

Novel agonists of the Piezo1 mechanosensing ion channel for placental drug development

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Background

Blood flow creates the mechanical force, fluid shear stress (FSS), which is required for placental vascular function and development. Piezo1 is a Ca²⁺-permeable ion channel activated by FSS. Piezo1 regulates vascular tone via mechanisms including coupling to nitric oxide (NO) signalling and is present in human placental endothelium. Failure to maintain normal placental blood flow causes fetal growth restriction, for which there is no effective treatment. The current Piezo1 agonist, Yoda1, lacks properties for in vivo use. We sought to develop new Piezo1 activators as a novel strategy towards improving placental blood flow. Our hypothesis is that novel Piezo1 agonists can be developed to provide a foundation for a novel therapeutic drug and its evaluation in complicated pregnancy, which can regulate NO by enhancing endothelial nitric oxide synthase (eNOS) activity.

Objectives

- Determine the response of Piezo1 in different vascular beds when treated with Piezo1 agonists
- Determine the potencies of novel Piezo1 agonists in healthy patients' cells

Methods

Yoda1 and three novel Piezo1 agonists **1**, **2** and **3** developed in our lab were tested on freshly isolated human umbilical vein and artery endothelial cells (HUVECs and HUAECs) (Fig1). Cells were isolated by vessel cannulation for collagenase digestion from patients delivering by caesarean section with no fetal or maternal concerns at Leeds Teaching Hospitals Trust (EA 18/LO/0067). Confluent cells at passages 2-5 were loaded with Fura2-AM Ca²⁺ indicator dye. Piezo1 channel activation was detected by changes in intracellular Ca²⁺ on a Flexstation plate reader (Molecular Devices).

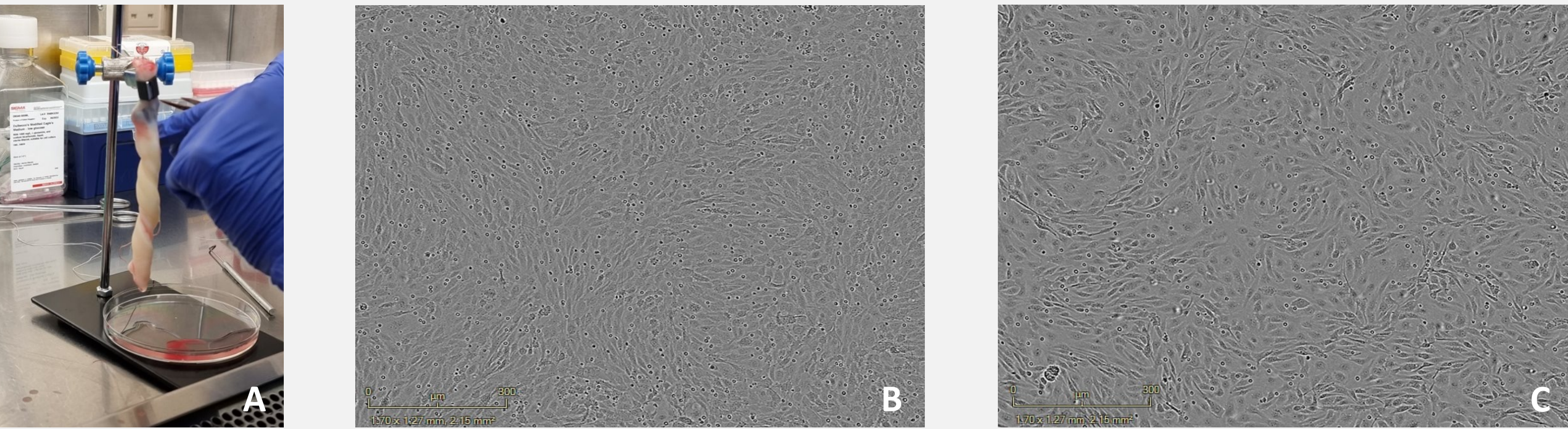


Figure 1. (A) Endothelial cells isolation, (B-C) IncuCyte™ imaging system was used to image both HUVECs and HUAECs at P0, (B) HUVECs, (C) HUAECs

