Loss of HS1 inhibits neutrophil extravasation during inflammation via disturbed PKA signaling

IV. Immunology Summit, Houston, 29.09.2015

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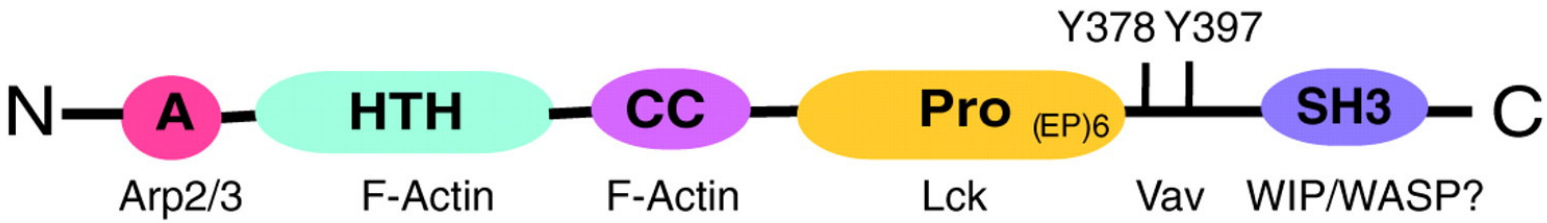
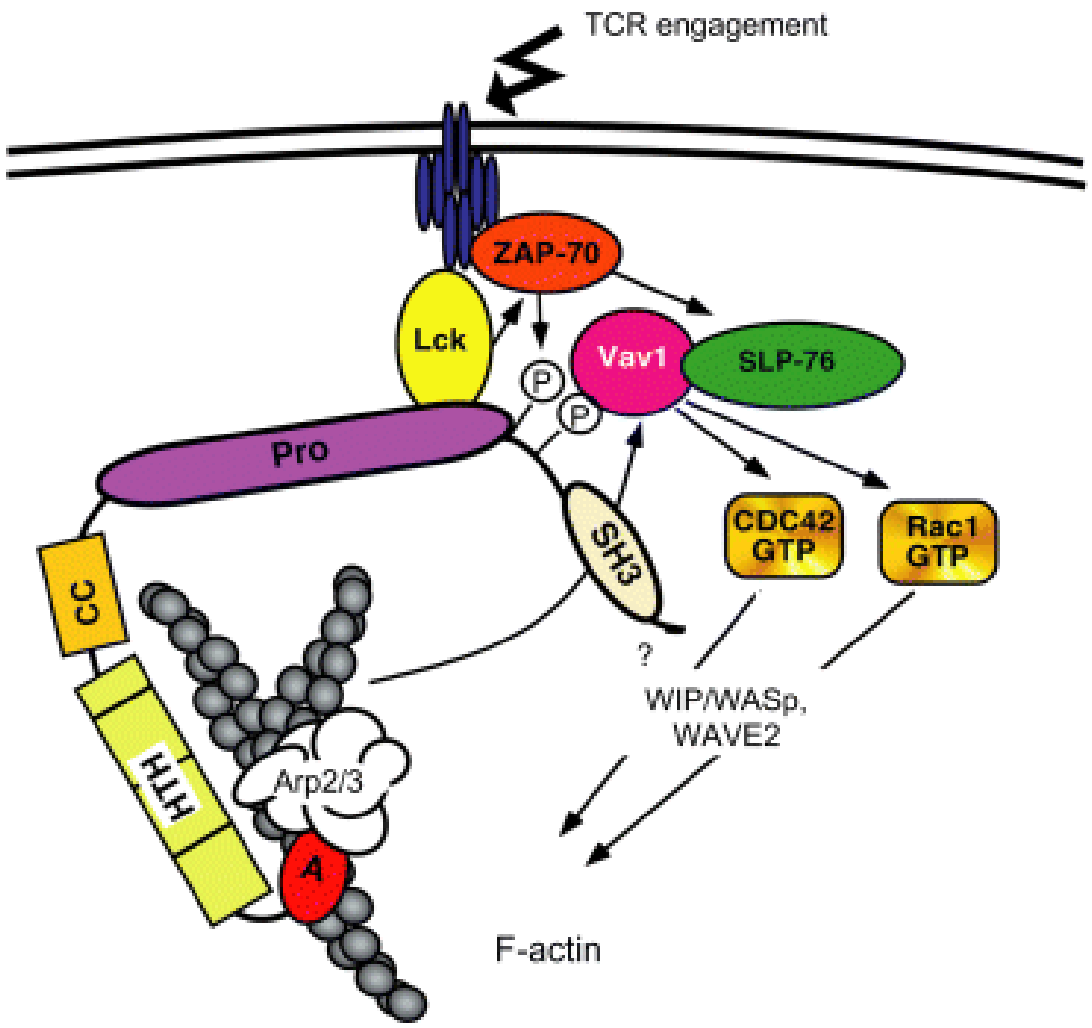
Domain structure of hematopoietic cell-specific lyn substrate (HS1)



Billadeau & Burkhardt, Traffic, 2006

HS1 is best studied at the immune synapse



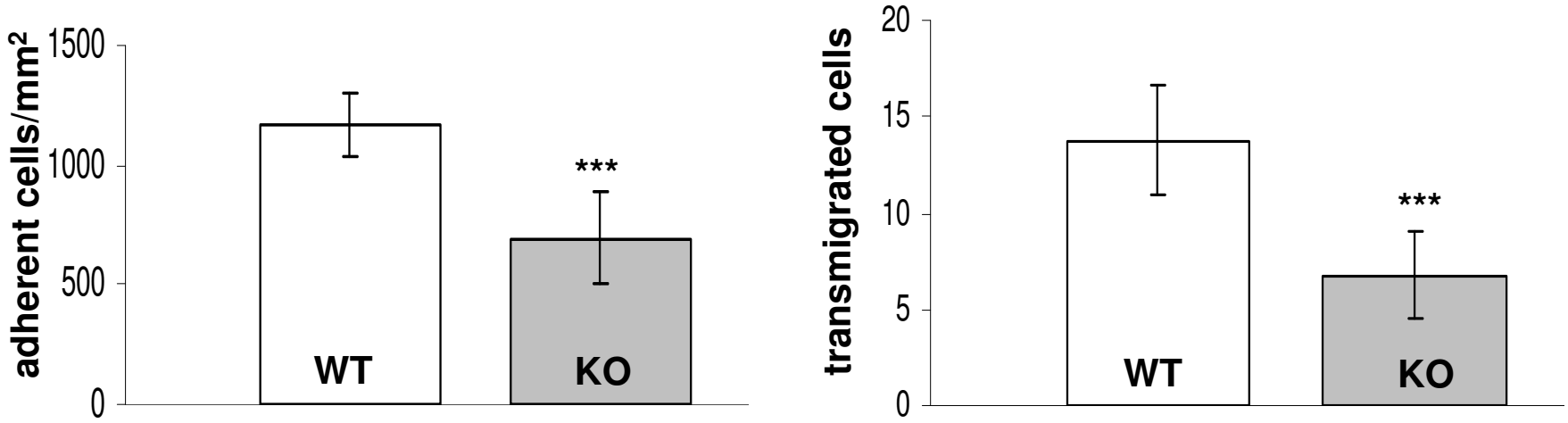
Billadeau & Burkhardt, Traffic, 2006

Summary of known HS1 functions

- can bind F-actin and actin nucleators such as Arp2/3 complex and WASP
- coordinates molecular components required for protrusion formation
- differential tyr-phosphorylation mediates adhesion to integrins and chemotaxis in NK cells
- is recruited to the immune synapse upon TCR engagement
- recruits Vav-1 to the IS and controls activation of cdc42 and Rac-1 upon TCR engagement
- HS1-KO mice are viable but show a defect in lymphocyte clonal expansion and B-cell homing

