

1 **Supplementary Fig 1. Time of HDM challenge effects AHR to methacholine,**
2 **measured as airway resistance (area under the curve, AUC, rather than**
3 **maximum resistance as in Fig 2) in wild type (WT) mice.**

4 **a.** Airway resistance to increasing doses of methacholine was measured by area under
5 the curve, AUC, in mice challenged with either HDM or PBS at ZT11 or ZT23. There
6 was a significant time of day difference in airway resistance in mice challenged with
7 HDM ($P = 0.005$), mixed linear modelling ($n = 8-9$, per treatment group). Mice
8 challenged with HDM at ZT11 showed increased airway resistance compared to those
9 challenged at ZT23.

10 **b.** HDM specific IgE was measured in serum from WT mice. There were significantly
11 increased levels of HDM specific IgE in WT mice treated with intranasal HDM
12 compared to PBS treated control mice ($* P < 0.05$ at ZT23 and $*** P < 0.001$ at ZT11).
13 There were no time of challenge differences in control or HDM treated groups (Mean
14 \pm SEM, ($n = 5-8$, per treatment group) Mann Whitney U).

15

16 **Supplementary Fig 2. REV-ERB α acts as a repressor of AHR**

17 **a.** Effect of time of HDM challenge on AHR in Rev-erb α ^{-/-} mice. Airway resistance
18 measured as area under the curve, AUC, rather than as maximum airway resistance
19 as in Fig 4. There was a significant increase in airway resistance (AUC) after HDM
20 challenge at ZT11 ($*** P < 0.001$) and ZT23 ($*** P < 0.001$), compared to control, PBS
21 challenged mice. There was no time of challenge difference in airway resistance
22 (AUC) after PBS challenge or after HDM challenge, mixed linear modelling, ($n = 7-9$ per
23 treatment group).

1 **b.** Maximum airway resistance (cmH₂O.s/ml) was measured in PBS challenged WT
2 and Rev-erb α ^{-/-} mice to increasing doses of nebulised methacholine. All
3 measurements of maximum airway resistance were increased in the Rev-erb α ^{-/-} mice
4 compared to WT mice.

5 **c.** HDM specific IgE was measured in serum from Rev-erb α ^{-/-} mice. HDM specific
6 serum IgE was significantly increased following HDM challenge, compared to control
7 mice (**P < 0.01 at ZT23 and ** P < 0.01 at ZT11). There were no time of challenge
8 differences in control or HDM treated groups (Mean \pm SEM (n=4-7 per treatment
9 group), Mann Whitney U).

10

11 **Supplementary Fig. 3. Genotype differences in airway and lung inflammatory**
12 **cells.**

13 a. BAL inflammatory cell counts measured as a percentage of the total cell count, were
14 analysed in WT and Rev-erb α ^{-/-} mice to determine genotype differences. There were
15 no significant differences between the groups. Mean \pm SEM (n= 8-12 per treatment
16 group), 1 way ANOVA, followed by Tukey multiple comparison test.

17

18 **Supplementary Fig 4. Expression of beta adrenoceptors in murine lung.**

19 **a.** Quantitative PCR for myosin light chain kinase 1 (*mlck1*) in mouse lung. There were
20 no time of day or genotype differences in expression between groups.

21 **b.** Quantitative PCR for smooth muscle myosin (*sm-mhc*) in mouse lung. There were
22 no time of day or genotype differences in expression between groups.

1 c. Quantitative PCR for smooth muscle actin (*acta*) in mouse lung. There were no time
2 of day or genotype differences in expression between groups.

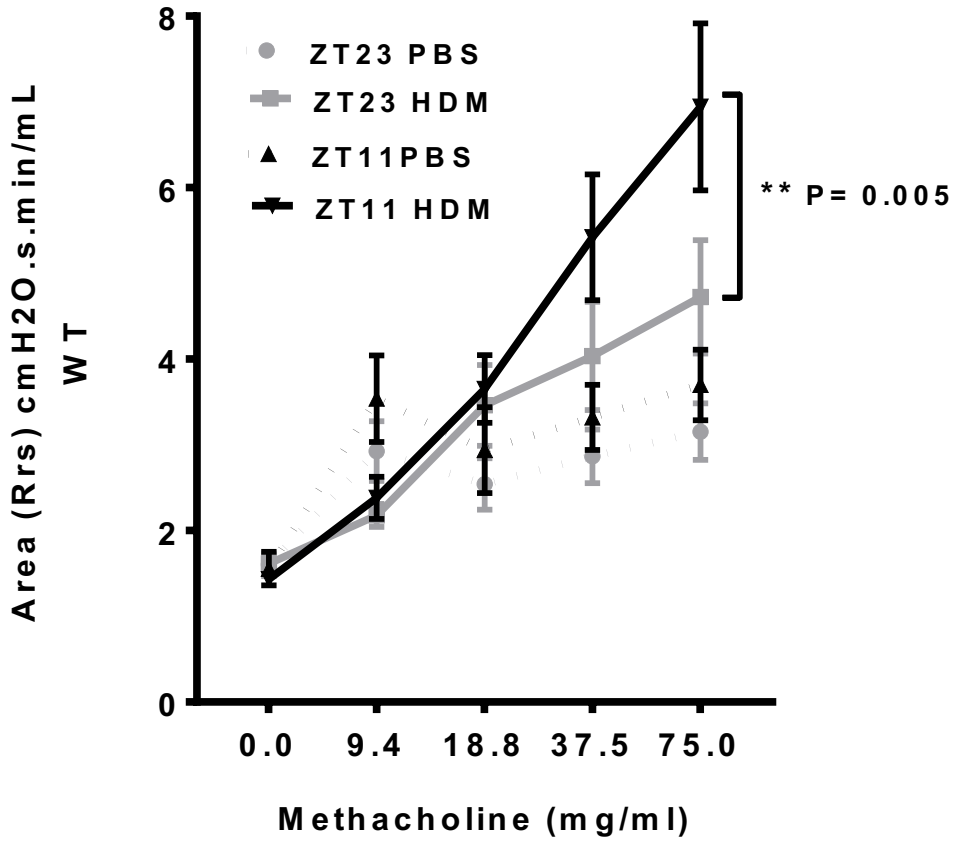
3 **d.** Quantitative PCR for Adrenoceptor Beta 1 (*Adrb1*) in mouse lung tissue. There were
4 no time of day or genotype differences in expression of *Adrb1*.

