

Supplementary Information

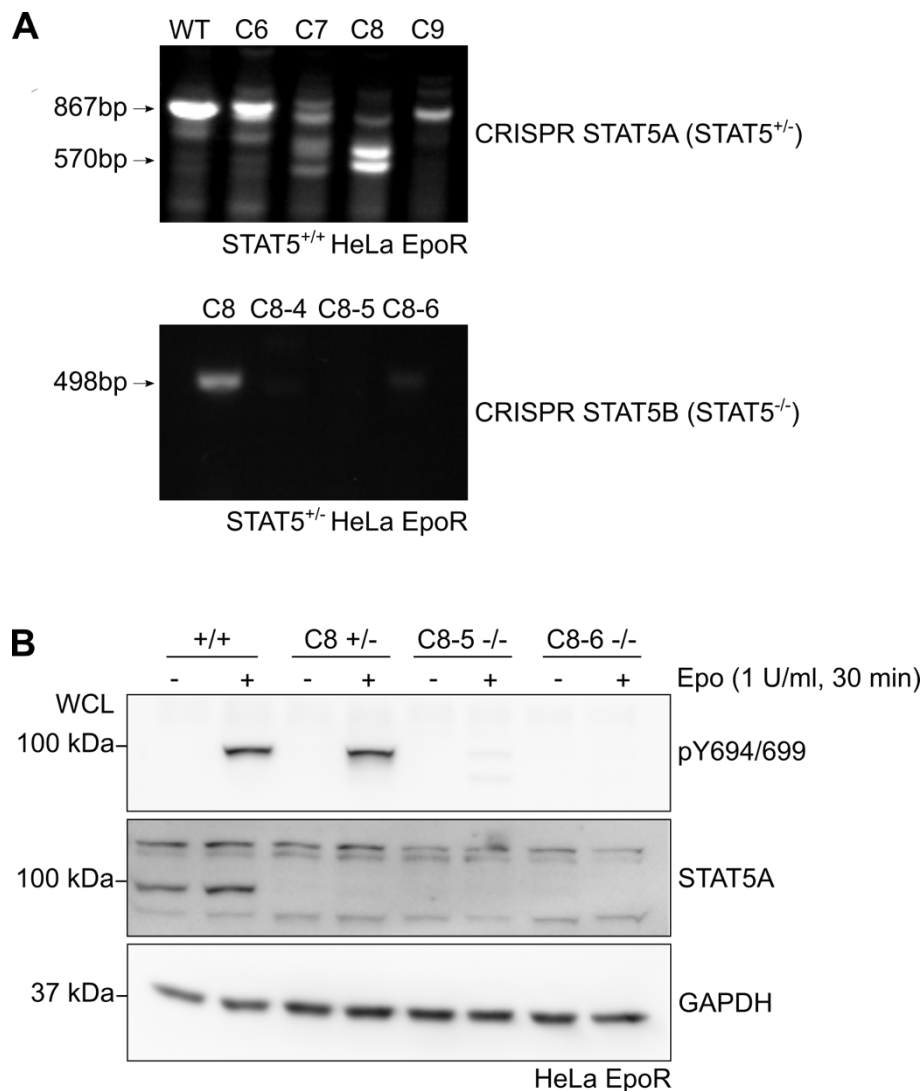
Unexpected functional role of the transactivation domain for nuclear import of STAT5

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Supplementary Figures

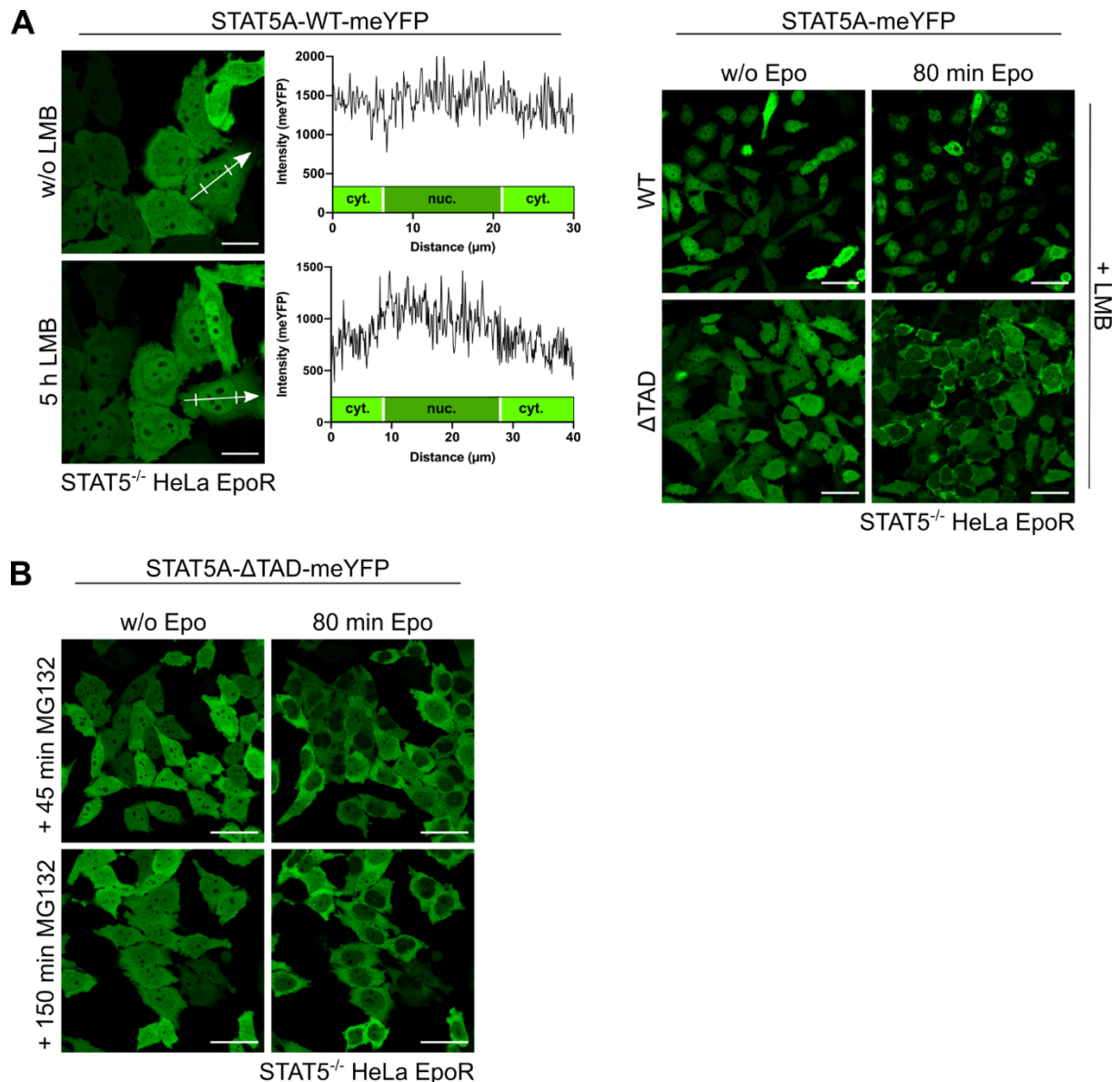


Suppl. Fig. 1: Generation of STAT5^{-/-} HeLa EpoR cells by CRISPR/Cas9-mediated gene targeting of STAT5A and STAT5B.

(A) Upper panel: A defined deletion in the STAT5A gene was introduced into STAT5^{+/+} HeLa T-REx HA-EpoR cells by transfection with four sgRNA/Cas9 expression plasmids (pIST5A 1-4). The presence of the intended genomic deletions was assessed by PCR using a STAT5A-specific primer pair (FP-ST5A-fwd1/rev1), followed by size analysis of the amplicons by agarose gel electrophoresis. The wild-type STAT5A locus yielded an 867 bp amplicon, whereas the deletion allele produced a shorter fragment of 570 bp, corresponding to the expected loss of 297 bp.

Lower panel: A targeted deletion in the STAT5B gene was introduced in STAT5^{+/-} HeLa T-REx HA-EpoR clone C8 cells using four sgRNA/Cas9 expression plasmids (pIST5B 1-4). Detection of deletions was performed by PCR amplification with the specific primer pair FP-ST5B-fwd1/rev1, followed by agarose gel electrophoresis analysis. The expected wild-type STAT5B amplicon was 498 bp in size, while mutant amplicons representing deletions were not detected under these conditions.

