*Supplementary Material*

*Article*

# Recombinant Human Prolidase (rhPEPD) Induces Wound Healing in Experimental Model of Inflammation Through Activation of EGFR Signalling in Fibroblasts

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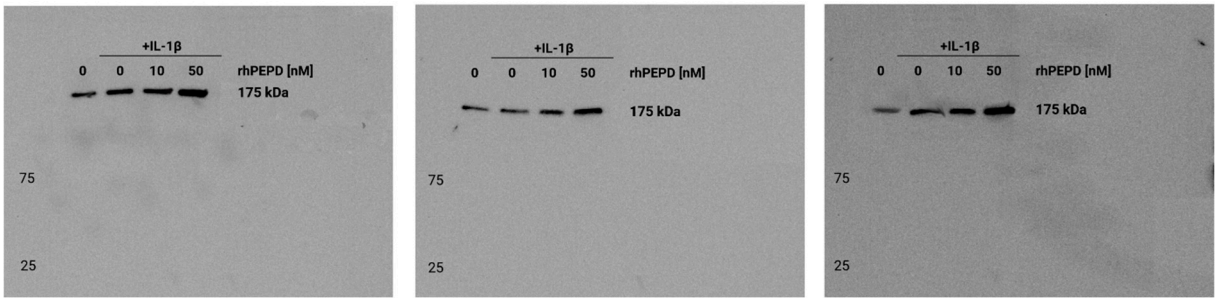
**Description of data**: The blots and images are described in the result section.
Supplementary data analysis presented- representative blots from Western blotting on Figures 3A,B and 4A,B.

## 1. Representative blots from Western blotting analysis presented in Figure 3A.

### 1.1. EGFR expression



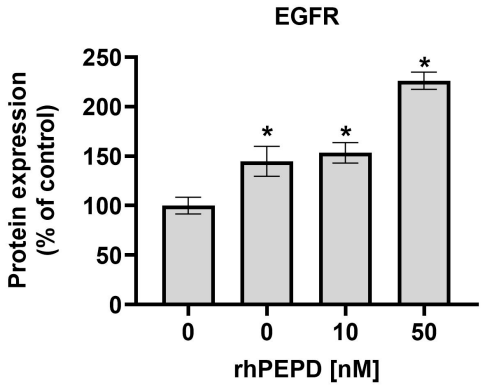
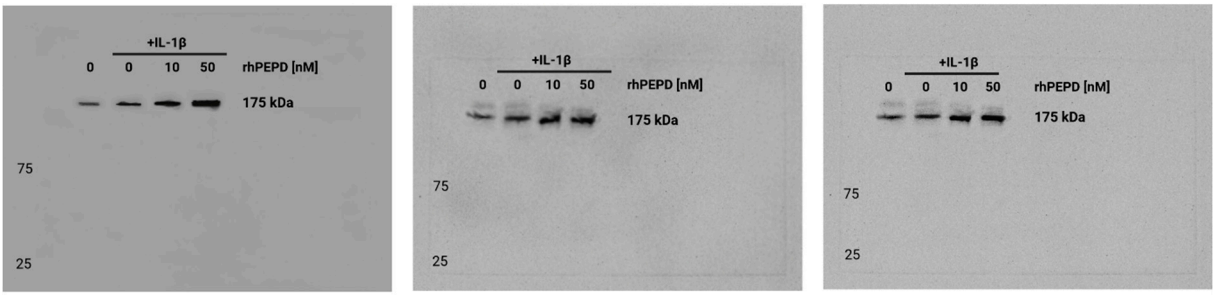



**Figure S1.** The EGFR expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.2. Phospho-EGFR expression



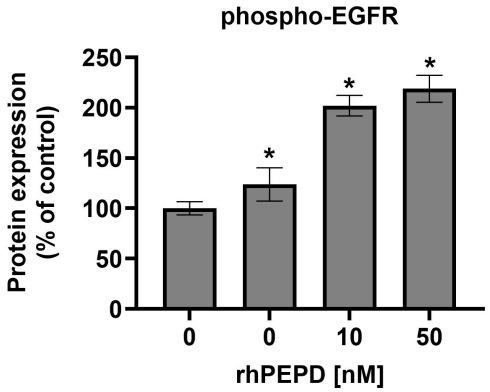
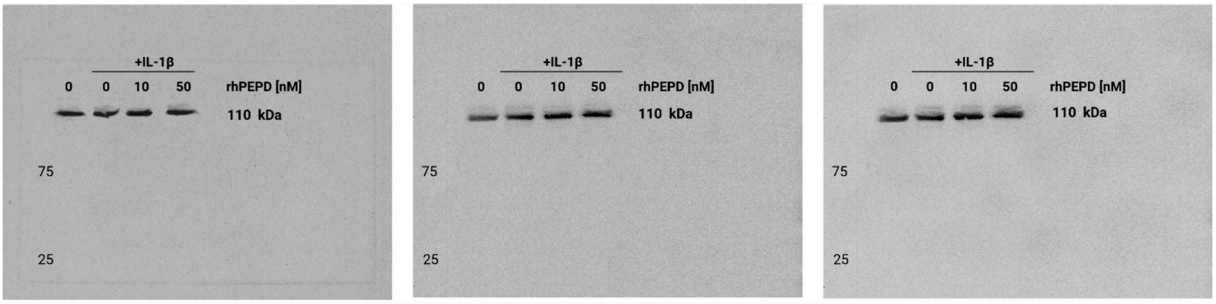




**Figure S2.** The phospho-EGFR expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.3. PI3K expression



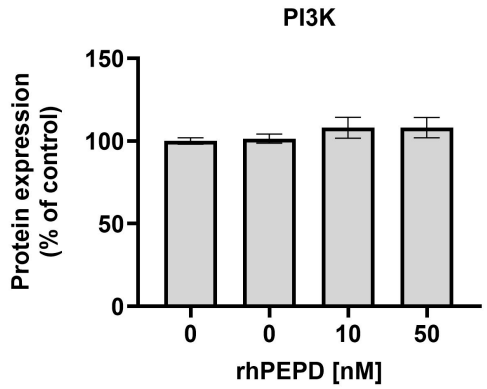
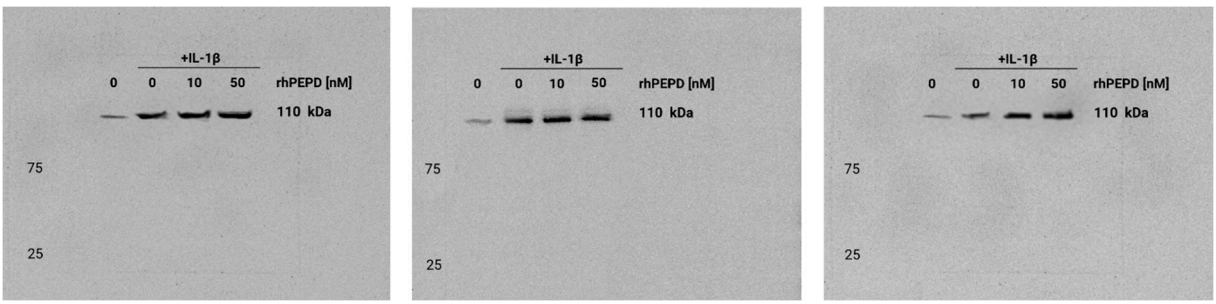




**Figure S3.** The PI3K expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.4. Phospho-PI3K expression



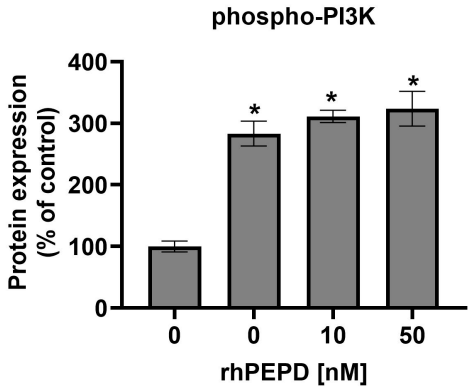
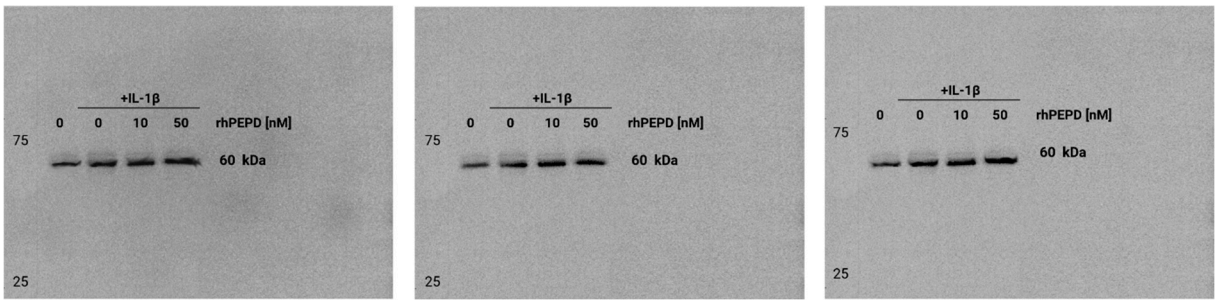



