

Lysine Catabolism Reprograms Tumour Immunity through Histone Crotonylation

Huairui Yuan¹, Xujia Wu¹, Qiulian Wu¹, Adam Chatoff², Emily Megill², Jinjun Gao³, Tengfei Huang¹, Tingting Duan¹, Kailin Yang⁴, Chunyu Jin⁵, Fanen Yuan¹, Shuai Wang¹, Linjie Zhao¹, Pascal O. Zinn^{1,6}, Kalil G. Abdullah^{1,6}, Yingming Zhao³, Nathaniel W. Snyder² & Jeremy N. Rich^{1,7}

¹Hillman Cancer Center and Department of Neurology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

²Department of Cardiovascular Sciences, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA.

³Ben May Department for Cancer Research, The University of Chicago, Chicago, IL, USA.

⁴Department of Radiation Oncology, Taussig Cancer Center, Cleveland Clinic, Cleveland, OH, USA.

⁵Department and School of Medicine, University of California, San Diego, CA, USA.

⁶Department of Neurosurgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

⁷Department of Neurology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

Correspondence should be addressed to J.N.R. (drjeremyrich@gmail.com)

This Supplementary information file contains:

- Supplementary Figures 1-9 legends **Page 1-3**
- Supplementary Tables 1-13 legends **Page 3**
- Supplementary Figures 1-9 **Page 4-12**

- Supplementary Figures 1-9 legends

Supplementary Fig. 1 Raw data for gels, related to Fig. 1 and Fig. 2. **Page 4**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Fig. 1d, 2c, 2e and 2h. Loading controls were run on the same blot.

Supplementary Fig. 2 Raw data for gels, related to Fig. 3. **Page 5**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Fig. 3b, 3f, 3h and 3j. Loading controls were run on the same blot.

Supplementary Fig. 3 Raw data for gels, related to Fig. 4. **Page 6**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Fig. 4b, 4c, 4e, 4g, 4h and 4i. Loading controls were run on the same blot.

The samples derive from the same experiment were run on same gel in Fig. 4d.

Supplementary Fig. 4 Raw data for gels and gating strategy of CD8⁺ T cells, related to Fig. 5. **Page 7**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Fig. 5g. Loading controls were run on the same blot.

Gating strategy of CD8⁺ T cells was used for Fig. 5i and Extended Data Fig. 10f.

Supplementary Fig. 5 Raw data for gels, related to Extended Data Fig. 1, Extended Data Fig. 2 and Extended Data Fig. 3. **Page 8**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Extended Data Fig. 1g, Extended Data Fig. 2q, 2r and Extended Data Fig. 3d, 3f. Loading controls were run on the same blot.

The samples derive from the same experiment were run on same gel in Extended Data Fig. 1l.

Supplementary Fig. 6 Raw data for gels, related to Extended Data Fig. 5. **Page 9**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Extended Data Fig. 5a, 5c and 5d. Loading controls were run on the same blot.

Supplementary Fig. 7 Raw data for gels, related to Extended Data Fig. 6. **Page 10**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Extended Data Fig. 6b, 6e, 6h and 6j. Loading controls were run on the same blot.

The samples derive from the same experiment were run on same gel in Extended Data Fig. 6d, 6f and 6g.

Supplementary Fig. 8 Raw data for gels, related to Extended Data Fig. 6 and Extended Data Fig. 7. **Page 11**

Due to similar molecular weight of some proteins, the samples derive from the same experiment were run on separate gels in Extended Data Fig. 6o and Extended Data Fig. 7j. Loading controls were run on the same blot.

The samples derive from the same experiment were run on same gel in Extended Data Fig. 6k and 6m.

Supplementary Fig. 9 Raw data for gels, related to Extended Data Fig. 8, Extended Data Fig. 9 and Extended Data Fig. 10. **Page 12**

Due to similar molecular weight of some proteins, the samples derive from the same

experiment were run on separate gels in Extended Data Fig. 8a, Extended Data Fig. 9e, 9l and Extended Data Fig. 10e. Loading controls were run on the same blot.

- Supplementary Tables 1-13 legends

Supplementary Table 1 Quantification of intracellular amino acids normalized to both ISTD and cell number using Mass spectrometry.

Supplementary Table 2 Increased amino acids in GSCs compared to both DGCs and NSCs ($p < 0.01$ and fold change > 2).

Supplementary Table 3 Quantification of intracellular amino acids upon SLC7A2 depletion after normalization to ISTD and cell number.

Supplementary Table 4 Differentially expressed genes upon GCDH depletion in GSCs (adjusted $p < 0.05$ and fold change > 2).

Supplementary Table 5 Differentially expressed genes upon ECHS1 loss in DGCs (adjusted $p < 0.05$ and fold change > 2).

Supplementary Table 6 Differentially expressed genes from GSCs cultured in media with low (0.2 mM) or high (2 mM) L-lysine (adjusted $p < 0.05$ and fold change > 2).

Supplementary Table 7 181 genes designated as "Lysine catabolism-repressed genes".

Supplementary Table 8 Identification of GCDH-interacting proteins based on MS-derived Peptide counts.

Supplementary Table 9 Identification of histone H4 crotonylation from GSCs cultured in media with low (0.2 mM) or high (2 mM) L-lysine.

Supplementary Table 10 Identification of enriched transcriptional factors with selective dependency in GSCs compared to DGCs and NSCs based on a computational biology framework "Lisa".

Supplementary Table 11 Differentially expressed retroelements from GSCs cultured in media with low (0.2 mM) or high (2 mM) L-lysine (adjusted $p < 0.05$ and fold change > 1.5).

Supplementary Table 12 Differentially expressed retroelements upon GCDH depletion in GSCs (adjusted $p < 0.05$ and fold change > 1.5).

Supplementary Table 13 List of siRNA, shRNA and qPCR primer.

Supplementary Fig. 1 Raw data for gels, related to Fig. 1 and Fig. 2.

