1.1 Title Page for Supplemental Data

Treatment of allergic asthma with Fenretinide formulation (LAU-7b) downregulates *Ormdl3* expression and normalizes ceramides imbalance

Mina Youssef[1,2], Juan B. De Sanctis[3], Juhi Shah[2,4], Daciana Catalina Dumut[2,5], Marian Hajduch[3], Anna K. Naumova[1,6], and Danuta Radzioch[1,2,3,5*]

Affiliations:

1 Department of Human Genetics, McGill University, Montreal, Quebec, Canada

2 Program in Infectious Diseases and Immunity in Global Health, McGill University Health Center, Montreal, Quebec, Canada

3 Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

4 Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

5 Division of Experimental Medicine, Department of Medicine, McGill University, Montreal, Quebec, Canada

6 Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada

1.2 Supplemental Data

1.2.1 Materials and Methods

1.2.1.1 Sensitization, Challenge, and Harvest Protocol

At the age of eight weeks, male and female KO and WT mice were sensitized by intraperitoneal (i.p.) injection of HDM allergen on a weekly basis for three weeks. 5µg of HDM protein extract (*Dermatophagoides Pteronyssinus*, Cat: XPB82D3A2.5, Greer lab., Lenoir, NC, USA) adsorbed to 1.5 mg of aluminum hydroxide adjuvant (Imject Alum, Pierce, Rockford, IL, USA) in a total volume of 200 µL sterile PBS was used for sensitization. One week following the third systemic sensitization with HDM, three daily successive challenges were achieved by intranasal (i.n.) exposures to 15 µL of 1 µg/µL HDM protein extract prepared in PBS. The animals were harvested 48 hours after the third challenge.

1.2.1.2 LAU-7b Treatment Protocol

The novel clinical oral formulation of Fenretinide, (LAU-7b), was developed by Laurent Pharmaceutical Inc., Montréal, Quebec, Canada. An oral dose of 10 mg/kg LAU-7b was tested in HDM sensitized and challenged mice. Allergen sensitized mice were treated with LAU-7b orally for 9 days by daily gavage starting from the day of the third sensitization until the day prior to harvest. 0.5% methyl cellulose in milliQ purified water was used as a drug vehicle to re-disperse the active ingredient (FEN) in the LAU-7b capsule plus the necessary excipients.

1.2.1.3 Primers Used for Gene Expression Measurements

Zpbp2 forward primer 5'-GGT TTG CTT CGC TCT GTA-3', and *Zpbp2* reverse primer 5'-CAA GCA TCA CGG CTC AG-3'. *Ormdl3* forward primer 5'-AGG AAG TTC TTA ACC ATC AC-3' and *Ormdl3* reverse primer 5'-AAG GAC ACA GTG TTG AGT AT-3'. *Il-4* forward primer 5'-CGA GCT CAC TCT CTG TGG TG-3' and *Il-4* reverse primer 5'-TGA ACG AGG

TCA CAG GAG AA-3'. *Il-5* forward primer 5'-CTC TGT TGA CAA GCA ATG AGA CG-3' and *Il-5* reverse primer 5'-TCT TCA GTA TGT CTA GCC CCT G-3'. *Il-13* forward primer 5'-CCT GGC TCT TGC TTG CCT T-3' and *Il-13* reverse primer 5'-GGT CTT GTG TGA TGT TGC TCA-3'. *Ccl11* forward primer 5'-CCA GAC ATT CGG CGG TTG-3' and *Ccl11* reverse primer 5'-CAG CAG CAG GCA CAT CAG-3'

