**Supplemental materials**

**Fig.S1. RACK1 enhances specifically IR/IGF1-R-mediated but not EGFR or EGFR-Ros chimeras-mediated STAT3 phosphorylation. A.** 293T cells were transfected with 500ng of EGFR, 150ng of STAT3 and 250ng of phEF-RACK1 or phEF-neo. **B.** NIH3T3/EB69 and **C.** NIH3T3/ES2, two stable clones of EGFR-Ros chimeras expressing cells, were transfected with 1 μg phEF-RACK1 or phEF-neo. After 24 h, cells were serum-starved for 12 hours and then stimulated with EGF (50 ng/ml) for 15 min or left unstimulated. SDS-PAGE and Western-blotting were similar to that in Fig.1. Results represent one typical experiment from two independent experiments.

**Fig.S2. Detergent and glycerol dependent association between RACK1 and IR.** For the top two panels, HEK293T cells were starved overnight, either left untreated or treated with insulin for 15 minutes; for the bottom two panels, HEK293T cells were transfected with 200 ng of IR. Starvation, stimulation with insulin total cell lysate (TCL) preparation and co-ip were performed as in Fig.1 with the indicated lysis buffer and antibodies, followed by SDS-PAGE and immunoblotting. Results represent one typical experiment from two independent experiments.

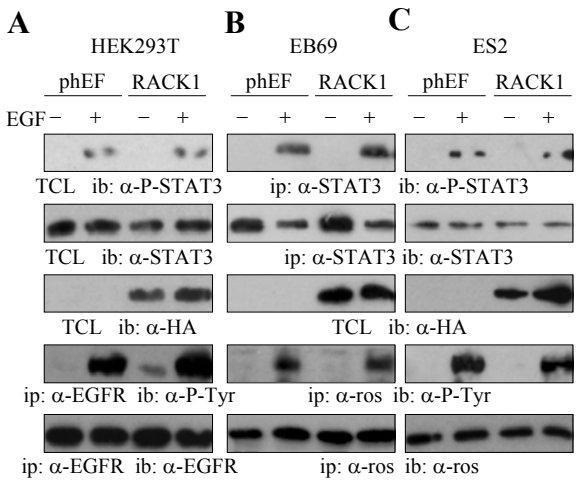
**Fig.S3. Overexpression of RACK1 or WD1-4 in HEK293T cells enhances IR-mediated STAT3 activation.** HEK293T cells were transfected with 150ng IR, 150ng STAT3 and 250ng of phEF-RACK1, phEF-WD1-4 or phEF-Neo. 24 hours post-transfection, cells were serum starved overnight, then either left untreated or treated with insulin or 10% FCS for 15 minutes. Cell lysate preparation, SDS-PAGE and immunoblotting were performed as in Fig.1 and analyzed with the indicated antibodies. Results represent one typical experiment from two independent experiments.

**Fig.S4. Triple complex detection of IR, RACK1 and STAT3.** Conditions are as described in Fig.3F. Proteins in 20 μl of each fraction were resolved by SDS-PAGE, followed by immunoblotting with the indicated antibodies. Results represent one typical experiment from three independent experiments.

**Fig.S5. Overexpression of RACK1 enhances the abundance of phospho-STAT3 in both cytoplasm and nucleus.** HEK293T cells were transfected with IR, STAT3 and RACK1 or phEF-Neo, followed by overnight serum starvation, insulin induction for different time periods and isolation of cytoplasmic and nuclear fractionations. 25 μg of samples were separated by SDS-PAGE and immunoblotted with the indicated antibodies, with β-tubulin and snRNP as cytoplasmic and nuclear markers, respectively.

