SUPPLEMENTARY DATA

The Insulin Receptor Negatively Regulates the Action of *Pseudomonas* Toxin-Based Immunotoxins and Native *Pseudomonas* Toxin

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Supplemental Figure Legends

Fig. S1 siRNA siIR-2, which does not knock down IR expression, did not affect SS1P toxicity. Control siRNA (GL2), siIR-1 and siIR-2 were transfected into KB31 cells, and viability of cells was measured at 72 hours by ATP assay after SS1P treatment (A); and 48 hours later total RNA was extracted and IR RNA level was analyzed by real time PCR (B).

Fig. S2 Treatment by IT reduced IR protein levels. KB31 cells were plated in 6 well plates overnight, cells were then incubated with HB21-PE40 or SS1P at indicated concentrations for 48 hours. Total cell lysates were analyzed with anti-IR and anti-actin antibody by western blot.

Fig. S3. LRP1B is expressed in KB31 cells. KB cells were transfected with siIR-1 for 48 hours. The levels of LRP1B and actin were analyzed with anti-LRP1B or anti-actin antibody by western blot and were unchanged.

Fig. S4. SS1P internalization was not enhanced in KB31 cells after IR knock down. KB cells were transfected with siIR-1(siIR) or control siRNA (siCo) for 48 hours. SS1P uptake at indicated time points was analyzed by FACS as described in Material and Methods.

Fig. S5. Inhibitors of the IR signal pathway did not affect SS1P induced toxicity. **A**. KB31 cells were transfected with siIR-1 (siIR) or siCo (GL2) for 48 hours. **B** and **C**. KB cells (5000 per well) were seeded in 96-well plates overnight, cells were then treated with 250 nM mTOR inhibitor rapamycin (Rapa), 10 μM PI3K inhibitor LY294002 (LY) or 10 μM of MAPK inhibitor PD98059 (PD) for 60 minutes before SS1P addition (**B** and **C**). **D**. KB cells (5000 per well) were incubated with 100 nM insulin or 1μM AGL 2263 for 24 hours before SS1P addition. ATP levels were measured 72 hours after SS1P (A-D). The inhibitors were present through out the assay. Cell viability was calculated as percentage of luminescence at the indicated SS1P concentration to that of the 'no SS1P' samples. In separate experiments, different concentrations of the inhibitors from 0.125-25 μM were added for 60 minutes to 16 hours before SS1P addition. In all the cases, cell viability was not affected with the combination of inhibitors and SS1P (data not shown).

Fig. S6. IR-A and IR-B isoform expression in cell lines. RNA from KB31 or A1847 cells was isolated and analyzed using IR primer pairs, IR2290 and IR-r2427, which span exon 11 by RT-PCR. The predicated IR-A isoform is 101 bp, and IR-B is 137 bp. The primer sequence of IR2290 is 5'- TTAGGAAGACGTTTGAGGAT-3'; and IRr2427 is 5'-GGCCACCGTCACATTCCCAA. The actin primers are listed in Table I.

SUPPLEMENTARY REFERENCES

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