➡ HS1 is involved in the regulation of granulopoeisis and it interacts with Arp2/3 in neutrophils to mediate chemotaxis

Cortactin deficiency affects leukocyte adhesion and transendothelial migration



Schnoor et al., 2011, J. Exp. Med., 208, 1721-35

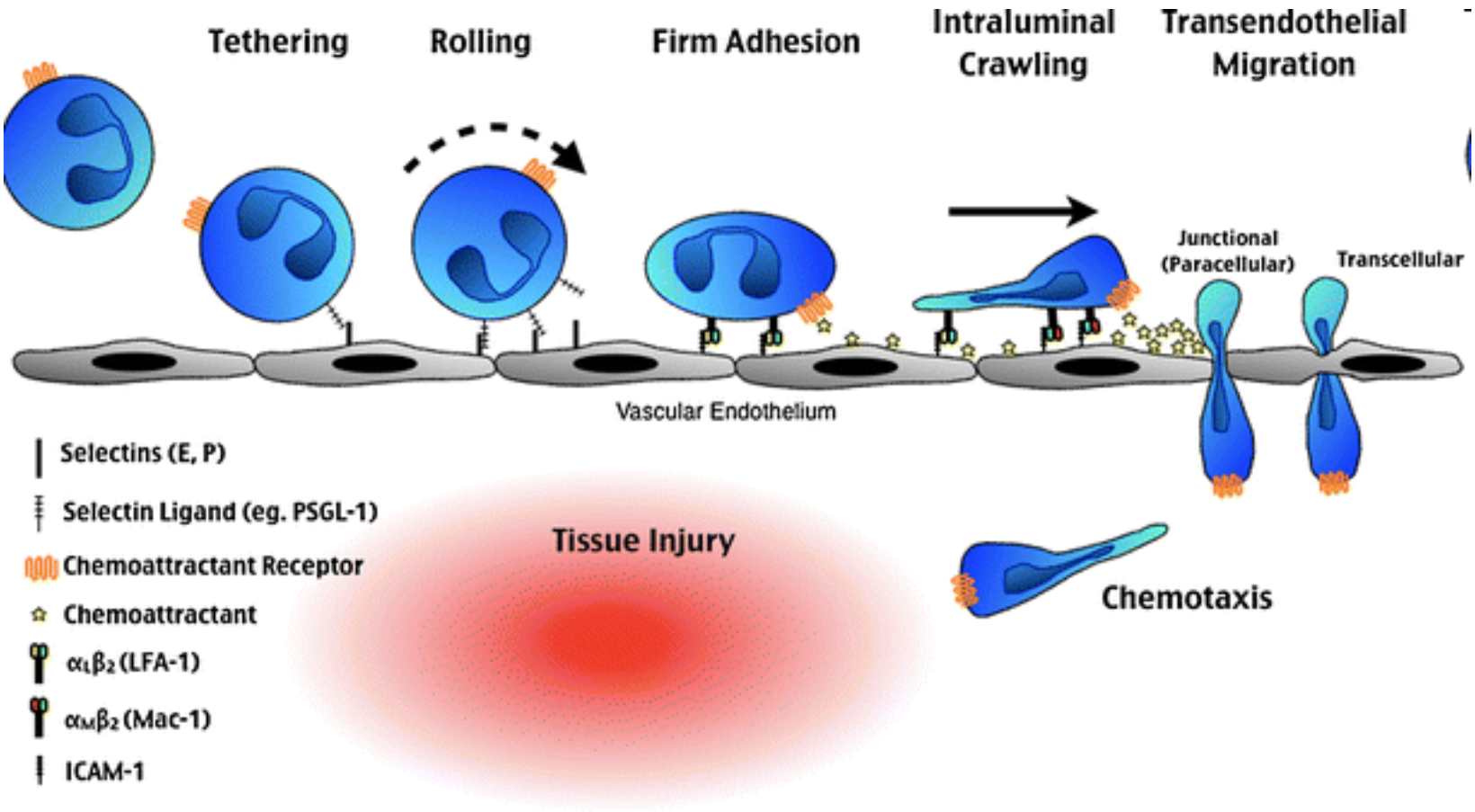
General Questions

Does HS1 influence leukocyte extravasation *in vivo*?

Which steps of the cascade are affected?

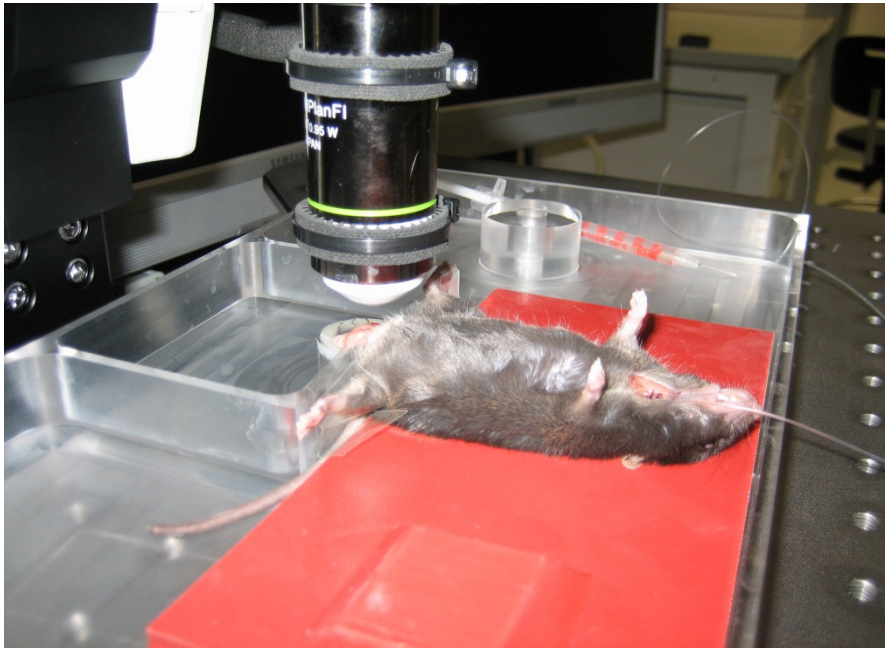
Which mechanisms are responsible for altered extravasation?

