SUPPLEMENTARY INFORMATION

miR-205 acts as a tumor radiosensitizer by targeting ZEB1 and Ubc13

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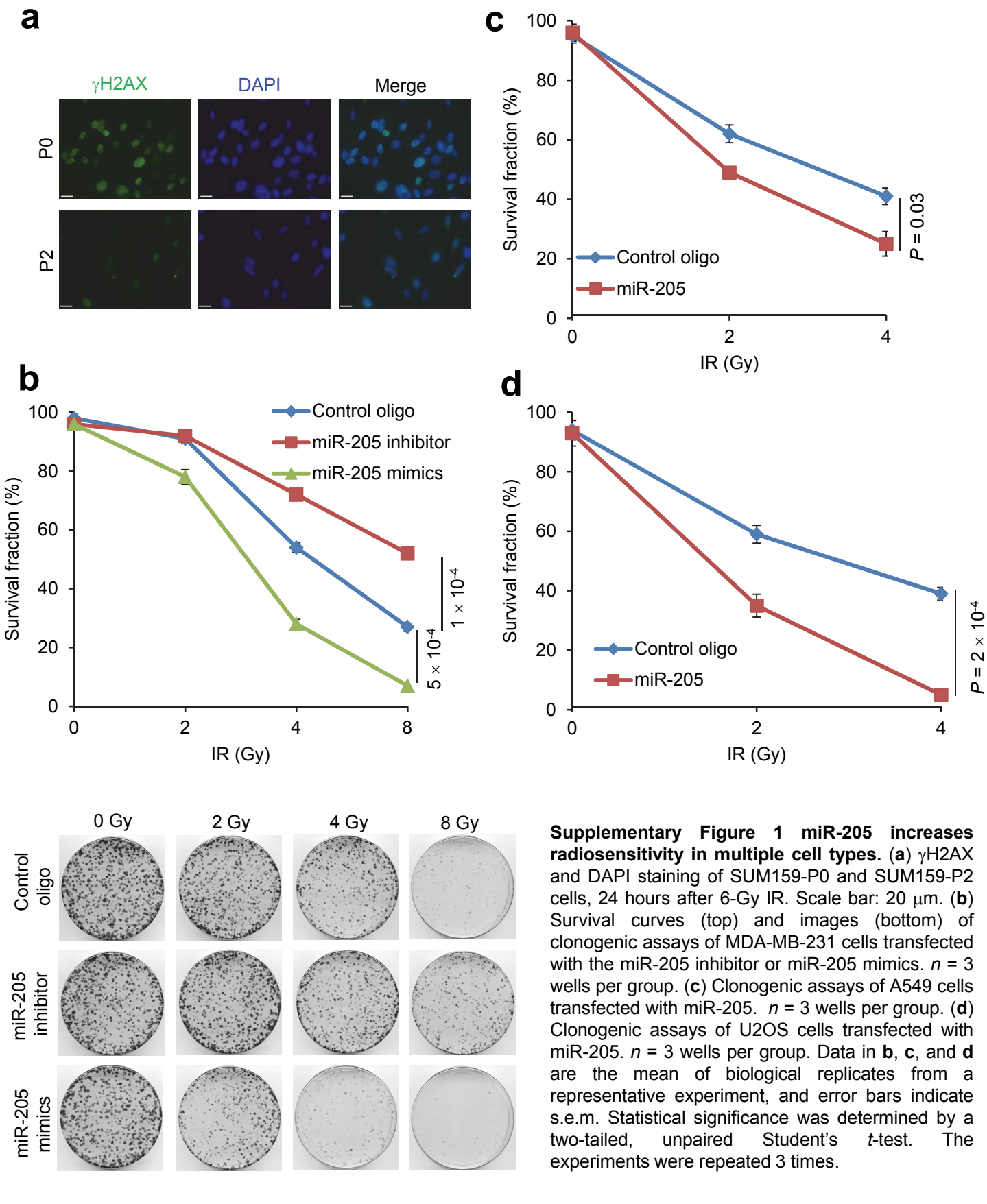
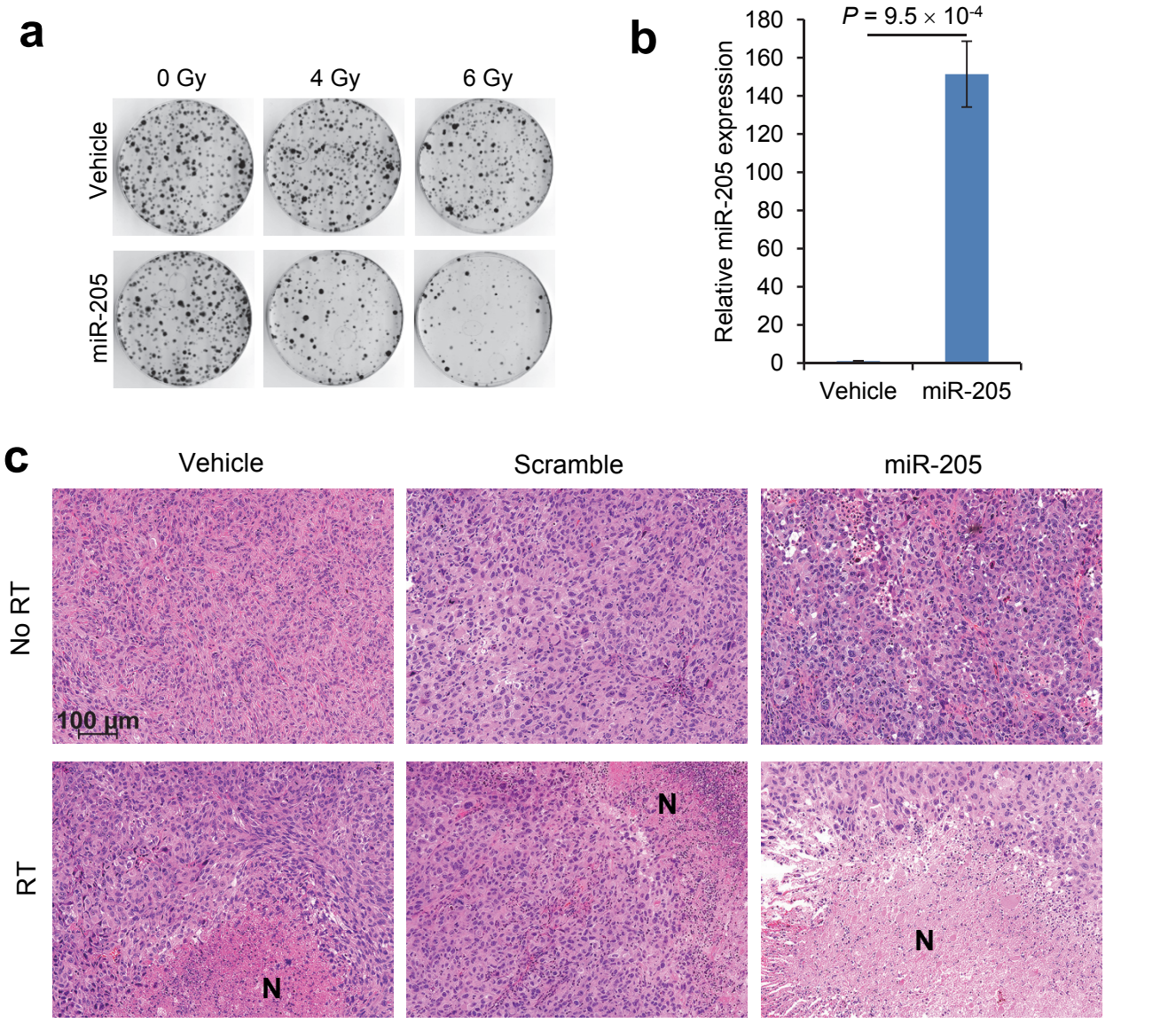
Supplementary Figure 1