**Fig.S6. Protein expression level in PA-1, SKOV3 and SW626 ovarian cancer cells and the effect of RACK1 expression on migration and invasion ability of PA-1 cells. A.** PA-1, SW626 and SKOV3 Cell lysates were prepared, followed by Western blotting as indicated. **B.** PA-1 cells were transfected with different plasmids (2μg of RACK1 or phEF-neo, 1μg of dnSTAT3) as indicated. Migration and invasion assays were performed after 48 hours post transfection supplemented with insulin and IGF-1.

Fig.S1


A
B
C
HEK293T
EB69
ES2
phEF
RACK1
phEF
RACK1
phEF
RACK1
EGF − + − + − + − + − + − +
TCL ib: α-P-STAT3
ip: α-STAT3 ib: α-P-STAT3
TCL ib: α-STAT3
ip: α-STAT3 ib: α-STAT3
TCL ib: α-HA
TCL ib: α-HA
ip: α-EGFR ib: α-P-Tyr
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ip: α-ros ib: α-ros

Fig.S2

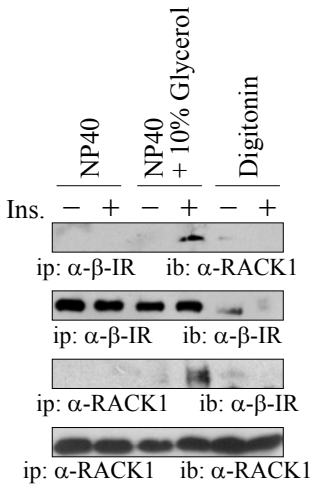

NP40
NP40 + 10% Glycerol
Digitonin
Ins. − + − + − +
ip: α-β-IR ib: α-RACK1
ip: α-β-IR ib: α-β-IR
ip: α-RACK1 ib: α-β-IR
ip: α-RACK1 ib: α-RACK1

Fig.S3

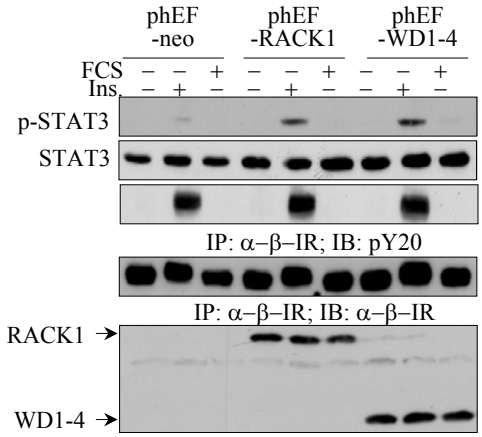

phEF
-neo
phEF
-RACK1
phEF
-WD1-4
FCS
Ins
p-STAT3
STAT3
IP: α–β–IR; IB: pY20
IP: α–β–IR; IB: α–β–IR
RACK1
WD1-4