1.2.2 Supplemental Figures

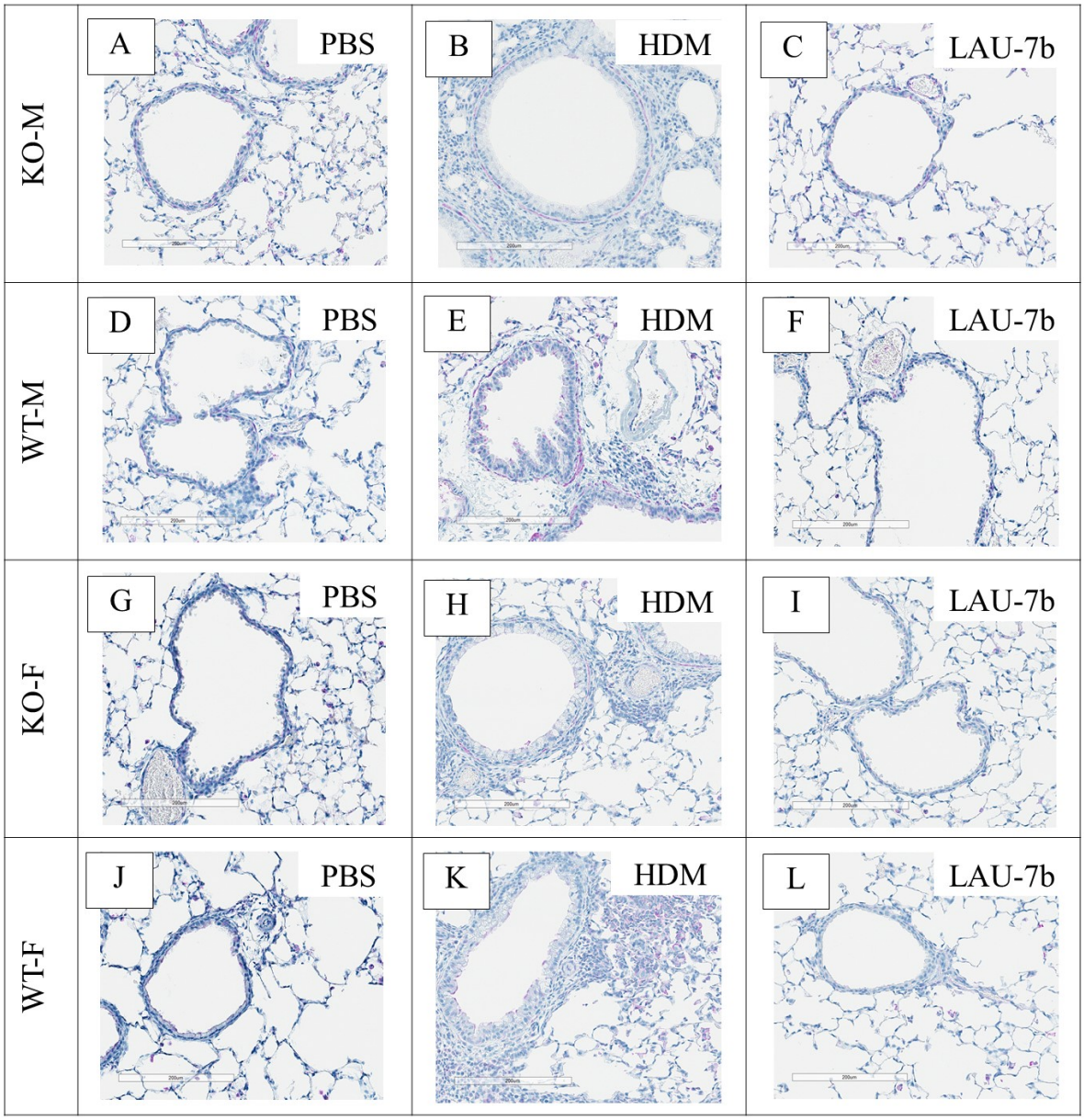

Figure E1 Staining of airway smooth muscle (ASM) mass in untreated and LAU-7b treated mice; α-smooth muscle actin antibody staining

All mice were sensitized with house dust mite (HDM) and challenged with either (PBS) or (HDM). Treatment group is marked as (LAU-7b). Panel A-C) *Zpbp2* KO males, D-F) WT males, G-I) *Zpbp2* KO females, and J-L) WT females.

