

**Figure 3 – figure supplement 2 Additional evidence supporting that SLC37A3 and ATRAID depend on each other for stable expression.** (**A**-**C**) Analysis ofIF images comparing the expression levels of SLC37A3-HA (**A**) or both isoforms of ATRAID-V5 (**B** and **C**) in their respective single knockout backgrounds and the KO2 background. The two images in each sub-figure were acquired with the same setting and adjusted to the same contrast. In each image a background area (turquoise or blue, inside nuclei, where no stain should be present) and a signal area (orange or red) were selected and the distribution of pixel values within each area was plotted in a histogram. The outlines in the histogram are color-coded to match the boarders of selected areas. (**D**-**E**)Polysome profiling experiment assessing the translation efficiency of *SLC37A3* transcripts in *SLC37A3*KO and KO2 backgrounds. Lysates from indicated cell lines were analyzed on a gradient station and fractionated into five fractions: untranslated transcripts (UT), small and large ribosome subunits (SL), lowly translated transcripts (LT), medially translated transcripts (MT) and highly translated transcripts (HT). The level of *SLC37A3* transcripts relative to the level of *TBP* (TATA-binding protein) transcripts in each fraction was measured and plotted. The SL fraction was excluded from the analysis. A total RNA fraction was included as a reference. No overall shift was observed in the distribution of *SLC37A3* transcripts in the KO2 background compared to that in the *SLC37A3*KO background, suggesting that the translation efficiency of *SLC37A3* is not affected by the absence of *ATRAID*. (**F**) Immunoblot comparing the glycosylation patterns of SLC37A3 in *SLC37A3*KO and KO2 backgrounds. Lysates from *SLC37A3*KO + SLC37A3-HA (lane 1-3) and KO2 + SLC37A3-HA (lane 4-6) HEK 293T cells were left untreated (lane 1 and 4), treated with PNGase-F (lane 2 and 5), or with Endo H (lane 3 and 6). The band corresponding to an un-glycosylated population of SLC37A3 that is present in the absence of ATRAID but not in the presence of ATRAID is marked with an asterisk. (**G**) Immunoblot comparing the glycosylation patterns of total cellular SLC37A3 and the sub-population of SLC37A3 that interacts with ATRAID. In KO2 HEK 293T cells over-expressing SLC37A3-HA and sATRAID-V5, proteins that interact with sATRAID-V5 were purified with immuno-precipitation against V5 epitope and compared with total proteins in the lysate. The pre-IP total lysate (lane 1-2) and anti-V5 IP eluate (lane 3-4) were either left untreated (lane 1 and 3) or treated with PNGase F (lane 2 and 4) and analyzed by blotting against SLC37A3-HA. IF: immunofluorescence. IB: immunoblot. KO2: *ATRAID*KO; *SLC37A3*KO. PNGase F: peptide: N-glycosidase F, an enzyme that removes all asparagine (N)-linked sugar modifications from glycoproteins. Endo H: endoglycosidase H, an enzyme that only removes high mannose sugar moieties on ER glycoproteins that have not been processed by the Golgi apparatus.