ONLINE SUPPLEMENTAL MATERIAL

Supplementary Figure E1. Siglec-7, Siglec-8, and Siglec-9 surface expression in human mast cell lines. (A-C) Representative flow cytometry analysis of Siglec-9 surface expression in human mast cell lines **(A)**, percentage of human mast cell lines expressing Siglec-7, Siglec-8, and Siglec-9 **(B),** and mean fluorescence intensity for Siglec-7, Siglec-8, and Siglec-9 in human mast cell lines **(C).** Flow cytometry data in **A** are representative of 3-5 experiments. Data in **B** (*n* = 3-5) and **C** (*n* = 3-5) are shown as mean + SEM with circles showing values from individual experiments. **P*< 0.05, *****P* < 0.0001.

Supplementary Figure E2. *SIGLEC7, SIGLEC8*, and *SIGLEC9* mRNA expression in human mast cells. Messenger RNA expression levels for the indicated transcripts are expressed as ΔCt values normalized against *GAPDH* as the reference transcript. Data are shown as mean + SEM of the average for duplicate specimens. Circles in **A** (*n* = 3) show values from individual experiments with human mast cell lines. Circles in **B** (*n* = 4) and **C** (*n* = 5) show values from individual experiments with cells generated from individual donors.

Supplementary Figure E3. Siglec-9 localization in LAD2 cells. (A-B) Representative confocal microscopy images show intracellular and cell surface staining for Siglec-9 in naïve **(A)** and IgE-dependent activated **(B)** LAD2 cells (green fluorescence in right panel). Nuclei were counterstained with DAPI (blue fluorescence). Negative control in **A** was performed with isotype control and secondary antibodies only (left panel). For LAD2 cell IgE-dependent activation, cells were sensitized with IgE (2 μg/ml) overnight and then were challenged with anti-human IgE (500 ng/ml) for 1 h. Scale bar equals 10μm. Confocal images are representative of 2 experiments.

Supplementary Figure E4. Siglec-9 internalization in LAD2 cells following antibody ligation. (A and **D)** Representative flow cytometry analysis of surface **(A)** and total **(D)** Siglec-9 expression in LAD2 cells treated with 5 μg/ml of either isotype control (Iso) or anti-Siglec-9 antibody (S9) for the indicated time points. Mean fluorescence intensity **(B** and **E)** and percentage of signal lost (**C** and **F**) at indicated points for surface (**B** and **C**) and total (**E** and **F**) Siglec-9 expression in LAD2 cells treated with 5 μg/ml of either APC-conjugated isotype control or APC-conjugated anti-Siglec-9 antibody (Anti-Sig9) for the indicated time points. **(G)** Mean fluorescence intensity at indicated time points for total Siglec-9 expression in LAD2 cells treated with 5 μg/ml isotype control. The percentage of signal lost was calculated as MFI for Siglec-9 at indicated point minus MFI for Siglec-9 at time 0. Flow cytometry data in **A** and **D** are representative of 3 experiments. Data in **B** (*n* = 5-6), **C** (*n* = 5-6), **E** (*n* = 6-7) **F** (*n* = 6-7) and **G** (*n* = 4) are shown as mean + SEM with circles showing values from individual experiments with LAD2 cells. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Supplementary Figure E5. Siglec-9 co-localizes with markers of the endosomal pathway in LAD2 cells after Siglec-9 engagement. Representative confocal microscopy images show intracellular and cell surface staining for Siglec-9, and intracellular staining for Rab5 and Rab7 in LAD2 cells treated with 5 μg/ml of either isotype control (Iso) or anti-Siglec-9 antibody (S9) for the indicated time points. Nuclei were counterstained with DAPI (blue fluorescence). Siglec-9 (green

fluorescence) co-localization with Rab 5 and Rab7 (red fluorescence) is indicated with white arrowheads. Scale bar equals 10μm. Confocal images are representative of 2 experiments.

Supplementary Figure E6. Expression of Siglec-9 ligands in LAD2 cells. (A-B) Representative flow cytometry analysis of Siglec-9 ligand expression (**A**) and percentage (**B**) of unedited and *SIGLEC9*-edited LAD2 cells expressing Siglec-9 ligands. Unedited LAD2 cells were maintained in medium alone or treated with sialidases (10 mU/ml) for 1 h. Flow cytometry data in **A** is representative of 3 experiments. Data in **B** (n = 3) are shown as mean + SEM with circles showing values from individual experiments with LAD2 cells. *P < 0.05, **P < 0.01, ***P < 0.001, ****P <0.0001.

Supplementary Figure E7. Expression of sialyltransferases and sialic acid biosynthesis enzymes in human mast cells. (A-B) Expression of sialyltransferases and sialic acid biosynthesis enzymes in human mast cells **(A)** and in unedited and *SIGLEC9*-edited LAD2 cells **(B).** Messenger RNA expression levels for the indicated transcripts are expressed as ΔCt values normalized against *GAPDH* as the reference transcript. Data are shown as mean + SEM of the average for duplicate specimens. Green triangles (n = 4-5) and red squares (n = 3-5) show values from individual experiments with cells generated from individual donors.

Supplementary Figure E8. Siglec-9 ligands inhibit degranulation in LAD2 cells and PBCMCs. Inhibition of β-hexosaminidase release in LAD2 cells **(A-D)** and reduction in LAMP-1 expression in PBCMCs **(E-H)** treated with either glycophorin A (GlycA) (25-100 μg/ml) or high molecular weight hyaluronic acid (HMW-HA) (20-100 μg/ml) for 20 min before stimulation. For LAD2 cell IgE-dependent activation, cells were sensitized with IgE (2 μg/ml) overnight and then were challenged with anti-human IgE (500 ng/ml) for 1 h. For PBCMC IgE-dependent activation, cells were treated with anti-FcεRIα antibodies (100 ng/ml) for 20 min. For IgE-independent mast cell activation, LAD2 cells and PBCMCs were stimulated with compound 48/80 (c48/80) (5 μg/ml) for 1 h and 20 min, respectively. Circles in **A-D** (n = 3-4) show values from individual experiments with LAD2 cells. Triangles in **E-H** (n = 4-6) show values from individual experiments with cells generated from individual donors.

