Skp2-mediated MLKL degradation confers cisplatin-resistant in non-small cell lung cancer cells

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Supplementary Table 1. Protein expression of Skp2 in NSCLC tissues and adjacent non-tumor tissues.

Tissue sample	No. of patients	Skp2		*p*- value
		Low	High	
Tumor	39	6	33	<0.0001*
Adjacent	39	28	11	

Chi-square test.

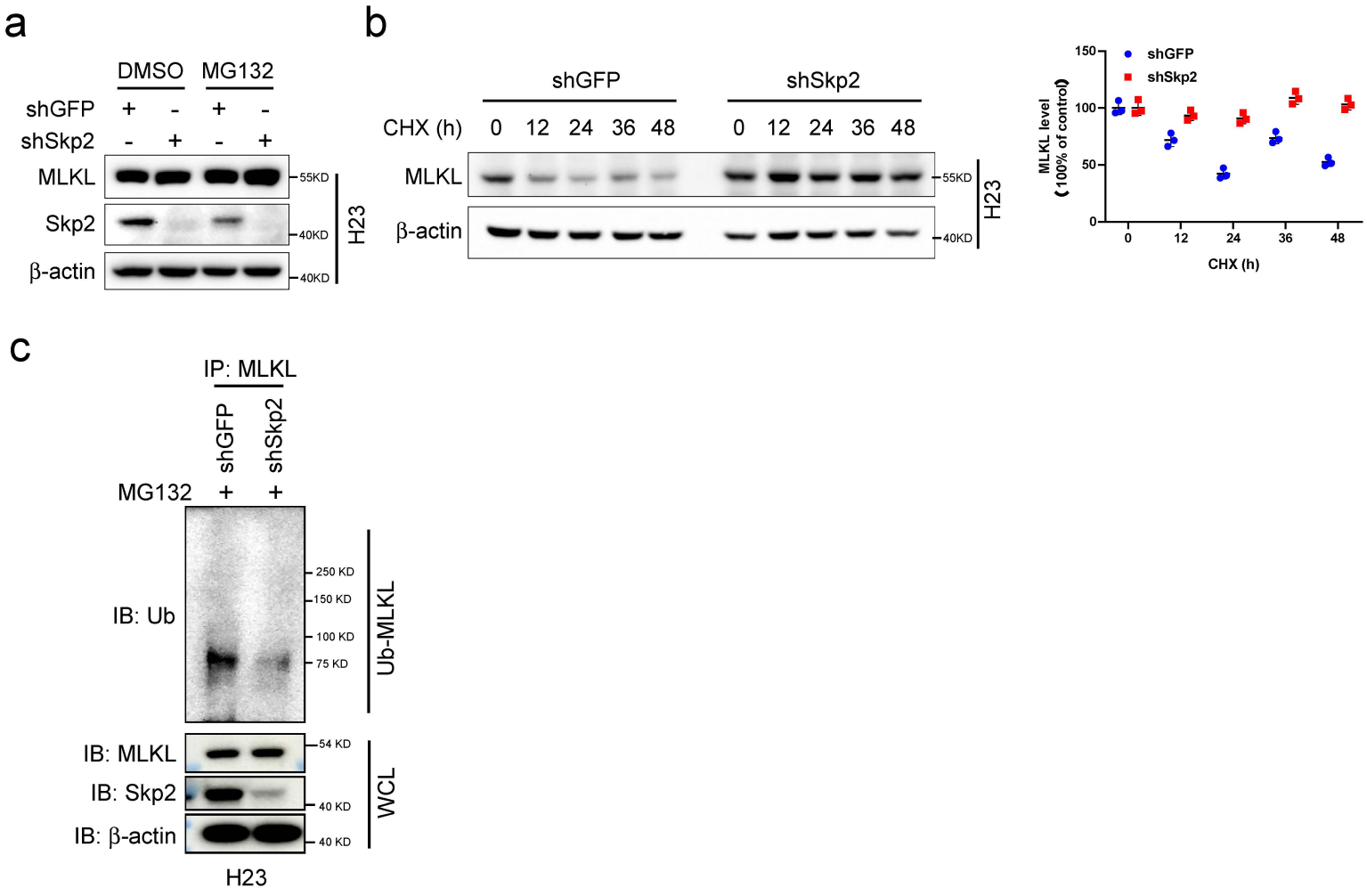
*$p<0.05$ indicates a significant association among the variables.

Supplementary Table 2. Protein expression of MLKL in NSCLC tissues and adjacent non-tumor tissues.

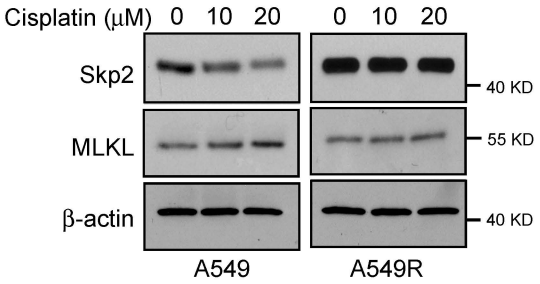
Tissue sample	No. of patients	MLKL		*p*- value
		Low	High	
Tumor	39	26	13	0.0002*
Adjacent	39	9	30	

Chi-square test.

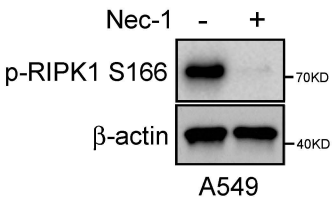
*$p<0.05$ indicates a significant association among the variables.



Supplementary Figure 1 Skp2 regulates the stability and ubiquitination of MLKL protein. a MLKL expression was analyzed in H23 cells expressing shGFP or shSkp2 and treated with MG132 (20 μ M) for 12 h. WCEs were subjected to Western blot analysis. b H23 cells expressing shGFP or shSkp2 were subjected to cycloheximide chase (25 μ g/mL for indicated times). WCEs were subjected to Western blot analysis. c Stable Skp2 knockdown H23 cells were treated with MG132 (20 mM) for 6 h, WCEs were prepared and subjected to MLKL ubiquitination analysis using an ubiquitin antibody.

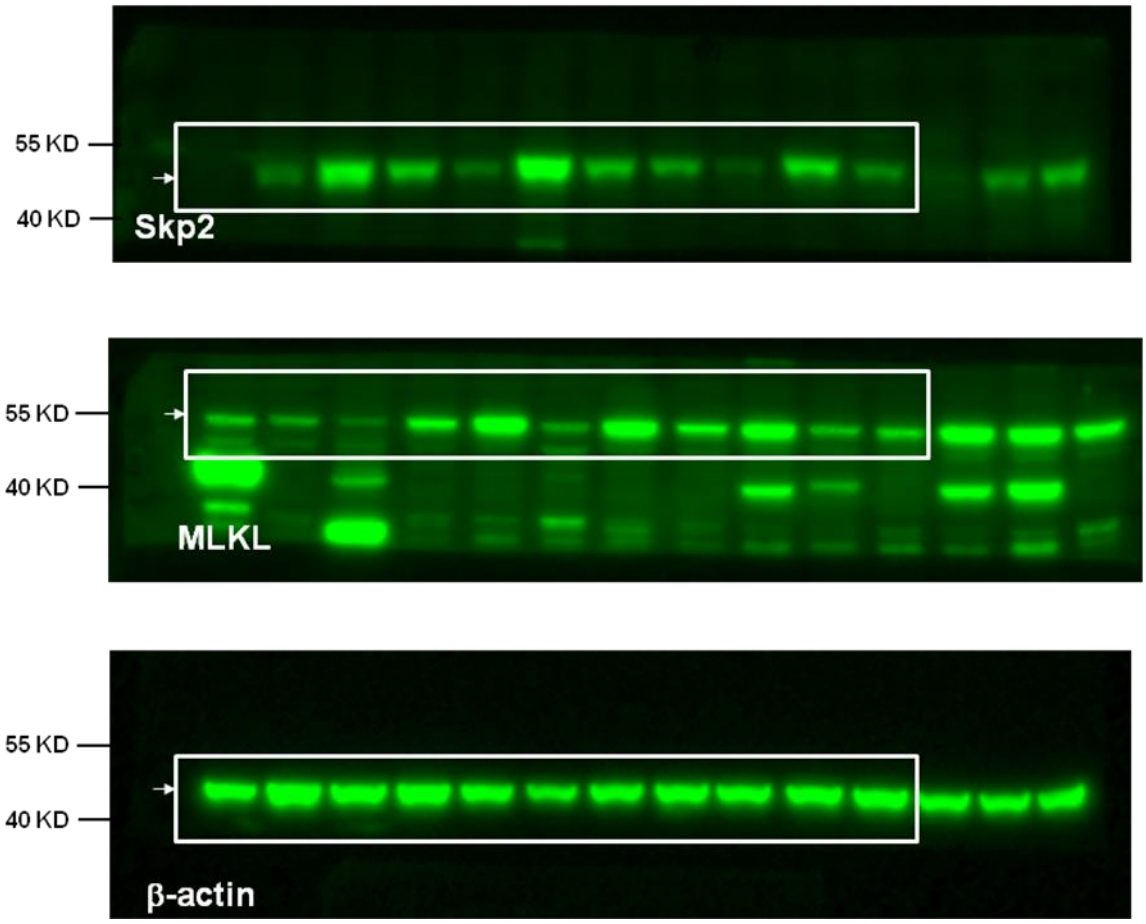


Supplementary Figure 2 Effect of acute exposure to cisplatin on Skp2 and MLKL protein levels in A549 and A549R cells. Cells were treated with indicated doses of cisplatin for 24 h, WCEs were subjected to Western blot analysis.



Supplementary Figure 3 Effect of necrostatin-1 (Nec-1) on the phosphorylation of RIPK1 at Ser166 in A549 cells. Cells were treated with DMSO or Nec-1 (50 μ M) for 24 h, WCEs were subjected to Western blot analysis.

