Supplemental data

Supplemental experimental procedures

T cell transfer

$CD4^+$ and $CD8^+$ T cells were isolated from pooled spleens and LNs of WT or $SLAT^{-/-}$ mice. The cells were then labeled for 10 min at 37°C with 0.5 or 5 μM CFSE (Molecular Probes), respectively. Then, $CD4^+$ or $CD8^+$ T cells from differentially CFSE-labeled WT and $SLAT^{-/-}$ mice were co-injected intravenously into WT recipients. Spleens, LNs and thymii were recovered on day 1 or 7 post-injection and stained for CD4 or CD8 expression. Percentages of $CFSE^+$ events among the $CD4^+$ or $CD8^+$ populations were determined.

Supplemental Figure 1

Normal maturation of thymocyte subsets in $SLAT^{-/-}$ mice. Thymocytes from $SLAT^{-/-}$and WT mice were stained with anti-CD4 and -CD8 mAbs, gated for DP (upper panels), SP $CD4^+$ (middle panels), and SP $CD8^+$ (lower panels), and expression of CD69 and/or CD62L and TCRβ was analyzed.

Supplemental Figure 2

SLAT protein expression in DN subsets. DN thymocytes were gated as described in Methods, and sorted based on their CD44 and CD25 expression. Cell lysates from total thymocytes or from the different DN subtypes were prepared and probed with an anti-SLAT Ab or with a Vav1-specific Ab as a loading control.

Supplemental Figure 3

T cell migration and survival in $SLAT^{-/-}$ mice. (**A**) $CD4^+$ and $CD8^+$ T cells from WT and $SLAT^{-/-}$ mice were purified and stained with CFSE (0.5 μM for WT and 5 μM for $SLAT^{-/-}$ cells). Equal number of WT and $SLAT^{-/-}$ $CD4^+$ or $CD8^+$ T cells were injected IV into WT mice. On day 1 and day 7, T cells were

recovered from the spleen and stained for CD4 and CD8 expression to evaluate the percentage of $CFSE^+$ CD4 or $CD8^+$ T cells. (**B**) Ratio of transferred $SLAT^{-/-}$/WT cells recovered in the spleen is shown.