Supplementary Figure E9. Siglec-9 ligands inhibit LAD2 cell degranulation by specifically engaging Siglec-9. (A) Mean fluorescence intensity for Glycophorin A (GlycA) in LAD2 treated with either isotype control or mouse anti-Siglec-9 (5 μg/ml) prior to incubation with GlycA (50 μg/ml) for 30 min. Data are shown as mean + SEM with circles showing values from individual experiments with LAD2 cells. **(B)** β-hexosaminidase release in *SIGLEC9*-edited LAD2 cells treated with either glycophorin A (GlycA) (25 μg/ml) or high molecular weight hyaluronic acid (HMW-HA) (50 μg/ml) for 20 min before stimulation. *SIGLEC9*-edited LAD2 cells were sensitized with IgE (2 μg/ml) overnight and then were challenged with anti-human IgE (500 ng/ml) for 1 h. Circles in **A** (n =5) and **B** (n = 4-8) are shown as mean + SEM with circles showing values from individual experiments. *P <0.05, **P <0.01, ****P <0.0001.

Supplementary Fig. E10. Siglec-9 expression upon engagement with Siglec-9 ligands. Mean fluorescence intensity at indicated time points for surface Siglec-9 expression in LAD2 cells

incubated in media alone or treated with either GlycA (50 μg/ml) or HMW-HA (50 μg/ml). Data are shown as mean + SEM with circles showing values from individual experiments (*n* = 4).

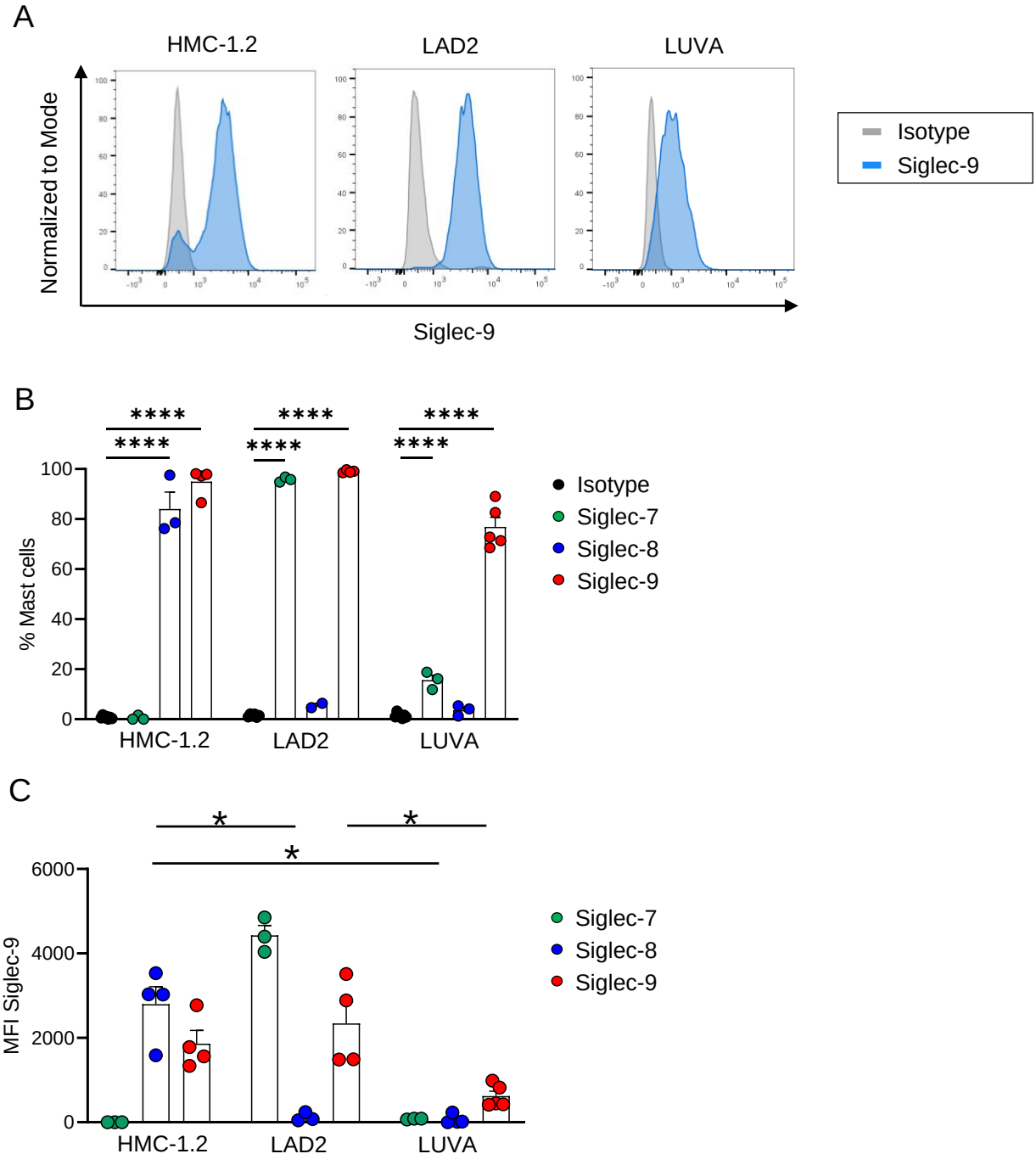
Supplementary Figure E11. Siglec-9 engagement with an anti-Siglec-9 antibody slightly inhibits LAD2 cell but not human primary mast cell degranulation. (A-B) β-hexosaminidase release in LAD2 cells sensitized with IgE (2 μg/ml) overnight and then challenged with either anti-human IgE (500 ng/ml) **(A)** or compound 48/80 (c48/80) (1 μg/ml) **(B)** for 1 h. **(C-E)** LAMP-1 expression in PBCMCs (**C** and **E**) and HSCMCs **(D)** treated with anti-FcεRIα antibodies (100 ng/ml) for 20 min. For Siglec-9 engagement, cells were pre-incubated with either isotype control or mouse anti-Siglec-9 (5 μg/ml) prior to activation. In **E**, PBCMCs incubated with anti-Siglec-9 or isotype control were exposed to 5 μg/ml goat anti-mouse IgG (Fc specific) F(ab')2 fragment antibody (5 μg/ml) for 2 min to cross-link Siglec-9 prior to activation. Data are shown as mean + SEM. Circles in **A** (*n* = 7-9) and **B** (*n* = 4) show values from individual experiments with LAD2 cells. Triangles in **C** (*n* = 7) and **E** (*n* = 3) and squares in **D** (*n* = 2) show values from individual experiments with cells generated from individual donors. **P* < 0.05, *****P* <0.0001.