Fig. 1a



By FluorChem FC2 imaging system (Alpha innotech, USA)

Fig. 1c

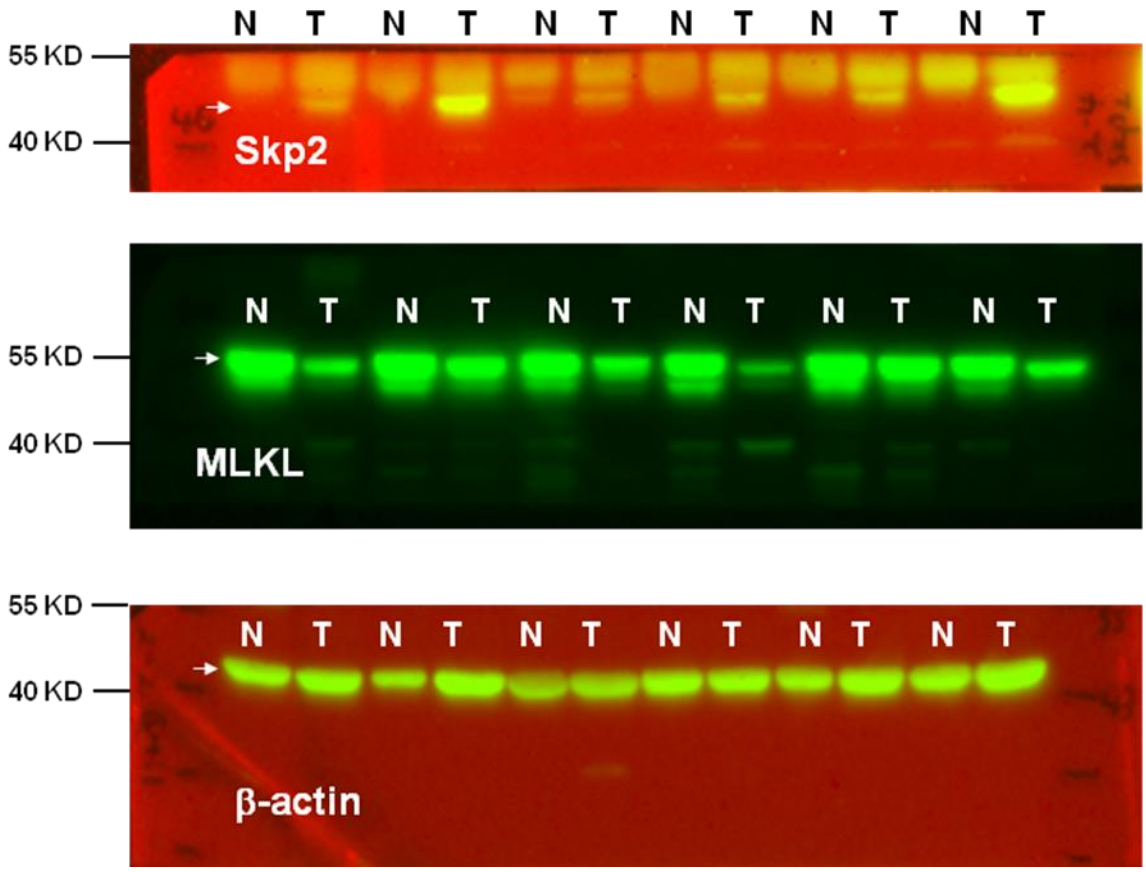


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Fig. 1e

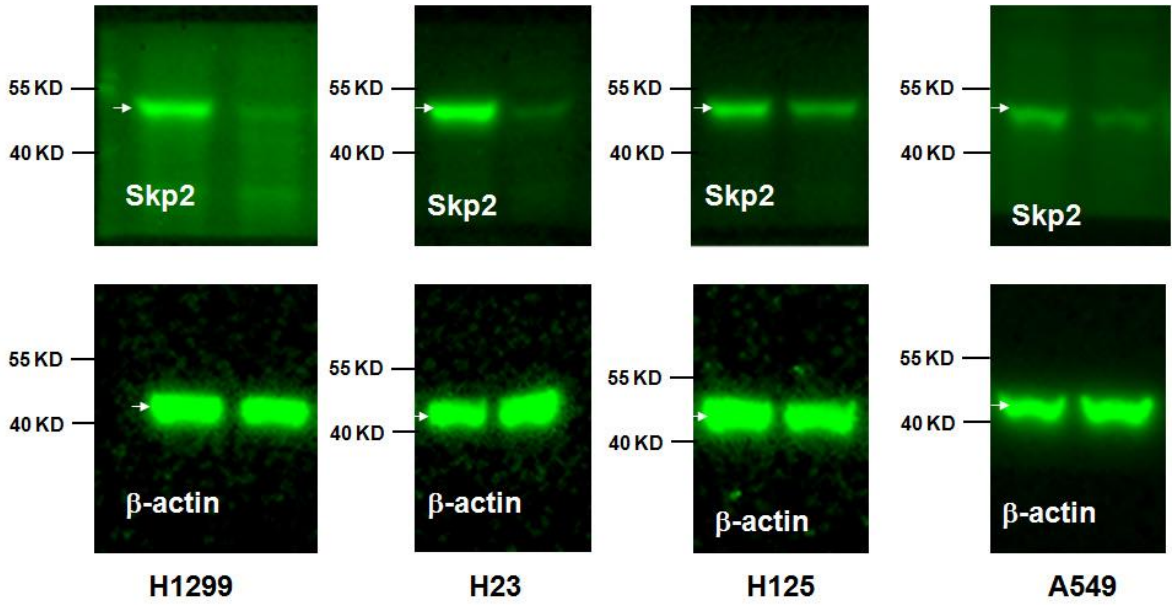

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Fig. 3a

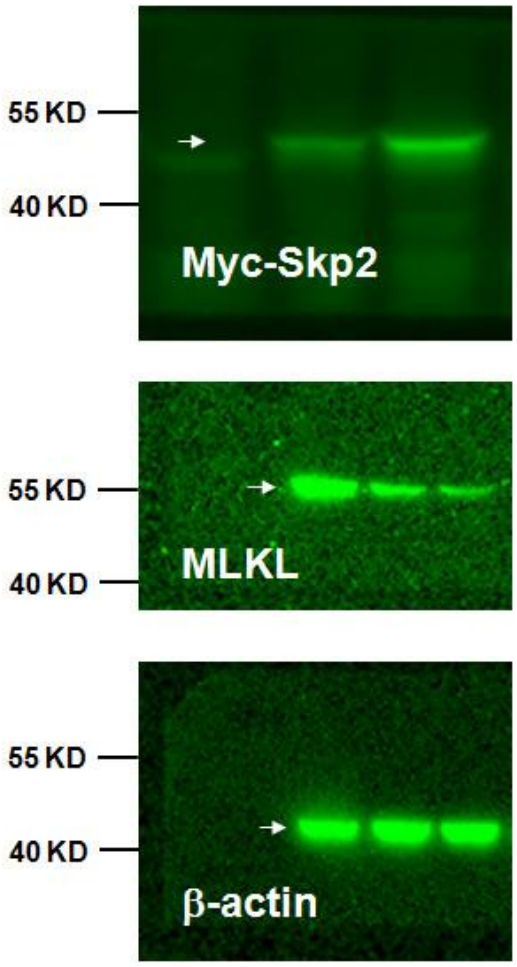

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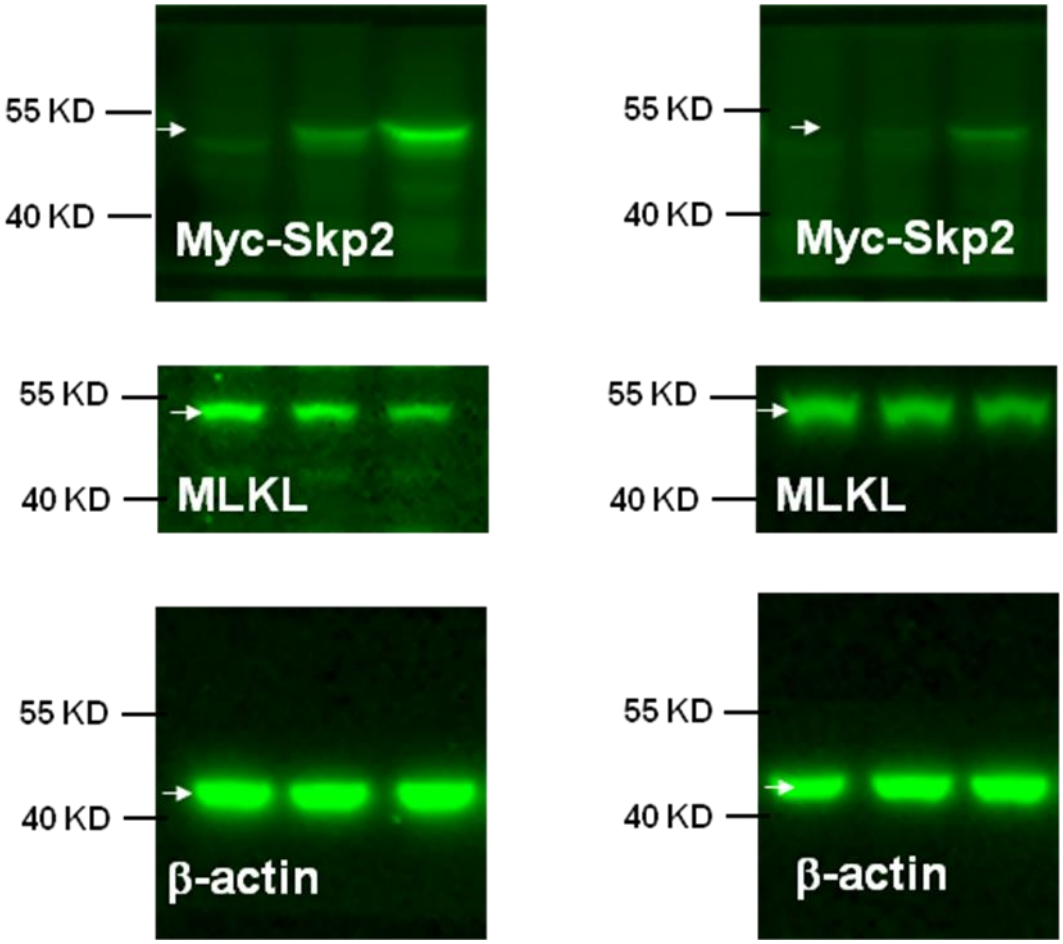
Fig. 3b



