Supplementary Information

Deptor Enhances Triple-negative Breast Cancer Metastasis and Chemoresistance *via* Coupling to Survivin Expression

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Running Title: Deptor Induces Survivin-dependent Chemoresistance

Table S1: Pharmacological inhibitors or chemicals

Name	Target	Concentration	Supplier
SB431542	TβR-I	10 μM	EMD Millipore (Billerica, MA, USA)
TβR-I Inh II	TβR-I	10 μg/ml	EMD Millipore
SB203580	p38 MAPK	10 μM	BD Biosciences (San Jose, CA, USA)
SIS3	Smad3	10 μM	EMD Millipore
5-Fluorouracil	Thymidylate synthase	10 μM	Sigma (St. Louis, MO, USA)
Hydrogen Peroxide	--	50 μM	Sigma
Tamoxifen	ERα	0.1 nM	Sigma
Fulvestrant (ICI)	ERα	1 μM	Calbiochem
Doxorubicin	DNA	50 nM	Cayman Chemicals
Paclitaxel	Microtubules	50 nM	LC Laboratories

Shown are the final concentrations of the pharmacological antagonists and used inhibit the indicated proteins or cellular targets. Also provided are the vendors where these reagents were obtained.

Table S2: Immunoblotting antibodies

Antibody	Dilution	Supplier
Deptor	1:1000	Novus Biologicals (Littleton, CO, USA)
Phospho-S473-Akt	1:1000	Cell Signaling Technologies (Danvers, MA, USA)
Akt	1:1000	Cell Signaling Technologies
Phospho-S6K	1:1000	Cell Signaling Technologies
S6K	1:1000	Cell Signaling Technologies
ERα	1:500	Santa Cruz Biotechnologies (Santa Cruz, CA, USA)

Twist	1:250	Abcam (Cambridge, MA, USA)
β3 Integrin	1:1000	Cell Signaling Technologies
Survivin	1:1000	Cell Signaling Technologies
β-actin	1:1000	Santa Cruz Biotechnologies

Shown are the antibodies and dilutions used to visualize the indicated proteins. Also provided are the vendors where these reagents were obtained.

TABLE S3: Real-time PCR primer pairs

Target	Application	Sequence (5' to 3')
GAPDH	PCR-Sense	5'-CAACTTTGGCATTGTGGAAGGGCTC
GAPDH	PCR-Antisense	5'-GCAGGGATGATGTTCTGGGCAGC
mDeptor	PCR-Sense	5'-TTCCAGATAGAAGGCTCCAGCACC
mDeptor	PCR-Antisense	5'-AAGCAACCCGTCAAAGCACTCG
hSurvivin	PCR-Sense	5'-TGACGACCCCATAGAGGAACA
hSurvivin	PCR-Antisense	5'-CAGTAGAGGAGCCAGGGACT
mE-cadherin	PCR-Sense	5'-CCCTACATACACTCTGGTGGTTCA
mE-Cadherin	PCR-Antisense	5'-GGCATCATCATCGGTCACTTTG
hE-cadherin	PCR-Sense	5'-CATCTTTGTGCCTCCTGAAA
hE-cadherin	PCR-Antisense	5'-TGGGCAGTGTAGGATGTGAT
mCK-19	PCR-Sense	5'-TTGGGTCAGGGGGTGTTTTC
mCK-19	PCR-Antisense	5'-TTCTCATTGCCAGACAGCAGC
hCK-19	PCR-Sense	5'-GACCTTGGAGGCAGACAAAT
hCK-19	PCR-Antisense	5'-GATAGTGAGCGGCAGAATCA