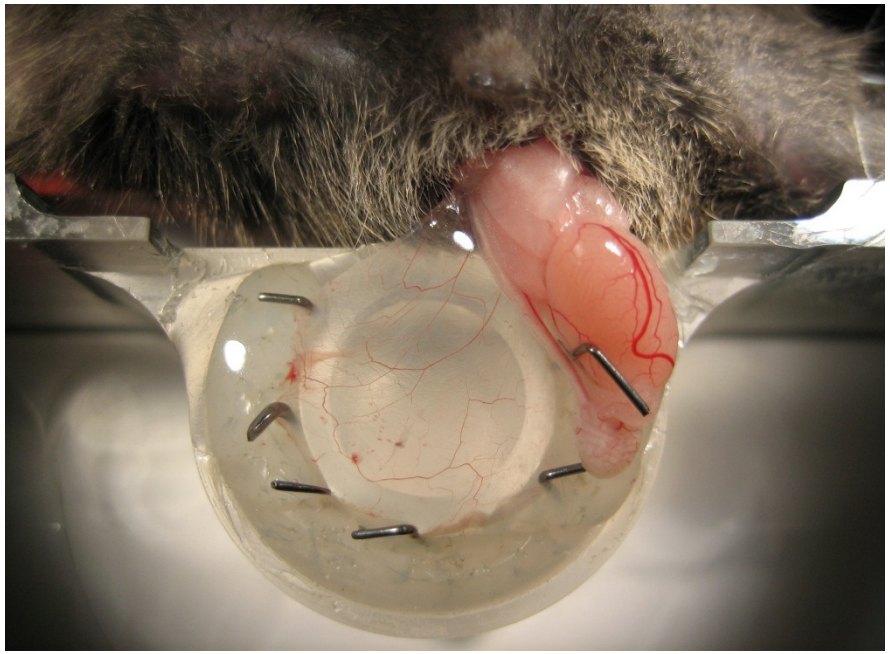
The leukocyte extravasation cascade



McDonald & Kubes, J Mol Med, 2011

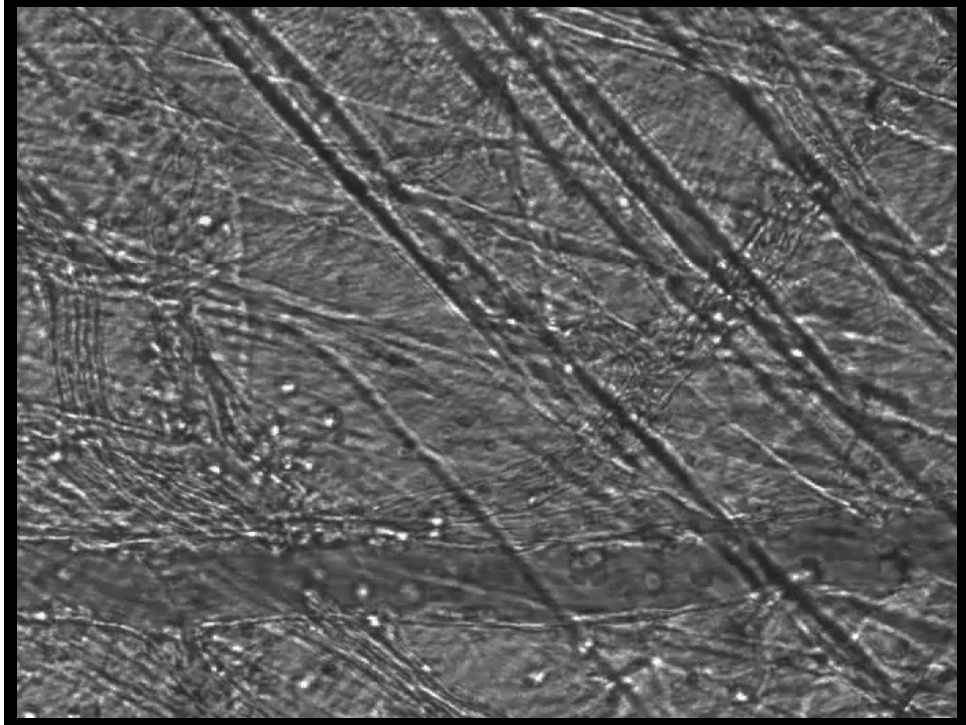
Intravital microscopy of inflamed cremaster venules