Supplementary Figure E12. Siglec-9 engagement with an anti-Siglec-9 antibody does not impact mast cell survival. Percentage of PI and annexin-V negative PBCMCs at the indicated time points after treatment with either isotype control or mouse anti-Siglec-9 (5 μg/ml). Data (*n* = 4) are shown as mean + SEM. Triangles show values from individual experiments with cells generated from individual donors.

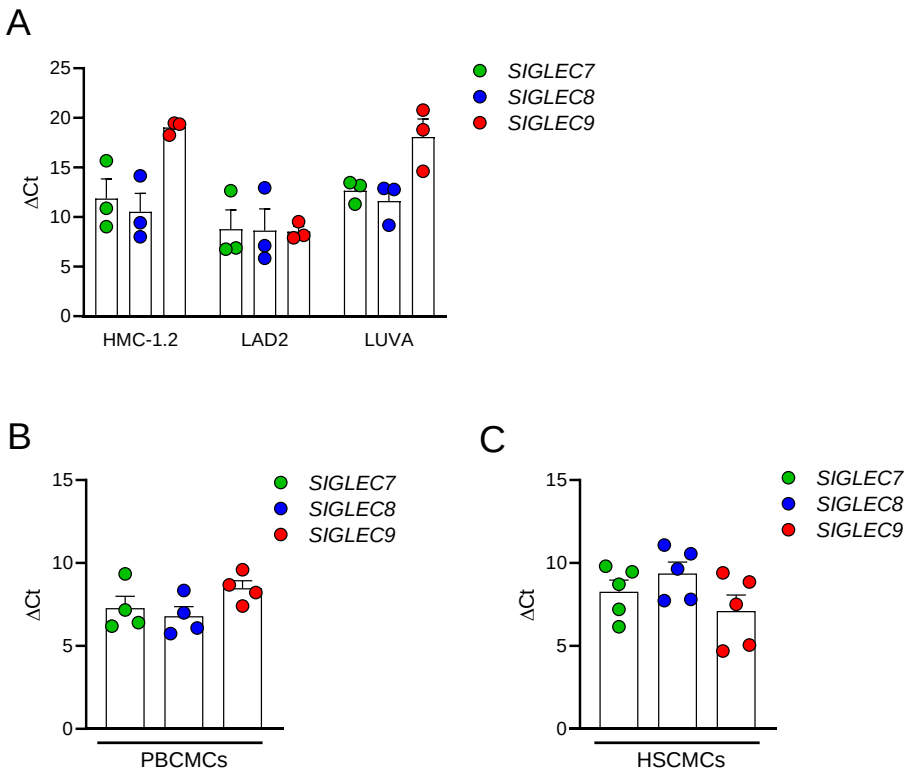
Supplementary Figure E13. Co-engagement of FcεRI and Siglec-9 inhibits production of arachidonic acid metabolites and IL-8 release in PBCMCs and HSCMCs. Cys-LT **(A** and **D**), PGD_2 **(B** and **E)** and IL-8 production (**C** and **F**) in PBCMCs (**A-C**) and HSCMCs (**D-F**) maintained in medium alone or incubated with either isotype control or mouse anti-Siglec-9 (5 μg/ml) and stimulated with anti-human FcεRIα (100 ng/ml) and a goat anti-mouse IgG (Fc specific) F(ab')2 fragment antibody (5 μg/ml) to cross-link FcεRIα and Siglec-9. Triangles in **A** (*n* = 4), **B** (*n* = 7) and **C** (*n* = 5), and squares in **D** (*n* = 4), **E** (*n* = 4) and **F** (*n* = 4), show values from individual experiments with cells generated from individual donors.

Supplementary Figure E14. Mouse mast cells express little or no Siglec-E. (A) Percentage of bone marrow derived-cultured mast cells (BMCMCs), fetal skin derived-cultured mast cells (FSCMCs) and peritoneal mast cells (PMCs) expressing Siglec-E. **(B)** Comparison of *Siglece* expression measured by RNAseq in blood neutrophils and mast cells from different tissues from ImmGen database (http://www.immgen.org/databrowser/). Data in **A** (*n* = 4-10) are shown as mean + SEM with circles showing values from individual experiments.