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Results

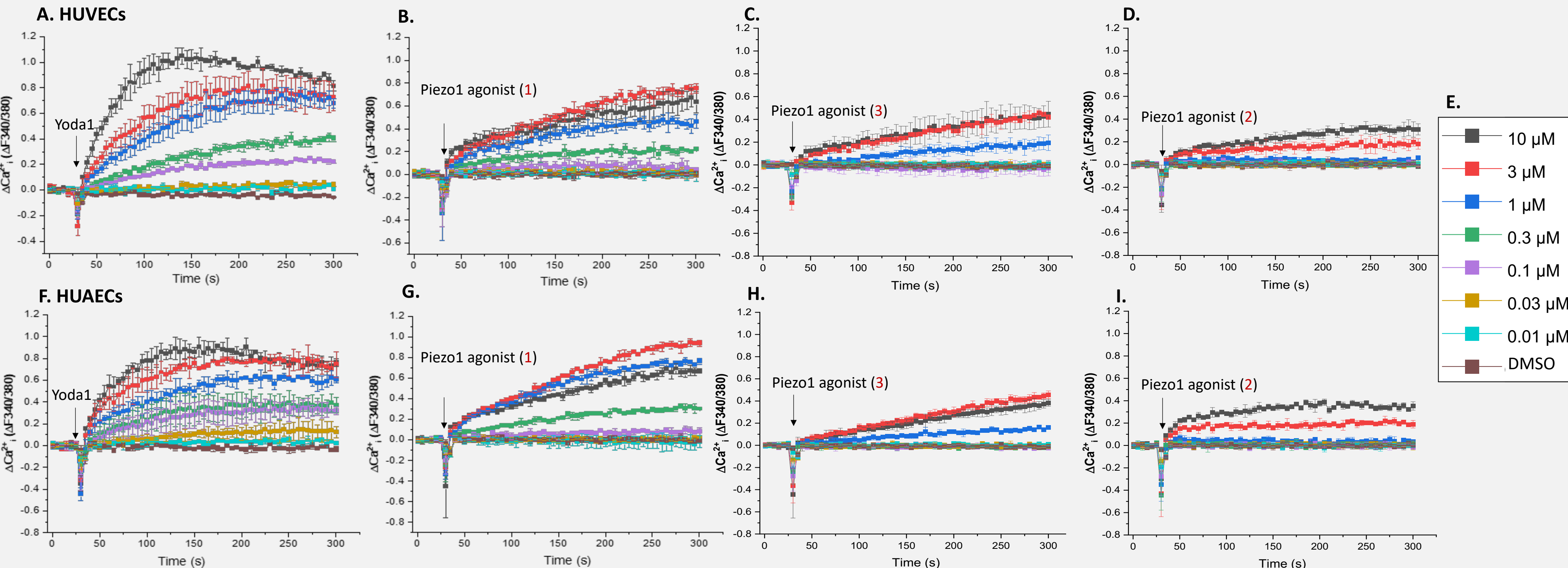


Figure 2. Representative traces of change in intracellular Ca²⁺ in HUVECs A-D (first row) and HUAECs F-I (second row) following the application of Yoda1 (A,F), Piezo1 agonist **1** (B,G), Piezo1 agonist **3**(C,H) and Piezo1 agonist **2** (D,I) at different dose concentrations compared with vehicle control (DMSO). The injection point is indicated by an arrow. At least three replicates were used for each trace.