Fig.S4

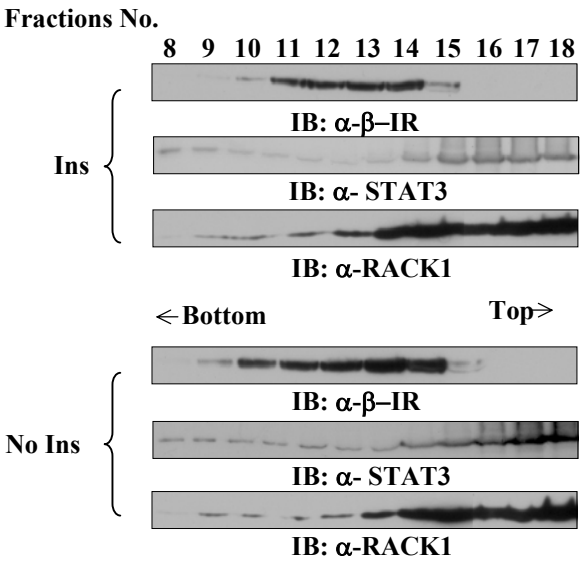
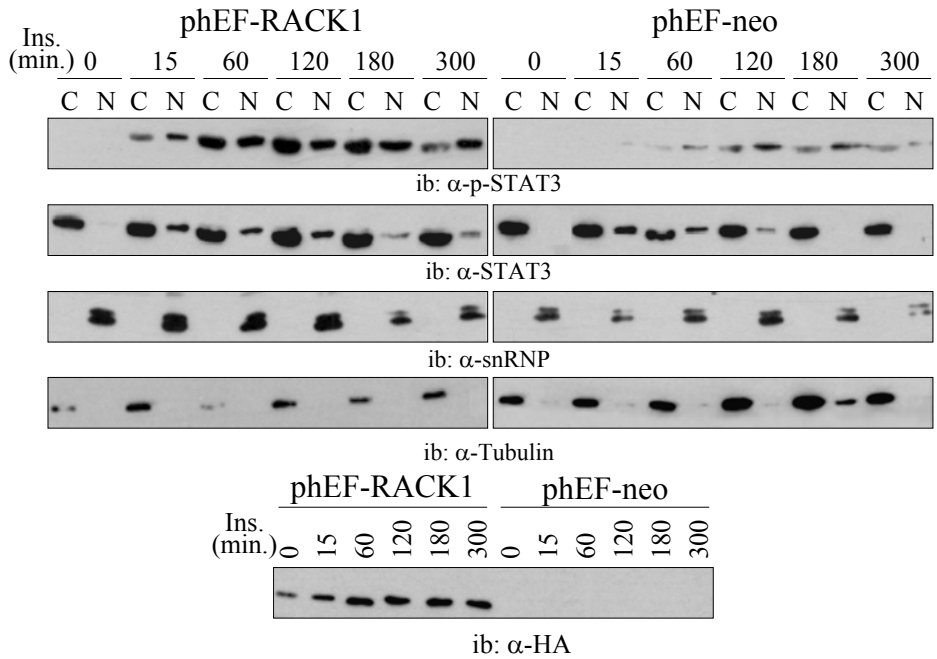

Fractions No.
8 9 10 11 12 13 14 15 16 17 18
Ins
IB: α-β–IR
IB: α- STAT3
IB: α-RACK1
←Bottom
Top→
No Ins
IB: α-β–IR
IB: α- STAT3
IB: α-RACK1

Fig.S5


phEF-RACK1
phEF-neo
Ins. (min.)
0 15 60 120 180 300 0 15 60 120 180 300
C N C N C N C N C N C N C N C N C N C N C N C N
ib: α-p-STAT3
ib: α-STAT3
ib: α-snRNP
ib: α-Tubulin
phEF-RACK1
phEF-neo
Ins. (min.)
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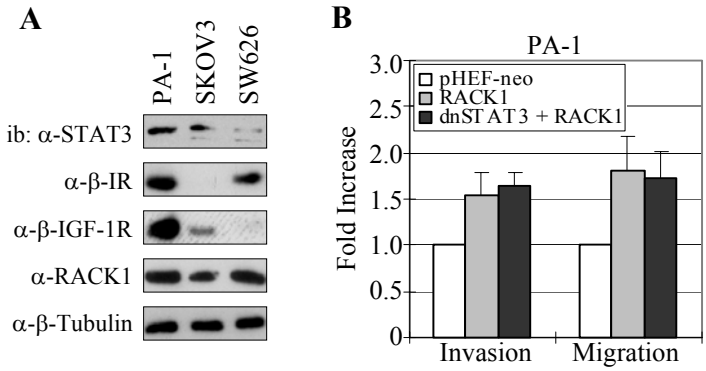
ib: α-HA

Fig.S6


A
PA-1
SKOV3
SW626
ib: α-STAT3
α-β-IR
α-β-IGF-1R
α-RACK1
α-β-Tubulin
B
PA-1
pHEF-neo
RACK1
dnSTAT3 + RACK1
Fold Increase
3.0
2.5
2.0
1.5
1.0
0.5
0
Invasion
Migration