**Figure S4.** The phospho-PI3K expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.5. AKT expression



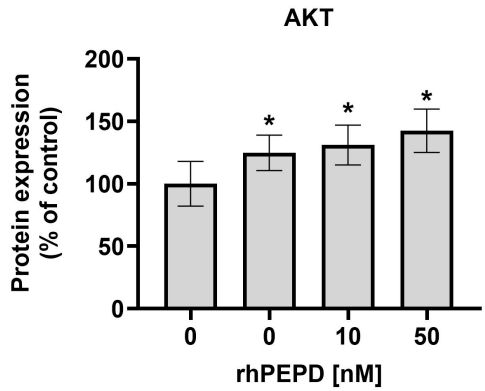
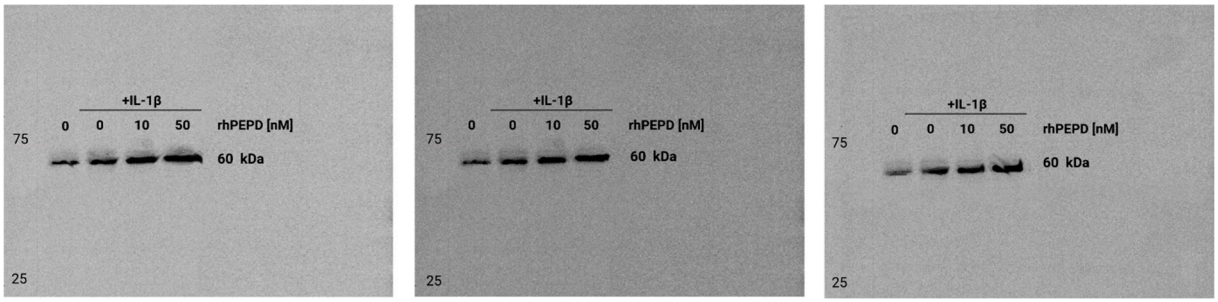




**Figure S5.** The AKT expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.6. Phospho-AKT expression



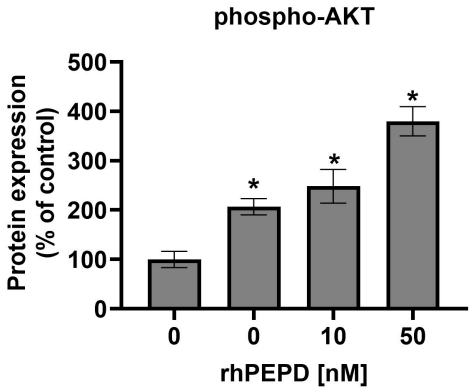
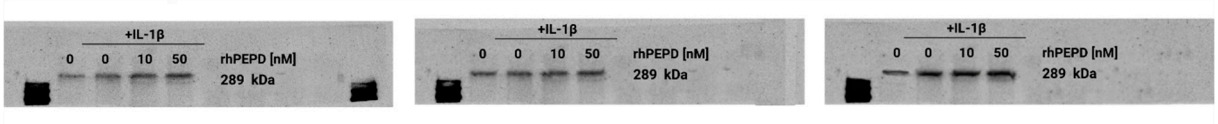




**Figure S6.** The phospho-AKT expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.7. mTOR expression



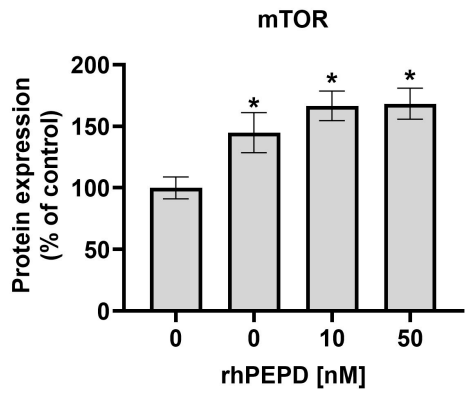



**Figure S7.** The mTOR expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.8. Phospho-mTOR expression

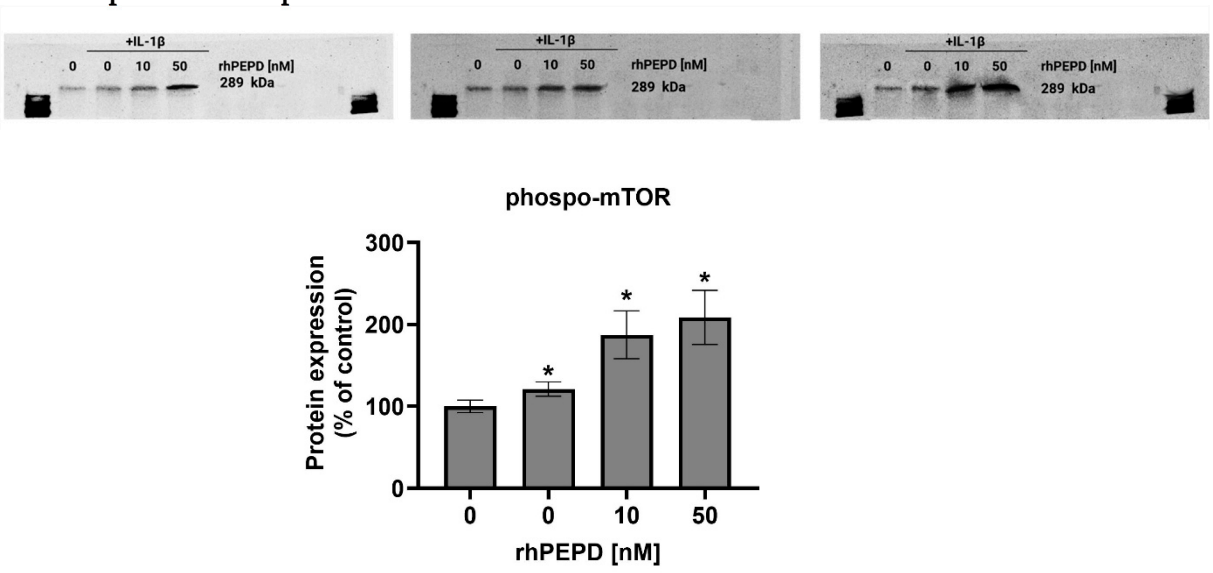
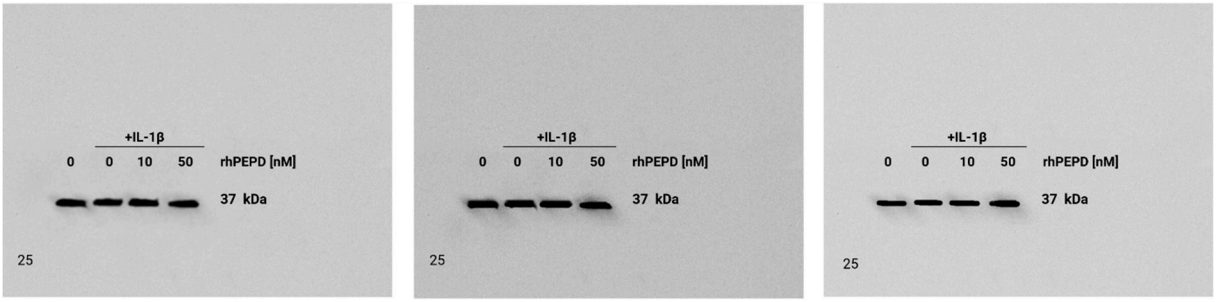



**Figure S8.** The phospho-mTOR expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 1.9. GAPDH expression



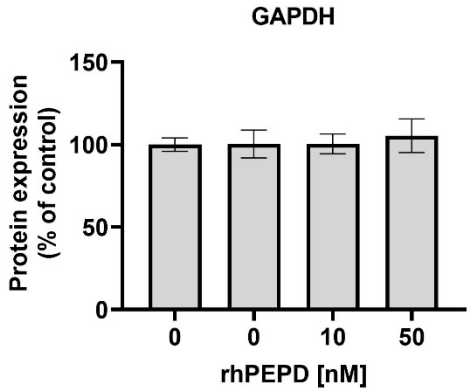
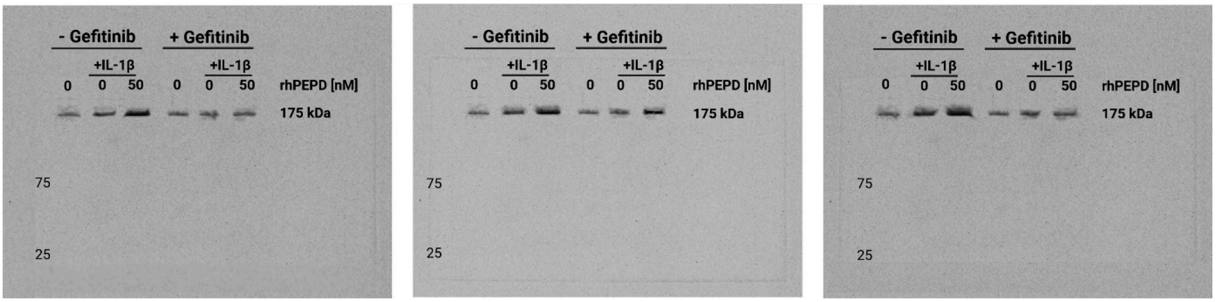



