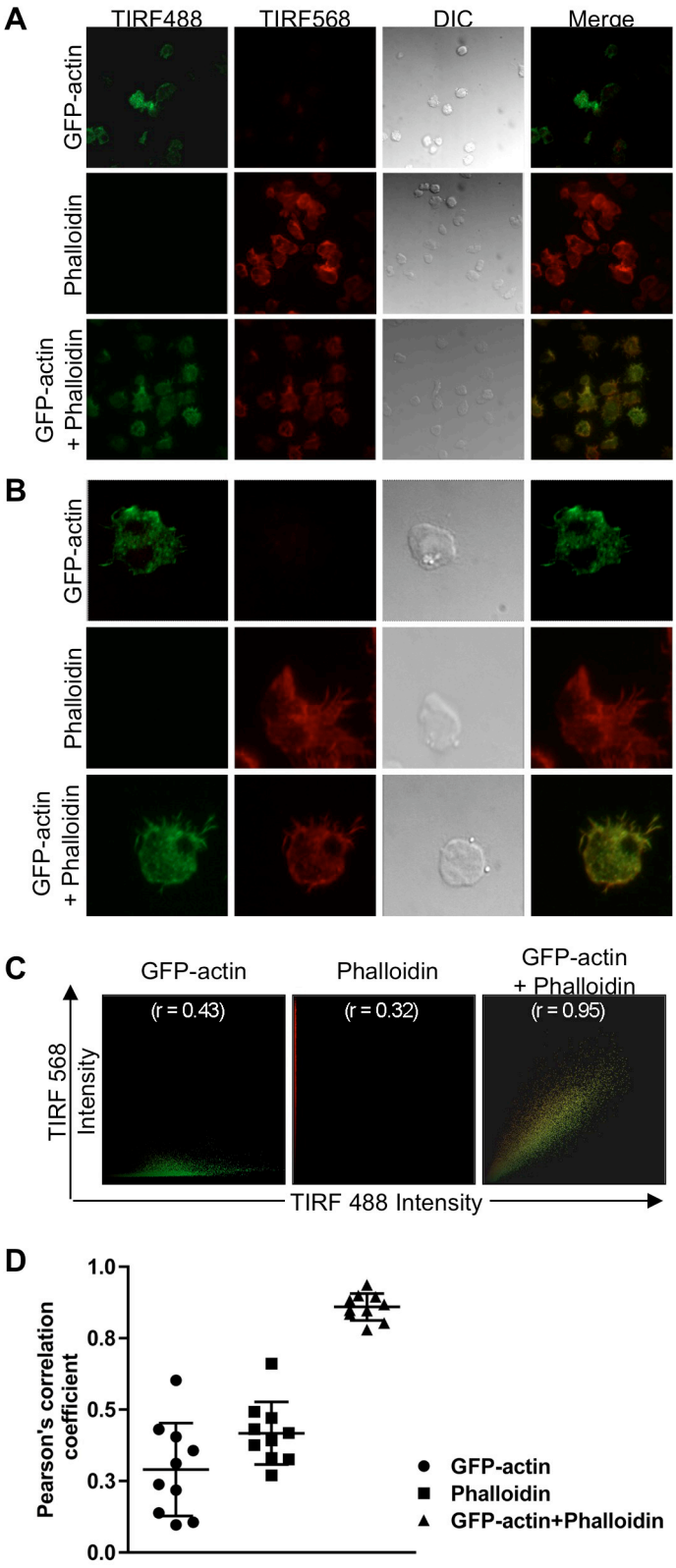
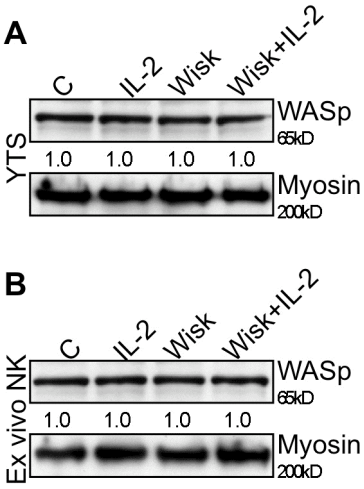
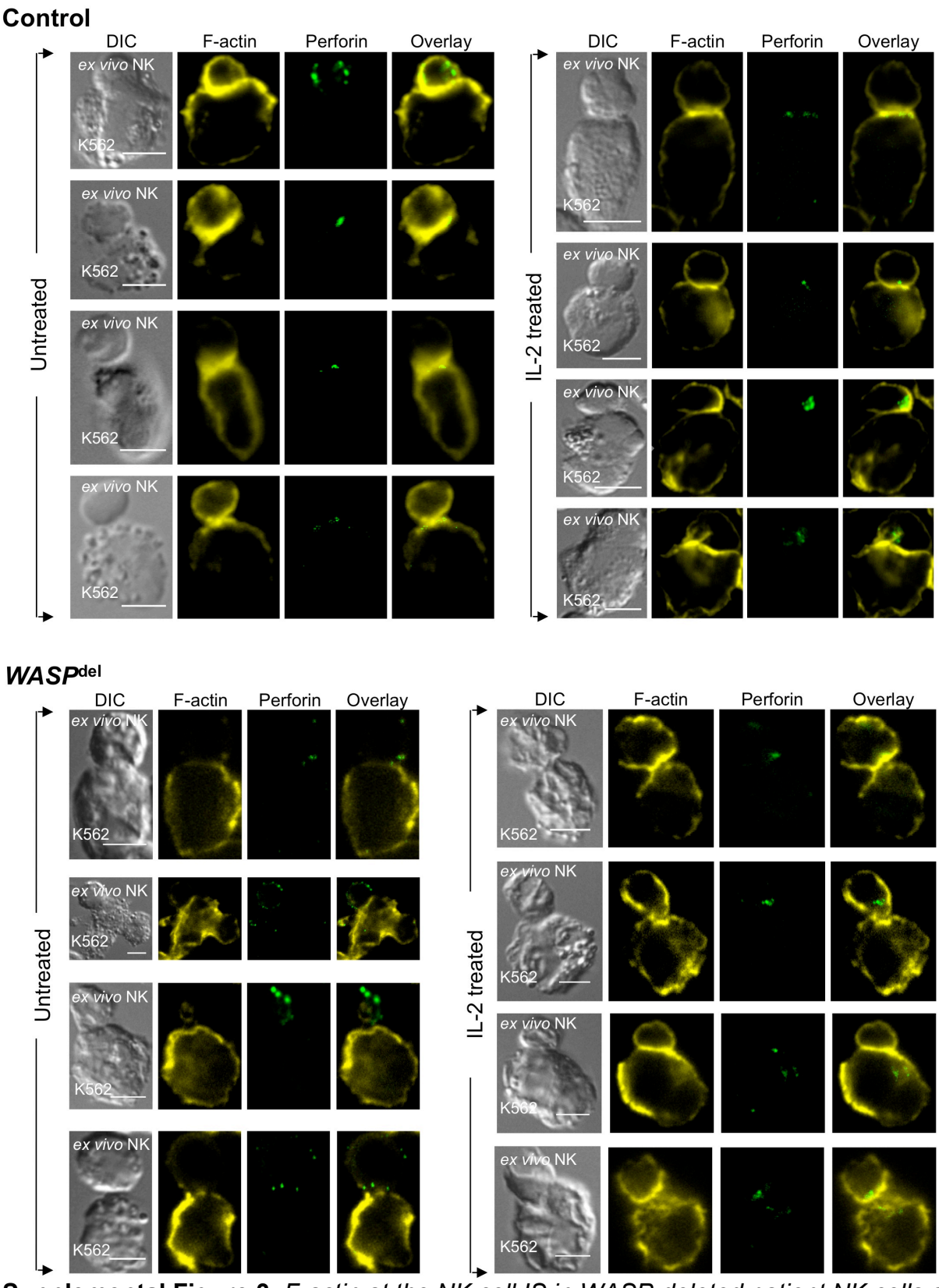
Supplemental Figures and Legends