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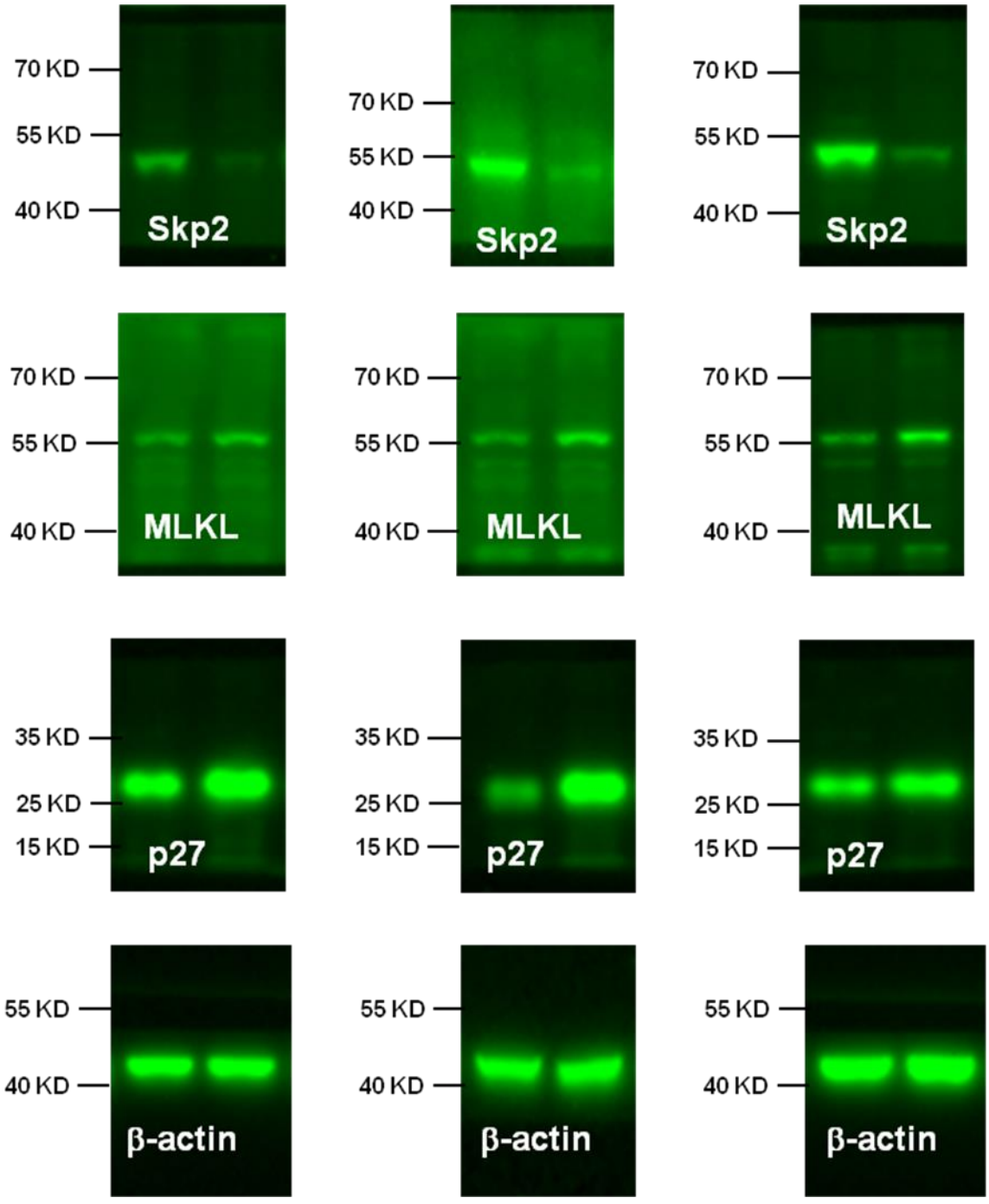

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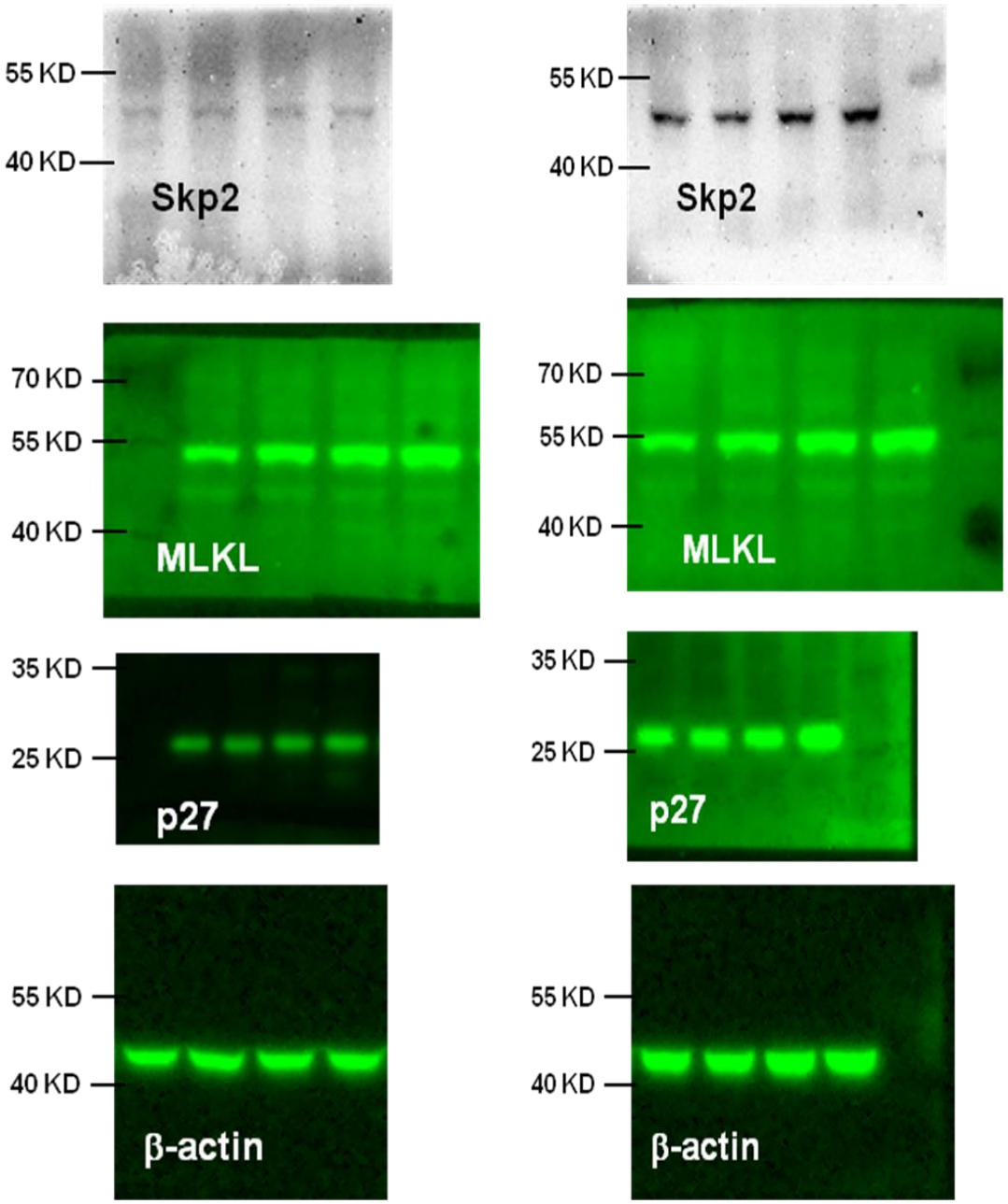
Fig. 3d



