

## SUPPLEMENTARY INFORMATION

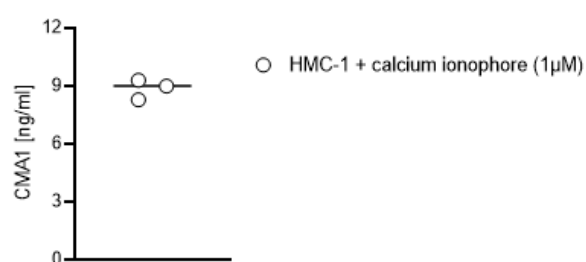
# Insights into early pregnancy mechanisms: mast cells and chymases CMA1 shape the phenotype and modulate the functionality of human trophoblast cells, vascular smooth muscle cells and endothelial cells.

Authors: Ningjuan Zhang<sup>1,2</sup>, Anne Schumacher<sup>1,2</sup>, Beate Fink<sup>1</sup>, Mario Bauer<sup>1</sup>, Ana Claudia Zenclussen<sup>1,2</sup> and Nicole Meyer<sup>1,2</sup>

Ningjuan Zhang <sup>1,2</sup>, Anne Schumacher <sup>1,2</sup>, Beate Fink <sup>1</sup>, Mario Bauer <sup>1</sup>, Ana Claudia Zenclussen <sup>1,2</sup> and Nicole Meyer <sup>1,2,\*</sup>

- <sup>1</sup> Department of Environmental Immunology, UFZ-Helmholtz Centre for Environmental Research Leipzig-Halle, 04318 Leipzig, Germany; ningjuan.zhang@ufz.de (N.Z.); anne.schumacher@ufz.de (A.S.); beate.fink@ufz.de (B.F.); mario.bauer@ufz.de (M.B.); ana.zenclussen@ufz.de (A.C.Z.)
- <sup>2</sup> Perinatal Immunology, Saxonian Incubator for Clinical Translation (SIKT), Medical Faculty, University Leipzig, 04103 Leipzig, Germany
- \* Correspondence: nicole.meyer@ufz.de; Tel.: +49-341-235-1542

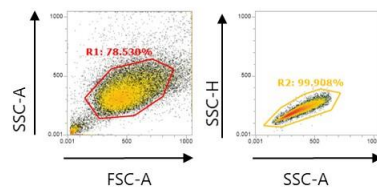
**Fig. S1**



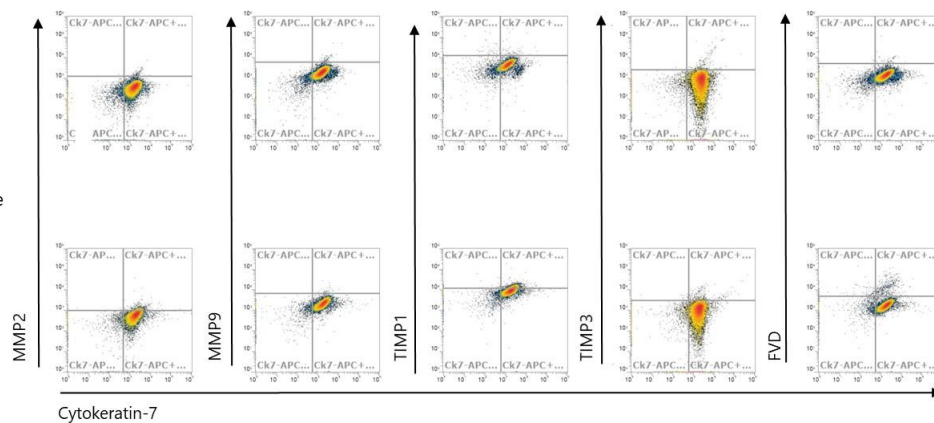
**Figure S1: CMA1 expression of calcium ionophore A23187-treated HMC-1 cells.** CMA1 expression (ng/ml) measured via ELISA in the supernatants of calcium ionophore A123187-treated HMC-1 cells.

