Novel IL-16/miR-125a axis controls neutrophil recruitment in pristane-induced lung inflammation.

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Supplementary Materials

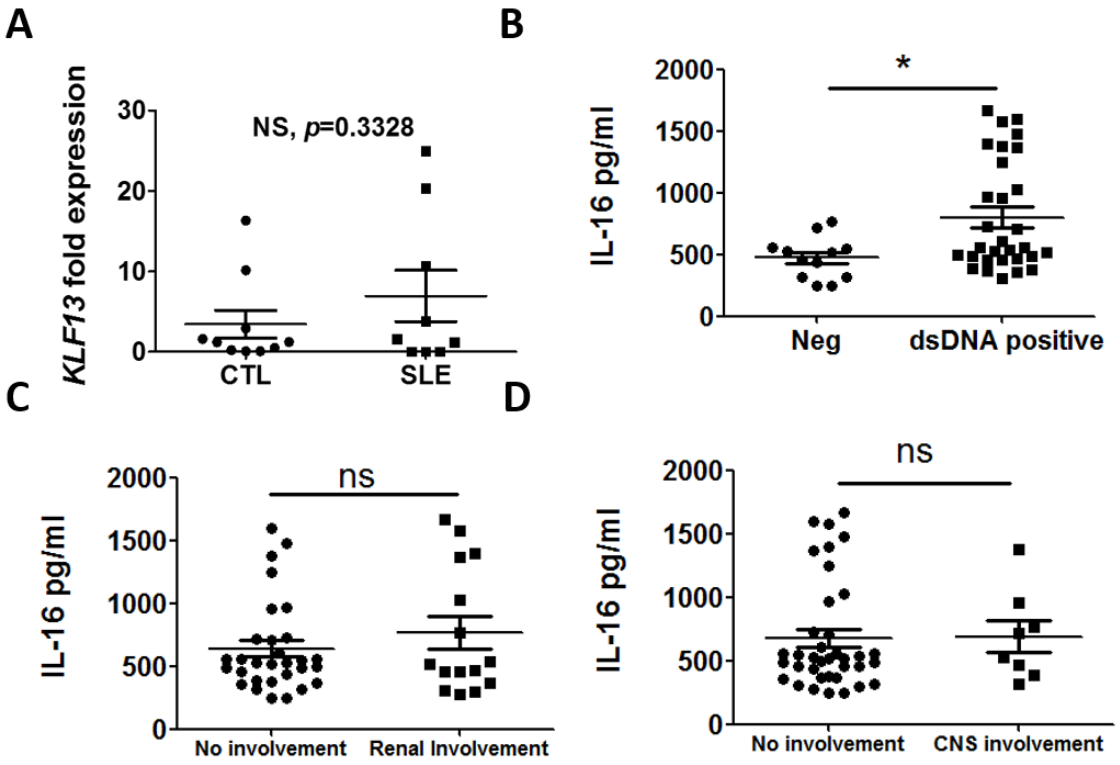
Supplementary Figure 1. miR-125a levels decreased in SLE monocytes compared to controls. Normalized read counts of miRNAs identified in the micro-array analysis were listed in these tables. miRNAs are arranged according to the SLE/CTL Ratio. Ratio: if the Ratio >2 (table 1) or <0.05 (table 2), the difference is recognized as significant.