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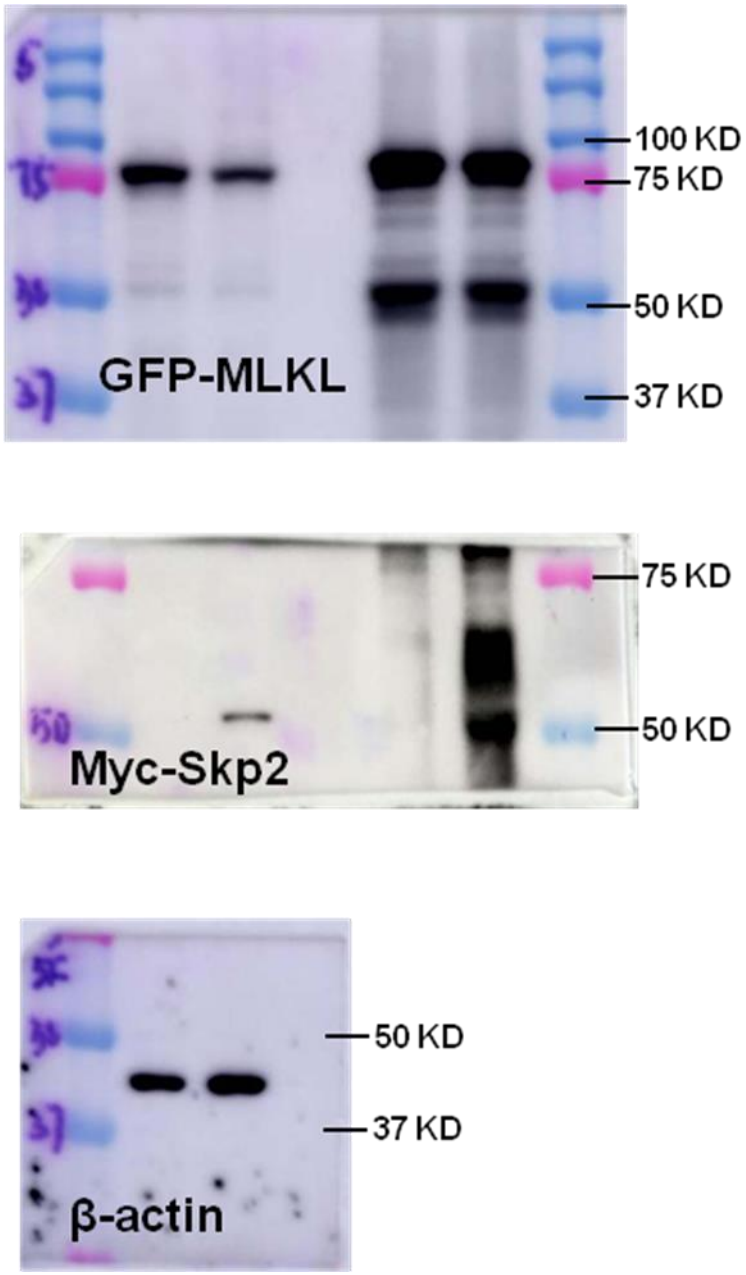

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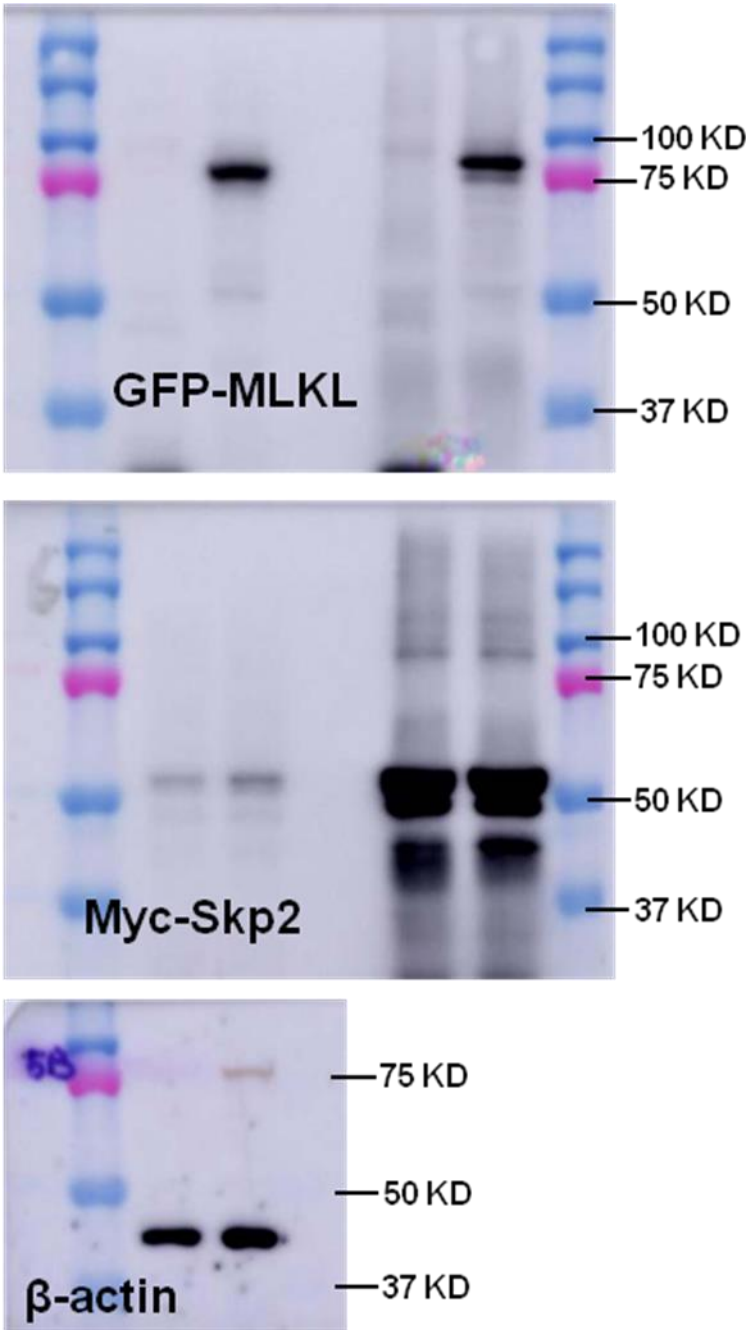

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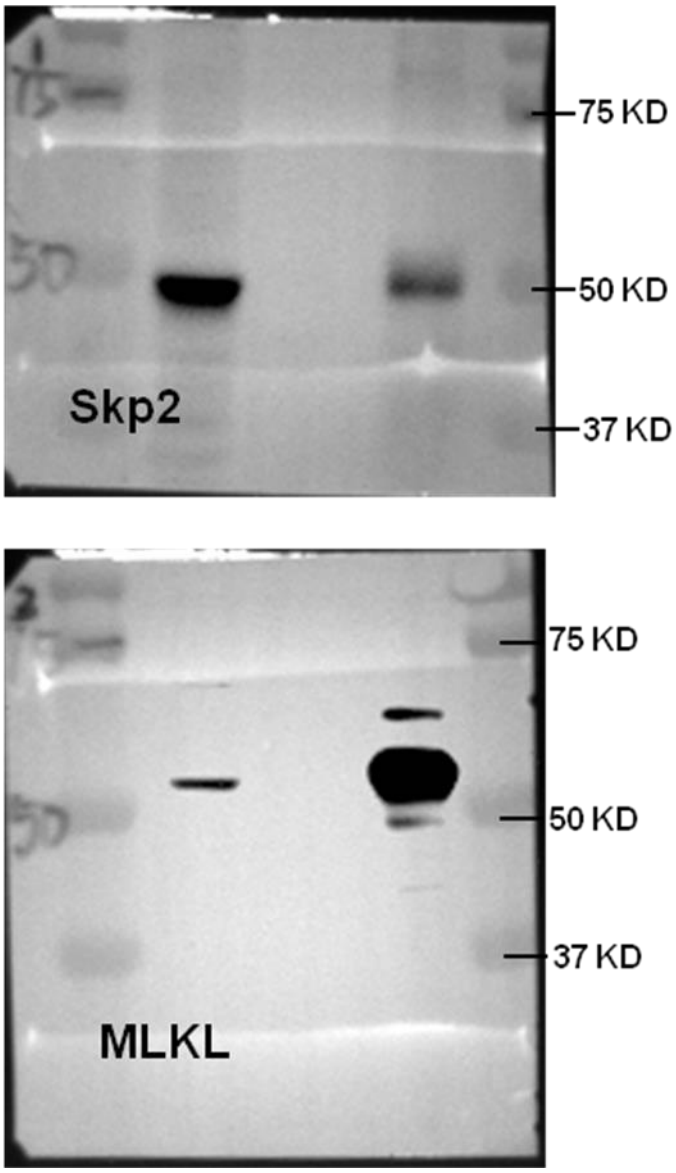

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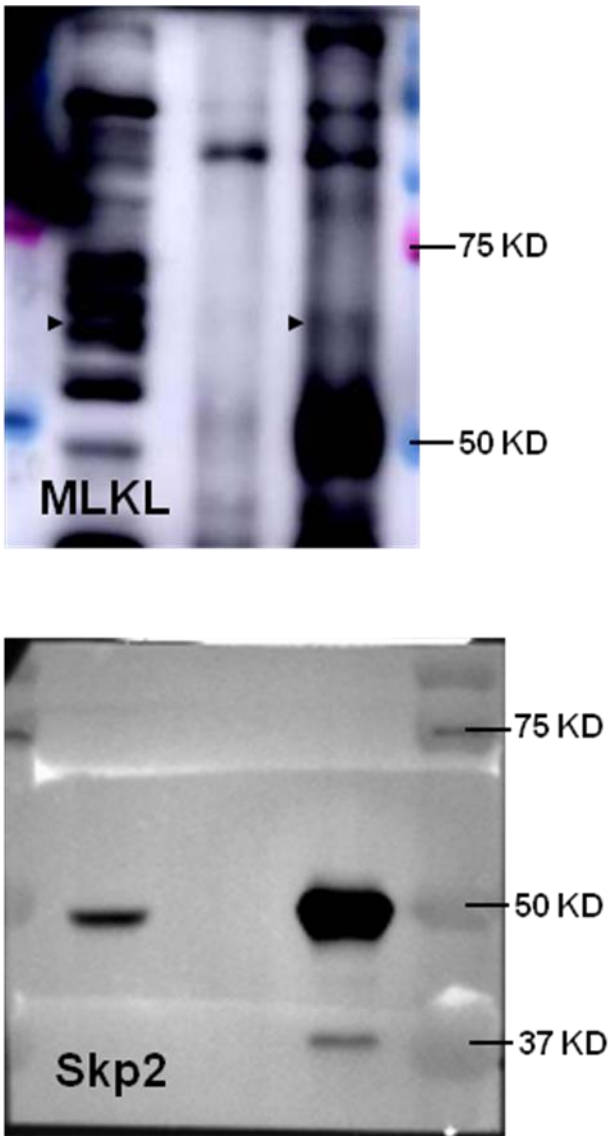