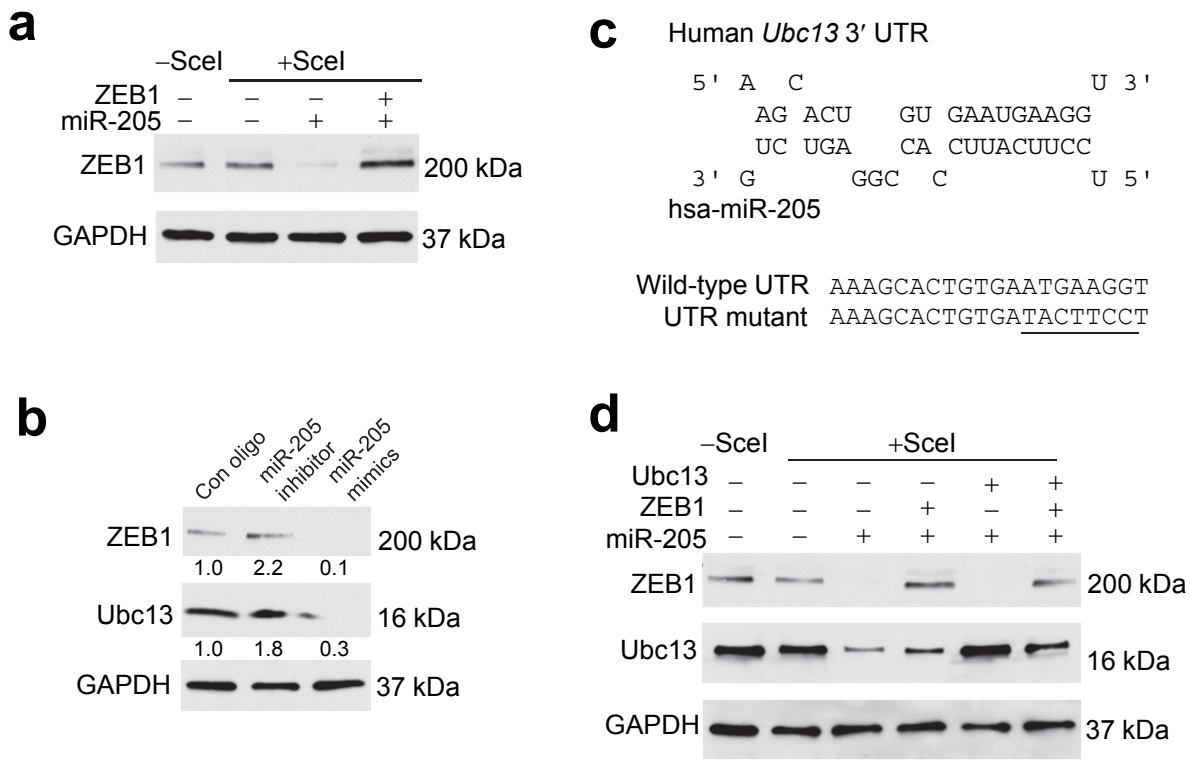
Supplementary Figure 1 miR-205 increases radiosensitivity in multiple cell types. (**a**) γH2AX and DAPI staining of SUM159-P0 and SUM159-P2 cells, 24 hours after 6-Gy IR. Scale bar: 20 μm. (**b**) Survival curves (top) and images (bottom) of clonogenic assays of MDA-MB-231 cells transfected with the miR-205 inhibitor or miR-205 mimics. *n* = 3 wells per group. (**c**) Clonogenic assays of A549 cells transfected with miR-205. *n* = 3 wells per group. (**d**) Clonogenic assays of U2OS cells transfected with miR-205. *n* = 3 wells per group. Data in **b**, **c**, and **d** are the mean of biological replicates from a representative experiment, and error bars indicate s.e.m. Statistical significance was determined by a two-tailed, unpaired Student's *t*-test. The experiments were repeated 3 times.