Fig. 1d

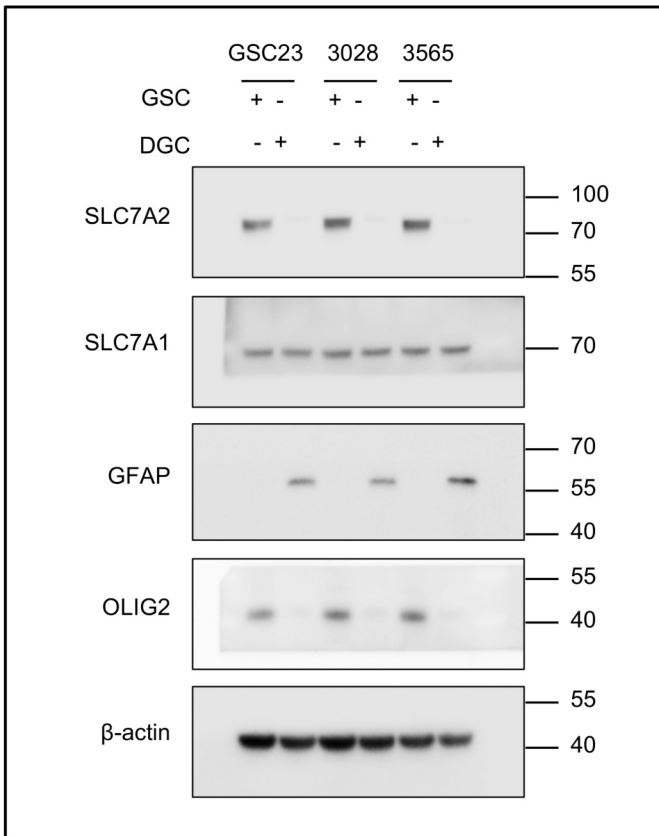


Fig. 2c

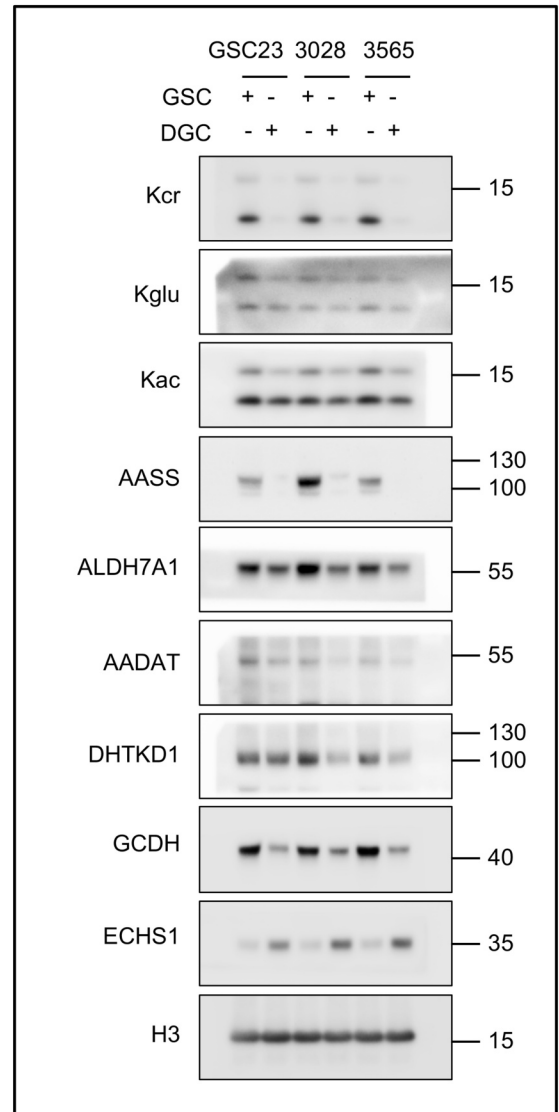


Fig. 2e

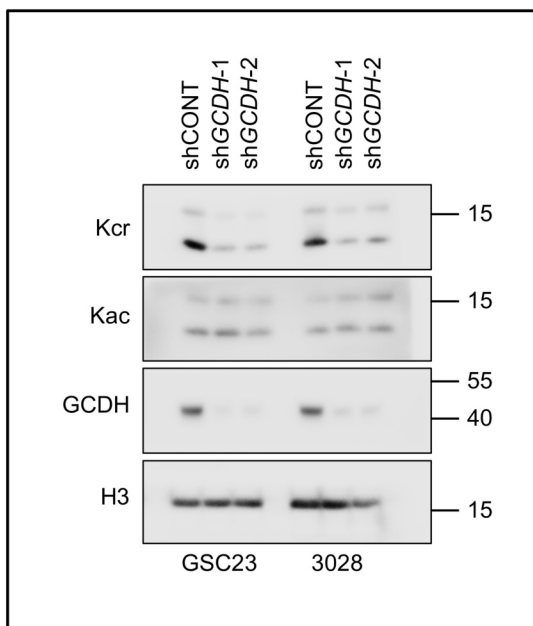


Fig. 2h

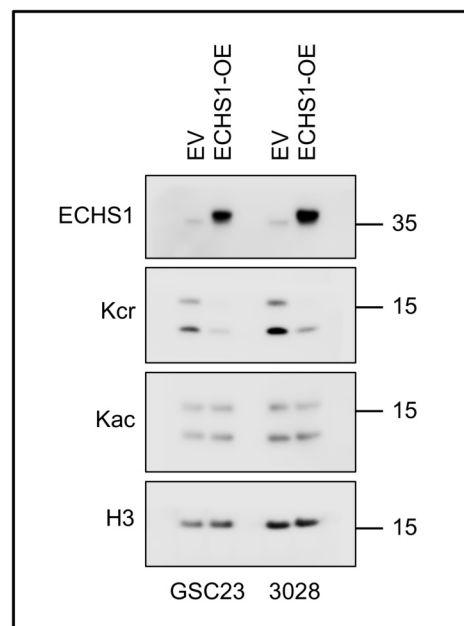


Fig. 3b

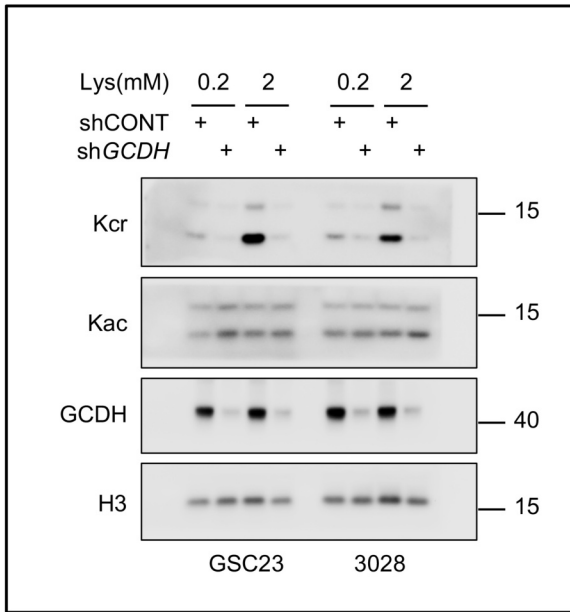


Fig. 3f

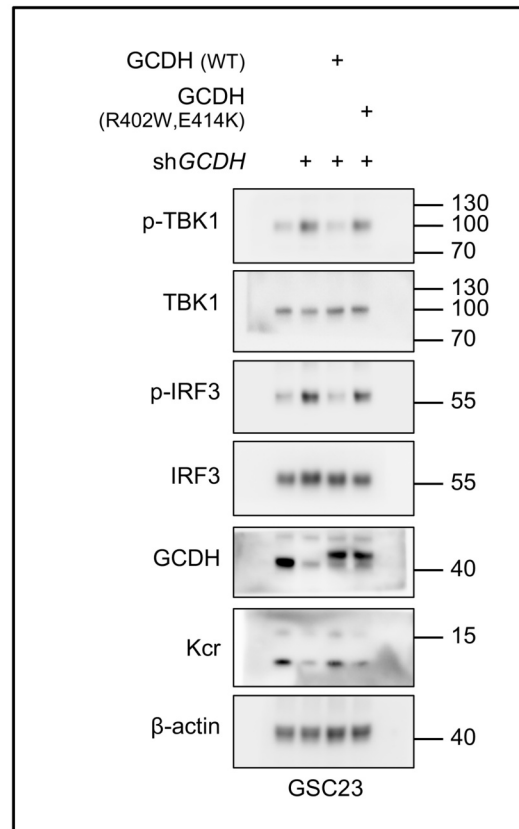