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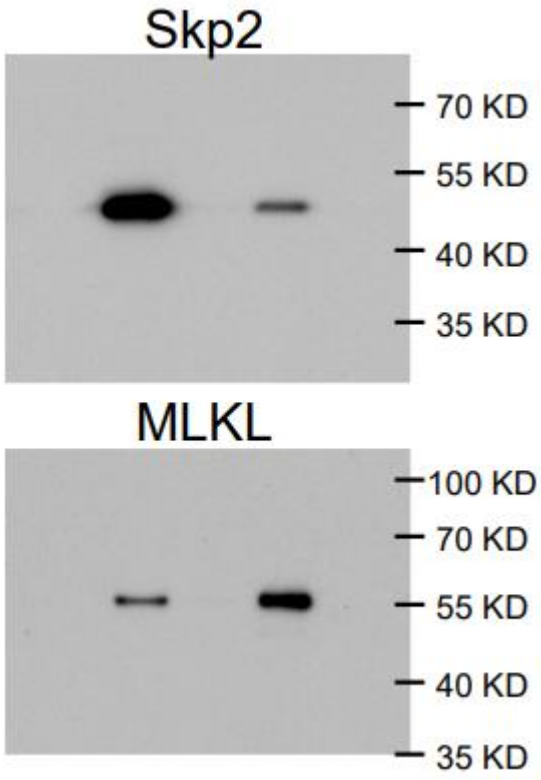
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MLKL
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Fig. 4f

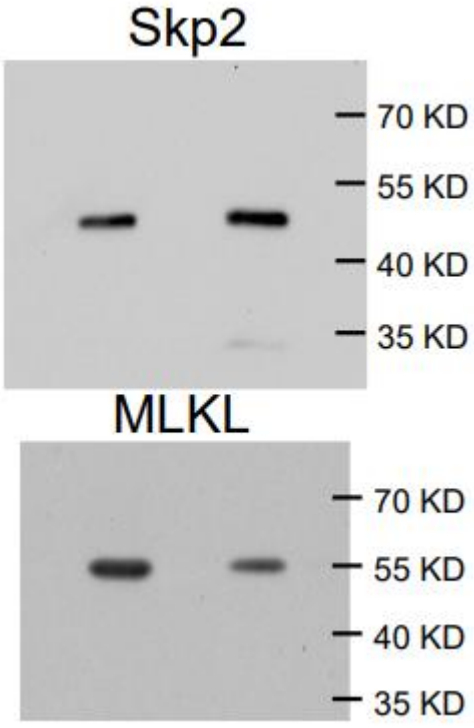
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Fig. 4g

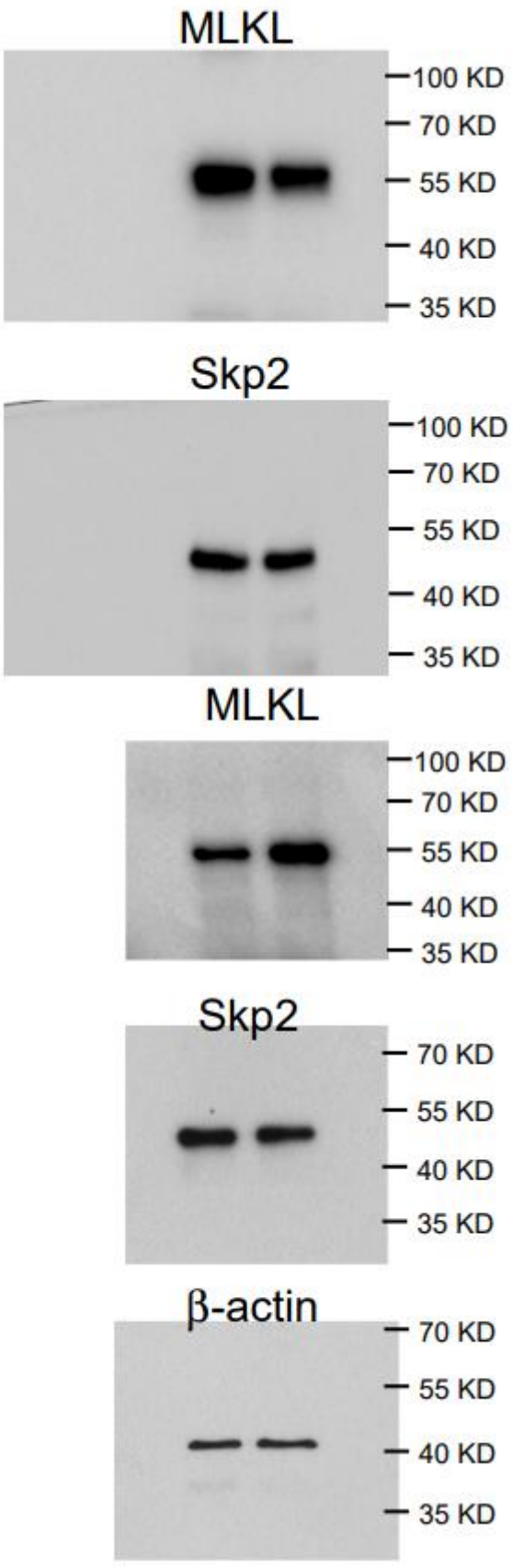
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Fig. 5a

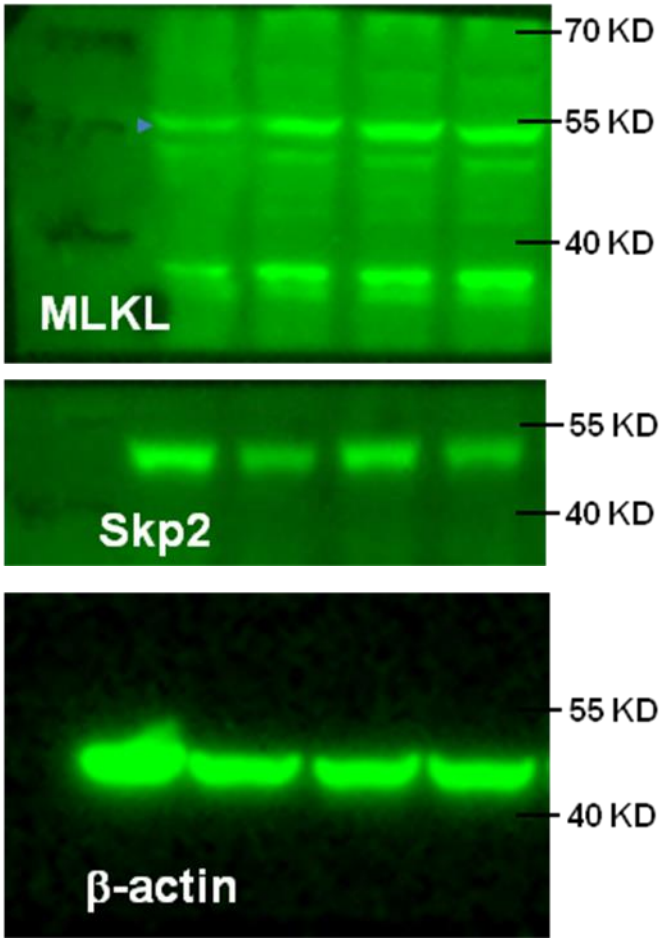

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Fig. 5b

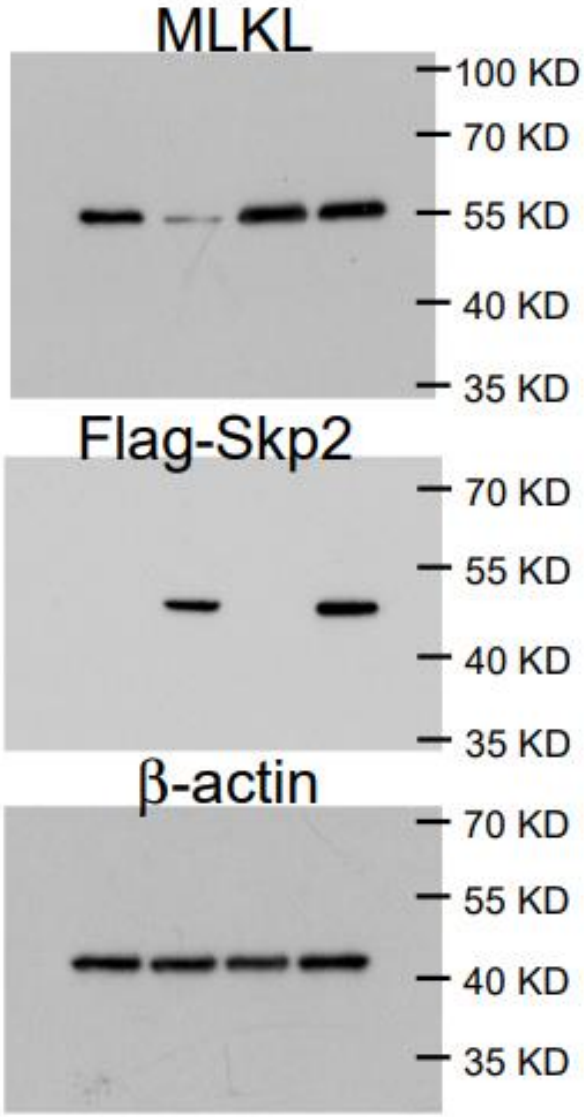
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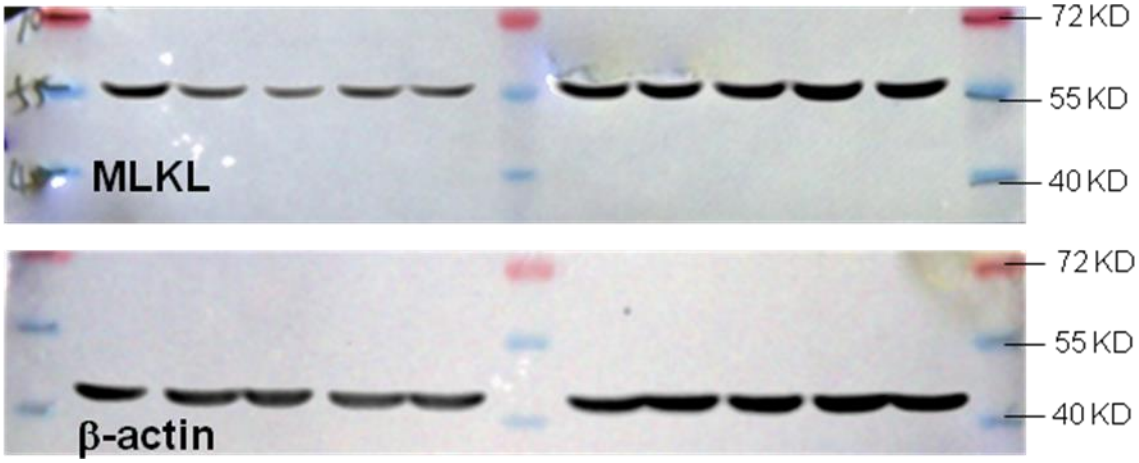
Fig. 5c



