

**Dear Author(s),**

***This document is divided into a number of sections in which you can add your comments to the video, voiceover, and online text/PDF. Please be aware that our policy is to do a single complimentary revision, so it is critical that all participants in this project offer their comments collectively. In addition, please make sure that your comments are easily interpreted and transparent.***

***Have fun!***

**Protocol Name: JoVE 59111, Buser and Spiess**

**Date: February 3, 2019 (Corrections)**

**Authors and Affiliations – Complete – no comments**

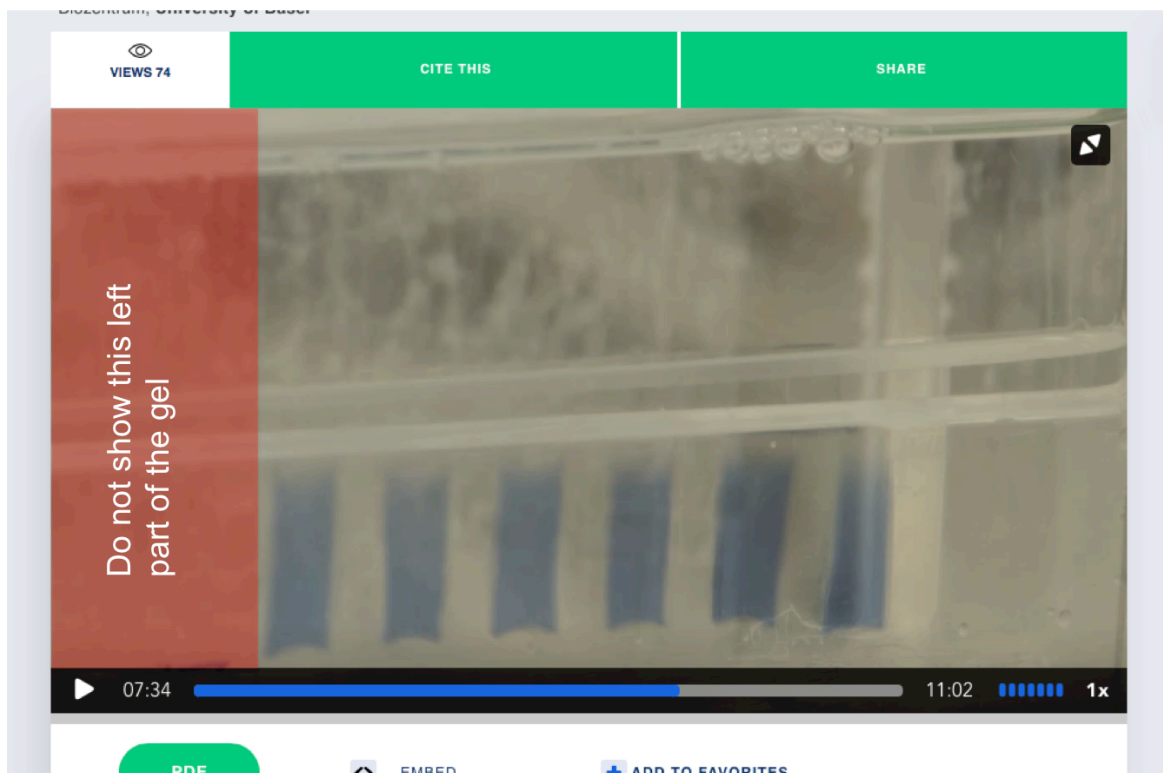
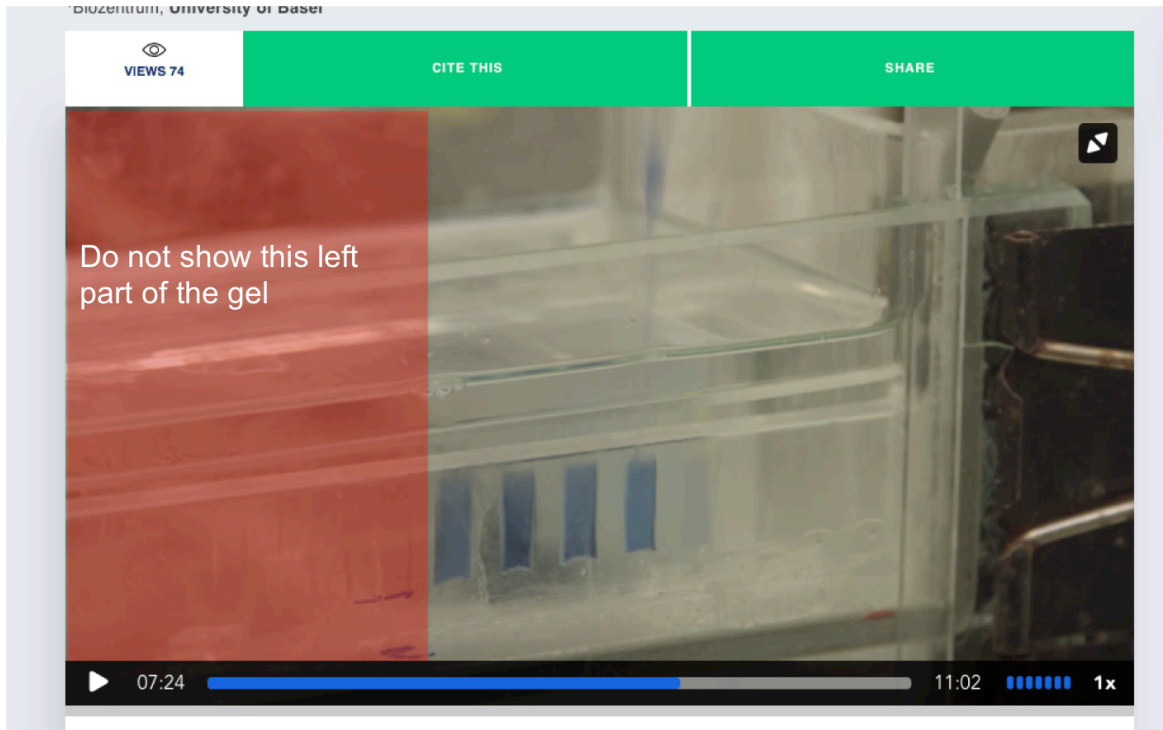
Please fill in any missing author information not included in the video.

