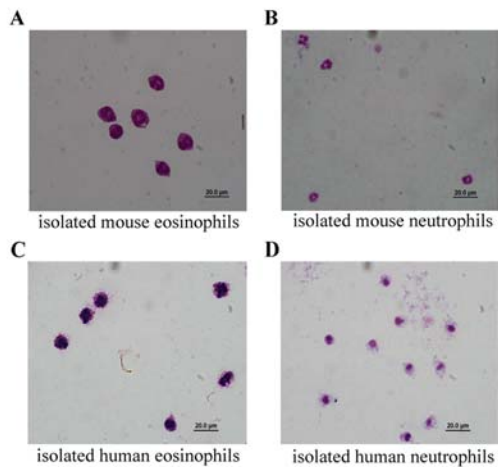


# Slit2 regulates attractive eosinophil and repulsive neutrophil chemotaxis through differential srGAP1 expression during lung inflammation

Bu-Qing Ye, Zhen H. Geng, Li Ma and Jian-Guo Geng

## Supplementary materials

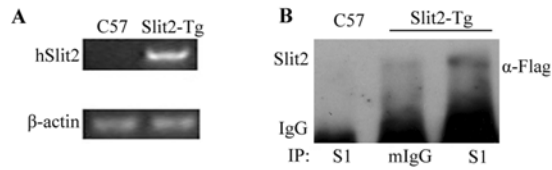
### Figure S1



### Figure S1 Wright's staining of isolated mouse and human leukocytes

Wright's staining of isolated mouse eosinophils (A) and neutrophils (B) or isolated human eosinophils (C) and human neutrophils (D). Scale bar, 20 µm. Data represent at least three independent experiments.

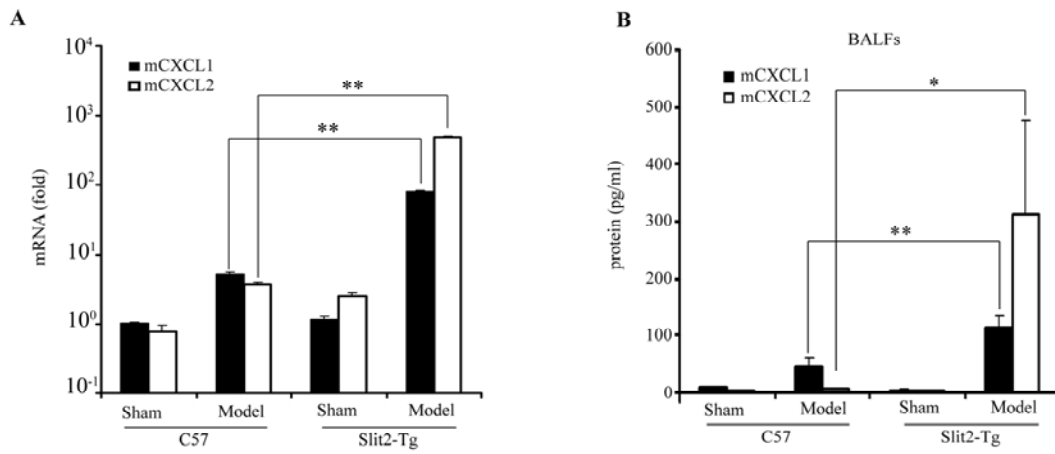
## Figure S2



### Figure S2 Generation of *Slit2*-Tg mice

(A) RT-PCR analysis of exogenous Flag-Slit2 transgene in C57 and *Slit2*-Tg mice. (B) Immunoprecipitation of the *Slit2* transgene protein from the lung tissue lysates of C57 and *Slit2*-Tg mice using mIgG or S1 (the anti-pan-Slit mAb) followed by immunoblotting with M2 (the anti-Flag mAb from Sigma-Aldrich). Data represent one of three independent experiments.

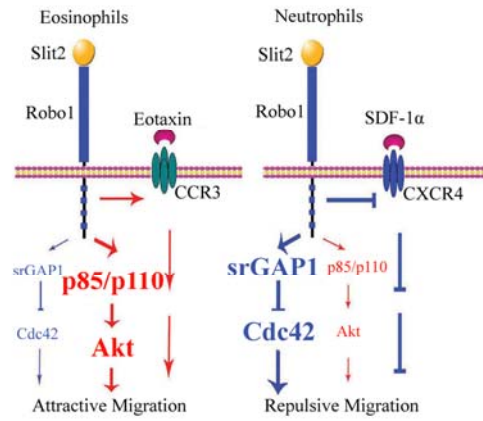
**Figure S3**



**Figure S3** Determinations of chemokines

Measurements of CXCL1 and CXCL2 mRNAs in the lungs (A) and proteins in BALFs (B) of *Slit2*-Tg versus C57 mice without (sham) with OVA sensitization (model). Data represent the mean  $\pm$  S.D. of two independent experiments (n=3-7 for each group). \*,  $p < 0.05$  and \*\*,  $p < 0.01$ .

