Supplementary Materials

Manuscript Title:

Knockdown of EPS8 expression may attenuate disease progression in enzalutamide-resistant prostate cancer.

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Supplementary Figure S1. Western blotting analysis for EPS8 expression in LNCaP, Enz-R, and LNCaP cells (n = 3).

Supplementary Figure S2. Western blotting analysis for the expression of EMT markers (E-cadherin, α-SMA, Snail, and Slug with β-Actin control) in LNCaP cells transfected with pCMV-EPS8 and pCMV-GFP (n = 3).

Supplementary Figure S3. Western blotting analysis for the expression of EMT markers (E-cadherin, α-SMA, Snail, and Slug with β-Actin control) in LNCaP and Enz-R cells transfected with pCMV-EPS8 and pCMV-GFP (n = 3).

Supplementary Figure S4. Western blotting analysis for the expression of EMT markers (E-cadherin, α-SMA, Snail, and Slug with β-Actin control) in LNCaP cells transfected with shRNA-EPS8 and shRNA-control (n = 3).

Supplementary Figure S5. Western blotting analysis for the expression of EMT markers (E-cadherin, α-SMA, Snail, and Slug with β-Actin control) in LNCaP and Enz-R cells transfected with shRNA-EPS8 and shRNA-control (n = 3)

Figure S6a

LNCap cell
E-cadherin 135KDa

Supplementary Figure S6a. Western blotting analysis for the expression of EMT markers (E-cadherin and α-SMA) in LNCaP cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors (n = 3).

Supplementary Figure S6b. Western blotting analysis for the expression of EMT markers (Snail and Slug) in LNCaP cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors (n = 3).

Supplementary Figure S6c. Western blotting analysis for the expression of β-actin (control) in LNCaP cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors (n = 3).

Figure S7a

LNCap Enz-R
E-cadherin 135KDa

pCMV-GFP

pCMV-Eps8

Repeat 1

Repeat 2

Repeat 3

LNCap Enz-R
α-SMA 42KDa

pCMV-GFP

pCMV-Eps8

Repeat 1

Repeat 2

Repeat 3

Supplementary Figure S7a. Western blotting analysis for the expression of EMT markers (E-cadherin and α-SMA) in LNCaP and Enz-R cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors (n

= 3).

Figure S7b

LNCap Enz-R

Snail 29KDa

pCMV-GFP

pCMV-Eps8

Repeat 1

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

Repeat 2

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa

Repeat 3

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa

Control(pCMV-Eps8)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

35KDa
25KDa

Control(pCMV-Eps8)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa

Control(pCMV-Eps8)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa

LNCap Enz-R

Slug 30KDa

pCMV-GFP

pCMV-Eps8

Repeat 1

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

35KDa
25KDa
15KDa

Repeat 2

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa

Repeat 3

Control(pCMV-GFP)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
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Buparlisib(1uM)

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Salirasib(20µM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
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Control(pCMV-Eps8)
Salirasib (20uM)
Nutlin-3 (10uM)
Baricitinib (20 µM)
Buparlisib(1uM)

75KDa
65KDa
45KDa
35KDa
25KDa
15KDa

Supplementary Figure S7b. Western blotting analysis for the expression of EMT markers (Snail and

Slug) in LNCaP and Enz-R cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors.

Figure S7c

Supplementary Figure S7c. Western blotting analysis for the expression of β-actin (control) in LNCaP and Enz-R cells after administration of Ras/p53/JAK/PI3K-signaling inhibitors.

Figure S8

Supplementary Figure S8. EPS8 transfection in xenograft tumor sections.
H&E staining for xenograft tumor sections 25 days after transfection. More aggressive infiltration was detected in tumor transfected with pCMV-EPS8, while less nucleus and infiltration was detected in tumor transfected with shRNA-EPS8. shRNA, short-hairpin RNA

Figure S9

Supplementary Figure S9. The EMT markers in xenograft tumors using IHC.
LNCaP xenograft tumors were analyzed for the expression of (A) EPS8, (B) α-SMA, and **(C)** E-cadherin by IHC 25 days after transfection. **(D)** Higher EPS8, higher α SMA, and lower E-cadherin were detected in the xenograft tumor cells transfected with pCMV-EPS8. In contrast, lower EPS8, lower α SMA, and higher E-cadherin were detected in the xenograft tumor cells transfected with shRNA-EPS8. [#]P < 0.05 vs. Control, *P < 0.05 vs. pCMV-GFP, + P < 0.05 vs. shRNA-control. α-SMA, α smooth muscle actin; EMT, epithelial-to-mesenchymal transition; GFP, green fluorescent protein; IHC, immunohistochemistry; shRNA, short-hairpin RNA