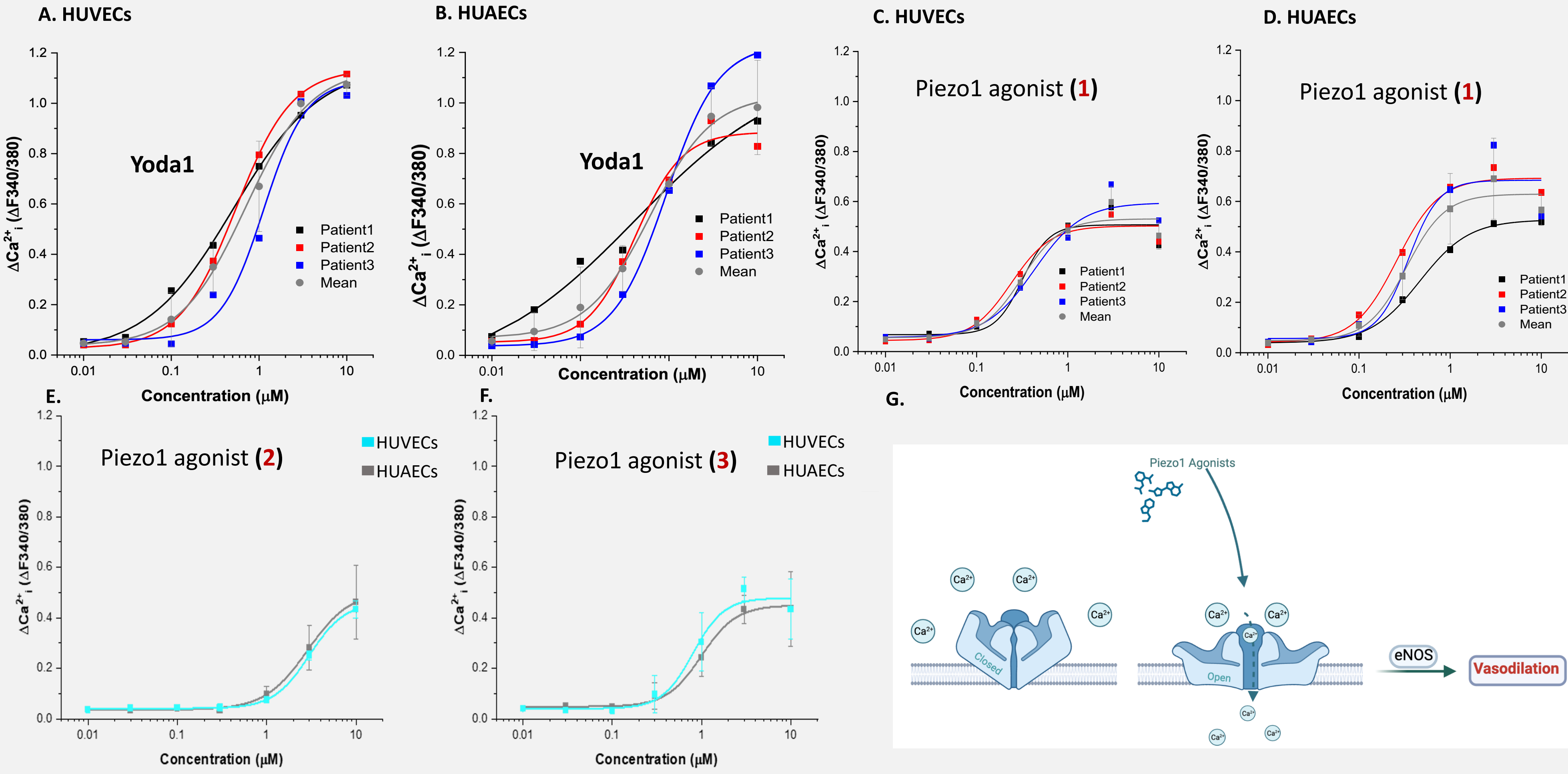


Figure 3. A-F Dose response of all tested Piezo1 agonists. Log dose-response curves from testing 7 different concentrations of each agonist demonstrated significantly lower half maximal effective concentrations (EC₅₀s) with **1** (C,D) compared to **2** (E), and **3** (F) in HUVECs and HUAECs. Both cell types were collected from three different patient samples N=3. G. Piezo1 ion channel can be activated by chemical stimulation that causes the opening of the Piezo1 ion channel, leading to evoked Ca²⁺ influx