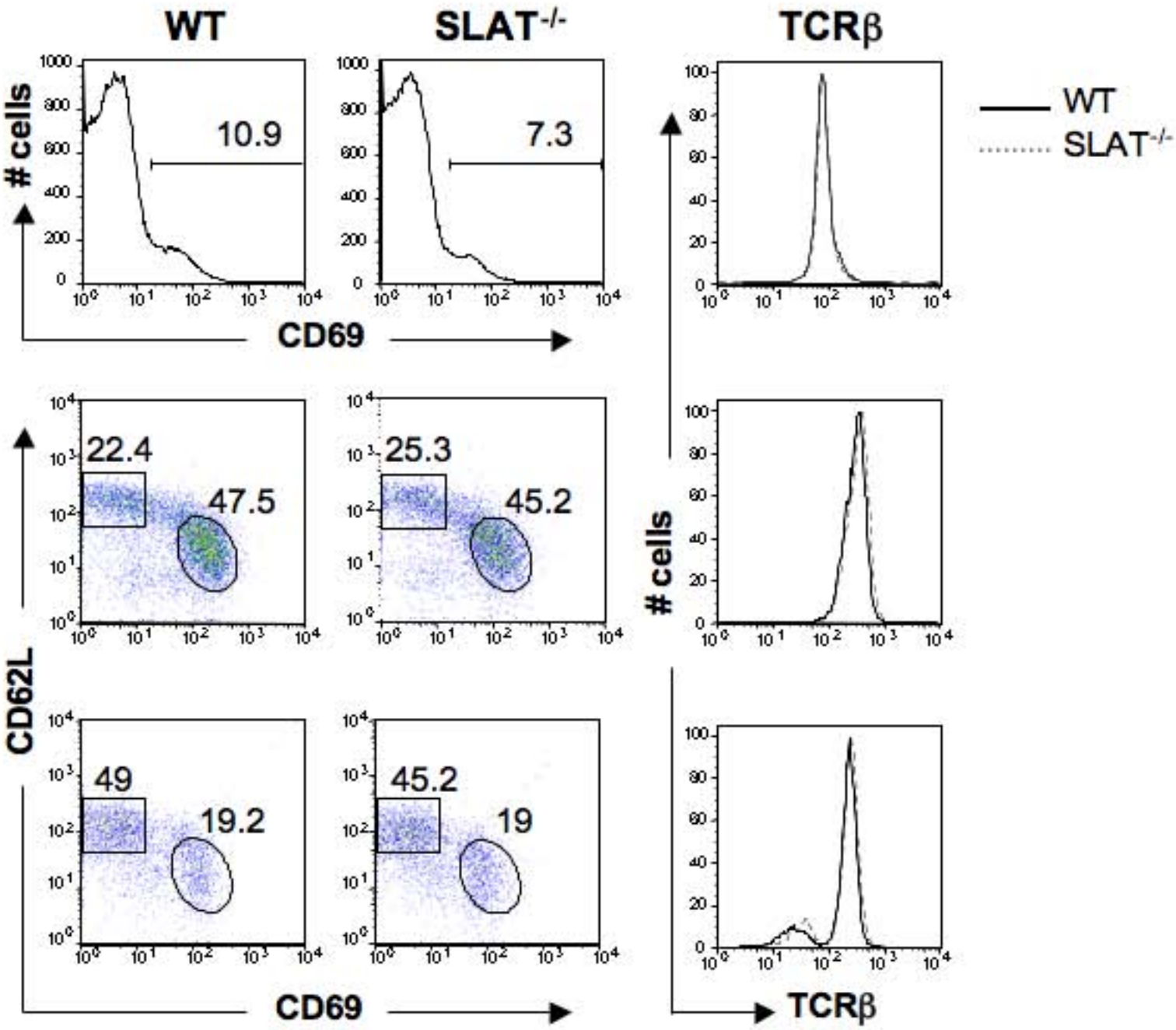
Supplemental Figure 4

Normal proliferation and IL-2 production of $SLAT^{-/-}$ $CD4^+$ T cells upon PMA and/or ionomycin stimulation. (**A**) Purified peripheral $CD4^+$ T cells from WT and $SLAT^{-/-}$ mice were stimulated with the indicated concentrations of PMA and/or ionomycin for 48h. [^{3}H]thymidine was added for the final 18 h of culture and proliferation was measured by tritium uptake. (**B**) IL-2 production was measured by an ELISA.

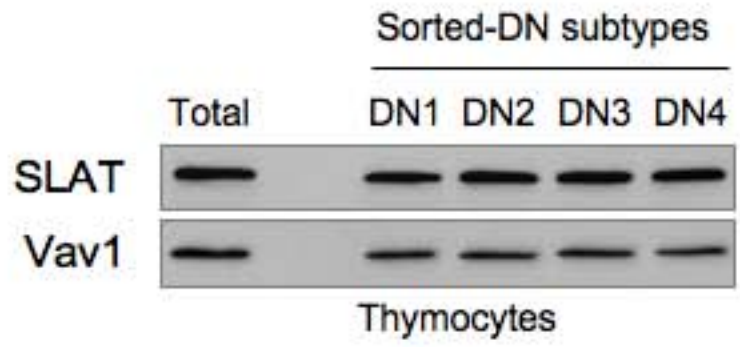
Supplemental Figure 5

SLAT protein expression in Th1 and Th2 lineage cells. WT $CD4^+$ T cells were differentiated under Th1- or Th2-inducing conditions, and lysates prepared at day 0 (Naïve) or day 6 (Th1 vs. Th2) after anti-CD3/CD28 stimulation were analyzed by immunoblotting as indicated. T-bet and GATA-3 protein expression are indicative of proper Th1 or Th2 differentiation, respectively. Reprobing with a Vav1-specific Ab is shown as a loading control.

