Chan et al., https://doi.org/10.1083/jcb.201704186

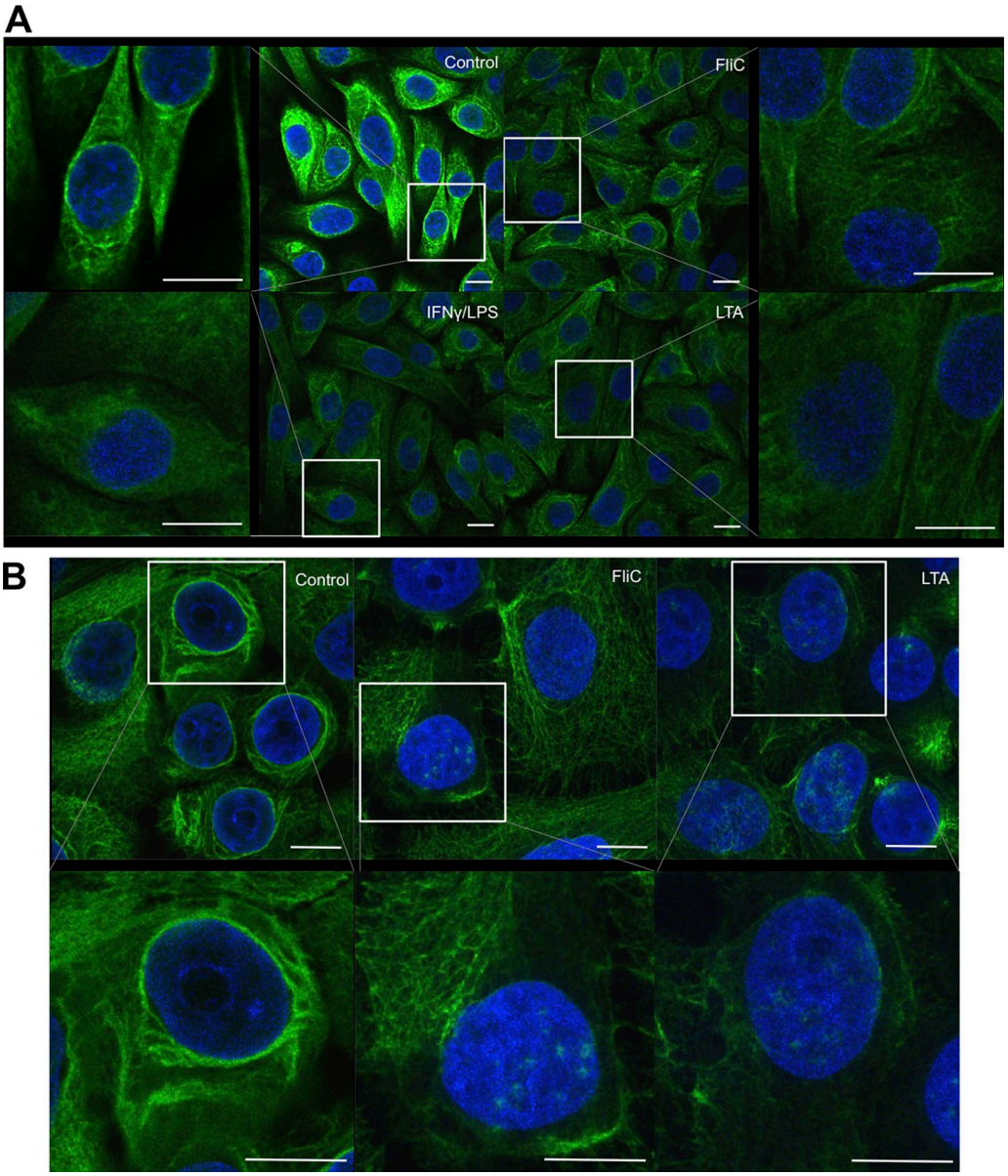


Figure S1. **Human mammary epithelial cells (hTERT-HME1) and ectocervical epithelial cells (Ect1/E6E7) demonstrate depolymerization of the filamentous K6a network in response to bacterial ligands. (A)** Confocal microscopic images of hTERT-HME1 cells treated for 16 h with vehicle control, LTA (1 µg/ml), flagellin (FliC; 0.5 µg/m) or LPS (1 µg/ml; pretreated with 250 ng/ml