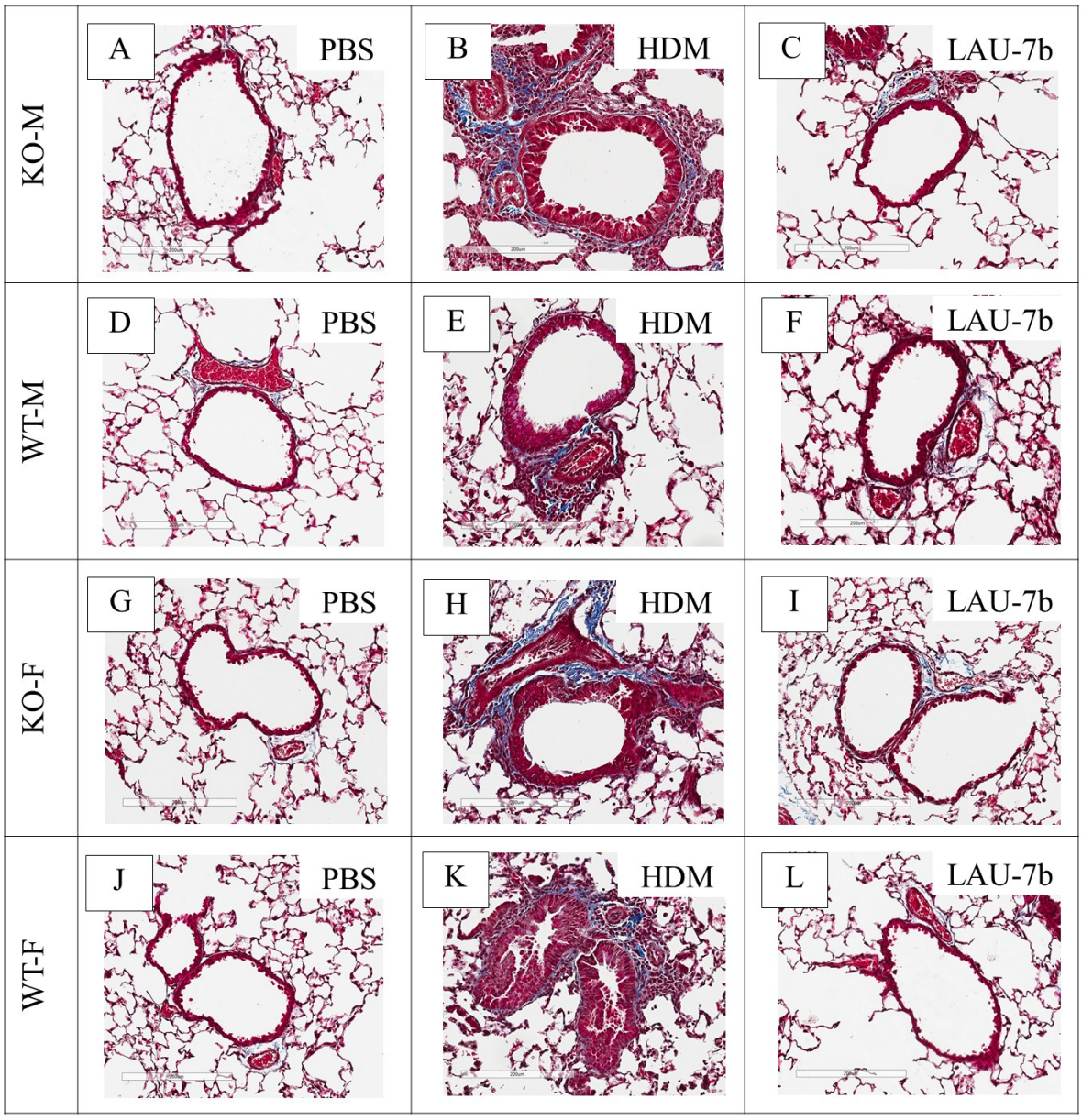

Figure E2 Assessment of collagen production in untreated and LAU-7b treated mice; Masson trichome staining

All mice were sensitized with house dust mite (HDM) and challenged with either (PBS) or (HDM). Treatment group is marked as (LAU-7b). Panel A-C) *Zpbp2* KO males, D-F) WT males, G-I) *Zpbp2* KO females, and J-L) WT females.