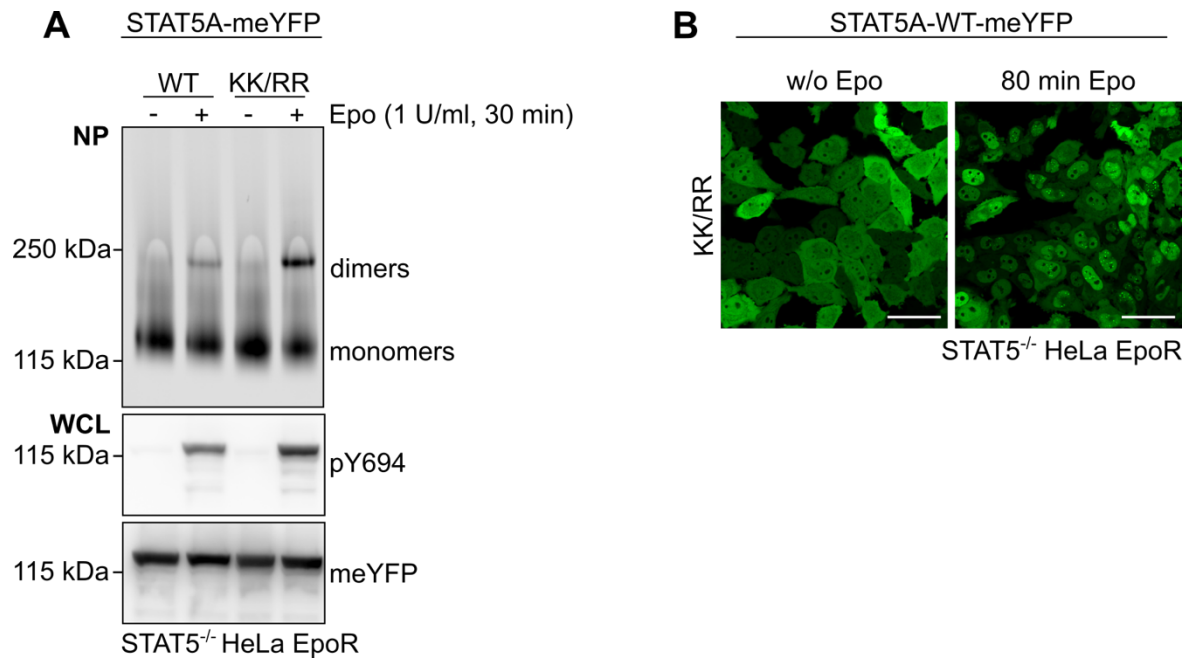
(B) Expression of STAT5A and Epo-induced phosphorylation of STAT5A and STAT5B were analyzed in HeLa T-REx HA-EpoR cells (STAT5^{+/+}), STAT5A-deficient clone 8 (C8^{+/-}) and two STAT5A/B-deficient subclones (C8-5^{-/-} and C8-6^{-/-}). Cells were stimulated with Epo as indicated. Whole cell lysates (WCL) were prepared and subjected to Western blot analysis. Phosphorylated STAT5A and STAT5B were detected using antibodies against phospho-STAT5 (pY694/699), while total STAT5A levels were assessed with a STAT5A-specific antibody. GAPDH served as a loading control.



Suppl. Fig. 2: Nucleocytoplasmic shuttling of STAT5A WT and Δ TAD deletion mutant after inhibition of CRM1-mediated nuclear export or MG132-mediated proteasome inhibition.

(A) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A-WT-meYFP were treated with 30 nM leptomycin B (LMB) as indicated. Left panel: meYFP fluorescence was detected via confocal live cell imaging. Scale bars: 20 μ m. Intensity profiles of meYFP fluorescence were generated along the white arrows depicted in the images, with cytoplasm (cyt.)/nucleus (nuc.) boundaries indicated. Right panel: STAT5^{-/-} HeLa EpoR cells transduced with STAT5A-WT-meYFP or STAT5A- Δ TAD-meYFP were preincubated with 30 nM LMB and subsequently stimulated with 1 U/ml Epo as indicated. meYFP fluorescence was detected via confocal live cell imaging. Scale bars: 50 μ m.

(B) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A- Δ TAD-meYFP were preincubated with 40 μ M MG132 and subsequently stimulated with 1 U/ml Epo as indicated. meYFP fluorescence was detected via confocal live cell imaging. w/o, without Epo; scale bars: 50 μ m.



Suppl. Fig. 3: Dimerization and subcellular localization of the STAT5A K696R/K700R mutant.

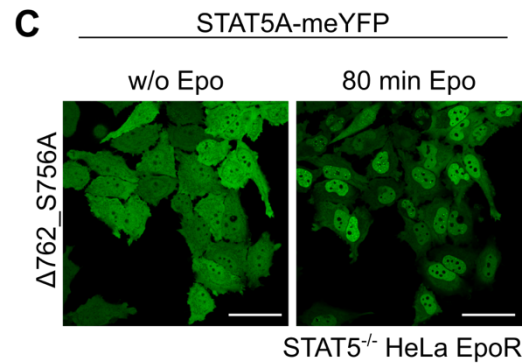
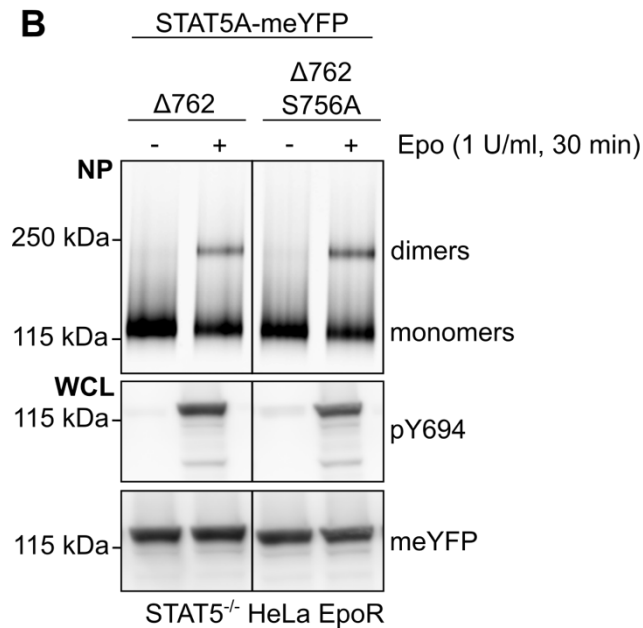
(A) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A-WT-meYFP or STAT5A-K696R/K700R-meYFP (KK/RR) were stimulated with Epo as indicated. Whole cell lysates (WCL) were analyzed by native PAGE (NP) or immunoblotting with the indicated antibodies.

(B) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A-K696R/K700R-meYFP (KK/RR) were stimulated with 1 U/ml Epo as indicated. meYFP fluorescence was detected via confocal live cell imaging. w/o, without; scale bars: 50 μ m.

STAT5A	Amino acid sequence			Localization before/after Epo stimulation	
	1-750	...751-762...	...793...	-	+
WT -(meYFP)	M1-E750	FDLDESMDVARH	793-STOP 793-meYFP-STOP		
ΔTAD -(meYFP)	M1-A717	----	STOP meYFP-STOP		
Δ750 -meYFP	M1-E750	----	meYFP-STOP		
Δ756 -meYFP	M1-E756	FDLDES	meYFP-STOP		
Δ762 -meYFP	M1-E750	FDLDESMDVARH	meYFP-STOP		
ΔTAD+12aa -meYFP	M1-A717	FDLDESMDVARH	meYFP-STOP		
12A -meYFP	M1-E750	AAAAAAAAAAAA	793-meYFP-STOP		
9WT3A -meYFP	M1-E750	FDLDESMDVAAA	793-meYFP-STOP		
3A9WT -meYFP	M1-E750	AAADESMDVARH	793-meYFP-STOP		
3A6WT3A -meYFP	M1-E750	AAADESMDVAAA	793-meYFP-STOP		
3WT6A3WT -meYFP	M1-E750	FDLAAAAAARH	793-meYFP-STOP		
2Dto2A -meYFP	M1-E750	FDLAESMAVARH	793-meYFP-STOP		
10Aand2D -meYFP	M1-E750	AAADAAADAAAA	793-meYFP-STOP		

Suppl. Fig. 4: Overview of STAT5A deletion and alanine-scanning constructs and schematic representation of their subcellular localizations.