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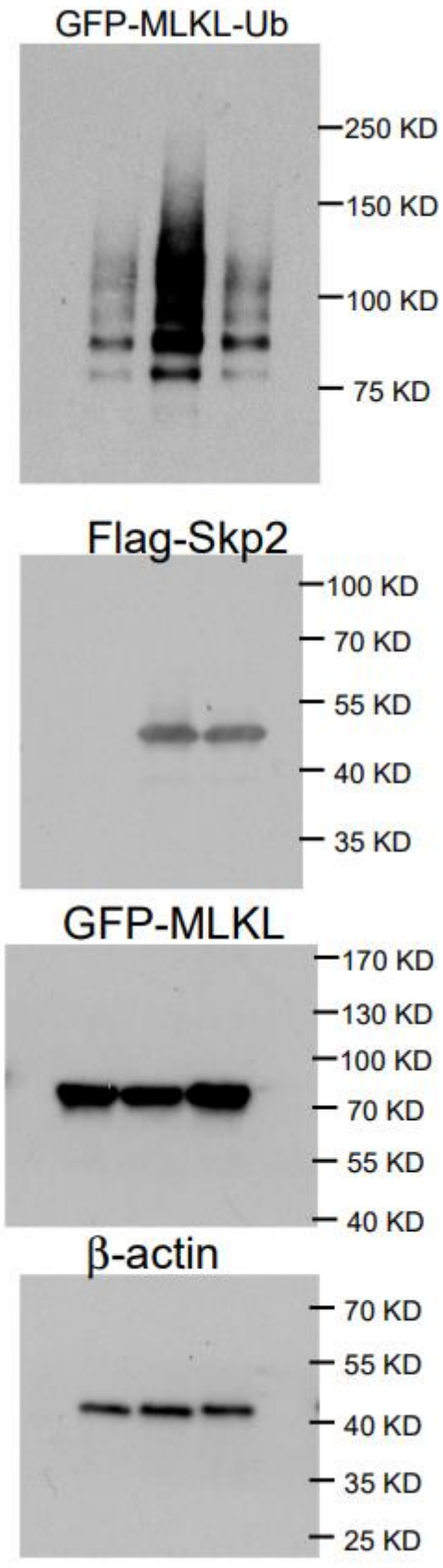
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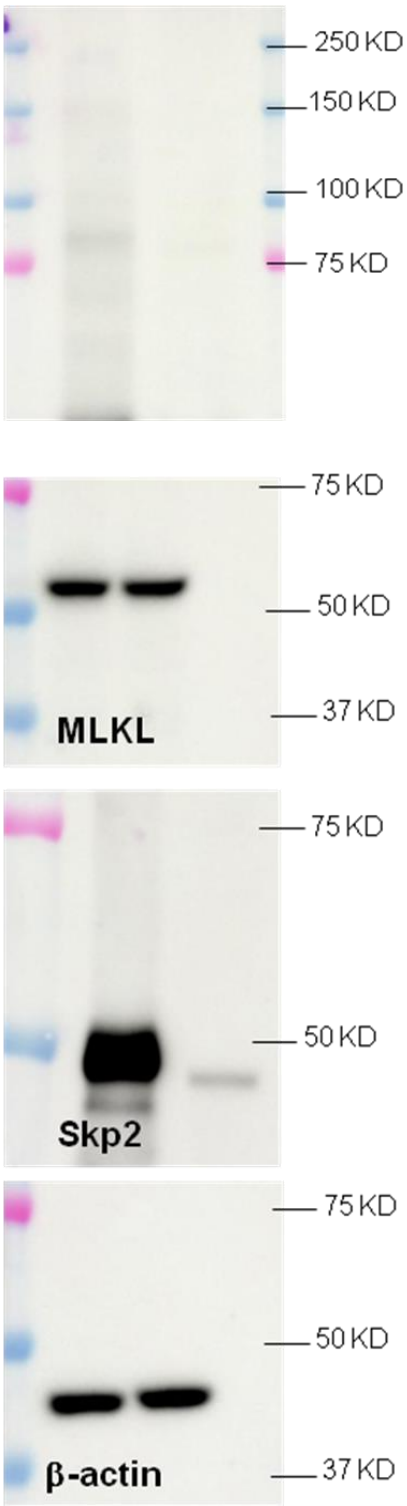
Fig. 5e



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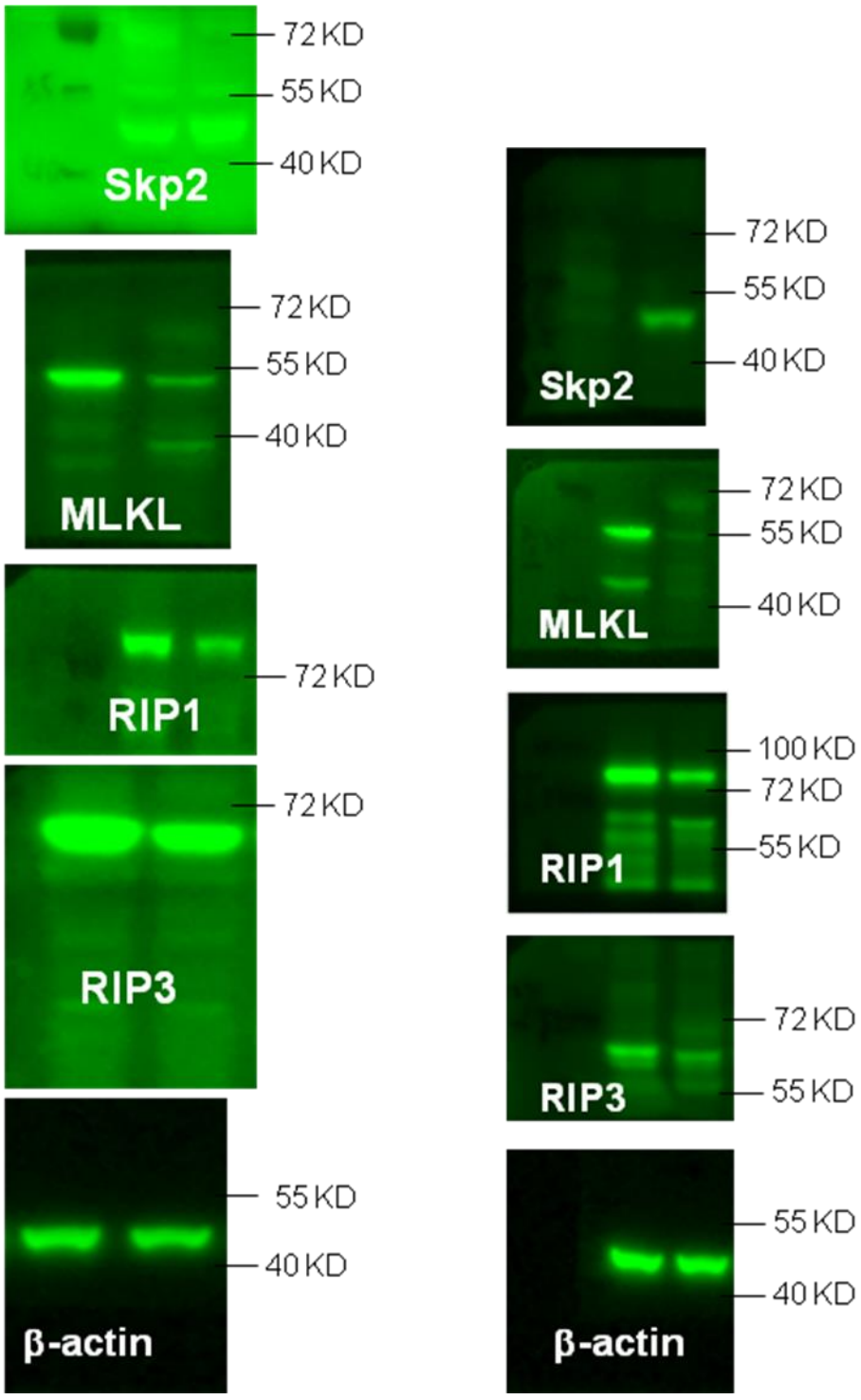