IFN-γ for 2 h). The intense filamentous K6a network staining surrounding the nucleus in the control cell (inset) became diffused and weak after stimulation with bacterial ligands. **(B)** Ect1/E6E7 cells were treated as described in A. Similar observations were made for Ect1/E6E7 cells. Note that Ect1/E6E7 cells do not express TLR-4. Bars, 10 µm.

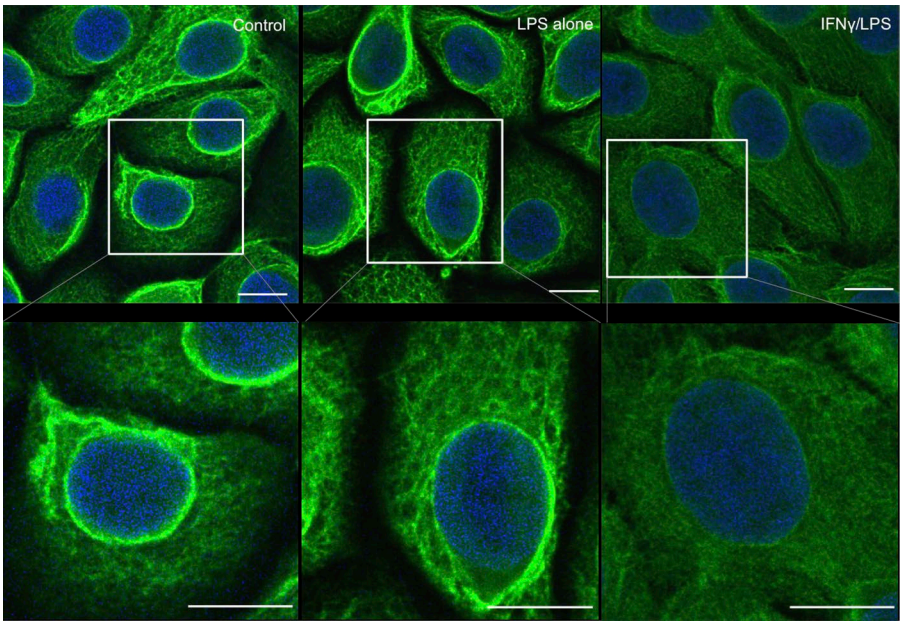
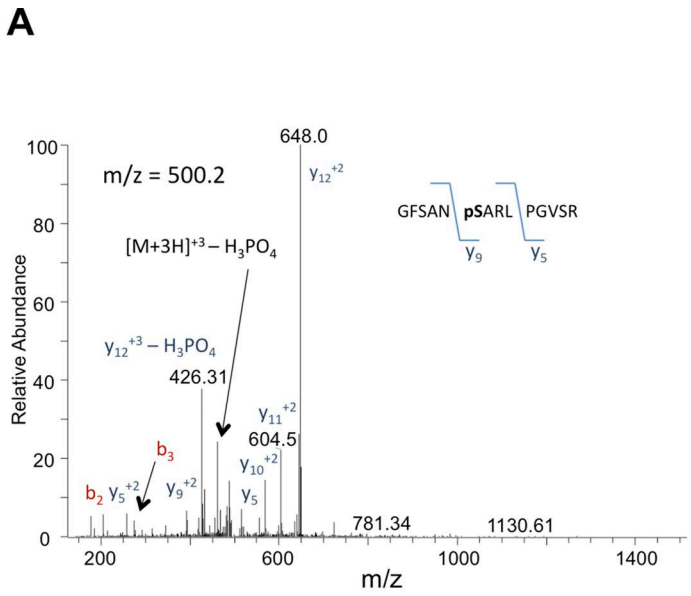
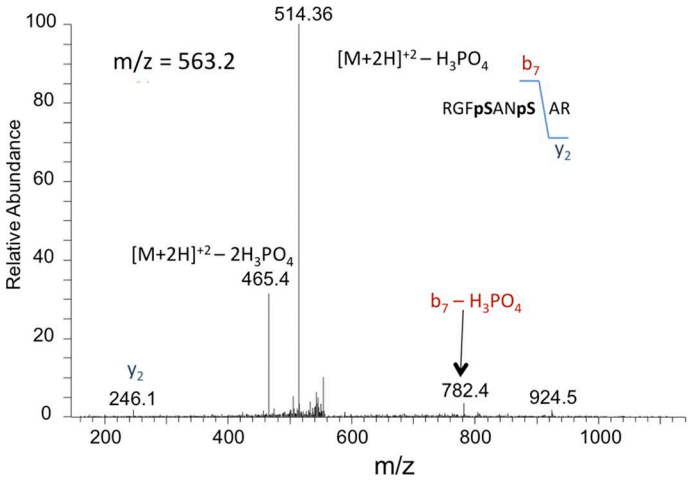