**Figure S9.** The GAPDH expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $*P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

## 2. Representative blots from Western blotting analysis presented in Figure 3B.

### 2.1. EGFR expression



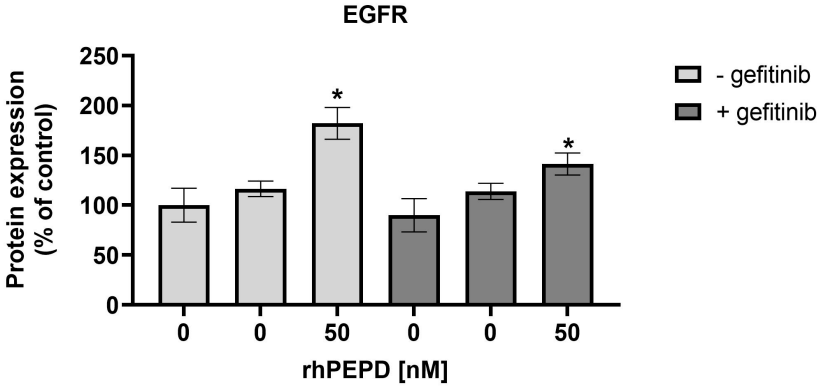




**Figure S10.** The EGFR expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The

densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as *$P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 2.2. Phospho-EGFR expression

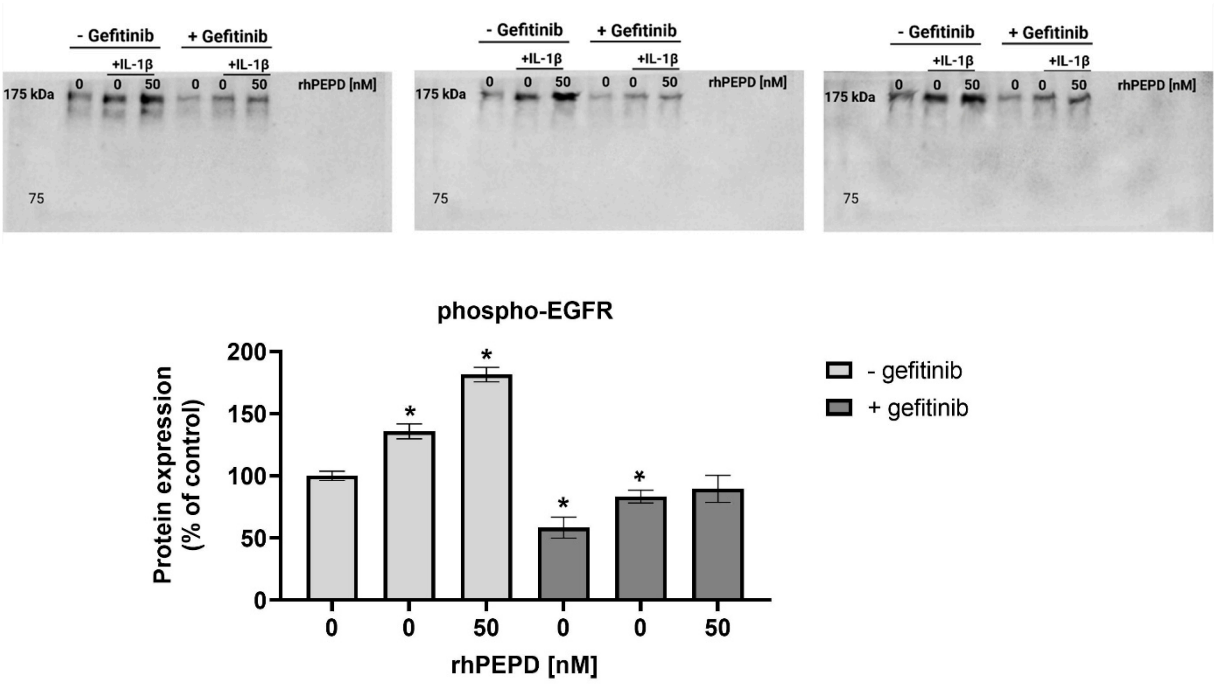
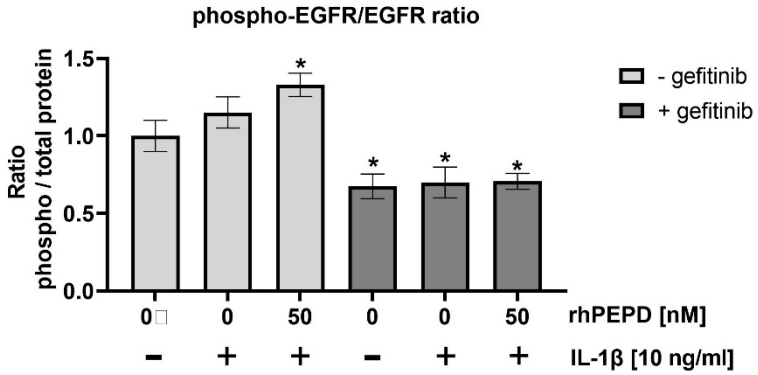



**Figure S11.** The phospho-EGFR expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as *$P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