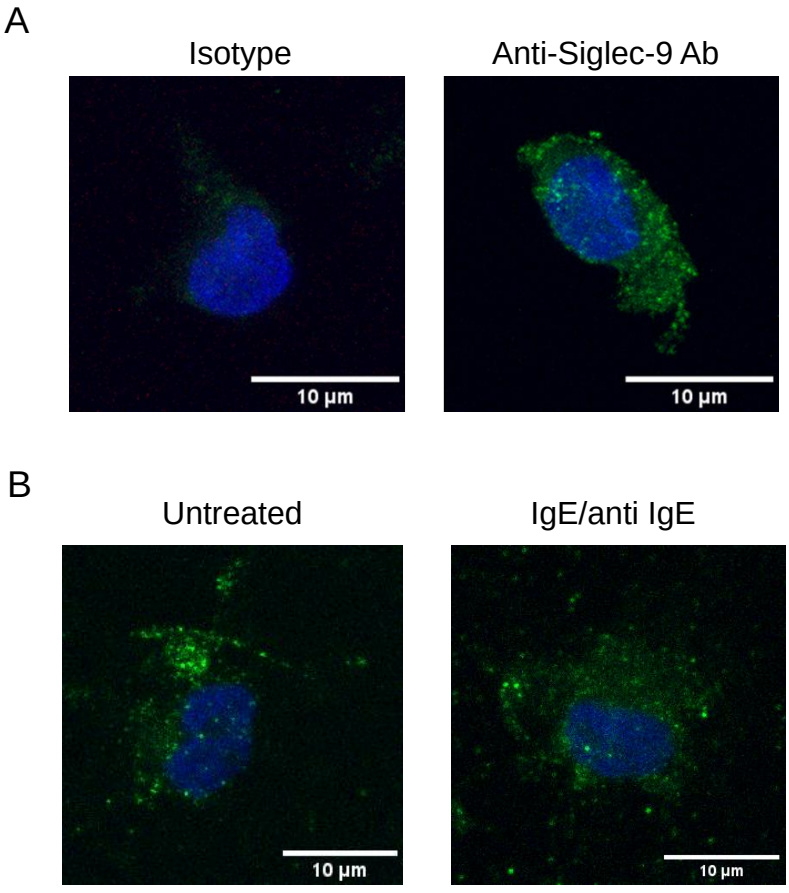
Supplemental Figure E1



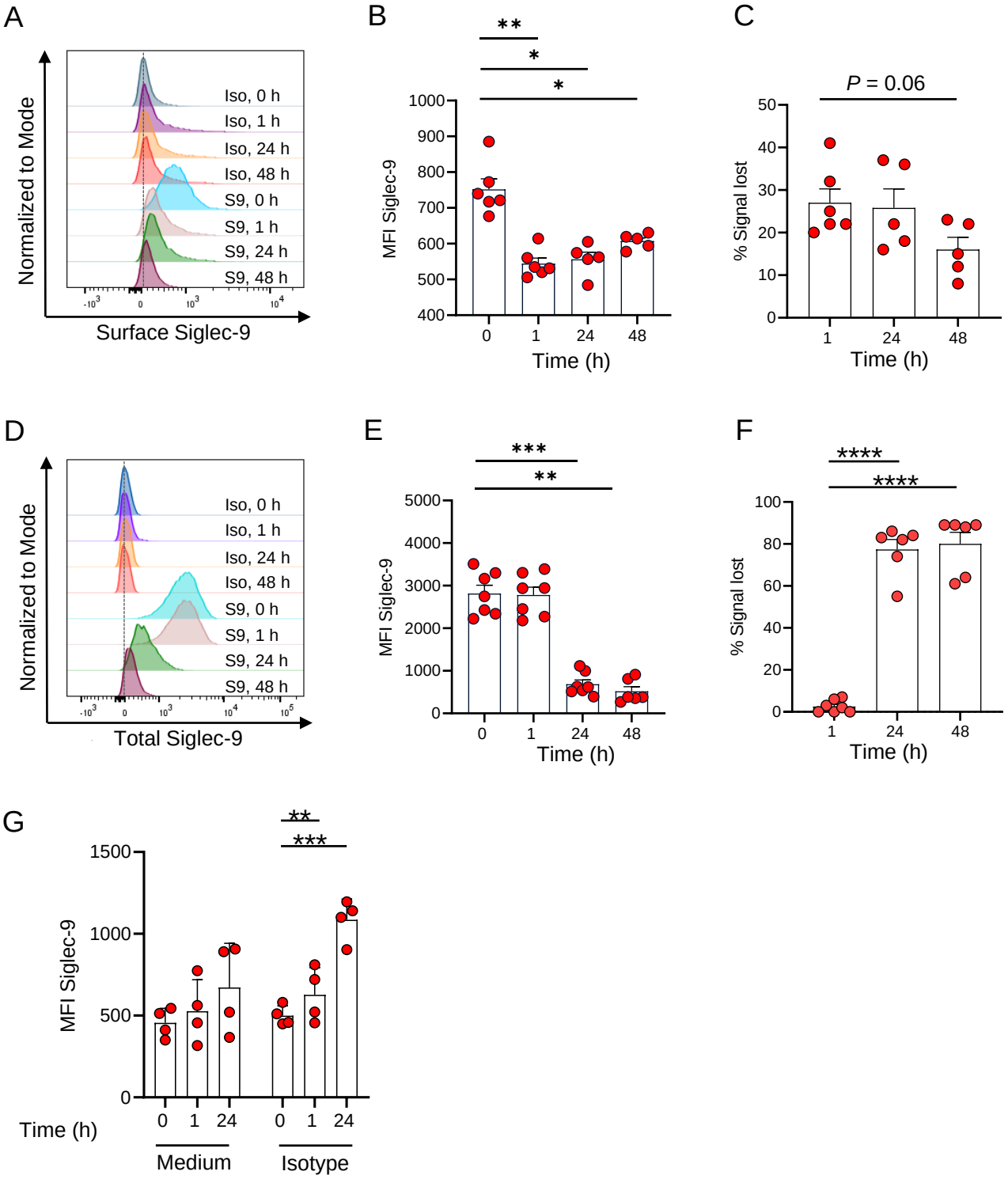
Supplemental Figure E2



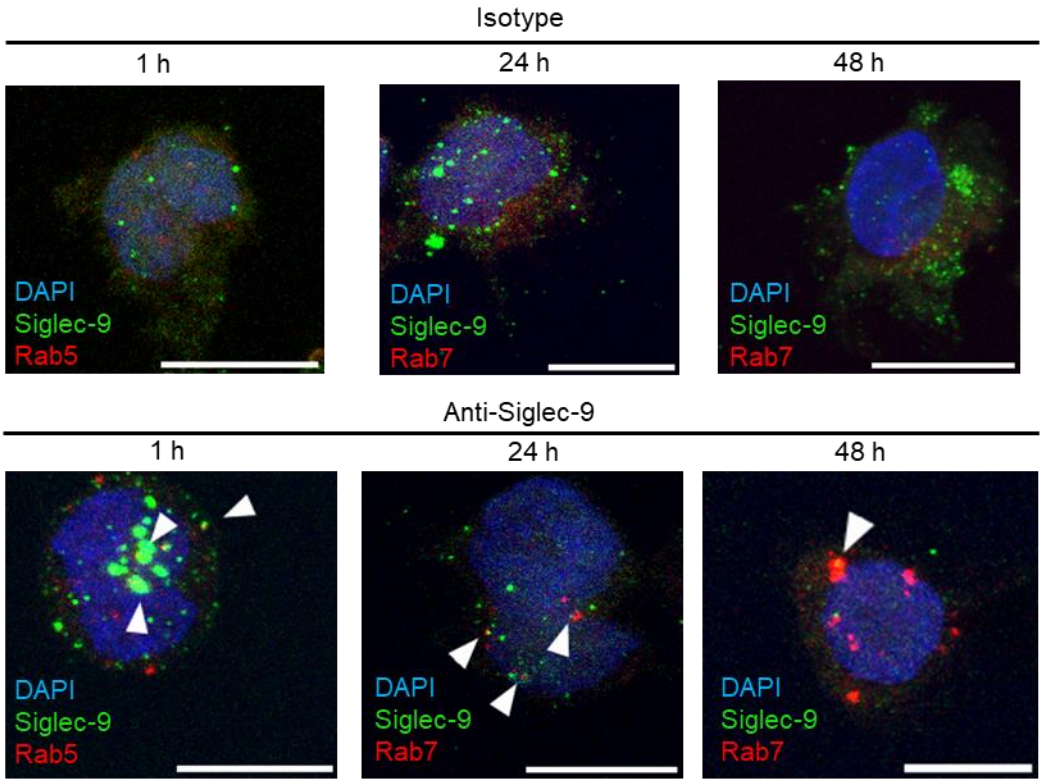