Figure S1: Parvani et al

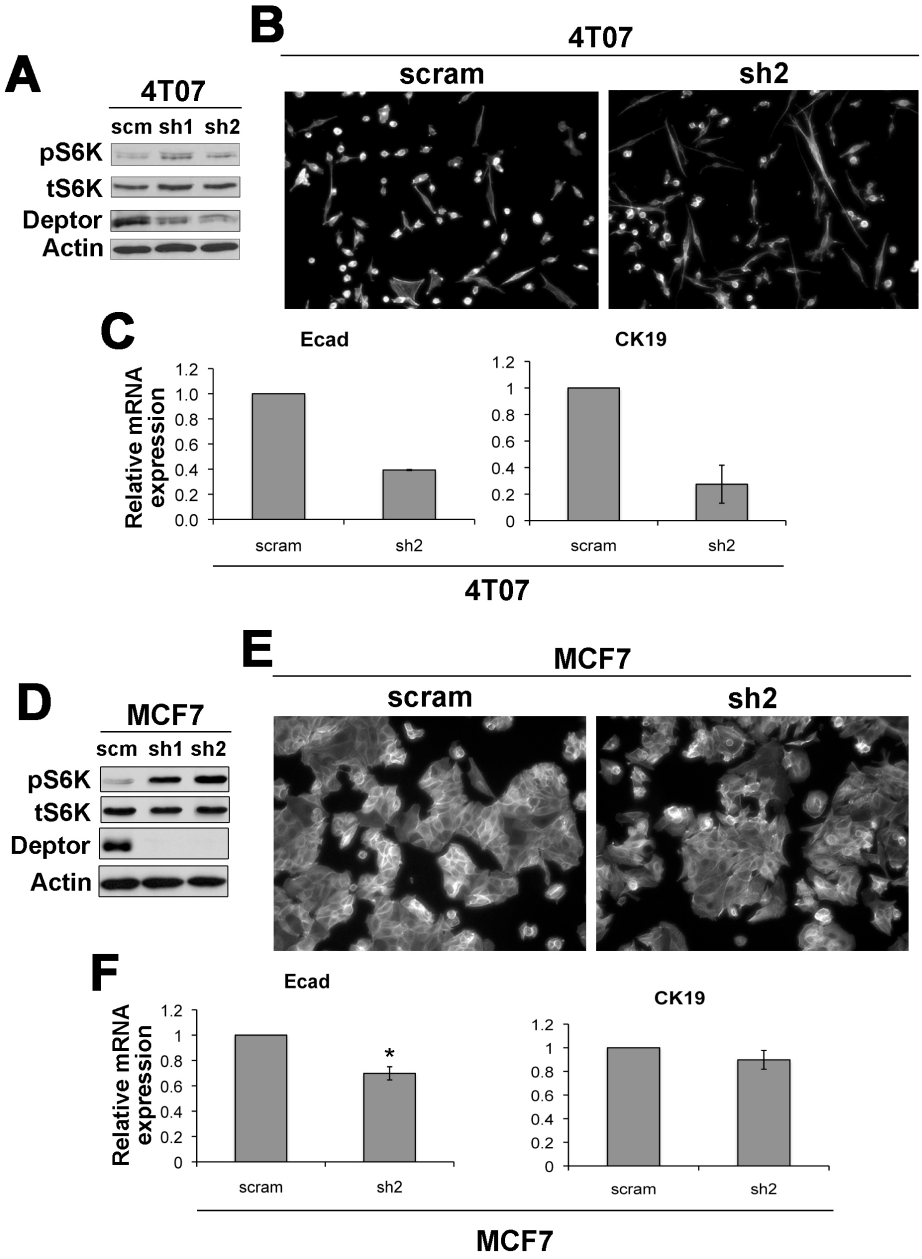

Figure S1. Deptor-deficiency induces EMT in malignant MECs. (A and D) Deptor-deficiency (sh1 and sh2) activates S6K activity in 4T07 (A) or MCF7 (D) cells. Immunoblots are representative images from 3 independent experiments. (B and E) Phalloidin staining of control (scram) and Deptor-deficient (sh2) 4T07 (B) or MCF7 (E) cells to monitor alterations in the actin cytoskeleton. Images are representative of 2 independent experiments. (C and F) Deptor-deficiency elicits loss of epithelial gene expression patterns, including that of E-cadherin (E-Cad) and cytokeratin-19 (CK19) as determined by semi-quantitative real-time PCR. Data are the mean (±SEM; n=3; **P*<0.05*).*