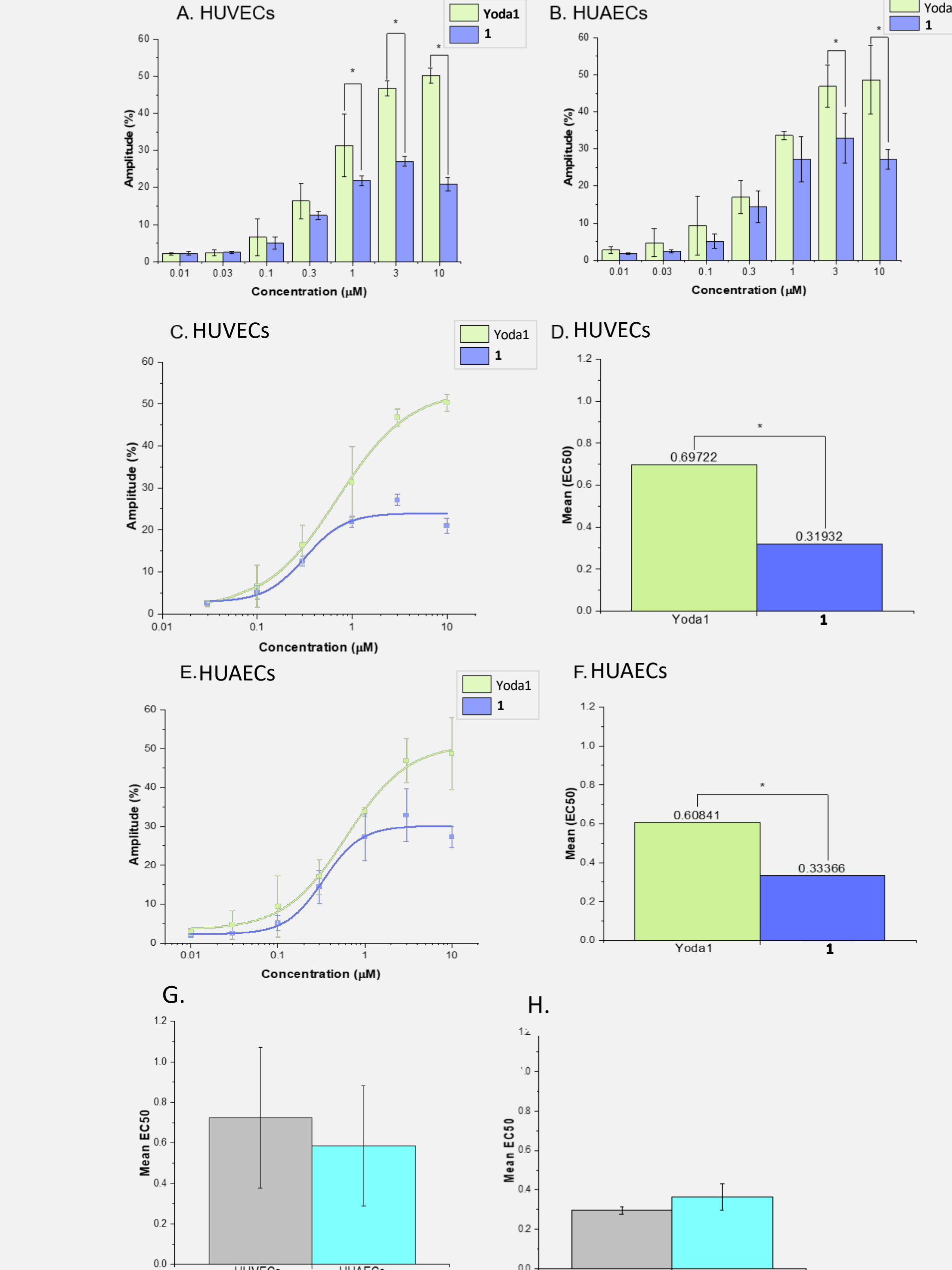


Figure 4. Pooled data comparing the amplitude of response to Yoda1 and **1** in HUVECs and HUAECs. (A,B) HUVECs and HUAECs in response to **1** and Yoda1 at different dose concentrations, with large variation observed at the high dose concentrations. The EC₅₀ for **1** is 0.32µM in HUVECs (D) and 0.33 µM in HUAECs (F), compared to 0.70 µM and 0.61 µM for Yoda1(C-F), indicating greater potency of **1**. No significant difference was found between arterial and venous cells in response to Yoda1 (G) nor to **1** (H). Data expressed as Mean ± SD. At least two independent experiments were included per cell type for each Piezo1 agonist.

Conclusion

1 named **Yoda2**, had better potency, aqueous solubility and stability than Yoda1 (Parsonage et al. (2022). Responses of Piezo1 agonists showed consistency across cell types and patients. Development of Piezo1 agonists with improved properties represents a step towards placental drug development targeting blood flow regulation.

References

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