Supplemental Figure 1. *Representation of F-actin by GFP-actin.* Parental or GFP-actin transduced NK92 cells were incubated on anti-CD18- and anti-NKp30-coated coverslips for 25 minutes then fixed, permeabilized, and stained where indicated with AlexaFluor 568-conjugated phalloidin. Cells were visualized using total internal reflection fluorescence (TIRF) and differential interference contrast (DIC) microscopy (original magnification 60×). A field **(A)** or single cell magnification **(B)** are shown. AlexaFluor568-conjugated streptavidin was used as a negative control for phalloidin (top row), and parental (non-GFP-containing) NK92 cells were used as a negative control for GFP-actin (middle row). Both GFP-actin and AlexaFluor 568-conjgated phalloidin were present in the images shown in the third row. An overlay of 488 and 568 TIRF fluorescence is provided in the right column. **(C)** Individual cells from (A) were selected and individual pixels evaluated for colocalization of green and red fluorescence using Volocity software. Representative analyses are shown for NK92 GFP-actin/SA-568 (left), NK92/568 phalloidin (middle) and NK92 GFP-actin/568 phalloidin (right). Pearson's correlation coefficient (r) was obtained for each analysis and is shown above each image. **(D)** Pearson's correlation coefficient was determined for individual cells from each condition (n = 10) and mean +SD of r-values plotted.