Table 1 miRNAs up-regulated in SLE compared to HC group

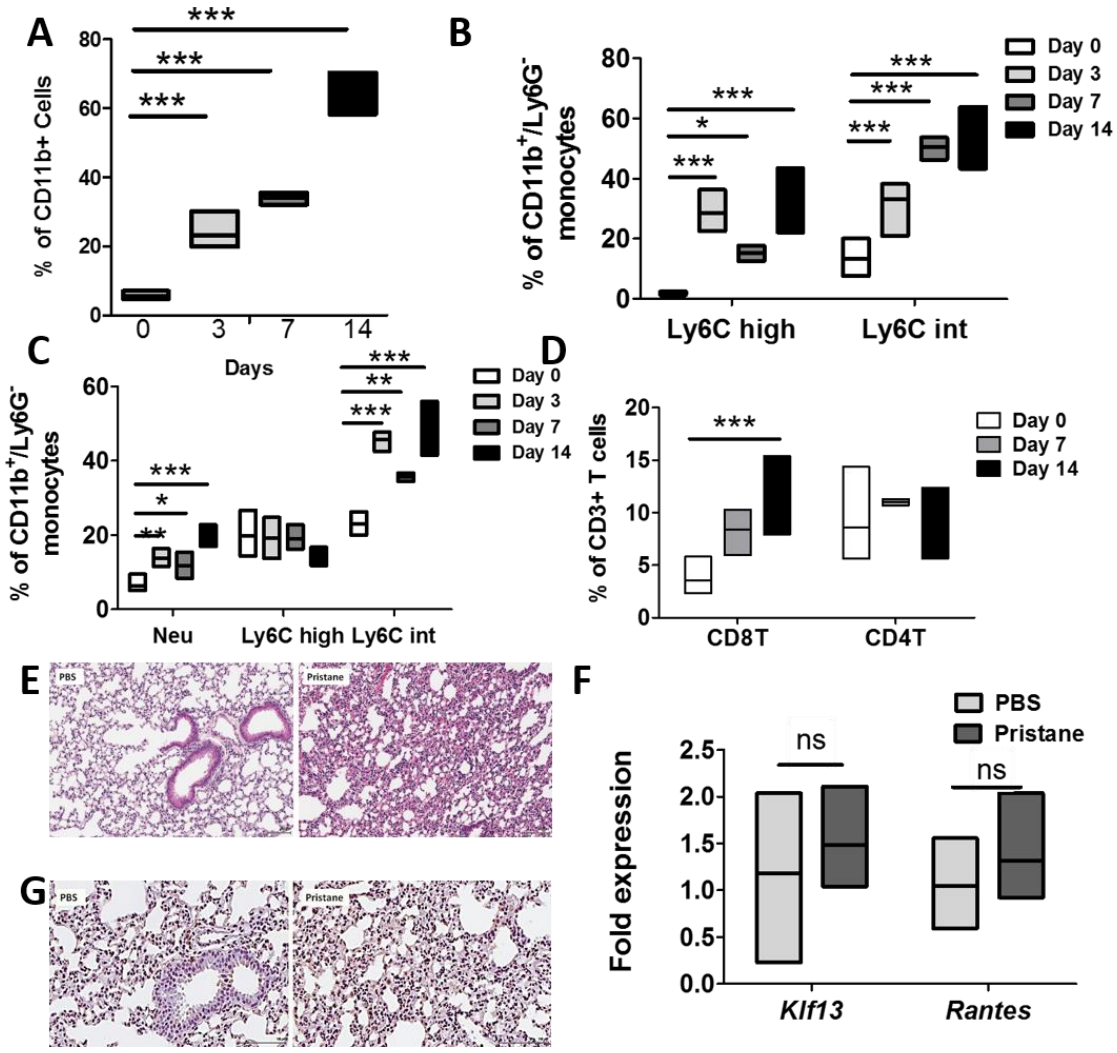
miRNA name	CTL	SLE	SLE/CTL ratio
hsa-miR-126-3p	809	4974	6.146582
hsa-miR-199a/b-3p	588	3223	5.479903
hsa-miR-130a-3p	179	932	5.218122
hsa-miR-27b-3p	67	244	3.634361
hsa-miR-19a-3p	95	279	2.934224
hsa-miR-199a-5p	110	314	2.862245
hsa-miR-221-3p	111	315	2.826032
hsa-miR-107	226	546	2.413349
hsa-miR-16-5p	1864	4327	2.320825
hsa-miR-19b-3p	380	845	2.223453
hsa-miR-148b-3p	654	1373	2.099974
hsa-miR-93-5p	870	1826	2.098561
hsa-let-7d-5p	451	923	2.048987
hsa-miR-23b-3p	100	203	2.035825

