**Supplementary Figure S1.** Killing of MDA-MB-231 by E75-CTL is HLA-A2 dependent. Calcein-AM cytotoxicity assay using E75-CTL show HLA-A2 (i.e. HLA-A*0201)-dependent killing of the HLA-A2+ MDA-MB-231 cells. Cell killing was HLA-A2 dependent since the incubation of MDA-MB-231 cells with the anti-HLA-A2 antibody BB7.2 attenuated the killing of MDA-MB-231 cells by E75-CTL (E75 is an HLA-A2-restricted peptide). Calcein-AM release was used to measure cytotoxicity as described in the materials and methods section.
Supplementary Figure S2.
NE uptake leads to increased surface expression of HLA class I in cancer cell lines. MEL526 (melanoma), H2023 (NSC lung cancer), OVCAR3 (ovarian cancer) and SW620 (colon cancer) cell lines were maintained in standard media +/- NE (10µg/mL). a NE uptake corresponded to an increase in HLA-ABC on the cell surface of MEL526 and b H2023 cells. NE uptake does not cause a significant increase in HLA-ABC on the surface of c OVCAR3 or d SW620 cells. Experiments were performed in triplicate; results are representative of 3 separate experiments. *P <0.05, **P <0.01, ***P <0.001 comparing NE treated to untreated, unless designated; MFI, median fluorescence intensity.
Supplementary Figure S3. MDA-MB-231 take up NE over time. a MDA-MB-231 cells were maintained in standard media +/- NE (10 µg/ml). Cells were treated for various time points and analyzed for intracellular NE using flow cytometry. Significant NE uptake was confirmed at all time points. b MDA-MB-231 cells were maintained in standard media +/- NE, with or without IFN-γ pretreatment. Cells that were treated with IFN-γ followed by NE had a greater NE uptake compared to cells treated with NE alone. Experiments were performed in triplicate; results are representative of 3 separate experiments. **P < 0.01, ***P < 0.001 comparing NE treated to untreated cells, unless designated. MFI, median fluorescence intensity.
Supplementary Figure S4. Breast cancer cell lines increase HLA class I surface expression after treatment with IFN-γ and NE. Breast cancer cell lines MDA-MB-468 and MCF-7, were maintained in standard media +/- IFN-γ. NE was added and cells were cultured overnight. a IFN-γ treatment of MDA-MB-468 cells led an increase in HLA-ABC surface expression compared with untreated cells. The addition of NE further increased HLA-ABC surface expression compared to IFN-γ treatment alone. b MCF-7 cells also showed an increase in HLA-A2 surface expression when treated with both IFN-γ and NE, compared to IFN-γ alone. Experiments were performed in triplicate; results are representative of 3 separate experiments. *P <0.01, **P <0.0001 comparing the different treatment groups, unless designated; MFI, median fluorescence intensity.
Supplementary Figure S5. Serine protease activity is inhibited by elafin and PMSF. NE was co-incubated with elafin or PMSF at 37°C for 1 hour. Protease activity was determined using an enzymatic fluorescence-based assay and analyzed by microplate fluorescence reader. Co-incubation with elafin or PMSF led to a significant decrease in protease activity as compared to NE alone. Experiments were performed in triplicate; results are representative of 3 separate experiments. **P < 0.01, ***P < 0.001 comparing serine protease inhibitor co-incubation to NE alone.