**Supplementary Information**

<u>Supplementary Figure Legends:</u>

**Figure S1.  FAK is dispensable for actin polymerization that drives TCR-induced spreading and that regulates TCR expression.**  Control and FAK-deficient Jurkat cells were stimulated on glass coverslips for 7.5 minutes and stained with FITC-phalloidin.  (A)  A representative phalloidin and DIC image at 100× is shown.  The white scale bar is equal to 5 µm. (B) The average intensity of phalloidin staining (top graph) and average cell area (bottom graph) from fifty cells taken from two independent experiments ± SD was calculated (n.s. $p > 0.05$).  (C)  Surface expression of the TCR in resting control or FAK-deficient Jurkat cells was analyzed by flow cytometry.  Data is representative of three independent experiments.  (D)  Jurkat cells were treated with Luc or Fak miRNAs and stimulated for various times with soluble anti-TCR (1µg/ml).  The surface expression of the TCR was then measured by flow cytometry.  Data is shown as percent surface expression as calculated in Supplemental Methods and is representative of three experiments.

**Figure S2.  FAK pY397 antibodies are not suitable for imaging in Jurkat cells.**  (A) Jurkat cells were treated with control and FAK-specific miRNAs and stimulated on glass coverslips for 7.5 minutes.  The cells were then stained using an anti-FAK pY397 primary antibody and anti-rabbit Alexafluor 488-conjugated secondary antibody.   A representative image from three independent experiments is shown.  (B) Western blot analysis of FAK tyrosine 397 phosphorylation (left panel) and total FAK expression (right panel) from Jurkat cell lysates stimulated for various times with soluble anti-CD3.  The arrow indicates the ~125 kilodalton band that corresponds to FAK.

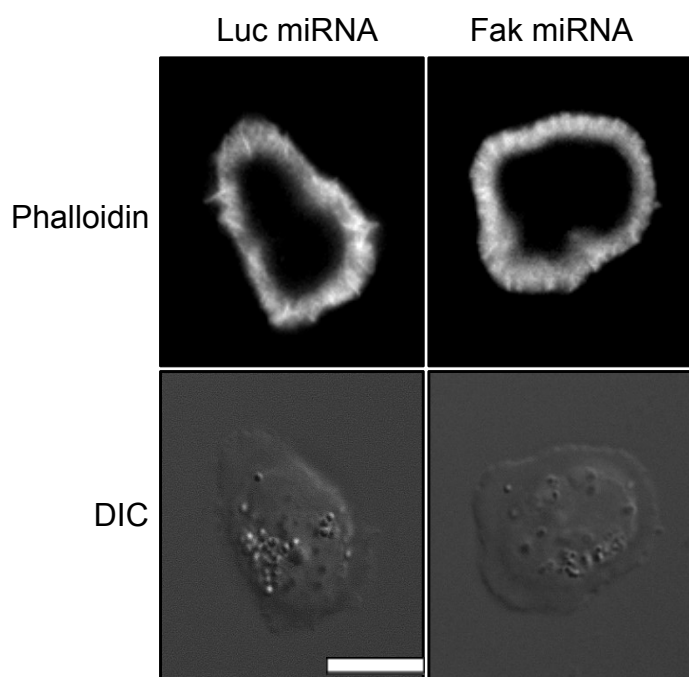**Figure S3.  Working model for FAK-mediated suppression of TCR function.**

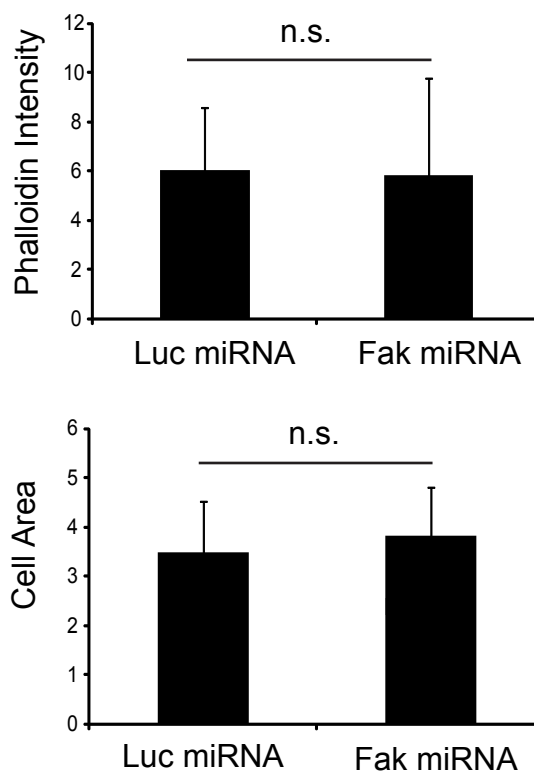**Table SI. Sequences of sense and antisense strands used to generate microRNA constructs.**