Supplementary Figure 2



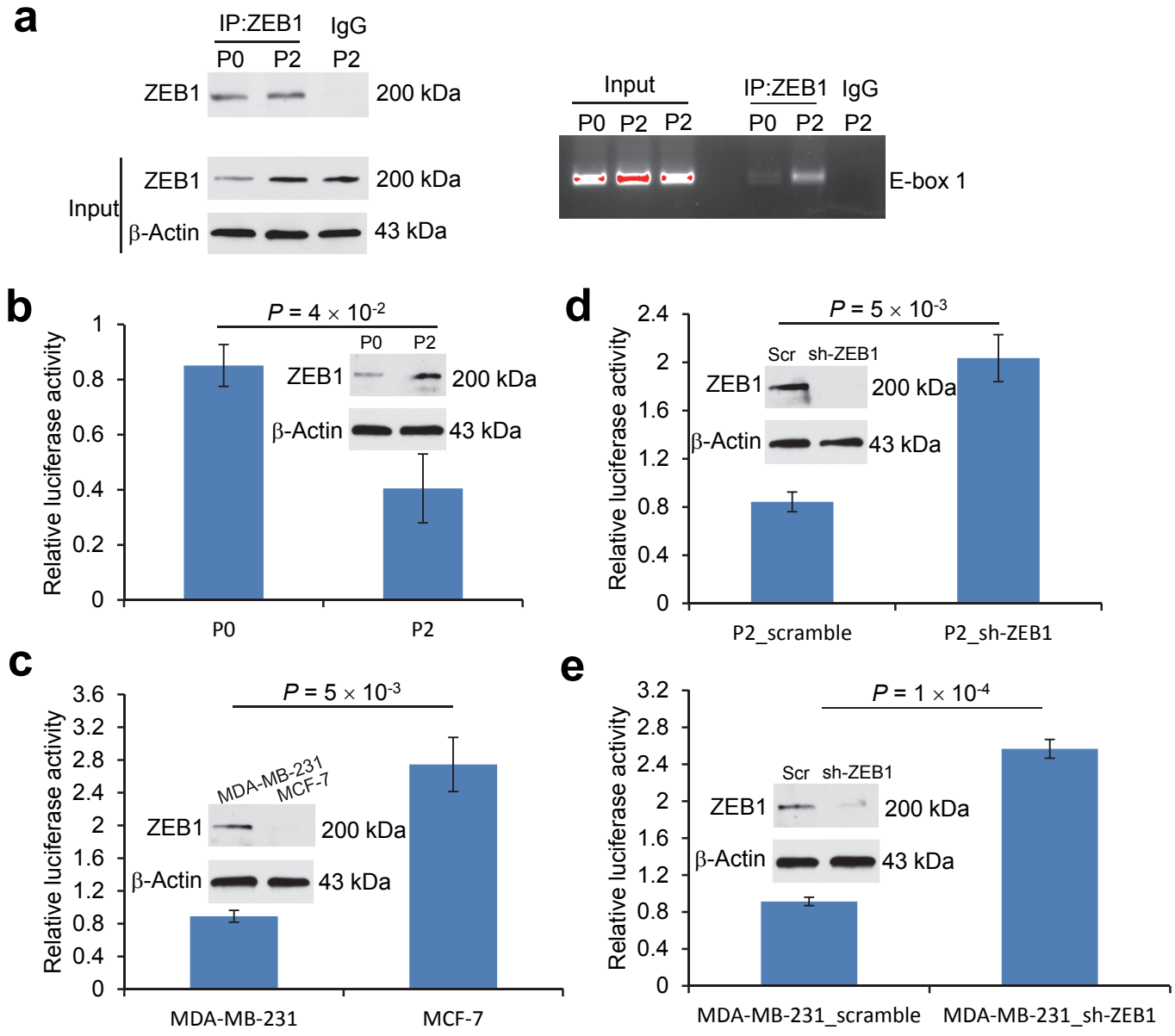
Supplementary Figure 2 Effect of nanoliposomal miR-205 mimics. (**a**) Images of clonogenic assays of SUM159-P2 cells incubated with 100 nM DOPC-encapsulated miR-205 mimics. (**b**) qPCR of miR-205 in SUM159-P2 cells incubated with 100 nM DOPC-encapsulated miR-205 mimics. *n* = 3 samples per group. (**c**) H&E staining of tumor tissues from the mice described in Fig. 2c. Scale bar: 100 μm. N: necrosis. (**d**) H&E staining of kidney and liver tissues from the mice described in Fig. 2c. Scale bar: 100 μm. Data in **b** are the mean of biological replicates from a representative experiment, and error bars indicate s.e.m. Statistical significance was determined by a two-tailed, unpaired Student's *t*-test. The experiments were repeated 3 times.