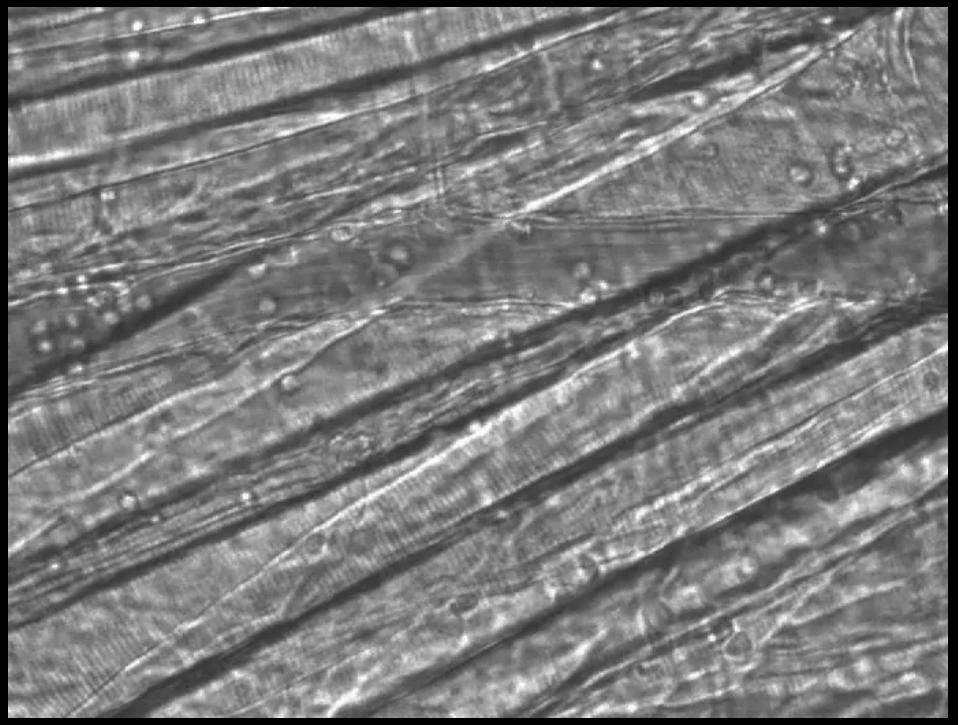


Intravital microscopy of inflamed cremaster venules

WT

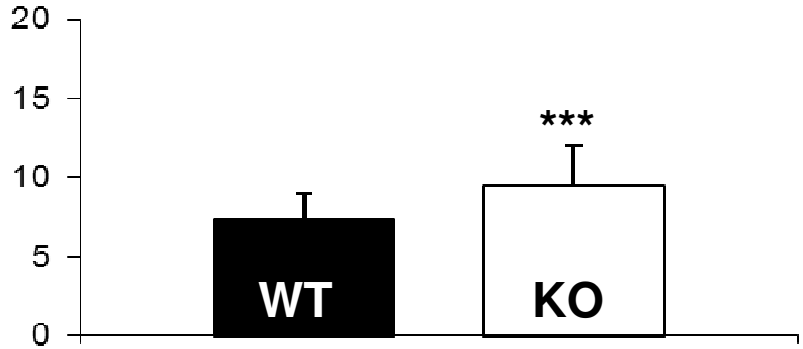


KO

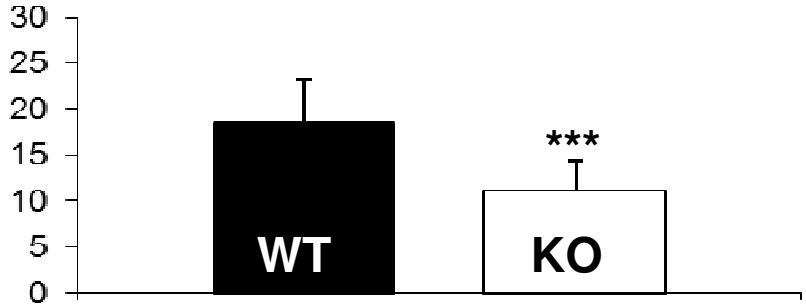


HS1-deficiency affects KC-induced leukocyte extravasation

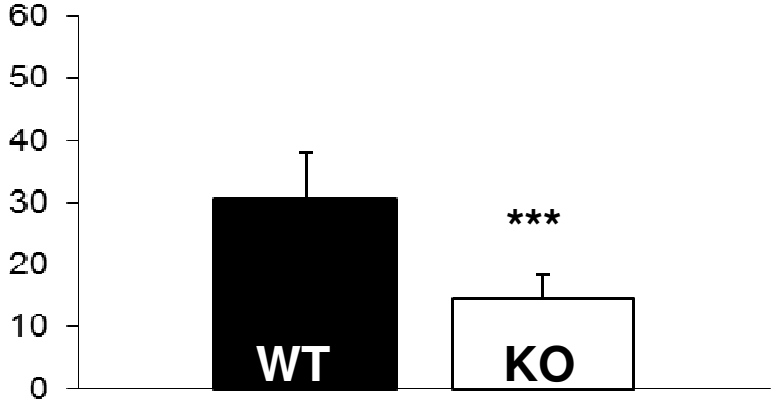
rolling velocity [µm/s]



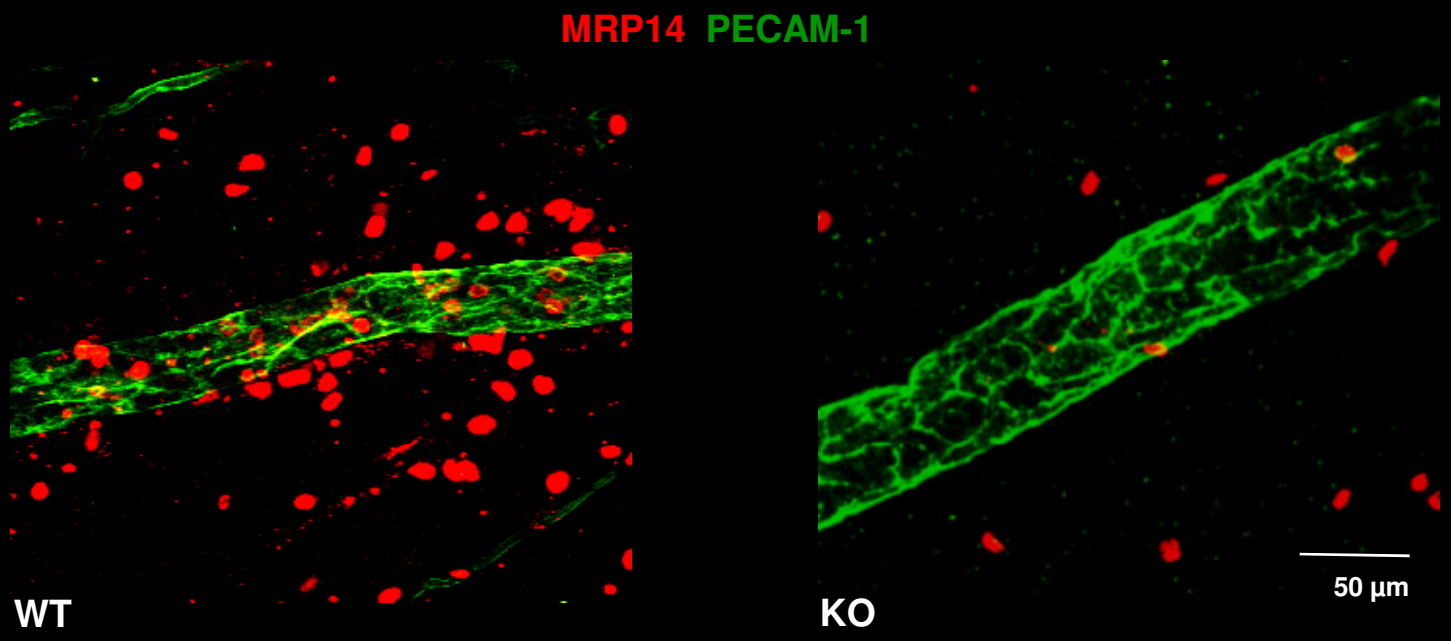
adhesion [x10^2 cells/mm^2]

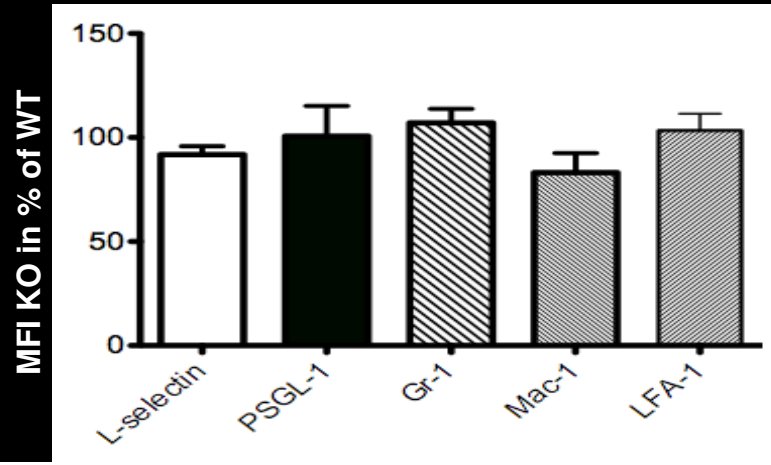


transmigration [cells]