**Ratio of phospho-EGFR/EGFR expression**



### 2.3. PI3K expression

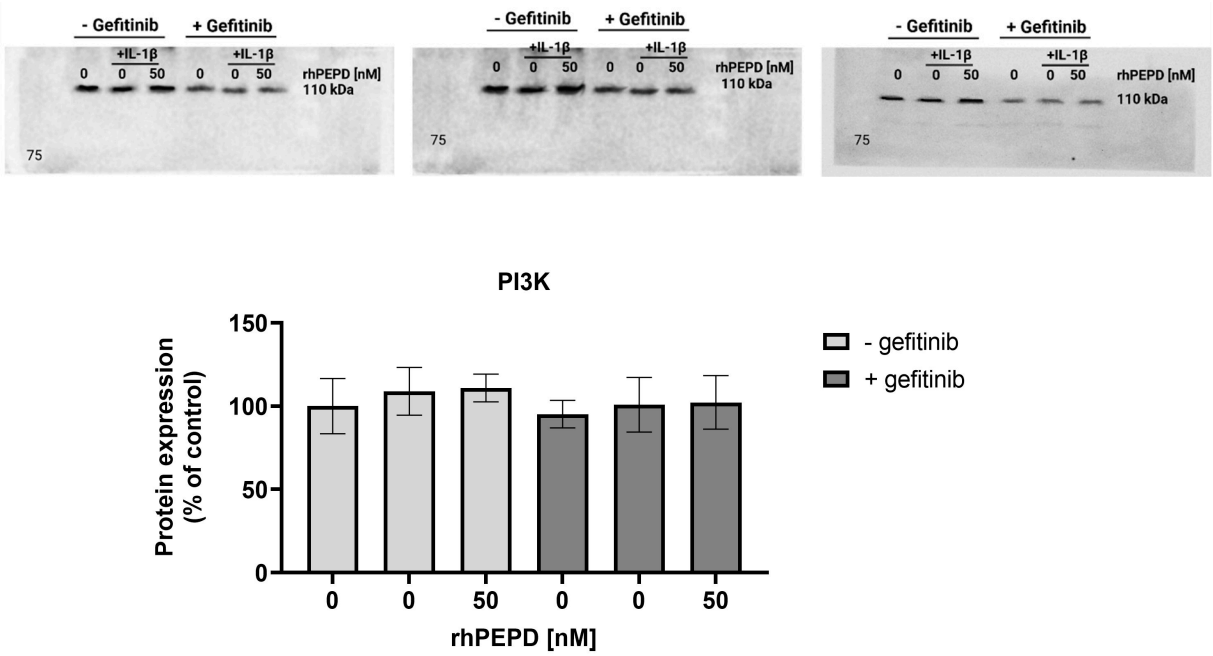



**Figure S12.** The PI3K expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 2.4. Phospho-PI3K expression

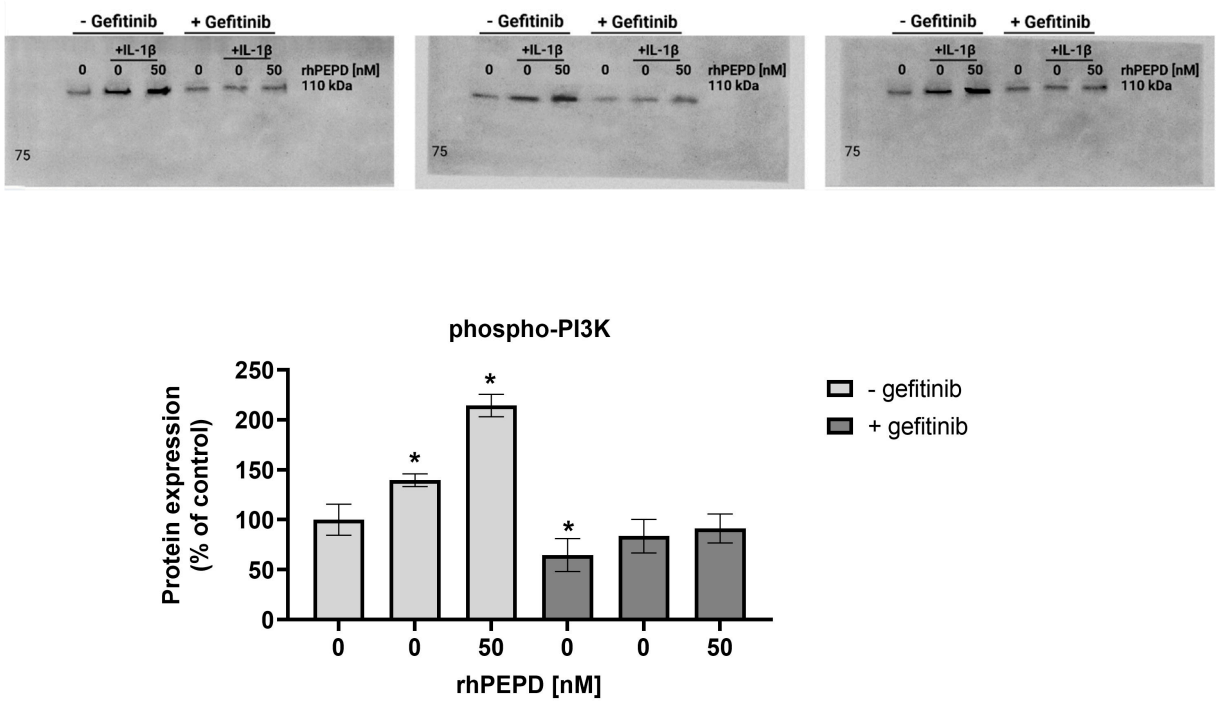
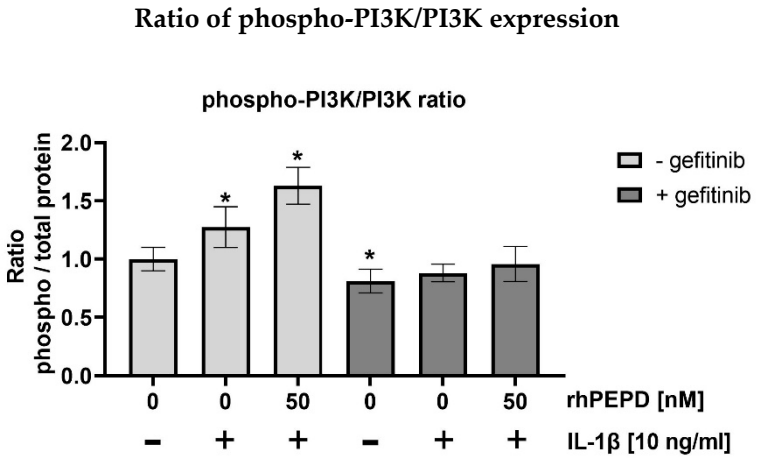
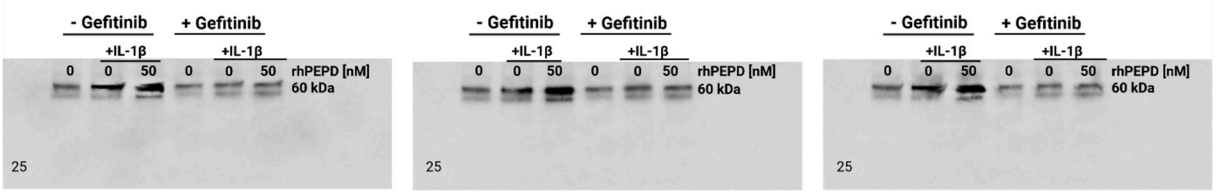


**Figure S13.** The phospho-PI3K expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).




## 2.5. AKT expression



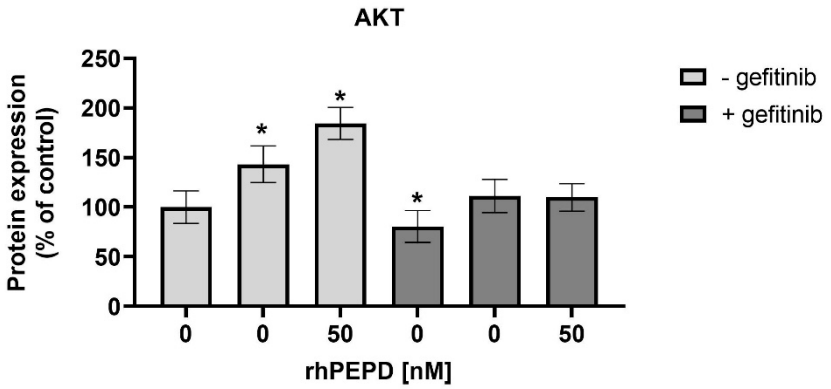




**Figure S14.** The AKT expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

## 2.6. Phospho-AKT expression

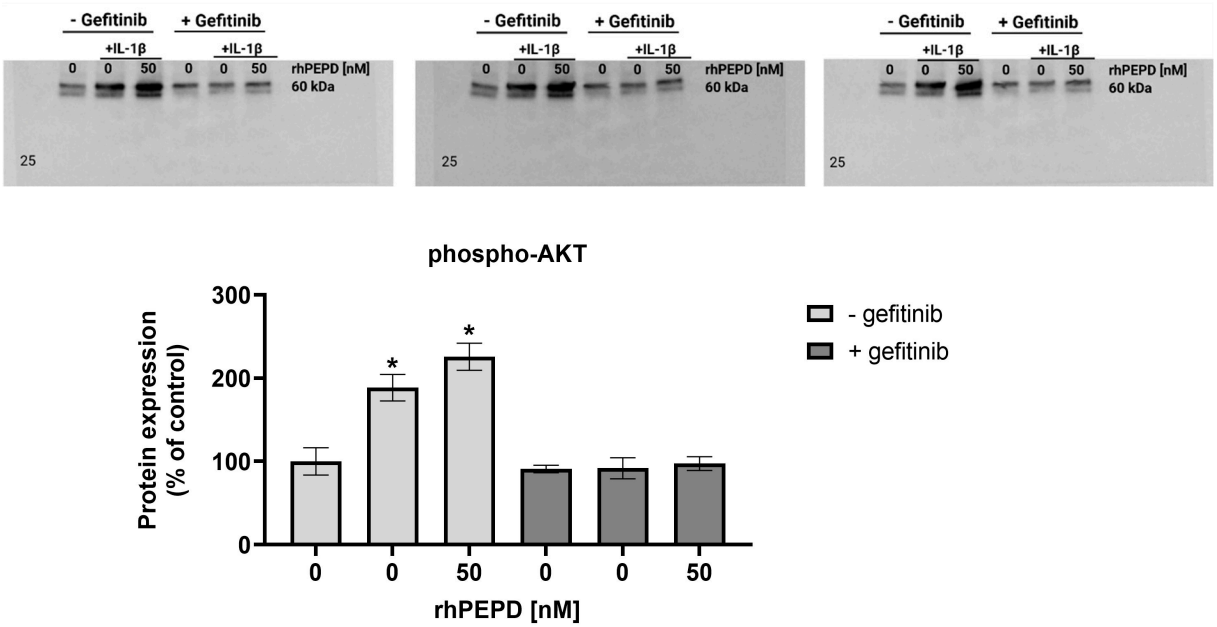
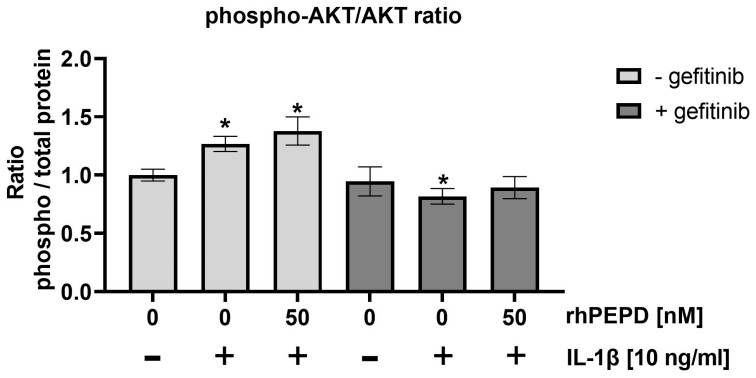
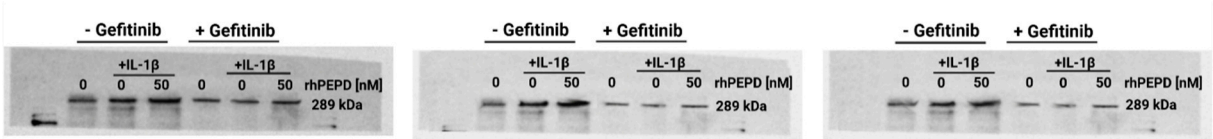