**Fig. S2**

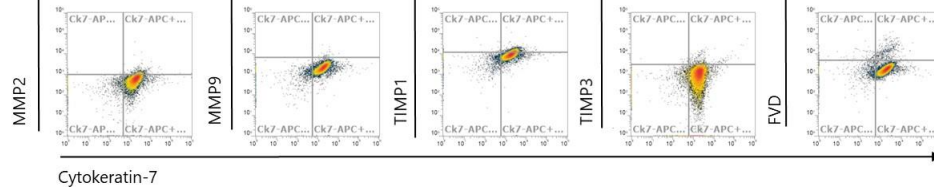
a) HTR-8 cell population



b) FMO



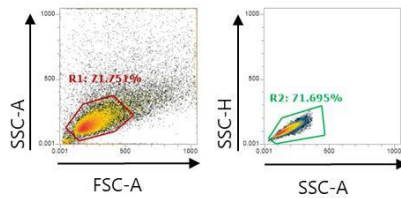
c) Sample



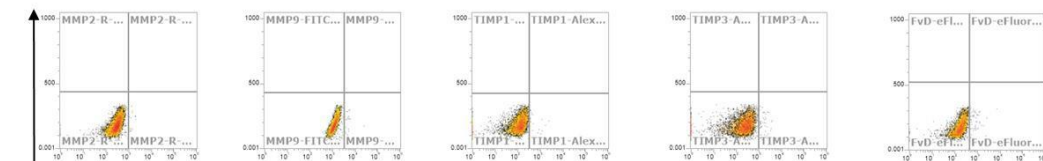
**Figure S2: Flow cytometry gating strategy: HTR-8 ± HMC-1 assay.** a) Representative gating strategy for HTR-8 cell population. b) Representative Fluorescence minus one (FMO) control or c) sample plots for MMP2, MMP9, TIMP1, TIMP3 or FVD of HTR-8 cells. HTR-8: HTR-8/SVneo cells; FMO; Fluorescence Minus One.

**Fig. S3**

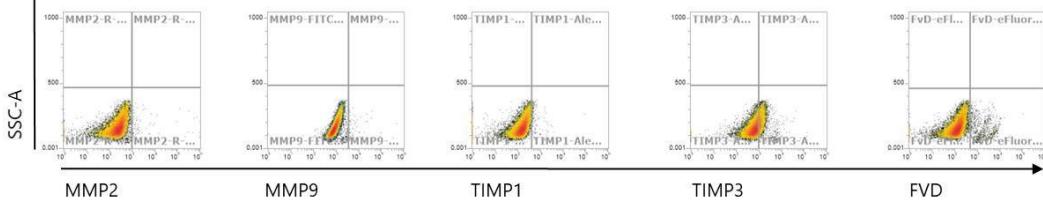
a) HTR-8 cell population



b) FMO



c) Sample



**Figure S3: Flow cytometry gating strategy: HTR-8  $\pm$  rhuCMA1 assay.** **a)** Representative gating strategy for HTR-8 cell population. **b)** Representative FMO control or **c)** sample plots for MMP2, MMP9, TIMP1, TIMP3 or FVD of HTR-8 cells stimulated  $\pm$  rhuCMA1. HTR-8: HTR-8/SVneo cells; rhuCMA1: recombinant human chymase; FMO; Fluorescence Minus One.

Tab. S1

Table S1: MMPs and TIMPs expression in supernatant of HTR-8 cells co-cultured with HMC-1 or treated with rhuCMA1

Median (95%CI) (pg/ml)	TIMP1	TIMP2	MMP2	MMP9
HTR-8+HMC-1 co-culture experiment 24h				
HTR-8	7313 (5503,7767)	n.d.	n.d.	n.d.
HTR-8+HMC-1 (1:1)	13674 (9005,18731)			
HTR-8+HMC-1 (1:5)	20403 (17937,20924) **			
HTR-8+rhuCMA1 experiment 24h				
HTR-8	11049 (8497,13737)	13238 (7805,18671)	n.d.	n.d.
HTR-8+rhuCMA1 (3ng/ml)	10864 (7688,13574)	3216		
HTR-8+rhuCMA1 (30ng/ml)	10902 (6953,13330)	12446 (6222,18671)		
HTR-8+rhuCMA1 (300ng/ml)	11666 (9911,12213)	5453 (1740,7805)		
HTR-8+HMC-1 co-culture experiment 48h				
HTR-8	16751 (15959,17792)	n.d.	n.d.	n.d.
HTR-8+HMC-1 (1:1)	23235 (22960,23617)			
HTR-8+HMC-1 (1:5)	22686 (20487,25317)			
HTR-8+rhuCMA1 experiment 48h				
HTR-8	15156 (11125,25767)	12934 (1740,14777)	n.d.	n.d.
HTR-8+rhuCMA1 (3ng/ml)	15081 (9761,21313)	11158 (8619,12038)		
HTR-8+rhuCMA1 (30ng/ml)	12540 (11279,24969)	7805 (7006,8619)		
HTR-8+rhuCMA1 (300ng/ml)	13825 (10515,29370)	n.d.		
HTR-8+HMC-1 co-culture experiment 72h				
HTR-8	34065 (24455,50822)	n.d.	n.d.	n.d.
HTR-8+HMC-1 (1:1)	38357 (29390,55236)			
HTR-8+HMC-1 (1:5)	27160 (24969,44776)			
HTR-8+rhuCMA1 experiment 72h				
HTR-8	24352 (21280,30972)	32835 (16829,53159)	n.d.	n.d.
HTR-8+rhuCMA1 (3ng/ml)	24591 (23323,27159)	35269 (22821,51336)		
HTR-8+rhuCMA1 (30ng/ml)	21080 (16553,33073)	45556 (29635,50729)		
HTR-8+rhuCMA1 (300ng/ml)	25485 (22471,27420)	47862 (29817,60014) *		