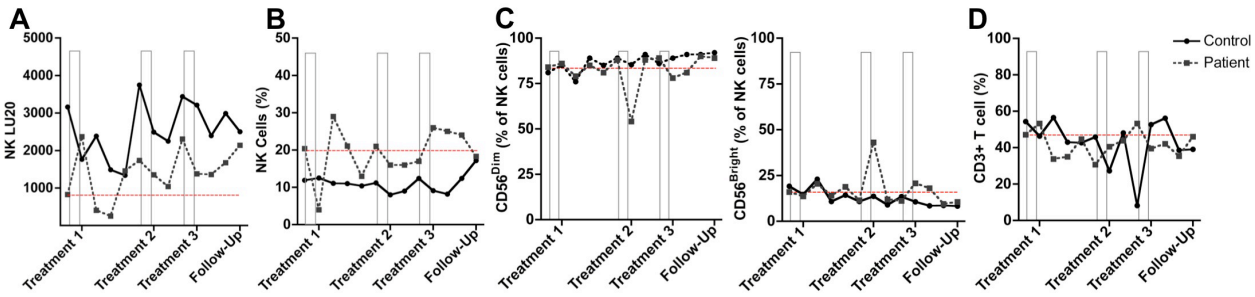


Supplemental Figure 2. *Effect of IL-2 upon cellular WASP content.* YTS **(A)** and ex vivo NK **(B)** cells were pretreated with vehicle (c - control), or Wiskostatin (Wisk) for 30min, followed by media or IL-2 for 30min prior to lysis. Whole cell lysates were evaluated for WASp content by Western blot analysis (top). Membranes were stripped and reprobed with for myosin-IIA as a loading control (bottom). Numbers beneath each lane represent densitometric ratios of WASp normalized to myosin-IIA.

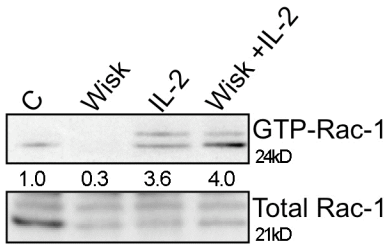


Supplemental Figure 3. *F-actin at the NK cell IS in WASP-deleted patient NK cells and the effect of in vitro IL-2.* Additional representative examples to those in Figure 2 of the

NK cell IS formed with K562 target cells in resting ex vivo NK cells from either a control donor (Control) or from a WAS patient having a complete deletion of the *WASP* gene (*WASP*^{del}) (left panels). The NK cell IS formed with K562 target cells was also evaluated in control or patient cells that had been stimulated in vitro for 30min with IL-2. Five separate examples of each are shown. Images show DIC (left most column) and confocal fluorescence microscopy for F-actin using phalloidin (yellow) and perforin (green) as well as an overlay of fluorescent channels (right most column). Scale bar=5μm.



Supplemental Figure 4. *Longitudinal NK cell function and percentage in a WAS patient during in vivo IL-2 treatment.* **(A)** NK cell cytotoxicity amongst PBMC was measured throughout the treatment course and was expressed as LU_{20} per NK cell present in the PBMC. The WAS patient values (dashed line) were compared to that in an individual healthy donor (not treated with IL-2 - solid line) throughout the study. The dashed red line denotes the patient's pre-treatment value and the open rectangles correspond to the 5d periods of IL-2 administration. These notations are held consistent throughout (B-D). **(B)** The percentage of total NK cells as defined by $CD3^-/CD56^+$ lymphocytes among PBMC as well the percentage of these that were **(C)** $CD56^{Dim}$ and **(D)** $CD56^{Bright}$. **(E)** The percentage of total T cells as defined by $CD3^+/CD56^-$ lymphocytes amongst PBMC in samples obtained throughout the protocol.



Supplemental Figure 5. *IL-2-induced Rac activation in NK cells*. YTS cells were pretreated with vehicle (c - control), or Wiskostatin (Wisk) for 30min, followed by media or IL-2 for 30min prior to lysis. Whole cell lysates were evaluated for total Rac-1 content by Western blot analysis (bottom). A portion of the lysate was used for immunoprecipitation of activated Rac using PAK-1 p21 binding domain GST fusion protein agarose beads. Precipitate was evaluated for the presence of Rac-1 by Western blot analysis (top). Numbers beneath each lane represent the activated densitometric ratio of the activated Rac-1 to that present in the control lane. Both bands shown in each lane were used for densitometric evaluation and take into account the relative tapering of the right-most lane in the top blot.