A STAT5A: 751-FDLDE**S**MDVARH-762
 STAT5B: 756-FDL**ED**TMDVAR**R**-767

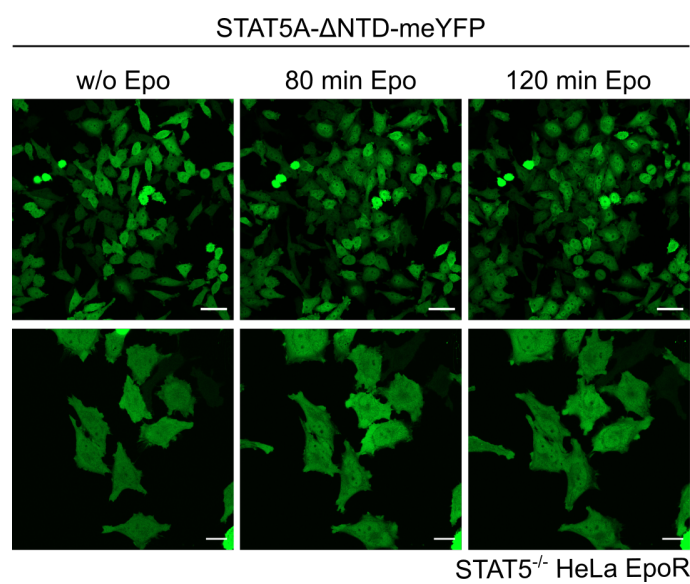


Suppl. Fig. 5: Dimerization and subcellular localization of the STAT5A $\Delta 762$ _S756A mutant.

(A) Amino acid sequences of STAT5A (residues Phe751 to His762) and STAT5B (residues Phe756 to Arg767) are shown. Serine 756 in STAT5A is highlighted in red. Conserved amino acid substitutions in STAT5B compared to STAT5A are indicated in blue.

(B) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A- $\Delta 762$ -meYFP or STAT5A- $\Delta 762$ _S756A-meYFP were stimulated with Epo as indicated. Whole cell lysates (WCL) were analyzed by native PAGE (NP) or immunoblotting with the indicated antibodies.

(C) STAT5^{-/-} HeLa EpoR cells transduced with STAT5A- $\Delta 762$ _S756A-meYFP were stimulated with 1 U/ml Epo as indicated. meYFP fluorescence was detected via confocal live cell imaging. w/o, without; scale bars: 50 μ m.

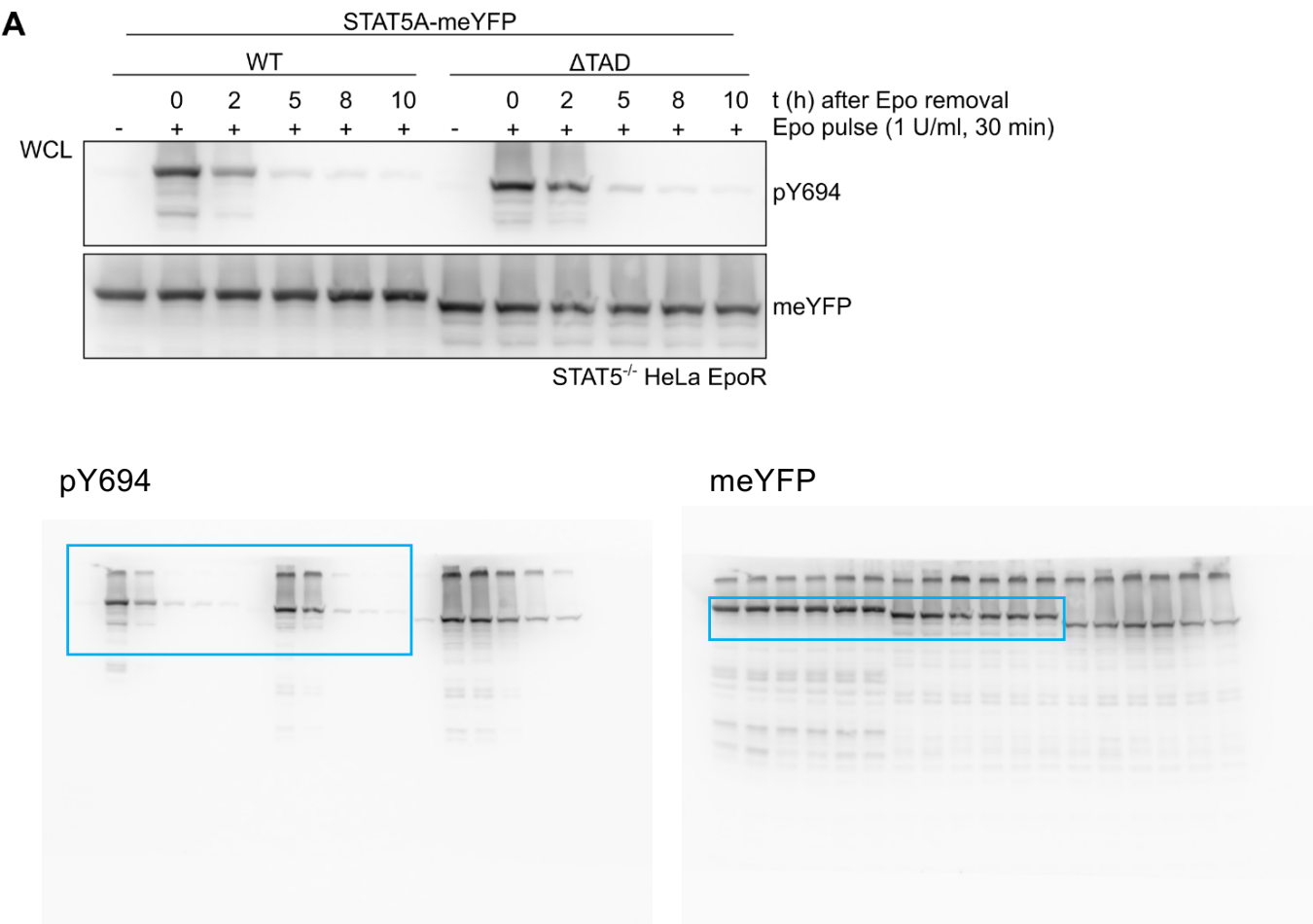


Suppl. Fig. 6: Subcellular localization of the STAT5A ΔNTD deletion mutant.

