and γ-Tubulin (cyan), with boxes surrounding the centrosomes and arrows indicating the corresponding panel at right for each cell; bar = 5 μm. Panels to the right are digitally magnified images of the boxes surrounding the centrosomes for each cell in the image at left; OAZ, red; γ-tubulin, green; bar = 1 μm. OAZ staining is reduced but not eliminated in GFP positive (GFP+) OAZ miR-expressing cells compared to adjacent GFP negative (GFP-) cells. (D) LacZ miR^{GFP} does not affect centrosomal OAZ. Shown are magnified images of centrosomes from a representative lacZ miR^{GFP}-expressing (GFP+) cell and its paired GFP- control cell; colors as in (A), bar = 1 μm. (E) AZI miR^{DsRed} effectively depletes centriolar AZI. Shown at left is a representative pair of AZI miR^{DsRed}-expressing (DsRed positive) and DsRed negative cells, showing DsRed (red), AZI (green), DNA (blue), and γ-tubulin (cyan), with boxes surrounding the centrosomes and arrows indicating the corresponding panel at right for each cell; bar = 5 μm. Panels to the right are digitally magnified images of the boxes surrounding the centrosomes for each cell in the image at left; AZI, green; γ-tubulin, red; bar = 1 μm. AZI can be detected in foci characteristic of centrioles (arrows) in DsRed negative (DsRed-) cells, but this staining is significantly reduced or absent in the DsRed positive (DsRed+) AZI miR^{DsRed}-expressing cell (carets indicate centrosome position as judged by γ-tubulin staining in the DsRed+ cell).

Supplemental Figure 4. The form of Mps1 that co-immunoprecipitates with GFP-OAZ contains the degradation signal.
(A) GFP-OAZ binds to a single Mps1 isoform in 293 cells that contains the Mps1 degradation signal, but fails to bind to $Mps1^{\Delta 12/13}$ that lacks the Mps1 degradation signal. After transfection of 293-$Mps1^{\Delta 12/13}$ cells with GFP or GFP-OAZ, cells were arrested in S-phase with a 24 hr HU treatment. GFP-OAZ complexes were immunoprecipitated with a mouse antibody against GFP and analyzed by dual channel immunoblotting on the LI-COR Odyssey scanner with the M02 and MDS antibodies against Mps1 (see Fig. 5 for a description of these antibodies) and an antibody against GFP. In the presence of