Supplemental Figure E3



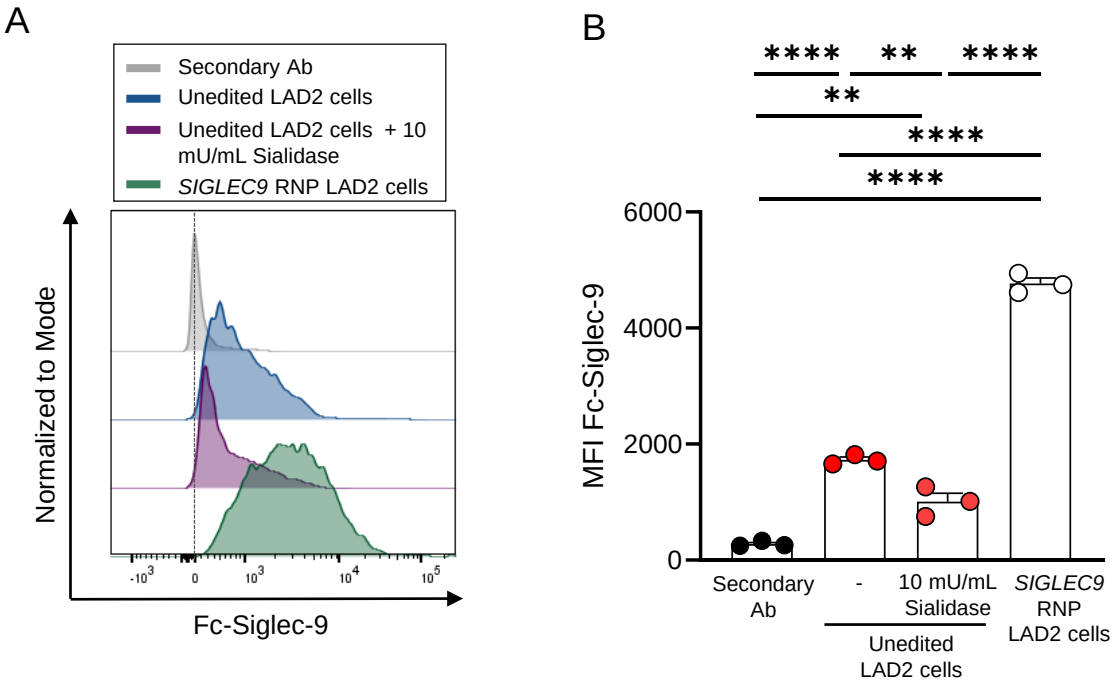
Supplemental Figure E4



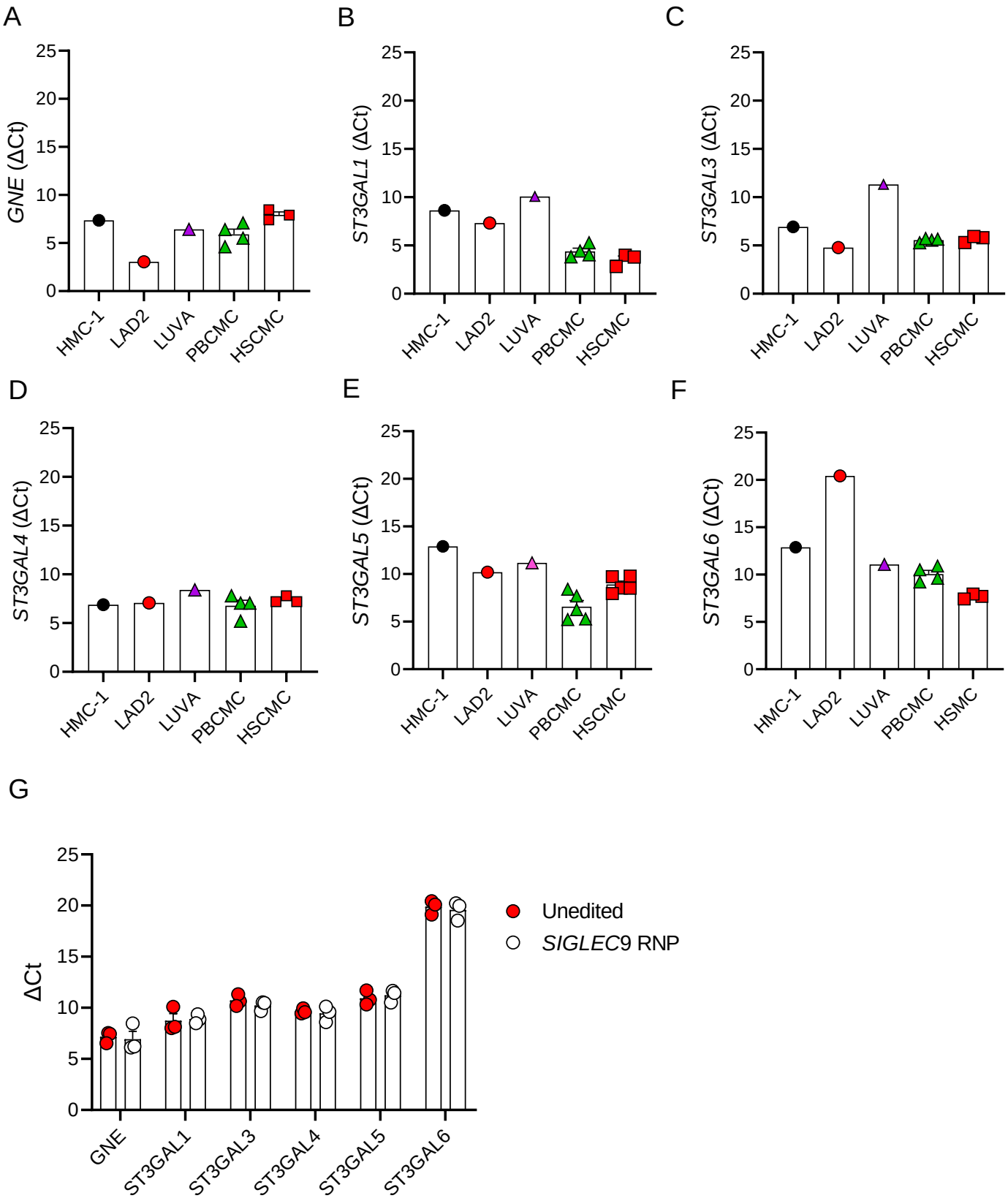
Supplemental Figure E5