**Figure S15.** The phospho-AKT expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

**Ratio of phospho-AKT/AKT expression**




## 2.7. mTOR expression



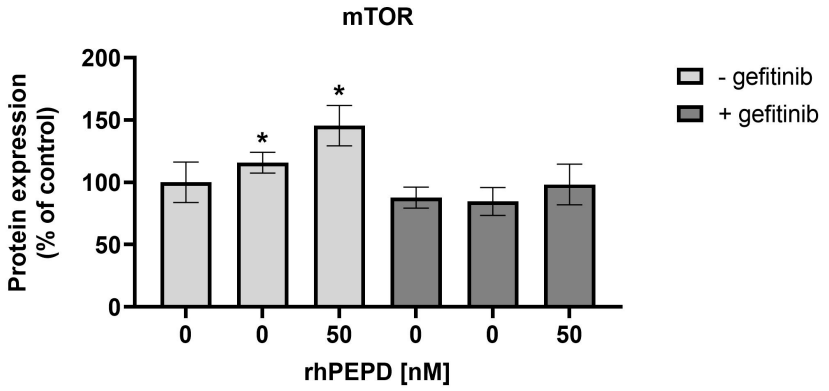
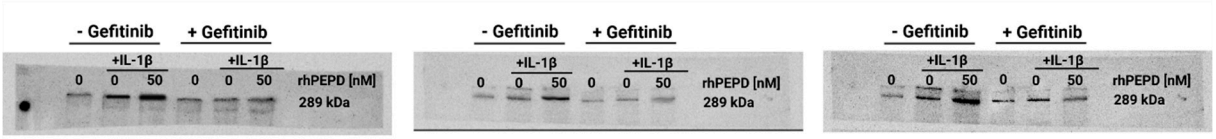



**Figure S16.** The mTOR expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

### 2.8. Phospho-mTOR expression



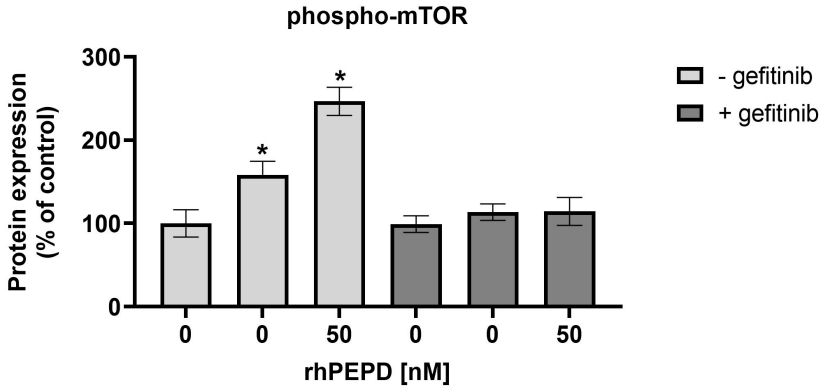
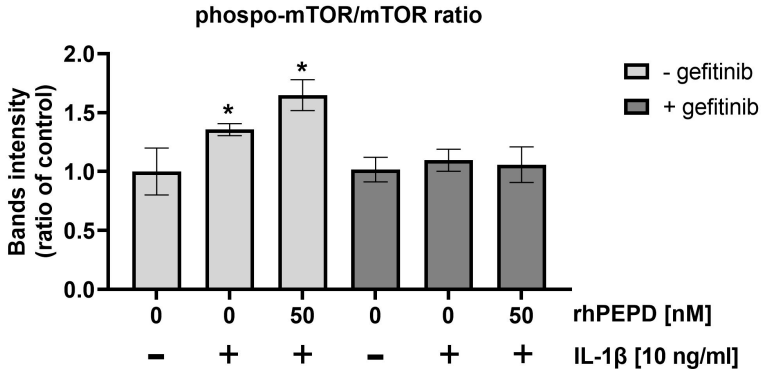
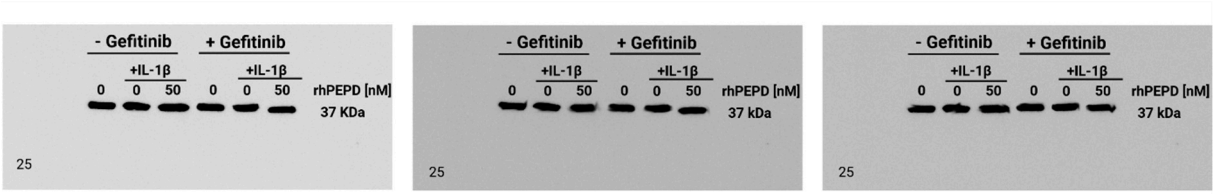




**Figure S17.** The phospho-mTOR expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

**Ratio of phospho-mTOR/mTOR expression**




## 2.9. GAPDH expression



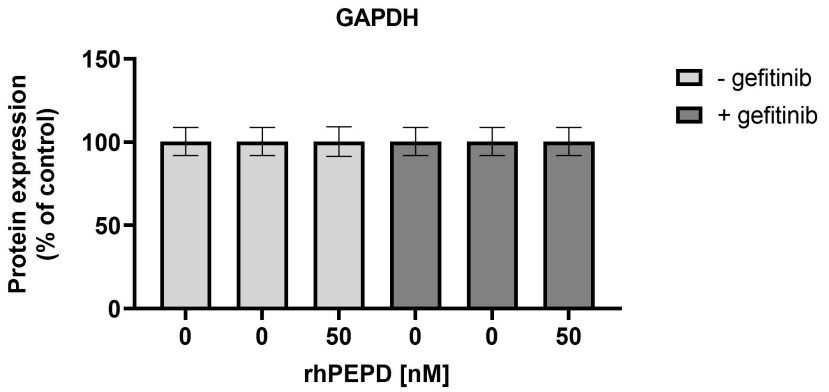
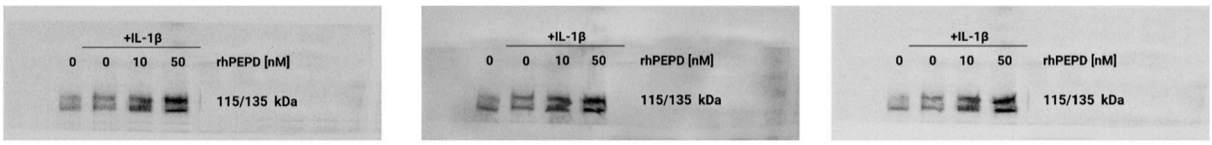




**Figure S18.** The GAPDH expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

## 3. Representative blots from Western blotting analysis presented in Figure 4A.

### 3.1. β1 integrin expression



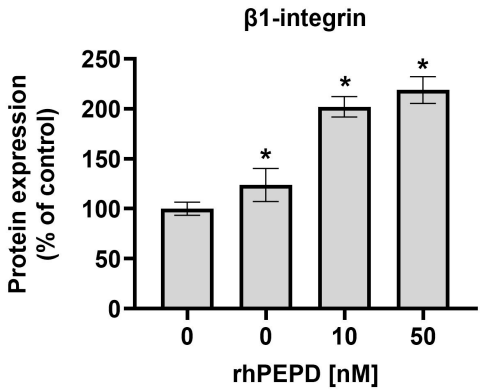
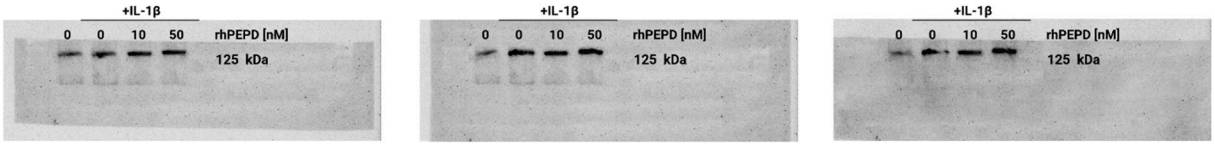




**Figure S19.** The β1 integrin expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $*P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 3.2. FAK expression