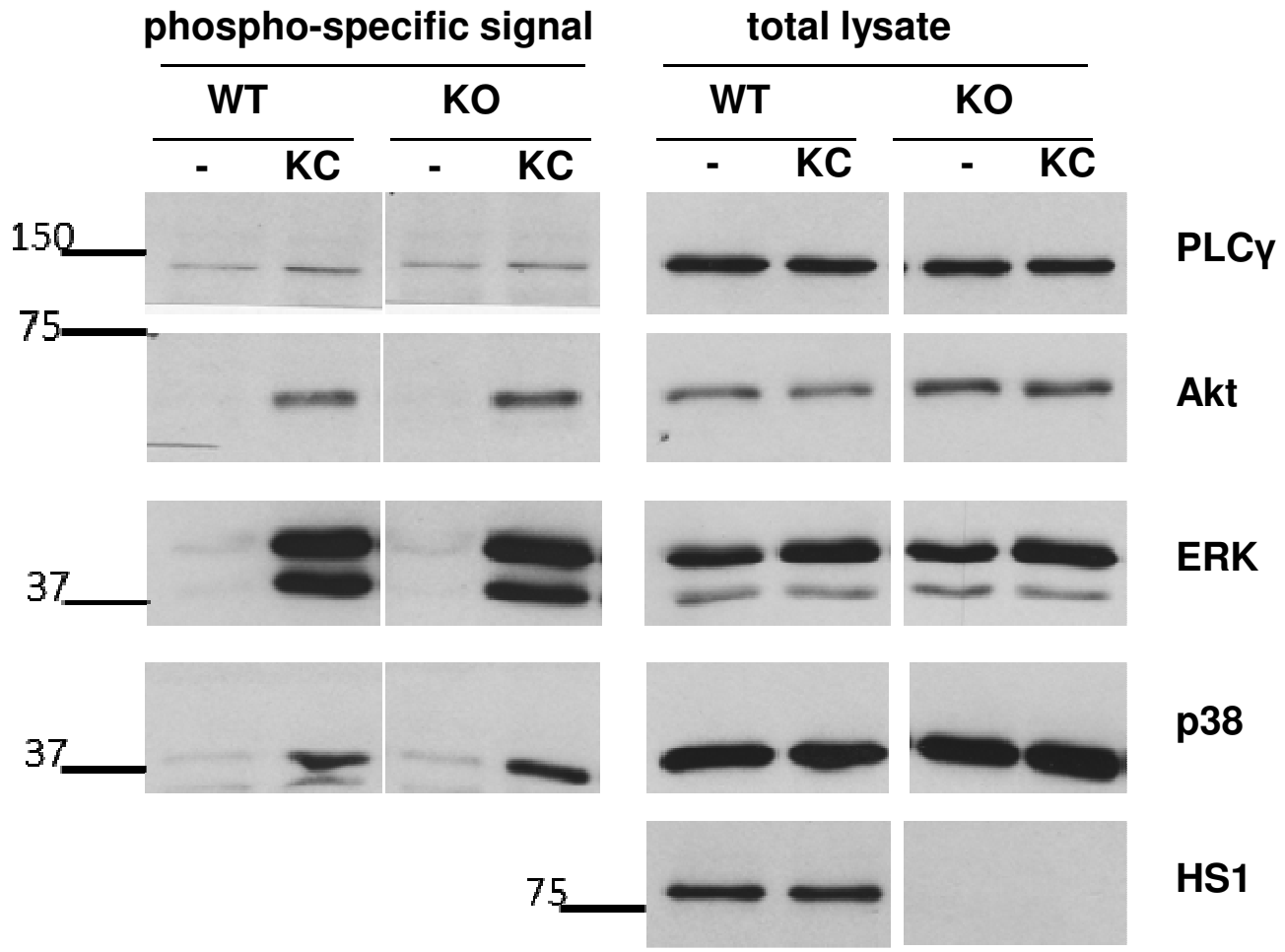


HS1-deficiency does neither affect vessel morphology in the cremaster nor surface expression of adhesion molecules





Reduced adhesion is not due to defective signaling mediated by PLC, Akt, Erk or p38 MAPK



PKA is involved in mediating HS1-dependent effects on transmigration

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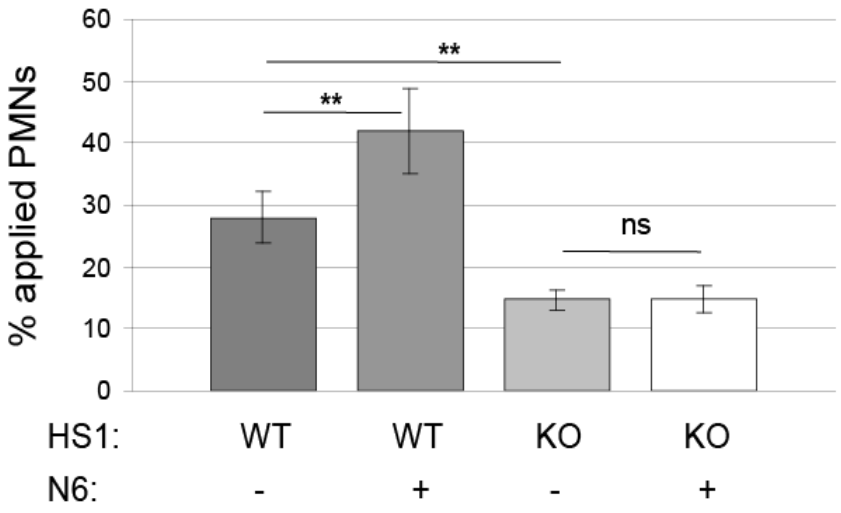
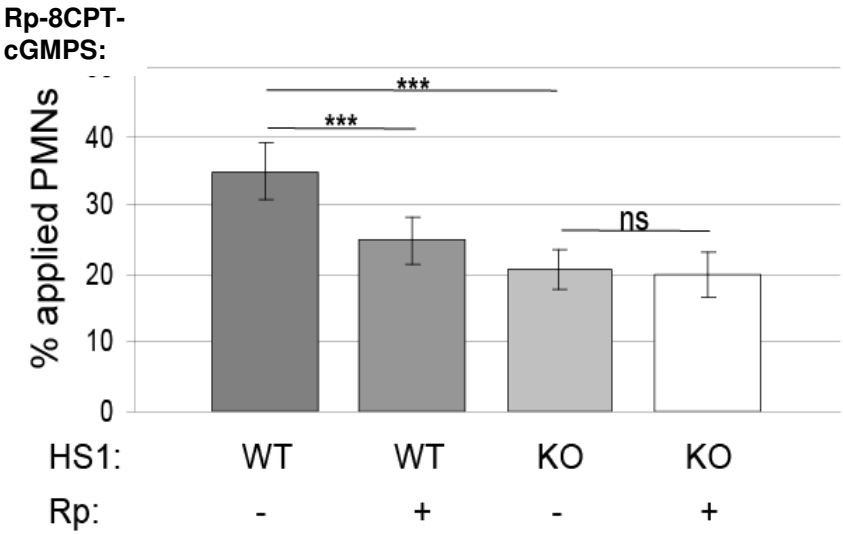
In vivo imaging reveals PKA regulation of ERK activity during neutrophil recruitment to inflamed intestines. Mizuno et al. 19.May.2014