Fig. 3h

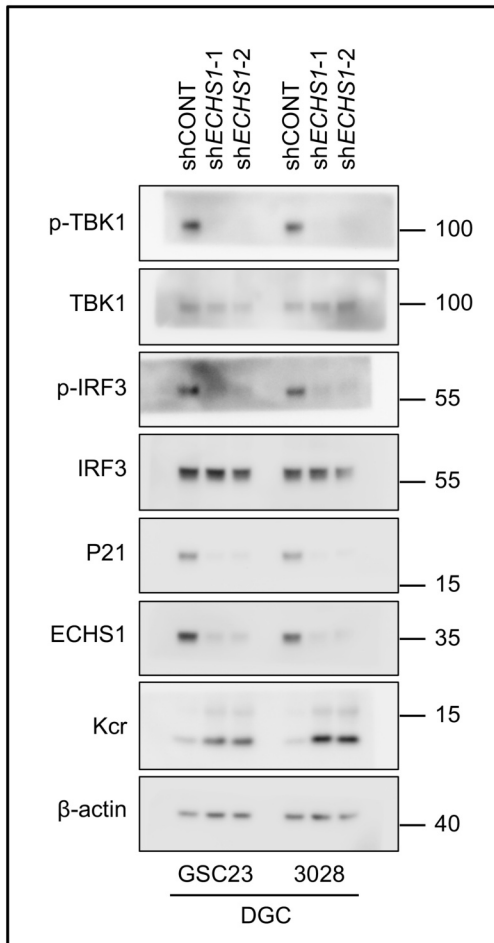


Fig. 3j

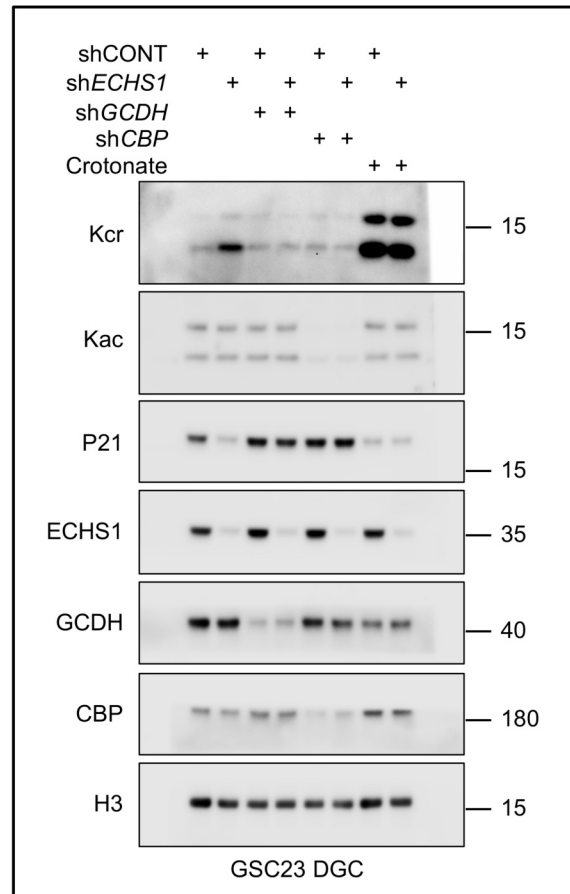


Fig. 4b

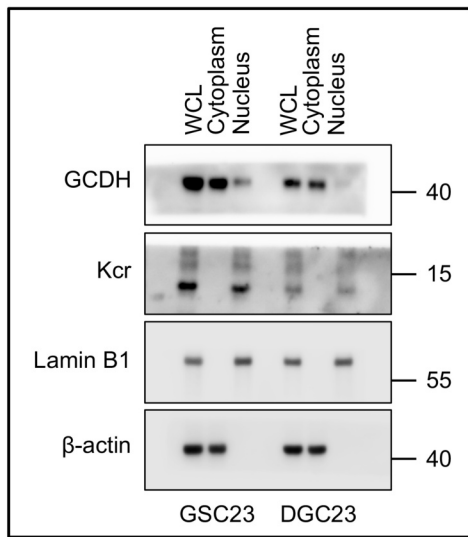


Fig. 4c

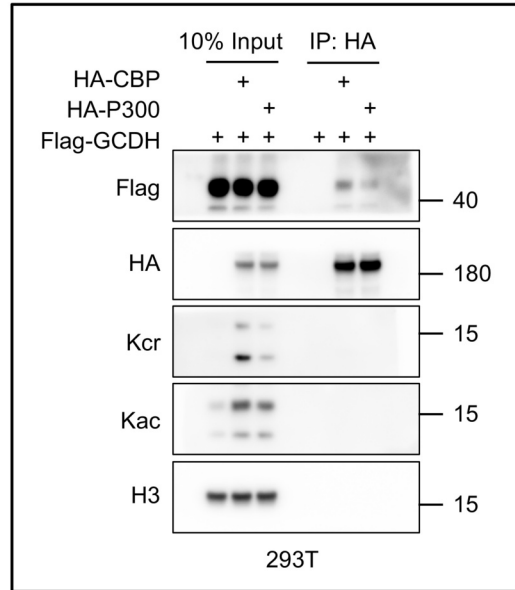


Fig. 4d

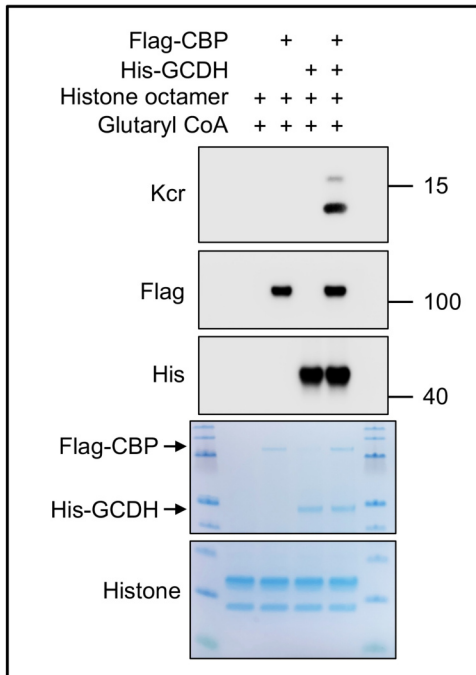


Fig. 4e