Figure S2. **LPS-induced depolymerization of the filamentous K6a network does not occur in epithelial cells with low expression of TLR-4 coreceptor MD-2.** Confocal microscopic images of hTCEpi cells treated for 16 h with vehicle control or LPS (1 µg/ml) with or without preincubation with IFN-γ (250 ng/ml; 2 h). Normal hTCEpi cells produce low levels of MD-2 and are unresponsive to LPS. A 2-h pretreatment with IFN-γ was used to induce MD-2 expression and LPS responsiveness. The filamentous K6a network staining (green) surrounding the nucleus (blue) in the control cells (left) and in cells treated with LPS alone (middle) was intense; on the contrary, the filamentous network staining in cells primed with IFN-γ before LPS stimulation (right) was diffused and weak. Bars, 10 µm.

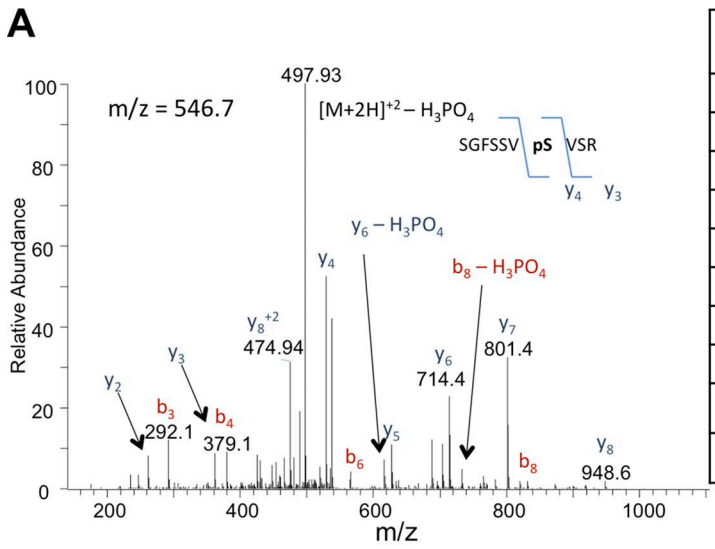


$b - H_3PO_4$ Da	b ion Da		y ion Da	$y - H_3PO_4$ Da
--	--	G	--	--
--	205	F	1441	1343
--	292	S	1294	1196
--	363	A	1207	1109
--	477	N	1136	1038
546	644	pS	1022	924
617	715	A	855	--
773	871	R	784	--
886	984	L	628	--
983	1081	P	515	--
1040	1138	G	418	--
1140	1238	V	361	--
1226	1324	S	262	--
--	--	R	175	--