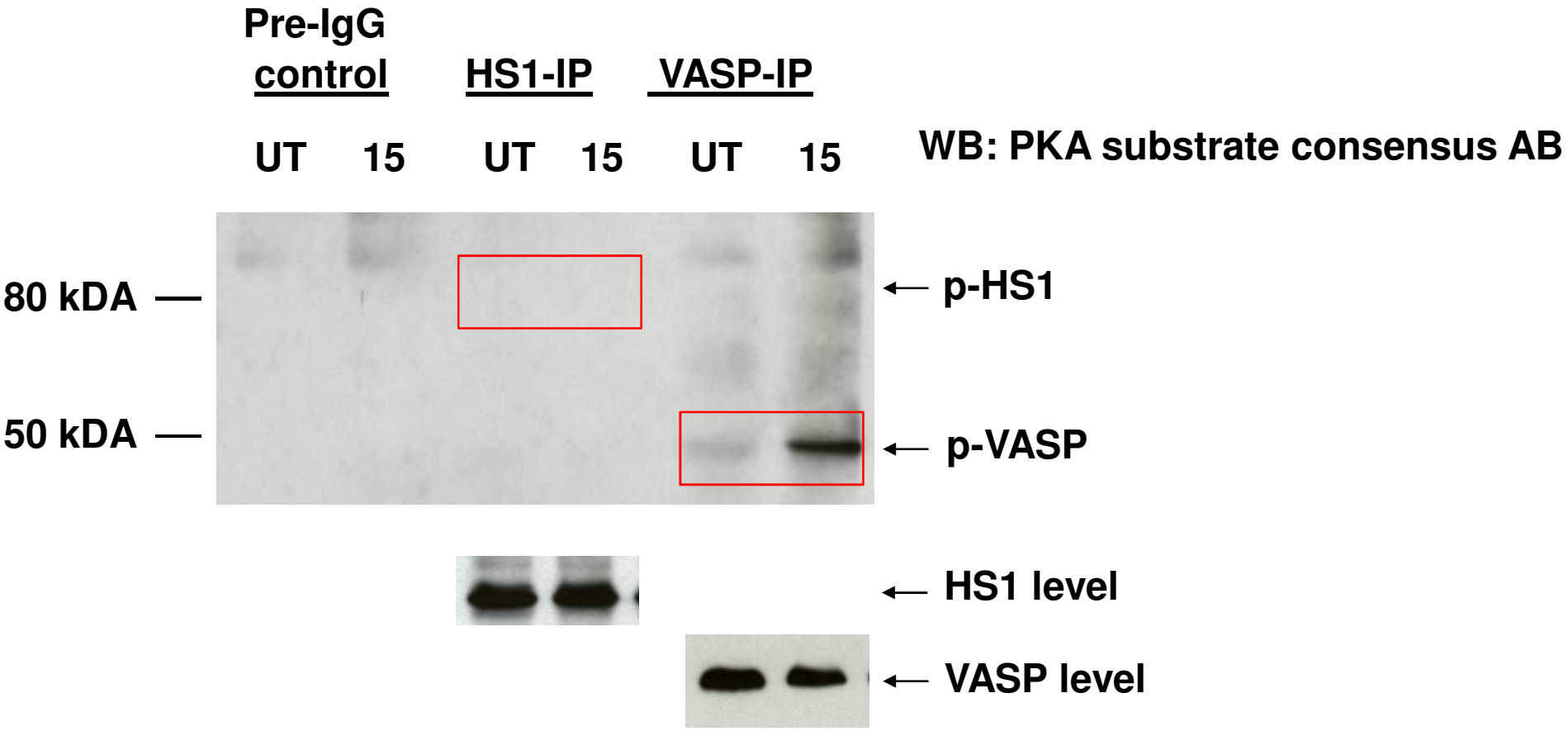
PKA activation increases TEM only in WT PMN



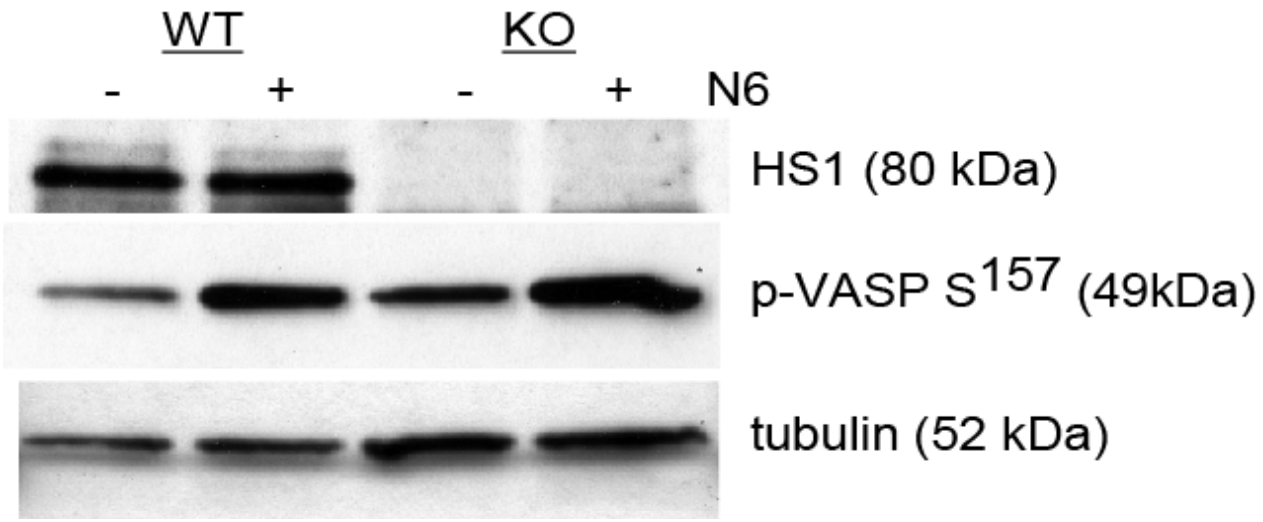
PKA inhibition reduces KC-triggered TEM only in WT PMN



