

Figure W1. Colocalization of c-Jun and PMSA5. Immunofluorescence staining of c-Jun and PMSA5 in MDA-MB-436 and 10 μ M MG132-treated MCF7 cells. DAPI was used to stain nucleus.

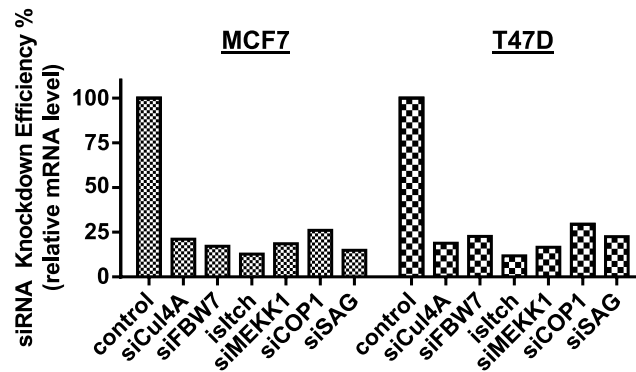


Figure W2. siRNA-mediated knockdown effect of c-Jun-targeting E3 ligases. MCF7 and T47D cells were transfected with 50 nM control or siRNA pool against CUL4, FBW7, ITCH, MEKK1, COP1, or SAG for 3 days. Total RNA was extracted from these cells and subjected to quantitative reverse transcription-polymerase chain reaction with the respective primers. β -Actin mRNA was used as the internal control for standardization.

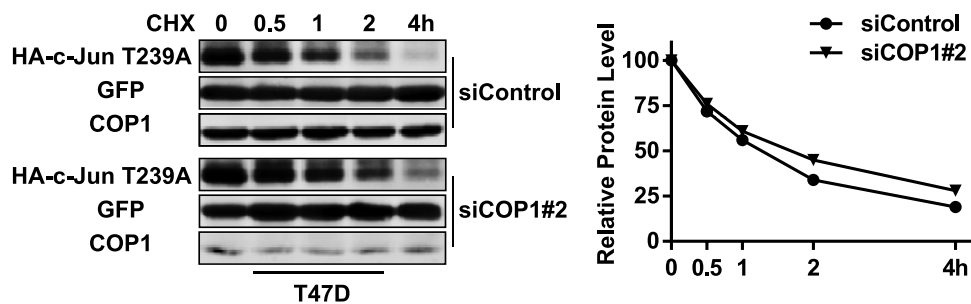


Figure W3. Depletion of COP1 does not alter protein stability of c-Jun-T239A. T47D cells were treated with control or COP1 siRNA for 2 days. HA-tagged c-Jun-T239A was then co-transfected into these cells with GFP for 1 day followed by the addition of 20 μ g/ml cycloheximide for varying times. Cells were harvested, and cell lysates were analyzed by immunoblot analysis to detect HA-tagged c-Jun and GFP. The relative HA-tagged c-Jun amount was standardized by the amount of GFP.

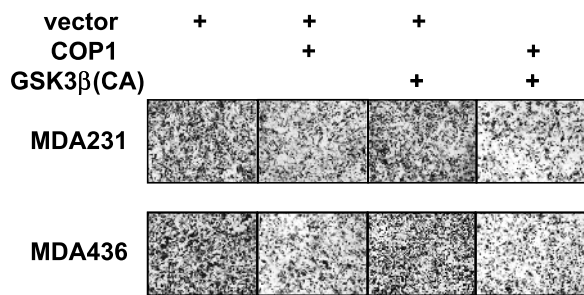


Figure W4. Simultaneously expressing COP1 and constitutively active GSK3β block cell migration of invasive breast cancer cells. COP1 and GSK3β(CA) were lentivirally transduced into MDA-MB-231 and MDA-MB-436 cells either alone or together. Populations of transduced cells were added into transwells and allowed to migrate for 4 hours. Images are crystal violet–stained migratory cells on the undersurface of transwells.

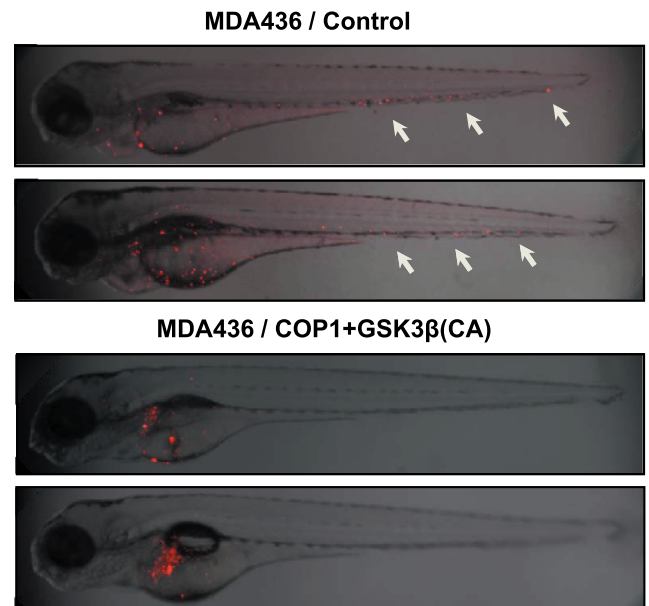


Figure W5. Simultaneously expressing COP1 and constitutively active GSK3β suppress breast cancer cell metastasis. MDA-MB-436 cells were lentivirally transduced with COP1 and constitutively active GSK3β together for 3 days. Control and COP1/GSK3βS9A-transduced MDA-MB-436 cells were then labeled with fluorescent dye CM-Dil and microinjected into the perivitelline space of 48-hpf zebrafish embryos. Embryos were imaged under a confocal microscope.

Table W1. Clinicopathologic Correlates of COP1 Expression in Human Breast Cancer.

Clinical Features	COP1 Expression*		P Value [†]
	Low (%)	High (%)	
Histologic grade			
1	5	6	.28
2-3	26	77	
Tumor diameter			.72
<2 cm	6	22	
≥2 cm	26	70	
Lymph node status			.71
Negative	14	48	
Positive	15	40	
ER receptor			.46
Negative	13	44	
Positive	20	46	
PR receptor			.89
Negative	15	42	
Positive	12	35	

PR indicates progesterone receptor.

*Low/high by median (low, COP1 < 0; high, COP1 ≥ 0 expression value).

[†]Pearson χ^2 test.