Supplementary Figure 3



Supplementary Figure 3 Expression levels of ZEB1 and Ubc13. (**a**) Immunoblotting of ZEB1 and GAPDH in U2OS_DR-GFP cells transfected with miR-205 alone or in combination with ZEB1. (**b**) Immunoblotting of ZEB1, Ubc13 and GAPDH in MDA-MB-231 cells transfected with the miR-205 inhibitor or miR-205 mimics. (**c**) Top: predicted duplex formation between human *Ubc13* 3' UTR and miR-205. Bottom: sequence of a mutant 3' UTR of human *Ubc13* containing mutations in the miR-205 binding site used for luciferase reporter assays in Fig. 3d. (**d**) Immunoblotting of Ubc13, ZEB1 and GAPDH in U2OS_DR-GFP cells transfected with miR-205 alone or in combination with ZEB1 or Ubc13, or both.

Supplementary Figure 4



Supplementary Figure 4 Endogenous ZEB1 regulates miR-205. (**a**) Left: SUM159-P0 and SUM159-P2 cells were immunoprecipitated with the ZEB1 antibody or control IgG and immunoblotted with the ZEB1 antibody. Right: ChIP assays of SUM159-P0 and SUM159-P2 cells. PCR was performed with primers specific for E-box 1. (**b-e**) Activity of a luciferase reporter fused to the putative human *mir-205* promoter in SUM159-P0 or SUM159-P2 cells (**b**), in MDA-MB-231 or MCF-7 cells (**c**), and in SUM159-P2 (**d**) or MDA-MB-231 (**e**) cells transduced with ZEB1 shRNA or a scrambled control. *n* = 3 wells per group. (**f**) Immunoblotting of ZEB1 and β-Actin in irradiated SUM159-P0 cells with or without pretreatment with the ATM inhibitor Ku55933 (10 μM, 1 hour). Data in **b-e** are the mean of biological replicates from a representative experiment, and error bars indicate s.e.m. Statistical significance was determined by a two-tailed, unpaired Student's *t*-test. The experiments were repeated 3 times.