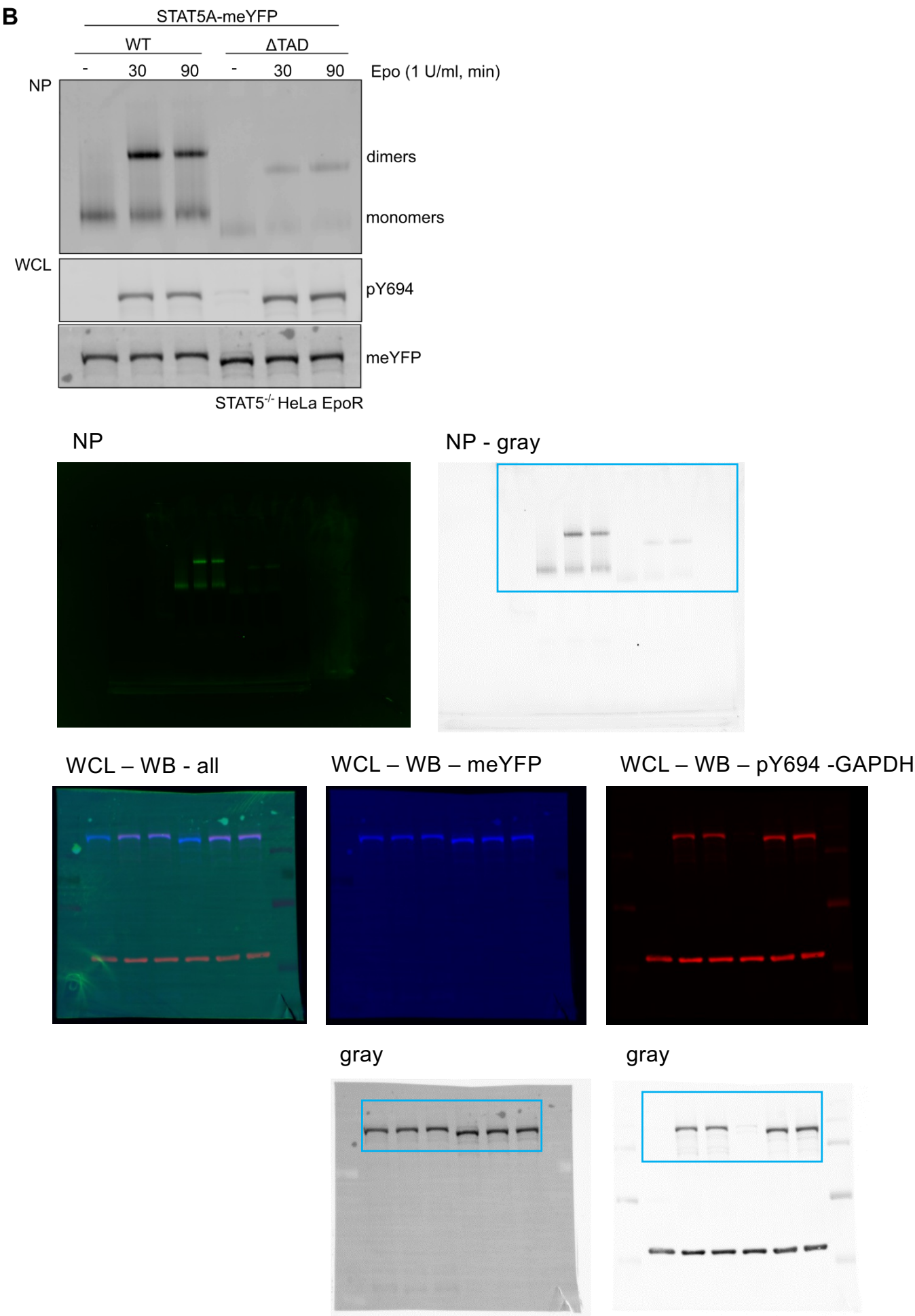
HeLa EpoR cells transduced with STAT5A-ΔNTD-meYFP were stimulated with 2 U/ml Epo as indicated. meYFP fluorescence was detected via confocal live cell imaging. w/o, without; scale bars: upper panel, 50 μm; lower panel, 20 μm.

Suppl. Fig. 7: Uncropped and unedited blot/gel images of all figures

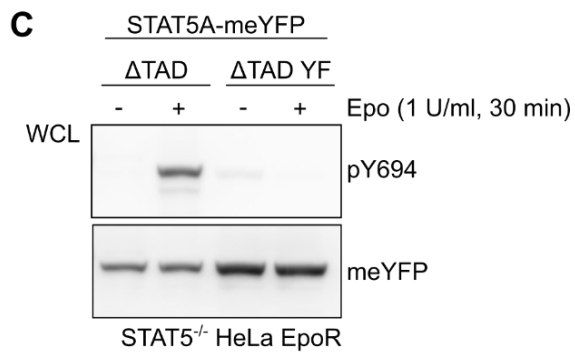
Uncropped and unedited blot/gel images of **Figure 3A**



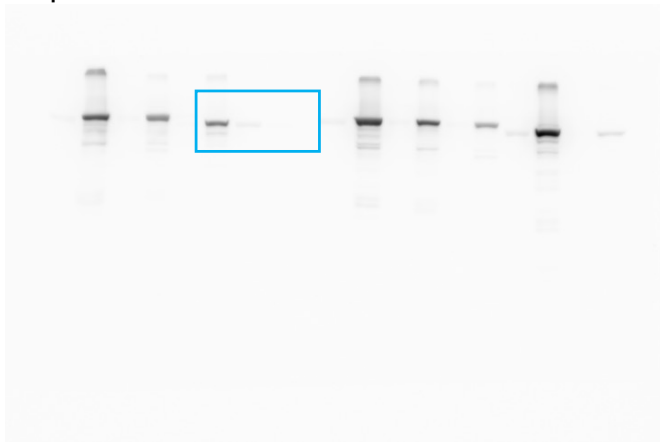
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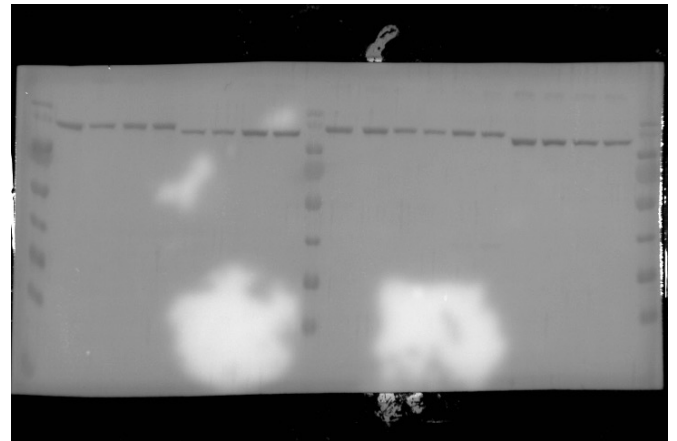
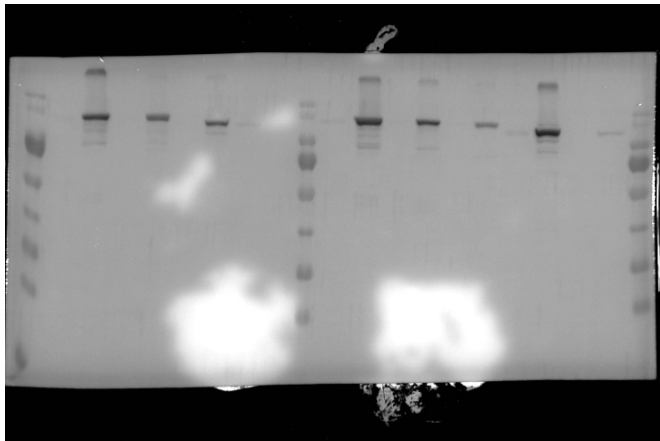
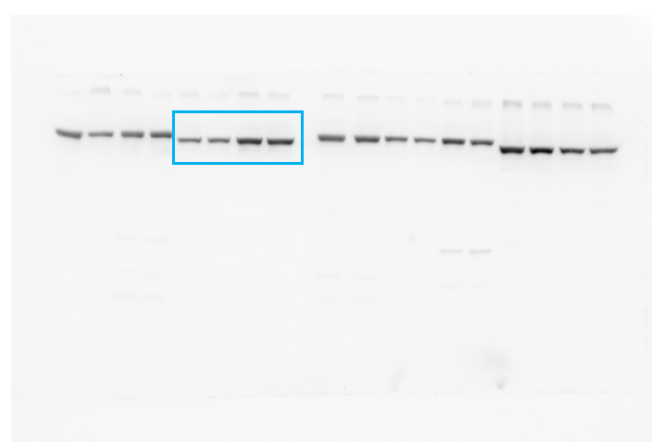
Uncropped and unedited blot/gel images of **Figure 3C**



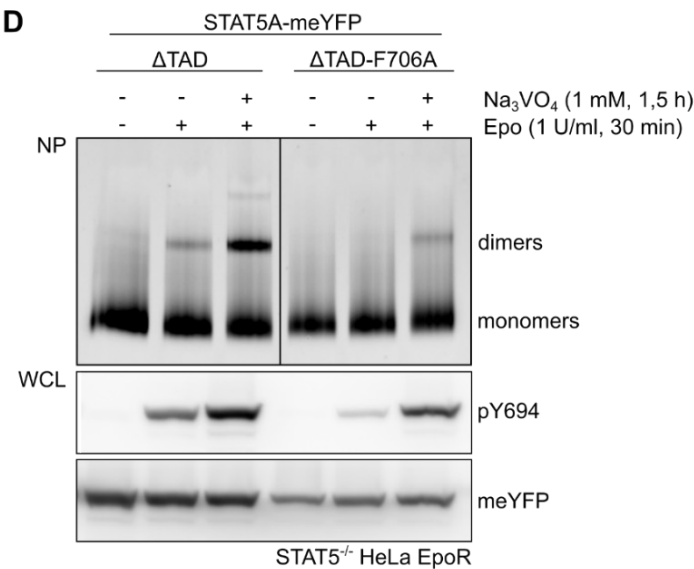
pY694



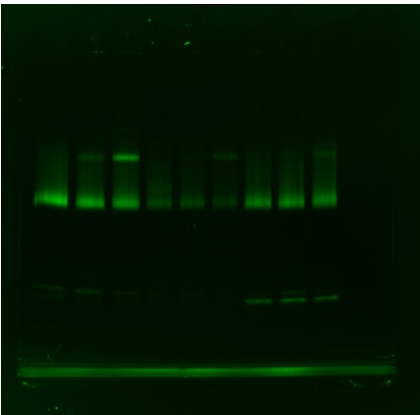
meYFP



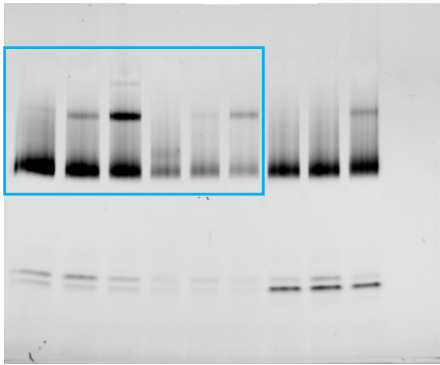
Uncropped and unedited blot/gel images of **Figure 3D**