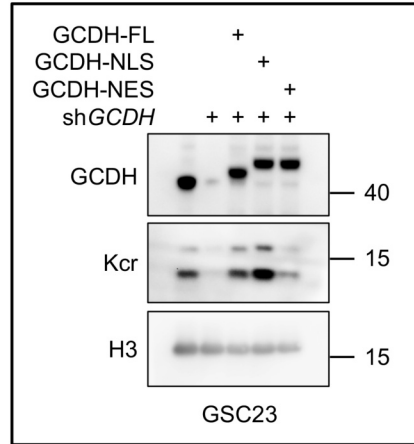


Fig. 4g

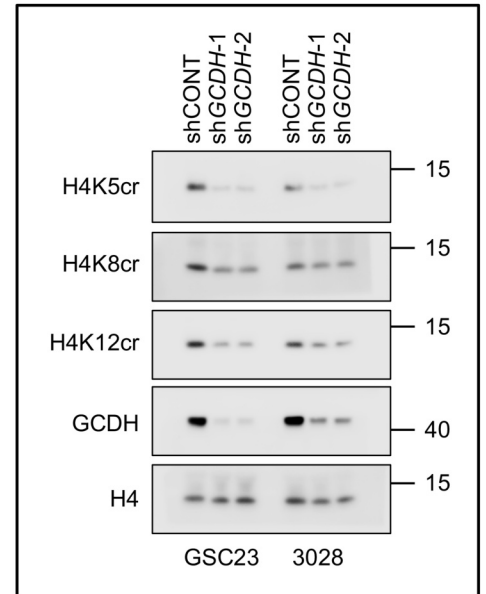


Fig. 4h

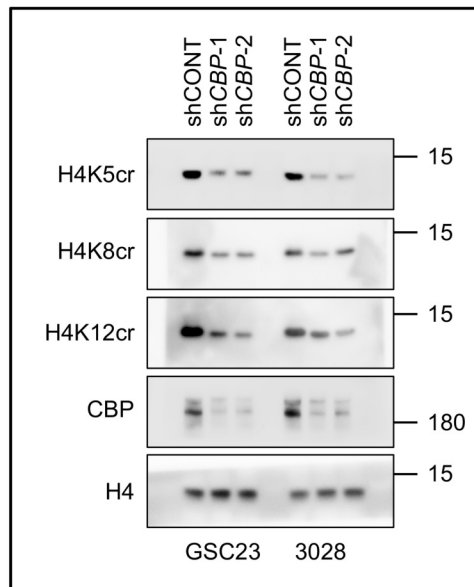
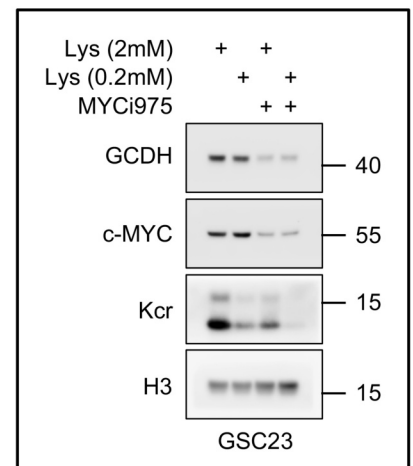


Fig. 4i



Supplementary Fig. 4 Raw data for gels and gating strategy of CD8⁺ T cells, related to Fig. 5.