Supplemental Figure 1

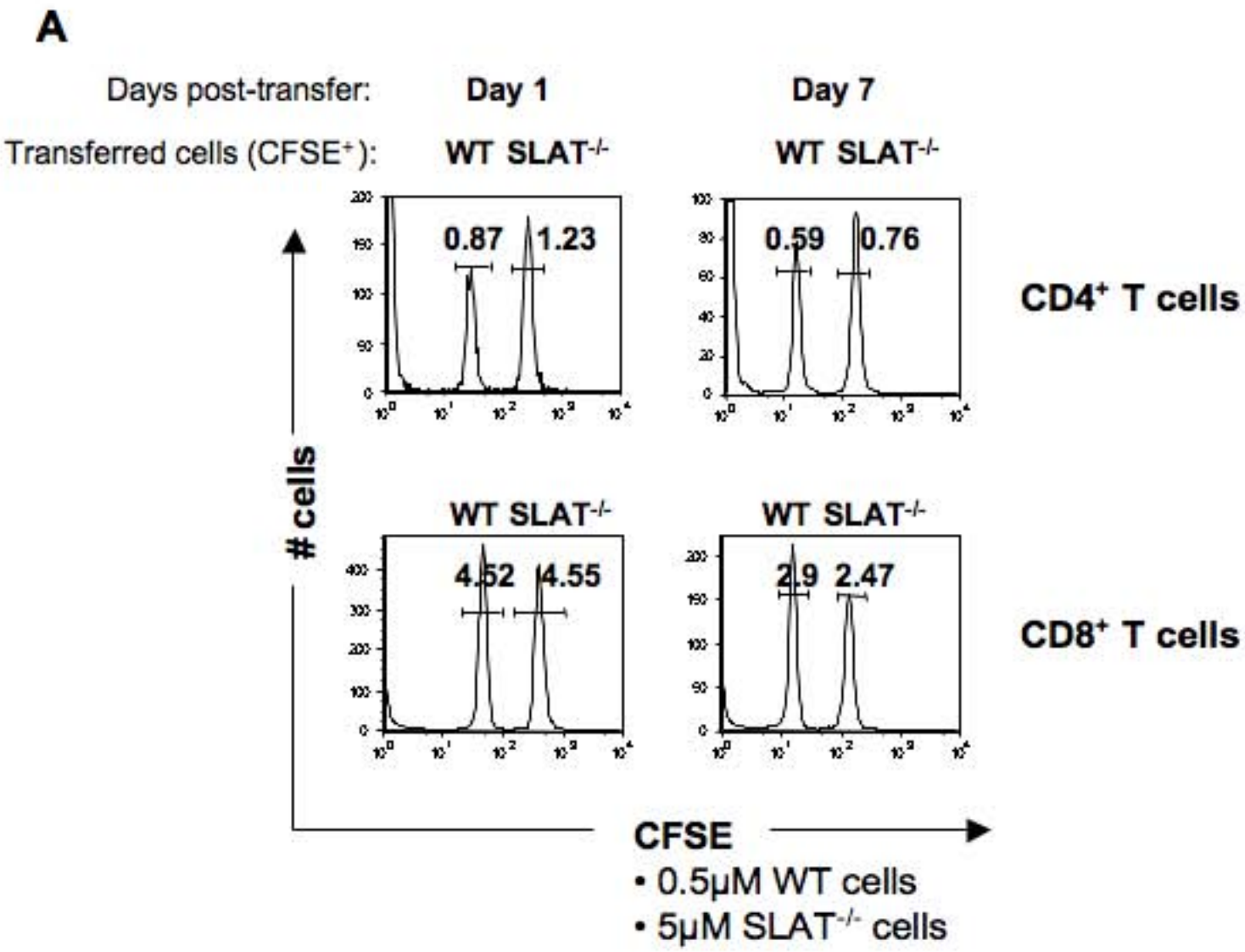


Supplemental Figure 2



Supplemental Figure 3

A

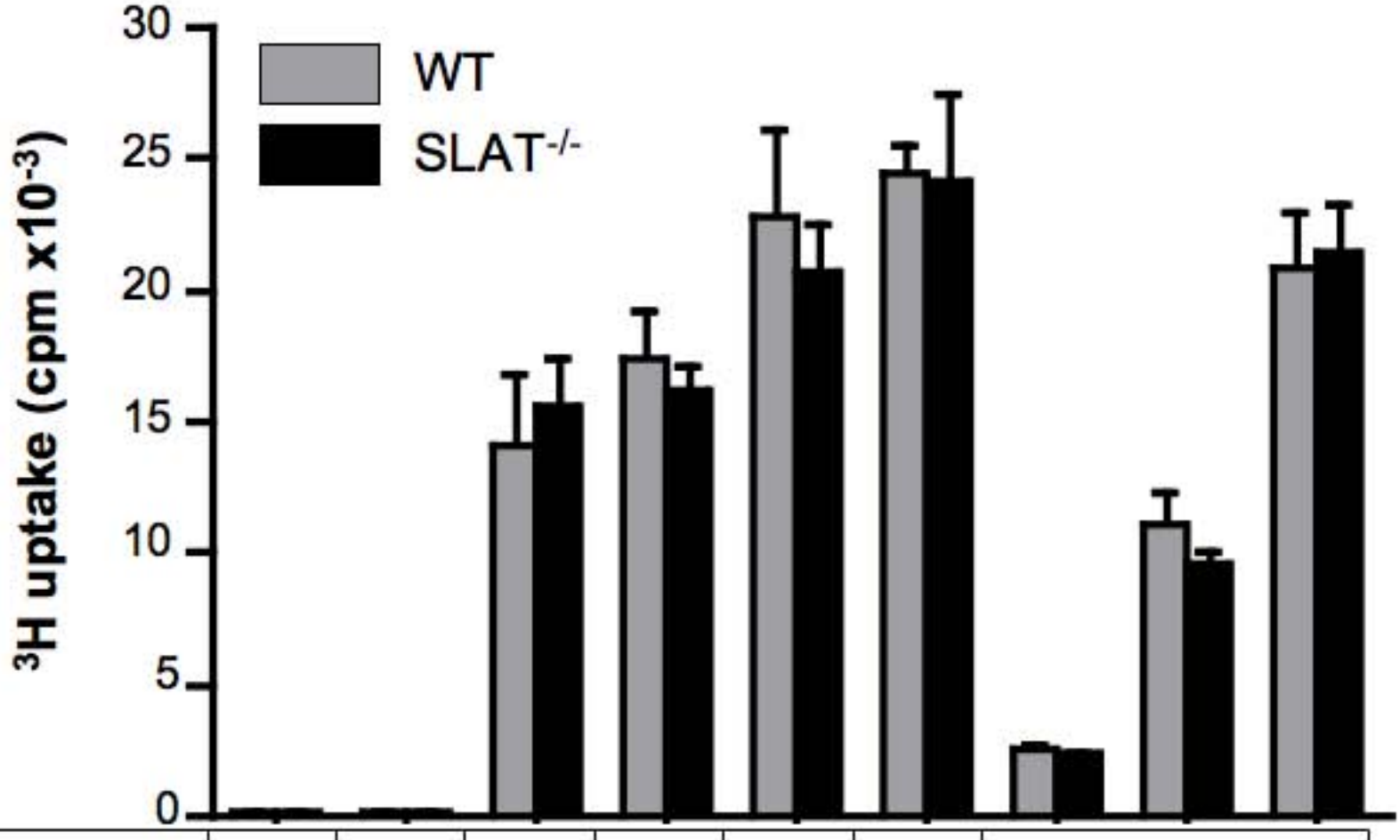


B

	Ratio ± SEM of transferred SLAT$^{-/-}$/WT cells recovered in spleen	
	Day 1 post-transfer	Day 7 post-transfer
CD4^{+} T cells	1.28 ± 0.12	1.1 ± 0.13
CD8^{+} T cells	0.9 ± 0.14	0.76 ± 0.12