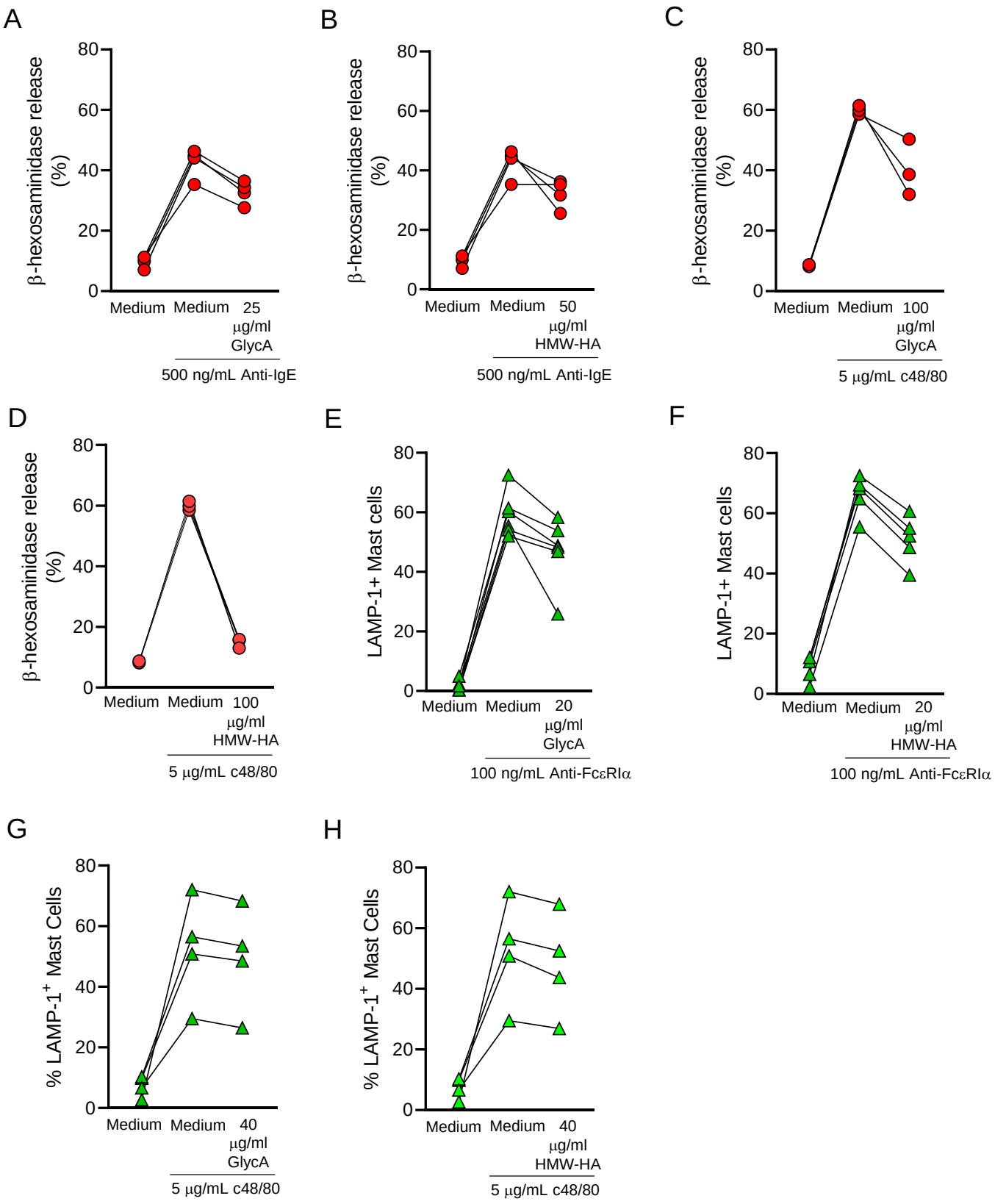
Supplemental Figure E6



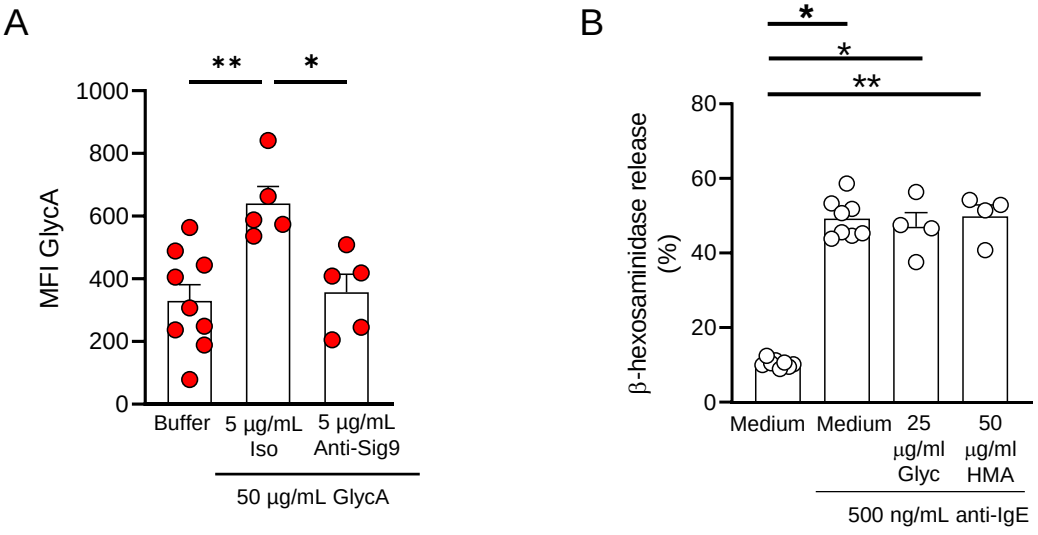
Supplemental Figure E7



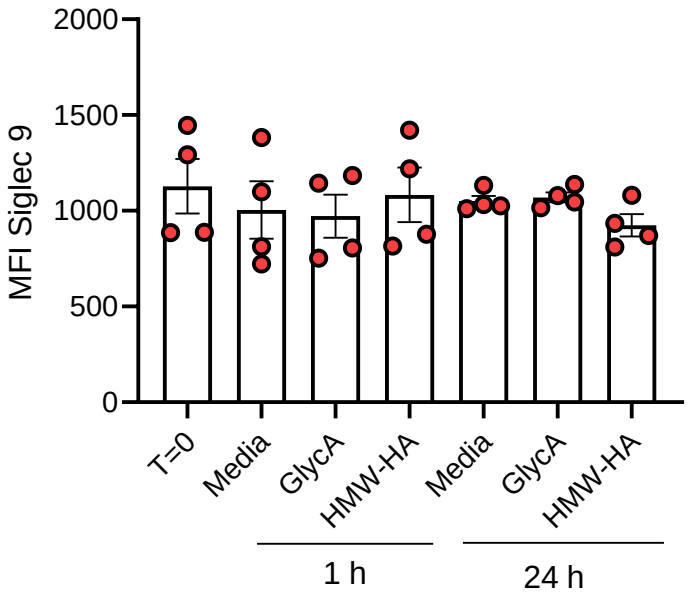
