

## S3 Figure. AGS cell IL-8 secretion induced by independent replicate clones of *H*. *pylori* isogenic CagL<sup>58/59</sup> substitution mutants.

Spent culture medium was collected at 6 or 24 hours post-inoculation with P12 wildtype (WT; D58K59), P12 $\Delta cagL$ , P12 $\Delta cagL$  knocked-in with 26695wt-derived *cagL* (NE) or two independent replicate clones (denoted by Arabic numerals) of P12 $\Delta cagL$ knocked-in with 26695-derived *cagL* carrying substitutions at amino acids 58 and/or 59 (NK, DE, DK or YE). Samples were assayed for IL-8 levels by ELISA and data from individual experiments were standardized as a percentage of IL-8 secretion by isogenic WT strain at 24 hours post-inoculation to correct for interassay variability in absolute IL-8 levels, and allow pooling of data from multiple independent experiments for statistical analysis; bars denote mean  $\pm$  SD from  $\geq$ 3 independent experiments (each performed in duplicate). \*\* P <0.01, \*\*\*\* *P* <0.0001 against all other isogenic strains at same time-point by two-way ANOVA with Tukey's multiple comparisons test; all other comparisons not significantly different (*P* >0.05).