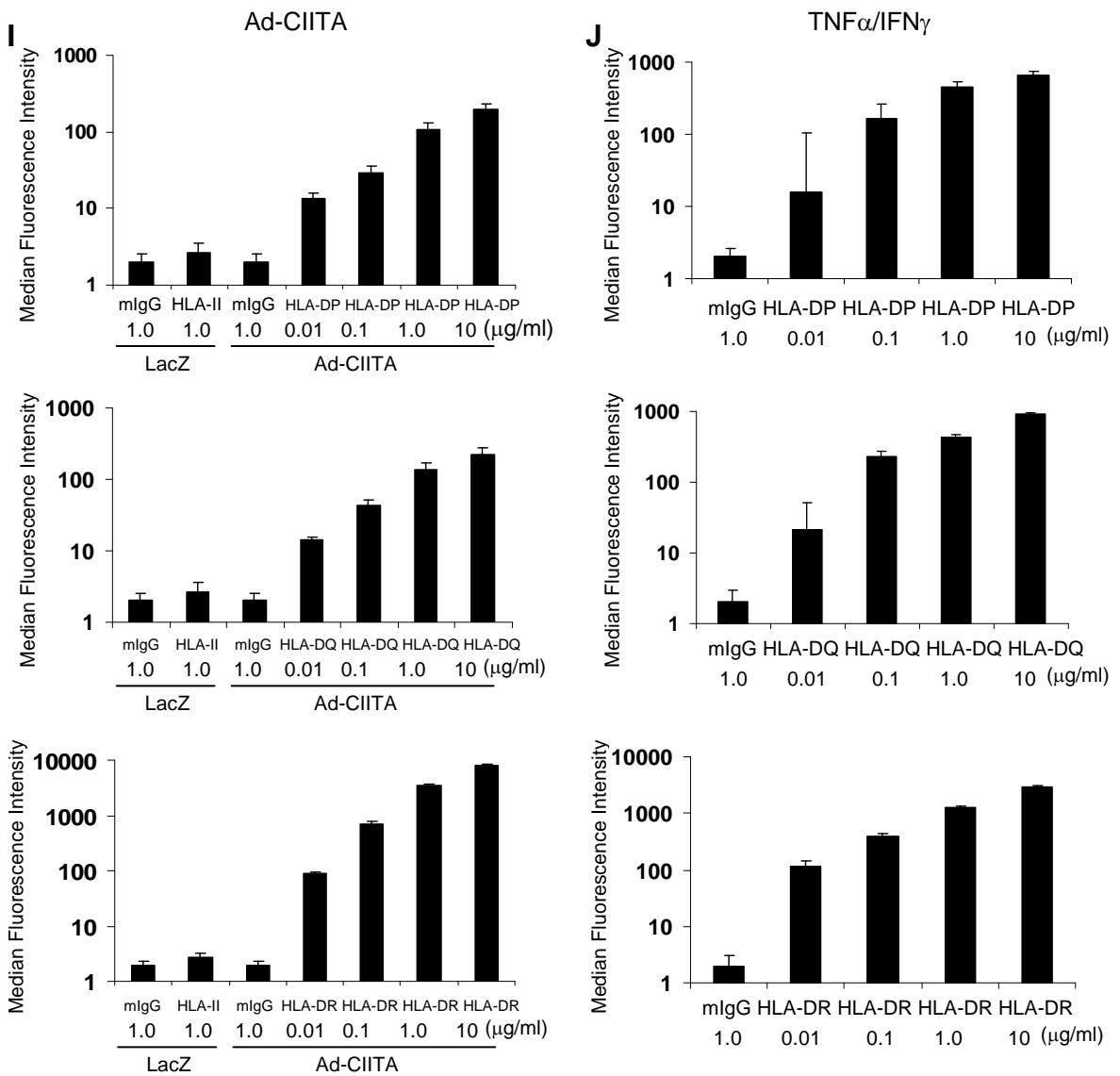


Figure S1



**Fig. S1. Induction of HLA class II molecules on human aortic endothelial cells induced by Ad-CIITA infection or TNF $\alpha$ /IFN $\gamma$  treatment.** *A*. Generation of adenovirus CIITA vector for HLA II molecule upregulation in primary human aortic endothelial cells (HAEC). HLA class II transactivator (CIITA) was sub-cloned from plasmid pcDNA3 myc-CIITA into pENTR 4 vector. The DNA fragment encoding CIITA from pENTR 4 vector was transferred into the adenovirus-based vector pAd/PL-DEST. Recombinant adenovirus encoding CIITA (Ad-CIITA) were amplified in HEK293A cells. HAEC were infected with Ad-CIITA or *B* pretreated with TNF $\alpha$ /IFN $\gamma$  for 48 h. *C, D*. Cells were lysed and proteins in the pre-cleared