Supplemental Figure E8



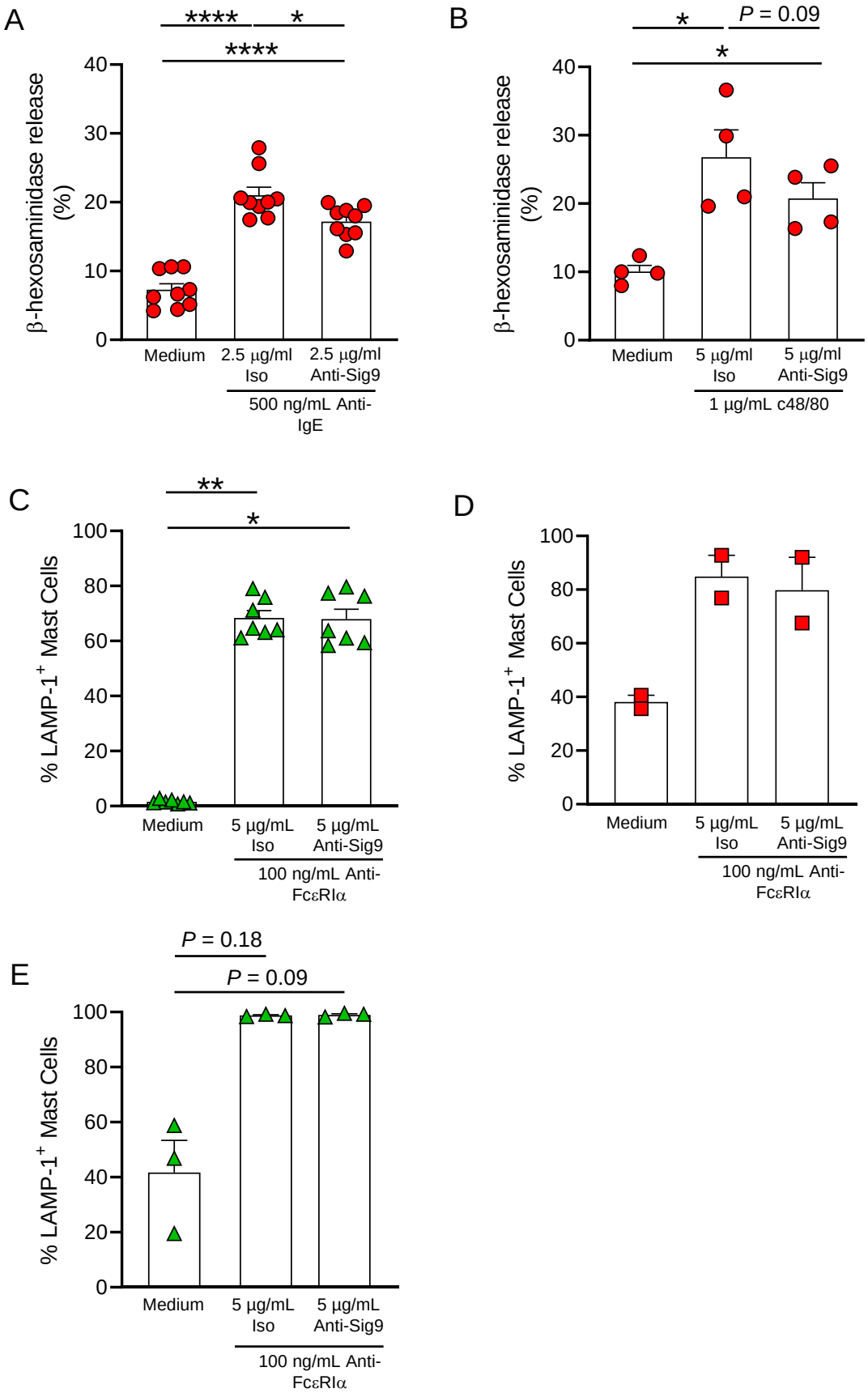
Supplemental Figure E9



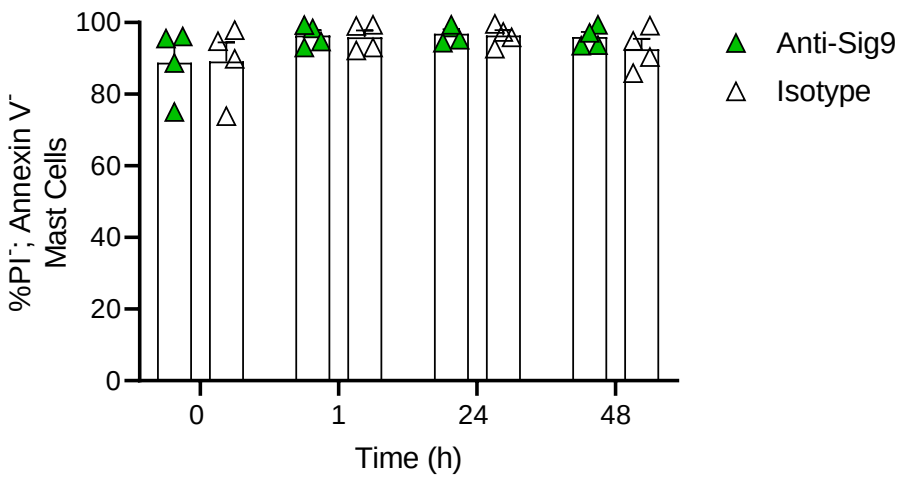
