**Supplementary material and methods**

*Culture of human mast cells*Mast cells were cultured from CD133+ progenitor cells isolated from human peripheral blood as described previously (1–3). Isolated CD133+ cells were cultured under optimal conditions for seven weeks resulting in mature mast cells. The cells were cultured in StemSpan serum-free medium containing 50 ng/ml rh-IL-6, 100 ng/ml rh-SCF and 100 µg/ml penicillin/streptomycin. The first three weeks the culture medium also contained 1 ng/ml rh-IL-3. From week 6 the culture medium was supplemented with 10% FCS. Once a week the cells were counted, metachromatic stained with Alcian blue, and passed on to fresh culture medium.

*Activation of mast cells, RNA extraction*

Mature human mast cells were sensitized with 2 µg/ml myeloma IgE (supernatant from the B-cell line U266) 24 hours before activation with 5 µg/ml anti-IgE (Dako, Denmark) for 30 min at 37 °C. After activation the cells were spun down and lysed with 700 µl QIAzol® (Qiagen, Denmark) and frozen at -80 °C. Total RNA including small RNA was purified and the integrity of the RNA was analyzed on an Agilent Bioanalyzer 2100 Expert.

Total RNA including small RNA was extracted by phase separation of the QIAzol homogenate followed by purification using silica membrane technology. In brief, the cells frozen in QIAzol were allowed to thaw at room temperature. Then 200 µl of chloroform was added and the samples were mixed thoroughly. Phase separation was achieved by centrifugation at 6.000 g for 15 min at 4 °C. The RNA was purified from the water phase by a semi-automated protocol based on the miRNeasy kit (Qiagen) implemented on a Biorobot Universal (Qiagen). The Samples were quantified using a BeckmanCoulter DTX880 platereader, and the integrity of the RNA analyzed on an Agilent Bioanalyzer 2100 Expert

The miRNA expression was profiled on Taqman® Low Density Arrays (TLDA) set (Cards A and B). The cDNA synthesis, pre-amplification and real-time PCR analysis was performed as described by the manufacturer. Briefly, RNA reverse transcription was accomplished according to the Megaplex RT Reaction protocol. For the reverse transcription the following thermal cycling conditions were used in the ABI 9700 thermal cycler: standard ramp, 16 °C for 2 min, 42 °C for 1 min (40 cycles), 50 °C for 1 sec, hold 85 °C 5 min, hold 4 °C. The resulting cDNA was used directly for preamplification according to the ABI TLDA preamplification protocol. This was conducted in the ABI 9700 thermal cycler using standard ramp speed and the following thermal cycling conditions: hold 95 °C 10 min; hold 55 °C 2 min; hold 72 °C 2 min; 12 cycles at 95 °C 15 sec and 60 °C 4 min; hold 4 °C.

The preamplified cDNA was diluted 1:4 in TE pH 8.0, loaded onto the TLDA cards and analyzed on ABI 7900HT Real-time PCR system. The PCR data were collected as relative data in ABI software SDS 2.2.2 and RQ Manager using identical thresholds (Ct = 40) and baseline for all assays. Non-detects were assigned a Ct value of 40.

*miRNA Data analysis*

The raw miRNA expression data was normalized by deltaCt normalization, as implemented in the Bioconductor R package “HTqPCR” (4). The raw expression data was normalized to the expression of 3 endogenous genes: mammU6, RNU44 and RNU48. Differential expression analysis was then performed using the Bioconductor package “Limma” where a model design was made to test for differential expression between resting and sensitized/activated, while correcting for interpersonal variability (5). P-values were adjusted for multiple testing by controlling the false discovery rate using the Benjamini Hochberg method. A miRNA was considered differentially expressed (DE) when the FDR p-value was < 0.05. For significant miRNAs, the ΔΔCt was calculated for each miRNA and fold change was calculated as FC = 2^-ΔΔCt. When FC < 1, down-regulation was calculated as down-regulation = -1/FC.