Fig. 5g

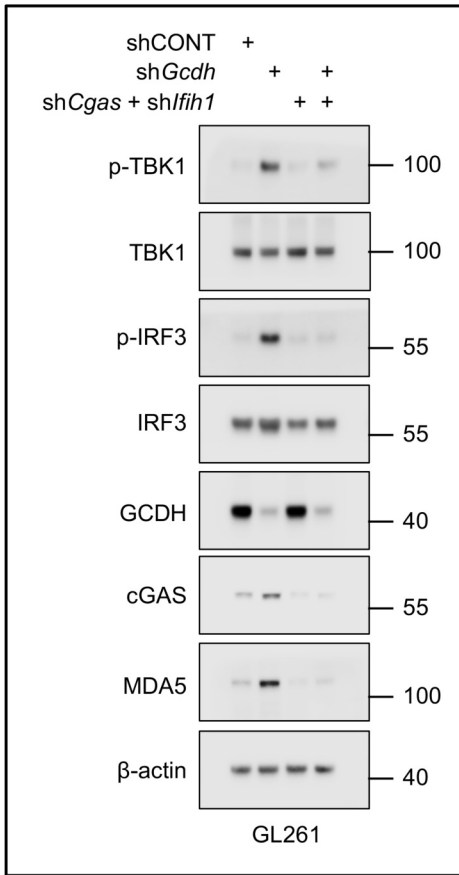
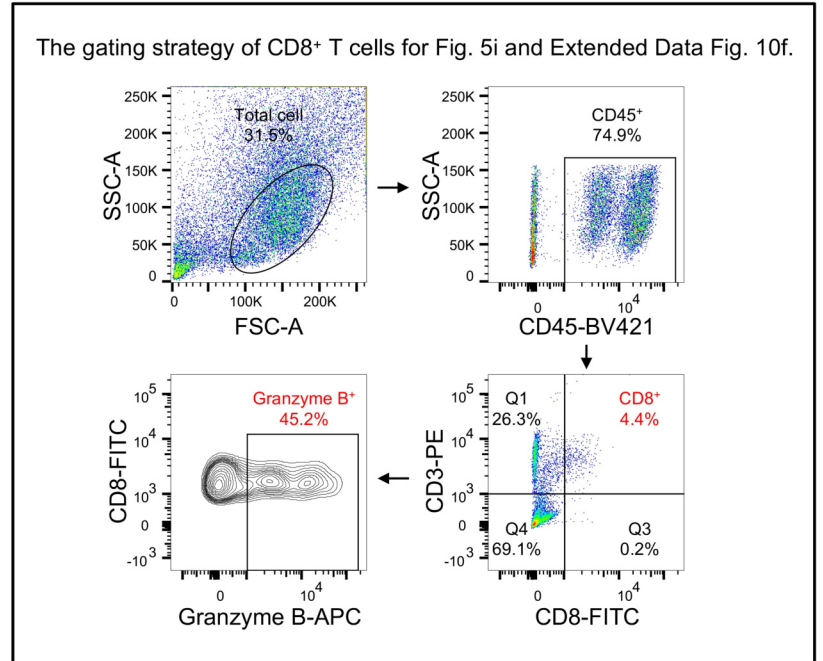
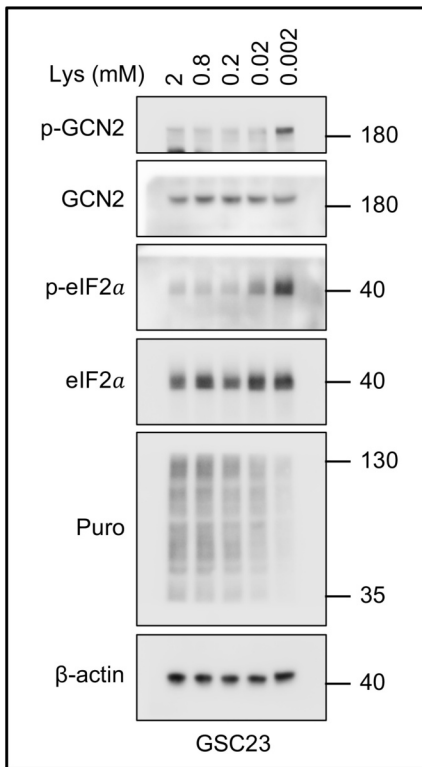


Fig. 5i and Extended Data Fig. 10f.

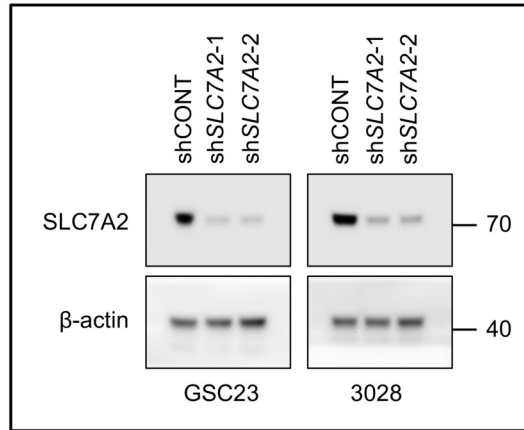


Supplementary Fig. 5 Raw data for gels, related to Extended Data Fig. 1, Extended Data Fig. 2 and Extended Data Fig. 3.