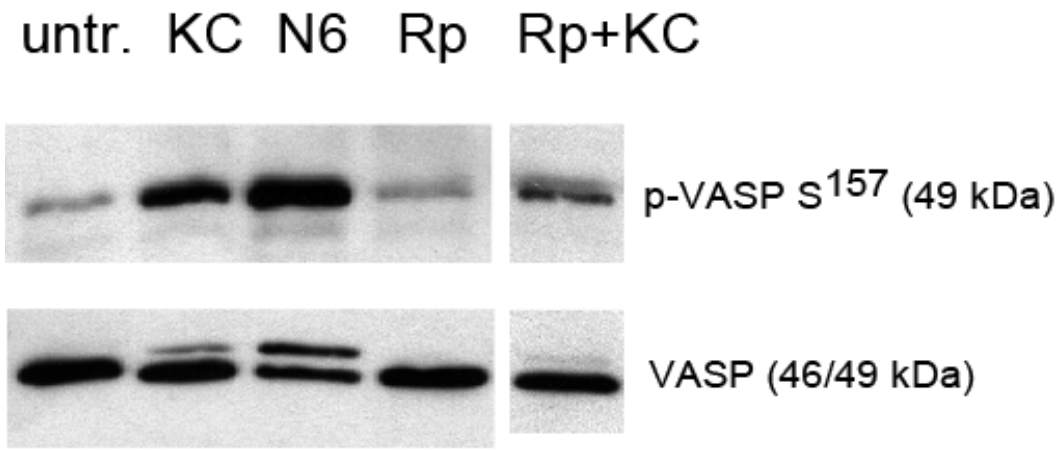
HS1 is not a direct PKA substrate



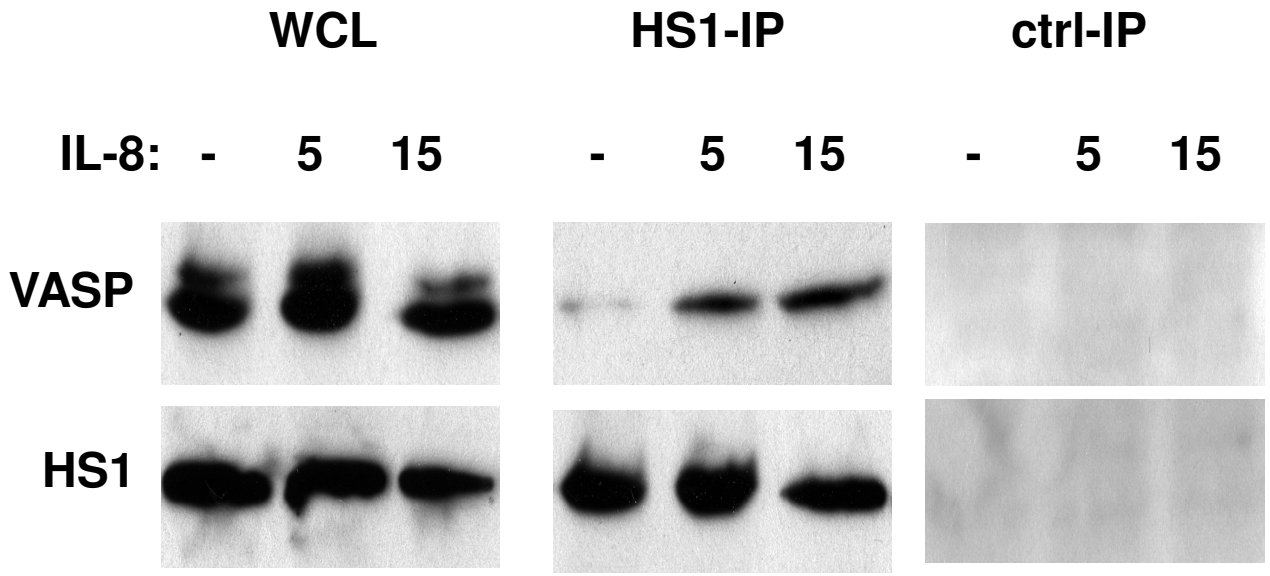
PKA is functional in the absence of HS1

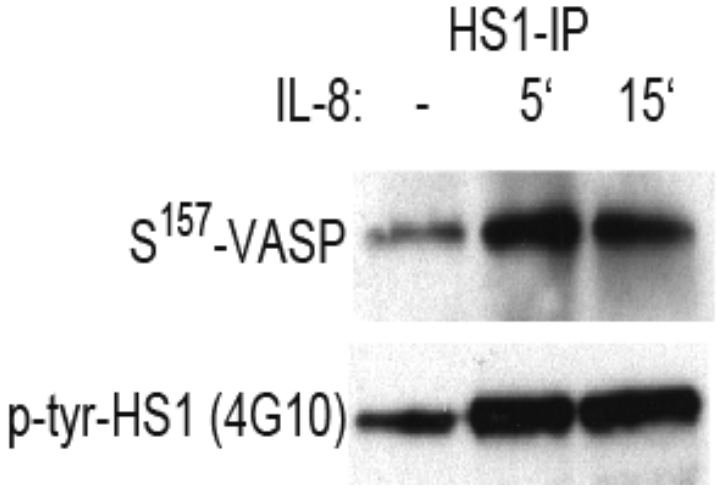


KC triggers PKA-mediated VASP phosphorylation

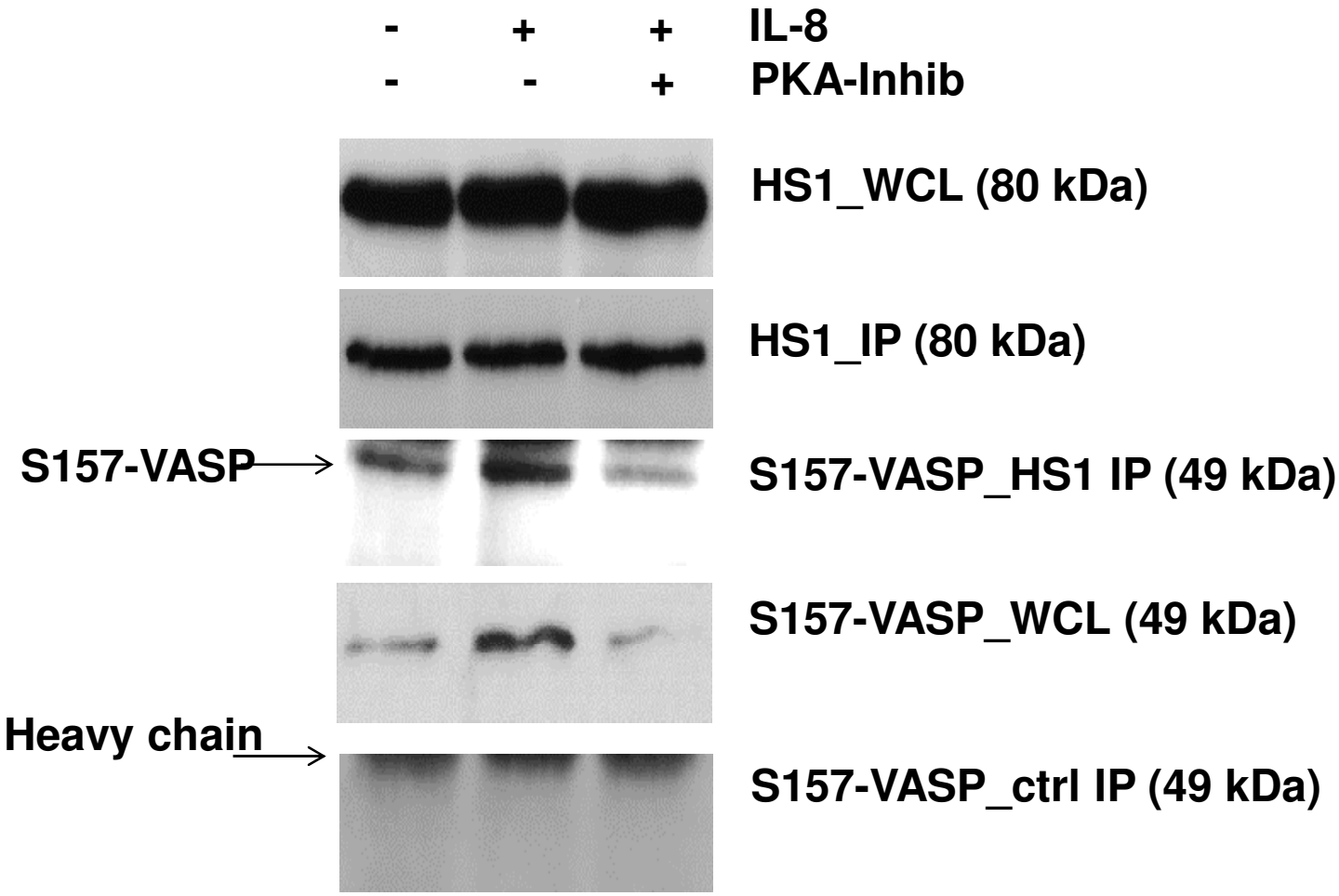


Phosphorylated HS1 and VASP co-precipitate

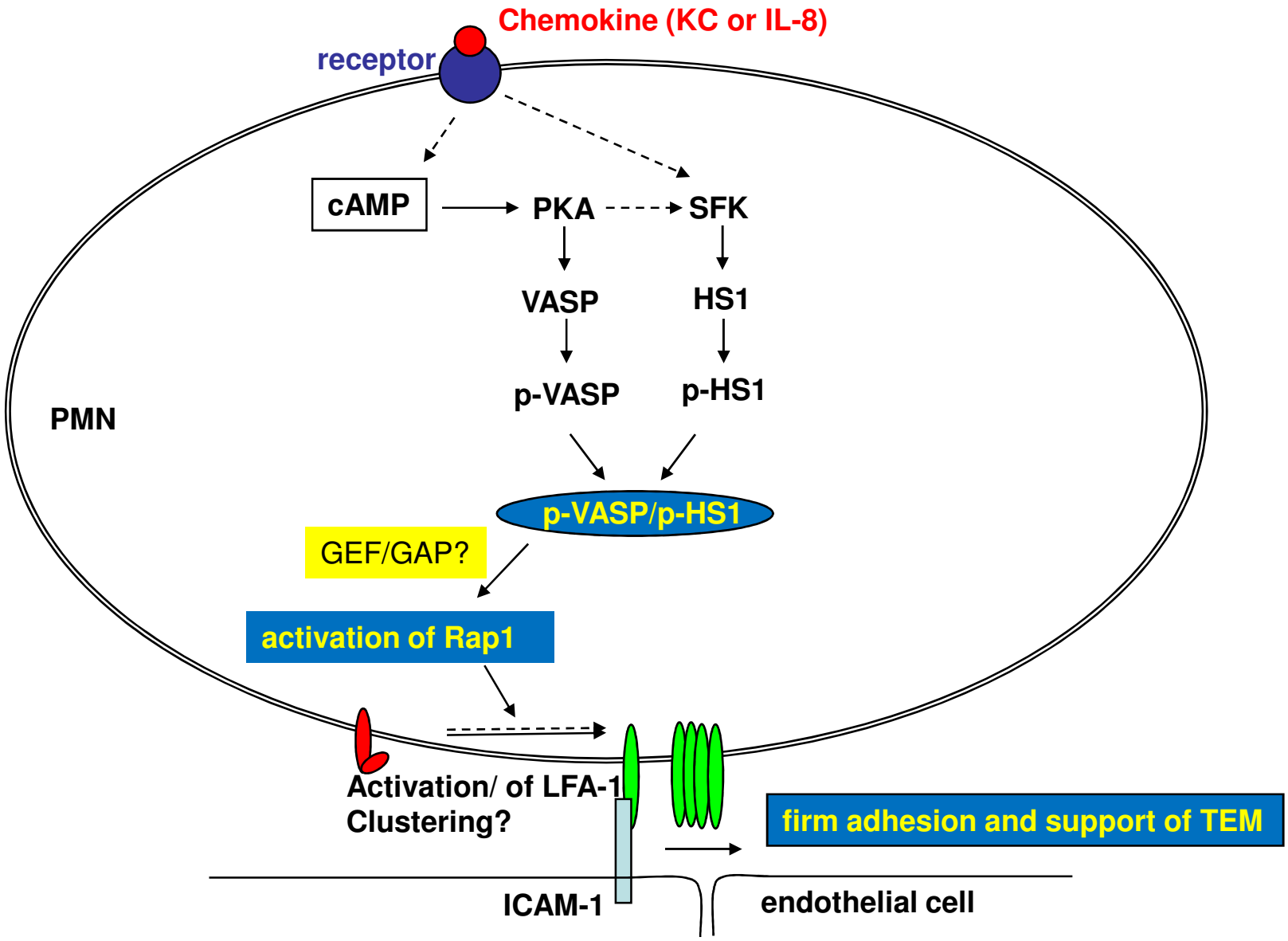




PKA inhibition prevents chemokine-induced phosphorylation of VASP and interaction with HS1



Possible function of HS1 in the regulation of leukocyte transmigration



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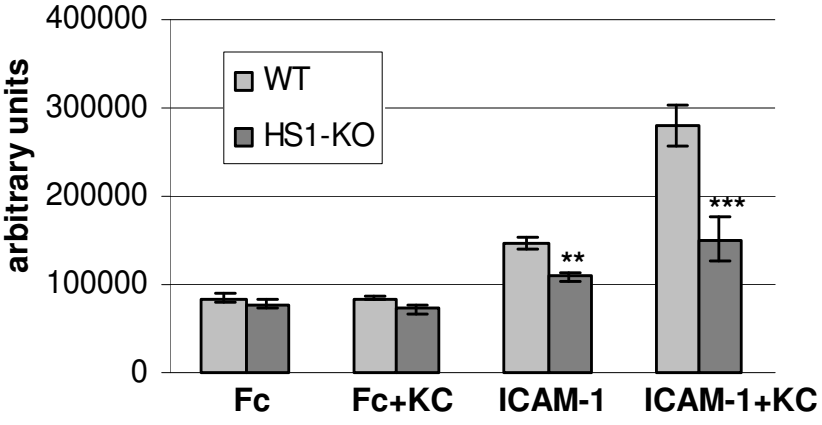
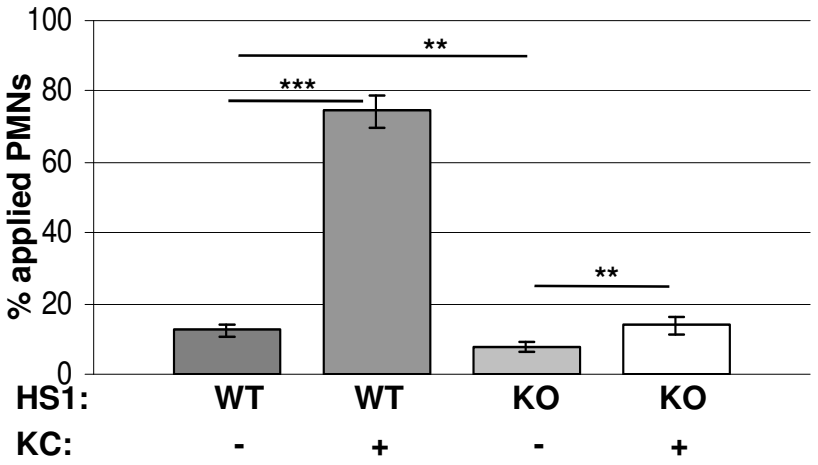
HS1 deficiency does not cause altered cell numbers in peripheral blood

	mean [WT]	SD	mean [KO]	SD	T-Test (n=8)
White blood cells [K/µl]	6.91064	1.0471219	7.55154	1.15649258	0.38516989
PMNs [K/µl]	1.32486	0.36698775	1.50042	0.60491002	0.59417357
Lymphocytes [K/µl]	4.80916	0.85250537	5.5869	1.05215898	0.23500117
Monocytes [K/µl]	0.17318	0.01581398	0.17088	0.06742749	0.94262764
Red blood cells [M/µl]	11.32396	1.13459566	11.5424	1.20527106	0.77544411
Platelets [K/µl]	990.244	144.671792	945.656	268.108192	0.75186585
Hemoglobin [g/dl]	16.31066	1.15588788	15.38868	2.25486595	0.4394048

KC triggers adhesion and transmigration of WT PMNs much stronger compared to KO





HS1-KO PMNs show the same morphology under resting conditions but unorganized protrusion formation after activation