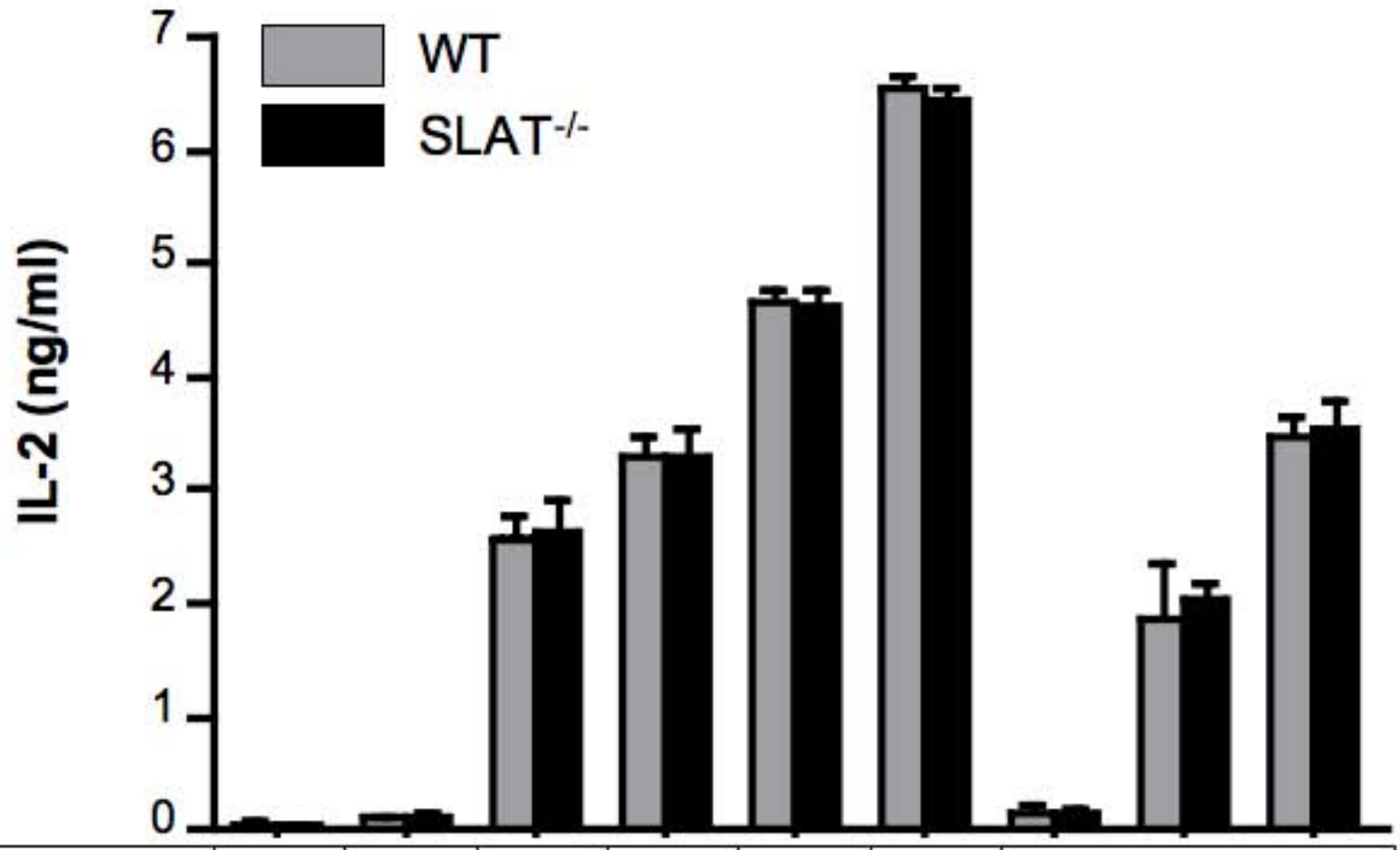
Supplemental Figure 4

A-



PMA (ng/ml)	0	0	2.5	5	10	20	20		
Iono (ng/ml)	0	500					0	125	250

B-



PMA (ng/ml)	0	0	2.5	5	10	20	20		
Iono (ng/ml)	0	500					0	125	250

Supplemental figure 5