

SUPPLEMENTAL MATERIAL

Liu et al., <http://www.jem.org/cgi/content/full/jem.20140508/DC1>**Table S1.** siRNA sequences for RNAi knockdown

Genes	Primers	Sequences
<i>Moesin</i>	#1 sense	5'-GATCCCCAGAGGGCATTCTCAATGATTTCAAGAGAATCATTGAGAATGCCCTCTTTTTTC-3'
	antisense	5'-TCGAGAAAAAGAGGGCATTCTCAATGATTCTTTGAAATCATTGAGAATGCCCTCTGGG-3'
	#2 sense	5'-GATCCAGAGGGCATTCTCAATGATTTCAAGAGAATCATTGAGAATGCCCTCTTTTTTG-3'
	antisense	5'-AATTCAAAAAAGAGGGCATTCTCAATGATTCTTTGAAATCATTGAGAATGCCCTCTG-3'
<i>PP1c</i>	#1 sense	5'-CCAGAUGAAAGCAUGAUGATT-3'
	antisense	5'-UCAUCAUGCUUUCUUCUGGTT-3'
	#2 sense	5'-CAAGCCUGAUUGUACUAGUTT-3'
	antisense	5'-ACUAGUACAAUCAGGCUUGTT-3'
	#3 sense	5'-CUACAUUGGUUGACUUGATT-3'
<i>MBS</i>	antisense	5'-UCUAAGUCAACCAUGUAGTT-3'
	#1 sense	5'-GCAAGGGGUUGAUUAGAATT-3'
	antisense	5'-UUCUAUAUCAACCCUUGCTT-3'
	#2 sense	5'-GAACGUAUGAUGAGACUUAATT-3'
	antisense	5'-UAAGUCUCAUCAUACGUUCTT-3'
<i>Vav1</i>	#3 sense	5'-CCAUCUCUUCACUUCUATT-3'
	antisense	5'-UAGAAAGUGAAGAGGAUGGTT-3'
	#1 sense	5'-CCAAGAUGACAGAGUAUGATT-3'
	antisense	5'-UCAUACUCUGUCAUCUUGGTT-3'
	#2 sense	5'-CUACCAGGUCUUCAUCAAAATT-3'
<i>Hem-1</i>	antisense	5'-UUUGAUGAAGACCUUGUAGTT-3'
	#3 sense	5'-GAGACCGAGACAACAAGAATT-3'
	antisense	5'-UUCUUGUUGUCUCGGUCUCTT-3'
	#1 sense	5'-GGAACCAUCUCUAGUAUTT-3'
	antisense	5'-AUACUUGAGAGAUGGUCCCTT-3'
<i>αPIX</i>	#2 sense	5'-GGAAUUUCGGGAUCAUGUATT-3'
	antisense	5'-UACAUGAUCCGAAUUCCTT-3'
	#3 sense	5'-CUCUCUGAAUCUAAACAATT-3'
	antisense	5'-UUGUUUGAGAUUCAGAGATT-3'
	#1 sense	5'-GGACGUUCCUCUUCUUAATT-3'
<i>PRG</i>	antisense	5'-UAAGAGAAGAGGAACGUCCTT-3'
	#2 sense	5'-GGAGCCUCCUCAAUUUAUATT-3'
	antisense	5'-UAUAAUUUGAGGAGGUCCTT-3'
	#3 sense	5'-GCACAGCCAUUAGAUUAUATT-3'
	antisense	5'-UAUAUCUAAAUGGCUGUGCTT-3'
<i>PRG</i>	#1 sense	5'-GAACCUGCCUGAACUCAUATT-3'
	antisense	5'-UAUGAGUUCAGGCAGGUUCTT-3'
	#2 sense	5'-CAAGAGCCUGGAUCUUAUATT-3'
	antisense	5'-UGUAAGAUCAGGCUCUUGTT-3'
	#3 sense	5'-CCUCAGACAUGCAAGUGAATT-3'
antisense	5'-UUCACUUGCAUGUCUGAGGTT-3'	

Table S2. The percentages of polarized HL60 cells stimulated with fMLF at the indicated times and distribution of moesin and MLC in uropod (Fig. 2 A) and PP1c in pseudopod (Fig. 8 A) in these polarized cells

Plasmids	Cells (<i>n</i>)	Polarization	Time			
			0 min	2 min	4 min	6 min
Moesin-YFP	102	Polarized cells (%)	6	55	78	86
		Moesin in uropod (%)	100	93	90	91
MLC-YFP	136	Polarized cells (%)	7	35	70	81
		MLC in uropod (%)	40	63	90	84
PP1c-YFP	91	Polarized cells (%)	7	71	91	92
		PP1c in pseudopod (%)	0	75	76	91

Table S3. The percentages of polarized HL60 cells (left untreated or blebbistatin or Y27632 treated) stimulated with fMLF at the indicated times (Fig. 3 A) and distribution of moesin or MLC in uropod in these polarized cells

Moesin-YFP/MLC-CFP	Cells (<i>n</i>)	Polarization	Time			
			0 min	2 min	4 min	6 min
Untreated	35	Polarized cells (%)	14	69	77	95
		Moesin in uropod (%)	100	92	93	97
		MLC in uropod (%)	0	17	56	83
Blebbistatin	22	Polarized cells (%)	9	83	91	91
		Moesin in uropod (%)	100	78	90	90
		MLC in uropod (%)	0	6	5	5
Y27632	22	Polarized cells (%)	14	59	82	91
		Moesin in uropod (%)	100	92	89	90
		MLC in uropod (%)	0	7	0	0

Table S4. The distribution of MLC to uropod in control or moesin RNAi-treated HL60 cells stimulated with fMLF at the indicated times (Fig. 3 C)

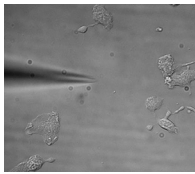
MLC-DsRed	Cells (<i>n</i>)	Polarization	Time			
			0 min	2 min	4 min	6 min
Control	71	Polarized cells (%)	4	31	52	93
		MLC in uropod (%)	0	77	84	94
Moesin RNAi	61	Polarized cells (%)	3	11	15	26
		MLC in uropod (%)	0	14	22	31

Table S5. Multiple pseudopod formation in control, moesin RNAi-, moesin + Vav1 RNAi-, or moesin + α PIX RNAi-treated HL60 cells stimulated with fMLF

Cell lines	Cells (<i>n</i>)	Pseudopods		
		0	1	≥ 2
		%	%	%
Control	98	14	76	10
Moesin RNAi	69	13	4	83
Moesin + Vav1 RNAi	66	12	33	55
Moesin + α PIX RNAi	60	15	73	12



Video 1. Left untreated, HL60 cells migrated toward a point source of fMLF (10 μ M, from a micropipette).



Video 2. Y27632-treated HL60 cells migrated toward a point source of fMLF (10 μ M, from a micropipette).



Video 3. Moesin RNAi-treated HL60 cells migrated toward a point source of fMLF (10 μ M, from a micropipette).