NP



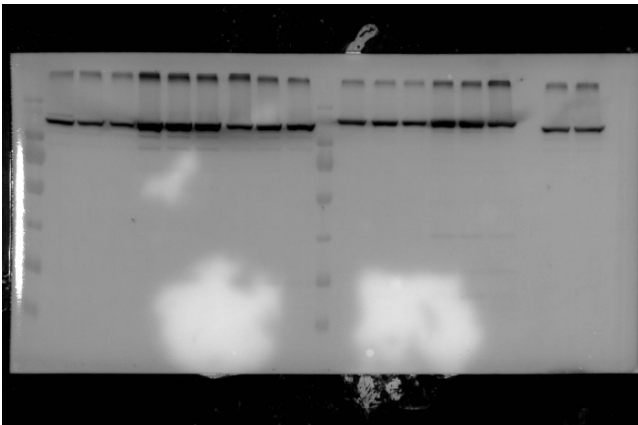
NP - gray



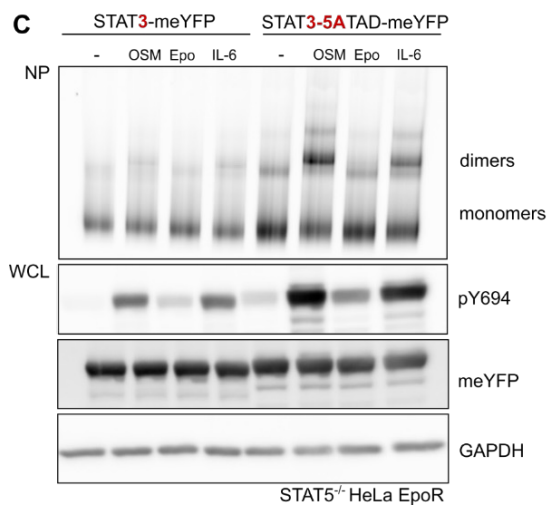
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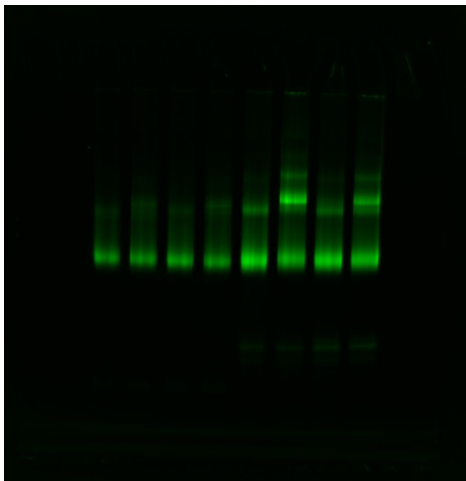
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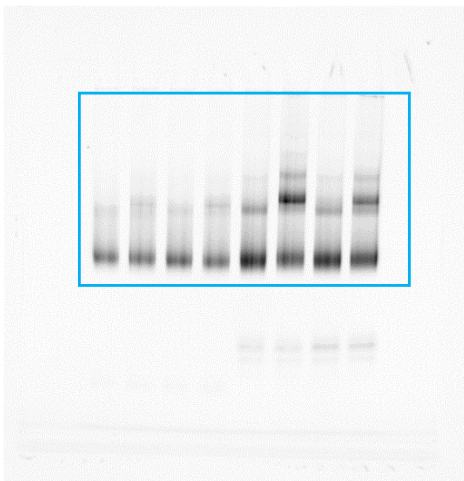
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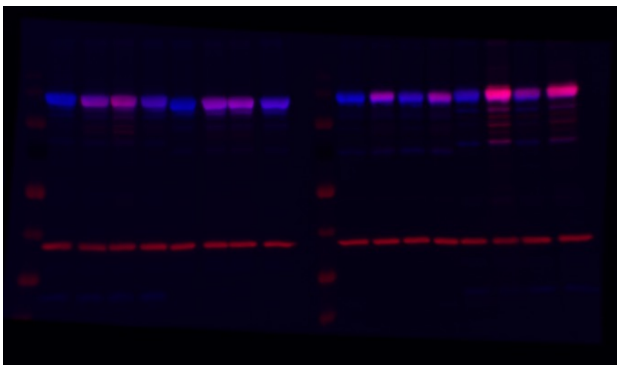
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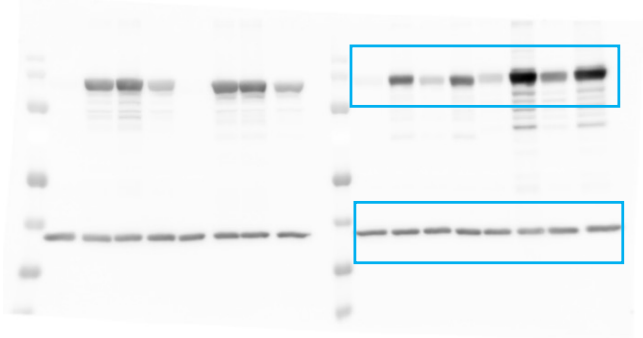
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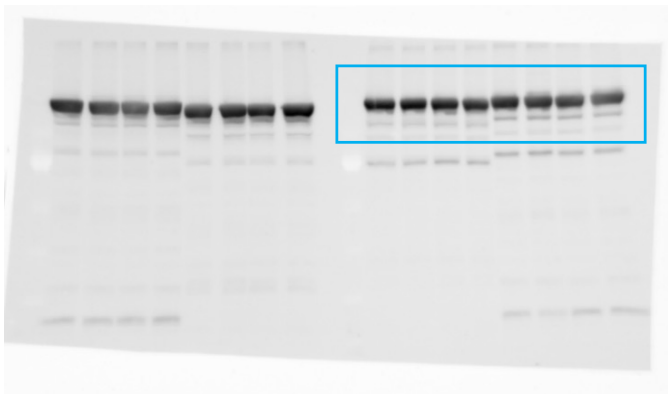
WCL – WB - all