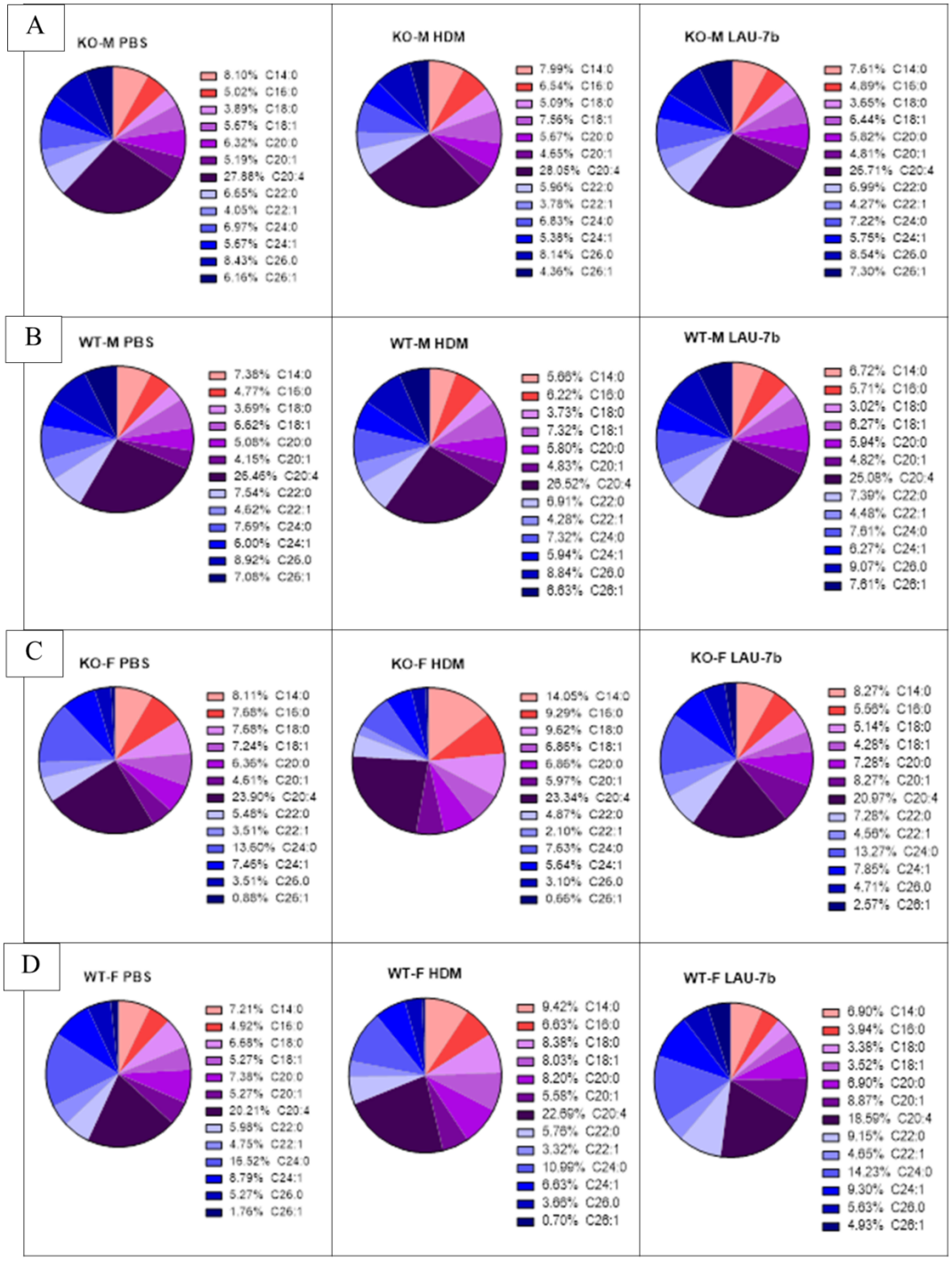
A
KO-M PBS
8.10% C14:0
5.02% C16:0
3.89% C18:0
5.67% C18:1
6.32% C20:0
5.19% C20:1
27.88% C20:4
6.65% C22:0
4.05% C22:1
6.97% C24:0
5.67% C24:1
8.43% C26:0
6.16% C26:1
KO-M HDM
7.99% C14:0
6.54% C16:0
5.09% C18:0
7.56% C18:1
5.67% C20:0
4.65% C20:1
28.05% C20:4
5.96% C22:0
3.78% C22:1
6.83% C24:0
5.38% C24:1
8.14% C26:0
4.36% C26:1
KO-M LAU-7b
7.61% C14:0
4.89% C16:0
3.65% C18:0
6.44% C18:1
5.82% C20:0
4.81% C20:1
26.71% C20:4
6.99% C22:0
4.27% C22:1
7.22% C24:0
5.75% C24:1
8.54% C26:0
7.30% C26:1
B
WT-M PBS
7.38% C14:0
4.77% C16:0
3.69% C18:0
6.62% C18:1
5.08% C20:0
4.15% C20:1
25.46% C20:4
7.54% C22:0
4.62% C22:1
7.69% C24:0
6.00% C24:1
8.92% C26:0
7.08% C26:1
WT-M HDM
5.66% C14:0
6.22% C16:0
3.73% C18:0
7.32% C18:1
5.80% C20:0
4.83% C20:1
26.52% C20:4
6.91% C22:0
4.28% C22:1
7.32% C24:0
5.94% C24:1
8.84% C26:0
6.63% C26:1
WT-M LAU-7b
6.72% C14:0
5.71% C16:0
3.02% C18:0
6.27% C18:1
5.94% C20:0
4.82% C20:1
25.08% C20:4
7.39% C22:0
4.48% C22:1
7.61% C24:0
6.27% C24:1
9.07% C26:0
7.81% C26:1
C
KO-F PBS
8.11% C14:0
7.68% C16:0
7.68% C18:0
7.24% C18:1
6.36% C20:0
4.61% C20:1
23.90% C20:4
5.48% C22:0
3.51% C22:1
13.60% C24:0
7.46% C24:1
3.51% C26:0
0.88% C26:1
KO-F HDM
14.05% C14:0
9.29% C16:0
9.62% C18:0
6.86% C18:1
6.86% C20:0
5.97% C20:1
23.34% C20:4
4.87% C22:0
2.10% C22:1
7.63% C24:0
5.64% C24:1
3.10% C26:0
0.66% C26:1
KO-F LAU-7b
8.27% C14:0
5.56% C16:0
5.14% C18:0
4.26% C18:1
7.28% C20:0
8.27% C20:1
20.97% C20:4
7.28% C22:0
4.56% C22:1