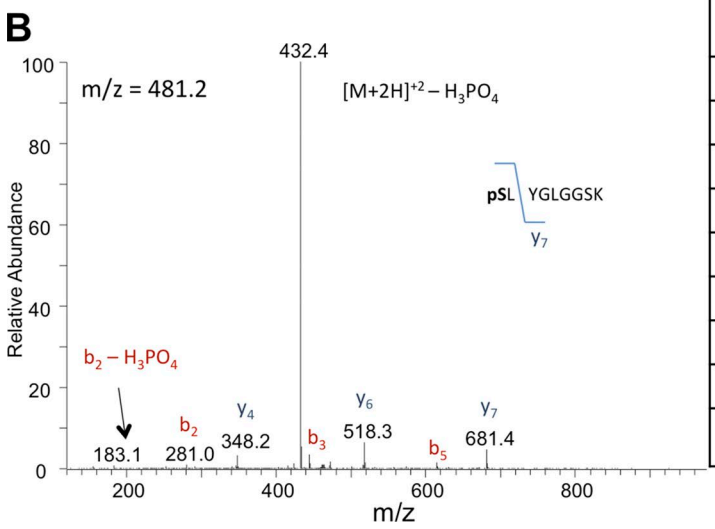


$b - H_3PO_4$ Da	b ion Da		y ion Da	$y - H_3PO_4$ Da
--	--	R	--	--
--	214	G	969	871
--	361	F	912	814
430	528	pS	765	667
501	599	A	598	501
615	713	N	527	429
782	880	pS	413	315
853	951	A	246	--
--	--	R	175	--

Figure S3. **Tandem mass spectrometry spectra of K6a phosphopeptides with a single phosphorylation at Ser-22 or double phosphorylation at Ser-19 and Ser-22. (A and B)** K6a was subjected to tryptic digestion, and these tryptic digests were analyzed by LC-MS looking for K6a phosphopeptides. **(A)** The tandem mass spectrometry spectrum for a phosphopeptide GFSANSARLPGVSR with a single phosphorylation at Ser-22 is shown. The m/z of 500.2 is consistent with the triply charged GFSANSARLPGVSR + PO_3 peptide. The difference of m/z values between y_9 and y_5 ions is consistent with a modification on S22. **(B)** The tandem mass spectrometry spectrum for a phosphopeptide RGFSANSAR with double phosphorylation at Ser-19 and Ser-22 is shown. The m/z of 563.2 is consistent with the doubly charged RGFSANSAR + 2 PO_3 peptide. The tandem mass spectrometry spectra are dominated by two spikes corresponding with one and two losses of H_3PO_4 from the $[M + 2 H]^{+2}$ ion (i.e., m/z 514.36 and 465.4). This is consistent with two sites of serine phosphorylation in this peptide. The m/z values of b_7-H_3PO_4 and y_2 ions are consistent with modifications at S19 and S22.