lysates were separated by SDS-PAGE followed by immunoblotting with anti-CIITA antibody. The membranes were re-probed with anti- $\beta$ -Tubulin antibody to confirm equal loading of total proteins. *E, F*. HAEC were detached and incubated with different concentrations of anti-HLA class II monoclonal antibody, or *I, J*. HLA-DR, DQ, or DP mAb for 30 min at 4°C with continuous mixing, after 3-time washes, incubated with FITC-conjugated goat anti-mouse IgG F(ab')<sub>2</sub> fragment for 30 min at 4°C. The fluorescence intensity was measured by LSRFortessa flow cytometry using FACSDiva program (Becton Dickinson, Mountain View, CA). Gates for forward and side scatter measurements were set on HAEC and a minimum of 10,000 events were acquired. Flow cytometric histogram overlay shows the amount of cellular antigen expression in median fluorescence of intensity (MFI). *G, H, I, J* The bar graphs show MFI values as the mean  $\pm$  SEM and calculated individually. Data represent at least three independent experiments.

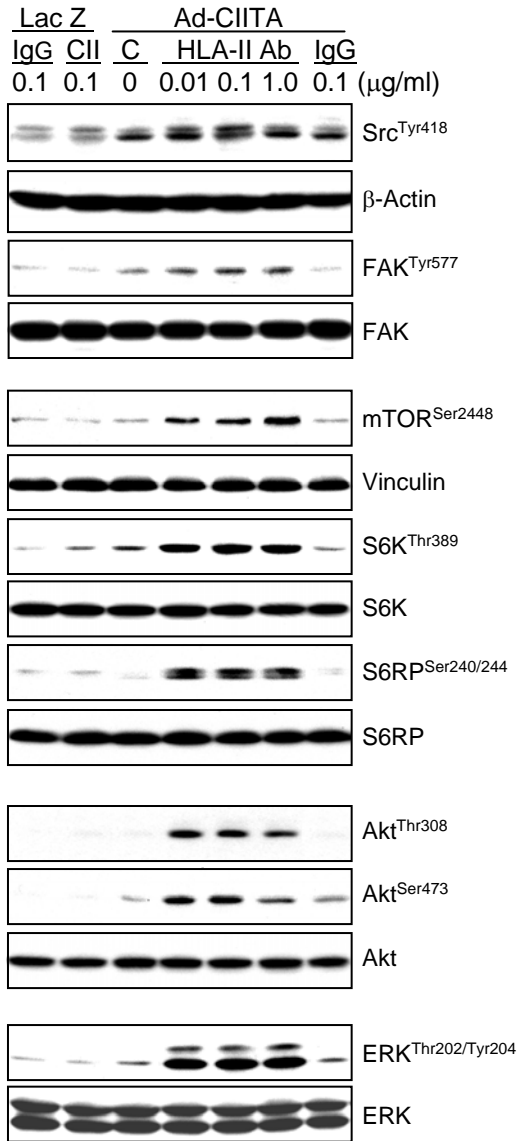
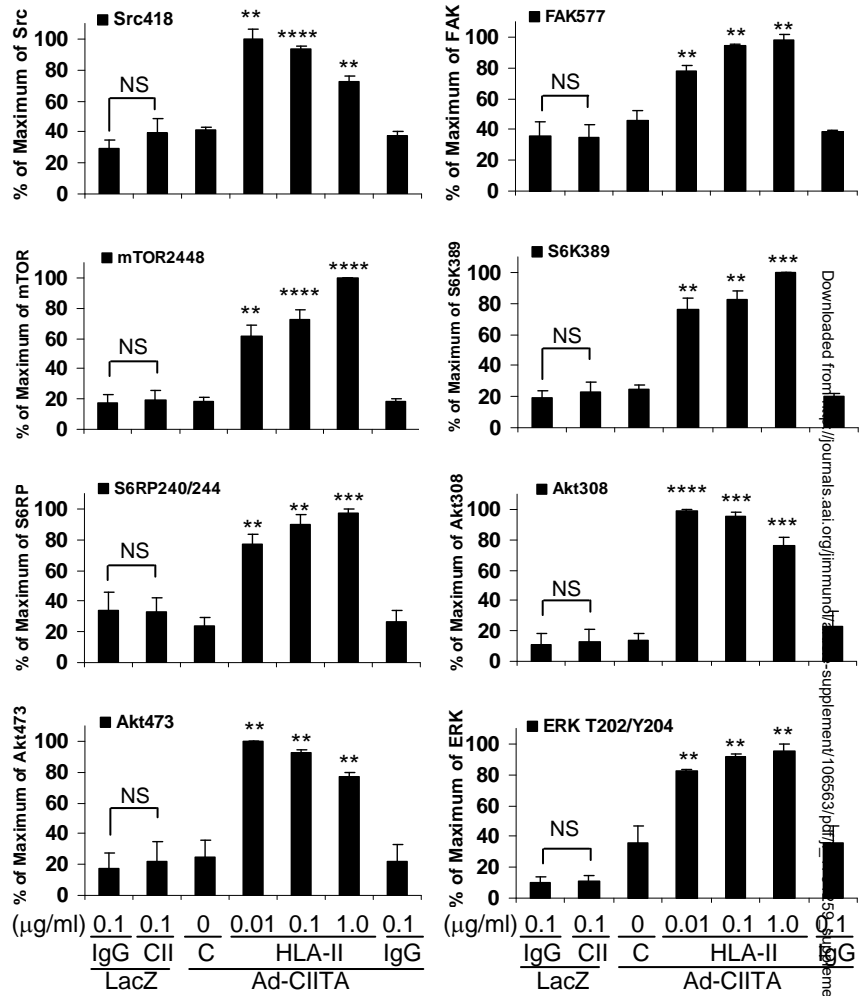
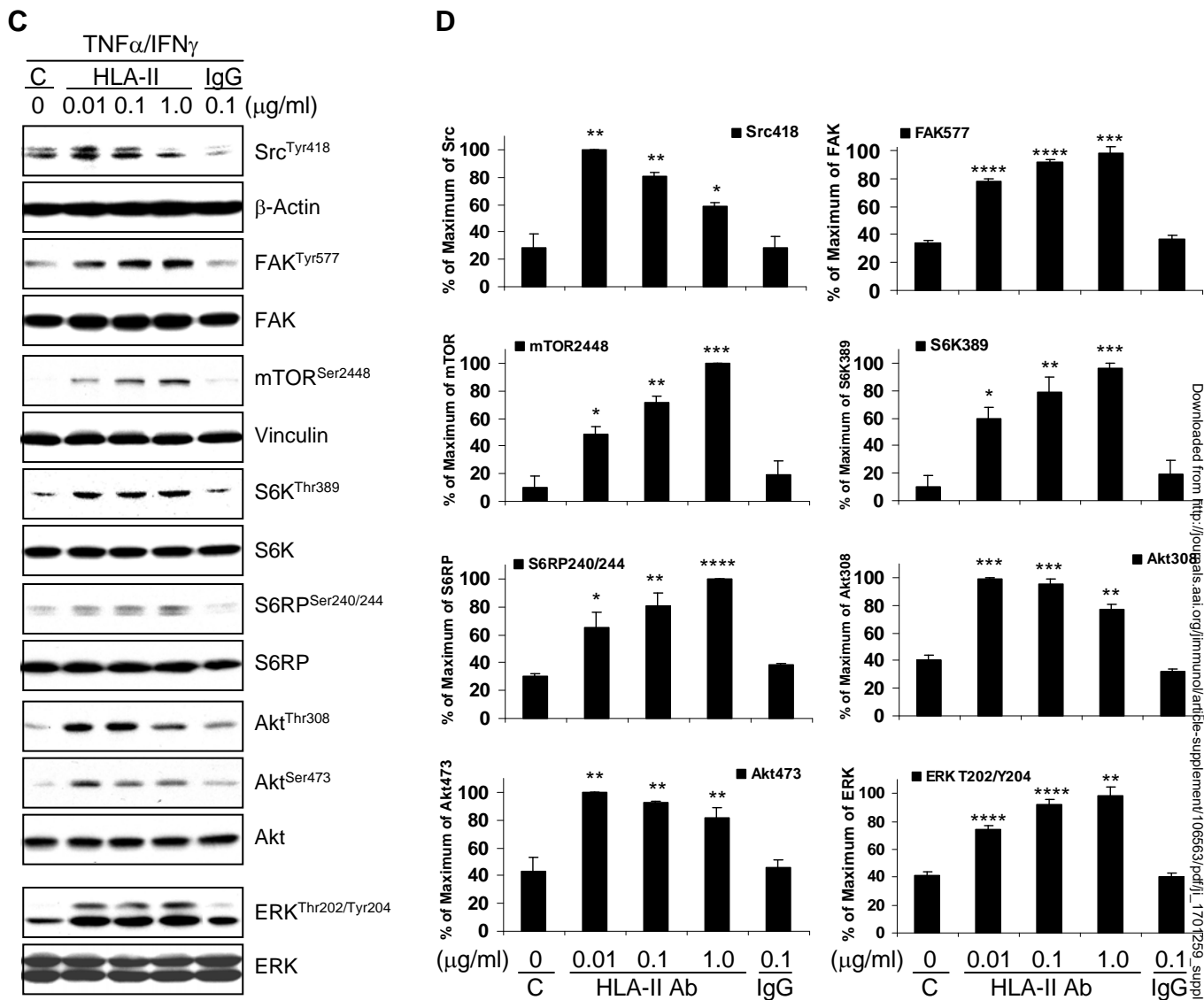
**A**

**B**


Figure S2



**Fig. S2. HLA class II antibody stimulates protein phosphorylation in HAEC in a dose-dependent manner.** **A.** HAEC were infected with Ad-LacZ or Ad-CIITA or **C.** pretreated with TNF $\alpha$ /IFN $\gamma$  in 60 min dishes coated with 0.1% gelatin for 48 h. Starved cells were stimulated with different concentration of HLA class II antibody or control mIgG for 15 min. Proteins in the precleared cell lysates were separated by 6~15% SDS-PAGE followed by immunoblotting with anti-phospho-Src Tyr418, FAK Tyr577, mTOR Ser2448, S6K Thr389, S6RP Ser240/244, Akt Thr308 and Ser473, or ERK1/2 Thr202/Tyr204 antibodies. The membrane was re-probed with anti- $\beta$ -actin, FAK, vinculin, S6K, S6RP, Akt or ERK total antibodies to confirm equal loading of proteins. **B, D.** Phosphorylated protein bands shown in **A** and **C** were quantified by densitometry scan analysis and results are expressed as the mean  $\pm$  SEM percentage of maximal increase in phosphorylation above control values. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, and \*\*\*\* $p$ <0.001 were analyzed by one way ANOVA with Fisher's LSD. Data represent at least three independent experiments.

