*Supplementary Materials*

# Ezrin Regulates the Cell Surface Localization of PD-L1 in HEC−151 Cells

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*Original source images for immunoblots.*

## PD−L1
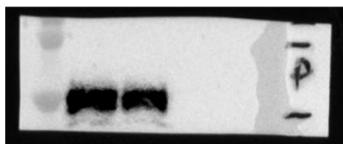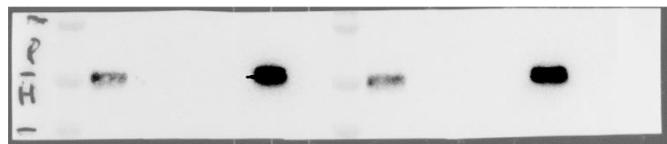


**Figure 1c**            **Figure 4**

**Ezrin**
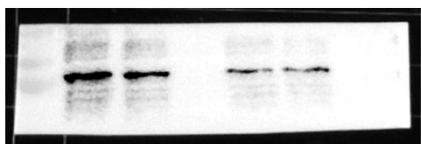


**Figure 1c**            **Figure 4**

## Radixin
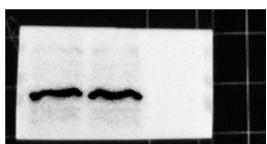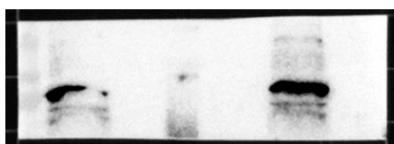


**Figure 1c**      **Figure 4**

## Moesin
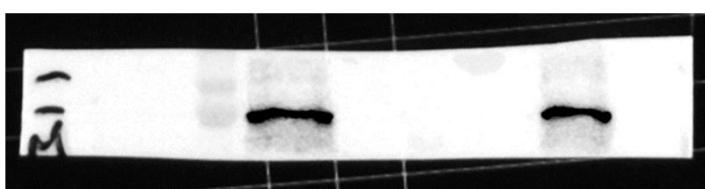


**Figure 1c**      **Figure 4**

## GAPDH
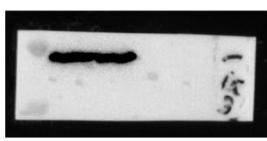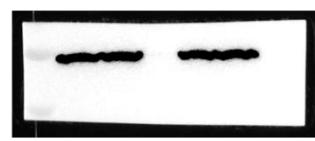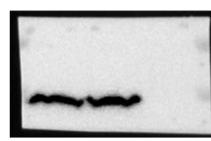


**Figure 1c**          **Figure 1c**          **Figure 1c**
**(PD−L1)**            **(Ezrin)**            **(Radixin)**
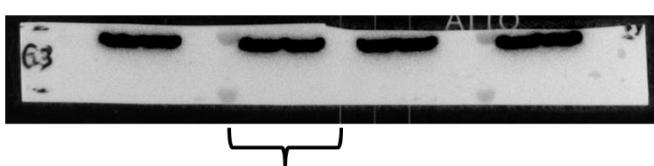


**Figure 1c**
**(Moesin)**

**Figure S1.** Original source images for immunoblots. The original western blotting membrane to detect the protein expression of programmed death ligand−1 (PD−L1), ezrin, radixin, and moesin as well as the corresponding glyceraldehyde−3−phosphate dehydrogenase (GAPDH) used as a loading control shown in Figure 1c or Figure 4. Blots are labeled according to the corresponding Figure panel within the main manuscript.

*RNA interference−mediated knockdown of target genes in HEC−151 cells.*
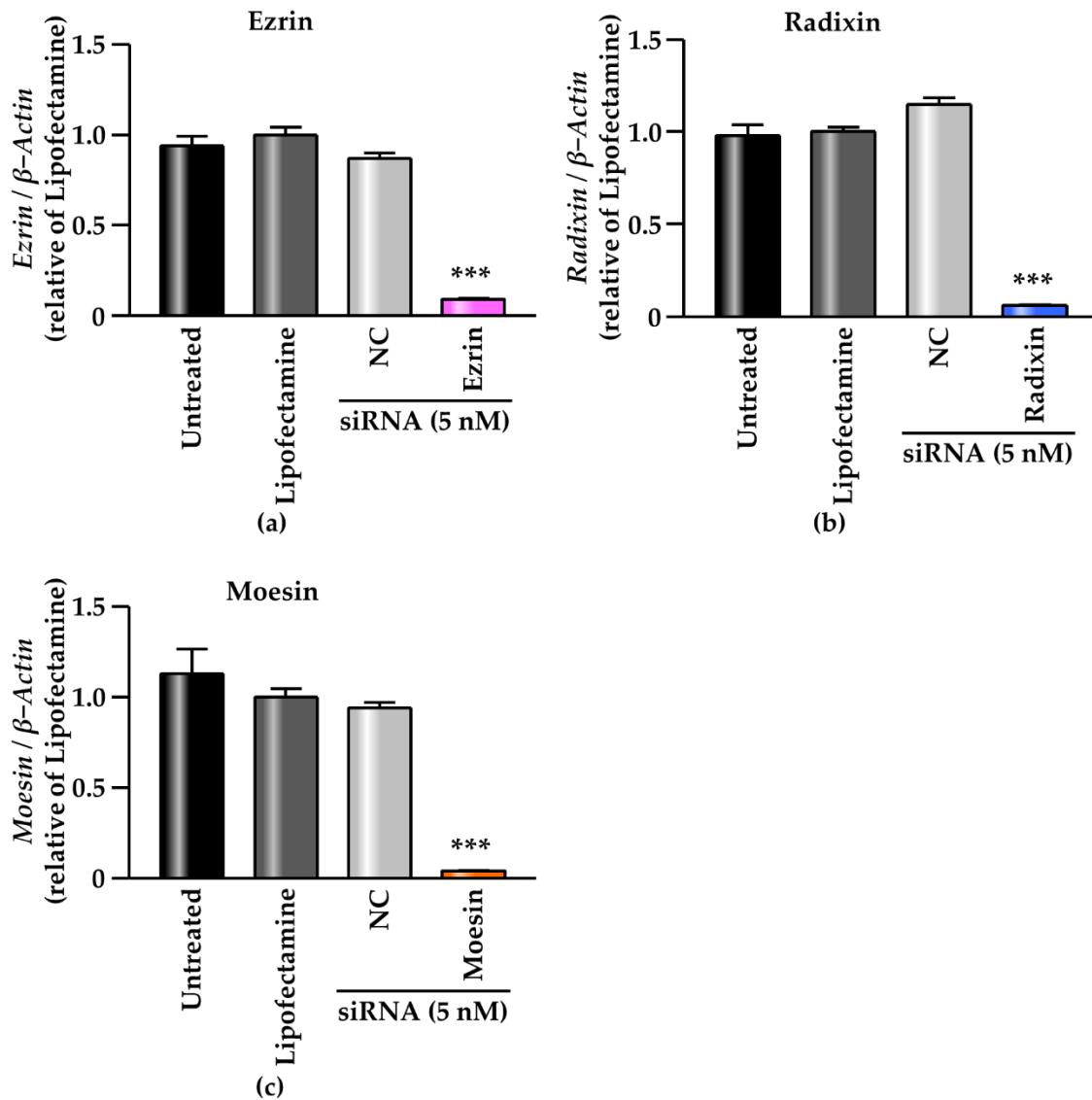