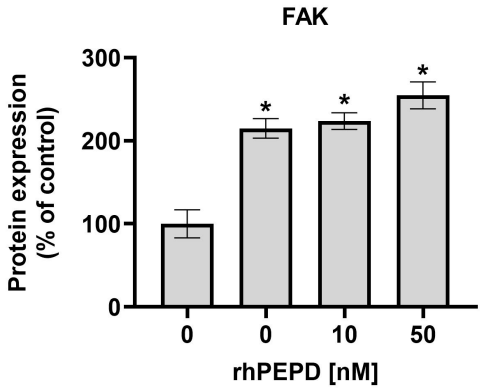



**Figure S20.** The FAK expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 3.3. Phospho-FAK expression

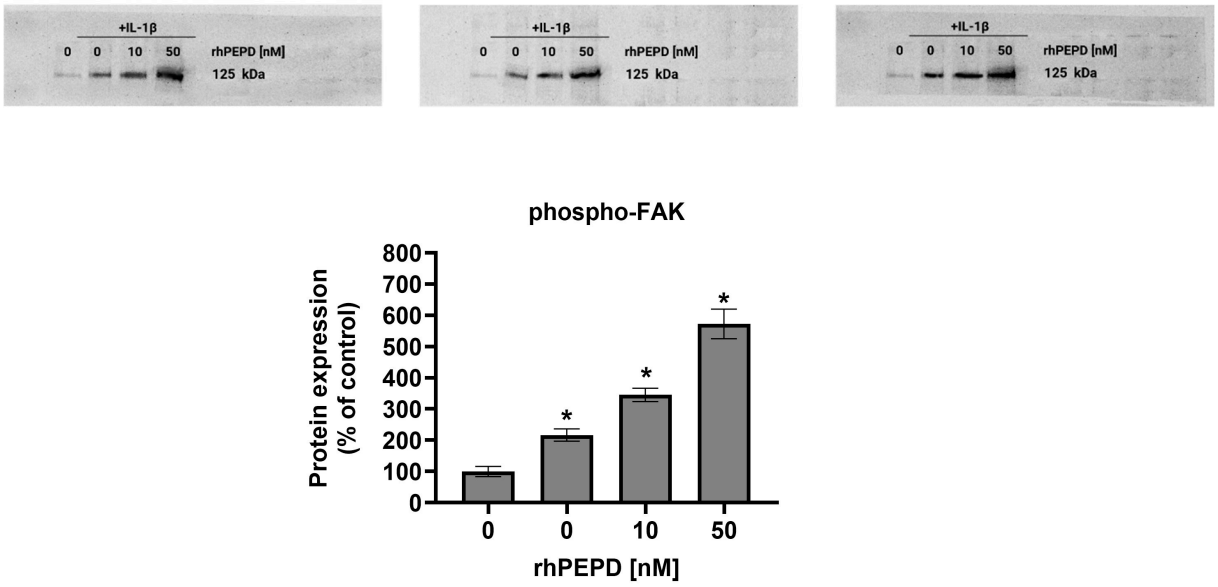
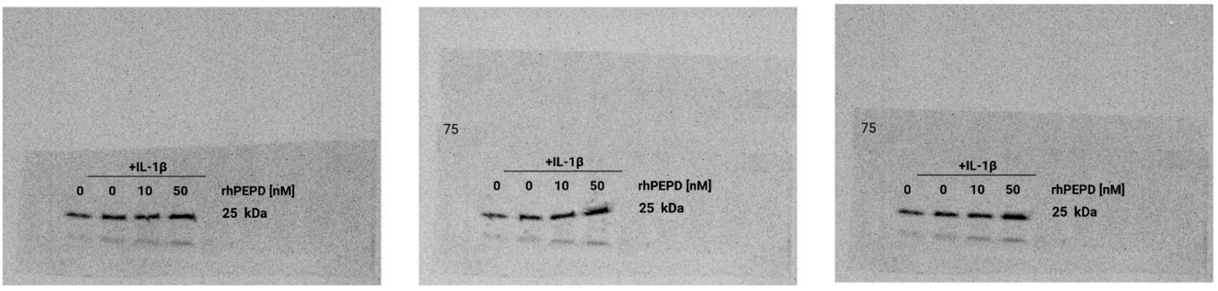



**Figure S21.** The phospho-FAK expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 3.4. Grb2 expression



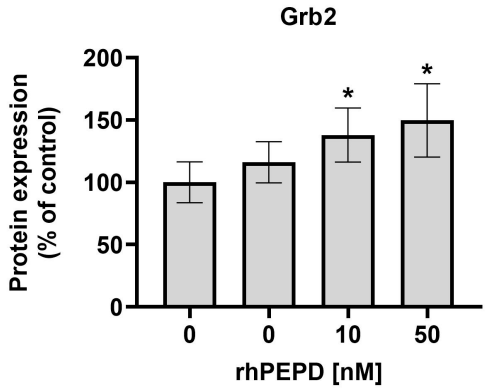
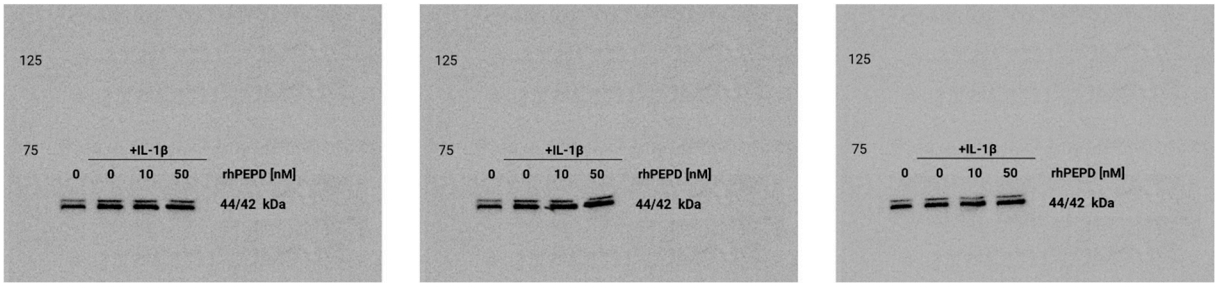



**Figure S22.** The Grb2 expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 3.5. ERK 1/2 expression



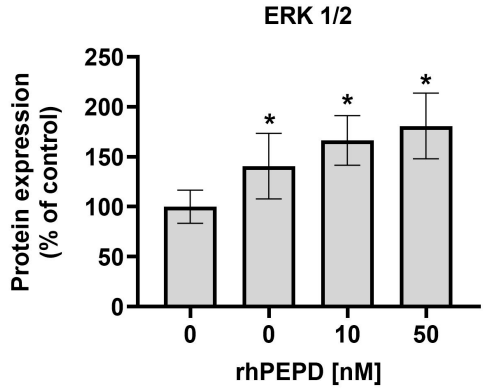
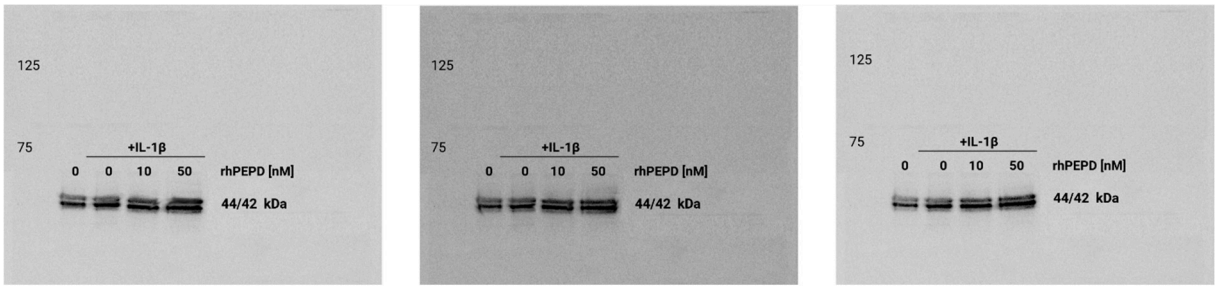




**Figure S23.** The ERK 1/2 expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min and 24 h in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 3.6. Phospho-ERK 1/2 expression



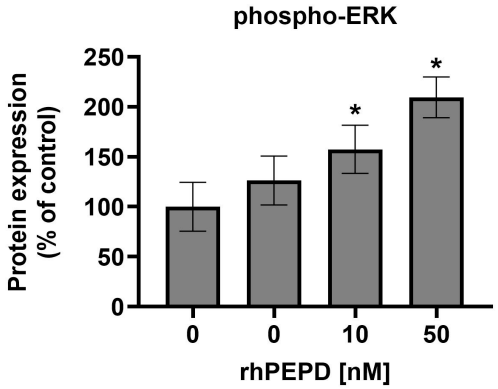
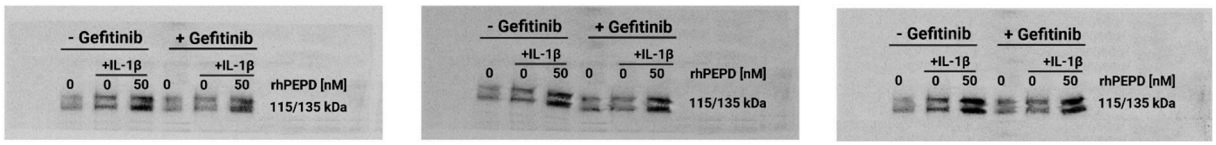




**Figure S24.** The phosphor-ERK 1/2 expressions in rhPEPD-treated fibroblasts (rhPEPD, 10, 50 nM) for 40 min in presence or absence of IL-1β (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $*P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