Table 2 miRNAs down-regulated in SLE compared to HC group

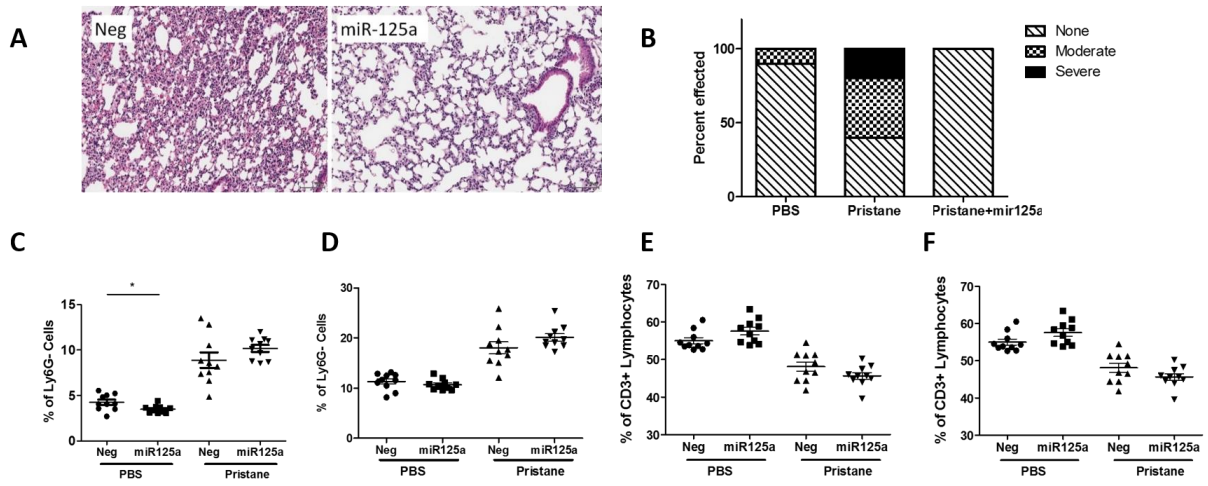
miRNA name	CTL	SLE	SLE/CTL ratio
hsa-miR-720	1604	224	0.139731
hsa-miR-155-5p	2079	352	0.169079
hsa-miR-132-3p	714	121	0.169945
hsa-miR-4516	706	137	0.193655
hsa-miR-4454	4549	1051	0.230912
hsa-miR-125a-5p	976	275	0.282125
hsa-miR-302d-3p	234	71	0.304648
hsa-miR-570-3p	167	57	0.339318
hsa-miR-4443	277	99	0.358941
hsa-miR-761	149	54	0.362423
hsa-miR-548al	195	71	0.362657
hsa-miR-29b-3p	3549	1294	0.364655
hsa-miR-188-5p	223	91	0.407118
hsa-let-7e-5p	285	118	0.414158
hsa-miR-548p	156	66	0.424248
hsa-miR-1183	192	82	0.428005
hsa-miR-99b-5p	292	127	0.434587
hsa-miR-631	149	66	0.442917



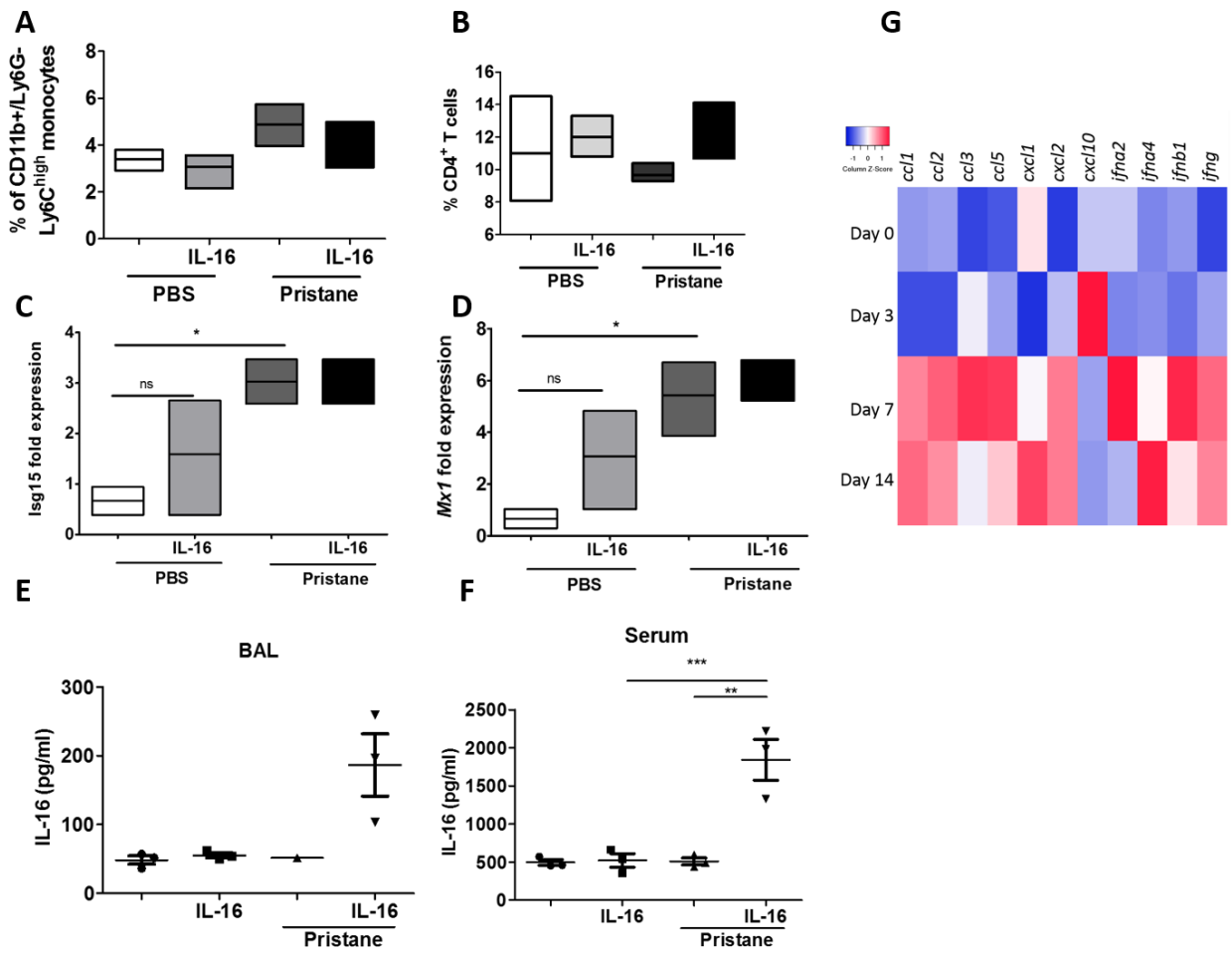
Supplementary Figure 2. *KLF13* levels not altered in SLE patient monocytes and IL-16 serum levels associate with dsDNA positivity in SLE patients. **A,** *KLF13* expression was determined by qPCR in SLE and healthy control monocytes, ns=non significant. **B,** Serum levels of IL-16 in SLE patients either negative (N=12) or positive (N=30) for the presence of dsDNA antibodies as determined by ELISA, ***=** $P \leq 0.05$. **C&D,** Serum levels of IL-16 in SLE patients with or without (C) renal (N=13 and 29, respectively) or (D) CNS (N=26 or 8, respectively) involvement as determined by ELISA, ns=non significant.