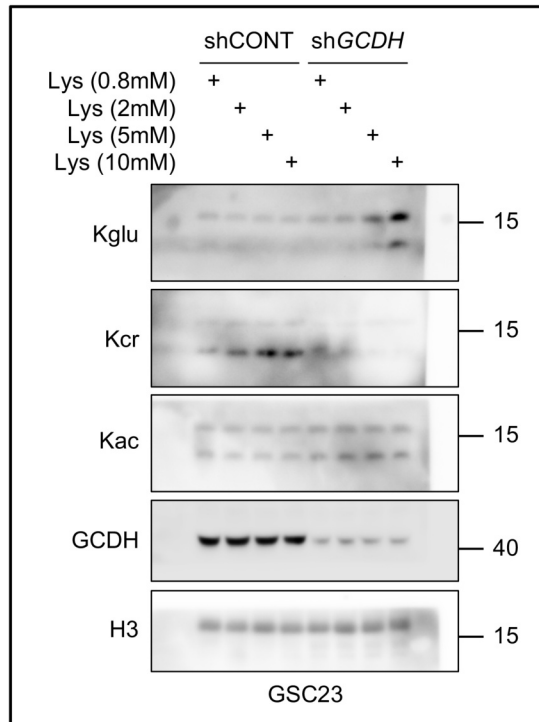
Extended Data Fig. 1g



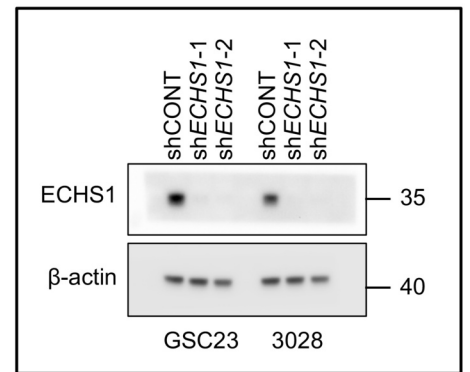
Extended Data Fig. 1i



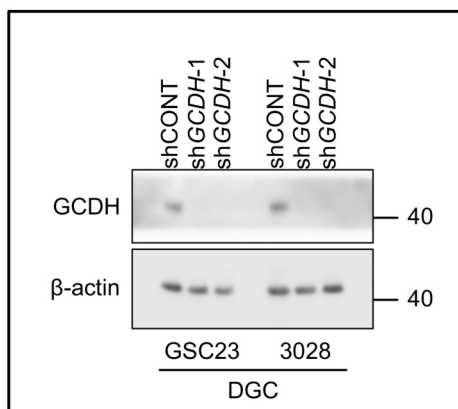
Extended Data Fig. 2q



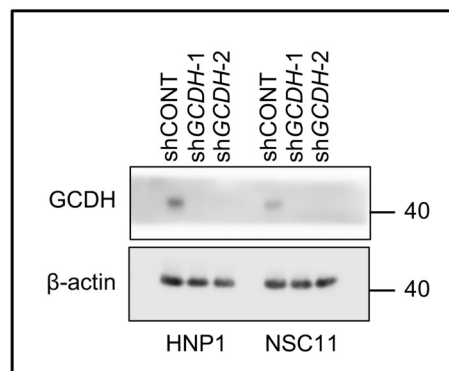
Extended Data Fig. 2r



Extended Data Fig. 3d

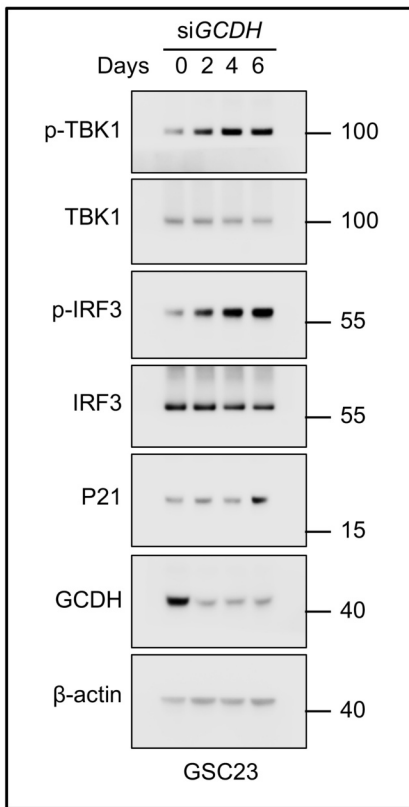


Extended Data Fig. 3f

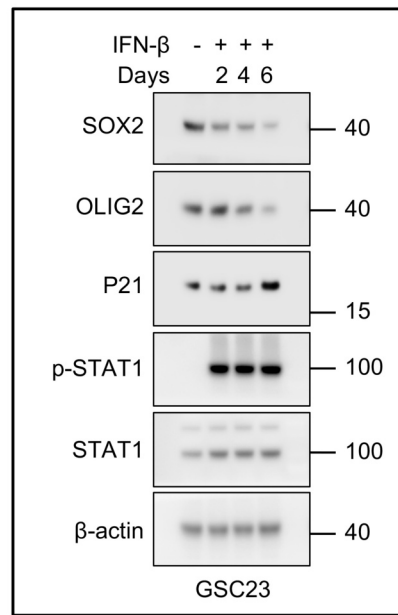


Supplementary Fig. 6 Raw data for gels, related to Extended Data Fig. 5.

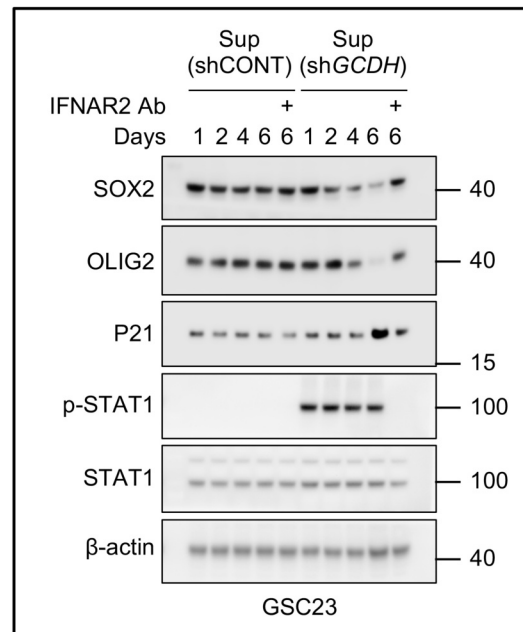
Extended Data Fig. 5a



Extended Data Fig. 5c

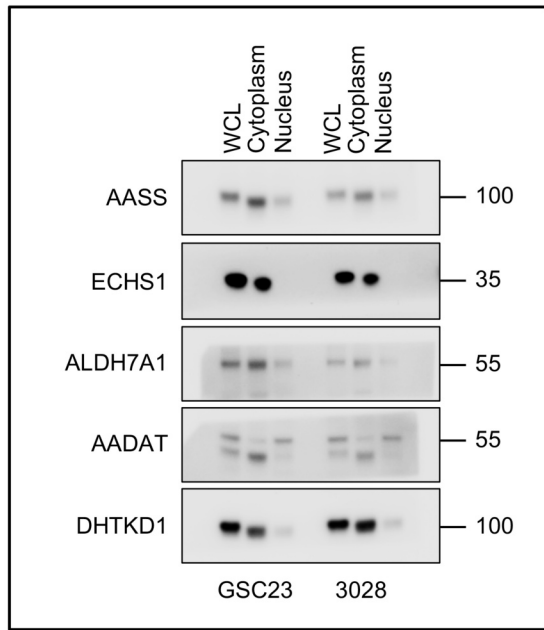


Extended Data Fig. 5d

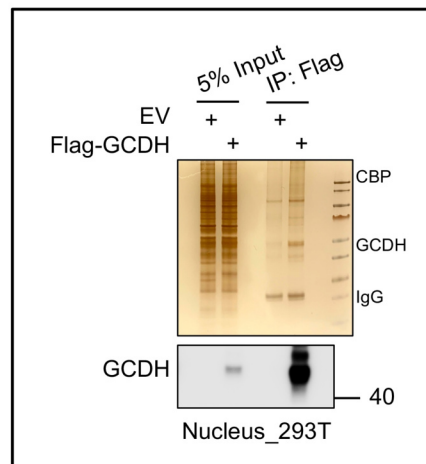


Supplementary Fig. 7 Raw data for gels, related to Extended Data Fig. 6.