5 **e.** Quantitative PCR for Adrenocpetor Beta 2 (*Adrb2*) in mouse lung tissue. There was
6 no time of day or genotype differences in expression of *Adrb2*. All data presented as
7 mean \pm SEM (n=5-9 per treatment group, in duplicate) and analysed by 1 way ANOVA,
8 followed by Tukey's multiple comparison test.

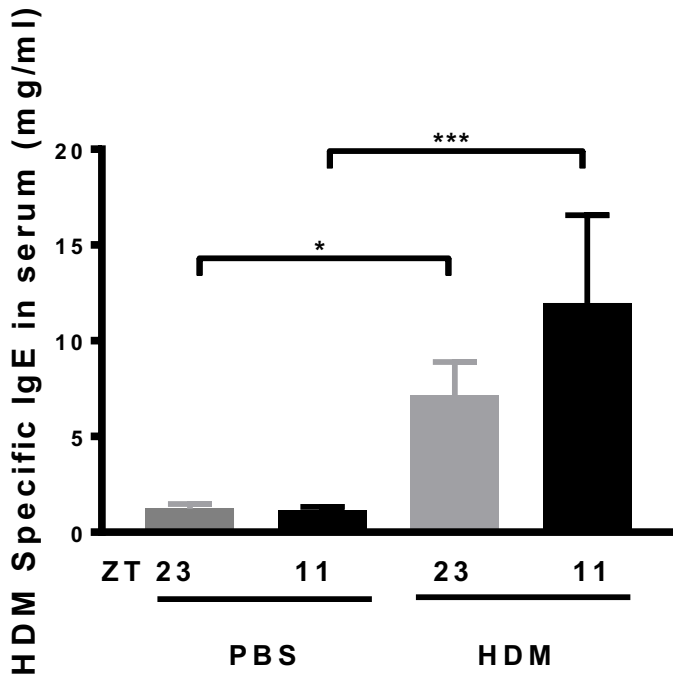
9 All QPCR data is compared to expression of the housekeeping gene *Gapdh* in WT
10 PBS challenged mice at ZT23. Black bars indicate challenge at ZT11 and grey bars
11 indicate challenge at ZT23.

Supplementary Fig 1.