*miRNA target prediction and enrichment analysis*

For target prediction, experimentally validated miRNA targets for miR-212/132 and miR-210 were retrieved from miRTarBase(6). Only targets with strong evidence were kept (reporter assay, western blot, qRT-PCR or qPCR). A miRNA-target interaction network was made using cytoscape (version 3.6.1). The target gene names of each miRNA were used as input in gene set over-representation analysis by the R-package “XGR” (7). Significantly enriched REACTOME pathways (8), adjusted p-value <0.05, were visualized according to fold enrichment.

*Mediator release from human mast cells*

Cultured mature mast cells were sensitized with 2 µg/ml myeloma IgE 24 hours before activation. They were activated with 5 µg/ml anti-IgE for 30 min at 37 °C. After 30 min the activation reaction was stopped with cold Pipes stock buffer (10 mM pipes, 150 mM Na-acetate, 5 mM K-acetate, 0.6 mM CaCl2, 1 mg/ml glucose, 0.3 mg/ml human serum albumin, 15 IE/ml heparin, pH 7.4). The cells were centrifuged for 5 min, 4 °C and 1500 rpm. For histamine release and PGD2 secretion 20,000 and 10,000 cells were used, respectively. For the analysis of histamine release, the samples were boiled for 10 minutes before centrifugation for 10 min, 4 °C and 14000 rpm. Afterwards the samples were loaded to microtiter plate containing microfiber pads that binds histamine. The released histamine from the activated mast cells were analyzed by use of a fluorescence based method (9). The release of histamine was expressed as a percentage of the total histamine content of the sample. In the absence of anti-IgE, the spontaneous histamine release amounted to 1-2% in all experiments. In order to analyze the secretion of PGD2, a Prostaglandin D2-MOX EIA kit purchased from Caymann Chemicals was used according to manufacturer's instructions. For both assays a parametric unpaired t-test was used to test for significant differences between mast cells isolated from allergic and healthy subjects.

*Correlation analysis*

The Pearson correlation between individual miRNA expression and PGD2 and HR was performed using the R-package “Hmisc” (10). For correlations with a p-value < 0.05, the correlation was plotted in Graphpad Prism (version 7.0) and linear regression with 95% confidence bands was calculated. Furthermore, the corresponding spearman correlation and p-value was calculated for each significant Pearson correlation. The raw miRNA expression data was normalized by rank invariant normalization, as implemented in the Bioconductor R package “HTqPCR”, to better model non-detects and reduce bias introduced by assigning Ct = 40 to non-detects.

References:

 1. Holm M, Andersen HB, Hetland TE, Dahl C, Hoffmann HJ, Junker S et al. Seven week culture of functional human mast cells from buffy coat preparations. *J Immunol Methods* Published Online First: 27 May 2008. doi:S0022-1759(08)00150-6

2. Andersen HB, Holm M, Hetland TE, Dahl C, Junker S, Schiøtz PO et al. Comparison of short term in vitro cultured human mast cells from different progenitors - Peripheral blood-derived progenitors generate highly mature and functional mast cells. *J Immunol Methods* Published Online First: 16 May 2008. doi:S0022-1759(08)00143-9

3. Hjort C, Schiøtz PO, Ohlin M, Würtzen PA, Christensen LH, Hoffmann HJ. The number and affinity of productive IgE pairs determine allergen activation of mast cells. *J Allergy Clin Immunol* 2017;**140**:1167-1170.e2.

4. Dvinge H, Bertone P. HTqPCR: high-throughput analysis and visualization of quantitative real-time PCR data in R. *Bioinforma Oxf Engl* 2009;**25**:3325–3326.

5. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;**43**:e47.

6. Chou C-H, Shrestha S, Yang C-D, Chang N-W, Lin Y-L, Liao K-W et al. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res* 2018;**46**:D296–D302.

7. Fang H, Knezevic B, Burnham KL, Knight JC. XGR software for enhanced interpretation of genomic summary data, illustrated by application to immunological traits. *Genome Med* 2016;**8**:129.

8. Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P et al. The Reactome Pathway Knowledgebase. *Nucleic Acids Res* 2018;**46**:D649–D655.

9. Skov PS, Mosbech H, Norn S, Weeke B. Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. Results compared with conventional leukocyte histamine release assay. *Allergy* 1985;**40**:213–218.

10. Harrell FEJ. *Hmisc: Harrell Miscellaneous*. 2018