EARLY-STAGE DEVELOPMENT OF AN IN VITRO SCREENING ASSAY TO CHARACTERIZE THE ACTIVATION OF THE HUMAN ADHESION GPCR ADGRE5

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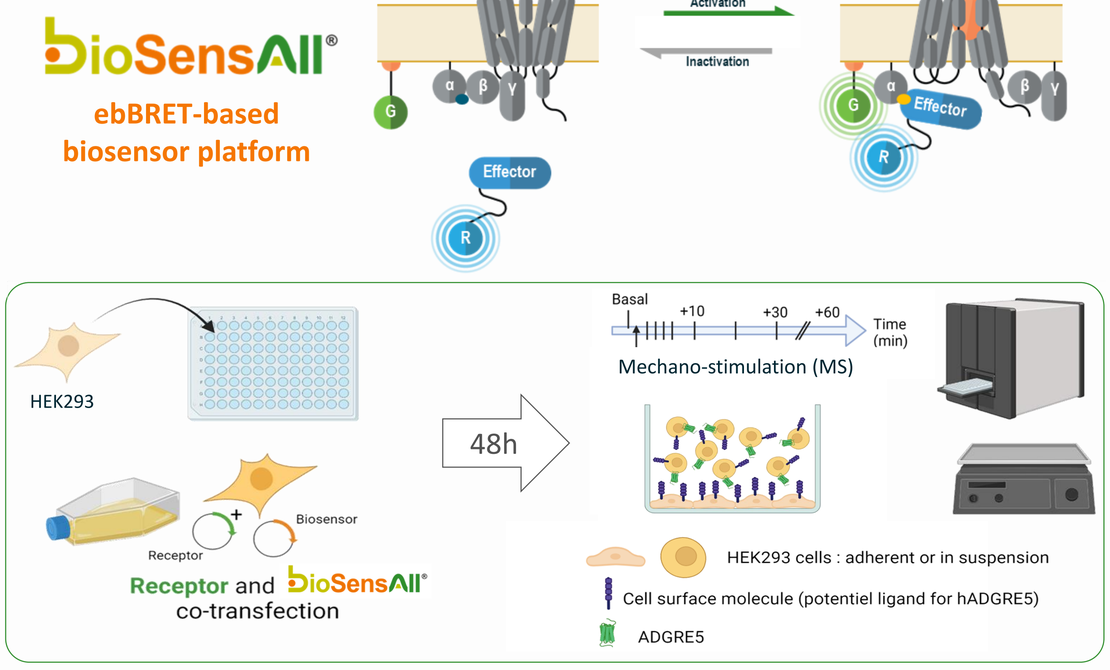
INTRODUCTION

ADGRE5 is a prototypical adhesion GPCR (aGPCR) that is expressed mainly on T lymphocytes, monocytes/macrophages, granulocytes, NK cells (especially CD56^{bright}) and smooth muscle cells. Importantly, ADGRE5 expression is upregulated in many cancers (e.g., gastric/colorectal/pancreatic carcinomas, various leukemias, glioblastoma), whereas the corresponding normal tissues express relatively little or no ADGRE5. Previous studies indicated that ADGRE5 plays an important role in modulating cell adherence/detaching, migration, invasion, and metastasis, making ADGRE5 a potential drug target in oncology/immuno-oncology. Yet, the development of assays enabling the discovery of drugs acting on aGPCRs has been hindered by the lack of functional agonists and the complex multimodal mechanisms believed to govern receptor activation. Current methods rely on artificial methods of activation that preclude the identification of allosteric modulators acting upon ADGRE5's tractable long extracellular N-terminal fragment.

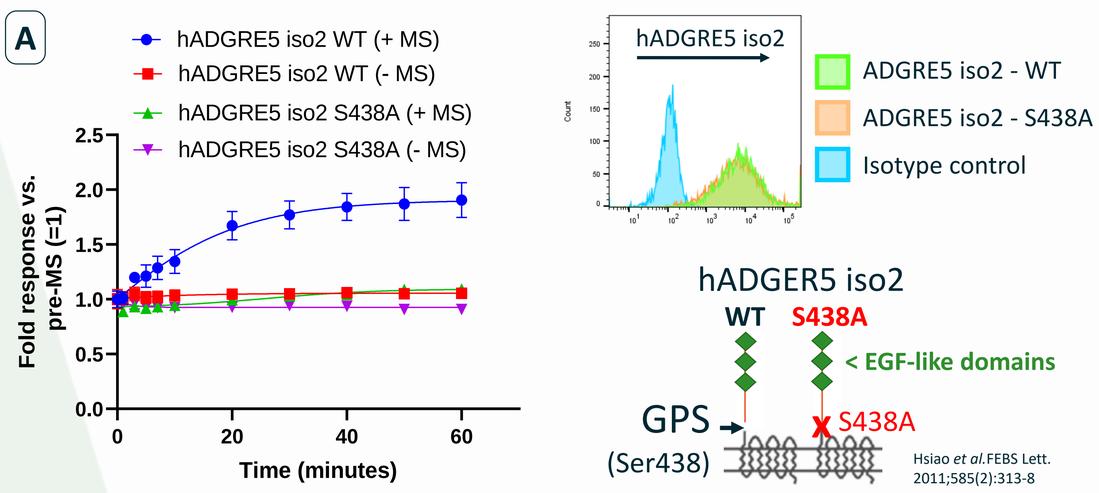
We describe herein a novel *in vitro* enhanced bystander (eb)BRET-based assay allowing to detect the activity of the full-length (native) form of human (h)ADGRE5 via its physiological activation mode (i.e., following mechanical stimulation).