Supplemental Figure E10



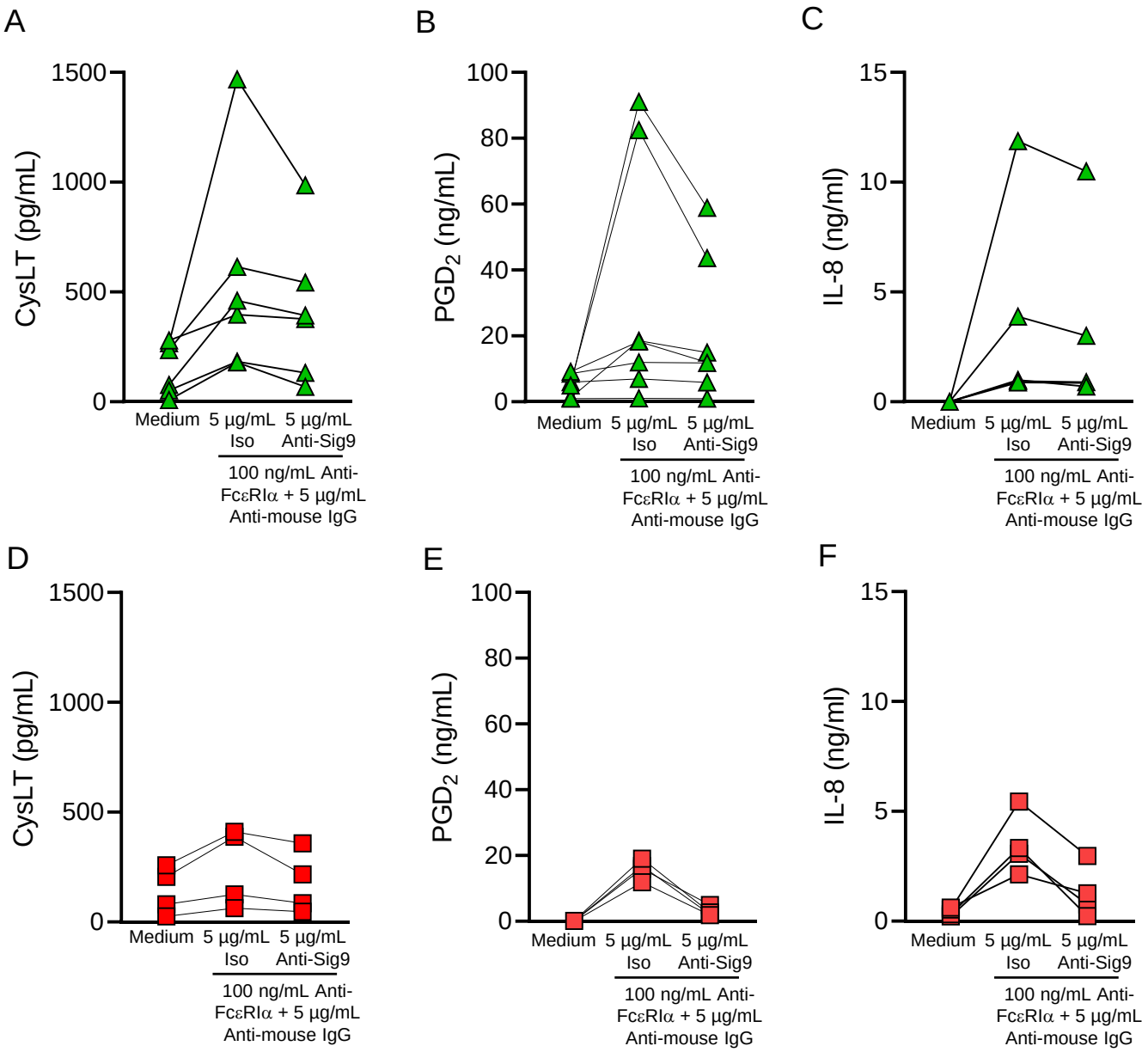
Supplemental Figure E11



Supplemental Figure E12



Supplemental Figure E13



Supplemental Figure E14