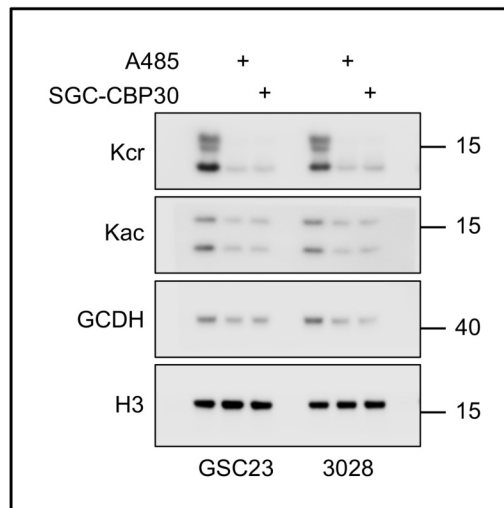
Extended Data Fig. 6b



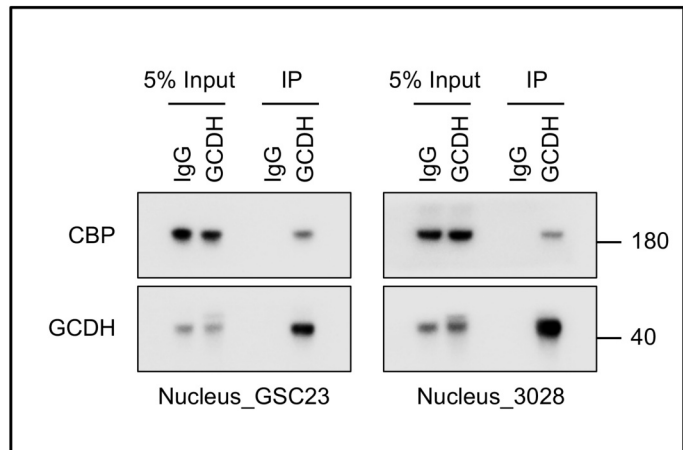
Extended Data Fig. 6d



Extended Data Fig. 6e

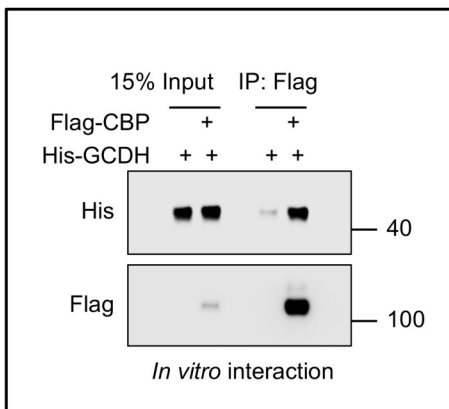


Extended Data Fig. 6f

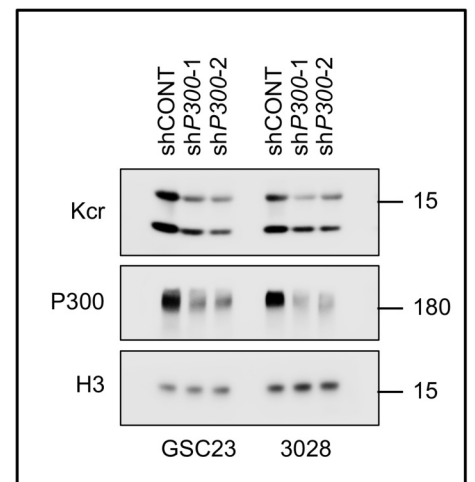
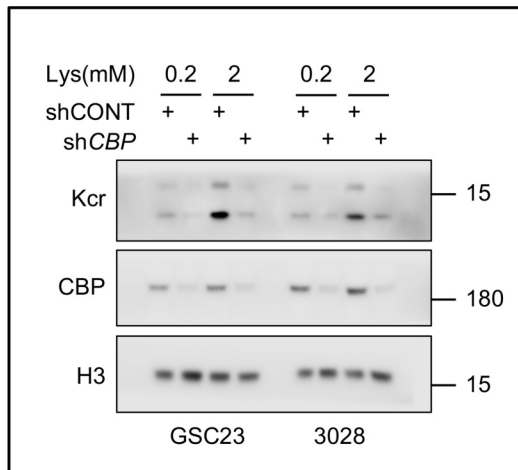


Extended Data Fig. 6j

Extended Data Fig. 6g

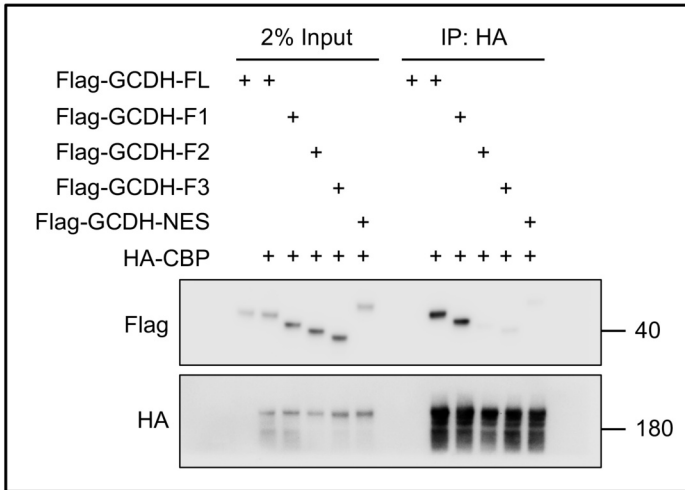


Extended Data Fig. 6h

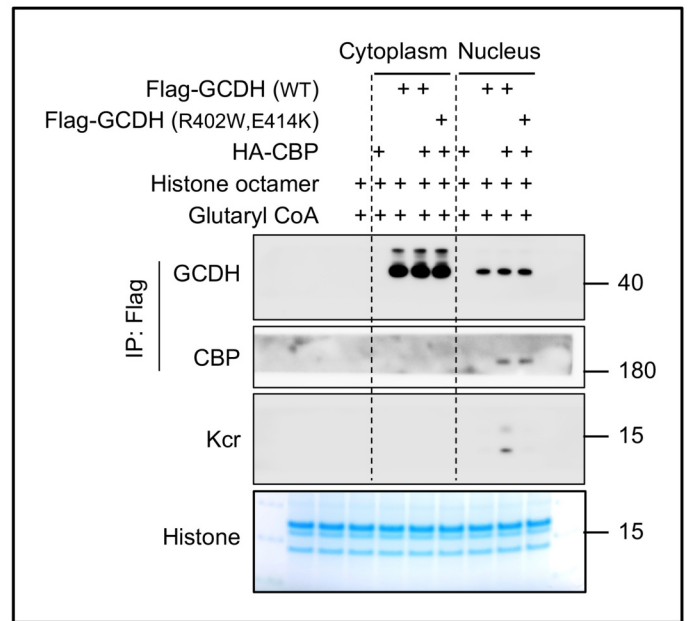


Supplementary Fig. 8 Raw data for gels, related to Extended Data Fig. 6 and Extended Data Fig. 7.