MATERIALS AND METHODS

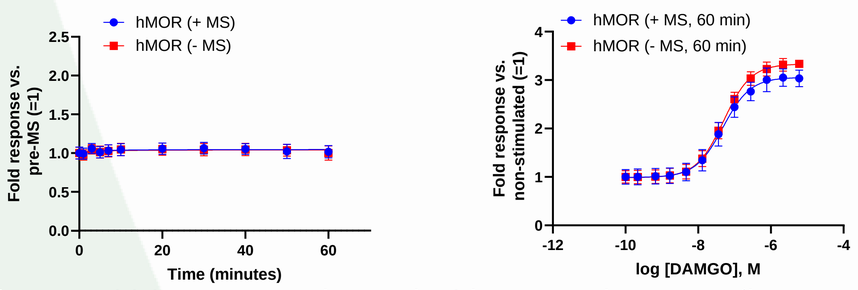


RESULTS

1 hADGRE5 iso2 recruits βArrestin 2 following mechano-stimulation (MS) via a GPCR proteolysis site (GPS)-dependent mechanism



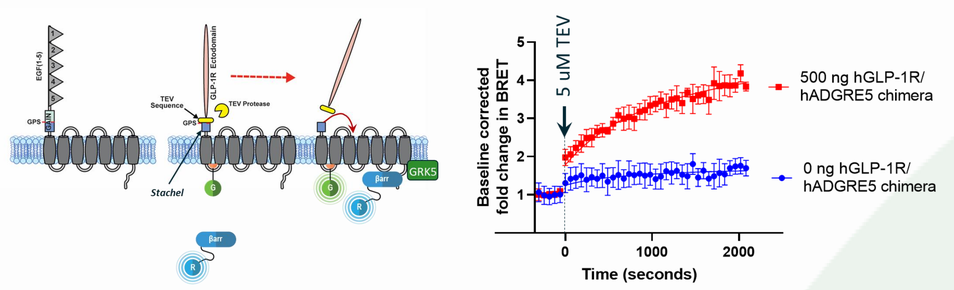
A. hADGRE5 iso2 WT, but not GPS point mutant S438A, recruits βArr2 following MS. The GPS is believed to play an important role in adhesion GPCR activation through the exposure of a tethered agonist following cleavage. Similar results obtained for iso1.



B. Mechanosensitivity was not observed with a control receptor (human mu opioid receptor, hMOR), despite its ability to engage βArr2 following ligand stimulation.

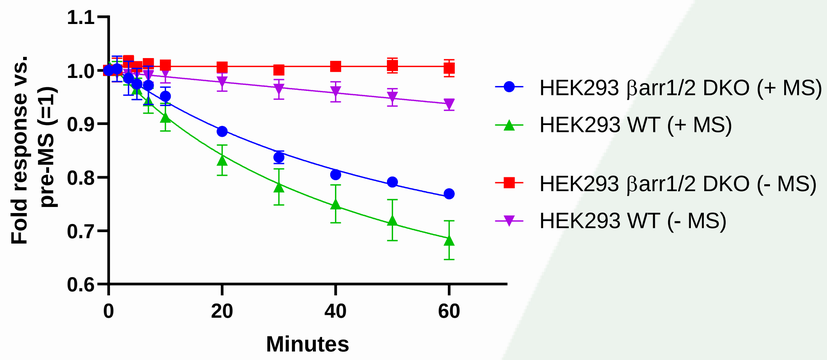
RESULTS

2 Cleavage of hADGRE5 at the GPS is sufficient to promote βarrestin2 recruitment to the receptor



> Receptor cleavage by TEV protease at the GPS results in the unmasking of a tethered agonist (*Stachel*), rendering it available for receptor engagement and activation.

