Supplemental Information

Inflammatory-Related P62 Triggers Malignant Transformation of Mesenchymal Stem Cells through the Cascade of CUDR-CTCF-IGFII-RAS Signaling

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Figure S1

A

B

C

Table below:

| Condition          | OD450 Value
|--------------------|-------------|
| GFP control + TNFα| 120         |
| GFP-P62 + TNFα    | 100         |
| RNAi control + TNFα| 80         |
| P62 + TNFα        | 60          |
| GFP control       | 40          |
| GFP-P62           | 20          |
| RNAi control      | 0           |
| P62 RNAi           | 0           |

BrdU positive cells (%):

| Condition          | % Positive Cells
|--------------------|-----------------|
| GFP control + TNFα| 90              |
| GFP-P62 + TNFα    | 80              |
| RNAi control + TNFα| 70             |
| P62 + TNFα        | 60              |
| GFP control       | 50              |
| GFP-P62           | 40              |
| RNAi control      | 30              |
| P62 RNAi           | 20              |
Figure S4

- IGFII: 20 KD
- Ras: 21 KD
- pRas: 21 KD
- β-actin: 42 KD
Figure S5
Figure S6

A

- GFP-Control+TNFα
- GFP-P62+TNFα
- GFP-P62-GFP-V-IGFII+TNFα

B

Colony formation rate(%)

C

Sphere formation rate(%)

D

Xenograft tumor weight(gram)

* *
Figure S7

A

GFP-P62 or GFP-P62(W338A)

P62
H-Ras
β-actin

B

C

colony formation rate (%)  

GFP-control  GFP-P62  GFP-P62(W338A)

Spheroid formation rate (%)  

GFP-control  GFP-P62  GFP-P62(W338A)
FIGURE LEGENDS

**Figure S1**: A. Immunohistochemical staining with anti-CEA (human) (DAB staining, original magnification×100). P, paracancerous liver tissue; C, cancer tissue. B. Cells growth assay using CCK8. Each value was presented as mean±standard error of the mean (SEM) (Student’s t-test). C. S phase cells assay using BrdU. Each value was presented as mean±standard error of the mean (SEM) (Student’s t-test).

**Figure S2**: TNFR knockdown abrogated the functions of P62 in human mesenchymal stem cells malignant transformation in vitro and in vivo. A. The Western blotting analysis with anti-TNFR in these human mesenchymal stem cells indicated in upper. β-actin as internal control. B. Cells soft agar colony formation assay in these human mesenchymal stem cells, including GFP-Control+TNFa, GFP-P62+TNFa, GFP-P62+pGFP-V-RS-TNFR+TNFa. C. Cells sphere formation ability. D. Tumorigenesis test in vivo. The wet weight of each tumor was determined for each mouse. Each value was presented as mean±standard error of the mean (SEM).

**Figure S3**: A portion of each tumor was fixed in 4% paraformaldehyde and embedded in paraffin for histological hematoxylin-eosin(HE) staining. (original magnification×100).

**Figure S4**: Western blotting with anti-IGFII, anti-Ras and anti-pRas in the TNFα treated mesenchymal stem cells, including GFP-Control+TNFa, GFP-P62+TNFa, GFP-P62+pGFP-V-RS-IGFIIR+TNFa. β-actin was used as an internal control.
**Figure S5:** A portion of each tumor was fixed in 4% paraformaldehyde and embedded in paraffin for histological hematoxylin-eosin (HE) staining.

**Figure S6:** IGFII knockdown abrogated the functions of P62 in human mesenchymal stem cells malignant transformation in *vitro* and in *vivo*. **A.** The cell growth assay in these human mesenchymal stem cells, including GFP-Control+TNFα, GFP-P62+TNFα, GFP-P62+pGFP-V-RS-IGFII+TNFα. **B.** Cells soft agar colony formation assay. **C.** Cells sphere formation ability. **D.** Tumorigenesis test *in vivo*. The wet weight of each tumor was determined for each mouse. Each value was presented as mean±standard error of the mean (SEM).

**Figure S7:** mutant P62 (W338A) lacks the functions of wild P62 in human mesenchymal stem cells. **A.** Cells soft agar colony formation assay in these human mesenchymal stem cells, including pCMV6-AC-GFP plus TNFα, pCMV6-AC-GFP-P62 plus TNFα, pCMV6-AC-GFP P62(W338A) plus TNFα. **B.** Cells sphere formation ability in these human mesenchymal stem cells, including pCMV6-AC-GFP plus TNFα, pCMV6-AC-GFP-P62 plus TNFα, pCMV6-AC-GFP-P62(W338A) plus TNFα.