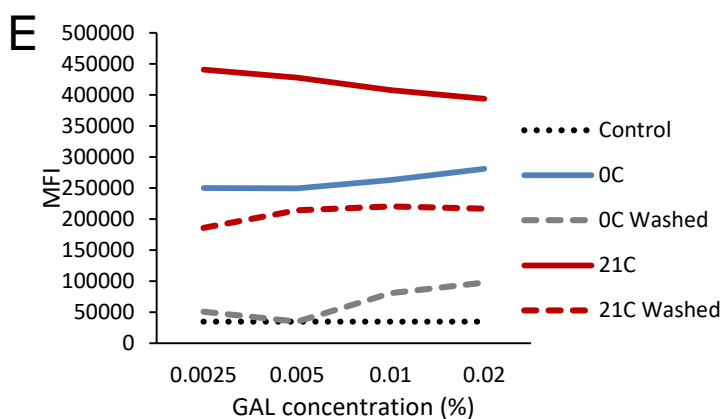
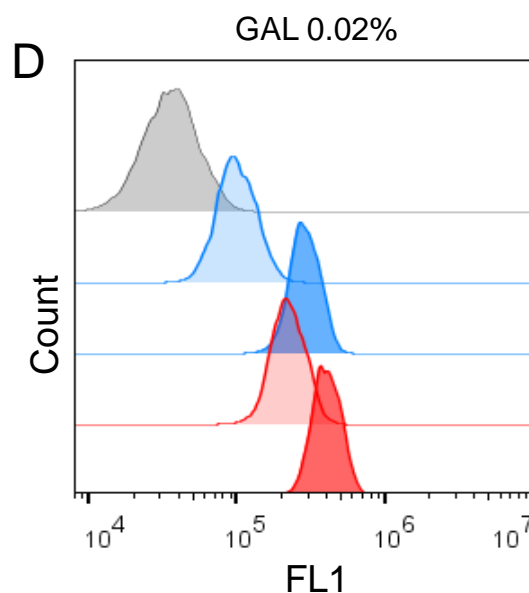
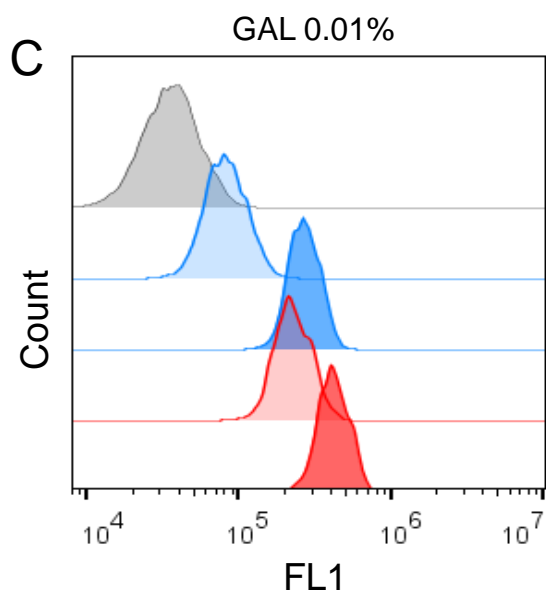
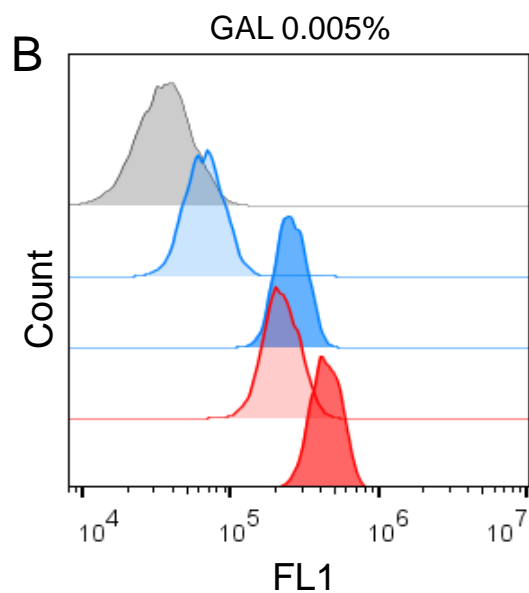
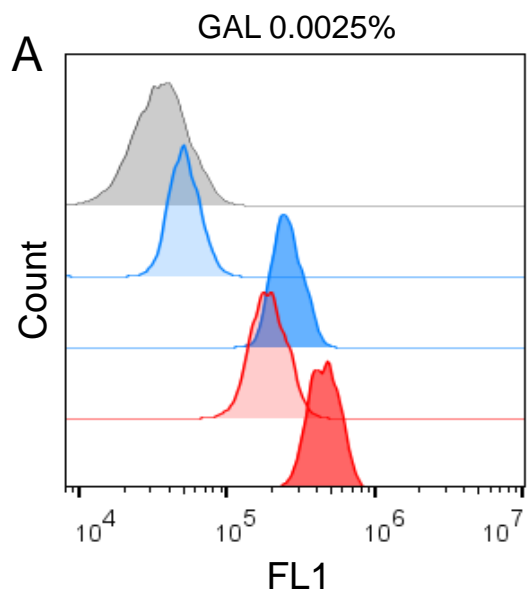
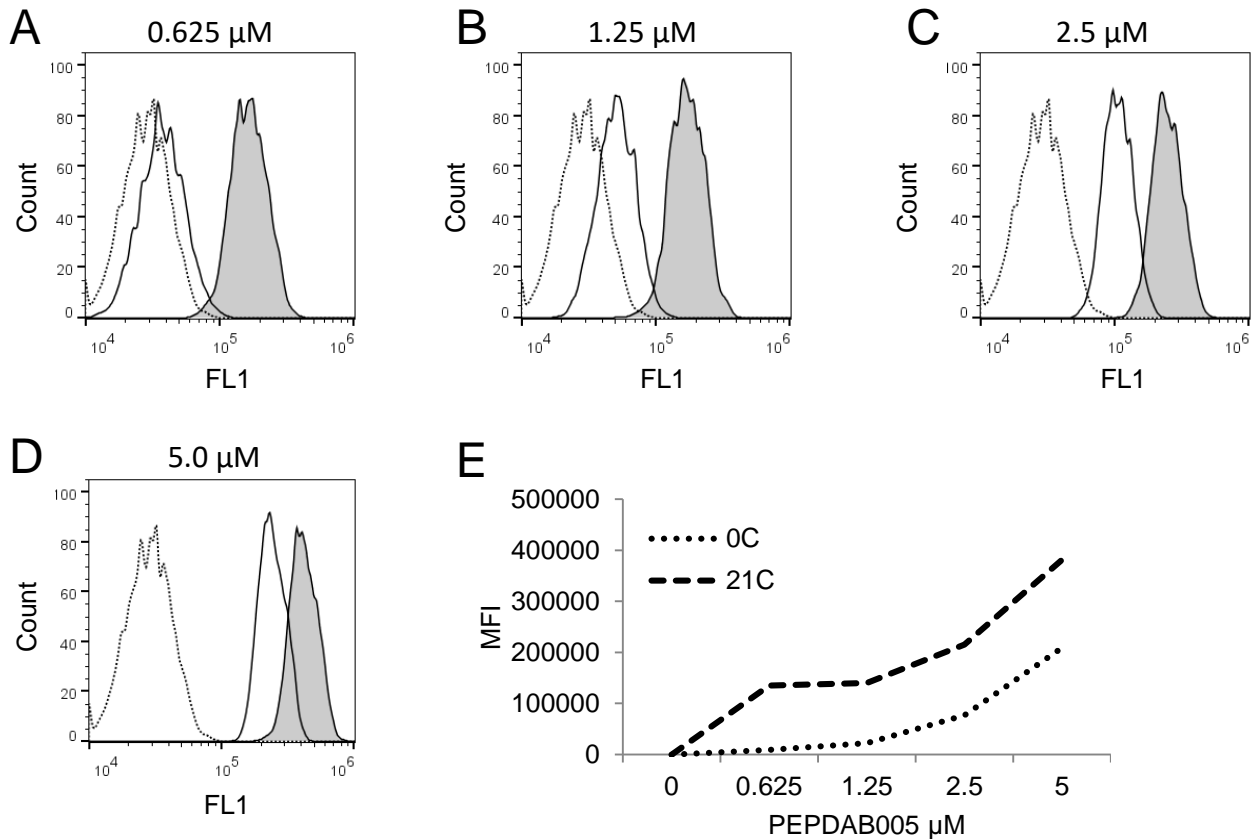


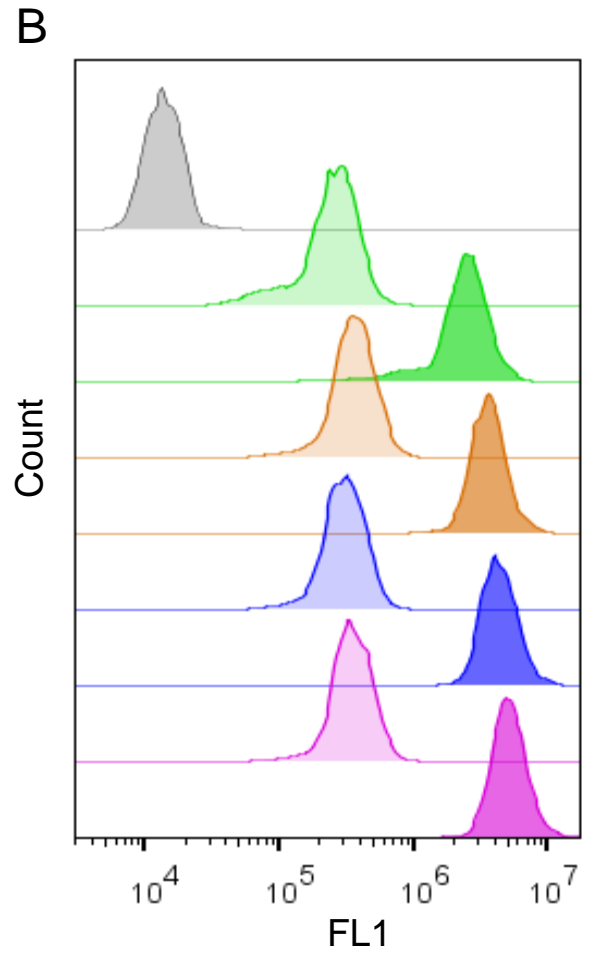
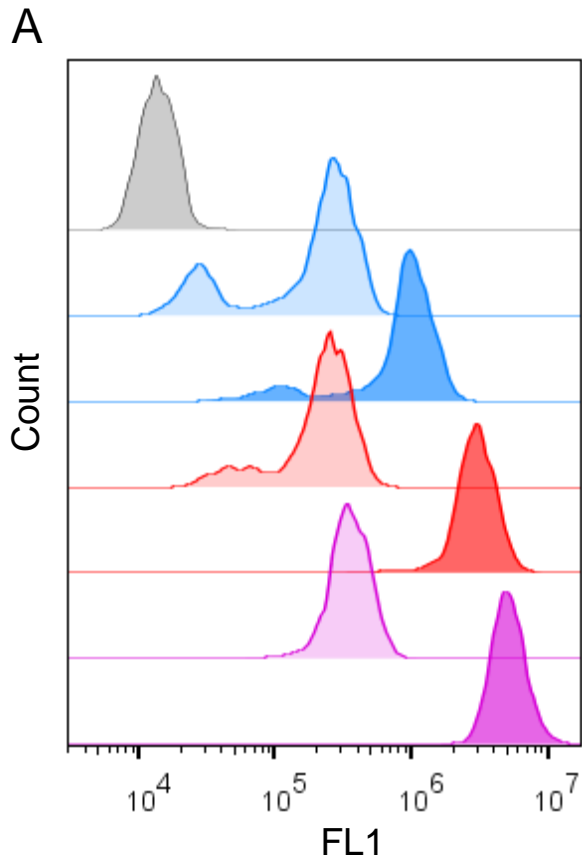
Supplemental Figure 1. *K562 cells express on plasma membrane high ADAM10 and low ADAM17 levels.* K562 cells were stained with PE-conjugated isotype control, anti-human ADAM10 and anti-human ADAM17 monoclonal antibodies, and examined by single-color flow cytometry. Presented data are histograms of fluorescence intensity.



Supplemental Figure 2. Low concentrations of GAL support PEPDAB005 processing and fix the processed fluorescent substrate to cell surface. K562 cells were incubated in the absence (grey histograms) and presence of 5 μ M PEPDAB005 at 0°C for 60 