**Figure S2.** RNA interference−mediated knockdown of target genes in HEC−151 cells. Cells were treated with the transfection medium (Untreated), transfection reagent (Lipofectamine), nontargeting control (NC) siRNA, and specific siRNAs for ezrin, radixin, or moesin at the concentrations of 5 nM, and then cultured for 4 days. Each column shows the mRNA expression levels of (**a**) ezrin, (**b**) radixin, and (**c**) moesin normalized with β−Actin in cells from each treatment group relative to that in cells treated with Lipofectamine alone as determined by real−time reverse transcription−polymerase chain reaction. *n* = 3, ***$p < 0.001$ vs. Lipofectamine. All data were expressed as the mean ± SEM and analyzed by one−way ANOVA followed by Dunnett's test.

**Table S1.** List of primers used in the present study.

| Gene | Primer sequence (5′→3′) |
| --- | --- |
| *h−β−Actin* (forward) | TGGCACCCAGCACAATGAA |
| *h−β−Actin* (reverse) | CTAAGTCATAGTCCGCCTAGAAGCA |
| *h−Ezrin* (forward) | ACCATGGATGCAGAGCTGGAG |
| *h−Ezrin* (reverse) | CATAGTGGAGGCCAAAGTACCACA |
| *h−Radixin* (forward) | GAATTTGCCATTCAGCCCAATA |
| *h−Radixin* (reverse) | GCCATGTAGAATAACCTTTGCTGTC |
| *h−Moesin* (forward) | CCGAATCCAAGCCGTGTGTA |
| *h−Moesin* (reverse) | GGCAAACTCCAGCTCTGCATC |
| *h−PD−L1* (forward) | CAATGTGACCAGCACACTGAGAA |
| *h−PD−L1* (reverse) | GGCATAATAAGATGGCTCCCAGAA |

**Table S2.** List of antibodies used in the present study.

| Antibodies | Source | Cat# | Dilution |
|---|---|---|---|
| rabbit anti−ezrin | Cell Signaling Technology | 3145 | 1:2,000 (WB) 1:50 (IF) |
| rabbit anti−radixin | Gene Tex | GTX105408 | 1:2,000 (WB) 1:100 (IF) |
| rabbit anti−moesin | Cell Signaling Technology | 3150 | 1:2,000 (WB) 1:50 (IF) |
| Alexa Fluor 488−conjugated rabbit anti−PD−L1 | Cell Signaling Technology | 25048 | 1:50 (IF) |
| Alexa Fluor 488−conjugated goat anti−rabbit IgG | Thermo Fisher Scientific | R37116 | 1:25 (IF) |
| Alexa Fluor 594−conjugated goat anti−rabbit IgG | Thermo Fisher Scientific | R37117 | 1:25 (IF) |
| HRP−conjugated rabbit anti−PD−L1 | Cell Signaling Technology | 51296s | 1:1,000 (WB) |
| mouse anti−GAPDH | Merck | MAB374 | 1:20,000 (WB) |
| HRP−conjugated anti−rabbit IgG (heavy + light) | SeraCare Life Sciences | 5220−0336 | 1:10,000 (WB) |
| HRP−conjugated anti−mouse IgG (heavy + light) | SeraCare Life Sciences | 5220−0341 | 1:10,000 (WB) |
| rabbit anti−PD−L1 | Cell Signaling Technology | 13684 | 1:30 (IP) |
| rabbit IgG isotype control | Cell Signaling Technology | 3900 | 1:30 (IP) |
| APC−conjugated mouse anti−human PD−L1 | BioLegend | 329708 | 4.0 µg/test (FC) |

western blotting; WB, immunofluorescence; IF, immunoprecipitation; IP, flow cytometry; FC, horseradish peroxidase; HRP, allophycocyanin; APC