Figure S2: Parvani et al

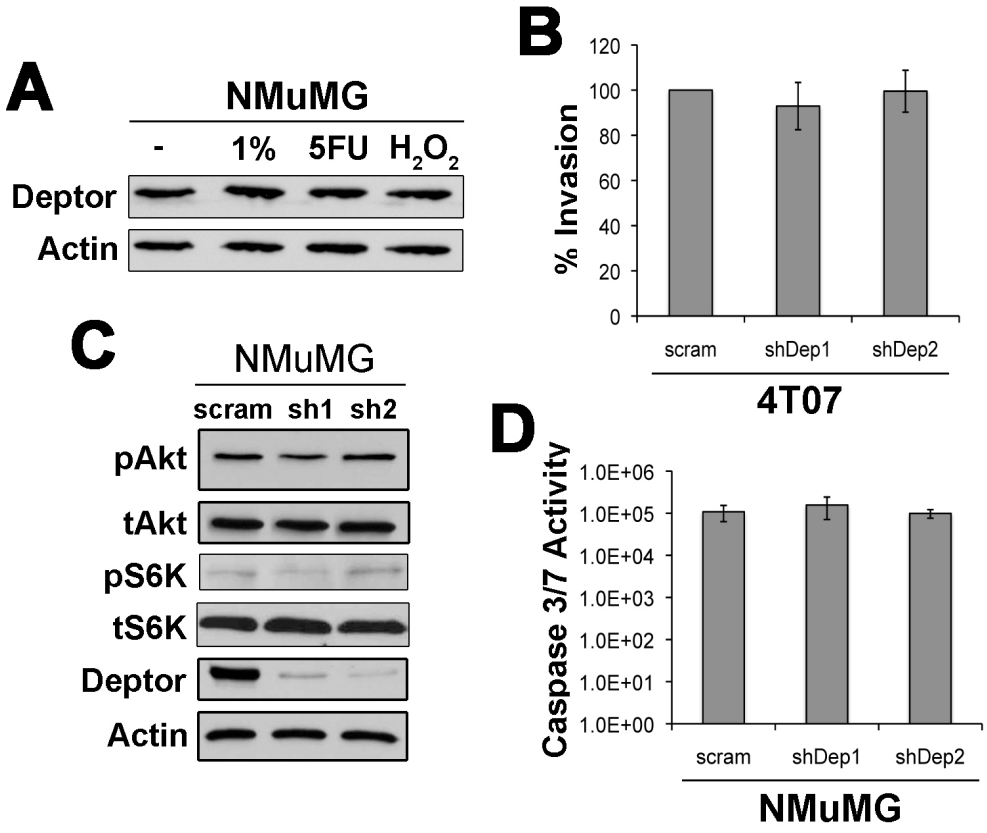

Figure S2. Deptor expression is unaffected by cellular stress, while Deptor-deficiency is insufficient to elicit EMT programs. (A) Deptor expression was evaluated in NMuMG cells that were cultured under different the following stress conditions: *(i)* untreated (-); *(ii)* serum-deprivation (% serum, 1%); *(iii)* 5-fluorouracil (5-FU, 10 μM); and *(iv)* hydrogen peroxide (H_2O_2, 50 μM). Immunoblots are representative of 2 independent experiments. Deptor-deficiency was insufficient in altering the invasion of 4T07 cells (B), the activation status of AKT and S6K in NMuMG cells (C), or the activity of caspase-3/7 in NMuMG cells (D). Data in *Panel B* are representative immunoblots from 3 independent experiments, while those in *Panels C* and *D* are the mean (±SEM; n=3).