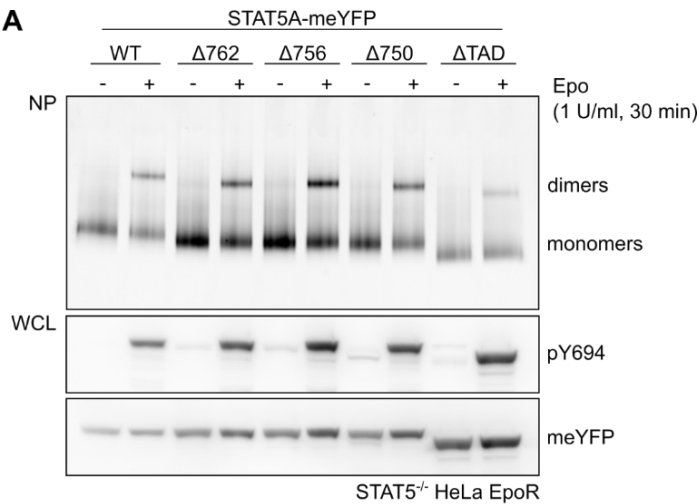
Gray- pY694 - GAPDH



Gray - meYFP



Uncropped and unedited blot/gel images of **Figure 5A**



NP



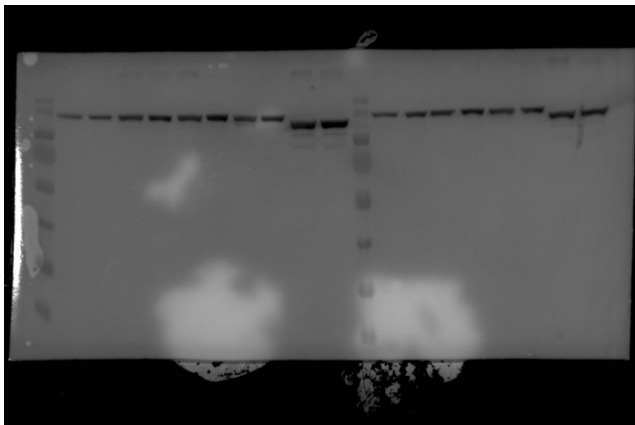
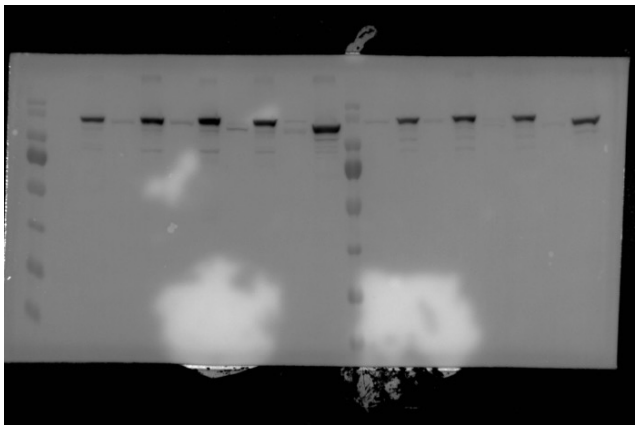
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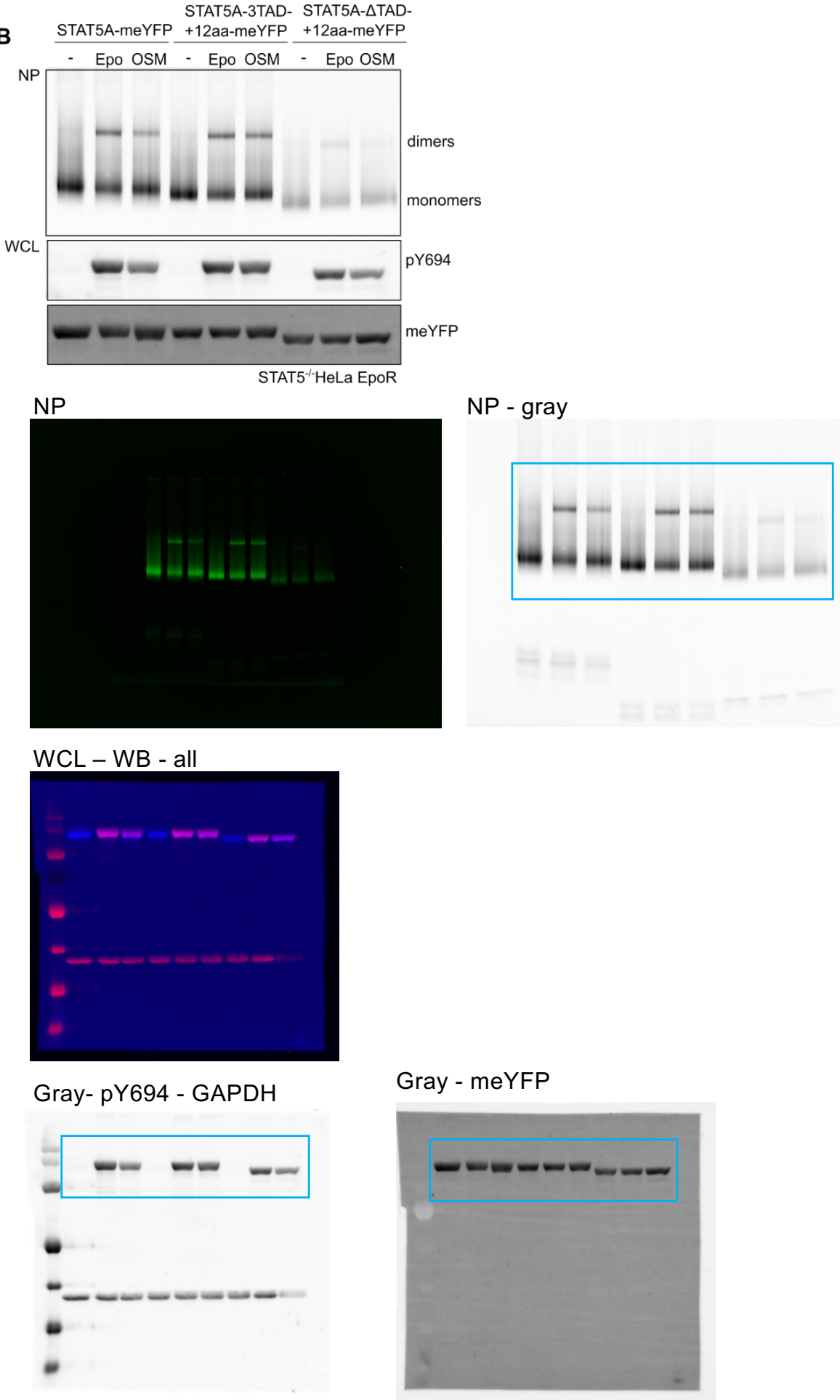
Gray- pY694