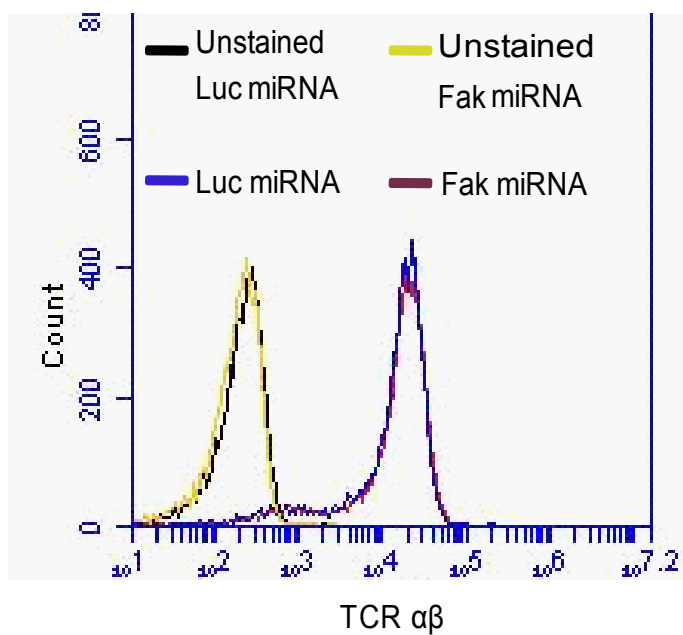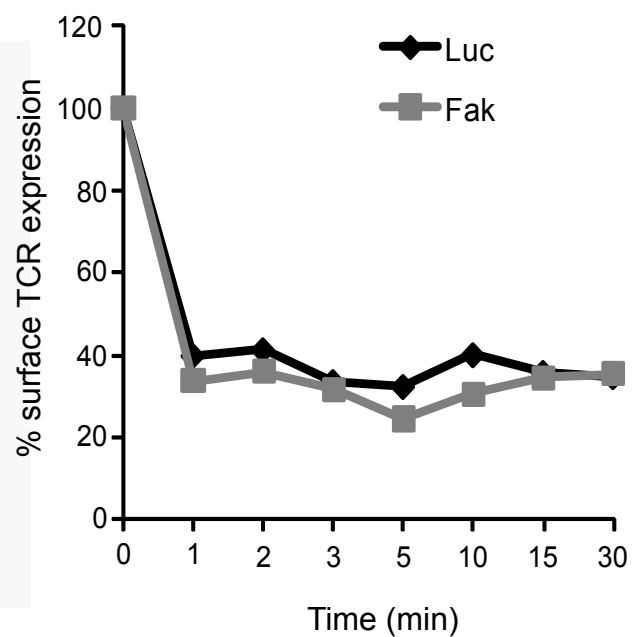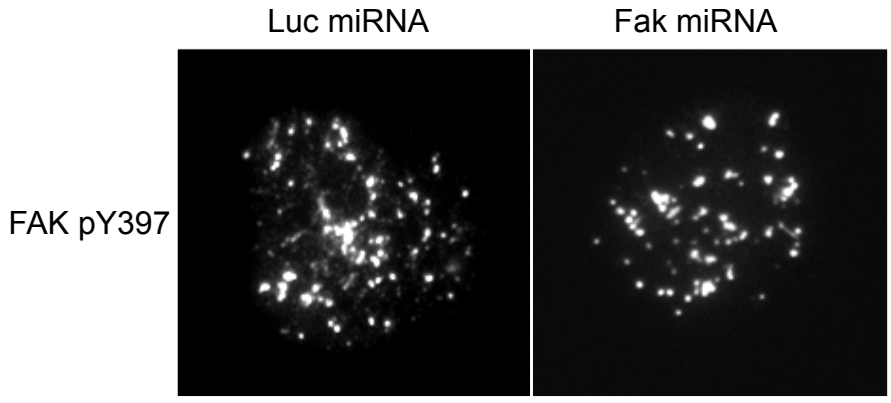| MicroRNA (miRNA) | Sense Strand | Antisense strand |
|---|---|---|
| Luc miR | GCCAGCGTCTAGAGACTTAGTA | TACTAGGTCTCTAGACGCTGGA |
| Fak miR-433 | CCAGCGTCTAGAGACTTAGTA | TACTAGGTCTCTAGACGCTGGA |
| Fak miR-580 | CCCAGAGGAGTGGAAATATGAA | TTCATATTTCCACTCCTCTGGT |
| Fak miR-1271 | CACTGCCGGCTGGTGAATGGAA | TTCCATTCACCAGCCGGCACTA |
| Fak miR-2224 | AAGGCGGCCCAGGTTTACTGAA | TTCAGTAAACCTGGGCCGCCTG |
| Fak miR-2436 | CCATGGTACAAACCAATCATTA | TAATGATTGGTTTGTACCATGT |
| Fak miR-2468 | AGCTACCCTGGTTCACATGGAA | TTCCATGTGAACCAGGGTAGCC |
| Fak miR-3183 | CGATGGCACAGAAGCTATTGAA | TTCAATAGCTTCTGTGCCATCT |

Figure S1

**A**

Luc miRNA   Fak miRNA

Phalloidin

DIC

**B**



**C**



- Unstained Luc miRNA
- Unstained Fak miRNA
- Luc miRNA
- Fak miRNA

TCR αβ

**D**

Figure S2

**A**

Luc miRNA    Fak miRNA

FAK pY397



**B**

anti-FAK pY397          anti-FAK

130

100

70

55

35

25

← FAK

Time (min):   0   2   5   7.5   10        0   2   5   7.5

Figure S3