## 4. Representative blots from Western blotting analysis presented in Figure 4B.

### 4.1. β1 integrin expression



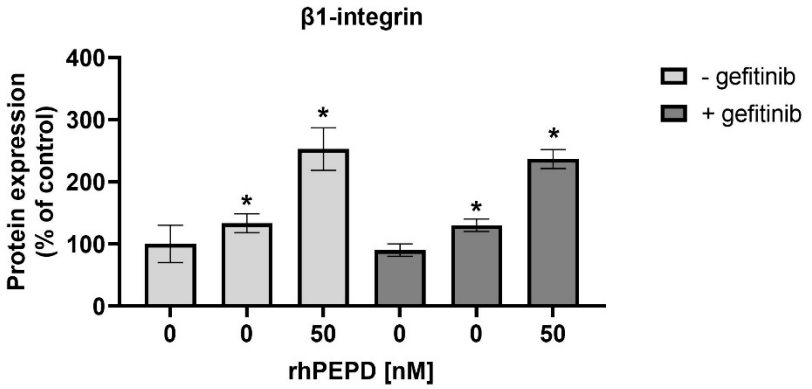



**Figure S25.** B1 integrin expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

### 4.2. Phospho-FAK expression

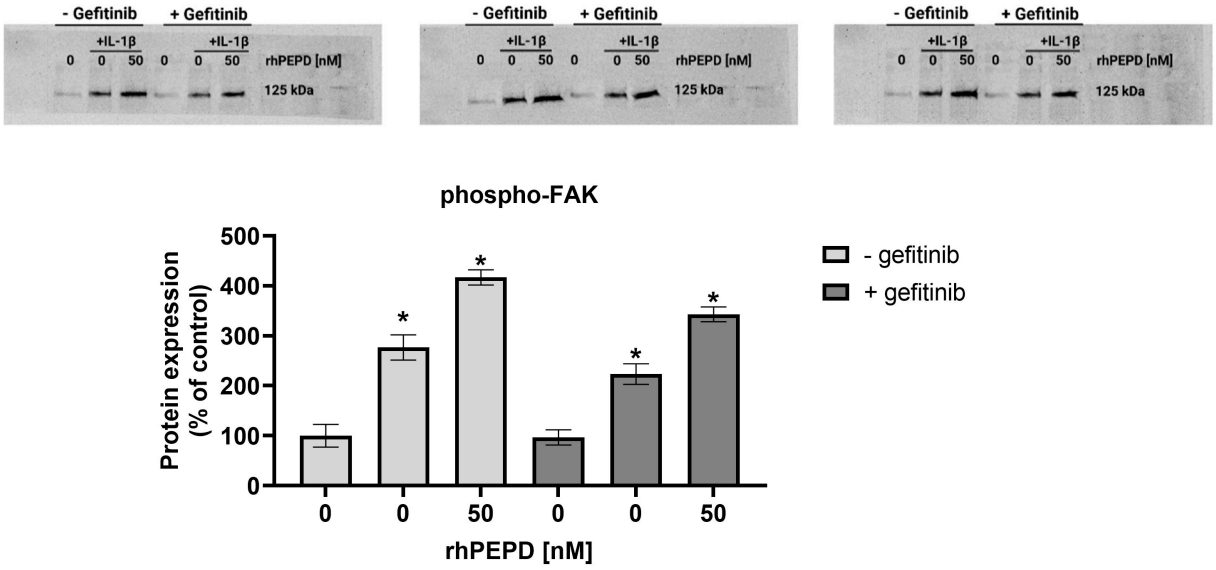



**Figure S26.** Phospho-FAK expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

### 4.3. Grb2 expression

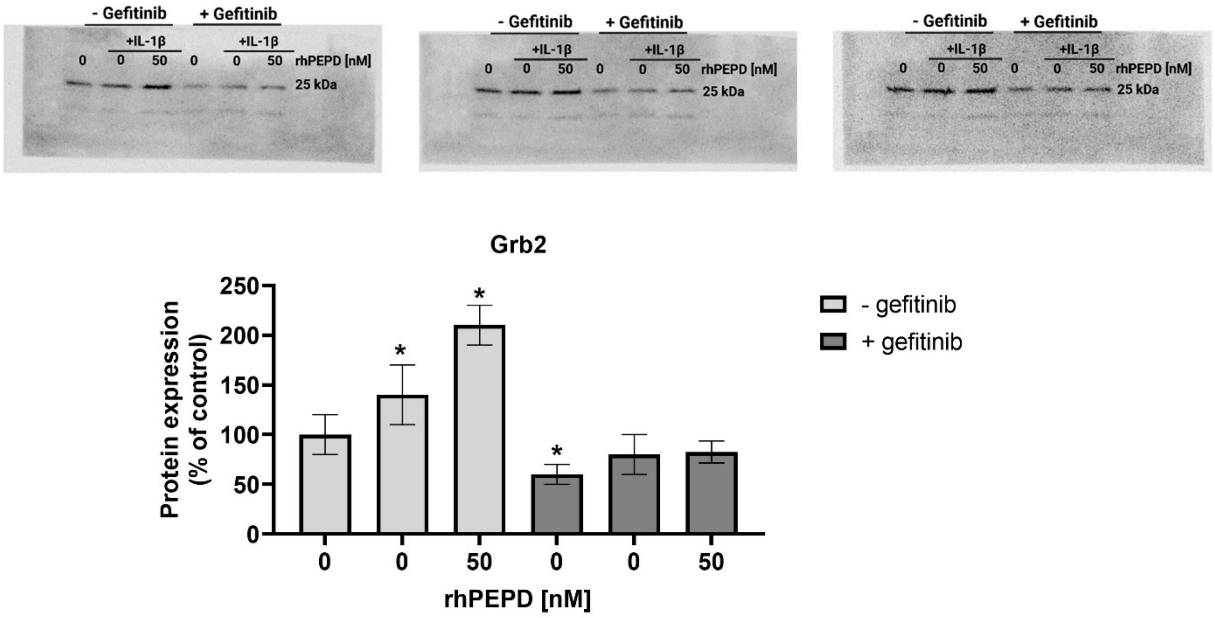



**Figure S27.** Grb2 expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as **P* < 0.05; vs. control cells (0 nM of PEPD, without IL-1β).

### 4.4. ERK 1/2 expression

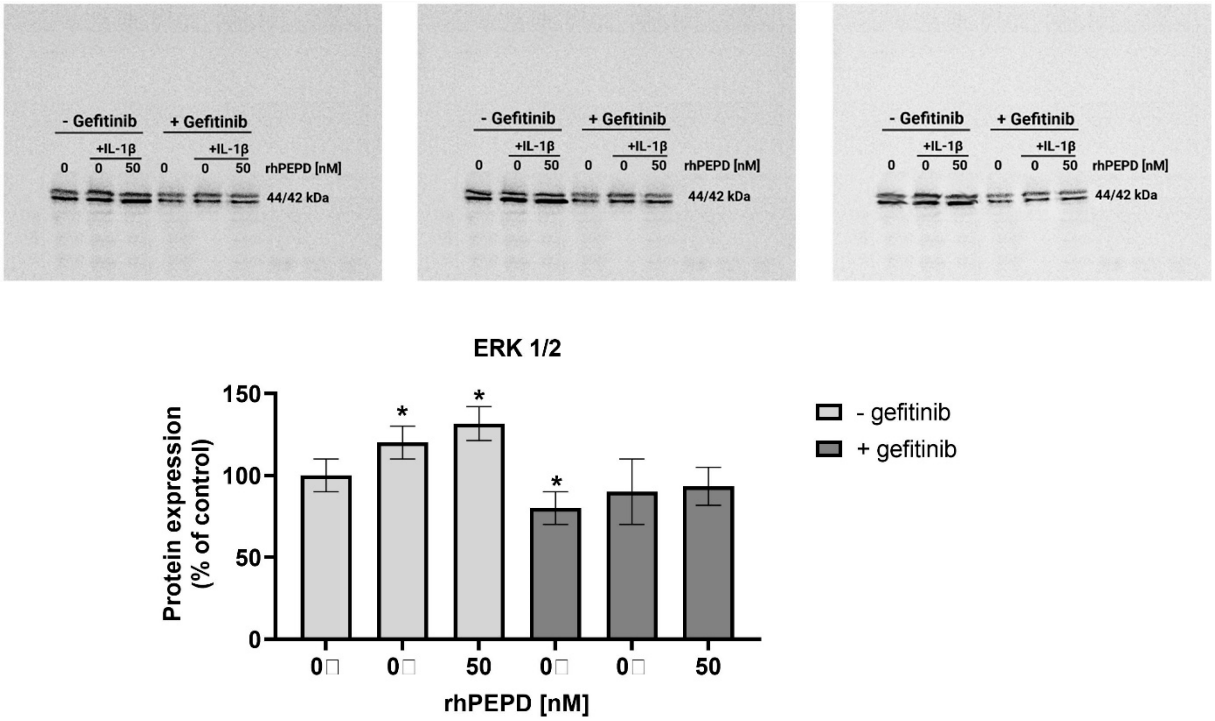


**Figure S28.** Erk1/2 expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 24 h in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