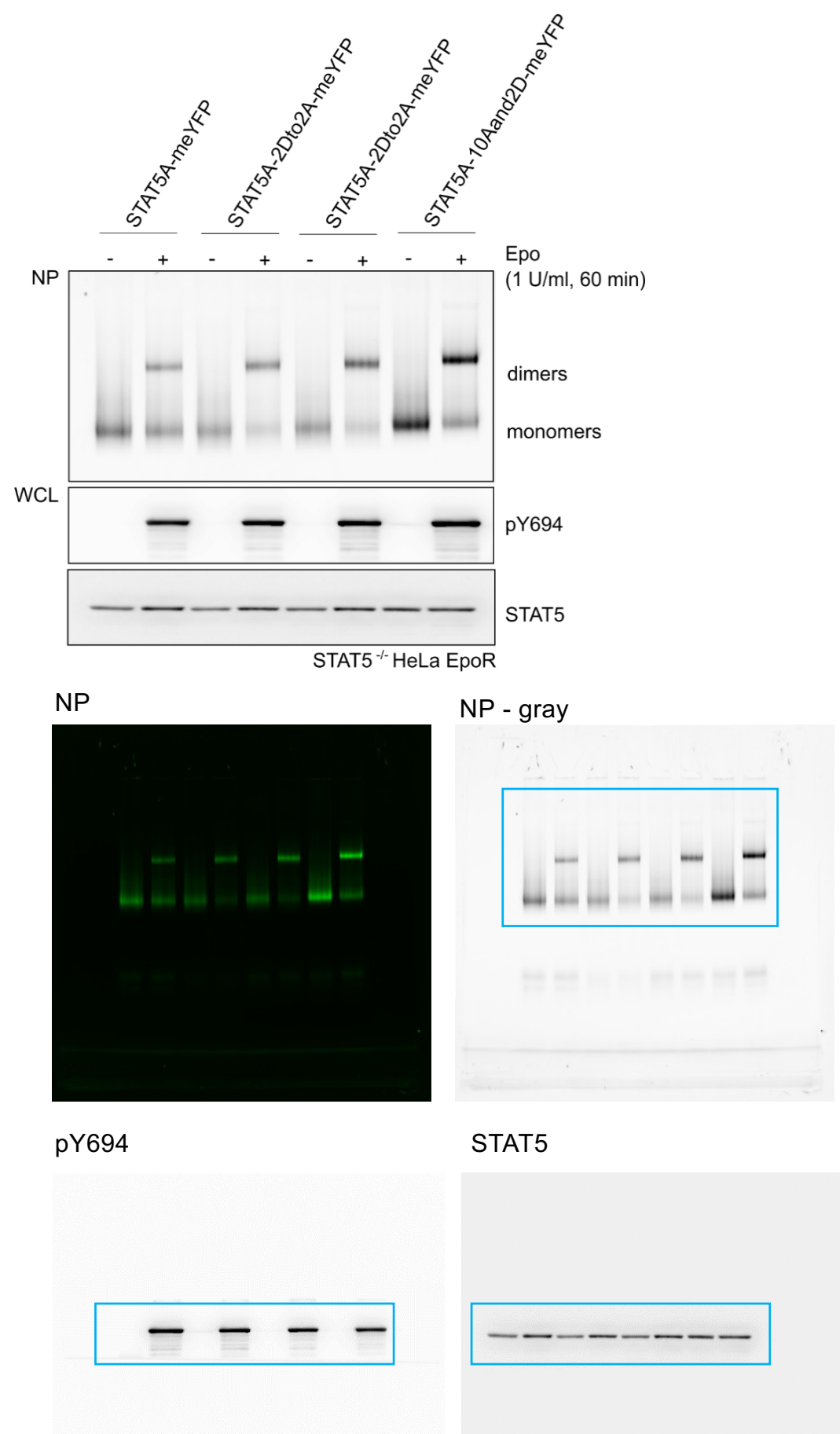
Gray - meYFP



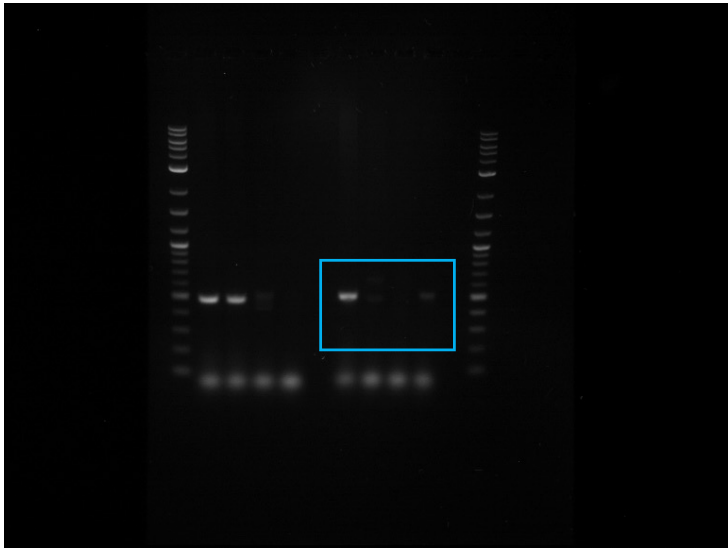
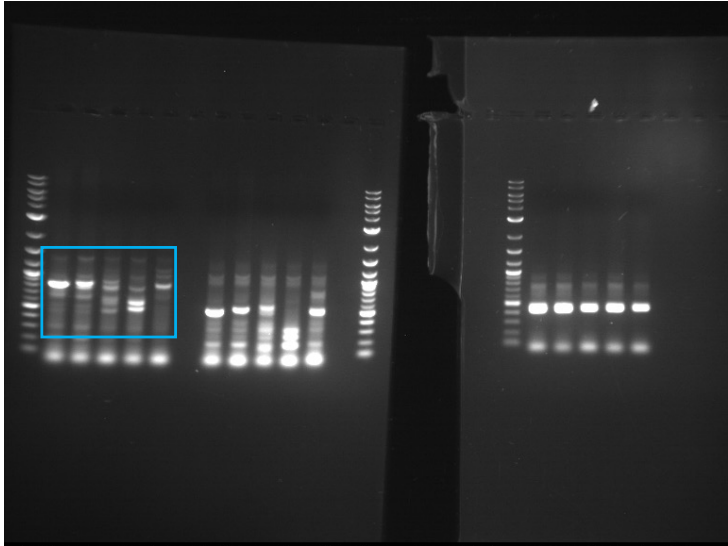
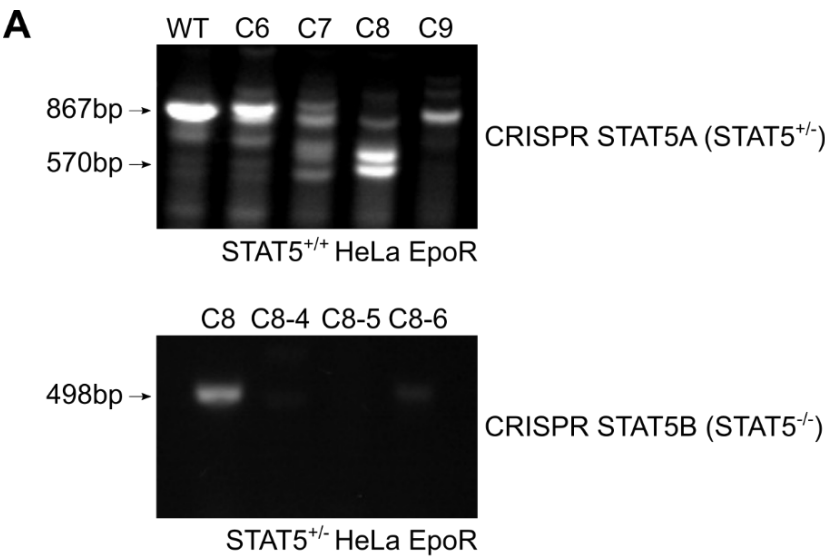
Uncropped and unedited blot/gel images of **Figure 6B**



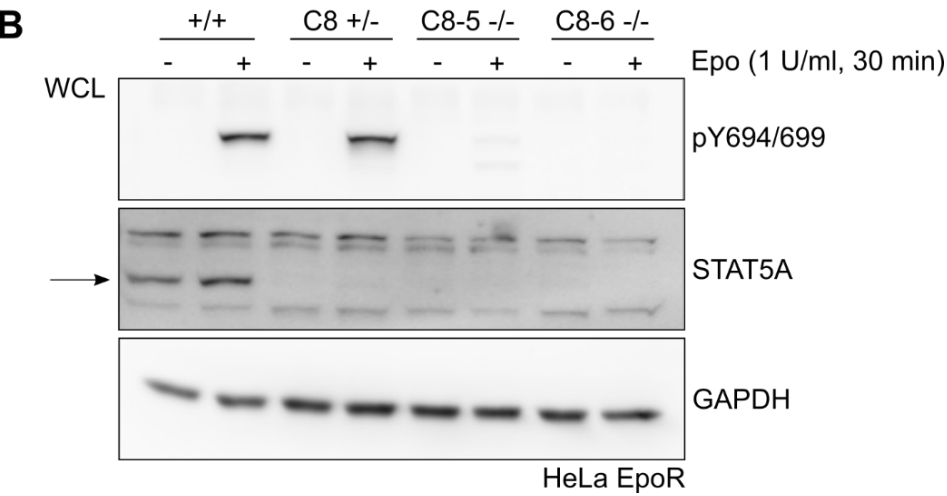
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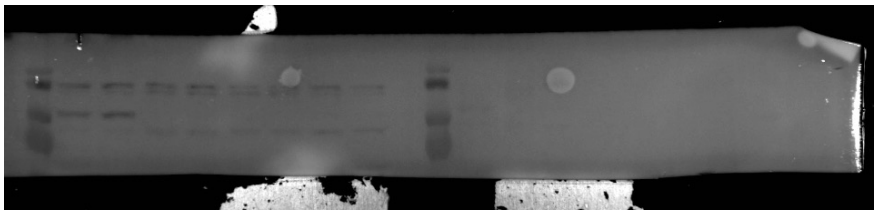
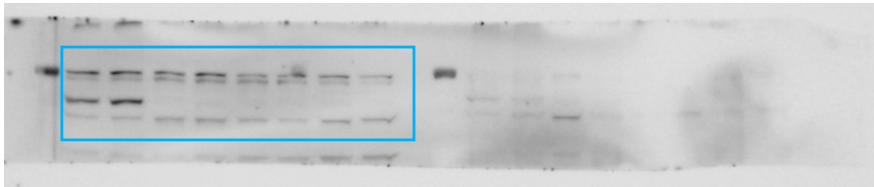
Uncropped and unedited blot/gel images of **Suppl. Fig. 1A**