Supplementary Figure 3. Increased cellular recruitment into the lungs and peritoneal cavity of pristane-treated mice. **A-B,** Flow cytometry analysis of cells in the peritoneal lavage of pristane-treated B6 mice. B6 mice were either left untreated or were injected with 0.5ml of pristane intraperitoneally and peritoneally lavaged after 3, 7 or 14 days. **A,** Flow cytometry analysis of neutrophils in the peritoneal lavage of pristane-treated B6 mice at the different timepoints are shown, ***= $P \leq 0.001$. **B,** Flow cytometry analysis of monocytes in the peritoneal lavage at the different timepoints are shown, *= $P \leq 0.05$, ***= $P \leq 0.001$. **C,** Flow cytometry analysis of neutrophils and monocytes in the lungs of pristane-treated B6 mice at the different timepoints are shown, *= $P \leq 0.05$, **= $P \leq 0.05$ ***= $P \leq 0.001$. **D,** Flow cytometry analysis of $CD8^+$ and $CD4^+$ T cells in the peritoneal lavage at the different timepoints are shown, ***= $P \leq 0.001$. **E,** Histological lung sections obtained from PBS and pristane-treated B6 mice stained with haematoxylin and eosin (H and E). Representative images from each group are shown (10x magnification). **F,** Expression of *Klf13* and *Rantes* were analysed in the lungs of 14d pristane-treated B6 mice as above. **G,** Histological lung sections obtained from PBS and pristane-treated B6 mice stained for IL-16 and counterstained with eosin. Representative images from each group are shown (20x magnification).



Supplementary Figure 4. Effects of miR-125a on pristane-induced lung pathology and recruitment of cell populations to the lung. **A-F,** Mice received a single intraperitoneal (IP) injection (10nmol) of miR-125a mimic or negative control mimic (n=10) for 1 day before IP injection of 500ml of pristane followed by sacrifice at 10 days for analysis. **(A)** A representative image of H&E stained lung sections from pristane-treated mice administered miR-125a mimic or negative control mimic (10x magnification); **(B)** Lungs from PBS, Pristane and Pristane+mir-125a treated mice were scored as shown: No, Moderate or Severe damage. **(C-F)** Flow cytometry analysis of **(C)** $Ly6C^{high}$ monocytes, **(D)** $Ly6C^{Int}$ monocytes, **(E)** CD4 T cells and **(F)** CD3- B cells in lungs of different treatment groups are shown. *****= $P \leq 0.05$.



Supplementary Figure 5. A-F, rmIL-16 (1mg) was administered to B6 mice 1 day after pristane challenge followed by a single injection every 4 days. Flow cytometry analysis of **(A)** Ly6C^{high} monocytes and **(B)** CD4^{+} T cells in lungs of different treatment groups at day 14 are shown. Expression of *Isg15* **(C)** and *Mx1* **(D)** in lung digests of the different treatment groups was measured by qPCR, ***= $P \leq 0.05$. **E&F,** IL-16 levels in the (E) BAL and (F) serum of the different treatment groups as measured by ELISA, ****= $P \leq 0.01$. **G,** Relative expression of a panel of chemokines and IFNs in lung digests following pristane administration over a 14 day period as measured by qPCR (n=5-7 mice per group).