**Figure S4**



**Figure S4** Schematic illustration of our model for differential regulation of leukocyte chemotaxis by Slit-Robo signaling

## Supplemental Video legends

### **Video 1** Effects of Slit2 on eotaxin-mediated eosinophil migration

Polarization and migration of freshly isolated mouse eosinophils in the presence of the control medium (A), the Slit2 medium (B), eotaxin (C), and eotaxin plus the Slit2 medium (D) were monitored in real-time in  $\mu$ -slides at 37°C. Images were recorded by a multi-dimensional live cell imaging workstation (Leica AS MDW) equipped with a heated enclosure to keep the chamber at 37°C. Data are representative of two or four independent experiments.

### **Video 2** Effects of Slit2 on SDF-1 $\alpha$ -mediated neutrophil migration

Polarization and migration of freshly isolated mouse neutrophils in the presence of the control medium (A), the Slit2 medium (B), SDF-1 $\alpha$  (C), and SDF-1 $\alpha$  plus the Slit2 medium (D) were monitored in  $\mu$ -slides. Data are representative of two or four independent experiments.

### **Video 3.** Effects of Slit2 on migration of human eosinophils or neutrophils

Polarization and migration of freshly isolated human eosinophils in the presence of control medium (A) or the Slit2 medium (B) were monitored in  $\mu$ -slides. Migration of isolated human neutrophils in the presence of control medium (C) or the Slit2 medium (D) were monitored in  $\mu$ -slides. Data are representative of two independent experiments.

### **Video 4.** Effects of R5 and PI3K inhibitors on Slit2-mediated eosinophil activation

Polarization and migration of freshly isolated mouse eosinophils in presence of Slit2 plus

R5 (A), Slit2 plus mIgG (B), Slit2 plus WT (C), Slit2 plus DMSO (D) or Slit2 plus AS (E) were monitored in  $\mu$ -slides. Data are representative of two or three independent experiments.

## Table S1

**Table S1** Real-time migratory features of eosinophils and neutrophils

Cell	Treatment	Accumulated	Euclidean	Velocity $\pm$ SD		Chemotaxis
		distance $\pm$ SD ( $\mu$ M)	distance $\pm$ SD ( $\mu$ M)	( $\mu$ M/sec)	Polarized ratio	ratio
Eosinophils	–	80.86 $\pm$ 27.83	33.67 $\pm$ 16.98	0.04 $\pm$ 0.02	0.23	0.00
Eosinophils	Slit2	94.08 $\pm$ 47.85	40.69 $\pm$ 29.8	0.05 $\pm$ 0.03	0.88	0.18
Eosinophils	eotaxin	45.48 $\pm$ 23.89	16.99 $\pm$ 10.58	0.03 $\pm$ 0.01	0.52	0.45
Eosinophils	eotaxin +Slit2	86.81 $\pm$ 32.03	34.38 $\pm$ 28.97	0.05 $\pm$ 0.02	0.83	0.67
Neutrophils	–	29.94 $\pm$ 15.96	21.88 $\pm$ 11.59	0.02 $\pm$ 0.01	0.00	0.00
Neutrophils	Slit2	28.45 $\pm$ 18.92	17.64 $\pm$ 12.21	0.02 $\pm$ 0.02	0.00	0.27
Neutrophils	SDF-1 $\alpha$	62.06 $\pm$ 30.63	42.5 $\pm$ 30.46	0.05 $\pm$ 0.03	0.05	0.77
Neutrophils	SDF-1 $\alpha$ +Slit2	37.89 $\pm$ 27.64	20.04 $\pm$ 19.32	0.03 $\pm$ 0.02	0.23	0.12
Eosinophils	Slit2+R5	65.16 $\pm$ 27.64	29.67 $\pm$ 16.4	0.04 $\pm$ 0.02	0.23	0.23
Eosinophils	Slit2+mIgG	109.24 $\pm$ 31.8	43.5 $\pm$ 22.71	0.07 $\pm$ 0.02	0.60	0.20
Eosinophils	Slit2+ wortmannin	56.61 $\pm$ 31.17	30.82 $\pm$ 23.37	0.03 $\pm$ 0.02	0.20	0.30
Eosinophils	Slit2+ AS605240	37.79 $\pm$ 31.46	24.69 $\pm$ 25.93	0.03 $\pm$ 0.05	0.17	0.17
Eosinophils	Slit2+DMSO	103.9 $\pm$ 44.24	22.56 $\pm$ 9.66	0.06 $\pm$ 0.02	0.60	0.20

### Supplementary Table Legends

**Table S1** Real-time migratory features of eosinophils and neutrophils

The parameters of accumulated distance, euclidean distance, velocity, polarized ratio and chemotaxis ratio of eosinophils and neutrophils in  $\mu$ -slides were statistically analyzed by software Image J chemotaxisTool plugin.