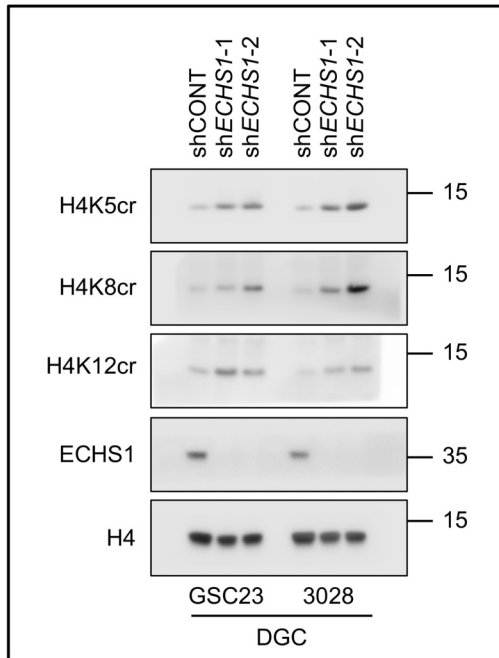
Extended Data Fig. 6k



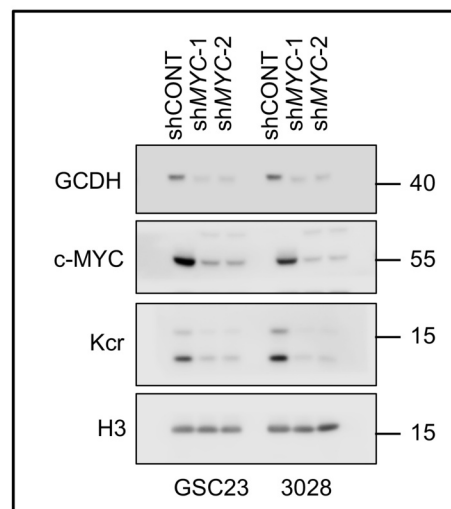
Extended Data Fig. 6m



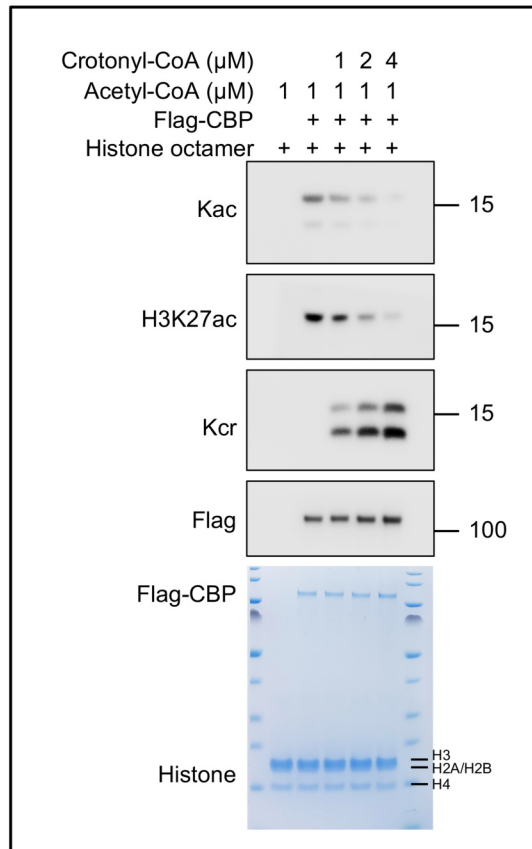
Extended Data Fig. 6o



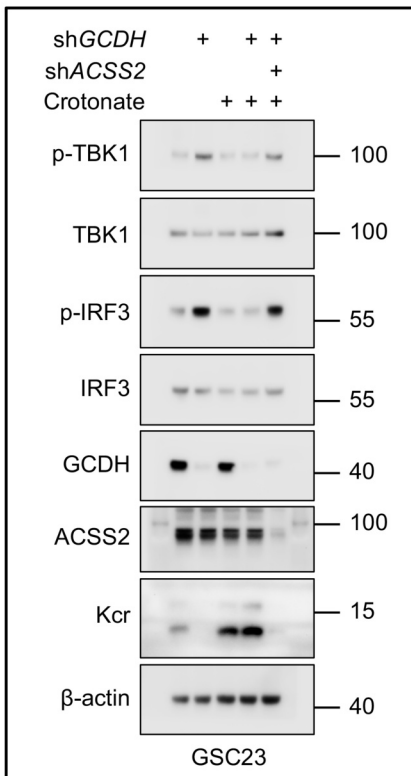
Extended Data Fig. 7j



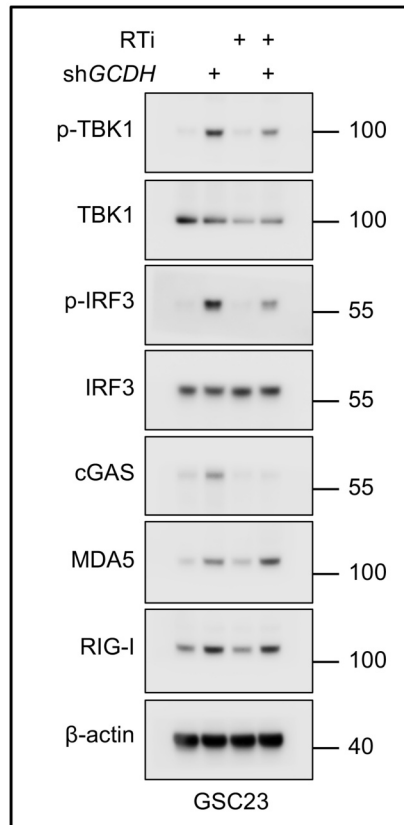
Extended Data Fig. 8a



Extended Data Fig. 9e



Extended Data Fig. 9i



Extended Data Fig. 10e