Order	Author	Affiliation	Email

## Video Comments

Please fill in any comments you wish to make using the table below using the example as a guide. If you need more space to write, please do so below the table. **DO NOT ADD CORRECTIONS TO THE NARRATION HERE. PLEASE DO THIS IN THE AUDIO COMMENTS SECTION.**

	<b>Time code</b>	<b>Comment</b>	<b>Requested Change</b>
<i>Example</i>	<i>2:52</i>	<i>Onscreen text says use 0.25 mM Fluo-4</i>	<i>Text should say use 0.50 mM Fluo-4</i>
1.	<i>2:25</i>	<i>Three centrifugations are bottles are shown. Please label them in the video with a text</i>	<i>Left bottle: <b>VHH-mCherry</b> Middle bottle: <b>VHH-APEX2</b> Right bottle: <b>VHH-2xTS</b></i>
2.	<i>7:23-7:34</i>	<i>Please do not show the part of the gel left from the first blue lane filled. Blue lanes should only be seen</i>	<i>Please process the video parts from 7:23-7:34 again so that the empty lanes on the left of the blue lanes cannot be seen (Please see snapshots below)</i>
3.	<i>8:56-10:05</i>	<i>It would be very good if there would be a legend shown for the abbreviations CNX, CDMPR and TfR</i>	<i>Please highlight in the video the abbreviations for what they stand for: <b>CNX: Calnexin</b> <b>CDMPR: Cation-dependent mannose-6-phosphate receptor</b> <b>TfR: Transferrin receptor</b></i>
4.	<i>10:57</i>	<i>Would it be possible to add a video acknowledgment at the end?</i>	<i>Add: <b>Acknowledgements: Karin Hinni</b></i>



## Audio Comments – Complete – no comments

This section is used to specify the changes that need to be made to the narration. Please follow the example below as a guide to list your changes. If there is a pronunciation change, please provide a phonetic pronunciation key.

	Time code	Comment	Step(s) in Shotlist	Rewritten Text or Corrected Pronunciation (highlight in bold)
<i>Example</i>	1:49	Original Script Text:  “Then, show the participant their electromyography, or EMG patterns, which correspond to eight specific and unique polar plots.”	2.2	Rewritten Script Text:  “Then, <b>show the participant the unique and specific polar plots, which correspond to their electromyography, or EMG (pronounced E-M-G) patterns.</b> ”
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## Online Text/PDF Protocol – MAJOR ISSUES

All indications in the protocol with micro ( $\mu$ ) were transformed into milli (m). Our original text was correct, but your online and PDF text has introduced these mistakes. They need to be urgently corrected. There are many  $\mu$  symbols in our text. Please use our word document (R3) as reference to accordingly copy our protocol into the online text/PDF protocol. It is easier to correct from the word file than listing mistakes below. In the Figures, the  $\mu$  has been apparently correctly introduced, but not in the protocol text.

Please use this table to address changes that need to be made to the online text/PDF document. Both the online text and PDF are generated from the HTML template of your article. Since the PDF is generated from the HTML by our conversion software, it may contain formatting errors. For major structural changes or more than 10 spelling or grammatical mistakes, we will require re-upload of the entire document.

See comment above

	Protocol Step	Comment	Requested Change (highlight in bold)
Example	1.1	Step says “Centrifuge lysate at 2,000 x g.”	Please correct to “Centrifuge lysate at <b>4,000</b> x g.”
1.	Figures 1-3	Low resolution of the Figures 1-3 in the preview or PDF	Include high resolution of the Figures 1-3
2.	General comment	Sometimes, there are empty lanes between the individual online/pdf protocol steps, e.g.  From 3.6 to 3.9  From 3.12 to 3.13  From 4.4 to 4.5  From 6.17 to 6.18	Please correct
3.	In the abstract	Please include a space after the dash before the indicated part starting with small	Now: anti-green fluorescent protein (GFP) nanobodies –small, monomeric, high-affinity protein binders ...

			<p>Correct to:</p> <p>anti-green fluorescent protein (GFP) nanobodies – small, monomeric, high-affinity protein binders ...</p>
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