



Supplemental Figure 3. Increasing levels of receptor expression, utilizing a human inducible mGlu5 cell line, result in a shift from pure PAM to agonist activity coupled with PAM activity for VU0360172. Cells were treated with vehicle or increasing concentrations of tetracycline (1-10 ng/ml) for 22 hours. Increasing levels of mGlu5 expression, determined by radioligand binding, were observed with increasing concentrations of tetracycline. Subsequently cells were treated with increasing concentrations of VU0360172 followed by the addition of an EC20 concentration of glutamate. The calcium response was normalized to a maximally effective concentration of glutamate (10  $\mu$ M). A) An increase in agonist activity was observed with increasing levels of receptor expression as a result of tetracycline induction. B) VU0360172 potentiated the response to a suboptimal (EC20) concentration of glutamate regardless of the level of receptor expression. Data represent the mean  $\pm$  SEM of five independent experiments performed in duplicate.