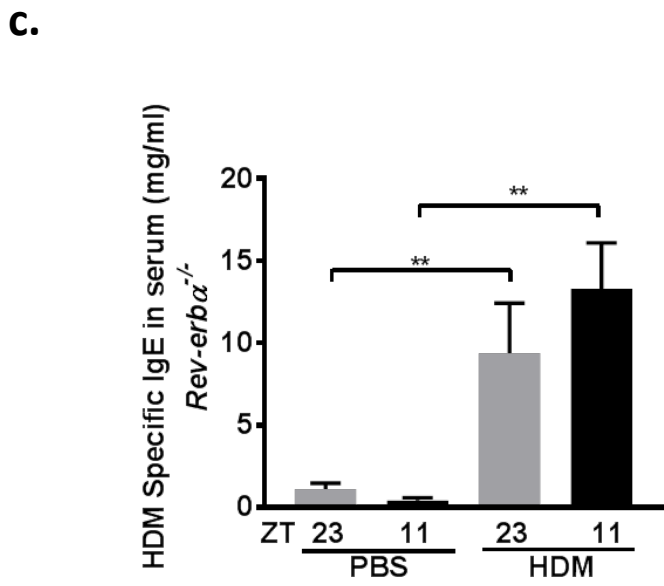
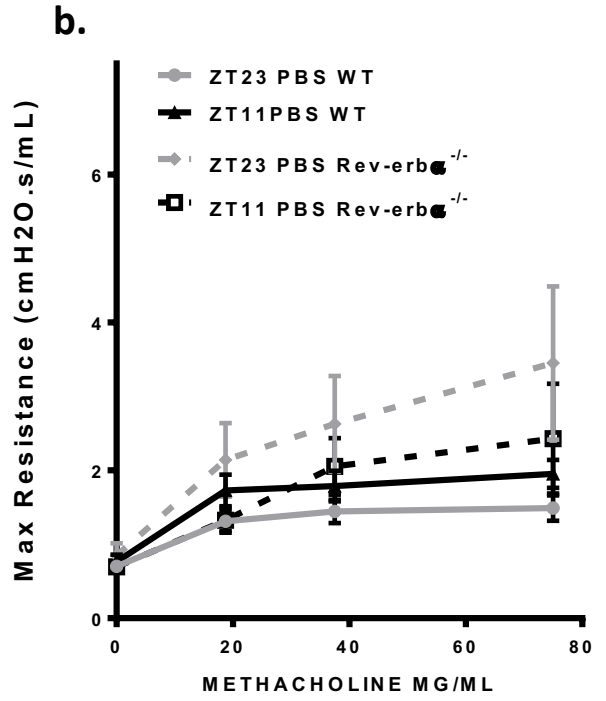
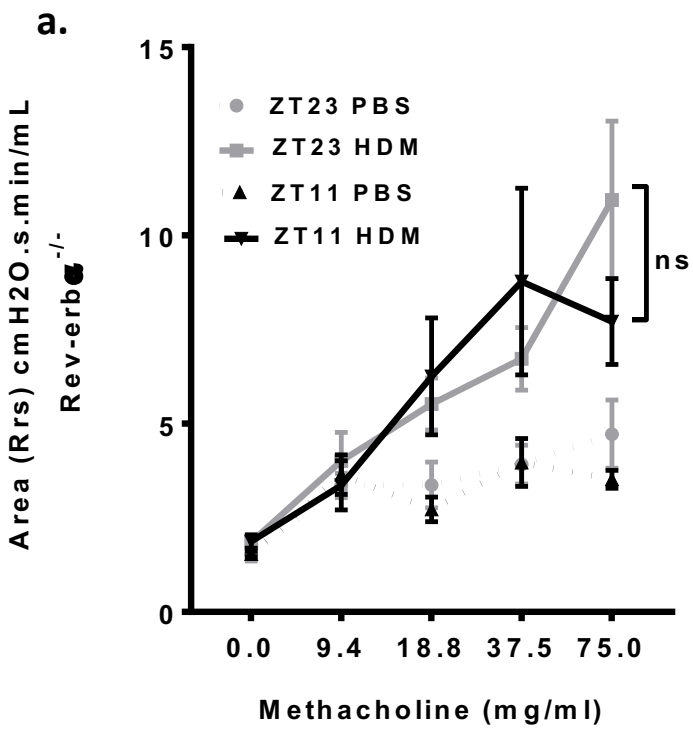
a.



b.

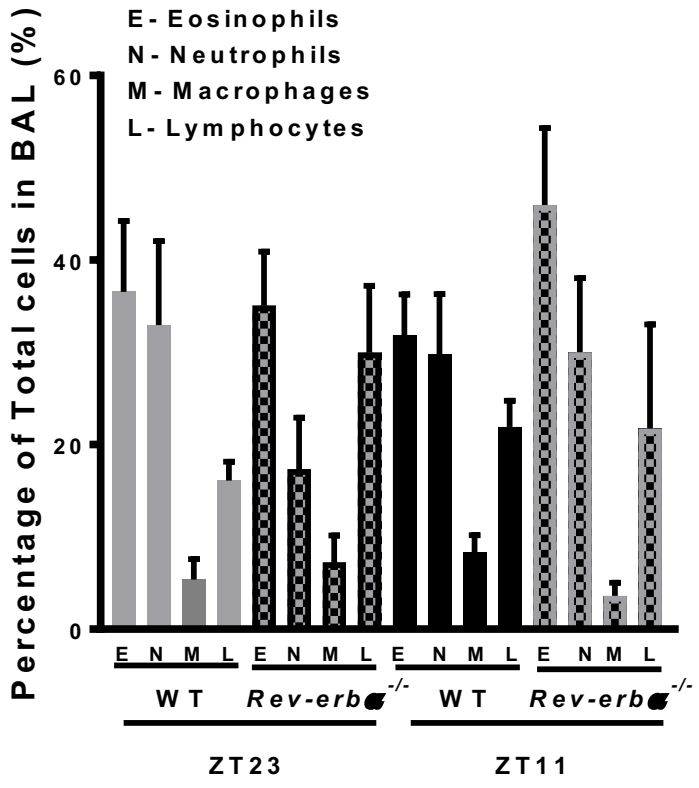


Supplementary Fig. 2



Supplementary Fig. 3

a.



Supplementary Fig. 4