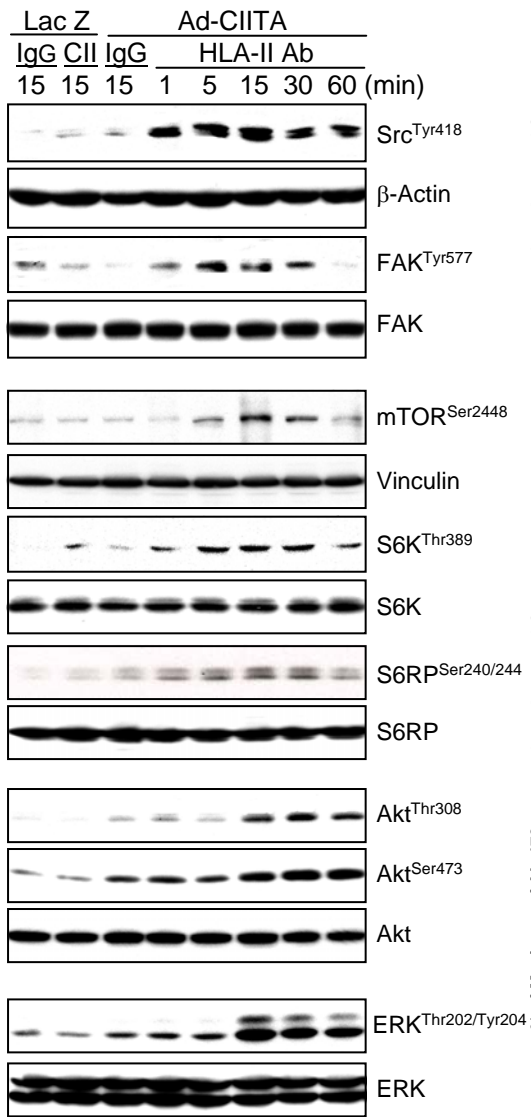
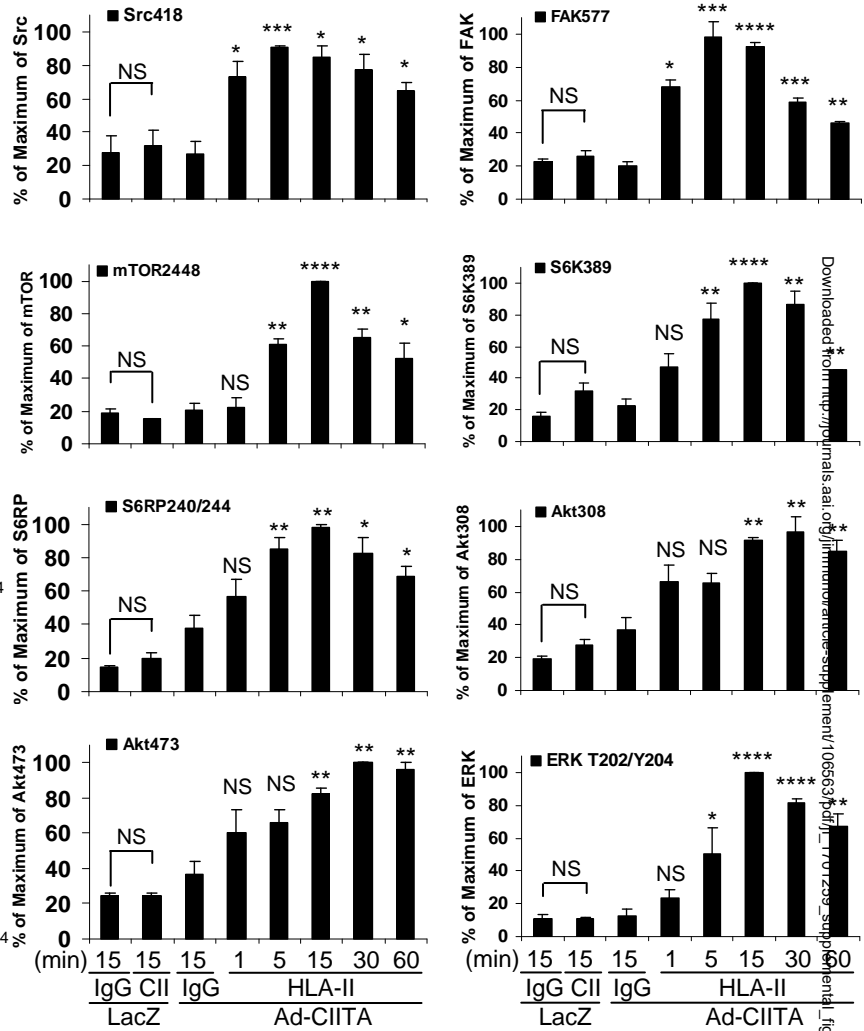
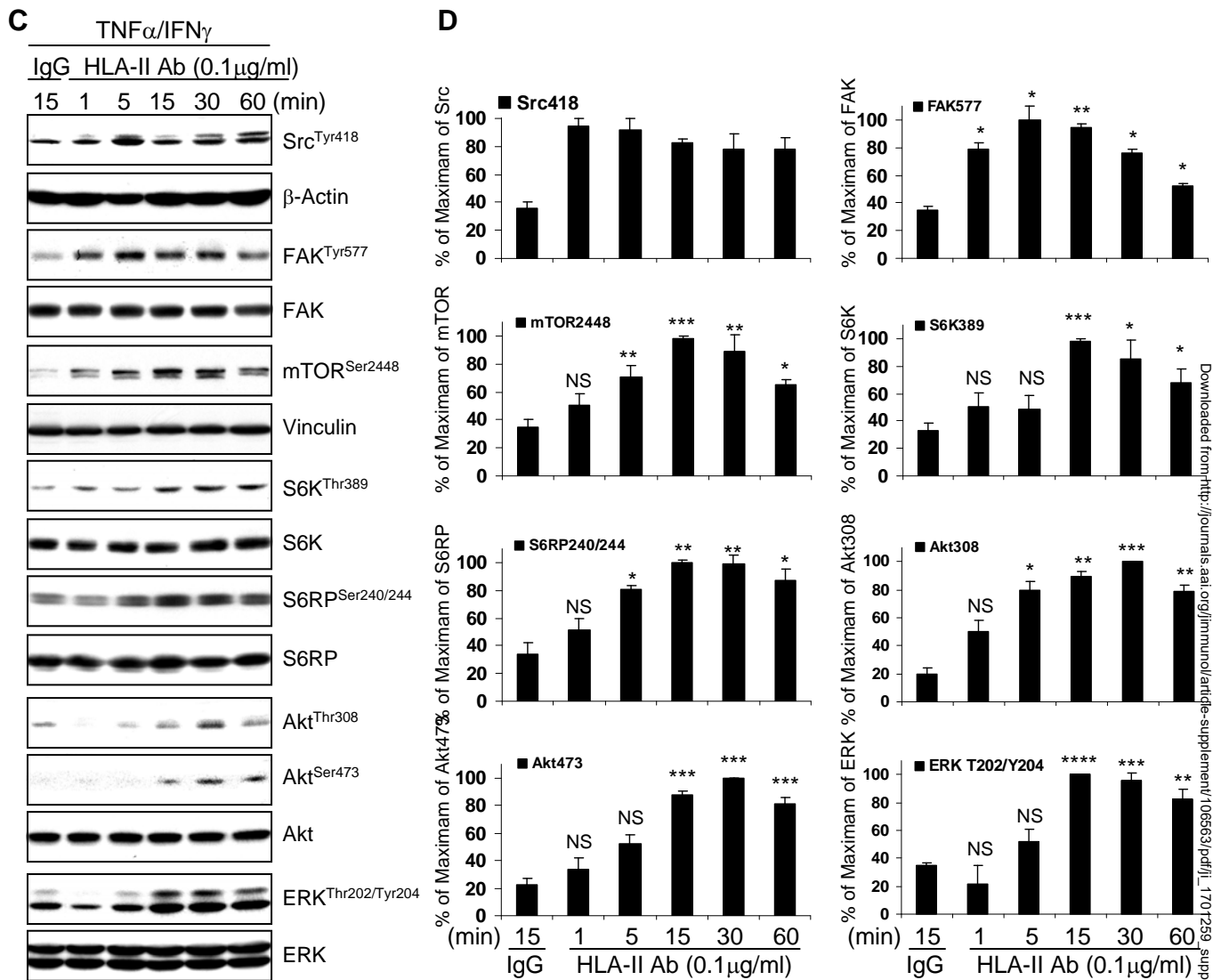
**A****B**

Figure S3



**Fig. S3. Time kinetics study in HLA class II antibody-stimulated activation of signal transduction molecules in HAEC.** **A.** HAEC were infected with Ad-LacZ or Ad-CIITA or **C.** pretreated with TNF $\alpha$ /IFN $\gamma$  in 60 mm dishes coated with 0.1% gelatin for 48 h. Starved cells were stimulated with 0.1  $\mu$ g/ml HLA class II antibody for various time points or mIgG for 15 min as negative control. Proteins in the pre-cleared cell lysates were separated by 6~15% SDS-PAGE followed by immunoblotting with anti-phospho-Src Tyr418, FAK Tyr577, mTOR Ser2448, S6K Thr389, S6RP Ser240/244, Akt Thr308, and Ser473, or ERK1/2 Thr202/Tyr204 antibodies. The membrane was re-probed with anti- $\beta$ -actin, FAK, vinculin, S6K, S6RP, Akt, or ERK total antibody to confirm equal loading of total proteins. **B, D.