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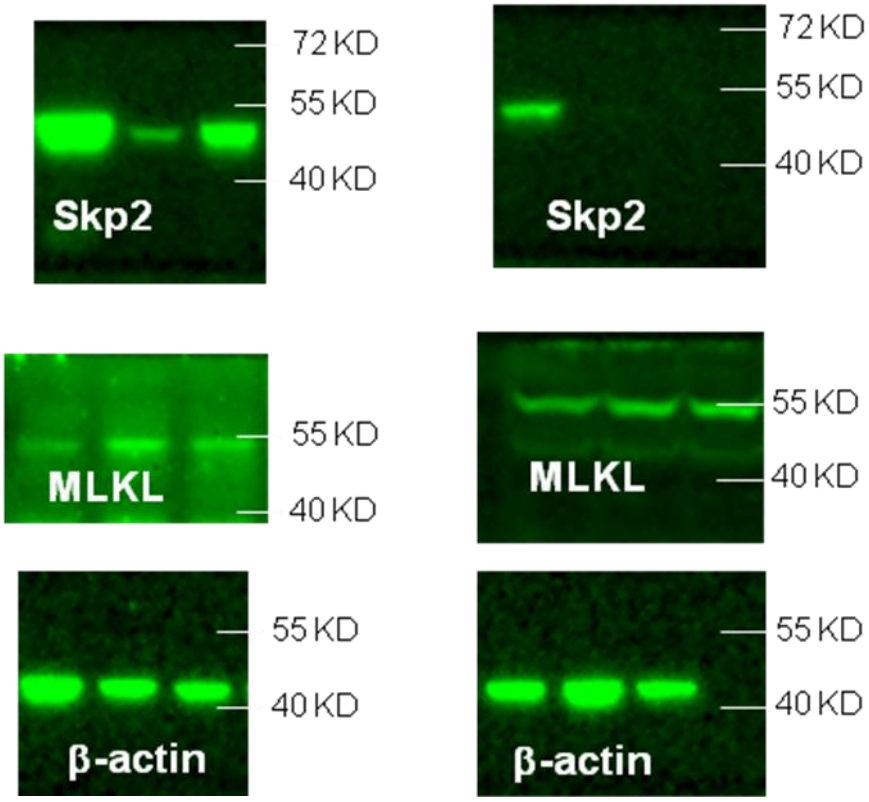
Fig. 6c



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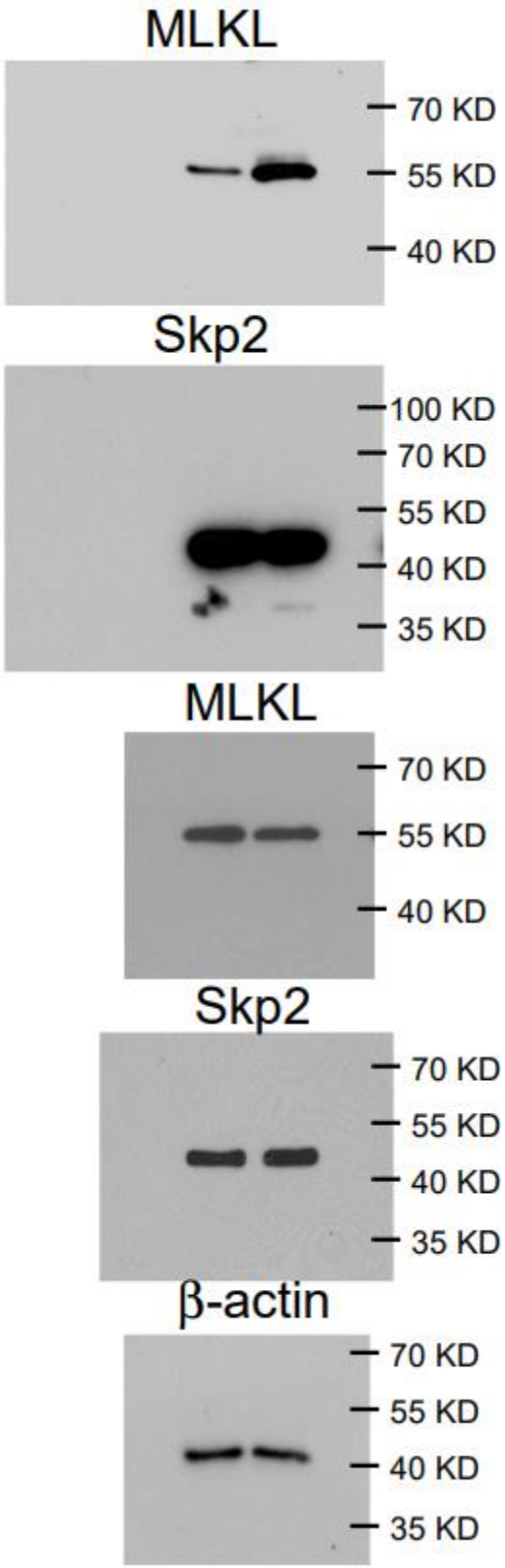
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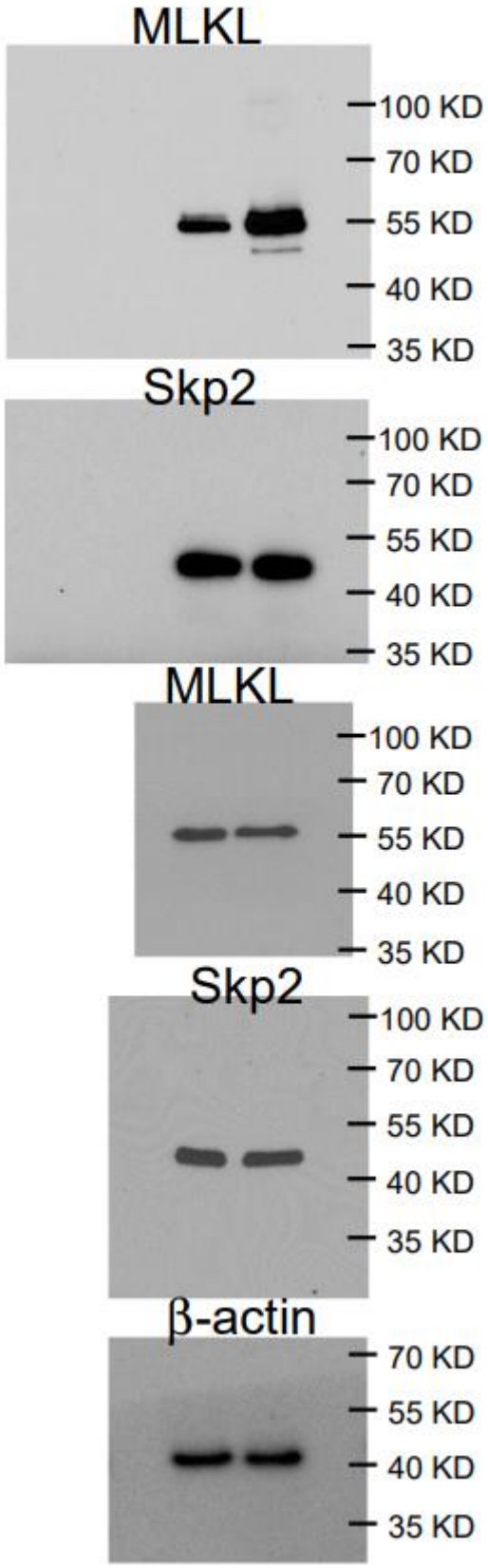
Fig. 6g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β-actin
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Fig. 7a

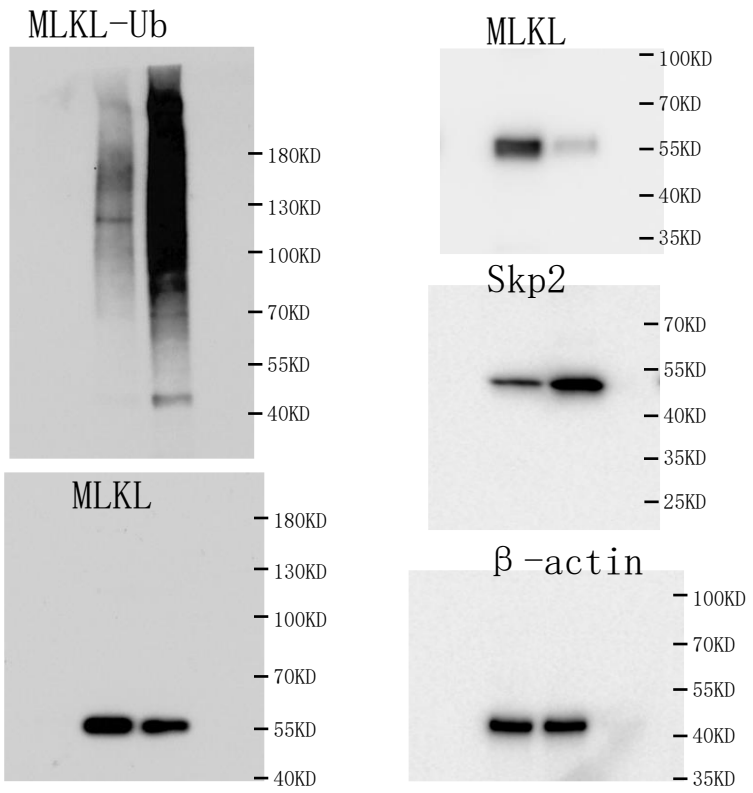
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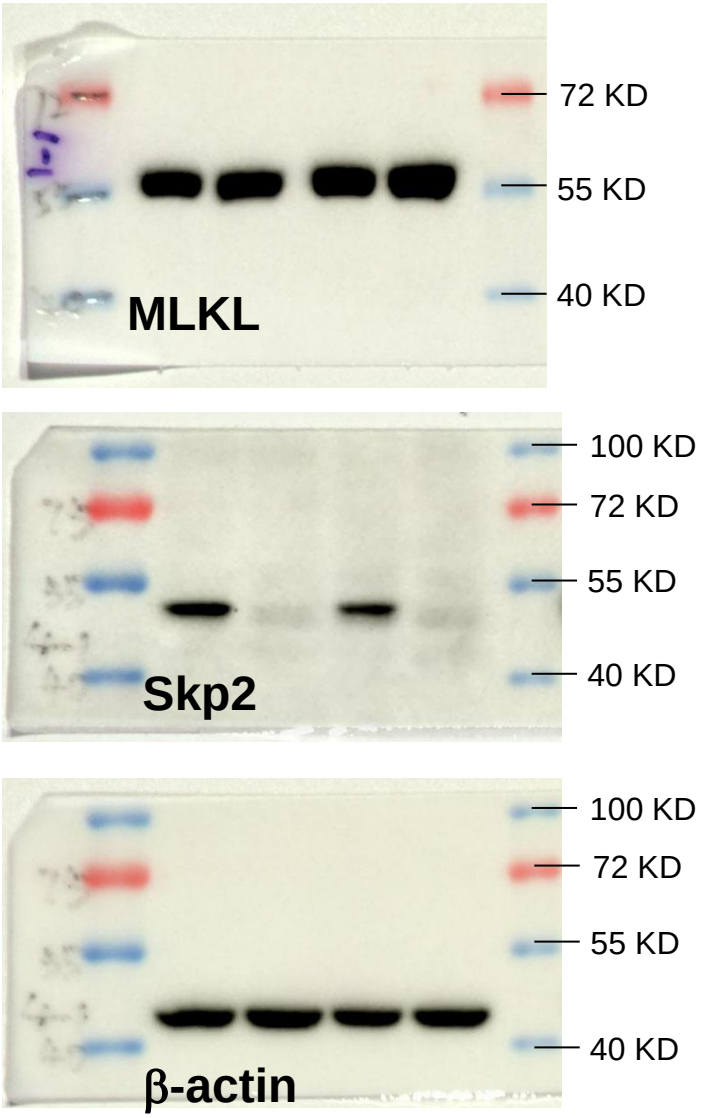
Fig. S1a