### 4.5. Phospho-ERK 1/2 expression

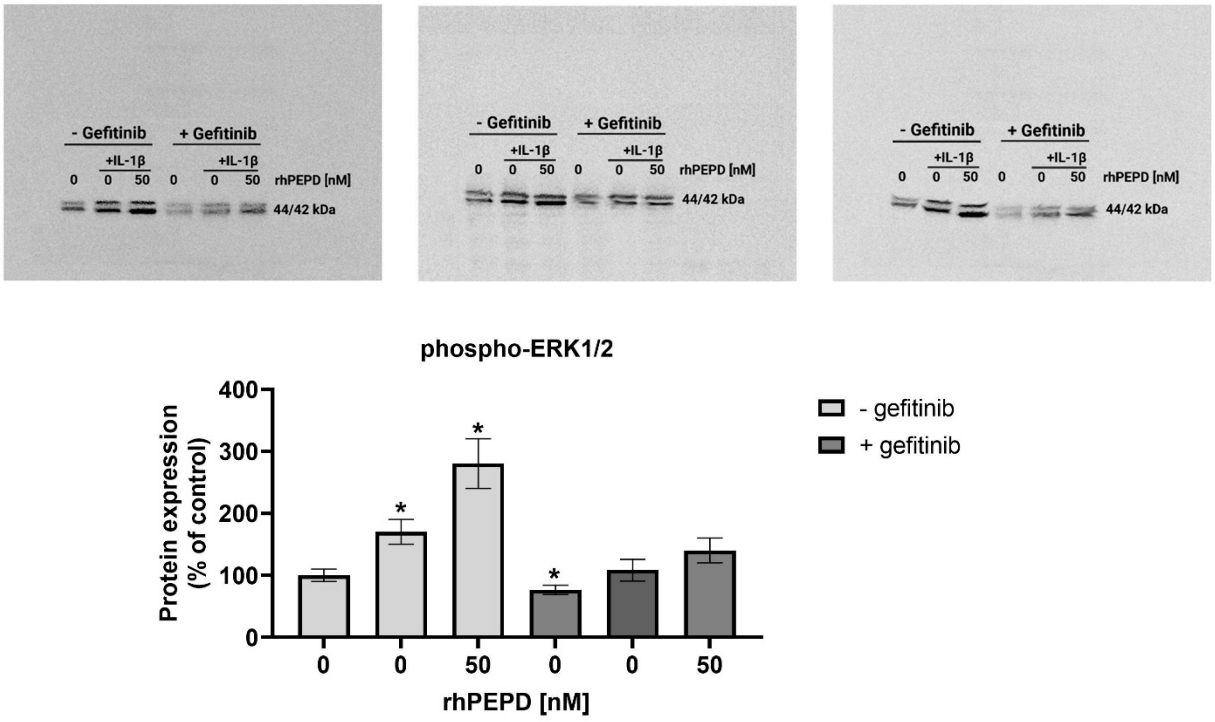



**Figure S29.** Phospho-Erk1/2 expressions in lysates of rhPEPD-treated fibroblasts (rhPEPD, 50 nM) and pretreated with an inhibitor of EGFR (gefitinib, 45 μM for 2 h) cultured for 40 min in presence or absence IL-1β. (10ng/ml). GAPDH expression was used as a loading control. The WB bands intensity of representative gels was quantified by densitometry and normalized to GAPDH. The densitometry values represent the mean (% of control) ± SD of three experiments. Statistical significances were expressed as $^{*}P < 0.05$; vs. control cells (0 nM of PEPD, without IL-1β).

## 5. CellTiter-Blue Cell Viability Assay

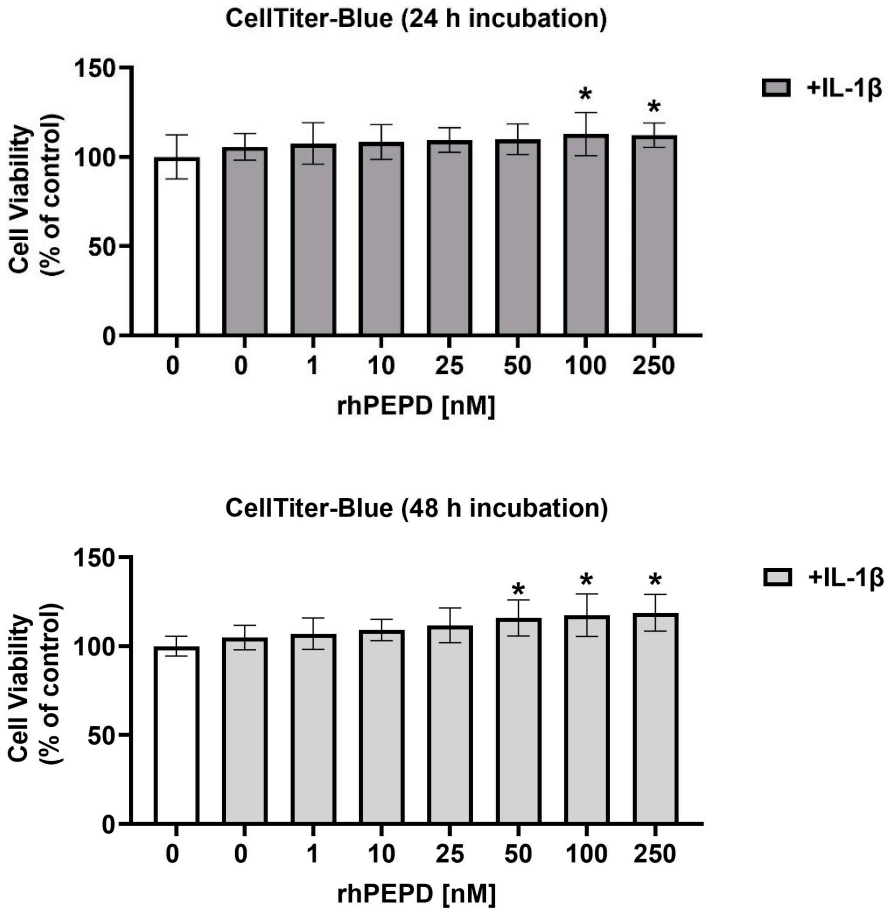



**Figure S30**. Cell viability of fibroblasts was measured using Cell Titer Blue assay as described in the manufacturer's protocol (Promega, Madison, WI, United States). Cells, seeded at $5 \times 10^3$ cells/well in a 96-well plate, were submitted to rhPEPD treatment at concentrations of 1–250 nM and IL-1$\beta$ (10 ng/ml)-for 24 h and 48 h. Briefly, cells were incubated with a resazurin-containing solution at 37°C for 2 h. Absorbance was read on TECAN Infinite® M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) at 570 and 600 nm as a reference wavelength. The results were presented as a percent of the control value. The mean values ± S.D. of three experiments done in duplicates are presented. The statistical significance was calculated vs. control (0 nM of rhPEPD, without IL-1$\beta$ ) and the results were considered significant at $^*P < 0.05$.

## 6. The influence of Gefitibin on the viability of fibroblasts

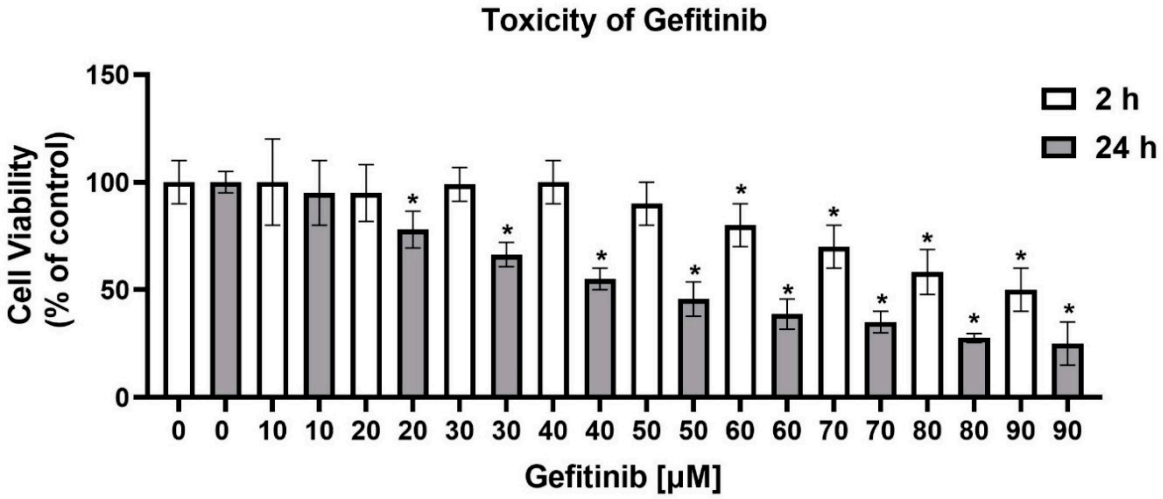



**Figure S31**. The influence of gefitinib on the viability of fibroblasts was measured using MTT test. Cells were treated of Gefitinib at concentrations of 0-90 μM for 2 h and 24 h. The results were presented as a percent of the control value. The mean values ± S.D. of three experiments done in duplicates are presented. The statistical significance was calculated vs. control (0 μM Gefitinib) and the results were considered significant at $^{*}P < 0.05$.