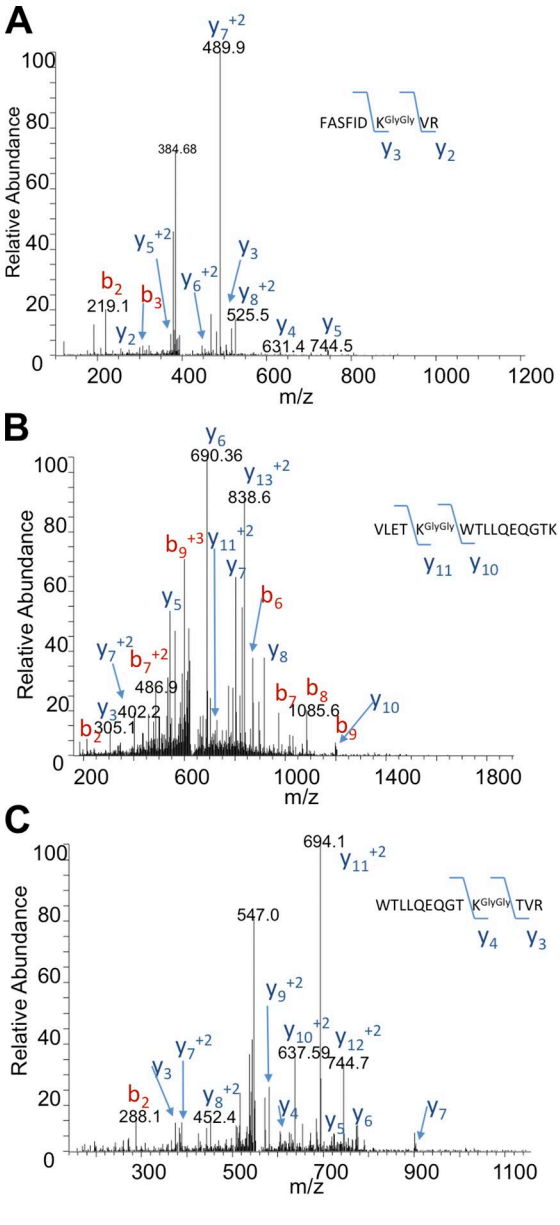


b – H_3PO_4 Da	b ion Da		y ion Da	y – H_3PO_4 Da
--	--	S	--	--
--	145	G	1005	907
--	292	F	948	850
--	379	S	801	703
--	466	S	714	616
--	565	V	627	529
634	732	pS	528	430
733	831	V	361	--
820	918	S	262	--
--	--	R	175	--



b – H_3PO_4 Da	b ion Da		y ion Da	y – H_3PO_4 Da
--	--	pS	--	--
183	281	L	794	--
346	444	Y	681	--
403	501	G	518	--
516	614	L	461	--
573	671	G	348	--
630	728	G	291	--
717	815	S	234	--
--	--	K	147	--

Figure S4. **Tandem mass spectrometry spectra of K6a phosphopeptides with a single phosphorylation at Ser-37 or Ser-60. (A and B)** K6a was subjected to tryptic digestion, and these tryptic digests were analyzed by LC-MS looking for K6a phosphopeptides. (**A**) The tandem mass spectrometry spectrum for a phosphopeptide SGFSSVSVSR with a single phosphorylation at Ser-37 is shown. The m/z of 546.7 is consistent with the doubly charged SGFSSVSVSR + PO_3 peptide. The difference of m/z values between y_3 and y_4 ions is consistent with a modification on S37. (**B**) The tandem mass spectrometry spectrum for a phosphopeptide SLYGLGGSK with a single phosphorylation at Ser-60 is shown. The m/z of 481.2 is consistent with the doubly charged SLYGLGGSK + PO_3 peptide. The m/z values of b_2 and y_7 ions are consistent with a modification on S60.



b ion Da		y ion Da
--	F	--
219	A	1049
306	S	978
453	F	891
566	I	744
681	D	631
923	K(gg)	516
1022	V	274
--	R	175

b ion Da		y ion Da
--	V	--
213	L	1788
342	E	1675
443	T	1546
685	K(gg)	1445
871	W	1203
972	T	1017
1085	L	916
1198	L	803
1326	Q	690
1455	E	562
1583	Q	433
1640	G	305
1741	T	248
--	K	147

b ion Da		y ion Da
--	W	--
288	T	1487
401	L	1386
514	L	1273
642	Q	1160
771	E	1032
899	Q	903
956	G	775
1057	T	718
1299	K(gg)	617
1400	T	375
1499	V	274
--	R	175

Figure S5. **Tandem mass spectrometry spectra of K6a peptides with ubiquitination at Lys-180, Lys-194, or Lys-204. (A–C)** K6a was subjected to tryptic digestion, and these tryptic digests were analyzed by LC-MS looking for K6a ubiquitinated peptides. (**A**) The tandem mass spectrometry spectrum for a triply charged peptide with an m/z of 399.6 is shown. The mass of this ion is consistent with the FASFIDKVR + GlyGly peptide. The mass difference between the y_3 and y_2 ions is consistent with modification at K180. **(B)** The tandem mass spectrometry spectrum for a triply charged peptide with an m/z of 630.0 is shown. The mass of this ion is consistent with the VLETKWTLLQEQGTK + GlyGly peptide. The mass difference between the C-terminal y_{11} and y_{10} ions is consistent with modification on K194. **(C)** The tandem mass spectrometry spectrum for a triply charged peptide with an m/z of 559.0 is shown. The mass of this ion is consistent with the WTLLQEQGTKTVR + GlyGly peptide. The mass difference between the C-terminal y_3 and y_4 ions is consistent with modification on K204.