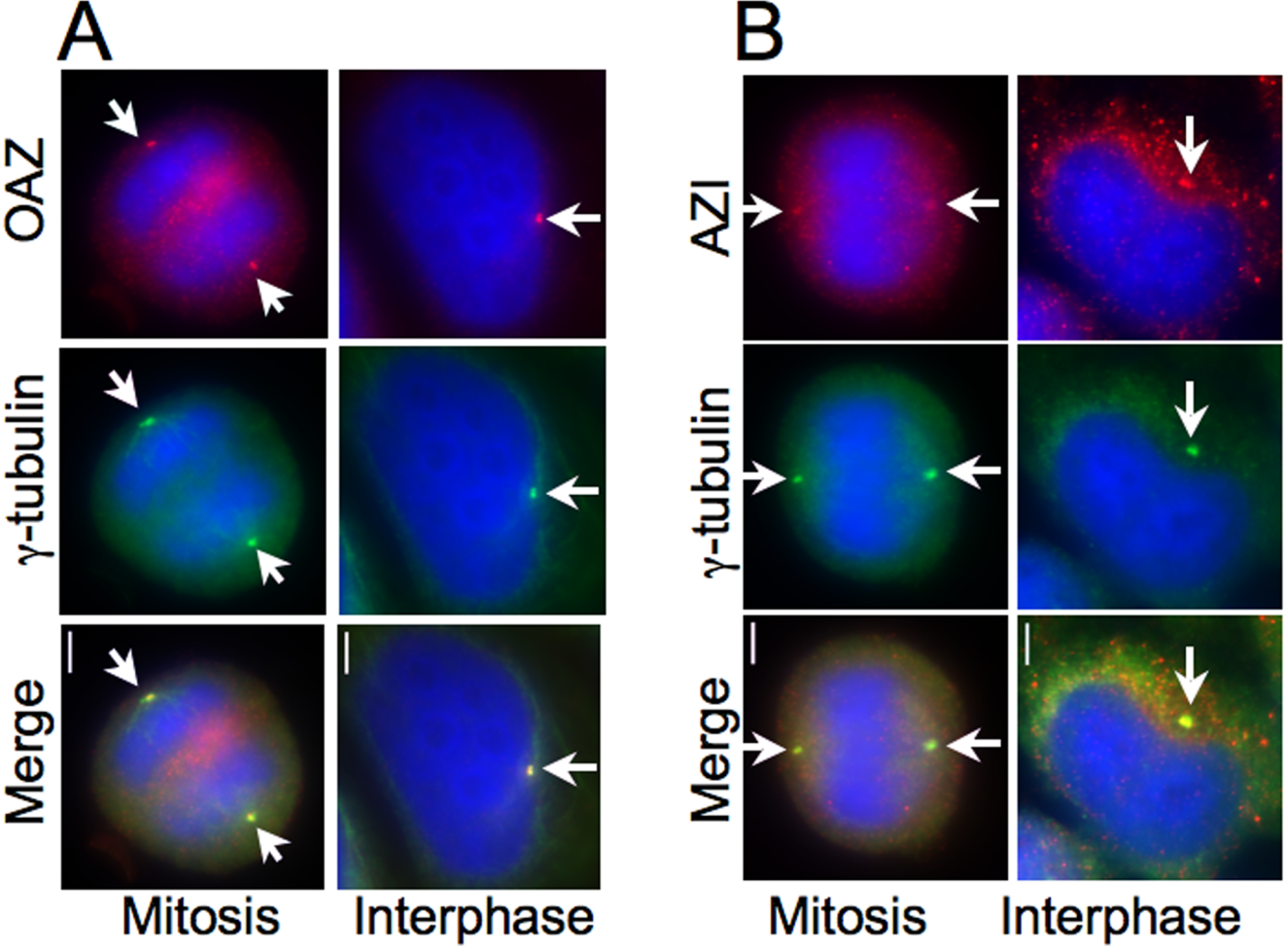
doxycycline (+Dox) untagged Mps1$^{\Delta 12/13}$ (indicated by an arrowhead) is expressed at roughly 50% the level of endogenous full-length Mps1, but Mps1$^{\Delta 12/13}$ is not detectable in uninduced cells (-Dox) (second panel, "WB: M02"). The MDS antibody recognizes full-length endogenous Mps1 (closed arrow) and a 293-specific Mps1 isoform (open arrow) that migrates faster than full-length Mps1, but does not bind to Mps1$^{\Delta 12/13}$ (MDS; top panel). The same Mps1 isoform binds to GFP-OAZ in both uninduced cells (where Mps1$^{\Delta 12/13}$ is not present) and induced cells, and is recognized by the Mps1 Degradation Signal antibody that binds to the region of Mps1 that is missing in Mps1$^{\Delta 12/13}$ (MDS; top panel). The failure of the MDS antibody to bind to Mps1$^{\Delta 12/13}$ is most notable in the "Merge" panel.

(B-D) GFP-OAZ complexes prepared as described in (A) were analyzed by dual color immunoblotting on the LI-COR Odyssey scanner with the MDS, N1, C-19, and M02 antibodies (see Fig. 5 for a description of these antibodies). All blots were incubated with Alexa680-donkey anti-rabbit and IRDye800-donkey anti-mouse secondary antibodies. Shown are the signals from both the 680 and 800 channels (only immunoprecipitates are shown, see (A) for controls). (B) Shown is a blot incubated with MDS as the only primary antibody followed by both anti-rabbit and anti-mouse secondary antibodies. Asterisks indicate a non-specific band (presumably non-denatured IgG from the immunoprecipitation) generated by IRDye800 anti-mouse. (C) The co-precipitated form of Mps1 is also recognized by N1. (D) The co-precipitated form of Mps1 is additionally recognized by C19 and M02. Although the N1 and M02 signals are weak, the absence of signal from the anti-mouse antibody in panel (A) shows that these signal are due to the presence of the N1 or M02 antibody.