** Phosphorylated protein bands shown in **A** and **C** were quantified by densitometry scan analysis and results are expressed as the mean  $\pm$  SEM percentage of maximal increase in phosphorylation above control values. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, and \*\*\*\* $p$ <0.001 were analyzed by one way ANOVA with Fisher's LSD. Data represent at least three independent experiments.

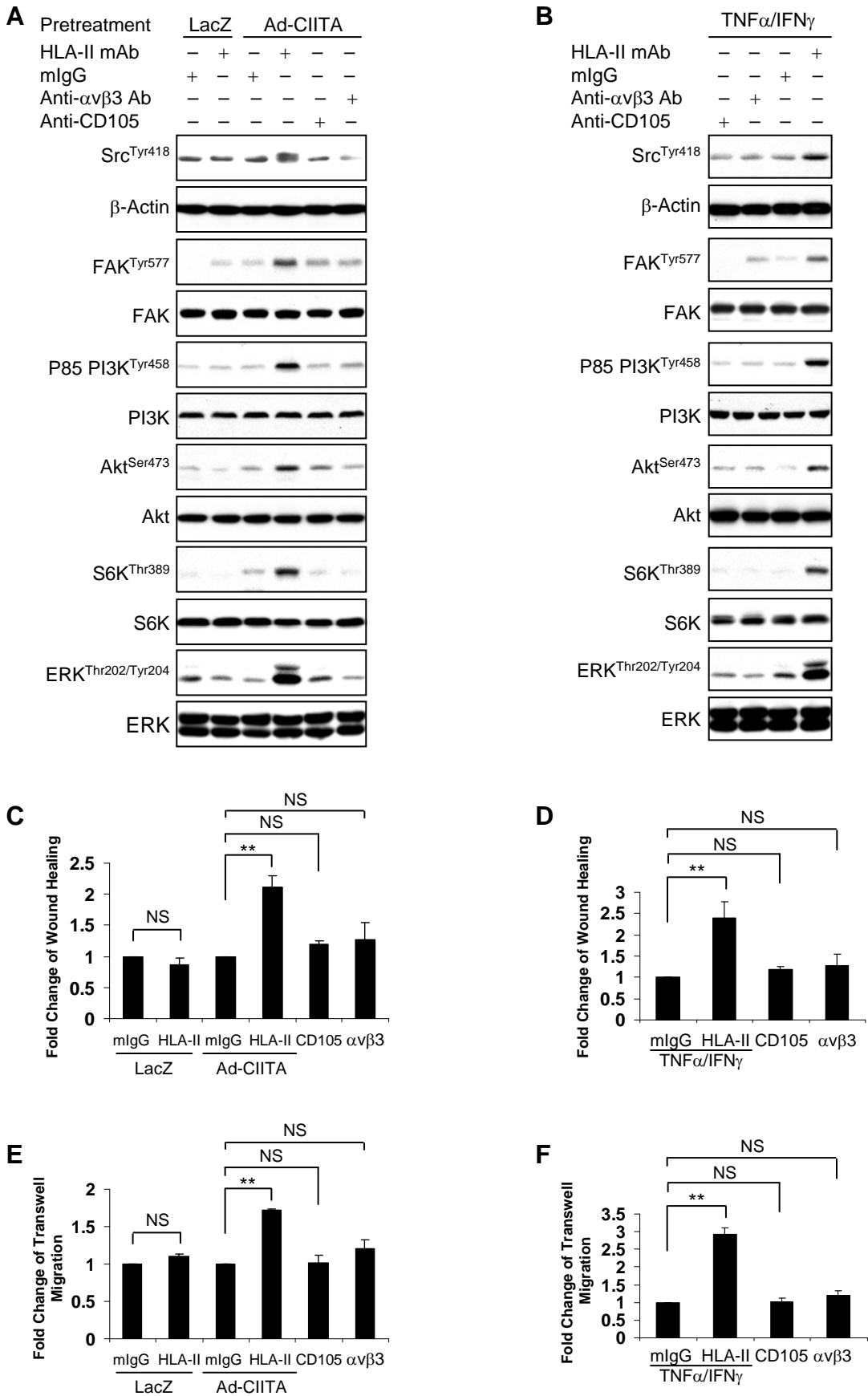


Figure S4

**Fig. S4. Effects of anti-CD105 and integrin  $\alpha\beta3$  antidotes as endothelial cell binding non-HLA antibody on signal transduction and cell migration.** EC were *A, C, E* infected with Ad-LacZ or Ad-CIITA or *B, D, F* pretreated with  $\text{TNF}\alpha/\text{IFN}\gamma$  for 48 h. *A, B* EC were stimulated with 0.1  $\mu\text{g}/\text{ml}$  of HLA class II antibody or 0.1  $\mu\text{g}/\text{ml}$  of anti-CD105, or 0.1  $\mu\text{g}/\text{ml}$  of anti-integrin  $\alpha\beta3$  antibody, or mIgG