min, washed, incubated in the presence of 0.0025% (**A**), 0.005% (**B**), 0.01% (**C**) and 0.02% (**D**) GAL at 0°C for 30 min, continued to incubate at 0°C (blue histograms) or 21°C (red histograms) for additional 30 min, post-fixed with 2% PFA, kept unwashed (dark-color histograms) or were washed before FACS analysis (light-color histograms). (**E**) MFI data presented in **A-D** figures are summarized.



Supplemental Figure 3. *In the presence of GAL, processed PEPDAB005 substrate can be detected on the cell surface using at least 2.5 μM PEPDAB005.* K562 cells were incubated in the absence (dashed line empty histograms) or presence of 0.625 μM (**A**), 1.25 μM (**B**), 2.5 μM (**C**) and 5.0 μM PEPDAB005 substrate (**D**) at 0°C for 60 min, washed, fixed with 0.005% GAL at 0°C for 30 min, incubated at 0°C (full line empty histograms) or 21°C (full line gray histograms) for 30 min, post-fixed with 2% PFA and analyzed by FACS. (**E**) MFI data presented in **A-D** figures are summarized.



Supplemental Figure 4. A significant portion of cell-associated processed PEPDAB005 substrate cannot be washed out and is dependent on temperature and incubation time. (**A**) H441 cells were incubated in the absence (grey histogram) or presence of 10 μ M PEPDAB005 at 0°C (blue histograms), 21°C (red histograms) and 37°C (purple histograms), fixed with 2% PFA, left unwashed (dark color histograms) or were washed (light color histograms), and analyzed by FACS. (**B**) H441 cells were incubated in the absence (grey histogram) or presence of 10 μ M PEPDAB005 at 37°C for 15 min (green histograms), 30 min (orange histograms), 45 min (blue histograms) and 60 min (purple histograms), fixed with 2% PFA, left unwashed (dark color histograms) or were washed (light color histograms), and analyzed by FACS.