Supplementary Figure 5

Fig. 1d

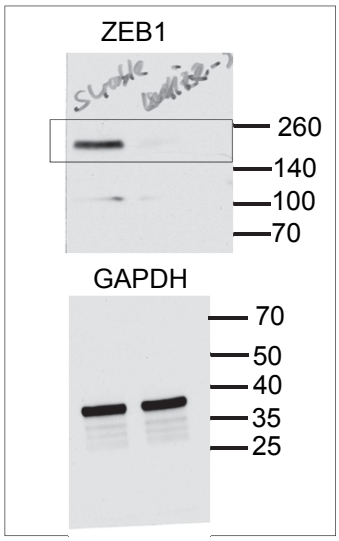


Fig. 1e

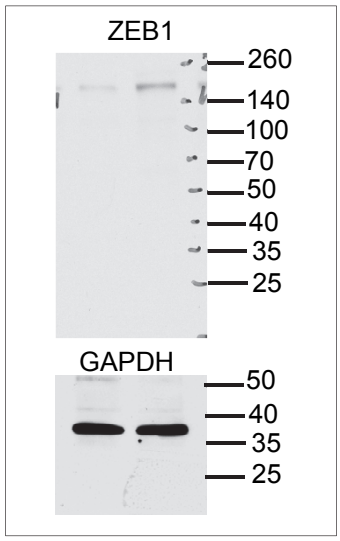


Fig. 3c

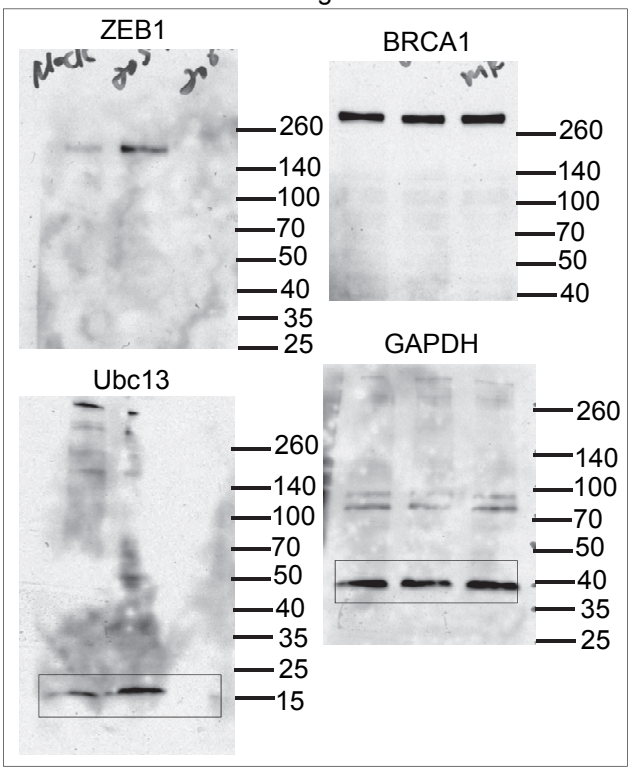


Fig. 2e

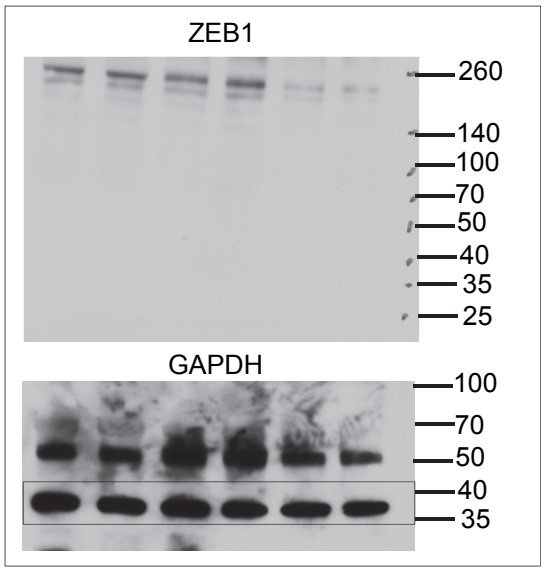


Fig. 3e

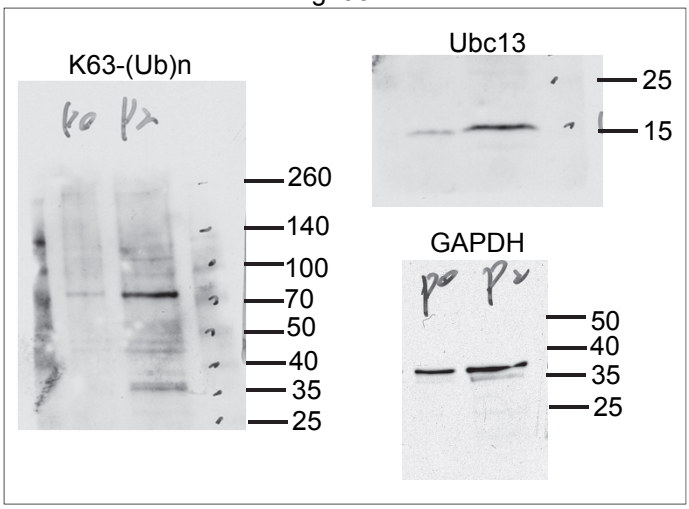
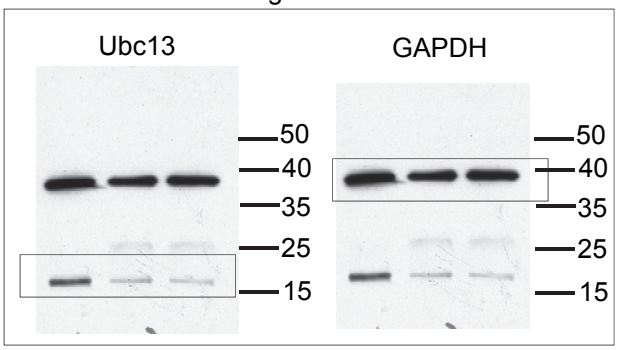


Fig. 3f



Supplementary Figure 5 Uncropped images of immunoblots.