Supplemental Figure 5. Modulating Antizyme activity does not affect the levels of cyclin D1 or cyclin A2.
(A) Overexpression of OAZ or AZI has no detectable effect on Cyclin D1. HeLa cells were transfected with GFP, GFP-OAZ, or GFP-AZI and collected 48 hrs after transfection. Immunoblot was performed on cell lysates using antibodies against GFP, α-tubulin, and cyclin D1. Quantification using the Licor-Odyssey showed no difference in cyclin D1 levels after normalization to α-tubulin. (B) Depletion of OAZ or AZI has no detectable effect on Cyclin A2. HeLa cells were transfected with control (siCon), OAZ-specific (siOAZ), or AZI-specific (siAZI) siRNAs, and collected 48 hrs after transfection. Immunoblot was performed on cell lysates using antibodies against α-tubulin and cyclin A2. Quantification using the Licor-Odyssey showed no difference in cyclin A levels after normalization to α-tubulin.

Supplemental Figure 6. Model for the Control of Mps1 Degradation by Antizyme.
OAZ binds to the Mps1 degradation signal (D.S), and the Mps1-OAZ complex is targeted to the proteasome for degradation. Cdk2 phosphorylates Mps1 at T468 within the degradation signal, and we hypothesize that this phosphorylation prevents OAZ from binding to Mps1. We further hypothesize that both the Mps1-OAZ interaction and Mps1 degradation occur at the centrosome. OAZ might prevent both premature initiation of centrosome duplication and centrosome reduplication by removing Mps1 from centrosomes when Cdk2 activity is low. When Cdk2 levels rise, Mps1 is protected from degradation, allowing centrosome duplication to proceed. An as yet unidentified