controls for 15 min. Proteins in the pre-cleared cell lysates were separated by 6~15% SDS-PAGE followed by immunoblotting with anti-phospho-Src Tyr418, FAK Tyr577, p85 PI3K Tyr458, Akt Ser 473, S6K Thr389, or ERK Thr202/Tyr204. The membranes were re-probed with anti-FAK, PI3K, Akt, S6K, ERK, or  $\beta$ -actin antibodies to confirm equal loading of proteins. *C, D* Cells were pretreated with 10  $\mu\text{g}/\text{ml}$  of mitomycin C for 2 h to inhibit cell proliferation before being assayed for their ability to migrate. A scratch wound was created with a sterile 200- $\mu\text{l}$  pipette tip. Wounded cells were stimulated with 1.0  $\mu\text{g}/\text{ml}$  of anti-HLA class II antibody, or mIgG, or anti-CD105 antibody or anti-integrin  $\alpha\beta3$  antibody for 16 h. The cell number between two initiated front edges was counted; migration rate was analyzed by calculating the cell number between two initiated front edges of class II-stimulated EC divided by the cell number between two initiated front edges of negative control EC. *E, F* Cell migration was measured in a transwell insert system. EC *E* infected with Ad-CIITA or *F* pretreated with  $\text{TNF}\alpha/\text{IFN}\gamma$  were seeded to the upper chamber of insert and stimulated with 1.0  $\mu\text{g}/\text{ml}$  HLA class II antibody or anti-CD105 antibody or anti-integrin  $\alpha\beta3$  antibody or mIgG for 16 h at 37°C. After incubation, the cells on the upper surface of the insert membrane were removed with a cotton swab, the migrated cells on the bottom of the insert membrane were fixed with methanol and stained with crystal violet, three middle fields per insert were photographed with 10 x objective lens, and migrated cells were counted. *C, D, E, F* The bar graph shows the mean  $\pm$  SEM number of migrated cells.  $**p < 0.01$  was analyzed by one way ANOVA with Fisher's LSD. Data represent at least three independent experiments.