Supplementary Figure 5 continued

Fig. 3g

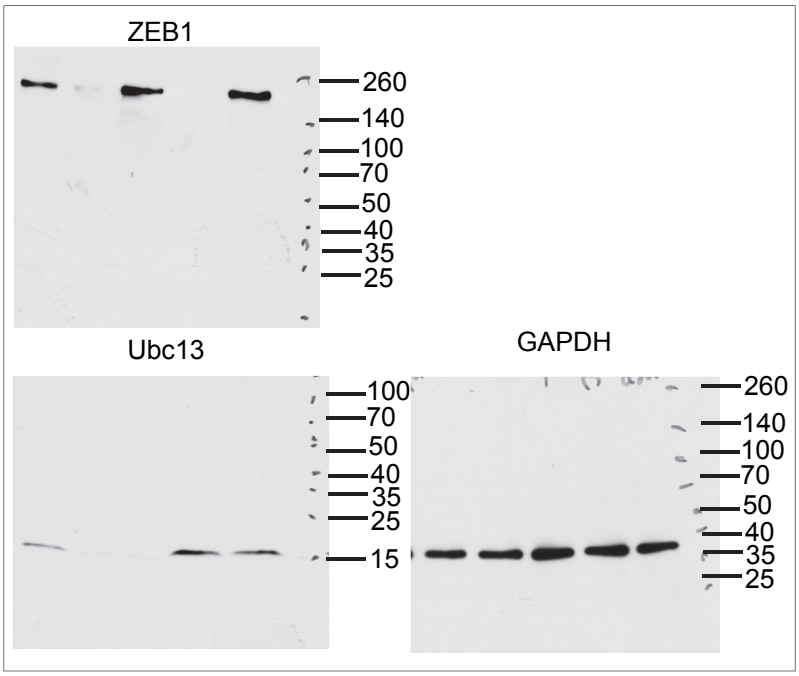


Fig. 4d

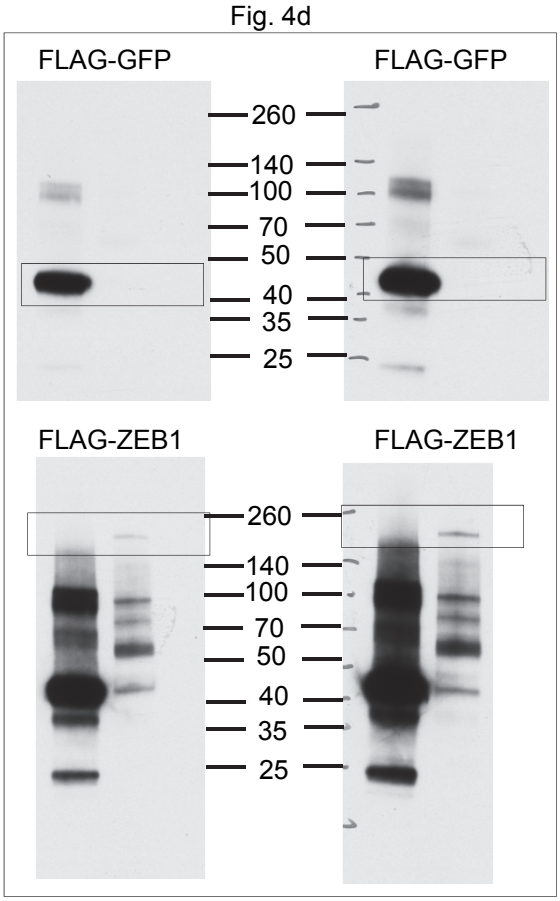


Fig. 4a

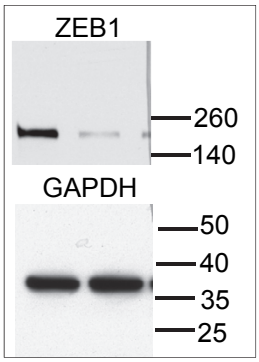
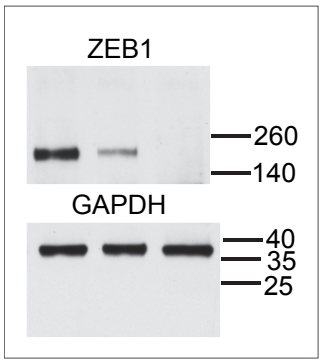


Fig. 4b



Supplementary Figure 5 Uncropped images of immunoblots (continued).

SUPPLEMENTARY TABLES

Supplementary Table 1 Layout of the miRNA PCR array.

Supplementary Table 2 Vectors used in this study.