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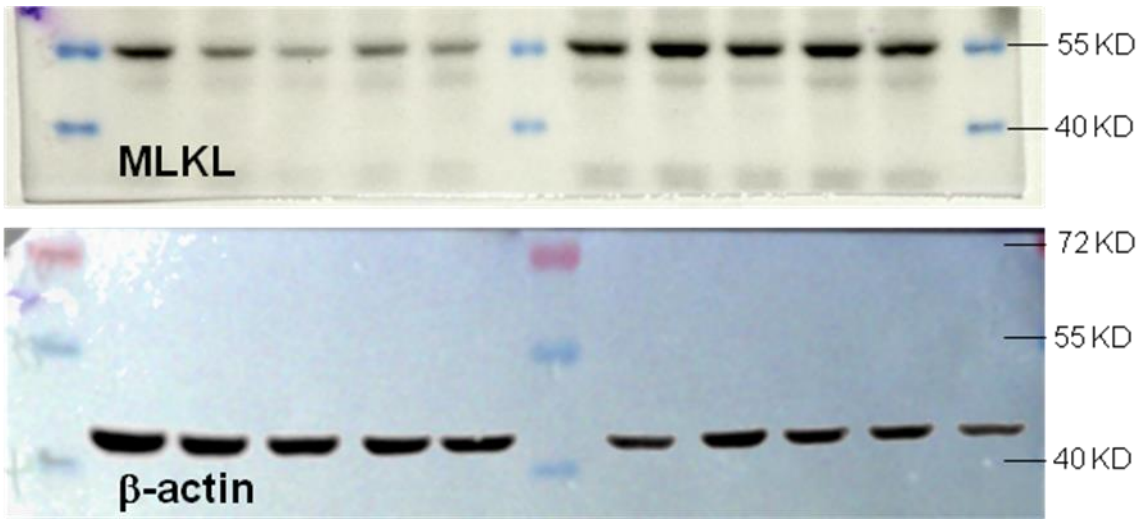

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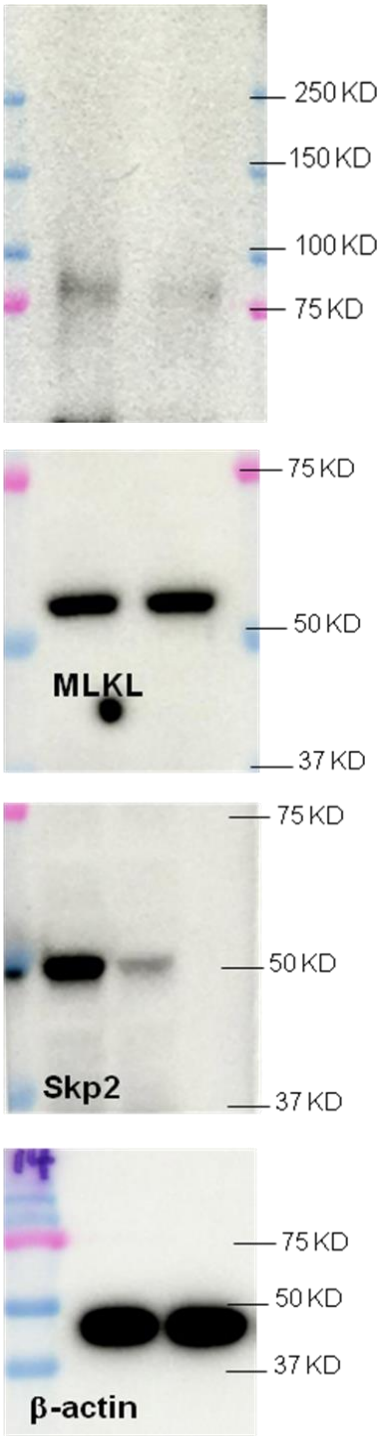

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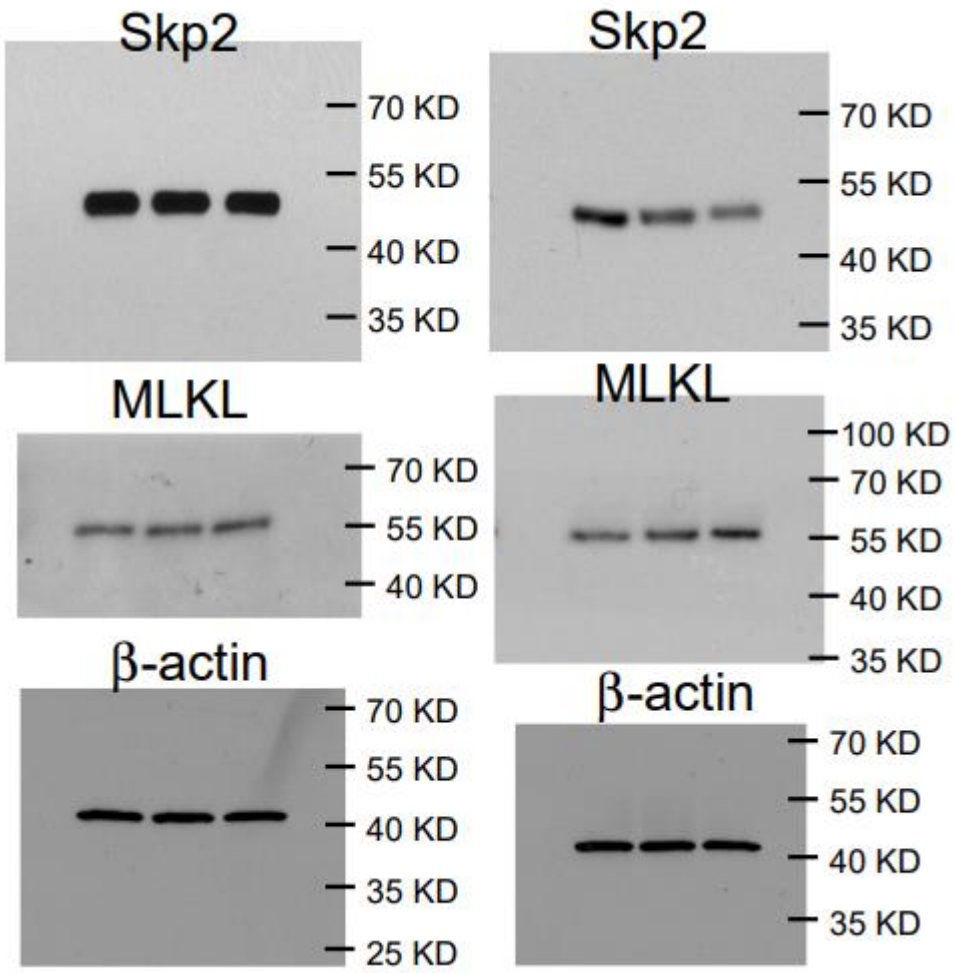
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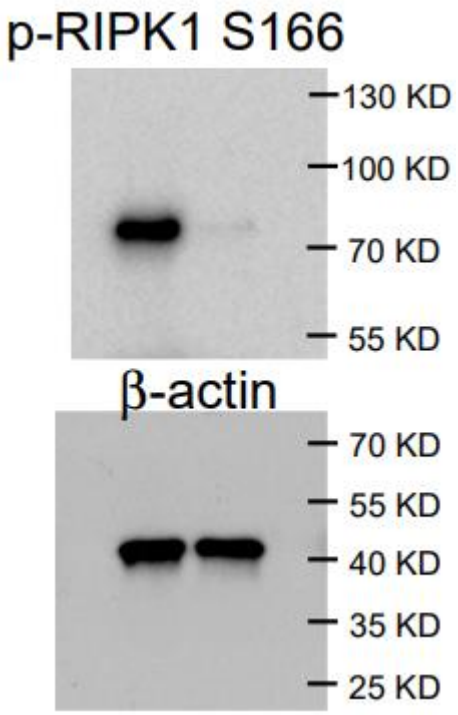
Fig. S3



Supplementary Figure 4 uncropped and unedited blots and gels.