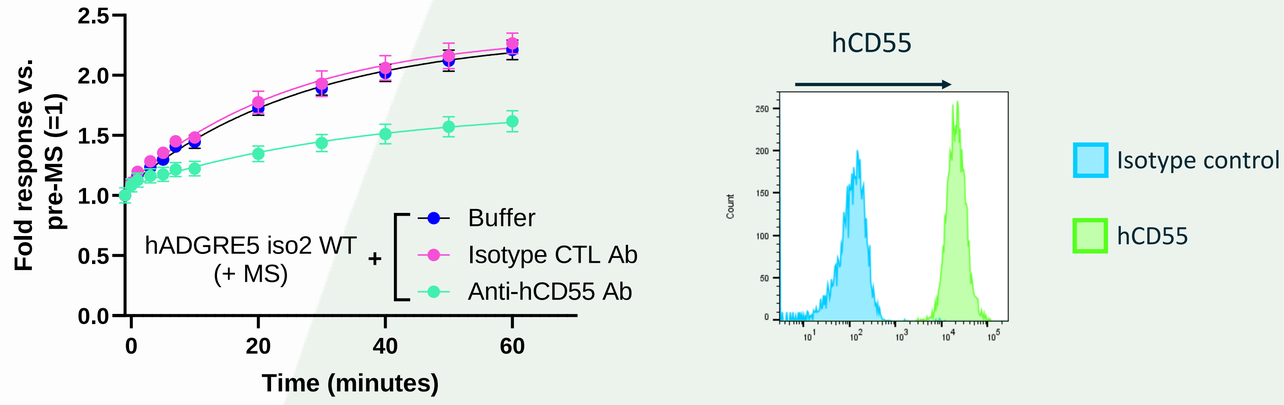
3 Mechano-stimulation (MS) provokes internalization of WT hADGRE5 iso2 largely via βArrestin-independent mechanisms



> Mechanostimulation led to hADGRE5 iso2 internalization but not that of a control receptor (SST2AR, data not shown).

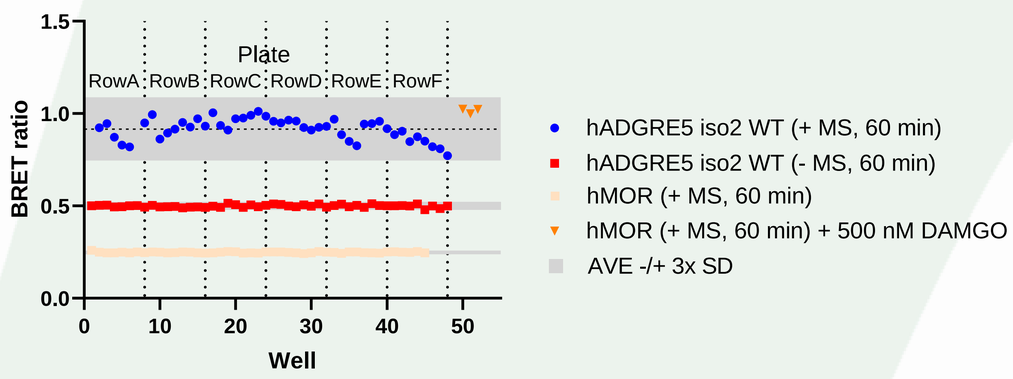
> Internalization was conserved in βArr1/2 double knockout cells.

4 Mechano-stimulation (MS) induced βArrestin 2 engagement by hADGRE5 iso2 involves receptor interaction with CD55



> CD55 is described to interact with hADGRE5's adhesive EGF-like domains
> CD55 is expressed endogenously by HEK293
> A neutralizing CD55 antibody dampened MS-induced hADGRE5 βArr2 recruitment

5 Mechano-stimulation (MS)-induced βArrestin 2 recruitment assay is robust and amenable to screening



Z' hADGRE5 iso2 WT (+ MS) vs hMOR (+ MS): **0.73**
Z' hADGRE5 iso2 WT (+ MS) vs hADGRE5 iso2 WT (- MS): **0.54**

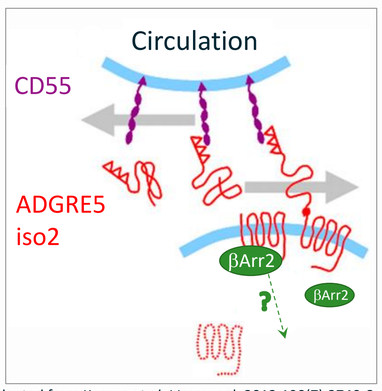
CONCLUSION

> Our ebBRET-based MS assay permits for the detection of physiologically-relevant hADGRE5 activation.

> We hypothesize that trans-signaling complexes between CD55 and hADGRE5 could mediate the hADGRE5 activity observed in our assays.

> The assay will facilitate the screening of entities acting orthosterically -OR- allosterically on hADGRE5's long N-term domain (e.g., antibodies).

> This protocol can be adapted to other biosensors and mechano-sensitive targets of interest.



Adapted from Karpus *et al.* J Immunol. 2013;190(7):3740-8