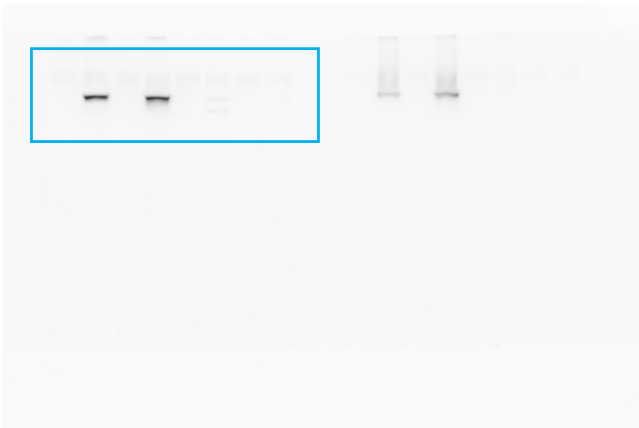
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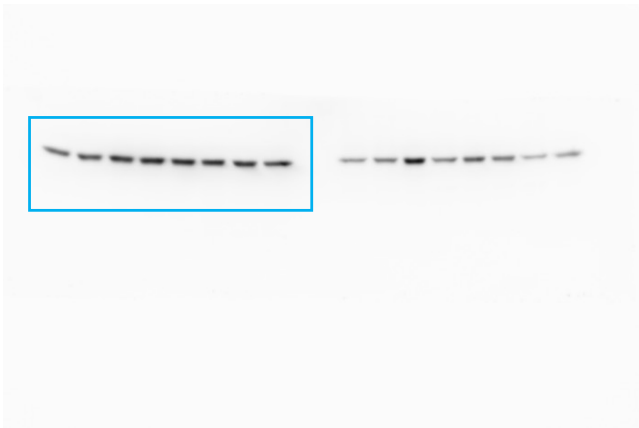
STAT5A



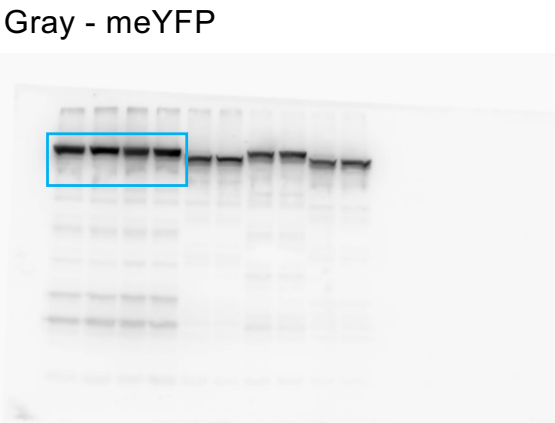
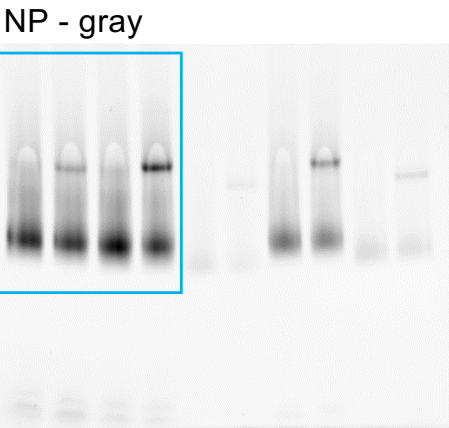
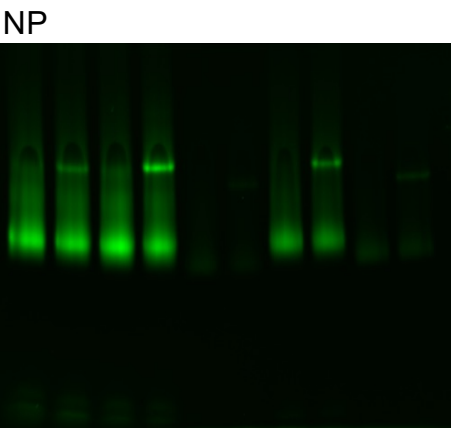
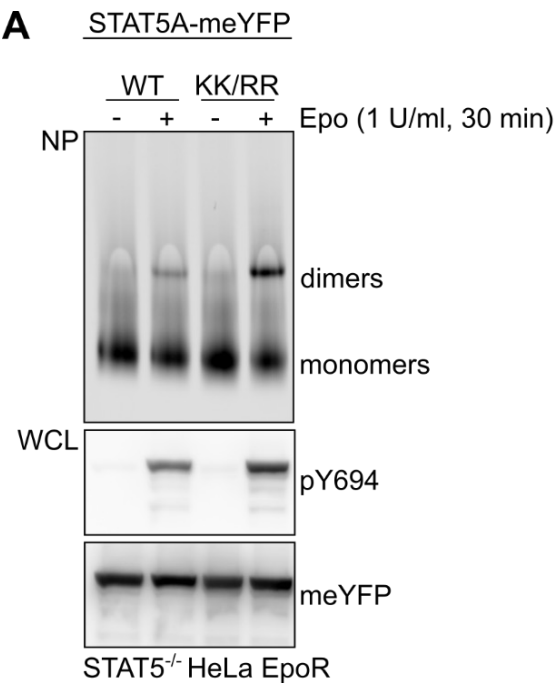
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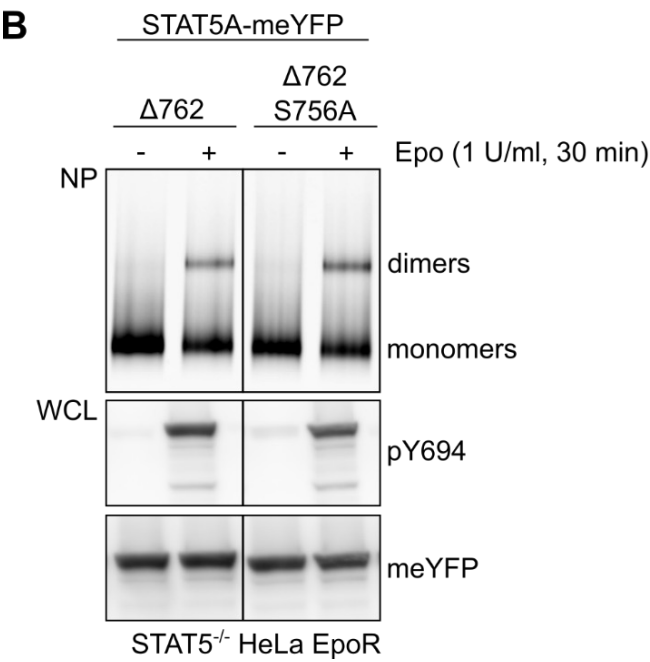
GAPDH



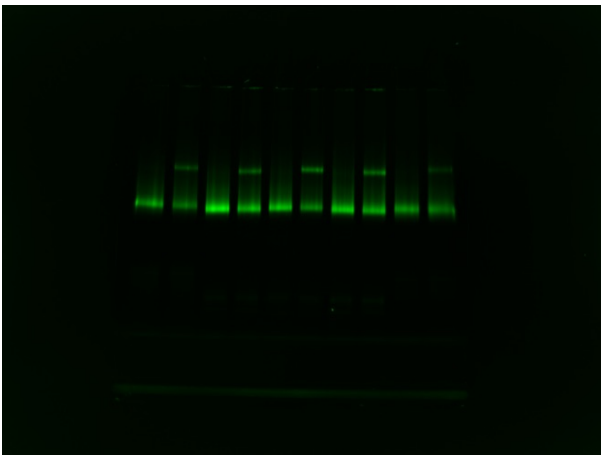
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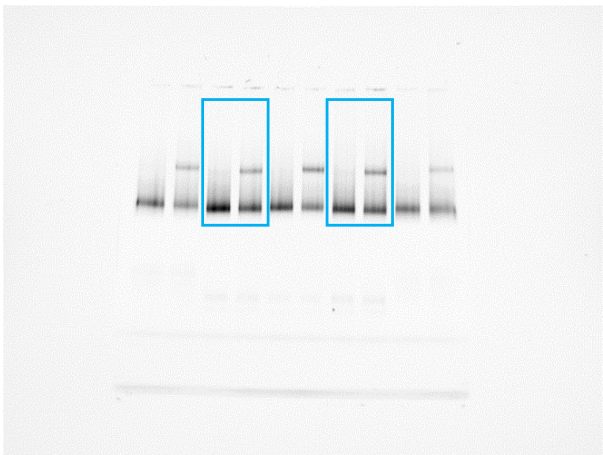
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NP



NP - gray



Gray- pY694



Gray - meYFP