phosphatase (indicated by a question mark) removes T468 phosphorylation, returning Mps1 to a state permissive for OAZ binding. Mps1 is represented by a white rectangle, with a black rectangle representing the Mps1 degradation signal (D.S; amino acids 420-507, encoded by exons 12 and 13 that are missing in $Mps1^{\Delta 12/13}$), and a grey rectangle representing the kinase domain. OAZ is represented as a light grey shape that binds to the Mps1 degradation signal.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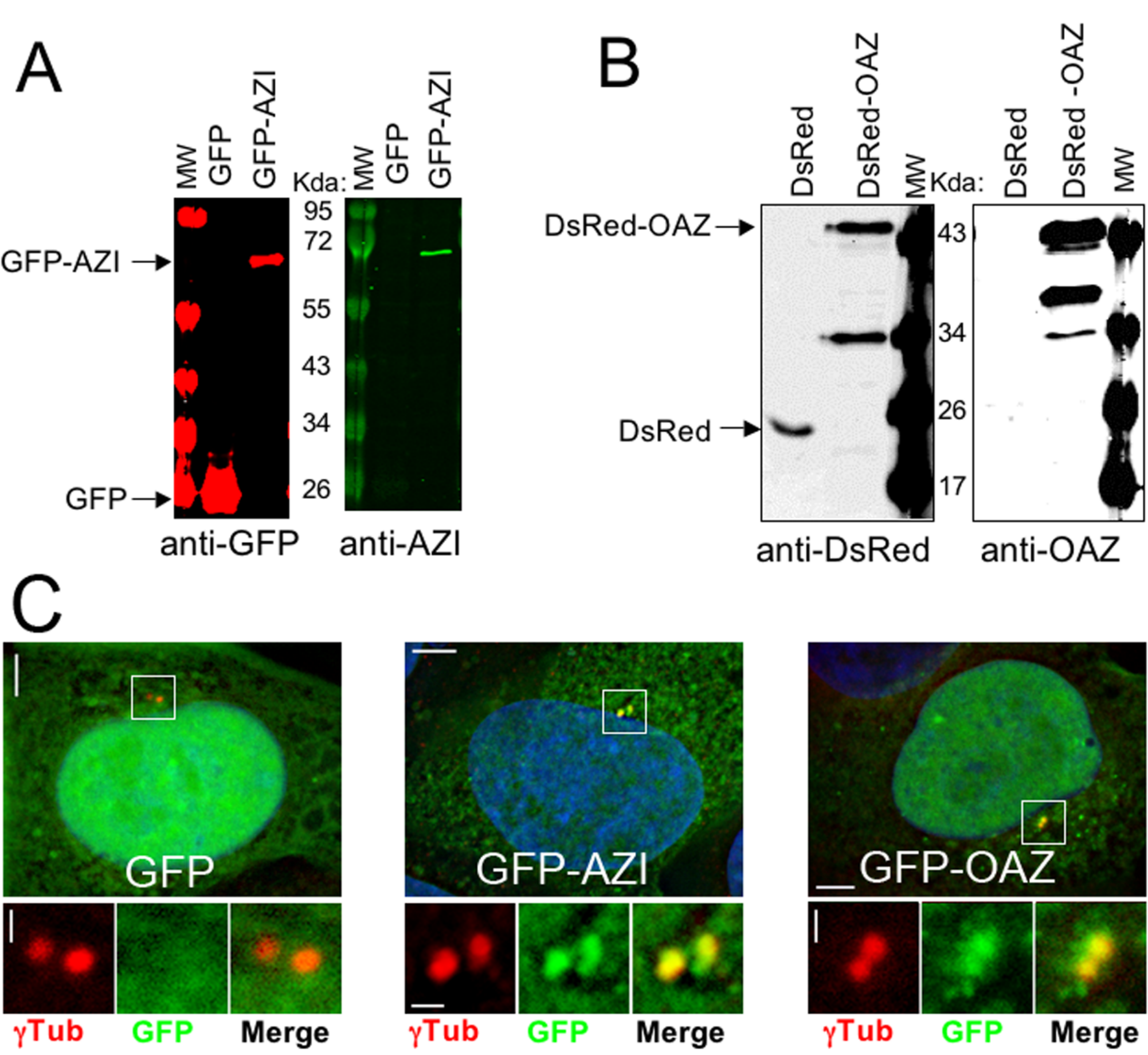
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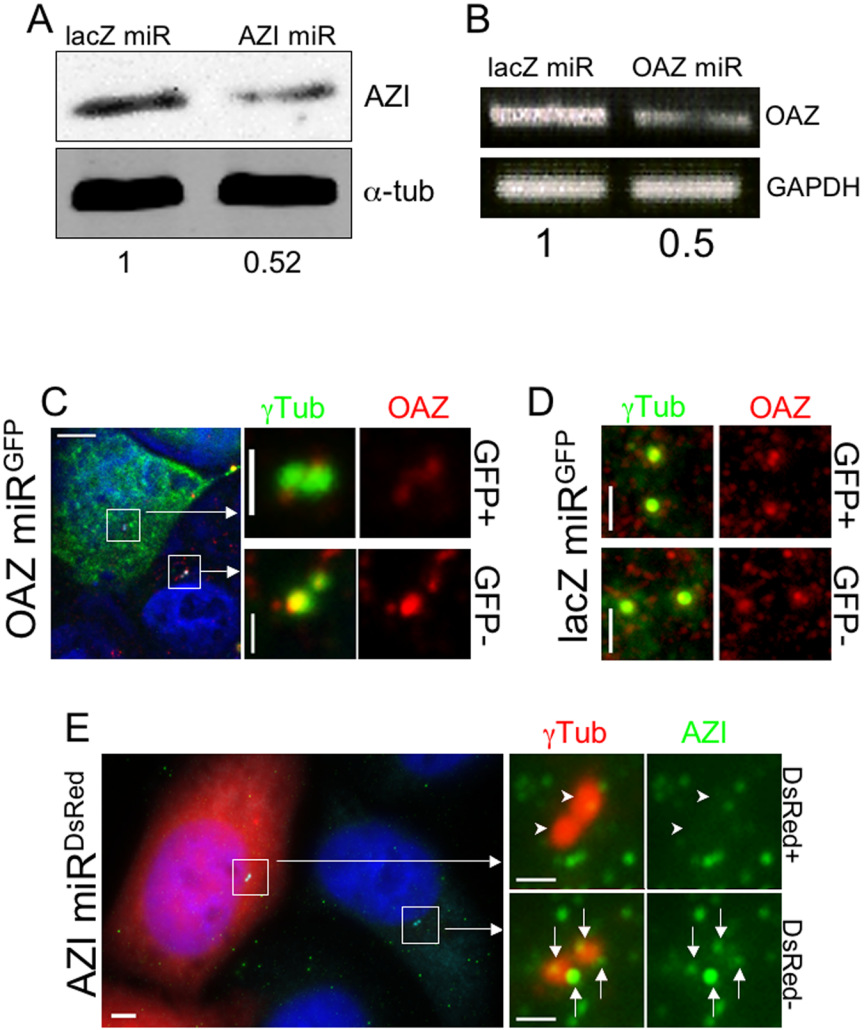
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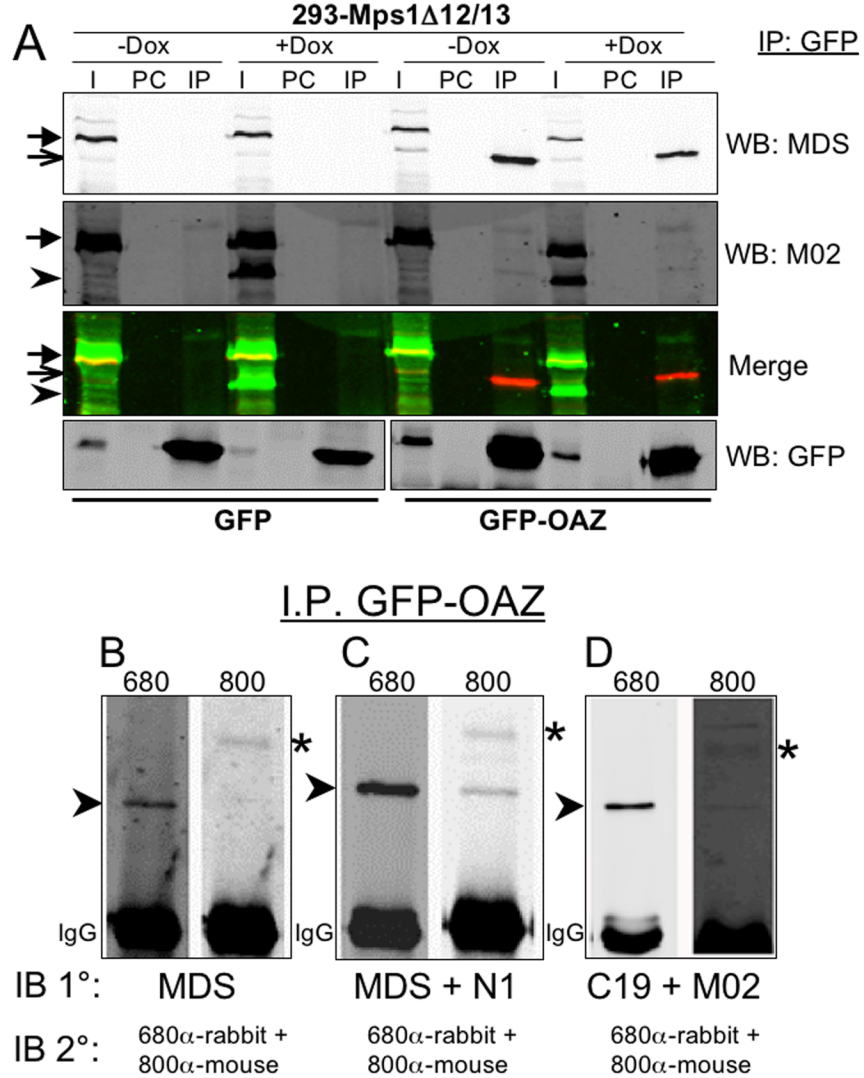
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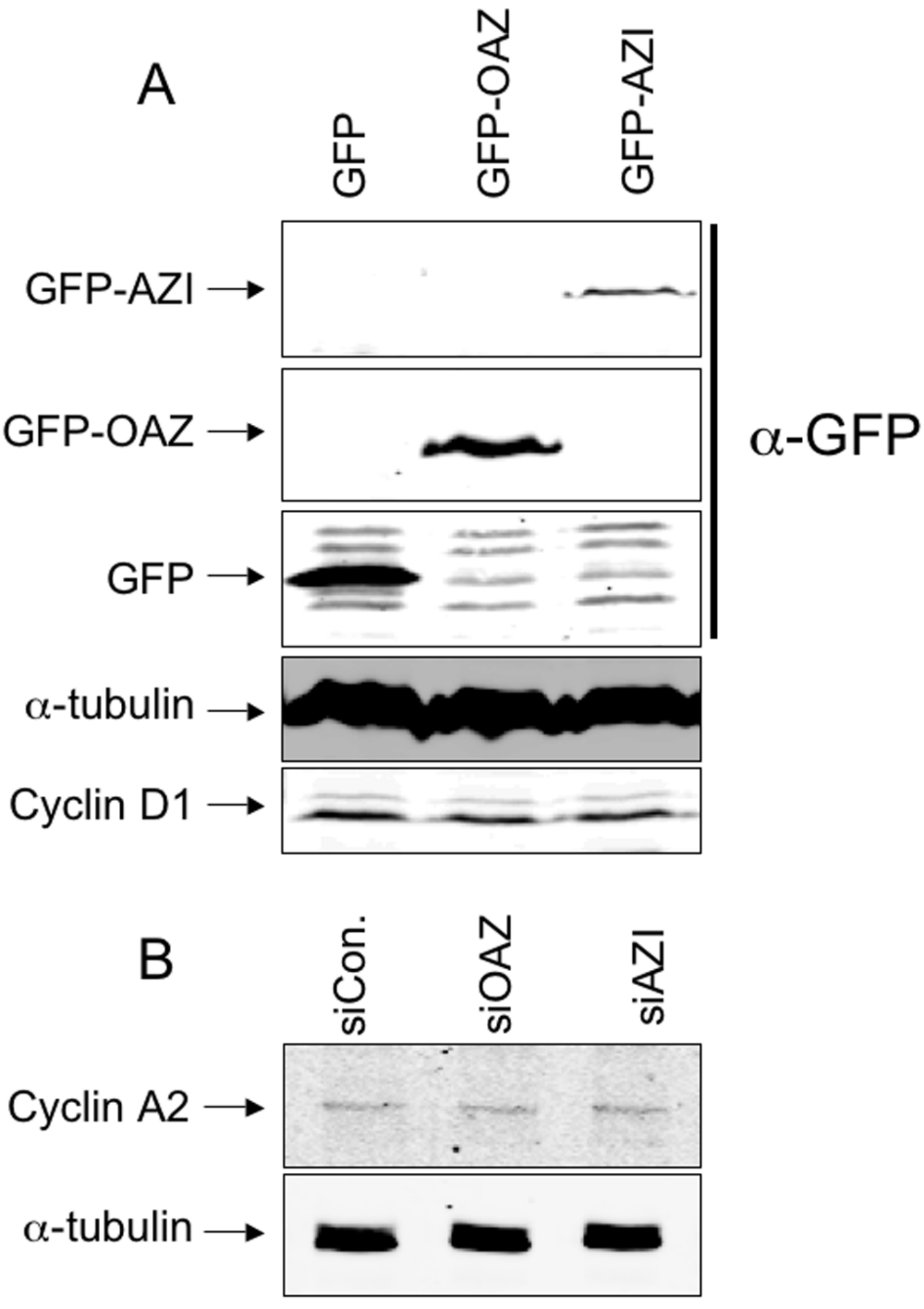
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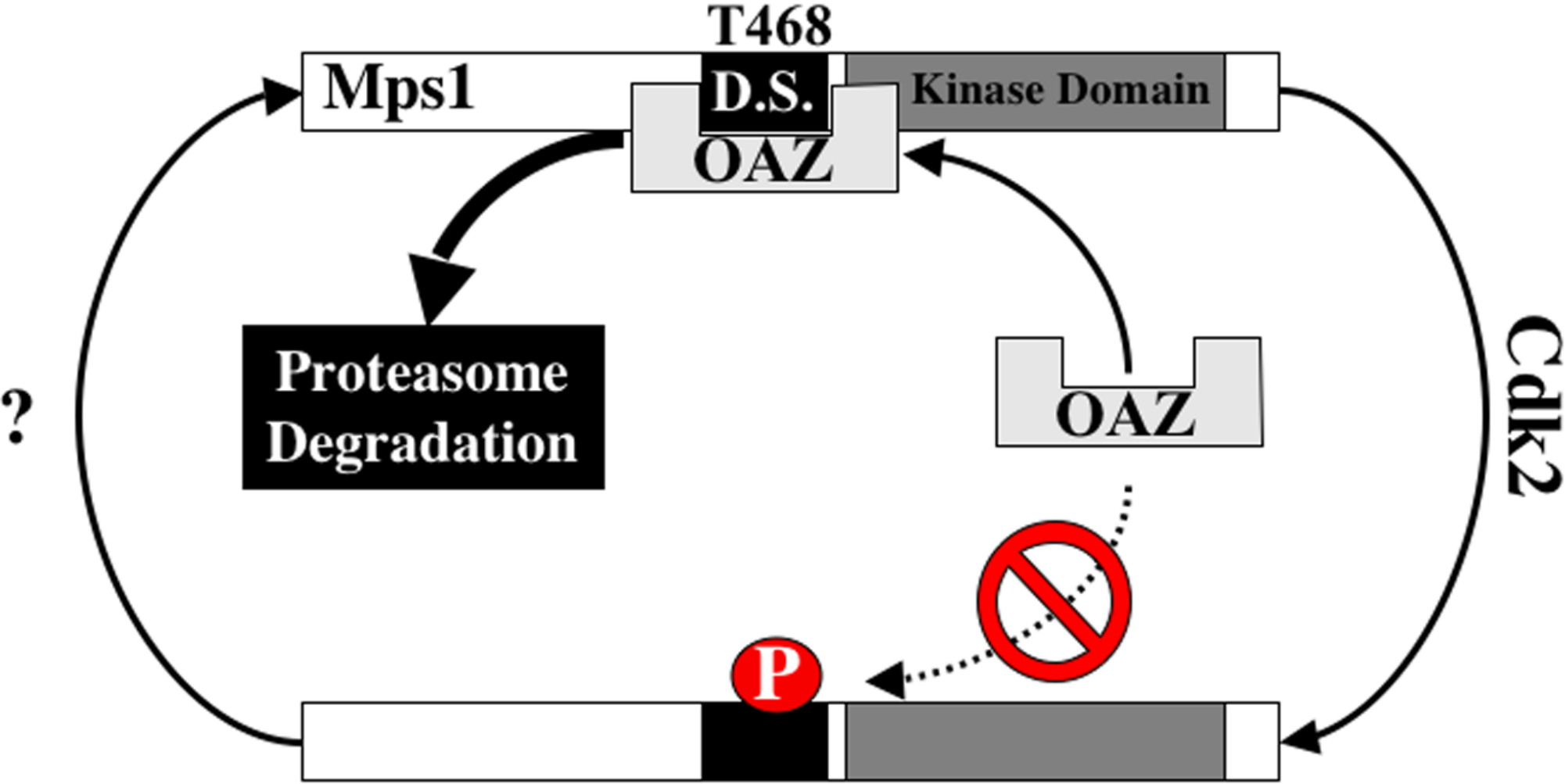
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P