13.27% C24:0
7.85% C24:1
4.71% C26:0
2.57% C26:1
D
WT-F PBS
7.21% C14:0
4.92% C16:0
6.68% C18:0
5.27% C18:1
7.38% C20:0
5.27% C20:1
20.21% C20:4
5.98% C22:0
4.75% C22:1
16.52% C24:0
8.79% C24:1
5.27% C26:0
1.76% C26:1
WT-F HDM
9.42% C14:0
6.63% C16:0
8.38% C18:0
8.03% C18:1
8.20% C20:0
5.58% C20:1
22.69% C20:4
5.78% C22:0
3.32% C22:1
10.99% C24:0
6.63% C24:1
3.66% C26:0
0.70% C26:1
WT-F LAU-7b
6.90% C14:0
3.94% C16:0
3.38% C18:0
3.52% C18:1
6.90% C20:0
8.87% C20:1
18.59% C20:4
9.15% C22:0
4.65% C22:1
14.23% C24:0
9.30% C24:1
5.63% C26:0
4.93% C26:1

Figure E3 Pie charts of ceramides levels in *Zpbp2* KO and WT, male and female, mice

Analysis of (long chain ceramides) LCCs and (very long chain ceramides) VLCCs species in A) *Zpbp2* KO male, B) WT male, C) *Zpbp2* KO female, and D) WT female mice. All mice were sensitized with house dust mite (HDM) and challenged with either (PBS) or (HDM). Treatment group is marked as (LAU-7b). Relative levels of VLCCs were diminished in HDM-challenged KO male and female mice compared to PBS-challenged KO mice. LAU-7b treated KO mice displayed higher levels of VLCCs and reduced levels of LCCs compared to untreated KO mice in both males and females. Compared to KO mice, the assessment of VLCCs demonstrated that WT male and female mice have higher levels of VLCCs for both PBS- and HDM-challenged groups. As observed in KO mice, LAU-7b treatment restored the levels of VLCCs to those typically observed in WT male and female mice after HDM challenge.